

1 **SUPPLEMENTARY INFORMATION**

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6 **Impaired bone morphogenetic protein (BMP) signaling pathways disrupt decidualization in**  
7 **endometriosis**

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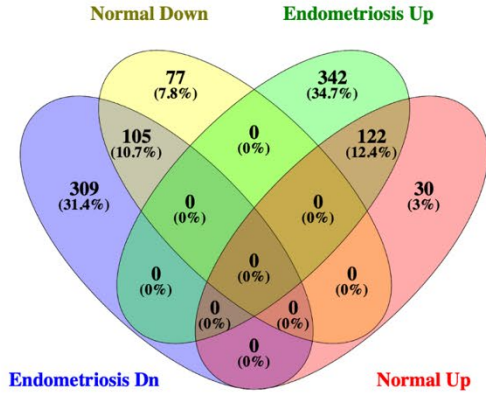
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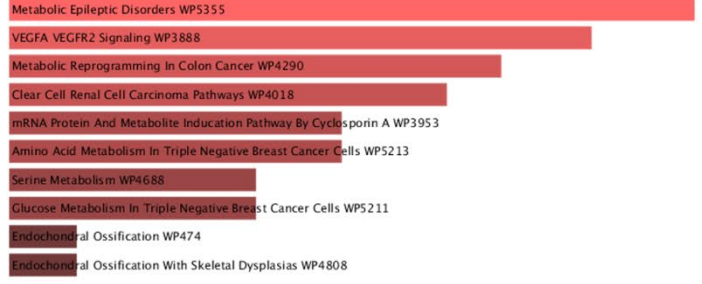
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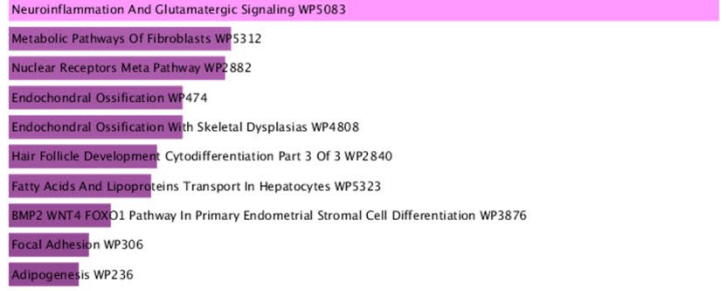
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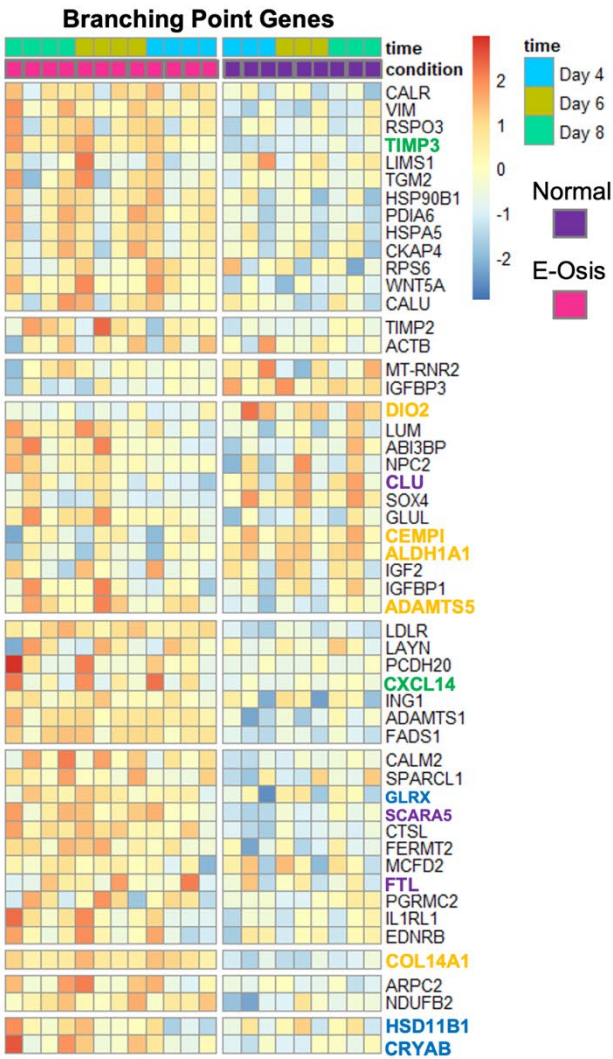
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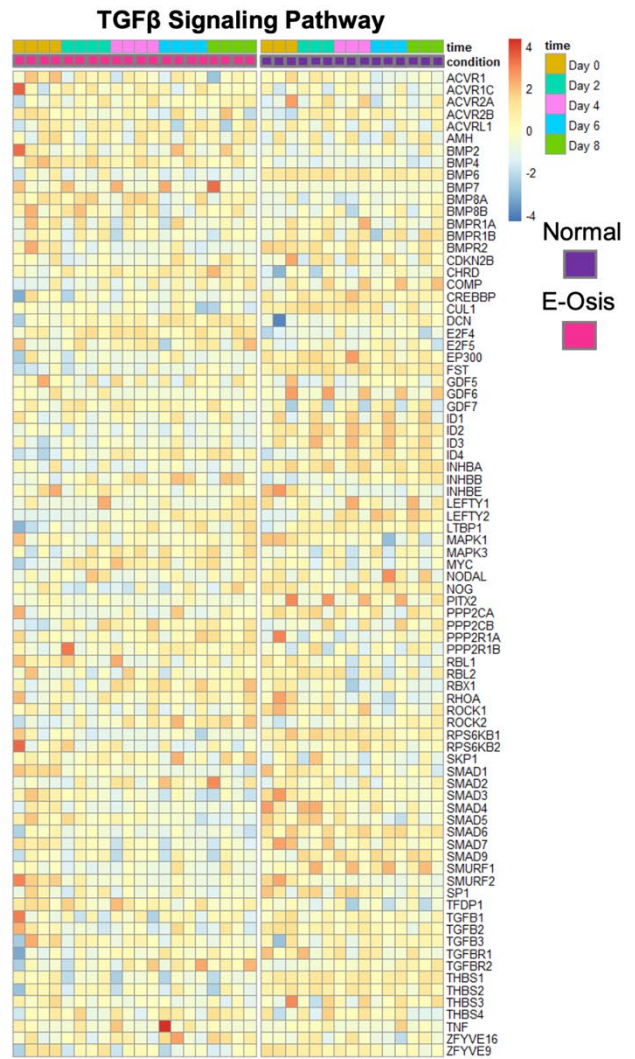
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**E**



38 **Supplemental Figure 1. Transcriptomic, gene ontology analysis and correlation with decidualization**  
39 **branchpoints of the time course decidualization datasets.** A) A Venn diagram was used to display the  
40 number of conserved genes between the normal and endometriosis groups during the time course  
41 decidualization program. Genes that showed a significant change on Day 8 of decidualization were used ( $>1.4$ ,  
42  $<0.4$ -fold change,  $FDR < 0.05$ ). B-C) Gene ontology analysis of the genes that showed a significant change  
43 ( $>1.4$ ,  $<0.4$ -fold change,  $FDR < 0.05$ ) on Day 8 of decidualization in the normal (B) or endometriosis (C)  
44 groups. D) DEGs from the Day 4, 6, 8 EPC treatment in normal and endometriosis stromal cells were  
45 correlated with the top 50 genes indicative of the senescence and non-senescent decidual branchpoints from  
46 Lucas ES et al. E) Heatmap displaying the gene expression levels of TGF $\beta$  family members in stromal cells  
47 from normal and endometriosis donors induced to decidualize over time.

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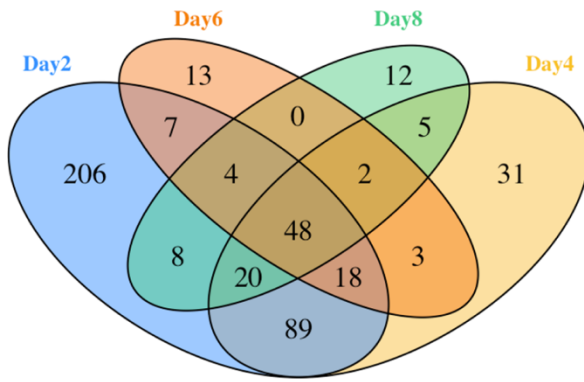
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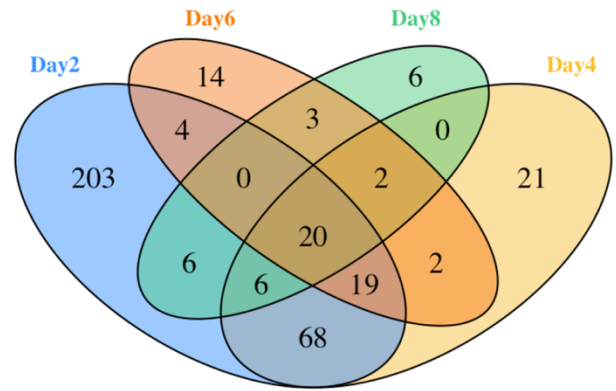
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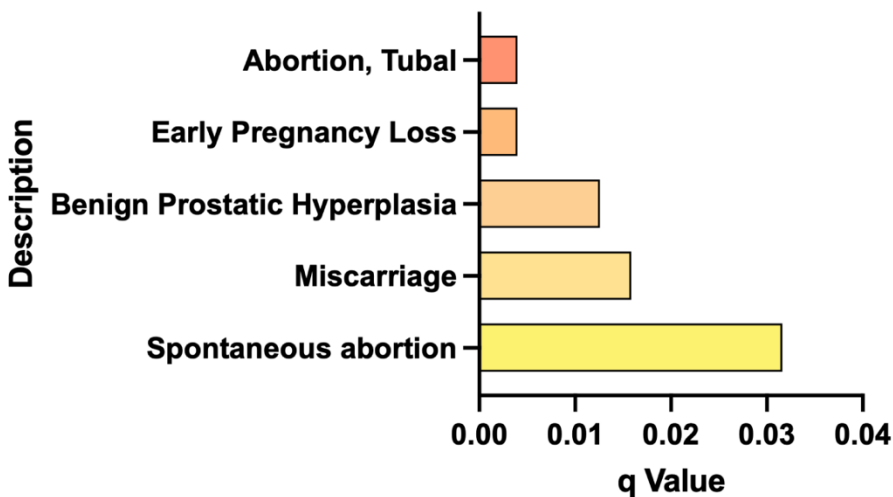
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**A**

Down-regulated genes

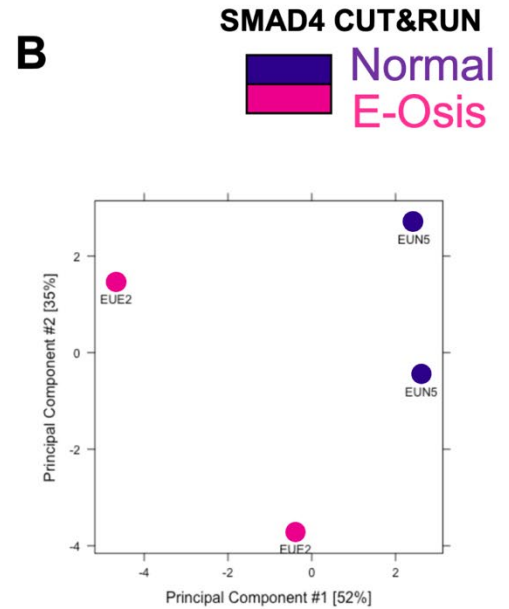
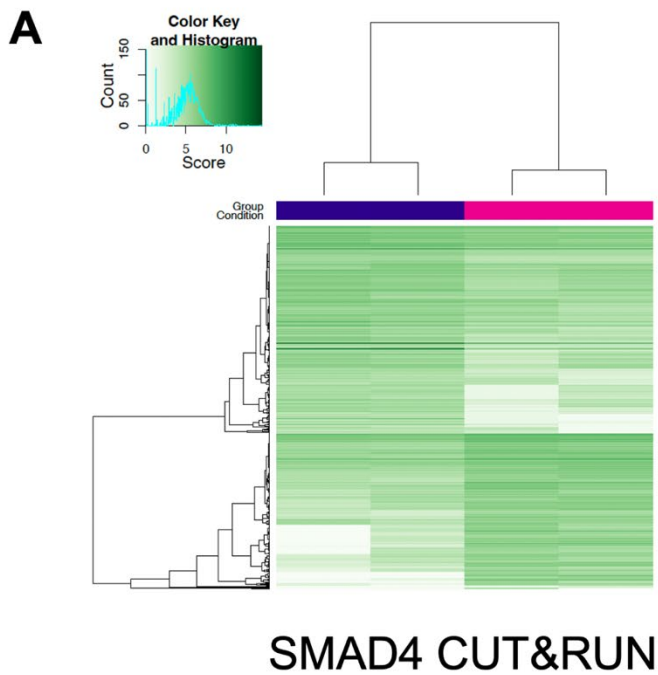
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Up-regulated genes

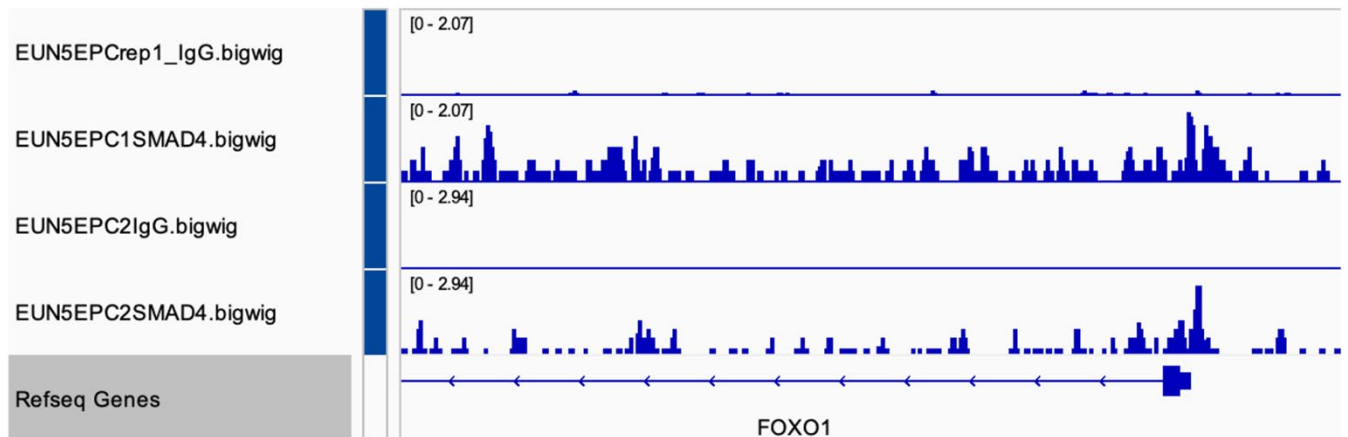
**C**

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56 **Supplemental Figure 2. Transcriptomic and classification of genes involved in the decidualization of**57 **endometrial stromal cells from individuals with and without endometriosis. A-B) A Venn diagram was**58 **used to display the number of conserved downregulated (A) and upregulated (B) genes in the time course**59 **decidualization of endometrial stromal cells from individuals with endometriosis. The analysis identified that 48**60 **genes were consistently down-regulated (A) and 20 genes were consistently up-regulated (B) regardless of the**61 **EPC treatment length. C) DisGeNET analysis for the 48 genes that were consistently down regulated in the**62 **stromal cells from individuals with endometriosis relative to individuals without endometriosis during the time**63 **course decidualization treatment.**

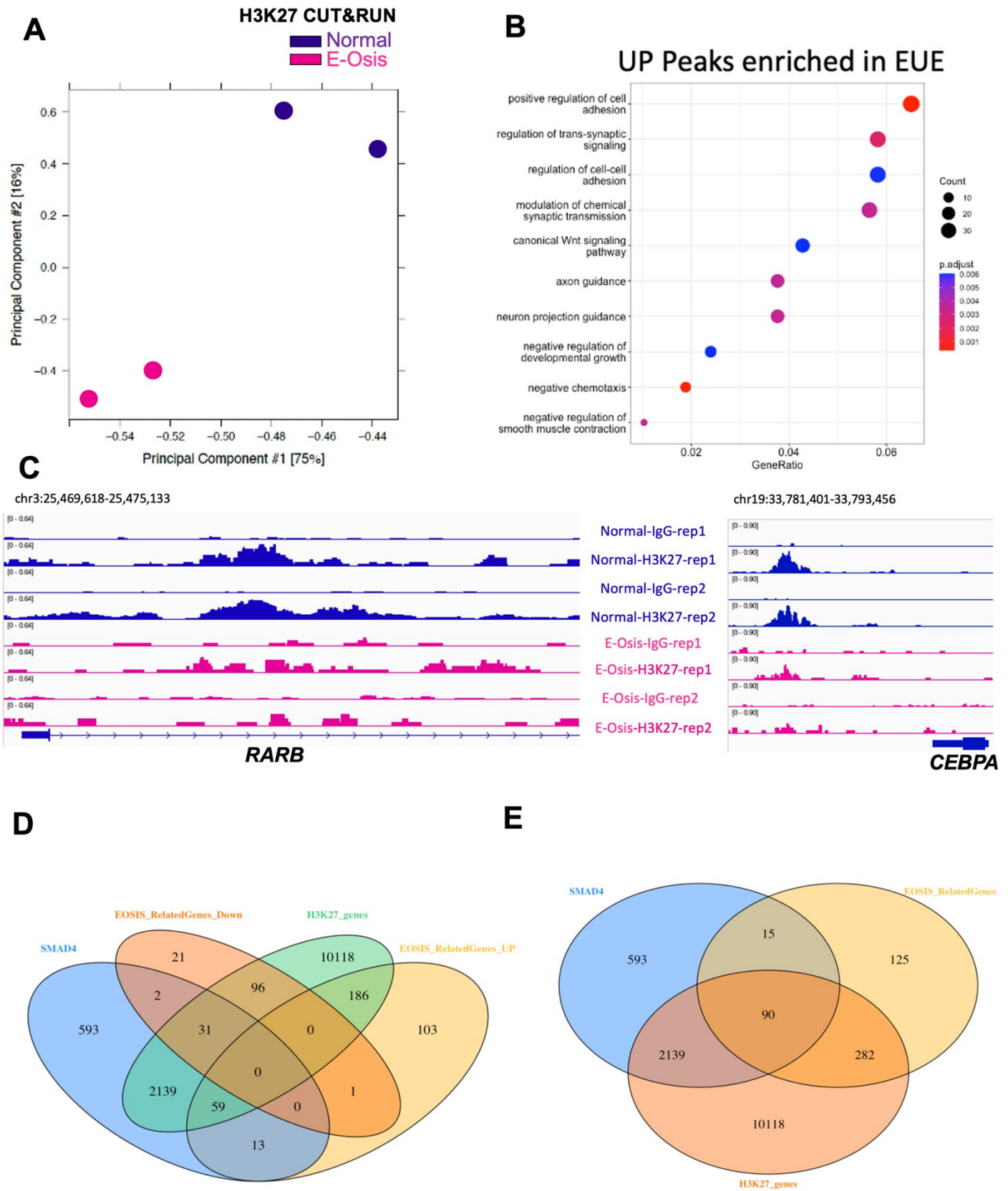


**C**



**Supplemental Figure 3. Patient-specific distribution of SMAD4 binding analysis during decidualization.**

A) SMAD4 CUT&RUN in endometrial stromal cells from individuals with and without endometriosis (E-Osis) after 4 days of EPC treatment. Differential peak signals obtained for the genome-wide SMAD4 distribution in normal versus endometriosis. B) PCA plot of the SMAD4 binding signals comparing the normal versus endometriosis sample replicates. C) Genome track view of the SMAD4 peaks located to the *FOXO1* TSS and gene body in stromal cells from normal individuals induced to decidualize for 4 days with EPC.



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**Supplemental Figure 4. Analysis of H3K27ac in the decidualizing stromal cells of individuals with and without endometriosis.** A) PCA plot for H3K27ac replicates of normal and endometriosis stromal cells, showing that the replicates are reproducible. B) Gene ontology classification of the H3K27ac peaks increased

75 in endometriosis stromal cells, showing that categories related to the regulation of cell adhesion were  
76 overrepresented. C) Genome track views for the distribution of H3K27ac peaks in the promoter regions of  
77 *RARB* and *CEBPA*, showing increased peak density in the endometrial stromal cells from individuals without  
78 endometriosis. D-E) Comparison of endometriosis-associated DEGs with SMAD4 and H3K27 bound genes.  
79 The endometriosis dataset was used from Day 4 EPC (FC>1.4, <0.4, FDR<0.05). The genes are visualized as  
80 Venn diagrams and grouped by Up- and Down-regulated (D) or as a Venn diagram without distinguishing  
81 between Up or Down-regulated genes (E).

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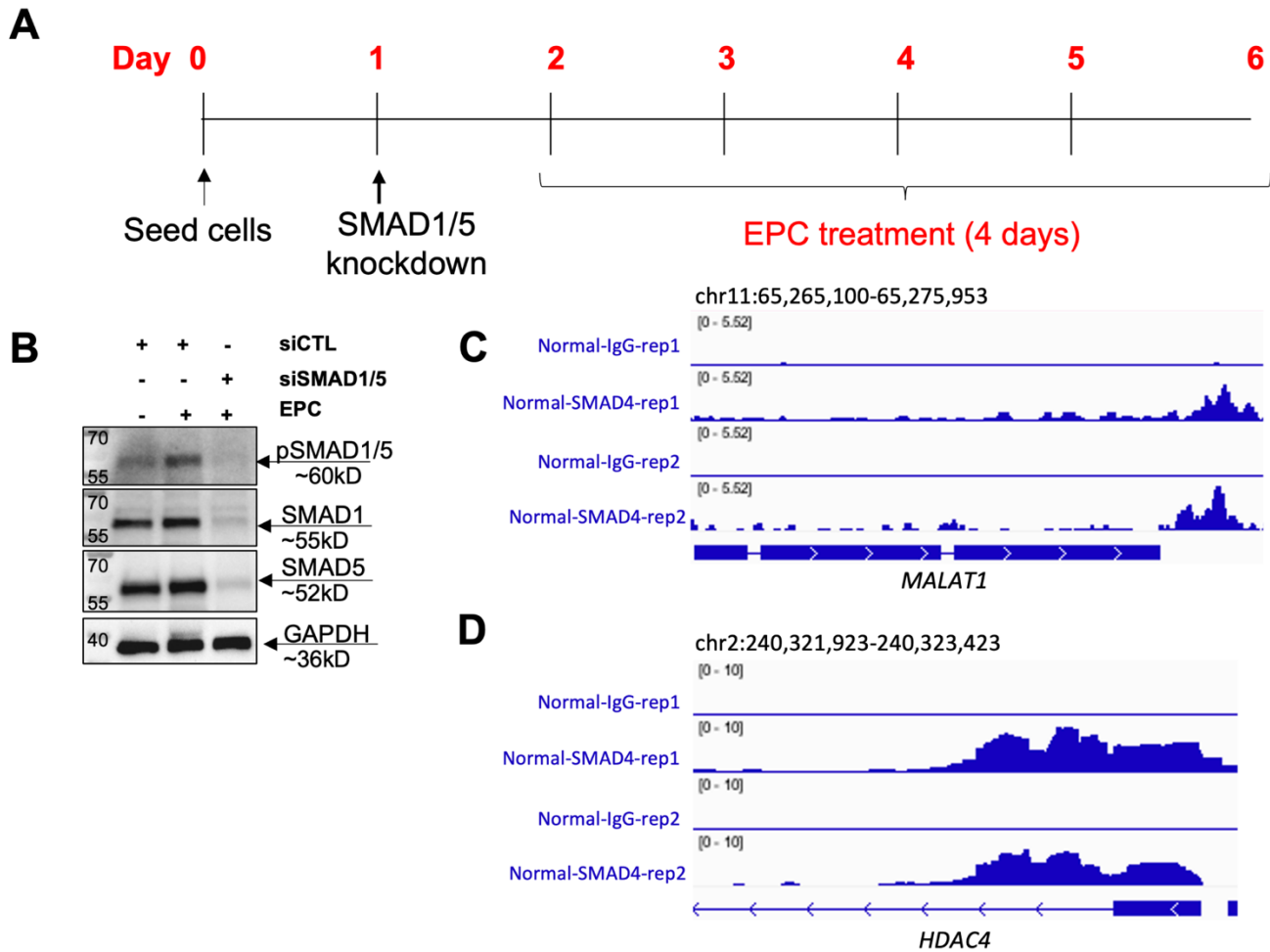
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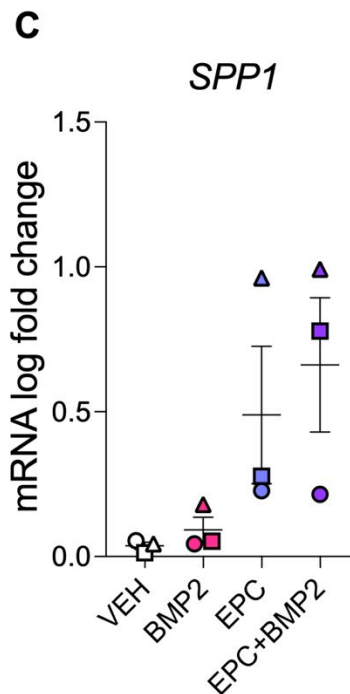
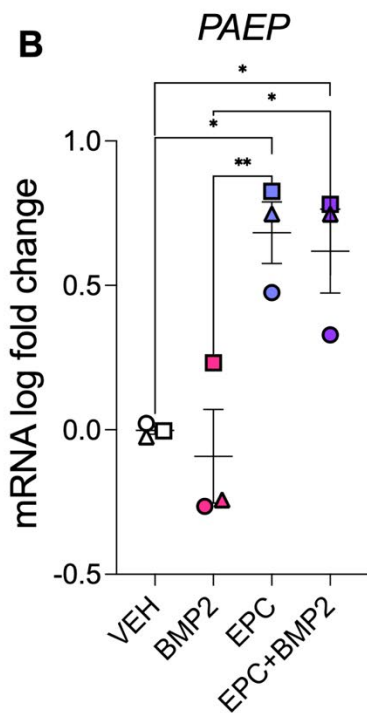
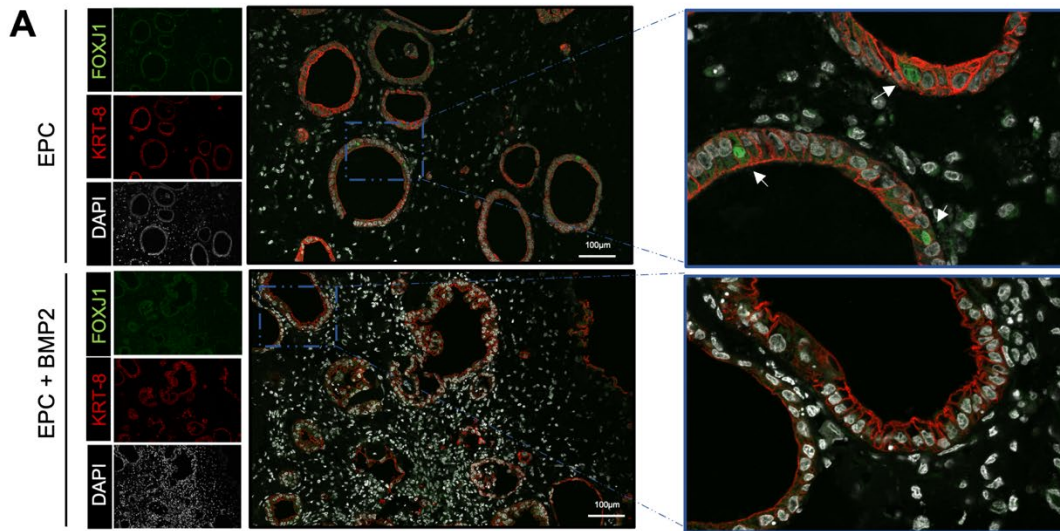
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**Supplemental Figure 5. siRNA knockdown of SMAD1 and SMAD5 perturbs decidualization.** A)

Experimental layout showing the time points at which endometrial stromal cells were plated, transfected with SMAD1/SMAD5 siRNAs, and induced to decidualize with 35nM estradiol, 1 $\mu$ M medroxyprogesterone acetate and 50 $\mu$ M cAMP (EPC) for 4 days. B) Western blot of endometrial stromal cells treated with non-targeting siRNAs (siCTL) or siRNAs targeting SMAD1 and SMAD5 and EPC. Membranes were probed with pSMAD1/5, total SMAD1, total SMAD5 and GAPDH antibodies to confirm knockdown of SMAD1/5 was successful. C-D) Genome track views of the *MALAT1* and *HDAC4* genes showing enrichment of the SMAD4 peaks.





98 **Supplemental Figure 6. Analysis of the ciliated cell marker, FOXJ1, in decidualizing 3D endometrial**

99 **assembloids from donors with endometriosis.** A) Immunostaining of the ciliated cell marker, FOXJ1

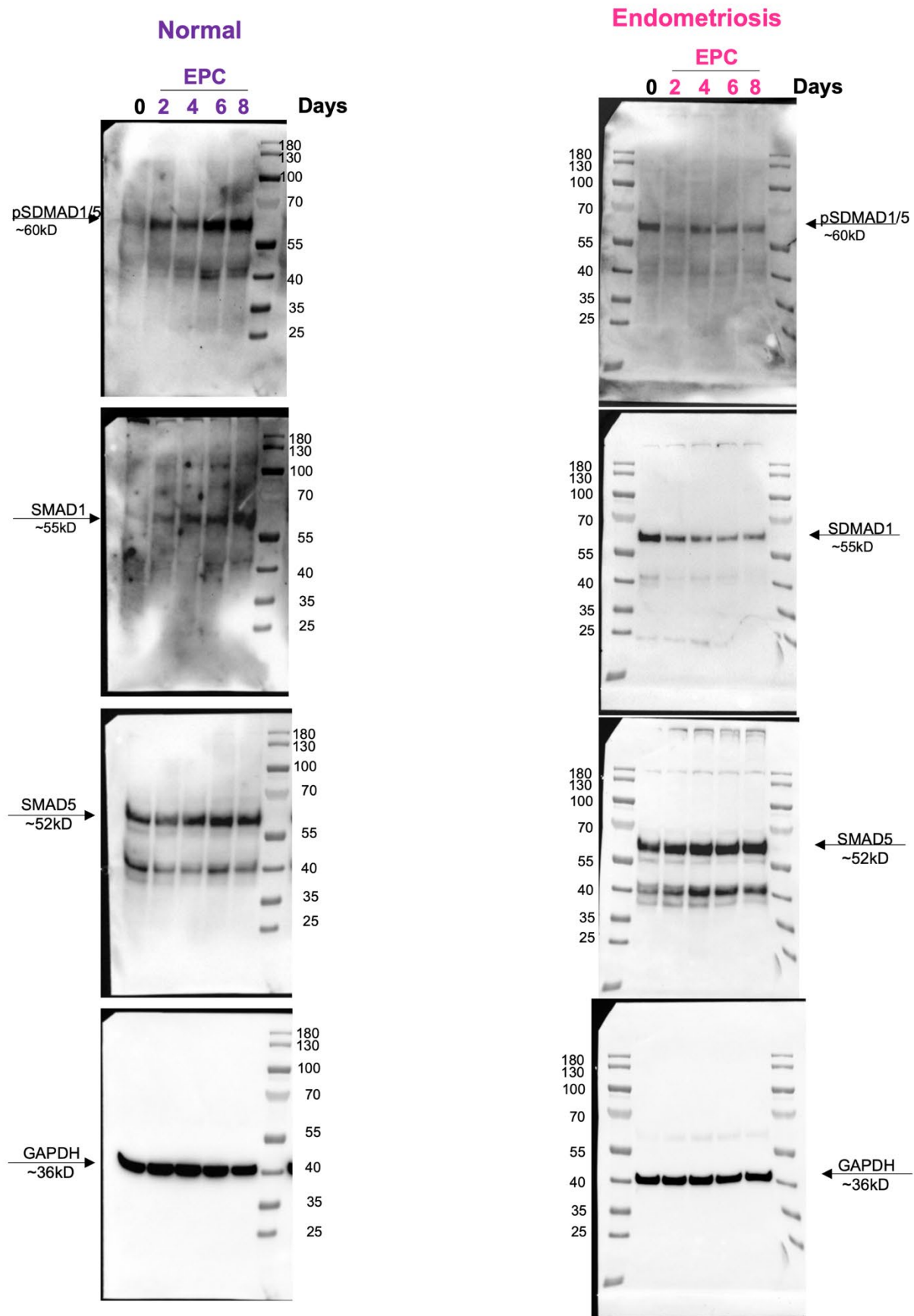
100 (green), the epithelial cell marker (cytokeratin 8, KRT-8, red) and DAPI (white) in endometrial assembloids

101 treated with EPC or EPC + BMP2, the hormonal stimuli. B-C) qRT-PCR analysis of glandular decidualization

102 markers, *PAEP* (B) or *SPP1* (D) in the endometrial assembloids treated with Vehicle, BMP2, EPC, or EPC +

103 BMP2. Plotted values represent mean +/- standard error of the mean, with the different symbols corresponding

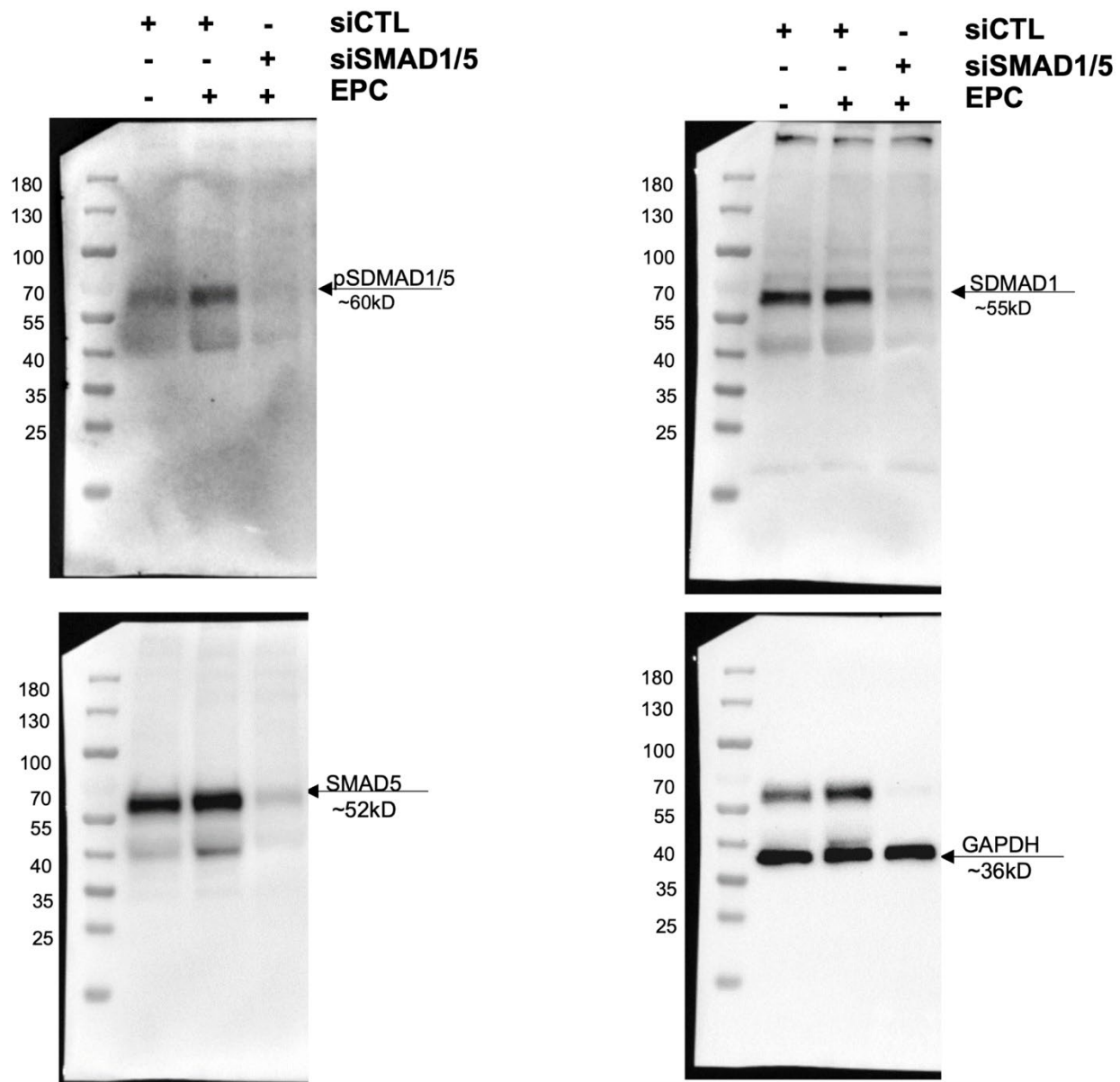
104 to each patient's trajectory. Data were analyzed using a one-way ANOVA with a Tukey's posthoc test.



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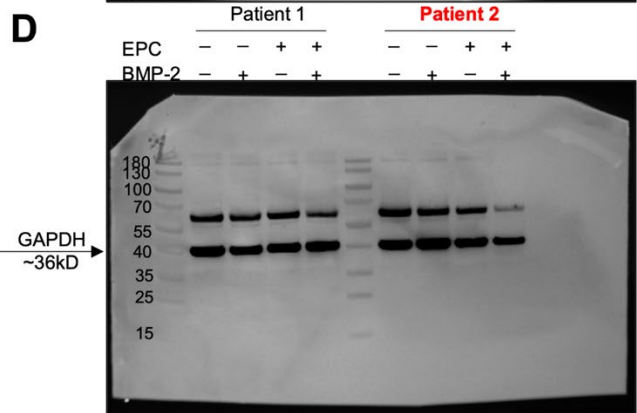
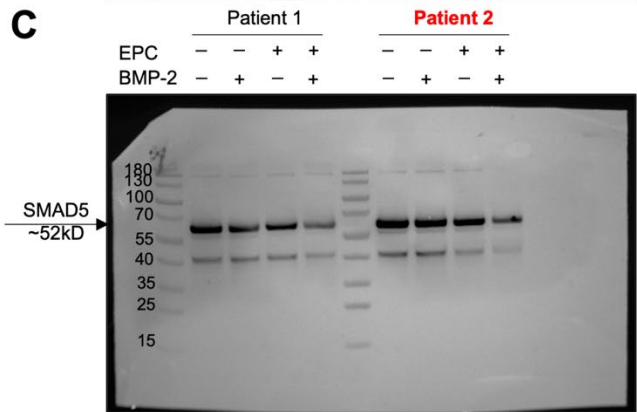
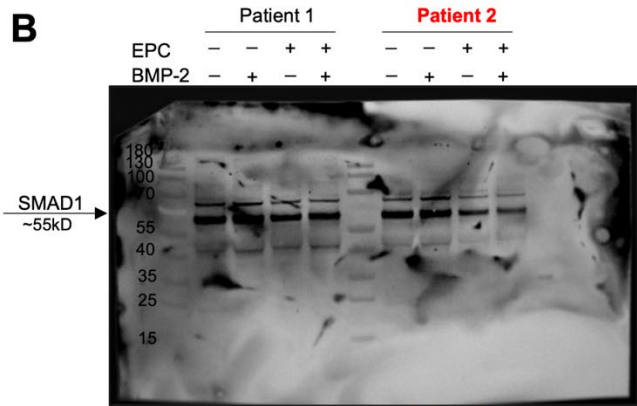
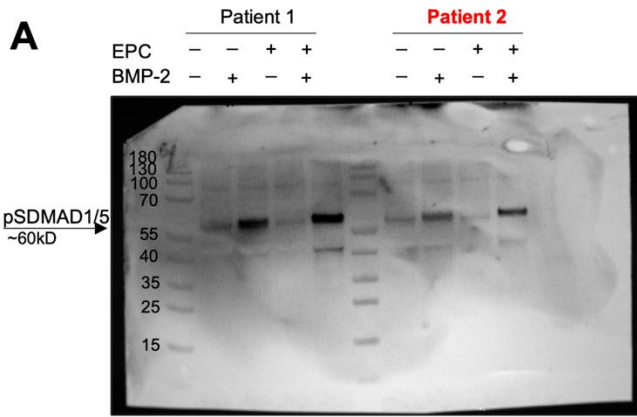
Supplemental Figure 7. Uncropped western blot images corresponding to Figure 3.



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**Supplemental Figure 8.** Uncropped western blot images corresponding to Supplemental Figure 5.



Patient 2 is displayed in Figure 6F

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Supplemental Figure 9. Uncropped western blot images corresponding to Figure 6.

## 114 SUPPLEMENTAL TABLES and DATA FILES

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## 116 Supplemental Table 1. qRT-PCR Primer Sequences

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<b>Gene</b>	<b>Forward (5'-3')</b>	<b>Reverse (5'-3')</b>
<i>BMP2</i>	ACCCGCTGTCTCTAGCGT	TTTCAGGCCGAACATGCTGAG
<i>IGFBP1</i>	TTGGGACGCCATCAGTACCTA	TTGGCTAAACTCTCTACGACTCT
<i>PRL</i>	AAGCTGTAGAGATTGAGGAGCAAAC	TCAGGATGAACCTGGCTGACTA
<i>FOXO1</i>	TGATAACTGGAGTACATTTTCGCC	CGGTCATAATGGGTGAGAGTCT
<i>WNT4</i>	CTCCACACTCGACTCCTTGC	CCGAAGAGATGGCGTACACG
<i>SPP1</i>	TGCAGCCTTCTCAGCCAAA	GGAGGCAAAGCAAATCACTG
<i>GAPDH</i>	ACAACCTTTGGTATCGTGGAAGG	GCCATCACGCCACAGTTTC

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147 **Supplemental Table 2.** Antibody list

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<b>Antibody</b>	<b>Vendor</b>	<b>Catalog</b>	<b>Application</b>
<b>pSMAD1/5</b>	<b>Cell Signaling</b>	<b>9516</b>	<b>WB 1:1000</b>
<b>SMAD1</b>	<b>Invitrogen</b>	<b>38-5400</b>	<b>WB 1:1000</b>
<b>SMAD5</b>	<b>Proteintech</b>	<b>12167-1-AP</b>	<b>WB 1:1000</b>
<b>GAPDH</b>	<b>Proteintech</b>	<b>HRP-60004</b>	<b>WB 1:5000</b>
<b>Vimentin</b>	<b>Cell Signaling</b>	<b>5741</b>	<b>IF 1:200</b>
<b>SMAD4</b>	<b>Abcam</b>	<b>ab40759</b>	<b>CUT&amp;RUN 1:50</b>
<b>K3K27ac</b>	<b>Cell Signaling</b>	<b>8173</b>	<b>CUT &amp; RUN 1:50</b>
<b>KRT-8</b>	<b>DSHB</b>	<b>TROMA-I</b>	<b>IF 1:50</b>
<b>FOXJ1</b>	<b>Sigma</b>	<b>HPA005714</b>	<b>IF 1:100</b>
<b>Antibody-Rabbit IgG (H+L) Highly Cross- Adsorbed_Alexa Fluor 488</b>	<b>ThermoFisher</b>	<b>A-21206</b>	<b>IF 1:250</b>
<b>Antibody-Rat IgG (H+L) Highly Cross- Adsorbed_Alexa Fluor 594</b>	<b>ThermoFisher</b>	<b>A-21209</b>	<b>IF 1:250</b>

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