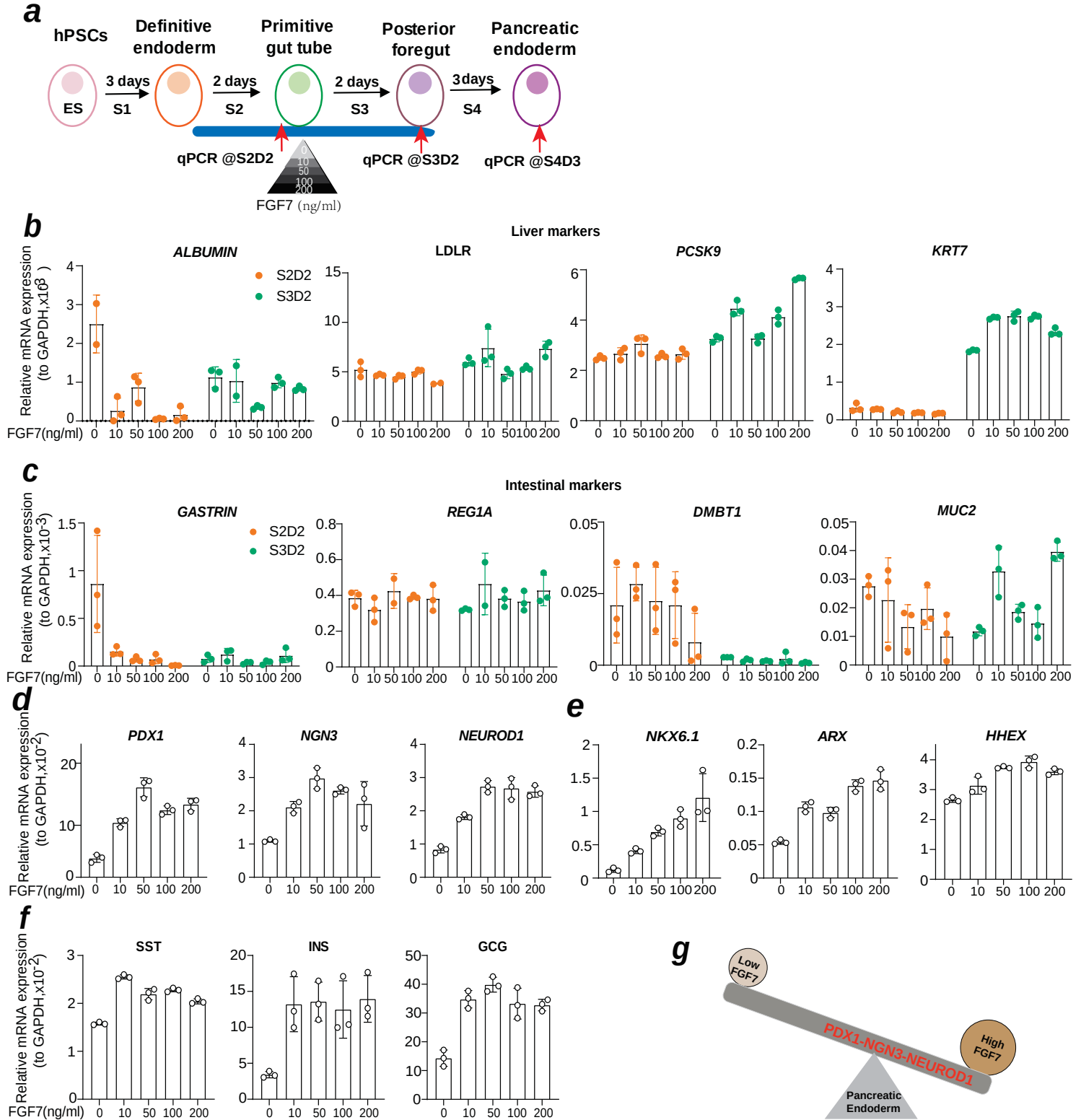
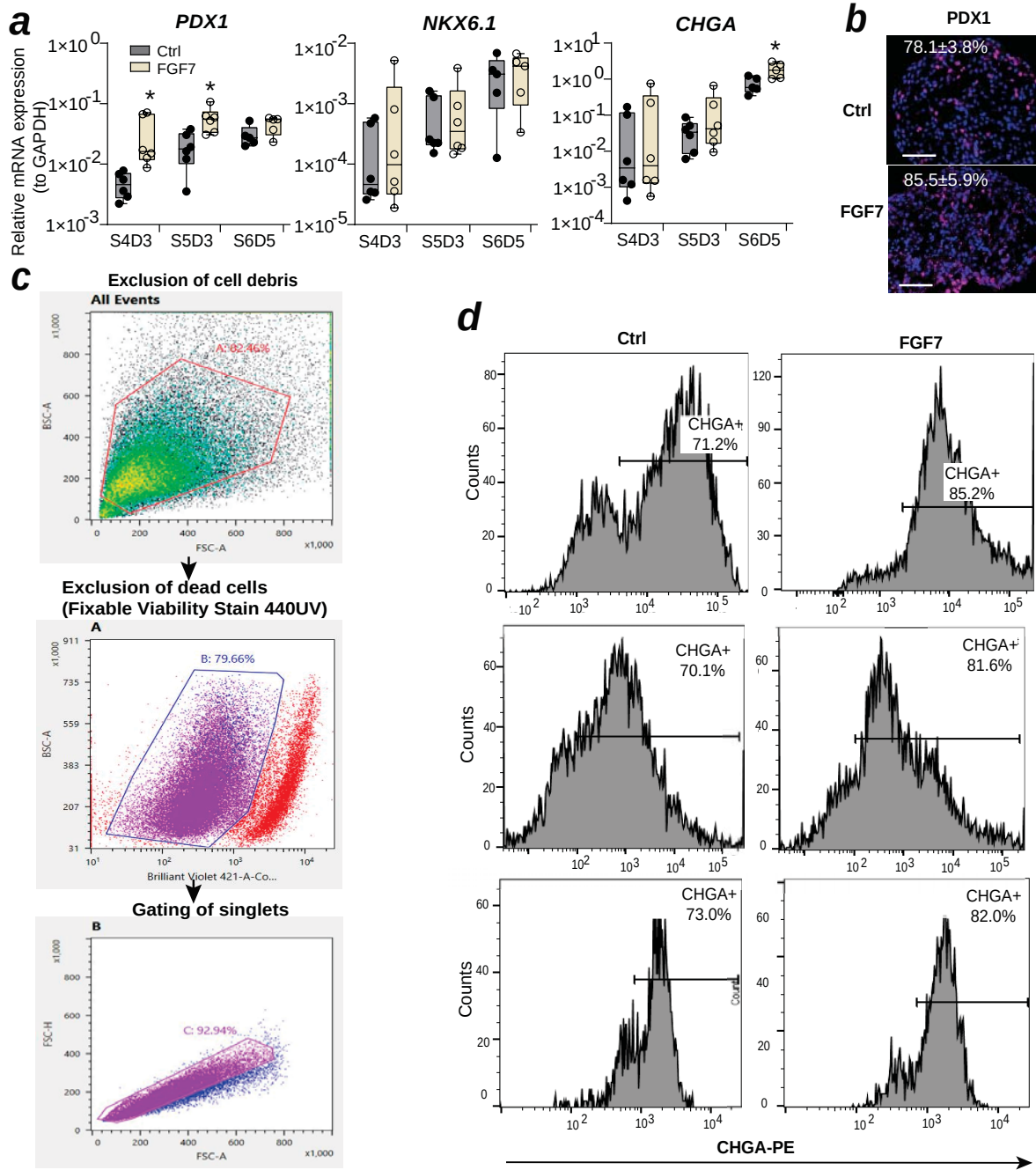




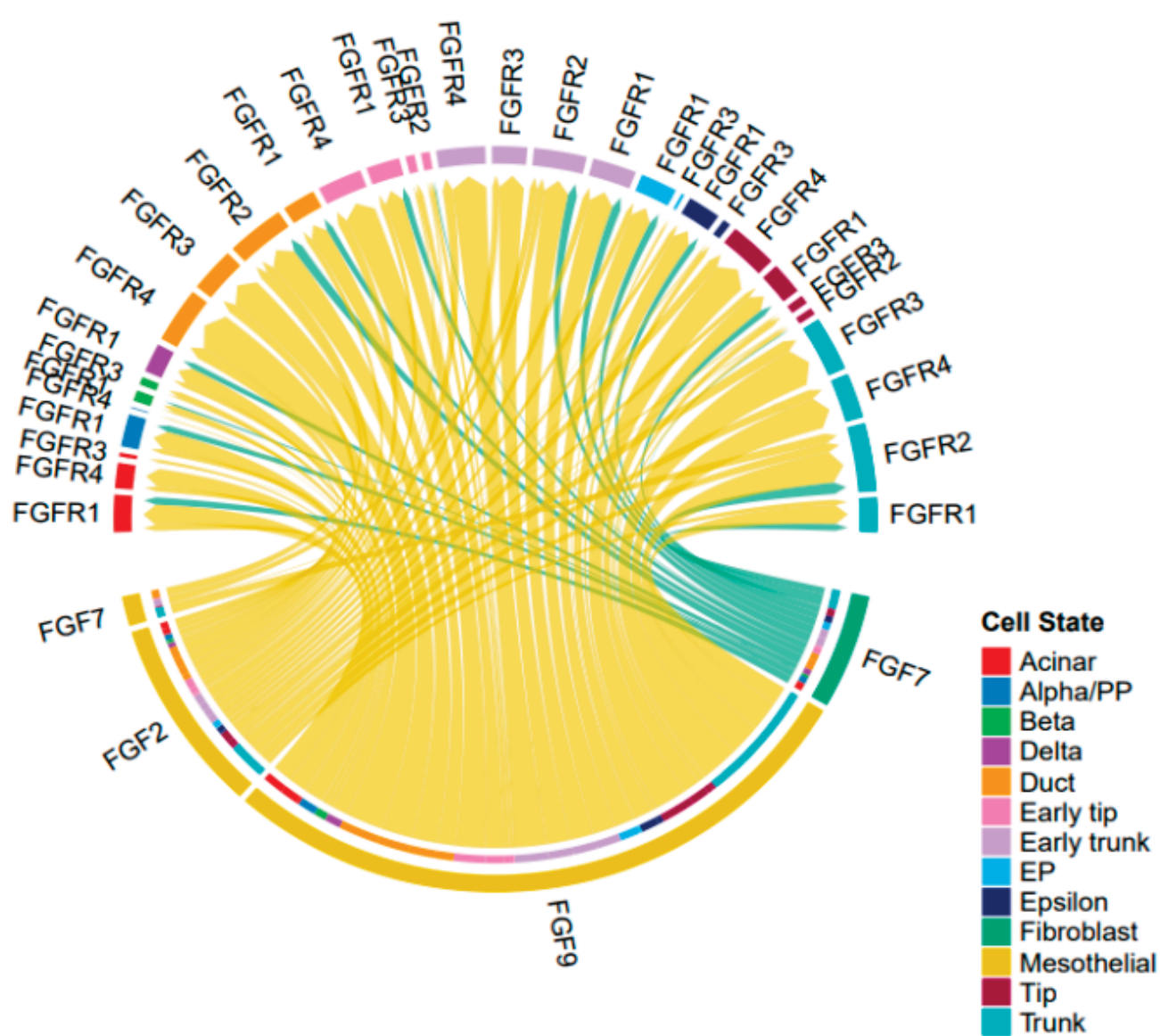
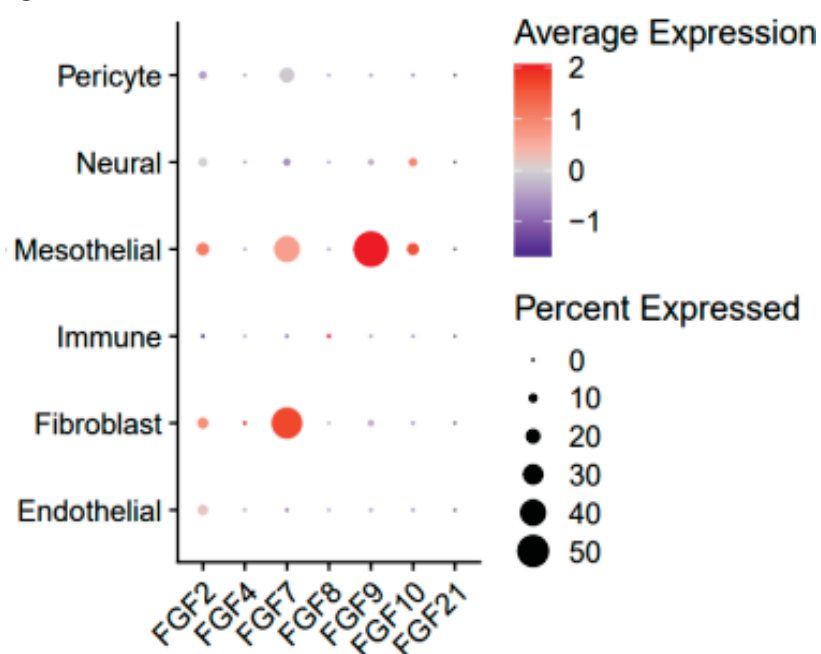
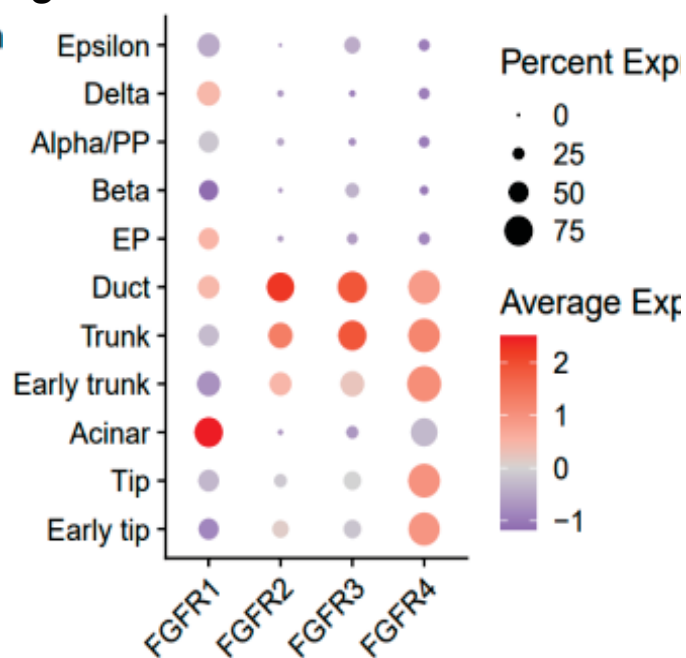
Supplementary Figure 1. FGF7 facilitates the induction of the pancreatic endodermal lineage rather than the liver and intestinal lineage.

a, Schematic of the basic differentiation protocol for producing pancreatic endoderm. **b-c**, RT-qPCR of liver lineage (*ALBUMIN*, *LDLR*, *PCSK9*, and *KRT7*) and intestinal lineage genes expression (*GASTRIN*, *REG1A*, *DMBT1*, and *MUC2*) at completion of stage 2 (S2D2) and stage 3 (S3D2). **d-f**, RT-qPCR of pancreatic endoderm and endocrine precursor genes expression (*PDX1*, *NEUROG3*, *NEUROD1*, *NKX6.1*, *HHEX*, *ARX*, *SST*, *INS*, and *GCG*) at completion of stage 4 (S4D3). Values are mean $\pm$ s.e.m. from 3 independent differentiations (**b-f**). **g**, Schematic diagram showing that FGF7 dose-dependently enhances pancreatic endoderm induction.



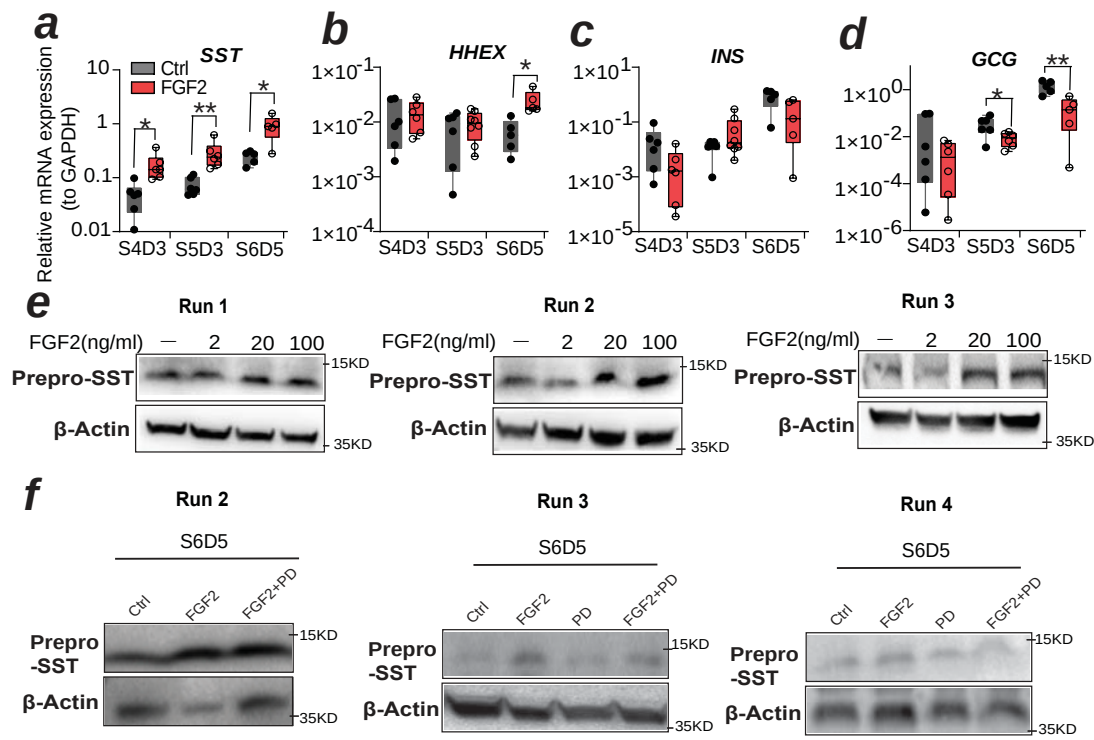
Supplementary Figure 2. Prolonged FGF7 treatment induces PDX1 and CHGA expression.

a, RT-qPCR of pancreatic genes expression (*PDX1*, *NKX6.1* and *CHGA*) the completion of stages 4, 5 and 6. Values are mean $\pm$ s.e.m. (n=6 independent differentiations for stages 4 and 5, n=5 for stage 6). * $p<0.01$; ** $p<0.01$ (unpaired two-tailed Student's t-test, FGF7 vs control at each timepoints). **b**, Immunostaining of cell clusters at the completion of stage 5 demonstrating that prolong treatment with FGF7 enhanced the PDX1 positive fraction. Scale bar, 50 μ m. **c**, Strategy for liver/dead cell separation and gating for FACS analyses. **d**, Flow cytometry results of cells at stage 6 day 5, with or without FGF7 treatment demonstrating that FGF7 increased the CHGA positive population (n=3, three independent differentiations).

a**b****c**

Supplementary Figure 3. Involvement of FGFs during human fetal pancreas development.

a, Interaction network of FGFs and FGF receptors during human fetal development (analyzed on published scRNA-Seq data from *Zhuo Ma et al., Nat. Commun., 2023*). **b**, FGF2 and FGF7 are both expressed in fibroblast and mesothelial cells, while FGF2, FGF7, FGF9 and FGF10 are abundant in mesothelial in the developing pancreas. **c**, FGFR1 is highly expressed in d-cells, compared to other endocrine cell types.



Supplementary Figure 4. FGF2 promotes SST expression and maintains pancreatic endocrine lineage
a-d, RT-qPCR of pancreatic endocrine cell genes expression (*SST*, *HHEX*, *INS* and *GCG*) at the completion of stages 4, 5 and 6. Values are mean $\pm$ s.e.m. (n=6 independent differentiations for stages 4 and 5, n=5 for stage 6). * p<0.05; ** p<0.01 (Unpaired two-sided Student's T test). **e**, Western blot experiments showed that FGF2 dose-dependently induced SST protein expression (three independent differentiations). **f**, Western blot experiments showed that FGF2-induced SST protein expression could be abolished by FGFR1 inhibition with PD1 (other batches in addition to data shown in Fig. 2j).

a siRNA strategy

H1 cells differentiated to S3D2 in 24-well plates



Transfection with cell suspension @ S3D2

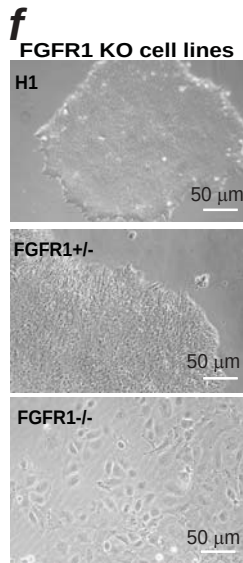
Reseed into 24-well plates, differentiated to S4D3



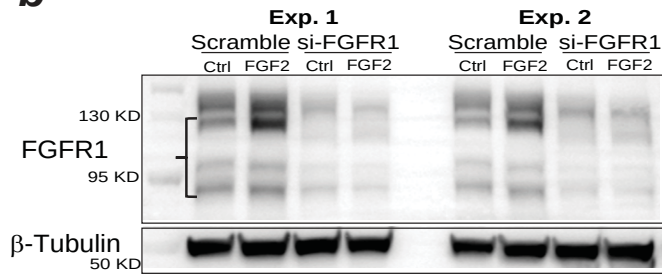
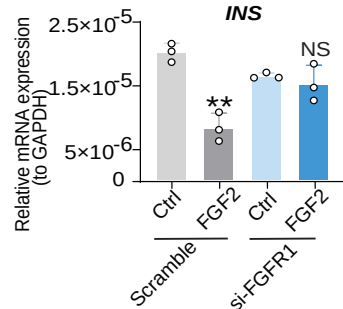
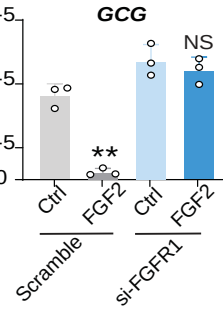
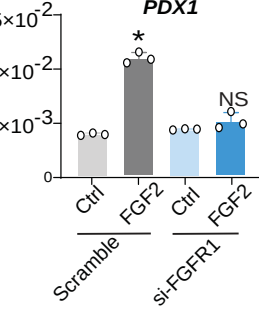
Transfection with cell suspension @ S4D3

Reseed into 24-well plates, differentiated to S5D3

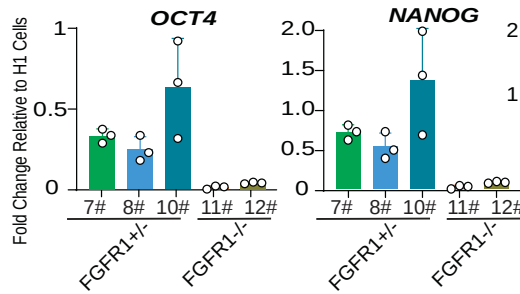
qPCR, WB @ S5D3

**b**

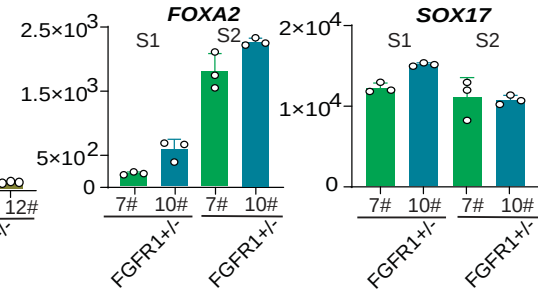
FGFR1 expression at S5D3

**c** Gene expression at S5D3**d****e****g**

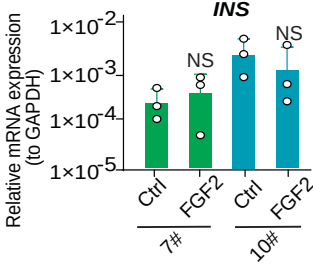
Stemness of FGFR1 KO cell lines

**h**

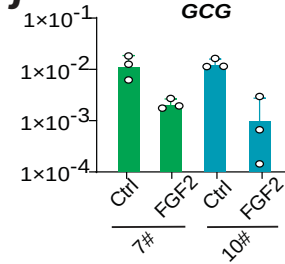
FGFR1+/- cell lines at S1D3 and S2D2

**i**

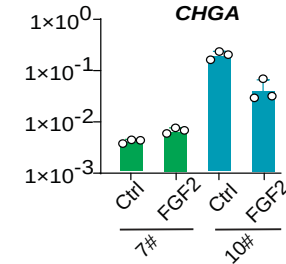
FGFR1+/- cell lines at S5D3

**j**

GCG

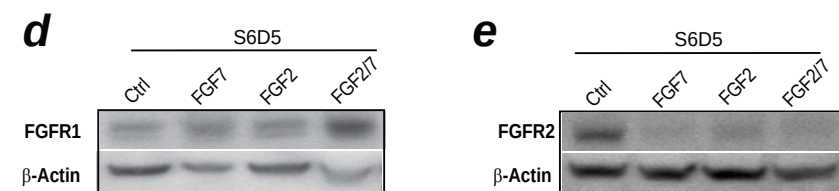
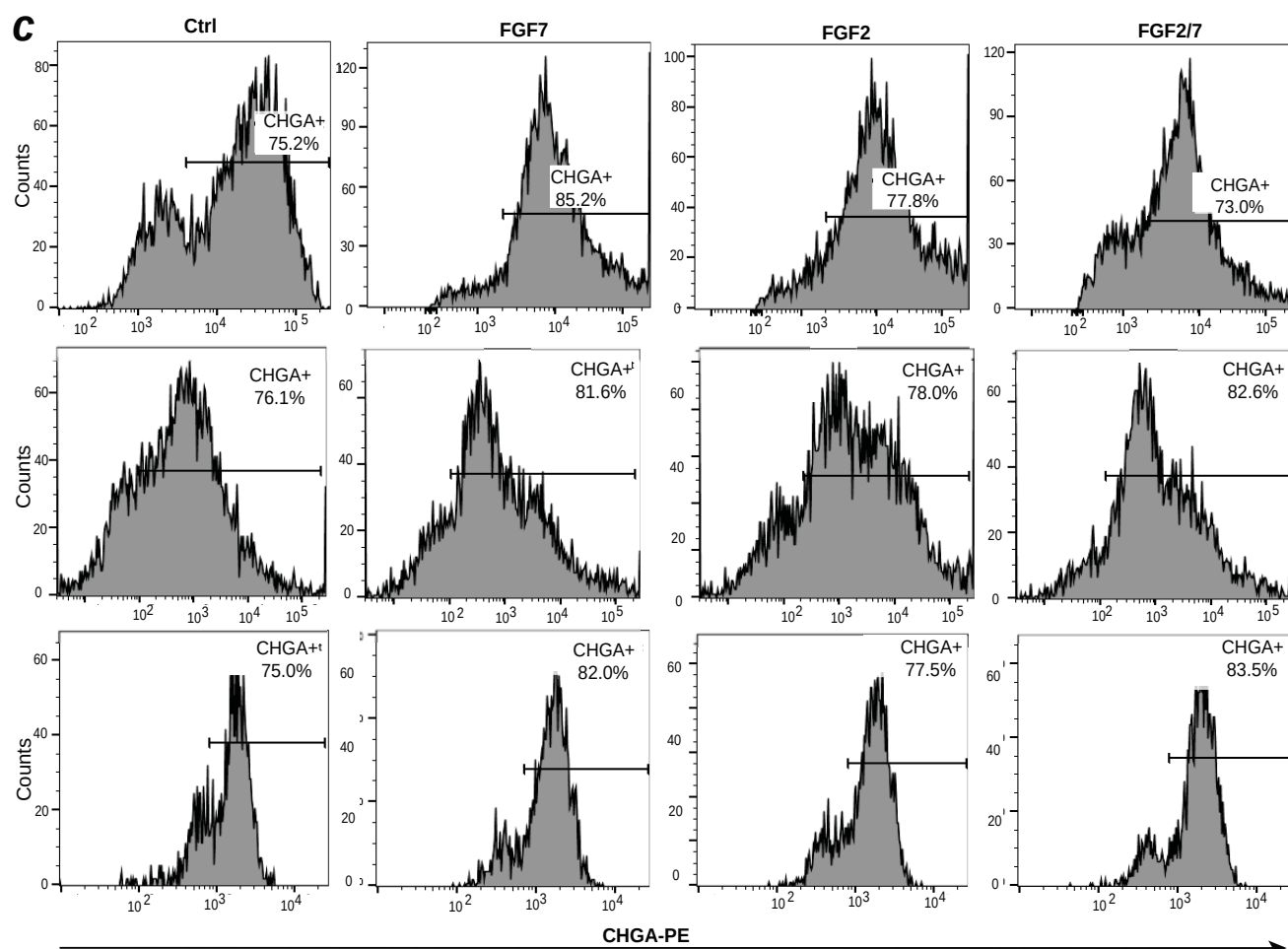
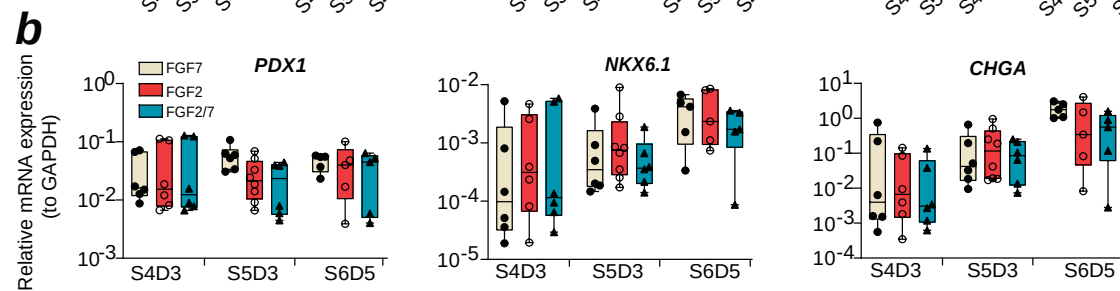
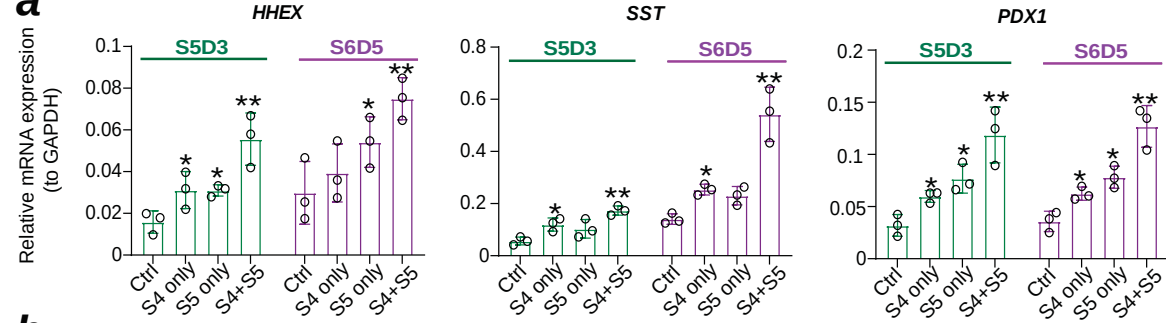
**k**

CHGA



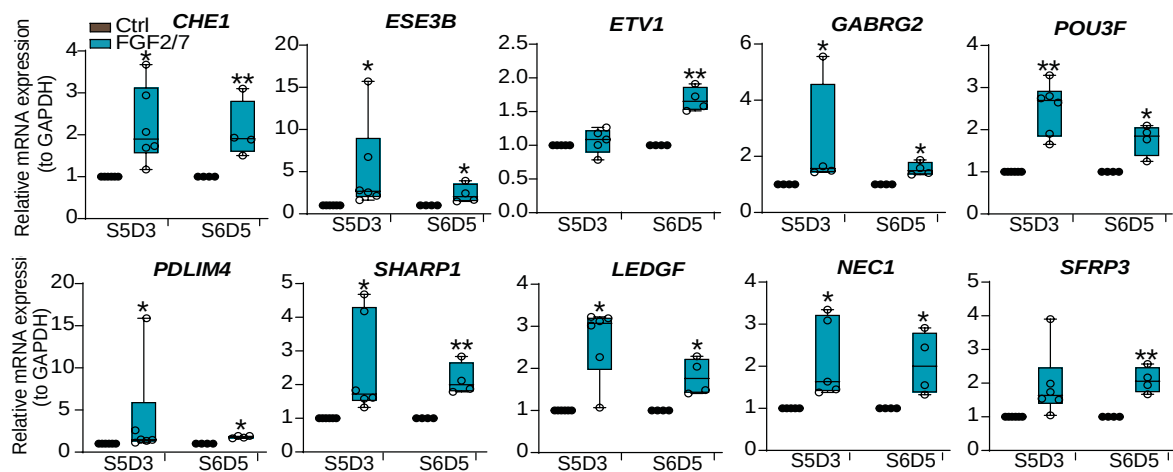
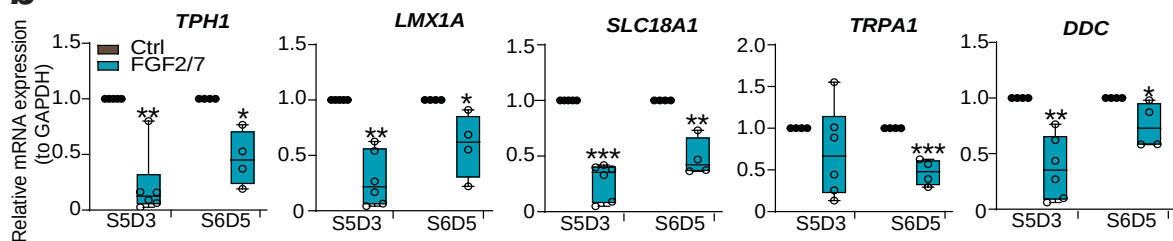
Supplementary Figure 5. FGFR1 knockdown and heterozygous knockout studies demonstrated that FGF2 induced δ -cell differentiation through FGFR1.

a, Strategy of small interfering RNA to knockdown FGFR1 during stages 4 and 5. **b**, Western blotting experiments to validate the knockdown of FGFR1 at S5D3. **c-e**, RT-qPCR of endocrine-cell genes (*INS*, *GCG* and *PDX1*) expression at the completion of stage 5. Values are mean $\pm$ SD from one representative experiment of 3 independent differentiations. * $p < 0.05$; ** $p < 0.01$; NS nonsignificant (unpaired two-side Student's t-test). **f**, Morphology of wildtype H1 and FGFR1 heterozygous and homozygous cell lines. **g**, RT-qPCR to assess the stemness of the FGFR1 heterozygous and homozygous cell lines. **h**, RT-qPCR to assess the differentiation capacity of the FGFR1 heterozygous and homozygous cell lines. Values are mean $\pm$ s.e.m. of 3 independent differentiations. **i-k**, RT-qPCR of endocrine-cell genes (*INS*, *GCG* and *CHGA*) expression at the completion of stage 5. Values are mean $\pm$ SD from one representative experiment of 3 independent differentiations. NS nonsignificant (unpaired two-sided Student's t-test).



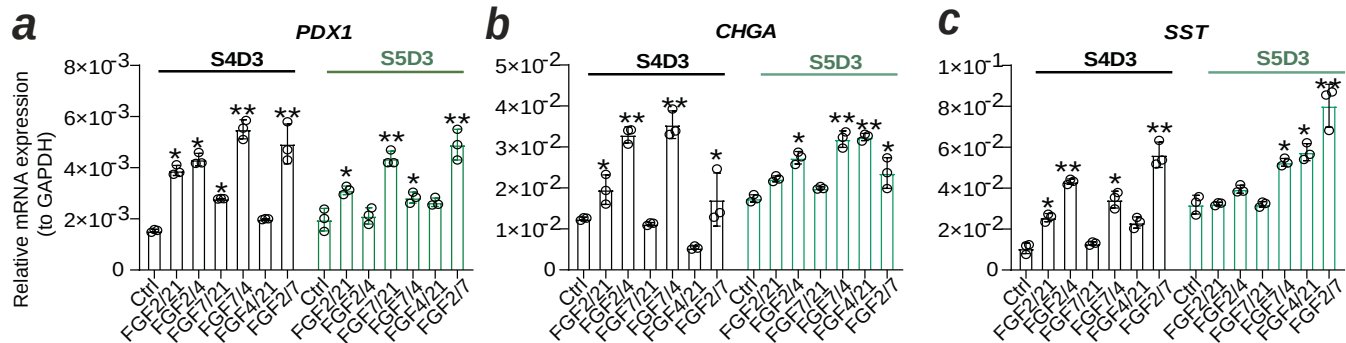
Supplementary Figure 6. FGF2/7 promotes SST expression and maintains pancreatic endocrine lineage

a, RT-qPCR of pancreatic genes expression (*HHEX*, *SST* and *PDX1*) at the completion of stages 5, and 6 treated by control, FGF2/7 during stage 4 (S4 only), stage5 (S5 only), and both stage 4 and 5 (S4+S5). Values are mean $\pm$ s.e.m. from 3 independent differentiations. Blue and purple indicate gene expression examined at S5D3 and S6D5, respectively. * $p < 0.05$; ** $p < 0.01$ (one way ANOVA followed by Tukey's multiple comparisons test). **b**, RT-qPCR of pancreatic genes expression (*PDX1*, *NKX6.1* and *CHGA*) at the completion of stages 4, 5 and 6. Values are mean $\pm$ s.e.m. from 6 (S4D3 and S5D3) or 5 (S6D5) independent differentiations (one way ANOVA followed by Tukey's multiple comparisons test). **c**, Flow cytometry results of cells at stage 6 day 5, with FGF7, FGF2 or FGF2/7 treatments demonstrating that FGF2, FGF2/7 did not significantly affect the CHGA positive endocrine fraction ($n=3$, three independent differentiations). **d-e**, Western blotting images showing that protein expression of FGFR1(**d**) was enhanced by FGF2/7, while all treatments decreased FGFR2 at the completion of stage 6 (**e**) protein expression. Images are one representative experiment from three independent differentiations.

a**Delta-cell markers****b****EC-cell markers**

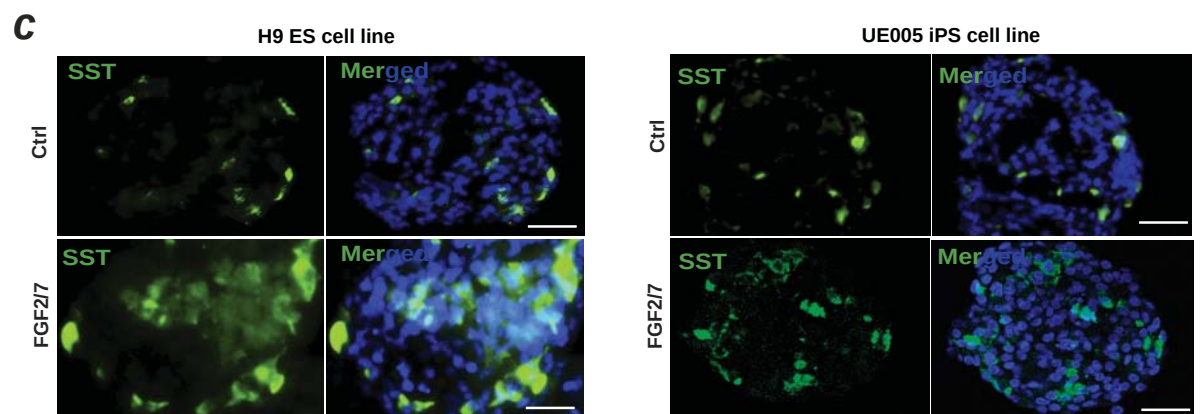
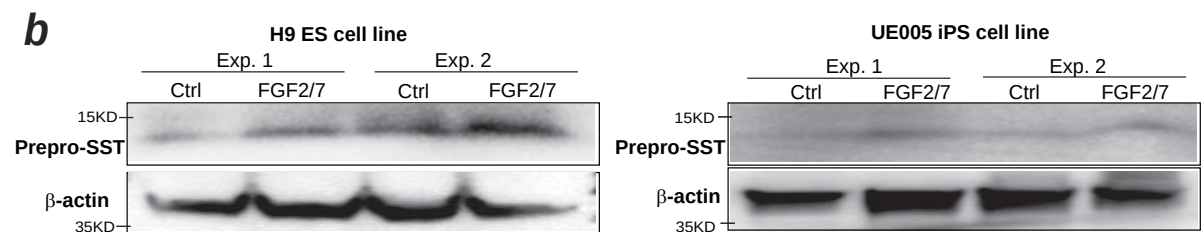
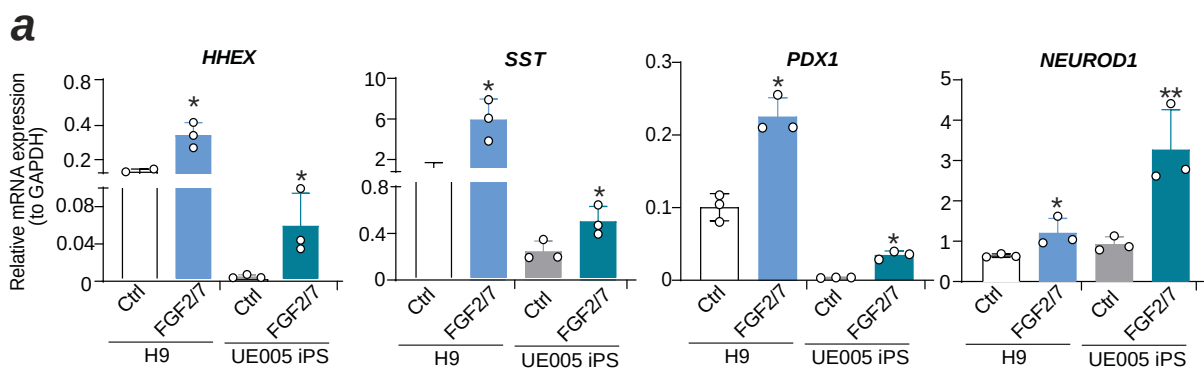
Supplementary Figure 7. FGF2/7 induces δ -cell marker gene expression while suppressing EC-cell markers.

a, RT-qPCR of pancreatic δ -cell marker gene expression at the completion of stage 5 and 6. Values are mean $\pm$ s.e.m. from 4 independent differentiations. **b**, RT-qPCR of pancreatic EC-cell marker gene expression at the completion of stage 5 and 6. Values are mean $\pm$ s.e.m. from 6 (S5D3) or 4 (S6D5) independent differentiations. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (unpaired two-side Student's t-test).



Supplementary Figure 8. Compared to FGF2/7, combinations of other FGFs had a lesser effect on δ -cell induction.

a-c, RT-qPCR of pancreatic endocrine cell gene (*PDX1*, *CHGA*) and δ -cell gene (*SST*) at the completion of stages 4 and 5. Values are mean $\pm$ s.e.m. from 3 independent differentiations. * $p < 0.05$; ** $p < 0.01$ (one way ANOVA followed by Dunnett's multiple comparisons test).

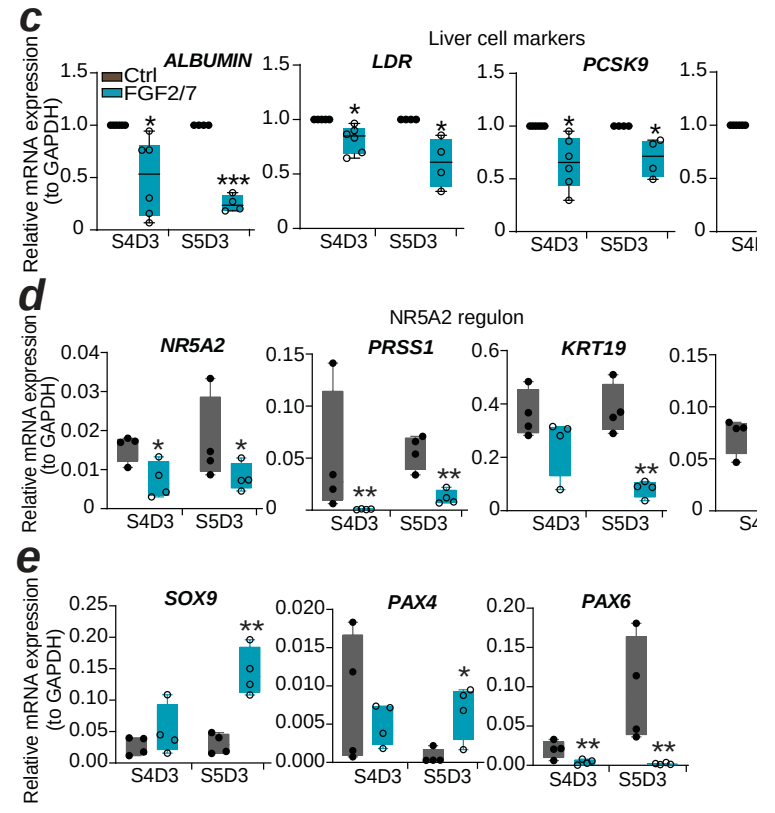
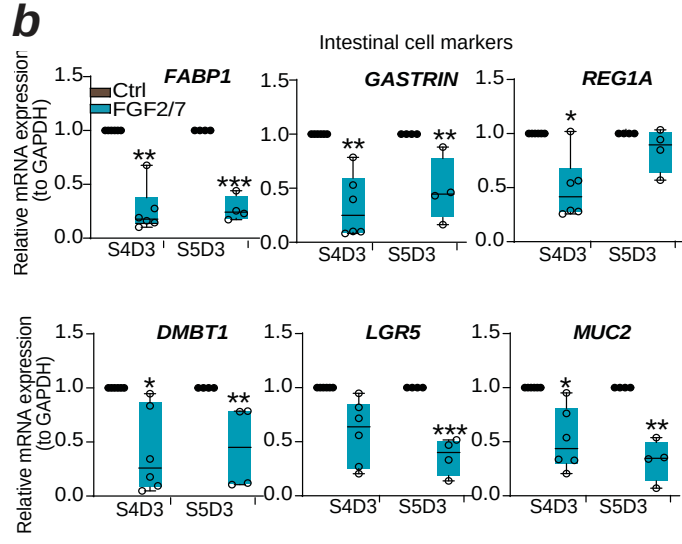
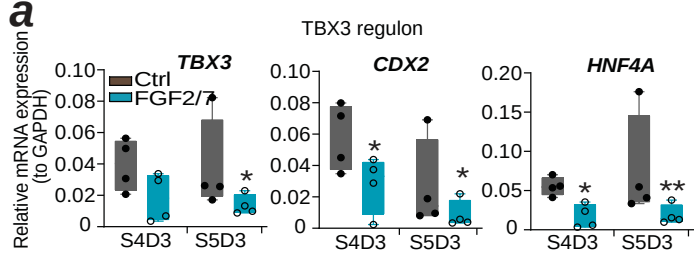


Supplementary Figure 9. FGF2/7 promotes SST expression in other pluripotent stem cell lines.

a, RT-qPCR of pancreatic δ -cell gene (*HHEX*, *SST*) and key endocrine marker gene (*PDX1*, *NEUROD1*) expression at the completion of stages 5 (H9 ES cells (left) and UE005 iPS cells (right)). Values are mean $\pm$ SD from one representative experiment of 3 independent differentiations. * $p < 0.05$; ** $p < 0.01$ (unpaired two-side Student's t-test). **b**, Western blotting images showing that protein expression of SST was enhanced by FGF2/7 in H9 ES cells (left) and UE005 iPS cells (right). Images are representative experiments from two independent differentiations. **c**, Immunostaining of cell clusters at stage 7 day 7 with SST (green). Results show that FGF2/7 greatly promoted δ -cell generation in H9 ES cells (left) and UE005 iPS cells (right). Scale bars, 50 μ m.

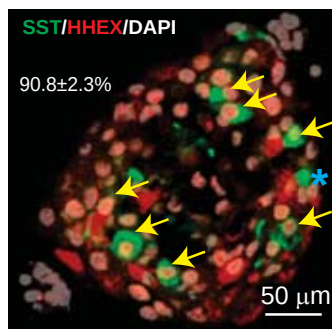
Supplementary Figure 10. Heat maps of other major pancreatic cell types

a-f, Heat map from bulk RNA sequencing of select genes from multiple pancreatic lineages including α cells, β cells, ϵ cells, γ cells, acinar cells and ductal cells. **g-h**, KEGG analyses for the cells differentiated into S5D3 (g) or S7D6 (h) that were treated with control or FGF2/7 during stages 4 and 5.



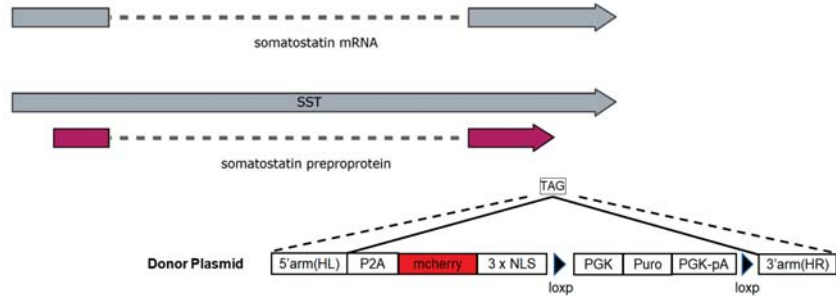
Supplementary Figure 11. FGF2/7 directed pancreatic endocrine lineage differentiation through downregulating master genes *TBX3* and *NR5A2*

a, RT-PCR of *TBX3*-regulated gene expression (*TBX3*, *CDX2* and *HNF4A*) at the complete of stage 5. Values are mean $\pm$ s.e.m. from 4 independent differentiations. * $p < 0.05$; ** $p < 0.01$ (FGF2/7 vs control, unpaired two-sided Student's t- test). **b-c**, RT-qPCR of intestinal lineage gene (*GASTRIN*, *REG1A*, *DMBT1*, and *MUC2*) and liver lineage gene (*ALBUMIN*, *LDLR*, *PCSK9*, and *KRT7*) expression at completion of stage 5 (S5D3, n=6) and stage 6 (S6D5, n=4). **d**, RT-PCR of *NR5A2*-regulated gene expression (*NR5A2*, *PRSS1*, *KRT19* and *GATA4*) at the complete of stage 5. Values are mean $\pm$ s.e.m. from 4 independent differentiations. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.01$ (FGF2/7 vs control, unpaired two-sided Student's t- test). **e**, RT-qPCR of *SOX9*, *PAX4* and *PAX6* gene expression at the complete of stage 4 (S4D3) and 5 (S5D3). Values are mean $\pm$ s.e.m. from 4 independent differentiations. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.01$ (FGF2/7 vs control, unpaired two-sided Student's t- test).

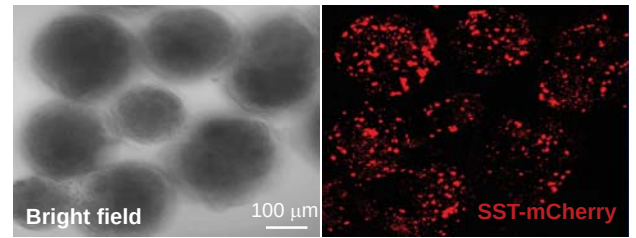
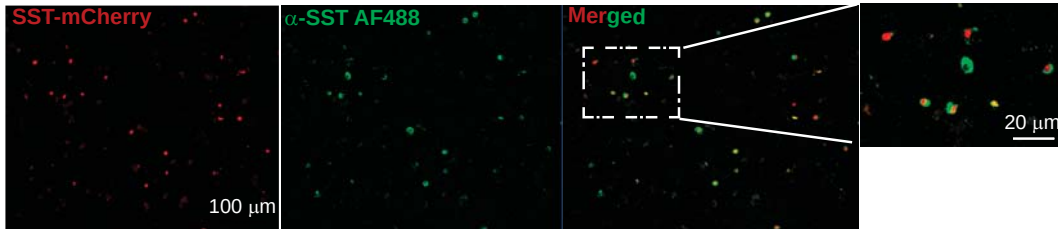


Supplementary Figure 12. Delta cells generated by FGF7 and FGF2/7 exhibited similar co-expression of HHEX and SST.

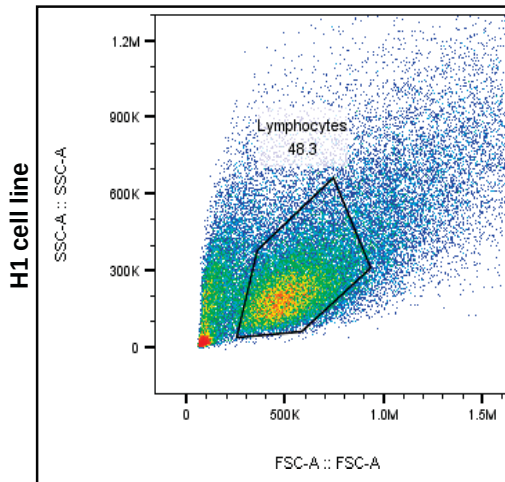
Immunostaining to characterize the generated δ cells at stage 7 day 6. SST (green) and HHEX (red) were co-stained for the clusters differentiated by using FGF7 at stages 4 and 5. The ratio indicates the percentage of SST positive cells that are also HHEX positive. Yellow arrows indicate the SST⁺/HHEX⁺ cells, a blue asterisk indicates the SST⁺/HHEX⁻ cell.

a Strategy**b**

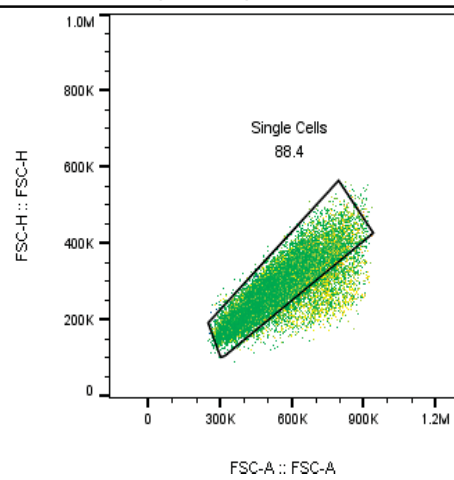
H1 SST-P2A-mCherry reporter line, S7D14

**c** H1 SST-P2A-mCherry reporter line, S7D14**d**

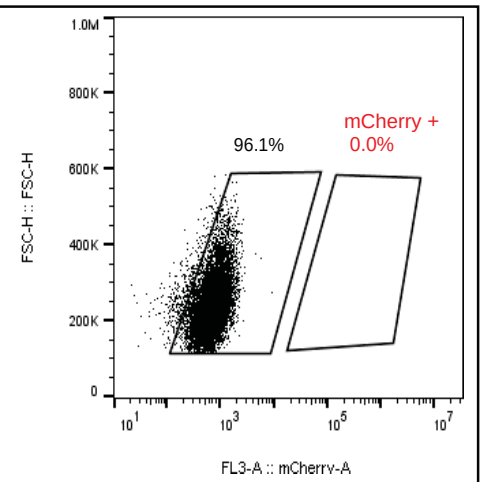
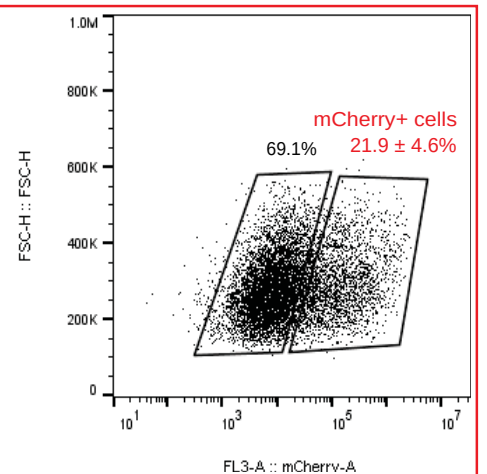
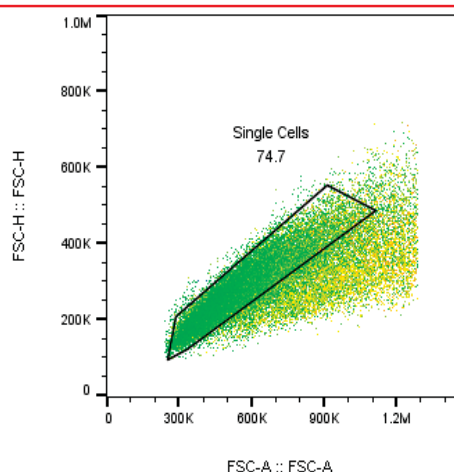
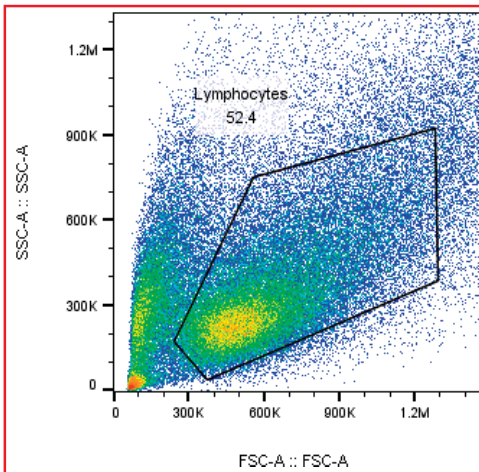
Exclusion of cell debris

**e**

Gating of singlets

**f**

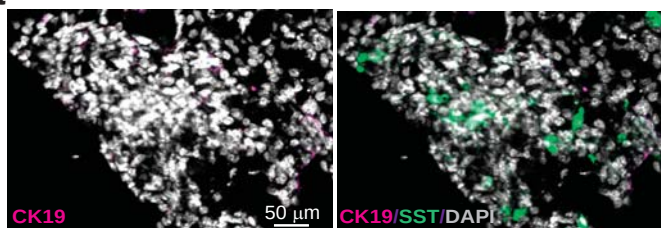
mCherry positive cells

**H1 SST-P2A-mCherry cell line**

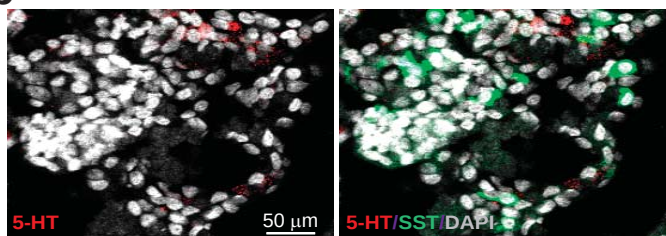
Supplementary Figure 13. Construction of the SST-P2A-mCherry reporter line.

a, Strategy of the construction of the SST-P2A-mCherry reporter line. **b-c**, Successful construction of the heterozygous SST-P2A-mCherry reporter line as validated by differentiation and immunostaining. **d-f**, FACS sorting strategy to purify the fraction of mCherry-positive δ cells. Values are mean $\pm$ SD from 3 independent differentiations.

a



b



Supplementary Figure 14. The delta cells do not co-express ductal cell or intestinal EC/ enteric serotonergic neuron cell markers

a-b, Immunostaining of cell clusters at stage 7 day 7 with SST (green) and ductal cell marker CK19 (**a**) or EC/neuron cell marker 5-HT (**b**). Results show that δ cells do not co-express CK19 or 5-HT.