

RACK1 regulates VEGF/Flt1-mediated cell migration via activation of a PI3K/Akt pathway: Supplemental Information

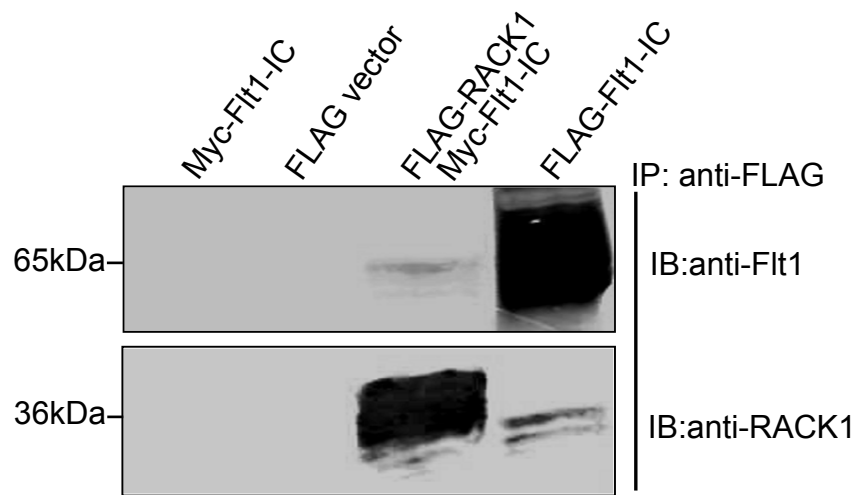
Supplementary Figure Legends.

Supplementary figure 1. Myc-Flt1-IC or FLAG peptide does not artificially interact with RACK1. HEK293T cells were transfected with plasmids expressing Myc-Flt1-IC, FLAG vector, FLAG-Flt1-IC, or co-expressing Myc-Flt1-IC and FLAG-RACK1. Cell lysates were subjected to immunoprecipitation (*IP*) with anti-FLAG antibody, and the FLAG-immunoprecipitates were probed with anti-Flt1 and anti-RACK1 antibodies.

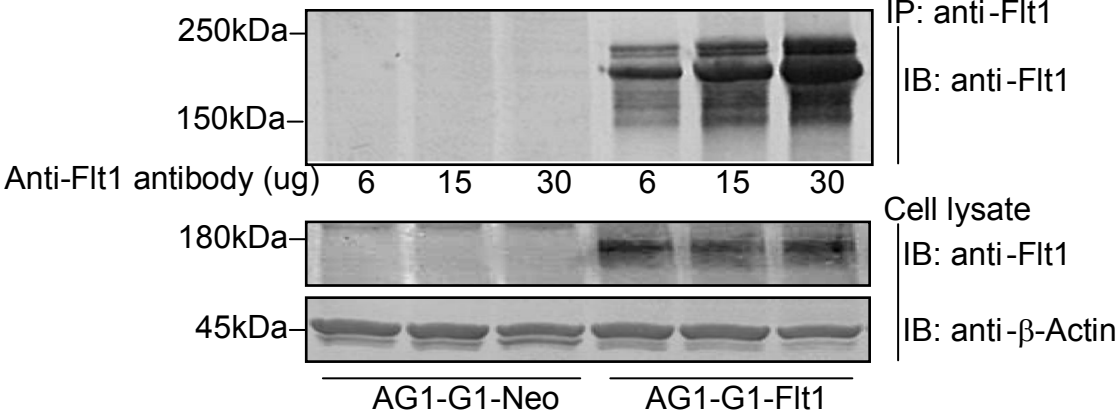
Supplementary figure 2. The AG1-G1-Neo cells do not express endogenous Flt1 at detectable levels. Equal amounts of cell lysate extracted from AG1-G1-Neo and AG1-G1-Flt1 cells were subjected to immunoprecipitation (*IP*) with 6 mg, 15 mg, or 30 mg anti-Flt1 antibody, and the immunoprecipitates and cell lysates were probed with anti-Flt1 or anti- β -actin antibody. Flt1 protein can be immunoprecipitated successfully from AG1-G1-Flt1 cells but not AG1-G1-Neo cells due to the low endogenous expression of Flt1 in the latter.

Supplementary figure 3. Flt1 does not interact with PI3K p85 protein at detectable levels. AG1-G1-Neo and AG1-G1-Flt1 cells were serum-starved and stimulated with VEGF (100 ng/ml) for 5 min. Equal amounts of cell lysate were subjected to immunoprecipitation (*IP*) with anti-Flt1 antibody. The cell lysates and immunoprecipitates were analyzed by Western blotting with anti-Flt1 or anti-PI3K p85 antibody.

Supplementary figure 1



Supplementary figure 2



Supplementary figure 3

