Supplementary Fig. 1: H3K9me3 at chromocenters in 2-cell to 16-cell stage embryos

(a) Upper panel row shows the Z-projection of a whole embryo, counterstained with DAPI. Scale bar represents 20µm. Bottom panels show a single section of a representative nucleus highlighted in Figure 1 after staining with DAPI (green) and H3K9me3 (red). Last row is the merge of the two signals. Scale bar represents 5µm for zoomed-in nuclei.
(b) The graphs show the intensity profiles of DAPI (green), H3K9me3 (red) and H3K27me3 (grey) signals across a single chromocenter highlighted by an arrowhead in **(a)**.
(c) Violin plots show the distribution of the Pearson's Correlation Coefficients (PCC) between DAPI and H3K9me3 profiles at chromocenters for each stage (149 chromocenters analysed).

Supplementary Fig. 2: H3K9me3 at chromocenters in the peri-implantation embryo, in embryonic compared to extra-embryonic lineages.

(a) Schematic representation of mouse peri-implantation development, lineages specification and their associated markers. **(b, e and h)** Embryos at E3.25 **(b)**, E3.75 **(e)**, and E4.25 **(h)** stained for the indicated markers. Upper left panel is a z-projection of the whole embryo showing DAPI counterstaining while other panels are a section encompassing the ICM/EPI region. A section of an embryo at E3.75 after staining with NANOG and GATA6, showing the spatial segregation between EPI and PrE cells is include **(e)**. **(c, f and i)** Embryos at E3.5 **(c)**, E4.0 **(f)**, and E5.5 **(i)** stained with H3K9me. **(d, g and j)** Graphs show the representative intensity profiles of DAPI (green) and H3K9me3 (red) signals across a single chromocenter in a nucleus of each lineage at E3.5 **(d)**, E4.0 **(g)** and E5.5 **(j)**.

Supplementary Fig. 3: Correlation dynamics between DAPI and H3K9me3 signals at chromocenters at peri-implantation stages.

(a, c and e) Pattern of H3K9me3 in a representative nucleus of EPI **(a)**, PrE **(c)** and TE **(e)** cells at different blastocyst stages (from E3.25 to E4.25). DNA is stained with DAPI. Scale bar represents 5µm. **(b, d and f)** Violin plots show the distribution dynamics of the Pearson's Correlation Coefficients (PCC) between DAPI and H3K9me3 profiles at chromocenters in EPI **(b)**, PrE **(d)** and TE **(f)** cells at various stages (196, 192 and 195 chromocenters analysed respectively).

Supplementary Fig. 4: Transcription dynamics of major satellite in 2-cell to 16-cell embryos assessed by RNA-FISH.

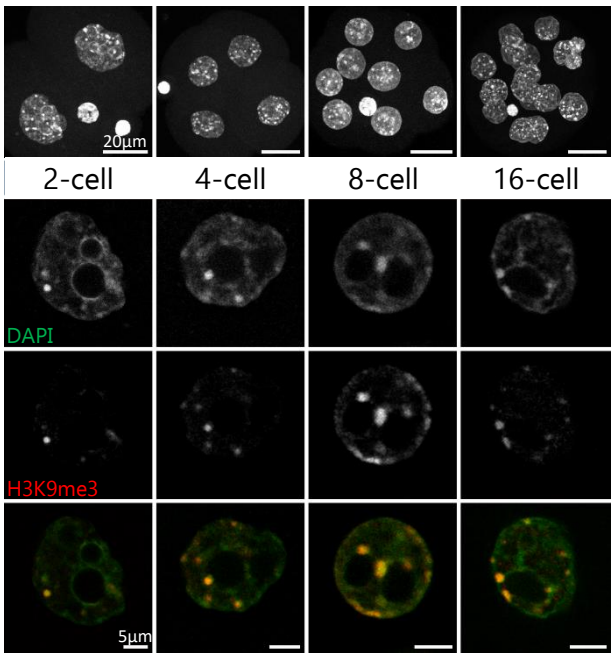
(A) Z-projection of whole embryos processed for immuno-RNA-FISH, with DAPI (blue) and Major satellite foci (red) staining. Scale bar represents 10µm. **(B)** Proportion of cells exhibiting 0, 1-2, 3-4 and 5 or more (5+) RNA-FISH foci per cell at each indicated stage (215 nuclei analysed).

Supplementary Fig. 5: H3K27me3 profile at chromocenters in TSCs and transcription dynamics of major satellite in each cell line assessed by RNA-FISH.

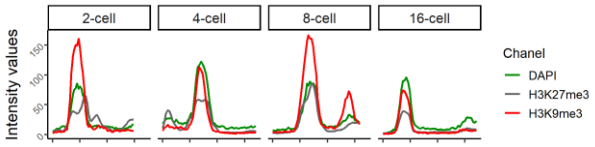
(a) FAXY-cultured TSCs colony stained for H3K27me3, DAPI and merge signals. **(b)** Profile of major satellite transcription (red) assessed by RNA-FISH in either 2i-LIF ESCs, cEpiSCs and FAXY TSCs. The proportion of cells exhibiting RNA-FISH foci is specified for each cell line in the zoomed-in nucleus panel (1297, 674 and 1286 nuclei analysed respectively). **(c)** Relative expression of genes associated with primed (left) or naïve (right) pluripotency during the conversion of ESCs to cEpiSCs presented in Fig. 5D.

Supplementary Figure 1

a



b



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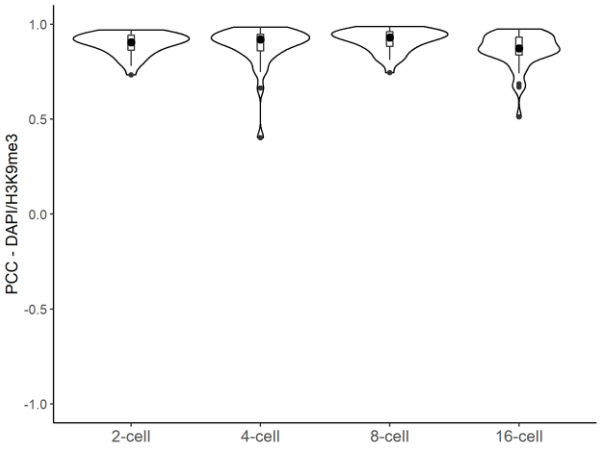
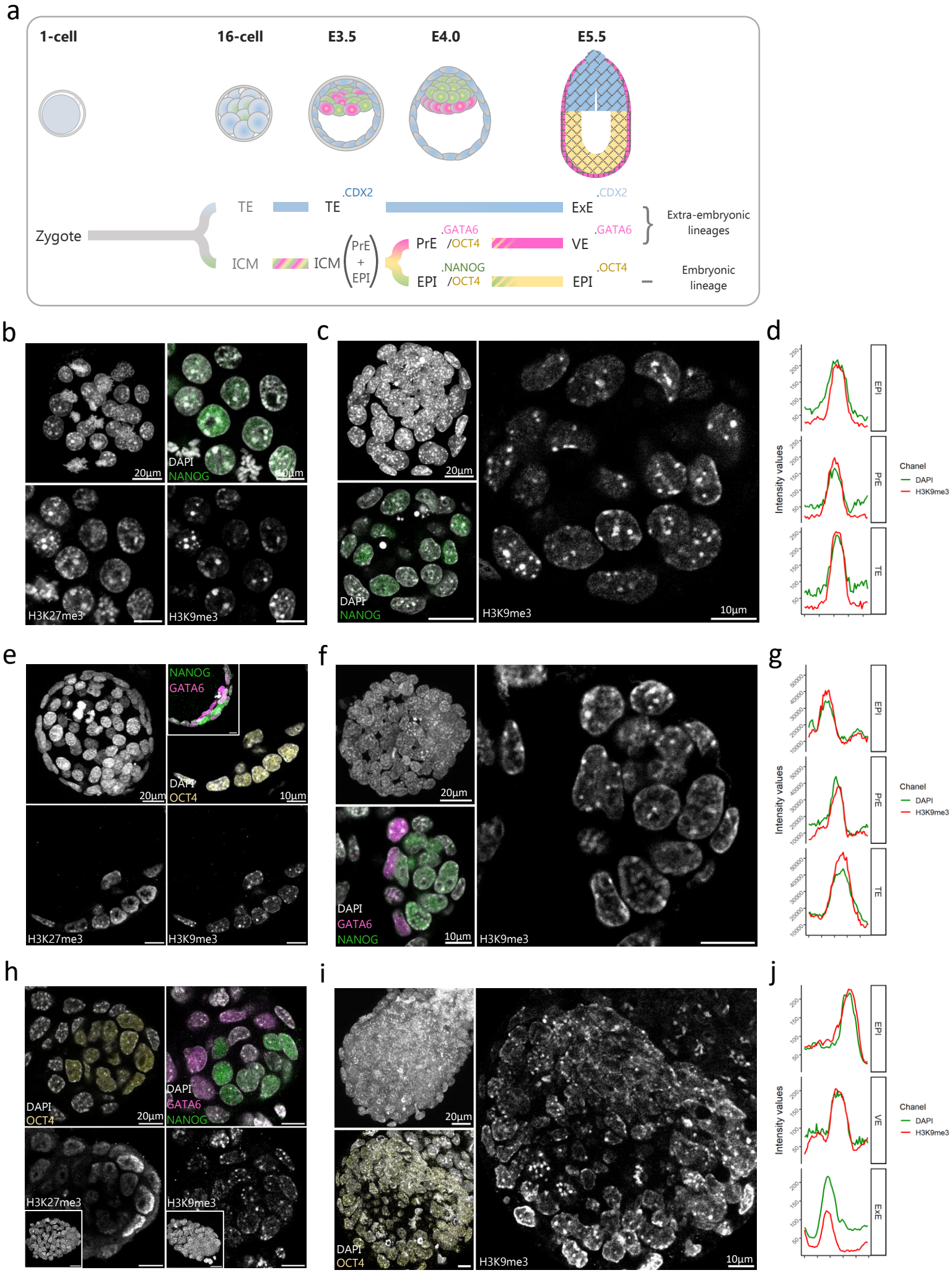
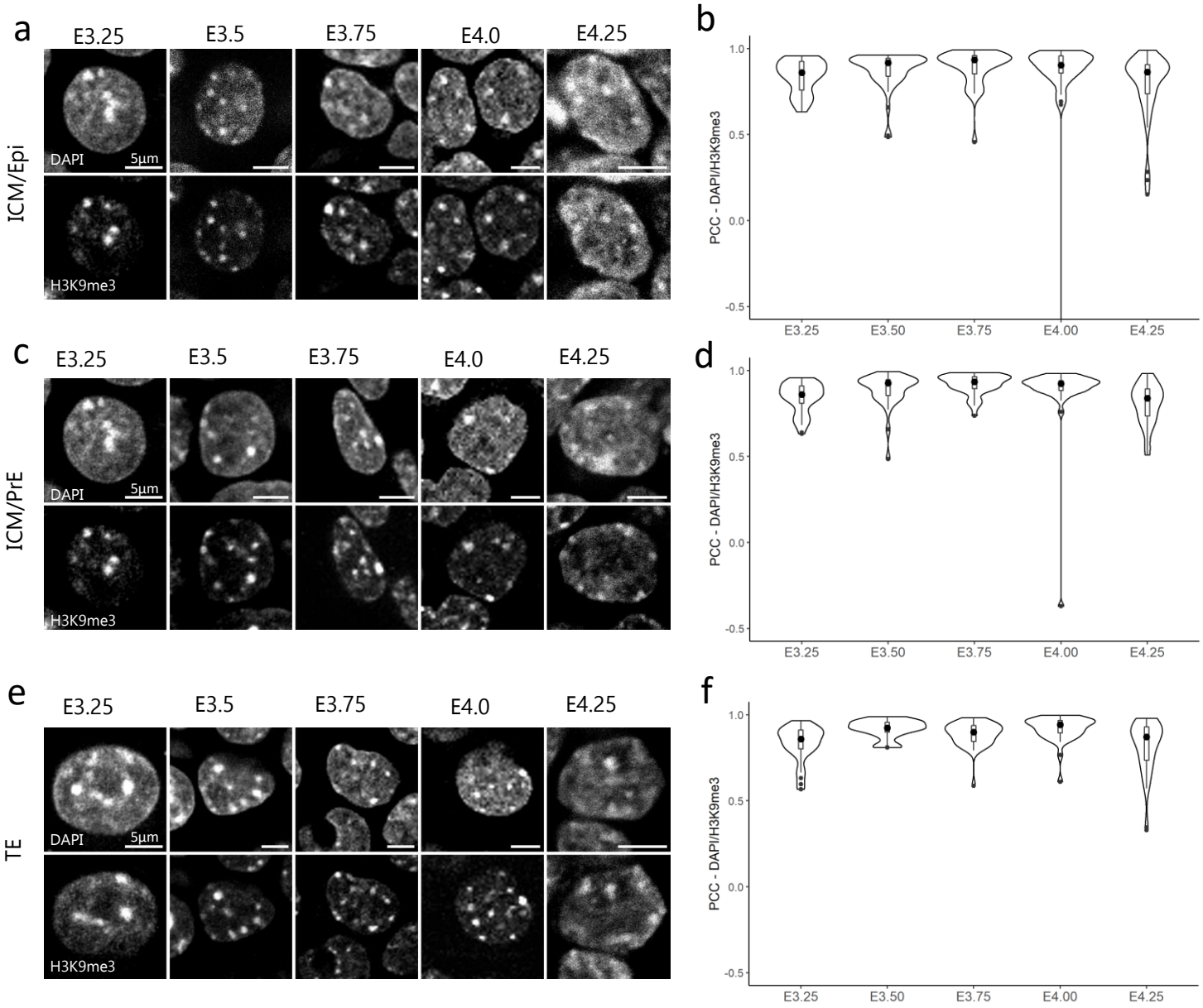


Supplementary Figure 2

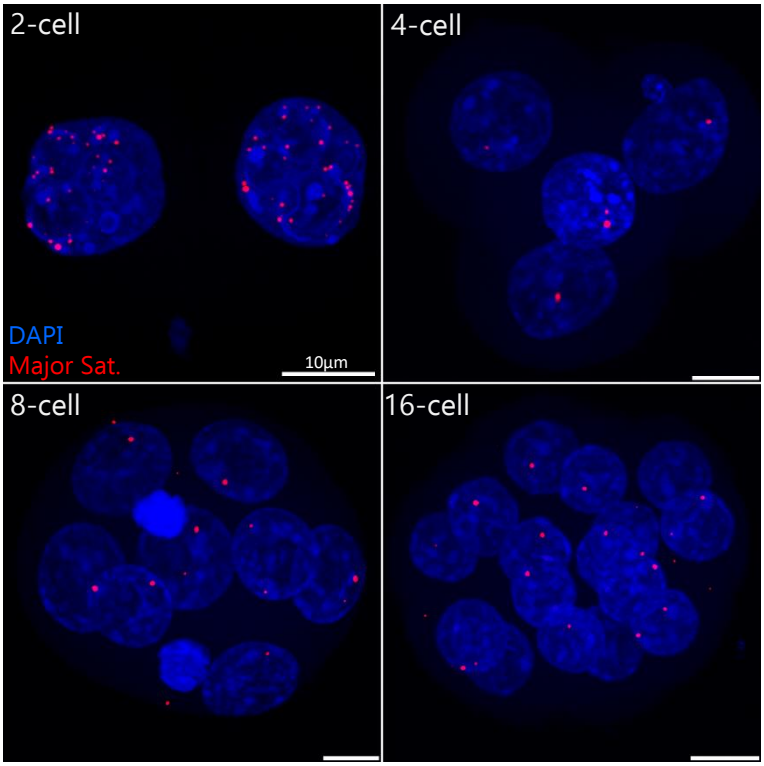


Supplementary Figure 3



Supplementary Figure 4

a



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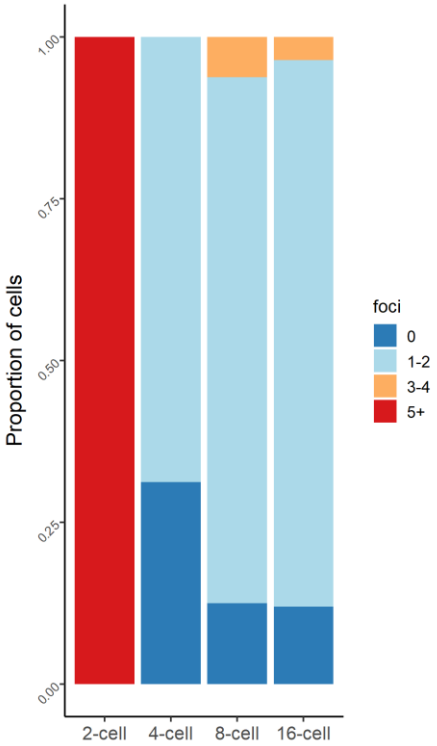

Supplementary Figure 5