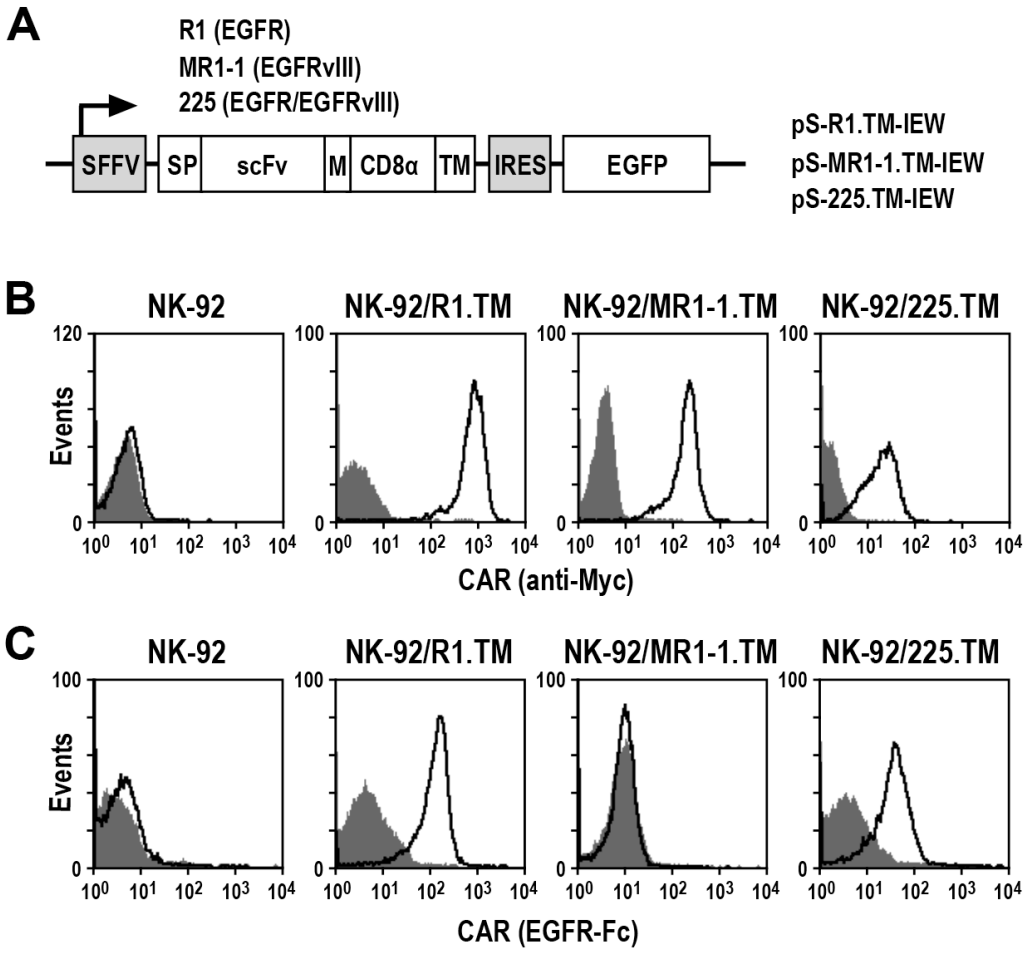

Figure S1. Generation of NK-92 cells expressing truncated chimeric antigen receptors. (**A**) Schematic representation of lentiviral transfer plasmids pS-R1.TM-IEW, pS-MR1-1.TM-IEW and pS-225.TM-IEW encoding under the transcriptional control of the Spleen Focus Forming Virus promoter (SFFV) chimeric antigen receptors which consist of an immunoglobulin heavy chain signal peptide (SP), scFv fragments derived from antibodies R1, MR1-1, or 225, followed by a Myc-tag (M), CD8α hinge region (CD8α), and the transmembrane domain of CD28 (TM). Enhanced green fluorescent protein (EGFP) cDNA separated from the CAR sequence by an internal ribosome entry site (IRES) served as a marker. (**B**) CAR surface expression on NK-92/R1.TM, NK-92/MR1-1.TM and NK-92/225.TM single cell clones was determined by flow cytometry with Myc-tag-specific antibody (open areas). CAR NK cells treated with isotype antibody (filled areas) and parental NK-92 cells treated with either antibody served as controls. (**C**) Binding of recombinant EGFR-Fc protein to the surface of CAR NK cells was measured by flow cytometry (open areas). CAR NK cells only treated with secondary antibody (filled areas) and parental NK-92 cells served as controls.

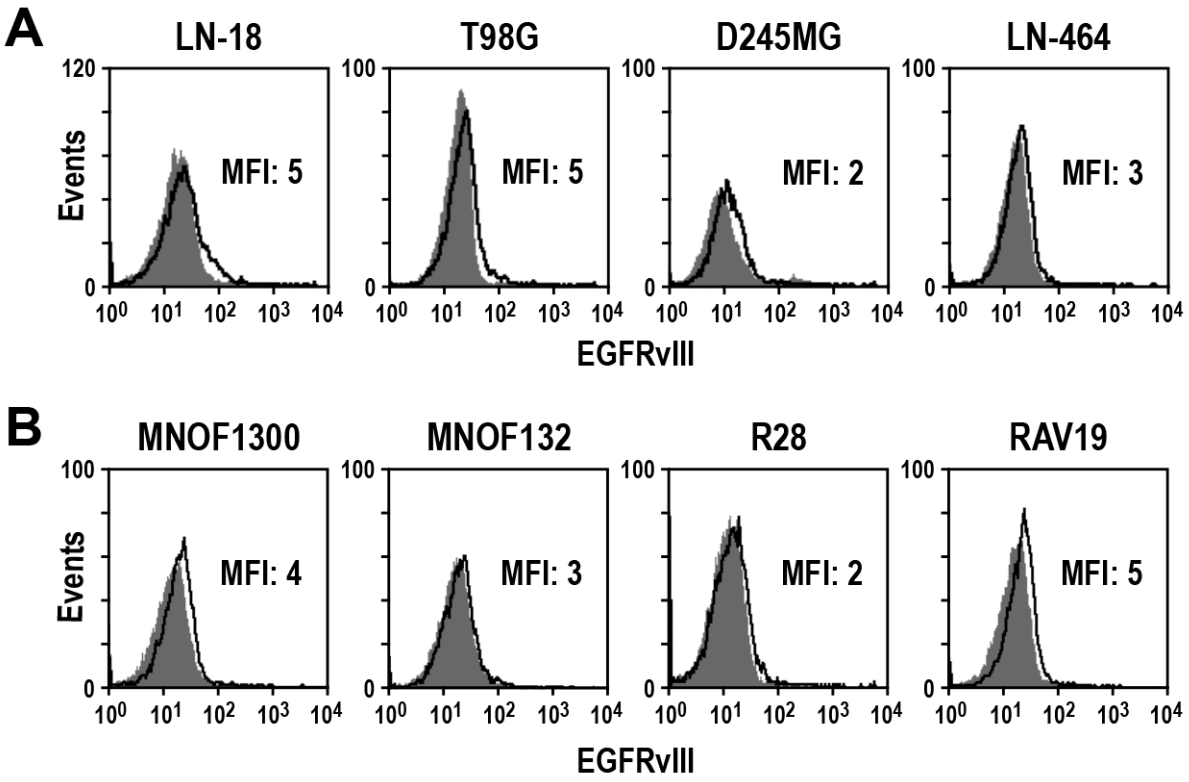

Figure S2. Analysis of EGFRvIII expression on the surface of established human glioblastoma cell lines and primary glioblastoma cell cultures. Expression of EGFRvIII on the surface of established LN-18, T98G, D245MG and LN-464 (**A**) and primary MNOF1300, MNOF132, R28 and RAV19 GBM cells (**B**) was determined by flow cytometry with EGFRvIII-specific antibody (open areas). Tumor cells only treated with secondary antibody (filled areas) served as controls. MFI: mean fluorescence intensity (geometric mean).

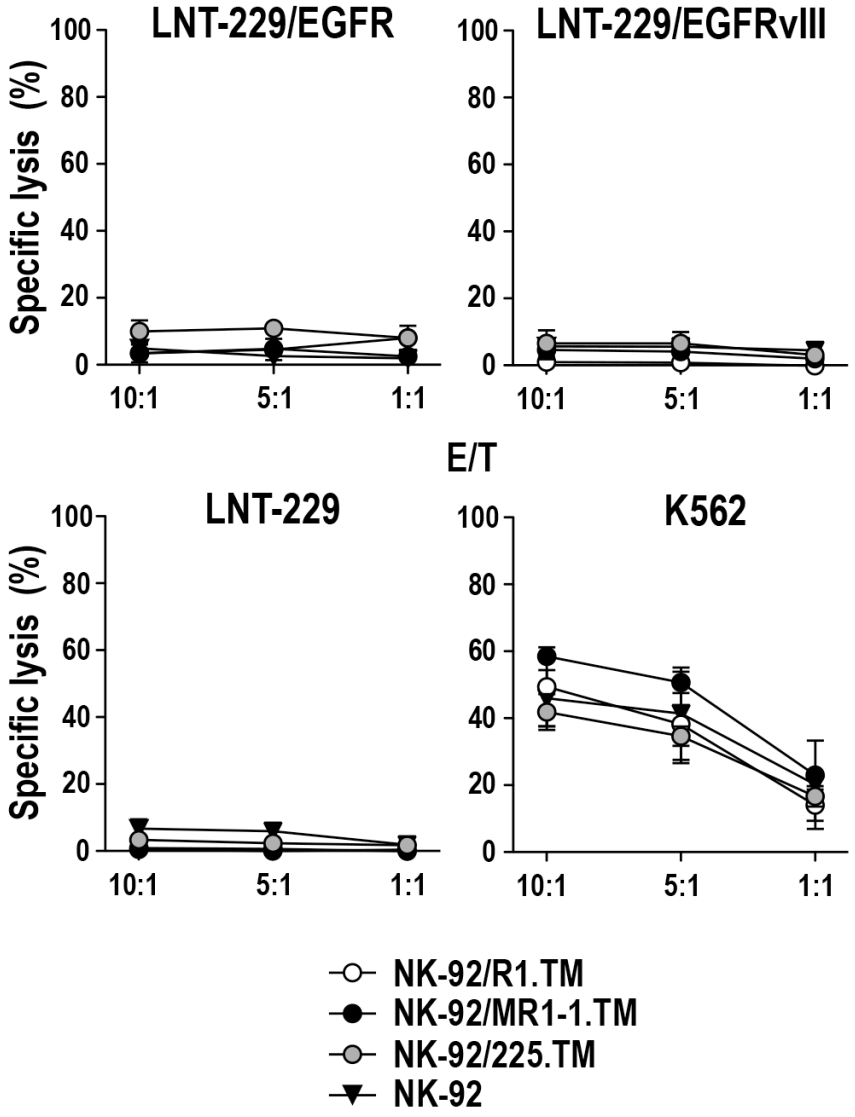

Figure S3. Cytotoxic activity of NK-92 cells expressing truncated chimeric antigen receptors. Cytotoxicity of NK-92/R1.TM, NK-92/MR1-1.TM and NK-92/225.TM cells expressing truncated CARs without signaling domains against EGFR-positive LNT-229/EGFR, EGFRvIII-positive LNT-229/EGFRvIII and parental LNT-229 GBM cells was investigated in FACS-based cytotoxicity assays after co-incubation of effector and target cells for 2 h at different E/T ratios. Parental NK-92 cells were included as a control. Mean values ± SEM are shown; n=3. For comparison K562 cells were included as targets which lack EGFR and EGFRvIII expression but are sensitive to the natural cytotoxicity of NK-92 cells.

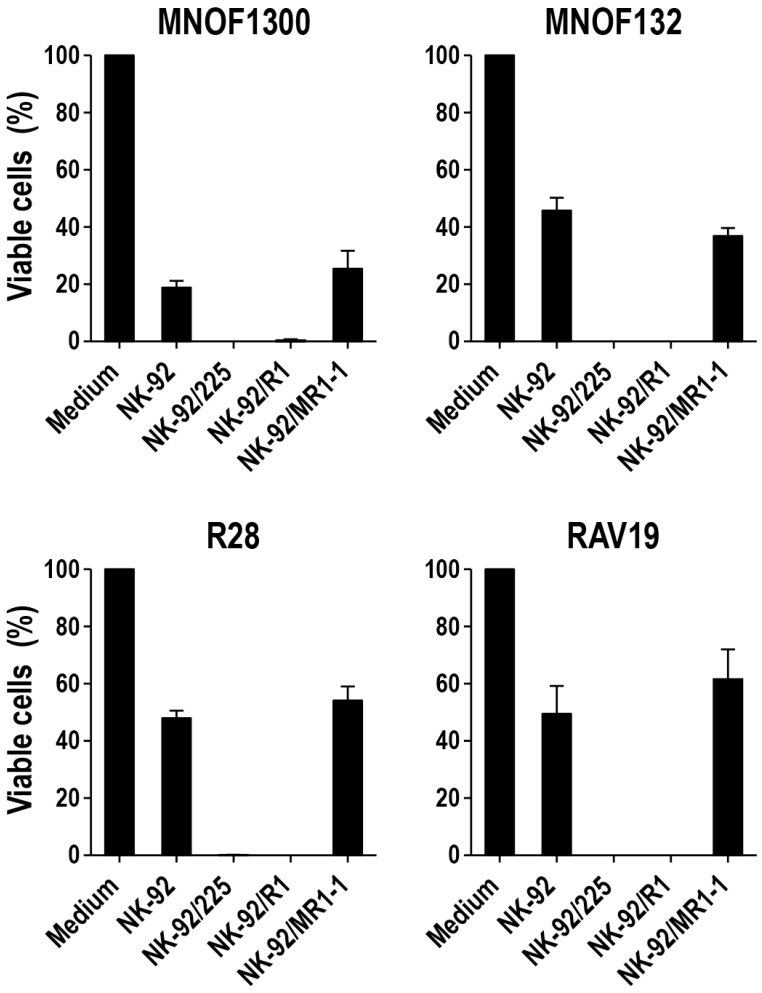

Figure S4. Cytotoxic activity of CAR NK cells against primary human glioblastoma cells upon extended co-culture. Cell killing of primary MNOF1300, MNOF132, R28 and RAV19 GBM cells by NK-92/R1, NK-92/MR1-1 and NK-92/225 cells was investigated in crystal violet cell viability assays after co-incubation of effector and target cells for 16 h at an E/T ratio of approximately 1:1. Parental NK-92 were included for comparison. Tumor cells cultured in NK cell medium without NK cells served as a standard. Mean values ± SEM are shown; n=4.

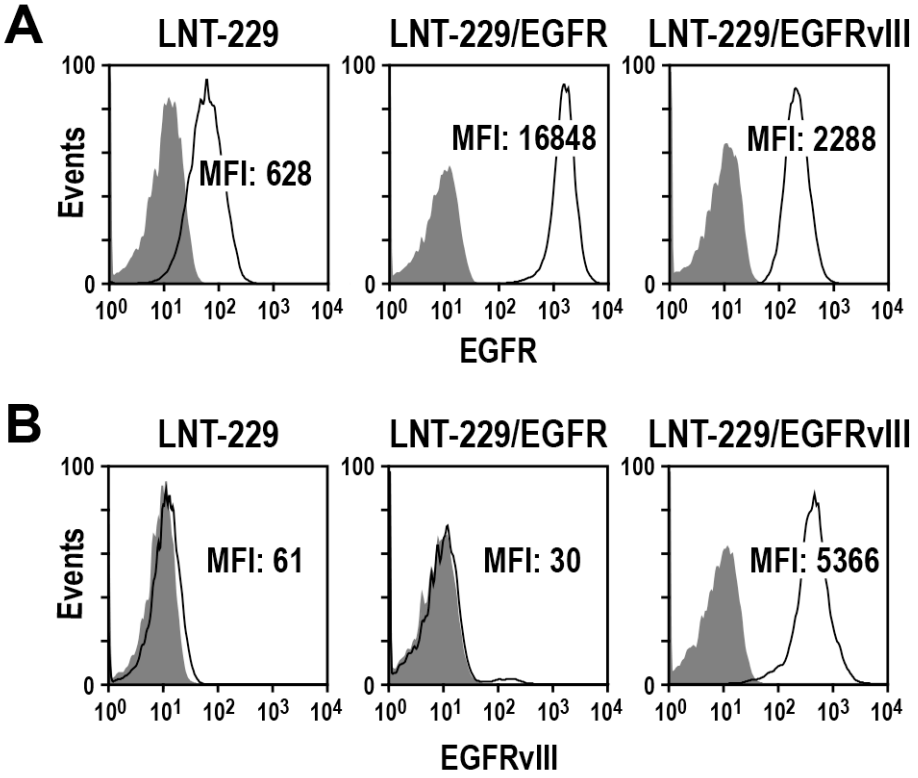

Figure S5. Analysis of EGFR and EGFRvIII expression on the surface of LNT-229 transfectants. (**A**) Expression of EGFR on the surface of LNT-229, LNT-229/EGFR and LNT-229/EGFRvIII cells was analyzed by flow cytometry with EGFR-specific antibody (open areas). Tumor cells treated with isotype antibody (filled areas) served as controls. **B**) Surface expression of EGFRvIII was analyzed by flow cytometry with EGFRvIII-specific antibody (open areas). Tumor cells only treated with secondary antibody (filled areas) served as controls. MFI: mean fluorescence intensity (geometric mean).

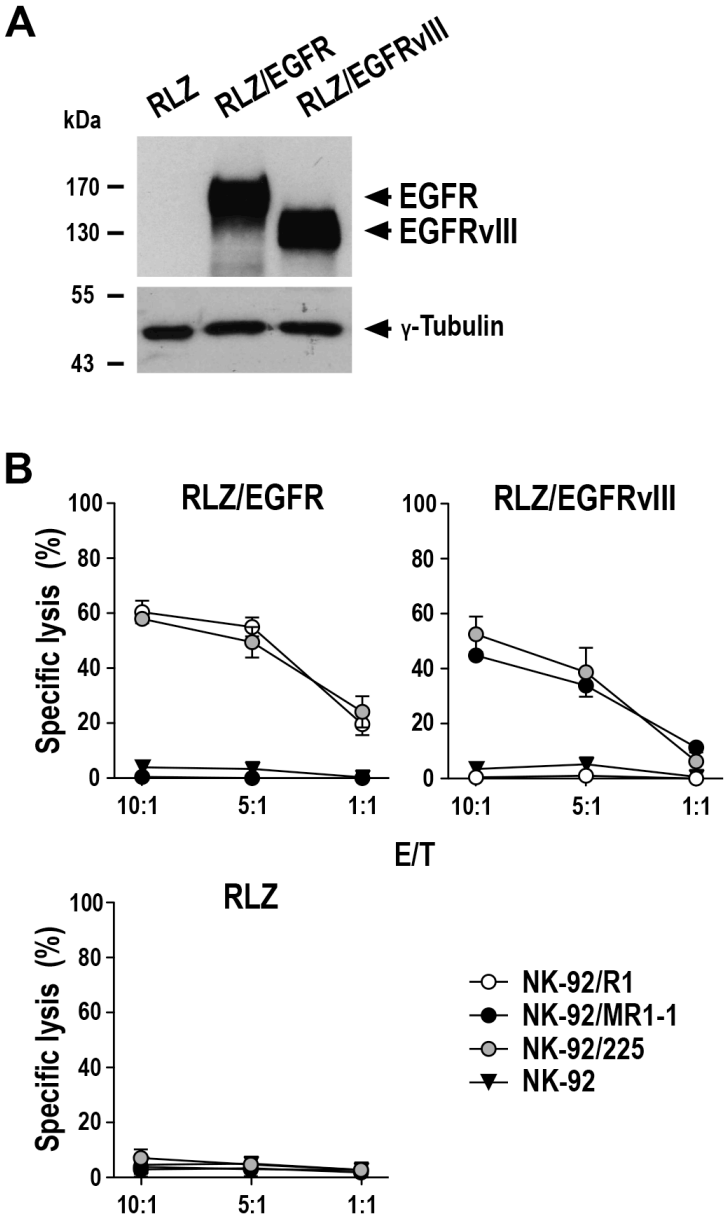

Figure S6. Specific cytotoxicity of CAR NK cells against EGFR- and EGFRvIII expressing cells. Specificity of NK-92/R1, NK-92/MR1-1 and NK-92/225 cells was tested using murine Renca-lacZ (RLZ) renal carcinoma cells stably expressing human EGFR (RLZ/EGFR) or EGFRvIII (RLZ/EGFRvIII) as targets. (**A**) EGFR (170 kDa) and EGFRvIII (140 kDa) were detected in cell lysates of RLZ/EGFR and RLZ/EGFRvIII cells by immunoblotting with an EGFR-specific antibody that binds to a C-terminal epitope preserved in EGFRvIII. Parental RLZ cells served as a control. γ-tubulin was analyzed as a loading control. (**B**) Cytotoxicity of CAR NK cells against RLZ/EGFR and RLZ/EGFRvIII cells was investigated in FACS-based cytotoxicity assays after co-incubation of effector and target cells for 2 h at different E/T ratios. Parental RLZ and NK-92 cells were included as controls. Mean values ± SEM are shown; n=3.

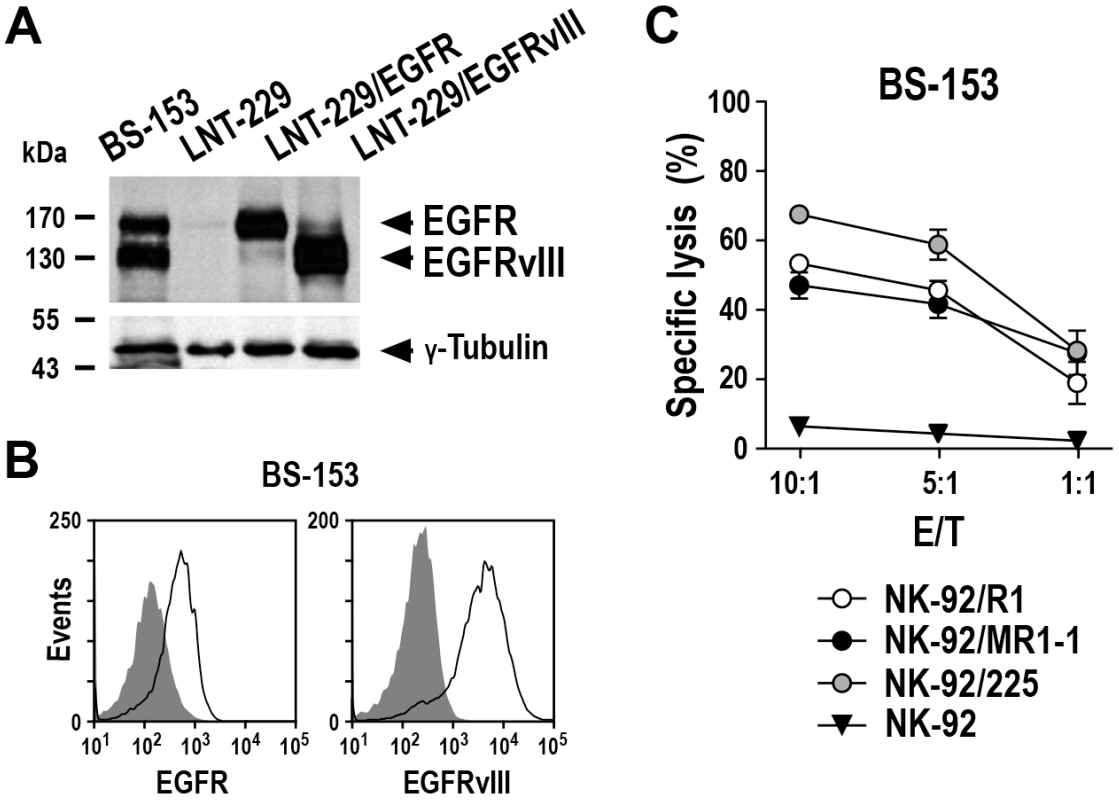

Figure S7. Cell killing activity of CAR NK cells against human BS-153 glioblastoma cells. (**A**) Endogenously expressed EGFR (170 kDa) and EGFRvIII (140 kDa) were detected in cell lysates of BS-153 cells by immunoblotting with an EGFR-specific antibody that binds to a C-terminal epitope preserved in EGFRvIII. LNT-229, LNT-229/EGFR and LNT-229/EGFRvIII cells were included for comparison. γ-tubulin was analyzed as a loading control. (**B**) Expression of EGFR and EGFRvIII on the surface of BS-153 cells was analyzed by flow cytometry with EGFR- and EGFRvIII-specific antibodies (open areas). Tumor cells treated with isotype antibody (EGFR) or only treated with secondary antibody (EGFRvIII) (filled areas) served as controls. (**C**) Cytotoxicity of CAR NK cells against BS-153 cells was investigated in FACS-based cytotoxicity assays after co-incubation of effector and target cells for 2 h at different E/T ratios. Mean values ± SEM are shown; n=3.

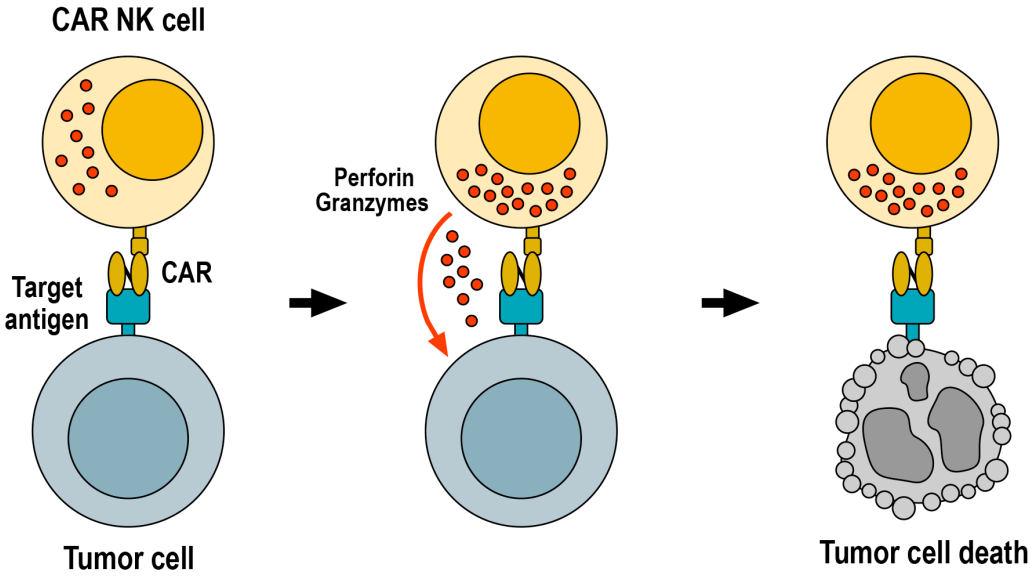

Figure S8. Killing of tumor cells by CAR NK cells. Specific binding of the chimeric antigen receptor (CAR) to its cognate target antigen on the surface of a tumor cell triggers CAR activation (left panel). This results in re-orientation of cytotoxic granules towards the immunological synapse formed between NK and target cell, followed by release of perforin and granzymes from cytotoxic granules into the synaptic cleft (middle panel). Perforin and granzymes taken up by the target tumor cell then trigger apoptotic cell death indicated by membrane blebbing and disintegration of the nucleus (right panel).

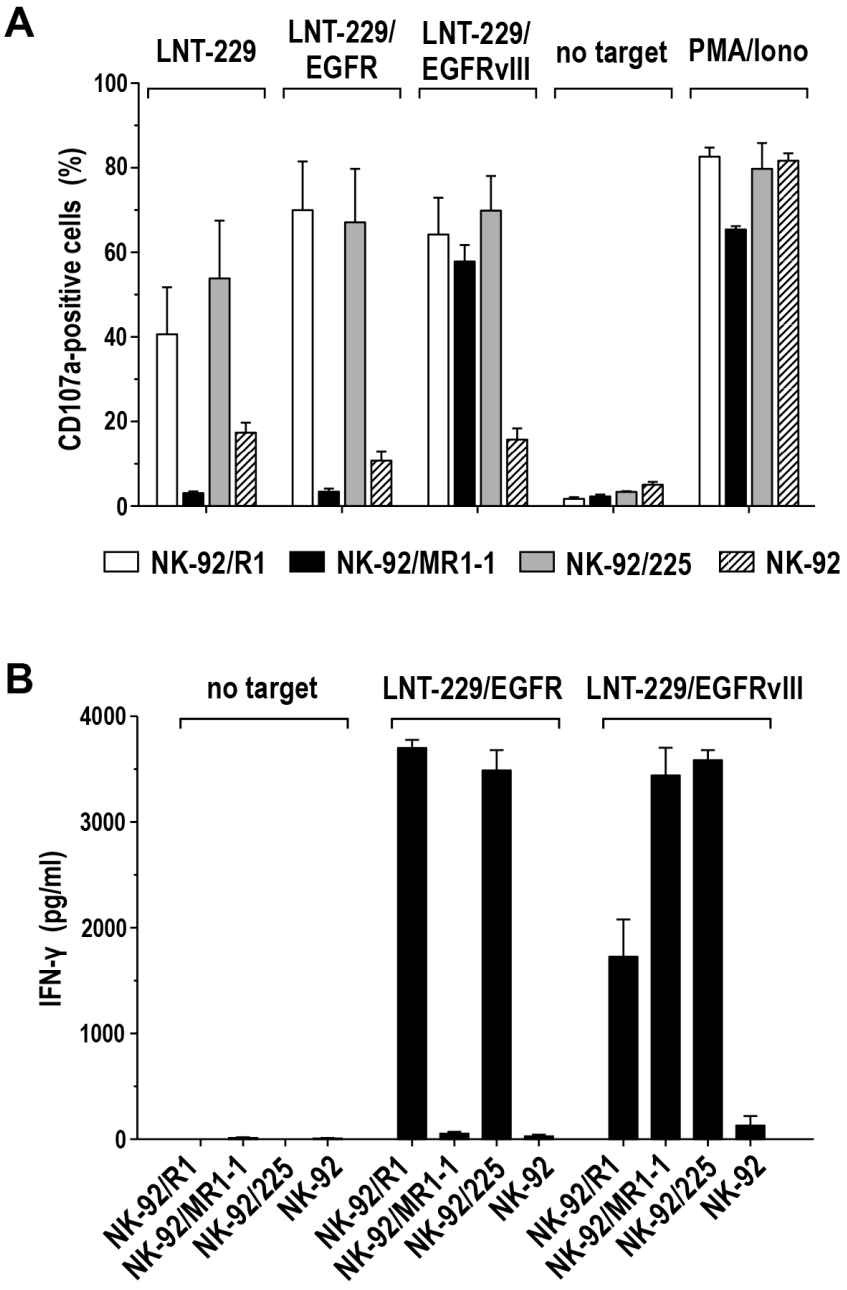

Figure S9. Degranulation of CAR NK cells and IFN-γ secretion upon activation by EGFR- and EGFRvIII expressing glioblastoma cells. (**A**) Degranulation of NK-92/R1, NK-92/MR1-1 and NK-92/225 cells was analyzed by flow cytometry determining CD107a surface expression after 5 h of co-culture with LNT-229, LNT-229/EGFR, or LNT-229/EGFRvIII GBM cells at an E/T ratio of 1:1. Parental NK-92 cells were included for comparison. NK cells kept without target cells or stimulated with 1 µg/mL phorbol 12-myristate 13-acetate (PMA) and 1 µg/mL ionomycin (Iono) served as controls. Mean values ± SEM are shown; n=3. (**B**) 5 x 10^5 CAR NK or parental NK-92 cells were incubated for 6 h with LNT-229/EGFR or LNT-229/EGFRvIII cells at an effector to target ratio of 1:1. Supernatants were collected and the level of IFN-γ was measured using cytometric beads. NK cells kept without target cells served as control. Mean values ± SEM are shown; n=3.