Figure legends for Supplementary Figures

Figure S1. The serum clearance and immunogenicity of VISTA-specific hamster monoclonal antibody 13F3. 300 μg of 13F3 were injected into naive mice, on day 0, 1, 2, 3, then every other day through day14. Mice were eye bled 24hr after each 13F3 injection and serum was harvested and analyzed by ELISA to detect serum level of 13F3, as well as the presence of IgGs that recognize 13F3. The serum concentration of 13F3 was calculated based on a standard curve using purified 13F3, shown by the left Y axis. The serum titer of 13F3-recognizing IgGs was calculated based on a total IgG standard curve, shown by the right Y axis.

Figure S2. VISTA mab did not directly alter the proliferation and apoptosis of tumor cells. B16 melanoma cells were cultured in triplicated wells in 6x well plate, in the presence of either control-Ig or 13F3. Cells were harvested on day+4 by trypsin digestion, when cells reach 80% confluence. Live cells were counted using a hemocytometer based on trypan-blue live/dead staining. The percentage of live cells was determined by calculating the amount of live and total cells. Similar conclusion could be reached by staining cells with live/dead dye 7-AAD (not shown). Shown are representative results from 2 independent experiments.

Figure S3. The distinct population of myeloid-derived suppressor cells (MDSCs) infiltrating the B16 melanoma and MB49 bladder tumors. B16 melanoma and MB49 bladder tumors were harvested from tumor bearing mice when tumors reached 7-9 mm diameter. Tumor-infiltrating leukocytes were harvested after collagenase digestion and percoll gradient centrifugation, and analyzed by flow cytometry. MDSCs were defined as $CD45^{+}CD11B^{+}CD11C^{-}$ TILs. Granulocytic and monocytic MDSCs were further distinguished by the expression of LY6G and LY6C.

Figure S4. VISTA mab (13F3) did not directly alter Foxp3 stability in cultured Tregs, as well as the proliferation and apoptosis of Tregs. Foxp3GFP$^+$ Tregs were sorted from naïve splenocytes (purity ~95%), and cultured in 96x well plates coated with polyclonal stimuli anti-CD3 (5 μg/mL), anti-CD28 (5 μg/mL), and recombinant human-IL2 (50 units/mL), in the presence of either control-Ig or anti-VISTA mab (13F3, 10 μg/mL). Cells were harvested on day+3 and analyzed by flow cytometry. The comparable amount of FOXP3 expression in cultured cells was shown by the percentage of Foxp3GFP$^+$ cells. The percentage of dead cells were examined by staining with 7-AAD. The number of live Foxp3GFP$^+$ Tregs from triplicated wells was shown. Shown are representative results from 2 independent experiments.

Figure S5. Representative graphs showing expression patterns of IFNγ, Granzyme B, CD107 and Ki67 on tumor-infiltrating T cells, and expression of IL12p40 and TNFα on tumor-infiltrating DCs.

Figure S6. Better long-term survival was achieved with a prime-boost vaccine regime when treating 7-day B16-BL6 melanoma in combination with VISTA mab (13F3). Mice bearing 7-day old B16-Bl6 tumors were treated either with 1 single vaccine dose on day+7, or with 2 vaccine doses on day+7 and day+14, as described in the methods. Mice were also concurrently treated with VISTA mab 13F3 (300 μg), starting on day+7, every day for 3 weeks. Tumor size was measured by caliper every 2-3 days. Shown are the representative survival curves from 2 independent experiments.

Figure S7. The therapeutic efficacy of vaccine/VISTA mab combination treatment requires T-cell mediated adaptive immune response.

(A) Mice (n=8 per group) were treated with 300 μg of $CD8^+$ T cell-depleting mab (clone 2.43) on day-2 and then every 4 days throughout the experiment. Mice were inoculated with B16-BL6 (20,000) on day0 and treated on day+2 post tumor inoculation with peptide vaccine as describe in methods. Mice were also treated with VISTA mab (300 μg) every day for 3 weeks. **(B)** Rag ko mice (n=7-9 per group) were inoculated with B16-BL6 (20,000). On day+2 post inoculation mice were treated with peptide vaccine and VISTA mab as indicated. Tumor size was measured with a caliper every 2 day and shown are representative results from 2 independent experiments.