

Figure W1. Down-regulation of EGFR expression in A549 cells by siRNA. A549 cells were transfected with EGFR siRNA using Lipofectamine 2000 reagent following the manufacturer's instruction for 24 hours. The transfected A549 cells were lysed in RIPA buffer and separated by sodium dodecyl sulfate—polyacrylamide gel electrophoresis on a 12% acrylamide gel. After transfer to a PVDF membrane, this was incubated with anti-EGFR or anti-GAPDH primary antibody and goat anti-rabbit IgG HRP-conjugated second antibody. Antibody binding was revealed by using the Enhanced Chemiluminescent Kit.

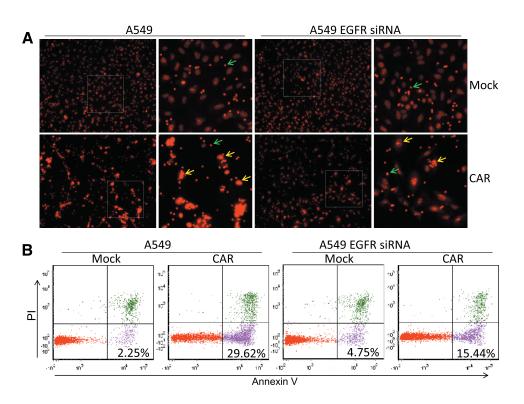


Figure W2. Visualization of the binding capacity and cytotoxicity of CAR-modified lymphocytes to tumor cells. A549 cells were transfected with EGFR siRNA using Lipofectamine 2000 reagent following the manufacturer's instruction for 24 hours. Then, the A549 cells were co-incubated with T lymphocytes. (A) Sixteen hours later, apoptosis or already dead A549 cells were identified by PI staining. A549 cells (yellow arrow) with bigger nuclei can be differed from lymphocytes (green arrow; original magnification, ×100). (B) Four hours later, the cells are double stained with annexin V and PI and detected by flow cytometry.