## SUPPLEMENTAL INFORMATION

## Supplemental Figures.



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Figure S1

**Supplemental Figure S1**. (A) Validation of junctional WAVE2 localisation by loss of junctional WAVE2 staining in WAVE2 KD cells. (B) Junctional localization of WAVE2-mCherry. mCherry-tagged WAVE2 was transiently expressed in Caco-2 cells; fixed samples were immunostained for Ecadherin and the transgene identified by its native mCherry fluorescence. Scale bar, 10µm.



Supplemental Figure S2. (A) Validation of WAVE2 knockdown (KD) by an siRNA

targeting the 3' untranslated region and reconstitution of WAVE2 expression by WAVE2-GFP. (B) Low (top panel) and high (bottom panel) magnification images of WAVE2 KD cells that have been transfected with RNAi-resistant WAVE2-GFP. Low magnification images show that the percentage of transfected cells is relatively low (<20%), which explains the low expression level of WAVE2-GFP in (A). High magnification images show restoration of F-actin localisation at contacts in cells expressing WAVE2-GFP (closed arrows) compared to non-transfected cells (open arrow) (C) Line scan quantitation of junctional F-actin in control, WAVE2 KD and reconstituted cells. Junctional F-actin was reduced in WAVE2 KD cells and restored to normal levels by expression of WAVE2-GFP, n=14, 16 and 10 for control, KD and reconstituted, respectively. Data are means ± SEM; \*\*\*, p<0.001. Scale bar, 20µm.



Figure S3

**Supplemental Figure S3.** Apical and basal confocal stacks of Myosin IIA and IIB in control and WAVE2 KD cells. In WAVE2 KD cells, Myosin II appeared to redistribute to the basal pool of actomyosin, in particular to stress fibres (arrows). Scale bar, 10µm.