Supporting Information

Knouse et al. 10.1073/pnas.1415287111

SI Materials and Methods

Tissue Sources. Frozen human liver was obtained from the Brigham and Women's Hospital Tissue and Blood Repository. Hepatocytes isolated from human cadaveric donors without liver disease were purchased from Life Technologies. Frontal lobe and epidermis were isolated from humans without neurologic disease or brain hypoxia upon autopsy at Massachusetts General Hospital through the Neuropathology Core of the Massachusetts Alzheimer Disease Research Center. All human procedures were approved by the Massachusetts Institute of Technology Committee on the Use of Humans as Experimental Subjects.

Murine liver, cerebral cortex, and epidermis were isolated from C57BL/6J mice purchased from the Jackson Laboratory. *BUB1B*$^{H/H}$ mice were obtained from Jan van Deursen (Mayo Clinic, Rochester, MN) (1), DRD2-GFP mice from David Housman (Massachusetts Institute of Technology, Cambridge, MA) (2), and nestin-GFP mice from David Scadden (Massachusetts General Hospital, Boston) (3). Trisomy 16 embryos were generated from Rb(6.16)24Lub and Rb(16.17)7Bnr mice purchased from the Jackson Laboratory. All animal procedures were approved by the Massachusetts Institute of Technology Committee on Animal Care.

Immunostaining and FISH. Tissues were fixed in 4% (wt/vol) paraformaldehyde in PBS for 16–24 h. Fixed tissues were washed with PBS, cryoprotected with 30% (wt/vol) sucrose, and frozen in O. C. T. Compound (Tissue-Tek). Slides with 10- or 30-μm-thick sections were prepared using a cryostat and stored at −80 °C until use. Slides were thawed, aged at room temperature for 16–24 h, rehydrated in PBS for 15 min, and boiled in sodium citrate buffer (10 mM trisodium citrate dihydrate, 0.05% Tween 20, pH 6.0) for 20 min. Slides were washed in PBS, dried briefly, and sections outlined with a hydrophobic pen. Sections were incubated with PBS containing 0.5% Triton X-100 (0.5% PBST) for 5 min, followed by incubation in blocking solution [4% (wt/vol) BSA, 10% (vol/vol) goat serum, 10% (vol/vol) donkey serum in 0.5% PBST] for 1 h at room temperature. Sections were incubated with a rabbit pan-cadherin antibody (Abcam) diluted 1:500 in block at 4 °C for 16–24 h. Sections were washed three times with 0.5% PBST for 5 min each. Sections were incubated with an AlexaFluor 647 goat anti-rabbit antibody (Life Technologies) diluted 1:1,000 in blocking solution at room temperature for 1 h. Sections were washed three times with 0.5% PBST for 5 min each. Sections were fixed with 2% (wt/vol) paraformaldehyde in PBS for 10 min and washed three times with PBS for 5 min each. Hydrophobic ink was removed and slides were incubated in 2× SSC for 5 min, followed by incubation in 50% (vol/vol) formamide, 2× SSC for 2 h. Fluorescently labeled probes targeting different regions of mouse chromosome 16 (RP23-354F11 and RP23-18M23) and human chromosome 7 (RP11-243E12 and RP11-377B19) (Empire Genomics) were diluted in hybridization buffer as per the manufacturer's instructions and applied to sections. Sections were sealed with a coverslip and rubber cement and incubated in the dark at 45 °C for 2 h to allow probes to infiltrate section. This was followed by incubation at 85 °C for 5 min to denature the DNA. Hybridization was performed in the dark at 37 °C for 48 h. Slides were then washed with 0.4× SSC containing 0.3% Nonidet P-40 for 2 min at 73 °C, followed by 2× SSC containing 0.1% Nonidet P-40 for 1 min at room temperature. Slides were incubated in 0.05 μg/mL DAPI in 2× SSC for 30 min and mounted with ProLong Gold Antifade Reagent (Life Technologies). Images were acquired on a spinning disk confocal microscope (PerkinElmer) and analyzed using the Volocity software package (PerkinElmer).

Tissue Dissociation. To isolate keratinocytes from human and mouse skin, skin was floated in 0.05% thermolysin in Hepes-buffered saline for 1–2 h at 37 °C. The epidermis was liberated from dermis using forceps, dissociated with 0.05% trypsin in 0.05% EDTA in PBS at 37 °C for 30 min, combined with an equal volume of DMEM with 10% (vol/vol) FBS, passed through a 40-μm filter, centrifuged at 100 × *g* for 5 min, and resuspended in DMEM with 10% (vol/vol) FBS.

To isolate neural progenitor cells from embryonic nestin-GFP mice and medium spiny neurons from adult DRD2-GFP mice, brains were dissociated as previously described (4) and GFP-positive cells isolated by flow cytometry. To isolate brain cells from adult human and mouse cortex, tissue pieces were processed as previously described (5). Fraction 3 was isolated to enrich for neurons.

To isolate nuclei from human hepatocytes, hepatocytes were washed with cold PBS, incubated in 0.2× PBS on ice for 10 min, lysed with a dounce homogenizer for 15 strokes, centrifuged at 1,000 × *g* at 4 °C for 10 min, resuspended in sucrose buffer (250 mM sucrose, 5 mM $MgCl_2$, 10 mM Tris·Cl, pH 7.4), and centrifuged and resuspended again. To isolate nuclei from mouse liver, liver lobes were dissected in PBS on ice, pressed through a 40-μM filter into sucrose buffer, centrifuged at 600 × *g* at 4 °C for 10 min, resuspended in sucrose buffer, and centrifuged and resuspended again.

Single Cell, Quantitative RT-PCR. A total of 2.5×10^3 dissociated cells were added to 20 mL of media or sucrose buffer containing 5% (vol/vol) FBS in a 15-cm Petri dish. Single cells were isolated using a homemade microaspirator and prepared for quantitative RT-PCR using the Single Cell-to-C_T Kit (Life Technologies). Specific transcripts were quantified using TaqMan Gene Expression Assays (Life Technologies) targeting mouse Rbfox3 (NeuN) (Mm01248771_m1), mouse GFAP (Mm01253033_m1), mouse MBP (Mm01266402_m1), and mouse GAPDH (Mm99999915_g1).

Single Cell, Whole-Genome Amplification. A total of 2.5×10^3 dissociated cells or nuclei were added to 20 mL of media or sucrose buffer containing 5% (vol/vol) FBS in a 15-cm Petri dish. Single cells and nuclei were isolated using a homemade microaspirator and transferred to 8 μL water in a 96-well plate. The microaspirator needle was cleaned with 10% (vol/vol) bleach, followed by water after transferring each cell. After up to 32 cells or nuclei had been isolated, cells were lysed and genomic DNA amplified using the GenomePlex Single Cell Whole Genome Amplification Kit (Sigma). All reagents were added using aerosol-resistant pipette tips in a laminar flow hood. After amplification, 4 μL of each sample were analyzed by agarose gel electrophoresis. Samples producing a smear ranging from 100 to 1,000 bp were sequenced.

Single Cell Sequencing. Products of whole-genome amplification were purified using the MinElute Cleanup kit (Qiagen). Purified DNA (50 ng) was prepared and barcoded using Nextera reagents (Illumina), and tagmented material was PCR amplified for seven cycles. Libraries were quantified using an AATI Fragment Analyzer and quantitative PCR before pooling. Between 15 and 24 cells were combined for sequencing in a single lane. Libraries were sequenced (40 or more nucleotide reads) on an Illumina HiSeq2000. Reads were demultiplexed using custom scripts allowing single mismatches within the reference barcode.

Copy Number Analysis. Sequence reads were trimmed to 40 nucleotides and aligned to genomes of human (hg19) or mouse (mm9) using BWA (0.6.1) with default options (6). HMMcopy (0.1.1) was used to detect copy number alterations by estimating

copy number in 500-kb bins controlling for mappability [downloaded from UCSC Genome Bioinformatics (http://hgdownload.cse.ucsc.edu/goldenPath/hg19/encodeDCC/wgEncodeMapability/ or http://hgdownload.cse.ucsc.edu/goldenPath/mm9/encodeDCC/wgEncodeMapability/)] and GC content (calculated by HMMcopy gcCounter) (7).

SDs of the corrected read copies ($\log_2$ based) from HMMcopy were computed within sliding windows (30 adjacent 500-kb bins) for all chromosomes, and the average was calculated for each chromosome. The average SDs of the three autosomes with highest variability were averaged to generate a variability score (VS). The distribution of VS for all cells had a positive skew, with a range of 0.14–2.01, a mean of 0.24, and an SD of 0.16. Cells with a VS exceeding 0.34 were excluded from analysis. This cutoff is 1.25 SDs from the minimum VS and resulted in exclusion of 55 of 544 cells. For autosomes in brain and skin cells, a $\log_2$ ratio exceeding 0.44 was considered a chromosome gain and a $\log_2$ ratio below −0.57 was considered a chromosome loss. For autosomes in hepatocyte nuclei, a $\log_2$ ratio exceeding 0.25 was considered a chromosome gain and a $\log_2$ ratio below −0.3 a loss. For the X chromosome in males (normal $\log_2$ ratio < −0.57), a $\log_2$ ratio greater than −0.23 was considered gain.

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Table S1. Prevalence of aneuploidy in somatic cells

Tissue	Total cells analyzed	Total aneuploid cells	Description of aneuploidy	Prevalence of aneuploidy (95% CI)
Mouse keratinocytes	37	1	Trisomy 12	2.7% (0.1–14.2%)
Human keratinocytes (48F, 68M)*	53 (29, 24)	0		0 (0–6.7%)
Mouse neural progenitor cells	36	0		0 (0–9.7%)
Mouse brain cells	43	1	Trisomy 15	2.3% (0.1–12.3%)
Mouse neurons	19	0		0 (0–17.6%)
Human brain cells (70M, 52M, 48F, 68M)	89 (20, 22, 22, 25)	2 (2, 0, 0, 0)	Monosomy 22 Trisomy 18 in tetraploid	2.2% (0.3–7.9%)
Mouse hepatocyte nuclei	66	0		0 (0–5.4%)
Human hepatocyte nuclei (46M, 51M)	100 (39, 61)	4 (1, 3)	Uninterpretable cell Pentasomy 5, 7 in tetraploid Pentasomy 7 in tetraploid Pentasomy 15 in tetraploid	4% (1.1–9.9%)

CI, confidence interval; F, female; M, male.
*Age and sex of analyzed individual.

Fig. S1. Sequence quality analysis and exclusion of uninterpretable cells. (*A*) Representative segmentation plots of two cells that were excluded from the analysis (*Top* and *Upper Middle*) and two cells that fulfilled quality standards and were therefore included in the analysis (*Lower Middle* and *Bottom*). Excluded cells had either or both a wide spread of read counts (first plot) or many segments with nondigital copy number states (second plot). Both cell types are characterized by high VS. The VS of an aneuploid cell (third panel) is no different from that of a euploid cell (fourth panel). (*B*) Box and whiskers plots of VS for all sequenced cells. Whiskers extend from minimum to maximum VS for each group of cells. Boxes extend from the 25th to the 75th percentile. Line within boxes indicates the median of the distribution. (*C*) Box and whiskers plots of VS of analyzed cells only. (*D*) Table summarizing number and percentage of cells that were excluded from the analysis.

Fig. S1

Fig. S2. Segmentation plots of euploid cells from normal mouse and human skin, brain, and liver. This figure includes all euploid cells not included in the main figures.

Fig. S2

Fig. S3. Segmentation plot of an aneuploid mouse keratinocyte. Segmentation plot of a mouse skin cell with trisomy 12.

Fig. S3

Fig. S4. Identity of brain cell preparations and segmentation plots of aneuploid brain cells. (*A*) Expression of the neuronal marker NeuN, the astrocyte marker GFAP, and the oligodendrocyte marker MBP in single mouse brain cells determined by single cell quantitative RT-PCR. (*B*) Segmentation plot of an aneuploid mouse brain cell with trisomy 15. (*C*) Segmentation plot of a tetraploid human brain cell with trisomy 18.

Fig. S4

Fig. S5. Ploidy of hepatocyte nuclei preparations and segmentation plots of aneuploid hepatocyte nuclei. (*A*) Ploidy of mouse hepatocyte nuclei inferred by FISH (chromosome 16 copy number) in tissue sections and nuclear diameter in dissociated nuclei. (*B*) Segmentation plots of tetraploid human hepatocyte nuclei with pentasomy 7 (*Upper*) and pentasomy 15 (*Lower*).

Fig. S5