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Supplemental Information

Rinf Regulates Pluripotency Network Genes and Tet Enzymes in Embryonic Stem Cells

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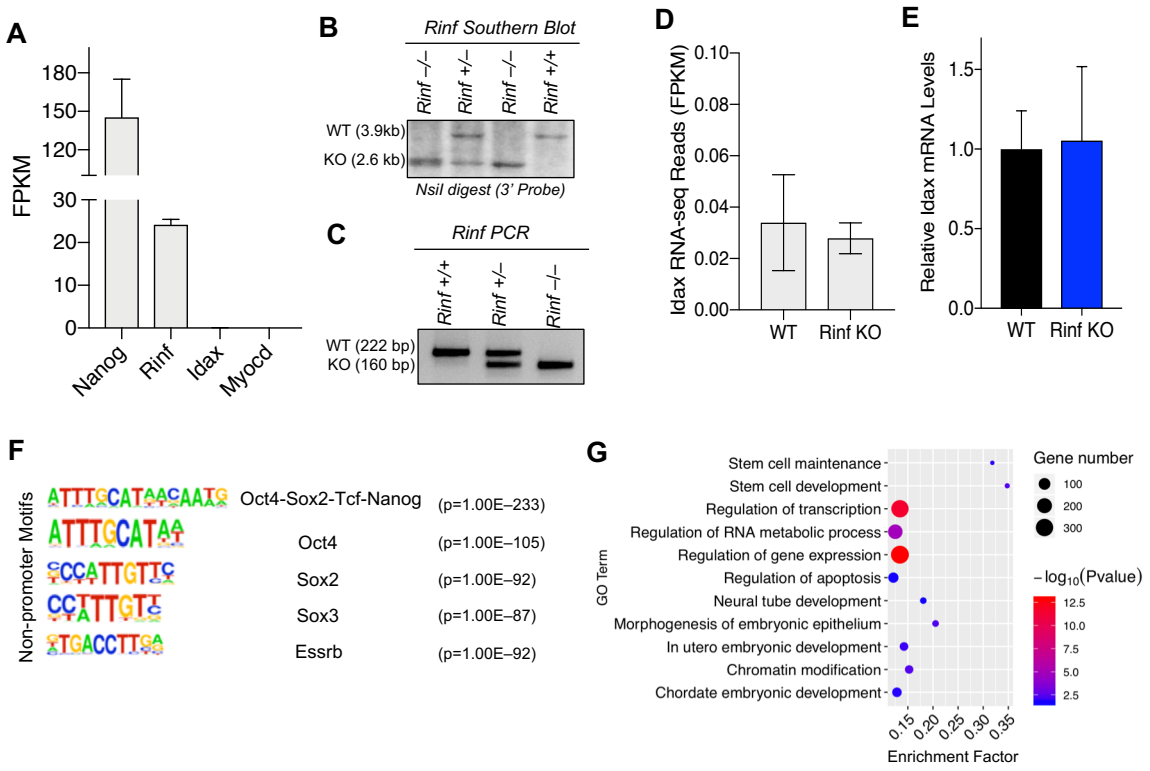

Figure S1 (Related to Figure 1): Expression and chromatin enrichment of Rinf in ESCs.

(A) RNA-seq read counts for indicated genes in ESCs. Note the absence of Idax transcripts in contrast to Rinf transcripts in ESCs. Nanog and Myocd are used as expressed and unexpressed gene controls in ESCs, respectively. Error bars = Stdev.

(B) Genotype confirmation of targeted ESCs by southern blot.

(C) Genotype confirmation of targeted ESCs by PCR.

(D) Idax RNA-seq read counts in ESCs of indicated genotypes. Note the very low FPKM values for Idax in both wild type and Rinf KO ESC. Error bars = Stdev.

(E) Quantification of Idax mRNA by RT-qPCR in wild type and *Rinf*$^{-/-}$ ESCs. Note that this is relative expression. Idax CT values were >33-34 for both WT and KO ESCs (no detectable transcript levels). Data normalized to Gapdh. Error bars = Stdev.

(F) Motif analysis of Rinf peaks revealing enrichment for pluripotency factor binding sites.

(G) Gene ontology analysis of Rinf bound genes.

Figure S2

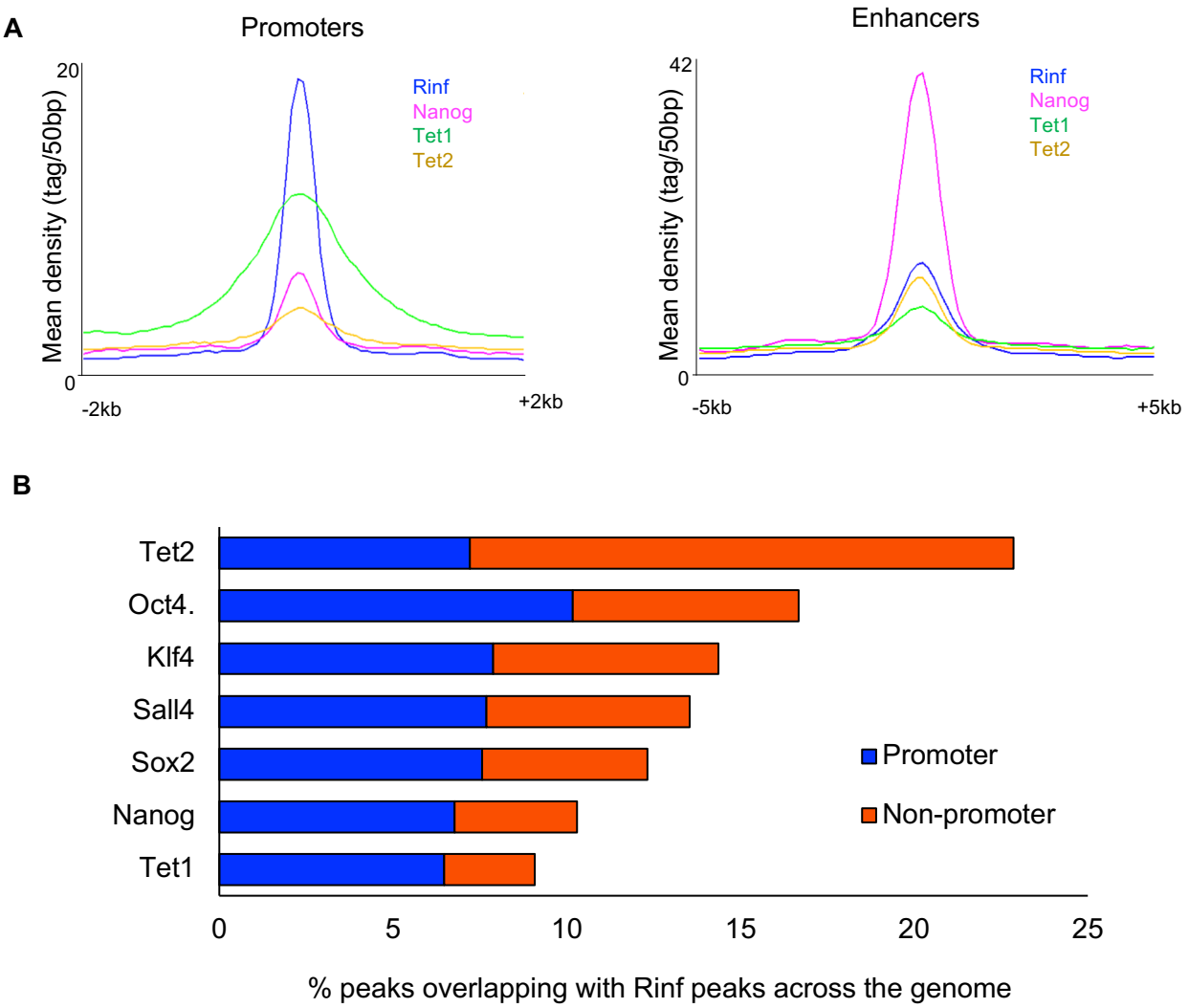

Figure S2 (Related to Figure 2): Co-occupancy of Rinf with pluripotency factors and Tet enzymes at gene regulatory elements

(A) ChIP-seq read densities at Rinf peaks presented as line graphs. The peaks are separated into those at promoters and enhancers. Centers are summit of Rinf peak.

(B) Fraction of pluripotency factor and Tet peaks that overlap with Rinf peaks at promoters and enhancers in ESCs.

A

Sample	Total reads	Trim galore	Align (concordant pairs)
WT-1	24089091	23973542	69.0%
WT-2	31096282	30969961	68.3%
KO-1	26947054	26844267	67.0%
KO-2	26331870	26195040	67.3%

B

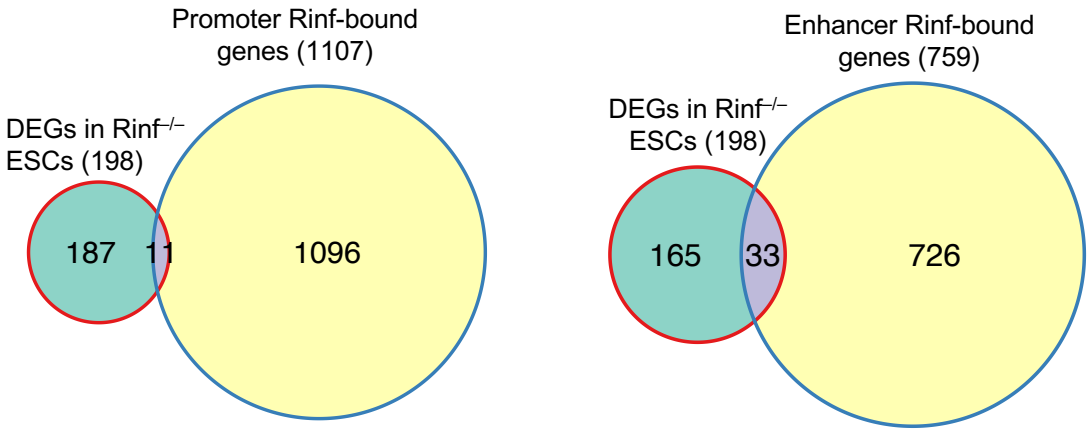

Figure S3 (Related to Figure 3): Gene expression analysis of Rinf deficient ESCs by RNA-seq.

(A) Summary table of number of RNA-seq reads in ESCs.

(B) Overlap of differentially expressed genes (DEGs) in Rinf-KO ESCs with Rinf bound genes in ESCs

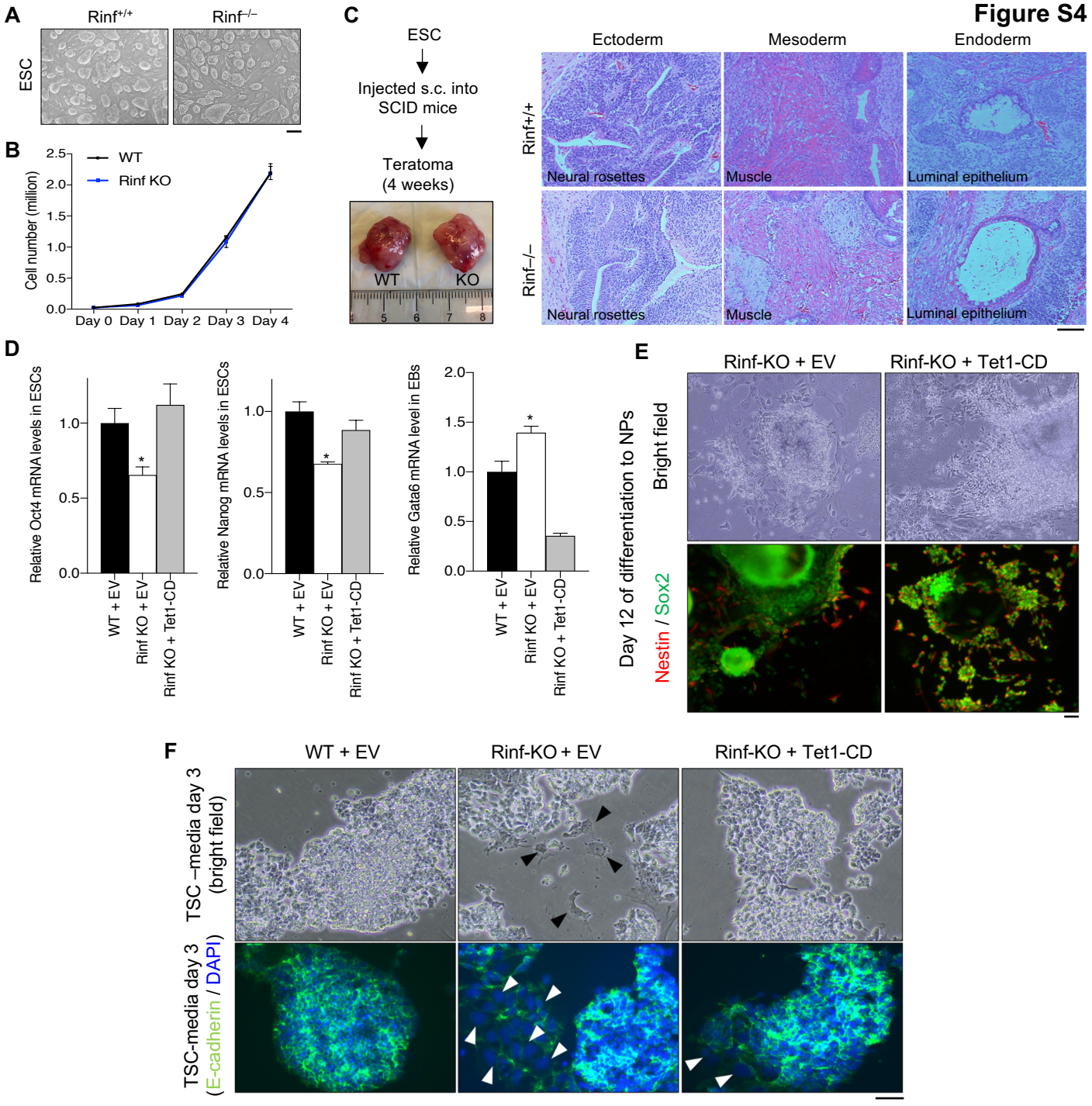

Figure S4 (Related to Figure 4): Characterization of wild type and *Rinf* $^{-/-}$ ESCs

(A) Brightfield images of ESCs of indicated genotypes. Bar = 200 μm

(B) Proliferation rate of ESCs. Cell count of 3 lines of each genotype plotted. Error bars = stdev.

(C) Gross images of teratomas (left) and histological analysis of teratomas by H&E staining (right). Bar = 100 μm

(D) Quantification of mRNA levels of indicated genes in ESCs or EBs of indicated genotypes. Data normalized to Gapdh. Error bars = Stdev. * Statistically significant ($p<0.05$).

(E) Bright field and immunofluorescence images of ESCs at day 12 of differentiation to neural progenitors (NPs). Note the improved differentiation of Rinf KO ESCs that express Tet1 catalytic domain (Tet1-CD). Bar = 50 μm

(F) Bright field and immunofluorescence images of ESCs cultured in Trophoblast stem cell media (TSC) for three days. Trophoblast giant cells are marked by flat morphology (black arrowheads) as well as negative E-cadherin staining and large nuclei (white arrowheads). Note that expression of Tet1 catalytic domain (Tet1-CD) in Rinf KO ESCs reduces skewed differentiation towards trophectoderm lineage. Bar = 50 μm

Table S1 (Related to STAR Methods): List of oligos used in study

Name	Sequence (5'-3')	Purpose	Source
Rinf gRNA Left For oligo	GTAATGCCTCATCAGACGTC	Gene targeting	This paper
Rinf gRNA Left Rev oligo	GACGTCTGATGAGGCATTAC	Gene targeting	This paper
Rinf gRNA Right For oligo	GCCAGCAAGCCATGGTTTGC	Gene targeting	This paper
Rinf gRNA Right Rev oligo	GCAAACCATGGCTTGCTGGC	Gene targeting	This paper
V5-Rinf gRNA oligo For	GCCACCGCCGAGGCTCGACA	Gene Targeting	This paper
V5-Rinf gRNA oligo Rev	TGTCGAGCCTCGGCGGTGGC	Gene Targeting	This paper
Rinf genotyping For	CGTGCTACACGCTCAACTCT	Genotyping	This paper
Rinf genotyping Rev	TGTTACTGCTGCTGCTACTGC	Genotyping	This paper
Rinf RT-qPCR For	CAGCTCAGGCAAGAAGAAGC	Real time qPCR	This paper
Rinf RT-qPCR Rev	GACGGAAGCATCACCTTCTC	Real time qPCR	This paper
Idax RT-qPCR For	CACTTCGCTAGAGAGAACACC	Real time qPCR	This paper
Idax RT-qPCR Rev	CTGGCCAATTCCCCAAACTTC	Real time qPCR	This paper
Tet1 RT-qPCR For	TGCACCTACTGCAAGAATCG	Real time qPCR	Dawlaty et al. Cell Stem Cell 2011
Tet1 RT-qPCR Rev	AAATTGGCATCACAGCTTCC	Real time qPCR	Dawlaty et al. Cell Stem Cell 2011
Tet2 RT-qPCR For	GTCAACAGGACATGATCCAGGAG	Real time qPCR	Zhe et al., Blood 2011
Tet2 RT-qPCR Rev	CCTGTTCCATCAGGCTTGCT	Real time qPCR	Zhe et al., Blood 2011
Tet3 RT-qPCR For	TCCGGATTGAGAAGGTCATC	Real time qPCR	Dawlaty et al. Developmental Cell 2014
Tet3 RT-qPCR Rev	CCAGGCCAGGATCAAGATAA	Real time qPCR	Dawlaty et al. Developmental Cell 2014
Dnmt3a RT-qPCR For	GACTCGCGTGCAATAACCTTAG	Real time qPCR	Yamaji et al., Cell Stem Cell 2013
Dnmt3a RT-qPCR Rev	GGTCACTTTCCCTCACTCTGG	Real time qPCR	Yamaji et al., Cell Stem Cell 2013
Dnmt3b RT-qPCR For	CTCGCAAGGTGTGGGCTTTTGTAAC	Real time qPCR	Yamaji et al., Cell Stem Cell 2013
Dnmt3b RT-qPCR Rev	CTGGGCATCTGTCATCTTTGCACC	Real time qPCR	Yamaji et al., Cell Stem Cell 2013
Dnmt3l RT-qPCR For	GCTCTAAGACCCTTGAAACCTTG	Real time qPCR	Litman et al., Nat Struct Mol Biol 2008
Dnmt3l RT-qPCR Rev	GCTGGTTCACTTTGACTTCGTA	Real time qPCR	Litman et al., Nat Struct Mol Biol 2008
Fgfr2 RT-qPCR For	CAAGGAGCTCTTGTTCTTCAGG	Real time qPCR	Yamaji et al., Cell Stem Cell 2013
Fgfr2 RT-qPCR Rev	TAACACTGCCGTTTATGTGTGG	Real time qPCR	Yamaji et al., Cell Stem Cell 2013
Fgfr1 RT-qPCR For	CTACCAACCCTGTCCCCAGT	Real time qPCR	Yamaji et al., Cell Stem Cell 2013
Fgfr1 RT-qPCR Rev	CACAGGAAGGCCTCAGTCAG	Real time qPCR	Yamaji et al., Cell Stem Cell 2013
Nanog RT-qPCR For	AAGCAGAAGATGCGGACTGT	Real time qPCR	Dawlaty et al. Developmental Cell 2014
Nanog RT-qPCR Rev	ATCTGCTGGGAGGCTGAGGTA	Real time qPCR	Dawlaty et al. Developmental Cell 2014
Pou5f1 RT-qPCR For	ACATCGCCAATCAGCTTGG	Real time qPCR	Dawlaty et al. Developmental Cell 2014
Pou5f1 RT-qPCR Rev	AGAACCATACTCGAACCACATCC	Real time qPCR	Dawlaty et al. Developmental Cell 2014
Sox2 RT-qPCR For	GCGGAGTGGAAACTTTTGTCC	Real time qPCR	Dawlaty et al. Developmental Cell 2014
Sox2 RT-qPCR Rev	CGGGAAGCGTGTACTTATCCTT	Real time qPCR	Dawlaty et al. Developmental Cell 2014
Prdm14 RT-qPCR For	ACAGCCAAGCAATTTGCACTAC	Real time qPCR	Yamaji et al., Cell Stem Cell 2013
Prdm14 RT-qPCR Rev	TTACCTGGCATTTTCATTGCTC	Real time qPCR	Yamaji et al., Cell Stem Cell 2013
Cdx2 RT-qPCR For	CGAGCCCTTGAGTCCTGTGA	Real time qPCR	Gu et al, Stem Cell Reports 2018
Cdx2 RT-qPCR Rev	AACCCCAGGGACAGAACC	Real time qPCR	Gu et al, Stem Cell Reports 2018
Gata4 RT-qPCR For	CAGAAGGCAGAGAGTGTGTC	Real time qPCR	This paper
Gata4 RT-qPCR Rev	AGTGGCATTGCTGGAGTTAC	Real time qPCR	This paper
Gata6 RT-qPCR For	GAGCTGGTGCTACCAAGAGG	Real time qPCR	Ito et al., Nature 2011
Gata6 RT-qPCR Rev	TGCAAAAGCCCATCTCTTCT	Real time qPCR	Ito et al., Nature 2011
Pax6 RT-qPCR For	AACAACCTGCCTATGCAACC	Real time qPCR	Dawlaty et al. Cell Stem Cell 2011
Pax6 RT-qPCR Rev	ACTTGGACGGGAACTGACAC	Real time qPCR	Dawlaty et al. Cell Stem Cell 2011
Gapdh RT-qPCR For	GTGTTCCTACCCCCAATGTGT	Real time qPCR	Dawlaty et al. Cell Stem Cell 2011
Gapdh RT-qPCR Rev	ATTGTCATACCAGGAAATGAGCTT	Real time qPCR	Dawlaty et al. Cell Stem Cell 2011
Tet1 Enhancer 1 For	TCAGAAAAGATCTGCCTGCCG	ChIP-qPCR	This paper
Tet1 Enhancer 1 Rev	TGGGGAAGGGTAGTCTCCAA	ChIP-qPCR	This paper
Tet1 Enhancer 2 For	AGGAATGACTGGTCTGCACC	ChIP-qPCR	This paper
Tet1 Enhancer 2 Rev	GAGACGCCTCTTGTTGAGGT	ChIP-qPCR	This paper
Tet1 Promoter1 For	CCTGGTCTACAGGAGACGCTA	ChIP-qPCR	This paper
Tet1 Promoter 1 Rev	AAGGGTGACCTTGAGCTTCC	ChIP-qPCR	This paper
Tet1 Promoter 2 For	GGCTGGCTACTGTCCTTGAT	ChIP-qPCR	This paper
Tet1 Promoter 2 Rev	CGTCCTTGGCAGGTGAATCC	ChIP-qPCR	This paper
Tet2 Enhancer 1 For	GTGAGTTTGCATCGGCCTAAC	ChIP-qPCR	This paper
Tet2 Enhancer 1 Rev	TGCAAACCACTGAGGGGAAG	ChIP-qPCR	This paper
Sox2 Enhancer 1 For	CTGGTGGTCGTCAAACTCTG	ChIP-qPCR	This paper
Sox2 Enhancer 1 Rev	GGGTTCCCCTCCTCTCCTAAT	ChIP-qPCR	This paper
Sox2 Enhancer 2 For	AAGCTAGGCAGGTTCCCCTC	ChIP-qPCR	This paper
Sox2 Enhancer 2 Rev	ATGTGTGAGCAAGAACTGTCG	ChIP-qPCR	This paper
Nanog Enhancer 1 For	CGCTCCCTGGATAGCGATGA	ChIP-qPCR	This paper
Nanog Enhancer 1 Rev	CTTGGGAGTGGCACTTTGGT	ChIP-qPCR	This paper
Nanog Enhancer 2 For	CCGGCTTAGAGCTTGAACCA	ChIP-qPCR	This paper
Nanog Enhancer 2 Rev	TCCCAAGGGCGACGTAATTT	ChIP-qPCR	This paper
Nanog Promoter 1 For	GTGGACCCAGAGGCAAGTTT	ChIP-qPCR	This paper
Nanog Promoter 1 Rev	TCCCAAGGGCGACGTAATTT	ChIP-qPCR	This paper
Oct4 Promoter 1 For	TGAACTGTGGTGGAGAGTGC	ChIP-qPCR	This paper
Oct4 Promoter 1 Rev	GTTATGCATCTGCCGTCTGC	ChIP-qPCR	This paper
Oct4 Promoter 2 For	GTTGGGGAGCAGGAAGTTGT	ChIP-qPCR	This paper
Oct4 Promoter 2 Rev	AATGGCCTTGGCTGGACAAT	ChIP-qPCR	This paper
ActB ChIP-qPCR For	GTGCTGAAGTTCCAGAGAACC	ChIP-qPCR	This paper
ActB ChIP-qPCR Rev	GTTTAGACACAGGCATGTGCAG	ChIP-qPCR	This paper
CYR61ChIP-qPCR-For	CATCGTTCAGACCACGTCTT	ChIP-qPCR	Wu et al.,2011
CYR61ChIP-qPCR-Rev	CAAGGACGCACTTCACAGAT	ChIP-qPCR	Wu et al., 2011

Table S2 (Related to STAR Methods): Genomic location of Rinf peaks and ChIPqPCR primers used

Gene Regulatory Region	chromosome	Peak Start	Peak Center	Peak End	ChIP-qPCR Forward Primer	ChIP-qPCR Reverse Primer	Product region	Product Size (bp)
Tet1 Enhancer 1 (E1)	chr10	62892803	62892980	62893200	TCAGAAAAGATCTGCCTGCCG	TGGGGAAGGGTAGTCTCCAA	62892850 - 62892999	150
Tet1 Enhancer 2 (E2)	chr10	62895488	62895684	62895876	AGGAATGACTGGTCTGCACC	GAGACGCCTCTTGTTGAGGT	62895639 - 62895733	95
Tet1 Promoter 1 (P1)	chr10	62896848	62897018	62897217	CCTGGTCTACAGGAGACGCTA	AAGGGTGACCTTGAGCTTCC	62895639 - 62895733	110
Tet1 Promoter 2 (P2)	chr10	62897931	62898324	62898483	GGCTGGCTACTGTCCTTGAT	CGTCCTTGGCAGGTGAATCC	62898304 - 62898394	91
Tet2 Enhancer (E1)	chr3	133532697	133532785	133532908	GTGAGTTTGCATCGGCCTAAC	TGCAAACCACTGAGGGGAAG	133532749 - 133532818	70
Nanog Enhancer 1 (E1)	chr6	122662791	122663012	122663176	CGCTCCCTGGATAGCGATGA	CTTGGGAGTGGCACTTTGGT	122662966 - 122663050	85
Nanog Enhancer 2 (E2)	chr6	122702474	122702941	122703231	GTGGACCCAGAGGCAAGTTT	TCCCAAGGGCGACGTAATTT	122702936 - 122703121	186
Nanog Promoter (P1)	chr6	122707333	122707483	122707579	ACAATGTCCATGGTGGACCC	ACCCTACCCACCCCCTATTC	122707422 - 122707527	106
Oct4 Enhancer (P1)	chr17	35503923	35504047	35504238	TGAACTGTGGTGGAGAGTGC	GTTATGCATCTGCCGTCTGC	35503925 - 35504059	135
Oct4 Promoter (P2)	chr17	35504766	35505096	35505356	GTTGGGGAGCAGGAAGTTGT	AATGGCCTTGGCTGGACAAT	35505059 - 35505175	117
Sox2 Enhancer 1 (E1)	chr3	34646228	34646394	34646529	CTGGTGGTCGTCAAACTCTG	GGGTTCCCCTCCTCTCCTAAT	34646276 - 34646407	132
Sox2 Enhancer 2 (E2)	chr3	34653945	34654029	34654245	AAGCTAGGCAGGTTCCCCTC	ATGTGTGAGCAAGAACTGTCG	34653984 - 34654113	130