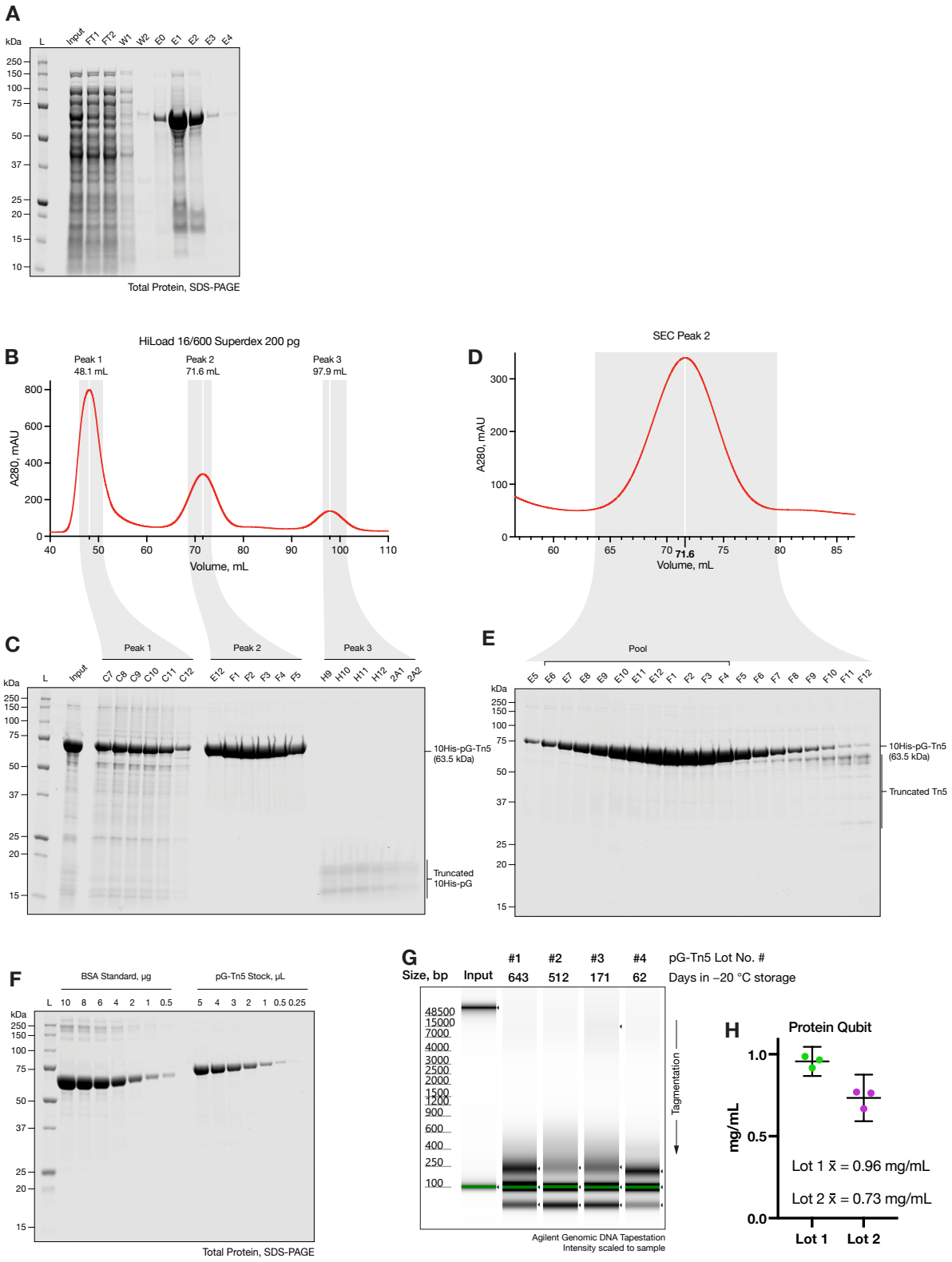

Figure S1: Legend on next page

Figure S1: Overview of pG-Tn5 purification, validation of stock concentration, stability testing. A. Profiling of total protein content from His-tag affinity purification fractions, InstantBlue stained SDS-PAGE. **B.** A280 profile of 10His-pG-Tn5 size exclusion chromatography (SEC) carried out on pooled His-tag affinity eluate fractions from panel A above, on HiLoad 16/600 Superdex 200 pg column using an ÄKTA FPLC instrument. **C.** SDS-PAGE analysis of highlighted SEC peak fractions corresponding to 10His-pG-Tn5 containing heterogeneous aggregates (peak 1), 10His-pG-Tn5 homodimer (peak 2) and truncated 10His-pG tag (peak 3). **D.** Detail of SEC peak 2, from the same purification run as panel B. **E.** SDS-PAGE of fractions corresponding to the highlighted region of SEC peak 2, showing contaminating 10His-pG-Tn5:Tn5 heterodimers containing truncated Tn5, which migrate slower than full length 10His-pG-Tn5 homodimer. SEC fractions containing pure, full-length 10His-pG-Tn5 homodimer chosen for pooling to generate stock are highlighted. **F.** Validation of protein concentration of 1 mg/mL 10His-pG-Tn5 final stock by dilution series against a BSA standard, analyzed by titration on SDS-PAGE with InstantBlue total protein staining. **G.** Assaying 10His-pG-Tn5 stock tagmentation activity after indicated time stored at –20 °C, showing negligible loss of λ gDNA tagmentation activity at 21 months. Equal volumes of stock were used to assemble 20 µM adapter loaded transposomes. **H.** Quantification of protein concentration of pG-Tn5 stock lots 1 and 2, demonstrating that the tagmented DNA product size is largely attributable to the variation in stock protein concentration.

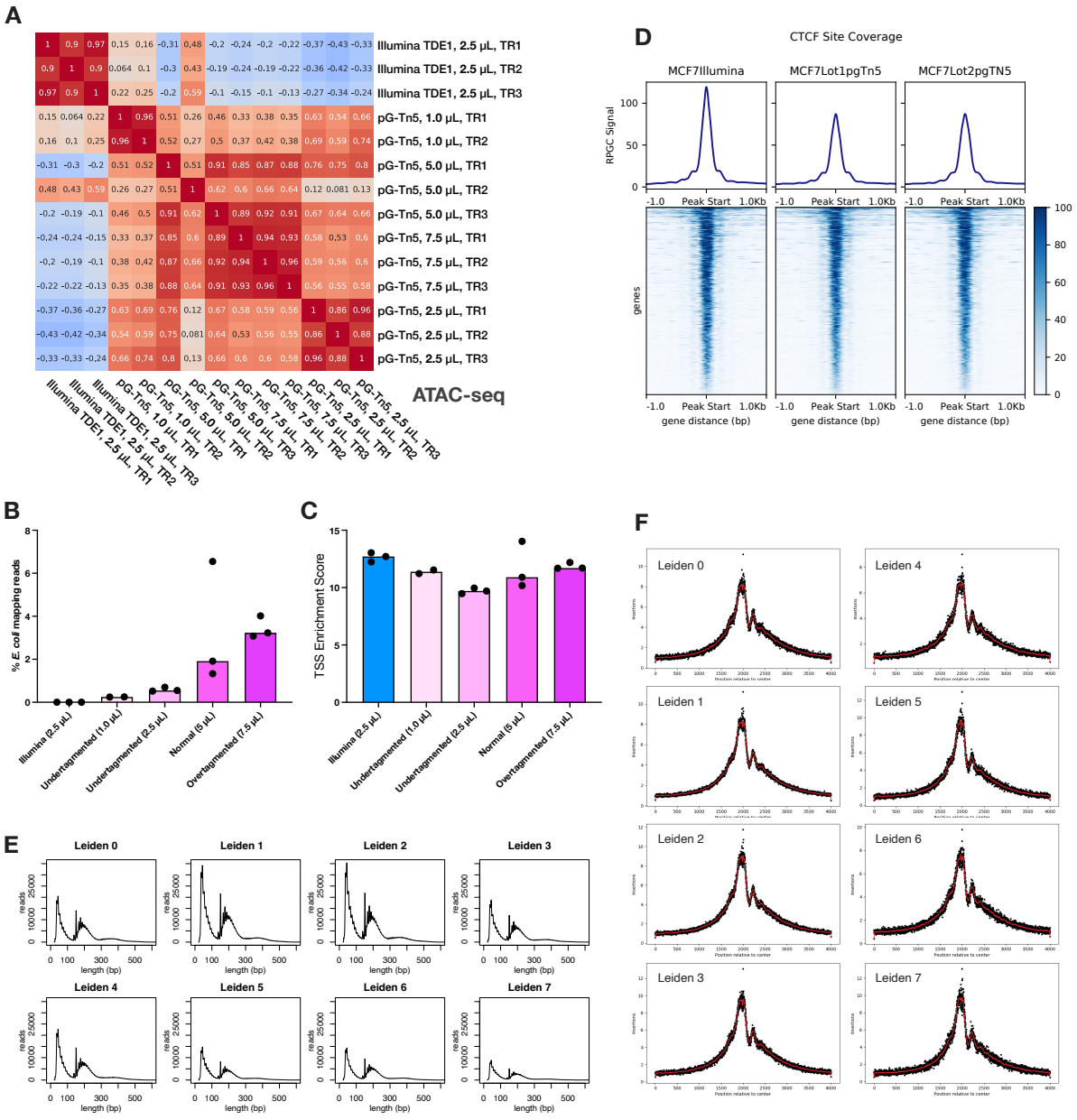

Figure S2: Validating pG-Tn5 in bulk and single-cell ATAC-seq performance using differing transposome volumes, bulk ATAC-seq coverage over individual CTCF sites, and EasySci-ATAC data quality metrics. A. Spearman rank correlation of ATAC-seq libraries generated in human K562 cells using different volumes of pG-Tn5 transposome, as compared to standard OmniATAC-seq protocol 2.50 µL volume of Illumina TDE1, calculated over 1 kb bins genome-wide. **B.** Relationship between pG-Tn5 transposome volume used per ATAC-seq reaction and % library reads aligning to *E. coli* genome, originating from carryover during purification, data from same experiment as in panel A above. **C.** Transcription start site (TSS) enrichment score of ATAC-seq libraries generated with Illumina TDE1 or differing pG-Tn5 transposome volumes, data from the same experiment as in panel A above. **D.** Heatmap of ATAC-seq signal coverage of individual CTCF-occupied sites in human MCF7 cells, as in Figure 1D-F. **E.** Fragment length distribution (FLD) plots of EasySci-ATAC individual Leiden annotations. **F.** Transcription start site (TSS) enrichment score plots of EasySci-ATAC individual Leiden annotations.