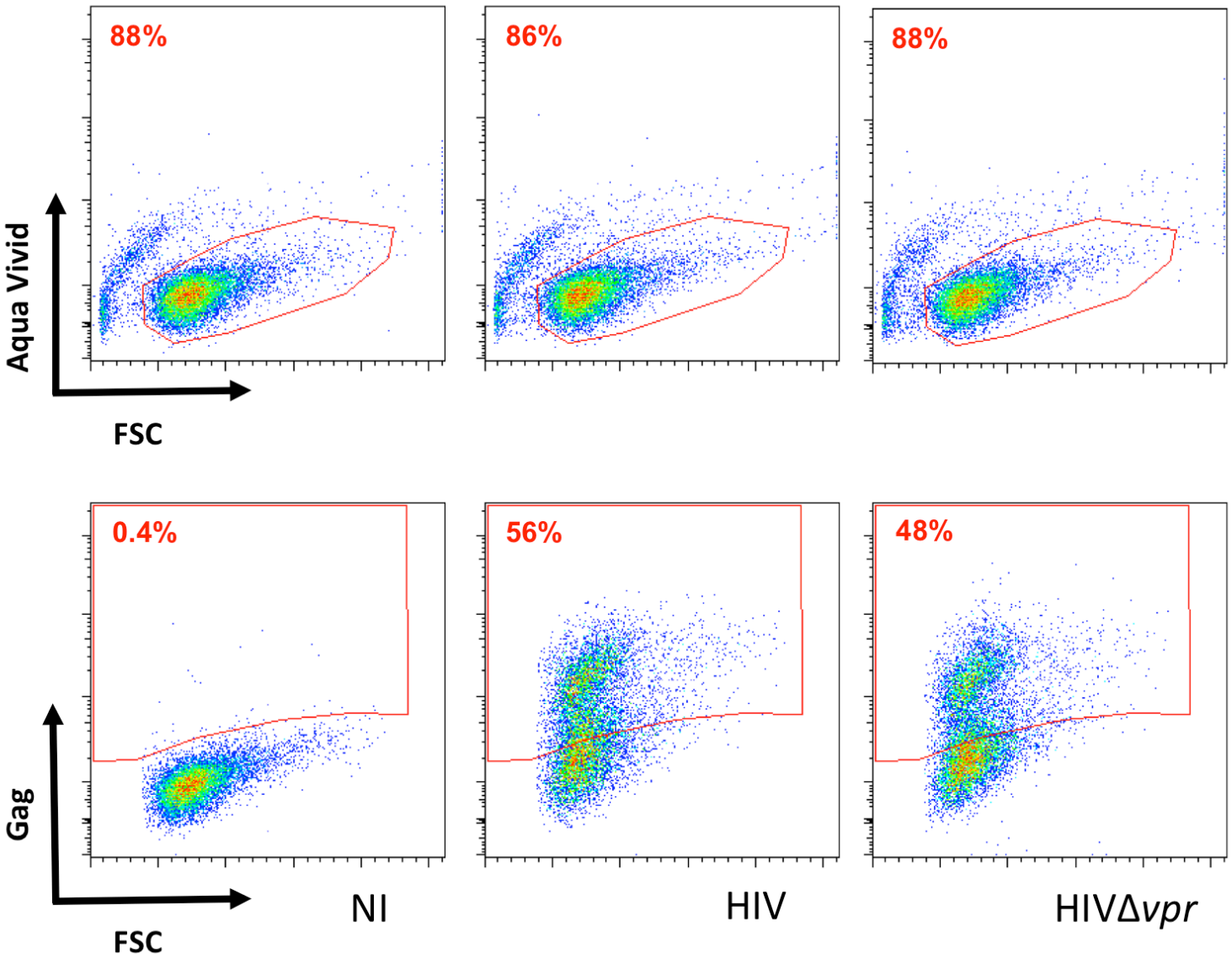
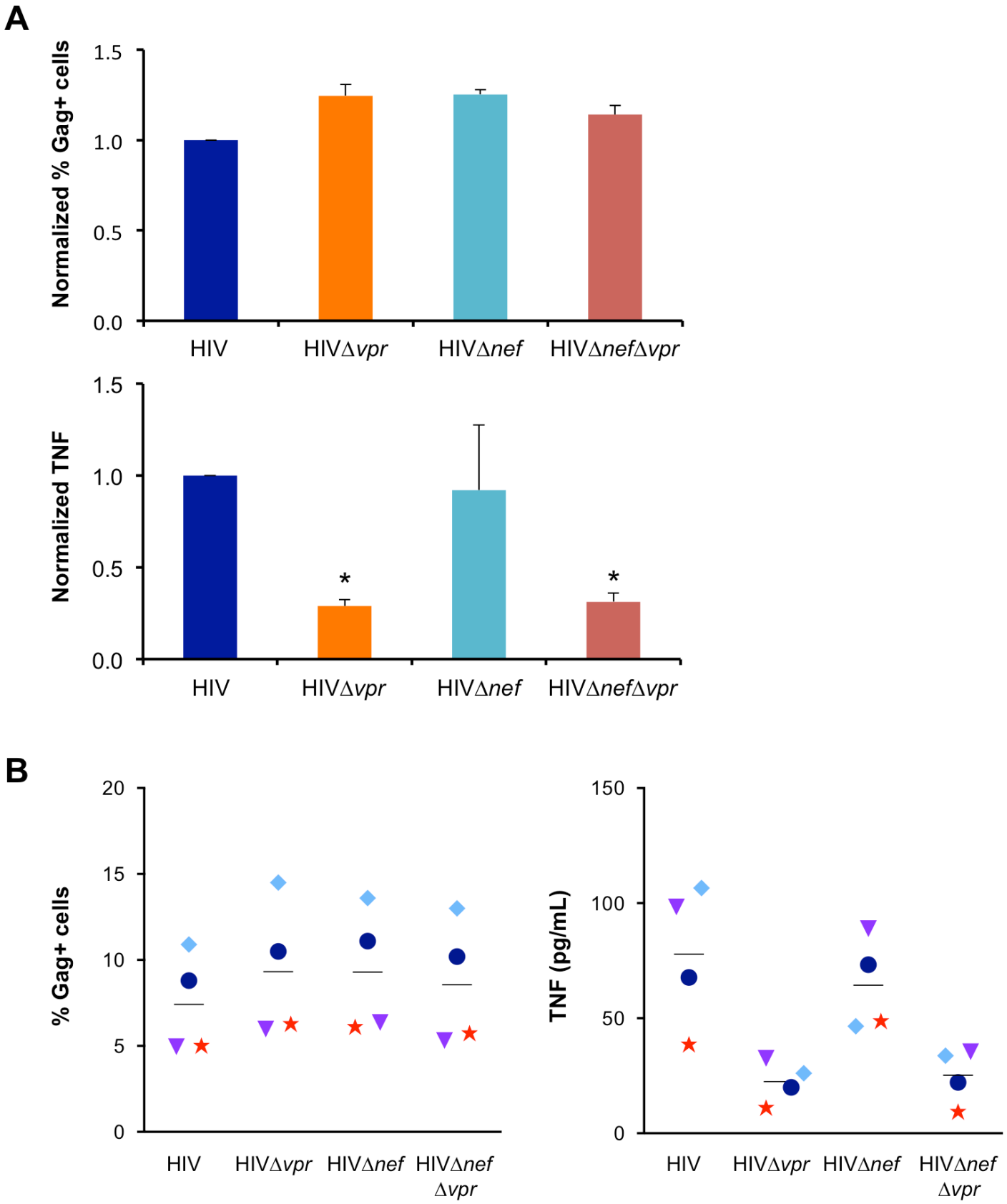
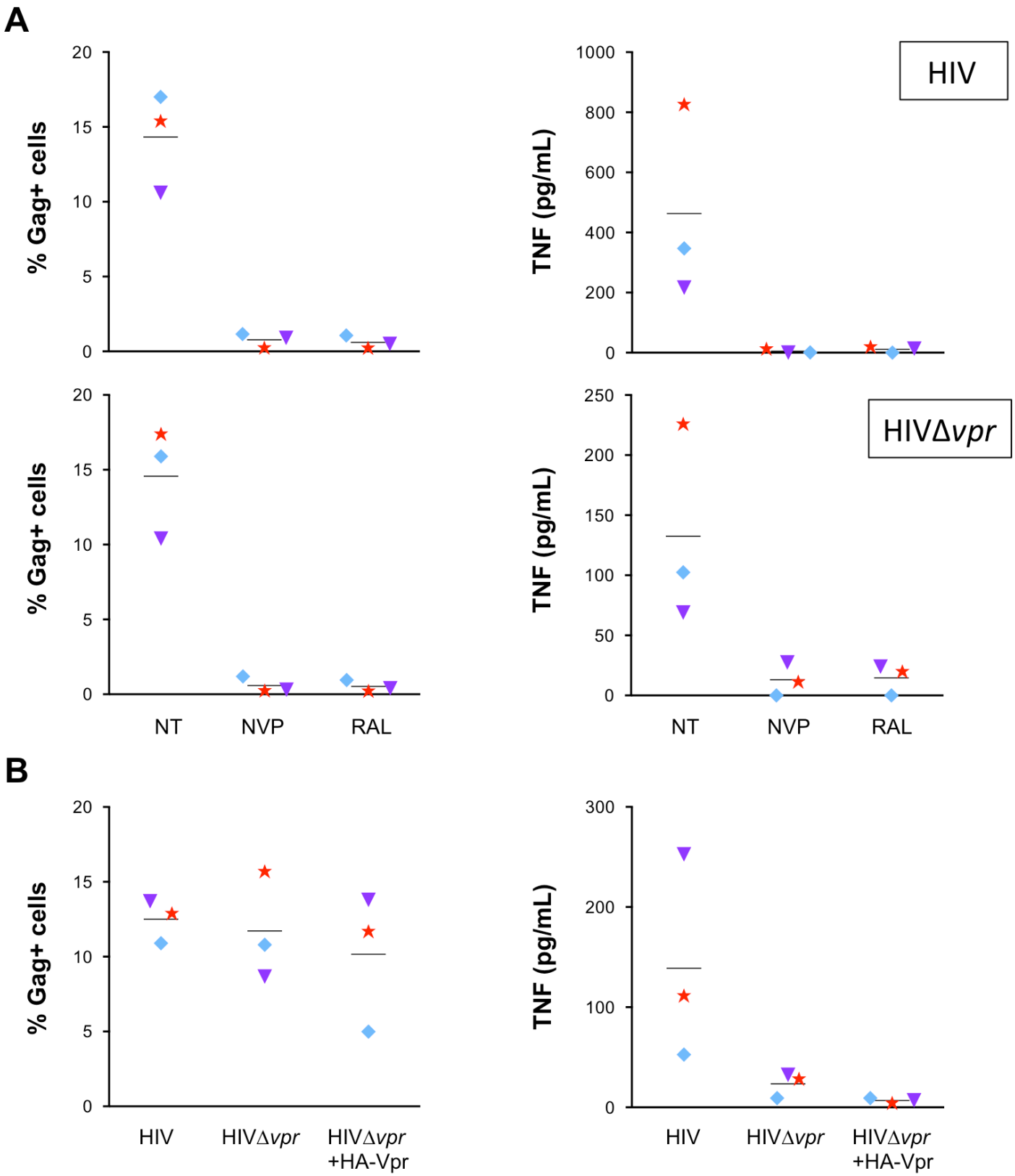


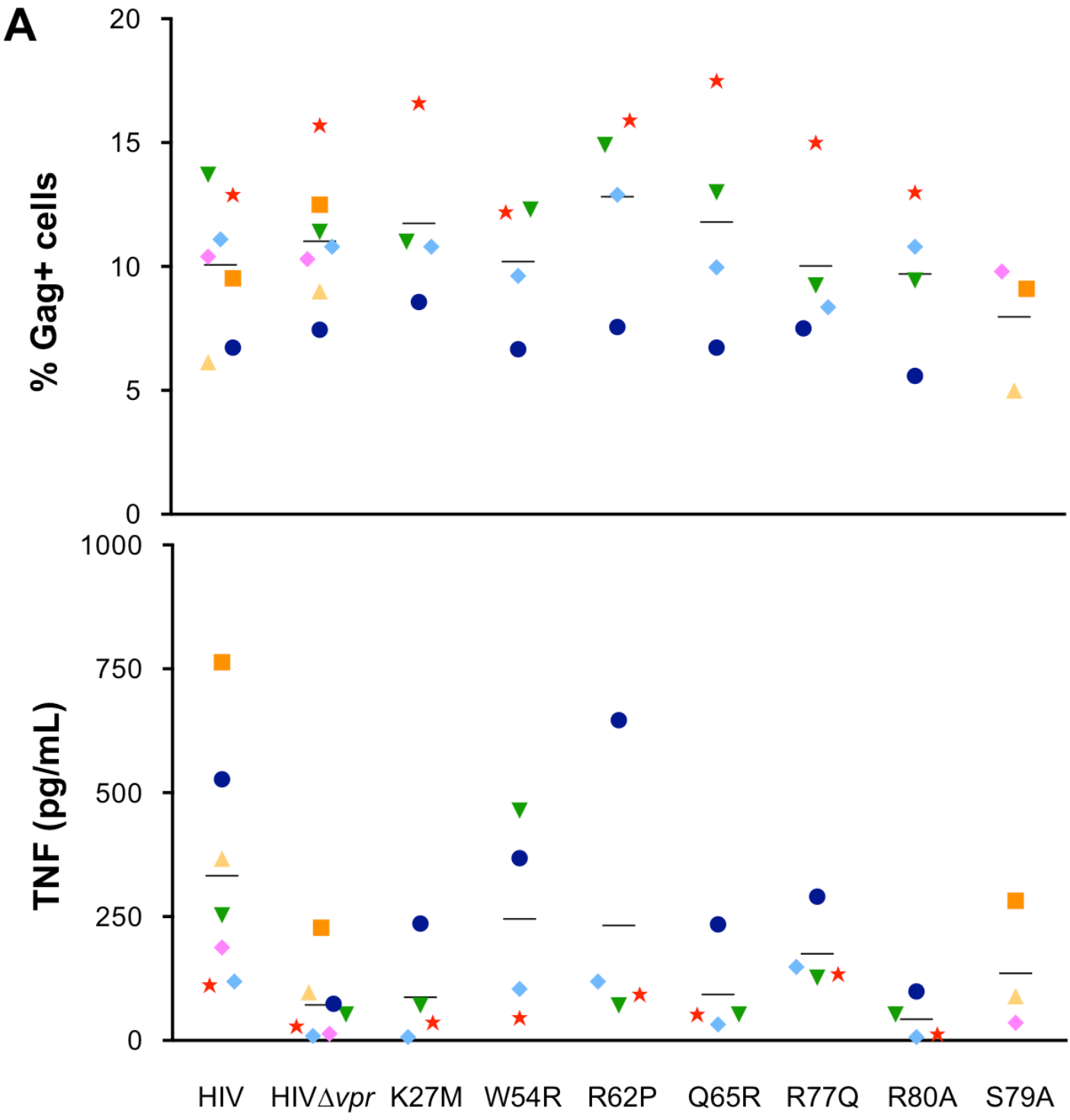
Supplementary Figure 1. Cell health profiles of infected MT4C5 cells.
MT4C5 were infected as described in Figure 1. Before fixation, cells were incubated with the viability marker Aqua Vivid. Dead cells were excluded based on Aqua Vivid incorporation and FSC profiles (upper panel). The levels of infection of cells were also determined by staining the viral protein Gag (lower panel).



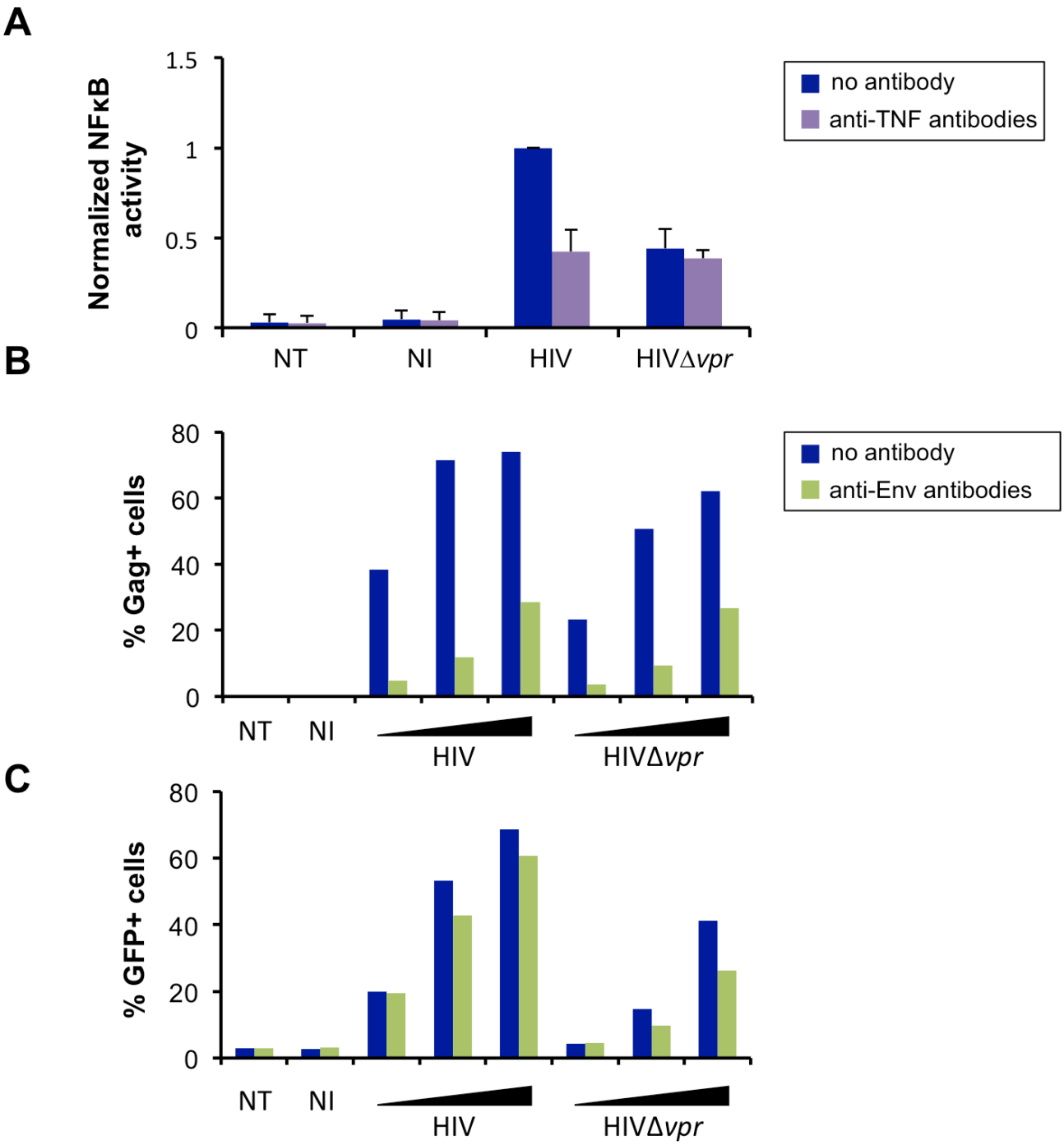
Supplementary Figure 2. Nef has no effect on TNF production in MT4C5 cells.
A : MT4C5 were infected with HIV-1 WT, Δ*nef*, Δ*vpr* or Δ*nef*Δ*vpr*. Infection levels at 24 h (upper panel) and TNF release at 48 h (lower panel) were measured as described in Figure 1. Gag and TNF levels obtained with wild type HIV were set at 1. * $p<0.05$, Mann Withney test. Results are mean ± SD of 4 independent experiments. **B :** Raw data for all experiments.



Supplementary Figure 3. Vpr neosynthesis is necessary for TNF production.
A : Raw data corresponding to Figure 2A. MT4C5 were either infected with wild type (upper panel) or *vpr* deleted HIV (lower panel). Infection levels and TNF production were measured at 24 and 48 h p.i., respectively. **B :** Raw data corresponding to figure 2C. MT4C5 were infected with HIV, HIVΔ*vpr*, or HIVΔ*vpr* complemented *in trans* with HA-Vpr. Infection levels and TNF production were measured at 24 h and 48 h p.i., respectively.



Supplementary Figure 4. Analysis of Vpr mutants.
A : MT4C5 were infected with HIV-1 strains carrying the indicated Vpr mutations. Levels of infection (upper panel) and TNF production (lower panel) were assessed at 24 h and 48 h p.i., respectively.



Supplementary Figure 5. Effect of Vpr on bystander cells.

A. Effect of anti-TNF antibodies on NFκB activity. Cocultures were performed as in Figure 5C, in presence of 1 µg/mL of anti-TNF antibodies. Luciferase was measured with a luminometer. HIV was set at 1. One representative experiment is shown.

B. Efficiency of anti-Env antibodies. Cocultures were performed as described in Figure 5E, but with Jurkat target cells instead of J-Lat cells, and in presence of the anti-Env broadly neutralizing antibody NIH 45-46 (50 nM). As a control, Jurkat cells were cultured alone (NT) or with non-infected MT4C5 (NI). The % of Gag + cells was determined by flow cytometry. One representative experiment is shown.

C. Effect of anti-Env antibodies on reactivation. Cocultures were performed as described in Figure 5E, the anti-Env broadly neutralizing antibody NIH 45-46 (50 nM). As a control, J-Lat cells were cultured alone (NT) or with non-infected MT4C5 (NI). The % of GFP+ cells was determined by flow cytometry. One representative experiment is shown.

	forward primer	reverse primer
K27M	5’-gagcttttagaggagcttATGagtgaagctgttagaca-3’	5’-tgtctaacagcttcactCATaagctcctctaaaagctc-3’
W54R	5’-ccactcctgcCCTagtatccccataagtttcataga-3’	5’-tctatgaaacttatggggatactAGGgcaggagtgg-3’
R62P	5’-gggcaggagtggaagccataataCCAattctgcaacaact-3’	5’-agttgttgcagaatTGGtattatggcttccactcctgccc-3’
Q65R	5’-agtggaagccataataagaattctgAGAcaactgctgtttattcatttcaga-3’	5’-tctgaaatgaataaacagcagttgTCTcagaattcttattatggcttccact-3’
R77Q	5’-gaattatgcctattctgctatgTTGacacccaattctgaaatgaata-3’	5’-tattcatttcagaattgggtgtCAAcatagcagaataggcataattc-3’
R80A	5’-gaattgggtgtcgacatagcGCAataggcataattcgacaga-3’	5’-tctgtcgaattatgcctatTGCgctatgtcgacacccaattc-3’

Supplementary Table 1
The primers used for site directed mutagenesis are indicated. The mutated codons are indicated in red capital letters.