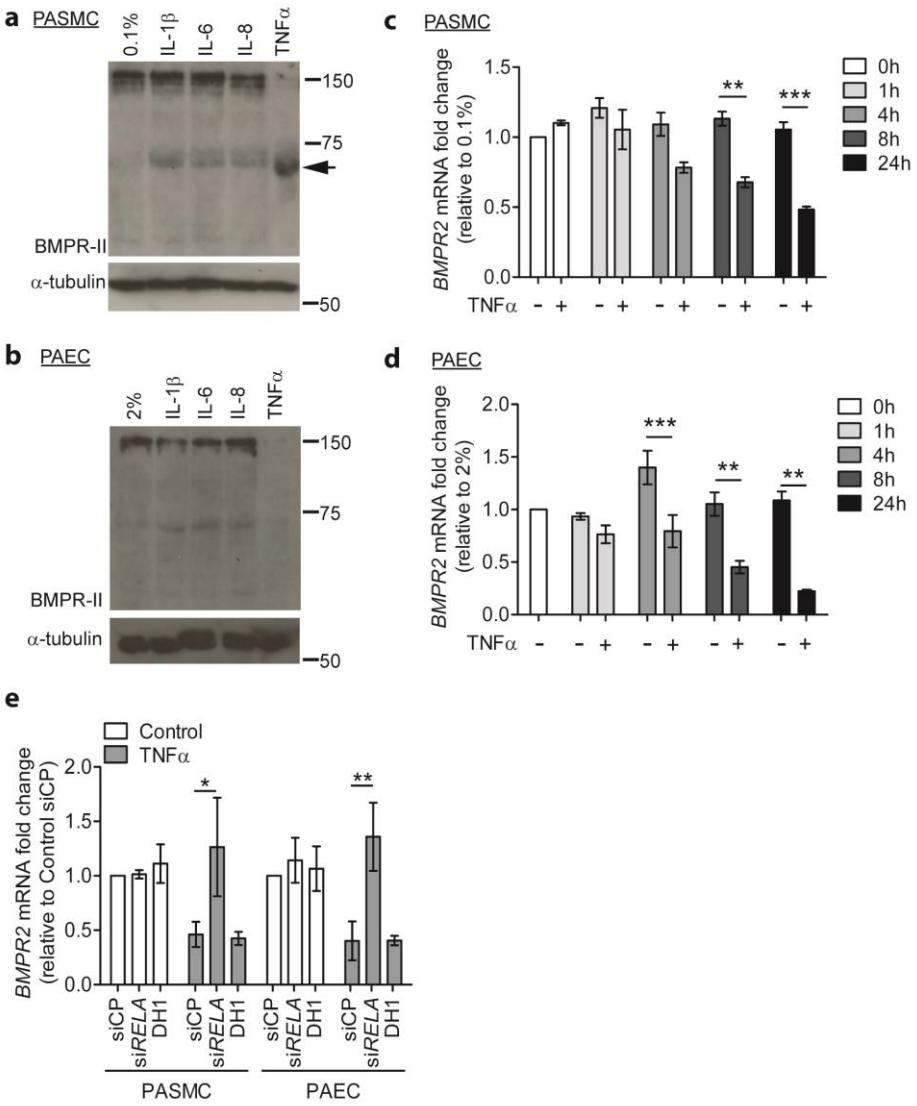
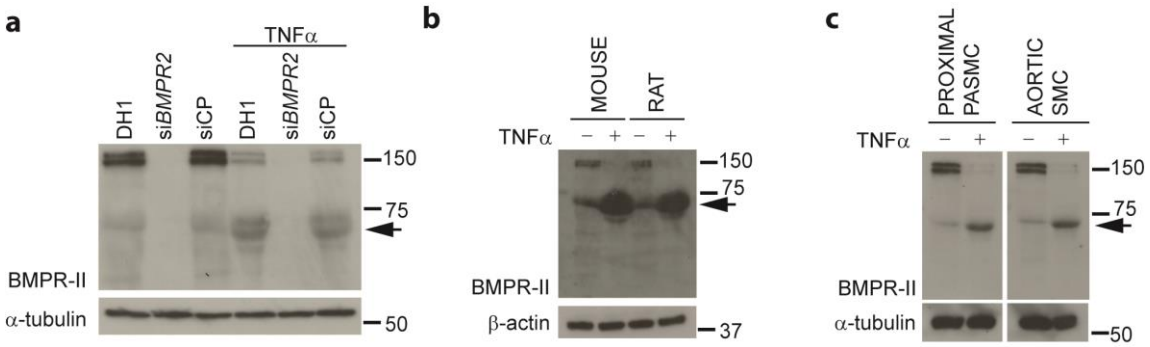
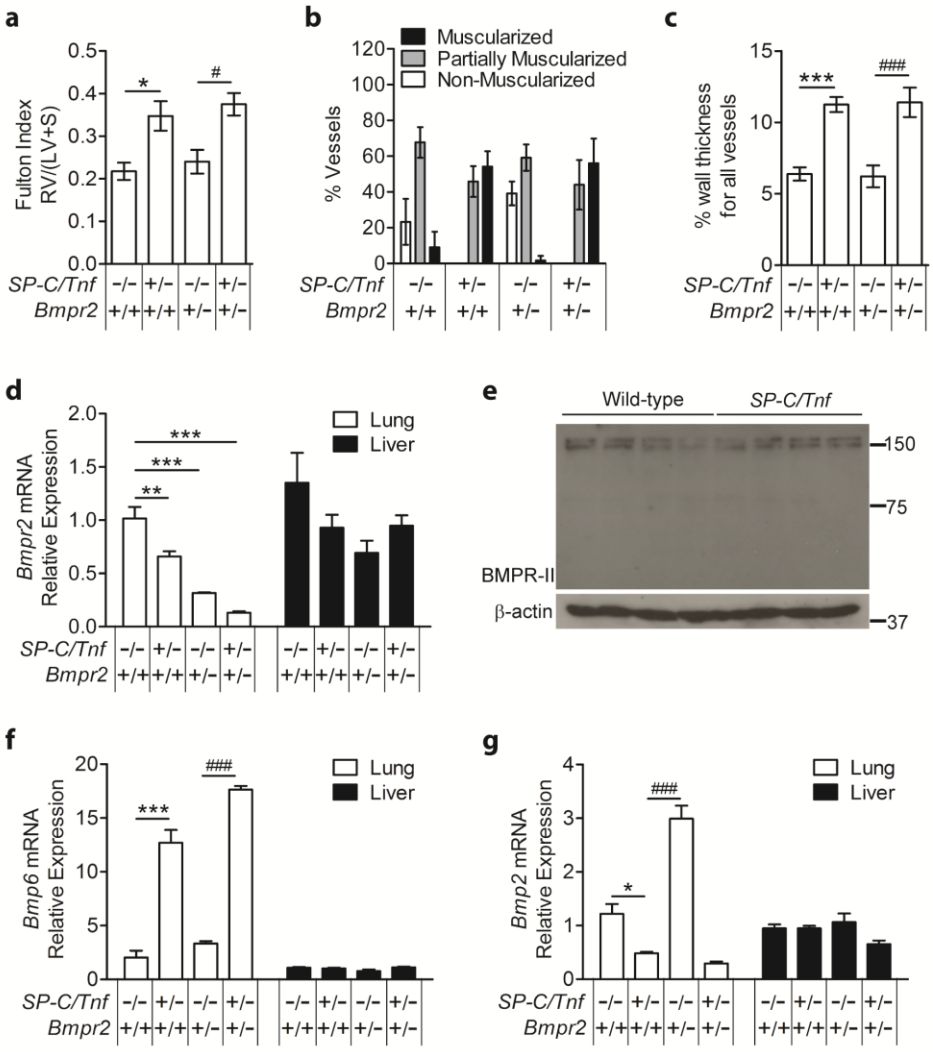


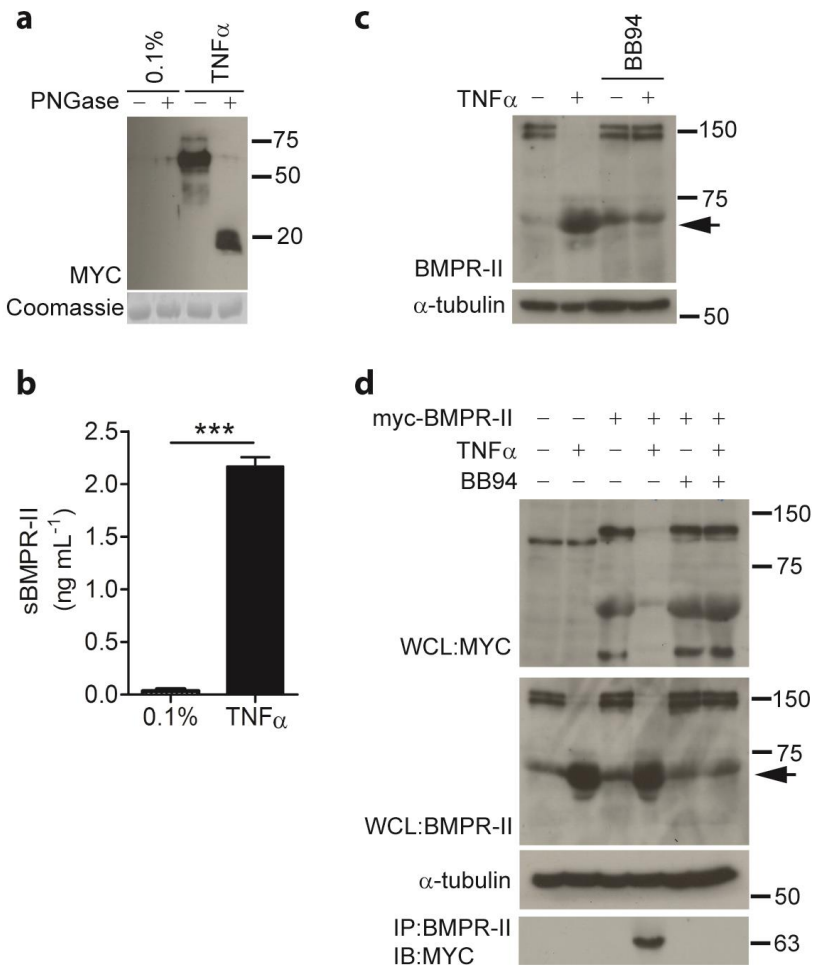
Supplementary Figure 1. TNFα reduces BMPR-II protein and mRNA expression via NF-κB/*RELA*. (**a** and **b**) Representative immunoblots of BMPR-II in human dPASMCs (**a**) and PAECs (**b**) treated with IL-1β (1 ng mL^{-1}), IL-6 (25 ng mL^{-1}), IL-8 (25 ng mL^{-1}) and TNFα (1 ng mL^{-1}) for 24 h. Blots were reprobed for α-tubulin to ensure equal loading. The data shown are representative of three experiments. (**c** and **d**) *BMPR2* mRNA expression, normalized to *ACTB*, in (**c**) human dPASMCs and (**d**) PAECs treated with TNFα (1 ng mL^{-1}) for 1, 4, 8 or 24 h (n = 3; Student's t-test). (**e**) *BMPR2* mRNA expression, normalized to *ACTB*, in human control dPASMCs and PAECs transfected with DharmaFECT1™ alone (DH1), si*RELA* or non-targeting siRNA control (siCP). Cells were treated with TNFα (1 ng mL^{-1}) for 24 h (n = 3; Student's t-test). *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$. Error bars represent mean ± s.e.m. Lower molecular mass BMPR-II is indicated by arrow.



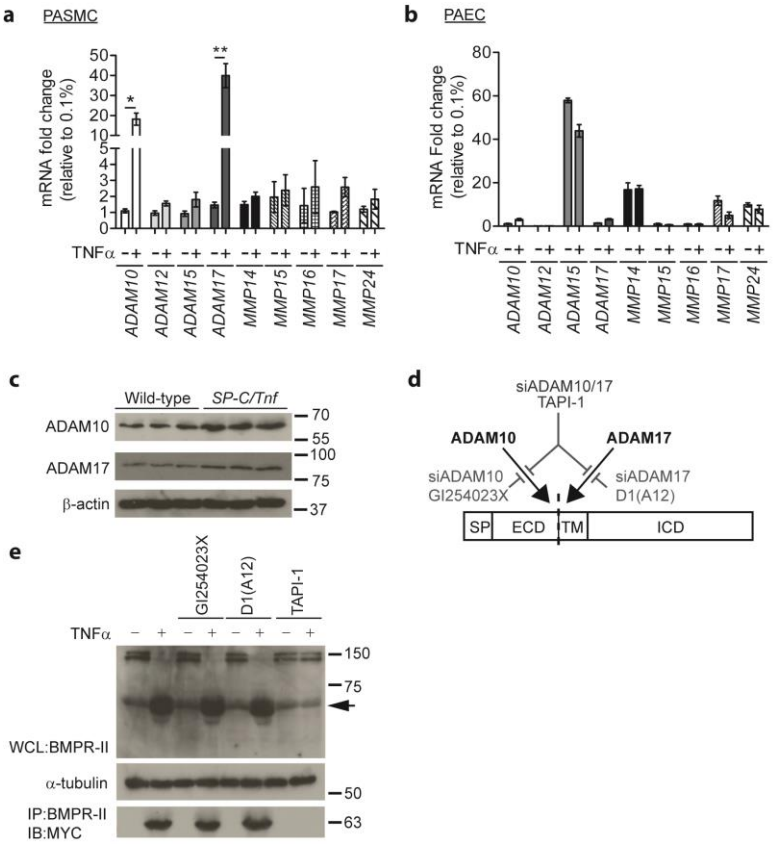
Supplementary Figure 2. TNFα reduces BMPR-II protein and generates a cleavage product in SMCs. (**a**) Representative immunoblots of BMPR-II in human control dPASMCs transfected with DharmaFECT1™ alone (DH1), si*BMPR2* or non-targeting siRNA control (siCP). Cells were treated with TNFα (1 ng mL^{-1}) for 24 h. Blots were reprobed for α-tubulin to ensure equal loading. The data shown are representative of three experiments. (**b**) Representative immunoblots of BMPR-II in mouse and rat PASMCs treated with TNFα (1 ng mL^{-1}) for 24 h. Reprobed for β-actin to ensure equal loading. The data shown are representative of three experiments. (**c**) Representative immunoblots of BMPR-II in human control proximal and aortic PASMCs treated with TNFα (1 ng mL^{-1}) for 24 h. Blots were reprobed for α-tubulin to ensure equal loading. The data shown are representative of three experiments. Lower molecular mass BMPR-II is indicated by an arrow.



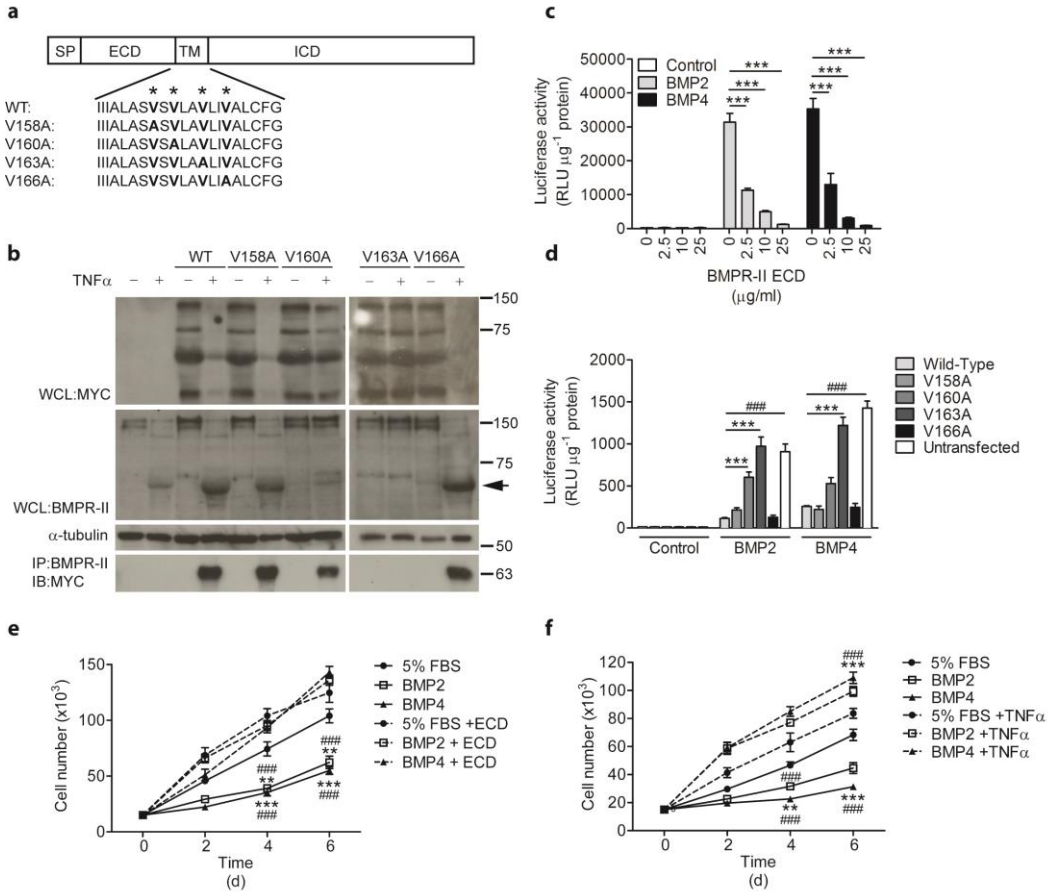
Supplementary Figure 3. Lung specific expression of TNFα induces pulmonary hypertension. *Bmpr2*$^{+/+}$, *SP-C/Tnf x Bmpr2*$^{+/+}$, *Bmpr2*$^{+/-}$ and *SP-C/Tnf x Bmpr2*$^{+/+}$ (n = 4 of each) transgenic mice were assessed at 8-9 weeks old. (**a**) Assessment of right ventricular hypertrophy (Fulton index (RV/LV+S)). (**b**) Quantification of the numbers of non-, partially and fully muscularized arteries as a percentage of total alveolar wall and duct arteries (n = 6 for all groups). (**c**) Assessment of pulmonary arterial wall thickness as a percentage of luminal diameter (n = 6 for all groups). (**d**) *Bmpr2* mRNA expression, normalized to *Actb*, in lungs and livers isolated from the above mice. (**e**) Representative immunoblot of BMPR-II expression in livers isolated from 8-9 week old *Bmpr2*$^{+/+}$ and *SP-C/Tnf x Bmpr2*$^{+/+}$ transgenic mice. Blots were reprobed for β-actin to ensure equal loading (n = 4). (**f**) *Bmp6* and (**g**) *Bmp2* mRNA expression, normalized to *Actb*, in lungs and livers isolated from the above mice. One-way ANOVA with post-hoc Tukey's for multiple comparisons used in **a**, **c**, **d**, **f** and **g**. */#$P \leq 0.05$, **$P \leq 0.01$, ***/###$P \leq 0.001$. Error bars represent mean ± s.e.m.



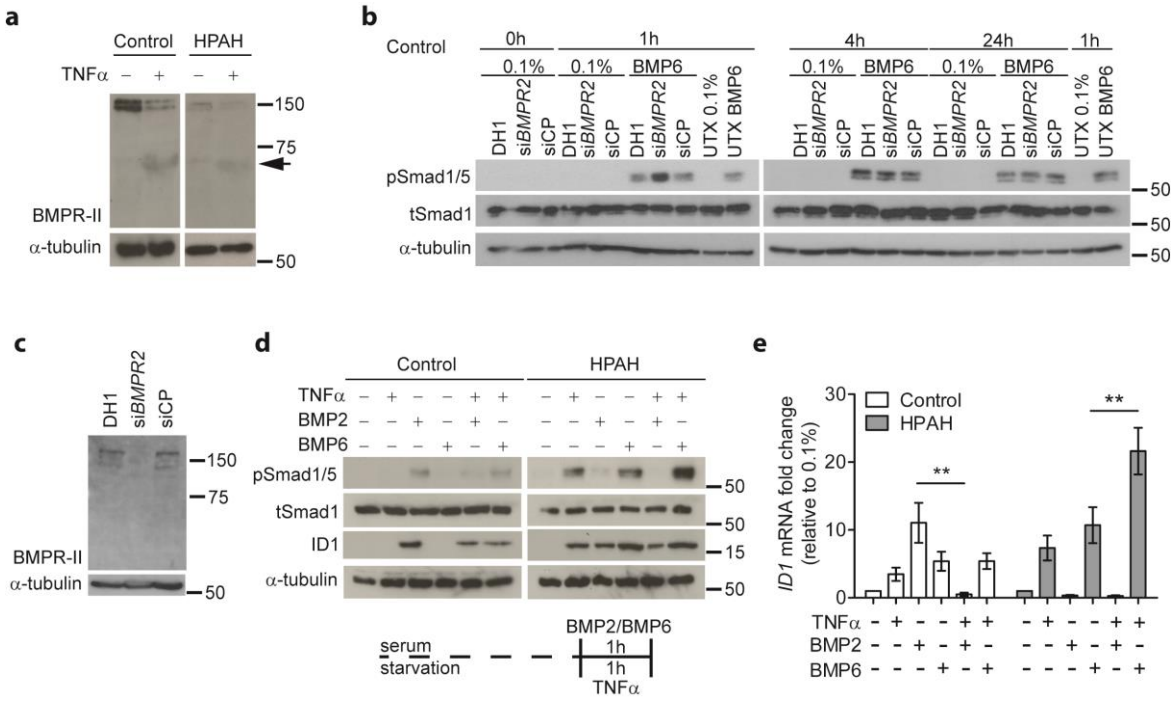
Supplementary Figure 4. Release of sBMPR-II can be inhibited by a pan-metalloprotease inhibitor. (**a**) Representative immunoblot of immunoprecipitated sBMPR-II in conditioned media from control human dPASMCs transfected with 5'-myc-tagged BMPR-II and treated with TNFα (1 ng mL^{-1}) for 24 h. Samples were deglycosylated with PNGaseF as indicated. Myc expression was assessed using immunoblotting. To ensure equal loading 0.1% FBS was loaded and assessed by Coomassie blue. (**b**) Assessment of sBMPR-II release by ELISA using control human dPASMCs treated with TNFα (1 ng mL^{-1}) for 24 h (n = 3; Student's t-test). (**c**) Representative immunoblot of BMPR-II expression of control human dPASMCs pre-treated with batimastat (BB94) (10 ng mL) for 30 m prior to TNFα (1 ng mL^{-1}) treatment for 24 h. (**d**) Representative immunoblots of BMPR-II, myc and immunoprecipitated sBMPR-II in conditioned media from control human dPASMCs transfected with 5'-myc-tagged BMPR-II and pre-treated with batimastat (BB94) (10 ng mL^{-1}) for 30 min prior to 24 h TNFα (1 ng mL^{-1}) treatment. Blots were reprobed for α-tubulin to ensure equal loading. The data shown are representative of three experiments. ***$P \leq 0.001$. Error bars represent mean ± s.e.m. Lower molecular mass BMPR-II is indicated by an arrow.



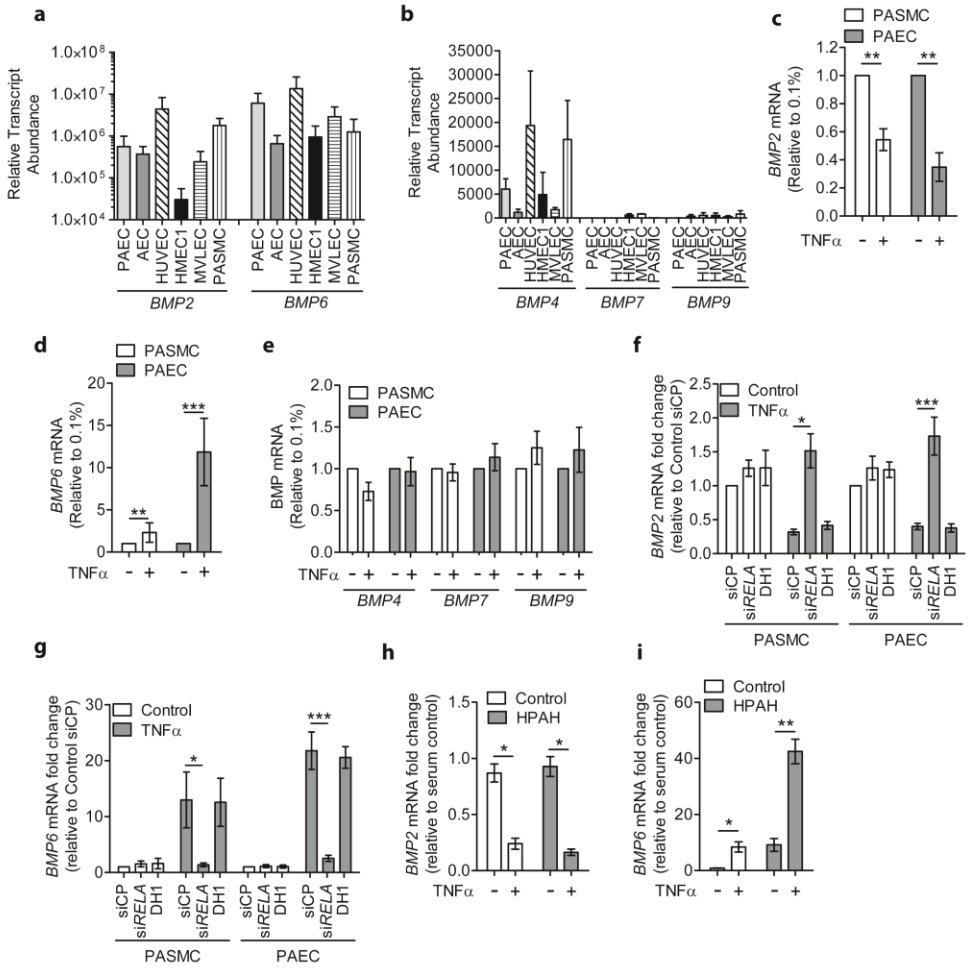
Supplementary Figure 5. TNFα induces *ADAM10/ADAM17* expression, promoting BMPR-II cleavage in SMCs. (**a** and **b**) *ADAM10*, *ADAM12*, *ADAM15*, *ADAM17*, *MMP14*, *MMP15*, *MMP16*, *MMP17* and *MMP24* mRNA expression, normalized to *ACTB*, in human control dPASMCs (**a**) and PAECs (**b**) treated with TNFα (1 ng mL^{-1}) for 24 h. (n = 4; Student's t-test). (**c**) Representative immunoblots of ADAM10 and ADAM17 expression in lungs isolated from 8 week old *Bmpr2*$^{+/+}$ (n = 3) and *SP-C/Tnf/Bmpr2*$^{+/+}$ (n = 3) transgenic mice. Blots were reprobed for β-actin to ensure equal loading. (**d**) Schematic of pharmacological and siRNA inhibition of ADAM10 and 17. (**e**) Representative immunoblots of BMPR-II, myc and immunoprecipitated sBMPR-II in conditioned media from control human dPASMCs transfected with 5'-myc-tagged BMPR-II and pre-treated with GI254023X (10 μM), D1(A12) (50 nM) or TAPI-1 (10 μM) for 30 min prior to 24 h TNFα (1 ng mL^{-1}) treatment. Blots were reprobed for α-tubulin to ensure equal loading. Data are representative of three experiments. *$P \leq 0.05$, **$P \leq 0.01$. Error bars represent mean ± s.e.m. Lower molecular mass BMPR-II is indicated by an arrow.



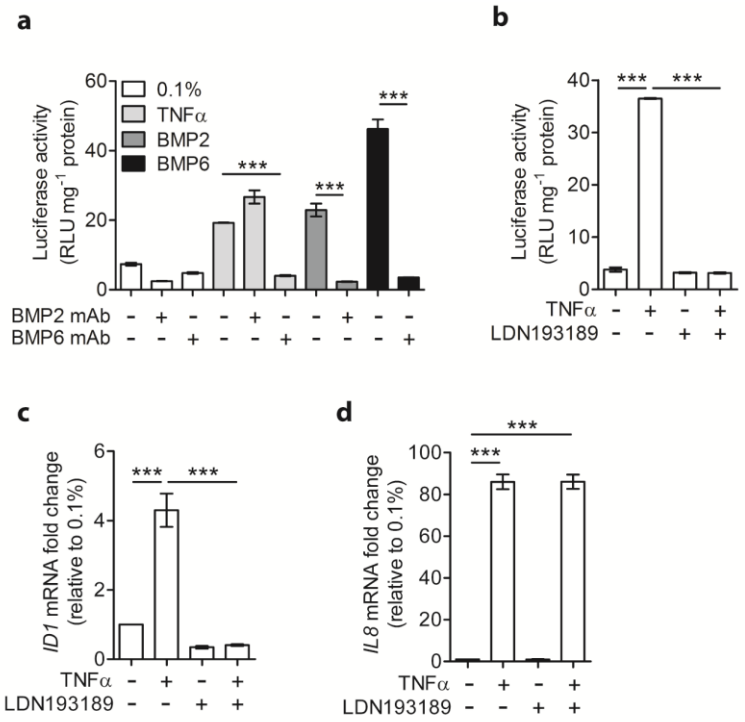
Supplementary Figure 6. Soluble BMPR-II acts as a ligand trap. (**a**) Schematic of full length BMPR-II (SP – signal peptide; ECD – ectodomain; TM – transmembrane; ICD - intracellular cytoplasmic domain). Valines (in bold) in 5'-myc-BMPR-II were converted to alanine (in bold) generating the V158A, V160A, V163A and V166A constructs (positions marked with "*"). (**b**) Representative immunoblots of BMPR-II, myc and immunoprecipitated sBMPR-II in conditioned media from control human dPASMCs transfected with wild-type and mutant 5'-myc-tagged BMPR-II constructs and treated with TNFα (1 ng mL^{-1}) for 24 h. Blots were reprobed for α-tubulin to ensure equal loading. The data shown are representative of three experiments. (**c** and **d**) Ligand trap assessment by measuring luciferase activity in C2C12-BRE cells. (**c**) C2C12-BRE cells were treated with a commercial glycosylated BMPR-II ectodomain (BMPR-II ECD) (2.5, 10 and 25 μg mL^{-1}) and BMP2 or BMP4 (10 ng mL^{-1}) for 1 h prior to measuring luciferase activity (n = 3). (**d**) C2C12-BRE cells were treated with conditioned media from control human dPASMCs transfected with wild type and the mutant 5'-myc-tagged BMPR-II constructs and treated with TNFα (1 ng mL^{-1}) for 24 h. C2C12-BRE cells were treated with conditioned media and BMP2 or BMP4 (10 ng mL^{-1}) for 1 h prior to measuring luciferase activity. (n = 3). (**e** and **f**) Proliferation assessment of human proximal PASMCs. (**e**) Cells were treated BMP2 or BMP4 (10 ng mL^{-1}) +/- BMPR-II-ECD (25 μg mL^{-1}) every 48 h for 6 days. Cells were counted on days 0, 2, 4 and 6 (n = 3; Student's t-test, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ compared with 5% FBS; $^{\#\#\#}P \leq 0.001$ compared with 5% FBS + BMPR-II ECD). (**f**) Cells were treated BMP2 or BMP4 (10 ng mL^{-1}) +/- TNFα (1 ng mL^{-1}) every 48 h for 6 days. Cells were counted on days 0, 2, 4 and 6 (n = 3; Student's t-test, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ compared with 5% FBS; $^{\#\#\#}P \leq 0.001$ compared with 5% FBS + TNFα). One-way ANOVA with post-hoc Tukey's for multiple comparisons used in **c** and **d**. $^{***/\#\#\#}P \leq 0.001$. Error bars represent mean ± s.e.m. Lower molecular mass BMPR-II is indicated by an arrow.



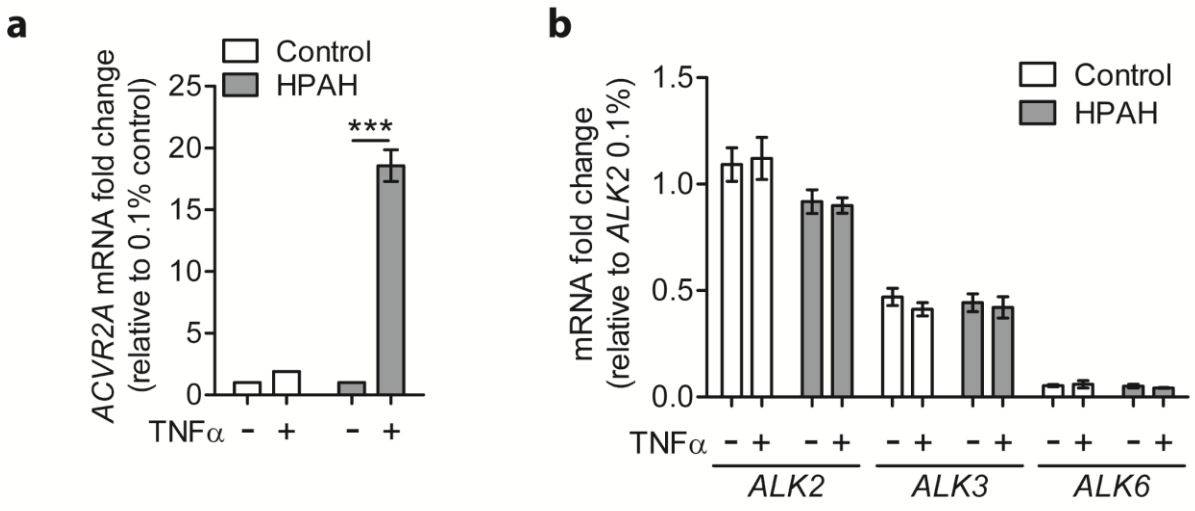
Supplementary Figure 7. TNFα changes BMP6 signaling dynamics consistent with BMPR-II siRNA. (**a**) Representative immunoblots of BMPR-II expression in human dPASMCs from disease-free controls and HPAH patients stimulated with TNFα (1 ng mL^{-1}) for 24 h. Blots were reprobed for α-tubulin to ensure equal loading. The data shown are representative of three control and HPAH cell lines. (**b** and **c**) Control human dPASMCs were transfected with *BMPR2* siRNA (si*BMPR2*) or control siRNA (siCP) using DharmaFECT1™ (DH1) followed by treatment with BMP6 (10 ng mL^{-1}) in 0.1% FBS for 1,4 or 24 hours. (**b**) Protein lysates were immunoblotted for phospho-Smad 1/5 and total Smad 1 followed by reprobing for α-tubulin to ensure equal loading. Data are representative of three separate experiments. (**c**) Immunoblotting for BMPR-II to confirm the loss of protein in siBMPR2-transfected PASMCs. Blots were reprobed for α-tubulin to ensure equal loading. Data are representative of three separate experiments. (**d**) Immunoblotting of phospho-Smad 1/5, total Smad 1 and ID1 expression in human dPASMCs from disease-free controls and HPAH patients co-stimulated with TNFα (1 ng mL^{-1}) and/or BMP2 or BMP6 (both 10 ng mL^{-1}) for 1 h. Blots were reprobed for α-tubulin to ensure equal loading. Data are representative of three control and HPAH cell lines. (**e**) *ID1* mRNA expression, normalized to *ACTB*, in human dPASMCs from disease-free controls and HPAH patients co-stimulated with TNFα (1 ng mL^{-1}) and/or BMP2 or BMP6 (both 10 ng mL^{-1}) for 1 h (n = 3; one-way ANOVA with post-hoc Tukey's for multiple comparisons). ** $P \leq 0.01$. Error bars represent mean ± s.e.m. Lower molecular mass BMPR-II is indicated by an arrow.



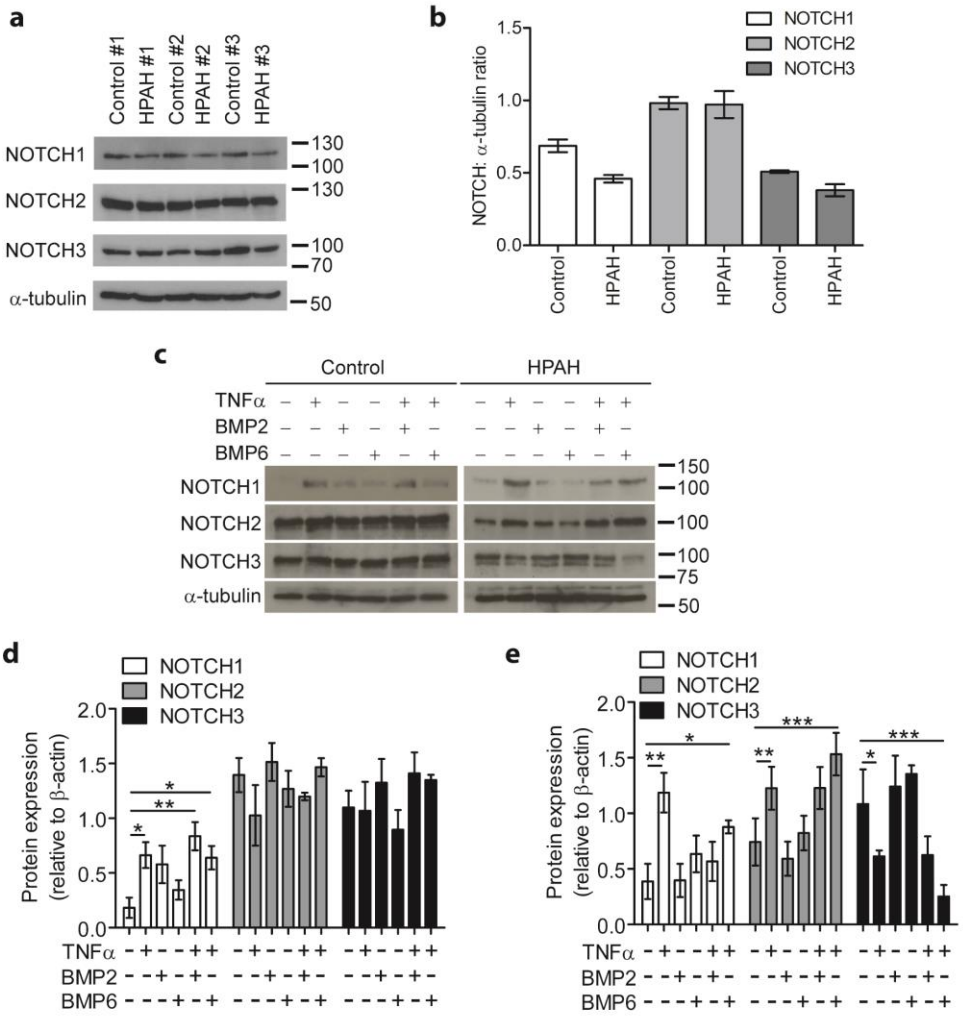
Supplementary Figure 8. *BMP2* and *BMP6* are the most abundantly expressed ligands in vascular cells. (**a** and **b**) Relative transcript abundance of *BMP2*, *BMP4*, *BMP6*, *BMP7* and *BMP9*, normalized to *B2M* and *ACTB* as detailed in the methods, in endothelial-derived cells (AECs, HMEC1s, HUVECs, HMVLECs, and PAECs) and distal smooth muscle cells (PASMCs) (n=3 independent experiments. (**c** - **e**) *BMP2* (**c**), *BMP6* (**d**), *BMP4*, *BMP7* and *BMP9* (**e**) mRNA expression, normalized to *ACTB*, in control human dPASMCs and PAECs treated with TNFα (1 ng mL^{-1}) for 24 h (n = 5; Student's t-test). (**f** and **g**) *BMP2* and *BMP6* mRNA expression, normalized to *ACTB*, in control human dPASMCs and PAECs following treatment with DharmaFECT1^{TM} alone (DH1), si*RELA* or non-targeting siRNA control (siCP) with or without 24 h TNFα (1 ng mL^{-1}) treatment (n = 3; one-way ANOVA with post-hoc Tukey's for multiple comparisons). (**h** and **i**) *BMP2* and *BMP6* mRNA expression, normalized to *ACTB*, in human dPASMCs from disease-free controls and HPAH patients stimulated with or without TNFα (1 ng mL^{-1}) for 24 h (n = 3; one-way ANOVA with post-hoc Tukey's for multiple comparisons). PAEC – human pulmonary artery endothelial cells; AEC - human aortic endothelial cells; HMEC1 - human microvascular endothelial cells; HUVEC – human umbilical vein endothelial cells; HMVLEC – human microvascular lung endothelial cells; PASMC – human distal pulmonary artery smooth muscle cells. *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$. Error bars represent mean ± s.e.m.



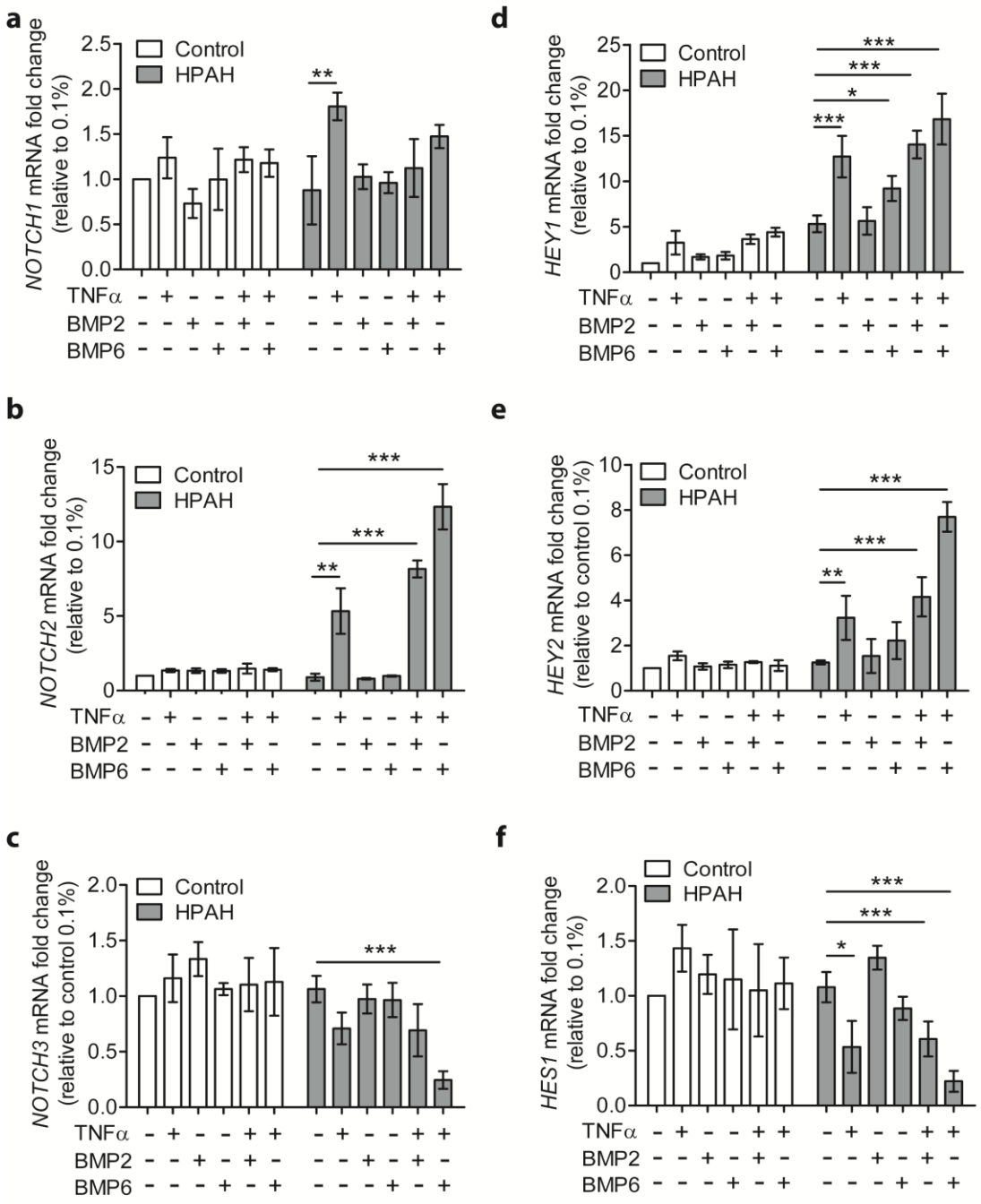
Supplementary Figure 9. TNFα promotes BMP signalling via BMP6 induction. (**a**) C2C12-BRE mouse myoblasts were treated with TNFα (1 ng mL^{-1}), BMP2 (10 ng mL^{-1}) or BMP6 (10 ng mL^{-1}) in the presence or absence of BMP2 mAb (1 µg mL^{-1}) or BMP6 mAb (1 µg mL^{-1}) for 24 h prior to measuring luciferase activity (n = 3; repeated measures ANOVA with post-hoc Tukey's test). (**b**) C2C12-BRE mouse myoblasts were treated with TNFα (1 ng mL^{-1}) and LDN193189 (250 nM) for 16 h prior to measuring luciferase activity (n = 3; repeated measures ANOVA with post-hoc Tukey's test). (**c** and **d**) *ID1* and *IL8* mRNA expression, normalized to *ACTB*, in control human dPASMCs treated with LDN193189 (250 nM) and/or TNFα (1 ng mL^{-1}) for 16 h as indicated. Data are expressed as the fold-change compared to the untreated control (n = 5; one-way ANOVA with post-hoc Tukey's for multiple comparisons). ***$P \leq 0.001$. Error bars represent mean ± s.e.m.



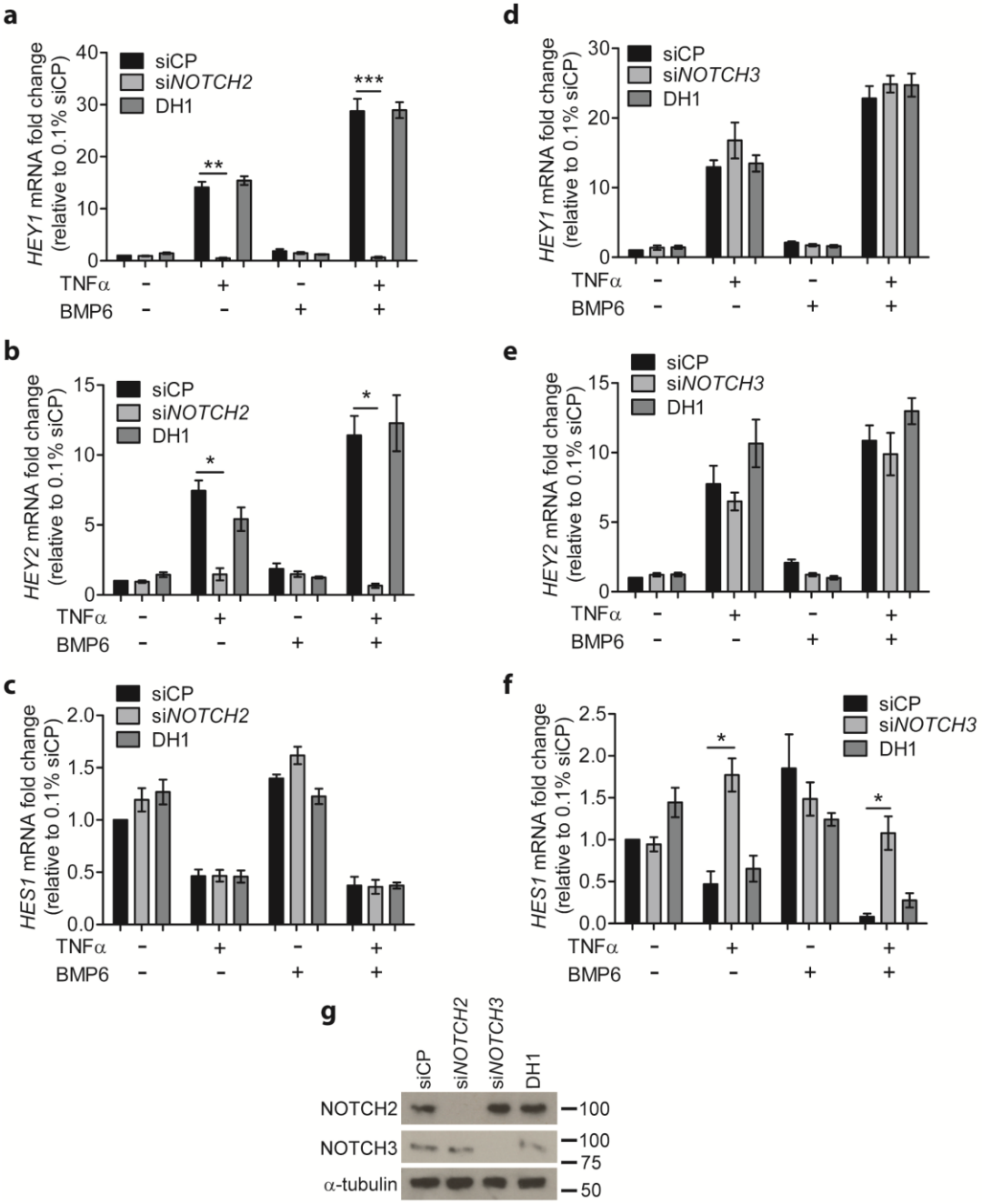
Supplementary Figure 10. TNFα induces *ACVR2A* expression in HPAH PASMCs. (**a**) *ACVR2A* mRNA expression, normalized to *ACTB*, in human dPASMCs from disease-free controls and HPAH patients stimulated with TNFα (1 ng mL^{-1}) for 24 h (n = 3; Student's t-test). (**b**) *ALK2*, *ALK3* and *ALK6* mRNA expression, normalized to *ACTB*, in human dPASMCs from disease-free controls and HPAH patients stimulated with TNFα (1 ng mL^{-1}) for 24 h. ***$P \leq 0.001$. Error bars represent mean ± s.e.m.



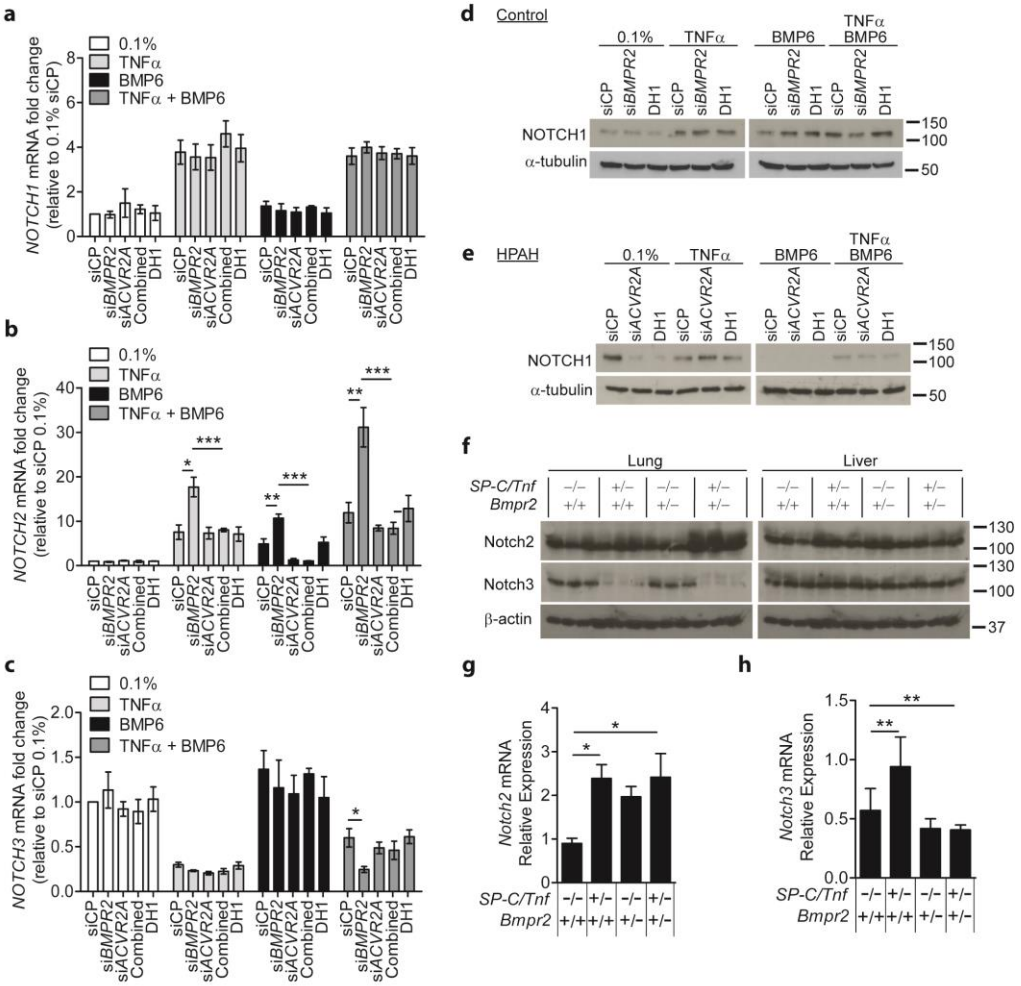
Supplementary Figure 11. TNFα selectively alters NOTCH expression in HPAH PASMCs. (**a**) Immunoblotting for cleaved/transmembrane intracellular NTM (NTM) regions for NOTCH1, NOTCH2 and NOTCH3 expression in control disease-free and HPAH dPASMCs (n=3 different lines for each). Blots were reprobed for α-tubulin to ensure equal loading. (**b**) Densitometry of NTM regions from blots in panel **a** relative to α-tubulin levels. (**c**) Immunoblotting for NTM regions for NOTCH1, NOTCH2 and NOTCH3 expression in control disease-free and HPAH dPASMCs treated with TNFα (1 ng mL^{-1}) and/or BMP2 or BMP6 (both 10 ng mL^{-1}) for 1 h as indicated. Blots were reprobed for α-tubulin to ensure equal loading. (**d** and **e**) Densitometry of NTM regions for immunoblots in panel **c** relative to α-tubulin levels. (n = 3; Mann-Whitney Test). *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$. Error bars represent mean ± s.e.m.



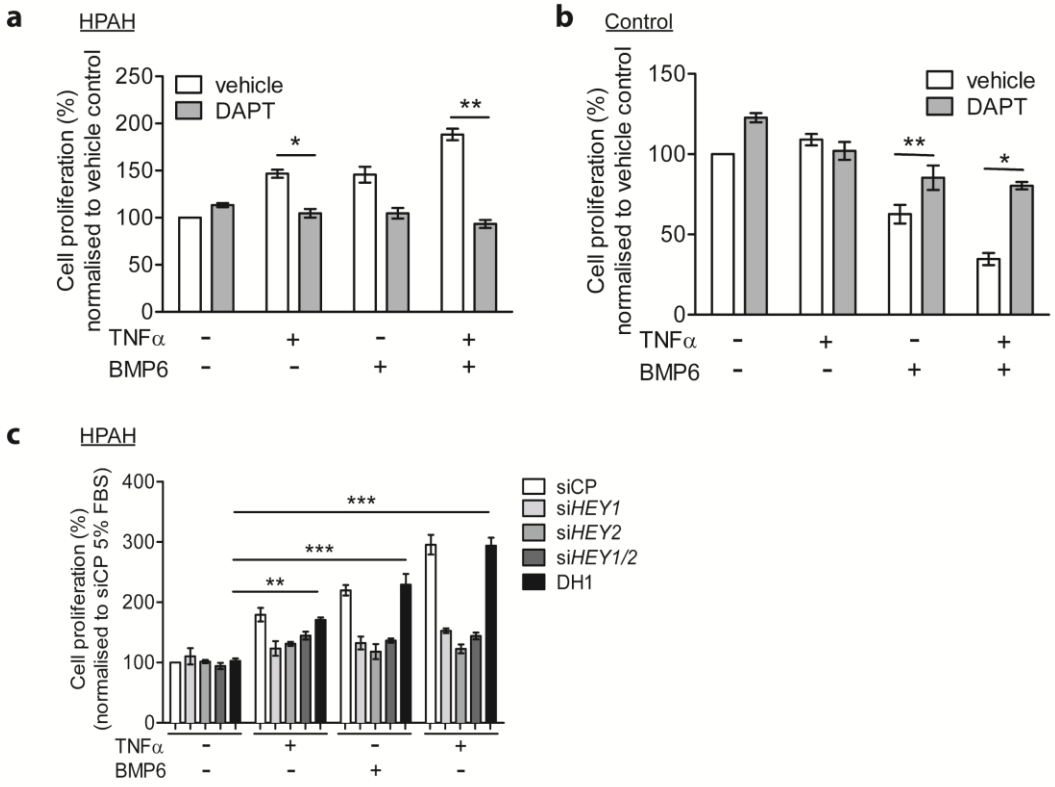
Supplementary Figure 12. *NOTCH* and its targets are altered by TNFα in HPAH PASMCs. (**a**-**f**) *NOTCH1* (**a**), *NOTCH2* (**b**), *NOTCH3* (**c**), *HEY1* (**d**), *HEY2* (**e**) and *HES1* (**f**) mRNA expression, normalized to *ACTB*, in human dPASMCs from disease-free controls and HPAH patients stimulated with TNFα (1 ng mL^{-1}) and/or BMP2 or BMP6 (both 10 ng mL^{-1}) for 1 h as indicated. One-way ANOVA with post-hoc Tukey's for multiple comparisons used in **a**, **b**, **c**, **d**, **e** and **f**. *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$. Error bars represent mean ± s.e.m.



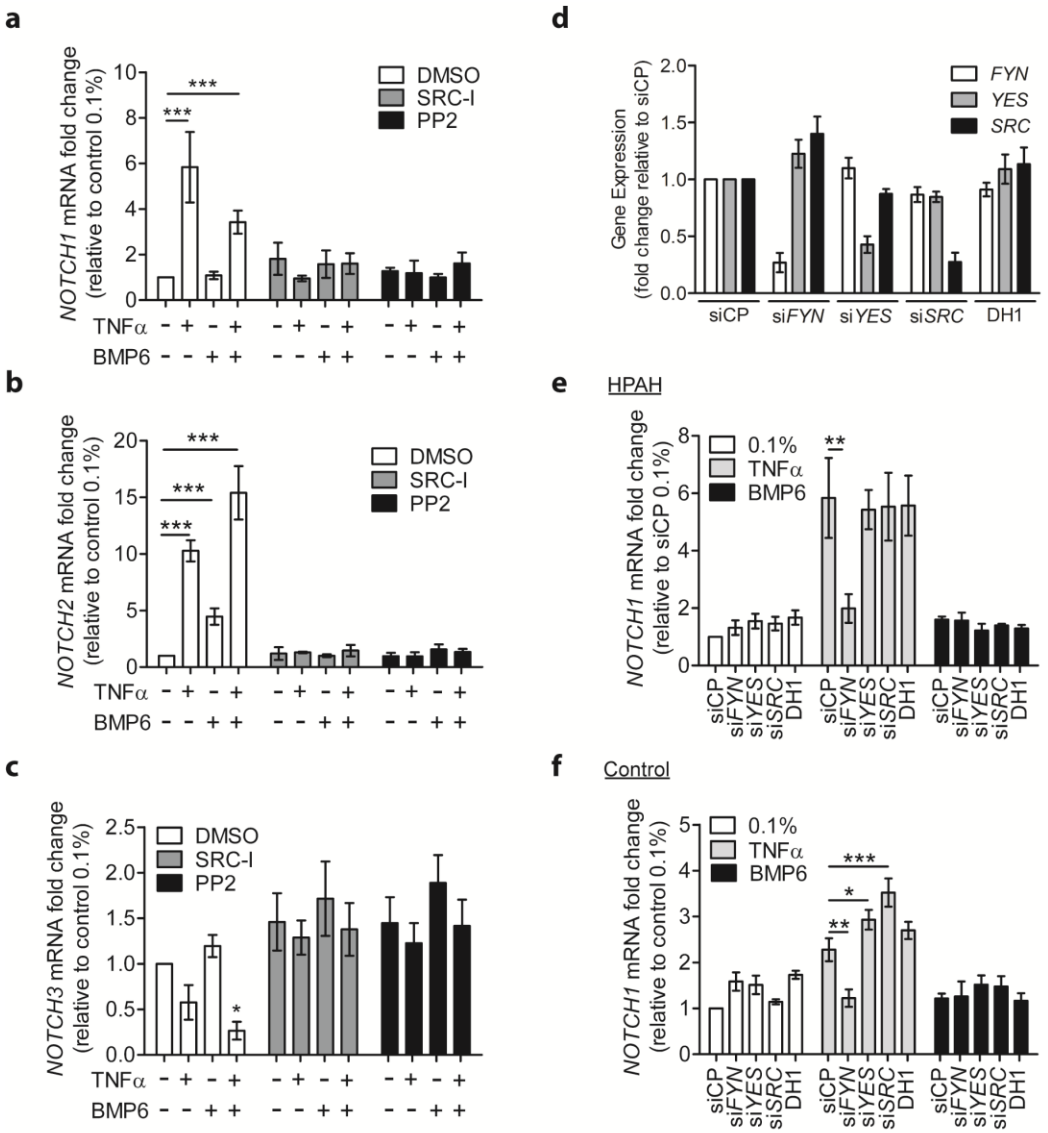
Supplementary Figure 13. *HEY1* and *HEY2* are targets of *NOTCH2*; *HES1* is a target of *NOTCH3*. (**a** - **c**) *HEY1* (**a**), *HEY2* (**b**) and *HES1* (**c**) mRNA expression, normalized to *ACTB*, in human HPAH dPASMCs transfected with DharmaFECT1™ alone (DH1), si*NOTCH2* or non-targeting siRNA control (siCP) and followed by treatment with TNFα (1 ng mL) and/or BMP6 (10 ng mL^{-1}) for 1 h as indicated (n = 3). (**d** - **f**) *HEY1* (**d**), *HEY2* (**e**) and *HES1* (**f**) mRNA expression, normalized to *ACTB*, in human HPAH dPASMCs transfected with DharmaFECT1™ alone (DH1), si*NOTCH3* or non-targeting siRNA control (siCP) followed by treatment with TNFα (1 ng mL^{-1}) and/or BMP6 (10 ng mL^{-1}) for 1 h as indicated (n = 3). (**g**) Immunoblot of NOTCH2 and NOTCH3 cleaved/transmembrane intracellular (NTM) regions in human HPAH dPASMCs transfected with DH1 alone , si*NOTCH2*, si*NOTCH3* or siCP . One-way ANOVA with post-hoc Tukey's for multiple comparisons used in **a**, **b** and **f**. *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$. Error bars represent mean ± s.e.m.



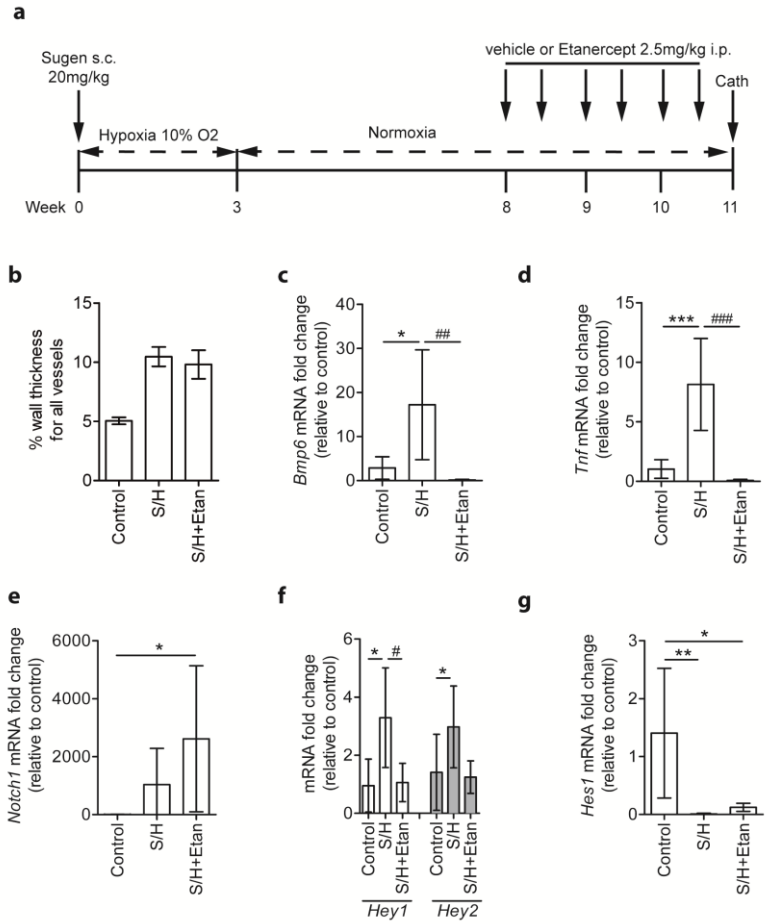
Supplementary Figure 14. TNFα alters *NOTCH* expression differentially in *BMPR2* deficiency. (**a** - **c**) *NOTCH1* (**a**), *NOTCH2* (**b**) and *NOTCH3* (**c**) mRNA expression, normalized to *ACTB*, in human control dPASMCs transfected with DharmaFECT1™ alone (DH1), si*BMPR2*, si*ACVR2A*, combined si*BMPR2* + si*ACVR2A* or non-targeting siRNA control (siCP) followed by treatment with TNFα (1 ng mL^{-1}) and/or BMP6 (10 ng mL^{-1}) for 1 h as indicated (n = 3; n = 4 for NOTCH3). (**d** and **e**) Representative immunoblots of NOTCH1 NTM region in (**d**) human disease-free control PASMCs following transfection with DH1 Alone, si*BMPR2*, or siCP or (**e**) HPAH dPASMCs following transfection with DH1 alone , si*ACVR2A*, or siCP followed by treatment with TNFα (1 ng mL^{-1}) and/or BMP6 (10 ng mL) for 1 h as indicated. Reprobed for α-tubulin to ensure equal loading. The data shown are representative of three experiments. Note that a consistent increase of NOTCH1 NTM region was observed with siCP transfection in HPAH PASMCs at baseline. (**f**) Immunoblots of Notch2 and Notch3 cleaved/transmembrane intracellular (NTM) regions for expression in lungs and livers isolated from 8-9 week old *Bmpr2*$^{+/+}$, *SP-C/Tnf/Bmpr2*$^{+/+}$, *Bmpr2*$^{+/-}$ and *SP-C/Tnf/Bmpr2*$^{+/-}$ transgenic mice (n = 3 per group). Blots were reprobed for β-actin to ensure equal loading. (**g** and **h**) *Notch2* and *Notch3* mRNA expression, normalized to *Actb*, in livers isolated from 8-9 week old *Bmpr2*$^{+/+}$, *SP-C/Tnf/Bmpr2*$^{+/+}$, *Bmpr2*$^{+/-}$ and *SP-C/Tnf/Bmpr2*$^{+/-}$ transgenic mice (n = 4). One-way ANOVA with post-hoc Tukey's for multiple comparisons used in **a**, **b**, **c**, **g** and **h**. *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$. Error bars represent mean ± s.e.m.



Supplementary Figure 15. DAPT inhibits TNFα influence on PASMC proliferation. (**a** and **b**) Day 6 assessment of human HPAH (**a**) and control (**b**) dPASMCs proliferation following pre-treatment with DAPT (5 µM) for 30 min before subsequent TNFα (1 ng mL^{-1}) and/or BMP6 (50 ng mL^{-1}) treatment for 24 h (n = 3; Student's t-test). (**c**) Proliferation of human HPAH dPASMCs on day 6 following transfection with DharmaFECT1^{TM} alone (DH1), si*HEY1*, si*HEY2*, si*HEY1*+si*HEY2* (si*HEY1/2*) or non-targeting siRNA control (siCP) and treatment every 48 h with TNFα (1 ng mL^{-1}) and/or BMP6 (50 ng mL^{-1}) as indicated (n = 3; one-way ANOVA with post-hoc Tukey's for multiple comparisons). *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$. Error bars represent mean ± s.e.m.

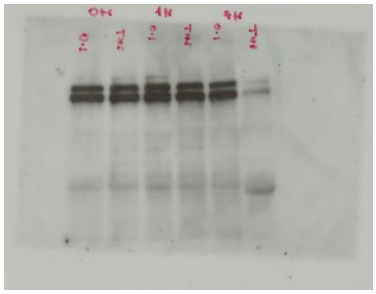


Supplementary Figure 16. Inhibition of SRC kinases reverses TNFα and BMP6 regulation of *NOTCH*. (**a** - **c**) *NOTCH1* (**a**), *NOTCH2* (**b**) and *NOTCH3* (**c**) mRNA expression, normalized to *ACTB*, in human HPAH dPASMCs pre-incubated with SRC-I (1μM) or PP2 (250 nM) in DMSO for 1 h before stimulation with TNFα (1 ng mL^{-1}) and/or BMP6 (10 ng mL^{-1}) for 1 h as indicated (n = 3; one-way ANOVA with post-hoc Tukey's for multiple comparisons). (**d**) Human *FYN*, *YES*, and *SRC* mRNA expression in control dPASMCs following transfection with DharmaFECT1™ alone (DH1), si*FYN*, si*YES*, si*SRC* or non-targeting siRNA control (siCP). (**e** and **f**) *NOTCH1* mRNA expression, normalized to *ACTB*, in control (**e**) and HPAH (**f**) human dPASMCs following transfection with DH1 alone, si*FYN*, si*YES*, si*SRC* or siCP and treated with TNFα (1 ng mL^{-1}) and/or BMP6 (10 ng mL^{-1}) for 1 h as indicated (n = 3; one-way ANOVA with post-hoc Tukey's for multiple comparisons). *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$. Error bars represent mean ± s.e.m.

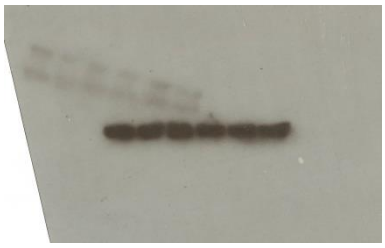


Supplementary Figure 17. Etanercept reverses established pulmonary hypertension in the Sugen-hypoxia model. (**a**) Rats were given vehicle injections and maintained in normoxia (Control, n = 6) or challenged with SU-5416 (20 mg/kg, s.c.) and 3 weeks of hypoxia (10% O2) before 5 weeks of normoxia and 3 weeks of biweekly treatment with saline vehicle (S/H, n = 9) or etanercept (S/H+Etan, n = 9; 2.5 mg/kg, i.p.). (**b**) Assessment of pulmonary arterial wall thickness as a percentage of luminal diameter. (**c** - **g**) *Bmp6* **(c)**, *Tnf* **(d)**, *Notch1* **(e)**, *Hey1* and *Hey2* **(f)** and *Hes1* **(g)** mRNA expression, normalized to *Actb*, in lungs isolated from control, S/H and S/H+Etan rats (n = 6). One-way ANOVA with post-hoc Tukey's for multiple comparisons. S/H = Sugen-hypoxia, S/H+Etan = Sugen-hypoxia + Etanercept */#$P \leq 0.05$, **/##$P \leq 0.01$, ***/###$P \leq 0.001$. Error bars represent mean $\pm$ s.e.m.

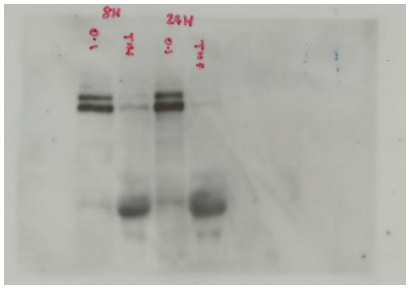
a) Figure 1c_BMPR-II (left panel)



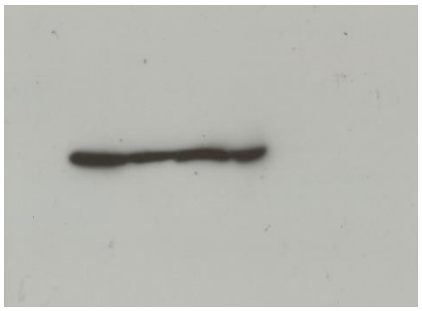
b) Figure 1c_alpha-tubulin (left panel)



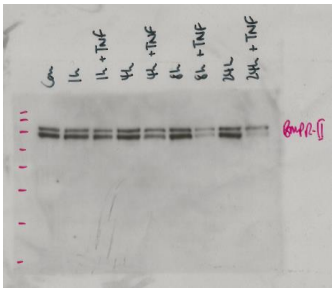
c) Figure 1c_BMPR-II (right panel)



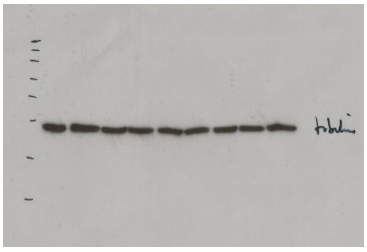
d) Figure 1c_alpha-tubulin (right panel)



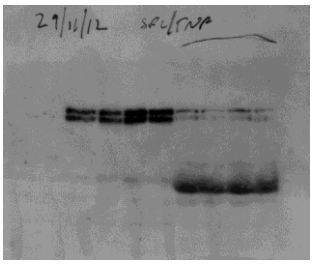
e) Figure 1d_BMPR-II



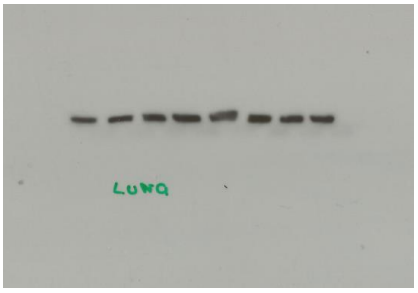
f) Figure 1d_alpha-tubulin



g) Figure 1g_BMPR-II

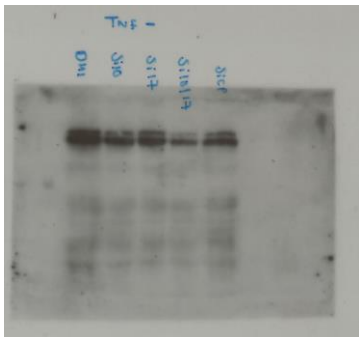


h) Figure 1g_alpha tubulin

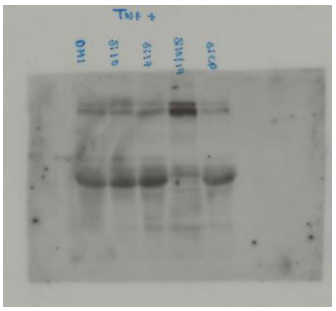


Supplementary Figure 18. Uncropped western blots for Figures 1c-g. The relevant figures are indicated in the blot titles.

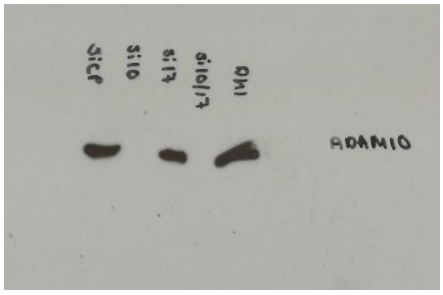
a) Figure 1h_BMPR-II (left panel)



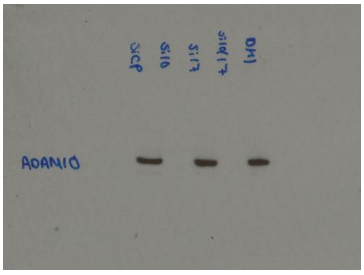
e) Figure 1h_BMPR-II (right panel)



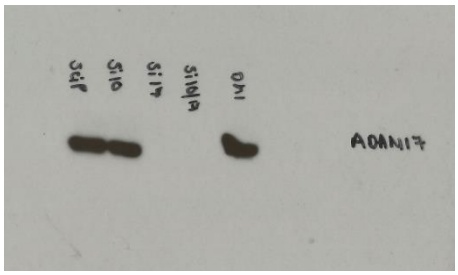
b) Figure 1h_ADAM10 (left panel)



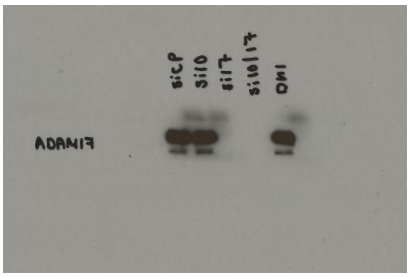
f) Figure 1h_ADAM10 (right panel)



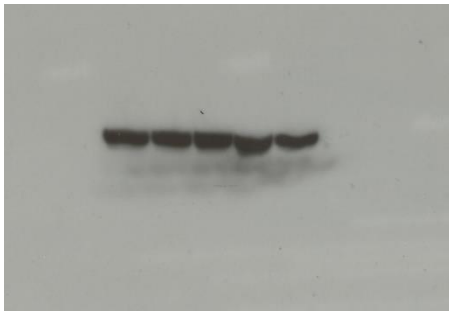
c) Figure 1h_ADAM17 (left panel)



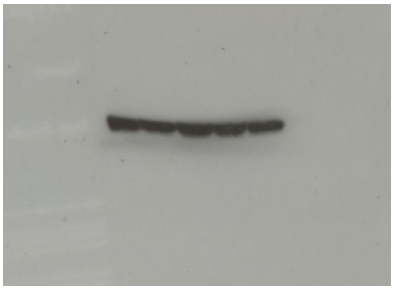
g) Figure 1h_ADAM17 (right panel)



d) Figure 1h_alpha tubulin (left panel)

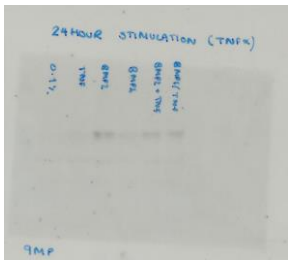


h) Figure 1h_alpha tubulin (right panel)

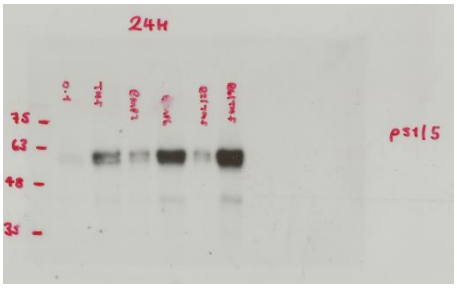


Supplementary Figure 19. Uncropped western blots for Figure 1h. The relevant figures are indicated in the blot titles.

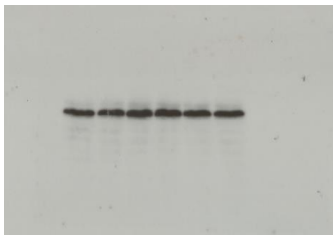
a) Figure 2b_pSMAD1/5 (left panel)



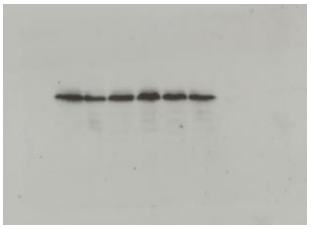
e) Figure 2b_pSMAD1/5 (right panel)



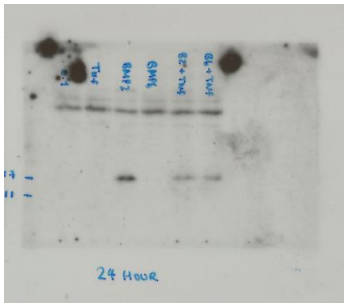
b) Figure 2b_tSMAD1 (left panel)



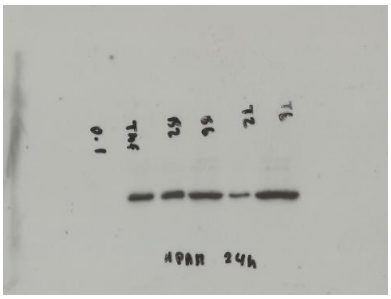
f) Figure 2b_tSMAD1 (right panel)



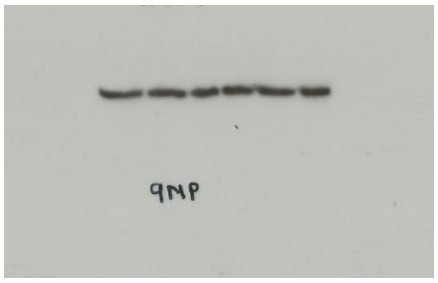
c) Figure 2b_ID1 (left panel)



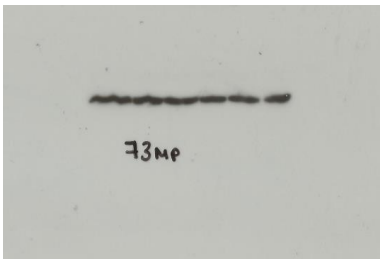
g) Figure 2b_ID1 (right panel)



d) Figure 2b_alpha tubulin (left panel)

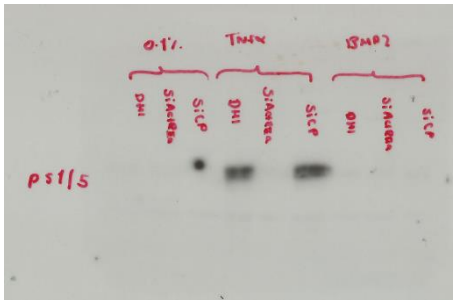


h) Figure 2b_alpha tubulin (right panel)

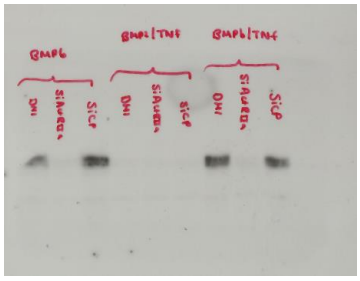


Supplementary Figure 20. Uncropped western blots for Figure 2b. The relevant figures are indicated in the blot titles.

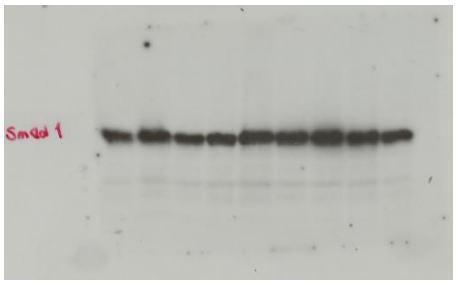
a) Figure 3e_pSMAD1/5 (left panel)



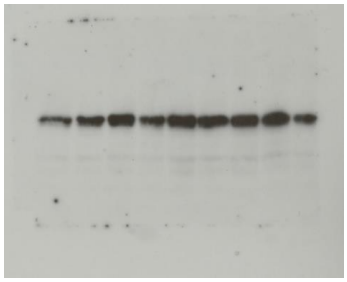
e) Figure 3e_pSMAD1/5 (right panel)



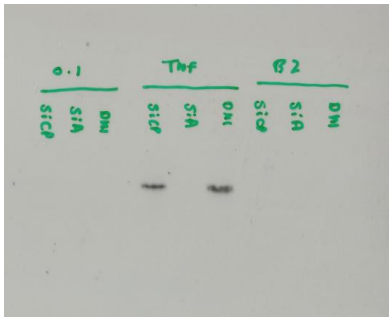
b) Figure 3e_tSMAD1 (left panel)



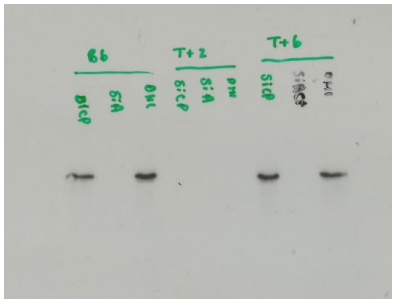
f) Figure 3e_tSMAD1 (right panel)



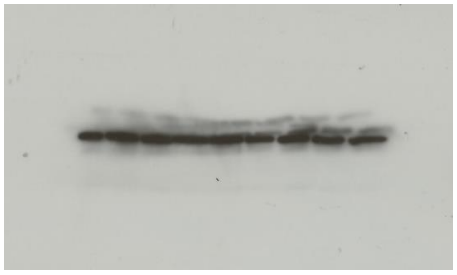
c) Figure 3e_ID1 (left panel)



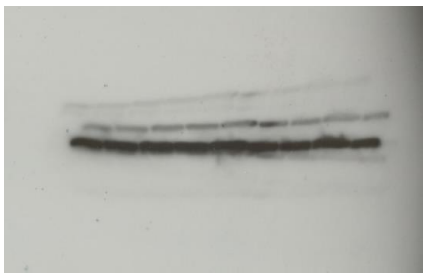
g) Figure 3e_ID1 (right panel)



d) Figure 3e_alpha tubulin (left panel)

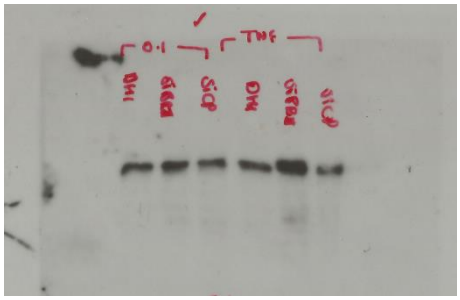


h) Figure 3e_alpha tubulin (right panel)

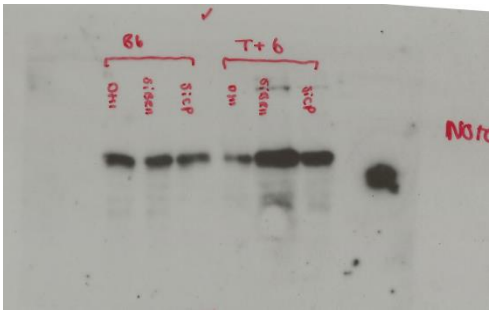


Supplementary Figure 21. Uncropped western blots for Figure 3e. The relevant figures are indicated in the blot titles.

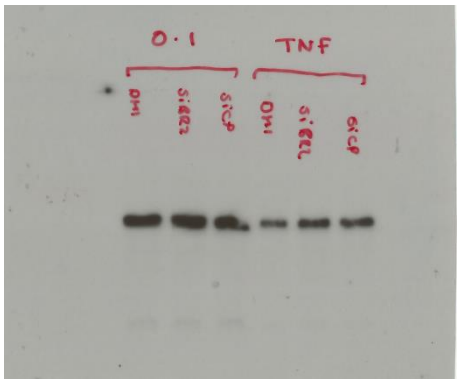
a) Figure 4a_NOTCH2 (left panel)



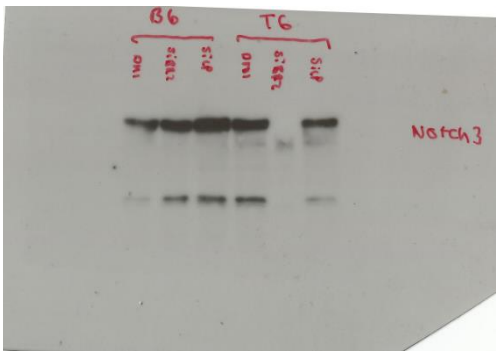
d) Figure 4a_NOTCH2 (right panel)



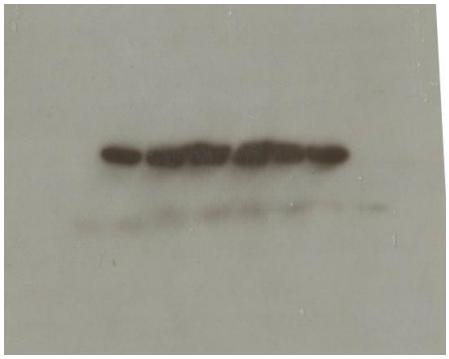
b) Figure 4a_NOTCH3 (left panel)



e) Figure 4a_NOTCH3 (right panel)



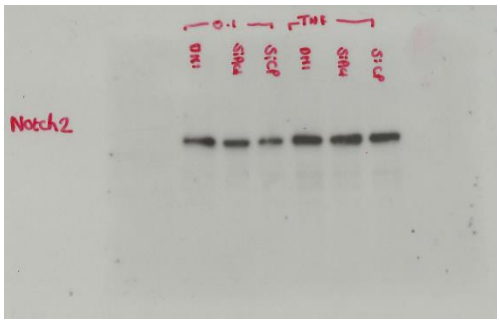
c) Figure 4a_alpha tubulin (left panel)



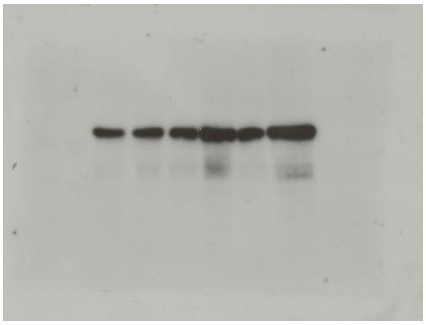
f) Figure 4a_alpha tubulin (right panel)

Supplementary Figure 22. Uncropped western blots for Figure 4a. The relevant figures are indicated in the blot titles.

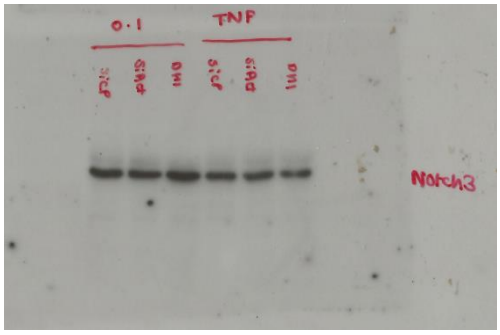
a) Figure 4b_NOTCH2 (left panel)



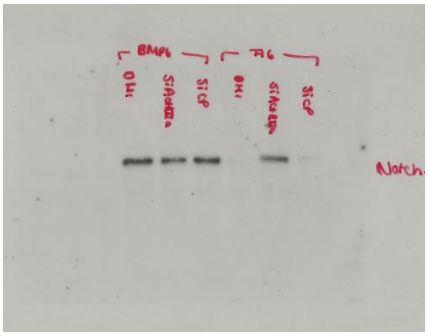
d) Figure 4b_NOTCH2 (right panel)



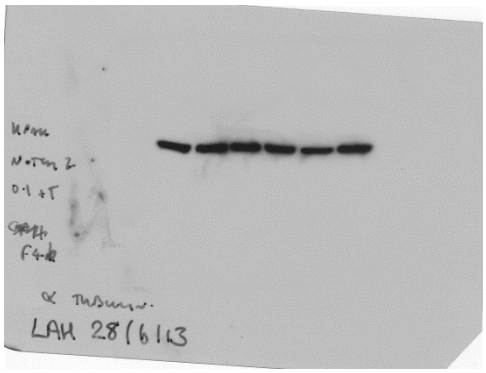
b) Figure 4b_NOTCH3 (left panel)



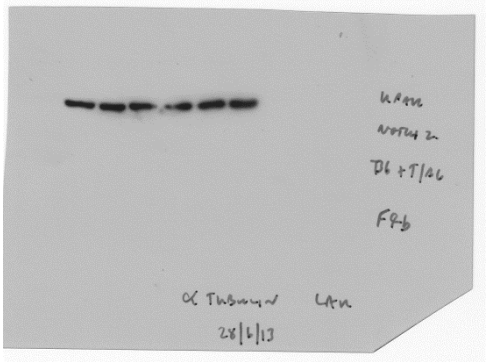
e) Figure 4b_NOTCH3 (right panel)



c) Figure 4b_alpha tubulin (left panel)

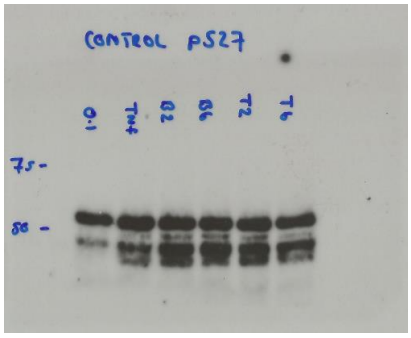


f) Figure 4b_alpha tubulin (right panel)

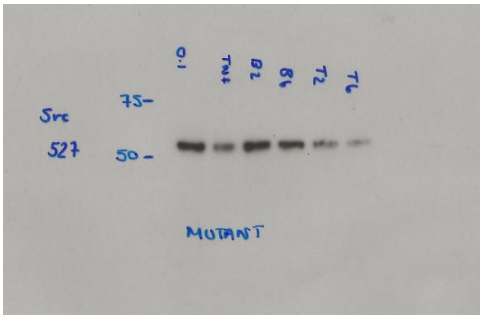


Supplementary Figure 23. Uncropped western blots for Figure 4b. The relevant figures are indicated in the blot titles.

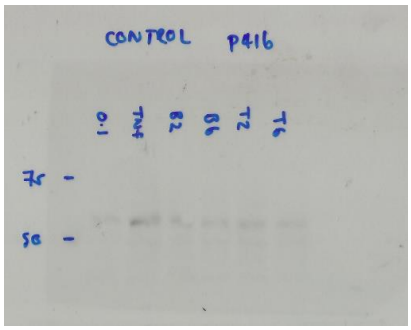
a) Figure 5b_pSRC(527) (left panel)



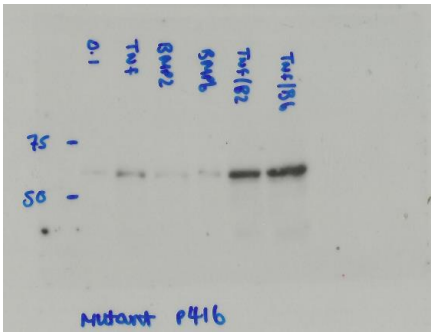
e) Figure 5b_pSRC(527) (right panel)



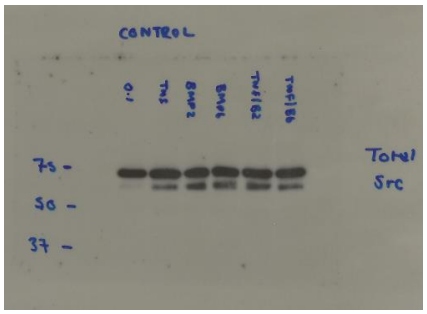
b) Figure 5b_pSRC(416) (left panel)



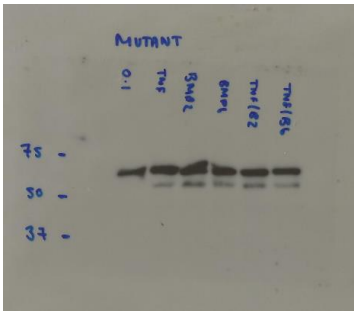
f) Figure 5b_pSRC(416) (right panel)



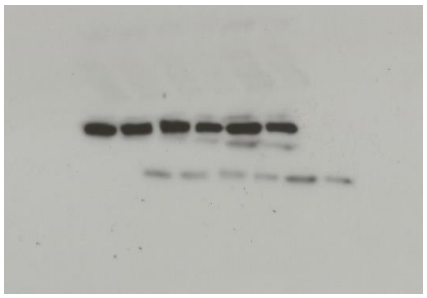
c) Figure 5b_SRC (left panel)



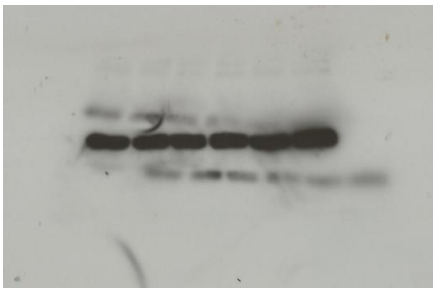
g) Figure 5b_SRC (right panel)



d) Figure 5b_alpha tubulin (left panel)

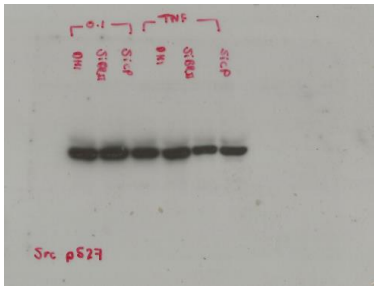


h) Figure 5b_alpha tubulin (right panel)

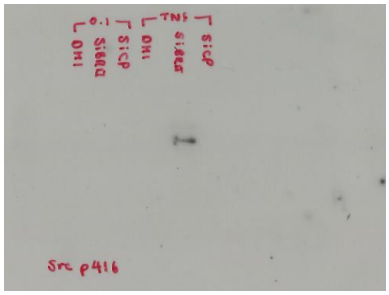


Supplementary Figure 24. Uncropped western blots for Figure 5b. The relevant figures are indicated in the blot titles.

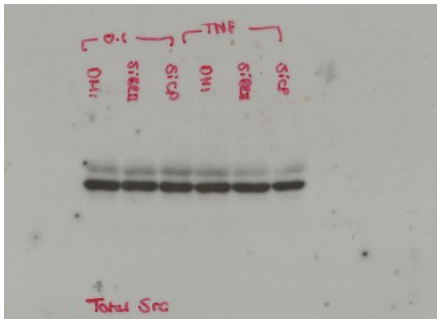
a) Figure 5c_pSRC(527) (left panel)



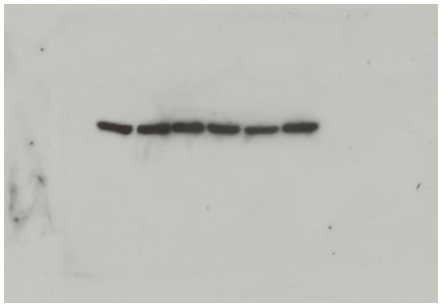
b) Figure 5c_pSRC(416) (left panel)



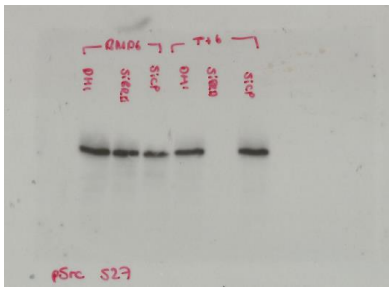
c) Figure 5c_SRC (left panel)



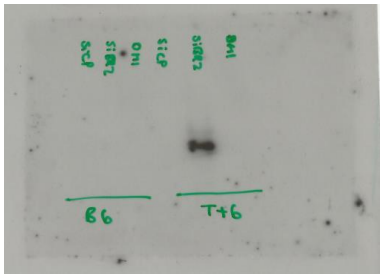
d) Figure 5c_alpha tubulin (left panel)



e) Figure 5c_pSRC(527) (right panel)

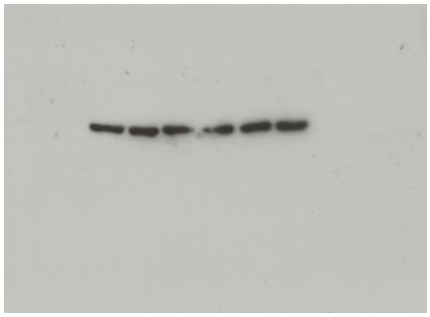


f) Figure 5c_pSRC(416) (right panel)



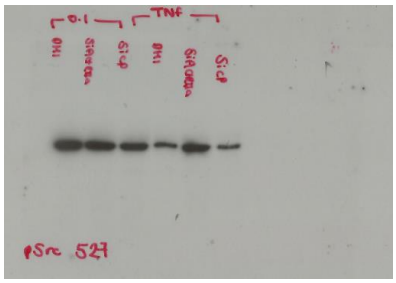
g) Figure 5c_SRC (right panel)

h) Figure 5c_alpha tubulin (right panel)

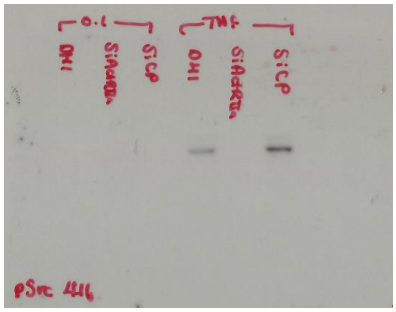


Supplementary Figure 25. Uncropped western blots for Figure 5c. The relevant figures are indicated in the blot titles.

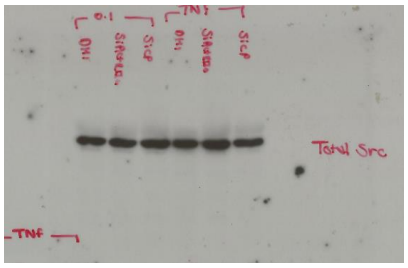
a) Figure 5d_pSRC(527) (left panel)



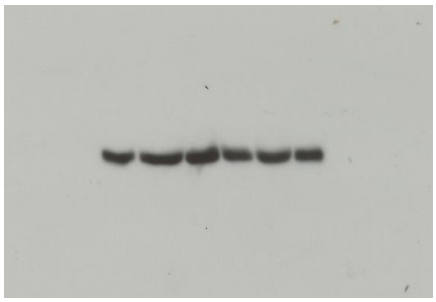
b) Figure 5d_pSRC(416) (left panel)



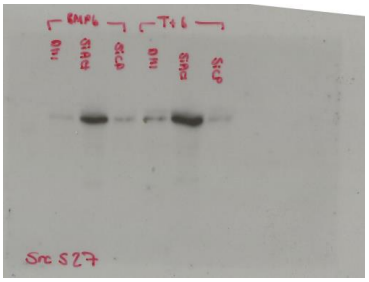
c) Figure 5d_SRC (left panel)



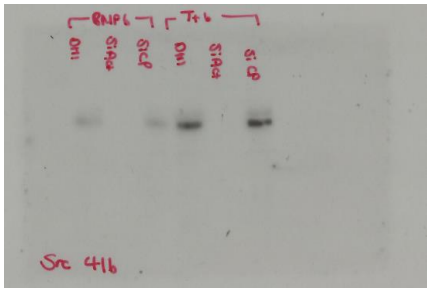
d) Figure 5d_alpha tubulin (left panel)



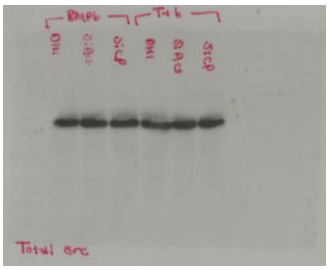
e) Figure 5d_pSRC(527) (right panel)



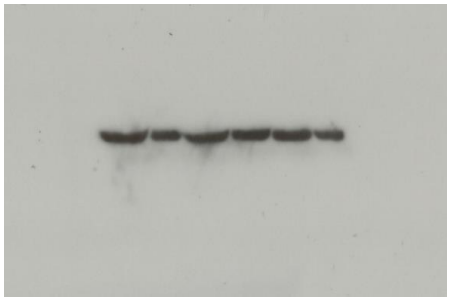
f) Figure 5d_pSRC(416) (right panel)



g) Figure 5d_SRC (right panel)

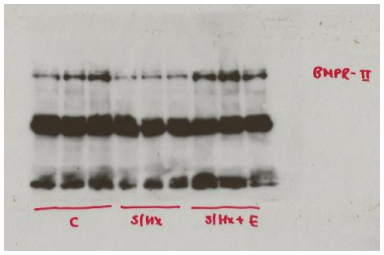


h) Figure 5d_alpha tubulin (right panel)

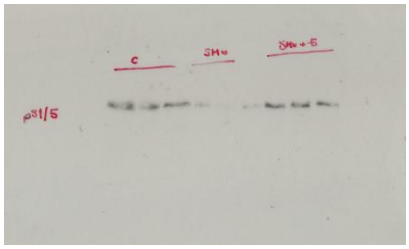


Supplementary Figure 26. Uncropped western blots for Figure 5d. The relevant figures are indicated in the blot titles.

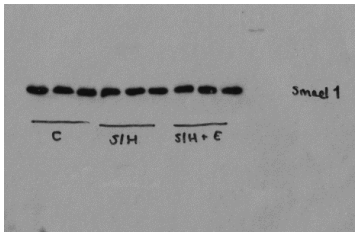
a) Figure 6e_BMPR-II



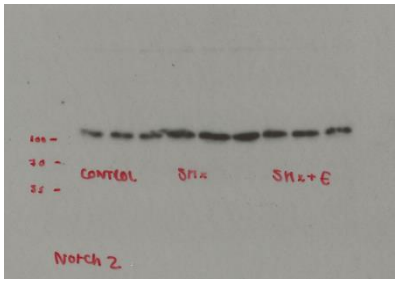
b) Figure 6e_pSmad1/5



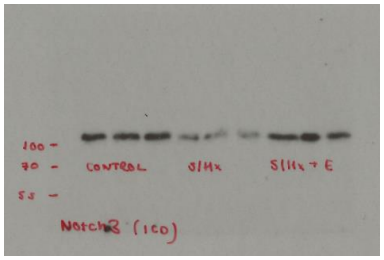
c) Figure 6e_Smad1



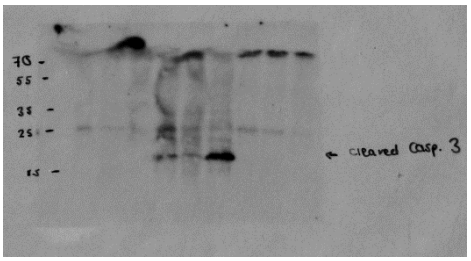
d) Figure 6e_Notch2



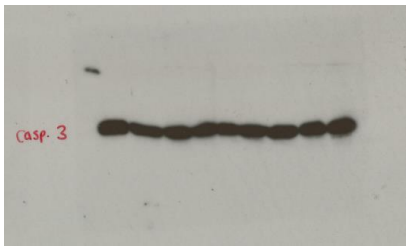
e) Figure 6e_Notch3



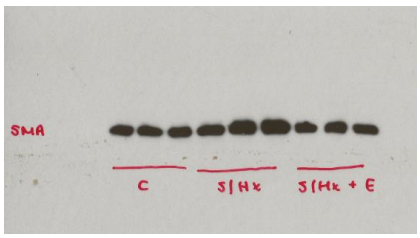
f) Figure 6e_Cleaved caspase-3



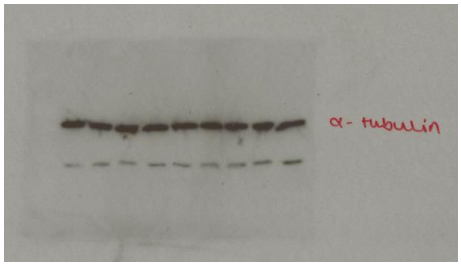
g) Figure 6e_caspase-3



h) Figure 6e_alpha-SMA

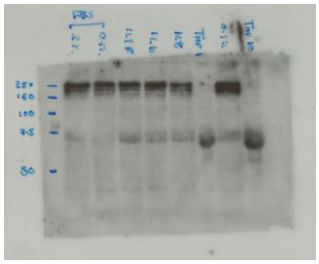


i) Figure 6e_alpha tubulin

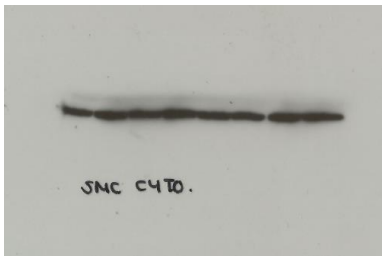


Supplementary Figure 27. Uncropped western blots for Figure 6e. The relevant figures are indicated in the blot titles.

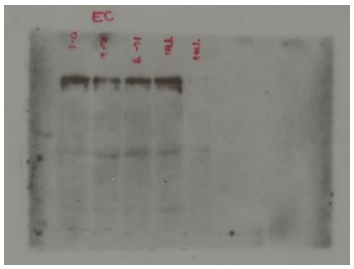
a) Supplement Fig 1a_BMPR-II



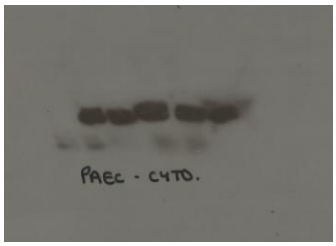
b) Supplement Fig 1a_alpha tubulin



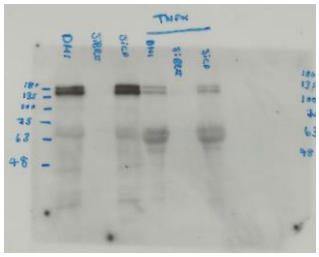
c) Supplement Fig 1b_BMPR-II



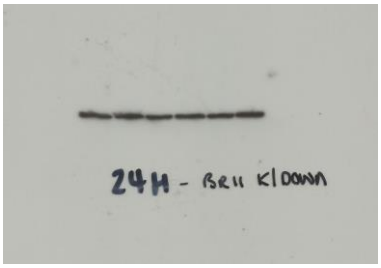
d) Supplement Fig 1b_alpha tubulin



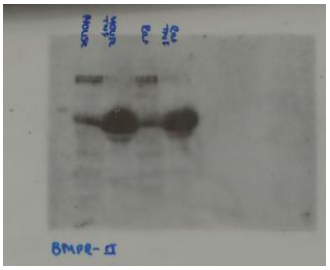
e) Supplement Fig 2a_BMPR-II



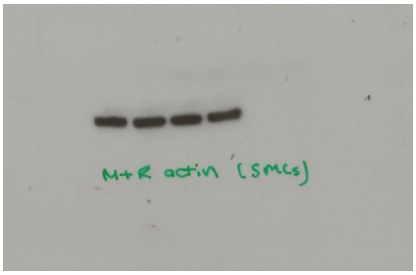
f) Supplement Fig 2a_alpha tubulin



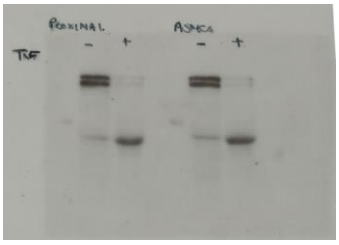
g) Supplement Fig 2b_BMPR-II



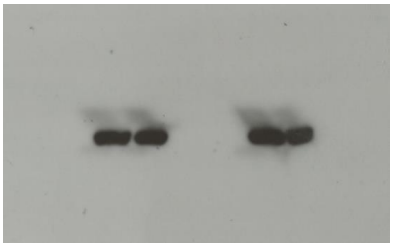
h) Supplement Fig 2b_alpha tubulin



i) Supplement Fig 2c_BMPR-II

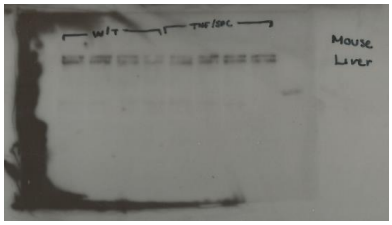


j) Supplement Fig 2c_alpha tubulin

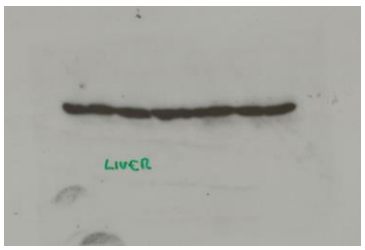


Supplementary Figure 28. Uncropped western blots for Supplementary Figures 1a, 1b, 2a, 2b and 2c. The relevant figures are indicated in the blot titles.

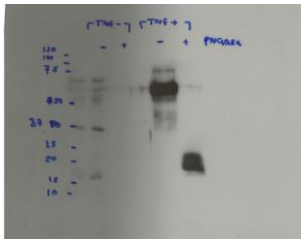
a) Supplement Fig 3e_BMPR-II



b) Supplement Fig 3e_alpha tubulin

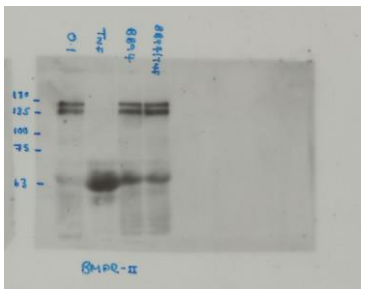


c) Supplement Fig 4a_MYC

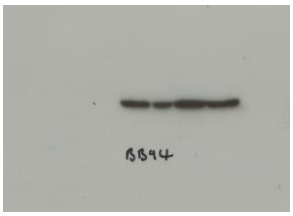


d) Supplement Fig 4a_Coomassie

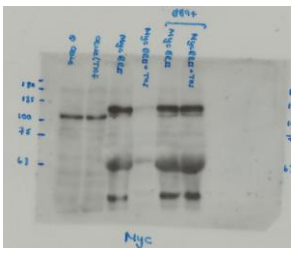
e) Supplement Fig 4c_BMPR-II IB:MYC



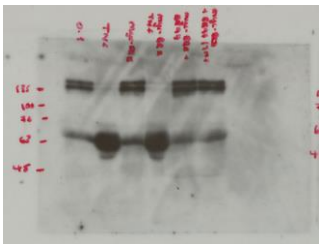
f) Supplement Fig 4c_alpha tubulin



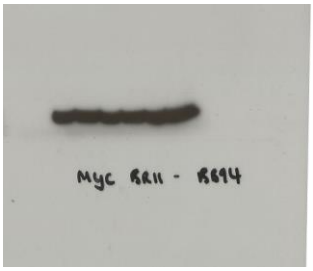
g) Supplement Fig 4d_WCL:MYC



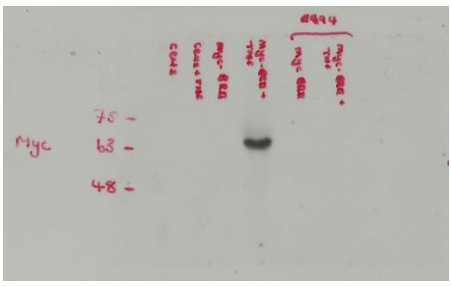
h) Supplement Fig 4d WCL:BMPR-II



i) Supplement Fig 4d_alpha tubulin

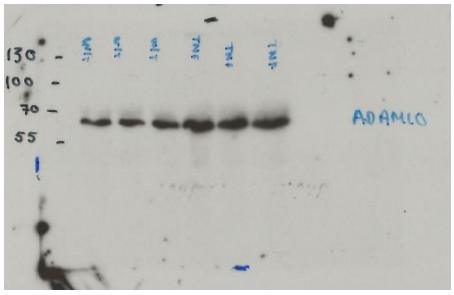


j) Supplement Fig 4d_IP:BMPR-II

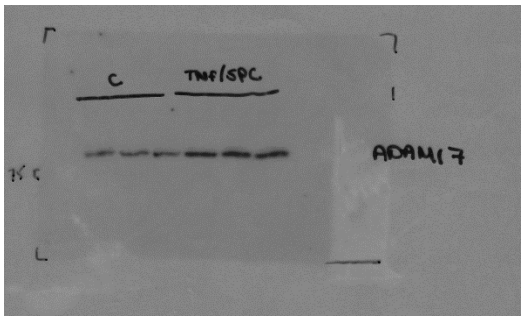


Supplementary Figure 29. Uncropped western blots for Supplementary Figures 3e, 4a, 4c and 4d. The relevant figures are indicated in the blot titles.

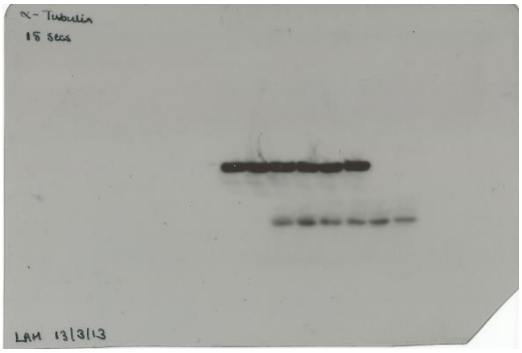
a) Supplement Fig 5c_ADAM10



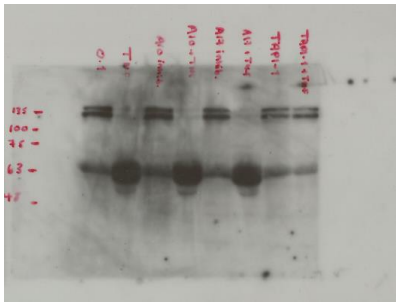
b) Supplement Fig 5c_ADAM17



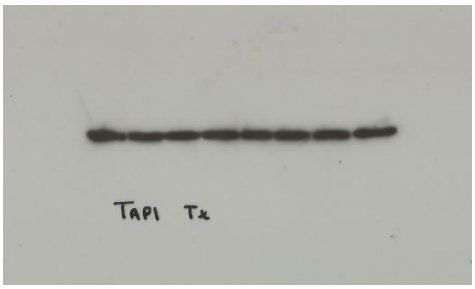
c) Supplement Fig 5c_beta actin



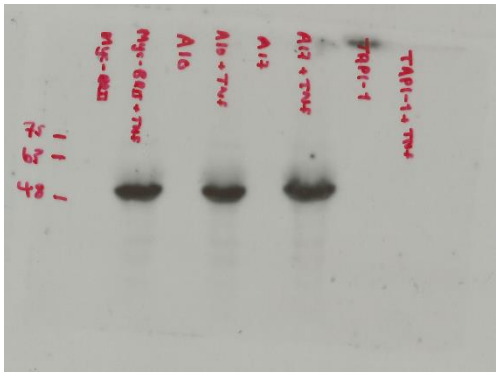
d) Supplement Fig 5e_BMPR-II



e) Supplement Fig 5e_alpha tubulin

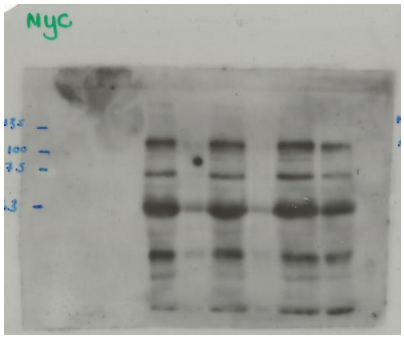


f) Supplement Fig 5e_IP:BMPR-II IB:MYC

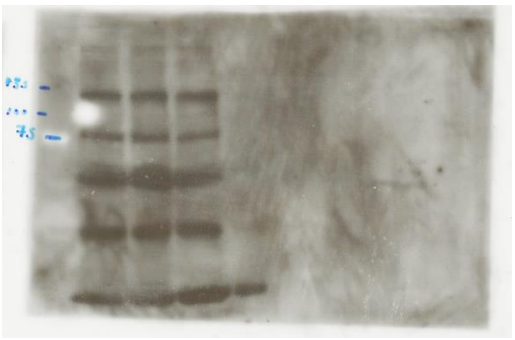


Supplementary Figure 30. Uncropped western blots for Supplementary Figures 5c and 5e. The relevant figures are indicated in the blot titles.

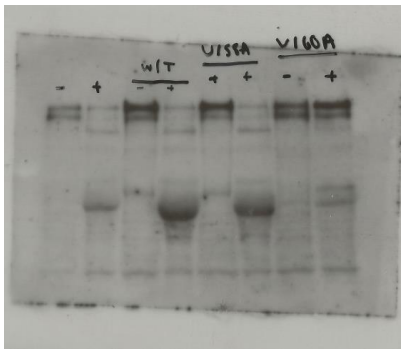
a) Supplement Fig 6b_WCL:MYC (left panel)



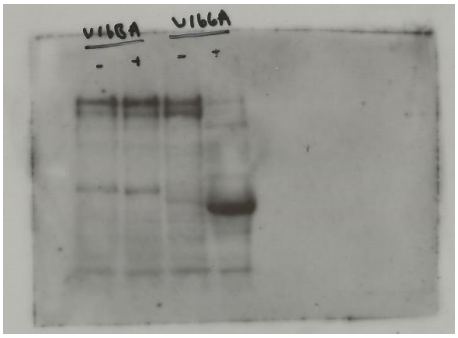
e) Supplement Fig 6b_WCL:MYC (right panel)



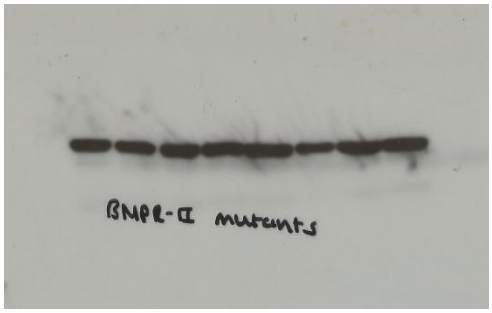
b) Supplement Fig 6b_BMPR-II (left panel)



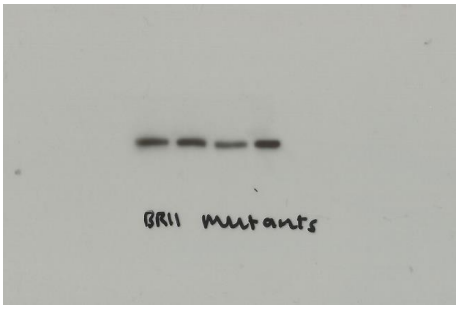
f) Supplement Fig 6b_BMPR-II (right panel)



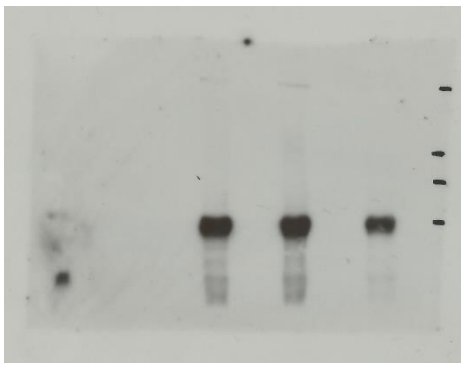
c) Supplement Fig 6b_alpha tubulin (left panel)



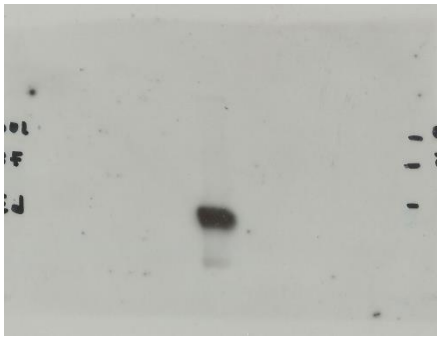
g) Supplement Fig 6b_alpha tubulin (right panel)



d) Supplement Fig 6b_IB:MYC (left panel)

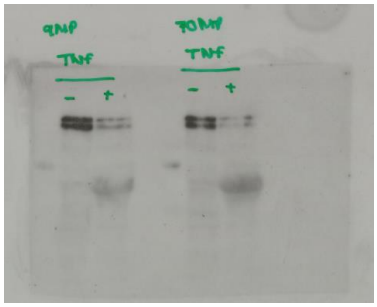


h) Supplement Fig 6b_IB:MYC (right panel)

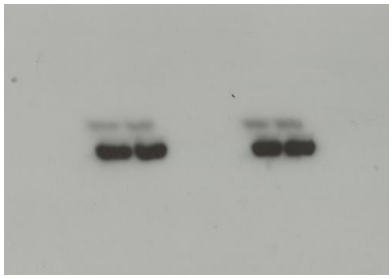


Supplementary Figure 31. Uncropped western blots for Supplementary Figures 5c and 5e. The relevant figures are indicated in the blot titles.

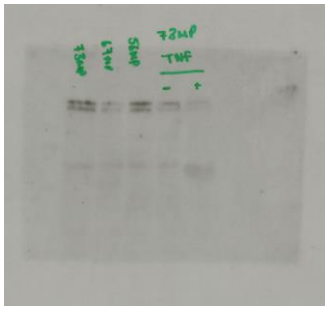
a) Supplement Fig 7a_BMPR-II (left panel)



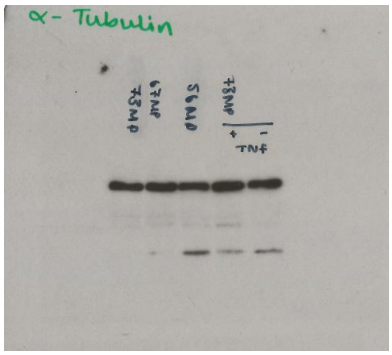
b) Supplement Fig 7a_alpha tubulin (left panel)



c) Supplement Fig 7a_BMPR-II (right panel)

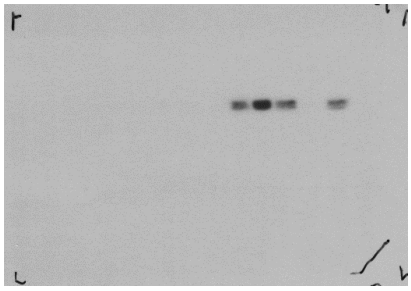


d) Supplement Fig 7a_alpha tubulin (right panel)

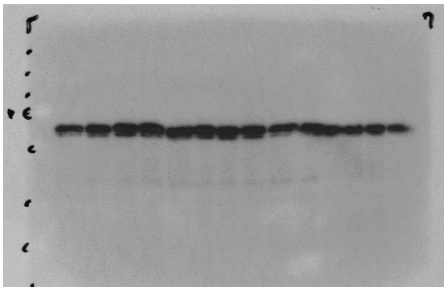


Supplementary Figure 32. Uncropped western blots for Supplementary Figures 7a. The relevant figures are indicated in the blot titles.

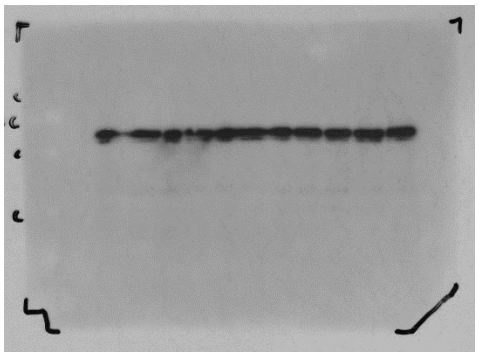
a) Supplement Fig 7b_pSMAD1/5 (left panel)



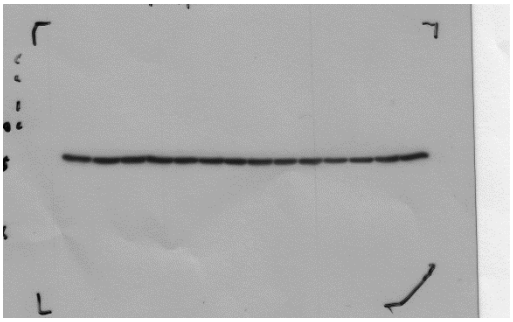
e) Supplement Fig 7b_tSMAD1 (right panel)



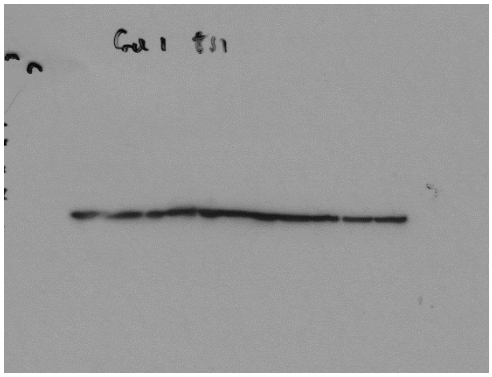
b) Supplement Fig 7b_tSMAD1 (left panel)



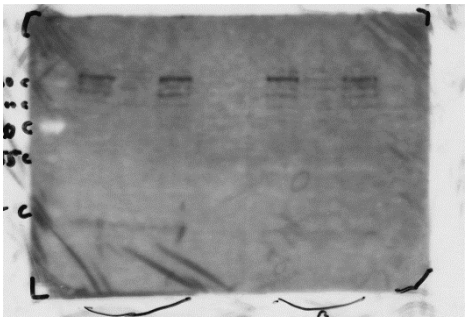
f) Supplement Fig 7b_alpha tubulin (right panel)



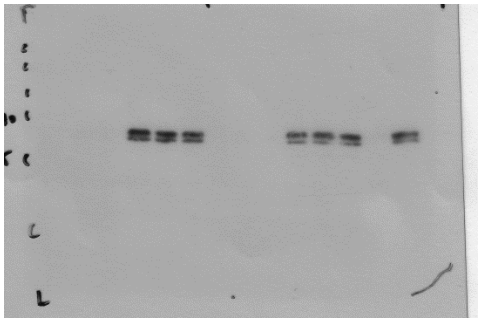
c) Supplement Fig 7b_alpha tubulin (left panel)



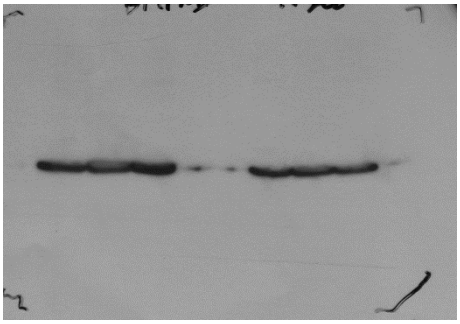
g) Supplement Fig 7c_BMPR-II



d) Supplement Fig 7b_pSMAD1/5 (right panel)

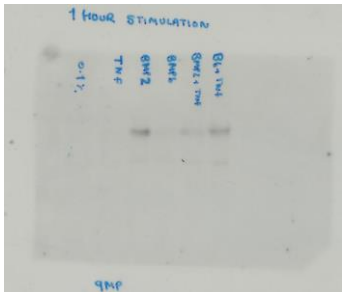


h) Supplement Fig 7c_alpha tubulin

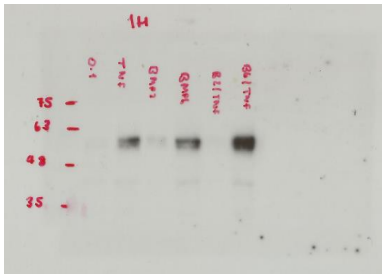


Supplementary Figure 33. Uncropped western blots for Supplementary Figures 7b and 7c. The relevant figures are indicated in the blot titles.

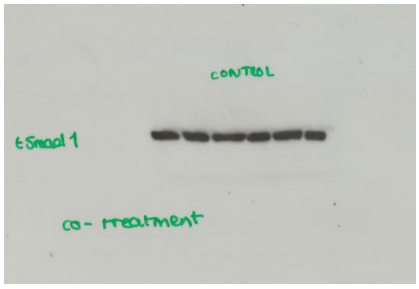
a) Supplement Fig 7d_pSMAD1/5 (right panel)



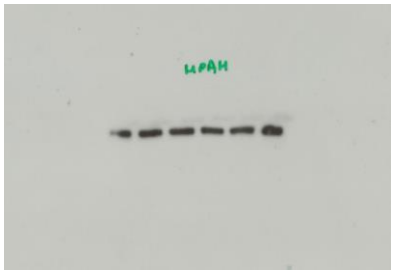
e) Supplement Fig 7d_pSMAD1/5 (left panel)



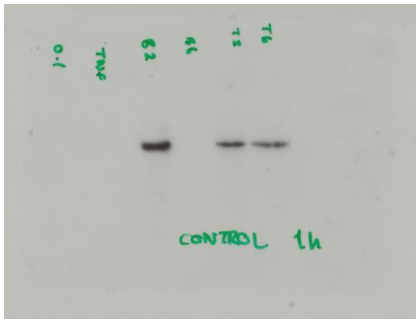
b) Supplement Fig 7d_tSMAD1 (right panel)



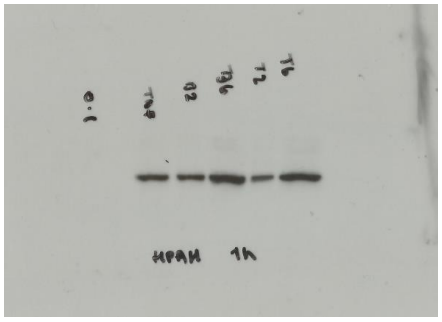
f) Supplement Fig 7d_tSMAD1 (left panel)



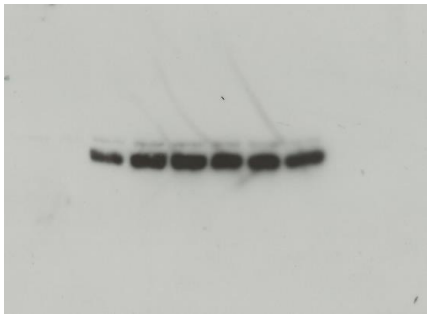
c) Supplement Fig 7d_ID1 (right panel)



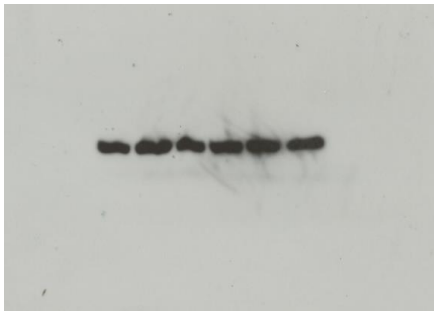
g) Supplement Fig 7d_ID1 (left panel)



d) Supplement Fig 7d_alpha tubulin (right panel)

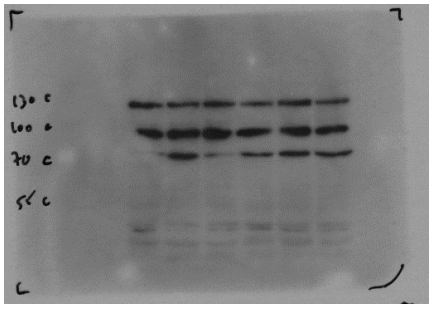


h) Supplement Fig 7d_alpha tubulin (left panel)

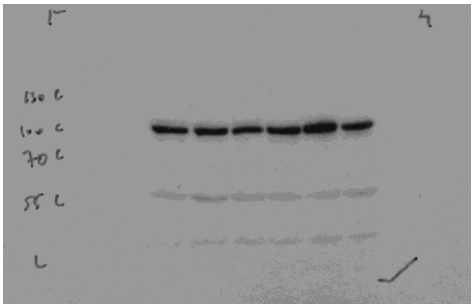


Supplementary Figure 34. Uncropped western blots for Supplementary Figure 7d. The relevant figures are indicated in the blot titles.

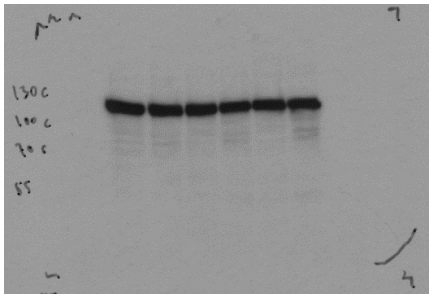
a) Supplement Fig 11a_NOTCH1



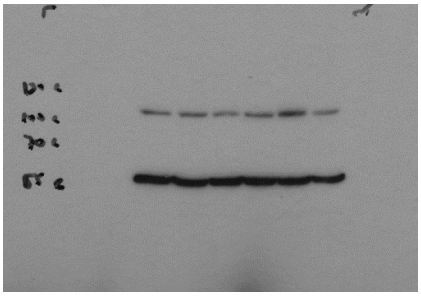
c) Supplement Fig 11a_NOTCH3



b) Supplement Fig 11a_NOTCH2

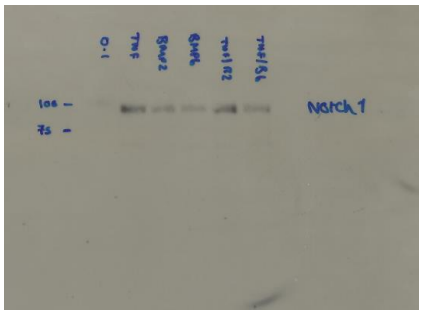


d) Supplement Fig 11a_alpha tubulin

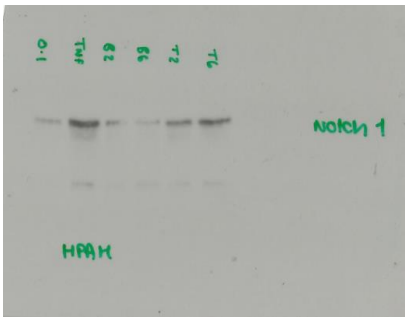


Supplementary Figure 35. Uncropped western blots for Supplementary Figure 11a. The relevant figures are indicated in the blot titles.

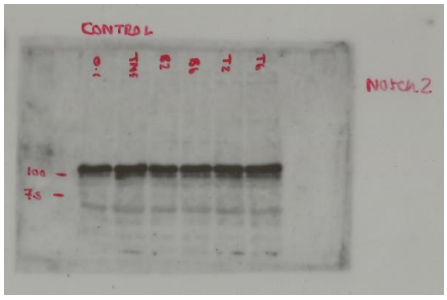
a) Supplement Fig 11c_NOTCH1 (left panel)



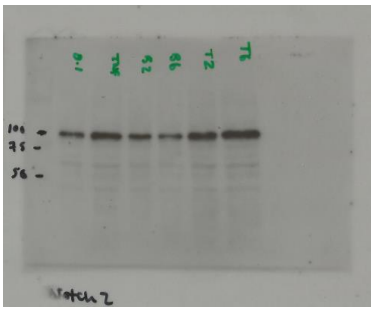
e) Supplement Fig 11c_NOTCH1 (right panel)



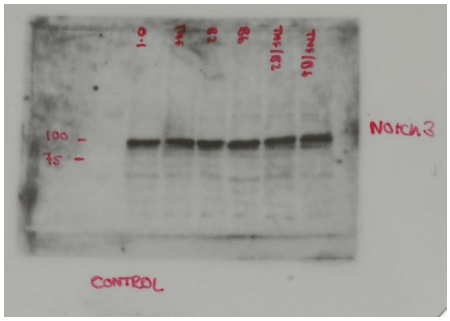
b) Supplement Fig 11c_NOTCH2 (left panel)



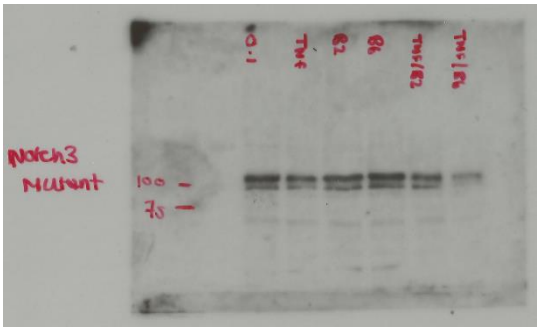
f) Supplement Fig 11c_NOTCH2 (right panel)



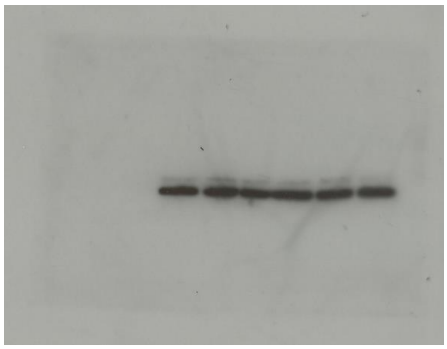
c) Supplement Fig 11c_NOTCH3 (left panel)



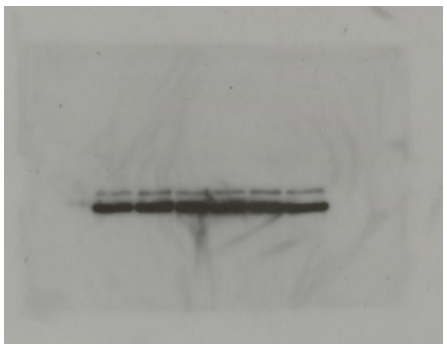
g) Supplement Fig 11c_NOTCH3 (right panel)



d) Supplement Fig 11c_alpha tubulin (left panel)

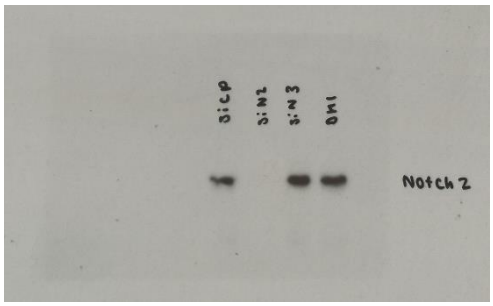


h) Supplement Fig 11c_alpha tubulin (right panel)

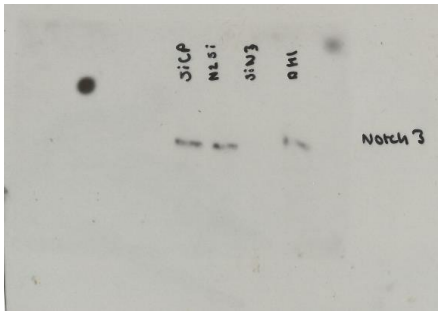


Supplementary Figure 36. Uncropped western blots for Supplementary Figure 11c. The relevant figures are indicated in the blot titles.

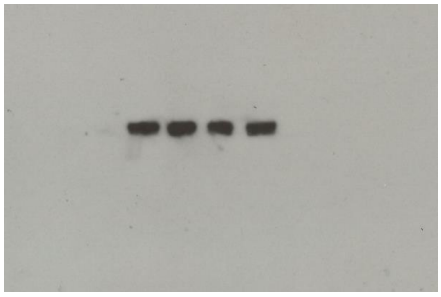
a) Supplement Fig 13g_NOTCH2 panel)



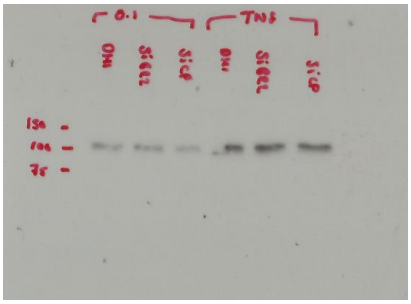
b) Supplement Fig 13g_NOTCH3



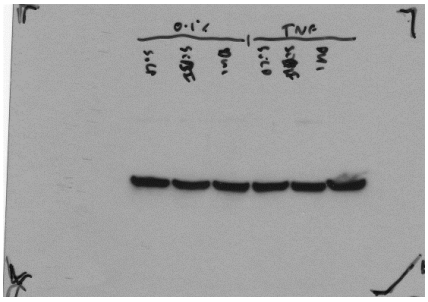
c) Supplement Fig 13g_alpha tubulin



d) Supplement Fig 14d_NOTCH1 (left

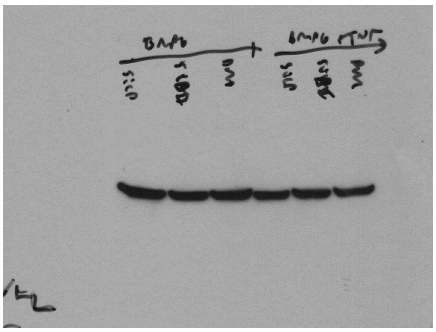


e) Supplement Fig 14d_alpha tubulin (left panel)



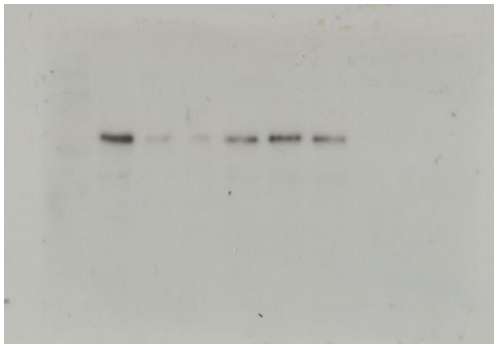
f) Supplement Fig 14d_NOTCH1 (right panel)

g) Supplement Fig 14d_alpha tubulin (right panel)

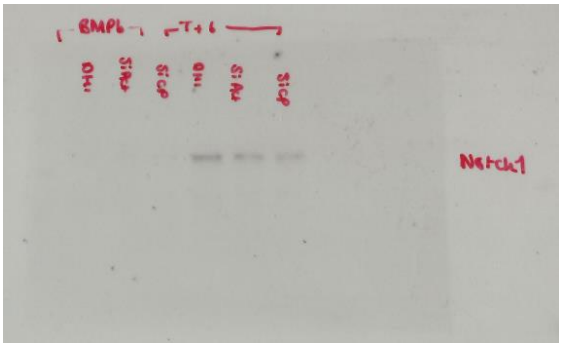


Supplementary Figure 37. Uncropped western blots for Supplementary Figures 13g and 14d. The relevant figures are indicated in the blot titles.

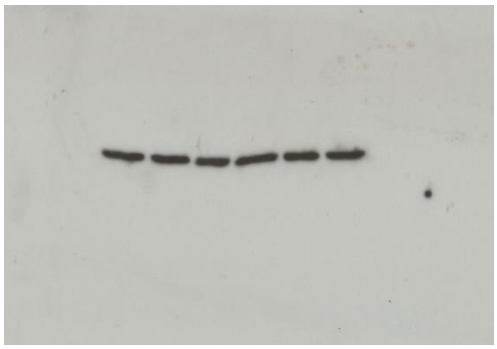
a) Supplement Fig 14e_NOTCH1 (left panel)



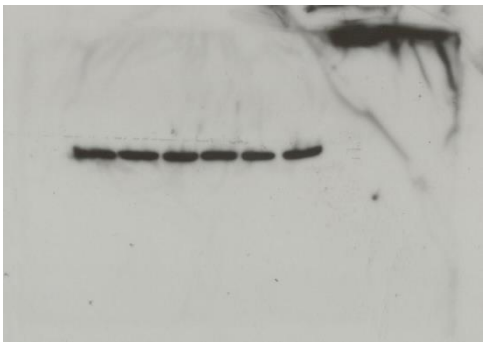
c) Supplement Fig 14e_NOTCH1 (right panel)



b) Supplement Fig 14e_alpha tubulin (left panel)

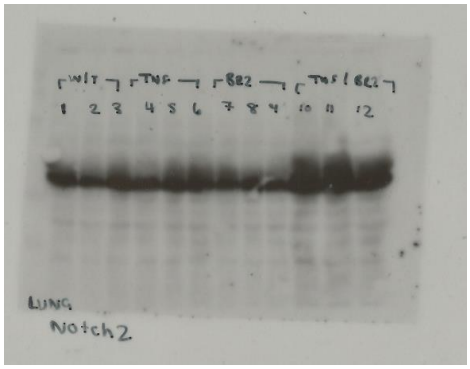


d) Supplement Fig 14e_alpha tubulin (right panel)

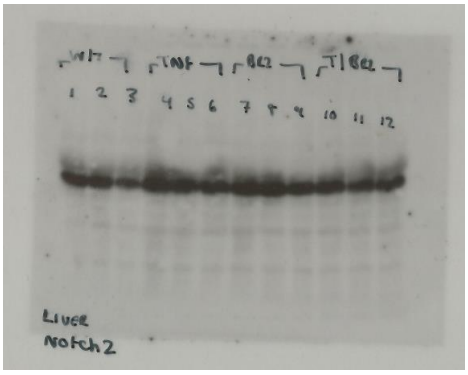


Supplementary Figure 38. Uncropped western blots for Supplementary Figure 14e. The relevant figures are indicated in the blot titles.

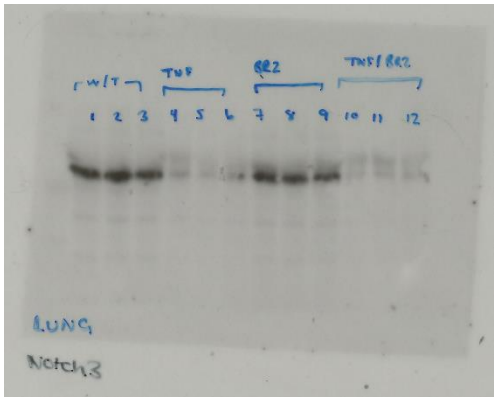
a) Supplement Fig 14f_NOTCH2 (left panel)



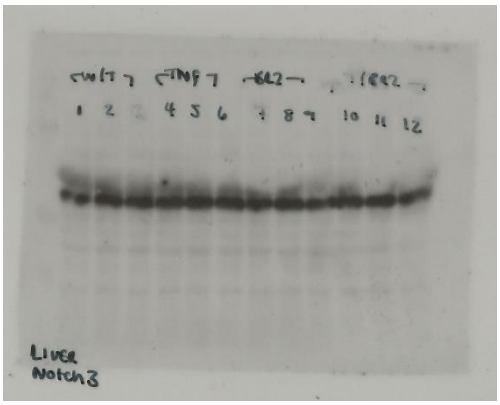
d) Supplement Fig 14f_NOTCH2 (right panel)



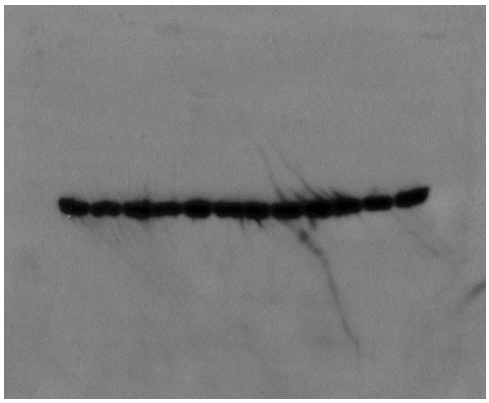
b) Supplement Fig 14f_NOTCH3 (left panel)



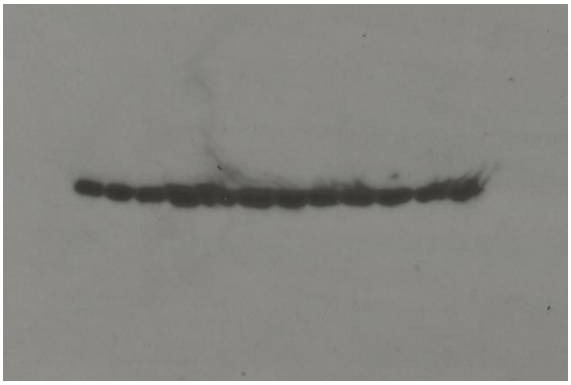
e) Supplement Fig 14f_NOTCH3 (right panel)



c) Supplement Fig 14f_alpha tubulin (left panel)



f) Supplement Fig 14f_alpha tubulin (right panel)



Supplementary Figure 39. Uncropped western blots for Supplementary Figure 14f. The relevant figures are indicated in the blot titles.

Supplementary Table 1: Left ventricular function in the Sugen-hypoxia rat model

		Sugen + Hypoxia - 11 weeks	
	Control	**Vehicle**	**Etanercept**
n	6	7	8
Heart rate (bpm)	445.3 ± 22.4	425.5 ± 45.9	373.1 ± 68.4*
LVESP (mmHg)	107.4 ± 10.7	104.5 ± 22.8	105.3 ± 14.5
LVEDP (mmHg)	21.59 ± 9.78	14.45 ± 8.36	17.18 ± 7.85
Stroke Volume (RVU)	19.23 ± 4.30	22.38 ± 5.34	23.04 ± 8.75
Cardiac Output (RVU/min)	8581 ± 2030	9450 ± 2360	8253 ± 2471
dP/dt max (mmHg s^{-1})	6835 ± 799	6363 ± 1574	6470 ± 1084
Blood Pressure (mmHg)	100.0 ± 3.69	101.0 ± 6.90	100.6 ± 10.0

+/- SEM, *P<0.05 relative to control
LVESP: Left Ventricular End-Systolic Pressure
LVEDP: Left Ventricular End-Diastolic Pressure
RVU: Relative volume units

Supplementary Table 2: Pulmonary arterial smooth muscle cell clinical information

Subject ID	**Sex**	**Age**	***BMPR2* Mutation**	**Clinical Details**
Control PASMCs				
9MP	M	72	-	Adenocarcinoma
32MP	F	58	-	Emphysema
70MP	M	66	-	Squamous cell carcinoma
HPAH PASMCs				
56MP	M	58	C347R	FPAH; transplant
67MP	M	22	W9X	FPAH; transplant
73MP	F	30	R899X	FPAH; transplant

Supplementary Table 3. Mutagenesis primers for myc-tagged BMPR-II valine-alanine plasmids.

Mutation	**Forward**	**Reverse**
V158A	AATAATCATTGCTTTGGCATCAG**C**C TCTGTATTAGCTGTTTTGATAG	CTATCAAAACAGCTAATACAGAG**G**CTGAT GCCAAAGCAATGATTATT
V160A	TGCTTTGGCATCAGTCTCTG**C**ATTA GCTGTTTTGATAGTTG	CAACTATCAAAACAGCTAAT**G**CAGAGACT GATGCCAAAGCA
V163A	CATCAGTCTCTGTATTAGCTG**C**TTTG ATAGTTGCCTTATGCTT	AAGCATAAGGCAACTATCAAA**G**CAGCTAA TACAGAGACTGATG
V166A	GTCTCTGTATTAGCTGTTTTGATAG**C** TGCCTTATGCTTTGGA	TCCAAAGCATAAGGCA**G**CTATCAAAACAG CTAATACAGAGAC

Supplementary Table 4. Quantitative RT-PCR primers for human.

Target	Forward	Reverse
ACTB	GCACCACACCTTCTACAATGA	GTCATCTTCTCGCGGTTGGC
ALK3	TTCGTATGACGGATCACTCG	AGCCCTACATCATGGCTGAC
ADAM10	TTCTGCTCCTCTCCTGGGCGG	GCTCTTTTGGCACGCTGGTGT
ADAM12	CACGGCAGAGGGGTGTGCAA	TCCGGCAGCAAGAAGACACAGG
ADAM15	GGCACTGAGGAGCAGCAGGC	CGGCCTGGGACCAACTCCCT
ADAM17	TAGCAGTGAGTGCCCGCCTCC	CATAGGGCACACAGCGGCCAG
B2M	CTCGCGCTACTCTCTCTTTCT	CATTCTCTGCTGGATGACGTG
BMPR2	CAAATCTGTGAGCCCAACAGTCAA	GAGGAAGAATAATCTGGATAAGGACCAAT
FYN	GGCGGGGAGAGGACCATGTGAG	GAGGCAGGACTGGTCTTTTTCCACG
HES1	GGAATCCCCCGTCTACCTCT	TGAGCAAGTGCTGAGGGTTT
HEY1	GGCTCTAGGTTCCATGTCCC	AGCAGATCCCTGCTTCTCAA
HEY2	GCCATACAGATGCCGACAGA	CAGTTACCGAGCTGCCTTGA
ID1	GACGGCCGAGGCGGCATG	GGGGAGACCCACAGAGCACG
NOTCH3	CCTAGACCTGGTGGACAA	ACACAGTCGTAGCGGTTG
MMP14	CGGACCATGTCTCCCGCCCC	GTGACTGGGGTGAGCGCTGTG
MMP15	CTTGGCGTAGCGGCCGAAGA	CTGGGCGGAACGCATGGTGG
MMP16	ACCTTCCACCGACTGACCCCA	GGTACACCGCATCGGGGCTTC
MMP17	GACAAGGTGCGCGTCTGGCA	CTGGGCCACCGCGTCAAAGT
MMP24	TCGCTGGTTCTGGCGTCTGC	GGGTACCCAGGCTCCACCGT
SRC	TCCACCGGGACCTTCGTGCA	AATTTGGCACCTTGCCGCGC
YES	GCGGCCGGAGGACAGATTTGAT	ACGGACATGGTGACACTGTAGTGGG

Supplementary Table 5. Quantitative RT-PCR primers for mouse

Target	Forward	Reverse
Bmpr2	AGATCTATCCTCTCCCTAAG	TTAGAATGGACTGCCCTGTC
Notch1	GTCGCAACTGTGAGAGTGA	TCGCAGAAGGCTGTGTTGAT
Notch2	AGCAGACTGGATGAACCGTG	AGCTGGAAAGTCACGATGGG
Notch3	GACTGCTCACTGAACGTGGA	CACACCGGCTGTTGTTGAAG

Supplementary Table 6. Quantitative RT-PCR primers for rat

Target	Forward	Reverse
Actb	CTGCCTGACGGTCAGGT	TGGATGCCACAGGATTCCAT
Alk2	TCTGTGCTAATGATGATGGCTCTCC	TTCGCGATCCAGGGAAGGATTTC
Acvr2a	ACTGCTGCAGATGGACCTG	AGCTCCAGTTCAGAGTCCC
Bmp6	AGCACAGAGACTCTGACCTATTTTTG	CCACAGATTGCTAGTTGCTGTGA
Hes1	CGACACCGGACAAACCAAA	GAATGTCTGCCTTCTCCAGCTT
Hey1	AAGCTGAGATCTTGCAGATG	GGCGGCGACAGTTTGGAG
Hey2	TGACATCCTCCATGTCCC	ACTGATAACGGTGGGCTG