Supplementary data:

Gene	*Entrez GeneID*	*Fold change*	*Log fold change*	*Adj. P value*
l7Rn6	67669	+1.953190	0.965833	0.00221
H19	14955	+2.055409	1.039425	0.00462
Rhox5/Pem	18617	+1.684209	0.752071	0.00818
Trpv6	64177	-1.685105	-0.752829	0.02326
Daam1	208846	-1.842641	-0.881775	0.04218

Table S1 related to Figure 1: Differentially expressed genes between β-$cat^{fl/fl}$ and β-$cat^{\Delta/\Delta}$ ESCs.

a

Gene	For	Rev
Mixl1	AGTTGCTGGAGCTCGTCTTC	CTCCCAGATCTCCCTGAGTG
Gata6	GCCAACTGTCACACCACAAC	CCTCTTGGTAGCACCAGCTC
Gata4	CTGTGCCAACTGCCAGACTA	GCGATGTCTGAGTGACAGGA
Gapdh	CAAATGGGGTGAGGCCGGTGCTGAGTAT	CGGCATCGAAGGTGGAAGAGTGGGAGTT
Pax6	AGGGGGAGAGAACACCAACT	GGTTGCATAGGCAGGTTGTT
Fgf5	GCGACGTTTTCTTCGTCTTC	GAAGTGGGTGGAGACGTGTT
Nestin	GATCGCTCAGATCCTGGAAG	GCTCTGGGAGGACACCAGTA
Brachyury	CTCTAAGGAACCACCGGTCA	CCATTGCTCACAGACCAGAG
Foxa2	GTTAAAGTATGCTGGGAGCCG	CGCCCACATAGGATGACATG

b

Gene	For	Rev
Mixl1	AGTTGCTGGAGCTCGTCTTC	AGGGCAATGGAGGAAAACTC
Gata6	ATTCACCAGCAGCGACTAGCAG	TCCAACCTGACTTTTGATTCCTCG
Foxa2	GTTAAAGTATGCTGGGAGCCG	CGCCCACATAGGATGACATG
Brachyury	CTCTAAGGAACCACCGGTCA	CCATTGCTCACAGACCAGAG
Cdx1	ACGCCCTACGAATGGATG	CTTGGTTCGGGTCTTACCG
Axin2	GCAGGAGCCTCACCCTTC	TGCCAGTTTCTTTGGCTCTT
Nanog	CCTCAGCCTCCAGCAGATGC	CCGCTTGCACTTCACCCTTTG
Oct4	GAAGCAGAAGAGGATCACCTTG	TTCTTAAGGCTGAGCTGCAAG
Sox2	GGCAGCTACAGCATGATGCAGGAGC	CTGGTCATGGAGTTGTACTGC
Rex1	GGCCAGTCCAGAATACCAGA	GAACTCGCTTCCAGAACCTG
Plakoglobin	CTGTGTGCCCTCTGTAAGCA	GAACTGTCCTCGCCTGAGAC
Hprt	AGCTACTGTAATGATCAGTCAACG	AGAGGTCCTTTTCACCAGCA

Table S2 Primer sequences for semiquantitative PCR (**a**) and qPCR (**b**).

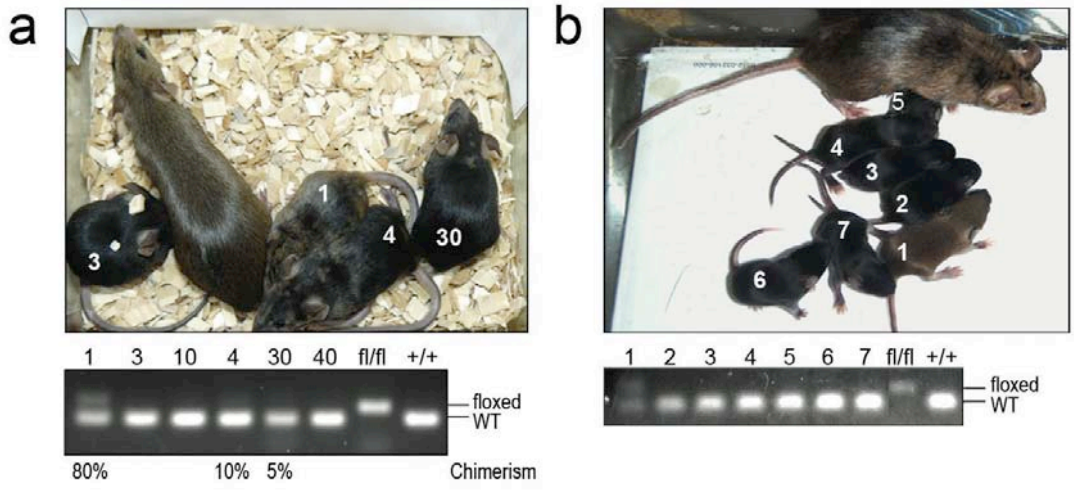


Figure S1: NLβ-12 *β-cat*$^{fl/fl}$ ESCs are fully pluripotent. (**a**) Chimeras generated from blastocyst injections of NLβ-12 *β-cat*$^{fl/fl}$ mESCs, with PCR genotyping results shown below. Estimated grade of chimerism based on coat color for the chimeric pups #1, #4 and #30 is indicated below. Note: pups #10 and #40 are not shown in the picture. (**b**) Litter from chimeric animal #1 (80% chimeric) after cross with C57Bl/6, showing for pup #1 transmission of the floxed allele through the germ line, based on coat color and genotyping PCR shown below.

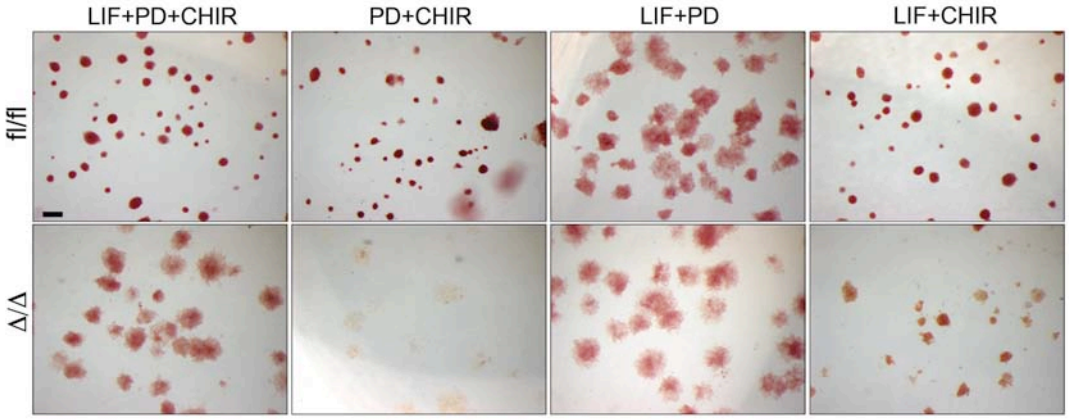


Figure S2 Alkaline phosphatase staining on β-$cat^{fl/fl}$ and β-$cat^{\Delta/\Delta}$ mESCs cultured in the following media LIF+2i, 2i (PD0325901+CHIR99021), LIF+PD0325901 and LIF+CHIR99021 for 5 days. Scale bar 200 μm.

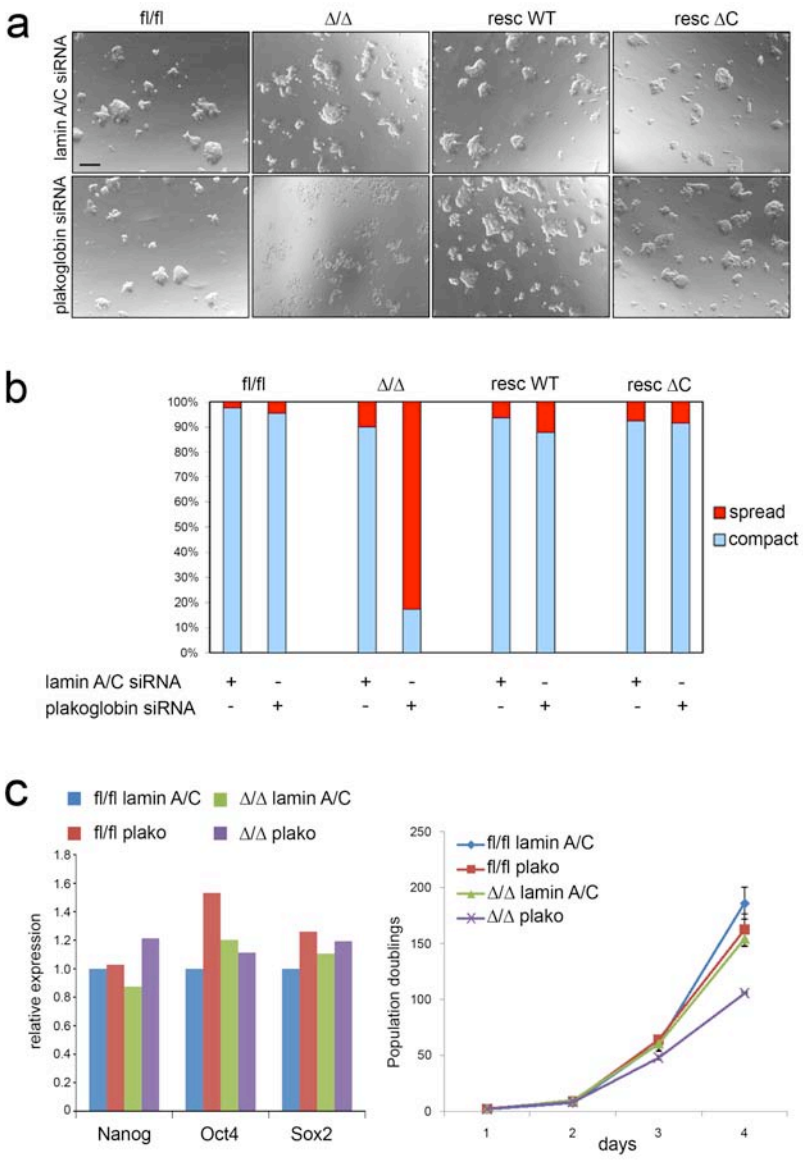


Figure S3 Quantification of morphological changes upon control lamin A/C siRNA and plakoglobin siRNA treatment. (**a**) Phase contrast images of β-cat$^{fl/fl}$, β-cat$^{\Delta/\Delta}$, β-cat^{rescWT} and β-cat$^{resc\Delta C}$ mESC cultures 48 hours after treatment with control lamin A/C or plakoglobin siRNA. Scale bar 200 µm. (**b**) Quantification of the percentage of mESC colonies showing morphological changes 48 hours after siRNA treatment; with their appearance being either spread (indicated by red color), similar to phase contrast image of β-cat$^{\Delta/\Delta}$ mESCs treated with plakoglobin siRNA shown in (A), or compact (indicated by blue color). (**c**) Histogram showing relative expression levels of *Nanog*, *Oct4* and *Sox2* on day 4 of culturing (n=2), and growth curve showing population doublings over 4 days of β-cat$^{fl/fl}$ and β-cat$^{\Delta/\Delta}$ mESC cultures treated with lamin A/C or plakoglobin siRNA performed in triplicates.

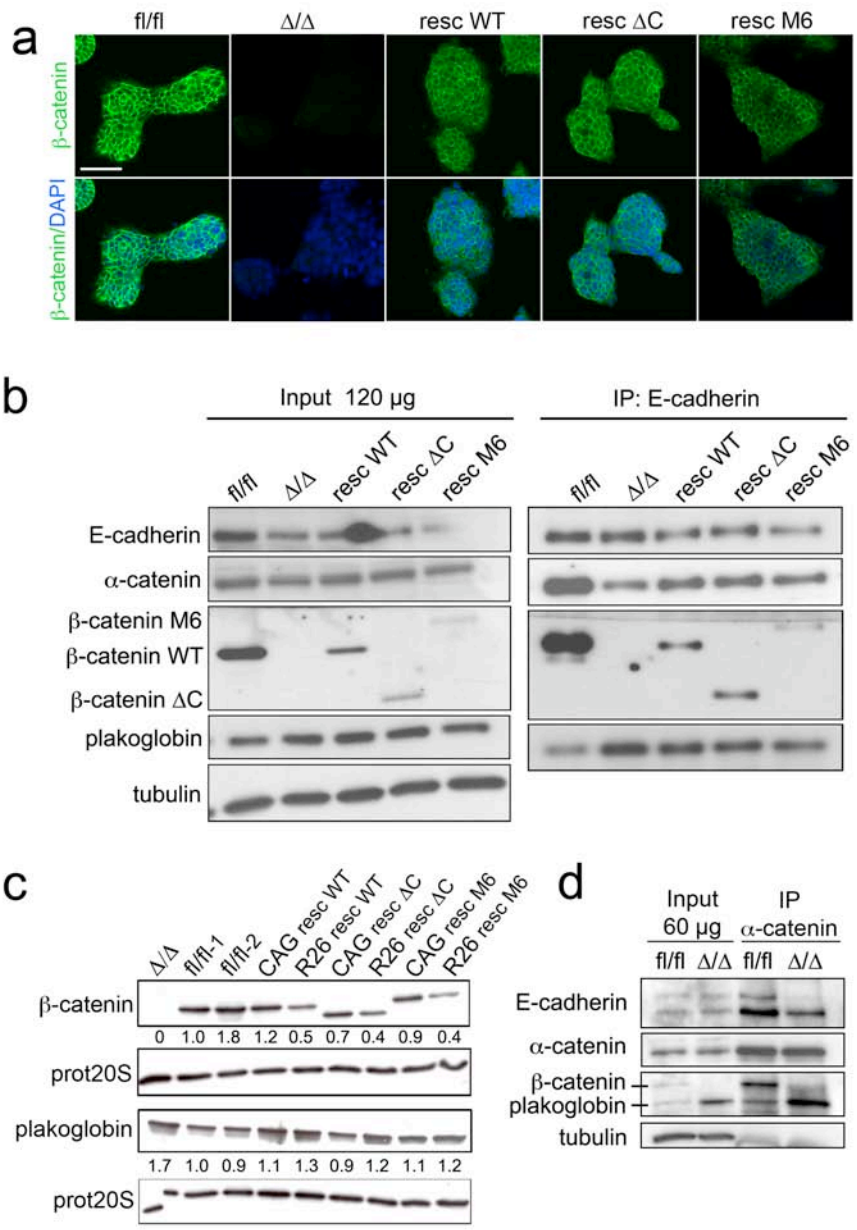


Figure S4 Characterization of β-catenin rescued mESCs with regard to cell-adhesion. (**a**) Confocal images (scale bar 50 µm) of immunofluorescent staining for β-catenin and β-catenin/DAPI on *β-cat*$^{fl/fl}$, *β-cat*$^{\Delta/\Delta}$, and Rosa26 *β-cat*^{rescWT}, *β-cat*$^{resc\Delta C}$ and *β-cat*^{rescM6} mESCs. (**b**) Western blot analyses for E-cadherin, α-catenin, β-catenin (WT, ΔC, M6), plakoglobin and tubulin showing input and E-cadherin immunoprecipitates (IP) in *β-cat*$^{fl/fl}$, *β-cat*$^{\Delta/\Delta}$, *β-cat*^{rescWT}, *β-cat*$^{resc\Delta C}$ and *β-cat*^{rescM6} mESCs. (**c**) Western blot showing β-catenin and plakoglobin protein levels in *β-cat*$^{fl/fl}$, *β-cat*$^{\Delta/\Delta}$, and pCAGGS and Rosa26 rescued mESCs compared to proteasome20S. (**d**) Western blot analyses for E-cadherin, α-catenin, β-catenin, plakoglobin and tubulin showing input and α-catenin immunoprecipitates in *β-cat*$^{fl/fl}$ and *β-cat*$^{\Delta/\Delta}$ mESCs.

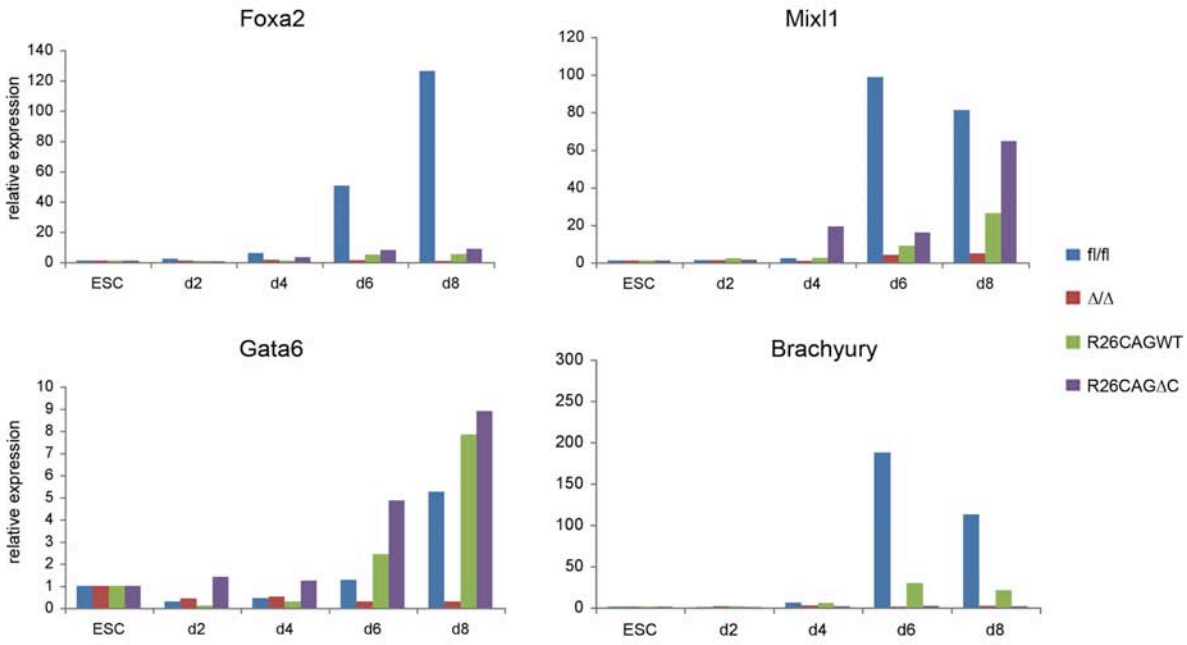
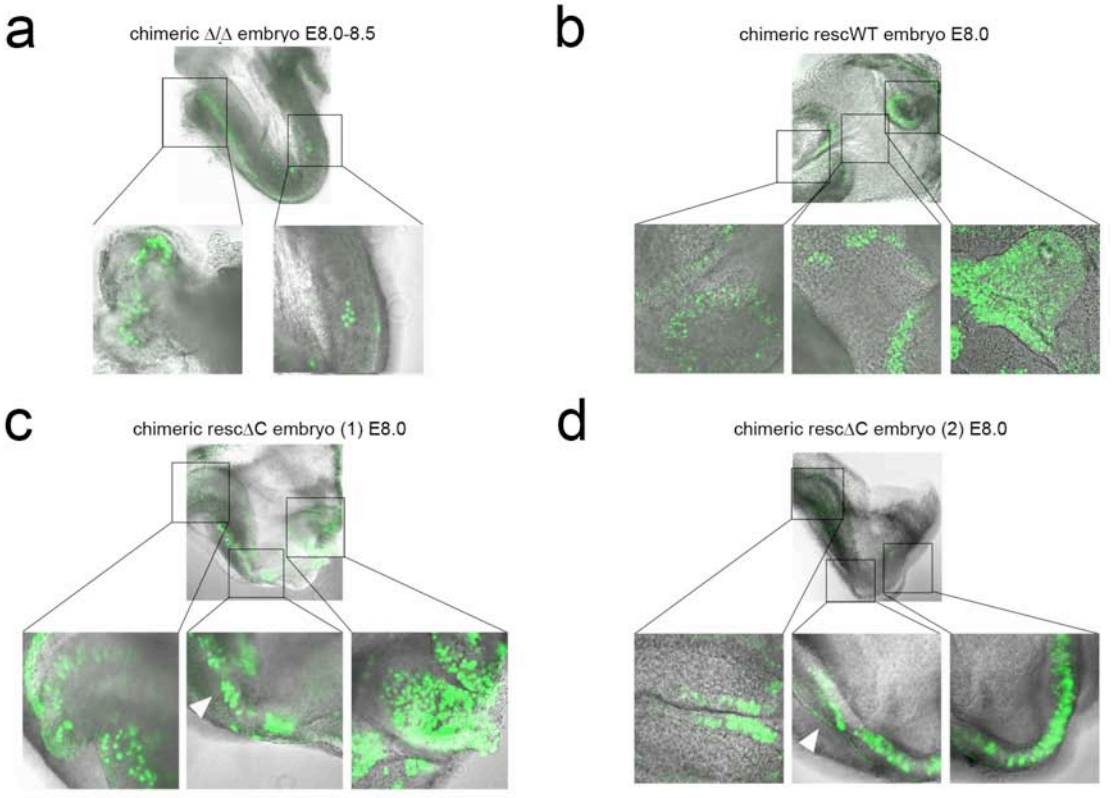


Figure S5 (related to Figure 7a): Histograms showing relative expression levels of endodermal markers Foxa2, Mixl1, Gata6 (n=1 each) and of the mesodermal marker Brachyury (n=2) in β-cat$^{fl/fl}$, β-cat$^{\Delta/\Delta}$, and Rosa26-CAG β-cat^{rescWT}, β-cat^{rescΔC} mESCs and during EB differentiation.



e

ESC clone	blastocysts injected	embryos recovered	E8.5	E8.0	E7.5	E7.0	GFP neg.	low [1]	medium [2]	high [3]
Δ/Δ	53	24	14	7	1	2	10	11	2	1*
rescWT	30	15	6	2	4	0	3	6	3	3
rescΔC	54	22	8	8	8	0	7	5	2	8

* E6.5/7.0 embryo;
[1] chimeric contribution similar to example shown in a; [2] similar to example schown in d; [3] similar to examples shown in b and c.

Figure S6 In vivo contribution analysis of mutant β-cat$^{\Delta/\Delta}$, and Rosa26 β-cat^{rescWT}, β-cat^{rescΔC} mESCs. Chimeric E8.0-8.5 embryos generated by injection of H2B-GFP marked mutant mESCs. Embryos are oriented head to the left, tail to the right. (**a**) Chimeric embryo showing contribution of β-cat$^{\Delta/\Delta}$ mESCs primarily to the head fold, with very few GFP-positive cells in the trunk region. (**b**) Chimeric embryo showing large contribution of Rosa26 β-cat^{rescWT} mESCs to the headfolds, trunk and tail regions. (**c,d**) Chimeric embryos showing large (**c**) and medium (**d**) contribution of Rosa26 β-cat^{rescΔC} mESCs to the headfolds, trunk and tail regions. Note: low contribution or absence of GFP-positive cells in the mesoderm layer (white arrowheads in c, d). Overview image (upper images) were taken with 10x, images in the boxed regions were taken with 25x objective. (**e**) Table summarizing the data from the analyses of chimeric embryos.

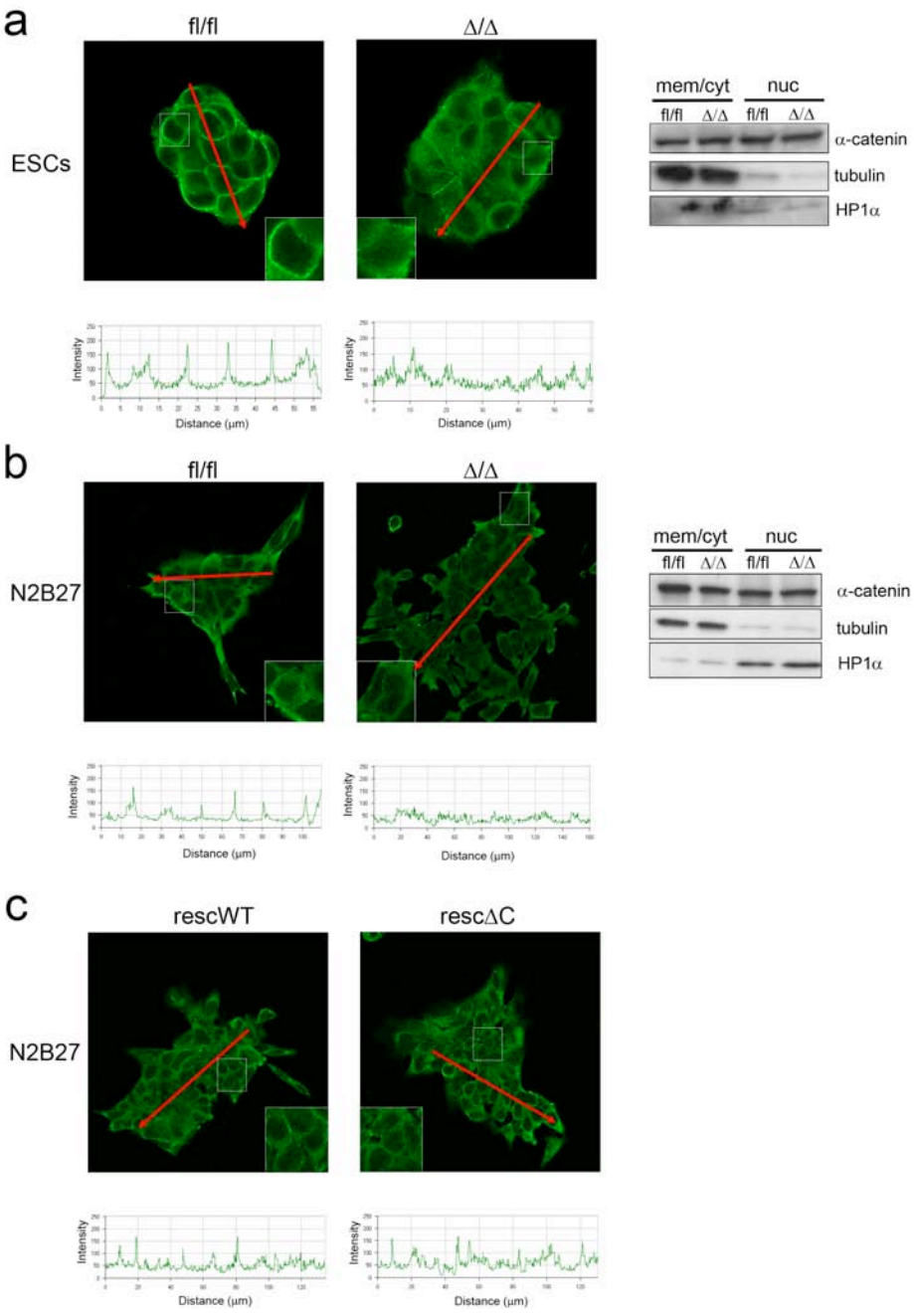


Figure S7 Distribution of α-catenin analysed by immunofluorescence and western blots. Line scan data for α-catenin are shown below each image. The red arrow indicates the scanned line.
(**a**) Immunofluorescent staining and western blot of membranous/cytoplasmic (mem/cyt) and nuclear (nuc) fractions for α-catenin of *β-cat*$^{fl/fl}$ and *β-cat*$^{Δ/Δ}$ mESCs under serum+LIF conditions. (**b**) Immunofluorescent staining and western blot of membranous/ cytoplasmic (mem/cyt) and nuclear (nuc) fractions for α-catenin of *β-cat*$^{fl/fl}$ and *β-cat*$^{Δ/Δ}$ mESCs that have been differentiating for 4 days in N2B27 medium. (**c**) Immunofluorescent staining for α-catenin in Rosa26 *β-cat*^{rescWT} and *β-cat*^{rescΔC} mESCs that have been differentiating for 4 days in N2B27 medium.

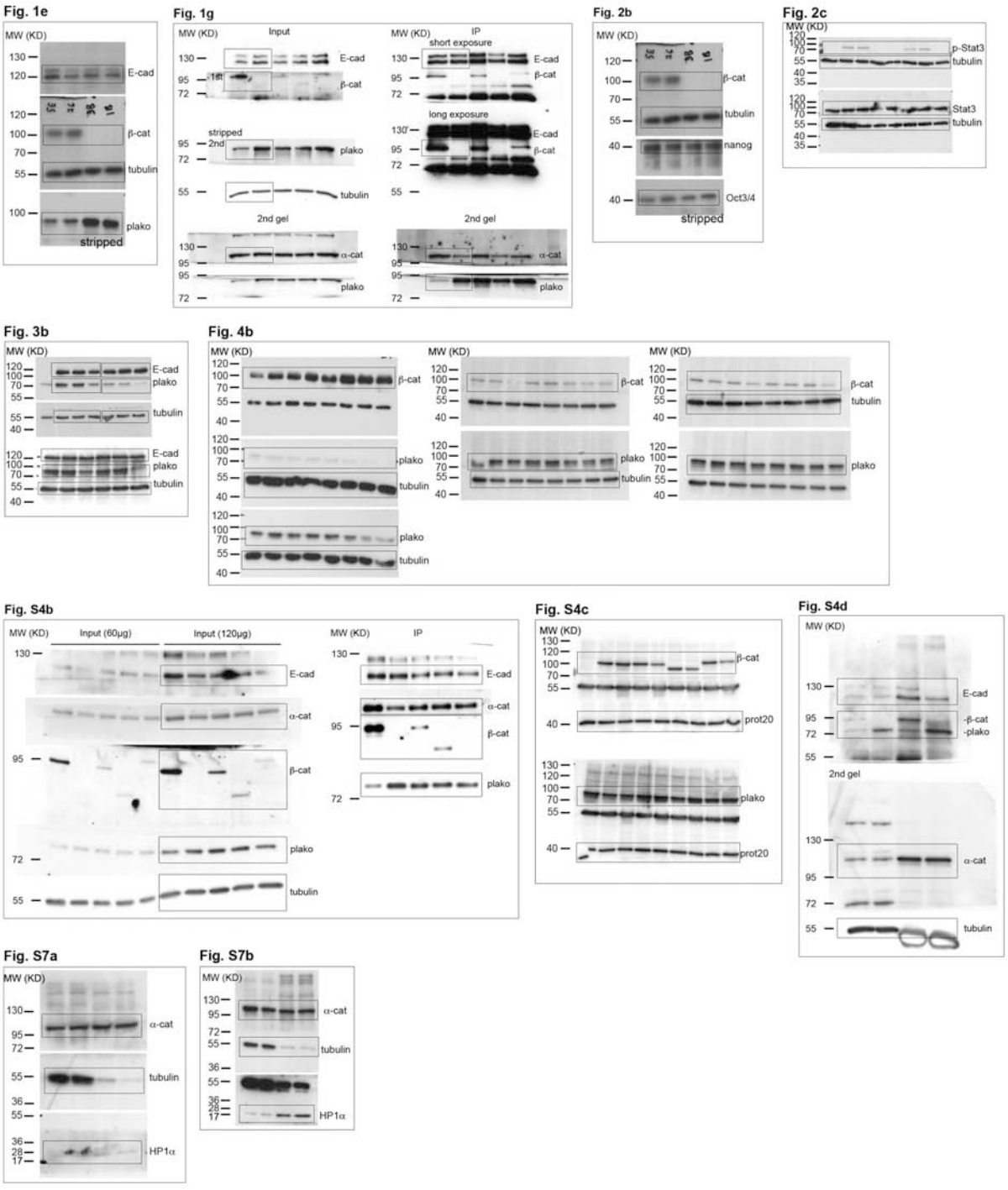


Figure S8 Full scan data of western blots. The bands shown in the figures are indicated by the boxed regions.