Werner et al., http://www.jcb.org/cgi/content/full/jcb.201312045/DC1

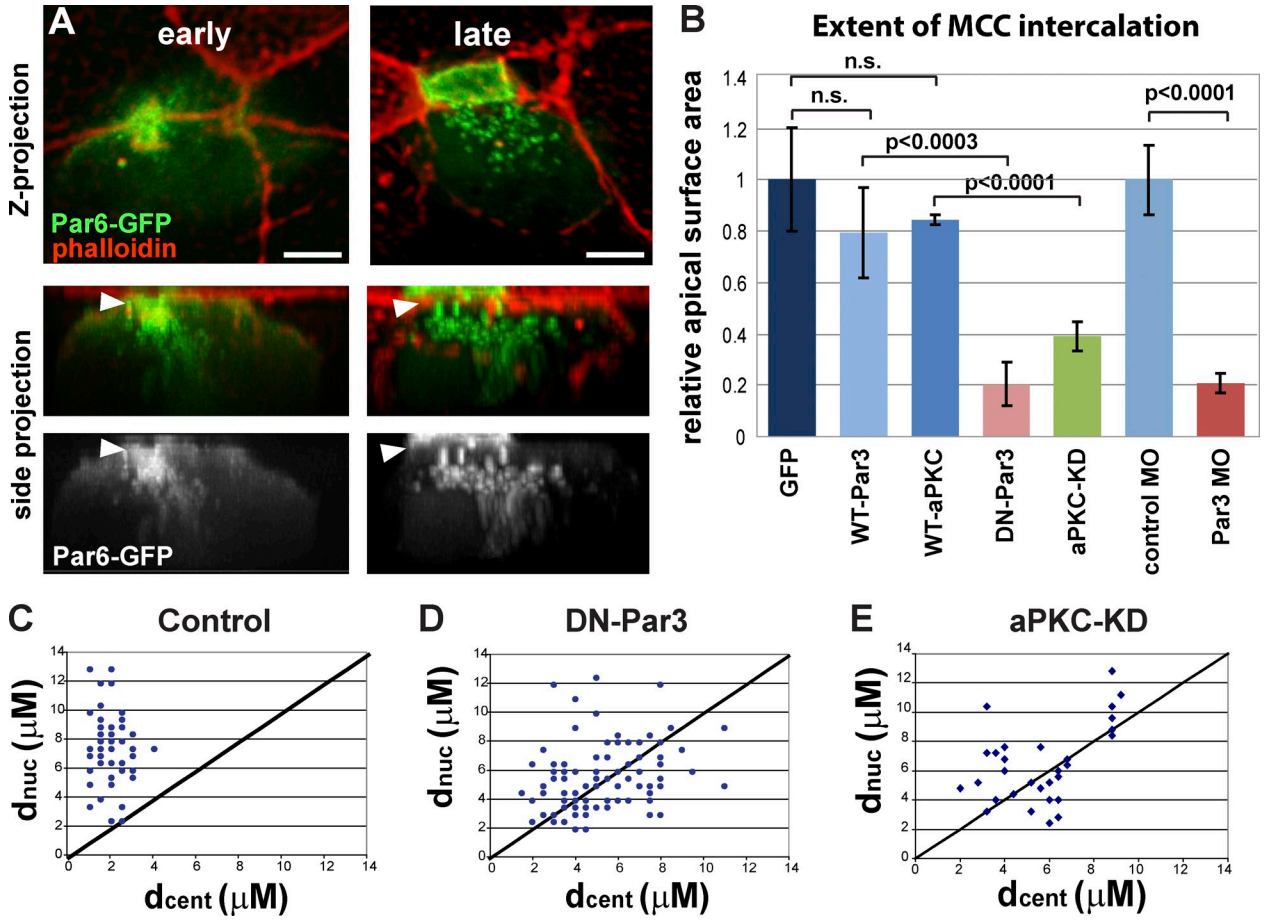


Figure S1. **The Par complex mediates apical positioning of centrioles and MCC intercalation.** (A) Apical localization (arrowheads) of Par6-GFP prior to intercalation (early) and during intercalation (late). (B) Quantification of apical surface area in MCCs from embryos injected with α-tub promoter-driven GFP (n = 523), Par3-GFP (n = 179), aPKC-GFP (n = 111), DN-Par3 (n = 1094), aPKC-KD (n = 533), control MO (n = 191), or Par-3 MO (n = 208; see Fig. 1 I). Statistical significance was determined using a t test. Error bars represent standard deviations. (C–E) Scatter plot showing the distance of the center of the centriole cluster (d_{cent}) and the distance of the center of the nucleus (d_{nuc}) from the apical surface in individual MCCs from embryos injected with α-tub promoter-driven GFP (C), DN-Par3 (D), and aPKC-KD (E; see Fig. 1, D–F). Scatter plots in C–E are based on at least 10 cells, each from a total of at least five embryos from at least two independent experiments. Bars, 5 µm.

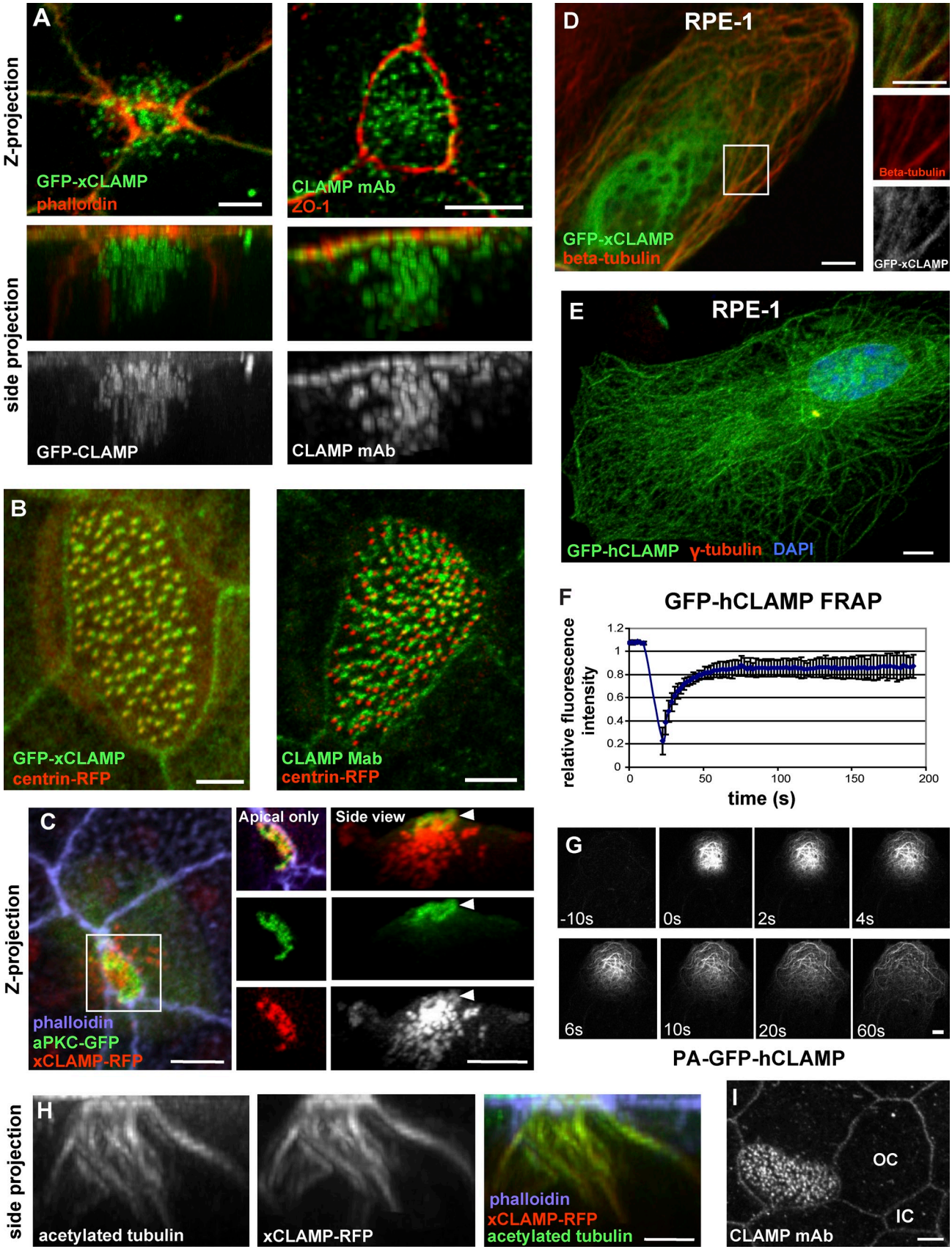


Figure S2. **CLAMP is enriched at centrioles and rootlets and dynamically associates with microtubules.** (A and B) Comparison of the localization of GFP-CLAMP (left) and CLAMP mAb staining (right) in MCCs during (A) and after completion (B) of intercalation. (C) Partial colocalization of xCLAMP-GFP and aPKC-GFP at the leading edge of intercalating MCCs (arrowheads), maximum intensity projection (left), apical optical section only (middle), and corresponding cross sections (right) are shown. (D) Colocalization of xCLAMP-GFP with MTs in RPE-1 cells stained with β-tubulin antibody. The boxed region is enlarged on the right. (E) Localization of hCLAMP-GFP in RPE-1 cells stained with γ-tubulin and DAPI. (F) Quantification of FRAP of hCLAMP-GFP in RPE-1 cells (see Video 1). The mean fluorescence intensity of three independent measurements is shown. Error bars represent the standard deviation. (G) Still frames of live imaging of photoactivatable hCLAMP-GFP (see Video 2). Time stamps are relative to photoactivation (0 s). (H) Colocalization of CLAMP-RFP with anti-acetylated tubulin staining in an intercalating IC. (I) Anti-CLAMP mAb staining highlighting CLAMP staining in OCs (OC) and ICs (IC). Cells were quantified from at least five embryos total, from at least two independent experiments. Bars, 5 µm.

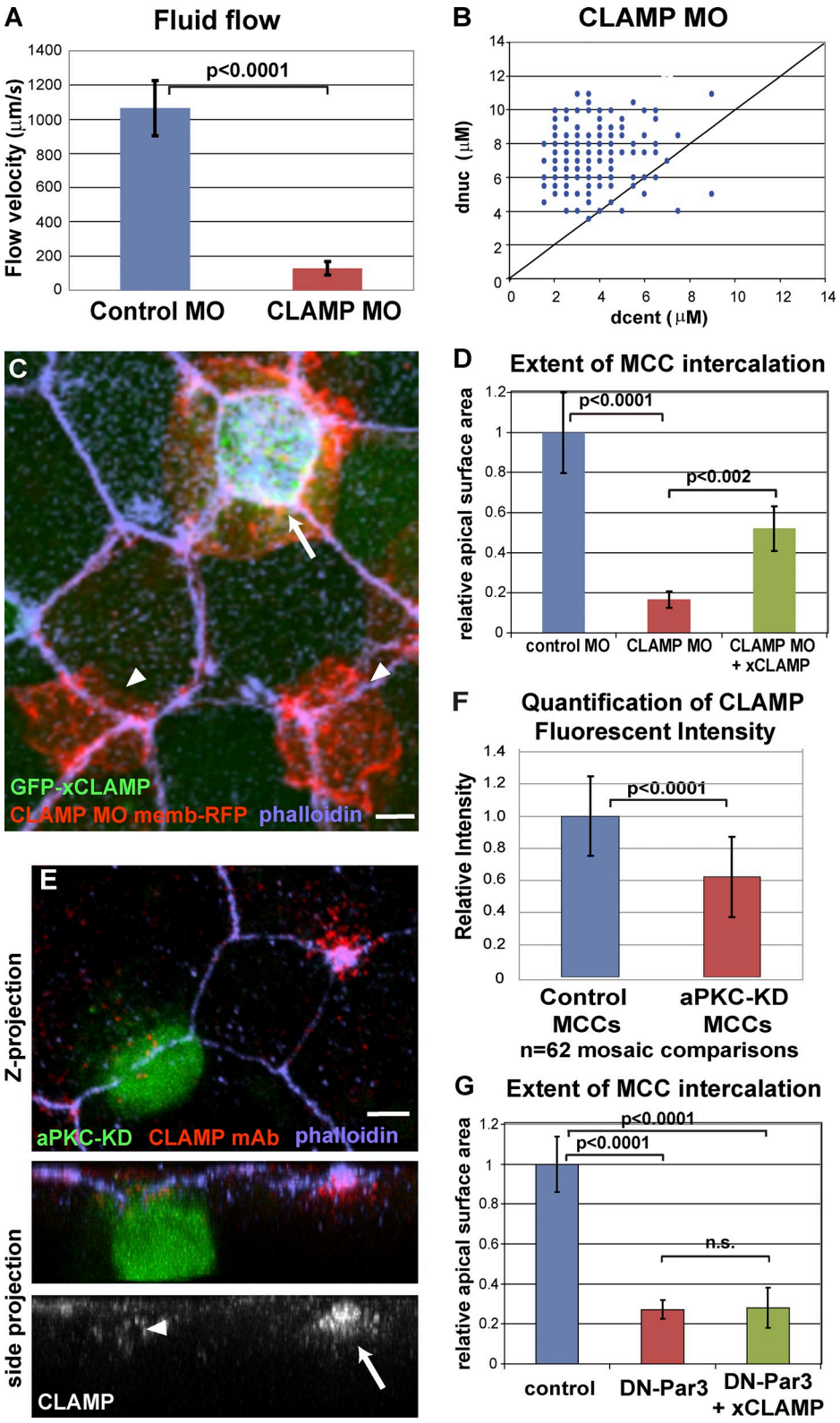
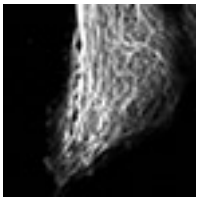


Figure S3. **CLAMP is required for MCC intercalation but not centriole positioning.** (A) Quantification of cilia-driven fluid flow in embryos injected with control MO (n = 10 embryos) and CLAMP MO (n = 10 embryos). (B) Analysis of centriole cluster versus nuclear position relative to the apical surface in MCCs from embryos injected with CLAMP MO showing distribution of individual cells (see Fig. 4 H). (C) Representative image of a mosaic embryo injected with CLAMP MO and membrane-RFP followed by separate injection of GFP-xCLAMP. Morphant MCCs not expressing GFP-xCLAMP (arrowheads) fail to intercalate whereas intercalation is rescue by expression of GFP-xCLAMP in morphant MCCs (arrow). (D) Quantification of apical surface size in MCCs from embryos injected with a control MO (n = 533), CLAMP MO (n = 429), or CLAMP MO together with GFP-xCLAMP (n = 140; see Fig. 4 D). (E and F) Representative image (E) and quantification (F) of apical CLAMP localization using anti-CLAMP mAb (red) in embryos mosaic for aPKC-KD-GFP (green, arrowheads) and wild-type (arrows). (G) Quantification of apical surface area in control (n = 533), DN-Par3-GFP (n = 564), or DN-Par3-GFP and CLAMP-RFP (n = 546) expressing MCCs. The scatter plot in B is based on at least 10 cells each from a total of five embryos from at least two independent experiments. Statistical significance in A, C, E, and G was determined using a t test. Bars, 5 µm. Error bars represent the standard deviation.

Table S1. **Statistics for the figures calculated either with a *t* test or with a χ^2 analysis as labeled**

Figure	Treatment	P-value (*t* test)	χ^2	P-value (χ^2)	Compared to
Fig.1 F	DN-par3		39.7144551	2.37747×10^{-9}	GFP
	aPKC-KD		35.31657559	2.1434×10^{-8}	GFP
Fig. 1 I	DN-par3		721.5177099	2.1107×10^{-157}	GFP
	aPKC-KD		262.142021	$1.19284332 \times 10^{-57}$	GFP
	DN-par3		348.3906623	2.22807×10^{-76}	Par3 GFP
	aPKC-KD		63.83993312	1.37194×10^{-14}	apKC GFP
	Par3 MO		390.5149424	1.58766×10^{-85}	GFP
Fig. 2 D	Nocodazole		327.5234061	$7.57174555 \times 10^{-72}$	DMSO
Fig. 2 F	Nocodazole		463.9809481	1.7694×10^{-101}	DMSO
Fig. 3 J	CLAMP MO	6.96913×10^{-8}			Control
Fig. 4 D	CLAMP MO		713.858355	$9.71913876 \times 10^{-156}$	Control MO
	CLAMP MO + xCLAMP		134.1267674	7.4945×10^{-30}	CLAMP MO
	CLAMP MO + hCLAMP		74.74213147	5.88779×10^{-17}	CLAMP MO
Fig. 4 F	CLAMP MO		146.1641109	1.82334×10^{-32}	Control MO
Fig. 4 H	CLAMP MO		2.888315647	0.2359447	Control MO
Fig. 4 J	DN-par3	0.008498493			Control
Fig. S1 B	DN-par3	4.59449×10^{-6}			GFP
	aPKC-KD	3.18137×10^{-5}			GFP
	DN-par3	0.000220952			Par3 GFP
	aPKC-KD	4.51732×10^{-5}			apKC GFP
	Par3 MO	1.17197×10^{-5}			control MO
Fig. S3 A	CLAMP MO	1.50306×10^{-6}			Control MO
Fig. S3 D	CLAMP MO	4.29704×10^{-5}			Control MO
	CLAMP MO + xCLAMP	0.001887014			CLAMP MO
Fig. S3 F	aPKC-KD	2.24784×10^{-6}			Control
Fig. S3 G	DN-par3	2.17682×10^{-5}			GFP
	DN-par3 + xCLAMP	1.32251×10^{-5}			GFP
	DN-par3 + xCLAMP	0.886014042			DN-par3



Video 1. **CLAMP dynamically interacts with MTs.** Representative FRAP experiment of an RPE-1 cell transfected with hCLAMP-GFP as quantified in Fig. 4 E. Images were collected using laser-scanning time-lapse confocal microscopy (A1R; Nikon). Frames were collected every 2 s.

Video 2. **CLAMP rapidly diffuses throughout the cytoplasm.** Movie of a photoactivation experiment in an RPE cell transfected with PA-GFP-hCLAMP shown in Fig. 4 F. Images were collected using laser-scanning time-lapse confocal microscopy (A1R; Nikon). Frames were collected every 2 s.