Supplementary Information

Acetylation of Aurora B by TIP60 ensures accurate chromosomal segregation

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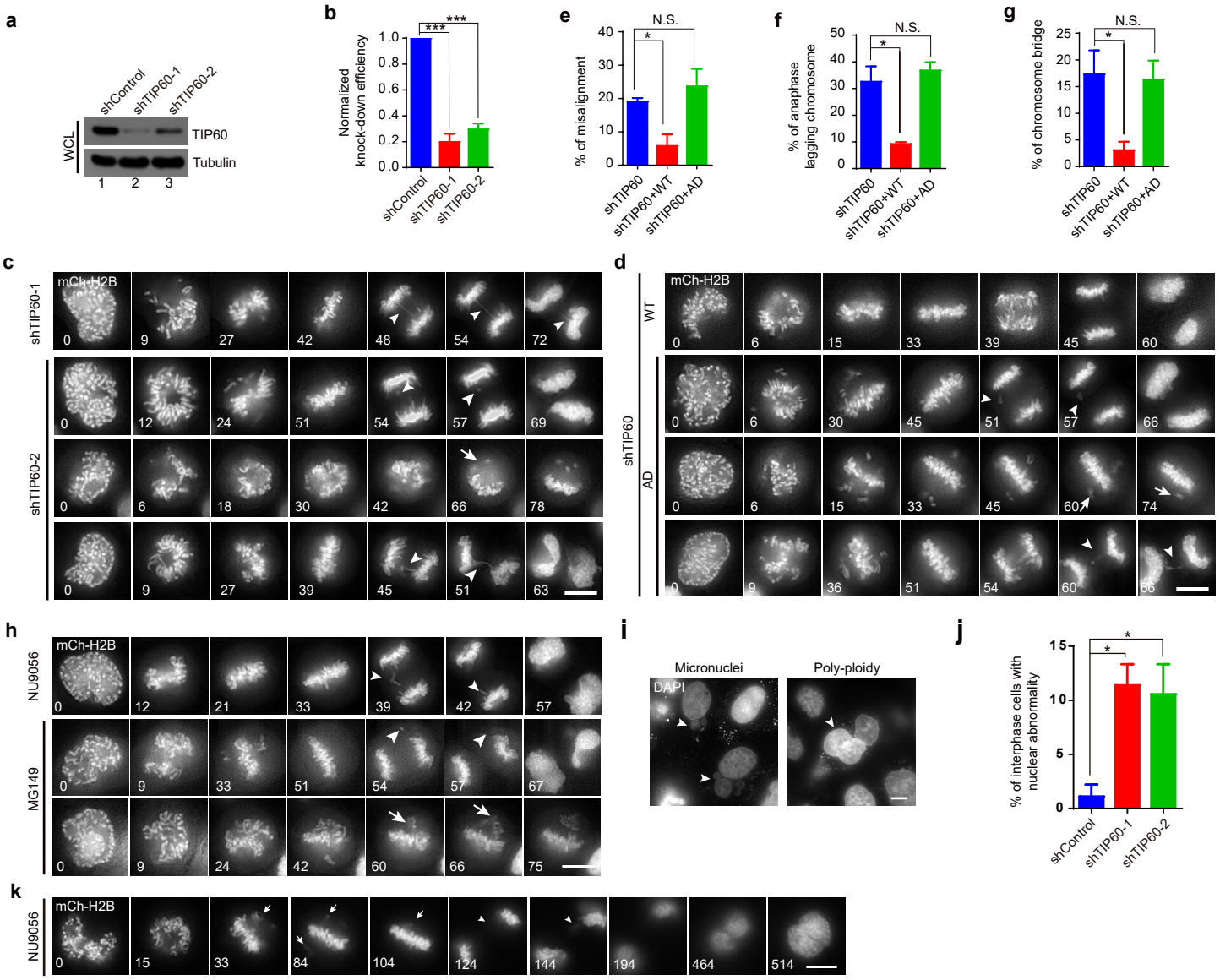
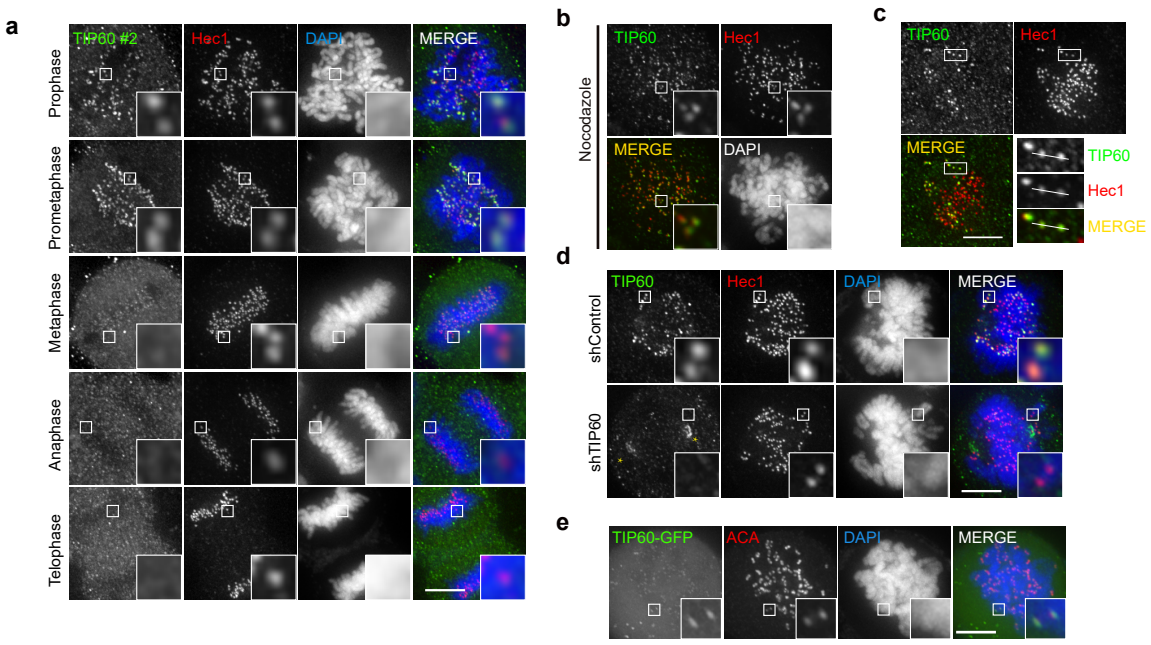
Supplementary Results
Supplementary Figure 1



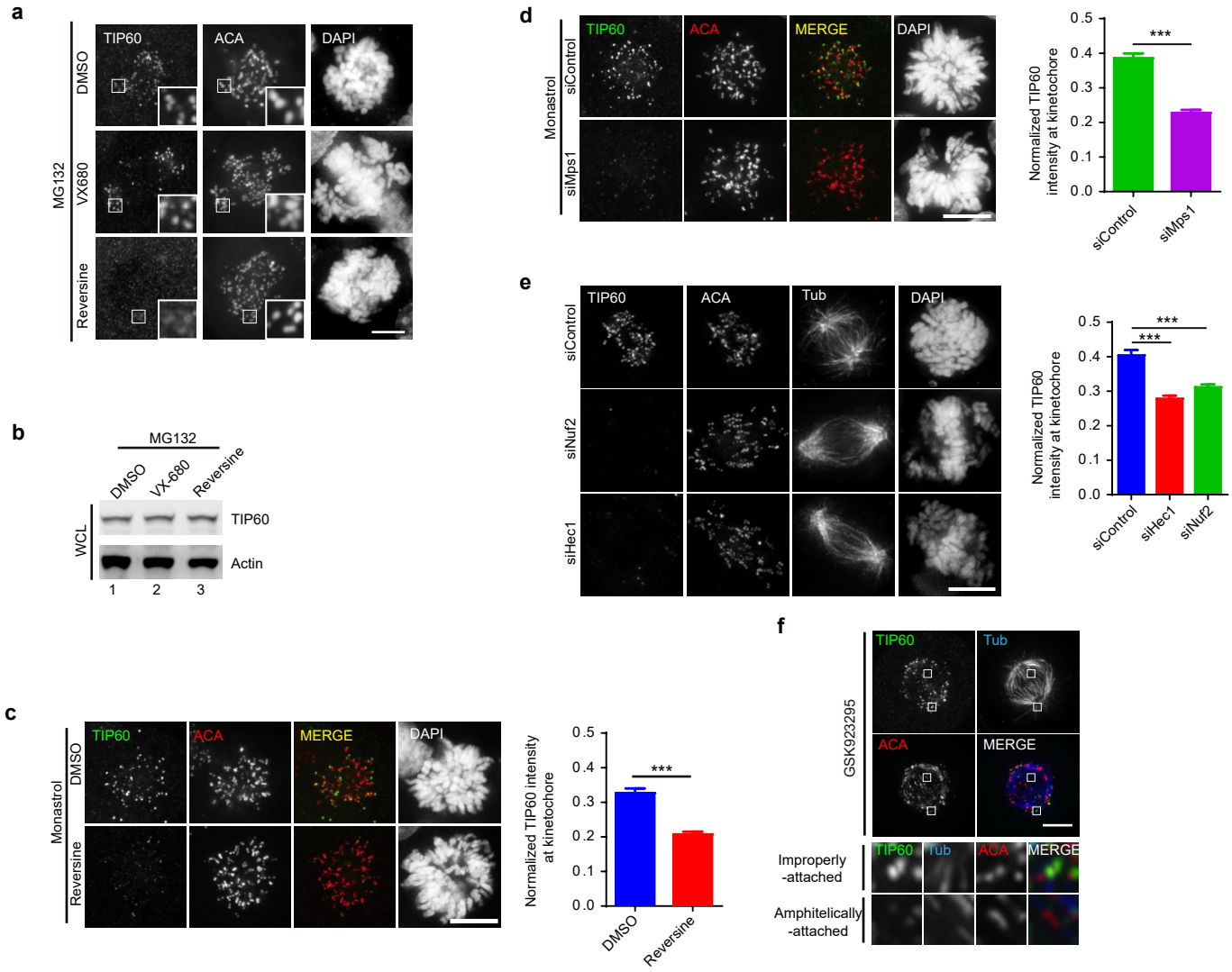
Supplementary Figure 1 | TIP60 was required for accurate chromosome segregation in mitosis.
(**a**) Knockdown efficiency of shTIP60-1 and shTIP60-2. Lysates of HeLa cells expressing control or TIP60 shRNA were probed with TIP60 and Tubulin antibodies. (**b**) Quantification of RNAi efficiency in **a**. Data represent Mean ± S.E.M from three independent experiments. (**c**) Time-lapse analysis of TIP60-depleted HeLa cells. Representative cells with chromosome bridge (arrow head), chromosome bridge (arrow head) or misalignment (arrow) were shown. (**d**) HeLa cells stably expressing mCherry-H2B were transiently transfected to express wild type (WT) and acetyltransferase-deficient (AD) TIP60 in the absence of endogenous TIP60 for time-lapse analysis. (**e-g**) Quantification of indicated phenotypes of TIP60-depleted HeLa cells rescued by TIP60-WT (n=113) or TIP60-AD (n=111). Data represented Mean ± S.E.M from three independent experiments. (**h**) Time-lapse imaging of HeLa cells treated with TIP60 inhibitor NU9056 (20 μM) or MG149 (100 μM) immediately after nuclear envelope breakdown. (**i**) Representative HeLa cells with micronucleus and polyploidy after TIP60 depletion. (**j**) Quantification of interphase HeLa cells with micronuclei and polyploidy as shown in **e**. Data represent Mean ± S.E.M of three independent experiments (n=104, shControl; n=123, shTIP60-1; n=122, shTIP60-2). (**k**) Time lapse analysis shown polyploidy generated from HeLa cells treated with 20 μM NU9056 immediately after nuclear envelope breakdown. Statistical significance was tested by two-sided *t*-test and represented by asterisks corresponding to *, $p < 0.05$; ***, $p < 0.001$ and N.S., $p > 0.05$. Scale bars, 5 μm.

Supplementary Figure 2



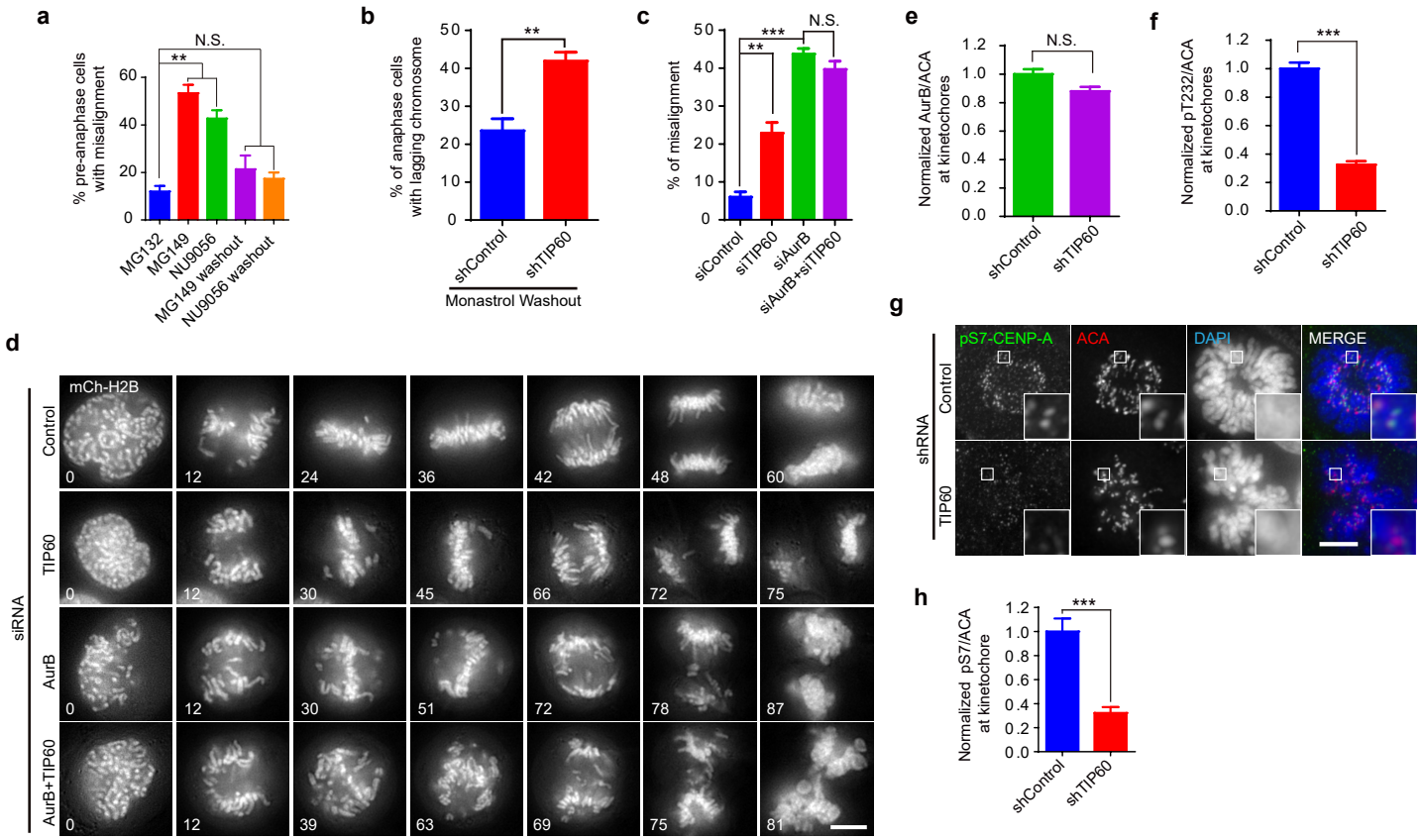
Supplementary Figure 2 | TIP60 localized to kinetochores in early mitosis.
(**a**) Immunostaining of HeLa cell with TIP60#2 antibody. Fixed HeLa cells were stained with TIP60#2 antibody, Hec1 antibody and DAPI. (**b**) An aliquot of HeLa cells was treated with nocodazole (100 ng/mL) followed by immunofluorescence staining. (**c**) Prometaphase HeLa cell labeled with TIP60 and Hec1 antibodies for line scan. (**d**) Validation of TIP60 antibody. HeLa cells expressing control shRNA or TIP60 shRNA were stained with TIP60 antibody, Hec1 antibody and DAPI. (*, unspecific staining) (**e**) Localization of TIP60-GFP in prometaphase HeLa cells. Scale bars, 5 μm.

Supplementary Figure 3



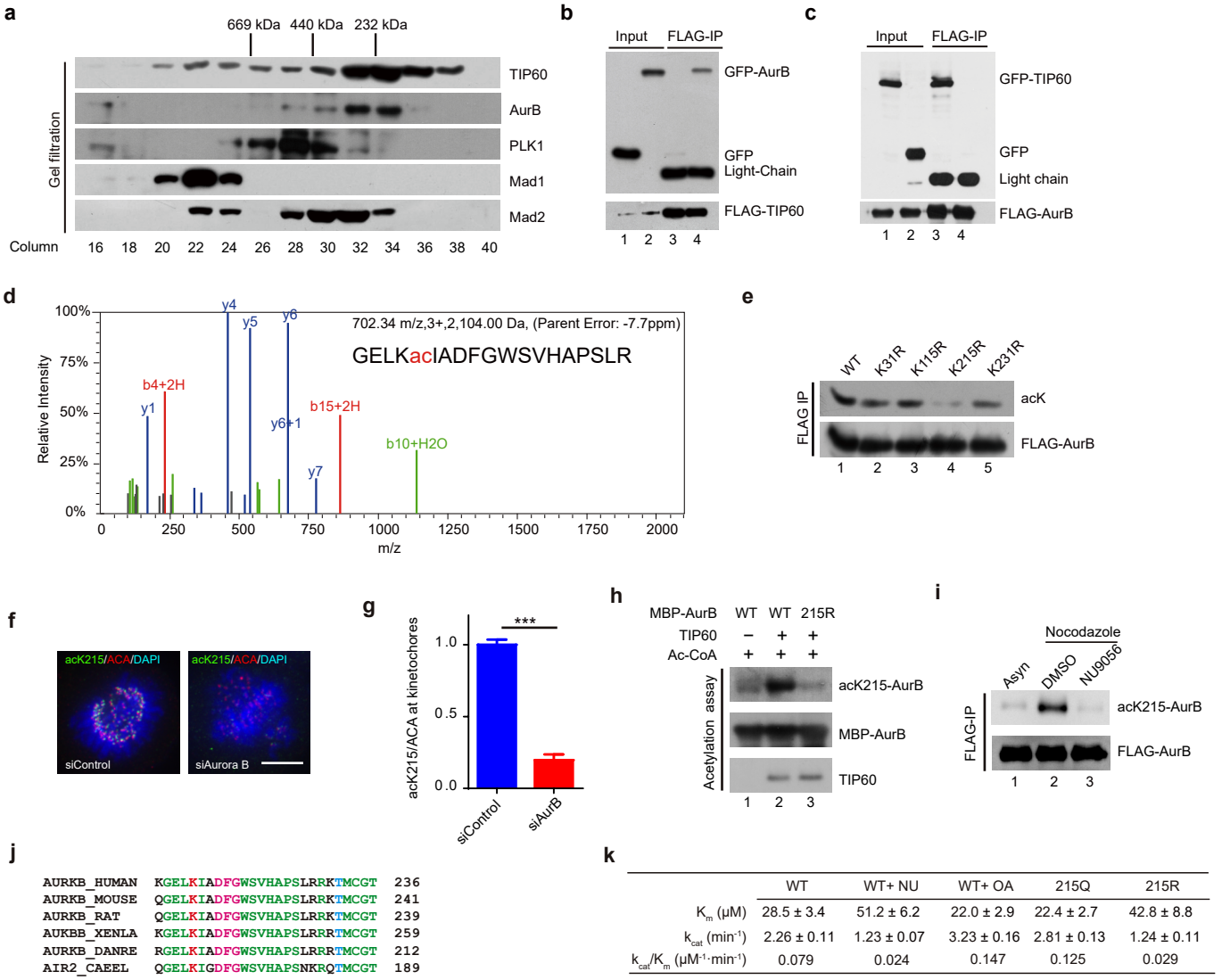
Supplementary Figure 3 | Mps1 activity was required for kinetochore localization of TIP60.
(**a**) Localization of TIP60 in Mps1 inhibited cells. HeLa cells were treated with MG132 plus either Aurora kinase inhibitor VX680 (500 nM) or Mps1 inhibitor Reversine (1 μM) before fixation. (**b**) TIP60 protein level in **a** was validated by Western blot assay. (**c**) Localization of TIP60 in Mps1 inhibited HeLa cells. Monastrol arrested HeLa cells was treated with MG132 (10 μM) plus DMSO or Reversine (1 μ M) before fixation. Relative TIP60 intensity at kinetochores was quantified in the right. Data represent Mean ± S.E.M. and were examined with two-sided *t*-test. Experiment was repeated for three times independently (n=100). (**d**) Localization of TIP60 in Mps1 knocked-down HeLa cells. Control and Mps1 siRNA transfected cells were stained with TIP60 and ACA antibodies. Relative TIP60 intensity at kinetochores was quantified in the right. Data represent Mean ± S.E.M. and were examined with two-sided *t*-test. Experiment was repeated for three times independently (n=100). (**e**) Localization of TIP60 in NDC80 knocked-down HeLa cells. HeLa cells transfected with Control, Hec1 or Nuf2 siRNAs were stained with TIP60 and ACA antibodies. Data represent Mean ± S.E.M. and were examined with two-sided *t*-test. Experiment was repeated for three times independently (n=100). (**f**) HeLa cells were treated with mitotic kinesin CENP-E inhibitor GSK923295 (50 nM) for 1 h to generate misaligned kinetochores. After fixation, cells were stained for ACA, tubulin, and TIP60. Statistical significance was tested by two-sided *t*-test and represented by asterisks corresponding to ***, $p < 0.001$. Scale bars, 5 μm.

Supplementary Figure 4



Supplementary Figure 4 | TIP60 promoted correction of erroneous attachment.
(**a**) HeLa cells were synchronized to mitosis by a thymidine block and release. The mitotic cells were incubated with monastrol (50 μM) for 1.5 h to generate erroneous attachments. The monastrol was washed out, and cells were treated with MG132 (10 μM) alone or in combination with NU9056 (20 μM) or MG149 (100 μM) for 1 h before fixation. In some cases, the NU9056 and MG149 were washed out, and cells were released into fresh media containing MG132 for another 1 h before fixation. In each condition, 75 kinetochores were examined from three independent experiments while data represented as Mean ± S.E.M. (**b**) HeLa cells expressing control shRNA or TIP60 shRNA were synchronized to mitosis by thymidine release. The mitotic cells were incubated with 50 μM monastrol for 1.5 h to generate erroneous attachment. After monastrol washout, the cells were released to fresh medium for 1 h before fixation. Cells with lagging chromosomes in anaphase were quantified under microscope. Error bars represent Mean ± S.E.M from three independent experiments (shControl, n=100; shTIP60, n=113). (**c-d**) Quantification of misalignment phenotypes in cells transfected with indicated siRNAs. Error bars represent Mean ± S.E.M from three independent experiments (shControl, n=67; shTIP60, n=76; shAurB, n=64; shAurB/TIP60, n=65). (**e-f**) Normalized pixel intensities of Aurora B (**e**) and pT232 (**f**) in Figure 2e in either control shRNA cells or TIP60 shRNA expressing cells were quantified and presented as Mean ± S.E.M. More than 62 kinetochores (67 kinetochores for shControl and 62 kinetochores for shTIP60) were examined from three independent experiments. (**g**) HeLa cells expressing TIP60 shRNA or Control shRNA was fixed and stained with indicated antibodies. (**h**) Quantification of immunofluorescence intensity in **g**. Data represent Mean ± S.E.M. and were examined with two-sided *t*-test. Experiment was repeated for three times independently (n=100). Statistical significance was tested by two-sided *t*-test and represented by asterisks corresponding to **, $p < 0.01$; ***, $p < 0.001$ and N.S., $p > 0.05$. Scale bars, 5 μm.

Supplementary Figure 5

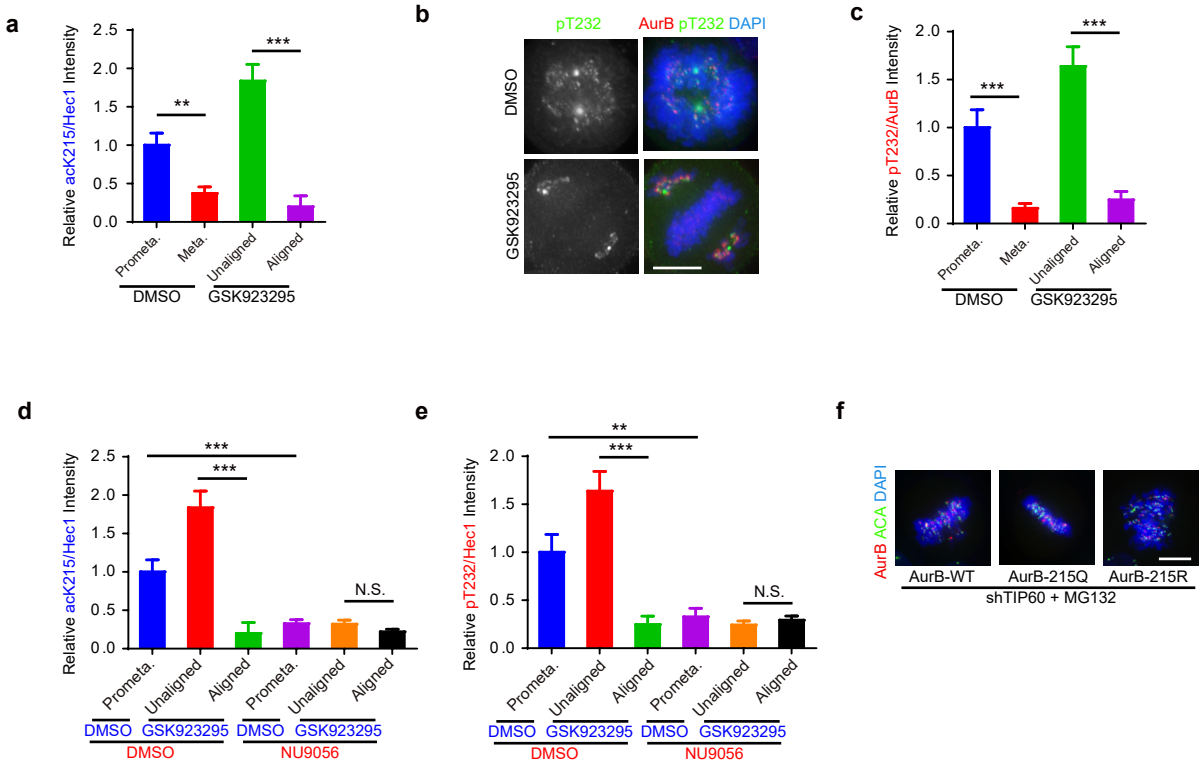


k

	WT	WT+ NU	WT+ OA	215Q	215R
K_m (μM)	28.5 ± 3.4	51.2 ± 6.2	22.0 ± 2.9	22.4 ± 2.7	42.8 ± 8.8
k_{cat} (min^{-1})	2.26 ± 0.11	1.23 ± 0.07	3.23 ± 0.16	2.81 ± 0.13	1.24 ± 0.11
k_{cat}/K_m ($\mu M^{-1} \cdot min^{-1}$)	0.079	0.024	0.147	0.125	0.029

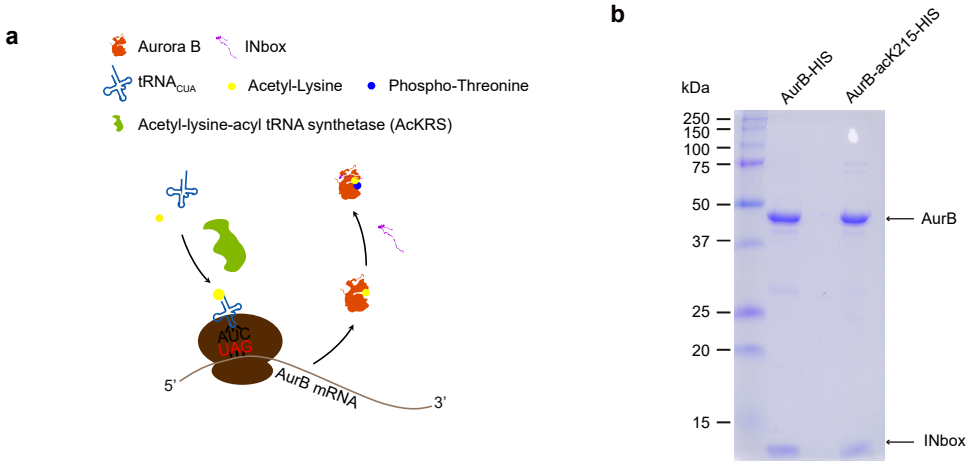
Supplementary Figure 5 | TIP60 forms a complex with Aurora B in mitotic cells.
(**a**) Analysis of TIP60-Aurora B complex using fast protein chromatography. Cell lysates were generated from mitotic HeLa cells, clarified and subjected to gel filtration chromatograph followed by Western blotting analyses with antibodies against TIP60, Aurora B, PLK1, Mad1 and Mad2. (**b**) Co-immunoprecipitation assay of FLAG-TIP60 and GFP-Aurora B in mitotic HeLa cells. (**c**) Co-immunoprecipitation assay of FLAG-Aurora B and GFP-TIP60 in mitotic HeLa cells.. (**d**) Mass spectrometry of Aurora B acetylation. (**e**) Mitotic cells expressing Flag-tagged wild-type or the indicated mutations of Aurora B were treated with Trichostatin A (TSA, 1 μM) and Nicotinamide (NAM, 5 mM) for 4 h to prevent deacetylation before harvest. FLAG-Aurora B was immunoprecipitated, and the acetylation levels were assessed with an anti-acetyl-lysine antibody. (**f**) Validation of acK215 antibody. HeLa cells transfected with control siRNA or Aurora B siRNA was stained with acK215 antibody and ACA. Scale bar, 5 μm. (**g**) Quantitative analyses of acK215/ACA levels at kinetochores in cells expressing control siRNA or Aurora B siRNA. Data represent Mean ± S.E.M. and were examined with two-sided *t*-test. Experiment was repeated for three times independently (n=73, siControl; n=68, siAurB). (**h**) Validation of Lys215 acetylation in vitro. MBP-Aurora B wild type (WT) or the K215R mutant was incubated with Ac-CoA and TIP60 for 2 h. Aurora B-acetylation levels were probed by a specific anti-acetyl-K215-Aurora B antibody. (**i**) FLAG-Aurora B was purified from asynchronized HeLa cell lysates (Asyn) or nocodazole synchronized mitotic HeLa cell lysates. In some cases, 20 μM NU9056 was added to the culture for 1 h before cell collection. The acetylation level of FLAG-Aurora B was analyzed by Western Blot. (**j**) Protein sequence alignment of Aurora B in different species. (**k**) The enzyme kinetics of Aurora B calculated according to the Michaelis-Menten equation. Statistical significance was tested by two-sided *t*-test and represented by asterisks corresponding to ***, $p < 0.001$. Scale bars, 5 μm.

Supplementary Figure 6



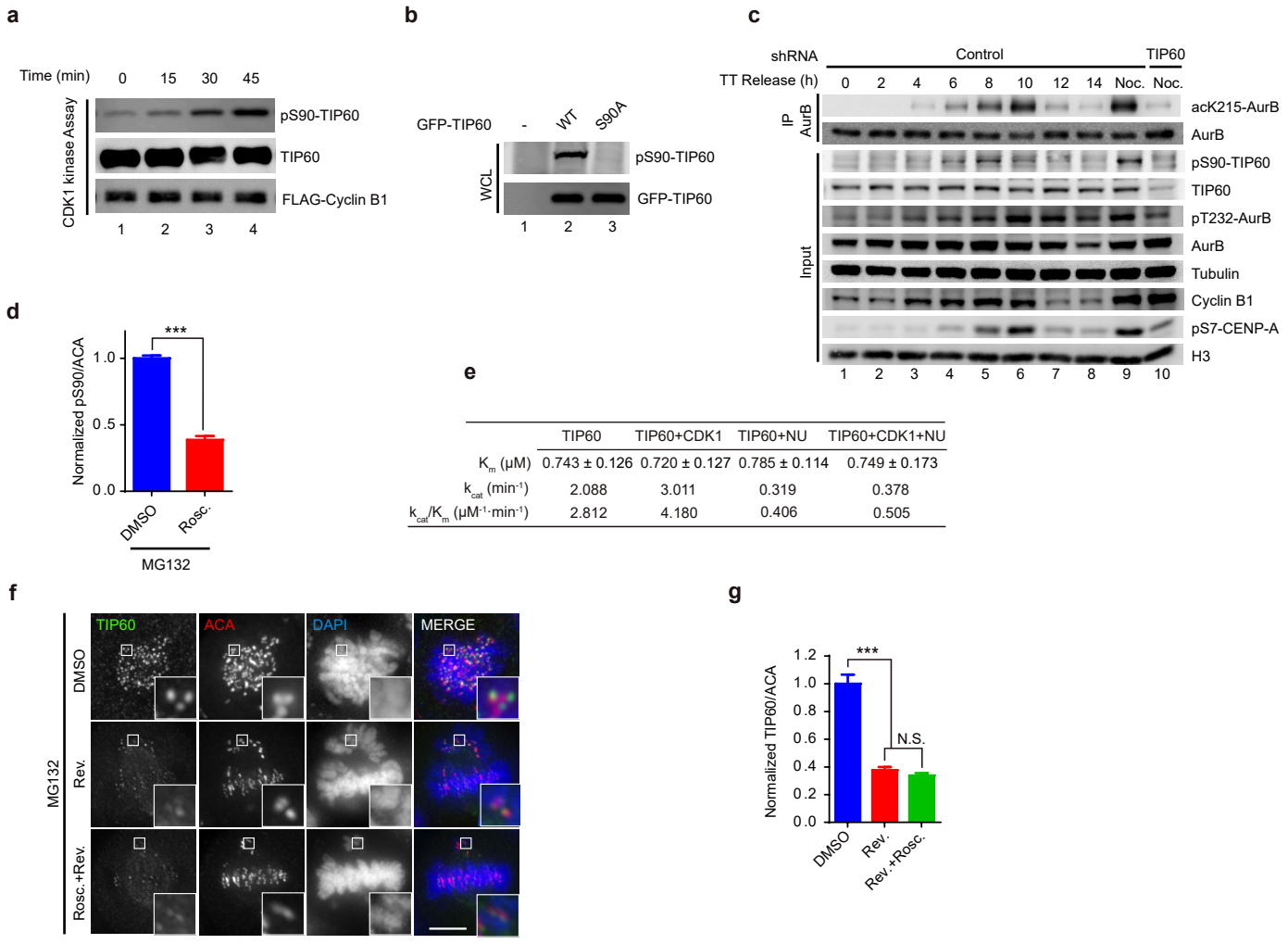
Supplementary Figure 6 | TIP60 promotes Aurora B activity at misaligned kinetochores.
(**a**) Quantitative analysis of relative intensity of acK215/Hec1 at kinetochores in HeLa cells treated with DMSO or GSK923295. Data represent Mean ± S.E.M. and were analyzed with two-sided t-test. A total of 50 kinetochores were examined from three independent experiments. (**b**) Immunofluorescence staining of pT232 and Aurora B, and with DAPI in DMSO or GSK923295 treated HeLa cells. (**c**) Quantitative analyses of pT232/Aurora B levels at indicated kinetochores. Data represent Mean ± S.E.M. and were examined with two-sided *t*-test. Experiment was repeated for three times independently (n=50). (**d-e**) Levels of acK215 or pT232 as a function of TIP60 activity. Quantitative analyses of acK215/Hec1, pT232/Aurora B levels at kinetochores of unaligned and apparently aligned chromosomes. Note that TIP60 inhibitor treatment abolished the immunofluorescence staining signals of acK215 and pT232 at unaligned kinetochores but did not correct the misalignment. Data represented Mean ± S.E.M. and were examined with two-sided *t*-test. Experiment was repeated for three times independently (n=50). (**f**) HeLa cells expressing TIP60 shRNA were transfected with Aurora B wild type or mutants. Statistical significance was tested by two-sided *t*-test and represented by asterisks corresponding to **, $p < 0.01$; ***, $p < 0.001$ and N.S., $p > 0.05$. Scale bars, 5 μm.

Supplementary Figure 7



Supplementary Figure 7 | K215 acetylated Aurora B was generated in vitro by an acetylated-protein recombinant system. (**a**) Schematic diagram showing the expression of acK215-Aurora B/INbox complex in *E. coli*. The UAG in the mRNA of Aurora B was decoded by tRNA$_{CUA}$ in the presence of N-ε-acetyl-lysine, giving rise to acK215-Aurora B. The acK215-Aurora B then formed a complex with INbox, which promoted auto-phosphorylation of Aurora B at Thr232. NAM (20 mM) was added to prevent deacetylation. (**b**) Validation of AurB-HIS and AurB-ac215-HIS protein via Coomassie Brilliant Blue staining.

Supplementary Figure 8



Supplementary Figure 8 | CDK1-Cyclin B1 phosphorylated TIP60 at Ser90.
(**a**) TIP60 was incubated with CDK1/Cyclin B1 for indicated time and analyzed by Western blot assay. (**b**) HeLa cells expressing wild-type GFP-TIP60 or S90A mutant was analyzed by TIP60-S90 antibody. (**c**) HeLa cells expressing either Control shRNA or TIP60 shRNA were arrested by nocodazole or synchronized to the indicated time points by double thymidine release. A portion of Aurora B kinase was immunoprecipitated and probed for its Lys215 acetylation level and other indicated antibodies. (**d**) Quantification of pS90 intensity at kinetochores in DMSO or Roscovitine treated HeLa cells. 50 kinetochores per group were examined from three independent experiments. The data were expressed as Mean ± S.E.M and analyzed with two-sided *t*-test. (**e**) The enzyme kinetics of TIP60 was calculated according to the Michaelis-Menten equation. (**f**) TIP60 localization in HeLa cells treated with indicated inhibitors or DMSO. (**g**) Quantification of fluorescence intensity in **f**. Experiment was repeated for three times independently (DMSO, n=58; Rev., n=60; Rev./Rosc., n=55). Statistical significance was tested by two-sided *t*-test and represented by asterisks corresponding to ***, $p < 0.001$ and N.S., $p > 0.05$. Scale bars, 5 μm. Rosc., Roscovitine; Rev., Reversine.

Supplementary Figure 9

Fig. 2f

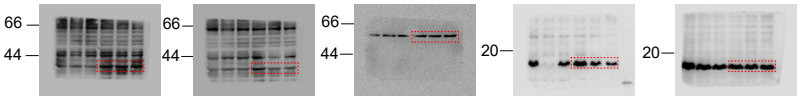


Fig. 3a

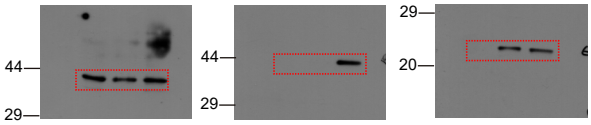


Fig. 3b

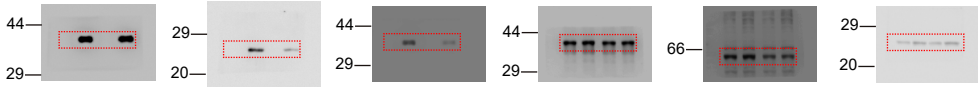


Fig. 3c

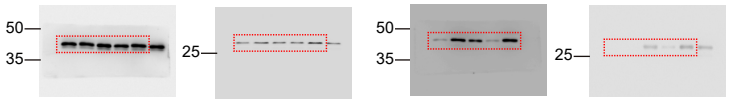


Fig. 4a

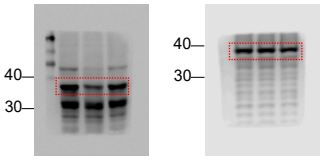


Fig. 4b

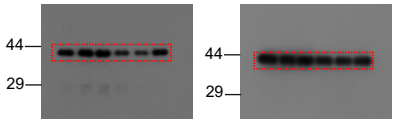


Fig. 4d

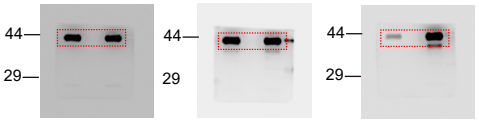


Fig. 4e

Fig. 4f

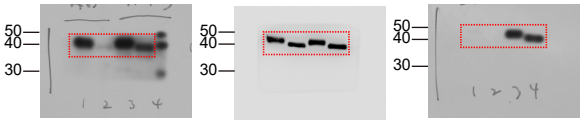


Fig. 4g

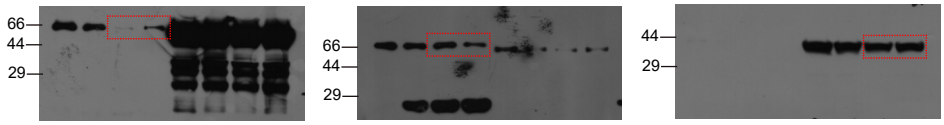


Fig. 4h

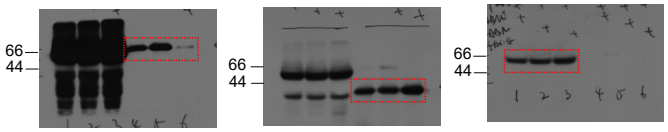


Fig. 4i

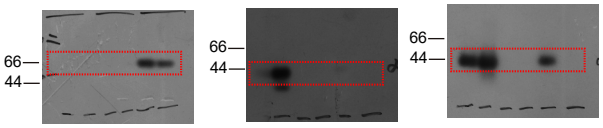


Fig. 5b

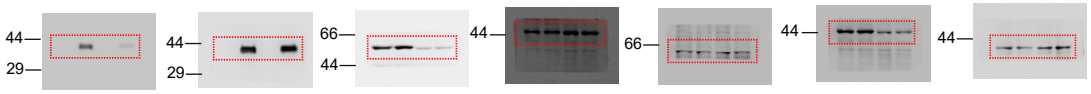
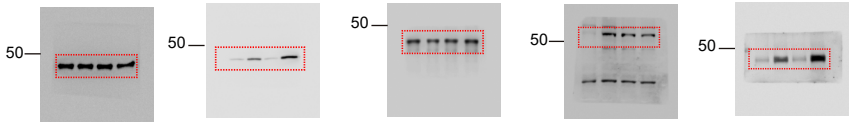
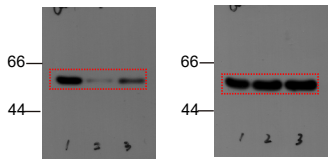


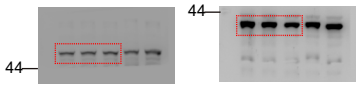
Fig. 5d



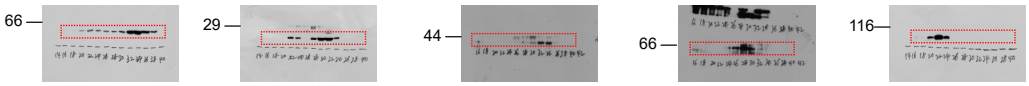
Supplementary Fig. 1a



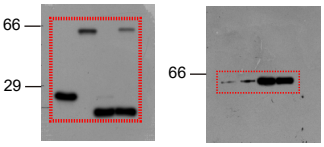
Supplementary Fig. 3b



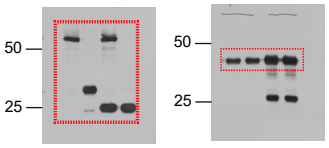
Supplementary Fig. 5a



Supplementary Fig. 5b



Supplementary Fig. 5c



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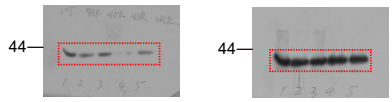
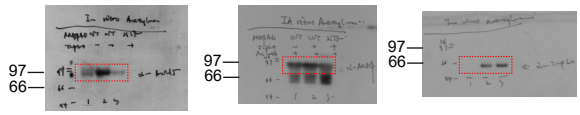
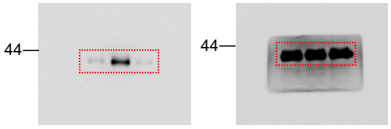


Supplementary Fig. 5h

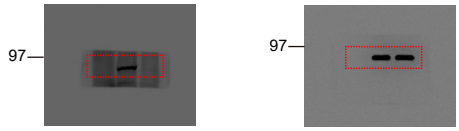


Supplementary Fig. 5i



Supplementary Fig. 8a

Supplementary Fig. 8b



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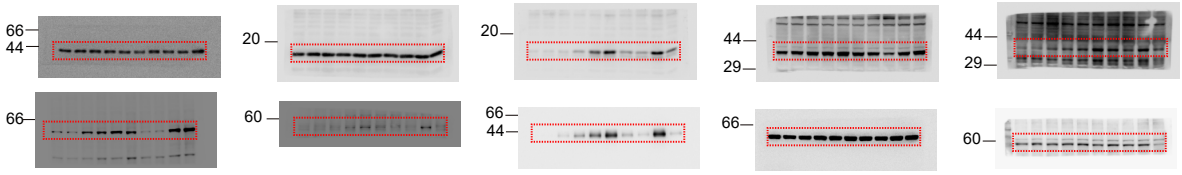

Supplementary Figure 9 | Full scans of original blots.