Supplemental Methods

Immunohistochemistry for MPR expression. Tumor tissues were excised and embedded in paraffin blocks. Four micron thick sections of the tissue were sliced and the sections were stained for MPR using rabbit MPR primary antibody (Abcam) and the Vectastain ABC kit. Hematoxylin was used for counterstaining the nucleus. The slides were processed for fixation and scanned using the Aperio™ (Vista, CA) ScanScope XT with a 200x/0.75NA objective lens at a rate of 2 minutes per slide via a Basler tri-linear-array detector. Image analysis was performed across the whole slide image by first segmenting the tumor from adjacent tissue using Aperio Genie® v1 customized algorithms. This training algorithm was quality controlled by a pathologist.

Immunopositivity of the mannose-6-phosphate biomarker was identified and quantitatively scored using Image Pro Plus v6.2 (Media Cybernetics, Bethesda, MD). The tumor region area was determined by pixel count. The number of strongly positive stained pixels was collected for all RBG pixels less than the established intensity threshold (<125) for the 8-bit image. The percent strong positive was calculated to be the number of strong positive pixels divided by total pixel number for the tumor regions.

Quantitative real-time polymerase chain reaction (qRT-PCR) and Western blotting. B16F10 and 4T1 cells were treated with TAX as described above. B16F10 tumor tissue from untreated and TAX treated mice was snap frozen in liquid nitrogen and homogenized. RNA was extracted with an RNase Mini kit and cDNA was synthesized using SuperScript III Reverse Transcriptase kit (QIAGEN, Valencia, CA). PCR was performed with 2.5 µl cDNA, 12.5 µl SYBR Master Mixture (Applied Biosystems, Foster City, CA), and target gene-specific primers(Forward primer: AGG TGA CCA TGG TGA ACG GAA CGC; Reverse primer TGG

GCT TCA CCT GGC AGA TGT TGG. Amplification of endogenous β-actin was used as an internal control.

Cytosolic and membrane proteins were extracted from cells using the Qproteome Cell Compartment kit (Qiagen). Samples (50 μg total protein per lane) were subjected to electrophoresis on 5% SDS-polyacrylamide gel followed by transfer to a polyvinylidene difluoride membrane. Membranes were blocked overnight with Western blocking reagent (Boehringer Mannheim) and then incubated with appropriate MPR or IGF2 (Abcam) primary antibodies overnight at 4°C followed by incubation with anti-goat IgG HRP-conjugated antibody (Santa Cruz) for 2 hr at room temperature. The bands were visualized by ECL Western plus kit (GE healthcare). To confirm equal loading of protein, the membranes for the TAX treated samples were probed for Cadherin or tubulin (Abcam) in addition to β- actin.

Luciferase murine model for bystander assay. For adoptive transfer using endogenous, vaccine-generated T cells, B16 tumor-bearing mice received $5x10^6$ $Trp2_{180}$-specific $CD8^+$T cells generated in B6 mice immunized with $Trp2_{180}$TriVax. $CD8^+$ T cells were purified from spleens using MACS LS columns (Miltenyi Biotec) 8 days after the last immunization. $CD8^+$ T cells were >40% tetramer-positive for $Trp2_{180}$. For TriVax immunizations, mice were injected i.v. with a mixture of 200 μg $Trp2_{180}$ (SVYDFFVWL) peptide, 50 μg anti-CD40 mAb (Clone; FGK-45.5), and a 50 μg poly-IC (Poly-ICLC/Hiltonol™, Oncovir, Inc.,).

B16-F10-luc-G5 cell line was combined with either B16F10 control shRNA kb- or B16F10 MPR shRNA kb- cell lines at 1:1 ratio of 0.15 $x10^6$ cells/ line/ mouse. These cells were injected s. c. to C57BL/6 mice on Day 0. A third experiment involved injecting the B16-F10-luc-G5 at the same numbers as above to the left flank and an equal

number of B16F10 control shRNA kb- cells to the right flank of mice. Mice were treated with $Trp2_{180}$-specific $CD8^+$ T cells on days 10 and 16 followed by vaccinations with Trp2 peptide on days 11 and 17. Mice in combination therapy groups received TAX 12.5mg/kg b.w. on Days 13 and 22. Tumor growth was monitored three times a week for 4 weeks by caliper measurement and twice a week by *in vivo* imaging. For imaging the tumors, a fresh stock solution of luciferin at 15mg/ml in DPBS was prepared and filter sterilized through a 0.2 um filter. Each mouse received 150 µg/kg body weight of luciferin 15 minutes prior to imaging.

Confocal imaging of patients samples. Bone marrow samples obtained from cancer patients were stained for MPR. Tumor cells were identified by kappa/lambda staining using biotinylated monoclonal kappa or monoclonal lambda antibodies (Abcam). Secondary antibodies Anti lambda Dylight 488 (Abcam) and Streptavidin FITC (BD Pharmingen) were used to stain tumor cells. MPR was stained using rabbit polyclonal primary (Abcam, 1:100) and Anti-rabbit Alexa 647 (Invitrogen, 1:100). Nuclei were counterstained using Vectastain DAPI (Vector Laboratories). The cells were scanned using a Leica SP5 laser scanning confocal microscope. 405nm and 633nm diode lasers, in combination with an argon laser tuned to excite 488nm were applied to excite the samples. Again, an AOBS was used in combination with sequential scanning to ensure fluorchrome detection specificity.