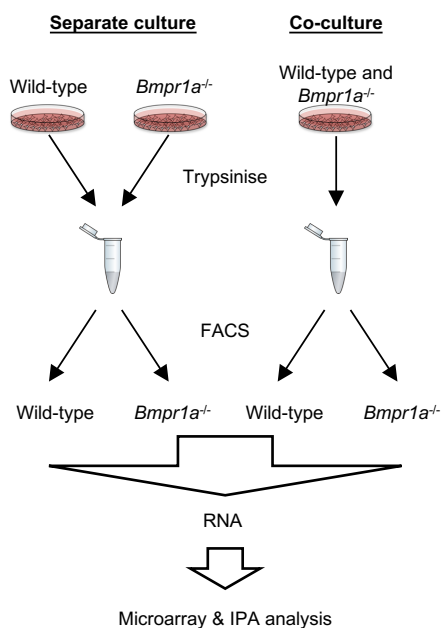
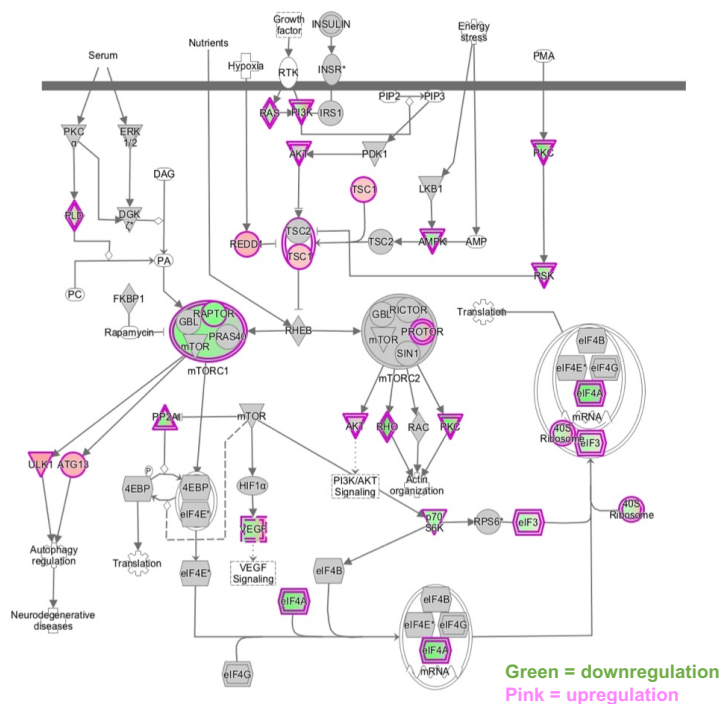


Supplementary information

A

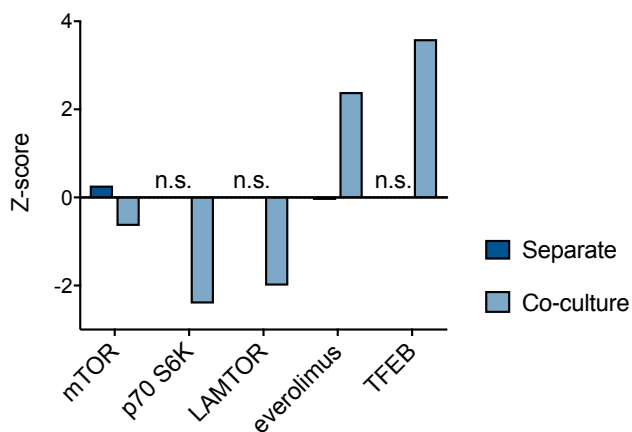


B



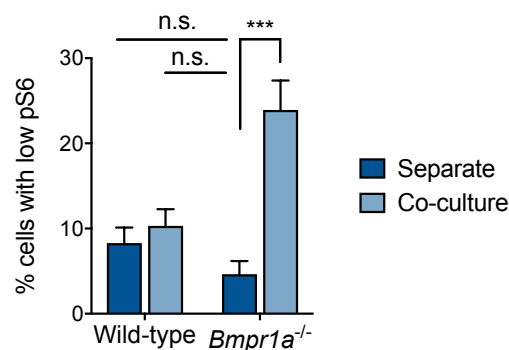
C

***Bmpr1a*^{-/-} vs wt**



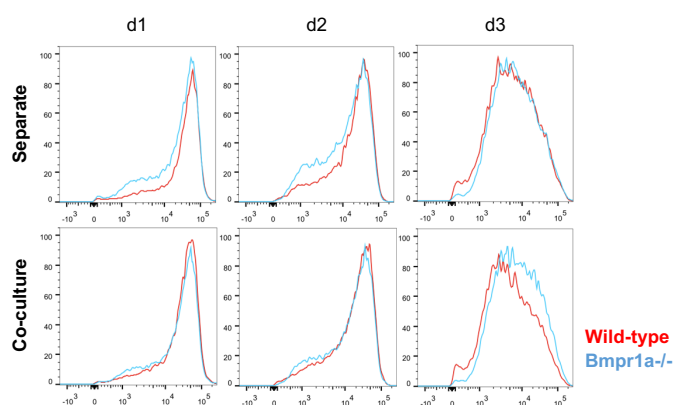
D

% cells low pS6

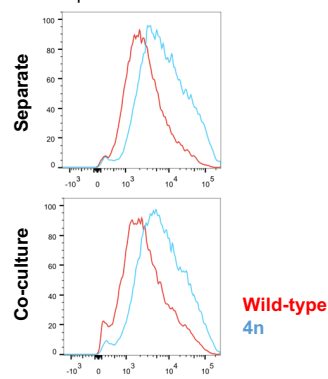


E

pS6 ESC media

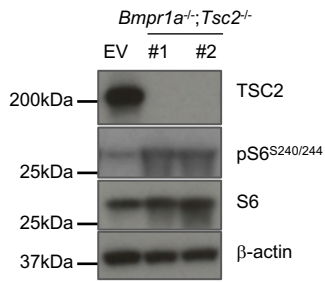
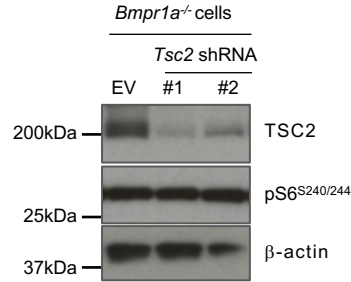
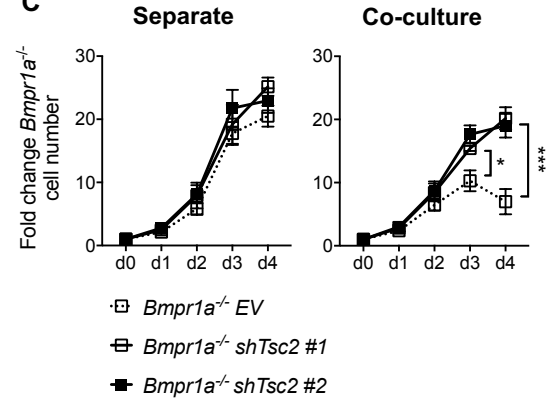
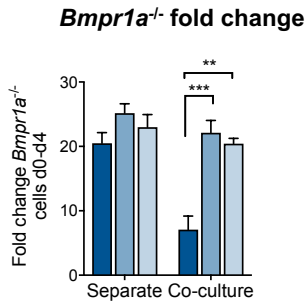
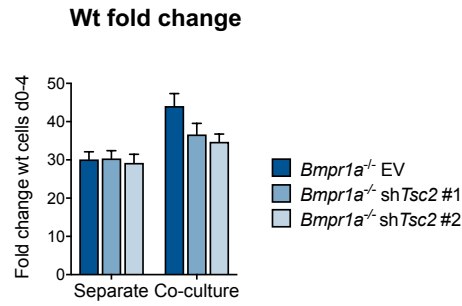
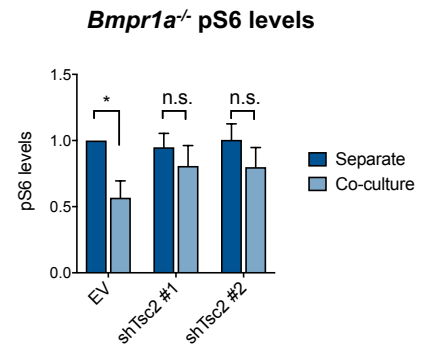
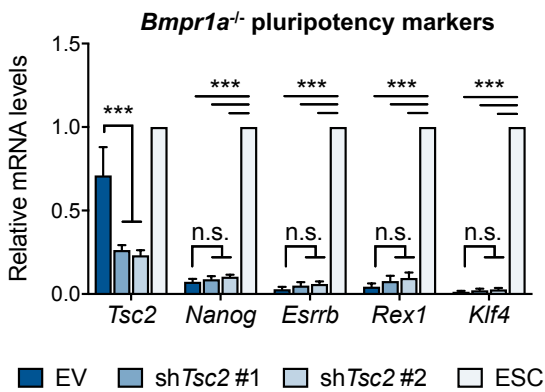
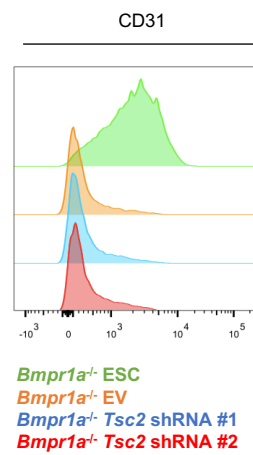
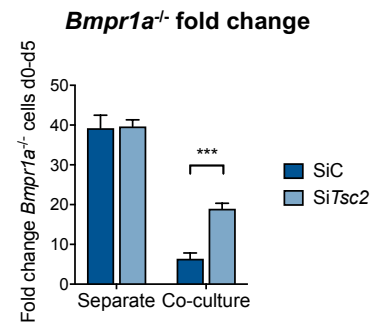
**F**

pS6 ESC media



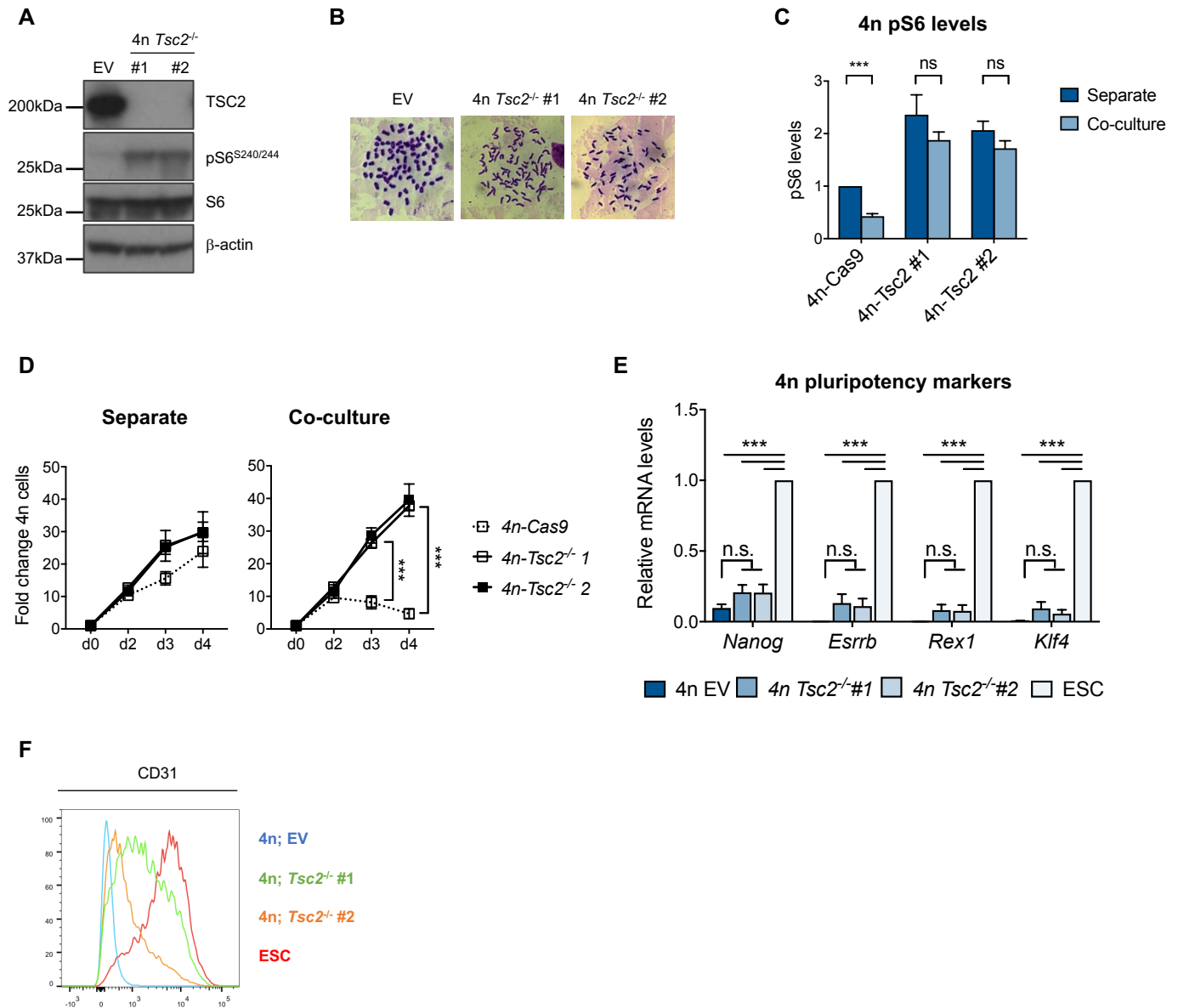
Supplementary Figure 1. Further analysis of mTOR activation during competition.

A. Schematic of the experimental setup, whereby cells were cultured for three days in N2B27 media before FACS, RNA collection, and gene expression analysis by microarray. B. Diagrammatic heat-map revealing mTOR pathway components differentially regulated in *Bmpr1a*^{-/-} compared to wild-type cells in competitive co-cultures. Green denotes pathway components which are downregulated; pink denotes pathway components which are upregulated. The schematic canonical pathway diagrams were generated through the use of IPA (QIAGEN Inc., <https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis>). C. IPA-derived Z-score of mTOR and related pathways in *Bmpr1a*^{-/-} compared with wild-type cells in separate and co-culture. D. Percentage of cells with low pS6 staining in wild-type and *Bmpr1a*^{-/-} cells cultured separately and together for 3 days in N2B27. ***, $p < 0.005$; ANOVA and Tukey's post-hoc test. E. Flow cytometry analysis of pS6^{S240/244} in wild-type and *Bmpr1a*^{-/-} cells over three days in ESC media, where pluripotency is maintained and competition is blocked. F. Flow cytometry analysis of pS6^{S240/244} in wild-type and 4n cells at day 3 in ESC media. $n=3$ for all studies.

A**B****C****D****E****F****G****H****I**

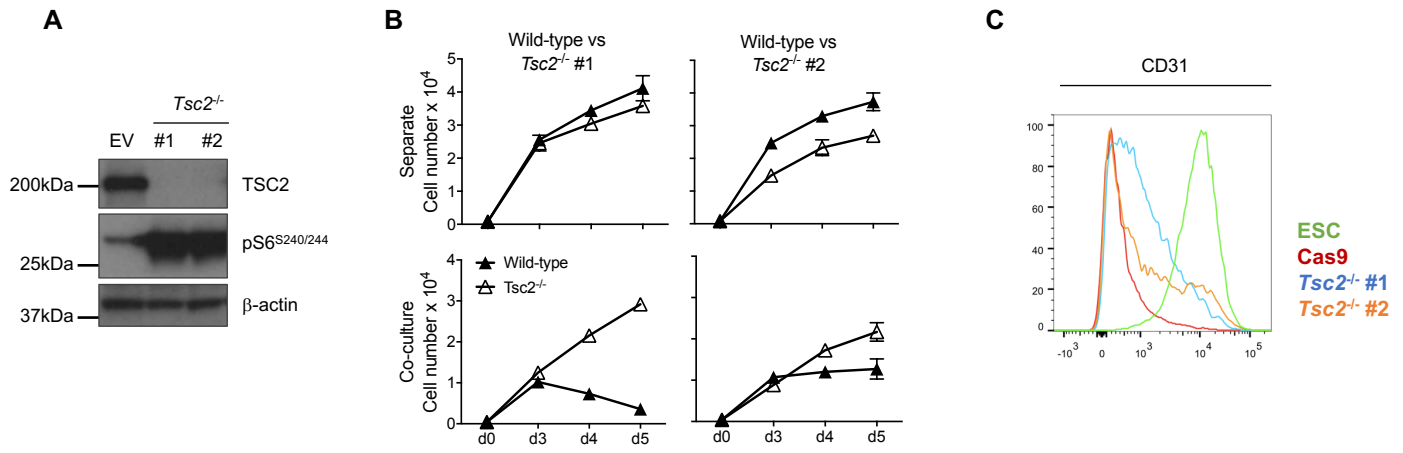
Supplementary Figure 2. mTOR pathway hyper-activation rescues elimination of BMP defective cells independently of differentiation.

A. TSC2 and pS6 levels in *Bmpr1a*^{-/-} cells and *Bmpr1a*^{-/-}; *Tsc2*^{-/-} cells where *Tsc2* has been mutated by CRISPR or (B) inhibited using two independent shRNAs. C. Growth curves of *Bmpr1a*^{-/-} cells with or without *Tsc2* shRNA inhibition in separate and co-culture with wild-type cells. D. Fold change in *Bmpr1a*^{-/-} and *Bmpr1a*^{-/-}; *Tsc2*^{shRNA} (two independent clones) cell numbers between d0-d4 when cultured alone (separate) or with wild-type cells in N2B27. E. Fold change in wild-type cell numbers between d0-d4 when cultured separately or co-cultured with *Bmpr1a*^{-/-} or *Bmpr1a*^{-/-}; *Tsc2*^{shRNA} cells. F. Levels (median fluorescence) of pS6^{S240/244} analysed by flow cytometry in wild-type and *Bmpr1a*^{-/-} cells infected with viruses expressing control or *Tsc2*-targetting shRNA. G. *Bmpr1a*^{-/-} cells were infected with control and *Tsc2*-targetting shRNAs, cultured for three days in N2B27, and expression levels of pluripotency genes *Nanog*, *Esrrb*, *Rex1* and *Klf4* were assessed by qPCR and compared *Bmpr1a*^{-/-} cultured in pluripotent conditions. H. Levels of cell-surface pluripotency marker CD31 were assessed by flow cytometry in pluripotent and differentiating *Bmpr1a*^{-/-} cells infected with control or *Tsc2*-targetting shRNAs. I. Fold change over d0-d5 in *Bmpr1a*^{-/-} cells in the presence of control (SiC) or *Tsc2*-targetting siRNA (Si*Tsc2*). n=3 for all studies. Error bars denote SEM. **p<0.01 and *** p<0.005; ANOVA and Tukeys post-hoc test (C-G and I).



Supplementary Figure 3. Loss of *Tsc2* rescues 4n cell elimination during competition independently of differentiation.

A. Two 4n clones targeted by CRISPR and lacking TSC2 were picked for competition analysis. B. Karyotypic analysis of 4n-*Tsc2*^{-/-} cells. C. Levels (median fluorescence) of pS6^{S240/244} analysed by flow cytometry in wild-type and control/ *Tsc2*^{-/-} 4n cells. D. Fold change in wild-type cells from d0-d4 when cultured alone and in the presence of 4n-Cas9 and the two 4n-*Tsc2*^{-/-} clones. E. Expression levels of pluripotency genes *Nanog*, *Esrrb*, *Rex1* and *Klf4* in control and *Tsc2*^{-/-} 4n cells cultured in N2B27 for 3 days were compared to pluripotent 4n cells by qPCR. Error bars denote SEM. F. Levels of cell-surface pluripotency marker CD31 were assessed by flow cytometry in pluripotent and differentiating control and *Tsc2*-knockout 4n cells. *** $p < 0.005$; ANOVA and Tukeys post-hoc test (C,D,E).



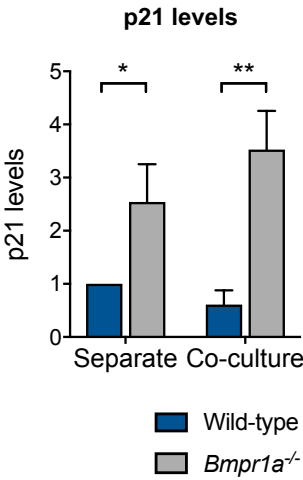
Supplementary Figure 4. Analysis of super-competition mediated by *Tsc2* deletion.

A. Two wild-type clones targeted by CRISPR and lacking TSC2 were picked for competition analysis. B. Growth curves of wild-type and two *Tsc2*^{-/-} cell clones cultured together and separately for 5 days. C. Levels of cell-surface pluripotency marker CD31 were assessed by FACS in pluripotent and differentiating control and *Tsc2*^{-/-} cells. n=3 for all studies.

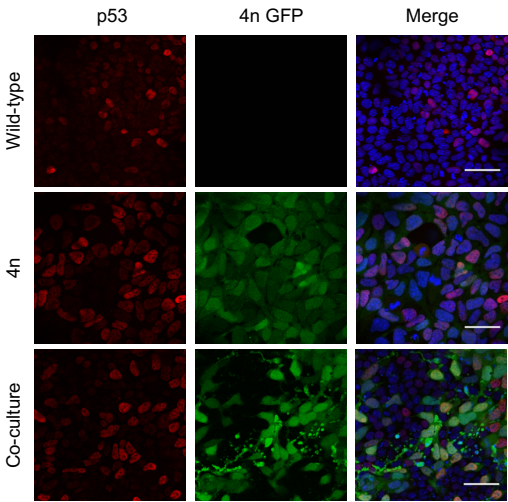
A

Upstream Regulator	Molecule Type	Predicted Activation State	Activation z-score	p-value of overlap
TP53	transcription regulator		0.849	6.73E-33
MYC	transcription regulator	Inhibited	-2.846	2.59E-18
L-dopa	chemical - endogenous mammalian		-1.895	3.87E-18
beta-estradiol	chemical - endogenous mammalian	Inhibited	-3.534	1.93E-17
ERBB2	kinase		0.097	2.55E-17
ESR1	ligand-dependent nuclear receptor		-1.12	2.90E-17
TGFB1	growth factor		-1.259	6.87E-15
HNF4A	transcription regulator	Activated	2.042	8.99E-15
NUPR1	transcription regulator	Activated	2.397	2.74E-13
PTEN	phosphatase		1.194	6.53E-13

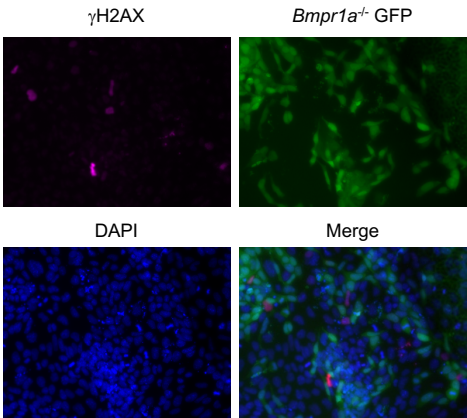
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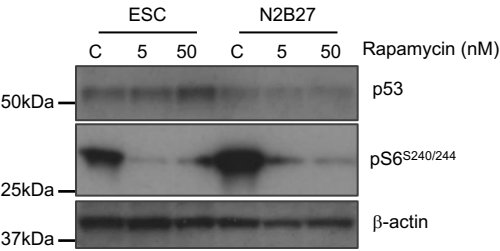
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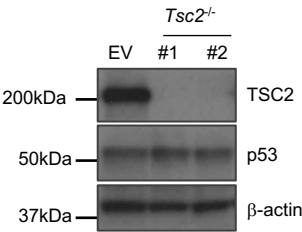
D



E

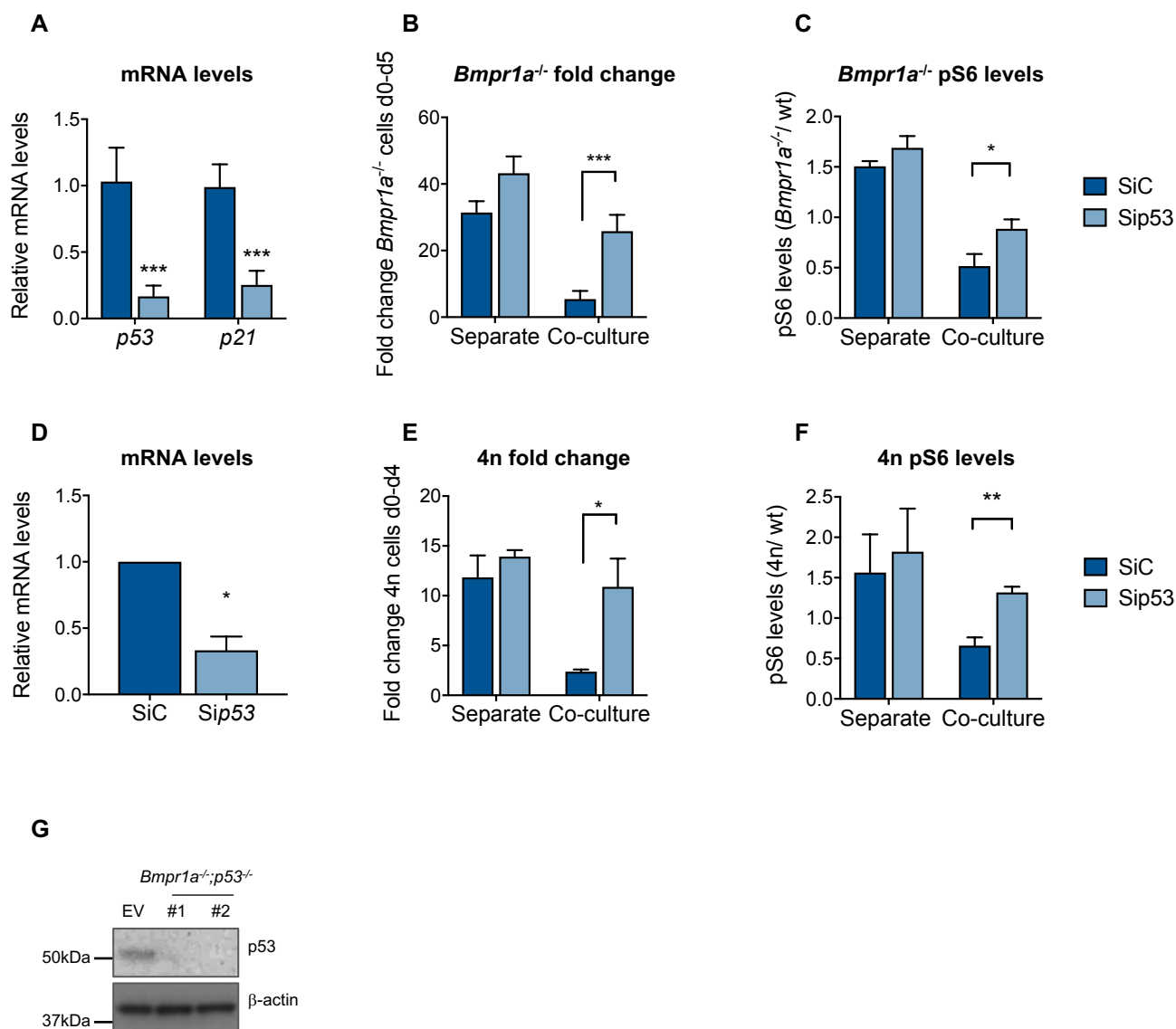


F



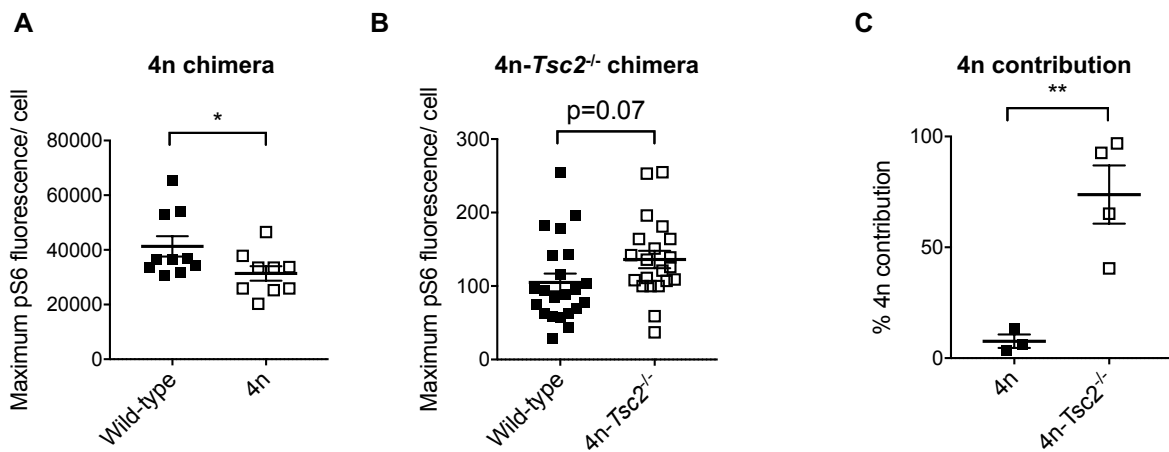
Supplementary Figure 5. Further analysis of p53 levels and possible activators in competition.

A. List of top 10 upstream regulators ranked by *p*-value with differential expression in *Bmpr1a*^{-/-} cells compared with wild-type cells during competition. B. Levels of p21 expression in wild-type and *Bmpr1a*^{-/-} cells in separate and co-culture assessed by qPCR. C. Levels of p53 in wild-type and 4n cells cultured separately and together were assessed by immunofluorescence. Scale bar=50μm. D. Protein levels of DNA double strand break marker gamma-H2AX in co-cultured wild-type and *Bmpr1a*^{-/-} cells were assessed by immunofluorescence. E. Cells were treated with rapamycin for 6 hours and levels of p53 were assessed by Western blot. F. Levels of p53 in *Tsc2*^{-/-} cells were assessed by Western blot. n=3 for all studies.



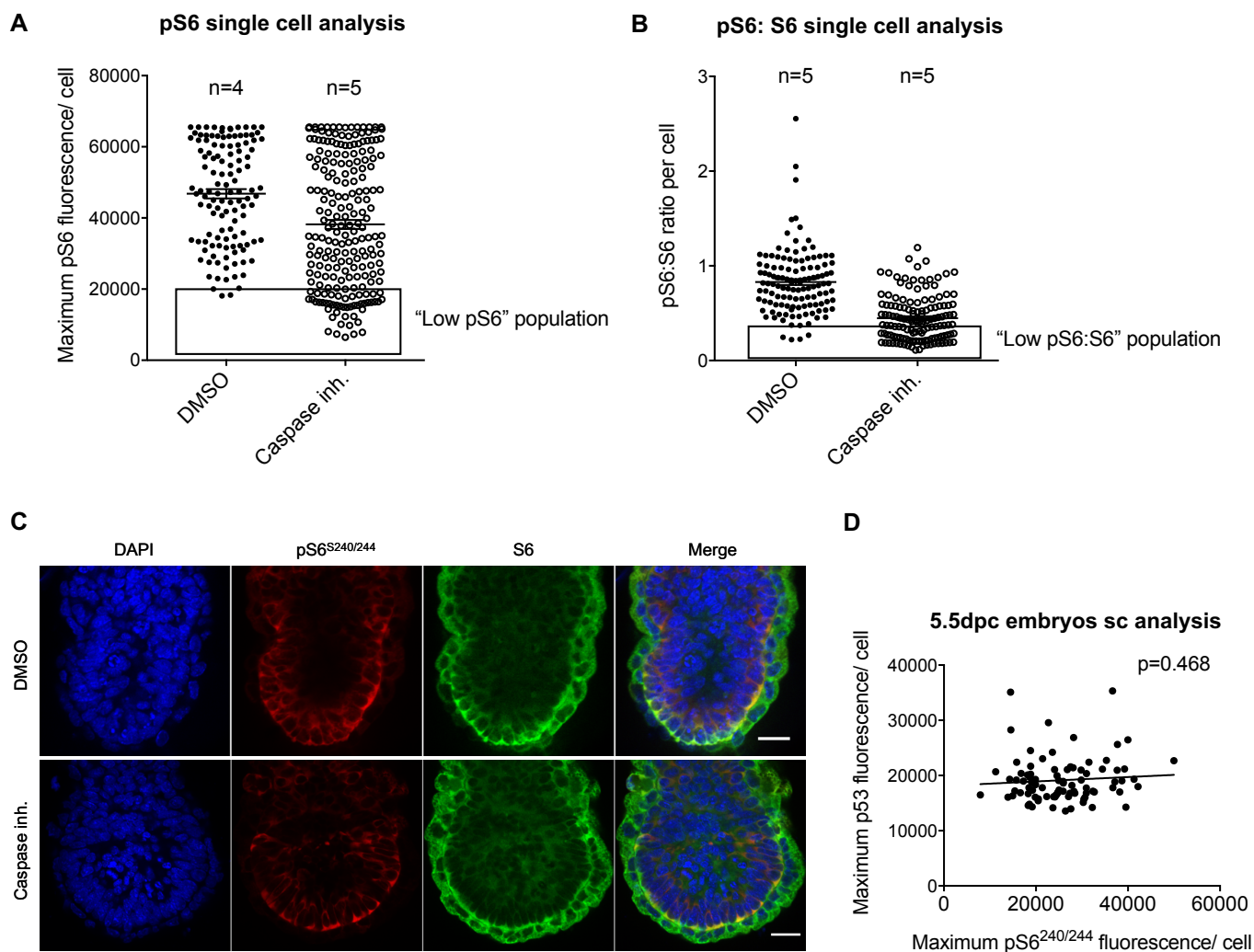
Supplementary Figure 6. *p53* knockdown rescues defective elimination and loss of mTOR activity during competition.

A. Knockdown efficiency of p53 siRNA in *Bmpr1a*^{-/-} cells was assessed by analysing mRNA levels of p53 and target p21 by qPCR. B. Fold change in *Bmpr1a*^{-/-} cell number from d0-d5 in cells cultured alone or with wild-type cells when transfected with a control siRNA or an siRNA targeting p53. C. Quantification of flow cytometry staining of pS6^{S240/244} levels in control or p53-knockdown cells at day 3 in competition. D. Knockdown efficiency of p53 siRNA in 4n cells was assessed by analysis of mRNA levels of p53 by qPCR. E. Fold change in 4n cell number from end of assay relative to initial seeding number in cells cultured alone or with wild-type cells when transfected with a control siRNA or an siRNA targeting p53. F. Quantification of median fluorescence of pS6^{S240/244} in 4n cells relative to wild-type cells when transfected with a control siRNA or an siRNA targeting p53 in separate and co-culture at day 3 in competition assessed by flow cytometry. G. Two independent *Bmpr1a*^{-/-};p53^{-/-} clones targeted by CRISPR were picked for further analysis. These had lost p53 expression as revealed by western blot analysis. n=3 for all studies. Error bars denote SEM. *p<0.05, **p<0.01 and *** p<0.005; unpaired, two-tailed *t*-test (A, E) or ANOVA and Tukeys post-hoc test (B,C,F,G).



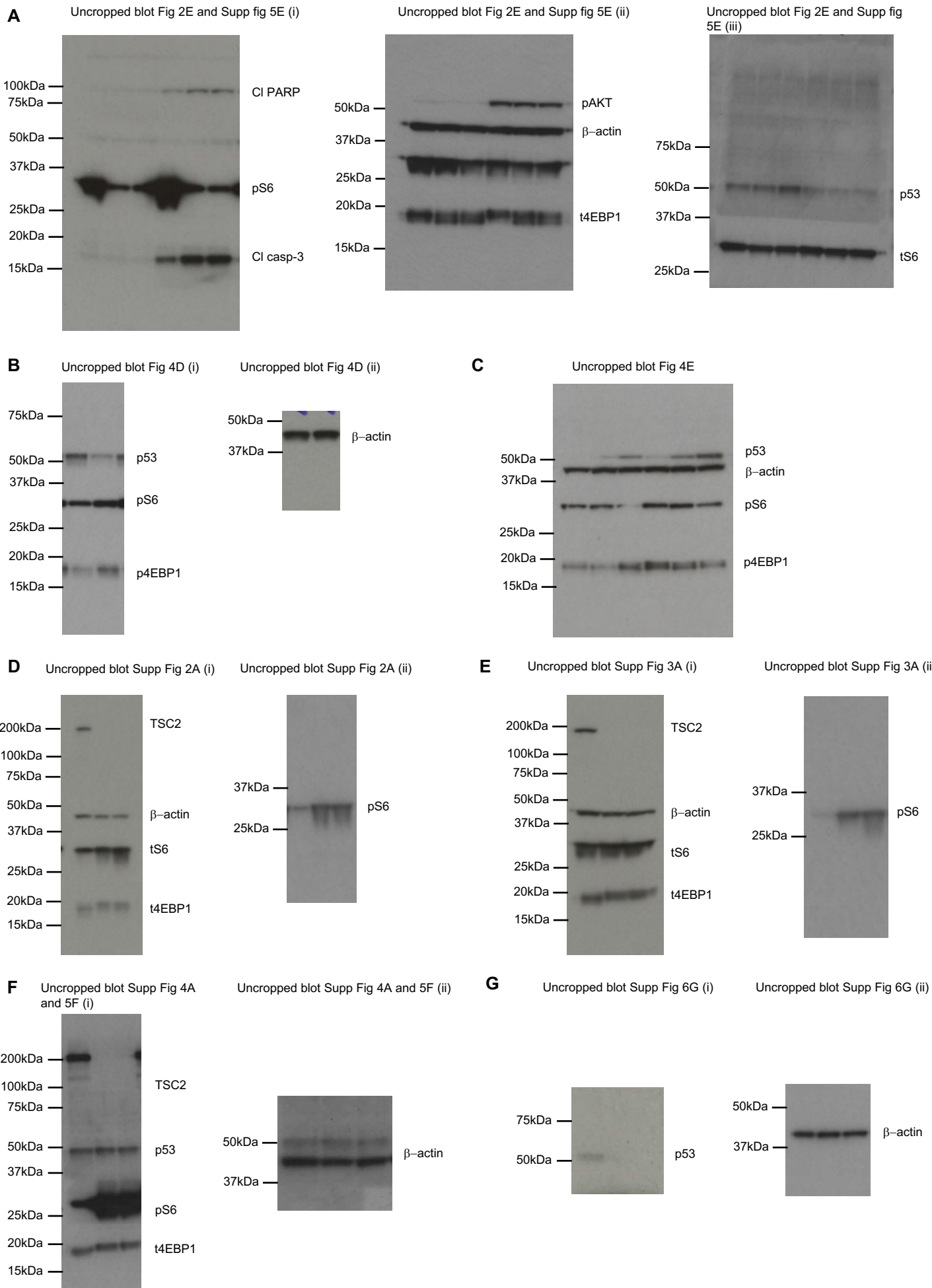
Supplementary Figure 7. Mutation of *Tsc2* rescues pS6 levels in 4n chimeras.

A. Analysis of pS6 levels detected by immunofluorescence in single cells from 4n/wild-type chimeras and (B) 4n-*Tsc2*^{-/-}/wild-type chimeras. C. Percentage contribution of 4n and 4n-*Tsc2*^{-/-} cells to wild-type embryos was quantified. *p<0.05, **p<0.01; unpaired, two-tailed *t*-test.



Supplementary Figure 8. Further analysis of the levels of pS6 and p53 in the early mouse embryo.

A. DMSO- or caspase inhibitor- treated embryos were stained for pS6 or (B) both pS6 and total S6 and levels of pS6 or pS6:S6 ratio in each epiblast cell of the embryo was quantified. The box indicates cut-off used to assign cells with low pS6 or low pS6:S6 levels. Data shown are from 1 litter (A: DMSO n=4, caspase inhibitor n=5; B: DMSO n=5, caspase inhibitor n=5). C. Immunofluorescence staining for total and phospho S6 levels in wild-type embryos cultured overnight with DMSO or a caspase inhibitor. D. Correlation at the single cell level of p53 and pS6 levels detected by immunofluorescence in 5.5dpc wild-type embryos. Scale bar= 25 μ m.



Supplementary Figure 9. Uncropped Western blots