

Figure S1

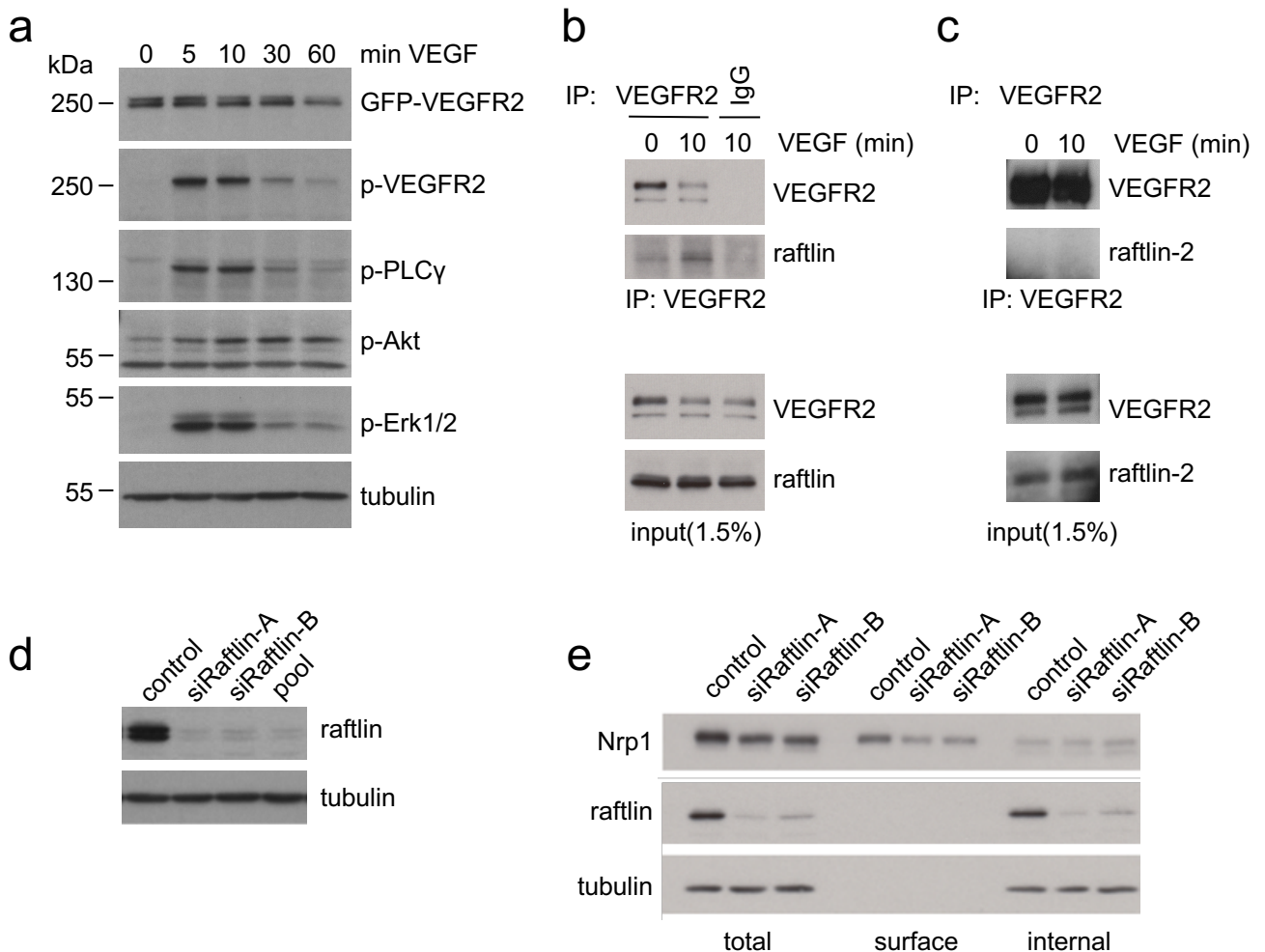


Fig. S1. Raftlin is recruited to activated VEGFR2. **a** CiGenC expressing GFP-VEGFR2 were stimulated with 40ng/ml VEGF over a 60min time course. The phosphorylation of downstream signaling partners was detected by western blotting. GFP-VEGFR2 was detected at its predicted molecular weight and was phosphorylated at Tyr-1175 on VEGF stimulation. Other downstream targets were phosphorylated as expected. **b** ECs were stimulated \pm 40ng/ml VEGF for 10min prior to harvesting and immunoprecipitation of endogenous VEGFR2. A small amount of endogenous raftlin was associated with the receptor in unstimulated cells, and this interaction increased significantly on VEGF stimulation. Control immunoprecipitation was with a non-specific IgG. **c** The experiment was repeated, and samples probed for raftlin-2. We did not detect raftlin-2 in these samples of purified VEGFR2 complex. **d** ECs were transfected with control siRNA, two independent raftlin siRNAs, or a pool of both. Effective silencing of endogenous raftlin was seen with both siRNAs. **e** HUVEC were transfected with raftlin siRNA or control. Surface proteins were biotinylated on ice and isolated using streptavidin beads. The panel shows a representative western blot. Silencing of raftlin caused a loss of surface Nrp1. Quantification is shown in Fig. 3d.