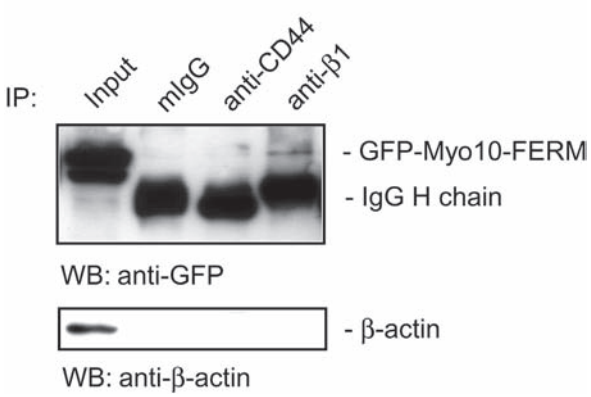

Figure S1 Immunoprecipitation of integrin β1, but not of CD44, brings down Myo10 and not β-actin. Upper: lysates containing GFP-Myo10-FERM expressed in 293T cells were immunoprecipitated by anti-integrin β1 mab, anti-CD44 mab or normal mouse IgG (mIgG) followed by Western blot against GFP. The left lane shows Western blot of 20 μg of the input lysate containing GFP-Myo10-FERM. Lower panel shows Western blot for β-actin with the same immunoprecipitated (or input) samples.

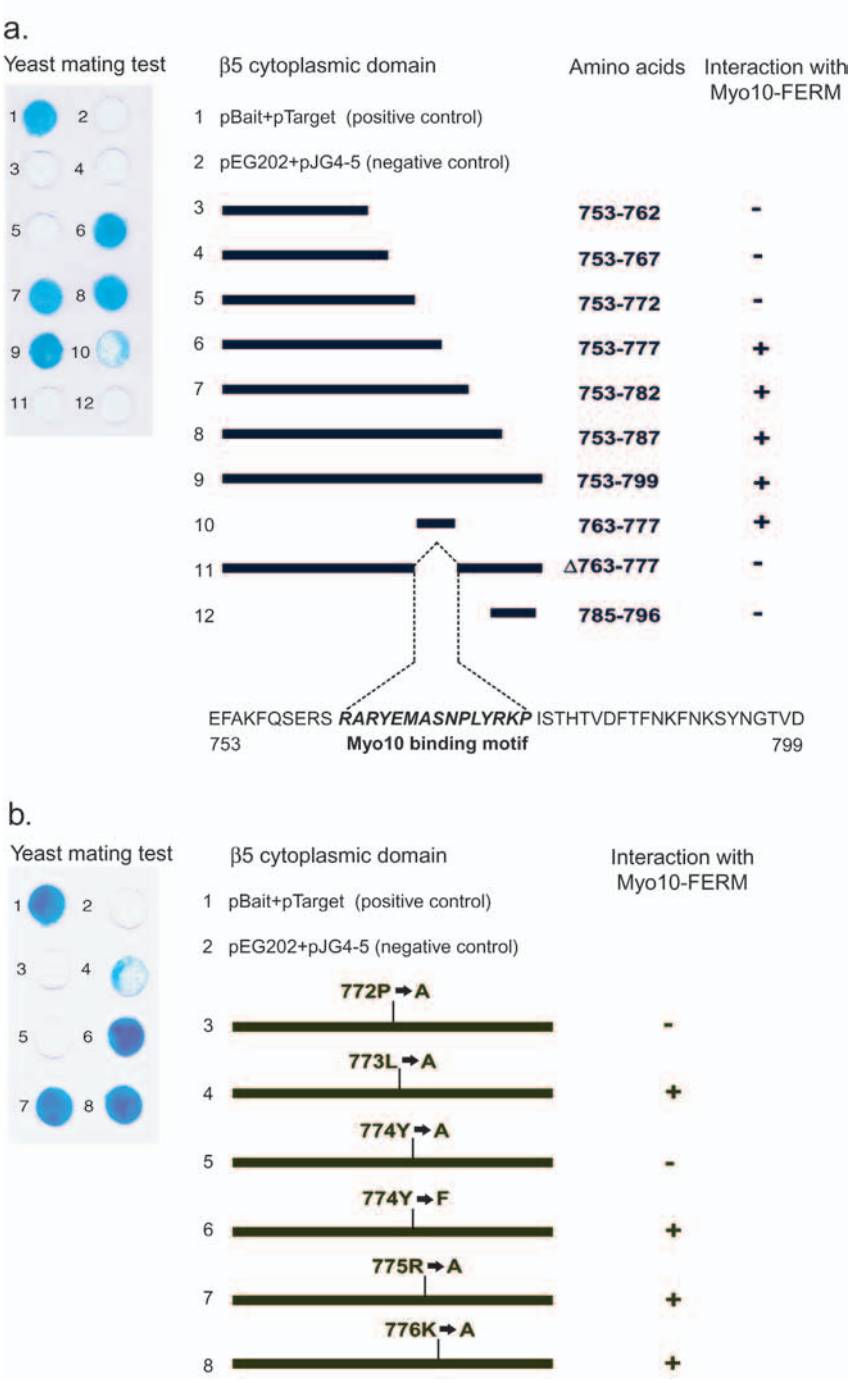

Figure S2 Yeast two-hybrid mating test mapping of the Myo10-binding site in the integrin β5 cytoplasmic domain. **a**, Yeast mating tests were employed to determine the Myo10-binding region within the integrin β5 cytoplasmic domain. Left: mating test results for each integrin β5 fragment where blue colour indicates a positive interaction. Middle and right: summary of the yeast mating test results displaying the various regions of the integrin β5 cytoplasmic domain that were tested for interaction with Myo10. Bottom: the Myo10-binding motif within the integrin β5 cytoplasmic domain is indicated. **b**, Left: mating test results for each mutation where blue colour indicates a positive interaction. Middle and right: summary of point mutations within a conserved NPXY-motif used for the mating tests and the mating test results. Mutation of 772P or 774Y to alanine disrupted the binding of β5 cytoplasmic domain to Myo10-FERM domain, while mutation of 774Y to phenylalanine that is not phosphorylatable did not affect the binding. Furthermore, mutation of 773L to alanine partially disrupted the Myo10 to integrin binding (spot #4) while the R775A and K776A mutants retained full binding to Myo10. This indicates that the NPXY-motif is required for binding of Myo10 to integrin β5.

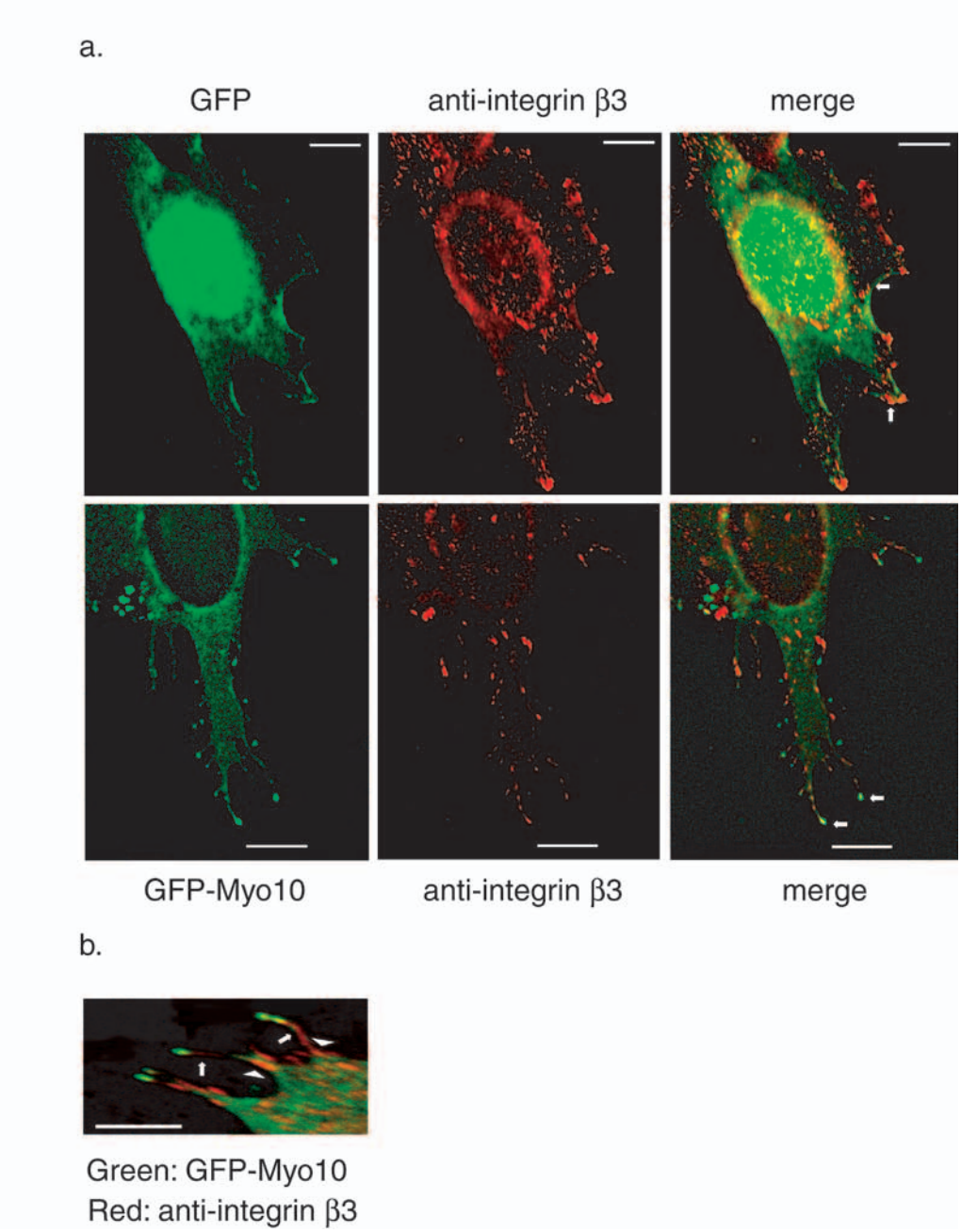

Figure S3 Myo10-mediated integrin relocalisation into filopodia. **a,** Upper panel: GFP-transfected M21 cells did not form filopodia and integrin β3 typically localised in focal adhesions within 3 h after replating onto VN, the displayed cell is stained 30 min after replating (green: GFP; red: integrin β3). Arrows mark integrin β3 staining at focal adhesions. Lower panel: GFP-Myo10 overexpression relocalised a small portion of integrin β3 to tips of filopodia, where Myo10 (green) and integrin β3 (red) colocalised (arrows) 30 min after replating onto VN. Note that in GFP-Myo10 transfected M21 cells plated on VN, approximately 80 % of transfected M21 cells showed GFP-Myo10 colocalisation with integrin β3 at tips of filopodia. While we did not detect integrin β3 in all filopodia, we were unable to determine if integrin β3 was not always present in Myo10-contaning filopodia or if the amount of integrin in filopodia was sometimes un-detectable by our fluorescent staining. However, in all cases where we detected integrin β3 in filopodia, Myo10 and integrin β3 colocalised at the tips of filopodia. Bars, 10 μm. **b,** An example of integrin β3 localisation within filopodia appearing like extensions from focal adhesions. Integrin β3-containing focal adhesions that filopodia appears to be extended from are indicated with arrowheads. Localisation of integrin β3 within the corresponding filopodia is indicated with arrows. Approximately 25 % GFP-Myo10 transfected M21 cells showed intrafilopodial colocalisation of Myo10 with integrin β3 while less than 5 % of GFP-Myo10 transfected M21 cells showed localization of Myo10 in integrin β3-containing focal adhesions. Bar, 10 μm.

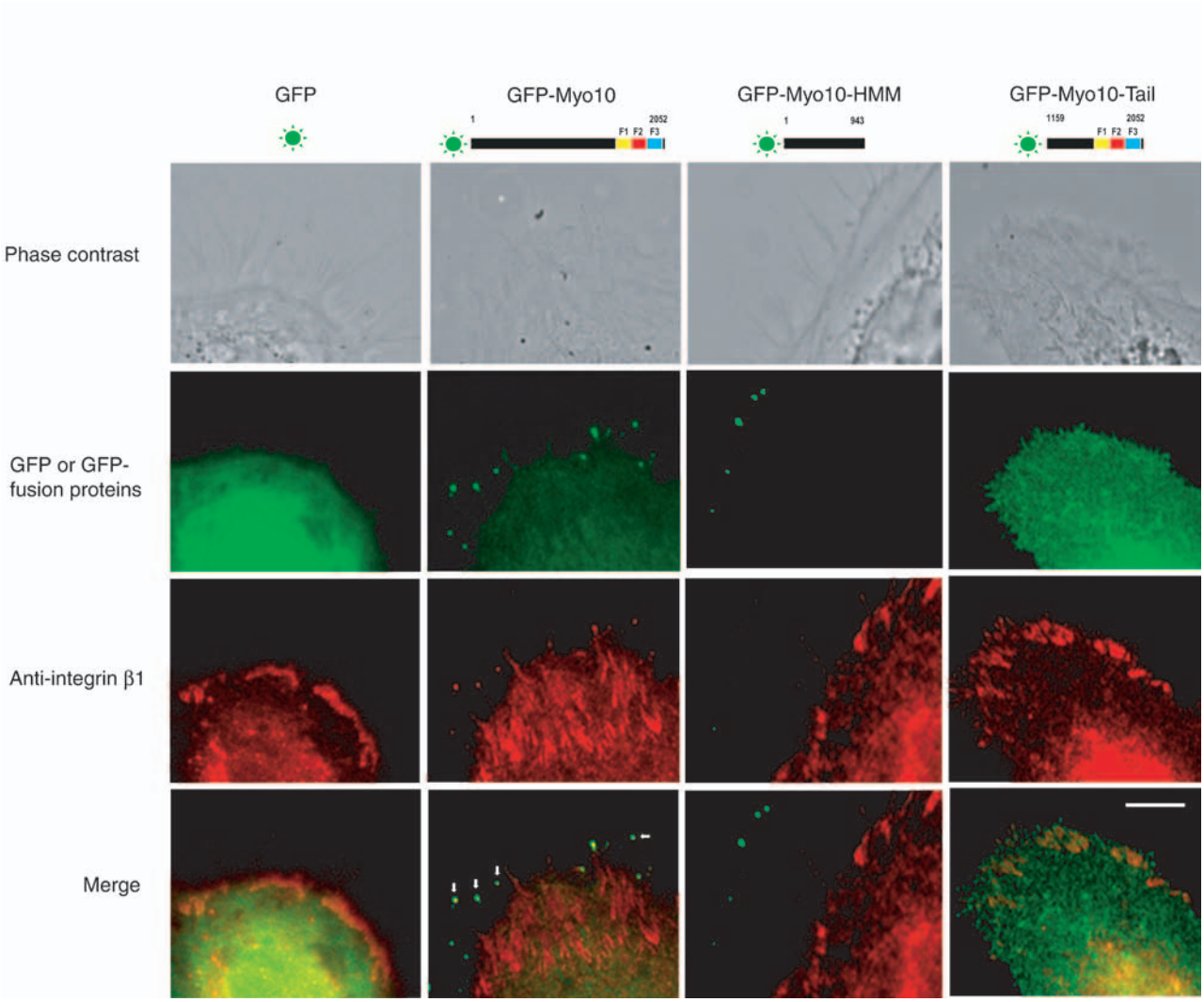

Figure S4 Overexpression of GFP-Myo10 in HeLa cells enhances integrin localization to tips of filopodia. HeLa cells were transfected with GFP, GFP-Myo10, GFP-Myo10-HMM, or GFP-Myo10-Tail (all green) and replated onto FN for 3 h and stained for integrin β1 (red). In contrast to the other cells tested, HeLa cells formed numerous filopodia even in the absence of exogenous Myo10 (GFP-transfected cells; leftmost). GFP-Myo10 appears to enhance integrin β1 staining at filopodial tips (middle left), while GFP (leftmost), GFP-Myo10-HMM (middle right) and GFP-Myo10-Tail (rightmost) does not. Myo10-HMM includes the actin-binding motor domain of Myo10 that localizes to tips of filopodia but does not increase the length or number of filopodia[6]. The results indicate that the integrity of Myo10 is important for its capacity to relocalise integrins into filopodia.