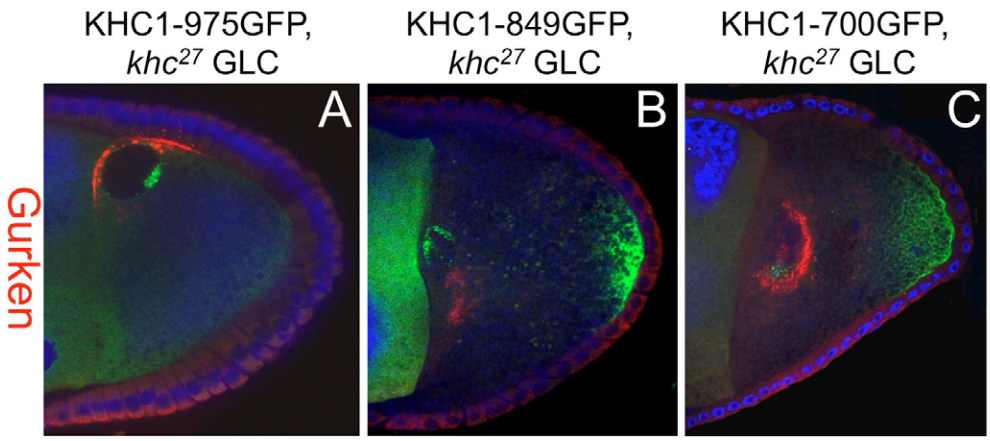
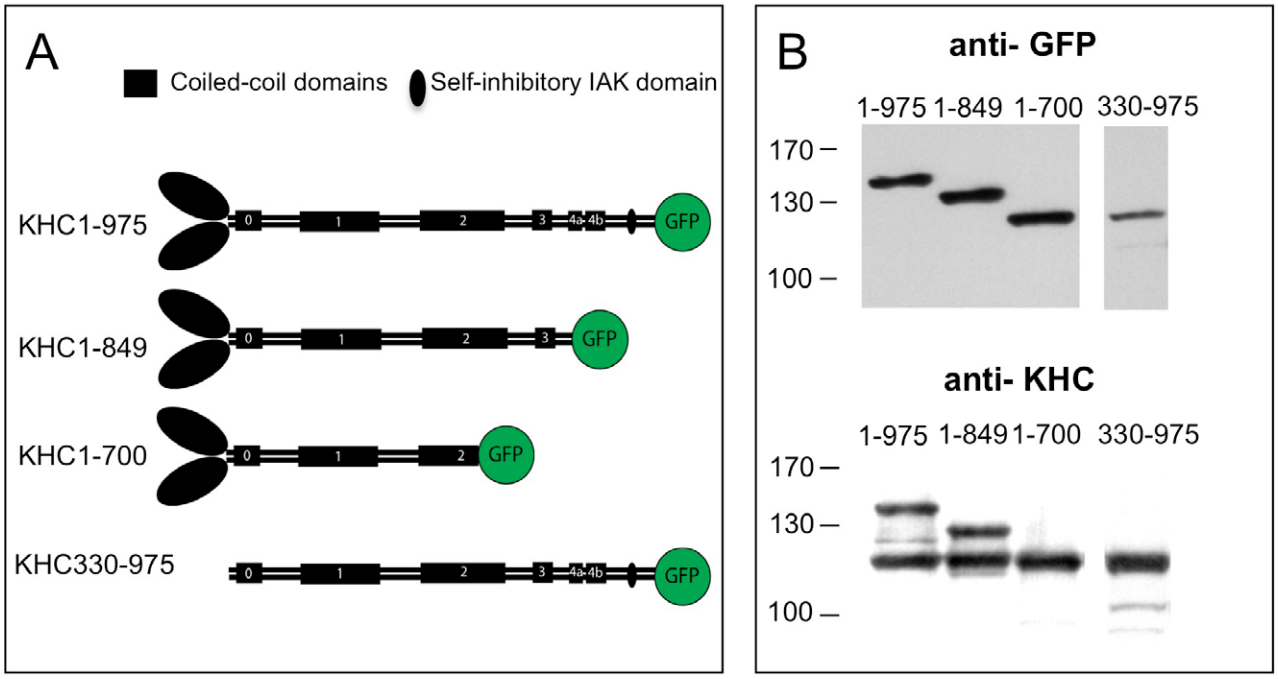


D

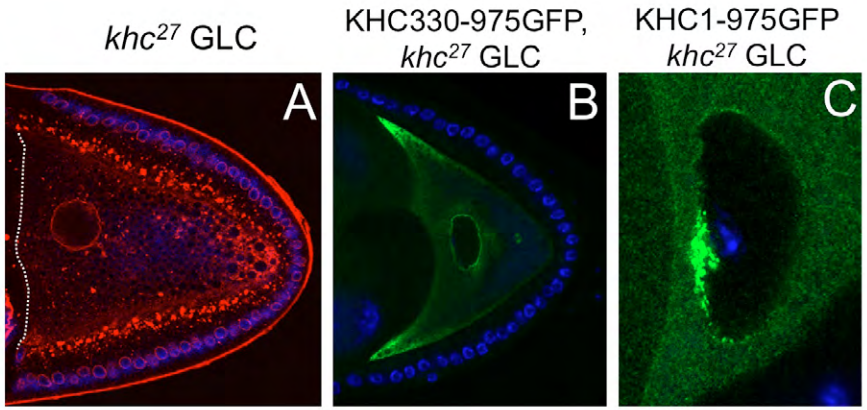
Gurken localization	**Dorsal crescent**
***khc*^{27} GLC; KHC1-975/+** (n=20)	100%
***khc*^{27} GLC; KHC1-849/+** (n=56)	14%*
***khc*^{27} GLC; KHC1-700/+** (n=7)	0%*
***khc*^{27} GLC** (n=27)	4%
***khc*^{27} GLC; KHC1-938/+** (n=20)	45%

Fig. S1. The tail of KHC is important for Gurken localization. (A-C) Gurken in st9 *khc*^{27} mutant oocytes (GLC) containing KHCGFP transgenes. A) KHC1-975 rescues Gurken localization, to a crescent at the anterior-dorsal corner, associated with the nucleus. B,C) KHC1-849 and KHC1-700 are unable to correctly localize Gurken protein to the nucleus (note that the nucleus is in contact with the anterior membrane (well positioned), but that the view axis is rotated 90 degrees). DAPI is in blue. D) Table showing quantification of the Gurken localization phenotype.*The statistical value between KHC1-700 and KHC1-849 is P (one-tailed) <0.0001



C	KHC1-975	KHC1-849	KHC1-700	KHC330-975	KHC330-850
Transgene expression relative to endogenous KHC	71%	66%	83%	30%	115%

Fig. S2. KHC transgenes and their expression levels *in vivo*. (A) Schematic of KHCs, coiled-coils are numbered 0 to 4a/4b, the IAK motif is shown by an oval. B) Western blots showing relative expression levels of the KHC transgenes. Top panel shows a blot against GFP, bottom panel shows a blot against KHC. Relative expression of KHC1-975 and KHC1-849 was calculated directly from anti-KHC blot. All others values were taken relative to KHC1-975GFP. C) Table showing relative expression levels of KHCs.



D Dorsal Appendages		Normal	Fused/ One	None
wildtype	(n=284)	97.5%	1.4%	1.1%
***khc*^{27} GLC; KHC1-975**	(n=79)	96.2%	3.8%	0%
***khc*^{27} GLC**	(n=401)	0.7%	17.0%	82.3%
***khc*^{27} GLC; KHC330-975**	(n=296)	0.7%	10.1%	89.1%

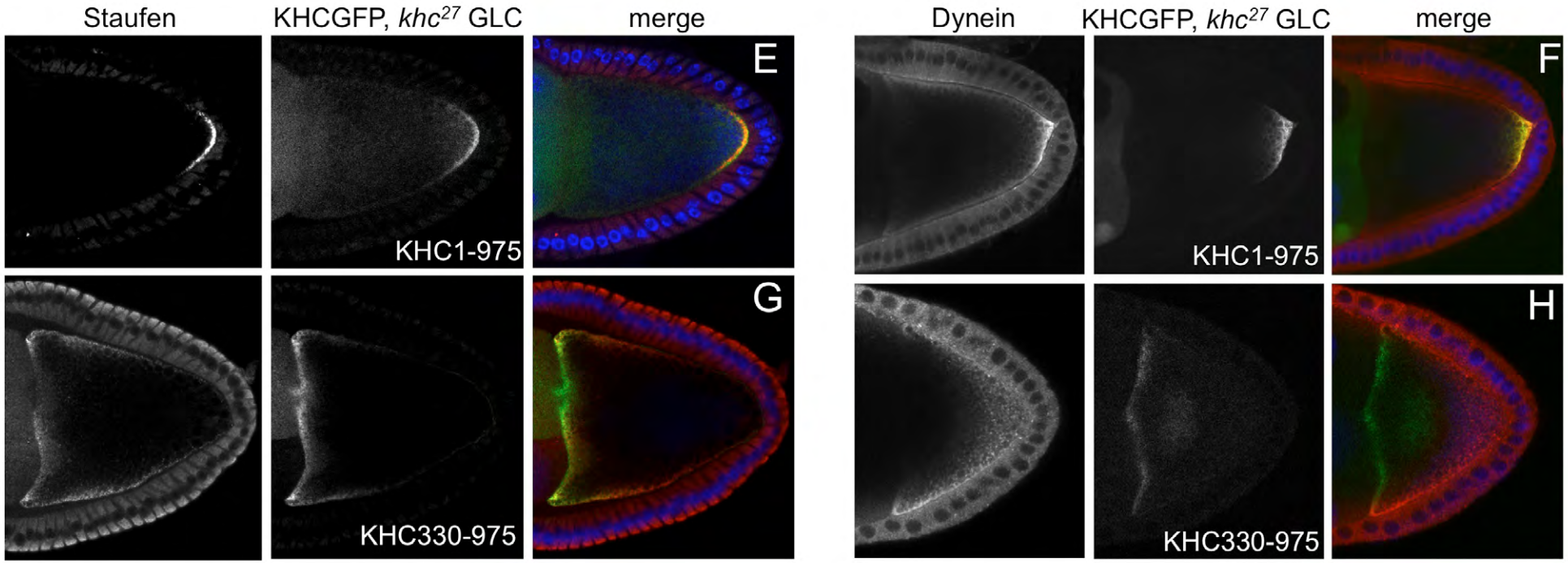
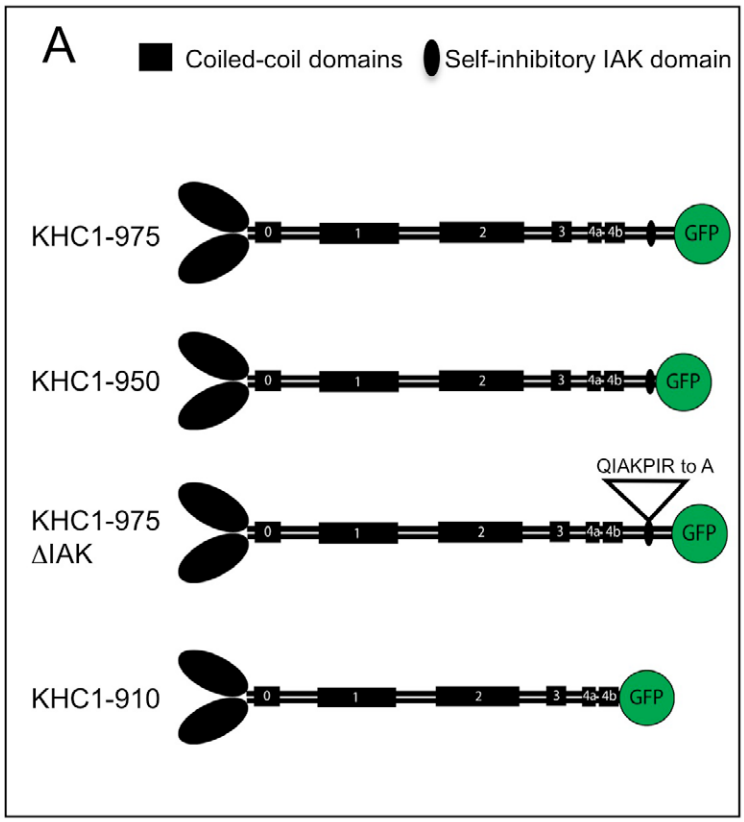
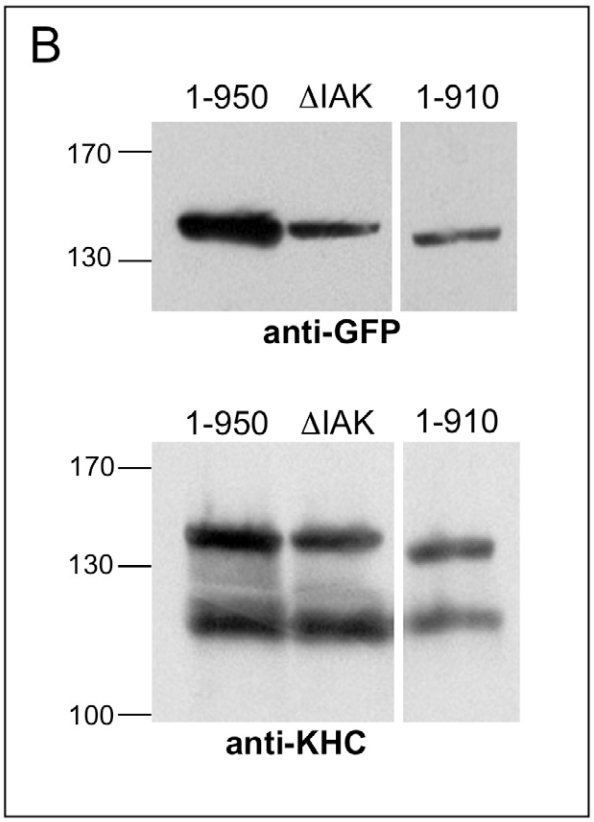


Fig. S3. The motor domain is essential for KHC function in the oocyte. (A,B) Nucleus positioning phenotypes in *khc*^{27} mutants (GLC) without (A, membrane stained with WGA in red) or with KHC330-975GFP (B). C) Image of a KHC1-975GFP oocyte nucleus showing both punctate localization and localization of KHC1-975GFP to the nuclear membrane. D) Table showing the DA phenotypes of eggs laid by mutant females of the indicated phenotypes. The phenotype of KHC330-975 eggs is stronger than that of the *khc* null. E-H) Staufen or Dynein (red in right panels, white in left panels) in st9 *khc*^{27} mutant oocytes (GLC) containing KHCs (green in right panels, white in middle panels). E,F) A full length KHCGFP rescues the posterior localization of Staufen and Dynein. A motorless KHC cannot transport Staufen (G) or Dynein (H) to the posterior. A-C,E-H) DAPI (DNA, blue).





C	KHC1-975	KHC1-950	KHCΔIAK	KHC1-910
Transgene expression relative to endogenous KHC	70%	100%	60%	90%

D	Staufen:	Normal	Dot/Crescent
+ / + ; KHC1-975ΔIAK / +	(n=42)	45%	55%
***khc*^{27} / + ; KHC1-975ΔIAK / +**	(n=45)	24%	76%
***khc*^{27} GLC ; KHC1-975ΔIAK / +**	(n=34)	3%	97%

Fig. S4. KHC tail mutant transgenes and their expression levels *in vivo*. (A) Schematic of KHC transgenes, coiled-coils are numbered 0 to 4a/4b, the IAK motif is shown by an oval. B) Western blots showing relative expression levels of the KHCs. Top panel shows a blot against GFP, bottom panel shows a blot against KHC. C) Table showing relative expression levels of KHCs calculated directly from anti-KHC blot. D) Quantification of the KHC1-975ΔIAK Staufen phenotype in the presence and absence of endogenous KHC.

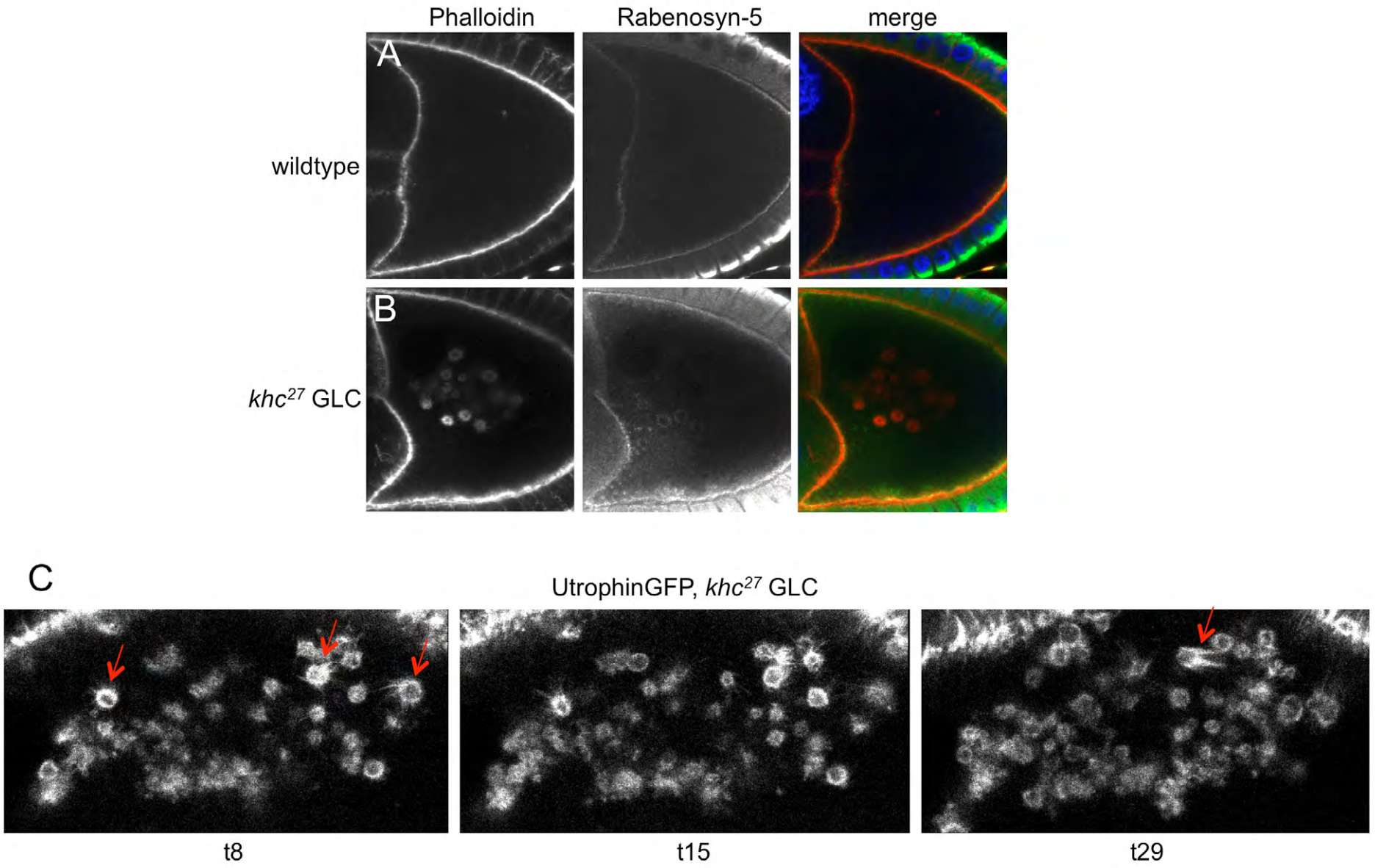


Fig. S5. Defective actin spheres in *khc* null oocytes correlates with the ectopic presence of the Rab5 effector Rabenosyn-5. (A,B) F-actin (Phalloidin, white in left panels, red in merge) and Rabenosyn-5 (white in middle panels, green in merge) in wildtype and khc^{27} st9 oocytes. DAPI (DNA, blue). C)Still frames of a movie of a st9 khc^{27} oocyte expressing Utrophin-GFP (the actin-binding domain of human Utrophin fused to GFP, and expressed under the *spaghetti-squash* promoter (Rauzi et al., 2010)), showing possible nucleation of actin from aberrantly distributed vesicles.

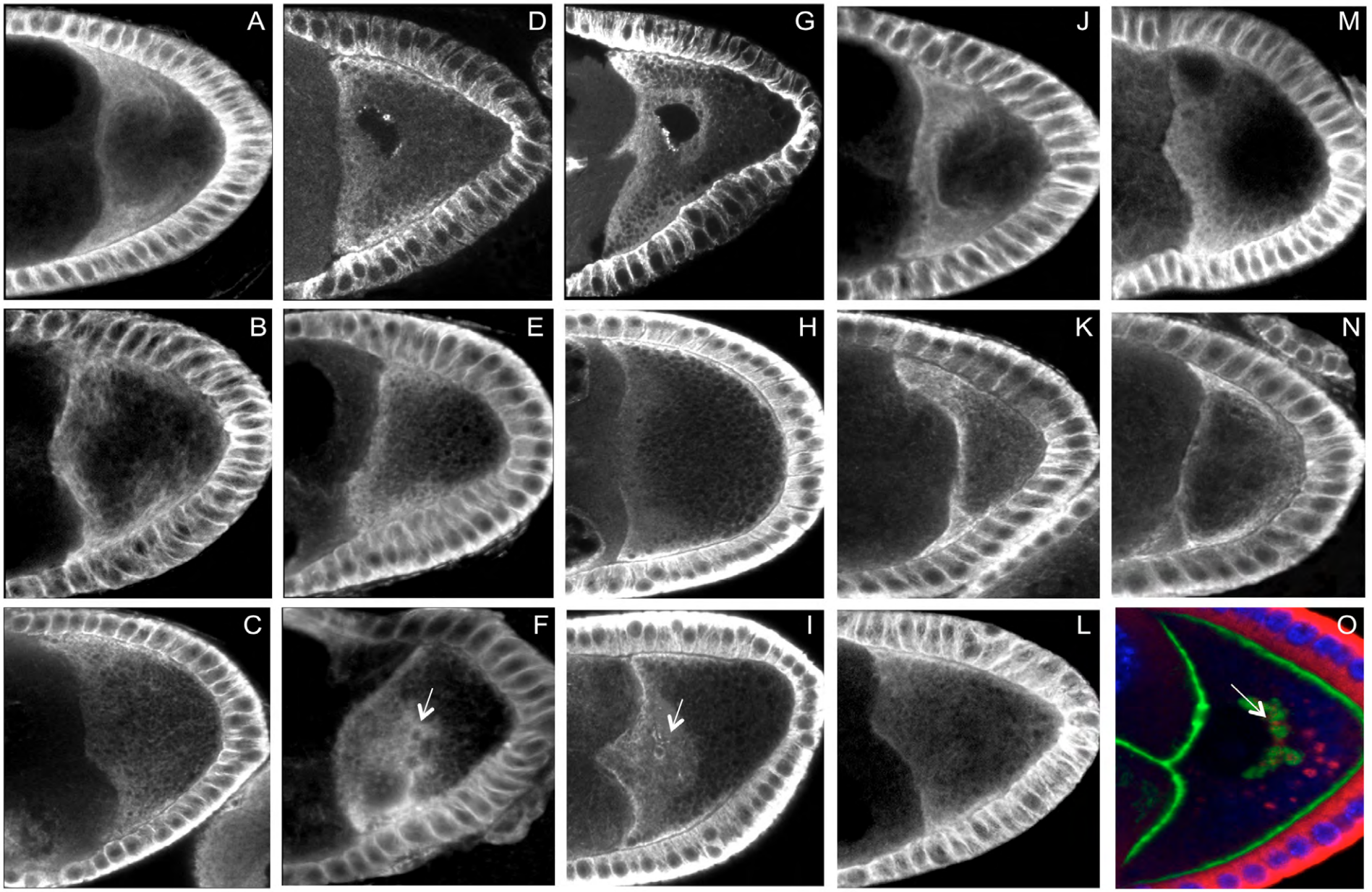
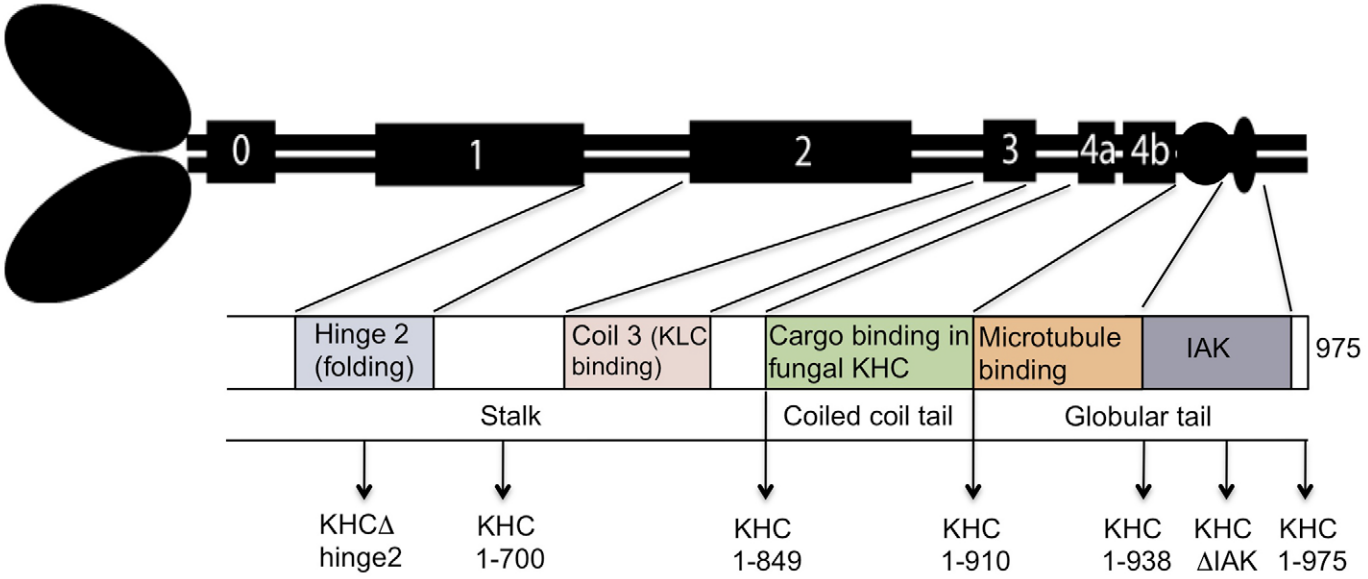


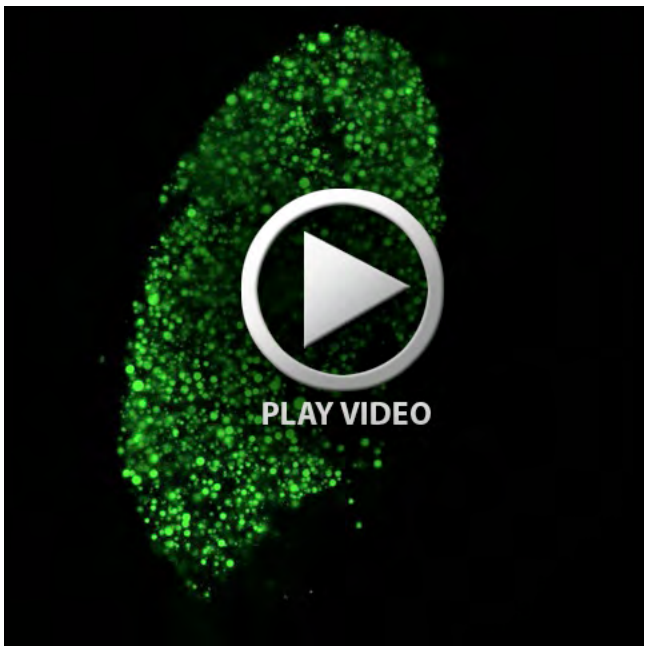
Fig. S6. (A-N) Alpha-tubulin in wildtype oocytes (A), or in *khc*^{27} oocytes that express KHC1-975 (B,C), KHC1-849 (D-F), KHC1-700 (G-I), KHC1-910 (J,K), KHCΔIAK (L), KHC1-938 (M,N). Tubulin stainings were performed using a FITC-conjugated alpha-tubulin antibody (A,B,D,G,J,L,M), or a rat alpha-tubulin antibody and 568nm-secondary antibody (C,E,F,H,I,K,N). The reason for using both antibodies is that even though the FITC-conjugated antibody renders higher quality images, the signal from the GFP-KHCs is not always gone, as it can be seen in D (dots around the nucleus). (O) Gurken protein (red), Phalloidin (F-actin, green) and DAPI (blue) in KHC1-938 oocytes. 45% of these mutant oocytes show aberrant actin-recruiting vesicles (*n*=11)



	KHCΔ hinge2	KHC 1-700	KHC 1-849	KHC 1-910	KHC 1-938	KHC ΔIAK	KHC 1-975
Dynein transport	nd	-	+	+	nd	+	+
Dynein wildtype localization	nd	-	-	-	nd	-	+
Nucleus positioning	nd	-	- (partial)	partial	partial	partial	+
Dorsal appendages	+ (partial)	-	-	-	-	-	+
Actin "vesicles"	+	-	-	nd	-	-	+
oskar transport	+	-	-	-	+	+	+
oskar wildtype localization	-	-	-	-	-	-	+
Stage 9 streaming	nd	-	-	-	nd	+	+

Fig. S7. Summary of the functional effects of the mutations. Dynein and *oskar* transport refers to the capacity of the motor to move the cargo from the anterior to the posterior pole. Dynein and oskar *wildtype* localization refers to the posterior crescent. Nd: not defined.

Movie 1. Cytoplasmic streaming in st11 wild-type oocyte



Movie 2. Cytoplasmic streaming in a st11 KHC1-849 oocyte