Supplemental Information

Mediator Condensates Localize Signaling

Factors to Key Cell Identity Genes

Figure S1

A

B

C

D

E
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):

Domains
Predicted disorder

PODNR VL3 score

STAT3 (770 AA):

SMAD3 (425 AA):

Domains
Predicted disorder

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

<table>
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<table>
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2 µm
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 microscope and 150x objective. Partition ratio was calculated for at least 10 images per condition.
Figure S4

A

B

Partition ratio

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*P < 0.05

Full length β-catenin Mediator

Full length aromatic mutant Mediator

Partition Ratio

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*P < 0.05
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 1.25μM 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

Control mES FL β-catenin
WT FL β-catenin
MUT FL β-catenin

tagged

endogenous

anti-β-catenin

anti-β-ACTIN

B

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C

Wt FL β-catenin
Mut FL β-catenin

D

Control Reporter

Fold activation

0 5 10 15

Control
Wt FL β-catenin
Mut FL β-catenin
**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
U2OS-2-6-3

LacI-MED1-IDR TCF1 Merge
U2OS-2-6-3

LacI-MED1-IDR TCF3 Merge
U2OS-2-6-3

LacI-MED1-IDR TCF7L2 Merge
U2OS-2-6-3

B

LacI-MED1-IDR Hp1α Merge
U2OS-2-6-3

C

HA-TCF7L2
Transfection
HEK 293T cells
Co-IP
WT MUT
Blot
10% Input
Lig IP Flag IP HA IP
WT MUT
anti-FLAG

D

TE 1

TE 2

E

β-catenin Wt Chimera Mut Chimera
anti-GFP
anti-β-actin

F

Control Reporter

G

CFP

Fold activation

Control Reporter

Normalized expression

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

β-catenin
armadillo
chimera

Actrt2
Fam168b
Figure S7. Related to Figure 7
S7A) Western blot of mES cells expressing 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.
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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.