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Supplementary Materials for

A transcriptional roadmap for 2C-like–to–pluripotent state transition

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Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/6/22/eaay5181/DC1)

Tables S1 to S6

Supplementary Materials

fig. S1

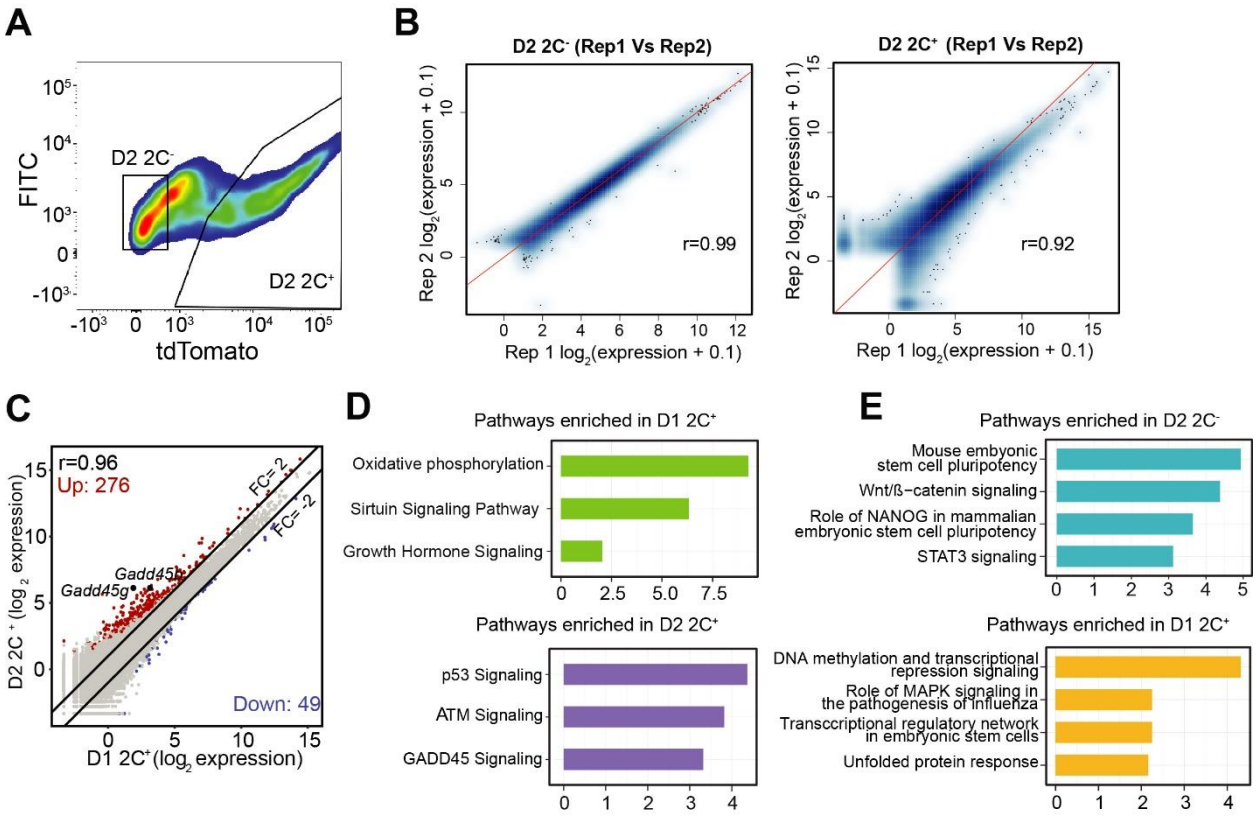

fig. S1. Bulk RNA-seq indicates that D2 2C$^+$ cells maintain their 2C-like state and D2 2C$^-$ cells exit from 2C-like state. (**A**) FACS gating for cell isolation. (**B**) Scatter plots showing good correlation (Pearson) between the transcriptomes of biological replicates of D2 2C$^-$ and D2 2C$^+$ cells. (**C**) Scatter plot comparing gene expression profiles between D2 2C$^+$ and D1 2C$^+$ population. Criteria: FC>2 and FDR < 0.001. Genes up-regulated in D2 2C$^+$ cells are indicated in red and genes up-regulated in D1 2C$^+$ are indicated in blue. Pearson correlation r=0.96. (**D**) Bar plot showing the -log$_{10}$(p-value) of the pathways enriched in D2 2C$^+$ and D1 2C$^+$ cells (right, right-tailed Fisher-exact test). (**E**) Bar plot showing the -log$_{10}$(p-value) of the pathways enriched in D1 2C$^+$ and D2 2C$^-$ cells (right, right-tailed Fisher-exact test).

fig. S2

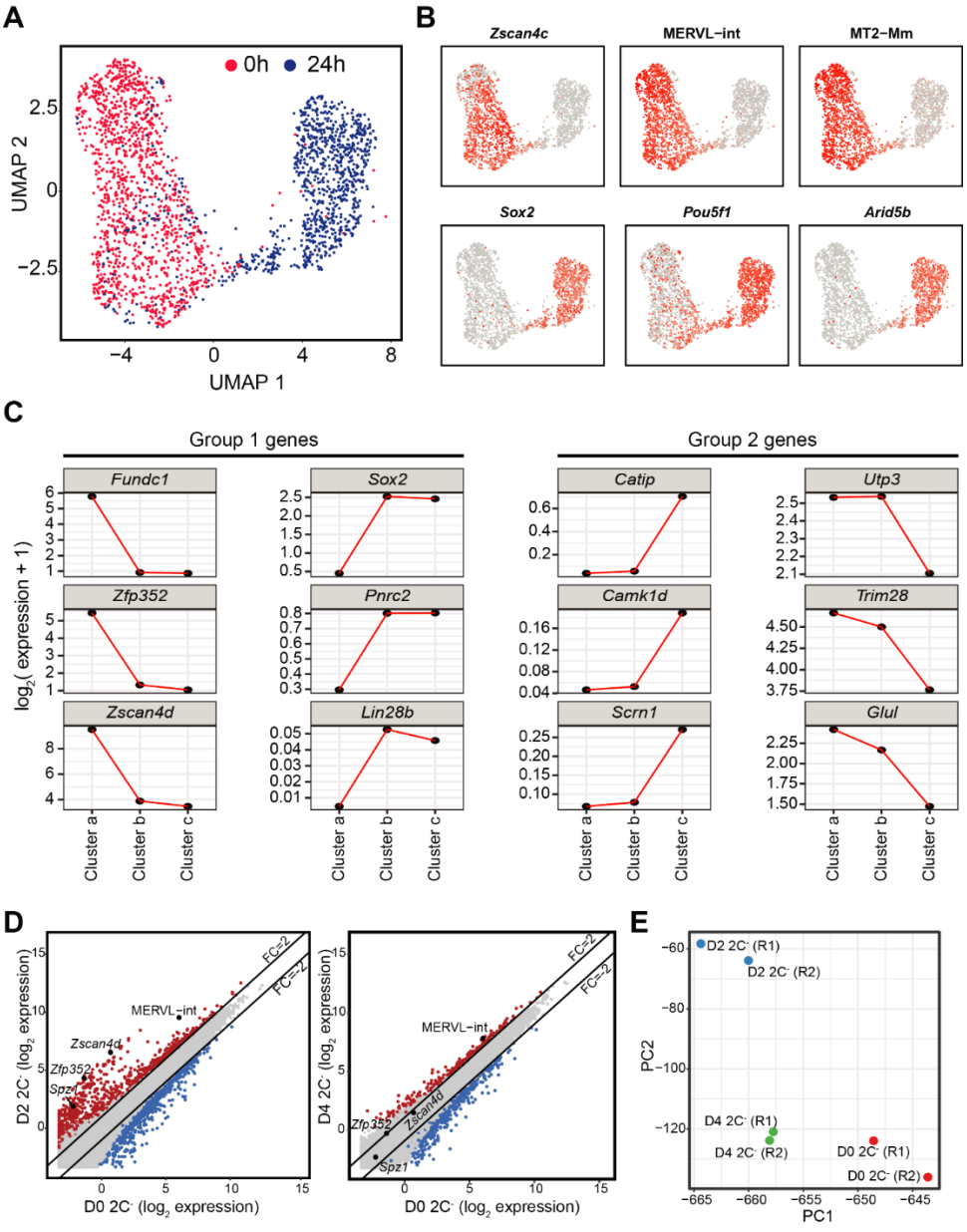

fig. S2. Single-cell RNA-seq reveals the existence of an intermediate cell cluster during 2C-like to pluripotent state transition. (**A**) UMAP plot showing the cells collected at 0h (red) and 24h (blue). (**B**) UMAP plot showing the expression of representative genes in each sequenced cell (n=2,872 cells). grey: no-expression, gradient red colors: expressed genes with bright red being the highest expression. (**C**) Mean expression of representative genes in each cell cluster. (**D**) Scatter plot comparing the mean expression of bulk RNA-seq between D2 2C$^-$ and D0 2C$^-$ cells (left panel) or between D4 2C$^-$ and D0 2C$^-$ cells (right panel). Criteria: FC>2 and FDR<0.001. (**E**) PCA projection of the bulk RNA-seq samples at D0 2C$^-$, D2 2C$^-$, and D4 2C$^-$

cells. R1 and R2 indicate two replicates. (D-E), D4 2C$^-$ cells refers to D2 2C$^-$ cells that are cultured for additional 48 h in the absence of doxycycline.

fig. S3

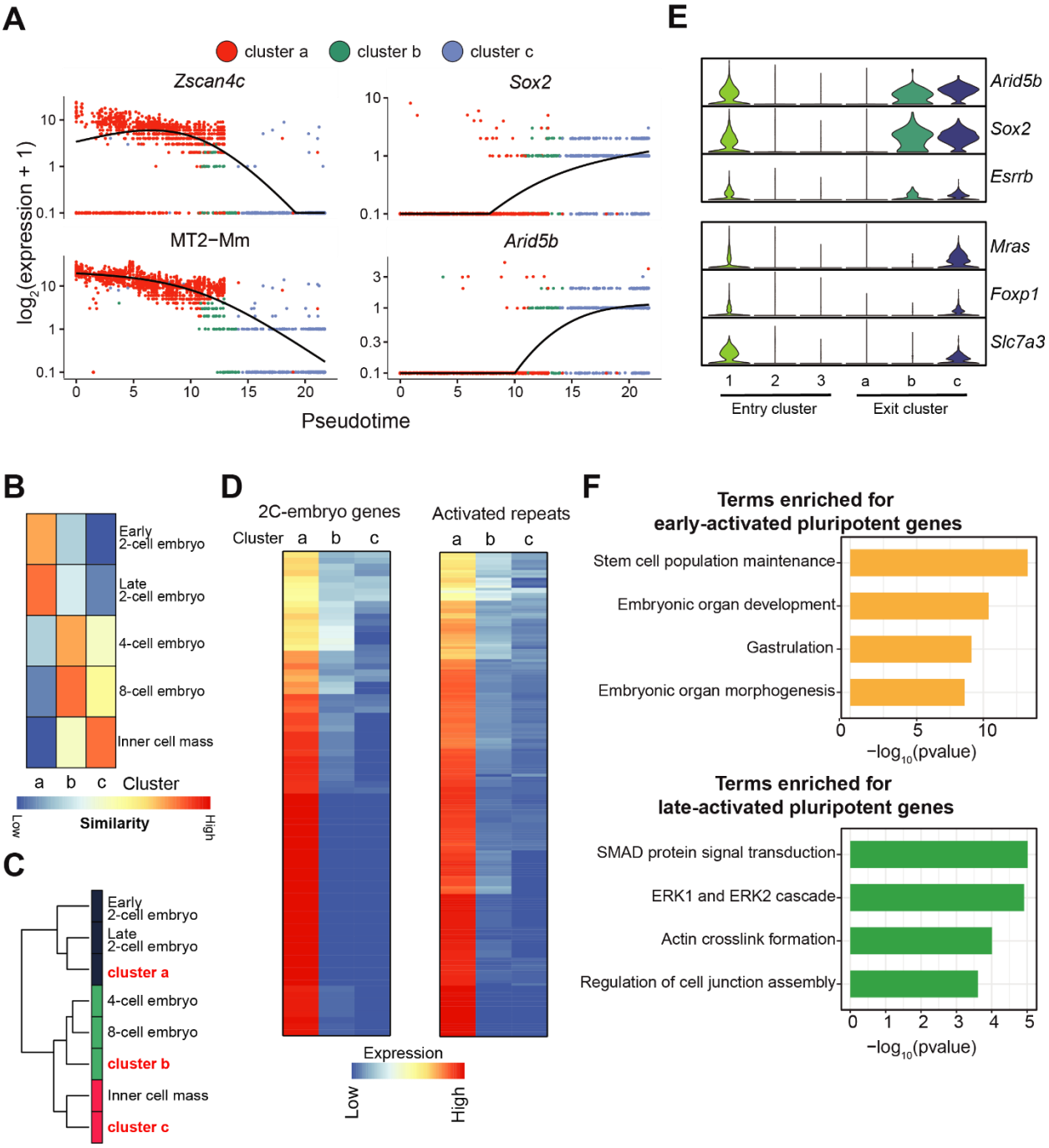

fig. S3. Transcriptional dynamics of pluripotent genes, 2C-embryo genes, and 2C^{+}-activated repeats during 2C-like to pluripotent state transition. (**A**) Jitter plots showing the expression pattern of representative genes and repeats along the pseudotime. Each dot represents the $\log_2$ expression level of the genes and repeats per-cell. The black line represents the fitted mean expression trend along the pseudotime. (**B**) Heatmap showing the degree of similarity between the transcriptional profiles of cell clusters derived from scRNA-seq and embryos of different preimplantation stages. (**C**) Hierarchical clustering (complete linkage) showing the

relationship between the scRNA-seq cell clusters and embryos of the different preimplantation stages. (**D**) Heatmap showing the normalized mean expression of 2C-embryo genes ($2C^+$-upregulated ZGA genes) and $2C^+$-activated repeats in each cell cluster. (**E**) Violin plot showing the expression of representative early-activated (top) and late-activated (bottom) pluripotent genes. (**F**) The $-\log_{10}$(p-value) of the GO terms enriched in early-activated and late-activated genes (right, right-tailed Fisher-exact test).

fig. S4

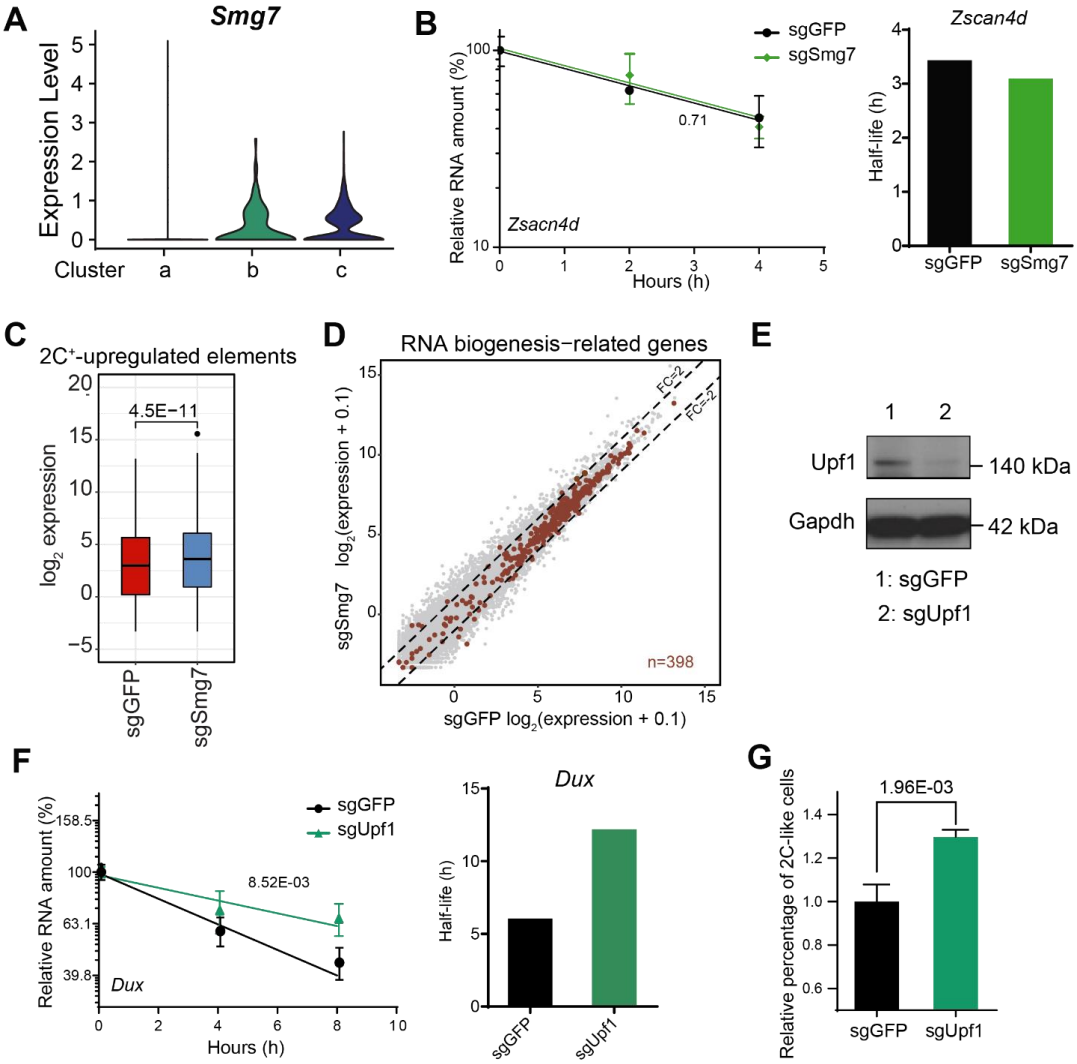

fig. S4. Smg7 destabilizes *Dux* mRNA directly through NMD. (**A**) Violin plot showing the expression of *Smg7* in each cluster. Cluster a in this figure only includes cells collected at 24h. (**B**) mRNA half-life measured by qRT-PCR. Left panel, relative *Zscan4d* mRNA level in sgGFP or sgSmg7 cells after actinomycin D treatment. Right panel, the half-life of *Zscan4d* mRNA calculated from the left panel. (**C**) Box plot showing the $\log_2$ level of $2C^+$-upregulated elements in Dux-activated mESCs infected with sgGFP or sgSmg7. P-values are calculated by two-tailed Mann-Whitney U-test. (**D**) Scatter plot showing the expression changes of 398 genes (marked in brown) involved in mRNA biogenesis (KEGG, BR:mmu03019) after Smg7 depletion. (**E**) Western-blot confirming efficient Upf1 knockdown. (**F**) Measurement of mRNA half-life by qRT-PCR. Left panel, relative *Dux* mRNA quantity in sgGFP or sgUpf1 cells after actinomycin D treatment. Right panel, the half-life of *Dux* mRNA calculated based on the data presented in the middle panel. The p-values in panels B and F are calculated by standard t-test of the degradation curves. (**G**) The percentage of 2C-like cells of sgUpf1-infected cells relative to control. P-value is calculated by unpaired t-test, two-tailed, two-sample unequal variance and is

shown as numbers in the figure. Data presented in panel **B**, **F**, **G** are mean ± SD, n=3. Data presented in panels **E-G** were from experiments repeated at least twice with similar results.