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Supplemental Information

Selective Export into Extracellular Vesicles and Function of tRNA Fragments during T Cell Activation

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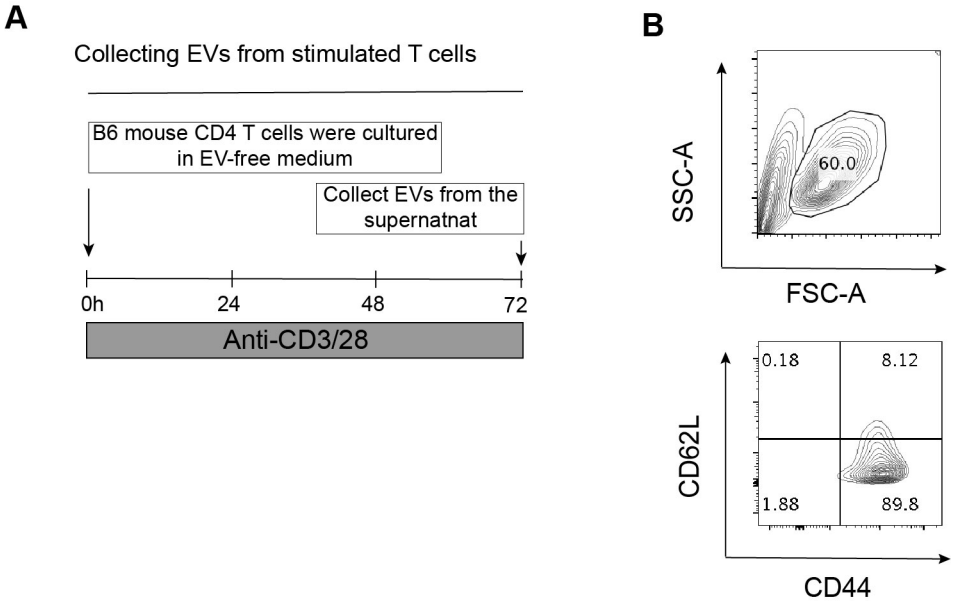


Figure S1. Stimulation by anti-CD3 and anti-CD28 antibodies induces T cell activation and apoptosis, related to Figure 1. (A) Schematic of workflow for EV collection from stimulated T cells. Primary mouse $CD4^+$ T cells were cultured in EV-depleted media with plate-bound anti-CD3 and anti-CD28 antibodies. After 3 days, cell culture supernatants were collected for EV isolation. (B) Flow cytometric analysis of representative T cells activated for 3 days. Light scatter analysis indicated that ~60% of cells were alive (top panel). Among live cells, nearly all exhibited an activated phenotype indicated by upregulated CD44 and downregulated CD62L (bottom panel).

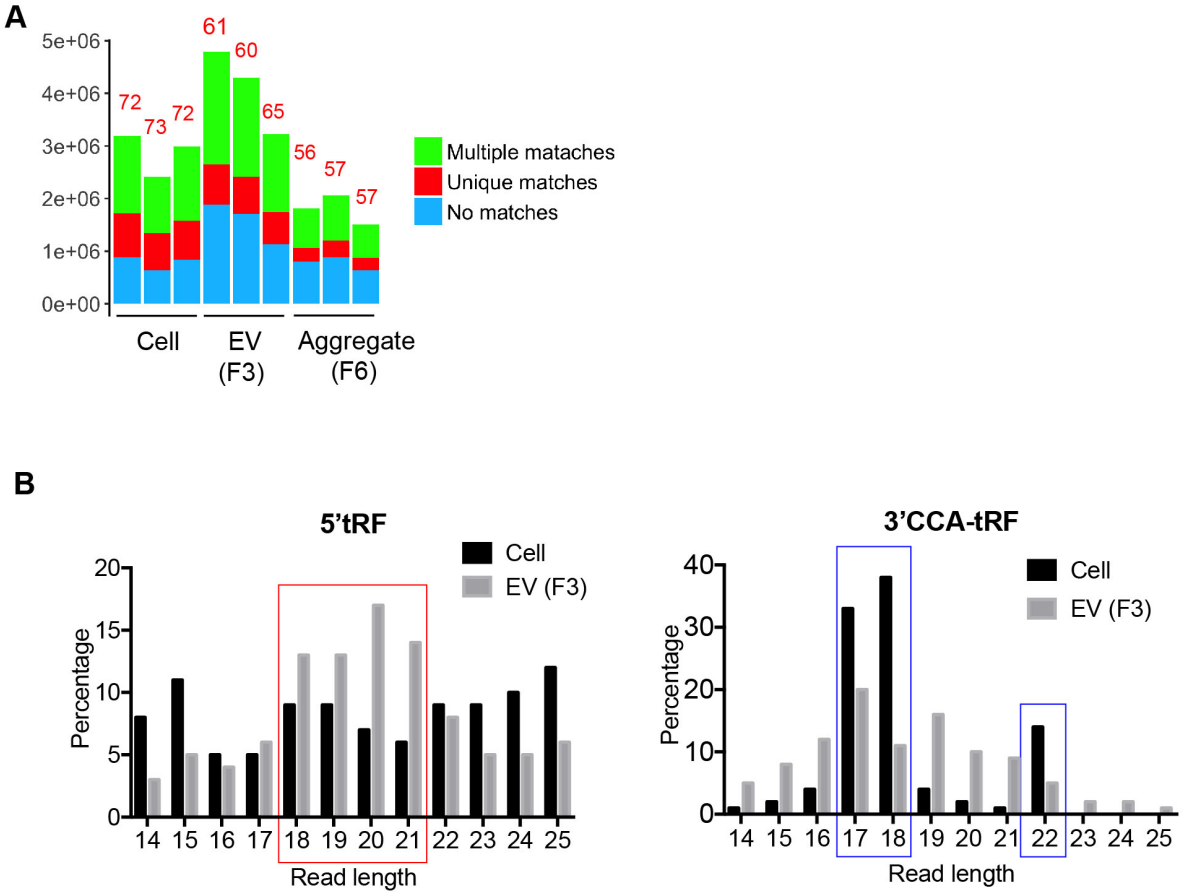


Figure S2. Read length distribution of tRFs in EVs versus in cells, related to Figure 2. (A) Total read numbers (y-axis) and the total percentage of mapped reads (number in red). Colored bars indicate reads that mapped to multiple genomic locations (green) or unique locations (red), and reads that could not be mapped (blue). (B) The length distribution of reads mapped to tRNA loci was determined for the indicated tRF classes in stimulated T cell (Cell) and fraction 3 (EV). The red box indicates the 5'tRF that are in 18-21 nt, which were specifically expressed higher in EVs then in cells. The blue box indicates the 3'CCA-tRF that are in 17-18 nt or 22 nt, which were specifically expressed lower in EVs then in Cells.

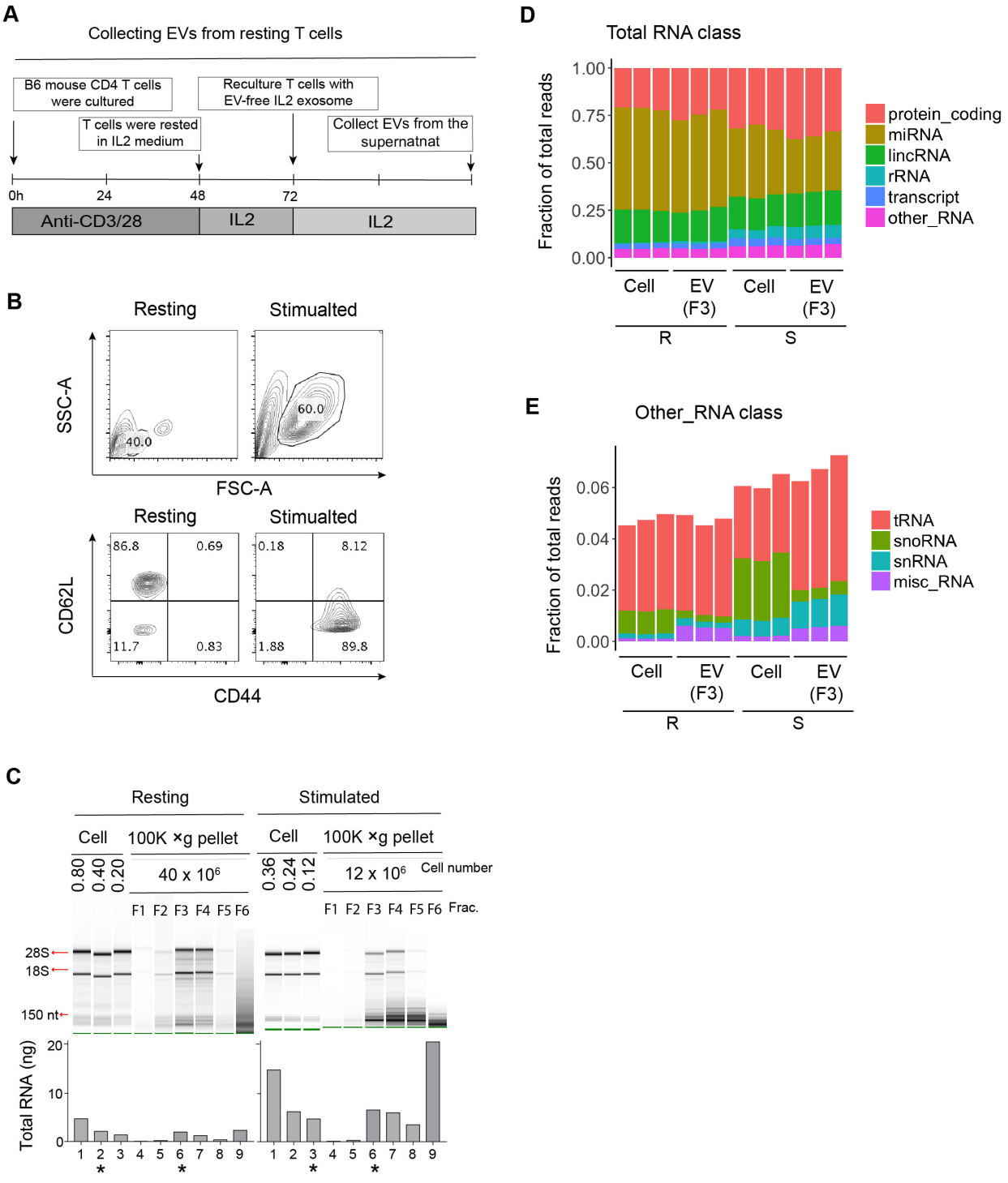


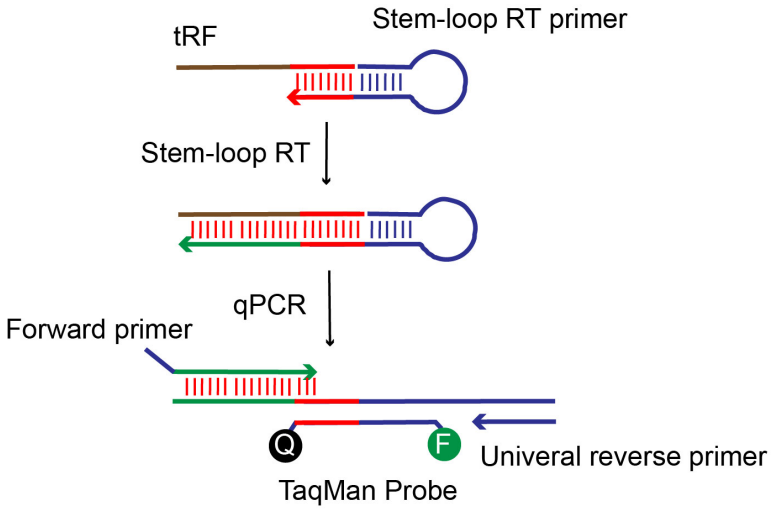
Figure S3. The enrichment of tRNA reads in T-cell EVs is dependent on activation, related to Figure 3. (A) Schematic of workflow for culturing T cells under resting conditions for EV isolation. Primary mouse T cells were activated for 2 days using anti-CD3 and anti-CD28 antibodies, and then expanded in media with IL-2 for 1 day. T cells were then centrifuged and resuspended in EV-free media with IL-2. After 2 days, cell culture supernatants were collected for EV isolation. (B) Flow cytometry analysis of resting and stimulated T cells. (C) Bioanalyzer analysis of RNA in fractions of the separated 100,000 ×g pellets. (D) Relative RNA composition of the reads in small RNA libraries of EVs and cells collected from resting and stimulated conditions. (E) Sub-classification of reads shown as "other_RNA" in (D) into tRNA, snoRNA, snRNA and misc RNA.

A

tRNA Leu-TAA: ACCAGAATGGCCGAGTGGTTAAGGCGTTGGACTTAAGATC-
CAATGGATTTATATCCGCGTGGGTTCGAACCCCACTTCTGGTA

tRNA Leu-TAG: GGTAGCGTGGCCGAGCGGTCtAAGGCGCTGGATTTAGGCTC-
CAGTCTCTTCGGAGGCGTGGGTTCGAATCCCACCGCTGCCA

B



C

Complemetary sequence for the stem-loop RT primer and TaqMan Probe regions shown in red in Fig. S4B

tRNA Leu-TAA: 5'tRF ACCAGAATGGC**CGAGTGGT**

Sequence for the forward primer region shown in green in Fig. S4B

tRNA Leu-TAG: 5'tRF GGTAGCGTGGC**CGAGCGGT**

tRNA Leu-TAA: 3'i-tRF TGGATTTATAT**CCGCGTGG**

Figure S4. Sequences of tRFs validated by oligo(dT) RT-qPCR and stem-loop RT-qPCR assays, related to Figure 4. (A) Sequences of full length tRNAs Leu-TAA and Leu-TAG. Red sequences are the 5'-tRFs that displayed activation-induced EE. Blue sequence is the 3'i-tRF that displayed activation-independent EE. These sequences were used as forward primers for oligo(dT) RT-qPCR assays. (B) Schematic of workflow for stem-loop RT-qPCR. Color coding indicates constant regions common to primers for analyzing all tRFs (blue), and tRF-specific regions within the stem-loop RT primer (red) and forward qPCR primer (green). (C) tRF-specific sequences used for primer design color coded as in (B). Red tRF sequences are complementary to the red region of the RT primers and TaqMan Probe. The tRF sequences underlined in green correspond to the green region of forward primers.

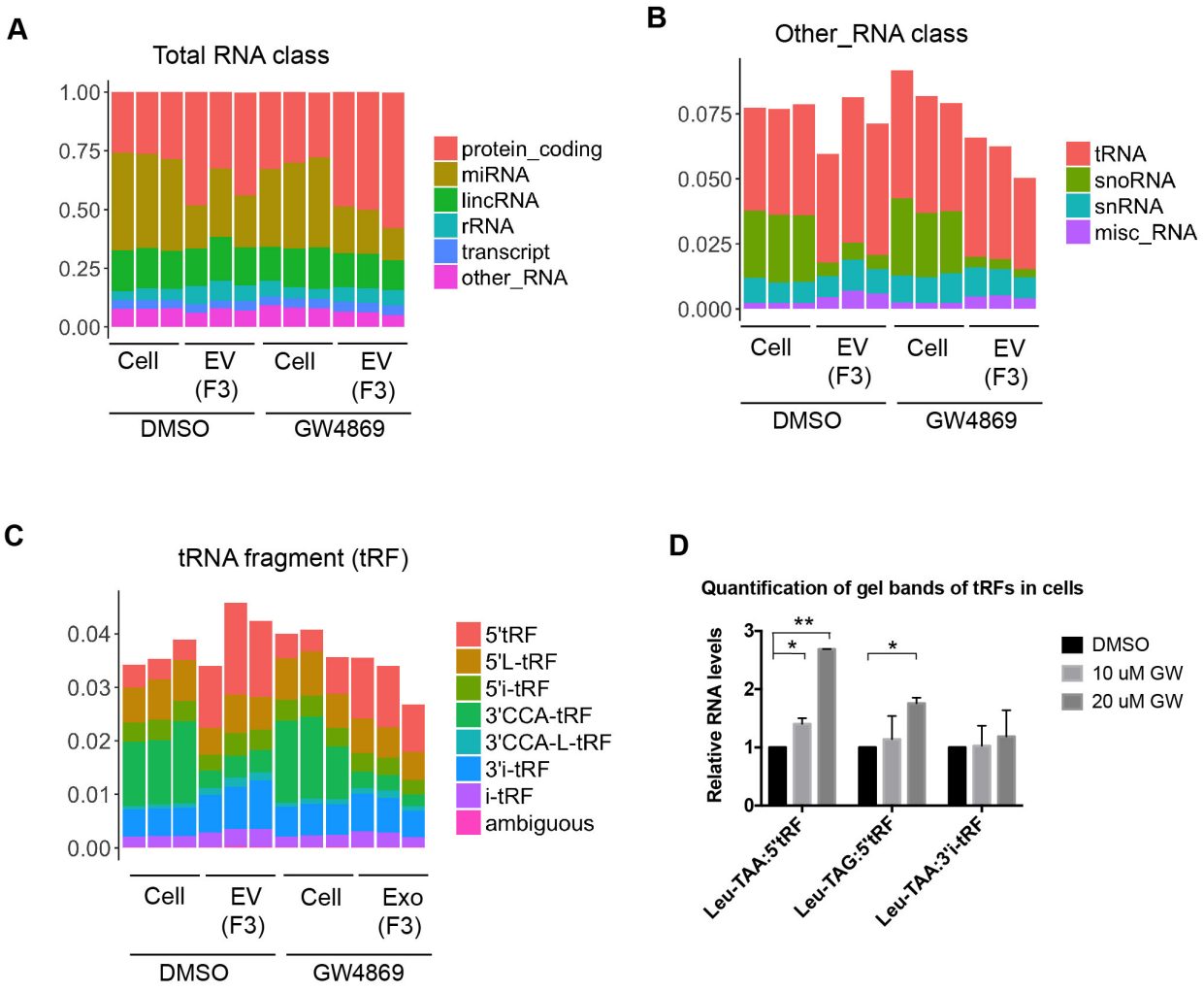


Figure S5. nSMase inhibitor treatment did not change the overall RNA composition of EVs and cells, related to Figure 5. (A) Relative RNA composition of reads in cellular RNA and EV RNA fractions of supernatants from T cells stimulated in the presence of DMSO vehicle alone or GW4869. (B) Sub-classification of reads shown as "other_RNA" in (A) into tRNA, snoRNA, snRNA and misc RNA. (C) Further classification of reads in the tRNA class as shown in (B) into the 7 tRF classes shown in Figure 2C and rare tRFs of ambiguous classification. (D) tRF abundance in cells stimulated with DMSO (black bars) and the indicated concentrations of GW4869 (grey bars) by quantifying the tRF bands in gels as shown in Figure 5E.

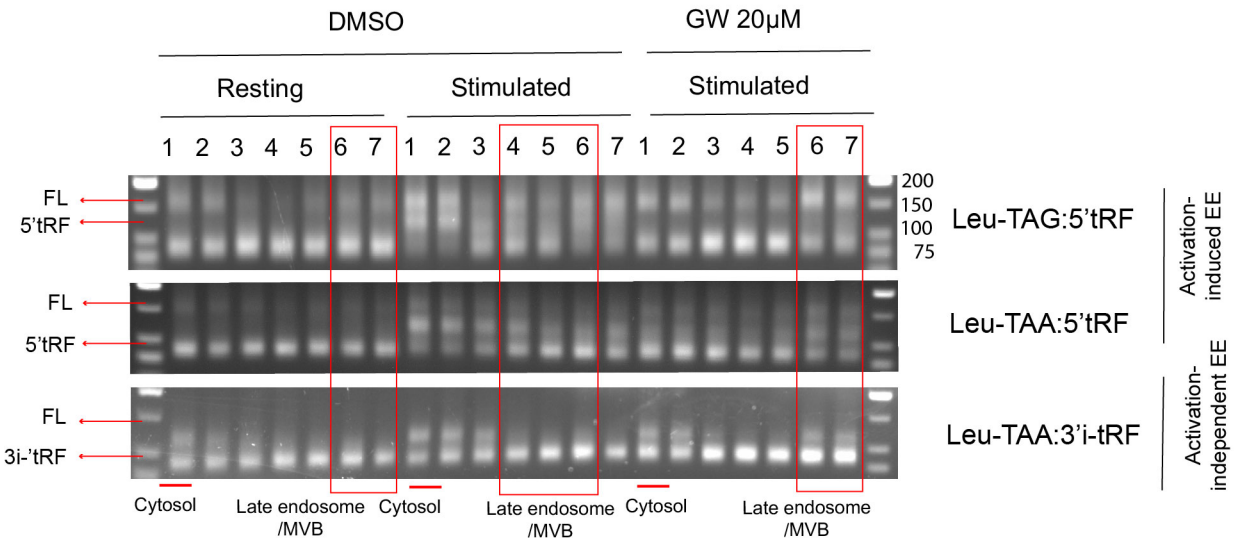


Figure S6. Qligo(dT) RT-PCR detected both full-length tRNAs and tRFs in key cellular Optiprep gradient fractions, related to Figure 6. Analysis of PCR products that were amplified by 40 cycles in the oligo(dT) RT-qPCR reaction across subcellular fractions obtained from resting conditions, or stimulated conditions treated with DMSO or GW4869. The red lines indicate the cytosol fractions and the red boxes indicate MVB fractions as determined by Figure 6B.

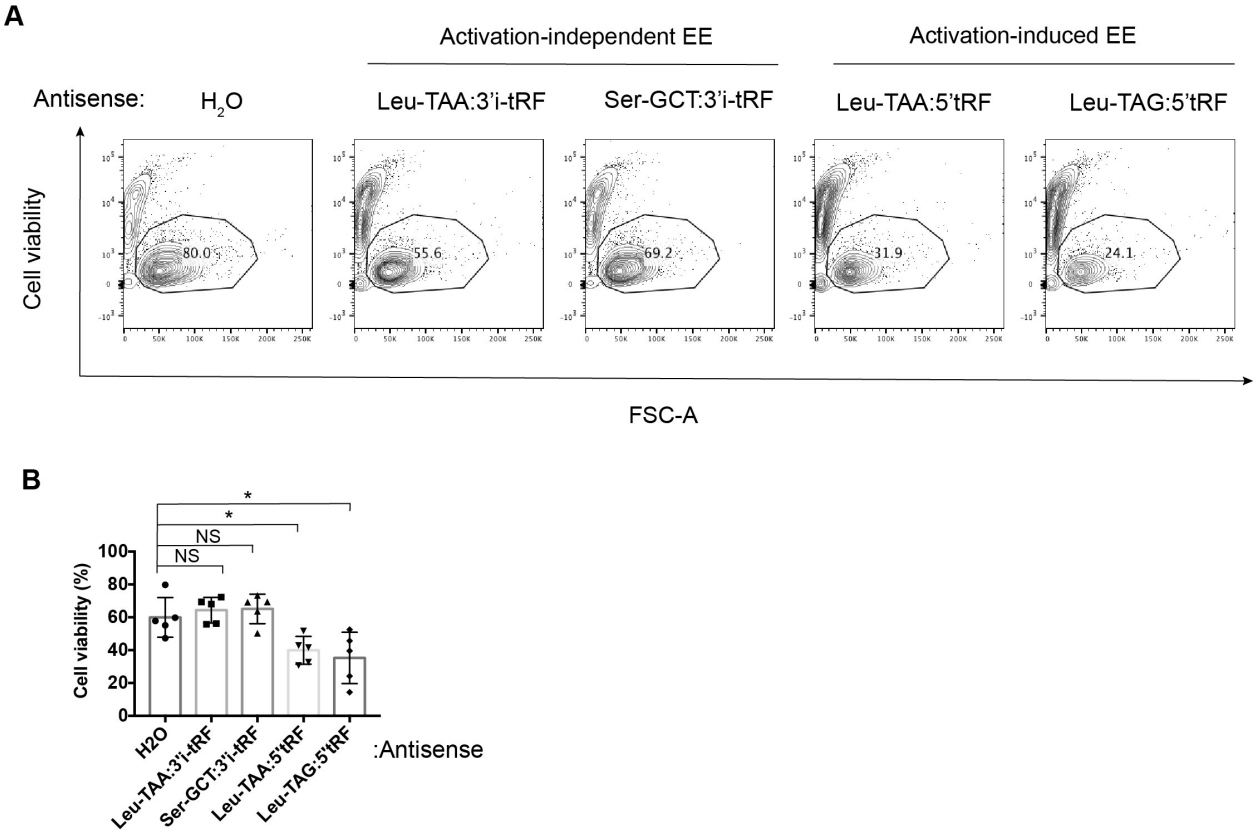


Figure S7. T cells that were transfected with antisense oligos against activation-induced EE tRFs displayed lower T cell viability after restimulation with PMA and Ionomycin, related to Figure 7. (A) Representative flow cytometric analysis of cell viability dye staining and forward scatter (FSC-A) of $CD4^+$ T cells transfected with antisense oligos complementary to Leu-TAA:3'i-tRF, Ser-GCT:3'i-tRF, Leu-TAA:5'tRF, or Leu-TAG:5'tRF, or water vehicle (H2O) control. (B) Quantified frequency of the indicated cell populations as shown in (A). Data are representative of at least three independent experiments. $^*P < 0.05$, one-tailed Student's *t* test,.Error bars indicate standard deviation of the mean.