Coon et al., http://www.jcb.org/cgi/content/full/jcb.201408103/DC1

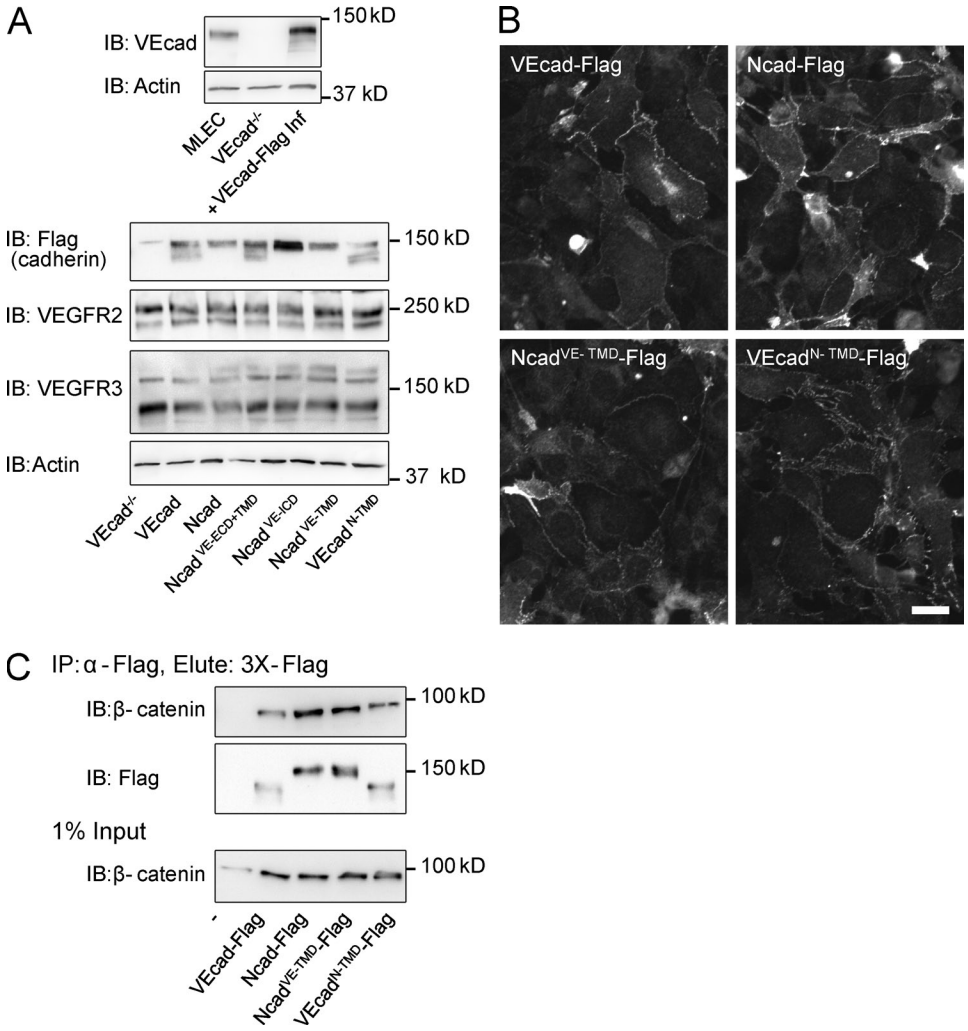

Figure S1. **Expression and localization of cadherins.** (A) MLEC and VEcad$^{-/-}$, WT, and chimeric cadherin-expressing cells were extracted and immunoblotted with the indicated antibodies. (B) VEcad$^{-/-}$ cells were infected with the indicated cadherin constructs using lentiviral vectors and sorted for equal cadherin expression. Cadherins were localized by staining with anti-Flag. Bar, 10 µm. (C) VEcad$^{-/-}$, WT, and chimeric cadherin-expressing cells were lysed, immunoprecipitated for Flag, and eluted with 3×-Flag peptide. Eluates were analyzed by SDS-PAGE and immunoblotting for Flag and β-catenin. IB, immunoblotting.

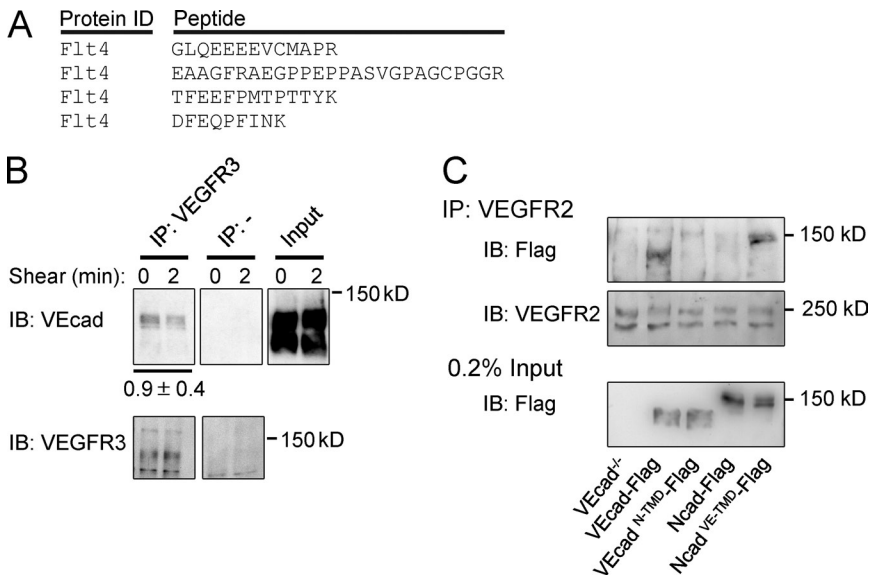

Figure S2. **The effect of shear stress and VEGFR homologue on the VEcad–VEGFR complex.** (A) VEGFR3 peptides within an Ncad$^{VE\text{-}TMD}$-Flag immunoprecipitation identified by mass spectrometry. (B) VEcad$^{-/-}$ and VEcad^{WT} cells were either left under static conditions or exposed to 12 dynes/cm^2 laminar shear for 2 min before collecting lysates and immunoprecipitating VEGFR3. Eluted protein was analyzed by SDS-PAGE and immunoblotting for VEcad and VEGFR3. Blots are representative of three independent experiments. (C) VEcad$^{-/-}$ and the indicated cadherin-reconstituted cells were lysed and immunoprecipitated with anti-VEGFR2 conjugated resin. Beads were washed and eluted protein was analyzed by SDS-PAGE and immunoblotting for Flag and VEGFR2. Blots are representative of two independent experiments. IB, immunoblotting; IP, immunoprecipitation.

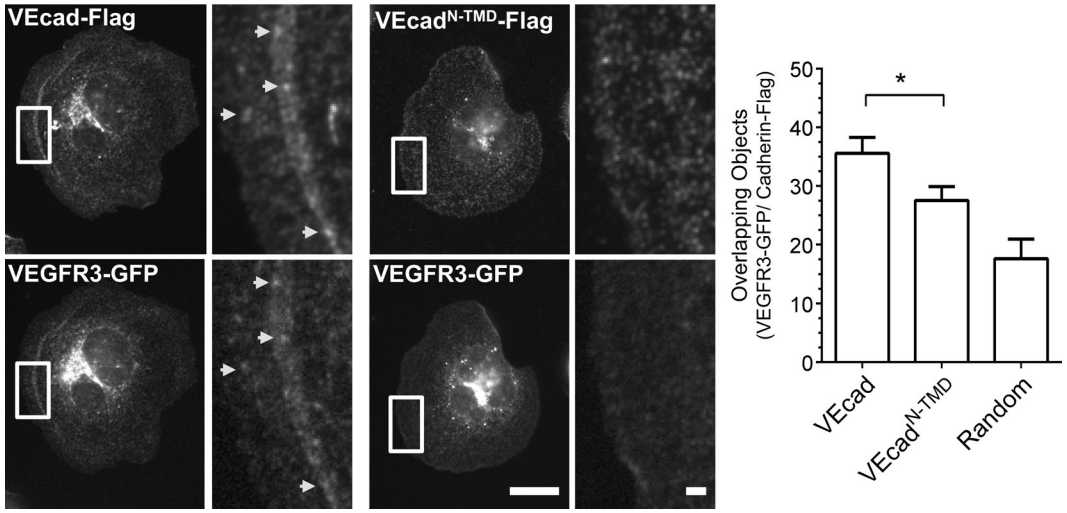

Figure S3. **TMD-dependent colocalization of VEcad with VEGFR3.** VEcad^{WT} and VEcad$^{N\text{-}TMD}$ cells were infected with VEGFR3-GFP adenovirus. Cells were resuspended with accutase and plated onto glass slides precoated with recombinant Fc-VE^{ECD}. Cells were fixed at 2 h, immunostained for the Flag-tagged cadherins, and visualized by fluorescence microscopy. The boxed regions are enlarged in the panels to their immediate right. Bars: (left) 10 µm; (right) 1 µm. Fluorescent objects were thresholded and analyzed for colocalization according to their center of mass by the ImageJ JACoP plugin. Data are means ± SEM (error bars), n = 15 cells. *, $P < 0.05$ using a t test.

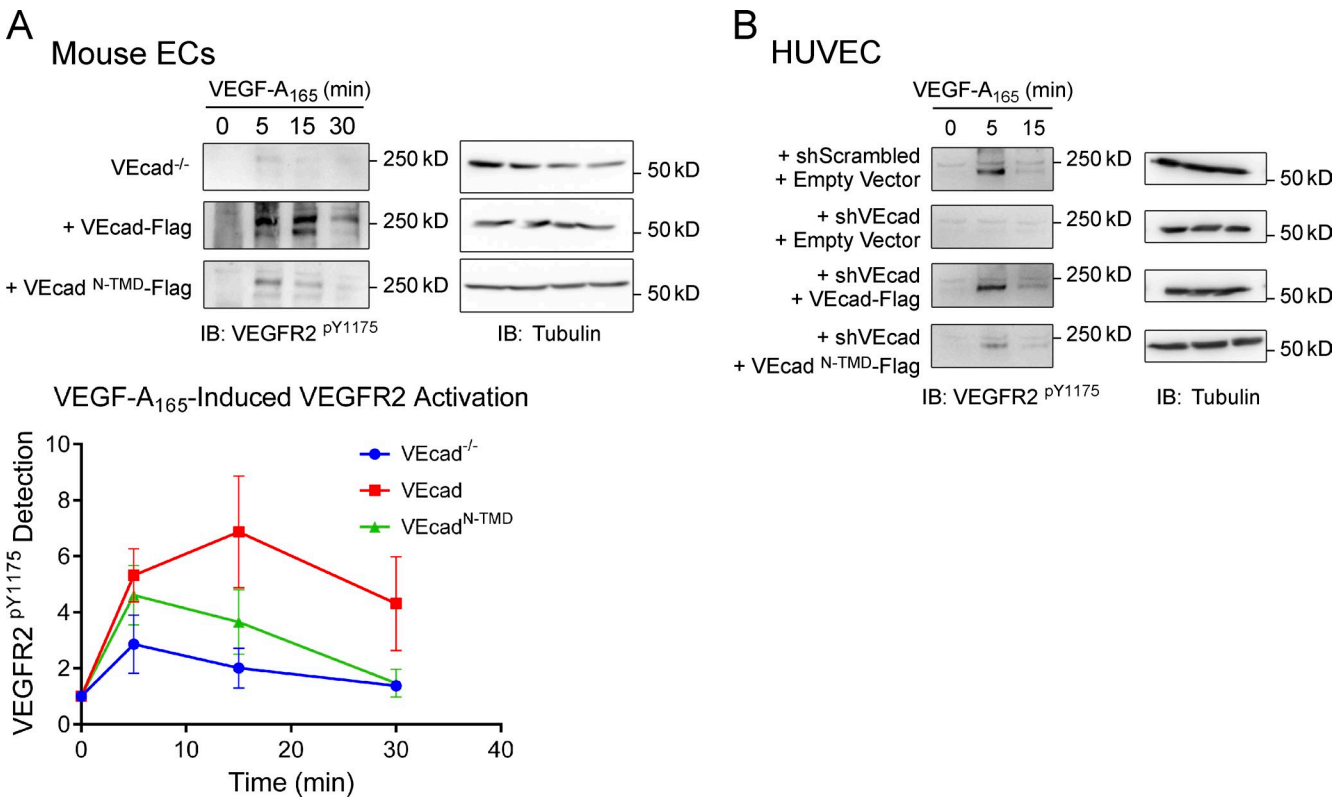

Figure S4. **Influence of the VE-cad TMD on VEGF-A165–mediated VEGFR2 activation.** (A) VEcad$^{-/-}$, VEcad^{WT}, and VEcad$^{N\text{-}TMD}$ cells were stimulated with 50 µg/ml VEGF-A165 for the indicated times. Lysates were collected and analyzed by SDS-PAGE and immunoblotting for VEGFR2^{pY1175} and actin. VEGFR2 activation was quantified by densitometry of the ~250/220 kD doublet. Values are means ± SEM (error bars) from three independent experiments. (B) HUVECs were infected with scrambled of VEcad shRNA lentivirus before infecting with empty vector, mouse VEcad, or mouse VEcad$^{N\text{-}TMD}$. Cells were stimulated with VEGF-A165 for 0, 5, or 15 min before collecting lysates and immunoblotting for VEGFR2^{pY1175}.

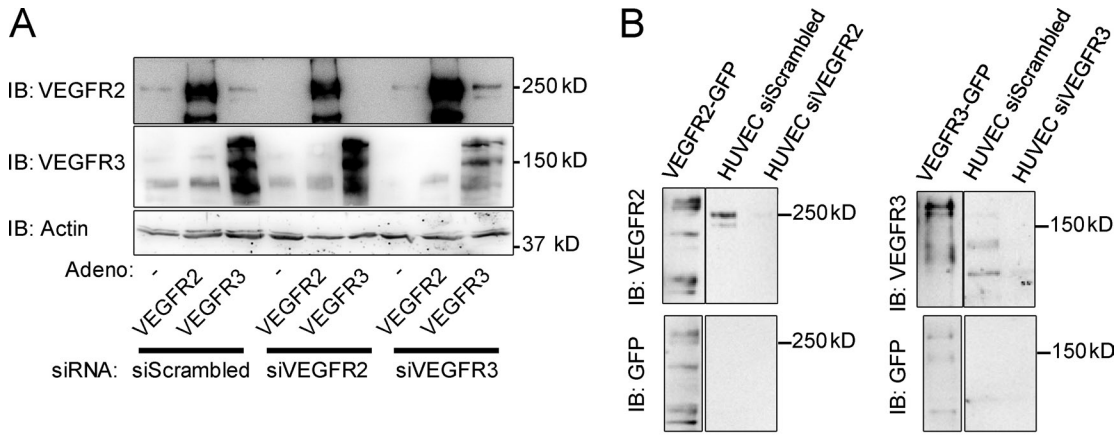

Figure S5. **Exogenous and endogenous VEGFR expression levels.** (A) HUVECs were infected with adenovirus encoding mCherry or VEGFR-GFP as in Fig. 6. Infection efficiency ranged from 60 to 90%. Lysates were blotted for VEGFR2 and VEGFR3 antibodies to compare the levels of endogenous and exogenous VEGFR-GFP expression. (B) 239T cells were transfected with either human VEGFR2-GFP– or human VEGFR3-GFP–expressing plasmids. HUVECs were also treated with the indicated siRNAs. Lysates were collected and immunoblotted for GFP (reference antibody), anti-VEGFR2, and anti-VEGFR3. Densitometry of the three signals reveals that the ratio of endogenous VEGFR2/VEGFR3 is between 75:25 and 60:40. IB, immunoblotting.