FIGURE 1A

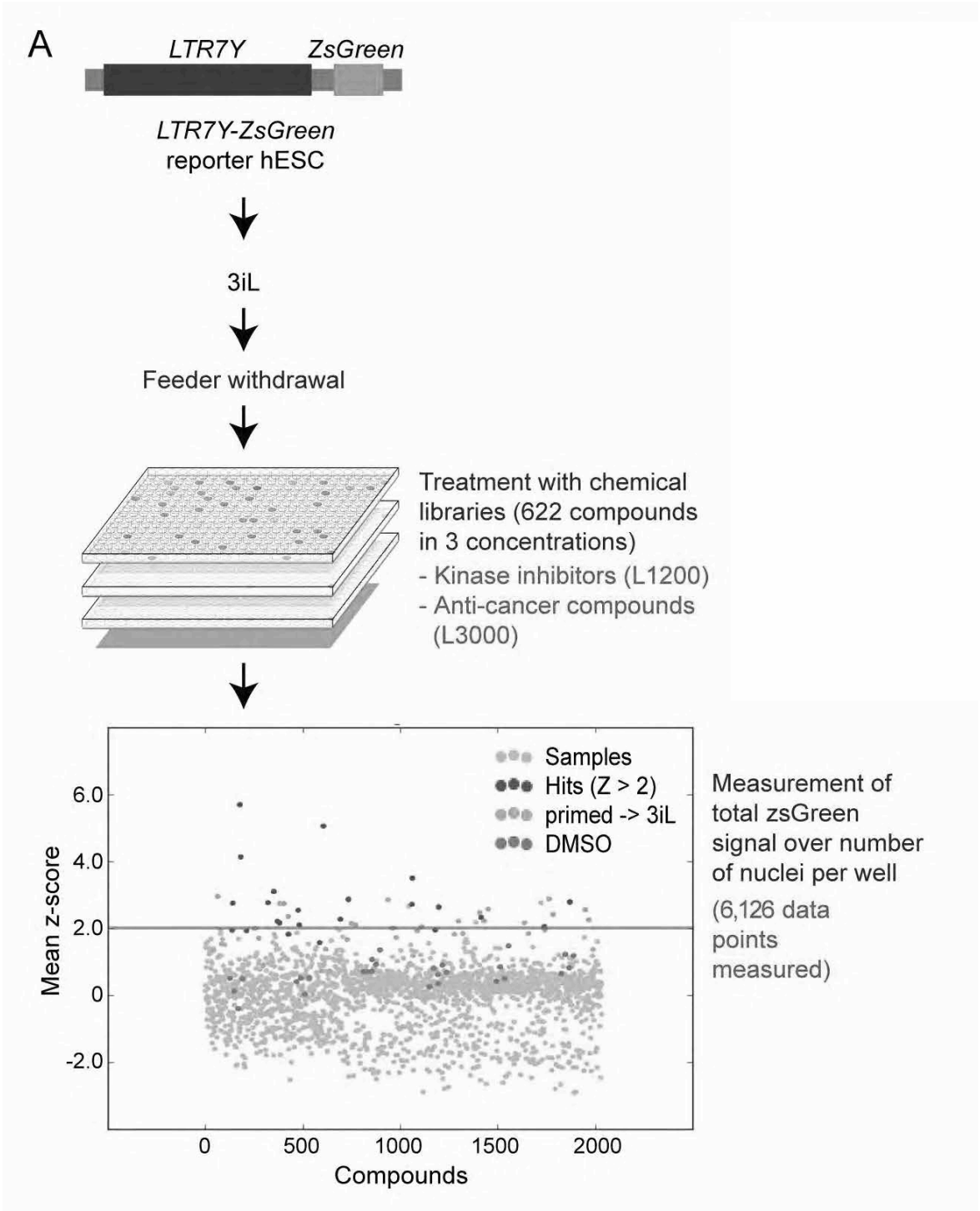

A
LTR7Y
ZsGreen
LTR7Y-ZsGreen
reporter hESC
3iL
Feeder withdrawal
Treatment with chemical libraries (622 compounds in 3 concentrations)
- Kinase inhibitors (L1200)
- Anti-cancer compounds (L3000)
Samples
Hits (Z > 2)
primed -> 3iL
DMSO
Mean z-score
6.0
4.0
2.0
0
-2.0
0
500
1000
1500
2000
Compounds
Measurement of total zsGreen signal over number of nuclei per well
(6,126 data points measured)

FIGURE 1B

Chemical name	Library	Conc (μM)	LTR7Y-zsG z-score	Pathway
Dasatinib (BMS-354825)*	L3000	1.0	2.86	Bcr-Abl, Src inh; Tyrosine kinase inh (e.g. EGFR, EphR)
	L1200	1.0	2.64	
	L3000	0.1	2.79	
	L1200	0.1	2.32	
CHIR-98014	L1200	1.0	3.50	GSK-3α/β inh
	L1200	0.1	2.05	
Saracatinib (AZD0530)	L1200	10.0	1.82	Bcr-Abl, Src inh
	L3000	1.0	2.27	
CHIR-99021 (CT99021) HCl	L3000	10.0	2.77	GSK-3α/β inh
Crenolanib (CP-868596)	L1200	1.0	2.72	Flt3, PDGFRα/β inh
Tandutinib (MLN518)	L1200	10.0	2.54	Flt3, PDGFR, Kit inh
Phenformin HCl*	L1200	10.0	2.21	AMPK activator
PF-04217903*	L1200	10.0	2.10	c-Met inh
AZD5438*	L1200	1.0	1.95	CDK1/2/9 inh
Enzastaurin (LY317615)	L3000	10.0	1.94	PKC inh
Vismodegib (GDC-0449)*	L3000	10.0	1.93	Smo inh

FIGURE 1C

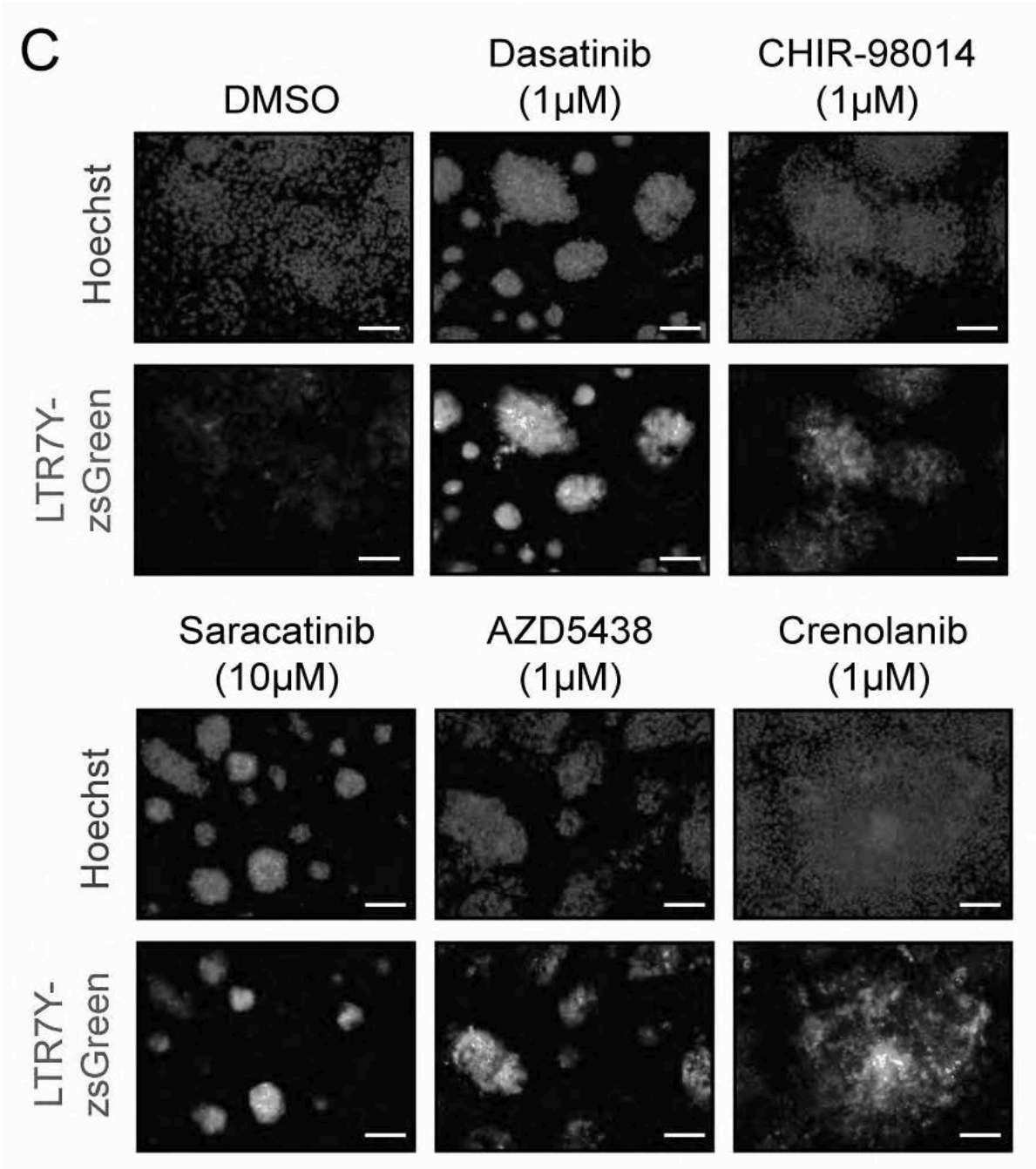

C
DMSO
Dasatinib (1μM)
CHIR-98014 (1μM)
Hoechst
LTR7Y-zsGreen
Saracatinib (10μM)
AZD5438 (1μM)
Crenolanib (1μM)
Hoechst
LTR7Y-zsGreen

FIGURE 1D

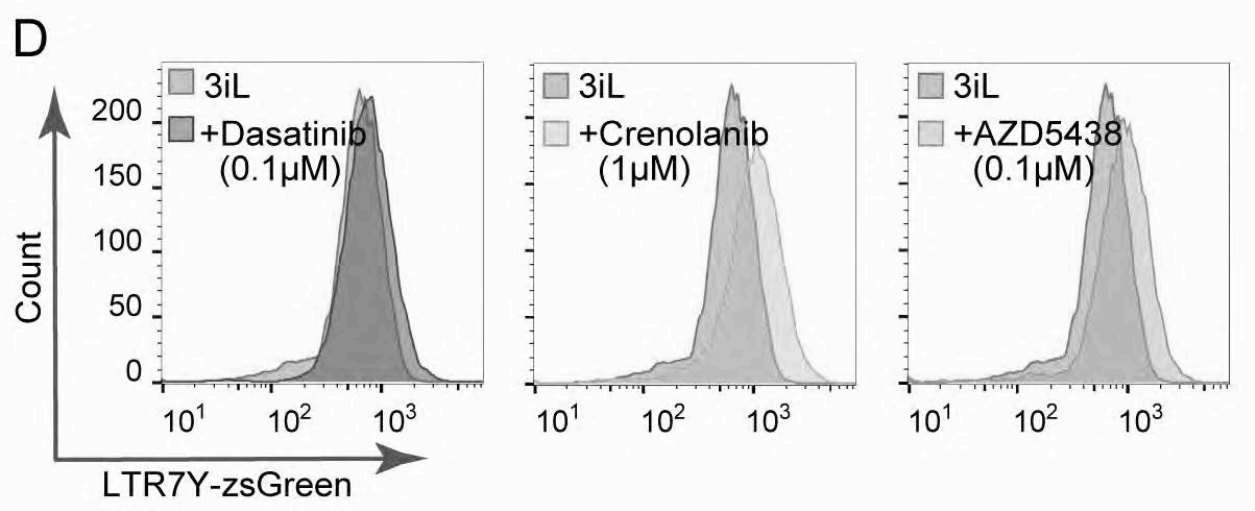

D
3iL
+Dasatinib
(0.1μM)
3iL
+Crenolanib
(1μM)
3iL
+AZD5438
(0.1μM)
Count
200
150
100
50
0
10^1
10^2
10^3
LTR7Y-zsGreen

FIGURE 2A

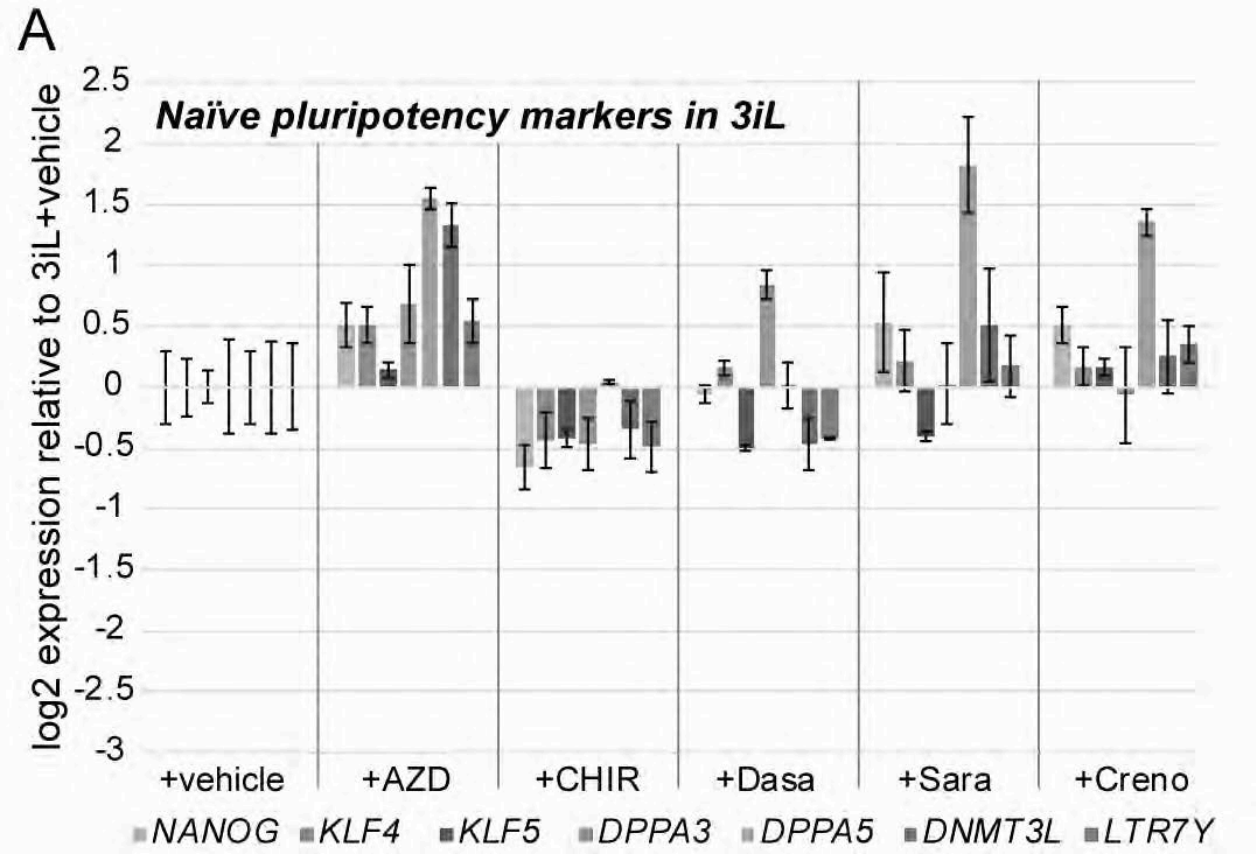

A
Naïve pluripotency markers in 3iL
log2 expression relative to 3iL+vehicle
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3
+vehicle
+AZD
+CHIR
+Dasa
+Sara
+Creno
NANOG
KLF4
KLF5
DPPA3
DPPA5
DNMT3L
LTR7Y

FIGURE 2B

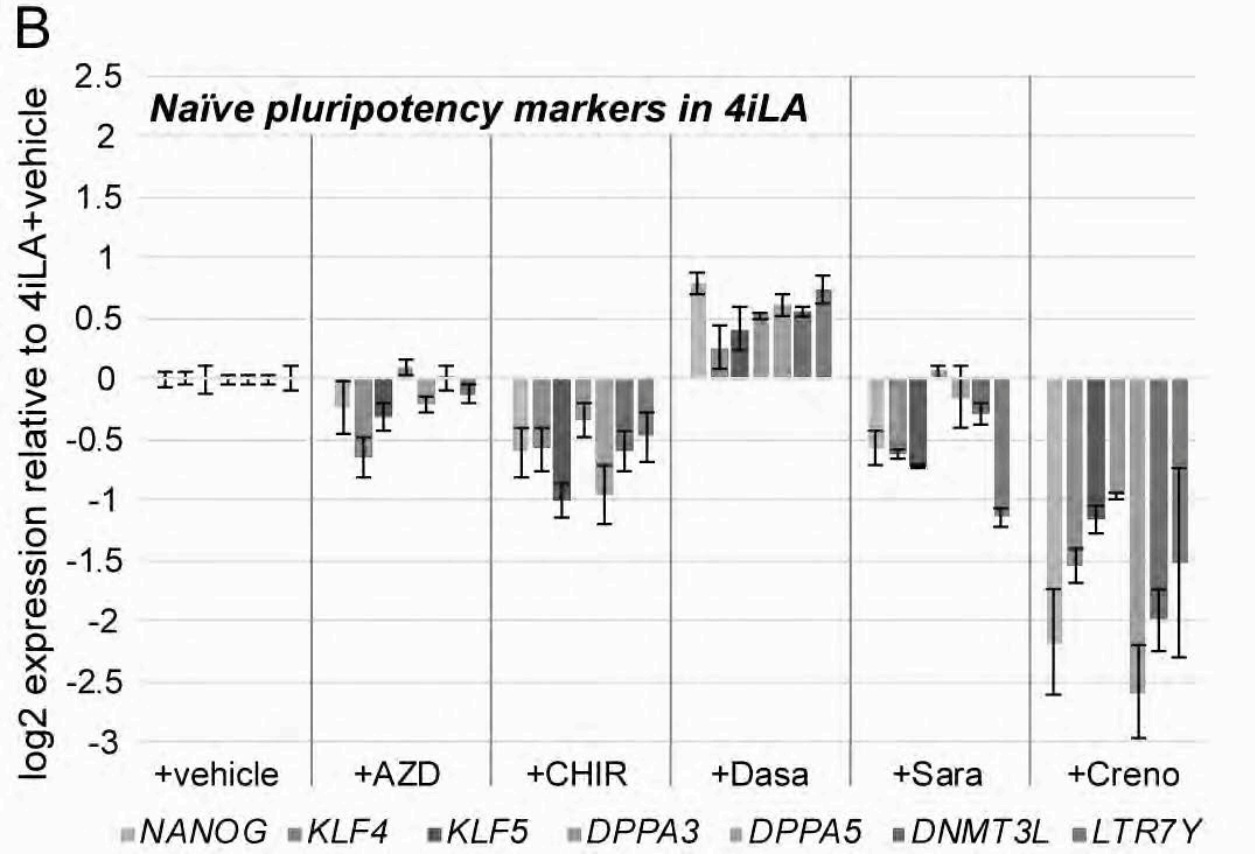

B
Naïve pluripotency markers in 4iLA
log2 expression relative to 4iLA+vehicle
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3
+vehicle
+AZD
+CHIR
+Dasa
+Sara
+Creno
NANOG
KLF4
KLF5
DPPA3
DPPA5
DNMT3L
LTR7Y

FIGURE 2C

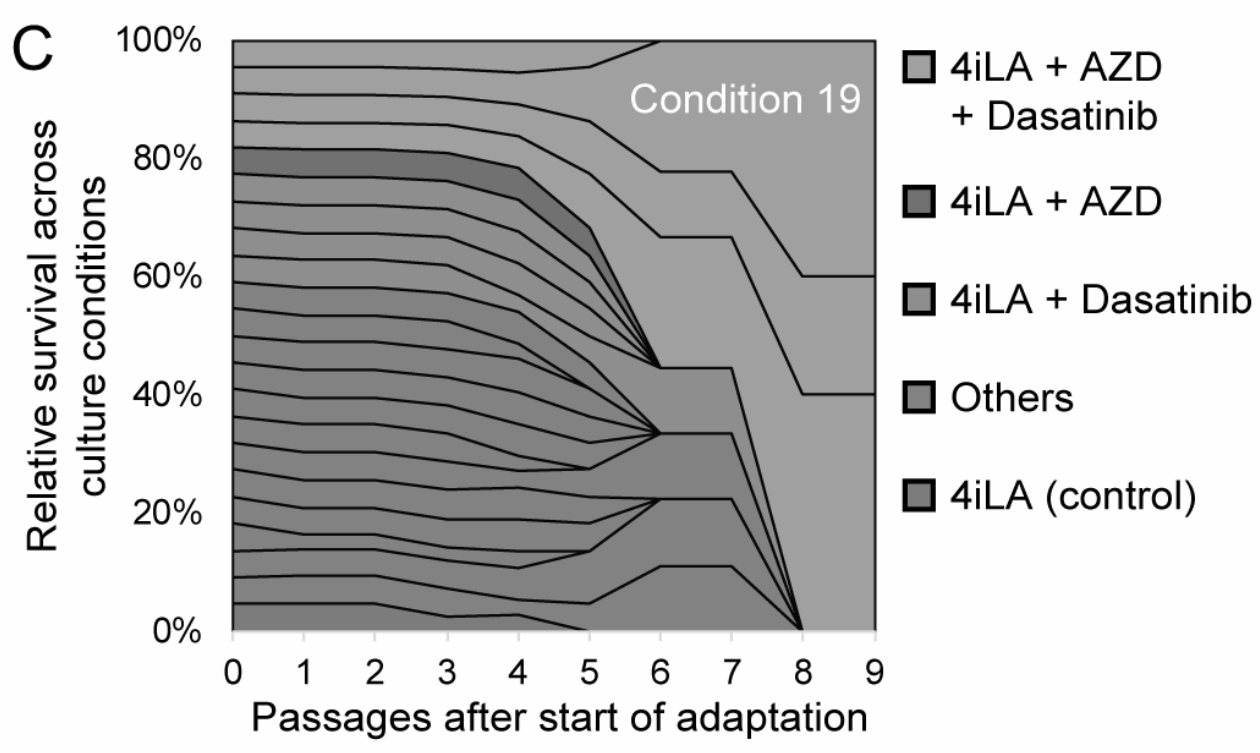

C
100%
80%
60%
40%
20%
0%
Relative survival across culture conditions
Condition 19
0 1 2 3 4 5 6 7 8 9
Passages after start of adaptation
4iLA + AZD + Dasatinib
4iLA + AZD
4iLA + Dasatinib
Others
4iLA (control)

FIGURE 2D

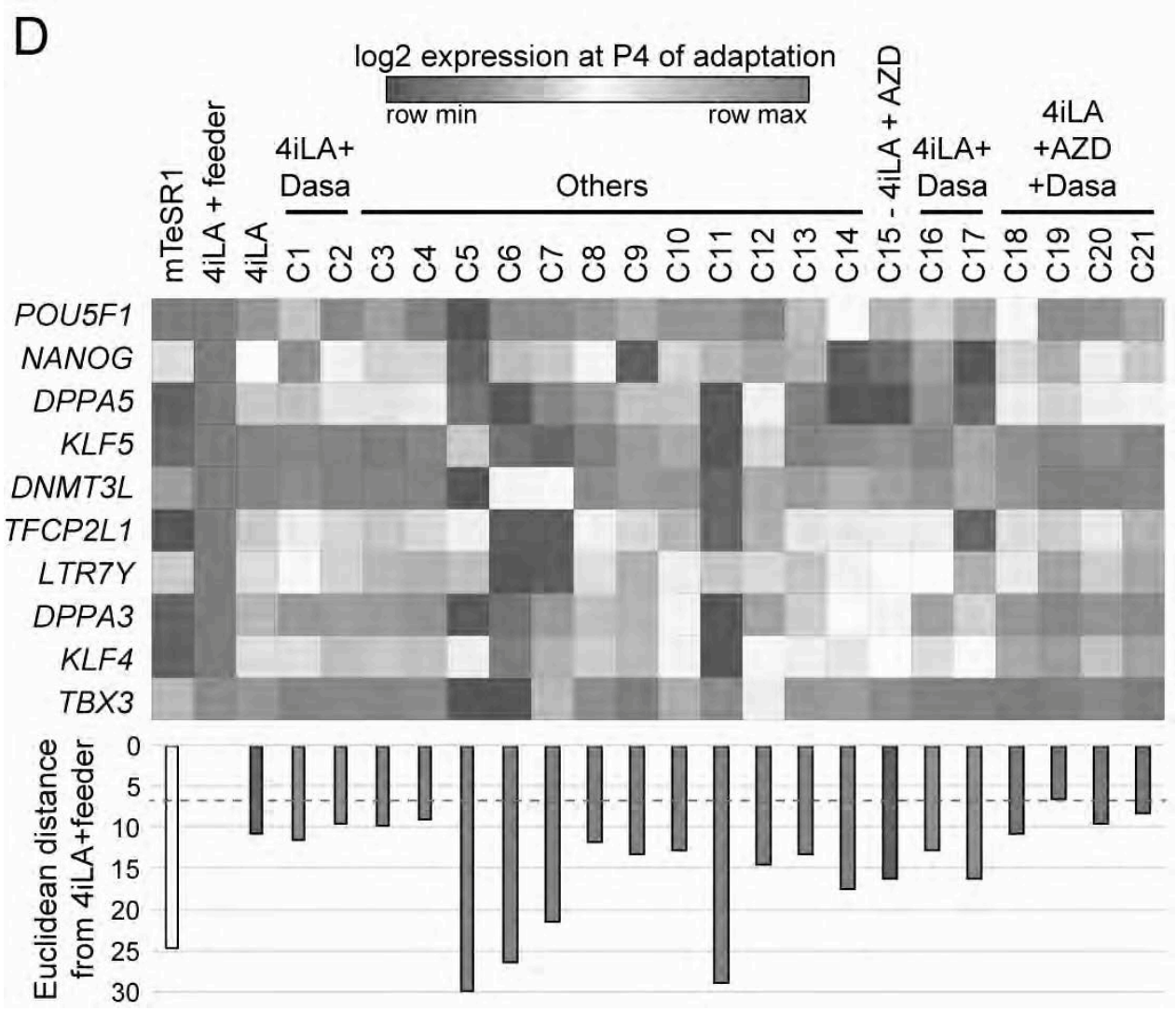

D
log2 expression at P4 of adaptation
row min
row max
mTeSR1
4iLA + feeder
4iLA
4iLA+ Dasa
Others
4iLA + AZD
4iLA+ Dasa
4iLA +AZD +Dasa
C1
C2
C3
C4
C5
C6
C7
C8
C9
C10
C11
C12
C13
C14
C15
C16
C17
C18
C19
C20
C21
POU5F1
NANOG
DPPA5
KLF5
DNMT3L
TFCP2L1
LTR7Y
DPPA3
KLF4
TBX3
Euclidean distance from 4iLA+feeder
0
5
10
15
20
25
30

FIGURE 2E

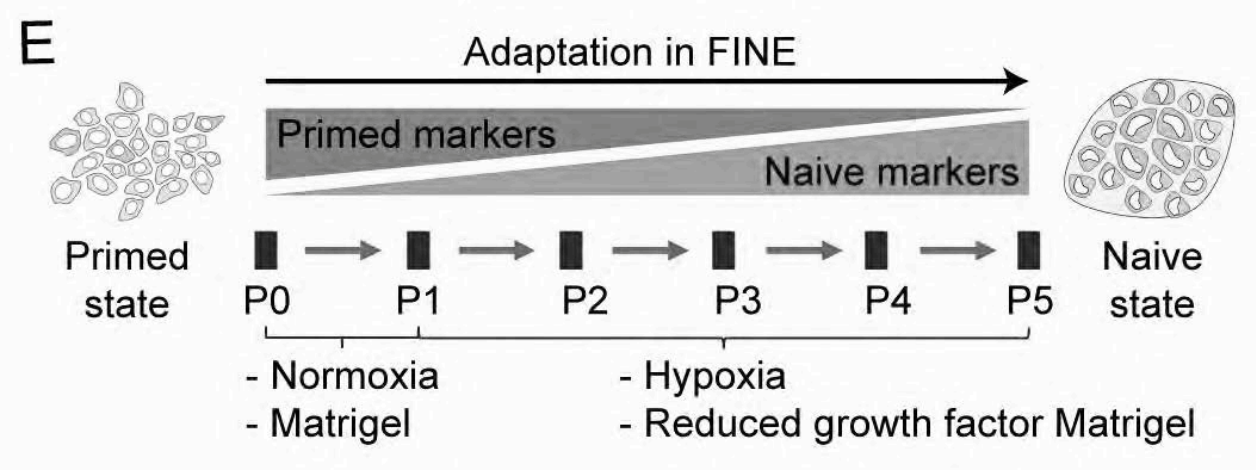

E
Adaptation in FINE
Primed markers
Naive markers
Primed state
Naive state
P0
P1
P2
P3
P4
P5
- Normoxia
- Matrigel
- Hypoxia
- Reduced growth factor Matrigel

FIGURE 2F

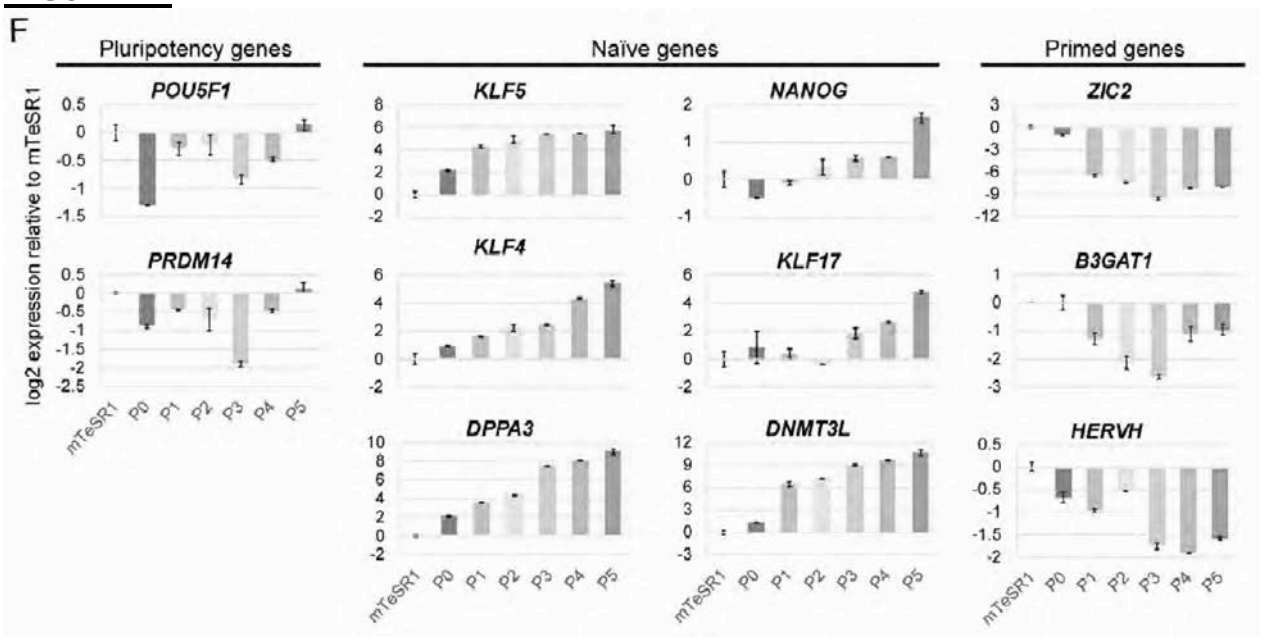

F
Pluripotency genes
Naïve genes
Primed genes
log2 expression relative to mTeSR1
POU5F1
PRDM14
KLF5
KLF4
DPPA3
NANOG
KLF17
DNMT3L
ZIC2
B3GAT1
HERVH
mTeSR1
P0
P1
P2
P3
P4
P5

FIGURE 3A

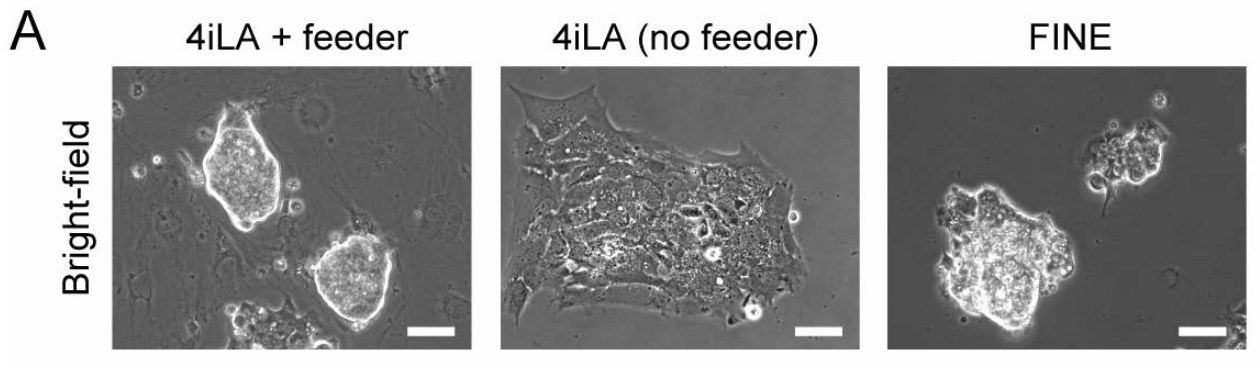


FIGURE 3B

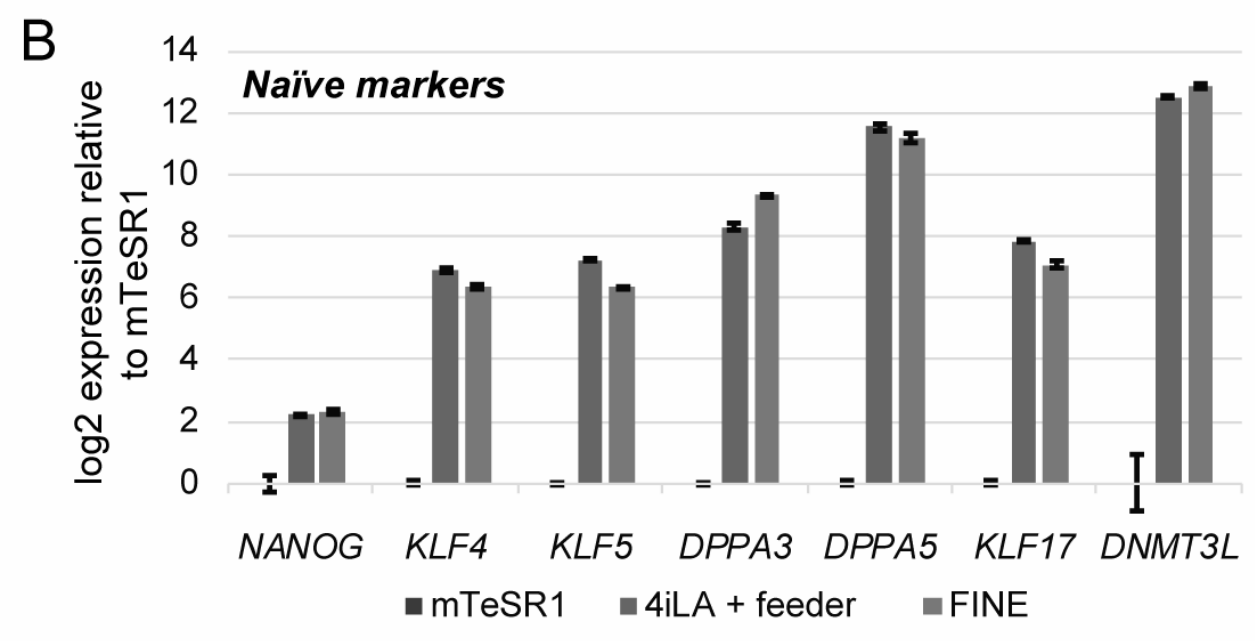

B
Naïve markers
log2 expression relative to mTeSR1
14
12
10
8
6
4
2
0
NANOG
KLF4
KLF5
DPPA3
DPPA5
KLF17
DNMT3L
mTeSR1
4iLA + feeder
FINE

FIGURE 3C

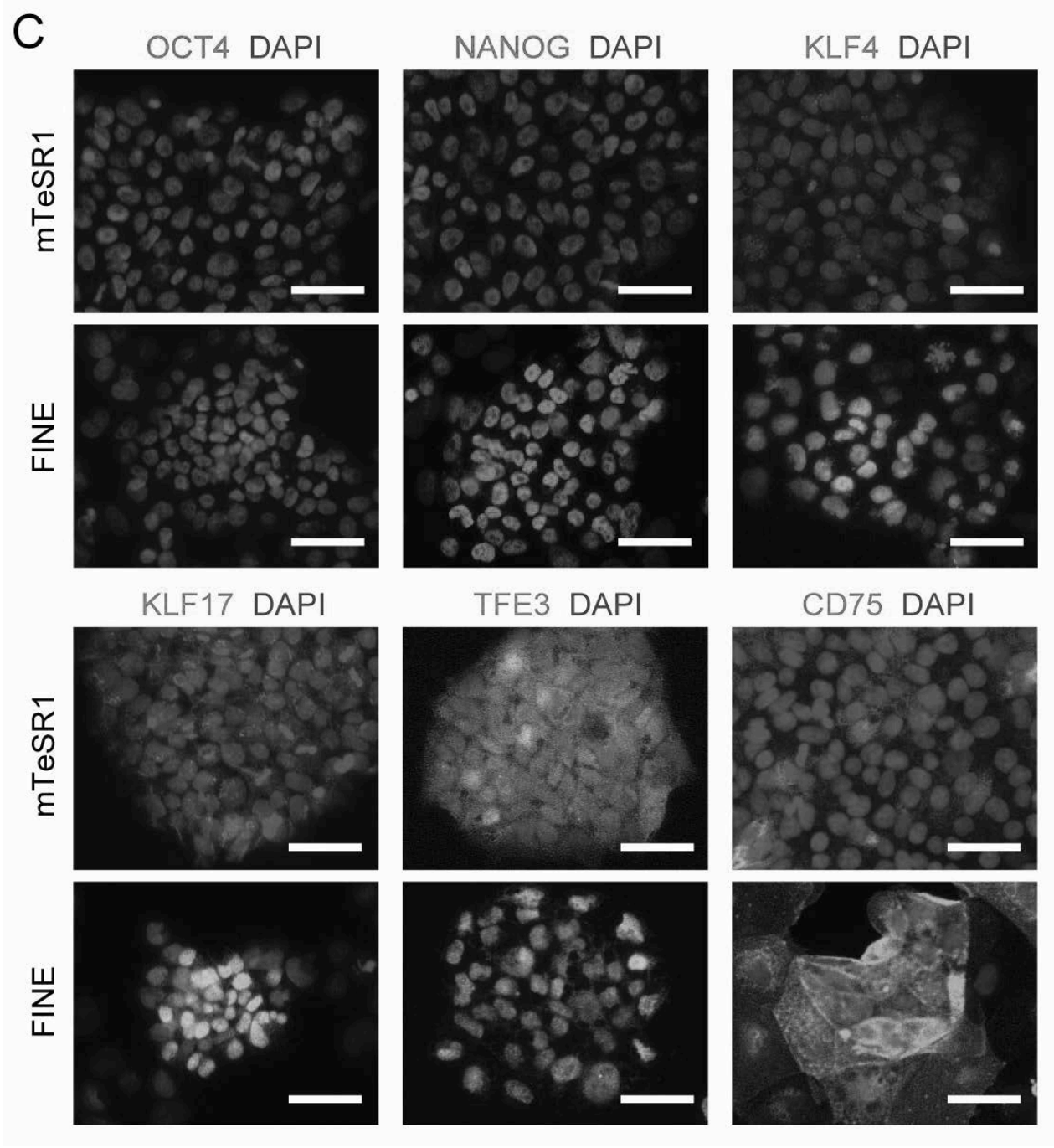

C
OCT4 DAPI
NANOG DAPI
KLF4 DAPI
mTeSR1
FINE
KLF17 DAPI
TFE3 DAPI
CD75 DAPI
mTeSR1
FINE

FIGURE 3D

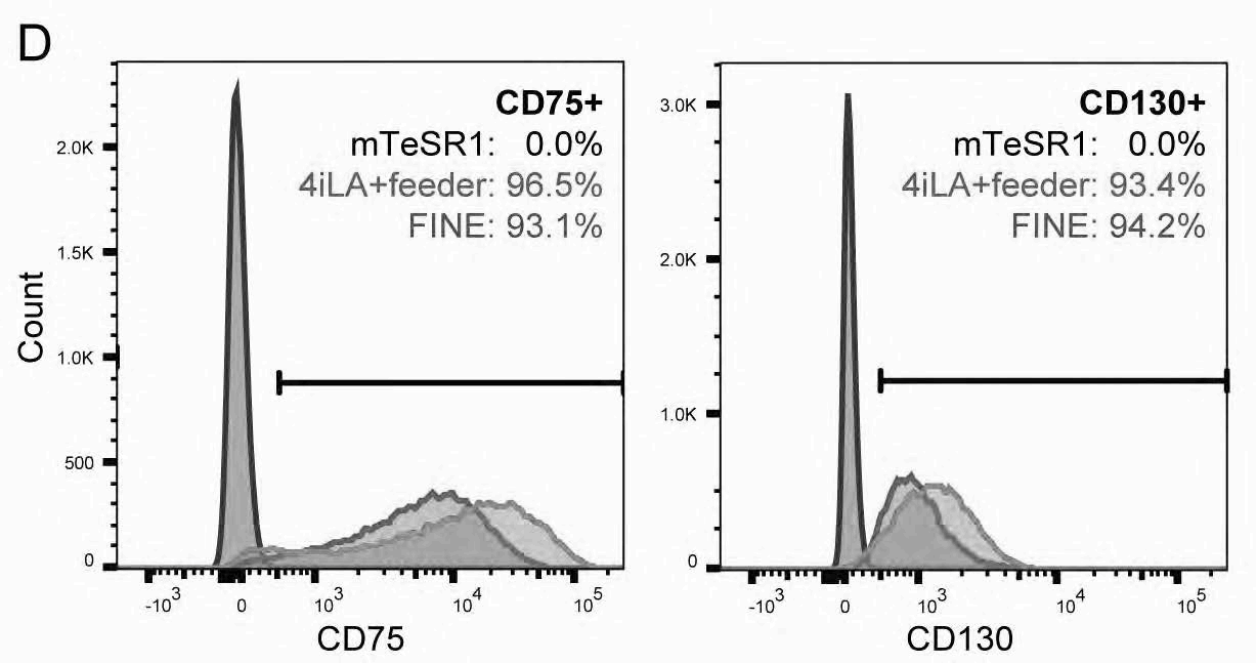

D
CD75+
mTeSR1: 0.0%
4iLA+feeder: 96.5%
FINE: 93.1%
Count
2.0K
1.5K
1.0K
500
0
-10^3
0
10^3
10^4
10^5
CD75
CD130+
mTeSR1: 0.0%
4iLA+feeder: 93.4%
FINE: 94.2%
3.0K
2.0K
1.0K
0
-10^3
0
10^3
10^4
10^5
CD130

FIGURE 3E

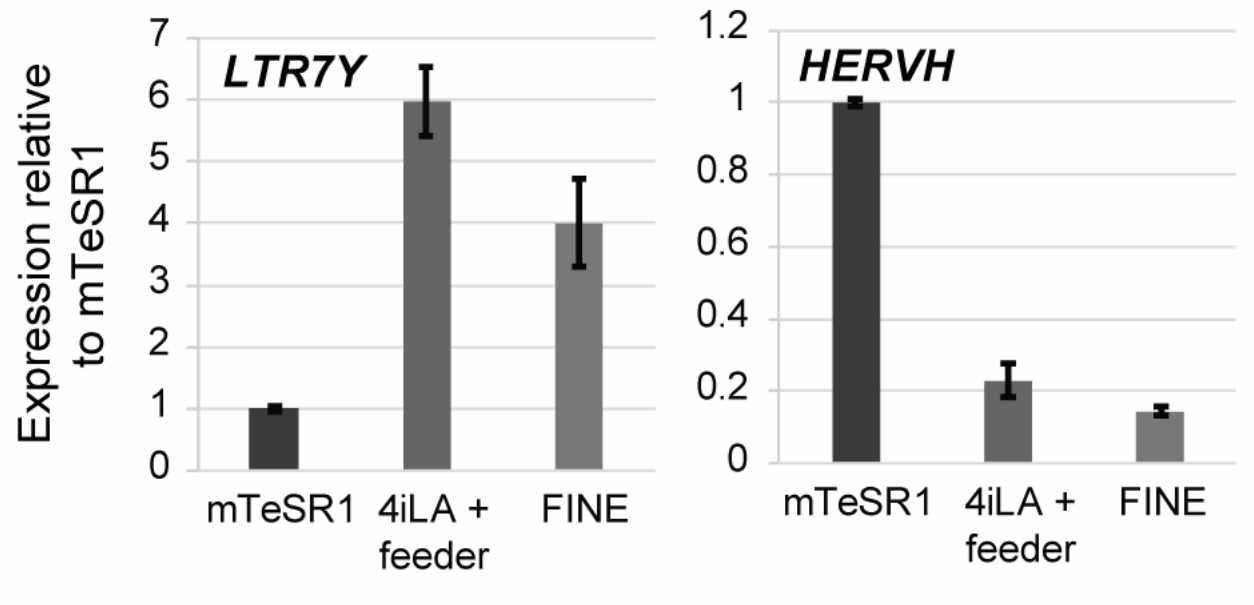

Expression relative to mTeSR1
LTR7Y
0
1
2
3
4
5
6
7
mTeSR1
4iLA + feeder
FINE
HERVH
0
0.2
0.4
0.6
0.8
1
1.2
mTeSR1
4iLA + feeder
FINE

FIGURE 3F

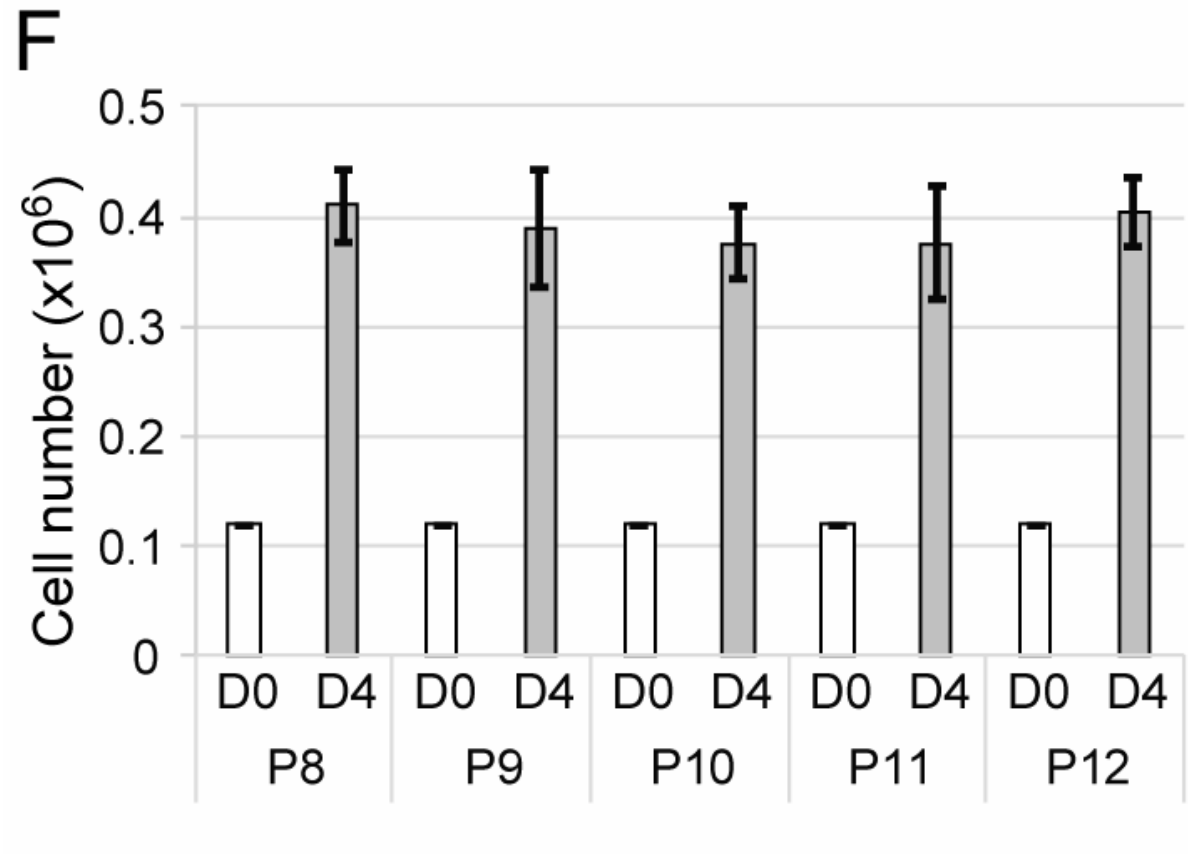


FIGURE 3G

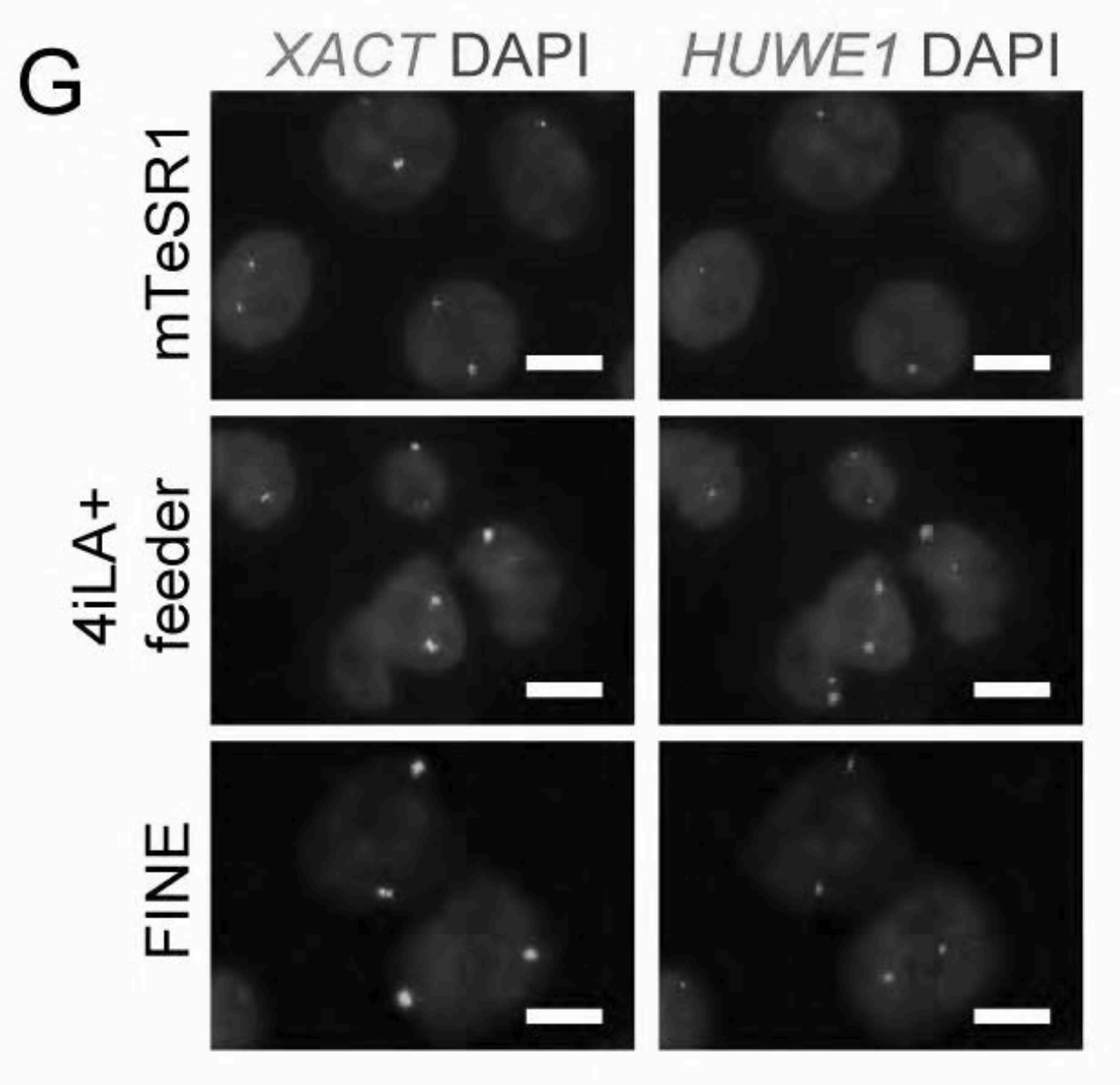


FIGURE 3H

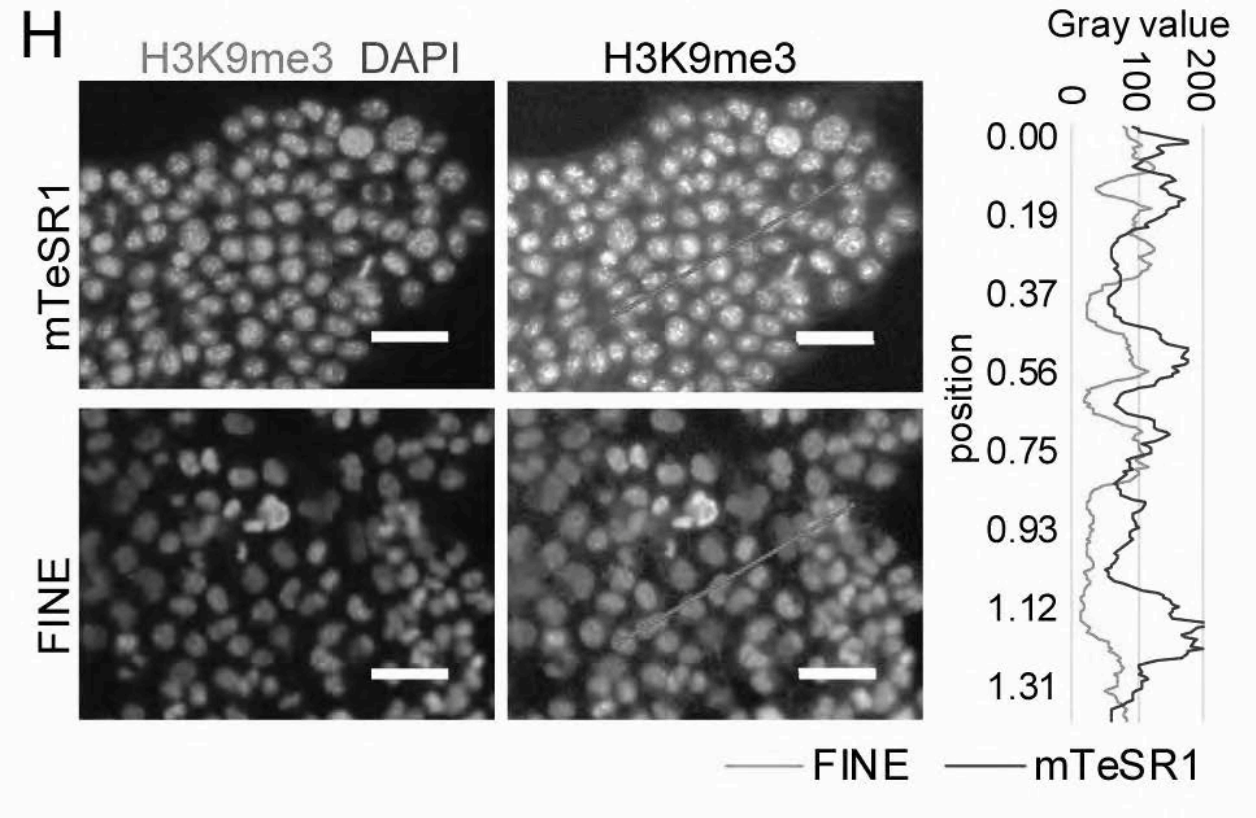

H
H3K9me3 DAPI
H3K9me3
mTeSR1
FINE
Gray value
0
100
200
position
0.00
0.19
0.37
0.56
0.75
0.93
1.12
1.31
FINE
mTeSR1

FIGURE 4A

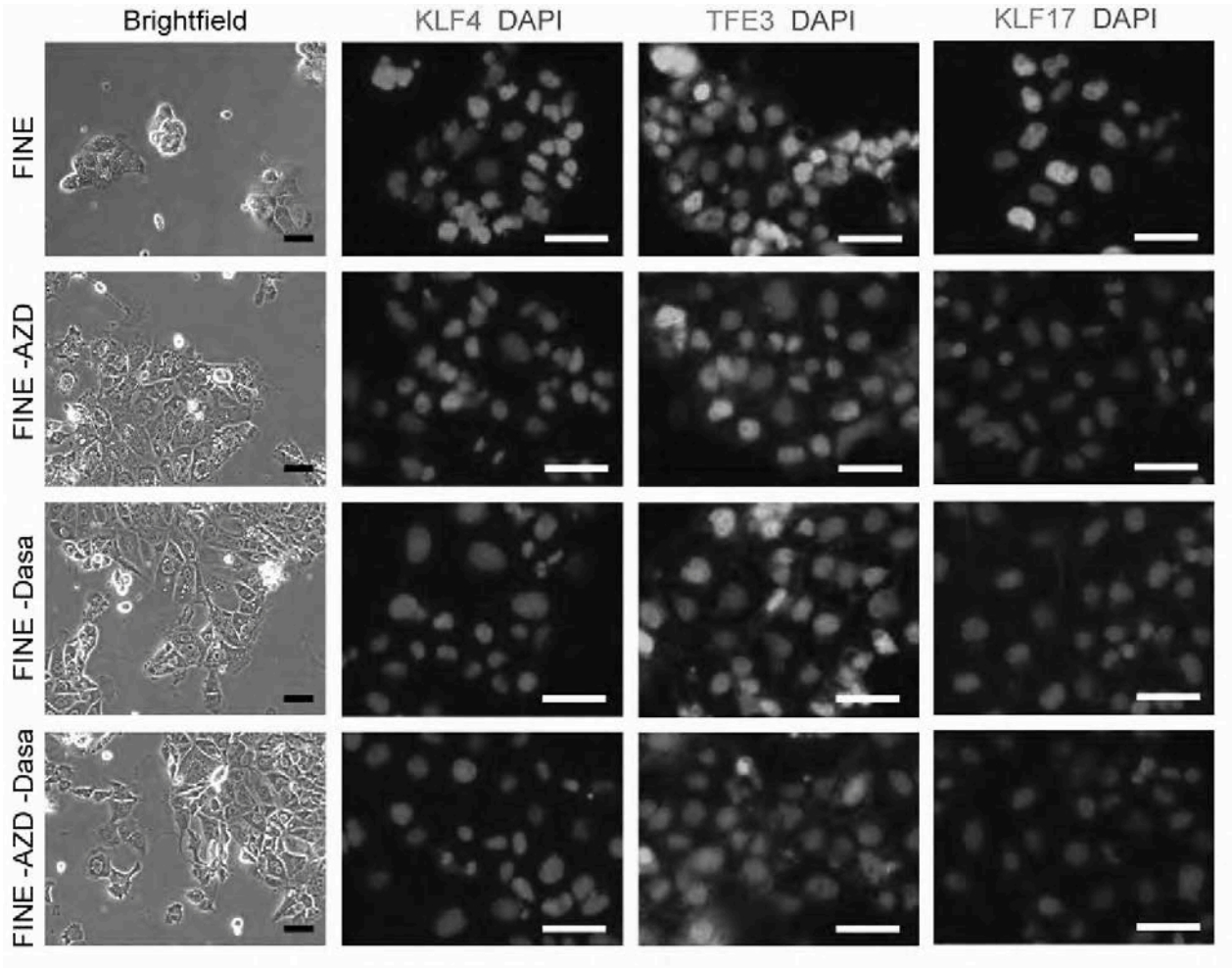

Brightfield
KLF4 DAPI
TFE3 DAPI
KLF17 DAPI
FINE
FINE -AZD
FINE -Dasa
FINE -AZD -Dasa

FIGURE 4B

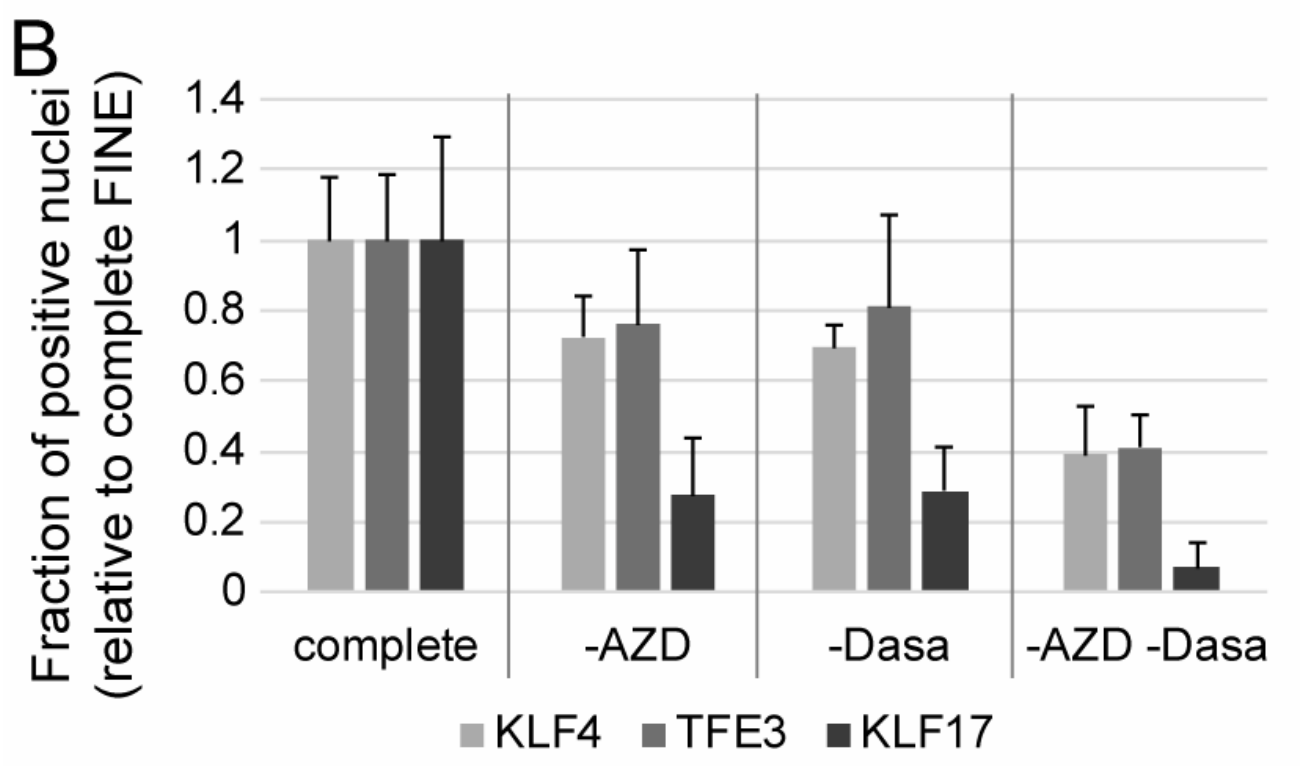

B
Fraction of positive nuclei
(relative to complete FINE)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
complete
-AZD
-Dasa
-AZD -Dasa
KLF4
TFE3
KLF17

FIGURE 4C

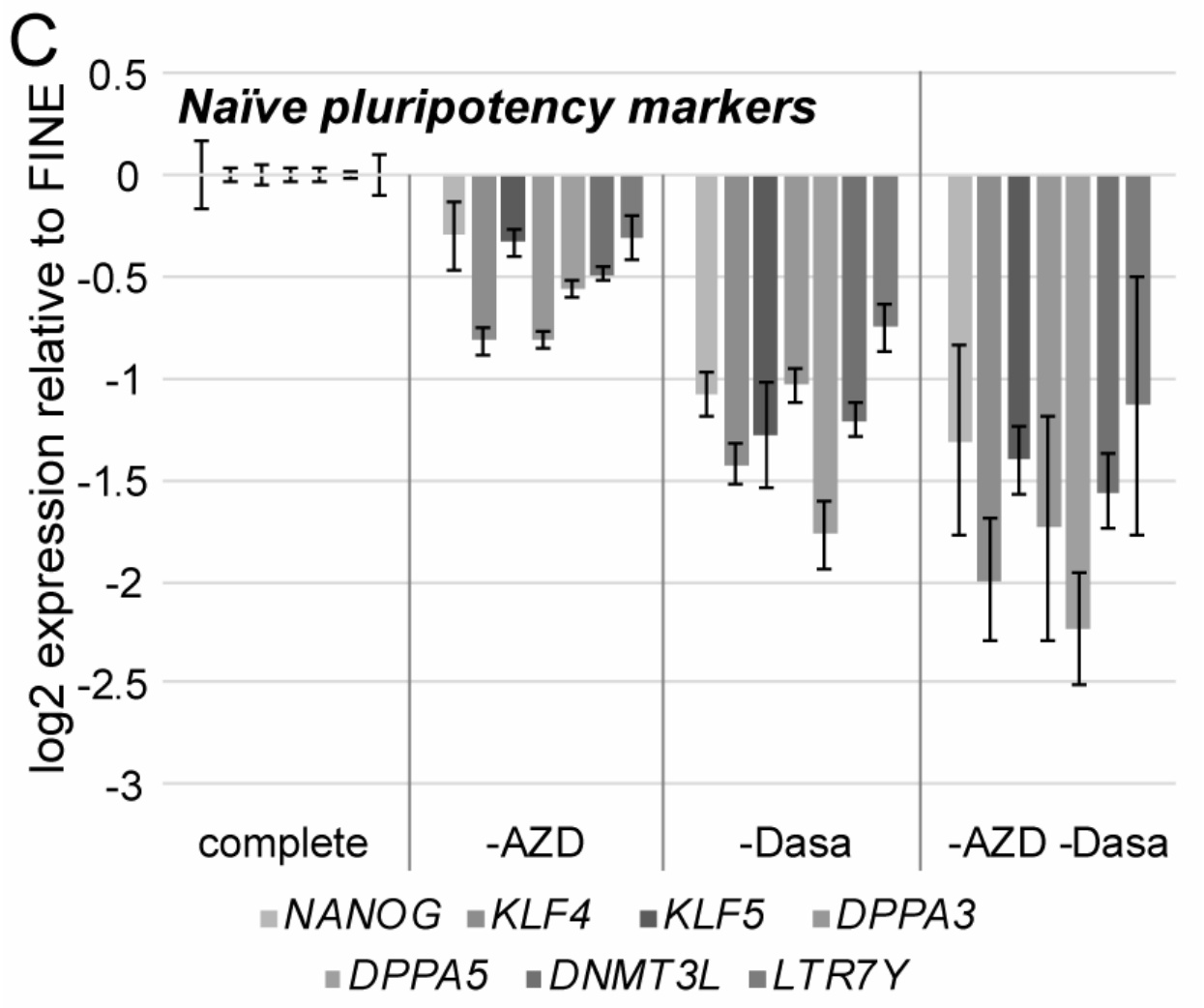

C
Naïve pluripotency markers
log2 expression relative to FINE
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3
complete
-AZD
-Dasa
-AZD -Dasa
NANOG
KLF4
KLF5
DPPA3
DPPA5
DNMT3L
LTR7Y

FIGURE 4D

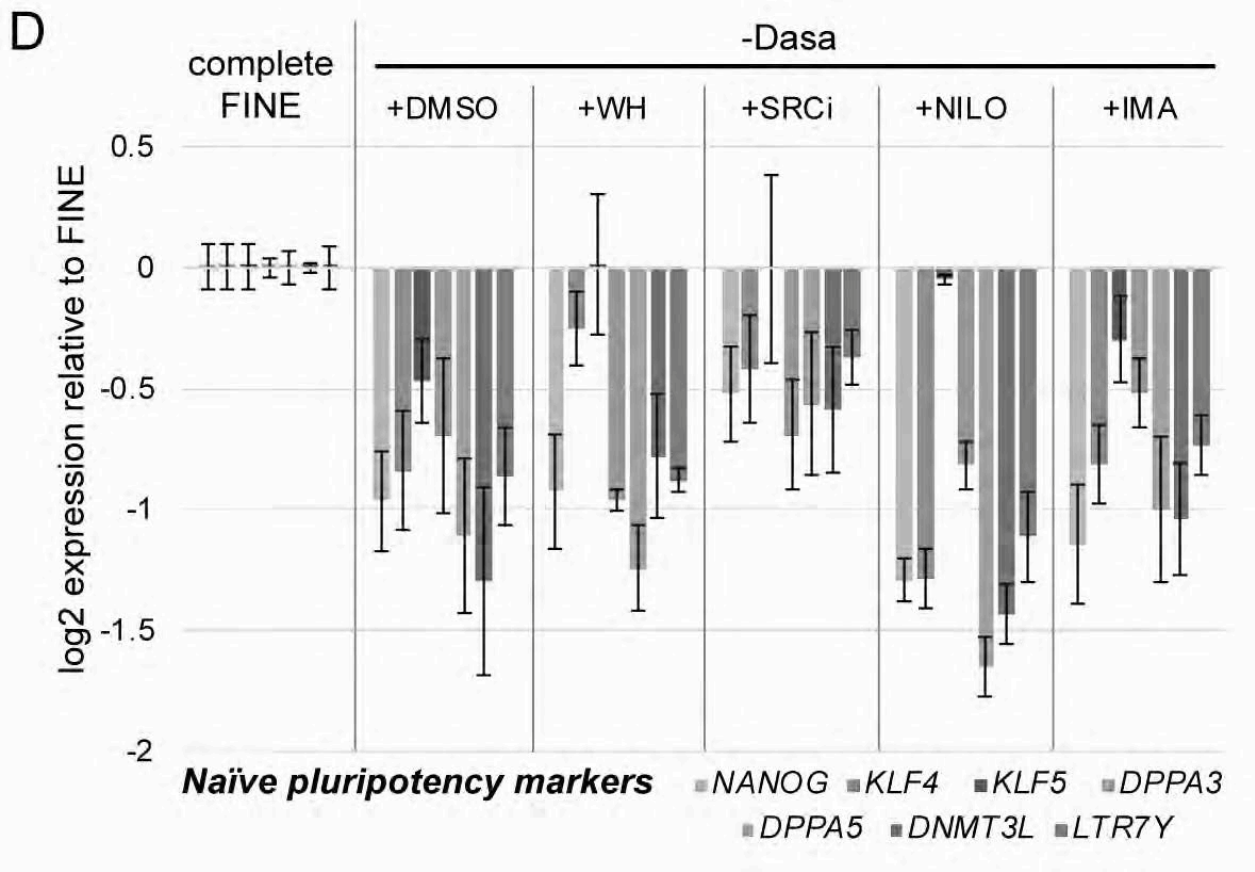

D
complete FINE
-Dasa
+DMSO
+WH
+SRCi
+NILO
+IMA
log2 expression relative to FINE
0.5
0
-0.5
-1
-1.5
-2
Naïve pluripotency markers
NANOG
KLF4
KLF5
DPPA3
DPPA5
DNMT3L
LTR7Y

FIGURE 4E

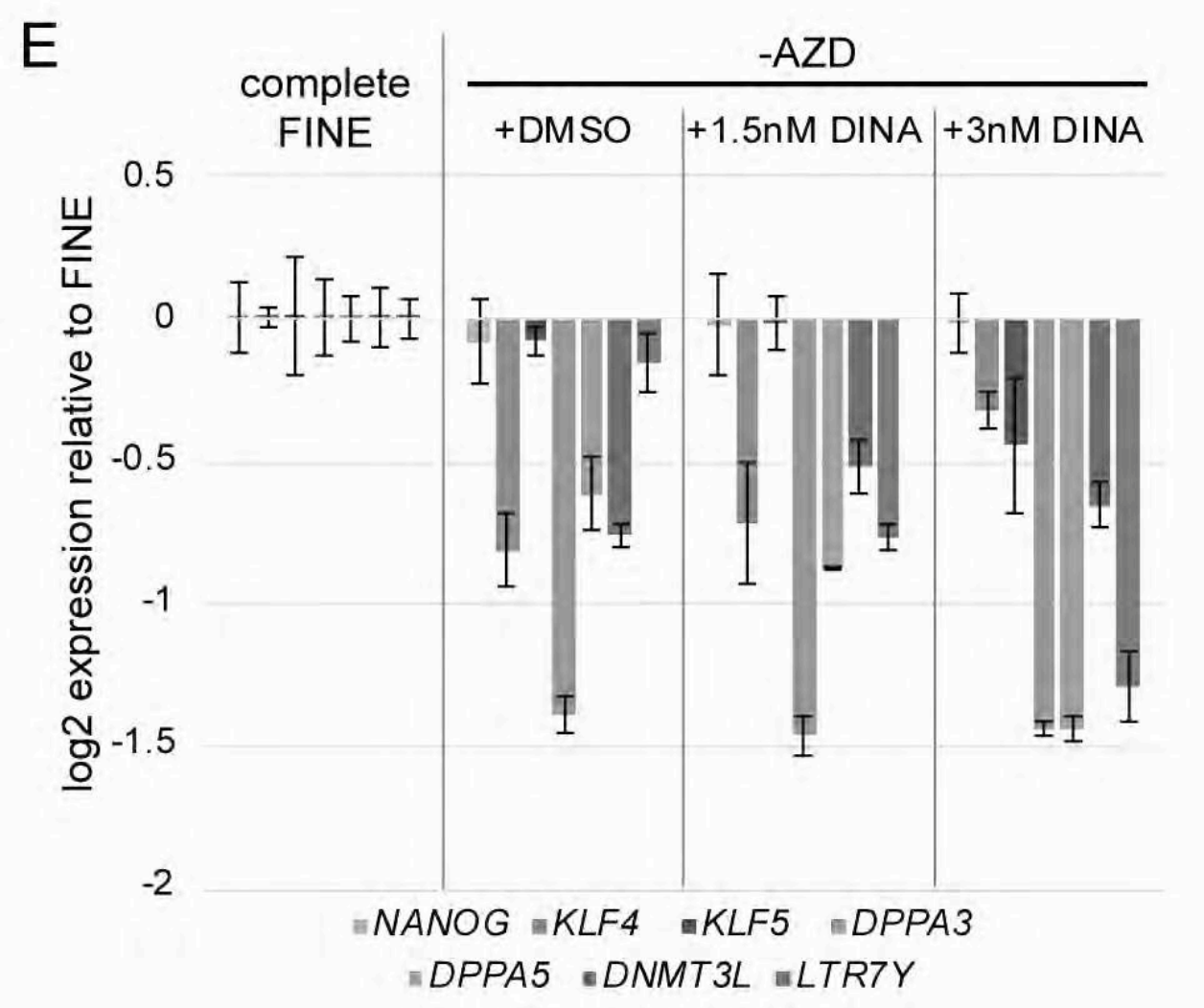

E
complete FINE
-AZD
+DMSO
+1.5nM DINA
+3nM DINA
log2 expression relative to FINE
0.5
0
-0.5
-1
-1.5
-2
NANOG
KLF4
KLF5
DPPA3
DPPA5
DNMT3L
LTR7Y

FIGURE 5A

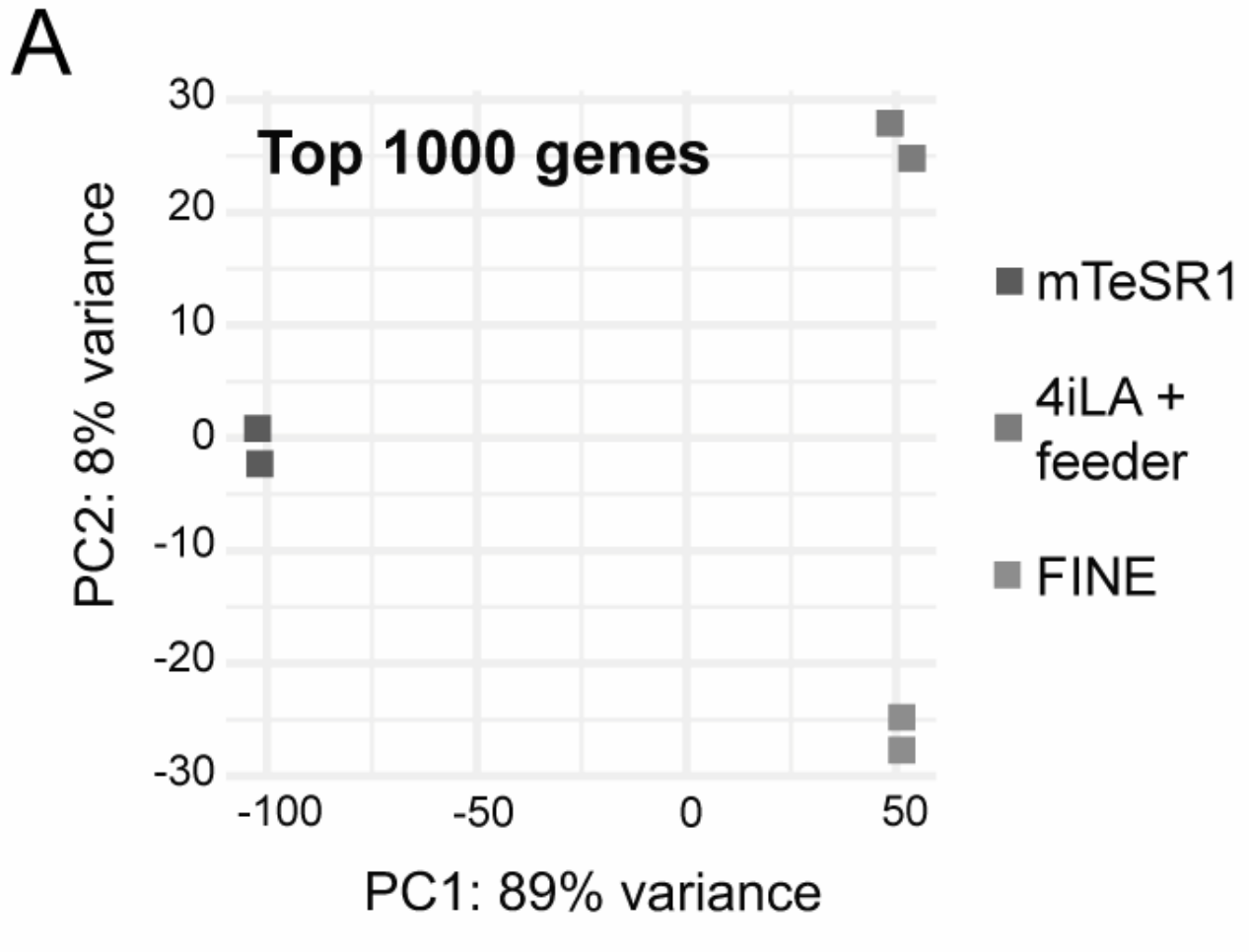

A
Top 1000 genes
PC2: 8% variance
30
20
10
0
-10
-20
-30
-100
-50
0
50
PC1: 89% variance
mTeSR1
4iLA + feeder
FINE

FIGURE 5B

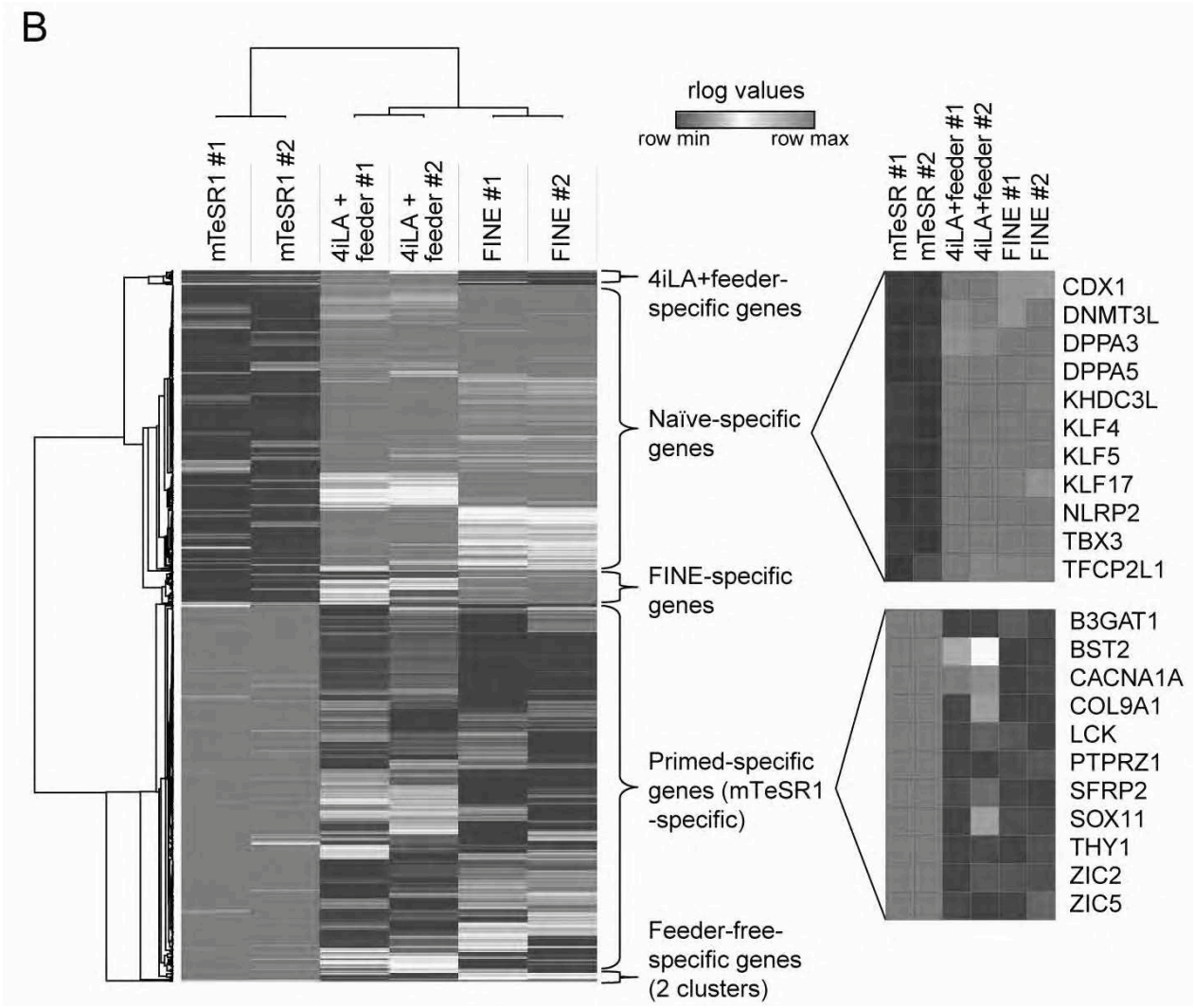

B
rlog values
row min
row max
mTeSR1 #1
mTeSR1 #2
4iLA + feeder #1
4iLA + feeder #2
FINE #1
FINE #2
4iLA+feeder-specific genes
Naïve-specific genes
FINE-specific genes
Primed-specific genes (mTeSR1 -specific)
Feeder-free-specific genes (2 clusters)
mTeSR #1
mTeSR #2
4iLA+feeder #1
4iLA+feeder #2
FINE #1
FINE #2
CDX1
DNMT3L
DPPA3
DPPA5
KHDC3L
KLF4
KLF5
KLF17
NLRP2
TBX3
TFCP2L1
B3GAT1
BST2
CACNA1A
COL9A1
LCK
PTPRZ1
SFRP2
SOX11
THY1
ZIC2
ZIC5

FIGURE 5C

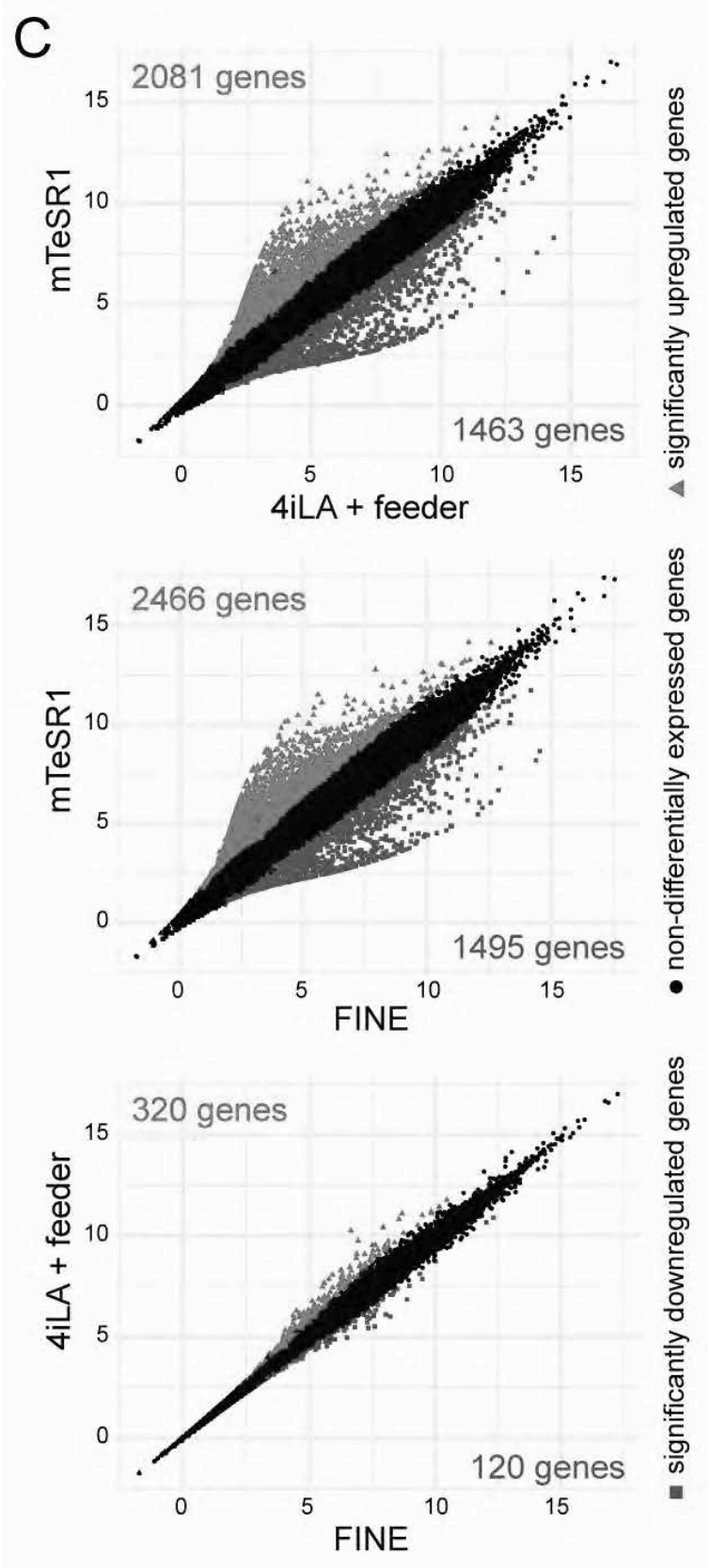

C
2081 genes
1463 genes
mTeSR1
4iLA + feeder
significantly upregulated genes
2466 genes
1495 genes
mTeSR1
FINE
non-differentially expressed genes
320 genes
120 genes
4iLA + feeder
FINE
significantly downregulated genes

FIGURE 5D

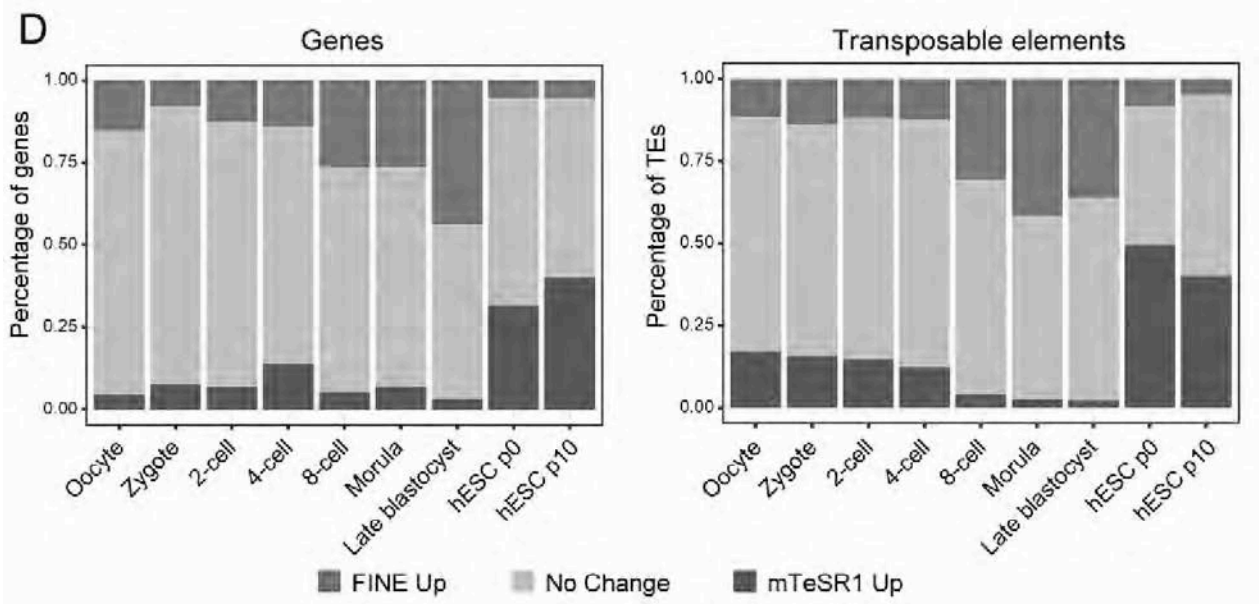

D
Genes
Transposable elements
Percentage of genes
Percentage of TEs
1.00
0.75
0.50
0.25
0.00
Oocyte
Zygote
2-cell
4-cell
8-cell
Morula
Late blastocyst
hESC p0
hESC p10
FINE Up
No Change
mTeSR1 Up

FIGURE 5E

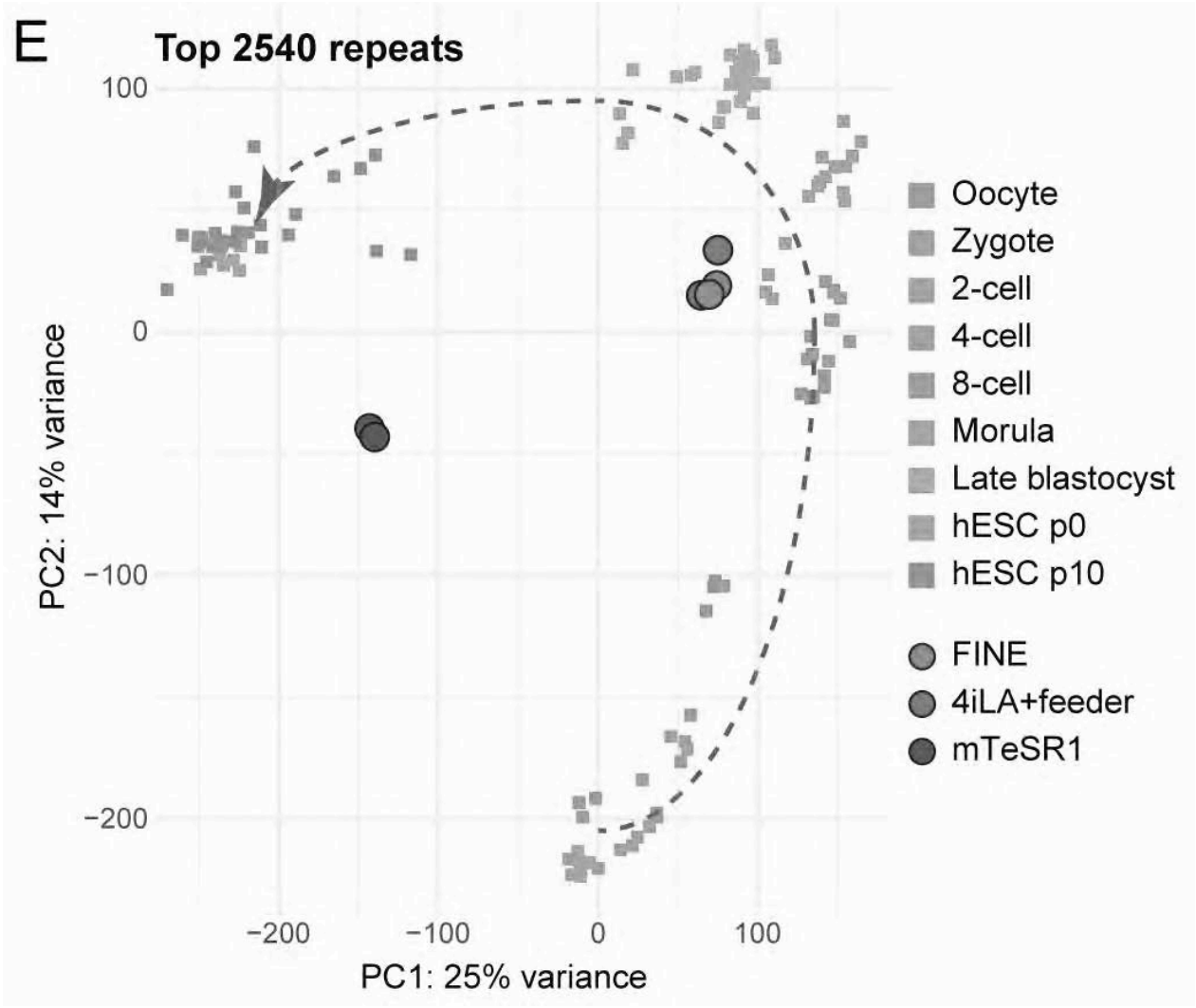

E
Top 2540 repeats
100
0
−100
−200
PC2: 14% variance
−200
−100
0
100
PC1: 25% variance
Oocyte
Zygote
2-cell
4-cell
8-cell
Morula
Late blastocyst
hESC p0
hESC p10
FINE
4iLA+feeder
mTeSR1

FIGURE 5F

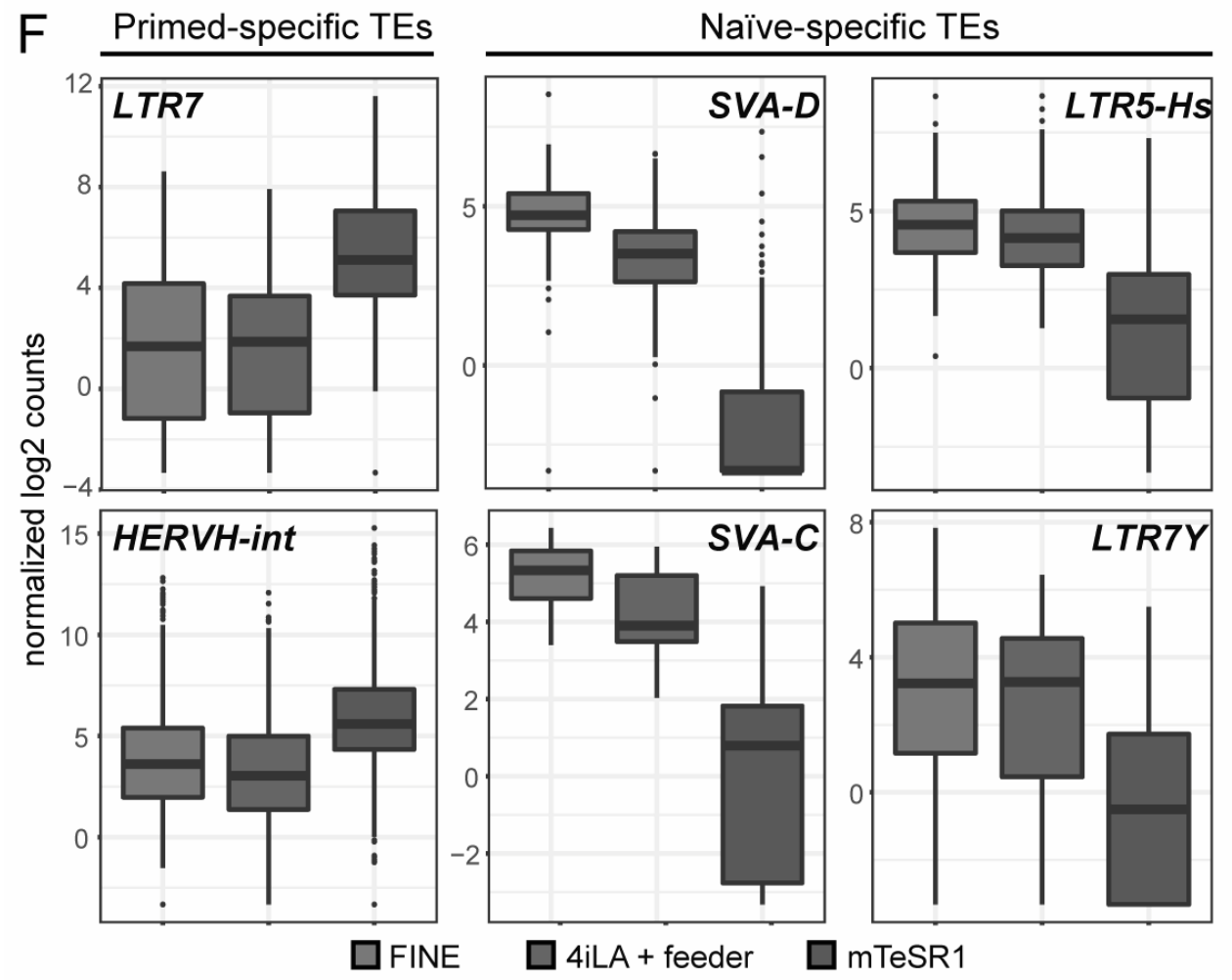

F
Primed-specific TEs
Naïve-specific TEs
LTR7
SVA-D
LTR5-Hs
HERVH-int
SVA-C
LTR7Y
normalized log2 counts
FINE
4iLA + feeder
mTeSR1

FIGURE 5G

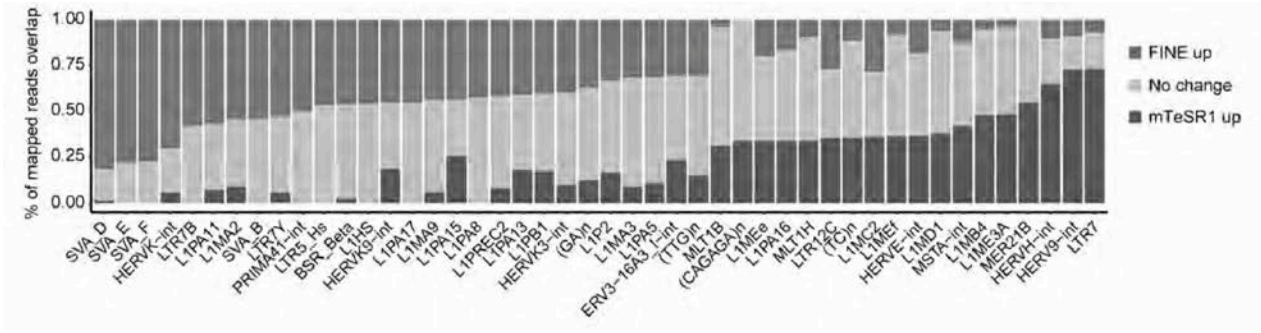

% of mapped reads overlap
1.00
0.75
0.50
0.25
0.00
FINE up
No change
mTeSR1 up
SVA_D
SVA_E
SVA_F
HERVK-int
LTR7B
L1PA11
L1MA2
SVA_B
LTR7Y
PRIMA41-int
LTR5_Hs
BSR_Beta
L1HS
HERVK9-int
L1PA17
L1MA9
L1PA15
L1PA8
L1PREC2
L1PA13
L1PB1
HERVK3-int
(GA)n
L1P2
L1MA3
L1PA5
ER/3-16A3_I-int
(TTG)n
MLT1B
(CAGAGA)n
L1MEe
L1PA16
MLT1H
LTR12C
(TC)n
L1MC2
L1MEf
HERVE-int
L1MD1
MSTA-int
L1MB4
L1ME3A
MER21B
HERVH-int
HERV9-int
LTR7

FIGURE 6A

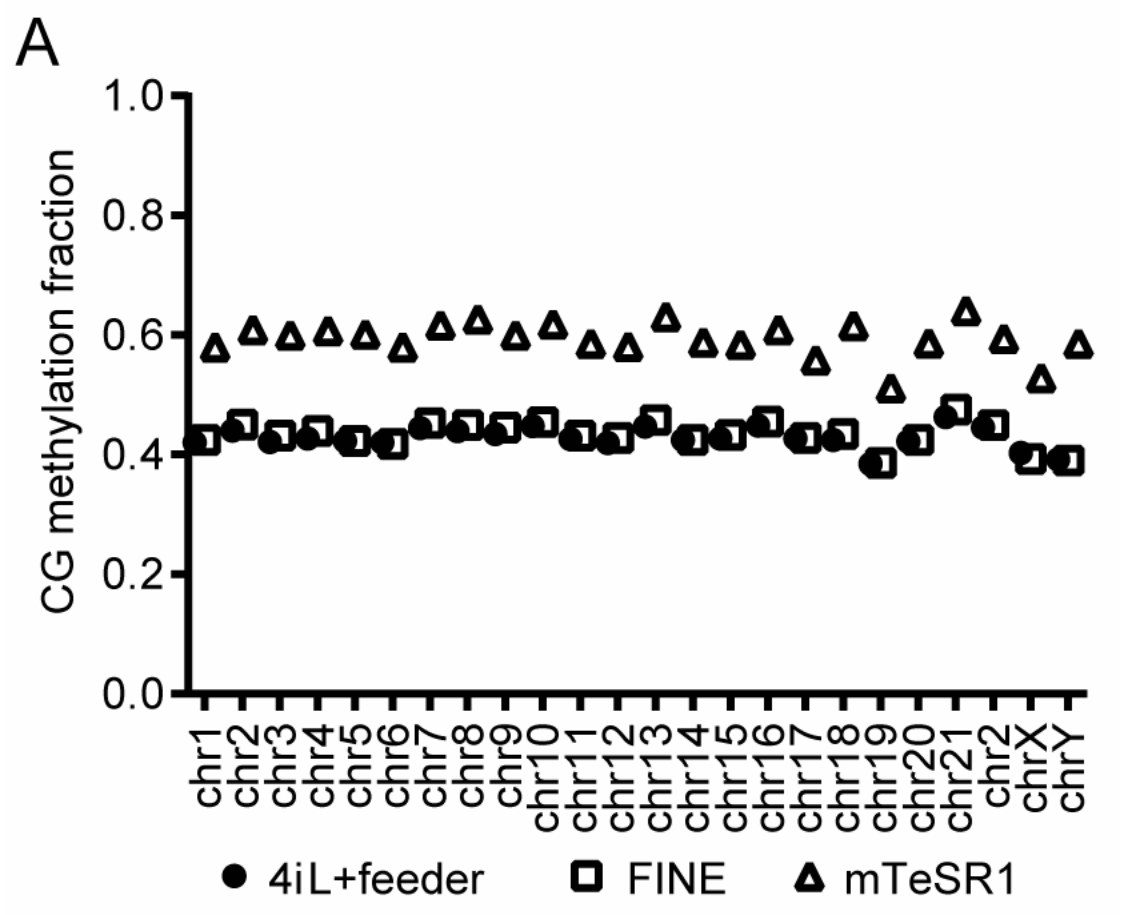

A
CG methylation fraction
1.0
0.8
0.6
0.4
0.2
0.0
chr1
chr2
chr3
chr4
chr5
chr6
chr7
chr8
chr9
chr10
chr11
chr12
chr13
chr14
chr15
chr16
chr17
chr18
chr19
chr20
chr21
chr2
chrX
chrY
4iL+feeder
FINE
mTeSR1

FIGURE 6B

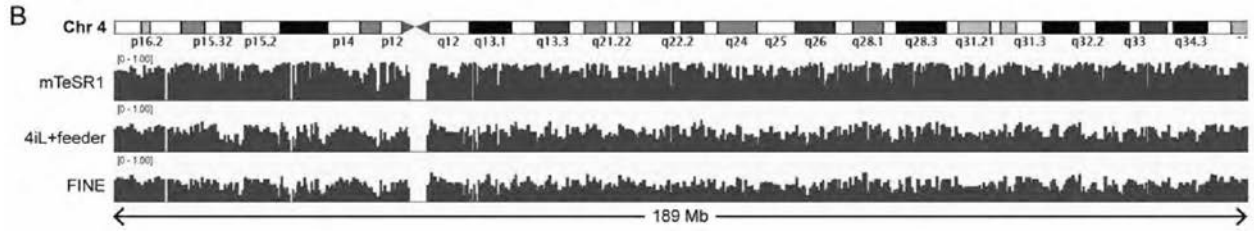

B
Chr 4
p16.2
p15.32
p15.2
p14
p12
q12
q13.1
q13.3
q21.22
q22.2
q24
q25
q26
q28.1
q28.3
q31.21
q31.3
q32.2
q33
q34.3
mTeSR1
4iL+feeder
FINE
189 Mb

FIGURE 6C

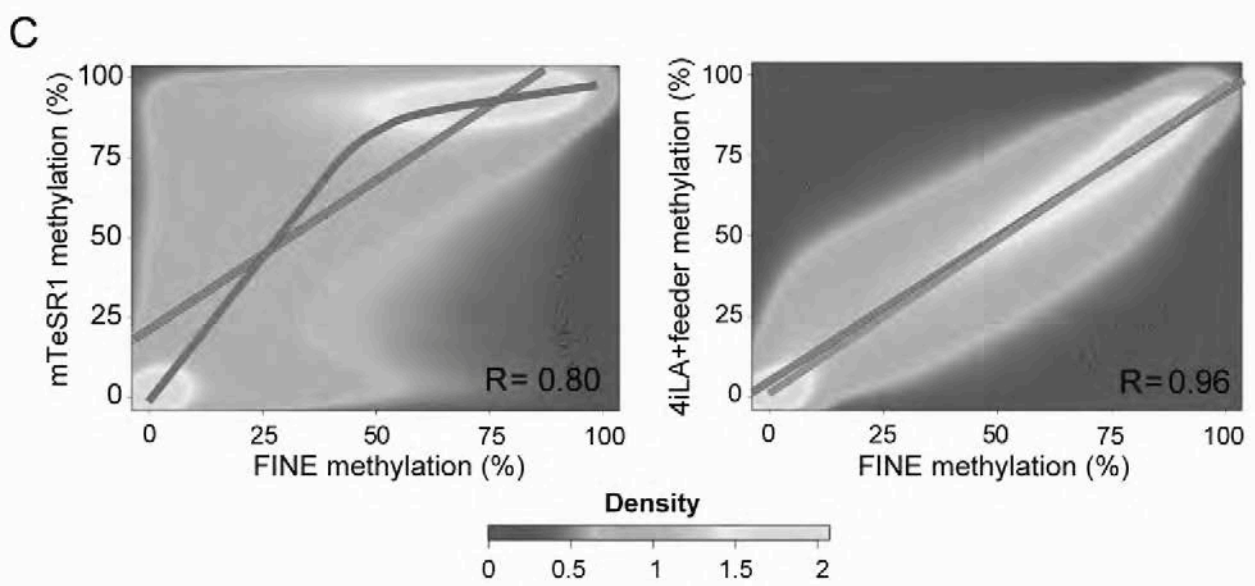

C
mTeSR1 methylation (%)
100
75
50
25
0
R= 0.80
0
25
50
75
100
FINE methylation (%)
4iLA+feeder methylation (%)
R= 0.96
Density
0
0.5
1
1.5
2

FIGURE 6D

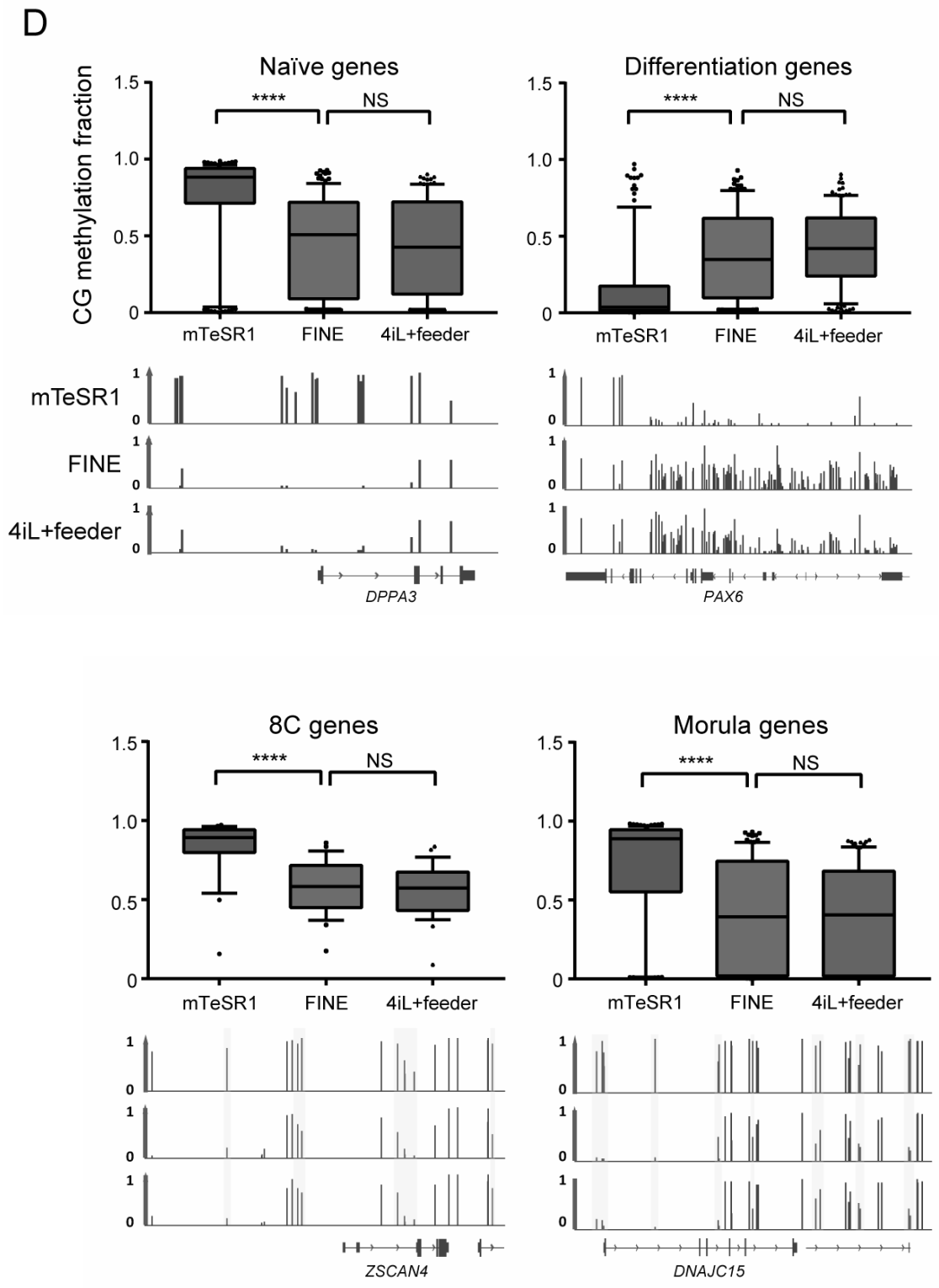

D
Naïve genes
Differentiation genes
CG methylation fraction
****
NS
mTeSR1
FINE
4iL+feeder
DPPA3
PAX6
8C genes
Morula genes
ZSCAN4
DNAJC15

FIGURE 7A

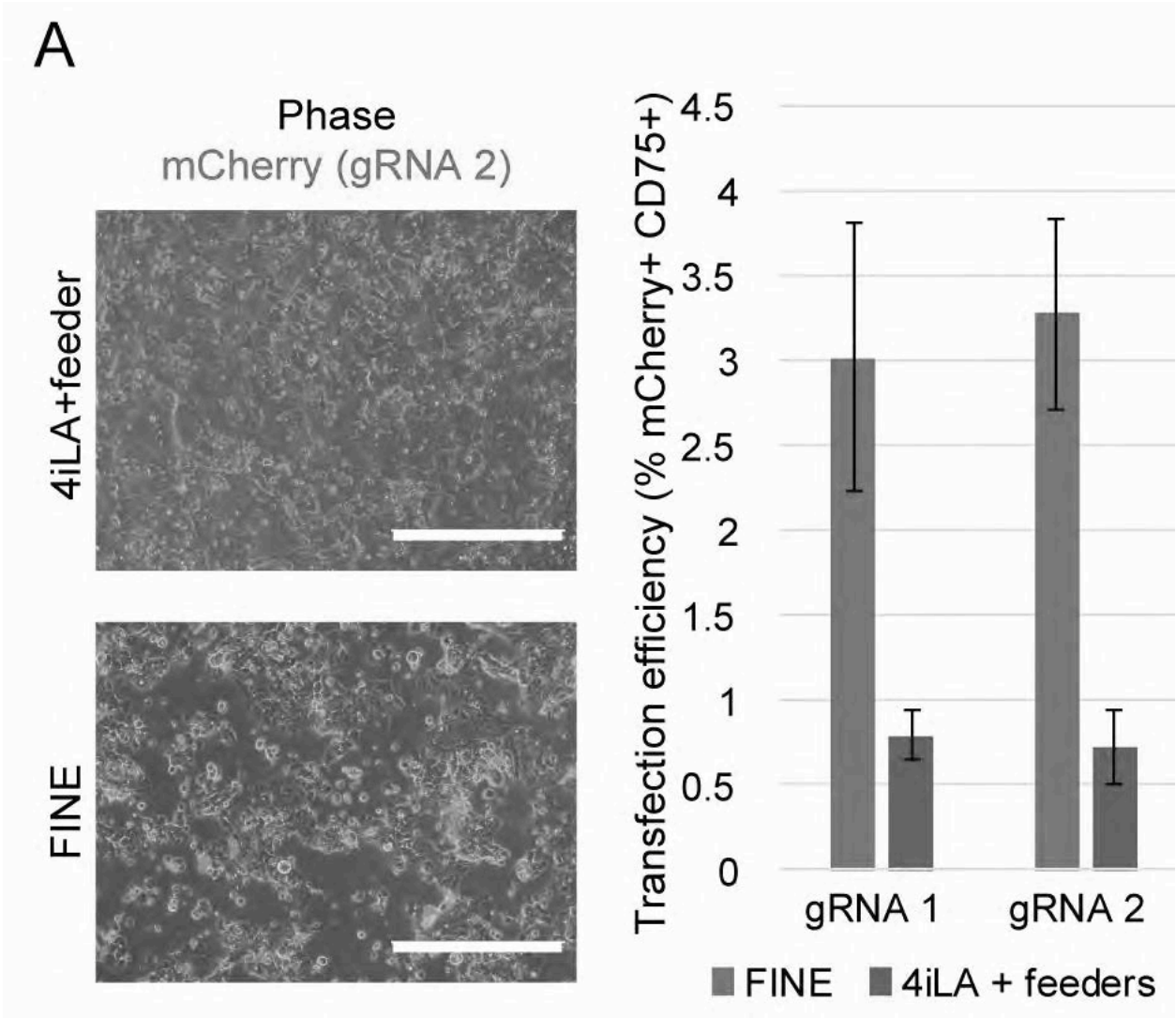

A
Phase
mCherry (gRNA 2)
4iLA+feeder
FINE
Transfection efficiency (% mCherry+ CD75+)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
gRNA 1
gRNA 2
FINE
4iLA + feeders

FIGURE 7B

B

Culture medium	P7	P12	P17	P25
4iLA + feeders	20/20 normal	9/20 isochromosomy ch21 11/20 normal		
FINE	20/20 normal	20/20 normal	3/20 trisomy ch6 3/20 trisomy ch7 14/20 normal	
FINE low PD03			20/20 normal	7/20 trisomy ch6 13/20 normal

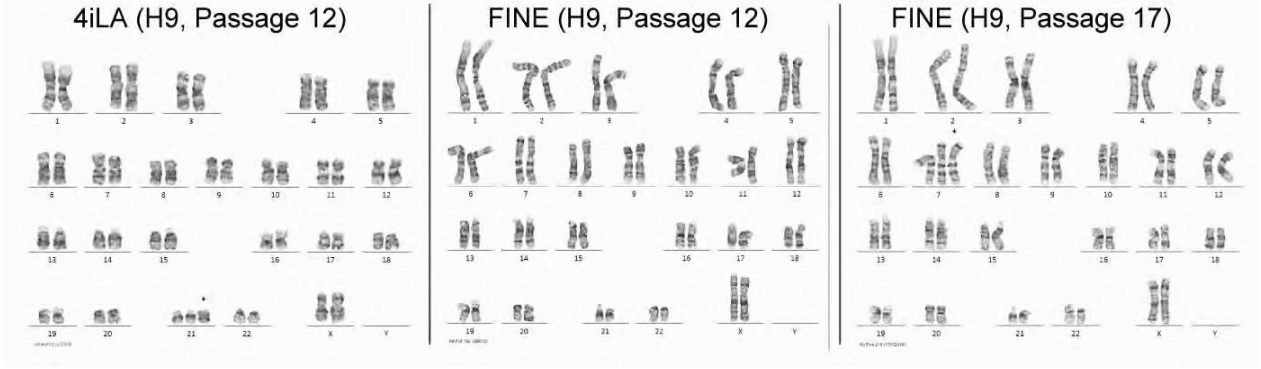


FIGURE 7C

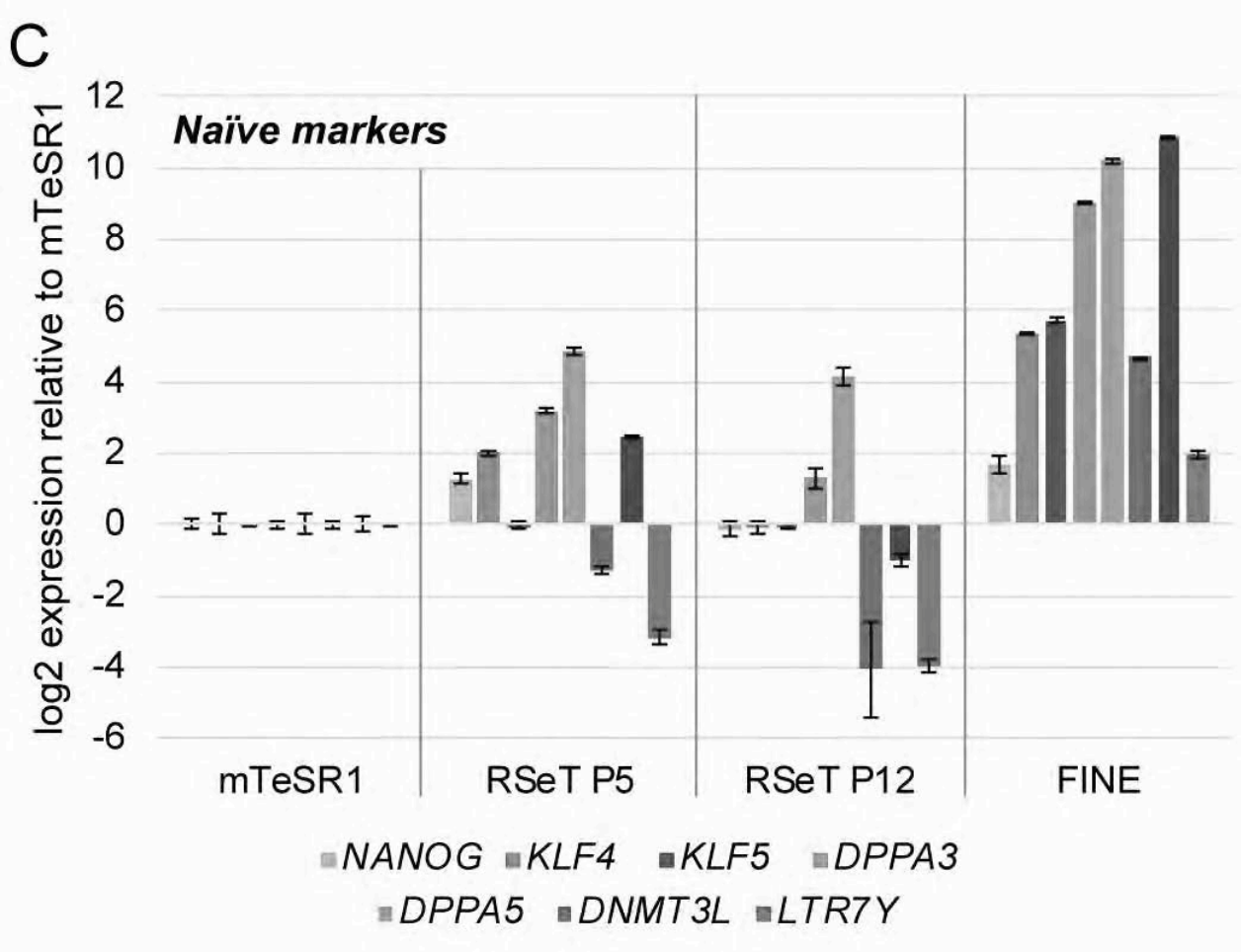

C
Naïve markers
log2 expression relative to mTeSR1
12
10
8
6
4
2
0
-2
-4
-6
mTeSR1
RSeT P5
RSeT P12
FINE
NANOG
KLF4
KLF5
DPPA3
DPPA5
DNMT3L
LTR7Y

FIGURE 7D

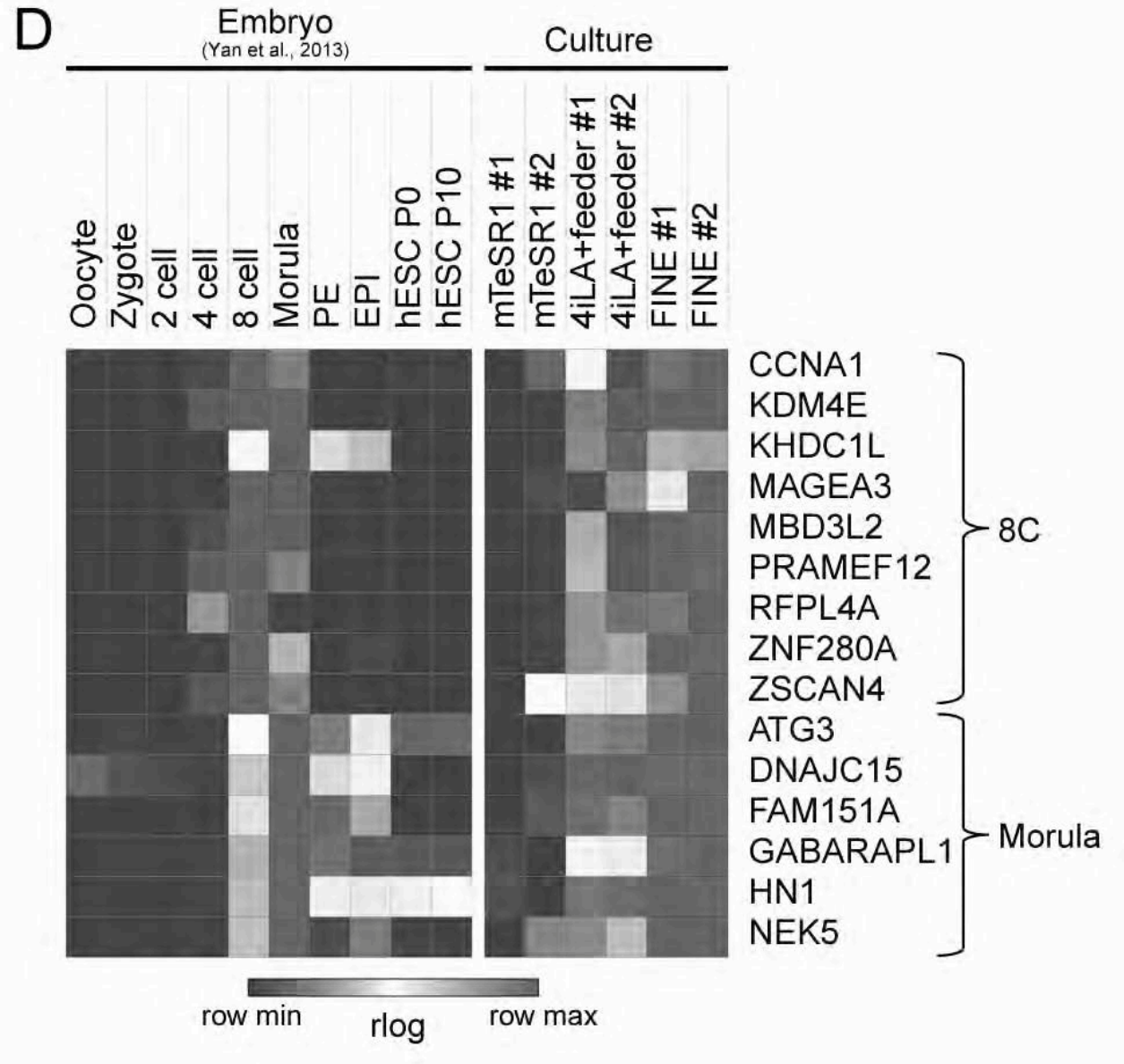

D
Embryo
(Yan et al., 2013)
Culture
Oocyte
Zygote
2 cell
4 cell
8 cell
Morula
PE
EPI
hESC P0
hESC P10
mTeSR1 #1
mTeSR1 #2
4iLA+feeder #1
4iLA+feeder #2
FINE #1
FINE #2
CCNA1
KDM4E
KHDC1L
MAGEA3
MBD3L2
PRAMEF12
RFPL4A
ZNF280A
ZSCAN4
8C
ATG3
DNAJC15
FAM151A
GABARAPL1
HN1
NEK5
Morula
row min
rlog
row max

FIGURE 8A

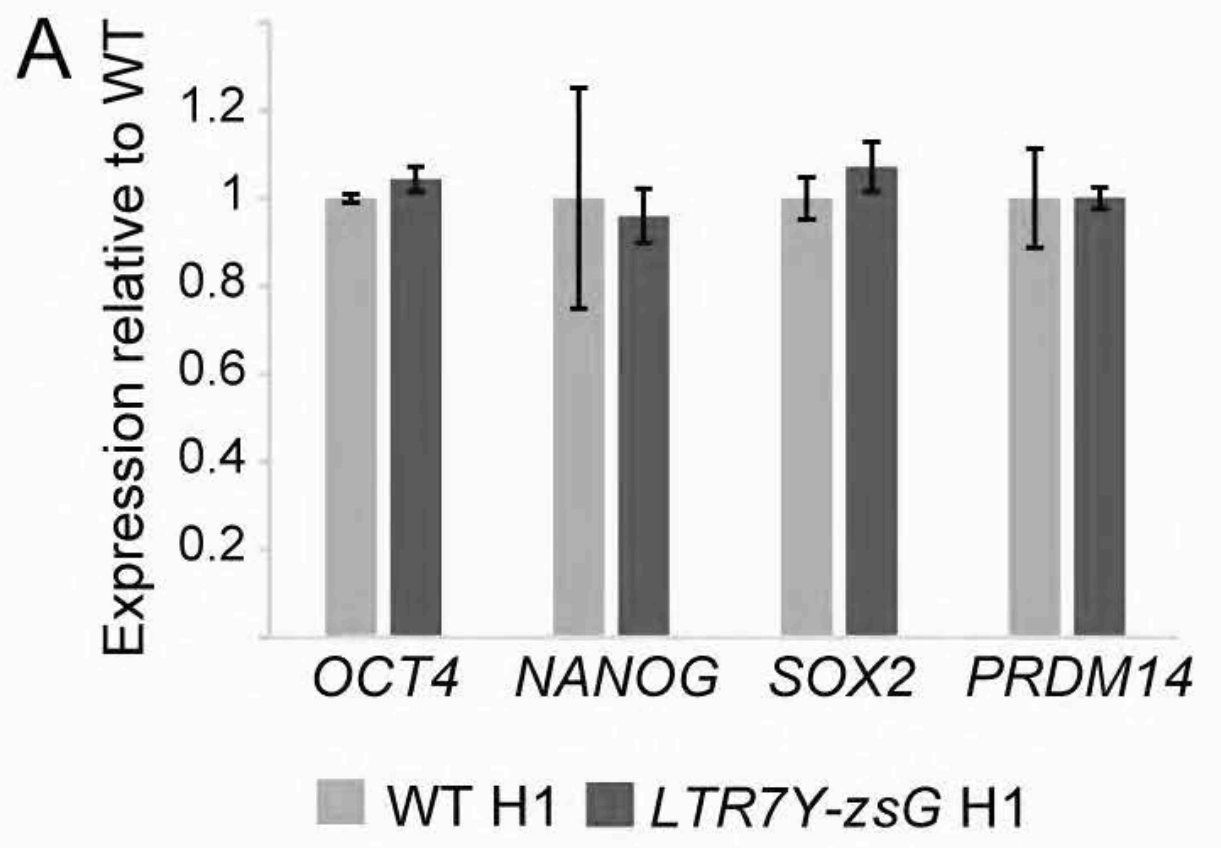

A
Expression relative to WT
1.2
1
0.8
0.6
0.4
0.2
OCT4
NANOG
SOX2
PRDM14
WT H1
LTR7Y-zsG H1

FIGURE 8B

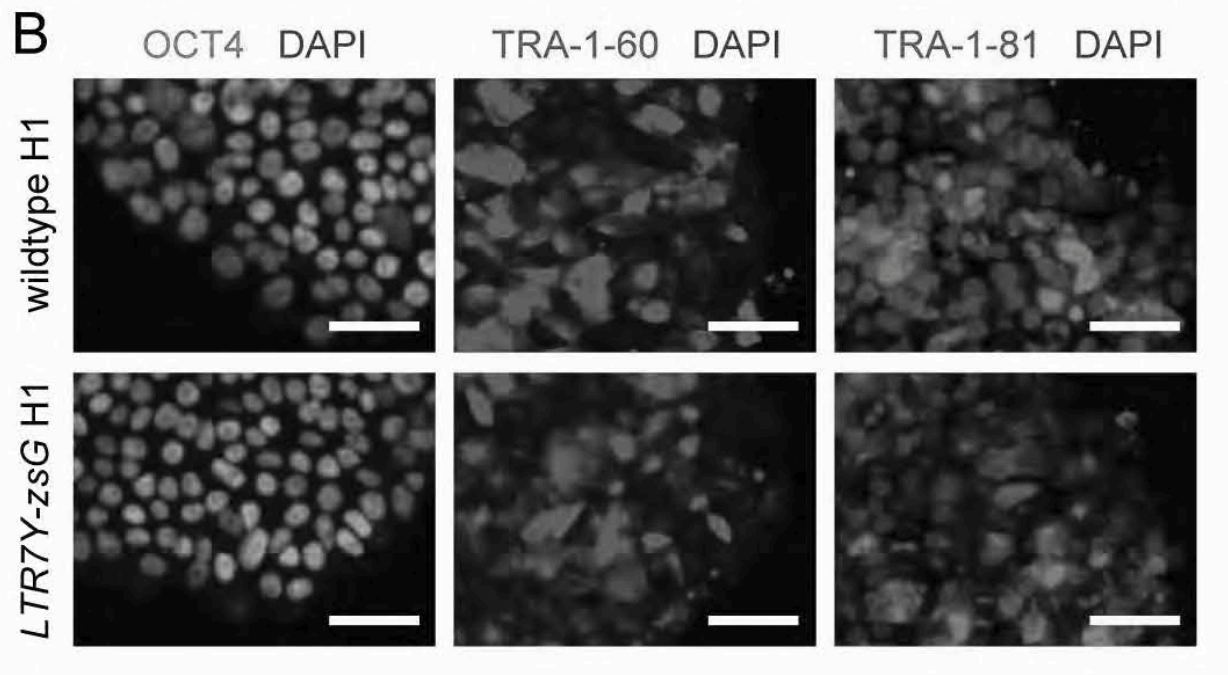


FIGURE 8C

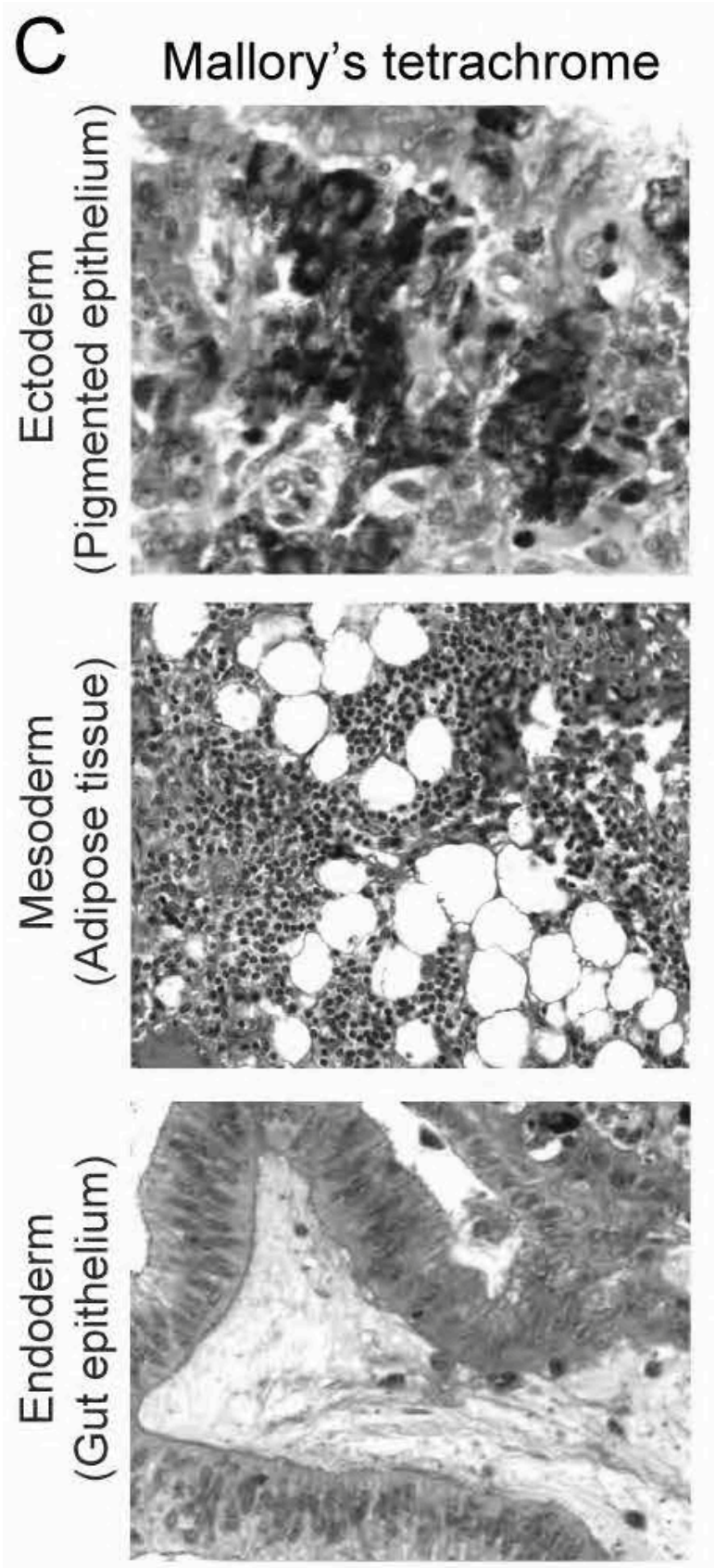

C
Mallory's tetrachrome
Ectoderm
(Pigmented epithelium)
Mesoderm
(Adipose tissue)
Endoderm
(Gut epithelium)

FIGURE 8D

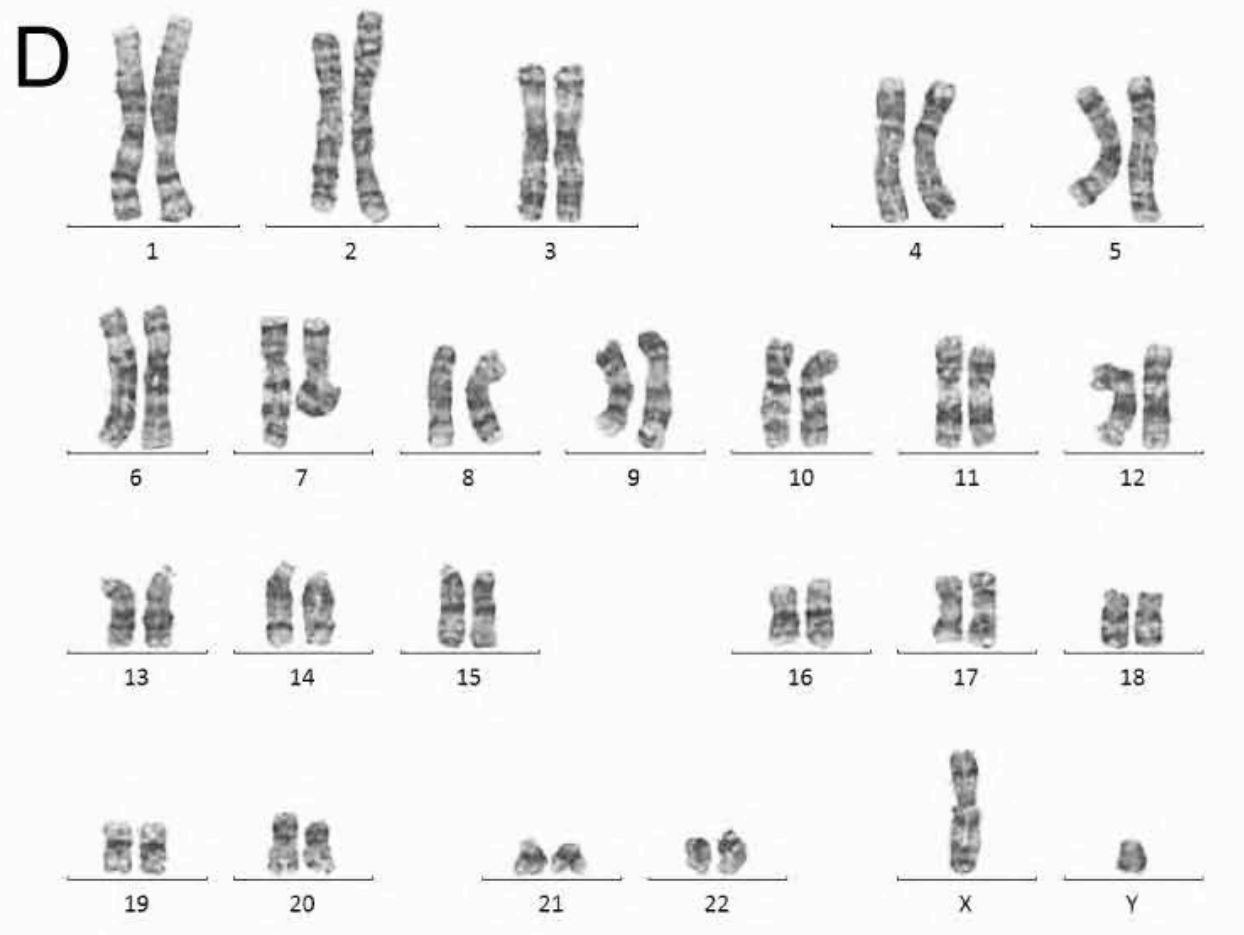


FIGURE 8E

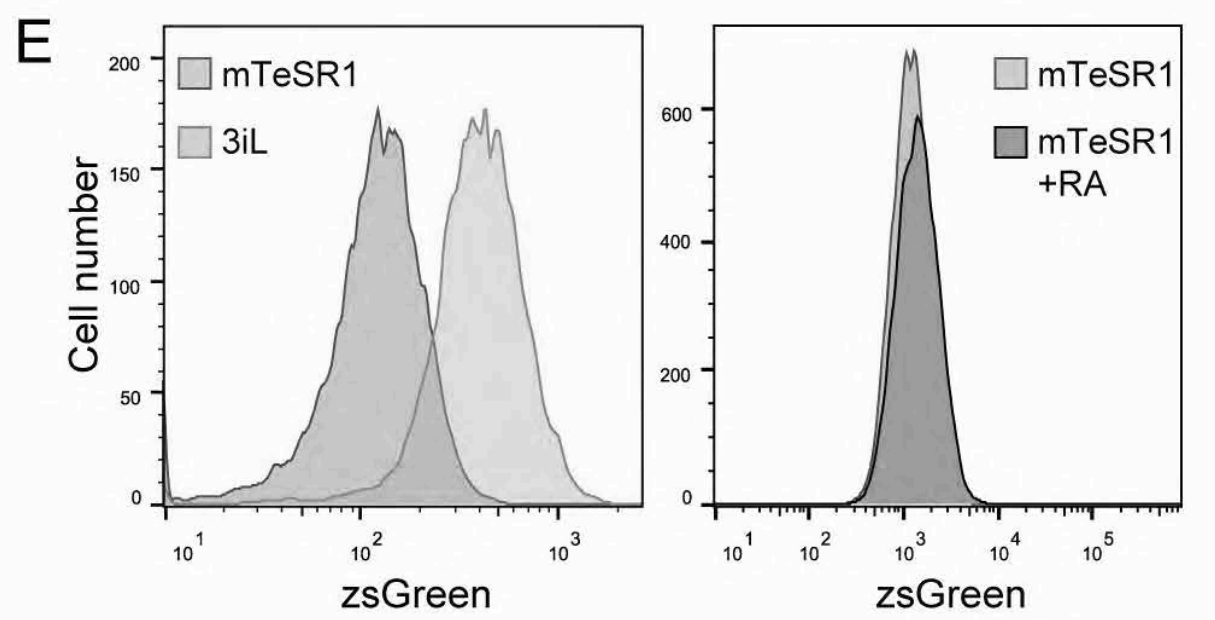

E
mTeSR1
3iL
Cell number
200
150
100
50
0
10^1
10^2
10^3
zsGreen
mTeSR1
mTeSR1
+RA
600
400
200
0
10^1
10^2
10^3
10^4
10^5
zsGreen

FIGURE 8F

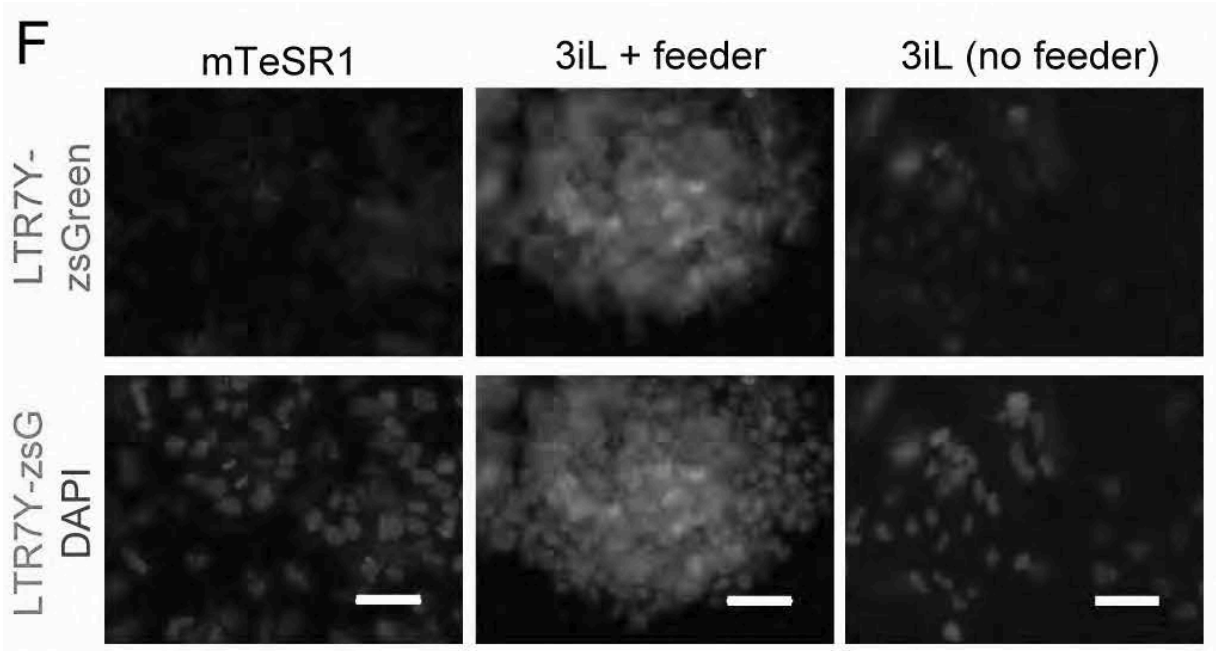

F
mTeSR1
3iL + feeder
3iL (no feeder)
LTR7Y-zsGreen
LTR7Y-zsG DAPI

FIGURE 8G

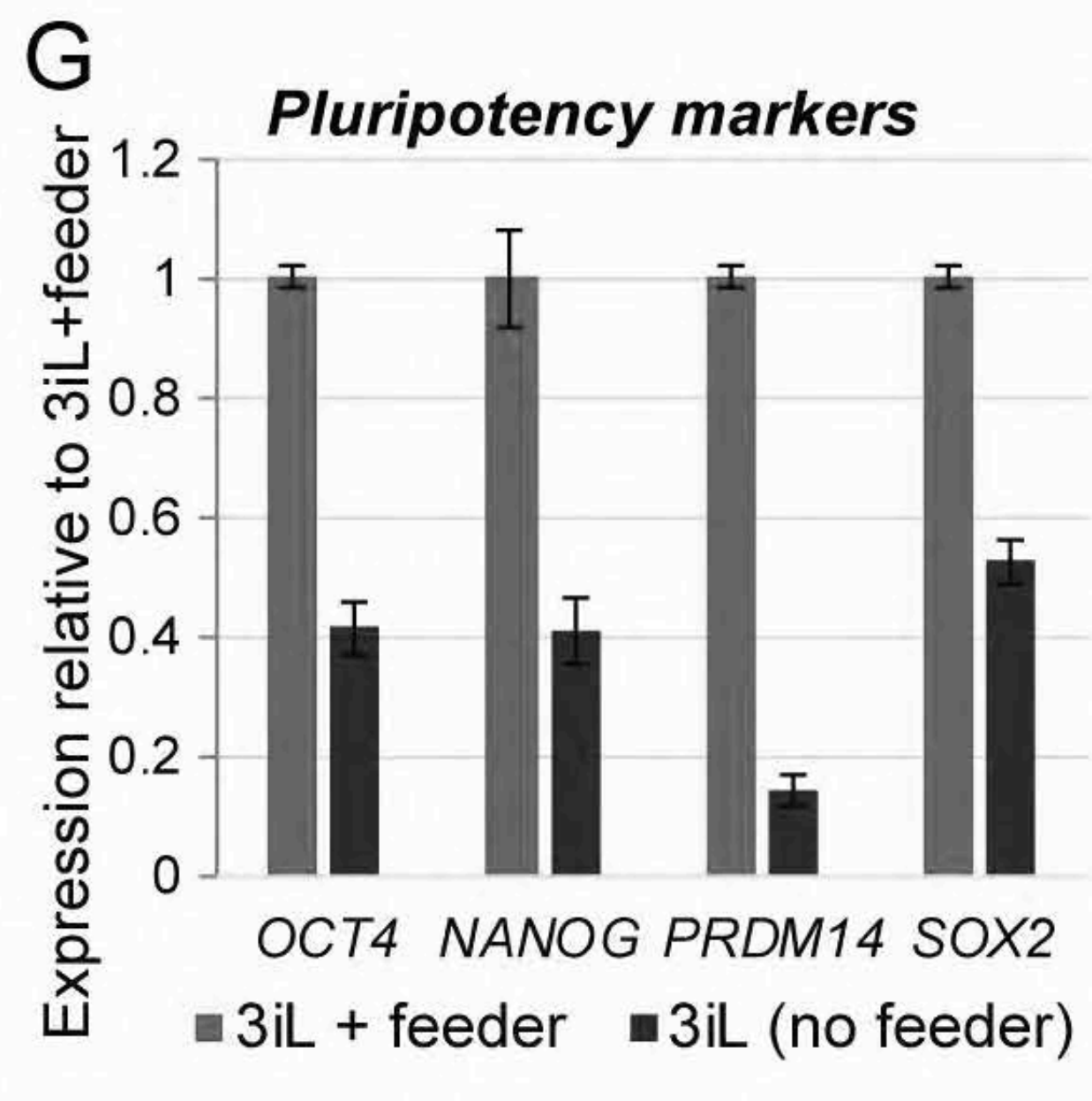

G
Pluripotency markers
Expression relative to 3iL+feeder
1.2
1
0.8
0.6
0.4
0.2
0
OCT4
NANOG
PRDM14
SOX2
3iL + feeder
3iL (no feeder)

FIGURE 8H

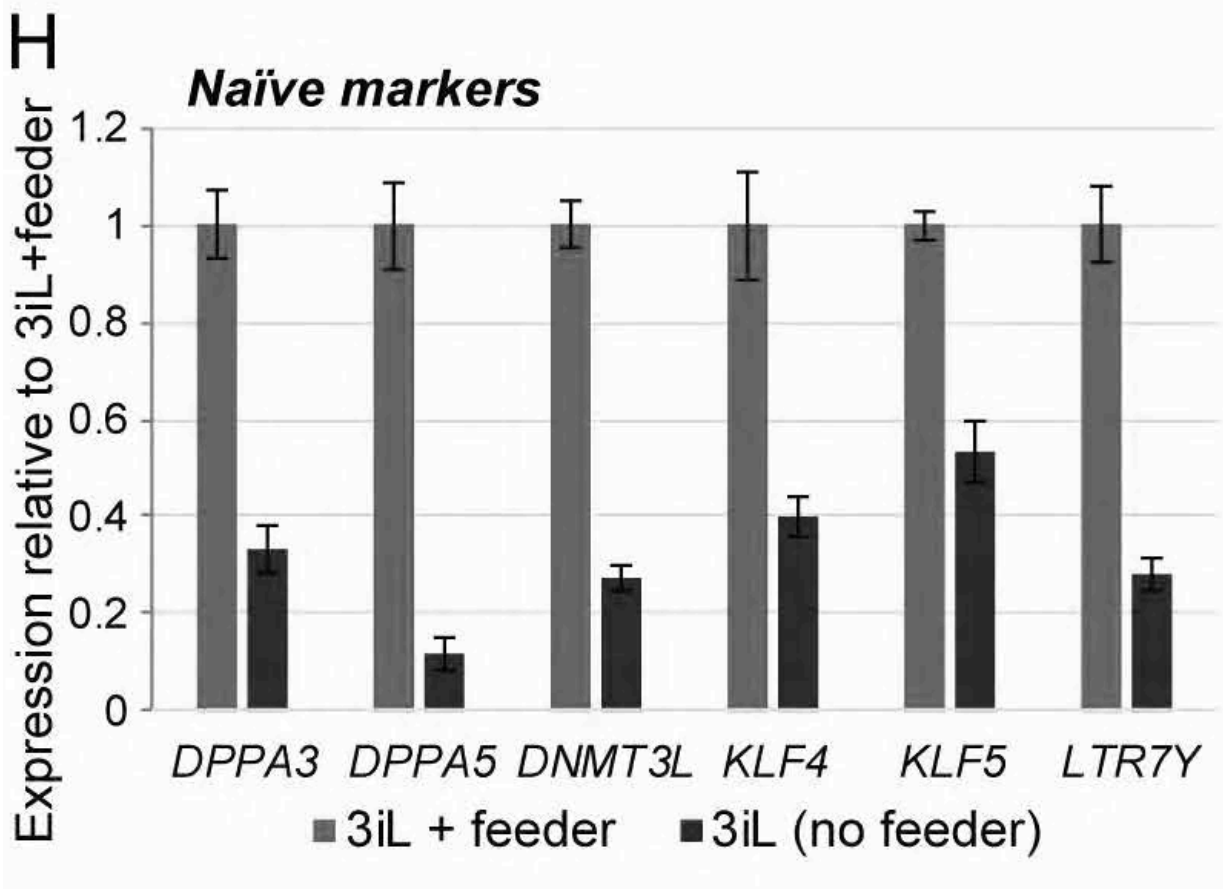

H
Naïve markers
Expression relative to 3iL+feeder
1.2
1
0.8
0.6
0.4
0.2
0
DPPA3
DPPA5
DNMT3L
KLF4
KLF5
LTR7Y
3iL + feeder
3iL (no feeder)

FIGURE 8I

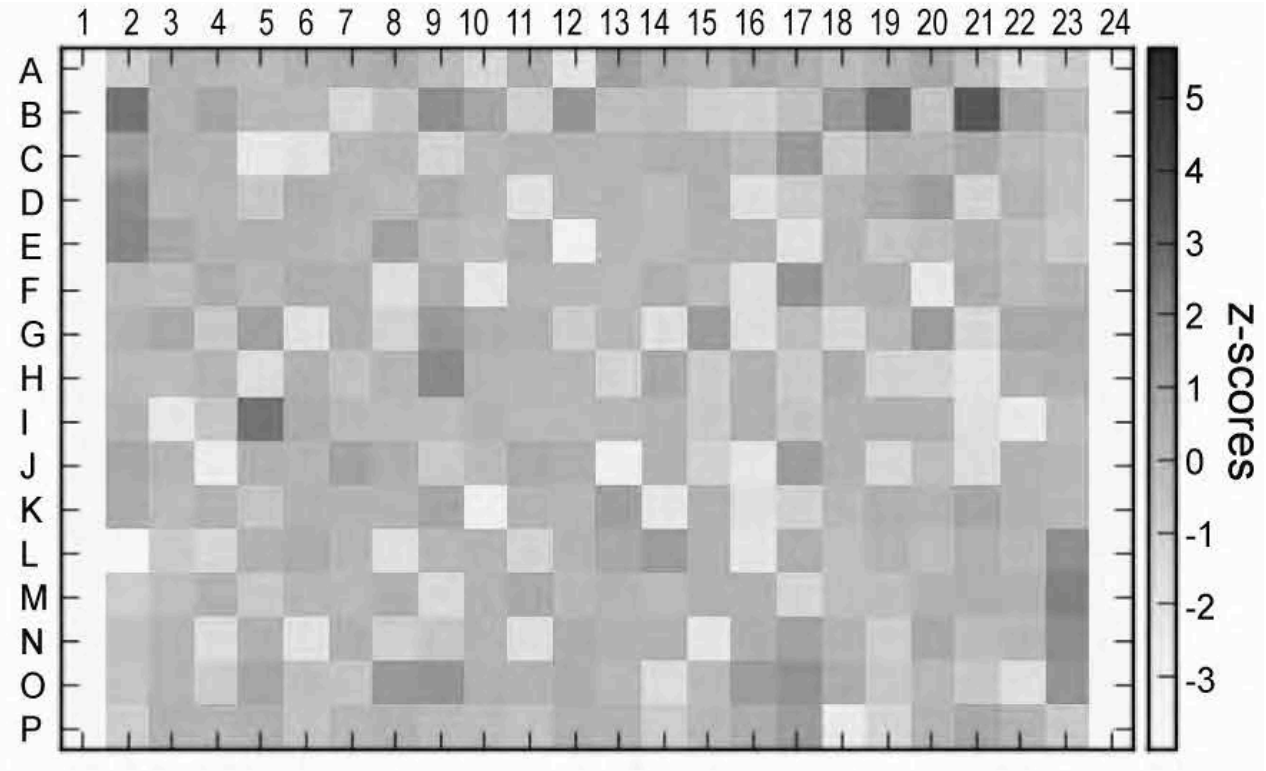

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
5
4
3
2
1
0
-1
-2
-3
Z-scores

FIGURE 8J

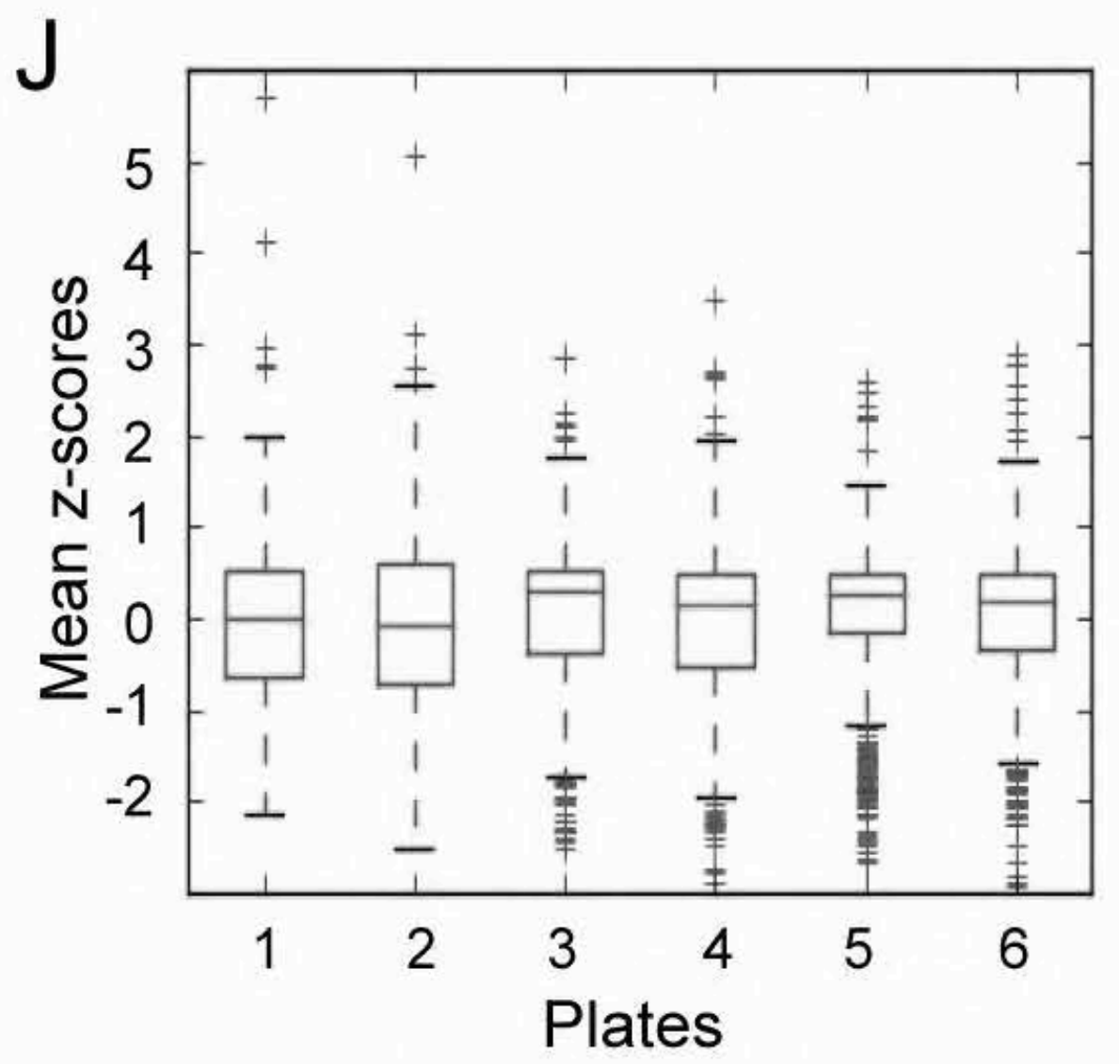


FIGURE 8K

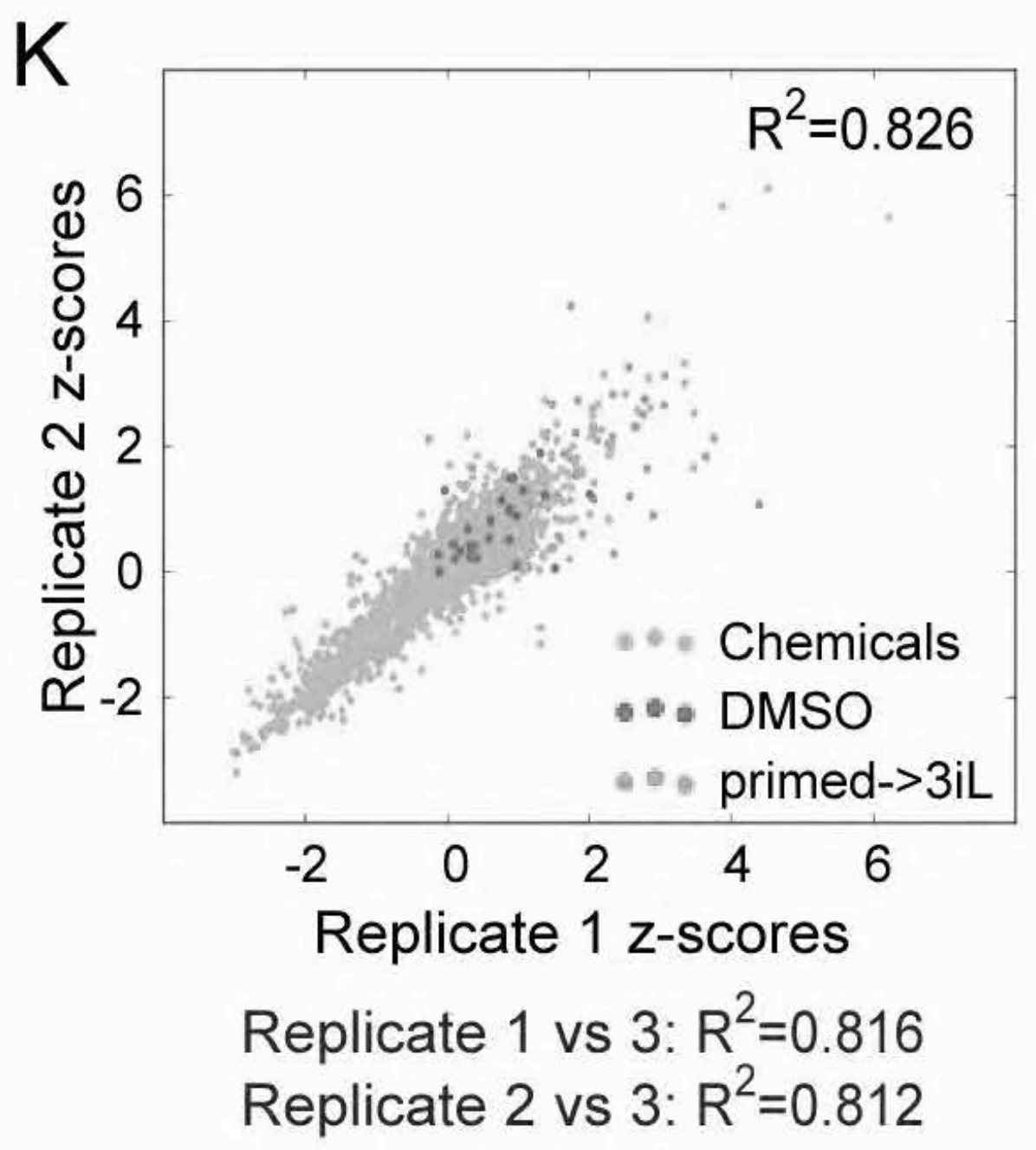

K
R^2=0.826
Replicate 2 z-scores
6
4
2
0
-2
Chemicals
DMSO
primed->3iL
-2
0
2
4
6
Replicate 1 z-scores
Replicate 1 vs 3: R^2=0.816
Replicate 2 vs 3: R^2=0.812

FIGURE 8L

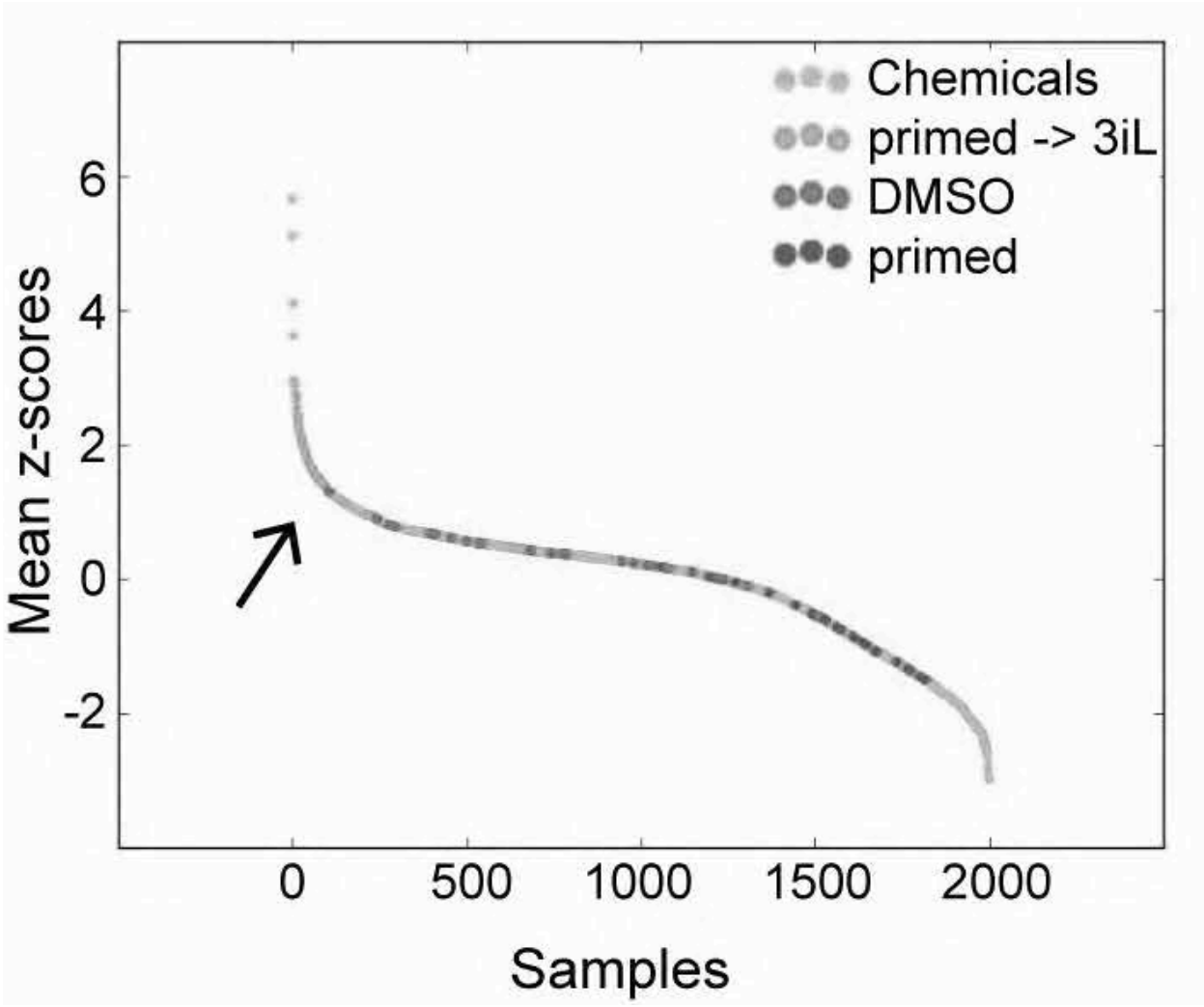

Chemicals
primed -> 3iL
DMSO
primed
Mean z-scores
6
4
2
0
-2
0
500
1000
1500
2000
Samples

FIGURE 8M

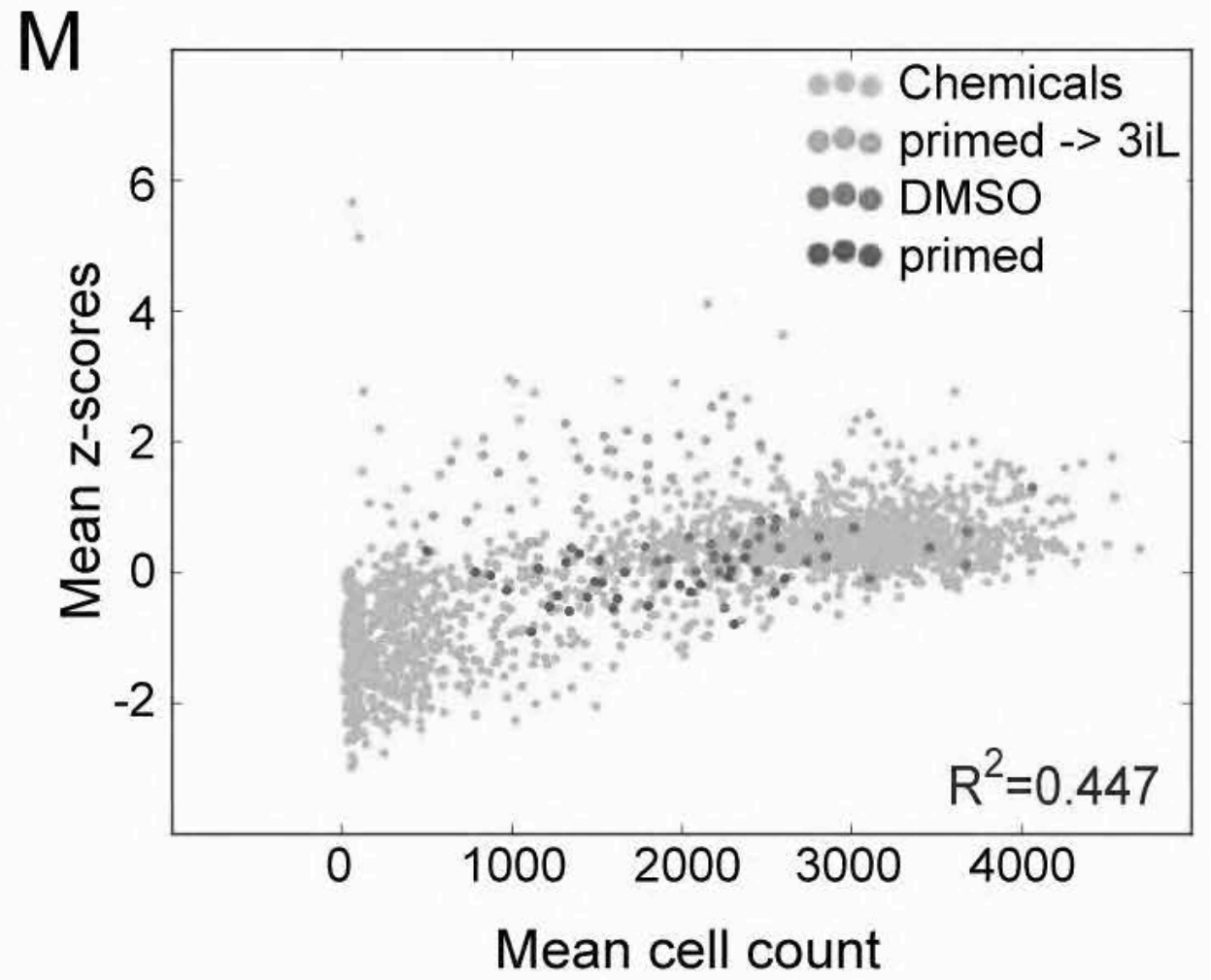

M
Chemicals
primed -> 3iL
DMSO
primed
Mean z-scores
6
4
2
0
-2
0
1000
2000
3000
4000
Mean cell count
R2=0.447

FIGURE 9A

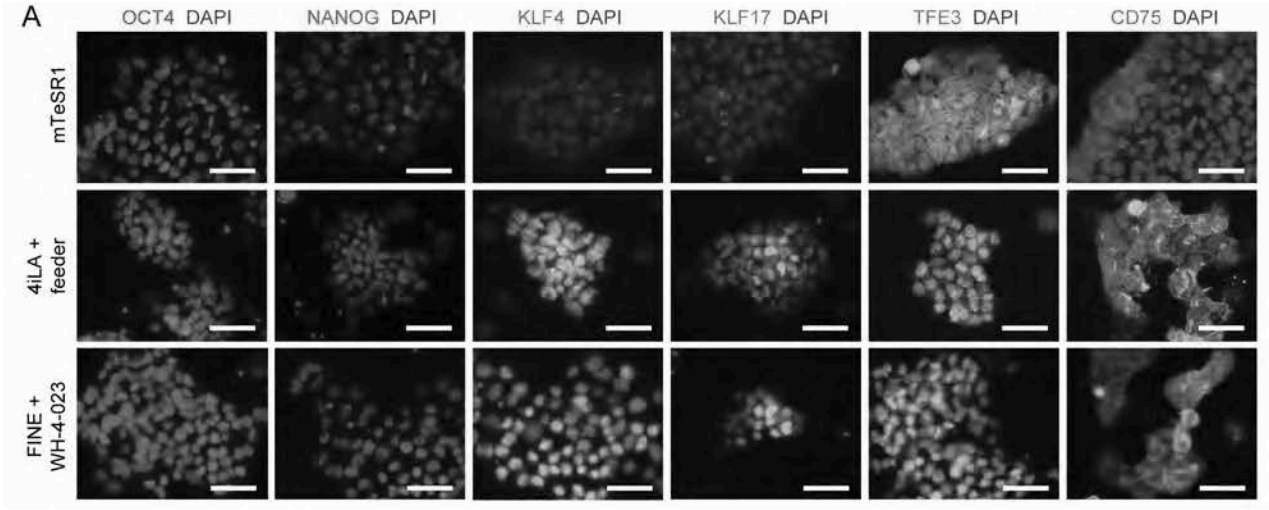

A
OCT4 DAPI
NANOG DAPI
KLF4 DAPI
KLF17 DAPI
TFE3 DAPI
CD75 DAPI
mTeSR1
4iLA + feeder
FINE + WH-4-023

FIGURE 9B

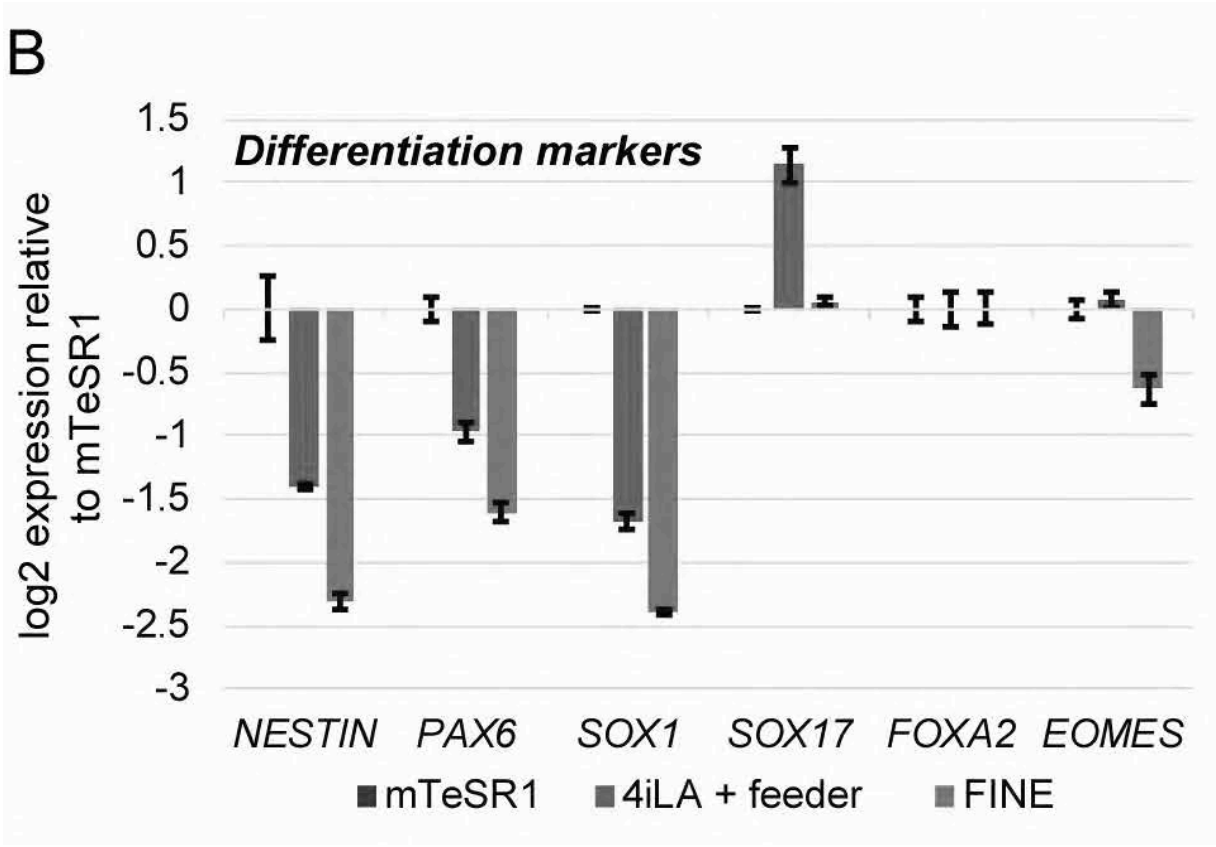

B
Differentiation markers
log2 expression relative to mTeSR1
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3
NESTIN
PAX6
SOX1
SOX17
FOXA2
EOMES
mTeSR1
4iLA + feeder
FINE

FIGURE 9C

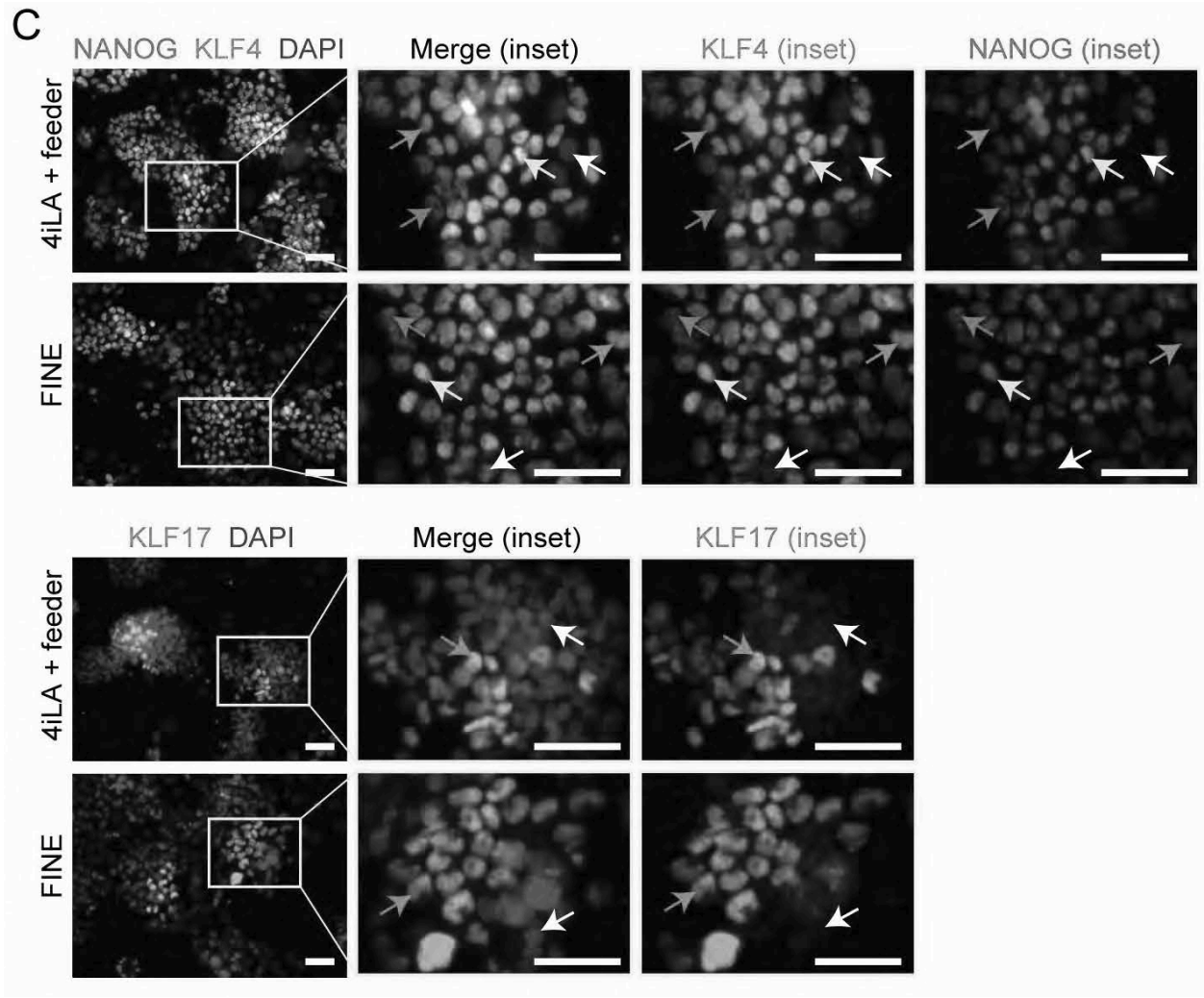

C
NANOG KLF4 DAPI
Merge (inset)
KLF4 (inset)
NANOG (inset)
4iLA + feeder
FINE
KLF17 DAPI
Merge (inset)
KLF17 (inset)
4iLA + feeder
FINE

FIGURE 9D

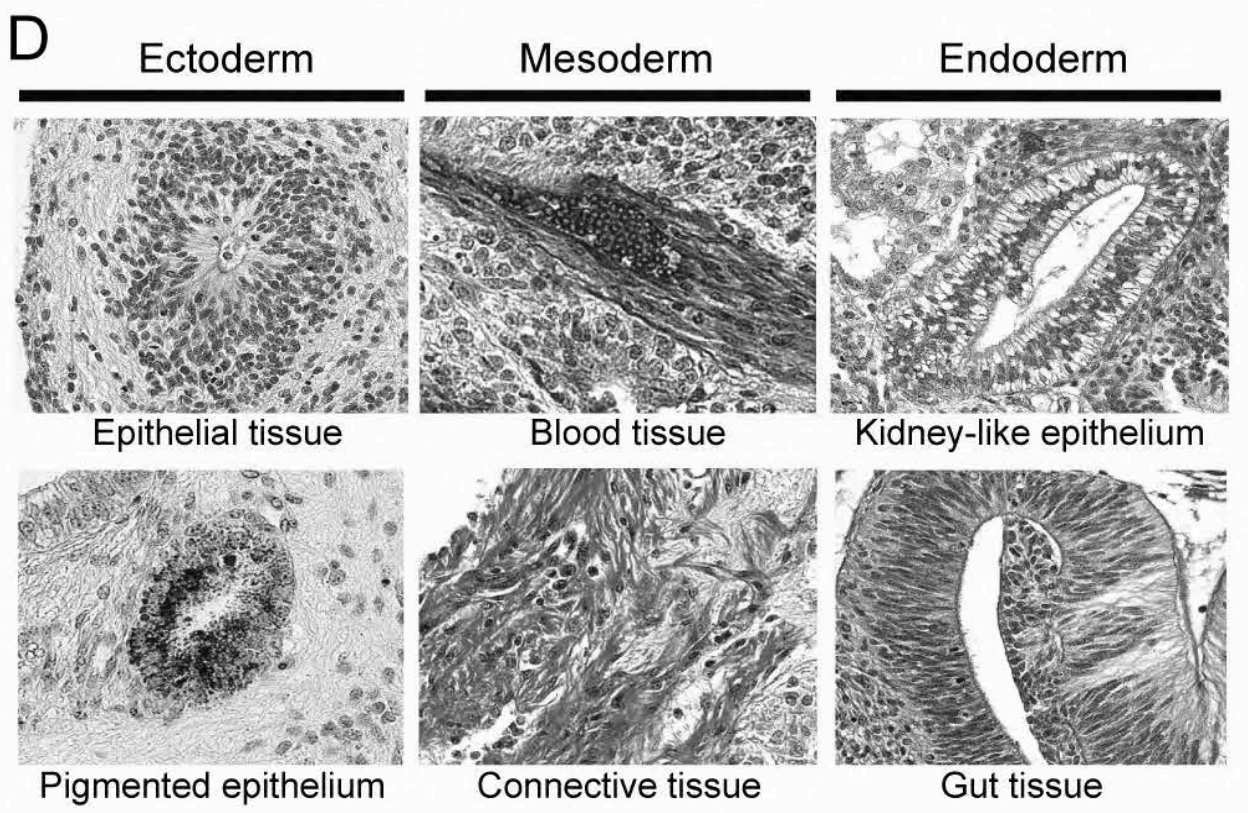

D
Ectoderm
Mesoderm
Endoderm
Epithelial tissue
Blood tissue
Kidney-like epithelium
Pigmented epithelium
Connective tissue
Gut tissue

FIGURE 9E

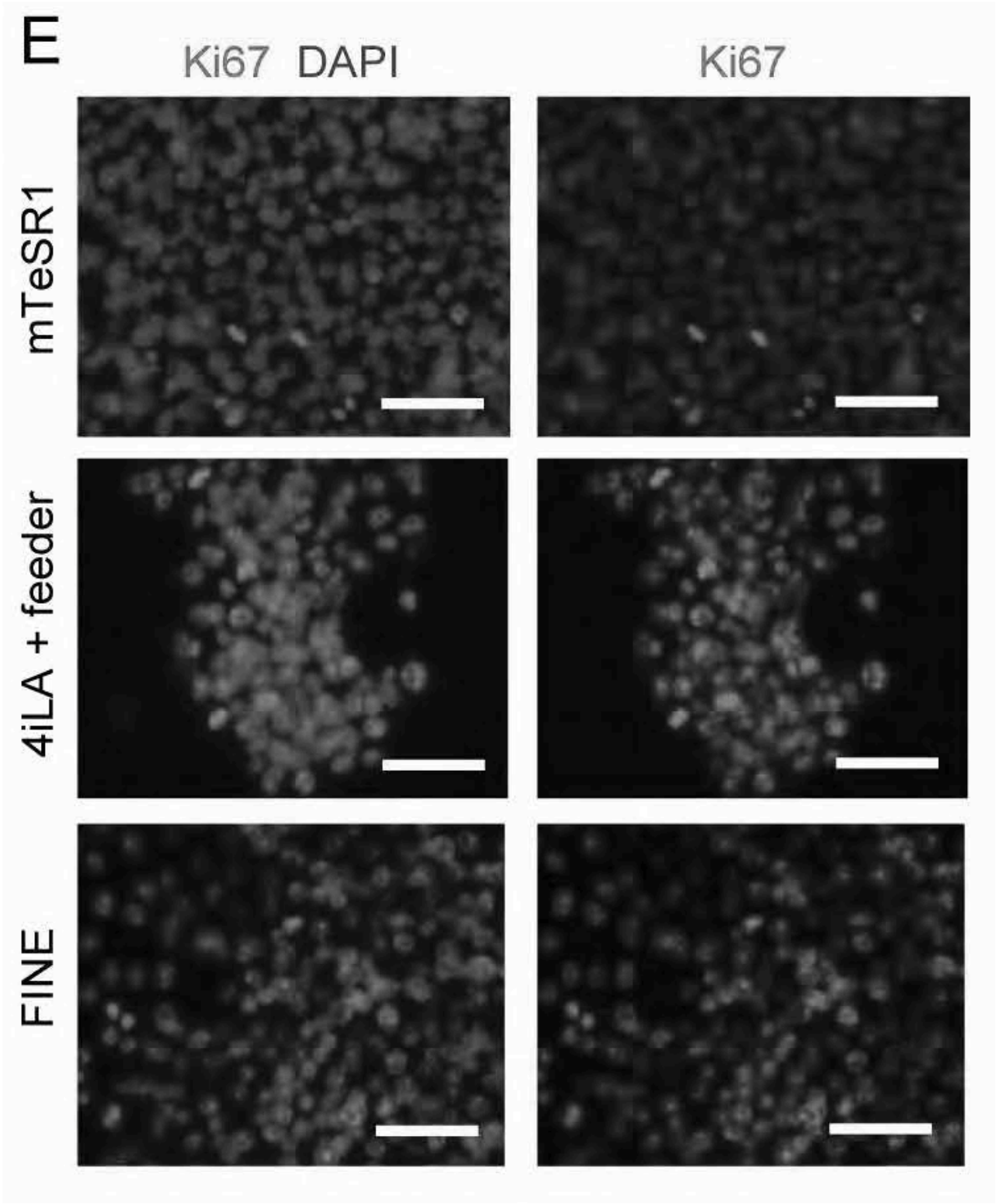


FIGURE 9F

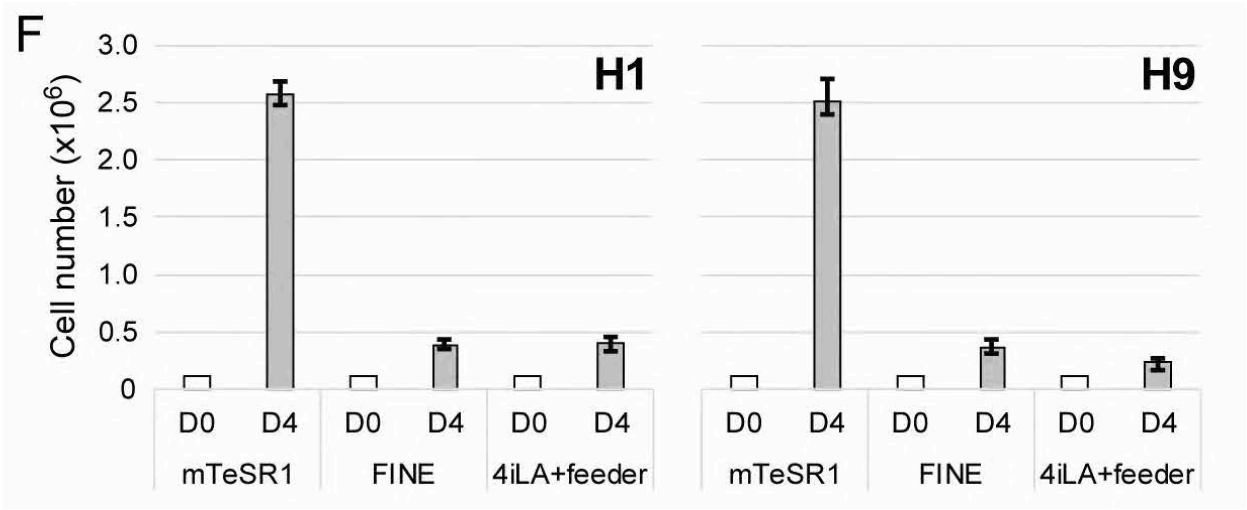

F
Cell number (x10^6)
3.0
2.5
2.0
1.5
1.0
0.5
0
H1
H9
D0
D4
mTeSR1
FINE
4iLA+feeder

FIGURE 9G

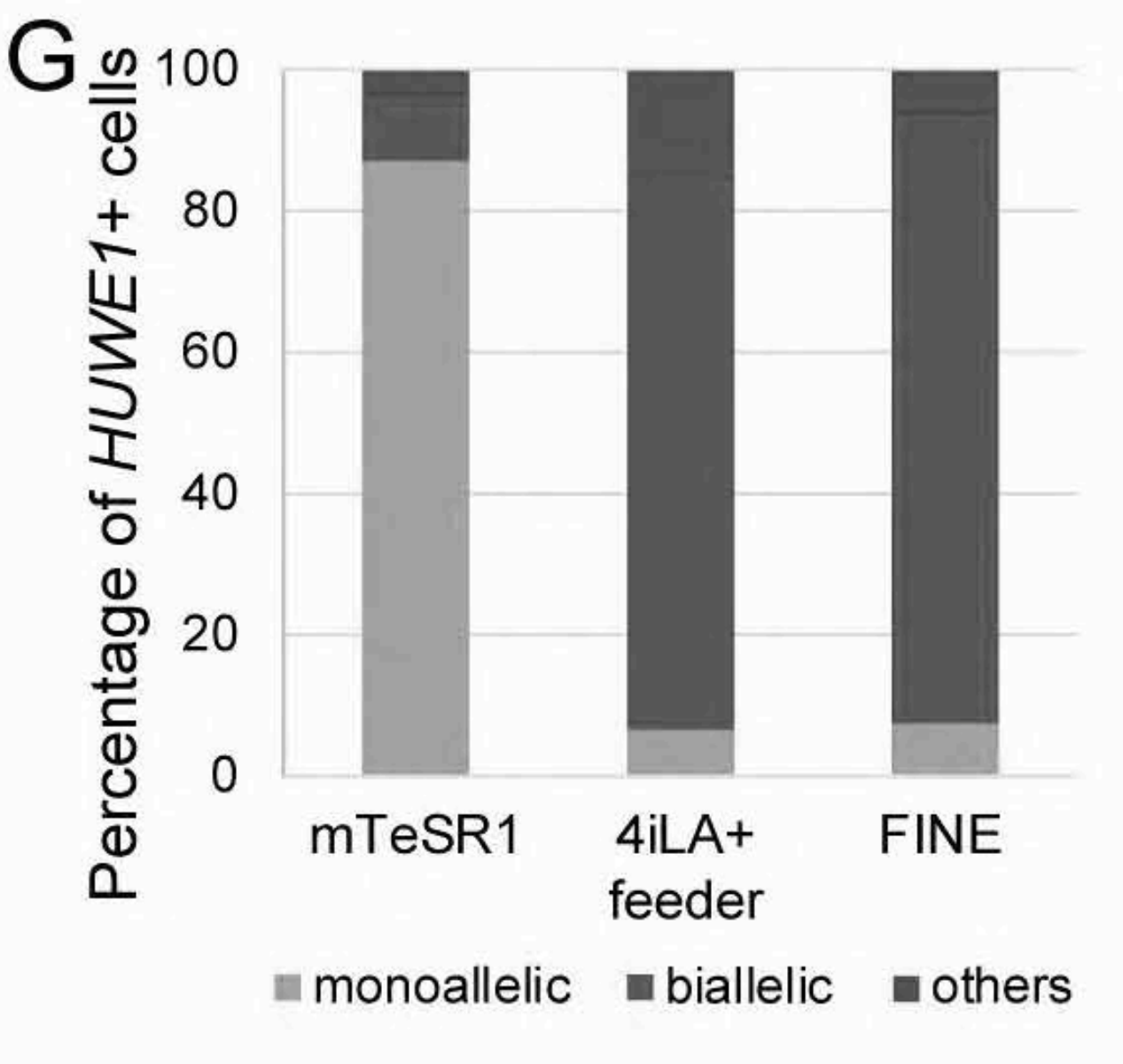

G
Percentage of HUWE1+ cells
100
80
60
40
20
0
mTeSR1
4iLA+ feeder
FINE
monoallelic
biallelic
others

FIGURE 9H

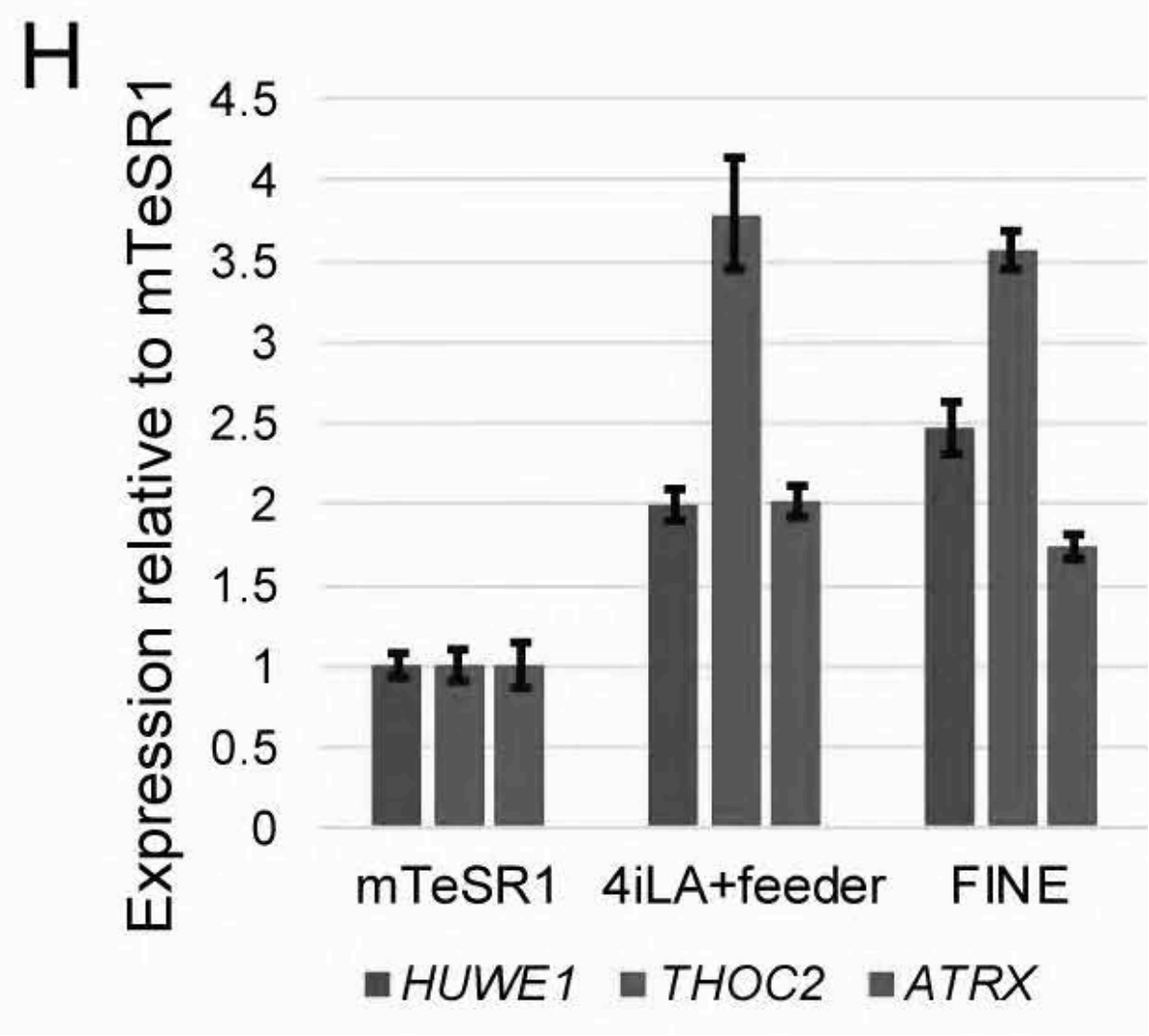

H
Expression relative to mTeSR1
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
mTeSR1
4iLA+feeder
FINE
HUWE1
THOC2
ATRX

FIGURE 10A

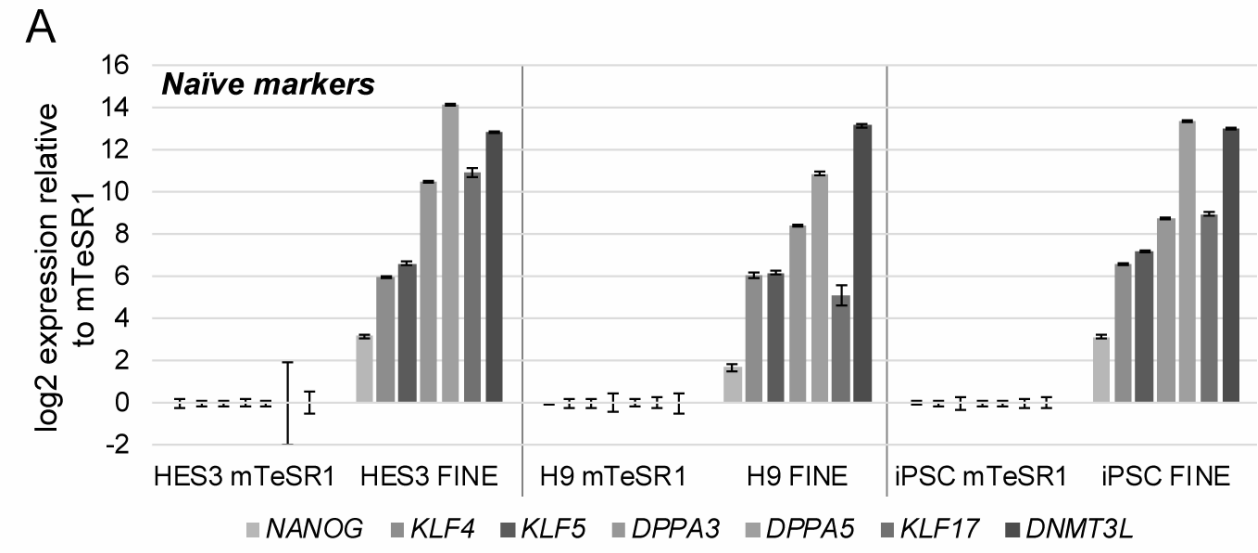

A
Naïve markers
log2 expression relative to mTeSR1
16
14
12
10
8
6
4
2
0
-2
HES3 mTeSR1
HES3 FINE
H9 mTeSR1
H9 FINE
iPSC mTeSR1
iPSC FINE
NANOG
KLF4
KLF5
DPPA3
DPPA5
KLF17
DNMT3L

FIGURE 10B

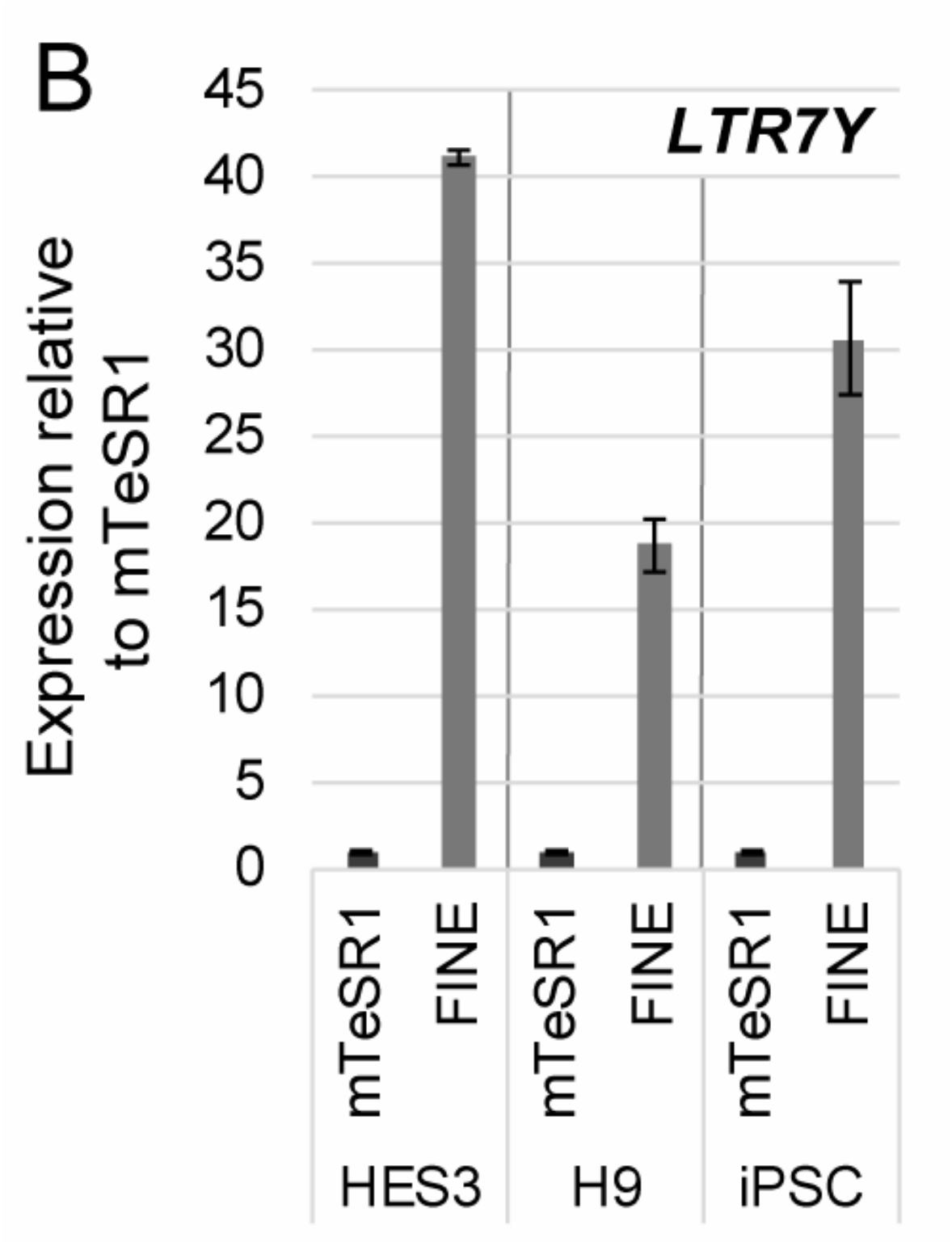

B
LTR7Y
Expression relative to mTeSR1
45
40
35
30
25
20
15
10
5
0
mTeSR1
FINE
mTeSR1
FINE
mTeSR1
FINE
HES3
H9
iPSC

FIGURE 10C

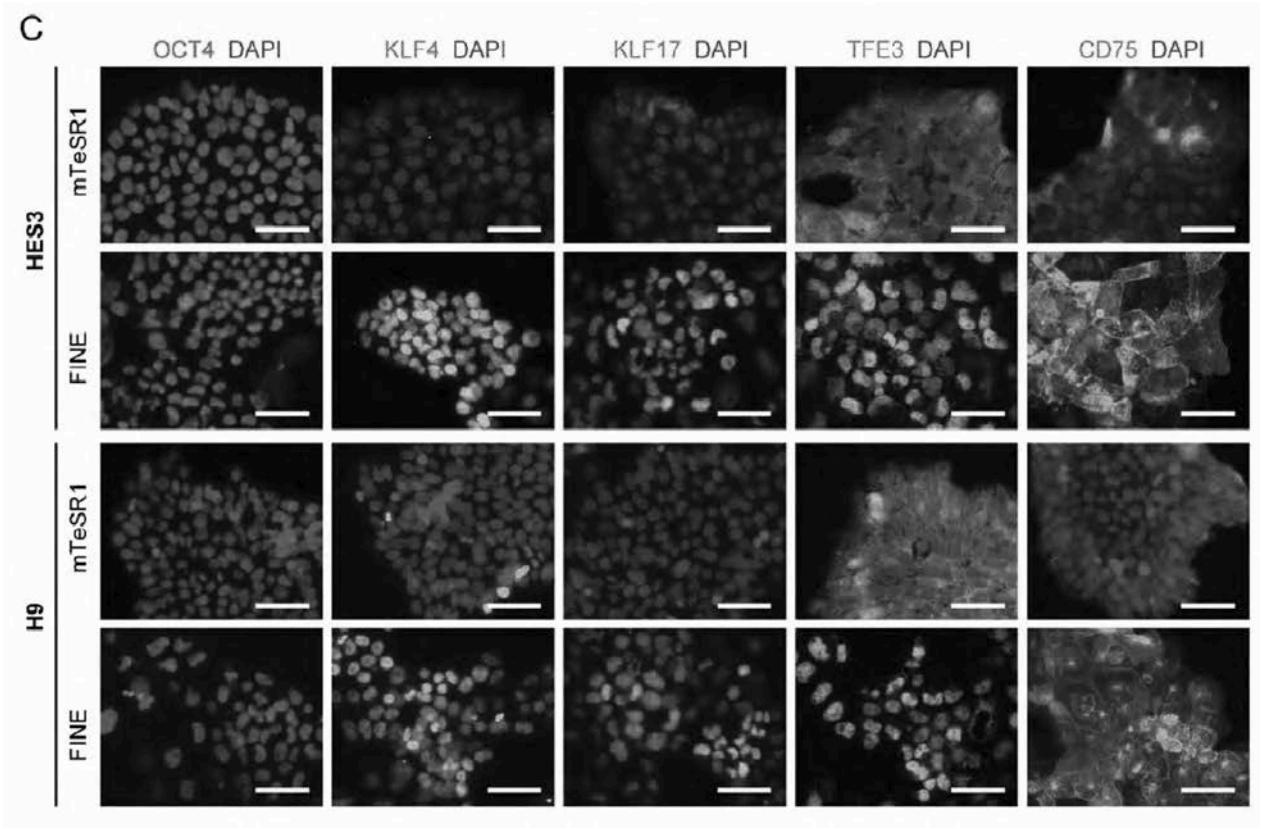

C
OCT4 DAPI
KLF4 DAPI
KLF17 DAPI
TFE3 DAPI
CD75 DAPI
HES3
mTeSR1
FINE
H9
mTeSR1
FINE

FIGURE 11A

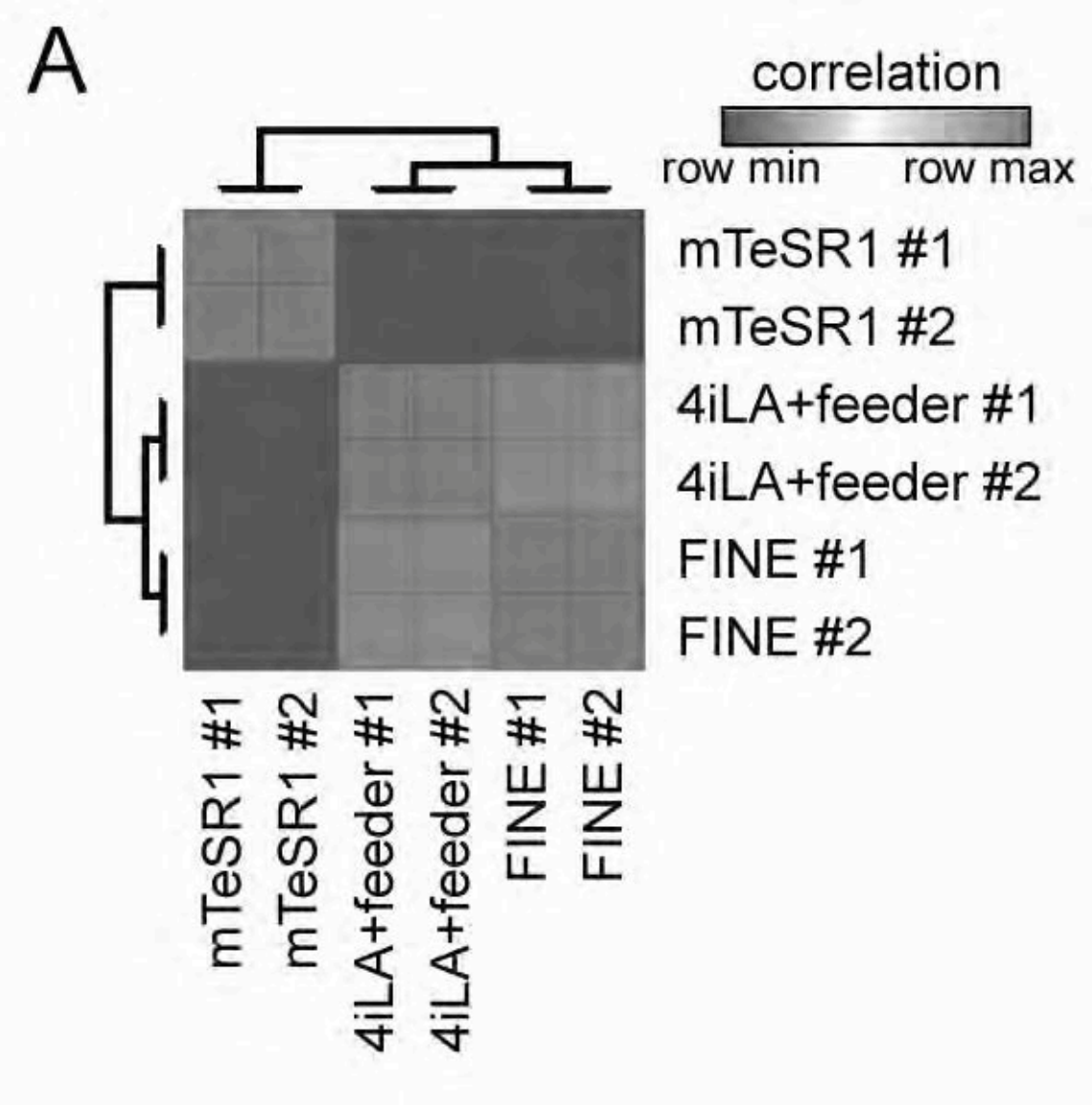

A
correlation
row min
row max
mTeSR1 #1
mTeSR1 #2
4iLA+feeder #1
4iLA+feeder #2
FINE #1
FINE #2
mTeSR1 #1
mTeSR1 #2
4iLA+feeder #1
4iLA+feeder #2
FINE #1
FINE #2

FIGURE 11B

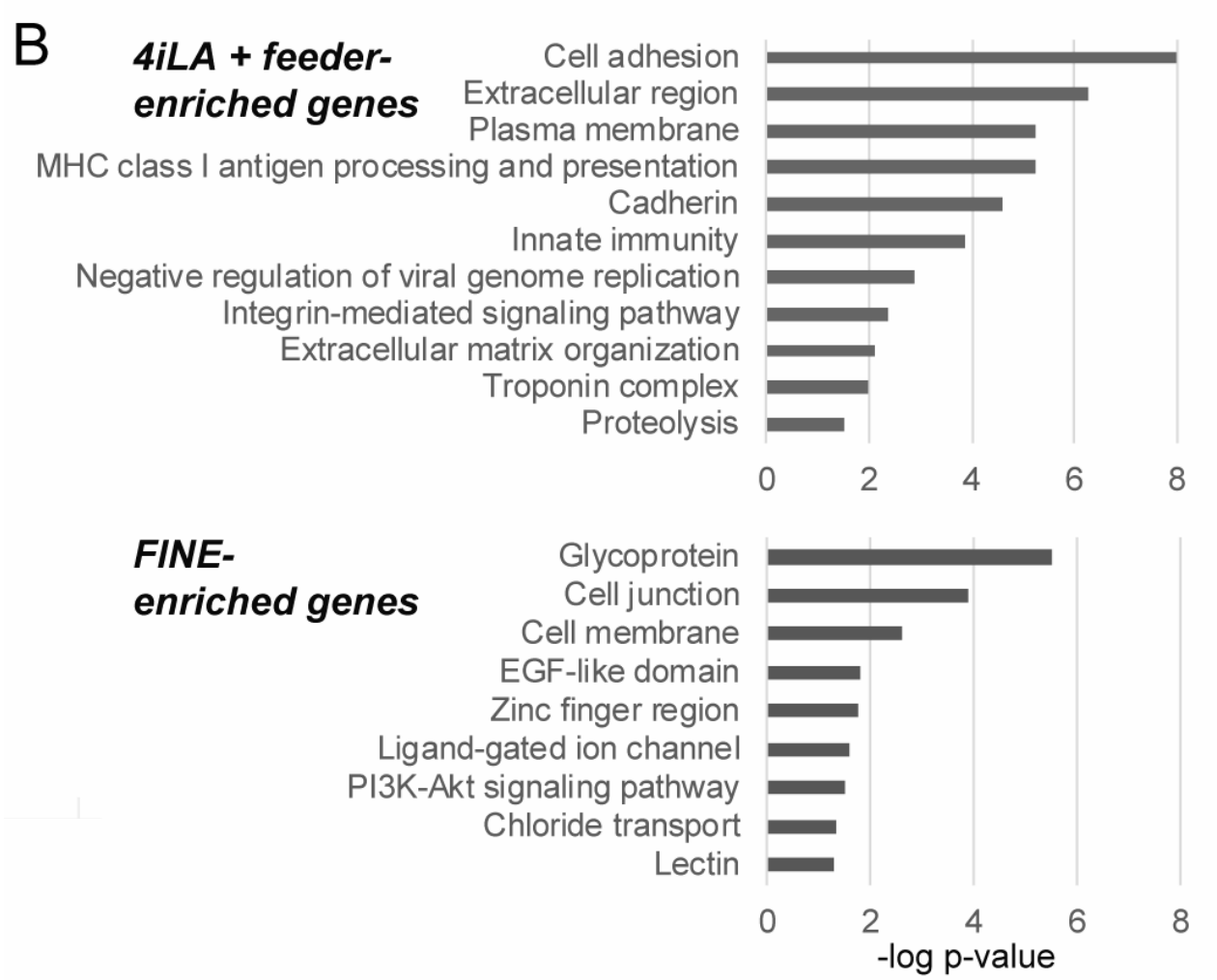

B
4iLA + feeder-enriched genes
Cell adhesion
Extracellular region
Plasma membrane
MHC class I antigen processing and presentation
Cadherin
Innate immunity
Negative regulation of viral genome replication
Integrin-mediated signaling pathway
Extracellular matrix organization
Troponin complex
Proteolysis
0
2
4
6
8
FINE-enriched genes
Glycoprotein
Cell junction
Cell membrane
EGF-like domain
Zinc finger region
Ligand-gated ion channel
PI3K-Akt signaling pathway
Chloride transport
Lectin
0
2
4
6
8
-log p-value

FIGURE 11C

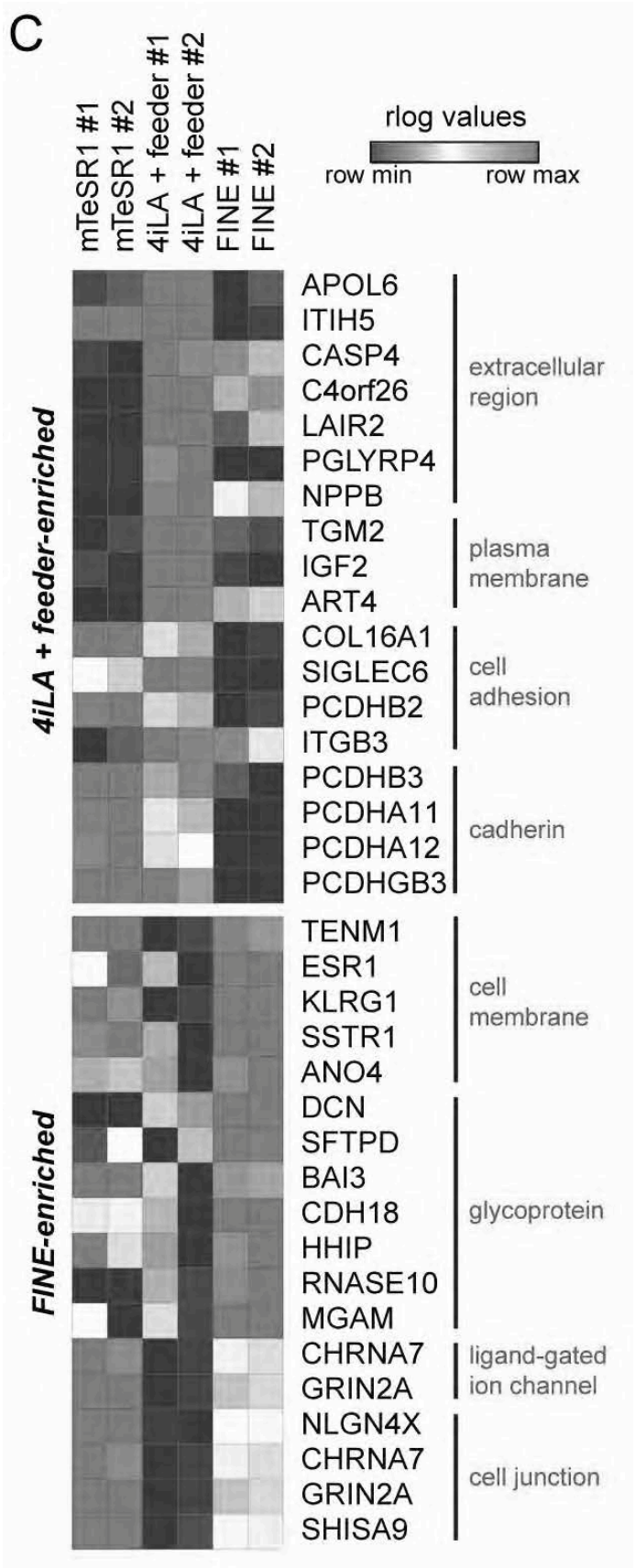

C
mTeSR1 #1
mTeSR1 #2
4iLA + feeder #1
4iLA + feeder #2
FINE #1
FINE #2
rlog values
row min
row max
4iLA + feeder-enriched
APOL6
ITIH5
CASP4
C4orf26
LAIR2
PGLYRP4
NPPB
extracellular region
TGM2
IGF2
ART4
plasma membrane
COL16A1
SIGLEC6
PCDHB2
ITGB3
cell adhesion
PCDHB3
PCDHA11
PCDHA12
PCDHGB3
cadherin
FINE-enriched
TENM1
ESR1
KLRG1
SSTR1
ANO4
cell membrane
DCN
SFTPD
BAI3
CDH18
HHIP
RNASE10
MGAM
glycoprotein
CHRNA7
GRIN2A
ligand-gated ion channel
NLGN4X
CHRNA7
GRIN2A
SHISA9
cell junction

FIGURE 11D

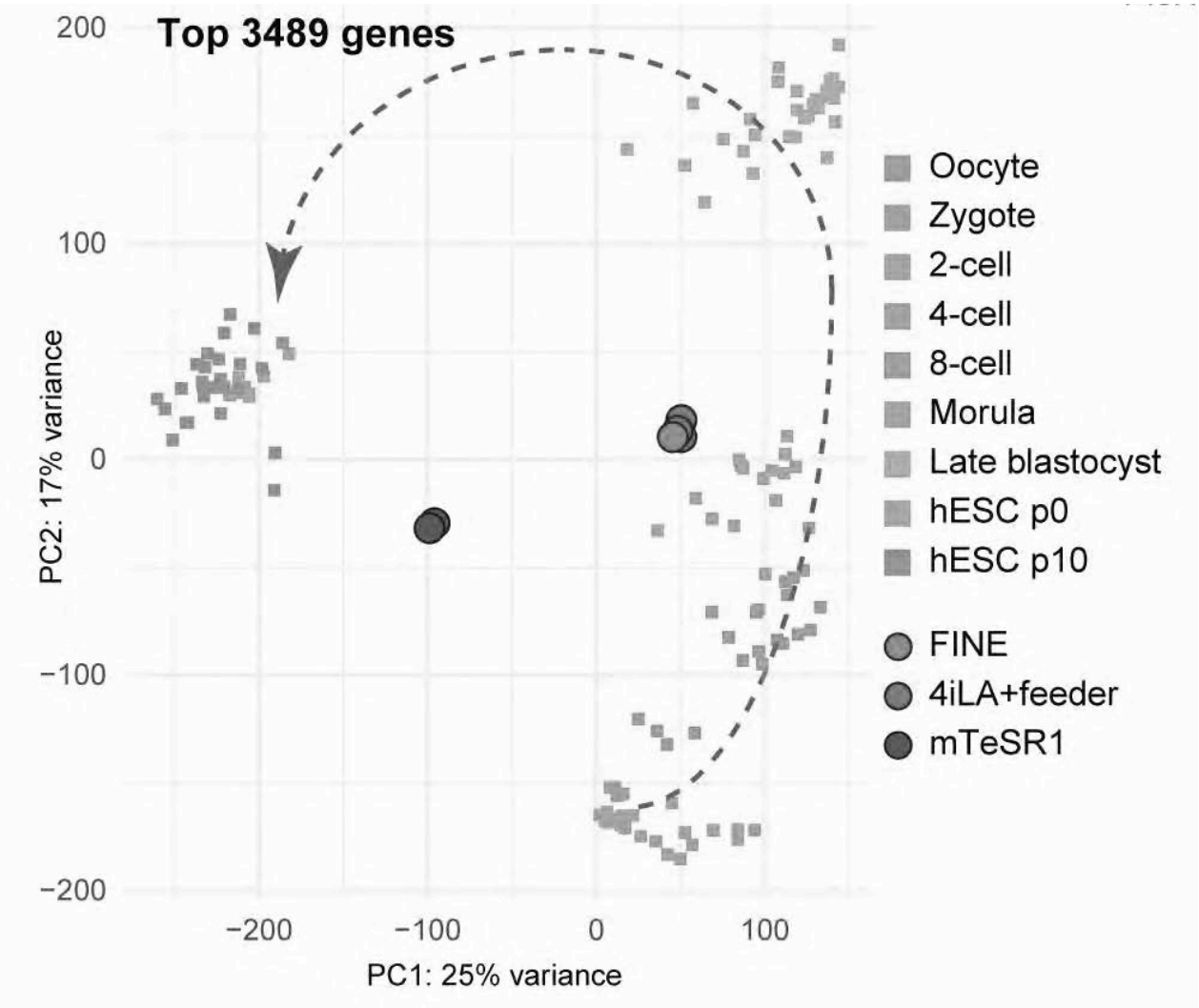

Top 3489 genes
200
100
0
−100
−200
PC2: 17% variance
−200
−100
0
100
PC1: 25% variance
Oocyte
Zygote
2-cell
4-cell
8-cell
Morula
Late blastocyst
hESC p0
hESC p10
FINE
4iLA+feeder
mTeSR1

FIGURE 11E

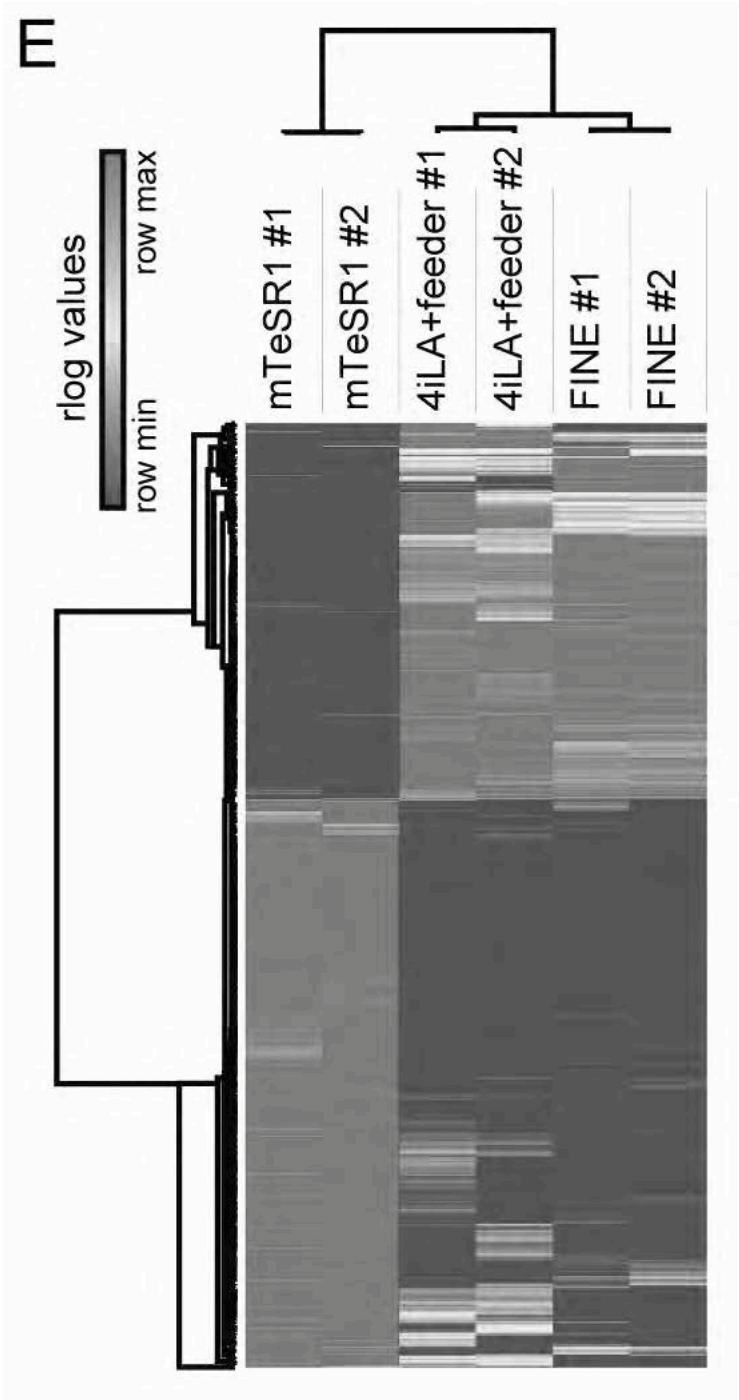

E
rlog values
row max
row min
mTeSR1 #1
mTeSR1 #2
4iLA+feeder #1
4iLA+feeder #2
FINE #1
FINE #2

FIGURE 12A

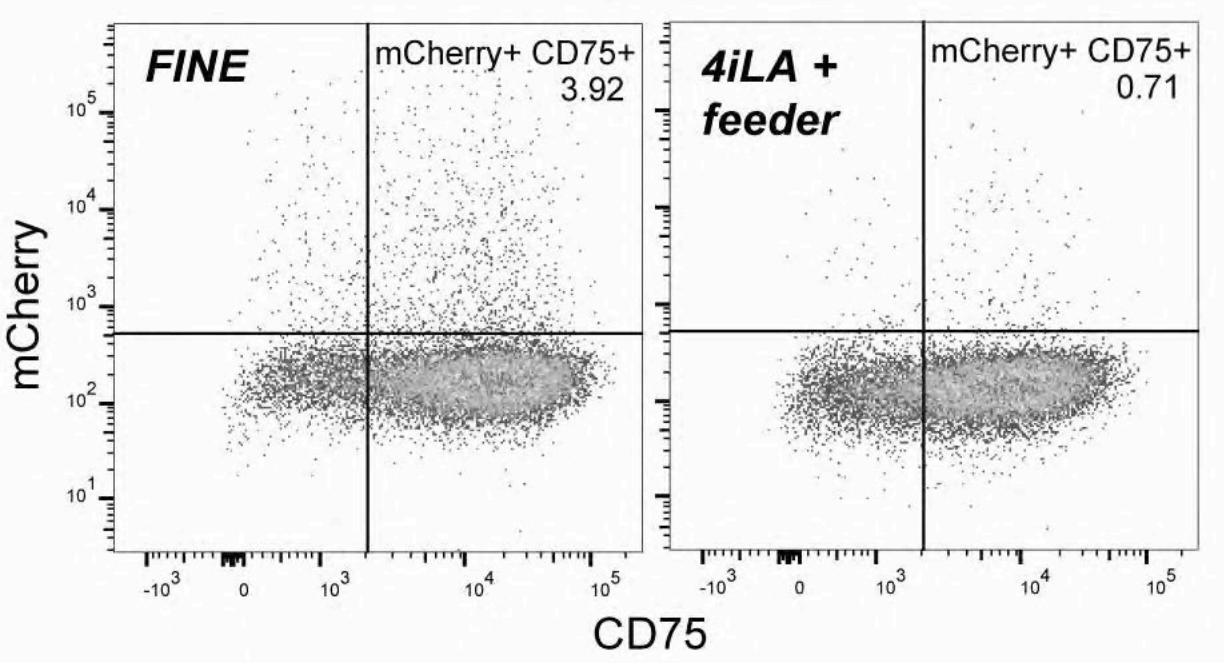

FINE
mCherry+ CD75+
3.92
4iLA +
feeder
mCherry+ CD75+
0.71
mCherry
CD75
10^5
10^4
10^3
10^2
10^1
-10^3
0
10^3
10^4
10^5

FIGURE 12B

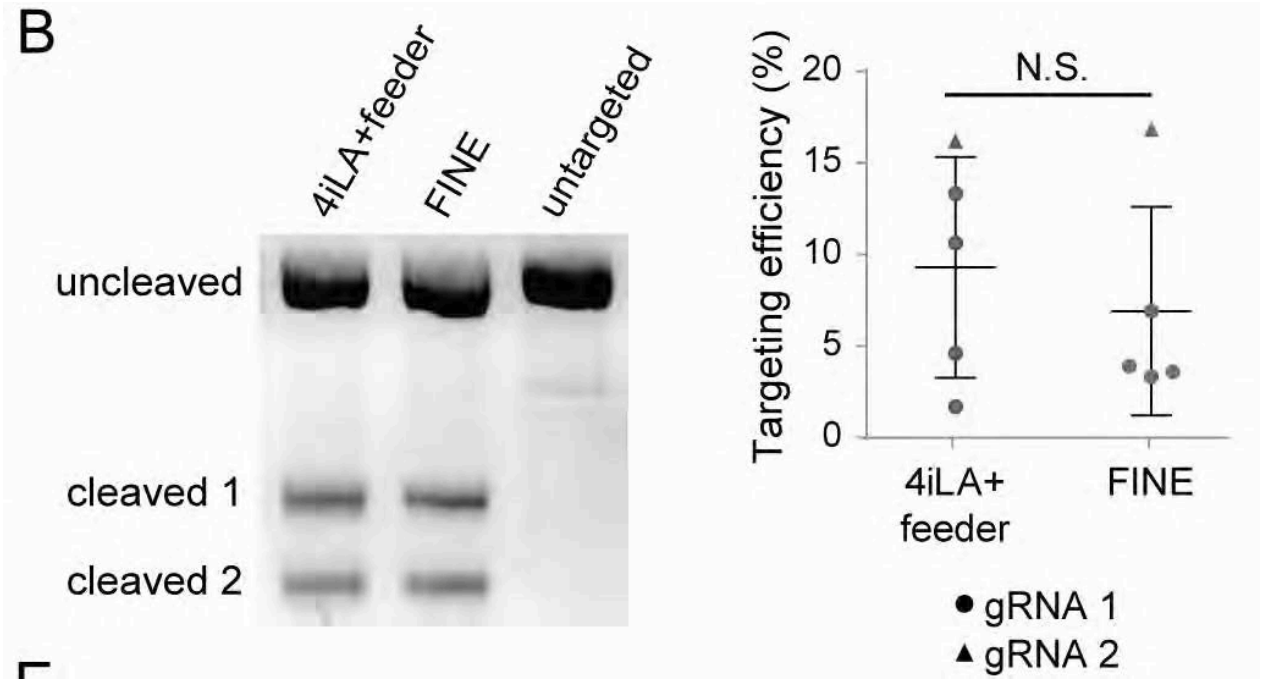

B
4iLA+feeder
FINE
untargeted
uncleaved
cleaved 1
cleaved 2
Targeting efficiency (%)
20
15
10
5
0
N.S.
4iLA+ feeder
FINE
gRNA 1
gRNA 2

FIGURE 12C

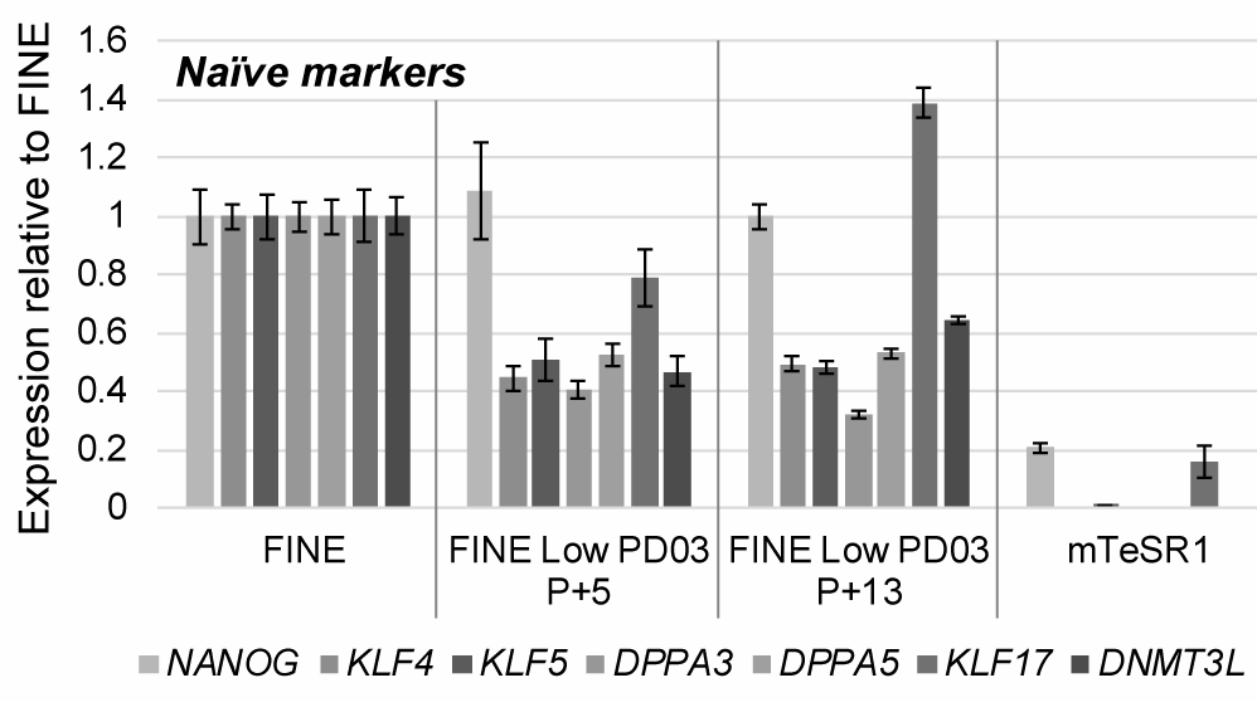

Naïve markers
Expression relative to FINE
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
FINE
FINE Low PD03 P+5
FINE Low PD03 P+13
mTeSR1
NANOG
KLF4
KLF5
DPPA3
DPPA5
KLF17
DNMT3L

FIGURE 12D

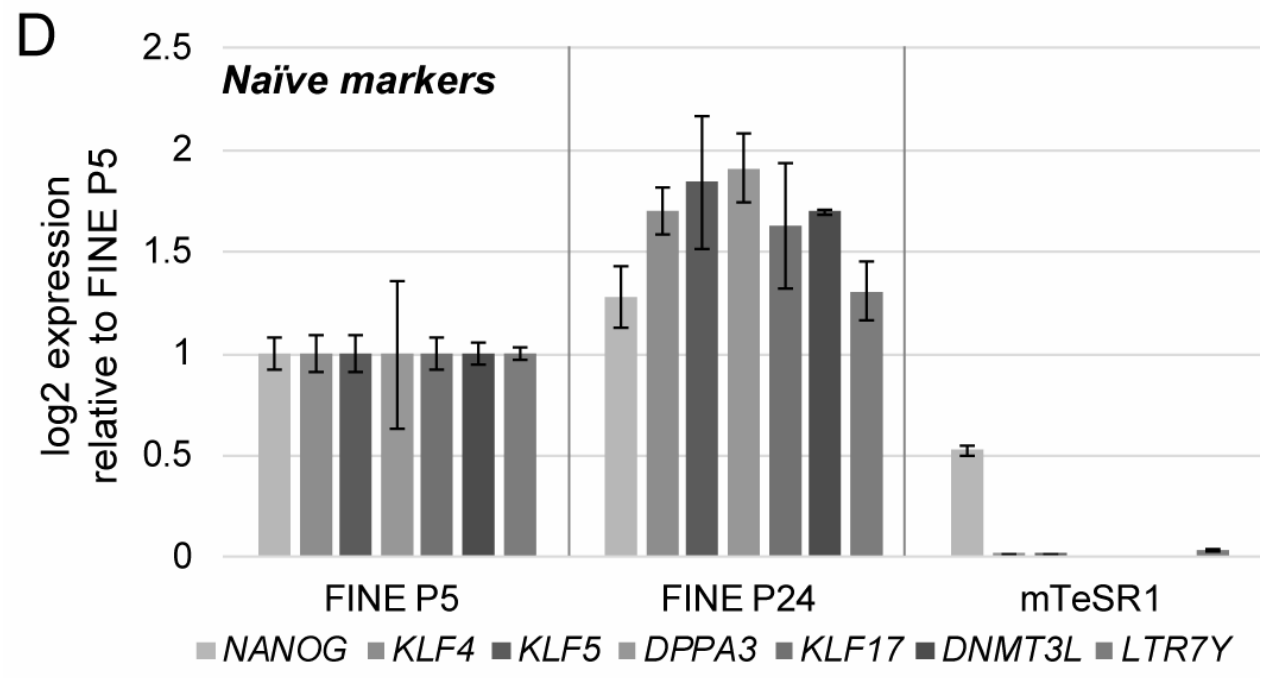
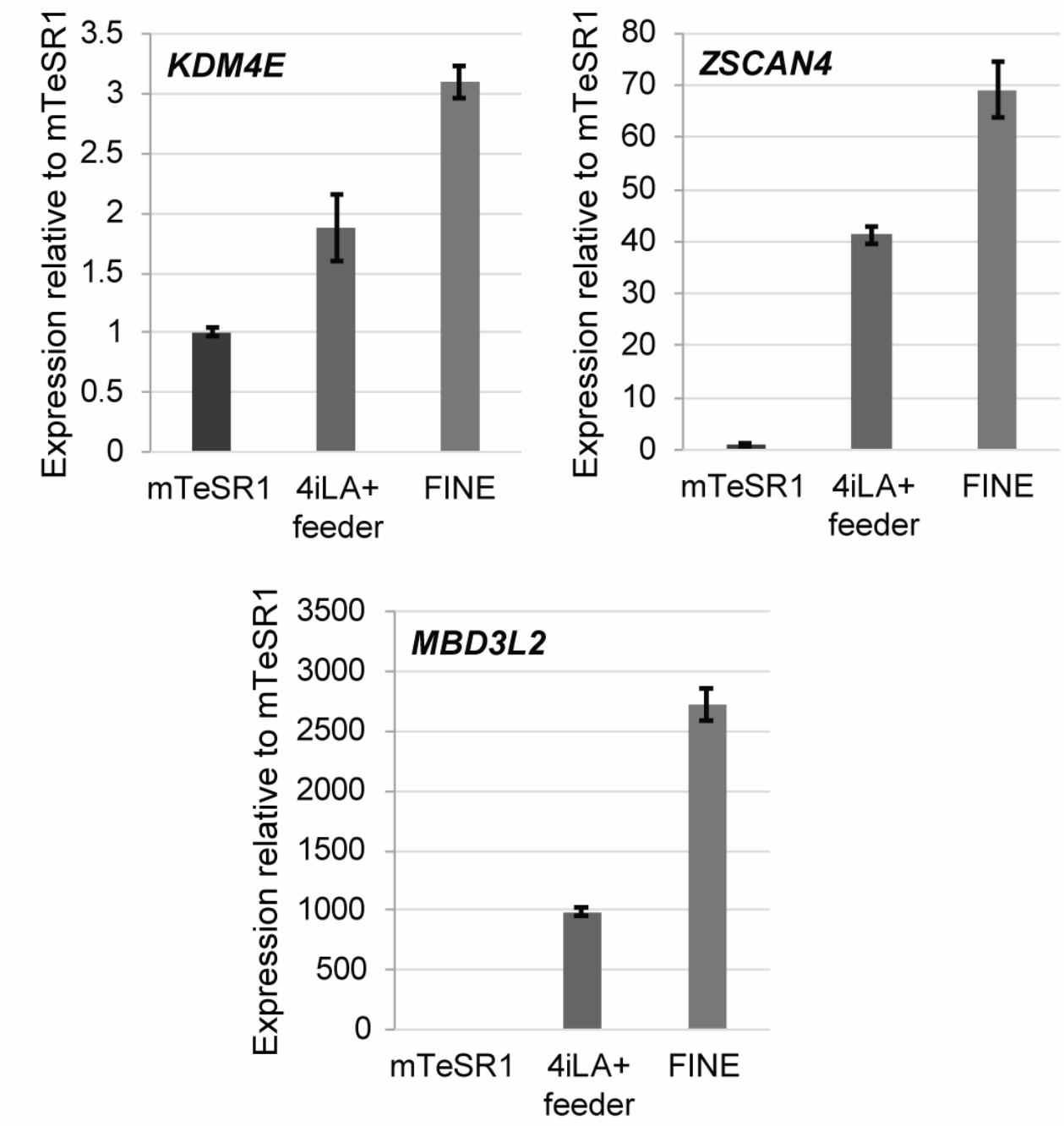

D
Naïve markers
log2 expression relative to FINE P5
2.5
2
1.5
1
0.5
0
FINE P5
FINE P24
mTeSR1
NANOG
KLF4
KLF5
DPPA3
KLF17
DNMT3L
LTR7Y

FIGURE 12E


KDM4E
Expression relative to mTeSR1
3.5
3
2.5
2
1.5
1
0.5
0
mTeSR1
4iLA+ feeder
FINE
ZSCAN4
Expression relative to mTeSR1
80
70
60
50
40
30
20
10
0
mTeSR1
4iLA+ feeder
FINE
MBD3L2
Expression relative to mTeSR1
3500
3000
2500
2000
1500
1000
500
0
mTeSR1
4iLA+ feeder
FINE