

of kymograph traces of VEGF-stimulated cells under DMSO vs CCG-1423 (5 M) treatment. **C)** Representative immunoblots of HmVEC extracts for VASP, ArpC2, Pfn1, and GAPDH (loading control) at various concentrations of CCG-1423 treatment (the numbers indicate the expression level relative to DMSO control averaged from 2 independent experiments). HmVEC lysates were prepared at the same time-point as the end-point cord formation or cell motility assay.

Figure 4. Pfn1 depletion inhibits angiogenic sprouting of EC. **A)** Pfn1 and tubulin (loading control) immunoblots HmVEC extracts 5 days after transfection with control or Pfn1 siRNA (5-day represents the end time-point of the parallel EC spheroid assay). **B-C)** Representative images (*panel B*) and quantification (*panel C*) of EC spheroid sprouting following treatment with either control or Pfn1 siRNA after 5 days (** $p < .01$). **D-E)** Representative images (*panel D*) and quantification (*panel E*) of aortic ring sprouting angiogenesis (in collagen-I) following treatment of aortic rings with either control or Pfn1-specific siRNA after 120 hours (vessels are identified by FITC lectin staining) (** $p < 0.01$; N indicates the number of independent experiments; in each experiment, aortic segments prepared from 1-2 mice was randomly distributed between the two groups). **F)** Genotyping confirmation of Pfn1^{+/-[EC]} mice (Tie2-Cre-mediated excision of floxed Pfn1 allele gives rise to a 700 bp knockout (KO) band in EC; EC were harvested from aortic segments). **G-H)** Representative images (*panel G*) and quantification (*panel H*) of sprouting angiogenesis in matrigel from WT vs Pfn1^{+/-[EC]} aortic segments (N: number of mice for each genotype; *: $p < 0.05$).

Supplemental Figure S1. Live/dead staining of HmVEC after treatment with either DMSO or CCG-1423 (10 M). Green depicts live cells and red (arrow) shows dead cells.

Supplemental Figure S2. Lectin and Pfn1 immunostaining of aortic rings treated with either control or Pfn1 siRNA (note that Pfn1-siRNA treated rings show an overall weaker staining Pfn1; white and yellow arrowheads depict sprouts with undetectable and intermediate levels of Pfn1).

Gau et al. Supplementary Fig S1

Live-dead stain of HmVEC



