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Reno1

Bahcc1

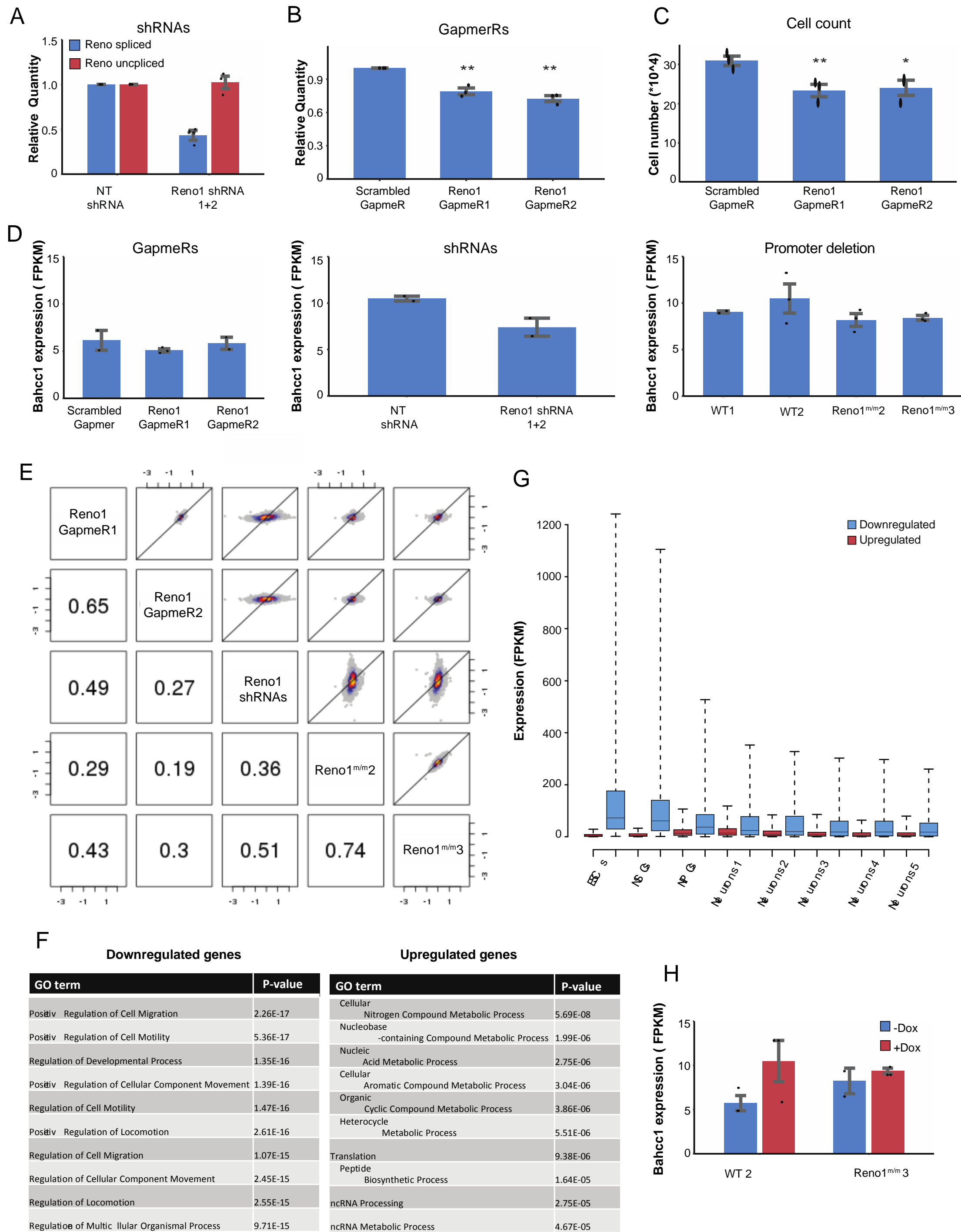
Expression

cerebellum
hippocampus
corpus striatum
cerebellar granule cells
cortex,
medulla oblongata
spinal cord,
diencephalon
corpora quadrigemina
visual cortex
olfactory brain
eyeball
forelimb
liver
kidney
stomach
intestine
lung
adrenal gland
pituitary gland
pancreas
heart
thymus
spleen
bone
skin
gonad
ovary
testis
accessory axillary lymph node
whole body

embryo E11, embryo E12, embryo E13, embryo E14, embryo E15, embryo E16, embryo E17, embryo E18, neonate N00, neonate N03, neonate N06, neonate N09, neonate N10, neonate N15, neonate N20, neonate N30, adult

Neurons – cortical
Neurons – hippocampal
Neurons – striatal
Neurons – dorsal spinal cord
Neurons – ventral spinal cord
Neurons – raphe
Neurons – substantia nigra
Astrocytes – cerebellar
Astrocytes – hippocampus
Astrocytes,
Microglia,
Schwann
Inner ear stem cells
Atoh+ Inner ear hair cells – organ of corti
Sox2+ Supporting cells – organ of corti
Mouse Renal epithelial cells
Meningeal cells
liver sinusoidal endothelial cells
hepatocyte,
hepatic stellate cells
Embryonic fibroblasts
Cardiac Myocytes
Granule cells
Mesenchymal stem cells – bone marrow derived,
Lgr5+ intestinal stem cells,
macrophage,
CD41+ megakaryocyte
granulocyte macrophage progenitor GMP
common myeloid progenitor CMP
stem cell (cKit+ Sca1+ lineage–) KSL,
CD19+ B Cells
CD4+ T Cells
CD8+ T Cells
CD4+CD25+ regulatory T cells
CD4+CD25+ regulatory T cells
CD4+CD25–CD44– naive conventional T cells
natural helper cells, naive
natural helper cells, IL33 treated
natural helper cells, IL2 treated
MC1+Gr1+ myeloid–derived suppressor cells
hematopoietic stem cell
CD326+ enterocyte,
CD326+ enterocyte isolated from mice
ES–OS25 embryonic stem cells, untreated control,
ES–46C embryonic stem cells, neuronal differentiation, day00,
ES–46C embryonic stem cells, neuronal differentiation, day01,
ES–46C embryonic stem cells, neuronal differentiation, day02,
ES–46C embryonic stem cells, neuronal differentiation, day03,
ES–46C embryonic stem cells, neuronal differentiation, day04,
ES–46C derived epistem cells, neuronal differentiation, day05,
ES–46C derived epistem cells, neuronal differentiation, day14,
ES–Ert2 embryonic stem cells, untreated control,
ES–Ert2 embryonic stem cells, tamoxifen treated,
ES–46C derived epistem cells, bio
ES–46C derived epistem cells, neuronal differentiation,

Appendix Figure S1. Expression of *Reno1* (left) and *Bahcc1* (right) in various mouse tissues at different developmental stages (top), and various cell types (bottom). All data obtained from the FANTOM5 project via the Zenbu browser.



Downregulated genes

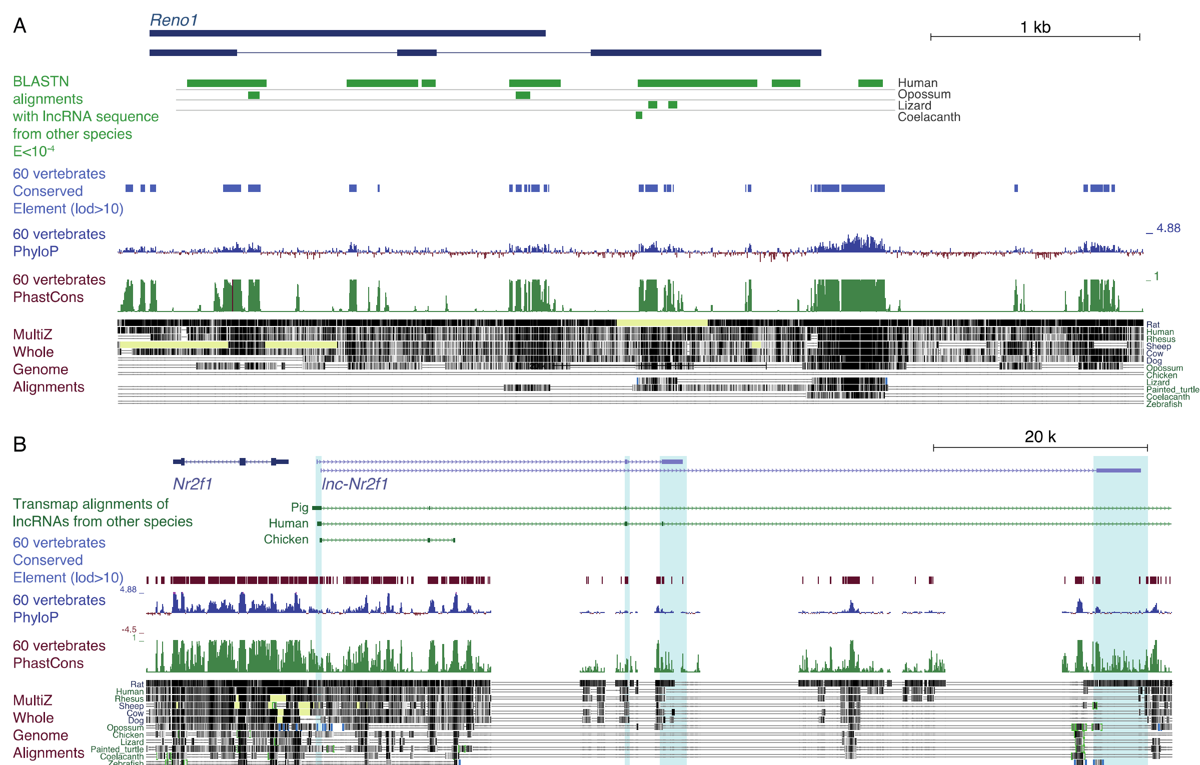
GO term	P-value
Positiv Regulation of Cell Migration	2.26E-17
Positiv Regulation of Cell Motility	5.36E-17
Regulation of Developmental Process	1.35E-16
Positiv Regulation of Cellular Component Movement	1.39E-16
Regulation of Cell Motility	1.47E-16
Positiv Regulation of Locomotion	2.61E-16
Regulation of Cell Migration	1.07E-15
Regulation of Cellular Component Movement	2.45E-15
Regulation of Locomotion	2.55E-15
Regulation of Multic llular Organismal Process	9.71E-15

Upregulated genes

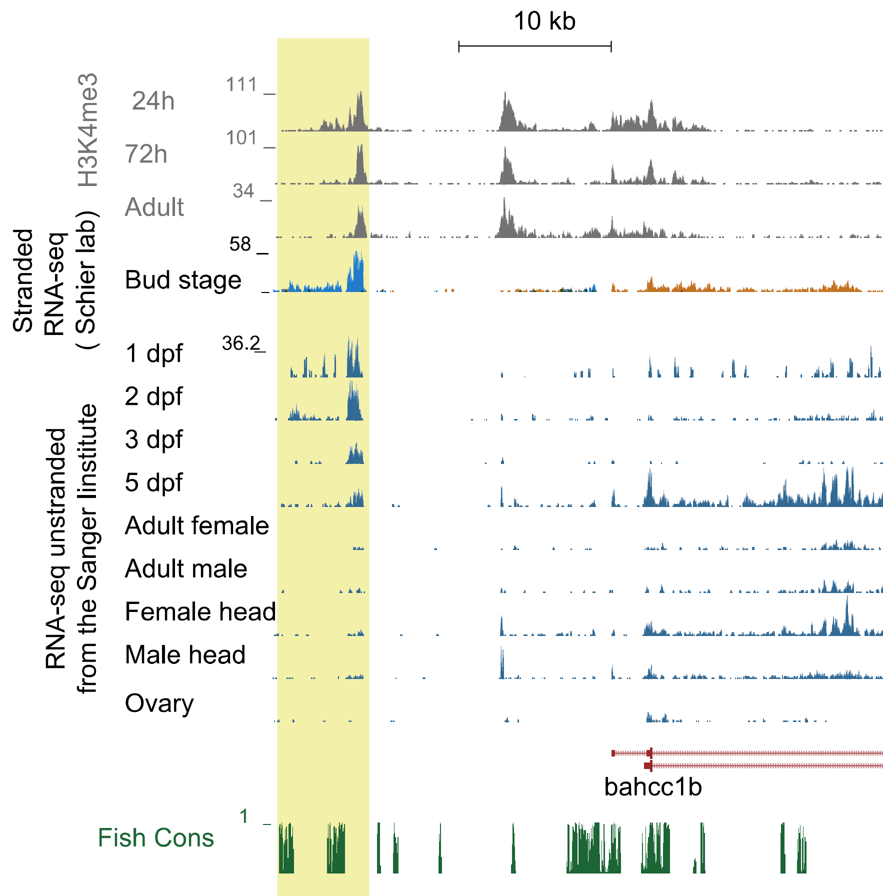
GO term	P-value
Cellular Nitrogen Compound Metabolic Process	5.69E-08
Nucleobase -containing Compound Metabolic Process	1.99E-06
Nucleic Acid Metabolic Process	2.75E-06
Cellular Aromatic Compound Metabolic Process	3.04E-06
Organic Cyclic Compound Metabolic Process	3.86E-06
Heterocycle Metabolic Process	5.51E-06
Translation	9.38E-06
Peptide Biosynthetic Process	1.64E-05
ncRNA Processing	2.75E-05
ncRNA Metabolic Process	4.67E-05

Appendix Figure S2. Transcriptional response to *Reno1* depletion after two and four days of differentiation. (A) qRT-PCR using the indicated primer pairs (see Methods) in cells at day 2 of differentiation following *Reno1* shRNA knockdown. Mean ± SEM is shown for three independent experiments. , ** P<0.01 (unpaired two sample t-test). **(B)** qRT-PCR of *Reno1* in ES cells transfected with LNA GapmeRs targeting *Reno1* or a scrambled GapmeR that was used for normalization, at day 2 of

differentiation. Mean ± SEM is shown for three independent experiments., * P<0.05, ** P<0.01 (unpaired two sample t-test). **(C)** Cell counts for cells transfected with LNA GapmeRs targeting *Reno1* or a scrambled GapmeR following four days of differentiation. Cells were harvested from one well of a 6-well plate, and counted using Orflo MOXI Z Mini Automated Cell Counter. Mean ± SEM is shown for three independent experiments. * P<0.05, ** P<0.01 (unpaired two sample t-test). **(D)** Expression levels of *Bahcc1* mRNA, measured using RNAseq, in cells where *Reno1* was depleted using GapmeRs (left), shRNAs (middle) or promoter deletion (right). Mean ± SEM is shown. The expression levels are not significantly different by DESeq2 analysis. **(E)** Pairwise scatterplots showing $\log_2$-transformed fold changes of cells with the indicated *Reno1* perturbation compared to their respective controls. Color indicates local point density. Numbers indicate Spearman correlation coefficient between the fold changes **(F)** Biological Process GO categories most enriched in genes significantly down- or upregulated in at least two *Reno1* perturbations, as identified using GOrilla (Eden et al, 2009). **(G)** Boxplots indicating the median, quartiles, and 5th and 95th percentiles of expression levels of genes which were significantly (P<0.05) downregulated (n=168) or upregulated (n=94) by at least two out of three *Reno1* perturbations at day 2 in fig. 5A, during the same neuronal differentiation time points described in Fig. 5C. **(H)** Expression levels of *Bahcc1* mRNA, measured using RNAseq, in WT and *Reno1*$^{m/m}$ cells following Dox addition. Mean ± SEM is shown. ** P<0.01 (DESeq2).



Appendix Figure S3. Sequence conservation of *Reno1* and lnc-*Nr2f1*. (A) *Reno1* lncRNA locus in human with the two isoforms show and with the regions alignable (E-value<10^{-4}) with lncRNAs from other species shown in Figure 3A, and conserved elements, conservation scores, and whole-genome alignments from the UCSC genome browser. **(B)** Transmap alignments of Ensembl transcripts from other species and genome conservation information from the UCSC genome browser for the *lnc-Nr2f1* lncRNA.



Appendix Figure S4. Zebrafish *Reno1* locus. H3K4me3 Chip-seq coverage from (Ulitsky et al, 2011), RNA-seq from (Howe et al, 2013; Pauli et al, 2012). Putative *Reno1* locus is shaded.