Cell Reports, Volume ■ ■

Supplemental information

Generation of salivary glands derived from pluripotent stem cells via conditional blastocyst complementation

Junichi Tanaka, Akihiro Miura, Yuko Shimamura, Youngmin Hwang, Dai Shimizu, Yuri Kondo, Anri Sawada, Hemanta Sarmah, Zurab Ninish, Kenji Mishima, and Munemasa Mori

Figure S1

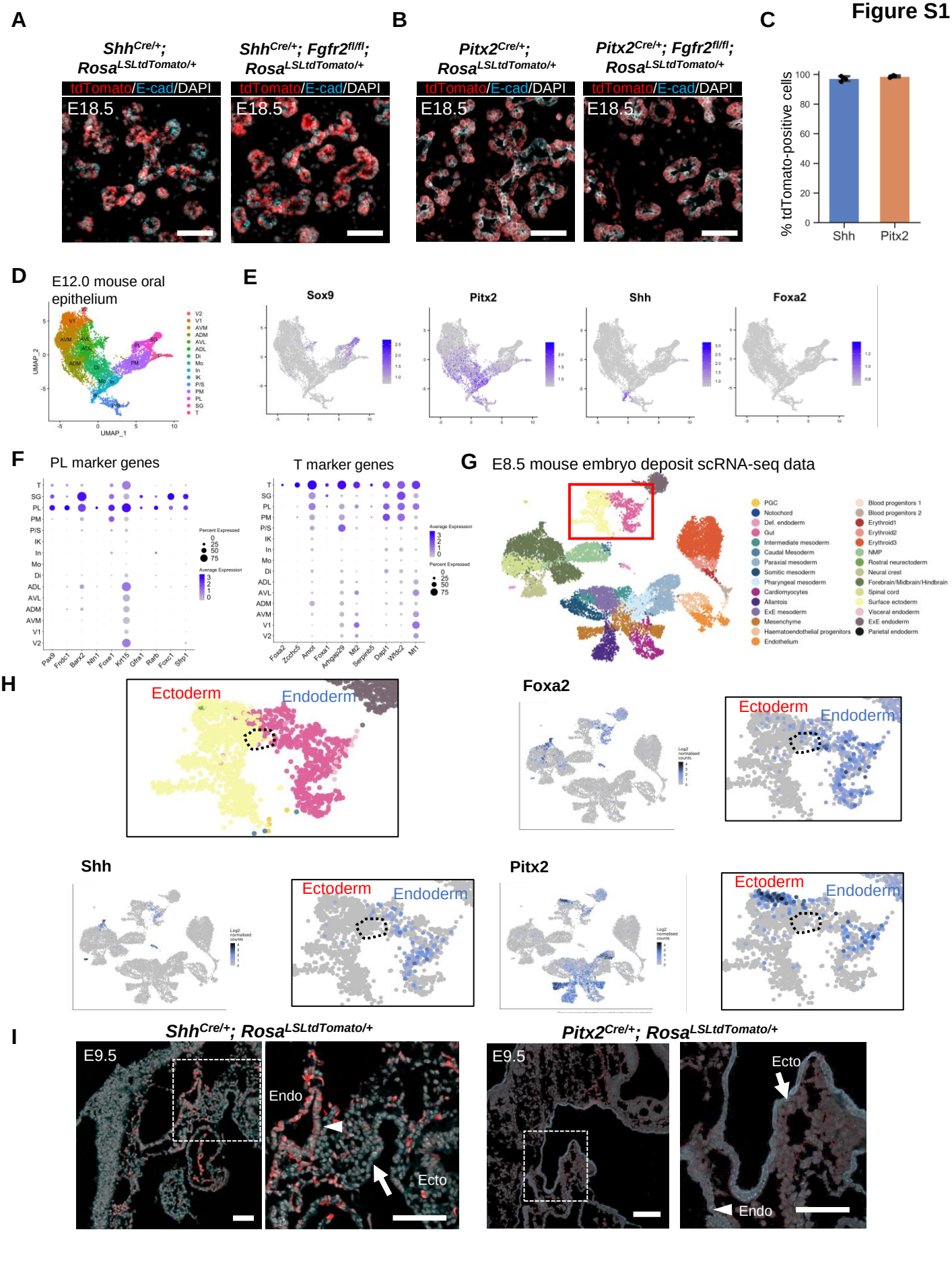
A
Shh^Cre/+; Rosa^LSLtdTomato/+
Shh^Cre/+; Fgfr2^fl/fl; Rosa^LSLtdTomato/+
tdTomato/E-cad/DAPI
E18.5
B
Pitx2^Cre/+; Rosa^LSLtdTomato/+
Pitx2^Cre/+; Fgfr2^fl/fl; Rosa^LSLtdTomato/+
C
% tdTomato-positive cells
Shh
Pitx2
D
E12.0 mouse oral epithelium
UMAP_1
UMAP_2
E
Sox9
Pitx2
Shh
Foxa2
F
PL marker genes
T marker genes
G
E8.5 mouse embryo deposit scRNA-seq data
H
Ectoderm
Endoderm
Foxa2
Shh
Pitx2
I
Shh^Cre/+; Rosa^LSLtdTomato/+
Pitx2^Cre/+; Rosa^LSLtdTomato/+
E9.5
Endo
Ecto

Figure S1. Lineage-tracing, Fgfr2 loss of function, and scRNA-seq analysis of Cre driver candidate genes for CBC, Related to Figure 1

(A) Representative confocal imaging of the salivary gland in the E18.5 *Shh$^{Cre/+}$; Rosa$^{LSL\text{-}tdTomato/+}$* (left) and *Shh$^{Cre/+}$; Fgfr2$^{flox/flox}$; Rosa$^{LSL\text{-}tdTomato/+}$* mouse (right). Immunostaining of tdTomato: red, E-cadherin (E-cad): cyan, DAPI: gray. (N=3) **(B)** Representative confocal imaging of the salivary gland in the E18.5 *Pitx2$^{Cre/+}$; Rosa$^{LSL\text{-}tdTomato/+}$* (left) and *Pitx2$^{Cre/+}$; Fgfr2$^{flox/flox}$; Rosa$^{LSL\text{-}tdTomato/+}$* mouse (right). Immunostaining of tdTomato: red, E-cadherin (E-cad): cyan, DAPI: gray. (N=3) **(C)** The morphometric analysis: % of tdTomato-positive cells in E-cad^{+} epithelial cells from the E18.5 Shh$^{Cre/+}$; Rosa$^{LSLtdTomato/+}$ or Pitx2$^{Cre/+}$; Rosa$^{LSLtdTomato/+}$ mice. (N = 3 per biological replicates, 5 fields per group). Error bars represent mean $\pm$ SD. **(D)** Annotated uniform manifold approximation projection (UMAP) plots of mouse oral epithelium at E12.0. Clusters assigned: ADL, anterodorsal-lateral; ADM, anterodorsal-medial; AVL, anteroventral-lateral; AVM, anteroventral-medial; Di, diastema; IK, initiation knot; In, incisor; Mo, molar; PL, posterior-lateral; PM, posterior-medial; P/S, periderm and suprabasal cells; SG, salivary gland; T, tongue; V1, ventral 1; V2, ventral 2. **(E)** Feature plots for the expression of candidate Cre-driver genes in mouse oral epithelium at E12.0. **(F)** Dot plots for top 10 differentially expressed genes of tongue (T) and posterior-lateral (PL) culsters. **(G)** Annotated UMAP plots of E8.5 mouse embryos. **(H)** Feature plots for the expression of candidate Cre-driver genes in E8.5 mouse embryos. Red dot aria is the boundary region of the oral cavity. **(I)** Representative confocal imaging of the oral cavity in the E9.5 *Shh$^{Cre/+}$; Rosa$^{LSL\text{-}tdTomato/+}$* (left) and E9.5 *Pitx2$^{Cre/+}$; Rosa$^{LSL\text{-}tdTomato/+}$* mouse (right). Each right panel is an enlarged image of a white dotted box. Shh lineage labeled only the endoderm (Endo) (arrowhead) but not ectoderm (Ecto) (arrow). Pitx2 lineage labeled neither of them. tdTomato: red, DAPI: gray. (N=3) Scale bars: 100 μm.

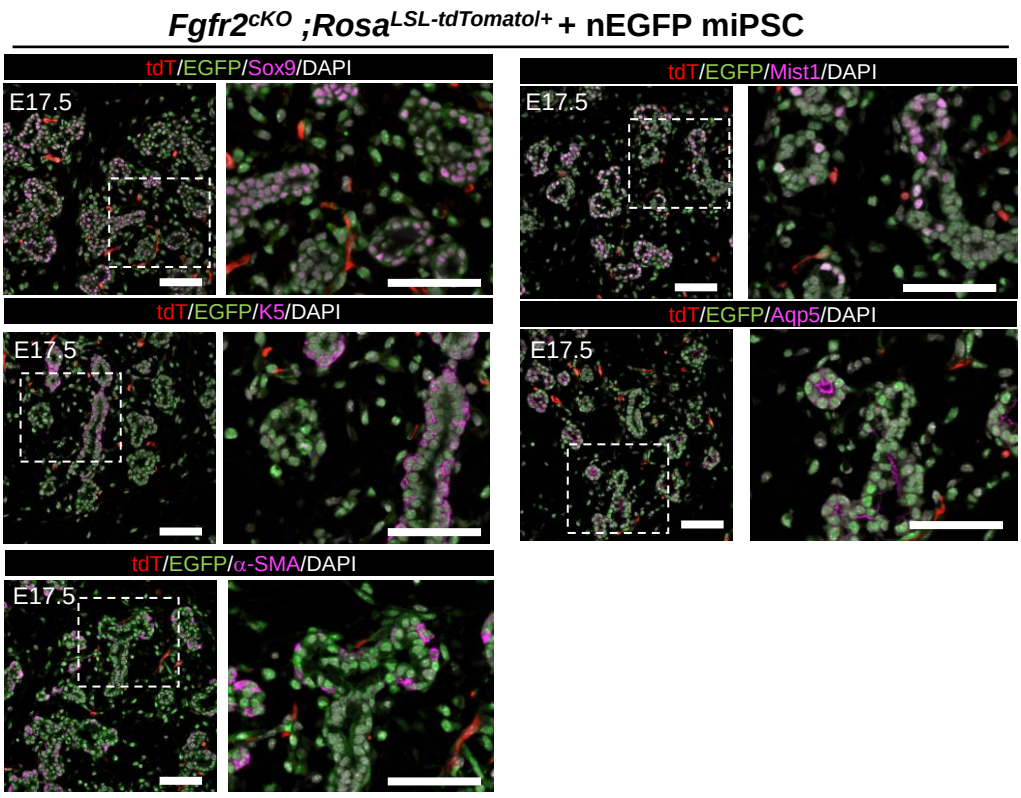

Figure S2. The developing salivary glands rescued by the donor cells had normal salivary gland structure and differentiation, Related to Figure 3

Representative IF-confocal imaging of complemented salivary gland from the E17.5 chimeric mice of *Fgfr2*^{cKO}+nEGFP miPSC. The right panel is an enlarged image of a white dotted box. Immunostaining of tdTomato: red, EGFP: green, each cell type maker: cyan, DAPI: gray. (N=3) Scale bars: 100 μm.

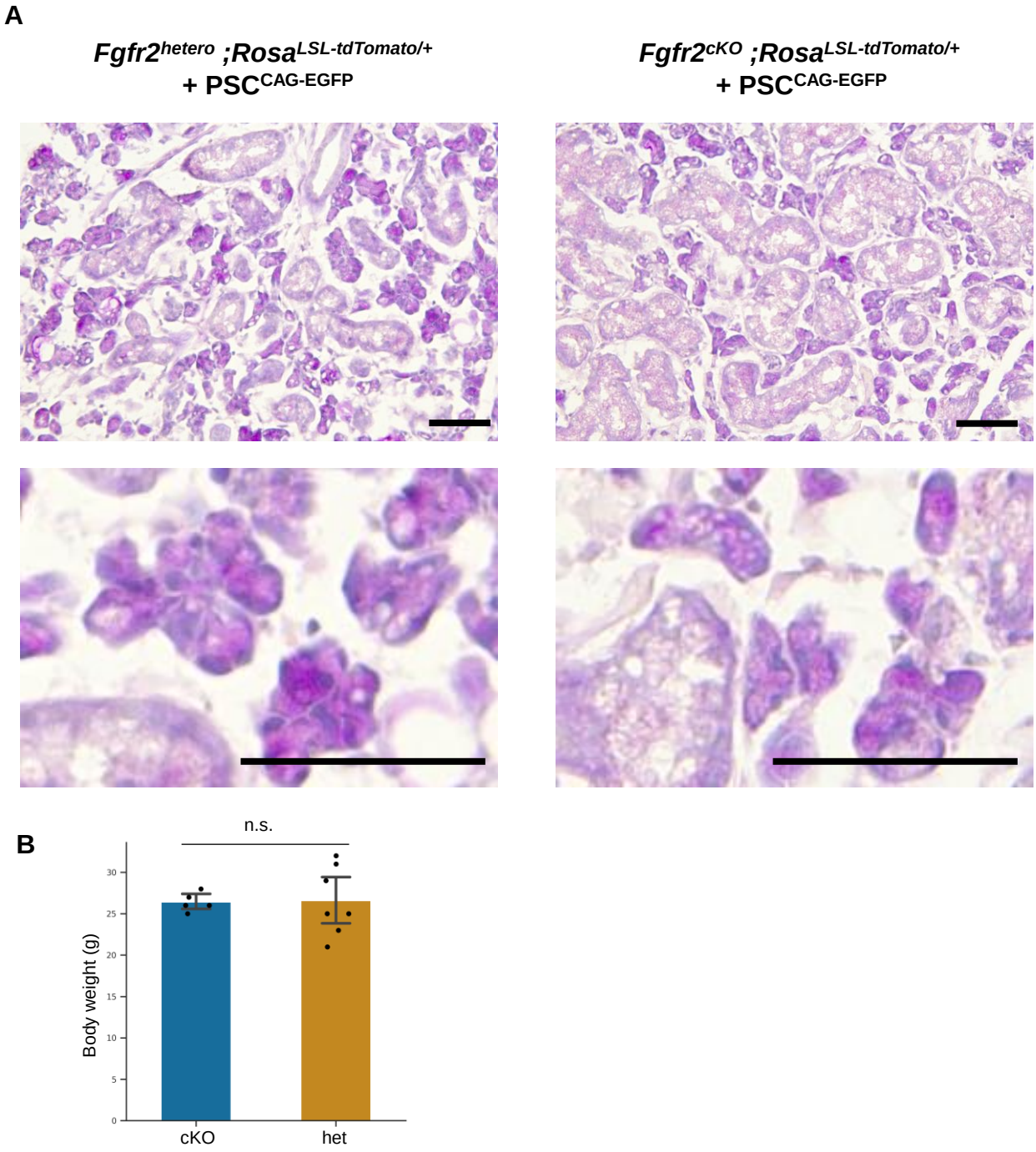

Figure S3. The complemented salivary glands in adulthood had normal functions, Related to Figure 4

(A) Representative PAS-staining images of complemented salivary gland from the 4 weeks-old chimeric mice of *Fgfr2*^{cKO}+ mPSCs or *Fgfr2*^{hetero}+ mPSCs. The bottom panel is an enlarged image. (N=3) Scale bars: 100 μm. **(B)** Analysis of body weight. Het: *Fgfr2*^{hetero}, PSC$^{CAG\text{-}EGFP}$, cKO: *Fgfr2*^{cKO}, PSC$^{CAG\text{-}EGFP}$. Statistical analyses: unpaired Student's t-test, significant: $p<0.05$. no significant change: n.s. Error bars represent mean ± SD.

Table S1. Summary for conditional blastocyst complementation, Related to Figure 3

Cell Line	host mice	Number of host blastocysts	% survival (number of pups or embryos/number of transferred blastocysts)	% survival at P0 (number of pups survived P0/number of pups born)	% chimera formation at analysis	Analysis	% cKO genotyping	% Salivary gland complementation (Number of complementation in Fgfr2 cKO genotype)
nGFP^{+} iPSC	*Foxa2*	16	75% (12/16)	-	83% (10/12)	E17.5	50% (5/10)	100% (5/5)
PSC$^{CAG-GFP}$	*Foxa2*	78	56% (44/78)	77% (34/44)	87% (27/31)	4 weeks	21% (5/24)	100% (5/5)

Table S2. E17.5 chimerism of Foxa2-driven Fgfr2 cKO conditional blastocyst complementation, Related to Figure 3

	Liver (%)	Hematopoietic cells (%)	Salivary galnd	
			Epithelium (%)	Mesenchyme (%)
Fgfr2 cKO	*56.2*	71.3	100	92.3
	54.5	64.9	100	59.4
	48.3	62.9	100	57.2
	14.4	21.1	100	48.4
Fgfr2 hetero	*60.2*	68.7	63.4	81.1
	55.8	70	63	68.2
	24.8	32	88.5	59.8
	9.7	12.6	84	42.1
Average Chimerism in Fgfr2 cKO (%)	***43.35***	***55.05***	***100***	***64.325***
Average in Fgfr2 hetero (%)	***37.625***	***45.825***	***74.725***	***62.8***