

## Legends to Supplementary Figures

**Figure S1. shRNA knockdown of *USF2* inhibits HIV-1 expression.** **A, C:** Jurkat mHIV-Luciferase cells were transduced with an pLKO empty vector control or shRNA targeting USF1 (A) or USF2 (C). Transduced cells were selected for with 3  $\mu\text{g/mL}$  puromycin for up to 8 days after which lysates were analyzed by immunoblotting using antibodies against USF1, USF2, and Tubulin. **B, D:** USF1 (B) or USF2 (D) shRNA transduced mHIV-Luciferase cells were left untreated (Ve, DMSO) or stimulated with 5 nM PMA for 4 hours prior to measuring luciferase activity ( $n = 3$ , mean  $\pm$  SD).

**Figure S2. *USF2* knockout limits proviral induction in TZM-bl cells.** **A:** *USF2* KO in the TZM-bl cell line was produced by using CRISPR-Cas9. Immunoblotting of cellular lysates was performed using antibodies against USF2 and Tubulin. **B:** Wildtype or *USF2* KO TZM-bl cells were left untreated (Ve, DMSO) or incubated with 5 nM PMA for 4 hours prior to measuring luciferase activity. The relative light units (RLU) are displayed ( $n = 3$ , mean  $\pm$  SD).

**Figure S3. Reincorporation of USF1 or USF2 rescues LTR transcription in *USF2* KO cells.**

**A:** Schematic representation of the LAI LTR-Tat-IRES-GFP reporter construct as well as the CMV-EV-EF1 $\alpha$ -RFP (EV), CMV-USF1-EF1 $\alpha$ -RFP (USF1), and CMV-USF2-EF1 $\alpha$ -RFP (USF2) expression vectors. **B:** Depiction of the flow cytometry scatter plot generated following co-transfection of LTR reporter and RFP expression vector. The bottom left quadrant contains un-transfected cells, negative fluorescent cells. The top left population is co-transfected by the

LTR reporter and the RFP expression vector but the LTR is silent. Cells in the top right quadrant are co-transfected by transcriptionally active LTR reporter as well as the RFP expression vector.

The bottom right quadrant are cells transfected only by the active LTR reporter. **C:**

Representative scatter plots following co-transfection of wildtype or *USF2* KO HEK293T cells with the LTR-Tat-IRES-GFP and either CMV-EV-EF1 $\alpha$ -RFP, CMV-USF1-EF1 $\alpha$ -RFP (USF1), or CMV-USF2-EF1 $\alpha$ -RFP (USF2) vector.

**Figure S4. Loss of USF1 or USF2 does not affect cellular growth.** **A, B:** Equivalent number of Wildtype, *USF1* KO (A), and *USF2* KO (B) Jurkat mHIV-Luc cells were plated, and cell number counted on the indicated day ( $n = 3$ , mean  $\pm$  SD). **C, D:** As in A, B but cellular viability was determined ( $n = 3$ , mean  $\pm$  SD).

**Figure S5. USF2 regulates HIV expression regardless of Tat.** **A:** Schematic representation of the CMV-EV-EF1 $\alpha$ -RFP vector and the LAI LTR-IRES-GFP (-Tat) and LTR-Tat-IRES-GFP (+Tat) reporter constructs. **B:** Depiction of the flow cytometry scatter plot generated following co-transfection of LTR reporter and RFP expression vector. See Figure S3B for further description. **C, D:** Representative scatter plots following co-transfection of wildtype or *USF2* KO HEK293T cell lines with RFP expression vector and either LTR-IRES-GFP (C) or LTR-Tat-IRES-GFP (D) LAI derived reporter construct.

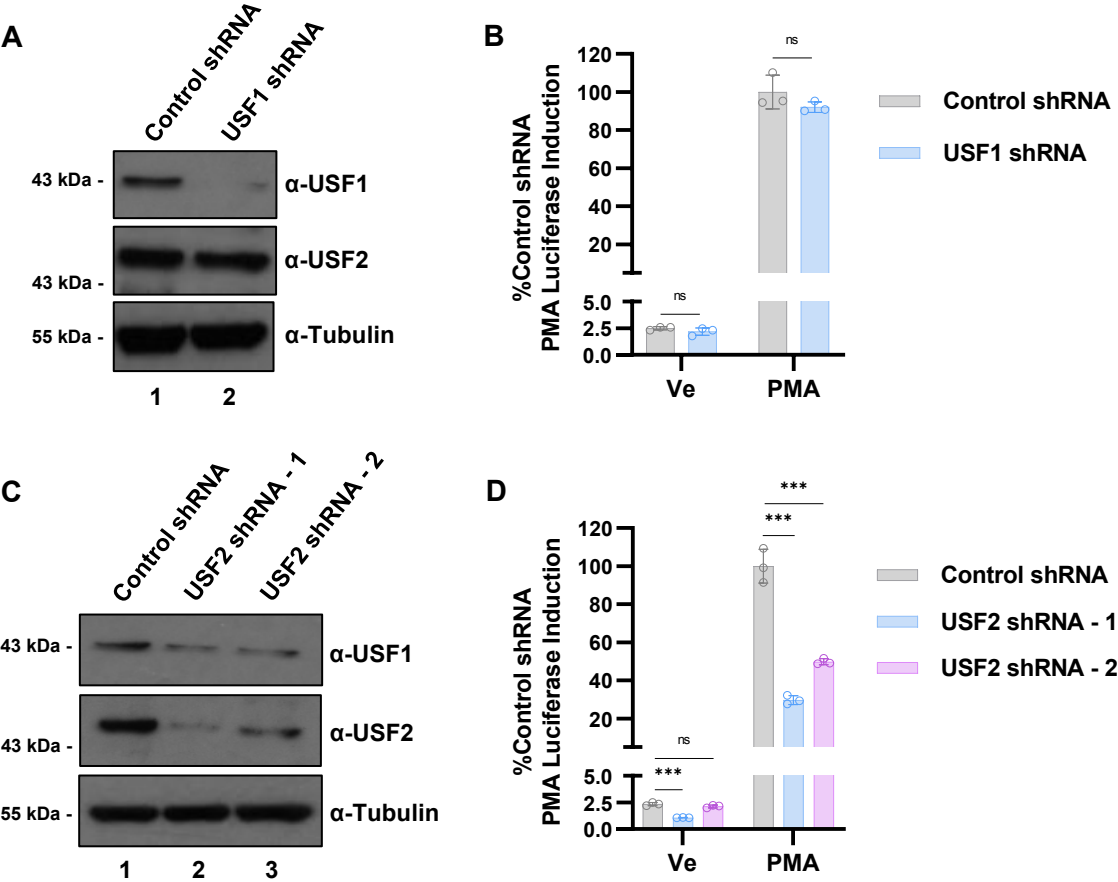
**Figure S6. Loss of either *USF1* or *USF2* impairs expression of genes downstream of multiple T cell activation pathways.** **A - D:** Hallmark GSEA plots of RNA-seq data analyzed

by DESeq2 of PMA/ionomycin stimulated *USF1* KO and *USF2* KO Jurkat T cells. Analysis was performed on  $n = 3$  RNA-seq samples.

**Figure S7. USF2 enhances HIV-1 infection of T cells.** **A:** Representative scatter plots of Wildtype, *USF1* KO, *USF2* KO Jurkat Tat T cells 3 days post-infection with Red-Blue-HIV-1 dual reporter virus [57]. LTR driven BFP expression is depicted on the x-axis while mCherry expression driven by the constitutive CMV promoter on the y-axis. **B:** Wildtype, *USF1* KO, or *USF2* KO Jurkat Tat cells were infected at the same multiplicity of infection (M.O.I.  $\sim 0.1$ ). 3 days post-infection, cells were analysed by flow cytometry and the infection rate was normalized to the number of wildtype cells that were infected ( $n = 3$ , mean  $\pm$  SD). **C:** Same as in B, but the ratio of productive to latent infection was determined ( $n = 3$ , mean  $\pm$  SD).

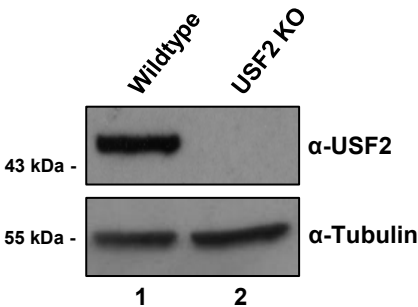
**Figure S8. Representative flow cytometry gating strategy.** **A, B:** Representative scatter plots depicting the gating strategy employed for HEK293T cell lines (A) and Jurkat T cell lines (B). Homogeneous populations of live cells were isolated and assessed by setting threshold forward scatter (FSC) and side scatter (SSC) settings.

# Supplementary Figure 1

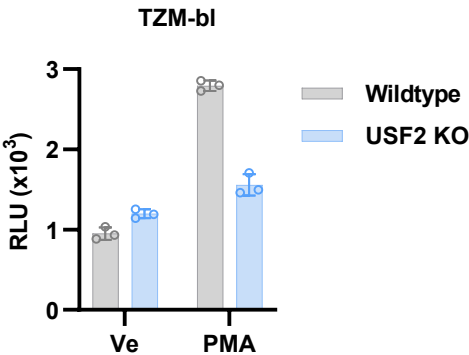


# Supplementary Figure 2

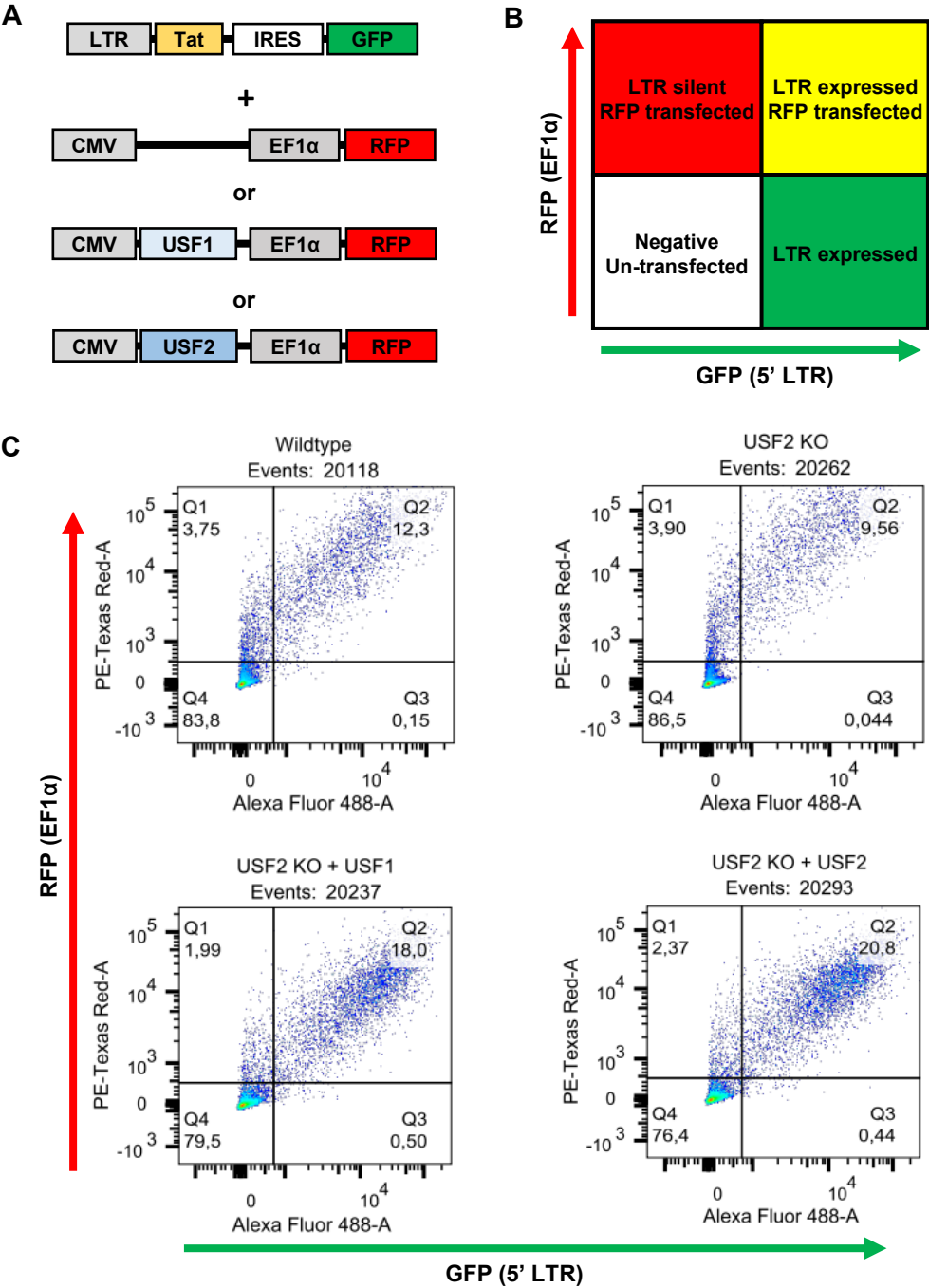
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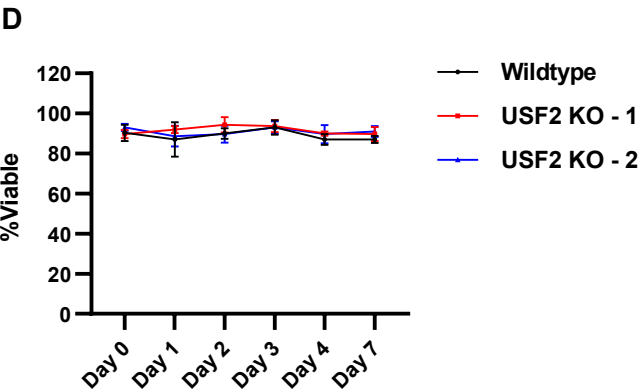
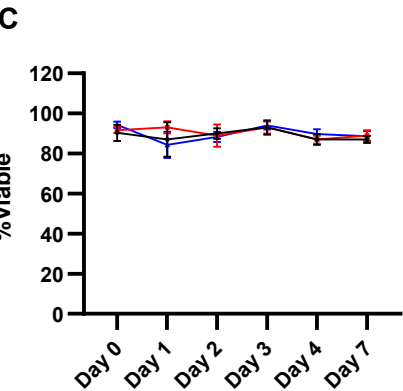
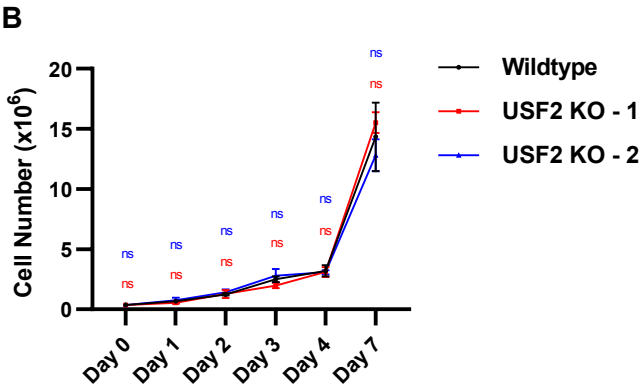
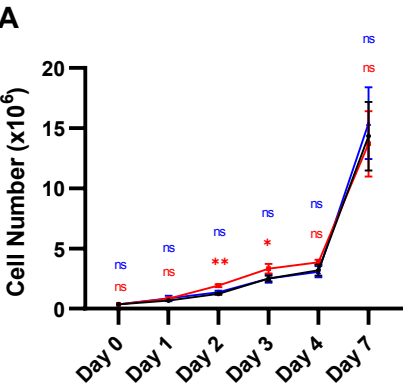
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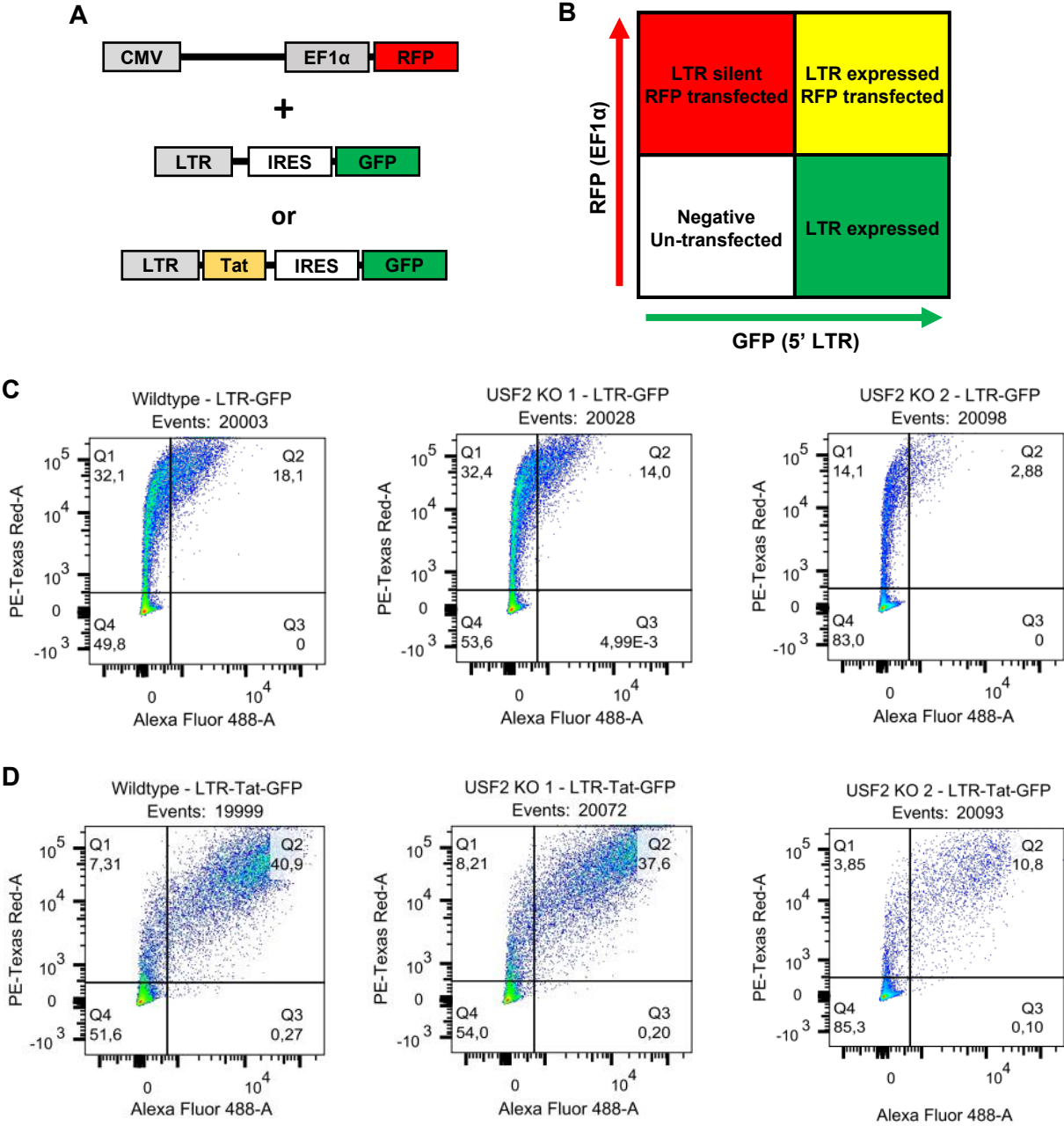
# Supplementary Figure 3



# Supplementary Figure 4

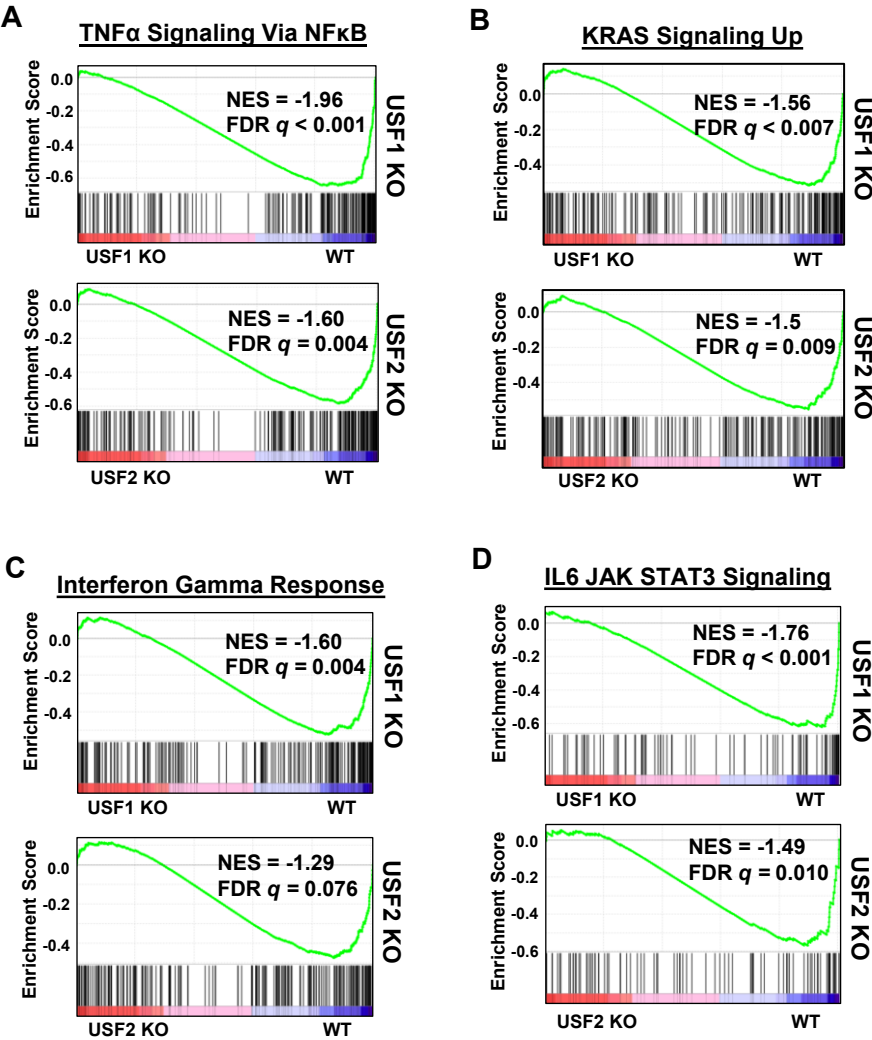


# Supplementary Figure 5

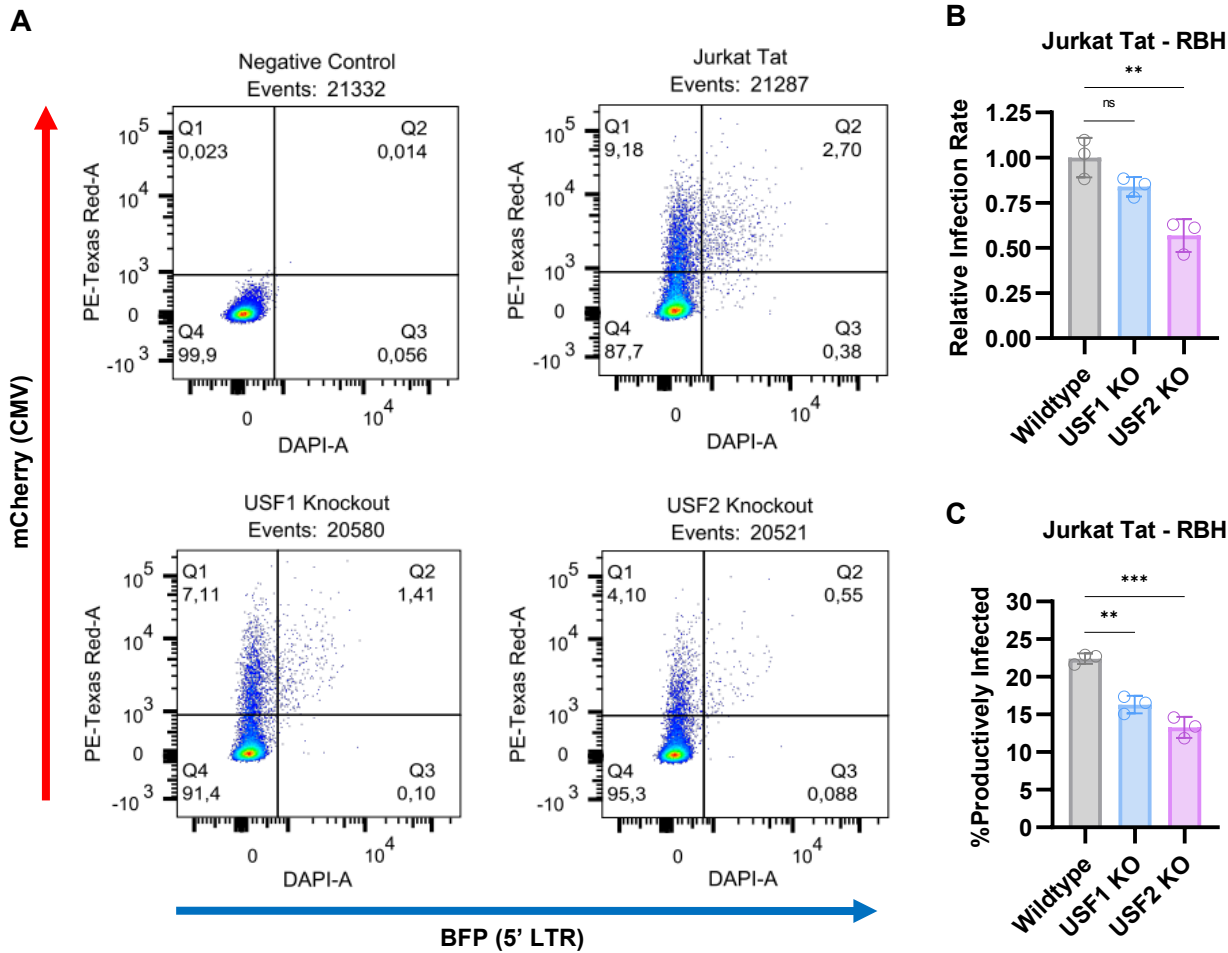




# Supplementary Figure 6

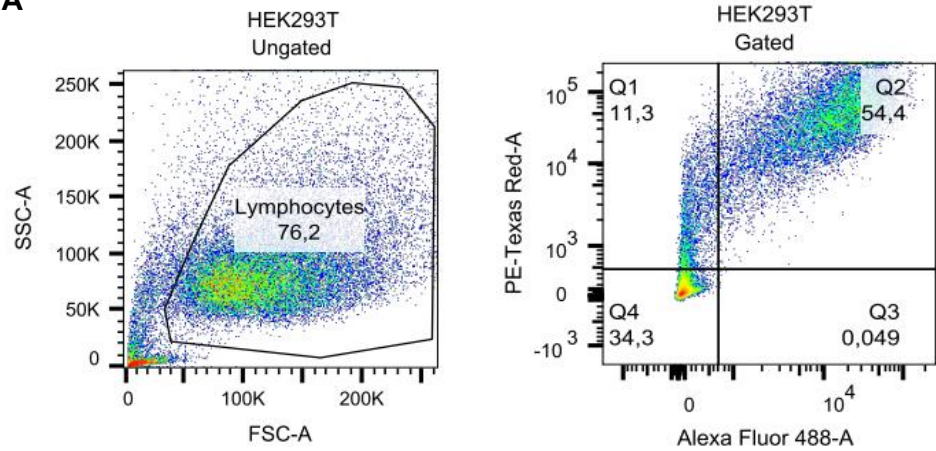


# Supplementary Figure 7



# Supplementary Figure 8

A



B

