Expanded View Figures

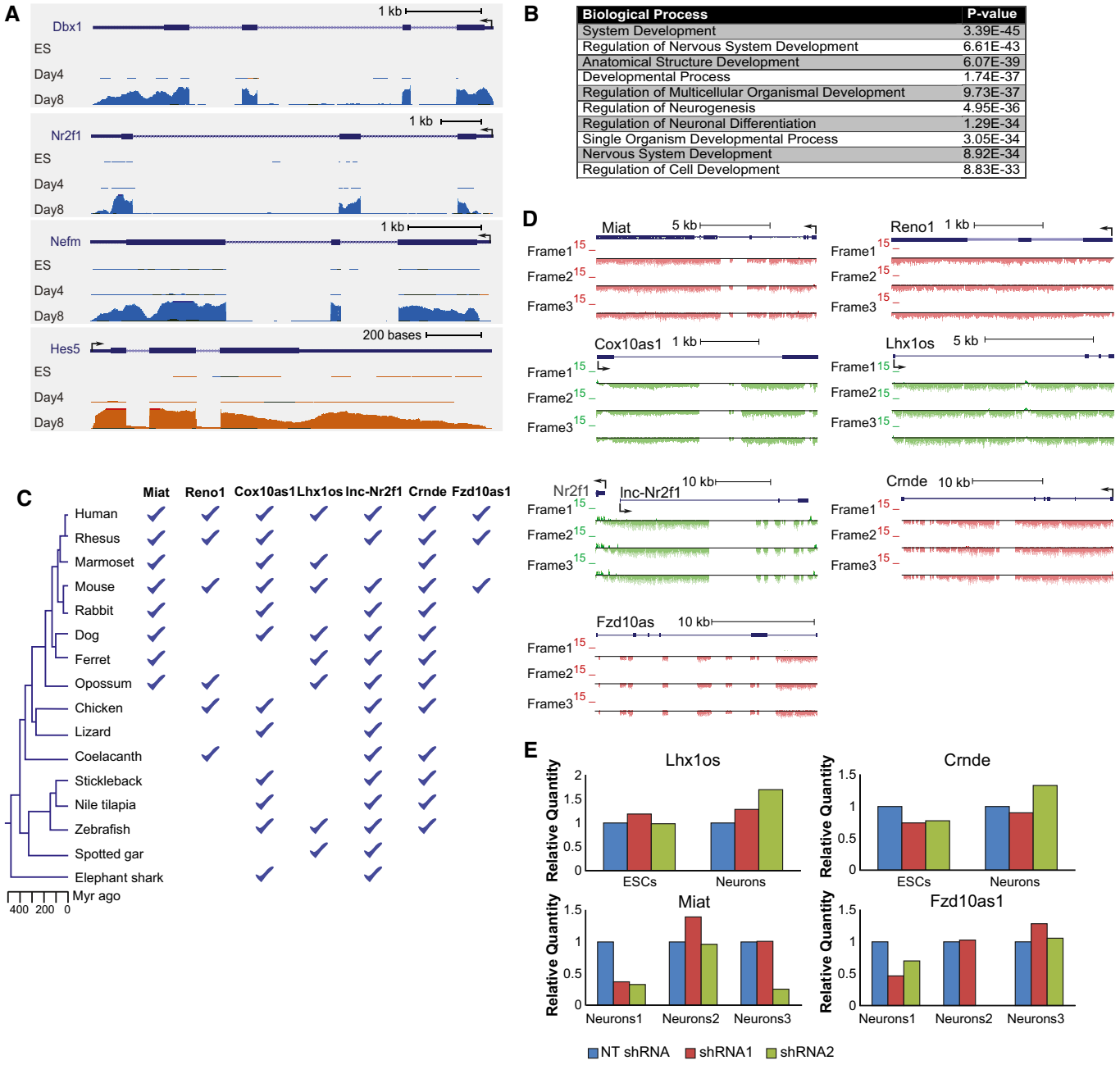


Figure EV1. Induction of neuronal and developmental genes during neuronal differentiation of ES cells.

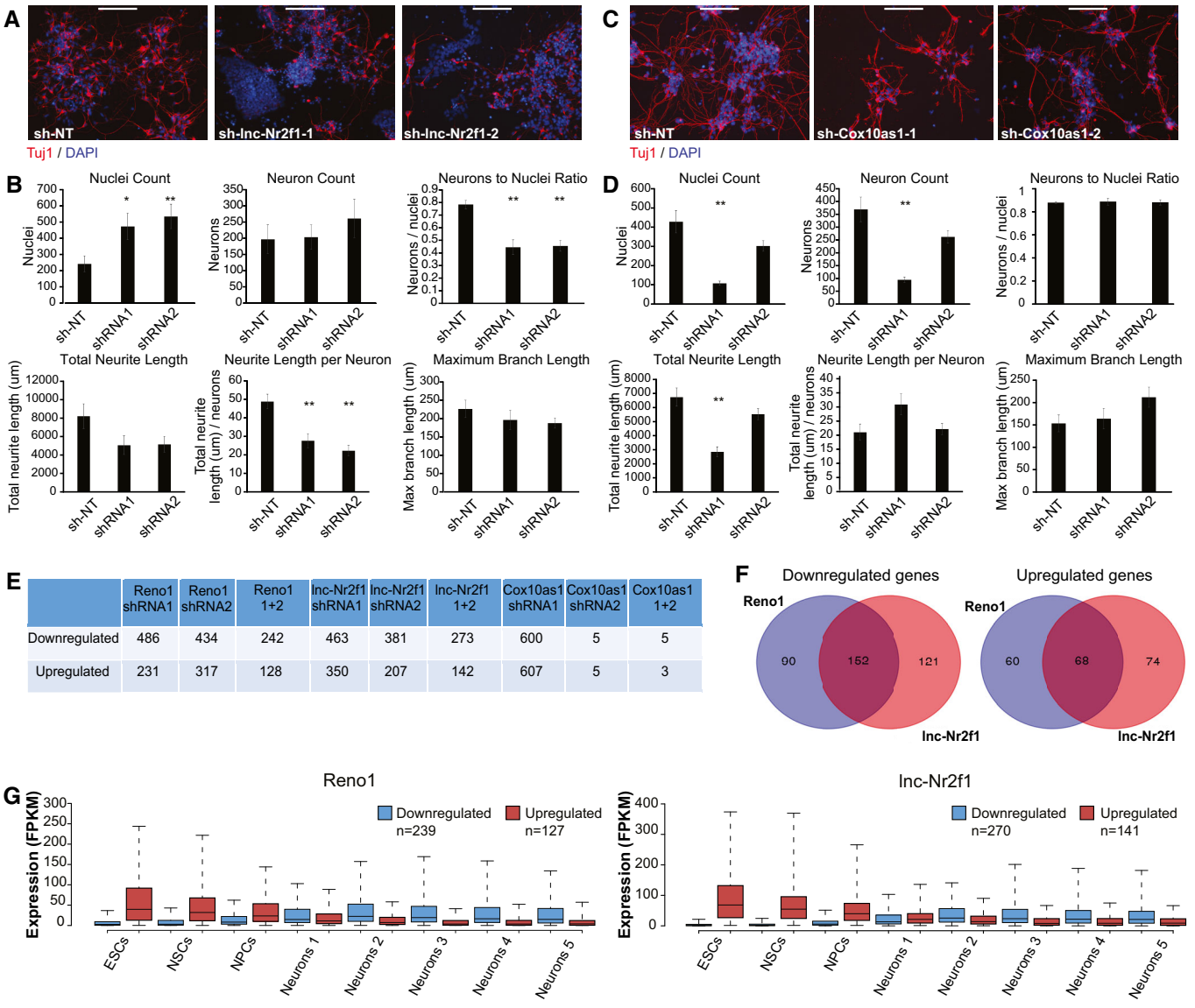
A RNA-seq read coverage at four of the protein-coding genes which were the most upregulated during differentiation. Colors and scaling are as described in Fig 1B.

B Top Biological Process Gene Ontology (GO) categories enriched in genes upregulated during neuronal differentiation, as identified using GOrilla (Eden *et al*, 2009).

C Species represented in clusters of sequence-similar lncRNAs containing each of the candidate lncRNAs, from Hezroni *et al* (2015).

D PhyloCSF scores (Lin *et al*, 2011) throughout the candidate lncRNAs loci in the three possible frames.

E qRT–PCR of *Lhx1os*, *Crnde*, *Miat*, and *Fzd10as1* following infection with two targeting shRNAs for KD in ES cells and following 8 days of neuronal differentiation (top), or three biological repeats of cells following 8 days of differentiation (bottom), all normalized to the non-targeting shRNA (sh-NT).



	Reno1 shRNA1	Reno1 shRNA2	Reno1 1+2	lnc-Nr2f1 shRNA1	lnc-Nr2f1 shRNA2	lnc-Nr2f1 1+2	Cox10as1 shRNA1	Cox10as1 shRNA2	Cox10as1 1+2
Downregulated	486	434	242	463	381	273	600	5	5
Upregulated	231	317	128	350	207	142	607	5	3

Figure EV2. Effects of *Reno1*, *lnc-Nr2f1*, and *Cox10as1* KD on neuronal differentiation.

A Immunostaining using anti-Tuj1 antibody of ES-cell-derived neurons following infection with either sh-NT, or two different shRNAs targeting *lnc-Nr2f1*. Scale bar: 200 μm.

B Quantification of cell numbers and neurite lengths of ten images of non-overlapping fields for each shRNA targeting *lnc-Nr2f1* or sh-NT. Mean ± SEM is shown, $*P < 0.05$, $**P < 0.01$ (unpaired two-sample *t*-test).

C Immunostaining using anti-Tuj1 antibody of ES-cell-derived neurons following infection with either sh-NT, or two different shRNAs targeting *Cox10as1*. Scale bar: 200 μm.

D Quantification of cell numbers and neurite lengths of 10 images of non-overlapping fields for each shRNA targeting *Cox10as1* or sh-NT. Mean ± SEM is shown, $**P < 0.01$ (unpaired two-sample *t*-test).

E Number of genes which were significantly ($P < 0.01$, DESeq2) down- or upregulated following KD of *Reno1*, *lnc-Nr2f1*, or *Cox10as1*.

F Overlap between genes that were significantly down- (left) or upregulated (right) following *Reno1* and *lnc-Nr2f1* KD.

G Boxplots indicating the median, quartiles, and 5th and 95th percentiles of expression levels of genes which were down- or upregulated following KD of *Reno1* or *lnc-Nr2f1* during the same neuronal differentiation time points described in Fig 2C. The numbers of genes in each group are indicated in the legend.

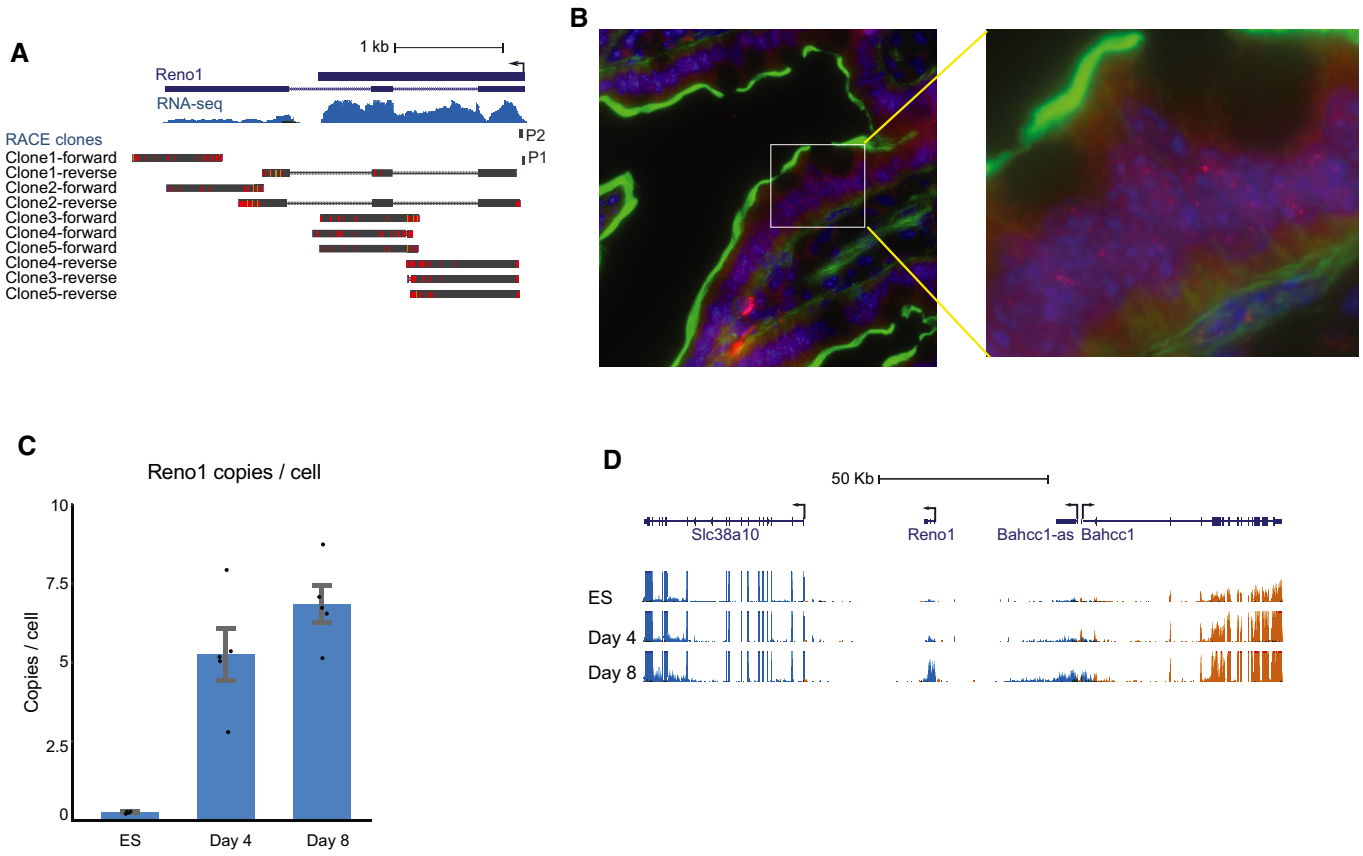


Figure EV3. Expression levels of *Reno1* and *Bahcc1* in various tissues and cells.

A RNA-seq read coverage and 3′ RACE on RNA extracted from cells following 8 days of differentiation. BLAT alignments of Sanger sequencing results of five independent 3′ RACE clones and the position of the primer used for 3′RACE (P1) are shown.

B *Reno1* smFISH signal (red), DAPI staining (blue) and phall (green) in mouse intestine, imaged using 100× objective.

C Absolute quantification of *Reno1* RNA copy number per cell, estimated by qRT–PCR. Mean ± SEM is shown for 3–4 independent experiments.

D RNA-seq read coverage at the *Reno1*, *Bahcc1*, and *Slc38a10* loci. Blue depicts transcripts from the "−" strand, and orange represents transcripts from the "+" strand. All tracks are scaled to the same level.

Figure EV4. Depletion of *Reno1* or *Bahcc1* inhibits neuronal differentiation.

A Genotyping of *Reno1*$^{m/m}$ ES cells using primers flanking the deleted region.

B RNA-seq read coverage in the *Reno1* locus in the RNA-seq data of differentiated cells in the indicated clones. Deletions validated by Sanger sequencing are shown—the thick boxes indicate Sanger-sequenced regions and the thin line the gap in the sequence.

C Ribo-seq and RNA-seq read coverage from multiple studies accumulated in the GWISP-vis database (Michel *et al*, 2014) for the first coding exon of Bahcc1 in human and mouse. Regions of the two possible start codons in frame with the main coding sequence are highlighted. An arrow indicates that position targeted by the gRNA used to generate *Bahcc1*$^{m/+}$ cells.

D RNA-seq read coverage around the first coding exon of *Bahcc1* in the RNA-seq data of differentiated cells in the indicated clones. Deletion validated by Sanger sequencing in the first clone is shown—the thick boxes indicate Sanger-sequenced regions and the thin line the gap in the sequence.

E Immunostaining using anti-Tuj1 antibody following 8 day differentiation of WT and *Reno1*$^{m/m}$ ES cells.

F Quantification of cell numbers and neurite lengths of 16 images of non-overlapping fields for each shRNA. Mean ± SEM is shown, $*P < 0.05$, $**P < 0.01$ (unpaired two-sample *t*-test).

G Overlap between genes that were significantly down- (left) or upregulated (right) in *Reno1*$^{m/m}$ or *Bahcc1*$^{m/+}$ cells at day 4 of differentiation.

H, I Changes in gene expression of pluripotency- and NPC-related genes (H) and genes from the indicated gene families (I) after 4 days of differentiation. Heatmaps show log_2-transformed fold changes of mutant clones compared to WT cells following 4 days of neuronal differentiation, and of day 4 of differentiation compared to undifferentiated ES cells.

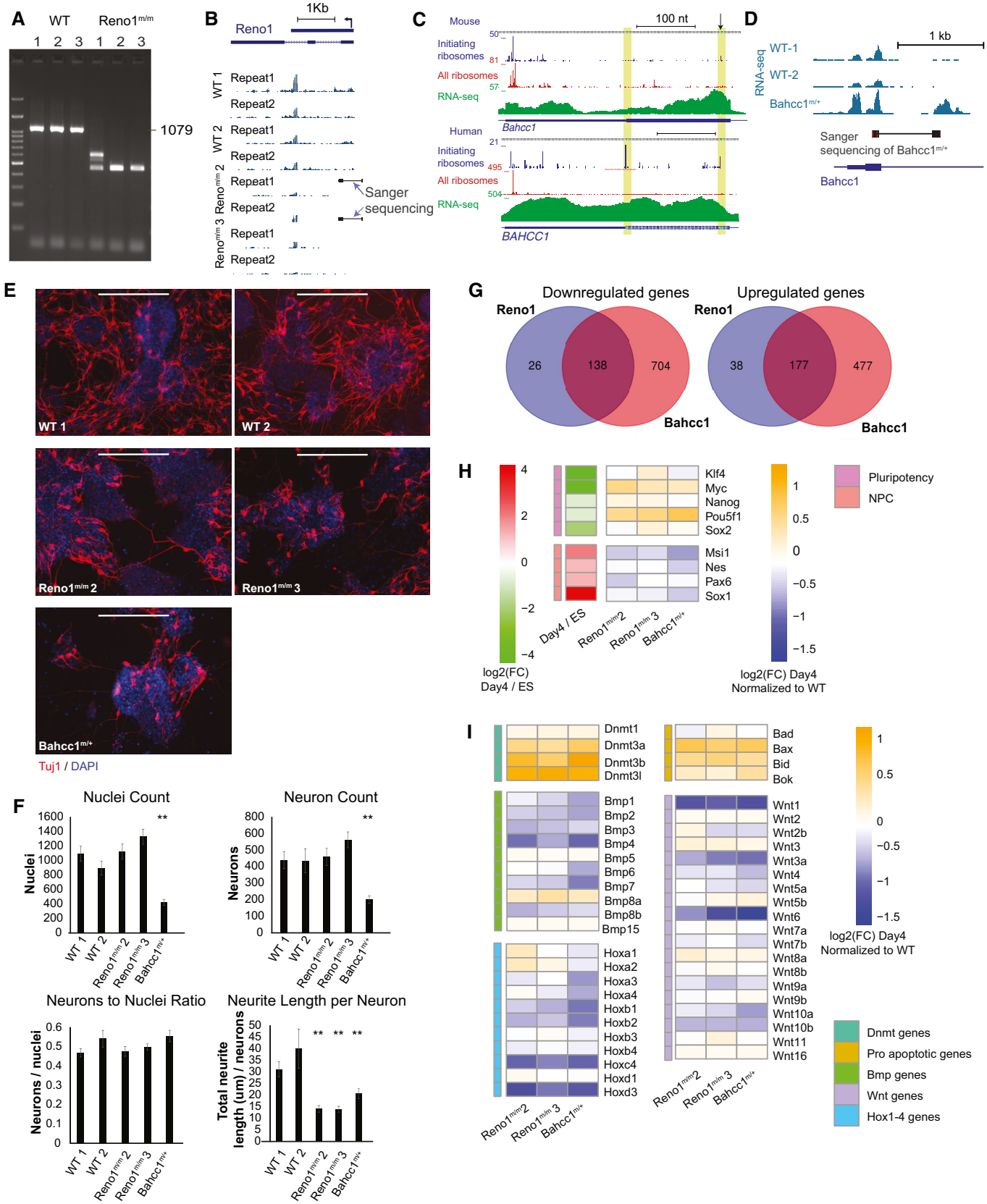


Figure EV4.

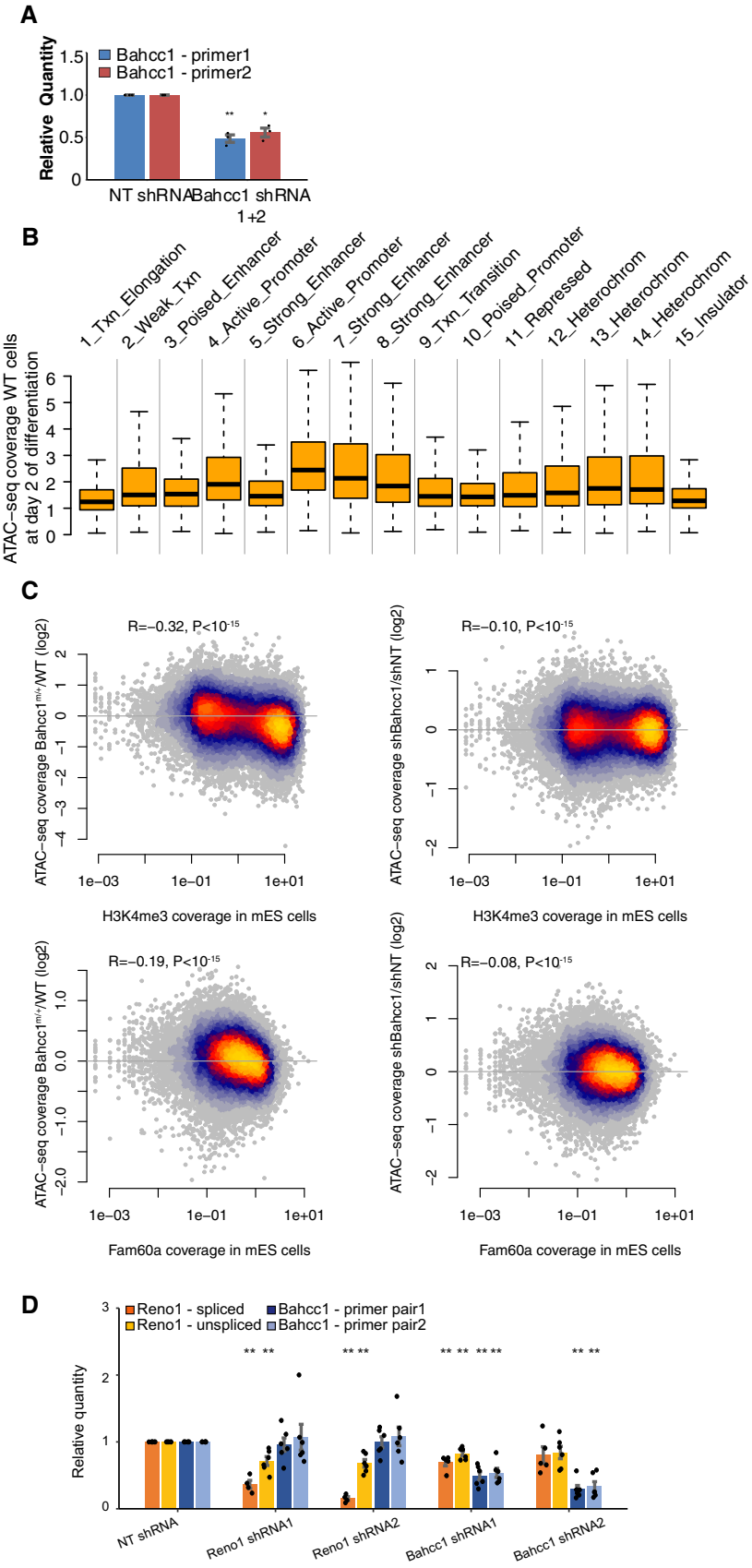


Figure EV5. Characterization of *Reno1*- and *Bahcc1*-perturbed cells.

A qRT–PCR using the indicated primer pairs (see Materials and Materials and Methods) in cells at day 2 of differentiation following *Bahcc1* shRNA KD. Mean ± SEM is shown for three independent experiments. $*P < 0.05$; $**P < 0.01$ (unpaired two-sample *t*-test).

B ATAC-seq accessibility in WT cells following 2 days of differentiation in peaks that overlap the indicated chromatin states in mouse ES cells.

C Correlation between changes in ATAC-seq signal following the indicated treatment and H3K4me3 ChIP-seq signal (top) or Fam60a ChIP-seq signal (bottom) in mouse ES cells (bottom).

D qRT–PCR of *Reno1* and *Bahcc1* following KD in N2a cells using two targeting shRNAs, normalized to a non-targeting shRNA (sh-NT Mean ± SEM is shown for 5–6 independent experiments. $**P < 0.01$ (unpaired two-sample *t*-test).