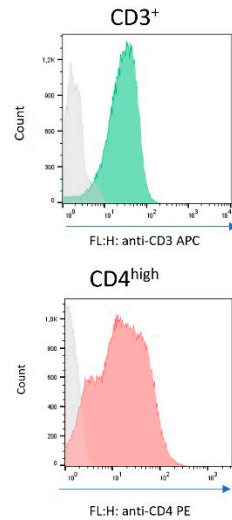
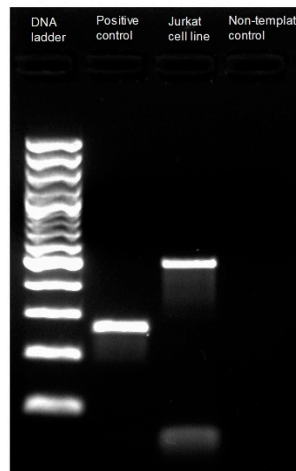


## Suppl. Fig. 1.

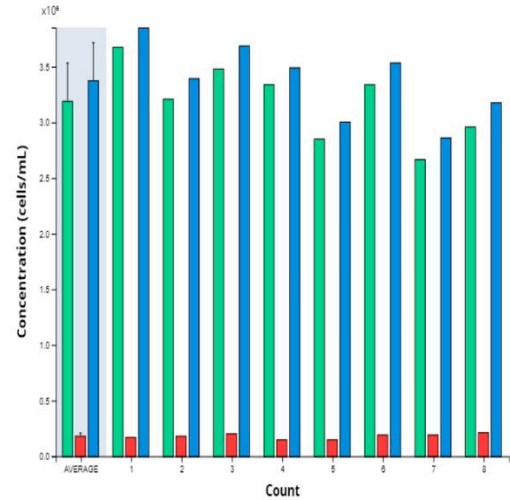
A. Jurkat T cell markers



B. Mycoplasma test



C. Post-transfection viability

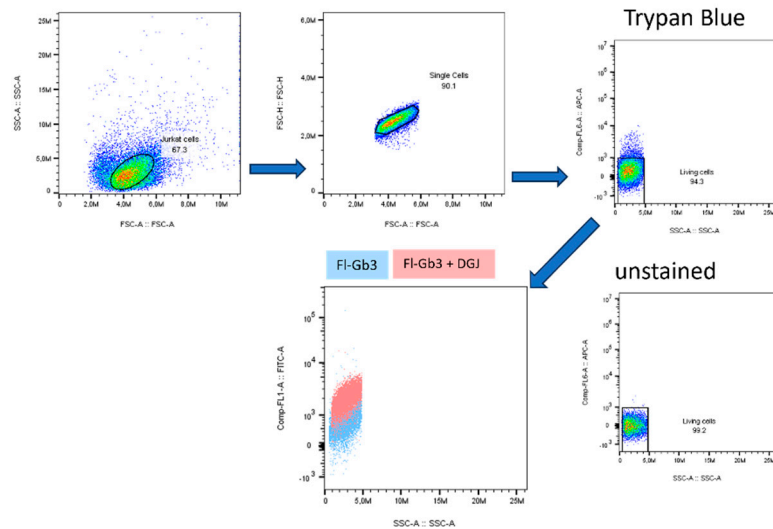


**Suppl.Fig.1.** Pre-transfection characterization of Jurkat cells and post-transfection viability of *GLA* KO Jurkat cells. **A.** Jurkat cells expressed CD3 and CD4 cell surface markers **B.** Jurkat cells were not contaminated by Mycoplasma spp. **C.** The viability of transfected *GLA* KO Jurkat cells 24 hours after transfection was above 95% (green bar- concentration of viable cells, red bar- concentration of dead cells, blue line – concentration of cell count). KO – knockout.

## Suppl. Fig. 2.

### Gating strategy

#### A. Gating strategy



**Suppl.Fig.2.** Gating strategy and effect of trypan blue quenching for AGAL enzymatic activity measurement. A. Morphologically intact Jurkat cells were gated, followed by single cell gate, afterwards a third gate of living cells (trypan blue negative cells) was applied. Enzymatic activity detection is based on the fluorescent channel 1 (FITC) signal intensity difference between fluorescently labelled globotriaosyl-ceramide (FI-Gb3) incubated cells and reversible AGAL inhibitor containing FI-Gb3 incubated cells. AGAL – alpha galactosidase A, FI-Gb3 – fluorescently labelled globotriaosyl-ceramide, DGJ - 1-deoxygalactonojirimycin.