Supporting Information

Janssens et al. 10.1073/pnas.0915075107

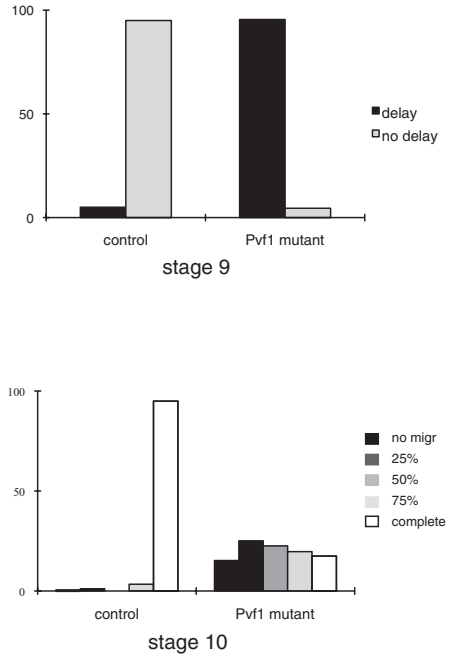


Fig. S1. Migration of border cell clusters in the control genotype used throughout this analysis (slbo-Gal/UAS-PVR) and with UAS-PVR in Pvf1 mutant background (Pvf1^{1624}/ Pvf1^{1624}; slbo-Gal/UAS-PVR) scored at stage 9 (*Upper*) when cells are normally migrating (n = 100, 111) and at stage 10 (*Lower*) when wild-type clusters have completed migration (n = 177, 315).

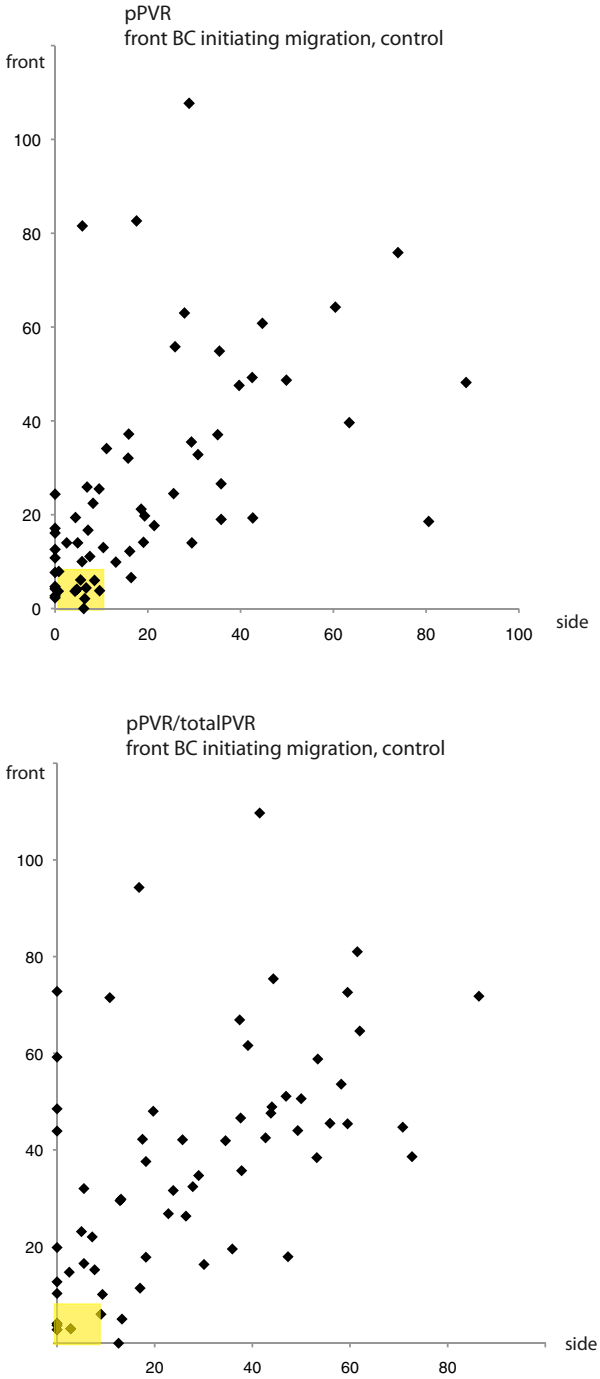


Fig. S2. Plots of pPVR signal and of pPVR/totalPVR at front and side membrane of control front border cells initiating migration. Each point represents measurements from one border cell cluster; $n = 63$. This is a completely independent set of data from that depicted in Fig. 3 *B* and *C*. Measurement areas were in this case not defined by a line but instead by a slim, hand-drawn box including the cell cortex (front or side) but avoiding underlying vesicles.

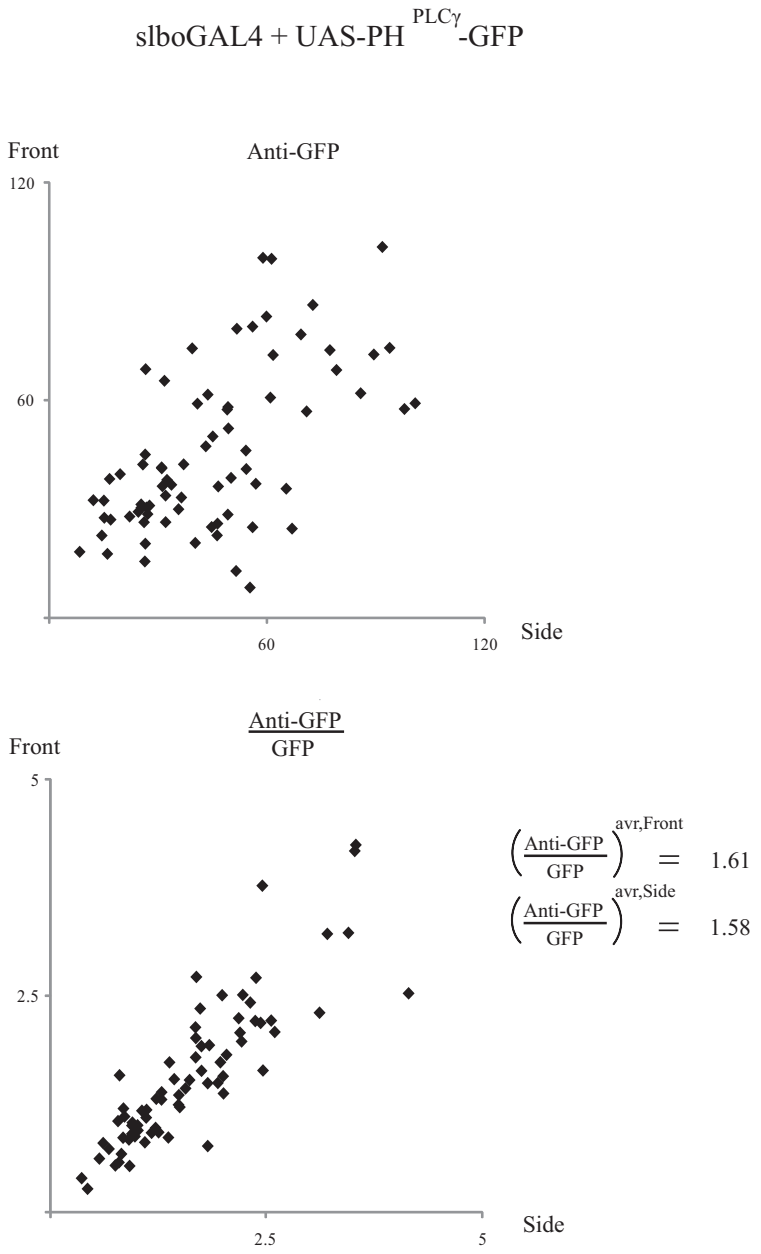
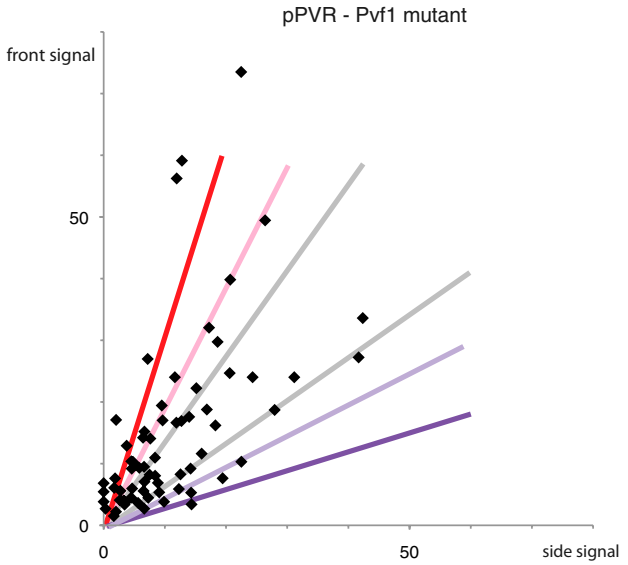


Fig. S3. Control for front and side bias. A generally membrane-associated $PH^{PLC\gamma}$-GFP fusion (1) was expressed in border cells using slbo-Gal4, stained with anti-GFP + Cy5 secondary AB, and front and side Cy5 signals measured in front border cells initiating migration (*Top*; measurements done as in Fig. 3). There is variability but no front bias. Comparing the average antibody signal to the average GFP (direct fluorescence) signal in the same experiment also showed no bias (values shown below). Bottom scatter plot shows anti-GFP/GFP for each measurement. This is not a constant value, probably because measurements are a short time after slbo-Gal4 initiates PH-GFP expression, so the amount of folded and fluorescent GFP has not reached steady state. Also, some egg chambers may show better overall antibody staining than others. The strong correlation between front and side values, however, supports that the more pronounced cell-to-cell variation between front and side values seen in the plot above (and in the pPVR and PVR antibody experiments) are due to real local protein accumulation differences. Each point represents measurements from one border cell cluster; $n = 74$.

1. Pinal N, et al. (2006) Regulated and polarized PtdIns(3,4,5)P3 accumulation is essential for apical membrane morphogenesis in photoreceptor epithelial cells. *Curr Biol* 16:140–149.



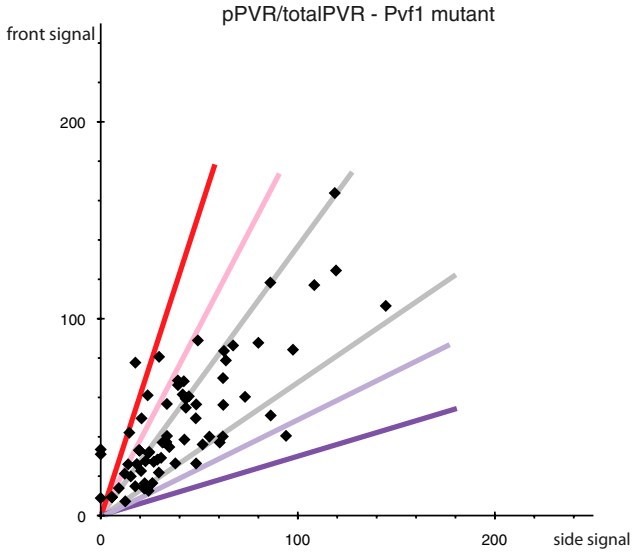


Fig. S4. Plots of pPVR signal and pPVR/totalPVR at front versus side membrane of a front border cell initiating migration as in Fig. 3, but in a *Pvf1/Pvf1* mutant background. Each point represents measurements from one border cell cluster; $n = 82$. Side measurements slightly below background were set to 0. Colored lines correspond to limits for classifications, with results depicted in Fig. 3*E*.

Table S1. Making and testing of potential pPVR mABs

PVR phosphopeptide	mAB clone	pPVR/PVR peptide signal in ELISA	Signal with PVR in COS cells	Signal with kinase-dead PVR-GFP in COS cells	In ovaries: UAS-PVR, also test UAS-EGFR
CGRSMYRGDNpYKKSENGKLP	4p-2C8	2	Signal?	Signal?	No staining
	4p-13B6	2.3	No signal	No signal	N/A
CGSHpYLDLNNPpYMQSNIEpYMKKQ	5p-20H8	5	Signal OK	No signal	No or weak staining
	5p-8A9	11.6	Signal OK	Low signal	Nonspecific phospho-Y
	5p-9F7	8.6	Signal OK	Low signal	UAS-EGFR also positive
	5p-2B10	3.6	No signal	No signal	N/A
	5p-6B5	4.7	No or very low signal	No signal	N/A
	5p-20B11	3.2	Some signal	No signal	Nonspecific phospho-Y
CGRIEELPDDpYMEMSRDSDPD	6p-6C11	3.5	No signal	No signal	N/A
	6p-19E2	3	No or very low signal	No signal	N/A
CGRRFNQALKQQpYVTPTPSPRH	7p-3F1	4.5	Variable signal	Low/variable signal	Poor staining
	7p-7E11	5	Signal ok	No signal	Poor staining
	7p-14D6	4	Signal ok	No signal	OK, specific
	7p-2G12	5	Variable signal	No signal	Poor, high background
CGEPSENpYVNMKPPRKNIP	8p-7G1	3	Variable signal	No signal	No staining
CGSNPSpYQPLSTVNEKEQRRpY	9p-9H5	4	Weak to OK signal	No signal	Nonspecific PM staining
Mix of 10p1 and 10p2 (below)	10p-17A6	1.5	No signal	No signal	N/A
10p1: CGRTGSGTATpYSYDRQMDT					
10p2: CGRTGSGTATYSpYDRQMDT					
CGTDpYLALMGSPDELAPAAPRpYVNG	12p-9B10	8.9	Weak signal	No signal	No staining
	12p-8E10	5.9	Weak + background	Weak signal	N/A
	12p-15B8	7.9	Some signal?	No signal	No staining
	12p-8B8	6.2	Signal?	Weak signal	N/A
	12p-15G8	3.9	Weak signal	No signal	N/A
	12p-5F1	3.8	Weak signal	No signal	No spec. signal
	12p-15E8	6.7	Strong + background	Weak + background	Nonspecific phospho-Y

Overview of phospho-PVR peptides (column 1) used to immunize mice and make monoclonal antibodies clones that could serve to detect pPVR (autophosphorylated, active PVR). Sequences are derived from the predicted C-terminal region of PVR (Flybase). Only clones that were found to be positive in the ELISA (specific phosphopeptide signal larger than corresponding peptide signal, column 3) are shown. Columns 4 and 5 list results of immunofluorescence tests on COS cells transfected to overexpress PVR (and thus have some active PVR) or kinase-dead PVR as shown in the example in Fig. 1*B*. This ensures that the site is a real PVR autophosphorylation site and that its phosphorylation can be detected by the mAB. In some cases, tyrosine-to-phenylalanine mutants of PVR (16) were also tested in COS cells. Positives from here were further tested by staining of ovaries with or without increased PVR expression (positive specificity control) or increased EGFR expression (negative control). Ovary staining protocols were optimized for individual positive clones. The clone used throughout this study (7p-14D6) is highlighted in yellow. Nonspecific phosY means staining at ring canals and nurse cell plasma membrane.