The role of YAP in epidermal homeostasis and tumourigenesis

Zoé I. Vincent-Mistiaen

University College London

and

The Francis Crick Institute

PhD Supervisor: Barry J. Thompson

A thesis submitted for the degree of

Doctor of Philosophy

University College London

September 2019

Declaration

I, Zoé I. Vincent-Mistiaen, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Abstract

The skin epidermis, a stratified squamous epithelium, is the main interface between the body and environment. A pool of resident progenitor keratinocytes continually renews this epithelium to maintain homeostasis in the face of constant physical, chemical and biological attacks. Furthermore, plasticity in the activity of these progenitor cells enables rapid wound healing to maintain barrier function. This flexibility is a double-edged sword, as many of the processes necessary for epithelial tissue repair are implicated in tumourigenesis. Indeed, epidermal carcinomas are the most common cancers worldwide, with incidence continuing to rise. The molecular mechanisms regulating epidermal progenitor cell fate, wound healing and tumour formation are not well understood. In this thesis, I highlight the central role of the transcriptional regulator YAP in these processes. I show that in normal skin, YAP is nuclear and transcriptionally active in basal layer progenitor cells and that this depends on contact with the basement membrane and consequent integrin-Src signalling. Thus, YAP couples progenitor cell adhesion to the basal extracellular matrix with the expression of genes involved in epidermal cell proliferation and hence skin renewal. I also show that nuclear YAP is elevated at the margin of healing wounds, where it stimulates proliferation; in the absence of YAP and its paralogue TAZ, cutaneous wound healing is impaired. However, pathological activation of YAP causes hyperproliferation of these basal layer cells, which can result in epidermal hyperplasia and squamous cell carcinoma (SCC). Indeed, YAP is widely expressed in human SCCs and overexpression of YAP drives SCC in mice. SCC can sometimes progress to a malignant metastatic subtype, characterised by atypical spindle-shaped mesenchymal cells, known as spindle cell SCC (spSCC). I find that whilst human spSCC is characterised by strongly nuclear YAP, epidermal-specific overexpression of activated YAP (NLS-YAP-5SA) in mice does not always cause spSCC, suggesting that another factor could be at play. It is thought that spSCC formation involves an epithelial-mesenchymal transition (EMT), but the molecular mechanism is not understood. I noted that whilst SCCs can arise randomly throughout the skin, spSCCs develop at scratch wound sites. I report that EMT transcription factor ZEB1 is widely expressed in human and murine spSCCs, but not in SCCs. During normal wound

healing YAP induces transient ZEB1 expression, whereas in the presence of constitutively active YAP, ZEB1 expression is sustained and a YAP-ZEB1 positive feedback loop drives spSCC formation. I propose that YAP synergises with the wound healing response to promote ZEB1-mediated EMT and metaplastic transformation of SCC to spSCC. Thus, YAP provides a link between epithelial damage and tissue repair, which can be hijacked by cancer. This could explain why spSCCs are so frequently associated with skin damage from trauma or UV radiation.

Highlights

Basal Integrin-Src signalling promotes nuclear YAP localisation in keratinocytes *in vivo*

YAP subcellular localisation across epithelia is regulated by antagonism between apical and basal signals

YAP synergises with the wound healing response to drive metastatic progression of squamous cell carcinoma to spindle cell squamous cell carcinoma via a ZEB1-mediated EMT

Impact Statement

The skin epidermis constitutes the outermost barrier between the body and external environment, providing protection against pathogens, radiation, abrasion and dehydration. The epidermis is continually renewed by a pool of stem/progenitor cells to maintain homeostasis. These progenitor cells are also crucial to heal wounds. However, their deregulation can lead to tumourigenesis. Epidermal carcinomas are the most common cancers worldwide, with incidence rising with increased UV exposure. Understanding the molecular mechanisms regulating the progenitor cells could help to develop better cancer therapies, as well as regenerative treatments. In my thesis, I show that the transcriptional regulator YAP is implicated in both epidermal wound healing and tumourigenesis. This suggests that YAP and/or its regulators could be exploited in regenerative medicine and targeted in tumour therapies.

Acknowledgement

First and foremost, thank you to Barry Thompson for giving me the opportunity to do this project, for being such a motivating supervisor, full of energy and ideas; always there for support and advice whilst allowing me my independence over my work. I have learned so much from him and will miss our discussions about YAP, science in general, politics, religion and everything else. Thanks to everyone in the Thompson lab for creating such a relaxed, fun atmosphere to work in. I am grateful and lucky to have worked with such bright, caring, hilarious and silly people. I will miss our coffees, gossips, karaoke and jokes. I am particularly indebted and grateful to Ahmed Elbediwy, my collaborator, mentor and friend. I could not have done this PhD without him, scientifically and emotionally. Huge thanks to the BRF technicians and Experimental Histopathology, especially Emma Nye and Brad Spencer-Dene. Thanks to George for being the best lab head and letting me show her one million photos of my cats without complaining. MC, Nerea y Mario: gracias por las risas. Thanks to Ilaria Malanchi for the constructive criticism and many great ideas. Thanks also to Dinis Calado, Sally Leevers, Stefanie Wculek, Rob, Graham, Hannah, Oriane, Hillary, the Tapon lab and the whole Crick fly community.
I could not have done this PhD without the unwavering support of my friends and family. Thanks to my parents Véronique and Jean-Paul for encouraging me to ask questions, always supporting me, believing in me and making me laugh. Alec, for being my brother and best friend. His inquisitive, patient, thoughtful and sensitive way of viewing the world and asking questions amazes and inspires me. Thanks to all of my friends, in particular: Emma for her constant support, love, wit, memes, and probably saving my life; Pavlina for library (/knights templar) sessions and being the funniest person I know; Poppy, my emo day 1, for the best times and best discussions; Juliana, mi hermana from the other side of the Atlantic; Laura and Alex, or they'll milkshake me. Thanks to Sarah Baichoo, for helping me to understand and not fear my brain. Finally, thank you to Rosie for her infectious curiosity about nature and life, her pure kindness, for being the best person I've ever met and the love of my life.

This work was funded by Cancer Research UK and the Wellcome Trust.

Table of Contents

Table of figures

Abbreviations

AJ	Adherens junctions
AMOT	Angiomotin
ANKRD1	Ankyrin Repeat Domain 1
AREG	Amphiregulin
AK	Actinic Keratoses
Akt	Also known as Protein Kinase B (PKB)
AXL	AXL receptor tyrosine kinase
BCC	Basal cell carcinoma
BIRC5	Baculoviral IAP Repeat Containing 5
BM	Basement membrane
BMPs	Bone morphogenetic proteins
CDC42	Cell division control protein 42
ChIP	Chromatin immunoprecipitation
CRB3	Crumbs3
Cre-ERt	Fusion protein of Cre recombinase and mutated estrogen receptor (tamoxifen-inducible)
CTGF	Connective tissue growth factor
CycE	Cyclin E
CYR61	Cysteine-rich angiogenic inducer 61
dKO	Double knockout
E-cad	E-cadherin
ECM	Extracellular matrix
EGFR	Epidermal growth factor receptor
EMT	Epithelial-mesenchymal transition
F-actin	Filamentous actin
FA	Focal adhesion
FAK	Focal adhesion associated kinase
GPCRs	G protein coupled receptor
H&E	Haemotoxylin and eosin
IFE	Interfollicular epidermis
iPS	Induced pluripotent stem cells

ITGB1	Integrin beta 1
K5	Keratin 5
K14	Keratin 14
LATS1/2	Large tumour suppressor-1/2 (Warts in *Drosophila*)
Lgl2	Lethal giant larvae 2
MAPK	Mitogen-activated protein kinase§
MAP4Ks	Mitogen activated protein kinase kinase kinase kinase
MOB1	Mps one binder
MST1/2	Mammalian sterile 20-like-1/2 (Hippo in *Drosophila*)
NF2	Neurofibromatosis type 2 (Merlin in *Drosophila*)
NLS	Nuclear localisation signal
NMSC	Non-melanoma skin cancer
NuRD	Nucleosome Remodeling Deacetylase
PCNA	Proliferating cell nuclear antigen
PCR	Polymerase chain reaction
PDK1	Phosphoinositide-dependent kinase
PDZ domain	Postsynaptic density 95/disc large/zonula occludens-1domain
RNA-seq	RNA sequencing
PI3K	Phosphoinositide-3 kinase
PP2A	Protein phosphatase 2A
Rho	Ras homolog gene family
RNAi	RNA interference
ROCK	Rho-associated protein kinase
RTK	Receptor tyrosine kinase
RT-PCR	Real Time PCR
SAV1	Salvador homolog 1
SCC	Squamous cell carcinoma
siRNA	Small interfering RNA
spSCC	Spindle cell squamous cell carcinoma
SWI/SNF	SWItch/Sucrose Non-Fermentable
TAZ	Transcriptional co-activator with PDZ-binding motif, also known as WWTR1 (WW domain-containing transcription regulator protein 1)
TEAD	Transcriptional enhancer factor TEF-1 (TEA *domain)*

UV	Ultraviolet
WBP2	WW domain binding protein 2
YAP	Yes-associated protein
YAP-5SA	YAP with the 5 HXRXXS serine motifs mutated to alanine
ZEB1	Zinc Finger E-Box Binding Homeobox 1

Chapter 1. Introduction

1.1. Overview

The word “tissue” is derived from the French verb “tisser”, meaning woven together. How do metazoa organize cells into biological tissues with such complexity and diversity in shapes and sizes? In his seminal work “On Growth and Form”, D’arcy Thompson proposed that shapes and patterns of biological structures were self-organising and determined by physical forces (Thompson 1917). Whilst Thompson did not predict the molecular determinants that were discovered over the following hundred years, his notions of tissue mechanics and self-assembly remain remarkably relevant.

The mechanisms governing tissue genesis and homeostasis are incredibly robust. Organ form is ultimately the sum of cell growth, proliferation, death and migration spatially and temporally coordinated at the tissue level. Even when these processes are significantly perturbed during development, tissues grow to the correct size. Furthermore, many tissues are able to regenerate following damage. How are cells able to sense where they are in a tissue as well as overall tissue architecture and integrity and then decide whether to divide or differentiate? The YAP/TAZ family of transcriptional co-activators have emerged as key regulators of tissue growth. Multiple biochemical, mechanical and polarity cues are known to converge on YAP/TAZ to direct changes in the transcription of pro-proliferative genes. However, the molecular mechanisms regulating YAP/TAZ are not fully understood.

The skin is the largest organ in the body. Cells lost from the surface are continuously replenished by self-renewal of epidermal stem cells to maintain intact barrier function. In this thesis, I sought to better understand how YAP/TAZ sense and direct changes in mammalian skin epidermis growth and integrity. Is the skin’s remarkable ability to heal wounds related to the fact that epidermal carcinomas are the most common human cancers? I begin this chapter by introducing the biology of epithelia, the archetypal and evolutionarily oldest organized tissue. Next, I

discuss YAP/TAZ as essential regulators of epithelial tissue growth. I then describe how mammalian skin epidermis is an ideal model in which to study these proteins. Finally, I introduce Dvorak's notion of cancer as "the wound that never heals" and how flexibility in epithelial tissue growth essential for homeostasis and regeneration can be co-opted in tumourigensis.

1.2. Introduction to epithelia

Epithelia are continuous sheets of tightly connected cells lining body surfaces, cavities and organs, constituting a protective and selective dynamic barrier between an organism's interior and exterior. The epithelium was likely the first organized multicellular tissue to emerge in evolution and is considered a hallmark of metazoa. Recent studies show that epithelial structures predate metazoa, with the finding of a non-cadherin polarised epithelium in the Amoebozian *Dictyostelium discoideum* (slime mould) (Dickinson, Nelson et al. 2011, Weis, Nelson et al. 2013). This pre-metazoan organism switches between a unicellular and multicellular existence, developing a single-cell tubular epithelium with apical sides of constituent junctionally-bound cells facing the lumen. By enabling the separation of a controlled internal milieu from the external environment, and later the functional compartmentalisation of body regions, epithelia enabled the evolution of complex multicellularity.

Life depends on the highly selective and directional exchange of molecules between cells and their environment. Over 150 years ago, Emil Du Bois-Reymond reported that a spontaneous electrical potential arose between two salt solutions separated by frog skin (Du Bois-Reymond 1848–1884). This notion of biological asymmetry is now widely established as polarity. In multicellular organisms coordinated organization of cellular asymmetry builds polarized epithelial tissues essential for specialised organ architecture and function. Whilst most eukaryotic cells are polarized, i.e. display structural and functional asymmetry, the polarity of an epithelial cell depends on its assimilation into the epithelium. Epithelial cells are tightly connected to their neighbours and to the underlying matrix, organised to form sheets of aligned polarities with distinct apical and basal surfaces. The apical

plasma membrane domain faces towards the external environment or luminal space whilst the basolateral surfaces contact adjacent cells and an underlying basal lamina. This coordinated sheet polarity is essential for barrier function and vectorial transport, as well as other specialised epithelial functions such as secretion. Cell-cell and cell-matrix adhesions and cell polarity are intimately linked; cells that lose adhesion usually lose their apicobasal polarity.

The epithelium is the first tissue to arise during embryogenesis and is considered the transcriptional default (Bryant and Mostov 2008). The other major animal tissue, mesenchyme, arises from epithelia by repression of the transcriptional program driving epithelial differentiation in a so-called “epithelial mesenchymal transition” (Frisch 1997, Tyler 2003). Mesenchyme is comprised of cells with a front-rear axis of polarity, of unaligned orientation, in contrast to the aligned polarity and defined tissue architecture of epithelia. In reality, the distinction between epithelia and mesenchyme is less black and white, with various reversible in-between phases. Conserved mechanisms regulating the interactions and transitions between epithelia and mesenchyme are used iteratively to build all organs of diverse functional and morphological complexity seen in the animal kingdom.

The third key structural feature of epithelial cells (aforementioned cell polarity and tight cell-cell adhesions being the first two) is the ability to orient the mitotic spindle. Symmetric divisions in the same plane of the sheet expand and maintain monolayers whilst asymmetric divisions give rise to different cell fates. Stratified epithelia form from simple epithelia by cell divisions perpendicular to the sheet. Thus, cell divisions along defined axes generate and maintain diverse and complex epithelial structures. Epithelial sheets exhibit a remarkable diversity of shapes. Sheets can comprise single or multiple layers, three-dimensional structures such as cysts or invaginations and adnexa such as glands or hair follicles. The three key structural features mentioned above are integral to the function of epithelia and their deregulation results in disruption of tissue architecture associated with tumourigenesis.

Epithelia are actively renewing tissues, with populations of rapidly dividing stem cells replenishing those constantly sheared off. For example, the rapid turnover of the human intestinal epithelium (differentiated cells renew every 4-5 days) maintains the tissue integrity in the face of constant wear and tear. Interfollicular epidermis of the skin renews roughly every four weeks. In addition, resident epithelial stem cells can increase their proliferative rate to repair tissue after injury (Crosnier, Stamataki et al. 2006, Vrana, Lavalle et al. 2013). Whilst stem cells endow epithelia with a remarkable plasticity, aberrant activation of pathways essential for development, homeostasis and regeneration can result in pathological conditions including tumourigensis. Indeed, 90% of all malignancies originate in epithelial tissues (Beyer, Weiss et al. 2013). The molecular mechanisms governing epithelial tissue growth control are not fully clear. Understanding the signals regulating the proliferation of epithelial cells has important implications for regenerative medicine and cancer treatments.

In summary, epithelial cells integrate biochemical and mechanical cues from apicobasal polarity, cell-cell contacts, cell-extracellular matrix (ECM) contacts and diffusible factors to know “who” and “where” they are within a tissue, as well as overall tissue integrity. These intrinsic and extrinsic signals converge to direct changes in gene expression that determine cell fate. The YAP/TAZ family of transcriptional co-activators have recently emerged as crucial sensors of tissue and cellular information regulating epithelial growth. In the next section, I introduce what is known about YAP/TAZ function and regulation in epithelial tissues. I then focus on their role in the context of the epidermis.

1.3. YAP/TAZ as regulators of epithelial growth

Although YAP and TAZ were discovered over 20 years ago, interest in these proteins only exploded when their sole *Drosophila* homolog Yorkie was identified as the downstream effector of the Hippo tumour suppressor pathway (Sudol 1994, Yagi, Chen et al. 1999, Kanai, Marignani et al. 2000, Huang, Wu et al. 2005). Since then, paralogues Yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ) have emerged as key regulators of tissue growth. YAP/TAZ play essential physiological roles in embryonic development, tissue

homeostasis and regeneration by modulating the expression of genes involved in cell proliferation, stemness, differentiation and survival. Their deregulation has been implicated in a range of pathological conditions including carcinogenesis.

1.3.1. Discovery and the core Hippo pathway

It is well established that within a species, humoral factors such as nutrients or hormones influence organ and global body size. For example, starved *Drosophila* larvae develop into smaller but perfectly proportioned adult flies. Nutrient deficiencies stunt organ growth via Target of Rapamycin (TOR) signalling in what is known as "organ extrinsic" size control (Edgar 2006, Grewal 2009, Hietakangas and Cohen 2009). Almost 100 years ago, Ross Harrison's classical salamander limb bud transplantation experiments suggested that a combination of extrinsic and intrinsic mechanisms control tissue size (Harrison 1924). Since then, it has been reported that mice foetal organs transplanted into adult mice and *Drosophila* imaginal discs transplanted into adult flies grow to their correct adult size (Metcalf 1963, Dittmer, Goss et al. 1974, Silber 1976, Bryant and Simpson 1984). Complete regeneration of rat liver after a two-thirds hepatectomy hinted that the organ-autonomous size sensing mechanism involved in development could also be activated during tissue repair (Michalopoulos and DeFrances 1997). The YAP/TAZ transcriptional co-activators and upstream Hippo pathway are now well established as crucial regulators of developmental and regenerative tissue growth in many tissues.

Components of the Hippo pathway were first characterised in a series of genetic mosaic screens in *Drosophila* for genes controlling organ size (Xu, Wang et al. 1995, Tapon, Harvey et al. 2002, Harvey, Pfleger et al. 2003, Udan, Kango-Singh et al. 2003, Wu, Huang et al. 2003, Lai, Wei et al. 2005, Harvey and Tapon 2007, Zhao, Li et al. 2010, Halder and Johnson 2011). The pathway was subsequently found to be highly evolutionarily conserved, with Hippo component human orthologues able to rescue loss-of-function phenotypes in *Drosophila* (Tao, Zhang et al. 1999, Wu, Huang et al. 2003, Lai, Wei et al. 2005). A yeast two-hybrid screen later identified Yorkie as the major effector of the Hippo pathway and homologue of human YAP (Huang, Wu et al. 2005). When unrestrained by Hippo signalling,

Yorkie induces the transcription of target genes involved in cell proliferation and evasion of apoptosis such as *diap1*, *cyclin E*, and *bantam* microRNA (Edgar 2006, Nolo, Morrison et al. 2006, Thompson and Cohen 2006). Loss-of-function mutations in *Yorkie* result in tissue atrophy whilst tissues overexpressing *Yorkie* exhibit massive overgrowth. Significantly, mammalian *YAP* was able to rescue the phenotype of mutant *Yorkie* (Wu, Liu et al. 2008, Zhang, Ren et al. 2008, Zhao, Ye et al. 2008). Similarly, mutation of Hippo pathway components or overexpression of *YAP* in adult mice liver resulted in a three-to-fourfold increase in liver mass. This increase in mass was found to be a result of increased cell numbers (Camargo, Gokhale et al. 2007, Dong, Feldmann et al. 2007, Zhou, Conrad et al. 2009, Lu, Li et al. 2010).

The canonical Hippo pathway comprises a core serine/threonine kinase module that culminates in the phosphorylation and inactivation of Yorkie/YAP/TAZ. In mammals, mammalian sterile 20-like (MST1/2) kinase, in complex with adaptor Salvador homologue 1 (SAV1), phosphorylates large tumour suppressor (LATS1/2), which together with activator protein Mps one binder (MOB1) directly phosphorylates YAP/TAZ on multiple serine residues. LATS1/2 also directly interacts with NF2/Merlin, which promotes LATS1/2 phosphorylation by MST1/2 (Yin, Yu et al. 2013). This facilitates subsequent autophosphorylation, activation and phosphorylation of YAP/TAZ (Chan, Nousiainen et al. 2005, Zhao, Wei et al. 2007). LATS1/2 can also be phosphorylated and activated by MAP4Ks (mitogen activated protein kinase kinase kinase kinase) (Meng, Moroishi et al. 2015, Zheng, Wang et al. 2015). LATS1/2-phosphorylated YAP/TAZ is marked for ubiquitin-mediated degradation (Zhao, Ye et al. 2008) or sequestered in the cytoplasm by binding to 14-3-3 proteins (Fig. 1.1) (Zhao, Wei et al. 2007, Lei, Zhang et al. 2008). Thus, when the Hippo pathway is on, YAP/TAZ is prevented from accessing the nucleus and activating the transcription of pro-proliferative and anti-apoptotic genes. In reality, YAP/TAZ regulation is likely far more dynamic than an “on/off” binary. YAP/TAZ rapidly travels between the cytoplasm and nucleus, with phosphorylation status determining time bound to cytoplasmic versus nuclear factors (Vassilev, Kaneko et al. 2001, Zhao, Wei et al. 2007, Zhang, Ren et al. 2008).

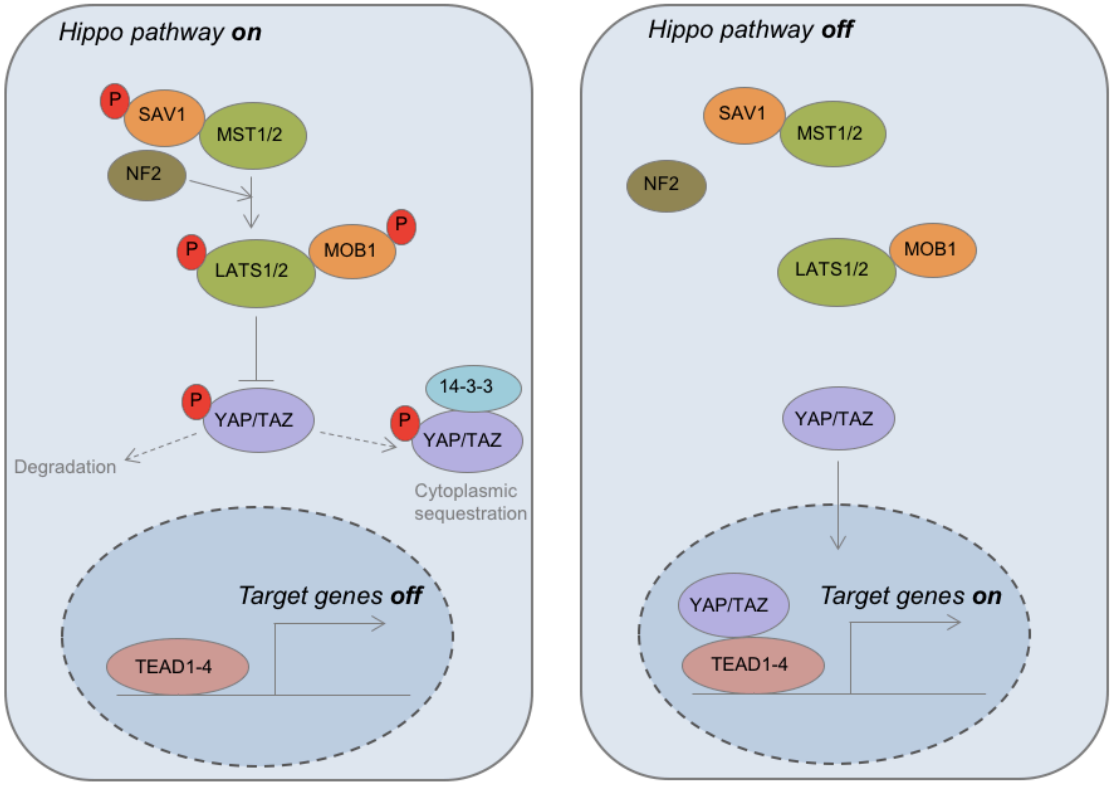


Figure 1.1. Schematic of YAP/TAZ regulation by the core Hippo kinase pathway. When the Hippo pathway is on, YAP/TAZ serine phosphorylation by LATS1/2 results in its degradation or cytoplasmic sequestration, excluding YAP/TAZ from the nucleus. When the Hippo pathway is off, YAP/TAZ is able to translocate into the nucleus, where they can interact with TEAD or other transcription factors to promote target gene expression.

1.3.2. YAP/TAZ structure and function

YAP (also known as YAP1 or YAP65) was initially identified in chicken as a proline-rich phosphoprotein bound to the SH3 domain of non-receptor tyrosine kinase Yes (Sudol 1994). First characterised as a 14-3-3 binding protein, TAZ is a YAP paralogue sharing ~50% amino acid sequence identity (Kanai, Marignani et al. 2000). YAP and TAZ proteins have a similar topology and domain organization, each comprising a TEAD-binding domain, a WW-domain, a transactivation domain and a C-terminal PDZ-binding domain (Fig. 1.2). In addition, YAP has an N-terminal proline-rich region, a second WW-domain and an SH3-binding motif.

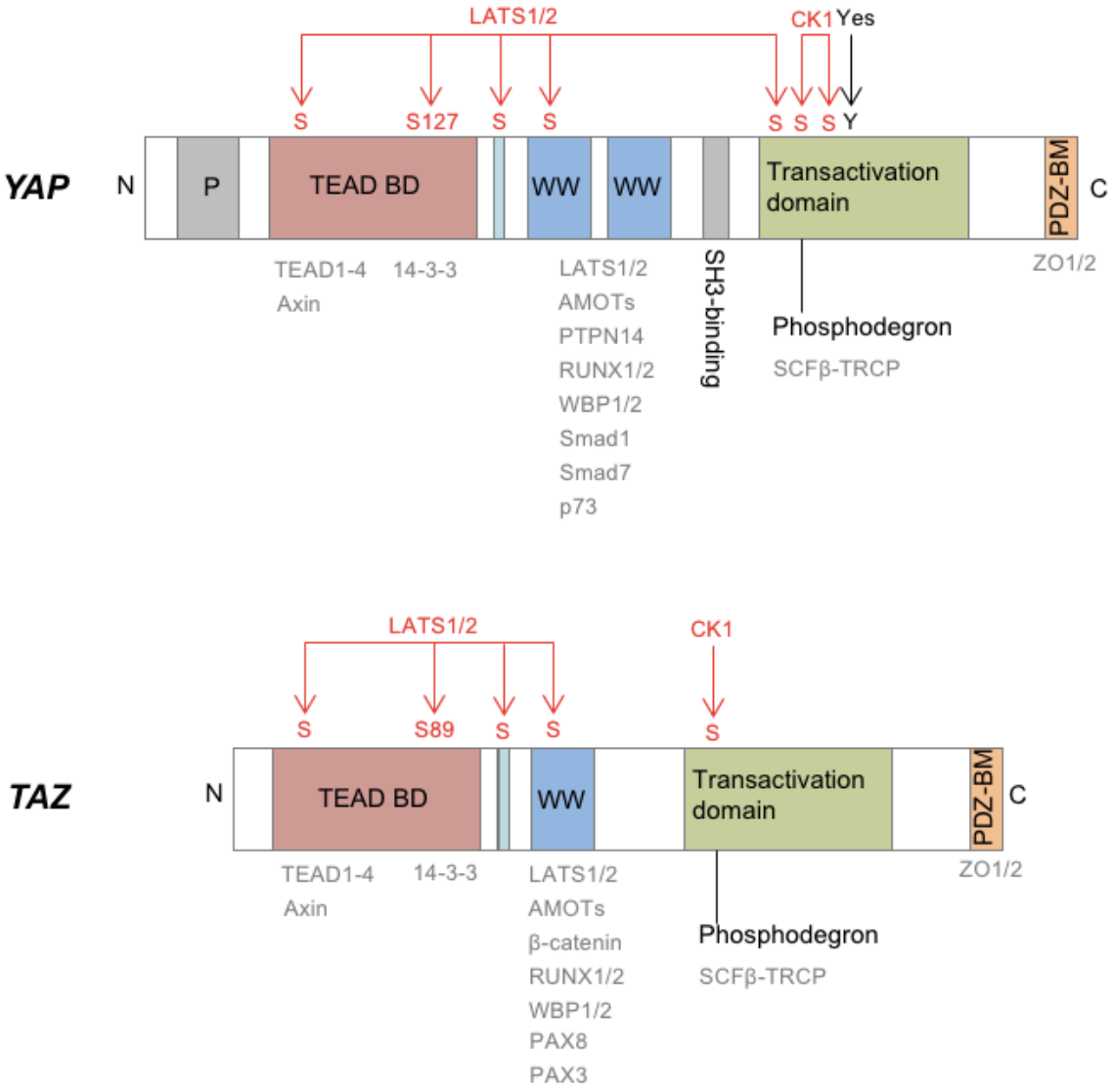


Figure 1.2. Schematic depiction of YAP and TAZ domain organization, phosphorylation sites and known interactors. Both YAP and TAZ have a TEAD binding domain, a WW domain (a domain with two tryptophan residues), a transactivation domain and a C-terminal PDZ-binding motif. In addition, YAP has an N-terminal proline-rich domain, a second WW domain (though this differs between the 8 YAP isoforms), and an SH3-binding domain. Inhibitory serine phosphorylation sites are shown in red whilst the activatory tyrosine phosphorylation site by Yes non-receptor tyrosine kinases is indicated in black. The LATS1/2 phosphorylation sites are indicated (five in YAP, four in TAZ), with the major site numbered. Published interactors are shown in grey below the corresponding domain.

LATS1/2 can phosphorylate YAP at five serine residues defined by the HXRXXS motif. TAZ has four such sites (Zhao, Li et al. 2010). LATS-phosphorylation of YAP and TAZ (particularly S127 and S89, respectively) promotes binding to 14-3-3 proteins and cytoplasmic sequestration YAP phosphorylation and binding to 14-3-3 is abolished by mutation of serine127 to alanine (S127A) (Zhao, Wei et al. 2007, Lei, Zhang et al. 2008). YAP S127A is still able to bind to TEAD and has normal transactivation activity. Thus, YAP S127A and YAP5SA (all five serines mutated to alanine) are useful mutants to study the effects of increased YAP nuclear localisation. In addition to stimulating their cytoplasmic localization, LATS1/2-phosphorylation imparts further inhibitory control of YAP/TAZ by reducing their stability. Phosphorylation of a serine residue in the C-terminal phosphodegron primes YAP/TAZ for casein kinase 1 (CK1) phosphorylation, recruitment of the SCFβ-TrCP lE3 ubiquitin ligase, ubiquitination and ultimately proteosomal degradation (Zhao, Li et al. 2010).

YAP/TAZ localization can also be influenced by interaction with various other proteins via distinct domains. The YAP/TAZ PDZ-binding motif binds the PDZ domains of many proteins, such as junctional protein ZO-2 which complexes with YAP to promote nuclear translocation (Oka and Sudol 2009, Remue, Meerschaert et al. 2010). Direct interaction between YAP/TAZ WW domains and PPXY motifs on PTPN14, LATS1/2 and angiomotin family of proteins (AMOTs) result in sequestration in the cytoplasm or junctional complexes (Zhao, Li et al. 2011, Liu, Yang et al. 2013). In addition, AMOTs can bind to LATS1/2 and YAP, promoting LATS1/2 autophosphorylation and subsequent phosphorylation of YAP (Mana-Capelli and McCollum 2018). Further layers of YAP/TAZ regulation are exerted by the cellular concentration and phosphorylation states of various scaffold proteins. For example, phosphorylation of AMOT at Serine 176 promotes the formation of a complex with YAP with tight junction proteins PALS and E-cadherin at the plasma membrane, whilst hypophosphorylated AMOT can accompany YAP into the nucleus (Moleirinho, Hoxha et al. 2017). Thus, the sum of a complex combination of posttranslational modifications and protein-protein interactions dictate YAP/TAZ stability and subcellular localization.

YAP/TAZ are transcriptional co-activators and as such lack a DNA-binding domain. When unrestrained by Hippo signalling, YAP/TAZ can accumulate in the nucleus where they interact with several transcription factors. TEA domain family 1-4 (TEAD1-4) were isolated as the main transcription factors bound by YAP/TAZ in mass spectrometry and ChIP experiments from various tissues (Zhao, Ye et al. 2008). YAP/TAZ in complex with TEAD drive the transcription of target genes involved in cell proliferation and survival (Lei, Zhang et al. 2008). Point mutations in the N-terminal TEAD-binding domain abolished *YAP/TAZ* overexpression phenotypes, highlighting the importance of TEAD interaction in YAP/TAZ function. (Vassilev, Kaneko et al. 2001, Ota and Sasaki 2008, Zhang, Ren et al. 2008, Chan, Lim et al. 2009, Zhao, Kim et al. 2009, Tian, Yu et al. 2010). Furthermore, a mouse model with a knock-in mutant *YAP* unable to bind with TEAD exhibited skin phenotypes reminiscent of *YAP* knockout mice (Schlegelmilch, Mohseni et al. 2011).

In addition to interacting with TEADs, YAP/TAZ can directly interact via their WW domains with PPXY motif-containing transcription factors such as Smads, RUNX1/2 and p73 (Fig. 1.2) (Yagi, Chen et al. 1999, Strano, Munarriz et al. 2001, Ferrigno, Lallemand et al. 2002, Zaidi, Sullivan et al. 2004, Varelas, Sakuma et al. 2008, Alarcon, Zaromytidou et al. 2009, Zhang, Liu et al. 2009, Varelas, Samavarchi-Tehrani et al. 2010, Grannas, Arngarden et al. 2015). By cooperatively binding with an array of factors, YAP/TAZ regulate specific cellular context-dependent transcriptional programs (Alarcon, Zaromytidou et al. 2009, Azzolin, Panciera et al. 2014).

Although YAP and TAZ share many transcription factor partners and target genes, there are some important differences. *YAP* knockout mice are embryonic lethal whilst TAZ knockout animals have renal defects but are viable (Morin-Kensicki, Boone et al. 2006, Hossain, Ali et al. 2007, Makita, Uchijima et al. 2008). Furthermore, in many tissues the *YAP* or *TAZ* knockout phenotype cannot be rescued by expression of the other (Hong, Hwang et al. 2005, Chan, Lim et al. 2008, Varelas, Sakuma et al. 2008, Zhao, Ye et al. 2008). This could be due to YAP and TAZ inducing different targets via interaction with different transcription factors or to differential regulation of YAP and TAZ proteins.

Whilst characterisation of targets in all tissues is incomplete, genome-wide analyses *in vitro* and *in vivo* have identified a YAP/TAZ transcriptional signature commonly used as a read-out for their activity. These include genes involved in cell proliferation and anti-apoptosis such as CTGF, CYR61, ANKRD1, BIRC5, AXL (Dong, Feldmann et al. 2007, Cordenonsi, Zanconato et al. 2011, Dupont, Morsut et al. 2011, von Gise, Lin et al. 2012, Calvo, Ege et al. 2013). However, the mechanism of YAP/TAZ transcriptional activation is not fully understood. ChIP data in *Drosophila* found that Yorkie physically interacts with the SWI/SNF complex component Brahma (Oh, Slattery et al. 2013). Other studies have since shown that Yorkie/TAP/TAZ recruit and directly interact with the SWI/SNF complex, GAGA factor, Mediator complex and NuRD complex (Bayarmagnai, Nicolay et al. 2012, Beyer, van Rensburg et al. 2013, Beyer, Weiss et al. 2013, Jin, Xu et al. 2013, Oh, Slattery et al. 2013, Qing, Yin et al. 2014, Skibinski, Breindel et al. 2014, Kim, Kim et al. 2015, Zhu, Li et al. 2015, Song, Herranz et al. 2017). Thus, an important mechanism of transcriptional activation by YAP/TAZ involves association with chromatin remodelling complexes and alteration of chromatin structure to enhance accessibility and transcription activity.

1.3.3. Regulation of YAP/TAZ activity

Whilst YAP/TAZ regulate gene transcription, relatively little is known about the transcriptional regulation of YAP/TAZ themselves. One study showed that YAP was transcriptionally downregulated during oxidative stress via direct binding of GABP to the YAP promoter (Wu, Xiao et al. 2013). Another found that YAP was transcriptionally upregulated in medulloblastoma (Fernandez, Northcott et al. 2009). However, the overwhelming majority of the literature on YAP/TAZ regulation highlights post-translational modifications and interaction with other proteins. As discussed above, YAP/TAZ have no nuclear localisation signal (NLS) and their activity is largely regulated by phosphorylation. The sum of multiple posttranslational modifications modulates the affinity of YAP/TAZ to various proteins to influence their stability, subcellular localisation, and transcription factor binding partners. Unlike most classical signalling pathways, Hippo/YAP/TAZ does not have a dedicated pathway ligand and receptor. YAP/TAZ activity has been

shown to be sensitive to contact inhibition of proliferation, cell-extracellular matrix contacts, cell polarity, force, cell metabolic status, tissue damage, as well as cross-talk with various other signalling pathways, including BMPs, GPCRs, Wnt, Notch, and Hedgehog. For simplicity, I divide some of the known upstream signals regulating YAP/TAZ into the following sections: 2.3.1 cell-cell adhesion, 2.3.2 apicobasal polarity, 2.3.3 cell-extracellular matrix interactions, 2.3.4 mechanotransduction and the actin cytoskeleton, 2.3.5 extracellular signalling molecules.

1.3.3.1. Cell-cell adhesion

LATS1/2-YAP/TAZ signalling has been shown to play an important role in cell-cell contact inhibition of proliferation. In mammalian cells cultured at high cell density, YAP is chiefly localised in the cytoplasm and exists in its phospho-YAPS127 state. Cell density-induced quiescence in post-confluent cells can be overcome by *LATS1/2* knockdown or *YAP* overexpression, which both result in increased unphosphorylated nuclear YAP (Zhao, Wei et al. 2007, Aragona, Panciera et al. 2013). This was shown to be TEAD-dependent. Thus LATS1/2-phosphorylation of YAP/TAZ represents one mechanism of contact inhibition of proliferation. Control of YAP-TEAD activity by contact inhibition has also been found to play a key role in development (Ota and Sasaki 2008, Nishioka, Inoue et al. 2009, Gumbiner and Kim 2014). YAP was nuclear in the peripheral cells of mouse blastocysts and cytoplasmic in inner layer cells which have more cell neighbours (Nishioka, Inoue et al. 2009). How does YAP/TAZ sense cell density?

Adherens junctions and tight junctions are strong intercellular adhesions crucial for epithelial integrity and barrier function. They are also essential for specifying and maintaining epithelial cell polarity, which is known to affect Hippo signalling (discussed in more detail in 2.3.2). Adherens and tight junctions comprise a transmembrane protein in complex with scaffold proteins bearing protein motifs capable of interacting with cytoplasmic proteins and neighbouring cell junctions. Treatment with E-cadherin (a major component of adherens junctions)-coated beads significantly reduced the proliferation of sparse cultured cells, whilst loss of some adherens junction components resulted in hyperproliferation and

tumourigenesis in mice (Vasioukhin, Bauer et al. 2001, Kobielak and Fuchs 2006, Lien, Klezovitch et al. 2006, Perez-Moreno, Davis et al. 2006, Perrais, Chen et al. 2007). E-cadherin-mediated restriction of proliferation was supressed by LATS1/2 knockdown or YAP overexpression. These data suggest that cells perceive their density via cell-cell adhesions, and that LATS1/2-YAP signalling transduce this information into changes in expression of genes involved in proliferation. Indeed, many Hippo pathway components localise at cell-cell junctions (Fig. 1.3) (Varelas, Samavarchi-Tehrani et al. 2010, Kim, Koh et al. 2011, Schlegelmilch, Mohseni et al. 2011, Silvis, Kreger et al. 2011, Yang, Graves et al. 2015).

Adherens junctions are calcium-dependent cell-cell adhesion complexes comprising cadherin, which link the cytoskeleton of neighbouring cells via associated catenins. In epithelia, homophilic interactions form between the extracellular domains of transmembrane protein E-cadherin. α/β-catenin interact with the cytoplasmic domains of E-cadherin and with the actin cytoskeleton, mechanically coupling adjacent cells (Liu, Tan et al. 2010, Collinet and Lecuit 2013) (I discuss mechanotransduction via cell-cell junctions in section 2.3.4) Disruption of adherens junctions induces YAP/TAZ nuclear localisation. The exact *in vivo* mechanism is not fully understood and may vary with tissue type. However, studies point to both LATS-dependent and independent adherens junction regulation of YAP/TAZ localisation (Varelas, Samavarchi-Tehrani et al. 2010, Kim, Koh et al. 2011, Schlegelmilch, Mohseni et al. 2011, Silvis, Kreger et al. 2011, Herr, Tsang et al. 2014). In human breast cells, E-cadherin-mediated contact inhibition and nuclear localization of YAP was dependent on α-catenin, β-catenin, as well as Hippo components Merlin, Kibra and LATS1/2 (Kim, Koh et al. 2011). Other groups found that under conditions of high cell density α-catenin stabilised the interaction of YAP with 14-3-3, thereby retaining YAP in the cytoplasm and inhibiting its dephosphorylation by protein phosphatase 2A (PP2A) (Schlegelmilch, Mohseni et al. 2011, Silvis, Kreger et al. 2011). In *Drosophila* and mammalian cells, tumour suppressor Merlin/NF2 predominantly localised to adherens junctions of confluent epithelial cells via α-catenin (Gladden, Hebert et al. 2010). Here, it functions as a scaffold, recruiting LATS1/2 to the cell membrane which promotes its phosphorylation by MST1/2 (Yin, Yu et al. 2013, Meng, Moroishi et al. 2016). Another adherens junction component, PTPN14, was found to bind directly to YAP

by its PPXY motif, resulting in nuclear exclusion (Wang, Huang et al. 2012, Liu, Yang et al. 2013, Michaloglou, Lehmann et al. 2013). YAP/TAZ is also sequestered in the cytoplasm by incorporation into the β-catenin destruction complex. Interestingly, mutant α-catenin unable to bind to E-cadherin could still form a cytoplasmic complex with YAP and hinder cell proliferation (Azzolin, Panciera et al. 2014). Thus, adherens junction components may function in the regulation of YAP/TAZ even when not associated with junctions.

Tight junctions are cell-cell adhesion complexes localised more apically than adherens junctions, forming a boundary between the apical and basal membrane domains by blocking lateral diffusion of integral membrane proteins. These multiprotein junctional complexes form a tight belt-like seal between adjacent cells and are essential for maintaining cell polarity, epithelial tissue integrity and regulating paracellular transport (Shin, Fogg et al. 2006, Balda and Matter 2008). Tight junction associated angiomotin (AMOT) proteins have been shown to negatively regulate YAP/TAZ. Direct binding of the YAP WW domain by AMOT PPXY motif resulted in localisation of YAP to the tight junctions and facilitated phosphorylation by LATS1/2 (Wells, Fawcett et al. 2006, Chan, Lim et al. 2011, Paramasivam, Sarkeshik et al. 2011, Wang, Huang et al. 2011, Zhao, Li et al. 2011). Conversely, tight junction protein ZO-2 was found to promote YAP nuclear localisation. ZO-2 interacts via its PDZ domain with the PDZ-binding motif of YAP to form a complex carrying YAP into the nucleus (Oka, Remue et al. 2010).

1.3.3.2. Apicobasal polarity

As mentioned above, cell-cell adhesion and apicobasal polarity are intimately linked. Disruption of cell junctions usually results in loss of cell polarity and vice versa (Wang, Ojakian et al. 1990, O'Brien, Zegers et al. 2002). Furthermore, multiple apicobasal polarity determinants associate with cell-cell junction complexes. Consequently, it can be difficult to separate the regulatory effects of adhesion and polarity on YAP/TAZ. However, various polarity determinants are known to directly regulate Hippo pathway components. Apicobasal polarity determinants, including the Crumbs complex, the aPKC-Par6-Par3 complex, and the basolateral Scribble complex have been reported to associate with and

facilitate activation of core Hippo kinases in *Drosophila* and mammalian cells (Chen, Gajewski et al. 2010, Grzeschik, Parsons et al. 2010, Varelas, Samavarchi-Tehrani et al. 2010, Chen, Schroeder et al. 2012, Hirate, Hirahara et al. 2013). Generally, apicobasal polarity proteins are thought to inhibit Yorkie/YAP/TAZ activity by regulating the spatial organization of Hippo pathway components.

FERM domain family proteins Merlin (*Drosophila* homologue of NF2) and Expanded form a complex with WW domain-containing protein Kibra at the apical plasma membrane. Here, the Mer/Ex/Kibra complex recruits and cooperatively activates Warts/LATS by facilitating its phosphorylation by Hippo/MST at the apical membrane (Fig 3) (Baumgartner, Poernbacher et al. 2010, Genevet, Wehr et al. 2010, Ho, Wei et al. 2010, Yu, Zheng et al. 2010, Yin, Yu et al. 2013). This promotion of kinase activation by recruitment of Hippo components at the apical compartment appears to be largely conserved in mammals (Benhamouche, Curto et al. 2010, Yin, Yu et al. 2013).

The evolutionarily conserved transmembrane protein Crumbs forms a complex with various proteins at apical junctions to establish and maintain the apical plasma domain of epithelial cells (Bazellieres, Assemat et al. 2009, Pocha and Knust 2013). Mammalian CRB3 accumulates at tight junctions, where it recruits and forms complexes with upstream Hippo components including AMOT, NF2, Kibra and FRMD6 (Wells, Fawcett et al. 2006, Chen, Gajewski et al. 2010, Ling, Zheng et al. 2010, Varelas, Samavarchi-Tehrani et al. 2010). AMOT can inhibit YAP by direct binding and retention at tight junctions and/or facilitating LATS-mediated phosphorylation of YAP in an NF2-dependent recruitment to the plasma membrane (Chen, Gajewski et al. 2010, Ling, Zheng et al. 2010, Varelas, Samavarchi-Tehrani et al. 2010, Robinson and Moberg 2011, Yi, Troutman et al. 2011, Li, Zhou et al. 2015). The literature suggest that CRB3 does not directly activate LATS1/2 kinase activity. Rather, by assembling multiprotein complexes at apical junctions, CRB3 favours the LATS1/2-YAP interaction and subsequent phosphorylation and inactivation of YAP (Fig. 1.3). Recent work has confirmed the requirement of apical CRB3 in the bronchiole epithelium to restrain YAP activity in an NF2- and LATS1/2-dependent manner in order to induce cell differentiation (Szymaniak, Mahoney et al. 2015).

1.3.3.3. Cell-extracellular matrix interactions

Far from a static scaffold structure, the extracellular matrix (ECM) is now understood to be a complex and dynamic network of secreted macromolecules including fibrous and glycosylated proteins. In epithelia, the ECM is essential for specifying the basal surface and maintaining apicobasal polarity. Cell-ECM interactions also play crucial roles in multiple cellular processes such as proliferation, differentiation and migration (Bosman and Stamenkovic 2003, Morrissey and Sherwood 2015). Specific cell adhesion receptors convey biochemical and mechanical signals from the ECM to intracellular signal transduction pathways and to the actin cytoskeleton. Studies found that cell detachment from the ECM resulted in cytoplasmic retention of YAP whilst increasing the area of contact between the cell surface and ECM increased YAP nuclear localisation and transcriptional activity (Wada, Itoga et al. 2011, Zhao, Li et al. 2012). The exact mechanisms relaying ECM information into YAP/TAZ activity remain unclear. Recent reports implicate integrins, a superfamily of transmembrane cell-ECM adhesion receptors that mediate outside-in and inside-out signalling. Large cell-ECM adhesion complexes called focal adhesions (FAs) mediate the connection between ECM-integrin and the actin cytoskeleton (Burridge and Chrzanowska-Wodnicka 1996, Dumbauld, Lee et al. 2013, Hirata, Sokabe et al. 2014). Attachment of cultured cells to a fibronectin-rich ECM matrix promoted YAP nuclear localization and activity via a LATS1/2-dependent β1-integrin–FAK–Src–PI3K–PDK1 axis (Kim and Gumbiner 2015). Integrins in FAs were also found to inactivate LATS1/2 in a Rho-GTPase and F-actin dependent pathway (Fig. 3) (Wada, Itoga et al. 2011, Zhao, Li et al. 2012). However, other groups have reported YAP/TAZ activation by transmission of FA tension across stress fibres in a LATS1/2-independent pathway (Dupont, Morsut et al. 2011, Aragona, Panciera et al. 2013) (discussed further in 2.3.4). Thus, there may be multiple LATS1/2-dependent and independent mechanisms of ECM-cell activation of YAP/TAZ.

Several bona fide YAP/TAZ target genes include ECM components including connective tissue growth factor (CTGF), cysteine-rich angiogenic inducer 61 (CYR61) and laminin subunit alpha 5 (Lama5) (Zhao, Ye et al. 2008, Chang, Goel

et al. 2015). CTGF and CYR61 are well-established ligands of integrins (Kireeva, Lam et al. 1998, Chen, Chen et al. 2000, Leu, Liu et al. 2003, Heng, Huang et al. 2006). Thus, is it conceivable that YAP/TAZ transcriptional activity induced by integrin engagement establishes a positive feedback loop.

1.3.3.4. Mechanotransduction and actin cytoskeleton

Epithelial cells are under mechanical tension arising from their environment. Specific cell adhesion complexes sense and transmit forces, mechanically coupling the actin cytoskeleton to that of neighbouring cells and to the ECM. These include cadherin-based cell-cell junctions and integrin-based cell-matrix adhesions (focal adhesions, FAs) (Chen, Li et al. 1999, Li, Butler et al. 2002, Geiger, Spatz et al. 2009, Jaalouk and Lammerding 2009, Mammoto and Ingber 2009). Remodelling of the actin cytoskeleton occurs in cells subjected to external mechanical forces. For example, cells can react to tension exerted by the ECM with an opposing force on F-actin at focal adhesions generated by non-muscle myosin II motor activity (Choquet, Felsenfeld et al. 1997, Chen, Tan et al. 2004, Vogel and Sheetz 2006, Fernandez-Gonzalez, Simoes Sde et al. 2009, Janmey and Miller 2011). This results in the formation of stress fibres and is dependent on Rho family GTPase RhoA (Torsoni, Marin et al. 2005, Liu, Nelson et al. 2007, Zhao, Laschinger et al. 2007, Cai and Sheetz 2009).

Actin cytoskeleton dynamics direct numerous cellular functions and states including morphology, adhesion, migration, as well as signalling events. Reorganisation of the cytoskeleton is induced by various mechanical cues, such as changes in cell geometry, density, and matrix stiffness (Discher, Janmey et al. 2005). Recent studies found that such mechanical cues also control YAP/TAZ subcellular localisation in an F-actin-dependent manner. Thus, it has been proposed that mechanical information perceived by the actin cytoskeleton can regulate YAP/TAZ, providing a missing link for how YAP/TAZ can convert tissue-level forces into transcriptional changes (Dupont, Morsut et al. 2011, Wada, Itoga et al. 2011, Yu, Zhao et al. 2012, Aragona, Panciera et al. 2013, Calvo, Ege et al. 2013). The exact mechanism is not fully understood, with different groups drawing different conclusions. YAP/TAZ were predominantly cytoplasmic in mammalian cells

cultured on a soft matrix or small micropatterned surfaces. Cell attachment to a stiff matrix or spreading on a large ECM surface resulted in YAP nuclear localisation and activation (Dupont, Morsut et al. 2011, Wada, Itoga et al. 2011, Aragona, Panciera et al. 2013, Tang, Rowe et al. 2013, Benham-Pyle, Pruitt et al. 2015). Interestingly, mechanical stretching can override contact inhibition of proliferation. YAP (usually cytoplasmic in densely cultured cells) relocalised to the nucleus of contact inhibited mammary epithelial cells, accompanied by reentry into the cell cycle when stretched (Aragona, Panciera et al. 2013, Codelia, Sun et al. 2014). YAP activity and nuclear localization on stiff matrices was dependent on actomyosin contractility and RhoA activity (Dupont, Morsut et al. 2011, Wada, Itoga et al. 2011, Aragona, Panciera et al. 2013). In many cell types and contexts induction of F-actin polymerisation induces nuclear accumulation of YAP/TAZ, whilst disruption of F-actin or inhibition of stress fibre formation by RhoA/mysosin II inhibition results in YAP/TAZ cytoplasmic localisation (Dupont, Morsut et al. 2011, Wada, Itoga et al. 2011, Zhao, Li et al. 2012, Aragona, Panciera et al. 2013, Calvo, Ege et al. 2013, Matsui and Lai 2013, Reginensi, Scott et al. 2013, Yu and Guan 2013). Thus, many lines of evidence point to regulation of YAP/TAZ by Rho-mediated actin stress fibre formation in response to artificial ECM forces in cultured cells. Less is known about the regulation of YAP/TAZ by physiological forces. Some *in vivo* evidence has been gleaned from *Drosophila* studies, where F-actin polymerisation promoted Yorkie activation and stimulated tissue overgrowth in a Warts/LATS-dependent mechanism (Fernandez, Gaspar et al. 2011, Sansores-Garcia, Bossuyt et al. 2011).

Studies in mammalian cells have yielded conflicting results on the involvement of Hippo signalling in the mechanical regulation of YAP/TAZ. LATS1/2 activity was increased upon contact inhibition of densely packed cells whilst *LATS1/2* knockdown hindered force-induced YAP activation (Zhao, Laschinger et al. 2007, Wada, Itoga et al. 2011, Zhao, Li et al. 2012). Furthermore, inhibition of actin polymerization or Rho GTPase resulted in increased LATS1/2-phosphorylation of YAP (Yu, Zhao et al. 2012, Yin, Yu et al. 2013). Conversely, Stefano Piccolo's group first provided evidence of a LATS1/2-independent mechanism for mechanical regulation of YAP (Dupont, Morsut et al. 2011, Aragona, Panciera et al. 2013). YAP/TAZ remained inactive and cytoplasmic despite loss of LATS1/2 in

human mammary epithelial cells and mesenchymal stem cells grown on a soft matrix (Dupont, Morsut et al. 2011). Thus, whilst the role of LATS1/2 remains unclear, the consensus is that various mechanical cues act via modulation of F-actin and Rho family GTPases to converge on YAP/TAZ activity (Dupont, Morsut et al. 2011, Fernandez, Gaspar et al. 2011, Sansores-Garcia, Bossuyt et al. 2011, Wada, Itoga et al. 2011, Aragona, Panciera et al. 2013, Gaspar and Tapon 2014).

Several groups have shown that integrin-Src signalling at FAs is required for YAP nuclear localisation and activity in cell culture (Wada, Itoga et al. 2011, Tang, Rowe et al. 2013, Kim and Gumbiner 2015). FAs are large integrin-based mechanosensitive cell-matrix adhesion complexes that physically link integrins to the actin cytoskeleton. Upon ECM engagement of integrin clusters, various structural and signalling proteins are recruited to form FAs (Zaidel-Bar, Itzkovitz et al. 2007, Zaidel-Bar and Geiger 2010, Kuo, Han et al. 2011). The maturation of FAs and formation of associated stress fibres is dependent on non-muscle myosin II motor activity and actin polymerization in response to mechanical cues (Legate, Wickstrom et al. 2009, Coyer, Singh et al. 2012, Bottcher and Fassler 2014, Humphrey, Dufresne et al. 2014). Kim and Gumbiner proposed that integrin-Src activation upon ECM attachment converges on growth factor signalling to activate YAP: adhesion of mammary epithelial cells to fibronectin activated the FAK-Src-PI3K-PDK1 (focal adhesion kinase-Src-phosphoinositide-3 kinase-phosphoinositide-dependent kinase) pathway and induced YAP nuclear localisation in a LATS1/2-dependent manner (Kim and Gumbiner 2015). LATS1/2 kinase activity was previously shown to be inhibited by growth factor-activated PI3K-PDK1 signalling (Strassburger, Tiebe et al. 2012, Fan, Kim et al. 2013, Gumbiner and Kim 2014). In an additional and/or parallel mechanism, YAP could be activated by direct tyrosine phosphorylation by Src (Taniguchi, Wu et al. 2015). However, it remains to be seen whether integrin-Src signalling activates YAP *in vivo*. In *Drosophila*, Yorkie can be activated indirectly by overexpression of *Src* (Fernandez, Jezowska et al. 2014).

Whilst there is a consensus that tension at FAs induces YAP nuclear localisation, the effect on YAP of actomyosin tension at AJs is less clear. Extracellular E-cadherin binding has been shown to be involved in both activation and inactivation

of YAP (Kim, Koh et al. 2011, Benham-Pyle, Pruitt et al. 2015). Severing of the actin cytoskeleton from the cadherin-catenin complex at AJs triggers YAP nuclear localisation and activity in confluent cells and in the mouse epidermis (Kim, Koh et al. 2011, Silvis, Kreger et al. 2011). FAs and AJs are not mechanically coupled and it is possible that actomyosin tension at these sites has different effects on YAP regulation.

Another potential mechanosensor upstream of YAP/TAZ is the contractile cytoskeletal protein spectrin. Spectrin forms a mesh-like network at the intracellular surface of the plasma membrane and is required for membrane tension and integrity (Machnicka, Czogalla et al. 2014). Loss of spectrin or dissociation from F-actin activated Yorkie/YAP in *Drosophila* and human cells. Deletion of apical spectrins in fly epithelia resulted in massive tissue overgrowth reminiscent of Hippo(/MST) or Warts(/LATS) mutants. (Deng, Wang et al. 2015, Fletcher, Elbediwy et al. 2015, Wong, Li et al. 2015). Apical upstream Hippo components Crumbs, Expanded, Merlin and Kibra associate with spectrins in the apical plasma membrane domain. Clustering of these apical components promotes recruitment and activation of Hippo and Warts kinase activity. It has been suggested that cell stretching could reduce the local concentration of apical components, thereby hindering Hippo activation and releasing its inhibition of Yorkie (Fletcher, Elbediwy et al. 2015).

In summary, numerous mechanical stimuli have been found to regulate YAP/TAZ subcellular localisation and activity via various LATS1/2-dependent and independent mechanisms. It is not yet clear whether different mechanical inputs act in parallel or feed into a central pathway. However, the actin cytoskeleton has been firmly established as a crucial intermediary for mechanical inputs converging on YAP/TAZ. Finally, much of the data identifying YAP/TAZ as mechanosensors was derived from cells in culture experiencing artificial forces. There is some evidence of Yorkie responding to mechanical force *in vivo*, but it will be interesting to see if these mechanisms are physiologically relevant in mammals.

1.3.3.5. Extracellular signalling molecules

YAP/TAZ are well established as potent inducers of tissue growth. Various hormones, cytokines and growth factors have been found to function upstream, including GPCR ligands, EGF, Wnts, TGFβ, Hedgehog and BMPs.

YAP/TAZ can be activated or repressed by GPCR agonists depending on the class of G protein coupled to the receptor (Yu, Zhao et al. 2012). Phospholipids sphingosine-1-phosphophate (S1P) and lysophosphatidic acid (LPA) potently induce nuclear localisation of YAP/TAZ in cell culture via RhoGTPase-modulation of actomysosin contractility (Miller, Yang et al. 2012, Yu, Zhao et al. 2012, Regue, Mou et al. 2013, Feng, Degese et al. 2014). Other GPCR ligands such as epinephrine and glucagon inhibit RhoA-GTPase via cAMP/PKA, resulting in suppression of YAP/TAZ activity (Miller, Yang et al. 2012, Mo, Yu et al. 2012, Yu, Zhao et al. 2012, Kim, Kim et al. 2013, Yu, Zhang et al. 2013, Wennmann, Vollenbroker et al. 2014, Zhou, Wang et al. 2015). There is also evidence of YAP/TAZ activation via GPCR-like Wnt receptor Frizzled and Rho GTPases (Park, Kim et al. 2015). YAP/TAZ have previously been shown to be regulated by Wnt and participate in the β-catenin destruction (Azzolin et al. 2012 Cell, Azzolin et al. 2014). Thus, there appears to be extensive cross-talk between the Wnt and Hippo pathways (Piccolo, Dupont et al. 2014, Hansen, Moroishi et al. 2015).

Modulation of Rho activity by other processes could also regulate YAP/TAZ via F-actin. For example, activation of RhoA and subsequent F-actin polymerisation by the mevalonate pathway resulted in increased YAP activity and migration ability of breast cancer cells (Wang, Wu et al. 2014). In fruit flies, Yorkie-induced tissue overgrowth could be rescued by blocking the mevalonate pathway (Sorrentino, Ruggeri et al. 2014). Metabolic processes are frequently deregulated in cancer. It will be interesting to see if future studies uncover further links between altered metabolism and YAP/TAZ oncogenic activity.

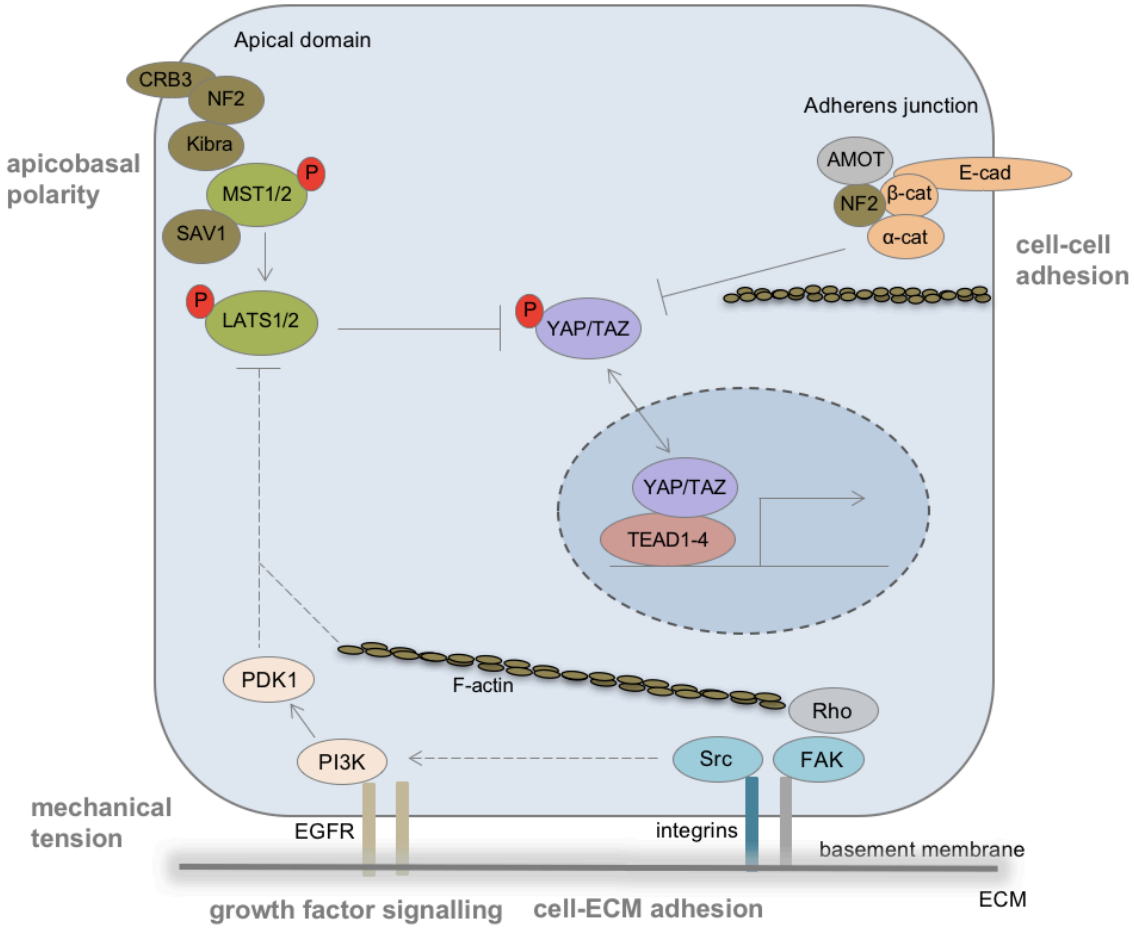
There is evidence of YAP/TAZ regulation by several receptor tyrosine kinase (RTK) families. RTKs are transmembrane proteins that upon activation trigger a

phosphorylation cascade to signal through multiple pathways, including PI3K and MAPK. RTK epidermal growth factor receptor (EGFR) signalling has been reported to activate YAP in mammalian cells and in *Drosophila*. EGFR was found to inhibit LATS activity via PI3K-PDK1 signalling or the Ras-Raf-MAPK pathway (Fan, Kim et al. 2013, Reddy and Irvine 2013). Interestingly, EGFR ligand – and YAP transcriptional target – amphiregulin (AREG) also activates YAP (Zhang, Ji et al. 2009). Further studies support the notion of a physiological positive feedback loop involving AREG and YAP (Han, Bai et al. 2014, Gregorieff, Liu et al. 2015). Conversely, other groups found no effect of EGF, PI3K, AKT or PDK1 loss on YAP activity (Zhao, Wei et al. 2007, Yu, Zhao et al. 2012). It has recently been proposed that MAP4Ks phosphorylate and activate LATS1/2 in a parallel and redundant fashion to MST1/2 (Meng, Moroishi et al. 2015, Zheng, Wang et al. 2015). Further *in vivo* work is needed to elucidate the physiological regulation of YAP/TAZ by RTKs.

1.3.3.6. Summary of YAP/TAZ regulation

In this section I have outlined some of the important regulators of YAP/TAZ, under the broad themes of cell-cell adhesion, apicobasal polarity, cell-extracellular matrix interactions, mechanotransduction and the actin cytoskeleton, and extracellular signalling molecules. It is by no means a comprehensive account of YAP/TAZ regulation, which is beyond the scope of this thesis. Moreover, the above regulatory modules overlap and interact to form a complex network. In this way, YAP/TAZ form a nexus on which a multitude of biochemical and mechanical extracellular and intracellular signals converge. These cues may synergise, antagonise or act in parallel to direct specific cell and context-dependent transcriptional responses. Delineating the molecular mechanisms underlying YAP/TAZ regulation by various cues will further our understanding of the role of YAP/TAZ in tissue regeneration or in pathological processes such as cancer or fibrosis. For example, Yorkie/YAP have been shown to be activated and to stimulate cell proliferation upon intestinal tissue damage (Cai, Zhang et al. 2010, Karpowicz, Perez et al. 2010, Shaw, Kohlmaier et al. 2010, Staley and Irvine 2010, Grusche, Degoutin et al. 2011, Azzolin, Panciera et al. 2014, Cai, Maitra et al. 2015, Gregorieff, Liu et al. 2015). Interestingly, loss of YAP and TAZ in mouse

intestine did not significantly affect normal homeostasis but loss of YAP alone severely impaired healing after intestinal damage. How YAP/TAZ mediates the response to tissue damage is unclear. Some of the cues regulating YAP/TAZ discussed above are known to be altered upon wounding. It is tempting to speculate that changes in cell contacts, actin dynamics and mechanical forces induced by injury could activate YAP/TAZ, enabling them to act as sensors of tissue integrity and architecture. In a pathological example, tumourigenesis is often accompanied by remodelling of the ECM and increased tissue stiffness (Levental, Yu et al. 2009). YAP/TAZ are activated by matrix stiffness and could conceivably contribute to tumour progression in this way. Our understanding of YAP/TAZ regulation has exploded in the last couple of decades, promising exciting novel therapeutic avenues. However, much of the data were derived from cell culture and



need to be tested in vertebrate models to ascertain physiological relevance.

Figure 1.3. Schematic summary of YAP/TAZ regulation. YAP/TAZ regulation by apical

signals, cell-cell adhesion, cell-ECM adhesion and growth factor signalling.

1.4. YAP/TAZ function in mammalian epidermis

The skin is the largest organ in the body. It is the main barrier between the body and the environment, providing protection against pathogens, radiation, abrasion and dehydration. Other functions include sensation, and temperature regulation mediated by hair and perspiration. The skin undergoes a nine-fold increase in surface area from neonate to adult and constantly replenishes cells shed from the surface with a turnover of four weeks. Furthermore, it is able to heal wounds. Clearly, the skin's capacity for growth is remarkably robust and flexible. It is established that a pool of resident stem cells endows the skin with its ability to maintain homeostasis in the face of constant physical, chemical and biological attacks. However, the molecular mechanisms controlling these cells are not fully understood. Deregulation of epidermal cell proliferation can result in skin diseases from epidermal thinning and improper barrier function to hyperproliferative disorders and carcinomas. Indeed, epidermal carcinomas are the most common cancers worldwide. In this thesis, I sought to investigate the function of YAP/TAZ in epidermal homeostasis and tumourigenesis.

1.4.1. Structure and function of mammalian epidermis: a stratified squamous epithelium

Epithelia are generally classified according to the morphology of constituent cells and the number of layers. Epithelial cells can be broadly divided into three morphologies: thin and flattened (squamous), cube-like (cuboidal), or tall and elongated (columnar) (Montell 2008). These can be arranged as a single layer of cells (simple epithelium) or multiple layers (stratified epithelium). Typically, the stem cells in stratified epithelia adhere to the basement membrane; in simple epithelia both stem cells and differentiated cells are attached to the basement membrane.

Mammalian skin comprises two main layers: the dermis and epidermis (Fig. 1.4). The epidermis is a stratified squamous epithelium that forms the outermost layer and consists of adnexal structures – hair follicles and various glands – and

intervening interfollicular epidermis (IFE). Epidermal adnexal structures vary in density in different regions of the body and among mammalian species; human

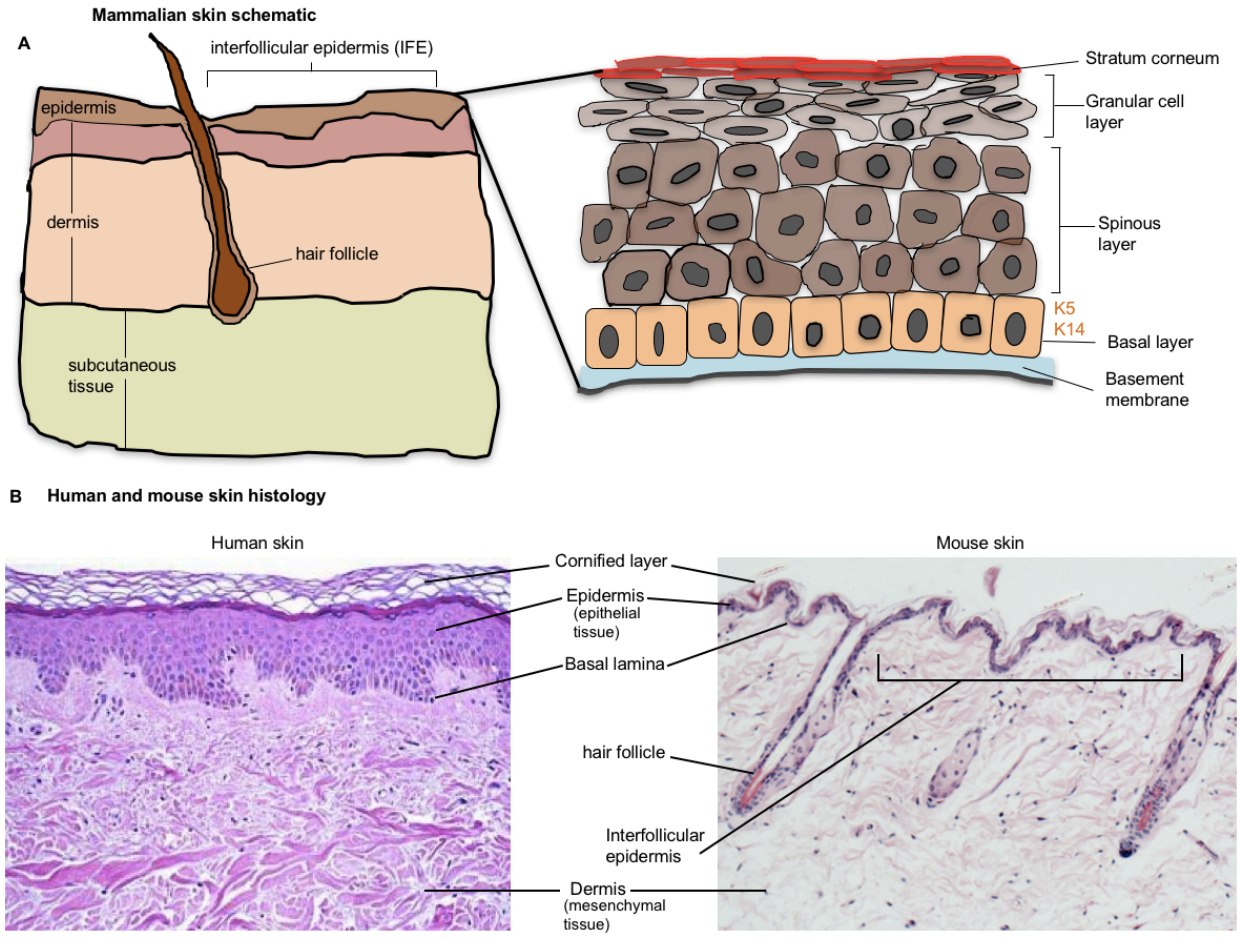


Figure 1.4. Structure of mammalian skin epidermis. (A) Schematic diagram of mammalian skin, with the epidermis magnified. **(B)** Histological sections of human and mouse skin stained for haemotoxylin and eosin. Note human epidermis is thicker and contains fewer hair follicles than mouse skin (no hair follicles are visible in this section). Human image from Dermatology, University of Iowa 1995.

epidermis is characterised by a much thicker IFE and far fewer hair follicles than mice (Khavari 2006). Keratinocytes constitute 90% of cells in the epidermis. The remainder consists of melanocytes, merkel cells, dendritic cells and other immune cells (Fuchs and Raghavan 2002, Simpson, Patel et al. 2011). Underlying the epidermis is the dermis: a much thicker mesenchymal layer composed predominantly of fibroblasts, adipocytes, macrophages and acellular matrix components like collagen. The dermis and epidermis are separated by the basement membrane (BM), to which the basal epidermal cells adhere. The BM is an ECM rich in growth factors, providing signalling information and polarity cues in addition to structural support for basal epidermal cells (Ryan, Christiano et al. 1996, Van Agtmael and Bruckner-Tuderman 2010, Watt and Fujiwara 2011). The BM is thought to promote cell survival and proliferation, as daughter cells that delaminate from the basement membrane stop proliferating and are committed to terminal differentiation. Suprabasal cells undergo dramatic transcriptional and morphological changes as they migrate upwards through spinous, granular and cornified layers, before eventually being shed from the surface (Watt 1984, Bohnert, Hornung et al. 1986, Barrandon and Green 1987, Watt and Hogan 2000, Fuchs and Raghavan 2002, Fuchs 2007, Simpson, Patel et al. 2011). Keratinocyte differentiation is characterised by significant remodelling of the cytoskeleton, changes in keratin expression and increase in keratin intermediate filaments. Basal layer progenitor cells express keratins 5 and 14 whilst spinous/granular layers express keratins 1 and 10 (Fuchs and Green 1980, Feng, Zhang et al. 2013, Homberg and Magin 2014). Finally, the outermost cornified layer is composed of terminally differentiated dead highly flattened enucleated water-impermeable squames (Candi, Schmidt et al. 2005, de Guzman Strong, Wertz et al. 2006, Koster 2009).

The balance between keratinocyte proliferation and differentiation must be tightly controlled to maintain homeostasis. It is well established that keratinocyte proliferation is restricted to cells in the IFE basal layer or the hair follicle (Blanpain and Fuchs 2009, Watt and Jensen 2009). Although attachment to the basement membrane is thought to be crucial in specifying stem/progenitor cell fate, the exact mechanisms are not fully understood. Contributions from integrin and growth factor signalling, as well as mechanical forces exerted by the basement membrane have

been reported (Sibilia, Fleischmann et al. 2000, Raghavan, Vaezi et al. 2003, Manohar, Shome et al. 2004, Dobereiner, Dubin-Thaler et al. 2005). Integrins mediate attachment of basal layer keratinocyte keratin filaments and actin cytoskeleton to the basement membrane via hemidesmosomes and focal adhesions (FAs), respectively (Jones and Watt 1993, Watt 2002).

Studies in human keratinocytes in culture and in mice found that β1 integrins play an essential role in adhesion, proliferation and survival of epidermal cells. Conditional knockout of *β1 integrin* in mouse epidermis resulted in significant defects in keratinocyte proliferation, whereas ectopic suprabasal integrin expression resulted in hyperproliferation of the epidermis and aberrant differentiation (Carroll, Romero et al. 1995, Jones, Harper et al. 1995, Brakebusch, Grose et al. 2000, Raghavan, Bauer et al. 2000, Grose, Hutter et al. 2002, Watt 2002). Engagement of αβ1 integrins at FAs with basement membrane components can trigger various downstream signalling events. Thus, integrin clustering activates FA associated kinase (FAK), integrin-linked kinase (ILK) and Src which in turn promote Ras–MAPK signalling, FA turnover, actin cytoskeleton remodelling and cell migration (Zervas, Gregory et al. 2001, Mackinnon, Qadota et al. 2002, Grashoff, Aszodi et al. 2003, Sakai, Li et al. 2003, Lorenz, Grashoff et al. 2007, Schober, Raghavan et al. 2007). Conditional epidermal ablation of FAK prevented chemically induced skin tumourigenesis (McLean, Komiyama et al. 2004, Ridgway, Serrels et al. 2012). These data point to a key role for basal integrin-FAK/Src signalling in maintaining epidermal homeostasis.

The precise definition of what qualifies as an epidermal stem cell has been variable and contentious. More recent lineage tracing studies have lead to the current consensus that under homeostasis multiple subsets of epidermal stem cell populations exist, differing in the markers they express and niches they occupy. Plasticity between these subpopulations is thought to underlie the remarkable capacity for epidermal regeneration (Jones, Harper et al. 1995, Taylor, Lehrer et al. 2000, Oshima, Rochat et al. 2001, Doupe, Klein et al. 2010, Mascre, Dekoninck et al. 2012, Tan, Jensen et al. 2013, Kretzschmar and Watt 2014, Schepeler, Page et al. 2014, Goodell, Nguyen et al. 2015). Regardless of niche or subset markers, keratinocyte progenitors share the common attributes of integrin-mediated

adhesion to the basement membrane and expression of keratins 5 and 14. The molecular mechanisms between integrin engagement and transcription of genes involved in epidermal cell proliferation and stemness remain unclear. In the next section, I discuss the possible role of YAP/TAZ as a link.

1.4.2. YAP/TAZ in the epidermis

Loss of core Hippo kinases MST1/2 or LATS1/2 (Fig. 1.1) in mouse liver or intestine results in nuclear translocalisation of YAP/TAZ and target gene expression. This indicates that the core Hippo kinases are necessary for YAP/TAZ inactivation in these tissues *in vivo* (Zhou, Conrad et al. 2009, Lee, Lee et al. 2010, Song, Mak et al. 2010, Zhou, Zhang et al. 2011, Imajo, Ebisuya et al. 2015, Lee, Park et al. 2016). Interestingly, in the mouse epidermis no phenotype was observed upon double knockdown of *MST1/2*. Knockdown of *MST1/2* or *LATS1/2* in keratinocytes had no effect on YAP S127 phosphorylation, nuclear localisation or reporter activity (Schlegelmilch, Mohseni et al. 2011). Furthermore, mechanoregulation of YAP in keratinocyte culture was found to be independent of LATS1/2. This suggests that canonical hippo signalling is dispensable for YAP/TAZ regulation in the skin (Aragona, Panciera et al. 2013). However, another group reported YAP activation and epidermal hyperplasia in *MOB1A/B* conditional double knockout mice (Nishio, Hamada et al. 2012). The effect of knocking out *LATS1/2*, the other core Hippo kinase, in epidermis remains to be investigated. Thus, whilst canonical Hippo signalling appears to be less important in the regulation of YAP/TAZ in mammalian epidermis, its contribution remains unclear. Why might YAP/TAZ regulation differ in epidermal cells?

In contrast to the classically polarized simple epithelium, epidermal cells lack a defined apical domain. Simple epithelial cells have distinct apical and basolateral membrane domains, separated by intercellular junctional complexes (Yeaman, Grindstaff et al. 1999, Nelson 2003, Bryant and Mostov 2008, Niessen and Gottardi 2008). Conversely, cells of the stratified epithelium of the epidermis do not have separate apical and basolateral domains. Instead, polarization of the tissue along the apicobasal axis is largely thought to be specified by basal membrane-integrin signalling and apical-lateral cell-cell junctions. Thus, the basement membrane and

the outermost cornified layer define the basal and apical surfaces, respectively (Nelson 2009, Green, Getsios et al. 2010, Huttenlocher and Horwitz 2011).

In cells that do develop an apical membrane domain, CRB3 is essential for restraining YAP activity in a LATS1/2-dependent manner; loss of CRB3 in cell culture or in mouse airway epithelium resulted in defective polarity and induction of YAP nuclear localisation (Varelas, Samavarchi-Tehrani et al. 2010, Szymaniak, Mahoney et al. 2015). Although the data on localisation of apical components such as CRB3 in stratified epithelia is sparse, it is likely that the role of these components differ in epidermal cells and simple epithelial cells. Therefore, mechanisms of YAP/TAZ inhibition other than LATS1/2-mediated phosphorylation ostensibly prevail in the epidermis. Indeed, two groups reported that whilst LATS1/2 were dispensable for YAP/TAZ inactivation in mouse epidermis, the adherens junction component, α-catenin, potently inhibited YAP activity (Schlegelmilch, Mohseni et al. 2011, Silvis, Kreger et al. 2011). The mechanism of negative regulation by α-catenin was proposed to involve AMOT, 14-3-3 and the PP2A phosphatase to promote YAP phosphorylation and cytoplasmic sequestration. Interestingly, 14-3-3σ is expressed in suprabasal layers of the epidermis but is largely absent from the basal keratinocytes, where YAP tends be nuclear (Dellambra, Golisano et al. 2000, Schlegelmilch, Mohseni et al. 2011, Silvis, Kreger et al. 2011)Therefore, junctional components seem to be key regulators of YAP activity in the epidermis.

In the wild-type mouse epidermis, YAP was reported to be highly expressed and nuclear in the majority of basal cells, whereas it was predominantly cytoplasmic in suprabasal cells (Zhang, Pasolli et al. 2011). Elevating nuclear YAP levels by expression of a S127A transgene under the control of a basal keratinocyte promoter (Keratin 14; K14) or by *α-catenin* knockdown resulted in epidermal hyperplasia. Keratinocyte proliferation was increased whilst differentiation and apoptosis were stunted (Schlegelmilch, Mohseni et al. 2011, Silvis, Kreger et al. 2011, Zhang, Pasolli et al. 2011). Conversely, conditional knockdown of *YAP* driven by a K14-Cre resulted in epidermal hypoplasia and disorganisation. The data on TAZ expression and function in the epidermis are scant.

Interaction with TEAD transcription factors is essential for YAP to function in epidermal progenitor cells. In human keratinocytes, RNAi knockdown of *YAP* abolished TEAD reporter activity and *TEAD* knockdown significantly diminished proliferation. Furthermore, disruption of YAP-TEAD interaction in mouse epidermis by knock-in of *YAP-S79A* resulted in epidermal hyperplasia reminiscent of *YAP* knockout (Zhang, Pasolli et al. 2011). A recent study reported that the YAP/TAZ-TEAD complex can also function as a transcriptional repressor of tumour suppressor genes *DDIT4* (DNA-damage-inducible transcript 4) and *Trail* (TNF-related apoptosis-inducing ligand) (Kim, Kim et al. 2015).

In summary, YAP is expressed and nuclear in basal progenitor keratinocytes, where it promotes proliferation and stemness. Loss of YAP results in impaired proliferation and thinning of the epidermis, whereas overexpression induces hyperproliferation and epidermal hyperplasia. Despite the documented importance of YAP in skin homeostasis, the data on *TAZ* overexpression or double *YAP/TAZ* knockout are lacking. How YAP is regulated is obviously a crucial question. There have been contradictory results regarding the importance of Hippo signalling in YAP regulation in the epidermis and negative regulation mediated by α-catenin is thought to predominate over LATS1/2-mediated phosphorylation. Differences in polarity and architecture of stratified epithelium compared to simple epithelia could contribute to differential regulation of YAP/TAZ in epidermis. Several studies report that YAP/TAZ transcriptional activity depends on its interaction with TEAD. Whilst *Cyr61* was found to be a YAP-TEAD target in keratinocytes (Zhang, Pasolli et al. 2011), *in vivo* targets remain unidentified. Identification of further YAP/TAZ target genes in the epidermis will shed more light on the mechanisms underlying their function.

1.5. Wound healing and cancer

1.5.1. YAP/TAZ in tissue repair

Some lower vertebrates – such as newts and salamanders – are able to regenerate entire limbs, jaws and eyes. Regenerative capacity is far more limited in mammals.

Only a few organs, such as the liver, intestine and skin have some potential for tissue repair (Whyte, Smith et al. 2012). Recently, Hippo-YAP/TAZ signalling has been implicated in the regeneration of several tissues following injury (Johnson and Halder 2014, Hong, Meng et al. 2016, Meng, Moroishi et al. 2016, Moya and Halder 2016, Xiao, Leach et al. 2016). One of the most striking examples of mammalian organ regenerative ability is seen in the liver, which can regain its original mass following a 70% hepatectomy ((Grijalva, Huizenga et al. 2014). Nuclear YAP is increased upon injury to rat liver, promoting proliferation and dedifferentiation of hepatocytes. YAP/TAZ activation is followed by activation of Hippo kinases and a return to baseline nuclear YAP/TAZ levels when the liver reaches its original size. Elevated YAP protein levels – but not mRNA levels – observed during liver regeneration suggest that YAP protein degradation is reduced in response to liver damage whilst transcription rate is unaffected. This would make YAP an ideal sensor of tissue damage as modulation of protein stability allows for rapid and reversible cellular responses (Bai, Zhang et al. 2012, Wang, Zhang et al. 2012, Grijalva, Huizenga et al. 2014, Su, Bondar et al. 2015).

Whilst the molecular mechanisms by which YAP/TAZ sense and direct repair is unclear, their regulation of cell proliferation, dedifferentiation and migration is thought to be important. YAP/TAZ are established as regulators of stem cells during embryogenesis and it is thought that tissue repair could represent a reactivation of developmental programs (Varelas 2014, Sasaki 2015). *YAP* overexpression or knockdown of Hippo kinases in cell culture promoted dedifferentiation, promotion of stem cell identity and iPS reprogramming (Quinn, Kunath et al. 2006, Lian, Kim et al. 2010, Qin, Blaschke et al. 2012, Panciera, Azzolin et al. 2016). Furthermore, multiple groups reported a physiological role for YAP/TAZ in induction of stem cell fate and expansion of stem cell populations in mouse intestine, lung and skin (Camargo, Gokhale et al. 2007, Cai, Zhang et al. 2010, Schlegelmilch, Mohseni et al. 2011, Zhang, Pasolli et al. 2011, Lee, Byun et al. 2014, Zhao, Fallon et al. 2014). Hippo-YAP/TAZ signalling has been reported to induce stem cell renewal and intestinal repair following injury. Interestingly, *YAP* knockout in mouse intestine showed that this protein is largely dispensable for homeostasis but is essential for regeneration (Cai, Zhang et al. 2010, Zhou, Zhang et al. 2011, Azzolin, Panciera et al. 2014, Gregorieff, Liu et al. 2015, Yu, Meng et

al. 2015). In the skin, wound healing depends on epidermal stem cells (Solanas and Benitah 2013, Goodell, Nguyen et al. 2015) and YAP/TAZ are known to regulate stem cell function during epidermal homeostasis. Additionally, ectopic YAP activity in neonatal and developing epidermis resulted in increased levels of progenitor cell markers accompanied by downregulation of differentiation markers (Silvis, Kreger et al. 2011, Zhang, Pasolli et al. 2011). Thus, it is conceivable that YAP/TAZ could function in wound healing by expansion of epidermal stem cell compartments. A recent study reported a slight delay in wound closure in mice treated topically with siRNAs against YAP/TAZ (Lee, Byun et al. 2014). However, with such an approach, *YAP/TAZ* knockdown was transient and non-cell-specific. Keratinocyte-restricted genetic knockdown will help elucidate the role of YAP/TAZ in epidermal tissue repair.

1.5.2. YAP/TAZ in cancer

Many of the cellular processes downstream of YAP/TAZ activity – such as proliferation, evasion of apoptosis, dedifferentiation, stemness – are hallmarks of cancer. Unsurprisingly, YAP/TAZ and upstream Hippo pathway components are frequently deregulated in human tumours. In normal human adult tissues, YAP nuclear localisation is rarely detected in differentiated cells and is generally restricted to stem/progenitor cells. Increased expression and nuclear localisation of YAP/TAZ has been widely reported in human cancers including liver, colorectal, ovarian, prostate, lung and breast, and is associated with poor prognosis (Zender, Spector et al. 2006, Dong, Feldmann et al. 2007, Zhao, Wei et al. 2007, Steinhardt, Gayyed et al. 2008, Xu, Yao et al. 2009, Hall, Wang et al. 2010, Pan 2010, Wang, Dong et al. 2010, Cordenonsi, Zanconato et al. 2011, Zhang, George et al. 2011, Su, Ma et al. 2012, Xie, Zhang et al. 2012, Kim, Kim et al. 2013, Yuen, McCrudden et al. 2013, Lau, Curtis et al. 2014). Intriguingly, although YAP/TAZ activity is commonly observed in many cancers, Hippo component mutations and *YAP/TAZ* genomic amplifications are rarely reported (Snijders-Keilholz, Ewing et al. 2005, Harvey, Zhang et al. 2013, Johnson and Halder 2014, Mannaerts, Leite et al. 2015). *NF2* and *LATS1/2* are mutated in a small number of rare tumours, such as schwannomas, and it is possible that tumour sequencing will yield further mutations or amplifications in Hippo components or *YAZ/TAZ*. As discussed in previous

sections, YAP/TAZ activity is sensitive to cues associated with cancer, such as loss of polarity and cell-cell adhesion, as well as cross-talk with other oncogenic pathways. Furthermore, matrix stiffening, which potently induces nuclear translocation of YAP/TAZ, is a near ubiquitous feature of solid tumours (Paszek, Zahir et al. 2005, Seewaldt 2014). Thus, it is conceivable that other tissue and cellular events involved in cancer initiation and progression induce YAP/TAZ activity in human tumours. How YAP/TAZ become oncogenic and the downstream effects of their activation in cancer remain unclear. Whilst ectopic YAP/TAZ activity drives overproliferation and hyperplasia in multiple murine tissues, it generally does not result in malignant tumour formation (Heallen, Zhang et al. 2011, Lavado, He et al. 2013, Chen, Zhang et al. 2014, Lin, Yao et al. 2015). Although YAP hyperactivity can induce hepatic cancer, not every cell expressing nuclear activated YAP becomes malignant (Lu, Li et al. 2010, Song, Mak et al. 2010). Evidence of YAP synergy with other oncogenic signals could explain why only some nuclear YAP+ cells result in malignant tumourigenesis (Aylon, Michael et al. 2006, Cancer Genome Atlas Research 2011, Zhang, Nandakumar et al. 2014, Zhang, Gao et al. 2015). Additionally, *YAP/TAZ* overexpression has been reported to potently drive tumour progression and malignancy of benign cancer cells bearing other oncogenic mutations (Bhat, Salazar et al. 2011, Cordenonsi, Zanconato et al. 2011, Lamar, Stern et al. 2012). Interestingly, some studies found YAP to have tumour supressing functions and promote apoptosis in response to DNA damage (Strano, Munarriz et al. 2001, Strano, Monti et al. 2005, Matallanas, Romano et al. 2007, Yuan, Tomlinson et al. 2008, Barry, Morikawa et al. 2013). YAP's preferential binding to different transcription factor partners (dictated by phosphorylation state) under different cellular conditions could explain some of its contradictory functions in tumourigenesis.

1.5.3. YAP/TAZ in epidermal cancers

In this thesis, I sought to investigate the role of YAP in epidermal tumourigenesis. Non-melanoma skin cancers (NMSCs) are the most common human cancers globally (Leiter, Eigentler et al. 2014). NMSCs can be divided into two major types: basal cell carcinoma (BCC) and cutaneous squamous cell carcinoma (SCC). The incidence of NMSC is rapidly rising, likely due to a combination of increased UV

exposure, ozone layer thinning, increased lifespan, changes in clothing and immunosuppressive medications. BCC, which accounts for 75% of cases of NMSC, has low invasive potential and rarely metastasises. Despite representing only 25%, SCC can metastasise, and is responsible for the majority of NMSC deaths. Furthermore, SCC contributes significant economic burden; it is the fifth most expensive cancer in the United States (Housman, Feldman et al. 2003, Smeets, Kuijpers et al. 2004, Trakatelli, Ulrich et al. 2007). SCC most commonly arises on the skin of the head and neck, with chronic UV exposure thought to be a major aetiological factor. Frequent use of tanning beds increases the risk of SCC 2.5-fold. SCCs exhibit significant heterogeneity in morphology, pathogenesis and clinical outcomes. SCCs can be characterised hyperproliferation of atypical epidermal cells, which sometimes invade the underlying stroma. Several subtypes can be classified based on their histology (Lohmann and Solomon 2001, Cassarino, Linden et al. 2005, Cassarino, Derienzo et al. 2006, Macbeth, Grindlay et al. 2011, Samarasinghe, Madan et al. 2011). Cutaneous SCCs are almost always associated with Actinic Keratoses (AK), from which they are thought to arise from. AKs are premalignant skin lesions linked to chronic UV exposure. They are characterised by aggregates of atypical keratinocytes with disrupted apicobasal polarity in the basal and suprabasal layers of the epidermis. Although the majority of AKs are benign, around one in ten can progress to metastatic SCC over the course of years (Alam and Ratner 2001, Rossi, Mori et al. 2007). Spindle cell SCC (spSCC) represents a rare and particularly invasive subtype of SCC. Histological analysis of spSCC reveals elongated spindle-shaped cells with atypical nuclei that can infiltrate the dermis, subcutaneous tissue, muscle and even bone (Morgan, Purohit et al. 2008). Why some AKs progress to SCCs, including highly invasive spSCCs, is not understood.

Nuclear localisation of YAP has been reported in murine and human BCCs and SCCs (Schlegelmilch, Mohseni et al. 2011, Silvis, Kreger et al. 2011, Zhang, Pasolli et al. 2011). These tumours were epidermal in nature, evidenced by their expression of Keratin 5. However, immunodeficient mice receiving transgenic skin grafts expressing YAP-S127A developed invasive mesenchymal-like SCCs (Schlegelmilch, Mohseni et al. 2011). Disruption of some of the known upstream regulators of YAP/TAZ promotes epidermal tumours in which YAP is highly nuclear.

For example, knockout of *14-3-3σ* resulted in epidermal hyperplasia and increased rate of tumourigenesis of mice treated with chemical carcinogens. Strong expression of nuclear YAP was observed in the resultant papillomas and cSCCs (Li, Sambandam et al. 2011). Genetic deletion of AJ components α-catenin or p120-catenin in mice also triggered epidermal hyperplasia and tumour formation (Vasioukhin, Bowers et al. 2001, Kobielak and Fuchs 2006, Perez-Moreno, Davis et al. 2006, Schlegelmilch, Mohseni et al. 2011, Silvis, Kreger et al. 2011). Thus, YAP nuclear localisation is clearly associated with BCCs and SCCs. However, the molecular mechanisms resulting in YAP activation and the subsequent events driving tumour formation and progression are not well understood.

1.5.4. Cancer as "the wound that never heals"

The notion of a link between cancer and wound-associated inflammation was first proposed by pathologist Rudolph Virchow over 150 years ago. Virchow noticed that neoplastic lesions arose at sites of chronic irritation and that these lesions were infiltrated by inflammatory cells. He hypothesised a causal relationship between inflammation and tumour formation (Virchow 1863). Over a century later, injections of chickens with Rous sarcoma virus (RSV) provided experimental evidence of a direct role for wounding in tumour formation. Chickens injected with the virus developed tumours solely at the injection site (Dolberg, Hollingsworth et al. 1985). It was subsequently reported that treating wound sites with topical anti-inflammatories could block tumour formation, suggesting that wound-induced inflammation drives tumourigenesis at these wound sites (Martins-Green, 1994 (Martins-Green, Boudreau et al. 1994). The relationship between infection and chronic inflammation is now well recognized: over 20% of cancers are thought to be triggered by infection-induced inflammation (Balkwill and Mantovani 2001, Wang, Hussain et al. 2002, Pawlotsky 2004, Hussain and Harris 2007, Hartnett and Egan 2012). Haddow surmised that tumourigenesis represents a wound overhealing. Noting histological similarities between the microenvironment of tumours and wounds, Dvorak subsequently proposed that tumours hijack the wound healing response, coining the term "wounds that do not heal" (Haddow 1972, Dvorak 1986). Since Dvorak's paper, interactions between tumour cells and the surrounding stroma have been found to be crucial for tumour growth. Many of

the stromal changes that accompany tumour development recapitulate those involved in wound healing.

Inflammation triggers an influx of inflammatory cytokines, growth factors, chemokines and leukocytes, secretion of matrix metalloproteases, remodelling of the ECM, activation of fibroblasts and neovascularization. These stromal changes are crucial for the proliferation, survival and migration of epithelial cells necessary to restore tissue integrity. However, the inflammatory milieu induced at wound sites has a dark side since it is permissive for neoplastic progression (Coussens and Werb 2002, De Wever and Mareel 2003, Karnoub and Weinberg 2006, Pietras, Pahler et al. 2008, Gabison, Huet et al. 2009, DeNardo, Andreu et al. 2010, Feng, Santoriello et al. 2010, Grivennikov and Karin 2010, Qian and Pollard 2010, Lu, Weaver et al. 2012). Whilst the molecular profiles of wound and tumour microenvironments are very similar, the return to baseline seen upon completion of tissue repair never takes place around tumours. Thus, whilst the normal wound healing process is self-limiting, constitutive activation of these processes in tumourigenesis – “the wound that does not heal” – drives their expansion and metastasis.

During epithelial wound healing, changes in epithelial cells accompany and are induced by changes in stromal cells. Re-epithelialization requires migration of cells at the wound edge to restore epithelial integrity over the newly deposited matrix. The transcriptional activity of immotile epithelial cells is transiently reprogrammed to enable a migratory mesenchymal phenotype in a process known as epithelial-mesenchymal transition (EMT) (Fig 1.5). EMT drives many developmental and morphogenetic processes and is controlled by a set of EMT-transcription factors (EMT-TFs). Key EMT-TF families ZEB, Snail and Twist potently decrease cell-cell adhesion by repression of E-cadherin expression. Epithelial genes such as keratins are downregulated whilst expression of mesenchymal markers Vimentin, fibronectin and N-cadherin increases. Cytoskeletal remodelling induced by loss of adherens junction and cell delamination from the basement membrane is accompanied by loss of apicobasal polarity. Cells become elongated and migrate along the ECM (Gurtner, Werner et al. 2008, Yang and Weinberg 2008, Kalluri and Weinberg 2009, Thiery, Acloque et al. 2009). Upon completion of wound repair,

keratinocytes revert to their epithelial phenotype. As such, wound healing-associated EMT is known as a partial EMT. The epidermal barrier is restored by concomitant degradation of excess ECM and apoptosis and phagocytosis of surplus fibroblasts. The precise mechanisms controlling cessation of wound healing pathways are not well understood (Shaw and Martin 2016). Similarly, cancer metastasis involves EMT and MET (mesenchymal to epithelial transition). Maintenance of an inflammatory microenvironment and aberrant ECM composition continues to supply tumour cells with growth and survival factors. Sustained disruption of apicobasal polarity and high migratory capability promotes tumour metastasis. The formation of a secondary tumour involves re-epithelialisation; the mechanisms regulating this process are not well understood, (Bilder 2004, Tse and Kalluri 2007, Hanahan and Weinberg 2011, Tiwari, Gheldof et al. 2012).

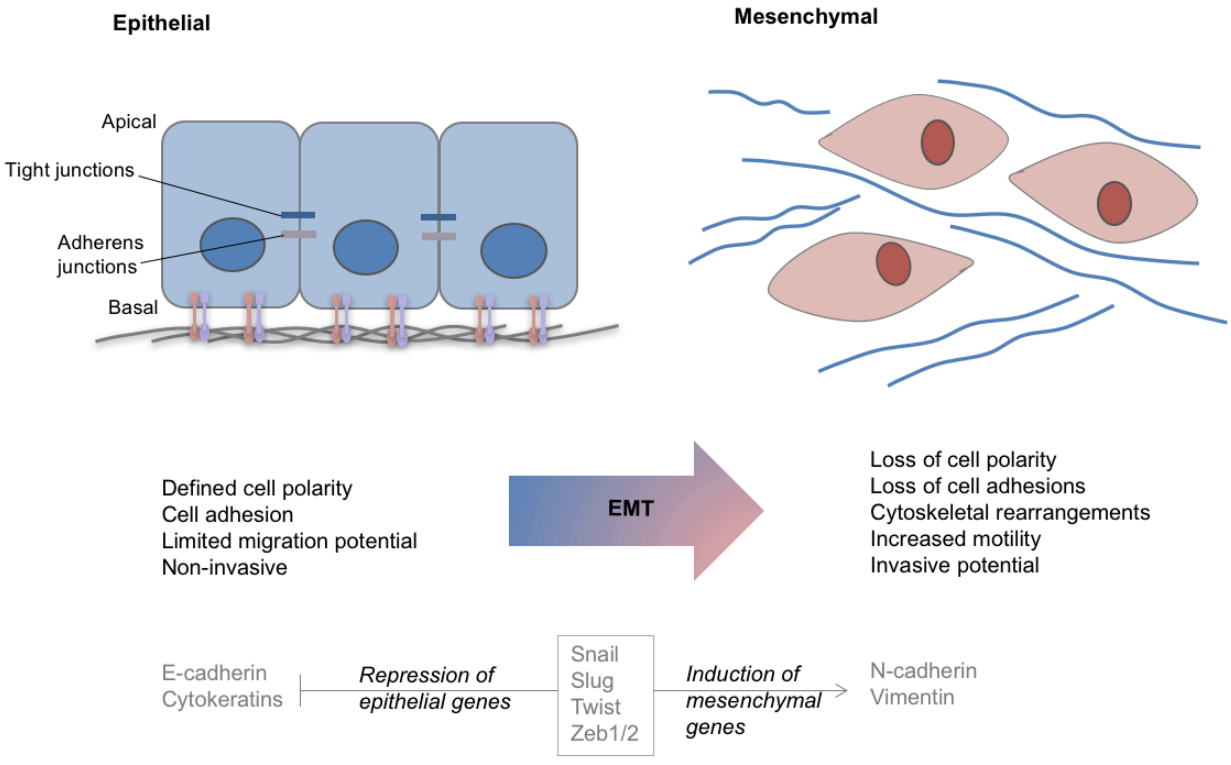


Figure 1.5. Schematic of morphological, functional and expression changes during epithelial-mesenchymal transition (EMT). See text for details.

YAP/TAZ are established as regulators of cellular processes important in tissue repair and tumourigenesis, such as cell proliferation, survival and stemness. Furthermore, many of the features of wound healing and cancer are thought to be potent activators of YAP/TAZ. Loss of cell-cell adhesion and polarity, matrix stiffening, cell spreading and enhanced growth factor signalling could represent upstream inducers of YAP/TAZ activity in the context of wound healing or cancer. For example, deposition of matrix components fibrin and fibronectin triggers recruitment and activation of myofibroblasts at wound sites. Wound tension has been reported to induce contractile closure of epithelial wounds in an integrin-dependent mechanism. In cell culture, fibronectin and integrins are required for invasion of cancer associated fibroblasts (CAFs). CAFs also promote ECM stiffening. Integrin clustering by fibronectin engagement and matrix stiffening is a potential activator of YAP via focal adhesion maturation and Src signalling (see section 2.3.3) (Levental, Yu et al. 2009, Liu, Xu et al. 2010, Jones and Ehrlich 2011). Connective tissue growth factor (CTGF), a known activator of fibroblasts in wound healing and the tumour microenvironment, is a bona fide YAP transcriptional target. Thus, sustained activation of YAP during wound healing or tumourigenesis could trigger a potential positive feedback loop sustaining a reciprocal relationship between the tumour and stroma (Grotendorst, Rahmanie et al. 2004, Lee, Shah et al. 2010, Alfaro, Deskins et al. 2013). In transgenic mice intestine and liver, YAP activation has been reported to synergize with tissue damage-induced inflammation to drive tumourigenesis (Cai, Zhang et al. 2010). Cutaneous wounds and SCCs were reported to exhibit highly similar expression profiles (Pedersen, Leethanakul et al. 2003). It will be interesting to see whether YAP plays a role in wound healing and tumourigenesis in the epidermis.

These considerations highlight the many parallels between wound healing and tumourigenesis and suggest that YAP could be a common denominator to these processes. This is a suggestion that I explored during my PhD.

Chapter 2. Materials & Methods

2.1. Mouse/human histology

2.1.1. Immunohistochemistry

Mouse backskin samples were harvested and fixed in neutral-buffered formaldehyde 10% vol/vol (sigma) and then embedded in paraffin in a head to tail orientation. The tissues were processed, embedded and sectioned at 4um and used for hematoxylin–eosin (H&E) staining and immunohistochemistry. Sections were de-waxed in xylene, dehydrated by passage through graded alcohols to water. If required for antigen retrieval, sections were microwaved in citrate buffer pH6 for 15 minutes and then transferred to PBS. Endogenous peroxidase was blocked using 1.6% hydrogen peroxide in PBS for 10 minutes followed by washing in distilled water. Species-specific blocking serum (Diluted to 10% in 1% BSA) was used to block non-specific staining in the tissue for 30 minutes. Slides were incubated with Primary antibody diluted to 1:100 in 1% BSA for 1 hour at room temperature. Sections were washed in PBS prior to applying the appropriate biotinylated secondary antibody for 45 min at room temperature. Sections were then washed in PBS and then incubated in ABC (Vector Laboratories PK-6100) for 30 minutes. Following washing in PBS, DAB solution was applied for 2-5 minutes with development of the colour reaction being monitored microscopically. Slides were washed in tap water, stained with a light haematoxylin, dehydrated, cleared and then mounted. Alternatively, slides were treated with a primary antibody. Images were acquired with a Zeiss light microscope using 40x and 20x objectives. Human samples were treated the same way. Additional images of human samples were obtained by data-mining the proteinatlas.org database (Berglund et al. 2008; Lundberg and Uhlén, 2010; Pontén et al. 2008; Uhlen et al. 2005, 2015, 2010).

2.1.2. Antibodies, image acquisition and quantification

Primary antibodies used for immunohistochemistry were: Vimentin (Abcam ab92547) 1/600, Zeb1 (Proteintech 21544-AP) 1/500, Keratin-5 (Abcam ab52635) 1/500, LacZ (Acris R1064P) 1/5000, E-Cadherin (Santa Cruz sc- 7870) 1/75 O/N,

YAP (Cell signalling 14074) 1/400 O/N; (Santa cruz sc-101199)1/200 O/N, Ki67 (Abcam ab16667) 1/350.
Samples were imaged with a Leica SP5 confocal microscope using a 63Å~ oil immersion objective and processed using Adobe Photoshop. Images were quantified for area or intensity of staining using ImageJ.

2.1.3. RNAscope (ACDbio)

Zeb1, Snai1, Snai2 probes was used according to manufacturer's instructions.

2.2. Mouse strains

All experiments were carried out in accordance with the United Kingdom Animal Scientific Procedures Act (1986) and UK Home Office regulations under project license number 70/7926. The *YAPfl/fl TAZfl/fl* mice were a gift from Axel Behrens (Francis Crick Institute). *K5-CreERt* mice were obtained from Ian Rosewell (Francis Crick Institute). *v-HA-Ras* transgene (TG.AC) mice were a gift from Ilaria Malanchi (Francis Crick Institute) and have been previously described (Leder et al. 1990). Wild-type mice were used in mixed background. All transgenic mice were in mixed background and used with littermate controls. APC p53 tumour sections from implanted nude mice were obtained from Owen Sansom (The Beatson Institute). *K14-Cre FAKfl/fl* mice and *Srcfl/fl*, *Fyn−/−*, *Yes−/−* mice were obtained from Val Brunton (University of Edinburgh) and were described previously (Marcotte et al. 2012; Ridgway et al. 2012). *Apc−/− p53−/−* (Apc580D/580D P53 Trp53**Δ**2−10 allele) mice were obtained from Owen Sansom and were previously described (Jonkers et al. 2001; Shibata et al. 1997). *AhCre* is previously described (Ireland et al. 2004). *K-rasG12D* allele was from Tyler Jacks (Jackson et al. 2001)

2.3. Mouse experiments

2.3.1. *YAP/TAZ* conditional deletion in adult epidermis

Tamoxifen (Sigma, 20 mg/ml in peanut oil) was injected intraperitoneally (IP) (5 µl/g body weight) for 5 consecutive days into 8- to 16-week-old controls or transgenic animals carrying *K5-CreERt YAPfl/fl TAZfl/fl* to induce *YAP/TAZ* knockdown and analysed for YAP/TAZ deficiency by immunohistochemistry 7 days thereafter. *K5-CreERt YAPfl/fl TAZfl/fl* mice used for long-term analysis were subsequently IP injected with tamoxifen every month for 3 consecutive days and analysed 8 weeks after the start of tamoxifen treatment. See fig. 2.2.

2.3.2. *YAP/TAZ* conditional deletion in neonate epidermis

100 µl 4 Hydroxytamoxifen (4OHT, Sigma) was topically applied per mouse using a pipette for 5 consecutive days at a dosage of 10 mg/ml in ethanol. Transgenic animals carrying *K5-CreERt YAPfl/fl TAZfl/fl* were used and controls were littermates carrying *K5-CreERt* or *YAPfl/fl TAZfl/fl*. See fig. 2.2.

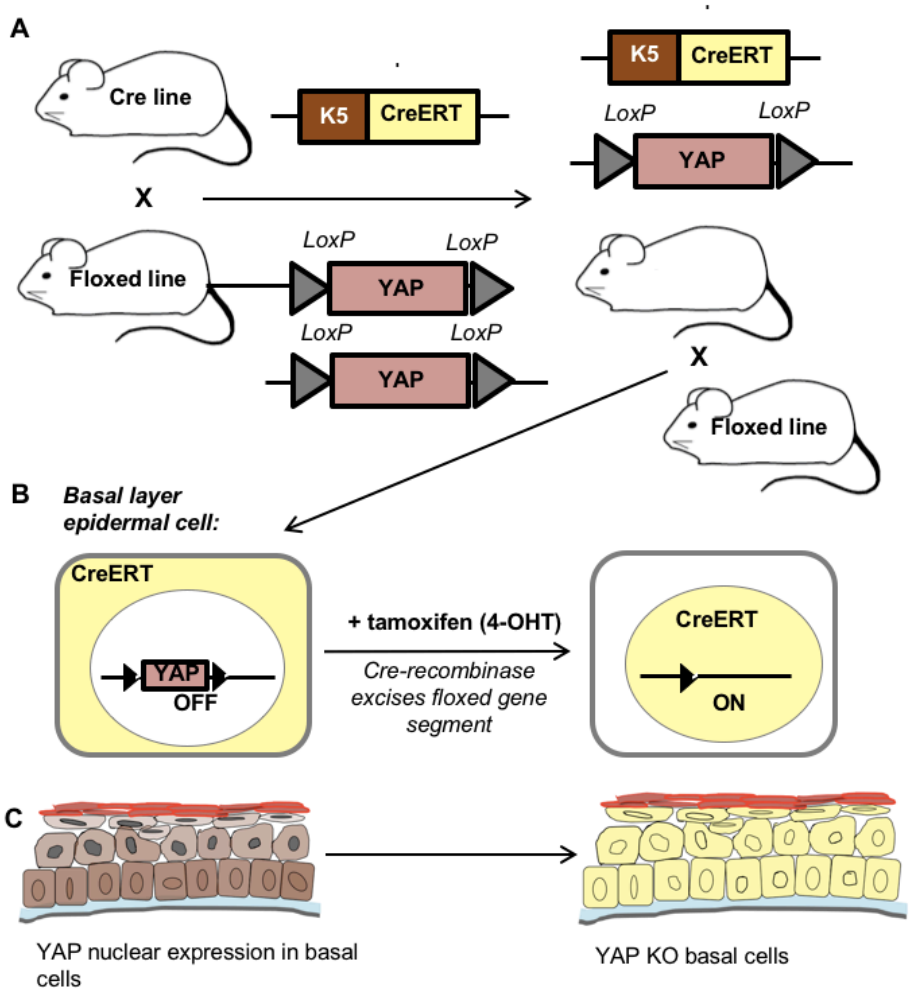


Figure 2.1. Conditional *YAP/TAZ* deletion. (A) The floxed mouse was generated by gene targeting to insert LoxP sites flanking the target sequence (in this case *YAP* or *TAZ*). The Cre transgenic mouse expressed the bacterial Cre-recombinase which recognises the palindromic loxP sequence under the control of the Keratin-5 promoter. Two generations are required to obtain offspring homozygous for the floxed allele and carrying the Cre recombinase. The Cre will be expressed in tissues expressing Keratin-5. **(B)** The time of deletion was controlled by using a tamoxifen-inducible recombinase: CreERT. The Cre-recombinase fused to a mutated form of the estrogen receptor is excluded from the nucleus. Only upon binding of the active tamoxifen metabolite 4-hydroxytamoxifen (4-OHT) is CreERT able to translocate from the cytoplasm into the nucleus, where it excises the floxed gene segment. **(C)** Epidermal basal cells express Keratin-5 and YAP. *YAZ* is deleted in the basal cells upon treatment of the K5-CreERT YAP floxed mice with tamoxifen. Since the basal cells are progenitor/stem cells which give rise to the suprabasal cells of the epidermis, the entire epidermis will be YAP-deficient upon epidermal turnover.

2.3.3. R26-YAP5SA-NLS transgene expression in adult epidermis

R26-YAP5SA-NLS mice were crossed to *K5-CreERt* mice. *K5-CreERt* mice were in mixed background. *ROSA26-YAP5SA-NLS K5-CreERt* mice were used with littermate controls. Expression of the transgene upon Cre-recombinase excision of the stop cassette was induced by tamoxifen application. Tamoxifen (Sigma, 20mg/ml in peanut oil) was injected intraperitoneally (IP) (5µl/g body weight) for 5 consecutive days into 8-16 week old controls or transgenic animals carrying *K5-CreERt R26-YAP5SA-NLS* to induce YAP5SA-NLS expression and analyzed for LacZ (lineage tracer) expression by immunohistochemistry from 3 days thereafter. *K5-CreERt R26-YAP5SA-NLS* mice used for tumour formation analysis were analyzed by immunohistochemistry from 10 days after the initial tamoxifen treatment start.

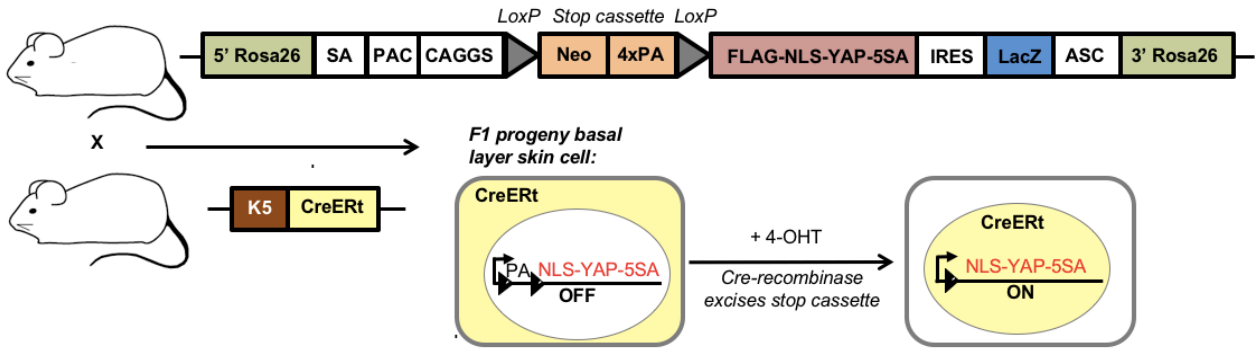


Figure 2.2. Schematic of R26-YAP5SA-NLS transgene expression in epidermal keratinocytes.

2.3.4. Wound healing experiments

Following the 5-day tamoxifen treatment, 4 hydroxytamoxifen (4OHT, sigma) was topically applied to shaved backskin for 5 consecutive days at a dosage of 10mg/ml in Ethanol and 100 µl was applied per mouse. Mice were anaesthetized with IsoFlo® (Isoflurane, Abbott Animal Health) and treated with the analgesics Vetergesic® (Alstoe Animal Health) and RimadylTM 266 (Pfizer Animal Health) for 2 days after wounding. A 4 mm punch wound was made in the backskin using a biopsy punch (Miltex) and mice were culled 48 hours later, with the wound section harvested and fixed immediately for immunohistochemical analysis.

2.3.5. Dasatinib treatment of skin

Mice between 8 and 14 weeks of age were topically treated with 150 µl Dasatinib (10 µM in DMSO, Selleck) onto shaved back skin for two consecutive days. Mice were analysed and the back skin harvested on the third day. Control mice were treated with DMSO.

2.3.6. Chemically-induced skin inflammation

Mice between 8 and 14 weeks of age were topically treated with 200 µl TPA (12-O-tetradecanoylphorbol-13-acetate, Sigma; stock dissolved in DMSO and diluted in acetone, 12.5 µg/mouse) onto shaved backskin for two consecutive days. Mice were analysed and the back skin harvested on the third day. Control mice were treated with DMSO and acetone.

2.3.7. Chemical carcinogenesis of skin

Chemical skin carcinogenesis was induced on 12-week-old *v-Ha-Ras* transgene (TG.AC)-expressing mice in mixed background by a single application of 100 µg/mouse DMBA [7,12-dimethylbenz(a)anthracene, Sigma] onto the shaved backskin followed by biweekly topical treatments with TPA (4 µg/mouse) starting 1 week after DMBA application. Skin papillomas were detectable 8 weeks after start of DMBA-TPA treatment and harvested at 13 weeks. For Dasatinib treatment, papillomas allowed to reach ~1 cm3. These established papillomas were treated topically with Dasatinib (10 µM in DMSO/acetone; 100 µl/papilloma) once and analysed 3 or 7 days thereafter. For generation of skin carcinomas, ~12-week-old DMBA-treated FVB/N wild-type mice were treated biweekly with 4 µg/mouse TPA onto shaved back skin for 10 weeks then weekly for a further 4 weeks before the carcinomas were harvested.

2.3.8. Intestinal experiments

Mice carrying the *AhCre* recombinase were induced by three intraperitoneal (i.p.) injections of 80 mg/kg **β**-Napthoflavone for 1 day. Intestinal phenotypes were analysed 4 or 7 days after transgene induction to assess homeostasis or regeneration, respectively. Intestinal regeneration was induced by irradiating mice with 14Gy gamma-irradiation 4 days after recombinase induction. Mice were

sacrificed 72 h post irradiation and the small intestine isolated and flushed with tap water. Ten 1 cm portions of small intestine were bound together with surgical tape and fixed in 4% neutral buffered formalin.

2.3.9. Organoid transplantation experiments

Intestinal crypts from *VillinCreER Apcfl/fl p53fl/fl* mice were removed 4 days following Cre induction with Tamoxifen (2 mg). This causes full recombination at both the *Apc* and *p53* loci and organoids now grow as spheres in an R-Spondin-independent manner (Sato et al. 2009). For transplantation of organoids, 50 organoids were transplanted subcutaneously into nude mice (see Valeri et al. 2014). A dose of 10 mg/kg dasatinib daily gavage was chosen as we have previously shown to cause a reduction in p-Src *in vivo* without toxicity (Morton et al. 2010). Mice were treated continuously from 10 days post injection of spheres.

2.4. Cell culture

Human HaCAT cells and A431 cells (Francis Crick Institute cell services) were grown in DMEM (Gibco 41966) with 10% FCS and Penicillin/Streptomycin. All cells are subject to mycoplasma testing.

2.4.1. siRNA

Human Caco-2, A431 or HaCAT cells were cultured as previously stated (Fletcher et al. 2015; Elbediwy et al. 2012). All siRNA transfections were performed using Lipofectamine RNAiMax transfection reagent (Invitrogen). Briefly, cells were seeded in 6-well plates and treated with the siRNA/transfection mix 2 h post seeding. A final concentration of 50-100 nM siRNA was used for transfections. The following day, another transfection was performed before the cells were trypsinised 4 h later and reseeded either for 2D or 3D culture. 2D siRNA treatments were left for a total of 72 h and 3D treatments were left for a total of 120 h. 3D cultures were prepared as previously stated (Elbediwy et al. 2012). siRNAs were used as siGenome pools (Dharmacon).

2.4.2. Treatment with inhibitor, blocking antibody and low-calcium medium

2D mammalian inhibitor treatments were for 4 h. They were as follows: 5 μM PF573228 (FAK); 5 μM saracatinib (Src); 5 μM dasatinib (Src/Abl); 5 μM BX795 (PDK1); 5 μM MK2206 (AKT); 2 μM GDC0941 (PI3K); 100 μM blebbistatin (myosin); 100 μM Y27632 (Rock); 2 μM latrunculin A (actin) and 3 μM everolimus (mTOR); reagents were supplied by Sigma-Aldrich and Stratech Scientific Ltd. Integrin **β**1 blocking antibody or control IgG antibody (gifts from Nancy Hogg, Francis Crick Institute) was incubated with the cells for 1 h at a concentration of 10 μg/ml before the cells were replated. Low calcium conditions were as previously reported (Elbediwy et al. 2012). 2D wound healing involved plating the cells at high density, causing a scratch and subsequent addition of Dasatinib for 4 h.

2.4.3. Cell culture scratch assay

Cells are transfected with siRNA as previously described (Dong et al. 2007), before being replated at high density and left for 24 hours. Cells are scratched with a P200 pipette tip and left for 4 hours before being fixed. Cells treated with dasatinib were treated and scratched simultaneously before being fixed. Cells were fixed with 4% PFA for 15 minutes before permeabilsing as previously described (Dong et al. 2007).

2.4.4. Cell culture antibodies, image acquisition and quantification

Primary antibodies used were: Rabbit Zeb1 Proteintech 21544-AP) 1/100 and Mouse YAP (Santa Cruz sc-101199) 1/100. Secondary antibodies were from Invitrogen, and used at 1:500 for 2 hours at room temperature along with DAPI. Cell culture samples were imaged with a Leica SP5 confocal microscope using a 63x oil immersion objective and processed using Adobe Photoshop. Cells were assessed over three independent experiments counting 200-300 cells per condition.

2.4.5. Plasmids

pCMV6 AC GFP ZEB-1 Transcript 1 (Origene) was transfected using Lipofectamine 3000 (Invitrogen). Quantification of extruded cells was performed by

analysing n342 transfected cells over three experiments and recording number of extruded cells versus cells present within the monolayer.

2.5. Molecular biology

2.5.1. RNAseq of HaCaTs and A431s and gene set enrichment analysis

A431 or HaCAT cell lysates transfected with empty vector, YAP1 S5A, control siRNA or YAP1 siRNAs were used. Sequencing was performed on biological triplicates on the Illumina HiSeq 2500 platform and generated ~69 million 100 bp paired end reads per sample (data deposited in GEO under accession number GSE80082). Sequenced reads were trimmed to 75 base pairs and mapped to the Refseq genome model, using RSEM (v.1.2.21). RSEM uses the bowtie2 alignment tool. Gene counts were filtered to remove genes with 10 or fewer mapped reads per sample. TMM (treated mean of M-values) normalisation and differential expression analysis using the negative binomial model was carried out with the RBioconductorpackage **'**EdgeR**'**. Genes with logCPM>1 and FDR<0.05 were judged to be differentially expressed. Enrichments of pathway, category and motif gene sets were assessed using GSEA with logFC preranked gene lists. Gene sets with an enrichment false discovery rate (FDR) value of less than 0.05 were judged to be strongly statistically significant and values of less than 0.25 significant.

2.5.2. Identification of TEAD1 binding sites

Predictions for TEAD1 binding sites were obtained by aligning the TEAD1 motif sequence (retrieved from the JASPAR-2014 database (motif ID: MA0090.1) to -/+ 1kB region around all transcription start sites annotated in ENSEMBLE from GSEA candidates. We used a positon-weight matrix (PWM) and set 85% of the maximum score as a cut-off. The predictions for the coding and antisense-strand of DNA were made separately (the candidate motif sequences are listed in Appendix 7.2). We picked one transcription start site for each gene (the one that had the most putative TEAD1 motifs close to the TSS) and highlighted the position of the TEAD1 site(s) as black boxes (Fig 3.7C).

2.5.3. RNA extraction of mouse skin and RT-PCR

Total RNA was extracted from homogenised mouse skin using an RNeasy Mini Kit

(Qiagen). cDNA synthesis for WT or dKO mice was performed using Superscript II (Invitrogen). Primers were purchased as Quantitect Primers (Qiagen). Gene samples were run in triplicate on a Quantstudio 12 Flex Thermocycler. Expression values were calculated using the ΔΔCT method relative to the housekeeping gene β-2 microglobulin (B2M). All error bars indicate s.e.m.

2.5.4. Chromatin Immunoprecipitation

ChIP was performed as previously described (Coda et al. 2017), with the exception of the use of the ChIP Clean and Concentrate kit (Zymo Research, USA) for clean-up of enriched chromatin according to the manufacturer's instructions. Quantitative PCR for enrichment at the ZEB1 promoter was performed using the following primers: Fwd: 5'-GATGGGGAAGTGAGACAAGC-3'; Rev: 5'CAGCTGGATTGAAAGAGAGGC-3'".

2.6. Key resources table

Key Resources Table				
Reagent type (species) or resource	**Designation**	**Source or reference**	**Identifiers**	**Additional information**
Cell Line (Human)	HaCAT	Cell Services (Francis Crick Institute) Pubmed ID: 26989177	RRID:CVCL_0038	
Antibody (Rabbit)	Vimentin	Abcam	ab92547	1/600 IHC
Antibody (Rabbit)	Zeb1	Proteintech	21544-AP	1/500 IHC / 1/100 IF
Antibody (Rabbit)	Keratin-5	Abcam	ab52635	1/500 IHC
Antibody (Rabbit)	LacZ	Acris	R1064P	1/5000 IHC
Antibody (Rabbit)	E-Cadherin	Santa Cruz	SC-7870	1/75 IHC
Antibody (Rabbit)	YAP	Cell Signalling Technology	14074	1/400 O/N IHC
Antibody (Rabbit)	Ki-67	Abcam	ab16667	1/350 IHC
Antibody (Mouse)	Tead-1	BD Biosciences	BD 610922	2ug CHIP
Antibody (Mouse)	YAP	Santa Cruz	SC-101199	1/100 IF
Antibody (Rabbit)	TAZ/WWTR1	Sigma	HPA007415	1/100 IHC
Transfection reagent	Lipofectamine RNAiMAX	Thermo Fisher	Cat no: 13778075	
siRNA	Tead 1	Dhamacon	Cat no: M-012603-01-0005	80 nM Final
siRNA	Tead 2	Dhamacon	M-012611-00-0005	80 nM Final
siRNA	Tead 3	Dhamacon	M-012604-01-0005	80 nM Final
siRNA	Tead 4	Dhamacon	M-019570-03-0005	80 nM Final
siRNA	YAP	Dhamacon	M-012200-00-0005	80 nM Final
Human Protein Atlas	Various	Pubmed ID: 16774037	https://www.proteinatlas.org/	
Human Cancer Samples	Vimentin/YAP	University of Southamption / Gareth Thomas		
Mouse strain	Rosa26-YAP5SA	Junhao Mao (University of Massachusetts Medical School)		mixed background
Mouse strain	K5-CreERT2	Ian Rosewell (Francis Crick Institute)		mixed background
Mouse strain	Yapfl/fl Tazfl/fl	Axel Behrens (Francis Crick Institute)		mixed background
Chemical compound, drug	12-O-tetradecanoylphorbol-13-acetate (TPA)	Sigma	P8139	12.5 µg/mouse dissolved in DMSO
Chemical compound, drug	12-dimethylbenz(a)anthracene (DMBA)	Sigma	D3254	100 µg/mouse
Chemical compound, drug	Dasatinib	Selleck Biochem	S1021	5µM Final
RNA target probe	RNAscope Probe - Mm-Zeb1	ACD	451201	
RNA target probe	RNAscope Probe - Mm-Snai1	ACD	451211	
RNA target probe	RNAscope Probe - Mm-Snai2	ACD	451191	
Chemical compound, drug	Tamoxifen	Sigma	T5648	IP 5 µl/g body weight of a 20mg/ml solution in peanut oil 5x consecutive days
Chemical compound, drug	4-Hydroxytamoxifen	Sigma	H7904	topical application of 200 µl oF 1.0 mg per 0.1 mL 4'OHT in DMSO on dorsal skin 5x consecutive days.

Chapter 3. YAP/TAZ regulate mammalian epidermal homeostasis

The skin is the largest organ in the body and functions as the major physical barrier to the external environment, protecting against insults from UV rays, chemicals, pathogens, as well as regulating temperature. Mammalian skin is comprised of two main layers: the epidermis and dermis, separated by a basement membrane (Fig 1.4). Dermal cells derive from mesenchyme to form a dense irregular connective tissue, whilst the epidermis is a stratified squamous epithelium primarily comprised of layers of keratinocytes. The basal layer keratinocytes adhere to the basement membrane and are the progenitor/stem cells of the epidermis. As basal cells proliferate, daughter cells move upwards and differentiate before eventually being shed from the skin surface (Fuchs 2007). This process of epidermal self-renewal must be very tightly regulated as too little proliferation results in thinning of the skin and improper barrier function whilst overproliferation can lead to hyperproliferative disorders or tumourigenesis. Additionally, the rate of keratinocyte proliferation must be flexible to be able to respond to epidermal injury. How do keratinocytes “know” when to proliferate or differentiate to maintain epidermal homeostasis? Since YAP and TAZ are known to be involved in epithelial tissue growth control, we began by examining their expression in skin to determine if they could be involved in regulating epidermal proliferation (see Introduction section 1.3 for more detail and references therein).

3.1. YAP/TAZ are expressed in epidermal basal layer progenitor cells of mouse and human skin

YAP and TAZ are transcriptional co-activators that are chiefly regulated by their subcellular localisation: active in the nucleus and inactive in the cytoplasm (Fig 3.1; see Introduction section 1.2 for more detail). Therefore, I began by recording both their expression and subcellular localisation in wild type mouse and human skin. YAP and TAZ proteins were both expressed in mouse skin epidermis at various stages of development: embryonic (Fig. 3.2A), neonatal (Fig 3.2B) and adult (Fig 3.2C). Both proteins were largely localised to the nucleus in basal layer epidermal keratinocytes whilst they were cytoplasmic in the differentiating suprabasal cells. At

all stages YAP expression was stronger and more prominently nuclear than TAZ in basal layer cells. Nuclear localisation of both proteins was particularly strong in the basal layer cells of the interfollicular epidermis and the hair follicle (Fig 3.2B,C). YAP and TAZ were also found to be nuclear in some highly flattened suprabasal squamous cells (Fig. 3.2A,B,C). Although the functional significance of such nuclear localisation is unclear, it is consistent with findings in cell culture that flattening cells or deforming the nucleus induces YAP and TAZ to accumulate in the nucleus (Dupont, Morsut et al. 2011, Elosegui-Artola, Andreu et al. 2017). YAP and TAZ showed a similar expression pattern and subcellular localisation in adult human skin (Fig 3.2D), with both proteins found to be nuclear in basal layer epidermal cells and cytoplasmic in suprabasal daughter cells. Similar to mouse skin, some nuclear YAP and TAZ was observed in the terminally differentiated highly flattened suprabasal squamous cells (Fig 3.2D).

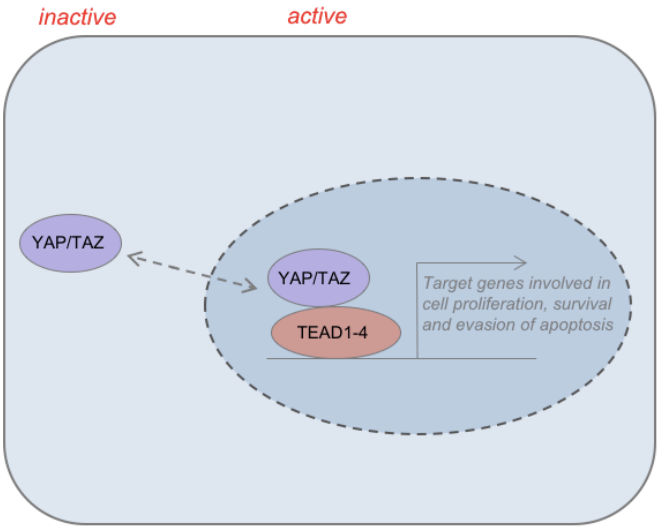


Figure 3.1. YAP and TAZ are transcriptional co-activators. Simplified schematic of YAP/TAZ translocation from the cytoplasm (inactive form) to the nucleus (active form), where it can interact with TEAD family transcription factors and other transcription factors to promote transcription of target genes involved in cell proliferation, survival and evasion of apoptosis.

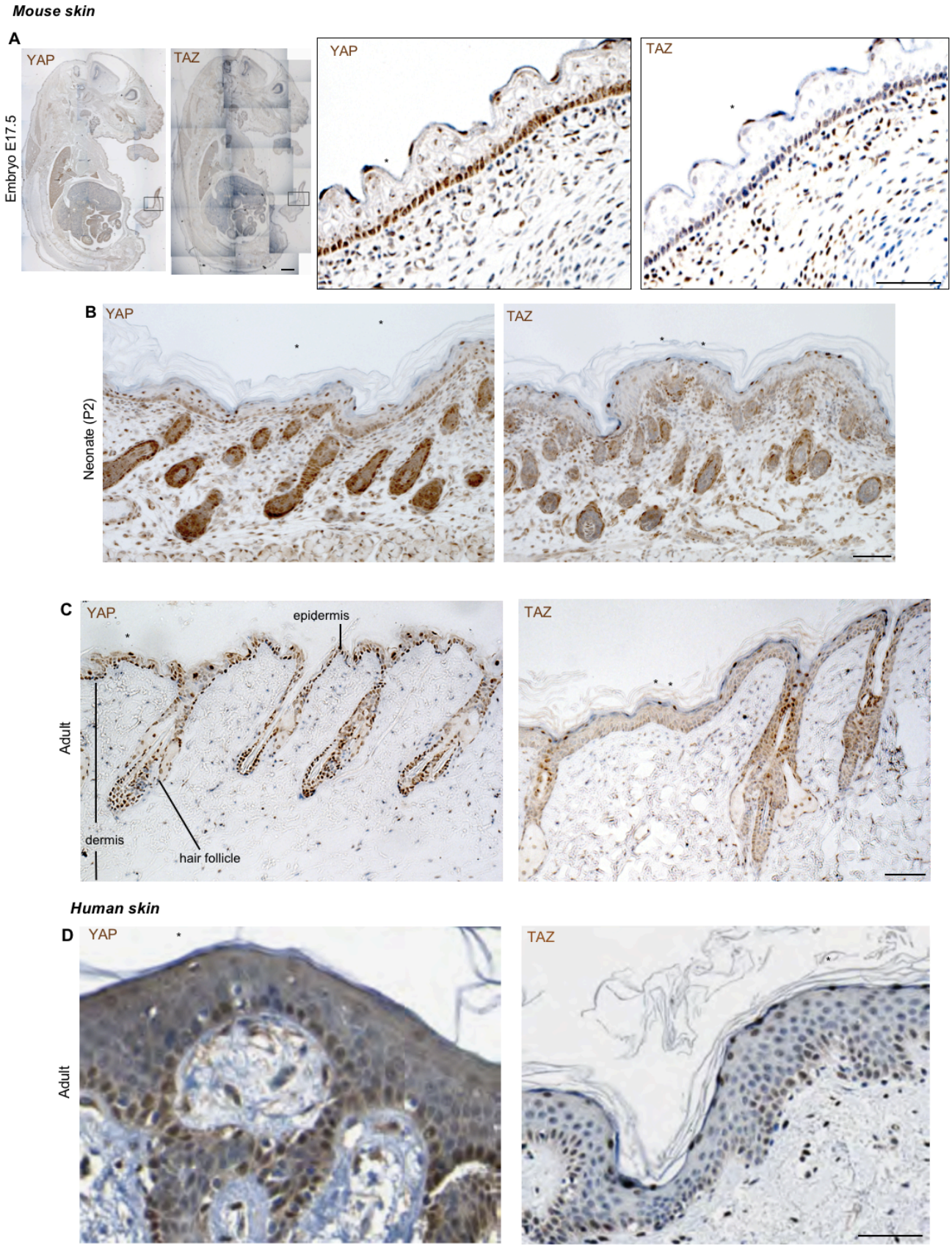


Figure 3.2. YAP and TAZ are nuclear localised in mouse and human basal layer progenitor cells. (Legend overleaf)

Figure 3.2. YAP and TAZ are nuclear localised in mouse and human basal layer progenitor cells. (A) Embryonic (E17.5) mouse skin tissue sections stained for either YAP or TAZ (brown immunostain) and co-stained for eosin (blue). Top panels show longitudinal section through whole embryo; bottom panels show magnified skin sections. Scale bars A' 1mm; A'' 200 μM; A''' 50 μM. **(B)** Neonatal (P2) mouse skin tissue sections stained for YAP or TAZ. Scale bar 50μm. **(C)** Adult (8 weeks) mouse tissue sections stained for YAP or TAZ. Scale bar 50 μM. **(D)** Adult human skin sections stained for YAP or TAZ, from Human Protein Atlas. Note the nuclear localisation of YAP in basal layer progenitor cells in both mouse and human epidermis, as well as some terminally differentiated flattened cells (asterisks). Other differentiating cells have cytoplasmic YAP localisation. TAZ is localised in a similar pattern, but expression is weaker than YAP. Scale bar 50 μM.

3.2. YAP/TAZ are required for skin homeostasis

Knockdown of *YAP* in various epithelial cell cultures is known to reduce cell proliferation and survival whilst YAP overexpression drives proliferation and suppresses apoptosis and differentiation (Zhang, Pasolli et al. 2011, Zhi, Zhao et al. 2012). *In vivo* studies found that overexpression or nuclear localisation of YAP in skin resulted in elevated epidermal cell proliferation, with expansion of the basal layer compartment and a thickened epidermis (Schlegelmilch, Mohseni et al. 2011, Zhang, Pasolli et al. 2011, Beverdam, Claxton et al. 2013). Epidermis lacking YAP is fragile and thin with reduced progenitor cell proliferation (Schlegelmilch, Mohseni et al. 2011, Zhang, Pasolli et al. 2011). Since YAP and TAZ show functional redundancy in other tissues it is possible that residual proliferation is driven by TAZ in YAP-deficient skin. One study used siRNA to knockdown *YAP* and *TAZ* in the skin but the results were inconclusive because the knockdown was transient and non-cell-specific (Lee, Byun et al. 2014). In order to better understand the physiological function of YAP and TAZ in the epidermis we generated double conditional knockout mice (dKO) and drove the tamoxifen-inducible Cre recombinase in the skin with the Keratin-5 promoter (*K5-CreERT YAP$^{fl/fl}$ TAZ$^{fl/fl}$*, Fig. 3.3A). Thus upon tamoxifen application *YAP* and *TAZ* were deleted specifically from the epidermis (Fig. 3.3B,C).

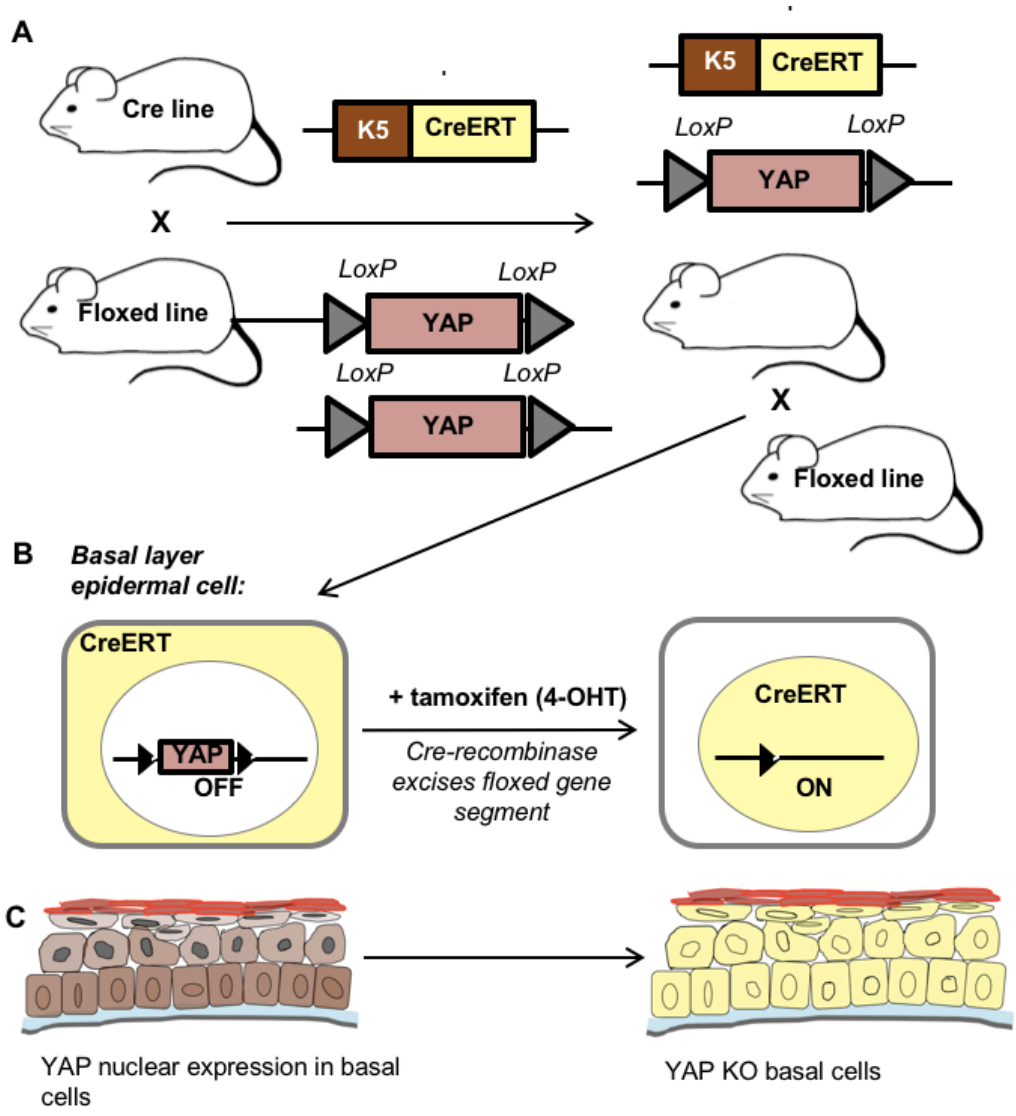


Figure 3.3. Conditional *YAP/TAZ* deletion. (A) The floxed mouse was generated by gene targeting to insert LoxP sites flanking the target sequence (in this case *YAP* or *TAZ*). The Cre transgenic mouse expressed the bacterial Cre-recombinase which recognises the palindromic loxP sequence under the control of the Keratin-5 promoter. Two generations are required to obtain offspring homozygous for the floxed allele and carrying the Cre recombinase, i.e. *K5-CreERT YAPfl/fl*. The Cre will be expressed in tissues expressing Keratin-5. **(B)** The time of deletion was controlled by using a tamoxifen-inducible recombinase: CreERT. The Cre-recombinase fused to a mutated form of the estrogen receptor is excluded from the nucleus. Only upon binding of the active tamoxifen metabolite 4-hydroxytamoxifen (4-OHT) is CreERT able to translocate from the cytoplasm into the nucleus, where it excises the floxed gene segment. **(C)** Epidermal basal cells express Keratin-5 and YAP. *YAZ* is deleted in the basal cells upon treatment of the K5-CreERT YAP floxed mice with tamoxifen. Since the basal cells are progenitor/stem cells which give rise to the suprabasal cells of the epidermis, the entire epidermis will be YAP-deficient upon epidermal turnover. These mice are crossed two generations to *TAZfl/fl* mice to obtain *K5-CreERT YAPfl/fl TAZfl/fl.*

Whilst the skin of control mice remained covered by a thick layer of hair (Fig. 3.4A), the dKO adult mice started to lose hair in patches two weeks after the first tamoxifen application (Fig. 3.4B,C). Neonatal dKO animals treated with tamoxifen at P1 (see Methods) failed to develop any hair (Fig. 3.4D). Histological sections confirmed the expression of YAP and TAZ in the control and the loss of both proteins in adult dKO one week after tamoxifen application. Proliferation of basal layer cells, marked by Ki-67, was significantly reduced in dKO skin (Fig. 3.4A,B,E). Despite the reduction in Ki-67-positive cells in dKO epidermis, the epidermal thickness was not significantly different (Fig. 3.4F). The *YAP/TA* dKO skin hair loss and impaired basal layer cell proliferation phenotypes are reminiscent of those seen in skin-specific *β1 integrin* conditional knockouts (Brakebusch, Grose et al. 2000, Raghavan, Bauer et al. 2000, Grose, Hutter et al. 2002, Piwko-Czuchra, Koegel et al. 2009, Singh, Chen et al. 2009). Notably, neither *YAZ* nor *TAZ* single knockout had an effect on hair or basal layer cell proliferation, confirming that these proteins act redundantly in the epidermis (data not shown).

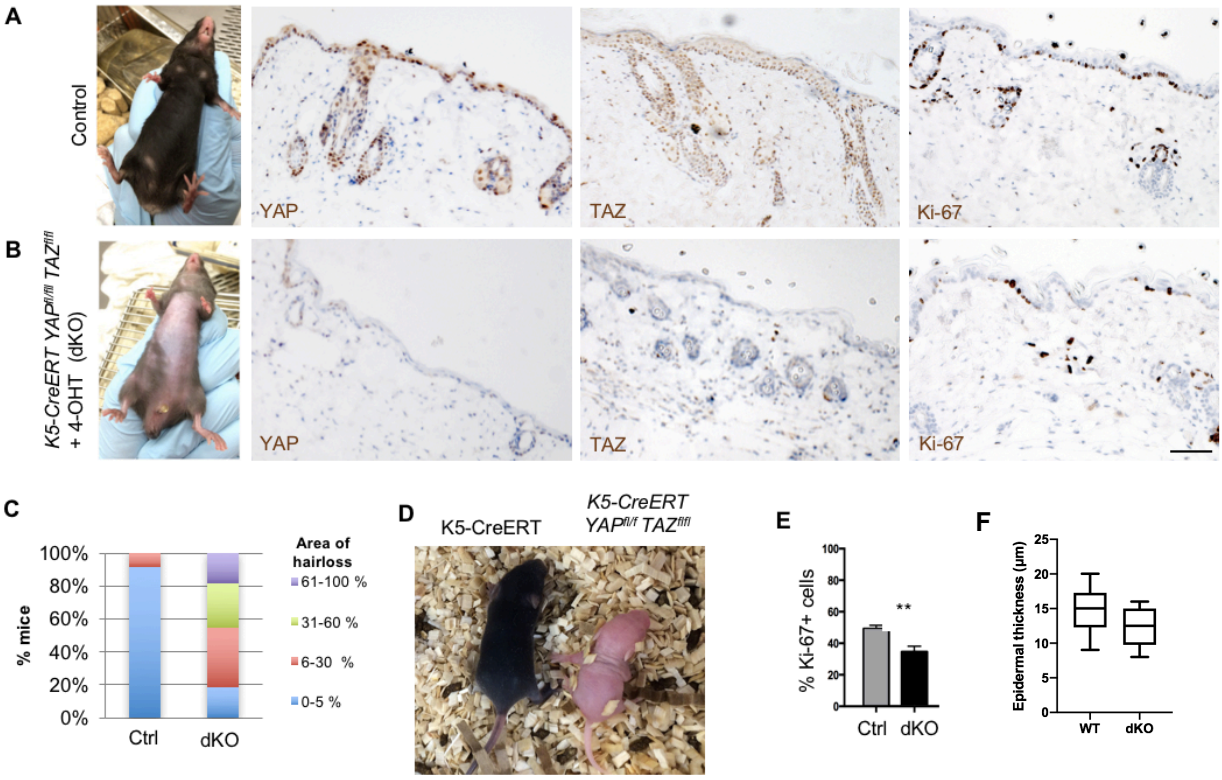


Figure 3.4. Epidermal deletion of *YAP* and *TAZ* results in impaired skin homeostasis. **(A)** Control mice have a thick layer of hair (fur) covering their skin. YAP, TAZ and Ki-67 are expressed in basal cells of the epidermis. **(B)** Double conditional *YAP/TAZ* knockout mice treated with tamoxifen as adults experience significant hair loss. Back skin sections stain negative for YAP and TAZ and Ki-67 staining is reduced. Representative histological skin sections are shown. Adult mice were 8-10 weeks old. Scale bar **50** µM. **(C)** Quantification of hair loss (Ctrl *n*=11; dKO *n*=12) **(D)** Representative control and dKO neonatal mice (P8) treated with tamoxifen at P1. Control mice develop hair whilst dKO mice do not. **(E)** Ki-67-positive cells quantified as a percentage of total interfollicular basal cells in each randomly selected 40Å~ field of view. *n*=757 control cells; *n*=896 dKO cells. Values are means±s.e.m. **P<0.01. **(F)** Epidermal thickness in µM of WT and dKO animals. *n*=3 samples each from 8 WT and 8 dKO animals. $p > 0.05$.

3.3. YAP/TAZ contribute to cutaneous wound healing

Given that deletion of *YAP* and *TAZ* resulted in reduced proliferation of basal layer cells, we next sought to examine the effect of their loss when increased proliferation of keratinocytes is in particularly high demand, during wound healing. A previous study found that siRNA knockdown of *YAP* and *TAZ* resulted in delayed wound closure (Lee, Byun et al. 2014). However, as the siRNAs were applied using a topical gel the knockdown was transient and affected other cell types including dermal cells. Using the conditional K5-CreERT to knock out *YAP* and *TAZ* we were able to dissect the roles of these proteins specifically in epidermal keratinocytes during wound healing.

I found that expression of both YAP and TAZ was increased in the epidermis of healing wounds, with strong nuclear staining of both proteins in the basal layer keratinocytes (Fig. 3.5A). Next, we sought to examine whether YAP and TAZ contribute to wound repair. To this end, we measured the time taken for small punch wounds (4mm) to close in control and dKO mouse back skin (Fig 3.5B; see Methods). We found that wounds in dKO animals took longer to close (12 days versus 10 days in controls. Fig. 3.5C). *Yap* and *Taz* single skin knockouts did not show a delay in wound closure compared to controls (data not shown). In control animals Ki-67 was strongly expressed in the basal layer cells of healing epidermis three days after wounding (Fig. 3.5D). The number of Ki-67-positive cells was significantly reduced in dKO epidermis, indicating that the delayed wound healing may be in part attributed to decreased basal layer keratinocyte proliferation in *YAP/TAZ* dKO animals (Fig. 3.5D). These data show that YAP and TAZ have an important physiological function in basal layer keratinocytes to promote proliferation.

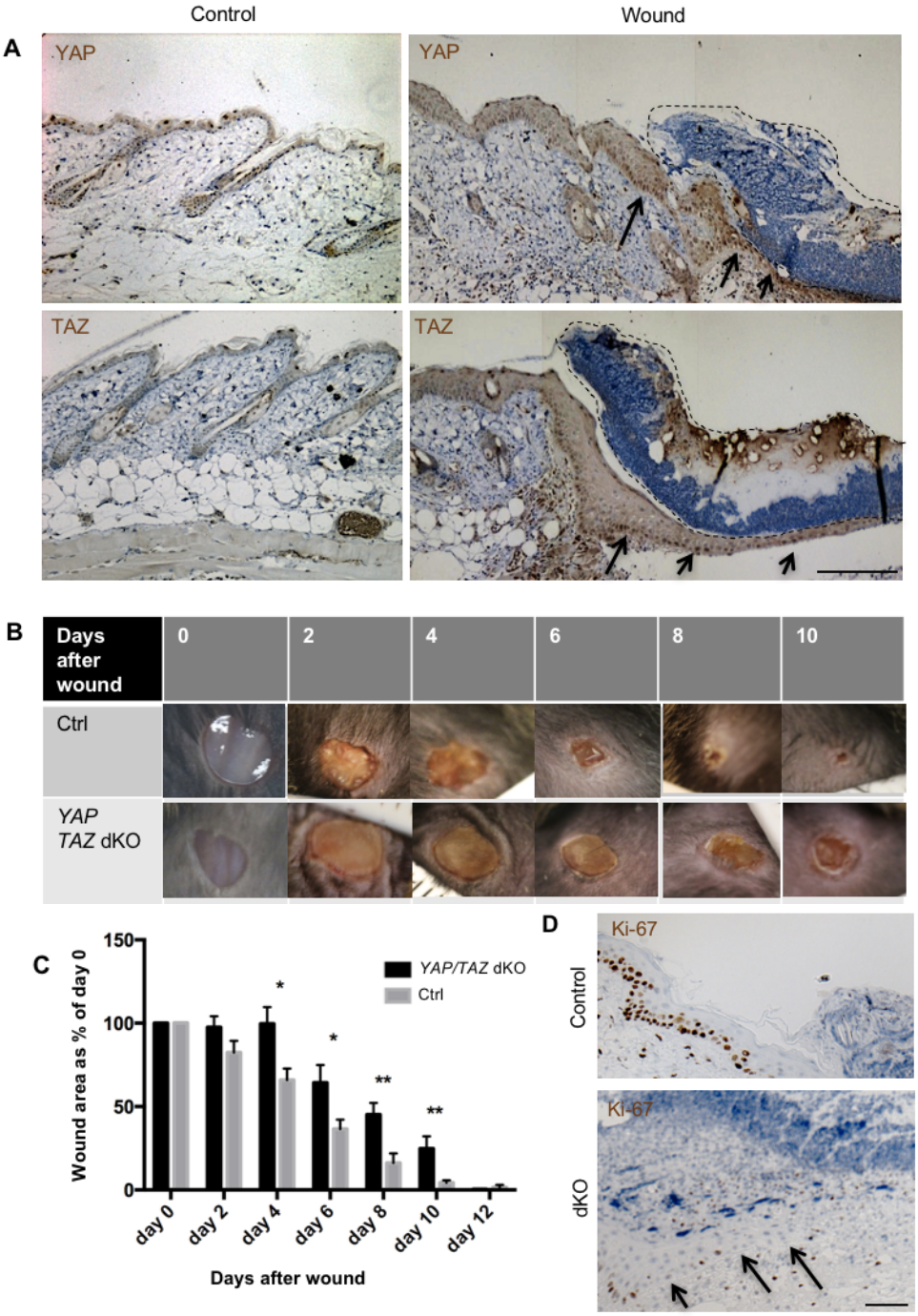


Figure 3.5. Epidermal-specific deletion of *YAP* and *TAZ* impairs wound healing. (A) Punch biopsy wound edge of wild type mouse skin stained for YAP and TAZ, taken 3 days after wounding. Dotted lines demarcate wound scab above newly formed epidermis. Arrows show strong nuclear expression of YAP and TAZ in basal cells of reepithelizing epidermis. Scale bar 100 μM. **(B)** Punch wound healing in *YAP/TAZ* double conditional knockout mice (dKO; *n*=8) is delayed compared to control (*n*=8). Representative images are shown. **(C)** Quantification of wound healing rates in control versus dKO mice. ImageJ was used to measure the wound area at each stage. Values are means±s.e.m. *P<0.05, **P<0.01. **(D)** Ki-67 staining is reduced in dKO healing epidermis (3 days post punch wound) compared to control animals. Representative sections shown. *n*=7 animals. Scale bar 50 μM.

Although *YAP/TAZ* dKO mice showed delayed wound healing, most wounds were fully re-epithelialized by 12-13 days after wounding (Fig 3.5D). I also noticed that some of the mice used for the hair loss experiment (Fig 3.4B) began to redevelop hair around 4 weeks after tamoxifen induction. It has been shown that following skin-specific knockout of *β1 integrin*, the few keratinocytes that escape Cre-mediated recombination can rapidly repopulate the mutant epidermis (Piwko-Czuchra, Koegel et al. 2009). Interestingly, the amplification of β1 integrin-positive cells escaping Cre-mediated recombination was more notable yet during wound closure. Similarly, we found that shortly after tamoxifen induction of Cre recombination the epidermis was mostly negative for YAP and TAZ though a small number of cells retain nuclear expression of the proteins (Fig. 3.6A,B). Yet, five weeks after tamoxifen administration YAP and TAZ were widely expressed throughout the epidermal basal layer keratinocytes, with expression levels similar to that of the wild type (Fig. 3.6C,D). This rapid repopulation of the epidermis by YAP-positive and TAZ-positive cells highlights their role in basal layer keratinocyte proliferation and suggests that YAP-positive and TAZ-positive cells escaping Cre-mediated recombination may be responsible for re-epithelialization of healing wounds in the dKO animals.

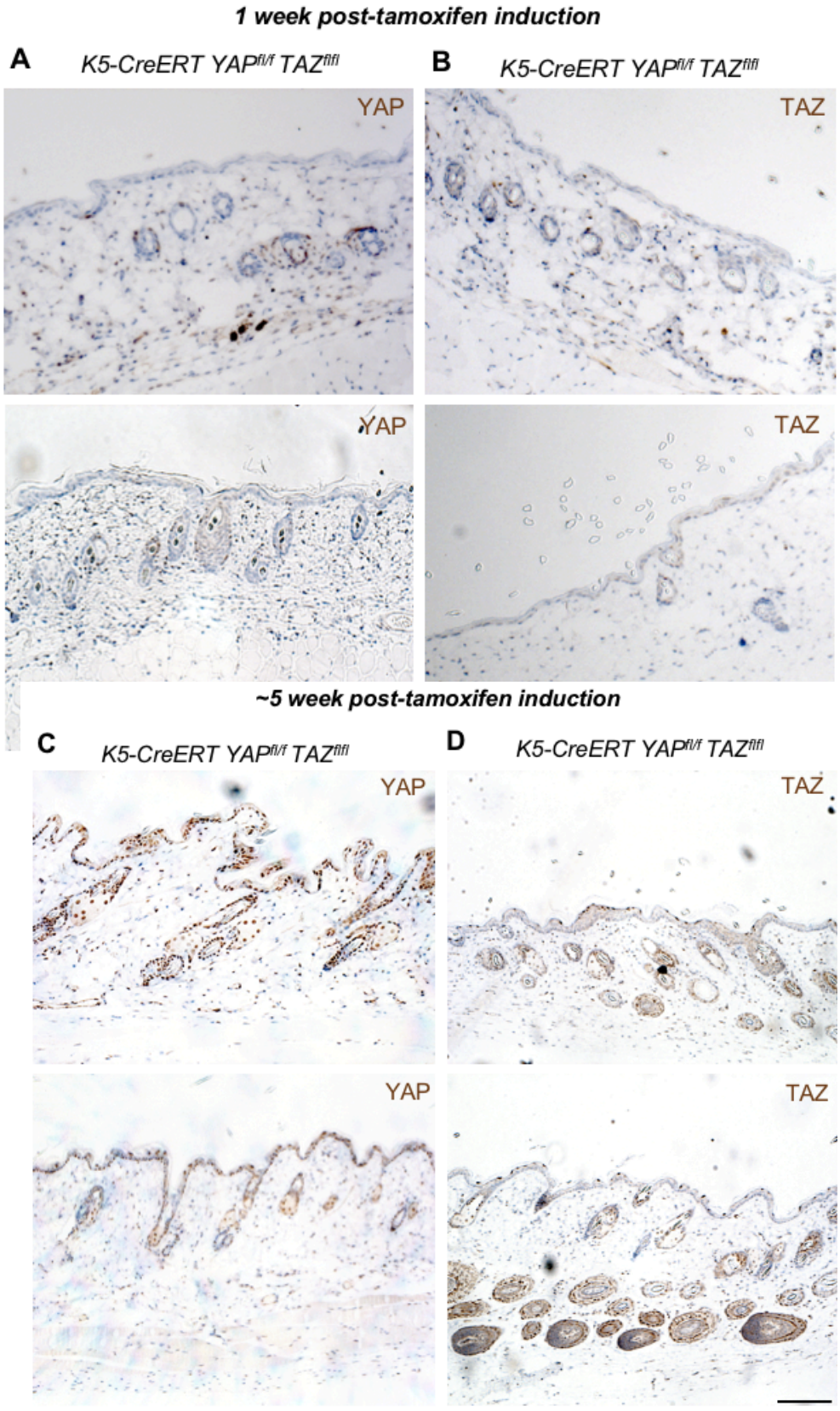


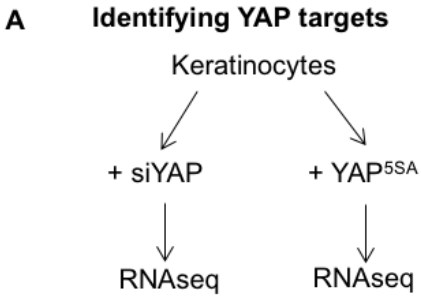
Figure 3.6. Repopulation of *YAP*/*TAZ* dKO skin by cells escaping Cre-mediated recombination. (Legend overleaf).

Figure 3.6. Repopulation of *YAP*/*TAZ* dKO skin by cells escaping Cre-mediated recombination. Histological sections of dKO back skin stained for YAP **(A)** and TAZ **(B)** at 1 week post-tamoxifen induction of K5-CreERT show very little epidermal expression. Note mosaic deletion. 4-6 weeks post-tamoxifen induction YAP **(C)** and TAZ **(D)** staining shows increased basal epidermal cell expression, similar to wild type expression. Scale bar 100 μM.

3.4. YAP regulates a set of target genes in epidermal basal layer keratinocytes

Having confirmed that YAP and TAZ promote proliferation in basal layer epidermal progenitor cells we sought to examine the downstream targets enacting this effect. As a first step we designed an experiment to identify YAP target genes in human keratinocytes. RNA sequencing (RNA-seq) was performed on mRNA isolated from A431 cells or HaCaT cells transfected with a mutant activated form of YAP (YAP^{5SA}) or siRNA against *YAP* (Fig. 3.7A,B). Differences in individual gene expression may be undetectable when comparing expression profiles for specific experimental conditions. Gene set enrichment analysis (GSEA) is a computational method that associates *a priori* defined aggregations of genes (gene sets defined by shared biological function or regulation) with an experimental condition (Subramanian, Tamayo et al. 2005). GSEA thus enables identification of gene sets that are statistically enriched or depleted between two expression profiles, providing a list of gene sets to prioritise for further study. GSEA of the RNA-seq data yielded several gene sets as YAP-regulated in human keratinocytes: the previously identified Hippo/YAP signalling reactomes; cell cycle reactomes (including PCNA and E2F targets and Cyclin E-associated genes); cell growth reactomes (including c-Myc, global translation regulators and related to regulation of ornithine decarboxylase); cancer signalling reactomes (including EGFR-Ras signalling targets); and cancer microenvironment/metastasis reactomes (including regulators of cellular-extra cellular matrix interactions) (see Methods 2.5.1). Gene sets identified were then analysed for the presence of TEAD-specific binding motifs within ±1 kb of their transcription start sites, since these would be likely to be direct YAP targets. These genes are listed in Fig. 3.7C. A limitation of only looking for direct targets is the likelihood of missing important functional effectors of YAP which are not direct transcriptional targets.

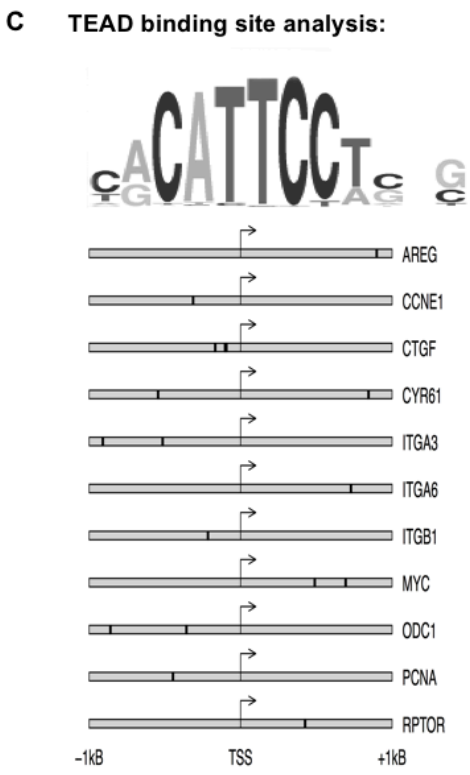
A



B

NAME	link.to.category	FDR_logFC_5SA_YAP_A431.top.txt	FDR_logFC_siYAP_siCTRL_A431.bottom.txt	FDR_logFC_siYAP_siCTRL_HaCAT.bottom.txt
YAP signalling				
REACTOME_SIGNALING_BY_HIPPO	http://www.broadinstitute.org/gsea/msig	0.27403983	0.0845902	0.016429855
REACTOME_YAP1_AND_WWTR1_TAZ_STIMULATED_GENE_EXPRESSION	http://www.broadinstitute.org/gsea/msig	0.09720611	0.27879855	0.2792244
Cell cycle				
REACTOME_G1_S_TRANSITION	http://www.broadinstitute.org/gsea/msig	0.02010638	0	0.000541
REACTOME_MITOTIC_M_M_G1_PHASES	http://www.broadinstitute.org/gsea/msig	0.02990606	0.000957	0
REACTOME_DNA_REPLICATION	http://www.broadinstitute.org/gsea/msig	0.030264564	7.26E-05	0.004145181
ZHOU_CELL_CYCLE_GENES_IN_IR_RESPONSE_6HR	http://www.broadinstitute.org/gsea/msig	0.031962935	0	0.011136545
REACTOME_ORC1_REMOVAL_FROM_CHROMATIN	http://www.broadinstitute.org/gsea/msig	0.032863572	0.00102613	0.009737373
REACTOME_M_G1_TRANSITION	http://www.broadinstitute.org/gsea/msig	0.033798005	0	0
REACTOME_S_PHASE	http://www.broadinstitute.org/gsea/msig	0.034246594	0	0.005542807
REACTOME_SYNTHESIS_OF_DNA	http://www.broadinstitute.org/gsea/msig	0.04779358	0	0.005340172
REACTOME_CELL_CYCLE_CHECKPOINTS	http://www.broadinstitute.org/gsea/msig	0.06410079	2.78E-05	0
GRAHAM_CML_DIVIDING_VS_NORMAL_QUIESCENT_UP	http://www.broadinstitute.org/gsea/msig	0.08075432	0	0.001056271
REACTOME_CYCLIN_E_ASSOCIATED_EVENTS_DURING_G1_S_TRANSITION_	http://www.broadinstitute.org/gsea/msig	0.08450879	0.021575017	0.024069246
BENPORATH_PROLIFERATION	http://www.broadinstitute.org/gsea/msig	0.0936338	0	0.001335144
GRAHAM_NORMAL_QUIESCENT_VS_NORMAL_DIVIDING_DN	http://www.broadinstitute.org/gsea/msig	0.10780007	0	0
REACTOME_CDK_MEDIATED_PHOSPHORYLATION_AND_REMOVAL_OF_CD	http://www.broadinstitute.org/gsea/msig	0.11104746	0.006053393	0.001100282
SGCGSSAAA_V$E2F1DP2_01	http://www.broadinstitute.org/gsea/msig	0.1132266	0.000446	0.36994377
REACTOME_REGULATION_OF_MITOTIC_CELL_CYCLE	http://www.broadinstitute.org/gsea/msig	0.12175222	0.030714292	0.00874929
REACTOME_E2F_MEDIATED_REGULATION_OF_DNA_REPLICATION	http://www.broadinstitute.org/gsea/msig	0.122322574	2.74E-05	0.01285284
REACTOME_APC_C_CDH1_MEDIATED_DEGRADATION_OF_CDC20_AND_OT	http://www.broadinstitute.org/gsea/msig	0.12268792	0.041439086	0.001123692
REACTOME_APC_C_CDC20_MEDIATED_DEGRADATION_OF_MITOTIC_PRO	http://www.broadinstitute.org/gsea/msig	0.124558374	0.05772412	0.00103512
V$E2F1_Q6	http://www.broadinstitute.org/gsea/msig	0.1419023	8.25E-05	0.21755916
REN_BOUND_BY_E2F	http://www.broadinstitute.org/gsea/msig	0.15824887	0	0.000168
V$E2F1DP2_01	http://www.broadinstitute.org/gsea/msig	0.1583431	0.000641	0.36111528
V$E2F4DP2_01	http://www.broadinstitute.org/gsea/msig	0.15922602	0.000316	0.308822
E2F1_UP.V1_UP	http://www.broadinstitute.org/gsea/msig	0.16001372	0.07726121	0.13206807
V$E2F1DP1_01	http://www.broadinstitute.org/gsea/msig	0.1625169	0.000331	0.3450875
V$E2F1_Q6_01	http://www.broadinstitute.org/gsea/msig	0.1640632	0.004975112	0.37715355
V$E2F_02	http://www.broadinstitute.org/gsea/msig	0.16427012	0.000364	0.30976173
ISHIDA_E2F_TARGETS	http://www.broadinstitute.org/gsea/msig	0.16454798	0	0
REACTOME_ACTIVATION_OF_THE_PRE_REPLICATIVE_COMPLEX	http://www.broadinstitute.org/gsea/msig	0.17069401	0	0.003772688
REACTOME_CELL_CYCLE_MITOTIC	http://www.broadinstitute.org/gsea/msig	0.18161228	0.001131917	0.024216514
V$E2F4DP1_01	http://www.broadinstitute.org/gsea/msig	0.18244343	0.000679	0.3290016
V$E2F1_Q3	http://www.broadinstitute.org/gsea/msig	0.1880097	0.007132111	0.5434143
V$E2F_Q4	http://www.broadinstitute.org/gsea/msig	0.18903509	0.000138	0.39689186
ZHOU_CELL_CYCLE_GENES_IN_IR_RESPONSE_24HR	http://www.broadinstitute.org/gsea/msig	0.19078194	0.008684116	0.002806893
REACTOME_G2_M_CHECKPOINTS	http://www.broadinstitute.org/gsea/msig	0.19329703	0	0.001109398
V$E2F_Q6	http://www.broadinstitute.org/gsea/msig	0.19384018	0.000136	0.3449235
WHITFIELD_CELL_CYCLE_LITERATURE	http://www.broadinstitute.org/gsea/msig	0.20452549	7.48E-05	0.003301728
REACTOME_G1_S_SPECIFIC_TRANSCRIPTION	http://www.broadinstitute.org/gsea/msig	0.20503585	6.67E-05	0.030643823
CHANG_CYCLING_GENES	http://www.broadinstitute.org/gsea/msig	0.21735805	0	0.000558
KEGG_DNA_REPLICATION	http://www.broadinstitute.org/gsea/msig	0.21759363	0	0.11667396
REACTOME_DNA_STRAND_ELONGATION	http://www.broadinstitute.org/gsea/msig	0.2220706	0	0.046146147
REACTOME_MITOTIC_G1_G1_S_PHASES	http://www.broadinstitute.org/gsea/msig	0.23380828	0	0.001282028
KONG_E2F3_TARGETS	http://www.broadinstitute.org/gsea/msig	0.23829126	0	0.000138
OLSSON_E2F3_TARGETS_DN	http://www.broadinstitute.org/gsea/msig	0.25124517	0.18841921	0.3222239
KAUFFMANN_DNA_REPLICATION_GENES	http://www.broadinstitute.org/gsea/msig	0.25620678	0	0.059563834
Cell growth				
REACTOME_MRNA_PROCESSING	http://www.broadinstitute.org/gsea/msig	0.012915834	0.002623442	0.001638745
BURTON_ADIPOGENESIS_PEAK_AT_16HR	http://www.broadinstitute.org/gsea/msig	0.035835117	0	0.08533237
REACTOME_TRNA_AMINOACYLATION	http://www.broadinstitute.org/gsea/msig	0.046439897	0.1984875	0.004750761
RNA_PROCESSING	http://www.broadinstitute.org/gsea/msig	0.04649598	0.001356013	0.011267367
KEGG_SPLICEOSOME	http://www.broadinstitute.org/gsea/msig	0.057477616	0.057894554	0.13498631
REACTOME_REGULATION_OF_ORNITHINE_DECARBOXYLASE_ODC	http://www.broadinstitute.org/gsea/msig	0.06119907	0.028434383	0.005405107
KEGG_AMINOACYL_TRNA_BIOSYNTHESIS	http://www.broadinstitute.org/gsea/msig	0.06144109	0.25855023	0.013828301
MRNA_PROCESSING_GO_0006397	http://www.broadinstitute.org/gsea/msig	0.08926187	0.011572194	0.012224521
REACTOME_TRANSCRIPTION	http://www.broadinstitute.org/gsea/msig	0.09406843	0	0.007614609
REACTOME_RNA_POL_II_TRANSCRIPTION	http://www.broadinstitute.org/gsea/msig	0.104830325	0.059167907	0.012420549
RNA_SPLICINGVIA_TRANSESTERIFICATION_REACTIONS	http://www.broadinstitute.org/gsea/msig	0.1983508	0.11179424	0.21043202
RNA_HELICASE_ACTIVITY	http://www.broadinstitute.org/gsea/msig	0.2004283	0.042425722	0.08311879
REACTOME_MRNA_SPLICING_MINOR_PATHWAY	http://www.broadinstitute.org/gsea/msig	0.20327862	0.028316427	0.017792491
REACTOME_RNA_POL_I_RNA_POL_III_AND_MITOCHONDRIAL_TRANSCRIP	http://www.broadinstitute.org/gsea/msig	0.23740312	0	0.04974437
REACTOME_TRANSPORT_OF_MATURE_MRNA_DERIVED_FROM_AN_INTRC	http://www.broadinstitute.org/gsea/msig	0.24279736	0.049280223	0.04197069
REACTOME_TRANSPORT_OF_RIBONUCLEOPROTEINS_INTO_THE_HOST_NU	http://www.broadinstitute.org/gsea/msig	0.26221517	0.052539285	0.04193959
SPLICEOSOME	http://www.broadinstitute.org/gsea/msig	0.2729094	0.22319931	0.052454144
REACTOME_PROCESSING_OF_CAPPED_INTRONLESS_PRE_MRNA	http://www.broadinstitute.org/gsea/msig	0.27634937	0.23457132	0.042890634
RNA_POLYMERASE_ACTIVITY	http://www.broadinstitute.org/gsea/msig	0.28282723	0.023127701	0.044442423
Cancer/metastasis				
GRADE_COLON_AND_RECTAL_CANCER_UP	http://www.broadinstitute.org/gsea/msig	0.02838891	0.21228471	0.003991324
KOBAYASHI_EGFR_SIGNALING_24HR_DN	http://www.broadinstitute.org/gsea/msig	0.0050721	0	0.000573
SCHLOSSER_MYC_TARGETS_REPRESSED_BY_SERUM	http://www.broadinstitute.org/gsea/msig	0.030234784	0.021466859	0
SOTIRIOU_BREAST_CANCER_GRADE_1_VS_3_UP	http://www.broadinstitute.org/gsea/msig	0.033990975	0	0
BOYAULT_LIVER_CANCER_SUBCLASS_G3_UP	http://www.broadinstitute.org/gsea/msig	0.043235235	0.004435256	0.018923517
NAKAMURA_CANCER_MICROENVIRONMENT_DN	http://www.broadinstitute.org/gsea/msig	0.046736367	0.000668	0.15767501
SARRIO_EPITHELIAL_MESENCHYMAL_TRANSITION_UP	http://www.broadinstitute.org/gsea/msig	0.06540228	0	0.031196702
WINNEPENNINCKX_MELANOMA_METASTASIS_UP	http://www.broadinstitute.org/gsea/msig	0.0807091	0.000266	0.001537526
RHODES_UNDIFFERENTIATED_CANCER	http://www.broadinstitute.org/gsea/msig	0.10512301	0.001352914	0.00111937
KAUFFMANN_MELANOMA_RELAPSE_UP	http://www.broadinstitute.org/gsea/msig	0.105220415	0	0.007739451
TOMIDA_METASTASIS_UP	http://www.broadinstitute.org/gsea/msig	0.10571032	0.041445088	0.21375494
VECCHI_GASTRIC_CANCER_EARLY_UP	http://www.broadinstitute.org/gsea/msig	0.2073714	0.020382322	0.05325398
ODONNELL_TARGETS_OF_MYC_AND_TFRC_DN	http://www.broadinstitute.org/gsea/msig	0.26682377	0.001433474	0.028167572
CROONQUIST_NRAS_SIGNALING_DN	http://www.broadinstitute.org/gsea/msig	0.23664127	0	0.000191
DANG_MYC_TARGETS_UP	http://www.broadinstitute.org/gsea/msig	0.22925662	0.16562039	0
BORCZUK_MALIGNANT_MESOTHELIOMA_UP	http://www.broadinstitute.org/gsea/msig	0.2360386	0.25912035	0.17807612
RHODES_CANCER_META_SIGNATURE	http://www.broadinstitute.org/gsea/msig	0.22573492	0.15227412	0.024029912
DANG_MYC_TARGETS_UP	http://www.broadinstitute.org/gsea/msig	0.22925662	0.16562039	0
BOYAULT_LIVER_CANCER_SUBCLASS_G123_UP	http://www.broadinstitute.org/gsea/msig	0.23370321	0.08395546	0.049637258
SHEDDEN_LUNG_CANCER_POOR_SURVIVAL_A6	http://www.broadinstitute.org/gsea/msig	0.23377588	0.000135	0.01669378
PUJANA_BREAST_CANCER_LIT_INT_NETWORK	http://www.broadinstitute.org/gsea/msig	0.27237272	0.003610279	0.024503974
DAIRKEE_CANCER_PRONE_RESPONSE_BPA	http://www.broadinstitute.org/gsea/msig	0.2751443	0.22182393	0.052520353
SMID_BREAST_CANCER_LUMINAL_A_DN	http://www.broadinstitute.org/gsea/msig	0.29719597	0.020338075	0.003284015

Figure 3.7. Gene set enrichment analysis of YAP target genes in keratinocytes. (A) Analysis of YAP-dependent gene expression by RNA-seq was performed by comparison of YAP loss and gain of function in keratinocytes (see materials and methods). Fig continued overleaf.



(B) A431 keratinocyte lysates were transfected with YAP5SA or siRNA against YAP. Results were compared to HaCaT keratinocytes. RNAseq analysis was performed on biological triplicates. After trimming of sequenced reads and mapping to the Refseq genome model, TMM (treated mean of M-values) normalisation and differential expression analysis using the negative binomial model was carried out with the RBioconductorpackage **'**EdgeR**'**. Enrichments of pathway, category and motif gene sets were assessed using GSEA with logFC preranked gene lists. Gene sets with an enrichment false discovery rate (FDR) value of less than 0.05 were judged to be strongly statistically significant and values of less than 0.25 significant. The table lists gene sets induced upon YAP5SA expression and decreased upon YAP siRNA treatment. Raw data available upon request. **(C)** The gene sets identified in (B) were then analysed for putative TEAD1-binding sites -/+ 1kB of transcription start sites. The TEAD1 motif sequence (retrieved from JASPAR-2014 database motif ID: MA0090.1) was aligned to the -/+ 1 kB region around transcription start sites using a stringency setting of 0.85. See Appendix 7.2 for TEAD binding raw data. Bioinformatic analyses were performed with the help of Stefan Boeing, Francis Crick Institute.

We next sought to validate candidate YAP target genes *in vivo*. Quantitative RT-PCR analysis found multiple YAP target genes (*CTGF*, *MYC*, *AREG1*, *PCNA1*, *CCNE1*) were indeed downregulated at the mRNA level in *YAP/TAZ* dKO skin (Fig. 3.8A). We confirmed that PCNA and CCNE1 were also downregulated at the protein level in dKO skin (Fig. 3.8B,C). The Human Protein Atlas dataset was mined to gain further information on the expression pattern of the YAP target genes identified in human keratinocyte culture: regulators of the cell cycle [CCNE2, PCNA, E2F1], cell growth [RPTOR, ODC1, ADC] and EGFR and integrin signalling (CYR61, CTGF, AREG, integrins α3, β1, β4). All YAP targets were strongly expressed in the basal cell layer of human skin epidermis. This is consistent with the notion that in this tissue YAP is transcriptionally active exclusively in the basal layer progenitor cells (Fig. 3.9).

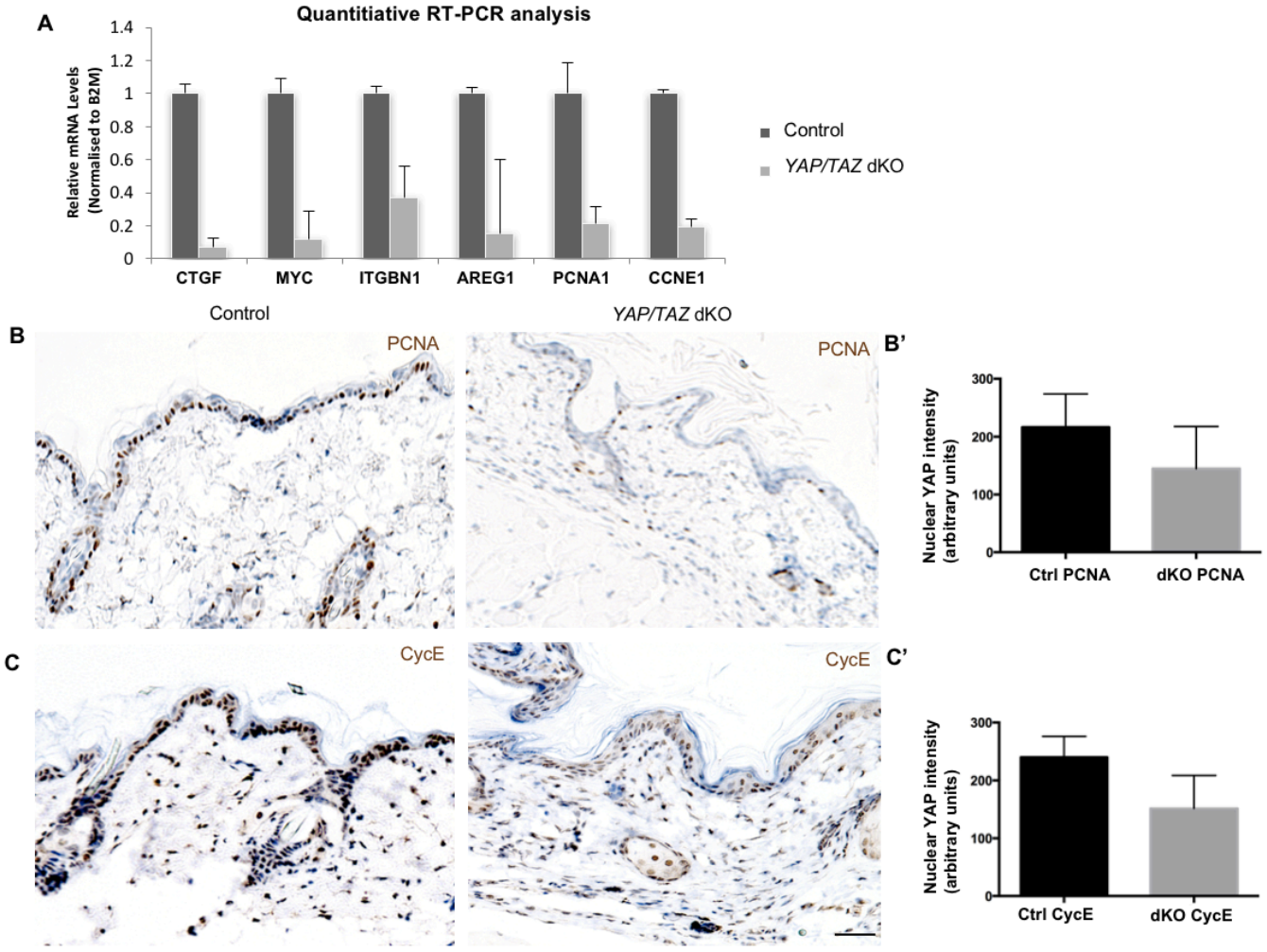


Figure 3.8. YAP/TAZ target gene expression is reduced in *YAP*/*TAZ* double knockout skin. Several YAP target genes identified in RNAseq experiments were selected for expression analysis in wild type versus *YAP/TAZ* dKO back skin. **(A)** Quantitative RT-PCR analysis shows downregulation of multiple YAP-dependent genes at the mRNA level in dKO skin compared to control. **(B)** Histological sections stained for PCNA, one of the putative target genes, show downregulation at the protein level in dKO back skin compared to control; quantified in **(B')** *n*=300 cells from at least 3 different mice. **(C)** Histological sections stained for CCNE1, another putative target gene, show downregulation at the protein level in dKO backskin compared to control; quantified in **(C')** *n*=300 cells from at least 3 different mice. Scale bar 50 μM.

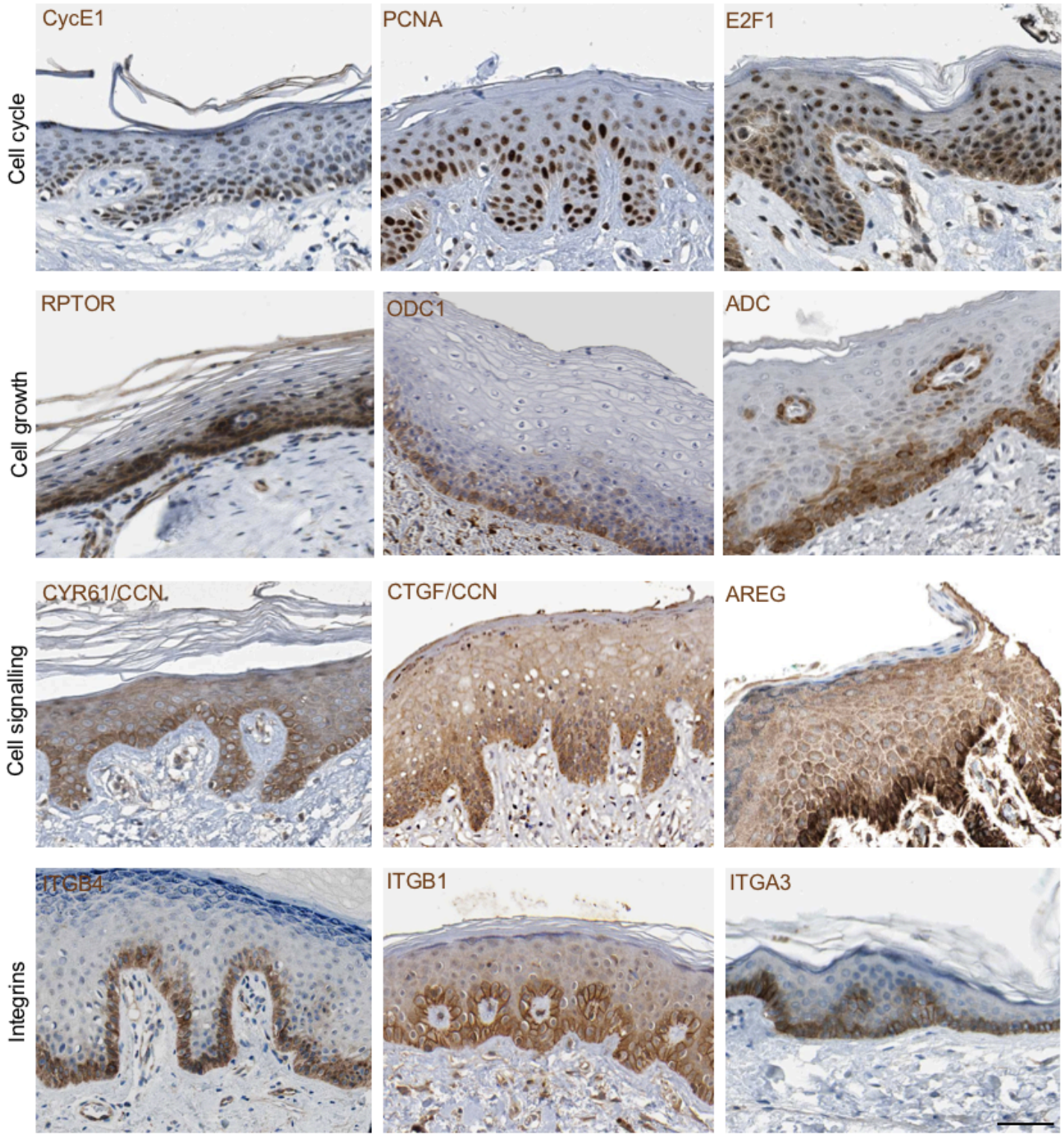


Figure 3.9. YAP-regulated genes in human skin.
YAP target genes identified in cell culture and mice show similar basal cell expression in human epidermis. Images from Human Protein Atlas. Scale bar 50 μM.

3.5. Summary

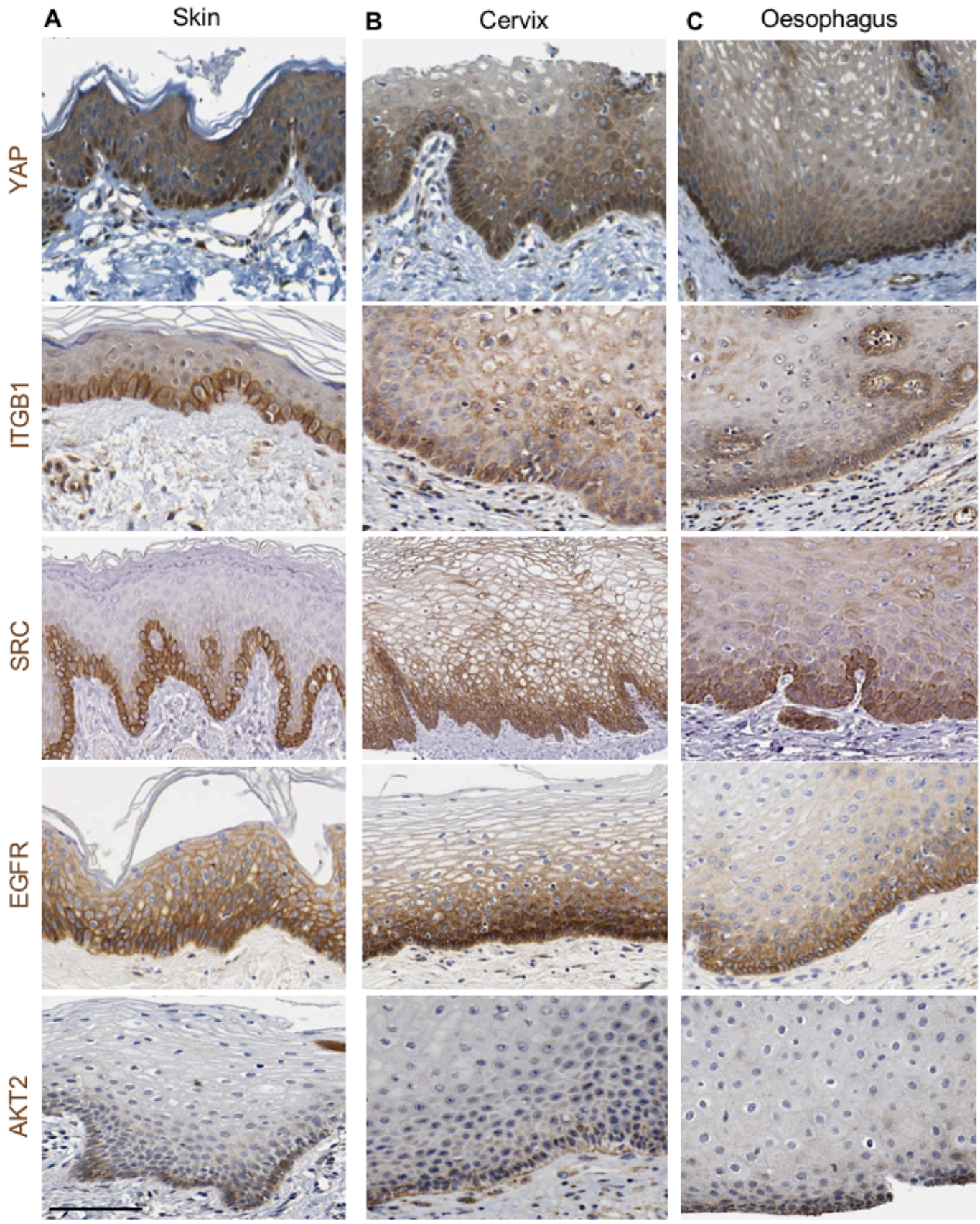
Our data indicate a physiological role for YAP and TAZ in driving basal layer progenitor keratinocyte cell proliferation and maintaining epidermal homeostasis (Fig. 3.2,3.3,3.4). *YAP/TAZ* conditional double knockout leads to hair loss and thinning of the epidermis. dKO animals show delayed cutaneous wound healing, due at least partly to reduced keratinocyte proliferation (Fig. 3.5). YAP and TAZ are nuclear localised in the basal layer progenitor cells, where they stimulate a transcriptional program comprising a set of genes involved in cell growth, cell cycle, cancer signalling and cell-matrix adhesion. (Fig. 3.2,3.6,3.7,3.8).

Chapter 4. YAP is regulated by basal integrin-Src signalling in keratinocytes

4.1. Epithelia under physiological conditions

4.1.1. Stratified squamous epithelia: integrin and Src signalling promote YAP nuclear localisation in keratinocytes

Having established that YAP nuclear localization and transcriptional activity is restricted to the basal layer progenitor cells of the epidermis, we sought to elucidate the mechanism regulating YAP subcellular localisation and ensuing activity in this tissue. Conditional knockout of *MST1/2* (known negative regulators of YAP in many organs) in the epidermis does not affect YAP localisation or activity and no phenotype is observed (Schlegelmilch, Mohseni et al. 2011). This suggests that mechanisms other than Hippo signalling are responsible for regulating YAP in this tissue. An obvious difference between the basal layer and suprabasal keratinocytes is that the former contact the basement membrane extracellular matrix via integrins whilst the latter do not. Integrins are membrane-spanning heterodimeric cell adhesion proteins that link the ECM to the cytoskeleton and function in outside-in signal transduction (Hynes 2002, Miranti and Brugge 2002). Others have shown that deletion of *β1 integrin* (ITGB1) in the epidermis results in hair loss and skin blistering; a phenotype reminiscent of *YAP/TAZ* dKO. ITGB1 mutant epidermis exhibit impaired proliferation, tissue architecture organisation, differentiation, and wound healing (Brakebusch, Grose et al. 2000, Raghavan, Bauer et al. 2000, Grose, Hutter et al. 2002).


A
Skin
B
Cervix
C
Oesophagus
YAP
ITGB1
SRC
EGFR
AKT2

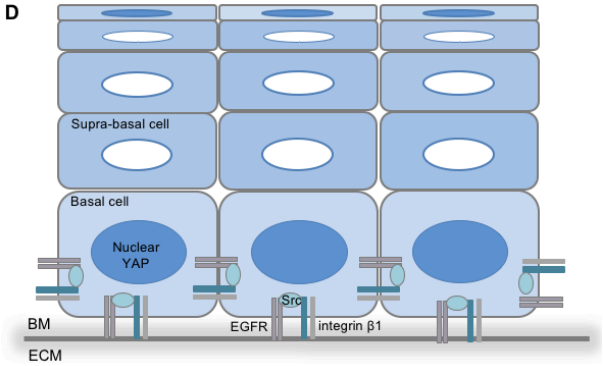


Figure 4.1. Integrin-Src & EGFR-PI3K localise in similar basal pattern as YAP in human stratified squamous epithelia. The Human Protein Atlas dataset was mined to identify genes that are expressed in the basal layer keratinocytes of human skin and could therefore act as regulators of YAP expression or nuclear localization in the epidermis. **(A)** YAP, ITGB1, SRC, EGFR and AKT2 immunostainings show basal layer expression in skin epidermis. **(B)** YAP, ITGB1, SRC, EGFR and AKT2 immunostainings show basal layer expression in cervix epithelium. **(C)** YAP, ITGB1, SRC, EGFR and AKT2 immunostainings show basal layer expression in oesophageal epithelium. Scale bar 100 μM. **(D)** Model for YAP regulation in stratified squamous epithelia.

Studies in cultured MCF10A breast cancer cells and in *Drosophila* showed that integrin-Src signalling and EGFR-PI3K signalling stimulate nuclear localisation of YAP (Enomoto and Igaki 2013, Fan, Kim et al. 2013, Kim and Gumbiner 2015). To investigate whether these pathways contribute to YAP regulation in skin we first examined their expression in human skin. By analysing the Human Protein Atlas dataset we found that ITGB1, SRC, EGFR and AKT2 (a marker of PI3K activation) are all strongly expressed in the epidermal basal layer of skin, while AKT2 is particularly enriched at the interface between basal layer cells and the underlying basement membrane extracellular matrix (Fig. 4.1A). These proteins show a similar pattern of basal layer expression in other squamous epithelia (cervix, Fig. 4.1B and oesophagus, Fig. 4.1C). These data suggest that integrin-Src and/or PI3K signalling could contribute to YAP nuclear localisation in basal layer cells in skin epidermis, and more generally across squamous epithelia (Fig. 4.1D). YAP is also known to function as a mechanosensor, with cell tension and spreading driving YAP into the nucleus (Dupont, Morsut et al. 2011, Wrighton 2011). Although the molecular mechanism is not fully understood, this process is dependent on actomyosin contractility. F-actin and non-muscle myosin II form bundles, regulated by RhoGTPase, that enable force generation and cell motility. Studies have shown that Yki/YAP in *Drosophila* and human cell culture was activated by F-actin polymerisation (Sansores-Garcia, Bossuyt et al. 2011, Reddy, Deguchi et al. 2013),

whilst treatment of cells with inhibitors of F-actin (latrunculin B) or myosin II ATPase (blebbistatin) decreased YAP nuclear localisation (Fernandez, Gaspar et al. 2011, Wada, Itoga et al. 2011, Zhao, Li et al. 2012, Fernandez, Jezowska et al. 2014, Deng, Wang et al. 2015, Fletcher, Elbediwy et al. 2015, Wong, Li et al. 2015).

To elucidate whether integrin-Src and/or PI3K signalling promote YAP nuclear localisation in keratinocytes we systematically inhibited components of these pathways in cultured human keratinocytes (Fig 4.2A-E). Inhibition of ITGB1 with siRNA or blocking antibodies or inhibition of the downstream effectors Src or FAK significantly hindered YAP nuclear localisation (Fig. 4.2A,B). Inhibition of PI3K similarly abolished YAP nuclear localisation. Interestingly inhibition of PI3K effectors AKT or TORC1 (at concentrations validated for this cell line) had no impact on YAP nuclear localisation, whereas inhibition of PDK1 partially impaired YAP nuclear localisation (Fig. 4.2B,C). Inhibition of F-actin significantly reduced YAP nuclear localisation, whilst the effect of myosin II or Rho kinase inhibition was moderate (Fig. 4.2D, quantified in 4.2E). Treatment of keratinocytes with either PI3K or Src inhibitors resulted in a significant increase in pYAP levels (YAP phosphorylated at S127; this form of YAP is retained in the cytoplasm and thereby is inactive. Fig. 2.3F). S127 is a major LATS phosphorylation site. This suggests that inhibiting Src or PI3K promotes Hippo (MST-LATS) signalling in keratinocytes. This is consistent with recent studies showing that Src inhibits Hippo signalling by tyrosine phosphorylation of LATS1 in multiple other cell lines (Si, Ji et al. 2017, Lamar, Xiao et al. 2019). EGFR via PI3K signalling has also been shown to negatively regulate hippo signalling in various cell culture lines (Fan, Kim et al. 2013, Azad, Janse van Rensburg et al. 2018). In a keratinocyte ‘scratch-wound’ assay YAP rapidly becomes nuclear at the leading edge of the wound. This nuclear localisation is abolished by treatment with Src inhibitor dasatinib (Fig. 4.2G). Overall, these data indicate that integrin-Src and EGFR-PI3K signalling are required for YAP nuclear localisation in keratinocytes (Fig. 4.2H). In line with our results, an earlier study found that Src directly phosphorylates YAP at three sites independent of LATS1/2 phosphorylation in keratinocytes, and that this phosphorylation is necessary for YAP nuclear localisation, interaction with TEAD and transcriptional activity (Li, Silvis et al. 2016)

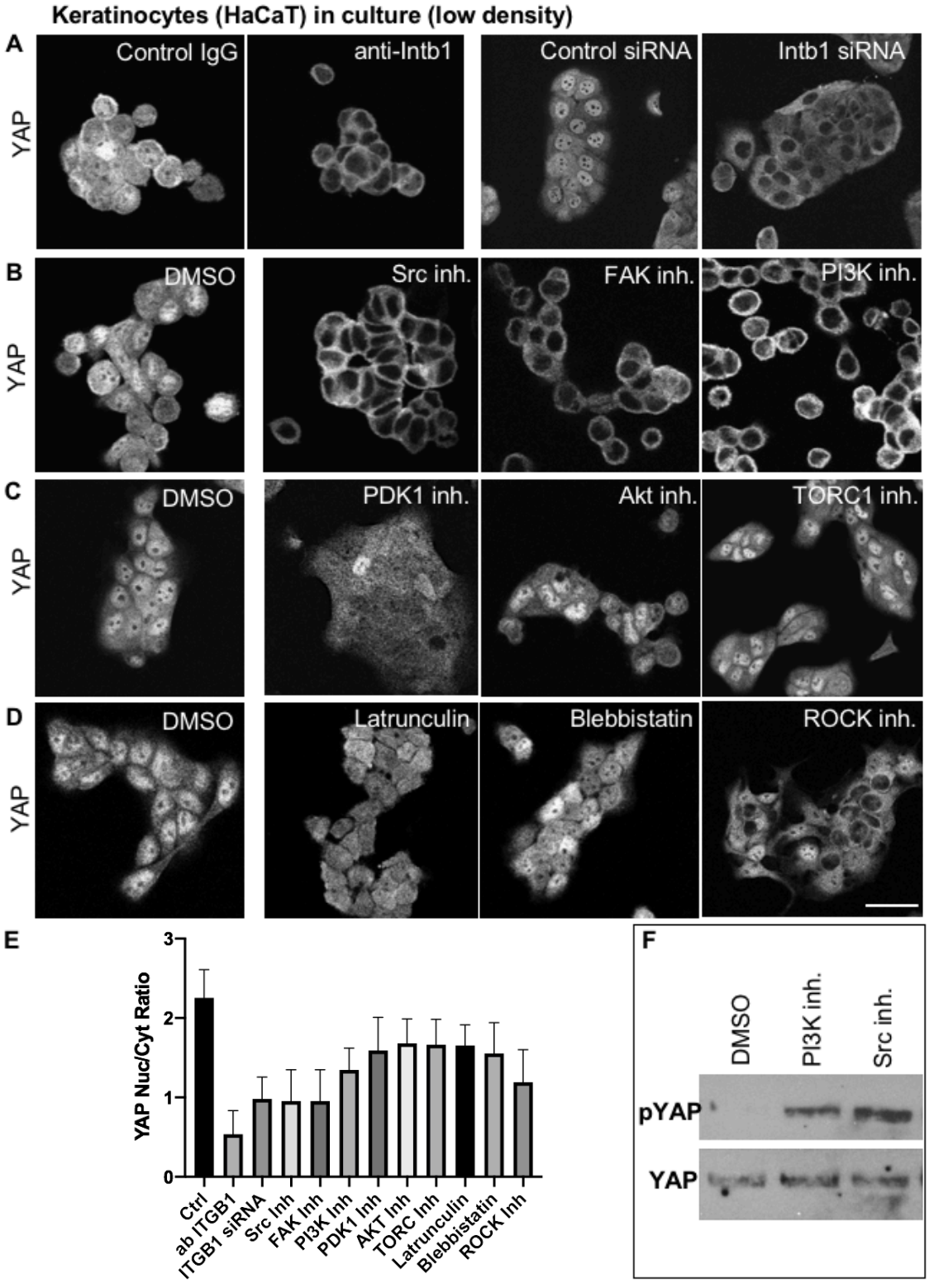


Figure 4.2. Blocking integrin-Src signalling inhibits YAP nuclear localisation in human HaCaT keratinocyte epithelial cells. (Fig. continued and legend on next page)

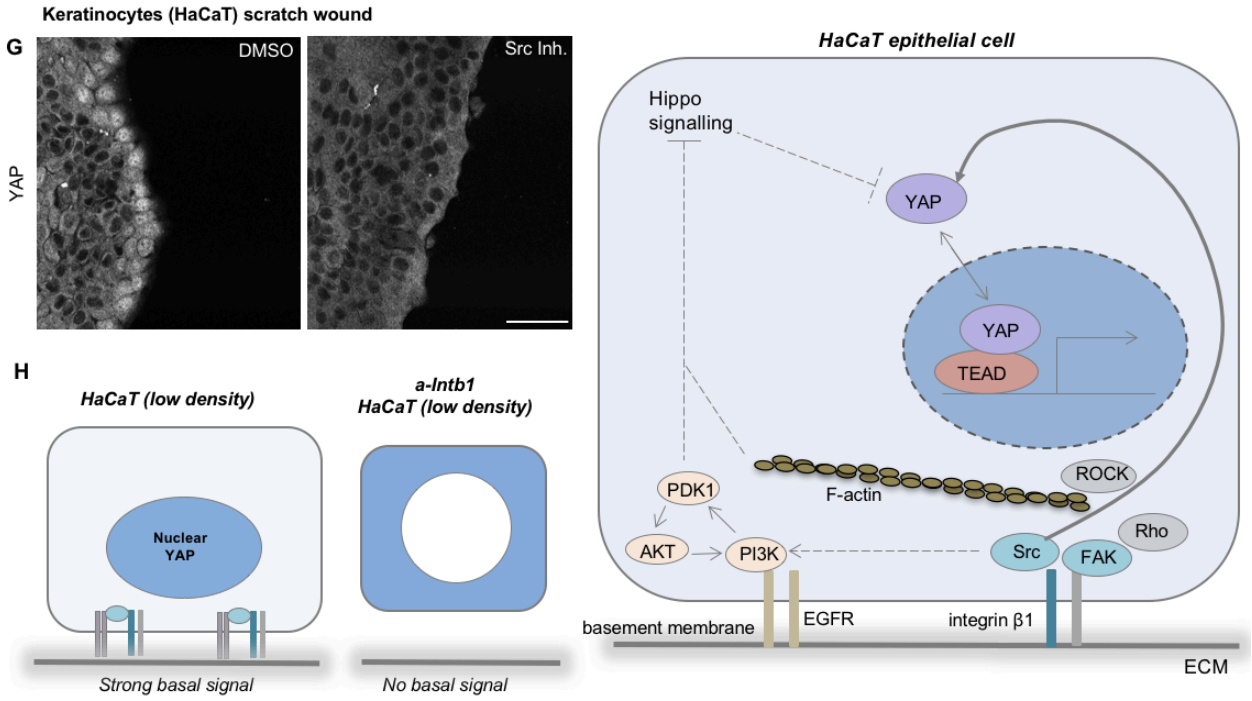
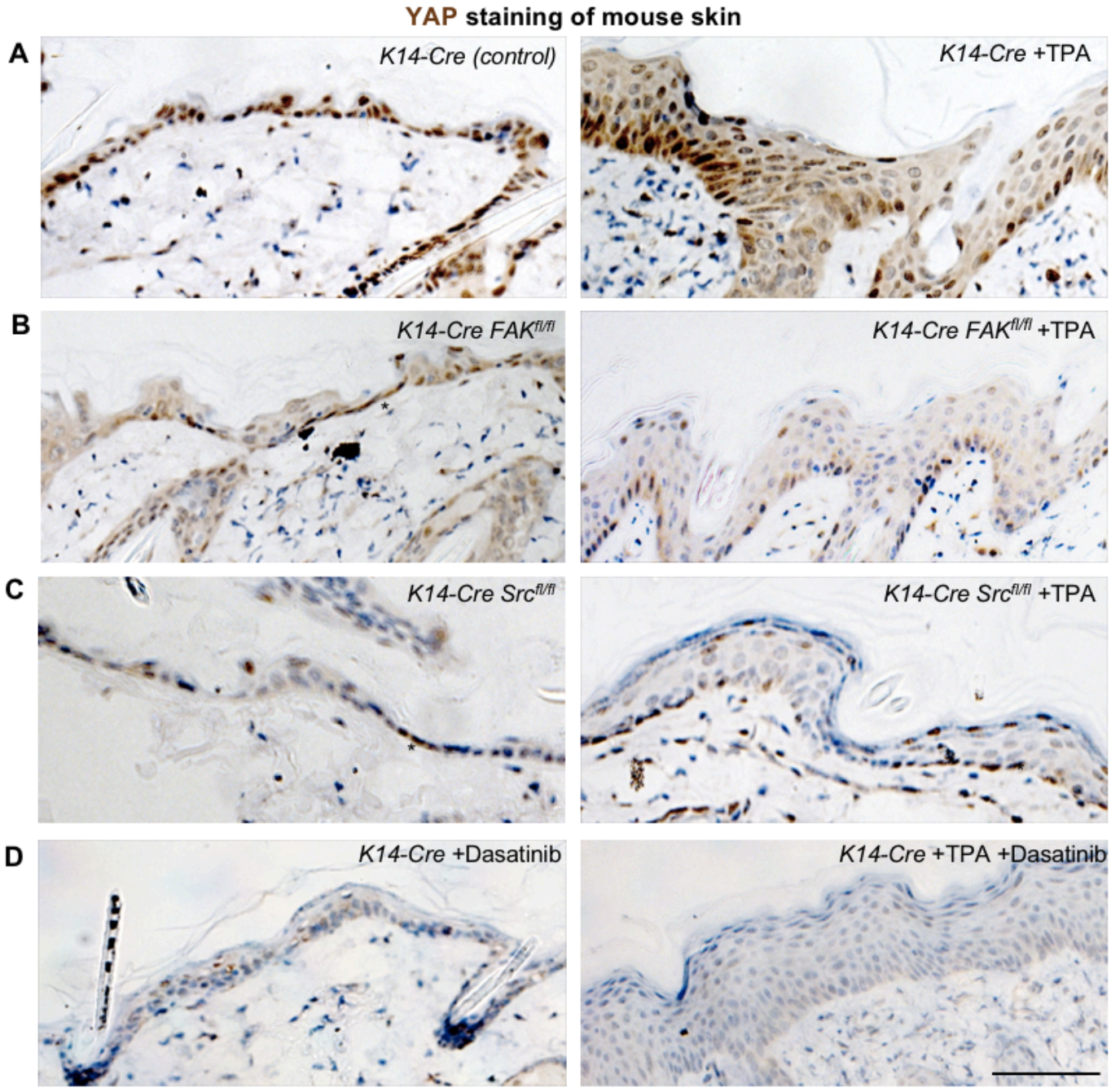


Figure 4.2. Blocking integrin-Src signalling inhibits YAP nuclear localisation in human HaCaT keratinocyte epithelial cells. (A) YAP is both nuclear and cytoplasmic localised in HaCaTs plated at low density treated with control IgG or control siRNA. YAP nuclear localisation is significantly reduced by treatment of keratinocytes with anti-ITGB1 antibodies (PD52) or by ITGB1 siRNA treatment. **(B)** YAP nuclear localisation is significantly reduced by treatment of keratinocytes with Src inhibitor dasatinib, by FAK inhibitor PF573228 or by PI3K inhibitor GDC0941, but not by treatment with DMSO solvent. **(C)** YAP nuclear localisation is reduced by treatment of keratinocytes with PDK1 inhibitor BX795, but not by AKT inhibitor MK2206, TORC1 inhibitor Everolimus or DMSO solvent. **(D)** YAP nuclear localisation is reduced by treatment of keratinocytes with F-actin destabilising drug Latrunculin, myosin II inhibitor Blebbistatin, or Rho-kinase inhibitor Y27632. Scale bar 25 μM. **(E)** Quantification of YAP nuclear/cytoplasmic ratio of A-D. Using ImageJ, the ROI was manually attached to the nucleus and the integrated intensity divided by that for the cytoplasm, yielding the nuclear/cytoplasmic ratio. Values are means±s.e.m. *n*=3 FOV, 5 repeats each condition. **(F)** Western blotting analysis of p-YAP levels in keratinocytes treated with either DMSO control, PI3K inhibitor or Src inhibitor. Total YAP levels are shown as a control. p-YAP is low keratinocytes treated with DMSO control and increased in keratinocytes treated with PI3k inhibitor or Src inhibitor **(G)** YAP nuclear localisation at the leading edge of a scratch wound in keratinocyte culture is prevented by treatment with Src inhibitor. Scale bar 50 μM. **(H)** Model of YAP regulation in keratinocytes.

We next sought to determine whether integrin-Src signalling plays a similar role in regulating YAP localisation *in vivo.* To this end, we examined genetic knockdown or chemical inhibition of pathway components in both mouse skin with and without TPA (the phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate) treatment. TPA is an inflammatory agent known to induce epidermal hyperplasia and elevated Src kinase activity in mouse skin (Matsumoto, Jiang et al. 2003). YAP nuclear intensity was slightly elevated upon TPA treatment of control skin (Fig. 4.3A), possibly mediated by Src signalling. Knockdown of either FAK or Src resulted in a significant decrease in YAP levels and nuclear YAP in both normal and TPA-induced hyperplastic epidermis (Fig. 4.3 A-E). A similar reduction in nuclear localisation of YAP and total YAP levels was observed after topical Src inhibitor (dasatinib) treatment of both normal or TPA-treated skin, as well as in skin papillomas (Fig. 4.3D and Fig. 4.10). These data suggest that integrin-Src signalling increases YAP nuclear localisation and YAP protein levels in basal layer epidermal keratinocytes. Analysis of YAP expression and cellular localization in β1 Integrin knockout epidermis would add weight to these data. The effect of integrin-Src signalling on YAP protein levels could be due to increased YAP stability upon Src tyrosine phosphorylation. It has been shown that phosphorylation of YAP on Y357 reduces the rate of YAP protein degradation in cell culture (Levy, Adamovich et al. 2008, Taniguchi, Wu et al. 2015).



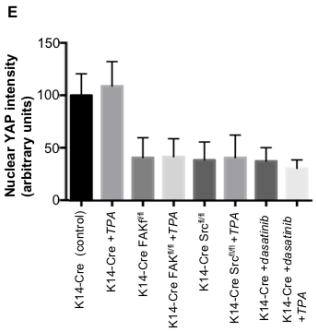


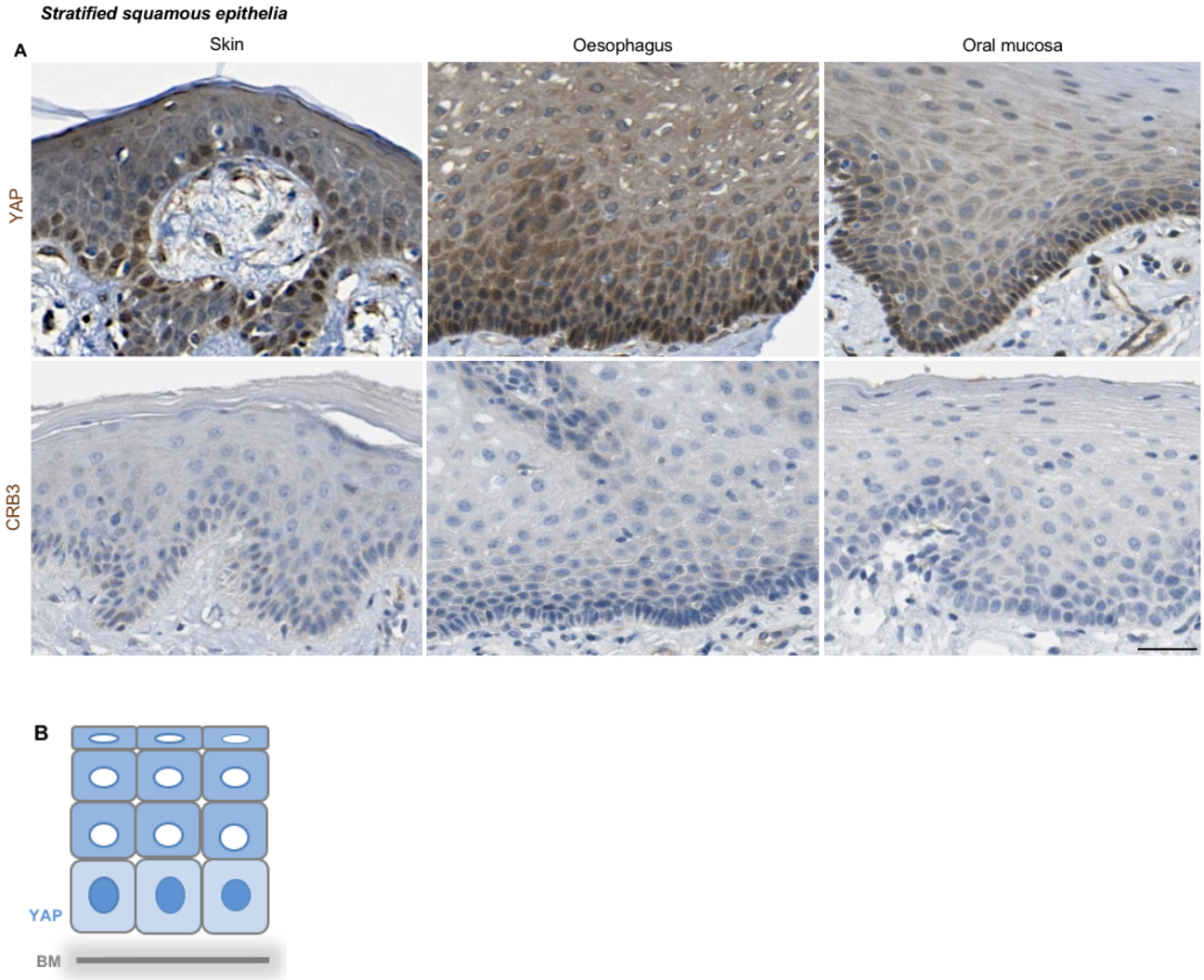
Figure 4.3. Blocking integrin-Src signalling reduces YAP nuclear localisation in mouse epidermis. The effect of the loss of FAK or Src on YAP expression and localisation was compared in normal and TPA-treated (to induce hyperplasia) back skin **(A)** control and TPA-treated skin stained for YAP **(B)** YAP staining is reduced in FAK conditional KO skin with or without TPA treatment. **(C)** YAP staining is reduced in Src conditional KO skin with or without TPA treatment. **(D)** YAP staining is reduced in dasatinib-treated skin with or without TPA treatment. Scale bar 50 μM. **(E)** Quantification of nuclear YAP intensity in A-D. Values are means±s.e.m, normalised to K14-Cre control. A students t test was performed on the data; $p<0.001$.

4.1.2. Columnar epithelia: Apical domain formation inhibits YAP nuclear localisation in columnar epithelia

Our and others' data suggest that proliferation of epidermal basal cells depends on contact with the basement membrane extracellular matrix and consequent integrin-Src and EGFR-PI3K signalling to promote YAP nuclear localisation and their self-renewing progenitor cell quality. According to this model, daughter cells would stop proliferating and differentiate by loss of contact with the basement membrane extracellular matrix and consequent loss of signalling (Fig 4.4B). Other types of stratified squamous epithelia show the same pattern of YAP subcellular localisation, suggesting that this may be a general mechanism of proliferation control (Fig. 4.4A). This model, however, is not expected to apply to columnar epithelia because differentiated columnar cells maintain contact with the basement membrane extracellular matrix. It has been well established that apical proteins associated with Crumbs (CRB3) promote Hippo (MST-LATS) signalling to promote YAP accumulation in the cytoplasm (Chen, Gajewski et al. 2010, Ling, Zheng et al. 2010, Varelas, Samavarchi-Tehrani et al. 2010, Fletcher, Elbediwy et al. 2015, Szymaniak, Mahoney et al. 2015). It is conceivable that the continuous presence of this apical signal in columnar epithelial cells overrides the basal signal, enabling these cells to differentiate.

To investigate the possible *in vivo* relevance of the model above (suppression of YAP by apical signals) we compared the subcellular localisation of YAP and the apical marker CRB3 in stratified squamous epithelia, pseudostratified columnar epithelia and simple columnar epithelia. Stratified squamous epithelial cells do not express CRB3 (Fig. 4.4A,B). In simple columnar epithelia, such as endometrial or gallbladder, columnar cells express apical CRB3 and YAP is localised cytoplasmically (Fig. 4.4E,F). In pseudostratified columnar epithelia, such as lung bronchus or salivary gland, YAP localises to the cytoplasm of columnar cells that express apical CRB3. Basal layer stem/progenitor cells that lack an apical domain and CRB3 expression retain nuclear YAP (Fig. 4.4C,D). Thus, in these tissues, CBR3 expression correlates with cytoplasmic localisation of YAP whilst absence of CRB3 expression *and* contact with the basement membrane extracellular matrix correlates with nuclear YAP. Key upstream Hippo pathway components

Figure 4.4. Human columnar epithelial cells that have an apical domain do not express nuclear YAP. Using the Human Protein Atlas dataset, the localisation of YAP was compared in cells with the presence or absence of an apical domain in different types of epithelia. **(A)** YAP is nuclear localised in the basal layer progenitor cells andcytoplasmic in the suprabasal cells of skin, oesophageal, oral mucosa, and uterine cervical stratified squamous epithelia. Scale bar 50 μM. **(B)** Schematic diagram of YAP localisation in stratified squamous epithelia. **(C)** YAP is nuclear localised in the basal layer progenitor cells, which lack a CRB3-positive apical domain, and cytoplasmic in columnar epithelial cells, which express a CRB3-positive apical domain, in the lung bronchus and salivary gland pseudostratified epithelia. Scale bar 50 μM. **(D)** Schematic diagram of YAP localisation in pseudostratified columnar epithelia. **(E)** YAP is cytoplasmic in simple columnar epithelial cells of the endometrium and gallbladder, which express a CRB3-positive apical domain. Scale bar 50 μM. **(F)** Schematic diagram of YAP localisation in simple columnar epithelia.



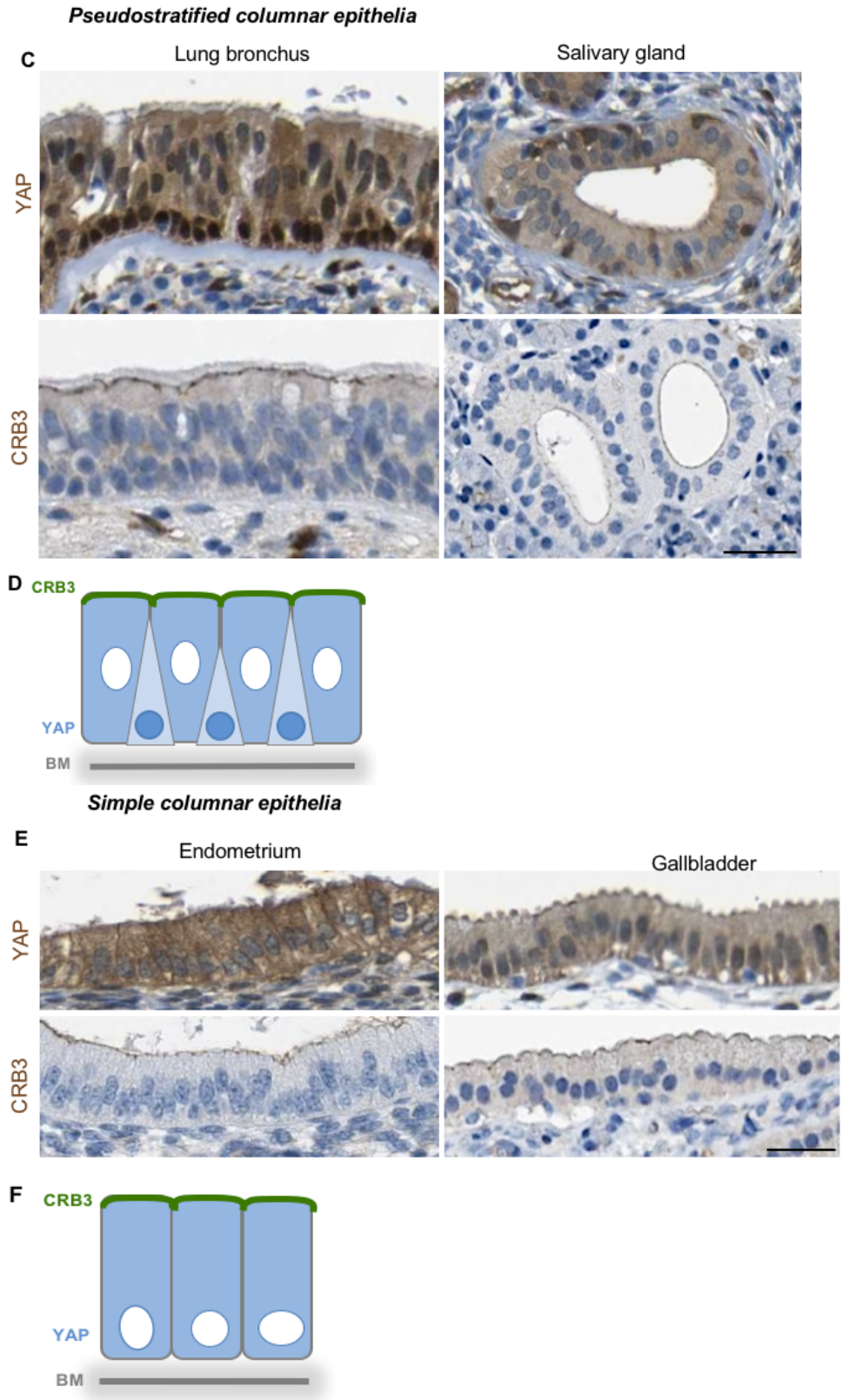
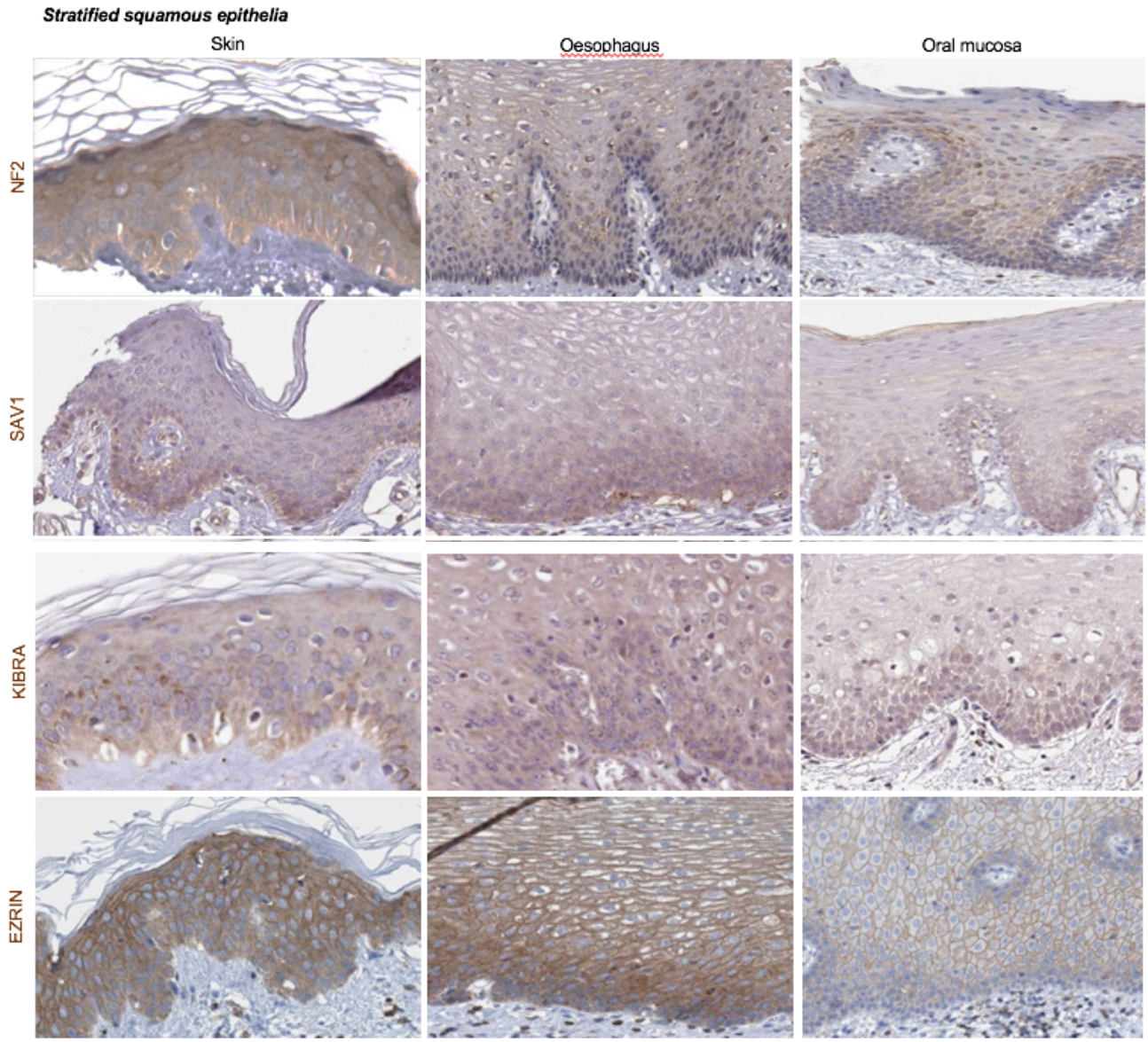
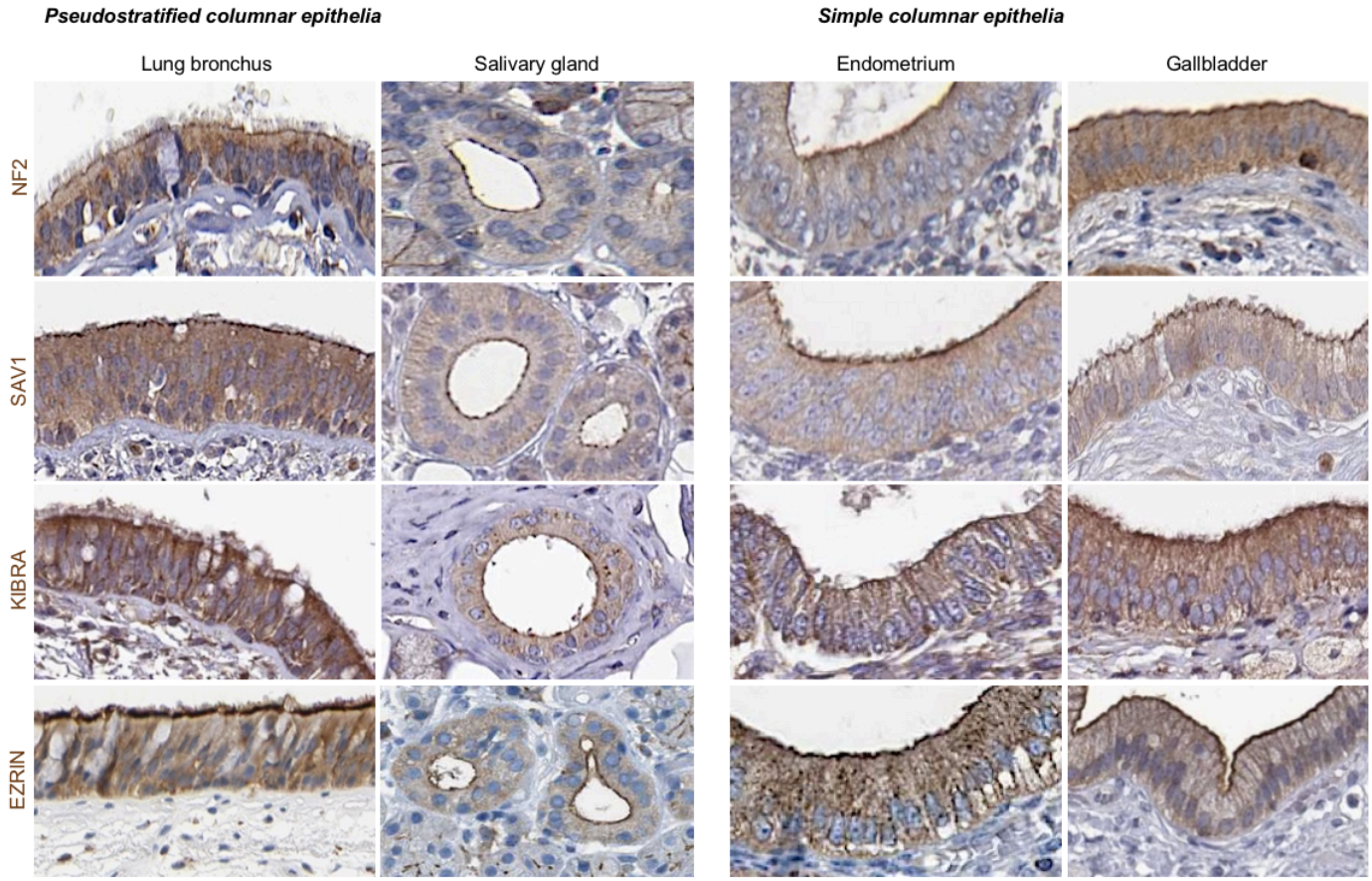

Pseudostratified columnar epithelia
C
Lung bronchus
Salivary gland
YAP
CRB3
D
CRB3
YAP
BM
Simple columnar epithelia
E
Endometrium
Gallbladder
YAP
CRB3
F
CRB3
YAP
BM

Figure 4.5. NF2, SAV1, KIBRA & EZRIN localise in the same apical fashion as CRB3. Human Protein Atlas data show that NF2, SAV1, KIBRA and EZRIN localise in the same apical fashion as CRB3 in columnar cells of various epithelia.





NF2, SAV1, KIBRA and EZRIN (known to function in cytoplasmic sequestration of YAP) colocalised apically with CRB3 in pseudostratified columnar and simple columnar epithelia (Fig 4.5) (Hamaratoglu, Willecke et al. 2006, Chen, Gajewski et al. 2010, Genevet, Wehr et al. 2010, Ling, Zheng et al. 2010, Yu, Zheng et al. 2010, Zhang, Bai et al. 2010, Yin, Yu et al. 2013).

In summary, these data suggest that YAP nuclear localisation requires signalling from contact with the basement membrane extracellular matrix but is inhibited by Hippo signalling arising from the apical surface. I suggest that when both signals are present, apical signalling dominates over basal signalling.

The above analysis is correlative. We therefore sought to test whether impairing the apical domain would prevent YAP from localising to the cytoplasm in columnar

epithelial cells. Human intestinal epithelial (Caco2) cells in culture are columnar cells that can form 3D cysts or 2D monolayers at high density. In both cases YAP is localised in the cytoplasm of these cells. SiRNA knockdown of either the apical protein *CDC42* or *LATS1/2* resulted in a similar relocalisation of YAP to the nucleus (Fig. 4.6A,B). Thus, at high density Hippo signalling via CDC42 and LATS1/2 results in YAP being sequestered in the cytoplasm. When apical Hippo signalling is abrogated YAP inhibition is released and YAP is driven into the nucleus (Fig. 4.6C,D).

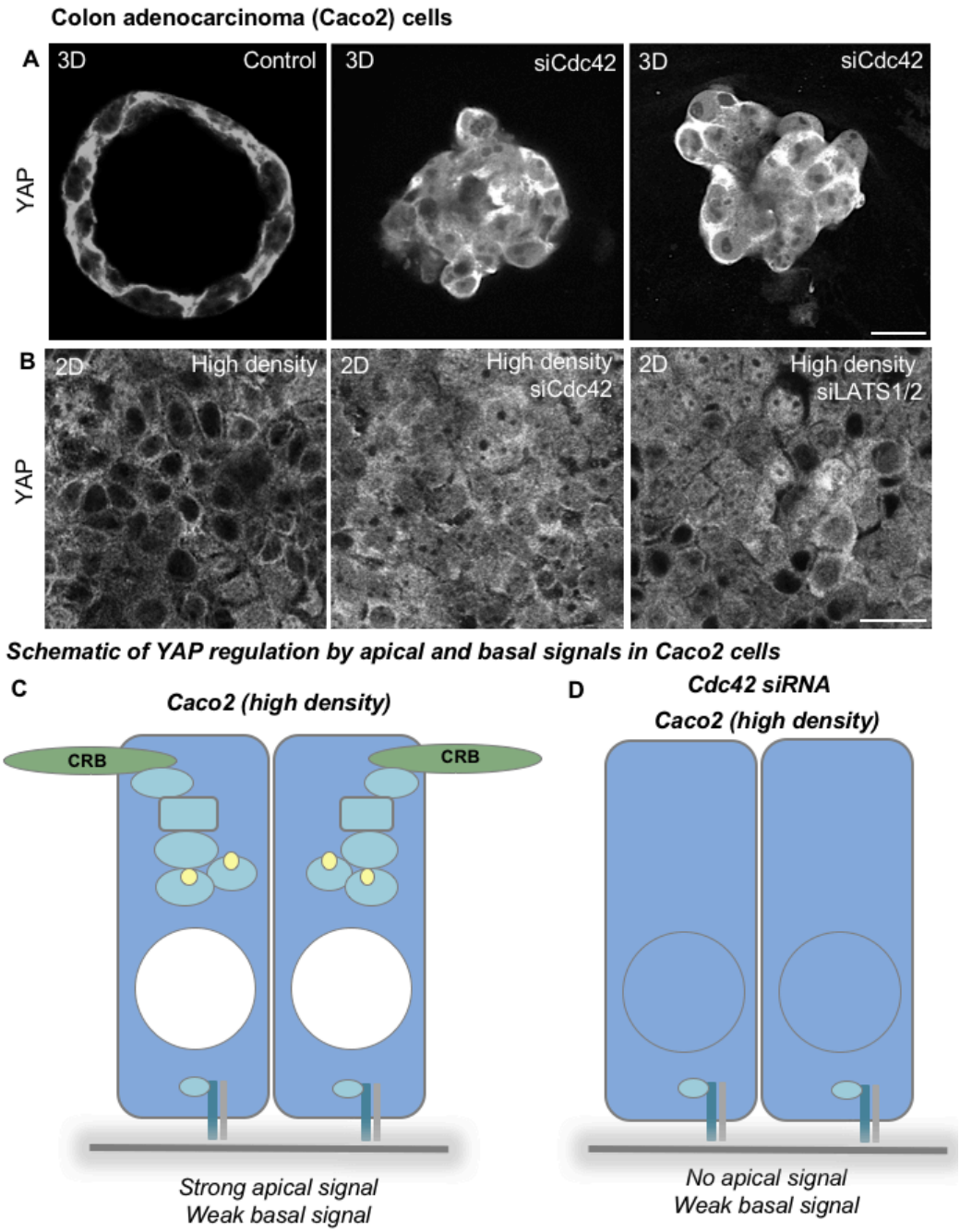


Figure 4.6. Apical domain formation inhibits YAP nuclear localisation in Caco2 cells. **(A)** Caco2 colon adenocarcinoma cells form 3D cysts in cell culture. YAP is cytoplasmic localised. siRNA knockdown of *CDC42* disrupts apical-basal polarity and YAP becomes more nuclear. **(B)** Caco2 colon adenocarcinoma cells form 2D epithelial monolayers at high density. siRNA knockdown of *CDC42* disrupts apical-basal polarity and YAP becomes more nuclear, similar to silencing of *LATS1/2*. Scale bar 10 μM. **(C)** Schematic diagram of YAP localisation regulation in Caco2 cells. This work was performed in collaboration with Ahmed Elbediwy.

When Caco2 cells are plated at low density they assume a more spread out morphology with a larger basal surface and are unable to differentiate an apical domain. YAP becomes nuclear (Fig. 4.7A). Inhibition of integrins (with low calcium media, anti-ITGB1 antibodies or *ITGB1* siRNA), Src or FAK resulted in YAP relocalising to the cytoplasm (Fig. 4.7A,B). This indicates that when apical signalling is blocked basal integrin-Src signalling similar to that in keratinocytes drives YAP into the nucleus in these cells. Inhibition of PI3K, PDK1 reduced YAP nuclear localisation to a similar extent as blocking F-actin or Rho. Neither AKT nor TORC1 inhibition had a significant effect (Fig. 4.7C,D,E). pYAP (phosphorylated at S127) levels increased after inhibition of ITGB1, Src, FAK or PI3K, indicating that integrin-Src signalling regulates YAP localisation at least in part via regulating LATS phosphorylation of YAP. One limitation of this experimental approach is that manipulating the apical domain could have an effect on the basal domin. Nonetheless, these findings are consistent with data from the Human Protein Atlas, which show YAP to be nuclear localised in basal progenitor cells that express ITGB1, Src and EGFR and cytoplasmic in cells that express these basal signals as well as an apical domain in pseudostratified columnar epithelia (Fig. 4.8A-E).

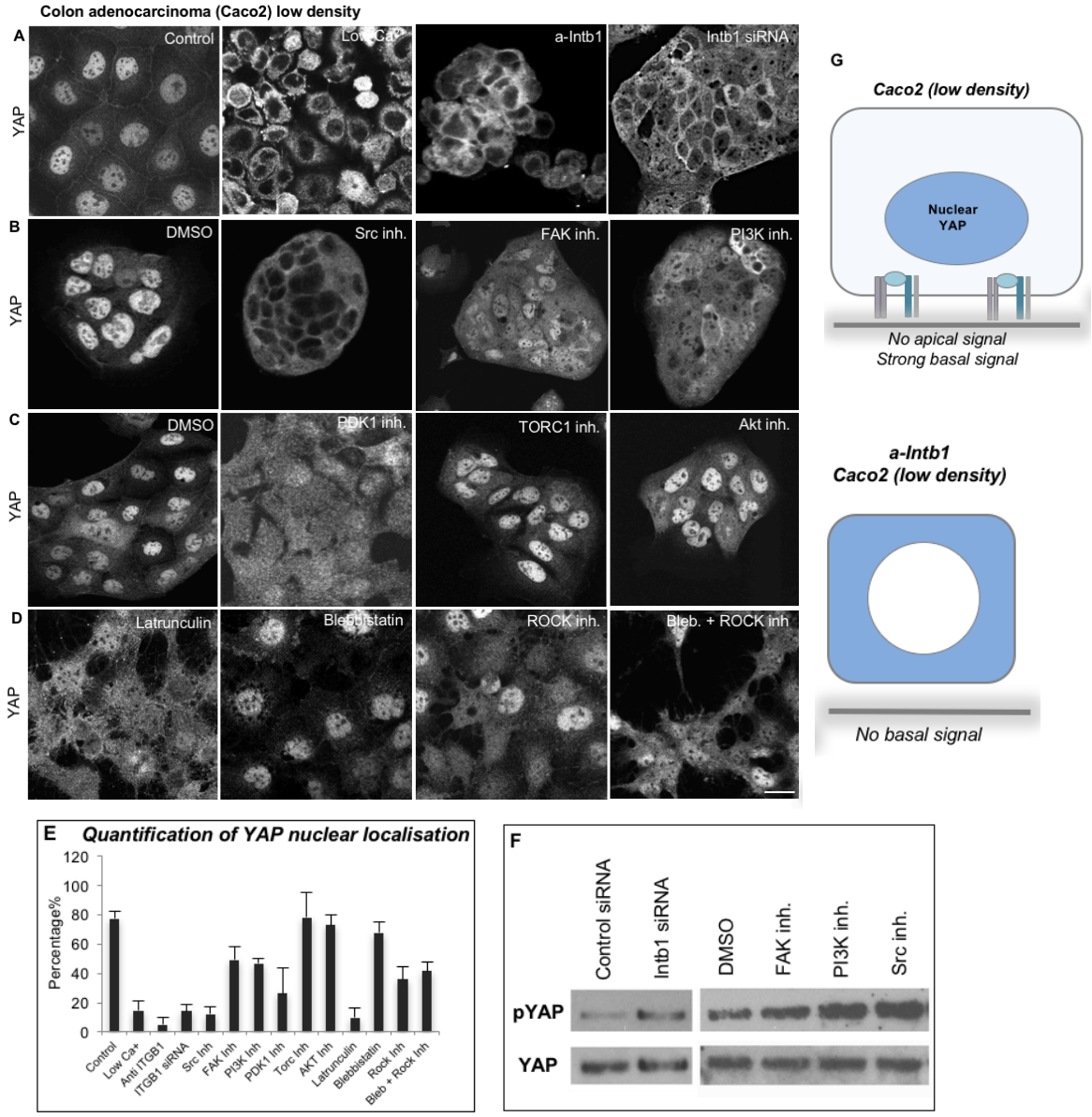
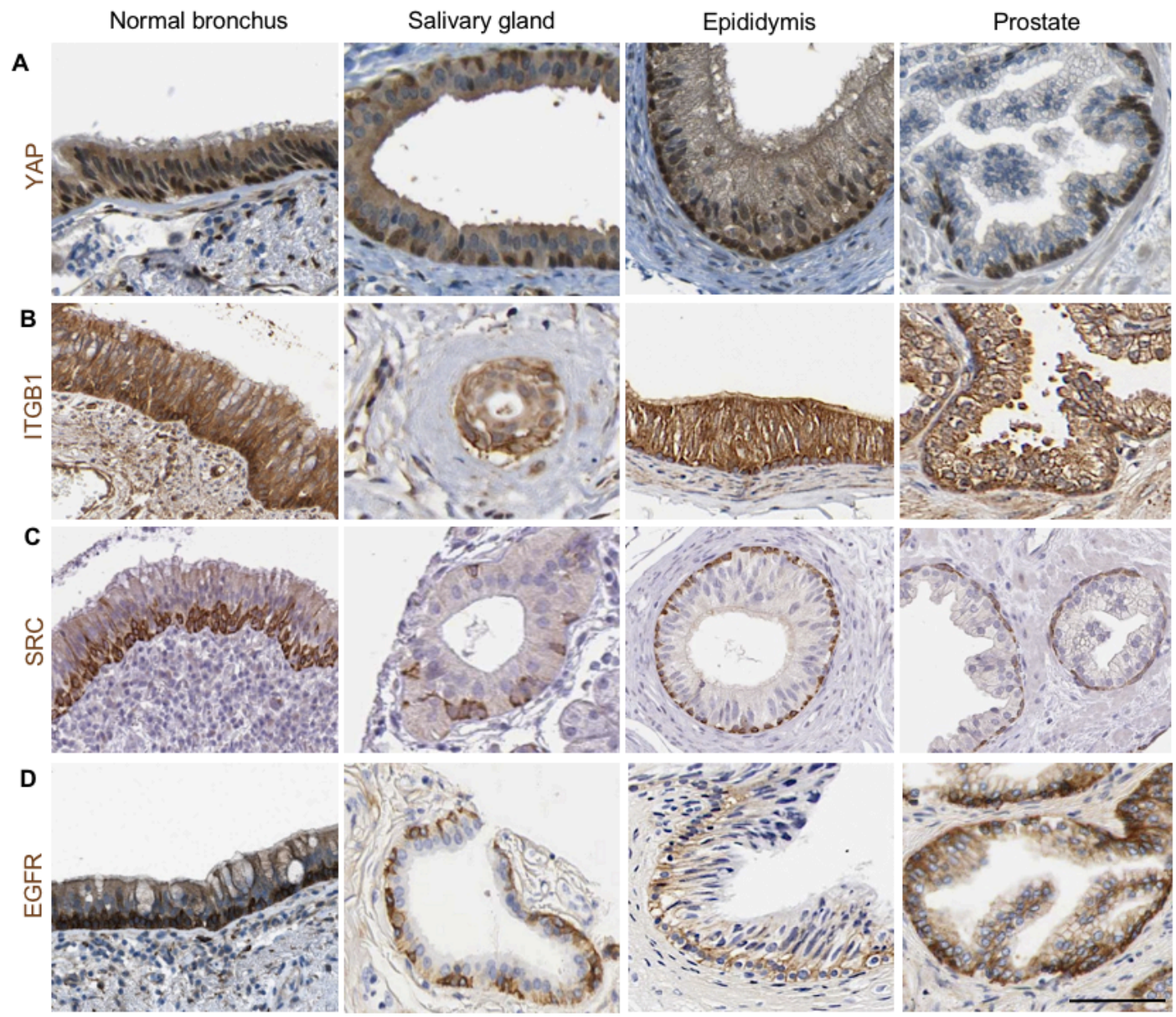


Figure 4.7. Blocking integrin-Src signalling inhibits YAP nuclear localisation in human Caco-2 cells when apical formation is blocked. Caco2 cells plated at low density to prevent apical domain formation show strong YAP nuclear localisation. **(A)** Treatment of Caco2 cells with low-calcium medium, anti-ITGB1 antibodies (PD52) or by *ITGB1* siRNA knockdown inhibits YAP nuclear localisation. **(B) T**reatment of Caco2 cells with Src inhibitor dasatinib, by FAK inhibitor PF573228 or by PI3K inhibitor GDC0941 reduces YAP nuclear localisation compared to treatment with DMSO solvent. **(C)** Treatment of Caco2 cells with PDK1 inhibitor BX795 reduces YAP nuclear localisation. YAP nuclear localisation is not changed by treatment with AKT inhibitor MK2206, TORC1 inhibitor Everolimus or DMSO solvent. **(D)** YAP nuclear localisation is reduced by treatment of Caco2 cells with F-actin destabilising drug Latrunculin, myosin II inhibitor Blebbistatin or Rho kinase inhibitor Y27632, or a combination of Blebbistatin and Y27532. Scale bar 20 μM. **(E)** Quantification of A-D. Values are means±s.e.m. **(F)** Western blotting analysis of p-YAP levels in Caco2 cells treated with control siRNAs, ITGB1 siRNAs, DMSO control, FAK inhibitor, PI3K inhibitor or Src inhibitor. Total YAP levels are shown as a control. **(G)** Schematic diagram of YAP cellular localisation regulation in Caco2 cells.



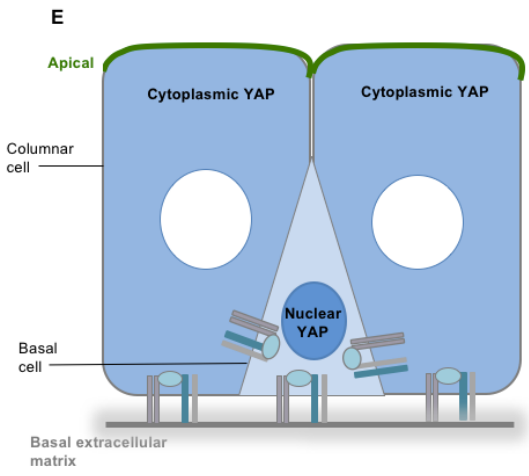


Figure 4.8. YAP is nuclear localised in basal progenitor cells that express integrin, Src and EGFR, and cytoplasmic in columnar cells that express an apical domain in human pseudostratified columnar epithelia. Using Human Protein Atlas data, the expression and localisation of YAP, ITGB1, SRC and EGFR were examined in pseudostratified epithelia. **(A)** YAP is nuclear localised in the basal layer progenitor cells of bronchus, salivary gland, epididymis and prostate. **(B)** ITGB1 is expressed in the basal layer progenitor cells of bronchus, salivary gland, epididymis and prostate. **(C)** SRC is expressed in the basal layer progenitor cells of bronchus, salivary gland, epididymis and prostate. **(D)** EGFR is expressed in the basal layer progenitor cells of bronchus, salivary gland, epididymis and prostate. Scale bar 100 μM. **(E)** Schematic diagram of YAP regulation in pseudostratified columnar epithelia.

In summary, the above analysis suggests an antagonistic system governing YAP subcellular localisation across epithelia in which apical determinants via Hippo signalling activate LATS resulting in YAP sequestration in the cytoplasm, whilst basal integrin-Src and PI3K signalling inhibit LATS, allowing YAP to localise to the nucleus.

4.2. Epithelia under pathological conditions

4.2.1. YAP in stratified squamous epithelial tumours

Disrupted cell polarity is a hallmark of epithelial cancers (Bilder 2004, Cavatorta, Fumero et al. 2004, Nakagawa, Yano et al. 2004, Navarro, Nola et al. 2005, Gardiol, Zacchi et al. 2006, Huang and Muthuswamy 2010). Although YAP mutations have not been found in human epithelial cancers, YAP expression and activity is associated with tumour formation and progression (Ma, Yang et al. 2015, Maugeri-Sacca, Barba et al. 2015, Zanconato, Cordenonsi et al. 2016, Bae, Kim et al. 2017). Since loss of polarity strongly induces YAP nuclear localisation, we sought to explore this relationship in epithelial tumours.

In human normal stratified squamous epithelia and squamous cell carcinoma YAP is nuclear in basal cells that are in contact with the basement membrane extracellular matrix and express integrin and Src whilst YAP is cytoplasmic in cells that do not contact the basement membrane extracellular matrix and lack integrin and Src expression (Fig. 4.9). Treatment of mouse DMBA/TPA-induced papillomas and squamous cell carcinomas with topical Src inhibitor (dasatinib) significantly reduced nuclear YAP and YAP levels in these tumours (Fig. 4.10). In line with these results, other groups have shown that the size and frequency of DMBA/TPA-induced tumours in mice can be significantly decreased by dasatinib treatment or genetic deletion of YAP (Serrels, Serrels et al. 2009, Creedon and Brunton 2012, Zanconato, Forcato et al. 2015).

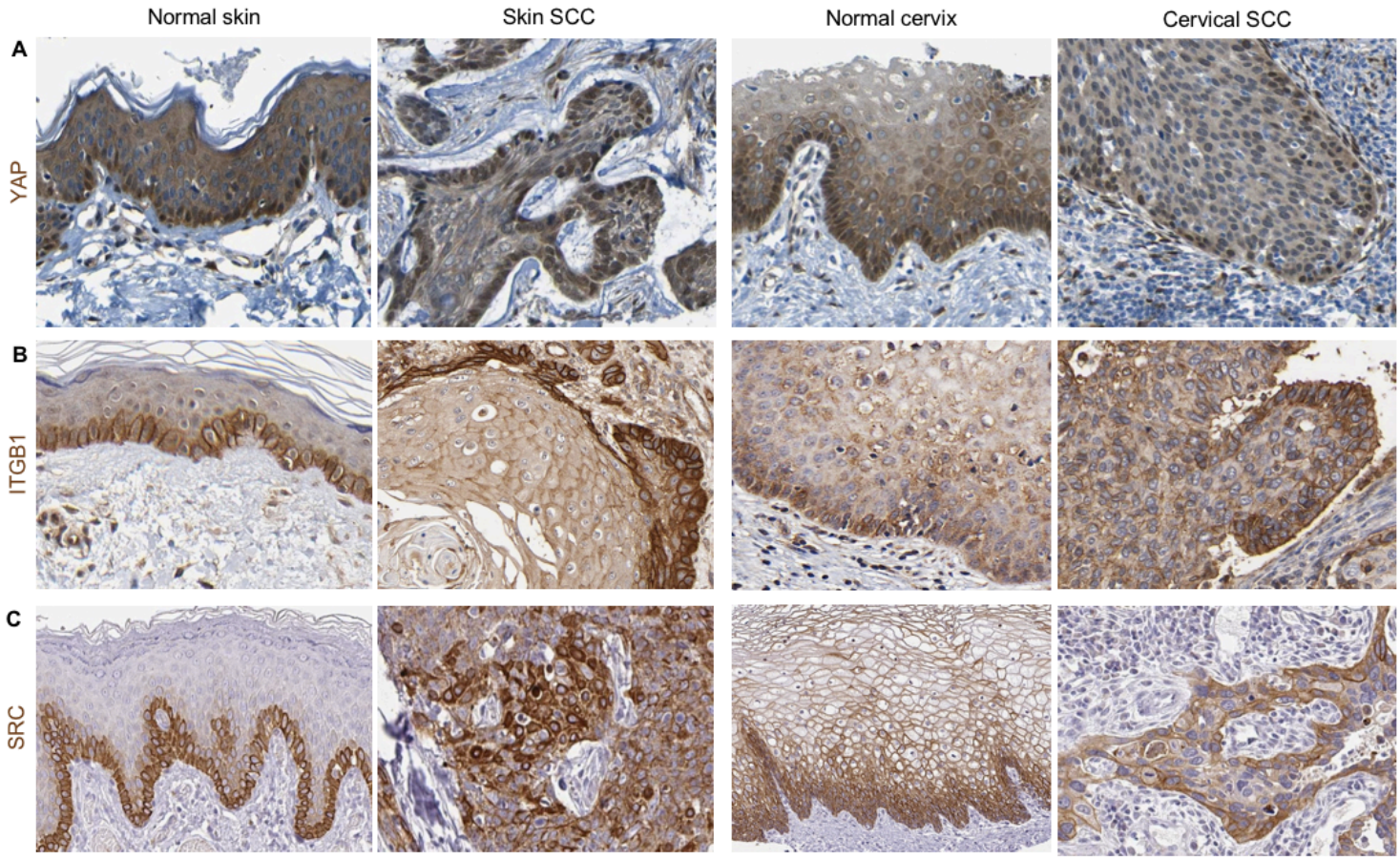


Figure 4.9. YAP is nuclear localised in cells that are in contact with the ECM and express integrin and Src in human normal stratified squamous epithelia and squamous cell carcinoma. Human Protein Atlas data were analysed to examine the expression and localisation of **(A)** YAP, **(B)** Integrin β1 and **(C)** Src in skin and cervical normal stratified squamous epithelia and squamous cell carcinoma.

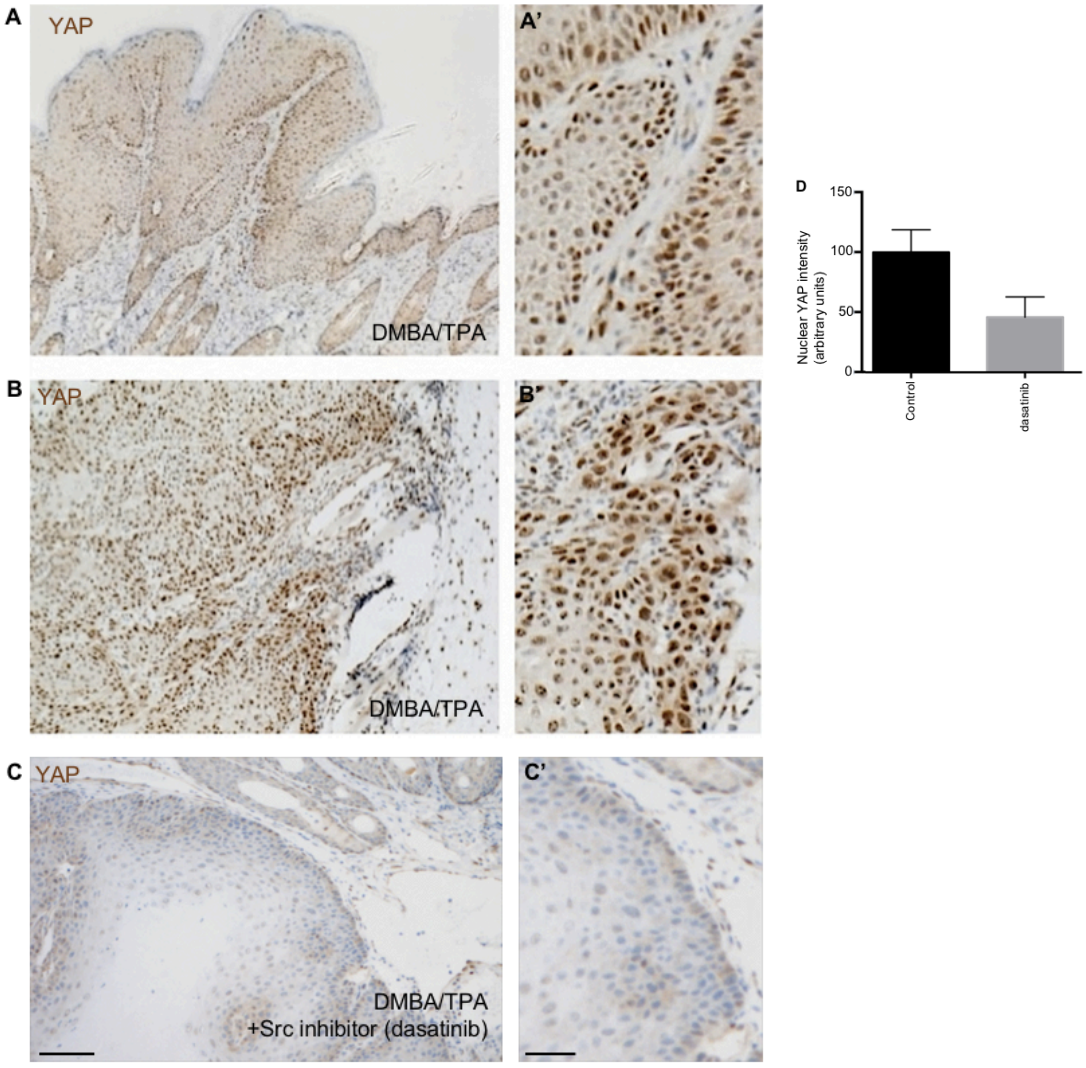
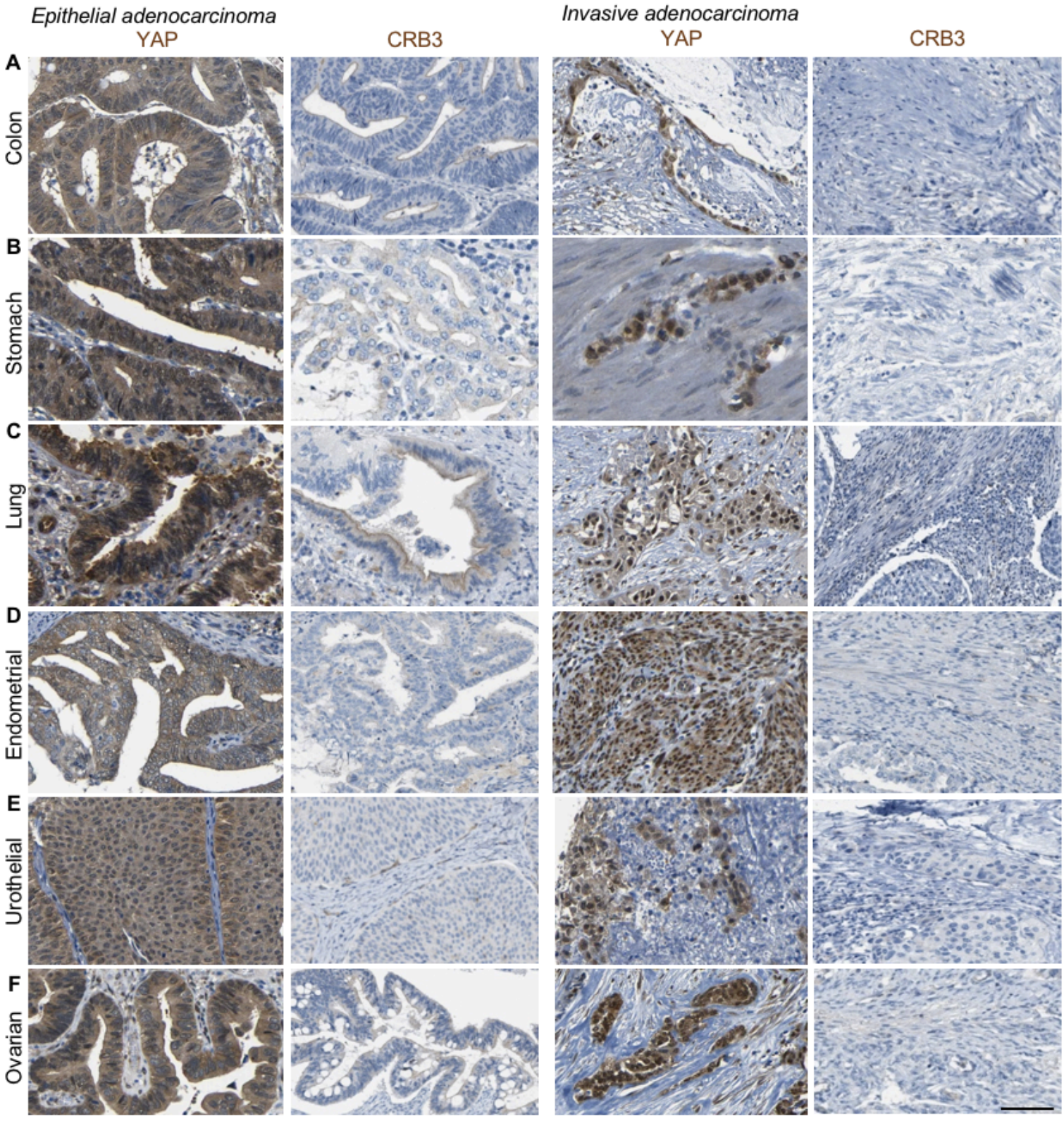


Figure 4.10. Nuclear YAP expression in mouse DMBA/TPA-induced cutaneous papilloma and squamous cell carcinoma is reduced by treatment with Src inhibitor dasatinib. (A) YAP staining of mouse skin papilloma induced by DMBA-TPA treatment of mice expressing v-Ha-Ras (see Materials and Methods). Note stronger nuclear localisation in the basal layer. A' higher magnification. **(B)** YAP staining of mouse skin squamous cell carcinoma induced by DMBA-TPA treatment of v-Ha-Ras-expressing mice. B' higher magnification. **(C)** YAP staining is strongly reduced by topical treatment of DMBA-TPA induced papillomas with Src inhibitor dasatinib (see Materials and Methods). C' higher magnification. Scale bars A,B,C 50 μM; A',B',C' 25 μM. **(D)** Quantification of nuclear YAP intensity in A-C. Values are means±s.e.m, normalised to control. A student's t test was performed on the data; $p<0.0001$. $n=3$ tumours each from 3 animals.

4.2.2. YAP in columnar epithelial tumours

We next sought to investigate the subcellular localisation of YAP in columnar epithelial tumours. In epithelial tumours of the colon, stomach, lung, endometrium, urothelium or ovary (Fig. 4.11A-F) YAP was localised cytoplasmically in cells that maintained their columnar epithelial morphology and expressed apical CRB3. Invasive adenocarcinomas of the same tissue origin displayed a dramatic loss of epithelial tissue architecture, epithelial cell morphology and CRB3 expression whilst nuclear YAP localisation increased (Fig. 4.11A-F). These data suggest that YAP becomes nuclear during tumour progression consequent to loss of cell polarity and apical determinant expression. Thus we next tested whether inhibition of integrin-Src signalling would reduce nuclear YAP in invasive adenocarcinomas. Apc-/- p53-/- mutant intestinal organoids subcutaneously transplanted into nude mice quickly yield adenocarcinomas that rapidly invade the surrounding tissue. We found that YAP was expressed throughout the implanted organoid, and was strongly nuclear in the invasive tumour cells infiltrating into the surrounding stroma (Fig. 4.11G). Mice with these invasive tumours were treated with Src inhibitor dasatinib. Nuclear YAP was significantly reduced, as was tumour growth and invasion (Fig. 4.11H,I). These data show that Src activity stimulates YAP nuclear localisation *in vivo*.

Fig. 4.11. YAP becomes nuclear in invasive adenocarcinomas, which are sensitive to dasatinib. (A-F) Comparison of YAP localisation in human epithelial and invasive adenocarcinomas. In the colon **(A)** and stomach **(B)** YAP is cytoplasmic in columnar epithelial cells in epithelial adenocarcinoma. These columnar cells have an apical domain expressing CRB3. YAP becomes nuclear in invasive adenocarcinoma cells, which have lost their columnar shape, lack CRB3 expression and lack a lumen. **(C)** In the bronchus YAP is nuclear in basal layer stem/progenitor cells and cytoplasmic in columnar epithelial cells that express apical CRB3 in epithelial adenocarcinoma. YAP is nuclear in invasive adenocarcinoma cells that have lost their columnar shape and lack CRB3 expression. **(D)** In the endometrial epithelium YAP is cytoplasmic in columnar epithelial cells in epithelial adenocarcinoma, and nuclear in invasive adenocarcinoma cells. **(E)** In urothelial epithelium YAP is cytoplasmic in pseudostratified columnar cells that express apical CRB3 in epithelial adenocarcinoma and nuclear in invasive adenocarcinoma cells which lack CRB3 expression. **(F)** In ovarian epithelium YAP is cytoplasmic in ovarian adenocarcinoma cells that express apical CRB3, and nuclear in invasive ovarian adenocarcinoma cells which lack CRB3 expression. (Fig overleaf).


Epithelial adenocarcinoma
YAP
CRB3
Invasive adenocarcinoma
YAP
CRB3
A
Colon
B
Stomach
C
Lung
D
Endometrial
E
Urothelial
F
Ovarian

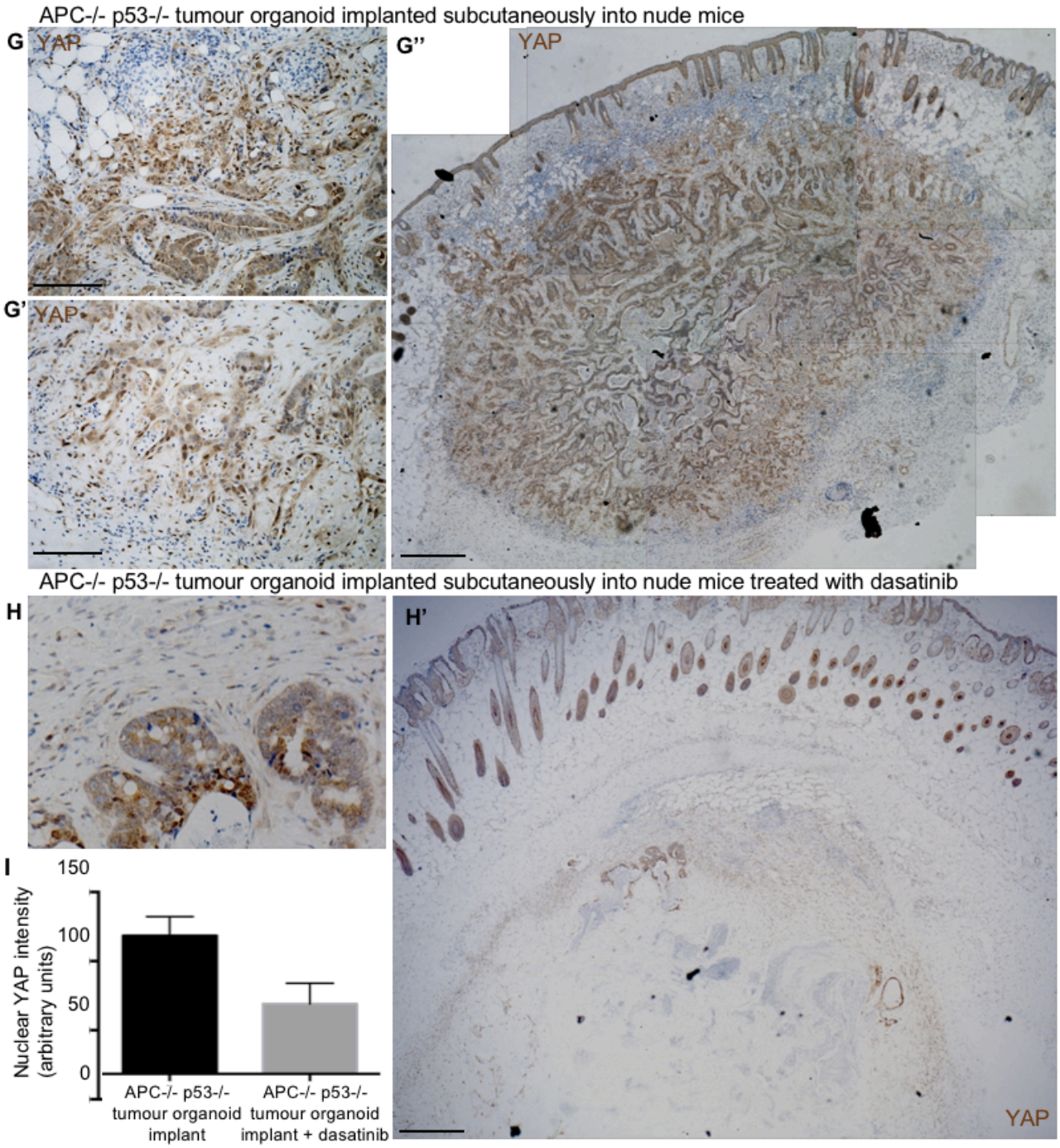


Figure 4.11. (G) YAP staining in Apc−/− p53−/− tumour organoids implanted subcutaneously under back skin into nude mice, which invade significantly into the surrounding tissue. Note that YAP is nuclear in cells at the invasive front, whereas columnar epithelial cells in the central regions of the tumour express cytoplasmic YAP. Scale bars G,G' 50μm; G''' 500 μM. **(H)** Treatment of Apc−/− p53−/− tumour organoids implanted subcutaneously into nude mice with Src inhibitor dasatinib strongly reduces YAP staining. Invasive tumour cells are not visible. Scale bars H 50 μM; H' 500 μM. **(I)** Quantification of nuclear YAP intensity in Apc−/− p53−/− tumour organoids implanted subcutaneously into nude mice with and without dasatinib treatment. Values are means±s.e.m, normalised to control. *n*=200 tumour cells, 3 samples for each condition. This work was performed in collaboration with Owen Samson, Beatson Institute, Glasgow.

It is known that YAP can be activated downstream of inflammatory cytokines, such as interleukin-6 (IL-6). Our results are consistent with a recent study showing that gp130 (the IL-6 corecptor) promotes YAP stability and nuclear translocation via Src kinases (Taniguchi et al. 2015). Others found that damage to intestinal epithelia activated IL-6, resulting in intestinal regeneration – a process in which Src and YAP are known to play a role (Cai, Zhang et al. 2010, Cordero, Ridgway et al. 2014, Kuhn, Manieri et al. 2014, Taniguchi, Wu et al. 2015). In line with this, we found that radiation injury to the intestine resulted in increased YAP levels, and importantly that this did not happen in Src-knockout intestine, indicating that Src is required for elevation of YAP levels upon intestinal injury (Fig. 4.12A) In wild-type irradiated intestines YAP remains largely nuclear in the basal crypt progenitor cells lacking an apical domain and cytoplasmic in columnar cells bearing and apical domain, suggesting that increased Src signalling upon inflammation is not sufficient to override the apical signalling retaining YAP in the cytoplasm (Fig. 4.12A).

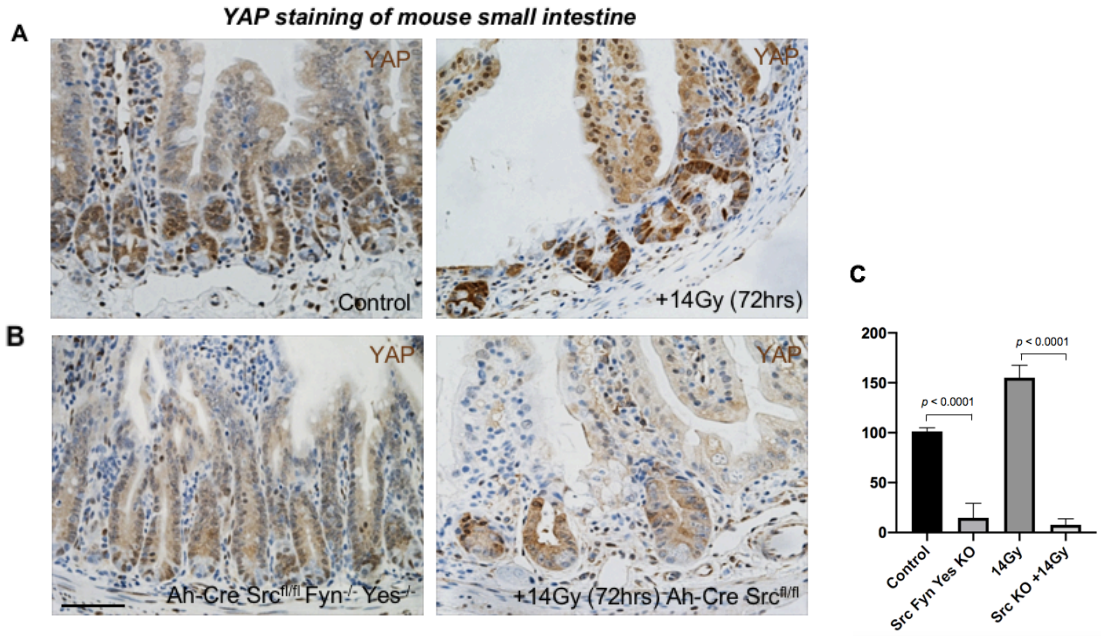


Figure 4.12. Src is required for YAP activation after intestinal irradiation. (A) Mouse small intestines stained for YAP before and after 72hrs of gamma-irradiation with 14Gy. Note increase in YAP in crypt progenitor cells. **(B)** Note decreased YAP levels and nuclear localisation in Src, Fyn, Yes triple knockout intestines or in Src single knockout intestines after 14Gy irradiation. Scale bar 100 μM. **(C)** Quantification of (A) and (B). Values are means±s.e.m, normalised to control. *n*=15 FOV each of 4 animals per condition. This work was performed in collaboration with Owen Samson, Beatson Institute, Glasgow.

4.3. Summary

In summary, apical-basal polarity signals play a role in controlling proliferation versus differentiation decisions in epithelial cells. We found that YAP, an oncogene known to promote proliferation/stemness in tumour cells, acts as a sensor of such polarity signals. We showed that integrin-Src and EGFR-PI3K – signalling molecules proposed to drive YAP to the nucleus in MCF10A cultured breast cells – are expressed in the basal layer keratinocytes of human and mouse epidermis and promote YAP nuclear localization in cultured keratinocytes and mouse basal layer cells *in vivo*. YAP induces expression of integrins, integrin ligands (*CTGF*, *CYR61*) and EGFR ligands (*AREG*) (Fig. 3.8). It is therefore conceivable that a positive feedback loop exists in which basal signals stimulate YAP nuclear localization, which in turn drives transcription of such signals. This loop would be broken when daughter cells lose contact with the basement membrane, thus creating a bistable system for determining progenitor versus differentiated cell fate. This model of YAP regulation appears to hold true for other squamous epithelia. Columnar epithelia, on the other hand, express an apical domain that overrides basal signals and results in cytoplasmic sequestration of YAP via MST/LATS kinase phosphorylation.

Chapter 5. YAP synergises with the wound healing response to drive ZEB1-mediated EMT and spindle cell squamous carcinoma formation

As discussed in the previous chapter, loss of polarity is linked to aberrant nuclear expression of YAP and progression to invasive tumours. To test whether increased and aberrant nuclear YAP expression induce tumour growth we expressed an activated nuclear YAP in the mouse epidermis and assayed the consequences.

YAP drives epidermal cell proliferation in the skin of mouse embryos (Zhang, Pasolli et al. 2011) and promotes squamous cell carcinoma (SCC) formation in embryonic mouse skin after transplantation into nude mice (Schlegelmilch, Mohseni et al. 2011). Conversely *Yap* knockout prevent Ras-driven skin SCC formation (Zanconato, Forcato et al. 2015, Debaugnies, Sanchez-Danes et al. 2018). YAP is furthermore known to exhibit recurrent amplifications in human SCC tumours (India Project Team of the International Cancer Genome 2013, Hiemer, Zhang et al. 2015) and to promote human SCC cell proliferation in culture (Walko, Woodhouse et al. 2017). SCC is an epithelial cancer that arises from keratinocytes in stratified squamous epithelia, such as skin, oesophagus, oral cavity, prostate, vagina or cervix. SCC is the most common cancer capable of progressing to metastatic malignancy and cutaneous SCC (cSCC) is the second most prevalent human cancer worldwide. SCC can progress to an aggressive and invasive morphologically distinct subtype called spindle cell squamous cell carcinoma (spSCC). SpSCC is characterized by elongated atypical spindle-shaped cells that infiltrate the dermis. Since spSCC arises in the same anatomical locations as SCC, the cell of origin is thought to be epidermal, even though the “sarcomatoid” appearance is reminiscent of mesenchymal fibroblasts of fibrosarcomas. SpSCC cells express mesenchymal markers such as Vimentin whilst downregulating epithelial markers such as cytokeratins and E-cadherin (Cassarino, Derienzo et al. 2006). The molecular mechanism for spSCC formation is not definitively known. One possibility is that the vimentin-expressing elongated spindle-shaped cells of spSCC could originate from epithelial cells of the epidermis via an epithelial to mesenchymal transition (EMT) (Yang and Weinberg 2008, Brabletz, Kalluri et al.

2018). EMT has been shown to occur in some mouse models of RasG12D-driven cutaneous carcinomas (Latil, Nassar et al. 2017). Unambiguous evidence of an epithelial cell of origin in spSCC requires new mouse models enabling lineage tracing.

5.1. YAP is nuclear localised in human cutaneous spindle cell squamous cell carcinoma

To address whether spSCC formation involves YAP we began by examining YAP expression in human cutaneous spSCC samples. In normal human skin, YAP is expressed predominantly in the keratinocytes of the epidermis and nuclear localised in the basal layer of progenitor cells (Fig. 5.1B,C). The epidermis is Keratin-5-positive whilst the underlying dermal stroma does not express Keratin-5 (K5; Fig. 5.1A). SpSCCs did not express K5, indicating that these tumours are not epidermal (Fig. 5.1A). Interestingly, despite the absence of epithelial marker, YAP was highly expressed and strongly nuclear throughout the tumours (Fig. 5.1B,C; Fig. 5.2A,B,C). These observations suggest that YAP accumulation in spSCCs correlates with the absence of Keratin-5 expression.

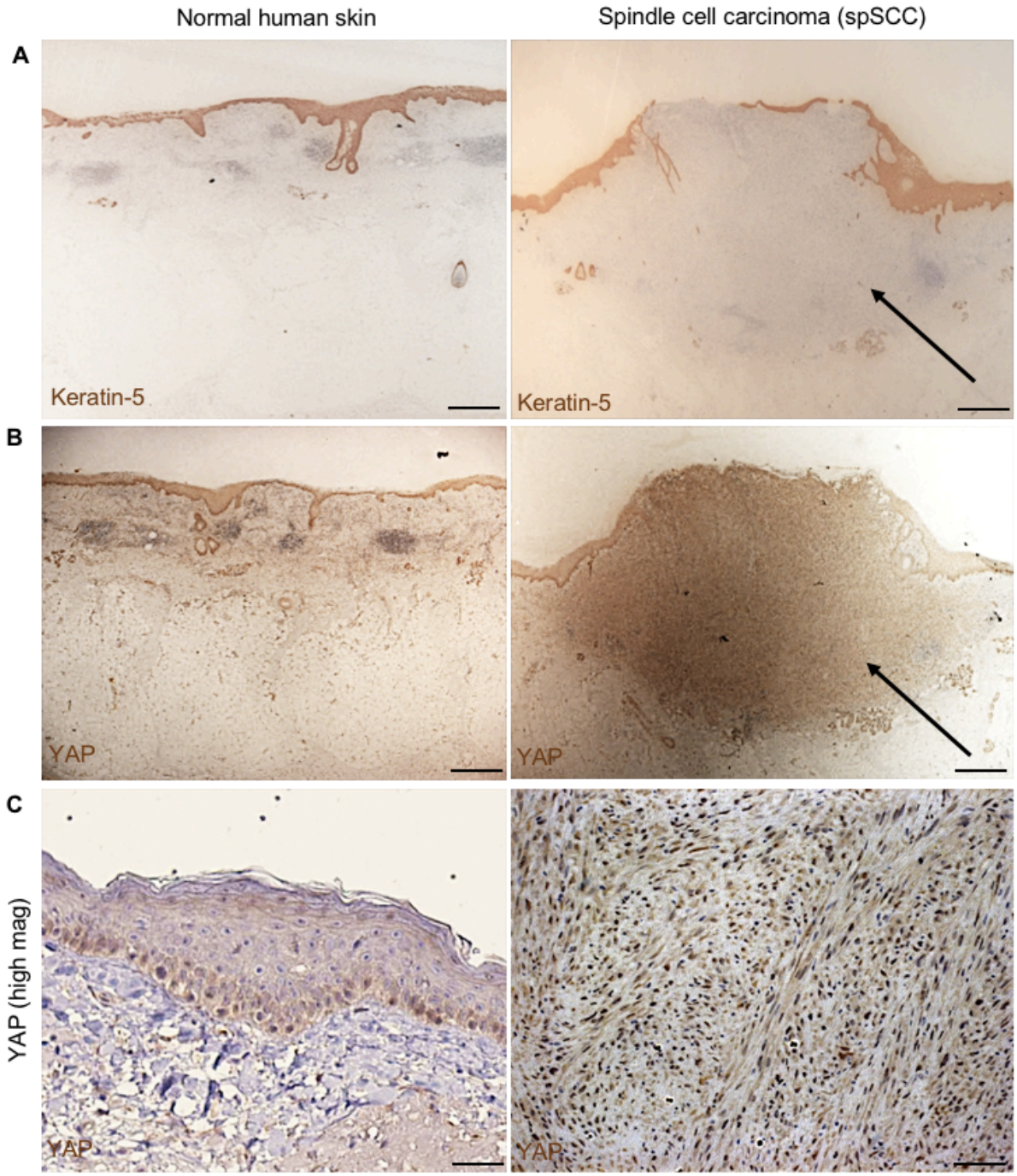


Figure 5.1. YAP is nuclear localised in human spindle cell squamous cell carcinoma, which is Keratin-5-negative. Histological sections of normal human skin and spindle cell carcinoma patient tumour. Representative images shown, see Fig 5.2 and Appendix 7.1 for more spSCC tumour sections stained for YAP. See Methods for protocol. **(A)** normal skin and spSCC stained for the epithelial marker Keratin-5. Note that mass of tumour (arrow) is negative for Keratin-5. Scale bar 200 μM. **(B)** normal skin and spSCC stained for YAP. Note tumour (arrow) is strongly positive for YAP. Scale bar 200 μM. **(C)** High magnification view of (B) (area indicated by arrow is magnified in the tumour). Note nuclear localisation of YAP protein in spSCC cells. Scale bar 50 μM.

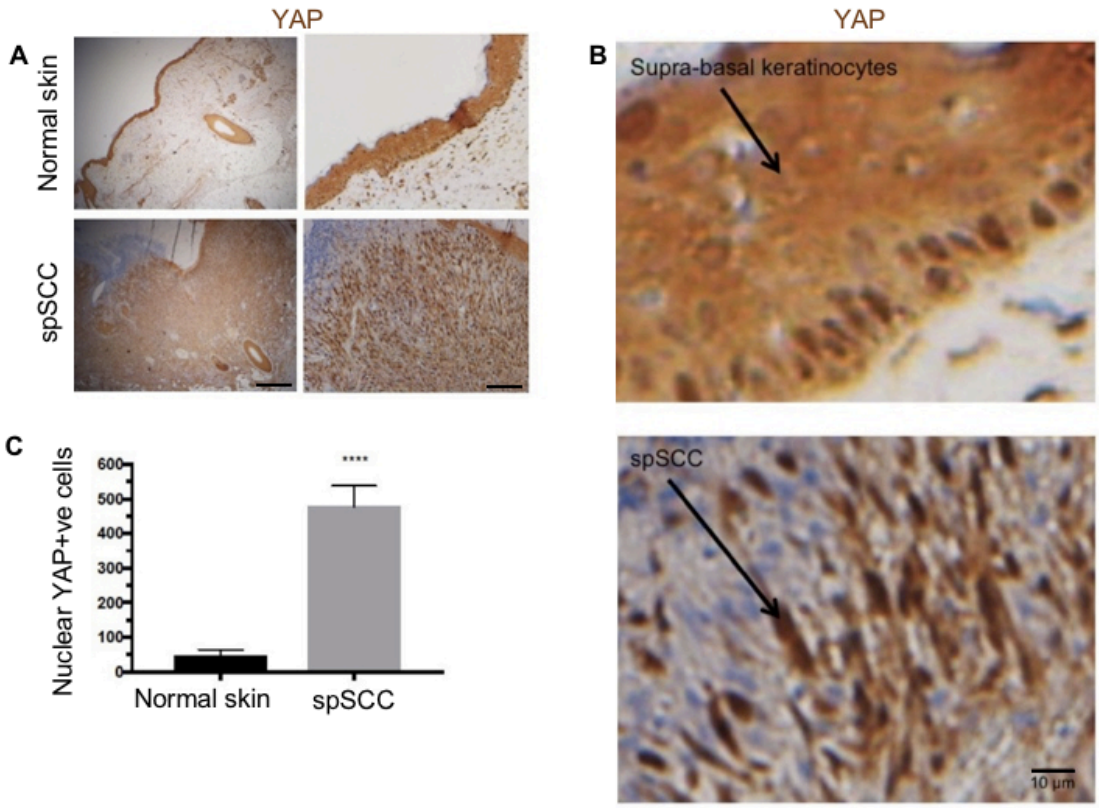


Figure 5.2. Human cutaneous spSCC tumours express high levels of nuclear YAP. **(A)** Normal human skin and human cutaneous spSCC immunostained for YAP Note widespread nuclear YAP localization in spSCC. Scale bars: left panels 200 µM, right panels 50 µM. **(B)** Higher magnification of normal skin and spSCC sections stained for YAP. Note in normal skin YAP is nuclear localised in the basal layer keratinocytes and cytoplasmic localised in the suprabasal keratinocytes of the epidermis. In spSCC Yap is nuclear in cells throughout the tumour. Scale bar 10 µM. For clarity only one sample is shown here, see Appendix 7.1 for gallery of spSCC tumour samples stained for YAP. **(C)** Quantitation of nuclear YAP-positive cells in normal skin versus spSCC. Values are nuclear positive cells per field of vision as means±s.e.m, *n*=22 samples, 3 fields of vision per sample.

5.2. Nuclear YAP drives formation of squamous cell carcinoma and spindle cell squamous cell carcinoma in mouse skin

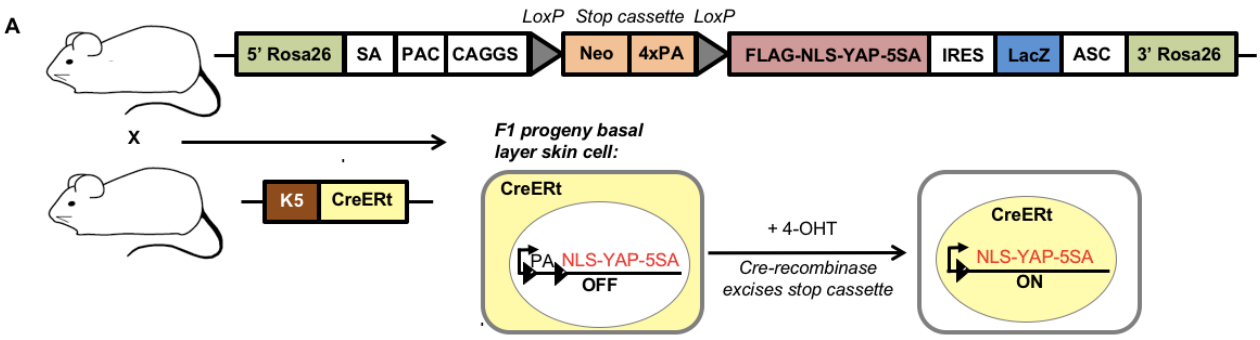
To test whether high levels of nuclear YAP can contribute to spSCC formation we generated a conditional inducible NLS-tagged activated YAP transgene (*Rosa26 LoxSTOPLox NLS-YAP-5SA IRES LacZ*). YAP5SA has five serine LATS phosphorylation sites mutated to alanine, rendering this form of YAP constitutively active. These mice were crossed to a strain expressing a tamoxifen-inducible Cre recombinase under the control of the Keratin-5 promoter, enabling conditional expression of nuclear activated YAP specifically in the epidermis (Fig. 5.3A). A LacZ gene comprising an IRES was included to allow lineage tracing of cells that undergo Cre-mediated excision of the STOP cassette and therefore express NLS-YAP-5SA. SCC as well as spSCC developed rapidly (2-4 weeks after tamoxifen administration) (Fig. 5.3B,D). SpSCCs were distinguished histologically from SCC; by their lack of Keratin-5 expression (Fig. 5.3B). SCCs were Keratin-5 positive, morphologically resembled hyperplastic epidermis, and arose randomly throughout the skin. spSCCs tended to be much larger in mass and tumour cells were dermal rather than epithelial in location and character (absence of K5 expression; Fig.5.3B). It is worth noting that spSCCs predominantly formed at grooming sites where the mice scratch their skin (top of the head, neck/shoulder and flank; Fig. 5.3C). SpSCCs were associated with epidermal wounds, as indicated by the disruption in the continuity of Keratin-5-positive epithelium above spSCC tumours (Fig. 5.3B). The results so far suggest that epidermal damage, in combination with nuclear YAP expression could lead to the transformation of K5-positive SCC into K5-negative spSCC.

In order to test whether the dermal spSCC tumour cells derived from the K5-positive epidermal cells expressing NLS-YAP-5SA, we stained SCCs and spSCCs for beta-Galactosidase (the product of the *LacZ* gene), which becomes expressed upon K5-CreERt-mediated recombination. Following Cre induction by tamoxifen administration nuclear beta-Galactosidase was expressed sporadically in patches of cells in the skin epidermis and in SCC-like epidermis (Fig. 5.3E). This indicated that only a small proportion of epidermal cells undergo Cre-mediated recombination and express NLS-YAP-5SA, along with beta-Galactosidase, and that this

keratinocyte population is sufficient to drive epidermal hyperplasia and SCC. In spSCCs, which arose solely at grooming scratch sites and were associated with epidermal wounding, beta-Galactosidase was strongly expressed throughout the dermal tumour cells and in some epidermal cells (Fig. 5.3E,G). Thus, the K5-negative dermal spSCC tumour cells are daughter cells of the K5-positive NLS-YAP-5SA-expressing keratinocytes. In SCCs, expression of the proliferation marker Ki-67 was largely confined to the basal layer cells of the epidermis, indicating that the epidermal hyperplasia results from over-proliferation of basal layer cells (Fig. 5.3F). By contrast Ki-67 was expressed irregularly throughout SpSCCs and these tumours were highly proliferative (Fig. 5.3F,G). Together these results suggest that:

1. Nuclear activated YAP expression can drive transformation of epidermal K5-positive epithelial cells into dermal K5-negative mesenchymal spindle cells in spSCC formation.
2. Epidermal wounding is involved in this epithelial to mesenchymal transformation process.

Figure 5.3. Epidermal nuclear YAP expression drives formation of SCC and spCC in mouse skin. (**A**) K5-CreERt mice were crossed to a line carrying a Lox-Stop-Lox cassette upstream of an NLS-YAP-5SA sequence to achieve epidermal-specific conditional expression of nuclear YAP. LacZ in the construct allows for lineage tracing of basal layer skin cells expressing the nuclear YAP. (Fig. continued on next page).



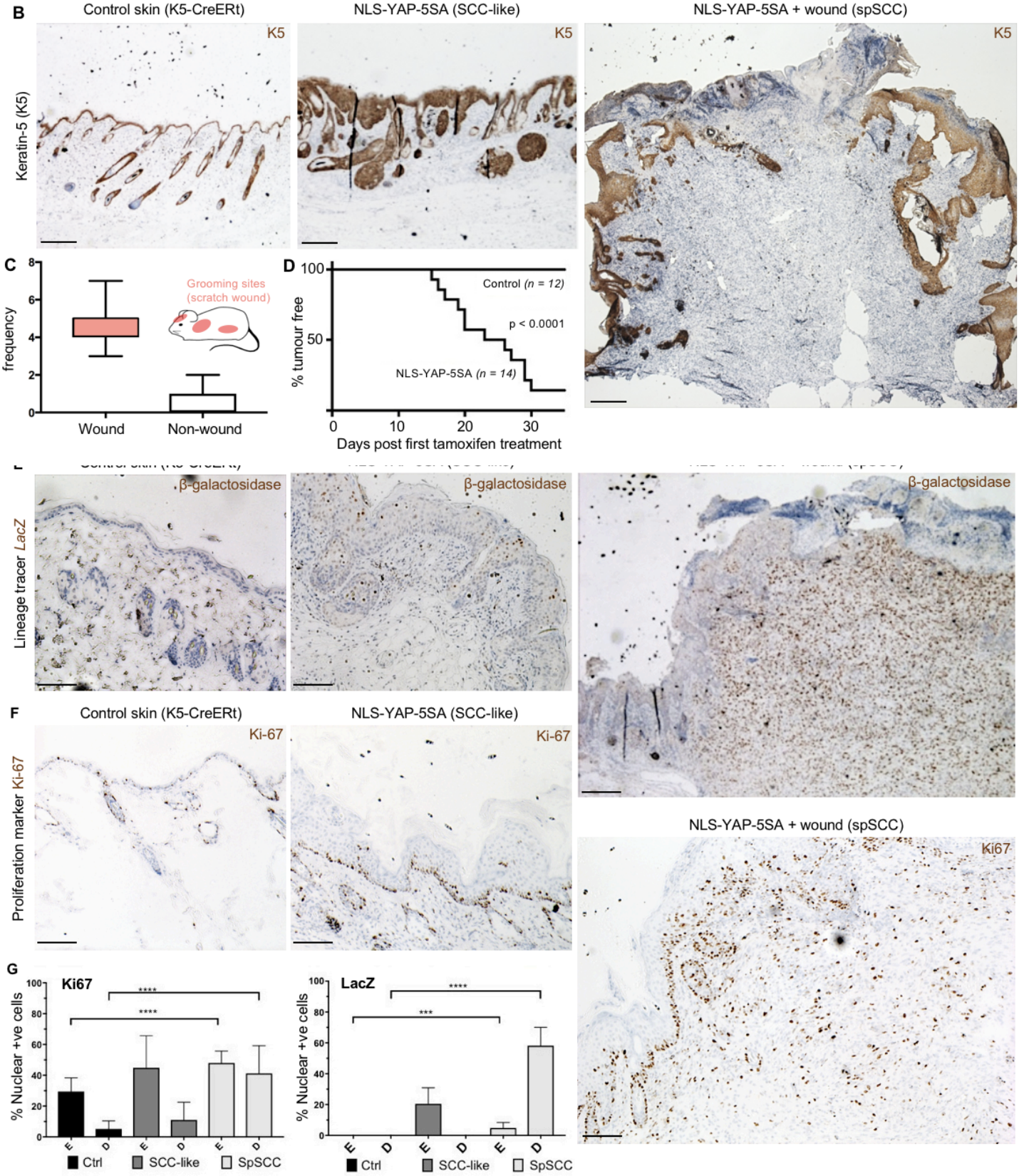


Figure 5.3. Epidermal nuclear YAP expression drives formation of SCC and spCC in mouse skin. (**B**) Expression of NLS-YAP-5SA in epidermal basal layer cells drives

formation of both SCC-like hyperplasia (Keratin-5 positive) and SpSCC-like tumours (mostly Keratin-5 negative). Note the disruption in the continuity of Keratin-5 positive epithelial layer above the SpSCC tumour, indicative of a wound-induced tumour. Scale bars 100 μM (*n*= 20). (**C**) Multiple SpSCC tumours arise per animal, but only in grooming sites (*n*= 22). (**D**) Kaplan-Meier analysis shows rapid tumour formation upon tamoxifen induction of NLS-YAP-5SA expression in epidermal basal layer cells (control *n*=12; NLS-YAP-5SA *n*=14). **(E)** Lineage tracing with LacZ (encoding nuclear beta-Gal immunostained in brown) shows that both SCC and SpSCC tumours arise from the K5-positive basal layer of the skin (*n*= 22). (**F**) Control, SCC and SpSCC sections stained for proliferation marker Ki-67 (*n*= 25). Scale bars E,F 100 μM. (**G**) Quantification of (E) and (F) in epidermal, E vs dermal, D compartments. Values are means+s.e.m. (See previous page).

5.3. YAP-driven mouse spindle cell squamous cell carcinoma formation involves transcriptional induction of Zeb1 and epithelial to mesenchymal transition

To confirm that the formation of nuclear activated YAP-driven SpSCC involves epithelial to mesenchymal transition (EMT) we stained NLS-YAP-5SA tumours for classic EMT biomarkers (Thiery and Sleeman 2006, Yang and Weinberg 2008, Kalluri and Weinberg 2009, Zeisberg and Neilson 2009, Lamouille, Xu et al. 2014). EMT was first characterised as a switch in transcriptional and signalling programs driving an ectodermal to mesodermal cell fate during normal embryonic development (Boulay, Dennefeld et al. 1987, Thisse, Stoetzel et al. 1988, Fortini, Lai et al. 1991, Leptin 1991, Nieto, Bennett et al. 1992, Broihier, Moore et al. 1998, Shook and Keller 2003). Epithelial cells lose their apicobasal polarity and cell-cell adhesion, acquiring the undifferentiated and motile potential of mesenchymal stem cells (Savagner 2015). During this process, which has since been well established as playing a role in tumour metastasis, epithelial markers such as keratins and E-cadherin are downregulated whilst mesenchymal markers including Vimentin and N-cadherin are upregulated and the cell becomes elongated. As discussed above, progression of activated nuclear YAP-driven tumours from SCC to spSCC following epidermal damage was accompanied by a loss of Keratin-5 expression (Fig. 5.3B). Concurrently, Vimentin was upregulated (Fig. 5.4B). High levels of Vimentin in tumours have been associated with elevated risk of metastasis and poor prognosis. There is growing evidence indicating that the role of Vimentin in EMT is causal as well as correlative: by inducing cytoskeletal reorganization, Vimentin effects changes in cell morphology, motility and adhesion (Satelli and Li 2011, Dauphin, Barbe et al. 2013, Yamashita, Tokunaga et al. 2013, Liu, Lin et al. 2015). Loss of cell adhesion via downregulation of E-cadherin and consequent loss of intercellular

junctions is another hallmark of EMT, causing cells to lose their apical-basal polarity and gain migration ability (Capaldo and Macara 2007, Baum and Georgiou 2011).

The classical inducers of EMT comprise three transcription factor families: the Snail, Twist and ZEB families. These factors bind to conserved enhancer box (E-box) sequences in the promoters of epithelial target genes, such as E-cadherin and other junctional proteins, directly repressing their transcription. ZEB1/2 (Zinc finger E-Box-binding homeobox) transcription factors are potent inducers of motility and stemness and are associated with aggressive invasive tumours with poor prognosis (Eger, Aigner et al. 2005, Aigner, Dampier et al. 2007, Krebs, Mitschke et al. 2017).

ZEB1 is known to be widely expressed in tissues undergoing developmental EMT (Funahashi, Sekido et al. 1993, Yang and Weinberg 2008, Thiery, Acloque et al. 2009, Nieto, Huang et al. 2016). Whilst a comprehensive analysis of ZEB1 expression in healthy human adult tissues is lacking, studies so far have found that ZEB1 is expressed in some mesodermal-derived cells, such as activated fibroblasts, but not in the majority of healthy adult post-mitotic epithelial cells (Hurt, Saykally et al. 2008, Furuya, Masuda et al. 2017). This suggests that expression of ZEB1 endows cells with mesenchymal properties such as migration and proliferation ability. Indeed, overexpression of ZEB1 in mammalian cancer cell lines can induce EMT and metastasis (Comijn, Berx et al. 2001, Eger, Aigner et al. 2005, Aigner, Dampier et al. 2007, Spaderna, Schmalhofer et al. 2008, Al Saleh, Sharaf et al. 2011, Hugo, Kokkinos et al. 2011, Ahn, Gibbons et al. 2012, Liu, Sun et al. 2012, Nieto 2013, Krebs, Mitschke et al. 2017). Furthermore, ZEB1 expression has been found in many human epithelial cancers and is associated with downregulation of E-cadherin and mestastasis (Guaita, Puig et al. 2002, Eger, Aigner et al. 2005, Pena, Garcia et al. 2005, Spoelstra, Manning et al. 2006, Witta, Gemmill et al. 2006, Peinado, Olmeda et al. 2007, Vandewalle, Van Roy et al. 2009, Wellner, Brabletz et al. 2010, Sanchez-Tillo, Siles et al. 2011, Jia, Liu et al. 2012, Okugawa, Toiyama et al. 2012, Zhou, Cao et al. 2012, Zhang, Zhou et al. 2013, Zhang, Lu et al. 2013, Bronsert, Kohler et al. 2014, Matsubara, Kishaba et al. 2014). Although well established as a transcriptional repressor of epithelial genes, ZEB1 can also function as a transcriptional activator of mesenchymal genes such

as vimentin, myosin, smooth muscle actin and collagens. (Chamberlain and Sanders 1999, Postigo 2003, Nishimura, Yasuhara et al. 2006, van Grunsven, Taelman et al. 2006, Lazarova and Bordonaro 2017). Furthermore, a recent study found that ZEB1 can interact directly with YAP and promote transcription of mesenchymal genes in cell culture (Lehmann, Mossmann et al. 2016). For these reasons, we decided to focus on the possible role of ZEB1 in the progression of SCC to spSCC.

We found neither *ZEB1* mRNA nor protein to be expressed in mouse skin epidermal cells (Fig. 5.4C,D). Notably, ZEB1 was not expressed in SCC but was strongly expressed at both the mRNA and protein level in NLS-YAP-5SA-driven spSCC (Fig. 5.4C,D,F). Similarly, *SNAIL1* mRNA was expressed in nuclear YAP-driven spSCC but not in normal epidermis nor in SCC (Fig. 5.4E). These data suggest that an EMT involving activation of ZEB1 expression could contribute to the formation of nuclear YAP-driven spSCC.

Figure 5.4. YAP-driven mouse spSCC formation involves transcriptional induction of Zeb1 expression and epithelial to mesenchymal transition. (**A**) Control skin and NLS-YAP-5SA-driven SCC-like and spSCC tumour sections immunostained for YAP (*n*= 32). (**B**) Control skin and NLS-YAP-5SA-driven SCC-like and spSCC tumour sections immunostained for Vimentin (mesenchymal marker). Note strong staining in spSCC (*n*= 30). (**C**) Control skin and NLS-YAP-5SA-driven SCC-like and spSCC tumour sections immunostained for ZEB1 (EMT inducing transcription factor) immunostaining of control skin as well as NLS-YAP-5SA driven SCC and spSCC tumours. Note strong nuclear staining in spSCC (*n*= 33). Scale bars controls 100 μM; SCC and spCCs 50 μM. (**D**) *ZEB1* mRNA *in situ* hybridisation of control skin and NLS-YAP-5SA driven SCC and SpSCC tumour sections. Note strong induction in SpSCC (*n*= 29). (**E**) *SNAIL1* mRNA *in situ* hybridisation of control skin and NLS-YAP-5SA driven SCC and spSCC tumour sections. Note strong induction in SpSCC (*n*= 8). Scale bars control 50 μM, SCC and SpCC 100 μM. (**F**) Quantitation of YAP and ZEB1 nuclear expression in samples from control skin and NLS-YAP-5SA-driven SCC-like and SpSCC-like mouse skin tumours in epidermal, E, and dermal, D, compartments. (*n*> 30 samples for each case). (Fig. on next page).

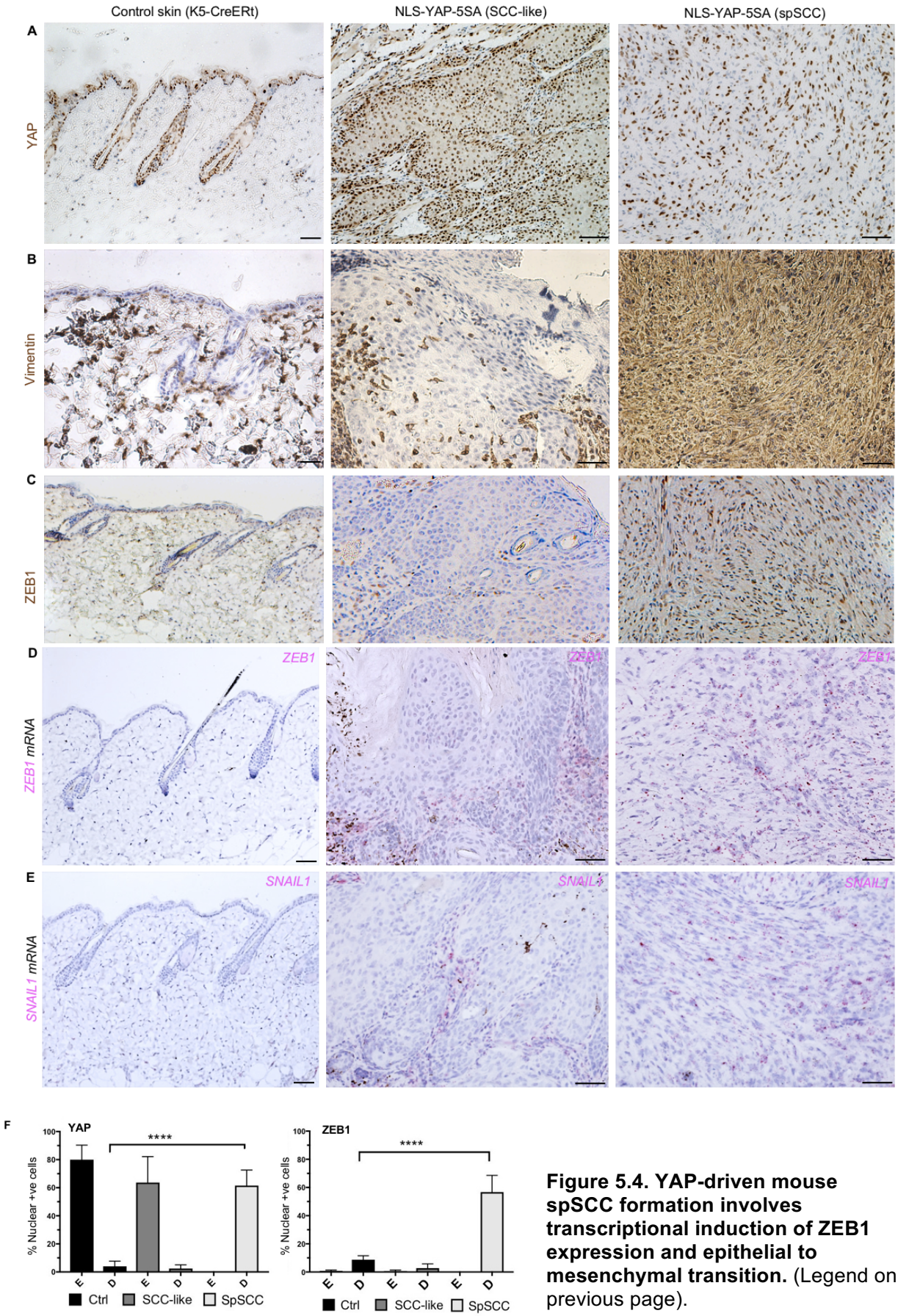


Figure 5.4. YAP-driven mouse spSCC formation involves transcriptional induction of ZEB1 expression and epithelial to mesenchymal transition. (Legend on previous page).

5.4. Human spindle cell squamous cell carcinomas express high levels of nuclear ZEB1

We next sought to verify whether activation of ZEB1 expression also occurs in human spSCC. Human spSCCs co-express high levels of nuclear YAP and ZEB1 with a concurrent gain of Vimentin and loss of E-cadherin (Fig. 5.5; Fig. 5.6). These data imply that in human, as well as in mice, spSCC formation could involve nuclear YAP expression plus ZEB1-mediated EMT.

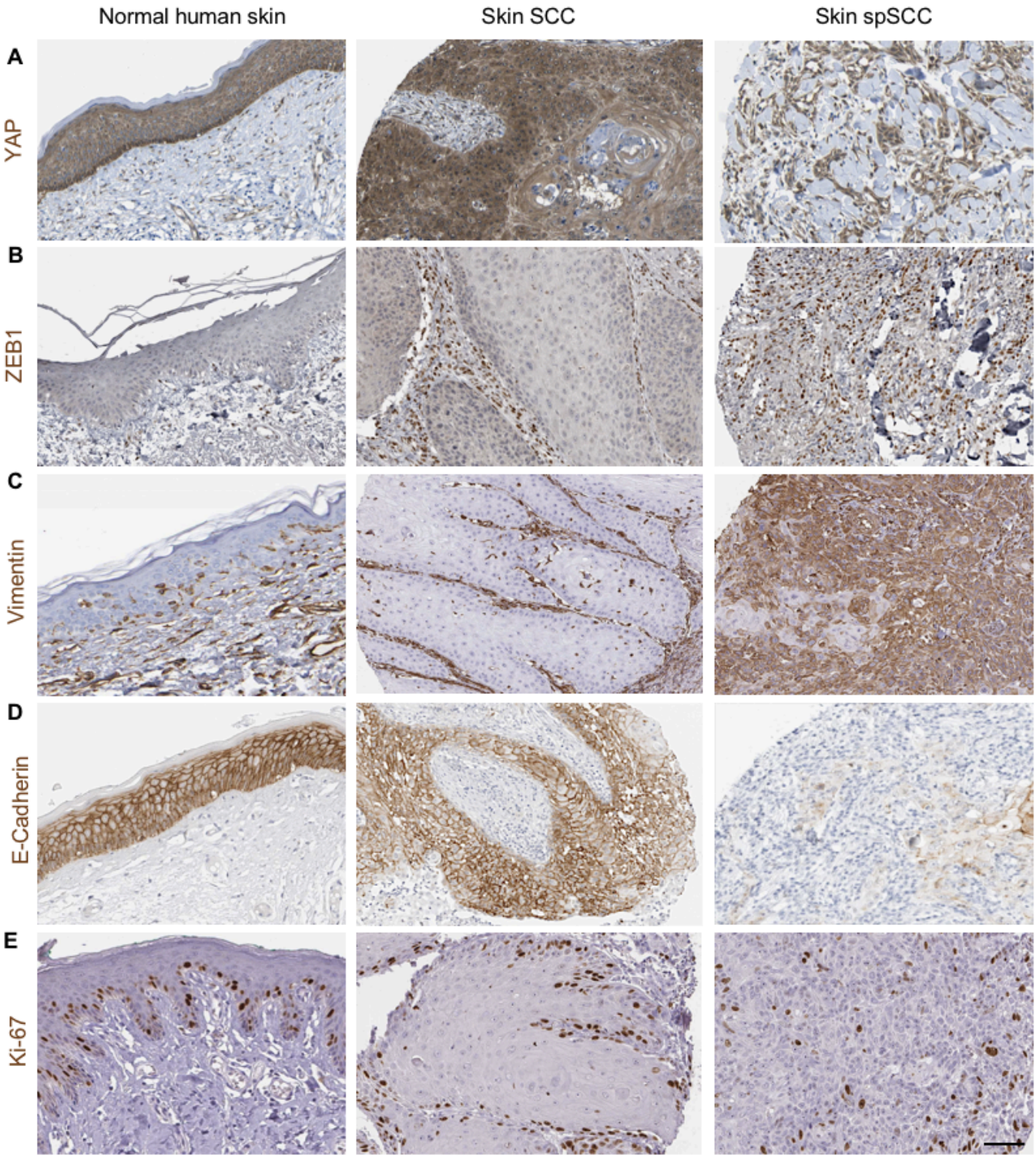


Figure 5.5. YAP and ZEB1 are co-expressed in human spSCC tumours. (**A**) YAP immunostaining of normal human skin, SCC and spSCC-like tumours. Note strong nuclear localisation in spindle-shaped spSCC tumour cells. (**B**) ZEB1 immunostaining of normal human skin, SCC and spSCC-like tumours. Note strong nuclear expression in spindle-shaped spSCC tumour cells. (**C**) Vimentin immunostaining of normal human skin, SCC and spSCC-like tumours. Note strong expression in spindle-shaped spSCC tumour cells. (**D**) E-cadherin immunostaining of normal human skin, SCC and spSCC-like tumours. Note absence of expression in spindle-shaped spSCC tumour cells. (**E**) Ki-67 immunostaining of

normal human skin, SCC and spSCC-like tumours. Scale bars 100 μM. Representative images shown; *n*= 20.

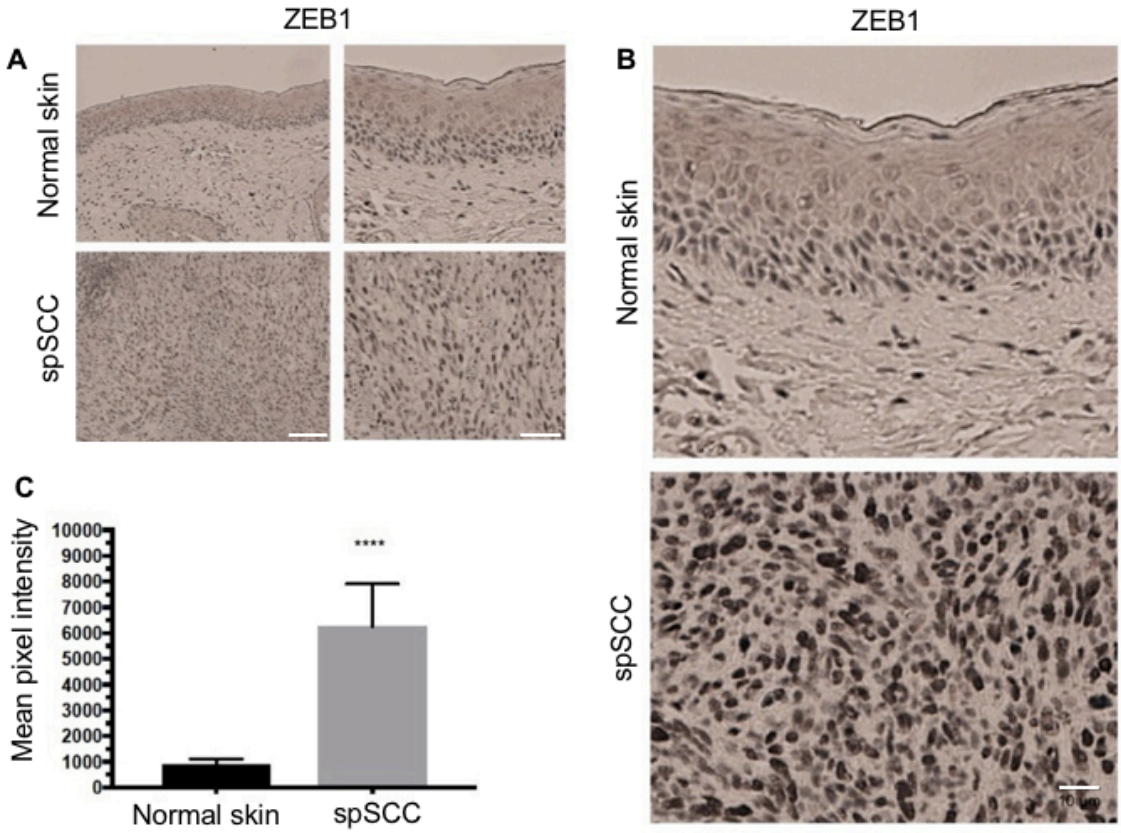


Figure 5.6. Human spSCC tumours express high levels of nuclear ZEB1. (A) Normal human skin and human cutaneous spSCC immunostained for ZEB1. Note widespread nuclear ZEB1 localisation in spSCC. Scale bars: left panels 200 μM, right panels 50 μM. Note widespread nuclear ZEB1 localisation. **(B)** Higher magnification of normal skin and spSCC sections stained for ZEB1. Note in normal skin ZEB1 is nuclear localised in some of the basal layer keratinocytes. In spSCC Yap is nuclear in cells throughout the tumour. Scale bar 10 μM. For clarity only one sample is shown here, see Appendix 7.1 for gallery of spSCC tumour samples stained for ZEB1. **(C)** Quantitation of mean pixel intensity of normal skin versus spSCC. Values are per field of vision means±s.e.m, *n*=22 samples, 3 fields of vision per sample.

5.5. ZEB1 becomes nuclear expressed after epidermal wounding in a YAP and TEAD-dependent manner

Why do some nuclear YAP-driven tumours express ZEB1 and progress to spSCC whilst others do not express ZEB1 and do not progress beyond SCC? As discussed previously, we found spSCCs to be associated with scratch wounds whilst SCCs arose randomly in the absence of wounding (Fig. 5.3C). Following epithelial tissue injury, cells at the wound edge lose their cell-cell adhesions, proliferating and migrating to close the wound. Thus, the wound healing response requires a partial and reversible EMT before reconstitution of epithelial sheet integrity (Arnoux et al. 2005, (Leopold, Vincent et al. 2012, Nieto, Huang et al. 2016). ZEB1 is expressed at the leading edge of healing wounds in various tissues and models (Chaturvedi, Reichert et al. 2017, Li, Lang et al. 2018). Studies in cell culture have shown that knockdown of *ZEB1* hinders closure of a monolayer of cells in a scratch wound healing assay whilst overexpression of *ZEB1* accelerates closure (Joseph, Conroy et al. 2014, Ran, Lin et al. 2015, Gu, Zhao et al. 2016, Orellana-Serradell, Herrera et al. 2018). This suggests that ZEB1 could be a key regulator of the transient EMT that accompanies the wound healing response.

In support of the suggestion that ZEB1 might contribute to wound healing, we found ZEB1 to be expressed in healing epidermal wounds in normal mouse skin (Fig. 5.7A). We previously showed that YAP is also upregulated during the wound healing process (Fig. 3.6A). Both YAP and ZEB1 were nuclear expressed at the leading edge of scratch wounded cultured keratinocyte monolayer (Fig. 5.7B).

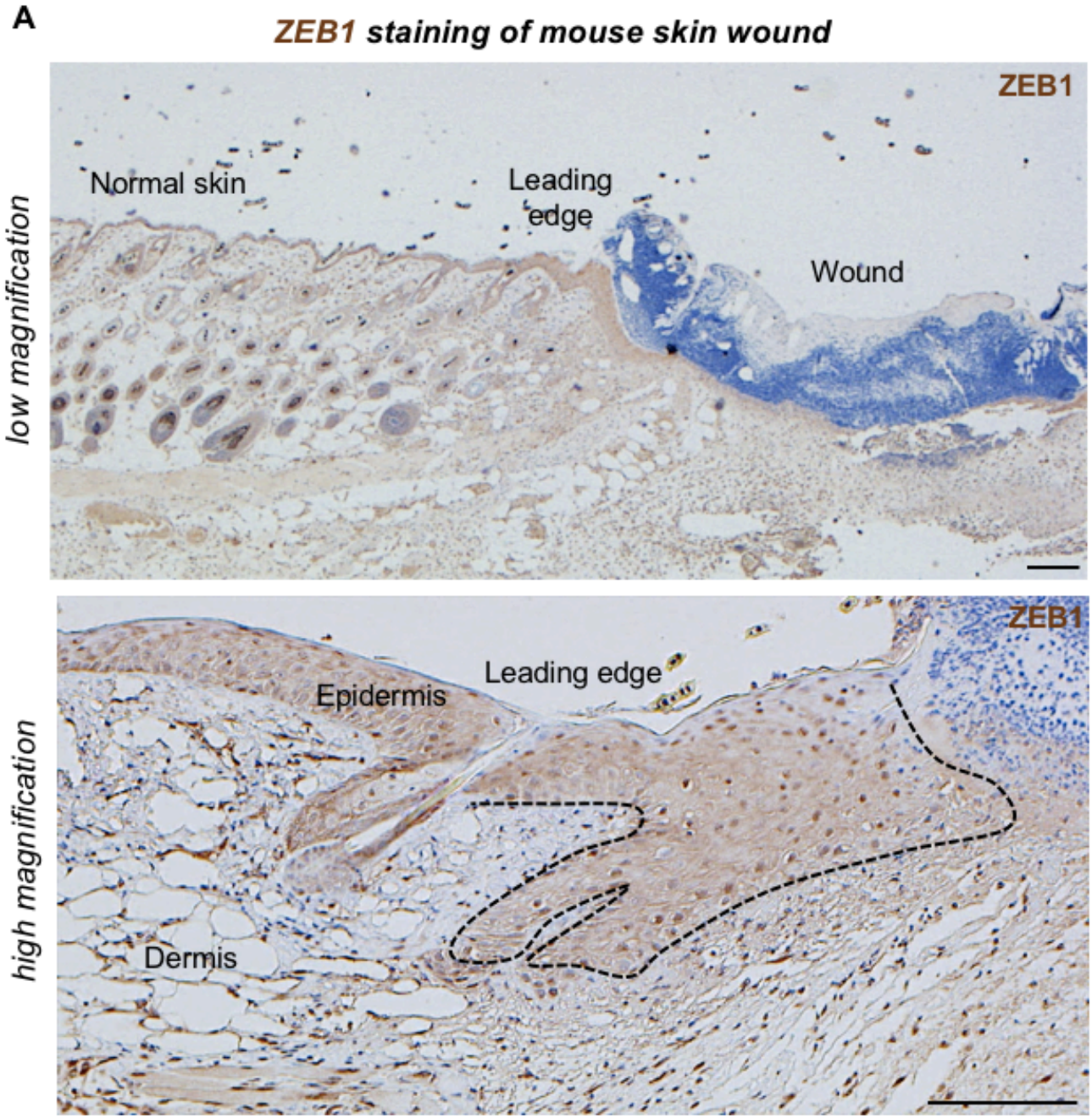


Figure 5.7. ZEB1 becomes nuclear at the leading edge of epidermal wounds. (A) Section of punch wounded of mouse skin immunostained for ZEB1 at lower and higher magnification. Note nuclear expression of ZEB1 in some of the leading edge cells of the healing epidermis. Sections taken 2 days post-wound. Scale bars 100 μM. *n*=8 animals. Fig continued on next page

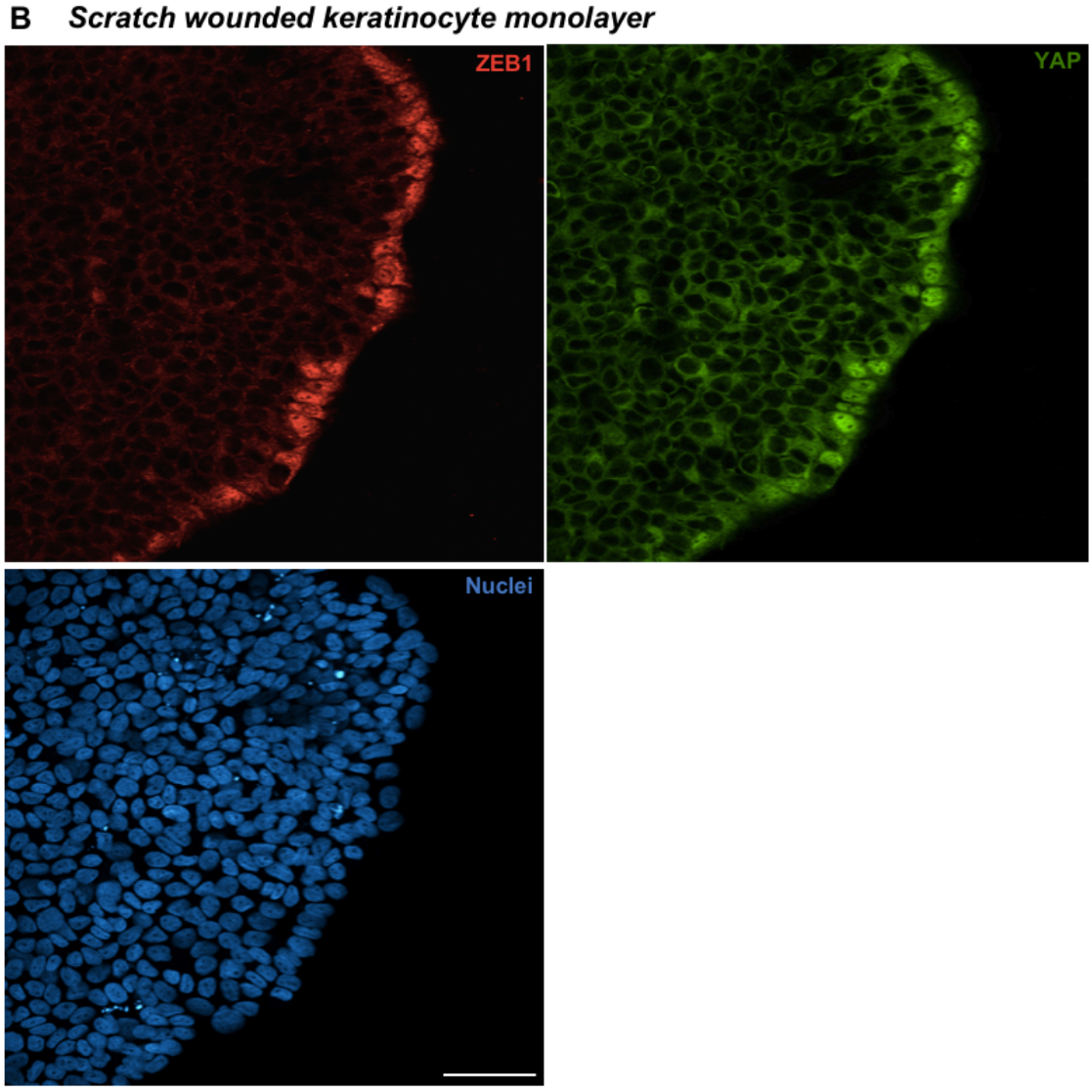


Figure 5.7. (B) Scratch wounding of human HaCaT keratinocytes in culture immunostained for ZEB1, YAP and DAPI. Note ZEB1 and YAP nuclear expression in leading edge cells. Scale bar 50 μM.

We used this system to test whether the induction of ZEB1 expression at the leading edge of healing wounds is YAP-dependent. ZEB1 expression was abolished in scratch-wounded keratinocyte monolayers treated with siRNAs against *YAP/TAZ* (Fig. 5.8A). Treatment of scratch-wounded keratinocyte monolayers with dasatinib, a Src family kinase inhibitor known to inhibit YAP activation (Kim and Gumbiner 2015, Elbediwy, Vincent-Mistiaen et al. 2016, Li, Silvis et al. 2016), similarly abrogated ZEB1 expression at four hours post-wounding (Fig. 5.8B). These results suggest that active YAP is required to maintain epidermal wound-induced ZEB1 expression. SiRNA knockdown of *TEAD1-4* also inhibited ZEB1 expression following scratch wounding, indicating that wound-induced ZEB1 expression depends on YAP-TEAD-mediated transcription (Fig. 5.8C). Chromatin Immunoprecipitation confirmed that TEAD1 binds to an enhancer upstream of *ZEB1* (Fig. 5.8D).

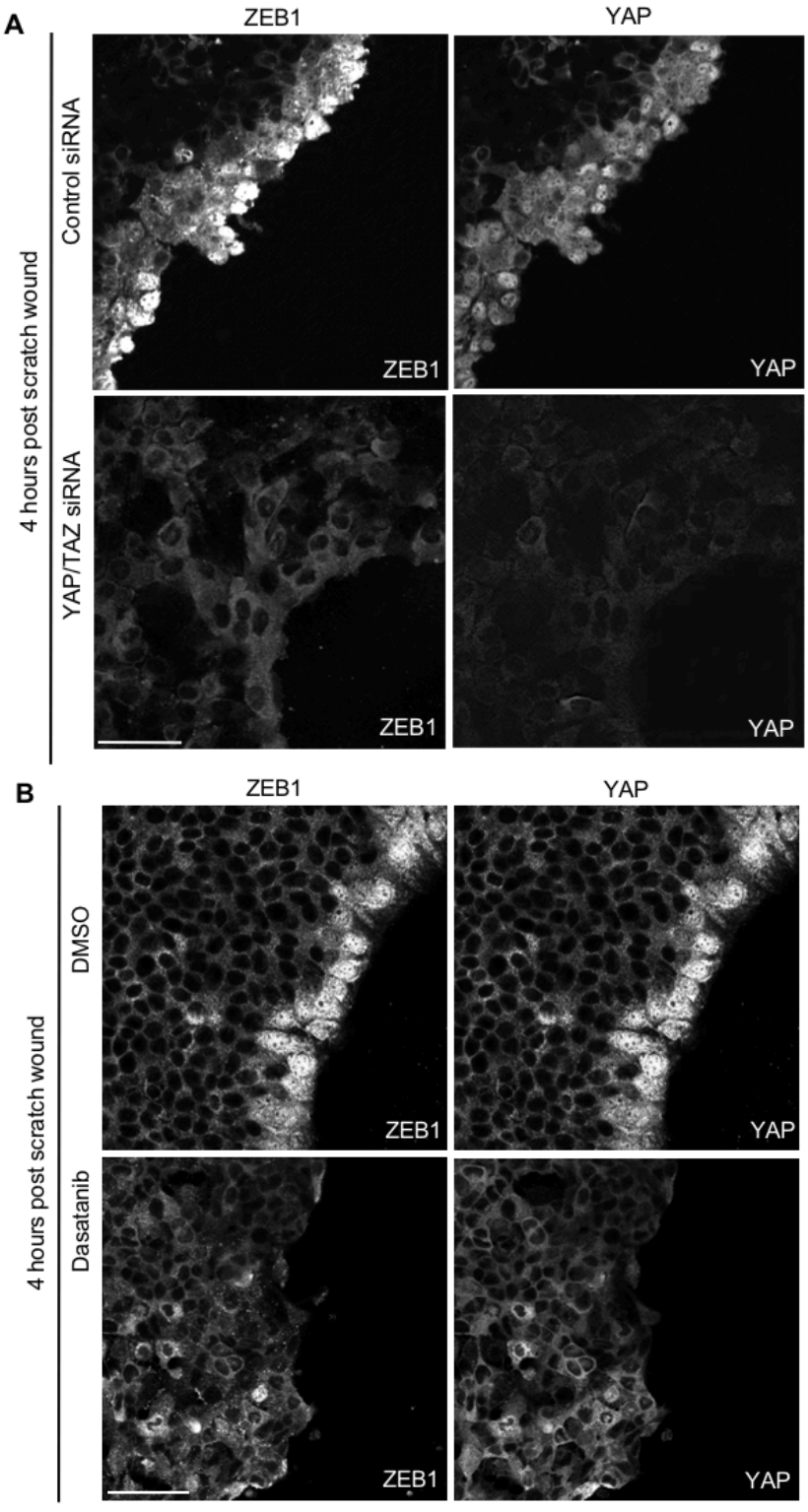


Figure 5.8. Blocking YAP or TEAD1-4 inhibits ZEB1 nuclear localisation after epidermal wounding. (A) Expression of ZEB1 at the leading edge of keratinocyte scratch wound is abolished by transfection with siRNAs against *YAP* and *TAZ*. (**B**) Expression of ZEB1 at the leading edge of keratinocyte scratch wound is abolished by treatment with dasatinib, a Src-family kinase inhibitor that prevents YAP activation. Fig continued on next page.

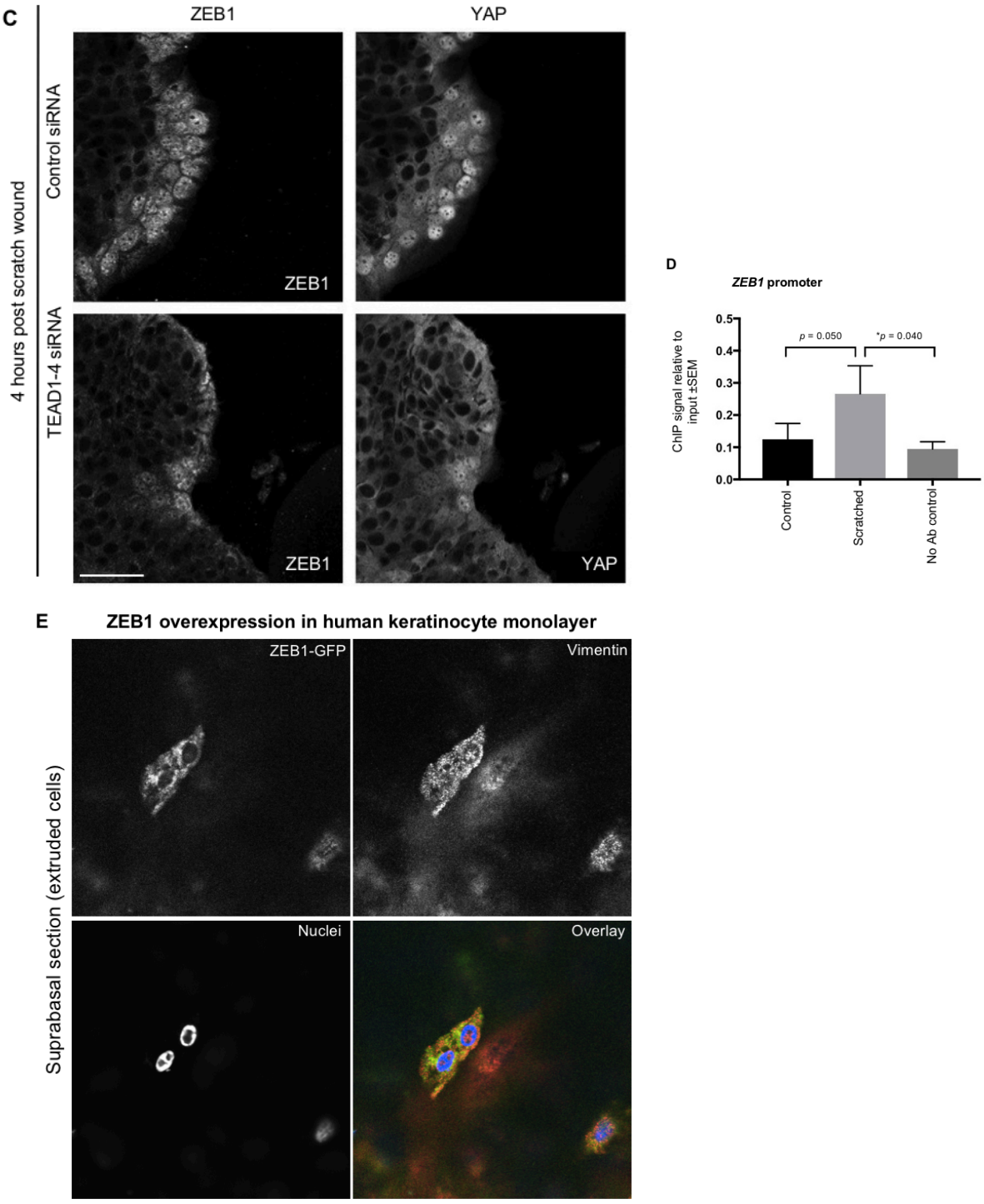


Figure 5.8. (C) Expression of ZEB1 at the leading edge of keratinocyte scratch wound is abolished by transfection with siRNAs against *TEAD1-4*. Scale bars A,B,C 50 μM. All confocal images were taken at the same setting. (A-C) *n*=3 FOV for each of 3 repeats per condition. **(D)** Chromatin Immunoprecipitation of TEAD1 at an upstream enhancer of the *ZEB1* gene in keratinocytes before or after scratch wounding. The weak enrichment may be explained by the small percentage of *ZEB1*-expressing cells in this experiment. Data were analysed by a Mann-Whitney Test *n*= 9 samples per experimental condition. **(E)** Overexpression of ZEB1-GFP in HaCaT cells causes cells to be extruded and assume an fibroblast-like morphology. This work was performed in collaboration with Ahmed Elbediwy

5.6. Summary

In summary, we found that epidermal basal layer cell expression of nuclear YAP resulted in the formation of both SCC and SpSCC. Lineage tracing confirmed that both SCC and SpSCC originate from basal layer cells. Hyperactivation of YAP results in SCC (essentially abnormal hyperproliferation of the epidermis) wherein tumour cells retain their epithelial characteristics. SpSCC formation, however, required hyperactivation of YAP in addition to wound-induced EMT driven by ZEB1.

Chapter 6. Discussion

In this thesis, I have described a physiological role for YAP/TAZ in driving proliferation of epidermal basal layer progenitor cells to maintain skin homeostasis and repair wounds. Nuclear YAP/TAZ levels were increased at the margin of healing wounds, correlating with an increase in proliferation marker Ki-67. This is functionally important since epidermal-specific *YAP/TAZ* double knockout resulted in reduced basal layer proliferation and delayed wound healing. I also confirmed that a set of YAP target genes previously identified in other tissues were expressed in keratinocytes in a YAP-dependent manner: These genes (associated with cell cycle progression, EGFR signalling and integrin-ECM adhesion) were downregulated *in vivo* in *YAP/TAZ* double knockout skin. Having established that YAP is physiologically important in the basal layer of the epidermis, we sought to understand how YAP becomes nuclear in these cells. We show that basal integrin-Src signalling promotes YAP nuclear localisation in basal layer cells. We suggest that YAP activity in progenitor cells is induced by basal contact with the ECM, which is increased upon loss of apicobasal polarity and cell spreading that occurs in both physiological wounding and tumourigenesis (6.1).

During epithelial tissue repair, this upregulation of YAP activity is transient. However, constitutive activation can result in tumour formation and progression: Expression of a constitutively active form of *YAP* in the epidermis resulted in the formation of squamous cell carcinoma (SCC) and the invasive subtype spindle cell SCC (spSCC). The molecular mechanism and cell of origin of the mesenchymal-like spSCC was not well understood. Using lineage tracing we show that YAP-driven spSCCs originate in epidermal basal layer cells and arise at scratch wound sites. We report nuclear expression of EMT transcription factor ZEB1 at the wound margin of wild-type mice and in human spSCC. Furthermore, we show that wound-induced ZEB1 expression depends on YAP-TEAD. Whilst completion of normal tissue repair is accompanied by a return to baseline of YAP, activated YAP could lock wound-induced ZEB1 into a constitutive positive feedback loop. We propose that sustained YAP activation synergises with the wound healing response to drive ZEB1-mediated EMT and malignant progression from SCC to spSCC (Fig. 6.1B, Fig. 6.2).

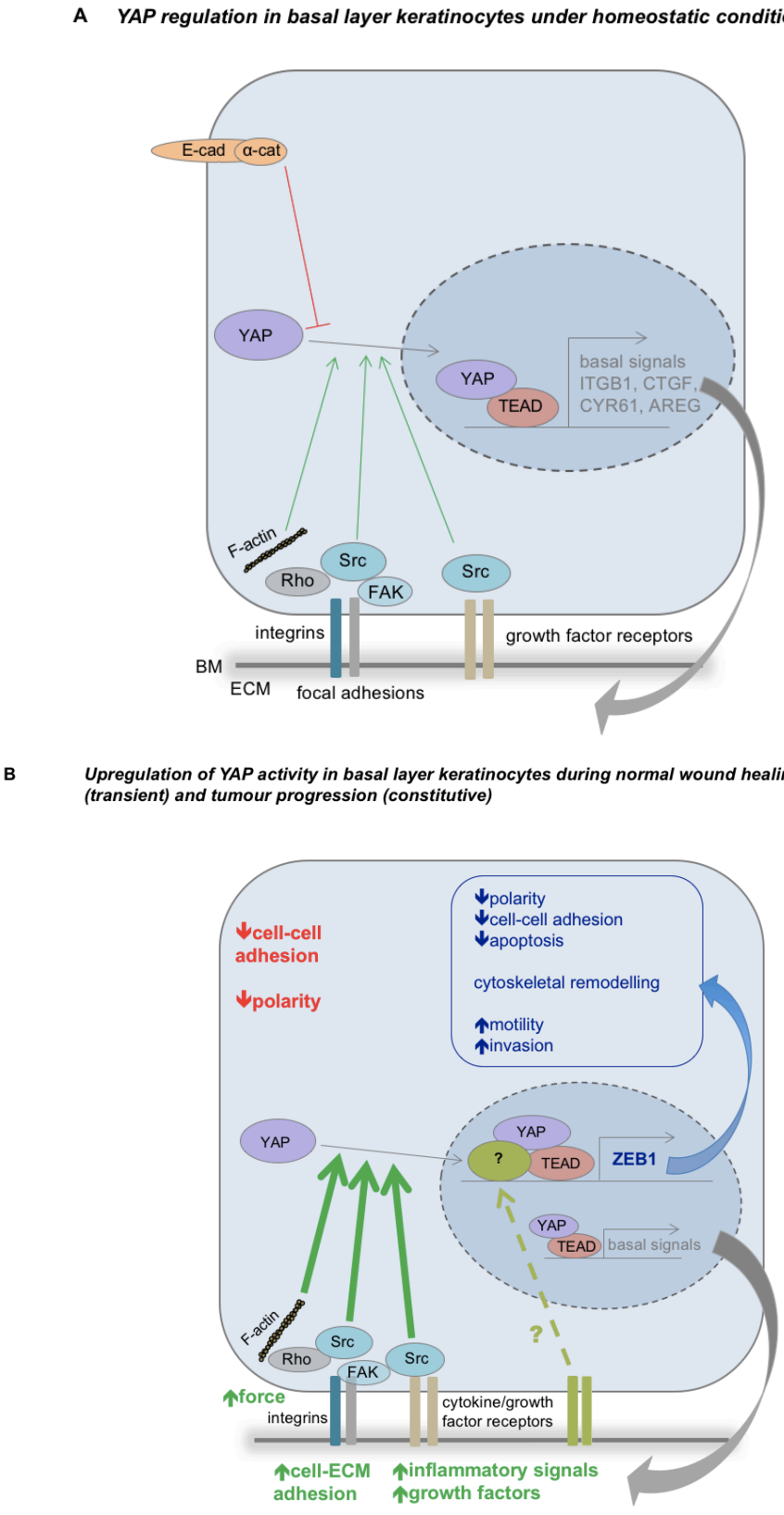


Figure 6.1. Model of YAP regulation in basal layer keratinocytes in normal epidermis and in wound healing/tumourigenesis. (Legend on next page).

Fig 6.1. Model of YAP regulation in basal layer keratinocytes in normal epidermis and in wound healing/tumourigenesis. (A) In uninjured epidermis, basal signals from integrin-Src signalling, actin cytoskeletal tension, and growth factor receptors promote YAP nuclear localisation whilst signals from adherens junctions components inhibit YAP. These inputs can function through multiple LATS-dependent and independent mechanisms. Nuclear YAP binds to TEAD to stimulate the expression of cell cycle genes (*CCNE1, PCNA*) EGFR ligand (*AREG*), integrins (I*TGB1*) and integrin ligands (*CTGF, CYR61*), thereby promoting basal layer cell proliferation and maintaining progenitor cell fate. **(B)** Following epidermal injury, loss of cell-cell adhesion (1) and apicobasal polarity (2) at the wound margin releases negative regulation of YAP by adherens junction contact inhibition and apical Hippo components. In addition, cell spreading over the basal surface increases integrin-Src activation (3) and mechanical strain on the actin cytoskeleton (4), both of which promote YAP nuclear translocation. Increased growth factors and cytokines (5) in the inflammatory wound healing milieu further activate YAP via Src tyrosine phosphorylation. An unidentified wound-induced signal (6) synergises with YAP-TEAD to drive the expression of EMT transcription factor ZEB1 (green in schematic). TGFβ –Smad, MRTF-SRF, Ras-AP1 are potential candidates. During normal wound healing, cessation of inflammatory signals is accompanied by a return to baseline of YAP and ZEB1 levels. However oncogenic mutations and chronic inflammation could YAP and ZEB1 in a positive feedback loop, with sustained activation driving malignant progression, EMT and metastasis. ((6) is discussed further in section 6.3)

6.1. YAP/TAZ play a physiological role in epidermal homeostasis and wound healing

The basal layer progenitor cells are known to endow the epidermis, a stratified squamous epithelium, with the ability for continual renewal required to maintain barrier function. The proliferation rate of these cells must be flexible whilst under tight control to meet the demands of developing skin and heal wounds, without resulting in tumourigenesis. Here we report heterogeneous nuclear localization of YAP/TAZ in adult epidermal basal layer cells, which increased significantly following wounding. YAP was nuclear in almost all foetal and neonatal basal layer cells, likely due to the higher rates of proliferation during epidermal development than in adult homeostasis. Epidermal-specific conditional double knockout (dKO) of *YAP/TAZ* (*K5-CreERt YAP*$^{fl/fl}$ *TAZ*$^{fl/fl}$) in adults resulted in reduced target gene expression accompanied by a reduction in basal layer cell proliferation. Whilst dKO in these adult mice showed slight epidermal thinning and patchy hair loss, they did not develop significant defects. When *YAP/TAZ* were deleted in neonates, these animals failed to develop any hair and were significantly smaller than wildtype, consistent with the higher levels of nuclear YAP and proliferative demand in neonatal compared to adult epidermis. Wound healing was moderately delayed in

dKO adults, partly due to reduced keratinocyte proliferation (Fig. 3.2-3.5). This was corroborated by recent work reporting upregulation of nuclear YAP and its cofactor WBP2 at the margin of epidermal wounds (Walko, Woodhouse et al. 2017). Loss of epidermal WBP2 similarly reduced basal layer proliferation and delayed wound healing. Reduced cell migration in *YAP/TAZ* dKO animals could also contribute to the delayed epidermal repair. Since activation of EGFR can disrupt adherens junctions by phosphorylation of the cadherin-catenin complex, enhanced YAP-dependent expression of EGFR ligand AREG could contribute to loss of cell-cell adhesion required for cell migration in wound closure (Fig. 3.7-8) (Bhora, Dunkin et al. 1995, Hudson and McCawley 1998, Repertinger, Campagnaro et al. 2004). Recent work in other tissues found that YAP promotes epithelial cell migration by a mechanism involving focal adhesion*s,* Rho, and the actin cytoskeleton (Qiao, Chen et al. 2017, Mason, Collins et al. 2019)*.* It would be interesting to explore the relevance of these mechanisms in the epidermis. For example, immunofluorescence could be used to compare stress fibre formation in wild-type and YAP/TAZ dKO healing wounds.

We found that YAP stimulates a transcriptional programme comprising a set of genes involved in cell cycle progression, EGFR signalling and integrin-matrix adhesion. We identified TEAD-specific binding motifs within 1kb of the transcription start sites in the promoters of several target genes identified by RNA-seq, including the cell cycle genes *CCNE1* and *PCNA*, EGFR ligand *AREG*, *integrin β1 and integrin ligands CTGF and CYR61.* These were validated *in vivo*, and were also found to be expressed in the basal layer keratinocytes of human epidermis using the Human Protein Atlas database (Fig. 3.2,3.6,3.7,3.8). Interestingly, a recent study of a psoriasis model found that YAP promoted hyperproliferation of keratinocytes in an AREG-dependent manner (Jia, Li et al. 2018). Our search for TEAD binding motifs, which was limited to those located within 1kb of the transcription start sites, is unlikely to have been comprehensive. In flies, Yorkie primarily binds to the promoter region of target genes and YAP/TAZ-TEAD complexes have been shown to bind to promoter regions (Lian, Kim et al. 2010, Oh, Slattery et al. 2013, Ikmi, Gaertner et al. 2014). However, recent ChIP-seq studies report predominant binding of YAP/TAZ-TEAD to distal enhancer regions in keratinocytes and other cell types (Galli, Carrara et al. 2015, Stein, Bardet et al.

2015, Zanconato, Forcato et al. 2015). Since enhancers tend to be located further than 1kb from transcription start sites, it is likely that further putative target genes could be identified by expanding the region searched for TEAD binding sites. Furthermore, whilst we searched solely for genes downregulated upon loss of *YAP/TAZ* and upregulated upon *YAP* overexpression, there is also evidence of YAP/TAZ acting as a transcriptional co-repressor of genes involved in terminal differentiation (Kim, Kim et al. 2015).

The classical binary model of regulation of YAP/TAZ activity defines on versus off by nuclear versus cytoplasmic subcellular localisation respectively. Recent work argues for a far more dynamic model, with the balance between nuclear import and export determining activity (Shreberk-Shaked and Oren 2019). Thus, a limitation of using immunohistochemistry to define YAP/TAZ activity by nuclear localisation is that only a static snapshot is captured. Furthermore, whilst nuclear localisation of YAP/TAZ is required for their transcriptional activity, it has not been definitively shown that nuclear localisation equals activation. Different phosphorylation states and interactions with co-factors could dictate their ability to bind to TEAD or other transcription factors. This could explain some of the discrepancies between nuclear YAP and proliferation marker Ki-67 (Fig. 3). Despite some uncertainty in whether all keratinocytes expressing nuclear YAP/TAZ are transcriptionally active, I am confident of their physiological relevance in epidermis as wound healing and target gene expression were both reduced in dKO animals.

6.2. YAP is a sensor of polarity and tissue damage

After establishing the function of YAP/TAZ in promoting epidermal basal layer cell proliferation, we sought to understand how their nuclear localization is regulated in these cells. We focused on YAP because TAZ nuclear expression was weaker and less clear, possibly due to antibody quality or more rapid ubiquitin-mediated degradation of TAZ protein (Liu, Zha et al. 2010, Cordenonsi, Zanconato et al. 2011). Why is YAP nuclear in basal cells and cytoplasmic in suprabasal cells? An obvious difference between these cells is whether they make direct contact with the basement membrane. Studies in epithelial breast cells reported induction of YAP

nuclear localization by integrin-Src and EGFR-PI3K signalling (Fan, Kim et al. 2013, Kim and Gumbiner 2015). Furthermore, the phenotype of *YAP/TAZ* dKO epidermis was reminiscent of that of *integrin β1* epidermal deletion (Fig. 3.2-3.5) (Brakebusch, Grose et al. 2000, Raghavan, Bauer et al. 2000, Grose, Hutter et al. 2002, Piwko-Czuchra, Koegel et al. 2009, Singh, Chen et al. 2009). We found that components of integrin-Src and EGFR-PI3K signalling were strongly expressed in the basal layer of human epidermis and that systematic inhibition of these components reduced YAP nuclear localization (Fig 4.1,4.2). Since YAP promotes expression of integrin β1, integrin ligands (CYR61, CTGF) and EGFR ligands (AREG) (Fig. 3.8), it is conceivable that a positive feedback loop exists in which basal signals stimulate YAP nuclear localization, which in turn drives transcription of such signals. Interestingly, ectopic expression of integrin β1 in suprabasal layers resulted in hyperproliferation and defective differentiation of keratinocytes and epidermal inflammation (Carroll, Romero et al. 1995). It would be interesting to determine if ectopic expression of integrin β1 triggers YAP nuclear localisation in these suprabasal keratinocytes. Genetic knockdown of Src or FAK or chemical inhibition of Src with dasatinib resulted in a significant decrease in nuclear YAP and YAP protein levels in normal and inflamed epidermis, as well as in skin papillomas and SCCs. These data suggest that Src signalling promotes YAP nuclear localization *in vivo*, and highlights the therapeutic potential of dasatinib in cutaneous tumours or inflammatory disorders (Fig. 4.3). In line with this, several recent studies report integrin-Src-mediated YAP activation various cell lines (Ames, Contois et al. 2016, Sabra, Brunner et al. 2017, Si, Ji et al. 2017, Ando, Charindra et al. 2018, Cui, Morales et al. 2018). YAP subcellular localization could be examined in *integrin β1* knockout epidermis with and without Dasatinib treatment to extend these findings *in vivo*.

We found an increase in pYAP-S127 upon inhibition of integrin β1, FAK or Src suggesting that integrin-Src signalling negatively regulates LATS1/2 to induce YAP nuclear localization (Fig. 4.2). Consistent with this, Src has been shown to activate YAP by inhibitory phosphorylation of LATS1/2 (Kim and Gumbiner 2015, Ames, Contois et al. 2016, Fisher, Kerr et al. 2016, Si, Ji et al. 2017, Ando, Charindra et al. 2018). Src has also been reported to activate YAP by direct phosphorylation of tyrosine residues (Y341/357/394) in the transactivation domain, which hinders YAP

binding to 14-3-3 proteins and promotes its binding to TEAD (Taniguchi, Wu et al. 2015, Vlahov, Scrace et al. 2015, Li, Silvis et al. 2016, Smoot, Werneburg et al. 2018). The decrease in YAP protein levels observed in epidermis lacking Src or FAK could be due to reduced rate of pYAP-Y357 proteasomal degradation seen in cultured cells (Levy, Adamovich et al. 2008, Shanzer, Adler et al. 2017). These studies suggest that Src can promote YAP nuclear localization in multiple tissues via LATS1/2-dependent and independent pathways. Li et al reported widespread phosphorylation of Y341/357/394 in endogenous YAP in human and mouse SCC (Li, Silvis et al. 2016). Furthermore, Src family kinase (SFK) inhibition significantly reduced SCC growth, in line with our observation of dasatinib treatment abolishing nuclear YAP in mouse SCC (Fig. 4.3). To ascertain the relative importance of these pathways in the epidermis, future work could examine YAP nuclear localization upon topical dasatinib treatment of transgenic mice expressing YAP5SA (resistant to LATS1/2 phosphorylation) or YAP3YF (resistant to SFK phosphorylation). Although the mechanisms by which tyrosine phosphorylation drives YAP nuclear localization is not fully understood, recent work point to the contribution of interaction with TEADs or SWI/SNF in the nucleus or the rate of nuclear export (Zhu, Li et al. 2015, Song, Herranz et al. 2017, Ege, Dowbaj et al. 2018).

Src activation by other receptors, besides integrins, has recently been reported to induce YAP nuclear localisation. For example, platelet derived growth factor receptor and the interleukin-6 (IL-6) co-receptor gp130 – both of which can be activated in response to inflammation – were shown to activate Src upstream of YAP activation. YAP activation by the IL-6-gp130 axis was required for regeneration of injured intestine in mice (Taniguchi, Wu et al. 2015, Smoot, Werneburg et al. 2018). Consistently, we observed a Src-dependent increase in YAP levels and nuclear localization upon TPA-induced inflammation of the epidermis (Fig. 4.3). Thus, Src activation of YAP could link inflammatory cues to a transcriptional response required for epithelial tissue repair. It would be interesting to test whether dasatinib treatment abrogates the elevated nuclear YAP and keratinocyte proliferation at the margin of healing epidermal wounds. Notably, the inflammatory pathways that activate Src-YAP in epithelial regeneration could be recapitulated in tumourigenesis. It is thought that YAP hyperactivity observed in many human tumours is due to pro-oncogenic and stromal inflammatory cues as

YAP mutations are rarely seen. Therefore, inhibition of YAP activators such as Src provides an attractive therapeutic avenue. Moreover, several FDA-approved Src inhibiting drugs already exist (Finn 2008, Kim, Song et al. 2009, Elias and Ditzel 2015).

In the above model of YAP regulation by basal ECM-integrin-Src signalling would induce YAP nuclear localization solely in the basal layer keratinocytes. In this way, progenitor cell specification and cell proliferation would depend on contact with the basement membrane. Nuclear YAP would be maintained in these cells by an integrin-Src-YAP-growth factor positive feedback loop. This loop would be broken when daughter cells lose contact with the basement membrane, thus creating a bistable system for determining progenitor versus differentiated cell fate. Cytokine signalling or mechanotransduction upon cell spreading induced by wounding would further increase nuclear YAP levels (Dupont, 2011). In this way YAP could act as a sensor for tissue damage.

This model appears to apply to other types of stratified squamous epithelia (Fig. 4.1). It is important to note however, that in columnar epithelia, YAP is cytoplasmic in differentiated cells that contact the basement membrane. Unlike squamous epithelia, columnar epithelia express apical domain components (CRB3, NF2, SAV1, KIBRA, EZRIN), which appear to override basal signals and result in cytoplasmic sequestration of YAP, ostensibly via MST/LATS kinase recruitment and phosphorylation at the apical membrane (Fig 4.4) (Hamaratoglu, Willecke et al. 2006, Baumgartner, Poernbacher et al. 2010, Chen, Gajewski et al. 2010, Genevet, Wehr et al. 2010, Ling, Zheng et al. 2010, Varelas, Samavarchi-Tehrani et al. 2010, Yu, Zheng et al. 2010, Yin, Yu et al. 2013, Fletcher, Elbediwy et al. 2015, Sun, Reddy et al. 2015, Szymaniak, Mahoney et al. 2015). In simple columnar epithelia, such as gallbladder or endometrium, in which all cells contact the basement membrane and feature an apical domain, YAP is cytoplasmic. In pseudostratified columnar epithelia, like lung bronchus or salivary gland, YAP is cytoplasmic in cells that possess a well-defined apical domain, whilst basal layer progenitor cells lack an apical domain and retain nuclear YAP (Fig. 4.4). A recent study in the mouse lung epithelium found that CRB3 was required for LATS1/2-dependent cytoplasmic retention of YAP in differentiated cells whilst progenitor/stem cells lacked CRB3

and featured nuclear YAP (Szymaniak, Mahoney et al. 2015). We found that disrupting the apical domain of confluent columnar intestinal cells in culture drove YAP from the cytoplasm to the nucleus in a LATS-dependent manner (Fig. 4.6), highlighting the opposing influences of the apical and basal domains. Interestingly, inhibition of integrins or Src in these cells triggered relocalisation of YAP to the cytoplasm. This suggests that when the apical domain in columnar cells is disrupted, basal integrin-Src signalling promotes YAP nuclear localization. Thus, in columnar cells that lose apicobasal polarity – such as in cancer – YAP "behaves" as it does in stem cells. In line with this, we found that when columnar epithelial tumours lose their apical domain and invade into the surrounding stroma, YAP becomes nuclear. Dasatinib treatment significantly reduced nuclear YAP and tumour invasion, suggesting that Src activation of YAP is important in these malignancies (Fig. 4.11). Loss of Hippo signalling upon polarity disruption, accompanied by increased contact with the surrounding ECM and consequent integrin-Src signalling could contribute to YAP activation and tumour progression. This conclusion is developed in a paper that I co-first authored with Ahmed Elbediwy, a post-doctoral fellow in the lab (Elbediwy, Vincent-Mistiaen et al. 2016).

In summary, our results suggest that integrin-Src signalling induced by contact with the basement membrane extracellular matrix promotes YAP nuclear localisation and progenitor cell fate. Conversely, YAP nuclear localization is inhibited by Hippo signalling arising from the apical surface. When both signals are present, apical signalling dominates over basal signalling to drive cell differentiation. Loss of apicobasal polarity, changes in cell morphology, ECM remodelling, inflammatory signals and crosstalk with other signalling pathways could activate YAP in the context of epithelial tissue repair or tumourigenesis.

6.3. Sustained YAP activation synergises with the wound healing response to drive ZEB1-mediated EMT and spindle cell squamous carcinoma formation

Nuclear YAP expression has been reported in murine and human squamous cell carcinomas (SCC), a type of epithelial cancer arising from stratified squamous epithelia of the body such as the skin, oesophagus or cervix (Schlegelmilch 2011

(Muramatsu, Imoto et al. 2011, Schlegelmilch, Mohseni et al. 2011, Silvis, Kreger et al. 2011, Liu, Liu et al. 2013, Xiao, Wu et al. 2014, He, Mao et al. 2015, Song, Honjo et al. 2015, Zanconato, Forcato et al. 2015, Jia, Li et al. 2016). Overexpression of *YAP* or deletion of negative regulator *α-catenin* in basal layer keratinocytes results in cutaneous SCC formation (Vasioukhin (Vasioukhin, Bauer et al. 2001, Kobielak and Fuchs 2006, Schlegelmilch, Mohseni et al. 2011, Silvis, Kreger et al. 2011). Furthermore, epidermal *YAP/TAZ* double knockout blocks formation of Ras-driven papilloma and SCC (Zanconato (Zanconato, Forcato et al. 2015, Jia, Li et al. 2016). These findings highlight the contribution of YAP to SCC formation. The majority of cutaneous SCCs are well-differentiated with limited invasive potential, however malignant progression to an aggressive subtype known as spindle cell SCC (spSCC) can lead to metastasis. SpSCC has a sarcomatoid appearance and is characterized by atypical spindle-shaped cells that infiltrate into the dermis. These spindled cells morphologically and functionally resemble mesenchymal fibroblasts of malignant fibrosarcomas, suggesting that formation of spSCC involves epithelial-mesenchymal transition (EMT) (Thiery, Acloque et al. 2009, Viswanathan, Rahman et al. 2010, Watson, Chernock et al. 2013, Bavle, Govinda et al. 2016, Nieto, Huang et al. 2016, Brabletz, Kalluri et al. 2018). However, the molecular mechanism underlying this EMT is not understood. Why do only some SCCs progress to spSCC?

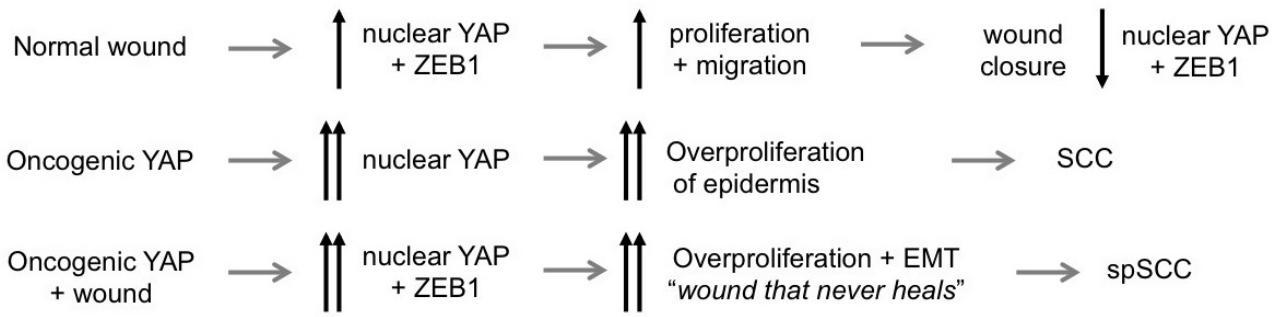
We found that YAP was strongly nuclear throughout human spSCCs, which lacked Keratin-5 (K5) expression, suggesting that YAP could be involved in the progression to spSCC (Fig. 5.1-2). K5-Cre-driven overexpression of activated YAP (NLS-YAP-5SA) resulted in rapid formation of both K5-positive SCC and K5-negative spSCC in mice. Lineage tracing confirmed that the mesenchymal spindled cells invading the dermis derived from NLS-YAP-5SA transgene-expressing basal layer epidermal cells. Interestingly, spSCCs arose at grooming (scratch wound) sites, whilst SCCs formed at unwounded skin (Fig. 5.3). This suggests that whilst YAP-driven SCC formation represents an exaggerated programme of normal basal layer proliferation, sustained YAP activation under conditions of tissue damage can promote metaplastic transformation of K5-positive epidermal epithelial cells into K5-negative mesenchymal spindle cells of spSCC. In

YAP-driven spSCCs, loss of K5 expression was accompanied by loss of E-cadherin and upregulation of Vimentin (Fig. 5.4)

To explore the mechanism of metaplastic transformation during oncogenic YAP-driven spSCC formation, we examined the expression of classical EMT markers (Fig. 5.4) (Yang and Weinberg 2008). A defining feature of EMT is the downregulation of epithelial markers such E-cadherin or Keratins and the upregulation of Vimentin. In nuclear YAP-driven tumours, K5 expression is inactivated and Vimentin expression is strongly induced upon transformation of SCC to spSCC following wounding. We found that the EMT transcription factor ZEB1 was not expressed in normal skin but was strongly expressed at the mRNA and protein level in spSCC (Fig. 5.4). Epithelial tissue repair is known to involve a reversible EMT, in which cells at the wound margin lose their cell-cell adhesions, proliferate and migrate to close the wound, before redifferentiating to restore the epithelial sheet integrity (Barriere, Fici et al. 2015, Stone, Pastar et al. 2016). ZEB1 has been reported to become expressed at the wound edge and accelerate closure *in vitro,* partly by downregulation of E-cadherin and consequent loss of cell adhesion (Kuwahara, Hatoko et al. 2001, Joseph, Conroy et al. 2014, Ran, Lin et al. 2015, Gu, Zhao et al. 2016, Chaturvedi, Reichert et al. 2017, Li, Lang et al. 2018, Orellana-Serradell, Herrera et al. 2018). We show that ZEB1 is expressed during the wound healing response in mouse epidermis and in cultured keratinocytes, suggesting that ZEB1 could be a regulator of the transient EMT induced following epithelial tissue damage. Knockdown of *YAP* or *TEAD1-4* in scratch-wounded keratinocytes prevented ZEB1 expression. Furthermore, we detected TEAD1 binding to an upstream enhancer at the *ZEB1* locus upon scratch wounding (Fig. 5.7-8). Taken together, these data suggest that wound-induced ZEB1 expression is dependent on YAP-TEAD transcription. In future work, this could be verified *in vivo* by examining whether ZEB1 is expressed in *YAP/TAZ* dKO skin wounds.

Significantly, the normal wound healing response is self-limiting, with cessation of inflammatory signals upon wound resolution accompanied by a return of YAP and ZEB1 to baseline levels. Conversely, sustained ZEB1 expression in the presence of NLS-YAP-5SA drives EMT and spSCC formation. ZEB1 becomes co-expressed

with YAP concurrent with a loss of E-cadherin and gain of Vimentin expression in human spSCC tumours, suggesting that this mechanism could be relevant in humans (Fig. 5.5-6). However, a limitation of this study is that exogenous overexpression of nuclear activated YAP may not accurately reflect the pathogenesis of human spSCC. As described in Vincent-Mistiaen et al, we propose that wound-induced ZEB1 expression synergises with aberrant activation of YAP by other oncogenic signals to form a positive-feedback loop driving tumour progression (Fig. 6.2) (Vincent-Mistiaen, Elbediwy et al. 2018). It would be interesting to test whether deletion of ZEB1 in NLS-YAP-5SA mice blocks spSCC formation (Brabletz, Lasierra Losada et al. 2017).



Figue 6.2. Sustained YAP activation synergises with wound-induced ZEB1 to drive EMT and tumour progression.

Aberrant ZEB1 expression has been reported in multiple human cancers of epithelial origin, and is associated with loss of E-cadherin and metastasis (Eger, Aigner et al. 2005, Spoelstra, Manning et al. 2006, Wellner, Brabletz et al. 2010, Jia, Liu et al. 2012, Zhang, Zhou et al. 2013, Zhang, Lu et al. 2013, Bronsert, Kohler et al. 2014, Matsubara, Kishaba et al. 2014). In addition to E-cadherin, several epithelial polarity genes – including CRB3 and Lgl2 – directly repressed by ZEB1 are known to be negative regulators of YAP (Aigner, Dampier et al. 2007). Furthermore, ZEB1 has been reported to induce the expression of mesenchymal markers and matrix metalloproteinases, driving further loss of polarity, ECM stiffening, and increase surface area of cell-ECM contact. Thus, whilst YAP promotes ZEB1 expression, downstream effects of ZEB1 activity on polarity, cell-cell adhesion and ECM feeds back onto YAP activity (Fig. 6.3).

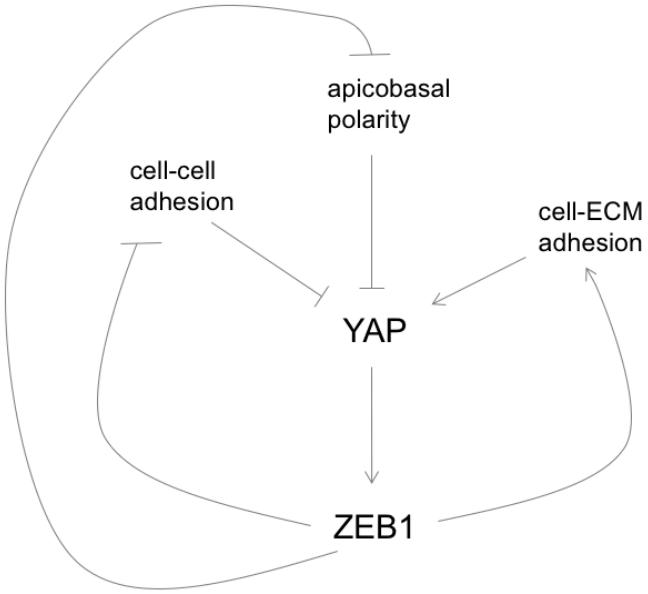


Figure 6.3. Model of YAP-ZEB1 positive feedback loop. Following epithelial injury, YAP induces ZEB1 expression. ZEB1-mediated repression of epithelial genes such as E-cadherin, CRB3 and Lgl2 would promote YAP activity. Activation of mesenchymal genes such Vimentin and N-cadherin as well as matrix metalloproteinases by ZEB1 would promote a permissive microenvironment for further YAP activation.

Interestingly, recent studies in cell culture report the direct interaction of ZEB1 and YAP to cooperatively drive a common set of target genes. Expression of this common YAP/ZEB1 target gene set in human breast cancer correlated with increased metastasis, therapy resistance and poor prognosis (Lehmann, Mossmann et al. 2016, Qiu, Wei et al. 2018, Kowalski-Chauvel, Gouaze-Andersson et al. 2019). This could explain why overexpression of YAP alone is usually not sufficient to induce malignant neoplasm formation, requiring a second hit from wound-induced ZEB1 expression.

How the wound-healing response synergises with YAP to induce ZEB1 transcription remains unclear. A preliminary future study to test the importance of inflammation in YAP-mediated transcription of ZEB1 could involve treating experimental mice with an anti-inflammatory and then observing whether these mice express ZEB1 and develop SpSCCs.

More specifically, binding sites for the effectors of various other wound-induced signalling pathways (TGFβ-SMAD, MRTF-SRF, Ras-AP1) have been found in the *ZEB1* regulatory sequence (Davies, Robinson et al. 2005, Yang and Weinberg 2008, Rasanen and Vaheri 2010, Bakiri, Macho-Maschler et al. 2015, David, Huang et al. 2016, Brabletz, Kalluri et al. 2018, Gasparics and Sebe 2018). YAP has been reported to form complexes with SMADS, SRF and AP1 in various cell types (Kim, Yang et al. 2015, Zanconato, Forcato et al. 2015, Kim, Jang et al. 2018). Thus, activation of these pathways in parallel with YAP activation could result in cooperative induction of ZEB1 transcription (Fig. 6.1). Also, inflammatory cytokines such as IL-6 have been reported to activate YAP by Src-mediated tyrosine phosphorylation of the YAP transactivation domain. This might potentiate YAP-TEAD binding to the *ZEB1* enhancer or interaction with other co-factors. A tyrosine phosphorylation-resistant form of YAP (YAP-3YF) could be used to test the relevance of this tyrosine phosphorylation for ZEB1 expression in a keratinocyte scratch assay.

6.4. Conclusion

In conclusion, YAP functions as a nexus integrating a multitude of biochemical and mechanical inputs to elicit cell and context-dependent transcriptional outputs. In basal layer progenitor cells of the epidermis YAP acts as a senor of polarity, with basal integrin-Src signalling upon contact with the basement membrane promoting YAP nuclear localization and cell proliferation to maintain epidermal homeostasis. YAP also acts as a sensor of tissue architecture, allowing for rapid reconstitution of barrier function following epithelial damage. Loss of apicobasal polarity and cell-cell adhesion, inflammation, increased cell-ECM contact and mechanical tension upon wounding would all be expected to induce YAP nuclear localization and activity. Importantly, all of these inputs are implicated in cancer. We propose that signals induced by tissue damage/inflammation synergise with YAP to induce transient ZEB1 expression and EMT necessary for tissue repair. Wound resolution is normally accompanied by downregulation of YAP and ZEB1, whereas constitutive activation results in a feed-forward loop and malignant progression (Fig. 6.1). We identify YAP as a driver of both cutaneous SCC and spSCC, originating in the basal keratinocytes of the epidermis. Sustained nuclear YAP results in abnormal acceleration of physiological epidermal proliferation and SCC formation, wherein tumour cells retain their epithelial attributes. In contrast, SpSCCs represent an alliance of sustained YAP activation and wound-induced ZEB1 EMT. Thus, YAP provides an important link between inflammation and tissue repair mechanisms that can be hijacked by cancer. In this thesis, I have begun to provide a molecular framework for why spSCC is frequently associated with chronic skin injury, infection and inflammation. My work suggests that aberrant YAP activation and sustained ZEB1 expression could be induced and maintained by oncogenic mutations and inflammation caused by chronic exposure to UV radiation.

Chapter 7. Appendix

7.1. Supplementary YAP and ZEB1 immunostaining in human spSCCs.

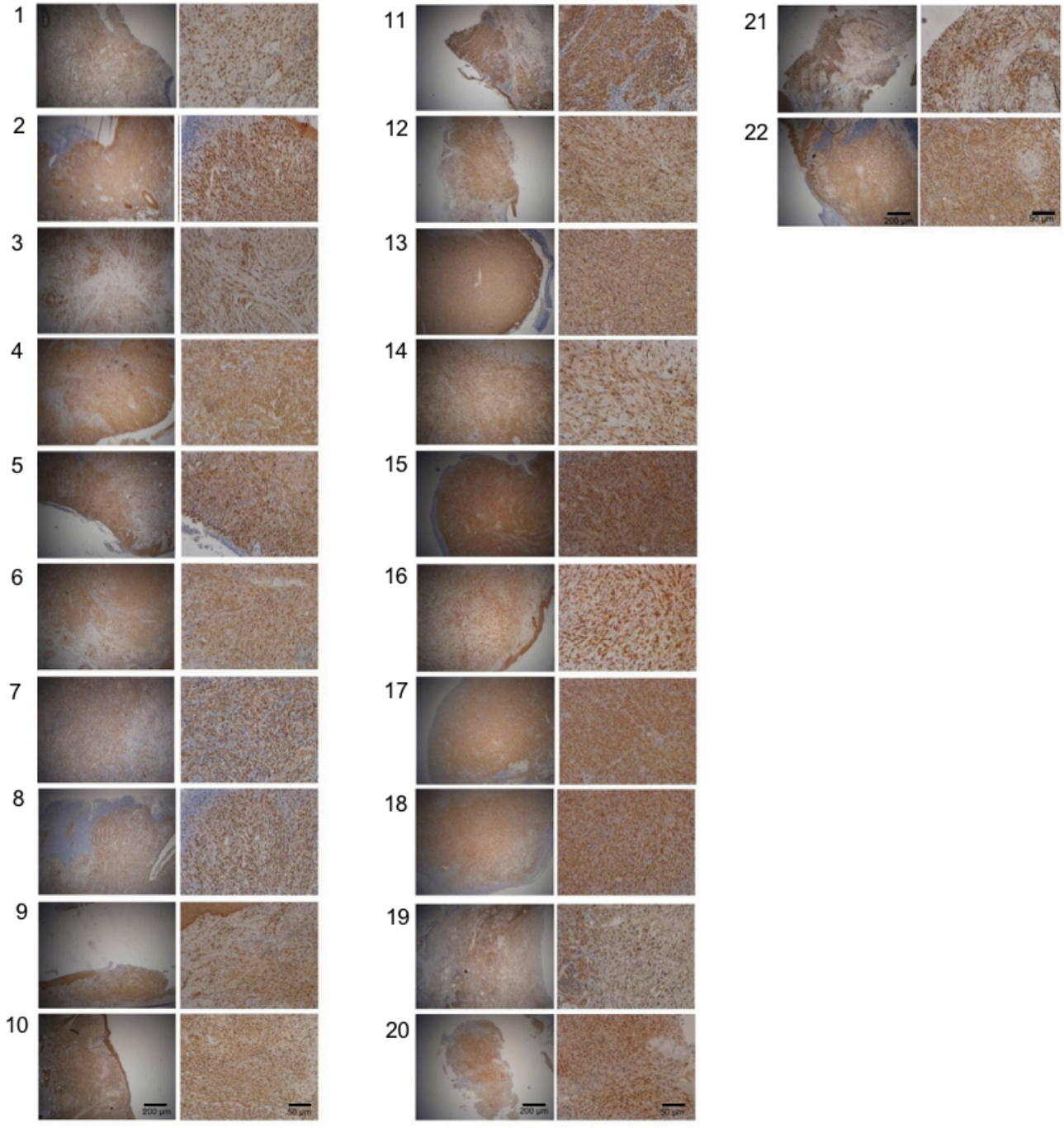


Figure 5.2 Supplementary YAP human spSCC. Panel of human cutaneous SpSCC tumours stained for YAP. Samples are labelled 1-22 and shown at two magnifications. Scale bars: left panels 200 μM; right panels 50 μM.

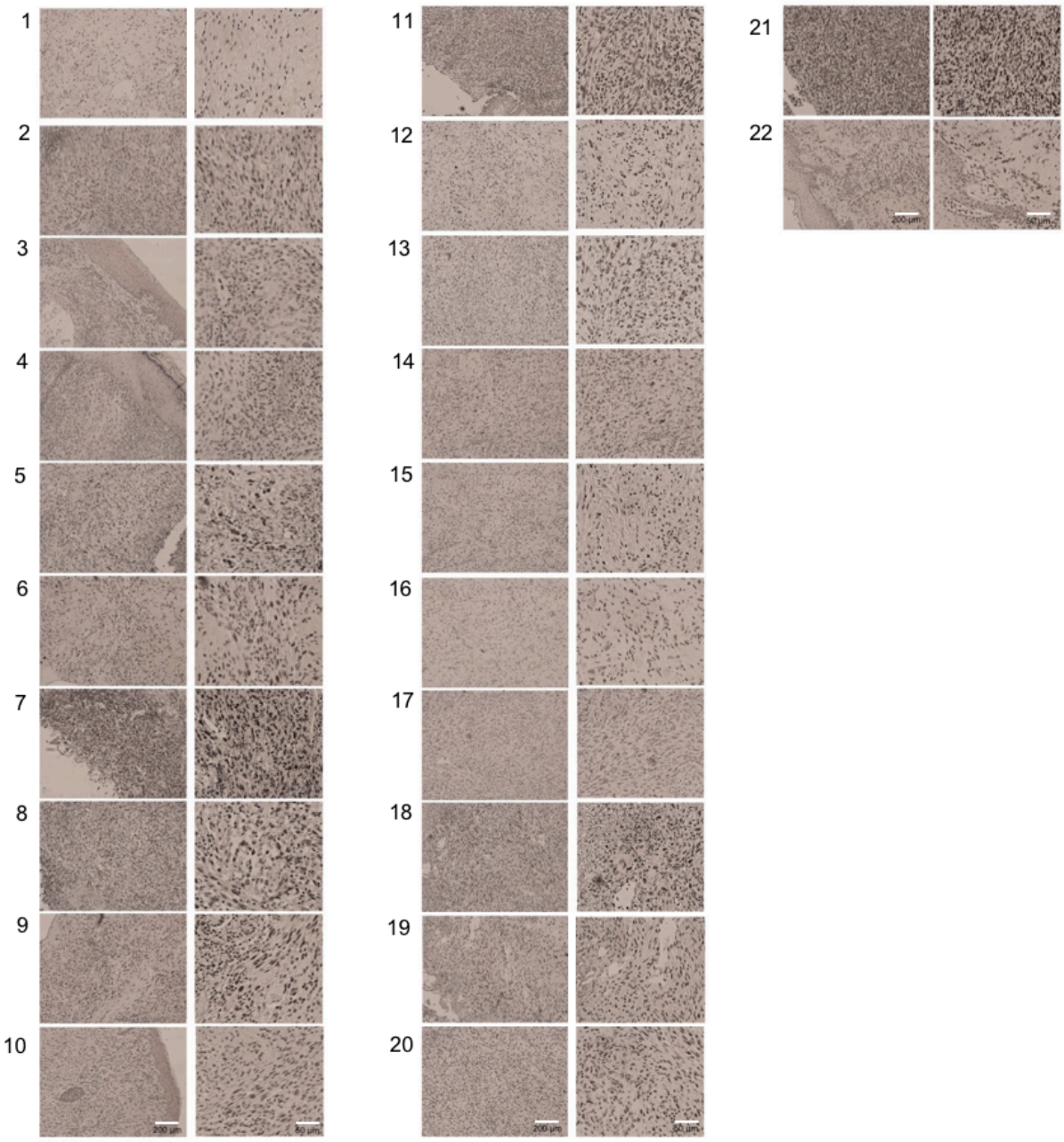


Figure 5.6 Supplementary human ZEB1 spSCC. Panel of human cutaneous SpSCC tumours stained for ZEB1. Samples are labelled 1-22 and shown at two magnifications. Scale bars: left panels 200 μM; right panels 50 μM.

7.2. Raw data from Chapter 3 Figure. 3.7C.

TEAD1 binding on transcript strand

ensembl_gene_id	hgnc_symbo	strar	chromosome_name	transcription_start_site	TEAD1_motif_position_relative_to	TEAD1_motif_candidate_sequence	TEAD1_motif_candidate_position
ENSG00000109321	AREG	1	4	74445134			
ENSG00000109321	AREG	1	4	74445302			
ENSG00000109321	AREG	1	4	74448639	18:29	AATATTCCTGGG	4:74448656-74448667
ENSG00000142920	AZIN2	1	1	33081104			
ENSG00000142920	AZIN2	1	1	33081113			
ENSG00000142920	AZIN2	1	1	33081148			
ENSG00000142920	AZIN2	1	1	33081150			
ENSG00000142920	AZIN2	1	1	33081155			
ENSG00000142920	AZIN2	1	1	33081161			
ENSG00000142920	AZIN2	1	1	33081163			
ENSG00000142920	AZIN2	1	1	33081164			
ENSG00000142920	AZIN2	1	1	33081165			
ENSG00000142920	AZIN2	1	1	33081167			
ENSG00000142920	AZIN2	1	1	33081169			
ENSG00000142920	AZIN2	1	1	33081176			
ENSG00000142920	AZIN2	1	1	33081659			
ENSG00000142920	AZIN2	1	1	33082250			
ENSG00000142920	AZIN2	1	1	33082906			
ENSG00000142920	AZIN2	1	1	33093242	-557:-546	GACATTCAAGCG	1:33092684-33092695
ENSG00000142920	AZIN2	1	1	33094644			
ENSG00000105173	CCNE1	1	19	29811898	-319:-308	TACATTCCACCC	19:29811578-29811589
ENSG00000105173	CCNE1	1	19	29812004	-425:-414	TACATTCCACCC	19:29811578-29811589
ENSG00000105173	CCNE1	1	19	29812654			
ENSG00000105173	CCNE1	1	19	29812725			
ENSG00000105173	CCNE1	1	19	29817203			
ENSG00000105173	CCNE1	1	19	29821732			
ENSG00000118523	CTGF	-1	6	131951373			
ENSG00000142871	CYR61	1	1	85580761	-617:-606; -111:-100	CACACTCCTCTT; AGCATTCCTGAG	1:85580143-85580154; 1:85580649-85580660
ENSG00000142871	CYR61	1	1	85581200	-550:-539; 839:850	AGCATTCCTGAG; TGCATTCCTCTG	1:85580649-85580660; 1:85582038-85582049
ENSG00000101412	E2F1	-1	20	33686404			
ENSG00000005884	ITGA3	1	17	50055968			
ENSG00000005884	ITGA3	1	17	50056068			
ENSG00000005884	ITGA3	1	17	50056110			
ENSG00000005884	ITGA3	1	17	50056440	879:890	TGCATTCCACAG	17:50057318-50057329
ENSG00000005884	ITGA3	1	17	50056487	832:843	TGCATTCCACAG	17:50057318-50057329
ENSG00000005884	ITGA3	1	17	50063538	-915:-904; -548:-537	TACATTCCTGAC; CAGATTCCACCA	17:50062622-50062633; 17:50062989-50063000
ENSG00000005884	ITGA3	1	17	50070873	250:261	CACAGTCCAGTG	17:50071122-50071133
ENSG00000005884	ITGA3	1	17	50071740	-617:-606	CACAGTCCAGTG	17:50071122-50071133
ENSG00000005884	ITGA3	1	17	50072160			
ENSG00000005884	ITGA3	1	17	50075460	-16:-5	TACATCCCTCCA	17:50075443-50075454
ENSG00000005884	ITGA3	1	17	50076625			
ENSG00000005884	ITGA3	1	17	50077400			
ENSG00000005884	ITGA3	1	17	50078004			
ENSG00000005884	ITGA3	1	17	50080317			
ENSG00000005884	ITGA3	1	17	50086329			
ENSG00000005884	ITGA3	1	17	50088213			
ENSG00000091409	ITGA6	1	2	172427354			
ENSG00000091409	ITGA6	1	2	172427418			
ENSG00000091409	ITGA6	1	2	172427555	980:991	TACATGCCTTTG	2:172428534-172428545
ENSG00000091409	ITGA6	1	2	172427789	746:757	TACATGCCTTTG	2:172428534-172428545
ENSG00000091409	ITGA6	1	2	172468998	-81:-70	CACATTCTAGTT	2:172468916-172468927
ENSG00000091409	ITGA6	1	2	172487962	126:137; 766:777	TACAATCCTCAT; CACATTCCTGAA	2:172488087-172488098; 2:172488727-172488738
ENSG00000091409	ITGA6	1	2	172488005	83:94; 723:734	TACAATCCTCAT; CACATTCCTGAA	2:172488087-172488098; 2:172488727-172488738
ENSG00000091409	ITGA6	1	2	172490792	-520:-509	CAGATTCCACAT	2:172490271-172490282
ENSG00000091409	ITGA6	1	2	172490929	-657:-646	CAGATTCCACAT	2:172490271-172490282
ENSG00000150093	ITGB1	-1	10	32908503			
ENSG00000150093	ITGB1	-1	10	32912064	-250:-261	CACACTCCATGG	10:32912313-32912324
ENSG00000150093	ITGB1	-1	10	32932717			
ENSG00000150093	ITGB1	-1	10	32935558			
ENSG00000150093	ITGB1	-1	10	32936251			
ENSG00000150093	ITGB1	-1	10	32955663	520:509	TACATTCGTCTT	10:32955142-32955153
ENSG00000150093	ITGB1	-1	10	32957794			
ENSG00000150093	ITGB1	-1	10	32957894			
ENSG00000150093	ITGB1	-1	10	32958196			
ENSG00000150093	ITGB1	-1	10	32958209			
ENSG00000150093	ITGB1	-1	10	32958218			
ENSG00000150093	ITGB1	-1	10	32958227			
ENSG00000150093	ITGB1	-1	10	32958232			
ENSG00000150093	ITGB1	-1	10	32960503			
ENSG00000150093	ITGB1	-1	10	32963283			
ENSG00000150093	ITGB1	-1	10	32992463	534:523	TACATTCCAGCA	10:32991928-32991939
ENSG00000150093	ITGB1	-1	10	33005792	-244:-255	GACATTCATCTG	10:33006035-33006046
ENSG00000136997	MYC	1	8	127735434	96:107	ATCATTCCTCCC	8:127735529-127735540
ENSG00000136997	MYC	1	8	127735519	11:22	ATCATTCCTCCC	8:127735529-127735540
ENSG00000136997	MYC	1	8	127736069	-539:-528; 646:657; 850:861	ATCATTCCTCCC; CCCATTCCTGCG; GACATTCCTGCT	8:127735529-127735540; 8:127736714-127736725; 8:127736918-127736929
ENSG00000136997	MYC	1	8	127736084	-554:-543; 631:642; 835:846	ATCATTCCTCCC; CCCATTCCTGCG; GACATTCCTGCT	8:127735529-127735540; 8:127736714-127736725; 8:127736918-127736929
ENSG00000136997	MYC	1	8	127736220	-690:-679; 495:506; 699:710	ATCATTCCTCCC; CCCATTCCTGCG; GACATTCCTGCT	8:127735529-127735540; 8:127736714-127736725; 8:127736918-127736929
ENSG00000136997	MYC	1	8	127736231	-701:-690; 484:495; 688:699	ATCATTCCTCCC; CCCATTCCTGCG; GACATTCCTGCT	8:127735529-127735540; 8:127736714-127736725; 8:127736918-127736929
ENSG00000115758	ODC1	-1	2	10447780			
ENSG00000115758	ODC1	-1	2	10447788			
ENSG00000115758	ODC1	-1	2	10448309			
ENSG00000115758	ODC1	-1	2	10448504	-854:-865	GGCATTCCAGCG	2:10449357-10449368
ENSG00000132646	PCNA	-1	20	5120026			
ENSG00000132646	PCNA	-1	20	5126626			
ENSG00000141564	RPTOR	1	17	80544819	123:134	CCCTTTCCTCCG	17:80544941-80544952
ENSG00000141564	RPTOR	1	17	80544831	111:122	CCCTTTCCTCCG	17:80544941-80544952
ENSG00000141564	RPTOR	1	17	80544877	65:76	CCCTTTCCTCCG	17:80544941-80544952
ENSG00000141564	RPTOR	1	17	80545268	-326:-315	CCCTTTCCTCCG	17:80544941-80544952
ENSG00000141564	RPTOR	1	17	80545468	-526:-515	CCCTTTCCTCCG	17:80544941-80544952
ENSG00000141564	RPTOR	1	17	80545666	-724:-713; 977:988	CCCTTTCCTCCG; CACATTTCAGTG	17:80544941-80544952; 17:80546642-80546653
ENSG00000141564	RPTOR	1	17	80708933			
ENSG00000141564	RPTOR	1	17	80743287	-304:-293	CACATTCCTAGT	17:80742982-80742993
ENSG00000141564	RPTOR	1	17	80922724	-18:-7	CCCATTCCTTTG	17:80922705-80922716
ENSG00000141564	RPTOR	1	17	80945567	919:930	CCCATTCCTGCA	17:80946485-80946496
ENSG00000141564	RPTOR	1	17	80948550			

ensembl_gene_id	hgnc_symbol	strand	chromosome_name	transcription_start_site	TEAD1_motif_position_relative_to	TEAD1_motif_candidate_sequence	TEAD1_motif_candidate_position
ENSG00000109321	AREG	1	4	74445134			
ENSG00000109321	AREG	1	4	74445302			
ENSG00000109321	AREG	1	4	74448639	18:29	AATATTCCTGGG	4:74448656-74448667
ENSG00000142920	AZIN2	1	1	33081104			
ENSG00000142920	AZIN2	1	1	33081113			
ENSG00000142920	AZIN2	1	1	33081148			
ENSG00000142920	AZIN2	1	1	33081150			
ENSG00000142920	AZIN2	1	1	33081155			
ENSG00000142920	AZIN2	1	1	33081161			
ENSG00000142920	AZIN2	1	1	33081163			
ENSG00000142920	AZIN2	1	1	33081164			
ENSG00000142920	AZIN2	1	1	33081165			
ENSG00000142920	AZIN2	1	1	33081167			
ENSG00000142920	AZIN2	1	1	33081169			
ENSG00000142920	AZIN2	1	1	33081176			
ENSG00000142920	AZIN2	1	1	33081659			
ENSG00000142920	AZIN2	1	1	33082250			
ENSG00000142920	AZIN2	1	1	33082906			
ENSG00000142920	AZIN2	1	1	33093242	-557:-546	GACATTCAAGCG	1:33092684-33092695
ENSG00000142920	AZIN2	1	1	33094644			
ENSG00000105173	CCNE1	1	19	29811898	-319:-308	TACATTCCACCC	19:29811578-29811589
ENSG00000105173	CCNE1	1	19	29812004	-425:-414	TACATTCCACCC	19:29811578-29811589
ENSG00000105173	CCNE1	1	19	29812654			
ENSG00000105173	CCNE1	1	19	29812725			
ENSG00000105173	CCNE1	1	19	29817203			
ENSG00000105173	CCNE1	1	19	29821732			
ENSG00000118523	CTGF	-1	6	131951373			
ENSG00000142871	CYR61	1	1	85580761	-617:-606; -111:-100	CACACTCCTCTT; AGCATTCCTGAG	1:85580143-85580154; 1:85580649-85580660
ENSG00000142871	CYR61	1	1	85581200	-550:-539; 839:850	AGCATTCCTGAG; TGCATTCCTCTG	1:85580649-85580660; 1:85582038-85582049
ENSG00000101412	E2F1	-1	20	33686404			
ENSG00000005884	ITGA3	1	17	50055968			
ENSG00000005884	ITGA3	1	17	50056068			
ENSG00000005884	ITGA3	1	17	50056110			
ENSG00000005884	ITGA3	1	17	50056440	879:890	TGCATTCCACAG	17:50057318-50057329
ENSG00000005884	ITGA3	1	17	50056487	832:843	TGCATTCCACAG	17:50057318-50057329
ENSG00000005884	ITGA3	1	17	50063538	-915:-904; -548:-537	TACATTCCTGAC; CAGATTCCACCA	17:50062622-50062633; 17:50062989-50063000
ENSG00000005884	ITGA3	1	17	50070873	250:261	CACAGTCCAGTG	17:50071122-50071133
ENSG00000005884	ITGA3	1	17	50071740	-617:-606	CACAGTCCAGTG	17:50071122-50071133
ENSG00000005884	ITGA3	1	17	50072160			
ENSG00000005884	ITGA3	1	17	50075460	-16:-5	TACATCCCTCCA	17:50075443-50075454
ENSG00000005884	ITGA3	1	17	50076625			
ENSG00000005884	ITGA3	1	17	50077400			
ENSG00000005884	ITGA3	1	17	50078004			
ENSG00000005884	ITGA3	1	17	50080317			
ENSG00000005884	ITGA3	1	17	50086329			
ENSG00000005884	ITGA3	1	17	50088213			
ENSG00000091409	ITGA6	1	2	172427354			
ENSG00000091409	ITGA6	1	2	172427418			
ENSG00000091409	ITGA6	1	2	172427555	980:991	TACATGCCTTTG	2:172428534-172428545
ENSG00000091409	ITGA6	1	2	172427789	746:757	TACATGCCTTTG	2:172428534-172428545
ENSG00000091409	ITGA6	1	2	172468998	-81:-70	CACATTCTAGTT	2:172468916-172468927
ENSG00000091409	ITGA6	1	2	172487962	126:137; 766:777	TACAATCCTCAT; CACATTCCTGAA	2:172488087-172488098; 2:172488727-172488738
ENSG00000091409	ITGA6	1	2	172488005	83:94; 723:734	TACAATCCTCAT; CACATTCCTGAA	2:172488087-172488098; 2:172488727-172488738
ENSG00000091409	ITGA6	1	2	172490792	-520:-509	CAGATTCCACAT	2:172490271-172490282
ENSG00000091409	ITGA6	1	2	172490929	-657:-646	CAGATTCCACAT	2:172490271-172490282
ENSG00000150093	ITGB1	-1	10	32908503			
ENSG00000150093	ITGB1	-1	10	32912064	-250:-261	CACACTCCATGG	10:32912313-32912324
ENSG00000150093	ITGB1	-1	10	32932717			
ENSG00000150093	ITGB1	-1	10	32935558			
ENSG00000150093	ITGB1	-1	10	32936251			
ENSG00000150093	ITGB1	-1	10	32955663	520:509	TACATTCGTCTT	10:32955142-32955153
ENSG00000150093	ITGB1	-1	10	32957794			
ENSG00000150093	ITGB1	-1	10	32957894			
ENSG00000150093	ITGB1	-1	10	32958196			
ENSG00000150093	ITGB1	-1	10	32958209			
ENSG00000150093	ITGB1	-1	10	32958218			
ENSG00000150093	ITGB1	-1	10	32958227			
ENSG00000150093	ITGB1	-1	10	32958232			
ENSG00000150093	ITGB1	-1	10	32960503			
ENSG00000150093	ITGB1	-1	10	32963283			
ENSG00000150093	ITGB1	-1	10	32992463	534:523	TACATTCCAGCA	10:32991928-32991939
ENSG00000150093	ITGB1	-1	10	33005792	-244:-255	GACATTCATCTG	10:33006035-33006046
ENSG00000136997	MYC	1	8	127735434	96:107	ATCATTCCTCCC	8:127735529-127735540
ENSG00000136997	MYC	1	8	127735519	11:22	ATCATTCCTCCC	8:127735529-127735540
ENSG00000136997	MYC	1	8	127736069	-539:-528; 646:657; 850:861	ATCATTCCTCCC; CCCATTCCTGCG; GACATTCCTGCT	8:127735529-127735540; 8:127736714-127736725; 8:127736918-127736929
ENSG00000136997	MYC	1	8	127736084	-554:-543; 631:642; 835:846	ATCATTCCTCCC; CCCATTCCTGCG; GACATTCCTGCT	8:127735529-127735540; 8:127736714-127736725; 8:127736918-127736929
ENSG00000136997	MYC	1	8	127736220	-690:-679; 495:506; 699:710	ATCATTCCTCCC; CCCATTCCTGCG; GACATTCCTGCT	8:127735529-127735540; 8:127736714-127736725; 8:127736918-127736929
ENSG00000136997	MYC	1	8	127736231	-701:-690; 484:495; 688:699	ATCATTCCTCCC; CCCATTCCTGCG; GACATTCCTGCT	8:127735529-127735540; 8:127736714-127736725; 8:127736918-127736929
ENSG00000115758	ODC1	-1	2	10447780			
ENSG00000115758	ODC1	-1	2	10447788			
ENSG00000115758	ODC1	-1	2	10448309			
ENSG00000115758	ODC1	-1	2	10448504	-854:-865	GGCATTCCAGCG	2:10449357-10449368
ENSG00000132646	PCNA	-1	20	5120026			
ENSG00000132646	PCNA	-1	20	5126626			
ENSG00000141564	RPTOR	1	17	80544819	123:134	CCCTTTCCTCCG	17:80544941-80544952
ENSG00000141564	RPTOR	1	17	80544831	111:122	CCCTTTCCTCCG	17:80544941-80544952
ENSG00000141564	RPTOR	1	17	80544877	65:76	CCCTTTCCTCCG	17:80544941-80544952
ENSG00000141564	RPTOR	1	17	80545268	-326:-315	CCCTTTCCTCCG	17:80544941-80544952
ENSG00000141564	RPTOR	1	17	80545468	-526:-515	CCCTTTCCTCCG	17:80544941-80544952
ENSG00000141564	RPTOR	1	17	80545666	-724:-713; 977:988	CCCTTTCCTCCG; CACATTTCAGTG	17:80544941-80544952; 17:80546642-80546653
ENSG00000141564	RPTOR	1	17	80708933			
ENSG00000141564	RPTOR	1	17	80743287	-304:-293	CACATTCCTAGT	17:80742982-80742993
ENSG00000141564	RPTOR	1	17	80922724	-18:-7	CCCATTCCTTTG	17:80922705-80922716
ENSG00000141564	RPTOR	1	17	80945567	919:930	CCCATTCCTGCA	17:80946485-80946496
ENSG00000141564	RPTOR	1	17	80948550			

TEAD1 binding on antisense strand

ensembl_gene_id	hgnc_symbol	strand	chromosome_name	transcription_start_site	as_strand_TEAD1_motif_position_relative_to_TSS	as_strand_TEAD1_motif_candidate_sequence	as_strand_TEAD1_motif_position
ENSG00000109321	AREG	1	4	74445134			
ENSG00000109321	AREG	1	4	74445302	893:904	CACATTCCAAAT	4:74446194-74446205
ENSG00000109321	AREG	1	4	74448639			
ENSG00000142920	AZIN2	1	1	33081104			
ENSG00000142920	AZIN2	1	1	33081113			
ENSG00000142920	AZIN2	1	1	33081148			
ENSG00000142920	AZIN2	1	1	33081150			
ENSG00000142920	AZIN2	1	1	33081155			
ENSG00000142920	AZIN2	1	1	33081161			
ENSG00000142920	AZIN2	1	1	33081163			
ENSG00000142920	AZIN2	1	1	33081164			
ENSG00000142920	AZIN2	1	1	33081165			
ENSG00000142920	AZIN2	1	1	33081167			
ENSG00000142920	AZIN2	1	1	33081169			
ENSG00000142920	AZIN2	1	1	33081176			
ENSG00000142920	AZIN2	1	1	33081659			
ENSG00000142920	AZIN2	1	1	33082250			
ENSG00000142920	AZIN2	1	1	33082906			
ENSG00000142920	AZIN2	1	1	33093242	-636:-625; -161:-150	TTCATTCCTCAA; CCCATTCCTGCA	1:33092605-33092616; 1:33093080-33093091
ENSG00000142920	AZIN2	1	1	33094644			
ENSG00000105173	CCNE1	1	19	29811898	-602:-591; 787:798	TCCATTCATCCG; CGCATCCCTGTG	19:29811295-29811306; 19:29812684-29812695
ENSG00000105173	CCNE1	1	19	29812004	-708:-697; 681:692	TCCATTCATCCG; CGCATCCCTGTG	19:29811295-29811306; 19:29812684-29812695
ENSG00000105173	CCNE1	1	19	29812654	31:42:00	CGCATCCCTGTG	19:29812684-29812695
ENSG00000105173	CCNE1	1	19	29812725	-40:-29	CGCATCCCTGTG	19:29812684-29812695
ENSG00000105173	CCNE1	1	19	29817203	-324:-313	CACATTCATTAG	19:29816878-29816889
ENSG00000105173	CCNE1	1	19	29821732			
ENSG00000118523	CTGF	-1	6	131951373	-88:-99; -97:-108; -109:-120; -162:-173	GACATTCCTCGC; CGCATTCCTCCC; CACCTTCCTGCC; AGCATTCCTCCG	6:131951460-131951471; 6:131951469-131951480; 6:131951481-1
ENSG00000142871	CYR61	1	1	85580761	765:776; 951:962	TGCATTCCAGCC; TGCATTCCAGAC	1:85581525-85581536; 1:85581711-85581722
ENSG00000142871	CYR61	1	1	85581200	326:337; 512:523	TGCATTCCAGCC; TGCATTCCAGAC	1:85581525-85581536; 1:85581711-85581722
ENSG00000101412	E2F1	-1	20	33686404	947:936; -295:-306	GACTTTCCTCCG; CGCATCCCACTG	20:33685456-33685467; 20:33686698-33686709
ENSG00000005884	ITGA3	1	17	50055968	-221:-210	CGCTTTCCTGCG	17:50055746-50055757
ENSG00000005884	ITGA3	1	17	50056068	-321:-310	CGCTTTCCTGCG	17:50055746-50055757
ENSG00000005884	ITGA3	1	17	50056110	-363:-352	CGCTTTCCTGCG	17:50055746-50055757
ENSG00000005884	ITGA3	1	17	50056440	-693:-682	CGCTTTCCTGCG	17:50055746-50055757
ENSG00000005884	ITGA3	1	17	50056487	-740:-729	CGCTTTCCTGCG	17:50055746-50055757
ENSG00000005884	ITGA3	1	17	50063538	-520:-509	TAGATTCCTCTG	17:50063017-50063028
ENSG00000005884	ITGA3	1	17	50070873			
ENSG00000005884	ITGA3	1	17	50071740	918:929	CACATCCCTGCC	17:50072657-50072668
ENSG00000005884	ITGA3	1	17	50072160	498:509	CACATCCCTGCC	17:50072657-50072668
ENSG00000005884	ITGA3	1	17	50075460	500:511; 509:520	CACTTTCCTTGG; CACACTCCTCAC	17:50075959-50075970; 17:50075968-50075979
ENSG00000005884	ITGA3	1	17	50076625	-665:-654; -656:-645	CACTTTCCTTGG; CACACTCCTCAC	17:50075959-50075970; 17:50075968-50075979
ENSG00000005884	ITGA3	1	17	50077400			
ENSG00000005884	ITGA3	1	17	50078004			
ENSG00000005884	ITGA3	1	17	50080317	318:329	AGCATTCCAGGG	17:50080634-50080645
ENSG00000005884	ITGA3	1	17	50086329			
ENSG00000005884	ITGA3	1	17	50088213	-351:-340	AACCTTCCACAG	17:50087861-50087872
ENSG00000091409	ITGA6	1	2	172427354			
ENSG00000091409	ITGA6	1	2	172427418			
ENSG00000091409	ITGA6	1	2	172427555			
ENSG00000091409	ITGA6	1	2	172427789			
ENSG00000091409	ITGA6	1	2	172468998			
ENSG00000091409	ITGA6	1	2	172487962	-925:-914; -450:-439	CACATCCCTCTT; CACAATCCACTC	2:172487036-172487047; 2:172487511-172487522
ENSG00000091409	ITGA6	1	2	172488005	-968:-957; -493:-482	CACATCCCTCTT; CACAATCCACTC	2:172487036-172487047; 2:172487511-172487522
ENSG00000091409	ITGA6	1	2	172490792			
ENSG00000091409	ITGA6	1	2	172490929			
ENSG00000150093	ITGB1	-1	10	32908503	867:856	CCCATTCCAGCC	10:32907635-32907646
ENSG00000150093	ITGB1	-1	10	32912064			
ENSG00000150093	ITGB1	-1	10	32932717			
ENSG00000150093	ITGB1	-1	10	32935558			
ENSG00000150093	ITGB1	-1	10	32936251			
ENSG00000150093	ITGB1	-1	10	32955663			
ENSG00000150093	ITGB1	-1	10	32957794	-612:-623	CGCATTCCTCTC	10:32958405-32958416
ENSG00000150093	ITGB1	-1	10	32957894	-512:-523	CGCATTCCTCTC	10:32958405-32958416
ENSG00000150093	ITGB1	-1	10	32958196	-210:-221	CGCATTCCTCTC	10:32958405-32958416
ENSG00000150093	ITGB1	-1	10	32958209	-197:-208	CGCATTCCTCTC	10:32958405-32958416
ENSG00000150093	ITGB1	-1	10	32958218	-188:-199	CGCATTCCTCTC	10:32958405-32958416
ENSG00000150093	ITGB1	-1	10	32958227	-179:-190	CGCATTCCTCTC	10:32958405-32958416
ENSG00000150093	ITGB1	-1	10	32958232	-174:-185	CGCATTCCTCTC	10:32958405-32958416
ENSG00000150093	ITGB1	-1	10	32960503			
ENSG00000150093	ITGB1	-1	10	32963283			
ENSG00000150093	ITGB1	-1	10	32992463			
ENSG00000150093	ITGB1	-1	10	33005792	183:172; 80:69; -398:-409	CACATACCAGAG; CGCATCCCTCCG; TCCATTCCACTT	10:33005608-33005619; 10:33005711-33005722; 10:33006189-330
ENSG00000136997	MYC	1	8	127735434	-952:-941; -74:-63	AACATTCTTCTC; TACCTTCCACCC	8:127734481-127734492; 8:127735359-127735370
ENSG00000136997	MYC	1	8	127735519	-159:-148	TACCTTCCACCC	8:127735359-127735370
ENSG00000136997	MYC	1	8	127736069	-709:-698	TACCTTCCACCC	8:127735359-127735370
ENSG00000136997	MYC	1	8	127736084	-724:-713	TACCTTCCACCC	8:127735359-127735370
ENSG00000136997	MYC	1	8	127736220	-860:-849	TACCTTCCACCC	8:127735359-127735370
ENSG00000136997	MYC	1	8	127736231	-871:-860	TACCTTCCACCC	8:127735359-127735370
ENSG00000115758	ODC1	-1	2	10447780	388:377	GACATTCCTGAG	2:10447391-10447402
ENSG00000115758	ODC1	-1	2	10447788	396:385	GACATTCCTGAG	2:10447391-10447402
ENSG00000115758	ODC1	-1	2	10448309	917:906; -547:-558	GACATTCCTGAG; CCCATTCCTCCG	2:10447391-10447402; 2:10448855-10448866
ENSG00000115758	ODC1	-1	2	10448504	-352:-363	CCCATTCCTCCG	2:10448855-10448866
ENSG00000132646	PCNA	-1	20	5120026	900:889; -441:-452	CACATTTCTCGC; AACATTCCCCCG	20:5119125-5119136; 20:5120466-5120477
ENSG00000132646	PCNA	-1	20	5126626			
ENSG00000141564	RPTOR	1	17	80544819			
ENSG00000141564	RPTOR	1	17	80544831			
ENSG00000141564	RPTOR	1	17	80544877			
ENSG00000141564	RPTOR	1	17	80545268			
ENSG00000141564	RPTOR	1	17	80545666			
ENSG00000141564	RPTOR	1	17	80708933			
ENSG00000141564	RPTOR	1	17	80743287			
ENSG00000141564	RPTOR	1	17	80922724			
ENSG00000141564	RPTOR	1	17	80945567	-485:-474	CAAATTCCTGGG	17:80945081-80945092
ENSG00000141564	RPTOR	1	17	80948550	420:431; 650:661	CCCATTCCTCTC; CACAATCCTGTC	17:80948969-80948980; 17:80949199-80949210

ensembl_gene_id	hgnc_symbol	strand	chromosome_name	transcription_start_site	as_strand_TEAD1_motif_position_relative_to_TSS	as_strand_TEAD1_motif_candidate_sequence	as_strand_TEAD1_motif_position
ENSG00000109321	AREG	1	4	74445134			
ENSG00000109321	AREG	1	4	74445302	893:904	CACATTCCAAAT	4:74446194-74446205
ENSG00000109321	AREG	1	4	74448639			
ENSG00000142920	AZIN2	1	1	33081104			
ENSG00000142920	AZIN2	1	1	33081113			
ENSG00000142920	AZIN2	1	1	33081148			
ENSG00000142920	AZIN2	1	1	33081150			
ENSG00000142920	AZIN2	1	1	33081155			
ENSG00000142920	AZIN2	1	1	33081161			
ENSG00000142920	AZIN2	1	1	33081163			
ENSG00000142920	AZIN2	1	1	33081164			
ENSG00000142920	AZIN2	1	1	33081165			
ENSG00000142920	AZIN2	1	1	33081167			
ENSG00000142920	AZIN2	1	1	33081169			
ENSG00000142920	AZIN2	1	1	33081176			
ENSG00000142920	AZIN2	1	1	33081659			
ENSG00000142920	AZIN2	1	1	33082250			
ENSG00000142920	AZIN2	1	1	33082906			
ENSG00000142920	AZIN2	1	1	33093242	-636:-625; -161:-150	TTCATTCCTCAA; CCCATTCCTGCA	1:33092605-33092616; 1:33093080-33093091
ENSG00000142920	AZIN2	1	1	33094644			
ENSG00000105173	CCNE1	1	19	29811898	-602:-591; 787:798	TCCATTCATCCG; CGCATCCCTGTG	19:29811295-29811306; 19:29812684-29812695
ENSG00000105173	CCNE1	1	19	29812004	-708:-697; 681:692	TCCATTCATCCG; CGCATCCCTGTG	19:29811295-29811306; 19:29812684-29812695
ENSG00000105173	CCNE1	1	19	29812654	31:42:00	CGCATCCCTGTG	19:29812684-29812695
ENSG00000105173	CCNE1	1	19	29812725	-40:-29	CGCATCCCTGTG	19:29812684-29812695
ENSG00000105173	CCNE1	1	19	29817203	-324:-313	CACATTCATTAG	19:29816878-29816889
ENSG00000105173	CCNE1	1	19	29821732			
ENSG00000118523	CTGF	-1	6	131951373	-88:-99; -97:-108; -109:-120; -162:-173	GACATTCCTCGC; CGCATTCCTCCC; CACCTTCCTGCC; AGCATTCCTCCG	6:131951460-131951471; 6:131951469-131951480; 6:131951481-1
ENSG00000142871	CYR61	1	1	85580761	765:776; 951:962	TGCATTCCAGCC; TGCATTCCAGAC	1:85581525-85581536; 1:85581711-85581722
ENSG00000142871	CYR61	1	1	85581200	326:337; 512:523	TGCATTCCAGCC; TGCATTCCAGAC	1:85581525-85581536; 1:85581711-85581722
ENSG00000101412	E2F1	-1	20	33686404	947:936; -295:-306	GACTTTCCTCCG; CGCATCCCACTG	20:33685456-33685467; 20:33686698-33686709
ENSG00000005884	ITGA3	1	17	50055968	-221:-210	CGCTTTCCTGCG	17:50055746-50055757
ENSG00000005884	ITGA3	1	17	50056068	-321:-310	CGCTTTCCTGCG	17:50055746-50055757
ENSG00000005884	ITGA3	1	17	50056110	-363:-352	CGCTTTCCTGCG	17:50055746-50055757
ENSG00000005884	ITGA3	1	17	50056440	-693:-682	CGCTTTCCTGCG	17:50055746-50055757
ENSG00000005884	ITGA3	1	17	50056487	-740:-729	CGCTTTCCTGCG	17:50055746-50055757
ENSG00000005884	ITGA3	1	17	50063538	-520:-509	TAGATTCCTCTG	17:50063017-50063028
ENSG00000005884	ITGA3	1	17	50070873			
ENSG00000005884	ITGA3	1	17	50071740	918:929	CACATCCCTGCC	17:50072657-50072668
ENSG00000005884	ITGA3	1	17	50072160	498:509	CACATCCCTGCC	17:50072657-50072668
ENSG00000005884	ITGA3	1	17	50075460	500:511; 509:520	CACTTTCCTTGG; CACACTCCTCAC	17:50075959-50075970; 17:50075968-50075979
ENSG00000005884	ITGA3	1	17	50076625	-665:-654; -656:-645	CACTTTCCTTGG; CACACTCCTCAC	17:50075959-50075970; 17:50075968-50075979
ENSG00000005884	ITGA3	1	17	50077400			
ENSG00000005884	ITGA3	1	17	50078004			
ENSG00000005884	ITGA3	1	17	50080317	318:329	AGCATTCCAGGG	17:50080634-50080645
ENSG00000005884	ITGA3	1	17	50086329			
ENSG00000005884	ITGA3	1	17	50088213	-351:-340	AACCTTCCACAG	17:50087861-50087872
ENSG00000091409	ITGA6	1	2	172427354			
ENSG00000091409	ITGA6	1	2	172427418			
ENSG00000091409	ITGA6	1	2	172427555			
ENSG00000091409	ITGA6	1	2	172427789			
ENSG00000091409	ITGA6	1	2	172468998			
ENSG00000091409	ITGA6	1	2	172487962	-925:-914; -450:-439	CACATCCCTCTT; CACAATCCACTC	2:172487036-172487047; 2:172487511-172487522
ENSG00000091409	ITGA6	1	2	172488005	-968:-957; -493:-482	CACATCCCTCTT; CACAATCCACTC	2:172487036-172487047; 2:172487511-172487522
ENSG00000091409	ITGA6	1	2	172490792			
ENSG00000091409	ITGA6	1	2	172490929			
ENSG00000150093	ITGB1	-1	10	32908503	867:856	CCCATTCCAGCC	10:32907635-32907646
ENSG00000150093	ITGB1	-1	10	32912064			
ENSG00000150093	ITGB1	-1	10	32932717			
ENSG00000150093	ITGB1	-1	10	32935558			
ENSG00000150093	ITGB1	-1	10	32936251			
ENSG00000150093	ITGB1	-1	10	32955663			
ENSG00000150093	ITGB1	-1	10	32957794	-612:-623	CGCATTCCTCTC	10:32958405-32958416
ENSG00000150093	ITGB1	-1	10	32957894	-512:-523	CGCATTCCTCTC	10:32958405-32958416
ENSG00000150093	ITGB1	-1	10	32958196	-210:-221	CGCATTCCTCTC	10:32958405-32958416
ENSG00000150093	ITGB1	-1	10	32958209	-197:-208	CGCATTCCTCTC	10:32958405-32958416
ENSG00000150093	ITGB1	-1	10	32958218	-188:-199	CGCATTCCTCTC	10:32958405-32958416
ENSG00000150093	ITGB1	-1	10	32958227	-179:-190	CGCATTCCTCTC	10:32958405-32958416
ENSG00000150093	ITGB1	-1	10	32958232	-174:-185	CGCATTCCTCTC	10:32958405-32958416
ENSG00000150093	ITGB1	-1	10	32960503			
ENSG00000150093	ITGB1	-1	10	32963283			
ENSG00000150093	ITGB1	-1	10	32992463			
ENSG00000150093	ITGB1	-1	10	33005792	183:172; 80:69; -398:-409	CACATACCAGAG; CGCATCCCTCCG; TCCATTCCACTT	10:33005608-33005619; 10:33005711-33005722; 10:33006189-330
ENSG00000136997	MYC	1	8	127735434	-952:-941; -74:-63	AACATTCTTCTC; TACCTTCCACCC	8:127734481-127734492; 8:127735359-127735370
ENSG00000136997	MYC	1	8	127735519	-159:-148	TACCTTCCACCC	8:127735359-127735370
ENSG00000136997	MYC	1	8	127736069	-709:-698	TACCTTCCACCC	8:127735359-127735370
ENSG00000136997	MYC	1	8	127736084	-724:-713	TACCTTCCACCC	8:127735359-127735370
ENSG00000136997	MYC	1	8	127736220	-860:-849	TACCTTCCACCC	8:127735359-127735370
ENSG00000136997	MYC	1	8	127736231	-871:-860	TACCTTCCACCC	8:127735359-127735370
ENSG00000115758	ODC1	-1	2	10447780	388:377	GACATTCCTGAG	2:10447391-10447402
ENSG00000115758	ODC1	-1	2	10447788	396:385	GACATTCCTGAG	2:10447391-10447402
ENSG00000115758	ODC1	-1	2	10448309	917:906; -547:-558	GACATTCCTGAG; CCCATTCCTCCG	2:10447391-10447402; 2:10448855-10448866
ENSG00000115758	ODC1	-1	2	10448504	-352:-363	CCCATTCCTCCG	2:10448855-10448866
ENSG00000132646	PCNA	-1	20	5120026	900:889; -441:-452	CACATTTCTCGC; AACATTCCCCCG	20:5119125-5119136; 20:5120466-5120477
ENSG00000132646	PCNA	-1	20	5126626			
ENSG00000141564	RPTOR	1	17	80544819			
ENSG00000141564	RPTOR	1	17	80544831			
ENSG00000141564	RPTOR	1	17	80544877			
ENSG00000141564	RPTOR	1	17	80545268			
ENSG00000141564	RPTOR	1	17	80545468			
ENSG00000141564	RPTOR	1	17	80545666			
ENSG00000141564	RPTOR	1	17	80708933			
ENSG00000141564	RPTOR	1	17	80743287			
ENSG00000141564	RPTOR	1	17	80922724			
ENSG00000141564	RPTOR	1	17	80945567	-485:-474	CAAATTCCTGGG	17:80945081-80945092
ENSG00000141564	RPTOR	1	17	80948550	420:431; 650:661	CCCATTCCTCTC; CACAATCCTGTC	17:80948969-80948980; 17:80949199-80949210

Predicted TEAD1 binding sites by stringency setting

gene.lis	N_predicted_TEAD1_binding_sites_score_0.90_or_more	N_predicted_TEAD1_binding_sites_score_0.85_or_more	N_predicted_TEAD1_binding_sites_score_0.80_or_more
AREG	0	1	2
AZIN2	0	0	3
CCNE1	1	1	4
CTGF	1	3	4
CYR61	1	2	5
E2F1	0	0	2
ITGA3	0	3	12
ITGA6	0	1	7
ITGB1	0	2	9
MYC	0	2	5
ODC1	2	3	3
PCNA	0	1	2
RPTOR	0	3	8

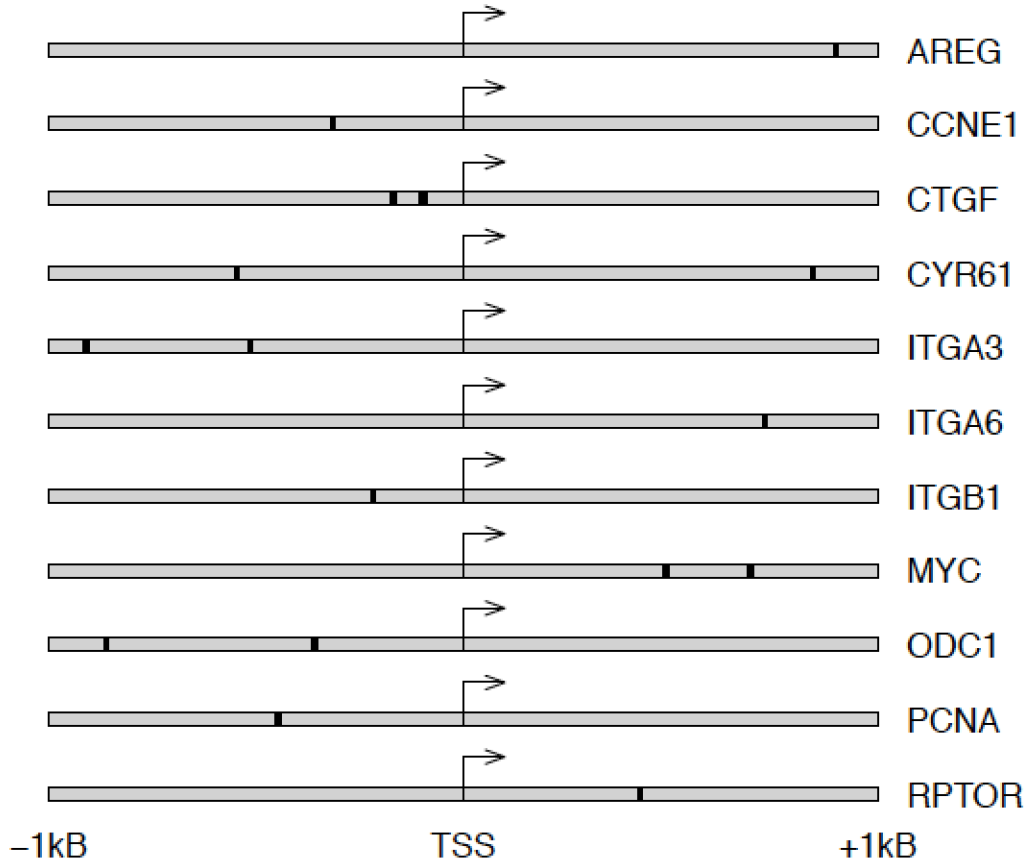
TEAD1 binding on both strands

ensembl_gene_id	hgnc_symbol	strand	chromosome_name	transcription_start_site	motif_position_relative_to_TSS	as_strand_motif_position_relative_to_TSS	motif_candidate_sequence(s)	as_strand_motif_candidate_sequence(s)	motif_position	as_strand_motif_position
ENSG00000109321	AREG	1	4	74445134						
ENSG00000109321	AREG	1	4	74445302		893:904		CACATTCCAAAT		4:74446194-74446205
ENSG00000109321	AREG	1	4	74446839						
ENSG00000142920	AZIN2	1	1	33081104						
ENSG00000142920	AZIN2	1	1	33081113						
ENSG00000142920	AZIN2	1	1	33081148						
ENSG00000142920	AZIN2	1	1	33081150						
ENSG00000142920	AZIN2	1	1	33081155						
ENSG00000142920	AZIN2	1	1	33081161						
ENSG00000142920	AZIN2	1	1	33081163						
ENSG00000142920	AZIN2	1	1	33081164						
ENSG00000142920	AZIN2	1	1	33081165						
ENSG00000142920	AZIN2	1	1	33081167						
ENSG00000142920	AZIN2	1	1	33081169						
ENSG00000142920	AZIN2	1	1	33081176						
ENSG00000142920	AZIN2	1	1	33081659						
ENSG00000142920	AZIN2	1	1	33082150						
ENSG00000142920	AZIN2	1	1	33082906						
ENSG00000142920	AZIN2	1	1	33093242						
ENSG00000142920	AZIN2	1	1	33094644						
ENSG00000105173	CCNE1	1	19	29811898	-319:-308		TACATTCCACCC		19:29811578-29811589	
ENSG00000105173	CCNE1	1	19	29812004	-425:-414		TACATTCCACCC		19:29811578-29811589	
ENSG00000105173	CCNE1	1	19	29812654						
ENSG00000105173	CCNE1	1	19	29817235						
ENSG00000105173	CCNE1	1	19	29817203						
ENSG00000105173	CCNE1	1	19	29821732						
ENSG00000118523	CTGF	-1	6	131951373		-88:-99; -97:-108; -162:-173		GACATTCCTCGC; CGCATTCCTCCC; AGCATTCCTCCG		6:131951460-131951471; 6:131951469-131951480; 6:131951534-131951545
ENSG00000142871	CYR61	1	1	85580761	-111:-100		AGCATTCCTGAG		1:85580649-85580660	
ENSG00000142871	CYR61	1	1	85581200	-550:-539; 839:850		AGCATTCCTGAG; TGCATTCCTCTG		1:85580649-85580660; 1:85582038-85582049	
ENSG00000201412	E2F1	-1	20	33684404						
ENSG00000005884	ITGA3	1	17	50055968						
ENSG00000005884	ITGA3	1	17	50056068						
ENSG00000005884	ITGA3	1	17	50056110						
ENSG00000005884	ITGA3	1	17	50056440	879:890		TGCATTCCACAG		17:50057318-50057329	
ENSG00000005884	ITGA3	1	17	50056487	832:843		TGCATTCCACAG		17:50057318-50057329	
ENSG00000005884	ITGA3	1	17	50063138	-915:-904	-520:-509	TACATTCTGAC	TAGATTCCTCTG	17:50063622-50063633	17:50063017-50063028
ENSG00000005884	ITGA3	1	17	50070873						
ENSG00000005884	ITGA3	1	17	50071740						
ENSG00000005884	ITGA3	1	17	50072160						
ENSG00000005884	ITGA3	1	17	50075460						
ENSG00000005884	ITGA3	1	17	50076625						
ENSG00000005884	ITGA3	1	17	50077400						
ENSG00000005884	ITGA3	1	17	50078004						
ENSG00000005884	ITGA3	1	17	50080317						
ENSG00000005884	ITGA3	1	17	50086329						
ENSG00000005884	ITGA3	1	17	50088213						
ENSG00000091409	ITGA6	1	2	172427354						
ENSG00000091409	ITGA6	1	2	172427418						
ENSG00000091409	ITGA6	1	2	172427555						
ENSG00000091409	ITGA6	1	2	172427789						
ENSG00000091409	ITGA6	1	2	172468998						
ENSG00000091409	ITGA6	1	2	172487962	766:777		CACATTCCTGAA		2:172488727-172488738	
ENSG00000091409	ITGA6	1	2	172488005	723:734		CACATTCCTGAA		2:172488727-172488738	
ENSG00000091409	ITGA6	1	2	172490792						
ENSG00000091409	ITGA6	1	2	172490929						
ENSG00000150093	ITGB1	-1	10	32908503						
ENSG00000150093	ITGB1	-1	10	32912004						
ENSG00000150093	ITGB1	-1	10	32932717						
ENSG00000150093	ITGB1	-1	10	32935558						
ENSG00000150093	ITGB1	-1	10	32939251						

ENSG00000150093	ITGB1	-1	10	32951663						
ENSG00000150093	ITGB1	-1	10	32957794		-612:-623		CGCATTCCTCTC		10:32958405-32958416
ENSG00000150093	ITGB1	-1	10	32957804		-512:-523		CGCATTCCTCTC		10:32958405-32958416
ENSG00000150093	ITGB1	-1	10	32958196		-210:-221		CGCATTCCTCTC		10:32958405-32958416
ENSG00000150093	ITGB1	-1	10	32958209		-197:-208		CGCATTCCTCTC		10:32958405-32958416
ENSG00000150093	ITGB1	-1	10	32958218		-188:-199		CGCATTCCTCTC		10:32958405-32958416
ENSG00000150093	ITGB1	-1	10	32958227		-179:-190		CGCATTCCTCTC		10:32958405-32958416
ENSG00000150093	ITGB1	-1	10	32958232		-174:-185		CGCATTCCTCTC		10:32958405-32958416
ENSG00000150093	ITGB1	-1	10	32960920						
ENSG00000150093	ITGB1	-1	10	32963283						
ENSG00000150093	ITGB1	-1	10	32993463	534:523		TACATTCCAGCA		10:32991928-32991939	
ENSG00000150093	ITGB1	-1	10	33005792						
ENSG00000136997	MYC	1	8	127735434						
ENSG00000136997	MYC	1	8	127735519						
ENSG00000136997	MYC	1	8	127736069	646:657; 850:861		CCCATTCCTGCG; GACATTCCTGCT		8:127736714-127736725; 8:127736918-127736929	
ENSG00000136997	MYC	1	8	127736084	631:642; 835:846		CCCATTCCTGCG; GACATTCCTGCT		8:127736714-127736725; 8:127736918-127736929	
ENSG00000136997	MYC	1	8	127736220	495:506; 699:710		CCCATTCCTGCG; GACATTCCTGCT		8:127736714-127736725; 8:127736918-127736929	
ENSG00000136997	MYC	1	8	127736231	484:495; 688:699		CCCATTCCTGCG; GACATTCCTGCT		8:127736714-127736725; 8:127736918-127736929	
ENSG00000115758	ODC1	-1	2	10447780		388:377		GACATTCCTGAG		2:10447391-10447402
ENSG00000115758	ODC1	-1	2	10447788		396:385		GACATTCCTGAG		2:10447391-10447402
ENSG00000115758	ODC1	-1	2	10448309		917:906; 647:-558		GACATTCCTGAG; CCCATTCCTCCG		2:10447391-10447402; 2:10448855-10448866
ENSG00000115758	ODC1	-1	2	10448504	-854:-865	-352:-363	GGCATTCCAGCG	CCCATTCCTCCG	2:10449357-10449368	2:10448855-10448866
ENSG00000132646	PCNA	-1	20	5120026		-441:-452		AACATTCCCCCG		20:5120466-5120477
ENSG00000132646	PCNA	-1	20	5126626						
ENSG00000141564	RPTOR	1	17	80544819						
ENSG00000141564	RPTOR	1	17	80544831						
ENSG00000141564	RPTOR	1	17	80544877						
ENSG00000141564	RPTOR	1	17	80545268						
ENSG00000141564	RPTOR	1	17	80545468						
ENSG00000141564	RPTOR	1	17	80545666						
ENSG00000141564	RPTOR	1	17	80708933						
ENSG00000141564	RPTOR	1	17	80743287	-304:-293		CACATTCCTAGT		17:80742982-80742993	
ENSG00000141564	RPTOR	1	17	80922724						
ENSG00000141564	RPTOR	1	17	80945567		-485:-474		CAAATTCCTGGG		17:80945081-80945092
ENSG00000141564	RPTOR	1	17	80948550		420:431		CCCATTCCTCTC		17:80948069-80948080

ensembl_gene_id	hgnc_symbol	strand	chromosome_name	transcription_start_site	motif_position_relative_to_TSS	as_strand_motif_position_relative_to_TSS	motif_candidate_sequence(s)	as_strand_motif_candidate_sequence(s)	motif_position	as_strand_motif_position
ENSG00000109321	AREG	1	4	74445134						
ENSG00000109321	AREG	-1	4	74443302		893:904		CACATTCCAAAT		4:74446194-74446205
ENSG00000109321	AREG	1	4	74446839						
ENSG00000142920	AZIN2	1	1	33081104						
ENSG00000142920	AZIN2	1	1	33081113						
ENSG00000142920	AZIN2	1	1	33081148						
ENSG00000142920	AZIN2	1	1	33081150						
ENSG00000142920	AZIN2	1	1	33081155						
ENSG00000142920	AZIN2	1	1	33081161						
ENSG00000142920	AZIN2	1	1	33081163						
ENSG00000142920	AZIN2	1	1	33081164						
ENSG00000142920	AZIN2	1	1	33081165						
ENSG00000142920	AZIN2	1	1	33081167						
ENSG00000142920	AZIN2	1	1	33081169						
ENSG00000142920	AZIN2	1	1	33081176						
ENSG00000142920	AZIN2	1	1	33081659						
ENSG00000142920	AZIN2	1	1	33082250						
ENSG00000142920	AZIN2	1	1	33082906						
ENSG00000142920	AZIN2	1	1	33093242						
ENSG00000142920	AZIN2	1	1	33094644						
ENSG00000105173	CCNE1	1	19	29811898	-319:-308		TACATTCCACCC		19:29811578-29811589	
ENSG00000105173	CCNE1	1	19	29812004	-425:-414		TACATTCCACCC		19:29811578-29811589	
ENSG00000105173	CCNE1	1	19	29812654						
ENSG00000105173	CCNE1	1	19	29817235						
ENSG00000105173	CCNE1	1	19	29817203						
ENSG00000105173	CCNE1	1	19	29821732						
ENSG00000118523	CTGF	-1	6	131951373		-88:-99; -97:-108; -162:-173		GACATTCCTCGC; CGCATTCCTCCC; AGCATTCCTCCG		6:131951460-131951471; 6:131951469-131951480; 6:131951534-131951545
ENSG00000142871	CYR61	1	1	85580761	-111:-100		AGCATTCCTGAG		1:85580649-85580660	
ENSG00000142871	CYR61	1	1	85581200	-550:-539; 839:850		AGCATTCCTGAG; TGCATTCCTCTG		1:85580649-85580660; 1:85582038-85582049	
ENSG00000101412	E2F1	-1	20	33088404						
ENSG00000005884	ITGA3	1	17	50053968						
ENSG00000005884	ITGA3	1	17	50056068						
ENSG00000005884	ITGA3	1	17	50056110						
ENSG00000005884	ITGA3	1	17	50056440	879:890		TGCATTCCACAG		17:50057318-50057329	
ENSG00000005884	ITGA3	1	17	50056487	832:843		TGCATTCCACAG		17:50057318-50057329	
ENSG00000005884	ITGA3	1	17	50063538	-915:-904	-520:-509	TACATTCCTGAC	TAGATTCCTCTG	17:50062622-50062633	17:50063017-50063028
ENSG00000005884	ITGA3	1	17	50070873						
ENSG00000005884	ITGA3	1	17	50071740						
ENSG00000005884	ITGA3	1	17	50072160						
ENSG00000005884	ITGA3	1	17	50075460						
ENSG00000005884	ITGA3	1	17	50076625						
ENSG00000005884	ITGA3	1	17	50077400						
ENSG00000005884	ITGA3	1	17	50078004						
ENSG00000005884	ITGA3	1	17	50080317						
ENSG00000005884	ITGA3	1	17	50086329						
ENSG00000005884	ITGA3	1	17	50088213						
ENSG00000091409	ITGA6	1	2	172427354						
ENSG00000091409	ITGA6	1	2	172427418						
ENSG00000091409	ITGA6	1	2	172427555						
ENSG00000091409	ITGA6	1	2	172427789						
ENSG00000091409	ITGA6	1	2	172468998						
ENSG00000091409	ITGA6	1	2	172487962	766:777		CACATTCCTGAA		2:172488727-172488738	
ENSG00000091409	ITGA6	1	2	172488005	723:734		CACATTCCTGAA		2:172488727-172488738	
ENSG00000091409	ITGA6	1	2	172490792						
ENSG00000091409	ITGA6	1	2	172490929						
ENSG00000150993	ITGB1	-1	10	32908503						
ENSG00000150993	ITGB1	-1	10	32912064						
ENSG00000150993	ITGB1	-1	10	32932717						
ENSG00000150993	ITGB1	-1	10	32935158						
ENSG00000150993	ITGB1	-1	10	32936251						
ENSG00000150993	ITGB1	-1	10	32953663						

ENSG00000150993	ITGB1	-1	10	32957794		-612:-623		CGCATTCCTCTC		10:32958405-32958416
ENSG00000150993	ITGB1	-1	10	32957894		-512:-523		CGCATTCCTCTC		10:32958405-32958416
ENSG00000150993	ITGB1	-1	10	32958196		-210:-221		CGCATTCCTCTC		10:32958405-32958416
ENSG00000150993	ITGB1	-1	10	32958209		-197:-208		CGCATTCCTCTC		10:32958405-32958416
ENSG00000150993	ITGB1	-1	10	32958218		-188:-199		CGCATTCCTCTC		10:32958405-32958416
ENSG00000150993	ITGB1	-1	10	32958227		-179:-190		CGCATTCCTCTC		10:32958405-32958416
ENSG00000150993	ITGB1	-1	10	32958232		-174:-185		CGCATTCCTCTC		10:32958405-32958416
ENSG00000150993	ITGB1	-1	10	32960503						
ENSG00000150993	ITGB1	-1	10	32963183						
ENSG00000150993	ITGB1	-1	10	32992463	534:523		TACATTCCAGCA		10:32991928-32991939	
ENSG00000150993	ITGB1	-1	10	33005792						
ENSG00000136997	MYC	1	8	127735434						
ENSG00000136997	MYC	1	8	127735519						
ENSG00000136997	MYC	1	8	127736089	646:657; 850:861		CCCATTCCTGCG; GACATTCCTGCT		8:127736714-127736725; 8:127736918-127736929	
ENSG00000136997	MYC	1	8	127736084	631:642; 835:846		CCCATTCCTGCG; GACATTCCTGCT		8:127736714-127736725; 8:127736918-127736929	
ENSG00000136997	MYC	1	8	127736220	495:506; 699:710		CCCATTCCTGCG; GACATTCCTGCT		8:127736714-127736725; 8:127736918-127736929	
ENSG00000136997	MYC	1	8	127736231	484:495; 688:699		CCCATTCCTGCG; GACATTCCTGCT		8:127736714-127736725; 8:127736918-127736929	
ENSG00000115758	ODC1	-1	2	10447780		388:377		GACATTCCTGAG		2:10447391-10447402
ENSG00000115758	ODC1	-1	2	10447788		396:385		GACATTCCTGAG		2:10447391-10447402
ENSG00000115758	ODC1	-1	2	10448309		917:906; -547:-558		GACATTCCTGAG; CCCATTCCTCCG		2:10447391-10447402; 2:10448855-10448866
ENSG00000115758	ODC1	-1	2	10448504	-854:-865	-312:-303	GGCATTCCAGCG	CCCATTCCTCCG	2:10448357-10448368	2:10448855-10448866
ENSG00000132646	PCNA	-1	20	5120026		-441:-452		AACATTCCCCCG		20:5120466-5120477
ENSG00000132646	PCNA	-1	20	5126626						
ENSG00000141564	RPTOR	1	17	80544819						
ENSG00000141564	RPTOR	1	17	80544831						
ENSG00000141564	RPTOR	1	17	80544877						
ENSG00000141564	RPTOR	1	17	80545268						
ENSG00000141564	RPTOR	1	17	80545468						
ENSG00000141564	RPTOR	1	17	80545666						
ENSG00000141564	RPTOR	1	17	80708833						
ENSG00000141564	RPTOR	1	17	80743287	-304:-293		CACATTCCTAGT		17:80742982-80742993	
ENSG00000141564	RPTOR	1	17	80922724						
ENSG00000141564	RPTOR	1	17	80945567		-485:-474		CAAATTCCTGGG		17:80945081-80945092
ENSG00000141564	RPTOR	1	17	80948550		420:431		CCCATTCCTCTC		17:80948969-80948980



Reference List

Ahn, Y. H., D. L. Gibbons, D. Chakravarti, C. J. Creighton, Z. H. Rizvi, H. P. Adams, A. Pertsemlidis, P. A. Gregory, J. A. Wright, G. J. Goodall, E. R. Flores and J. M. Kurie (2012). "ZEB1 drives prometastatic actin cytoskeletal remodeling by downregulating miR-34a expression." J Clin Invest **122**(9): 3170-3183.
Aigner, K., B. Dampier, L. Descovich, M. Mikula, A. Sultan, M. Schreiber, W. Mikulits, T. Brabletz, D. Strand, P. Obrist, W. Sommergruber, N. Schweifer, A. Wernitznig, H. Beug, R. Foisner and A. Eger (2007). "The transcription factor ZEB1 (deltaEF1) promotes tumour cell dedifferentiation by repressing master regulators of epithelial polarity." Oncogene **26**(49): 6979-6988.
Al Saleh, S., L. H. Sharaf and Y. A. Luqmani (2011). "Signalling pathways involved in endocrine resistance in breast cancer and associations with epithelial to mesenchymal transition (Review)." Int J Oncol **38**(5): 1197-1217.
Alam, M. and D. Ratner (2001). "Cutaneous squamous-cell carcinoma." N Engl J Med **344**(13): 975-983.
Alarcon, C., A. I. Zaromytidou, Q. Xi, S. Gao, J. Yu, S. Fujisawa, A. Barlas, A. N. Miller, K. Manova-Todorova, M. J. Macias, G. Sapkota, D. Pan and J. Massague (2009). "Nuclear CDKs drive Smad transcriptional activation and turnover in BMP and TGF-beta pathways." Cell **139**(4): 757-769.
Alfaro, M. P., D. L. Deskins, M. Wallus, J. DasGupta, J. M. Davidson, L. B. Nanney, A. G. M, M. Gannon and P. P. Young (2013). "A physiological role for connective tissue growth factor in early wound healing." Lab Invest **93**(1): 81-95.
Ames, J. J., L. Contois, J. M. Caron, E. Tweedie, X. Yang, R. Friesel, C. Vary and P. C. Brooks (2016). "Identification of an Endogenously Generated Cryptic Collagen Epitope (XL313) That May Selectively Regulate Angiogenesis by an Integrin Yes-associated Protein (YAP) Mechano-transduction Pathway." J Biol Chem **291**(6): 2731-2750.
Ando, T., D. Charindra, M. Shrestha, H. Umehara, I. Ogawa, M. Miyauchi and T. Takata (2018). "Tissue inhibitor of metalloproteinase-1 promotes cell proliferation through YAP/TAZ activation in cancer." Oncogene **37**(2): 263-270.
Aragona, M., T. Panciera, A. Manfrin, S. Giulitti, F. Michielin, N. Elvassore, S. Dupont and S. Piccolo (2013). "A mechanical checkpoint controls multicellular growth through YAP/TAZ regulation by actin-processing factors." Cell **154**(5): 1047-1059.
Aylon, Y., D. Michael, A. Shmueli, N. Yabuta, H. Nojima and M. Oren (2006). "A positive feedback loop between the p53 and Lats2 tumor suppressors prevents tetraploidization." Genes Dev **20**(19): 2687-2700.
Azad, T., H. J. Janse van Rensburg, E. D. Lightbody, B. Neveu, A. Champagne, A. Ghaffari, V. R. Kay, Y. Hao, H. Shen, B. Yeung, B. A. Croy, K. L. Guan, F. Pouliot, J. Zhang, C. J. B. Nicol and X. Yang (2018). "A LATS biosensor screen identifies VEGFR as a regulator of the Hippo pathway in angiogenesis." Nat Commun **9**(1): 1061.
Azzolin, L., T. Panciera, S. Soligo, E. Enzo, S. Bicciato, S. Dupont, S. Bresolin, C. Frasson, G. Basso, V. Guzzardo, A. Fassina, M. Cordenonsi and S. Piccolo (2014). "YAP/TAZ incorporation in the beta-catenin destruction complex orchestrates the Wnt response." Cell **158**(1): 157-170.
Bae, J. S., S. M. Kim and H. Lee (2017). "The Hippo signaling pathway provides novel anti-cancer drug targets." Oncotarget **8**(9): 16084-16098.

Bai, H., N. Zhang, Y. Xu, Q. Chen, M. Khan, J. J. Potter, S. K. Nayar, T. Cornish, G. Alpini, S. Bronk, D. Pan and R. A. Anders (2012). "Yes-associated protein regulates the hepatic response after bile duct ligation." Hepatology **56**(3): 1097-1107.
Bakiri, L., S. Macho-Maschler, I. Custic, J. Niemiec, A. Guio-Carrion, S. C. Hasenfuss, A. Eger, M. Muller, H. Beug and E. F. Wagner (2015). "Fra-1/AP-1 induces EMT in mammary epithelial cells by modulating Zeb1/2 and TGFbeta expression." Cell Death Differ **22**(2): 336-350.
Balda, M. S. and K. Matter (2008). "Tight junctions at a glance." J Cell Sci **121**(Pt 22): 3677-3682.
Balkwill, F. and A. Mantovani (2001). "Inflammation and cancer: back to Virchow?" Lancet **357**(9255): 539-545.
Barrandon, Y. and H. Green (1987). "Three clonal types of keratinocyte with different capacities for multiplication." Proc Natl Acad Sci U S A **84**(8): 2302-2306.
Barriere, G., P. Fici, G. Gallerani, F. Fabbri and M. Rigaud (2015). "Epithelial Mesenchymal Transition: a double-edged sword." Clin Transl Med **4**: 14.
Barry, E. R., T. Morikawa, B. L. Butler, K. Shrestha, R. de la Rosa, K. S. Yan, C. S. Fuchs, S. T. Magness, R. Smits, S. Ogino, C. J. Kuo and F. D. Camargo (2013). "Restriction of intestinal stem cell expansion and the regenerative response by YAP." Nature **493**(7430): 106-110.
Baum, B. and M. Georgiou (2011). "Dynamics of adherens junctions in epithelial establishment, maintenance, and remodeling." J Cell Biol **192**(6): 907-917.
Baumgartner, R., I. Poernbacher, N. Buser, E. Hafen and H. Stocker (2010). "The WW domain protein Kibra acts upstream of Hippo in Drosophila." Dev Cell **18**(2): 309-316.
Bavle, R. M., G. Govinda, P. G. Venkataramanaiah, S. Muniswamappa and R. Venugopal (2016). "Fallacious Carcinoma- Spindle Cell Variant of Squamous Cell Carcinoma." J Clin Diagn Res **10**(7): ZD05-08.
Bayarmagnai, B., B. N. Nicolay, A. B. Islam, N. Lopez-Bigas and M. V. Frolov (2012). "Drosophila GAGA factor is required for full activation of the dE2f1-Yki/Sd transcriptional program." Cell Cycle **11**(22): 4191-4202.
Bazellieres, E., E. Assemat, J. P. Arsanto, A. Le Bivic and D. Massey-Harroche (2009). "Crumbs proteins in epithelial morphogenesis." Front Biosci (Landmark Ed) **14**: 2149-2169.
Benham-Pyle, B. W., B. L. Pruitt and W. J. Nelson (2015). "Cell adhesion. Mechanical strain induces E-cadherin-dependent Yap1 and beta-catenin activation to drive cell cycle entry." Science **348**(6238): 1024-1027.
Benhamouche, S., M. Curto, I. Saotome, A. B. Gladden, C. H. Liu, M. Giovannini and A. I. McClatchey (2010). "Nf2/Merlin controls progenitor homeostasis and tumorigenesis in the liver." Genes Dev **24**(16): 1718-1730.
Beverdam, A., C. Claxton, X. Zhang, G. James, K. F. Harvey and B. Key (2013). "Yap controls stem/progenitor cell proliferation in the mouse postnatal epidermis." J Invest Dermatol **133**(6): 1497-1505.
Beyer, I., R. van Rensburg and A. Lieber (2013). "Overcoming physical barriers in cancer therapy." Tissue Barriers **1**(1): e23647.
Beyer, T. A., A. Weiss, Y. Khomchuk, K. Huang, A. A. Ogunjimi, X. Varelas and J. L. Wrana (2013). "Switch enhancers interpret TGF-beta and Hippo signaling to control cell fate in human embryonic stem cells." Cell Rep **5**(6): 1611-1624.
Bhat, K. P., K. L. Salazar, V. Balasubramaniyan, K. Wani, L. Heathcock, F. Hollingsworth, J. D. James, J. Gumin, K. L. Diefes, S. H. Kim, A. Turski, Y. Azodi,

Y. Yang, T. Doucette, H. Colman, E. P. Sulman, F. F. Lang, G. Rao, S. Copray, B. D. Vaillant and K. D. Aldape (2011). "The transcriptional coactivator TAZ regulates mesenchymal differentiation in malignant glioma." Genes Dev **25**(24): 2594-2609.
Bhora, F. Y., B. J. Dunkin, S. Batzri, H. M. Aly, B. L. Bass, A. N. Sidawy and J. W. Harmon (1995). "Effect of growth factors on cell proliferation and epithelialization in human skin." J Surg Res **59**(2): 236-244.
Bilder, D. (2004). "Epithelial polarity and proliferation control: links from the Drosophila neoplastic tumor suppressors." Genes Dev **18**(16): 1909-1925.
Blanpain, C. and E. Fuchs (2009). "Epidermal homeostasis: a balancing act of stem cells in the skin." Nat Rev Mol Cell Biol **10**(3): 207-217.
Bohnert, A., J. Hornung, I. C. Mackenzie and N. E. Fusenig (1986). "Epithelial-mesenchymal interactions control basement membrane production and differentiation in cultured and transplanted mouse keratinocytes." Cell Tissue Res **244**(2): 413-429.
Bosman, F. T. and I. Stamenkovic (2003). "Functional structure and composition of the extracellular matrix." J Pathol **200**(4): 423-428.
Bottcher, R. T. and R. Fassler (2014). "Membrane tension drives ligand-independent integrin signaling." EMBO J **33**(21): 2439-2441.
Boulay, J. L., C. Dennefeld and A. Alberga (1987). "The Drosophila developmental gene snail encodes a protein with nucleic acid binding fingers." Nature **330**(6146): 395-398.
Brabletz, S., M. Lasierra Losada, O. Schmalhofer, J. Mitschke, A. Krebs, T. Brabletz and M. P. Stemmler (2017). "Generation and characterization of mice for conditional inactivation of Zeb1." Genesis **55**(4).
Brabletz, T., R. Kalluri, M. A. Nieto and R. A. Weinberg (2018). "EMT in cancer." Nat Rev Cancer **18**(2): 128-134.
Brakebusch, C., R. Grose, F. Quondamatteo, A. Ramirez, J. L. Jorcano, A. Pirro, M. Svensson, R. Herken, T. Sasaki, R. Timpl, S. Werner and R. Fassler (2000). "Skin and hair follicle integrity is crucially dependent on beta 1 integrin expression on keratinocytes." EMBO J **19**(15): 3990-4003.
Broihier, H. T., L. A. Moore, M. Van Doren, S. Newman and R. Lehmann (1998). "zfh-1 is required for germ cell migration and gonadal mesoderm development in Drosophila." Development **125**(4): 655-666.
Bronsert, P., I. Kohler, S. Timme, S. Kiefer, M. Werner, O. Schilling, Y. Vashist, F. Makowiec, T. Brabletz, U. T. Hopt, D. Bausch, B. Kulemann, T. Keck and U. F. Wellner (2014). "Prognostic significance of Zinc finger E-box binding homeobox 1 (ZEB1) expression in cancer cells and cancer-associated fibroblasts in pancreatic head cancer." Surgery **156**(1): 97-108.
Bryant, D. M. and K. E. Mostov (2008). "From cells to organs: building polarized tissue." Nat Rev Mol Cell Biol **9**(11): 887-901.
Bryant, P. J. and P. Simpson (1984). "Intrinsic and extrinsic control of growth in developing organs." Q Rev Biol **59**(4): 387-415.
Burridge, K. and M. Chrzanowska-Wodnicka (1996). "Focal adhesions, contractility, and signaling." Annu Rev Cell Dev Biol **12**: 463-518.
Cai, J., A. Maitra, R. A. Anders, M. M. Taketo and D. Pan (2015). "beta-Catenin destruction complex-independent regulation of Hippo-YAP signaling by APC in intestinal tumorigenesis." Genes Dev **29**(14): 1493-1506.
Cai, J., N. Zhang, Y. Zheng, R. F. de Wilde, A. Maitra and D. Pan (2010). "The Hippo signaling pathway restricts the oncogenic potential of an intestinal regeneration program." Genes Dev **24**(21): 2383-2388.

Cai, Y. and M. P. Sheetz (2009). "Force propagation across cells: mechanical coherence of dynamic cytoskeletons." Curr Opin Cell Biol **21**(1): 47-50.
Calvo, F., N. Ege, A. Grande-Garcia, S. Hooper, R. P. Jenkins, S. I. Chaudhry, K. Harrington, P. Williamson, E. Moeendarbary, G. Charras and E. Sahai (2013). "Mechanotransduction and YAP-dependent matrix remodelling is required for the generation and maintenance of cancer-associated fibroblasts." Nat Cell Biol **15**(6): 637-646.
Camargo, F. D., S. Gokhale, J. B. Johnnidis, D. Fu, G. W. Bell, R. Jaenisch and T. R. Brummelkamp (2007). "YAP1 increases organ size and expands undifferentiated progenitor cells." Curr Biol **17**(23): 2054-2060.
Cancer Genome Atlas Research, N. (2011). "Integrated genomic analyses of ovarian carcinoma." Nature **474**(7353): 609-615.
Candi, E., R. Schmidt and G. Melino (2005). "The cornified envelope: a model of cell death in the skin." Nat Rev Mol Cell Biol **6**(4): 328-340.
Capaldo, C. T. and I. G. Macara (2007). "Depletion of E-cadherin disrupts establishment but not maintenance of cell junctions in Madin-Darby canine kidney epithelial cells." Mol Biol Cell **18**(1): 189-200.
Carroll, J. M., M. R. Romero and F. M. Watt (1995). "Suprabasal integrin expression in the epidermis of transgenic mice results in developmental defects and a phenotype resembling psoriasis." Cell **83**(6): 957-968.
Cassarino, D. S., D. P. Derienzo and R. J. Barr (2006). "Cutaneous squamous cell carcinoma: a comprehensive clinicopathologic classification. Part one." J Cutan Pathol **33**(3): 191-206.
Cassarino, D. S., K. G. Linden and R. J. Barr (2005). "Cutaneous keratocyst arising independently of the nevoid basal cell carcinoma syndrome." Am J Dermatopathol **27**(2): 177-178.
Cavatorta, A. L., G. Fumero, D. Chouhy, R. Aguirre, A. L. Nocito, A. A. Giri, L. Banks and D. Gardiol (2004). "Differential expression of the human homologue of drosophila discs large oncosuppressor in histologic samples from human papillomavirus-associated lesions as a marker for progression to malignancy." Int J Cancer **111**(3): 373-380.
Chamberlain, E. M. and M. M. Sanders (1999). "Identification of the novel player deltaEF1 in estrogen transcriptional cascades." Mol Cell Biol **19**(5): 3600-3606.
Chan, E. H., M. Nousiainen, R. B. Chalamalasetty, A. Schafer, E. A. Nigg and H. H. Sillje (2005). "The Ste20-like kinase Mst2 activates the human large tumor suppressor kinase Lats1." Oncogene **24**(12): 2076-2086.
Chan, S. W., C. J. Lim, Y. F. Chong, A. V. Pobbati, C. Huang and W. Hong (2011). "Hippo pathway-independent restriction of TAZ and YAP by angiomotin." J Biol Chem **286**(9): 7018-7026.
Chan, S. W., C. J. Lim, K. Guo, C. P. Ng, I. Lee, W. Hunziker, Q. Zeng and W. Hong (2008). "A role for TAZ in migration, invasion, and tumorigenesis of breast cancer cells." Cancer Res **68**(8): 2592-2598.
Chan, S. W., C. J. Lim, L. S. Loo, Y. F. Chong, C. Huang and W. Hong (2009). "TEADs mediate nuclear retention of TAZ to promote oncogenic transformation." J Biol Chem **284**(21): 14347-14358.
Chang, C., H. L. Goel, H. Gao, B. Pursell, L. D. Shultz, D. L. Greiner, S. Ingerpuu, M. Patarroyo, S. Cao, E. Lim, J. Mao, K. K. McKee, P. D. Yurchenco and A. M. Mercurio (2015). "A laminin 511 matrix is regulated by TAZ and functions as the ligand for the alpha6Bbeta1 integrin to sustain breast cancer stem cells." Genes Dev **29**(1): 1-6.

Chaturvedi, D., H. Reichert, R. D. Gunage and K. VijayRaghavan (2017). "Identification and functional characterization of muscle satellite cells in Drosophila." Elife **6**.
Chen, C. L., K. M. Gajewski, F. Hamaratoglu, W. Bossuyt, L. Sansores-Garcia, C. Tao and G. Halder (2010). "The apical-basal cell polarity determinant Crumbs regulates Hippo signaling in Drosophila." Proc Natl Acad Sci U S A **107**(36): 15810-15815.
Chen, C. L., M. C. Schroeder, M. Kango-Singh, C. Tao and G. Halder (2012). "Tumor suppression by cell competition through regulation of the Hippo pathway." Proc Natl Acad Sci U S A **109**(2): 484-489.
Chen, C. S., J. Tan and J. Tien (2004). "Mechanotransduction at cell-matrix and cell-cell contacts." Annu Rev Biomed Eng **6**: 275-302.
Chen, K. D., Y. S. Li, M. Kim, S. Li, S. Yuan, S. Chien and J. Y. Shyy (1999). "Mechanotransduction in response to shear stress. Roles of receptor tyrosine kinases, integrins, and Shc." J Biol Chem **274**(26): 18393-18400.
Chen, N., C. C. Chen and L. F. Lau (2000). "Adhesion of human skin fibroblasts to Cyr61 is mediated through integrin alpha 6beta 1 and cell surface heparan sulfate proteoglycans." J Biol Chem **275**(32): 24953-24961.
Chen, Q., N. Zhang, R. S. Gray, H. Li, A. J. Ewald, C. A. Zahnow and D. Pan (2014). "A temporal requirement for Hippo signaling in mammary gland differentiation, growth, and tumorigenesis." Genes Dev **28**(5): 432-437.
Choquet, D., D. P. Felsenfeld and M. P. Sheetz (1997). "Extracellular matrix rigidity causes strengthening of integrin-cytoskeleton linkages." Cell **88**(1): 39-48.
Codelia, V. A., G. Sun and K. D. Irvine (2014). "Regulation of YAP by mechanical strain through Jnk and Hippo signaling." Curr Biol **24**(17): 2012-2017.
Collinet, C. and T. Lecuit (2013). "Stability and dynamics of cell-cell junctions." Prog Mol Biol Transl Sci **116**: 25-47.
Comijn, J., G. Berx, P. Vermassen, K. Verschueren, L. van Grunsven, E. Bruyneel, M. Mareel, D. Huylebroeck and F. van Roy (2001). "The two-handed E box binding zinc finger protein SIP1 downregulates E-cadherin and induces invasion." Mol Cell **7**(6): 1267-1278.
Cordenonsi, M., F. Zanconato, L. Azzolin, M. Forcato, A. Rosato, C. Frasson, M. Inui, M. Montagner, A. R. Parenti, A. Poletti, M. G. Daidone, S. Dupont, G. Basso, S. Bicciato and S. Piccolo (2011). "The Hippo transducer TAZ confers cancer stem cell-related traits on breast cancer cells." Cell **147**(4): 759-772.
Cordero, J. B., R. A. Ridgway, N. Valeri, C. Nixon, M. C. Frame, W. J. Muller, M. Vidal and O. J. Sansom (2014). "c-Src drives intestinal regeneration and transformation." EMBO J **33**(13): 1474-1491.
Coussens, L. M. and Z. Werb (2002). "Inflammation and cancer." Nature **420**(6917): 860-867.
Coyer, S. R., A. Singh, D. W. Dumbauld, D. A. Calderwood, S. W. Craig, E. Delamarche and A. J. Garcia (2012). "Nanopatterning reveals an ECM area threshold for focal adhesion assembly and force transmission that is regulated by integrin activation and cytoskeleton tension." J Cell Sci **125**(Pt 21): 5110-5123.
Creedon, H. and V. G. Brunton (2012). "Src kinase inhibitors: promising cancer therapeutics?" Crit Rev Oncog **17**(2): 145-159.
Crosnier, C., D. Stamataki and J. Lewis (2006). "Organizing cell renewal in the intestine: stem cells, signals and combinatorial control." Nat Rev Genet **7**(5): 349-359.

Cui, X., R. T. Morales, W. Qian, H. Wang, J. P. Gagner, I. Dolgalev, D. Placantonakis, D. Zagzag, L. Cimmino, M. Snuderl, R. H. W. Lam and W. Chen (2018). "Hacking macrophage-associated immunosuppression for regulating glioblastoma angiogenesis." Biomaterials **161**: 164-178.
Dauphin, M., C. Barbe, S. Lemaire, B. Nawrocki-Raby, E. Lagonotte, G. Delepine, P. Birembaut, C. Gilles and M. Polette (2013). "Vimentin expression predicts the occurrence of metastases in non small cell lung carcinomas." Lung Cancer **81**(1): 117-122.
David, C. J., Y. H. Huang, M. Chen, J. Su, Y. Zou, N. Bardeesy, C. A. Iacobuzio-Donahue and J. Massague (2016). "TGF-beta Tumor Suppression through a Lethal EMT." Cell **164**(5): 1015-1030.
Davies, M., M. Robinson, E. Smith, S. Huntley, S. Prime and I. Paterson (2005). "Induction of an epithelial to mesenchymal transition in human immortal and malignant keratinocytes by TGF-beta1 involves MAPK, Smad and AP-1 signalling pathways." J Cell Biochem **95**(5): 918-931.
de Guzman Strong, C., P. W. Wertz, C. Wang, F. Yang, P. S. Meltzer, T. Andl, S. E. Millar, I. C. Ho, S. Y. Pai and J. A. Segre (2006). "Lipid defect underlies selective skin barrier impairment of an epidermal-specific deletion of Gata-3." J Cell Biol **175**(4): 661-670.
De Wever, O. and M. Mareel (2003). "Role of tissue stroma in cancer cell invasion." J Pathol **200**(4): 429-447.
Debaugnies, M., A. Sanchez-Danes, S. Rorive, M. Raphael, M. Liagre, M. A. Parent, A. Brisebarre, I. Salmon and C. Blanpain (2018). "YAP and TAZ are essential for basal and squamous cell carcinoma initiation." EMBO Rep **19**(7).
Dellambra, E., O. Golisano, S. Bondanza, E. Siviero, P. Lacal, M. Molinari, S. D'Atri and M. De Luca (2000). "Downregulation of 14-3-3sigma prevents clonal evolution and leads to immortalization of primary human keratinocytes." J Cell Biol **149**(5): 1117-1130.
DeNardo, D. G., P. Andreu and L. M. Coussens (2010). "Interactions between lymphocytes and myeloid cells regulate pro- versus anti-tumor immunity." Cancer Metastasis Rev **29**(2): 309-316.
Deng, H., W. Wang, J. Yu, Y. Zheng, Y. Qing and D. Pan (2015). "Spectrin regulates Hippo signaling by modulating cortical actomyosin activity." Elife **4**: e06567.
Dickinson, D. J., W. J. Nelson and W. I. Weis (2011). "A polarized epithelium organized by beta- and alpha-catenin predates cadherin and metazoan origins." Science **331**(6022): 1336-1339.
Discher, D. E., P. Janmey and Y. L. Wang (2005). "Tissue cells feel and respond to the stiffness of their substrate." Science **310**(5751): 1139-1143.
Dittmer, J. E., R. J. Goss and C. E. Dinsmore (1974). "The growth of infant hearts grafted to young and adult rats." Am J Anat **141**(1): 155-160.
Dobereiner, H. G., B. J. Dubin-Thaler, G. Giannone and M. P. Sheetz (2005). "Force sensing and generation in cell phases: analyses of complex functions." J Appl Physiol (1985) **98**(4): 1542-1546.
Dolberg, D. S., R. Hollingsworth, M. Hertle and M. J. Bissell (1985). "Wounding and its role in RSV-mediated tumor formation." Science **230**(4726): 676-678.
Dong, J., G. Feldmann, J. Huang, S. Wu, N. Zhang, S. A. Comerford, M. F. Gayyed, R. A. Anders, A. Maitra and D. Pan (2007). "Elucidation of a universal size-control mechanism in Drosophila and mammals." Cell **130**(6): 1120-1133.

Doupe, D. P., A. M. Klein, B. D. Simons and P. H. Jones (2010). "The ordered architecture of murine ear epidermis is maintained by progenitor cells with random fate." Dev Cell **18**(2): 317-323.
Dumbauld, D. W., T. T. Lee, A. Singh, J. Scrimgeour, C. A. Gersbach, E. A. Zamir, J. Fu, C. S. Chen, J. E. Curtis, S. W. Craig and A. J. Garcia (2013). "How vinculin regulates force transmission." Proc Natl Acad Sci U S A **110**(24): 9788-9793.
du Bois-Reymond E (1848-84). Untersuchungen iiber thierische Elektricitdt, 2 vols., Berlin
Dupont, S., L. Morsut, M. Aragona, E. Enzo, S. Giulitti, M. Cordenonsi, F. Zanconato, J. Le Digabel, M. Forcato, S. Bicciato, N. Elvassore and S. Piccolo (2011). "Role of YAP/TAZ in mechanotransduction." Nature **474**(7350): 179-183.
Dvorak, H. F. (1986). "Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing." N Engl J Med **315**(26): 1650-1659.
Edgar, B. A. (2006). "From cell structure to transcription: Hippo forges a new path." Cell **124**(2): 267-273.
Edgar, B. A. (2006). "How flies get their size: genetics meets physiology." Nat Rev Genet **7**(12): 907-916.
Ege, N., A. M. Dowbaj, M. Jiang, M. Howell, S. Hooper, C. Foster, R. P. Jenkins and E. Sahai (2018). "Quantitative Analysis Reveals that Actin and Src-Family Kinases Regulate Nuclear YAP1 and Its Export." Cell Syst **6**(6): 692-708 e613.
Eger, A., K. Aigner, S. Sonderegger, B. Dampier, S. Oehler, M. Schreiber, G. Berx, A. Cano, H. Beug and R. Foisner (2005). "DeltaEF1 is a transcriptional repressor of E-cadherin and regulates epithelial plasticity in breast cancer cells." Oncogene **24**(14): 2375-2385.
Elbediwy, A., Z. I. Vincent-Mistiaen, B. Spencer-Dene, R. K. Stone, S. Boeing, S. K. Wculek, J. Cordero, E. H. Tan, R. Ridgway, V. G. Brunton, E. Sahai, H. Gerhardt, A. Behrens, I. Malanchi, O. J. Sansom and B. J. Thompson (2016). "Integrin signalling regulates YAP and TAZ to control skin homeostasis." Development **143**(10): 1674-1687.
Elias, D. and H. J. Ditzel (2015). "The potential of Src inhibitors." Aging (Albany NY) **7**(10): 734-735.
Elosegui-Artola, A., I. Andreu, A. E. M. Beedle, A. Lezamiz, M. Uroz, A. J. Kosmalska, R. Oria, J. Z. Kechagia, P. Rico-Lastres, A. L. Le Roux, C. M. Shanahan, X. Trepat, D. Navajas, S. Garcia-Manyes and P. Roca-Cusachs (2017). "Force Triggers YAP Nuclear Entry by Regulating Transport across Nuclear Pores." Cell **171**(6): 1397-1410 e1314.
Enomoto, M. and T. Igaki (2013). "Src controls tumorigenesis via JNK-dependent regulation of the Hippo pathway in Drosophila." EMBO Rep **14**(1): 65-72.
Fan, R., N. G. Kim and B. M. Gumbiner (2013). "Regulation of Hippo pathway by mitogenic growth factors via phosphoinositide 3-kinase and phosphoinositide-dependent kinase-1." Proc Natl Acad Sci U S A **110**(7): 2569-2574.
Feng, X., M. S. Degese, R. Iglesias-Bartolome, J. P. Vaque, A. A. Molinolo, M. Rodrigues, M. R. Zaidi, B. R. Ksander, G. Merlino, A. Sodhi, Q. Chen and J. S. Gutkind (2014). "Hippo-independent activation of YAP by the GNAQ uveal melanoma oncogene through a trio-regulated rho GTPase signaling circuitry." Cancer Cell **25**(6): 831-845.
Feng, X., H. Zhang, J. B. Margolick and P. A. Coulombe (2013). "Keratin intracellular concentration revisited: implications for keratin function in surface epithelia." J Invest Dermatol **133**(3): 850-853.

Feng, Y., C. Santoriello, M. Mione, A. Hurlstone and P. Martin (2010). "Live imaging of innate immune cell sensing of transformed cells in zebrafish larvae: parallels between tumor initiation and wound inflammation." PLoS Biol **8**(12): e1000562.
Fernandez, B. G., P. Gaspar, C. Bras-Pereira, B. Jezowska, S. R. Rebelo and F. Janody (2011). "Actin-Capping Protein and the Hippo pathway regulate F-actin and tissue growth in Drosophila." Development **138**(11): 2337-2346.
Fernandez, B. G., B. Jezowska and F. Janody (2014). "Drosophila actin-Capping Protein limits JNK activation by the Src proto-oncogene." Oncogene **33**(16): 2027-2039.
Fernandez, L. A., P. A. Northcott, J. Dalton, C. Fraga, D. Ellison, S. Angers, M. D. Taylor and A. M. Kenney (2009). "YAP1 is amplified and up-regulated in hedgehog-associated medulloblastomas and mediates Sonic hedgehog-driven neural precursor proliferation." Genes Dev **23**(23): 2729-2741.
Fernandez-Gonzalez, R., M. Simoes Sde, J. C. Roper, S. Eaton and J. A. Zallen (2009). "Myosin II dynamics are regulated by tension in intercalating cells." Dev Cell **17**(5): 736-743.
Ferrigno, O., F. Lallemand, F. Verrecchia, S. L'Hoste, J. Camonis, A. Atfi and A. Mauviel (2002). "Yes-associated protein (YAP65) interacts with Smad7 and potentiates its inhibitory activity against TGF-beta/Smad signaling." Oncogene **21**(32): 4879-4884.
Finn, R. S. (2008). "Targeting Src in breast cancer." Ann Oncol **19**(8): 1379-1386.
Fisher, M. L., C. Kerr, G. Adhikary, D. Grun, W. Xu, J. W. Keillor and R. L. Eckert (2016). "Transglutaminase Interaction with alpha6/beta4-Integrin Stimulates YAP1-Dependent DeltaNp63alpha Stabilization and Leads to Enhanced Cancer Stem Cell Survival and Tumor Formation." Cancer Res **76**(24): 7265-7276.
Fletcher, G. C., A. Elbediwy, I. Khanal, P. S. Ribeiro, N. Tapon and B. J. Thompson (2015). "The Spectrin cytoskeleton regulates the Hippo signalling pathway." EMBO J **34**(7): 940-954.
Fortini, M. E., Z. C. Lai and G. M. Rubin (1991). "The Drosophila zfh-1 and zfh-2 genes encode novel proteins containing both zinc-finger and homeodomain motifs." Mech Dev **34**(2-3): 113-122.
Frisch, S. M. (1997). "The epithelial cell default-phenotype hypothesis and its implications for cancer." Bioessays **19**(8): 705-709.
Fuchs, E. (2007). "Scratching the surface of skin development." Nature **445**(7130): 834-842.
Fuchs, E. and H. Green (1980). "Changes in keratin gene expression during terminal differentiation of the keratinocyte." Cell **19**(4): 1033-1042.
Fuchs, E. and S. Raghavan (2002). "Getting under the skin of epidermal morphogenesis." Nat Rev Genet **3**(3): 199-209.
Funahashi, J., R. Sekido, K. Murai, Y. Kamachi and H. Kondoh (1993). "Delta-crystallin enhancer binding protein delta EF1 is a zinc finger-homeodomain protein implicated in postgastrulation embryogenesis." Development **119**(2): 433-446.
Furuya, M., H. Masuda, K. Hara, H. Uchida, K. Sato, S. Sato, H. Asada, T. Maruyama, Y. Yoshimura, H. Katabuchi, M. Tanaka and H. Saya (2017). "ZEB1 expression is a potential indicator of invasive endometriosis." Acta Obstet Gynecol Scand **96**(9): 1128-1135.
Gabison, E. E., E. Huet, C. Baudouin and S. Menashi (2009). "Direct epithelial-stromal interaction in corneal wound healing: Role of EMMPRIN/CD147 in MMPs induction and beyond." Prog Retin Eye Res **28**(1): 19-33.

Galli, G. G., M. Carrara, W. C. Yuan, C. Valdes-Quezada, B. Gurung, B. Pepe-Mooney, T. Zhang, G. Geeven, N. S. Gray, W. de Laat, R. A. Calogero and F. D. Camargo (2015). "YAP Drives Growth by Controlling Transcriptional Pause Release from Dynamic Enhancers." Mol Cell **60**(2): 328-337.
Gardiol, D., A. Zacchi, F. Petrera, G. Stanta and L. Banks (2006). "Human discs large and scrib are localized at the same regions in colon mucosa and changes in their expression patterns are correlated with loss of tissue architecture during malignant progression." Int J Cancer **119**(6): 1285-1290.
Gaspar, P. and N. Tapon (2014). "Sensing the local environment: actin architecture and Hippo signalling." Curr Opin Cell Biol **31**: 74-83.
Gasparics, A. and A. Sebe (2018). "MRTFs- master regulators of EMT." Dev Dyn **247**(3): 396-404.
Geiger, B., J. P. Spatz and A. D. Bershadsky (2009). "Environmental sensing through focal adhesions." Nat Rev Mol Cell Biol **10**(1): 21-33.
Genevet, A., M. C. Wehr, R. Brain, B. J. Thompson and N. Tapon (2010). "Kibra is a regulator of the Salvador/Warts/Hippo signaling network." Dev Cell **18**(2): 300-308.
Gladden, A. B., A. M. Hebert, E. E. Schneeberger and A. I. McClatchey (2010). "The NF2 tumor suppressor, Merlin, regulates epidermal development through the establishment of a junctional polarity complex." Dev Cell **19**(5): 727-739.
Goodell, M. A., H. Nguyen and N. Shroyer (2015). "Somatic stem cell heterogeneity: diversity in the blood, skin and intestinal stem cell compartments." Nat Rev Mol Cell Biol **16**(5): 299-309.
Grannas, K., L. Arngarden, P. Lonn, M. Mazurkiewicz, A. Blokzijl, A. Zieba and O. Soderberg (2015). "Crosstalk between Hippo and TGFbeta: Subcellular Localization of YAP/TAZ/Smad Complexes." J Mol Biol **427**(21): 3407-3415.
Grashoff, C., A. Aszodi, T. Sakai, E. B. Hunziker and R. Fassler (2003). "Integrin-linked kinase regulates chondrocyte shape and proliferation." EMBO Rep **4**(4): 432-438.
Green, K. J., S. Getsios, S. Troyanovsky and L. M. Godsel (2010). "Intercellular junction assembly, dynamics, and homeostasis." Cold Spring Harb Perspect Biol **2**(2): a000125.
Gregorieff, A., Y. Liu, M. R. Inanlou, Y. Khomchuk and J. L. Wrana (2015). "Yap-dependent reprogramming of Lgr5(+) stem cells drives intestinal regeneration and cancer." Nature **526**(7575): 715-718.
Grewal, S. S. (2009). "Insulin/TOR signaling in growth and homeostasis: a view from the fly world." Int J Biochem Cell Biol **41**(5): 1006-1010.
Grijalva, J. L., M. Huizenga, K. Mueller, S. Rodriguez, J. Brazzo, F. Camargo, G. Sadri-Vakili and K. Vakili (2014). "Dynamic alterations in Hippo signaling pathway and YAP activation during liver regeneration." Am J Physiol Gastrointest Liver Physiol **307**(2): G196-204.
Grivennikov, S. I. and M. Karin (2010). "Dangerous liaisons: STAT3 and NF-kappaB collaboration and crosstalk in cancer." Cytokine Growth Factor Rev **21**(1): 11-19.
Grose, R., C. Hutter, W. Bloch, I. Thorey, F. M. Watt, R. Fassler, C. Brakebusch and S. Werner (2002). "A crucial role of beta 1 integrins for keratinocyte migration in vitro and during cutaneous wound repair." Development **129**(9): 2303-2315.
Grotendorst, G. R., H. Rahmanie and M. R. Duncan (2004). "Combinatorial signaling pathways determine fibroblast proliferation and myofibroblast differentiation." FASEB J **18**(3): 469-479.

Grusche, F. A., J. L. Degoutin, H. E. Richardson and K. F. Harvey (2011). "The Salvador/Warts/Hippo pathway controls regenerative tissue growth in Drosophila melanogaster." Dev Biol **350**(2): 255-266.
Grzeschik, N. A., L. M. Parsons, M. L. Allott, K. F. Harvey and H. E. Richardson (2010). "Lgl, aPKC, and Crumbs regulate the Salvador/Warts/Hippo pathway through two distinct mechanisms." Curr Biol **20**(7): 573-581.
Gu, Y., Y. Zhao, Y. Zhou, Y. Xie, P. Ju, Y. Long, J. Liu, D. Ni, F. Cao, Z. Lyu, Z. Mao, J. Hao, Y. Li, Q. Wan, Q. Kanyomse, Y. Liu, D. Ren, Y. Ning, X. Li, Q. Zhou and B. Li (2016). "Zeb1 Is a Potential Regulator of Six2 in the Proliferation, Apoptosis and Migration of Metanephric Mesenchyme Cells." Int J Mol Sci **17**(8).
Guaita, S., I. Puig, C. Franci, M. Garrido, D. Dominguez, E. Batlle, E. Sancho, S. Dedhar, A. G. De Herreros and J. Baulida (2002). "Snail induction of epithelial to mesenchymal transition in tumor cells is accompanied by MUC1 repression and ZEB1 expression." J Biol Chem **277**(42): 39209-39216.
Gumbiner, B. M. and N. G. Kim (2014). "The Hippo-YAP signaling pathway and contact inhibition of growth." J Cell Sci **127**(Pt 4): 709-717.
Gurtner, G. C., S. Werner, Y. Barrandon and M. T. Longaker (2008). "Wound repair and regeneration." Nature **453**(7193): 314-321.
Haddow, A. (1972). "Molecular repair, wound healing, and carcinogenesis: tumor production a possible overhealing?" Adv Cancer Res **16**: 181-234.
Halder, G. and R. L. Johnson (2011). "Hippo signaling: growth control and beyond." Development **138**(1): 9-22.
Hall, C. A., R. Wang, J. Miao, E. Oliva, X. Shen, T. Wheeler, S. G. Hilsenbeck, S. Orsulic and S. Goode (2010). "Hippo pathway effector Yap is an ovarian cancer oncogene." Cancer Res **70**(21): 8517-8525.
Hamaratoglu, F., M. Willecke, M. Kango-Singh, R. Nolo, E. Hyun, C. Tao, H. Jafar-Nejad and G. Halder (2006). "The tumour-suppressor genes NF2/Merlin and Expanded act through Hippo signalling to regulate cell proliferation and apoptosis." Nat Cell Biol **8**(1): 27-36.
Han, S. X., E. Bai, G. H. Jin, C. C. He, X. J. Guo, L. J. Wang, M. Li, X. Ying and Q. Zhu (2014). "Expression and clinical significance of YAP, TAZ, and AREG in hepatocellular carcinoma." J Immunol Res **2014**: 261365.
Hanahan, D. and R. A. Weinberg (2011). "Hallmarks of cancer: the next generation." Cell **144**(5): 646-674.
Hansen, C. G., T. Moroishi and K. L. Guan (2015). "YAP and TAZ: a nexus for Hippo signaling and beyond." Trends Cell Biol **25**(9): 499-513.
Harrison, R. G. (1924). "Some Unexpected Results of the Heteroplastic Transplantation of Limbs." Proc Natl Acad Sci U S A **10**(2): 69-74.
Hartnett, L. and L. J. Egan (2012). "Inflammation, DNA methylation and colitis-associated cancer." Carcinogenesis **33**(4): 723-731.
Harvey, K. and N. Tapon (2007). "The Salvador-Warts-Hippo pathway - an emerging tumour-suppressor network." Nat Rev Cancer **7**(3): 182-191.
Harvey, K. F., C. M. Pfleger and I. K. Hariharan (2003). "The Drosophila Mst ortholog, hippo, restricts growth and cell proliferation and promotes apoptosis." Cell **114**(4): 457-467.
Harvey, K. F., X. Zhang and D. M. Thomas (2013). "The Hippo pathway and human cancer." Nat Rev Cancer **13**(4): 246-257.
He, C., D. Mao, G. Hua, X. Lv, X. Chen, P. C. Angeletti, J. Dong, S. W. Remmenga, K. J. Rodabaugh, J. Zhou, P. F. Lambert, P. Yang, J. S. Davis and C. Wang (2015). "The Hippo/YAP pathway interacts with EGFR signaling and HPV

oncoproteins to regulate cervical cancer progression." EMBO Mol Med **7**(11): 1426-1449.
Heallen, T., M. Zhang, J. Wang, M. Bonilla-Claudio, E. Klysik, R. L. Johnson and J. F. Martin (2011). "Hippo pathway inhibits Wnt signaling to restrain cardiomyocyte proliferation and heart size." Science **332**(6028): 458-461.
Heng, E. C., Y. Huang, S. A. Black, Jr. and P. C. Trackman (2006). "CCN2, connective tissue growth factor, stimulates collagen deposition by gingival fibroblasts via module 3 and alpha6- and beta1 integrins." J Cell Biochem **98**(2): 409-420.
Herr, K. J., Y. H. Tsang, J. W. Ong, Q. Li, L. L. Yap, W. Yu, H. Yin, R. L. Bogorad, J. E. Dahlman, Y. G. Chan, B. H. Bay, R. Singaraja, D. G. Anderson, V. Koteliansky, V. Viasnoff and J. P. Thiery (2014). "Loss of alpha-catenin elicits a cholestatic response and impairs liver regeneration." Sci Rep **4**: 6835.
Hiemer, S. E., L. Zhang, V. K. Kartha, T. S. Packer, M. Almershed, V. Noonan, M. Kukuruzinska, M. V. Bais, S. Monti and X. Varelas (2015). "A YAP/TAZ-Regulated Molecular Signature Is Associated with Oral Squamous Cell Carcinoma." Mol Cancer Res **13**(6): 957-968.
Hietakangas, V. and S. M. Cohen (2009). "Regulation of tissue growth through nutrient sensing." Annu Rev Genet **43**: 389-410.
Hirata, H., M. Sokabe and C. T. Lim (2014). "Molecular mechanisms underlying the force-dependent regulation of actin-to-ECM linkage at the focal adhesions." Prog Mol Biol Transl Sci **126**: 135-154.
Hirate, Y., S. Hirahara, K. Inoue, A. Suzuki, V. B. Alarcon, K. Akimoto, T. Hirai, T. Hara, M. Adachi, K. Chida, S. Ohno, Y. Marikawa, K. Nakao, A. Shimono and H. Sasaki (2013). "Polarity-dependent distribution of angiomotin localizes Hippo signaling in preimplantation embryos." Curr Biol **23**(13): 1181-1194.
Ho, L. L., X. Wei, T. Shimizu and Z. C. Lai (2010). "Mob as tumor suppressor is activated at the cell membrane to control tissue growth and organ size in Drosophila." Dev Biol **337**(2): 274-283.
Homberg, M. and T. M. Magin (2014). "Beyond expectations: novel insights into epidermal keratin function and regulation." Int Rev Cell Mol Biol **311**: 265-306.
Hong, A. W., Z. Meng and K. L. Guan (2016). "The Hippo pathway in intestinal regeneration and disease." Nat Rev Gastroenterol Hepatol **13**(6): 324-337.
Hong, J. H., E. S. Hwang, M. T. McManus, A. Amsterdam, Y. Tian, R. Kalmukova, E. Mueller, T. Benjamin, B. M. Spiegelman, P. A. Sharp, N. Hopkins and M. B. Yaffe (2005). "TAZ, a transcriptional modulator of mesenchymal stem cell differentiation." Science **309**(5737): 1074-1078.
Hossain, Z., S. M. Ali, H. L. Ko, J. Xu, C. P. Ng, K. Guo, Z. Qi, S. Ponniah, W. Hong and W. Hunziker (2007). "Glomerulocystic kidney disease in mice with a targeted inactivation of Wwtr1." Proc Natl Acad Sci U S A **104**(5): 1631-1636.
Housman, T. S., S. R. Feldman, P. M. Williford, A. B. Fleischer, Jr., N. D. Goldman, J. M. Acostamadiedo and G. J. Chen (2003). "Skin cancer is among the most costly of all cancers to treat for the Medicare population." J Am Acad Dermatol **48**(3): 425-429.
Huang, J., S. Wu, J. Barrera, K. Matthews and D. Pan (2005). "The Hippo signaling pathway coordinately regulates cell proliferation and apoptosis by inactivating Yorkie, the Drosophila Homolog of YAP." Cell **122**(3): 421-434.
Huang, L. and S. K. Muthuswamy (2010). "Polarity protein alterations in carcinoma: a focus on emerging roles for polarity regulators." Curr Opin Genet Dev **20**(1): 41-50.

Hudson, L. G. and L. J. McCawley (1998). "Contributions of the epidermal growth factor receptor to keratinocyte motility." Microsc Res Tech **43**(5): 444-455.
Hugo, H. J., M. I. Kokkinos, T. Blick, M. L. Ackland, E. W. Thompson and D. F. Newgreen (2011). "Defining the E-cadherin repressor interactome in epithelial-mesenchymal transition: the PMC42 model as a case study." Cells Tissues Organs **193**(1-2): 23-40.
Humphrey, J. D., E. R. Dufresne and M. A. Schwartz (2014). "Mechanotransduction and extracellular matrix homeostasis." Nat Rev Mol Cell Biol **15**(12): 802-812.
Hurt, E. M., J. N. Saykally, B. M. Anose, K. R. Kalli and M. M. Sanders (2008). "Expression of the ZEB1 (deltaEF1) transcription factor in human: additional insights." Mol Cell Biochem **318**(1-2): 89-99.
Hussain, S. P. and C. C. Harris (2007). "Inflammation and cancer: an ancient link with novel potentials." Int J Cancer **121**(11): 2373-2380.
Huttenlocher, A. and A. R. Horwitz (2011). "Integrins in cell migration." Cold Spring Harb Perspect Biol **3**(9): a005074.
Hynes, R. O. (2002). "Integrins: bidirectional, allosteric signaling machines." Cell **110**(6): 673-687.
Ikmi, A., B. Gaertner, C. Seidel, M. Srivastava, J. Zeitlinger and M. C. Gibson (2014). "Molecular evolution of the Yap/Yorkie proto-oncogene and elucidation of its core transcriptional program." Mol Biol Evol **31**(6): 1375-1390.
Imajo, M., M. Ebisuya and E. Nishida (2015). "Dual role of YAP and TAZ in renewal of the intestinal epithelium." Nat Cell Biol **17**(1): 7-19.
India Project Team of the International Cancer Genome, C. (2013). "Mutational landscape of gingivo-buccal oral squamous cell carcinoma reveals new recurrently-mutated genes and molecular subgroups." Nat Commun **4**: 2873.
Jaalouk, D. E. and J. Lammerding (2009). "Mechanotransduction gone awry." Nat Rev Mol Cell Biol **10**(1): 63-73.
Janmey, P. A. and R. T. Miller (2011). "Mechanisms of mechanical signaling in development and disease." J Cell Sci **124**(Pt 1): 9-18.
Jia, B., H. Liu, Q. Kong and B. Li (2012). "Overexpression of ZEB1 associated with metastasis and invasion in patients with gastric carcinoma." Mol Cell Biochem **366**(1-2): 223-229.
Jia, J., C. Li, S. Luo, F. Liu-Smith, J. Yang, X. Wang, N. Wang, B. Lai, T. Lei, Q. Wang, S. Xiao, Y. Shao and Y. Zheng (2016). "Yes-Associated Protein Contributes to the Development of Human Cutaneous Squamous Cell Carcinoma via Activation of RAS." J Invest Dermatol **136**(6): 1267-1277.
Jia, J., C. Li, J. Yang, X. Wang, R. Li, S. Luo, Z. Li, J. Liu, Z. Liu and Y. Zheng (2018). "Yes-associated protein promotes the abnormal proliferation of psoriatic keratinocytes via an amphiregulin dependent pathway." Sci Rep **8**(1): 14513.
Jin, Y., J. Xu, M. X. Yin, Y. Lu, L. Hu, P. Li, P. Zhang, Z. Yuan, M. S. Ho, H. Ji, Y. Zhao and L. Zhang (2013). "Brahma is essential for Drosophila intestinal stem cell proliferation and regulated by Hippo signaling." Elife **2**: e00999.
Johnson, R. and G. Halder (2014). "The two faces of Hippo: targeting the Hippo pathway for regenerative medicine and cancer treatment." Nat Rev Drug Discov **13**(1): 63-79.
Jones, C. and H. P. Ehrlich (2011). "Fibroblast expression of alpha-smooth muscle actin, alpha2beta1 integrin and alphavbeta3 integrin: influence of surface rigidity." Exp Mol Pathol **91**(1): 394-399.
Jones, P. H., S. Harper and F. M. Watt (1995). "Stem cell patterning and fate in human epidermis." Cell **80**(1): 83-93.

Jones, P. H. and F. M. Watt (1993). "Separation of human epidermal stem cells from transit amplifying cells on the basis of differences in integrin function and expression." Cell **73**(4): 713-724.
Joseph, J. V., S. Conroy, T. Tomar, E. Eggens-Meijer, K. Bhat, S. Copray, A. M. Walenkamp, E. Boddeke, V. Balasubramanyian, M. Wagemakers, W. F. den Dunnen and F. A. Kruyt (2014). "TGF-beta is an inducer of ZEB1-dependent mesenchymal transdifferentiation in glioblastoma that is associated with tumor invasion." Cell Death Dis **5**: e1443.
Kalluri, R. and R. A. Weinberg (2009). "The basics of epithelial-mesenchymal transition." J Clin Invest **119**(6): 1420-1428.
Kanai, F., P. A. Marignani, D. Sarbassova, R. Yagi, R. A. Hall, M. Donowitz, A. Hisaminato, T. Fujiwara, Y. Ito, L. C. Cantley and M. B. Yaffe (2000). "TAZ: a novel transcriptional co-activator regulated by interactions with 14-3-3 and PDZ domain proteins." EMBO J **19**(24): 6778-6791.
Karnoub, A. E. and R. A. Weinberg (2006). "Chemokine networks and breast cancer metastasis." Breast Dis **26**: 75-85.
Karpowicz, P., J. Perez and N. Perrimon (2010). "The Hippo tumor suppressor pathway regulates intestinal stem cell regeneration." Development **137**(24): 4135-4145.
Khavari, P. A. (2006). "Modelling cancer in human skin tissue." Nat Rev Cancer **6**(4): 270-280.
Kim, G. J., H. Kim and Y. N. Park (2013). "Increased expression of Yes-associated protein 1 in hepatocellular carcinoma with stemness and combined hepatocellular-cholangiocarcinoma." PLoS One **8**(9): e75449.
Kim, L. C., L. Song and E. B. Haura (2009). "Src kinases as therapeutic targets for cancer." Nat Rev Clin Oncol **6**(10): 587-595.
Kim, M., M. Kim, S. Lee, S. Kuninaka, H. Saya, H. Lee, S. Lee and D. S. Lim (2013). "cAMP/PKA signalling reinforces the LATS-YAP pathway to fully suppress YAP in response to actin cytoskeletal changes." EMBO J **32**(11): 1543-1555.
Kim, M., T. Kim, R. L. Johnson and D. S. Lim (2015). "Transcriptional co-repressor function of the hippo pathway transducers YAP and TAZ." Cell Rep **11**(2): 270-282.
Kim, M. K., J. W. Jang and S. C. Bae (2018). "DNA binding partners of YAP/TAZ." BMB Rep **51**(3): 126-133.
Kim, N. G. and B. M. Gumbiner (2015). "Adhesion to fibronectin regulates Hippo signaling via the FAK-Src-PI3K pathway." J Cell Biol **210**(3): 503-515.
Kim, N. G., E. Koh, X. Chen and B. M. Gumbiner (2011). "E-cadherin mediates contact inhibition of proliferation through Hippo signaling-pathway components." Proc Natl Acad Sci U S A **108**(29): 11930-11935.
Kim, T., S. J. Yang, D. Hwang, J. Song, M. Kim, S. Kyum Kim, K. Kang, J. Ahn, D. Lee, M. Y. Kim, S. Kim, J. Seung Koo, S. Seok Koh, S. Y. Kim and D. S. Lim (2015). "A basal-like breast cancer-specific role for SRF-IL6 in YAP-induced cancer stemness." Nat Commun **6**: 10186.
Kireeva, M. L., S. C. Lam and L. F. Lau (1998). "Adhesion of human umbilical vein endothelial cells to the immediate-early gene product Cyr61 is mediated through integrin alphavbeta3." J Biol Chem **273**(5): 3090-3096.
Kobielak, A. and E. Fuchs (2006). "Links between alpha-catenin, NF-kappaB, and squamous cell carcinoma in skin." Proc Natl Acad Sci U S A **103**(7): 2322-2327.
Koster, M. I. (2009). "Making an epidermis." Ann N Y Acad Sci **1170**: 7-10.
Kowalski-Chauvel, A., V. Gouaze-Andersson, L. Baricault, E. Martin, C. Delmas, C. Toulas, E. Cohen-Jonathan-Moyal and C. Seva (2019). "Alpha6-Integrin Regulates

FGFR1 Expression through the ZEB1/YAP1 Transcription Complex in Glioblastoma Stem Cells Resulting in Enhanced Proliferation and Stemness." Cancers (Basel) **11**(3).
Krebs, A. M., J. Mitschke, M. Lasierra Losada, O. Schmalhofer, M. Boerries, H. Busch, M. Boettcher, D. Mougiakakos, W. Reichardt, P. Bronsert, V. G. Brunton, C. Pilarsky, T. H. Winkler, S. Brabletz, M. P. Stemmler and T. Brabletz (2017). "The EMT-activator Zeb1 is a key factor for cell plasticity and promotes metastasis in pancreatic cancer." Nat Cell Biol **19**(5): 518-529.
Kretzschmar, K. and F. M. Watt (2014). "Markers of epidermal stem cell subpopulations in adult mammalian skin." Cold Spring Harb Perspect Med **4**(10).
Kuhn, K. A., N. A. Manieri, T. C. Liu and T. S. Stappenbeck (2014). "IL-6 stimulates intestinal epithelial proliferation and repair after injury." PLoS One **9**(12): e114195.
Kuo, J. C., X. Han, C. T. Hsiao, J. R. Yates, 3rd and C. M. Waterman (2011). "Analysis of the myosin-II-responsive focal adhesion proteome reveals a role for beta-Pix in negative regulation of focal adhesion maturation." Nat Cell Biol **13**(4): 383-393.
Kuwahara, M., M. Hatoko, H. Tada and A. Tanaka (2001). "E-cadherin expression in wound healing of mouse skin." J Cutan Pathol **28**(4): 191-199.
Lai, Z. C., X. Wei, T. Shimizu, E. Ramos, M. Rohrbaugh, N. Nikolaidis, L. L. Ho and Y. Li (2005). "Control of cell proliferation and apoptosis by mob as tumor suppressor, mats." Cell **120**(5): 675-685.
Lamar, J. M., P. Stern, H. Liu, J. W. Schindler, Z. G. Jiang and R. O. Hynes (2012). "The Hippo pathway target, YAP, promotes metastasis through its TEAD-interaction domain." Proc Natl Acad Sci U S A **109**(37): E2441-2450.
Lamar, J. M., Y. Xiao, E. Norton, Z. G. Jiang, G. M. Gerhard, S. Kooner, J. S. A. Warren and R. O. Hynes (2019). "SRC tyrosine kinase activates the YAP/TAZ axis and thereby drives tumor growth and metastasis." J Biol Chem **294**(7): 2302-2317.
Lamouille, S., J. Xu and R. Derynck (2014). "Molecular mechanisms of epithelial-mesenchymal transition." Nat Rev Mol Cell Biol **15**(3): 178-196.
Latil, M., D. Nassar, B. Beck, S. Boumahdi, L. Wang, A. Brisebarre, C. Dubois, E. Nkusi, S. Lenglez, A. Checinska, A. Vercauteren Drubbel, M. Devos, W. Declercq, R. Yi and C. Blanpain (2017). "Cell-Type-Specific Chromatin States Differentially Prime Squamous Cell Carcinoma Tumor-Initiating Cells for Epithelial to Mesenchymal Transition." Cell Stem Cell **20**(2): 191-204 e195.
Lau, A. N., S. J. Curtis, C. M. Fillmore, S. P. Rowbotham, M. Mohseni, D. E. Wagner, A. M. Beede, D. T. Montoro, K. W. Sinkevicius, Z. E. Walton, J. Barrios, D. J. Weiss, F. D. Camargo, K. K. Wong and C. F. Kim (2014). "Tumor-propagating cells and Yap/Taz activity contribute to lung tumor progression and metastasis." EMBO J **33**(5): 468-481.
Lavado, A., Y. He, J. Pare, G. Neale, E. N. Olson, M. Giovannini and X. Cao (2013). "Tumor suppressor Nf2 limits expansion of the neural progenitor pool by inhibiting Yap/Taz transcriptional coactivators." Development **140**(16): 3323-3334.
Lazarova, D. and M. Bordonaro (2017). "ZEB1 Mediates Drug Resistance and EMT in p300-Deficient CRC." J Cancer **8**(8): 1453-1459.
Lee, C. H., B. Shah, E. K. Moioli and J. J. Mao (2010). "CTGF directs fibroblast differentiation from human mesenchymal stem/stromal cells and defines connective tissue healing in a rodent injury model." J Clin Invest **120**(9): 3340-3349.
Lee, D. H., J. O. Park, T. S. Kim, S. K. Kim, T. H. Kim, M. C. Kim, G. S. Park, J. H. Kim, S. Kuninaka, E. N. Olson, H. Saya, S. Y. Kim, H. Lee and D. S. Lim (2016).

"LATS-YAP/TAZ controls lineage specification by regulating TGFbeta signaling and Hnf4alpha expression during liver development." Nat Commun **7**: 11961.
Lee, K. P., J. H. Lee, T. S. Kim, T. H. Kim, H. D. Park, J. S. Byun, M. C. Kim, W. I. Jeong, D. F. Calvisi, J. M. Kim and D. S. Lim (2010). "The Hippo-Salvador pathway restrains hepatic oval cell proliferation, liver size, and liver tumorigenesis." Proc Natl Acad Sci U S A **107**(18): 8248-8253.
Lee, M. J., M. R. Byun, M. Furutani-Seiki, J. H. Hong and H. S. Jung (2014). "YAP and TAZ regulate skin wound healing." J Invest Dermatol **134**(2): 518-525.
Legate, K. R., S. A. Wickstrom and R. Fassler (2009). "Genetic and cell biological analysis of integrin outside-in signaling." Genes Dev **23**(4): 397-418.
Lehmann, W., D. Mossmann, J. Kleemann, K. Mock, C. Meisinger, T. Brummer, R. Herr, S. Brabletz, M. P. Stemmler and T. Brabletz (2016). "ZEB1 turns into a transcriptional activator by interacting with YAP1 in aggressive cancer types." Nat Commun **7**: 10498.
Lei, Q. Y., H. Zhang, B. Zhao, Z. Y. Zha, F. Bai, X. H. Pei, S. Zhao, Y. Xiong and K. L. Guan (2008). "TAZ promotes cell proliferation and epithelial-mesenchymal transition and is inhibited by the hippo pathway." Mol Cell Biol **28**(7): 2426-2436.
Leiter, U., T. Eigentler and C. Garbe (2014). "Epidemiology of skin cancer." Adv Exp Med Biol **810**: 120-140.
Leopold, P. L., J. Vincent and H. Wang (2012). "A comparison of epithelial-to-mesenchymal transition and re-epithelialization." Semin Cancer Biol **22**(5-6): 471-483.
Leptin, M. (1991). "twist and snail as positive and negative regulators during Drosophila mesoderm development." Genes Dev **5**(9): 1568-1576.
Leu, S. J., Y. Liu, N. Chen, C. C. Chen, S. C. Lam and L. F. Lau (2003). "Identification of a novel integrin alpha 6 beta 1 binding site in the angiogenic inducer CCN1 (CYR61)." J Biol Chem **278**(36): 33801-33808.
Levental, K. R., H. Yu, L. Kass, J. N. Lakins, M. Egeblad, J. T. Erler, S. F. Fong, K. Csiszar, A. Giaccia, W. Weninger, M. Yamauchi, D. L. Gasser and V. M. Weaver (2009). "Matrix crosslinking forces tumor progression by enhancing integrin signaling." Cell **139**(5): 891-906.
Levy, D., Y. Adamovich, N. Reuven and Y. Shaul (2008). "Yap1 phosphorylation by c-Abl is a critical step in selective activation of proapoptotic genes in response to DNA damage." Mol Cell **29**(3): 350-361.
Li, D., W. Lang, C. Zhou, C. Wu, F. Zhang, Q. Liu, S. Yang and J. Hao (2018). "Upregulation of Microglial ZEB1 Ameliorates Brain Damage after Acute Ischemic Stroke." Cell Rep **22**(13): 3574-3586.
Li, P., M. R. Silvis, Y. Honaker, W. H. Lien, S. T. Arron and V. Vasioukhin (2016). "alphaE-catenin inhibits a Src-YAP1 oncogenic module that couples tyrosine kinases and the effector of Hippo signaling pathway." Genes Dev **30**(7): 798-811.
Li, Q., S. A. Sambandam, H. J. Lu, A. Thomson, S. H. Kim, H. Lu, Y. Xin and Q. Lu (2011). "14-3-3sigma and p63 play opposing roles in epidermal tumorigenesis." Carcinogenesis **32**(12): 1782-1788.
Li, S., P. Butler, Y. Wang, Y. Hu, D. C. Han, S. Usami, J. L. Guan and S. Chien (2002). "The role of the dynamics of focal adhesion kinase in the mechanotaxis of endothelial cells." Proc Natl Acad Sci U S A **99**(6): 3546-3551.
Li, Y., H. Zhou, F. Li, S. W. Chan, Z. Lin, Z. Wei, Z. Yang, F. Guo, C. J. Lim, W. Xing, Y. Shen, W. Hong, J. Long and M. Zhang (2015). "Angiomotin binding-induced activation of Merlin/NF2 in the Hippo pathway." Cell Res **25**(7): 801-817.

Lian, I., J. Kim, H. Okazawa, J. Zhao, B. Zhao, J. Yu, A. Chinnaiyan, M. A. Israel, L. S. Goldstein, R. Abujarour, S. Ding and K. L. Guan (2010). "The role of YAP transcription coactivator in regulating stem cell self-renewal and differentiation." Genes Dev **24**(11): 1106-1118.
Lien, W. H., O. Klezovitch and V. Vasioukhin (2006). "Cadherin-catenin proteins in vertebrate development." Curr Opin Cell Biol **18**(5): 499-506.
Lin, C., E. Yao and P. T. Chuang (2015). "A conserved MST1/2-YAP axis mediates Hippo signaling during lung growth." Dev Biol **403**(1): 101-113.
Ling, C., Y. Zheng, F. Yin, J. Yu, J. Huang, Y. Hong, S. Wu and D. Pan (2010). "The apical transmembrane protein Crumbs functions as a tumor suppressor that regulates Hippo signaling by binding to Expanded." Proc Natl Acad Sci U S A **107**(23): 10532-10537.
Liu, C. Y., H. H. Lin, M. J. Tang and Y. K. Wang (2015). "Vimentin contributes to epithelial-mesenchymal transition cancer cell mechanics by mediating cytoskeletal organization and focal adhesion maturation." Oncotarget **6**(18): 15966-15983.
Liu, C. Y., Z. Y. Zha, X. Zhou, H. Zhang, W. Huang, D. Zhao, T. Li, S. W. Chan, C. J. Lim, W. Hong, S. Zhao, Y. Xiong, Q. Y. Lei and K. L. Guan (2010). "The hippo tumor pathway promotes TAZ degradation by phosphorylating a phosphodegron and recruiting the SCF{beta}-TrCP E3 ligase." J Biol Chem **285**(48): 37159-37169.
Liu, S., S. W. Xu, K. Blumbach, M. Eastwood, C. P. Denton, B. Eckes, T. Krieg, D. J. Abraham and A. Leask (2010). "Expression of integrin beta1 by fibroblasts is required for tissue repair in vivo." J Cell Sci **123**(Pt 21): 3674-3682.
Liu, T., Y. Liu, H. Gao, F. Meng, S. Yang and G. Lou (2013). "Clinical significance of yes-associated protein overexpression in cervical carcinoma: the differential effects based on histotypes." Int J Gynecol Cancer **23**(4): 735-742.
Liu, W. F., C. M. Nelson, J. L. Tan and C. S. Chen (2007). "Cadherins, RhoA, and Rac1 are differentially required for stretch-mediated proliferation in endothelial versus smooth muscle cells." Circ Res **101**(5): e44-52.
Liu, X., N. Yang, S. A. Figel, K. E. Wilson, C. D. Morrison, I. H. Gelman and J. Zhang (2013). "PTPN14 interacts with and negatively regulates the oncogenic function of YAP." Oncogene **32**(10): 1266-1273.
Liu, Z., B. Sun, L. Qi, H. Li, J. Gao and X. Leng (2012). "Zinc finger E-box binding homeobox 1 promotes vasculogenic mimicry in colorectal cancer through induction of epithelial-to-mesenchymal transition." Cancer Sci **103**(4): 813-820.
Liu, Z., J. L. Tan, D. M. Cohen, M. T. Yang, N. J. Sniadecki, S. A. Ruiz, C. M. Nelson and C. S. Chen (2010). "Mechanical tugging force regulates the size of cell-cell junctions." Proc Natl Acad Sci U S A **107**(22): 9944-9949.
Lohmann, C. M. and A. R. Solomon (2001). "Clinicopathologic variants of cutaneous squamous cell carcinoma." Adv Anat Pathol **8**(1): 27-36.
Lorenz, K., C. Grashoff, R. Torka, T. Sakai, L. Langbein, W. Bloch, M. Aumailley and R. Fassler (2007). "Integrin-linked kinase is required for epidermal and hair follicle morphogenesis." J Cell Biol **177**(3): 501-513.
Lu, L., Y. Li, S. M. Kim, W. Bossuyt, P. Liu, Q. Qiu, Y. Wang, G. Halder, M. J. Finegold, J. S. Lee and R. L. Johnson (2010). "Hippo signaling is a potent in vivo growth and tumor suppressor pathway in the mammalian liver." Proc Natl Acad Sci U S A **107**(4): 1437-1442.
Lu, P., V. M. Weaver and Z. Werb (2012). "The extracellular matrix: a dynamic niche in cancer progression." J Cell Biol **196**(4): 395-406.
Ma, Y., Y. Yang, F. Wang, Q. Wei and H. Qin (2015). "Hippo-YAP signaling pathway: A new paradigm for cancer therapy." Int J Cancer **137**(10): 2275-2286.

Macbeth, A. E., D. J. Grindlay and H. C. Williams (2011). "What's new in skin cancer? An analysis of guidelines and systematic reviews published in 2008-2009." Clin Exp Dermatol **36**(5): 453-458.
Machnicka, B., A. Czogalla, A. Hryniewicz-Jankowska, D. M. Boguslawska, R. Grochowalska, E. Heger and A. F. Sikorski (2014). "Spectrins: a structural platform for stabilization and activation of membrane channels, receptors and transporters." Biochim Biophys Acta **1838**(2): 620-634.
Mackinnon, A. C., H. Qadota, K. R. Norman, D. G. Moerman and B. D. Williams (2002). "C. elegans PAT-4/ILK functions as an adaptor protein within integrin adhesion complexes." Curr Biol **12**(10): 787-797.
Makita, R., Y. Uchijima, K. Nishiyama, T. Amano, Q. Chen, T. Takeuchi, A. Mitani, T. Nagase, Y. Yatomi, H. Aburatani, O. Nakagawa, E. V. Small, P. Cobo-Stark, P. Igarashi, M. Murakami, J. Tominaga, T. Sato, T. Asano, Y. Kurihara and H. Kurihara (2008). "Multiple renal cysts, urinary concentration defects, and pulmonary emphysematous changes in mice lacking TAZ." Am J Physiol Renal Physiol **294**(3): F542-553.
Mammoto, A. and D. E. Ingber (2009). "Cytoskeletal control of growth and cell fate switching." Curr Opin Cell Biol **21**(6): 864-870.
Mana-Capelli, S. and D. McCollum (2018). "Angiomotins stimulate LATS kinase autophosphorylation and act as scaffolds that promote Hippo signaling." J Biol Chem **293**(47): 18230-18241.
Mannaerts, I., S. B. Leite, S. Verhulst, S. Claerhout, N. Eysackers, L. F. Thoen, A. Hoorens, H. Reynaert, G. Halder and L. A. van Grunsven (2015). "The Hippo pathway effector YAP controls mouse hepatic stellate cell activation." J Hepatol **63**(3): 679-688.
Manohar, A., S. G. Shome, J. Lamar, L. Stirling, V. Iyer, K. Pumiglia and C. M. DiPersio (2004). "Alpha 3 beta 1 integrin promotes keratinocyte cell survival through activation of a MEK/ERK signaling pathway." J Cell Sci **117**(Pt 18): 4043-4054.
Martins-Green, M., N. Boudreau and M. J. Bissell (1994). "Inflammation is responsible for the development of wound-induced tumors in chickens infected with Rous sarcoma virus." Cancer Res **54**(16): 4334-4341.
Mascre, G., S. Dekoninck, B. Drogat, K. K. Youssef, S. Brohee, P. A. Sotiropoulou, B. D. Simons and C. Blanpain (2012). "Distinct contribution of stem and progenitor cells to epidermal maintenance." Nature **489**(7415): 257-262.
Mason, D. E., J. M. Collins, J. H. Dawahare, T. D. Nguyen, Y. Lin, S. L. Voytik-Harbin, P. Zorlutuna, M. C. Yoder and J. D. Boerckel (2019). "YAP and TAZ limit cytoskeletal and focal adhesion maturation to enable persistent cell motility." J Cell Biol **218**(4): 1369-1389.
Matallanas, D., D. Romano, K. Yee, K. Meissl, L. Kucerova, D. Piazzolla, M. Baccarini, J. K. Vass, W. Kolch and E. O'Neill (2007). "RASSF1A elicits apoptosis through an MST2 pathway directing proapoptotic transcription by the p73 tumor suppressor protein." Mol Cell **27**(6): 962-975.
Matsubara, D., Y. Kishaba, T. Yoshimoto, Y. Sakuma, T. Sakatani, T. Tamura, S. Endo, Y. Sugiyama, Y. Murakami and T. Niki (2014). "Immunohistochemical analysis of the expression of E-cadherin and ZEB1 in non-small cell lung cancer." Pathol Int **64**(11): 560-568.
Matsui, Y. and Z. C. Lai (2013). "Mutual regulation between Hippo signaling and actin cytoskeleton." Protein Cell **4**(12): 904-910.

Matsumoto, T., J. Jiang, K. Kiguchi, L. Ruffino, S. Carbajal, L. Beltran, D. K. Bol, M. P. Rosenberg and J. DiGiovanni (2003). "Targeted expression of c-Src in epidermal basal cells leads to enhanced skin tumor promotion, malignant progression, and metastasis." Cancer Res **63**(16): 4819-4828.
Maugeri-Sacca, M., M. Barba, L. Pizzuti, P. Vici, L. Di Lauro, R. Dattilo, I. Vitale, M. Bartucci, M. Mottolese and R. De Maria (2015). "The Hippo transducers TAZ and YAP in breast cancer: oncogenic activities and clinical implications." Expert Rev Mol Med **17**: e14.
McLean, G. W., N. H. Komiyama, B. Serrels, H. Asano, L. Reynolds, F. Conti, K. Hodivala-Dilke, D. Metzger, P. Chambon, S. G. Grant and M. C. Frame (2004). "Specific deletion of focal adhesion kinase suppresses tumor formation and blocks malignant progression." Genes Dev **18**(24): 2998-3003.
Meng, Z., T. Moroishi and K. L. Guan (2016). "Mechanisms of Hippo pathway regulation." Genes Dev **30**(1): 1-17.
Meng, Z., T. Moroishi, V. Mottier-Pavie, S. W. Plouffe, C. G. Hansen, A. W. Hong, H. W. Park, J. S. Mo, W. Lu, S. Lu, F. Flores, F. X. Yu, G. Halder and K. L. Guan (2015). "MAP4K family kinases act in parallel to MST1/2 to activate LATS1/2 in the Hippo pathway." Nat Commun **6**: 8357.
Metcalf, D. (1963). "The Autonomous Behaviour of Normal Thymus Grafts." Aust J Exp Biol Med Sci **41**: SUPPL437-447.
Michaloglou, C., W. Lehmann, T. Martin, C. Delaunay, A. Hueber, L. Barys, H. Niu, E. Billy, M. Wartmann, M. Ito, C. J. Wilson, M. E. Digan, A. Bauer, H. Voshol, G. Christofori, W. R. Sellers, F. Hofmann and T. Schmelzle (2013). "The tyrosine phosphatase PTPN14 is a negative regulator of YAP activity." PLoS One **8**(4): e61916.
Michalopoulos, G. K. and M. C. DeFrances (1997). "Liver regeneration." Science **276**(5309): 60-66.
Miller, E., J. Yang, M. DeRan, C. Wu, A. I. Su, G. M. Bonamy, J. Liu, E. C. Peters and X. Wu (2012). "Identification of serum-derived sphingosine-1-phosphate as a small molecule regulator of YAP." Chem Biol **19**(8): 955-962.
Miranti, C. K. and J. S. Brugge (2002). "Sensing the environment: a historical perspective on integrin signal transduction." Nat Cell Biol **4**(4): E83-90.
Mo, J. S., F. X. Yu, R. Gong, J. H. Brown and K. L. Guan (2012). "Regulation of the Hippo-YAP pathway by protease-activated receptors (PARs)." Genes Dev **26**(19): 2138-2143.
Moleirinho, S., S. Hoxha, V. Mandati, G. Curtale, S. Troutman, U. Ehmer and J. L. Kissil (2017). "Regulation of localization and function of the transcriptional co-activator YAP by angiomotin." Elife **6**.
Montell, D. J. (2008). "Morphogenetic cell movements: diversity from modular mechanical properties." Science **322**(5907): 1502-1505.
Morgan, M. B., C. Purohit and T. R. Anglin (2008). "Immunohistochemical distinction of cutaneous spindle cell carcinoma." Am J Dermatopathol **30**(3): 228-232.
Morin-Kensicki, E. M., B. N. Boone, M. Howell, J. R. Stonebraker, J. Teed, J. G. Alb, T. R. Magnuson, W. O'Neal and S. L. Milgram (2006). "Defects in yolk sac vasculogenesis, chorioallantoic fusion, and embryonic axis elongation in mice with targeted disruption of Yap65." Mol Cell Biol **26**(1): 77-87.
Morrissey, M. A. and D. R. Sherwood (2015). "An active role for basement membrane assembly and modification in tissue sculpting." J Cell Sci **128**(9): 1661-1668.

Moya, I. M. and G. Halder (2016). "The Hippo pathway in cellular reprogramming and regeneration of different organs." Curr Opin Cell Biol **43**: 62-68.
Muramatsu, T., I. Imoto, T. Matsui, K. Kozaki, S. Haruki, M. Sudol, Y. Shimada, H. Tsuda, T. Kawano and J. Inazawa (2011). "YAP is a candidate oncogene for esophageal squamous cell carcinoma." Carcinogenesis **32**(3): 389-398.
Nakagawa, S., T. Yano, K. Nakagawa, S. Takizawa, Y. Suzuki, T. Yasugi, J. M. Huibregtse and Y. Taketani (2004). "Analysis of the expression and localisation of a LAP protein, human scribble, in the normal and neoplastic epithelium of uterine cervix." Br J Cancer **90**(1): 194-199.
Navarro, C., S. Nola, S. Audebert, M. J. Santoni, J. P. Arsanto, C. Ginestier, S. Marchetto, J. Jacquemier, D. Isnardon, A. Le Bivic, D. Birnbaum and J. P. Borg (2005). "Junctional recruitment of mammalian Scribble relies on E-cadherin engagement." Oncogene **24**(27): 4330-4339.
Nelson, W. J. (2003). "Adaptation of core mechanisms to generate cell polarity." Nature **422**(6933): 766-774.
Nelson, W. J. (2009). "Remodeling epithelial cell organization: transitions between front-rear and apical-basal polarity." Cold Spring Harb Perspect Biol **1**(1): a000513.
Niessen, C. M. and C. J. Gottardi (2008). "Molecular components of the adherens junction." Biochim Biophys Acta **1778**(3): 562-571.
Nieto, M. A. (2013). "Epithelial plasticity: a common theme in embryonic and cancer cells." Science **342**(6159): 1234850.
Nieto, M. A., M. F. Bennett, M. G. Sargent and D. G. Wilkinson (1992). "Cloning and developmental expression of Sna, a murine homologue of the Drosophila snail gene." Development **116**(1): 227-237.
Nieto, M. A., R. Y. Huang, R. A. Jackson and J. P. Thiery (2016). "Emt: 2016." Cell **166**(1): 21-45.
Nishimura, T., Y. Yasuhara and T. Hayashi (2006). "Highly selective 1,6-addition of aryl boronic acids to alpha,beta,gamma,delta-unsaturated carbonyl compounds catalyzed by an iridium complex." Angew Chem Int Ed Engl **45**(31): 5164-5166.
Nishio, M., K. Hamada, K. Kawahara, M. Sasaki, F. Noguchi, S. Chiba, K. Mizuno, S. O. Suzuki, Y. Dong, M. Tokuda, T. Morikawa, H. Hikasa, J. Eggenschwiler, N. Yabuta, H. Nojima, K. Nakagawa, Y. Hata, H. Nishina, K. Mimori, M. Mori, T. Sasaki, T. W. Mak, T. Nakano, S. Itami and A. Suzuki (2012). "Cancer susceptibility and embryonic lethality in Mob1a/1b double-mutant mice." J Clin Invest **122**(12): 4505-4518.
Nishioka, N., K. Inoue, K. Adachi, H. Kiyonari, M. Ota, A. Ralston, N. Yabuta, S. Hirahara, R. O. Stephenson, N. Ogonuki, R. Makita, H. Kurihara, E. M. Morin-Kensicki, H. Nojima, J. Rossant, K. Nakao, H. Niwa and H. Sasaki (2009). "The Hippo signaling pathway components Lats and Yap pattern Tead4 activity to distinguish mouse trophectoderm from inner cell mass." Dev Cell **16**(3): 398-410.
Nolo, R., C. M. Morrison, C. Tao, X. Zhang and G. Halder (2006). "The bantam microRNA is a target of the hippo tumor-suppressor pathway." Curr Biol **16**(19): 1895-1904.
O'Brien, L. E., M. M. Zegers and K. E. Mostov (2002). "Opinion: Building epithelial architecture: insights from three-dimensional culture models." Nat Rev Mol Cell Biol **3**(7): 531-537.
Oh, H., M. Slattery, L. Ma, A. Crofts, K. P. White, R. S. Mann and K. D. Irvine (2013). "Genome-wide association of Yorkie with chromatin and chromatin-remodeling complexes." Cell Rep **3**(2): 309-318.

Oka, T., E. Remue, K. Meerschaert, B. Vanloo, C. Boucherie, D. Gfeller, G. D. Bader, S. S. Sidhu, J. Vandekerckhove, J. Gettemans and M. Sudol (2010). "Functional complexes between YAP2 and ZO-2 are PDZ domain-dependent, and regulate YAP2 nuclear localization and signalling." Biochem J **432**(3): 461-472.
Oka, T. and M. Sudol (2009). "Nuclear localization and pro-apoptotic signaling of YAP2 require intact PDZ-binding motif." Genes Cells **14**(5): 607-615.
Okugawa, Y., Y. Toiyama, K. Tanaka, K. Matsusita, H. Fujikawa, S. Saigusa, M. Ohi, Y. Inoue, Y. Mohri, K. Uchida and M. Kusunoki (2012). "Clinical significance of Zinc finger E-box Binding homeobox 1 (ZEB1) in human gastric cancer." J Surg Oncol **106**(3): 280-285.
Orellana-Serradell, O., D. Herrera, E. A. Castellon and H. R. Contreras (2018). "The transcription factor ZEB1 promotes an aggressive phenotype in prostate cancer cell lines." Asian J Androl **20**(3): 294-299.
Oshima, H., A. Rochat, C. Kedzia, K. Kobayashi and Y. Barrandon (2001). "Morphogenesis and renewal of hair follicles from adult multipotent stem cells." Cell **104**(2): 233-245.
Ota, M. and H. Sasaki (2008). "Mammalian Tead proteins regulate cell proliferation and contact inhibition as transcriptional mediators of Hippo signaling." Development **135**(24): 4059-4069.
Pan, D. (2010). "The hippo signaling pathway in development and cancer." Dev Cell **19**(4): 491-505.
Panciera, T., L. Azzolin, A. Fujimura, D. Di Biagio, C. Frasson, S. Bresolin, S. Soligo, G. Basso, S. Bicciato, A. Rosato, M. Cordenonsi and S. Piccolo (2016). "Induction of Expandable Tissue-Specific Stem/Progenitor Cells through Transient Expression of YAP/TAZ." Cell Stem Cell **19**(6): 725-737.
Paramasivam, M., A. Sarkeshik, J. R. Yates, 3rd, M. J. Fernandes and D. McCollum (2011). "Angiomotin family proteins are novel activators of the LATS2 kinase tumor suppressor." Mol Biol Cell **22**(19): 3725-3733.
Park, H. W., Y. C. Kim, B. Yu, T. Moroishi, J. S. Mo, S. W. Plouffe, Z. Meng, K. C. Lin, F. X. Yu, C. M. Alexander, C. Y. Wang and K. L. Guan (2015). "Alternative Wnt Signaling Activates YAP/TAZ." Cell **162**(4): 780-794.
Paszek, M. J., N. Zahir, K. R. Johnson, J. N. Lakins, G. I. Rozenberg, A. Gefen, C. A. Reinhart-King, S. S. Margulies, M. Dembo, D. Boettiger, D. A. Hammer and V. M. Weaver (2005). "Tensional homeostasis and the malignant phenotype." Cancer Cell **8**(3): 241-254.
Pawlotsky, J. M. (2004). "Pathophysiology of hepatitis C virus infection and related liver disease." Trends Microbiol **12**(2): 96-102.
Pedersen, T. X., C. Leethanakul, V. Patel, D. Mitola, L. R. Lund, K. Dano, M. Johnsen, J. S. Gutkind and T. H. Bugge (2003). "Laser capture microdissection-based in vivo genomic profiling of wound keratinocytes identifies similarities and differences to squamous cell carcinoma." Oncogene **22**(25): 3964-3976.
Peinado, H., D. Olmeda and A. Cano (2007). "Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype?" Nat Rev Cancer **7**(6): 415-428.
Pena, C., J. M. Garcia, J. Silva, V. Garcia, R. Rodriguez, I. Alonso, I. Millan, C. Salas, A. G. de Herreros, A. Munoz and F. Bonilla (2005). "E-cadherin and vitamin D receptor regulation by SNAIL and ZEB1 in colon cancer: clinicopathological correlations." Hum Mol Genet **14**(22): 3361-3370.

Perez-Moreno, M., M. A. Davis, E. Wong, H. A. Pasolli, A. B. Reynolds and E. Fuchs (2006). "p120-catenin mediates inflammatory responses in the skin." Cell **124**(3): 631-644.
Perrais, M., X. Chen, M. Perez-Moreno and B. M. Gumbiner (2007). "E-cadherin homophilic ligation inhibits cell growth and epidermal growth factor receptor signaling independently of other cell interactions." Mol Biol Cell **18**(6): 2013-2025.
Piccolo, S., S. Dupont and M. Cordenonsi (2014). "The biology of YAP/TAZ: hippo signaling and beyond." Physiol Rev **94**(4): 1287-1312.
Pietras, K., J. Pahler, G. Bergers and D. Hanahan (2008). "Functions of paracrine PDGF signaling in the proangiogenic tumor stroma revealed by pharmacological targeting." PLoS Med **5**(1): e19.
Piwko-Czuchra, A., H. Koegel, H. Meyer, M. Bauer, S. Werner, C. Brakebusch and R. Fassler (2009). "Beta1 integrin-mediated adhesion signalling is essential for epidermal progenitor cell expansion." PLoS One **4**(5): e5488.
Pocha, S. M. and E. Knust (2013). "Complexities of Crumbs function and regulation in tissue morphogenesis." Curr Biol **23**(7): R289-293.
Postigo, A. A. (2003). "Opposing functions of ZEB proteins in the regulation of the TGFbeta/BMP signaling pathway." EMBO J **22**(10): 2443-2452.
Qian, B. Z. and J. W. Pollard (2010). "Macrophage diversity enhances tumor progression and metastasis." Cell **141**(1): 39-51.
Qiao, Y., J. Chen, Y. B. Lim, M. L. Finch-Edmondson, V. P. Seshachalam, L. Qin, T. Jiang, B. C. Low, H. Singh, C. T. Lim and M. Sudol (2017). "YAP Regulates Actin Dynamics through ARHGAP29 and Promotes Metastasis." Cell Rep **19**(8): 1495-1502.
Qin, H., K. Blaschke, G. Wei, Y. Ohi, L. Blouin, Z. Qi, J. Yu, R. F. Yeh, M. Hebrok and M. Ramalho-Santos (2012). "Transcriptional analysis of pluripotency reveals the Hippo pathway as a barrier to reprogramming." Hum Mol Genet **21**(9): 2054-2067.
Qing, Y., F. Yin, W. Wang, Y. Zheng, P. Guo, F. Schozer, H. Deng and D. Pan (2014). "The Hippo effector Yorkie activates transcription by interacting with a histone methyltransferase complex through Ncoa6." Elife **3**.
Qiu, B., W. Wei, J. Zhu, G. Fu and D. Lu (2018). "EMT induced by loss of LKB1 promotes migration and invasion of liver cancer cells through ZEB1-induced YAP signaling." Oncol Lett **16**(5): 6465-6471.
Quinn, J., T. Kunath and J. Rossant (2006). "Mouse trophoblast stem cells." Methods Mol Med **121**: 125-148.
Raghavan, S., C. Bauer, G. Mundschau, Q. Li and E. Fuchs (2000). "Conditional ablation of beta1 integrin in skin. Severe defects in epidermal proliferation, basement membrane formation, and hair follicle invagination." J Cell Biol **150**(5): 1149-1160.
Raghavan, S., A. Vaezi and E. Fuchs (2003). "A role for alphabeta1 integrins in focal adhesion function and polarized cytoskeletal dynamics." Dev Cell **5**(3): 415-427.
Ran, J., D. L. Lin, R. F. Wu, Q. H. Chen, H. P. Huang, N. X. Qiu and S. Quan (2015). "ZEB1 promotes epithelial-mesenchymal transition in cervical cancer metastasis." Fertil Steril **103**(6): 1606-1614 e1601-1602.
Rasanen, K. and A. Vaheri (2010). "TGF-beta1 causes epithelial-mesenchymal transition in HaCaT derivatives, but induces expression of COX-2 and migration only in benign, not in malignant keratinocytes." J Dermatol Sci **58**(2): 97-104.

Reddy, B. V. and K. D. Irvine (2013). "Regulation of Hippo signaling by EGFR-MAPK signaling through Ajuba family proteins." Dev Cell **24**(5): 459-471.
Reddy, P., M. Deguchi, Y. Cheng and A. J. Hsueh (2013). "Actin cytoskeleton regulates Hippo signaling." PLoS One **8**(9): e73763.
Reginensi, A., R. P. Scott, A. Gregorieff, M. Bagherie-Lachidan, C. Chung, D. S. Lim, T. Pawson, J. Wrana and H. McNeill (2013). "Yap- and Cdc42-dependent nephrogenesis and morphogenesis during mouse kidney development." PLoS Genet **9**(3): e1003380.
Regue, L., F. Mou and J. Avruch (2013). "G protein-coupled receptors engage the mammalian Hippo pathway through F-actin: F-Actin, assembled in response to Galpha12/13 induced RhoA-GTP, promotes dephosphorylation and activation of the YAP oncogene." Bioessays **35**(5): 430-435.
Remue, E., K. Meerschaert, T. Oka, C. Boucherie, J. Vandekerckhove, M. Sudol and J. Gettemans (2010). "TAZ interacts with zonula occludens-1 and -2 proteins in a PDZ-1 dependent manner." FEBS Lett **584**(19): 4175-4180.
Repertinger, S. K., E. Campagnaro, J. Fuhrman, T. El-Abaseri, S. H. Yuspa and L. A. Hansen (2004). "EGFR enhances early healing after cutaneous incisional wounding." J Invest Dermatol **123**(5): 982-989.
Ridgway, R. A., B. Serrels, S. Mason, A. Kinnaird, M. Muir, H. Patel, W. J. Muller, O. J. Sansom and V. G. Brunton (2012). "Focal adhesion kinase is required for beta-catenin-induced mobilization of epidermal stem cells." Carcinogenesis **33**(12): 2369-2376.
Robinson, B. S. and K. H. Moberg (2011). "Cell-cell junctions: alpha-catenin and E-cadherin help fence in Yap1." Curr Biol **21**(21): R890-892.
Rossi, R., M. Mori and T. Lotti (2007). "Actinic keratosis." Int J Dermatol **46**(9): 895-904.
Ryan, M. C., A. M. Christiano, E. Engvall, U. M. Wewer, J. H. Miner, J. R. Sanes and R. E. Burgeson (1996). "The functions of laminins: lessons from in vivo studies." Matrix Biol **15**(6): 369-381.
Sabra, H., M. Brunner, V. Mandati, B. Wehrle-Haller, D. Lallemand, A. S. Ribba, G. Chevalier, P. Guardiola, M. R. Block and D. Bouvard (2017). "beta1 integrin-dependent Rac/group I PAK signaling mediates YAP activation of Yes-associated protein 1 (YAP1) via NF2/merlin." J Biol Chem **292**(47): 19179-19197.
Sakai, T., S. Li, D. Docheva, C. Grashoff, K. Sakai, G. Kostka, A. Braun, A. Pfeifer, P. D. Yurchenco and R. Fassler (2003). "Integrin-linked kinase (ILK) is required for polarizing the epiblast, cell adhesion, and controlling actin accumulation." Genes Dev **17**(7): 926-940.
Samarasinghe, V., V. Madan and J. T. Lear (2011). "Management of high-risk squamous cell carcinoma of the skin." Expert Rev Anticancer Ther **11**(5): 763-769.
Sanchez-Tillo, E., L. Siles, O. de Barrios, M. Cuatrecasas, E. C. Vaquero, A. Castells and A. Postigo (2011). "Expanding roles of ZEB factors in tumorigenesis and tumor progression." Am J Cancer Res **1**(7): 897-912.
Sansores-Garcia, L., W. Bossuyt, K. Wada, S. Yonemura, C. Tao, H. Sasaki and G. Halder (2011). "Modulating F-actin organization induces organ growth by affecting the Hippo pathway." EMBO J **30**(12): 2325-2335.
Sasaki, H. (2015). "Position- and polarity-dependent Hippo signaling regulates cell fates in preimplantation mouse embryos." Semin Cell Dev Biol **47-48**: 80-87.
Satelli, A. and S. Li (2011). "Vimentin in cancer and its potential as a molecular target for cancer therapy." Cell Mol Life Sci **68**(18): 3033-3046.

Savagner, P. (2015). "Epithelial-mesenchymal transitions: from cell plasticity to concept elasticity." Curr Top Dev Biol **112**: 273-300.
Schepeler, T., M. E. Page and K. B. Jensen (2014). "Heterogeneity and plasticity of epidermal stem cells." Development **141**(13): 2559-2567.
Schlegelmilch, K., M. Mohseni, O. Kirak, J. Pruszak, J. R. Rodriguez, D. Zhou, B. T. Kreger, V. Vasioukhin, J. Avruch, T. R. Brummelkamp and F. D. Camargo (2011). "Yap1 acts downstream of alpha-catenin to control epidermal proliferation." Cell **144**(5): 782-795.
Schober, M., S. Raghavan, M. Nikolova, L. Polak, H. A. Pasolli, H. E. Beggs, L. F. Reichardt and E. Fuchs (2007). "Focal adhesion kinase modulates tension signaling to control actin and focal adhesion dynamics." J Cell Biol **176**(5): 667-680.
Seewaldt, V. (2014). "ECM stiffness paves the way for tumor cells." Nat Med **20**(4): 332-333.
Serrels, B., A. Serrels, S. M. Mason, C. Baldeschi, G. H. Ashton, M. Canel, L. J. Mackintosh, B. Doyle, T. P. Green, M. C. Frame, O. J. Sansom and V. G. Brunton (2009). "A novel Src kinase inhibitor reduces tumour formation in a skin carcinogenesis model." Carcinogenesis **30**(2): 249-257.
Shanzer, M., J. Adler, I. Ricardo-Lax, N. Reuven and Y. Shaul (2017). "The nonreceptor tyrosine kinase c-Src attenuates SCF(beta-TrCP) E3-ligase activity abrogating Taz proteasomal degradation." Proc Natl Acad Sci U S A **114**(7): 1678-1683.
Shaw, R. L., A. Kohlmaier, C. Polesello, C. Veelken, B. A. Edgar and N. Tapon (2010). "The Hippo pathway regulates intestinal stem cell proliferation during Drosophila adult midgut regeneration." Development **137**(24): 4147-4158.
Shaw, T. J. and P. Martin (2016). "Wound repair: a showcase for cell plasticity and migration." Curr Opin Cell Biol **42**: 29-37.
Shin, K., V. C. Fogg and B. Margolis (2006). "Tight junctions and cell polarity." Annu Rev Cell Dev Biol **22**: 207-235.
Shook, D. and R. Keller (2003). "Mechanisms, mechanics and function of epithelial-mesenchymal transitions in early development." Mech Dev **120**(11): 1351-1383.
Shreberk-Shaked, M. and M. Oren (2019). "New insights into YAP/TAZ nucleo-cytoplasmic shuttling: new cancer therapeutic opportunities?" Mol Oncol **13**(6): 1335-1341.
Si, Y., X. Ji, X. Cao, X. Dai, L. Xu, H. Zhao, X. Guo, H. Yan, H. Zhang, C. Zhu, Q. Zhou, M. Tang, Z. Xia, L. Li, Y. S. Cong, S. Ye, T. Liang, X. H. Feng and B. Zhao (2017). "Src Inhibits the Hippo Tumor Suppressor Pathway through Tyrosine Phosphorylation of Lats1." Cancer Res **77**(18): 4868-4880.
Sibilia, M., A. Fleischmann, A. Behrens, L. Stingl, J. Carroll, F. M. Watt, J. Schlessinger and E. F. Wagner (2000). "The EGF receptor provides an essential survival signal for SOS-dependent skin tumor development." Cell **102**(2): 211-220.
Silber, S. J. (1976). "Growth of baby kidneys transplanted into adults." Arch Surg **111**(1): 75-77.
Silvis, M. R., B. T. Kreger, W. H. Lien, O. Klezovitch, G. M. Rudakova, F. D. Camargo, D. M. Lantz, J. T. Seykora and V. Vasioukhin (2011). "alpha-catenin is a tumor suppressor that controls cell accumulation by regulating the localization and activity of the transcriptional coactivator Yap1." Sci Signal **4**(174): ra33.
Simpson, C. L., D. M. Patel and K. J. Green (2011). "Deconstructing the skin: cytoarchitectural determinants of epidermal morphogenesis." Nat Rev Mol Cell Biol **12**(9): 565-580.

Singh, P., C. Chen, S. Pal-Ghosh, M. A. Stepp, D. Sheppard and L. Van De Water (2009). "Loss of integrin alpha9beta1 results in defects in proliferation, causing poor re-epithelialization during cutaneous wound healing." J Invest Dermatol **129**(1): 217-228.
Skibinski, A., J. L. Breindel, A. Prat, P. Galvan, E. Smith, A. Rolfs, P. B. Gupta, J. LaBaer and C. Kuperwasser (2014). "The Hippo transducer TAZ interacts with the SWI/SNF complex to regulate breast epithelial lineage commitment." Cell Rep **6**(6): 1059-1072.
Smeets, N. W., D. I. Kuijpers, P. Nelemans, J. U. Ostertag, M. E. Verhaegh, G. A. Krekels and H. A. Neumann (2004). "Mohs' micrographic surgery for treatment of basal cell carcinoma of the face--results of a retrospective study and review of the literature." Br J Dermatol **151**(1): 141-147.
Smoot, R. L., N. W. Werneburg, T. Sugihara, M. C. Hernandez, L. Yang, C. Mehner, R. P. Graham, S. F. Bronk, M. J. Truty and G. J. Gores (2018). "Platelet-derived growth factor regulates YAP transcriptional activity via Src family kinase dependent tyrosine phosphorylation." J Cell Biochem **119**(1): 824-836.
Snijders-Keilholz, A., P. Ewing, C. Seynaeve and C. W. Burger (2005). "Primitive neuroectodermal tumor of the cervix uteri: a case report -- changing concepts in therapy." Gynecol Oncol **98**(3): 516-519.
Solanas, G. and S. A. Benitah (2013). "Regenerating the skin: a task for the heterogeneous stem cell pool and surrounding niche." Nat Rev Mol Cell Biol **14**(11): 737-748.
Song, H., K. K. Mak, L. Topol, K. Yun, J. Hu, L. Garrett, Y. Chen, O. Park, J. Chang, R. M. Simpson, C. Y. Wang, B. Gao, J. Jiang and Y. Yang (2010). "Mammalian Mst1 and Mst2 kinases play essential roles in organ size control and tumor suppression." Proc Natl Acad Sci U S A **107**(4): 1431-1436.
Song, S., H. Herranz and S. M. Cohen (2017). "The chromatin remodeling BAP complex limits tumor-promoting activity of the Hippo pathway effector Yki to prevent neoplastic transformation in Drosophila epithelia." Dis Model Mech **10**(10): 1201-1209.
Song, S., S. Honjo, J. Jin, S. S. Chang, A. W. Scott, Q. Chen, N. Kalhor, A. M. Correa, W. L. Hofstetter, C. T. Albarracin, T. T. Wu, R. L. Johnson, M. C. Hung and J. A. Ajani (2015). "The Hippo Coactivator YAP1 Mediates EGFR Overexpression and Confers Chemoresistance in Esophageal Cancer." Clin Cancer Res **21**(11): 2580-2590.
Sorrentino, G., N. Ruggeri, V. Specchia, M. Cordenonsi, M. Mano, S. Dupont, A. Manfrin, E. Ingallina, R. Sommaggio, S. Piazza, A. Rosato, S. Piccolo and G. Del Sal (2014). "Metabolic control of YAP and TAZ by the mevalonate pathway." Nat Cell Biol **16**(4): 357-366.
Spaderna, S., O. Schmalhofer, M. Wahlbuhl, A. Dimmler, K. Bauer, A. Sultan, F. Hlubek, A. Jung, D. Strand, A. Eger, T. Kirchner, J. Behrens and T. Brabletz (2008). "The transcriptional repressor ZEB1 promotes metastasis and loss of cell polarity in cancer." Cancer Res **68**(2): 537-544.
Spoelstra, N. S., N. G. Manning, Y. Higashi, D. Darling, M. Singh, K. R. Shroyer, R. R. Broaddus, K. B. Horwitz and J. K. Richer (2006). "The transcription factor ZEB1 is aberrantly expressed in aggressive uterine cancers." Cancer Res **66**(7): 3893-3902.
Staley, B. K. and K. D. Irvine (2010). "Warts and Yorkie mediate intestinal regeneration by influencing stem cell proliferation." Curr Biol **20**(17): 1580-1587.

Stein, C., A. F. Bardet, G. Roma, S. Bergling, I. Clay, A. Ruchti, C. Agarinis, T. Schmelzle, T. Bouwmeester, D. Schubeler and A. Bauer (2015). "YAP1 Exerts Its Transcriptional Control via TEAD-Mediated Activation of Enhancers." PLoS Genet **11**(8): e1005465.
Steinhardt, A. A., M. F. Gayyed, A. P. Klein, J. Dong, A. Maitra, D. Pan, E. A. Montgomery and R. A. Anders (2008). "Expression of Yes-associated protein in common solid tumors." Hum Pathol **39**(11): 1582-1589.
Stone, R. C., I. Pastar, N. Ojeh, V. Chen, S. Liu, K. I. Garzon and M. Tomic-Canic (2016). "Epithelial-mesenchymal transition in tissue repair and fibrosis." Cell Tissue Res **365**(3): 495-506.
Strano, S., O. Monti, N. Pediconi, A. Baccarini, G. Fontemaggi, E. Lapi, F. Mantovani, A. Damalas, G. Citro, A. Sacchi, G. Del Sal, M. Levrero and G. Blandino (2005). "The transcriptional coactivator Yes-associated protein drives p73 gene-target specificity in response to DNA Damage." Mol Cell **18**(4): 447-459.
Strano, S., E. Munarriz, M. Rossi, L. Castagnoli, Y. Shaul, A. Sacchi, M. Oren, M. Sudol, G. Cesareni and G. Blandino (2001). "Physical interaction with Yes-associated protein enhances p73 transcriptional activity." J Biol Chem **276**(18): 15164-15173.
Strassburger, K., M. Tiebe, F. Pinna, K. Breuhahn and A. A. Teleman (2012). "Insulin/IGF signaling drives cell proliferation in part via Yorkie/YAP." Dev Biol **367**(2): 187-196.
Su, L. L., W. X. Ma, J. F. Yuan, Y. Shao, W. Xiao and S. J. Jiang (2012). "Expression of Yes-associated protein in non-small cell lung cancer and its relationship with clinical pathological factors." Chin Med J (Engl) **125**(22): 4003-4008.
Su, T., T. Bondar, X. Zhou, C. Zhang, H. He and R. Medzhitov (2015). "Two-signal requirement for growth-promoting function of Yap in hepatocytes." Elife **4**.
Subramanian, A., P. Tamayo, V. K. Mootha, S. Mukherjee, B. L. Ebert, M. A. Gillette, A. Paulovich, S. L. Pomeroy, T. R. Golub, E. S. Lander and J. P. Mesirov (2005). "Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles." Proc Natl Acad Sci U S A **102**(43): 15545-15550.
Sudol, M. (1994). "Yes-associated protein (YAP65) is a proline-rich phosphoprotein that binds to the SH3 domain of the Yes proto-oncogene product." Oncogene **9**(8): 2145-2152.
Sun, S., B. V. Reddy and K. D. Irvine (2015). "Localization of Hippo signalling complexes and Warts activation in vivo." Nat Commun **6**: 8402.
Szymaniak, A. D., J. E. Mahoney, W. V. Cardoso and X. Varelas (2015). "Crumbs3-Mediated Polarity Directs Airway Epithelial Cell Fate through the Hippo Pathway Effector Yap." Dev Cell **34**(3): 283-296.
Tan, D. W., K. B. Jensen, M. W. Trotter, J. T. Connelly, S. Broad and F. M. Watt (2013). "Single-cell gene expression profiling reveals functional heterogeneity of undifferentiated human epidermal cells." Development **140**(7): 1433-1444.
Tang, Y., R. G. Rowe, E. L. Botvinick, A. Kurup, A. J. Putnam, M. Seiki, V. M. Weaver, E. T. Keller, S. Goldstein, J. Dai, D. Begun, T. Saunders and S. J. Weiss (2013). "MT1-MMP-dependent control of skeletal stem cell commitment via a beta1-integrin/YAP/TAZ signaling axis." Dev Cell **25**(4): 402-416.
Taniguchi, K., L. W. Wu, S. I. Grivennikov, P. R. de Jong, I. Lian, F. X. Yu, K. Wang, S. B. Ho, B. S. Boland, J. T. Chang, W. J. Sandborn, G. Hardiman, E. Raz, Y. Maehara, A. Yoshimura, J. Zucman-Rossi, K. L. Guan and M. Karin (2015). "A

gp130-Src-YAP module links inflammation to epithelial regeneration." Nature **519**(7541): 57-62.
Tao, W., S. Zhang, G. S. Turenchalk, R. A. Stewart, M. A. St John, W. Chen and T. Xu (1999). "Human homologue of the Drosophila melanogaster lats tumour suppressor modulates CDC2 activity." Nat Genet **21**(2): 177-181.
Tapon, N., K. F. Harvey, D. W. Bell, D. C. Wahrer, T. A. Schiripo, D. Haber and I. K. Hariharan (2002). "salvador Promotes both cell cycle exit and apoptosis in Drosophila and is mutated in human cancer cell lines." Cell **110**(4): 467-478.
Taylor, G., M. S. Lehrer, P. J. Jensen, T. T. Sun and R. M. Lavker (2000). "Involvement of follicular stem cells in forming not only the follicle but also the epidermis." Cell **102**(4): 451-461.
Thiery, J. P., H. Acloque, R. Y. Huang and M. A. Nieto (2009). "Epithelial-mesenchymal transitions in development and disease." Cell **139**(5): 871-890.
Thiery, J. P. and J. P. Sleeman (2006). "Complex networks orchestrate epithelial-mesenchymal transitions." Nat Rev Mol Cell Biol **7**(2): 131-142.
Thisse, B., C. Stoetzel, C. Gorostiza-Thisse and F. Perrin-Schmitt (1988). "Sequence of the twist gene and nuclear localization of its protein in endomesodermal cells of early Drosophila embryos." EMBO J **7**(7): 2175-2183.
Thompson, B. J. and S. M. Cohen (2006). "The Hippo pathway regulates the bantam microRNA to control cell proliferation and apoptosis in Drosophila." Cell **126**(4): 767-774.
Thompson, D. A. W. (1917). On growth and form. Cambridge Eng., University press.
Tian, W., J. Yu, D. R. Tomchick, D. Pan and X. Luo (2010). "Structural and functional analysis of the YAP-binding domain of human TEAD2." Proc Natl Acad Sci U S A **107**(16): 7293-7298.
Tiwari, N., A. Gheldof, M. Tatari and G. Christofori (2012). "EMT as the ultimate survival mechanism of cancer cells." Semin Cancer Biol **22**(3): 194-207.
Torsoni, A. S., T. M. Marin, L. A. Velloso and K. G. Franchini (2005). "RhoA/ROCK signaling is critical to FAK activation by cyclic stretch in cardiac myocytes." Am J Physiol Heart Circ Physiol **289**(4): H1488-1496.
Trakatelli, M., C. Ulrich, V. del Marmol, S. Euvrard, E. Stockfleth and D. Abeni (2007). "Epidemiology of nonmelanoma skin cancer (NMSC) in Europe: accurate and comparable data are needed for effective public health monitoring and interventions." Br J Dermatol **156 Suppl 3**: 1-7.
Tse, J. C. and R. Kalluri (2007). "Mechanisms of metastasis: epithelial-to-mesenchymal transition and contribution of tumor microenvironment." J Cell Biochem **101**(4): 816-829.
Tyler, S. (2003). "Epithelium--the primary building block for metazoan complexity." Integr Comp Biol **43**(1): 55-63.
Udan, R. S., M. Kango-Singh, R. Nolo, C. Tao and G. Halder (2003). "Hippo promotes proliferation arrest and apoptosis in the Salvador/Warts pathway." Nat Cell Biol **5**(10): 914-920.
Van Agtmael, T. and L. Bruckner-Tuderman (2010). "Basement membranes and human disease." Cell Tissue Res **339**(1): 167-188.
van Grunsven, L. A., V. Taelman, C. Michiels, K. Opdecamp, D. Huylebroeck and E. J. Bellefroid (2006). "deltaEF1 and SIP1 are differentially expressed and have overlapping activities during Xenopus embryogenesis." Dev Dyn **235**(6): 1491-1500.

Vandewalle, C., F. Van Roy and G. Berx (2009). "The role of the ZEB family of transcription factors in development and disease." Cell Mol Life Sci **66**(5): 773-787.
Varelas, X. (2014). "The Hippo pathway effectors TAZ and YAP in development, homeostasis and disease." Development **141**(8): 1614-1626.
Varelas, X., R. Sakuma, P. Samavarchi-Tehrani, R. Peerani, B. M. Rao, J. Dembowy, M. B. Yaffe, P. W. Zandstra and J. L. Wrana (2008). "TAZ controls Smad nucleocytoplasmic shuttling and regulates human embryonic stem-cell self-renewal." Nat Cell Biol **10**(7): 837-848.
Varelas, X., P. Samavarchi-Tehrani, M. Narimatsu, A. Weiss, K. Cockburn, B. G. Larsen, J. Rossant and J. L. Wrana (2010). "The Crumbs complex couples cell density sensing to Hippo-dependent control of the TGF-beta-SMAD pathway." Dev Cell **19**(6): 831-844.
Vasioukhin, V., C. Bauer, L. Degenstein, B. Wise and E. Fuchs (2001). "Hyperproliferation and defects in epithelial polarity upon conditional ablation of alpha-catenin in skin." Cell **104**(4): 605-617.
Vasioukhin, V., E. Bowers, C. Bauer, L. Degenstein and E. Fuchs (2001). "Desmoplakin is essential in epidermal sheet formation." Nat Cell Biol **3**(12): 1076-1085.
Vassilev, A., K. J. Kaneko, H. Shu, Y. Zhao and M. L. DePamphilis (2001). "TEAD/TEF transcription factors utilize the activation domain of YAP65, a Src/Yes-associated protein localized in the cytoplasm." Genes Dev **15**(10): 1229-1241.
Virchow, R (1863) Die Krankhaften Geschwülste, Berlin, Germany,
Viswanathan, S., K. Rahman, S. Pallavi, J. Sachin, A. Patil, P. Chaturvedi, A. D'Cruz, J. Agarwal and S. V. Kane (2010). "Sarcomatoid (spindle cell) carcinoma of the head and neck mucosal region: a clinicopathologic review of 103 cases from a tertiary referral cancer centre." Head Neck Pathol **4**(4): 265-275.
Vlahov, N., S. Scrace, M. S. Soto, A. M. Grawenda, L. Bradley, D. Pankova, A. Papaspyropoulos, K. S. Yee, F. Buffa, C. R. Goding, P. Timpson, N. Sibson and E. O'Neill (2015). "Alternate RASSF1 Transcripts Control SRC Activity, E-Cadherin Contacts, and YAP-Mediated Invasion." Curr Biol **25**(23): 3019-3034.
Vogel, V. and M. Sheetz (2006). "Local force and geometry sensing regulate cell functions." Nat Rev Mol Cell Biol **7**(4): 265-275.
von Gise, A., Z. Lin, K. Schlegelmilch, L. B. Honor, G. M. Pan, J. N. Buck, Q. Ma, T. Ishiwata, B. Zhou, F. D. Camargo and W. T. Pu (2012). "YAP1, the nuclear target of Hippo signaling, stimulates heart growth through cardiomyocyte proliferation but not hypertrophy." Proc Natl Acad Sci U S A **109**(7): 2394-2399.
Vrana, N. E., P. Lavalle, M. R. Dokmeci, F. Dehghani, A. M. Ghaemmaghami and A. Khademhosseini (2013). "Engineering functional epithelium for regenerative medicine and in vitro organ models: a review." Tissue Eng Part B Rev **19**(6): 529-543.
Wada, K., K. Itoga, T. Okano, S. Yonemura and H. Sasaki (2011). "Hippo pathway regulation by cell morphology and stress fibers." Development **138**(18): 3907-3914.
Walko, G., S. Woodhouse, A. O. Pisco, E. Rognoni, K. Liakath-Ali, B. M. Lichtenberger, A. Mishra, S. B. Telerman, P. Viswanathan, M. Logtenberg, L. M. Renz, G. Donati, S. R. Quist and F. M. Watt (2017). "A genome-wide screen identifies YAP/WBP2 interplay conferring growth advantage on human epidermal stem cells." Nat Commun **8**: 14744.
Wang, A. Z., G. K. Ojakian and W. J. Nelson (1990). "Steps in the morphogenesis of a polarized epithelium. I. Uncoupling the roles of cell-cell and cell-substratum

contact in establishing plasma membrane polarity in multicellular epithelial (MDCK) cysts." J Cell Sci **95 (Pt 1)**: 137-151.
Wang, C., L. Zhang, Q. He, X. Feng, J. Zhu, Z. Xu, X. Wang, F. Chen, X. Li and J. Dong (2012). "Differences in Yes-associated protein and mRNA levels in regenerating liver and hepatocellular carcinoma." Mol Med Rep **5**(2): 410-414.
Wang, W., J. Huang and J. Chen (2011). "Angiomotin-like proteins associate with and negatively regulate YAP1." J Biol Chem **286**(6): 4364-4370.
Wang, W., J. Huang, X. Wang, J. Yuan, X. Li, L. Feng, J. I. Park and J. Chen (2012). "PTPN14 is required for the density-dependent control of YAP1." Genes Dev **26**(17): 1959-1971.
Wang, X. W., S. P. Hussain, T. I. Huo, C. G. Wu, M. Forgues, L. J. Hofseth, C. Brechot and C. C. Harris (2002). "Molecular pathogenesis of human hepatocellular carcinoma." Toxicology **181-182**: 43-47.
Wang, Y., Q. Dong, Q. Zhang, Z. Li, E. Wang and X. Qiu (2010). "Overexpression of yes-associated protein contributes to progression and poor prognosis of non-small-cell lung cancer." Cancer Sci **101**(5): 1279-1285.
Wang, Z., Y. Wu, H. Wang, Y. Zhang, L. Mei, X. Fang, X. Zhang, F. Zhang, H. Chen, Y. Liu, Y. Jiang, S. Sun, Y. Zheng, N. Li and L. Huang (2014). "Interplay of mevalonate and Hippo pathways regulates RHAMM transcription via YAP to modulate breast cancer cell motility." Proc Natl Acad Sci U S A **111**(1): E89-98.
Watson, R. F., R. D. Chernock, X. Wang, W. Liu, X. J. Ma, Y. Luo, H. Wang, S. K. El-Mofty and J. S. Lewis, Jr. (2013). "Spindle cell carcinomas of the head and neck rarely harbor transcriptionally-active human papillomavirus." Head Neck Pathol **7**(3): 250-257.
Watt, F. M. (1984). "Selective migration of terminally differentiating cells from the basal layer of cultured human epidermis." J Cell Biol **98**(1): 16-21.
Watt, F. M. (2002). "Role of integrins in regulating epidermal adhesion, growth and differentiation." EMBO J **21**(15): 3919-3926.
Watt, F. M. and H. Fujiwara (2011). "Cell-extracellular matrix interactions in normal and diseased skin." Cold Spring Harb Perspect Biol **3**(4).
Watt, F. M. and B. L. Hogan (2000). "Out of Eden: stem cells and their niches." Science **287**(5457): 1427-1430.
Watt, F. M. and K. B. Jensen (2009). "Epidermal stem cell diversity and quiescence." EMBO Mol Med **1**(5): 260-267.
Weis, W. I., W. J. Nelson and D. J. Dickinson (2013). "Evolution and cell physiology. 3. Using Dictyostelium discoideum to investigate mechanisms of epithelial polarity." Am J Physiol Cell Physiol **305**(11): C1091-1095.
Wellner, U., T. Brabletz and T. Keck (2010). "ZEB1 in Pancreatic Cancer." Cancers (Basel) **2**(3): 1617-1628.
Wells, C. D., J. P. Fawcett, A. Traweger, Y. Yamanaka, M. Goudreault, K. Elder, S. Kulkarni, G. Gish, C. Virag, C. Lim, K. Colwill, A. Starostine, P. Metalnikov and T. Pawson (2006). "A Rich1/Amot complex regulates the Cdc42 GTPase and apical-polarity proteins in epithelial cells." Cell **125**(3): 535-548.
Wennmann, D. O., B. Vollenbroker, A. K. Eckart, J. Bonse, F. Erdmann, D. A. Wolters, L. K. Schenk, U. Schulze, J. Kremerskothen, T. Weide and H. Pavenstadt (2014). "The Hippo pathway is controlled by Angiotensin II signaling and its reactivation induces apoptosis in podocytes." Cell Death Dis **5**: e1519.
Whyte, J. L., A. A. Smith and J. A. Helms (2012). "Wnt signaling and injury repair." Cold Spring Harb Perspect Biol **4**(8): a008078.

Witta, S. E., R. M. Gemmill, F. R. Hirsch, C. D. Coldren, K. Hedman, L. Ravdel, B. Helfrich, R. Dziadziuszko, D. C. Chan, M. Sugita, Z. Chan, A. Baron, W. Franklin, H. A. Drabkin, L. Girard, A. F. Gazdar, J. D. Minna and P. A. Bunn, Jr. (2006). "Restoring E-cadherin expression increases sensitivity to epidermal growth factor receptor inhibitors in lung cancer cell lines." Cancer Res **66**(2): 944-950.
Wong, K. K., W. Li, Y. An, Y. Duan, Z. Li, Y. Kang and Y. Yan (2015). "beta-Spectrin regulates the hippo signaling pathway and modulates the basal actin network." J Biol Chem **290**(10): 6397-6407.
Wrighton, K. H. (2011). "Mechanotransduction: YAP and TAZ feel the force." Nat Rev Mol Cell Biol **12**(7): 404.
Wu, H., Y. Xiao, S. Zhang, S. Ji, L. Wei, F. Fan, J. Geng, J. Tian, X. Sun, F. Qin, C. Jin, J. Lin, Z. Y. Yin, T. Zhang, L. Luo, Y. Li, S. Song, S. C. Lin, X. Deng, F. Camargo, J. Avruch, L. Chen and D. Zhou (2013). "The Ets transcription factor GABP is a component of the hippo pathway essential for growth and antioxidant defense." Cell Rep **3**(5): 1663-1677.
Wu, S., J. Huang, J. Dong and D. Pan (2003). "hippo encodes a Ste-20 family protein kinase that restricts cell proliferation and promotes apoptosis in conjunction with salvador and warts." Cell **114**(4): 445-456.
Wu, S., Y. Liu, Y. Zheng, J. Dong and D. Pan (2008). "The TEAD/TEF family protein Scalloped mediates transcriptional output of the Hippo growth-regulatory pathway." Dev Cell **14**(3): 388-398.
Xiao, H., L. Wu, H. Zheng, N. Li, H. Wan, G. Liang, Y. Zhao and J. Liang (2014). "Expression of Yes-associated protein in cervical squamous epithelium lesions." Int J Gynecol Cancer **24**(9): 1575-1582.
Xiao, Y., J. Leach, J. Wang and J. F. Martin (2016). "Hippo/Yap Signaling in Cardiac Development and Regeneration." Curr Treat Options Cardiovasc Med **18**(6): 38.
Xie, M., L. Zhang, C. S. He, J. H. Hou, S. X. Lin, Z. H. Hu, F. Xu and H. Y. Zhao (2012). "Prognostic significance of TAZ expression in resected non-small cell lung cancer." J Thorac Oncol **7**(5): 799-807.
Xu, M. Z., T. J. Yao, N. P. Lee, I. O. Ng, Y. T. Chan, L. Zender, S. W. Lowe, R. T. Poon and J. M. Luk (2009). "Yes-associated protein is an independent prognostic marker in hepatocellular carcinoma." Cancer **115**(19): 4576-4585.
Xu, T., W. Wang, S. Zhang, R. A. Stewart and W. Yu (1995). "Identifying tumor suppressors in genetic mosaics: the Drosophila lats gene encodes a putative protein kinase." Development **121**(4): 1053-1063.
Yagi, R., L. F. Chen, K. Shigesada, Y. Murakami and Y. Ito (1999). "A WW domain-containing yes-associated protein (YAP) is a novel transcriptional co-activator." EMBO J **18**(9): 2551-2562.
Yamashita, N., E. Tokunaga, H. Kitao, Y. Hisamatsu, K. Taketani, S. Akiyoshi, S. Okada, S. Aishima, M. Morita and Y. Maehara (2013). "Vimentin as a poor prognostic factor for triple-negative breast cancer." J Cancer Res Clin Oncol **139**(5): 739-746.
Yang, C. C., H. K. Graves, I. M. Moya, C. Tao, F. Hamaratoglu, A. B. Gladden and G. Halder (2015). "Differential regulation of the Hippo pathway by adherens junctions and apical-basal cell polarity modules." Proc Natl Acad Sci U S A **112**(6): 1785-1790.
Yang, J. and R. A. Weinberg (2008). "Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis." Dev Cell **14**(6): 818-829.

Yeaman, C., K. K. Grindstaff and W. J. Nelson (1999). "New perspectives on mechanisms involved in generating epithelial cell polarity." Physiol Rev **79**(1): 73-98.
Yi, C., S. Troutman, D. Fera, A. Stemmer-Rachamimov, J. L. Avila, N. Christian, N. L. Persson, A. Shimono, D. W. Speicher, R. Marmorstein, L. Holmgren and J. L. Kissil (2011). "A tight junction-associated Merlin-angiomotin complex mediates Merlin's regulation of mitogenic signaling and tumor suppressive functions." Cancer Cell **19**(4): 527-540.
Yin, F., J. Yu, Y. Zheng, Q. Chen, N. Zhang and D. Pan (2013). "Spatial organization of Hippo signaling at the plasma membrane mediated by the tumor suppressor Merlin/NF2." Cell **154**(6): 1342-1355.
Yu, F. X. and K. L. Guan (2013). "The Hippo pathway: regulators and regulations." Genes Dev **27**(4): 355-371.
Yu, F. X., Z. Meng, S. W. Plouffe and K. L. Guan (2015). "Hippo pathway regulation of gastrointestinal tissues." Annu Rev Physiol **77**: 201-227.
Yu, F. X., Y. Zhang, H. W. Park, J. L. Jewell, Q. Chen, Y. Deng, D. Pan, S. S. Taylor, Z. C. Lai and K. L. Guan (2013). "Protein kinase A activates the Hippo pathway to modulate cell proliferation and differentiation." Genes Dev **27**(11): 1223-1232.
Yu, F. X., B. Zhao, N. Panupinthu, J. L. Jewell, I. Lian, L. H. Wang, J. Zhao, H. Yuan, K. Tumaneng, H. Li, X. D. Fu, G. B. Mills and K. L. Guan (2012). "Regulation of the Hippo-YAP pathway by G-protein-coupled receptor signaling." Cell **150**(4): 780-791.
Yu, J., Y. Zheng, J. Dong, S. Klusza, W. M. Deng and D. Pan (2010). "Kibra functions as a tumor suppressor protein that regulates Hippo signaling in conjunction with Merlin and Expanded." Dev Cell **18**(2): 288-299.
Yuan, M., V. Tomlinson, R. Lara, D. Holliday, C. Chelala, T. Harada, R. Gangeswaran, C. Manson-Bishop, P. Smith, S. A. Danovi, O. Pardo, T. Crook, C. A. Mein, N. R. Lemoine, L. J. Jones and S. Basu (2008). "Yes-associated protein (YAP) functions as a tumor suppressor in breast." Cell Death Differ **15**(11): 1752-1759.
Yuen, H. F., C. M. McCrudden, Y. H. Huang, J. M. Tham, X. Zhang, Q. Zeng, S. D. Zhang and W. Hong (2013). "TAZ expression as a prognostic indicator in colorectal cancer." PLoS One **8**(1): e54211.
Zaidel-Bar, R. and B. Geiger (2010). "The switchable integrin adhesome." J Cell Sci **123**(Pt 9): 1385-1388.
Zaidel-Bar, R., S. Itzkovitz, A. Ma'ayan, R. Iyengar and B. Geiger (2007). "Functional atlas of the integrin adhesome." Nat Cell Biol **9**(8): 858-867.
Zaidi, S. K., A. J. Sullivan, R. Medina, Y. Ito, A. J. van Wijnen, J. L. Stein, J. B. Lian and G. S. Stein (2004). "Tyrosine phosphorylation controls Runx2-mediated subnuclear targeting of YAP to repress transcription." EMBO J **23**(4): 790-799.
Zanconato, F., M. Cordenonsi and S. Piccolo (2016). "YAP/TAZ at the Roots of Cancer." Cancer Cell **29**(6): 783-803.
Zanconato, F., M. Forcato, G. Battilana, L. Azzolin, E. Quaranta, B. Bodega, A. Rosato, S. Bicciato, M. Cordenonsi and S. Piccolo (2015). "Genome-wide association between YAP/TAZ/TEAD and AP-1 at enhancers drives oncogenic growth." Nat Cell Biol **17**(9): 1218-1227.
Zeisberg, M. and E. G. Neilson (2009). "Biomarkers for epithelial-mesenchymal transitions." J Clin Invest **119**(6): 1429-1437.

Zender, L., M. S. Spector, W. Xue, P. Flemming, C. Cordon-Cardo, J. Silke, S. T. Fan, J. M. Luk, M. Wigler, G. J. Hannon, D. Mu, R. Lucito, S. Powers and S. W. Lowe (2006). "Identification and validation of oncogenes in liver cancer using an integrative oncogenomic approach." Cell **125**(7): 1253-1267.
Zervas, C. G., S. L. Gregory and N. H. Brown (2001). "Drosophila integrin-linked kinase is required at sites of integrin adhesion to link the cytoskeleton to the plasma membrane." J Cell Biol **152**(5): 1007-1018.
Zhang, G. J., T. Zhou, H. P. Tian, Z. L. Liu and S. S. Xia (2013). "High expression of ZEB1 correlates with liver metastasis and poor prognosis in colorectal cancer." Oncol Lett **5**(2): 564-568.
Zhang, H., C. Y. Liu, Z. Y. Zha, B. Zhao, J. Yao, S. Zhao, Y. Xiong, Q. Y. Lei and K. L. Guan (2009). "TEAD transcription factors mediate the function of TAZ in cell growth and epithelial-mesenchymal transition." J Biol Chem **284**(20): 13355-13362.
Zhang, H., H. A. Pasolli and E. Fuchs (2011). "Yes-associated protein (YAP) transcriptional coactivator functions in balancing growth and differentiation in skin." Proc Natl Acad Sci U S A **108**(6): 2270-2275.
Zhang, J., J. Y. Ji, M. Yu, M. Overholtzer, G. A. Smolen, R. Wang, J. S. Brugge, N. J. Dyson and D. A. Haber (2009). "YAP-dependent induction of amphiregulin identifies a non-cell-autonomous component of the Hippo pathway." Nat Cell Biol **11**(12): 1444-1450.
Zhang, J., C. Lu, J. Zhang, J. Kang, C. Cao and M. Li (2013). "Involvement of ZEB1 and E-cadherin in the invasion of lung squamous cell carcinoma." Mol Biol Rep **40**(2): 949-956.
Zhang, L., F. Ren, Q. Zhang, Y. Chen, B. Wang and J. Jiang (2008). "The TEAD/TEF family of transcription factor Scalloped mediates Hippo signaling in organ size control." Dev Cell **14**(3): 377-387.
Zhang, N., H. Bai, K. K. David, J. Dong, Y. Zheng, J. Cai, M. Giovannini, P. Liu, R. A. Anders and D. Pan (2010). "The Merlin/NF2 tumor suppressor functions through the YAP oncoprotein to regulate tissue homeostasis in mammals." Dev Cell **19**(1): 27-38.
Zhang, W., Y. Gao, F. Li, X. Tong, Y. Ren, X. Han, S. Yao, F. Long, Z. Yang, H. Fan, L. Zhang and H. Ji (2015). "YAP promotes malignant progression of Lkb1-deficient lung adenocarcinoma through downstream regulation of survivin." Cancer Res **75**(21): 4450-4457.
Zhang, W., N. Nandakumar, Y. Shi, M. Manzano, A. Smith, G. Graham, S. Gupta, E. E. Vietsch, S. Z. Laughlin, M. Wadhwa, M. Chetram, M. Joshi, F. Wang, B. Kallakury, J. Toretsky, A. Wellstein and C. Yi (2014). "Downstream of mutant KRAS, the transcription regulator YAP is essential for neoplastic progression to pancreatic ductal adenocarcinoma." Sci Signal **7**(324): ra42.
Zhang, X., J. George, S. Deb, J. L. Degoutin, E. A. Takano, S. B. Fox, A. S. group, D. D. Bowtell and K. F. Harvey (2011). "The Hippo pathway transcriptional co-activator, YAP, is an ovarian cancer oncogene." Oncogene **30**(25): 2810-2822.
Zhao, B., J. Kim, X. Ye, Z. C. Lai and K. L. Guan (2009). "Both TEAD-binding and WW domains are required for the growth stimulation and oncogenic transformation activity of yes-associated protein." Cancer Res **69**(3): 1089-1098.
Zhao, B., L. Li, Q. Lei and K. L. Guan (2010). "The Hippo-YAP pathway in organ size control and tumorigenesis: an updated version." Genes Dev **24**(9): 862-874.
Zhao, B., L. Li, Q. Lu, L. H. Wang, C. Y. Liu, Q. Lei and K. L. Guan (2011). "Angiomotin is a novel Hippo pathway component that inhibits YAP oncoprotein." Genes Dev **25**(1): 51-63.

Zhao, B., L. Li, L. Wang, C. Y. Wang, J. Yu and K. L. Guan (2012). "Cell detachment activates the Hippo pathway via cytoskeleton reorganization to induce anoikis." Genes Dev **26**(1): 54-68.
Zhao, B., X. Wei, W. Li, R. S. Udan, Q. Yang, J. Kim, J. Xie, T. Ikenoue, J. Yu, L. Li, P. Zheng, K. Ye, A. Chinnaiyan, G. Halder, Z. C. Lai and K. L. Guan (2007). "Inactivation of YAP oncoprotein by the Hippo pathway is involved in cell contact inhibition and tissue growth control." Genes Dev **21**(21): 2747-2761.
Zhao, B., X. Ye, J. Yu, L. Li, W. Li, S. Li, J. Yu, J. D. Lin, C. Y. Wang, A. M. Chinnaiyan, Z. C. Lai and K. L. Guan (2008). "TEAD mediates YAP-dependent gene induction and growth control." Genes Dev **22**(14): 1962-1971.
Zhao, R., T. R. Fallon, S. V. Saladi, A. Pardo-Saganta, J. Villoria, H. Mou, V. Vinarsky, M. Gonzalez-Celeiro, N. Nunna, L. P. Hariri, F. Camargo, L. W. Ellisen and J. Rajagopal (2014). "Yap tunes airway epithelial size and architecture by regulating the identity, maintenance, and self-renewal of stem cells." Dev Cell **30**(2): 151-165.
Zhao, X. H., C. Laschinger, P. Arora, K. Szaszi, A. Kapus and C. A. McCulloch (2007). "Force activates smooth muscle alpha-actin promoter activity through the Rho signaling pathway." J Cell Sci **120**(Pt 10): 1801-1809.
Zheng, Y., W. Wang, B. Liu, H. Deng, E. Uster and D. Pan (2015). "Identification of Happyhour/MAP4K as Alternative Hpo/Mst-like Kinases in the Hippo Kinase Cascade." Dev Cell **34**(6): 642-655.
Zhi, X., D. Zhao, Z. Zhou, R. Liu and C. Chen (2012). "YAP promotes breast cell proliferation and survival partially through stabilizing the KLF5 transcription factor." Am J Pathol **180**(6): 2452-2461.
Zhou, D., C. Conrad, F. Xia, J. S. Park, B. Payer, Y. Yin, G. Y. Lauwers, W. Thasler, J. T. Lee, J. Avruch and N. Bardeesy (2009). "Mst1 and Mst2 maintain hepatocyte quiescence and suppress hepatocellular carcinoma development through inactivation of the Yap1 oncogene." Cancer Cell **16**(5): 425-438.
Zhou, D., Y. Zhang, H. Wu, E. Barry, Y. Yin, E. Lawrence, D. Dawson, J. E. Willis, S. D. Markowitz, F. D. Camargo and J. Avruch (2011). "Mst1 and Mst2 protein kinases restrain intestinal stem cell proliferation and colonic tumorigenesis by inhibition of Yes-associated protein (Yap) overabundance." Proc Natl Acad Sci U S A **108**(49): E1312-1320.
Zhou, X., S. Wang, Z. Wang, X. Feng, P. Liu, X. B. Lv, F. Li, F. X. Yu, Y. Sun, H. Yuan, H. Zhu, Y. Xiong, Q. Y. Lei and K. L. Guan (2015). "Estrogen regulates Hippo signaling via GPER in breast cancer." J Clin Invest **125**(5): 2123-2135.
Zhou, Y. M., L. Cao, B. Li, R. X. Zhang, C. J. Sui, Z. F. Yin and J. M. Yang (2012). "Clinicopathological significance of ZEB1 protein in patients with hepatocellular carcinoma." Ann Surg Oncol **19**(5): 1700-1706.
Zhu, Y., D. Li, Y. Wang, C. Pei, S. Liu, L. Zhang, Z. Yuan and P. Zhang (2015). "Brahma regulates the Hippo pathway activity through forming complex with Yki-Sd and regulating the transcription of Crumbs." Cell Signal **27**(3): 606-613.