Stem Cell Reports, Volume *11*

Supplemental Information

Genetic Engineering of Human Embryonic Stem Cells for Precise Cell Fate Tracing during Human Lineage Development

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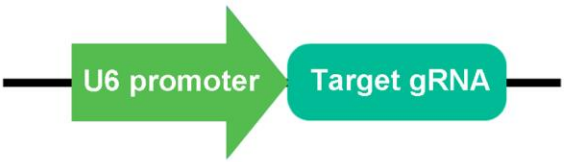
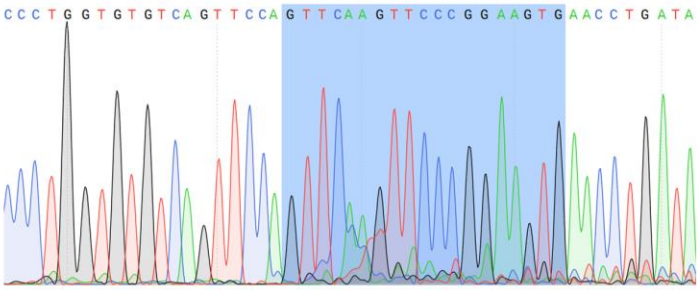
Supplemental Figure 1



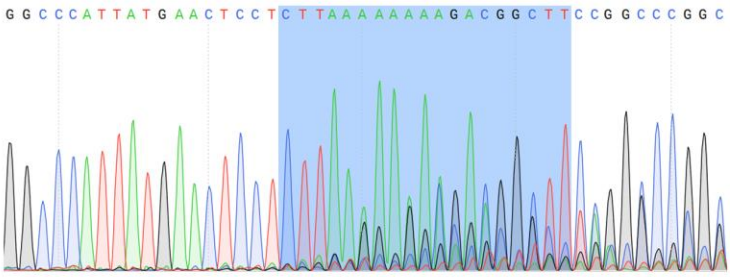
gRNA designed near the TAA stop codon of PAX6:

... ...TCCAGTTCAAGTTCCCGGAAGTGAACCTGATATGTCTCAATACTGGCCAAGATTACAGTAAAAAA... ...



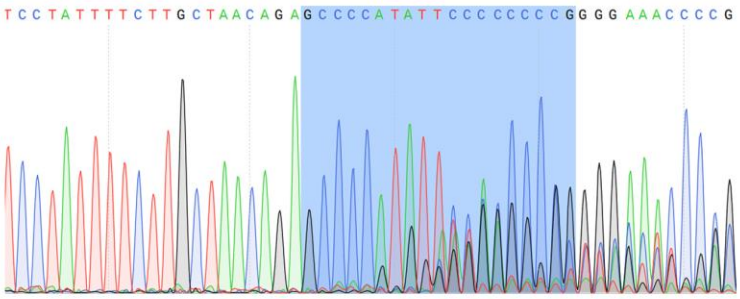
gRNA designed near the TAA stop codon of FOXA2:

... ...AGGGGGTGTACTCCCGGCCCATTATGAACTCCTCTTAAGAAGACGACGGCTTCAGGCCCGGCTA... ...



gRNA designed near the ATG start codon of PAX6:

... ...ACAGAGCCCCATATTCGAGCCCCGTGGAATCCCGCGGCCCCCAGCCAGAGCCAGCATGCAGAA... ...



gRNA designed near the ATG start codon of FOXA2:

... ...CCATGCACTCGGCTTCCAGTATGCTGGGAGCGGTGAAGATGGAAGGGCACGAGCCGTCCGACT... ...

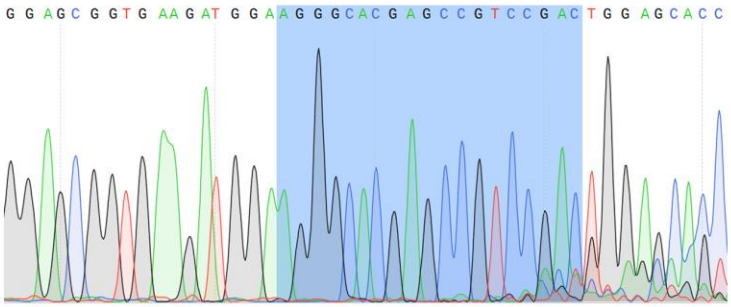


Figure S1. gRNA Design and Targeting Efficiency Verification. Related to Figure 2.

gRNAs targeting the stop codon (TAA) regions and start codon (ATG) regions of PAX6 and FOXA2 are designed and underlined as shown. Stop and start codons are marked in red. The targeting gRNA and Cas9 plasmids are co-transfected into HEK293 cells for 72 hours, and genomic DNA of each group is extracted. Sequencing of the PCR amplified genomic DNA surrounds the targeting sites identifies overlapped peaks, which represents non-homologous end joining repair after correct DNA targeting.

Supplemental Figure 2

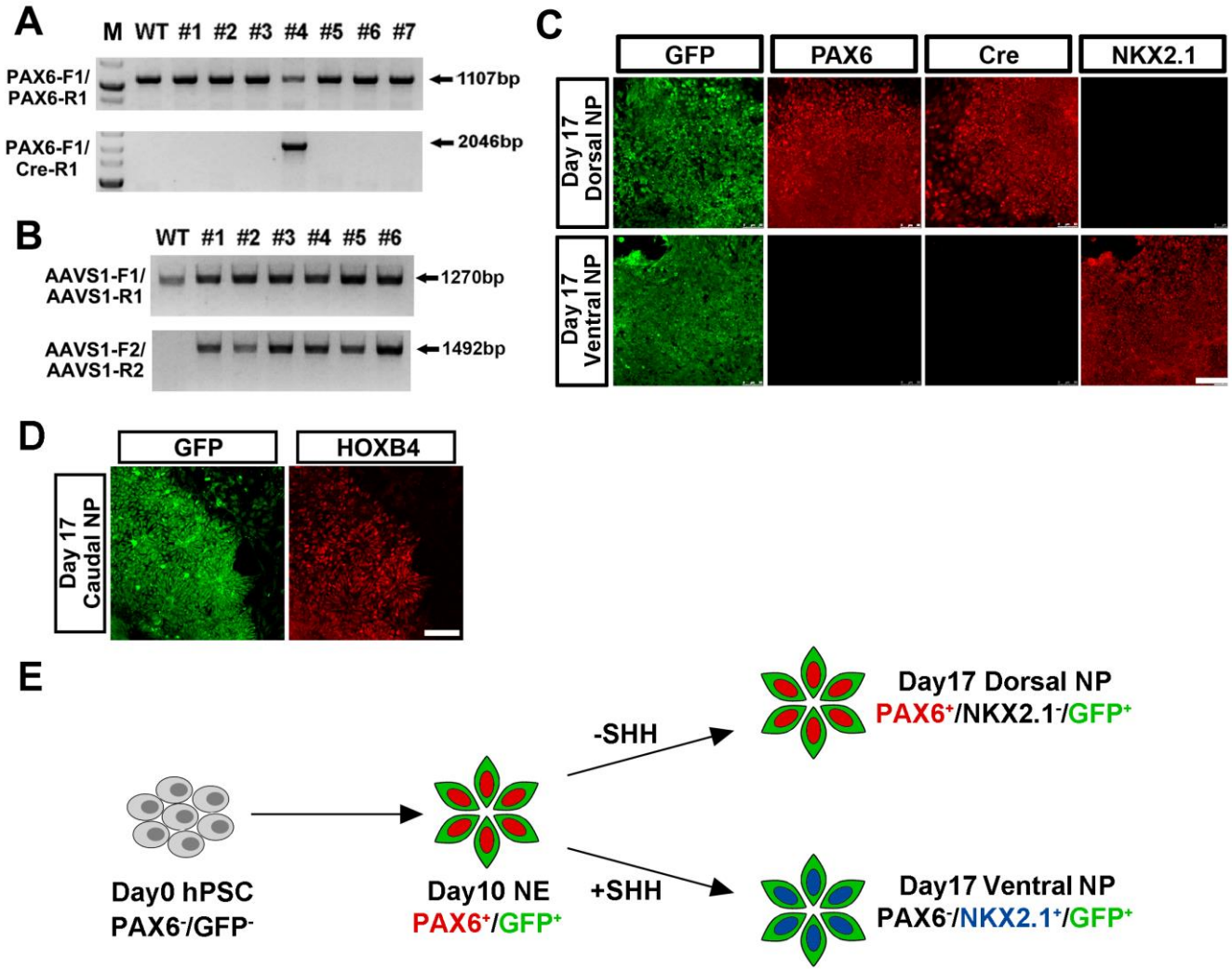


Figure S2. Tracing PAX6 Expressing NE *in vitro*. Related to Figure 3.

(**A-C**) Two-step engineering of PAX6-P2A-Cre/AAVS1-LSL-GFP cassettes in H7 hESCs and subsequent tracing results reveal both dorsal and ventral NPs are originated from PAX6 expressing NE. Scale bar, 100μm.

(**D)** Immunolabeling of GFP and HOXB4 in PAX6-P2A-Cre/AAVS1-LSL-GFP lines at day 17 caudal NPs after retinoid acid patterning reveals their exclusive origin of PAX6 expression NE. Scale bar, 100μm.

(**E**) Human day 10 NE uniformly expresses PAX6. With no patterning morphogens, human NE is defaulted to a dorsal NP identity with retained PAX6 expression. While under SHH patterning, human NE is ventralized to a medial ganglionic eminence ventral NP identity with NKX2.1 expression. Lineage tracing studies *in vitro* and *in vivo* reveal both dorsal and ventral NPs are derived from PAX6 expressing NE.

Supplemental Figure 3

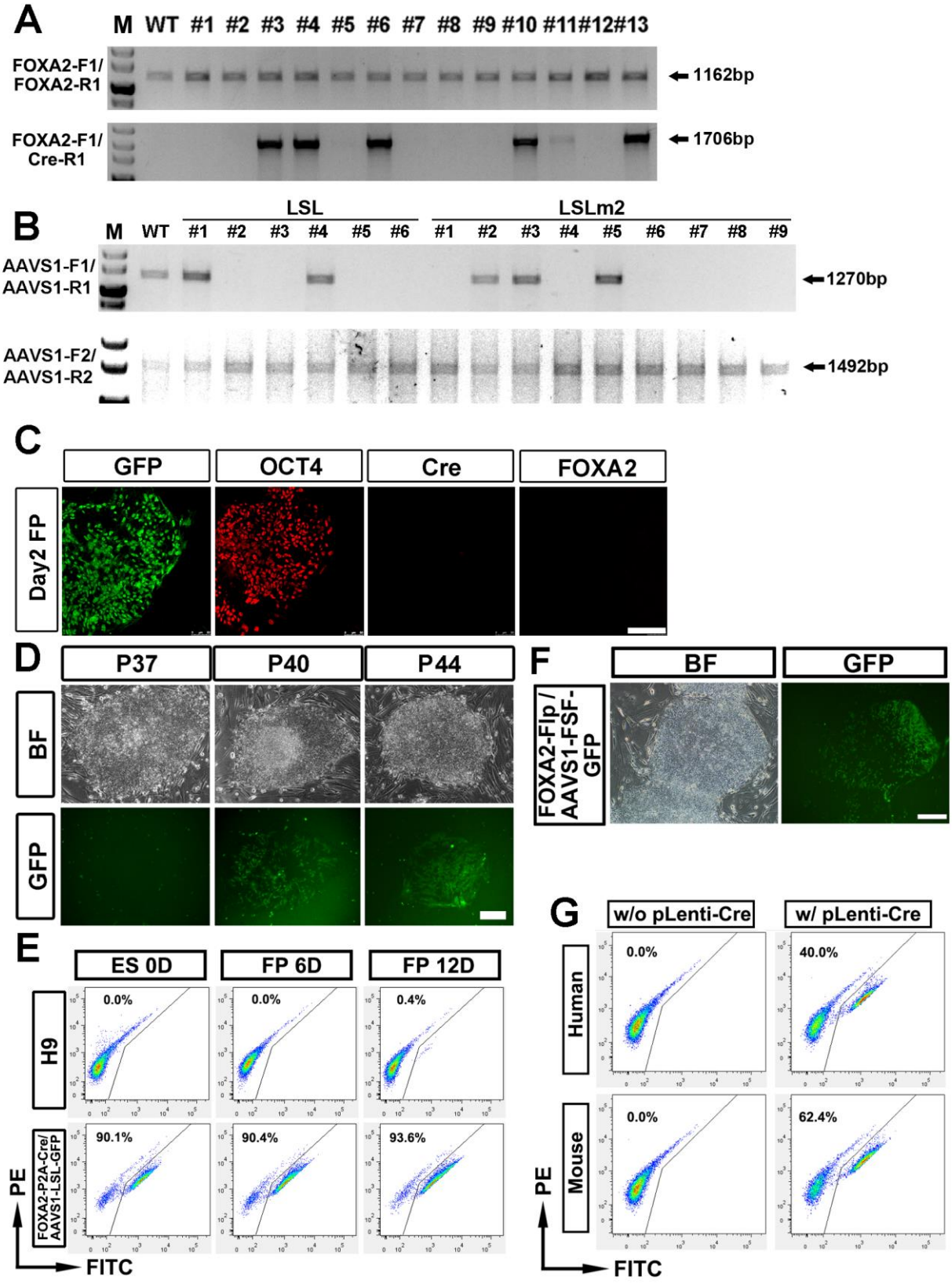


Figure S3. Modifying Recombination Sensitivity through LoxP Sequence Mutations. Related to Figure 4.

(**A**) Constructing of FOXA2-P2A-Cre cassette in H7 hESCs. Genomic DNA PCR results identify 5 monoallelic HR colonies after genetic engineering.

(**B**) Constructing of AAVS1-LSL-GFP or AAVS1-LSLm2-GFP in characterized FOXA2-P2A-Cre H7 hESC line. Genomic DNA PCR analysis identifies monoallelically and biallelically targeted colonies in both systems.

(**C**) Immunolabeling of GFP, OCT4, Cre and FOXA2 in FOXA2-P2A-Cre/AAVS1-LSL-GFP H7 hESC line differentiated toward a FP fate for 2 days. Inaccurate GFP labeling of $OCT4^{+}$ cells is seen. Scale bar, 100μm.

(**D**) Bight field and fluorescent images of FOXA2-P2A-Cre/AAVS1-LSL-GFP H7 hESC line at different passages indicated. Scale bar, 100μm.

(**E**) Representative FACS plots of WT and FOXA2-P2A-Cre/AAVS1-LSL-GFP H9 hESCs (passage 45) at pluripotent state or differentiated to a FP fate for 6 or 12 days.

(**F**) Bight field and fluorescent images of FOXA2-P2A-Flp/AAVS1-FSF-GFP H9 hESC line after 10 extra passages in vitro. Scale bar, 100μm.

(**G**) Representative FACS plots of GFP quantification of fibroblast-like cells derived from AAVS1-LSL-GFP H9 hESCs and fibroblasts derived from Rosa26-LSL-GFP mouse after pLenti-Cre plasmid (500ng) electroporation.

Supplemental Figure 4

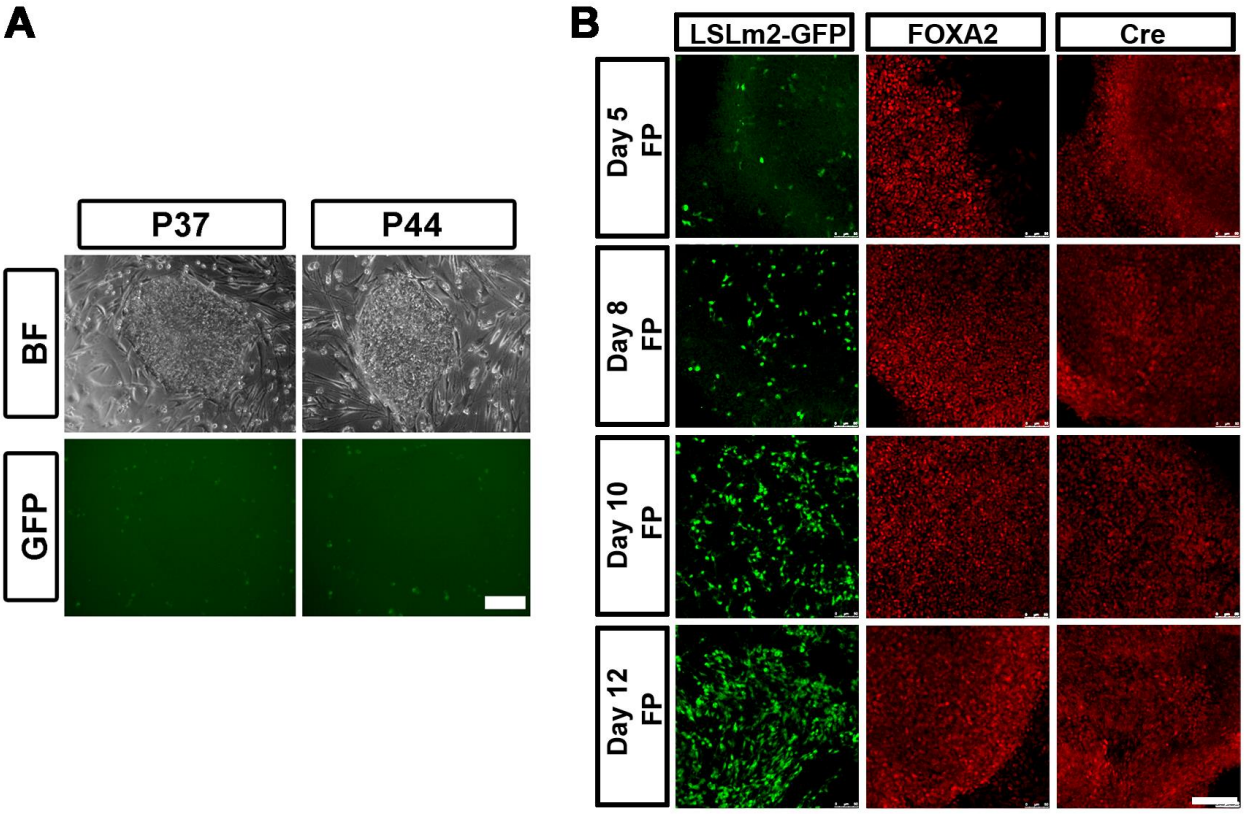


Figure S4. A Series of LSL Mutants are Designed with Variable Recombination Sensitivities. Related to Figure 5.

(**A**) Bight field and fluorescent images of FOXA2-P2A-Cre/AAVS1-LSLm2-GFP H7 hESC line at different passages indicated. No inaccurate GFP labeling is seen. Scale bar, 100μm.

(**B**) Immunolabeling of GFP, FOXA2 and Cre in FOXA2-P2A-Cre/AAVS1-LSLm2-GFP H7 hESC line differentiated toward a FP fate for 5, 8, 10 and 12 days. Sale bar, 100μm.

Supplemental Figure 5

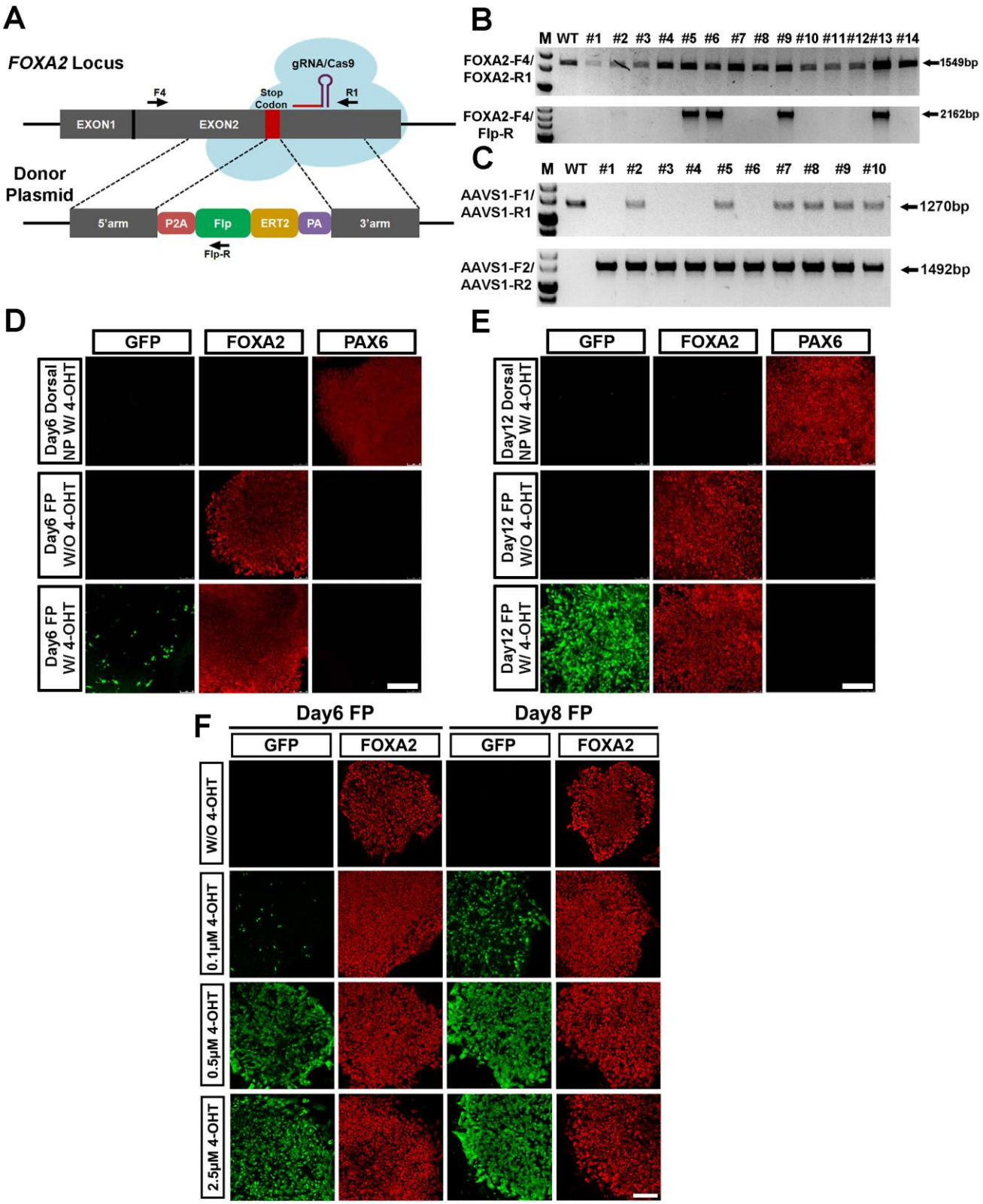


Figure S5. Trancing FP Lineage through FOXA2-P2A-Flp^{ERT2}/AAVS1-FSF-GFP Line. Related to Figure 6.

(**A**) Schematic diagram for constructing FOXA2-P2A-Flp^{ERT2} cassette in hPSCs through gRNA-guided CRISPR/Cas9 system. Genotyping PCR primer sets are labeled with arrows.

(**B, C**) Genomic DNA PCR results showing recombination of P2A-Flp^{ERT2} near the TAA stop codon region of FOXA2 and CAG-FSF-GFP cassette within the AAVS1 intron.

(**D, E**) Immunolabeling of GFP, FOXA2 and PAX6 in dorsal NP and FP cells at day 6 (D) and day 12 (E) in FOXA2-Flp^{ERT2}/AAVS1-FSF-GFP line treated with or without 4-OHT. 4-OHT was administered through day 4-6 (D) and day 10-12 (E). Scale bars, 100μm.

(**F**) Immunolabeling of GFP and FOXA2 in FP cells at day 6 (left) and day 8 (right) in FOXA2-Flp^{ERT2}/AAVS1-FSF-GFP line treated with or without 4-OHT through day 4-6 (left) and day 4-8 (right) at different concentrations (0, 0.1μM, 0.5μM and 2.5μM). Scale bar, 100μm.

Supplemental Figure 6

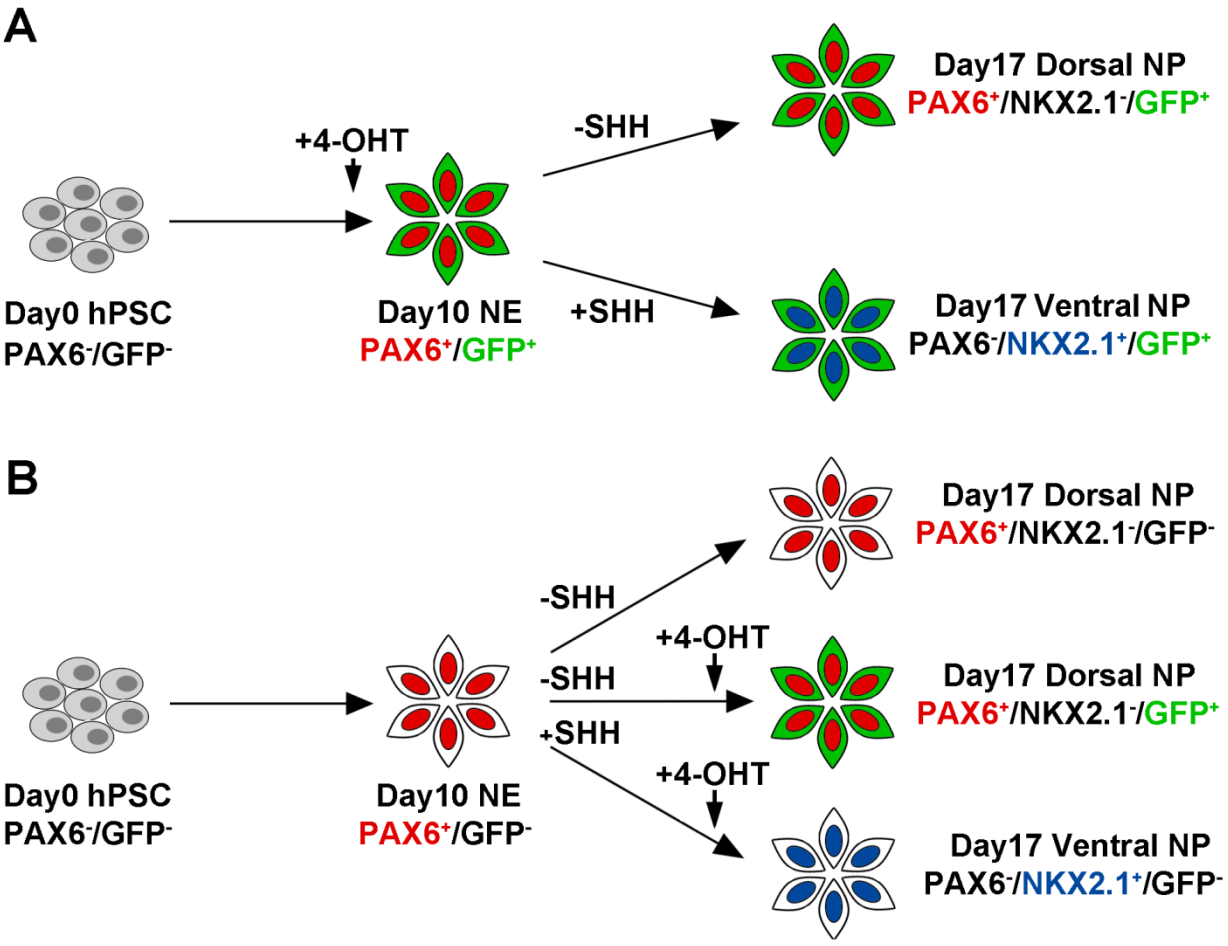


Figure S6. Temporal Tracing of Pax6 Expression Cells Identifies their Sequential NE and Dorsal NP Identities. Related to Figure 7.

(**A, B**) Human day 10 NE uniformly expresses PAX6. With no patterning morphogens, human NE is defaulted to a dorsal NP identity which retained PAX6 expression. While under SHH patterning, human NE is ventralized to a medial ganglionic eminence ventral NP identity with NKX2.1 expression. Dynamically tracing of the PAX6-Cre^{ERT2}/AAVS1-LSL-GFP hESC line reveals distinct NE (through day 8) and cortical NP states (through day 15) of PAX6 expression cells. Tamoxifen administration at day 8-10, both $PAX6^{+}/NKX2.1^{-}$ cortical NPs and $PAX6^{-}/NKX2.1^{+}$ ventral NPs are GFP labeled (A). While tamoxifen treatment at day 15-17, cortical NPs but not ventral cells are labeled with GFP (B).