

Supplementary Fig. 1. Integrin αv knockdown reduces VANGL2 membrane protein levels (plasma and intracellular). Western blot of fractionated cellular protein from non-targeting (NT) control and integrin αv (ITGAV) #10 single siRNA transfected cells. Pan-cadherin antibody labeling shows enrichment for membrane proteins. The VANGL2 blot was re-probed with GAPDH antibody to serve as a loading control and to show enrichment for cytoplasmic proteins (experiment performed three times, n = 3 biological replicates).

Supplementary Fig. 2. Integrin αv does not influence PRICKLE1 or VANGL1 protein levels and VANGL2 is unaffected by integrin $\alpha 5\beta 1$ knockdown. (A) Western blots of total protein from non-targeting (NT) control and integrin αv (ITGAV) knockdown HT-1080 cells. (B) Box

plot shows median value \pm standard deviation (whiskers) and the interquartile range (boxes) of likely variation. Integrin αv knockdown cells have normal PRICKLE1 and VANGL1 protein levels (experiments performed three times, $n = 16$ (PRICKLE1) and $n = 10$ (VANGL1) biological replicates). **(C)** Western blots of total protein from NT control and integrin $\alpha 5$ (ITGA5) knockdown cells. Integrin $\alpha 5$ protein is reduced by 76%. **(D)** Box plot showing integrin $\alpha 5$ knockdown cells have normal VANGL2 protein levels (experiment performed three times, $n = 8$ biological replicates). **(E)** Western blots of total protein from NT control and integrin $\beta 1$ (ITGB1) knockdown cells. Integrin $\beta 1$ protein is reduced by 100%. **(F)** Box plot showing integrin $\beta 1$ knockdown cells have normal VANGL2 protein levels (experiment performed three times, $n = 8$ biological replicates).

Supplementary Fig. 3. Early endosomal VANGL2 localization in integrin αv knockdown cells.

(A) Non-targeting (NT) control siRNA transfected cells expressing GFP-VANGL2 and immunolabeled for early endosome antigen 1 (EEA1). Nuclei are labeled with DAPI (blue). Arrows denote plasma membrane expression. **(B)** Integrin αv (ITGAV) siRNA transfected cells expressing GFP-VANGL2 and with EEA1 and DAPI labeling. Certain double-positive vesicles are highlighted with arrowheads. Scale bars = 5 μ m.

Supplementary Fig. 4. VANGL2 protein expression in cells grown on different substrates.

Western blots of total VANGL2 protein levels in HT-1080 cells plated 48 h on untreated plastic, fibronectin (Fn), and vitronectin (Vn). Box plot shows median value \pm standard deviation (whiskers) and the interquartile range (boxes) of likely variation (experiment performed three

times, n = 8 biological replicates). $**P < 0.01$; P values are versus VANGL2 expression in cells grown on untreated plastic; two-tailed unpaired t -test.