

SUPPLEMENTAL TABLES

Table S1: Summary of bulk calling cards experiments, related to Figures 1, 2, 4, S1 and Supplemental Methods

| Sample | Construct | Modality | Replicates^a | Insertions | Reads | Mean coverage |
|---------------|------------------|------------------|-------------------------------|-------------------|--------------|----------------------|
| HCT-116 | SP1-PBase | DNA ^b | 1 | 31,001 | 21,975,948 | 708.9 |
| HCT-116 | SP1-PBase | RNA ^b | 1 | 62,500 | 14,993,901 | 239.9 |
| HCT-116 | PBase | RNA | 10 | 1,521,048 | 58,316,389 | 38.3 |
| HCT-116 | SP1-PBase | RNA | 10 | 410,588 | 35,526,586 | 86.5 |
| HCT-116 | HyPBase | RNA | 12 | 5,771,207 | 47,572,324 | 8.2 |
| HCT-116 | SP1-HyPBase | RNA | 11 | 2,029,931 | 40,214,827 | 19.8 |
| HCT-116 | SB100X | RNA | 12 | 26,515,072 | 67,650,985 | 2.6 |
| OCM-1A | HyPBase | RNA | 10 | 5,951,669 | 261,476,361 | 43.9 |
| OCM-1A | BAP1-HyPBase | RNA | 10 | 5,740,754 | 293,332,813 | 51.1 |

^a Biological replicates. ^b These experiments were used to assess DNA- vs. RNA-based recovery (Figure 1C).

Table S2: Summary of single cell calling cards experiments, related to Figures 3, 4, 5, 6, 7, S2, S3, S4, S5, S6, and S7

| Sample | Construct | Libraries | Cells | Insertions | Reads | Mean coverage | Mean IPC | Median IPC | % cells with ≥ 1 insertion |
|---------------|------------------|------------------|--------------|-------------------|--------------|----------------------|-----------------|-------------------|---------------------------------------------------|
| HCT-116 & N2a | HyPBase | 1 ^a | 6,068 | 33,223 | 1,710,525 | 51.5 | 5.4 | 4 | 91.8 |
| HCT-116 | HyPBase | 4 ^b | 12,891 | 37,774 | 4,768,230 | 126.2 | 3.0 | 2 | 93.4 |
| K562 | HyPBase | 4 ^b | 11,912 | 107,385 | 10,404,042 | 96.9 | 9.5 | 6 | 96.9 |
| HCT-116 | SP1-HyPBase | 4 | 30,411 | 77,210 | 9,874,157 | 127.9 | 2.6 | 2 | 83.8 |
| K562 | SP1-HyPBase | 4 | 21,554 | 327,465 | 44,851,070 | 137.0 | 15.3 | 9 | 95.8 |
| HepG2 | HyPBase | 3 | 17,195 | 144,176 | 20,035,606 | 139.0 | 8.4 | 6 | 96.1 |
| HepG2 | FOXA2-HyPBase | 3 | 16,623 | 105,000 | 15,677,152 | 149.3 | 6.3 | 4 | 96.0 |
| OCM-1A | HyPBase | 3 | 23,978 | 150,707 | 19,794,848 | 131.3 | 6.3 | 4 | 96.2 |
| OCM-1A | BAP1-HyPBase | 3 | 19,572 | 215,330 | 27,666,808 | 128.5 | 11.0 | 7 | 97.6 |
| Mouse cortex | HyPBase | 9 ^c | 35,950 | 111,382 | 12,204,369 | 109.6 | 3.1 | 3 | 73.7 |

^a This library was from a species-mixing experiment (Figures 3B and S2). ^b These libraries were demultiplexed from a cell line-mixing experiment (Figures 3C-D and S3). ^c This experiment is further stratified by cell type in Table S3. IPC: insertions per cell.

Table S3: Breakdown of cortical cell types and scCC HyPBase insertions per cluster, related to Figures 6, 7, and S7

| Cluster | Cells | Insertions | Mean IPC |
|----------------------------|--------------|-------------------|-----------------|
| Astrocyte | 4,727 | 16,791 | 3.6 |
| Astro_Neuron_Doublet | 394 | 1,653 | 4.2 |
| Ependymal | 107 | 153 | 1.4 |
| Microglia | 569 | 238 | 0.4 |
| Neuroblast_SVZ | 369 | 1,084 | 2.9 |
| Neuron_Cajal-Retzius | 552 | 4,363 | 7.9 |
| Neuron_Excit_AON | 1,939 | 8,190 | 4.2 |
| Neuron_Excit_Indeterminate | 3,660 | 6,377 | 1.7 |
| Neuron_Excit_L2-4 | 9,083 | 29,465 | 3.2 |
| Neuron_Excit_L5 | 5,544 | 26,437 | 4.8 |
| Neuron_Excit_L6 | 1,436 | 5,169 | 3.6 |
| Neuron_Granule_DG | 535 | 1,674 | 3.1 |
| Neuron_Inhibitory | 2,409 | 6,564 | 2.7 |
| Oligo_Mature | 2,740 | 1,729 | 0.6 |
| Oligo_NewlyForming | 959 | 674 | 0.7 |
| Oligo_Progenitor | 504 | 477 | 0.9 |
| Vascular_endothelial | 196 | 69 | 0.4 |
| Vascular_meningeal | 227 | 275 | 1.2 |

IPC: insertions per cell.

Table S4: Oligonucleotides referenced in this work, related to STAR Methods

| Name | Sequence | Purification | Notes |
|---------------------------------|--------------------------------------------------------------------------------------|-----------------|-------------------------------------------------------------------------------------------------------|
| SMART_dT18VN | AAGCAGTGGTATCAACGCAGAGTACGTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTVN | Standard desalt | RT primer for bulk RNA calling card recovery |
| SMART | AAGCAGTGGTATCAACGCAGAGT | Standard desalt | PCR primer for bulk RNA calling card amplification |
| SRT_PAC_F1 | CAACCTCCCCTTCTACGAGC | Standard desalt | Puromycin marker in SRT |
| SRT_tdTomato_F1 | TCCTGTACGGCATGGACGAG | Standard desalt | tdTomato marker in SRT |
| Raff_ACTB_F | CCTCGCCTTTGCCGATCCG | Standard desalt | Human ACTB primer (for RT control) |
| Raff_ACTB_R | GGATCTTCATGAGGTAGTCAGTCAGGTCC | Standard desalt | Human ACTB primer (for RT control) |
| OM-PB-ACG | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTACGTTTACGCAGACTATCTTTCTAG | Standard desalt | For use with <i>piggyBac</i> SRTs |
| OM-PB-CTA | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCTATTTACGCAGACTATCTTTCTAG | Standard desalt | For use with <i>piggyBac</i> SRTs |
| OM-PB-GAT | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGATTTTACGCAGACTATCTTTCTAG | Standard desalt | For use with <i>piggyBac</i> SRTs |
| OM-PB-TGC | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTGCTTTTACGCAGACTATCTTTCTAG | Standard desalt | For use with <i>piggyBac</i> SRTs |
| OM-PB-TAG | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTAGTTTACGCAGACTATCTTTCTAG | Standard desalt | For use with <i>piggyBac</i> SRTs |
| OM-PB-ATC | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTATCTTTTACGCAGACTATCTTTCTAG | Standard desalt | For use with <i>piggyBac</i> SRTs |
| OM-PB-CGT | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCGTTTTTACGCAGACTATCTTTCTAG | Standard desalt | For use with <i>piggyBac</i> SRTs |
| OM-PB-GCA | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGCATTTTACGCAGACTATCTTTCTAG | Standard desalt | For use with <i>piggyBac</i> SRTs |
| OM-SB-ACG | AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTACGTAAGTGTATGTAAACTTCCGACTTCAA | Standard desalt | For use with <i>Sleeping Beauty</i> SRTs |
| OM-SB-CTA | AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTCTATAAGTGTATGTAAACTTCCGACTTCAA | Standard desalt | For use with <i>Sleeping Beauty</i> SRTs |
| OM-SB-GAT | AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTGATTAAGTGTATGTAAACTTCCGACTTCAA | Standard desalt | For use with <i>Sleeping Beauty</i> SRTs |
| OM-SB-TGC | AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTTGCTAAGTGTATGTAAACTTCCGACTTCAA | Standard desalt | For use with <i>Sleeping Beauty</i> SRTs |
| OM-SB-TAG | AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTTAGTAAGTGTATGTAAACTTCCGACTTCAA | Standard desalt | For use with <i>Sleeping Beauty</i> SRTs |
| OM-SB-ATC | AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTATCTAAGTGTATGTAAACTTCCGACTTCAA | Standard desalt | For use with <i>Sleeping Beauty</i> SRTs |
| OM-SB-CGT | AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTCGTTAAGTGTATGTAAACTTCCGACTTCAA | Standard desalt | For use with <i>Sleeping Beauty</i> SRTs |
| OM-SB-GCA | AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTGCATAAGTGTATGTAAACTTCCGACTTCAA | Standard desalt | For use with <i>Sleeping Beauty</i> SRTs |
| N7 indexed primer | CAAGCAGAAGACGGCATAACGAGAT [index]GTCTCGTGGGCTCGG | Standard desalt | Uniquely identifies each bulk RNA calling card library in conjunction with barcoded transposon primer |
| 10x_TSO | AAGCAGTGGTATCAACGCAGAGTACATrGrGrG | Standard desalt | For continuing 10x scRNA-seq prep after splitting first RT product in half |
| Bio_Illumina_Seq1_scCC_10X_3xPT | /5Phos/ACACTCTTTCCC/iBiodT/ACACGACGCTCTTCCGA*T*C*T | HPLC | Single cell calling card primer for use with 10x Chromium 3' v2 kit |
| Bio_Long_PB_LTR_3xPT | /5Phos/GCGTCAATTTTACGCAGAC/iBiodT/ATCTTTC*T*A*G | HPLC | Single cell calling card primer for use with <i>piggyBac</i> SRTs |

| | | | |
|---------------------|-------------------------------------------------------------------------|-----------------|------------------------------------------------------------------------------------------|
| scCC_P5_adapter | AATGATACGGCGACCACCGAGATCTTCACTCATTCCACACGACTCCTTGCCAGTCTC*T | Standard desalt | Adapter for scCC (needs to be pre-annealed with scCC_P7_adapter) |
| scCC_P7_adapter | /5Phos/GAGACTGGCAAGTACACGTCGCACTCACCATGA[index]ATCTCGTATGCCGCTTTCTGCTTG | Standard desalt | Adapter for scCC (needs to be pre-annealed with scCC_P5_adapter) |
| scCC_P5_primer | AATGATACGGCGACCACCGAGATC | Standard desalt | For final scCC library PCR |
| scCC_P7_primer | CAAGCAGAAGACGGCATAACGAGAT | Standard desalt | For final scCC library PCR |
| scCC_PB_CustomRead2 | CGTGTAGGGAAAGAGTGTGCGTCAATTTTACGCAGACTATCTTTCTAG | PAGE | For custom sequencing of <i>piggyBac</i> scCC libraries; read 2 should begin with GGTTAA |
| scCC_CustomIndex1 | GAGACTGGCAAGTACACGTCGCACTCACCATGA | PAGE | For custom sequencing of scCC libraries |
| ACTB_PrimerBank_F | CATGTACGTTGCTATCCAGGC | Standard desalt | For qRT-PCR |
| ACTB_PrimerBank_R | CTCCTTAATGTCACGCACGAT | Standard desalt | For qRT-PCR |
| CD24_PrimerBank_F | CTCCTACCCACGCAGATTTATTC | Standard desalt | For qRT-PCR |
| CD24_PrimerBank_R | AGAGTGAGACCACGAAGAGAC | Standard desalt | For qRT-PCR |
| MYC_PrimerBank_F | GTCAAGAGGCGAACACACAAC | Standard desalt | For qRT-PCR |
| MYC_PrimerBank_R | TTGGACGGACAGGATGTATGC | Standard desalt | For qRT-PCR |
| BRD2_PrimerBank_F | AATGGCACAAACGCTGGAAAA | Standard desalt | For qRT-PCR |
| BRD2_PrimerBank_R | CACTGGTAACACTGCCCTG | Standard desalt | For qRT-PCR |
| BRD3_PrimerBank_F | TGCAAGCGAATGTATGCAGGA | Standard desalt | For qRT-PCR |
| BRD3_PrimerBank_R | CATCTGGGCCACTTTTGTAGAA | Standard desalt | For qRT-PCR |
| BRD4_PrimerBank_F | GAGCTACCCACAGAAGAAACC | Standard desalt | For qRT-PCR |
| BRD4_PrimerBank_R | GAGTCGATGCTTGAGTTGTGTT | Standard desalt | For qRT-PCR |
| BRD4 CRISPRi gRNA | GCGGCTGCCGGCGGTGCCCG | N/A | For knockdown of BRD4 with CRISPRi |
| NT CRISPRi gRNA | GGAGGCGAGGTAAGACGCGG | N/A | Control non-targeting gRNA for CRISPRi |