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Supplemental Data

EphA2 Mediates Ligand-Dependent Inhibition and Ligand-Independent Promotion of Cell Migration and Invasion via a Reciprocal Regulatory Loop with Akt

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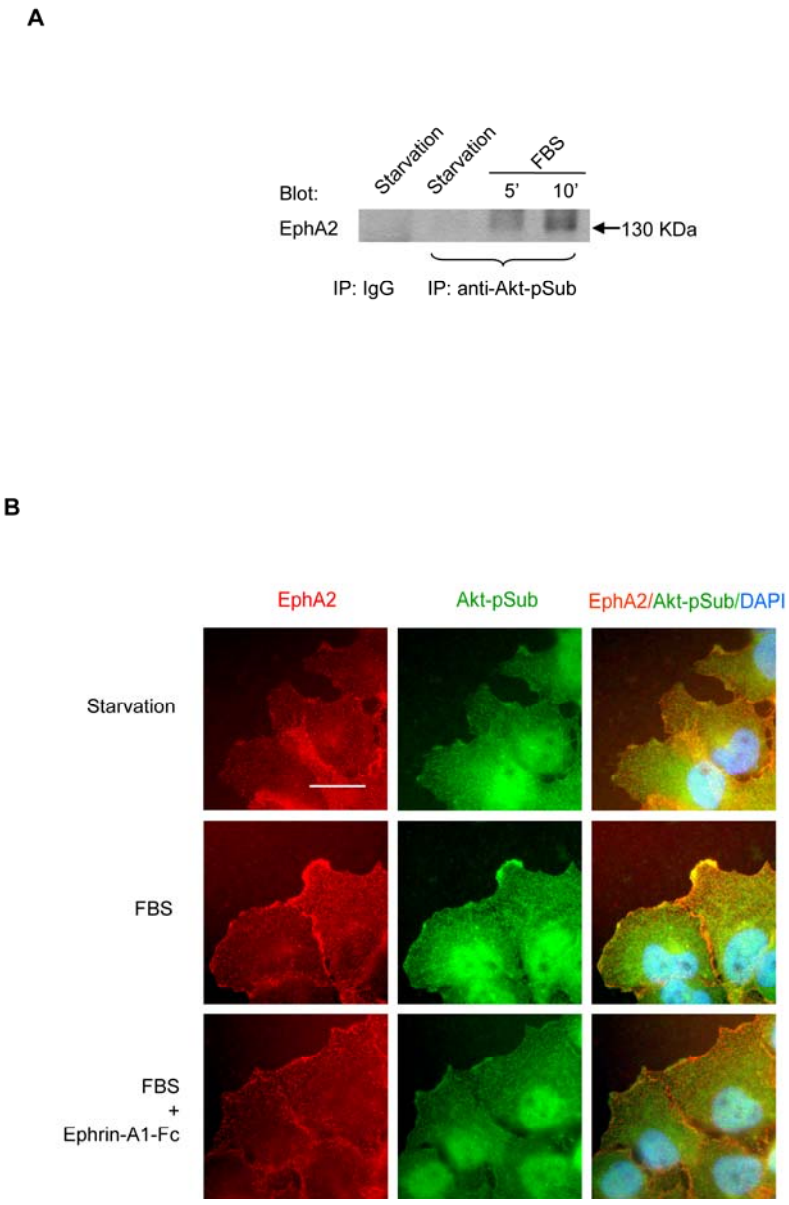

Figure S1. **A**. Detection of EphA2 in Akt phospho-substrate immunoprecipitates. Cell lysates were subjected to immunoprecipitation with anti-Akt-pSub antibodies. The precipitated materials were probed with an antibody against EphA2. **B**. EphA2 colocalizes with Akt phospho-substrates at leading edge of migrating cells upon serum stimulation, which is inhibited upon EphA2 kinase activation by ephrin-A1 stimulation. U373 cells were starved, wounded as descried in Methods. After stimulation for 5 min with FBS in the absence and presence of ephrin-A1, cells were fixed and stained with mouse polyclonal anti-EphA2 and rabbit polyclonal anti-Akt-pSub, followed by detection with fluorescence-conjugated secondary antibodies. Scale bar, 25 μm.

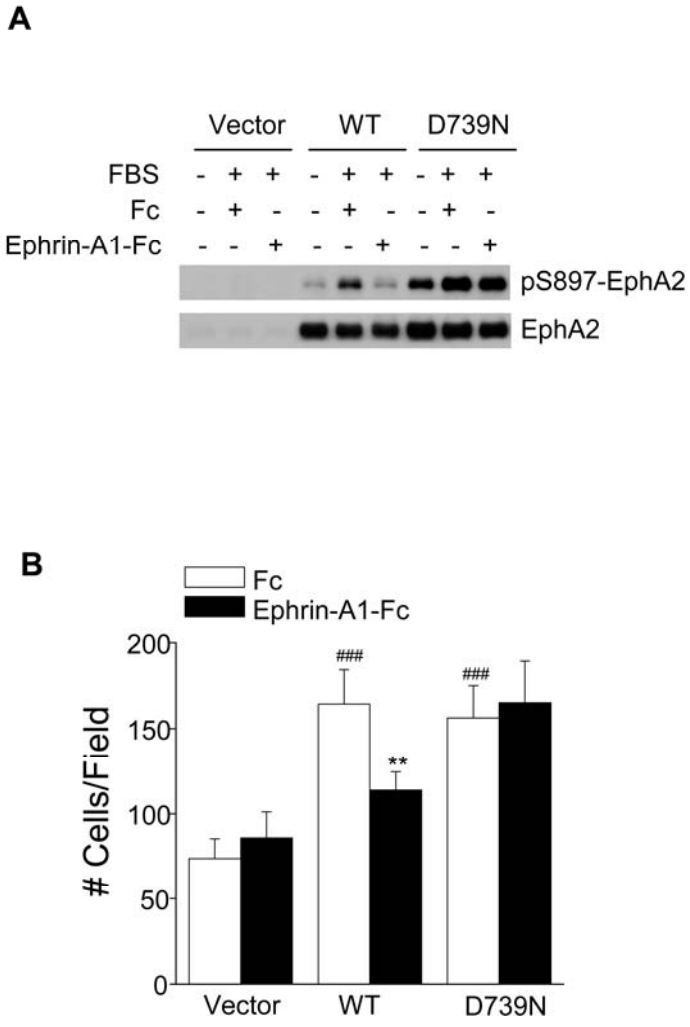

Figure S2. EphA2 kinase activity is not required for phosphorylation of S897-EphA2 and promotion of cell migration. WT- or D739N-EphA2 were introduced into HEK 293 cells via retroviral infection. A. Cells were starved and stimulated with FBS in the presence of Fc or ephrin-A1-Fc for 10 min. Cell lysates were probed for pS897-EphA2 and total EphA2. B. Cells were subjected to Boyden chamber migration assay. Numbers represent mean ± S.D. from 6 random fields. **, $p<0.01$ compared to Fc control of the same cell line. ###, $p<0.001$ compared to Fc control of vector cells.

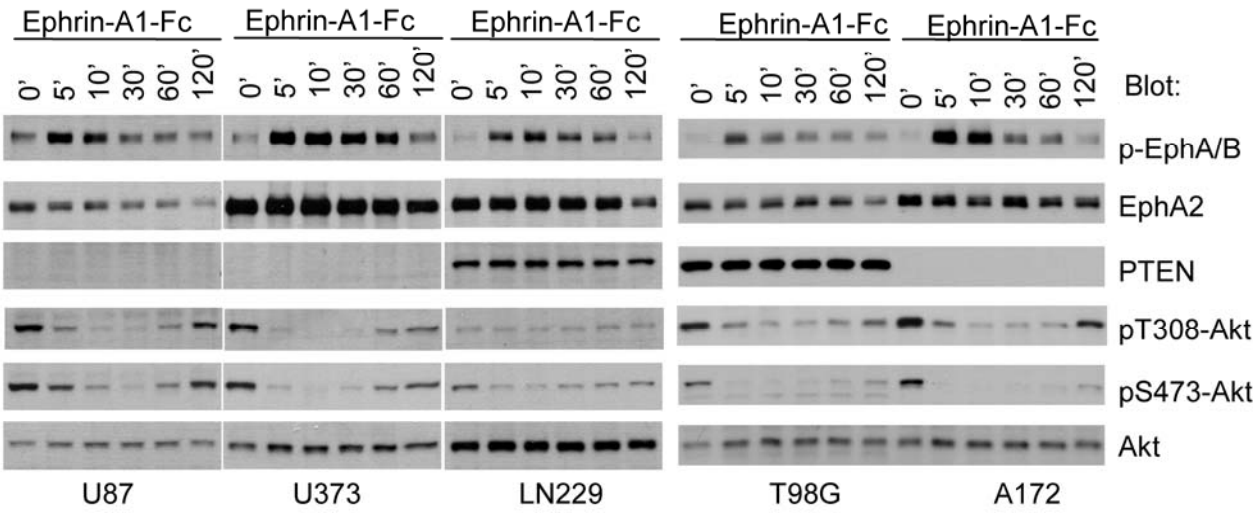

Figure S3. Ephrin-A1 stimulation of EphA2 preferentially inhibits Akt activation in PTEN-deficient glioma cells. Subconfluent cells were stimulated with ephrin-A1-Fc for the indicated times and lysed. Total cell lysates were analyzed by immunoblotting with the antibodies as indicated.

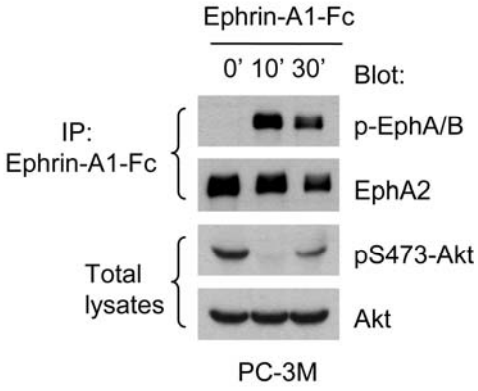

Figure S4. EphA2 kinase activation by ephrin-A1 stimulation inhibits Akt activation in migrating PC-3M cells. Confluent cells were scratch-wounded to induce cell migration. Four hours after wounding cells were stimulated with ephrin-A1-Fc for the indicated times. Total cell lysates were subjected to immunoblot with the indicated antibodies.

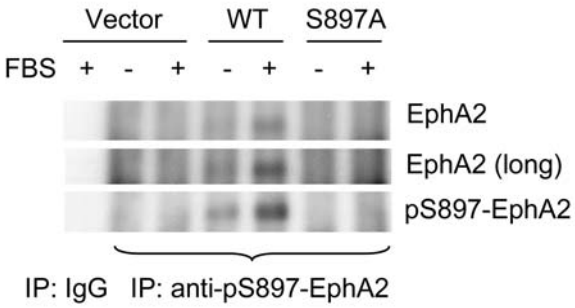

Figure S5. Detection of EphA2 in pS897-EphA2 immunoprecipitates. Serum-starved HEK 293 cells that express vector, WT-EphA2, or S897A-EphA2 were stimulated with FBS for 10 min. Total cell lysates were subjected to immunoprecipitation with rabbit polyclonal anti-pS897-EphA2. The precipitated materials were analyzed by immunoblotting with anti-EphA2 and anti-pS897-EphA2.

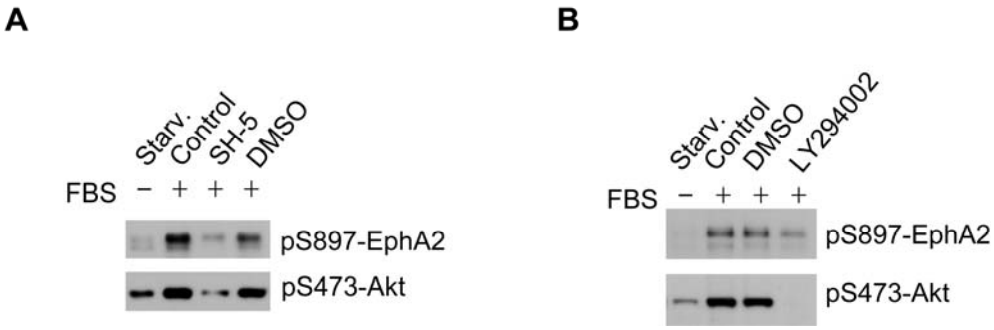

Figure S6. Inhibition of Akt or PI3K abolishes serum-induced pS897-EphA2. Serum-starved U373 cells were pretreated with 10 μM Akt inhibitor II (SH-5, A) for 30 min or 10 μM LY294002 (B) for 1 hour, then stimulated with FBS for 10 min. Cell lysates were analyzed by immunoblotting with polyclonal anti-pS897-EphA2 and pS473-Akt.

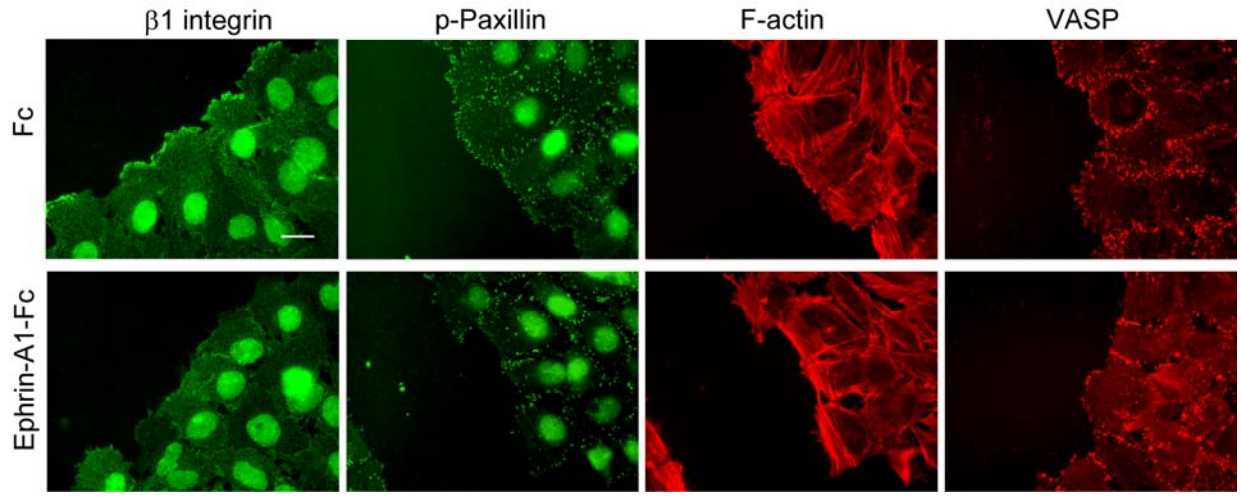

Figure S7. Ephrin-A1 stimulation of glioma cell line U373 inhibits β1-integrin localization at leading edge and actin cytoskeletal structure during cell migration. Freshly confluent U373 cells were scratch-wounded to induced cell migration. Four hours after wounding, cells were stimulated with ephrin-A1-Fc or Fc for 10 min. Cells were fixed and stained with rabbit polyclonal anti-β1-integrin and anti-p-paxillin, or mouse monoclonal anti-VASP. F-actin was stained with Texas Red-conjugated phalloidin. Scale bar, 25 μm.

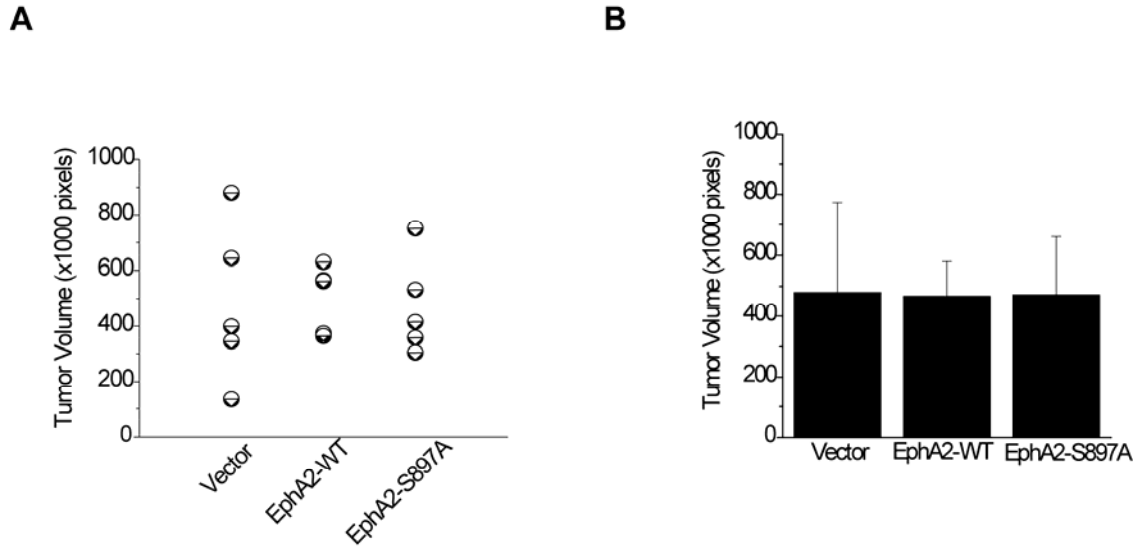

Figure S8. Overexpression of EphA2 did not affect intracranial growth of U87 tumors. U87 cells expressing vector, WT or mutant EphA2 were implanted stereotactically into brains of nude mice. Brain sections were prepared 3 weeks post implantation and stained with rabbit anti-human vimentin. Tumor volumes were estimated from consecutive sections. **A**. Tumor volumes from each individual mouse. **B**. The average tumor volumes of each group of mice. Numbers represent mean ± S.D, n = 5.