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## Supplemental Data

# Truncated SALL1 Impedes Primary Cilia Function in Townes-Brocks Syndrome

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**Figure S1**

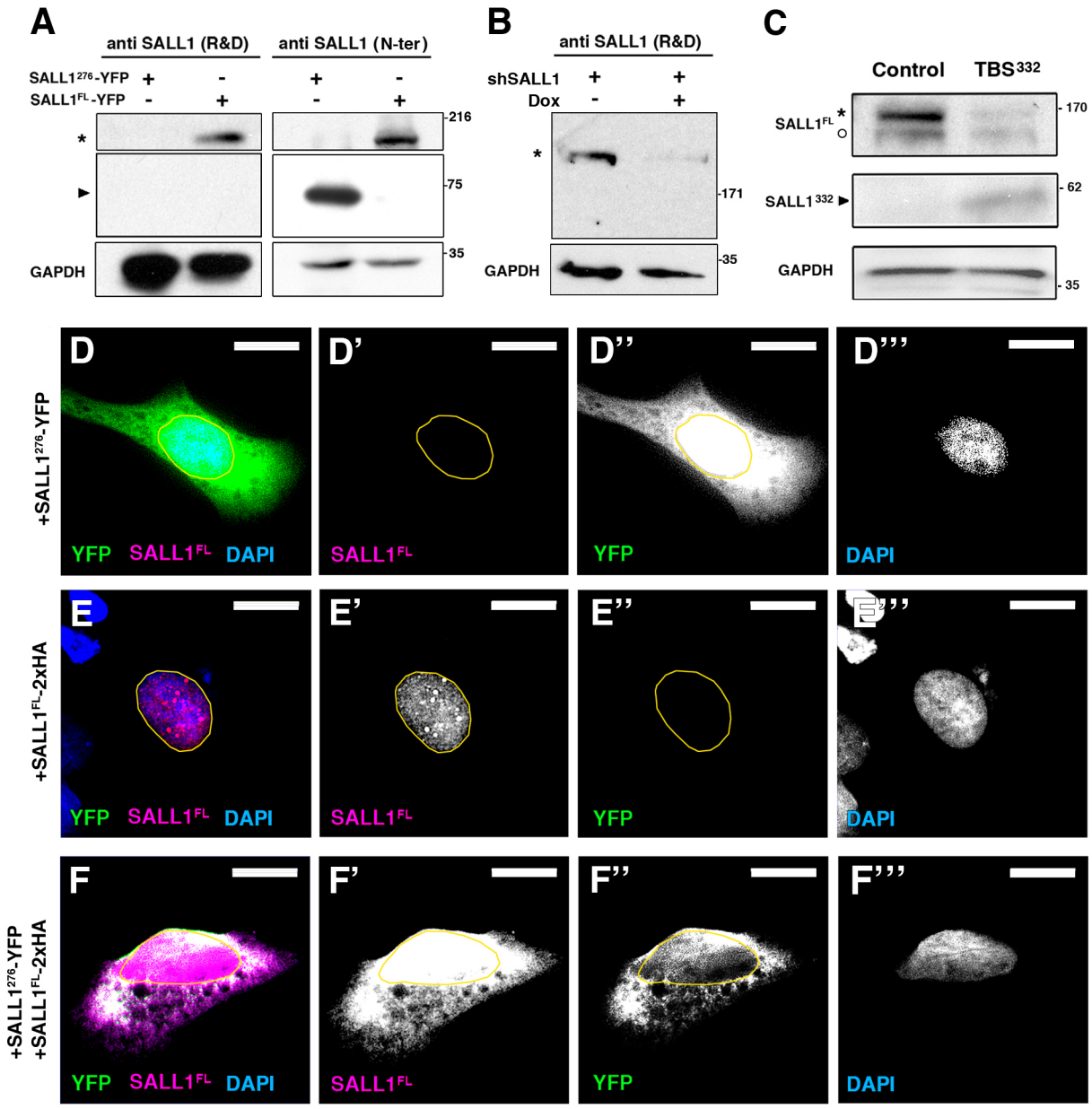
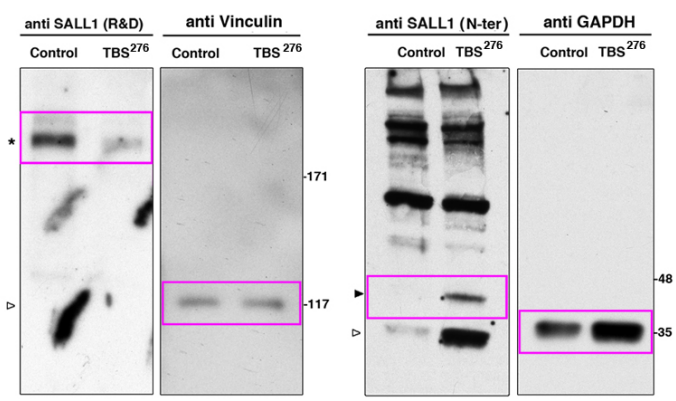



**Figure S1. Truncated SALL1 interacts with SALL1$^{FL}$ in the cytoplasm.** (**A**) Western blot analysis of total lysates of HEK 293FT cells transfected with *SALL1$^{c.826C>T}$(SALL1$^{276}$)-YFP* or *SALL1$^{FL}$-YFP*. Samples were run in duplicate on the same gel and probed against SALL1 using two different antibodies. Anti-SALL1 antibody from R&D specifically recognizes SALL1$^{FL}$ (asterisk), but not the truncated form (black arrowhead), while anti-SALL1 N-terminal specific detects both. Molecular

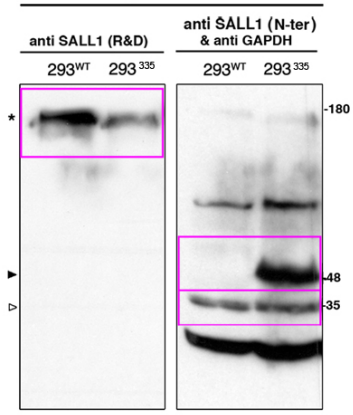
weights in kDa are shown to the right. **(B)** Western blot analysis of HEK 293FT lysates expressing SALL1 specific shRNA under the control of a doxycycline inducible promoter. Cells were subjected to doxycycline (+) or DMSO treatment (-) for 72h. $SALL1^{FL}$ protein was detected using anti-SALL1 antibody from R&D (asterisk) and GAPDH was used as a loading control. (**C**) Western blot analysis of human control or $TBS^{332}$ fibroblast lysates, showing reduction in the levels of the endogenous FL protein (asterisk, detected with R&D antibody) and expression of a truncated protein (black arrowhead, detected with N-terminal specific antibodies) in patients-derived cells. Empty circle indicates an unspecific band. Molecular weights in kDa are shown to the right. (**D-F**) Confocal micrographs of U2OS cells transfected with *$SALL1^{276}$-YFP* (**D**), *$SALL1^{FL}$–HA* (**E**) or both (**F**). $SALL1^{FL}$-HA is in purple, $SALL1^{276}$-YFP in green and nuclei in blue (DAPI). $SALL1^{FL}$ is recognized with FL specific antibodies (R&D) that do not recognize $SALL1^{276}$, which localizes diffusely thorough nucleus and cytoplasm (**D**). $SALL1^{FL}$ exhibits a nuclear pattern (**E**) that is impaired when $SALL1^{276}$ is present (**F**). Black and white images show the single purple (**D'-F'**), green (**D''-F''**) and blue channels **(D´´´-F´´´)**. Images were taken with a Zeiss Axioimager D1, 63x objective. Scale bar, 10 μm.

# Figure S2

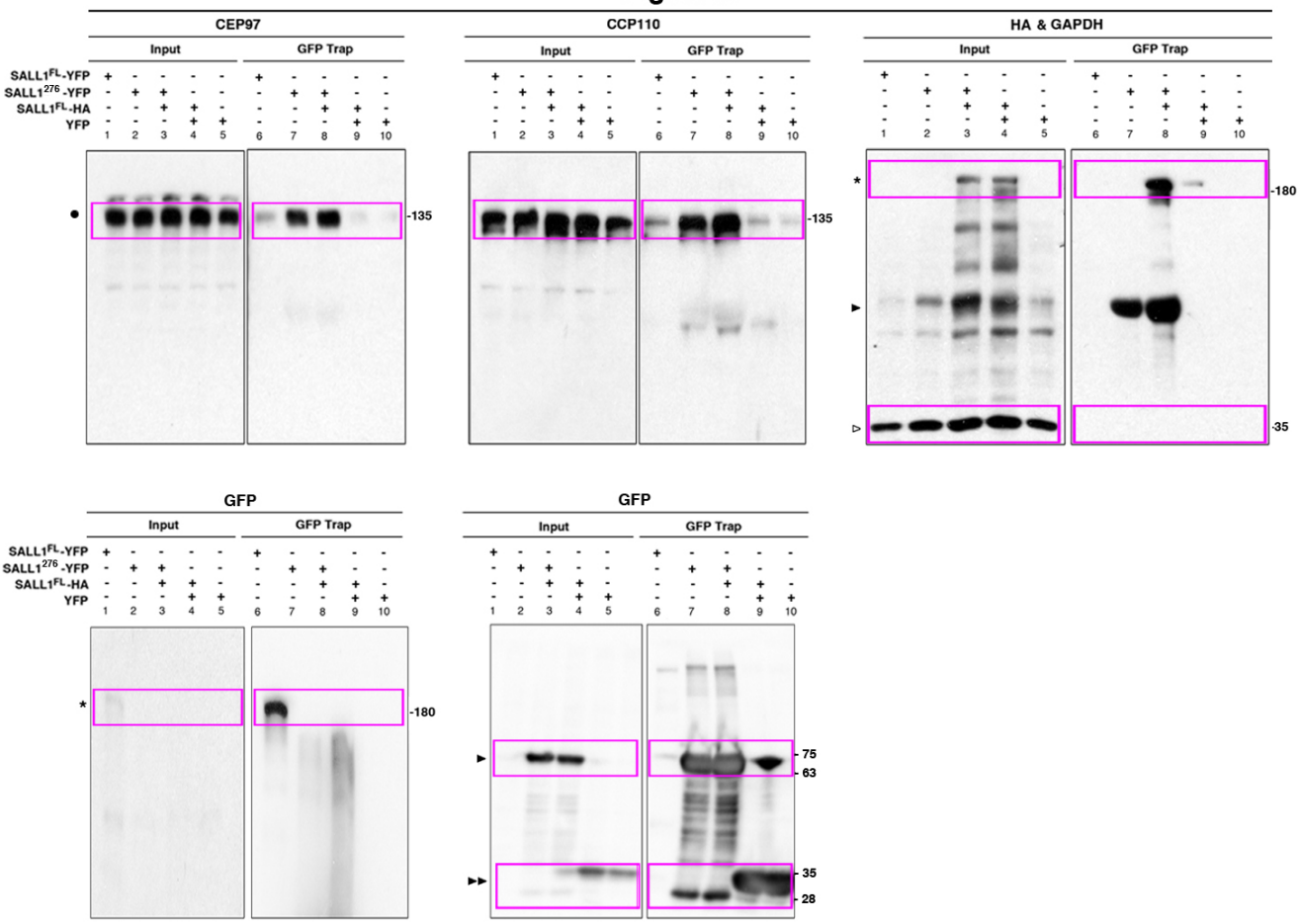
## Figure 3B




## Figure 7A




## Figure 5F



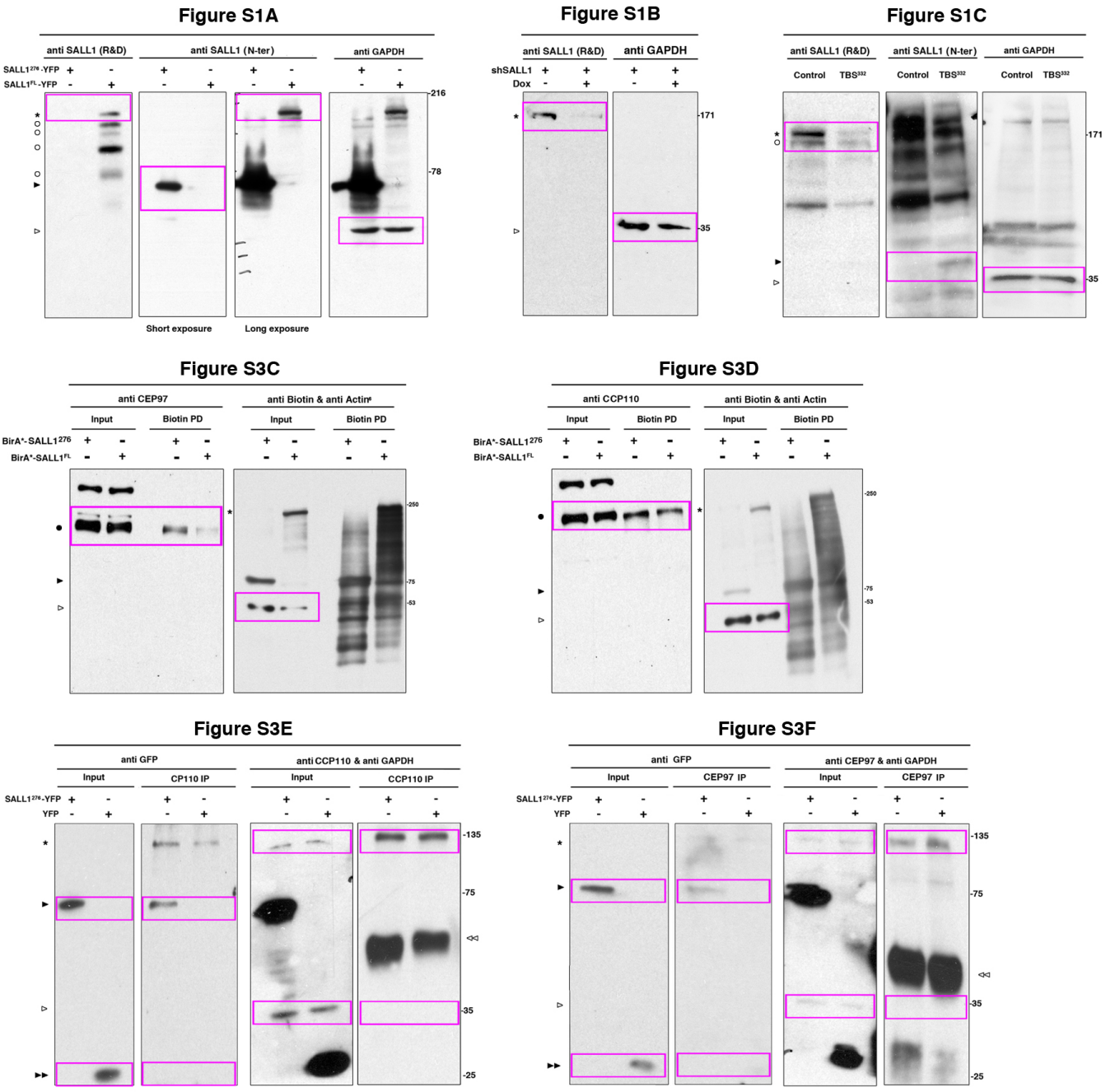



**Figure S2. Complete Western blot gel pictures.** Titles indicate the Figure where each Western blot belongs to; purple boxes show the region of the gel that was used to build their respective figures on each gel. SALL1$^{FL}$ protein is indicated by one asterisk, SALL1 truncated forms by one black arrowhead, CCP110 or CEP97 by a black circle, GAPDH, Vinculin or actin by an empty arrowhead and GFP or YFP by two black arrowheads. SALL1$^{FL}$ protein breakdowns or unspecific bands are indicated by empty circles.

**Figure S3**

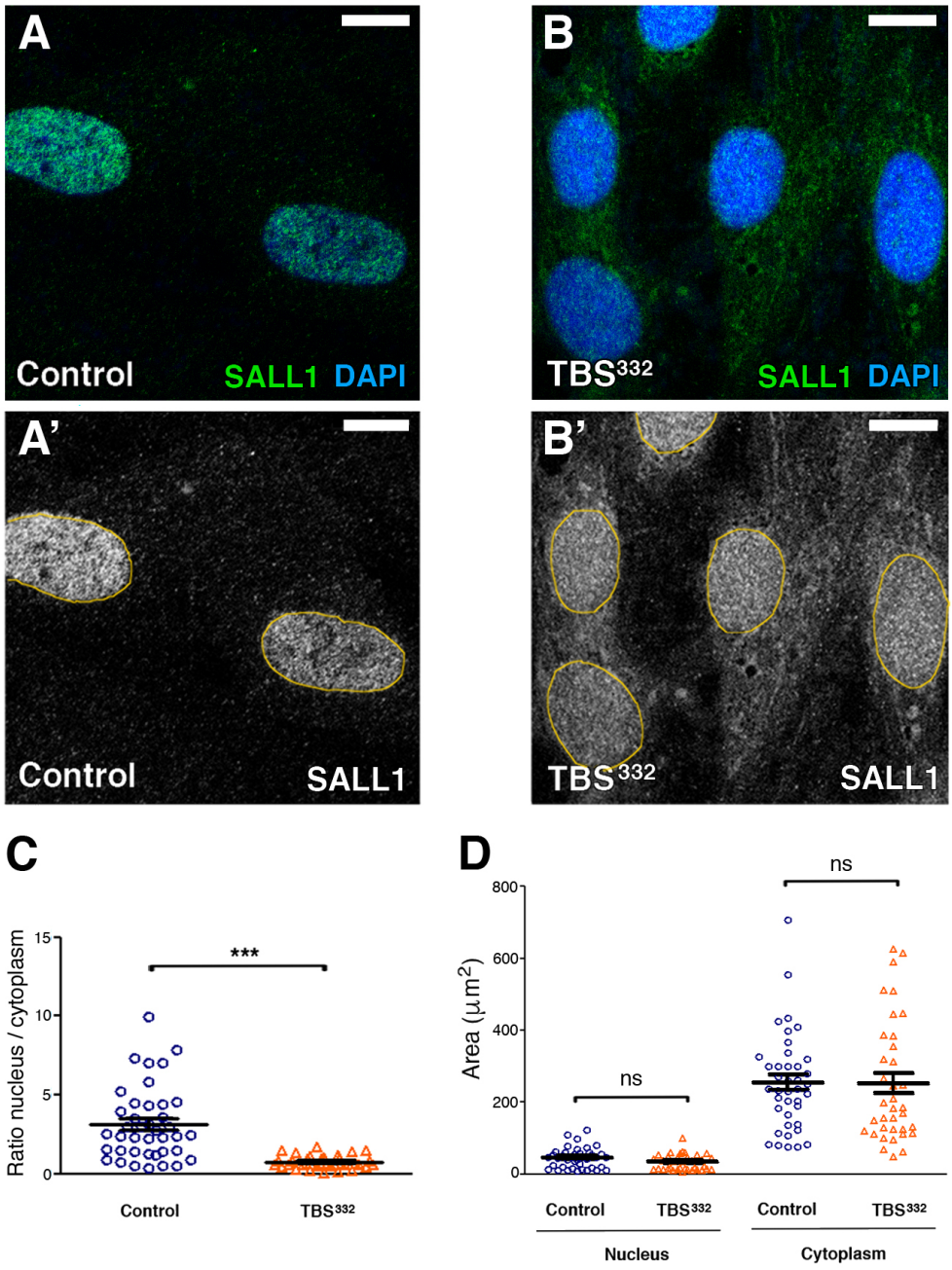



**Figure S3. Truncated SALL1 recruits SALL1$^{FL}$ to the cytoplasm.** (**A**,**B**) Confocal micrographs showing SALL1$^{FL}$ detected by FL specific antibodies (R&D) in control HFF (**A**) or TBS$^{p.Pro332Hisfs*10}$ fibroblasts (TBS$^{332}$) (**B**). Nuclei were labeled by DAPI (blue). Single green channel is shown as black and white images (A',B'). (**C**,**D**) Graphical representation of three independent experiments showing the ratio between the fluorescence quantification of the levels of SALL1$^{FL}$ in the nucleus or cytoplasm of

control HFF (n=40 cells; blue circles) or TBS$^{332}$-derived fibroblasts (n=36 cells; orange triangles) (**C**) or the area in μm$^2$ of control (n=33 cells, blue circles) or TBS$^{332}$ (n=33 cells, orange triangles) (**D**). The P-values were calculated with the Mann-Whitney two-tailed test. Median and interquartile range of the median are represented. All graphs represent the mean and SEM. (*) P-value < 0.05, (***) P-value < 0.0001. Scale bars, 10 μm.

**Figure S4**

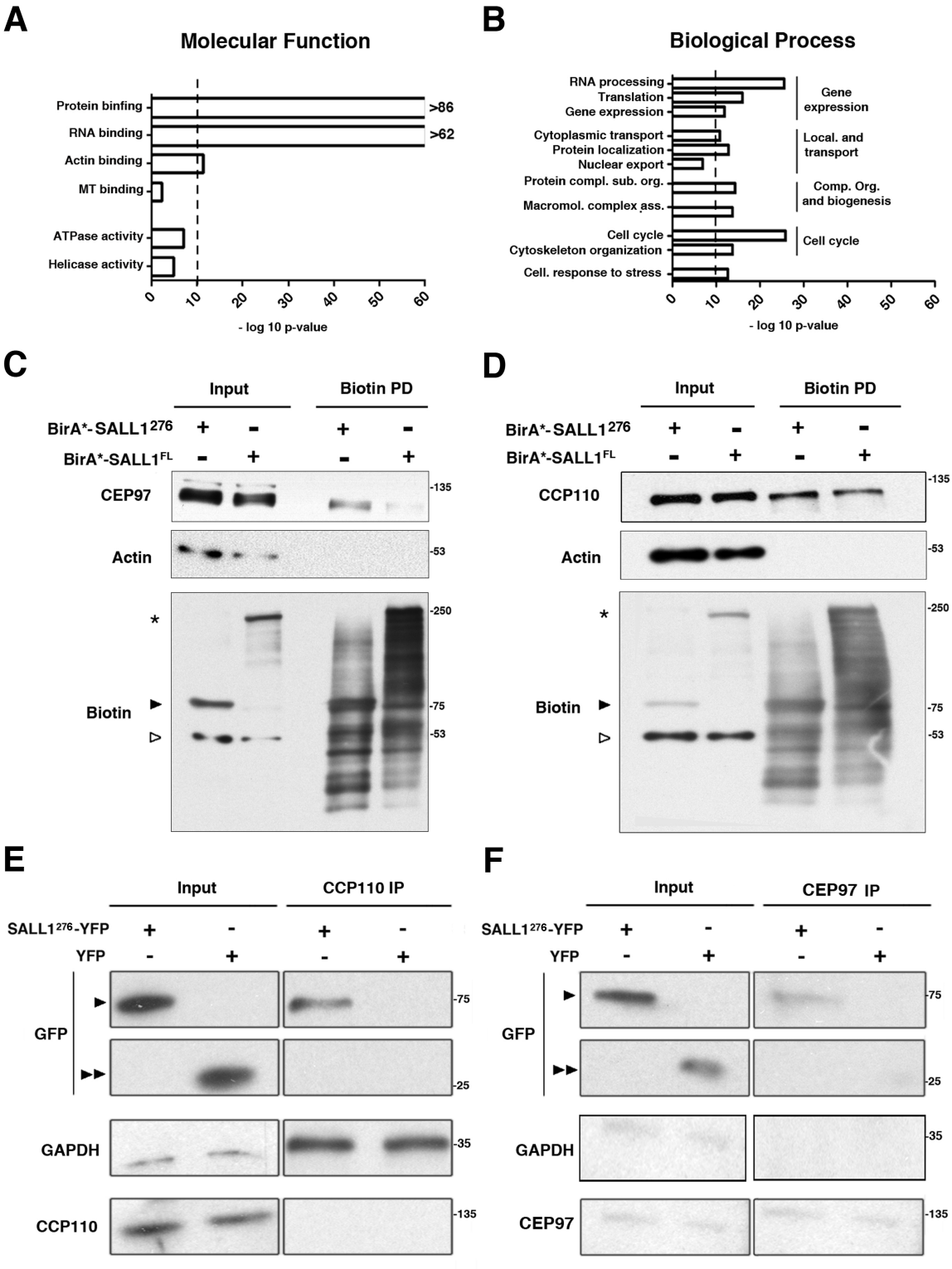



**Figure S4. Specific protein interactions by truncated or FL SALL1. (A,B)** Graphical representation of the -$\log_{10}$ of the P-value for each of the represented

Molecular Function (A) or Biological Process (B) GO terms in the BirA*-SALL1$^{p.Arg276*}$proteome (SALL1$^{276}$). Cell. response to stress, cellular response to stress; Comp. org. and biogenesis, component organization and biogenesis; Local. and transport, localization and transport; MT, microtubules; Macromol. complex as., macromolecular complex assembly; Protein compl. sub. org., protein complex subunit organization. (**C,D**) Western blot analysis of BioID, biotin pulldowns (PD) of HEK 293FT cells transfected with Myc-tagged *BirA*-SALL1$^{FL}$* or *BirA*-SALL1$^{c.826C>T}$* (BirA*-SALL1$^{276}$). Specific antibodies against the endogenous proteins CEP97 (C) or CCP110 (D) were used. Actin was used as loading control. Biotin antibody detected the most biotinylated proteins in the inputs and pulldowns, which are the self-biotinylated forms of BirA*-SALL1$^{FL}$ (asterisks) and BirA*-SALL1$^{276}$ (one black arrowhead), as well as other interactors in the pulldowns. White arrowheads indicate actin signal from previous probing. Blots shown are representative of three independent experiments. (**E-F**) Western blot of inputs and CCP110 or CEP97 IPs performed in HEK 293FT cells transfected with *SALL1$^{c.826C>T}$-YFP* or *YFP* alone. Endogenous CCP110 and CEP97 in the IP interacted with conjugating G sepharose beads linked to specific antibodies. One black arrowhead marks SALL1$^{276}$-YFP and two arrowheads YFP alone. GAPDH was used as loading control and CCP110 and CEP97 antibodies to control the IP. Molecular weight markers are shown to the right.

**Figure S5**

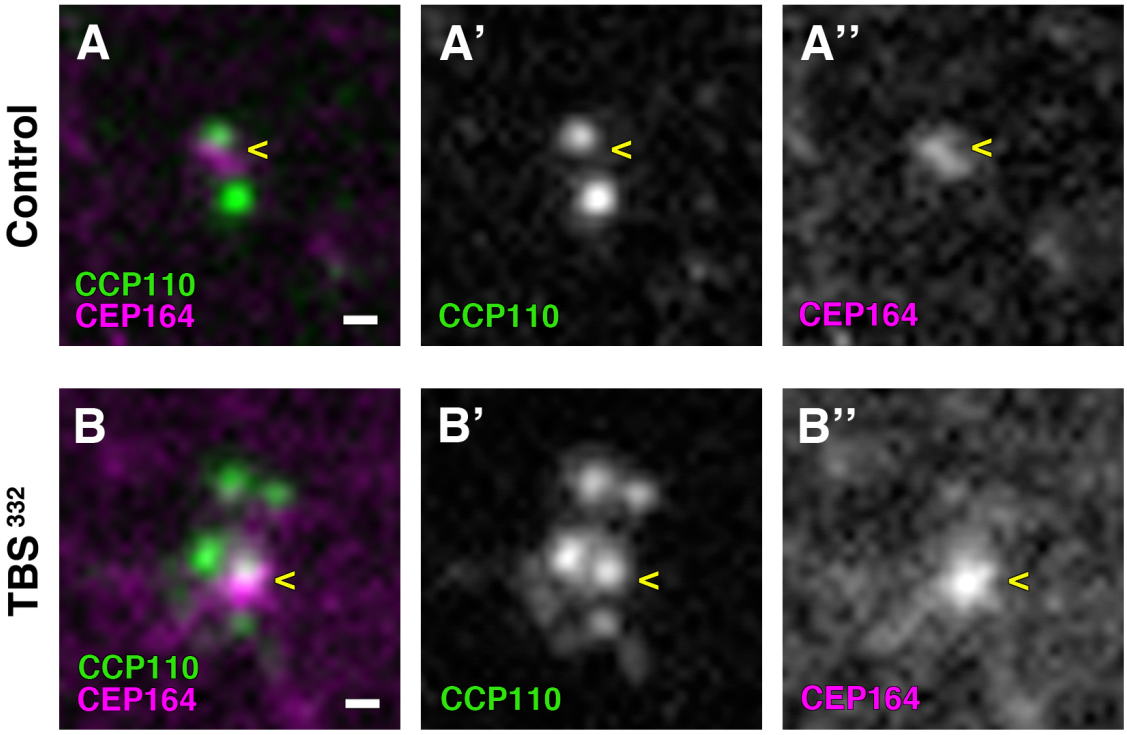



**Figure S5. CCP110 localizes aberrantly in multiple foci in TBS $^{p.Pro332Hisfs*10}$ cells.** Representative fluorescence micrograph of a control (**A**) and a TBS$^{p.Pro332Hisfs*10}$ (TBS$^{332}$) centrosome (**B**) showing CCP110 (green) in a cloudy pattern around the mother centriole (yellow arrow) marked by CEP164 (purple). Black and white images show the single green and purple channels. Pictures were taken using a Zeiss Axioimager D1, 63x objective. Scale bar, 1 µm.