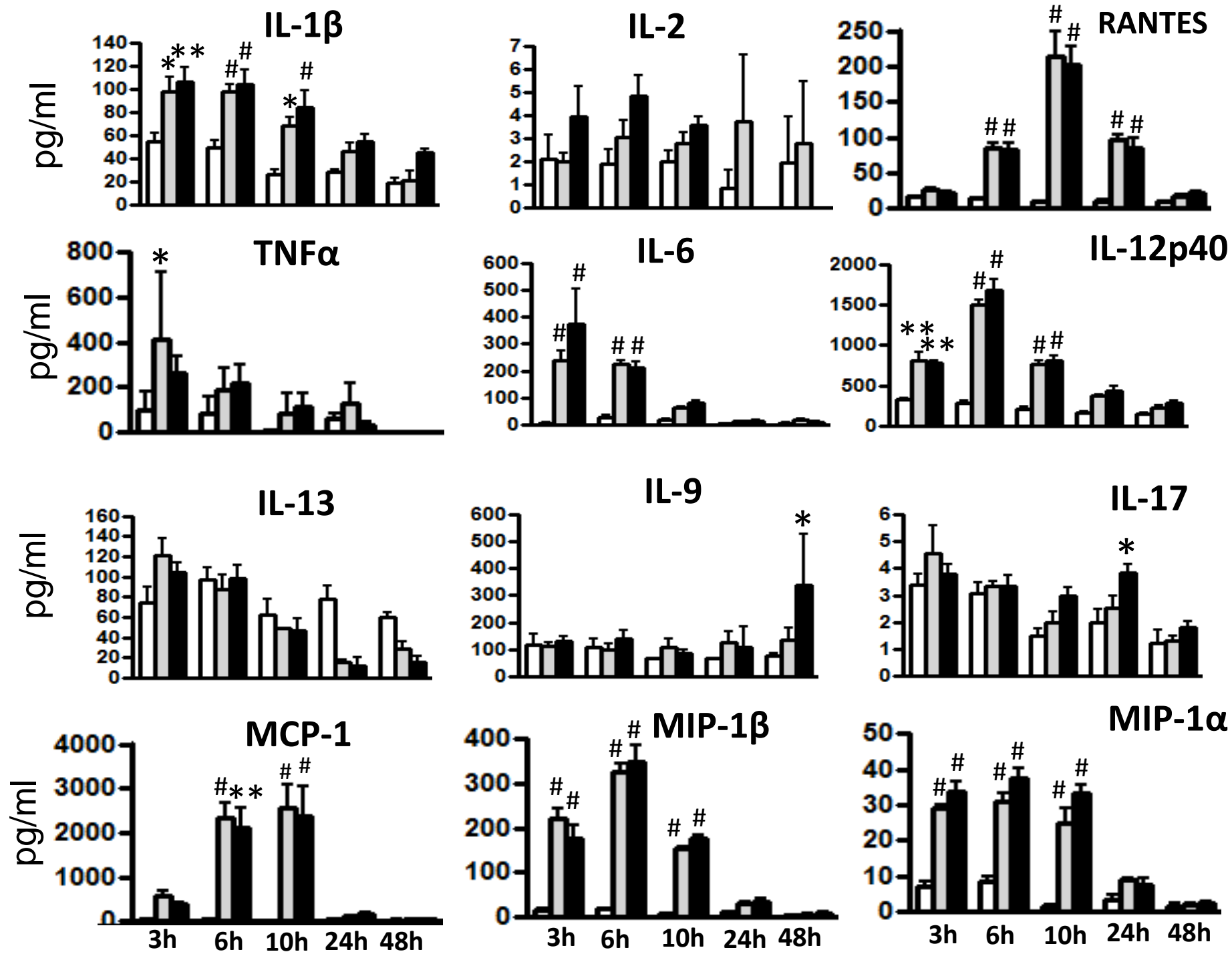
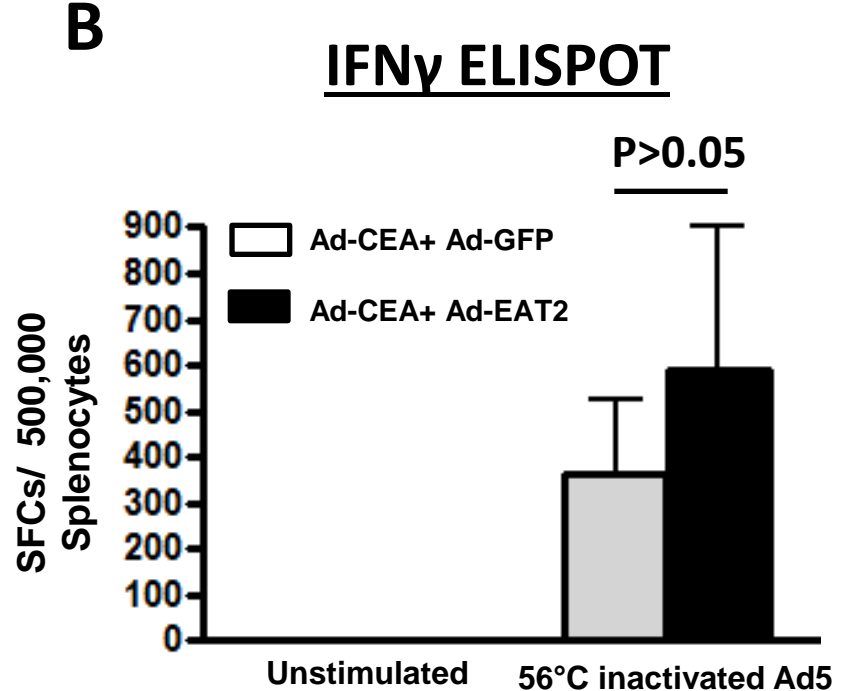
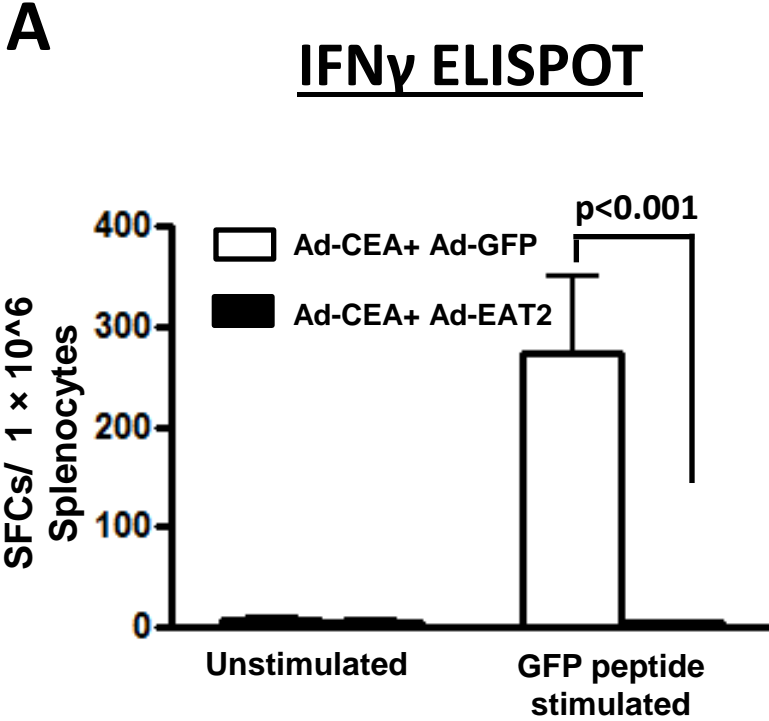


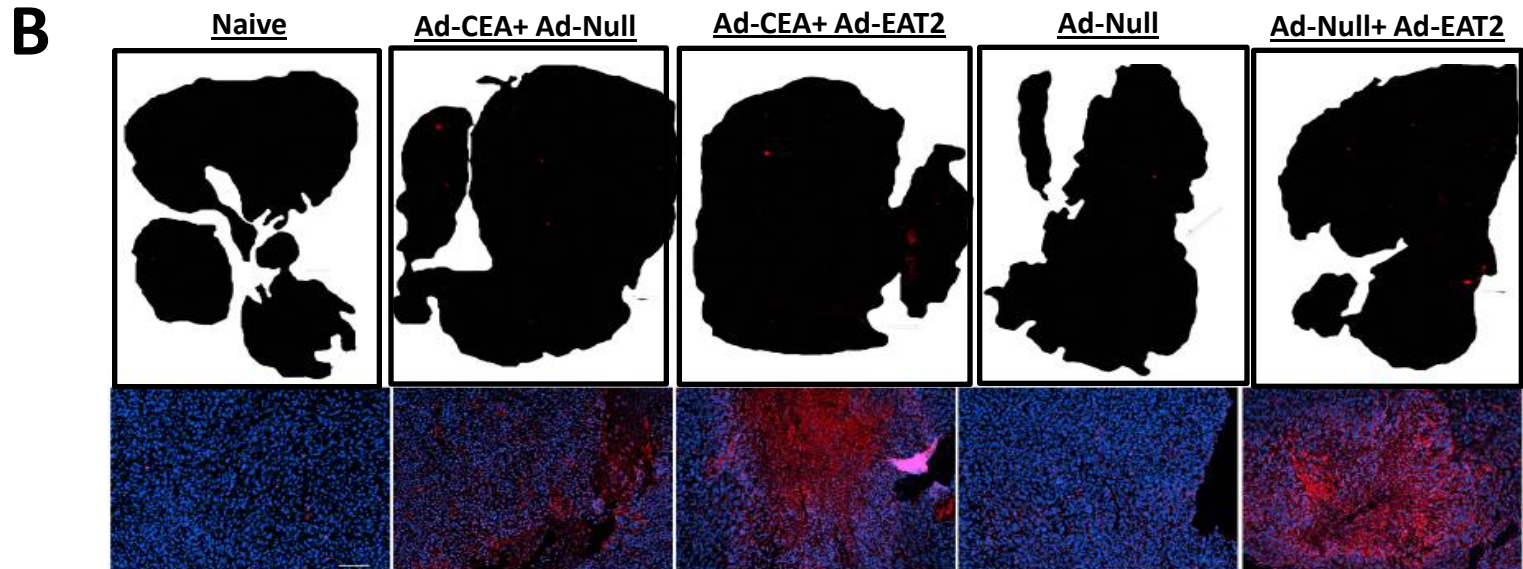
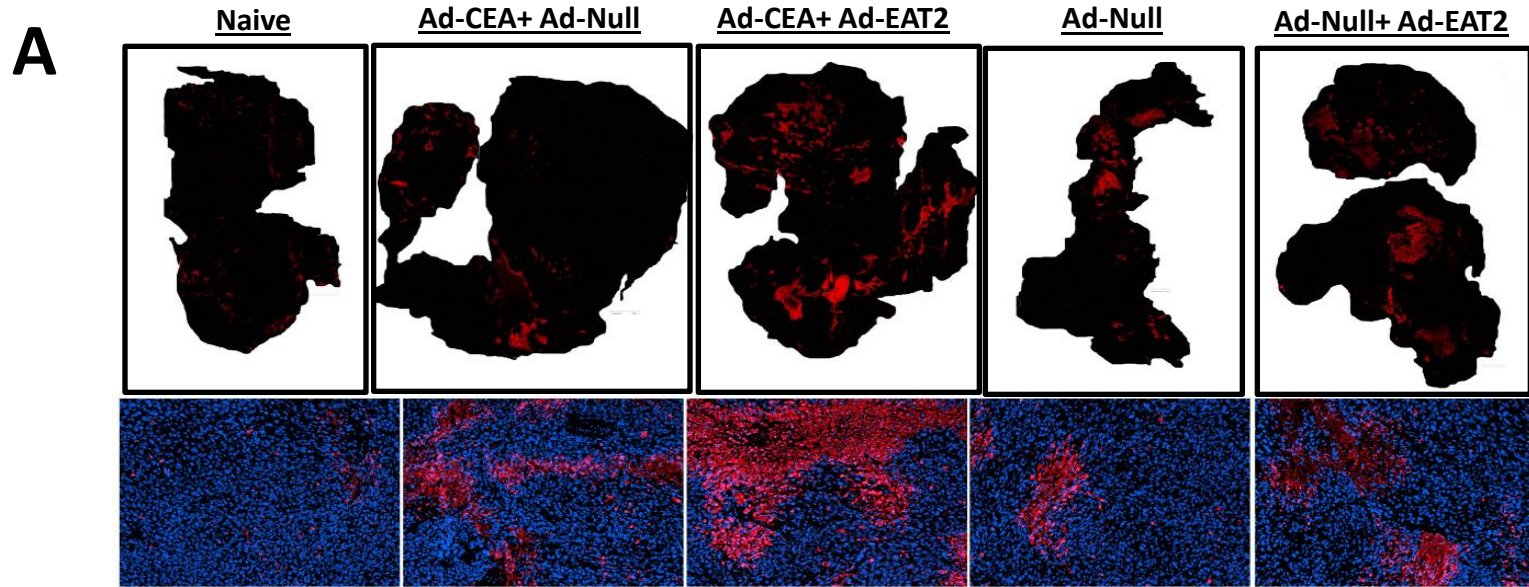
Supplemental Figure 1: Naive rAd5-Null rAd5-EAT2



Supplemental Figure: 2



Supplemental Figure 3:



Supplemental Figure 1: Kinetics of pro-inflammatory cytokine and chemokine production following rAd5-EAT2 administration. WT C57BL/6 mice (n=8) were either mock injected, or intravenously injected with 7.5×10^{10} vps of either rAd5-EAT2 or rAd5-Null control vectors. Plasma was harvested at 3, 6, 10, 24, and 48h after virus injection. Cytokine induction was evaluated using a 23-plex multiplexed bead array based quantitative system. The bars represent mean \pm SD. Statistical analysis was completed using a Two Way ANOVA with a Student-Newman-Keuls post-hoc test, $p < 0.05$ was deemed a statistically significant difference. *, denotes $p < 0.05$, ** denotes $p < 0.01$, # denotes $p < 0.001$, statistically significant different from mock injected animals.

Supplemental Figure 2: Vaccine elicited GFP and Ad5-specific T cell immune responses. WT C57BL/6 mice (n=4) were co-immunized intramuscularly in the tibialis anterior with equivalent viral particles of rAd5-CEA mixed with either rAd5-GFP or rAd5-EAT2 (total of 1×10^9 vps mixed prior to injection). At 14 dpi, splenocytes were harvested and stimulated *ex vivo* with the GFP-specific peptide (a) or heat (55°C) inactivated Ads (b), followed by IFN γ ELISPOT, performed as described in Materials and Methods. Spot forming cells (SFCs) were quantified using an ELISPOT reader. Data are presented as mean \pm SD. Statistical analysis was completed using One-Way ANOVA with a Bonferroni post-hoc test, $p < 0.05$ was deemed a statistically significant difference.

Supplemental Figure 3: Tumor tissues derived from rAd5-EAT2 and rAd5-EAT2 co-vaccinated mice have increased caspase-3 activation and granzyme B expression.

Immunohistochemistry for active Caspase-3 (a) and granzyme B (b) (red) was performed on

tumor tissues (whole tumor section (top) or 10 X magnifications (bottom)) derived from rAd5-CEA+ rAd5-EAT2 co-injected animals at the end of the study (18 dpi). Tumor tissues derived from rAd5-EAT2 co-injected mice show a strong signal for active caspase-3 and granzyme B.

Supplemental Table #1:

Cytokine/Chemokine	Lower detection limit (pg/ml)
IL-1 α	1.44
IL-1 β	3.61
IL-2	3.58
IL-3	1.6
IL-4	6.53
IL-5	2.77
IL-6	1.1
IL-9	4.3
IL-10	3.75
IL-12(p40)	1.1
IL-12(p70)	5.44
IL-13	5.45
IL-17	2.03
Eotaxin	41.98
G-CSF	2.32
GM-CSF	1.96
IFN- γ	1.33
KC	3.21
MCP-1	4.88
MIP-1 α	2.45
MIP-1 β	1.3
RANTES	0.7
TNF- α	10.9

Assay range for the lower and upper detection limit for the 23-

Upper detection limit (pg/ml)
27885.71
46323.47
57140.74
20478.46
99593.26
11780.96
13045.58
30650.2
17348.83
20036.45
20390.28
41923.87
30411.16
37427.59
43675.94
2255.34
5483.35
36304.5
11350.52
63935.68
23066.95
3246.71
53383.53

plex mouse cytokines and chemokines