cancers

Supplementary Materials: Anti-apoptotic c-FLIP reduces the anti-tumour activity of chimeric antigen receptor T cells

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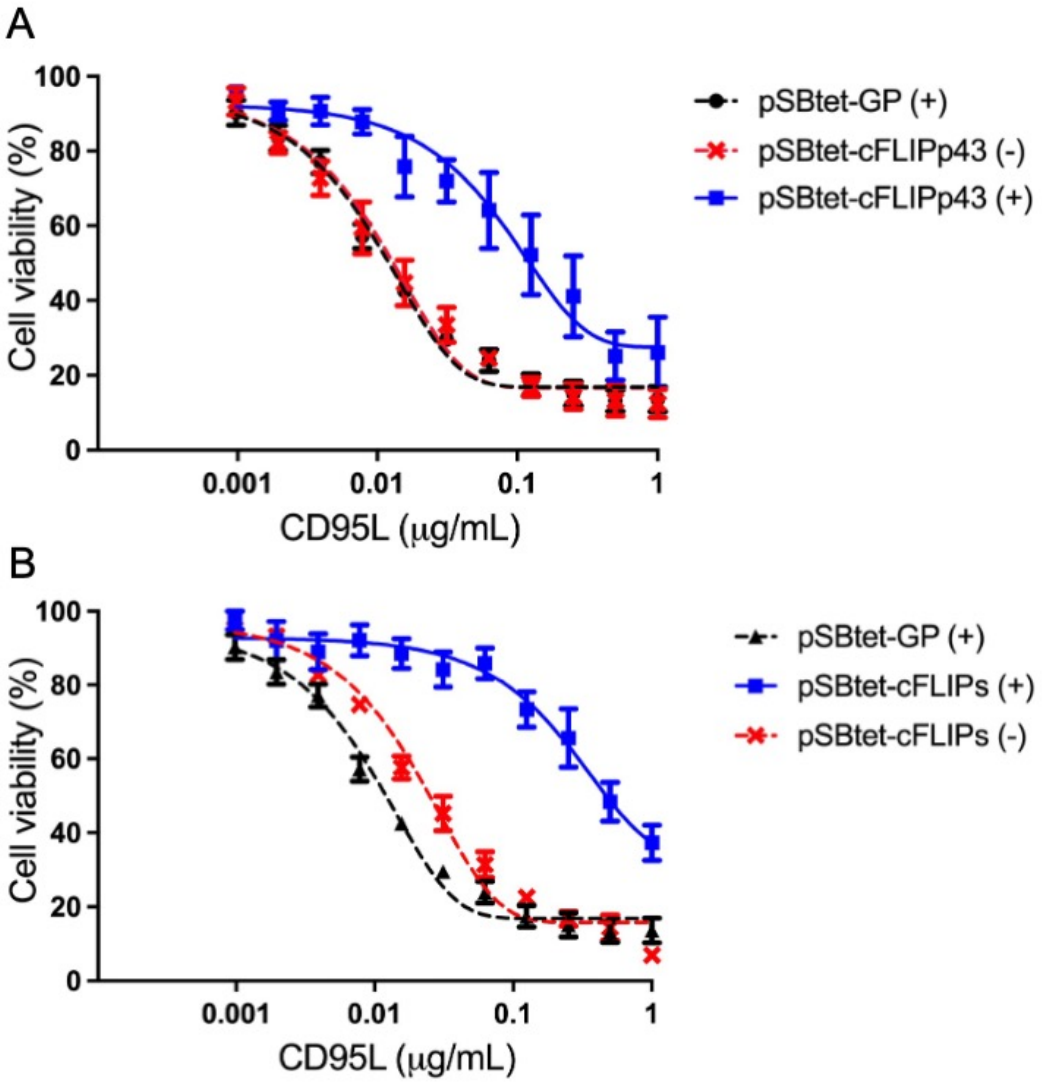

Figure S1. Tet-On induction of c-FLIP protects Jurkat T cells against cell-death induced by CD95L challenge. Jurkat cells were transposed with Tet-On pSB-Tet-On [1,2] constructs using the SB100X transposase vector containing no insert (pSB-GP) or the same vector with the indicated insert. Stable cell lines were generated using puromycin selection. Stably transfected Jurkat cells expressing c-FLIP isoforms with (+) or without (-) induction with 5 µg/mL doxycycline treatment were challenged with 500 ng/mL of LZ-CD95L for 24h to mimic AICD. Cell viability was determined by resazurin assay [3]. Cells were incubated with resazurin solution and quantified as previously described [3]. IC_{50} curve were plotted as relative cell viability percentage against untreated cells. Error bars represent mean ± SEM of triplicate samples (n=3).

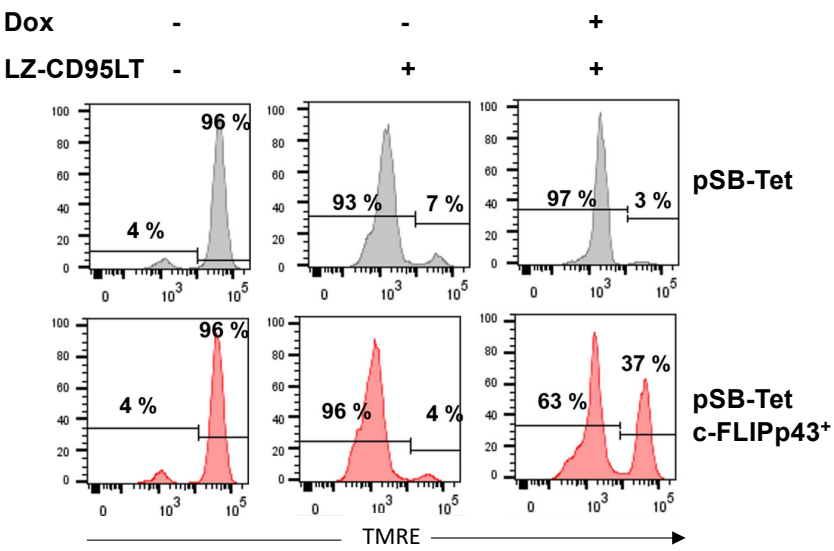

Figure S2. Tet-On induction of c-FLIP protects Jurkat T cells against mitochondrial depolarisation mediated by CD95L challenge. Jurkat cells were transfected with Tet-On pSB-Tet-On [1,2] constructs and selected using puromycin. Stably transfected Jurkat cells expressing c-FLIP with (+) or without (-) induction with 5 μg/mL doxycycline treatment were challenged with 500 ng/mL of LZ-CD95L for 24h to mimic AICD. Cell viability was determined by TMRE assay [3]. Cells were stained with TMRE as previously described [4,5]. Histograms displays cell count of GFP^+ gated cells TMRE staining (x-axis). $TMRE^+$ events (right gate) represent cells with high Ψ_M while $TMRE^-$ (left gate) are cells with depolarised mitochondria. Flow charts are a representative of three independent repeats.

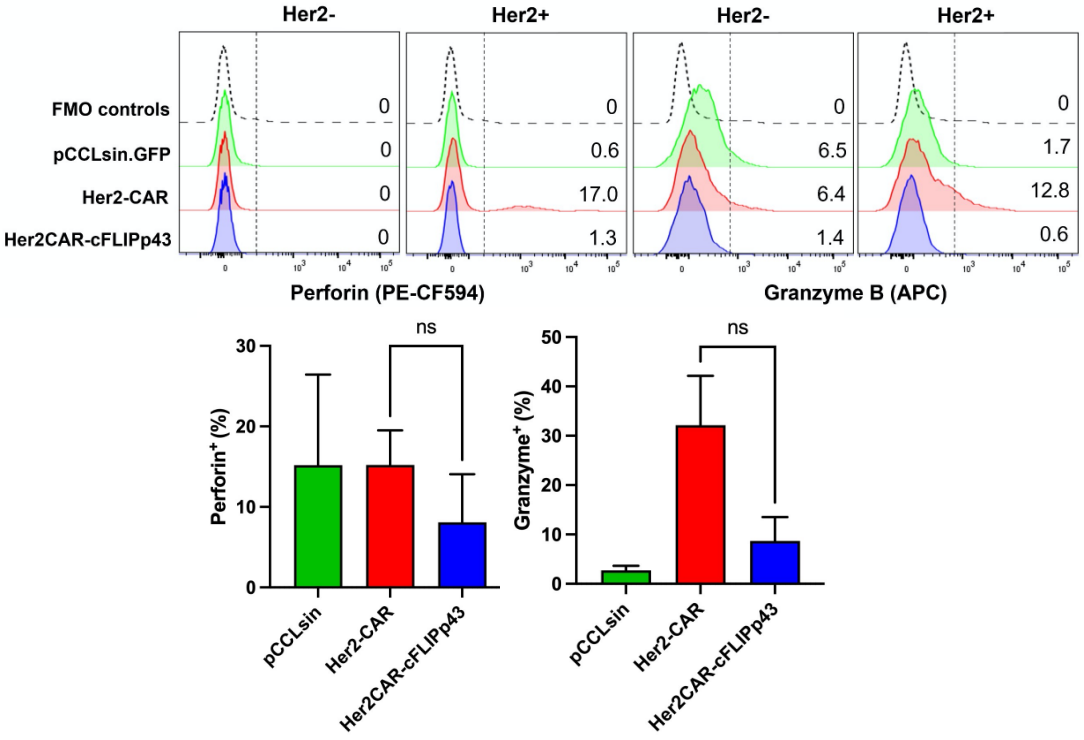

Figure S3. Perforin and granzyme B production of c-FLIP-expressing CAR T cells. Negative control, pCCLsin, CAR control Her2-CAR or Her2CAR-cFLIPp43 transduced primary human T cells were cocultured at a 2:1 ratio for six hours with Her2-target MCF-7 with four final hours of Brefeldin A treatment. The cells were fixed, and intracellularly stained with perforin and granzyme B antibody for flow cytometric analysis. Representative donor flow plots (n=3) of cell count versus perforin or granzyme B with Her2+ antigen and without Her2+ antigen. (lower). Bar graph values represent the mean values ± SEM from three independent experiments where each experiment indicates different donor (n=3). Statistical analysis: two-tailed paired t test.

References

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