

Supplemental Figure S1. The number of lung metastases per mouse was determined in histological lung sections of all five lung lobes. Each point represents lung metastases in one mouse. Midline and whiskers are average and S.D. N = 6. P = 0.006, Student's T-test.



Supplemental Figure S2. A. Kaplan-Meier curve representing the percentage of MMTV-PyVmT mice per group remaining tumor free. n.s., not significant,

B. Total mammary burden was measured as (total mammary weight ÷ mouse weight) X 100. Multi-focal mammary tumors that arise in MMTV-PyVmT grow into each other, making individual tumor volume measurements in live mice unfeasible by current means.



Supplemental Figure S3. Proliferating cell nuclear antigen (PCNA) was used as a molecular marker of cycling tumor cells. **A.** Immunohistochemical detection was performed in tumors harvested from 71 d old virgin mice and 71 d old mice at Inv d7. **B.** The percentage of the tumor population that was PCNA positive was calculated (average \pm S.D., N = 4, assessing 5 randomly chosen 400X fields per sample).



Supplemental Figure S4. Hematoxylin and eosin-stained sections of mammary tumors harvested from age-matched mice that were either nulliparous (top row) or parous (bottom row). Representative images are shown. N = 4-6 per group. Original magnification of images was taken at 100 X, using low power to demonstrate the widespread histological features of the mammary tumors.



Supplemental Figure S5. A. Western analysis of tumors harvested at 71 d of age. **B**. Tumor latency in virgin $MerTK^{+/+}$ and $MerTK^{-/-}$ mice. **C.** Total tumor burden in virgin mice at 64, 71, 85, and 104 d of age, average \pm S.D., N = 4. **D**. IHC for PCNA in tumors from 71 d old virgin mice. Qantitation shows the percentage of PCNA+ tumor cells (average, \pm S.D., N = 6. Student's T-Test, P = n.s.). **E.** Quantitation of TUNEL analysis performed on tumor sections from virgin $MerTK^{+/+}$ and $MerTK^{-/-}$ mice at the ages indicated. Values shown are average \pm S.D. n.s. = not significant. Student's T-test. **F.** Hematoxylin and eosin-stained sections of tumors harvested from virgin mice at ages indicated above each panel. **G.** PyVmT primary tumor cells harvested from $MerTK^{+/+}$ and $MerTK^{+/-+}$ mice cultured in 10% serum or serum-free media (right panels) for 48 hours were cultured with Annexin V-FITC (5 μ g/ml) for the final 3 h . Images were captured by live fluorescence microscopy at the end of the culture period. Cells were harvested and assessed by flow cytometry to detect FITC+ cells.



Supplemental Figure S6. A. Total tumor burden in parous mice at 64, 71, 85, and 104 d of age, average \pm S.D., N = 4. **B.** Hematoxylin and eosin-stained sections of tumors harvested from parous mice at ages indicated above each panel. **C.** TUNEL analysis of tumor sections from parous mice harvested at ages indicated above each panel. Genotype for MerTK is indicated to the left.



Supplemental Figure S7. Cytokine antibody array of whole tumor protein lysates harvested from agematched virgin and post-partum (involution day 7) mice. Representative blots are shown. The upper panels show the names and locations of protein species of interest. The lower panel shows the original scan of the two antibody arrays, from the same film exposure.



Supplemental Figure S8. A. Cytokine antibody array was performed using whole tumor lysates from 71 day old parous mice (involution day 7) (N = 4). Relative cytokine levels were quantified using Image J. **B.** Cytokine antibody array was performed using whole mammary lysates (from a normal, non-tumor-bearing mammary gland) from 71 day old parous mice (involution day 7) (N = 4). Relative cytokine levels were quantified using Image J.



Supplemental Figure S9. Annexin V staining of MMTV-PyVmT primary mammary tumor cells (A) and MCF7 human breast cancer cells (B) cells treated with gancyclovir for 24 hours. Cells were infected with adenovirus expressing HSV-TK (Ad.HSV-TK) or adenovirus expressing led fluorescent protein (Ad.RFP). At 7 days post-infection, cells were treated with or without gancyclovir (0.5 µg/ml) for 0-36 hours. Live cells were cultured in the presence of Annexin V-FITC (5 µg/ml) for the final 1 hour of culture prior to photo washing, photo-documentation under live fluorescence, and quantitation of fluorescence.



Supplemental Figure S10 Western analysis of Phospho-tyrosine immunoprecipitates from Raw264.7 macrophages treated 16 h with the goat anti-mouse MerTK antibody. Cells were acid-washed to remove residual goat anti-mouse MerTK, neutralized, then lysed. Whole cell lysates were used for immunoprecipitation with PT66 (mouse monoclonal anti-phosphotyrosine). Immune complexes were assessed by western analysis for MerTK using a goat anti-mouse MerTK antibody and anti-goat secondary antibody. Whole cell lysates used for input were assessed by western analysis for β -actin as an input control.

MCF7 breast cancer cell death



Supplemental Figure S11. Annexin V staining of MCF7 cells treated with BKM-120 (1 μ M) ± ABT263 (2 μ M) for 4 hours in suspension.



Supplemental Figure S12. Murine IL-10 was quantitated by ELISA in cultured media harvested from Raw264.7 murine macrophages cultured alone for 16 hours in the presence or absence of the goat anti-mouse MerTK neutralizing antibody, or co-cultured with dying MCF7 cells (DCs) for 16 hours in the presence and absence of the MerTK antibody. Cultured media was collected, passed through a 0.2 micron filter, and used neat for ELISA on pre-coated 96-well plates.



Supplemental Figure S13. qRT-PCR to detect *NOS2* transcripts in whole tumor RNA harvested from MMTV-PyVmT mice at 71 days of age. Mice were either virgin, or were at Inv d7. Values shown are the average of 4 tumor RNA samples, each analyzed in six replicates. P = not significant, Student's T-test.



Supplemental Figure S14. A. qRT-PCR to detect *IL4, IL-10 and Tgfb1* transcripts in whole tumor RNA harvested from 71 d old MMTV-PyVmT mice at 71 days of age treated with 11B11 (anti-IL4 antibody) from Inv d1 through Inv d14. Values shown are the average of 4 tumor RNA samples, each analyzed in six replicates. P = values calculated using Student's T-test. **B.** Western analysis of whole tumor RNA harvested from mice treated with 11B11 (anti-IL4 antibody) from Inv d1 through Inv d14.



Supplemental Figure S15. Western analysis of whole tumor lysates harvested from mice at 71 d of age (Inv d7).