CD13 Activation Assembles Phosphoinositide (PI) Signaling Complexes to Regulate the Actin Cytoskeleton

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Running title: CD13 activation promotes actin-based protrusions

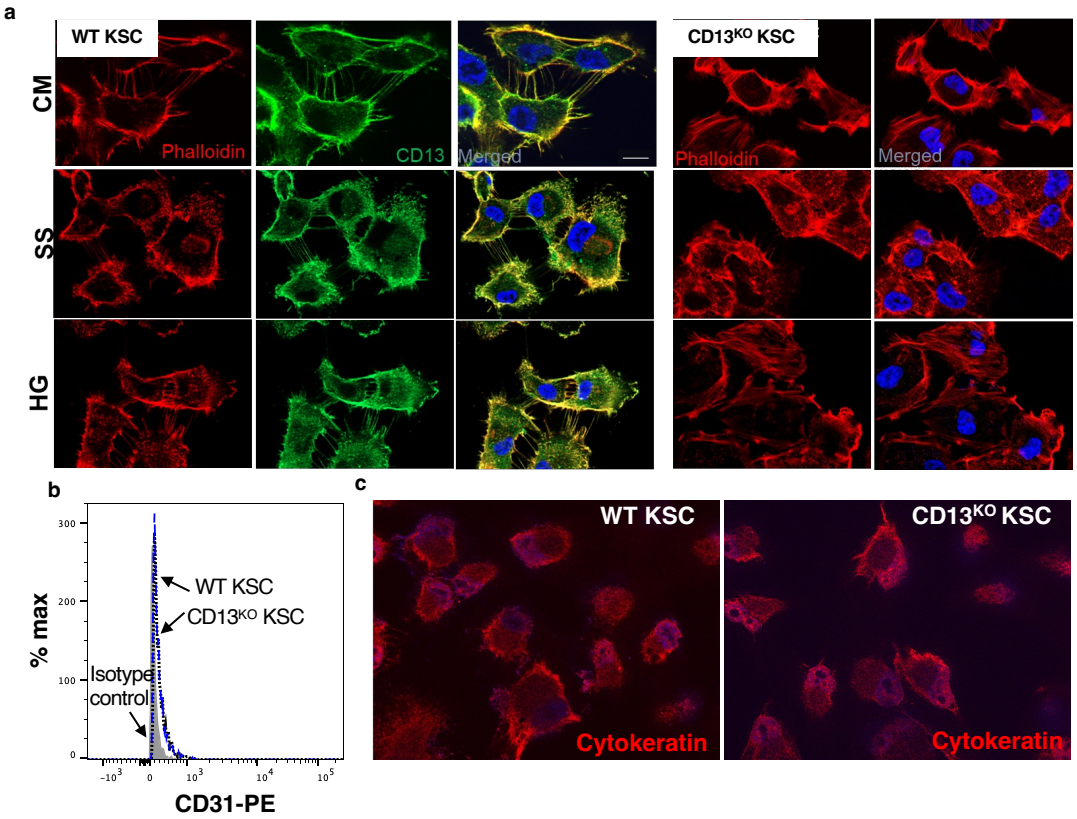

FIG S1. (**a**). Split-channel comparison of WT and CD13^{KO} KSCs forming protrusion. Phalloidin (red) and CD13 (green) channels separated across rows followed by merged images with DAPI (blue) for WT and CD13^{KO} KSCs. Rows represent culture conditions with complete media (CM), serum starvation (SS) or high glucose (HG) . Scale bar: 10μm. (**b**). Flow cytometric analysis of endothelial marker, CD31. Grey area (isotype control), black dotted line (WT) and blue dotted line (CD13^{KO}), indicated that KSCs express low levels of endothelial marker, CD31. (**c**). Immunofluorescence analysis indicated expression of epithelial cell marker, cytokeratin (red) in WT and CD13^{KO} KSCs.

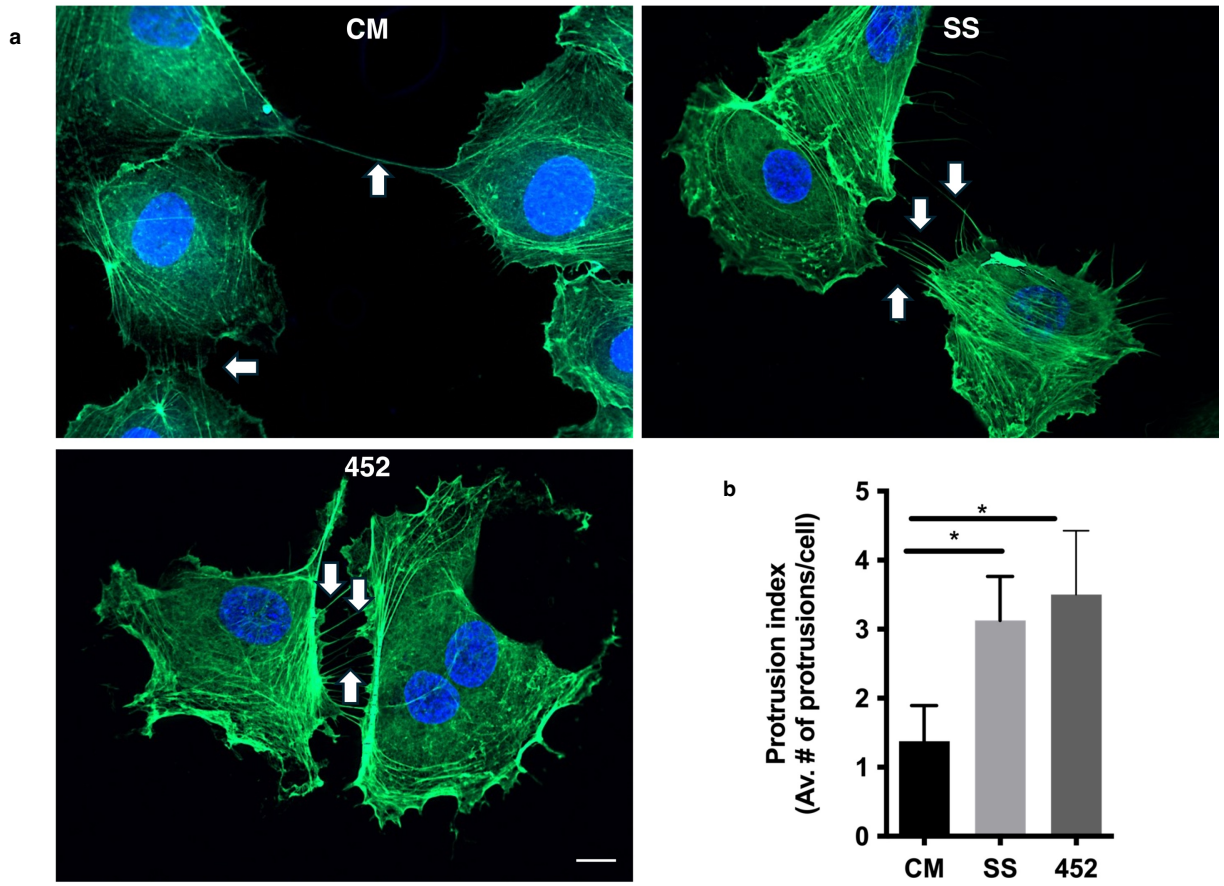

FIG S2. Serum starvation and CD13 mAb 452 treatment induce actin-based protrusion formation in HUVEC. (**a**). HUVEC were grown in complete medium (CM) or serum starved for 1h (SS) or treated with 1.8μg/mL CD13 mAb for 1h (452). (**b**). Quantification of actin^{+} protrusion (Phalloidin; green) formation highlighted by white arrows. Data are represented as mean ± standard error. * = $p<0.05$. Scale bar: 10μm.

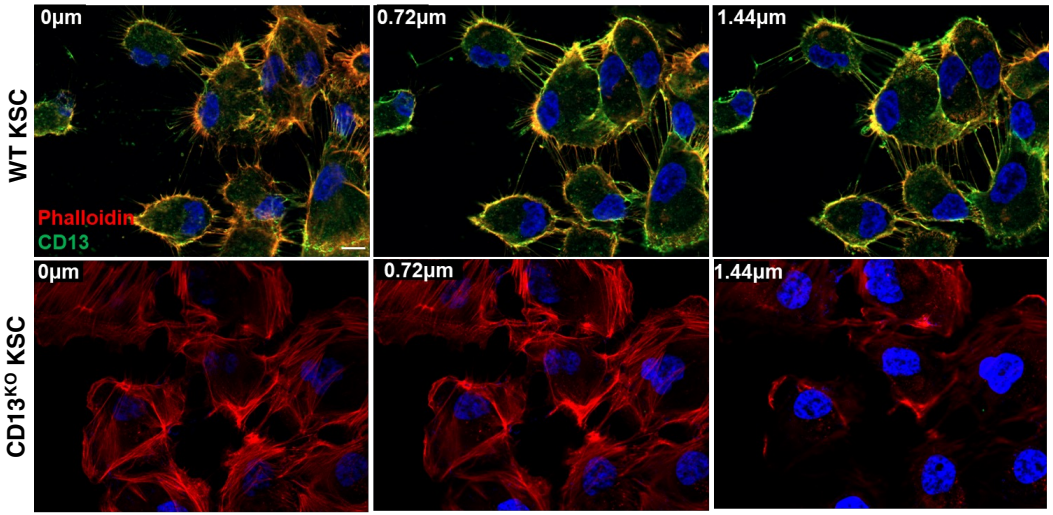

FIG S3. Protrusions in WT cells form further from the substrate than in $CD13^{KO}$ cells. Representative z-stack images of WT and $CD13^{KO}$ cells with protrusions spanning between 0.48-1.44μm above the substrate in WT KSCs. Scale bar: 10μm.

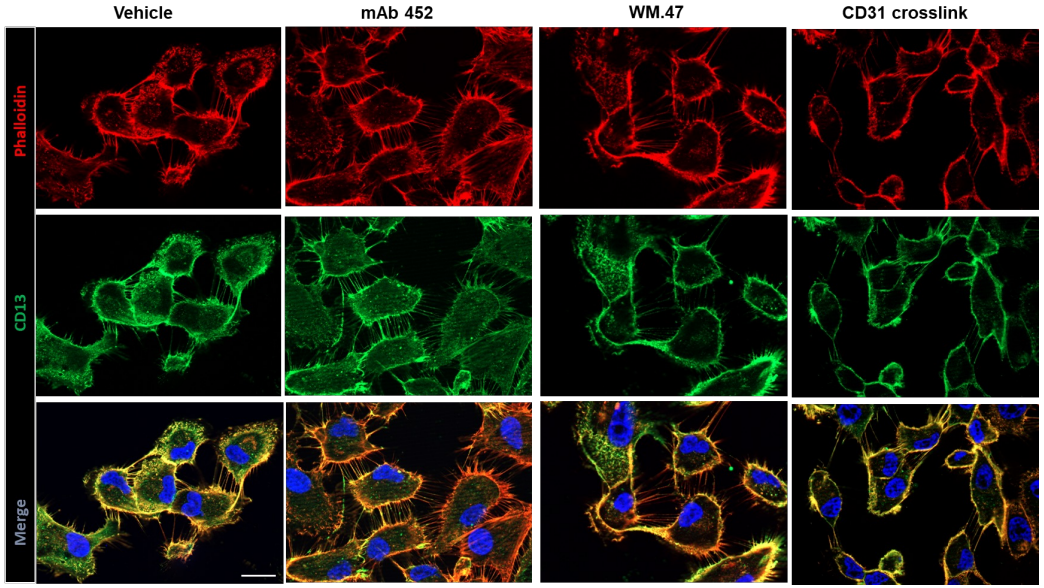

FIG S4. Split-channel comparison of CD13 and phalloidin staining in WT KSCs treated with crosslinking or non-crosslinking antibodies. Phalloidin (red) and CD13 (green) channels separated across rows followed by merged images with DAPI (blue) for WT KSCs. Rows represent culture conditions with vehicle control (Vehicle; complete media), mAb 452 (1.8μg/mL), CD13 mAb WM4.7 (1:100), or CD31 crosslink (1μg/mL). Scale bar: 10μm.

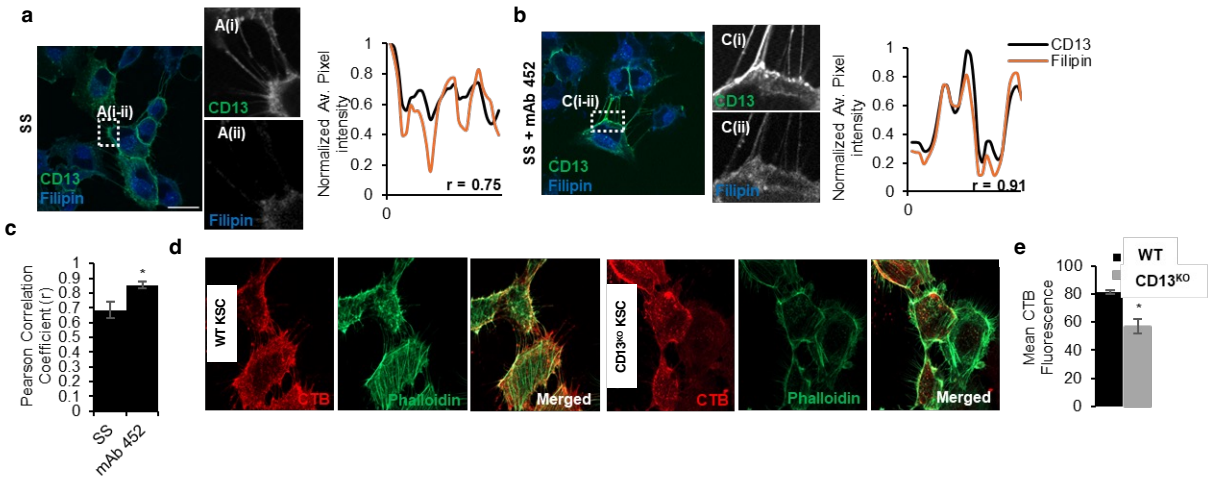

FIG S5. CD13 may localize membrane lipids at the base of protrusions. (**a-b**) Representative images and overlaid membrane line scans for localization of CD13 (green) and filipin (blue) at the base of protrusions in WT KSCs in serum-starved (a; SS) or mAb 452 (b; 1.8µg/mL) treatment conditions. (**c**) Comparison of the average Pearson Correlation Coefficient in cells under SS or mAb 452 conditions. (**d**) Representative images of CTB staining in WT versus CD13^{KO} KSCs. (**e**) Quantification of mean CTB fluorescence on WT versus CD13^{KO} KSCs. Scale bar: 10µm. Data are represented as mean ± standard error. * $p<0.05$.

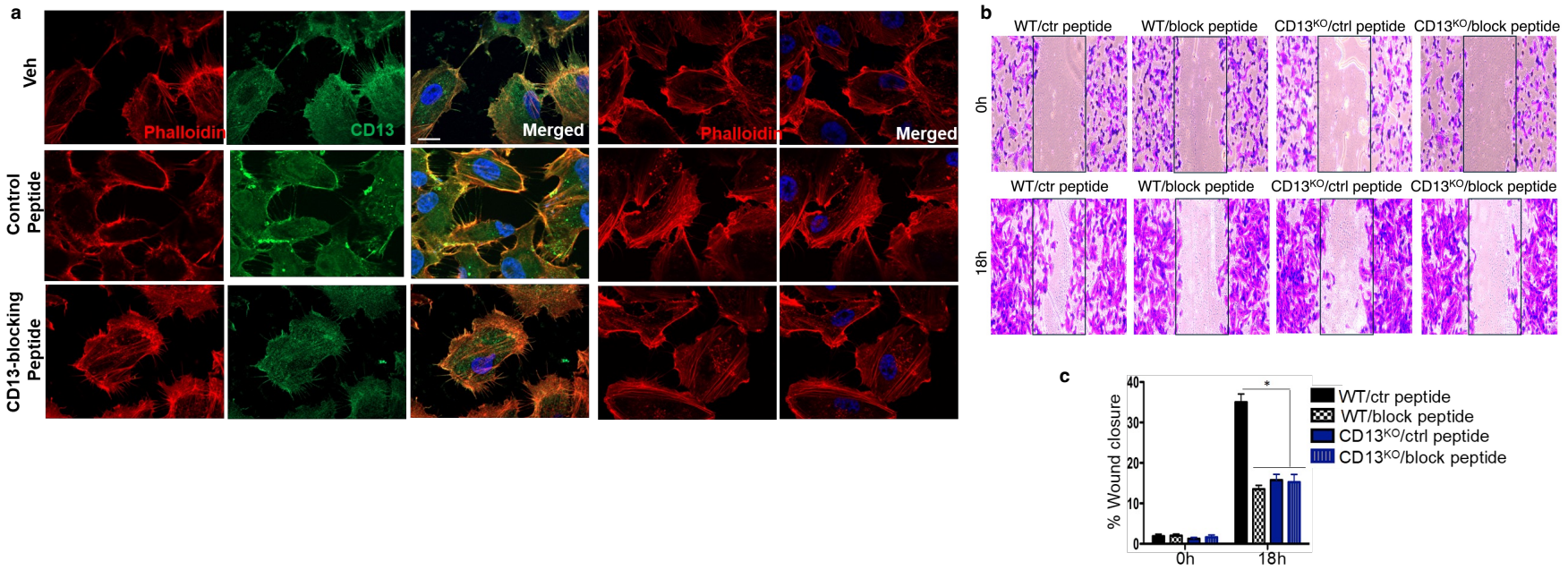

FIG S6. CD13-blocking peptide effectively mimics CD13^{KO} KSCs in wound-closure assay. (**a**) Images of separated channels of WT and CD13^{KO} KSCs, phalloidin (red), CD13 (green) merged with DAPI (blue) of vehicle control, control (1µg/mL) or CD13-blocking (1µg/mL) peptide loaded cells. (**b-c**) Representative image (b) and quantification (c) of wound healing assay in WT and CD13^{KO} KSCs in presence of control or CD13-blocking peptide. CD13 has been shown to regulate cell migration during wound healing. Blocking CD13 with this peptide significantly inhibited wound closure, like in CD13^{KO} KSCs. Data are represented as mean ± standard error. *p<0.05. Scale bar: 10µm.

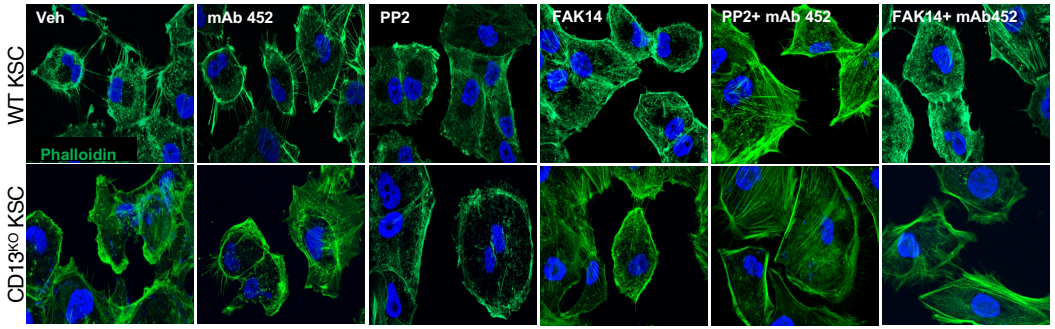

FIG S7. Inhibition of FAK/Src signaling abrogates protrusion formation. WT and $CD13^{KO}$ KSCs were treated with a FAK inhibitor 14 (FAK14; 20μM) or Src inhibitor (PP2; 10μM) with or without mAb 452(1.8μg/mL), for 1hr prior to staining with phalloidin (green) and DAPI (blue). Scale bar: 10μm.