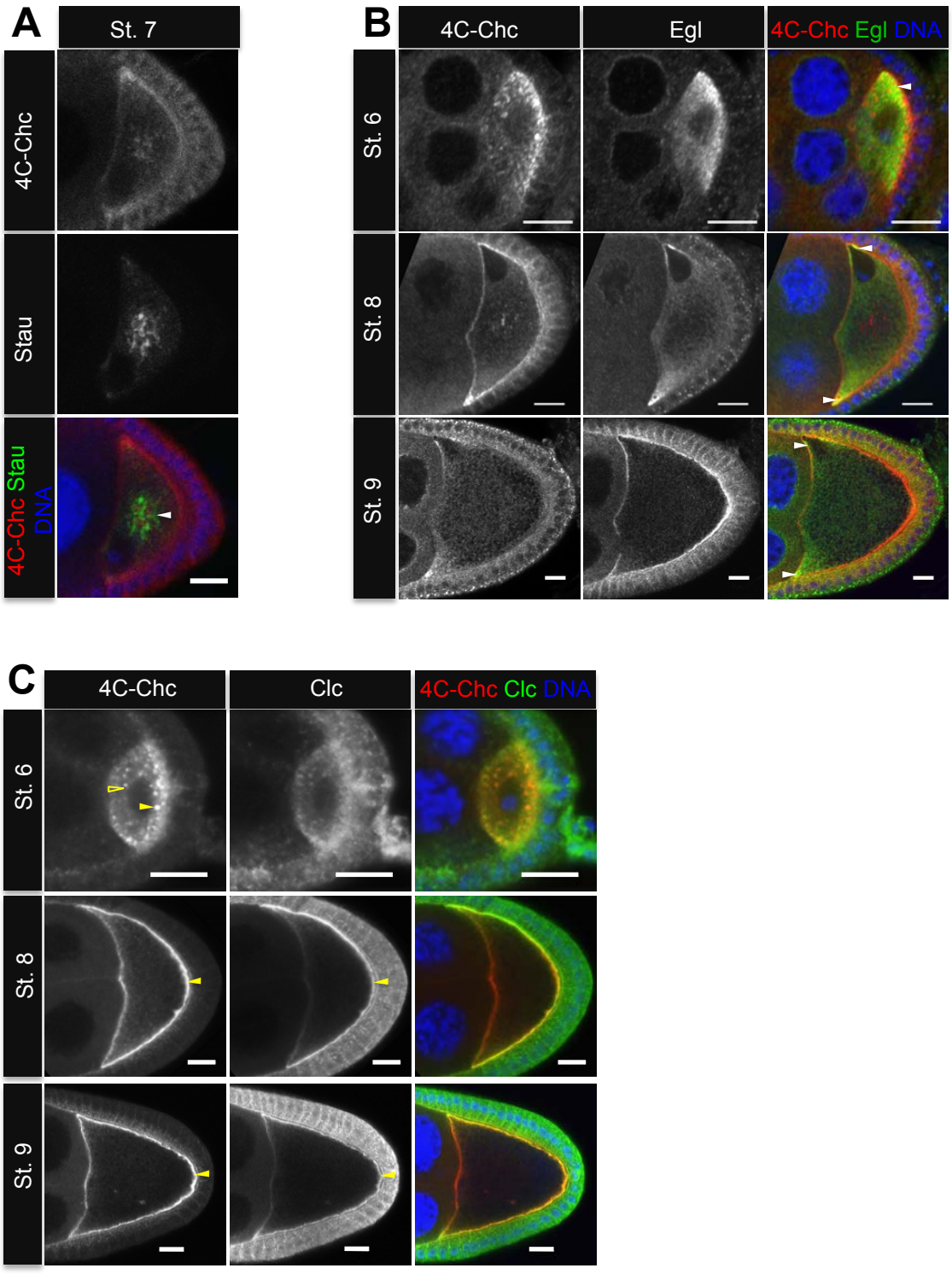
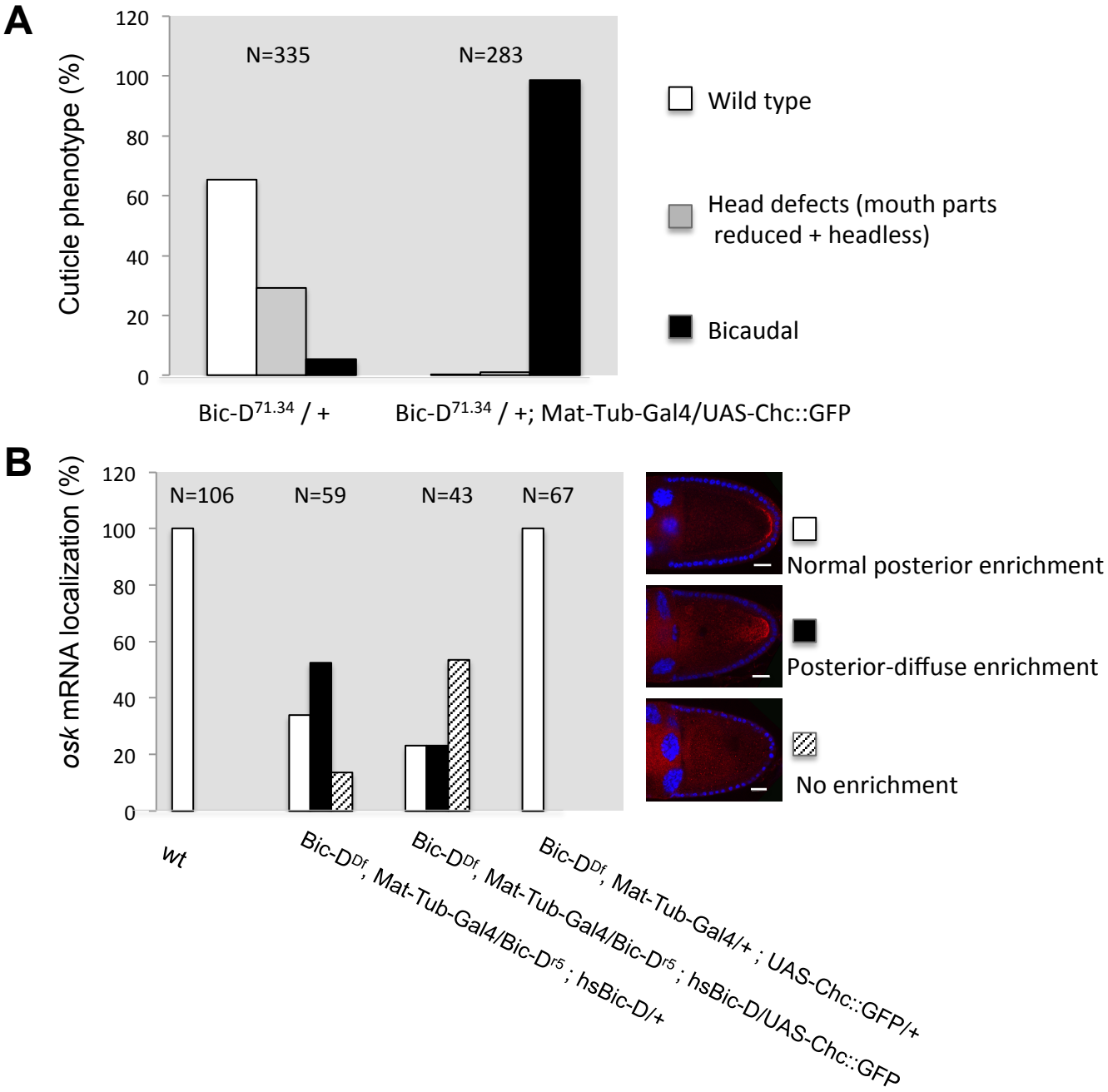
Supplementary Material S1:



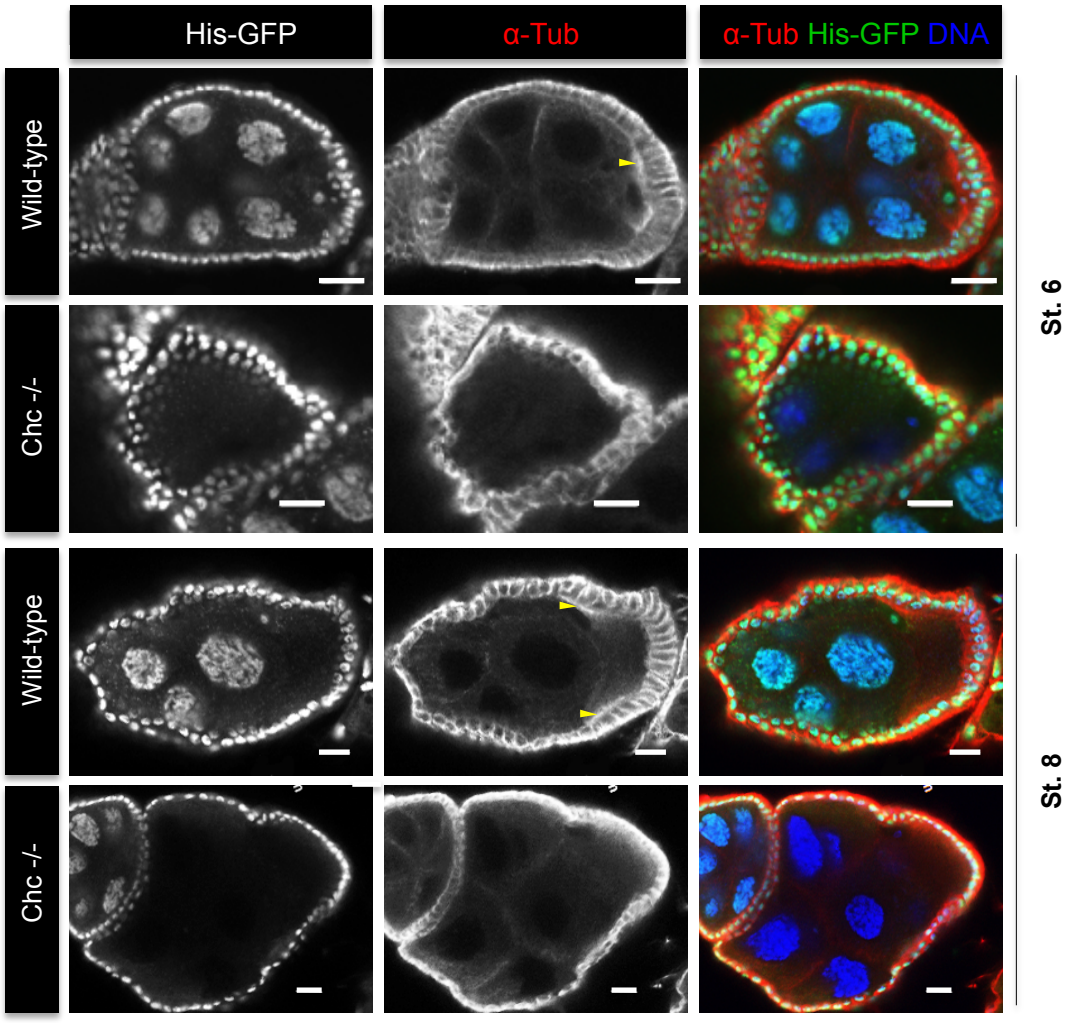
Legend for Supplementary Material S1:
(A) Immunostaining of 4C-CHC expressing ovarioles with anti-Flag (to detect Flag::Chc) and anti-Staufen antibodies. In stage 7-8, a Chc enrichment was detected in the center of the oocyte (see also Figure 1E, H). This was seen when probing for endogenous Chc, UAS-Chc::GFP and 4C-Chc, but it was not seen with Myc::Chc and Chc::V5 staining (see Figure 1E, H; data not shown). We have not tested whether the differences are due to differences in expression levels. Interestingly, this localization pattern resembles the one of Stau and several endosomal proteins (Tanaka and Nakamura, 2008). Stau and Chc are both enriched in the center of the oocyte by stage 6-8. However, the two do not completely co-localize in this central structure. **(B)** Immunostaining of 4C-CHC expressing ovarioles with anti-Flag and anti-Egl antibodies. In stage 6 oocytes Chc shows a partial co-localization with Bic-D and Egl at the posterior cortex (arrowhead) (see also Figure 1H). A second partial co-localization between Bic-D / Egl and Chc is evident at the anterior cortex in stage 8 egg chambers (arrowhead, see also Figure 1H). However, in stage 7-8 egg chambers Bic-D and Egl are excluded from the central region where Chc additionally accumulates (see also Figure 1H). **(C)** Immunostaining of 4C-CHC expressing ovarioles with anti-Flag (to detect Chc) and anti-Clc antibodies. In stage 6 Clc and Chc accumulate in the oocyte and both show an enrichment towards the posterior cortex. However co-localization is not complete. Particles showing strong Chc and Clc staining (open arrowhead), but also others showing only strong Chc enrichment (arrowhead) are observed. Colocalization at the oocyte cortex is also observed in st. 8-9 oocytes. However, while Chc shows an enrichment at the posterior cortex, Clc shows a more uniform cortical accumulation (arrowheads in stage 8 and 9). Endogenous Clc is also strongly expressed in the somatic follicle cells around the oocyte. Scale bars: 10 μm.

Supplementary Material S2:



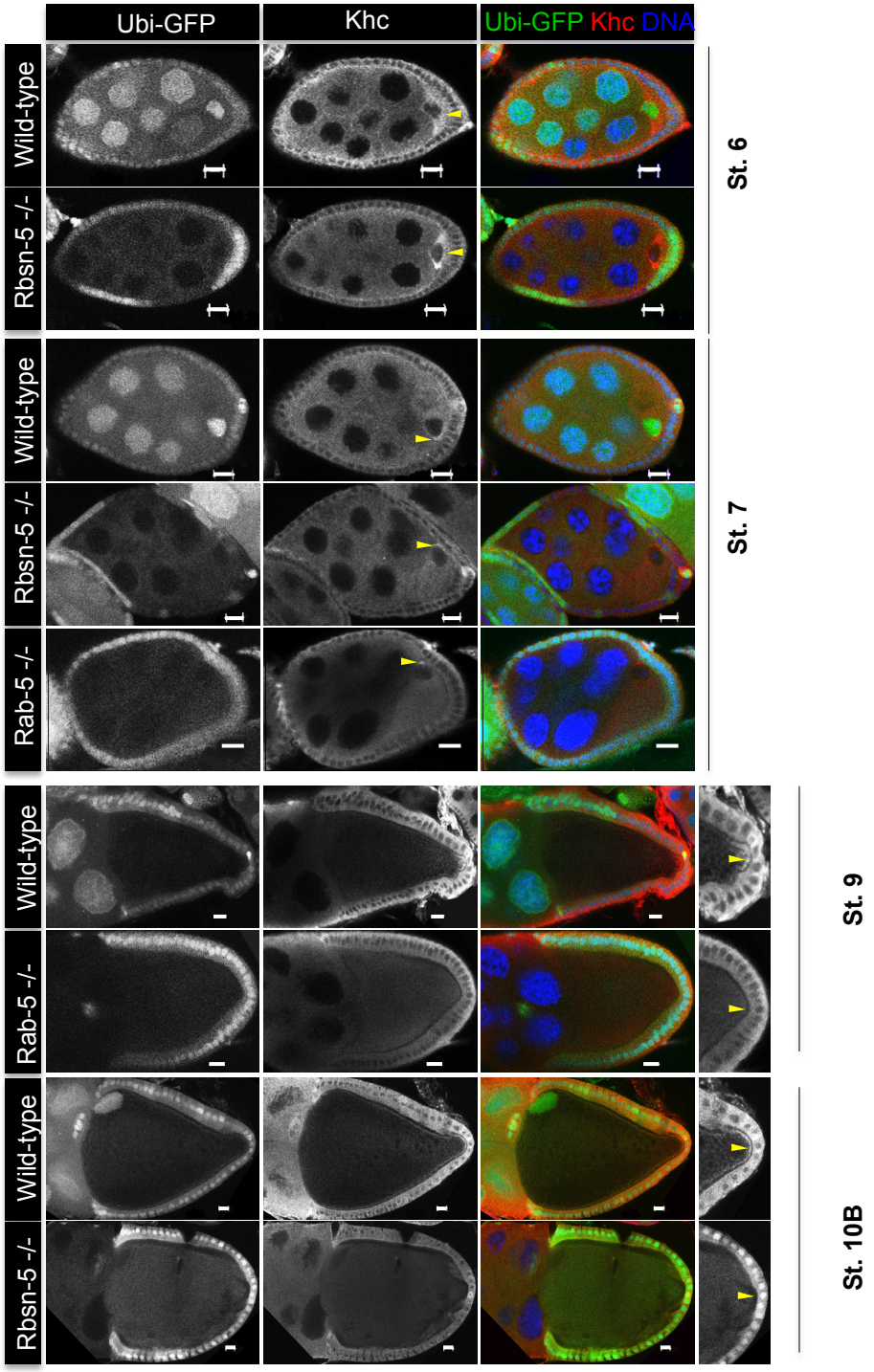
Legend for Supplementary Material S2:

Genetic interaction between *Bic-D* and *Chc*. Overexpression of *Chc::GFP* enhances the *osk* mislocalization phenotypes in *Bic-D* dominant and in *Bic-D*^{null} backgrounds.
(A) Cuticles were prepared from embryos laid by mothers of the depicted genotypes, and embryonic phenotypes were scored as indicated. Overexpression of the Chc::GFP fusion protein enhanced the bicaudal phenotype of the *Bic-D*$^{71.34}$ dominant allele**.** A possible reason for this could be that the overexpression leads to slightly enhanced endocytosis and thereby to enhanced cortical anchoring of ectopic *osk* mRNA.
(B) *In situ* hybridization to ovaries of the indicated genotypes using digoxigenin-labeled antisense RNA probes against *osk* mRNA. Localization of *osk* mRNA in stage 9-10 egg chambers was scored as depicted. *osk* mRNA signal is in red, DNA staining in blue. In *wild-type* oocytes *osk* signal is strong and focused at the posterior pole (white bars). In *Bic-D*^{mom} oocytes *osk* signal was overall weaker and many egg chambers showed more diffuse posterior signals or uniform/no signal (black and dashed bars). The mutant phenotype was enhanced by overexpressing Chc::GFP. This was not due to overexpression of Chc::GFP by itself, since overexpression in egg chambers containing one functional *Bic-D* copy did not alter *osk* mRNA localization (4th genotype). Competition for Bic-D by the two different cargoes *osk* mRNA/Egl and Chc protein could account for the reduced posterior localization of *osk* mRNA. Scale bars: 20µm.



Supplementary Material S3:

Oocyte microtubule polarity is affected in *Chc*^{null} germline clones. Germline clones for a null allele of *Chc*, *Chc*^{GF23} were generated and stained with anti-alpha tubulin antibodies (red). Mutant clones are marked by the absence of His-GFP. Focusing of microtubules (arrowheads) stained with alpha-tubulin is affected in mutant egg chambers. Ovarioles were also stained with Hoechst (blue) to visualize the DNA. Scale bars: 10 µm.



Supplementary Material S4:

Oocyte microtubule polarity is not affected during early oogenesis in *Rab5* and *Rbsn-5* mutants. *Rab5*^{2} and *Rbsn-5*^{C241} germline clones were generated and stained with anti-Khc antibodies (red). Mutant clones are marked by the absence of GFP (green). Accumulation of Khc (arrowheads) is only affected in mutant egg chambers from stage 9 on when the signal becomes fainter at the posterior cortex (arrowheads in the magnification). Ovarioles were also stained with Hoechst (blue) to visualize the DNA. Scale bars: 10 µm.