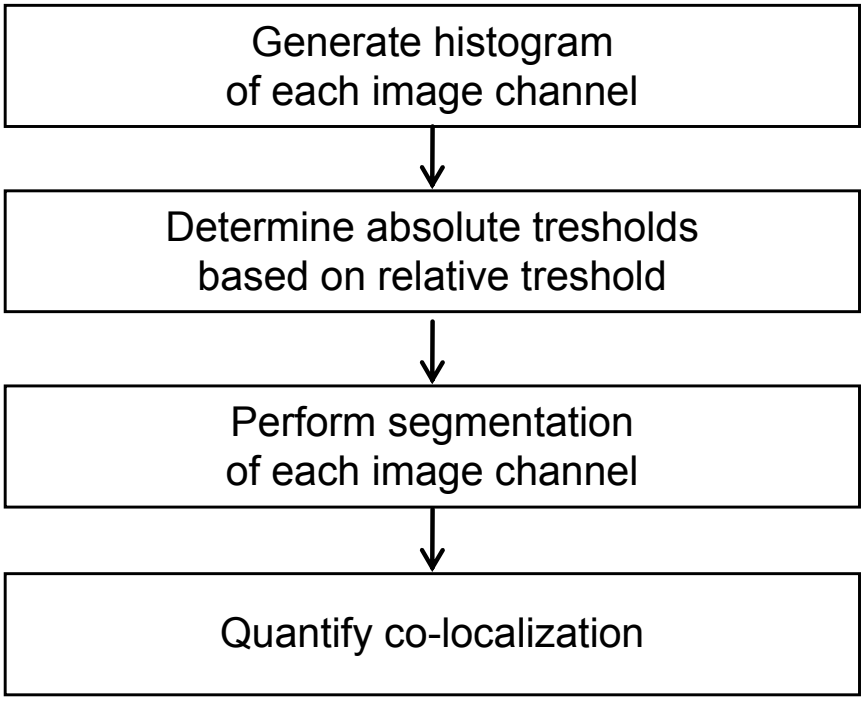
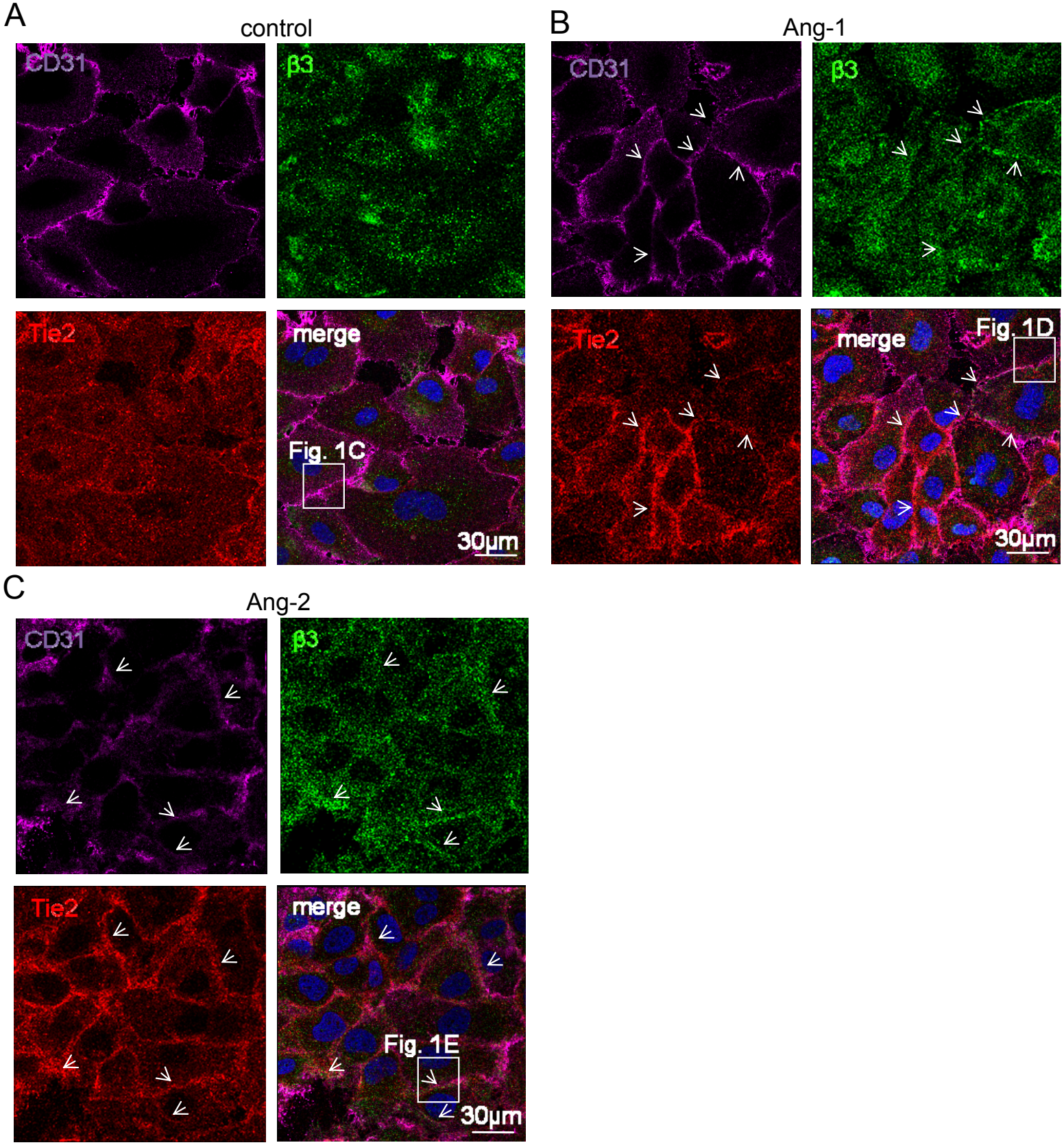
Thomas et al., Supplementary Figure 1



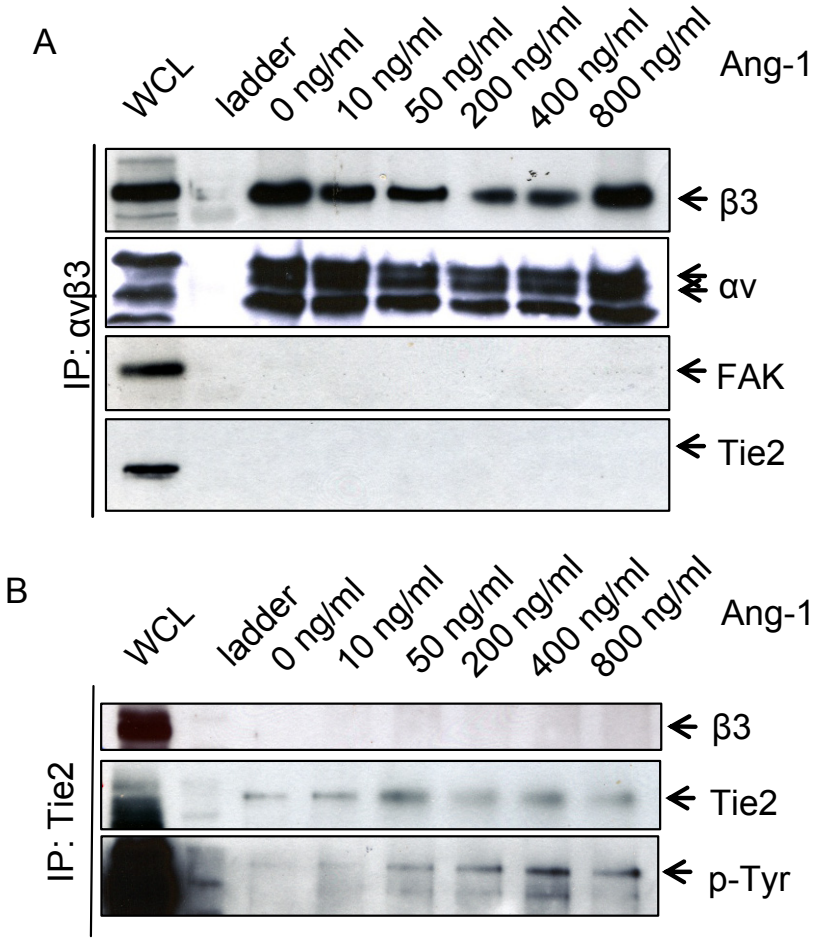
Approach for co-localization quantification

Thomas et al., Supplementary Figure 2



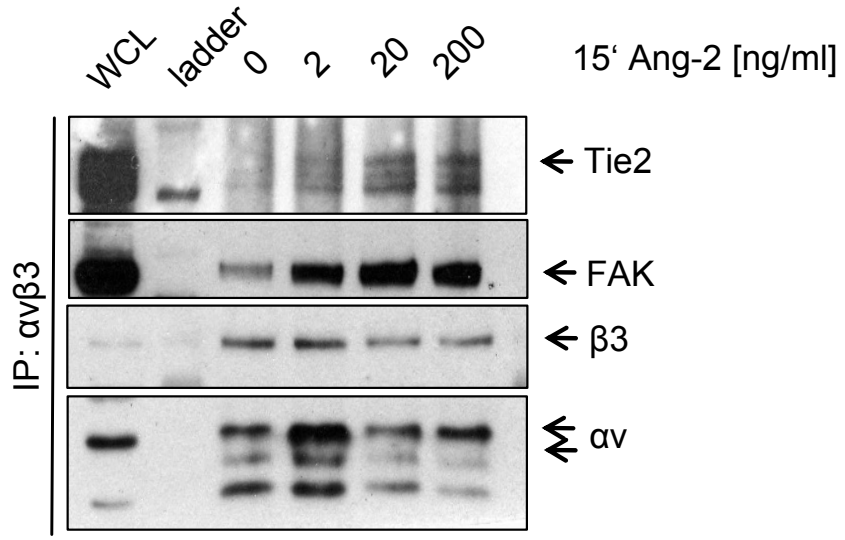
Angiopoietins induce translocation of Tie2 & β3 integrins in cell-cell-junctions. HUVEC were grown to confluence on fibronectin-coated chambered coverglass and stimulated for 15 min with either 200ng/ml Ang-1 or 200ng/ml Ang-2. Cells were fixed with 10% PFA, followed by incubation with primary antibody against CD31, Tie2 and β3 integrin over night at 4°C. After secondary incubation, pictures were taken with confocal microscopy.

Thomas et al., Supplementary Figure 3



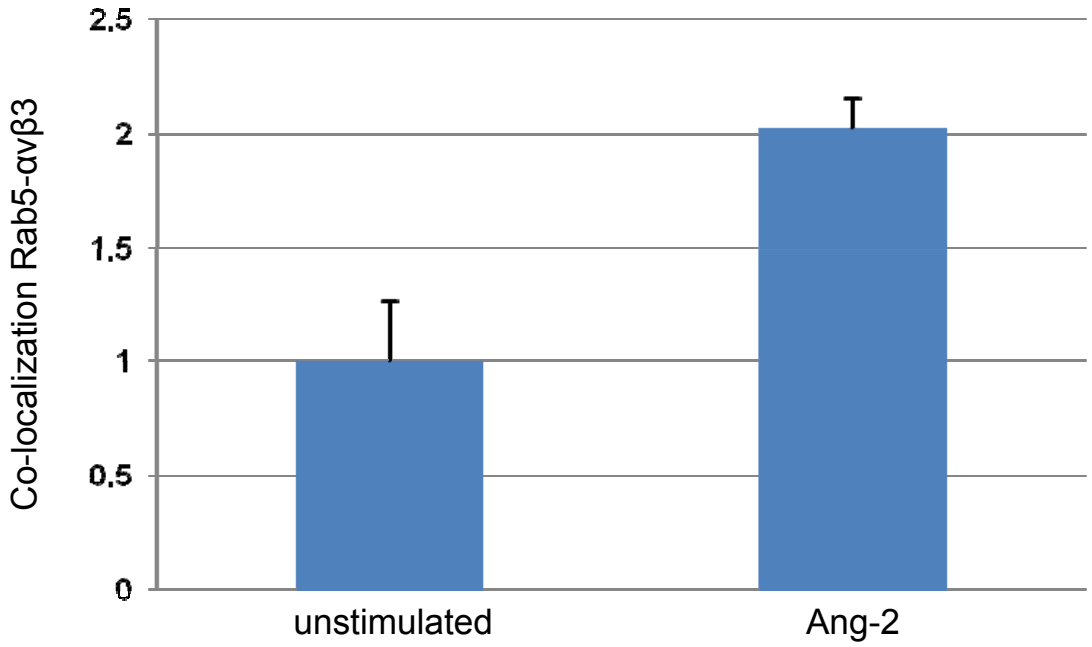
*Ang-1 does not induce complex formation between Tie2 and αvβ3 integrin (*A) HUVEC were stimulated with different concentrations of Ang-1 (15 min). Protein lysates were immunoprecipitated for αvβ3 integrin, Western blotted and the membrane was cut horizontally at 50 kDa into two pieces. The upper piece of the membrane was probed for Tie2, stripped and reblotted for FAK, followed by β3 integrin analyses, αv integrin was detected on the lower part of the membrane. (B) Whole cell lysates were immunoprecipitated for Tie2, Western blotted and the membrane was cut horizontally at 50 kDa into two pieces. The upper piece of the membrane was probed for β3 integrin, stripped and reblotted for p-Tyr followed by Tie2 analyses, αv integrin was detected on the lower part of the membrane. Whole cell lysates (WCL) was used as a positive control.

Thomas et al., Supplementary Figure 4



Ang-2 induces dose-dependently complex-formation between Tie2-αvβ3 integrin-FAK. Confluent HUVEC were stimulated with different concentrations of Ang-2 for 15 min. Protein lysates were immunoprecipitated against αvβ3 integrin and Western blotted. The membrane was horizontally cut under Ponceau Red control at 70 and 35kDa. The upper part was probed for Tie2, stripped and reprobed against FAK and β3 integrin. The lower part was probed for αv integrin. Whole cell lysate of HUVE cells was used as a positive control (WCL).

Thomas et al., Supplementary Figure 5



Ang-2 induces co-localization between Rab5 and αvβ3 integrin Confluent HUVEC were stimulated with 200ng/ml Ang-2 for 30 min followed by staining against αvβ3 integrin and Rab5. Pictures were taken by confocal microscopy (and analyzed with the newly developed co-localization software [see materials and methods]) . S.E. Standard Error.