Supplemental Information

Identifying Division Symmetry of Mouse Embryonic Stem Cells: Negative Impact of DNA Methyltransferases on Symmetric Self-Renewal

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Inventory of Supplemental Information

Supplemental Figures

Figure S1: Single molecule FISH assays were initially conducted to determine the level of division symmetry and the limitation lead us to establish assays based on single cell RNA analyses (related to Figure 1).

Figure S2: Evaluation of our single assay system is shown (related to Figure 2).

Figure S3: Effects of LIF & BMP4 and 2i on cellular phenotypes are shown (related to Table 1).

Figure S4: Co-regulation of ESC genes at single cell levels is shown. The corresponding data for reprogramming factors are shown in Figure 3.

Supplemental Tables

Table S1: Probe sequences for single cell molecule FISH assays, related to Figure 1.

Table S2: List of 48 genes that were analyzed in Biomark qPCR (Figures 1, 2, 3, and 4, and Table 1).

Table S3: Primer sequences and higher thresholds for reliable Cq values in Biomark qPCR analyses (Figures 1, 2, 3, and 4, and Table 1).
Table S4: Biomark qPCR expression data are shown (Figure 1, 2, 3, and 4 and Table 1).

Table S5: Correlation of expression levels between 1,128 gene pairs are shown (related to Figure 3).

**Supplemental Movie**

Movie S1: A movie capturing the sister cell microdissection process (related to Figure 1).
Figure S1, Single molecule FISH analyses with *Klf4* and *Pou5f1* oligoprobes (5’ TAMRA labelled), related to Figure 1. ESCs cultured in 2i conditions were examined with the confocal microscopy. An image from one of confocal sections is shown for each probe. Bars, 5µm. Scatter plots indicate pair-wise comparisons of numbers of RNA spots throughout z-stacks between sister cells. Between sister cells, cells expressing lower and higher levels of relevant genes were called “sister cells A” and “sister cells B”, respectively, on the plot. Data points closer to, or on the central line indicate similar expression levels between sister cells (N=10 and 9 for *Pou5f1* and *Klf4*, respectively).
Figure S2, Evaluation of experimental systems, related to Figure 2. A, Scatter plots between dataset derived from anti-E-cadherin-treated and untreated ESC RNA (1ng). Average Cq values for 5 replicates are plotted for 48 genes. B and C, Alkaline phosphatase (AP) staining. ESCs were cultured with (C) or without (B) nocodazole for 6 hours, and then cultured for five days in LIF & BMP4 media prior to analyzing AP activity. Bars, 200µm. D, Single cell-equivalent 30 pg of ESC RNA was reversely transcribed, amplified, and then analyzed on Biomark systems. Distribution of correlation coefficients between expression levels of pairs of 48 RNA samples is shown. E, Scatter plots between two independent Biomark assays. F, Histogram of Cq differences observed between two replicates on Biomark platform.
Figure S3. ES cell differentiation, related to Table 1. A, Alkaline phosphatase (AP) staining. ESCs were cultured for five days in each medium prior to analyzing AP activity. LIF and BMP4 (left), no LIF and BMP4 (middle), and 2i+LIF and BMP4 (right). Bars, 200µm. B, Probabilities (left) and levels (right) of expression for four reprogramming factors in three conditions: media without LIF and BMP4 for 24 hours (black bars: Differentiated states), media with LIF and BMP4 (gray bars: Pluripotent states), and media with LIF, BMP4, and 2i (white bars: Ground pluripotent states). The p-values of statistically different groups are shown in the graph (the Fisher’s exact test). Mean Cq values with 95% confidence intervals are plotted. C, Correlation coefficients of expression levels of 48 ESC markers between sister cells cultured in media without LIF and BMP4.
Figure S4. Co-regulation of ESC genes at single cell levels, related to Figure 3. A, Scatter plots show the correlation between expression levels (Cq values) of top 6 combinations among 1,128 possible gene pairs. The number with (*) in each graph indicates the correlation coefficient (r) of expression levels that are statistically significant after applying the Bonferroni correction tests. Full lists of correlated gene pairs are available in Table S5. B, The distribution of correlation coefficients between expression levels for 1,128 gene pairs for media without LIF and BMP4 (black bars), media with LIF and BMP4 (gray bars), and media with LIF, BMP4, and 2i (white bars) conditions.
Supplemental Movies
Movie S1, Sister cell microdissection, related to Figure 1
The glasspipette tip (shown as the circle at the center) provided pressure on the junction between sister cells in order to separate sister cells (0-39s). The first (40s) and second sister cells (56s) were subsequently isolated.