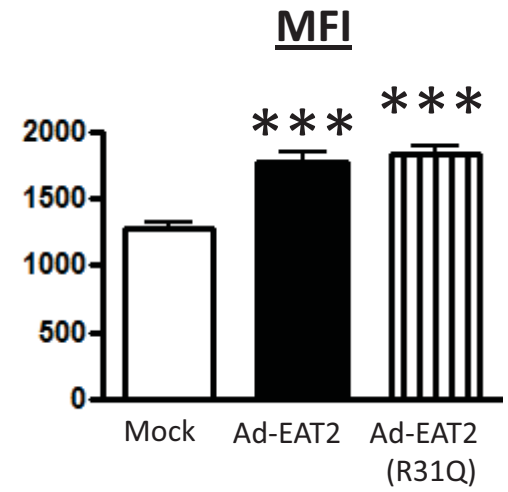
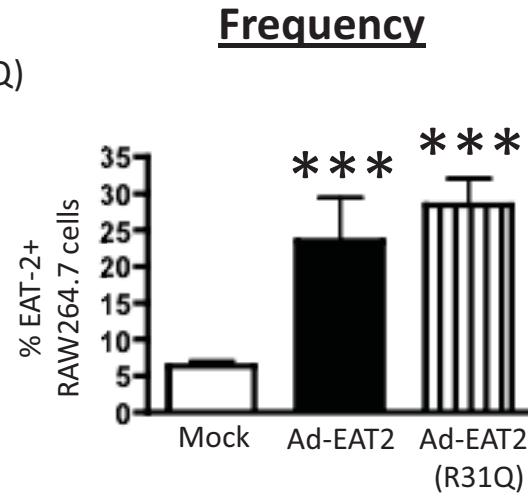
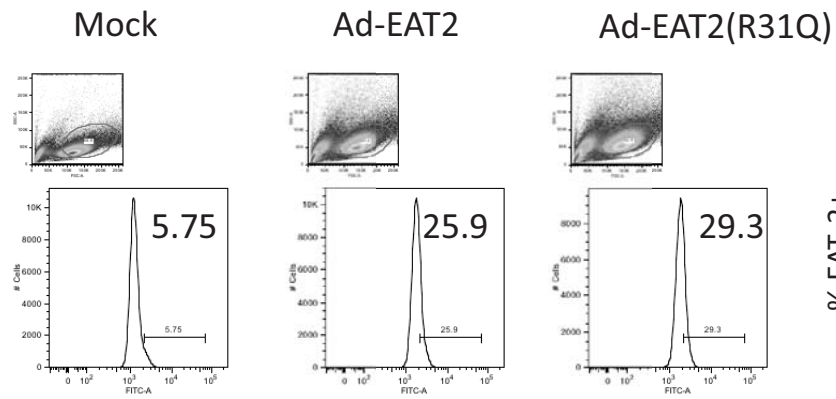
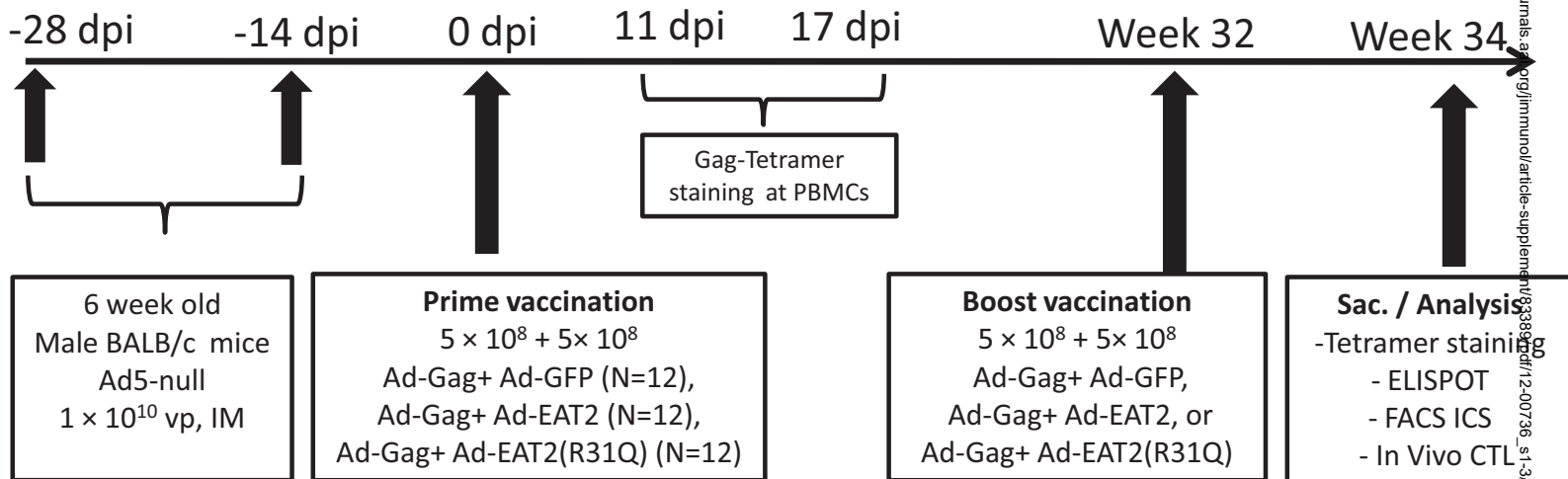


# Supplemental Figure: 1

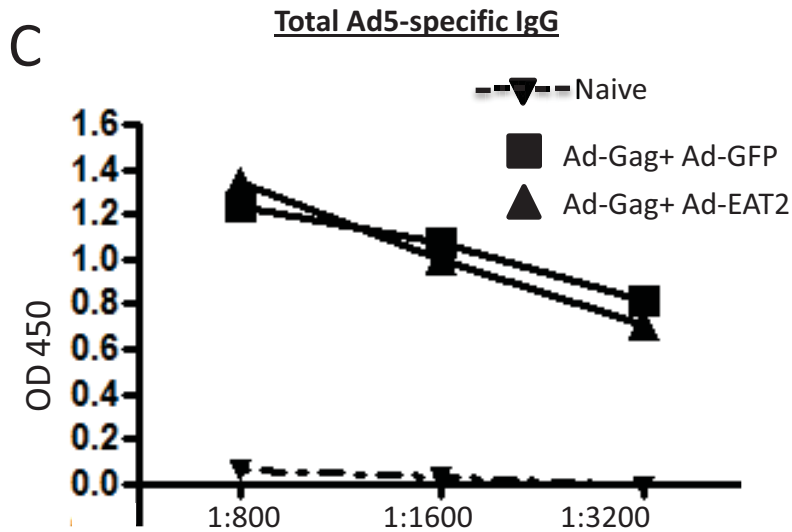
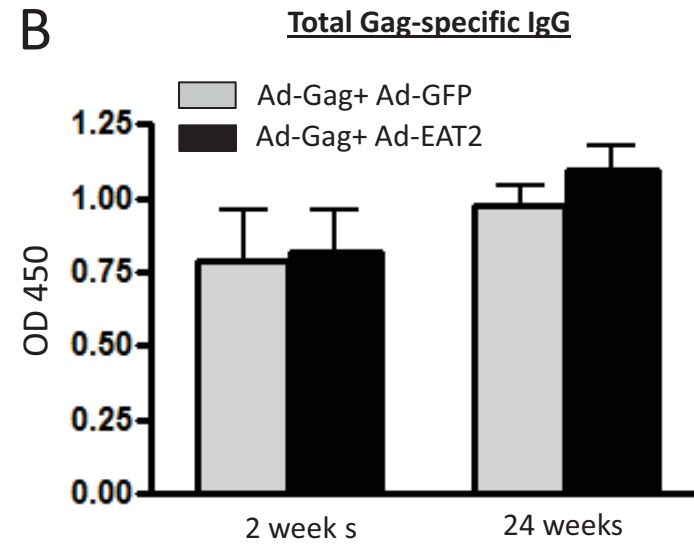
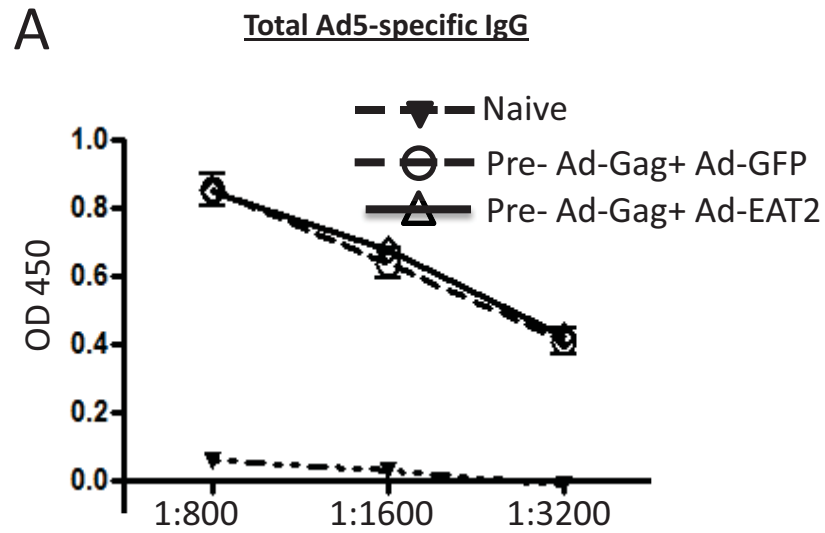


## Supplemental Figure: 2

### Vaccination scheme



# Supplemental Figure: 3



## Supplemental materials

**Supplementary Figure 1: rAd5-EAT-2(R31Q) vector produces stable protein levels of EAT-2 protein.** In vitro cultured RAW264.7 macrophages (300,000 cells/ well) were mock infected or infected with the rAd5-EAT2 (black) or rAd5-EAT2(R31Q) (dash lines) for 72 hours at the multiplicity of infection (MOI) of 20,000 vector particles/ cell. At 72 hpi, cells were stained with Alexa Fluore488-conjugated EAT-2 specific antibody and analyzed on an LSR-II flow cytometer. (A) Expression of CRACC receptor on RAW264.7 following infection with rAd5-EAT2 or rAd-EAT-2(R31Q) is shown. Statistical analysis was completed using One Way ANOVA with a student- Newman-Keuls post-hoc test,  $p < 0.05$  was deemed a statistically significant difference. Samples were plated in quadruplicate and are expressed as mean  $\pm$  SD. \*\*\* denotes  $P < 0.001$ , statistically different from mock treated cells.

**Supplementary Figure 2: Vaccination scheme.** Balb/c mice (N=12) were intramuscularly vaccinated with  $1 \times 10^{10}$  vps/ mouse of Ad5-Null 28 and 14 day before the prime vaccination. At day 0, Ad5-immune mice were co-vaccinated with  $5 \times 10^8 + 5 \times 10^8$  (total of  $1 \times 10^9$  vps/ mouse) of Ad-Gag+ Ad-GFP, Ad-Gag+ Ad-EAT2, or Ad-Gag+ Ad-EAT2(R31Q). PBMCs (pool of three mice) were collected from naïve or co-vaccinated mice at day 11 and 17 for initial evaluation of Gag-specific responses. At week 32, mice were boosted with  $5 \times 10^8 + 5 \times 10^8$  (total of  $1 \times 10^9$  vps/ mouse) of experimental and control vectors. Mice were then sacrificed at week 34 and PBMCs and splenocytes were prepared for Gag-specific T cell response analysis. In a different set of experiments, Ad5-immune mice were primed with  $5 \times 10^7 + 5 \times 10^7$  (total of

$1 \times 10^8$  vps/ mouse) of experimental and control vectors. Mice were then boosted at week 22 with  $1 \times 10^8$  vps/ mouse and Gag-specific responses were analyzed at week 24.

**Supplementary Figure 3: Humoral immune responses in rAd5 pre-immune Balb/c mice**

**following rAds vaccination.** Plasma from naïve (n=4) or rAd5 pre-immune vaccinated Balb/c mice (n=12) was collected at 27 dpi following rAd5-Null injection (A), two or twenty four weeks following vaccination (B and C). (A) Ad5-specific total IgG levels were measured as detailed in material and methods. (B) HIV/Gag-specific total IgG levels measure in mice at week 2 following prime vaccination or at week 24 following prime-boost vaccination. (C) Ad5-specific total IgG levels at week 24 following prime-boost vaccination.