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**Supplemental Information**

**Erk5 Is a Key Regulator of Naive-Primed  
Transition and Embryonic Stem Cell Identity**

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## Supplemental Information

### Supplemental Figure legends

**Figure S1, related to Figure 2** A) Erk5<sup>+/+</sup> mESCs were treated with XMD8-92 at varying concentrations for 2 days, and relative cell survival determined. Data are represented as average  $\pm$  SD from 3 independent experiments. B) mESCs were treated with the indicated concentrations of AZD4547, Ruxolitinib, XMD8-92 and JWG-071 for 48h, and levels of Nanog, Dnmt3b and Erk1/2 determined by immunoblotting. Intervening lanes were removed, as indicated by a dotted line (n=3). C) mESCs were treated with 1 $\mu$ M AZD4547, Ruxolitinib and JWG-071 for 48h, and Dnmt3b mRNA levels determined by qRT-PCR. Data are represented as average  $\pm$  SD from a representative experiment (n=3). D) mESCs were transfected with control non-targeting siRNA or siRNA targeting Dnmt3b. Dnmt3b and Sox2 were analysed by immunoblotting (n=3). E) mESCs were transfected with Cas9 D10A and either control gRNAs or gRNAs specifically targeting Brd4, Grb2 or Stat3 and levels of Brd4, Grb2, Stat3 and Erk1/2 determined by immunoblotting (n=3).

**Figure S2, related to Figure 3.** A) Erk5<sup>+/+</sup> or Erk5<sup>-/-</sup> mESCs were transfected with either empty vector or Erk5 cDNA, and Klf2 mRNA (left panel) or Erk5 and Erk1/2 expression (right panel) determined by immunoblotting. Data are represented as average  $\pm$  SD from a representative experiment (n=3). B) Erk5<sup>+/+</sup> mESCs were treated with indicated concentrations of Mek5 inhibitor BIX 02189 or Erk5 inhibitor JWG-071. Klf2 and Erk5 levels were determined by immunoblotting (n=3). C) Erk5<sup>+/+</sup> mESCs were transfected with empty vector or Mek5DD cDNA and Fgf5 mRNA levels at indicated days post transfection determined by qRT-PCR. The ratio of Mek5DD/Control is represented as average  $\pm$  SD from 3 independent experiments. D) Erk5<sup>-/-</sup> mESCs were transfected with either empty vector or Erk5 cDNA, and Klf4, Erk5 and Erk1/2 expression determined by immunoblotting (n=3). E) Erk5<sup>+/+</sup> or Erk5<sup>-/-</sup> mESCs were LIF deprived for the indicated time and Klf4, Erk5 and Erk1/2 expression determined by immunoblotting. Intervening lanes were removed, as indicated by a dotted line (n=3).

**Figure S3, related to Figure 4.** A) Representative images of alkaline phosphatase stained Erk5<sup>+/+</sup> and Erk5<sup>-/-</sup> mESC colonies B) Erk5<sup>+/+</sup>, Erk5 $\Delta$ N<sup>-/-</sup> and Erk5<sup>-/-</sup> mESCs were counted over 5 days to determine relative proliferation rate (n=3). C) A single Erk5<sup>-/-</sup> mESC clone was used to generate clones of Erk5<sup>-/-</sup> mESCs re-expressing Erk5, and percentage beating compared to parental Erk5<sup>-/-</sup> and Erk5 $\Delta$ N<sup>-/-</sup> mESC lines. Data are represented as average  $\pm$  SD for 4 independent clones. D) Representative images of Erk5<sup>+/+</sup> and Erk5<sup>-/-</sup> embryoid bodies.

Figure S1, related to Figure 2

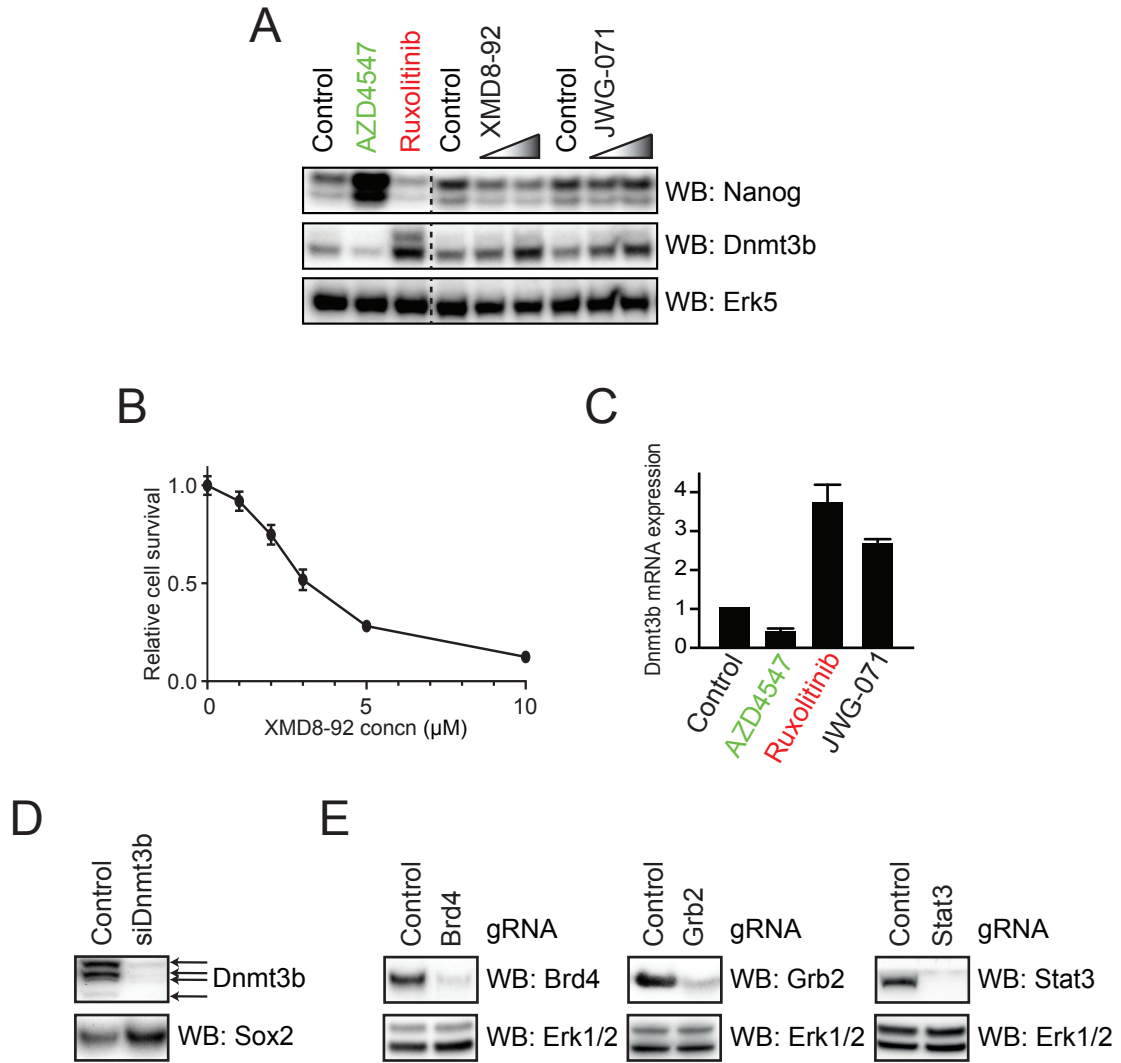


Figure S2, related to Figure 3

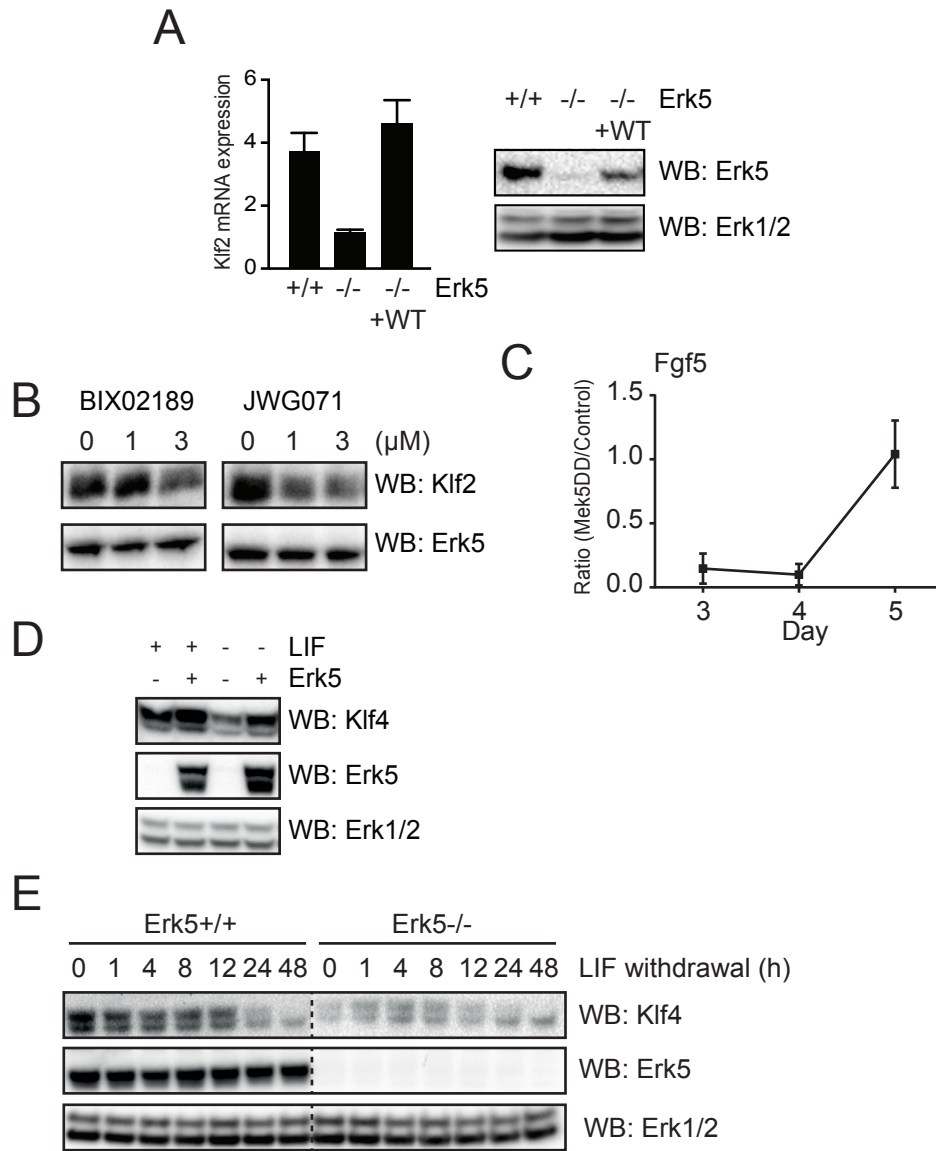
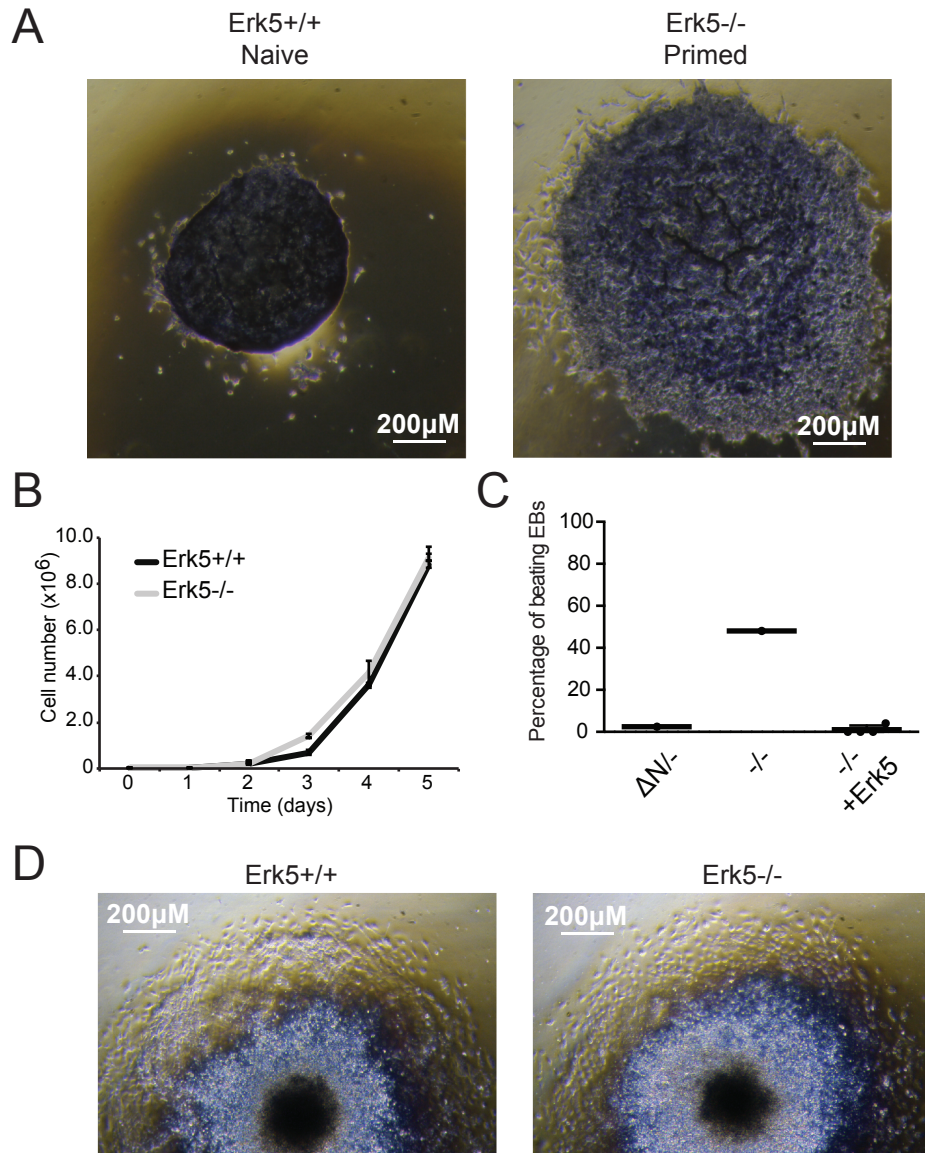




Figure S3, related to Figure 4



## Supplemental Tables

**Table S1. Full list of primed hits from screen, related to Figure 1**

### Primed Hits (<0.5 Nanog:Dnmt3b)

Inhibitor	Primary Target(s)	Comments
Ruxolitinib	JAK	Known pluripotency regulator
GDC-0879	B-Raf	Known pluripotency regulator
17-AAG	Hsp90	Broad spectrum kinase inhibitor
BX795	TBK1/PDK1	Known pluripotency regulator
TPCA-1	IKK2, STAT3	Known pluripotency regulator
VX-745	p38 MAPK	Known pluripotency regulator
OSI-930	c-Kit, VEGFR2	Broad spectrum kinase inhibitor
XL765 (SAR245409)	mTOR/PI3K	Known pluripotency regulator
D4476	CK1, ALK5 (TGF $\beta$ RI)	Known pluripotency regulator
KIN001-269	FMS	Broad spectrum kinase inhibitor
LDN193189	ALK2 (BMPR)	Known pluripotency regulator
SB203580	p38 MAPK	Known pluripotency regulator
ABT-869 (Linifanib/AL-39324)	FLT-3	Broad spectrum kinase inhibitor
KIN001-236	Tie2	Broad spectrum kinase inhibitor
PIK-93	PI3K, PI4KIII $\beta$	Known pluripotency regulator
A769662	AMPK	Known pluripotency regulator
TGX221	PI3K	Known pluripotency regulator
SU6656	Src	Broad spectrum kinase inhibitor
BS-181	CDK7	Broad spectrum kinase inhibitor
KIN001-260 (Bayer IKK $\beta$ inhibitor)	IKK $\beta$	Known pluripotency regulator
<b>XMD8-85</b>	<b>ERK5</b>	
Brivanib	VEGFR, PDGFR	Broad spectrum kinase inhibitor
CC-401	JNK	Known pluripotency regulator
CYT387	JAK	Known pluripotency regulator
NVP-BHG712	EPHB4	Broad spectrum kinase inhibitor
ABT-737	Bcl-2	Non-kinase
Regorafenib (BAY 73-4506)	c-KIT, VEGFR2, B-Raf	Broad spectrum kinase inhibitor
BAY-439006	B-Raf	Known pluripotency regulator
RAF-265 (CHIR-265)	B-Raf	Known pluripotency regulator
BIRB-796 (Doramapimod)	p38 MAPK	Known pluripotency regulator

**Table S2. Full list of naïve hits from screen, related to Figure 1****Naïve Hits (>2 Nanog:Dnmt3b)**

<b>Inhibitor</b>	<b>Primary Target(s)</b>	<b>Comments</b>
AZD8330	MEK1/2	Known pluripotency regulator
GSK-1120212 (JTP-74057)	MEK1/2	Known pluripotency regulator
BIBF-1120 (Vargatef)	VEGFR, PDGFR and FGFR	Known pluripotency regulator
WZ-4-145	CSF1R/DDR1/EGFR/TIE1/ PDGFR2	Broad spectrum kinase inhibitor
PD173074	FGFR	Known pluripotency regulator
HG-6-64-01	Tyrosine kinases	Broad spectrum kinase inhibitor
WZ-7043	CSF1R/DDR1/FGFR/TAO1	Known pluripotency regulator
AP24534 (Ponatinib)	BCR-ABL	Broad spectrum kinase inhibitor
<b>NU7441 (KU 57788)</b>	<b>DNA-PK</b>	<b>Under investigation</b>
AZD4547	FGFR	Known pluripotency regulator
KIN001-043	GSK3	Known pluripotency regulator
BGJ398	FGFR	Known pluripotency regulator
HG-14-10-04	ALK	Broad spectrum kinase inhibitor
GW786034 (Pazopanib)	VEGFR1	Broad spectrum kinase inhibitor
AZD6244 (Selumetinib)	MEK1/2	Known pluripotency regulator
MK2206	AKT1	Known pluripotency regulator
GDC-0941	PI3K	Known pluripotency regulator
R406	Syk	Broad spectrum kinase inhibitor
AZD0530 (Saracatinib)	Src	Broad spectrum kinase inhibitor
PD0325901	MEK1/2	Known pluripotency regulator
HG-5-113-01	LOK/LTK/TRCB/ABL(T315I)	Broad spectrum kinase inhibitor
Dasatinib	Src family	Broad spectrum kinase inhibitor
AZD6482	PI3K	Known pluripotency regulator

**Table S3. Sequences of Erk5 gene targeted mESC lines, related to Figure 2.**

Erk5 Line	Allele Summary	DNA Sequence
+/+	wild-type	A C C A A T G C C A A A C G G A C C C T C A G G G A G C T G A A G A T C C T C A A A C A C T T C A A A C A G G A C A A T A T C A T C G C C A T C A A G G A C A T C C T G A A G C C T A C T G T G
ΔN/-	A1 in frame	A C C A A T G C C A A A C G G A C C C T C A G G G A G C T G A A G A T C C T C A A A C
	A2 large insertion	A C C A A T G C C A A A C G G A C C C T C A G G G A G C T G A A G A T C C T C A A A C A C T T C A A A C A G G A C A A T A T C A T C G C C A T C A A G G A C A T C C T G A A G C C T A C T G T G
ΔN/-	A1 in frame	A C C A A T G C C A A A C G G A C C C T C A G G G A G C T G A A G A T C C T C A A A C A C T T C A A A C A G G A C A A T A T C A T C G C C A T C A A G G A C A T C C T G A A G C C T A C T G T G
	A2 frameshift	A C C A A T G C C A A A C G G A C C C T C A G G G A G C T G A A G A T C C T C A A A C A C T T C A A A C A G G A C A A T A T C A T C G C C A T C A A G G A C A T C C T G A A G C C T A C T G T G
-/-	A1 frameshift	A C C A A T G C C A A A C G G A C C C T C A G G G A G C T G A A G A T C C T C A A A C A C T T C A A A C A G G A C A A T A T C A T C G C C A T C A A G G A C A T C C T G A A G C C T A C T G T G
	A2 frameshift	A C C A A T G C C A A A C G G A C C C T C A G G G A G C T G A A G A T C C T C A A A C A C T T C A A A C A G G A C A A T A T C A T C G C C A T C A A G G A C A T C C T G A A G C C T A C T G T G
ΔN/-	A1 in frame	A C C A A T G C C A A A C G G A C C C T C A G G G A G C T G A A G A T C C T C A A A C A C T T C A A A C A G G A C A A T A T C A T C G C C A T C A A G G A C A T C C T G A A G C C T A C T G T G
	A2 large insertion	A C C A A T G C C A A A C G G A C C C T C A G G G A G C T G A A G A T C C T C A A A C A C T T C A A A C A G G A C A A T A T C A T C G C C A T C A A G G A C A T C C T G A A G C C T A C T G T G
-/-	A1 frameshift	A C C A A T G C C A A A C G G A C C C T C A G G G A G C T G A A G A T C C T C A A A C A C T T C A A A C A G G A C A A T A T C A T C G C C A T C A A G G A C A T C C T G A A G C C T A C T G T G
	A2 frameshift	A C C A A T G C C A A A C G G A C C C T C A G G G A G C T G A A G A T C C T C A A A C A C T T C A A A C A G G A C A A T A T C A T C G C C A T C A A G G A C A T C C T G A A G C C T A C T G T G
-/-	A3 frameshift	A C C A A T G C C A A A C G G A C C C T C A G G G A G C T G A A G A T C C T C A A A C A C T T C A A A C A G G A C A A T A T C A T C G C C A T C A A G G A C A T C C T G A A G C C T A C T G T G
	A1 frameshift	A C C A A T G C C A A A C G G A C C C T C A G G G A G C T G A A G A T C C T C A A A C A C T T C A A A C A G G A C A A T A T C A T C G C C A T C A A G G A C A T C C T G A A G C C T A C T G T G
-/-	A2 large insertion	A C C A A T G C C A A A C G G A C C C T C A G G G A G C T G A A G A T C C T C A A A C A C T T C A A A C A G G A C A A T A T C A T C G C C A T C A A G G A C A T C C T G A A G C C T A C T G T G

N.B. large insertions were not sequenced

## Supplemental Experimental Procedures

**Table S4. Primers used for qRT-PCR, related to Experimental Procedures**

<b>Gene</b>	<b>Forward</b>	<b>Reverse</b>
Nanog	CTCATCAATGCCTGCAGTTTTTCA	CTCCTCAGGGCCCTTGTCAGC
Oct4	AGCTGCTGAAGCAGAAGAGG	AGATGGTGGTCTGGCTGAAC
Klf2	CTCAGCGAGCCTATCTTGCC	CACGTTGTTTAGGTCCTCATCC
Rex1	CCCTCGACAGACTGACCCTAA	TCGGGGCTAATCTCACTTTCAT
Essrb	AACCGAATGTCGTCCGAAGAC	GTGGCTGAGGGCATCAATG
Fgf5	GCTGTGTCTCAGGGGATTGT	CACTCTCGGCCTGTCTTTTC
Brachyury	TCCCGAGACCCAGTTCATAG	TTCTTTGGCATCAAGGAAGG
Pdgfra	TCCATGCTAGACTCAGAAAGTCAA	TCCCGGTGGACACAATTTTTC
Flk1	TCCAGAATCCTCTTCCATGC	AAACCTCCTGCAAGCAAATG
GAPDH	CTCGTCCCGTAGACAAAA	TGAATTTGCCGTGAGTGG
Sox1	TTCCCAGGACTCCGAGGCG	GCTGTGTGCCTCCTCTGCGG
Nkx2-5	ACCTTTAGGAGAAGGGCGATGACT	AAGTGGGATGGATCGGAGAAAGGT
Nppa	CAGAATCGACTGCCTTTTCC	GGGGGTAGGATTGACAGGAT
Tnt	ACCCTCAGGCTCAGGTTCA	GTGTGCAGTCCCTGTTTCA

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