Stem Cell Reports, Volume *3*

Supplemental Information

Local BMP-SMAD1 Signaling Increases LIF Receptor-Dependent STAT3 Responsiveness and Primed-to-Naive Mouse Pluripotent Stem Cell Conversion Frequency

Kento Onishi, Peter D. Tonge, Andras Nagy, and Peter W. Zandstra

Figure S1

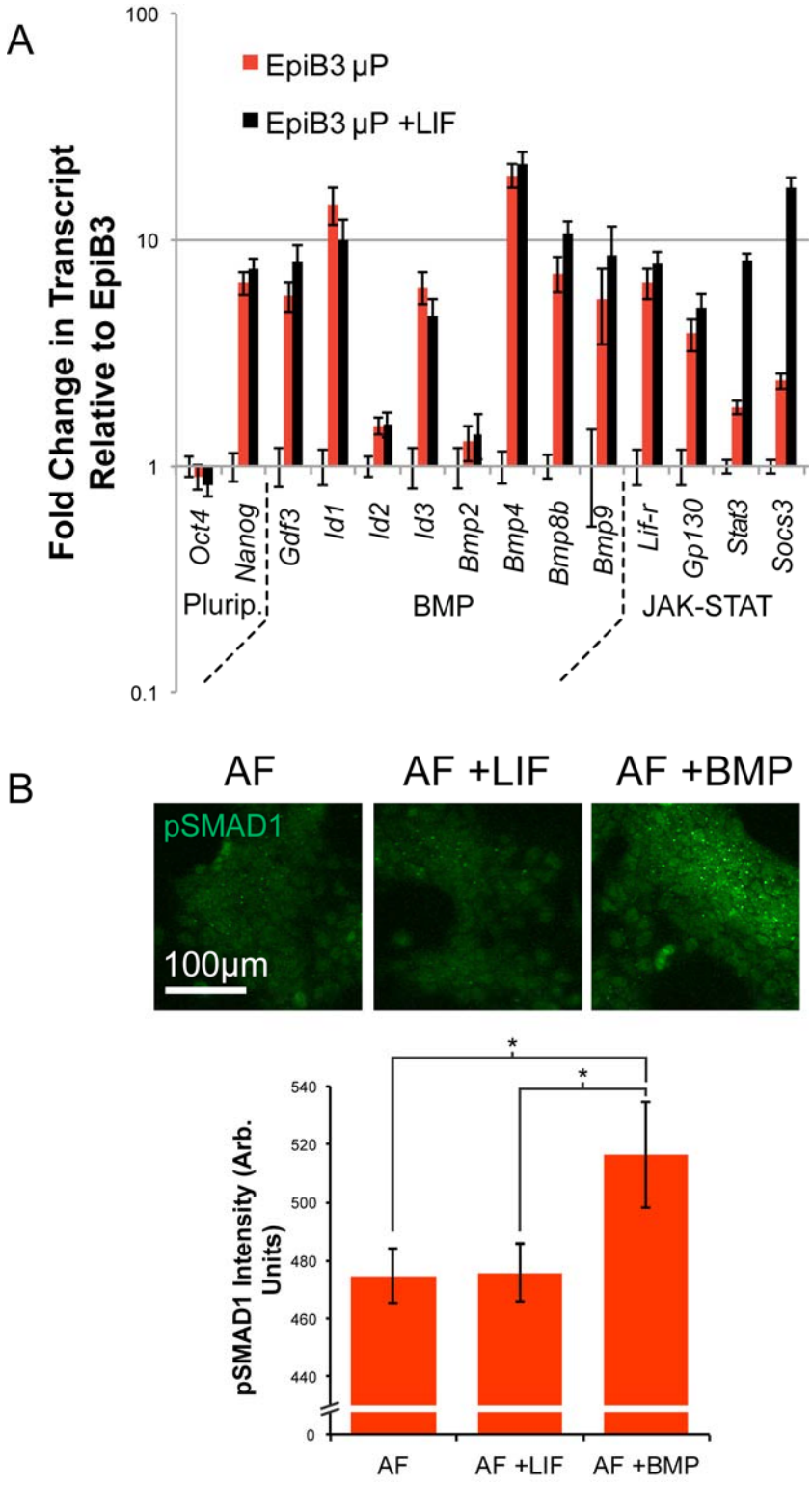

Figure S1. BMP gene expression in EpiB3 after µP, Related to Figure 1. A) EpiB3 cells were µP and assayed for gene expression of Pluripotency genes (Plurip.) *Oct4* and

Nanog, BMP-signaling genes, *Gdf3*, *Id1*, *Id2*, *Id3*, *BMP2*, *4*, *8*, and *9*, and JAK-STAT genes, *Lif-r* and *Socs3* normalized to housekeeping gene, *Gapdh*. Representative plot, error bars represent standard deviation n=3 technical replicates (3 wells). B) LIF alone does not activate SMAD1 signaling in non-patterned EpiSCs. Representative plot, error bars represent standard deviation n=6 technical replicates (6 wells). *$p<0.05$ as measured by two-tailed student's t-test.

Figure S2

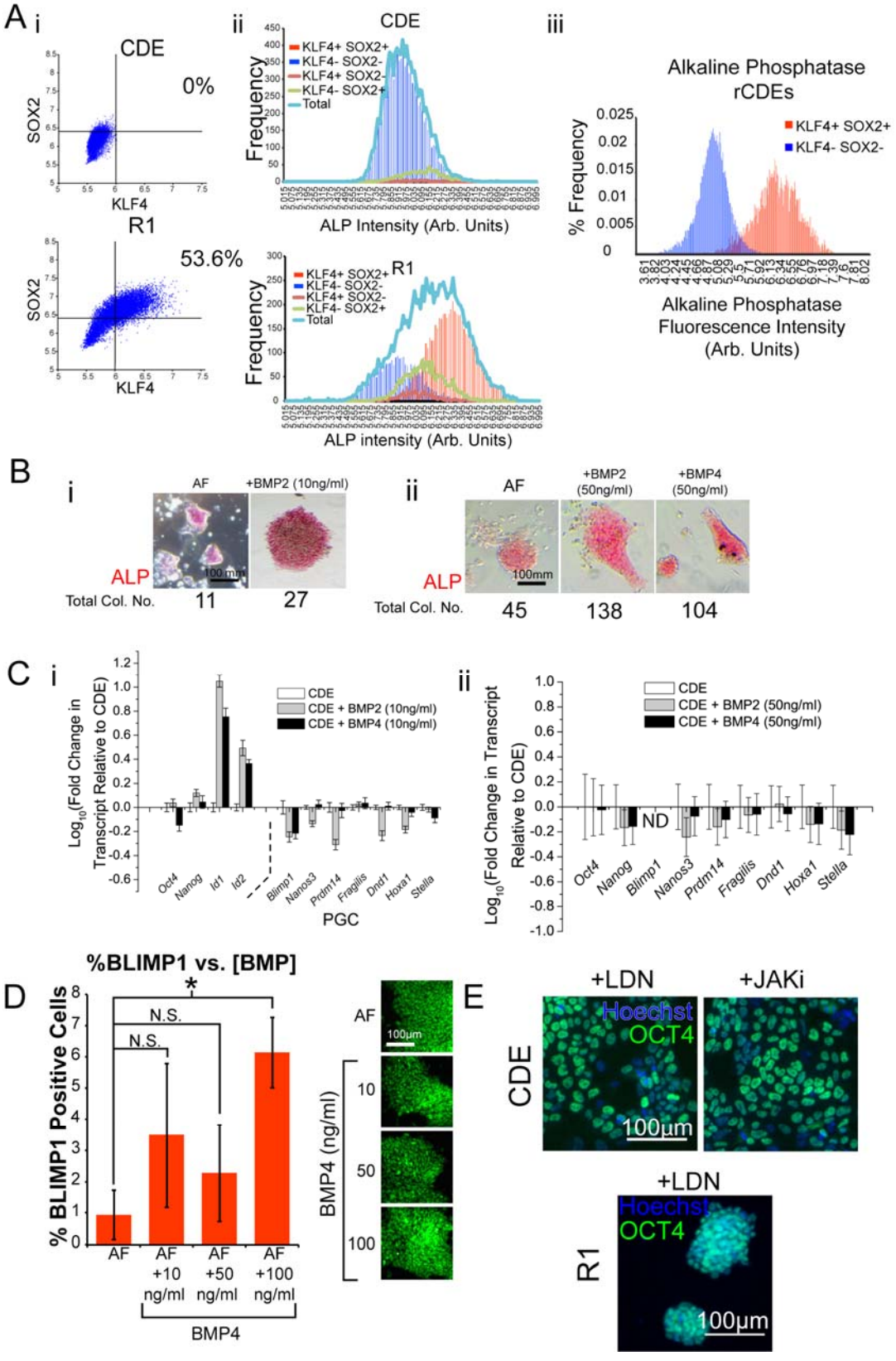
A
i
CDE
0%
SOX2
KLF4
R1
53.6%
ii
CDE
KLF4+ SOX2+
KLF4- SOX2-
KLF4+ SOX2-
KLF4- SOX2+
Total
Frequency
ALP Intensity (Arb. Units)
R1
ALP intensity (Arb. Units)
iii
Alkaline Phosphatase
rCDEs
% Frequency
Alkaline Phosphatase
Fluorescence Intensity
(Arb. Units)
B
i
AF
+BMP2 (10ng/ml)
ALP
Total Col. No.
11
27
ii
AF
+BMP2
(50ng/ml)
+BMP4
(50ng/ml)
ALP
100mm
Total Col. No.
45
138
104
C
i
CDE
CDE + BMP2 (10ng/ml)
CDE + BMP4 (10ng/ml)
Log10(Fold Change in
Transcript Relative to CDE)
Oct4
Nanog
Id1
Id2
Blimp1
Nanos3
Prdm14
Fragilis
Dnd1
Hoxa1
Stella
PGC
ii
CDE
CDE + BMP2 (50ng/ml)
CDE + BMP4 (50ng/ml)
Log10(Fold Change in Transcript
Relative to CDE)
ND
D
%BLIMP1 vs. [BMP]
% BLIMP1 Positive Cells
N.S.
N.S.
*
AF
AF
+10
ng/ml
AF
+50
ng/ml
AF
+100
ng/ml
BMP4
AF
100µm
BMP4 (ng/ml)
10
50
100
E
+LDN
+JAKi
CDE
Hoechst
OCT4
100µm
+LDN
R1
Hoechst
OCT4
100µm

Figure S2. BMP2 or 4 exert similar effects on EpiSCs without upregulation of PGC genes, Related to Figure 2. A) i) Expression of KLF4 and SOX2 in EpiSCs (CDE) vs mESCs (R1) demonstrates the total absence of double positive cells (0% s. 53.6%, respectively) ii) Total population-level ALP expression (cyan line) divided into sub-populations of KLF4-SOX2 double positive (DP - red bars), double negative (DN - blue bars), and single positive (green and red lines) demonstrates high ALP expression correlates with DP cells and low expression with DN cells. iii) Similar results for a population of reverted CDEs. ALP expression is only observed in DP cells. B) i) ALP positive colonies emerge at a higher frequency from CDEs treated for 1 day with 10ng/ml BMP2 than from untreated CDEs. Total colony count (Total Col. No.) listed below images. ii) The same experiment was performed with a higher concentration of BMP4 (50ng/ml). Increased ALP colonies formation relative to baseline is again observed upon 50ng/ml of BMP2 or BMP4 treatment for one day prior to seeding in 2iL. Total colony count (Total Col. No.) listed below images. C) qRT-PCR on CDEs demonstrates a lack of primordial germ cell (PGC) genes that emerge as a result of BMP2 or BMP4 i) 10ng/ml treatment or ii) 50ng/ml for one day. ND - not detected in any sample D) Percentage of BLIMP1 positive cells as assayed by immunostaining of cells cultured in Activin A and FGF media (AF) supplemented with 10ng/ml, 50ng/ml, or 100ng/ml of BMP4. Representative images of BLIMP1 staining shown for these conditions. E) CDEs were able to maintain Oct4 levels in long-term (5 passages) culture in 3μM LDN-193189 (LDN), a BMP4 inhibitor, or 2μM JAK inhibitor (JAKi). Similarly, R1 cells maintain Oct4 expression upon culture in 0.1 μM LDN in 2iL media. Error bars in panels C and D represent standard deviations of n=3 technical replicates (wells within the same experiment). Significance determined by two-tailed student's T-test. *$p<0.05$.

Figure S3

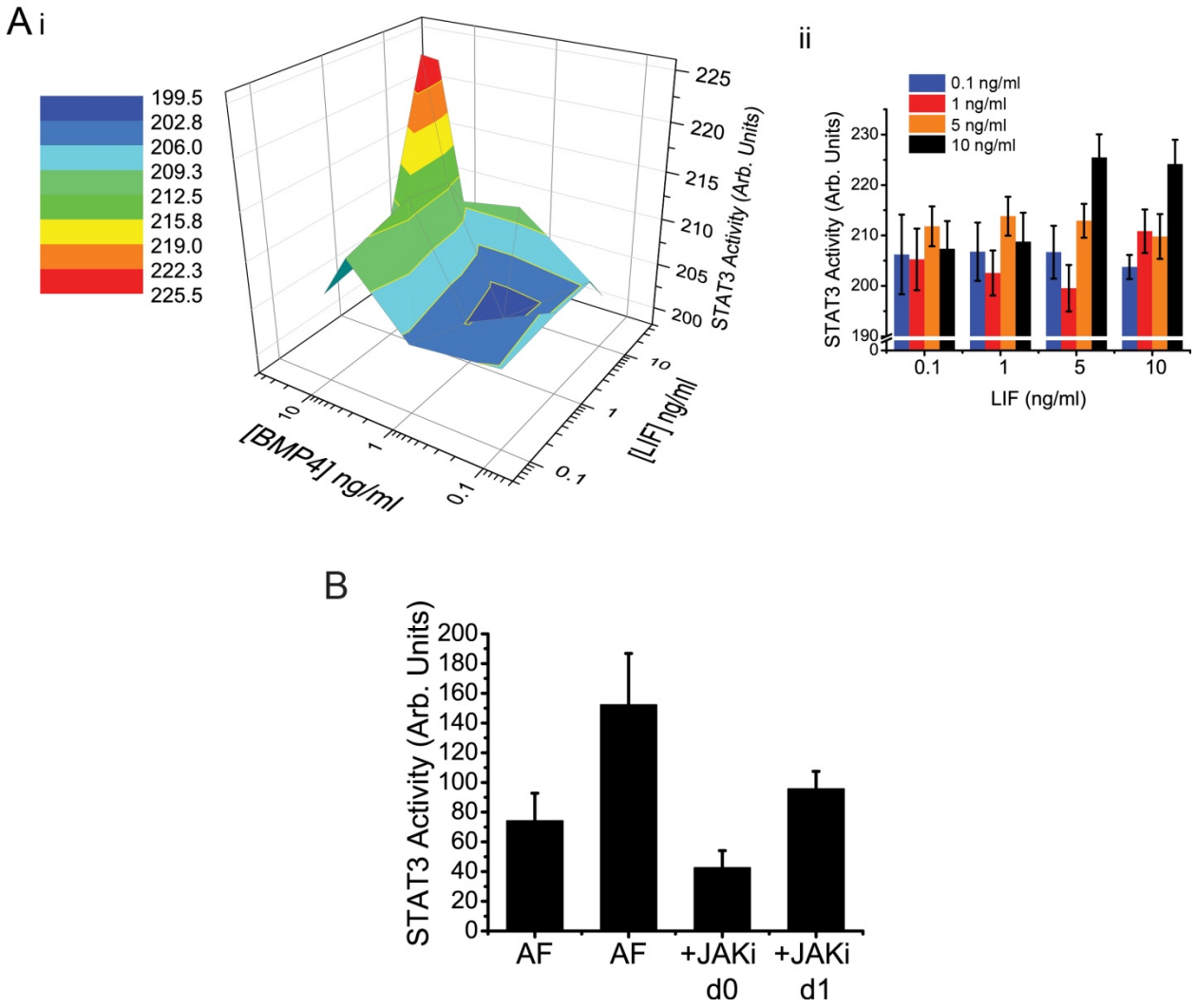

Figure S3. Dose curve of LIF and BMP4 reveals threshold of BMP signaling to recover LIF responsiveness in EpiSCs, Related to Figure 3. A) Treatment with 0.1, 1, 5, or 10ng/ml of each of LIF or BMP4 and subsequent measurement of LIF responsiveness (STAT3 activation) in EpiSCs depicted as a i) 3D surface and as ii) a set of bar graphs. B) JAKi was administered to μP EpiSCs at the seeding step (d0) or 1 day after seeding (d1) and assayed for LIF responsiveness. Error bars represent standard deviation n=4 in A and n=6 in B of technical replicates (wells within the same experiment).

Figure S4

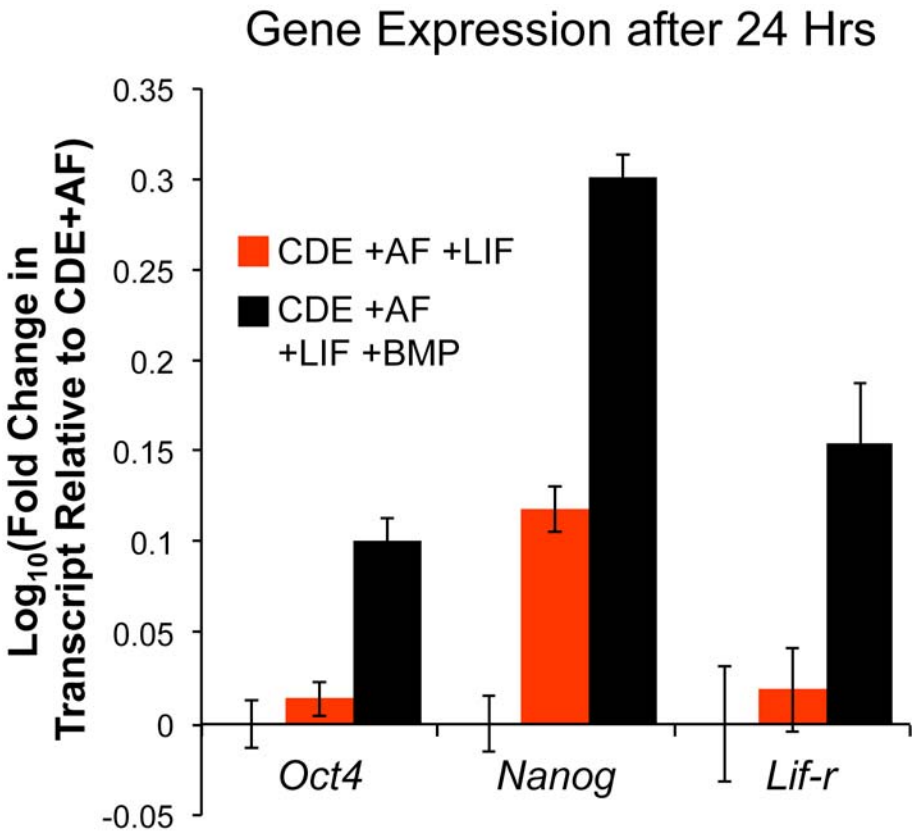

Figure S4. Transcription of *Oct4, Nanog,* and *Lif-r* in response to LIF and/or BMP in 24hrs, Related to Figure 4. Treatment with LIF alone for 24 hrs (red bars) does not result in a significant increase in transcription of *Lif-r*. In contrast, LIF and BMP (black bars) in combination result in robust transcription of *Lif-r*. *Oct4* and *Nanog* remain at or above levels observed in CDEs (Baseline – Y=0) demonstrating maintenance of population-level pluripotency during this treatment period. Representative plot, n=3 technical replicates, error bars represent standard deviation.

Table S1

Primer name	Forward	Reverse	Amplicon Size
LIF-R-enh_chip	CGAGGTATTCCAGGGCCAAG	AGTTCAGGGTCAAATATGAGGCA	373
LIF-R-pro1_chip1	GGTAGGGGACACAAGCAAGA	TGACCCACATGACAGTGACC	411
LIF-R-pro1_chip2	GCTGTGAGTTGGTTTGCTCC	ATAAGACAGAATGCCCCGCT	492
LIF-R-pro1_chip3	AGCGGGGCATTCTGTCTTAT	GTAACTGGCTGCCAAGGTCT	423
LIF-R-pro1_chip4	TGTTAGCCCTCAAACCAGGG	TGTCTCCAAGCTTTCTCAGTT	418
LIF-R-pro1_chip5	ACTGAGAAAGCTTGGAGACAA	GCACAGCTTAACACACCCAG	552
LIF-R-pro2_chip1	ACAAGTGTATTCCCCTGGGC	AGCAAAGGGGACAGCCATAA	503
LIF-R-pro2_chip2	TCATGGCACGTCTGTCTTGT	GCACAGAGAACCAAGGACCA	471
LIF-R-pro2_chip3	GTTCATGCCGAGTCTCCCTC	TCGGGCTTCCCATTTGAGAC	478
LIF-R-pro2_chip4	GTCTCAAATGGGAAGCCCGA	AGCTTGCCGTTTGCTTTGAG	430

Table S1. Primers used for ChIP on regions upstream of *Lif-r*, Related to Figure 4.