PNAS
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Supplementary Information for

Functions of paralogous RNA Polymerase III subunits POLR3G and POLR3GL in mouse development

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Supplementary Figures (Figures S1 to S4)
Supplementary Tables (Table S1 and S2)

Supplementary Figures

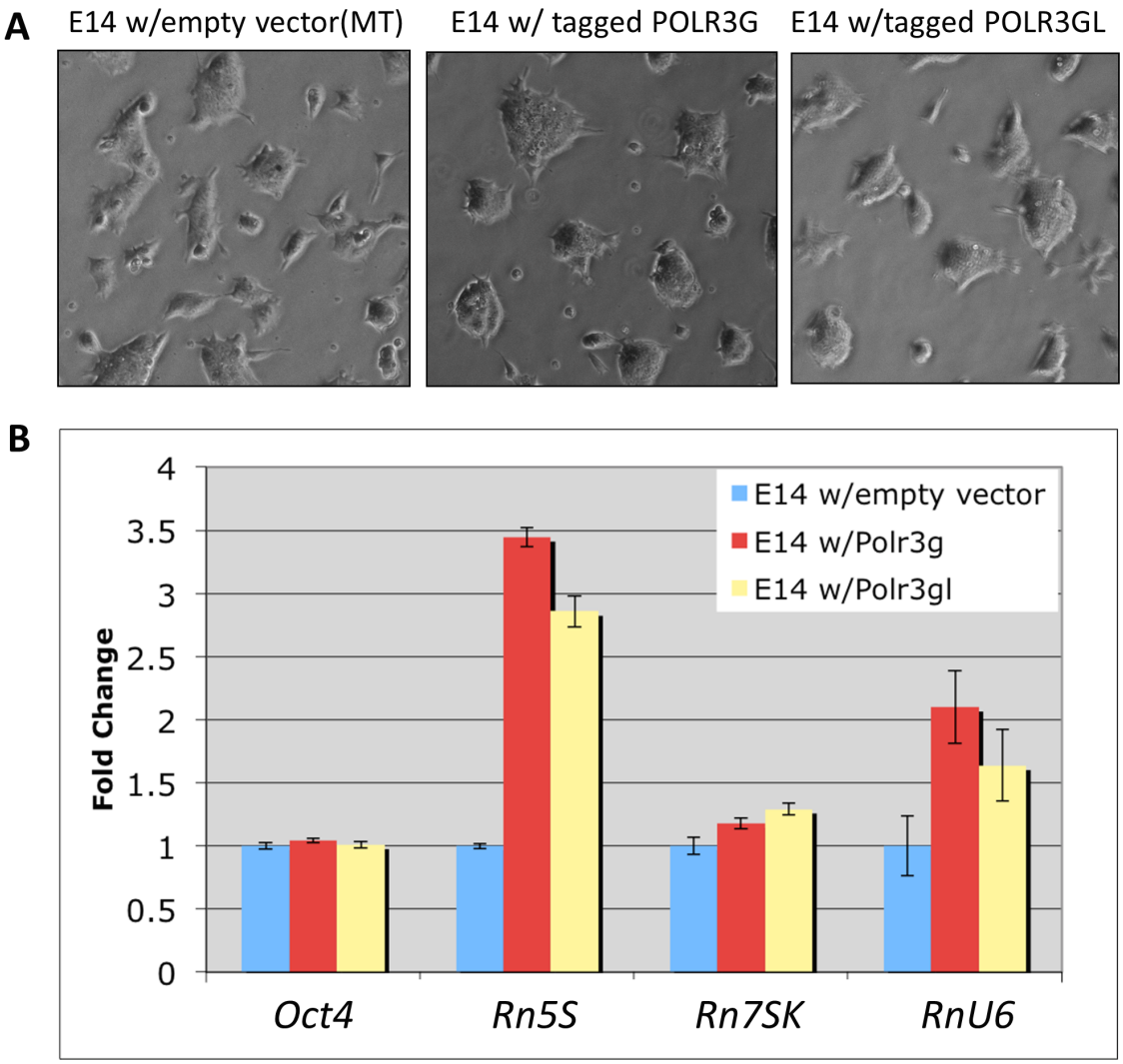

Fig. S1. **(A)** Overexpression of either POLR3G or POLR3GL does not alter stem cell morphology as observed microscopically. **(B)** RT-qPCR analysis of the ESC pluripotency marker *Pou5f1* and expression of Pol III-transcribed *Rn5S*, *Rn7SK* and *RnU6* genes in control (E14TG2a w/ empty vector) and in POLR3G- and POLR3GL-overexpressing cells. The results indicate that overexpression of POLR3G or POLR3GL does not alter expression of Pol II-transcribed pluripotency marker *Oct4* but slightly, moderately or significantly increases expression, respectively, of the *Rn7SK, RnU6, and Rn5S* genes, consistent with earlier results indicating in vitro transcription of these genes by human Pol III isoforms (14). However, and most importantly, the differences for these genes between POLR3G- and POLR3GL-overexpressing cells are marginal.

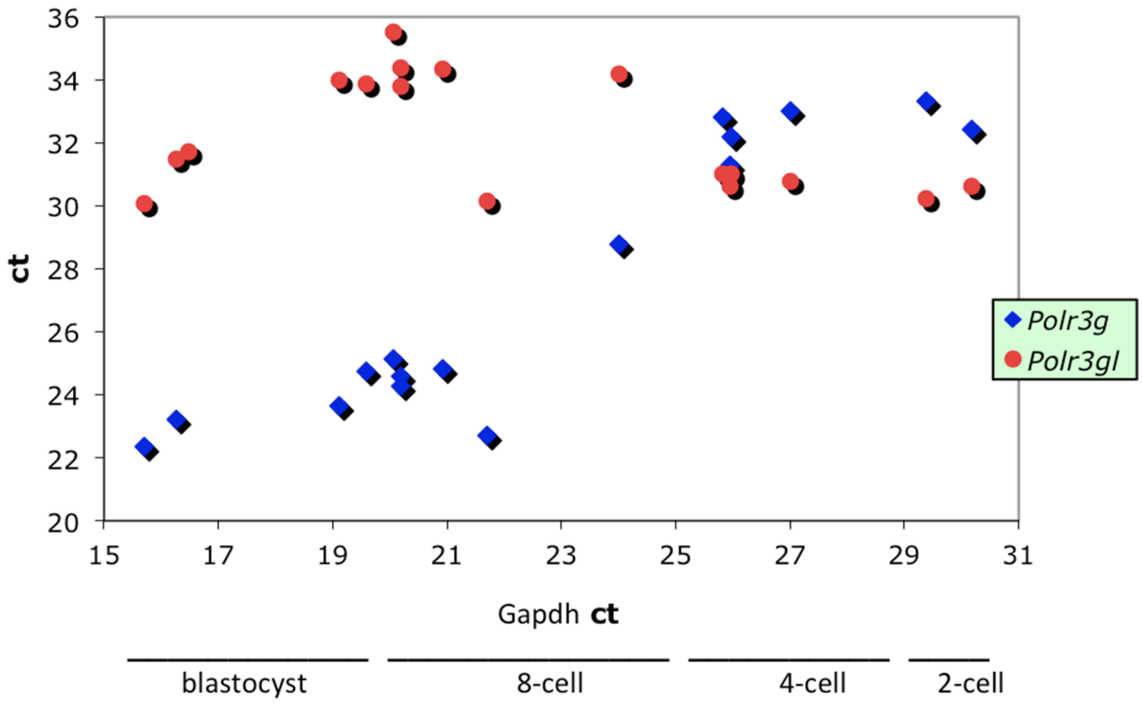

Fig. S2. Early RNA expression of *Polr3g* and *Polr3gl* genes detected by single cell to CT experiments from 2-cell stage to blastula stage embryos. Each dot represents one embryo. The X-axis shows the CT value for *Gapdh*. The Y-axis shows the the CT value for *Polr3g* (blue) or *Polr3gl* (red). *Gapdh* expression is increased during early development (as reflected by reduced CT number).

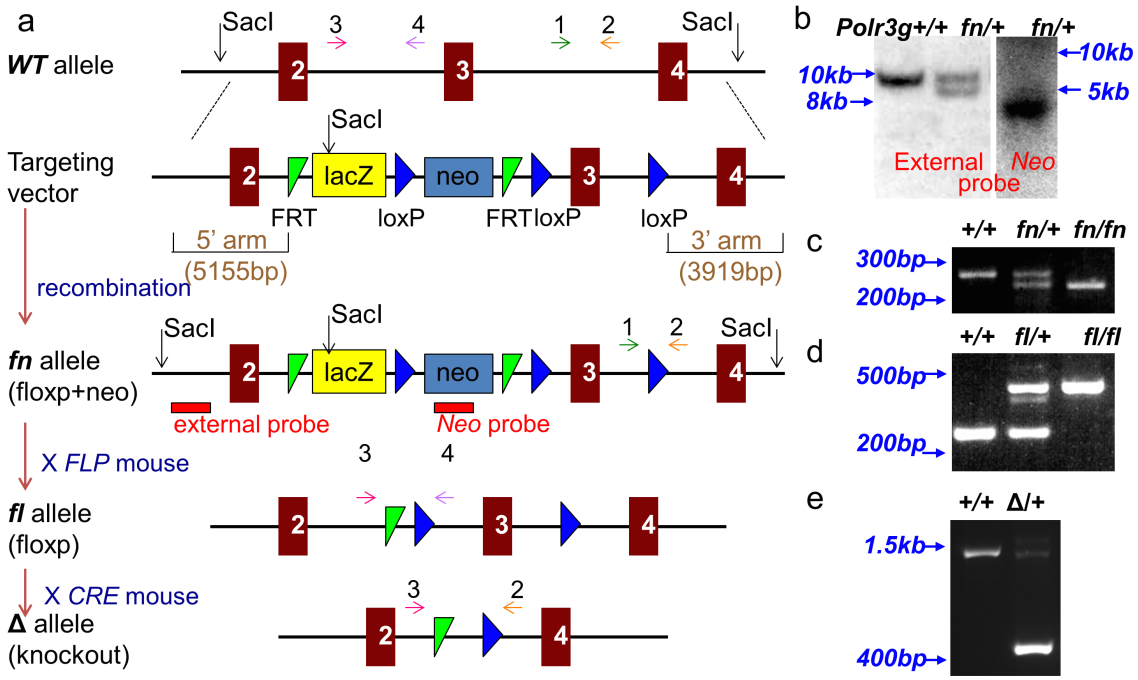
a
WT allele
SacI
3
4
1
2
SacI
2
3
4
Targeting vector
SacI
lacZ
neo
FRT
loxP
FRT
loxP
loxP
5' arm
(5155bp)
3' arm
(3919bp)
recombination
fn allele
(floxp+neo)
external probe
Neo probe
X FLP mouse
fl allele
(floxp)
X CRE mouse
Δ allele
(knockout)
b
Polr3g+/+
fn/+
fn/+
10kb
8kb
10kb
5kb
External probe
Neo probe
c
+/+
fn/+
fn/fn
300bp
200bp
d
+/+
fl/+
fl/fl
500bp
200bp
e
+/+
Δ/+
1.5kb
400bp

Fig. S3

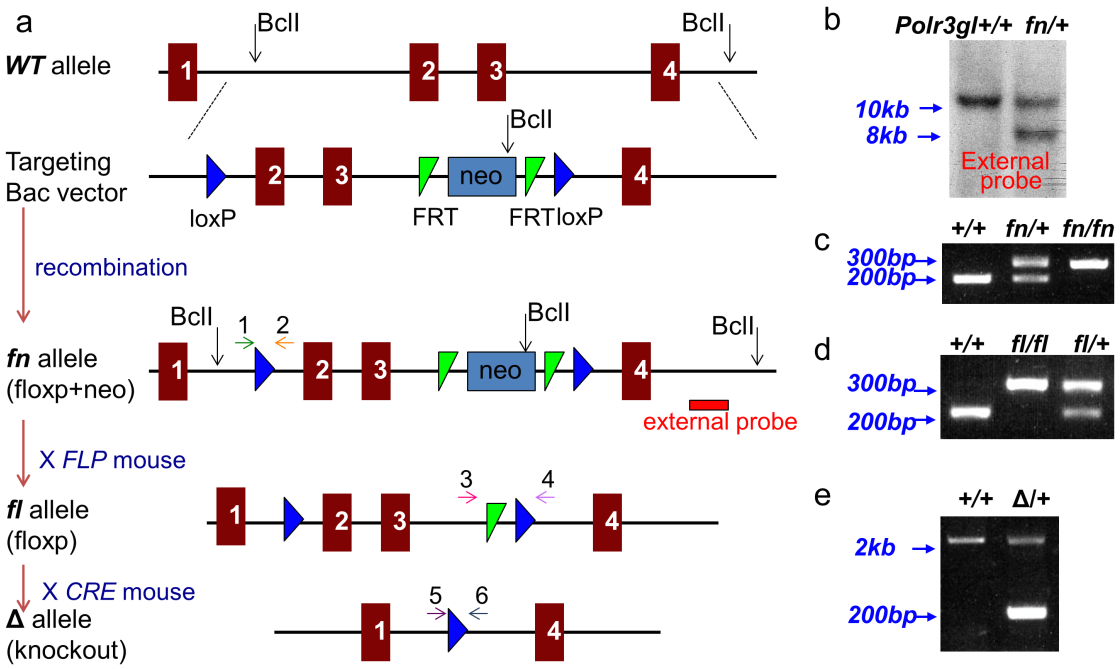

Fig. S4

Fig. S3 and S4. Generation and validation of *Polr3g* (Fig. S3) and *Polr3gl* (Fig. S4) conditional knockout mice.
(A) Diagram of gene targeting and mice crossing procedures. Both targeting vectors were generated by inserting loxP sites (blue triangle) flanking exon(s) and a neomycin cassette (*neo*) flanked by FRT sites (green triangle). Locations of PCR primers are shown by arrows. **(B)** "Knockout-first" alleles (fn alleles) were confirmed by Southern blot analysis using both external and neo probes. **(C)** "Knockout-first" alleles (fn alleles) were also confirmed by PCR genotyping. **(D)** Mice with fn alleles were crossed to transgenic FLP mice to generate conditional knockout (CKO) mice (f allele), which were confirmed by PCR genotyping. Both *Polr3g* and *Polr3gl* fl homozygous mice were viable without any notable defects. **(E)** The knockout potentials of f allele mice were further tested by crossing to transgenic EIIA-CRE mice. After breeding one more generation, it was possible to produce heterozygous Δ alleles. PCR genotyping confirmed the deletion sites.

Supplementary Tables

Table S1. MS analysis showing proteins differentially co-immunoprecipitated with POLR3G versus POLR3GL antibodies.

Table S2. Summary of number of reads and peaks detected in ChIP-seq experiments.

Dataset S1. Lists of peaks detected in Fig. 2C.

Dataset S2. Lists of peaks detected in Fig. 2D.

Dataset S3. Lists of primers

Table S1: Summary of non-Pol III proteins detected in immunoprecipitates of POLR3G or POLR3GL

Detected only in POLR3G pull-down			
Accession	Description	Score	Coverage
191765	alpha-fetoprotein	52.69	3.11
81878656	L-threonine 3-dehydrogenase, mitochondrial precursor	3786.922	63.81
62201487	Glyceraldehyde-3-phosphate dehydrogenase [Mus musculus]	2861.807	35.44
6756039	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, theta polypeptide [Mus musculus]	289.5953	13.47
123228024	*peptidyl prolyl isomerase H [Mus musculus]*	*70.932*	*8.86*
149257773	*PREDICTED: peptidyl-prolyl cis-trans isomerase A-like [Mus musculus]*		
148685494	mCG22383, isoform CRA_b [Mus musculus] (TIM_phosphate_binding)	102.972	10.29
197725012	(ubiquitin) Chain B, Crystal Structure Of Human Amsh-Lp Dub Domain In Complex With Lys63-Linked Ubiquitin Dimer	463.5471	50

Detected only in POLR3GL Pull-down			
Accession	Description	Score	Coverage
29612546	Transcription elongation factor B (SIII), polypeptide 3 [Mus musculus] (Elongin-A)	33.44	1.94
74211667	Hsp73	474.8507	24.59

Table S2: Summary of number of reads and peaks detected in ChIP-seq experiments

[a] peak called by Homer program
[b] identified peaks were examined by IGV visualization to set the thresholds of

ChIP-seq experiment	Total reads	Uniquely aligned reads	Number of peaks Called[a]	Validated[b]	Mapping Efficiency (%)
input_ES	30,275,598	18,465,288			61
3C_ES	19,956,879	12,147,386	1,021	317[c]	61
3G_ES	31,651,234	19,674,803	533	243[d]	62
3GL_ES	35,987,871	21,016,069	195	13[d]	58
Pol II_ES	37,389,466	25,369,634			68
input_pMEF	29,392,063	14,496,555			49
3C_pMEF	29,360,199	18,843,549	397	275[e]	64
3G_pMEF	30,934,711	20,319,371	272	264[d]	66
3GL_pMEF	30,402,871	20,000,609	190	177[d]	66
Pol II_pMEF	34,423,268	24,648,991			72

Normalized Tag Count for each ChIP-seq experiment.
[c] the threshold of Normalized Tag Count is 260
[d] the threshold of Normalized Tag Count is 15
[e] the threshold of Normalized Tag Count is 40