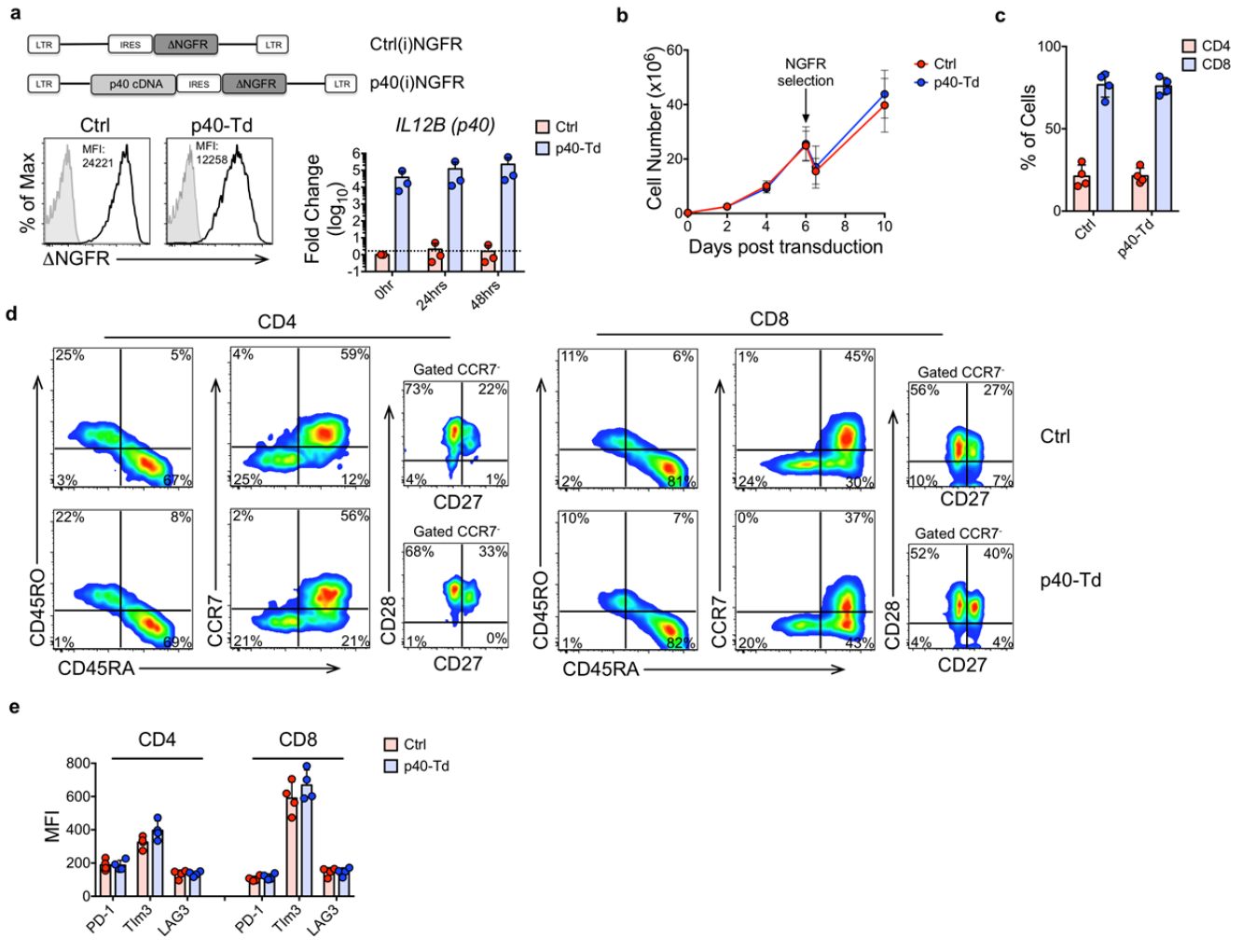
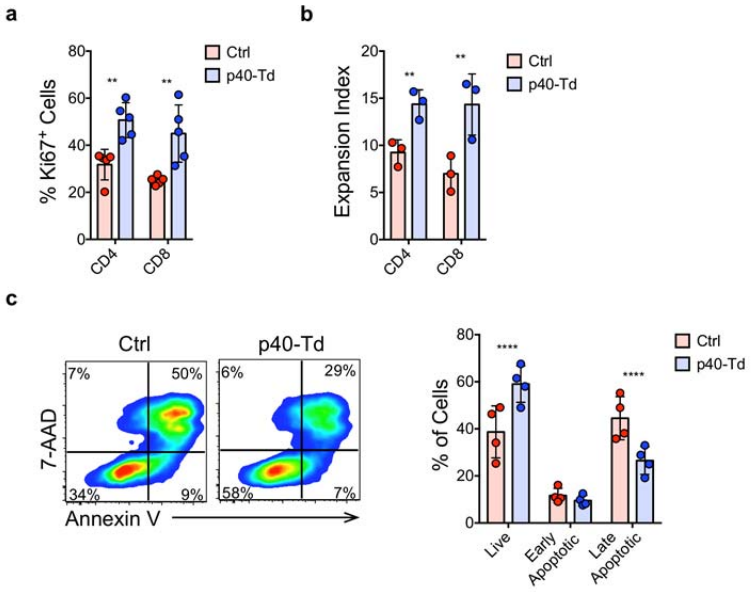



**Supplementary Figure 1**

**Phenotypic analysis of p40-Td cells.**

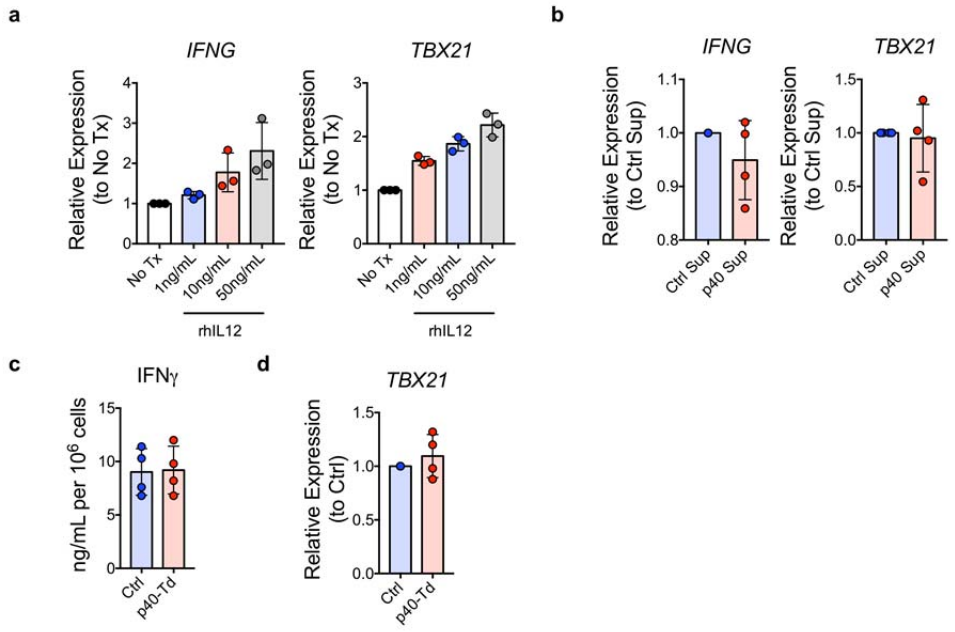
(**a**) Schematic representation of the retroviral vectors (upper panel) and representative flow cytometry plots illustrating the transduction efficiency of T cells measured by ΔNGFR expression (left panel) and qRT-PCR (right panel). Data shown as representative data and mean ± SD (n =4). (**b**) Cell counts of Ctrl cells and p40-Td cells cultured with IL-7 and IL-15. ΔNGFR$^+$ T cells were sorted on day 5 post-transduction. Data shown as mean ± SD (n = 4). (**c**) Frequency of CD4$^+$ and CD8$^+$ T cells of Ctrl cells and p40-Td cells at day 12 post-transduction. Data shown as representative data and mean ± SD (n = 4). (**d**) Representative flow cytometry plots illustrating the expression of memory markers in Ctrl cells and p40-Td cells at day 12 post-transduction (n = 4). (**e**) Mean fluorescence intensity (MFI) of exhaustion and inhibitory receptors in Ctrl cells and p40-Td cells at day 12 post-transduction. Data shown as mean ± SD (n = 4).




**Supplementary Figure 2**

**p40-Td cells show enhanced proliferation and survival upon activation.**

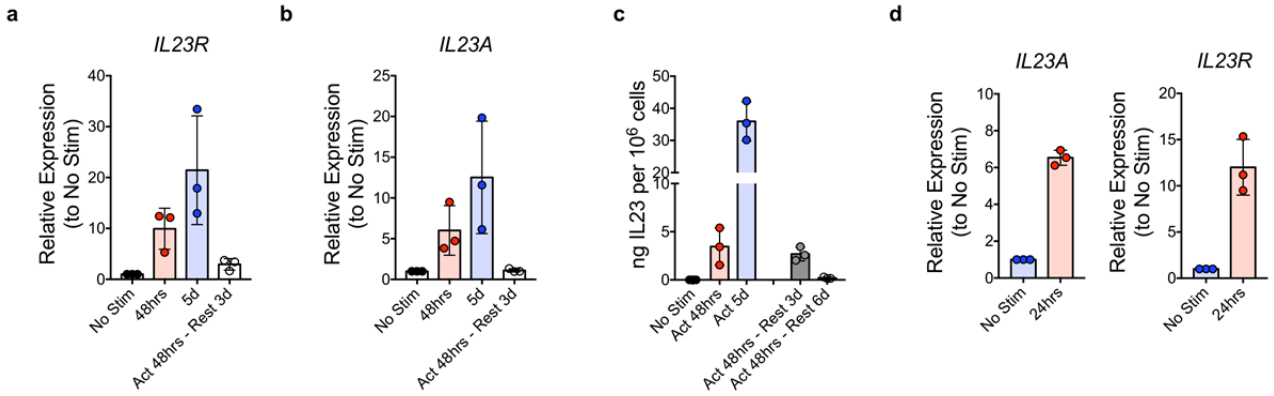
(**a**) Frequency of Ki67$^+$ cells in CD4$^+$ and CD8$^+$ T cells of Ctrl cells and p40-Td cells stimulated with αCD3 and αCD28 Abs for 3 days. Data shown as individual value, mean ± SD (n = 5). p=0.0051 for CD4$^+$T cells; p=0.0038 for CD8$^+$ T cells determined by repeated measure 2-way ANOVA with Sidak post hoc test. (**b**) Expansion index of CellTrace Violet labeled Ctrl cells and p40-Td cells stimulated with αCD3 and αCD28 Abs for 5 days. Data shown as individual value, mean ± SD (n = 3). **p=0.0056 for CD4$^+$; **p=0.0014 for CD8$^+$T cells determined by repeated measure 2-way ANOVA with Sidak post hoc test. (**c**) Representative flow plots (left panel) and summary (right panel) of Annexin V and 7AAD staining of Ctrl cells and p40-Td cells stimulated with αCD3 and αCD28 Abs for 5 days. Data shown as individual value, mean ± SD (n = 4). ****p<0.0001 determined by repeated measure 2-way ANOVA with Sidak post hoc test.




**Supplementary Figure 3**

**IL12 produced by p40-Td cells is insufficient to induce IL12 signaling.**

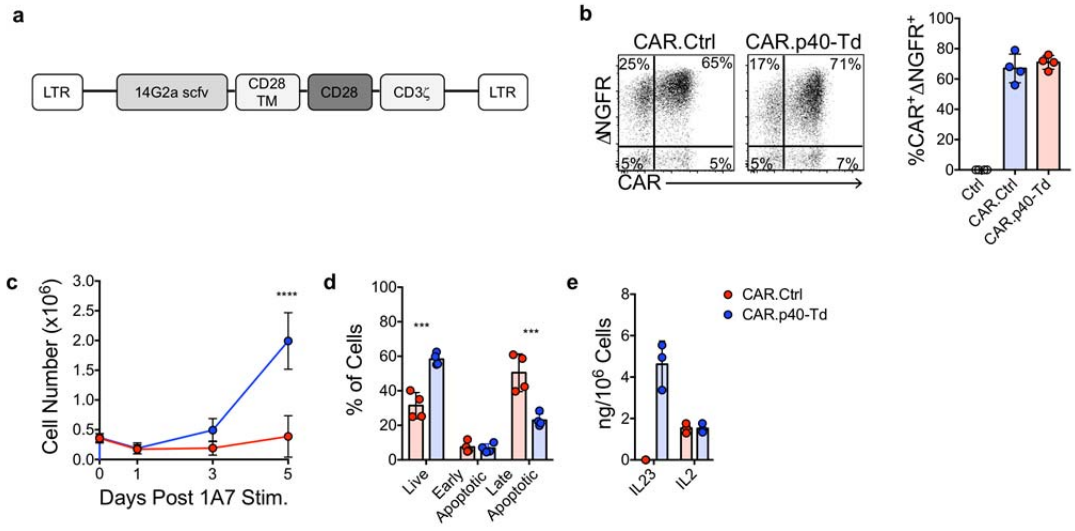
Naïve $CD4^+$ T cells were isolated from human peripheral blood mononuclear cells and activated with αCD3 and αCD28 Abs for 2 days with addition of (**a**) recombinant human IL12 in escalating concentration or (**b**) supernatant of activated Ctrl cells or p40-Td cells. Gene expression of IL12 target genes *IFNG* and *TBX21* was measured by qRT-PCR. Data shown as individual values and mean ± SD (n = 3 for **(a**) and n = 4 for (b)). (**c**) Ctrl cells and p40-Td cells were activated with αCD3 and αCD28 Abs, and IFN-γ secretion was measured at 24 hrs post activation by ELISA. Data shown as individual values and mean ± SD (n = 4). (**d**) Ctrl cells and p40-Td cells were activated with αCD3 and αCD28 Abs for 2 days, and expression of *TBX21* gene was measured by qPCR. Data shown as individual values and mean ± SD (n = 4).




**Supplementary Figure 4**

**Activation-inducible *IL23R/IL23A* gene expression and IL23 secretion.**

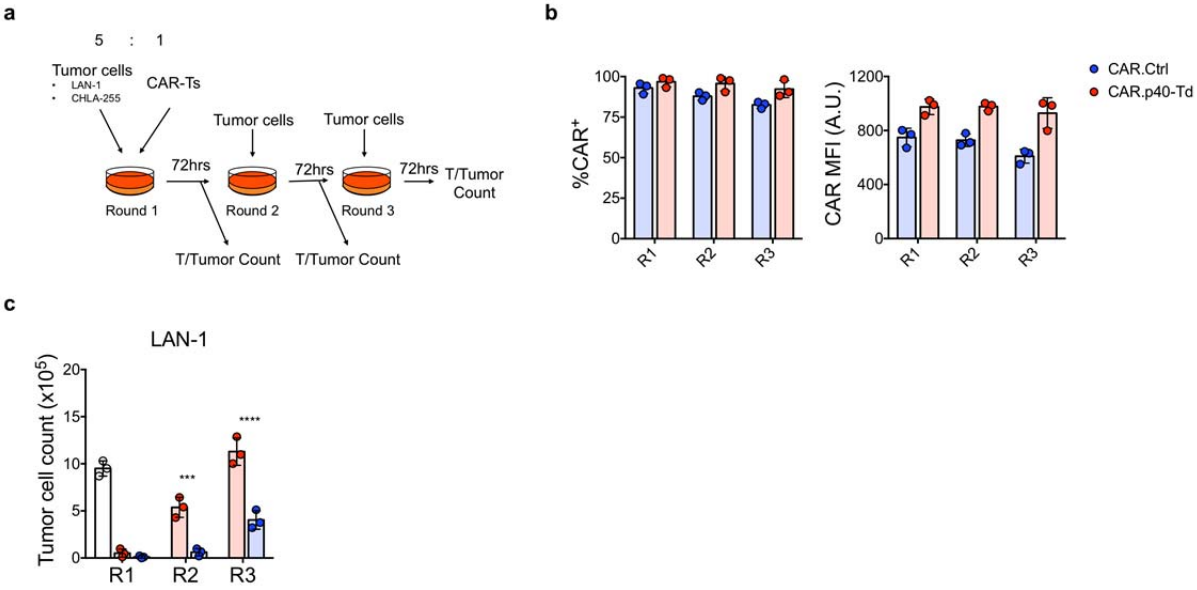
Ex-$T_M$ cells (**a-b**) and p40-Td cells (**c**) were rested or stimulated with αCD3 and αCD28 Abs for 48 hrs and 5 days or stimulated for 48 hrs and then rested for additional 3 days (**a-b**) and 6 days (**c**). Gene expression of *IL23R* and *IL23A* was measured by qPCR at the indicated time points (**a-b**). IL23 protein in the supernatant was measured by ELISA (**c**). Data shown as individual values, mean ± SD (n = 3). (**d**) T cells transduced with the tyrosinase-specific TCR were rested (No stim) or stimulated with T2 cells pulsed with 1μM tyrosinase peptide for 24 hrs. Gene expression of *IL23R* and *IL23A* was measured by qPCR. Data shown as individual values, mean ± SD from 3 experiments using T cells obtained from a healthy donor.




**Supplementary Figure 5**

**p40 expression enhances the proliferation of CAR T cells targeting neuroblastoma.**

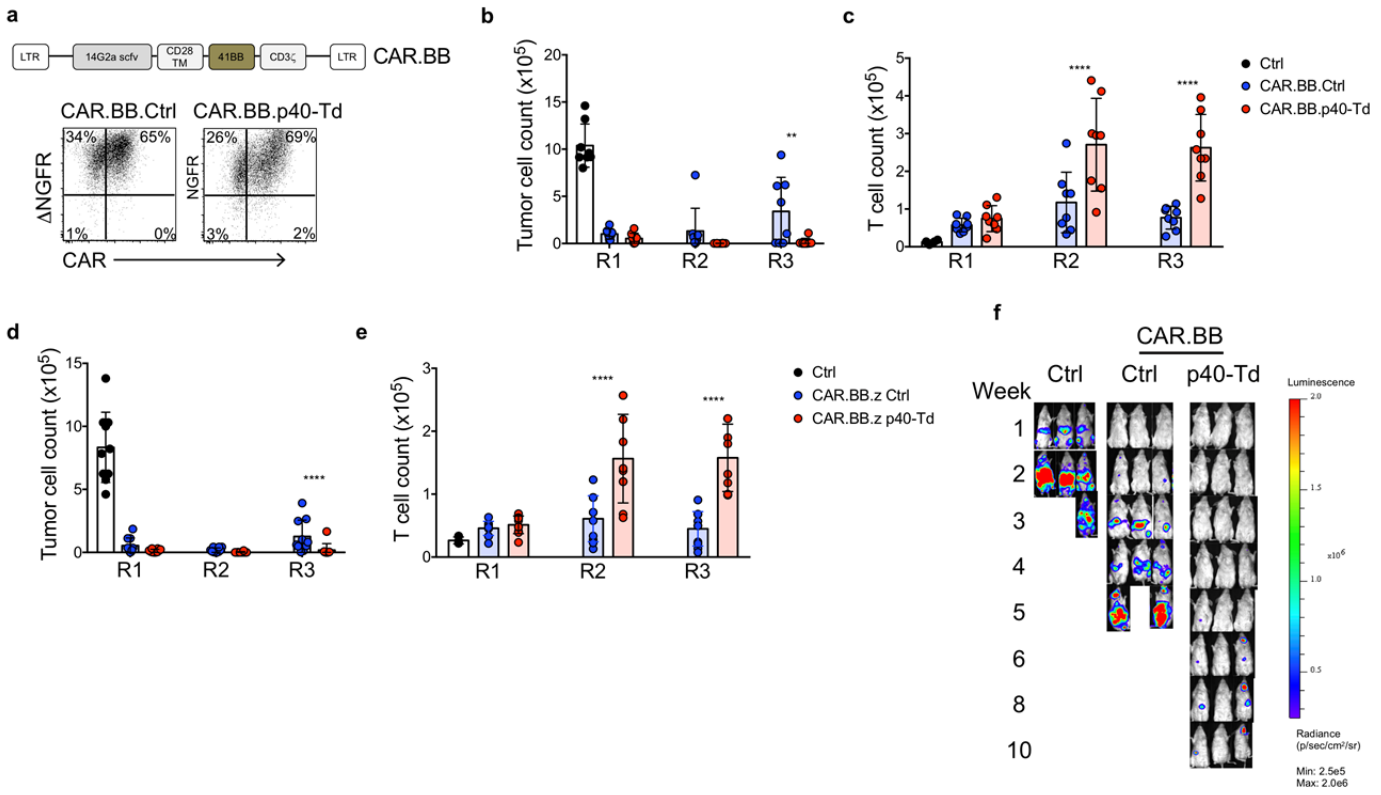
(**a**) Schematic of the CAR design of the GD2-specific CAR (CAR) targeting neuroblastoma. (**b**) Representative flow plots (left panel) and summary (right panel) illustrating the co-expression of CAR and ΔNGFR in Ctrl CAR cells (CAR.Ctrl) and CAR cells expressing p40 (CAR.p40-Td). Data shown as individual values, mean ± SD (n = 4). (**c**) T cell counts of CAR.Ctrl cells or CAR.p40-Td cells activated by plate-bound anti- idiotype 1A7 Ab. Data shown as mean ± SD (n = 5). ****p<0.0001 determined by repeated measure 2-way ANOVA with Sidak post hoc test. (**d**) Frequency of apoptotic cells in CAR.Ctrl cells and CAR.p40-Td cells activated by the 1A7 Ab for 5 days using the Annexin V/7AAD staining. Data shown as individual values and mean ± SD (n = 4). ***p=0.0006 for live cells; ***p=0.0005 for late apoptotic cells determined by repeated measure 2-way ANOVA with Sidak post hoc test. (**e**) Detection of IL23 and IL2 in the supernatant of CAR.Ctrl cells and CAR.p40-Td cells post-stimulation with the 1A7 Ab. Data shown as individual values and mean ± SD (n = 3).




**Supplementary Figure 6**

**p40 expression enhances the anti-tumor activity of CAR T cells targeting neuroblastoma.**

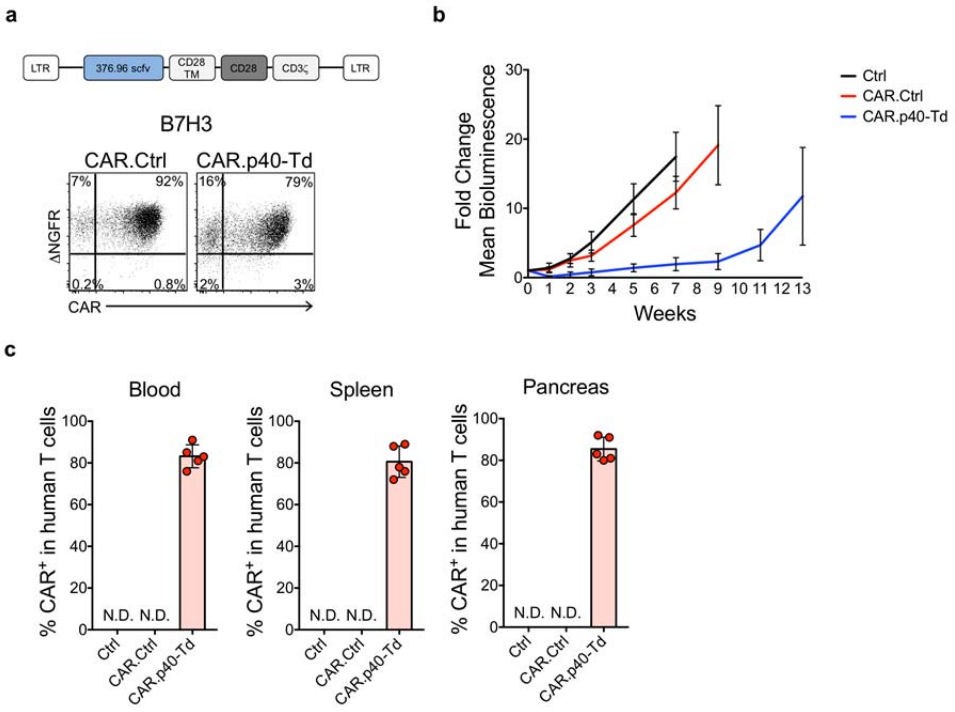
(**a**) Schematics of the repetitive coculture experiment. (**b**) CAR expression in T cells upon repetitive coculture. Data shown as individual value, mean ± SD (n = 3). (**c**) Tumor cell counts after multiple round coculture (R1, R2 and R3) of LAN-1 cells with CAR T cells cultured in IL2 instead of IL7 and IL15. Data shown as individual value, mean ± SD (n = 3). ***p=0.0007; ****p<0.0001 determined by repeated measure 2-way ANOVA with Sidak post hoc test.




**Supplementary Figure 7**

**p40 expression enhances the anti-tumor activity of CAR T cells encoding the 4-1BB endodomain.**

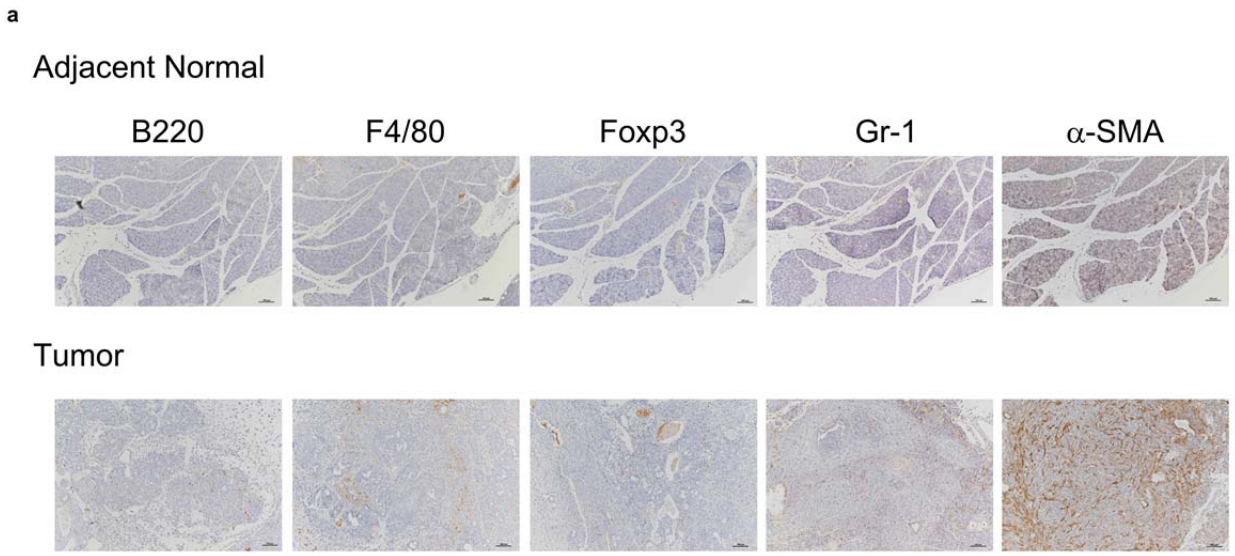
(**a**) Schematics of CAR design of the GD2-specific CAR encoding the 4-1BB endodomain (GD2.CAR) and CAR expression in T cells. Data shown are representative of 10 independent experiments. (**b-e**) Counts of neuroblastoma tumor cells (LAN-1 (b-c) and CHLA-255 (d-e)) and CAR T cells after each round of repetitive coculture (R1, R2 and R3) with either control (Ctrl.) T cells, GD2.CAR T cells with 4-1BB endodomain (CAR.BB.Ctrl) or GD2.CAR T cells with 4-1BB endodomain coexpressing p40 (CAR.BB.p40-Td). Data shown as individual values and mean ± SD (n = 8 for LAN1, n =10 for CHLA). **p=0.008; ****p<0.0001 determined by repeated measure 2-way ANOVA with Sidak post hoc test. (**f**) Tumor bioluminescence (BLI) in mice engrafted with the neuroblastoma cell line CHA-225 and treated with 2 x $10^6$ Ctrl cells, CAR.BB.Ctrl cells or CAR.BB.p40-Td cells (n = 3 mice/group).




**Supplementary Figure 8**

**p40 expression enhances the antitumor activity of B7-H3-specific CAR T cells.**

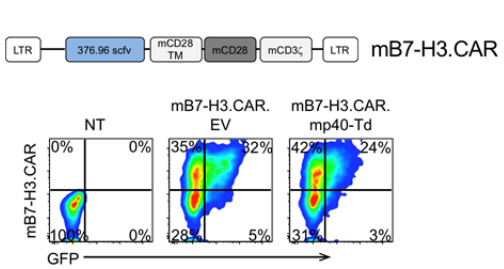
(**a**) Schematics of the B7-H3-specific CAR design and CAR expression in T cells. Data shown are representative of 5 independent experiments. (**b**) Longer follow-up for the experiment described in **Fig. 4c**. Data shown are collected from 1 mouse experiment with 5 mice per group. (**c**) CAR expression in human T cells collected at the end of the in vivo experiment in the BXPC3 pancreatic tumor model. N.D.: not determined due to insufficient T cell events for analysis. Data shown as individual value and mean ± SD (n = 5 mice/group).




**Supplementary Figure 9**

**Immunohistochemistry staining determining infiltrating immune cells and tumor-associated fibroblasts in the tumor model in which KPC-mB7H3 tumor cells are orthotopically engrafted in immunocompetent mice.**

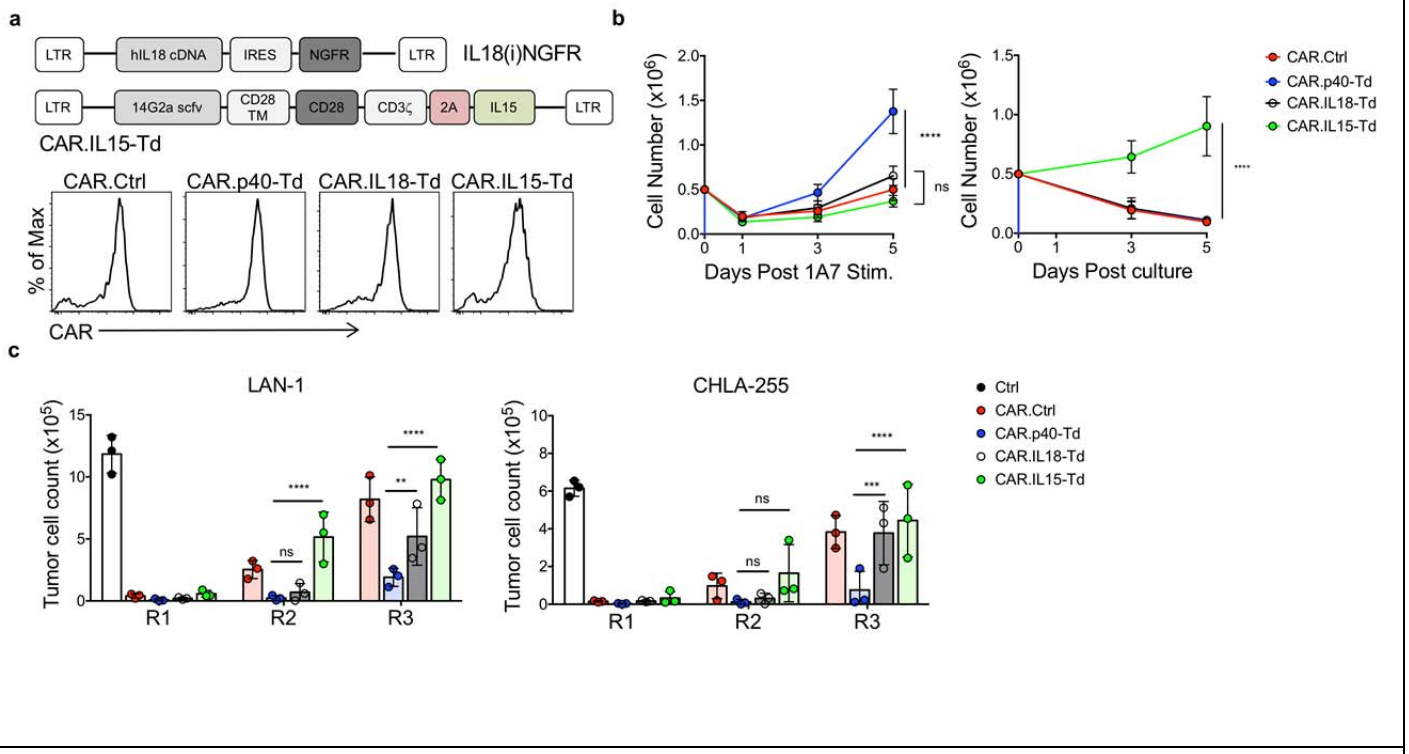
(**a**) C57BL/6 mice were orthotopically engrafted with the KPC-mB7H3 pancreatic tumor cells. Tumors were collected at day 40 post-tumor engraftment and immunohistochemistry staining was performed using the indicated Abs. Section of tumor and adjacent normal tissue are shown. The scale bars indicate 100 μm. Data representative of 2 mice of 2 independent experiments.




**Supplementary Figure 10**

**Murine CAR T cells targeting murine B7-H3.**

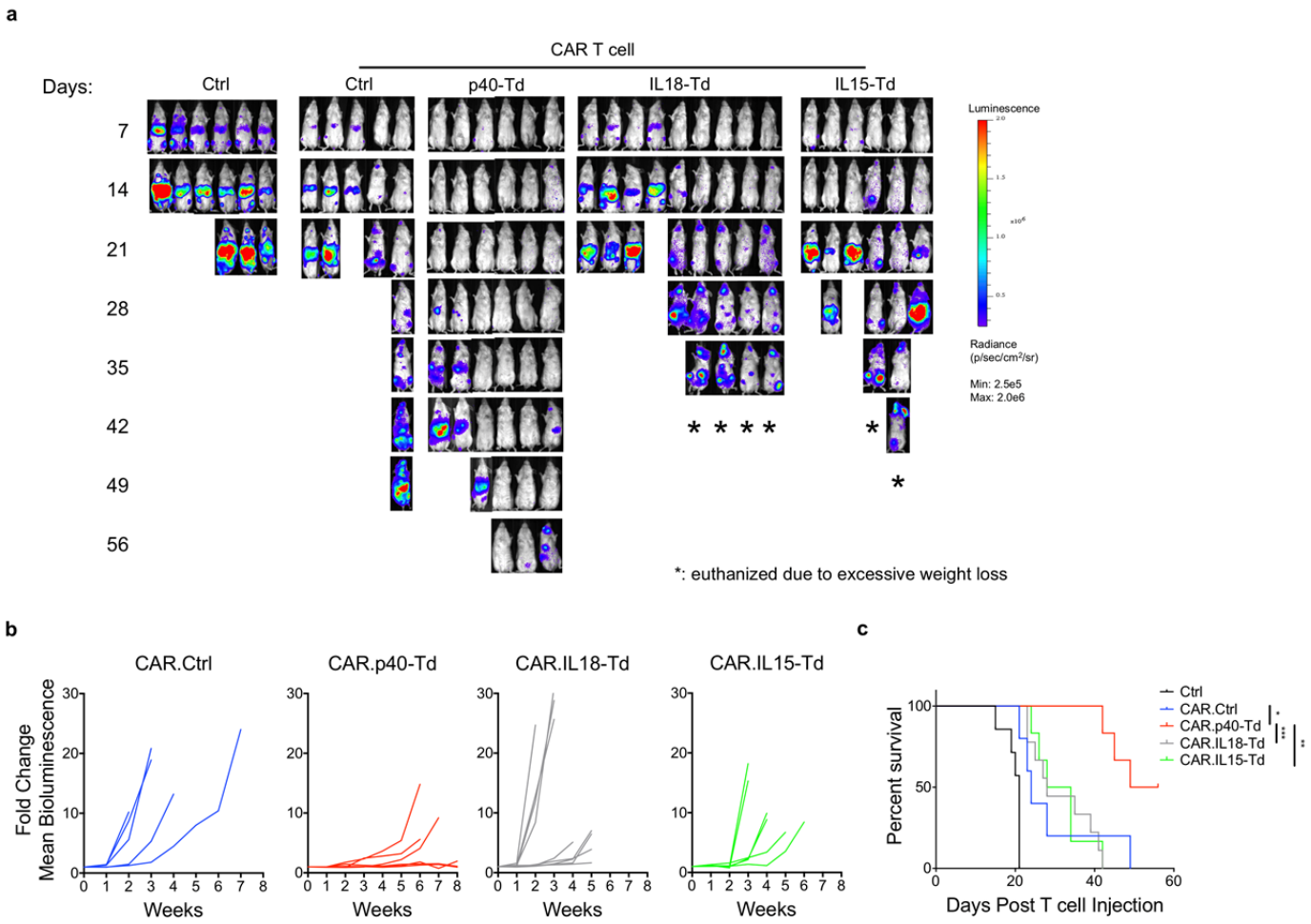
(**a**) Schematics of the B7-H3-specific CAR targeting murine B7-H3 and encoding murine CD28 and CD3ζ chain (mB7-H3.CAR) (upper panel). CAR expression in murine T cells co-transduced with an empty vector (EV) or a vector encoding murine p40 (mp40-Td). NT indicate control T cells non transduced. Data shown are representative of 3 independent experiments.




**Supplementary Figure 11**

**CAR T cells equipped with p40 show superior proliferative capacity upon stimulation and anti-tumor effects *in vitro* compared to CAR T cells engineered with IL18 or IL15**.

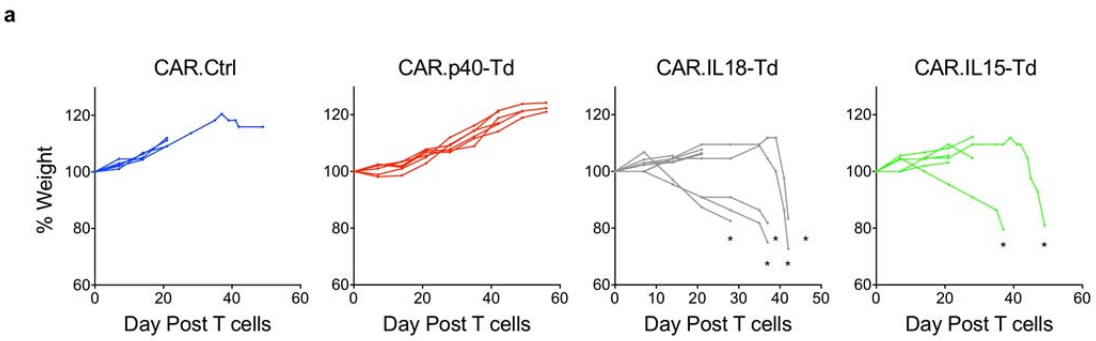
(**a**) Schematics of IL18 expression vector and CAR.IL15-Td and expression of CAR molecule on CAR.Ctrl cells, CAR.p40-Td cells, CAR.IL18-Td cells and CAR.IL15-Td cells. Data shown are representative of 3 independent experiments. (**b**) Cell counts of CAR.Ctrl cells, CAR.p40-Td cells, CAR.IL18-Td cells and CAR.IL15-Td cells upon stimulation with the 1A7 Ab or cultured in media without addition of cytokines for the indicated days. Data shown as mean ± SD (n = 4). ****p<0.0001 determined by repeated measure 2-way ANOVA with Sidak post hoc test for cell count at day 5 post stimulation/culture. (**c**) Repetitive coculture assay described in S.Figure 6a with LAN-1 and CHLA-255 neuroblastoma tumor cells and GD2 specific CAR.Ctrl cells, CAR.p40-Td cells, CAR.IL18-Td cells and CAR.IL15-Td cells. Tumor cell numbers after each round of coculture (R1, R2 and R3) are shown. Data presented as individual values, mean ± SD (n = 3). **p=0.0028'; ***p=0.0003; **** p<0.0001 determined by repeated measure 2-way ANOVA with Sidak post hoc test.




**Supplementary Figure 12**

**CAR T cells equipped with p40 show superior anti-tumor effects *in vivo* as compared to CAR T cells engineered with IL18 or IL15**.

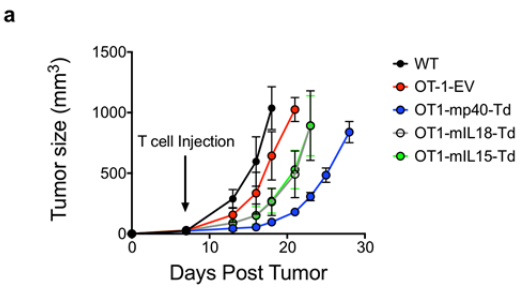
NSG mice were systemically engrafted with the neuroblastoma cell line CHLA-255. Two weeks later, mice were treated with 2 x $10^6$ CAR T cells. Data shown are collected from 2 independent experiments (n = 6 for Ctrl cells, n = 5 for CAR.Ctrl cells, n = 6 for CAR.p40-Td cells, n = 9 for CAR.IL18Td cells, n = 6 for CAR.IL15-Td cells). (**a-b**) Tumor progression indicated by tumor BLI signal was monitored by IVIS imaging. Mice euthanized unrelated to tumor growth are indicated by "*", and weight loss for each mice is shown in S.Fig 13. (**c**) Kaplan-Meier survival curve. *:p=0.0159; **p=0.0014; ***p=0.0004 determined by Log-rank (Mantel-Cox) test. Deaths due to tumor progression or weight loss were recorded.




**Supplementary Figure 13**

**CAR T cells equipped with p40 show superior safety profile as compared to CAR T cells engineered with IL18 or IL15**.

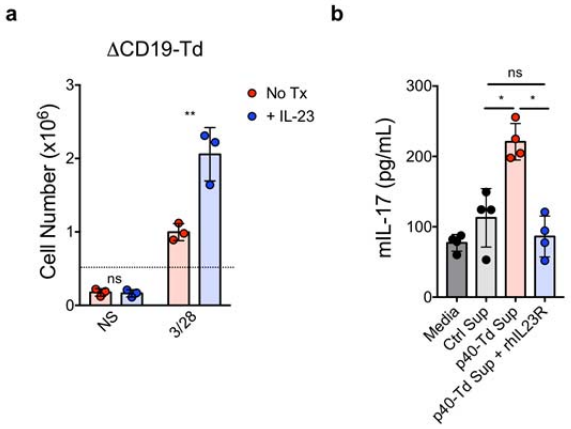
(**a**) Weight loss of mice engrafted with CHLA-255 and treated with CAR T cells. * Indicates euthanasia due to excessive weight loss. Data shown are cumulative measurements from 2 independent experiments (n = 5 for CAR.Ctrl cells, n = 6 for CAR.p40-Td cells, n = 9 for CAR.IL18Td cells, n = 6 for CAR.IL15-Td cells).




**Supplementary Figure 14**

**OT-1-specific T cells co-expressing p40 promote superior antitumor effects as compared to OT-1 T cells co-expressing IL18 or IL15 *in vivo***.

(**a**) B16-OVA model described in **Fig.5e**. Tumor bearing mice were treated with 2 x $10^6$ OT-1 T cells modified to express murine p40 (OT1-mp40-Td), murine IL18 (OT1-mIL18-Td) or murine IL15 (OT1-mIL15-Td). Data shown as mean ± SD tumor volume post tumor engraftment from 2 independent experiments. Data shown as mean tumor volume ± SD (n = 7 for WT cells, OT1-EV cells, OT1-mIL18-Td cells and OT1-mIL15-Td cells, n = 6 for OT1-mp40-Td cells). EV indicates empty vector.




**Supplementary Figure 15**

**Engineered IL23 functions predominantly through an autocrine mode of action**.

(**a**) ΔCD19-Td cells were rested or activated with αCD3 and αCD28 Abs for 7 days with or without 50 ng/mL recombinant human IL23 (rhIL23). Cell number were numerated by beads counting. Data shown as individual values and mean ± SD (n = 3). **p=0.0018 determined by repeated measure 2-way NAOVA with Sidak post hoc test. (**b**) Supernatants were collected from Ctrl cells or p40-Td cells incubated with murine splenocytes with or without recombinant human IL23 receptor (rhIL23R) protein. Murine IL17 released by the splenocytes in response to IL23 was measured by ELISA. Data are presented as individual values and mean ± SD (n = 4). p=0.0122 for Ctrl cells vs. p40-Td cells; p=0.0308 for p40-Td cells vs. p40-Td cells plus rhIL23R determined by repeated measure 1-way NAOVA with Sidak post hoc test.

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