

Stem Cell Reports, Volume 6

Supplemental Information

**Early Developmental Perturbations in a Human Stem Cell Model of
MODY5/HNF1B Pancreatic Hypoplasia**

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SUPPLEMENTAL INFORMATION

SUPPLEMENTAL FIGURE LEGENDS

“Figure S1. Establishment of a stem cell model for MODY5, Related to Figure 1”

(A) S148L (C443T in exon 2) mutation (or lack thereof) in *HNF1B* has been verified in iN805 hiPSCs at least thrice. (B) Expression of pancreas-related (*NEUROD1*, *HNF6*, *DLK1*, *SOX4* and *MAFB*) markers in hiPSCs differentiated for 17 days. All error bars indicate standard deviation of three biological replicates in an independent experiment. (C) Immunostaining for HNF1B, PAX6, ISL1, SOX9 and PDX1 on hiPSCs differentiated for 12 days. Scale bar: 200 μ m. (D) The percentage of PDX1⁺, SOX9⁺, HNF1B⁺, HNF1A⁺ and PTF1A⁺ cells after differentiation of hiPSCs for 17 days.

“Figure S2. Transcriptional perturbations in MODY5-hiPSC-derived pancreatic progenitors, Related to Figures 1, 2 and 3”

(A) Gene ontology (GO) analyses of differentially up- and down-regulated genes (fold change >2) in control hiPSCs differentiated for 10 days. (B) Gene expression of *SOX17*, *FOXA2*, *ISL1*, *HLXB9*, *SOX9* and *RFX6* in non-family control and iN805 hiPSCs differentiated for 0, 12, 14 and 17 days. Data are representative of at least 2 independent hiPSC lines per subject. The experiment was replicated at least twice. All error bars indicate standard deviation of three biological replicates in an independent experiment. (C) Immunostaining for HNF1B, SOX9 and ISL1 on iN805 hiPSCs differentiated for 12 days. Data are

representative of at least 2 independent hiPSC lines per subject. The experiment was replicated at least twice. Scale bar: 200 μm .

“Figure S3. Further characterization of pancreatic differentiation model for

MODY5-hiPSCs, Related to Figures 2 and 3” (A) Gene expression heat map of

representative pancreas-related genes in undifferentiated (first four columns), Day 12 differentiated (middle four columns) and Day 17 differentiated (last four columns) control and iN805 hiPSCs (up-regulation in red, down-regulation in blue). The colors in the heat map depict gene expression in units of standard deviation from the mean across all samples. GO analyses of differentially up- and down-regulated genes (fold change >2) in iN805 versus control cells on (B) Day 12 or (C) Day 17. (D) ChIP demonstrates that HNF1B is bound onto genomic loci of *HLXB9* and *HNF1B* on Day 12. (E) Cell count was performed on control and iN805 hiPSCs differentiated for 17 days. All error bars indicate standard deviation of three biological replicates in an independent experiment.

“Figure S4. HNF1B has no impact on *HNF4A* or *SOX9* transcription, Related to

Figure 4” (A) Luciferase assay showing the effect of *HNF1B* overexpression on *HNF4A*

transcriptional activity in hiPSCs differentiated for 12 days. Expression of *SOX9* in hiPSCs differentiated for 10 days and overexpressing increasing amounts of (B) mutant *HNF1B*^{S148L} or (C) *HNF1B* from Day 7-10. Control experiments demonstrating that the overexpression of (D) HNF1B or (E) HNF1A is successful. Experiments were replicated

at least twice. All error bars indicate standard deviation of three biological replicates in an independent experiment.

SUPPLEMENTAL TABLE LEGENDS

“Table S1. Microarray performed on undifferentiated and Day 10 differentiated control-hiPSCs, Related to Figure 1”

“Table S2. Microarray performed on undifferentiated, Day 12 and Day 17 differentiated control and iN805 hiPSCs, Related to Figures 2 and 3”

“Table S3. Primers and antibodies used, Related to Figures 1, 2, 3, 4 and 5”

Figure S1: Teo et al.,

A **MODY5 (*HNF1B*) (S148L)**

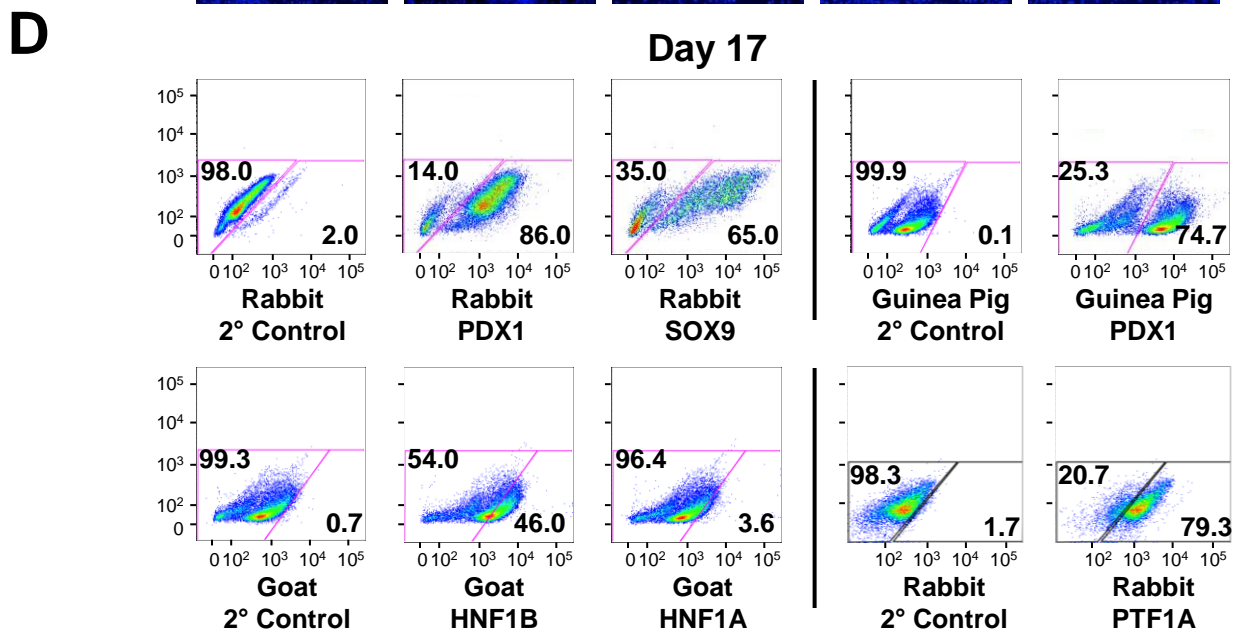
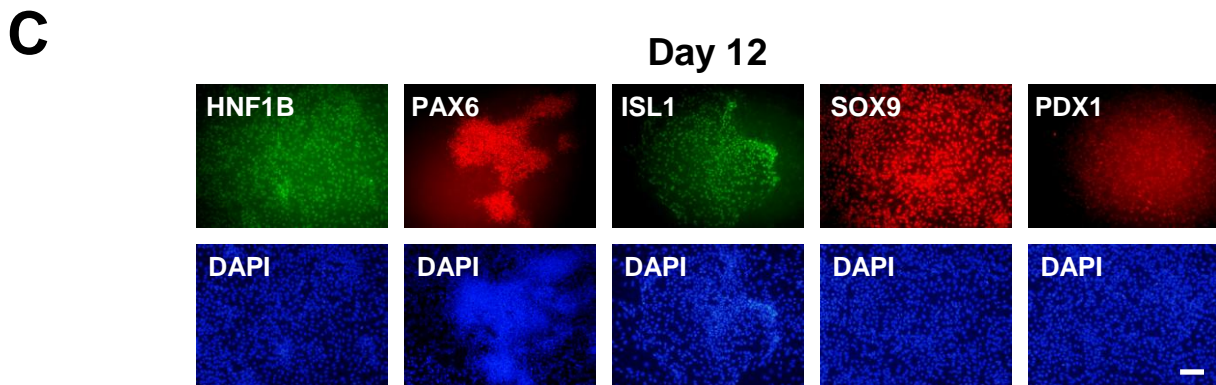
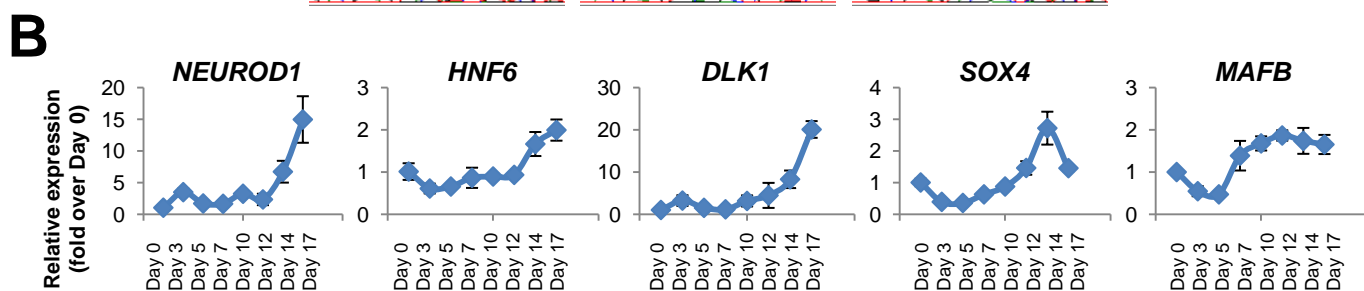
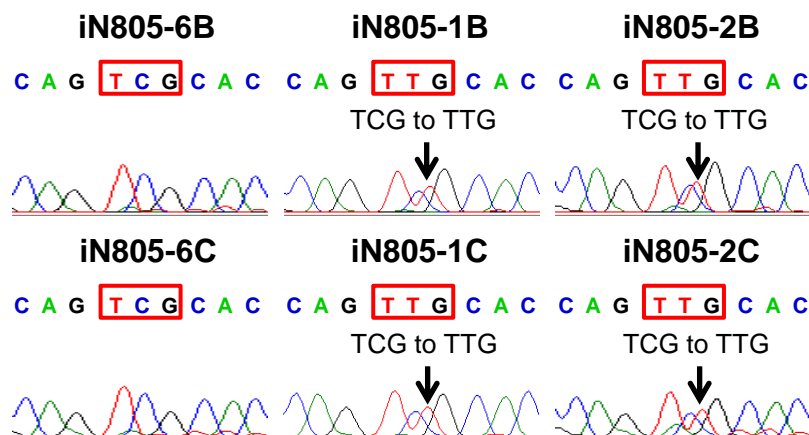
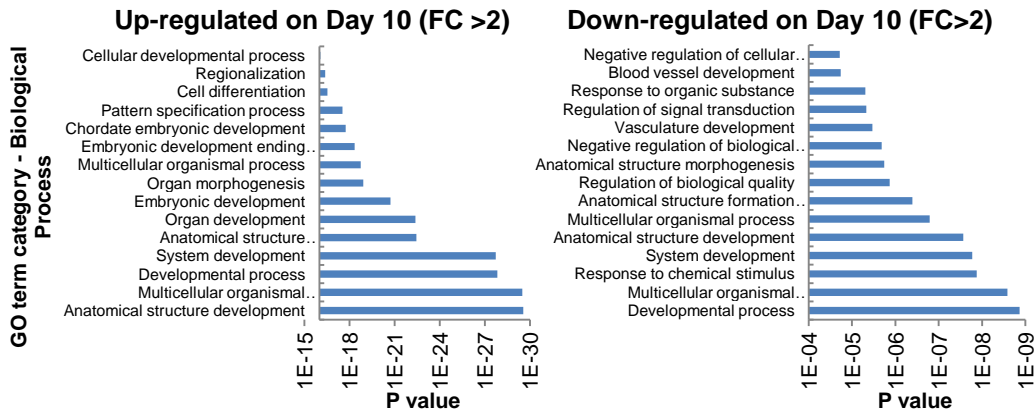
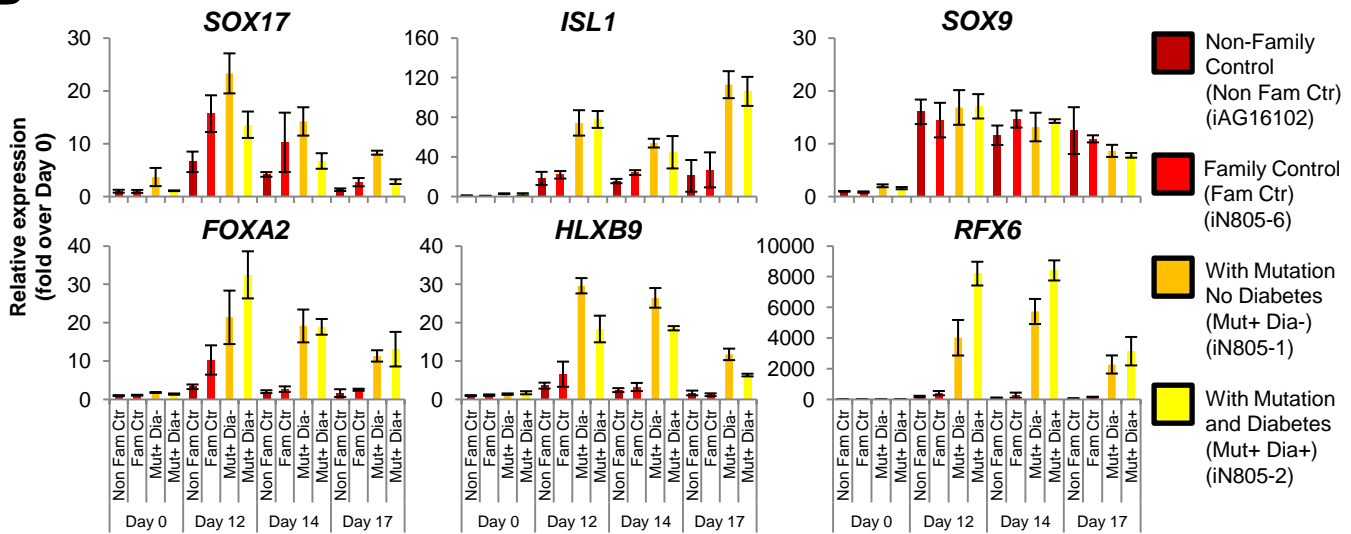


Figure S2: Teo et al.,

A



B



C

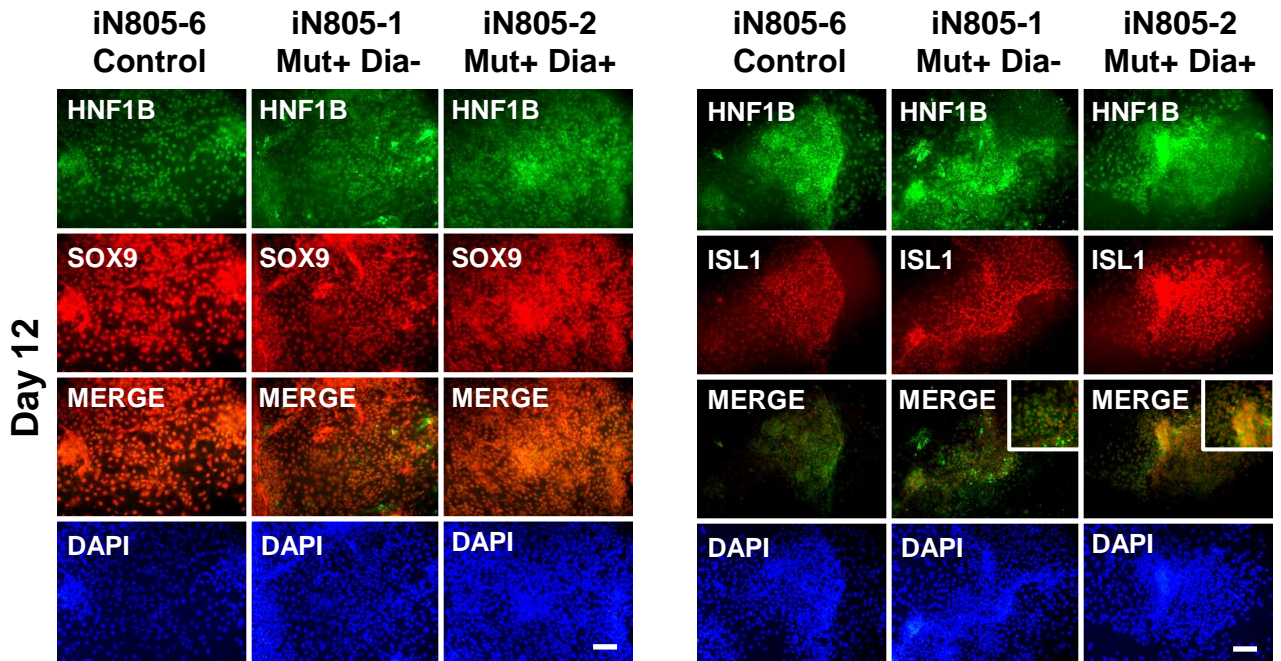
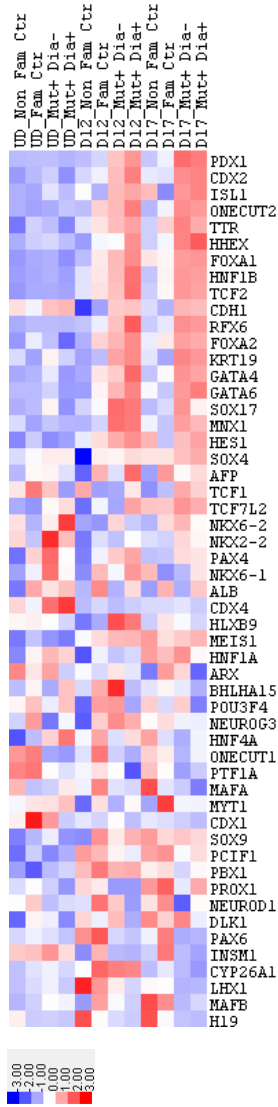


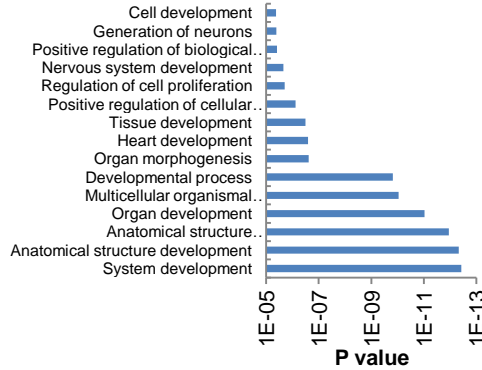
Figure S3: Teo et al.,

A

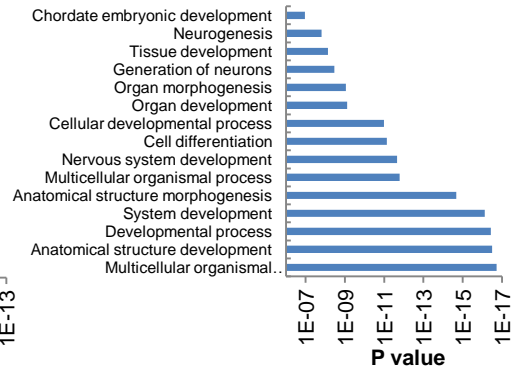


B

Up-regulated in MODY5 on Day 12 (FC >2)

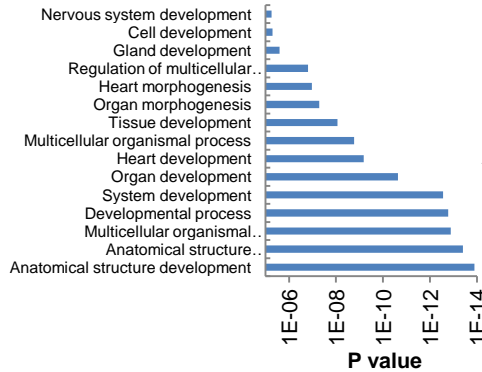


Down-regulated in MODY5 on Day 12 (FC >2)

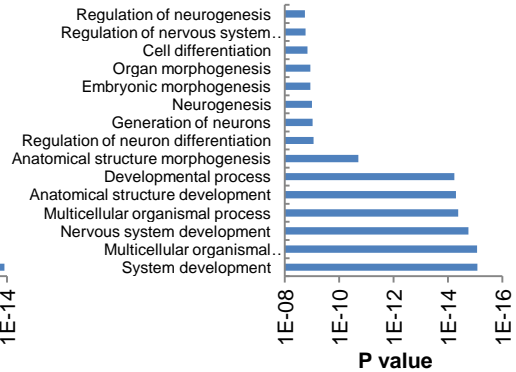


C

Up-regulated in MODY5 on Day 17 (FC >2)

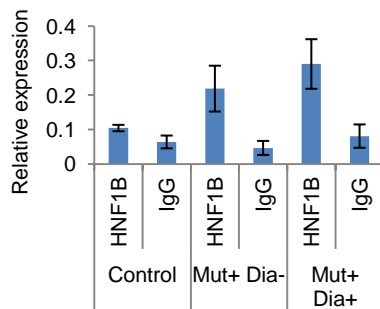


Down-regulated in MODY5 on Day 17 (FC >2)

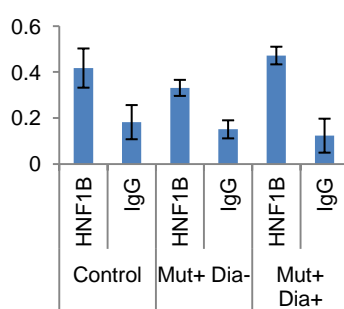


D

HLXB9 genomic locus



HNF1B genomic locus



E

Cell count during differentiation

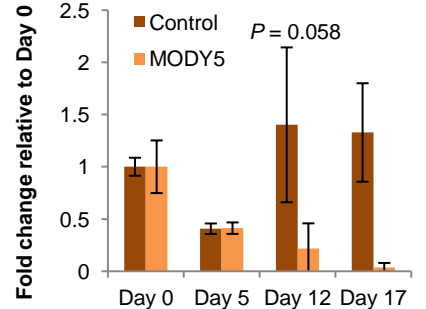
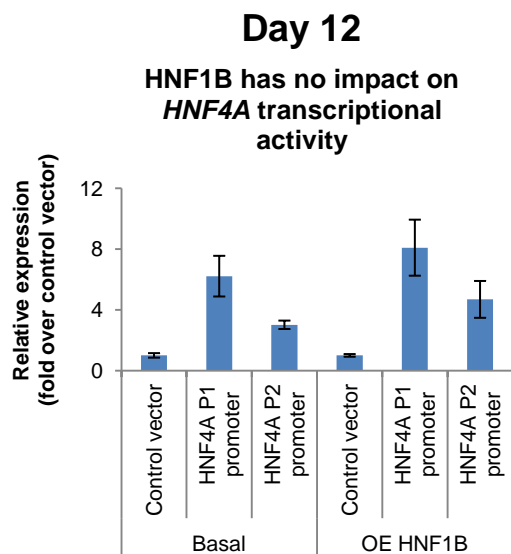
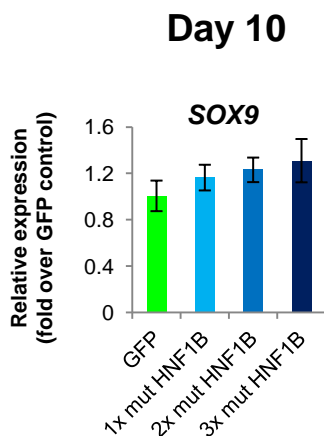


Figure S4: Teo et al.,

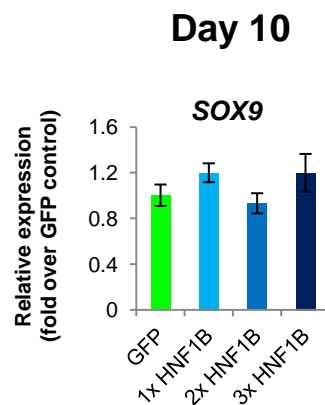
A



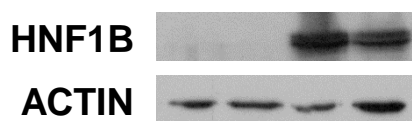
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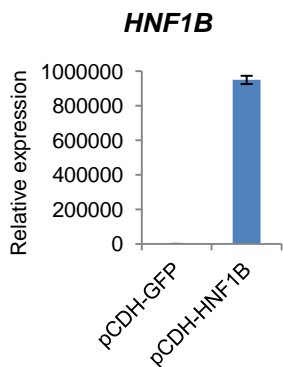
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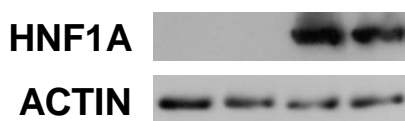
D



PCDH-GFP
PCDH-GFP
PCDH-HNF1B
PCDH-HNF1B



E



PCDNA3.1
PCDNA3.1
PCDNA3.1-HNF1A
PCDNA3.1-HNF1A

