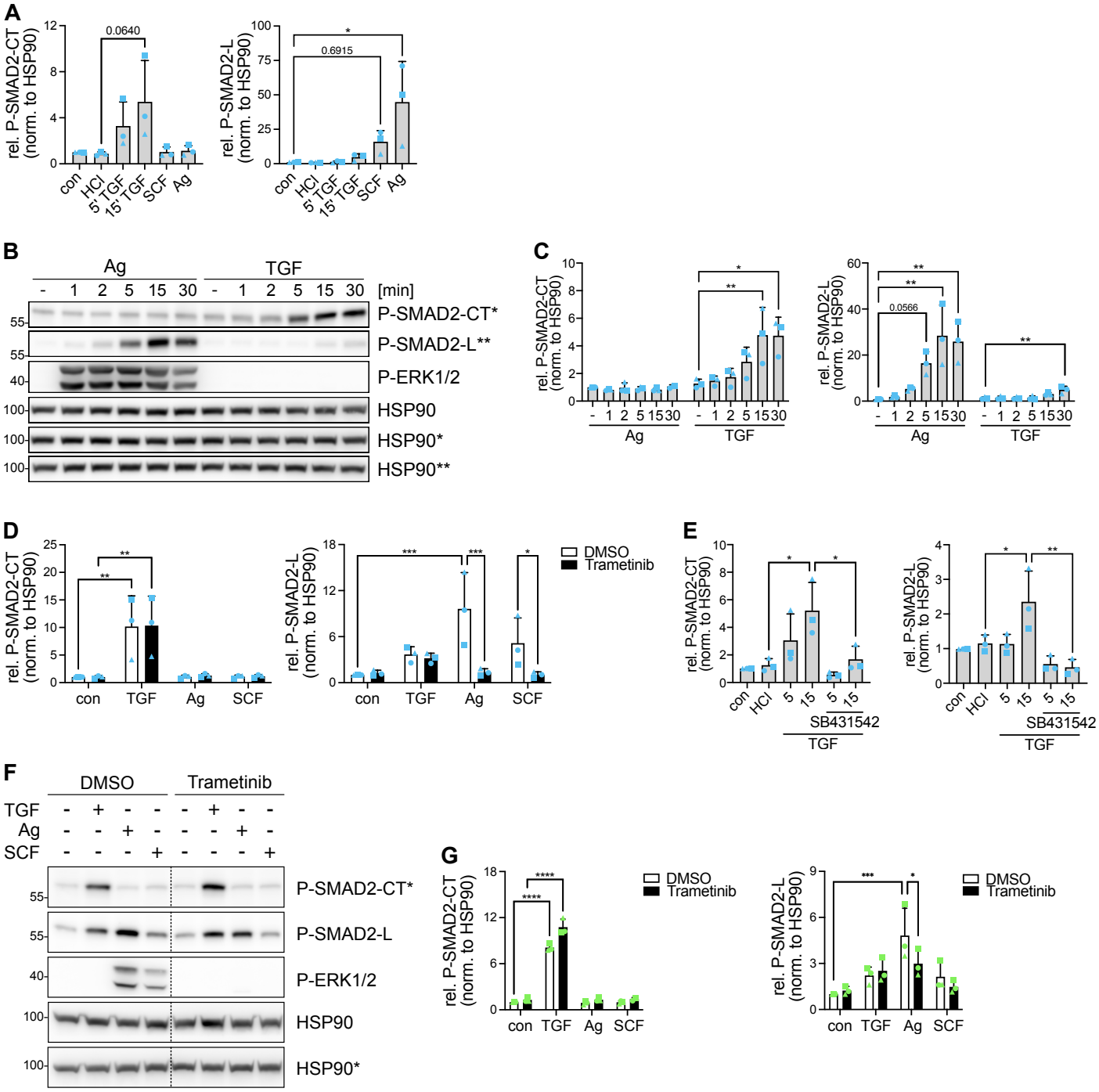
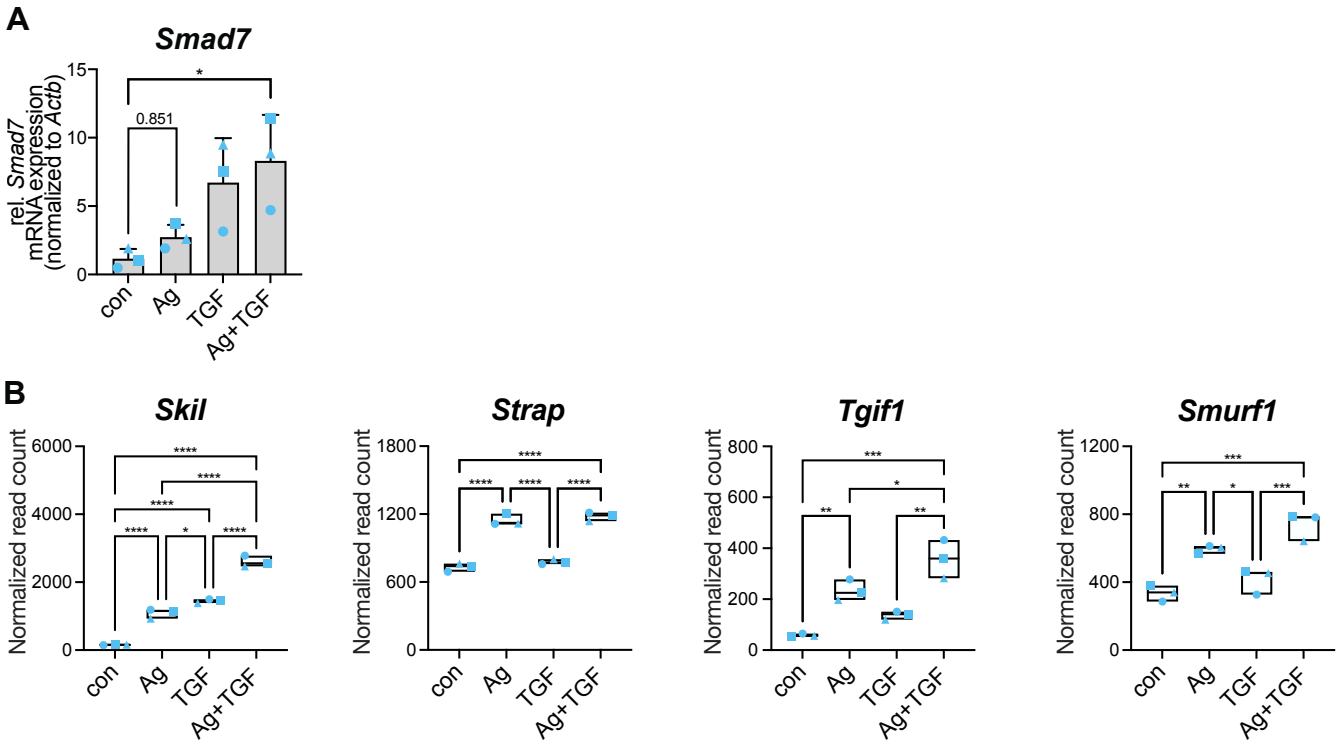


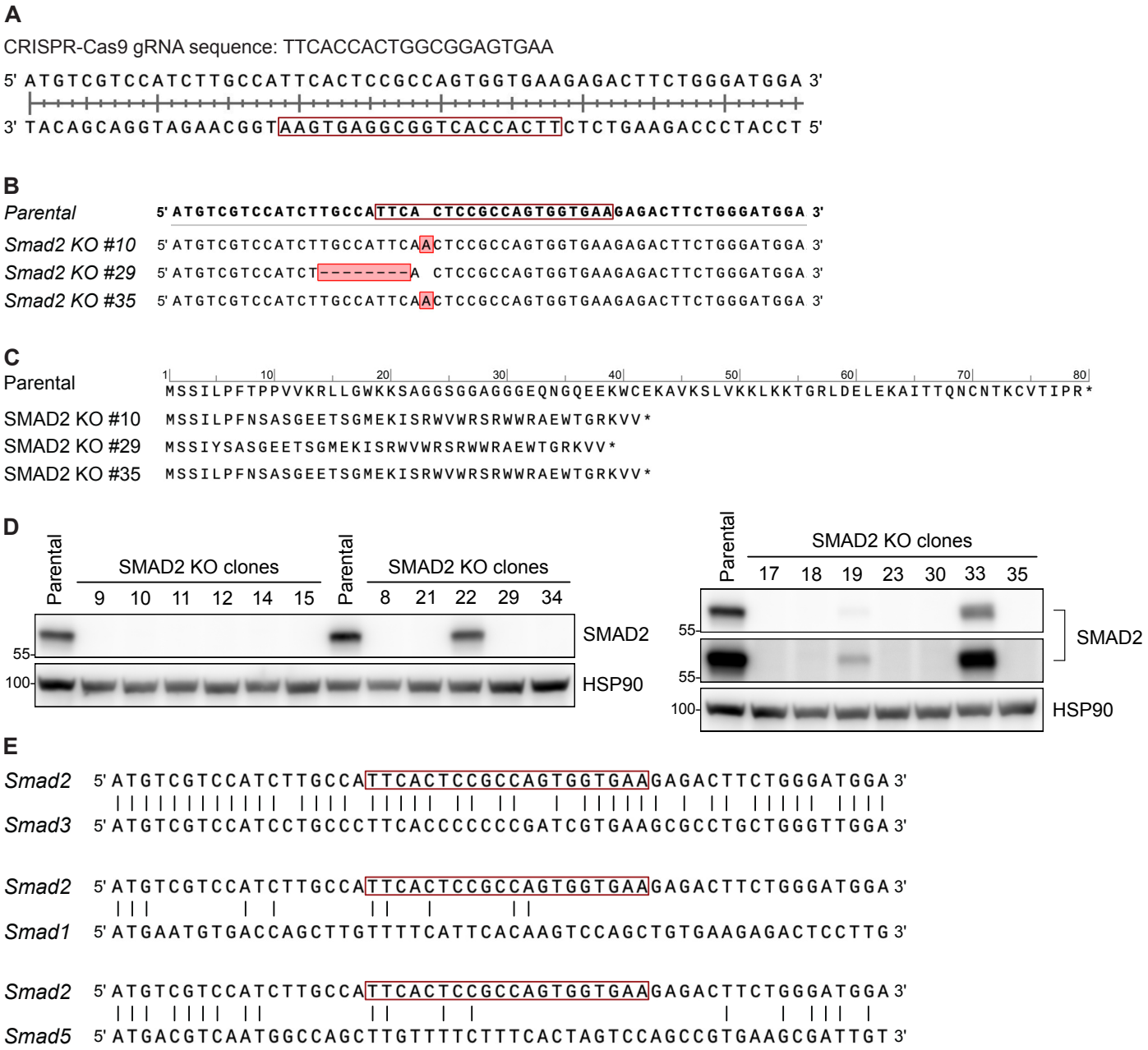
Bronneberg et al. – Supplement Fig. 1

(A) Quantitative analysis of P-SMAD2-CT and P-SMAD2-L protein levels relative to HSP90 (corresponding to Fig. 1A) (B) Representative Western blot for P-SMAD2-CT, P-SMAD2-L, and P-ERK1/2 in BMMCs in response to Ag (20 ng/mL) and TGF-β (1 ng/mL) for the indicated time points. HSP90 served as loading control. Asterisks indicate detection on the same membrane. (C) Quantitative analysis of P-SMAD2-CT and P-SMAD2-L levels relative to HSP90. n=3 (D and E) Quantitative analysis of P-SMAD2-CT and P-SMAD2-L protein levels relative to HSP90. Graphs depicted in (D) correspond to Fig. 1B and graphs depicted in (E) correspond to Fig. 1C. (F) Representative Western blot for P-SMAD2-CT, P-SMAD2-L, and P-ERK1/2 in PMC-306 cells in response to 15 min TGF-β (1 ng/mL), 15 min Ag (2 ng/mL), and 15 min SCF (30 ng/mL) with pre-incubation of 30 min Trametinib (50 nM) or DMSO control. HSP90 served as loading control. Asterisks indicate detection on the same membrane. Non-relevant lanes were removed from the blot indicated by a dashed line. (G) Quantitative analysis of P-SMAD2-CT and P-SMAD2-L levels relative to HSP90. n=3. Bar graphs are shown as mean + SD. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 by one-way ANOVA and Tukey's multiple comparison test (A, E), one-way ANOVA and Dunnett's multiple comparison test (C), or two-way ANOVA and Tukey's multiple comparison test (D, G).



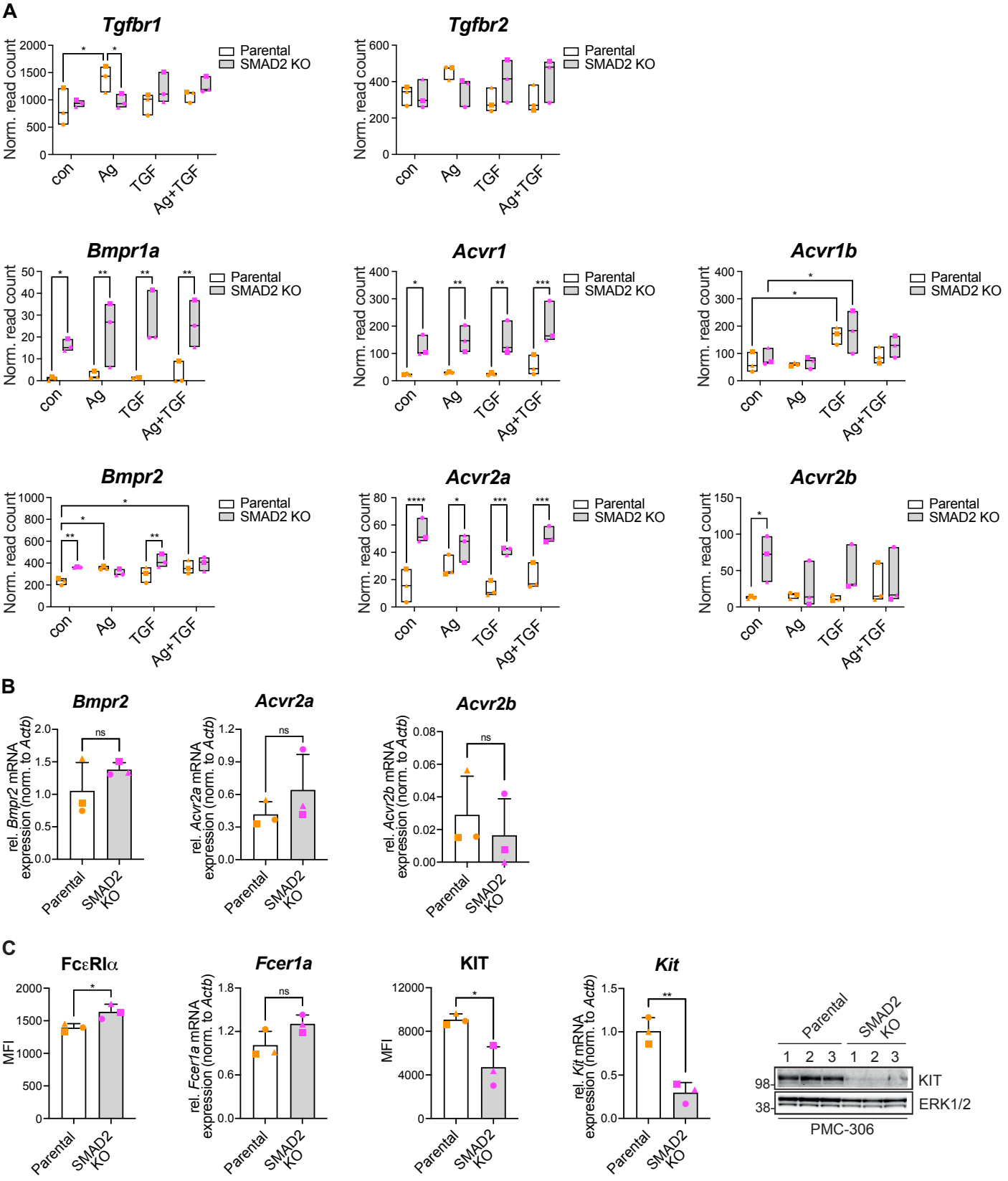
Bronneberg et al. – Supplement Fig. 2

(A) mRNA expression of *Smad7* was assessed by RT-qPCR in BMMCs in response to 90 min Ag, TGF-β and Ag+TGF-β and was normalized to *Actb*. n=3. (B) Differentially expressed genes from BMMCs in response to Ag (2 ng/mL), TGF-β, and Ag+TGF β for 90 min. Floating bars display the normalized read counts (NGS analysis). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 by one-way ANOVA and Dunnett's multiple comparison test (A) or one-way ANOVA and Tukey's multiple comparison test (B).



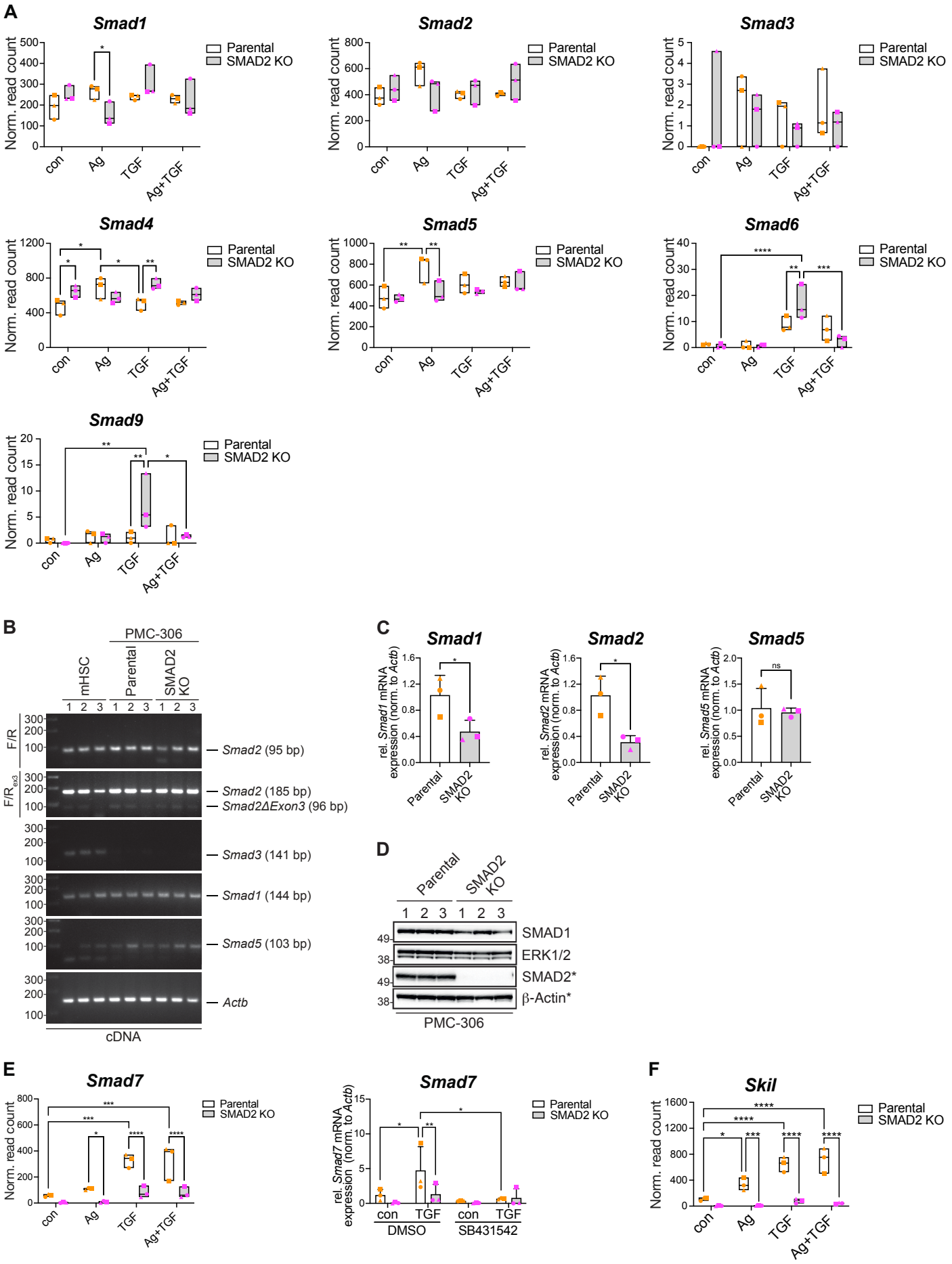
Bronneberg et al. – Supplement Fig. 3

(A) The depicted CRISPR-Cas9 gRNA sequence (red box) of *Smad2* was used to generate PMC-306 SMAD2 KO cells. (B, C) Multiple clones were collected from which number 10, 29 and 35 were sequenced (B) and subsequently selected for experiments. Clone 10 and 35 had the same single nucleotide insertion, while clone 29 had an 8 nucleotide deletion. Those changes result in frame shifts and cause premature stop codons (nonsense mutations) in the amino acid sequence (C, indicated with asterisks). (D) Western blots for SMAD2 using protein extracts from PMC-306 parental and S2KO cells, with HSP90 serving as the loading control. (E) Sequence alignment of *Smad2* with *Smad3*, *Smad1* and *Smad5*, respectively. The red box marks the binding site of the CRISPR-Cas9 gRNA.



Bronneberg et al. – Supplement Fig. 4

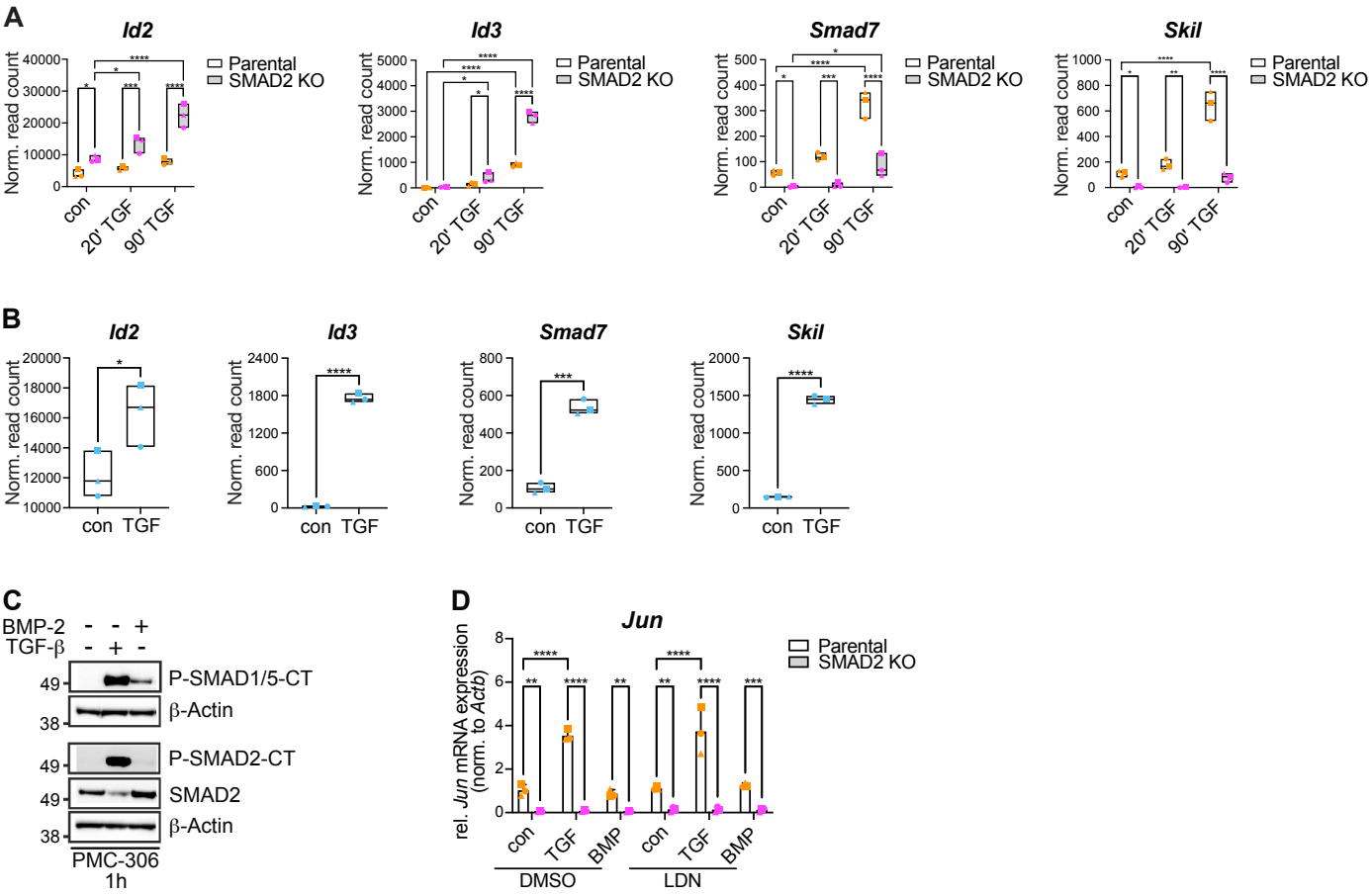
(A) Differentially expressed receptor genes in PMC-306 parental and S2KO cells in response to 90 min of Ag, TGF-β, and Ag + TGF-β. The floating bars display the normalized read counts from NGS analysis, with each replicate represented by a unique symbol. (B) mRNA expression of *Bmpr2, Acvr2a* and *Acvr2b* was assessed (RT-qPCR) in PMC-306 parental and S2KO cells and normalized to *Actb*. n=3. (C) Flow cytometric analysis of cell surface expression of FcεR1α and KIT in PMC-306 parental and S2KO cells. Data from three independent experiments are depicted as mean fluorescence intensity (MFI) + SD. mRNA expression of *Fcer1a* and *Kit* was assessed (RT-qPCR) and normalized to *Actb*. Expression of KIT was analyzed by Western blot, ERK1/2 served as loading control. Bar graphs are shown as mean + SD. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 by unpaired t-test (B, C) and two-way ANOVA and Tukey's multiple comparison test (A).



Bronneberg et al. – Supplement Fig. 5

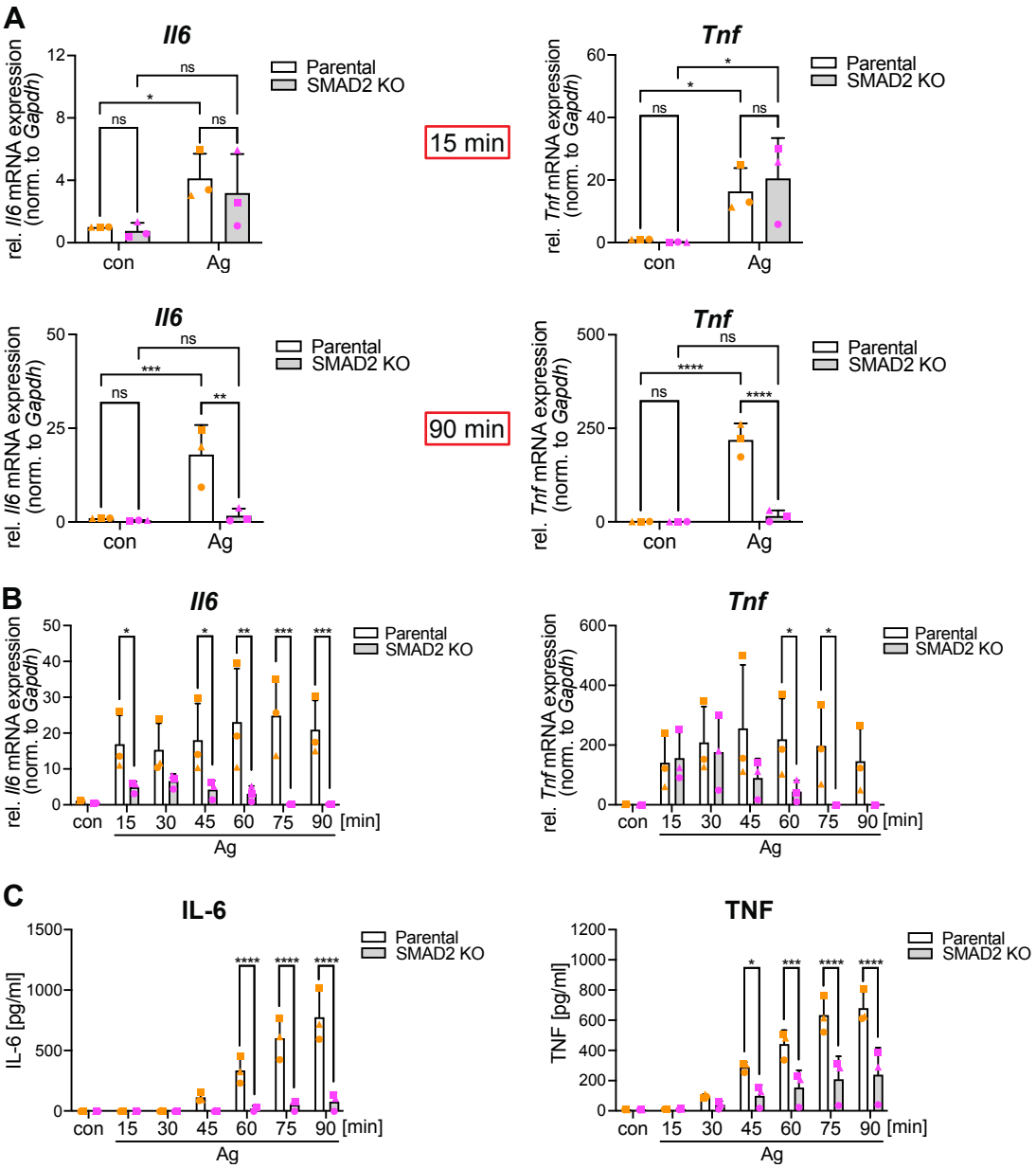
(A) Differentially expressed *Smad* genes in PMC-306 parental and S2KO cells in response to 90 min stimulation with Ag, TGF-β, and Ag + TGF-β. The floating bars display the normalized read counts from NGS analysis. (B) The expression of *Smad2*, *Smad2ΔExon3*, *Smad3*, *Smad1* and *Smad5* was assessed in murine primary hepatic stellate cells (mHSCs) and PMC-306 parental and S2KO cells using RT-PCR. *Actb* was used as the loading

control. (C) mRNA expression of *Smad1, Smad2, and Smad5* was assessed (RT-qPCR) in PMC-306 parental and S2KO cells and normalized to *Actb*. n=3. (D) Western blot analysis for SMAD1 and SMAD2 from PMC-306 parental and S2KO cells. ERK1/2 and β-Actin served as loading controls. Asterisks indicate detection on the same membrane. (E) (Left) Expression of *Smad7* mRNA was identified in PMC-306 parental and S2KO cells in response to 90 min Ag, TGF-β, and Ag+TGF-β. The floating bars display the normalized read counts (NGS analysis). (Right) The mRNA expression of *Smad7* was assessed by RT-qPCR in BMMCs in response to 90 min TGF-β with pre-incubation of 60 min SB431542 (5 μM) or DMSO control. The mRNA expression was normalized to *Actb*. n=3. (F) Expression of *Skil* mRNA was identified in PMC-306 parental and S2KO cells in response to 90 min Ag, TGF-β, and Ag+TGF-β. The floating bars display the normalized read counts (NGS analysis). Bar graphs are shown as mean + SD. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 by unpaired t-test (C) and two-way ANOVA and Tukey's multiple comparison test (A, E, F).



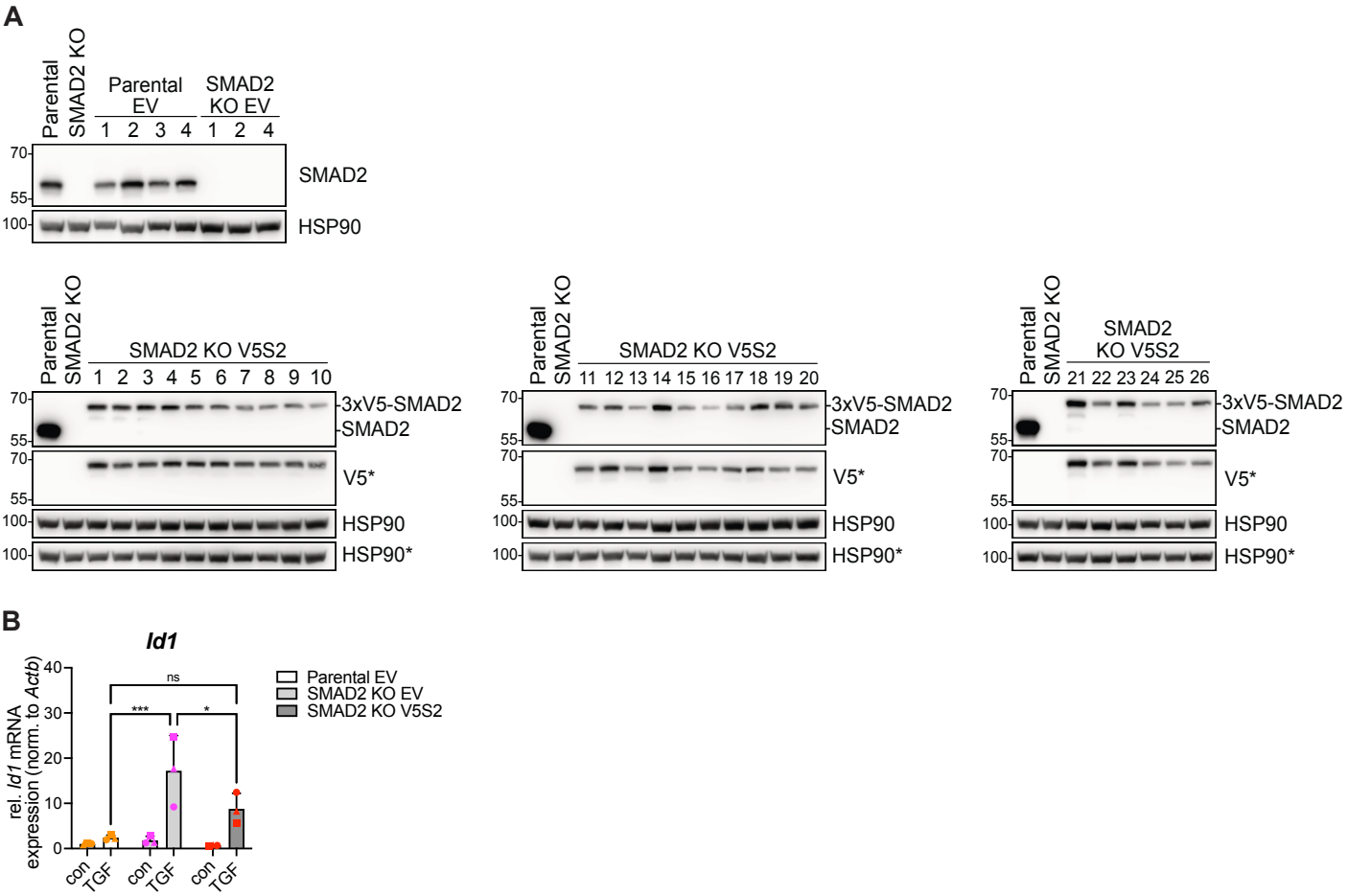
Bronneberg et al. – Supplement Fig. 6

(A) Differentially expressed genes (*Id2*, *Id3*, *Smad7*, *Skil*) from PMC-306 parental and S2KO cells in response to 20 and 90 min TGF-β. The floating bars display the normalized read counts (NGS analysis). (B) Differentially expressed genes (*Id2*, *Id3*, *Smad7*, *Skil*) from BMMCs in response to 90 min TGF-β stimulation. The floating bars display the normalized read counts (NGS analysis). (C) Western blot analysis of PMC-306 cells stimulated or not with TGF-β or BMP-2 (50 ng/mL) for 1 hr and detection of P-SMAD1/5-CT, P-SMAD2-CT and SMAD2, with β-Actin serving as the loading control. (D) mRNA expression of *Jun* was assessed (RT-qPCR) in PMC-306 parental and S2KO cells in response to 60 min TGF-β or BMP-2 with pre-incubation of 60 min LDN193189 (10 μM) or DMSO control and normalized to *Actb*. n=3. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 by unpaired t-test (B) and two-way ANOVA and Tukey's multiple comparison test (A, D).



Bronneberg et al. – Supplement Fig. 7

(A, B) The mRNA expression of *Il6* and *Tnf* was assessed by RT-qPCR in PMC-306 parental and S2KO cells in response to either 15 min and 90 min (A) or the indicated time points (B) of Ag. The mRNA expression was normalized to *Gapdh*. n=3 (C) The secretion of IL-6 and TNF from PMC-306 parental and S2KO cells in response to Ag for the indicated time points was measured by ELISA. n=3. Bar graphs are shown as mean + SD. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 by two-way ANOVA and Fisher's LSD test (A) or two-way ANOVA and Tukey's multiple comparison test (B, C).



Bronneberg et al. – Supplement Fig. 8

(A) Representative Western blots for SMAD2 and V5-tag in protein extracts from single-colony PMC-306 parental empty vector (EV), S2KO EV and S2KO 3xV5-Smad2 (V5S2) cells. HSP90 served as loading control. Asterisks indicate detection on the same membrane. (B) The mRNA expression of *Id1* was evaluated by RT-qPCR in PMC-306 parental EV, S2KO EV, and S2KO V5S2 cells after 90 min with or without TGF-β treatment. The mRNA expression was normalized to *Actb*. Three independent experiments are presented as mean + SD. *p<0.05, **p<0.01, ***p<0.001 by two-way ANOVA and Tukey's multiple comparison test.