

Supplemental Information

**Human naive epiblast cells possess
unrestricted lineage potential**

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SUPPLEMENTAL TABLE

Table S1 gRNA sequences for targeting in Cas9 expressing naïve cells, Related to Star Methods

<i>gRNA target</i>	<i>Sequence</i>
<i>POU5F1-g1</i>	GCCACCAAATAGAACCCCCA
<i>POU5F1-g2</i>	GCCACCAAATAGAACCCCCA
<i>SOX2-g1</i>	GTTATAAATACCGGCCCCGG
<i>SOX2-g2</i>	GCAGGGCGCTCACGTCGTAG
<i>NANOG-g1</i>	GGTTCACCAGGCATCCCTGG
<i>NANOG-g2</i>	GGTCGCAAAAAGGAAGACA
<i>TFAP2C-g1</i>	ATATTCGGCGACTCCAGTGT
<i>TFAP2C-g2</i>	GCTTAAATGCCTCGTTAC
<i>YAP-g1</i>	GACGTTTCATCTGGGACAGCA
<i>TAZ-g1</i>	TACGAGCTCATCGAGAAGCG
<i>GFP-g1</i>	GAGCTGGACGGCGACGTAAA

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Trophectoderm formation, Related to Figure 1

- A. qRT-PCR assay for hypoblast markers after 5 days in indicated conditions.
- B. qRT-PCR assay for naïve and trophectoderm markers after 5 days in indicated conditions.
- C. qRT-PCR assay for trophectoderm markers after 5 days in N2B27 or PD03 of human naïve PSC lines; HNES1, cR-NCRM2 and cR-Shef6
- D. qRT-PCR assay for core pluripotency and trophectoderm marker expression in mouse ES cells cultured for 3 days in indicated conditions.
- E. Phospho-Smad2/3 immunoblot for conventional H9 hPSCs and naïve cells in indicated conditions.
- F. Phospho-Smad2/3 immunoblot for naïve cells in absence or presence of A83
- G. Phase and fluorescence images of GATA3:mKO2 cells in PXGL with or without A83 for 4 passages. Scale bar, 100µM
- H. Flow cytometry analysis of GATA3:mKO2 cells in PXGL or in PXGL plus A83 for 4 passages.
- I. qRT-PCR assay for *GATA2* and *GATA3* expression in human naïve cells cultured in PXGL with A83. X-axis indicates passages in PXGL with A83.
- J. Flow cytometry analysis of GATA3:mKO2 cells in N2B27 or with A83 for 3 days.
- K. Phase contrast and fluorescence time lapse stills of GATA3:mKO2 cells in PD+A83. (See also Supplemental Movies 1 and 2).

Figure S2. Trophectoderm differentiation and cytotrophoblast stem cells, Related to Figure 2

- A. Wide field images of cysts formed in adherent culture in PD+A83 immunostained for aPKC ι and PAR6B.
- B. Two examples of suspension cysts outgrown for 3 days in N2B27 and immunostained for CK7 and extravillous trophoblast marker HLA-G.
- C. qRT-PCR analysis of gene expression in placenta and naïve stem cell derived TSCs. Naïve stem cell derived TSCs include two independent cultures derived from HNES1 and one each from naïve iPSC lines niPSC2 and niPSC4. CT27 is a placental cytotrophoblast TSC line (Okada et al, 2018). Error bars from technical duplicates.
- D. qRT-PCR analysis of gene expression during differentiation of placenta and naïve stem cell derived TSCs using syncytiotrophoblast (SCT) and extravillous trophoblast (EVT) protocols. Error bars from technical duplicates.

Figure S3. Whole transcriptome analysis, Related to Figure 3

- A. One-way hierarchical clustering of early blastocyst (E5) single cells (Petropoulos et al., 2016) computed with lineage marker genes (Stirparo et al., 2018).
- B. One-way hierarchical clustering of E6 and E7 single cells (Petropoulos et al., 2016) computed with lineage marker genes (Stirparo et al., 2018).
- C. PCA computed for all the genes expressed in early ICM (cluster 4, FIG.S2A), early TE (cluster 5, Figure S2A), epiblast (Stirparo et al., 2018) and late TE (cluster 2, Figure S2 B, C, D). In red, cells expressing more than 6000 genes.
- D. PCA computed as in C for filtered cells expressing >6000 genes (log₂expression >0, n=18694)

- E. PCA for filtered cells, computed with differentially expressed genes in human embryo (n= 4507).
- F. As Figure 3B for cR-H9 cells.
- G. PCA computed with all orthologues (average macaque dataset, ICM, EPI, postE, postL, early TE, late TE and post PA.TE, Nakamura et al., 2016) of expressed protein coding genes (log2 expression in time-course > 0 & orthologues, n= 12992).

Figure S4. Single cell analysis, Related to figure 4

- A. Expression of early trophectoderm genes coloured on Figure 4A UMAP
- B. Expression of cytotrophoblast, syncytiotrophoblast and extravillous trophoblast lineage markers on Figure 4A UMAP
- C. Expression of mid (E9-10) and late (E12-14) post-implantation epiblast markers on Figure 4A UMAP
- D. Expression of additional hypoblast markers on Figure 4E UMAP
- E. Immunostaining of cells cultured in PD+A83 for 24h followed by 48h in A83 or N2B27.

Figure S5. Genetic perturbations, Related to Figure 5

- A. Alkaline phosphatase (AP) staining after indicated Cas9/gRNA RNP transfection and culture in PXGL on MEF for 4 days. Separate experiments were performed on parental HNES1 or HNES1-GATA3:mKO2 cells. Controls were transfected with GFP gRNA.
- B. AP staining after indicated gRNA plasmid transfection in Cas9 expressing HNES1-GATA3:mKO2 cells. Cells were maintained in PXGL on MEF with puromycin selection for 7 days. Controls were transfected with GFP gDNA.
- C. Flow cytometry analysis of GATA3:mKO2 cells after gRNA plasmid transfection and culture for 4 days in N2B27 alone or with A83.
- D. Flow cytometry analysis of GATA3:mKO2 expression after Cas9 RNP transfection with GFP or TFAP2C gRNA.

Figure S6. Fates of naïve versus primed stem cells, Related to Figure 6

- A. Flow cytometry analysis of naïve GATA3:mKO2 cells in indicated culture conditions for three days. LDN, BMP receptor inhibitor LDN-193189.
- B. Phospho-Smad1/5 immunoblot on naïve cells and primed HNES1 cells in indicated conditions. P, PD03; B, BMP2, number indicates BMP2 concentration, ng/ml; L, human LIF.
- C. Flow cytometry analysis of naïve and primed GATA3:mKO2 cells in indicated culture conditions for 5 days.
- D. qRT-PCR assay of trophectoderm and amnion markers in human naïve and primed cell differentiated in PD+A83 with or without LDN for 5 days. Error bars from technical duplicates.
- E. qRT-PCR assay for neural markers in naïve or primed PSCs differentiated in indicated conditions for 5 days with or without LDN. Error bars from technical duplicates.
- F. PCA of human naïve and conventional primed cells (Stirparo et al., 2018), naïve cells from this study, and hEPSCs (Gao et al., 2019; Yang et al., 2017) computed using most variable genes, log2expression > 1, cv>0.5, n=3510.
- G. 3D PCA of human naïve and conventional primed cells, naïve cells from this study, hEPSCs, and human embryo in vitro development (Xiang et al., 2019), computed with variable genes in embryo development, n=1517.

- H. Two-dimensional kernel density estimation of amnion genes from human differentiation in vitro (expression >1, Zheng et al., 2019) in Figure 6D PCA.
- I. Two-dimensional kernel density estimation of amnion genes from *macaca* embryo cultures (Ma et al., 2019) in Figure 6D PCA.
- J. Density of placental cytotrophoblast TSC (Okae, 2019)(left) and AME (right) enriched genes in Figure 6F PCA.
- K. Violin plot of z-score for TSC- and AME-enriched genes in differentiated hEPSCs (group 1) and naïve stem cell derived and placental TSCs (group 2) (see Figure 6F).
- L. Bootstrap Spearman correlation (iteration 100, number of genes=50) of naïve or EPSC differentiation timecourses with differentiated populations induced by BAP treatment.
- M. As Figure 6H with addition of BAP cell expression values.

Figure S7. Plasticity of human embryo pre-implantation epiblast, Related to Figure 7

- A. Phase images of human blastocysts cultured for 24h and classified as fully expanded (E6 or E7) for use in this study.
- B. Phase contrast and immunofluorescence images of immunosurgically isolated ICM explants after 5 days in N2B27 or PD+A83.
- C. Time lapse stills at 0h and 48h with endpoint immunostaining of immunosurgically isolated ICM explants in PD+A83 plus LDN.
- D. Images of microdissected E6 embryo with epiblast labelled by live cell immunostaining for SUSD2-FITC after culture for 16h. Arrow points to SUSD2 negative cells. Subsequent images show that cells persist as SUSD2 staining fades.
- E. Immunostaining of SUSD2 labelled microdissected ICM with polar trophectoderm in Figure 7C after 5 days in PD+A83.
- F. WGA (Alexa 594 conjugate) labelling of intact fully expanded blastocysts. Scale bar, 200µM.

Figure S1

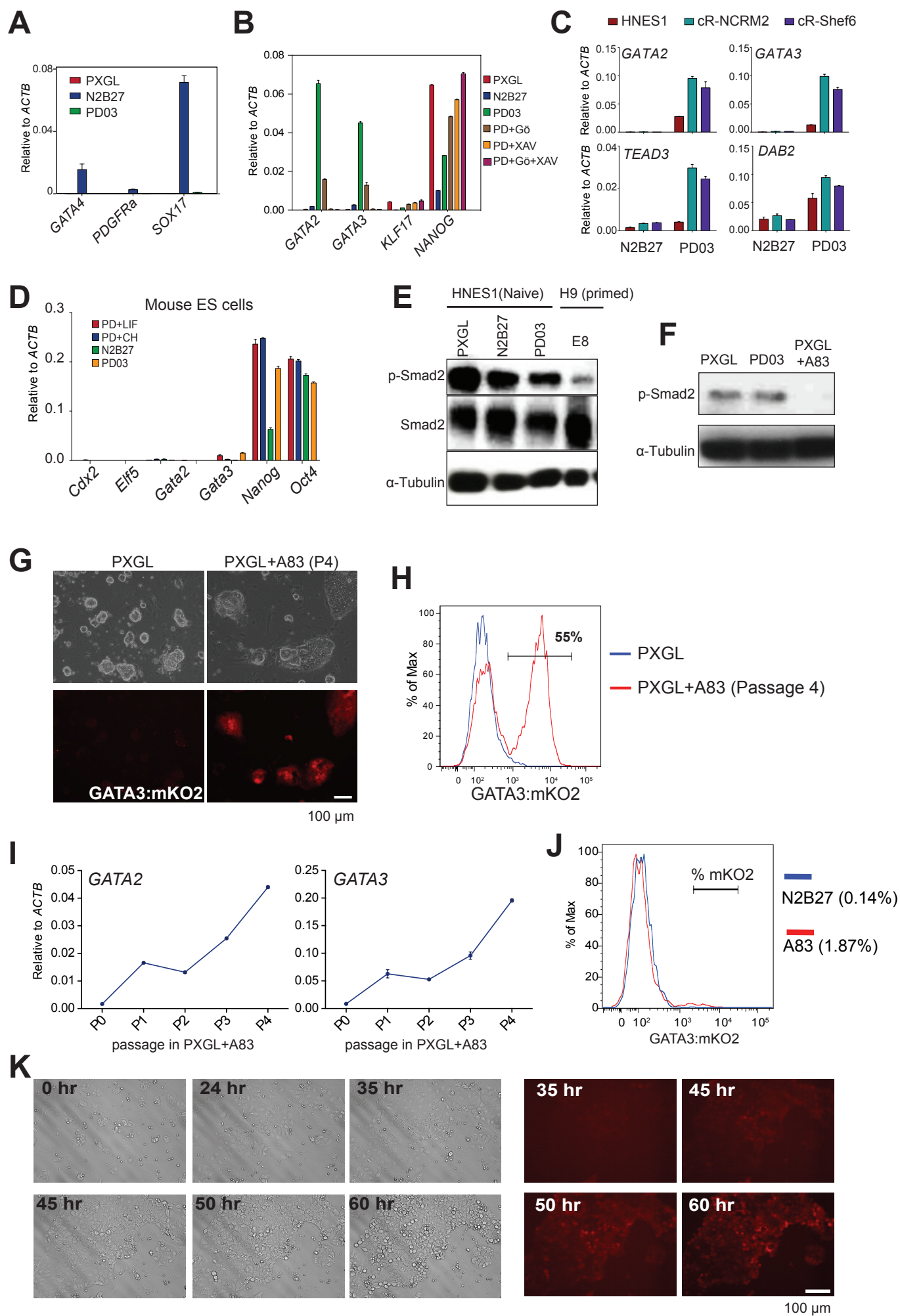
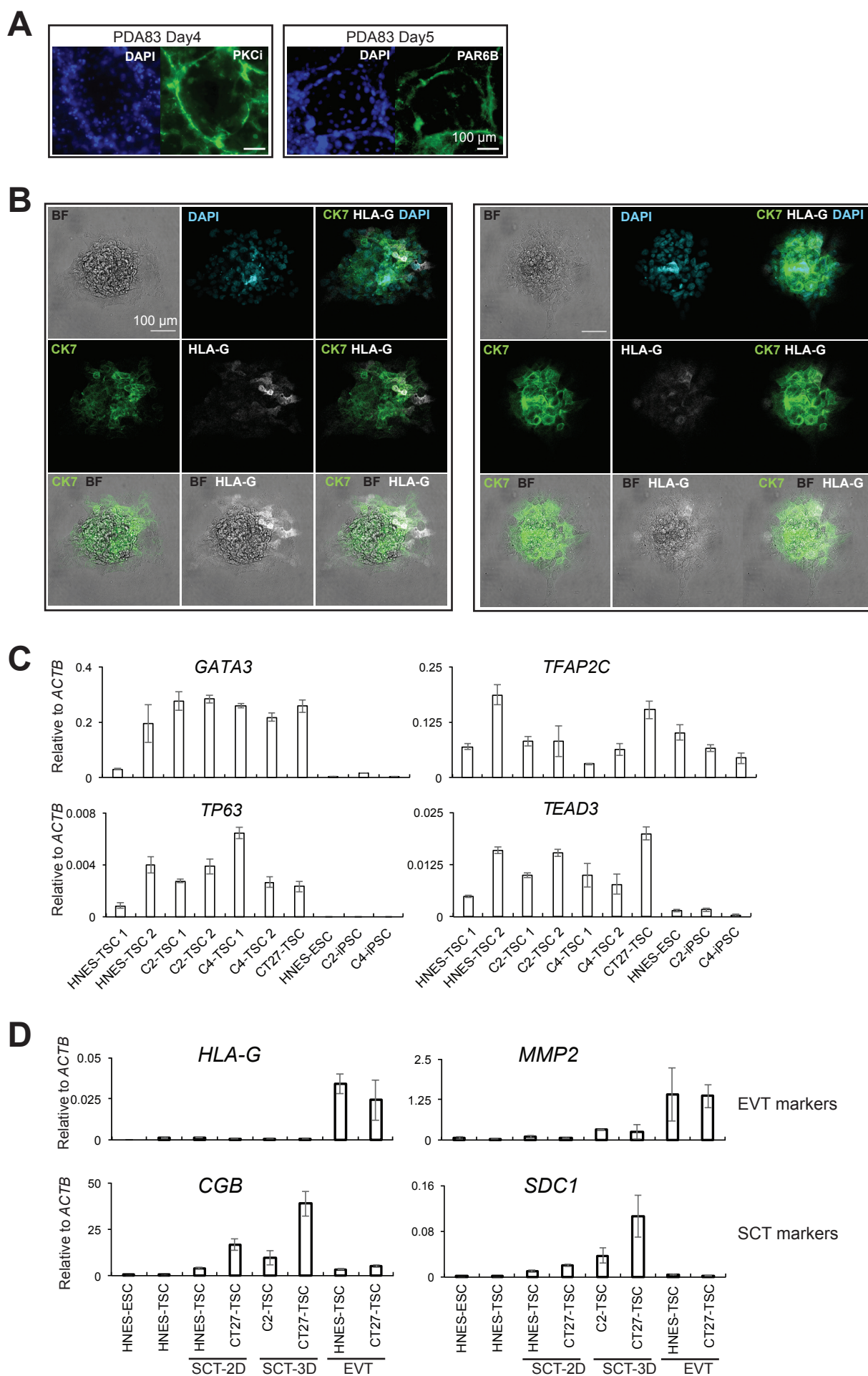
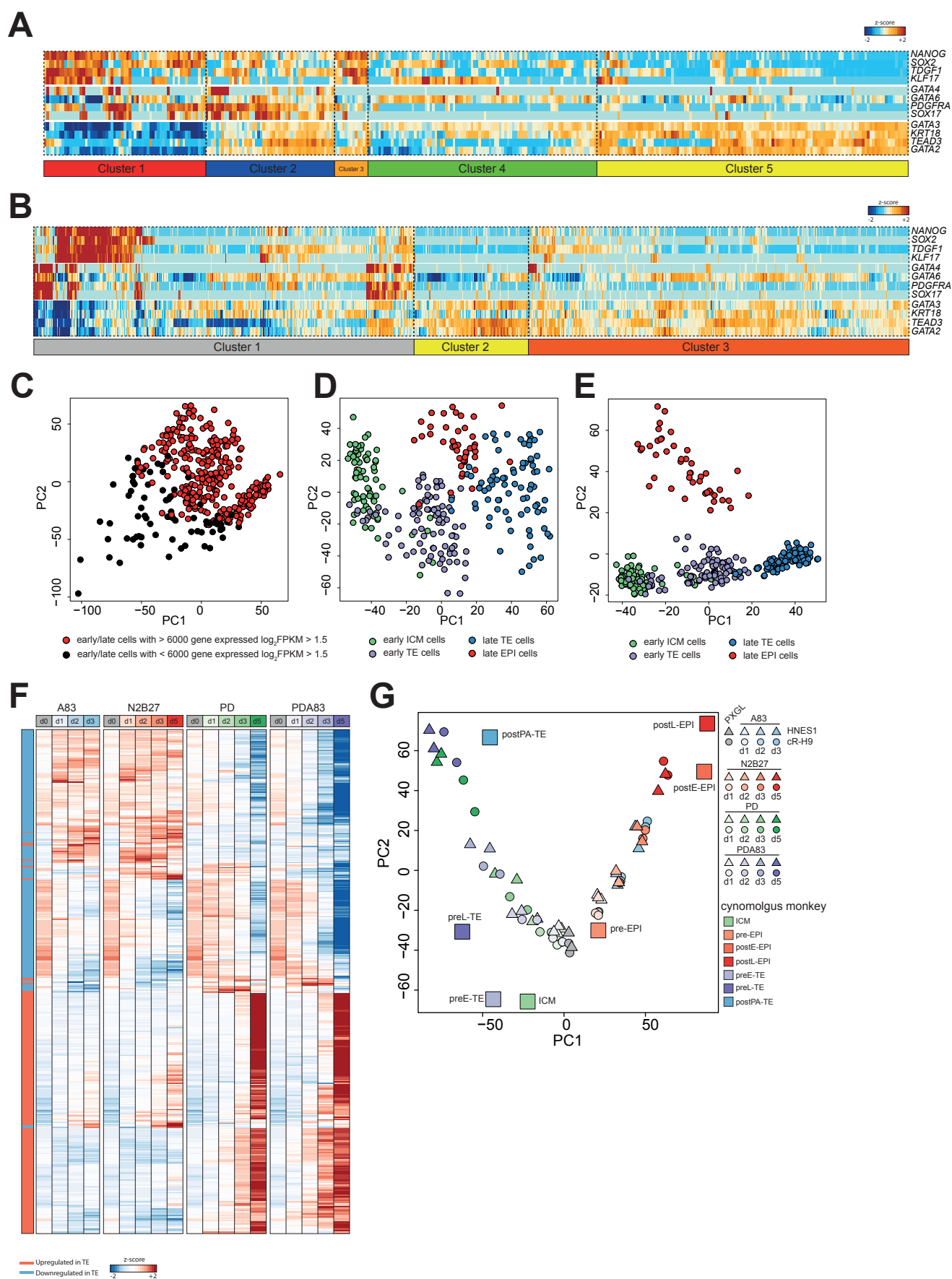
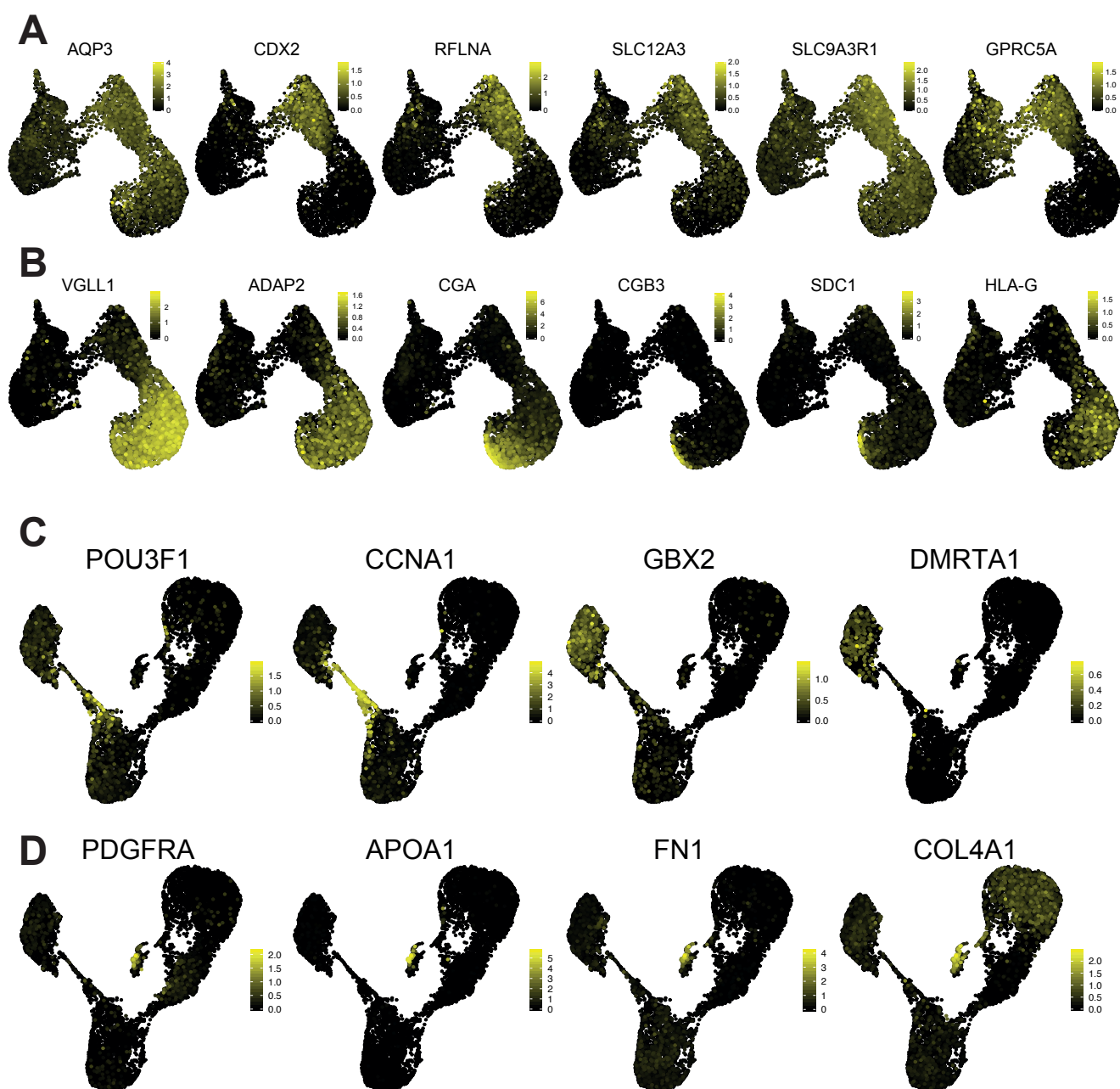


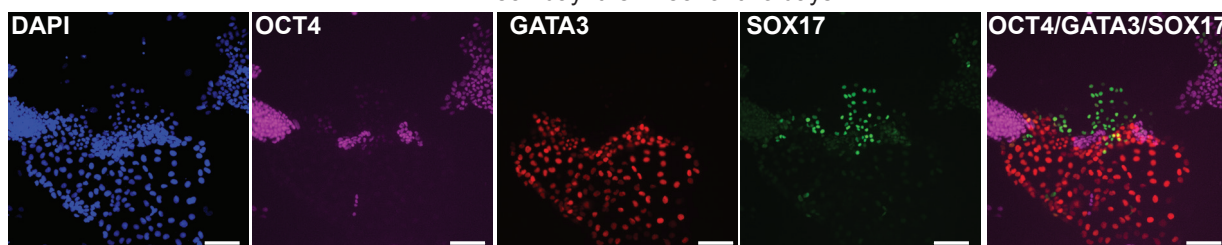
Figure S2





**E**

PDA83 1day then A83 for two days



PDA83 one day then N2B27 for two days

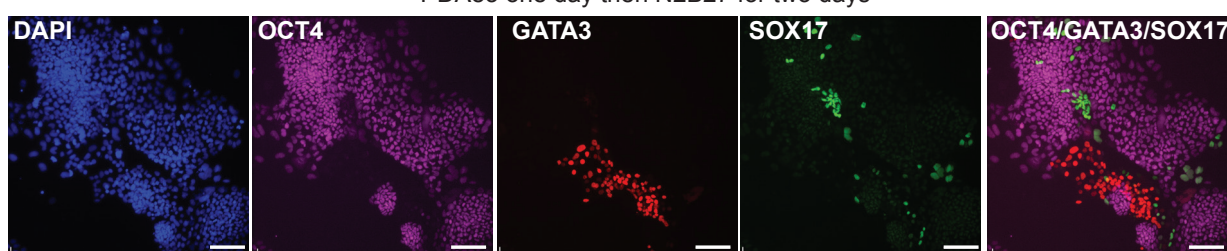
100 μ m

Figure S5

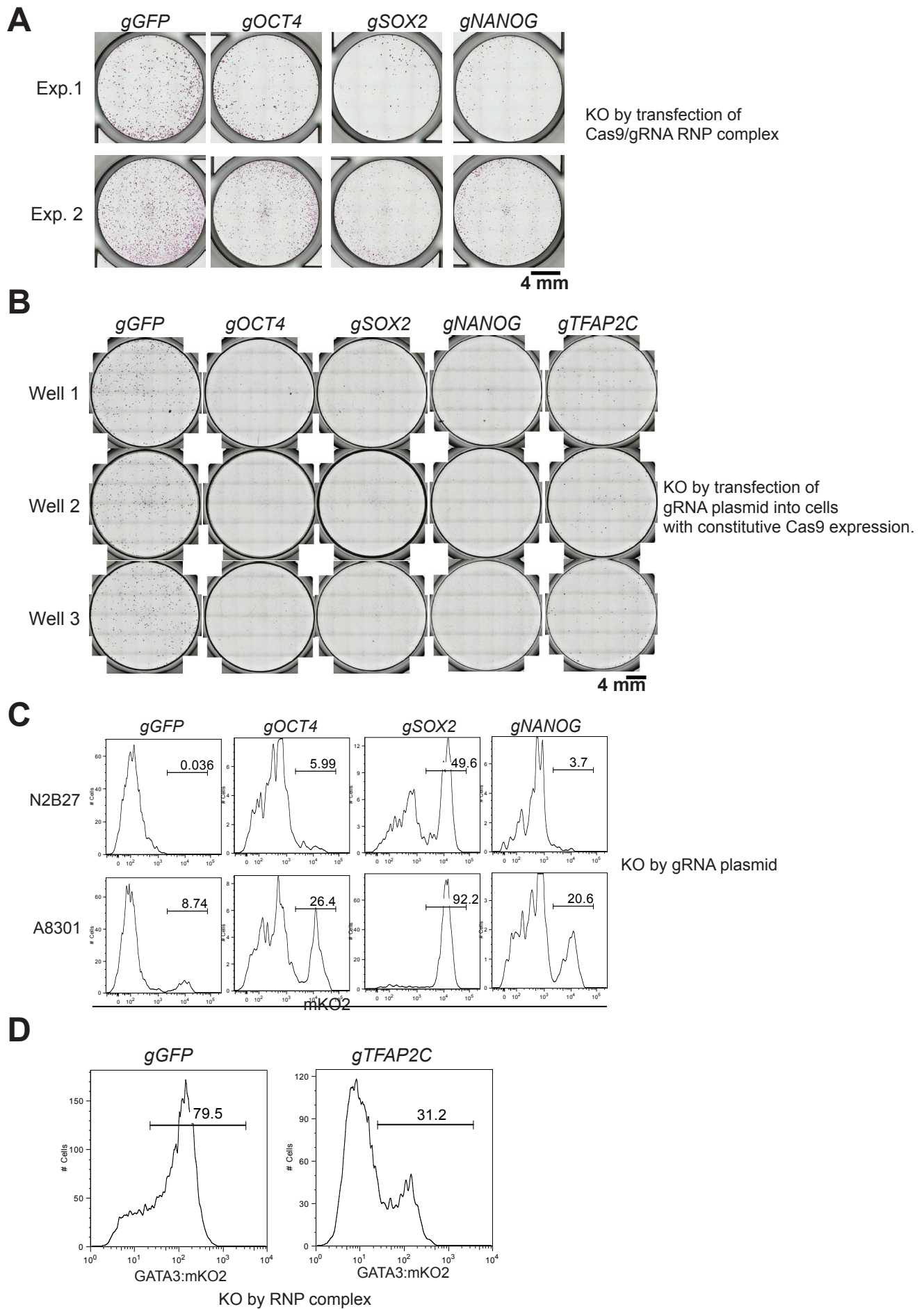


Figure S6

