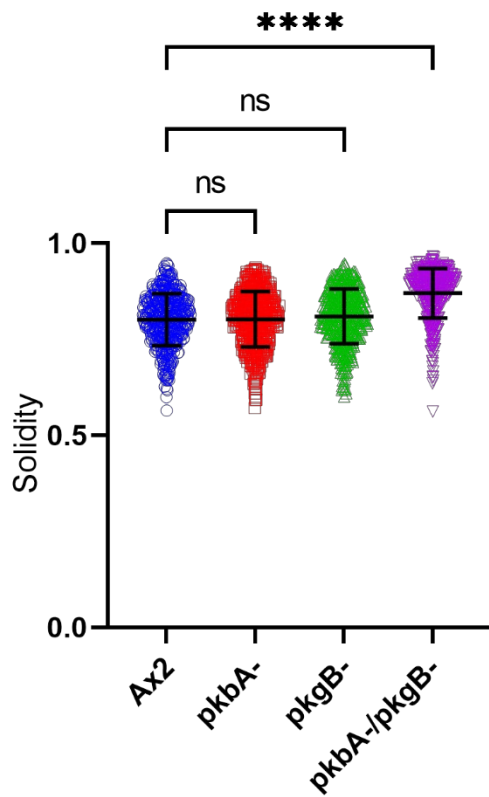
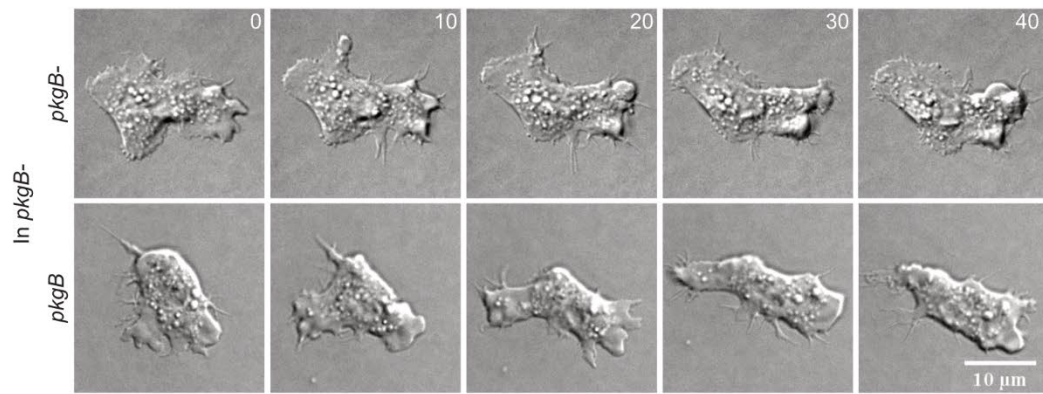


## Supplementary materials

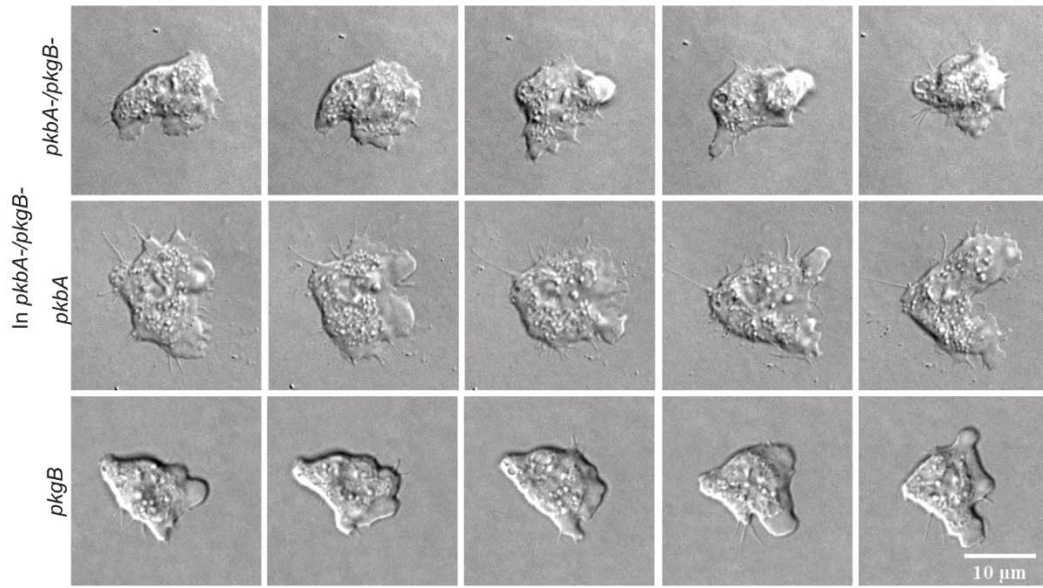


**Figure S1. Change in cell solidity in Ax2, and *pkbA*-, *pkgB*-, and *pkbA*-/*pkgB*- cells.** Ratio of total cell area and convex cell areas was calculated using a custom-made Image J plugin. (mean  $\pm$  SD;  $n = 416$  Ax2,  $474$  *pkbA*-,  $458$  *pkgB*- and  $366$  *pkbA*-/*pkgB*- over 3 independent experiments, \*\*\*\* $p \leq 0.0001$ , 1-way ANOVA, Dunn's multiple comparison test).

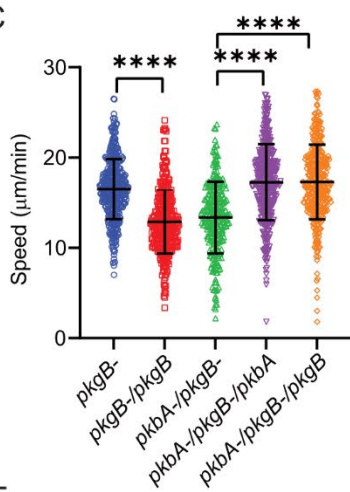
A



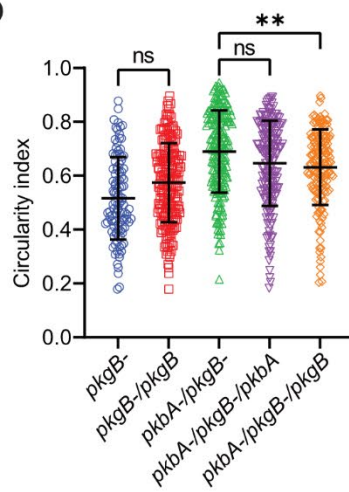
B



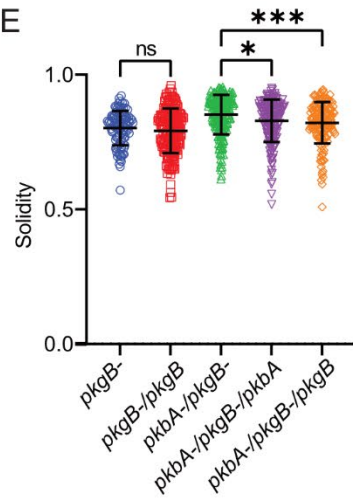
C



D



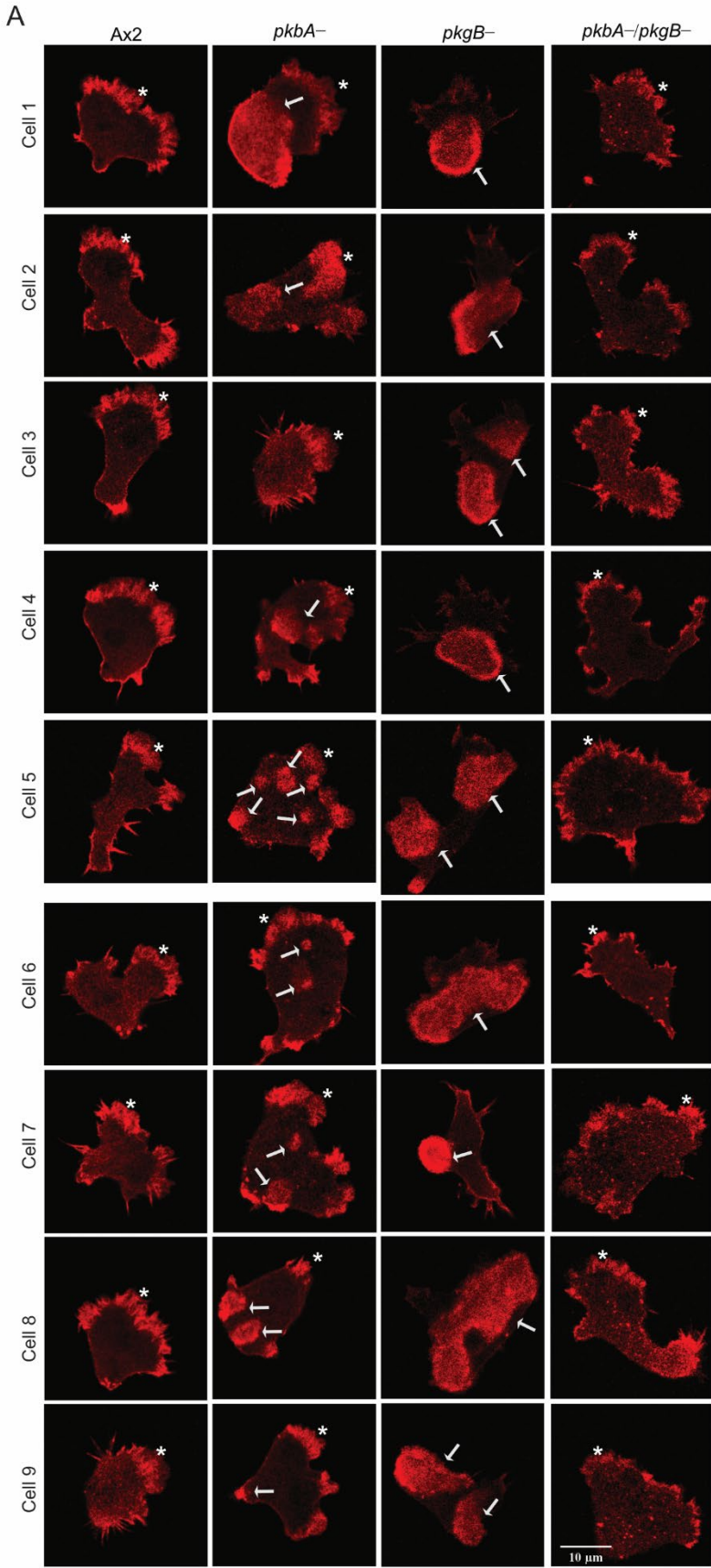
E



F

Cells	Speed(μm/min; mean±SD)	Circularity index	Solidity
<i>pkgB</i> -	16.62±3.3	0.51±0.15	0.8±0.06
<i>pkgB</i> -/ <i>pkgB</i>	12.9±3.5	0.57±0.14	0.79±0.08
<i>pkbA</i> -/ <i>pkgB</i> -	13.3±3.9	0.68±0.15	0.85±0.07
<i>pkbA</i> -/ <i>pkgB</i> -/ <i>pkbA</i> -	17.2±4.2	0.64±0.15	0.82±0.07
<i>pkbA</i> -/ <i>pkgB</i> -/ <i>pkgB</i> -	17.3±4.1	0.63±0.14	0.82±0.07

**Figure S2. Rescue of pseudopodia formation in *pkgB*- and *pkbA*-/*pkgB*-.** (A) Extrachromosomal expression of *pkgB* rescues pseudopodia formation in *pkgB*- cells. (B) Extrachromosomal expression of *pkbA* (panel 2) and *pkgB* (panel 3) rescues pseudopodia formation in *pkbA*-/*pkgB*- cells. (C) Quantification of speed of *pkgB*-, *pkbA*-/*pkgB*- and rescued cells. (mean  $\pm$  SD; n >300 cells over 3 independent experiments, \*\*\*\*p  $\leq$  0.0001, 1-way ANOVA, Dunn's multiple comparison test). (D&E) Quantification of circularity index and solidity of *pkgB*-, *pkbA*-/*pkgB*- and rescued cells, (mean  $\pm$  SD; n>50 cells over 3 independent experiments, \*p  $\leq$  0.05, \*\*p  $\leq$  0.01, \*\*\*p  $\leq$  0.001, 1-way ANOVA, Dunn's multiple comparison test). (F) Quantitative measurement of speed, circularity index and solidity.

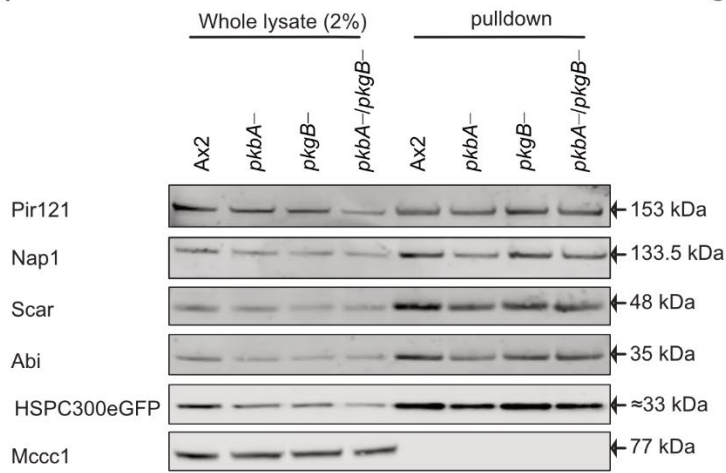


**B**

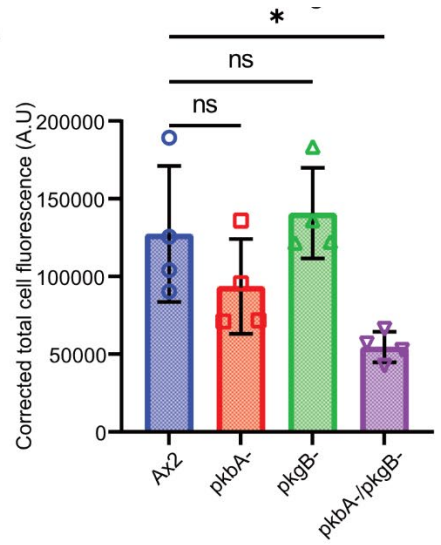
Cells	% of cells
Ax2 (n=19)	32
<i>pkbA</i> <sup>-</sup> (n=17)	88
<i>pkgB</i> <sup>-</sup> (n=12)	100
<i>pkbA</i> <sup>-</sup> / <i>pkgB</i> <sup>-</sup> (n=19)	32

**Figure S3: Comparison of F-Actin distribution in multiple cells of Ax2 and mutant cells by AiryScan confocal microscopy.** (A) Panel of cells showing F-actin accumulation in protrusions, cell body and rear. Life-act-mRFPmars2 was expressed in Ax2, and mutant cells. Cells were allowed to migrate under agarose mediated by a folate gradient and imaged by AiryScan confocal microscopy at a frame interval of 3 seconds (1f/3s). Ax2 and *pkbA*- cells show F-actin in pseudopodia (asterisks). F-actin accumulation is absent in *pkgB*- protrusions, and highly reduced in *pkbA*-/*pkgB*- cells (asterisks). Static F-actin in the cell body is mainly present in both *pkbA*- and *pkgB*- cells (arrows). (B) Percentage of cells with F-actin accumulation at the rear or cell body. n= number of cells <sup>19</sup>Ax2, <sup>17</sup>*pkbA*-, <sup>12</sup>*pkgB*- and <sup>19</sup>*pkbA*-/*pkgB*-.

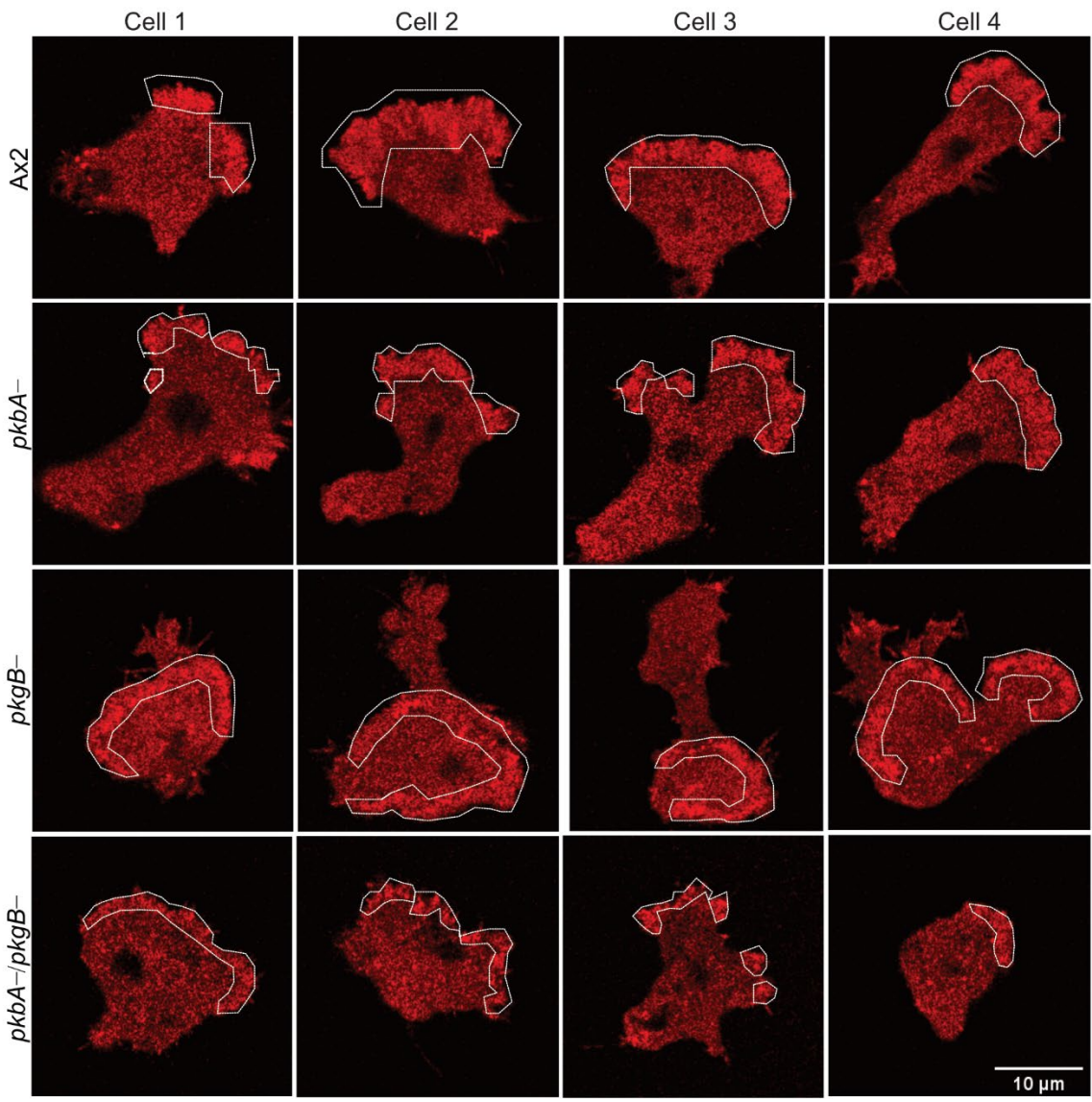
A



C



B



**Figure S4: Stable Scar/WAVE complex formation in Ax2 and mutants.** (A) The Scar/WAVE complex was purified from cells expressing HSPC300-eGFP by GFP trap. Whole cell lysate and pulldown samples were analysed for the expression of, Pir121, Nap, Scar, Abi, 1 and GFP by western blotting. Mccc1 was used as loading control. This experiment was repeated thrice. (B) Recruitment of Arp2/3 complex in cell protrusions of multiple cells over 3 experiments. Ax2, *pkbA*<sup>-</sup>, *pkgB*<sup>-</sup> and *pkbA*<sup>-</sup>/*pkgB*<sup>-</sup> cells were labelled with mRFPmars2-ArpC4 (Arp2/3 complex), allowed to migrate under agarose following a folate gradient and imaged by AiryScan confocal microscopy at a frame interval of 3 seconds (1f/3s). The Arp2/3 complex is enriched broadly in the pseudopodia of Ax2 and *pkbA*<sup>-</sup> cells, in the cell body of *pkgB*<sup>-</sup>, but is highly reduced in the *pkbA*<sup>-</sup>/*pkgB*<sup>-</sup> cells (dotted lines). (C) Integrated fluorescent intensities of the Arp2/3 complex were calculated using image and normalized to cell area is represented. Reduced Arp2/3 complex intensity in the protrusions of *pkbA*<sup>-</sup> and *pkbA*<sup>-</sup>/*pkgB*<sup>-</sup> cells. Mean and SD, n=4, , \*p ≤ 0.05.

## Video legends

**Video S1. Pseudopodia formation in Ax2, *pkbA*<sup>-</sup>, *pkgB*<sup>-</sup> and *pkbA*<sup>-</sup>/*pkgB*<sup>-</sup> cells during directed migration.** All cells were allowed to migrate under agarose chasing a folate gradient and observed by DIC microscopy. Filmed at 1 frame/2 seconds, movie shows 10 frames/second.

**Video S2. Rescue of pseudopodia in *pkgB*<sup>-</sup> by extrachromosomal expression of *pkgB*.** *pkgB*<sup>-</sup> cells expressing *pkgB* were allowed to migrate under agarose up a folate gradient and observed by DIC microscopy. Filmed at 1 frame/2 seconds, movie shows 10 frames/second.

**Video S3. Rescue of pseudopodia in *pkbA*<sup>-</sup>/*pkgB*<sup>-</sup> by extrachromosomal expression of *pkbA* or *pkgB*.** *pkbA*<sup>-</sup>/*pkgB*<sup>-</sup> cells expressing *pkbA* or *pkgB* were allowed to migrate under agarose up a folate gradient and observed by DIC microscopy. Filmed at 1 frame/2 seconds, movie shows 10 frames/second.

**Video S4. Localization of F-actin in Ax2 and mutant cells.** Cells expressing Lifeact-mRFPmars2 were allowed to migrate under agarose up a folate gradient and observed by AiryScan confocal microscopy. **Filmed at 1 frame/3 seconds, movie shows 10 frames/second.**

**Video S5. Recruitment of the Scar/WAVE and Arp2/3 complexes in Ax2 and mutant cells.** Cells expressing HSPC300-eGFP and mRFPmars2-ArpC4 were allowed to migrate under agarose towards a folate gradient and observed by AiryScan confocal microscopy. Filmed at 1 frame/3 seconds, movie shows 10 frames/second.