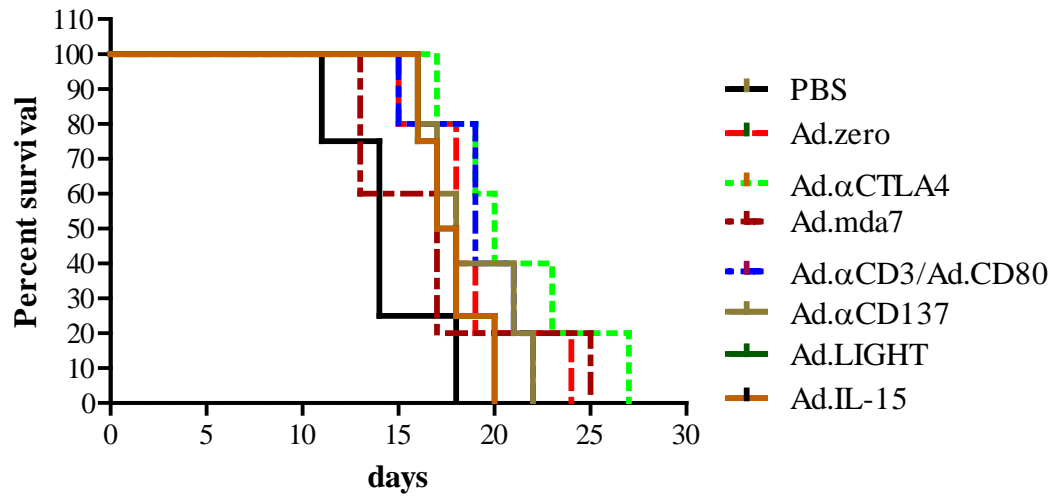
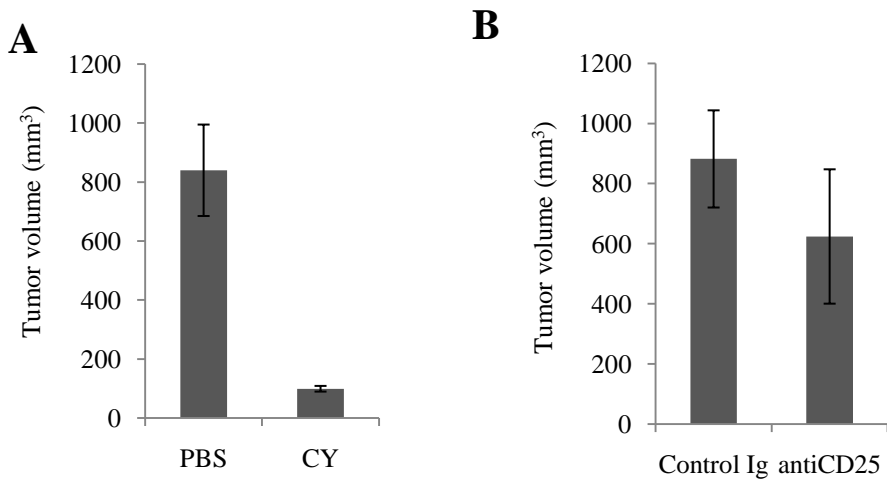


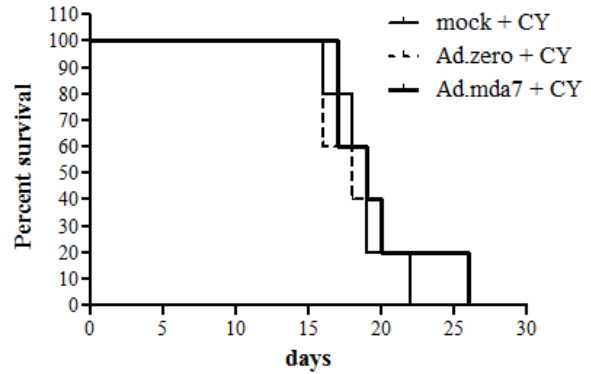
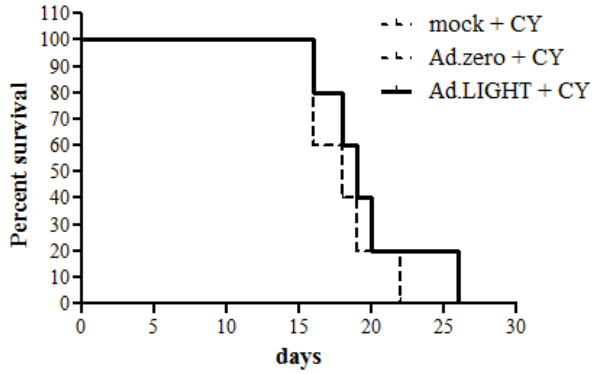
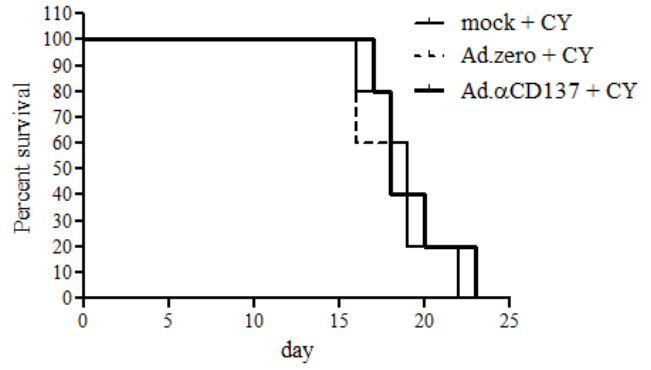
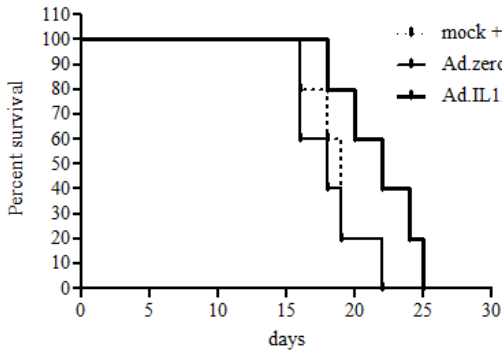
Suppl. Fig.1. Genomic structure of Ad vectors. All vectors are based on E1/E3 deleted serotype 5 Ads and contained the Rous sarcoma virus long terminal repeat (RSV) promoter and bidirectional SV40 polyadenylation signal (pA). Ad.zero lacks a transgene. Ad.bGal, Ad.IL-15, Ad.LIGHT, and Ad.mda7 contain the coding sequence for E.coli b-galactosidase, mouse interleukin 15; mouse tumor necrosis factor (ligand) superfamily member 14 (LIGHT), and human melanoma differentiation-associated gene-7 (mda7), respectively. Ad.alphaCD3 contains membrane-bound anti-mouse CD3scFv (hybridoma 2-C11) fusion protein including the hinge and CH2 and CH3 domains of the human IgG1 heavy chain (IgG Fc), the murine B7-1 (CD80) transmembrane (TM) domain and cytoplasmic tail (CT), a murine immunoglobulin κ chain signal peptide (SP), and a 9 amino acid HA epitope (HA). Ad.alphaCD137 contains the membrane-bound scFv against mouse CD137 (hybridoma 1D8), the IgG Fc, and the B7-1 TM and CT region. Ad.alphaCTLA4 contains the scFv against CTLA4 (hybridoma UC10-F10-11), the IgG Fc, the signal peptide and both an HA and myc tag. Notably, CTLA4-scFv is secreted. Ad.CD80 contains the coding sequence for murine CD80.



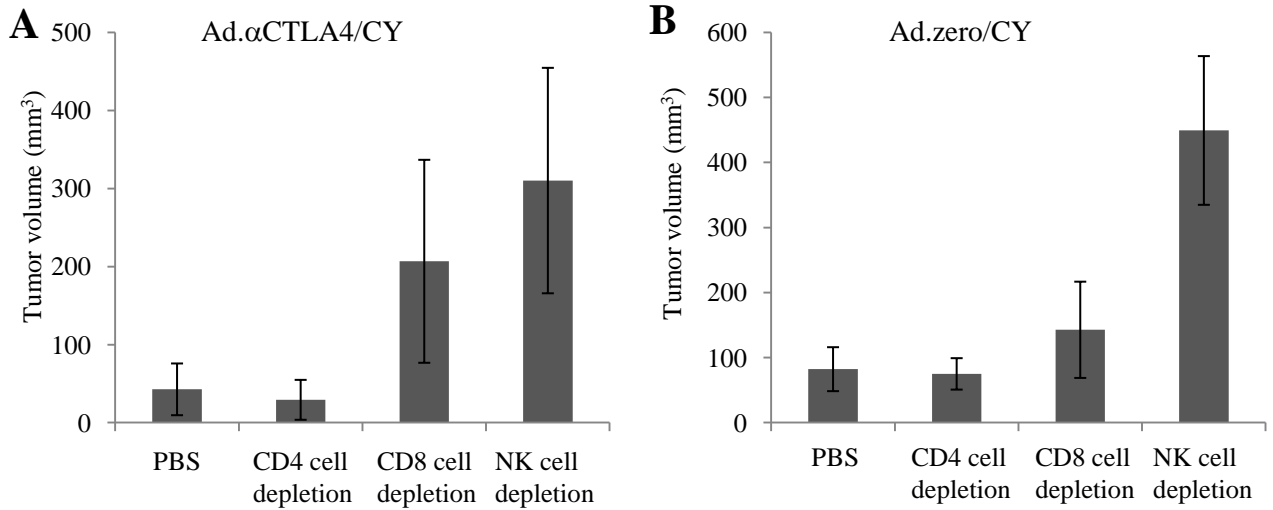
Suppl. Fig.2 A total of 5×10^5 MMC cells in 100ul PBS were subcutaneously injected into *neu-tg* mice. When tumors reached a size of 4x3mm (usually at day 6 after cell injection), Ad vectors (1×10^9 pfu) were injected intratumorally. Tumor size was measured every other day. The day tumors reached a volume of 250mm^3 represented the endpoint in Kaplan-Meier survival studies. Medium survival was calculated using GraphPad-Prizm4 software. N=5.



Suppl. Fig.3 Therapy studies with MMC tumors in CB17-SCID/beige mice. A) MMC tumor cells were implanted and low-dose CY (100mg/kg) or PBS was injected i.p. six days later. Shown are tumor volumes at day 16 after MMC cell transplantation. PBS vs CY: $p < 0.001$. **B)** In another experiment, tumor-bearing CB17-SCID/beige mice received 250 μ g of antiCD25 or control antibody 6 days after MMC cell implantation. Shown is the tumor volume at day 16. Control Ig vs antiCD25: $p = 0.07$



Suppl. Fig. 4 Therapy studies with TC-1 tumors. Survival of TC-1 tumor bearing mice after treatment with CY (100mg/ml) 4 days before PBS or Ad injection. The day tumors reached a volume of 200mm³ represented the endpoint in Kaplan-Meier survival studies. N=5.



Suppl. Fig.5 Mechanisms of Ad. α CTLA4 stimulated anti-tumor immune response in TC-1 model. A total of 1×10^4 TC-1 cells were subcutaneously injected into the left inguinal flank of C57Bl/6 mice at day 0. At day 5, mice received an intraperitoneal injection of CY. At day 9, 1×10^6 Ad.zero or Ad. α CTLA4 transduced TC-1 cells were injected into the right inguinal flank. Four days before vaccination with Ad. α CTLA4 (A) or Ad.zero (B) transduced cells, mice received intraperitoneal injection of CD4, CD8 or NK-cell depleting antibodies. Injections were repeated every 3 days for the time of monitoring. Shown is the tumor volume at day 22 after TC1 cell implantation.