

Legends to Supplementary figures

Supplementary figure 1. A) Intradermal injection of untargeted-Ad-GFP leads to massive transduction of large CD11c⁻ cells (GFP⁺, green) 24h post-injection, predominantly in the marginal zones of draining LN. DC were detected by anti-CD11c (red, magnification 400x). B) Autofluorescent hair follicles (H, green) surrounded by MHC class II positive DC (DC, red) in the murine skin are shown. Cell nuclei were visualized using DAPI (blue, magnification 400x). Data shown are representative of three independent experiments with n=2-3 per experimental group.

Supplementary figure 2. CD40-Ad-induced immune responses mostly depend on DC in the skin. Mice were i.d. injected with Ad-GFP, untargeted Ad-GFP-TRP2_{aa180-188} or CD40-Ad-GFP-TRP2_{aa180-188} (10x10⁶ iu/mice) on the flank. A) The skin vaccination site was surgically excised after 4h. Rates of IFN- γ producing CD8⁺ T cells in peripheral blood leukocytes (14d post vaccination) were determined by flowcytometry after in vitro restimulation with TRP2_{aa180-188} peptide. B) Growth kinetics of B16F10 melanomas in vaccinated mice with or without surgical excision after 4h of the vaccination site (n=6). *number of tumor bearing mice/total number of mice is indicated. C) Depletion of regional macrophages by liposome-complexed clodronate prior to i.d. vaccination significantly enhanced TRP2 specific CD8⁺ T cells in the PBL of mice vaccinated with untargeted Ad-GFP-TRP2_{aa180-188} but not with CD40-Ad-GFP-TRP2_{aa180-188}. Means \pm SEM are plotted (n=3-6). The data were analyzed by ANOVA. Differences were considered significant when *P<0.05.

Supplementary figure 3. Blocking of CTLA-4 during vaccination or depletion of regulatory T cells (Tregs) prior to vaccination, do not enhance therapeutic efficacy of CD40-Ad5-gp100. Mice were vaccinated on the contralateral flank with CFm40L, Ad-GFP, Ad-gp100 or CD40-Ad-gp100 (i.d. 1x10⁹ iu/mouse) once B16F10 tumors (3mm) were established; booster

vaccination was given on day 9. A) Two groups intraperitoneally (i.p.) received either anti-CTLA-4 (250ug/mouse) or an equal quantity of isotype matched control (ctrl) mAb. B) Tregs were depleted by injecting anti-CD25 mAb (200ug/mouse, i.p.) 24h prior to vaccination. Shown are mean tumor volumes \pm SEM (n=4 per group).