

Supplemental material

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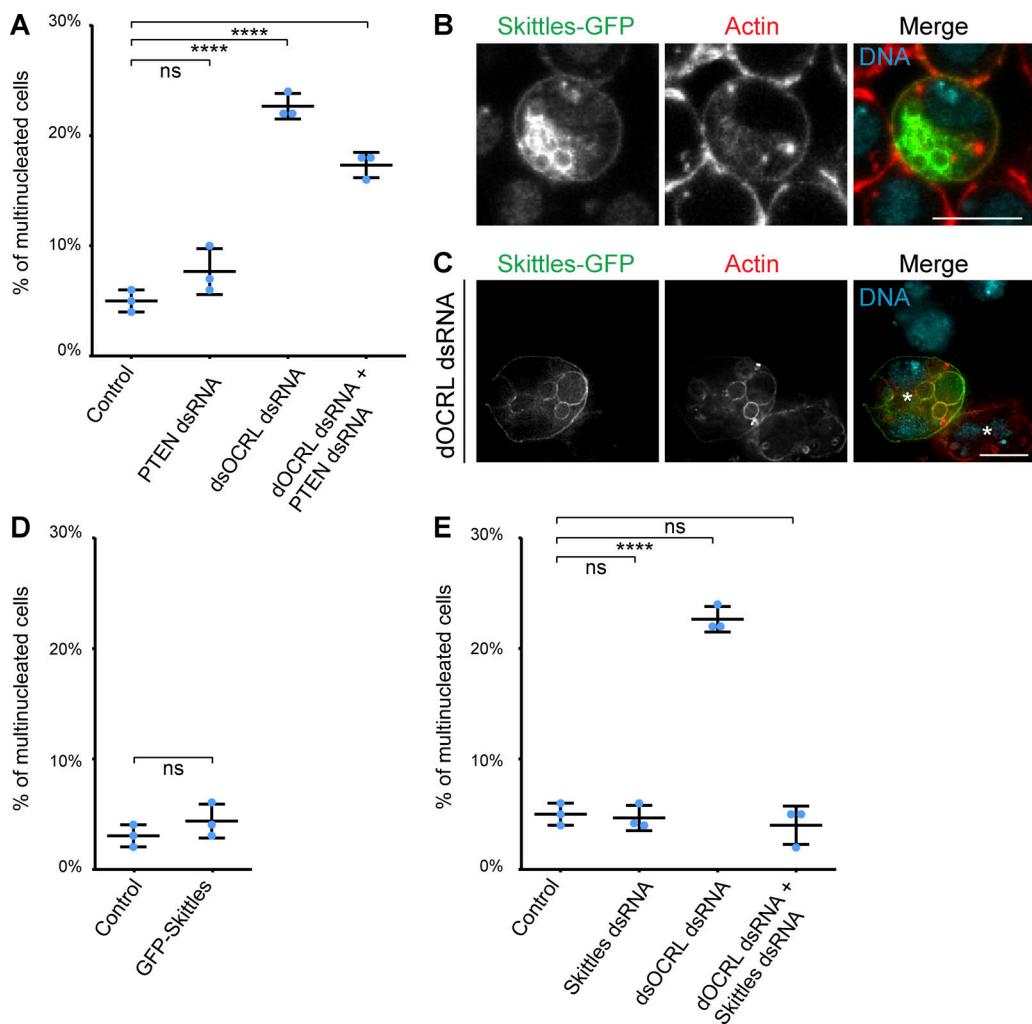


Figure S1. A balance of Skittles and dOCRL activities regulates PtdIns(4,5)P₂ on endomembranes and cytokinesis outcome. **(A, D, and E)** Percentage of multinucleated S2 cells quantified following the indicated treatments; blue dots show individual independent experiment with ≥ 300 cells/experiment (bars represent mean and SD). P values were calculated using one-way ANOVA, Tukey's multiple comparisons test with a single pooled variance. ns, not significant. **(B)** S2 cells were transiently transfected with Skittles-GFP, fixed, and labeled for F-actin (red) and DNA (blue). Note that Skittles expression triggers endosome enlargement. **(C)** dOCRL-depleted S2 cells were transiently transfected with Skittles-GFP, fixed, and labeled for F-actin (red) and DNA (blue). Bars, 10 μm. ****, P < 0.0001.

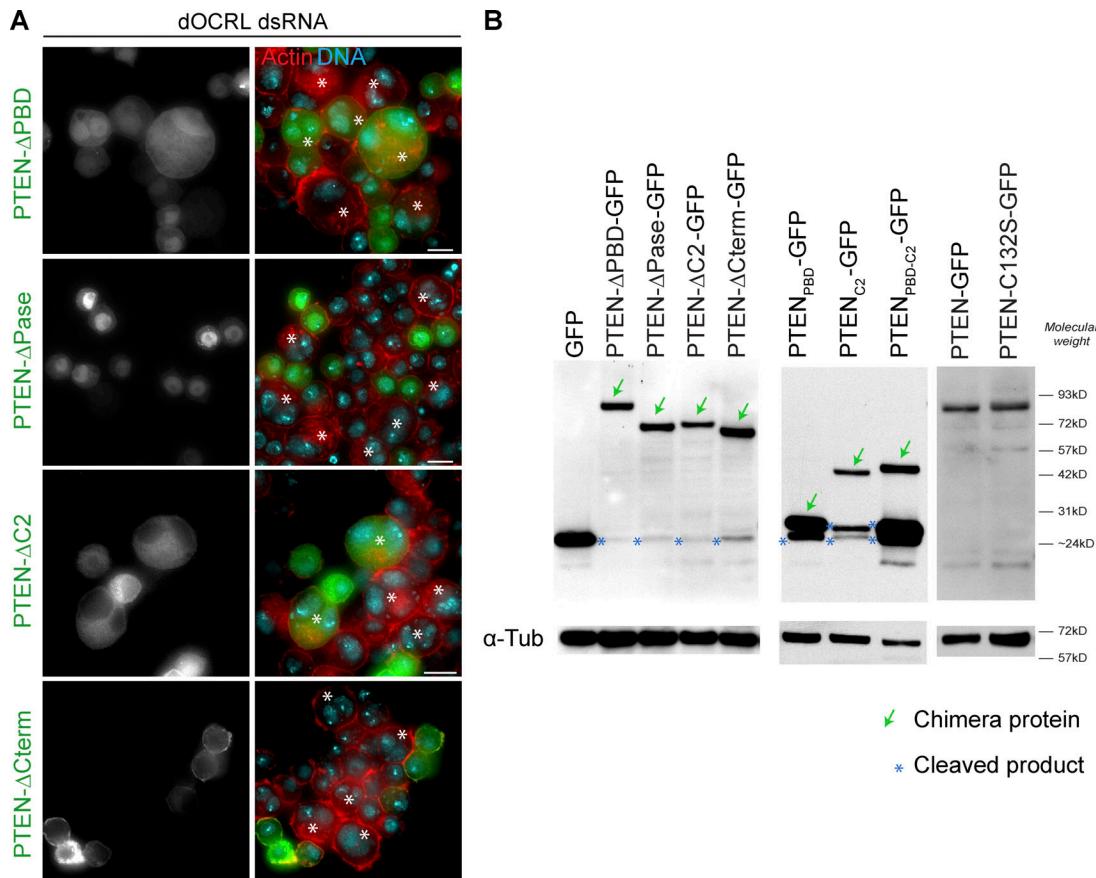


Figure S2. The PTEN_{PBD-C2} are necessary to rescue dOCRL depletion. **(A)** dOCRL dsRNA-treated cells were transfected by the indicated cDNA after 4 d of dsRNA treatment and fixed 2 d after. Cells were labeled for F-actin (red) and DNA (blue). Asterisks show multinucleated cells. **(B)** Western blot on cell extract transiently transfected by the PTEN constructs used in Fig. 1 and Fig. 2. The chimera were revealed using an α -GFP antibody. Note that some chimeras are cleaved, and the Western blot revealed cleaved GFP. Bars, 10 μ m.

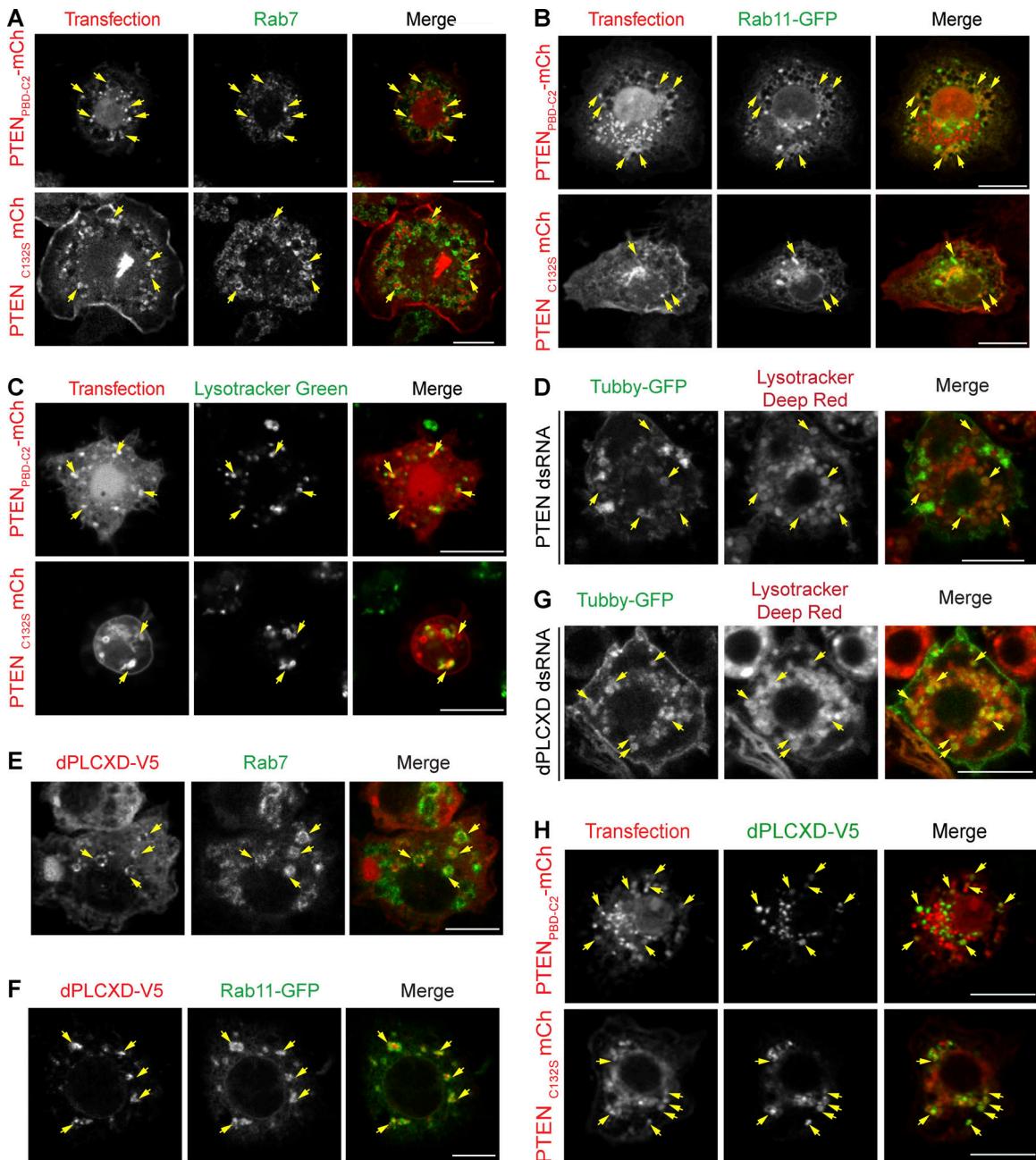


Figure S3. PTEN, dPLCXD, and the PtdIns(4,5)P₂ biosensor Tubby-GFP colocalize with endosome markers. **(A)** S2 cells expressing PTEN_{PBD-C2}mCh or PTEN_{C132S}mCh (red) were immunostained for Rab7 (green). Arrows show colocalization of the indicated proteins on endosomes. ns, not significant. **(B)** S2 cells coexpressing PTEN_{PBD-C2}mCh or PTEN_{C132S}mCh (red) and GFP-Rab11 (green). Arrows show colocalization of the indicated proteins on endosomes. ns, not significant. **(C)** S2 cells expressing PTEN_{PBD-C2}mCh or PTEN_{C132S}mCh (red) were incubated with LysoTracker Green (green). Arrows show acidic vesicles where PTEN constructs and LysoTracker colocalize. ns, not significant. **(D)** S2 cells stably expressing low levels of the PtdIns(4,5)P₂ biosensor Tubby-GFP (green), were treated with PTEN dsRNA and were incubated with LysoTracker Deep Red (red). Arrows show acidic vesicles positive for the PtdIns(4,5)P₂ biosensor Tubby-GFP. **(E)** S2 cells expressing dPLCXD-V5 were immunostained for V5 (red) and Rab7 (green). Arrows show colocalization of the indicated proteins on endosomes. **(F)** S2 cells coexpressing dPLCXD-V5 and GFP-Rab11 (green) were immunostained for V5 (red). Arrows show colocalization of the indicated proteins on endosomes. **(G)** S2 cells stably expressing low levels of the PtdIns(4,5)P₂ biosensor Tubby-GFP (green), were treated with dPLCXD dsRNA and were incubated with LysoTracker Deep Red (red). Arrows show acidic vesicles positive for the PtdIns(4,5)P₂ biosensor Tubby-GFP. **(H)** S2 cells coexpressing PTEN_{PBD-C2}mCh or PTEN_{C132S}mCh (red) and dPLCXD-V5 were immunostained for V5 (green). Arrows show vesicles where both indicated proteins localize. Bars, 10 μ m.

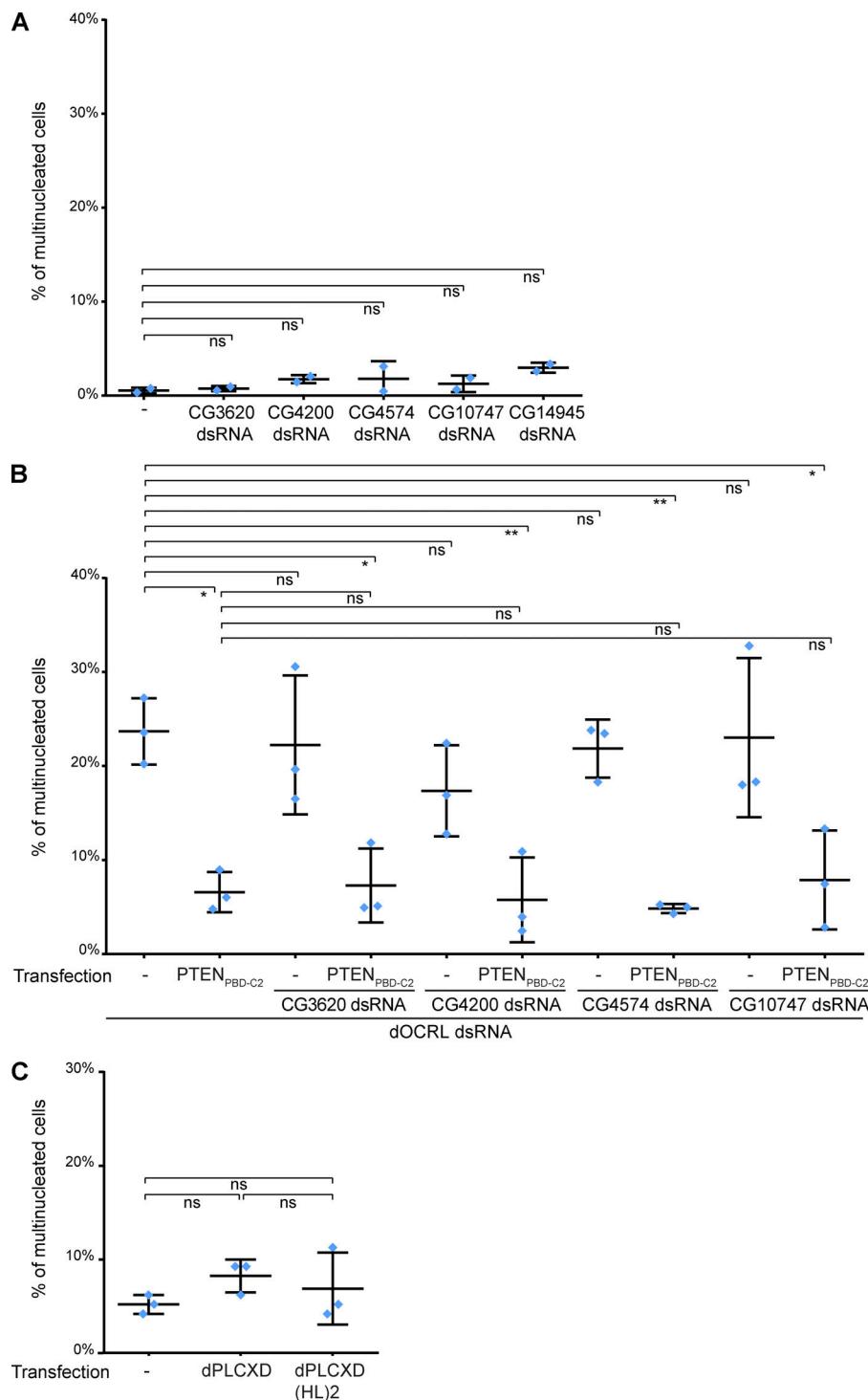


Figure S4. Depletion of PLCs other than dPLCXD does not affect PTEN rescue of dOCRL depletion. **(A)** Percentage of multinucleated S2 cells quantified following the indicated treatments; blue diamonds show the percentage of individual independent experiments with $n > 300$ cells (bars represent mean and SD). P values were calculated using unpaired and parametric ordinary one-way ANOVA, Tukey's multiple comparisons test with a single pooled variance. **(B)** Percentage of multinucleated cells quantified following the indicated treatments; blue diamonds show the percentage of individual independent experiments, $n > 300$ cells (bars represent mean and SD). P values were calculated using unpaired and parametric ordinary one-way ANOVA, Tukey's multiple comparisons test with a single pooled variance. ns, not significant. **(C)** Percentage of multinucleated S2 cells quantified following the indicated treatments; blue diamonds show the percentage of individual independent experiments with $n > 300$ cells (bars represent mean and SD). P values were calculated using unpaired and parametric ordinary one-way ANOVA, Tukey's multiple comparisons test with a single pooled variance. *, $P < 0.05$; **, $P < 0.01$.

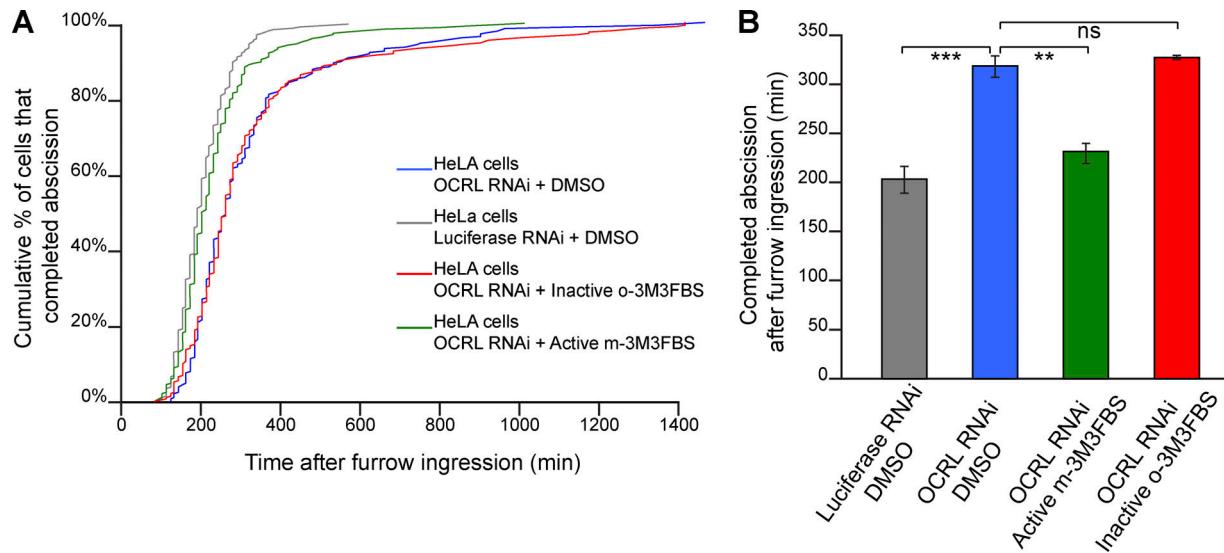


Figure S5. Chemical activation of PLC rescues delayed abscission in HeLa cells treated with OCRL RNAi. (A) DMSO (Vehicle), the PLC activator m-3M3FBS, or its inactive analogue o-3M3FBS was added to HeLa cells treated with the indicated RNAi. Cell divisions were recorded by time-lapse microscopy. The curves represent the distribution of the abscission times in the indicated cell populations. **(B)** Mean abscission times were measured on time-lapse videos quantified in A. P values were calculated using paired and parametric Student's *t* test, $n = 3$. **, $P < 0.01$; ***, $P < 0.001$.