SUPPLEMENTAL MATERIAL

Strauss et al., http://www.jem.org/cgi/content/full/jem.20082363/DC1

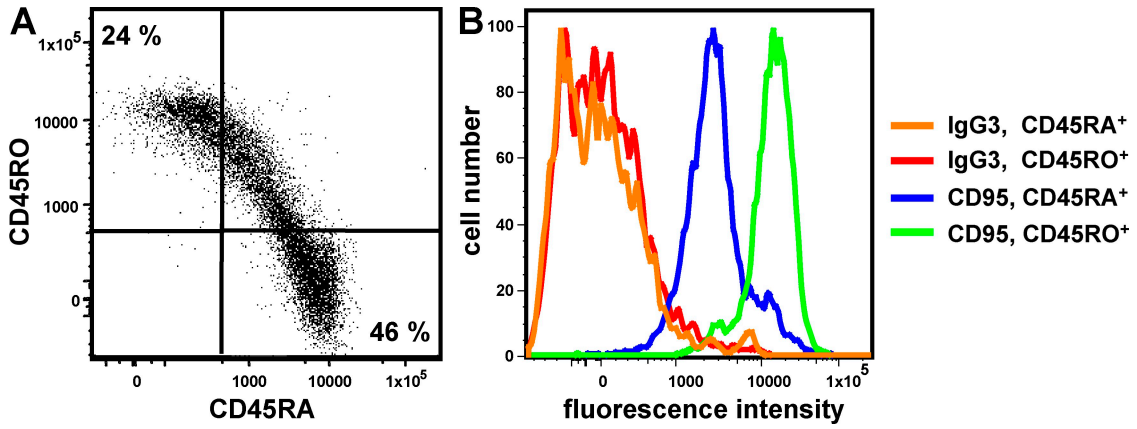

Figure S1. CD95 is up-regulated on preactivated, CD45RO$^+$ T cells. Purified T cells isolated from a buffy coat of a healthy donor were stained with CD45RA-FITC, CD45RO-PE (A), and APO-1 (anti-CD95) supernatant or IgG3 isotype control, followed by biotinylated polyclonal rabbit anti-mouse F(ab)$_2$ and streptavidin-APC. Cells were counterstained with 7-AAD to gate on live cells. Expression of CD95 and IgG3 is shown on the CD45RA$^+$ and CD45RO$^+$ T cell population (B). Experiment is representative of two experiments carried out with T cells from different donors.

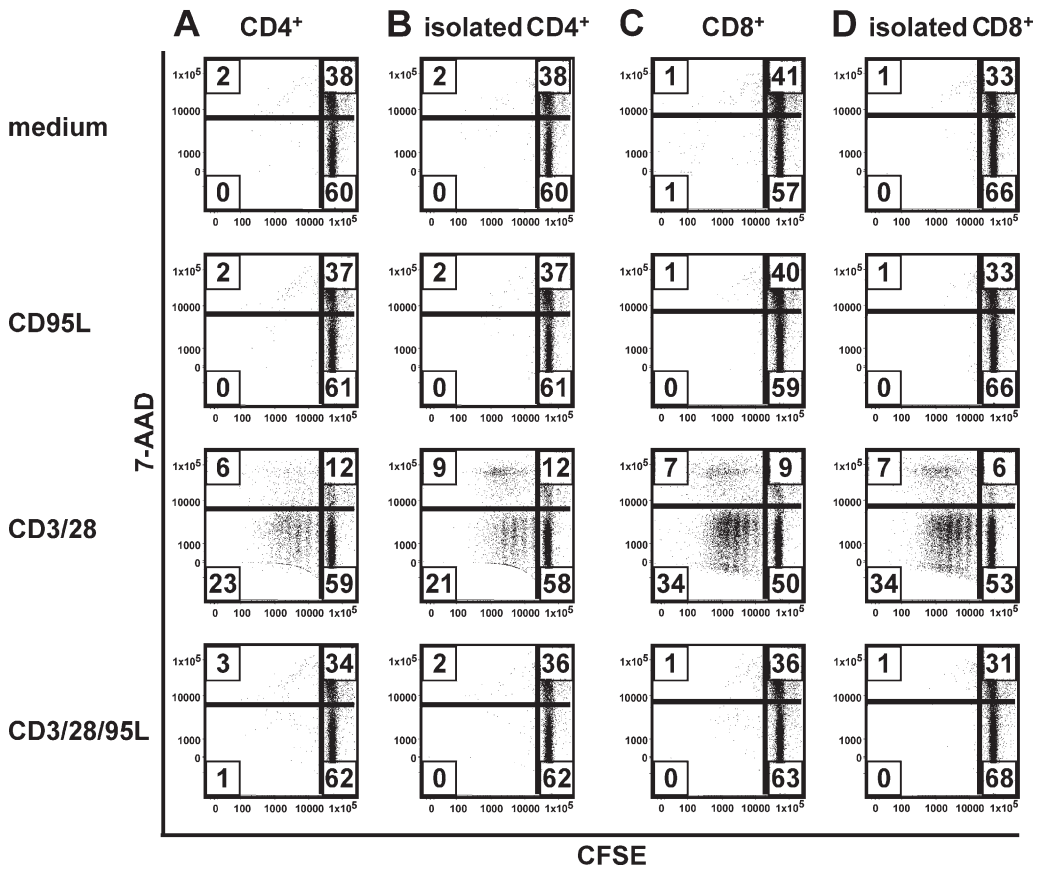

Figure S2. Costimulation of CD95 blocks T cell proliferation in isolated and co-cultured CD4+ and CD8+ T cell subsets. Naive T cells were isolated from a buffy coat of a healthy donor. Subsequently, CD4+ and CD8+ T cells were isolated from total, naive T cells by incubation with OKT8 (anti-CD8) supernatant to obtain CD4+ T cells or HP2/6 (anti-CD4) supernatant to obtain CD8+ T cells, followed by treatment with goat anti–mouse IgG magnetic beads. Total T cells (A and C) and isolated CD4+ (B) and CD8+ (D) T cells were CFSE labeled and stimulated with immobilized anti-CD3 plus anti-CD28 antibody (0.2 µg/ml) in the absence (CD3/28) or presence (CD3/28/95L) of CD95L or with CD95L or medium alone. After 6 d, naive T cells were stained with CD4-Pacific blue; CD8-PE-Cy7, the isolated CD4+ T cells, were stained with CD4-Pacific blue; and isolated CD8+ T cells were stained with CD8-PE-Cy7. Proliferation and cell death in total naive T cells was determined by gating on CD4+ (A) and CD8+ (C) T cells and compared with isolated CD4+ (B) and isolated CD8+ (D) T cells. This experiment is representative of two experiments.

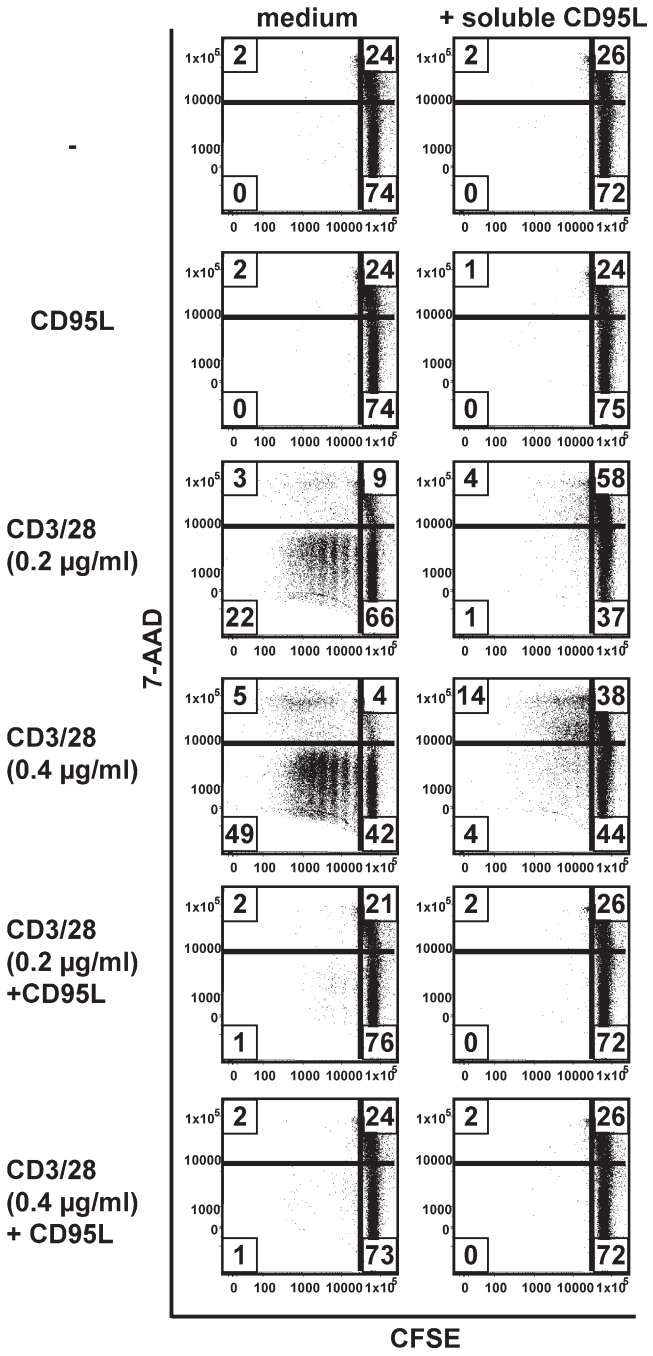

Figure S3. Soluble CD95L does not reverse the antiproliferative effect of immobilized CD95L during T cell activation. CFSE-labled naive T cells were activated with immobilized anti-CD3 and anti-CD28 antibodies (CD3/28), immobilized CD95L (CD95L; 2 µg/ml), and immobilized CD3/28 + immobilized CD95L (CD3/28 + CD95L) or left untreated (-) in the absence (medium) or presence of soluble CD95L (2 µg/ml). After 6 d, cells were harvested and stained with CD2-APC and 7-AAD, and proliferation and apoptosis induction were analyzed in the $CD2^+$ population. Experiment is representative of two experiments.

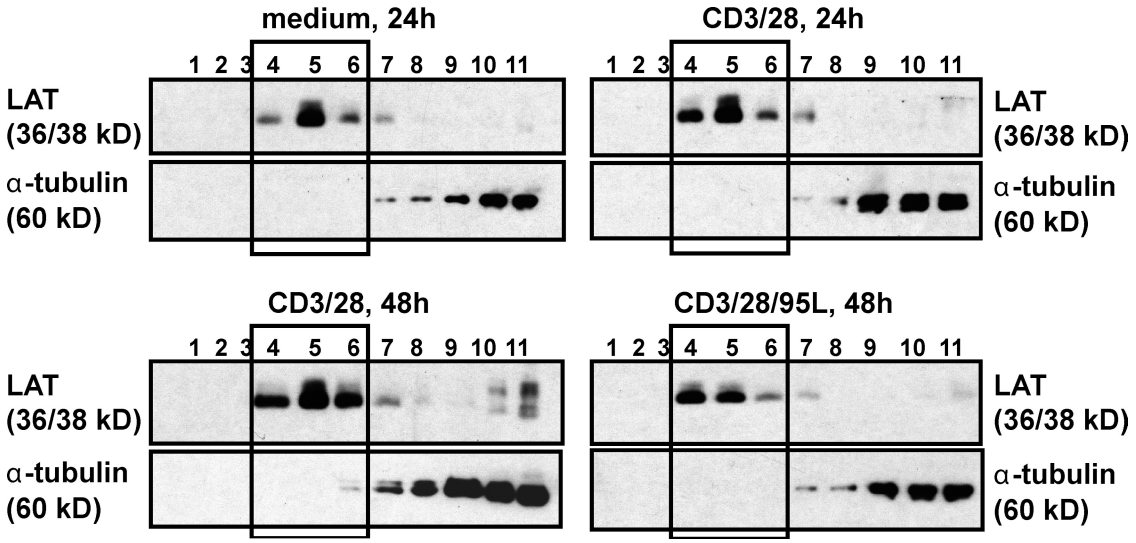

Figure S4. Determination of the raft and nonraft fraction of stimulated T cells after discontinuous sucrose gradient. Purified T cells isolated from a buffy coat of a healthy donor were left untreated (medium) or activated with immobilized anti-CD3 and -CD28 antibodies in the absence (CD3/28) or presence (CD3/28/95L) of CD95L for 24 or 48 h. Subsequently, 5×10^7 T cells were lysed and lysates were loaded on a discontinuous sucrose gradient. 11 1-ml fractions were collected after 20 h of centrifugation and 25 µl of each fraction was immediately subjected to Western blot analysis. Gels were stained for the raft marker LAT and the cytoplasmic protein α-tubulin (DM1A). The experiment is representative of three independent experiments.

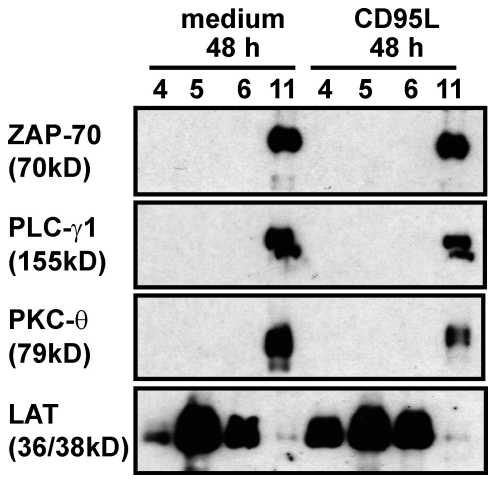

Figure S5. Untreated and CD95L–treated T cells do not exhibit differences in the recruitment of TCR-associated signal molecules into lipid rafts. Purified T cells isolated from a buffy coat of a healthy donor were left untreated (medium) or were stimulated in the presence of CD95L for 48 h. Precipitated raft (4–6) and nonraft fractions (Mitsiades, C.S., V. Poulaki, G. Fanourakis, E. Sozopoulos, D. McMillin, Z. Wen, G. Voutsinas, S. Tseleni-Balafouta, and N. Mitsiades. 2006. *Clin. Cancer Res.* 12:3705–3712) were subjected to Western blot analysis. The results are representative for one experiment out of two.

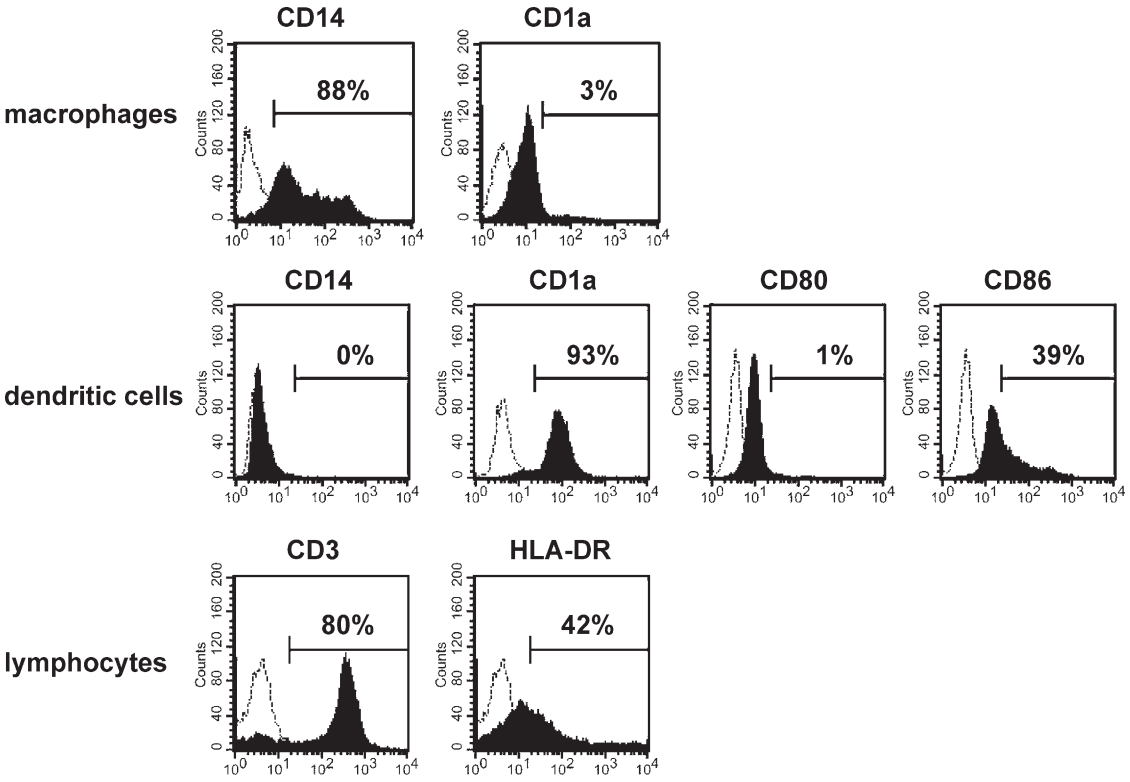

Figure S6. Phenotypic characterization of primary macrophages, DCs, and lymphocytes before infection. Flow cytometry analysis was performed in lymphocytes 3 d after PHA-activation and in DCs and macrophages 5–7 d after differentiation. Dotted lines represent staining with appropriate isotype controls. The experiment is representative of three independent experiments done on different blood donors.