

1 **Supplementary File**

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3 **Supplementary Figure S1**

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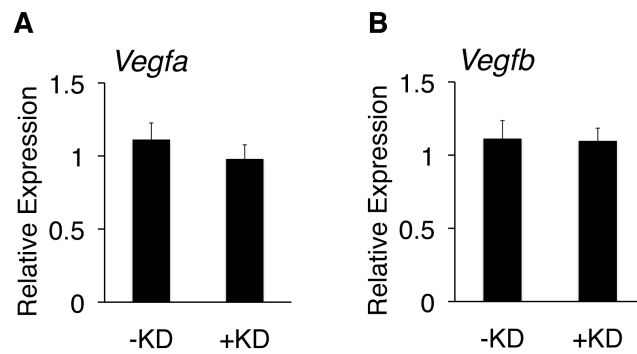
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12 **Supplementary Figure S1.** No significant changes in the levels of *Vegfa* (A) and *Vegfb* (B)  
13 were observed in the wild type mouse granulosa cells with *Smad1/5* knockdown.

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14 **Supplementary Figure S2**

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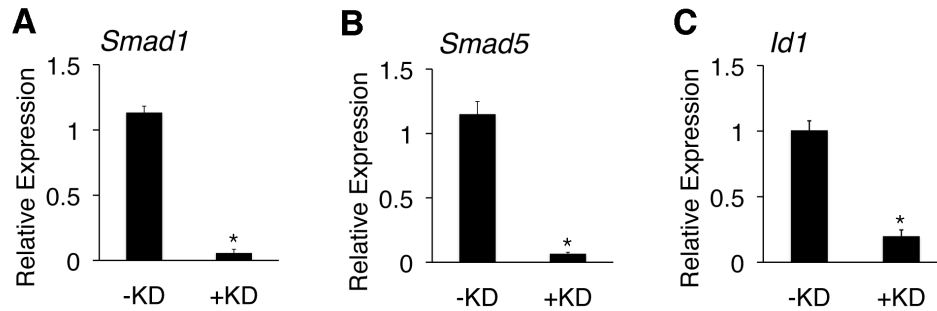
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23 **Supplementary Figure S2.** Loss of *Smad1* and *Smad5* results in the downregulation of *Id1* gene

24 expression. siRNA-mediated knockdown (+KD) of *Smad1* and *Smad5* in mouse granulosa cells

25 resulted in a significant reduction in *Smad1* (A) and *Smad5* (B) transcript levels compared to the

26 control (scrambled siRNA) transfected cells (-KD). A significant decrease in *Id1* (C) expression

27 is observed following *Smad1/5* knockdown. Asterisks indicate statistical significance (n=4;  $P <$

28 0.05).

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29 **Supplementary Figure S3**

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44 **Supplementary Figure S3.** Loss of SMAD1/5 results in the upregulation of PDGFA. Western

45 blot analysis of whole cell lysates harvested from COV434 cells transfected with siRNA to

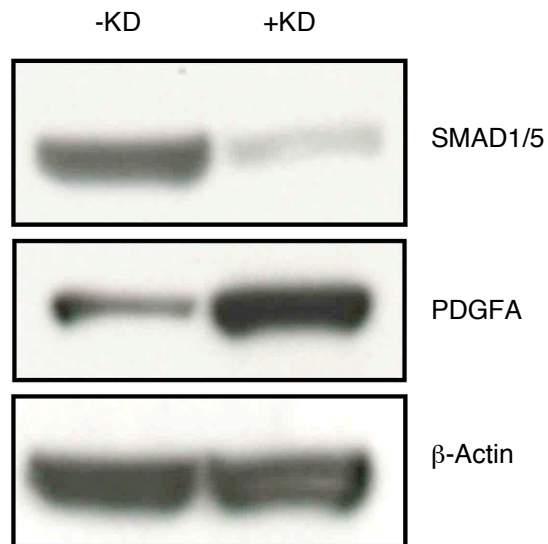
46 SMAD1 and SMAD5. siRNA-mediated knockdown (+KD) of *Smad1* and *Smad5* in mouse

47 granulosa cells resulted in a significant reduction in SMAD1/5 and upregulation of PDGFA

48 protein levels compared to the control (scrambled siRNA) transfected cells (-KD). The

49 membrane was stripped and reprobbed with anti-actin antibody to ensure equal protein loading.

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50 **Supplementary Figure S4**

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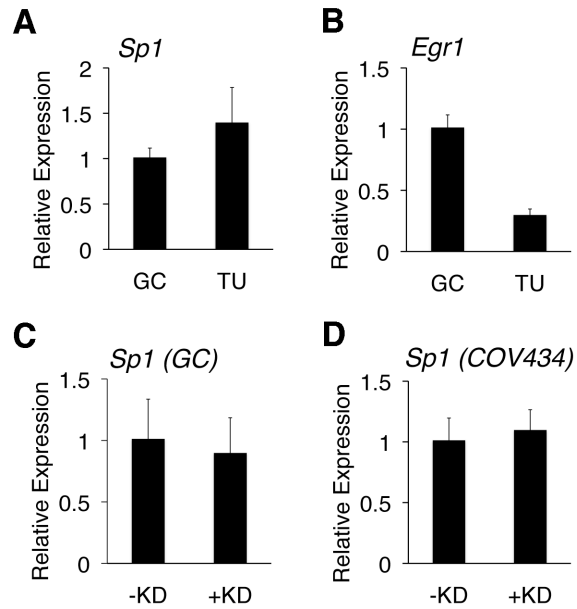
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62 **Supplementary Figure S4.** Expression analysis of *Sp1* and *Egr1*. Quantitative PCR was used  
63 to determine expression levels of *Sp1* (A) and *Egr1* (B) in wild type mouse granulosa cells (GC)  
64 and *Smad1/5* dKO tumors (TU). No significant change is observed in the levels of *Sp1* wild  
65 type mouse granulosa cells (C) or COV434 cells (D) following *Smad1/5* knockdown (+KD)  
66 compared to control siRNA transfected cells (-KD)

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67 **Supplementary Figure S5**

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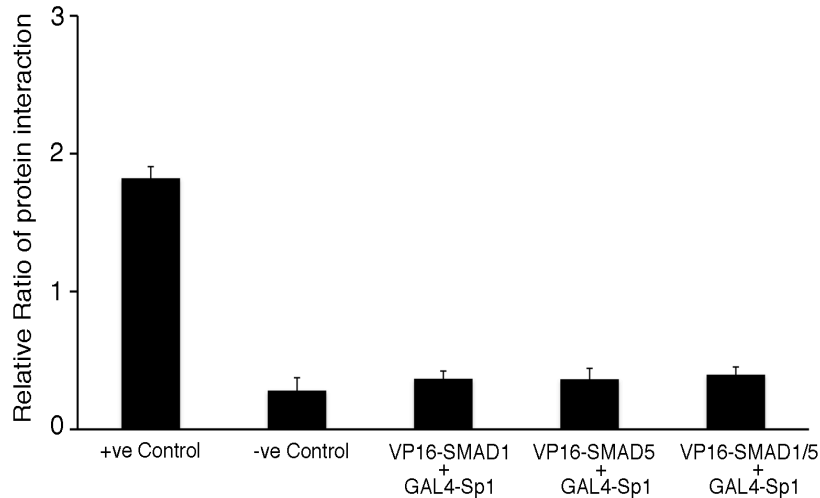
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77 **Supplementary Figure S5.** Mammalian two-hybrid assay indicates that BR-SMADs do not

78 interact with Sp1. COV434 cells were transiently transfected with the respective plasmids

79 indicated in the figure. pACT-MyoD and pBIND-Id provided in the kit served as positive

80 control (+ve con) where as empty pACT and pBIND, which lack fusion proteins, were used as

81 negative controls (-ve con) for protein interaction. No interaction was observed in the cells co-

82 transfected pACT vector expressing SMAD1 and SMAD5 fused to a transcriptional activation

83 domain (VP16-SMAD1 and VP16-SMAD5) and the pBIND vector expressing Sp1 fused to a

84 DNA-binding domain (GAL4-Sp1). YFP was used for the normalization of the transfections.

85 The relative interaction of proteins is determined by the mCherry/YFP ratio. Experiments were

86 repeated three times with same results.

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88 **Supplementary Table 1**

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Gene Name	Species	Primer Name	Primer Sequence (5' to 3')	Application
<i>Smad1</i>	Mouse	m <i>Smad1</i> -Sybr For	GCTTCGTGAAGGGTTGGGG	Real-time PCR
		m <i>Smad1</i> -Sybr Rev	CGGATGAAATAGGATTGTGGGG	
<i>Smad5</i>	Mouse	m <i>Smad5</i> -Sybr For	TTGTCAGAGTAGGAACTGCAAC	Real-time PCR
		m <i>Smad5</i> -Sybr Rev	GAAGCTGAGCAAACCTCTGAT	
<i>Pdgfa</i>	Mouse	m <i>Pdgfa</i> -Sybr For	GAGGAAGCCGAGATACCCC	Real-time PCR
		m <i>Pdgfa</i> -Sybr Rev	TGCTGTGGATCTGACTTCGAG	
<i>Pdgfb</i>	Mouse	m <i>Pdgfb</i> -Sybr For	CATCCGCTCCTTTGATGATCTT	Real-time PCR
		m <i>Pdgfb</i> -Sybr Rev	GTGCTCGGGTCATGTTCAAGT	
<i>Vegfa</i>	Mouse	m <i>Vegfa</i> -Sybr For	GCACATAGAGAGAATGAGCTTCC	Real-time PCR
		m <i>Vegfa</i> -Sybr Rev	CTCCGCTCTGAACAAGGCT	
<i>Vegfb</i>	Mouse	m <i>Vegfb</i> -Sybr For	GCCAGACAGGGTTGCCATAC	Real-time PCR
		m <i>Vegfb</i> -Sybr Rev	GGAGTGGGATGGATGATGTCAG	
<i>Sp1</i>	Mouse	m <i>Sp1</i> -Sybr For	CCTATTGCAAAGACAGTGAG	Real-time PCR
		m <i>Sp1</i> -Sybr Rev	CTTGATGTGACAAATGTGCTG	
<i>Erg1</i>	Mouse	m <i>Erg1</i> -Sybr For	TCCTCAAGGGGAGCCGAGCG	Real-time PCR
		m <i>Erg1</i> -Sybr Rev	TGTTGCCACTGTTGGGTGCGG	
<i>Gapdh</i>	Mouse	m <i>Gapdh</i> -Sybr For	AGGTCGGTGTGAACGGATTTG	Real-time PCR
		m <i>Gapdh</i> -Sybr Rev	TGTAGACCATGTAGTTGAGGTCA	
<i>SMAD1</i>	Human	h <i>SMAD1</i> -Sybr For	TTGGTTCCAAGCAGAAGGAGGTCT	Real-time PCR
		h <i>SMAD1</i> -Sybr Rev	AACTGAGCTAAGAGGCTGTGCTGA	
<i>SMAD5</i>	Human	h <i>SMAD5</i> -Sybr For	TTGGTGGAGAGGTGTATGCGGAAT	Real-time PCR
		h <i>SMAD5</i> -Sybr Rev	ACAGATTGAGCCAGAAGCTGAGCA	
<i>PDGFA</i>	Human	h <i>PDGFA</i> -Sybr For	TCGATGAGATGGAGGGTCCG	Real-time PCR
		h <i>PDGFA</i> -Sybr Rev	ACCCGGACAGAAATCCAGTCT	
<i>PDGFB</i>	Human	h <i>PDGFB</i> -Sybr For	TGGGCCTCGGGGACCCCAT	Real-time PCR

<i>GAPDH</i>	Human	h <i>PDGFB</i> -Sybr Rev h <i>GAPDH</i> -Sybr For h <i>GAPDH</i> -Sybr Rev	CGTGCAGCAGGCGTTGGAGA GGACCTGACCTGCCGTCTAGAA GGTGTCGCTGTTGAAGTCAGAG	Real-time PCR
<i>SPI</i>	Human	h <i>SPI</i> -Sybr For h <i>SPI</i> -Sybr Rev	TTGAAAAAGGAGTTGGTGGC TGCTGGTTCTGTAAGTTGGG	Real-time PCR
<i>SMAD1</i>	Human	h <i>SMAD1</i> - For h <i>SMAD1</i> - Rev	GCTCAGTTATTGGCACAGTCT GGTCAAGTATTATCAAGGCTCC	PCR
<i>SMAD5</i>	Human	h <i>SMAD5</i> - For h <i>SMAD5</i> - Rev	CCAGTTCAGAAATTTGGCATTG GCACTGTCAGTACTATAAAG	PCR
<i>PDGFA</i>	Human	h <i>PDGFA</i> - For h <i>PDGFA</i> - Rev	CCCCTGCCCATTCGGAGGAAGAG AAGTTGGCGGACGTGGGGTCGA	PCR
<i>PDGFB</i>	Human	h <i>PDGFB</i> - For h <i>PDGFB</i> - Rev	GATCCGCTCCTTTGATGATC GTCTCACACTTGCATGCCAG	PCR
<i>PDGFRA</i>	Human	h <i>PDGFRA</i> - For h <i>PDGFRA</i> - Rev	ATCAATCAGCCCAGATGGAC TTCACGGGCAGAAAGGTACT	PCR
<i>PDGFRB</i>	Human	h <i>PDGFRB</i> - For h <i>PDGFRB</i> - Rev	AATGTCTCCAGCACCTTCGT AGCGGATGTGGTAAGGCATA	PCR
<i>BMPRI1</i>	Human	h <i>BMPRI1</i> - For h <i>BMPRI1</i> - Rev	AGCACCAGAGGATACCTTGC AATGAGCAAAACCAGCCATA	PCR
<i>BMPRI2</i>	Human	h <i>BMPRI2</i> - For h <i>BMPRI2</i> - Rev	GTTGTAAATGCCACCACCATT GTCTGGTTTCTTGTCTTTTAT	PCR
<i>ACVR1</i>	Human	h <i>ACVR1</i> - For h <i>ACVR1</i> - Rev	TTCCACCTCCTGACACAACA GGCAATGTTGTCATGTTCCA	PCR
<i>ACVR2A</i>	Human	h <i>ACVR2A</i> - For h <i>ACVR2A</i> - Rev	GCATTCCCAGAGCACCAATC CTGTGAGTCTTGCGGATGGA	PCR
<i>BMP2</i>	Human	h <i>BMP2</i> - For h <i>BMP2</i> - Rev	TCCTGCTCTATTCCTTGGTG CTTCATTCCAGGCAAAGTGT AACGGACATTCGGTCCTTGC CGCAACTCGAACTCGCTCAG	PCR

<i>BMP4</i>	Human	<i>hBMP4</i> - For	TGAGCCTTTCCAGCAAGTTT	PCR
		<i>hBMP4</i> - Rev	CCAGACTGAAGCCGGTAAAG	
<i>BMP7</i>	Human	<i>hBMP7</i> - For	TTCCCCTCCCTATCCCCAACTTT	PCR
		<i>hBMP7</i> - Rev	TTTTCTTTTCGCACAGACACC	
<i>PBGD</i>	Human	<i>hPBGD</i> - For	ACACAGCCTACTTTCCAAGCGGAGCCAT	PCR
		<i>hPBGD</i> - Rev	TCTTGTCCCCTGTGGTGGACATAGCAAT	
<i>PDGFA</i>	Human	<i>hPDGFA</i> pro-For	AGGATTGCAGCTGGCACTGGAGGG	ChIP
		<i>hPDGFA</i> pro-Rev	CCCGCCTCCCCCGGATTCCGG	
Neg Con		Neg Con -For	GGGAACCAGGGAAAGAGGA	ChIP
		Neg Con -For	GGGAAATAGGCACCCGATAA	
SP1	Human	<i>hSP1</i> pro- For	TGCGGGTCCCAGGCCCGGAAT	ChIP
		<i>hSP1</i> pro- Rev	GGCGGCGGGGAGAGGGTTATA	
<i>SMAD1</i>	Human	<i>hSMAD1</i> -MTH- For	TATTCTAGAATGAATGTGACAAGTTTATTTTCC	Cloning into pACT vector
		<i>hSMAD1</i> -MTH- Rev	ATAGGTACCAGATACAGATGAAATAGGATTA	
<i>SMAD5</i>	Human	<i>hSMAD5</i> -MTH- For	TATTCTAGAATGACGTCAATGGCCAGCTTG	Cloning into pACT vector
		<i>hSMAD5</i> -MTH- Rev	ATAGGTACCTTATGAAACAGAAGATATGGGGTT	
<i>SP1</i>	Human	<i>hSP1</i> -MTH- For	TATTCTAGA ATGAGCGACCAAGATCACTCCA	Cloning into pBIND vector
		<i>hSP1</i> -MTH- Rev	ATAGGTACCTCAGAAGCCATTGCCACTGATA	
<i>PDGFA</i>	Human	<i>hPDGFA</i> -mutA-For	GGAATCCGGGGCTGGCGGGGGGGG	Site-directed mutagenesis
		<i>hPDGFA</i> -mutA-Rev	CCCCCCCCGCCAGCCCCGGATTCC	

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