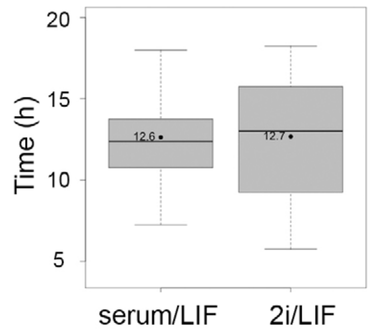


Figure S1. Time-lapse imaging of Nd cells analysis. (A) Box plots comparing the cell cycle time of Nd mESCs grown in serum/LIF (*n*=37 cells) or 2i/LIF (*n*=49 cells) culture conditions. No significant differences are observed for the average time of a single cell cycle between the two different conditions. The medians are shown as solid black lines within the box and mean values as full black circles. The edges of the box indicate the 25th and 75th percentiles and the whiskers indicate the range of non-outliers data points.

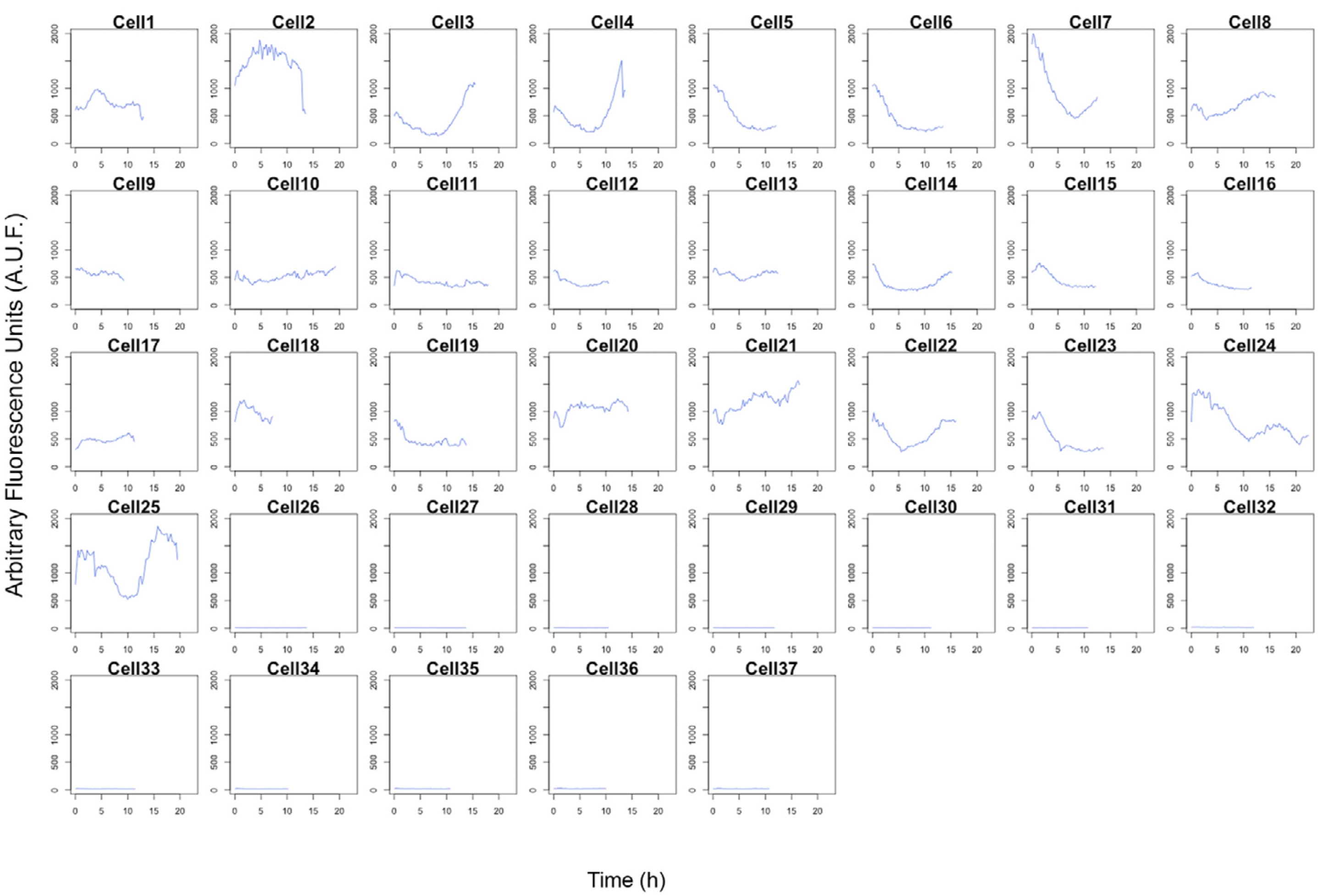


Figure S2. Individual graphs for time-lapse imaging of Nd cells grown in serum/LIF. Individual plots of long-term kinetics of Nanog:VNP expression of 37 cells grown in serum/LIF, with fluorescence intensity plotted for individual cells against time during the interphase of a single cell cycle. Cells were imaged every 15min.

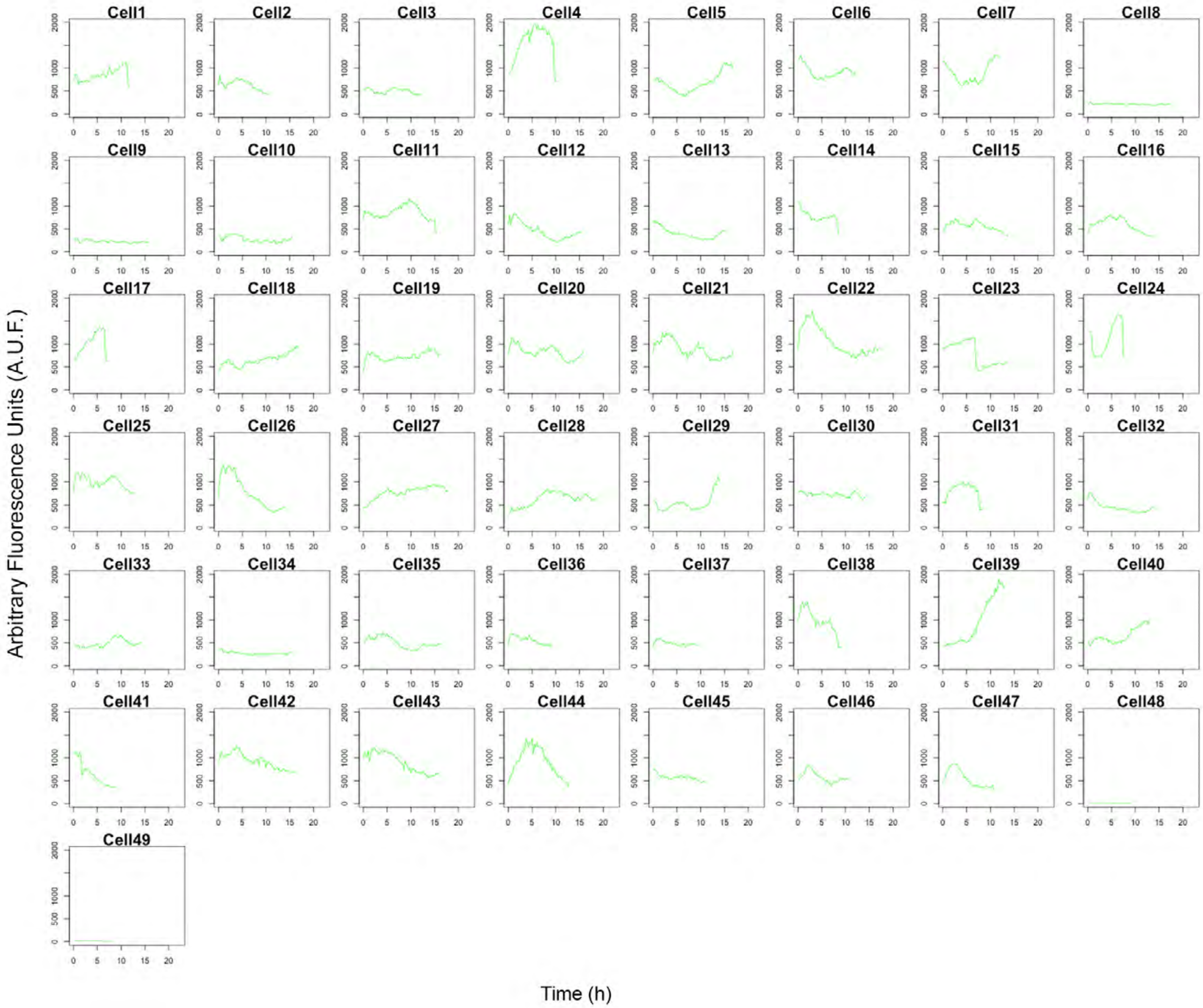


Figure S3. Individual graphs for time-lapse imaging of Nd cells grown in 2i/LIF. Individual plots of long-term kinetics of Nanog:VNP expression of 49 cells grown in 2i/LIF, with fluorescence intensity plotted for individual cells against time during the interphase of a single cell cycle. Cells were imaged every 15min.

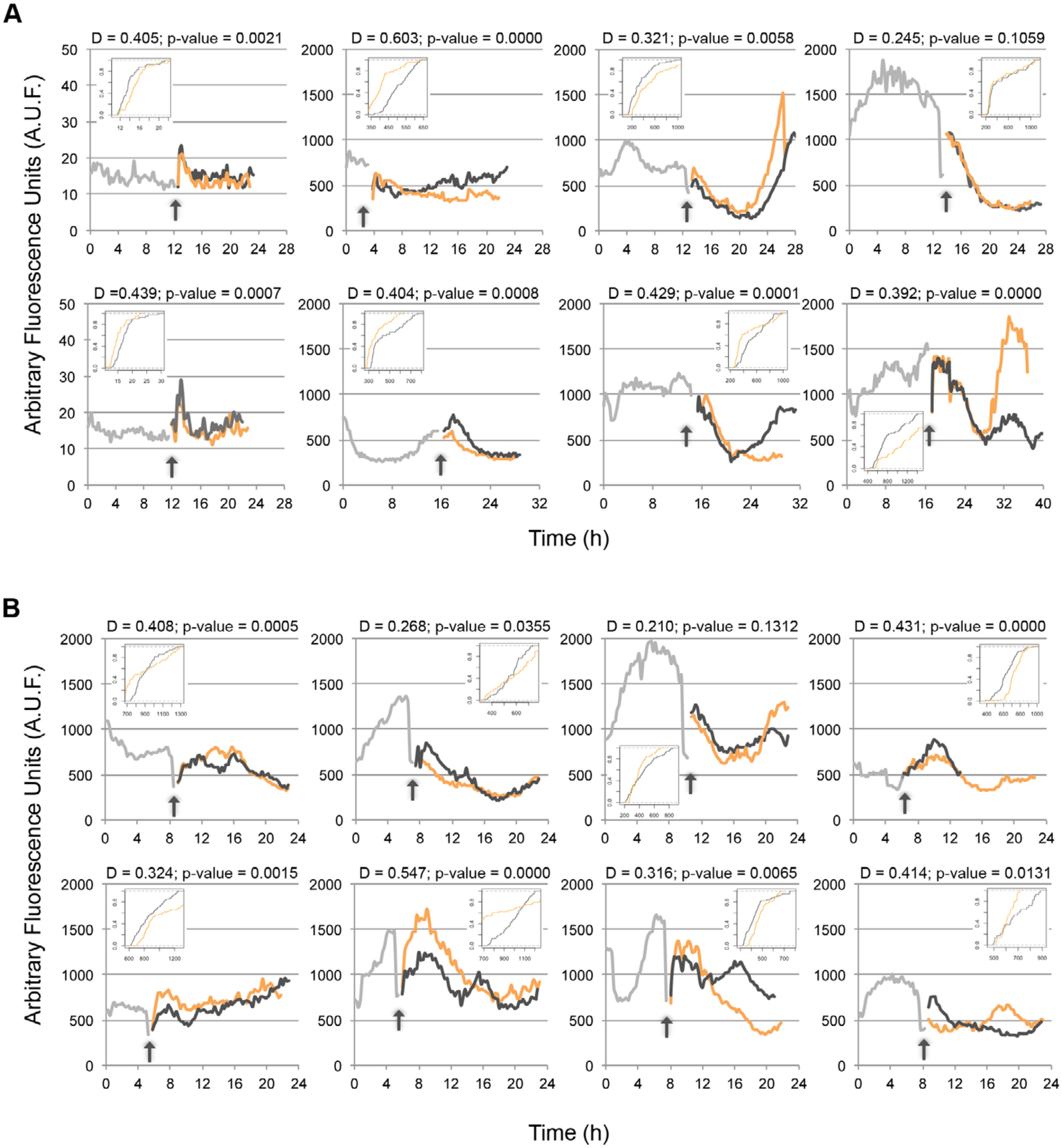
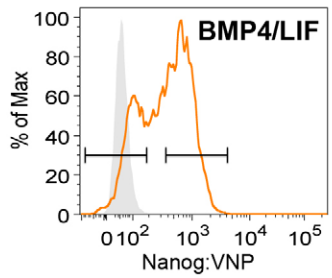


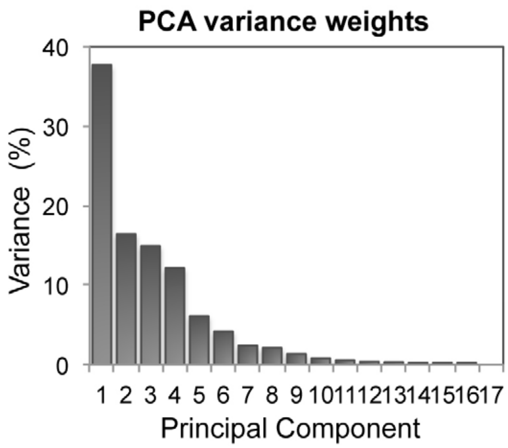
Figure S4. Time-lapse imaging of Nd cells progeny. (A) Kinetics of Nanog:VNP expression of 8 mother cells and respective daughter cells, grown in serum/LIF conditions, with fluorescence intensity plotted for individual cells against time. Arrows indicate division time. Cells were imaged every 15min. Inset plots show the empirical cumulative distribution functions for each sister cell. The Kolmogorov-Smirnov (K-S) test was used to compare the behaviour of sister cells and the obtained results (D and p-value) are depicted in the top of each graph. For the 8 pairs of sister cells analysed, the K-S test only found no significant differences in one pair of cells (p=0.1059), suggesting that similarities between sister cells are reduced. (B) Same as (A) for cells grown in 2i/LIF conditions. Similarly to serum/LIF conditions, only one pair of sisters cells showed p-values higher than 0.05, suggesting differences in Nanog:VNP expression between sister cells even in "ground state" conditions . Overall, the observed data suggest that dissimilarities between sister cells exist in the pluripotent state, regardless of the culture environment.

Figure S5. Colony types observed in clonal assays (undifferentiated, mixed and differentiated colony types).

A

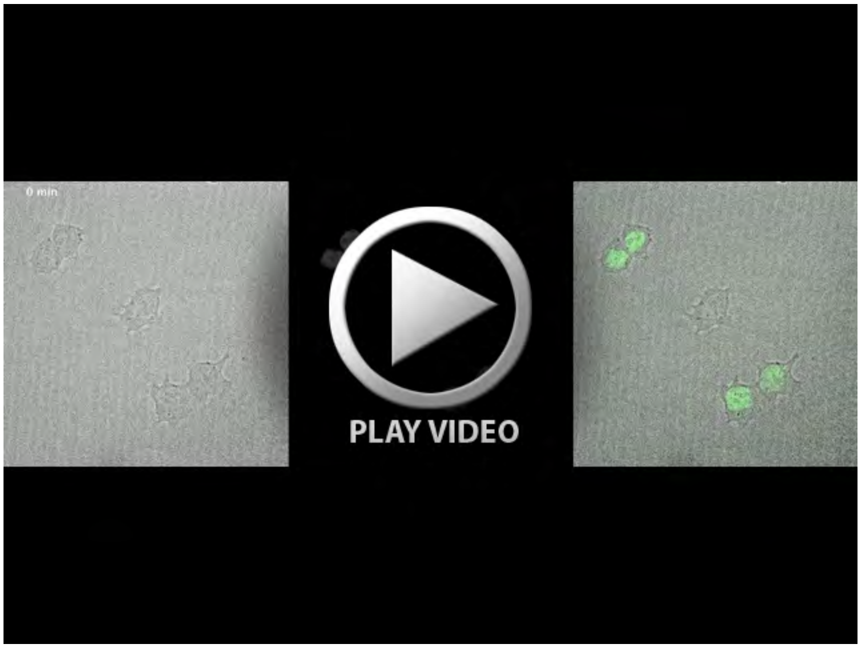


B

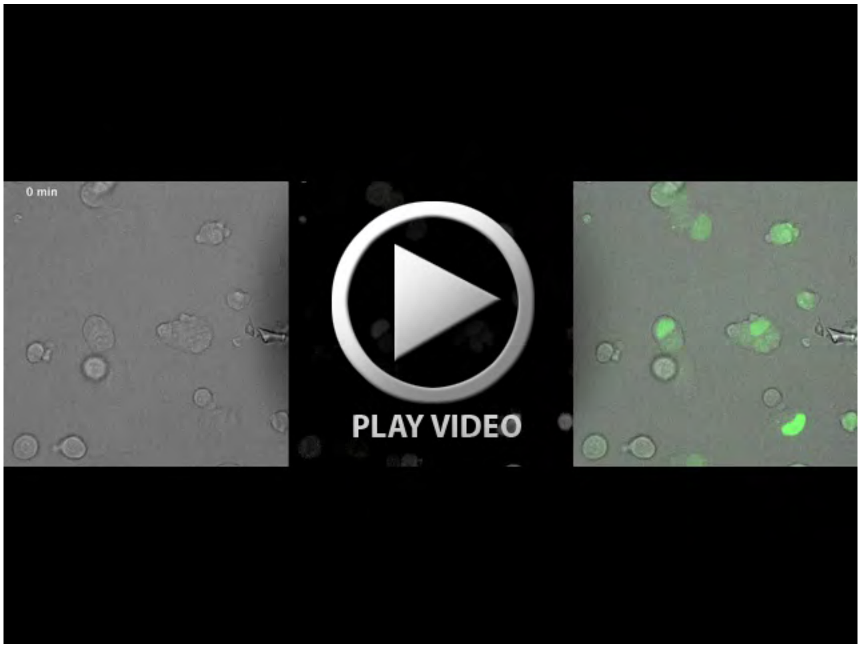


Principal Component	Eigenvalues	Variance (%)	Cumulative Variance (%)
1	**11.68**	**37.69**	**37.69**
2	**5.09**	**16.41**	**54.10**
3	4.62	14.92	69.02
4	3.76	12.12	81.15
5	1.90	6.13	87.28
6	1.30	4.20	91.48
7	0.75	2.42	93.90
8	0.66	2.14	96.04
9	0.43	1.37	97.41
10	0.26	0.83	98.25
11	0.18	0.58	98.83
12	0.12	0.39	99.22
13	0.11	0.34	99.57
14	0.07	0.23	99.79
15	0.04	0.14	99.93
16	0.02	0.07	100.00
17	8.68E-31	0.00	100.00

Figure S6. (A) Representative histogram of FACS-sorted Nd subpopulations grown in BMP4/LIF. VNP-low (VNP_L) and VNP-high (VNP_H) subpopulations were collected for posterior analysis, as well as non-sorted bulk populations (All). (B) Plot representing the variances associated with each principal component. Corresponding eigenvalues and variances are indicated on the table. The first two principal components explain 54.1% of the observed differences.



Movie 1: Fluctuations in Nanog:VNP expression in mESCs cultured in serum/LIF. mESCs were imaged on an Andor spinning disk confocal microscope with the time interval between each acquisition set to 15 min. The duration of acquisition ranged from 18 to 39h.



Movie 2: Fluctuations in Nanog:VNP expression in mESCs cultured in 2i/LIF. mESCs were imaged on a Andor spinning disk confocal microscope with the time interval between each acquisition set to 15 min. The duration of acquisition ranged from 18 to 39h.

Table S1. *Nanog* oligos used for FISH.

Sequence name	Sequence (5' to 3')
nanog_1	aaatcagcctatctgaaggc
nanog_2	cagaaagagcaagacaccaa
nanog_3	gaagtcagaaggaagtgagc
nanog_4	actcagtgtctagaaggaaa
nanog_5	ggttttaggcaacaaccaaa
nanog_6	cgagggaagggatttctgaa
nanog_7	cacactcatgtcagtgtgat
nanog_8	cagaactaggcaaactgtgg
nanog_9	ttcccagaattcgatgcttc
nanog_10	aaaaactgcaggcattgatg
nanog_11	agcaagaatagttctcggga
nanog_12	cagagcatctcagtagcaga
nanog_13	gaagaggcaggtcttcagag
nanog_14	tgggactggtagaagaatca
nanog_15	tcaggacttgagagcttttg
nanog_16	cttgttctcctcctcctcag
nanog_17	gagaacacagtccgcatctt
nanog_18	ctgtccttgagtgcacacag
nanog_19	tgaggtacttctgcttctga
nanog_20	gagagttcttgcatctgctg
nanog_21	atagctcaggttcagaatgg
nanog_22	gaaaccaggtcttaacctgc
nanog_23	ttgcacttcatcctttggtt
nanog_24	tcaaccactggtttttctgc
nanog_25	ttctgaatcagaccattgct
nanog_26	gatactccactggtgctgag
nanog_27	ggatagctgcaatggatgct
nanog_28	cagatgcgttcaccagatag
nanog_29	aagttgggttggtccaagtc
nanog_30	gtctggttgttccaagttgg
nanog_31	aaagtcctccccgaagttat

Table S2. DCt values describing gene expression in 17 cell samples: Diff (n=3); VNP_L and VNP_H from serum/LIF (SL) cultures (n=3); VNP_L and VNP_H from 2i/LIF (2iL) cultures (n=2); and VNP_L and VNP_H from BMP4/LIF (BL) cultures (n=2).

Name	Diff #1	Diff #2	Diff #3	$VNP_{H,}$ 2iL #1	$VNP_{H,}$ 2iL #2	$VNP_{H,}$ BL #1	$VNP_{H,}$ BL #2	$VNP_{H,}$ SL #1	$VNP_{H,}$ SL #2	$VNP_{H,}$ SL #3	$VNP_{L,}$ 2iL #1	$VNP_{L,}$ 2iL #2	$VNP_{L,}$ BL #1	$VNP_{L,}$ BL #2	$VNP_{L,}$ SL #1	$VNP_{L,}$ SL #2	$VNP_{L,}$ SL #3
actb	1.3	1.4	1.2	1.3	1.5	1.1	1.2	1.3	1.3	1.3	1.4	1.3	1	0.6	1.2	1.1	0.9
gapdh	-1.3	-1.4	-1.2	-1.3	-1.5	-1.1	-1.2	-1.3	-1.3	-1.3	-1.4	-1.3	-1	-0.6	-1.2	-1.1	-0.9
cmyc	5.2	5.7	6.9	10.1	9.6	6.8	7.3	6.9	6.8	7.3	9.9	9.4	6.4	6.4	5.5	6	6.2
stat3	9	9.3	9.1	7.9	7.1	6.9	6.8	6.7	7.5	7.9	8.1	7.5	6.7	7.2	7.4	7.2	8.7
sox13	7.2	7.4	6.9	6.8	5.6	6.6	6.7	6.4	6.2	6.7	7.2	6.5	5.8	7	6.4	6.6	7
sall4	3.6	3.9	4	4.4	3.9	3.8	3.9	3.1	3	3.8	4.7	4.5	3.5	4.4	4	4.1	4.6
pou5f1	1.5	1.6	1.6	1.4	0.8	1.4	1.1	1.1	1	1.3	1.9	1.7	1.2	3.5	1.5	1.6	2
lin28	3.6	3.6	4.5	6.9	7.1	5.4	5.1	3.9	6.1	5	6.9	7.9	5.2	5.1	3.7	3.7	5.1
klf4	7.7	7.2	5.4	3.5	2.4	2.7	2	2.6	3.1	3.5	3.4	3.1	4	4.7	4.5	5.2	5.9
esrrb	7	7.6	4.6	2.3	1.4	2.7	2.1	1.7	1.6	2.7	2.8	2.4	4.2	5.4	4.3	4.7	6.3
zfp42	11.1	11.2	12.1	7.1	5.7	9.5	7.5	5.4	5	7.5	8.4	7.3	9.3	10.4	9	9.7	12.4
nanog	7.2	7.7	7.6	5.1	4.3	5.6	5.4	4.5	4.5	6.3	6.1	5.3	7.4	8.6	7.9	7.8	9.8
pecam1	7.2	7.4	5.1	3.7	2.6	4.9	4.6	3.7	3.8	4.5	3.9	3.7	5.6	7.6	5.7	6.2	6.8
fgf4	10.6	10.1	10.2	8.5	8.8	9.5	9.4	7.5	7.7	10.1	9.6	9	10.1	12.7	10	10	12.1
rbl2	10.6	10.9	10.7	9.3	7.9	9.3	8.7	8.3	8.4	10	9.7	8.8	13.4	10.6	9.3	9.3	11.4
tcfap2a	9.2	10.1	18.1	14.9	14.3	12.6	12	15.8	8.7	18.1	13.7	13.3	12.3	12.2	12.7	14.9	15.3
cbx8	9.9	11	12.5	11.4	10	13.3	13	12.5	13.8	14.3	10.8	9.5	11.6	11.2	11.6	11.7	13.1
gata3	8.2	9.7	15.3	17.4	16	15.2	14.4	11.2	11.2	16.7	16.7	15.7	12.4	11.1	10.1	10.1	14.9
tead4	10.4	11.1	10.4	7.2	7	8.2	7.3	8	7.5	8.9	7.3	7.6	8.7	8.1	9.5	9.7	10.3
gata4	16.2	14.9	16.7	16.5	16.1	17.1	18.4	16.9	16.9	16.8	16	15.9	15.3	12.4	15.9	16.4	17.2
gata6	14.5	14.3	15.8	16.6	14.9	18.4	18.4	13.4	15	16.7	14.8	15.3	14.4	11.1	12.7	13.8	14.7
pdgfra	13	12.5	14	13.2	11.3	14.3	13.4	14.5	12.5	15.2	8.2	8.7	10.7	7.2	12.4	12.9	14.4
fgfr2	9.7	10	10	12.1	13	13.8	12.8	8.4	8.3	10.1	11.8	12.1	13.2	13.2	9.2	8.7	10.9
creb3l2	7.5	8	11.3	7.8	9.9	9.2	8.4	3.8	3.7	10.6	7.5	7.6	10.5	8.6	6.7	6.9	11.3
brachyury	8.1	8.7	11	10.2	9.7	9	7.5	11.9	11.2	13.1	9	9.9	6.2	5.5	10.5	10.7	11.7
lefty1	3.6	4.2	3.9	3.6	3.5	6	5.8	4.8	5.6	5.1	3.9	4	6.2	5.2	3.8	3.7	4
msx1	12.2	12.3	19.3	20.8	19.1	20.3	18.9	16.9	16.9	19.9	18.1	18.4	13.7	11.1	17	16.4	20
tbx6	12.9	12.6	13.2	14	12.9	13.8	12.6	12.6	9.7	13.9	12.6	13	12.5	11.1	11.5	11.6	13.3
nestin	6	6.5	6.8	8.1	7.3	9.3	8.6	8.7	8.8	9	7.4	7.1	6.7	5.8	4.8	5.1	5.5
pax3	16.2	13.5	16.3	15.8	13.8	20.3	17.4	16.9	16.9	19.9	11	11.2	12.8	12	13.1	13	14.6
crabp2	9.2	9.2	10.3	11.8	12.3	13.6	11.8	11.5	11.7	12.2	10.4	10.4	10.4	9.9	10.6	10	11.1