

**Title**

Stochastic promoter activation affects Nanog expression variability in mouse embryonic stem cells

**Authors**

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\* Corresponding author

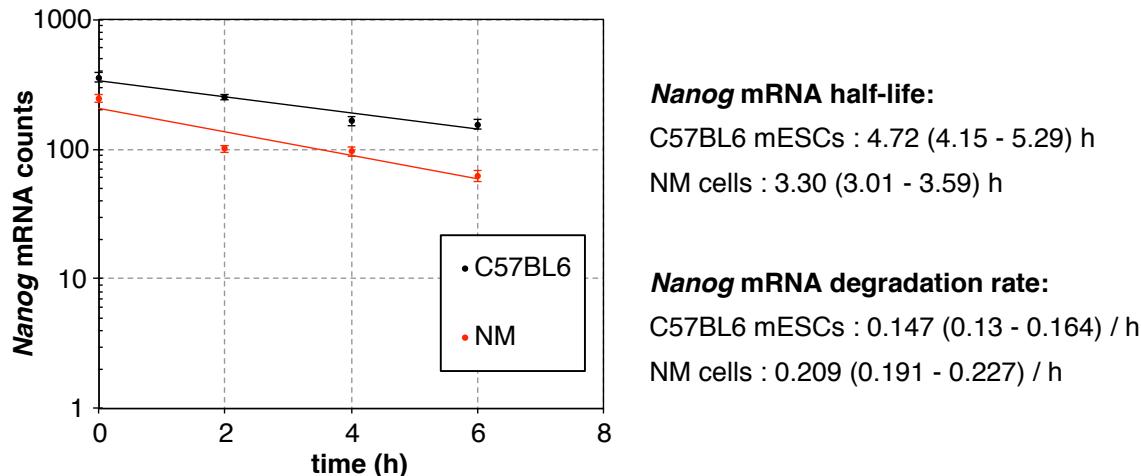
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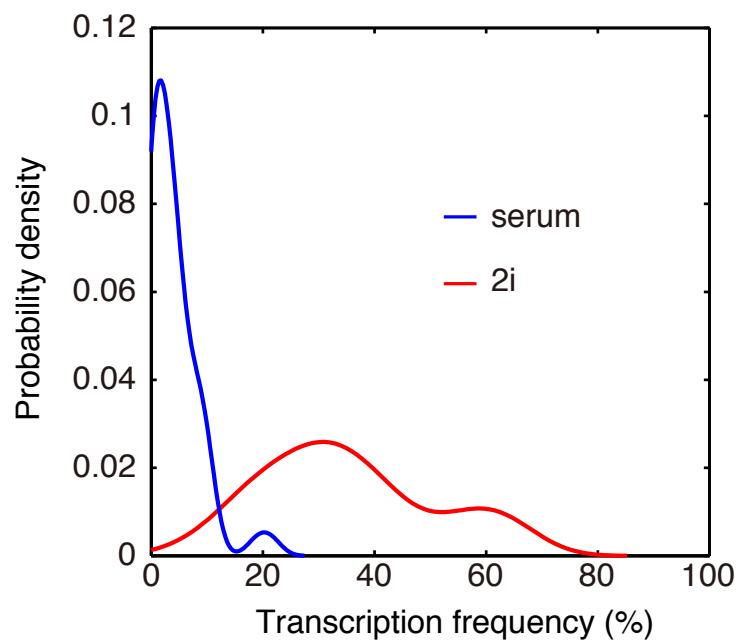
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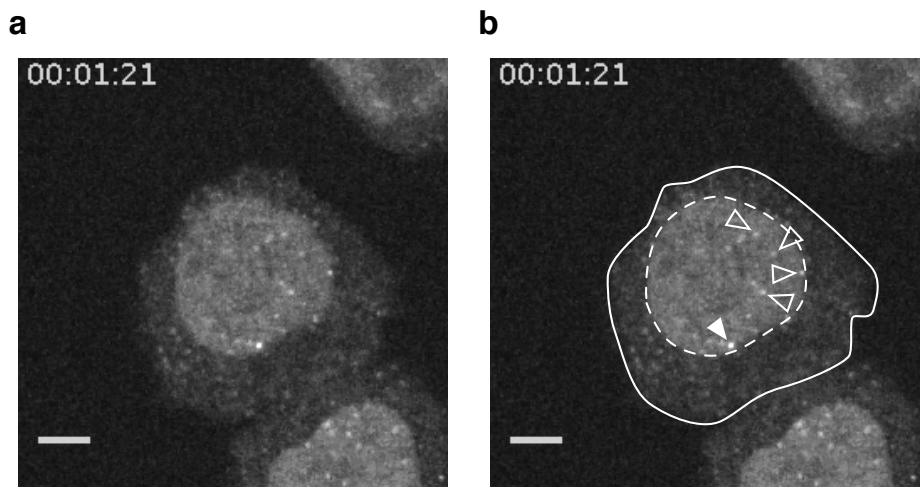
## Supplementary Figures



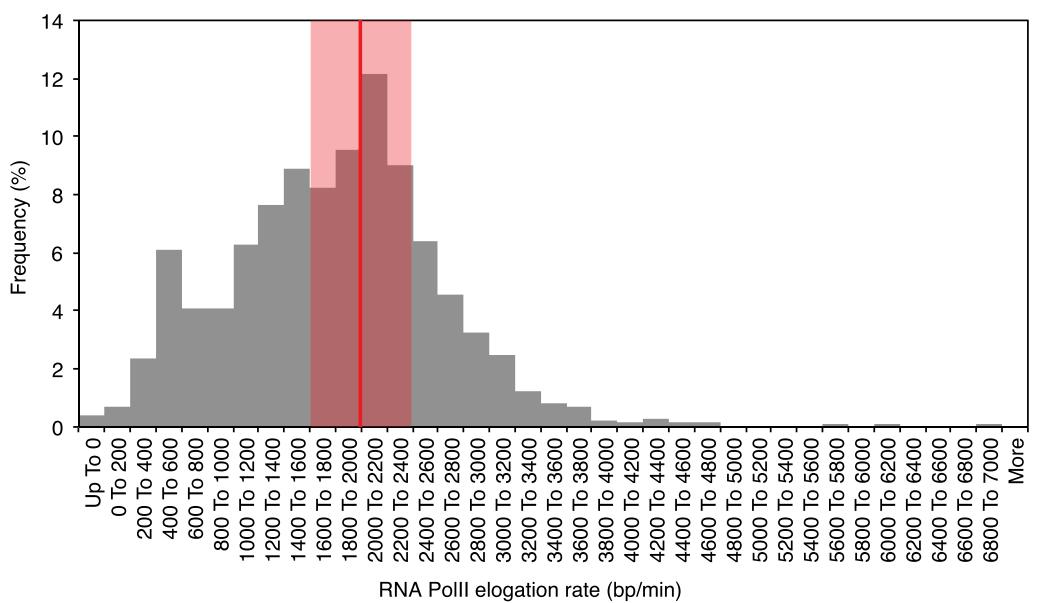
**Supplementary Figure S1, Half-lives of WT and MS2-integrated *Nanog* mRNA.**  
*Nanog* mRNAs in NM and wild-type (WT) C57BL6 mESCs were counted by smFISH using *Nanog* exonic probes over 6 hours after transcription inhibition with actinomycin D (averages and 95% confidence intervals are shown). Half-lives and degradation rates are shown at the right side of the Figure. n > 100 sample points. 95% confidence limits are in parentheses.



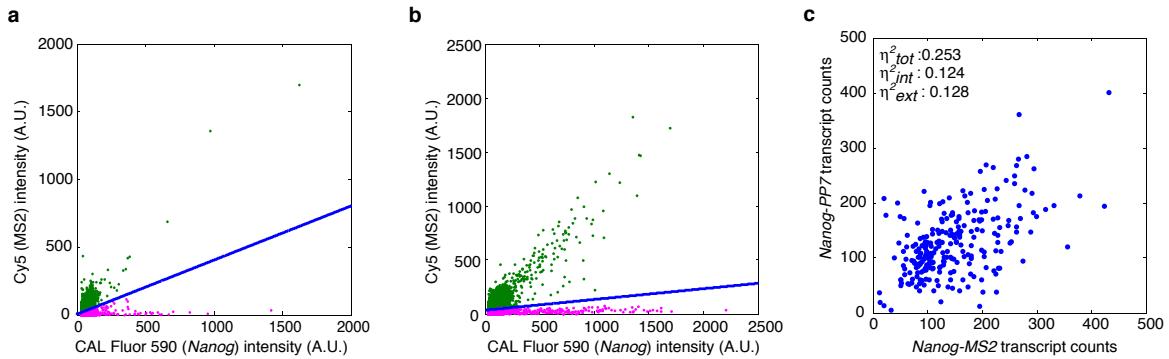
**Supplementary Figure S2, Multidmodal distributions of transcription frequency.**  
Probability density functions of transcription frequency over 4 hours in NM-G cells cultured in serum and 2i conditions (Figure 3c) are shown. Continuous probability density functions were generated using a kernel smoothed density estimate (ksdensity, Matlab).



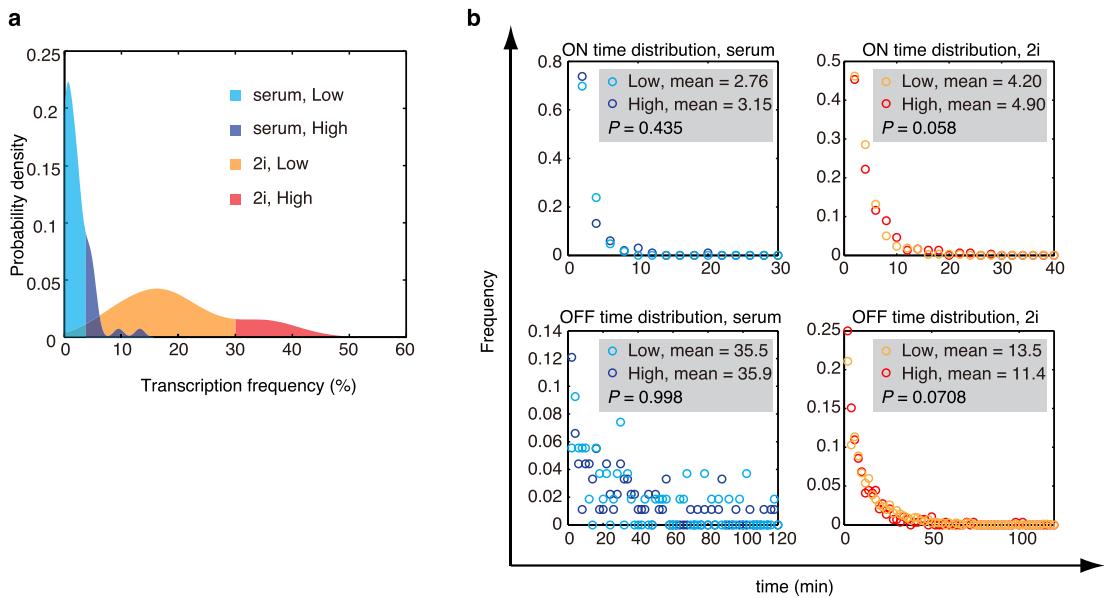
**Supplementary Figure S3, Simultaneous visualization of individual mRNA molecules and transcription site.** (a, b) The images are the same snapshot of live imaging of an NM-G cell with (b) or without (a) annotations (Supplementary Video S3). Solid and dashed lines indicate the edges of cell and nuclear membranes, respectively. Filled and unfilled arrowheads point to a transcription site and individual mRNA spots, respectively. Scale bar, 5  $\mu$ m.



**Supplementary Figure S4, Distribution of RNA Pol II elongation rate.** Histogram shows distribution of RNA Pol II elongation rate in mESCs determined by Jonker et al. (Jonker et al., *eLife*, 2014). Red line and light red rectangle represent the mean (1.99 kb/min) and standard deviation ( $\pm 0.39$  kb/min) of RNA Pol II elongation rate estimated in this study.



**Supplementary Figure S5, Allele-specific single-molecule fluorescence *in situ* hybridization (smFISH) in a reporter mESC line.** (a, b) smFISH was performed in NMP cells cultured in serum (a) or 2i conditions (b) using *Nanog* exonic probes (CAL Fluor 590 conjugated) and MS2 probes (Cy5 conjugated). The fluorescence intensities of CAL Fluor 590 above the threshold and Cy5 of individual mRNA spots were plotted. Among the *Nanog* probe-positive spots, those with an MS2 probe signal intensity above a given threshold (blue line) were assumed to be mRNAs expressed from an MS2-integrated allele (green dots); otherwise, mRNAs were assumed to be expressed from PP7 alleles (magenta dots). The threshold lines were manually determined to equally separate all dots. (c) Scatter plot of *Nanog*-MS2 and -PP7 transcripts determined by smFISH using MS2 and PP7 probes.



**Supplementary Figure S6, ON and OFF time for *Nanog* transcription is not significantly affected by differences in state.** (a) Probability density functions of transcription frequency over 4 hours in NM-G cells cultured in serum and 2i conditions (Figure 3c) are shown (see also Supplementary Figure S2). The functions are divided into two groups with higher or lower transcription frequencies relative to threshold values (3.75% and 30% for serum and 2i conditions, respectively). (b) Distributions of ON and OFF time for *Nanog* transcription in cells with higher or lower transcription frequencies. Mean values are displayed in each graph. Differences between the two groups within the same culture conditions were not significant ( $P > 0.05$ , two-sample Kolmogorov–Smirnov test).

mNanog-TALEN-L

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LGGSRKPDGAIYVGSPIDYGVIDTKAYSGGYNLPIGQADEMERYVEENQTRDKHLNPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHI  
TNCNGAVLSVEELLIGEMIKAGTTLTEEVRRKFNNGEINFRS\*

mNanog-TALEN-R

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TNCNGAVLSVEELLIGEMIKAGTTLTEEVRRKFNNGEINFRS\*

**Supplementary Figure S6, Amino acid sequences of TALENs.**

The amino acid sequences of TALENs (mNanog-TALEN-L and mNanog-TALEN-R) used in this study are shown. Repeat variable diresidues (RVDs) are shown in red.

### Supplementary Fig. S7, Nucleotide sequence of pTV-mNanog-PMS used in this study.

## Restriction map of pTV-mNanog-PMS

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 BspMII  
 MspI  
 BseAI  
 Kpn2I  
 Aor13HI  
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**Supplementary Fig. S7, continued**

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BoxI  
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BstEII  
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Supplementary Fig. S7, continued

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Bss1107I

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HindIII
< M13-rev
< LacO
< lac
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AssI
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**Supplementary Fig. S7, continued**

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↑  
Amp prom  
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**Features :**

T3	: [6355 : 6336 - CCW]
M13-fwd	: [599 : 616 - CW]
M13-rev	: [6393 : 6373 - CCW]
T7	: [626 : 645 - CW]
ColE1 origin	: [7389 : 6761 - CCW]
F1 ori	: [13 : 453 - CW]
LacZ alpha	: [528 : 460 - CCW]
LacO	: [6421 : 6399 - CCW]
PuroR	: [2923 : 3522 - CW]
LoxP	: [1696 : 1729 - CW]
LoxP	: [3755 : 3788 - CW]
Amp prom	: [8460 : 8426 - CCW]
lacZ	: [625 : 6426 - CCW]
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MS2 stem loop	: [3928 : 4029 - CW]
MS2 stem loop	: [4036 : 4137 - CW]
MS2 stem loop	: [4144 : 4245 - CW]
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Z2 peptide	: [2869 : 2922 - CW]
hsvTK	: [1732 : 2868 - CW]
FactorXa site	: [1639 : 1650 - CW]
Nanog-5' homology arm	: [673 : 1641 - CW]
Nanog-3' homology arm	: [5153 : 6305 - CW]

**Supplementary Fig. S8 Nucleotide sequence of pTV-mNanog-HPP used in this study.**

## Restriction map of pTV-mNanog-HPP

Supplementary Fig. S8, continued

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BstAUI  
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>Esp3I  
>BsmBI  
CGTAGAGGAAAATCCTGGTCCATGAAAAAGCTGAACCTACCGCGACGCTGCTGAGAAGTTCTGATGACATGAAAAGTCGAGACGCTCCGACCTGATG < 3000

CAGCTCTCGGAGGGCGAAGAAATCTCGTCTTCAGCTTCAGTGTAGGGGGCTGGATATGCTCTGGGTAATAGCTGCCTGCGATGGTTCTCAAAG < 3100

ATCGTTATTTATCGGCACTTTGATCGGCCGCGCTCCGATTCGGAGTGCTTGACATTGGGAATTCAAGCAGAGCCTGACCTATTGATCTCCG < 3200

AsisI  
RgaI  
Bsp19I  
SgfI  
NcoI  
SfaAI  
CCGTGCACAGGGTGTACGTTGAAAGACCTGCCGTGAAACCGAACCTGCCGCTTCTGAGCCGGTGCAGGGCATGGATGCGATGCTGCCGCG < 3300

CpoI  
CspI  
RsrII  
RsrII  
CTTAGCCGACAGAGCGGGTCCGGCCATTGGACCCGAAAGGATCGGTAACATACACTACATGGCGTGTATTCATATGCGGATITGCTGATCCCACATGTT < 3400

>Hg9R  
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>MaqI  
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<AjuI  
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CGACGCAATCGTCCGATCCGGAGCCGGACTGTCGGCGTACACAAATGCCGCGAGAGCGCGGGCTGACGGCTGTGATGAGTACTCGCC < 3900

>LoxP  
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>PP7 stem loop  
GGTTAAACGAATTGCCCTCGCGGATCCTAAGGTACCTAATTGCTAGAAAGGAGCAGACGATATGGCGTCGCTCCCTGAGGTCGACTCTAGAAC < 4100

CAGCAGAGCATATGGGCTCGCTGGCTGCAGTATTCCGGGTTCAATTAGATCTTAAGGTACCTAATTGCTAGAAAGGAGCAGACGATATGGCGTCGCTCC < 4200

>PP7 stem loop  
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>PP7 stem loop  
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>PP7 stem loop  
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>PP7 stem loop  
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>PP7 stem loop  
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>PP7 stem loop  
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>PP7 stem loop  
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>PP7 stem loop  
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>PP7 stem loop  
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CTCTAGAACACAGCAGACGATATGGGCTCGCTGGCTGCAGTATTCCGGGTTCAATTAGATCTTAAGGTACCTAATTGCTAGAAAGGAGCAGACGATATG < 5400

>PP7 stem loop  
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**Supplementary Fig. S8, continued**

TTTCAATTATGTAAGTACACTGTAGCTGTCAGAACCTCCAGAAGGGGGCTCAGAGTCTGTTACGTTGAGCTATGGTTGAGGCCACCATGTTGCTGGGA < 5700

>Alfi

TTTGAACCTCTGACCTTCGGAAGAGCAGCTGGTGCTTATCCACTGAGCCATCTCACAGGCCCTGGTTATTTTTAAATTATTGCTTTTG < 5800

TTATCAAGACAGGGTTCTCGATAGCTAATTGCTTTGAACTAGCTCTGCAGACCAGCCGGCTGAACTCAGAGATCTGCCACTTATCTTGC < 5900

<TstI

AspA2I  
XmaJI  
AvrII  
BlnI

CTCTGAAATGCTGGGACCAAAGGTGGCATACCACACCTGGCATATAATTGTTATTCTATTGGTGCAGAGCAAACCTAGGAC < 6000

>Nanog-3' homology arm

TTAGAACATGCTGGGACCAACTCAACTCTGAGCTCTATTACAACCTGGTGTAGTGTATTGCTTAGTCTGAATTGCTCTTTTTAGTGT < 6100

AACTCTAGGCTTTGGAGACAGTGAGGTGGCATATACTCTCTCCAAAGAATAAGTGTGAAACACCCCTACCCACGCCACCCACCCATGCTAGTCTT < 6200

BssNAI  
Bst1107I  
BstZ17I

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ATGAGACAGAAGGACCAAGGAGTTGAGGGTAGCTCAGATATGCAATAAGTCAGGCCAACCTGTACTATGTTAAATAGTAAGACAGCATCTCGATAAA < 6400

HindIII

ATAATAAAAACCTAAAGTCTCAACAAAATAAAGCTTACCTTAAAGGTGCTTGCTTGGAGTCCCCAAGAGTAAGTGTATGTTAAATATCTGT < 6500

BcUI  
SpeI  
AhiI

AGAAAAGATGTTTATTTGACTGTACCATGTAACCGATGCCAGCTGGACTAGTTAACAAAATTAACACTAATTACCTTAACTGTGTCTTATCTT < 6600

OliI  
AeiI

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<F3  
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BspLU1II  
PciI  
PscI

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ACCCGACAGGACTATAAGATACCAGGCTTCCCCCTGGAGCTCCCTCGTGTGCTCTCTGGACCTGCGCTACCGGATACCTGTCCGCTT < 7300

TCTCCCTTGGGAGCGTGGCTTCTCATAGCTCACGCTGTAGGTATCTCAGTTGGTGTAGGTGTGCTCCAGCTGGCTGTGTGCAAGAACCC < 7400

<ColE1 origin  
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GCTGAAGCCAGTTACCTCGGAAAAAGAGTTGGTAGCTTGTGGCTGGGAAACAAACACCCGCTGGTAGCGGGTGGTTTTGTTGCAAGCAGCAGATT < 7700

ACCGCAGAAAAAGGATCTCAAGAAGATCCTTGATCTTCA CGGGTCTGACGCTCACTGGAACGAAAACACGTTGAGTGGTCTGAGCTTACCA < 7800

GATTATCAAAAGGATCTCACCTAGATCCTTTAAATTAAAGTAACTAAAGTATATGAGTAACTGGTCTGACAGTTACCA < 7900

BmeRI  
Eam1105I  
DriI  
AspEI  
AhdI

ATGCTTAACTAGTGGAGCACCTATCAGCGATCTGCTATTGCTCATCCATAGTGTGCTGACTCCCGTGTAGATAACTACGATACGGGAGGGC < 8000

TTACCATCTGGCCCCAGTGTGCAATGATACCGCGCTGCCAGCTCACGGCTCCAGATTATCAGCAATAAACAGCCAGCGGAAGGGCGAGCGCA < 8100

GAAGTGGCTCTGCAACTTATCCGCTCAGCTTGTAGAGTAACTGGCCAGCTAGAGTAAGTGTGGCCAGTTATGTTGGCAACCGTTGT < 8200

TGCCATTGCTACAGGCATCGTGGTGTACGCTCGTGTGGTACAGCTGGTGTGGCTTACCGTCCAGATCAAGGCAGTTACATGATCCCCATG < 8300

TTGTGCAAAAAGCGGTTAGCTCTCGGCTCCGATCTGTCAGAAGTAAGTGGCCAGTGTATCAGTGTGTTATGGCAGACTGCTGATAATT < 8400

CTCTTACTGTGTCATGCCATCGCAAGATGCTTCTGTAAGTGTGACTGGTAGACTCAACCAAGTCATTGAGAATAGTGTATGGCCAGGAGTGTCTTG < 8500

**Supplementary Fig. S8, continued**

CCCGGCCGTCATAACGGGATAATACCGCGCCACATAGCAGAACCTTAAAGTGCATCATGGAAAACGTTCTCGGGGCAAAACTCTAAGGATCTTA < 8600

CCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTCAGCACCCAACTGATCTTCAGCATCTTACTTCACCAGCCTCTGGGTGAGCAAAACAGGA < 8700

GGCAAAATGCCGAAAAAGGGATAAGGGCAGACGGAAATGGTAATCTCATCTTCCTTTCAATATTATTGAAGCATTATCAGGGTT**ATTG** < 8800

<Amp\_prom

TCTCATGAGCGGATACATATTGAATGTATTAGAAAAAAACAAATAGGGTCCGCGCACATTCCCAGAAAGTGCAC < 8883

**Features :**

T3	: [6712 : 6693 - CCW]
M13-fwd	: [599 : 616 - CW]
M13-rev	: [6750 : 6730 - CCW]
T7	: [626 : 645 - CW]
ColE1 origin	: [7746 : 7118 - CCW]
F1 ori	: [13 : 453 - CW]
LacZ alpha	: [528 : 460 - CCW]
LacO	: [6778 : 6756 - CCW]
LoxP	: [6691 : 1725 - CW]
LoxP	: [336 : 339 - CW]
Amp_prom	: [8025 : 8797 - CW]
lac	: [6812 : 6783 - CCW]
PP7 stem loop	: [4032 : 4146 - CW]
PP7 stem loop	: [4153 : 4267 - CW]
PP7 stem loop	: [4274 : 4388 - CW]
PP7 stem loop	: [4395 : 4509 - CW]
PP7 stem loop	: [4516 : 4630 - CW]
PP7 stem loop	: [4637 : 4751 - CW]
PP7 stem loop	: [4758 : 4872 - CW]
PP7 stem loop	: [4879 : 4993 - CW]
PP7 stem loop	: [5000 : 5114 - CW]
PP7 stem loop	: [5121 : 5235 - CW]
PP7 stem loop	: [5242 : 5356 - CW]
PP7 stem loop	: [5363 : 5477 - CW]
2A peptide	: [1642 : 1695 - CW]
2A peptide	: [2869 : 2922 - CW]
hsVTK	: [1732 : 2868 - CW]
HygR	: [2923 : 3978 - CW]
FactorXa site	: [1639 : 1650 - CW]
Nanog-5' homology arm	: [673 : 1641 - CW]
Nanog-3' homology arm	: [5509 : 6662 - CW]

**Supplementary Table S1, Concentrations of chemical inhibitors.**

Concentrations of inhibitors ( $\mu$ M)		mean # of mRNA	Intrinsic noise	95% CI
	PD0325901			
Serum condition	0	0	35.915	0.606
2i	0.2	0.6	146.620	0.316
	0.5	1.5	217.626	0.218
	0.75	2.25	151.372	0.246
	1	3	238.511	0.128
PD0325901	0.2	0	93.616	0.337
	0.5	0	211.453	0.196
	0.75	0	308.127	0.112
	1	0	319.824	0.126

**Supplementary Table S2, Nucleotide sequences of smFISH probes used in this study.**

<i>Nanog</i> intronic probes		Dye: CAL Fluor Red 590
Probe #	Probe (5'-> 3')	
1	TTACTGGGTTCTTCGGGGAC	
2	TTTTTCTACTCTTACCTA	
3	AGAAGCAATAACCCTTCAGC	
4	CCCGCTTATGTTAATGACTA	
5	GGGTTTCCAGAAGAGTGATA	
6	CAGACTAGAAGGCCAACGTA	
7	TTATATTGCTCCGTCTGTG	
8	TAGGATGTTAGGTCTCCCTG	
9	AAATGGGGTGCTCATTCCTA	
10	CTAACTGTATAACCTCACCA	
11	AAACGGCCATTGGGCAAAT	
12	AATGCTAACTGCTCTGCTG	
13	TAAGTGACATCCATATTCCC	
14	TGAGCTCACAAACCCAGAAC	
15	CTCCAGATGCTAGCTATAAG	
16	AGACAATGAGCTTCAGACCT	

<i>Nanog</i> exonic probes		Dye: CAL Fluor Red 590
Probe #	Probe (5'-> 3')	
1	AGGTTTAGGCAACAACCAA	
2	ATGGCGAGGGAAAGGGATTTC	
3	AACTAGGCCTACTGTGGGGA	
4	TTCCCAGAATTGATGCTTC	
5	AACTGCAGGCATTGATGAGG	
6	AGCAAGAATAGTTCTCGGGGA	
7	AGAGCATCTCAGTAGCAGAC	
8	TTCAGAGGAAGGGCGAGGAG	
9	GAATCAGGGCTGCCTTGAAG	
10	CTTTGTTGGACTGGTAG	
11	TGTCAGCCTCAGGACTTGAG	
12	TGAGAGAACACAGTCCGCAT	
13	CTGTCCTTGAGTGCACACAG	
14	GAGGTACTTCTGCTTCTGAA	
15	GAGAGTTCTGCATCTGCTG	
16	ATAGCTCAGGTTCAGAACATGG	
17	GAAACCAGGTCTAACCTGC	
18	TTGCACTTCATCCTTGGTT	
19	TCTTCAACCACTGGTTTTTC	
20	TTCTGAATCAGACCATTGCT	

21	GATACTCCACTGGTGCTGAG
22	GGGGATAGCTGCAATGGATG
23	ACATGGAAAGGCTTCCAGAT
24	AAGTTGGGTTGGTCCAAGTC
25	AGGTCTGGTTGGTCCAAGTT
26	GAAGTTATGGAGCGGAGCAG
27	CTGTACGTAAGGCTGCAGAA
28	CTGGCAGAGAAGTTTGCTG
29	CTTCAAATTACACCTCCAAA
30	TAAAATGCGCATGGCTTCC
31	ATAATTCCAAGGCTTGTGGG
32	GTAAGTCTCATATTCACCT
33	TGACTTTAACGCCAGATGTT
34	CATGTTCTAACGTCTAGGTT
35	AGCTCAGAAGTTGAGTTGGT
36	ACACTAACACACCAAGTTGT
37	TCTCCAAAGCCTAGAGTTAA
38	GAGAGAGTATATGCACCTCA
39	GGTGTCAAGCACTTATTCT
40	AGACCCACGCTTCTAAGAAA
41	AAACCTCACCCCTCAAAATG
42	CCACCATATCGTTACTTT
43	TTCTGTCTCATCCTCGAGAG
44	TATCTGAGCTACCCCTCAAAC
45	CGAGATGCTGTCTTACTATT
46	GCAAGCACCTTAATAGGTGA
47	ACATAGCAGTTACTCTTGGG
48	ATCGGTTCATCATGGTACAG

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MS2 probes*		Dye: Cy5
Probe #	Probe (5'-> 3')	Probe (5'-> 3')
1	TTTCTTGGCAATAAGTACCGTA	
2	TTTGAAGATTGACCTGGAG	
3	GATGAACCTGGAATACTGGAG	

\*each probe is labeled at both ends

PP7 probes*		Dye: Cy3
Probe #	Probe (5'-> 3')	Probe (5'-> 3')
1	TTCTAGGCAATTAGGTACCTTA	
2	TTTCTAGAGTCGACCTGCAG	
3	AATGAACCCGGGAATACTGCAG	

\*each probe is labeled at both ends

**Supplementary Video S1, Live imaging of NM-G cells cultured in serum condition.**

NM-G cells cultured in serum condition were subjected to live imaging with a spinning-disc confocal microscope at 2-min intervals for 4 h. Z series of 41 focal planes with a step size of 0.25  $\mu$ m were acquired. Maximum-intensity image projections are shown. Scale bar, 10  $\mu$ m.

**Supplementary Video S2, Live imaging of NM-G cells cultured in 2i condition.**

NM-G cells cultured in 2i condition were subjected to live imaging with a spinning-disc confocal microscope at 2-min intervals for 4 h. Z series of 41 focal planes with a step size of 0.25  $\mu$ m were acquired. Maximum-intensity image projections are shown. Scale bar, 10  $\mu$ m.

**Supplementary Video S3, Simultaneous visualization of individual mRNA molecules and transcription site in the NM-G cell line.** NM-G cells cultured in 2i conditions were subjected to live imaging with a spinning-disc confocal microscope using a 100 $\times$  objective. Z series of 3 focal planes with a step size of 0.15  $\mu$ m were continuously acquired at minimum intervals with 30-ms exposure time for about 2.5 s. The approximated collection time is indicated. Maximum-intensity image projections are shown. Scale bar, 5  $\mu$ m.