

Supplementary Methods:

High Throughput Screen:

Libraries of FDA approved drugs and bioactive compounds containing 3756 unique compounds (NIH-CC, LOPAC, Biomol ICCB Known Bioactives, Microsource spectrum, Biomol FDA approved drug library) were available from the High-Throughput-Bioscience-Center at Stanford University. Some compounds were present in more than one drug library. The C2C12 mouse myoblast cell line that stably expresses a **BMP Response Element** from the Id1 promoter linked to luciferase (BRE-luc) (1) was used as a reporter cell line for BMP activation. The bioassay can detect luciferase activity after stimulation with as low as 3 pM BMP4 (our ligand of choice due to the excellent assay sensitivity) and has been validated for BMP2, BMP6 and BMP7; however, other members of the TGF- β family, i.e., TGF- β 1, 2, 3 or TGF- β unrelated growth factors such as FGF-2 and VEGF, do not induce luciferase activity. We optimized a number of conditions (cell number, BMP4 concentration, time-course of plating cells, adding stimulus, adding luciferase substrate *Brightglo*[®] (Promega) and the library of interest). C2C12 BRE-luc cells were grown in DMEM (4.5 g/L D-Glucose, L-Glutamine, 10% FBS, 1% Penicillin/Streptomycin) up to 80% confluency. Cells were trypsinized, centrifuged and re-suspended in complete medium. Cells were counted in a Coulter counter (size range 11-21 μ m) and 1500 cells/60 μ l were plated in each well of a 384-well plate (EK-30098, clear bottom plate, E&K Scientific) using the Matrix Wellmate. Cells were placed in the Liconix automated incubator at 37 $^{\circ}$ C for 24 h. Ten microliters of the stimulant BMP4 was added to columns 1-22 of every other plate by the multidrop (250 pM final concentration) on the Staccato system and 100 nL of the compounds were added (in duplicate +/- BMP4) to plates using the Pin Tool on the SciClone ALH3000. The plates were incubated for an additional 24 hours and 10 μ l Bright-Glow (Promega) was added to each well and the plates were read immediately afterwards in the AnalystGT (0.2 second read per well). Hits were determined as compounds that either increase the BMP4 induced signal (weak activators) or increase the un-induced signal (strong activators) without exogenous BMP4 and only endogenously produced BMP4 around. The intra-plate and inter-plate variability in a pilot screen using 10 x 384 well plates showed a coefficient of variance of 4.7% and 7.1% respectively. The Z' of the screen was above 0.5 showing a robust and reproducible response from BMP4 activation. "Hits" were validated with respect to their potential to activate luciferase to detect false positive signals and promising hits were tested in 6 serial dilutions.

Immunoprecipitation:

To determine the interaction between TGF- β type I receptors and FKBP12, 293T cells were transfected with low amounts of Flag-tagged FKBP12 and/or BMP type I receptor (ALK1, -2 or -3)-HA tagged plasmid. Medium was changed 12 hour after transfection, and 48h after transfection cells were stimulated with FK506 (100ng/ml) or vehicle for 30 min. prior to harvest. Cells were then washed with PBS and lysed with 1ml lysis buffer (10mM Tris-HCl at pH 7.5, 1mM EDTA, 150mM NaCl and 0.5% NP-40) plus protease inhibitors (Sigma) for 10min at 4°C. Lysates were clarified by centrifugation and equal amounts of lysate were incubated with anti-Flag agarose beads (A2220, Sigma) for 1hour at 4°C before the beads were washed three times with washing buffer (50mM Tris-HCl at pH8.0, 150mM NaCl, 0.5% Nonidet P-40, 0.5%sodium deoxycholate and 0.1%SDS) and re-suspended in lysis buffer. Total protein expression was detected by immunoblotting with anti-Flag (M2, Sigma) or anti-HA (Y-11, SC-805, Santa Cruz biotechnology) antibodies. The immune complexes were eluted with sample buffer containing 1% SDS for 5min at 95°C, and separated by SDS-PAGE. Western blotting was performed with indicated antibodies and secondary anti-mouse or anti-rabbit antibodies conjugated with horseradish peroxidase (Amersham Biosciences). Visualization was obtained with chemiluminescence detection

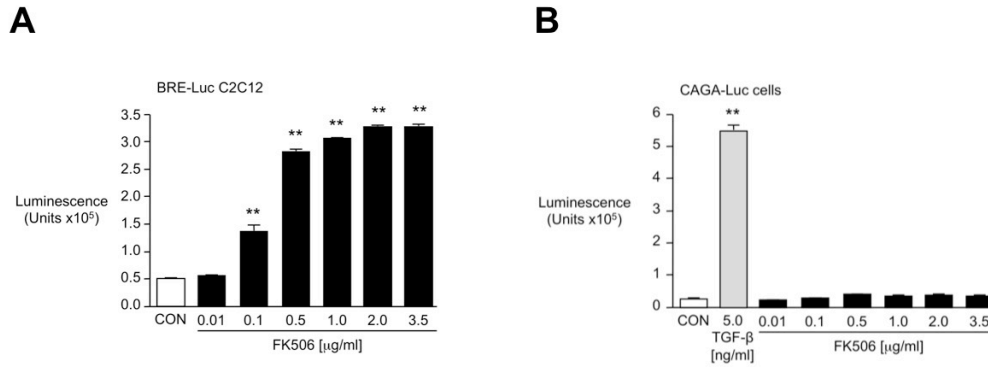
While upon co-expression FKBP12 and BMP type I receptor were readily detected by Western blot analysis, when expressed alone, FKBP12 or BMP type I receptor expression levels were below the detection limit. A possibility for the higher expression of FKBP12 and BMP type I receptor, when co-expressed, is that FKBP12 stabilizes the complex via its chaperone activity. FK506 short-term treatment potently inhibited the association between FKBP12 and BMP type I receptor.

	WT				BMPR2 KO				Significance value
	Normoxia VEH	Normoxia FK	Hypoxia VEH	Hypoxia FK	Normoxia VEH	Normoxia FK	Hypoxia VEH	Hypoxia FK	
Cardiac Output (ml/min)	25.3 ± 2.5	-	16.1 ± 3.9*	11 ± 0.8*	33.9 ± 4.3	-	12.72 ± 2.8*	15.6 ± 2.6*	*p < 0.05
Fractional Shortening (%)	25.3 ± 2.5	-	34.6 ± 1.7	33.1 ± 4.7	33.9 ± 4.3	-	36.6 ± 3.0	31.8 ± 2.2	ns
LVDd (cm)	0.44 ± 0.01	-	0.36 ± 0.01	0.33 ± 0.03	0.36 ± 0.01	-	0.39 ± 0.02	0.37 ± 0.03	ns
WBC total (Tsd)	6.3 ± 0.3	6.3 ± 1	3.8 ± 1.6	5.0 ± 2	6.2 ± 2.5	3.8 ± 1	2.5 ± 0.2	3.3 ± 0.1	ns
Neutrophils (%)	14 ± 5	4 ± 0	44 ± 5*	26 ± 5	26 ± 6	22 ± 3	33 ± 8	44 ± 7*	*p < 0.05
Lymphocytes	70 ± 0	79 ± 9	48 ± 1	68 ± 2	64 ± 2	70 ± 4	62 ± 7	50 ± 9	ns
HCT (%)	41 ± 5	42 ± 0	64 ± 4*	62 ± 2*	44 ± 2	43 ± 1	59 ± 1*	62 ± 4*	*p < 0.01

Supplementary Table 1: Characterization of mice with a conditional endothelial specific deletion in BMPR2 in Normoxia and Hypoxia ± Vehicle (VEH) or FK-506 in terms of echocardiographic as well as laboratory parameters. (2-way ANOVA and Bonferroni's post test, N=8, *p<0.05 vs Normoxia VEH. ns=No significant difference between treated and untreated groups). All data mean ± s.e.m.

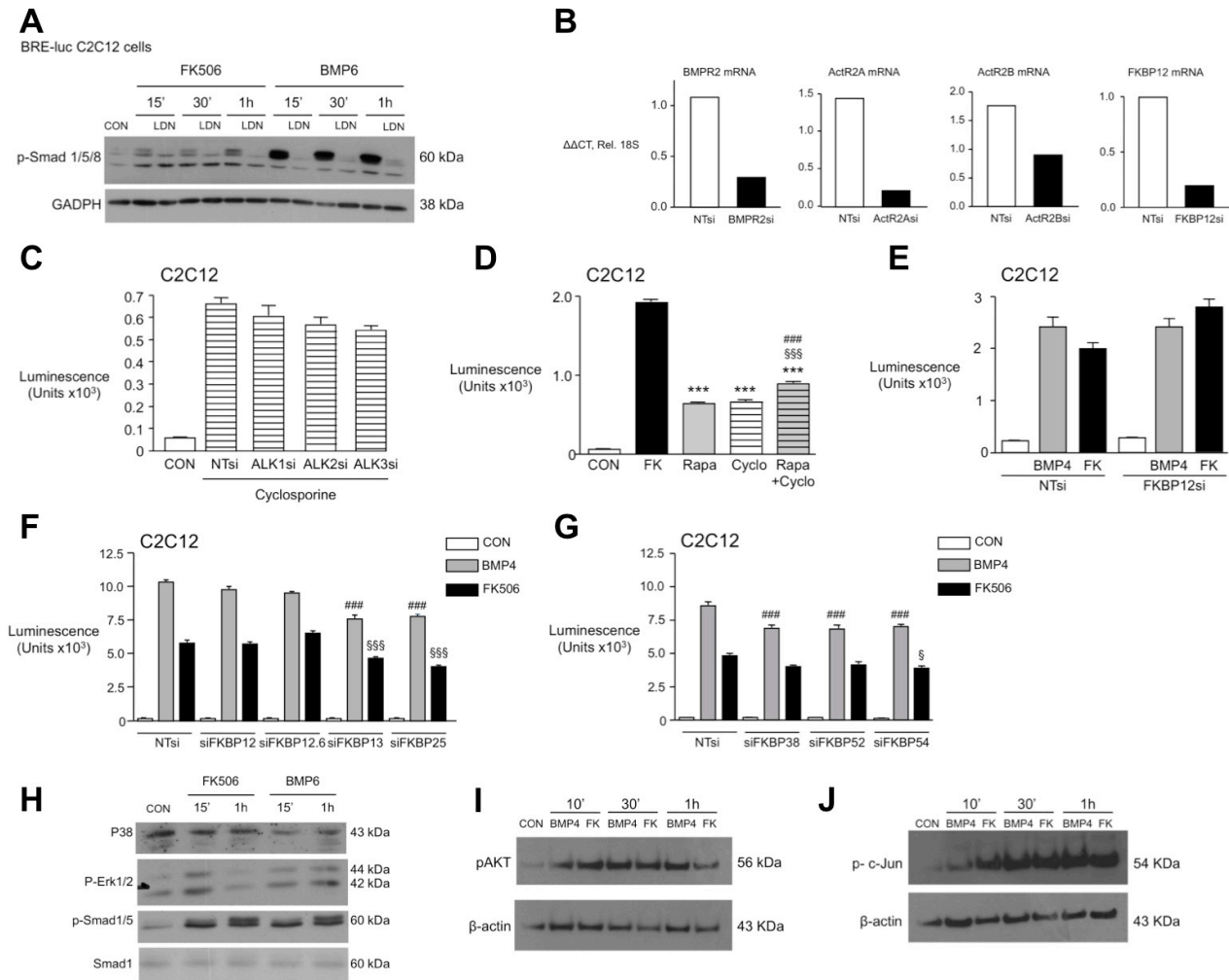
	Control Rats	SUGEN Hypoxia 8 weeks	SUGEN Hypoxia 11 weeks Vehicle	SUGEN Hypoxia 11 weeks FK-506	Significance value
Cardiac Output (ml/min)	148.5 ± 3.14	104.7 ± 15.9	110.5 ± 21.5	140.4 ± 18.7	ns
Cardiac Output/ 100g bodyweight (ml/min/g)	42.9 ± 1.0	34.2 ± 7.1	22.5 ± 3.8	28.6 ± 2.8	ns
Fractional Shortening (%)	58.3 ± 8.5	40.3 ± 1.8*	37.3 ± 1.9*	43.3 ± 2.4	*p < 0.05
LVDD (cm)	0.53 ± 0.17	0.69 ± 0.06	0.82 ± 0.02	0.82 ± 0.02	ns

Supplementary Table 2: Echocardiographic cardiac output measurements in control versus SUGEN/Hypoxia treated rats with and without treatment with FK506. (N=8, 1-way ANOVA, Bonferroni comparison all pairs, *p<0.05 vs. control). All data mean ± s.e.m.

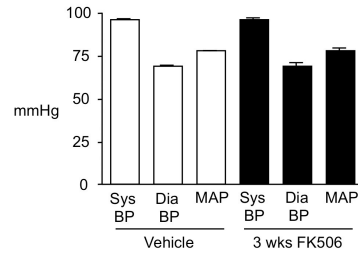


Supplement Figure 1: FK506 in a dose dependent manner induces BMP but not TGF- β signaling

(A) BRE-luciferase activity in C2C12 cells at different concentrations of FK506 (0.01 and 3.5 μ g/ml) (N=6, **p<0.01 vs CON, one-way ANOVA, Dunnett's post-test). **(B)** Luciferase activity in CAGA-Luc cells with TGF- β (5ng/ml) and different concentrations of FK506 (0.01 and 3.5 μ g/ml) (N=6, **p<0.01 vs CON, one-way ANOVA, Dunnett's post-test). Bars are mean \pm s.e.m.

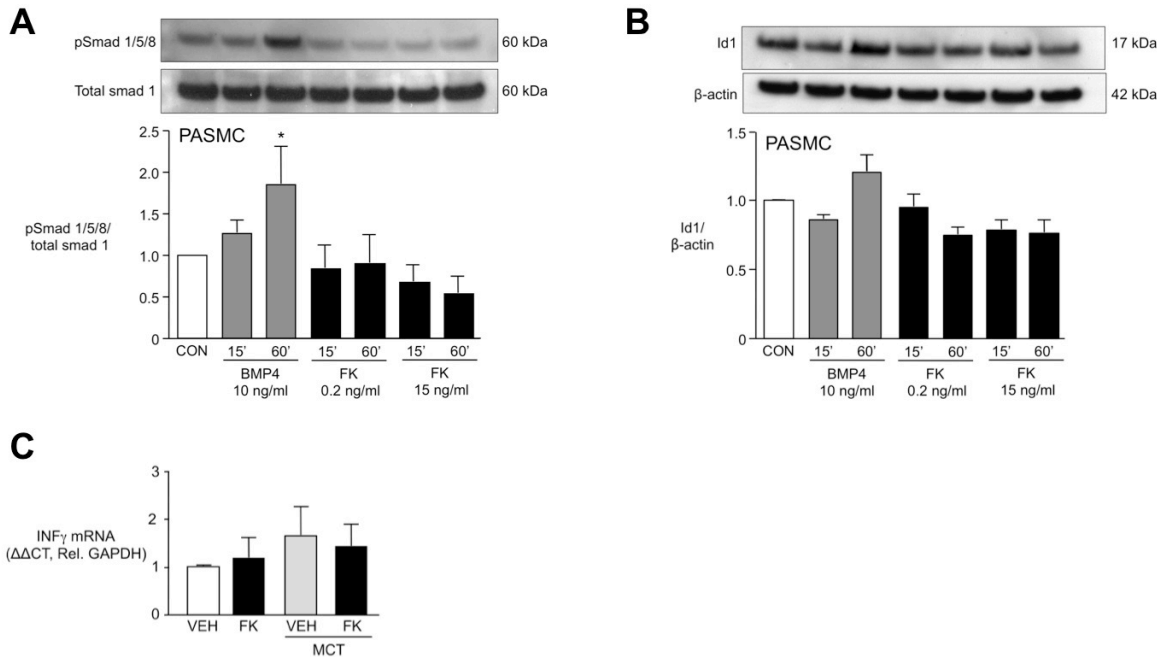


Supplement Figure 2: Mechanism of FK506 induced BMP signaling related to FKBP12 release from type I receptors. (A) Representative immunoblot showing expression of phospho Smad1/5/8 and GAPDH in C2C12 cells, pretreated with LDN-193189 (120 nM) for 30 min and stimulated with BMP6 (50ng/ml) and FK506 (1 μ g/ml) for 15 min, 30 min and 1 h. (B) Panels showing knockdown efficiency of, BMPR2si, ActivinR 2Asi as well as ActivinR 2Bsi FKBP12si in C2C12 BRE-luc cells. (C) BRE-luciferase activity in C2C12 cells treated with non-targeting (NT)si, ALK1si, ALK2si and ALK3si and stimulated with Cyclosporine (2 μ g/ml) (N=8, NS vs. NTsi with cyclosporine, One-way ANOVA). (D) BRE-luciferase activity in C2C12 cells after FK506 (2 μ g/ml), Rapamycin (4 μ g/ml), Cyclosporine (2 μ g/ml) and Rapamycin combined with Cyclosporine (same conc. as above) (N=8, ***p<0.001 vs. FK; ###p<0.01 vs. Rapa; and \$\$\$p<0.001 vs. Cyclo one-way ANOVA, Bonferroni's Multiple Comparison Test). (E) BRE-luciferase activity in C2C12 cells after knocking down FKBP12 by siRNA and stimulation with FK506 (2 μ g/ml) and BMP4 (250 pM) (N=16, **p<0.01 NTsi + FK vs. FKBP12 si + FK, two-way ANOVA, Bonferroni's Multiple Comparison Test). (F and G) BRE-luciferase activity in C2C12 cells after knocking down different FK Binding Proteins (FKBPs) by siRNA and no stimulation or stimulation with BMP4 (250 pM) or FK506 (2 μ g/ml): FKBP1A (=FKBP12), FKBP1B (=FKBP12.6), FKBP2 (=FKBP13), FKBP3 (=FKBP25), FKBP8 (=FKBP38), FKBP4 (=FKBP52), FKBP5 (=FKBP54). (H) Representative immunoblot showing phospho p38, phospho ERK, Phospho Smad1/5 and Smad1 in PAECs stimulated with BMP6 (50 ng/ml) and FK506 (15 ng/ml) for 15 min and 1 h. (I) Representative immunoblot showing phospho AKT and β -actin in PAECs stimulated with BMP4 (40 ng/ml) and FK506 (15 ng/ml) for 10 min, 30 min and 1 h. (J) Representative immunoblot showing phospho c-Jun and β -actin in PAECs stimulated with BMP4 (40 ng/ml) and FK506 (15 ng/ml) for 10 min, 30 min and 1 h. Bars are mean \pm s.e.m.



Supplement Figure 3: 3-week treatment with low dose FK506 does not increase the systemic blood pressure in mice.

Systemic blood pressure measurements via carotid artery in C57Bl6 mice (8-10 weeks of age) after 3 weeks treatment with vehicle or FK506 (0.05mg/kg/d) via mini osmotic pump (N=3 in each group). Bars are mean \pm s.e.m.

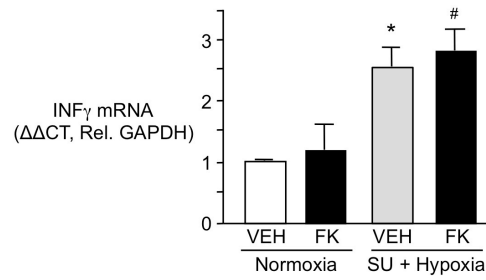


Supplement Figure 4 (A,B): FK506 does not induce Smad1/5 phosphorylation or Id1 expression in human pulmonary artery smooth muscle cells (hPASCs).

(A) Representative immunoblot and densitometric analysis of pSmad1/5/8 relative to total Smad-1 protein expression and (B) Id1 protein relative to β -actin at 15 min and 60 min after stimulation with BMP4 (10 ng/ml), FK506 (0.2 ng/ml and 15 ng/ml) in PASCs. (In A and B, for N=3, *p< 0.05 vs. CON, 1-way ANOVA, Dunnett's post-test). Bars are mean \pm s.e.m.

Supplement Figure 4 (C): Low dose FK506 does not reduce NFAT dependent $\text{INF}\gamma$ expression in monocrotaline treated rats.

(C) $\text{INF}\gamma$ expression in whole lungs of rats 21 d after MCT treatment (60 mg sc once) and \pm 3 week treatment with either vehicle (VEH) or low dose FK506 (N=6-8, non significant, 2-way ANOVA). Bars are mean \pm s.e.m.



Supplement Figure 5: Low dose FK506 does not decrease NFAT dependent INF γ expression in whole lungs of SUGEN/hypoxia rats

INF γ expression in whole lungs of rats in normoxia (\pm VEH or low-dose FK506) as well as in rats after hypoxia/Sugen/normoxia sacrificed at 11 w (\pm 3 w treatment of VEH or low dose FK506). (N=8, *p<0.05 VEH normoxia vs VEH hypoxia, #p<0.05 FK normoxia vs FK hypoxia, two-way ANOVA, Bonferroni post-test). Bars are mean \pm s.e.m.