Supplementary figure legends

FIGURE S1: Eps8 interacts with the F-box protein Fbxw5

(a) Flag-Fbxw5 and interacting proteins were enriched via anti-flag immunoprecipitation from five 15 cm dishes of transiently transfected HEK 293T cells followed by flag peptide elution. As a control, the IP was also performed from HEK 293T cells transfected with pCDNA3.1 vector. Flag-Fbxw5 and control immunoprecipitates were separated on a gradient gel and Coomassie stained.

(b) IP fractions shown in (a) were subjected to mass spectrometry analysis. The table summarizes a selection of proteins specifically identified in the flag-Fbxw5 IP. Listed are proteins with a MASCOT protein score higher than 20.

(c) Anti-Flag IPs were performed as in (a) from HEK 293T cells stably expressing flag-Fbxw5 and analyzed by immunoblotting with indicated antibodies.

(d) Extracts from HeLa suspension cells (108 cells per sample) were subjected to IP using rabbit pre-immune serum or affinity-purified rabbit Fbxw5 antibodies, and analyzed by immunoblotting with indicated antibodies.

(e) Extracts of HEK293T cells (five 10 cm dishes per sample) transiently transfected with pCDNA3.1 (control), pCDNA3.1-Flag-Fbxw5, or pCDNA3.1-Flag-Fbxw5ΔF-box were subjected to anti-flag IP and analyzed by immunoblotting with indicated antibodies.

FIGURE S2: Fbxw5 knock down increases steady state levels of Eps8

(a) Immunoblot analysis of HeLa cells transfected with non-targeting siRNA (si nt) or siFbxw5#1,#3, or #4 for 42 h with indicated antibodies. 24 h after siRNA treatment, cells were treated with taxol or mock-treated with DMSO for 18 h.

(b) Immunoblot analysis of HeLa cells transfected with non-targeting siRNA (si nt) or siRNAs targeting Fbxw5 (siFbxw5 #1-4) for 72 h with indicated antibodies. Eps8 levels were quantified relative to α-tubulin levels; relative Eps8 levels of control cells set to 100 %.

* cross-reactive band

FIGURE S3: Flag-Fbxw5ΔF-box interacts with components of CRL4 but not SCF complexes

(a) Schematic representation of SCF and CRL4 complexes.

(b) Extracts of HEK293T cells (five 10 cm dishes per sample) transiently transfected with pCDNA3.1 (control), pCDNA3.1-Flag-Fbxw5, or pCDNA3.1-

Flag-Fbxw5ΔF-box were subjected to anti-flag IP and analyzed by immunoblotting with indicated antibodies. Same samples as in Supplementary Figure 1e, for reference the anti-flag immunoblot is shown again.

FIGURE S4: Downregulation of Fbxw5 delays progression into metaphase but has no dramatic impact on the cytoplasmic or cortical actin cytoskeleton in pro-metaphase

(a) Z-stack maximum intensity projection of representative Hela cells in prometaphase treated with non-targeting siRNA (si nt) or siRNA targeting Fbxw5 (si Fbxw5) for 72 h. Z-stack images (step with=0.25 µM) of Hela cells stably expressing Lifeact-GFP and H2B-RFP were taken and maximum intensity projection images were generated using ImageJ after deconvulotion. Scale bar = 20 µm

(b) Stills from fluorescence time lapses of HeLa cells stably expressing a-tub-GFP (green) and H2B-RFP (red) and transfected with si nt or si Fbxw5 #3. Scale bar = 10 µm

(c) Quantification of prometaphase duration (nuclear envelope breakdown - chromosome alignment in metaphase) in cells stably expressing a-tub-GFP and H2B-RFP transfected with si nt or si Fbxw5 #3 (mean +/- standard deviation of 8 cells).

(d) Quantification of prometaphase duration (nuclear envelope breakdown - chromosome alignment in metaphase) in HeLa cells stably expressing Lifeact-GFP and H2B-RFP transfected with si nt or si Fbxw5 #3 (mean +/- standard deviation of 25 cells).

FIGURE S5: Downregulation of Fbxw5 or Eps8 does not significantly change the mitotic index

(a) HeLa cells were treated with non-targeting siRNA (si nt) or siRNAs targeting Fbxw5 (si Fbxw5 #3) for 72 h. Cells were fixed, stained with HOECHST and anti-α-tubulin and anti-g-tubulin antibodies. Representative images of different mitotic stages are depicted. Classification of mitotic stages shown in Fig. 4c was performed according to these pictures. Scale bar = 10 µm.

(b) Same experiment as shown in Figure 4a-c. The percentage of mitotic and interphase cells is shown (mean +/- s.e.m. of three independent experiments, ~1000 cells were counted per condition and experiment).

FIGURE S6: Eps8 phosphorylation is not required for SCFFbxw5-mediated ubiquitylation, and phospho-mimicking and -deficient mutants of Fbxw5S151 exhibit no major changes in SCF complex formation, Eps8 binding, or ubiquitylation activity towards Eps8

(a) In vitro dephosphorylation of His-Eps8 (5 µM) purified from SF9 cells with 10 U CIP (+ phosphatase) at 37°C for 1 h followed by immunoblotting.

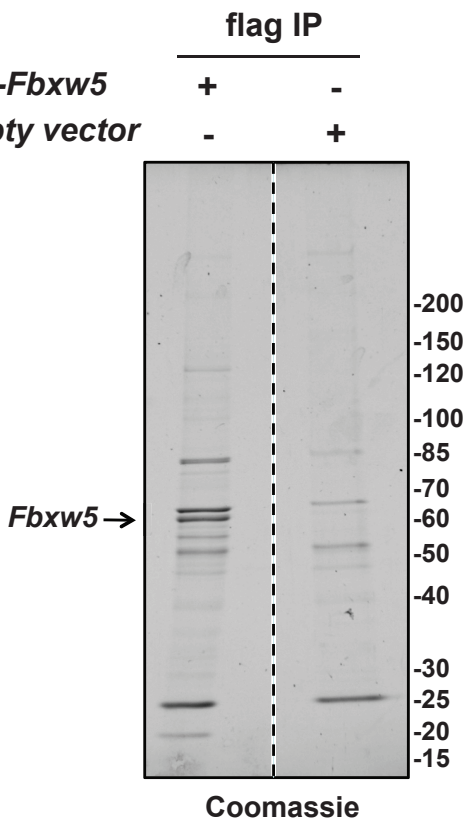
(b) In vitro ubiquitylation of +/-phosphatase-treated His-Eps8 (0.2µM) incubated with 75 µM His-Ubiquitin, 170 nM Ube1, 1 µM UbcH5b, 150 nM SCFFbxw5, and 5 mM ATP at 30°C for 30 and 60 min.

(c) Extracts of HEK293T cells transiently transfected with pCDNA3.1 (control), pCDNA3.1-Flag-Fbxw5, pCDNA3.1-Flag-Fbxw5S151E or pCDNA3.1-Flag-Fbxw5S151A were subjected to anti-flag IP and analyzed by immunoblotting with indicated antibodies.

(d) In vitro ubiquitylation reaction of His-Eps8 (0.2 µM) with 75 µM His-Ubiquitin, 170 nM Ube1, 1 µM UbcH5b, 5 mM ATP, and control (= flag-IPs from non-transfected cells), flag-Fbxw5, flag-Fbxw5S151E or flag-Fbxw5S151A immunoprecipitates at 30°C. Reactions were stopped at indicated time points and analyzed by immunoblotting with indicated antibodies.

FIGURE S7: Un-cropped blots of key experiments

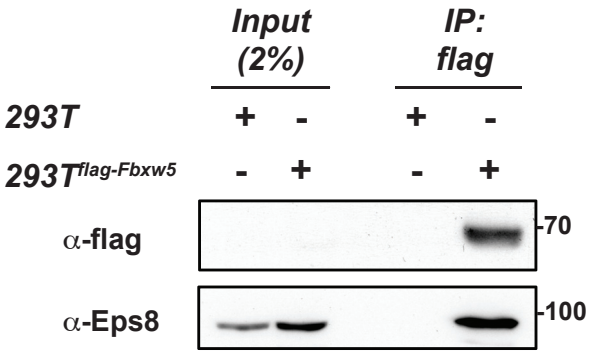
a



b

Protein	Score	Peptides identified
Fbxw5	760	15
Cul1	510	16
Eps8	90	3
SGN1	78	3
SGN4	72	2
DDB1	22	1
Ubiquitin	22	1

c



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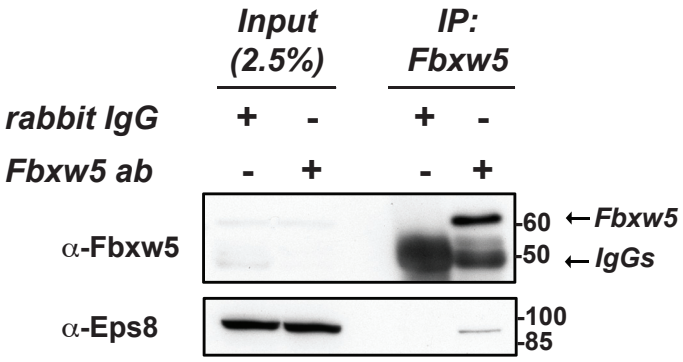

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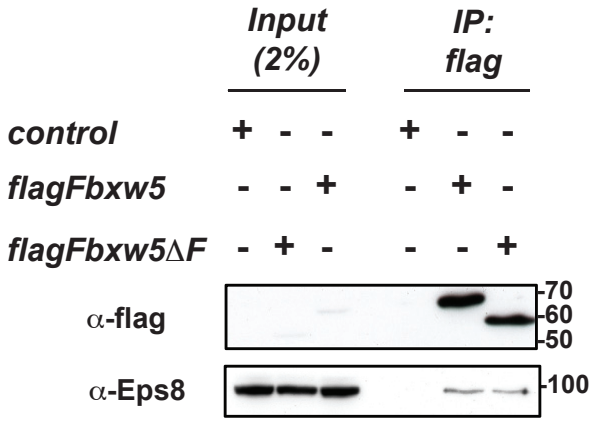
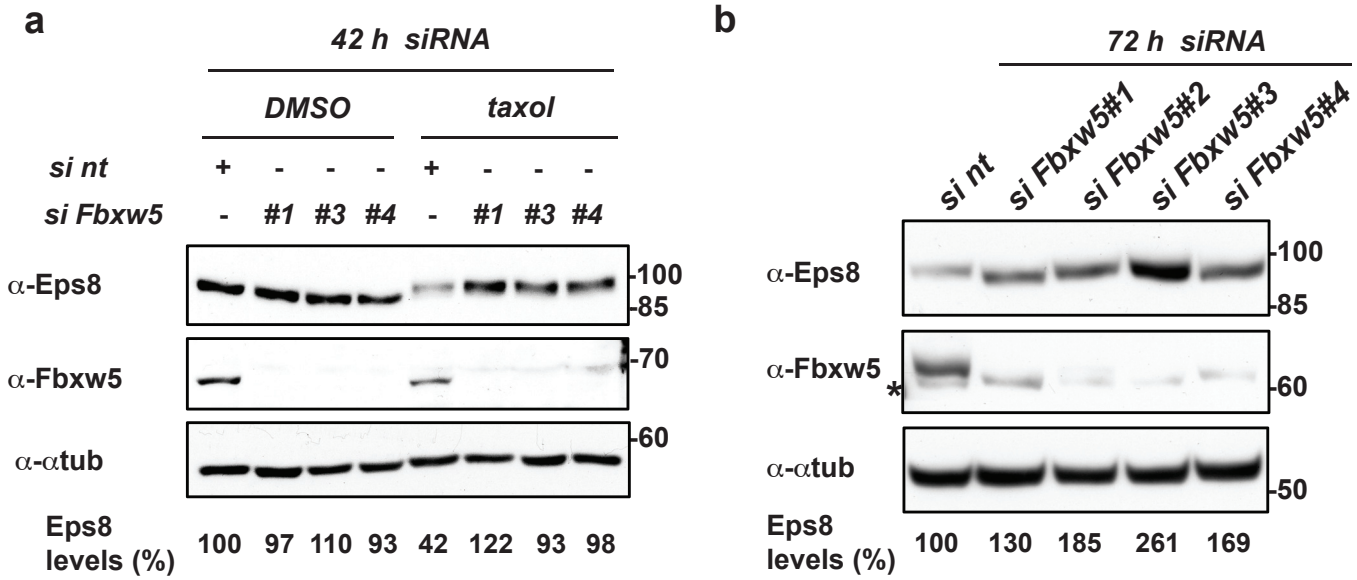


SUPPL FIGURE 1 (Melchior)



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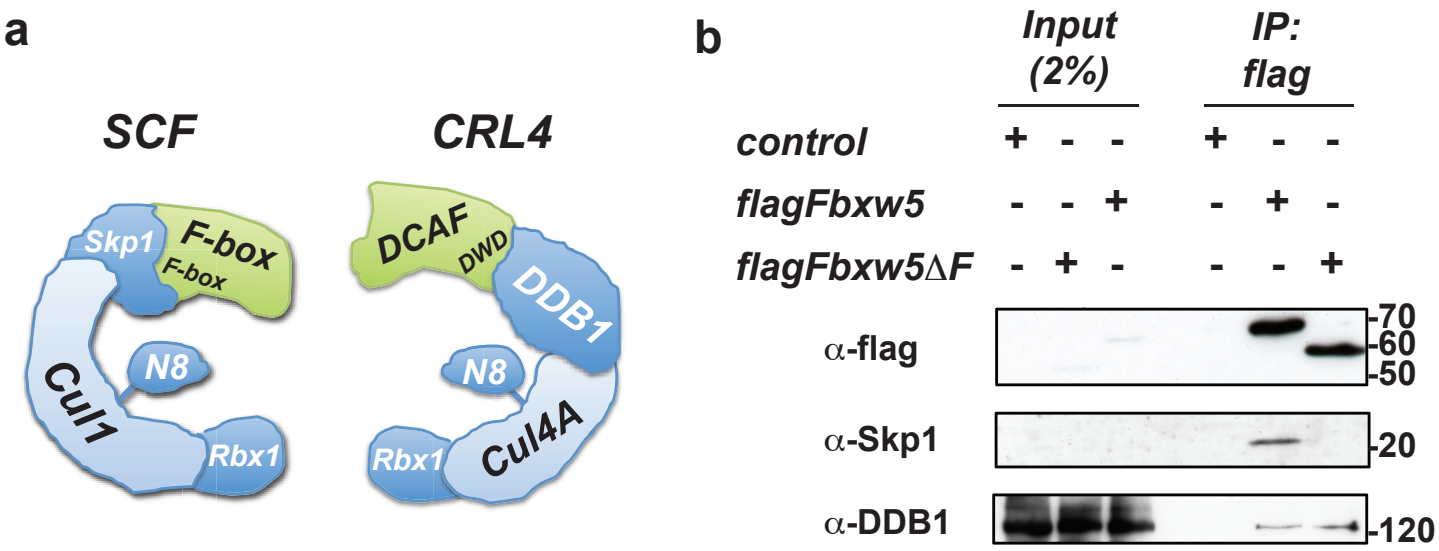

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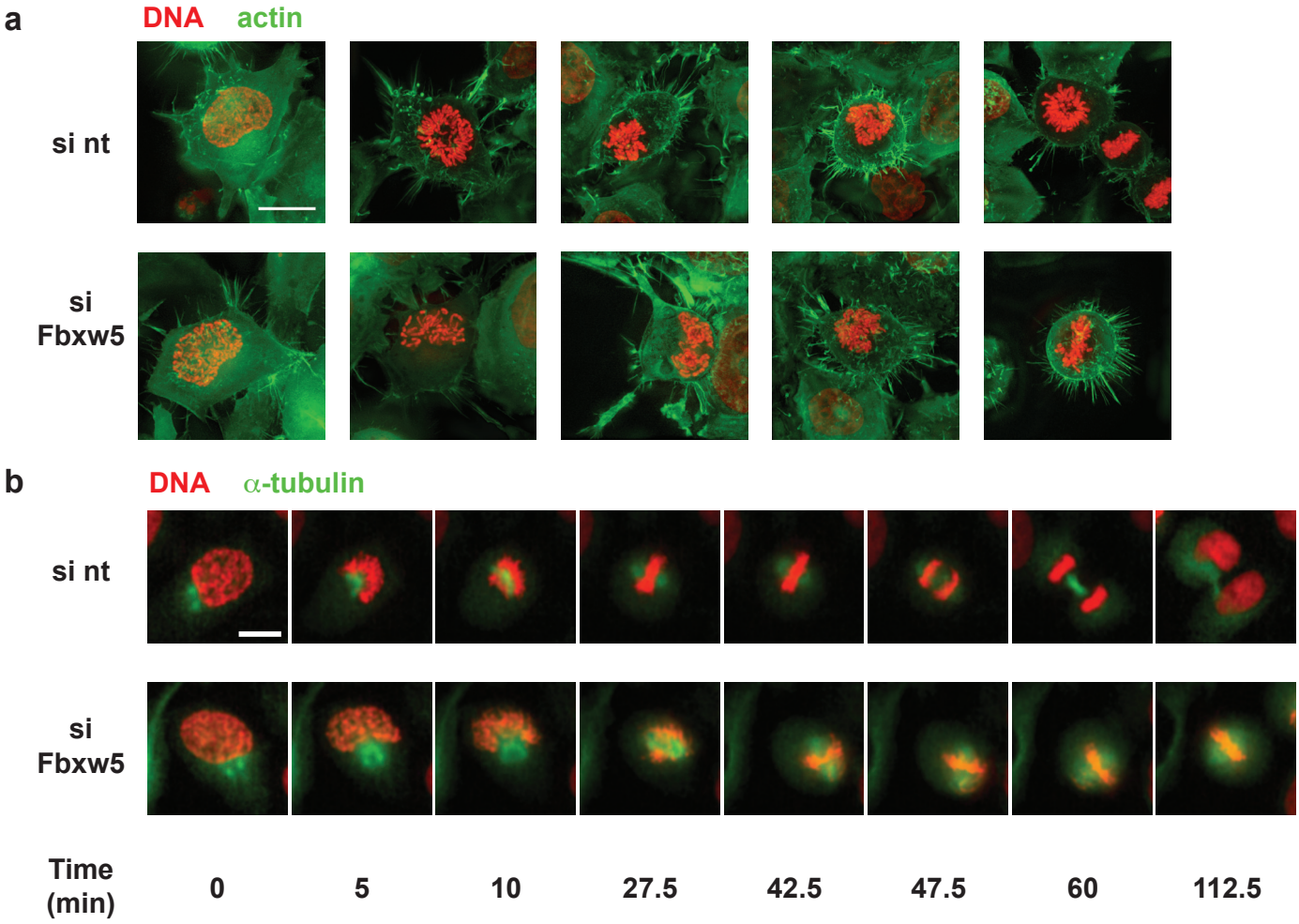
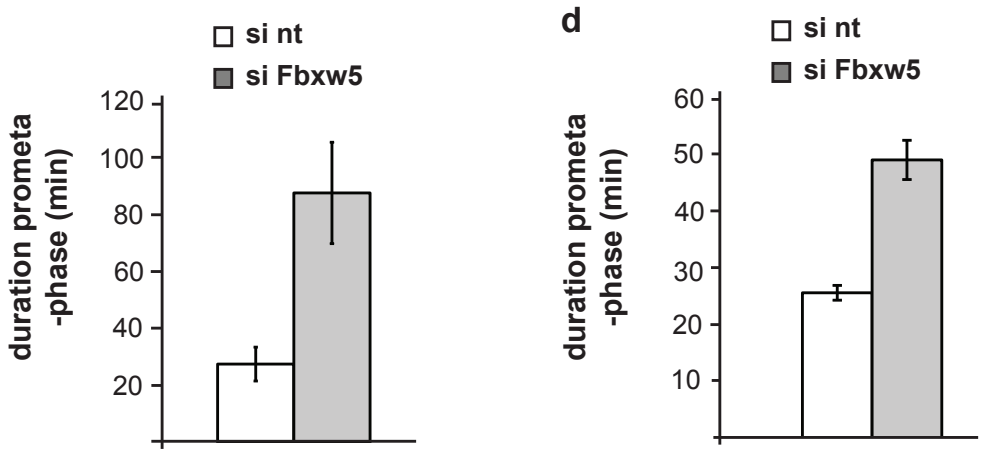
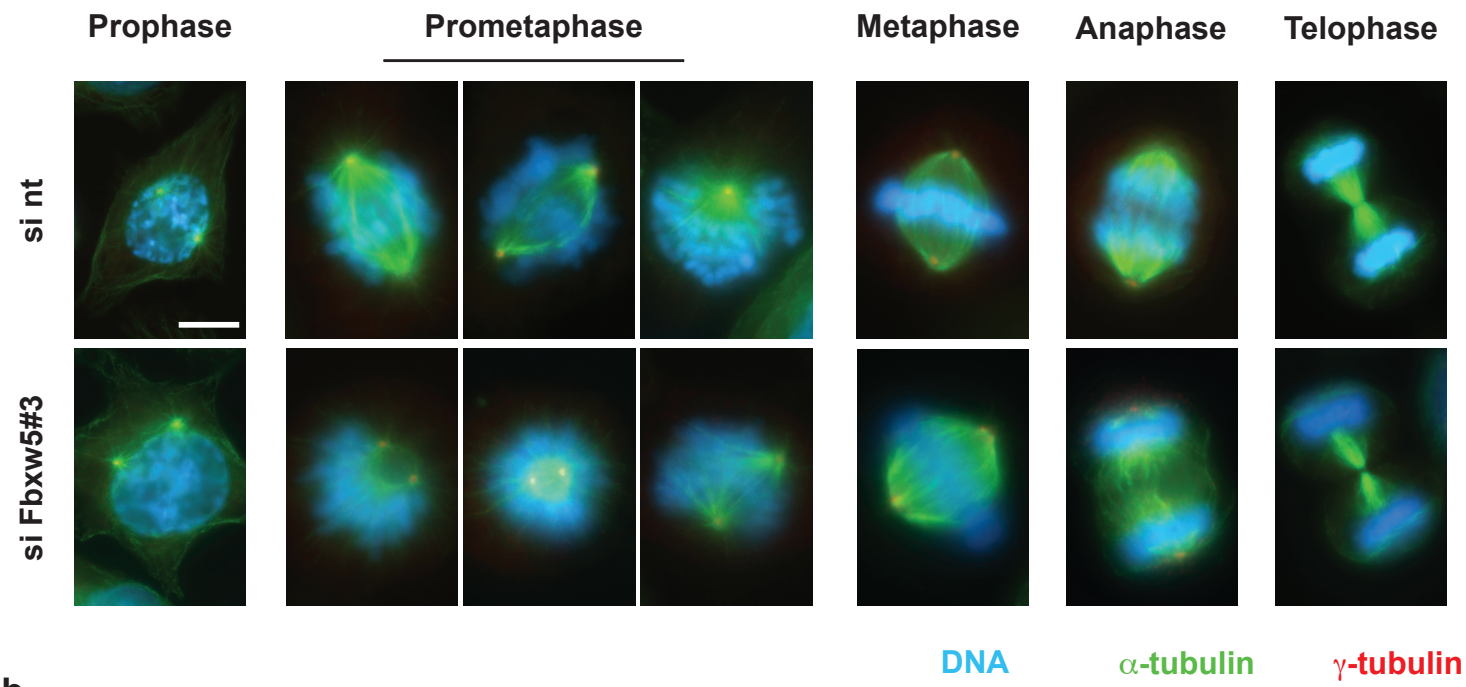




SUPPL FIGURE 4 (Melchior)

a



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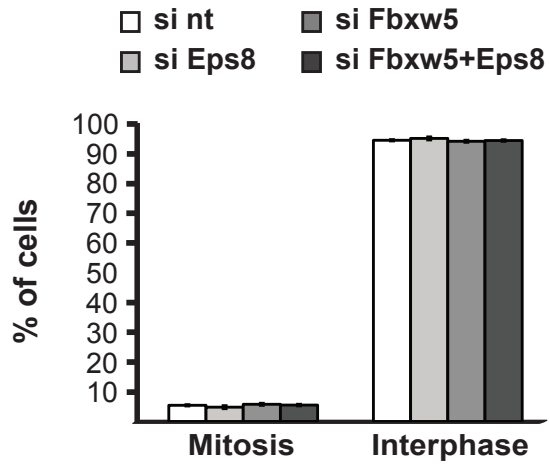

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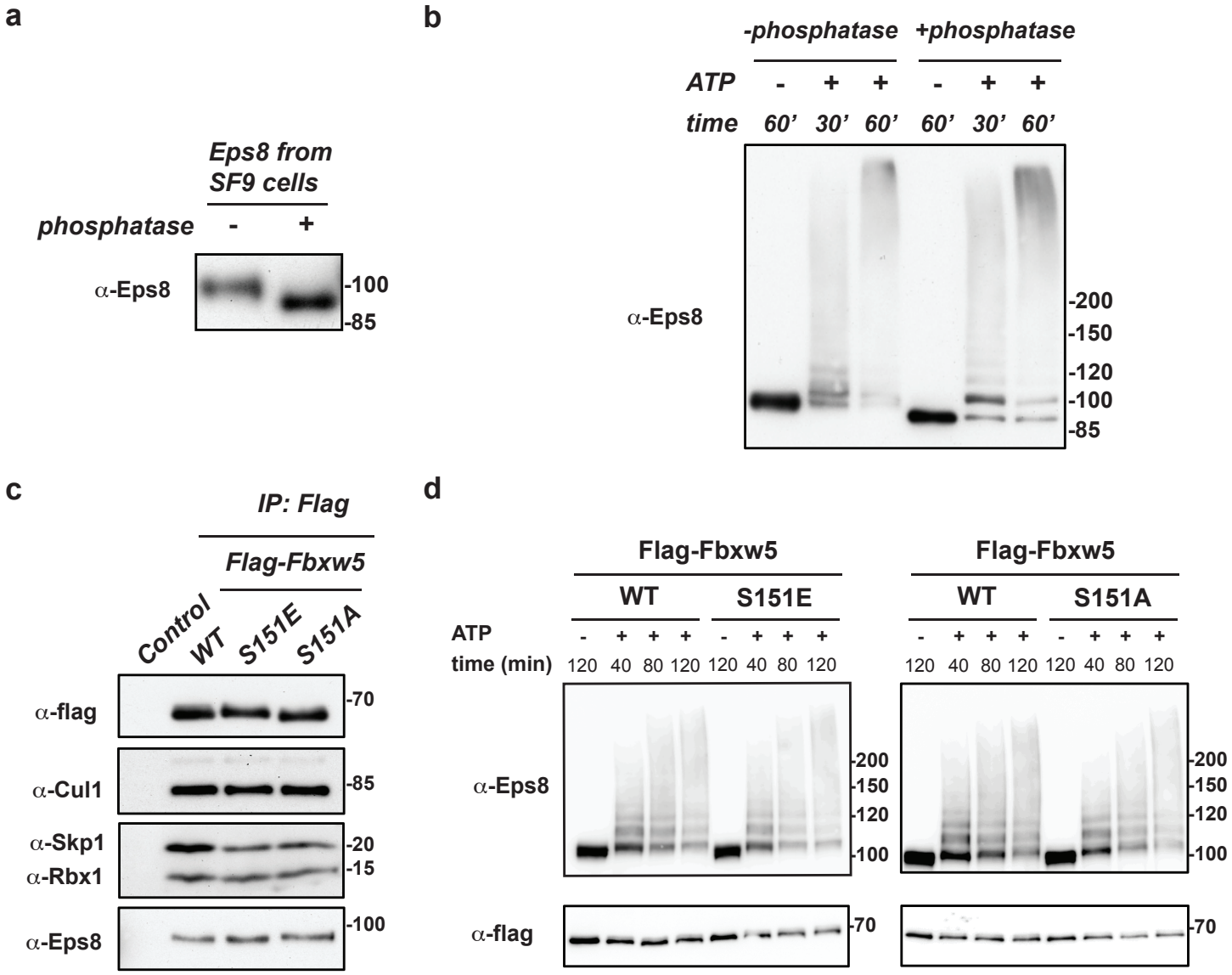


SUPPL FIGURE 6 (Melchior)

Figure 1 a

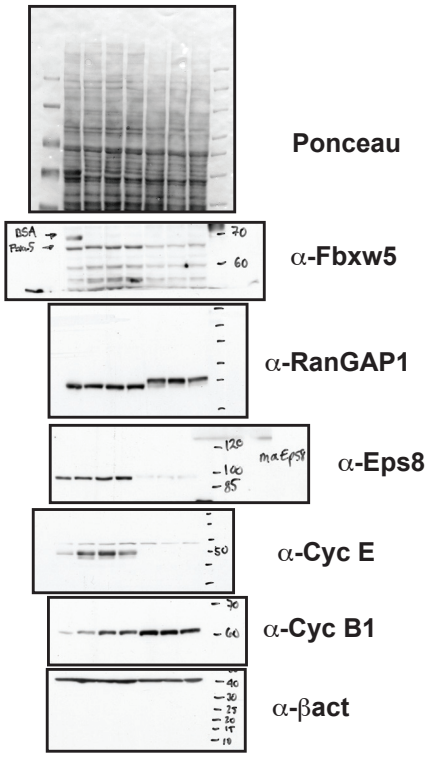


Figure 1 b

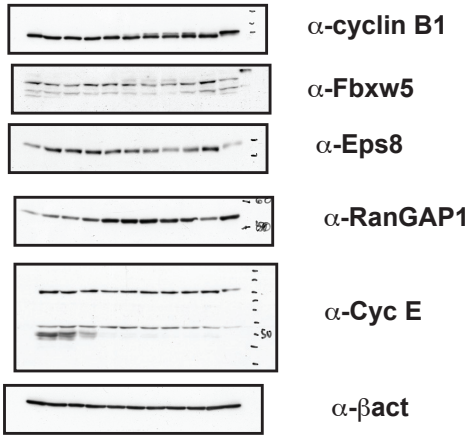


Figure 1c

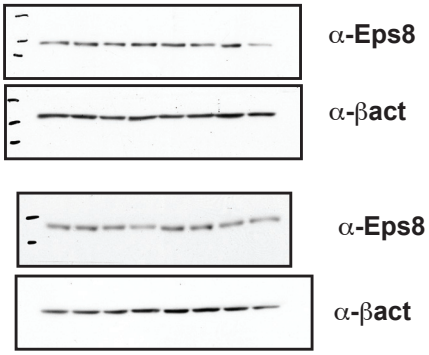


Figure 1 d

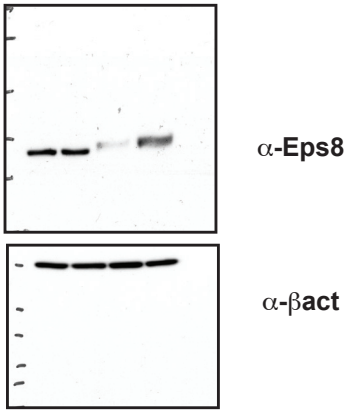


Figure 3a

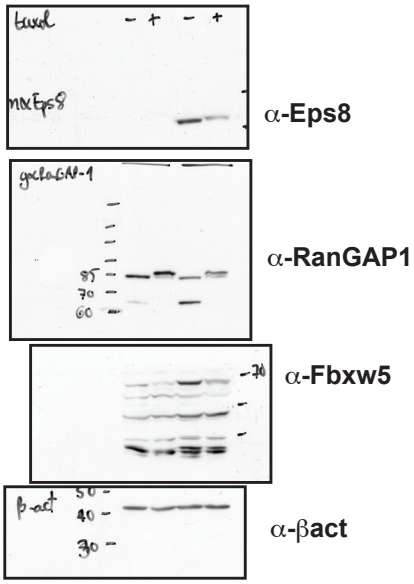


Figure 4b

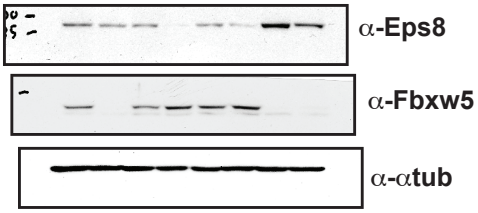
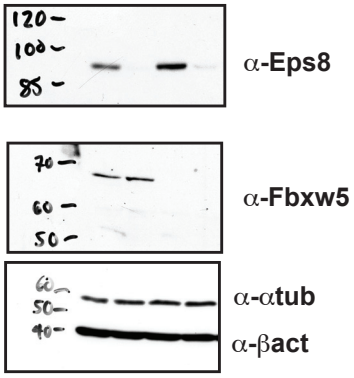


Figure 5a



SUPPL FIGURE 7 (Melchior)