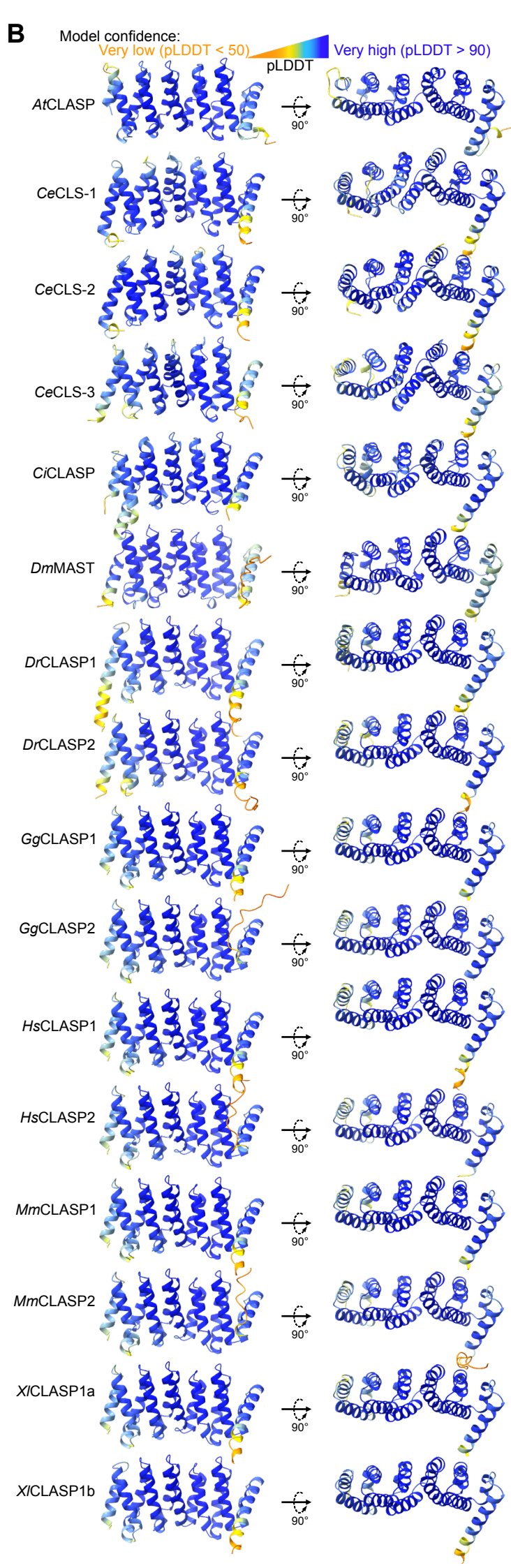
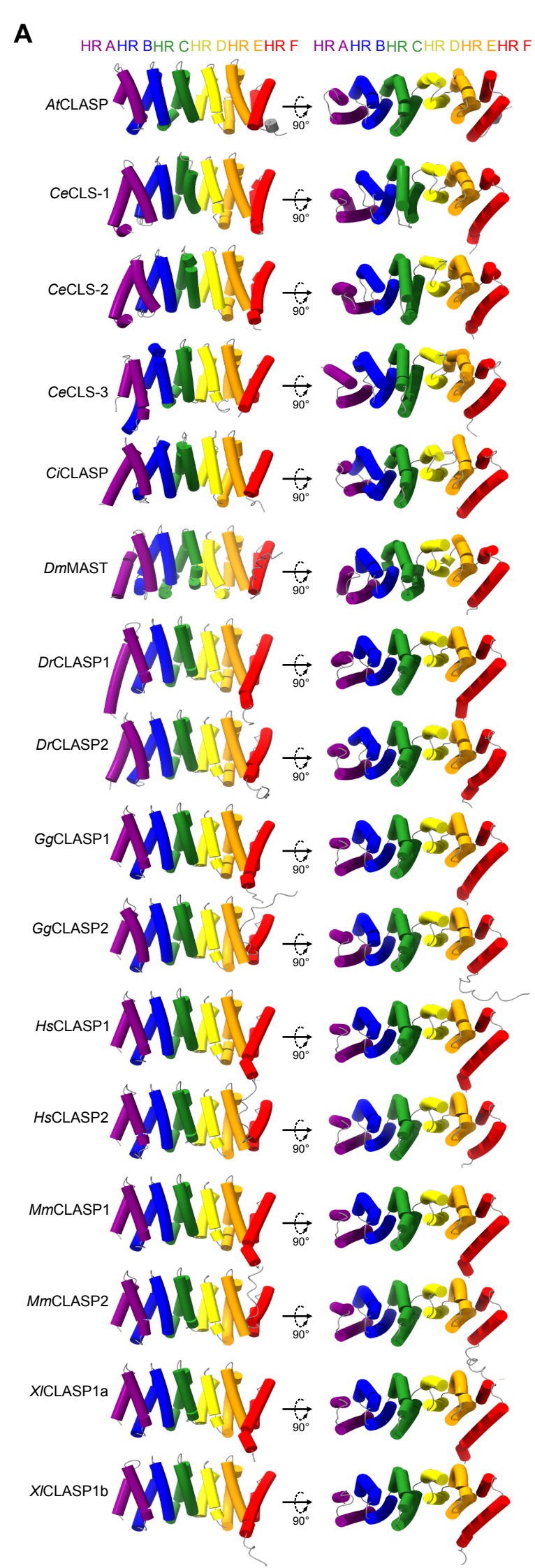


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**Supplemental Information**

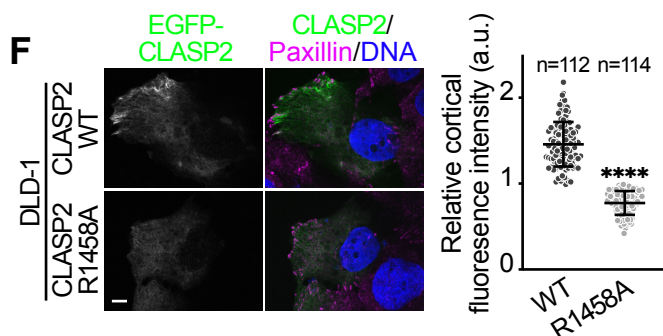
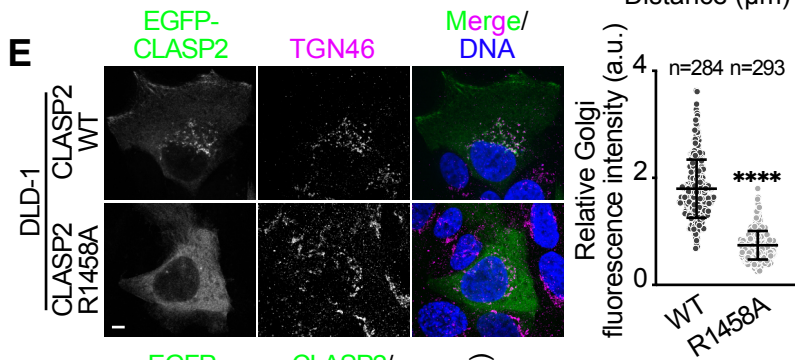
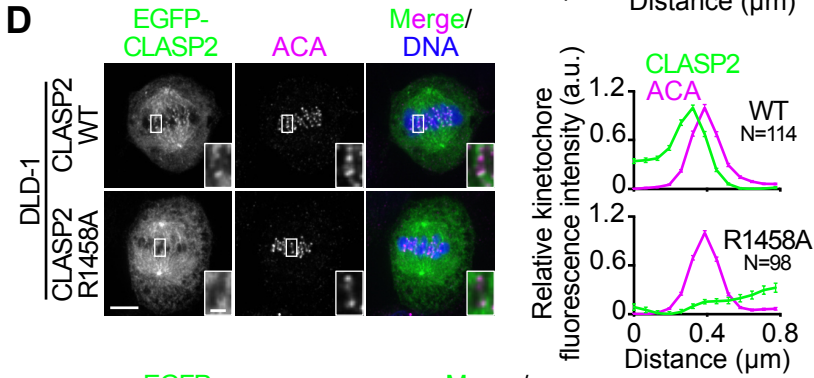
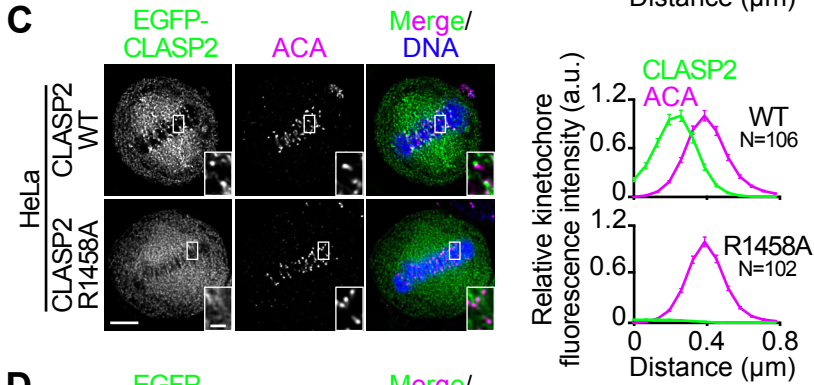
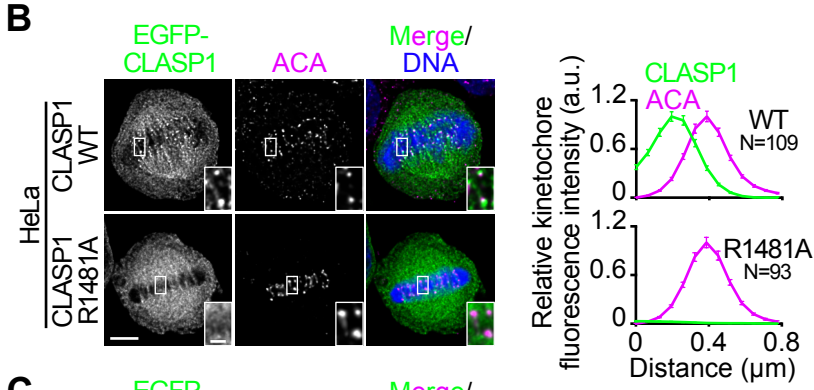
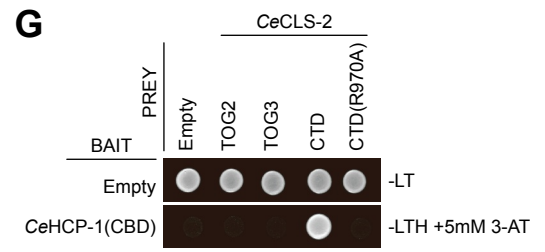
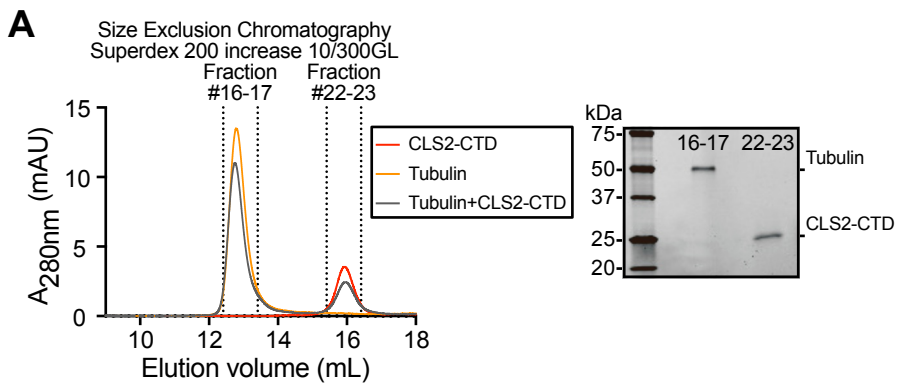
**An unconventional TOG domain  
is required for CLASP localization**

**Nelly Gareil, Alison Gervais, Nicolas Macaisne, Guillaume Chevreux, Julie C. Canman, Jessica Andreani, and Julien Dumont**



**FIGURE S1 related to Figure 1. The CTD of CLASP proteins folds like a TOG domain.**

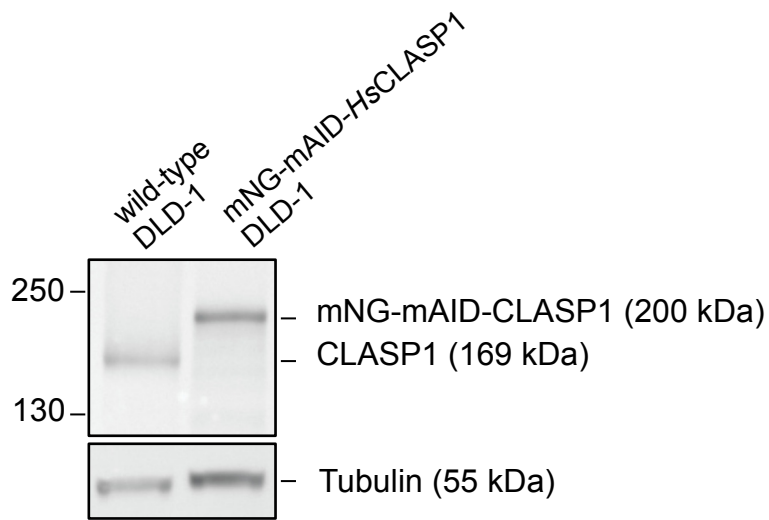
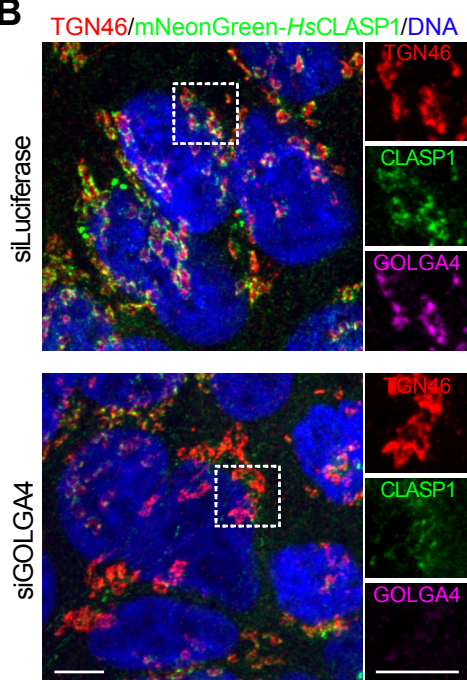
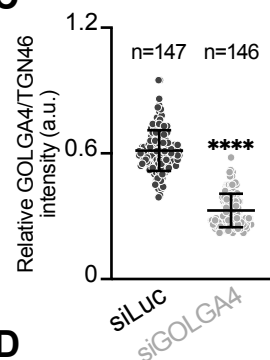
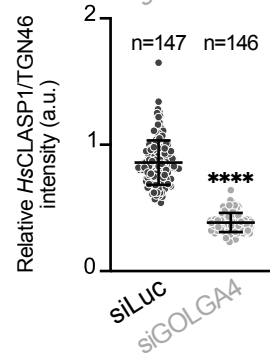
(A-B) AlphaFold2 3D structure prediction of the CTDs of 16 CLASP proteins from various species. Left: side view. Right: end-on view. (A) Heat Repeats (HR) A-F are color-coded. (B) Model confidence is color-coded based on the AlphaFold2 pLDDT score (Local Distance Difference Test)<sup>32</sup>. (*At*: *Arabidopsis thaliana*, *Ce*: *Caenorhabditis elegans*, *Ci*: *Ciona intestinalis*, *Dm*: *Drosophila melanogaster*, *Dr*: *Danio rerio*, *Gg*: *Gallus gallus*, *Hs*: *Homo sapiens*, *Mm*: *Mus musculus*, *Xl*: *Xenopus laevis*).



**FIGURE S2 related to Figure 2. A conserved arginine in the CTD of CLASP proteins is essential for their proper sub-cellular localizations.**

(A) Size exclusion chromatography of the CLS-2-CTD with or without tubulin, and of tubulin alone. Coomassie staining of the fractions of interest is shown on the right. (B-F) Left: Immunofluorescence images of (B,C) HeLa or (D-F) DLD-1 cells transiently expressing (B) WT or R1481A EGFP-*HsCLASP1*, (C-F) WT or R1458A EGFP-*HsCLASP2*, and stained for (B-D) the centromeric marker ACA (bottom right: zoom on one kinetochore pair. Scale bar on zoom, 1  $\mu\text{m}$ ), (E) the trans-Golgi marker TGN46, (F) the focal adhesion marker Paxillin. Scale bars, 5  $\mu\text{m}$ . Right: Quantification of mean intensity of WT or mutant CLASPs at (B-D) kinetochores, (E) the Golgi and (F) the cell cortex. Error bars: SEM for B-D, SD for E,F. Sample sizes (N kinetochores, n areas) are indicated on each graph. Unpaired t-tests,  $p < 0.0001$ . (G) Yeast-two-hybrid interaction tests between the CLS-2-binding domain (CBD) of CeHCP-1, and the TOG2, TOG3 and wild-type or R970A CTD of CeCLS-2. In the presence of 5 mM 3-AT (3-Amino-1,2,4-Triazole), only the wild-type CeCLS-2 CTD interacts with the CBD of CeHCP-1.



**A****B****C****D**

**FIGURE S3 related to Figure 3. GOLGA4 is required for *HsCLASP1* Golgi localization.**

(A) Western blot of wild-type and CRISPR/Cas9-engineered mNG-mAID-*HsCLASP1* DLD-1 cells using an anti-*HsCLASP1* antibody. (B) Immunofluorescent images of the trans-Golgi marker TGN46 (red), endogenously-tagged mNeonGreen-*HsCLASP1* (green), the Golgin protein GOLGA4 (magenta) and DNA (blue) in DLD-1 cells transfected with Luciferase- (top) or GOLGA4-targeting siRNAs (bottom). Scale bars, 5  $\mu$ m. (C) Quantification of GOLGA4 intensity at the Golgi relative to TGN46 in siLuciferase or siGOLGA4-transfected DLD-1 cells. Unpaired t-test,  $p < 0.0001$ . (D) Quantification of mean mNeonGreen-*HsCLASP1* intensity at the Golgi relative to TGN46 in siLuciferase or siGOLGA4-transfected DLD-1 cells. Error bars, SD. Sample sizes (n areas) are indicated on each graph. Unpaired t-test,  $p < 0.0001$ .