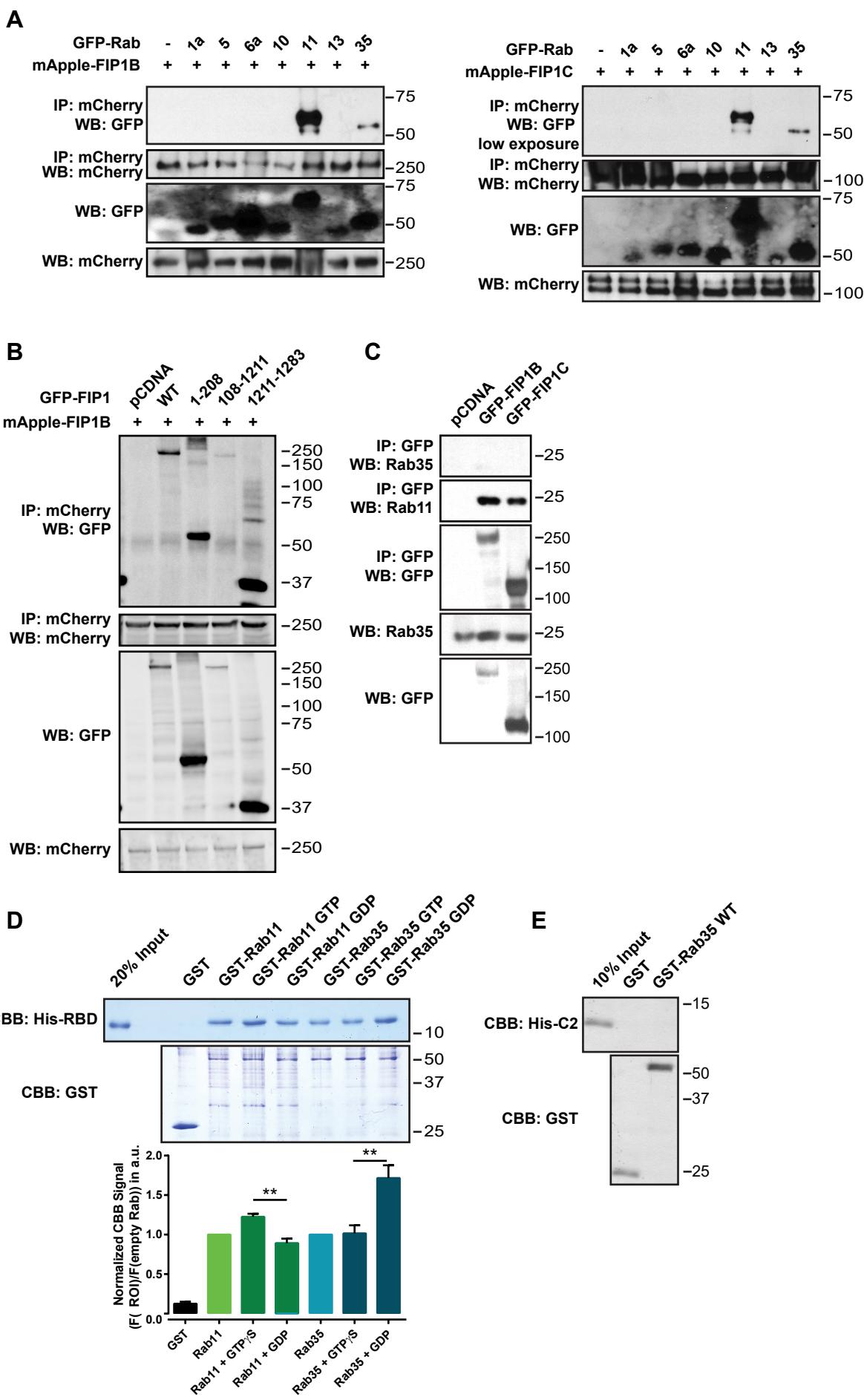


**Fig. S1. Supplementary information pertaining to figure 1**

A) Quantifications of average cellular binucleation. siNT=504, siFIP1+siFIP2 n=90, siFIP1+siFIP5 n=15, siFIP2+siFIP5 n=27. B) Quantification of cellular binucleation following depletion of FIP1 in indicated cell lines. siNT in U2OS, 293T, HCT116, A375, A549 and MCF7 respectively n=504, 230, 112, 175, 367, 378 and 100. siFIP1 in U2OS, 293T, HCT116, A375, A549 and MCF7 respectively n=766, 375, 137, 265, 447, 327, 200. C) (Left) Representative images of cells treated with either siNT or siFIP1 wherein DNA is stained in cyan,  $\beta$ -Tubulin in magenta, and either (upper two panels) MKLP1 in green or (lower two panels) RhoA-binding-domain-of-anillin in green. Inverted black and white images of the green and magenta channels of MKLP1/RhoA-binding-domain-of-anillin and  $\beta$ -Tubulin are shown in the lower right corner zooms. Arrows indicate either the accumulation of MKLP1 at the midbody or the localization of RhoA at the midbody. (right) Representative Western blot showing FIP1 knockdown and GFP expression of the GFP-RhoA-binding-domain-of-anillin in both conditions. FIP1 antibody has non-specific bands and a single asterisk denotes FIP1B, and a double asterisk denotes FIP1C. D) Dot plot of every value for cytokinesis timing of indicated conditions corresponding to figure 1E and figure 1H. siNT n=70, siFIP1 n=133, siFIP2 n=70, siFIP1 + GFP-FIP1A n=18, siFIP1 + GFP-FIP1B n=55, siFIP1 + GFP-FIP1C n=62. Bars correspond to average value and upper and lower bars correspond to inter-quartile range. E) Representative images of cells treated with either siNT or siFIP1 wherein DNA is stained in cyan,  $\beta$ -Tubulin in magenta, and either (left two panels) FIP3 in green or (right two panels) CHMP4B in green. Inverted black and white images of the green and magenta channels of FIP3/CHMP4B and  $\beta$ -Tubulin are shown in the lower right corner zooms. (lower) Quantification of either FIP3 or CHMP4B average fluorescence intensity signal enrichment in a ROI around the ICB, measured as the region of the  $\beta$ -Tubulin staining and surrounding area during FIP1 depletion in either early bridges or abscission sites. For FIP3 siNT n=14, siFIP1 n=14. For CHMP4B siNT n=23, siFIP1 n=21. \*P<0.05; \*\*P<0.01; \*\*\*P< 0.001; \*\*\*\*P<0.0001; n.s., not significant, n.a., not measurable, Wilcoxon signed-rank test for S1D, unpaired two-tailed Student t-test for the rest.

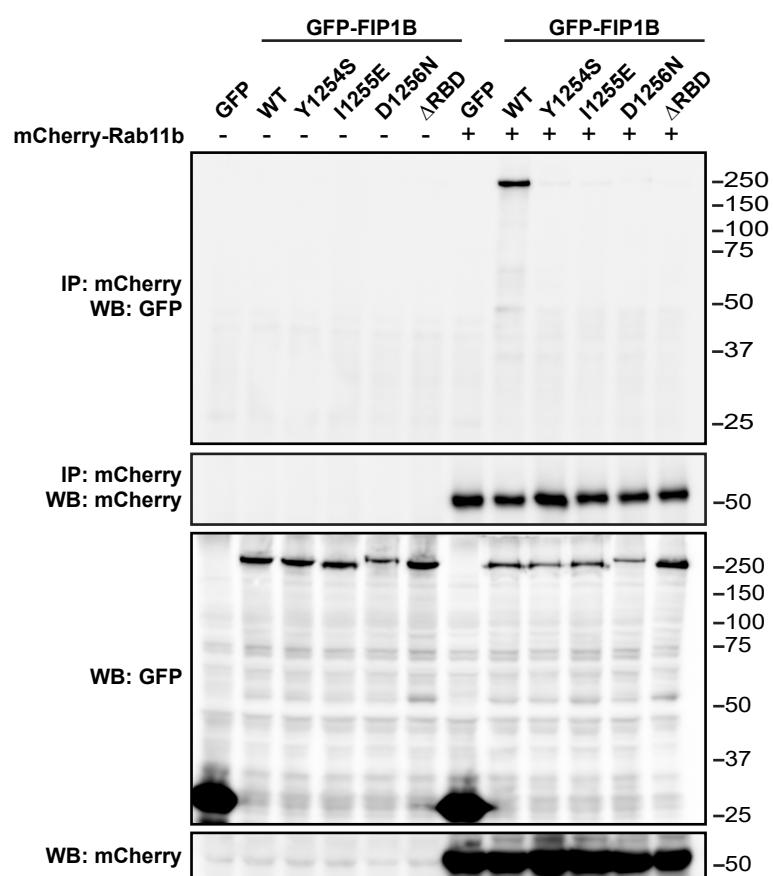


**Fig. S2. Supplementary information pertaining to figure 3**

A) (left) Immunoprecipitation of GFP-Rab1a, GFP-Rab5, GFP-Rab6a, GFP-Rab10, GFP-Rab11, GFP-Rab13 and GFP-Rab35 by mApple-FIP1B (left) and mApple-FIP1C (right). B) Immunoprecipitation of FIP1 constructs from figure 3C baited to full-length mApple-FIP1B showing dimerization capacity for indicated domains. C) Immunoprecipitation of endogenous Rab35 or Rab11 by GFP-FIP1B or GFP-FIP1C. D) (upper) GST-pulldown of recombinant His-RBD by recombinant GST, GST-Rab11 or GST-Rab35 either loaded or not with GTP or GDP. (lower) Quantification of 4 independent GST-Rab pulldowns of His-RBD. E) GST-pulldown of recombinant His-C2 by recombinant GST or GST-Rab35 and revealed by CBB staining.

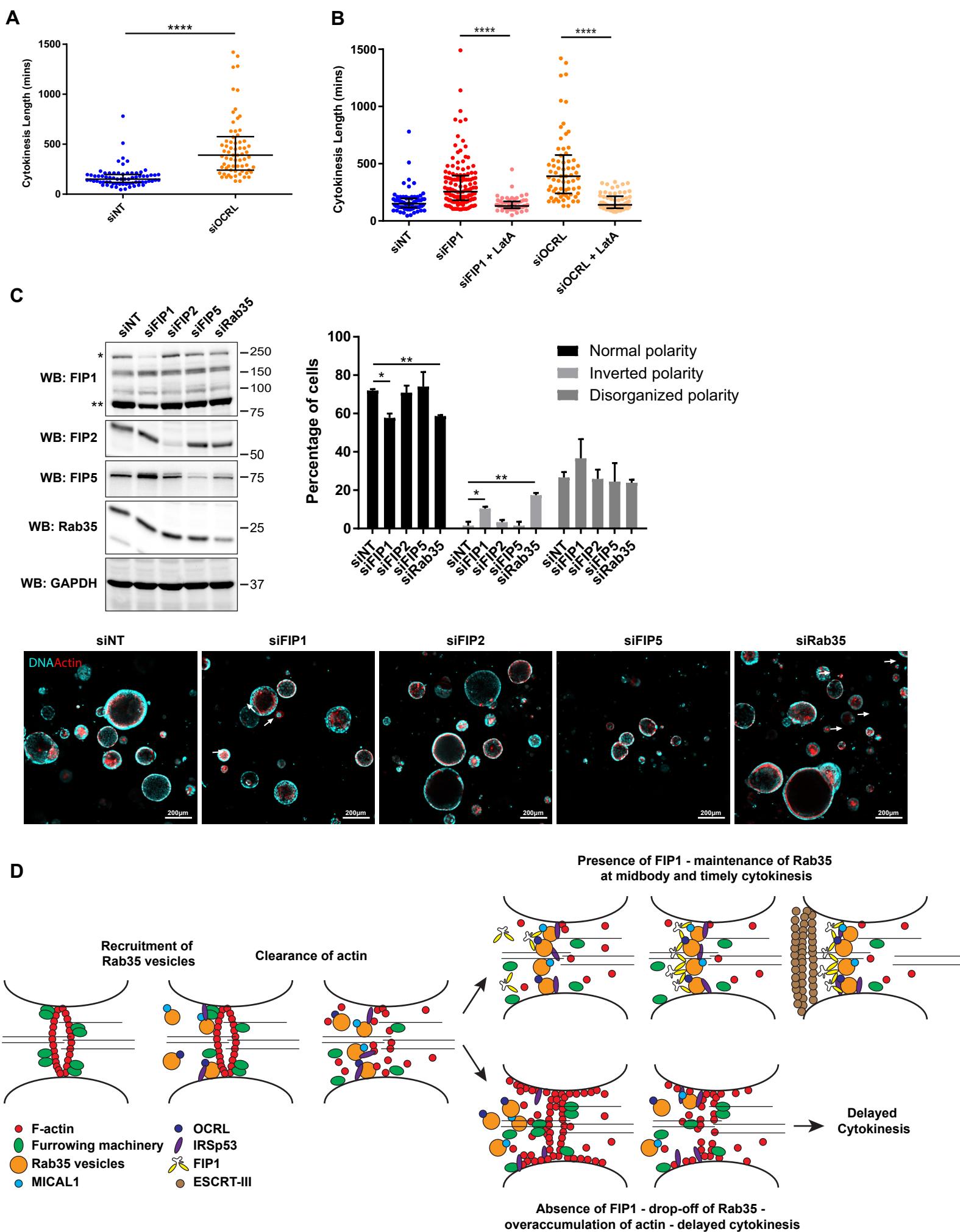
\*\*P<0.01; Paired two-tailed Student t-test.

A



**Fig. S3. Supplementary information pertaining to figure 4**

A) Immunoprecipitation of GFP-FIP1 RBD mutants by mCherry-Rab11b.



**Fig. S4. Supplementary information pertaining to figure 6**

A) Dot plot of every value for cytokinesis timing of indicated conditions corresponding to figure 6B. siNT n=70, siOCRL n=70. Bars correspond to average value and upper and lower bars correspond to inter-quartile range. B) Dot plot of every value for cytokinesis timing of indicated conditions corresponding to figure 6E. siNT n=70, siFIP1 n=133, siFIP1 + LatA n=60, siOCRL n=70, siOCRL + LatA n=61. Bars correspond to average value and upper and lower bars correspond to inter-quartile range. C) (upper left) Western blot showing protein depletions after treatment with siNT, siFIP1, siFIP2, siFIP5 or siRab35. FIP1 antibody has non-specific bands and a single asterisk denotes FIP1B, and a double asterisk denotes FIP1C. (upper right) Quantification of different polarities in each condition, either normal, inverted or disorganized polarity. siNT total n=116, siFIP1 total n=92, siFIP2 total n=126, siFIP5 total n=86, siRab35 total n=148. (lower) Representative images of single Z-planes of Caco-2 cysts depleted for indicated proteins. DNA is shown in cyan and Actin is stained in red. Arrows point to cells showing either complete or partial inverted polarity represented as Actin on the basal side of the cysts. D) Schematic representation of FIP1 function in cytokinesis. \*P<0.05; \*\*P< 0.01; \*\*\*\*P<0.0001; Wilcoxon signed-rank test for S4A and S4B, unpaired two-tailed Student t-test for S4C.