**Additional experimental sample processing details**

* Fixation using formaldehyde prior to lysis: Different concentrations of formaldehyde, 0% (no fixation), 0.1% and 1% were tested and compared within the scope of experiments presented in new **Figure 1** and **Extended Data Figure 1**. Fixation time was kept constant at 10min at room temperature. We did observe 1% formaldehyde to show minimal cross contamination of mtDNA, to not introduce mutational artifacts and yield high quality accessible chromatin data. Aside from **Figure 1** and **Extended Data Figure 1**, where respective deviations have been appropriately labeled, all data generated in this manuscript relied on 1% formaldehyde fixation for 10 min at room temperature.
* Lysis buffer: We tested different lysis buffers where the base composition was kept constant (10mM Tris-HCL pH 7.4, 10mM NaCl, 3mM MgCl2, 1% BSA), but detergent content was varied. Specifically, we compared the so called Omni-ATAC conditions (Corces *et al.*, Nature Methods 2017, doi:10.1038/nmeth.4396) containing 0.1% NP40, 0.1% Tween20 and 0.01% Digitonin in the lysis buffer (abbreviated Omni) to the buffer that we recommend containing only 0.1% NP40 (abbreviated NP40) as used in one of the original descriptions of the ATAC-seq protocol, that yielded a substantially higher fraction of reads mapping to mtDNA (as also used in Ludwig *et al.*, Cell 2019, doi:10.1016/j.cell.2019.01.022). These comparisons were conducted in the experiments described in **Figure 1** and **Extended Data Figure 1**. Specific samples are labeled using the abbreviations Omni and NP40. Aside from **Figure 1** and **Extended Data Figure 1**, all data generated in this manuscript relied on 0.1% NP40 lysis.
* Lysis time: 3min on ice for PBMCs and all primary cells throughout the manuscript. Time was increased to 5 min on ice for cell lines as recommended by 10x Genomics.
* Wash buffer: The base composition of the buffer was kept constant (10mM Tris-HCL pH 7.4, 10mM NaCl, 3mM MgCl2, 1% BSA), and the effects of absent or addition of 0.1% Tween20 were then compared in experiments presented in **Figure 1** and **Extended Data Figure 1**. Consistent with previous observations, the addition of Tween20 significantly reduced the yield of reads mapping to mtDNA (Corces *et al*., Nature Methods 2017, doi:10.1038/nmeth.4396). Aside from **Figure 1** and **Extended Data Figure 1**, where the absence or addition of Tween20 has been indicated, all data generated in this manuscript relied on a wash buffer that contained no Tween20.
* Decrosslinking time after GEM generation prior to the PCR 72C elongation step. To supposedly facilitate decrosslinking of fixed DNA we tested the effects of introducing a 60C decrosslinking step, the effect of which was compared in **Figure 1b,c** and **Extended Data Figure 1a,b**. However, this did not appear to significantly affect single cell library complexities. Presumably, the high temperatures during PCR, varying from 59-98C are sufficient to facilitate decrosslinking. Given the generally very good results, we did not further test variations of this parameter. Aside from these experiments that are labeled as such, we used the regular PCR conditions as recommended by 10x Genomics data, including for all primary cell data presented in the manuscript. No additional modifications were made to the following library preparation steps.