Functional analysis of the threonine motif in the β1 integrin cytoplasmic tail in mice

Christopher Stremmel

Aus dem Max-Planck-Institut für Biochemie, Martinsried
Abteilung für Molekulare Medizin
Leitung: Prof. Dr. Reinhard Fässler

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1 Abbreviations

Aa	Amino acids
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
ADP	Adenosine diphosphate
Akt/ PKB	Protein kinase B
APP	Amyloid precursor protein
ARF6	ADP ribosylation factor 6
BAR	Bin/amphiphysin/rvs
BM	Basement membrane
BSA	Bovine serum albumin
Ca	Calcium
CCD	Charge-coupled device
Cdc42	Cell division cycle 42
cDNA	Complementary DNA
CNS	Central nervous system
Dab	Disabled
Dab2	Disabled homolog 2
DAPI	4',6-diamidino-2-phenylindole
DNA	Deoxyribonucleic acid
E7.5	Day 7.5 of embryonic development
EB	Embryoid body
EBS	Epidermolysis bullosa syndrome
ECM	Extracellular matrix
EDTA	Ethylenediaminetetraacetic acid
EEA1	Early endosome antigen 1
eGFP	Enhanced green fluorescent protein
EGFR	Epidermal growth factor receptor
EGTA	Ethylene glycol tetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
ERK	Extracellular-signal-regulated kinase

ES	Embryonic stem
ESCRT	Endosomal sorting complex required for transport
FA	Focal adhesion
FACS	Fluorescence-activated cell sorting
F-actin	Filamentous actin
FAK	Focal adhesion kinase
FBS	Fetal bovine serum
FC	Focal complex
FERM	Band4.1/ezrin/radixin/moesin
FGFR	Fibroblast growth factor receptor
FITC	Fluorescein isothiocyanate
FN	Fibronectin
GAP	GTPase activating protein
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GEF	Guanine nucleotide exchange factor
GID	Guanine nucleotide dissociation inhibitor
GST	Glutathione S-transferase
GTPase	Guanosine triphosphatase
HAX1	HCLS1-associated protein X-1
HCLS1	Hematopoietic lineage cell-specific protein
HE	Haematoxilin and eosin
HEK	Human embryonic kidney
HRP	Horseradish peroxidase
IAA	Iodoacetamide
ICAM-1	Intracellular adhesion molecule-1
IF	Immunofluorescence
Ig	Immunoglobulin
ILV	Intraluminal vesicle
IP	Immunoprecipitation
IPTG	Isopropyl β-D-1-thiogalactopyranoside
ITR	Inverted terminal repeat
Krit1	Krev-interaction trapped 1

LAD	Leukocyte-adhesion deficiency
Lamp1	Lysosomal-associated membrane protein 1
LAP	Latency associated peptide
LDLR	Low density lipoprotein receptor
LDV	Leucine-aspartic acid-valine
LFA1	Lymphocyte function-associated antigen 1, integrin αLβ2
LIF	Leukemia inhibitory factor
LN	Laminin
LRP1	Low density lipoprotein receptor related protein 1
mAb	Monoclonal antibody
MAdCAM-1	Mucosal addressin cell adhesion molecule-1
mCherry	Monomeric Cherry
MEF	Mouse embryonic fibroblasts
Mesna	Sodium 2-mercaptoethanesulfonate
Mg	Magnesium
MIDAS	Metal-ion dependent adhesion sites
Mn	Manganese
mRFP	Monomeric red fluorescent protein
mRNA	Messenger ribonucleic acid
MS	Multiple sclerosis
MVB	Multivesicular body
NFκB	Nuclear factor κ-light-chain-enhancer of activated B cells
NHS-LC-biotin	Succinimidyl-6-(biotinamido)hexanoate
NHS-SS-biotin	Succinimidyl-2-(biotinamido)-ethyl-1,3'-dithiopropionate
NLS	Nuclear localization signal
NRP1	Neuropilin 1
PBS	Phosphate-buffered saline
PBSST	0.1% Triton-X, 5% BSA in PBS
PBS-T	PBS + Tween 20
PCR	Polymerase chain reaction
PE	Phycoerythrin
PFA	Paraformaldehyde

PH	Pleckstrin homology
PI3	Phosphatidylinositol 3
PLCγ1	Phospholipase C γ1
PML	Progressive multifocal leukoencephalopathy
PNRC	Perinuclear recycling compartment
PNS	Peripheral nervous system
PTB	Phosphotyrosin-binding
PtdInsP, PIP	Phosphatidylinositol phosphate
PX	Phox homology
qRT	Quantitative real-time
Rab	Ras-associated binding
Rac1	Ras-related C3 botulinum toxin substrate 1
Ras	Rat sarcoma
RCP	Rab-coupling protein
RGD	Arginine-glycine-aspartic acid
RhoA	Ras homolog gene family, member A
RIPA	Radioimmunoprecipitation assay
RNA	Ribonucleic acid
Rpm	Revolutions per minute
RT	Room temperature
RTK	Receptor tyrosin kinase
SD	Standard deviation
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEM	Standard error of the mean
SH3	Src Homology 3
shRNA	Short hairpin RNA
SILAC	Stable isotope labeling by amino acids in cell culture
siRNA	Small interfering RNA
SLIC	Sequence- and ligation-independent cloning
SNX	Sorting nexin
SV40	Simian virus 40
TBS	Tris-buffered saline

TCR	T-cell receptor
Tfr	Transferrin receptor
TGFβ	Transforming growth factor β
TGN	Trans-Golgi network
THD	Talin head domain
TSG101	Tumor susceptibility gene 101
UB	Ureteric bud
VCAM-1	Vascular cell adhesion molecule-1
VEGF	Vascular endothelial growth factor
VN	Vitronectin
WB	Western blotting
Wcl	Whole-cell lysate
Wt	Wild type

2 Introduction

2.1 Integrin adhesion receptors

Integrins are major type I transmembrane receptors mediating adhesion to the extracellular matrix (ECM) as well as other cells. One of 18 α and one of 8 β subunits are non-covalently associated and form a plethora of at least 24 different integrin heterodimers (Hynes 2002), each of them specific for certain ECM components such as fibronectin (FN), laminin (LN) or collagen as well as soluble ligands or cell surface components (Humphries et al. 2006) (Figure 1).

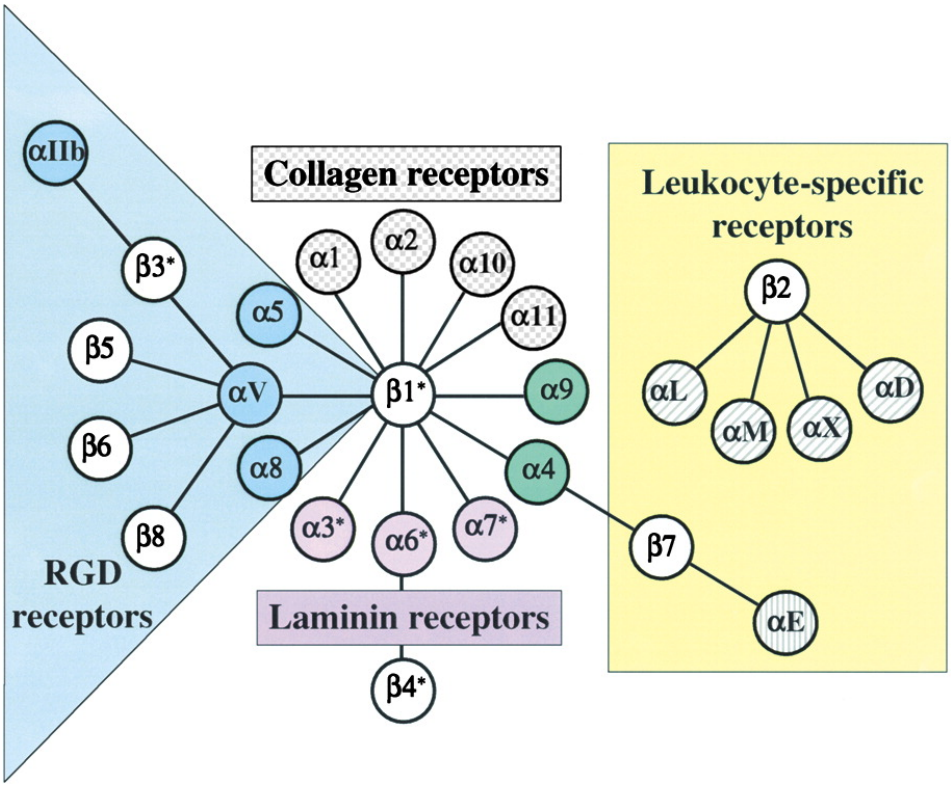


Figure 1: The integrin receptor family
Depicted are the 18 α and 8 β subunits and their 24 potential heterodimer associations divided into four subclasses of integrin receptors according to major ligands or leukocyte-specific expression (adapted from Hynes 2002 (Hynes 2002)).

Integrin receptors can be classified into four distinct groups according to specific ligands or cell-type specific expression patterns. The first three groups are characterized by their main ligand including collagen, LN and RGD (arginine-glycine-aspartic acid)-motif specific integrins, which bind ECM components such as FN, vitronectin (VN), thrombospondin, osteopontin and tenascin. However, the collagen receptors α1β1, α2β1, α10β1 and α11β1 have also been described to bind LN (Humphries et al. 2006). The fourth group is defined by its cell-type specific expression on leukocytes and recognizes the tripeptide motif LDV (leucine-aspartic acid-valine) or similar motifs, which are typically present in endothelial ligands such as VCAM-1 (vascular cell adhesion molecule-1), ICAM-1 (intracellular adhesion molecule-1) and MAdCAM-1 (mucosal addressin cell adhesion molecule-1) as well as the unrelated protein E-cadherin. Moreover, leukocyte integrins can also recognize plasma proteins such as FN or the complement component iC3b. Minor exceptions with simultaneous characteristics of two integrin subclasses are the two heterodimers α9β1 and α4β1 as they recognize the LDV-motif in FN and they associate with receptors such as VCAM-1, an Ig-superfamily counter-receptor (Hynes 2002).

Although the production of α and β chains within any given cell type may not be balanced, only intact heterodimeric integrins are found on the cell surface in cell-type dependent patterns – so called integrin profiles. Once integrins are bound to ECM components bidirectional signaling events influence cell adhesion, proliferation, migration, polarity, differentiation and survival, making integrins indispensible for the development and homeostasis of multicellular organisms (Sheppard 2000; Hynes 2002; Humphries et al. 2006). Due to the unique and essential features of integrins it is not surprising that there has been an evolutionary conservation in metazoans. Along with increasing organism complexity the

number of integrin heterodimers increased from two in *Caenorhabditis elegans* via five in *Drosophila melanogaster* to 24 in mammals with partially overlapping substrate specificity (Hynes 2002; Lowell and Mayadas 2012).

2.2 Integrins have bidirectional signaling capacity

The name "integrins" refers to their ability to integrate signals from the surrounding extracellular environment with the interior of the cell and vice-versa (Tamkun et al. 1986). This bidirectional signaling capacity is usually referred to as inside-out and outside-in signaling, respectively.

2.2.1 Inside-out signaling

2.2.1.1 Integrin conformation dictates receptor affinity

The large extracellular integrin domain (>700 aa) binds ECM components and is linked via a transmembrane region (25-29 aa) to a short cytoplasmic tail (about 20-50 aa) (Arnaout et al. 2005; Luo et al. 2007). In the case of inside-out signaling the intracellular adaptor molecules talin and kindlin initiate a conformational change of the extracellular integrin domain from a bent inactive state to an extended active state (Hynes 2002; Moser et al. 2009). This structural integrin modification was described as the switchblade model and involves an additional separation of the cytoplasmic tails and the transmembrane region to further upregulate receptor affinity (Luo et al. 2007) (Figure 2).

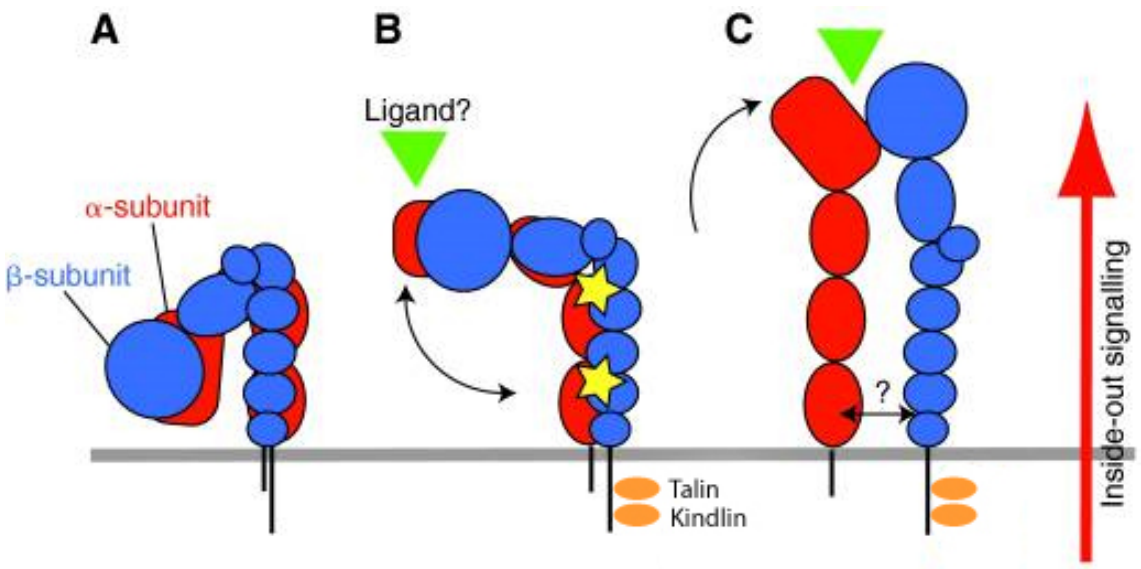


Figure 2: Conformational affinity regulation of integrins
The bent inactive extracellular integrin domain (A) extends upon Talin and kindlin recruitment to the cytoplasmic integrin tail (B) followed by an additional increase in receptor affinity by transmembrane separation (C) (adapted and modified from Askari, 2009 (Askari et al. 2009)).

Full integrin activation is prevented by a super weak salt bridge interaction between the α and the β subunit in the transmembrane region. In the platelet integrin αIIbβ3 for example, this salt bridge was identified to be established by the α subunit arginine 995 and the β subunit aspartic acid 723 (Hughes et al. 1996). Mutations of corresponding residues in the conserved GFFKR motif in α4 and αL disrupted the interaction and led to integrin activation (Lu and Springer 1997; Imai et al. 2008). However, an aspartic acid to alanine mutation of the salt bridge residue in the β1 cytoplasmic tail in mice did not show any obvious phenotype questioning the role of a putative salt bridge *in vivo* (Czuchra et al. 2006). Therefore, recent studies suggest an inner and outer membrane clasp (Lau et al. 2009) which might be primarily responsible for tail association with an only minor contribution of a potential salt bridge (Kim et al. 2009). Despite of these discrepancies regarding a mechanistic explanation, the overall

concept of transmembrane association and separation is widely accepted. Moreover, artificial models have demonstrated that integrin affinity downregulation is driven by transmembrane association (Lu et al. 2001; Luo et al. 2004; Zhu et al. 2008) and that upregulation is separation-dependent (Hughes et al. 1996; Gottschalk 2005; Li et al. 2005; Luo et al. 2005; Partridge et al. 2005).

Conformational changes of the integrin molecule are mainly initiated by the recruitment of integrin activating molecules. However, there is an alternative mechanism of integrin activation, which is often used to verify defects in conventional integrin activation. Mg^{2+} or Ca^{2+} physiologically occupy metal-ion dependent adhesion sites (MIDAS) or related regions, whereas substitutions of these ions by Mn^{2+} cause a shift to the high affinity integrin conformation (Hynes 2002; Arnaout et al. 2005; Luo et al. 2007).

2.2.1.2 Talin and Kindlin regulate integrin receptor affinity

Receptor affinity of integrins is regulated by conformational changes initiated by the two FERM-domain-containing proteins talin and kindlin.

Talin consists of a flexible rod domain and a FERM-domain-containing talin head domain (THD) (Rees et al. 1990; Calderwood et al. 2002; Garcia-Alvarez et al. 2003), which enables it to bind to the conserved membrane proximal NPxY motif as well as additional membrane proximal residues (Figure 3).

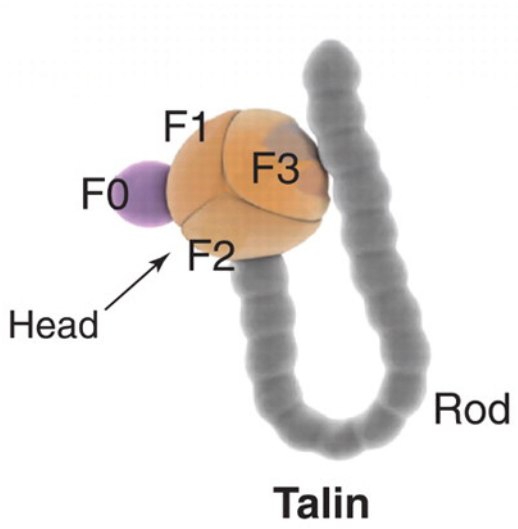


Figure 3: The talin molecule
Depicted are the FERM-domain-containing THD and the flexible talin rod. The THD is composed of the F0- to F3-FERM-subdomains (adapted from Moser 2009 (Moser et al. 2009)).

Upon talin binding, cytoplasmic tails and transmembrane regions separate – a process that is referred to as the final common step in integrin activation (Tadokoro et al. 2003; Simonson et al. 2006; Lim et al. 2007; Nieswandt et al. 2007; Petrich et al. 2007). Whereas the phosphotyrosine-binding (PTB) F3-FERM-subdomain was described to be sufficient for β3 integrin activation (Calderwood et al. 2002), a complete THD association is required for β1 integrin activation (Bouaouina et al. 2008). Although the talin rod domain is not necessary for integrin activation, it plays a central role in focal adhesion (FA) formation and possibly also in integrin clustering by establishing connections to the actin cytoskeleton and by potential homodimerization via its second integrin binding site (Critchley and Gingras 2008; Zhang et al. 2008). Multiple studies have shown that a disruption of the NPxY motif in the cytoplasmic integrin domain inhibits talin binding and prevents integrin activation (Knezevic et al. 1996; Vinogradova et al. 2002; Ulmer et al. 2003; Wegener et al. 2007). Additionally, there is an autoinhibitory association between the talin head and rod domains, which functions as a

regulatory mechanism to prevent excessive integrin activation and which is disrupted by phosphatidylinositol-4,5-bisphosphate binding (Martel et al. 2001; Goksoy et al. 2008). Until today it is not entirely clear if phosphorylation of the integrin NPxY motif plays a role in the recruitment of talin to the cytoplasmic tail. *In vivo* studies on non-phosphorylatable β1 integrins showed no obvious phenotype (Chen et al. 2006; Czuchra et al. 2006), while analogue mutations in the β3 tail resulted in mild bleeding defects (Law et al. 1999) and pathological angiogenesis (Mahabeleshwar et al. 2006).

Recent studies revealed that there is an additional requirement of kindlin besides the essential integrin activator talin (Ma et al. 2008; Montanez et al. 2008; Ussar et al. 2008). The kindlin family consists of kindlin-1, which is mainly expressed in epithelial cells, kindlin-2, which is widely expressed, and the hematopoietic kindlin-3 (Jobard et al. 2003; Siegel et al. 2003; Ussar et al. 2006; Meves et al. 2009). The structure of kindlin resembles the THD with a PH (pleckstrin homology)-domain inserted into the F2-FERM-subdomain (Kloeker et al. 2004; Goult et al. 2009; Meves et al. 2009). As described for talin, kindlin uses its F3-FERM-subdomain to bind to the distal NxxY motif and the adjacent threonines or serines depending on the β integrin subunit (Shi et al. 2007; Ma et al. 2008; Montanez et al. 2008; Moser et al. 2008; Ussar et al. 2008; Moser et al. 2009) (Figure 4).

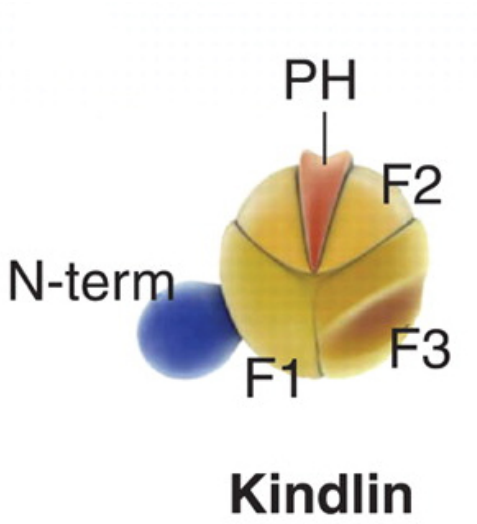


Figure 4: The kindlin molecule
Depicted is the kindlin molecule with its N-terminus and the C-terminal F1- to F3-FERM-subdomains including an inserted PH-domain in the F2-FERM-subdomain (adapted from Moser 2009 (Moser et al. 2009)).

Functional consequences of defective kindlin-binding on integrin-mediated processes were demonstrated in *in vivo* knockout studies in mice. Loss-of-function mutations in human kindlin-1 are causative of a rare autosomal recessive skin disease characterized by congenital skin blistering, photosensitivity, progressive poikiloderma, cutaneous atrophy and potential skin cancer formation (Jobard et al. 2003; Siegel et al. 2003; Meves et al. 2009). While similar effects were also observed in kindlin-1-null mice, additional intestinal defects were more pronounced and caused perinatal death due to ulcerative colitis (Ussar et al. 2008). Interestingly, this gastrointestinal phenotype has later also been observed in humans (Sadler et al. 2006; Kern et al. 2007). Kindlin-2 deletion in mice results in early embryonic lethality due to severe adhesion and polarization defects on a defective BM at the peri-implantation stage (Montanez et al. 2008). Most likely, the severe consequences of kindlin-2 dysfunction are the reason why analogue mutations have not been identified in humans so far. Finally, kindlin-3

deletion in the hematopoietic system caused severe bleedings and defective platelet aggregation (Moser et al. 2008). Additionally, kindlin-3 was shown to be required for leukocyte adhesion and extravasation (Moser et al. 2009). Kindlin-3 mutations were identified to be responsible for a rare genetic leukocyte abnormality called leukocyte-adhesion deficiency (LAD) type III characterized by bleedings as well as defects in leukocyte adhesion and extravasation (Mory et al. 2008; Kuijpers et al. 2009; Malinin et al. 2009; Svensson et al. 2009).

2.2.1.3 Concepts of integrin activation

Despite of a widely accepted synergistic integrin activation model including talin and kindlin, mechanistic details remain largely elusive. Although simultaneous binding is theoretically possible, there are also alternative concepts of sequential binding or integrin transactivation (Moser et al. 2009) (Figure 5). The transactivation concept refers to the binding of one integrin activator to one integrin heterodimer while the other activator binds an associated second heterodimer.

Moreover there might also be substantial differences in the role of integrin activation for different integrin heterodimers. While the platelet integrin αIIbβ3 requires extremely fast activation upon vascular injury, β1 integrins in epithelial cells might tolerate slower changes in receptor affinity. All things considered, there is a widely accepted general concept of integrin activation. However, it is not entirely clear until today how the activation works in detail and if there are cell-type specific differences in receptor affinity modulation.

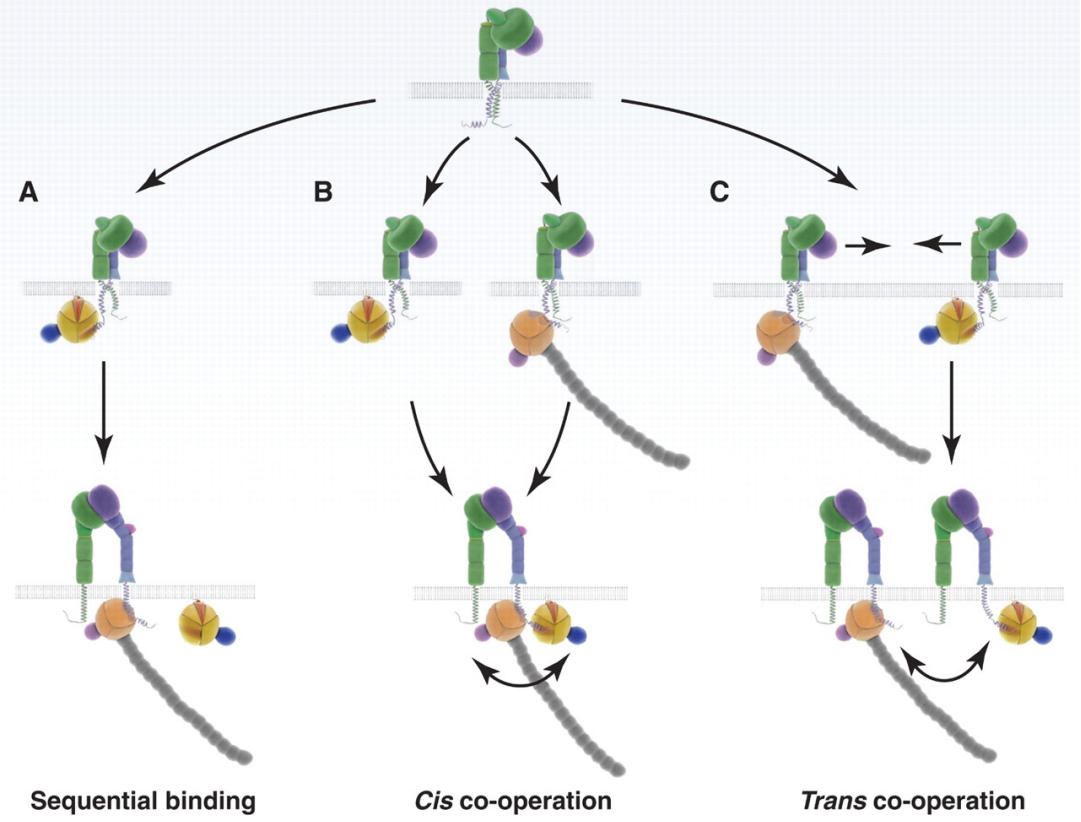


Figure 5: Potential concepts of integrin activation
Depicted are three potential concepts of talin- and kindlin-mediated integrin activation including sequential binding (A), simultaneous binding (B) or transactivation (C) (adapted from Moser, 2009 (Moser et al. 2009)).

2.2.2 Outside-in signaling

Integrins possess bidirectional signaling capacity as they cannot only convey signals from the inside to the outside of the cell, as described for integrin activation, but also vice-versa from the outside to the inside. Due to a lack of any enzymatic activity integrins do not only establish connections to the actin cytoskeleton but they also recruit numerous adaptor proteins that modulate cell behavior like cell cycle stimulation via ERK and cyclin D1, inhibition of apoptosis via PI3-kinase, Akt and NFκB as well as shape, polaritiy and motility modulation via protein tyrosine kinases, phosphatases and members of the Rho family of small GTPases

(Luo et al. 2007; Legate et al. 2009; Lowell and Mayadas 2012). This network of adaptor proteins is usually referred to as integrin adhesome and comprises about 40 direct and 140 indirect integrin binding proteins (Zaidel-Bar et al. 2007; Zaidel-Bar and Geiger 2010; Schiller et al. 2011) most of which are recruited to the β subunit (Legate et al. 2009). This highly complex network undergoes a continuous process of reorganization involving molecular assembly and disassembly. The major functions of this network are signal transduction and actin cytoskeleton interactions (Figure 6). This is mediated by direct integrin-binding proteins with actin-binding capability such as talin, α-actinin, filamin and tensin. However, this network is supplemented by additional actin-binding molecules such as vinculin, which are indirectly recruited to the tail via adaptor molecules. Although talin is capable of direct actin recruitment indirect connections via vinculin have also been described (Humphries and Humphries 2007; Legate et al. 2009).

The actin cytoskeleton undergoes continuous reorganization processes initiated by Rho GTPase recruitment, a member of the Ras (rat sarcoma) superfamily. In humans there are over 22 Rho GTPases including the most prominent representatives RhoA, Rac1 (Ras-related C3 botulinum toxin substrate 1) and Cdc42 (cell division cycle 42). Rho GTPases are regulated by GEFs (guanine nucleotide exchange factor), GAPs (GTPase activating proteins) and GIDs (guanine nucleotide dissociation inhibitor) to allow highly dynamic regulations of not only actin dynamics, but also gene expression, membrane trafficking, microtubule dynamics, proliferation and cytokinesis (Hynes 2002; Jaffe and Hall 2005; Heasman and Ridley 2008).

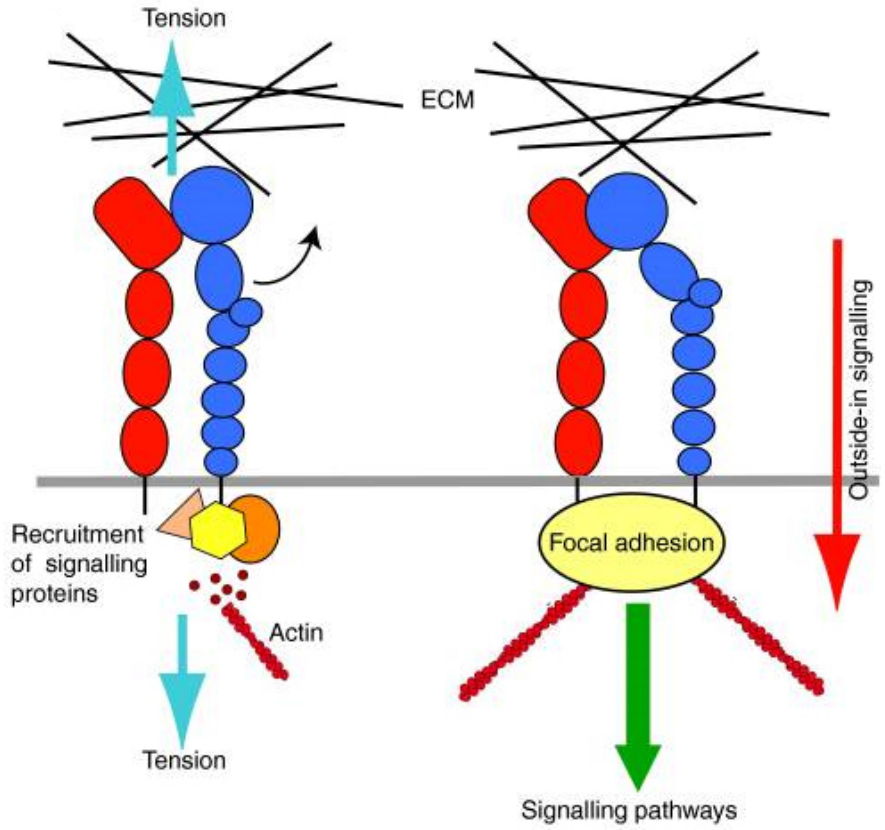


Figure 6: Integrin outside-in signaling
Integrins bind the ECM to establish dynamic actin cytoskeleton interactions and to convey signals by adaptor protein recruitment to their cytoplasmic tails (adapted and modified from Askari 2009 (Askari et al. 2009)).

Since individual integrin-ligand interactions are relatively weak, multiple connections need to be established for proper ECM adhesion. This involves clustering of integrins into adhesive units in a synergistic process known as avidity. Based on size, morphology, localization and composition several types of adhesions can be distinguished in cultured cells including nascent adhesion (Choi et al. 2008), focal complexes (FCs), focal adhesions (FAs) and fibrillar adhesions (Geiger and Bershadsky 2001). Special adhesive structure such as

podosomes and invadopodia are cell-type specific and exclusively found in monocytic and tumor cells, respectively (Linder 2009).

Furthermore integrins modulate growth factor signaling and trafficking, as described for the epidermal growth factor receptor (EGFR) or the fibroblast growth factor receptor (FGFR) (Walker et al. 2005), as well as lipid raft trafficking (del Pozo et al. 2005; Balasubramanian et al. 2007). Taken together integrins are much more than a purely mechanic link between the ECM and the actin cytoskeleton. Bidirectional signaling and actin dynamics make integrins indispensible for multiple cellular processes (Schwartz 2001; Hynes 2002; Ramsay et al. 2007; Ulrich and Heisenberg 2009).

2.3 Integrin trafficking

Fundamental cellular processes such as cell adhesion, proliferation and migration do not only depend on bidirectional signaling but also on continuous integrin synthesis, internalization, recycling and degradation – a process called integrin trafficking (Figure 7).

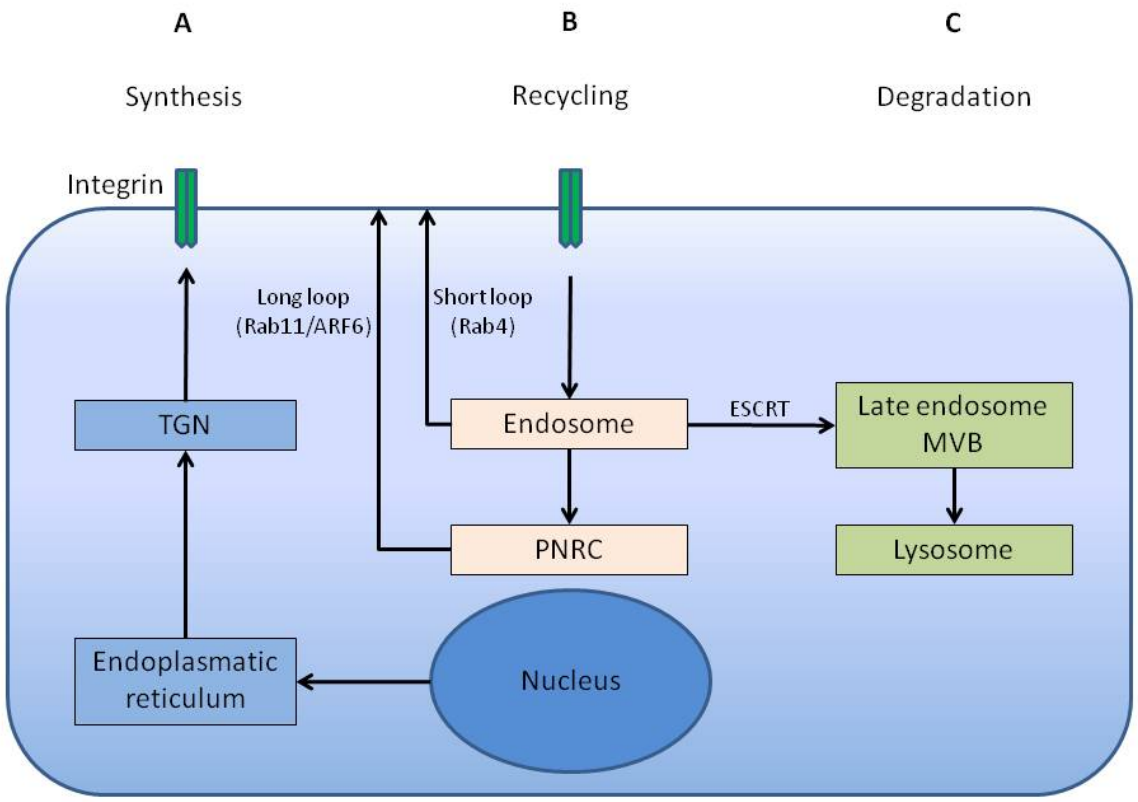


Figure 7: Integrin trafficking
(A) Integrin mRNA is transcribed in the nucleus and translated in ribosomes at the endoplasmic reticulum prior to exocytosis to the plasma membrane via the TGN. (B) Upon internalization into the endosomal compartment integrins are either recycled via the Rab4-dependent short loop pathway or via the Rab11/ARF6-dependent long loop recycling pathway through the PNRC. (C) A minor proportion of integrins leaves this recycling machinery and enters ECSRT-mediated lysosomal degradation.

2.3.1 Internalization

In a continuous process integrin DNA is transcribed to acquire mRNA for ribosomal protein synthesis. These newly synthesized integrin polypeptides mature by various modifications such as glycosylations as they travel through the trans-Golgi network (TGN) to the cell surface where they exhibit their function as adhesion receptors (Figure 7A). Initial misfolding leads to proteasomal degradation (Romisch 2005). Integrins are internalized in a continuous

process, which is either clathrin- or caveolin-dependent or entirely independent of these two proteins (Mosesson et al. 2008; Caswell et al. 2009; Doherty and McMahon 2009; Grant and Donaldson 2009; Sorkin and von Zastrow 2009). There is substantial evidence that integrins, analogue to the EGFR, can use all of these three routes (Caswell et al. 2009; Shin et al. 2012) (Galvez et al. 2004; Pellinen et al. 2008; Shi and Sottile 2008). Even a specific integrin heterodimer can use different routes of endocytosis depending on localization, cell type and cell state (Caswell and Norman 2008; Caswell et al. 2009). There is contradicting evidence regarding the requirement of a functional NxxY motif for clathrin-mediated endocytosis (Vignoud et al. 1994; Pellinen et al. 2008). Multiple clathrin-adaptor proteins like HAX1 (Ramsay et al. 2007), Dab/ Dab2 (Calderwood et al. 2003; Chao and Kunz 2009; Teckchandani et al. 2009) and Numb (Calderwood et al. 2003; Nishimura and Kaibuchi 2007) have been identified as integrin binding partners in previous studies, while the latter two were shown to bind integrin NxxY motifs. Moreover there is contradicting evidence with respect to the activation state of internalized integrins. Internalization of active (Nishimura and Kaibuchi 2007; Chao and Kunz 2009) as well as inactive integrins or activation-independent endocytosis (Teckchandani et al. 2009) is controversially discussed. One study suggests that in certain cell types an association of integrins with neuropilin 1 (NRP1) selectively promotes active integrin internalization (Valdembri et al. 2009). The authors suggest this mechanism to be crucial for ECM reorganization. It is known that α5β1 integrins bind monomeric FN and expose hidden domains by mechanical force transduction, which is essential for FN polymerization (Zhong et al. 1998). Localized integrin trafficking is substantial for focal adhesion reorganization and strengthening (Shi and Sottile 2008; Valdembri et al. 2009).

2.3.2 Recycling

Upon internalization integrins are routed back to the plasma membrane, a process called integrin recycling (Figure 7B). Only a minor proportion of integrins leaves this cycle for lysosomal degradation. This highly efficient spatiotemporally regulated integrin recycling does not only save energy, which would otherwise be necessary for synthesis, but also favors a continuous redistribution of integrins being an essential prerequisite for directed migration (Bretscher 1996; Caswell and Norman 2006; Caswell et al. 2007; Pellinen et al. 2008), adhesion, polarity, proliferation and signaling (Caswell and Norman 2006; Caswell et al. 2009; Sorkin and von Zastrow 2009; Scita and Di Fiore 2010). Detailed analyses, especially of cell migration, could identify additional contributing factors such as Rho GTPase signaling and interactions with growth factor receptors (White et al. 2007; Caswell et al. 2008; Reynolds et al. 2009).

Recent studies demonstrated that integrin trafficking is rather a local event than being an *en masse* redistribution of integrin molecules. Shortly after internalization local accumulations of integrins were visualized in sorting endosomes at the leading edge of the cell (Pierini et al. 2000), where also clathrin-mediated endocytosis was shown to be highly pronounced (Rappoport and Simon 2003) Afterwards a majority of endosomes enters the perinuclear recycling compartment (PNRC) (Laukaitis et al. 2001; Nishimura and Kaibuchi 2007) before being transported back to the plasma membrane. Both, newly synthesized as well as recycling integrins were shown to be mainly targeted to the cell front (Hopkins et al. 1994; Schmoranzer et al. 2003; Prigozhina and Waterman-Storer 2004). Having this in mind it seems rather unlikely that an *en masse* redistribution of integrins is the driving force of cell migration. Instead, local integrin reorganization seems to focus signaling events at the leading

edge. However, a Rab21-driven *en masse* relocalization of integrins does occur during cell division and the following repolarization of the cell (Pellinen et al. 2006; Pellinen et al. 2008). Yet, this has to be considered as an exception since similar *en masse* integrin redistributions have not been observed in any other cellular process.

Integrin trafficking is regulated by a number of small Rab (Ras-associated binding) proteins of the family of small GTPases, which function as molecular switches in vesicular transport (Jones et al. 2006; Chia and Tang 2009; Stenmark 2009). So far approximately 70 Rab GTPases have been identified (Mitra et al. 2011) most of which are involved in intracellular protein trafficking. After Rab21-mediated integrin endocytosis two competing routes target integrins back to the plasma membrane either via the Rab11 and/or ADP ribosylation factor 6 (ARF6) dependent long loop pathway through the PNRC or the Rab4-dependent short loop recycling pathway through early endosomes (Caswell and Norman 2006; Jones et al. 2006; Pellinen and Ivaska 2006; Caswell and Norman 2008) (Figure 7B). Remarkably, integrins can co-recycle with other receptors as it has been shown for the EGFR-1 in a Rab-coupling protein (RCP) mediated process (Caswell et al. 2008).

2.3.3 Degradation

Upon internalization integrins are mainly recycled back to the plasma membrane. However, a minor part is targeted for lysosomal degradation (Caswell et al. 2009; Lobert et al. 2010). Like almost all transmembrane receptors, including the extensively studied receptor tyrosine kinases (RTKs) and the EGFR, integrins are ubiquitinated prior to endosomal sorting complex required for transport (ESCRT)-mediated lysosomal degradation (Lobert et al. 2010). To this end, integrins are separated from cytoplasmic adaptor proteins by sorting them into

intraluminal vesicles (ILVs) in multivesicular bodies (MVBs). Afterwards these fuse with the lysosomal compartment and exposed integrins to degrading enzymes such as proteases and lipases (Figure 7C).

Numerous studies attribute ESCRT-mediated protein degradation a central role in cell migration. For example, a knockout of the ESCRT-1 component TSG101 (Tumor susceptibility gene 101) impairs cell migration and leads to intracellular integrin accumulation (Lobert et al. 2010) as well as reduced FAK and Src signaling (Tu et al. 2010). Since integrins were reported to be lysosomally degraded (Lobert et al. 2010) the reduced cell migration is likely to be caused by defective integrin trafficking in these cells. Moreover, regulated integrin degradation has been identified as a substantial factor in integrin signaling (Huveneers and Danen 2009).

Despite extensive research on integrin trafficking and deep insights in the recycling machinery, there is only little known about integrin degradation. Yet, tightly regulated integrin degradation is indispensible for multiple cellular processes and proper integrin homeostasis. There is increasing evidence that integrin internalization is more than just a simple starting point of integrin recycling and reorganization. Most likely, internalization targets integrins to a point of quality control from which functional integrins enter the recycling process, while defective molecules are targeted for lysosomal degradation. An alternative concept of lysosomal integrin trafficking was described by Lobert et al. identifying this pathway as a potential mechanism to degrade integrin-bound FN in the sense of constant ECM remodeling and subsequent recycling of free integrins back to the plasma membrane (Lobert et al. 2010).

2.4 The family of PX-domain proteins and sorting nexins

The whole pathway of protein trafficking from synthesis via endocytosis and recycling to degradation requires a tightly controlled sorting machinery to assure proper protein targeting. Recently, scientists found that there is more to integrin trafficking than pure protein sorting, since it was also reported to be necessary for other cellular processes such as cell signaling (Sorkin and von Zastrow 2009; Scita and Di Fiore 2010).

Essential factors in the molecular sorting process are members of the phox homology (PX)-domain protein family whose salient feature is to bind phosphatidylinositol phospahte (PtdInsP) lipids of cytoplasmic vesicles. Until today there are 49 members to this family including the most prominent representatives known as sorting nexins (SNXs) (Seet and Hong 2006; Cullen 2008). The name SNX was coined by Kurten et al. as they described SNX1 for the first time and it became the name of a whole protein family with at least 50% sequence homology to the PX-domain of SNX1 (Teasdale et al. 2001; Worby and Dixon 2002). Based on this rather arbitrary classification Teasdale et al. suggest to assign all new PX-domain proteins a successive SNX number (Teasdale and Collins 2012). The unique features of PX-domain proteins make them indispensible in membrane trafficking, signaling, membrane remodeling and in cell organelle trafficking. Almost all PX-domain proteins bind PtdIns3P, a lipid which is typically found in the limiting membrane of endosomal vesicles and therefore recruits PX-domain proteins to these compartments (Teasdale and Collins 2012).

Co-incidental detection of membrane lipid binding domains such as bin/amphiphysin/rvs (BAR), band4.1/ezrin/radixin/moesin (FERM) or pleckstrin homology (PH) proteins as well as other cargo proteins such as transmembrane receptors or Rabs can increase specific binding

to certain cellular structures (Carlton and Cullen 2005; Teasdale and Collins 2012). With respect to integrin trafficking SNX17, SNX27 and SNX31 are highly interesting since they do not only posses a PX-domain but also a FERM-domain, which is typically known to bind NxxY motifs as found in the cytoplasmic β1 integrin tail (Ghai et al. 2011; Teasdale and Collins 2012) (Figure 8). Previous studies demonstrated the role of SNX17 in endosomal trafficking of different transmembrane receptors such as the low density lipoprotein receptor related protein 1 (LRP1) (Stockinger et al. 2002; van Kerkhof et al. 2005; Betts et al. 2008; Donoso et al. 2009), the low density lipoprotein receptor (LDLR) (Stockinger et al. 2002; Burden et al. 2004), P-selectin (Florian et al. 2001; Williams et al. 2004; Knauth et al. 2005) and the amyloid precursor protein (APP) (Lee et al. 2008). However, the molecular mechanism how SNX17 functions in sorting these cargo proteins is not entirely clear. For the LDL receptor it was shown that SNX17 promotes endocytosis (Stockinger et al. 2002) and recycling back to the plasma membrane (van Kerkhof et al. 2005; Donoso et al. 2009). Yet, in the case of P-selectin it prevents lysosomal degradation (Knauth et al. 2005). An association with the retromer complex transporting endosomal proteins to the TGN has not been identified for SNX17 so far as opposed to most other SNX protein family members. From a clinical point of view SNX17 came into the focus of interest due to its inhibition of APP processing to the toxic amyloid Aβ (Lee et al. 2008), which is a major component in the pathogenesis of the Alzheimer's disease. Besides its typical association as a FERM domain protein with NxxY motifs, SNX17 binds also Krit1 (krev-interaction trapped 1), which promotes endosomal recruitment (Czubayko et al. 2006) as well as SH3-domain proteins such as Src-related tyrosin kinases and PLCγ1 (phospholipase C γ1) (Wu et al. 2007) (Figure 8).

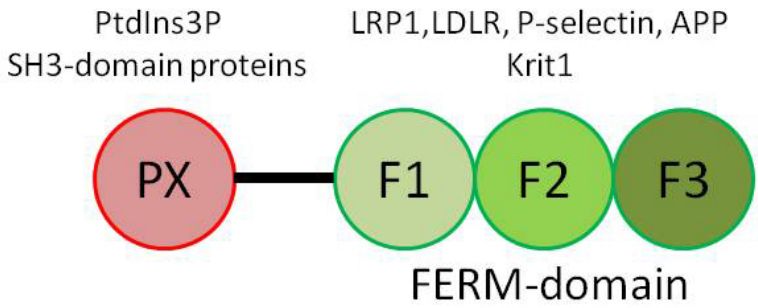


Figure 8: Molecular structure and interactions of SNX17
Depicted is a model of SNX17 with annotated previously reported binding partners.

2.5 Integrins in a medical context

Integrins are not only of fundamental interest in basic research but they also have broad implications in medical contexts. Every single integrin subunit has now been deleted in mice providing substantial knowledge about the specialized functions of integrins *in vivo* (Sheppard 2000). The phenotypes resulting from these deletions are grouped according to the most effected organ system.

2.5.1 Vasculature

Interactions of integrins with growth factor receptors such as VEGF and of endothelial cell integrins with the ECM are of critical importance in vascular formation during embryonic development and adult blood vessel integrity (Weis 2007; McCarty 2009) as well as in pathological angiogenesis in neoplastic tissues or chronic inflammation. The most prominent subclass of integrin adhesion receptors in vasculogenesis are the RGD-motif recognizing heterodimers, like integrin α5β1 with its ligand FN (Astrof and Hynes 2009). Therefore, it is

not surprising that severe mesodermal defects and poor vascularization of the yolk sac and the embryo itself were described in both α5 integrin knockout mice (Yang et al. 1993) and even more pronounced in FN-depleted mice (Hynes 2007). β1-null mice suffer from dramatic defects in gastrulation with early peri-implantation lethality due to the loss of many integrin heterodimers (Fassler and Meyer 1995; Stephens et al. 1995). Interestingly, Tie2-cre-mediated deletion of the β1 integrin subunit in endothelial cells displays a very similar phenotype to α5-null mice underlining the predominant role of endothelial α5β1 integrin in vascular morphogenesis (Carlson et al. 2008).

In pathological vessel formation as present in tumor growth and wound healing the VEGF- and VEGF receptor-interacting αvβ3 integrin plays a central role (Brooks et al. 1994; Soldi et al. 1999; De et al. 2005; Mahabeleshwar et al. 2006). This makes αvβ3 integrins an ideal therapeutic target in tumor neovascularization with only minor interference with physiological vascularization mainly maintained by α5β1 integrins. In line with this, first successes have been described in animal tumor models using a monoclonal αvβ3-blocking antibody (Brooks et al. 1994). Knockout studies on αv integrins have identified its role in vascular parenchyma organization with severe vascular malformations and hemorrhages, predominantly present in the brain (Bader et al. 1998; McCarty et al. 2002). Consistent with this localized function, endothelial deletion of αv integrins has no effect on vascular development (McCarty et al. 2005). Similar defects have been observed upon β8 integrin deletion pointing towards a predominant role of the αvβ8 heterodimer (Zhu et al. 2002; Proctor et al. 2005).

The vascular defect in α4-null mice arises later as a consequence of a failed chorion-allantois fusion during placentation with accompanying cardiac abnormalities, most likely due to the deficient interactions with VCAM-1 (Kwee et al. 1995; Yang et al. 1995; Grazioli et al.

2006). Moreover, α9β1 integrins and their interactions with VEGF-C and VEGF-D have been identified to be essential for lymphatic vessel development (Huang et al. 2000; Vlahakis et al. 2005; Bazigou et al. 2009).

2.5.2 Nervous system

Within the central nervous system (CNS) the eye is the best-studied organ with respect to integrin-related functions. Lens development depends on proper FN, collagen and LN assembly by functional interactions with the corresponding integrin heterodimers, especially those containing a β1 subunit (Simirskii et al. 2007; Walker and Menko 2009). Moreover, integrins guide the migration of neuronal precursor cells along glial fibers as required for retina development (Milner and Campbell 2002). Associations of cortical structures along the developing meningeal BM dictate the inner architecture of the brain by functional β1 integrin-LN interactions (Miyagoe-Suzuki et al. 2000; Graus-Porta et al. 2001). Similarly, neuronal synapse stability depends on β3 integrin heterodimers (Benson et al. 2000). Neural crest migration during the development of the peripheral nervous system (PNS) depends on β1 integrin heterodimer-directed migration along the embryonic ECM, while functional abnormalities in this system lead to abnormal nerve arborization, Schwann cell defects and impaired neuromuscular junction differentiation (Feltri et al. 2002; Pietri et al. 2004; Denda and Reichardt 2007). Additionally, αvβ5 integrins on retinal pigment epithelial cells were identified as phagocytic receptors to mediate uptake of shed photoreceptor particles while β5-null mice lack this clearing system and develop age-related blindness (Nandrot et al. 2004).

Integrin signaling has also been implicated to effect neuronal survival in degenerative diseases such as Parkinson's or Alzheimer's disease. And finally and most obvious, integrins

have also been associated with neuroinflammatory disease such as multiple sclerosis as they are absolutely essential for immune response (Denda and Reichardt 2007).

2.5.3 Immunity

The immune system is probably the best-studied field of integrin biology and most functional concepts are based on observations in this system. For example processes such as bidirectional integrin signaling were first described and analyzed in leukocytes and platelets. The major task of integrins in this process is to localize the immune response to the site of inflammation (Evans et al. 2009). Especially leukocyte exit from the vasculature into the lymph nodes or the site of inflammation and injury are critically dependent on integrins. The leukocyte adhesion cascade with subsequent diapedesis is initiated by selectin-mediated adhesion and rolling as well as chemokine-induced integrin activation. Afterwards high-affinity integrins lead to full cell arrest on the endothelium and finally diapedesis (Ley et al. 2007). However, this model is extremely simplified and for example also α4β1 integrin-driven rolling has been observed (Berlin et al. 1995). Nevertheless, the overall concept is well established and β2-deficient mice show severe immunodeficiency in support of this model (Scharffetter-Kochanek et al. 1998). Similarly, patients lacking the β2 integrin subunit suffer from a disease called leukocyte adhesion deficiency (LAD) type I characterized by recurrent infections without pus formation, wound healing defects and variable forms of infectious gingivitis (Etzioni 2010). Recently, LAD type III, a disease characterized by bleedings, immunodeficiency and osteopetrosis-like bone defects, has also been associated with integrins and their inside-out signaling capacity. Activation of β1, β2 and β3 integrins in hematopoietic cells is critically dependent on kindlin-3 recruitment to the distal cytoplasmic integrin tail which was identified to be mutated in these patients (Mory et al. 2008; Kuijpers et al. 2009;

Malinin et al. 2009; Moser et al. 2009; Svensson et al. 2009). A lack of β1 integrins on blood cells impairs hematopoietic stem cell colonization to the fetal liver or spleen during development (Potocnik et al. 2000) and causes defects in leukocyte migration to peripheral organs (Sixt et al. 2006).

Although it is widely accepted that immune cell migration is absolutely dependent on integrin function, studies on mice lacking all leukocyte integrins questioned this simplistic concept. Dendritic cells lacking all these integrin adhesion molecules migrated normally through the skin and into lymph nodes where they initiated immune response by antigen presentation (Lammermann et al. 2008). Therefore, we have to question the overall implication of integrins in immune cell migration, although they remain indispensible for multiple processes such as leukocyte extravasation.

Beyond the role of integrins in immune cell migration, they have also been described to be involved in outside-in signaling and to stimulate T-cell receptor (TCR) response (Pribila et al. 2004; Abram and Lowell 2009; Smith-Garvin et al. 2009). Analogue modulations were observed in neutrophils (Berton et al. 1996). Interestingly, this activation of leukocyte integrins is practically even more important than the simple migratory processes. For example, bleedings induced by endotoxin and chemokine exposure in a model of thrombohemorrhagic vasculitis can be minimized in the absence of αMβ2 integrins (Hirahashi et al. 2006). Although αMβ2-deficient neutrophils are recruited to the site of vascular inflammation they do not release proteases. This is due to a defective recognition of complement deposits and subsequently reduced activation by outside-in signaling events.

Therapeutic concepts of autoimmune disorders include antagonists for two integrins. First, the monoclonal antibody (mAb) natalizumab that binds the α4 subunit is approved for the treatment of multiple sclerosis (MS) (Rice et al. 2005) and Crohn's disease (Targan et al. 2007). Second, there is the mAb efalizumab targeting αLβ2 (also known as LFA1) with indications in severe psoriasis treatment (Frampton and Plosker 2009). However, these two antibodies have severe potentially fatal side effects due to their immune suppressive properties leading to progressive multifocal leukoencephalopathy (PML) (Warnke et al. 2010). Therefore, natalizumab was withdrawn from the market in 2005 and reintroduced due to its high efficacy in reducing the rate of relapses in MS in 2006. However, efalizumab was withdrawn from the market in 2009 and never reintroduced (Major 2010).

And last but not least, various infectious agents have been reported to utilize integrins and subsequent co-internalization to enter the intracellular milieu (Fitzgerald et al. 2006; Stewart and Nemerow 2007).

2.5.4 Platelets and thrombosis

The first process to be associated with integrins was thrombosis. Upon vascular injuries, the platelet integrin αIIbβ3 is exposed to thrombotic stimuli and becomes activated – a process which is regard as the initial step of clot formation (White et al. 2004). Subsequently, integrins mediate platelet-platelet interactions by FN bridges between each other (Coller and Shattil 2008). This serves as an essential mechanism to prevent blood loss. However, excessive thrombus formation might cause a heart attack or stroke.

Absence of the β3 subunit as an essential component of the platelet αIIbβ3 integrin leads to severe bleedings in mice (Hodivala-Dilke et al. 1999). The corresponding disease in humans

is usually referred to as Glanzmann's thrombasthenia (George et al. 1990; Lefkovits et al. 1995; Bennett 2005; Wegener et al. 2007; Harburger et al. 2009; Moser et al. 2009; Moser et al. 2009).

This detailed knowledge about integrins in platelet aggregation drove the development of new therapeutic strategies. In the 1990s, αIIbβ3 integrin was the first one to be pharmacological inhibited by three different intravenous drugs namely the antibody fragment abciximab and the small-molecule inhibitors eptifibatide and tirofiban, while the development of orally active antagonists failed so far (Chew et al. 2001). Initial indications were the prevention of ischemic events in patients with acute coronary syndromes and those undergoing percutaneous coronary intervention (Bonaca et al. 2009; Cox et al. 2010). However, ADP receptor antagonists such as clopidogrel or prasugrel mainly dominate the global pharmaceutical market, while indications for integrin inhibitors are limited to high-risk patients.

2.5.5 Skin

Integrins link keratinocytes to the underlying BM via α6β4 integrin-containing hemidesmosomes and to each other directly (Watt 2002). Conditional Kreatin5- or Keratin14-driven deletion of β1 integrin heterodimers in the skin leads to incomplete perinatal lethality with some mice surviving up to 6 weeks (Brakebusch et al. 2000; Raghavan et al. 2000). These mice lose their hair completely and show poor keratinocyte proliferation and fibrosis due to chronic inflammation. Interestingly, a loss of either α2β1 or α3β1integrin alone can be compensated by the other heterodimer indicating redundant functions (DiPersio et al. 1997; Chen et al. 2002). Inducible β1 integrin deletions in the skin or in cell culture revealed an

increase in proliferation and differentiation suggesting a suppressive role of β1 integrins under these circumstances (Grose et al. 2002; Muller et al. 2008). Functional β1 integrin signaling is of critical importance in the skin since a loss of function mutation of the epithelial-expressed integrin activator kindlin-1 has been identified to cause a rare genetic skin disease in humans called Kindler syndrome characterized by blistering, progressive poikiloderma and potential skin cancer formation (Meves et al. 2009).

Defects in α6β4 integrins lead to dermal and epidermal separation and alter immune response by influencing TGFβ signaling (DiPersio et al. 2000). In line with these functional abnormalities in the hemidesmosome integrin, a very similar phenotype can also be observed in humans in a rare skin disease called junctional epidermolysis bullosa syndrome (EBS) characterized by extreme blistering and skin fragility. The EBS is frequently combined with an intestinal occlusion, termed pyloric atresia, leading to early postnatal lethality. Occlusions of the esophagus, urethra or pylorus result from detachment and subsequent fusion of epithelial linings (Hogg and Bates 2000; Wilhelmsen et al. 2006).

2.5.6 Lung

Similar to epithelial abnormalities in the skin, defects in integrin receptors such as the α3β1 heterodimer lead to pulmonary impairments due to defective BM formation and poor cell adhesion (Kreidberg et al. 1996). The RGD-motif-binding αvβ6 integrin is typically expressed in epithelial cells of the lung, skin and kidney where it binds FN and associates with the latency associated peptide (LAP) which is responsible of preventing newly secreted TGFβ to bind to its receptor (Aluwihare and Munger 2008). Interactions of αvβ6 integrins with LAP lead to TGFβ dissociation and subsequent binding to its receptor on epithelial cells and

pulmonary macrophages. This initiates a strong anti-inflammatory response which prevents lung injury by inappropriate macrophage activation (Takabayshi et al. 2006). Therefore, mice lacking either the β6 subunit or TGFβ develop a strong pulmonary inflammation with accompanying emphysema formation (Huang et al. 1996; Morris et al. 2003). Additionally, these mice are not capable of surfactant recycling due to defects in macrophage functionality (Koth et al. 2007). Similarly, αvβ8 integrin-TGFβ interactions regulate the proliferative and inflammatory response of epithelial cells in the upper airway (Fjellbirkeland et al. 2003). Finally, αvβ5 binds and activates VEGF, which increases vascular permeability and leads to pulmonary edema formation after ischemia-reperfusion or hyperventilation (Su et al. 2007). This process is driven by actin stress fiber formation and cell retraction – a phenotype that is absent in αvβ5 integrin deficient mice.

2.5.7 Muscles and skeleton

In muscle tissue integrin focal adhesion sites allow the establishment of actin/myosin structures (Sparrow and Schock 2009). It is clear that a number of RGD-motif-, LN- and collagen-binding integrins are involved like the α1β1 or the α2β1 heterodimer. However, the exact individual heterodimers are less well defined. α5β1 integrins were associated with a progressive skeletal muscle dystrophy with diffuse muscle cell degeneration and apoptosis due to defective bidirectional signaling (Taverna et al. 1998). Additionally, a loss of the ECM-integrin connection leads to a special form of apoptosis usually referred to as anoikis. Interestingly, in neoplastic tissues or in the case of metastasis formation, cells can overcome this process of anoikis and survive (Frisch and Screaton 2001). LN receptors such as dystrophin, utrophin or α7β1 integrin link LN of the ECM to the muscle cell actin cytoskeleton with partially overlapping functions and reciprocal compensation (Campbell

1995). Abnormalities in this anchorage lead to a well-known disease called Duchenne's muscular dystrophy – a progressive myocyte degeneration caused by a loss of dystrophin. A similar but less pronounced impairment was reported in α7-null mice (Hayashi et al. 1998). These mice presented an additional defect in neuromuscular junctions as well as in muscle-tendon junctions indicating a role of α7 integrins in these structures (Nawrotzki et al. 2003). This shows that although LN receptors are dispensable for muscle development, they play a major role in the maintenance of myocyte integrity and function.

Skeletal development is primarily dependent on RGD-motif and collagen receptors. Osteoclast signaling events initiated by αvβ3 integrin-osteopontin and bone sialoprotein interactions regulate bone morphogenesis by continuous remodeling processes mediated by bone-resorbing proteases (Ross and Teitelbaum 2005). Therefore, a lack of β3 integrins or downstream signaling proteins like Src kinases causes an excess of bone material called osteopetrosis (Horton et al. 2003; Faccio et al. 2005; Ross and Teitelbaum 2005). However, in patients with Glanzmann's thrombasthenia, which is characterized by a lack of β3 integrins, this phenotype has not been observed, most likely due to a compensating overexpression of β1 integrins (Horton et al. 2003). Nevertheless minor skeletal abnormalities or bone cysts might be present in these patients. Collagen binding integrins such as α10β1 and α11β1 only have a minor effect on musculoskeletal development characterized by dental development abnormalities and retarded growth of long bones in the limbs due to cartilage defects in the growth plate (Bengtsson et al. 2005; Popova et al. 2007).

2.5.8 Kidney

Multiple β1 integrins have been reported to be involved in renal development and kidney diseases. For example, a lack of the α8β1 heterodimer or its renal ligand nephronectin leads to frequent complete renal agenesis due to the disability of epithelial ureteric bud (UB) cells to invade into the surrounding mesenchyme and subsequently form the metanephric kidney (Muller et al. 1997; Brandenberger et al. 2001; Linton et al. 2007). The α8β1 integrin-nephronectin interaction functions not only as a guiding structure for directed migration, but also induces bidirectional signaling and especially growth factor signaling of the TGFβ superfamily (Costantini and Shakya 2006). α3β1 integrin is another heterodimer involved in renal development with special implications in the branching morphogenesis of the renal tubular collecting system (Kreidberg et al. 1996; Liu et al. 2009). Since an entire deletion of all β1 integrins causes a much more severe phenotype than deletions of the individual heterodimers mentioned above, it is very likely that additional integrin heterodimers dictate renal development and function (Zhang et al. 2009). Interestingly, a late deletion of β1 integrins does not impair renal development but negatively effects renal response upon injury in adult mice, most likely do to defective growth factor interactions (Zhang et al. 2009). Finally, renal glomerular structures composed of mesangial cells and podocytes interacting with the BM in fine vascular capillaries are dependent on β1 integrins as selective knockdowns in podocytes cause massive proteinuria and rapid death (Kanasaki et al. 2008). Moreover, glomerular injury, for example as a result of immune complex deposition, leads to mesangial cell proliferation induced by integrin-growth factor interactions (Kagami and Kondo 2004). In a mouse model of Alport's syndrome with mice lacking α1β1 integrins, the

proliferative response and the subsequent glomerular injury could be dramatically reduced (Cosgrove et al. 2000).

2.5.9 Cancer

Carcinogenesis is highly dependent on integrins with their unique feature to modulate all steps of cancer progression including detachment from and invasion of the ECM, blood vessel infiltration, extravasation in distant organs and metastasis formation (Brooks et al. 1996; Felding-Habermann et al. 2001; Mercurio et al. 2001; Bates et al. 2005; Makrilia et al. 2009). Moreover, they are also involved in signaling events of neoplastic tissues by RTK modulation. Therefore, it is not surprising that integrins have attracted considerable attention as potential targets for cancer therapy and diagnosis (Kenny et al. 2008; Axelsson et al. 2010).

Being a known trigger for cell survival (Chudakova et al. 2008), scientists identified the highest therapeutic potential in targeting αvβ3 integrins (Nemeth et al. 2007). There are numerous ongoing clinical trials with multiple αvβ3 inhibitors for different indications: The mAb etaracizumab for melanoma (Hersey et al. 2010; Moschos et al. 2010) and solid tumors (Delbaldo et al. 2008), the orally active non-peptide MK 0429 for prostate cancer (Rosenthal et al. 2010) as well as the cyclic peptide cilengitide for glioblastoma (Reardon et al. 2008; Stupp et al. 2010) and other brain cancers (MacDonald et al. 2008) which has just recently entered phase III trials as the first anti-integrin therapy for cancer (Carter 2010). However, first in vivo studies with the αvβ3 integrin inhibitor cilengitide showed disappointing results, which is most likely due to increased recycling of other integrin heterodimers as well as RTKs (Nisato et al. 2003; Maubant et al. 2006; Tucker 2006; Stupp and Ruegg 2007; Caswell et al. 2009).

Another integrin of interest with respect to cell survival, migration and angiogenesis is α5β1 integrin (Kim et al. 2000; Kim et al. 2000). Therefore, the development of the α5-specific antibody volociximab for solid tumors was initiated (Ricart et al. 2008; Ng et al. 2010). A potential co-administration of αvβ3 as well as α5β1 inhibitors might optimize the effects on tumor angiogenesis (Laurens et al. 2009).

3 Aims of the thesis

3.1 Aim 1: Generation of a β1 TT/AA mouse model

Integrins are adhesion receptors with fundamental functions in cell adhesion, proliferation and migration. These functions critically depend on modifications in receptor affinity by the integrin-activating proteins talin and kindlin. Mutations in their binding sites have been identified to cause severe impairments in cellular functions. In this thesis an *in vivo* mouse model was established to analyze the effects of a defective kindlin-binding site by introducing a threonine 788/789 to alanine mutation in the β1 integrin cytoplasmic tail.

Aim1: Generation of a mouse model to analyze the *in vivo* consequence of a β1 TT/AA mutation, which was described to dramatically reduce kindlin-binding *in vitro*.

3.2 Aim 2: SILAC-screen for new β1 integrin binding partners

Detailed analyses of the β1 TT/AA mutation *in vivo* revealed substantial defects in integrin function due to reduced integrin activation as predicted by previous *in vitro* studies as well as due to dramatically reduced integrin expression by increased lysosomal degradation.

Aim 2: To identify new cytoplasmic β1 integrin binding partners a SILAC-screen was applied.

3.3 Aim 3: Characterization of SNX17 in β1 integrin trafficking and degradation

We identified SNX17 as a new β1 integrin binding partner. This PX-domain protein has previously been associated with endosomal sorting of transmembrane receptors and has a potential role in integrin trafficking.

Aim 3: Analysis of SNX17-mediated integrin trafficking and lysosomal integrin degradation.

4 Methods

The method section was adapted and modified from Böttcher and Stremmel et al. 2012 (Bottcher et al. 2012).

4.1 Mouse strains

Mice were basically generated as described previously (Czuchra et al. 2006). The loxP-flanked neomycin cassette was inserted upstream of β1 integrin exon 16 and the following point mutations were introduced using the AlteredSites II *in vitro* mutagenesis system (Promega): threonines 788 and 789 to alanines (β1 TT/AA) and tyrosine 795 to alanine (β1 Y795A). These targeting constructs were introduced into R1 ES cells and clones that underwent homologous recombination were isolated. BamHI digested genomic DNA was tested with an external and internal probe by Southern blotting and injected into blastocysts to generate germline chimeras. These mice were intercrossed with deleter-Cre transgenic mice (Betz et al. 1996) to obtain heterozygous mutants. Homozygous mutants were embryonic lethal. For fibroblast isolations heterozygous mutants were intercrossed with mice carrying a floxed β1 integrin gene (β1 floxed) (Brakebusch et al. 2000). Genotyping was performed by Southern blotting or PCR using DNA isolated from tail biopsies. All animal studies were approved by the Regierung von Oberbayern.

4.2 Antibodies

The following antibodies were used for western blotting (WB) and immunofluorescence (IF):

Antibody	Company (Clone)	Catalogue #	Dilution	Used for
Actin	Sigma	A-2066	1:3000	WB
β1 integrin	Chemicon (MB1.2)	MAB1997	1:400	IF
β3 integrin	Millipore (EP2417Y)	04-1060	1:1000	WB
β-catenin	Sigma	C2206	1:800	IF
E-cadherin	Zymed (ECCD-2)	13-1900	1:200	IF
Laminin111	Abcam	ab30320	1:400	IF
EEA1	BD Transduction Laboratories	610457	1:100	IF
GAPDH	Calbiochem (6C5)	CB1001	1:5000	WB
Kindlin-2	Sigma	K3269	1:1000	WB
Lamp1	By L. Huber (Medical University Innsbruck, Austria)		1:100	IF
Paxillin	BD Transduction Laboratories (349)	610051	1:400	IF
SNX17	Proteintech	10275-1-AP	1:1000	WB
Talin-1	Sigma (8d4)	T3287	1:1000	WB
Transferrin receptor	Invitrogen (H68.4)	13-6800	1:1000	WB
Ubiquitin	Cell Signaling (P4D1)	3936	1:1000	WB
Kindlin-1	Homemade (Ussar et al. 2006)		1:5000	WB
Kindlin-3	Homemade (Ussar et al. 2006)		1:5000	WB
β1 integrin	Homemade (Azimifar et al. 2012)		1:10000	WB
Phalloidin	Molecular Probes	A12379 and A22287	1:400	IF
DAPI	Sigma			IF

The following antibodies were used for flow cytometry:

Antibody	Company	Clone	Catalogue #	Dilution
β1 integrin PE	BioLegend	HMbeta1-1	102207	1:400
β1 integrin biotin	BD Pharmingen	Ha2/5	555004	1:400
β1 integrin 9EG7	BD Pharmingen	9EG7	550531	1:100
β3 integrin PE	eBioscience	2C9.G3	12-0611	1:400
β4 integrin PE	Serotec	346-11A	MCA2369	1:400
α2 integrin FITC	BD Pharmingen	Ha1/29	554999	1:400
α5 integrin PE	BD Pharmingen	5H10-27	557447	1:400
α5 integrin biotin	BD Pharmingen	5H10-27	557446	1:400
α6 integrin PE	BD Pharmingen	GoH3	555736	1:400
αv integrin PE	BD Pharmingen	RMV-7	551187	1:400

4.3 Plasmids and constructs

The following point mutations were introduced into the corresponding cDNAs by site directed mutagenesis: β1 integrin (TT788/789AA, Y783A, Y795A, 8xKR (K752R, K765R, K768R, K770R, K774R, K784R, K794R, K798R), 8xKR+TT788/789AA), α5 integrin (4xKR (K1022R, K1027R, K1038R, K1042R)) and SNX17 (QW360/361AA). Stable cDNA expression was achieved by cloning of the following cDNAs into the retroviral expression vector pCLMFG or pLZRS: β1 integrin (wt, TT788/789AA, Y783A, Y795A, 8xKR, 8xKR+TT/AA), human α5 integrin (wt, 4xKR, α5-eGFP) as well as mouse SNX17 (wt, Flag-tagged, eGFP-tagged, and mCherry-tagged). Recombinant expression of GST-tagged SNX17,

wt SNX17 and SNX17 QW360/361AA cDNA was achieved by cloning into the pGEX-6P-1 vector (GE Healthcare). Lamp1-mRFP, Rab7-mRFP vectors were obtained from J. Norman (Beaton Institute, Glasgow), Rab5a-GFP was provided by L. Huber (Medical University Innsbruck, Austria) and transiently expressed by transfection with Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol.

SNX17 expression was stably depleted by shRNAs: 5'-GTACATGCAAGCTGTTCGG-3' (shSNX17-1), 5'-GATTGTGCTCAGAAAGAGT-3' (shSNX17-2). Target sequences were introduced into the pSuper.Retro vector (OligoEngine, USA) to produce retroviral particles.

To obtain a GFP-tagged kindlin-2, the kindlin-2 cDNA (Ussar et al. 2006) was ligated in frame with the GFP using SLIC cloning (Li and Elledge 2007). The CAG promoter, GFP-fusion and SV40 polyA were flanked by ITR elements, thus co-transfection of this construct with a sleeping beauty SB100x expression vector (Mates et al. 2009) resulted in transposase mediated genomic integration of this DNA sequence.

4.4 Cell lines

Heterozygous β1 TT/AA mice were intercrossed with homozygous β1 floxed mice. Mouse embryonic fibroblasts (MEFs) were isolated from E9.5 embryos and immortalized by retroviral transduction of the SV40 large T antigen. Cells were cloned by low-density plating and subsequent picking. The floxed β1 integrin allele was removed by adenoviral Cre transduction. Disruption of the β1 allele and expression of the β1 TT/AA were checked by PCR. To generate β1-null rescue cell lines, β1 wt or β1 mutant variants were virally re-expressed in β1-null fibroblasts derived from adenoviral Cre transduced floxed β1 parental cells.

Fibroblasts homozygous for floxed kindlin-1 and -2 genes were isolated from kidneys of 21 day-old double-floxed mice (whose generation will be described elsewhere). After immortalization by retroviral transduction of the SV40 large T antigen, cells were cloned by limiting dilutions. The floxed kindlin alleles were removed by adenoviral Cre transduction to obtain kindlin-1 and -2 double-null cells (provided by M. Widmaier).

4.5 Transient and stable transfection/transduction

Cells were transiently transfected with Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. To generate stable cell lines, VSV-G pseudotyped retroviral vectors were produced by transient transfection of HEK293T (human embryonic kidney) cells. Viral particles were concentrated from cell culture supernatant as described (Pfeifer et al. 2000) and used for infection.

4.6 Embryo isolation and histological analysis

For whole-mount analyses and bright field images, staged embryos (E6.5 to E9.5) were dissected in ice-cold PBS. For histological analysis, decidua swellings were isolated, fixed in 4% paraformaldehyde overnight and embedded in paraffin. Sections were stained with haematoxilin and eosin or antibodies as indicated.

4.7 Whole-mount immunohistochemistry

Embryos were dissected in cold PBS and fixed in 4% paraformaldehyde (PFA) for 2 hours at 4°C. Samples were incubated in 0.5% NP-40/PBS for 20 minutes and in PBSST (0.1% Triton-X, 5% BSA in PBS) for 2 hours at 4°C for permeabilization. Staining was performed by incubation with primary overnight at 4°C followed by the secondary antibody overnight at

4°C. Nuclei were stained with DAPI solution for about 30 minutes. Finally, embryos were dehydrated by increasing methanol concentrations, cleared in benzyl alcohol/benzyl benzoate (1:2) and imaged with a confocal microscope (DMIRE2; Leica) using Leica Confocal Software (version 2.5 Build 1227). After imaging embryos were rehydrated for genotyping by PCR.

4.8 ES cells and EBs

ES cells were isolated and cultured as previously described (Montanez et al. 2007). Briefly, ES cells were isolated from the inner cell mass of E3.5 embryos called blastocysts and cultured on a monolayer of growth-inactivated MEFs (feeder cells) (Talts et al. 1999) in the presence of LIF to prevent ES cell differentiation.

EBs were generated as previously described (Montanez et al. 2007). ES cells were trypsinized and separated from feeder cells by repetitive platings. Afterwards small ES cells aggregates of three to four cells were formed with the help of a fine-tip Pasteur pipette. These aggregates were subsequently cultured in simple untreated petri dishes in LIF-free ES cell medium to allow EB formation for approximately 5 days. Afterwards EBs were used for bright field images or stainings with antibodies as indicated.

4.9 Metabolic labeling

80% confluent cells were incubated for 30 min at 37°C in methionine/cysteine-free labeling media containing 10% dialyzed FBS and pulse-labeled for 30 min at 37°C in labeling medium containing ^{35}S-methionine/cysteine (200 µCi/10 cm plate, EasyTag Express ^{35}S Protein Labeling Mix, PerkinElmer). Afterwards, the cells were either immediately harvested (time 0)

or chased for 4 h, 8 h and 22 h in regular growth medium containing 10% FBS. Cells were lysed in IP buffer (50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% Triton X-100, 0.1% sodium deoxycholate, 1 mM EDTA and protease inhibitors) prior to incubation with β1 integrin antibodies for 1h on ice, followed by incubation with Protein G sepharose (Sigma) for 2 h at 4°C for β1 immunoprecpiptation. After several washes with lysis buffer proteins were eluted from the beads by boiling with Laemmli sample buffer and subjected to SDS-PAGE. The gels were fixed, dried and exposed to film.

4.10 Turnover of surface integrins

The half-life of surface proteins was determined by biotinylation. Fibroblasts were grown to 80% confluence, washed twice in cold PBS and surface-biotinylated with 0.2 mg/ml sulfo-NHS-LC-biotin (Thermo Scientific) in PBS for 45 min at 4°C. Afterwards cells were washed with cold PBS to remove excessive biotin prior to incubation in regular growth medium for 0, 5, 10 and 15 h at 37°C. Cells were lysed in IP buffer (50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% Triton X-100, 0.1% sodium deoxycholate, 1 mM EDTA and protease inhibitors) and biotinylated proteins were pulled down with streptavidin-Sepharose (GE Healthcare). After three washes with lysis buffer, samples were analyzed by SDS-PAGE and western blotting. For capture ELISA, cells were lysed in 100 µl of lysis buffer (75 mM Tris, 200 mM NaCl, 7.5 mM EDTA and 7.5 mM EGTA, 1.5% Triton X-100, 0.75% Igepal CA-630 and protease inhibitors).

4.11 Integrin trafficking assays

Integrin trafficking assays were performed as described previously (Roberts et al. 2001). Cells were grown to 80% confluency and pre-incubated with serum free medium for 45 min.

Afterwards cells were washed with ice-cold PBS, surface-labeled with 0.13 mg/ml sulfo-NHS-SS-biotin (Thermo Scientific) for 30 min at 4°C and washed again with ice-cold PBS to remove excessive biotin. Labeled surface proteins were internalized in serum-free medium at 37°C for different time points. The remaining surface label was stripped with sodium 2-mercaptoethanesulfonate (Mesna, Sigma) for 30 min at 4°C. Afterwards Mesna was quenched with iodoacetamide (IAA, Sigma) for 20 minutes at 4°C. Cells were washed with PBS and lysed in 100 µl of lysis buffer (75 mM Tris, 200 mM NaCl, 7.5 mM EDTA and 7.5 mM EGTA, 1.5% Triton X-100, 0.75 % Igepal CA-630 and protease inhibitors). The amount of biotinylated integrins was quantified by capture ELISA.

For recycling assays, internalization was performed for 30 min and the remaining surface label was stripped with Mesna. Afterwards cells were incubated in regular growth medium containing 10% FBS for different time points at 37°C followed by a second stripping and subsequent quenching with IAA. Finally, the remaining β1 integrin biotin label was measured in lysed cells by capture ELISA.

4.12 Capture ELISA

Maxisorb 96-well plates (Life Technologies) were coated overnight with anti-β1 integrin antibody (MAB1997, Chemicon) in carbonate buffer at 4°C. Plates were washed with PBS and unspecific binding was blocked by 5% BSA in PBS/0.1% Tween-20 (PBS-T) for 2 h at RT. Cell lysates (50 µl each) were added to the wells and incubated overnight at 4°C to capture integrins. Following extensive washes with PBS-T, plates were incubated with streptavidin-HRP in 1% BSA in PBS-T for 1 hour at 4°C. Biotinylated β1 integrin was

detected after several washing steps by chromogenic reaction with ABTS peroxidase substrate (Vector Laboratories).

4.13 Selective immunoprecipitations

Selective isolation of β1 integrins on the cell surface and in endocytic vesicles was achieved by immunoprecipitation. Cell surface β1 integrins of living cells were labeled with a rabbit anti-β1 integrin antibody (homemade, 1:1500) for 1 h on ice and subsequently washed with ice-cold PBS to remove unbound antibody. Then, cells were incubated for 20 min in medium with or without 0.5 mM primaquine to inhibit integrin recycling to the cell surface (Roberts et al. 2001). After washing with PBS cells were lysed in IP buffer (50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% Triton X-100, 0.1% sodium deoxycholate, 1 mM EDTA and protease inhibitors) and cleared by centrifugation. β1 integrin immune-complexes were pulled down by incubation with protein G Sepharose (Sigma) for 2 h at 4°C. After several washes with lysis buffer, proteins were subjected to SDS-PAGE and western blot analysis.

4.14 SILAC-based peptide pull-downs

Pull-downs were performed as previously described (Meves et al. 2011) with the following cytoplasmic tail peptides: β1 wt tail peptide (758-798: HDRREFAKFEKEKMNAKWDTGENPIYKSAVTTVVNPKYEGK-OH), β1 TT/AA tail peptide (HDRREFAKFEKEKMNAKWDTGENPIYKSAVAAVVNPKYEGK-OH), β1 Y795A tail peptide (HDRREFAKFEKEKMNAKWDTGENPIYKSAVTTVVNPKAEGK-OH), a scrambled peptide (EYEFEPDKVDTGAKGTKMAKNEKKFRNYTVHNIWESRKVAP-OH) and α5 peptide (KLGFFKRSLPYGTAMEKAQLKPPATSDA-OH). All peptides were desthiobiotinylated.

Prior to use, peptides were immobilized on 75 µl Dynabeads MyOne Streptavidine C1 (10 mg/ml, Invitrogen).

Cell lysates were generated with Mammalian Protein Extraction Reagent (Thermo Scientific) from fibroblasts cultured in the presence of normal or L-$^{13}C_6{}^{15}N_4$-arginine (Arg10)-, L-$^{13}C_6{}^{15}N_2$-lysine (Lys8)-labeled medium. 1 mg of unlabeled or Arg10-, Lys8-labeled supernatant was incubated with either control or β1 peptide overnight at 4 °C. After washing with lysis buffer beads of corresponding peptide pairs were combined and proteins were eluted by incubating the beads in 16 mM biotin (Sigma-Aldrich) in PBS (pH 7.0) for 30 min at 30 °C followed by precipitation with cold acetone at −20°C overnight. The protein pellet was dissolved in SDS-PAGE sample buffer and separated on a 4–15% gradient SDS-PAGE gel. The gel was stained with Coomassie using the Gel Code Blue Safe Protein Stain reagent (Thermo Scientific) and used for mass analysis.

4.15 Expression and purification of recombinant proteins

Plasmids encoding GST-SNX17 or GST-SNX17QW/AA were transformed into BL21(DE3) Arctic Express *Escherichia coli*. One colony was grown in a 5 ml starter culture overnight at 37°C, which was used to inoculate a 500 ml culture. Once the 500 ml culture reached OD_{600}=0.6, protein expression was induced with 1 mM IPTG and the cells were grown for 3 h at 30°C. Cells were pelleted, washed once with PBS, lysed in TBS (50 mM Tris HCl, with 150 mM NaCl, pH 7.4) containing protease inhibitors (Protease Inhibitor Cocktail Tablets, Merck), sonicated with a tip sonicator on setting “3” for 30 seconds 3 times, and thereafter, incubated with 100 µg/ml Lysozyme (Sigma) and 50 µg/ml DNaseI (Sigma) at 4°C for 3 hours. Lysates were centrifuged with 14000 rpm at 4°C for 20 min. Supernatants were then

incubated with 1 ml glutathione Sepharose beads (GST-binding resin, Novagen) for 3 h at 4°C. GST-tagged proteins were bound and eluted according to the manufacturer's instruction.

Plasmid encoding for His-tagged kindlin-2 was transformed into BL21 T1 pRARE cells. Supernatants were generated as described above and then incubate with His-Select Ni Affinity gel (Sigma) for 2.5 h at 4°C. His-tagged kindlin-2 eluted was eluted from the beads and subjected to gel filtration to further purify the recombinant protein.

For pull-downs, synthetic peptides were immobilized on 20 µl Dynabeads My One Streptavidine C1 (10 mg/ml, Invitrogen) for 3 h at 4°C, incubated with 2% BSA in Mammalian Protein Extraction Reagent (Thermo Scientific) for 30 minutes to block unspecific binding prior to adding 50 ng recombinant GST-tagged SNX17 protein and further incubation on a rotator for 2h at 4°C. For competition experiments, 50 ng GST-SNX17 and 500 ng His-kindlin-2 or 500 ng BSA were incubated with the tail peptides for 3 h at 4°C. After 3 washes with RIPA buffer, proteins were eluted from the beads by boiling with 80 µl SDS-PAGE sample buffer for 5 minutes, separated by SDS-PAGE gel and blotted with SNX17 antibody.

4.16 qRT-PCR

RNA was isolated from cells using the RNeasy mini kit (Qiagen) and 1 µg of total RNA was transcribed into cDNA using the iScript-cDNA Synthesis kit (Bio-Rad). Quantitative PCR assays were performed with the iCycler-iQ (Bio-Rad) using SYBR green and the following primers: β1 integrin-fwd (5'-atgccaaatcttgcggagaat-3'), β1 integrin-rev (5'-tttgctgcgattggtgacatt-3'), β3 integrin-fwd (5'-ccacacgaggcgtgaactc-3'), β3 integrin-rev (5'-

cttcaggttacatcggggtga-3'), GAPDH-fwd (5'-tcgtggatctgacgtgccgcctg-3'), GAPDH-rev (5'-caccaccctgttgctgtagccgtat-3').

4.17 Immunofluorescence

For immunostaining, cells were cultured on glass coated with 10 µg/ml FN (Calbiochem). For the detection of endosomes, cells were fixed with 4% PFA/PBS for 15 minutes on ice, washed with PBS and permeabilised with 0.01% saponin/PBS for 10 minutes on ice. Cells were blocked with 3% BSA/PBS for 1 hour followed by incubation with the primary antibody in 3% BSA/0.01% saponin/PBS overnight at 4°C. After several washes with PBS, the fluorescently labeled secondary antibodies were incubated for 1 hour at RT in the dark. For other stainings, cells were fixed with 4% PFA/PBS for 15 minutes on ice and permeabilized with 0.2% TritonX-100/PBS for 10 minutes on ice. After fixation and permeabilization, the cells were incubated with 3% BSA in PBS for 1 hour followed by incubation with the primary antibody for 1 hour at RT or overnight at 4°C. Secondary antibodies were incubated for 1 hour at RT.

To determine the endocytic trafficking of β1 integrins from the cell surface by surface labeling, cells were washed with ice-cold PBS and incubated with an anti-β1 integrin antibody (HMbeta1-1, BioLegend) for 30 min on ice. Then, cells were washed again to remove excessive antibody. Surface-bound antibody was allowed to internalize for different time points at 37°C in regular growth medium. At each time point, the samples were washed with cold PBS and the remaining antibody at the cell surface was removed by two acid washes (0.2 M acetic acid/0.5 M NaCl/PBS) for 2 min on ice. Subsequently, the cells were fixed, permeabilized and stained as described above.

Images were collected at RT by confocal microscopy (DMIRE2; Leica) with a 63x/1.4 objective using the Leica Confocal Software (version 2.5, build 1227) or collected with an AxioImager Z1 microscope (Zeiss) with a 63x/1.4 oil objective.

4.18 Time-lapse video microscopy of cell spreading and migration

Cell spreading and single-cell migration assays were done as described (Montanez et al. 2007). Briefly, 250,000 cells were plated on a FN-coated six-well plate and immediately imaged by live cell microscopy to determine spreading kinetics for indicated time points. Afterwards, adherent cells were monitored to analyze cell migration. Cell spreading was additionally measured with cells seeded on FN-coated glass slides, fixed with 4% paraformaldehyde at 37°C at indicated time points and stained with phalloidin-Alexa488 for F-actin. Images were taken with an AxioImager Z1 microscope (Zeiss) with a 20x objective and the spreading area was calculated using MetaMorph 7 imaging software (Molecular Devices).

For single-cell migration the acquired images were analyzed using the manual tracking plugin of ImageJ and the Chemotaxis and Migration Tool (v2.0) of the QWT project.

Cell wounding assays were performed with confluent monolayers of cells cultured in FN-coated 6-well dishes. Cells were serum-starved overnight before wounds were applied with a 200 µl plastic micropipette followed by thorough washing with PBS. Wound closure was imaged in serum-free medium at 15 min intervals overnight. The acquired images were analyzed using the manual tracking plugin of ImageJ and the Chemotaxis and Migration Tool (v2.0) of the QWT project.

Images of live cells were recorded at 37°C and 5% CO_2 on a Zeiss Axiovert 200 M (Zeiss) equipped with 10x/.3, 20x/.4 and 40x/.6 objectives, a motorized stage (Märzhäuser) and an environment chamber (EMBL Precision Engineering) with a cooled CCD camera (Roper Scientific). Image acquisition and microscope control were carried out with MetaMorph software (Molecular Devices).

4.19 Statistics

Statistical analysis was performed using the GraphPad Prism software (version 5.00, GraphPad Software). Statistical significance was determined by the unpaired *t*-test or Mann-Whitney U-test as indicated. Results are expressed as the mean ± SD if not indicated otherwise.

4.20 Figures and figure legends

Figures and figure legends were adapted and modified from Böttcher and Stremmel et al. 2012 (Bottcher et al. 2012).

5 Results

5.1 Early embryonic lethality by β1 integrin TT788/789AA and Y795A mutations

The threonines 788/789 and the tyrosine 795 in the distal NxxY motif of the murine β1 integrin tail are essential for kindlin binding and their disruption leads to severe functional integrin defects (Ma et al. 2008; Moser et al. 2008; Ussar et al. 2008). Therefore, we established an *in vivo* mouse model in which we substituted these amino acids by alanines causing massive impairments in early mouse development. While heterozygous mice developed normally, their intercrosses failed to produce living offspring (TT/AA: +/+: 35%, [TT/AA]/+: 65%, [TT/AA]/[TT/AA]: 0%, N=100; Y795A: +/+: 33%, [Y795A]/+: 67%, [Y795A]/[Y795A]: 0%, N=100). Timed-matings revealed extremely malformed or resorbed embryos on day 7.5 of embryonic development (E7.5) (+/+: 25%, [TT/AA]/+: 53%, [TT/AA]/[TT/AA]: 17%, resorbed: 5%, N=36) with defects in cell polarity, laminin111 (LN111) distribution and cavitation (Figure 9A, B). Due to almost identical abnormalities in β1 TT/AA and β1 Y795A mice, we focused our work on the β1 TT/AA mutation.

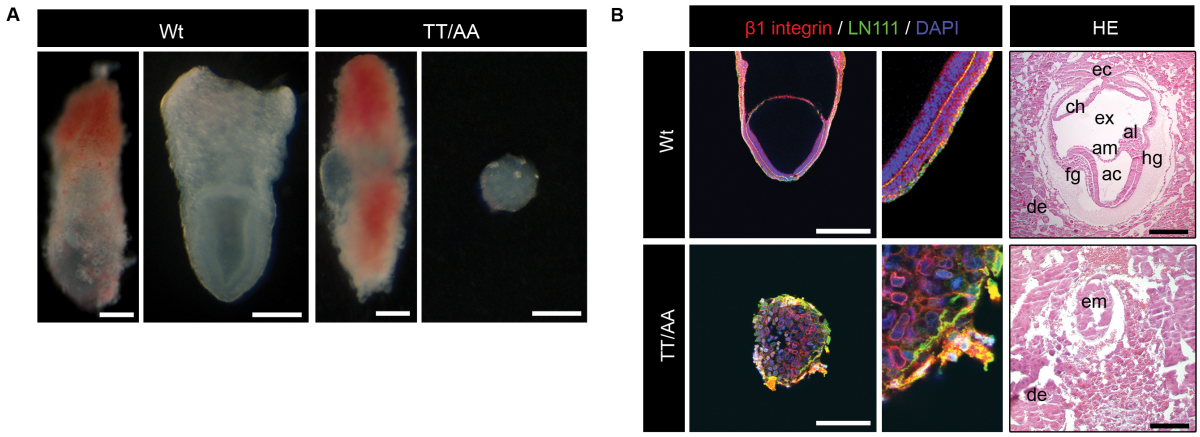


Figure 9: β1 TT/AA mutation causes early embryonic lethality
(A) Bright field images of E7.5 embryos with and without implantation chambers. (B) Left and central panels show whole-mount immunostainings of E7.5 embryos for β1 integrin (red) and LN111 (green). Nuclei were counterstained with DAPI (blue). Right panels show haematoxilin and eosin (HE) stainings of E7.5 embryo sections. em, embryo; de, decidua; ac, amniotic cavity; am, amnion; al, allantois; ch, chorion; ec, ectoplacental cone; fg, foregut; hg, hindgut; ex, exocoelomic cavity. Scale bars 100 µm. (C. Stremmel)

To further analyze the *in vivo* effects, we isolated embryonic stem cells (ES cells) from blastocysts of β1 wild type (β1 wt) and littermate homozygous TT/AA (β1 TT/AA) mice. These ES cells were subsequently used to generate so-called embryoid bodies (EBs) *in vitro,* mimicking the early embryonic development in the pre-implantation period. After 2-4 days in suspension culture β1 wt ES cells formed first EBs consisting of an outer layer of primitive endoderm on a basement membrane (BM) with an undifferentiated core in the center, which differentiated in a pseudostratified ectoderm surrounding a central cavity by day 4-6. β1 TT/AA ES cells formed compact cell aggregates surrounded by a discontinuous BM with single endodermal cells attached to it. These aggregates did neither show a polarized distribution of β-catenin, E-cadherin or F-actin nor a central cavity (Figure 10A, B).

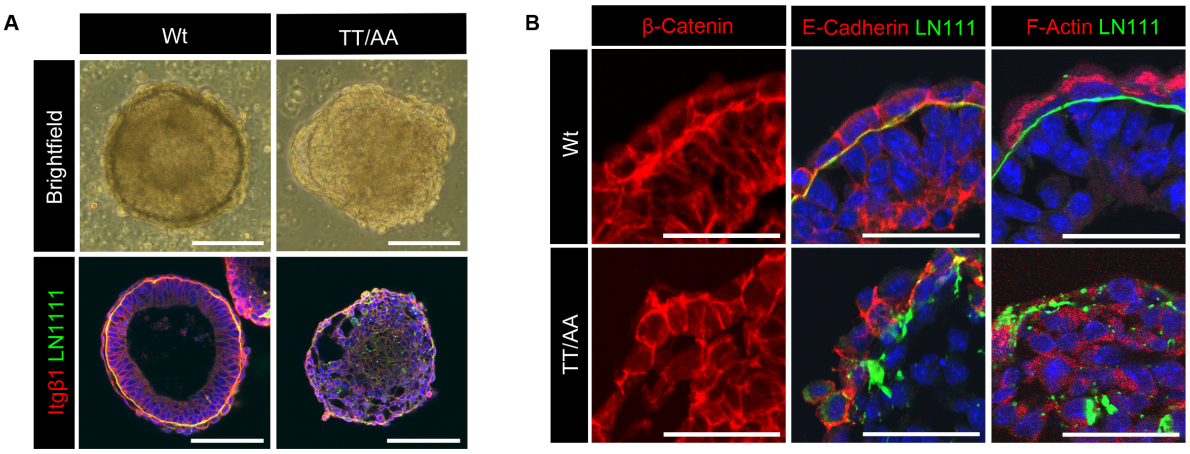


Figure 10: Defective β1 TT/AA embryoid body formation
(A) Upper panels show bright field images of EBs on the 5th day of suspension culture. Lower panels show cryo sections of EBs on the 5th day of suspension culture stained for β1 integrin (red) and LN111 (green). Nuclei were counterstained with DAPI (blue). (C) Cryo sections of EBs on the 5th day of suspension culture stained for β-catenin (red), E-cadherin (red), F-actin (red) and LN111 (green). Nuclei were counterstained with DAPI (blue). Scale bars 100 µm (A), 50 µm (B). (C. Stremmel)

The analysis of β1 TT/AA ES cells revealed significant adhesion defects in culture on feeder cells (Figure 11A) as well as a dramatic reduction of the mature β1 integrin form (125 kDa), while the expression of talin-1 und kindlin-2 was unaffected (Figure 11B). These results highly correlated with fluorescence activated cell sorting (FACS) experiments, showing a reduction of β1 surface expression down to about 40%. Corresponding integrin α subunits, α5 and α6, were similarly reduced, while β3 and αv levels were unchanged (Figure 11C). We used a 9EG7-epitope-recognizing antibody to study the activation state of β1 integrins. In line with previous studies demonstrating the requirement of an intact kindlin-binding site for proper integrin activation (Montanez et al. 2008), we found clearly reduced integrin activation levels in β1 TT/AA ES cells, which could be normalize by manganese treatment (Figure 11D). Moreover, we performed quantitative real-time PCR (qRT-PCR) analyses and found significantly elevated β1 mRNA levels in TT/AA ES cells, indicating that the reduced surface

expression was not due to insufficient transcription (Figure 11E). These defects had also functional consequences as β1 TT/AA ES cells showed significant adhesion defects on ECM substrates (Figure 11F). Similar results regarding integrin expression and activation were observed in β1 Y795A ES cells.

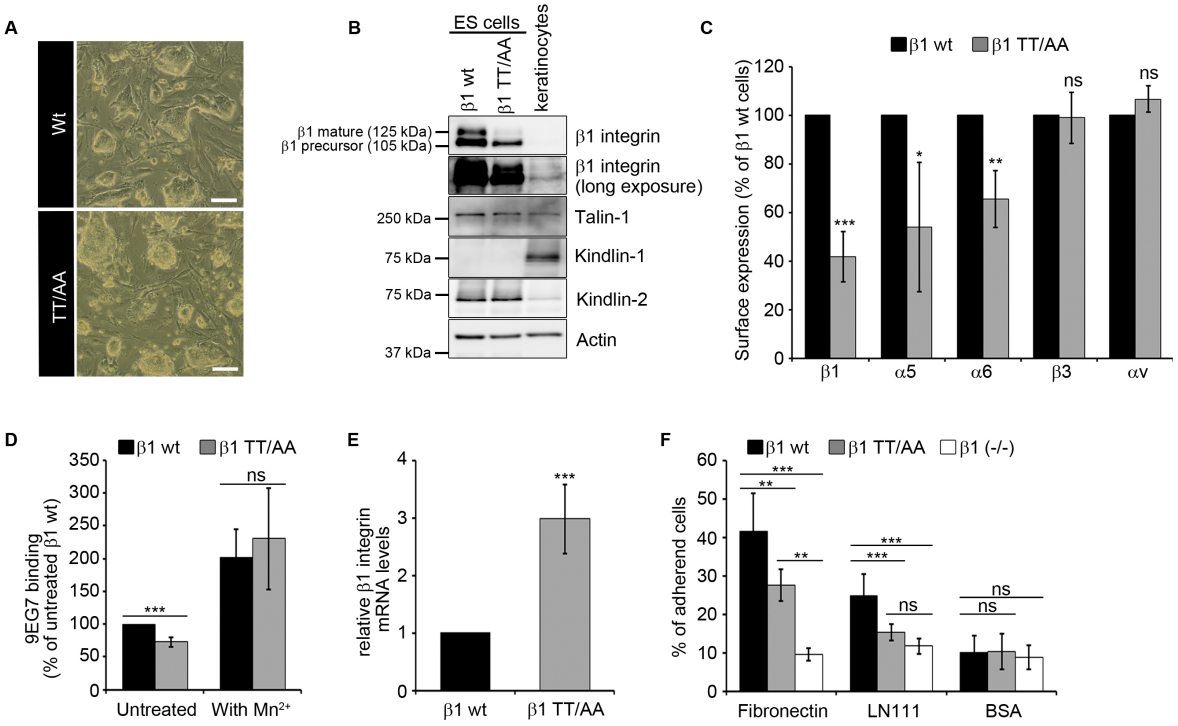


Figure 11: Reduced expression and activation of β1 TT/AA ES cells
(A) Bright field images of ES cell colonies on feeder cells. (B) Western blot for β1 integrin, Talin-1, Kindlin-1, Kindlin-2 and actin of ES cell lysates including a control lane for Kindlin-1 from a wt keratinocyte lysate. (C) Expression of integrin subunits in ES cells determined by FACS (mean±SD, n=4, *P=0.0134, **P=0.0011, ***P<0.0001). (D) Integrin activation on ES cells measured by 9EG7 binding and corrected for total β1 integrin expression (mean±SD, n=4, ***P=0.0003). (E) Expression of β1 integrin mRNA in ES cells measured by quantitative real-time PCR (n=2). (F) Adhesion assay of ES cells on different substrates (mean±SD, n=3, **P<0.01 and ***P<0.005). P values: Student's t-test; ns=not significant. (C. Stremmel)

To test whether these effects could also be observed in other cellular systems, we compared β1 wt and β1 TT/AA or β1 Y795A fibroblasts with each other. The cell lines were generated in two different ways: On the one hand we generated β1-null fibroblasts from β1 floxed mice and rescued these with β1 wt, β1 TT/AA or β1 Y795A cDNAs (Figure 12A). On the other hand, we isolated littermate β1 flox/wt and β1 flox/TT/AA fibroblasts and removed the floxed allele by adenoviral Cre transduction (Figure 12B). Fibroblasts from both cell systems showed analogue results in all assays.

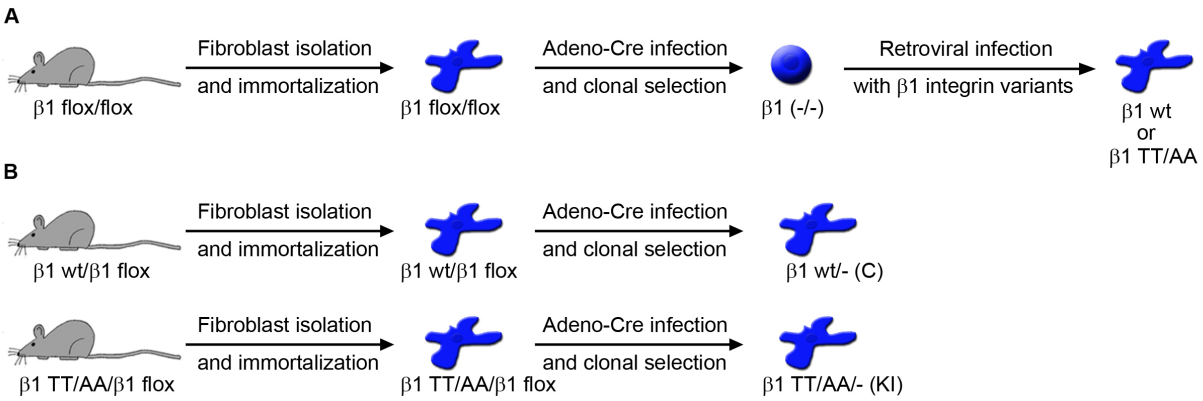


Figure 12: Generation of fibroblast cell lines
Scheme depicting the generation of β1 wt and β1 TT/AA fibroblasts. β1 wt and β1 TT/AA fibroblasts were either obtained by retroviral expression of β1 variants in β1-null cells (A) or by immortalization of fibroblasts from mouse embryos and subsequent adenoviral Cre transduction (B). ((A) R. Böttcher mainly contributed, (B) C. Stremmel)

Despite elevated mRNA levels, the expression of mature β1 integrin (125 kDa) was clearly reduced in β1 TT/AA fibroblasts, while a relative increase in expression of the immature form (105 kDa) in relation to β1 wt cells was observed in western blot experiments (Figure 13A-E). Again, 9EG7 antibody binding was substantially reduced in mutant cells (Figure 13F). In summary, these observations indicate that TT/AA or Y795A mutations reduce β1 integrin expression by processing or turnover alterations.

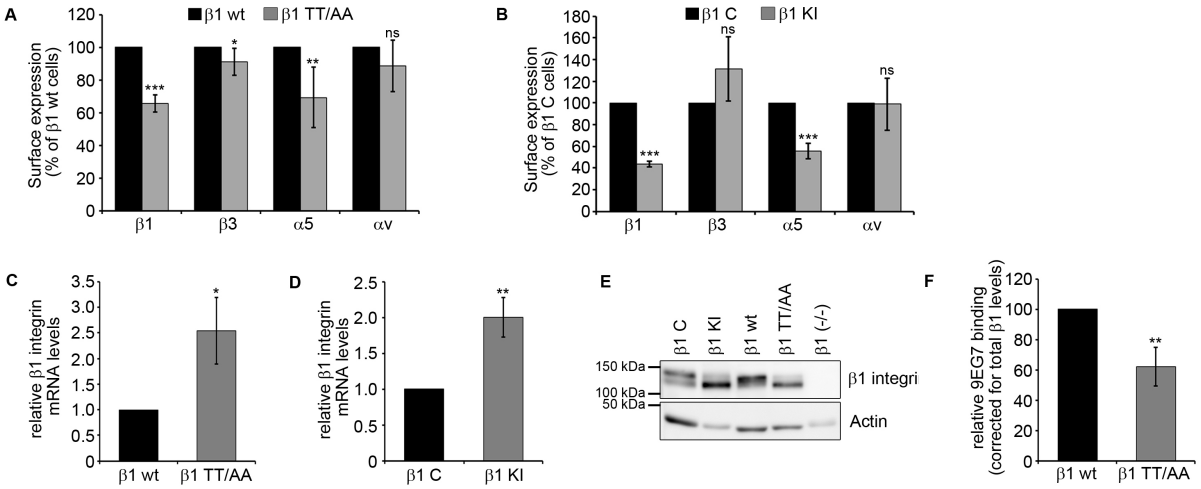


Figure 13: Characterization of β1 TT/AA fibroblasts
(A, B) Surface expression of different α and β integrin subunits on β1 wt and β1 TT/AA fibroblasts determined by FACS (mean±SD, n=6 (A), n=4 (B), *P<0.05, **P=0.0022, ***P<0.0001). (C, D) β1 integrin mRNA levels determined by qRT-PCR (mean±SD, n=3 with two independent cDNAs, *P=0.015, **P=0.0032). (E) Western blot analysis of fibroblast cell lines with antibodies against β1 integrin and actin. (F) β1 integrin activation measured by 9EG7 binding and corrected for β1 integrin expression (mean±SD, n=4; **P=0.0097). P values: Student's t-tests, ns=not significant. (C. Stremmel)

5.2 Integrin turnover is controlled by the distal β1 integrin tail

The determinants of integrin surface expression are synthesis, maturation, surface presentation, endocytosis, recycling and degradation. Synthesis-related causes for the reduced integrin surface expression in β1 TT/AA mutants were excluded by mRNA expression analyses. Therefore, we investigated integrin maturation in its secretion pathway by pulsed labeling with ^{35}S-methionine/cystein. According to the higher integrin synthesis rate in β1 TT/AA fibroblasts we found a pronounced 105 kDa band representing the immature integrin in these cells. Yet, the relative decrease in band intensity for the immature integrin was comparable in β1 wt and β1 TT/AA fibroblasts indicating normal processing of the β1

integrin polypeptide despite of the introduced mutation. The significantly higher expression of immature integrin (105 kDa) in β1 TT/AA fibroblasts paired with longer absolute persistence of mature integrin (125 kDa) in β1 wt cells, provides clear evidence for mutation-related differences in integrin turnover (Figure 14).

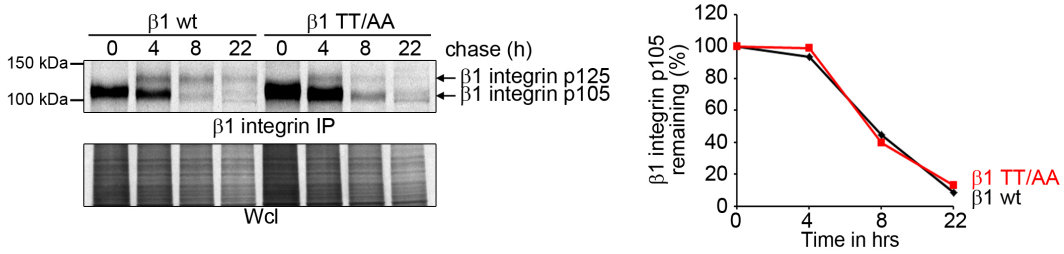


Figure 14: β1 TT/AA integrin maturation
Pulse-chase analyses of β1 integrin maturation in cells expressing β1 wt or TT/AA integrins. Cells were collected and analysed by immunoprecipitation after metabolic labeling with ^{35}S-methionine/cysteine and chased for the indicated time points. Maturation curves show immature β1 integrin as a percentage of the total β1 integrin (right) and were drawn from densitometric scans of the autoradiograms (left). (R. Böttcher mainly contributed)

Further analyses of integrin turnover were performed by internalization and recycling assays after surface labeling with cleavable biotin. In doing so, we found a dramatically reduced recycling rate back to the plasma membrane in β1 TT/AA fibroblasts, while internalization kinetics were unaffected (Figure 15A, B). To test whether the reduced recycling rate is due to increased integrin degradation, we used the lysosomal inhibitors chloroquine and bafilomycin or the proteasomal inhibitor MG132 respectively. While lysosomal inhibition rescued β1 TT/AA surface expression including the corresponding α5 integrin subunit, proteasomal inhibition failed to rescue the defects (Figure 15C).

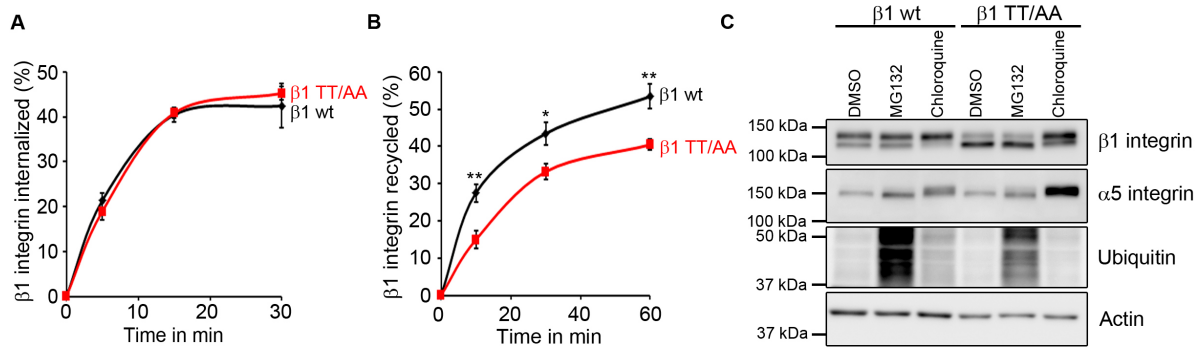


Figure 15: Reduced recycling and enhanced lysosomal degradation of β1 TT/AA integrins
(A) Quantification of β1 integrin internalization in β1 wt- and β1 TT/AA-expressing cells by capture ELISA (mean±SD, n=4). (B) Quantification of β1 integrin recycling in β1 wt and β1 TT/AA-expressing fibroblasts by capture ELISA (mean±SD, n=5, *P < 0.05 and **P < 0.01). (C) Western blot analysis of β1 wt- and β1 TT/AA-expressing cells treated with either a proteasome (MG132) or a lysosome inhibitor (chloroquine) for 20 h. Actin served as a loading control. ((A, B) C. Stremmel, (C) C. Stremmel/ R. Böttcher equally contributed)

Degradation kinetics were quantified by surface labeling with biotin followed by streptavidin pull-downs and western blotting or capture ELISA, an experimental technique utilizing β1 integrin antibody-coated ELISA plates. We measured integrin half-lives of about 20 hours for the wild type protein and of about 4-5 hours for the TT/AA mutant. The observed effects were specific for the β1 mutation, since turnover rates of β3 integrin or the transferrin receptor (Tfr) were unchanged (Figure 16A-C). Analogue experiments were performed for the tyrosine to alanine mutation in both β1 integrin NxxY motifs. While the β1 Y783A mutation in the membrane proximal NxxY motif did not influence integrin stability, the β1 Y795A mutation in the distal motif revealed a dramatic reduction in integrin stability comparable to the β1 TT/AA mutant (Figure 16D). These results let us draw the following conclusion: Mutating the threonines 788/789 or the distal NxxY motif respectively, leads to a dramatic increase in integrin turnover and lysosomal degradation.

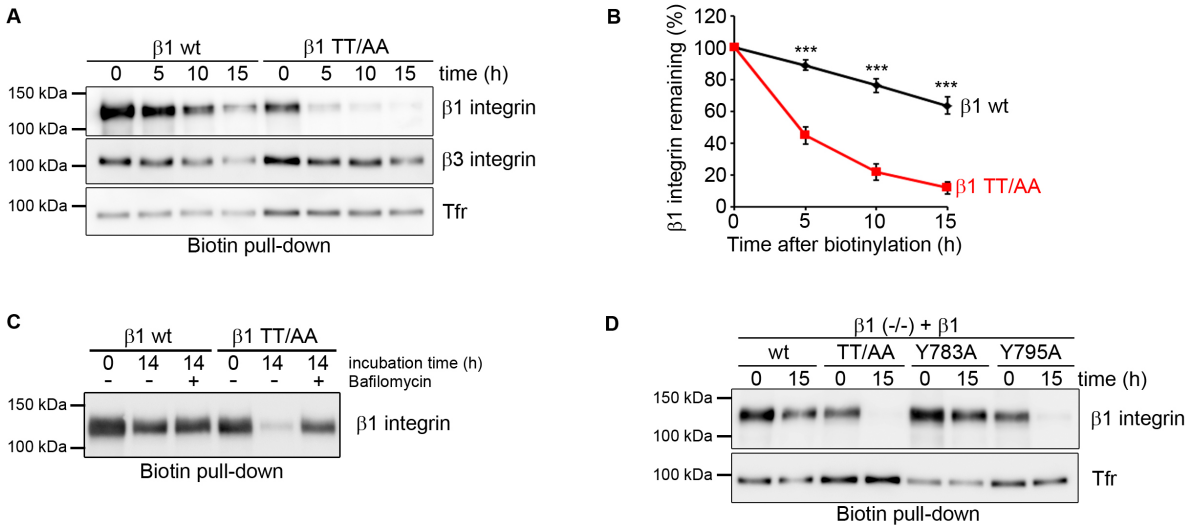


Figure 16: Lysosomal integrin degradation kinetics
(A, B) Degradation of cell surface integrins was determined by biotinylating cell surface proteins and incubating for the indicated time points, followed by biotin pull-down and western blot analysis (A) or quantification by capture ELISA (mean±SD, n=4, ***P<0.0001) (B). (C) Lysosomal inhibition by bafilomycin prevents degradation of β1 TT/AA integrin. Degradation was measured by surface biotinylation and incubation of the cells for 14 h in the presence or absence of bafilomycin. (D) Degradation of β1 integrin mutants (wt, TT/AA, Y783A and Y795A) was determined by biotinylating cell surface proteins and incubating for 0 h and 15 h followed by biotin pull-down and western blot analysis. P values: Student's t - test. (R. Böttcher mainly contributed)

5.3 Kindlin-2 regulates β1 integrin surface expression by β1 mRNA level alterations

The two threonines 788/789 and the distal NxxY motif are essential for kindlin binding and mutations within this region dramatically reduce the binding (Ma et al. 2008; Moser et al. 2008; Ussar et al. 2008). In line with a previous study (Harburger et al. 2009), we demonstrated that kindlin2-eGFP overexpression in β1 wt fibroblasts clearly increases β1 integrin expression. Interestingly, this increase could not be observed in β1 TT/AA fibroblasts, which is most likely due to the preexisting mRNA upregulation in these cells

(Figure 17A). Vice versa, cells lacking kindlin-1 and -2 (Figure 17H) showed a clear reduction in β1 integrin surface expression (Figure 17B). These obvious correlations between kindlin and integrin expression drove us to perform internalization, recycling and stability assays in kindlin-2-overexpressing cells all of which not showing any differences when compared to β1 wt fibroblasts (Figure 17C-E). Similarly, kindlin-1 and -2 knockout cells were not altered in degradation kinetics (Figure 17F). Internalization and recycling assays were technically impossible due to poor adhesion on ECM substrates. Based on these experiments, we were able to exclude kindlin-related trafficking processes as causative for the alterations in integrin expression. However, qRT-PCR analyses of kindlin-2 overexpressing cells showed a significant increase in β1 mRNA levels revealing a conclusive explanation for the observed relation between kindlin and integrin expression (Figure 17G).

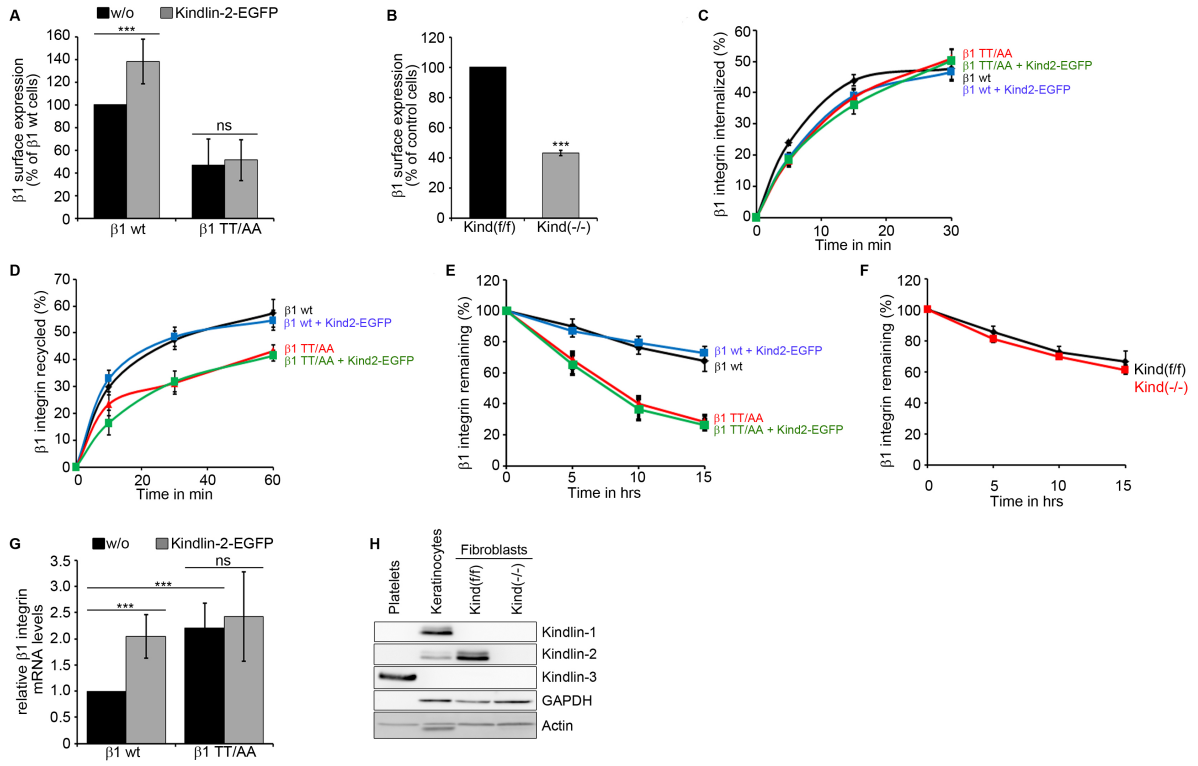


Figure 17: Kindlin-2-dependent regulation of β1 integrin surface levels
(A) β1 integrin surface expression in β1 wt- and β1 TT/AA-expressing fibroblasts after overexpression of kindlin-2-eGFP determined by FACS (mean±SD, n=11, ***P < 0.0001). (B) β1 surface levels in control (Kind (f/f)) and kindlin-1 and -2-double-null (Kind(-/-)) fibroblasts determined by FACS (mean±SD, n=3, ***P < 0.0001). Quantification of β1 internalization (C) and recycling (D) in β1 wt and β1 TT/AA cells with and without kindlin-2-eGFP overexpression by capture ELISA (mean±SEM, n=8 (C) and n=10 (D)). (E, F) Quantification of surface β1 integrin stability in kindlin-2-eGFP-overexpressing (E) and Kind(-/-) cells (F). Degradation of cell surface β1 integrin was determined by biotinylating cell surface proteins and incubating for the indicated time points, followed by capture ELISA (mean±SD, n=4 (E), n=3 (F)). (G) β1 integrin mRNA expression in kindlin-2-eGFP-overexpressing fibroblasts measured by quantitative real-time PCR (n=2). (H) Western blot analysis of cell lysates from platelets, keratinocytes and Kind (-/-) fibroblasts with antibodies against the three kindlin family members. Actin and GAPDH served as loading control. P values: Student's t-test, ns=not significant. ((A-D, G) C. Stremmel, (E, F, H) R. Böttcher mainly contributed)

5.4 SNX17 interacts with the TT788/789-Y795-motif in the cytoplasmic β1 integrin tail

To clarify how the threonines 788/789 and the membrane distal NxxY motif regulate surface expression, recycling and degradation, we screened for a new interactor with the β1 integrin cytoplasmic tail. Therefore, we used the SILAC (stable isotope labeling by amino acids in cell culture) (Ong et al. 2002) method in combination with subsequent pull-down experiments with synthesized full-length cytoplasmic domains of β1 wt and β1 TT/AA integrins as well as a scrambled peptide to identify unspecific interactions. Afterwards we performed mass spectrometry-based proteomics (Mann 2006) and identified the known β1 integrin interactors talin-1 and -2, kindlin-2, Dab2 and ILK as well as the new binding partner SXN17. Simultaneous analyses for the β1 TT/AA peptide showed a massive reduction in kindlin-2- and SNX17-binding due to the introduced mutation (Figure 18A).

SNX17 is a member of PX-domain proteins, which play a central role in endosomal trafficking and associate predominantly with PtdIns3P-rich membranes. Besides, SNX17 is characterized by a FERM-domain with a QW-motif in the F3-subdomain, which is also present in the famous integrin adaptor proteins talin and kindlin and typically binds NxxY motifs as there are two of them in the β1 integrin cytoplasmic tail (Tadokoro et al. 2003; Ghai et al. 2011). An important role of SNX17 in endosomal trafficking has already been demonstrated for the transmembrane receptors LDLR, LRP1, P-selectin and APP (Stockinger et al. 2002; Burden et al. 2004; Knauth et al. 2005; van Kerkhof et al. 2005; Lee et al. 2008).

We confirmed our proteomics data in peptide pull-down experiments and detected kindlin-2 and SNX17 binding to β1 wt, but not to β1 TT/AA, α5 or scrambled peptides (Figure 18B). Moreover, we used this experimental setting to show that SNX17 binding to β1 integrins is

direct and specific. We used recombinant wild type GST-SNX17 (GST-SNX17) and compared it to GST-SNX17 in which the QW-motif in the FERM-F3-subdomain was mutated to alanines (GST-SNX17QW/AA). In doing so, we detected SNX17 with the GST-SNX17 peptide, while there was no binding detectable in case of the QW/AA mutation (Figure 18C).

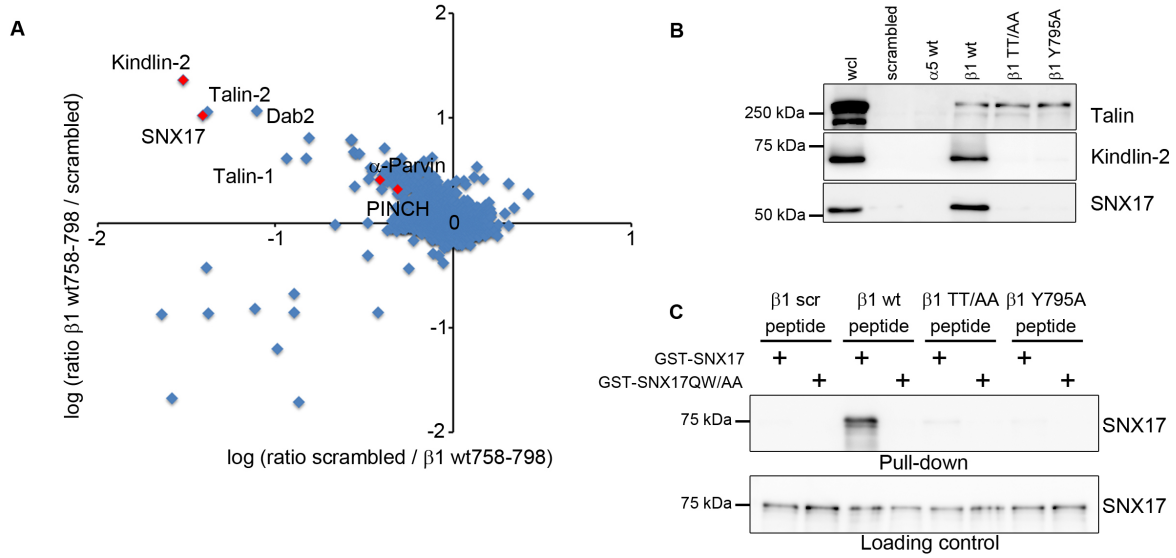


Figure 18: SNX17 binds the distal cytoplasmic integrin tail
(A) Scatter plot of β1 wt tail peptide versus scrambled-peptide pull-down results. The log2 SILAC ratio of proteins identified with at least 2 unique peptides in each mass spectrometry run is plotted as the forward pull-down (x axis) against the reverse labeling pull-down (y axis). Specific interaction partners show inverse ratios between forward and reverse experiment, grouping them into the upper left quadrant. Red dots indicate those proteins that failed to bind to the β1 TT/AA tail peptide in a separate experiment. (B) Western blot showing talin-1, kindlin-2 and SNX17 binding to the biotinylated peptides indicated. Wcl=whole-cell lysate. (C) Streptavidin-bead pull-down assay with the indicated biotinylated β1 integrin cytoplasmic tail peptides and recombinant GST-tagged SNX17 or a GST-tagged SNX17QW/AA. ((A) A. Meves mainly contributed, (B, C) R. Böttcher mainly contributed)

Immunofluorescence experiments showed co-localization of the early endosomal marker early endosomal antigen 1 (EEA1) (Figure 19A) as well as with the transferrin receptor as a marker for recycling endosomes (Figure 19B), while we were not able to detect any co-localization with the lysosomal markers lysosomal-associated membrane protein 1 (Lamp1) (Figure 19C)

or Rab7 (Figure 19D). Furthermore co-localization of kindlin-2 and SNX17 was not detected, pointing towards a spatially separated binding to β1 integrins in different cellular compartments (Figure 19E). However, both proteins kindlin-2 and SNX17 compete for the same binding region in the distal cytoplasmic integrin tail (Figure 19J).

Due to a lack of suitable antibodies, we used live-cell microscopy to visualize SNX17-integrin co-localization. To this end, we preincubated our cells with fluorescent integrin antibodies and allowed receptor internalization for defined time periods. Afterwards, we successfully visualized co-localization of β1 integrin and SNX17 in SNX17-eGFP transfected cells (Figure 19F). Not surprisingly, SNX17 was also recruited to β1 TT/AA positive endosomes, although we excluded direct interactions of these proteins (Figure 19G). Therefore, this observation is most likely due to interactions with other cargo proteins in integrin-carrying endosomes as LRP1 for example. With the help of antibodies against the 9EG7-epitope we visualized integrins in focal adhesions, but not in SNX17-positive endosomes suggesting a predominant binding to low-affinity conformation integrins (Figure 19G). To demonstrate interactions also with endogenous proteins, we enriched endosomal β1 integrins with the recycling inhibitor primaquine (van Weert et al. 2000) and co-precipitated this with endogenous SNX17. Also in the endogenous setting, the interaction was destroyed by the introduction of the β1 TT/AA mutation (Figure 19H). In summary, the new β1 integrin-binding protein SNX17 associates with β1 integrins in the kindlin-binding region and is located in endosomes.

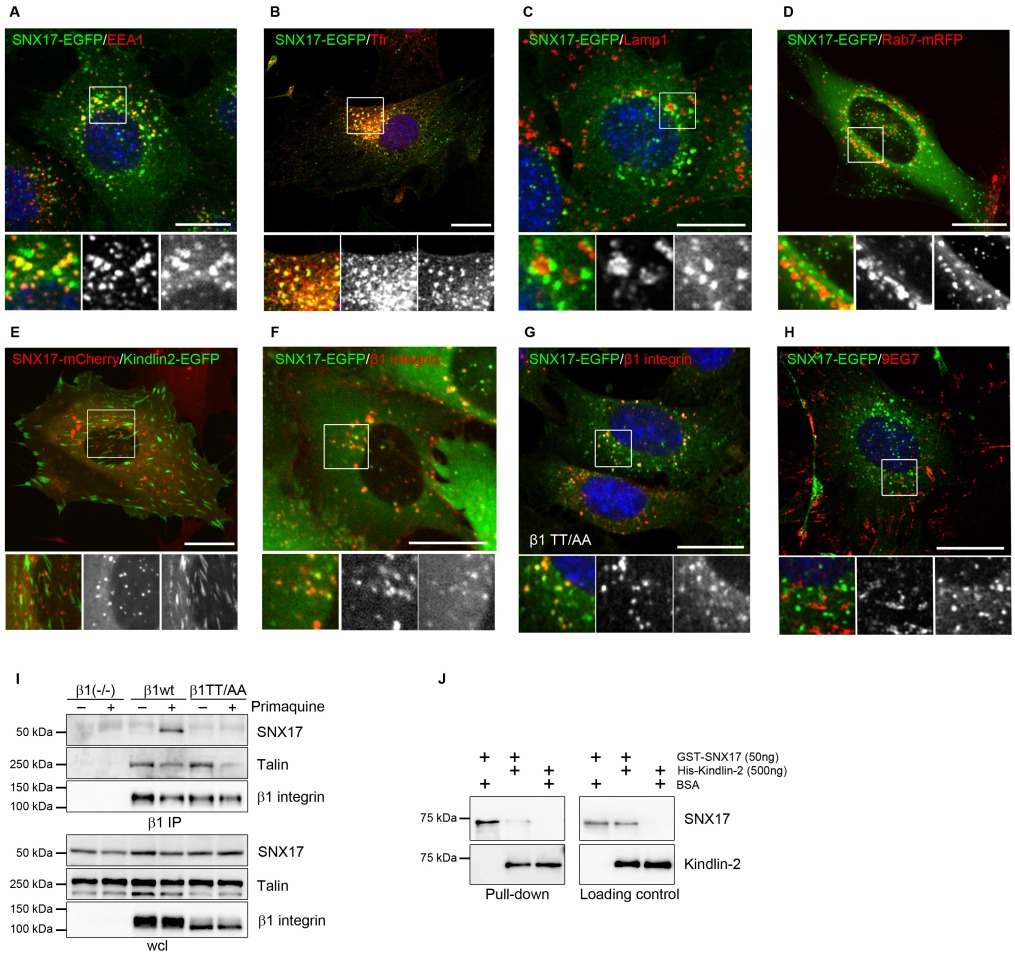


Figure 19: SNX17 binds the distal cytoplasmic integrin tail in endosomes
(A, C) Immunostaining of SNX17-eGFP-expressing cells with EEA1 (A), Tfr (B) and Lamp1 (C) antibodies. Nuclei were counterstained with DAPI (blue). Correlation coefficient 0.300±0.116 (EEA1), 0.032±0.121 (Tfr), 0.457±0.141 (Lamp1), mean±SD, n=44 (A), n=13 (B), n=25 (C). (D) SNX17-eGFP-expressing cells were transfected with Rab7-mRFP and the fluorescence distribution was determined in living cells by spinning disk confocal microscopy. Stills of movies are shown (correlation coefficient Rab7-mRFP 0.454±0.106, mean±SD, n=15. (E) SNX17-mCherry-expressing cells were transfected with kindlin-2-eGFP and fluorescence distribution was determined in living cells by spinning disk confocal microscopy. Stills of movies are shown. (F) Localization of endogenous β1 integrin after surface labeling with an anti-β1 integrin antibody and internalization for 15 min in SNX17-eGFP-expressing cells. The fluorescence intensity was determined in living cells by spinning disk confocal microscopy. A still of a movie is shown. In A to H, lower panels show an enlargement of the area indicated by the white rectangle. (I) Co-immunoprecipitation of endogenous β1 integrin and SNX17 from β1 wt- and β1 TT/AA-expressing cells pre-treated with or without primaquine. Wcl=whole-cell lysate. Scale bars 20 µm. (J) Streptavidin-bead pull-down assay with a biotinylated wild type β1 integrin cytoplasmic tail peptide and recombinant GST-tagged SNX17 and His-tagged kindlin-2. (R. Böttcher mainly contributed)

5.5 SNX17 controls recycling and stability of β1 integrins

To analyze the function of SNX17 in integrin trafficking and/or degradation, we depleted SNX17 by retroviral expression of shRNAs (shRNA17-1 and shSNX17-2). This depletion caused of clear reduction of the mature integrin form in western blotting as well a 40% reduction in β1 integrin surface expression (Figure 20A, B). In line with our previous results, we were able to rescue this reduction by bafilomycin treatment. SNX17-depelted cells showed an excessive integrin degradation in western blotting as well as capture ELISA compared to control fibroblasts (shCtr). This degradation was specific since neither the Tfr nor β1 TT/AA integrins, which cannot bind SNX17, were affected by the SNX17-depletion (Figure 20D-F). Additionally, re-expression of shRNA-resistant SNX17 rescued the defects, while SNX17QW/AA failed in doing so (Figure 20C, G). Similar to β1 TT/AA cells, trafficking assays did not show any internalization defects in SNX17-depleted cells, despite of significant defects in integrin recycling. Therefore, we classify SNX17 as a promoter of integrin recycling back to the cell surface, which inhibits degradation (Figure 20H).

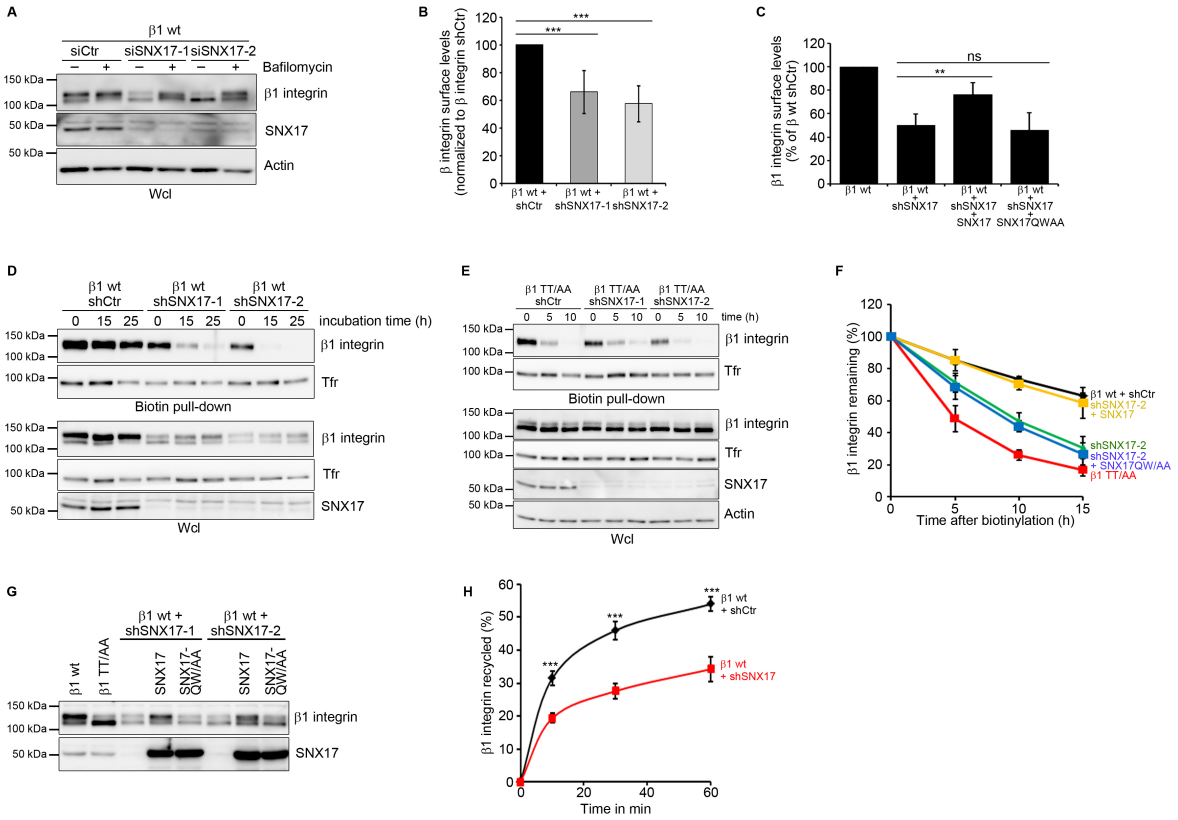


Figure 20: Depletion of SNX17 reduces surface expression of β1 integrins
(A) Western blot analysis of control (siCtr) or two siSNX17-transfected cells (siSNX17-1 and siSNX17-2) treated with or without bafilomycin for 20 h. Actin served as loading control. (B) Quantification of β1 surface levels in control and SNX17-depleted cells determined by FACS (mean±SD, n=9, ***P < 0.0001). (C) β1 integrin surface levels in SNX17-depleted cells were restored by re-expressing siRNA-insensitive wt SNX17 but not with SNX17QW/AA (mean±SD, n=3, **P=0.0079). (D, F) Degradation of cell surface integrins was determined by biotin pull-down and western blot analysis (D) or capture ELISA (F) (mean±SD, n=5). (E) SNX17-depleted β1 TT/AA expressing cells were surface-biotinylated and incubated for the indicated time points before biotinylated proteins were pulled down with streptavidin-Sepharose and analyzed by western blotting. (G) Western blot analysis of cell lysates derived from the indicated β1 integrin-expressing and SNX17-depleted cells. SNX17 levels were restored in the SNX17-depleted cell lines by re-expressing either siRNA-insensitive wt SNX17 or SNX17QW/AA. (H) Quantification of β1 integrin recycling in SNX17-depleted cells by capture ELISA (mean±SEM, n=7, ***P<0.0008). Note the strong reduction in the level of β1 integrin recycling after SNX17 depletion. P values: Student's t-test, ns=not significant. ((A, D-G) R. Böttcher mainly contributed, (B, C, H) C. Stremmel)

To further analyze the early endocytosis pathway, we surface-labeled integrins with fluorescent antibodies prior to internalization for different time points. After 10 minutes both β1 wt and β1 TT/AA fibroblasts showed an evenly distributed punctuated integrin pattern in the early endosomal compartment. After 30 minutes the fluorescent signal was clearly reduced in β1 wt cells, indicative for an effective recycling back to the plasma membrane. However, in β1 TT/AA and SNX17-depeleted cells the fluorescent signal co-localized with Lamp1, a marker for the lysosomal compartment. Therefore, we strengthened our conclusion that a functional β1 integrin-SNX17 interaction promotes integrin recycling and prevents its degradation (Figure 21).

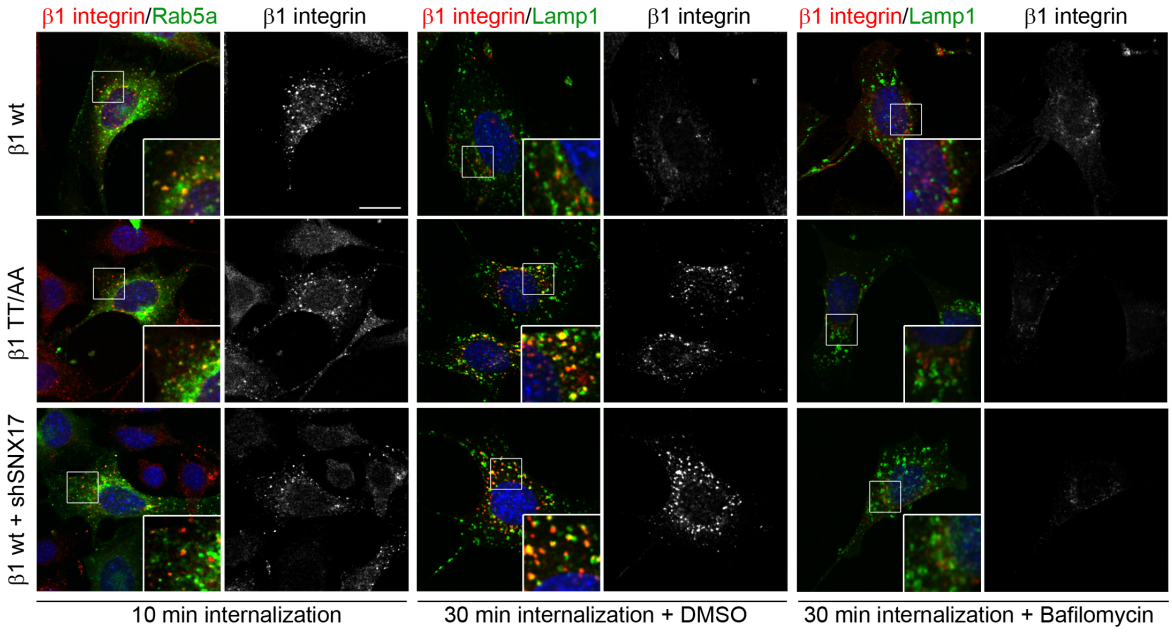


Figure 21: Immunostaining of lysosomal β1 integrin degradation
Localization of endogenous β1 integrin after surface labeling with an anti-β1 integrin antibody and internalization for 10 and 30 min in β1 wt, β1 TT/AA and SNX17-depleted cells, respectively, treated with or without bafilomycin. Cells either expressed Rab5a-GFP or were fixed and stained with antibodies against Lamp1. Nuclei were counterstained with DAPI (blue). Scale bar 20 µm. (R. Böttcher mainly contributed)

Further analyses focused on the question whether SNX17 primarily promotes recycling or inhibits degradation of integrins. We performed recycling experiments with β1 wt and β1 TT/AA or SNX-depleted cells in the presence or absence of bafilomycin. In doing so, we demonstrated that bafilomycin treatment can normalize the recycling defects as well as the β1 integrin surface expression in both β1 TT/AA and SNX17-depleted cells to β1 wt levels (Figure 22A, D, E). Integrin antibody internalization assays showed that the previously described accumulation of integrins in the lysosomal compartment of mutant cells was abrogated by bafilomycin treatment (Figure 21). Additionally, we substituted all α5 and β1 integrin cytoplasmic lysine residues by arginines (α5 4xKR, β1 8xKR±TT/AA) to prevent ubiquitin-mediated lysosomal degradation. While integrin internalizations was unaffected by the mutations, we could rescue the stability and recycling defects in β1 TT/AA fibroblasts by simultaneous expression of β1 8xKR+TT/AA and α5 4xKR (Figure 22B, C). Remarkably, expression of β1 8xKR+TT/AA alone did not fully rescue the defects, indicating that α5β1 integrin degradation cannot only be mediated by the β but also by the α subunit. In this study, we show that SNX17 primarily prevents integrin degradation, while the effects on integrin recycling are secondary.

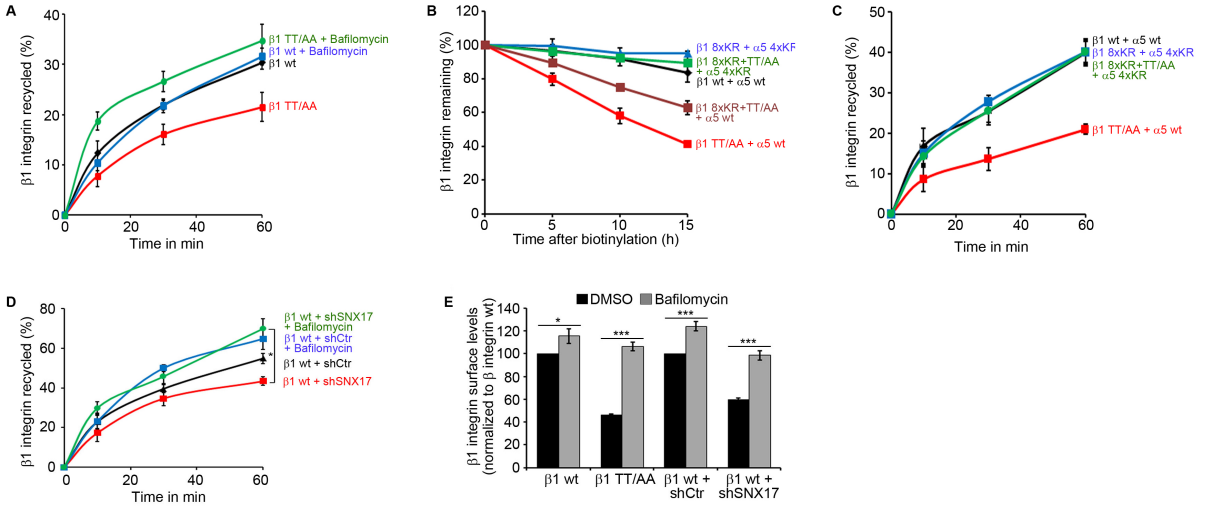


Figure 22: β1 TT/AA and SNX17 regulate lysosomal integrin degradation
(A) β1 integrin recycling is restored to wt levels in β1 TT/AA cells treated with bafilomycin. The quantity of biotinylated β1 integrin remaining within the cells was determined by capture ELISA using β1 integrin-specific antibodies (mean±SEM, n=3). (B) Quantification of the stability of cell surface β1 integrins by capture ELISA. The indicated cell lines were surface biotinylated and incubated under starving conditions (mean±SD, n=3). (C) Quantification of β1 integrin recycling determined by capture ELISA. Cells express either α5 wt and β1 wt or α5β1 with K > R-mutant tails (mean±SEM, n=4). (D) Bafilomycin normalizes the recycling of β1 wt in SNX17-depleted cells. The quantity of biotinylated β1 integrin remaining within the cells was determined by capture ELISA using β1 integrin specific antibodies (mean±SEM, n=3). (E) Quantification of β1 surface levels by FACS in indicated cell lines treated for 8 h with and without bafilomycin (mean±SD, n=3, *P=0.0133, ***P<0.0006) P values: Student's t-test, ns=not significant. ((A, C-E) C. Stremmel, (B) R. Böttcher mainly contributed)

5.6 SNX17 regulates integrin-mediated cell functions

Since kindlin and SNX17 share the same binding site, mutations within this region affect both kindlin- and SNX17-mediated cell functions. To overcome this analytical problem, we studied cell spreading and migration in SNX17-depleted cells. Reduced spreading and cell motility was found in β1 TT/AA and SNX17-depleted cells compared to β1 wt fibroblasts. Moreover, *in vitro* wound healing assays revealed a reduced cell migration velocity in these cells. Parallel analyses of β1-null fibroblasts showed almost no migration in these cells (Figure

23A-F). In summary, we show that SNX17 exhibits important functional tasks and is a key modulator of multiple integrin-mediated processes.

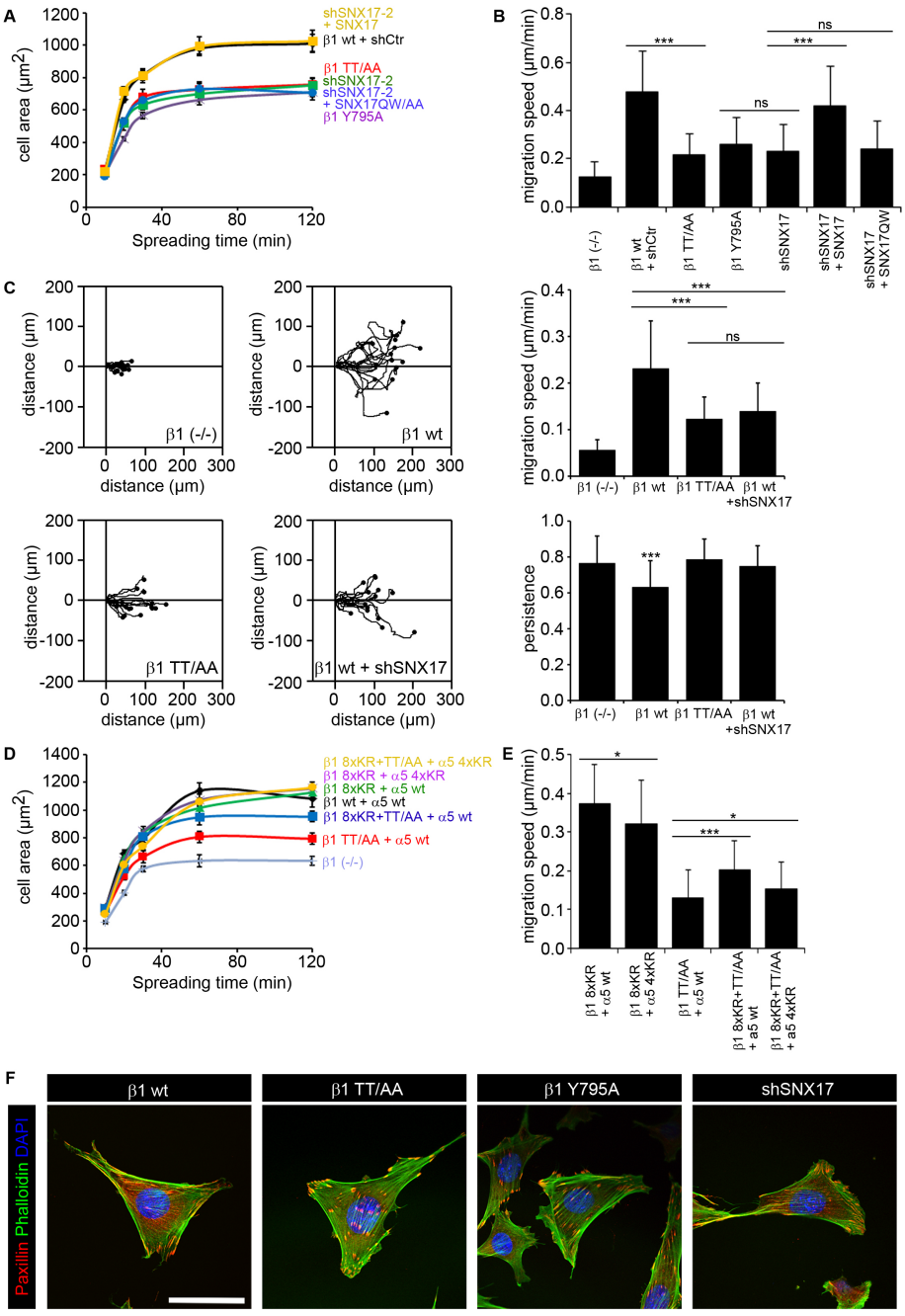


Figure 23: SNX17 is required for β1 integrin function
(A) Quantification of the spreading area of the indicated cell lines (mean±SEM of two independent experiments, n=40 cells). (B) Quantification of single-cell migration velocity of the indicated cell lines extracted from time-lapse microscopy recordings by single-cell tracking (mean±SD of three independent experiments, n=82 cells, ***P < 0.0001). (C) Migration analysis of β1 wt, β1 (-/-), β1 TT/AA and SNX17-depleted cells in a scratch assay by time-lapse video microscopy. The movement of individual cells into the wound was followed using cell-tracking software and representative trajectories are shown (left panels). The speed (distance migrated per minute, upper right) and persistence of migration of cells (lower right) were quantified from the track plots (means±SD of 52 cells analyzed in six individual wounds of two independent experiments, ***P<0.0001). (D) Quantification of the spreading area of the indicated cell lines (mean±SEM of two independent experiments, n=40 cells). (E) Quantification of single-cell migration velocity of the indicated cell lines extracted from time-lapse microscopy recordings by single-cell tracking (mean±SD of three independent experiments, n=60 cells, *P < 0.0325, ***P < 0.0001). (F) β1 wt, β1 TT/AA, β1 Y795A and SNX17-depleted β1 wt cells were stained with an antibody against paxillin (red) and fluorescently labeled phalloidin to visualize F-actin (green). Nuclei were counterstained with DAPI (blue). Scale bar 50 μm. P values: Mann-Whitney U-test, ns=not significant. ((A, D, F) C. Stremmel, (B, C, E) R. Böttcher mainly contributed)

6 Discussion

Integrin functions critically depend on receptor affinity regulation for ligands and efficient trafficking through endosomal compartments. In this thesis, we identified the new cytoplasmic β1 integrin adaptor protein SNX17, which binds integrins in the kindlin-binding region in the endosomal compartment and thus prevents lysosomal degradation. For the first time, we demonstrated a coupling of affinity regulation by kindlin and of integrin turnover by SNX17.

We showed that both the β1 TT/AA and the β1 Y795A mutation in the kindlin-binding site lead to early embryonic lethality – phenotypically similar as described for kindlin-2 knockout mice (Montanez et al. 2008). In ES cells, diverse fibroblast cell lines and keratinocytes (Stremmel and Fässler, unpublished) with β1 TT/AA or β1 Y795A mutations, we detected reduced integrin activity and surface expression despite of elevated mRNA levels. Therefore, the kindlin-binding site serves as a determining factor with respect to integrin stability. We identified excessive lysosomal integrin degradation as causative for the reduced surface expression leading to a secondary reduction in integrin recycling back to the plasma membrane. In search of a mechanistic explanation for the reduced β1 TT/AA surface expression, we identified a control machinery in which the threonines 788/789 and the distal NxxY motif in the cytoplasmic β1 integrin tail regulate integrin affinity at the plasma membrane and endosomal integrin trafficking back to the cell surface. Kindlin binds these motifs with its F3-FERM-subdomain and the plasma membrane with its PH-domain to increase integrin receptor affinity and to establish and modulate links to the actin cytoskeleton (Meves et al. 2009; Liu et al. 2011). Upon internalization, kindlin leaves the cytoplasmic integrin tail and enables early endosomal SNX17-binding to inhibit lysosomal β1 integrin

degradation. This special sequence couples affinity regulation by kindlin with SNX17 as a mediator of integrin degradation via the same binding site and is a potential major mechanism of integrin quality control. Therefore, integrins with defects in their distal cytoplasmic domain, which fail to bind kindlin and SNX17 are subsequently impaired in integrin activation and lysosomally degraded.

SNX17 is a member of the large family of sorting nexin proteins, which are characterized by a PX-domain. This domain is specialized on phosphoinositide-binding, especially PtdIns3P, which is typically found in endosomal membranes (Cullen 2008). Besides its N-terminal PX-domain, SNX17 has a C-terminal FERM-domain binding to NxxY motifs as they are found in the cytoplasmic integrin tail (Ghai et al. 2011). Due to these unique features, it is not surprising that SNX17 has already been described to be involved in transmembrane receptor trafficking such as LDLR (Stockinger et al. 2002; Burden et al. 2004), LRP1 (van Kerkhof et al. 2005; Donoso et al. 2009), P-selectin (Williams et al. 2004; Knauth et al. 2005) and APP (Lee et al. 2008). However, it remains unclear if SNX17 is primarily responsible for recycling back to the plasma membrane or inhibits lysosomal degradation. In this thesis, we show that lysosomal inhibition rescues the recycling defect of β1 TT/AA integrins, providing clear evidence of primary degradation defects, which consequently cause reduced recycling of integrins back to the cell surface. How SNX17 regulates degradation on a molecular basis remains unclear and needs to be addressed in future studies. One possibility is that SNX17 prevents that integrins enter inner vesicles of multivesicular bodies – an essential step in lysosomal degradation. This could work via the recruitment of a deubiquitinase that removes ubiquitin-residues form integrins and thus prevents access of the ESCRT-machinery (Raiborg and Stenmark 2009).

The early lethality of β1 TT/AA and β1 Y795A mice results from a combination of defects including impaired integrin activation, abnormal actin dynamics and reduced integrin recycling. Moreover, adhesion, spreading and migration defects of SNX17-depleted fibroblasts indicate dramatic abnormalities in integrin function resulting from deregulated integrin degradation. Additionally, reduced β1 TT/AA integrin surface expression may also affect other cellular processes such as expression and signaling of growth factor receptors or co-internalization and resecretion of fibronectin and possibly also other ECM proteins (Caswell et al. 2008; Sung et al. 2011).

One cycle of integrin trafficking, from internalization to recycling back to the cell surface, takes almost one hour in our experiments. This seems rather long compared to fast cellular processes such as adhesion, spreading and migration which require integrin-dependent changes within seconds to minutes. This goes in line with previous studies suggesting integrin trafficking as a means of local restructuring (Caswell et al. 2009), which might possibly be involved in integrin quality control via SNX17. Going one step further, one could suggest that integrins with defects in their kindlin-binding site are not only prone to SNX17-mediated degradation but they also liberate kindlin molecules. These could translocate to the nuclear region to bind promoter regions and unregulated β1 mRNA levels in order to replace degraded integrin heterodimers. We demonstrated for the first time that kindlin is in principle capable of increasing mRNA levels. Additionally, previous studies identified a nuclear localization signal (NLS) in the kindlin-2 protein and kindlin-2 was shown to be present in the nucleus in immunofluorescence experiments (Meves et al. 2009).

In this study, we provide fundamental insight into integrin trafficking and especially integrin degradation. This knowledge is not only of interest for basic research but has also got a broad

medical impact, since integrin-mediated physiological and pathological processes play a central role in every cell. Especially cancer, infections, thrombosis and autoimmune disorders are tightly associated with integrin functions. So far pharmacological inhibitors have been approved for three integrins. Recent advances in our understanding of integrins and downstream signaling events have created a vast potential for new therapeutic strategies.

For example, cancer is linked to integrin functions in multiple ways. Integrins mediate cell growth by ECM interactions with continuous bidirectional signaling events, which modulate proliferation and differentiation. This signaling cascade is further affected by integrin-growth factor interactions. Detachment of cancer cells, migration through the tissue, blood vessel invasion and metastases formation do all critically depend on integrins. Therefore, it is obvious that a fundamental knowledge about integrins is absolutely essential in cancer treatment and the development of new therapeutic strategies.

At the same time, this publication, along with many others, illustrates that integrins are essential for every cell and that they are integrated into a highly complex network. Changes in this system have severe, partially unpredictable, consequences, which make integrin-related therapeutic interventions very challenging. Moreover, the redundancy among integrins, low dose agonist-like activity of integrin inhibitors (Cox et al. 2000; Reynolds et al. 2009; Weis et al. 2009), as well as the importance of integrins in key physiological systems, make therapeutic interventions rather difficult. It will be challenging to unravel how individual heterodimers signal their diverse functions and how the same integrin can exert environment-specific tasks. This knowledge will allow us to establish integrin-targeted therapeutic strategies to modulate processes in which integrins have been heavily implicated such as cancer formation, inflammation and thrombosis (Lowell and Mayadas 2012).

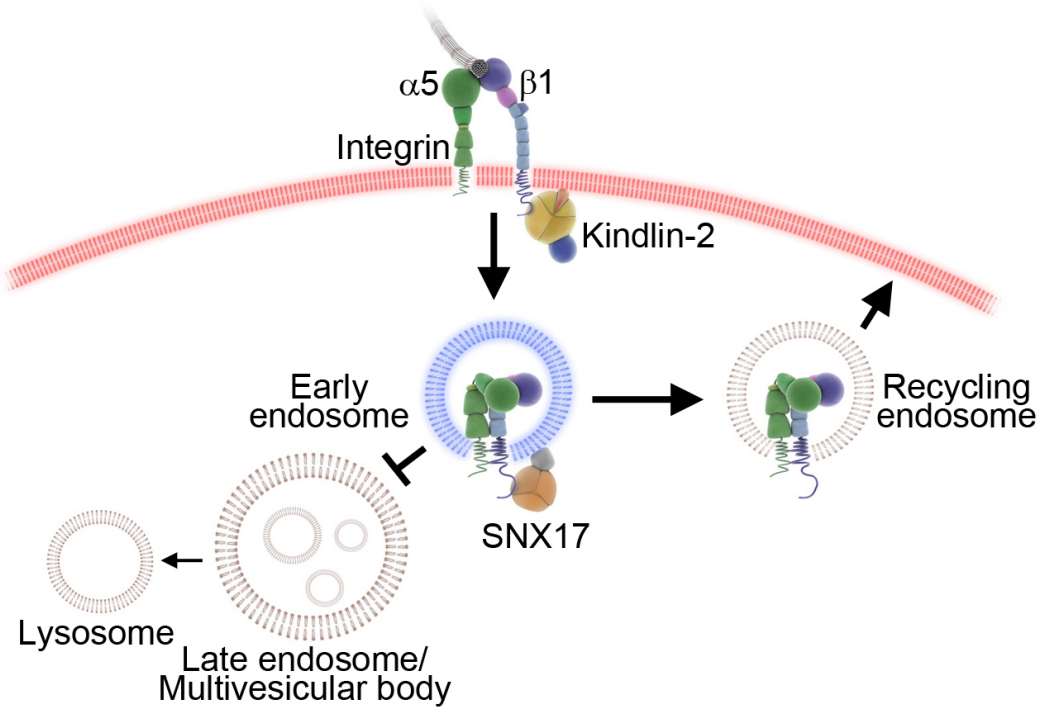
7 Summary

Integrins are ubiquitously expressed adhesion receptors with important functions in cellular adhesion, proliferation, migration and signaling. These functions are determined by integrin trafficking through endosomal compartments and receptor affinity regulation. In this thesis, we identified the distal NxxY motif of the β1 integrin cytoplasmic tail as a molecular switch modulating a spatiotemporally controlled binding of two FERM-domain proteins in different cellular compartments. Kindlins mediate integrin activation at the plasma membrane and they dislodge upon internalization. In the endosomal compartment, the free cytoplasmic domain is subsequently bound by sorting nexin 17 (SNX17) to inhibit integrin degradation. We identified SNX17 as a new β1 integrin adaptor protein, which uses the kindlin-binding site in endosomal compartments to stabilize integrins and to promote their recycling back to the plasma membrane.

8 Zusammenfassung

Integrine sind ubiquitär exprimierte Adhäsionsmoleküle mit entscheidender Bedeutung für die zelluläre Adhäsion, Wachstum, Migration und Signalgebung. Diese Funktionen werden durch konstantes Integrintrafficking durch endosomale Kompartimente sowie durch Modulation der Rezeptoraffinität kontrolliert. Im Rahmen der vorliegenden Dissertation wurde das distale NxxY-Motiv im zytoplasmatischen β1 Integrinschwanz als molekularer Schalter identifiziert, der die Bindung zweier FERM-Domänen-Proteine in unterschiedlichen zellulären Kompartimenten vermittelt. Während Kindline an der Zellmembran die Integrinaktivität regulieren, dislozieren sie nach der Internalisierung und Sorting Nexin 17 (SNX17) wird an den nun freien β1 Integrinschwanz rekrutiert, um die Integrindegradation zu hemmen. Wir haben SNX17 als neuen β1 Integrin-Bindungspartner identifiziert, der die Kindlin-Bindungsstelle im endosomalen Kompartiment verwendet, um Integrine zu stabilisieren und ihr Recycling zurück an die Zelloberfläche zu fördern.

9 Graphical summary



Graphical summary
Kindlin binds the β1 integrin subunit with its FERM-domain to regulate integrin receptor affinity. Upon integrin internalization it dislodges and SNX17 is recruited to the free cytoplasmic tail. This endosomal binding prevents lysosomal integrin degradation and promotes integrin recycling back to the plasma membrane. Specific lipid membrane compositions promote the spatial regulation of FERM-domain protein recruitment via additional motifs. The kindlin PH-domain is recruited to the PIP2/PIP3 enriched plasma membrane (red), whereas the SNX17 PX-domain preferably binds PtdIns3P in endosomal membranes (blue).

Grafische Zusammenfassung
Kindlin bindet die β1 Integrin-Untereinheit an der Plasmamembran und reguliert so die Rezeptoraffinität. Nach der Internalisierung verlässt Kindlin den zytoplasmatischen Integrinschwanz und SNX17 wird rekrutiert. Diese endosomale Bindung verhindert den lysosomalen Integrinabbau und fördert das Integrin-Recycling zurück an die Plasmamembran. Spezifische Zusammensetzungen der Lipidmembranen fördern die lokale Bindungsregulation der FERM-Domänen-Proteine über zusätzliche Motive. Die Kindlin PH-Domäne wird vorzugsweise an die PIP2/PIP3-haltige Plasmamembran (rot) rekrutiert, während die SNX17 PX-Domäne vorzugsweise PtdIns3P in endosomalen Membranen (blau) bindet.

10 References

Abram, C. L. and C. A. Lowell (2009). "The ins and outs of leukocyte integrin signaling." Annu Rev Immunol **27**(19302044): 339-362.

Aluwihare, P. and J. S. Munger (2008). "What the lung has taught us about latent TGF-beta activation." Am J Respir Cell Mol Biol **39**(18927350): 499-502.

Arnaout, M. A., B. Mahalingam, et al. (2005). "Integrin structure, allostery, and bidirectional signaling." Annu Rev Cell Dev Biol **21**: 381-410.

Askari, J. A., P. A. Buckley, et al. (2009). "Linking integrin conformation to function." J Cell Sci **122**(Pt 2): 165-170.

Astrof, S. and R. O. Hynes (2009). "Fibronectins in vascular morphogenesis." Angiogenesis **12**(2): 165-175.

Axelsson, R., T. Bach-Gansmo, et al. (2010). "An open-label, multicenter, phase 2a study to assess the feasibility of imaging metastases in late-stage cancer patients with the alpha v beta 3-selective angiogenesis imaging agent 99mTc-NC100692." Acta Radiol **51**(1): 40-46.

Azimifar, S. B., R. T. Bottcher, et al. (2012). "Induction of membrane circular dorsal ruffles requires co-signalling of integrin-ILK-complex and EGF receptor." J Cell Sci **125**(Pt 2): 435-448.

Bader, B. L., H. Rayburn, et al. (1998). "Extensive vasculogenesis, angiogenesis, and organogenesis precede lethality in mice lacking all alpha v integrins." Cell **95**(9827803): 507-519.

Balasubramanian, N., D. W. Scott, et al. (2007). "Arf6 and microtubules in adhesion-dependent trafficking of lipid rafts." Nat Cell Biol **9**(12): 1381-1391.

Bates, R. C., D. I. Bellovin, et al. (2005). "Transcriptional activation of integrin beta6 during the epithelial-mesenchymal transition defines a novel prognostic indicator of aggressive colon carcinoma." J Clin Invest **115**(2): 339-347.

Bazigou, E., S. Xie, et al. (2009). "Integrin-alpha9 is required for fibronectin matrix assembly during lymphatic valve morphogenesis." Dev Cell **17**(19686679): 175-186.

Bengtsson, T., A. Aszodi, et al. (2005). "Loss of alpha10beta1 integrin expression leads to moderate dysfunction of growth plate chondrocytes." J Cell Sci **118**(15713743): 929-936.

Bennett, J. S. (2005). "Structure and function of the platelet integrin alphaIIbbeta3." J Clin Invest **115**(12): 3363-3369.

Benson, D. L., L. M. Schnapp, et al. (2000). "Making memories stick: cell-adhesion molecules in synaptic plasticity." Trends Cell Biol **10**(11050419): 473-482.

Berlin, C., R. F. Bargatze, et al. (1995). "alpha 4 integrins mediate lymphocyte attachment and rolling under physiologic flow." Cell **80**(7532110): 413-422.

Berton, G., S. R. Yan, et al. (1996). "Neutrophil activation by adhesion: mechanisms and pathophysiological implications." Int J Clin Lab Res **26**(8905448): 160-177.

Betts, G. N., P. van der Geer, et al. (2008). "Structural and functional consequences of tyrosine phosphorylation in the LRP1 cytoplasmic domain." J Biol Chem **283**(23): 15656-15664.

Betz, U. A., C. A. Vosshenrich, et al. (1996). "Bypass of lethality with mosaic mice generated by Cre-loxP-mediated recombination." Curr Biol **6**(10): 1307-1316.

Bonaca, M. P., P. G. Steg, et al. (2009). "Antithrombotics in acute coronary syndromes." J Am Coll Cardiol **54**(19729112): 969-984.

Bottcher, R. T., C. Stremmel, et al. (2012). "Sorting nexin 17 prevents lysosomal degradation of beta1 integrins by binding to the beta1-integrin tail." Nat Cell Biol **14**(6): 584-592.

Bouaouina, M., Y. Lad, et al. (2008). "The N-terminal domains of talin cooperate with the phosphotyrosine binding-like domain to activate beta1 and beta3 integrins." J Biol Chem **283**(10): 6118-6125.

Brakebusch, C., R. Grose, et al. (2000). "Skin and hair follicle integrity is crucially dependent on beta 1 integrin expression on keratinocytes." EMBO J **19**(10921880): 3990-4003.

Brandenberger, R., A. Schmidt, et al. (2001). "Identification and characterization of a novel extracellular matrix protein nephronectin that is associated with integrin alpha8beta1 in the embryonic kidney." J Cell Biol **154**(11470831): 447-458.

Bretscher, M. S. (1996). "Moving membrane up to the front of migrating cells." Cell **85**(4): 465-467.

Brooks, P. C., R. A. Clark, et al. (1994). "Requirement of vascular integrin alpha v beta 3 for angiogenesis." Science **264**(5158): 569-571.

Brooks, P. C., A. M. Montgomery, et al. (1994). "Integrin alpha v beta 3 antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels." Cell **79**(7): 1157-1164.

Brooks, P. C., S. Stromblad, et al. (1996). "Localization of matrix metalloproteinase MMP-2 to the surface of invasive cells by interaction with integrin alpha v beta 3." Cell **85**(5): 683-693.

Burden, J. J., X. M. Sun, et al. (2004). "Sorting motifs in the intracellular domain of the low density lipoprotein receptor interact with a novel domain of sorting nexin-17." J Biol Chem **279**(16): 16237-16245.

Calderwood, D. A., Y. Fujioka, et al. (2003). "Integrin beta cytoplasmic domain interactions with phosphotyrosine-binding domains: a structural prototype for diversity in integrin signaling." Proc Natl Acad Sci U S A **100**(5): 2272-2277.

Calderwood, D. A., B. Yan, et al. (2002). "The phosphotyrosine binding-like domain of talin activates integrins." J Biol Chem **277**(24): 21749-21758.

Campbell, K. P. (1995). "Three muscular dystrophies: loss of cytoskeleton-extracellular matrix linkage." Cell **80**(5): 675-679.

Carlson, T. R., H. Hu, et al. (2008). "Cell-autonomous requirement for beta1 integrin in endothelial cell adhesion, migration and survival during angiogenesis in mice." Development **135**(12): 2193-2202.

Carlton, J. G. and P. J. Cullen (2005). "Coincidence detection in phosphoinositide signaling." Trends Cell Biol **15**(10): 540-547.

Carter, A. (2010). "Integrins as target: first phase III trial launches, but questions remain." J Natl Cancer Inst **102**(10): 675-677.

Caswell, P. and J. Norman (2008). "Endocytic transport of integrins during cell migration and invasion." Trends Cell Biol **18**(6): 257-263.

Caswell, P. T., M. Chan, et al. (2008). "Rab-coupling protein coordinates recycling of alpha5beta1 integrin and EGFR1 to promote cell migration in 3D microenvironments." J Cell Biol **183**(1): 143-155.

Caswell, P. T. and J. C. Norman (2006). "Integrin trafficking and the control of cell migration." Traffic **7**(1): 14-21.

Caswell, P. T., H. J. Spence, et al. (2007). "Rab25 associates with alpha5beta1 integrin to promote invasive migration in 3D microenvironments." Dev Cell **13**(4): 496-510.

Caswell, P. T., S. Vadrevu, et al. (2009). "Integrins: masters and slaves of endocytic transport." Nat Rev Mol Cell Biol **10**(12): 843-853.

Chao, W. T. and J. Kunz (2009). "Focal adhesion disassembly requires clathrin-dependent endocytosis of integrins." FEBS Lett **583**(8): 1337-1343.

Chen, H., Z. Zou, et al. (2006). "In vivo beta1 integrin function requires phosphorylation-independent regulation by cytoplasmic tyrosines." Genes Dev **20**(8): 927-932.

Chen, J., T. G. Diacovo, et al. (2002). "The alpha(2) integrin subunit-deficient mouse: a multifaceted phenotype including defects of branching morphogenesis and hemostasis." Am J Pathol **161**(12107118): 337-344.

Chew, D. P., D. L. Bhatt, et al. (2001). "Increased mortality with oral platelet glycoprotein IIb/IIIa antagonists: a meta-analysis of phase III multicenter randomized trials." Circulation **103**(11208677): 201-206.

Chia, W. J. and B. L. Tang (2009). "Emerging roles for Rab family GTPases in human cancer." Biochim Biophys Acta **1795**(2): 110-116.

Choi, C. K., M. Vicente-Manzanares, et al. (2008). "Actin and alpha-actinin orchestrate the assembly and maturation of nascent adhesions in a myosin II motor-independent manner." Nat Cell Biol **10**(9): 1039-1050.

Chudakova, D. A., Y. H. Zeidan, et al. (2008). "Integrin-associated Lyn kinase promotes cell survival by suppressing acid sphingomyelinase activity." J Biol Chem **283**(18682390): 28806-28816.

Coller, B. S. and S. J. Shattil (2008). "The GPIIb/IIIa (integrin alphaIIbbeta3) odyssey: a technology-driven saga of a receptor with twists, turns, and even a bend." Blood **112**(18840725): 3011-3025.

Cosgrove, D., K. Rodgers, et al. (2000). "Integrin alpha1beta1 and transforming growth factor-beta1 play distinct roles in alport glomerular pathogenesis and serve as dual targets for metabolic therapy." Am J Pathol **157**(11073824): 1649-1659.

Costantini, F. and R. Shakya (2006). "GDNF/Ret signaling and the development of the kidney." Bioessays **28**(16435290): 117-127.

Cox, D., M. Brennan, et al. (2010). "Integrins as therapeutic targets: lessons and opportunities." Nat Rev Drug Discov **9**(10): 804-820.

Cox, D., R. Smith, et al. (2000). "Evidence of platelet activation during treatment with a GPIIb/IIIa antagonist in patients presenting with acute coronary syndromes." J Am Coll Cardiol **36**(11079651): 1514-1519.

Critchley, D. R. and A. R. Gingras (2008). "Talin at a glance." J Cell Sci **121**(Pt 9): 1345-1347.

Cullen, P. J. (2008). "Endosomal sorting and signalling: an emerging role for sorting nexins." Nat Rev Mol Cell Biol **9**(7): 574-582.

Czubayko, M., P. Knauth, et al. (2006). "Sorting nexin 17, a non-self-assembling and a PtdIns(3)P high class affinity protein, interacts with the cerebral cavernous malformation related protein KRIT1." Biochem Biophys Res Commun **345**(3): 1264-1272.

Czuchra, A., H. Meyer, et al. (2006). "Genetic analysis of beta1 integrin "activation motifs" in mice." J Cell Biol **174**(6): 889-899.

De, S., O. Razorenova, et al. (2005). "VEGF-integrin interplay controls tumor growth and vascularization." Proc Natl Acad Sci U S A **102**(15897451): 7589-7594.

del Pozo, M. A., N. Balasubramanian, et al. (2005). "Phospho-caveolin-1 mediates integrin-regulated membrane domain internalization." Nat Cell Biol **7**(9): 901-908.

Delbaldo, C., E. Raymond, et al. (2008). "Phase I and pharmacokinetic study of etaracizumab (Abegrin), a humanized monoclonal antibody against alphavbeta3 integrin receptor, in patients with advanced solid tumors." Invest New Drugs **26**(1): 35-43.

Denda, S. and L. F. Reichardt (2007). "Studies on integrins in the nervous system." Methods Enzymol **426**: 203-221.

DiPersio, C. M., K. M. Hodivala-Dilke, et al. (1997). "alpha3beta1 Integrin is required for normal development of the epidermal basement membrane." J Cell Biol **137**(9151677): 729-742.

DiPersio, C. M., R. van der Neut, et al. (2000). "alpha3beta1 and alpha6beta4 integrin receptors for laminin-5 are not essential for epidermal morphogenesis and homeostasis during skin development." J Cell Sci **113 (Pt 17)**: 3051-3062.

Doherty, G. J. and H. T. McMahon (2009). "Mechanisms of endocytosis." Annu Rev Biochem **78**: 857-902.

Donoso, M., J. Cancino, et al. (2009). "Polarized traffic of LRP1 involves AP1B and SNX17 operating on Y-dependent sorting motifs in different pathways." Mol Biol Cell **20**(1): 481-497.

Etzioni, A. (2010). "Defects in the leukocyte adhesion cascade." Clin Rev Allergy Immunol **38**(19437145): 54-60.

Evans, R., I. Patzak, et al. (2009). "Integrins in immunity." J Cell Sci **122**(19118214): 215-225.

Faccio, R., S. L. Teitelbaum, et al. (2005). "Vav3 regulates osteoclast function and bone mass." Nat Med **11**(15711558): 284-290.

Fassler, R. and M. Meyer (1995). "Consequences of lack of beta 1 integrin gene expression in mice." Genes Dev **9**(15): 1896-1908.

Felding-Habermann, B., T. E. O'Toole, et al. (2001). "Integrin activation controls metastasis in human breast cancer." Proc Natl Acad Sci U S A **98**(4): 1853-1858.

Feltri, M. L., D. Graus Porta, et al. (2002). "Conditional disruption of beta 1 integrin in Schwann cells impedes interactions with axons." J Cell Biol **156**(11777940): 199-209.

Fitzgerald, J. R., T. J. Foster, et al. (2006). "The interaction of bacterial pathogens with platelets." Nat Rev Microbiol **4**(16710325): 445-457.

Fjellbirkeland, L., S. Cambier, et al. (2003). "Integrin alphavbeta8-mediated activation of transforming growth factor-beta inhibits human airway epithelial proliferation in intact bronchial tissue." Am J Pathol **163**(12875973): 533-542.

Florian, V., T. Schluter, et al. (2001). "A new member of the sorting nexin family interacts with the C-terminus of P-selectin." Biochem Biophys Res Commun **281**(4): 1045-1050.

Frampton, J. E. and G. L. Plosker (2009). "Efalizumab: a review of its use in the management of chronic moderate-to-severe plaque psoriasis." Am J Clin Dermatol **10**(1): 51-72.

Frisch, S. M. and R. A. Screaton (2001). "Anoikis mechanisms." Curr Opin Cell Biol **13**(5): 555-562.

Galvez, B. G., S. Matias-Roman, et al. (2004). "Caveolae are a novel pathway for membrane-type 1 matrix metalloproteinase traffic in human endothelial cells." Mol Biol Cell **15**(2): 678-687.

Garcia-Alvarez, B., J. M. de Pereda, et al. (2003). "Structural determinants of integrin recognition by talin." Mol Cell **11**(1): 49-58.

Geiger, B. and A. Bershadsky (2001). "Assembly and mechanosensory function of focal contacts." Curr Opin Cell Biol **13**(5): 584-592.

George, J. N., J. P. Caen, et al. (1990). "Glanzmann's thrombasthenia: the spectrum of clinical disease." Blood **75**(7): 1383-1395.

Ghai, R., M. Mobli, et al. (2011). "Phox homology band 4.1/ezrin/radixin/moesin-like proteins function as molecular scaffolds that interact with cargo receptors and Ras GTPases." Proc Natl Acad Sci U S A **108**(19): 7763-7768.

Goksoy, E., Y. Q. Ma, et al. (2008). "Structural basis for the autoinhibition of talin in regulating integrin activation." Mol Cell **31**(1): 124-133.

Gottschalk, K. E. (2005). "A coiled-coil structure of the alphaIIbbeta3 integrin transmembrane and cytoplasmic domains in its resting state." Structure **13**(5): 703-712.

Goult, B. T., M. Bouaouina, et al. (2009). "The structure of the N-terminus of kindlin-1: a domain important for alphaiibbeta3 integrin activation." J Mol Biol **394**(5): 944-956.

Grant, B. D. and J. G. Donaldson (2009). "Pathways and mechanisms of endocytic recycling." Nat Rev Mol Cell Biol **10**(9): 597-608.

Graus-Porta, D., S. Blaess, et al. (2001). "Beta1-class integrins regulate the development of laminae and folia in the cerebral and cerebellar cortex." Neuron **31**(11516395): 367-379.

Grazioli, A., C. S. Alves, et al. (2006). "Defective blood vessel development and pericyte/pvSMC distribution in alpha 4 integrin-deficient mouse embryos." Dev Biol **293**(16529735): 165-177.

Grose, R., C. Hutter, et al. (2002). "A crucial role of beta 1 integrins for keratinocyte migration in vitro and during cutaneous wound repair." Development **129**(11959837): 2303-2315.

Harburger, D. S., M. Bouaouina, et al. (2009). "Kindlin-1 and -2 directly bind the C-terminal region of beta integrin cytoplasmic tails and exert integrin-specific activation effects." J Biol Chem **284**(17): 11485-11497.

Hayashi, Y. K., F. L. Chou, et al. (1998). "Mutations in the integrin alpha7 gene cause congenital myopathy." Nat Genet **19**(9590299): 94-97.

Heasman, S. J. and A. J. Ridley (2008). "Mammalian Rho GTPases: new insights into their functions from in vivo studies." Nat Rev Mol Cell Biol **9**(9): 690-701.

Hersey, P., J. Sosman, et al. (2010). "A randomized phase 2 study of etaracizumab, a monoclonal antibody against integrin alpha(v)beta(3), + or - dacarbazine in patients with stage IV metastatic melanoma." Cancer **116**(20108344): 1526-1534.

Hirahashi, J., D. Mekala, et al. (2006). "Mac-1 signaling via Src-family and Syk kinases results in elastase-dependent thrombohemorrhagic vasculopathy." Immunity **25**(16872848): 271-283.

Hodivala-Dilke, K. M., K. P. McHugh, et al. (1999). "Beta3-integrin-deficient mice are a model for Glanzmann thrombasthenia showing placental defects and reduced survival." J Clin Invest **103**(9916135): 229-238.

Hogg, N. and P. A. Bates (2000). "Genetic analysis of integrin function in man: LAD-1 and other syndromes." Matrix Biol **19**(10936446): 211-222.

Hopkins, C. R., A. Gibson, et al. (1994). "In migrating fibroblasts, recycling receptors are concentrated in narrow tubules in the pericentriolar area, and then routed to the plasma membrane of the leading lamella." J Cell Biol **125**(6): 1265-1274.

Horton, M. A., H. M. Massey, et al. (2003). "Upregulation of osteoclast alpha2beta1 integrin compensates for lack of alphavbeta3 vitronectin receptor in Iraqi-Jewish-type Glanzmann thrombasthenia." Br J Haematol **122**(12956766): 950-957.

Huang, X. Z., J. F. Wu, et al. (1996). "Inactivation of the integrin beta 6 subunit gene reveals a role of epithelial integrins in regulating inflammation in the lung and skin." J Cell Biol **133**(8666675): 921-928.

Huang, X. Z., J. F. Wu, et al. (2000). "Fatal bilateral chylothorax in mice lacking the integrin alpha9beta1." Mol Cell Biol **20**(10866676): 5208-5215.

Hughes, P. E., F. Diaz-Gonzalez, et al. (1996). "Breaking the integrin hinge. A defined structural constraint regulates integrin signaling." J Biol Chem **271**(12): 6571-6574.

Humphries, J. D., A. Byron, et al. (2006). "Integrin ligands at a glance." J Cell Sci **119**(Pt 19): 3901-3903.

Humphries, J. D. and M. J. Humphries (2007). "CD14 is a ligand for the integrin alpha4beta1." FEBS Lett **581**(4): 757-763.

Huveneers, S. and E. H. Danen (2009). "Adhesion signaling - crosstalk between integrins, Src and Rho." J Cell Sci **122**(Pt 8): 1059-1069.

Hynes, R. O. (2002). "Integrins: bidirectional, allosteric signaling machines." Cell **110**(6): 673-687.

Hynes, R. O. (2007). "Cell-matrix adhesion in vascular development." J Thromb Haemost **5 Suppl 1**: 32-40.

Imai, Y., E. J. Park, et al. (2008). "Genetic perturbation of the putative cytoplasmic membrane-proximal salt bridge aberrantly activates alpha(4) integrins." Blood **112**(13): 5007-5015.

Jaffe, A. B. and A. Hall (2005). "Rho GTPases: biochemistry and biology." Annu Rev Cell Dev Biol **21**: 247-269.

Jobard, F., B. Bouadjar, et al. (2003). "Identification of mutations in a new gene encoding a FERM family protein with a pleckstrin homology domain in Kindler syndrome." Hum Mol Genet **12**(8): 925-935.

Jones, M. C., P. T. Caswell, et al. (2006). "Endocytic recycling pathways: emerging regulators of cell migration." Curr Opin Cell Biol **18**(5): 549-557.

Kagami, S. and S. Kondo (2004). "Beta1-integrins and glomerular injury." J Med Invest **51**(15000250): 1-13.

Kanasaki, K., Y. Kanda, et al. (2008). "Integrin beta1-mediated matrix assembly and signaling are critical for the normal development and function of the kidney glomerulus." Dev Biol **313**(18082680): 584-593.

Kenny, L. M., R. C. Coombes, et al. (2008). "Phase I trial of the positron-emitting Arg-Gly-Asp (RGD) peptide radioligand 18F-AH111585 in breast cancer patients." J Nucl Med **49**(6): 879-886.

Kern, J. S., C. Herz, et al. (2007). "Chronic colitis due to an epithelial barrier defect: the role of kindlin-1 isoforms." J Pathol **213**(4): 462-470.

Kim, C., T. L. Lau, et al. (2009). "Interactions of platelet integrin alphaIIb and beta3 transmembrane domains in mammalian cell membranes and their role in integrin activation." Blood **113**(19): 4747-4753.

Kim, S., K. Bell, et al. (2000). "Regulation of angiogenesis in vivo by ligation of integrin alpha5beta1 with the central cell-binding domain of fibronectin." Am J Pathol **156**(10751360): 1345-1362.

Kim, S., M. Harris, et al. (2000). "Regulation of integrin alpha vbeta 3-mediated endothelial cell migration and angiogenesis by integrin alpha5beta1 and protein kinase A." J Biol Chem **275**(10944524): 33920-33928.

Kloeker, S., M. B. Major, et al. (2004). "The Kindler syndrome protein is regulated by transforming growth factor-beta and involved in integrin-mediated adhesion." J Biol Chem **279**(8): 6824-6833.

Knauth, P., T. Schluter, et al. (2005). "Functions of sorting nexin 17 domains and recognition motif for P-selectin trafficking." J Mol Biol **347**(4): 813-825.

Knezevic, I., T. M. Leisner, et al. (1996). "Direct binding of the platelet integrin alphaIIbbeta3 (GPIIb-IIIa) to talin. Evidence that interaction is mediated through the cytoplasmic domains of both alphaIIb and beta3." J Biol Chem **271**(27): 16416-16421.

Koth, L. L., B. Alex, et al. (2007). "Integrin beta6 mediates phospholipid and collectin homeostasis by activation of latent TGF-beta1." Am J Respir Cell Mol Biol **37**(17641300): 651-659.

Kreidberg, J. A., M. J. Donovan, et al. (1996). "Alpha 3 beta 1 integrin has a crucial role in kidney and lung organogenesis." Development **122**(8951069): 3537-3547.

Kuijpers, T. W., E. van de Vijver, et al. (2009). "LAD-1/variant syndrome is caused by mutations in FERMT3." Blood **113**(19): 4740-4746.

Kwee, L., H. S. Baldwin, et al. (1995). "Defective development of the embryonic and extraembryonic circulatory systems in vascular cell adhesion molecule (VCAM-1) deficient mice." Development **121**(7539357): 489-503.

Lammermann, T., B. L. Bader, et al. (2008). "Rapid leukocyte migration by integrin-independent flowing and squeezing." Nature **453**(18451854): 51-55.

Lau, T. L., C. Kim, et al. (2009). "The structure of the integrin alphaIIbbeta3 transmembrane complex explains integrin transmembrane signalling." EMBO J **28**(9): 1351-1361.

Laukaitis, C. M., D. J. Webb, et al. (2001). "Differential dynamics of alpha 5 integrin, paxillin, and alpha-actinin during formation and disassembly of adhesions in migrating cells." J Cell Biol **153**(7): 1427-1440.

Laurens, N., M. A. Engelse, et al. (2009). "Single and combined effects of alphavbeta3- and alpha5beta1-integrins on capillary tube formation in a human fibrinous matrix." Angiogenesis **12**(19449108): 275-285.

Law, D. A., F. R. DeGuzman, et al. (1999). "Integrin cytoplasmic tyrosine motif is required for outside-in alphaIIbbeta3 signalling and platelet function." Nature **401**(6755): 808-811.

Lee, J., C. Retamal, et al. (2008). "Adaptor protein sorting nexin 17 regulates amyloid precursor protein trafficking and processing in the early endosomes." J Biol Chem **283**(17): 11501-11508.

Lefkovits, J., E. F. Plow, et al. (1995). "Platelet glycoprotein IIb/IIIa receptors in cardiovascular medicine." N Engl J Med **332**(23): 1553-1559.

Legate, K. R., S. A. Wickstrom, et al. (2009). "Genetic and cell biological analysis of integrin outside-in signaling." Genes Dev **23**(4): 397-418.

Ley, K., C. Laudanna, et al. (2007). "Getting to the site of inflammation: the leukocyte adhesion cascade updated." Nat Rev Immunol **7**(17717539): 678-689.

Li, M. Z. and S. J. Elledge (2007). "Harnessing homologous recombination in vitro to generate recombinant DNA via SLIC." Nat Methods **4**(3): 251-256.

Li, W., D. G. Metcalf, et al. (2005). "A push-pull mechanism for regulating integrin function." Proc Natl Acad Sci U S A **102**(5): 1424-1429.

Lim, J., A. Wiedemann, et al. (2007). "An essential role for talin during alpha(M)beta(2)-mediated phagocytosis." Mol Biol Cell **18**(3): 976-985.

Linder, S. (2009). "Invadosomes at a glance." J Cell Sci **122**(Pt 17): 3009-3013.

Linton, J. M., G. R. Martin, et al. (2007). "The ECM protein nephronectin promotes kidney development via integrin alpha8beta1-mediated stimulation of Gdnf expression." Development **134**(17537792): 2501-2509.

Liu, J., K. Fukuda, et al. (2011). "Structural basis of phosphoinositide binding to kindlin-2 protein pleckstrin homology domain in regulating integrin activation." J Biol Chem **286**(50): 43334-43342.

Liu, Y., N. Chattopadhyay, et al. (2009). "Coordinate integrin and c-Met signaling regulate Wnt gene expression during epithelial morphogenesis." Development **136**(19176588): 843-853.

Lobert, V. H., A. Brech, et al. (2010). "Ubiquitination of alpha 5 beta 1 integrin controls fibroblast migration through lysosomal degradation of fibronectin-integrin complexes." Dev Cell **19**(1): 148-159.

Lowell, C. A. and T. N. Mayadas (2012). "Overview: studying integrins in vivo." Methods Mol Biol **757**: 369-397.

Lu, C., J. Takagi, et al. (2001). "Association of the membrane proximal regions of the alpha and beta subunit cytoplasmic domains constrains an integrin in the inactive state." J Biol Chem **276**(18): 14642-14648.

Lu, C. F. and T. A. Springer (1997). "The alpha subunit cytoplasmic domain regulates the assembly and adhesiveness of integrin lymphocyte function-associated antigen-1." J Immunol **159**(1): 268-278.

Luo, B. H., C. V. Carman, et al. (2007). "Structural basis of integrin regulation and signaling." Annu Rev Immunol **25**: 619-647.

Luo, B. H., C. V. Carman, et al. (2005). "Disrupting integrin transmembrane domain heterodimerization increases ligand binding affinity, not valency or clustering." Proc Natl Acad Sci U S A **102**(10): 3679-3684.

Luo, B. H., T. A. Springer, et al. (2004). "A specific interface between integrin transmembrane helices and affinity for ligand." PLoS Biol **2**(6): e153.

Ma, Y. Q., J. Qin, et al. (2008). "Kindlin-2 (Mig-2): a co-activator of beta3 integrins." J Cell Biol **181**(3): 439-446.

MacDonald, T. J., C. F. Stewart, et al. (2008). "Phase I clinical trial of cilengitide in children with refractory brain tumors: Pediatric Brain Tumor Consortium Study PBTC-012." J Clin Oncol **26**(6): 919-924.

Mahabeleshwar, G. H., W. Feng, et al. (2006). "Integrin signaling is critical for pathological angiogenesis." J Exp Med **203**(11): 2495-2507.

Major, E. O. (2010). "Progressive multifocal leukoencephalopathy in patients on immunomodulatory therapies." Annu Rev Med **61**(19719397): 35-47.

Makrilia, N., A. Kollias, et al. (2009). "Cell adhesion molecules: role and clinical significance in cancer." Cancer Invest **27**(10): 1023-1037.

Malinin, N. L., L. Zhang, et al. (2009). "A point mutation in KINDLIN3 ablates activation of three integrin subfamilies in humans." Nat Med **15**(3): 313-318.

Mann, M. (2006). "Functional and quantitative proteomics using SILAC." Nat Rev Mol Cell Biol **7**(12): 952-958.

Martel, V., C. Racaud-Sultan, et al. (2001). "Conformation, localization, and integrin binding of talin depend on its interaction with phosphoinositides." J Biol Chem **276**(24): 21217-21227.

Mates, L., M. K. Chuah, et al. (2009). "Molecular evolution of a novel hyperactive Sleeping Beauty transposase enables robust stable gene transfer in vertebrates." Nat Genet **41**(6): 753-761.

Maubant, S., D. Saint-Dizier, et al. (2006). "Blockade of alpha v beta3 and alpha v beta5 integrins by RGD mimetics induces anoikis and not integrin-mediated death in human endothelial cells." Blood **108**(9): 3035-3044.

McCarty, J. H. (2009). "Integrin-mediated regulation of neurovascular development, physiology and disease." Cell Adh Migr **3**(2): 211-215.

McCarty, J. H., A. Lacy-Hulbert, et al. (2005). "Selective ablation of alphav integrins in the central nervous system leads to cerebral hemorrhage, seizures, axonal degeneration and premature death." Development **132**(15576410): 165-176.

McCarty, J. H., R. A. Monahan-Earley, et al. (2002). "Defective associations between blood vessels and brain parenchyma lead to cerebral hemorrhage in mice lacking alphav integrins." Mol Cell Biol **22**(12370313): 7667-7677.

Mercurio, A. M., I. Rabinovitz, et al. (2001). "The alpha 6 beta 4 integrin and epithelial cell migration." Curr Opin Cell Biol **13**(5): 541-545.

Meves, A., T. Geiger, et al. (2011). "Beta1 integrin cytoplasmic tyrosines promote skin tumorigenesis independent of their phosphorylation." Proc Natl Acad Sci U S A **108**(37): 15213-15218.

Meves, A., C. Stremmel, et al. (2009). "The Kindlin protein family: new members to the club of focal adhesion proteins." Trends Cell Biol **19**(10): 504-513.

Milner, R. and I. L. Campbell (2002). "The integrin family of cell adhesion molecules has multiple functions within the CNS." J Neurosci Res **69**(3): 286-291.

Mitra, S., K. W. Cheng, et al. (2011). "Rab GTPases implicated in inherited and acquired disorders." Semin Cell Dev Biol **22**(1): 57-68.

Miyagoe-Suzuki, Y., M. Nakagawa, et al. (2000). "Merosin and congenital muscular dystrophy." Microsc Res Tech **48**(10679965): 181-191.

Montanez, E., A. Piwko-Czuchra, et al. (2007). "Analysis of integrin functions in peri-implantation embryos, hematopoietic system, and skin." Methods Enzymol **426**: 239-289.

Montanez, E., A. Piwko-Czuchra, et al. (2007). "Analysis of integrin functions in peri-implantation embryos, hematopoietic system, and skin." Methods Enzymol. **426**: 239-289.

Montanez, E., S. Ussar, et al. (2008). "Kindlin-2 controls bidirectional signaling of integrins." Genes Dev **22**(10): 1325-1330.

Morris, D. G., X. Huang, et al. (2003). "Loss of integrin alpha(v)beta6-mediated TGF-beta activation causes Mmp12-dependent emphysema." Nature **422**(12634787): 169-173.

Mory, A., S. W. Feigelson, et al. (2008). "Kindlin-3: a new gene involved in the pathogenesis of LAD-III." Blood **112**(6): 2591.

Moschos, S. J., C. A. Sander, et al. (2010). "Pharmacodynamic (phase 0) study using etaracizumab in advanced melanoma." J Immunother **33**(20445352): 316-325.

Moser, M., M. Bauer, et al. (2009). "Kindlin-3 is required for beta2 integrin-mediated leukocyte adhesion to endothelial cells." Nat Med **15**(3): 300-305.

Moser, M., K. R. Legate, et al. (2009). "The tail of integrins, talin, and kindlins." Science **324**(5929): 895-899.

Moser, M., B. Nieswandt, et al. (2008). "Kindlin-3 is essential for integrin activation and platelet aggregation." Nat Med **14**(3): 325-330.

Mosesson, Y., G. B. Mills, et al. (2008). "Derailed endocytosis: an emerging feature of cancer." Nat Rev Cancer **8**(11): 835-850.

Muller, E. J., L. Williamson, et al. (2008). "Outside-in signaling through integrins and cadherins: a central mechanism to control epidermal growth and differentiation?" J Invest Dermatol **128**(18268536): 501-516.

Muller, U., D. Wang, et al. (1997). "Integrin alpha8beta1 is critically important for epithelial-mesenchymal interactions during kidney morphogenesis." Cell **88**(9054500): 603-613.

Nandrot, E. F., Y. Kim, et al. (2004). "Loss of synchronized retinal phagocytosis and age-related blindness in mice lacking alphavbeta5 integrin." J Exp Med **200**(15596525): 1539-1545.

Nawrotzki, R., M. Willem, et al. (2003). "Defective integrin switch and matrix composition at alpha 7-deficient myotendinous junctions precede the onset of muscular dystrophy in mice." Hum Mol Genet **12**(12588796): 483-495.

Nemeth, J. A., M. T. Nakada, et al. (2007). "Alpha-v integrins as therapeutic targets in oncology." Cancer Invest **25**(7): 632-646.

Ng, C. M., S. Bai, et al. (2010). "Mechanism-based receptor-binding model to describe the pharmacokinetic and pharmacodynamic of an anti-alpha5beta1 integrin monoclonal antibody (volociximab) in cancer patients." Cancer Chemother Pharmacol **65**(2): 207-217.

Nieswandt, B., M. Moser, et al. (2007). "Loss of talin1 in platelets abrogates integrin activation, platelet aggregation, and thrombus formation in vitro and in vivo." J Exp Med **204**(13): 3113-3118.

Nisato, R. E., J. C. Tille, et al. (2003). "alphav beta 3 and alphav beta 5 integrin antagonists inhibit angiogenesis in vitro." Angiogenesis **6**(2): 105-119.

Nishimura, T. and K. Kaibuchi (2007). "Numb controls integrin endocytosis for directional cell migration with aPKC and PAR-3." Dev Cell **13**(1): 15-28.

Ong, S. E., B. Blagoev, et al. (2002). "Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics." Mol Cell Proteomics **1**(5): 376-386.

Partridge, A. W., S. Liu, et al. (2005). "Transmembrane domain helix packing stabilizes integrin alphaIIbbeta3 in the low affinity state." J Biol Chem **280**(8): 7294-7300.

Pellinen, T., A. Arjonen, et al. (2006). "Small GTPase Rab21 regulates cell adhesion and controls endosomal traffic of beta1-integrins." J Cell Biol **173**(5): 767-780.

Pellinen, T. and J. Ivaska (2006). "Integrin traffic." J Cell Sci **119**(Pt 18): 3723-3731.

Pellinen, T., S. Tuomi, et al. (2008). "Integrin trafficking regulated by Rab21 is necessary for cytokinesis." Dev Cell **15**(3): 371-385.

Petrich, B. G., P. Marchese, et al. (2007). "Talin is required for integrin-mediated platelet function in hemostasis and thrombosis." J Exp Med **204**(13): 3103-3111.

Pfeifer, A., T. Kessler, et al. (2000). "Suppression of angiogenesis by lentiviral delivery of PEX, a noncatalytic fragment of matrix metalloproteinase 2." Proc Natl Acad Sci U S A **97**(22): 12227-12232.

Pierini, L. M., M. A. Lawson, et al. (2000). "Oriented endocytic recycling of alpha5beta1 in motile neutrophils." Blood **95**(8): 2471-2480.

Pietri, T., O. Eder, et al. (2004). "Conditional beta1-integrin gene deletion in neural crest cells causes severe developmental alterations of the peripheral nervous system." Development **131**(15253938): 3871-3883.

Popova, S. N., M. Barczyk, et al. (2007). "Alpha11 beta1 integrin-dependent regulation of periodontal ligament function in the erupting mouse incisor." Mol Cell Biol **27**(17420280): 4306-4316.

Potocnik, A. J., C. Brakebusch, et al. (2000). "Fetal and adult hematopoietic stem cells require beta1 integrin function for colonizing fetal liver, spleen, and bone marrow." Immunity **12**(10894165): 653-663.

Pribila, J. T., A. C. Quale, et al. (2004). "Integrins and T cell-mediated immunity." Annu Rev Immunol **22**(15032577): 157-180.

Prigozhina, N. L. and C. M. Waterman-Storer (2004). "Protein kinase D-mediated anterograde membrane trafficking is required for fibroblast motility." Curr Biol **14**(2): 88-98.

Proctor, J. M., K. Zang, et al. (2005). "Vascular development of the brain requires beta8 integrin expression in the neuroepithelium." J Neurosci **25**(16251442): 9940-9948.

Raghavan, S., C. Bauer, et al. (2000). "Conditional ablation of beta1 integrin in skin. Severe defects in epidermal proliferation, basement membrane formation, and hair follicle invagination." J Cell Biol **150**(10974002): 1149-1160.

Raiborg, C. and H. Stenmark (2009). "The ESCRT machinery in endosomal sorting of ubiquitylated membrane proteins." Nature **458**(7237): 445-452.

Ramsay, A. G., M. D. Keppler, et al. (2007). "HS1-associated protein X-1 regulates carcinoma cell migration and invasion via clathrin-mediated endocytosis of integrin alphavbeta6." Cancer Res **67**(11): 5275-5284.

Ramsay, A. G., J. F. Marshall, et al. (2007). "Integrin trafficking and its role in cancer metastasis." Cancer Metastasis Rev **26**(3-4): 567-578.

Rappoport, J. Z. and S. M. Simon (2003). "Real-time analysis of clathrin-mediated endocytosis during cell migration." J Cell Sci **116**(Pt 5): 847-855.

Reardon, D. A., K. L. Fink, et al. (2008). "Randomized phase II study of cilengitide, an integrin-targeting arginine-glycine-aspartic acid peptide, in recurrent glioblastoma multiforme." J Clin Oncol **26**(18981465): 5610-5617.

Rees, D. J., S. E. Ades, et al. (1990). "Sequence and domain structure of talin." Nature **347**(6294): 685-689.

Reynolds, A. R., I. R. Hart, et al. (2009). "Stimulation of tumor growth and angiogenesis by low concentrations of RGD-mimetic integrin inhibitors." Nat Med **15**(4): 392-400.

Ricart, A. D., J. D. Berlin, et al. (2008). "Phase I, pharmacokinetic and biological correlative study of OSI-7904L, a novel liposomal thymidylate synthase inhibitor, and cisplatin in patients with solid tumors." Clin Cancer Res **14**(19047127): 7947-7955.

Rice, G. P., H. P. Hartung, et al. (2005). "Anti-alpha4 integrin therapy for multiple sclerosis: mechanisms and rationale." Neurology **64**(8): 1336-1342.

Roberts, M., S. Barry, et al. (2001). "PDGF-regulated rab4-dependent recycling of alphavbeta3 integrin from early endosomes is necessary for cell adhesion and spreading." Current Biol. **11**(18): 1392-1402.

Romisch, K. (2005). "Endoplasmic reticulum-associated degradation." Annu Rev Cell Dev Biol **21**: 435-456.

Rosenthal, M. A., P. Davidson, et al. (2010). "Evaluation of the safety, pharmacokinetics and treatment effects of an alpha(nu)beta(3) integrin inhibitor on bone turnover and disease activity in men with hormone-refractory prostate cancer and bone metastases." Asia Pac J Clin Oncol **6**(20398037): 42-48.

Ross, F. P. and S. L. Teitelbaum (2005). "alphavbeta3 and macrophage colony-stimulating factor: partners in osteoclast biology." Immunol Rev **208**(16313343): 88-8105.

Sadler, E., A. Klausegger, et al. (2006). "Novel KIND1 gene mutation in Kindler syndrome with severe gastrointestinal tract involvement." Arch Dermatol **142**(12): 1619-1624.

Scharffetter-Kochanek, K., H. Lu, et al. (1998). "Spontaneous skin ulceration and defective T cell function in CD18 null mice." J Exp Med **188**(9653089): 119-131.

Schiller, H. B., C. C. Friedel, et al. (2011). "Quantitative proteomics of the integrin adhesome show a myosin II-dependent recruitment of LIM domain proteins." EMBO Rep **12**(3): 259-266.

Schmoranzer, J., G. Kreitzer, et al. (2003). "Migrating fibroblasts perform polarized, microtubule-dependent exocytosis towards the leading edge." J Cell Sci **116**(Pt 22): 4513-4519.

Schwartz, M. A. (2001). "Integrin signaling revisited." Trends Cell Biol **11**(12): 466-470.

Scita, G. and P. P. Di Fiore (2010). "The endocytic matrix." Nature **463**(7280): 464-473.

Seet, L. F. and W. Hong (2006). "The Phox (PX) domain proteins and membrane traffic." Biochim Biophys Acta **1761**(8): 878-896.

Sheppard, D. (2000). "In vivo functions of integrins: lessons from null mutations in mice." Matrix Biol **19**(3): 203-209.

Shi, F. and J. Sottile (2008). "Caveolin-1-dependent beta1 integrin endocytosis is a critical regulator of fibronectin turnover." J Cell Sci **121**(Pt 14): 2360-2371.

Shi, X., Y. Q. Ma, et al. (2007). "The MIG-2/integrin interaction strengthens cell-matrix adhesion and modulates cell motility." J Biol Chem **282**(28): 20455-20466.

Shin, S., L. Wolgamott, et al. (2012). "Integrin trafficking and tumor progression." Int J Cell Biol **2012**: 516789.

Siegel, D. H., G. H. Ashton, et al. (2003). "Loss of kindlin-1, a human homolog of the Caenorhabditis elegans actin-extracellular-matrix linker protein UNC-112, causes Kindler syndrome." Am J Hum Genet **73**(1): 174-187.

Simirskii, V. N., Y. Wang, et al. (2007). "Conditional deletion of beta1-integrin from the developing lens leads to loss of the lens epithelial phenotype." Dev Biol **306**(17493607): 658-668.

Simonson, W. T., S. J. Franco, et al. (2006). "Talin1 regulates TCR-mediated LFA-1 function." J Immunol **177**(11): 7707-7714.

Sixt, M., M. Bauer, et al. (2006). "Beta1 integrins: zip codes and signaling relay for blood cells." Curr Opin Cell Biol **18**(16919433): 482-490.

Smith-Garvin, J. E., G. A. Koretzky, et al. (2009). "T cell activation." Annu Rev Immunol **27**: 591-619.

Soldi, R., S. Mitola, et al. (1999). "Role of alphavbeta3 integrin in the activation of vascular endothelial growth factor receptor-2." EMBO J **18**(10022831): 882-892.

Sorkin, A. and M. von Zastrow (2009). "Endocytosis and signalling: intertwining molecular networks." Nat Rev Mol Cell Biol **10**(9): 609-622.

Sparrow, J. C. and F. Schock (2009). "The initial steps of myofibril assembly: integrins pave the way." Nat Rev Mol Cell Biol **10**(19190670): 293-298.

Stenmark, H. (2009). "Rab GTPases as coordinators of vesicle traffic." Nat Rev Mol Cell Biol **10**(8): 513-525.

Stephens, L. E., A. E. Sutherland, et al. (1995). "Deletion of beta 1 integrins in mice results in inner cell mass failure and peri-implantation lethality." Genes Dev **9**(15): 1883-1895.

Stewart, P. L. and G. R. Nemerow (2007). "Cell integrins: commonly used receptors for diverse viral pathogens." Trends Microbiol **15**(17988871): 500-507.

Stockinger, W., B. Sailler, et al. (2002). "The PX-domain protein SNX17 interacts with members of the LDL receptor family and modulates endocytosis of the LDL receptor." EMBO J **21**(16): 4259-4267.

Stupp, R., M. E. Hegi, et al. (2010). "Phase I/IIa study of cilengitide and temozolomide with concomitant radiotherapy followed by cilengitide and temozolomide maintenance therapy in patients with newly diagnosed glioblastoma." J Clin Oncol **28**(16): 2712-2718.

Stupp, R. and C. Ruegg (2007). "Integrin inhibitors reaching the clinic." J Clin Oncol **25**(13): 1637-1638.

Su, G., M. Hodnett, et al. (2007). "Integrin alphavbeta5 regulates lung vascular permeability and pulmonary endothelial barrier function." Am J Respir Cell Mol Biol **36**(17079779): 377-386.

Sung, B. H., X. Zhu, et al. (2011). "Cortactin controls cell motility and lamellipodial dynamics by regulating ECM secretion." Curr Biol **21**(17): 1460-1469.

Svensson, L., K. Howarth, et al. (2009). "Leukocyte adhesion deficiency-III is caused by mutations in KINDLIN3 affecting integrin activation." Nat Med **15**(3): 306-312.

Tadokoro, S., S. J. Shattil, et al. (2003). "Talin binding to integrin beta tails: a final common step in integrin activation." Science **302**(5642): 103-106.

Takabayshi, K., M. Corr, et al. (2006). "Induction of a homeostatic circuit in lung tissue by microbial compounds." Immunity **24**(16618605): 475-487.

Talts, J. F., C. Brakebusch, et al. (1999). "Integrin gene targeting." Methods Mol Biol **129**: 153-187.

Tamkun, J. W., D. W. DeSimone, et al. (1986). "Structure of integrin, a glycoprotein involved in the transmembrane linkage between fibronectin and actin." Cell **46**(2): 271-282.

Targan, S. R., B. G. Feagan, et al. (2007). "Natalizumab for the treatment of active Crohn's disease: results of the ENCORE Trial." Gastroenterology **132**(17484865): 1672-1683.

Taverna, D., M. H. Disatnik, et al. (1998). "Dystrophic muscle in mice chimeric for expression of alpha5 integrin." J Cell Biol **143**(9813102): 849-859.

Teasdale, R. D. and B. M. Collins (2012). "Insights into the PX (phox-homology) domain and SNX (sorting nexin) protein families: structures, functions and roles in disease." Biochem J **441**(1): 39-59.

Teasdale, R. D., D. Loci, et al. (2001). "A large family of endosome-localized proteins related to sorting nexin 1." Biochem J **358**(Pt 1): 7-16.

Teckchandani, A., N. Toida, et al. (2009). "Quantitative proteomics identifies a Dab2/integrin module regulating cell migration." J Cell Biol **186**(1): 99-111.

Tu, C., C. F. Ortega-Cava, et al. (2010). "Endosomal-sorting complexes required for transport (ESCRT) pathway-dependent endosomal traffic regulates the localization of active Src at focal adhesions." Proc Natl Acad Sci U S A **107**(37): 16107-16112.

Tucker, G. C. (2006). "Integrins: molecular targets in cancer therapy." Curr Oncol Rep **8**(2): 96-103.

Ulmer, T. S., D. A. Calderwood, et al. (2003). "Domain-specific interactions of talin with the membrane-proximal region of the integrin beta3 subunit." Biochemistry **42**(27): 8307-8312.

Ulrich, F. and C. P. Heisenberg (2009). "Trafficking and cell migration." Traffic **10**(7): 811-818.

Ussar, S., M. Moser, et al. (2008). "Loss of Kindlin-1 causes skin atrophy and lethal neonatal intestinal epithelial dysfunction." PLoS Genet **4**(12): e1000289.

Ussar, S., H. V. Wang, et al. (2006). "The Kindlins: subcellular localization and expression during murine development." Exp Cell Res **312**(16): 3142-3151.

Valdembri, D., P. T. Caswell, et al. (2009). "Neuropilin-1/GIPC1 signaling regulates alpha5beta1 integrin traffic and function in endothelial cells." PLoS Biol **7**(1): e25.

van Kerkhof, P., J. Lee, et al. (2005). "Sorting nexin 17 facilitates LRP recycling in the early endosome." EMBO J **24**(16): 2851-2861.

van Weert, A. W., H. J. Geuze, et al. (2000). "Primaquine interferes with membrane recycling from endosomes to the plasma membrane through a direct interaction with endosomes which does not involve neutralisation of endosomal pH nor osmotic swelling of endosomes." Eur J Cell Biol **79**(6): 394-399.

Vignoud, L., Y. Usson, et al. (1994). "Internalization of the alpha 5 beta 1 integrin does not depend on "NPXY" signals." Biochem Biophys Res Commun **199**(2): 603-611.

Vinogradova, O., A. Velyvis, et al. (2002). "A structural mechanism of integrin alpha(IIb)beta(3) "inside-out" activation as regulated by its cytoplasmic face." Cell **110**(5): 587-597.

Vlahakis, N. E., B. A. Young, et al. (2005). "The lymphangiogenic vascular endothelial growth factors VEGF-C and -D are ligands for the integrin alpha9beta1." J Biol Chem **280**(15590642): 4544-4552.

Walker, J. and A. S. Menko (2009). "Integrins in lens development and disease." Exp Eye Res **88**(18671967): 216-225.

Walker, J. L., A. K. Fournier, et al. (2005). "Regulation of growth factor signaling and cell cycle progression by cell adhesion and adhesion-dependent changes in cellular tension." Cytokine Growth Factor Rev **16**(4-5): 395-405.

Warnke, C., T. Menge, et al. (2010). "Natalizumab and progressive multifocal leukoencephalopathy: what are the causal factors and can it be avoided?" Arch Neurol **67**(20697042): 923-930.

Watt, F. M. (2002). "Role of integrins in regulating epidermal adhesion, growth and differentiation." EMBO J **21**(12145193): 3919-3926.

Wegener, K. L., A. W. Partridge, et al. (2007). "Structural basis of integrin activation by talin." Cell **128**(1): 171-182.

Weis, S. M. (2007). "Evaluating integrin function in models of angiogenesis and vascular permeability." Methods Enzymol **426**: 505-528.

Weis, S. M., D. G. Stupack, et al. (2009). "Agonizing integrin antagonists?" Cancer Cell **15**(5): 359-361.

White, D. J., S. Puranen, et al. (2004). "The collagen receptor subfamily of the integrins." Int J Biochem Cell Biol **36**(15147720): 1405-1410.

White, D. P., P. T. Caswell, et al. (2007). "alpha v beta3 and alpha5beta1 integrin recycling pathways dictate downstream Rho kinase signaling to regulate persistent cell migration." J Cell Biol **177**(3): 515-525.

Wilhelmsen, K., S. H. M. Litjens, et al. (2006). "Multiple functions of the integrin alpha6beta4 in epidermal homeostasis and tumorigenesis." Mol Cell Biol **26**(16581764): 2877-2886.

Williams, R., T. Schluter, et al. (2004). "Sorting nexin 17 accelerates internalization yet retards degradation of P-selectin." Mol Biol Cell **15**(7): 3095-3105.

Worby, C. A. and J. E. Dixon (2002). "Sorting out the cellular functions of sorting nexins." Nat Rev Mol Cell Biol **3**(12): 919-931.

Wu, C., M. H. Ma, et al. (2007). "Systematic identification of SH3 domain-mediated human protein-protein interactions by peptide array target screening." Proteomics **7**(11): 1775-1785.

Yang, J. T., H. Rayburn, et al. (1993). "Embryonic mesodermal defects in alpha 5 integrin-deficient mice." Development **119**(4): 1093-1105.

Yang, J. T., H. Rayburn, et al. (1995). "Cell adhesion events mediated by alpha 4 integrins are essential in placental and cardiac development." Development **121**(7539359): 549-560.

Zaidel-Bar, R. and B. Geiger (2010). "The switchable integrin adhesome." J Cell Sci **123**(Pt 9): 1385-1388.

Zaidel-Bar, R., S. Itzkovitz, et al. (2007). "Functional atlas of the integrin adhesome." Nat Cell Biol **9**(8): 858-867.

Zhang, X., G. Jiang, et al. (2008). "Talin depletion reveals independence of initial cell spreading from integrin activation and traction." Nat Cell Biol **10**(9): 1062-1068.

Zhang, X., G. Mernaugh, et al. (2009). "beta1 integrin is necessary for ureteric bud branching morphogenesis and maintenance of collecting duct structural integrity." Development **136**(19710172): 3357-3366.

Zhong, C., M. Chrzanowska-Wodnicka, et al. (1998). "Rho-mediated contractility exposes a cryptic site in fibronectin and induces fibronectin matrix assembly." J Cell Biol **141**(2): 539-551.

Zhu, J., B. H. Luo, et al. (2008). "Structure of a complete integrin ectodomain in a physiologic resting state and activation and deactivation by applied forces." Mol Cell **32**(6): 849-861.

Zhu, J., K. Motejlek, et al. (2002). "beta8 integrins are required for vascular morphogenesis in mouse embryos." Development **129**(12050137): 2891-2903.

11 Publications

Abstracts were adapted from the orginal publications.

11.1 Publication 1: Meves et al. 2009 (Meves et al. 2009)

Kindlins are a group of proteins that have recently attracted attention for their ability to bind and activate integrins. Moreover, they have also been linked to inherited and acquired human diseases including Kindler syndrome, leukocyte adhesion deficiency, and cancer. Although most studies have focused on kindlins as key regulatory components of cell-extracellular matrix junctions such as focal adhesions, preliminary data suggest the involvement of additional cellular compartments in mediating their functions, particularly at cell-cell contacts and the nucleus. Investigating the many roles of kindlins is likely to expand and sharpen our view on the versatility of integrin-mediated cell adhesion, the nuclear function of focal adhesion proteins, and the crosstalk between cell-cell and cell-matrix adhesions in health and disease.

11.2 Publication 2: Böttcher et al. 2012 (Bottcher et al. 2012)

Integrin functions are controlled by regulating their affinity for ligand, and by the efficient recycling of intact integrins through endosomes. Here we demonstrate that the kindlin-binding site in the β1 integrin cytoplasmic domain serves as a molecular switch enabling the sequential binding of two FERM-domain-containing proteins in different cellular compartments. When β1 integrins are at the plasma membrane, kindlins control ligand-binding affinity. However, when they are internalized, kindlins dissociate from integrins and sorting nexin 17 (SNX17) is recruited to free β1 integrin tails in early endosomes to prevent β1 integrin degradation, leading to their recycling back to the cell surface. Our results identify SNX17 as a β1 integrin tail-binding protein that interacts with the free kindlin-binding site in endosomes to stabilize β1 integrins, resulting in their recycling to the cell surface where they can be reused.