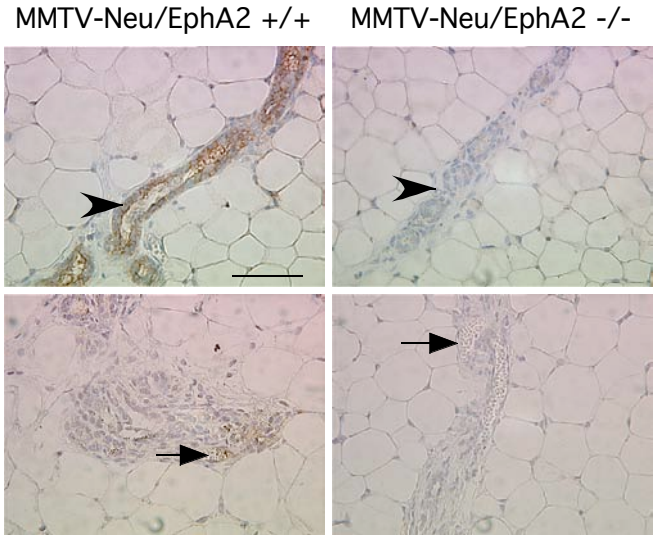
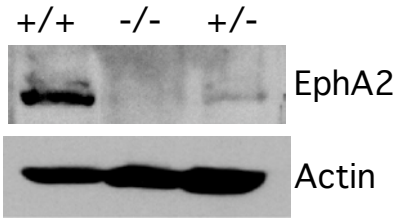
A


Whole-mount

B

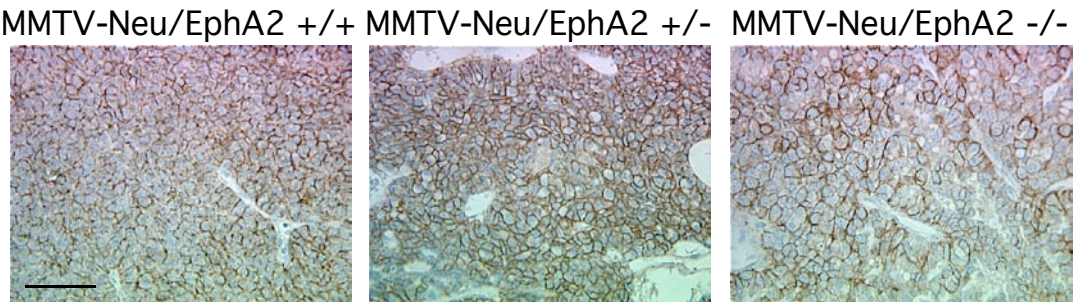



Anti-EphA2

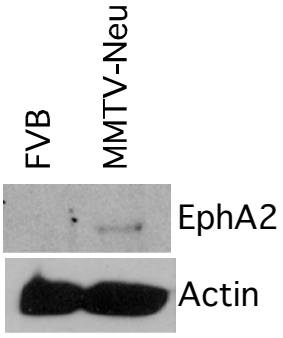



Mammary Gland Lysate

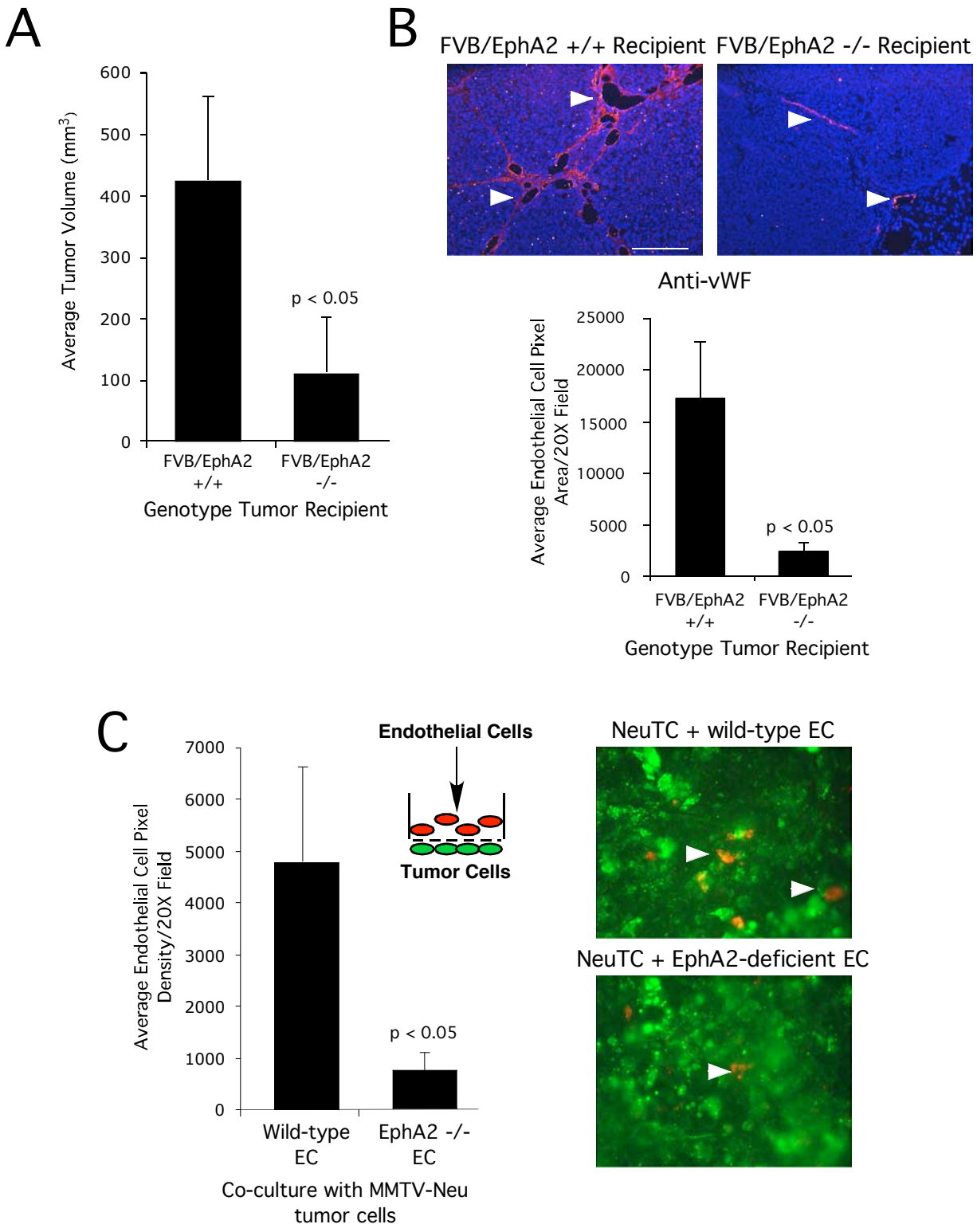
C




Anti-ErbB2

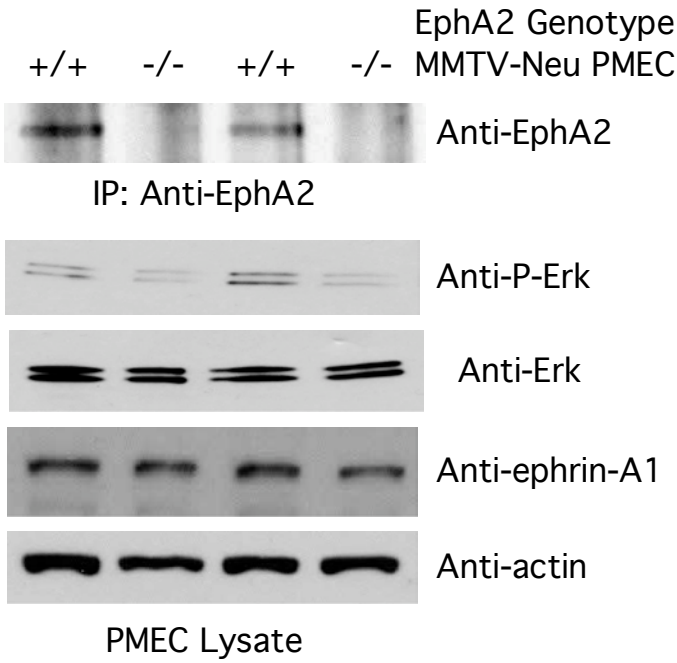



Brantley-Sieders et al.,
Supplemental Figure 1

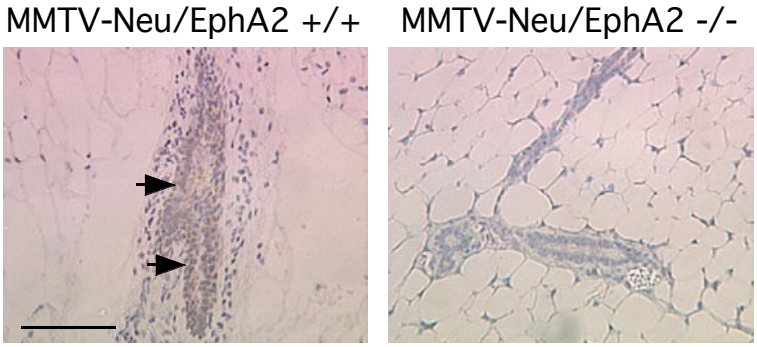



Brantley-Sieders et al.
Supplemental Figure 2

A



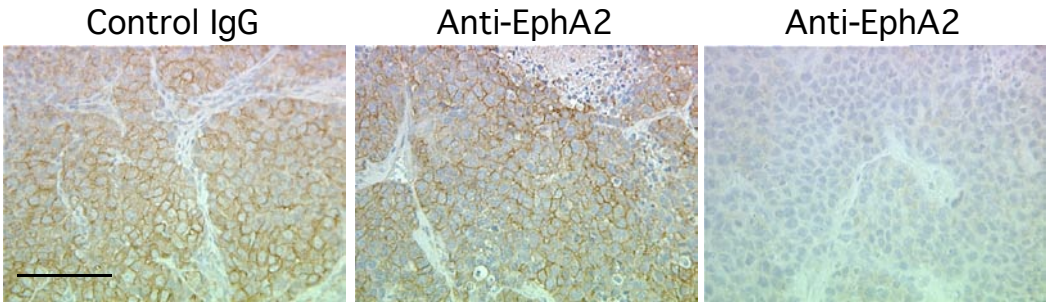

B




Anti-P-Erk

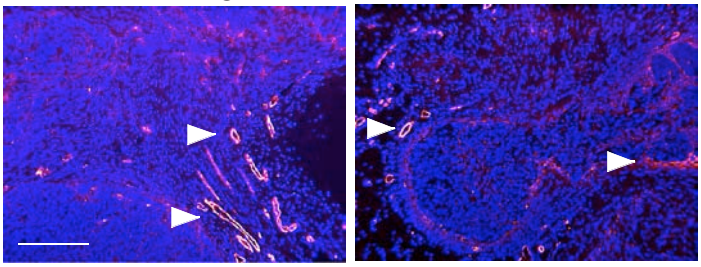
Brantley-Sieders et al.,
Supplemental Figure 3

A



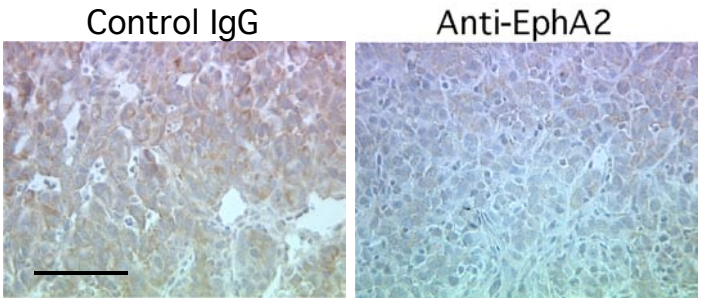

Anti-ErbB2 rIgG Control

B




Anti-vWF

C




Anti-EphA2

Brantley-Sieders et al.,
Supplemental Figure 4

**Supplemental Figure Legends**

**Supplemental Figure 1. EphA2-deficiency impairs mammary epithelial penetration of the surrounding fat pad in a subset of MMTV-Neu animals.** (A) Whole-mount hematoxylin staining of number 4 inguinal mammary glands collected from EphA2 +/+ and -/- MMTV-Neu female transgenic animals 8 months after birth reveals failure of the mammary epithelium to fully penetrate the mammary fat pad (dashed line shows extent of penetration in the far right panel) past the inguinal lymph node (*), a phenotype observed in approximately 30% of -/- animals. The left and middle panels show whole-mount preparations from +/+ and an independent -/- mammary gland, respectively, for comparison. (B) Immunohistochemical staining for EphA2 was used to confirm loss of EphA2 protein expression in mammary epithelium (arrowheads, upper panels) and mammary blood vessels (arrows, lower panels) in EphA2-deficient tissue samples relative to wild-type controls. Scale bar = 50 μm. Immunoblot analysis of mammary gland lysates confirmed loss of EphA2 protein in EphA2-deficient animals, and loss of approximately half of EphA2 protein in heterozygous littermates. (C) Immunohistochemical staining for ErbB2 revealed no apparent differences in expression or localization of ErbB2 between EphA2 +/+, +/-, or -/- MMTV-Neu tumors. Scale bar = 50 μm.

**Supplemental Figure 2. Vascular defects observed in MMTV-Neu/EphA2-deficient tumors are due in part to loss of EphA2 expression in host endothelium.** (A) Tumor cells derived from MMTV-Neu animals were orthotopically transplanted into cleared mammary fat pads wild-type or EphA2-deficient FVB host animals. Relative to wild-type controls, we observed a significant decrease in tumor volume in tumors collected from EphA2-deficient host animals 5 weeks after transplantation ($p<0.05$; single factor ANOVA). (B) Consistent with previous studies, we observed significantly reduced ($p<0.05$; ANOVA) microvascular density in tumors isolated from EphA2-deficient hosts versus wild-type controls based on quantification of vWF immunofluorescence (arrowheads indicate vWF+ blood vessels). Scale bar = 100 μm. (C) To determine if the defects observed in vascular recruitment were due to loss of EphA2 expression in host

endothelium, we performed tumor cell-endothelial cell co-culture migration assays (see diagram). Wild-type MMTV-Neu tumor cells labeled with a green fluorescent marker were seeded on the lower surface of a Matrigel-coated transwell. Endothelial cells derived from wild-type or EphA2-deficient animals were labeled with a red fluorescent dye and added to the upper chamber of the transwell and recruitment of endothelial cells to the lower surface by tumor-derived signals was measured. After 5 hours, we observed significantly fewer ($p<0.05$; 2-tailed, paired student's T-test) EphA2-deficient endothelial cells on the lower surface of the transwell than control wild-type endothelial cells (arrows indicate endothelial cells that migrated to the lower surface of the transwell).

**Supplemental Figure 3. EphA2-deficiency reduces Erk phosporylation in primary mammary epithelial cells (PMEC) derived from MMTV-Neu mice and phospho-Erk in mammary epithelium.** (A) Consistent with our observations in primary tumor cells, PMEC isolated from EphA2-deficient MMTV-Neu animals displayed lower basal levels of phospho-Erk than control PMEC derived from wild-type animals in the absence of changes in the expression levels of ephrin-A1 ligand. EphA2-deficiency was confirmed by immuoprecipitation of EphA2 from PMEC lysates. (B) Consistent with these observations, we observed lower expression of p-Erk in tissue sections prepared from EphA2-deficient versus wild-type MMTV-Neu mammary glands. Scale bar = 50 μm.

**Supplemental Figure 4. Treatment with Anti-EphA2 antibody downregulates EphA2 expression in MMTV-Neu and MMTV-PyV-mT tumor cells but does not affect expression of ErbB2 in MMTV-Neu tumors.** (A) Immunohistochemical staining for ErbB2 revealed no apparent differences in expression or localization in MMTV-Neu tumors harvested from animals treated with control IgG versus anti-EphA2 antibodies. Staining specificity for ErbB2 was confirmed by probing adjacent sections with control rabbit IgG. Scale bar = 50 μm. (B) Treatment with anti-murine EphA2 antibody had no impact on microvascular density in MMTV-PyV-mT tumors relative to controls based on vWF fluorescence (arrowheads indicate vWF+ blood vessels). Scale bar = 100 μm. (C) Treatment with anti-murine EphA2 antibody diminishes EphA2 expression in MMTV-PyV-mT tumors relative to control tumor-bearing animals treated with IgG. Scale bar = 50 μm.