

Supplementary Figure Legends

Supplementary Figure 1. Survivin peptide vaccine, SurVaxM, induced antigen-specific CD8 effector cells in the tumor bearing animals. Tetramer analysis of splenocytes obtained from mice immunized with SurVaxM. Splenocytes were stained with anti-CD8 antibodies and survivin-specific MHC classI tetramers for flow cytometric analysis. Results are based upon gating of CD8⁺ T cells and indicate the percent of double labeled cells (CD8⁺/Tetramer⁺) with respect to specific tetramer.

Supplementary Figure 2. Tasquinimod modulation of MDSC subpopulations in CR Myc-CaP tumor bearing FVB mice. **A.** FACS analysis of blood samples with MDSCs surface marker (Gr1 and CD11b) staining. **B.** MDSC granulocytic and monocytic subpopulation analysis with blood samples. **C.** FACS analysis of splenocyte samples with MDSCs surface marker (Gr1 and CD11b) staining. **D.** Infiltrating MDSC granulocytic and monocytic subpopulation analysis. (** $p < 0.01$; t-test. Error bars indicate s.e.m.)

Supplementary Figure 3. CD11b⁺ in tumor and MDSC subpopulations in spleen from B16-h5T4 tumor bearing C57/B16 mice. **A.** CD11b⁺ frequency of viable cells in tumor tissue. **B.** Total CD11b⁺ cells in spleen ($\times 10^6$). **C.** MDSC subpopulations in spleen (CD11b⁺). (** $p < 0.01$; t-test. Error bars indicate s.e.m.)

Supplementary Figure 4. The effect of tasquinimod on T cell proliferation and regulatory T cells. **A.** T cells were isolated from spleens of CR Myc-CaP tumor bearing mice that had received indicated treatment and activated in vitro in CD3 and CD28-coated plates for 65-72 hours, ³H-thymidine was added to culture for the last 12 hours. T cell proliferation was measured by ³H-thymidine incorporation. **B.** T cells were isolated from naïve or B16-h5T4 tumor bearing

C57Bl/6 mice, labelled with CFSE and stimulated with anti-CD3 and anti-CD28 coated beads in the presence or absence of indicated concentrations of tasquinimod. After 3 days of culture, cell division was measured as the median fluorescence intensity (MFI) of CFSE in CD4⁺ and CD8⁺ T cells respectively by FACS analysis. The frequencies of CD4⁺Foxp3⁺ Treg cells were measured by FACS analysis in spleen and tumor from CR Myc-CaP (C.) and B16-h5T4 (D.) tumor bearing mice.

Supplementary Figure 5. The effect of tasquinimod treatment on CD11c⁺ dendritic cells. A.

The frequencies of CD11c⁺ dendritic cells in spleen from B16-h5T4 tumor bearing mice that had received indicated treatment. **B.** CD11c⁺ dendritic cells isolated from spleens from vehicle- or tasquinimod treated B16-h5T4 tumor bearing mice were used to act as APCs for CFSE-labelled naïve T cells *in vitro*. The T cells were stimulated with the superantigen SEA in the presence or absence of dendritic cells at different ratios. After 3 days of culture, T cells division was measured as CFSE expression by FACS analyses. Percentage of divided cells were calculated based on the CFSE expression of undivided T cells.

Supplementary Figure 6. The effect of tasquinimod on nitric oxide synthase activity in infiltrating myeloid cells.

Lysates were prepared from infiltrating myeloid cells from vehicle or tasquinimod treated mice and were tested with a nitric oxide synthase kit (see method). The enzyme activity was measured as concentration of its end product, nitrite. (**p* < 0.05, t-test. Error bars indicate s.e.m.)