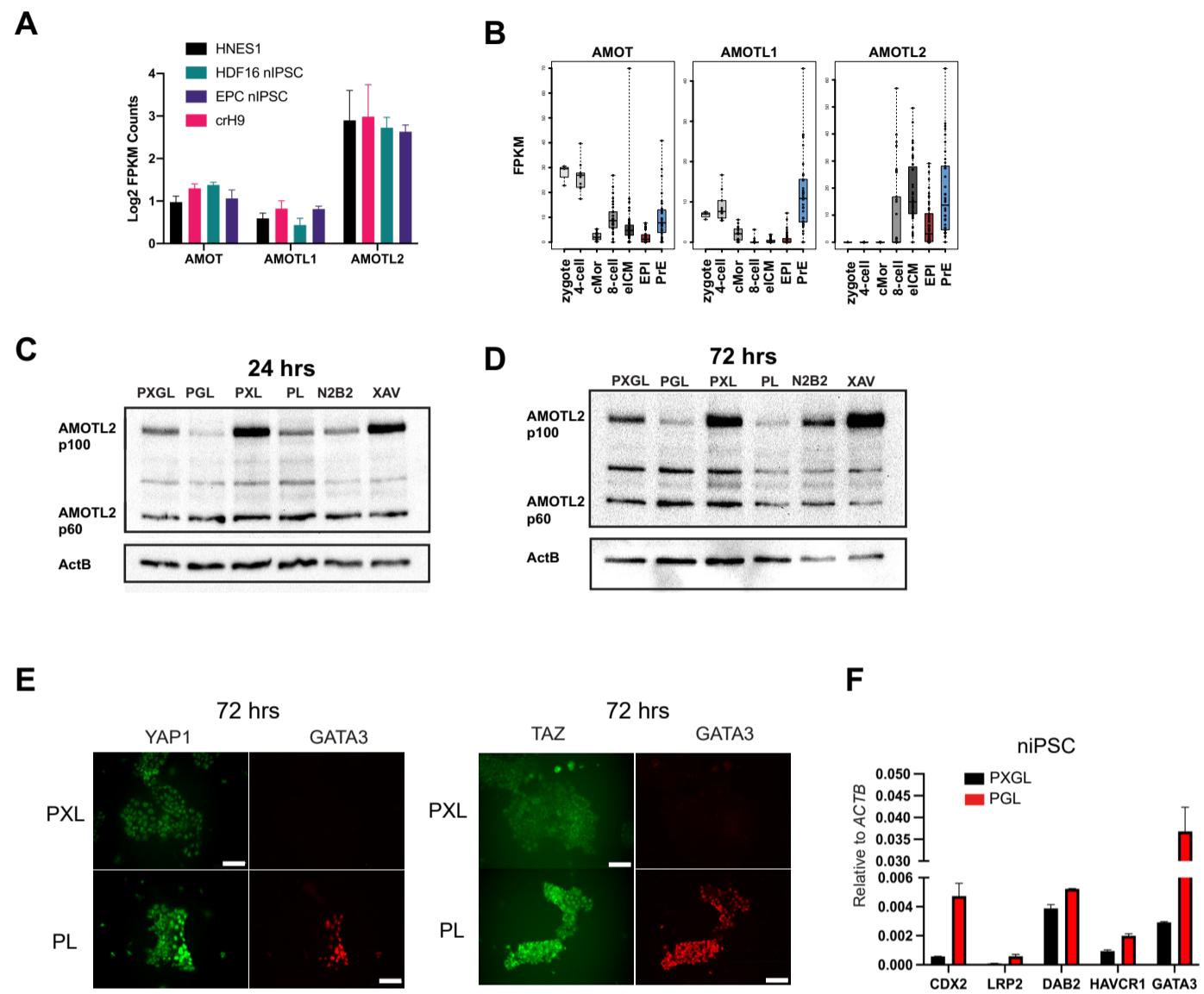


- Fig. S1.** **A.** qRT-PCR for TE marker *GATA3* and WNT target gene *TBX3* following 3 days transfer from PXGL to indicated culture conditions. Error bars indicate s.d. of PCR duplicates.
- B.** Schematic of strategy for generating and validating *CTNNB1* KO human nPSCs.
- C.** Flow cytometry analysis following  $\beta$ -catenin immunostaining in GFP and *CTNNB1* gRNA transfected pools expanded for 10 passages in PXGL.
- D.** Immunofluorescence staining for  $\beta$ -catenin in wild-type and clonal *CTNNB1* KO nPSCs. Scale bar: 20  $\mu$ m.
- E.** qRT-PCR for pluripotency markers in wild-type and *CTNNB1* KO cells. Error bars indicate s.d. of PCR duplicates.
- F.** Immunofluorescence staining for Plakoglobin and *GATA3* in WT and *CTNNB1*  $^{-/-}$  cells following culture in N2B27 containing PD03 and LIF (PL) for 3 days. Scale bar: 100  $\mu$ m.
- G.** Immunofluorescence-staining for naïve marker *KLF17* and Plakoglobin in wild-type and *CTNNB1* KO nPSCs cultured in PXGL on MEF. Scale bar: 100  $\mu$ m.



**Fig. S2. A.** FPKM counts for AMOT paralogs in naïve PSCs in published dataset GSE150933 (Stirparo et al., 2021).

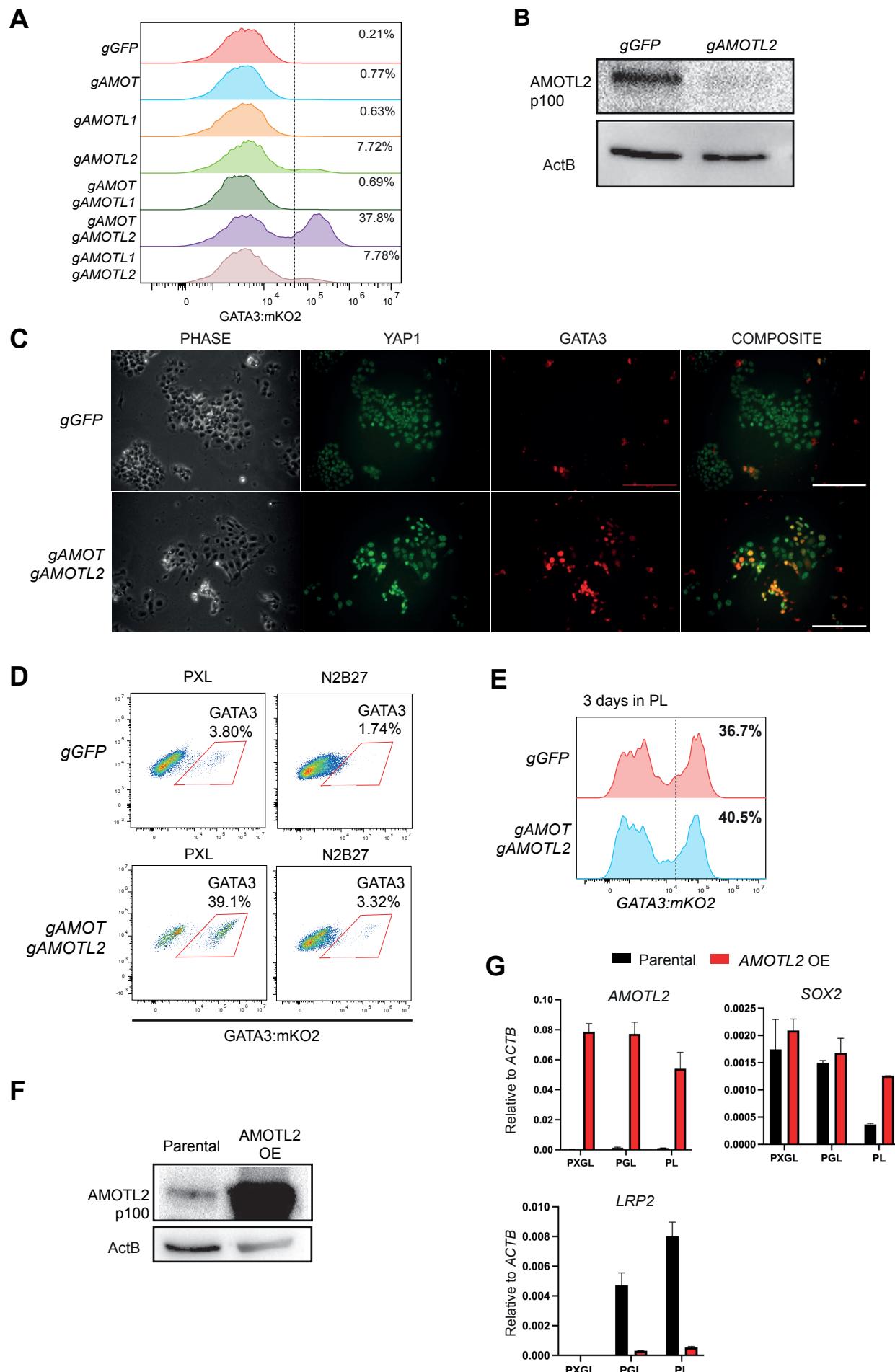
**B.** FPKM expression values of AMOT family genes derived from scRNA-seq datasets for early human embryo. Graphs taken from the GRAPPA web app (Boroviak et al., 2018) and indicate the first and third quartile of expression levels. The midline identifies the median. Each dot corresponds to an individual cell.

**C.** Complete western blot for Fig. 2C showing AMOTL2 expression in indicated conditions for 24 hrs following transfer from PXGL.

**D.** Western blot showing AMOTL2 expression in indicated conditions for 72 hrs following transfer from PXGL.

**E.** Immunofluorescence staining of YAP1 or TAZ and GATA3 after 72 hrs in N2B27 containing PD03, XAV, LIF (PXL) or PD03 and LIF (PL). Scale bar: 100 μm.

**F.** Expression of YAP/TEAD target genes in niPSCs cultured in PXGL or PGL for 3 days.



**Fig. S3. A.** Flow cytometry analysis for *GATA3:mKO2* reporter in cells transfected with indicated AMOT gRNAs. Cells were cultured in PXGL 5 days following gRNA transfection and puromycin selection.

**B.** Western blot of AMOTL2 six days following GFP or AMOTL2 gRNA transfection. Cells were cultured in PXGL prior to protein collection.

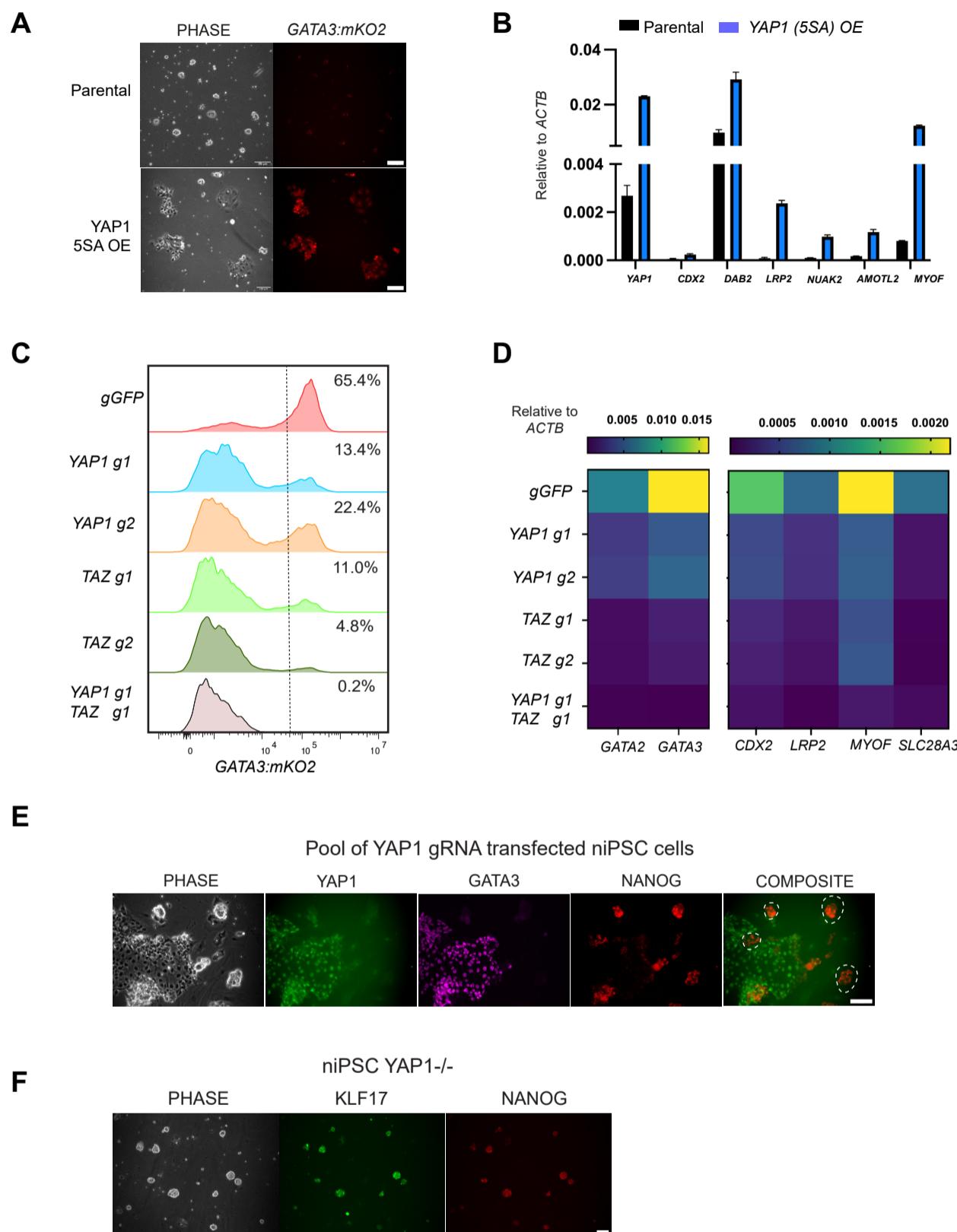
**C.** Immunofluorescence staining for YAP1 and GATA3 five days following transfection with *GFP* and *AMOT/AMOTL2* gRNAs in PXL. Scale bar: 200 µm.

**D.** Flow cytometry for *GATA3:mKO2* reporter expression following *AMOT/AMOTL2* double gRNA or *GFP* gRNA transfection. Cells were cultured in N2B27 or N2B27 containing PD03, XAV, LIF (PXL) for 4 days.

**E.** Flow cytometry for *GATA3:mKO2* reporter expression following *AMOT/AMOTL2* double gRNA or *GFP* gRNA transfection, and culture in PL for 3 days.

**F.** Western blot for AMOTL2 in parental and in cells expressing AMOTL2 transgene (AMOTL2 OE).

**G.** qRT-PCR assays for trophectoderm and naïve pluripotency markers for parental and AMOTL2 OE cells in PL 3 days following transfer from PXGL. Data are mean± s.d. of PCR duplicates.



**Fig. S4. A.** Phase and fluorescence images of naïve cell cultures in PXGL in parental and in cells 3 days after transfection with *YAP1 (5SA)* OE vectors. Scale bar: 100 µm

**B.** Trophectoderm and YAP-TEAD target gene expression in cells 5 days following transfection of *YAP1 (5SA)* OE vectors.

**C.** Flow cytometry analysis for *GATA3:mKO2* reporter in cells transfected with indicated gRNAs. Cells were cultured in PD03 for 4 days.

**D.** Heatmap of the expression of TE markers in gRNA transfected pools as in Fig. 4A. Expression levels are means of duplicate qRT-PCR assays.

**E.** Immunofluorescence staining in naïve iPSCs (niPSC) after *YAP1* gRNA/Cas9 protein complex transfection and 4 days' culture in PL. Circles indicate *YAP1* -/- naïve-like colonies lacking both *GATA3* and *YAP1* expression. Scale bar: 100 µm

**F.** Phase and immunofluorescence images of a *YAP1* -/- niPSC clone after 4 days' culture in PL. Colonies remained dome-shaped and expressed naïve pluripotency markers *KLF17* and *NANOG*. Scale bar: 100 µm

**Table S1. gRNA oligoes, qRT-PCR assays and antibodies**

[Click here to download Table S1](#)