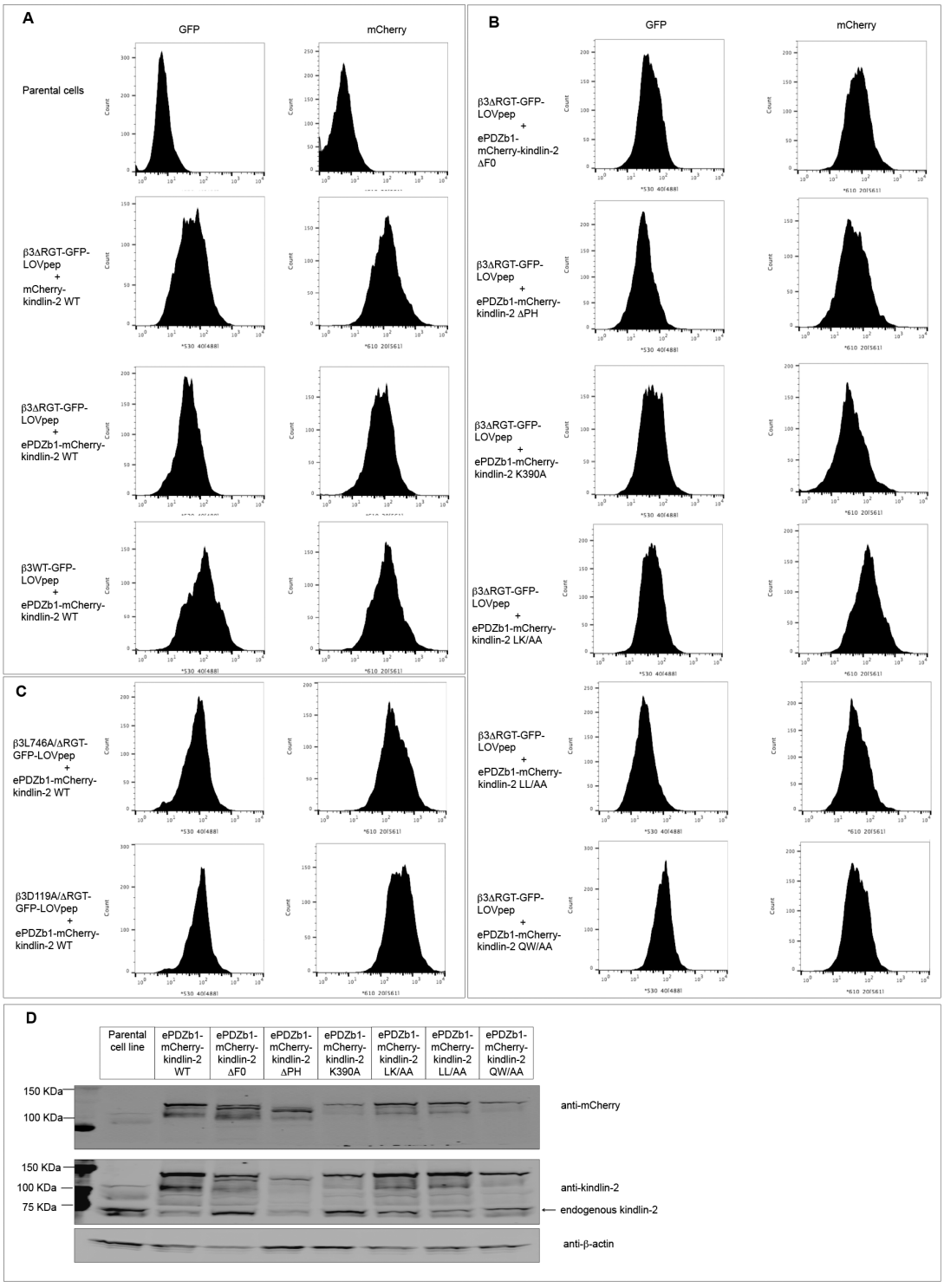

Fig. S1. Cellular expression of β3ΔRGT-GFP-LOVpep and ePDZb1-mCherry-kindlin-2 in endothelial cells. β3-null immortalized murine lung endothelial cells were transduced with lentivirus encoding the indicated fusion proteins. (A-C) Flow cytometry was used to

detect the β3 (GFP) and kindlin-2 (mCherry) fusion proteins. Non-transduced $\beta3^{-/-}$ immortalized endothelial cells were used as a negative control. (D) Expression of ePDZb1-mCherry-kindlin-2 and mutants were detected by Western blotting.

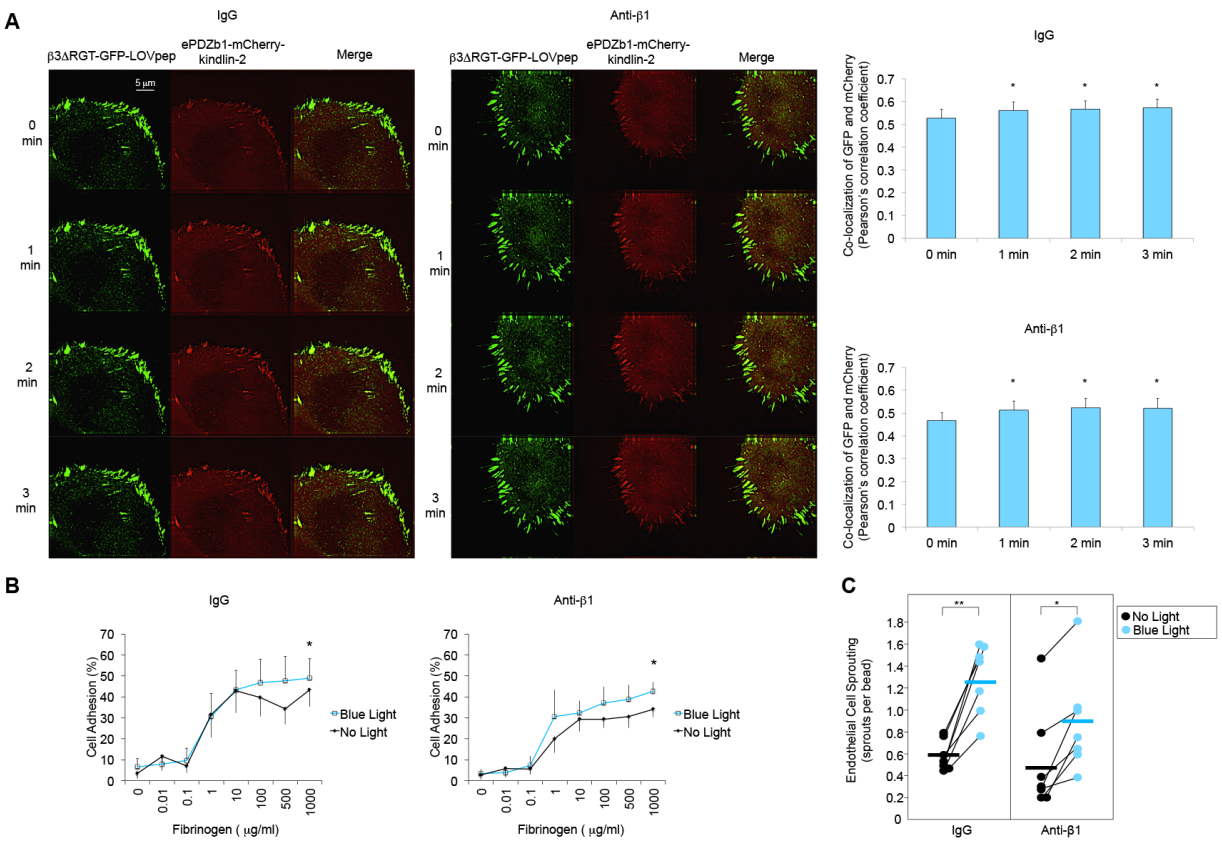

Fig. S2. β1 integrin blockade does not prevent endothelial cell responses to optogenetically-induced interaction of kindlin-2 with αVβ3. (A) $\beta3^{-/-}$ immortalized lung endothelial cells expressing β3ΔRGT-GFP-LOVpep and ePDZb1-mCherry-kindlin-2 were treated with 25 μg/ml of the anti-mouse β1 blocking antibody, HMβ1-1 (Biolegend; San Diego, CA) or with an IgG isotype control for one hour before plating cells on fibrinogen. (A) Images were obtained with time-lapse TIRF microscopy. Blue laser light was used to stimulate the interaction of β3ΔRGT-GFP-LOVpep and ePDZb1-mCherry-kindlin-2 at 100-150 ms illumination every min. Scale bar, 5 μm. Co-localization between GFP and mCherry channels was quantified and shown as Pearson's correlation coefficient on the right. Data represent means ± SEM for 7-10 cells in 3 experiments. *, $P < 0.05$ (paired Student *t* test). (B) Cell adhesion was examined as described in Materials and Methods and depicted as percentage of adherent cells over total input. Data represent means ± SEM of 5 experiments. *, $P < 0.05$ (paired Student *t* test). (C) Cytodex beads coated with $\beta3^{-/-}$ immortalized lung endothelial cells expressing β3ΔRGT-GFP-LOVpep and ePDZb1-mCherry-kindlin-2 were embedded in fibrin gel containing 25

μg/ml HMβ1-1 or IgG isotype control. After overnight incubation in the dark or in the presence of blue light, angiogenic sprouts were quantified. Data represent means of 7 experiments. **, $P < 0.01$; *, $P < 0.05$ (paired Student *t* test).

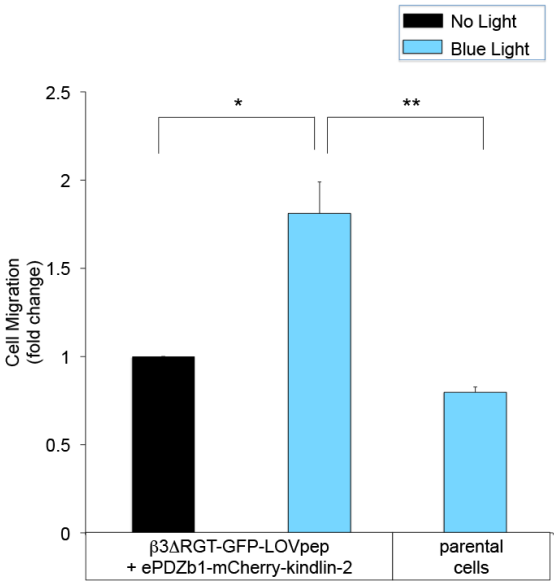

Fig. S3. Optogenetically-induced interaction of kindlin-2 with integrin β3 promotes migration of lung endothelial cells derived from "β3ΔRGT" knock-in mice. Lentiviruses encoding β3ΔRGT-GFP-LOVpep and ePDZb1-mCherry-kindlin-2 were transduced into β3ΔRGT endothelial cells. Transwells were coated with 10 μg/ml anti-human β3 antibody, SSA6. Cell migration across SSA6-coated transwells was measured as described in Materials and Methods. Migration of cells in the dark was arbitrarily assigned a fold-change of 1. Data represent means ± SEM of 4 experiments. *, $P < 0.05$; **, $P < 0.01$ (paired Student *t* test).

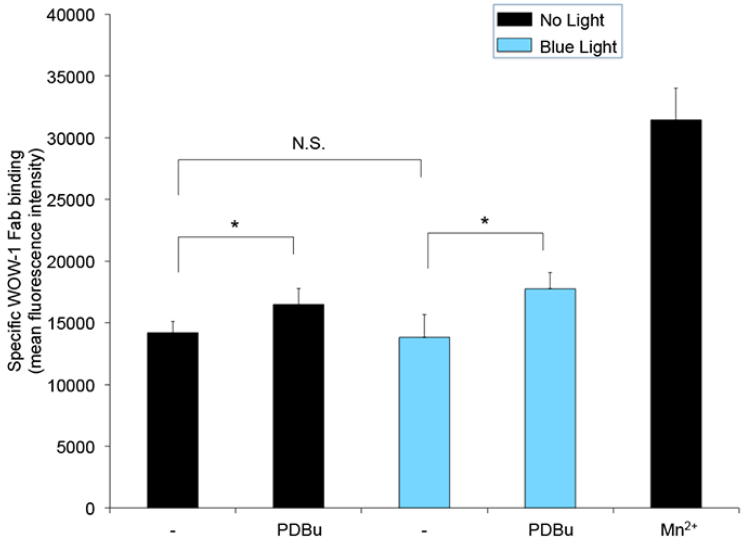

Fig. S4. Optogenetic interaction of kindlin-2 with β3ΔRGT does not affect αVβ3 affinity. $\beta3^{-/-}$ immortalized lung endothelial cells expressing β3ΔRGT-GFP-LOVpep and ePDZb1-mCherry-kindlin-2 were maintained in the dark or illuminated with blue light for 5 min before incubation with WOW-1 Fab in the presence or absence of 200 nM phorbol myristate acetate (PDBu). As a positive control, an aliquot of cells was incubated separately with 1 mM $MnCl_2$ to stimulate integrin affinity extrinsically. Note that the presence of blue light did not influence WOW-1 Fab binding. Data represent mean ± SEM of specific WOW-1 Fab binding, defined as binding inhibited by EDTA (4 experiments). *, $P < 0.05$; N.S., not significant (paired Student *t* test).

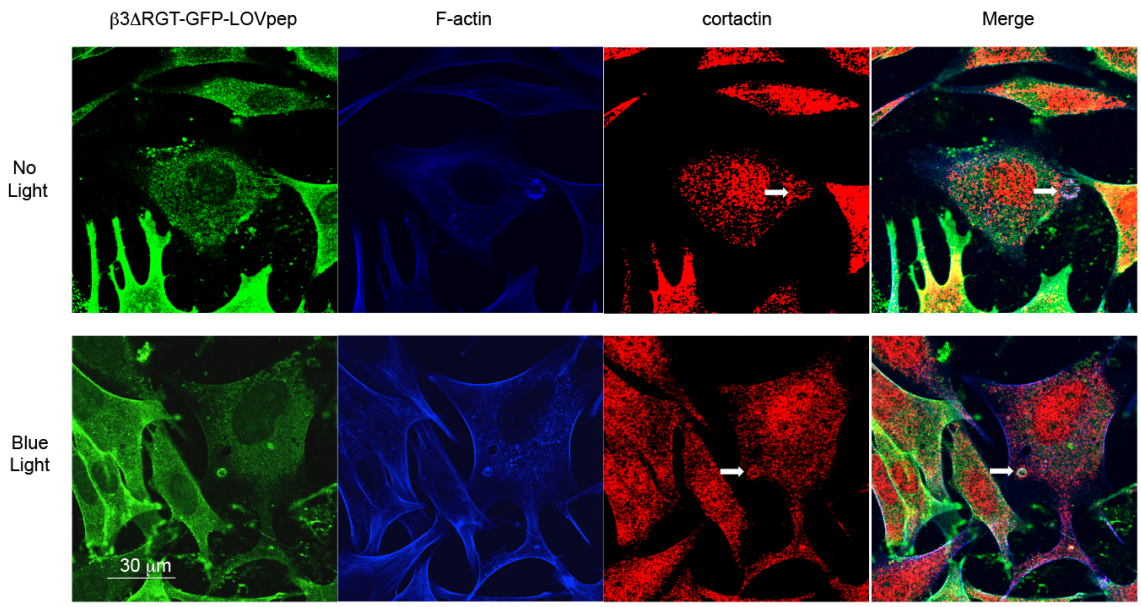

Fig. S5. Localization of cortactin to podosomes formed by endothelial cells. Endothelial cells expressing β3ΔRGT-GFP-LOVpep and ePDZb1-mCherry-kindlin-2 were seeded onto 10 ng/ml vitronectin-coated coverslips and incubated overnight with or without blue light illumination. Cells were fixed and stained with anti-cortactin (red pseudocolor) and Alexa Fluo 647-conjugated phalloidin (blue pseudocolor). Scale bar, 30 μm.

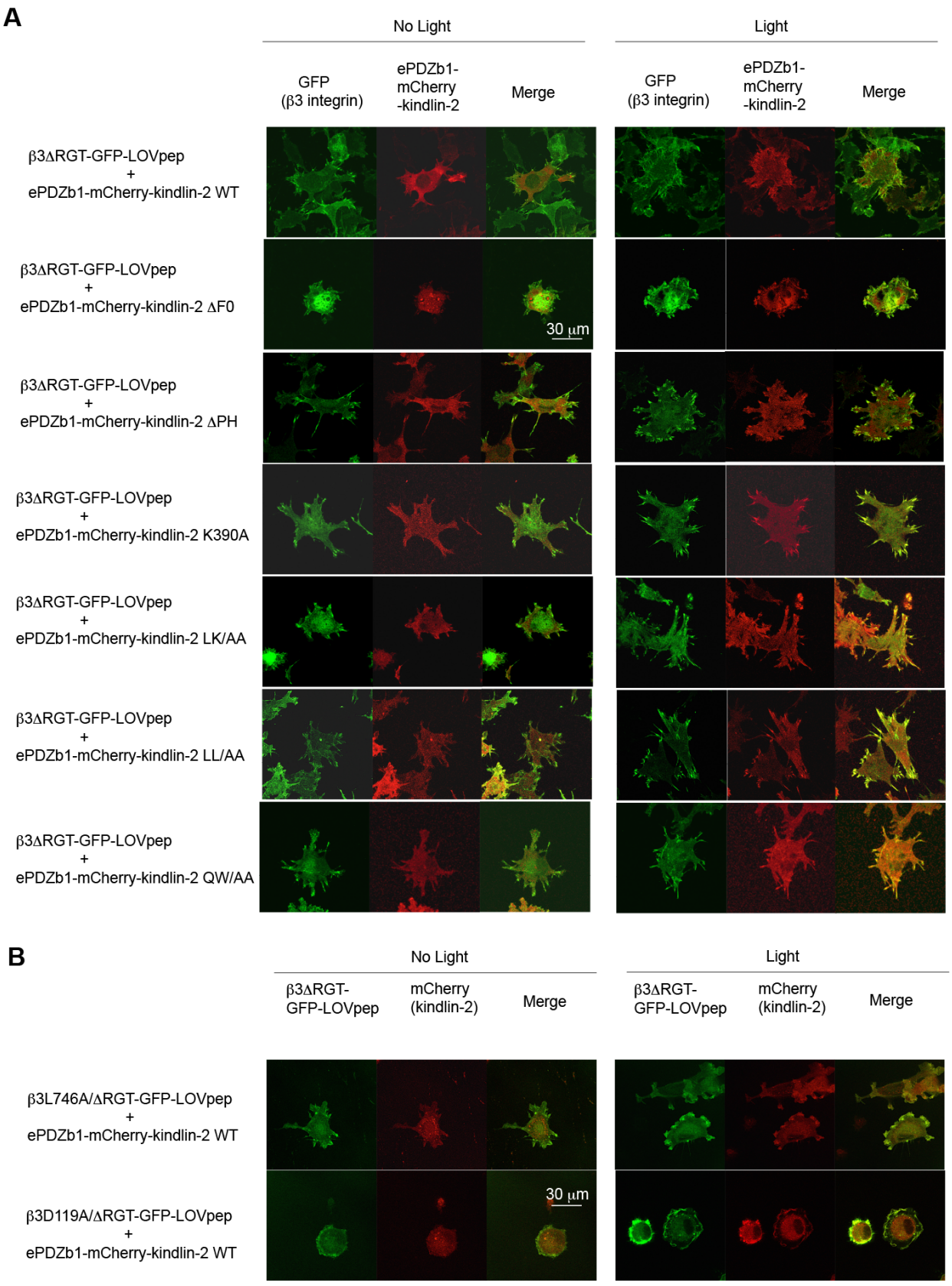

Fig. S6. Optogenetic co-localization of β3ΔRGT-GFP-LOVpep and ePDZb1-mCherry-

kindlin-2 mutants by confocal imaging. (A) Blue light-induced co-localization of β3ΔRGT-GFP-LOVpep (shown in green) and ePDZb1-mCherry-kindlin-2 mutants (shown in red). Cells were plated onto coverslips coated with fibrinogen and allowed to spread for 30 min with or without blue light illumination (0.5s illumination every minute) as indicated. Scale bar, 30 µm. (B) Blue light-induced co-localization of either β3L746A/ΔRGT-GFP-LOVpep or β3D119A/ΔRGT-GFP-LOVpep with ePDZb1-mCherry-kindlin-2. Scale bar, 30 µm.

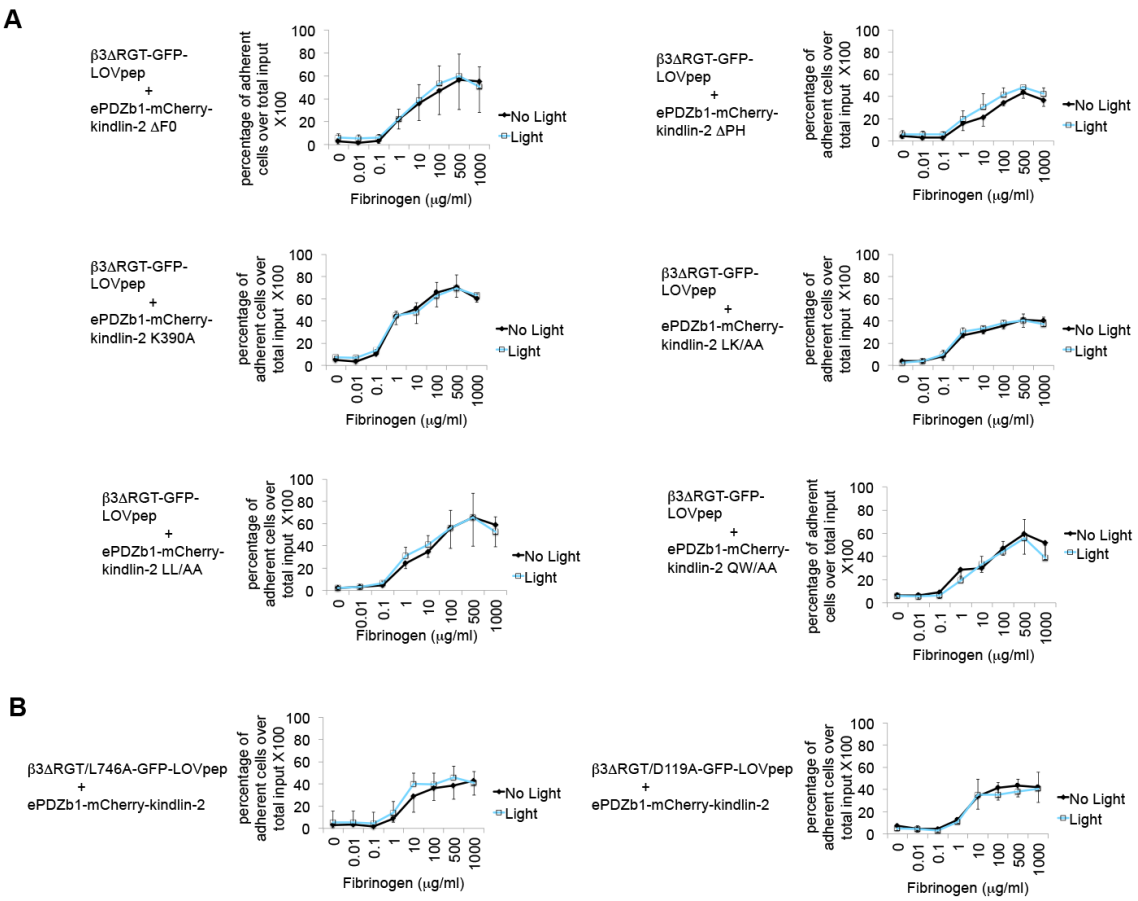

Fig. S7. The indicated kindlin-2 mutants or β3 mutants failed to promote endothelial cell adhesion to fibrinogen in response to blue light. Cell adhesion is depicted as percentage of adherent cells over total input. (A) Adhesion of β3 null endothelial cells expressing β3ΔRGT-GFP-LOVpep and one of the indicated ePDZb1-mCherry-kindlin-2 mutants. (B) Adhesion of β3 null endothelial cells expressing ePDZb1-mCherry-kindlin-2 with either β3L746A/ΔRGT-GFP-LOVpep or β3D119A/ΔRGT-GFP-LOVpep. Black line, without 450 nm illumination; blue line, with 450nm illumination.