Targeted shock-and-kill HIV-1 gene therapy approach combining CRISPR activation, suicide gene tBid and retargeted adenovirus delivery

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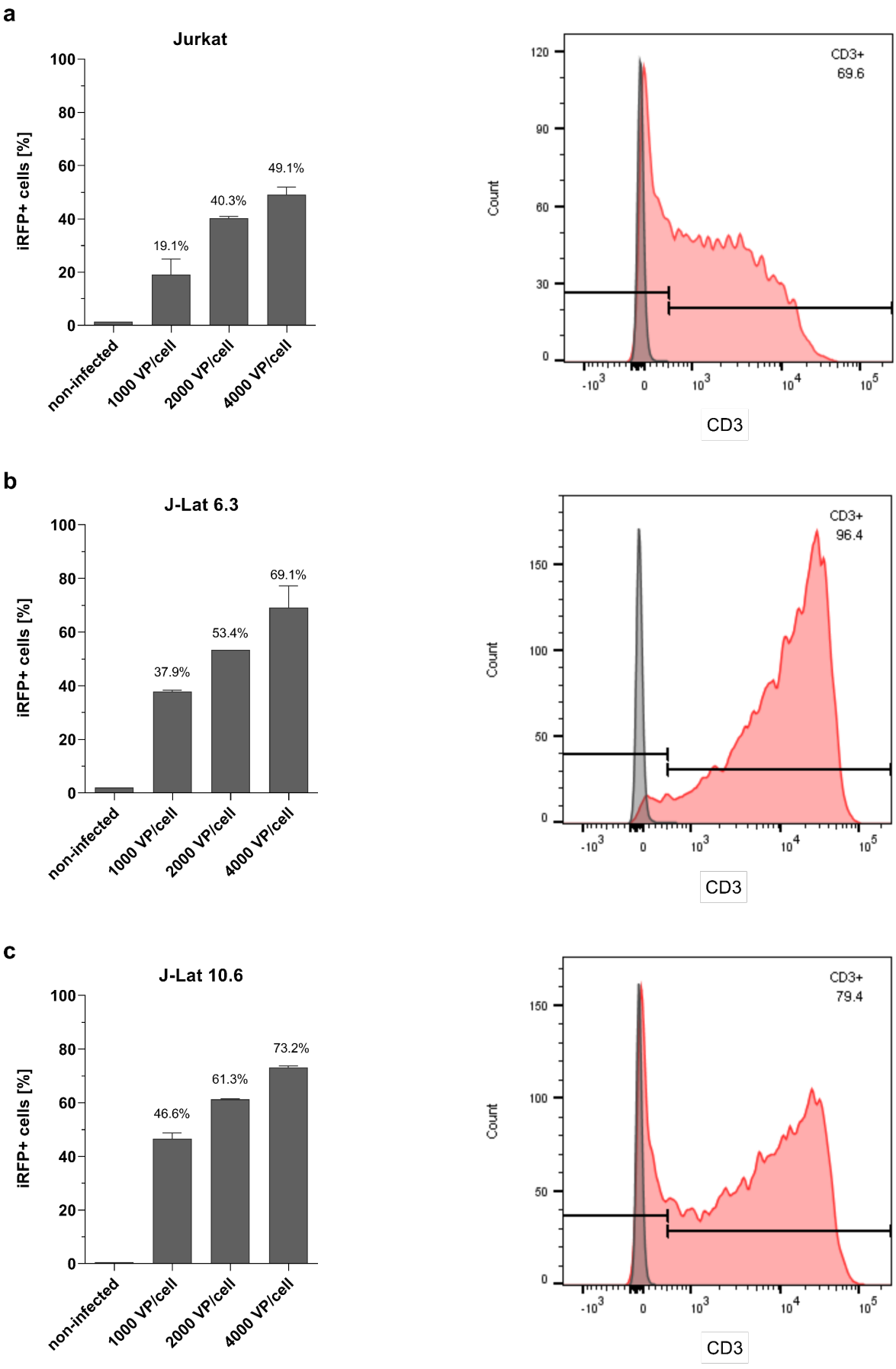
Supplementary Material

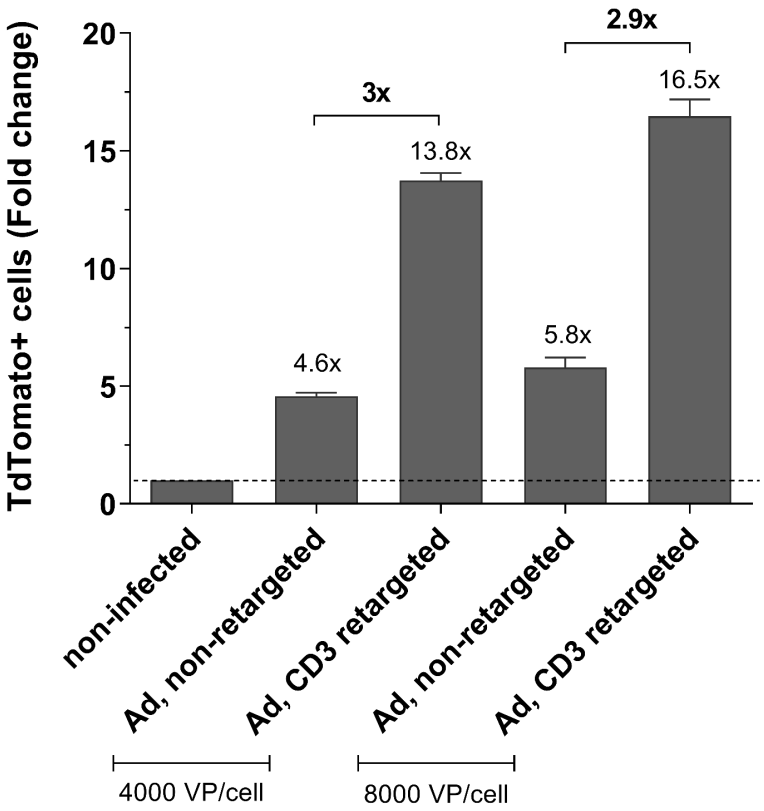
Table S1: Oligonucleotides (Microsynth, Switzerland)

Primer name	**Sequence (5'-3')**
gRNA-Con fw	CACCATGTACACTCGGCGCAAAGT
gRNA-Con rev	AAACACTTTGCG CCGAGTGTACATC
dCasVPR_U6gRNA_fw	GCTTGACCGACAATTCGGTACCGAGGGCCTATTTCCC ATGATTCC
U6gRNA_dCas9VPR_rev	ATTCTTCATGCAATTGGAAAAAAAGCACCGACTCG
PS1_CRISPRa_fw	ATAGCGCGTAATACTGGAGGGCCTATTTCCCATGATT CC
PS1_CRISPRa_rev	ACTCGAGGCG GCCGCGGAAGCGGCCTTAGTTATTCAGCG
GibA_tBid-PS1_fw	TACTCATAGCGCGTAATACTGGTACCAGTTCTACTTA CACCAGGAAAGG
GibA_tBid-PS1_rev	TCTAGACTCGAGGCGGCCGCGGTACTCACTGCAACC TCTACCTC
InFusion_polyA_fw	CTAGAGCGGCCTCGAAATAAAAGATCTTTATTTTCAT TAGATCTGTG
InFusion_polyA_rev	TATCTCTAGACTCGACTCTAGACACACAAAAAACCA ACAC
GibsAss_iRFP670_fwd	CCGAACAGGGACTTGAAAGCGAAAGGCGGCCGCAT GGCC AGAAAGGTGGAC
GibsAss_iRFP670_rev	CCGCGCGCTTCAGCAAGCCGAGTCCCGATCGT CATCATCTCTGGTGGTGAG

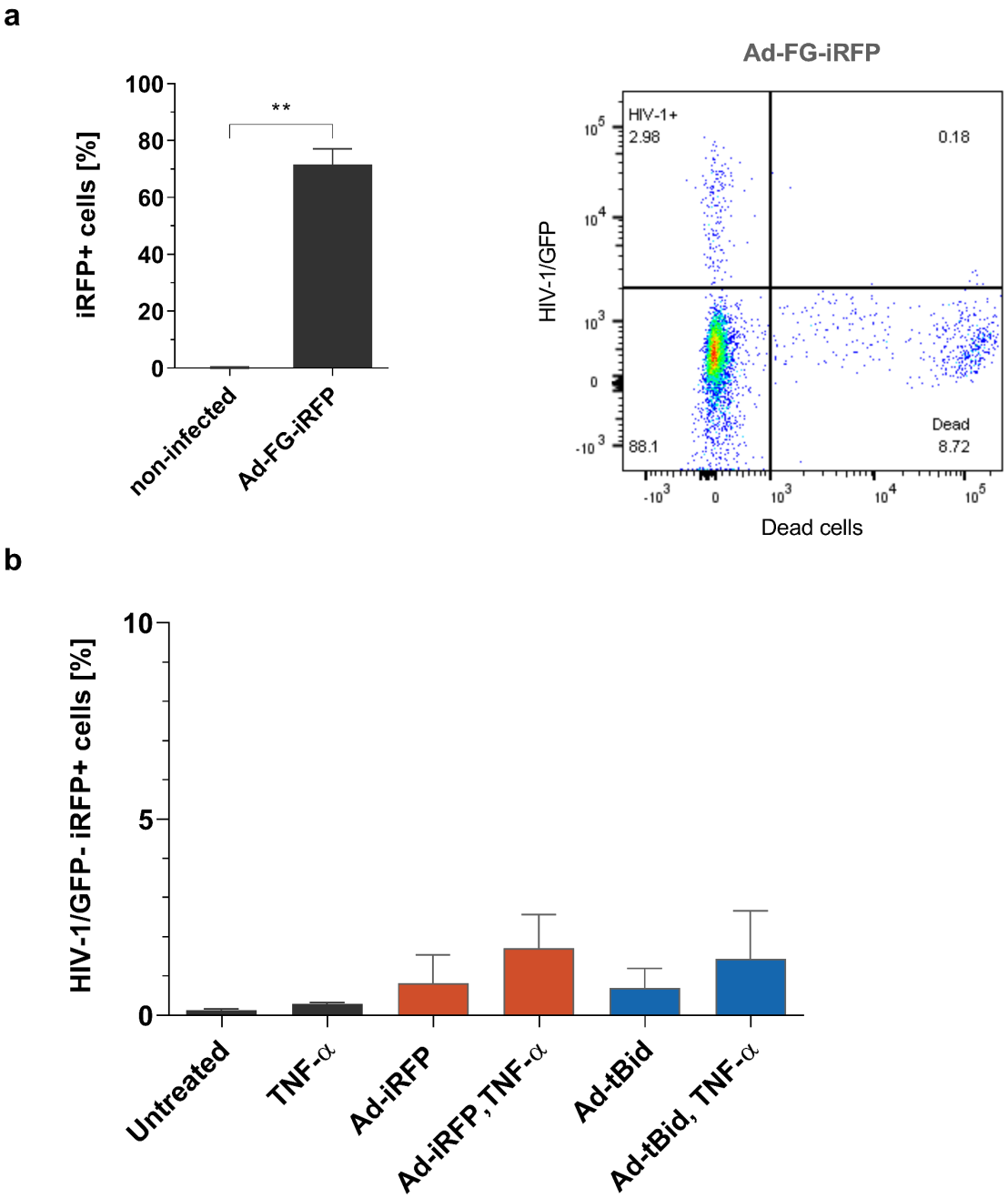


Supplementary figure S1: Adenovirus transduction with CD3-retargeting adapters of various CD3-expressing T cell lines. (a) Jurkat cells, (b) J-Lat 6.3 cells and (c) J-Lat 10.6 cells

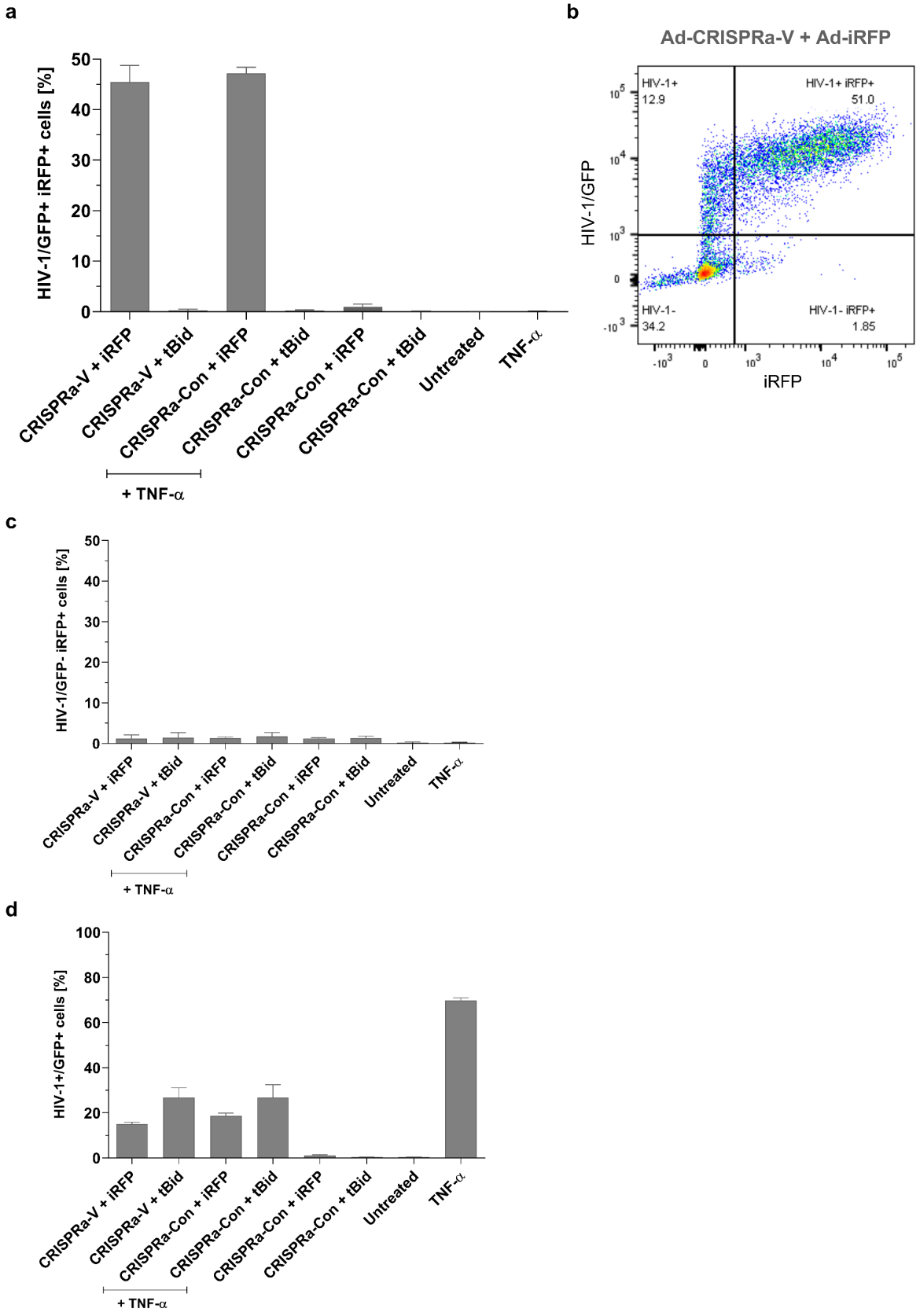
were stained with a CD3 antibody (CD3 (HIT3a clone)-APC, Biolegend 300312) for 30 min at 4°C to determine CD3 expression levels. Prior to and after staining, cells were washed twice with PBS and measured by flow cytometry. Ad transduction was assessed by transducing $1x10^5$ cells with $1x10^3$, $2x10^3$ or $4x10^3$ VP/cell of CD3-retargeted Ad-FG-iRFP. Ad coating was performed by preincubating Ads with CD3-retargeting adapters in a 50-fold molar excess over adenovirus fiber knob for 1.5 h on ice before addition to cells. Flow cytometry was performed at 48 h post-transduction measuring iRFP expression to asses Ad transduction efficiency and is shown as iRFP+cells [%] ±SD, n=2.



Supplementary figure S2: Effect of CD3-retargeting on Ad transduction efficiency. $1x10^5$ Jurkat cells were transduced with $4x10^3$ or $8x10^3$ VP/cell of CD3-retargeted or non-retargeted Ad-TdTomato. Ad coating was performed by preincubating Ads with CD3-retargeting adapters in a 50-fold molar excess over adenovirus fiber knob for 1.5 h on ice before addition to cells. Ad transduction efficiency was measured by flow cytometry 48 h post-transduction. Shown is the fold change of Ad transduction efficiency over untreated cells as TdTomato+ cells (fold change) ±SD with n=2.

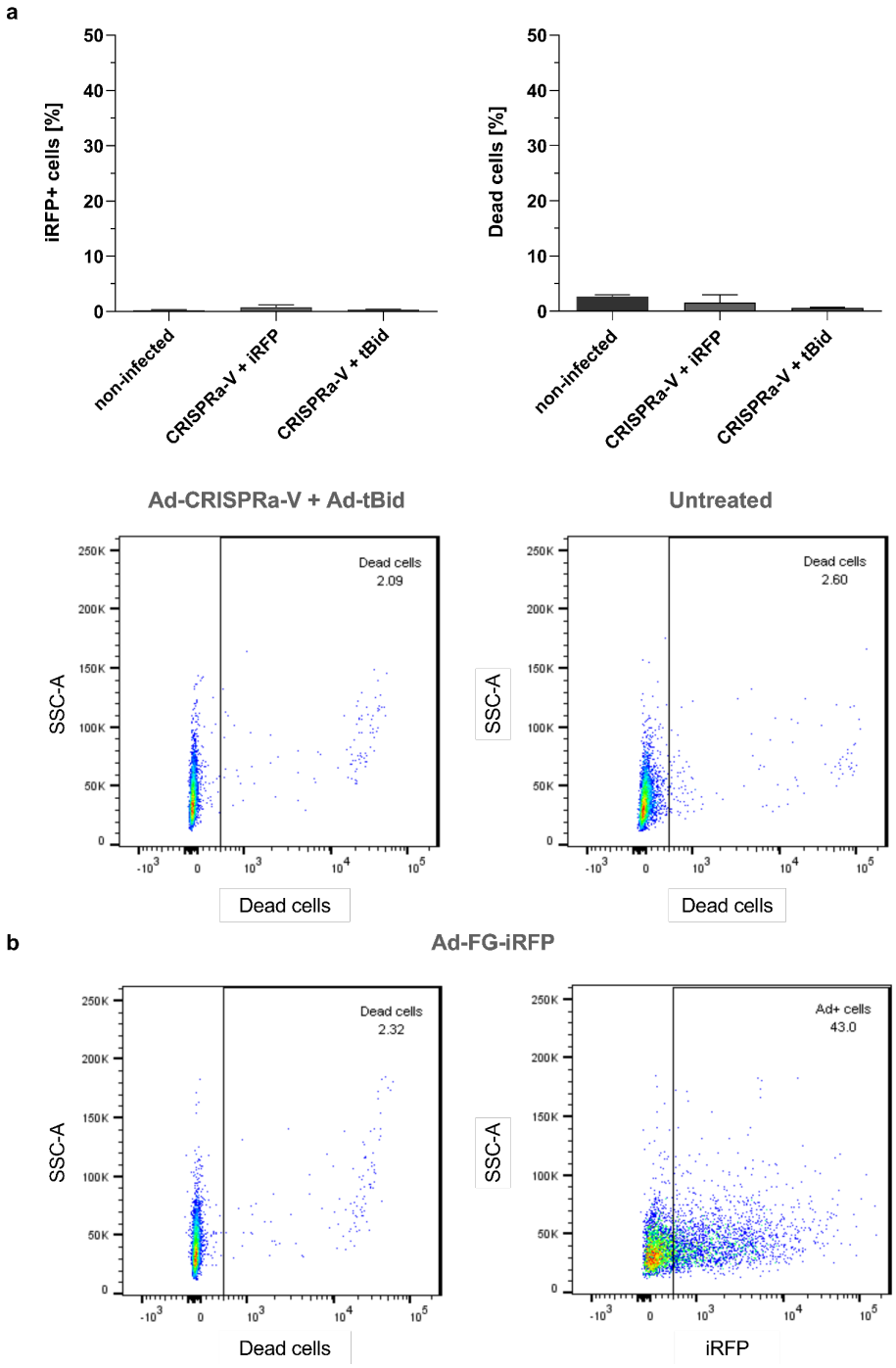


Supplementary figure S3: HIV-1 specific killing by Ad-tBid in latently infected J-Lat 10.6 cells. 1x10^5 J-Lat 10.6 cells were transduced with 4x10^3 VP/cell of retargeting adapter-coated Ad-iRFP, Ad-tBid or Ad-FG-iRFP. Ad coating was performed by preincubating Ads with CD3-retargeting adapters in a 50-fold molar excess over adenovirus fiber knob for 1.5 h on ice before addition to cells. HIV-1 latency reversal was achieved by adding TNF-α [10 ng/ml] 24 h post transduction. 48 h post-transduction cells were stained with the dead cell zombie dye and HIV-1 latency reversal as well as suicide construct transgene activation (iRFP or tBid) and cell death were measured by flow cytometry. (a) Shown is the Ad transduction efficiency ±SD with the Ad-FG-iRFP reporter virus as iRFP+ cells with n=2 from independent experiments, as well as an exemplary flow cytometry plot of HIV-1 latency reversal and cell death of the same sample. *P<0.033 and **P<0.002 indicate statistical significance between two samples by paired, two-tailed t-test.(b) No leaky expression of the iRFP transgene is observed shown as HIV-1/GFP negative iRFP+ single-positive cells [%] ±SD with n=3. Black bars (Untreated, TNF- α) show non-infected cells. Data shown from three independent experiments.



Supplementary figure S4: Targeted shock and kill with CD3-retargeted Ads in latently infected J-Lat 10.6 cells. 1x10^5 J-Lat 10.6 cells were co-transduced with a total of 8x10^3 VP/cell with two different retargeted Ads at the same time, either Ad-tBid or Ad-iRFP and Ad-CRISPRa-V or Ad-CRISPRa-Con. Ad coating was performed by preincubating Ads with CD3-

retargeting adapters in a 50-fold molar excess over adenovirus fiber knob for 1.5 h on ice before addition to cells. HIV-1 latency reversal in the CRISPRa-Con and Cell controls was achieved by adding TNF-α [10 ng/ml] 24 h post transduction. At 48 h post-transduction cells were stained with the dead cell zombie dye and measured by flow cytometry. (a) Shown are simultaneous HIV-1 latency reversal and iRFP transgene activation as HIV-1+/GFP+ iRFP+ double-positive cells [%] ±SD with n=3 from three independent experiments.(b) Exemplary flow cytometry plots of HIV-1+/GFP+ iRFP+ double-positive cells in Ad-CRISPRa-V and Ad-iRFP co-transduced cells. (c) No leaky expression of the iRFP transgene is observed shown as HIV-1/GFP negative iRFP+ single-positive cells [%] ±SD with n=3 from three independent experiments. (d) Single Ad-CRISPRa-V transduced cells observed as HIV-1/GFP+ single positive cells [%] ±SD with n=3 from three independent experiments. Black bars (Untreated, TNF- α) show non-infected cells.



Supplementary figure S5: Ad-CRISPRa-V in combination with Ad-tBid or Ad-iRFP does not activate the expression of iRFP and tBid from the suicide vector. 1x10^5 Jurkat cells were co-transduced with a total of 8x10^3 VP/cell with CD3-retargeted Ad-CRISPRa-V and either Ad-tBid or Ad-iRFP. Ad-FG-iRFP control was transduced with a total of 4x10^3 VP/cell. Ad coating was performed by preincubating Ads with CD3-retargeting adapters in a 50-fold molar excess over adenovirus fiber knob for 1.5 h on ice before addition to cells. HIV-1 latency reversal in the CRISPRa-Con and Cell controls was achieved by adding TNF-α [10 ng/ml] 24 h post transduction. At 48 h post-transduction cells were stained with the dead cell zombie dye and measured by flow cytometry. (a) Shown are iRFP transgene expression as iRFP+ cells [%] and cell death as dead cells [%] in co-transduced cells, ±SD with n=3 as well as exemplary flow cytometry plots. (b) Flow cytometry plots of Ad-FG-iRFP transduction control showing cell death and Ad transduced cell population as iRFP+ cells.