

Titratable Pharmacological Regulation of CAR T Cells Using Zinc Finger-Based Transcription Factors

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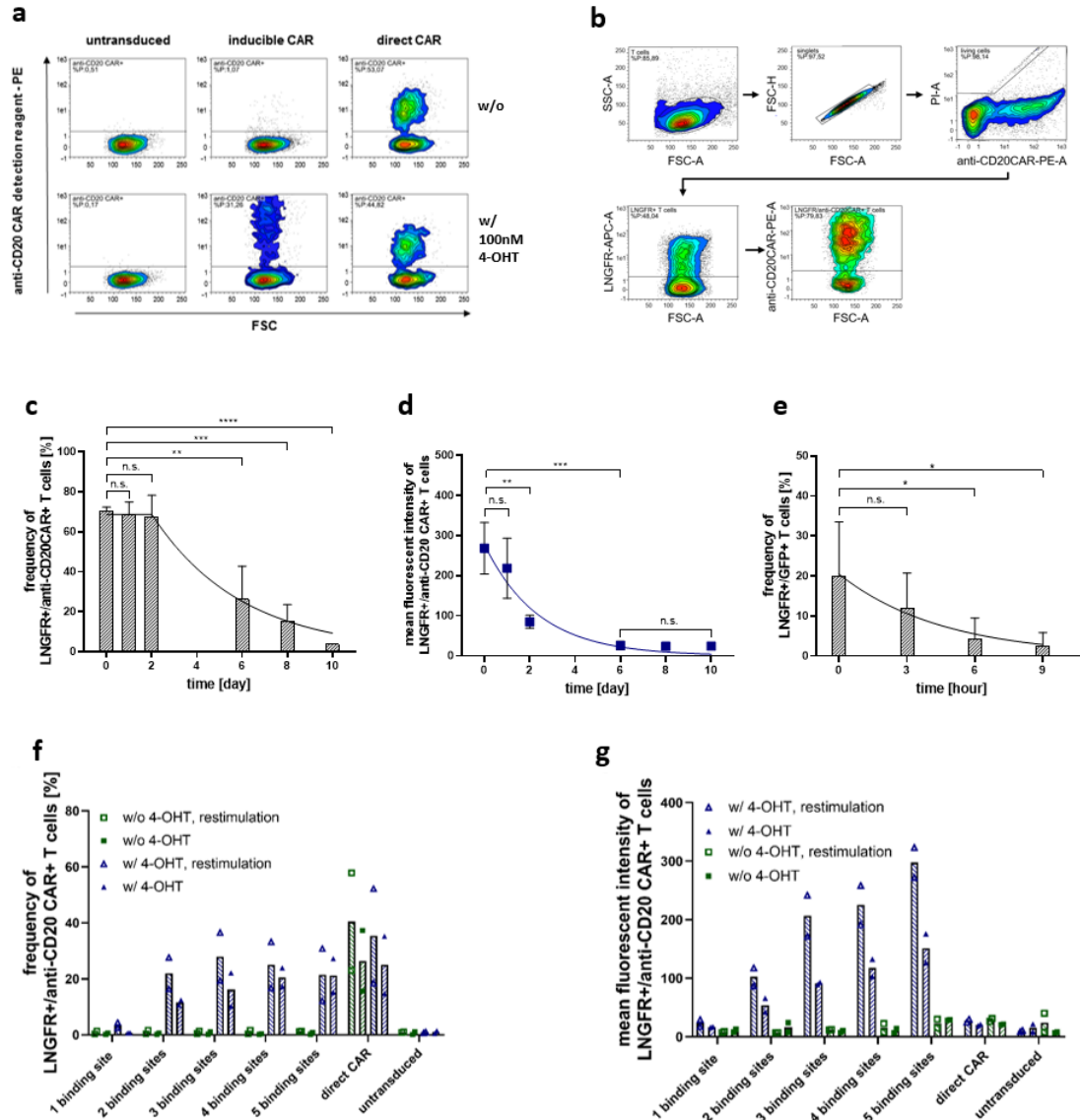


Figure S1. 4-OHT dependent induction of anti-CD20 CAR expression. (a) Representative flow plots show the percentage of anti-CD20 CAR positive T cells upon induction with 100 nM 4-OHT for 40 h in comparison to the conventional constitutively expressed CAR and untransduced T cells. (b) Cells were gated on size, singularity, viability, and Δ LNGFR expression before analyzing anti-CD20 CAR expression. (c) Data points from figure 1f representing the frequency of anti-CD20 CAR+ T cells following inducer drug discontinuation were fitted to a plateau followed by one-phase decay model and the half-life was calculated as $t_{1/2} \approx 2.8$ days. (d) Data points from figure 1f representing the MFI of anti-CD20 CAR+ T cells following inducer drug discontinuation were fitted to a one-phase decay model and the half-life was calculated as $t_{1/2} \approx 1.6$ days. (e) Data points from figure 1h representing the frequency of GFP+ T cells following inducer drug discontinuation were fitted to a one-phase decay model and the half-life was calculated as $t_{1/2} \approx 3.2$ h. (f, g) Inducible CAR T cells bearing

one to five binding sites for the synthetic transcription factor, direct CAR T cells and untransduced cells were stimulated with TransAct and induced with 100 nM 4-OHT. Cell surface expression of anti-CD20 CAR was determined after 48 h by flow cytometry using anti-CD20 CAR detection reagent-PE. Data for frequency (f) and MFI (g) of anti-CD20 CAR T cells are normalized to the expression of the transduction marker Δ LNGFR. Graphs show data from $n = 3$ (c, d, e); $n = 2$ (f, g) different donors. Data shown are mean values \pm s.d. with $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$ by two-way analysis of variance (ANOVA).

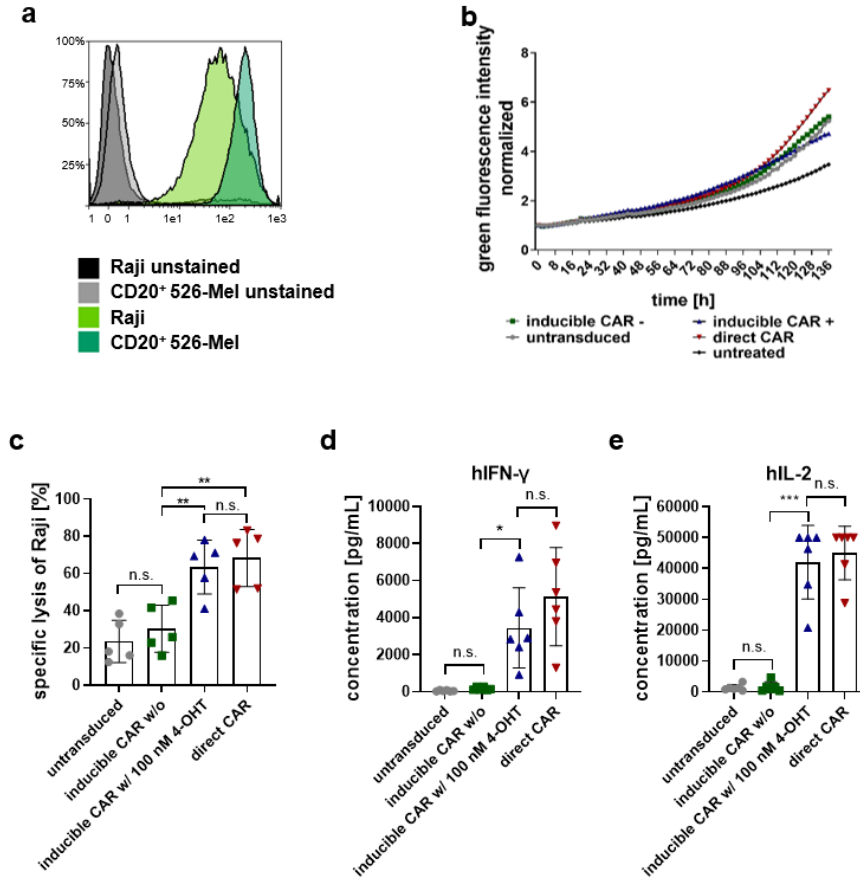


Figure S2. Cytotoxic activity of inducible anti-CD20 CAR T cells against Raji and antigen negative 526-Mel cells in vitro. (a) Flow histogram depicting the cell surface staining of the tumor cell lines GFP⁺ Raji and GFP⁺ CD20⁺ 526-Mel for the target antigen CD20 using anti-CD20-PE. (b) Inducible CAR T cells were co-cultured with antigen negative GFP⁺ 526-Mel at an E:T ratio of 1:1 in the absence (-) or presence of 100 nM 4-OHT (+) added at the start of the assay. Growth of GFP⁺ CD20⁺ 526-Mel was monitored in 2 h intervals using a live-cell imaging device. (c, d, e) Inducible CAR T cells comprising five binding sites for the synthetic transcription factor were co-cultured with GFP⁺ Raji at an E:T ratio of 1:1 in the presence of 100 nM 4-OHT added at the start of the assay. (c) Specific lysis of tumor cells was measured 40 h after co-culture setup by the quantification of living GFP⁺ Raji via MACS Quant Analyzer. (d, e) The concentration of human IFN- γ (d) and IL-2 (e) was analyzed in the supernatant of 40 h co-cultures by MACSplex Cytokine 12 Kit. Graphs show data from $n = 1$ (b), $n = 5$ (c), $n = 6$ (d, e), different donors. Data shown are mean values \pm s.d. with $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$ by one-way analysis of variance (ANOVA).

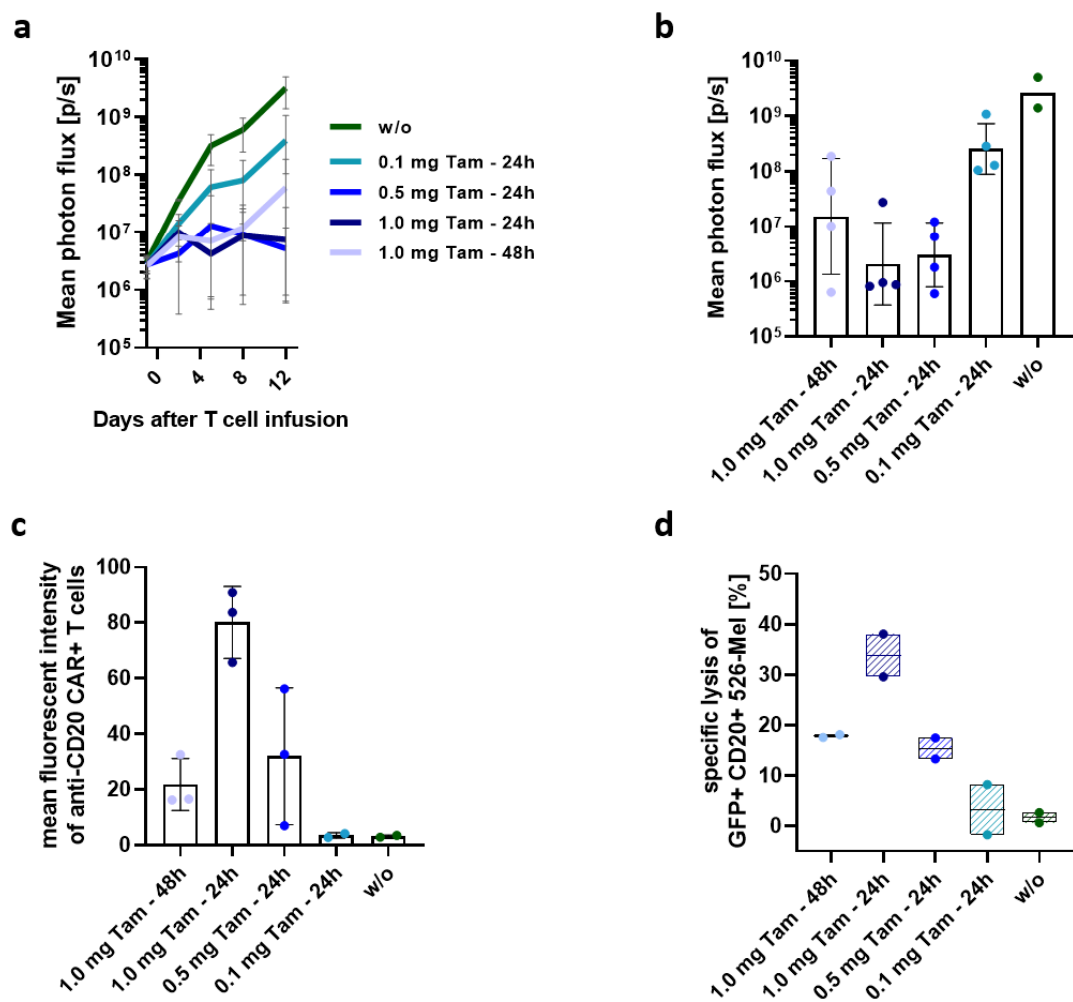


Figure S3. Modulation of inducible anti-CD20 CAR T cells in vivo by inducer dose and injection interval. NSG mice were inoculated with 4×10^5 Raji^{ffLuc} cells via tail-vein injection on day -5. On day 0, following randomization, mice were treated with 3×10^6 inducible anti-CD20 CAR T cells and a total of 1.1×10^7 total T cells. Individual groups received 1, 0.5 or 0.1 mg tamoxifen either at a 24 h or 48 h administration interval by i.p. injections starting on day -1. (a) Tumor burden expressed in mean photon flux [photons/sec] was determined by in vivo BLI for individual inducible CAR groups on day -1 (randomization) and days 2, 5, 8, and 12 after T cell injection (b) Photon flux [photons/sec] of individual mice plotted for day 12 after T cell injection. (c) For ex vivo analysis, cells were isolated from the bone marrow of femur and tibia either on day 12 (0.1 mg - 24 h, w/o) or on day 20 (1 mg - 48 h, 1 mg - 24 h, 0.5 mg - 24 h) after T cell infusion. Isolated cells were stained for anti-CD20 CAR expression using the anti-CD20 CAR detection reagent and analyzed by flow cytometry. MFI of anti-CD20 CAR⁺ T cells is plotted for individual mice. (d) Inducible CAR T cells were co-cultured with GFP⁺ CD20⁺ 526-Mel in the presence of 10 μ L plasma from mice treated with tamoxifen at indicated doses and injection intervals. Specific lysis of GFP⁺ CD20⁺ 526-Mel was measured 40 h after co-culture setup.

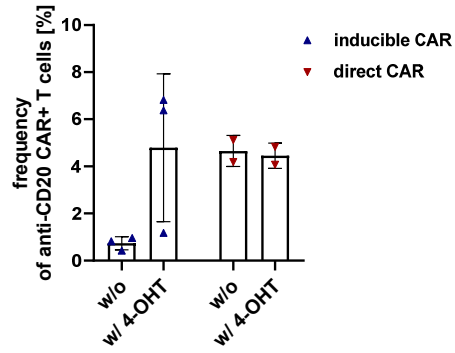


Figure S4. Induction of anti-CD20 CAR expression in ex vivo cultivated T cells Ex vivo cultivated T cells isolated via CD4⁺/CD8⁺ enrichment from the spleen of individual mice were induced with 100 nM 4-OHT for 46 h and analyzed for anti-CD20 CAR expression by flow cytometry. Data represent the frequency of anti-CD20 CAR expressing cells.

Table S1. Comparison of transduction frequencies of inducible and conventional (direct) anti-CD20 CAR T cells.

	inducible anti-CD20 CAR	direct anti-CD20 CAR
frequency of LNGFR ⁺ T cells	30.1 ± 10.9	94.4 ± 1.9
post enrichment [%]	(range 18.1 – 52.8, n=10)	(range 90.9 – 97.0, n=10)

Frequencies of LNGFR⁺ T cells post enrichment include data from $n = 10$ different donors.