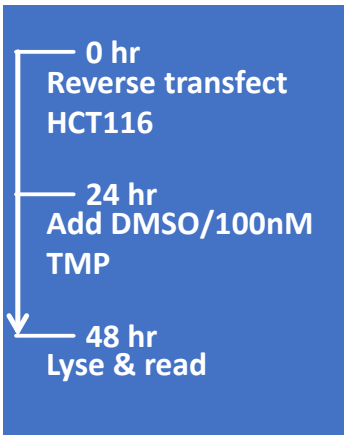
A



B

Switch	Uninduced activity (%Rluc)	Induced activity (%Rluc)	Max fold change	Time to max (hr)
NDHFR	5%	24%	6	12
CDHFR	34%	91%	3	12

C

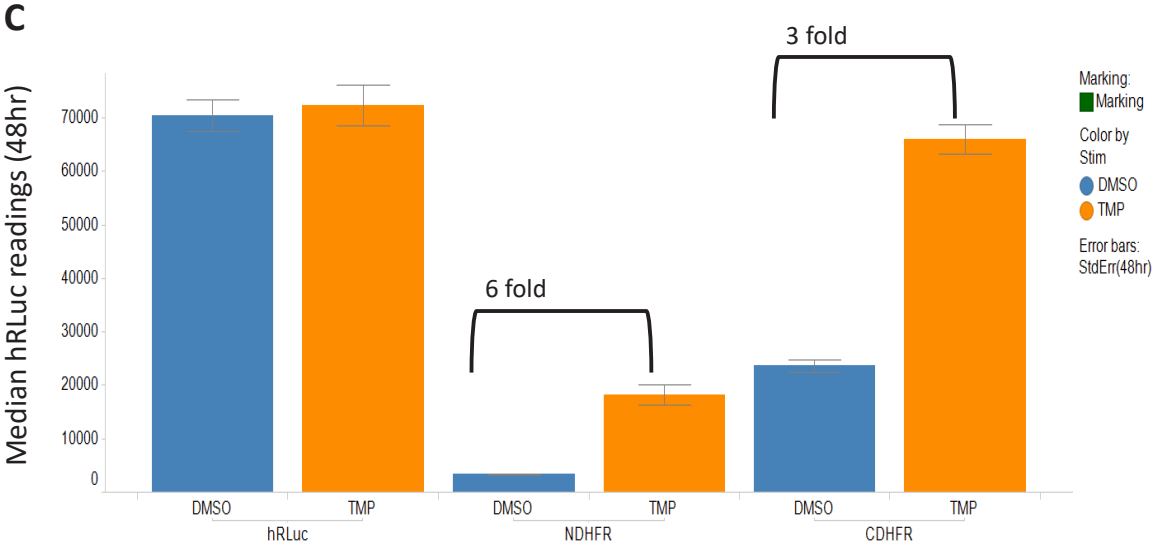


Figure S1 Compared the regulation efficacy and potency of NDHFR and CDHFR in HCT116 cells. (A) Outline of the experiment procedure. (B) Table to compare the efficacy and potency of NDHFR and CDHFR. (C) Bar chart of hRLuc readings at 48hr post TMP or DMSO (vehicle) treatment.

https://dx.doi.org/10.21037/atm-21-443

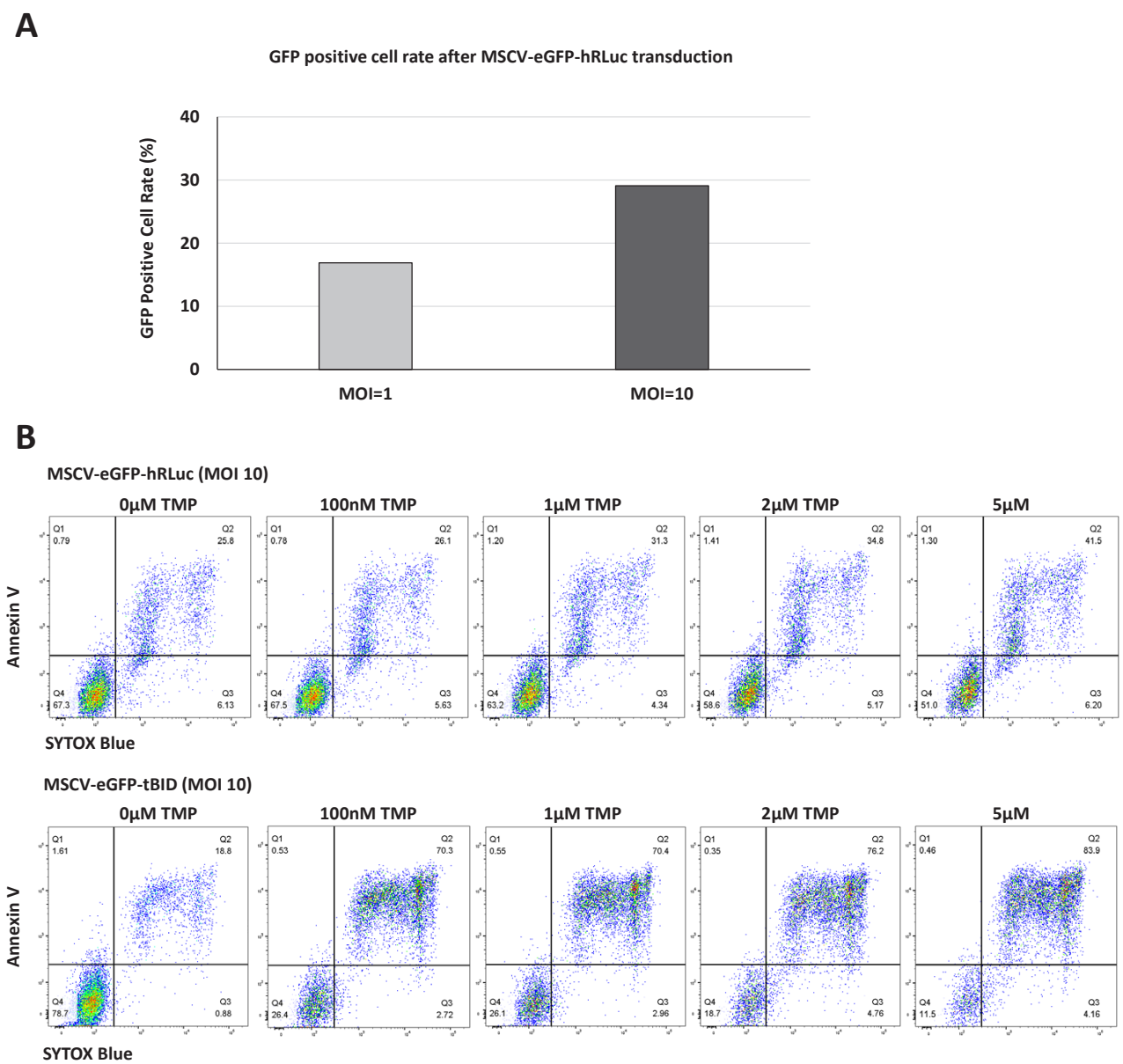


Figure S2 Optimization of lentivirus transduction MOI and TMP dosage. (A) Comparison of lentivirus transduction efficacy in Jurkat cells at MOI 1 and MOI 10 by using *MSCV-eGFP-hRLuc*. The transduction efficacy was determined by the rate of GFP positive cells obtained from flow cytometry analysis. (B) Dosage test of TMP for activating tBID safety switch in Jurkat cells. The Jurkat cells transduced by *MSCV-eGFP-hRLuc* lentivirus or *MSCV-eGFP-tBID* lentivirus were treated with TMP at numerous concentrations for two days. The viability of GFP positive transduced Jurkat cells were quantified through flow cytometry analysis after staining with Annexin V and SYTOX Blue.

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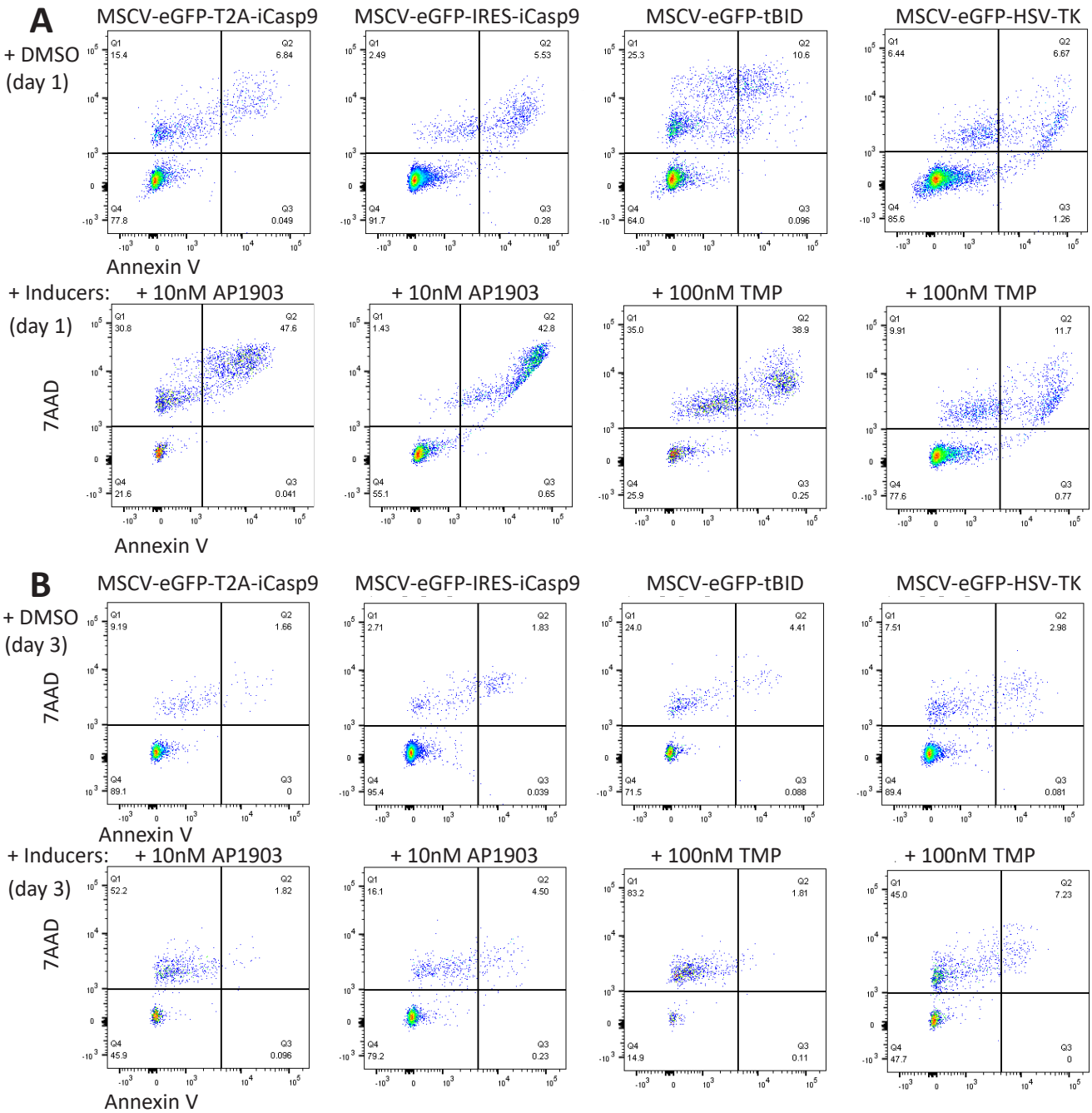

Figure S3 The killing efficacy of NDHFR-tBID safety switch in Jurkat cells. (A) Flow cytometry analysis of Jurkat cells transduced with lentivirus vectors described in *Figure 1A* on day 1 post treatment. (B) Flow cytometry analysis of Jurkat cells transduced with lentivirus vectors described in *Figure 1A* on day 3 post treatment.

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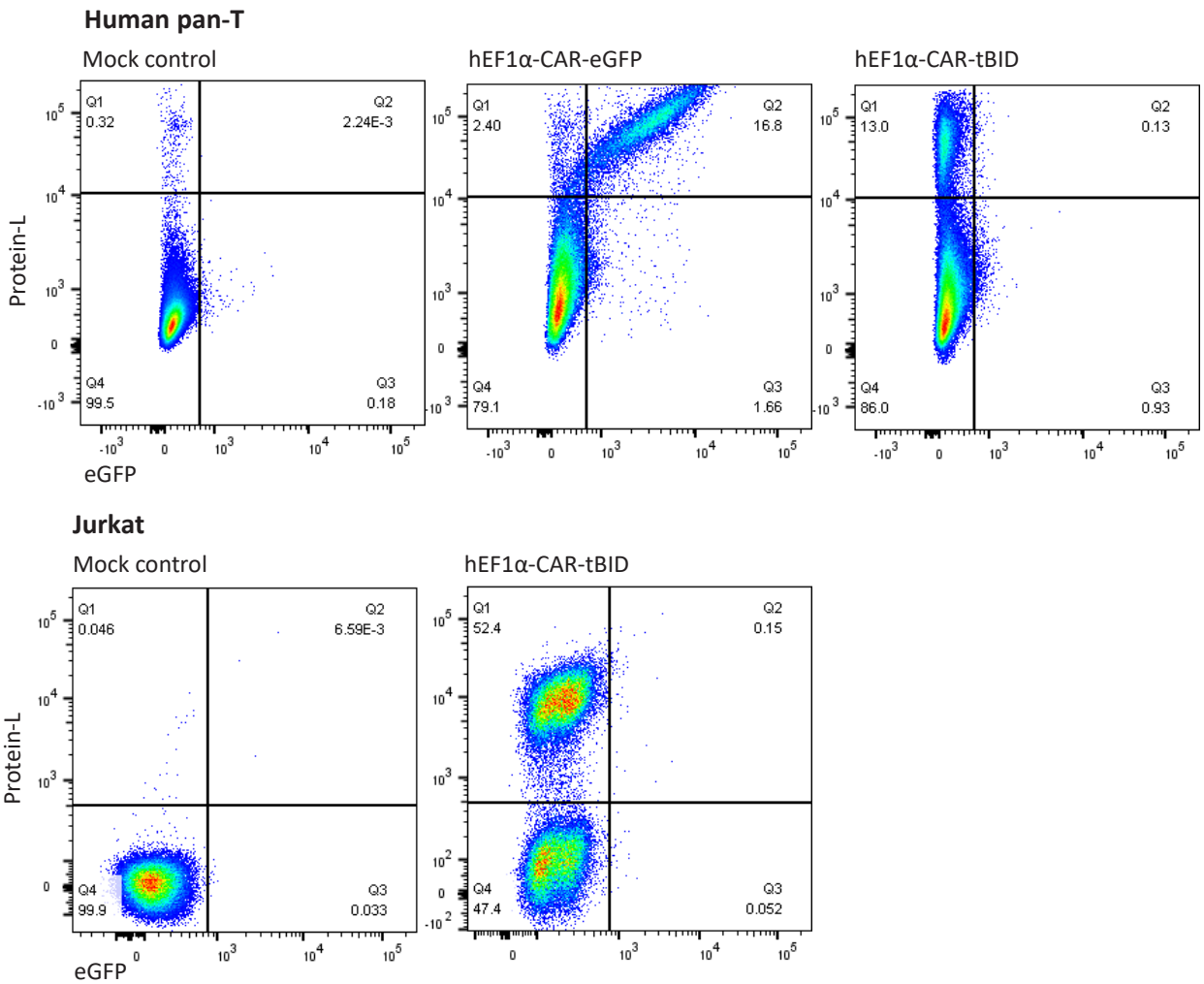

Figure S4 Transduction efficiency of lentiviral vectors in human pan-T cells and Jurkat cells. Lentiviral vectors *bEF1α-CAR-eGFP* and *bEF1α-CAR-tBID* were transduced into human pan-T cells at MOI =10. Lentiviral vector *bEF1α-CAR-tBID* was transduced into Jurkat cells at MOI =10. Both human pan-T cells and Jurkat cells were stained with Annexin V and Protein L followed by flow cytometry analysis on day 3 post transduction. The Annexin V negative single cells were further categorized for eGEP expression and positive Protain L staining.

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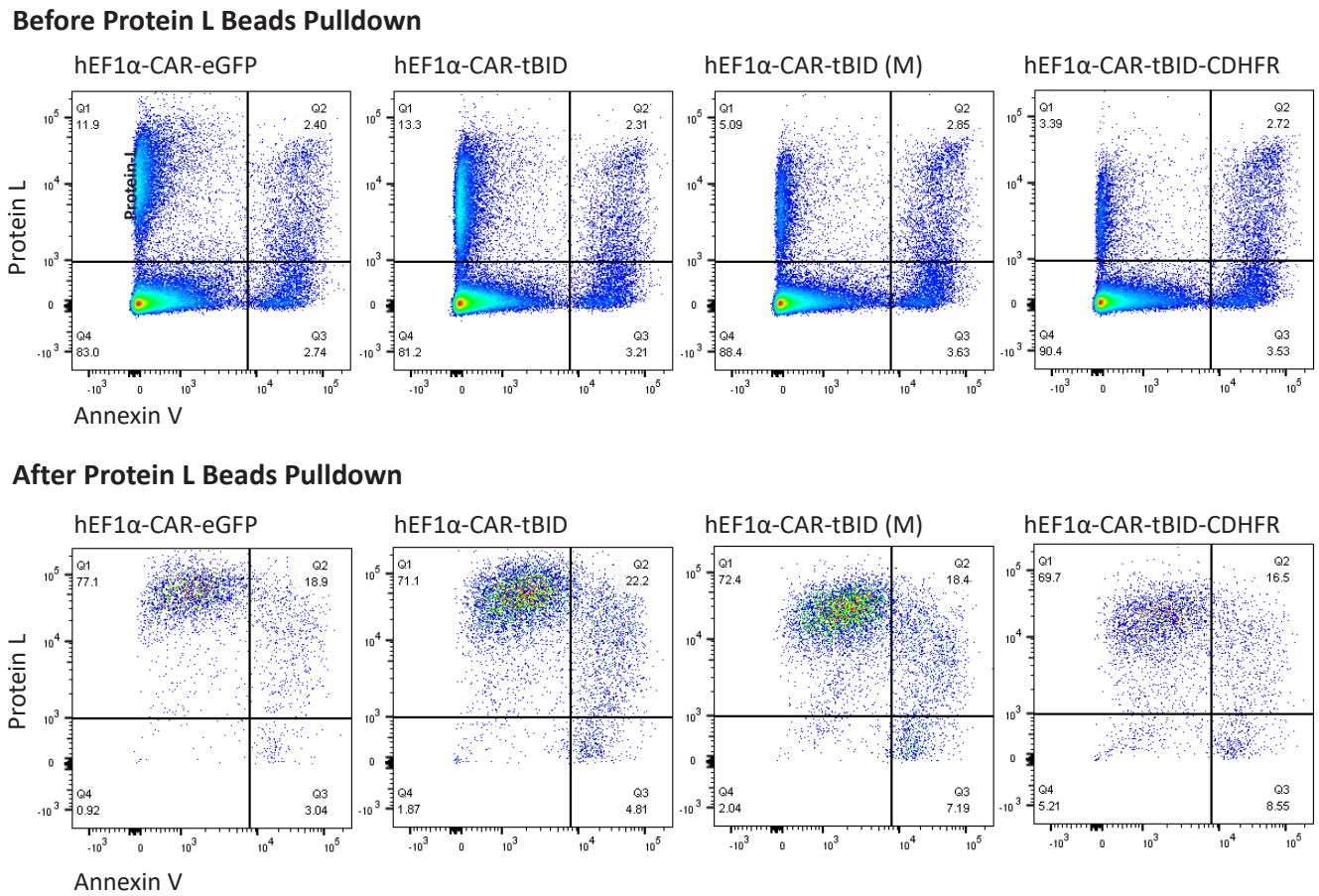

Figure S5 Protein L beads purification of lentiviral vector transduced human pan-T cells.

Table S1 Comparison of HEK293 cell viability during lentivirus packaging

Lentiviral Prep Name	In-HouseP24 (VP/mL)	In-House HEK293 Cell Viability (%) Day of Harvest
CAR-NDHFR-tBID	4.63E+11	72.0
CAR-iCasp9	8.80E+09	59.7
CAR-eGFP	5.43E+11	73.3

https://dx.doi.org/10.21037/atm-21-443