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Supplemental Information

RAS Regulates the Transition from Naive to Primed Pluripotent Stem Cells

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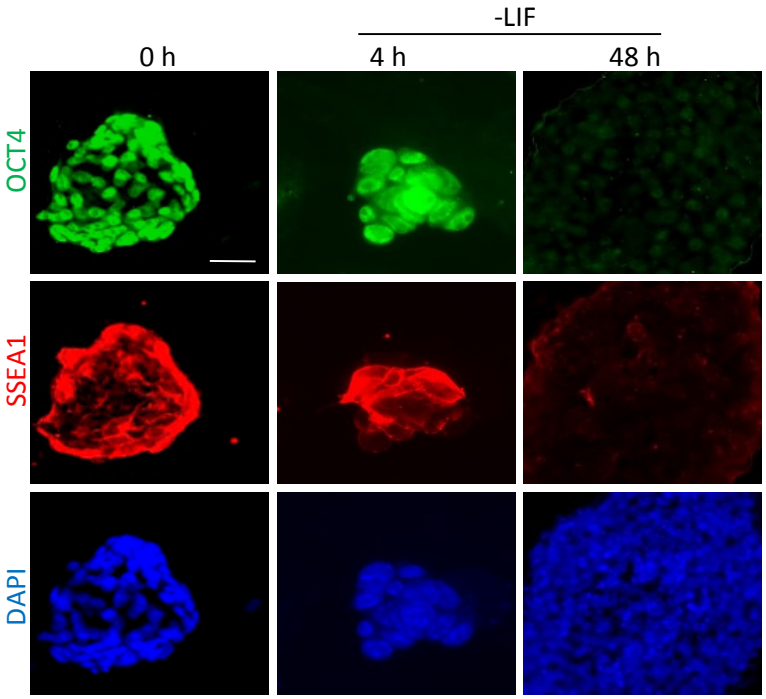


Figure S1 (Related to figure 1). Quantification of Oct4 and SSEA1 expression. Cells were differentiated for the indicated time points in the absence of LIF, and immunostained using the indicated antibodies. Representative images are shown. Scale bar 50 µm. Data shown represent 3 independent experiments.

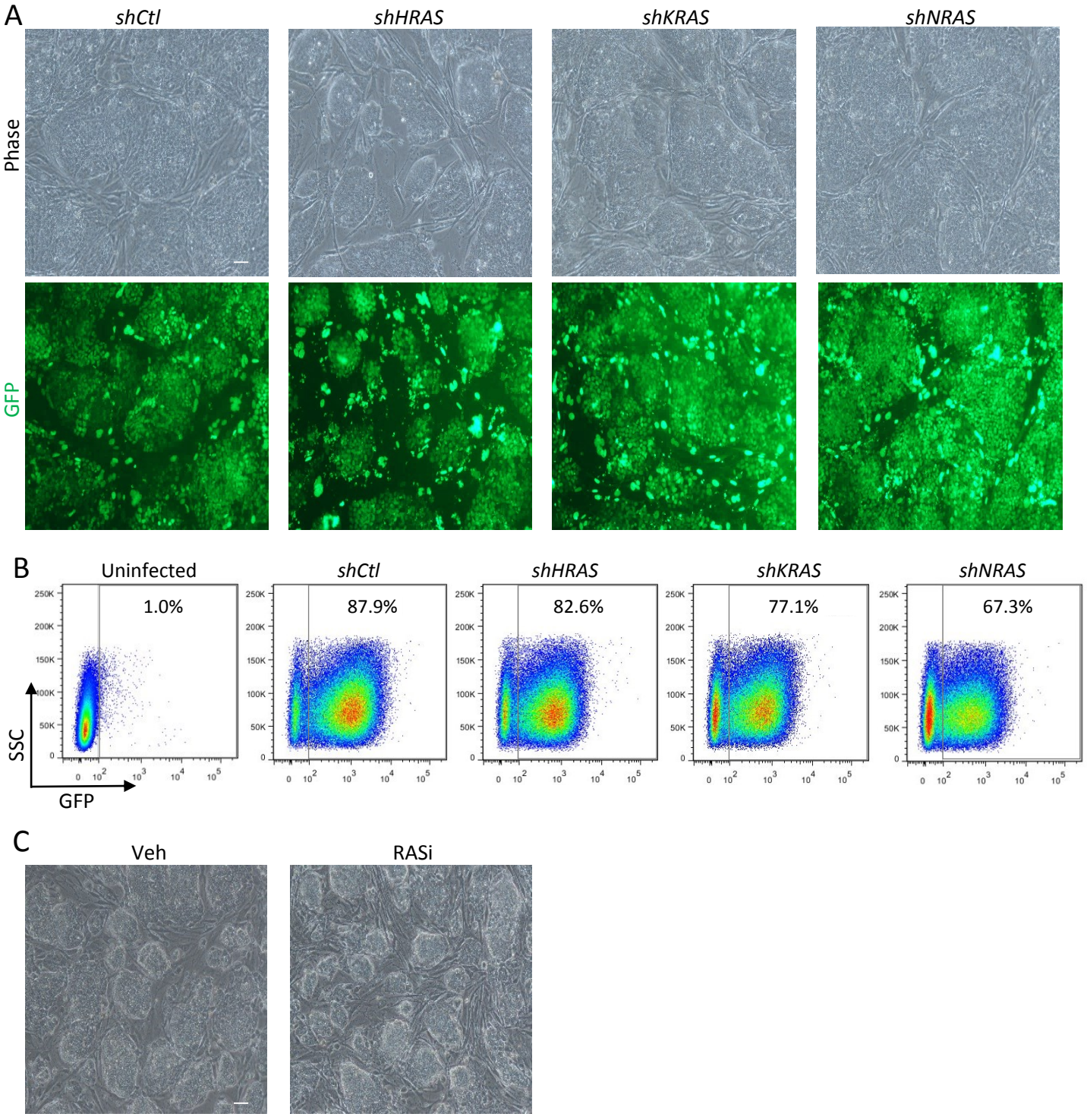
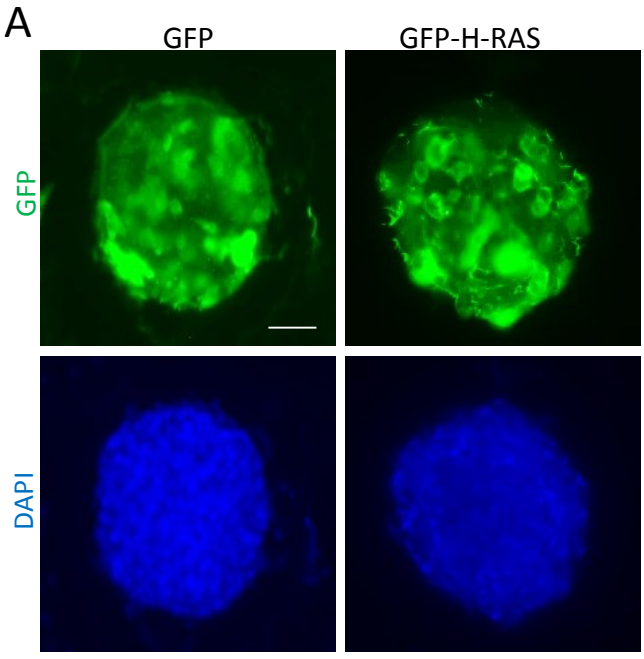


Figure S2 (Related to figure 2). Lentiviral infection with virus containing shRNA against RAS isoforms. mESCs were grown in KSR/bFGF and infected with the indicated virus against H-RAS (shHRAS), K-RAS (shKRAS) or N-RAS (shNRAS), or control shRNA (shCtl) which contained nuclear GFP reporter. Infected cells were imaged by bright field (A) fluorescent microscopy (B) and examined by flow cytometry analysis showing high infection rates. (C) Bright field image of mESCs that were grown in KSR/bFGF in the presence of the RAS inhibitor (75μM) or vehicle (Veh) as control for 48h. Scale bar 50μm. Data shown represent 3 independent experiments.



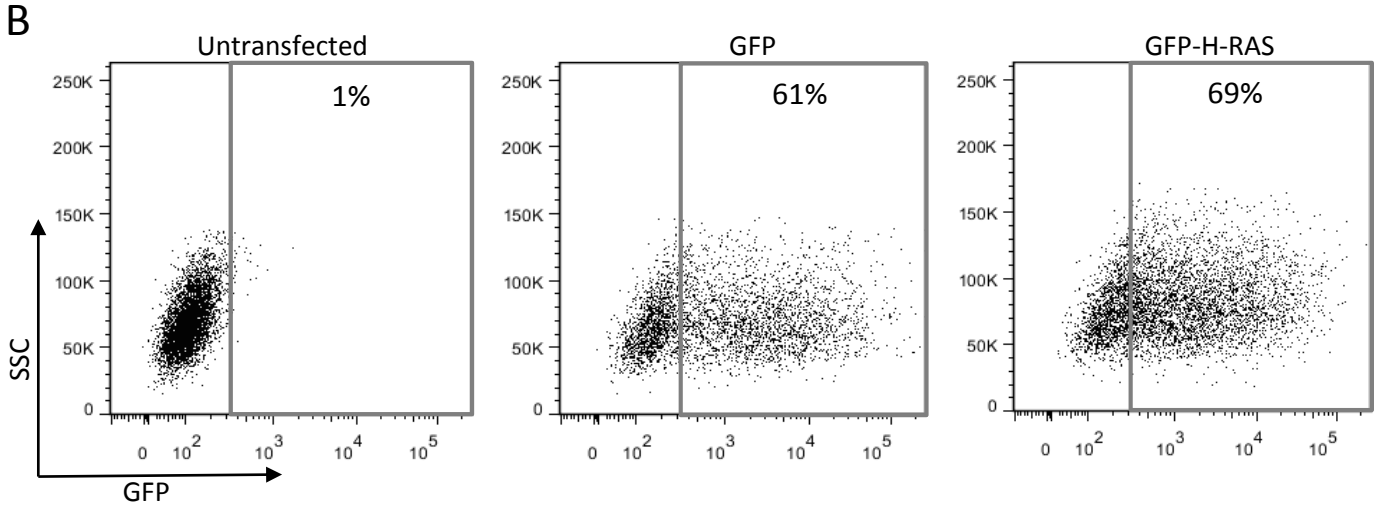


Figure S3 (Related to figure 2). Over expression of RAS. mESCs were grown in 2i/LIF and transfected with a plasmid encoding for GFP fused to H-RAS (GFP-H-RAS) or GFP alone. Fluorescent microscopy (A) and Flow cytometry analysis (B) show high transfection rates. Scale bar 50µm. Data shown represent 3 independent experiments.

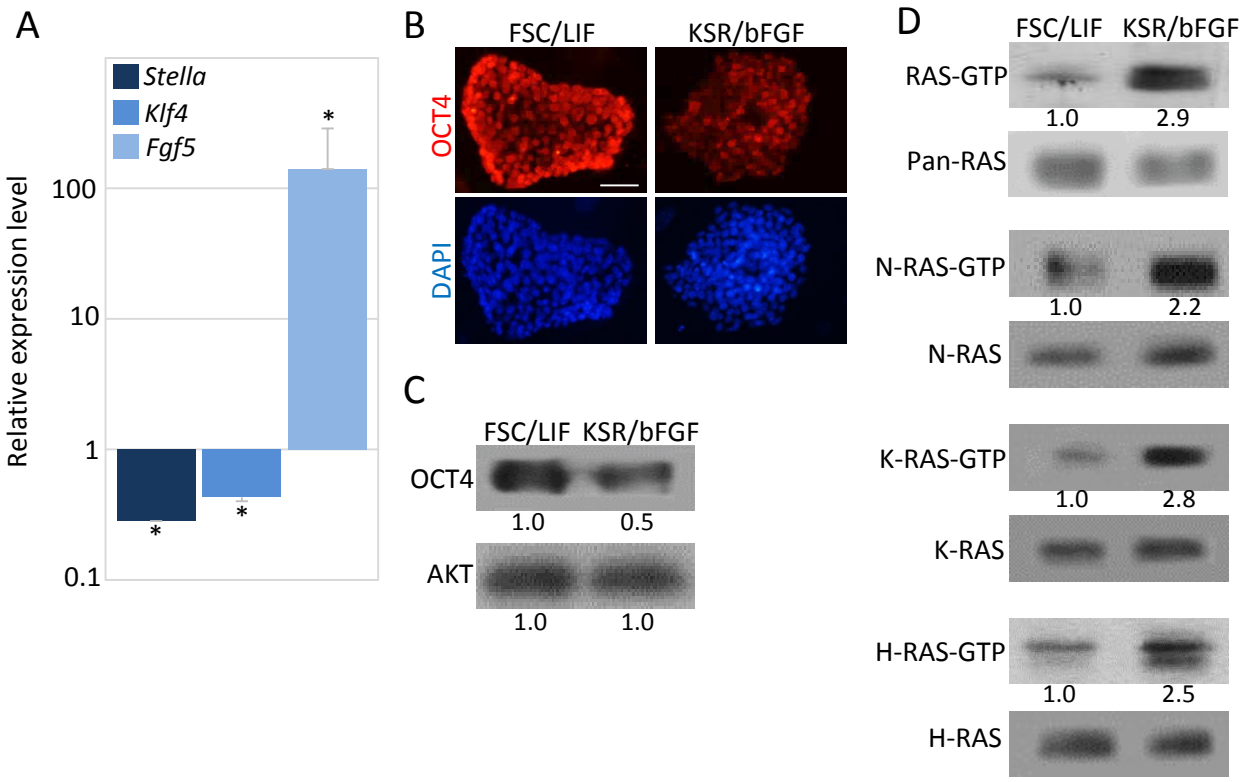


Figure S4 (Related to figure 3). RAS is induced during the transition from the naïve (FCS/LIF) to the primed state. mESCs which were grown in FCS/LIF were switched to KSR/bFGF medium and grown for 10 passages and then subjected to real time PCR analysis (A), immunofluorescence staining (B) or Western blot analysis (C-D) of the indicated proteins. (C-D) Values represent densitometry analysis of three independent experiments. Scale bar 50 µm. Data shown are mean ± standard deviation from 3 independent experiments. **P* <0.05 statistically significant by student's *t*-test.

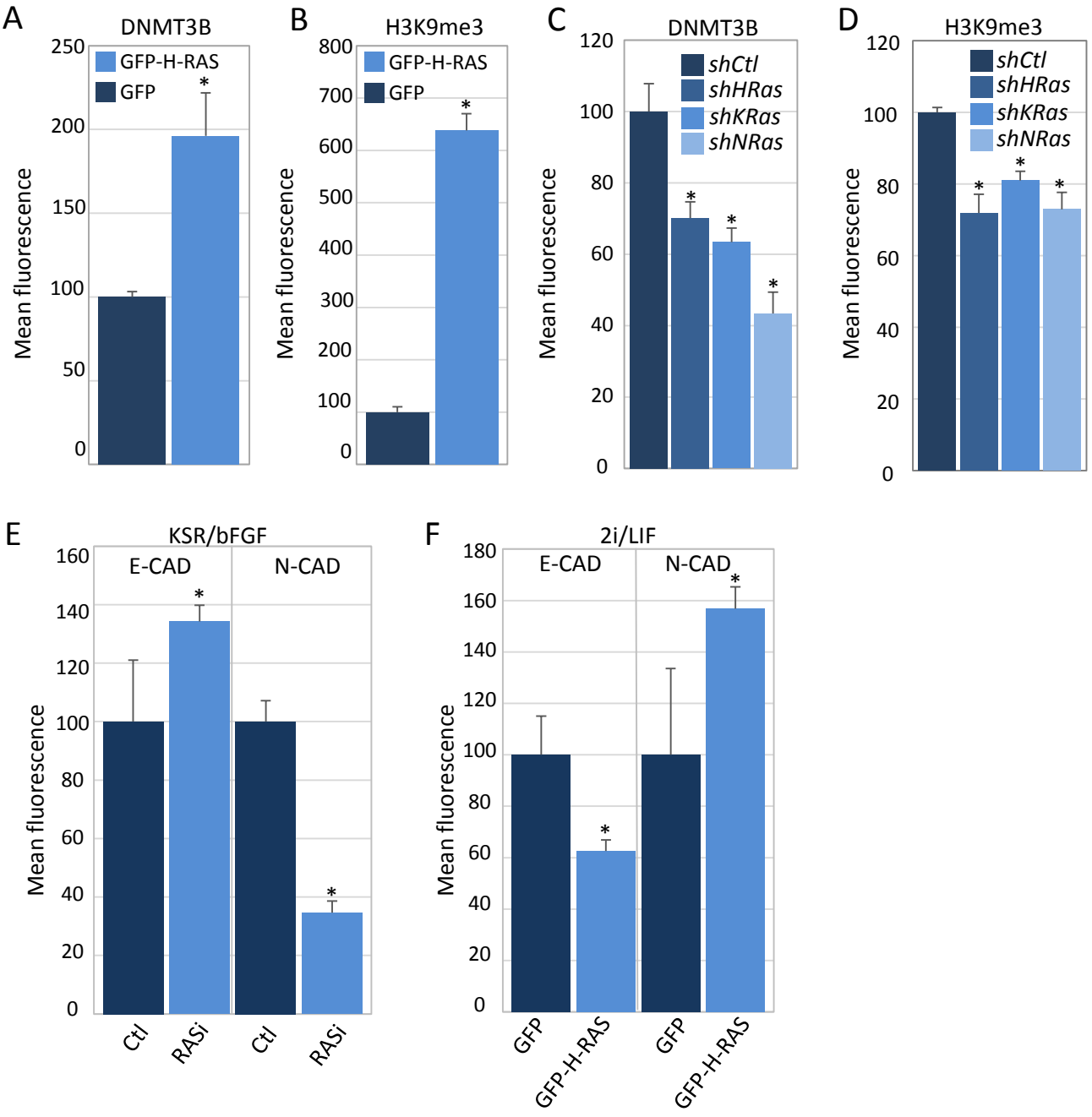


Figure S5 (Related to figure 4 and 5). Quantification of immunofluorescent staining.
mESCs were grown in 2i/LIF and transfected with the indicated plasmids (A-B), or grown in KSR/bFGF and infected with the indicated viral vectors that also contained nuclear GFP reporter (C-D). Immunostaining DNMT3B and H3K9me3 was performed (shown in Fig. 4A-B and in Fig. 4E-F) and quantification shown here was performed using Nikon Nis-elements D, as detailed Methods. mESCs were grown in KSR/bFGF and incubated with RASi (75 μM) or vehicle (Veh) (E), or grown in 2i/LIF and transfected with the indicated plasmids (F). Immunostaining E-CADHERIN (E-CAD) and N-CADHERIN (N-CAD) followed by imaging is shown in Fig. 5C-D, 5F-G and quantification shown here was performed using Nikon Nis-elements D, as detailed Methods. Data shown are mean ± standard deviation from 3 independent experiments. *P <0.05 statistically significant by student's t-test.

A

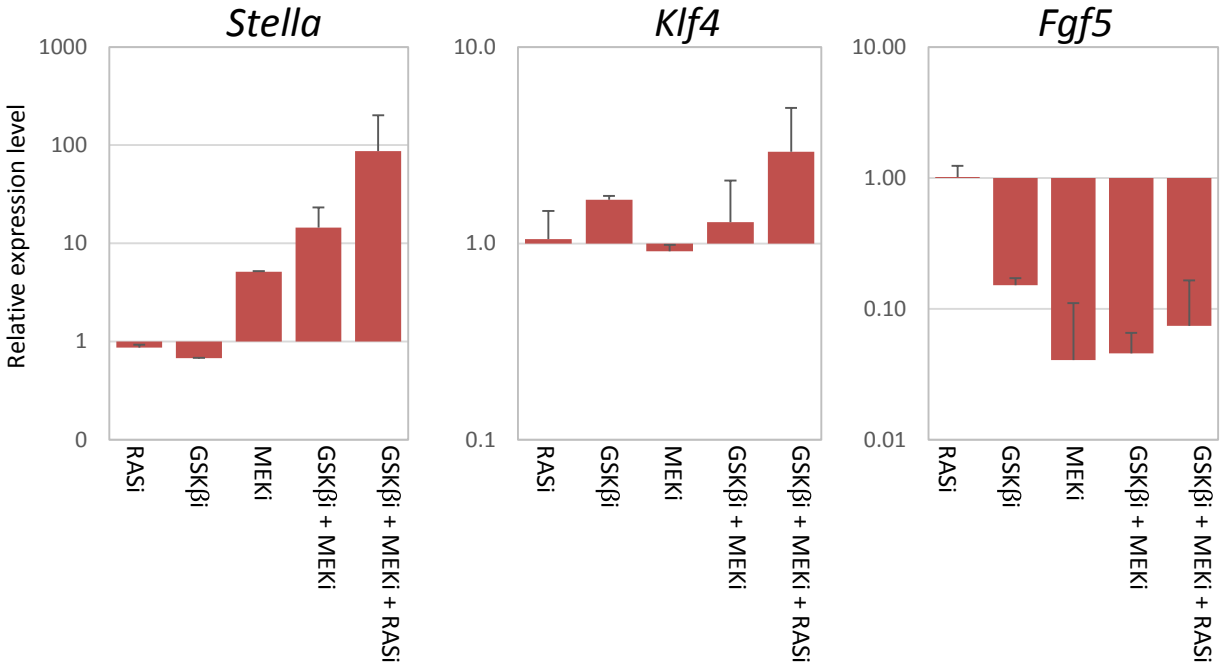


Figure S6 (Related to figure 4). Quantitative Real Time PCR of combinations of inhibitors of MEK and GSKβ (2i) together with Ras depletion.

(A) mESCs were grown in KSR/bFGF and treated with RASi (75 μM), GSKβ (CHIR99021-3μM), MEKi (PD0325901-1μM), combination of MEKi with GSKβ or combination of RASi together with MEKi and GSKβ for 7 days. Quantitative Real Time PCR analysis were performed for the indicated markers. Data shown are mean ± standard deviation from 3 independent experiments.

Gene	Sense primer (5'-3')	Antisense primer (5'-3')
Gapdh	CTTCCCATTCTCGGCCTTG	TGACCTCAACTACATGGTCTACA
Klf4	GCTGGACGCAGTGTCTTCTC	GGCGAGTCTGACATGGCTG
Fgf5	CTGGAAACTGCTATGTTCCGAG	AAGTAGCGCGACGTTTTCTTC
Stella	TTCTTCCCGATTTTCGCATTCT	CAGTGAGCCATTCAGATGTCTC
Dnmt3b	GGGAGCATCCTTCGTGTCTG	AGCGGGTATGAGGAGTGCAT
H-Ras	CGTGAGATTCGGCAGCATAAA	GACAGCACACATTTGCAGCTC
K-Ras	CAAGAGCGCCTTGACGATACA	CCAAGAGACAGGTTTCTCCATC
N-Ras	ACTGAGTACAAACTGGTGGTGG	TCGGTAAGAATCCTCTATGGTGG
Brachury	GACTTCGTGACGGCTGAC	CGAGTCTGGGTGGATGTA
Tbx5	CCAGCTGGGCGAAGGATGTTT	CCGACGCCGTGTACCGAGTGAT
FoxA2	CCCTACGCCAACATGAAG	GTTCTGCCGGTAGAAAGC
Gata4	TCCCCACAAGGCTATGCAT	CCGACGCCGTGTACCGAGTGAT
K18	TAGATGCCCCCAAATCTCA	CTCATGGAGTCCAGGTCGAT
Nestin	CCCTGAAGTCGAGGAGCTG	CTGCTGCACCTCTAAGCGA
Nanog	AGCAGAAGATGCGGACTGTGT	TCAGGTTCAGAATGGAGGAGAGTT
Oct4	CGGCTTCAGACTTCGCCTC	AACCTGAGGTCCACAGTAC
GAPDH	GCCAAGGTCATCCATGACAAC	CTCCACCACCCTGTTGCTGTA
STELLA	TTAATCCAACCTACTTCCAGGG	AGGGGAAACAGATTCGCTACTA
KLF4	CGGACATCAACGACGTGAG	GACGCCTTCAGCACGAACT
FGF5	GTAACCAATCCAGTGAATAGA	TATGTCCAGCAGTCAGTAT
DNMT3B	ACCTCGTGTGGGGAAAGATCA	CCATCGCCAAACCACTGGA

Table S1: A list of primers that were used for real time PCR.