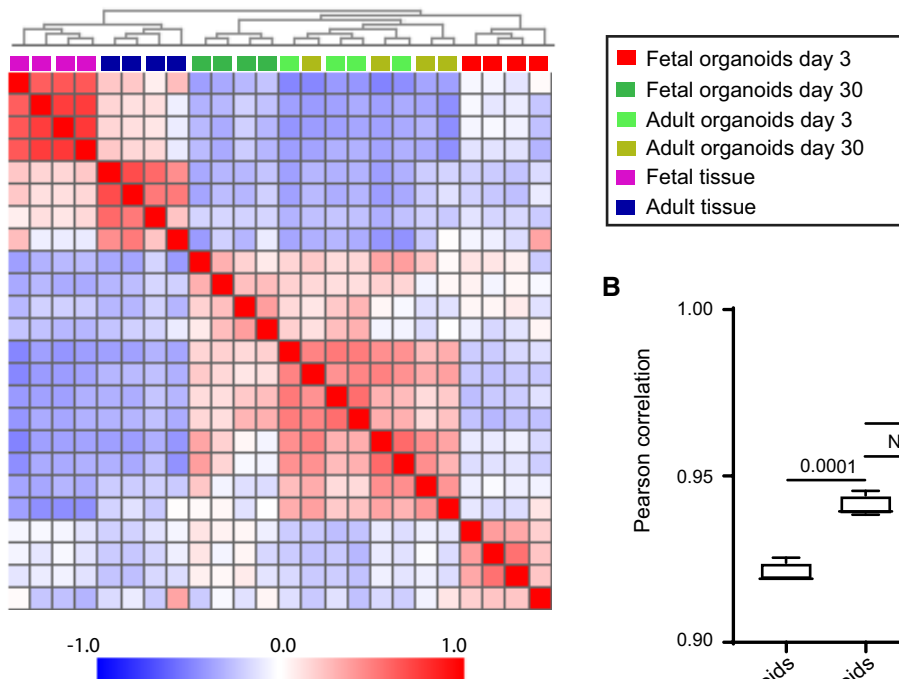
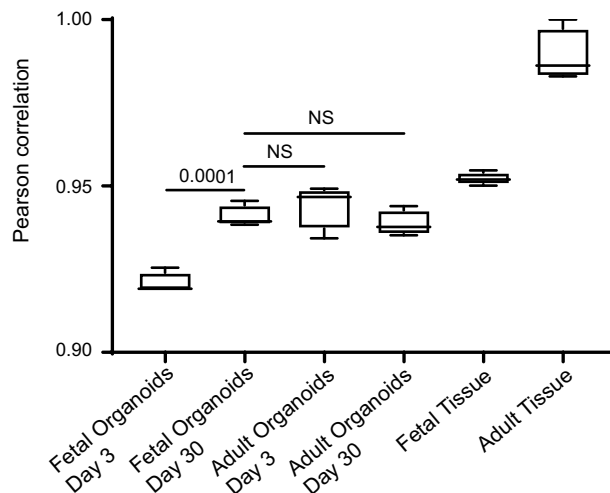


Expanded View Figures

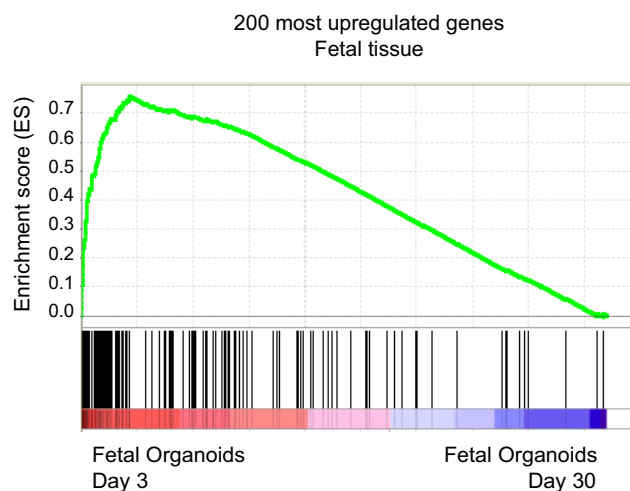
A



B



C



D

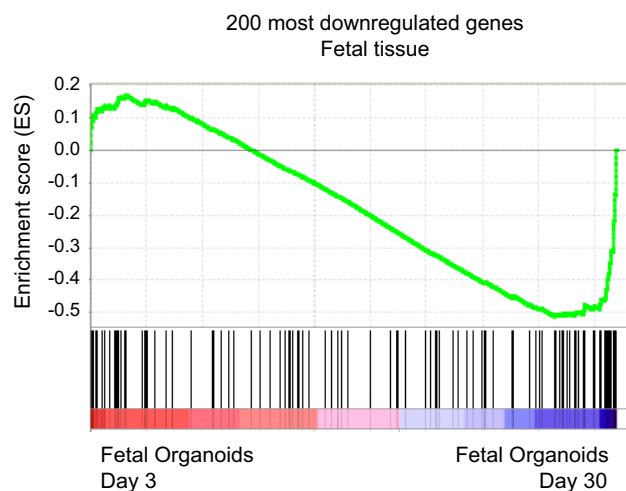


Figure EV1. Comparison of organoid with tissue maturation.

- A Pearson's correlation matrix including hierarchical clustering for whole-genome normalized microarray probes expressed in organoids/tissues indicated in the colored figure legend.
- B Pearson's correlation coefficient for the comparison of whole-genome normalized microarray probes between each of the sample listed on the x-axis and adult tissue. *P*-values indicate the results of Student's *t*-test. NS is not significant. *n* = 4.
- C, D Gene set enrichment analyses of 200 most (C) up- and (D) downregulated genes from mouse primary fetal versus adult epithelium (GSE35596) across fetal organoid maturation dataset. Vertical lines below x-axis display relative distribution of expression per gene included in the geneset.

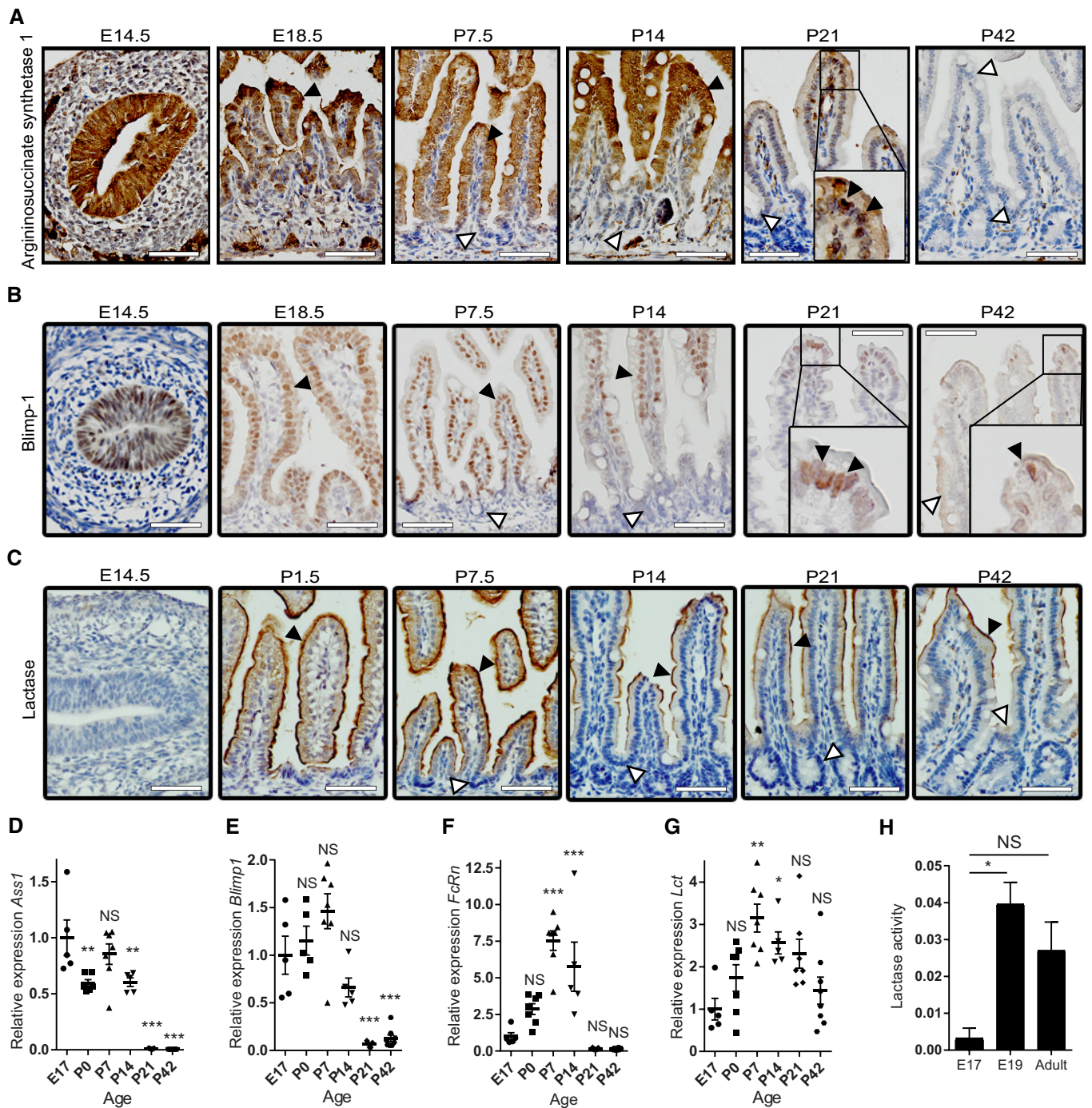


Figure EV2. In vivo expression of neonatal intestinal epithelial markers.

A–C Immunohistochemistry of neonatal markers: (A) *Ass1*, (B) *Blimp-1*, and (C) *Lct*. Insets represent higher magnification of the rectangle. White arrowheads indicate negative cells, and black arrowheads indicate positive cells. Scale bars: 50 μ m.

D–G Whole tissue real-time qPCR on (D) *Ass1*, (E) *Blimp-1*, (F) *FcRn*, and (G) *Lct* ($n = 5$ –8 individual intestinal specimens generated from offspring of single pregnant mice for E17 and P0–21, $n = 8$ independent intestines of adult mice for P42).

H Enzyme activity assay of fetal and adult whole tissue lysates for lactase ($n = 3$ –5 individual intestinal specimens, generated from offspring of single pregnant mice for E17–19, $n = 5$ independent intestines of adult mice for P42).

Data information: Data are presented as mean \pm SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS: not significant, in (D–G) relative to expression level at E17 (one-way ANOVA).

Figure EV3. *In vivo* expression of adult intestinal epithelial markers.

- A, B Immunohistochemistry of adult markers (A) *Sis* and (B) *Arg2*. Insets represent higher magnification of the rectangle. White arrowheads indicate negative cells, and black arrowheads indicate positive cells. Scale bars: 50 μm .
- C–H Whole tissue real-time qPCR on (C) *Sis*, (D) *Arg2*, (E) *Treh*, (F) *Lyz1*, (G) *Defcr1*, and (H) *Defcr5* ($n = 5$ –8 individual intestinal specimens generated from offspring of single pregnant mice for E17 and P0–21, $n = 4$ –8 independent intestines of adult mice for P42).
- I–L Enzyme activity assay of fetal and adult whole tissue lysates for (I) sucrase, (J) maltase, (K) trehalase, and (L) arginase activities ($n = 3$ –5 individual intestinal specimens, generated from offspring of single pregnant mice for E17–19, $n = 5$ independent intestines of adult mice for P42).

Data information: Data are presented as mean \pm SEM. ND not detected, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS: not significant, in (C–H) relative to expression level at E17 (one-way ANOVA).

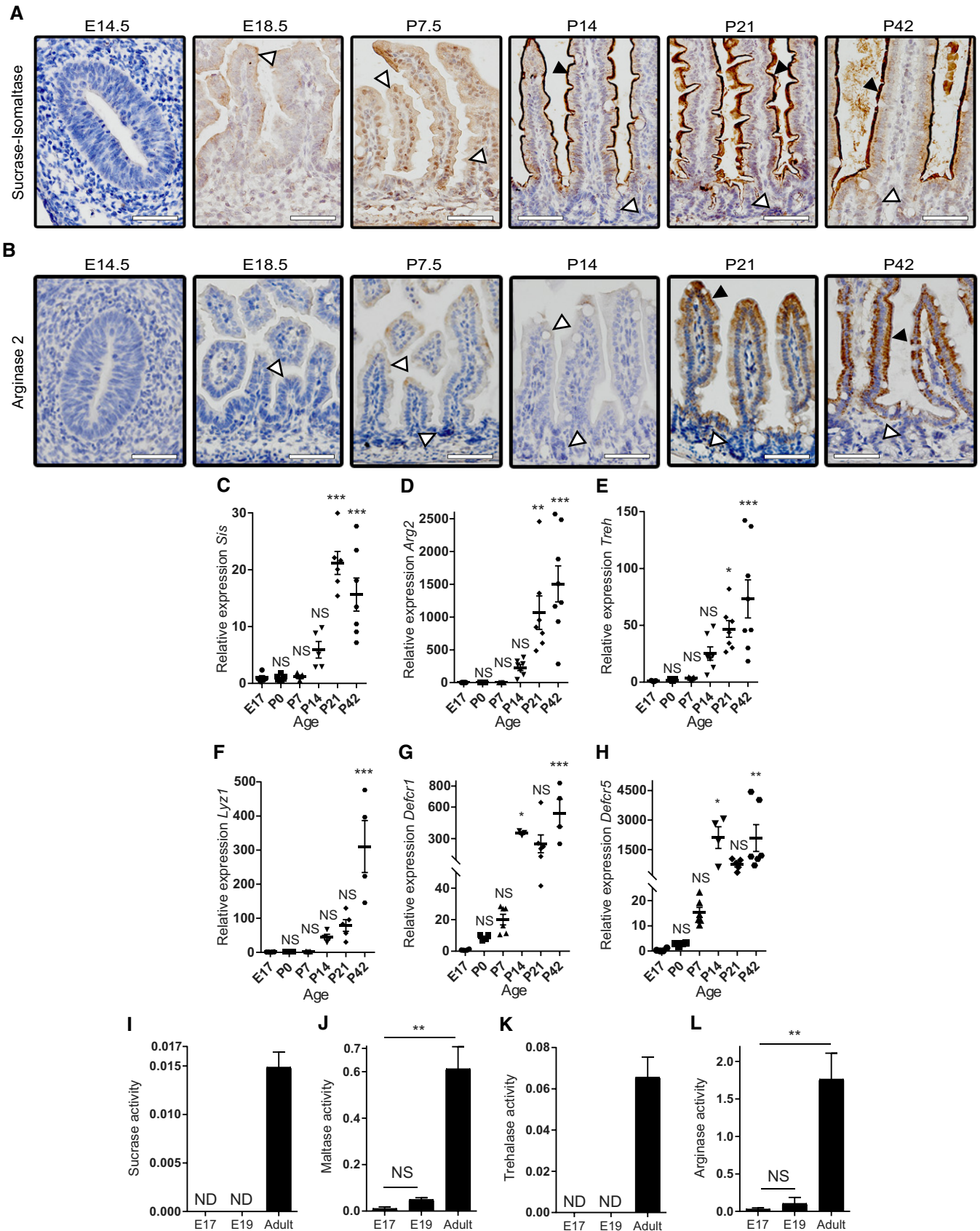


Figure EV3.

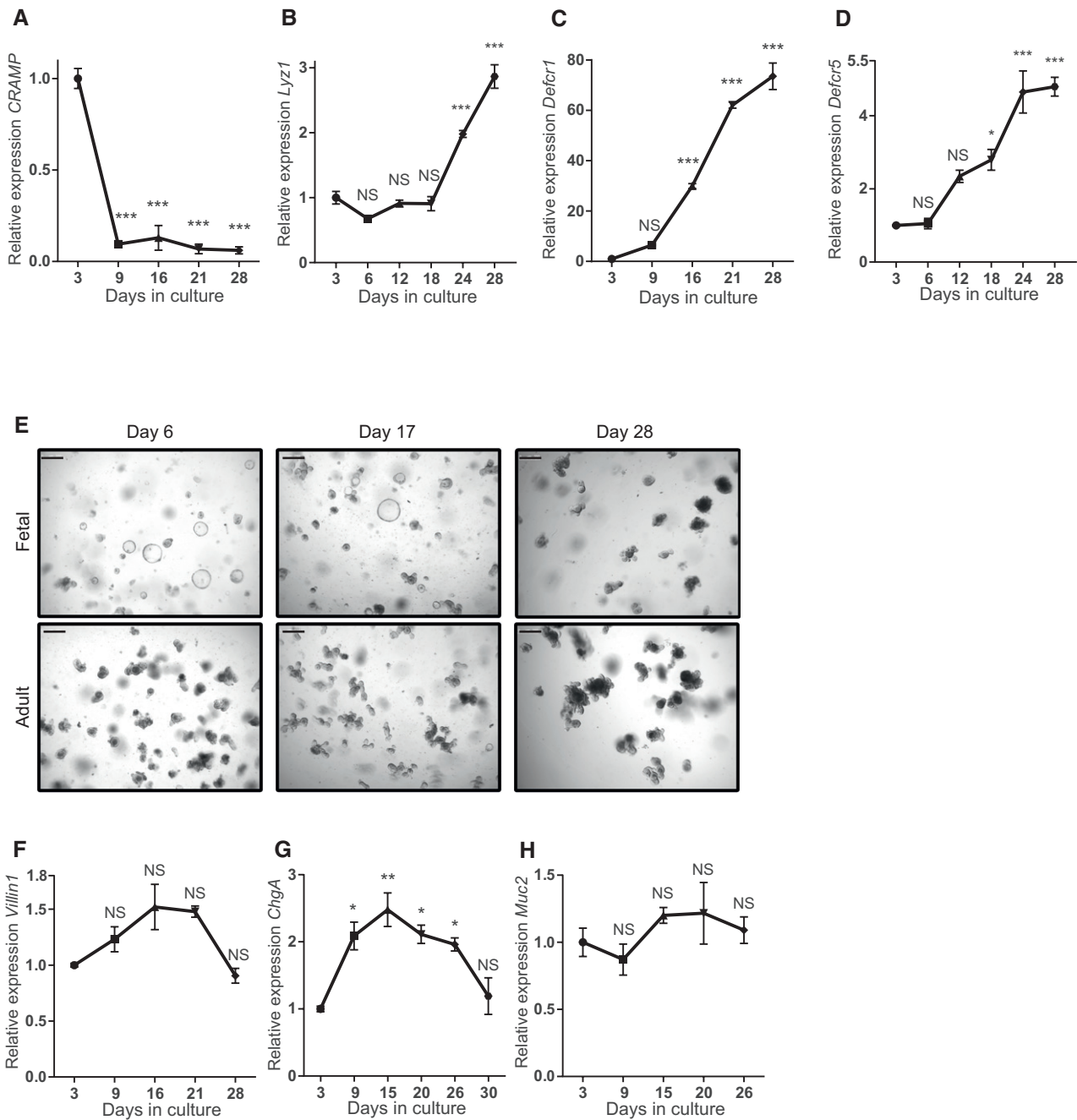


Figure EV4. Fetal organoids mature *in vitro*.

A–D Real-time qPCR analysis of fetal organoids cultured for 1 month showing decrease in relative expression of (A) *CRAMP* and increase in the Paneth cell markers (B) *Lyz1*, (C) *Defcr1*, and (D) *Defcr5* ($n = 3$ individual wells from single organoid culture (see Materials and Methods); experiment was repeated four times with similar results).

E Microscopic images of fetal and adult organoids at days 6, 17, and 28 of culture. Scale bars: 250 μ m.

F–H Real-time qPCR of (F) *Villin1*, (G) *ChgA*, and (H) *Muc2* ($n = 3$ individual wells from single organoid culture (see Materials and Methods); experiment was repeated four times with similar results).

Data information: Data are presented as mean \pm SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS: not significant, relative to expression level at day 3 (one-way ANOVA).

Figure EV5. Spheroid and organoid culture follow the same dynamics as mixed culture.

- A Scheme of culture separation.
- B–D Percentage of spheroids versus organoids in (B) mixed culture, (C) spheroids, and (D) organoids ($n = 3$ individual wells from single organoid culture (see Materials and Methods); experiment was repeated four times with similar results).
- E Microscopic images of mixed culture at days 1, 3, and 4 post-passage (p.p.), showing the transition of spheroids 1 and 2 into organoids between passage 1 and passage 2. Scale bars: 500 μm .
- F, G Microscopic images of (F) spheroid and (G) organoid culture at days 0, 1, 4, and 5 post-passage (p.p.), showing the conversion of spheroids 1, 2, and 3 into organoids between passage 1 and passage 2. Scale bars: 500 μm .

Data information: Data are presented as mean \pm SEM.

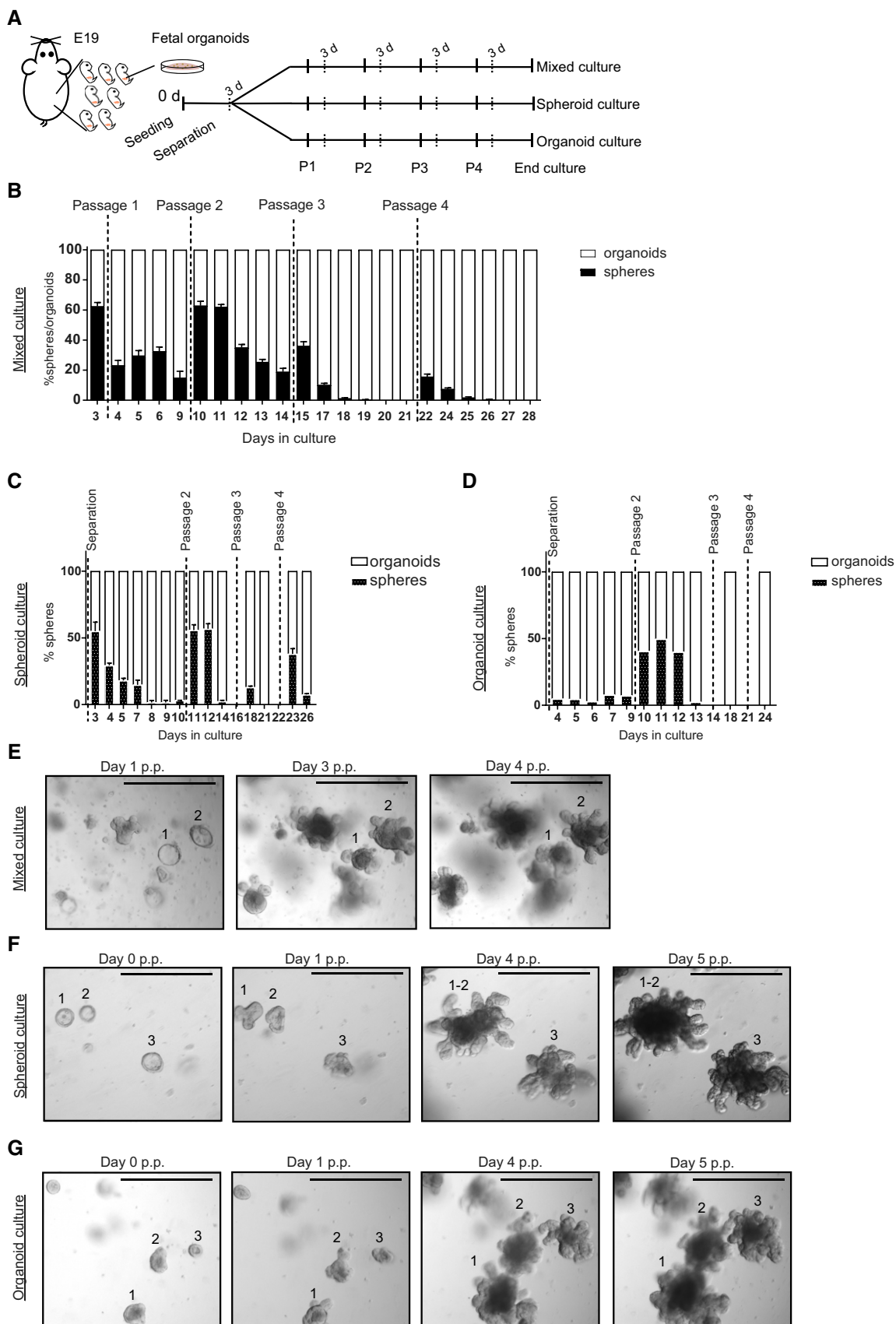


Figure EV5.