Supporting Information

Hillerdal et al. 10.1073/pnas.1209042109

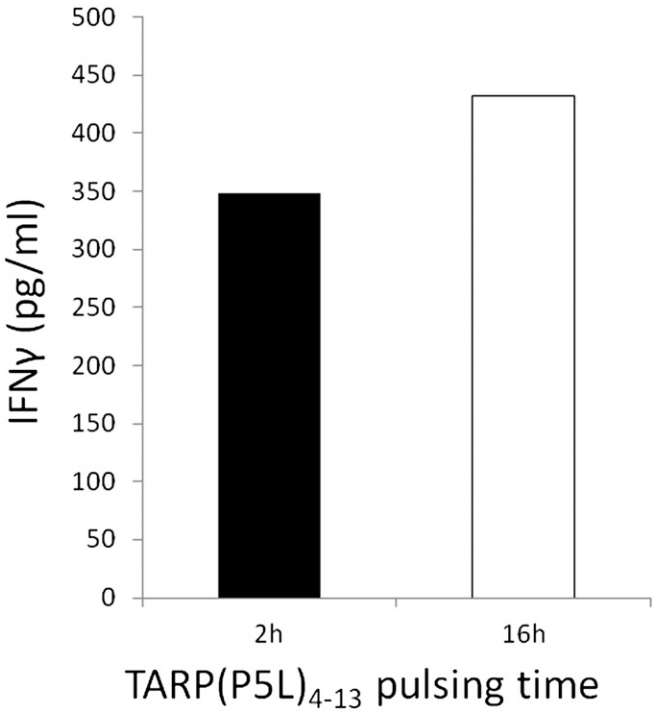

Fig. S1. IFN-γ production from T-cell receptor (TCR) γ-chain alternate reading-frame protein (TARP)-TCR–engineered T cells after coculture with peptide-pulsed T2 target cells. Antigen processing-deficient, HLA-A2$^+$ T2 cells were resuspended at 1 million cells/mL and pulsed with 50 μg/mL HLA-A2-restricted TARP $(P5L)_{4–13}$ peptide for either 2 or 16 h in presence of 3 μg/mL β2 microglobulin (Sigma-Aldrich). The target cells were then cocultured overnight at a 1:1 ratio with TARP-TCR–engineered T cells. An ELISA kit (Mabtech) was used to measure IFN-γ released into the supernatants.

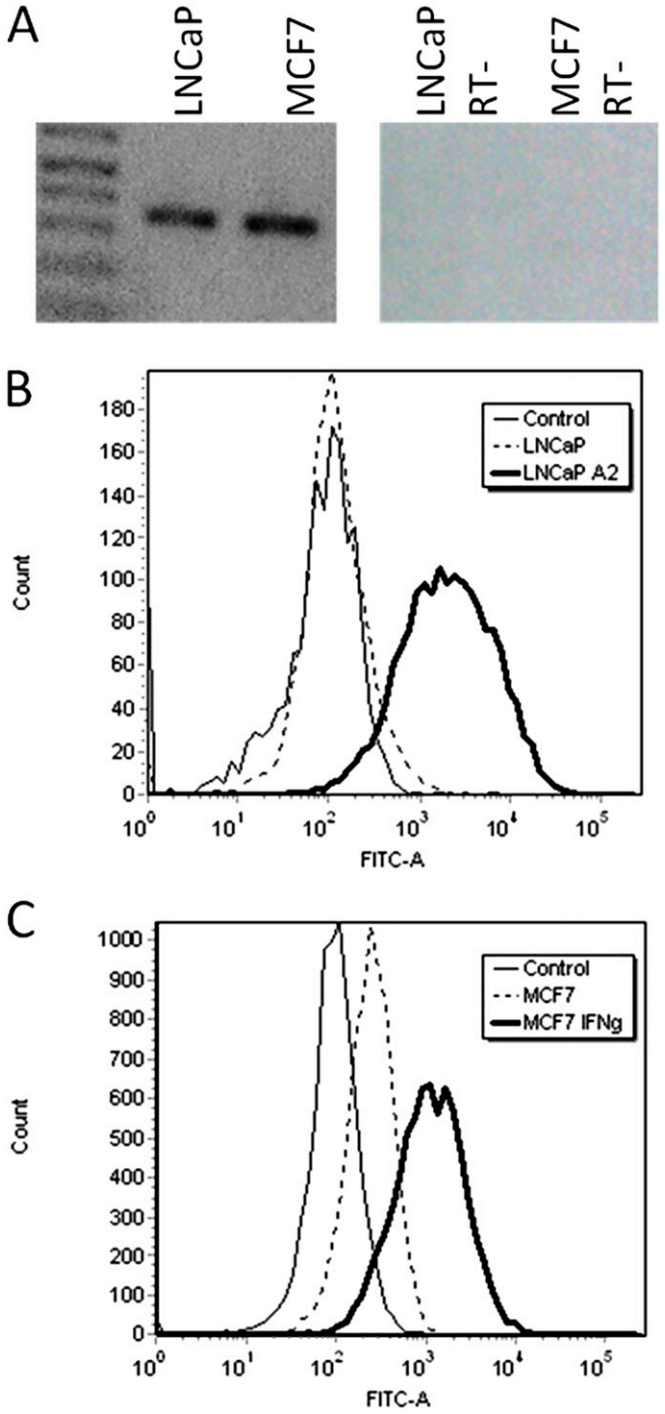

Fig. S2. TARP mRNA expression and HLA-A2 protein cell surface expression in the prostate cancer cell line LNCaP and the breast cancer cell line MCF7. (*A*) Total RNA was isolated from LNCaP and MCF7 using RNeasy Mini Kit (Qiagen) and cDNA was subsequently synthesized using SuperScript II reverse transcriptase (Invitrogen). Thirty cycles of PCR were performed with the TARP-specific primer pair: 5′-GGG AAC ACC ATG AAG ACT AAC GAC AC-3′ and 5′-TTT CTC TCC ATT GCA GCA GAA AGC CG-3′. The PCR products were analyzed on a 1.5% agarose gel. As negative controls, the same PCRs were performed leaving out the reverse transcriptase during the cDNA synthesis step (reverse transcriptase). (*B*) LNCaP cells were transduced with a lentiviral vector encoding HLA-A2. The transduced and nontransduced LNCaP cells were then evaluated for HLA-A2 expression by flow cytometry using a FITC-conjugated antibody against HLA-A2 (BioLegend). (*C*) MCF7 cells were treated with IFN-γ (1,000 IU/mL) for 72 h. The treated and nontreated MCF7 cells were then evaluated for HLA-A2 expression by flow cytometry using a FITC-conjugated antibody against HLA-A2 (BioLegend).