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SUPPLEMENTARY INFORMATION

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Single-nucleus analysis of accessible chromatin in developing mouse forebrain reveals cell-type-specific transcriptional regulation

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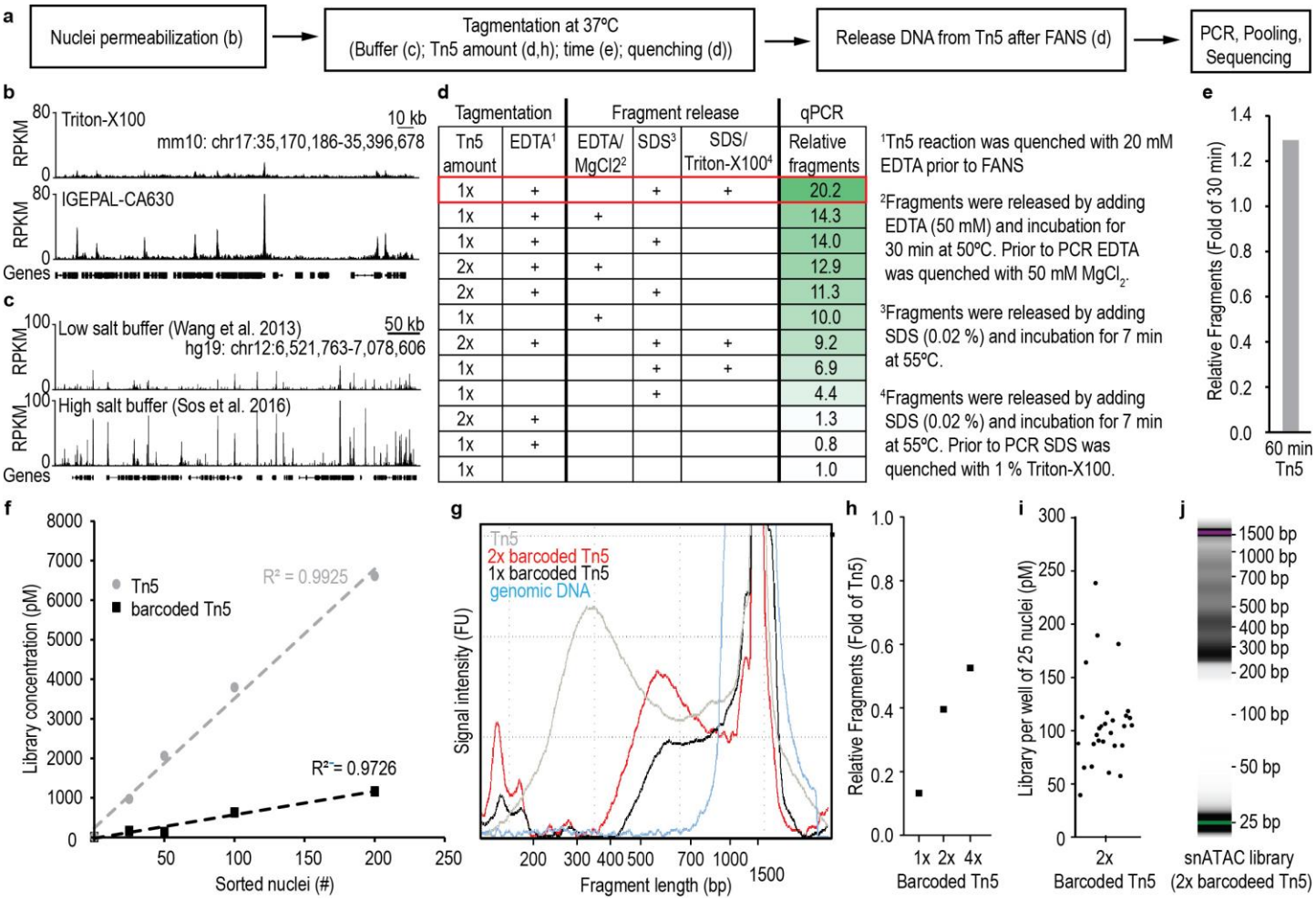

Supplementary Figure 1

snATAC-seq protocol optimization.

a. Overview of critical steps for the snATAC-seq procedure for nuclei from frozen tissues. **b.** IGEPAL-CA630 but not Triton-X100 was sufficient for tagmentation of frozen tissues (n = 1 experiment). **c.** Tagmentation was facilitated by high salt concentrations in reaction buffer (n = 1 experiment; Wang, Q. *et al. Nature protocols, 2013,* doi:10.1038/nprot.2013.118: Sos, B. C. *et al. Genome biology*, 2016, doi:10.1186/s13059-016-0882-7). **d.** Maximum amount of fragments per nucleus could be recovered when quenching Tn5 by EDTA prior to FANS and denaturation of Tn5 after FANS by SDS. Finally, SDS was quenched by Triton-X100 to allow efficient PCR amplification. **e.** Increasing tagmentation time from 30 min to 60 min can result in more DNA fragments per nucleus (n = 1 experiment). **f.** Number of sorted nuclei was highly correlated with the final library concentration. Tn5 loaded with barcoded adapters showed less efficient tagmentation as compared to Tn5 without barcodes. Wells were amplified for 13 cycles, purified and libraries quantified by qPCR using standards with known molarity (n = 1 experiment). **g.** Tagmentation with barcoded Tn5 was less efficient and resulted in larger fragments than Tn5 (550 bp vs. 300 bp). Ratio for barcoded Tn5 was based on concentration of regular Tn5. **h.** Doubling the concentration of barcoded Tn5 increased the number of fragments per nucleus by 3 fold. Further increase resulted only in minor improvements (n = 1 experiment). **i.** Dot blot illustrating the amount of library from 25 nuclei per well. Each well was amplified for 11 cycles and quantified by qPCR. This output was used to calculate the number of required PCR cycles for snATAC-seq libraries to prevent overamplification (n = 28 wells). **j.** Size distribution of a successful snATAC-seq library from a mixture of E15.5 forebrain and GM12878 cells shows a nucleosomal pattern. SnATAC-seq was performed including all the optimization steps described above with barcoded Tn5 in 96 well format (n = 1 experiment; snATAC libraries for forebrain samples showed comparable nucleosomal patterns: n = 16 experiments).