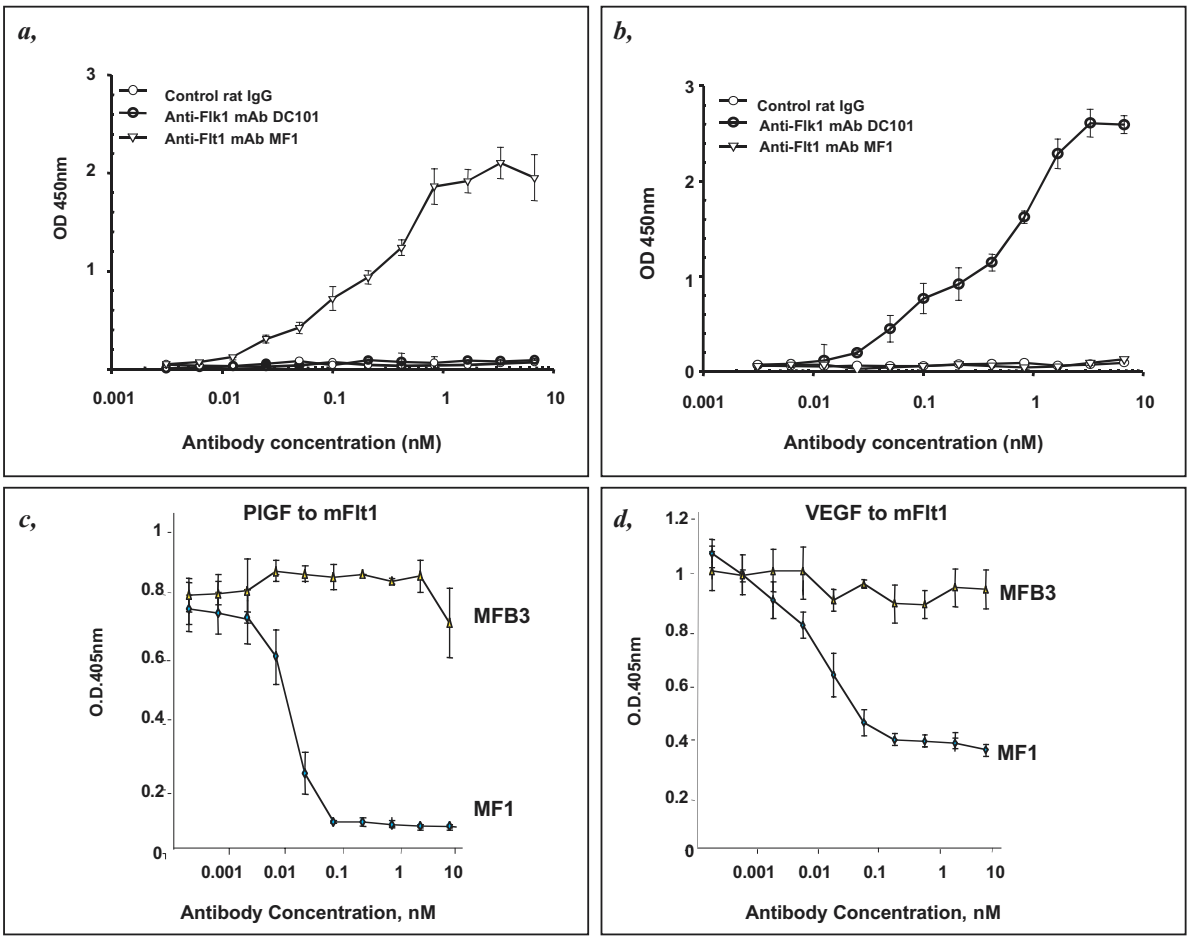

Supplementary Fig. A: Anti-Flt1 specifically blocks binding of VEGF and PlGF to Flt1. a and b, anti-Flt1 (MF1) specifically bound Flt1 (a) but not Flk1 (b), whereas anti-Flk1 (DC101) specifically bound Flk1 (b) but not Flt1 (a). c and d, anti-Flt1 significantly inhibited binding of PlGF (c) and VEGF (d) to Flt1 with IC50 of 0.1 nM and 0.3 nM, respectively, whereas a control MFB3 antibody did not interfere with ligand binding. Methods: Generation of anti-Flt1: Lewis rats (Harlan Sprague–Dawley Inc., Indianapolis, Indiana) were primed with a subcutaneous injection of murine Flt1-Fc (100 mg; extracellular domain of Flt1 fused to Fc-fragment; generated at Imclone) emulsified in complete Freund's adjuvant (Sigma). Rats received 4 booster intraperitoneal injections at 2–3-wk intervals with 100 mg of Flt1-Fc. Rats showing highest titer of blocking antibody in VEGF/Flt1-Fc blocking assays (see below) were boosted intravenously with 50 mg of Flt1-Fc. 5 d later, splenocytes were harvested and fused to mouse myeloma cells P3-X63-Ag8.653. Generation of hybridomas and subcloning was performed according to standard protocols. Hybridomas secreting anti-Flt1 were selected for binding to soluble Flt1-Fc and negative binding to Fc protein alone in ELISA. The anti-Flt1 was selected for inhibition of Flt1-Fc/ligand binding as described below. The binding kinetics of anti-Flt1 (Kd = 2 × 10–6 M) were measured using a BIAcore biosensor (Pharmacia Biosensor). Anti-Flt1 was produced by continuous feed fermentation of hybridoma cells in serum-free medium. Anti-Flt1 was purified from conditioned media by a multi-step chromatography process and assessed for purity by SDS-PAGE and immunoreactivity with soluble Flt1 receptor in ELISA. The negative control rat IgG was purchased from Jackson ImmunoResearch Laboratories (West Grove, Pennsylvania). Protein concentration of antibodies was determined using the BCA method. Characterization: To determine whether anti-Flt1 efficiently bound to VEGF-receptor 1 (Flt1), we performed binding assays in Flt1-AP protein-coated plates (Flt1-AP: extracellular domain of Flt1 fused to human secretory alkaline phosphatase (SEAP)). After sequential incubation with various concentrations of anti-Flt1, goat anti-rat IgG-HRP and colorigenic substrate, we quantified binding by reading on a microtiter plate reader at 450 nm. Subsequently, we measured the efficiency of anti-Flt1 to block binding of Flt1 ligands to their receptor by Flt1/VEGF or Flt1/PlGF blocking assays in plates coated with VEGF or PlGF. After sequential incubation with Flt1-AP (that we preincubated with various concentrations of anti-Flt1) and colorigenic substrate, we measured binding by reading at 405 nm.