

1.1 Supplementary Figure S1: Chromatin accessibility variation can exist even if total accessibility is the same between cells.

Measurements of an ensemble of cells can show similar total level of accessibility within cells (*i.e.* comparable number of accessible sites in a cell) yet accessibility can occur at non-overlapping regulatory elements. In this hypothetical case, red represents an accessible sequence in a given cell, and blue represents an inaccessible sequence. Each cell has 3 accessible sequences (red) total, or a total accessibility of 3. Thus, the total accessibility is the same between cells. But each cell is accessible at completely different DNA sequences, which may have different functions. Existing algorithms, such as chromVAR and Buenrostro *et.al* (2015)'s workflows, are built on the standard deviation of total accessibility, hence they cannot measure variation in these cases.

1.2 Supplementary Figure S2: PRISM outperforms chromVAR for multiple values of peak number for background peaks.

PRISM outperforms chromVAR when 40 or 50 background peaks are selected in calculating variability in mouse forebrain tissue, mouse double-positive T cells and human AML cells.

1.3 Supplementary Figure S3: PRISM outperforms chromVAR under subtype B when cells with low chromatin accessibility are selected.

PRISM outperforms chromVAR under subtype B when cells with low chromatin accessibility are selected in mouse double-positive T cells and human AML cells.