Supplementary Figure 2. Stages of gating involved in the PIGA mutation assay.

Cells analysed for PIGA mutation were gated by a number of steps to assure the detection of true PIGA mutants and minimal false-negative events. (A) Gating of cells based on Forward Scatter and Side Scatter to exclude cell clumps and cell debris. (B) Gating of cells with CD19-APC antibody, a B-cell surface marker as a positive control for successful antibody binding. (C) Gating of cells based on the exclusion of propidium iodide to exclude dead cells. (D) Gating of cells for CD55-PE and CD59-PE, PIGA-anchored cell surface proteins. The geometric mean of fluorescence in the FL-2 channel where CD55-PE and CD59-PE were detected (Y axis) was calculated and the threshold point for calculating PIGA mutants was defined as any events that had less than 4% of the geometric mean of the average of the whole population were considered to be PIGA mutants (boxed in panel D). (E) MT1 cells were used as a positive control for the PIGA assay. MT1 is a derivative of TK6 cells and have a significantly elevated mutation rate due to compound heterozygous mutations in the MSH6 DNA mismatch repair gene. Cells were gated and PIGA mutant cells determined as described in panels A-D.