## Supplementary Figures

## Supplementary Fig. 1

a

b


a 3D matrigel invasion assay of spheroids in the presence of control, BMP2 or BMP9 (10nM) (left). quantitation of spheroid invasion for the indicated time in PA1 cells (right). b Growth curve of PA1 cells grown under attached 2D conditions in the presence of control, BMP2 or BMP9. c Body weight in grams of NOD-SCID mice receiving either vehicle or BMP9 at indicated doses for a 21 day period ( $\mathrm{n}=2$ per group). d Absorbance units of Alanine Transaminase (ALT) as a measure of liver function measured from plasma ( $\mathrm{n}=2$ per group). e Representative Necrotic region in tumors from rhBMP9 vs vehicle receiving mice injected with SKOV3-luc-GFP (Scale bar= $50 \mu \mathrm{M}$ ). f Elisa of BMP9 in plasma from mice in vehicle and BMP9 treated groups in PA1-luc-GFP mice. Data are presented as mean $\pm$ SEM, ${ }^{*} p<$ $0.05,{ }^{* *} p<0.01,{ }^{* * *} p<0.001$ (a) ANOVA followed by Dunnett's multiple comparisons test. (b-f) unpaired Student's ttest

## Supplementary Fig. 2


a Heatmap of transcription profile of 48,226 genes in PA1 cells treated with BMP9 for 24 hr under anchorage independence. b REACTOME pathway analysis of genes from data in (a). c Relative qRT-PCR analysis of Sox2 after BMP treatment for 24 hr under anchorage independence (3D) in PA1 cells. d qRT-PCR analysis of Oct4 and Nanog after BMP9 treatment under anchorage independence (3D) condition in PA1 cells. e Representative images of PA1 cells cultured under anchorage independence for 48 hrs , and subsequently treated with either vehicle control or with 10nM BMP4 for 24 hrs. Live/dead cell ratios were assessed by staining with Calcein-AM (green=live cells) and Ethidium homodimer dye (red= dead cells) and images taken by confocal microscopy. Scale bar $=50 \mu \mathrm{~m}$. ( $\mathrm{n}=5$ ). f Representative images of PA1 cells cultured under anchorage independence for 48 hrs , and subsequently treated with either vehicle control or with 10nM BMP10 for 24 hrs. ( $n=8$ ). g qRT-PCR analysis of Sox2 in tumors from vehicle and BMP9 treated groups in SKOV3-luc-GFP mice ( $n=2$ per condition). Data are presented as mean $\pm$ SEM, ${ }^{*} p<0.05$, ** $p<$ $0.01,{ }^{* * *} p<0.001$; (c-d) ANOVA followed by Dunnett's multiple comparison test; and (e-g) unpaired Student's t test.

## Supplementary Fig. 3


a Western blot (left) and relative qRT-PCR (right) of Sox2 expression under attached (2D) versus under anchorage independence (3D) conditions in OVCAR3 cells after 72 hr under 3D condition. b Western blot of Sox2 expression under 2D versus 3D condition in PA1 cells after 72 hr under 3D condition. Data are presented as mean $\pm$ SEM, * $p<$ $0.05,{ }^{* *} p<0.01,{ }^{* * *} p<0.001$

## Supplementary Fig. 4


a Relative qRT-PCR analysis of Sox2 after TGF $\beta 1$ treatment for 24 hrs under anchorage independence (3D) condition in OVCAR3 cells. b Live-dead analysis of cells under anchorage independence after TGF $\beta 1$ and activin treatment for 24 hr in indicated cells ( $\mathrm{n}=3$ to 5 ). c Western blot of Sox2 after combined treatment of equimolar (10nM) activin and BMP2/9 for 24 hr in PA1 cells ( $\mathrm{n}=2$ ). Data are presented as mean $\pm$ SEM, * $p<$ $0.05,{ }^{* *} p<0.01,{ }^{* * *} p<0.001$. (ANOVA followed by Sidak's multiple comparisons test).

## Supplementary Fig. 5


a qRT-PCR analysis of Sox2 expression in OVCAR3 cells pretreated with $5 \mu \mathrm{M}$ Dorsomorphin (DM) and $5 \mu \mathrm{M}$ SB431542 for 1hr, followed by treatment with BMP9 for 24 hrs . Data are normalized to DMSO vehicle controls. b qRT-PCR analysis of Sox2 expression in OVCAR3 cells pretreated with $5 \mu \mathrm{M}$ Dorsomorphin (DM) and $5 \mu \mathrm{M}$ SB431542 for 1hr, followed by treatment with BMP2 for 24 hrs . Data are normalized to DMSO vehicle controls. c Western blot of Sox2 expression in OVCAR3 cells pretreated with $3 \mu \mathrm{M}$ ALK1,2 inhibitor ML347 and $0.8 \mu \mathrm{M}$ ALK2,3 LDN193189 for 1hr, followed by treatment with BMP2/9 for 24 hrs . Data are normalized to vehicle controls presented.

## Supplementary Fig. 6


a Representative relative qRT-PCR of indicated regions (primer sites) after chromatin immunoprecipitation of SMAD3 to sites on Sox2 proximal chromosomal regions with or without 1 hr of activin A treatment, expressed as the ratio over IgG controls normalized to untreated cells. b Western blot (left) and qRT-PCR (right) of Sox2 upon either 10ng/ $\mu \mathrm{L}$ Cycloheximide (CHX) or BMP2 or BMP9 treatment as indicated normalized to DMSO control in PA1 cells. (ANOVA followed by Dunnett's multiple comparison test ( $n=3$ ). c Relative qRT-PCR analysis of Sox2 in the presence or absence of $0.5 \mathrm{ng} / \mathrm{mL}$ Actinomycin D (Act D) with or without BMP2 or BMP9 as indicated normalized to DMSO control for 12 hrs in PA1 cells. (ANOVA followed by Sidak's multiple comparison test). d Western blot of Sox2 expression in PA1 cells pretreated for 1 hr with $0.5 \mu \mathrm{M} \mathrm{MG132} ,\mathrm{followed} \mathrm{by} \mathrm{BMP2/9}$. mean $\pm$ SEM, ${ }^{*} p<0.05,{ }^{* *} p<0.01$, ${ }^{* * *} p<0.001$.

## Supplementary Fig. 7



## C



a Heatmap of 21 common differentially expressed genes between the microarray from GEO \#GSE185924, Fig. 2a and RNA sequencing analysis GEO GSE185932, Fig. 8c. b LASAGNA analysis of Sox2 binding motifs for the 21 DEGs from (a). c-d qRT-PCR analysis of indicated candidate genes in siNTC and siSox2 normalized to siNTC in either PA1 cells or OVCAR3 as indicated Data are presented as mean $\pm$ SEM, ${ }^{*} p<0.05$, ${ }^{* *} p<0.01$, ${ }^{* * *} p<0.001$.

