Molecular Cell, Volume *76*

Supplemental Information

Mediator Condensates Localize Signaling Factors to Key Cell Identity Genes

Alicia V. Zamudio, Alessandra Dall'Agnese, Jonathan E. Henninger, John C. Manteiga, Lena K. Afeyan, Nancy M. Hannett, Eliot L. Coffey, Charles H. Li, Ozgur Oksuz, Benjamin R. Sabari, Ann Boija, Isaac A. Klein, Susana W. Hawken, Jan-Hendrik Spille, Tim-Michael Decker, Ibrahim I. Cisse, Brian J. Abraham, Tong I. Lee, Dylan J. Taatjes, Jurian Schuijers, and Richard A. Young

Figure S1

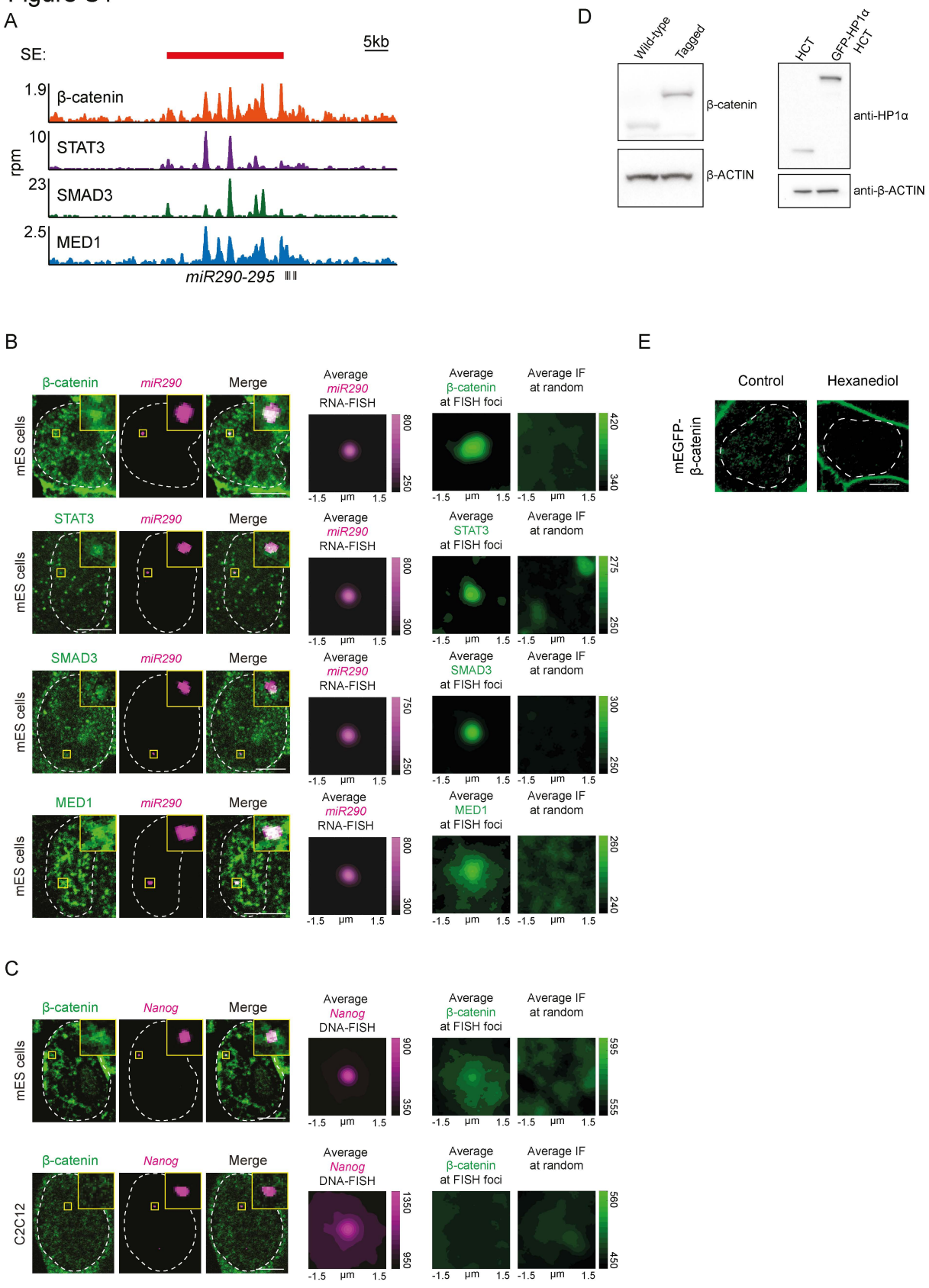

A
SE:
5kb
1.9
β-catenin
10
STAT3
rpm
23
SMAD3
2.5
MED1
miR290-295
D
Wild-type
Tagged
β-catenin
β-ACTIN
HCT
GFP-HP1α HCT
anti-HP1α
anti-β-ACTIN
B
β-catenin
miR290
Merge
mES cells
Average miR290 RNA-FISH
Average β-catenin at FISH foci
Average IF at random
STAT3
SMAD3
MED1
-1.5 μm 1.5
E
Control
Hexanediol
mEGFP-β-catenin
C
Nanog
C2C12
Average Nanog DNA-FISH

Figure S1. Related to Figure 1

S1A) ChIP-seq tracks displaying occupancy of β-catenin, STAT3, SMAD3 and MED1 at the super-enhancer of the *miR290* gene. Reads densities are displayed in reads per million per bin (rpm/bin) and the super-enhancer is indicated with a red bar.

S1B.) Immunofluorescence for β-catenin, STAT3, SMAD3 and MED1 with concurrent RNA-FISH for *miR290* nascent RNA demonstrating the presence of condensed nuclear foci of the signaling factors at the *miR290* super-enhancer in mES cells. Cells were grown for 24 hours in the presence of CHIR99021, LIF or Activin A prior to fixation. Hoechst staining was used to determine the nuclear periphery, highlighted with a dotted line. 100x objective was used for imaging on a spinning disk confocal microscope. Average RNA-FISH signal and average IF signal centered on the RNA-FISH focus for each signaling factor from at least 10 images is shown. Average signaling factor IF signal at randomly selected nuclear positions is displayed in the right most panel. Scale bars indicate 5 μm.

S1C) Immunofluorescence for β-catenin with concurrent DNA-FISH for *Nanog* demonstrating the absence of nuclear foci of the signaling factors at the *Nanog* super-enhancer in C2C12 cells. Cells were grown for 24 hours in the presence of CHIR99021 prior to fixation. Hoechst staining was used to determine the nuclear periphery, highlighted with a dotted line.100x objective was used for imaging on a spinning disk confocal microscope. Average DNA-FISH signal and average IF signal centered on the DNA-FISH focus for each signaling factor from at least 10 images is shown. Average signaling factor IF signal at randomly selected nuclear positions is displayed in the right most panel. Scale bar indicates 5 μm.

S1D) Western blot showing levels of endogenously tagged mEGFP- β-catenin in comparison to endogenous β-catenin in HCT116 cells, and mEGFP-Hp1α in engineered HCT116 cells compared to Hp1α in wild type cells.

S1E) Live-cell imaging of HCT116 with endogenously-tagged mEGFP-β-catenin before and after treatment with 10% 1,6-hexanediol or vehicle for 30 seconds. Hexanediol treatment led to a sharp reduction in bright -β-catenin foci *in vivo*. Images were obtained using a Zeiss LSM880 confocal microscope with and Airyscan detector at 63x magnification. Scalebar indicates 2um.

Figure S2

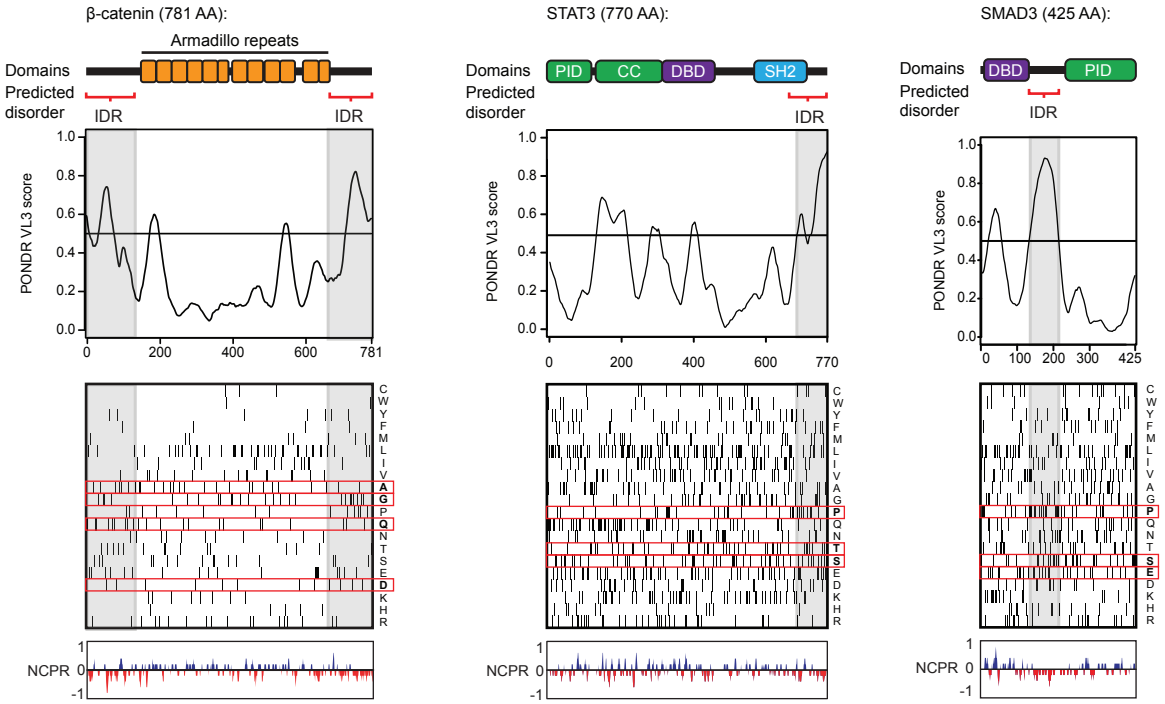
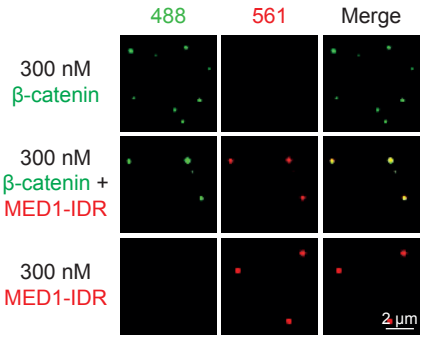

β-catenin (781 AA):
Armadillo repeats
Domains
Predicted disorder
IDR
IDR
PONDR VL3 score
1.0
0.8
0.6
0.4
0.2
0.0
0
200
400
600
781
C W Y F M L I V A G P Q N T S E D K H R
NCPR
1
0
-1
STAT3 (770 AA):
Domains
Predicted disorder
PID
CC
DBD
SH2
IDR
PONDR VL3 score
0
200
400
600
770
C W Y F M L I V A G P Q N T S E D K H R
NCPR
SMAD3 (425 AA):
Domains
Predicted disorder
DBD
PID
IDR
PONDR VL3 score
0
100
200
300
425
C W Y F M L I V A G P Q N T S E D K H R
NCPR

Figure S2. Related to Figure 2

Domain structures of β-catenin, STAT3 and SMAD3 the signaling factors used in this manuscript. DBD: DNA binding domain, PID: protein interaction domain, CC: coiled coil domain, DD: dimerization domain, SH2: *Src* homology domain 2 . The predicted intrinsically disordered regions (IDR) marked in red. PONDR VL3 score per amino acid was used to predict disorder and is plotted below. Barcode plots indicate the location of different amino acids below. Red boxes indicate the top 3 over-represented amino acids in the predicted IDRs of the protein. Lowest panel shows the net charge per residue (NCPR) for the indicated protein.

Figure S3

A


488
561
Merge
300 nM
β-catenin
300 nM
β-catenin +
MED1-IDR
300 nM
MED1-IDR
2 μm

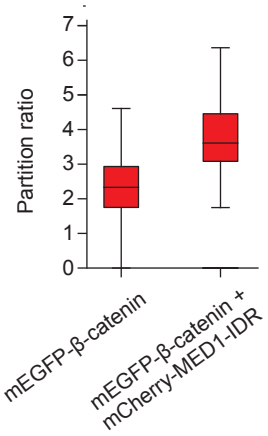
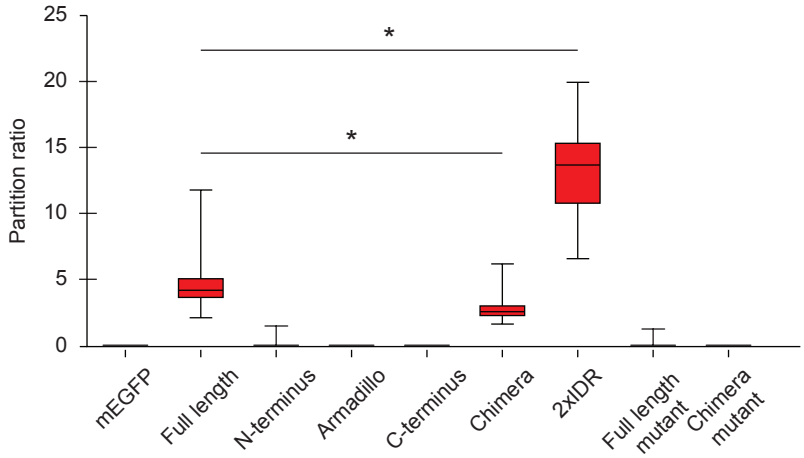

Partition ratio
0
1
2
3
4
5
6
7
mEGFP-β-catenin
mEGFP-β-catenin +
mCherry-MED1-IDR

Figure S3. Related to Figure 3

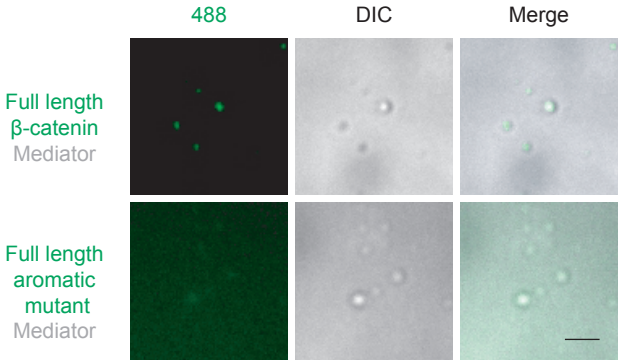
S3A) β-catenin forms droplets with MED1-IDR at nanomolar concentrations of both factors. Droplet assays were formed in 10% PEG-8000 and 125mM NaCl and imaged using a spinning disk confocal miscroscope and 150x objective. Partition ratio was calculated for at least 10 images per condition.

Figure S4

A


Partition ratio
25
20
15
10
5
0
*
*
mEGFP
Full length
N-terminus
Armadillo
C-terminus
Chimera
2xIDR
Full length mutant
Chimera mutant

B


488
DIC
Merge
Full length β-catenin Mediator
Full length aromatic mutant Mediator

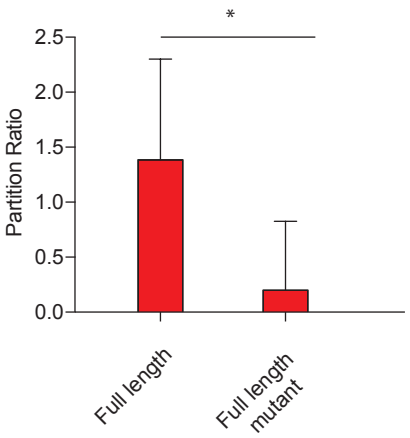

Partition Ratio
2.5
2.0
1.5
1.0
0.5
0.0
*
Full length
Full length mutant

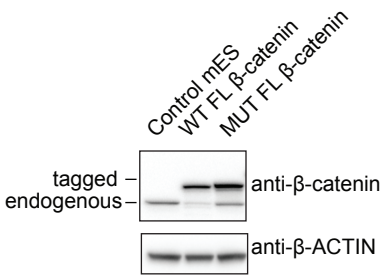
Figure S4. Related to Figure 4

S4A) Partition ratio was calculated by dividing the average fluorescence signal inside the droplets by the average fluorescence signal outside the droplets for at least 10 acquired images for the condition in which 1.25uM concentration of all factors were used for this assay.

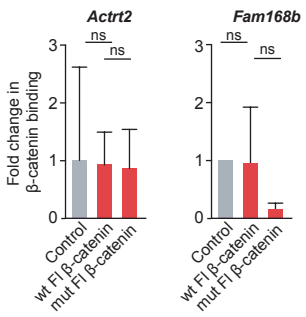
S4B) Representative images of *in vitro* droplet assays of wild type full length β-catenin and mutant full length β-catenin with purified Mediator showing the ability of β-catenin to interact and partition into Mediator droplets compared to that of the mutant β-catenin. Reactions were performed in the presence of 10% PEG-8000 and 300 nM β-catenin and imaged using a spinning disk confocal microscope with a 150x objective. Partition ratio was calculated for 10 acquired images in each condition. Scale bars indicate 2 μm.

Figure S5

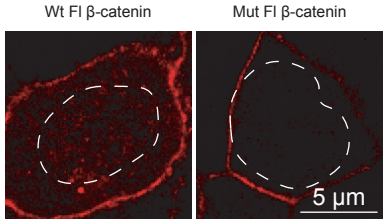
A



B



C



D

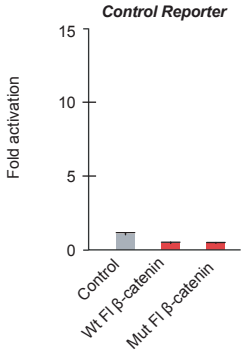


Figure S5. Related to Figure 5

S5A) Western blot showing expression levels of wild type and mutant β-catenin that were integrated in mES cells under a doxycycline inducible promoter compared to those of endogenous β-catenin in wild type mES cells. Cell were induced with 1µg/ml doxycycline for 24 hours and an inhibitor of the WNT pathway to replace endogenous nuclear β-catenin with Td-Tomato- β-catenin or Td-Tomato-aromatic-mutant- β-catenin.

S5B) ChIP-qPCR for two typical enhancer-driven genes showing that β-catenin and its mutant form are not targeted to non-WNT responsive genes. Ns indicates a t-test with a p-value of > 0.05.

S5C) Representative images of Td-Tomato- β-catenin or Td-Tomato-aromatic-mutant- β-catenin integrated into mES cells imaged using the Zeiss LSM 880 confocal microscope with Airyscan detector with a 63x objective.

S5D) Luciferase assay for a WNT unresponsive reporter showing no activation in two overexpressed versions of β-catenin. Quantification was performed for three biological replicates.

Figure S6

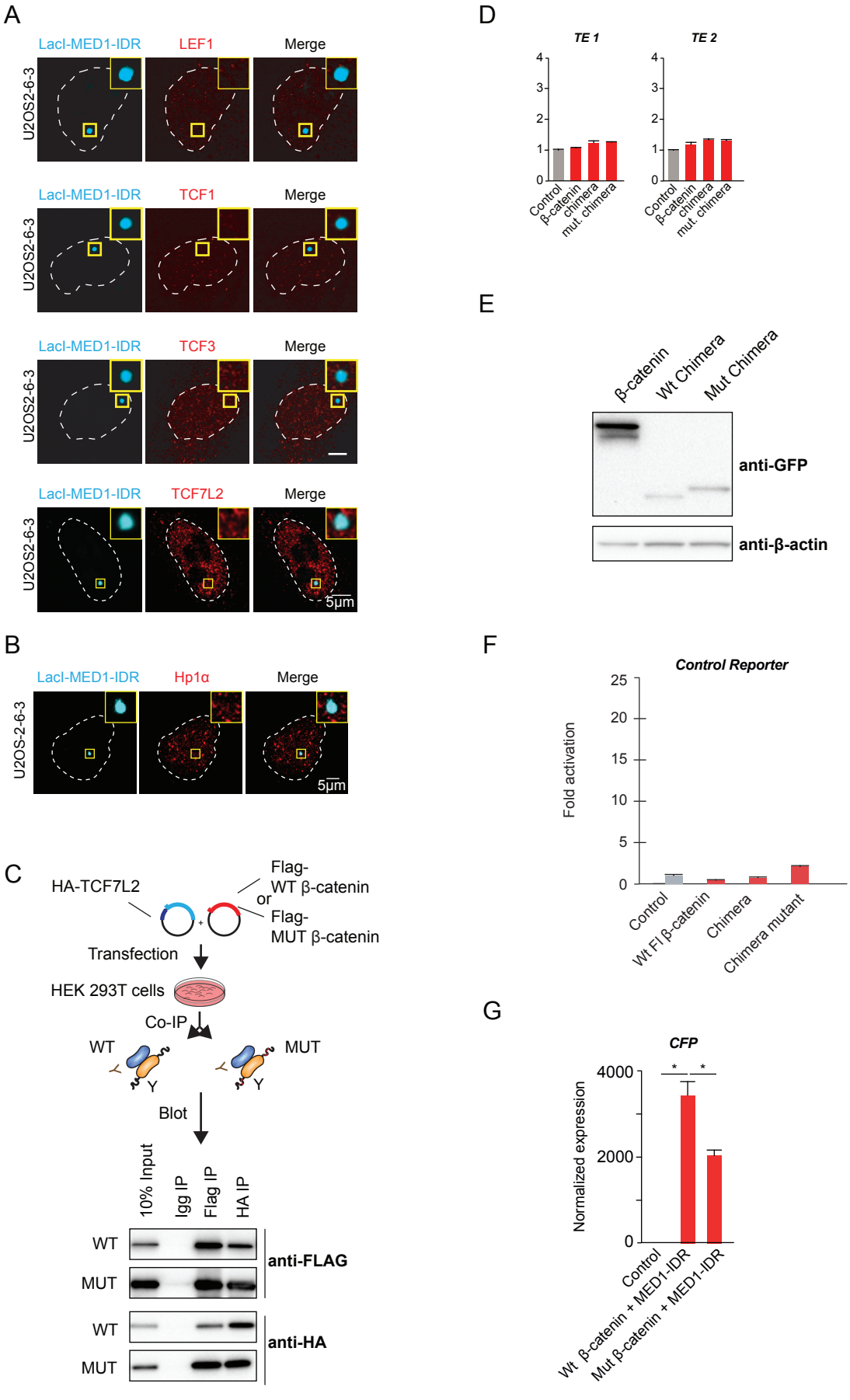

A
LacI-MED1-IDR
LEF1
Merge
U2OS2-6-3
LacI-MED1-IDR
TCF1
Merge
U2OS2-6-3
LacI-MED1-IDR
TCF3
Merge
U2OS2-6-3
LacI-MED1-IDR
TCF7L2
Merge
U2OS2-6-3
5μm
B
LacI-MED1-IDR
Hp1α
Merge
U2OS-2-6-3
5μm
C
HA-TCF7L2
Flag-
WT β-catenin
or
Flag-
MUT β-catenin
Transfection
HEK 293T cells
Co-IP
WT
MUT
Y
Y
Blot
10% Input
Igg IP
Flag IP
HA IP
WT
MUT
anti-FLAG
WT
MUT
anti-HA
D
TE 1
TE 2
4
3
2
1
0
Control
β-catenin
chimera
mut. chimera
E
β-catenin
Wt Chimera
Mut Chimera
anti-GFP
anti-β-actin
F
Control Reporter
Fold activation
25
20
15
10
5
0
Control
Wt Fl β-catenin
Chimera
Chimera mutant
G
CFP
Normalized expression
4000
2000
0
*
*
Control
Wt β-catenin + MED1-IDR
Mut β-catenin + MED1-IDR

Figure S6. Related to Figure 6

S6A) IF of LEF1, TCF1, TCF3, and TCF7L2 in Lac-U2OS cells transfected with a Lac binding domain-CFP-MED1-IDR construct showing no accumulation of TCF/LEF family members in the Lac spot. Images were obtained using a 100x objective on a spinning disk confocal microscope. Scale bars indicate 5μm.

S6B) IF of HP1α in U2OS2-6-3 cells transfected with a Lac binding domain-CFP-MED1-IDR construct. Images were obtained using a 100x objective on a spinning disk confocal microscope. Scale bars indicate 5μm.

S6C) Co-immunoprecipitation of wild type and mutant forms of β-catenin show both factors can interact with TCF7L2. Top: Diagram of experimental setup where HEK293T cells were co-transfected with flag-tagged-wild type or flag-tagged-mutant β -catenin and ha-tagged-TCF7L2 and either the flag-tagged factor or ha-tagged factor immunoprecipitated and blotted for the reciprocal tag. Bottom: Western blot of immunoprecipitated material.

S6D) ChIP-qPCR for two typical enhancer that are not normally bound by β-catenin shows no enrichment of β -catenin, chimera or mutant chimera at this gene.

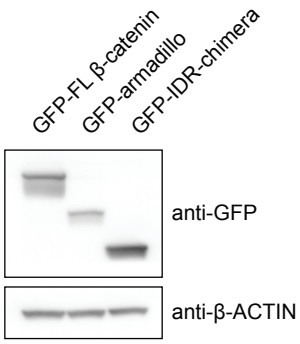
S6E) Western blot showing the levels of wild type β-catenin, chimera or chimera mutant protein in HEK293T cells compared to endogenous β-catenin. β -actin was used as a loading control.

S6F) Luciferase assay for a WNT unresponsive reporter showing no activation of luciferase expression in the presence of wt, chimera and chimera mutant forms of β-catenin. Untransfected control and WT FL- β-catenin came from the same experiment and are the same as in Figure S5, but displayed in two different graphs.

S6G) Expression of the Lac array is lower in the presence of mutant β-catenin than in wild-type β-catenin. RT-qPCR for CFP for engineered U2OS cells untransfected (control), or transfected with wild-type or mutant forms of β-catenin and MED1-IDR. Star represents a p-value of <0.05 in a t-test.

Figure S7

A


GFP-FL β-catenin
GFP-armadillo
GFP-IDR-chimera
anti-GFP
anti-β-ACTIN

B

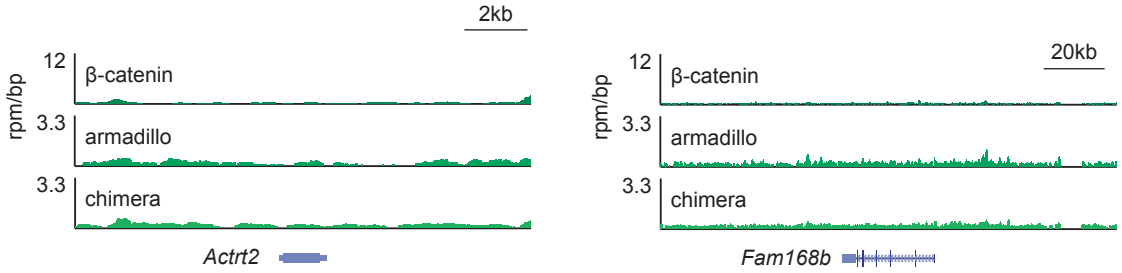

2kb
12
β-catenin
rpm/bp
3.3
armadillo
3.3
chimera
Actrt2
20kb
12
β-catenin
rpm/bp
3.3
armadillo
3.3
chimera
Fam168b

Figure S7. Related to Figure 7

S7A) Western blot of mES cells expresssing mEGFP-tagged- β-catenin, mEGFP-tagged-armadillo-repeats (armadillo) and mEGFP-tagged-IDRs (chimera). The endogenous β-catenin locus was tagged and the armadillo-repeats and IDRs integrated into mES cells using a transposable system.

S7B) ChIP-seq tracks of two typical-enhancer-associated genes show no association of β-catenin, armadillo or chimera with these sites.

Table S1. Oligonucleotides used in this study

ChIP-qPCR	
ChIP-negative-FWD	ACACAACATCTGCCCAAACA
ChIP-negative-REV	TGAGATCCTGGTGTGACCAA
Klf4-1-FWD	AGGGTGATGAATGGATCAGG
Klf4-1-REV	CTCTCCCCACGAATTAACGA
Myc -1-FWD	CCAGTGAACAAAAGTGCAA
Myc -1-REV	TCCAGGCACATCTCAGTTTG
Sp5 -1-FWD	GGAGCTCGCTTTAGTCCTCA
Sp5 -1-REV	CCCCCACTTGCAATTAAAGA
ChIP-negative-hu-FWD	CTCCCTTCCATCTTCCCTTC
ChIP-negative-hu-REV	TGCTTTCTTGGGGCATTAAC
SOX9-FWD	CTGTTGGGAATTCAGCCAAT
SOX9-REV	AATGAAGGGAGTGCAGGATG
SMAD7-FWD	AAATCCATCGGGTATCTGGA
SMAD7-REV	AGGCGGCCTCTTTTGTTTAT
KLF9-FWD	GCTCTGAAACCTGGCTCATC
KLF9-REV	ATTCTCTTGTCGGGTTGCAG
huTE1_F	GAGGGTAGGCTTTGTGGTGA
huTE1_R	AGGCTCATCTCACCTCTGGA
huTE2_F	GCTGTAGACCGGAGCTGTTC
huTE2_R	GGGACATGCTTTTTGATGCT
Actrt2_enhancer_FWD	actgctactgctgcccactt
Actrt2_enhancer_REV	tcctttgtttgggtcaggtc
Fam168b qPCR Primer F	CGGTGGTGATGGAAACTTCT
Fam168b qPCR Primer 2	TCCCAAATGTTGGGGTTAAA
RT-qPCR	
human gapdh rt primer f	TGCACCACCAACTGCTTAGC
human gapdh rt primer r	GGCATGGACTGTGGTCATGAG
CFP rt primer f	CGTGACCACCCTGACCTGG
CFP rt primer r	TCCTGGACGTAGCCTTCGG
mGapdh-FWD	CCATGTAGTTGAGGTCAATGAAGG
mGapdh-REV	TGGTGAAGGTCGGTGTGAAC
mKlf4-FWD	CTCCCGTCCTTCTCCACGTT
mKlf4-REV	TTCCTCACGCCAACGGTTA

Table S1. Related to Figures 5 & 6 and STAR Methods.
Oligonucleotides used in this study.

Movie S1 Related to Figure 3.
Timelapse imaging of preformed MED1-IDR droplets in 10% PEG-8000 to which 10 μM dilute β-catenin was added. Time interval 30 seconds. Magnification 150x.

Movie S2 Related to Figure 3.
Timelapse imaging of preformed MED1-IDR droplets in 10% PEG-8000 to which 10 μM dilute mEGFP-STAT3 was added. Time interval 30 seconds. Magnification 150x.

Movie S3 Related to Figure 3.
Timelapse imaging of preformed MED1-IDR droplets in 10% PEG-8000 to which 10 μM dilute mEGFP-SMAD3 was added. Time interval 30 seconds. Magnification 150x.