Supporting Information

Adams et al. 10.1073/pnas.1017146108

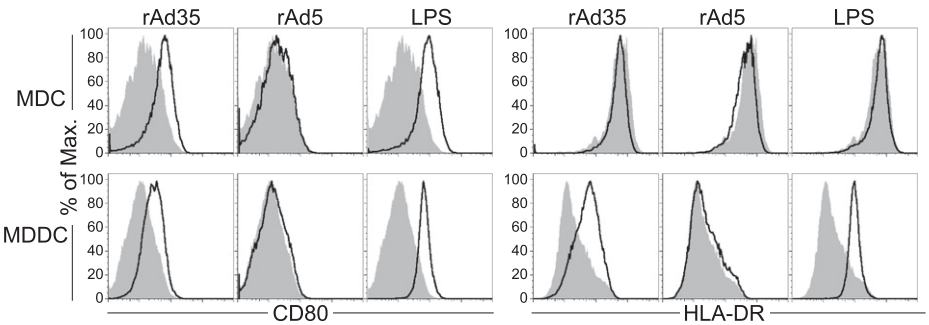

Fig. S1. Induction of phenotypic maturation of DC subsets following rAd exposure. Human MDCs and MDDCs were exposed to either mock, rAd35 (10 ip/cell), rAd5 (100 ip/cell), or LPS (100 ng/mL) treatment for 24 h at 37 °C. Surface expression of CD80 and HLA-DR from all exposed DCs was assessed by flow cytometry. Histograms depict fluorescence intensity of CD80 or HLA-DR on mock (gray-filled) or stimulated (black line) DCs of one representative donor from three independent experiments.

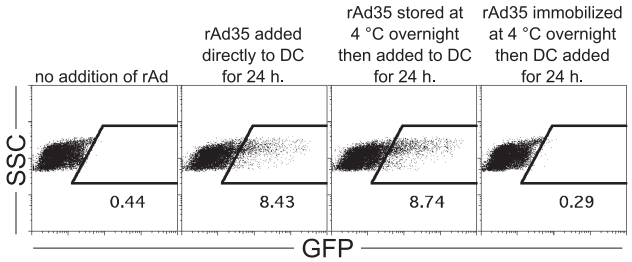

Fig. S2. Immobilization renders rAd35 vectors unable to infect DCs. MDDCs were exposed to either mock treatment or to rAd35 or rAd35 that had been stored overnight at 4 °C or rAd35 that had been immobilized overnight at 4 °C. All vectors were used at 10 ip/cell. Flow cytometry plots show the frequency of GFP^+ DCs after 24 h. One representative experiment is shown.

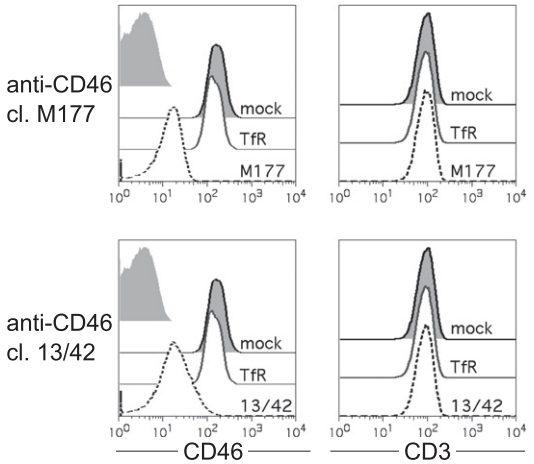

Fig. S3. CD46 is down-regulated by anti-CD46 mAb (targeting the SCR1 and SCR2 domains). Anti-CD46 mAb (cl. 13/42 or M177) or anti-TfR mAb was immobilized in 96-well plates overnight before washing. Total $CD4^+$ T cells were incubated with immobilized mAb for 24 h, at which time surface CD3 and CD46 expression was assessed by flow cytometry. Histograms show fluorescence intensity of CD3 and CD46 following indicated treatments. One representative experiment is shown. Gray histogram is the fluorescence minus one (FMO) staining control. cl., clone.

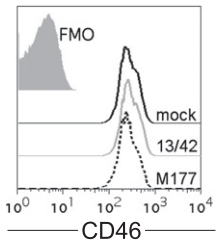

Fig. S4. Immobilized anti-CD46 mAb clones 13/42 and M177 do not interfere with staining with anti-CD46 mAb clone 8E2. To determine whether binding of immobilized anti-CD46 mAb interfered with binding of the staining mAb used in flow cytometry, we incubated T cells for 20 min at 4 °C on immobilized anti-CD46 mAb (clones 13/42 and M177). These conditions allow for Ab receptor binding but block receptor internalization. The T cells were then stained for surface CD46, and expression was assessed by flow cytometry. Histograms depict fluorescence intensity of mock-treated (black line), 13/42-treated (gray line), or M177-treated (black dashed line) cells. One representative experiment is shown. FMO, fluorescence minus one.

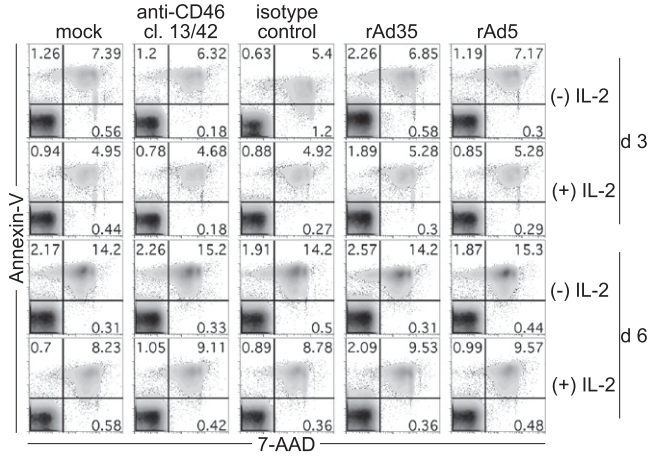

Fig. S5. CD46 ligation does not affect normal survival kinetics or IL-2 responsiveness of naive CD4$^+$ T cells. Flow cytometry plots of naive CD4$^+$ T cells exposed to immobilized anti-CD46 mAb, isotype control, rAd35, or rAd5 (100 ip/cell) for 1–5 d with or without IL-2 are shown. At indicated time points, cells were stained for Annexin-V and 7-AAD to assess viability. One representative donor from three independent experiments is shown. cl., clone.

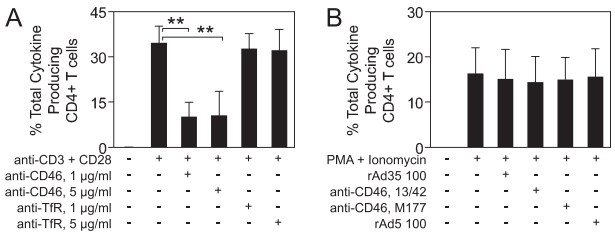

Fig. S6. CD46 ligation specifically interferes with cytokine production induced by anti-CD3/CD28 mAb. (*A*) Total CD4$^+$ T cells were stimulated with CD3/CD28 with or without immobilized anti-CD46 mAb (13/42) or isotype-matched anti-TfR mAb. The bar graph summarizes mean ± SD from three donors of total IL-2– and IFN-γ–producing CD4$^+$ T cells. Paired *t* test: $^{**}P < 0.01$. (*B*) CD4$^+$ T cells were exposed to immobilized rAd vectors or anti-CD46 mAb and stimulated with PMA and ionomycin in the presence of Brefeldin A for 5 h, permeabilized, and stained for intracellular IL-2 and IFN-γ. The bar graph summarizes mean ± SD from three donors of total IL-2– and IFN-γ–producing CD4$^+$ T cells. Paired *t* test: $P > 0.05$.

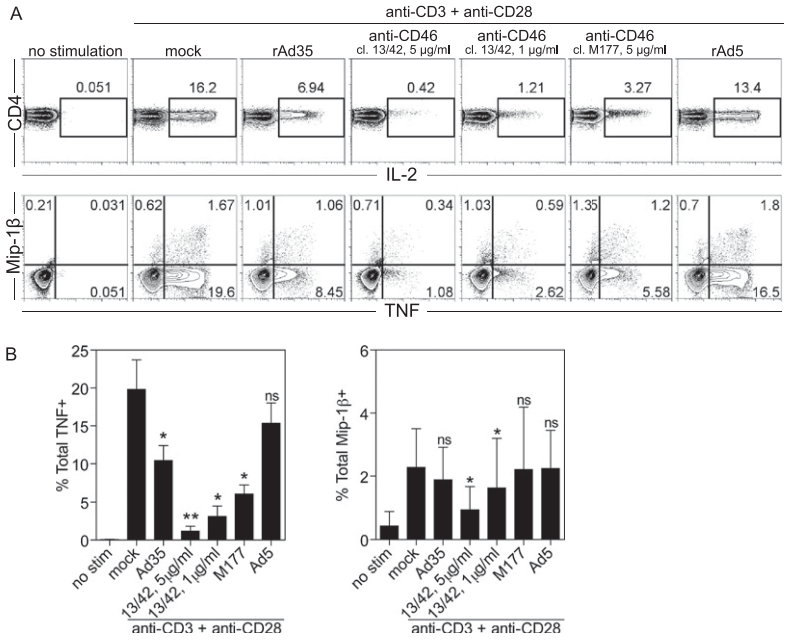

Fig. S7. rAd35 inhibits TNF but not Mip-1β expression in naive CD4$^+$ T cells. Sorted total CD4$^+$ T cells were stimulated with CD3/CD28 with or without immobilized anti-CD46 mAb clone 13/42 or M177 at the indicated concentrations, rAd35, or rAd5 (100 ip/cell). After 5 h in the presence of Brefeldin A, cells were permeabilized and stained for intracellular IL-2, TNF, and Mip-1β simultaneously. (*A*) One representative donor from three independent experiments is shown. (*B*) Summary of data from three donors (mean ± SD) is depicted in the graphs. Paired *t* test: $*P < 0.05$, $**P < 0.01$, nonsignificant (n.s.) $P > 0.05$.