633

Figure S1. Related to Figure 1

(A) Confocal images of hPSC-amnion stained with indicated markers, outlining nuclear

aspect ratio quantitation.

(B) Expression of pluripotency markers, SOX2, NANOG and POU5F1, superimposed

onto the Gel-3D UMAP plot.

639 (C) A UMAP plot displaying the original single cell transcriptome coordinates of the CS7

640 human embryo described in Tyser *et al.*, shown with the original annotations.

641 (D,E) Expression of indicated broad (D) and late (E) amnion markers superimposed

642 onto the Tyser UMAP plot.

643 (F) UMAP plots showing the integrated single cell transcriptomes of d1-d4 Gel-3D and

644 Tyser *et al.* CS7 embryo datasets with individual Tyser annotations.

645

646 **Figure S2. Validation of selected specified and maturing markers in a cynomolgus**

647 **macaque embryo staged between CS12 and CS13**

648 (A,B) CS12/13 cynomolgus macaque embryo sections were stained for indicated

649 markers (A: *GABRP* – green, *SESN3* – red, *ISL1* – magenta; B: *ADAMTS18* – green,

650 *SESN3* – red, *ISL1* – magenta) using RNA *in situ* hybridization. (i) – (vii) indicate insets:

651 individual channels are shown in gray scale to aid visualization (from left to right:

652 *GABRP*, *SESN3*, *ISL1* and merge in (A), *ADAMTS18*, *SESN3*, *ISL1* in (B)), and dotted

653 lines indicate a layer of amniotic epithelium and mesenchyme. Most amnion cells are

654 positive for *GABRP, SESN3* and *ISL1. ADAMTS18* expression is restricted to a fraction

655 of cells. In (iv), there are four *ADAMTS18^{high}* cells, while *ADAMTS18* is largely

656 undetected in the amnion in (v). In (vi) and (vii), most cells are *ADAMTS18^{high}*.

657 FB; forebrain, HB, hindbrain; FG, foregut; C; cardiac tissue; NT, neural tube; S, somite.

658 * indicates an *ISL1^{+}* tissue that is likely of genital tubercle lineage. Scale bars = 500μm.

659 (C) An overview image of the CS12/13 embryo implantation site shown in (A). Only

660 nuclear staining is shown in grayscale. Scale bar = 2mm.

661

662 **Figure S3. Additional expression analyses**

663 (A) Expression of *TBXT* and *CLDN10* superimposed onto the uncropped Tyser *et al.*

664 CS7 human embryo UMAP plot.

665 (B) A UMAP plot displaying the GD14 cynomolgus macaque single cell transcriptome

666 with six identified general clusters (epiblast – light blue; transition – purple; amnion –

667 gold; mesoderm – green; endoderm – teal; extraembryonic mesoderm – salmon). Arrow

668 indicates the likely trajectory of amnion differentiation based on the data in Yang *et al.*

669 as well as the expression analysis in (C). Note that, although not identical to the

670 published UMAP plot in Yang *et al.*, general characteristics are well recapitulated.

671 Trophectoderm cells have been omitted as performed in Yang *et al.*.

672 (C) Expression of indicated markers superimposed onto the Yang *et al.* UMAP plot. Late

673 amnion markers (EPAS1 and GABRP) are expressed at the tip of the TFAP2A and ISL1

674 double-positive amnion cluster. CLDN10 positive cells show a weak TBXT expression

675 level.

676 (D,E) Individual channels of the whole embryo images in **Fig. 3C** and **3D**, respectively.

677 (F) An overview image of the CS10 (**Fig. 3C**) embryo implantation sites (DNA signal

678 shown in gray scale, Scale bar = 2mm).

679

680 **Figure S4. Validation of two distinct *CLDN10*-KO hPSC lines**

681 (A) Sequenced genotyping results of *CLDN10*-KO #1 and #2 lines. KO lines #1 and #2

682 have similar phenotype, both displaying an increased formation of PGC-LC and

683 defective cyst organization. The second coding sequence of *CLDN10* was targeted.

684 (B) Representative sequence traces for each mutations.

685

Table 1. Lists of differentially expressed genes in pluripotency-exiting (1506), early progenitor (692), late progenitor (697), specified (944), maturing (868), PGC-LC (1181) and advM-LC (746) clusters

689

Table 2. List of antibodies, RNAscope probes, primers and plasmids used in this study

Figure S1

A
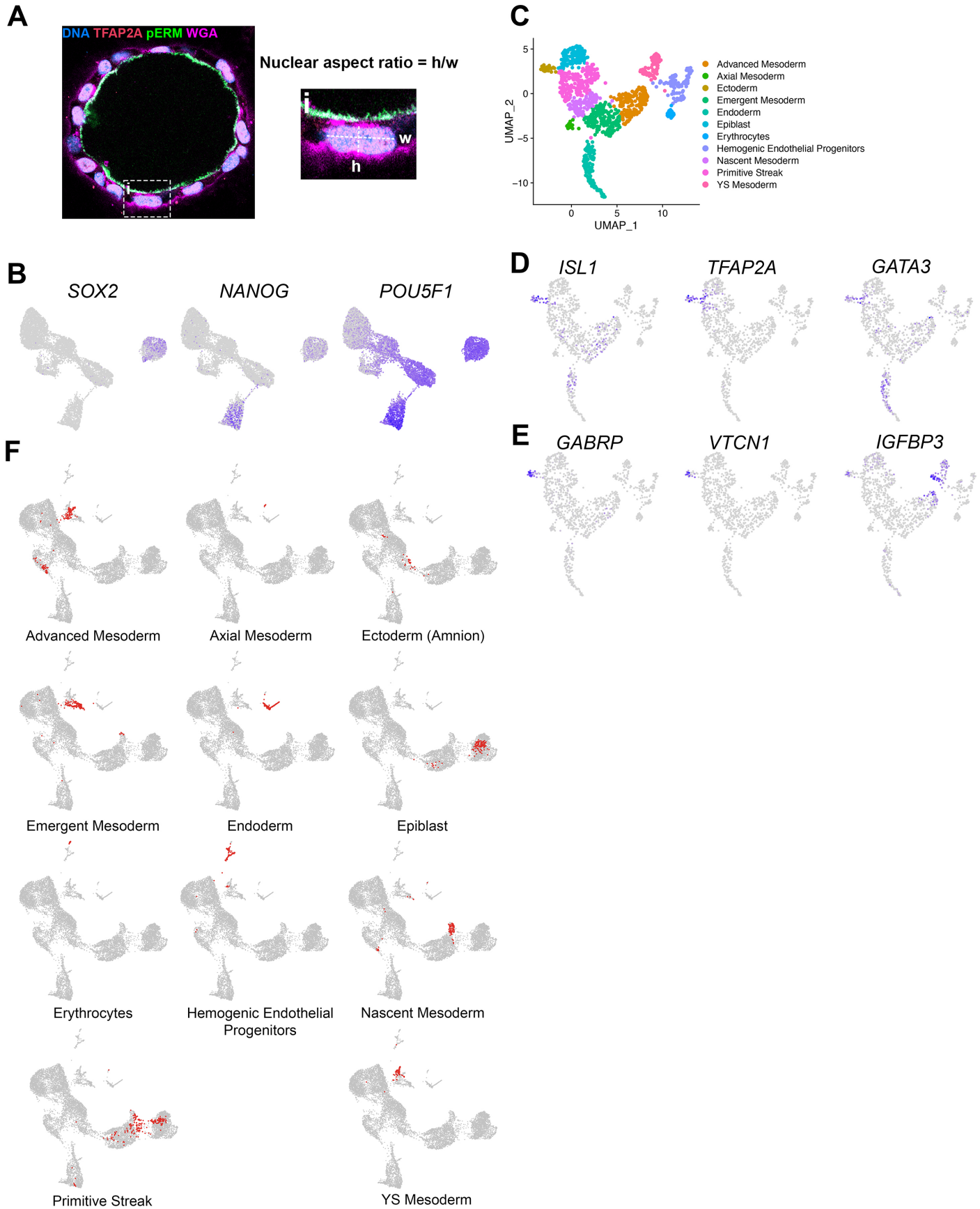
DNA TFAP2A pERM WGA

Nuclear aspect ratio = h/w

C

- Advanced Mesoderm
- Axial Mesoderm
- Ectoderm
- Emergent Mesoderm
- Endoderm
- Epiblast
- Erythrocytes
- Hemogenic Endothelial Progenitors
- Nascent Mesoderm
- Primitive Streak
- YS Mesoderm

B

SOX2   NANOG   POU5F1

D

ISL1   TFAP2A   GATA3

E

GABRP   VTCN1   IGFBP3

F

Advanced Mesoderm   Axial Mesoderm   Ectoderm (Amnion)

Emergent Mesoderm   Endoderm   Epiblast

Erythrocytes   Hemogenic Endothelial Progenitors   Nascent Mesoderm

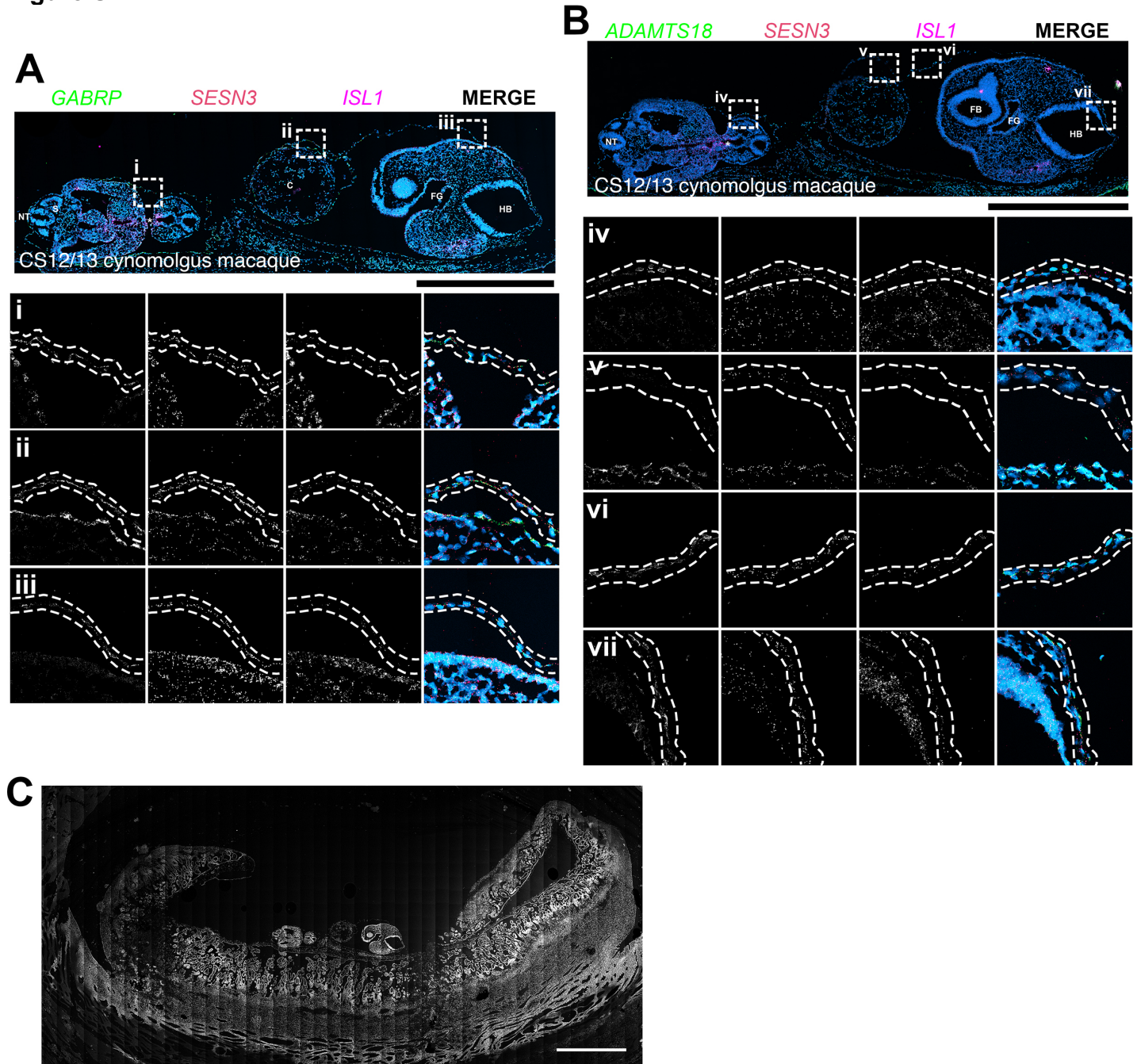Primitive Streak   YS Mesoderm

Figure S2
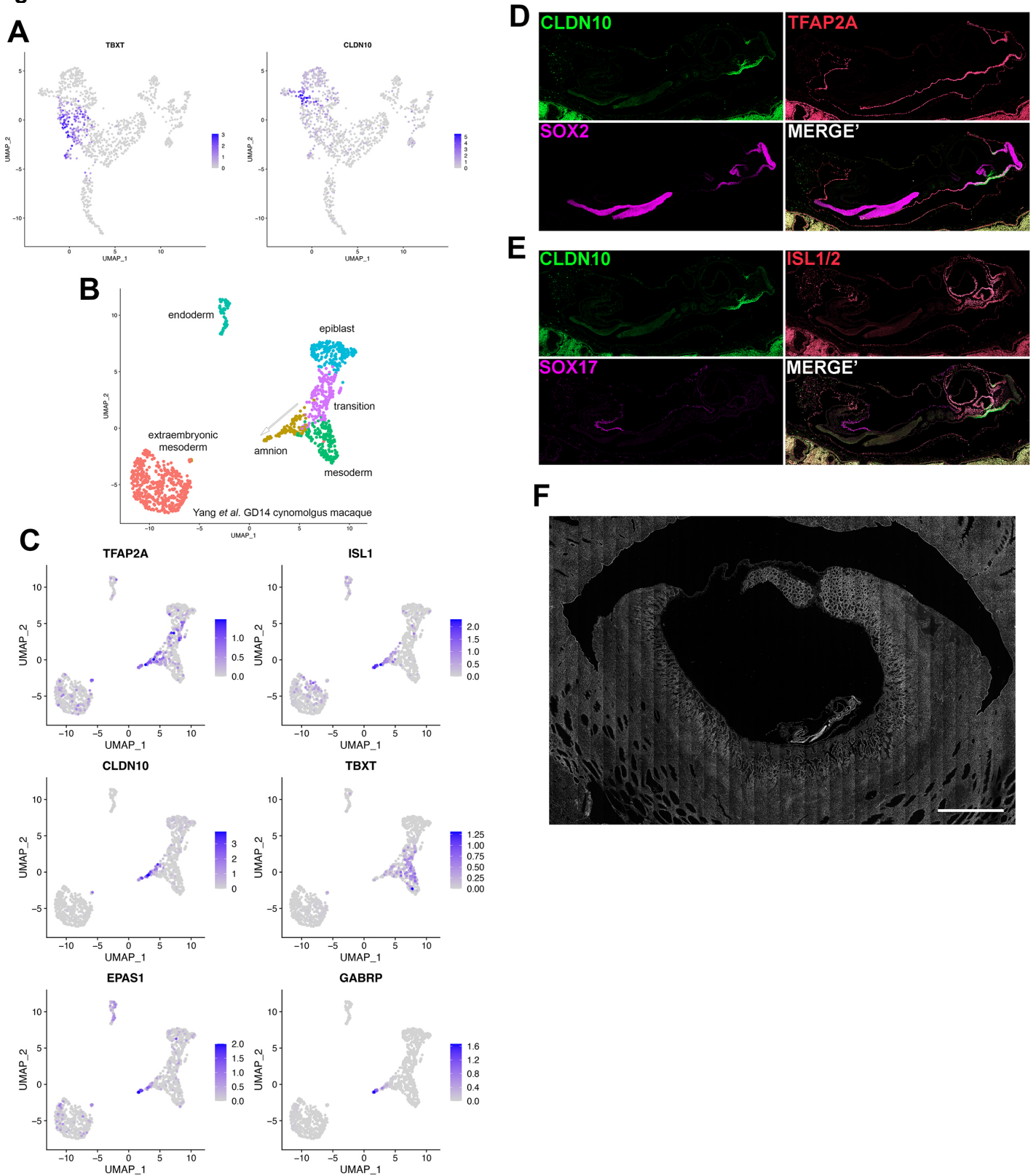
Figure S3

Figure S4

A   **Green: PAM**   **Red: Target sequence**   **--- : Deletion**

CLDN10-WT
1. ATGAAGTGTACCAAAGTCGGAGGCTCCGATAAAGCCAAAGCTAAA
2. ATGAAGTGTACCAAAGTCGGAGGCTCCGATAAAGCCAAAGCTAAA

CLDN10-KO #1
1. ATGAAGTGTAC- - - - - - - - - - - - -TCCGATAAAGCCAAAGCTAAA (13bp deletion)
2. ATGAAGTGTACCAAA- -CGGAGGCTCCGATAAAGCCAAAGCTAAA (2bp deletion)

CLDN10-KO #2
1. ATGAAGTGTACCAAA-TCGGAGGCTCCGATAAAGCCAAAGCTAAA (1bp deletion)
2. ATGAAGTGTACCAAA-TCGGAGGCTCCGATAAAGCCAAAGCTAAA (1bp deletion)