**SUPPORTING INFORMATION**

**Title:** Sense-and-respond payload delivery using a novel antigen-inducible promoter improves suboptimal CAR-T activation

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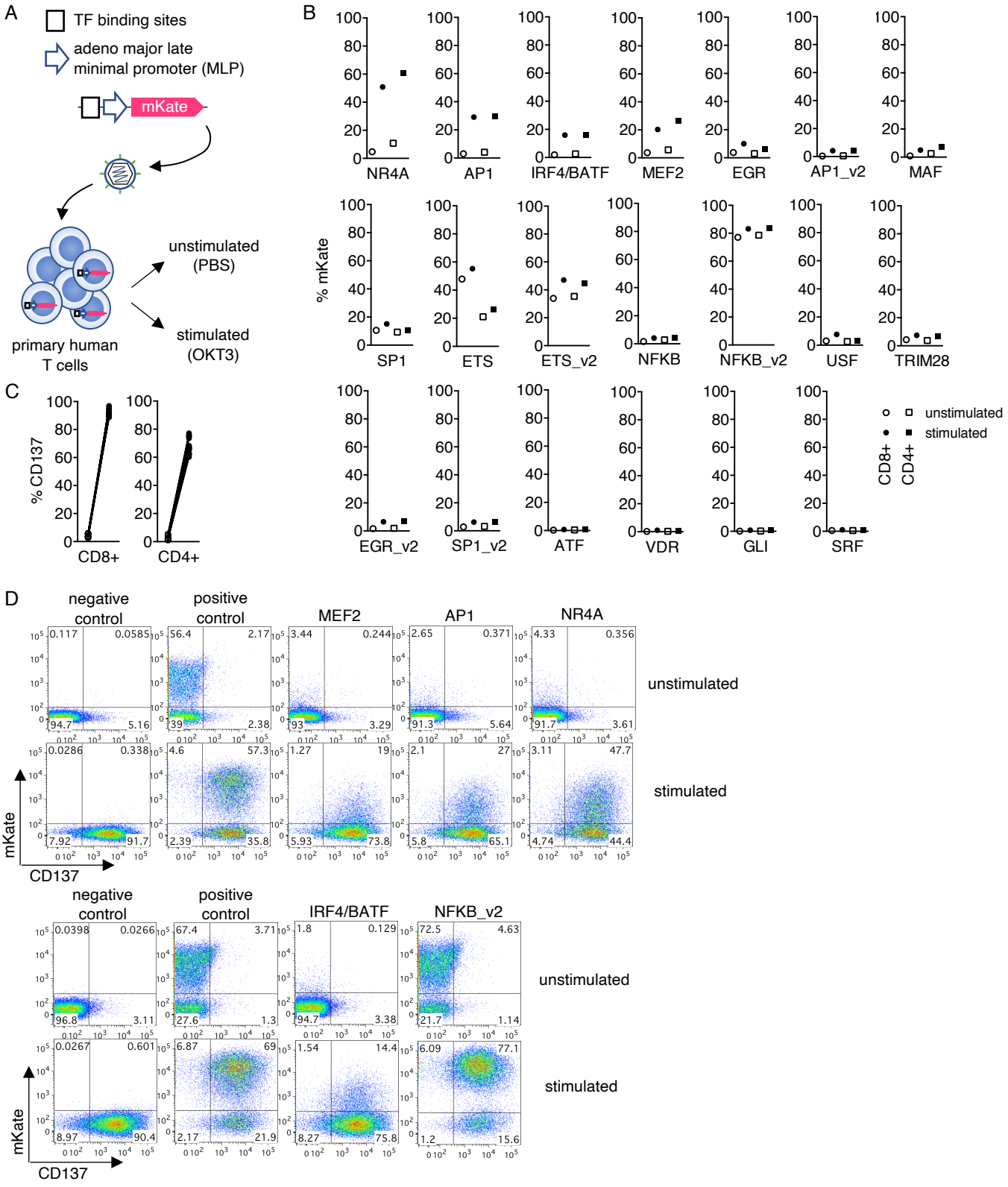



**Figure S1. Screening for novel TCR-inducible response elements.** (**a**) Schematics for vector design from the SPECS library and experimental setup. (**b**) Primary human T cells transduced with a panel of vectors were treated with PBS (unstimulated, open symbols) or OKT3 (stimulated, filled symbols) for 24 hours, and mKate fluorescence in the CD8+ (circles) or CD4+ (squares) subset was quantified. (**c**) CD137 expression after PBS (open) or OKT3 (filled) treatment. Measurements are matched by the same vector. (**d**) Raw flow plots gated on CD8+ T cells for several notable promoters are shown. The positive control vector encodes the constitutive UBC promoter to express mKate, whereas the negative control vector lacks mKate. n=1 for each promoter.

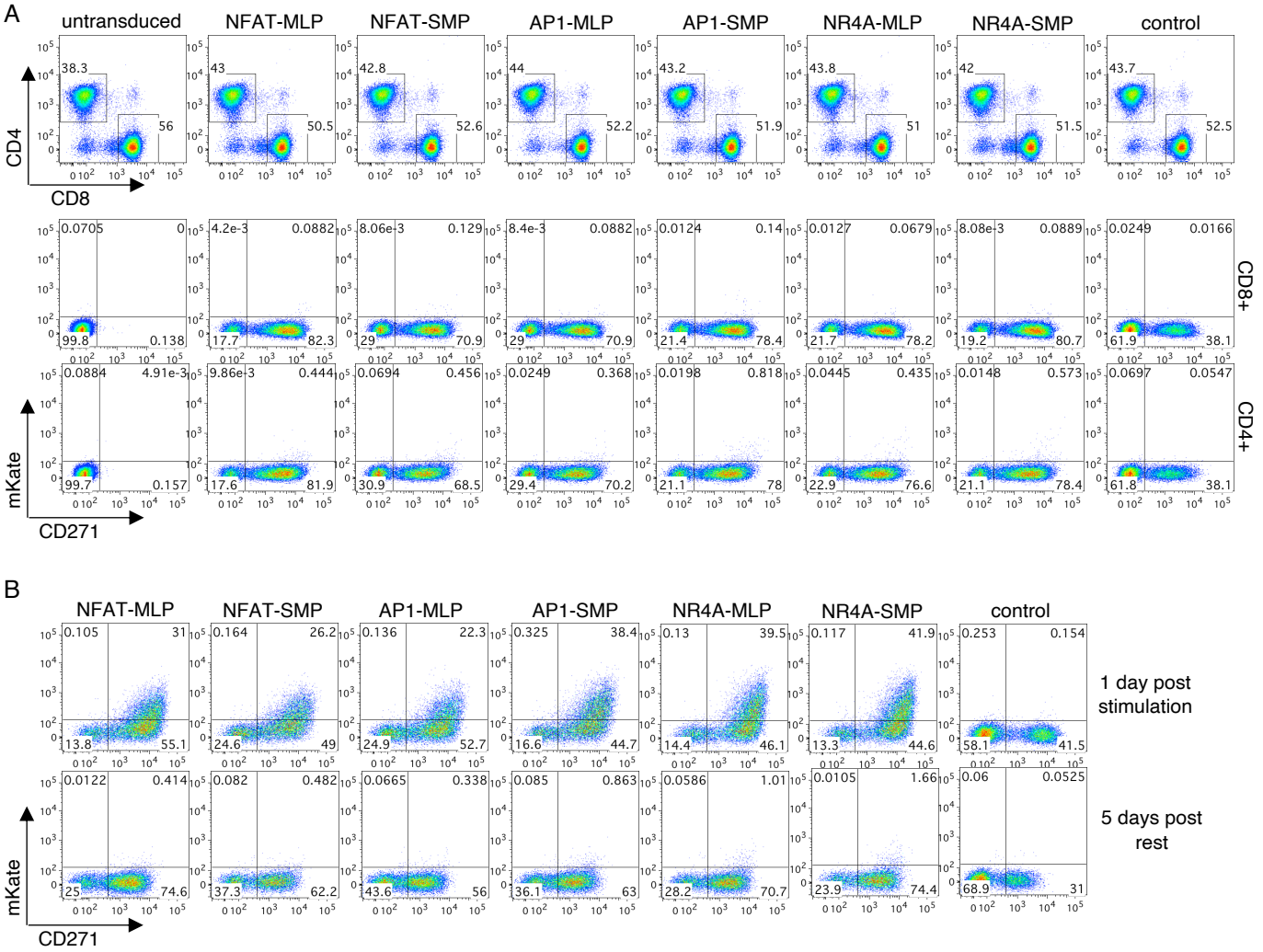



**Figure S2. Gating strategy, transduction efficiency, and mKate stability.** (**a**) CD4+ or CD8+ T cells are gated as shown on the top row. Transduction efficiency measured by CD271 positivity is shown on the bottom rows. (**b**) Fluorescence of the mKate reporter after one day of OKT3 stimulation and after 5 days of rest upon transfer to fresh wells is shown for representative CD8+ cells.

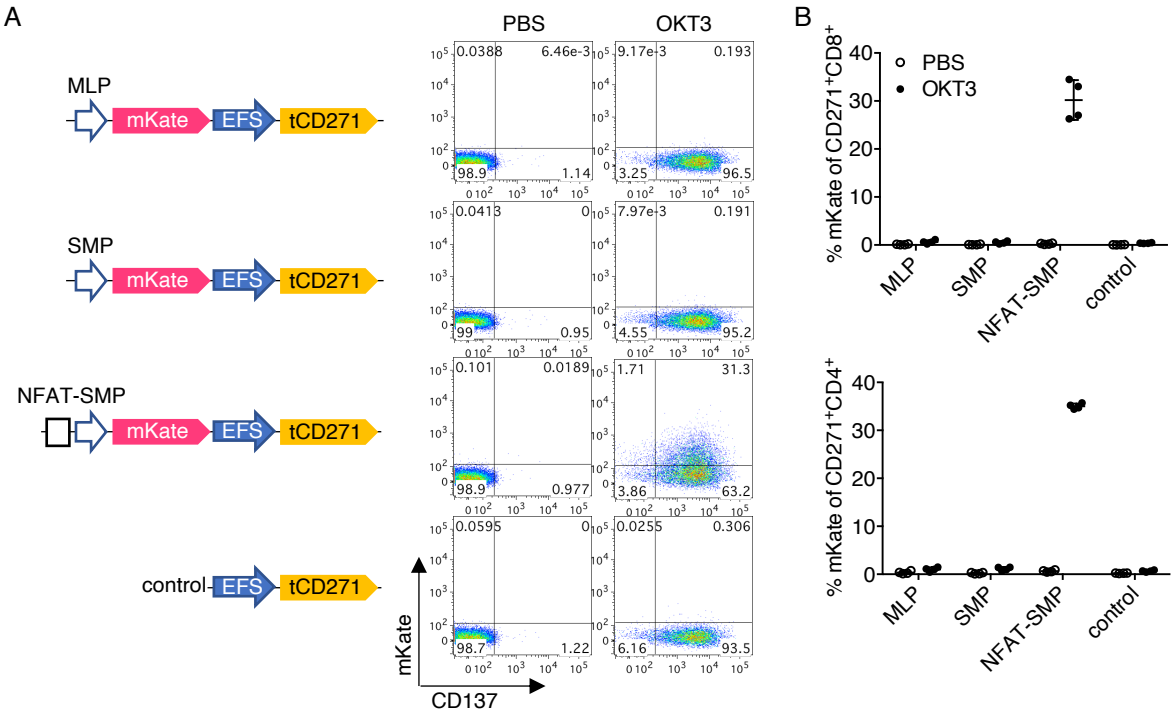



**Figure S3. The EFS constitutive promoter does not possess detectable enhancer-like activity.** (**a**) Primary human T cells transduced with the indicated vector shown on the left were treated with PBS or OKT3. CD137 and mKate upregulation was measured after 24 hours. Representative flow plots gated on CD271+CD8+ cells are shown on the right. (**b**) Quantification of data shown in panel **a**. Lines and error bars denote mean ± standard deviation. n=4 from 2 independent donors tested in 2 technical replicates.

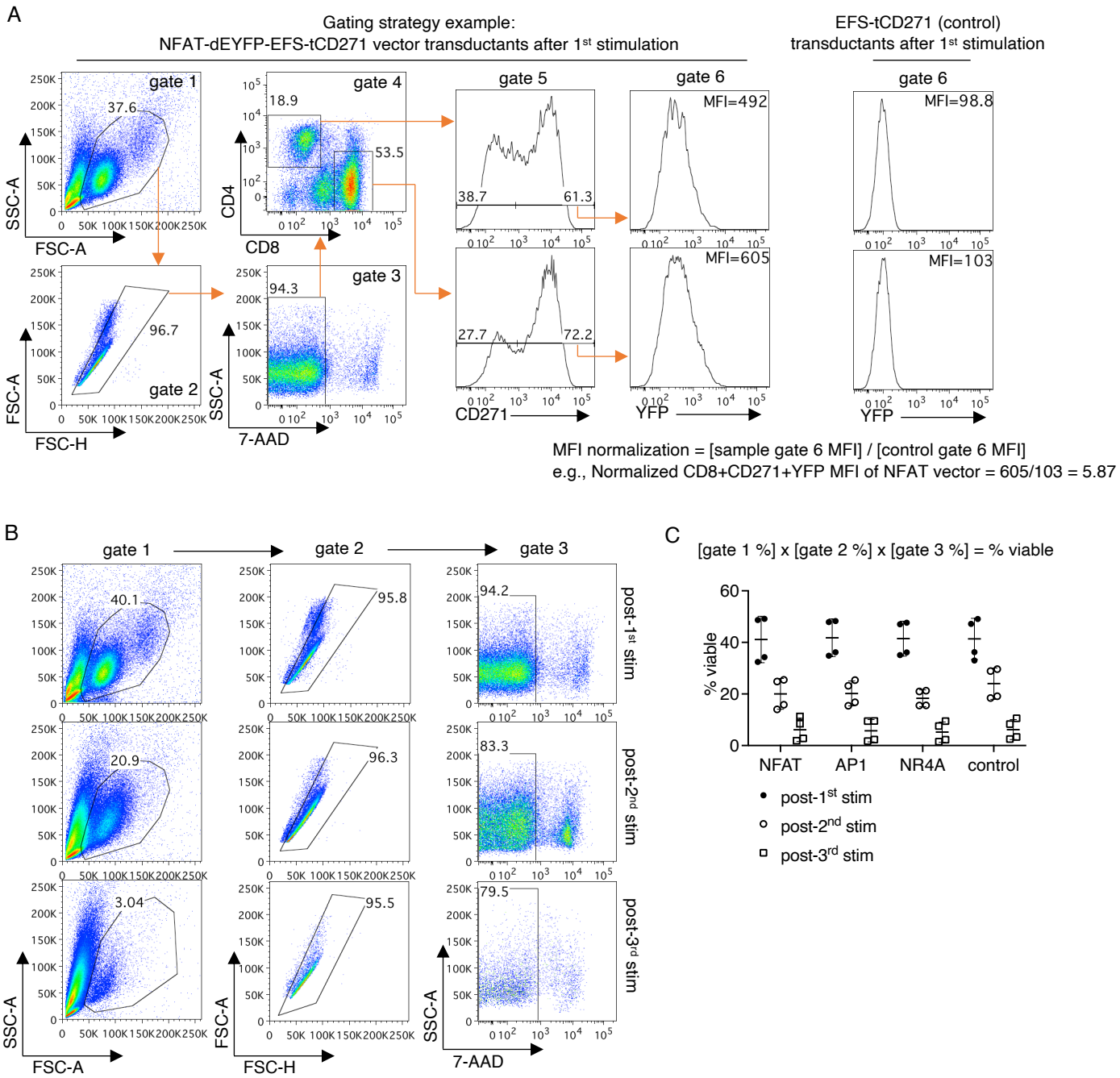



**Figure S4. Gating strategy and viability analyses for repeated stimulation of T cells transduced with inducible promoters.** (**a**) Gating strategy for analyzing experiments described in Figure 3. An example of YFP MFI normalization is shown on the right. (**b**) Representative viability gates for one NFAT promoter sample after each round of stimulation. Post stimulation here corresponds to the "OKT3" condition in Figure 3. (**c**) Quantification of sample viability based on the formula shown on the top. Lines and error bars denote mean ± standard deviation. n=4 from 2 independent donors tested in 2 technical replicates.

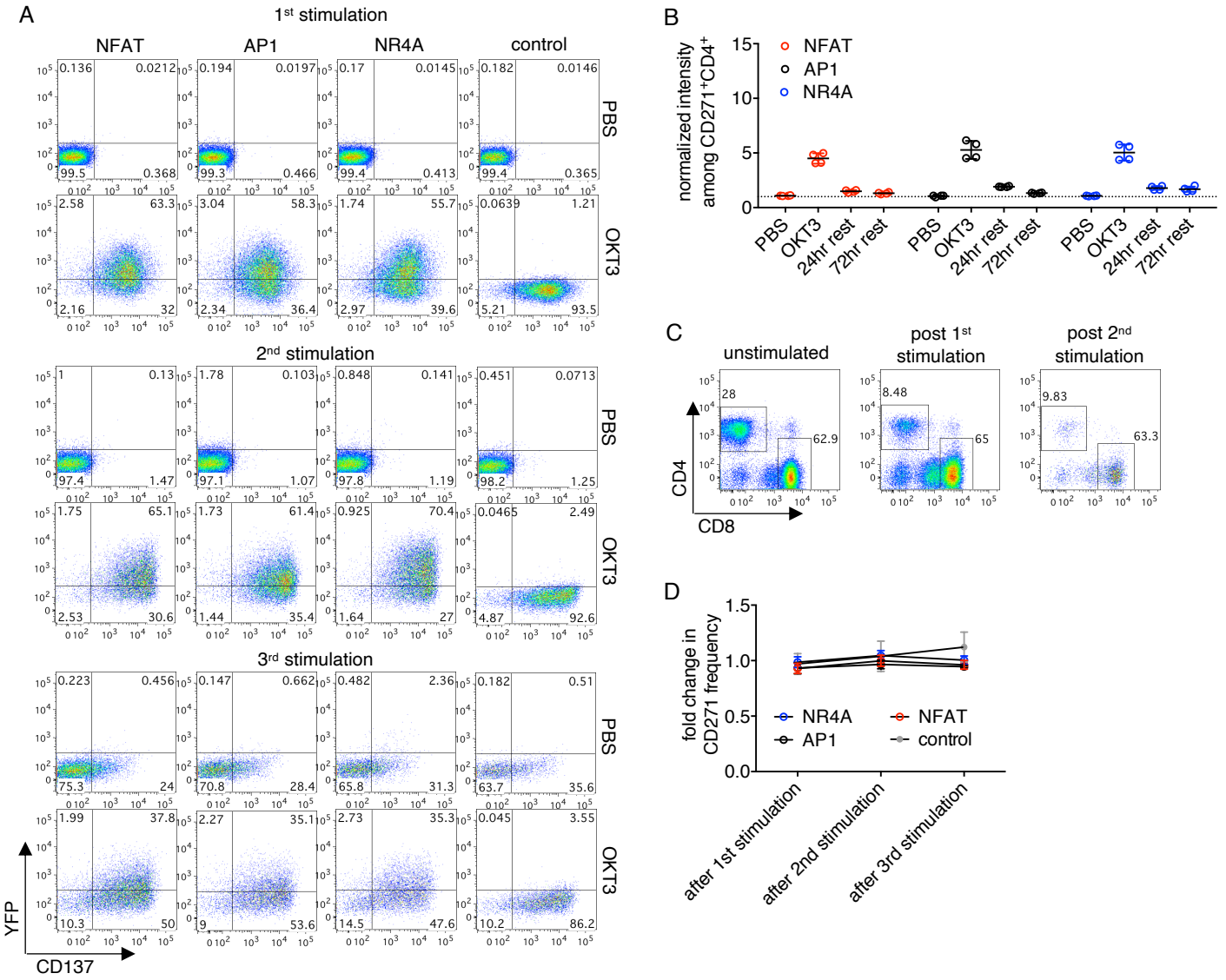



**Figure S5. Inducible promoters can be repeatedly stimulated without causing cellular toxicity.** (**a**) Representative flow plots gated on CD271+CD8+ cells for the PBS and OKT3 conditions in Figure 3b-d are shown. (**b**) Reversible promoter responses among CD271+CD4+ T cells were measured, as described in the caption of Figure 2, after the first round of stimulation. (**c**) Representative frequency of CD4+ and CD8+ subsets in culture after repeated stimulation. Gated on live cells. Due to the activation-induced cell death and biased outgrowth of CD4– cells, CD4+ cells could not be reliably analyzed after the first round of stimulation. (**d**) Change in percent of transduced (CD271+) cells was tracked through the three rounds of stimulation and remained constant. Frequency was normalized to that at the start of the experiment (prior to first stimulation). Lines and error bars denote mean ± standard deviation. n=4 from 2 independent donors tested in 2 technical replicates.

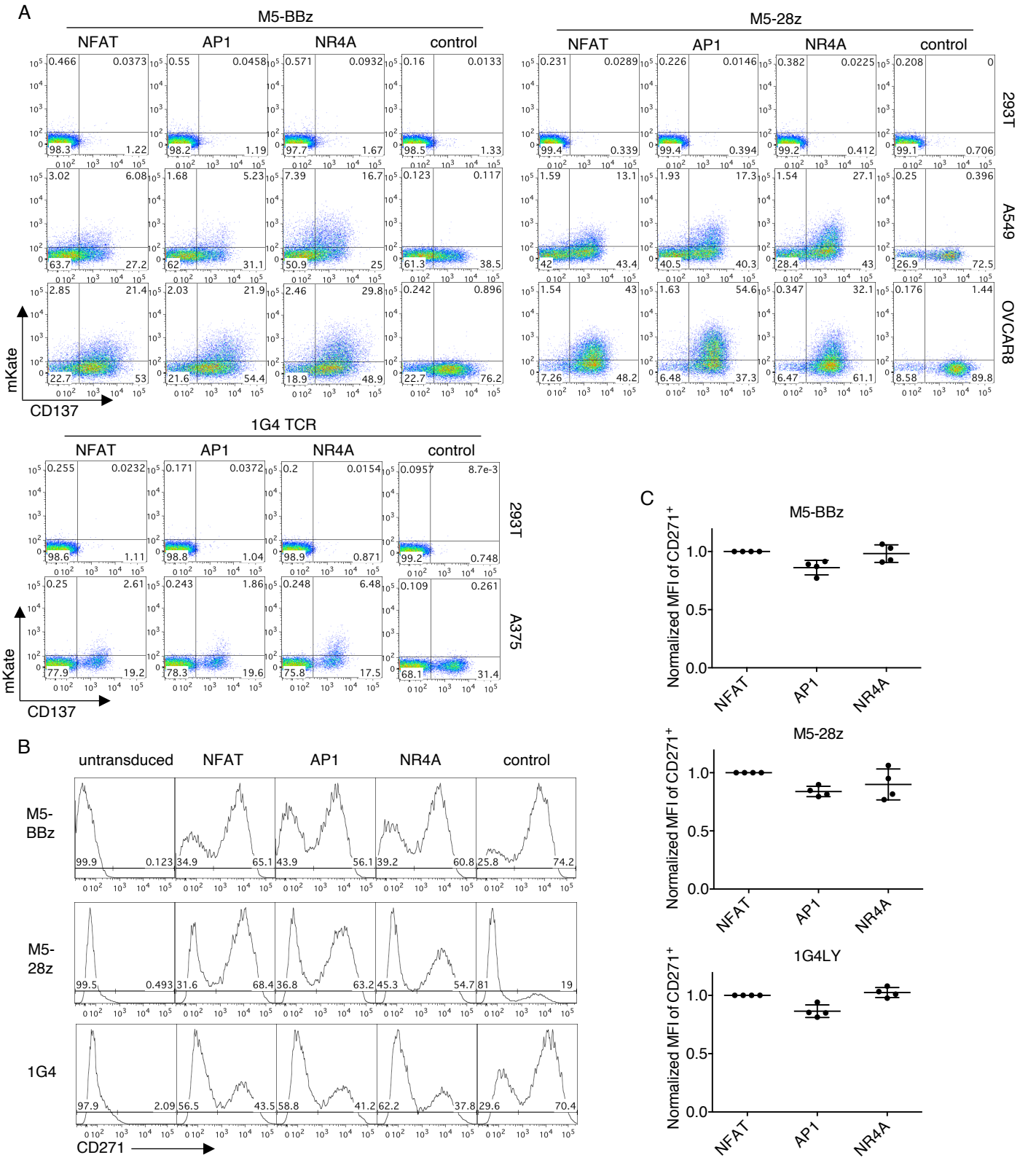



**Figure S6. Inducible promoter activities in TCR/CAR-T models.** (**a**) Representative flow plots gated on CD271+CD8+ cells for data in Figure 4 are shown. (**b**) Representative transduction efficiencies of the TCR/CAR vectors. Gated on total live cells. Note that the control M5-28z transduction was unexpectedly low. However, this does not affect the interpretation of the data, as the control M5-28z vector mainly serves as a gating control for mKate encoding vectors. (**c**) CD271 mean fluorescent intensities (MFIs) among primary T cells transduced with the vectors shown in Figure 3 were normalized to that of the respective NFAT vector.

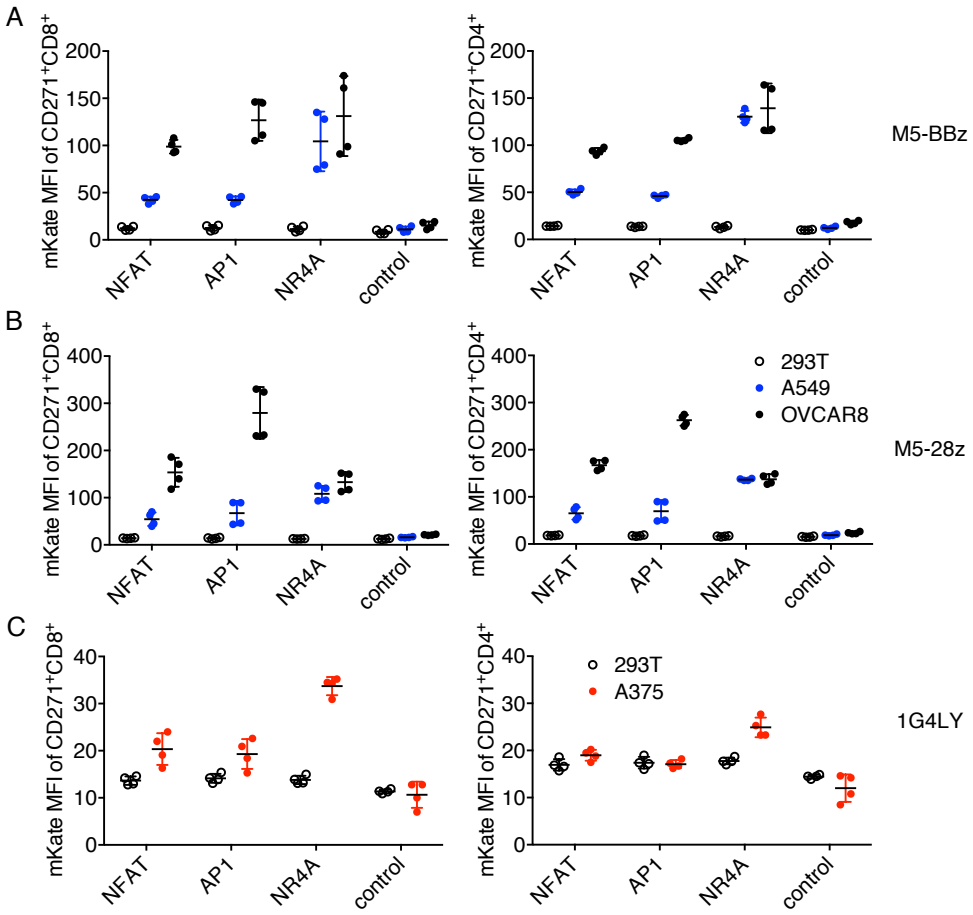



**Figure S7. Mean fluorescent intensities of mKate reporter responses.** MFIs for data shown in Figure 4b (**a**), Figure 4d (**b**), and Figure 4f (**c**). Lines and error bars denote mean ± standard deviation. n=4 from 2 independent donors tested in 2 technical replicates.

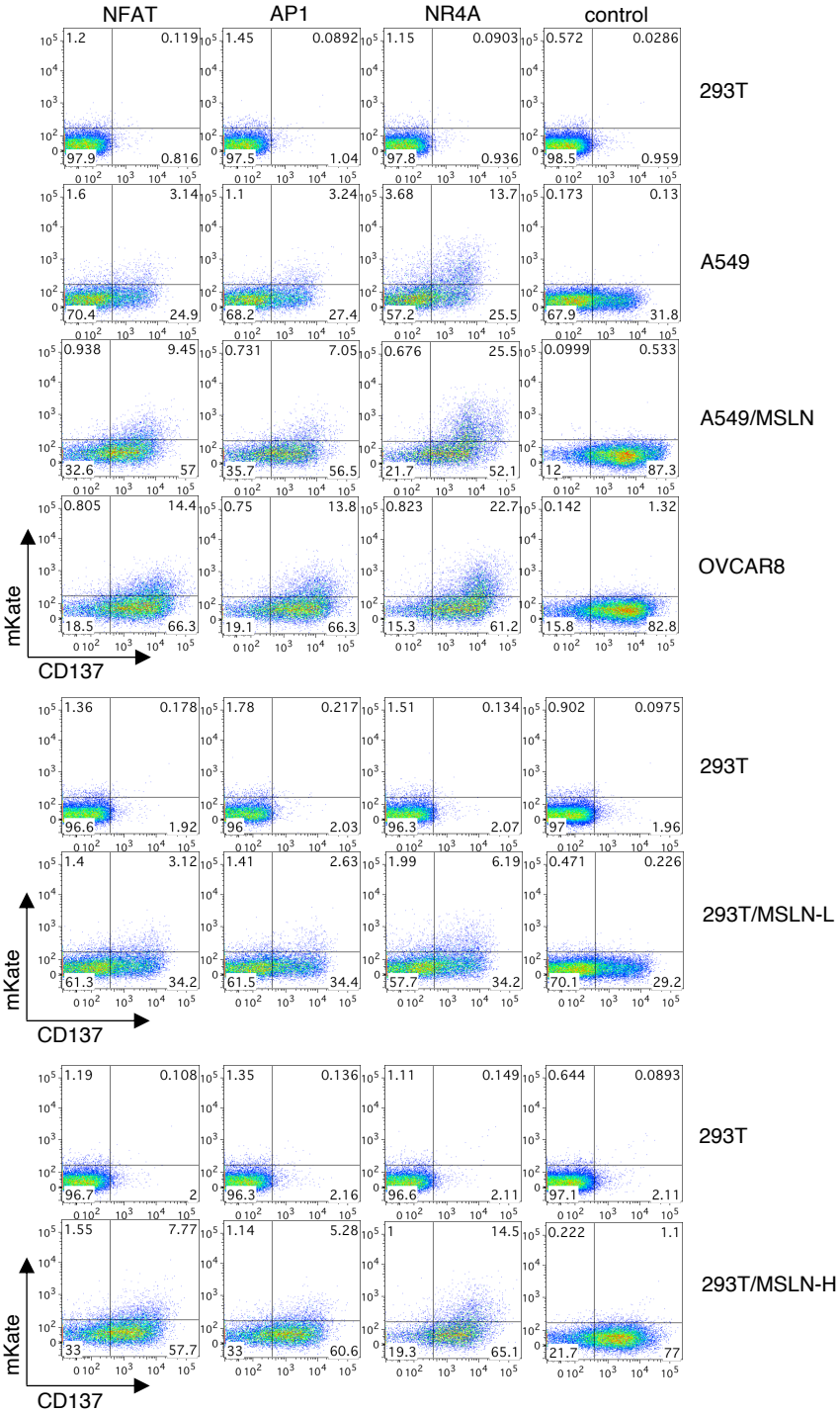
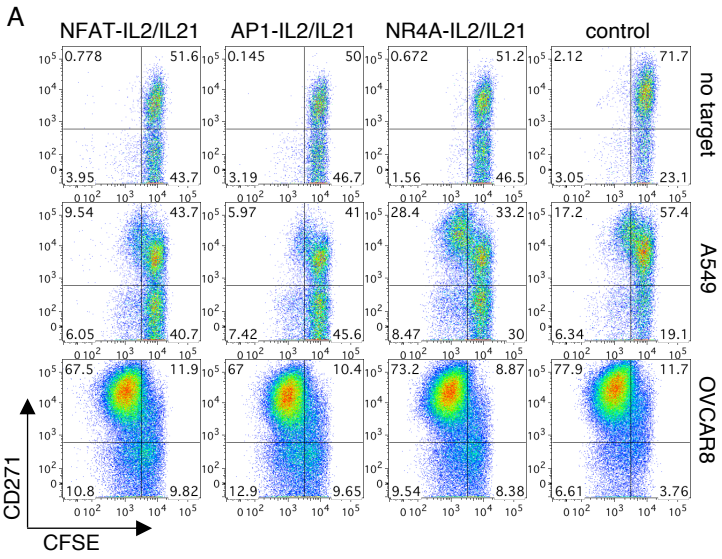



**Figure S8. M5-BBz CAR-T responses after stimulating with various mesothelin-expressing target cell lines.** Representative flow plots gated on CD271+CD8+ cells for data in Figures 5b, 5d, and 5e are shown.



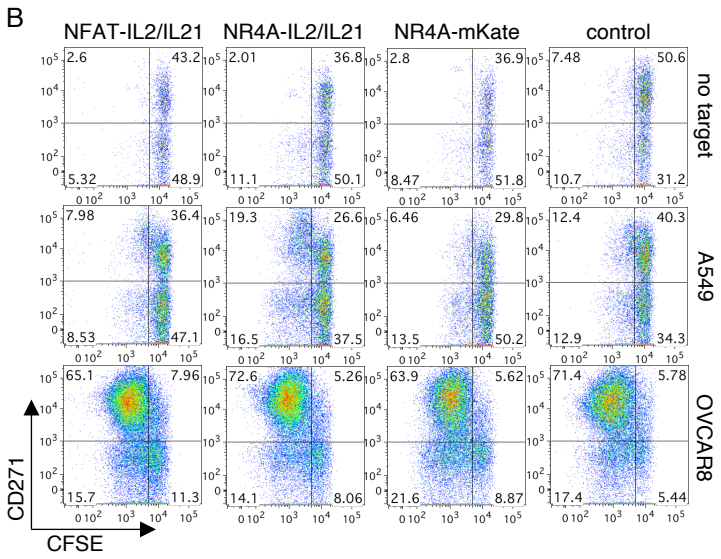




**Figure S9. Inducible expression of cytokines augments weak antigen-specific response in CAR-T cells.** (**a**) Representative flow plots gated on CD8+ cells for data in Figure 6a-c are shown. (**b**) Representative flow plots gated on CD8+ cells for data in Figure 6d-f are shown.

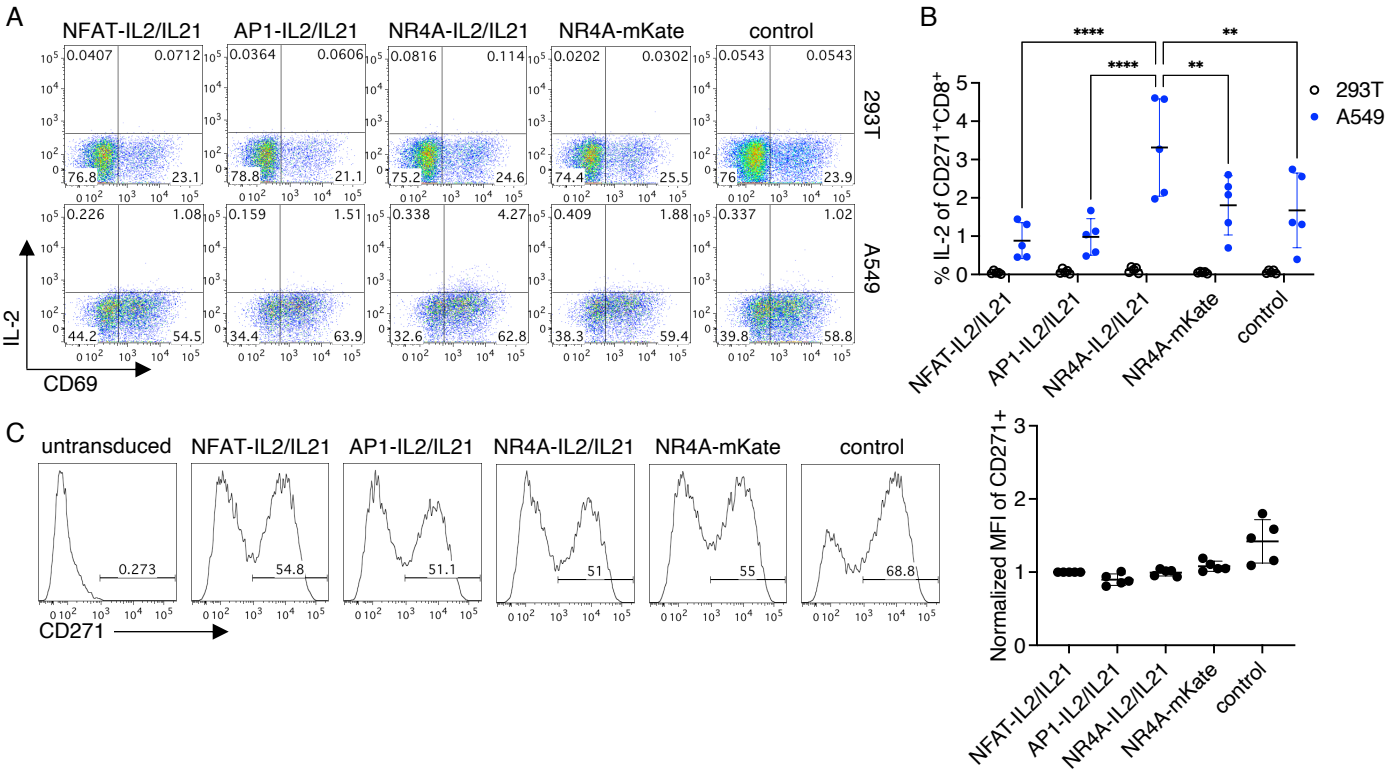



**Figure S10. Analyses of inducible payload expression in stimulated CAR-T cells.** (**a**) CAR-T cells transduced with inducible IL-2/IL-21 or control constructs were stimulated with 293T or A549 for 18 hours. Monesin was then added, and cells were cultured for another 6 hours before staining. Representative flow plots gated on CD271+CD8+ cells are shown. (**b**) Quantification for data shown in **a**. **P<0.01, ****P<0.0001 by two-way ANOVA adjusted for all possible comparisons using Tukey's test. n=5 from 3 independent donors, with 2 donors tested in 2 technical replicates. (**c**) Representative transduction efficiencies of vectors used in this experiment and Figure 6. Quantification of CD271 MFIs among transduced cells shown on the left were normalized to that of the NFAT vector. Lines and error bars denote mean ± standard deviation.

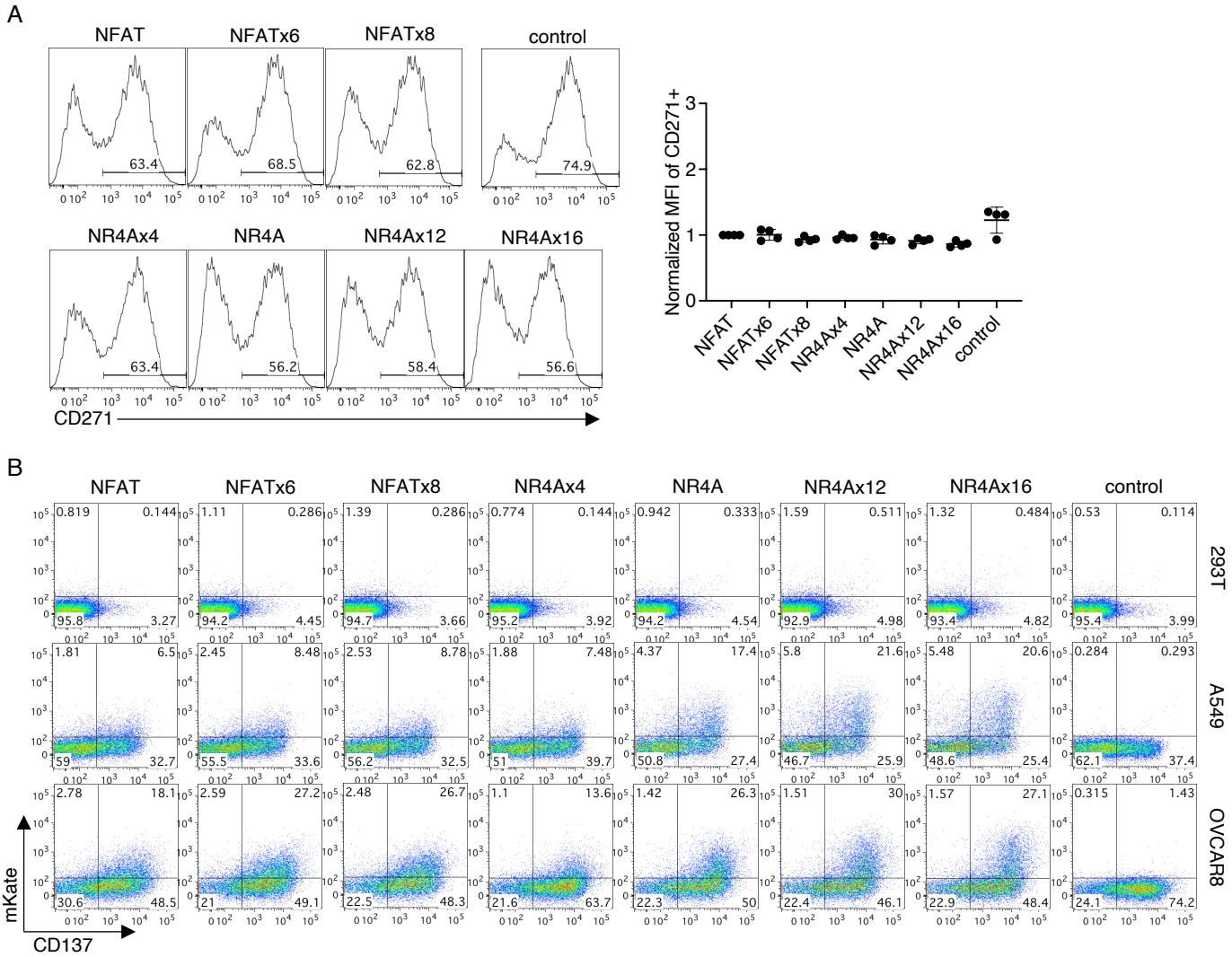



**Figure S11. NFAT and NR4A promoters with varying number of transcription factor binding sites.** (**a**) Representative transduction efficiency for T cells transduced with the NFAT, NR4A, or control constructs shown in Figure 7a. MFI of CD271 among transduced cells were normalized to that of the NFAT vector and are quantified on the right. n=4 from 2 independent donors in technical replicates. Lines and error bars denote mean ± standard deviation. (**b**) Representative plots of CD271+CD8+ cells for data shown in Figure 7b-d.

| ID | Ref. | Full sequence | Length (bp) | Motif |
|---|---|---|---|---|
| **AP1** | **26** | **TTGAGTCAAGATTGAGTCATCGTTGAGTCAGACTTGAGTCACTATTGAGTCAACTTTGAGTCATGCTTGAGTCAGTATTGAGTCA** | **85** | **TTGAGTCA** |
| AP1_v2 | | GGTGACTCATGAGAGGTGACTCATGTCGGGTGACTCATGGACGGTGACTCATGCTAGGTGACTCATGACTGGTGACTCATGTGCGGTGACTCATGGTAGGTGACTCATG | 109 | GGTGACTCATG |
| ATF | 27 | CTCTGACGTCAAGACTCTGACGTCATCGCTCTGACGTCAGACCTCTGACGTCACTACTCTGACGTCAACTCTCTGACGTCATGCCTCTGACGTCAGTACTCTGACGTCA | 109 | CTCTGACGTCA |
| EGR | 33 | CCGCCCACGCAGACCGCCCACGCTCGCCGCCCACGCGACCCGCCCACGCCTACCGCCCACGCACTCCGCCCACGCTGCCCGCCCACGCGTACCGCCCACGC | 101 | CCGCCCACGC |
| EGR_v2 | | TGCGTGGGCGAGATGCGTGGGCGTCGTGCGTGGGCGGACTGCGTGGGCGCTATGCGTGGGCGACTTGCGTGGGCGTGCTGCGTGGGCGGTATGCGTGGGCG | 101 | TGCGTGGGCG |
| ETS | 28 | CCGGAAGAGACCGGAAGTCGCCGGAAGGACCCGGAAGCTACCGGAAGACTCCGGAAGTGCCCGGAAGGTACCGGAAG | 77 | CCGGAAG |
| ETS_v2 | | CCGGAAGTGGCAGACCGGAAGTGGCTCGCCGGAAGTGGCGACCCGGAAGTGGCCTACCGGAAGTGGCACTCCGGAAGTGGCTGCCCGGAAGTGGCGTACCGGAAGTGGC | 109 | CCGGAAGTGGC |
| GLI | 34 | CCTGGGTGGTCCAGACCTGGGTGGTCCTCGCCTGGGTGGTCCGACCCTGGGTGGTCCCTACCTGGGTGGTCCACTCCTGGGTGGTCCTGCCCTGGGTGGTCCGTACCTGGGTGGTCC | 117 | CCTGGGTGGTCC |
| IRF4/BATF | 35 | GAAATGAGTCAAGAGAAATGAGTCATCGGAAATGAGTCAGACGAAATGAGTCACTAGAAATGAGTCAACTGAAATGAGTCATGCGAAATGAGTCAGTAGAAATGAGTCA | 109 | GAAATGAGTCA |
| MAF | 36 | TGCTGACTCAGCAAGATGCTGACTCAGCATCGTGCTGACTCAGCAGACTGCTGACTCAGCACTATGCTGACTCAGCAACTTGCTGACTCAGCATGCTGCTGACTCAGCAGTATGCTGACTCAGCA | 125 | TGCTGACTCAGCA |
| MEF2 | 29 | ACTATAAATAGAAGAACTATAAATAGATCGACTATAAATAGAGACACTATAAATAGACTAACTATAAATAGAACTACTATAAATAGATGCACTATAAATAGAGTAACTATAAATAGA | 117 | ACTATAAATAGA |
| **NFAT** | **22** | **GGAGGAAAAACTGTTTCATACAGAAGGCGTGGAGGAAAAACTGTTTCATACAGAAGGCGTGGAGGAAAAACTGTTTCATACAGAAGGCGTGGAGGAAAAACTGTTTCATACAGAAGGCGT** | **120** | **GGAGGAAAAACTGTTTCATACAGAAGGCGT** |
| NFKB | 30 | GGGGAAATTCCCCTAGAGGGGAAATTCCCCTTCGGGGGAAATTCCCCTGACGGGGAAATTCCCCTCTAGGGGAAATTCCCCTACTGGGGAAATTCCCCTTGCGGGGAAATTCCCCTGTA | 119 | GGGGAAATTCCCCT |
| NFKB_v2 | | AGGGGATTTCCAAGGAGAAGGGGATTTCCAAGGTCGAGGGGATTTCCAAGGGACAGGGGATTTCCAAGGCTAAGGGGATTTCCAAGGACTAGGGGATTTCCAAGGTGCAGGGGATTTCCAAGGGTA | 126 | AGGGGATTTCCAAGG |
| **NR4A** | **37** | **AAAGGTCACAGAAAAGGTCACTCGAAAGGTCACGACAAAGGTCACCTAAAAGGTCACACTAAAGGTCACTGCAAAGGTCACGTAAAAGGTCAC** | **93** | **AAAGGTCAC** |
| SP1 | 38 | AAGTGGGCGTGGCCAGAAAGTGGGCGTGGCCTCGAAGTGGGCGTGGCCGACGGCGTGGCCCTAAAGTGGGCGTGGCCACTAATGGGCGTGGCCTGCAAGTGGGCGTGGCCGTA | 113 | AAGTGGGCGTGGCC |
| SP1_v2 | | CCCTCCCCAAGGCTAGACCCTCCCCAAGGCTTCGCCCTCCCCAAGGCTGACCCCTCCCCAAGGCTCTACCCTCCCCAAGGCTACTCCCTCCCCAAGGCTTGCCCCTCCCCAAGCTGTA | 118 | CCCTCCCCAAGGCT |
| SRF | 31 | ATGCCCATATATGGAAGAATGCCCATATATGGATCGATGCCCATATATGGAGACATGCCCATATATGGACTAATGCCCATATATGGAACTATGCCCATATATGGATGCATGCCCATATATGGAGTA | 126 | ATGCCCATATATGGA |
| TRIM28 | 39 | GGTTTCTCTAGAGGTTTCTCTTCGGGTTTCTCTGACGGTTTCTCTCTAGGTTTCTCTACTGGTTTCTCTTGCGGTTTCCTCTGTAGGTTTCTCT | 94 | GGTTTCTCT |
| USF | 32 | GGTCACGTGACAGAGGTCACGTGACTCGGGTCACGTGACGACGGTCACGTGACCTAGGTCACGTGACACTGGTCACGTGACTGCGGTCACGTGACGTAGGTCACGTGAC | 109 | GGTCACGTGAC |
| VDR | 40 | GGGTTCACCGGGAGAGGGTTCACCGGGTCGGGGTTCACCGGGGACGGGTTCACCTGGCTAGGGTTCACCGGGACTGGGTTCACCGGGTGCGGGTTCACCGGGGTAGGGTTCACCGGG | 117 | GGGTTCACCGGG |

**Table S1. Transcription factor (TF) binding site sequences.** Sequences and length for the promoters studied in Supplementary Figure S1 and the conventional NFAT promoter are shown. Bolded promoters were studied in the main figures. References for association of respective TF pathway with TCR signaling are listed.

| Vector Component | Nucleotide Sequences |
|---|---|
| EFS promoter | GGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGATCCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTG GCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACA |
| Synthetic minimal promoter | TCGCGGAGACTCTAGAGGGTATATAATGGTTTAAACTTAAGCTTGGTACCGGGCCCCCGAAGCC |
| Major late minimal promoter | AGACGCTAGCGGGGGGCTATAAAAGGGGGTGGGGGCGTTCGTCCTCACTCT |
| NFATx6 | GGAGGAAAAACTGTTTCATACAGAAGGCGTGGAGGAAAAACTGTTTCATACAGAAGGCGTGGAGGAAAAACTGTTTCATACAGAAGGCGTGGAGGAAAAACTGTTTCATACAGAA GGCGTGGAGGAAAAACTGTTTCATACAGAAGGCGTGGAGGAAAAACTGTTTCATACAGAAGGCGT |
| NFATx8 | GGAGGAAAAACTGTTTCATACAGAAGGCGTGGAGGAAAAACTGTTTCATACAGAAGGCGTGGAGGAAAAACTGTTTCATACAGAAGGCGTGGATCCTAGTCTCAGGAGGAAAAACTGTTTCATACAGAAGGCGTGGAGGAAAAACTGTTTCATACAGAAGGCGTGGAGGAAAAACTGTTTCATACAGAAGGCGTGGAGGA AAAACTGTTTCATACAGAAGGCGT |
| NR4Ax4 | AAAGGTCACAGAAAAGGTCACTCGAAAGGTCACGACAAAGGTCAC |
| NR4Ax12 | AAAGGTCACAGAAAAGGTCACTCGAAAGGTCACGACAAAGGTCACCTAAAAGGTCACACTAAAGGTCACTGCAAAGGTCACGTAAAAGGTCACCATAAAGGTCACGCTAAAGGTC ACCTGAAAGGTCACGATAAAGGTCAC |
| NR4Ax16 | AAAGGTCACAGAAAAGGTCACTCGAAAGGTCACGACAAAGGTCACCTAAAAGGTCACACTAAAGGTCACTGCAAAGGTCACGTAAAAGGTCACCATAAAGGTCACGCTAAAGGTC ACCTGAAAGGTCACGATAAAGGTCACACTAAAGGTCACTACAAAGGTCACGAGAAAGGTCACCGTAAAGGTCAC |
| | |
| | Amino Acid Sequences |
| Truncated CD271 | MDGPRLLLLLLLGVSLGGAKEACPTGLYTHSGECCKACNLGEGVAQPCGANQTVCEPCLDSVTFSDVVSATEPCKPCTECVGLQSMSAPCVEADDAVCRCAYGYYQDETTGRCEA CRVCEAGSGLVFSCQDKQNTVCEECPDGTYSDEANHVDPCLPCTVCEDTERQLRECTRWADAECEEIPGRWITRSTPPEGSDSTAPSTQEPEAPPEQDLIASTVAGVVTTVMGSS QPVVTRGTTDNLIPVYCSILAAVVVGLVAYIAFKRWNS |
| mKate | MVSKGEELIKENMHMKLYMEGTVNNHHFKCTSEGEGKPYEGTQTMRIKVVEGGPLPFAFDILATSFMYGSKTFINHTQGIPDFFKQSFPEGFTWERVTTYEDGGVLTATQDTSLQ DGCLIYNVKIRGVNFPSNGPVMQKKTLGWEASTEMLYPADGGLEGRSDMALKLVGGGHLICNLKTTYRSKKPAKNLKMPGVYYVDRRLERIKEADKETYVEQHEVAVARYCDLPS KLGHKLN |
| Destabilized EYFP | MVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTFGYGLQCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKF EGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSYQSKLSKDPNEKRDHMVLLEFVTAAGI TLGMDELYKGSTGSSHGFPPEVEEQDDGTLPMSCAQESGMDRHPAACASARINV |
| M5 single-chain variable fragment | MALPVTALLLPLALLLHAARPQVQLVQSGAEVEKPGASVKVSCKASGYTFTDYYMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVY YCASGWDFDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSDIVMTQSPSSLSASVGDRVTITCRASQSIRYYLSWYQQKPGKAPKLLIYTASILQNGVPSRFSGSGSGTDFTLT ISSLQPEDFATYYCLQTYTTPYFGPGTKVEIK |
| 28z | IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSA DAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR |
| BBz | PAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRS ADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR |
| 1G4LY TCR alpha chain | METLLGLLILWLQLQWVSSKQEVTQIPAALSVPEGENLVLNCSFTDSAIYNLQWFRQDPGKGLTSLLLIQSSQREQTSGRLNASLDKSSGRSTLYIAASQPGDSATYLCAVRPLY GGSYIPTFGRGTSLIVHPYIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDV KLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLWSS |
| 1G4 TCR beta chain | MSIGLLCCAALSLLWAGPVNAGVTQTPKFQVLKTGQSMTLQCAQDMNHEYMSWYRQDPGMGLRLIHYSVGAGITDQGEVPNGYNVSRSTTEDFPLRLLSAAPSQTSVYFCASSYV GNTGELFFGEGSRLTVLEDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQV QFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSESYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDSRG |
| IL2-P2A-IL21 | MYRMQLLSCIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLE LKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLTRAKRSGSGATNFSLLKQAGDVEENPGPMWLQSLLLLGTVACSISQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAP EDVETNCEWSAFSCFQKAQLKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLERFKSLLQKMIHQHLSSRTHGSEDS |

**Table S2. Additional vector component sequences.** Nucleotide sequences for regulatory element not listed in Supplementary Table S1, and protein amino acid sequences used in this study are shown.