Supplementary Figures

hnRNPK S379 phosphorylation participates in migration regulation of triple negative MDA-MB-231 cells

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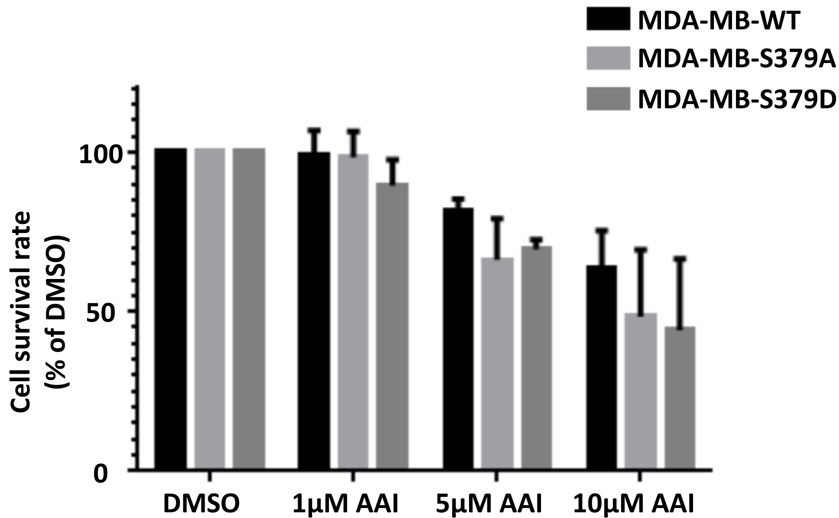
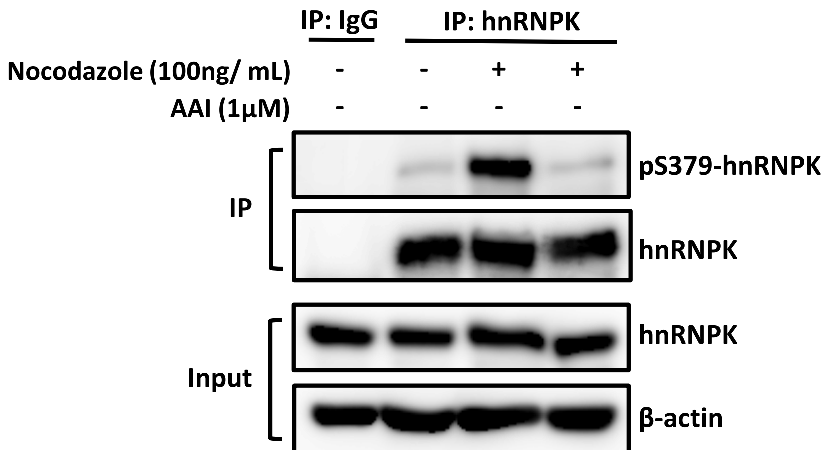
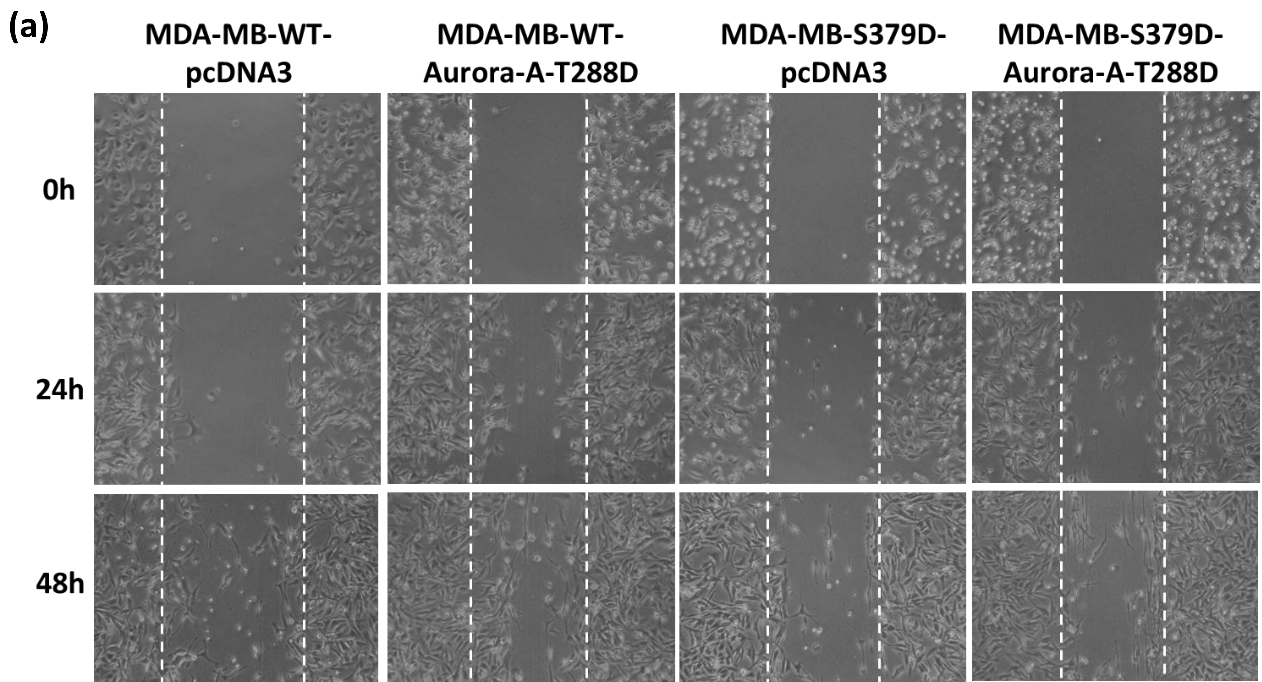
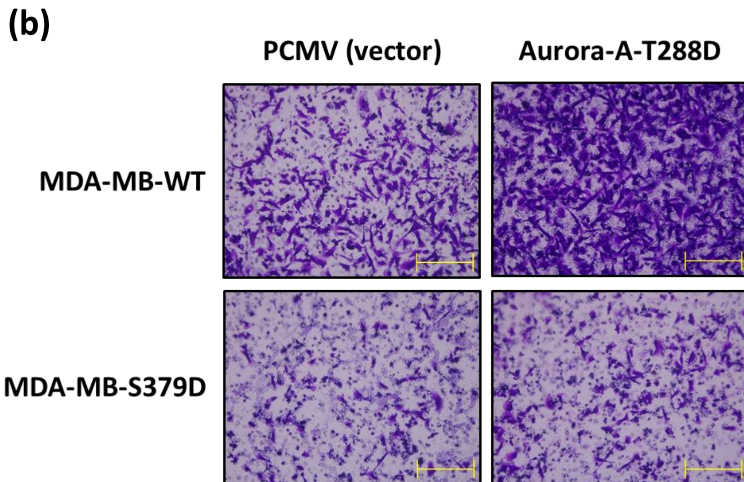
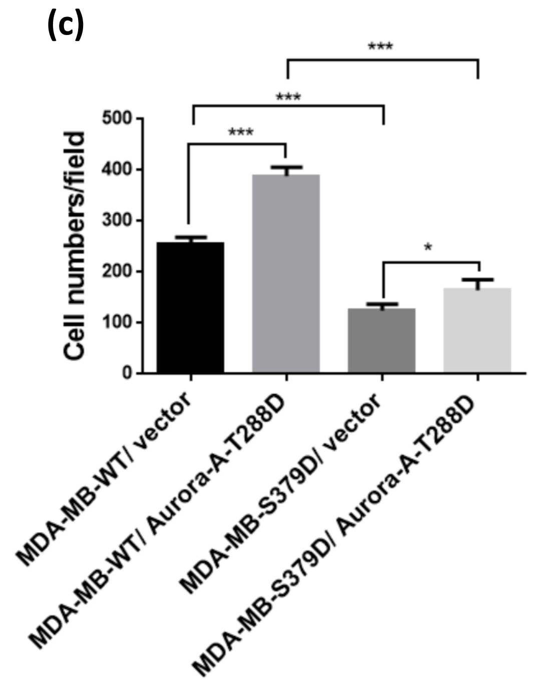


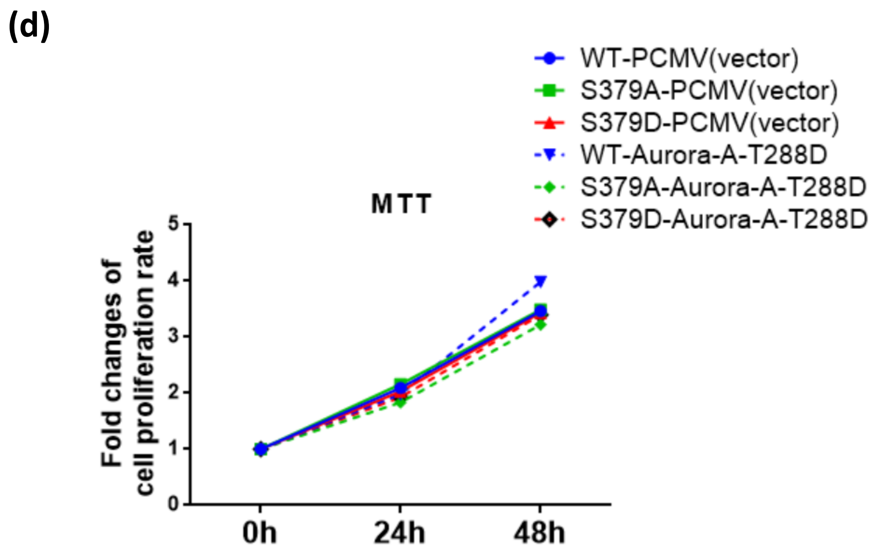


Supplementary Figure 1. Comparison of the cell survival rate and hnRNPK S379 phosphorylation across MDA-MB-WT, MDA-MB-S379A and MDA-MB-S379D cells upon inhibition of Aurora-A activity. (a) MDA-MB-WT, MDA-MB-S379A and MDA-MB-S379D cells exhibit similar cell survival rates after treatment with DMSO or 1, 5, 10μM of AAI treatment. MDA-MB-WT, MDA-MB-S379A and MDA-MB-S379D cells were respectively cultured in the present of DMSO only, or in the presence of 1μM, 5μM or 10μM of AAI for 20h. MTT assays were used to determine the cell survival rates of all three groups. **(b) S379 phosphorylation of hnRNPK is elevated by nocodazole treatment in MDA-MD-23 cells and this elevation is able to be suppressed by inhibition of Aurora-A activity.** MDA-MB-231 cells were cultured with DMSO or nocodazole in the presence or absence of AAI (1μM) for 20h. The hnRNPK in each sample was immunoprecipitated by anti-hnRNPK antibodies. The levels of hnRNPK S379 phosphorylation were then determined by Western blot analysis using the S379 phosphorylation-specific antibodies.

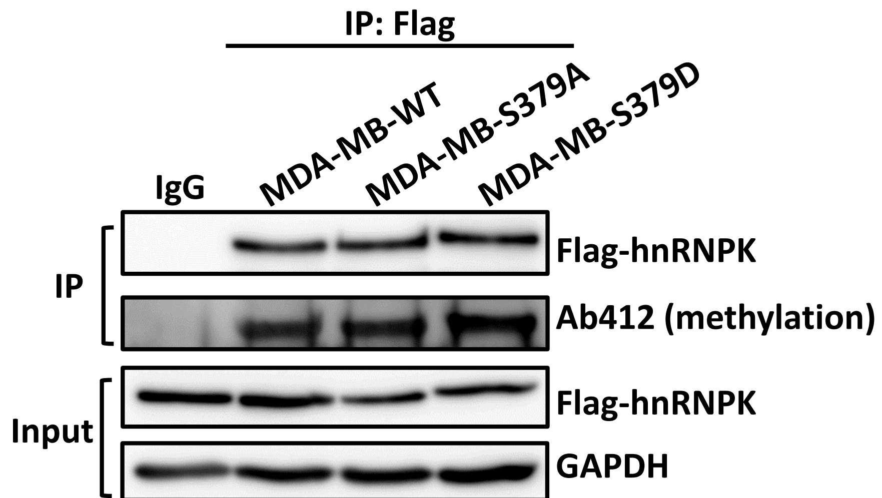

(a)
MDA-MB-WT-pcDNA3
MDA-MB-WT-Aurora-A-T288D
MDA-MB-S379D-pcDNA3
MDA-MB-S379D-Aurora-A-T288D
0h
24h
48h


(b)
PCMV (vector)
Aurora-A-T288D
MDA-MB-WT
MDA-MB-S379D

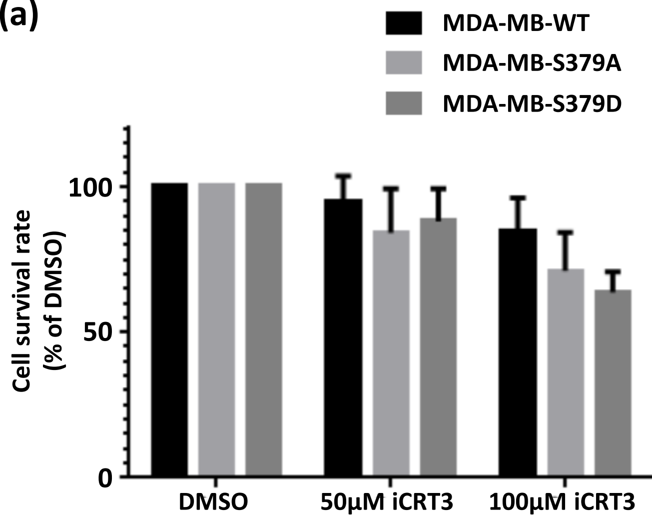

(c)
Cell numbers/field
500
400
300
200
100
0
***
***
***
*
MDA-MB-WT/ vector
MDA-MB-WT/ Aurora-A-T288D
MDA-MB-S379D/ vector
MDA-MB-S379D/ Aurora-A-T288D

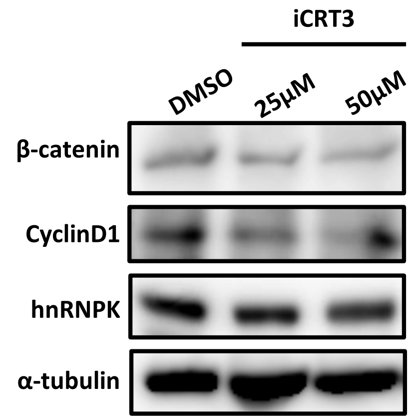

(d)
WT-PCMV(vector)
S379A-PCMV(vector)
S379D-PCMV(vector)
WT-Aurora-A-T288D
S379A-Aurora-A-T288D
S379D-Aurora-A-T288D
MTT
Fold changes of cell proliferation rate
5
4
3
2
1
0
0h
24h
48h

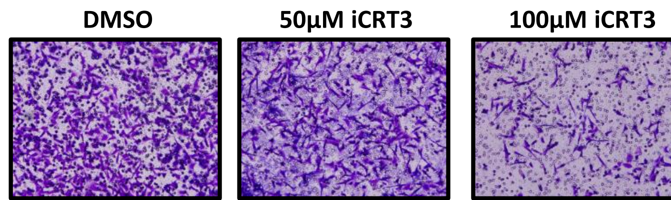
Supplementary Figure 2. MDA-MB-S379D cells overexpressed with T288D Aurora-A exhibit elevated migration ability but not increased proliferation. (a) Comparison of wound healing ability of MDA-MB-WT and MDA-MB-S379D cells upon T288D Aurora-A-transfection. A total of $5x10^5$ T288D Aurora-A-transfected MDA-MB-WT and MDA-MB-S379D cells were independently seeded into a 6-well plate and incubated for 24h. This was followed by scratching an area with fixed width on the plate using a 10μL tip. The two types of cell were allowed to migrate into the scratched area. The migrated cells were photographed using a microscope at 24h or 48h after the scratch was created. **(b) Comparison of migration ability of MDA-MB-WT and MDA-MB-S379D cells upon T288D Aurora-A-transfection.** A total of $3 X 10^5$ T288D Aurora-A-transfected MDA-MB-WT and MDA-MB-S379D cells were seeded into trans-wells for invasion assay for 20h. **(c)** The quantitative result for (b). **(d) Comparison of proliferation rates of MDA-MB-WT and MDA-MB-S379D cells upon T288D Aurora-A-transfection.** Measurement of proliferation rates was performed using the MTT assay at 0, 24 and 48h after seeding.

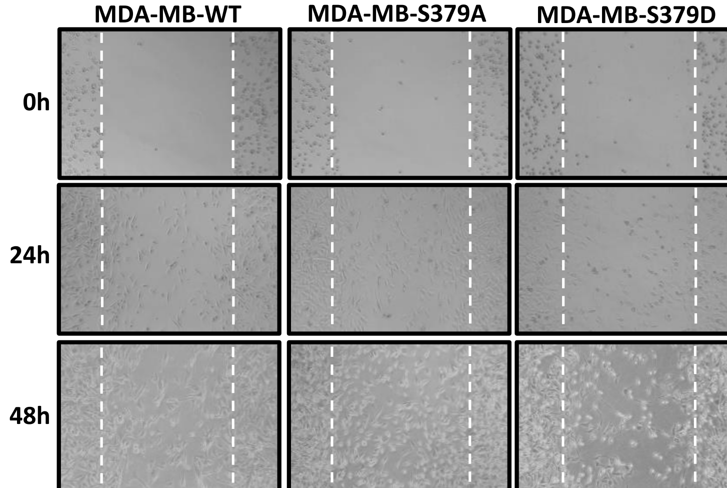


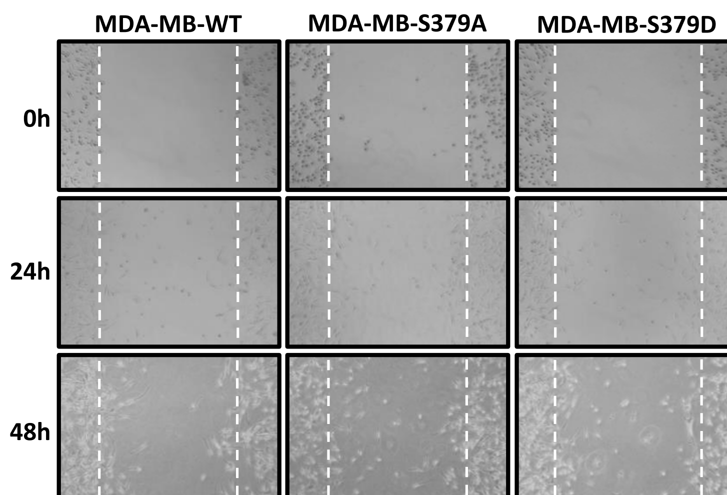
Supplementary Figure 3. S379 phosphorylation of hnRNPK does not affect the methylation level of hnRNPK in MDA-MB-231 cells. All cellular hnRNPKs from the MDA-MB-WT, MDA-MB-S379A and MDA-MB-S3979D cells was respectively precipitated for the detection of their arginine methylation levels using anti-arginine-methylation antibody (AB412). The methylation levels of hnRNPKs are nearly the same among all three cells.


(a)
MDA-MB-WT
MDA-MB-S379A
MDA-MB-S379D
Cell survival rate
(% of DMSO)
100
50
0
DMSO
50μM iCRT3
100μM iCRT3


(b) MDA-MB-231
iCRT3
DMSO
25μM
50μM
β-catenin
CyclinD1
hnRNPK
α-tubulin


(c) MDA-MB-231
DMSO
50μM iCRT3
100μM iCRT3


(d) DMSO
MDA-MB-WT
MDA-MB-S379A
MDA-MB-S379D
0h
24h
48h


50μM iCRT3
MDA-MB-WT
MDA-MB-S379A
MDA-MB-S379D
0h
24h
48h

Supplementary Figure 4. Inhibition of β-catenin transcription activity suppresses the migration of MDA-MB-WT, MDA-MB-S379A and MDA-MB-S379D cells. (a) MDA-MB-WT, MDA-MB-S379A and MDA-MB-S379D cells exhibit similar survival rates upon iCRT3 treatment. The cell survival rates of MDA-MB-WT, MDA-MB-S379A and MDA-MB-S379D cells upon DMSO treatment (control) or iCRT3 treatment (50μM or 100μM) were measured using the MTT assay. Bar graphs of the relative fold changes in cell survival rates for the MDA-MB-WT, MDA-MB-S379A and MDA-MB-S379D cells upon DMSO (control) or iCRT3 are shown. **(b) Protein levels of Cyclin D1, a transcriptional target of β-catenin, are decreased upon iCRT3 treatment using MDA-MB-231 cells.** MDA-MB-231 cells were independently treated with DMSO, 25μM or 50μM iCRT3 for 20h. The protein levels of Cyclin D1 in all three groups were determined using Western blot analysis. **(c) Migration ability of MDA-MB-231 cells was suppressed by inhibition of β-catenin transcriptional activity.** A total of $3x10^4$ MDA-MB-231 cells were seeded into Transwells for the migration assay under DMSO (control) or 50μM/100μM of iCRT3. After 20h, the migrated cells were fixed using 10% formaldehyde and stained with crystal violet for image analysis. **(d) MDA-MB-WT, MDA-MB-S379A and MDA-MB-S379D cells, upon inhibition of β-catenin transcription activity, exhibit similar migration abilities using the wound healing assay.** A total of $5x10^5$ MDA-MB-WT, MDA-MB-S379A and MDA-MB-S379D cells were independently seeded into 6-well plates for 24h under DMSO (control) or 50μM iCRT3 treatment and this was followed by scratching out an area of ah fixed width on the plate using a 10μL tip. The three cell types were allowed to proliferate and migrated into the scratched area. The migrated cells were photographed at 48h after the scratch using a microscope.

For Figure 1a

GST-hnRNPK + +
Aurora-A + +
ATP - +

pS379-hnRNPK

GST-hnRNPK + +
Aurora-A + +
ATP - +

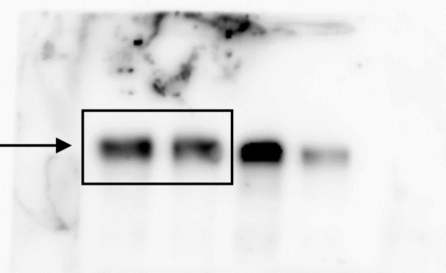
hnRNPK

Supplementary figure 5. Raw images of the cropped blots presented in the main figure 1.

For Figure 1a

GST-hnRNPK + +
Aurora-A + +
ATP - +

Aurora-A



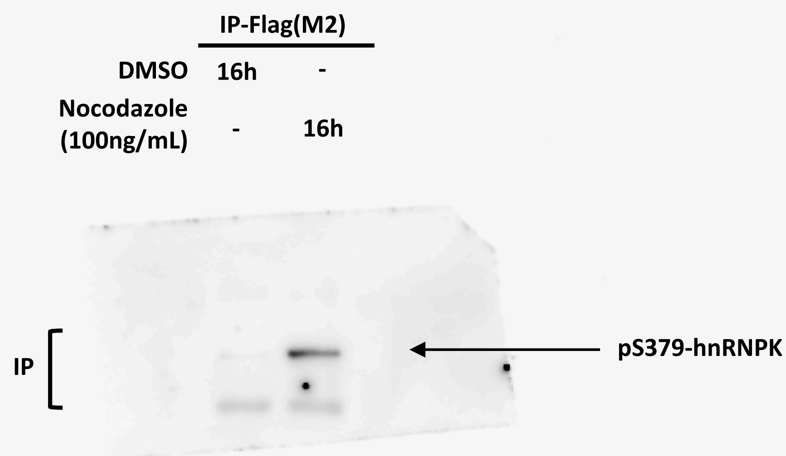
For Figure 1b

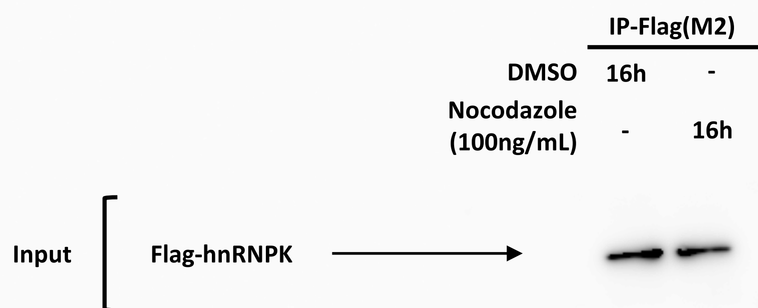
IP-Flag(M2)
DMSO 16h -
Nocodazole (100ng/mL) - 16h

IP Flag-hnRNPK

Supplementary figure 5. Continued.

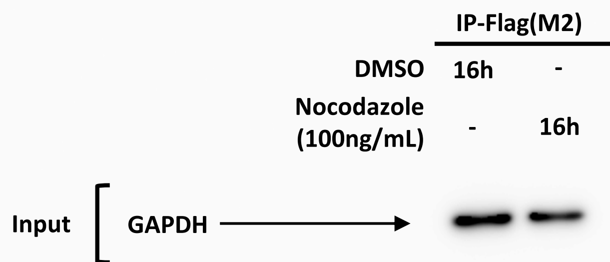
For Figure 1b





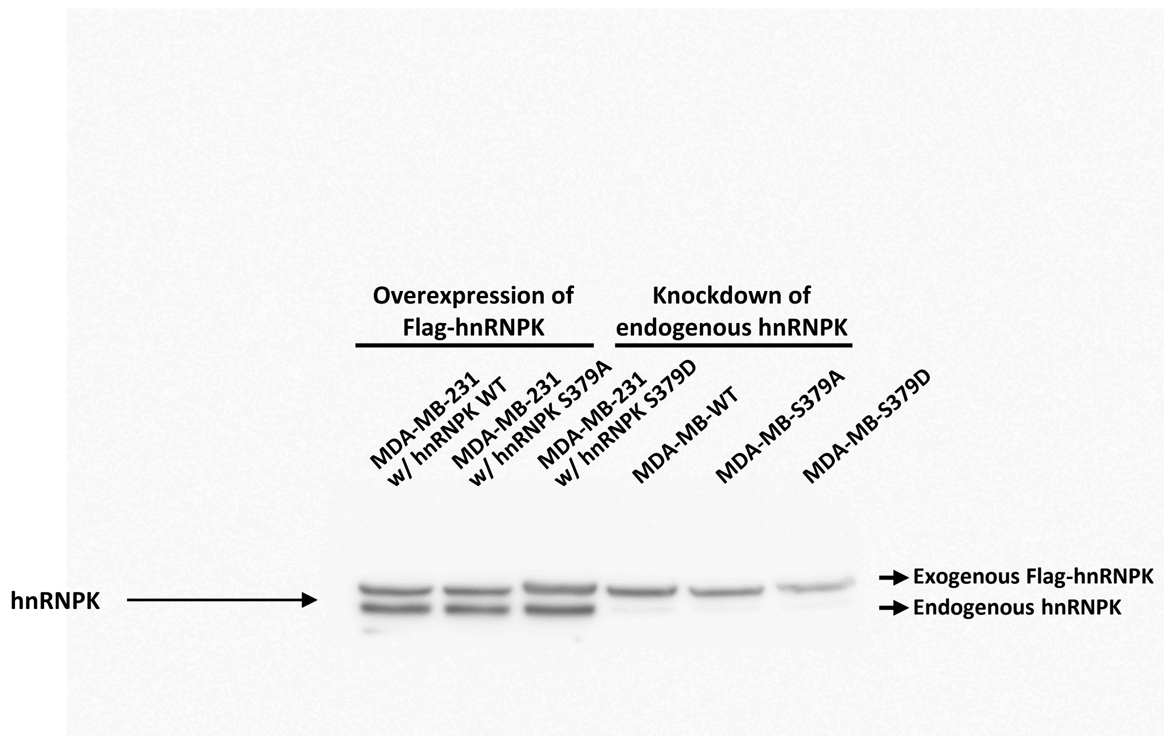
Supplementary figure 5. Continued.

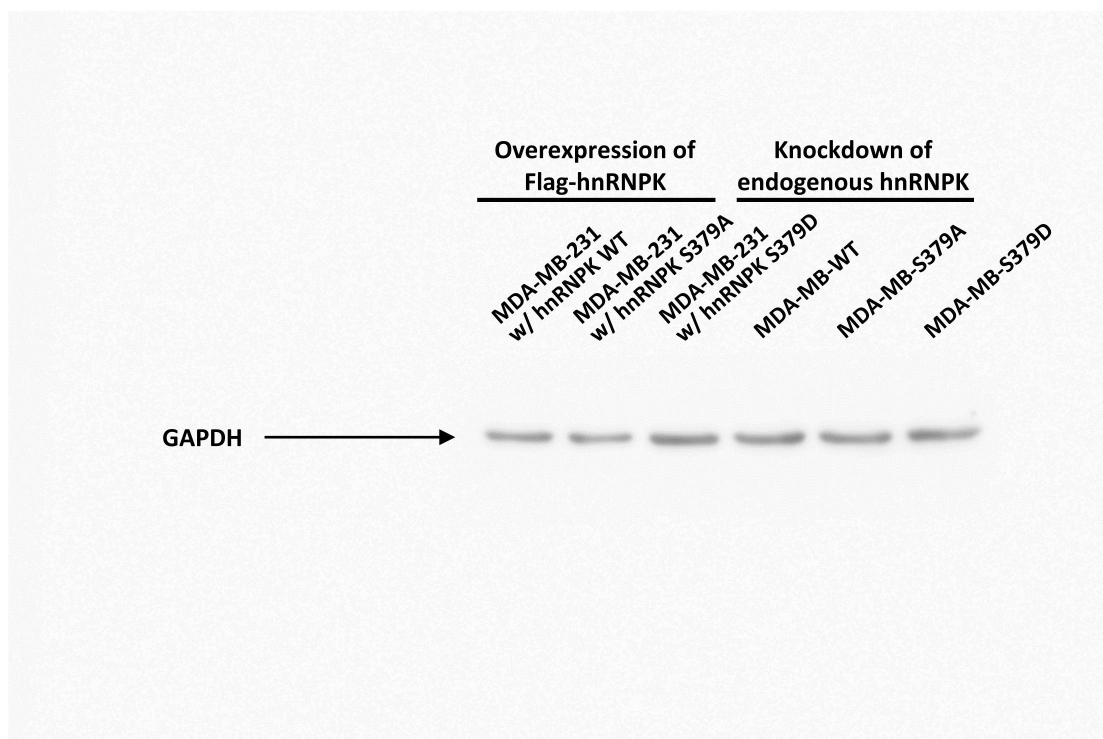
For Figure 1b



Supplementary figure 5. Continued.

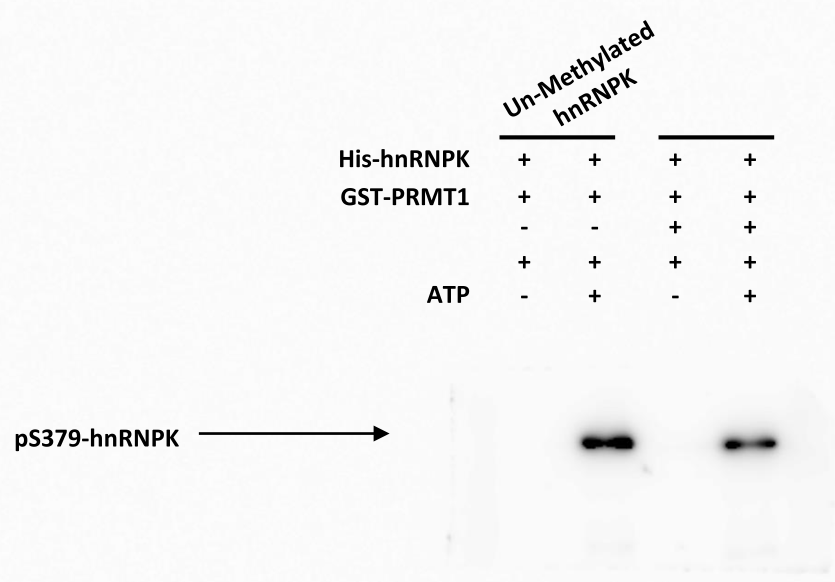
For Figure 2a

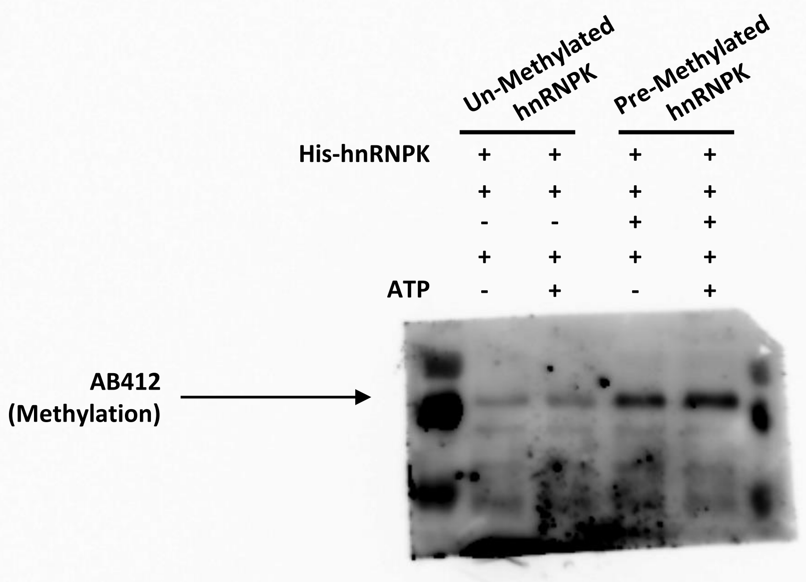




Supplementary figure 6. Raw images of the cropped blots presented in the main figure 2.

For Figure 4a



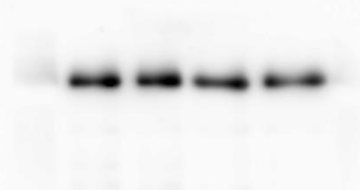


Supplementary figure 7. Raw images of the cropped blots presented in the main figure 4.

For Figure 4a

	Un-Methylated hnRNPK		Pre-Methylated hnRNPK	
His-hnRNPK	+	+	+	+
	+	+	+	+
	-	-	+	+
	+	+	+	+
ATP	-	+	-	+

hnRNPK

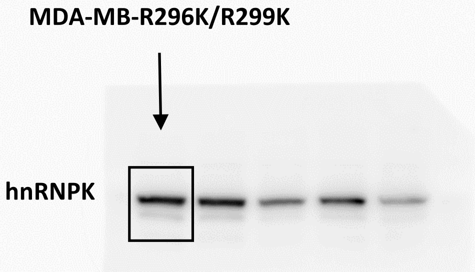


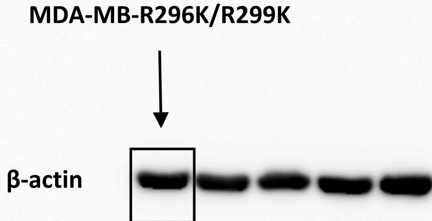
	Un-Methylated hnRNPK		Pre-Methylated hnRNPK	
His-hnRNPK	+	+	+	+
	+	+	+	+
	-	-	+	+
	+	+	+	+
ATP	-	+	-	+

Aurora-A

Supplementary figure 7. Continued.

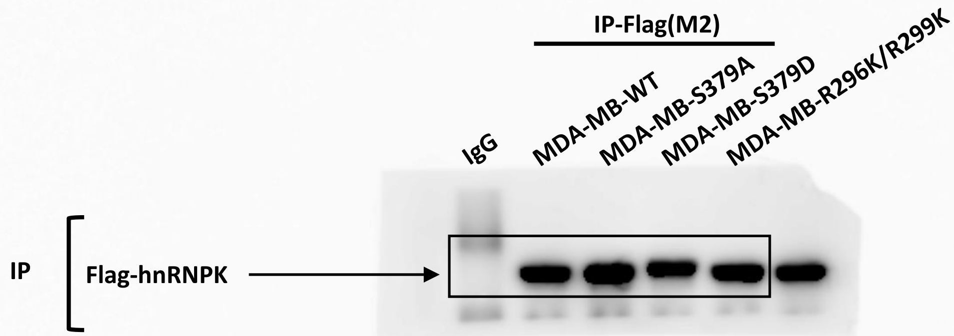
For Figure 4c

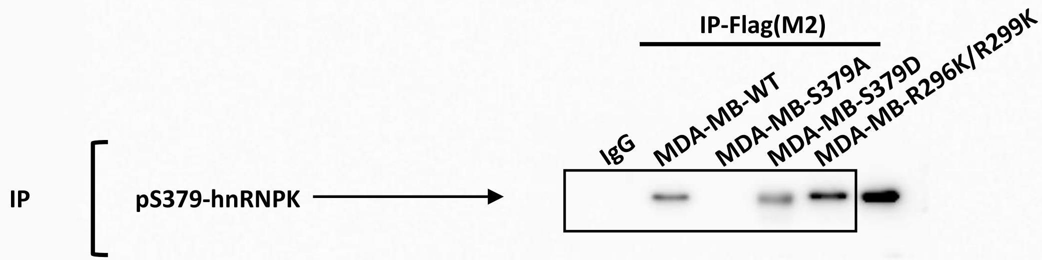




Supplementary figure 7. Continued.

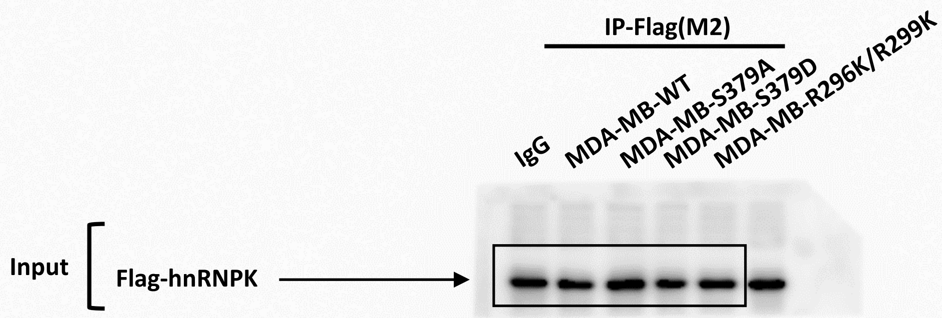
For Figure 4d

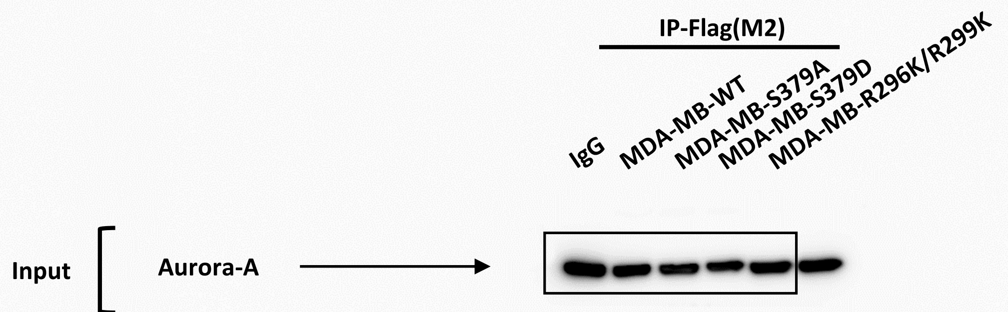




Supplementary figure 7. Continued.

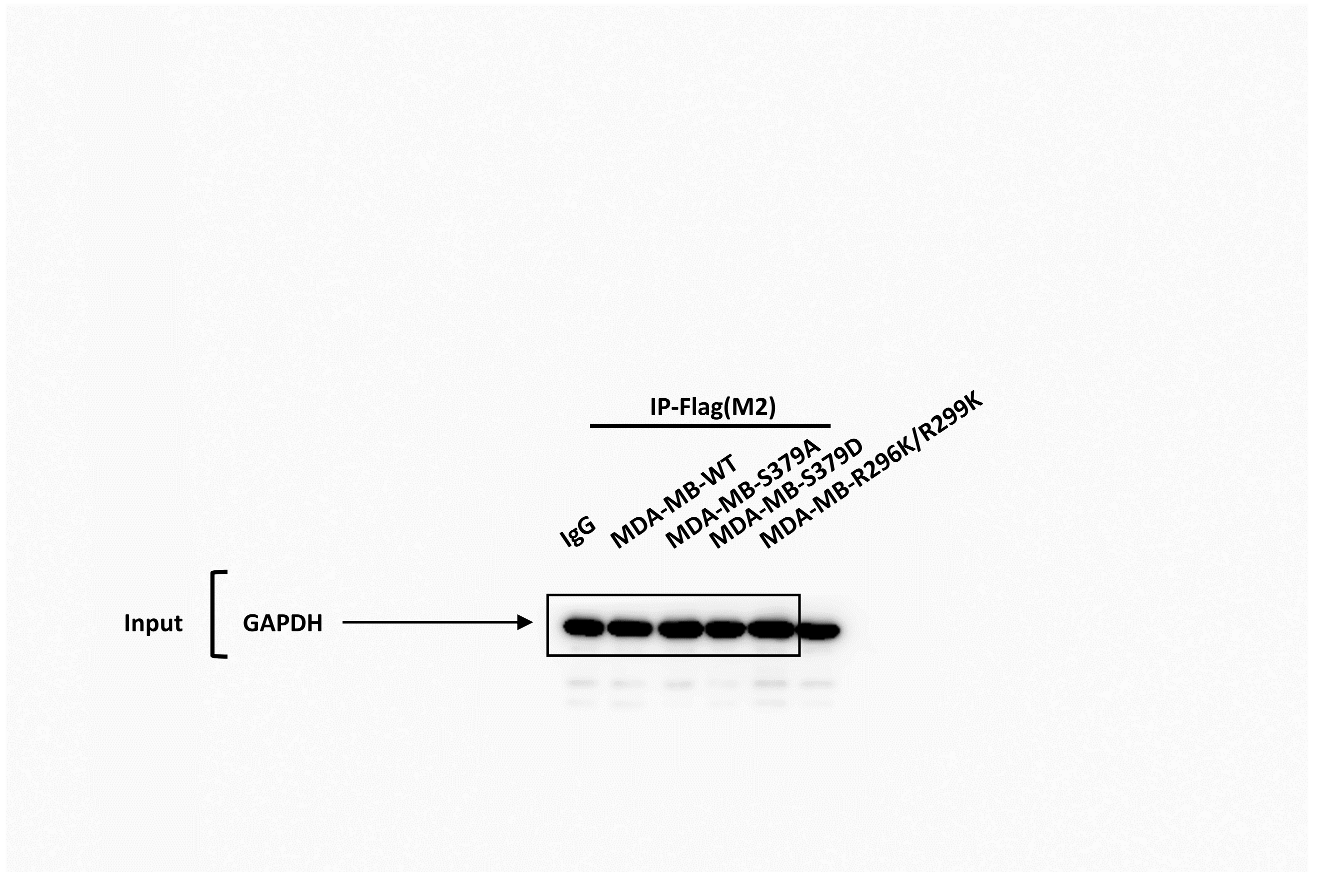
For Figure 4d





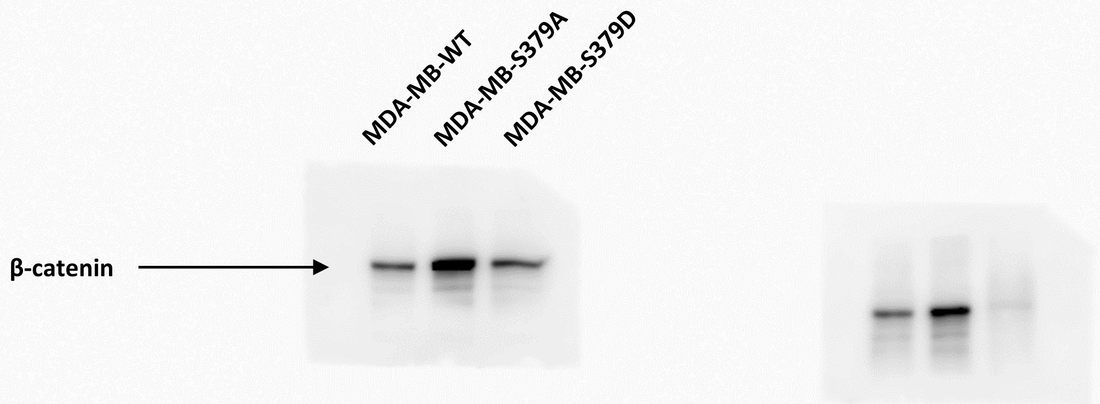
Supplementary figure 7. Continued.

For Figure 4d



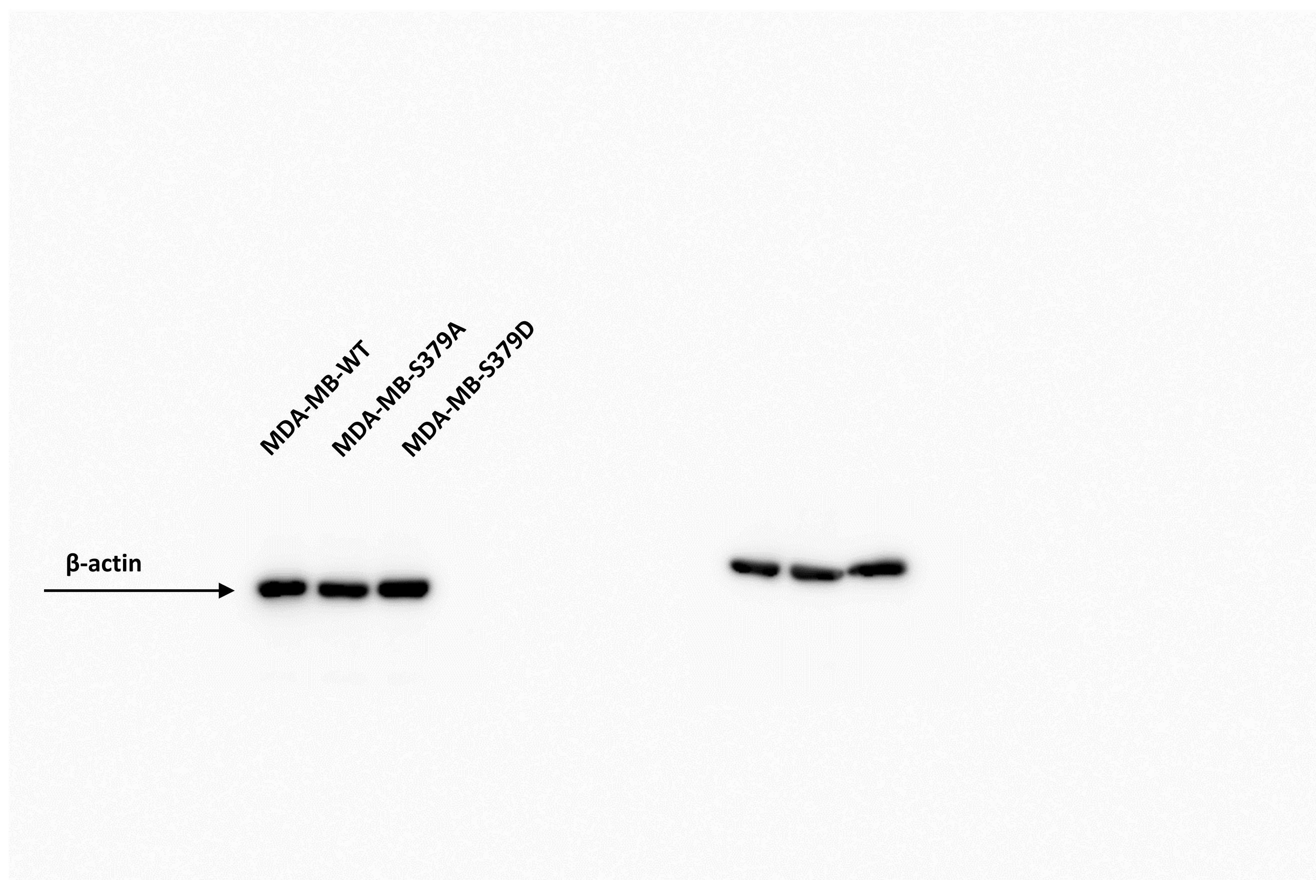
Supplementary figure 7. Continued.

For figure 5b



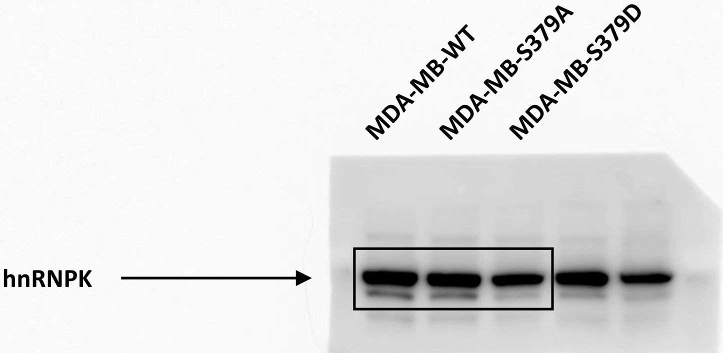
Supplementary figure 8. Raw images of the cropped blots presented in the main figure 5.

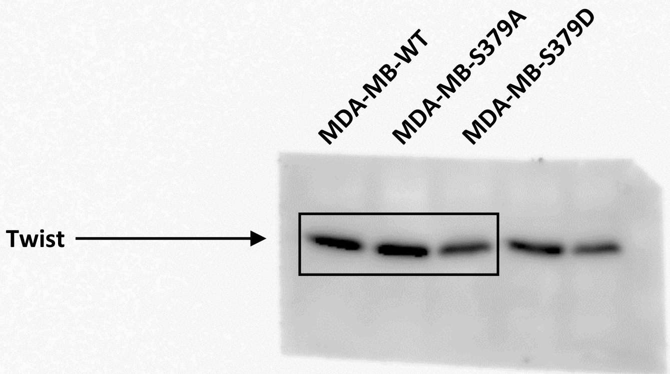
For figure 5b



Supplementary figure 8. Continued.

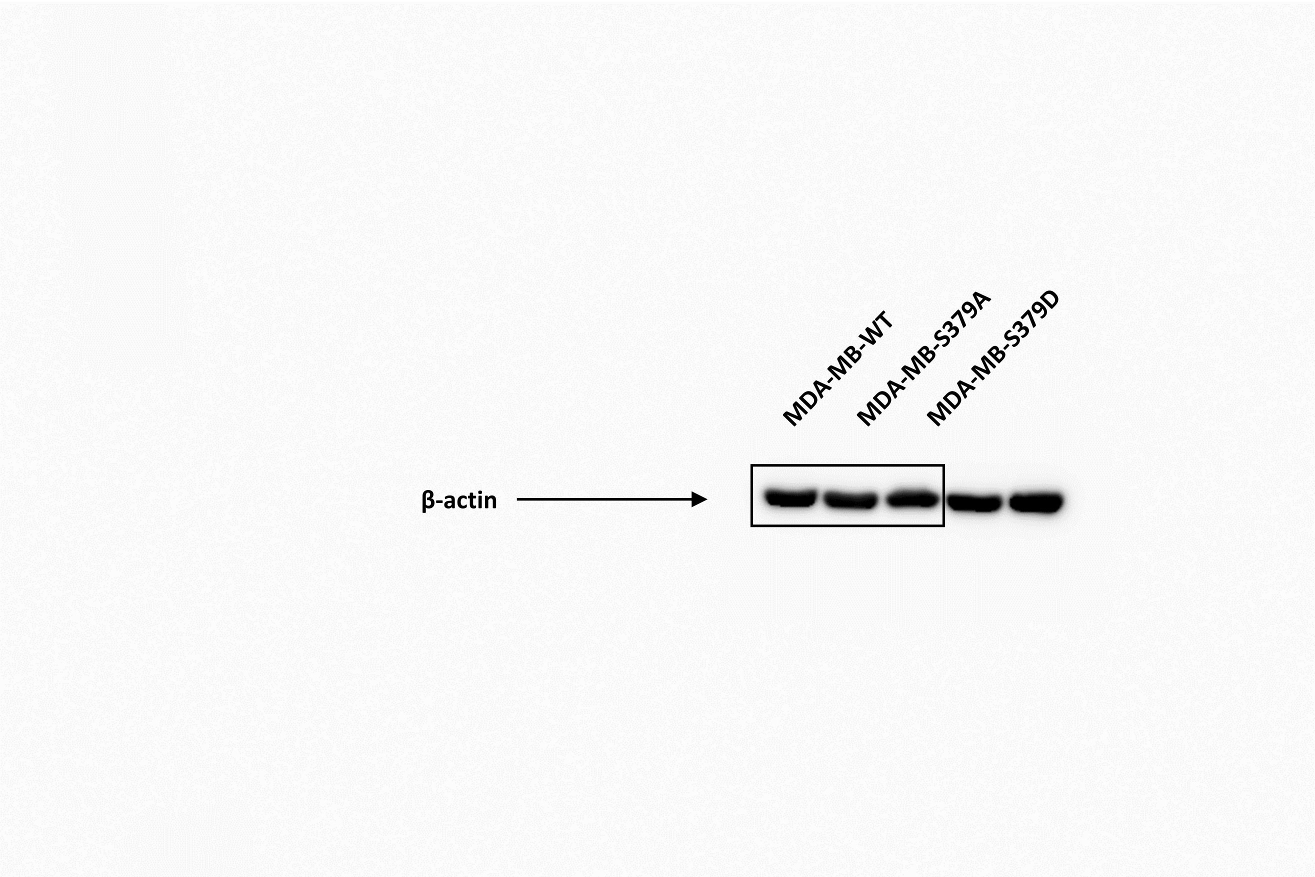
For figure 5b





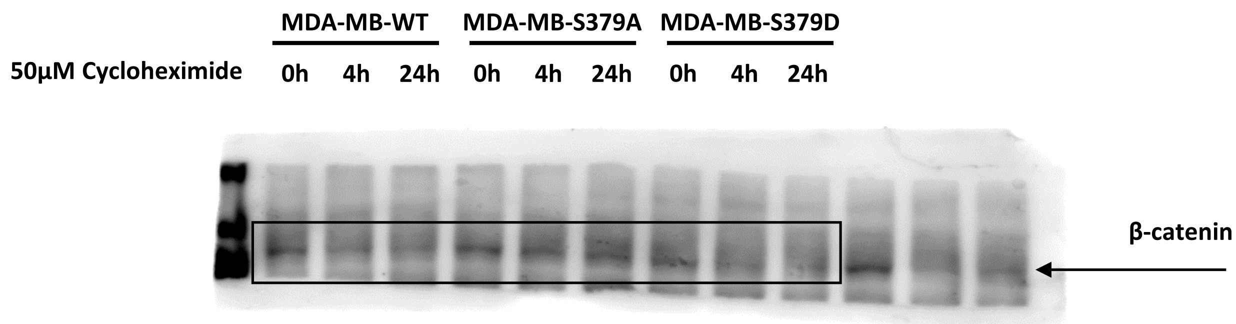
Supplementary figure 8. Continued.

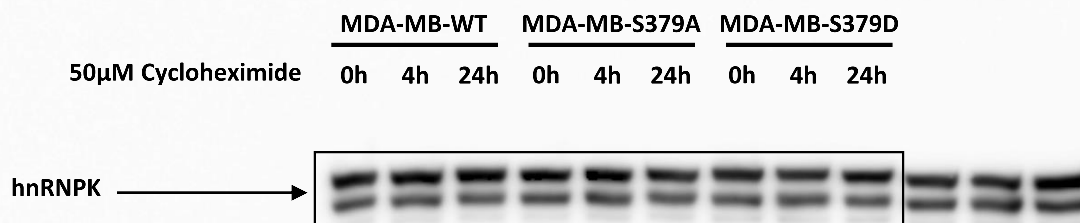
For figure 5b



Supplementary figure 8. Continued.

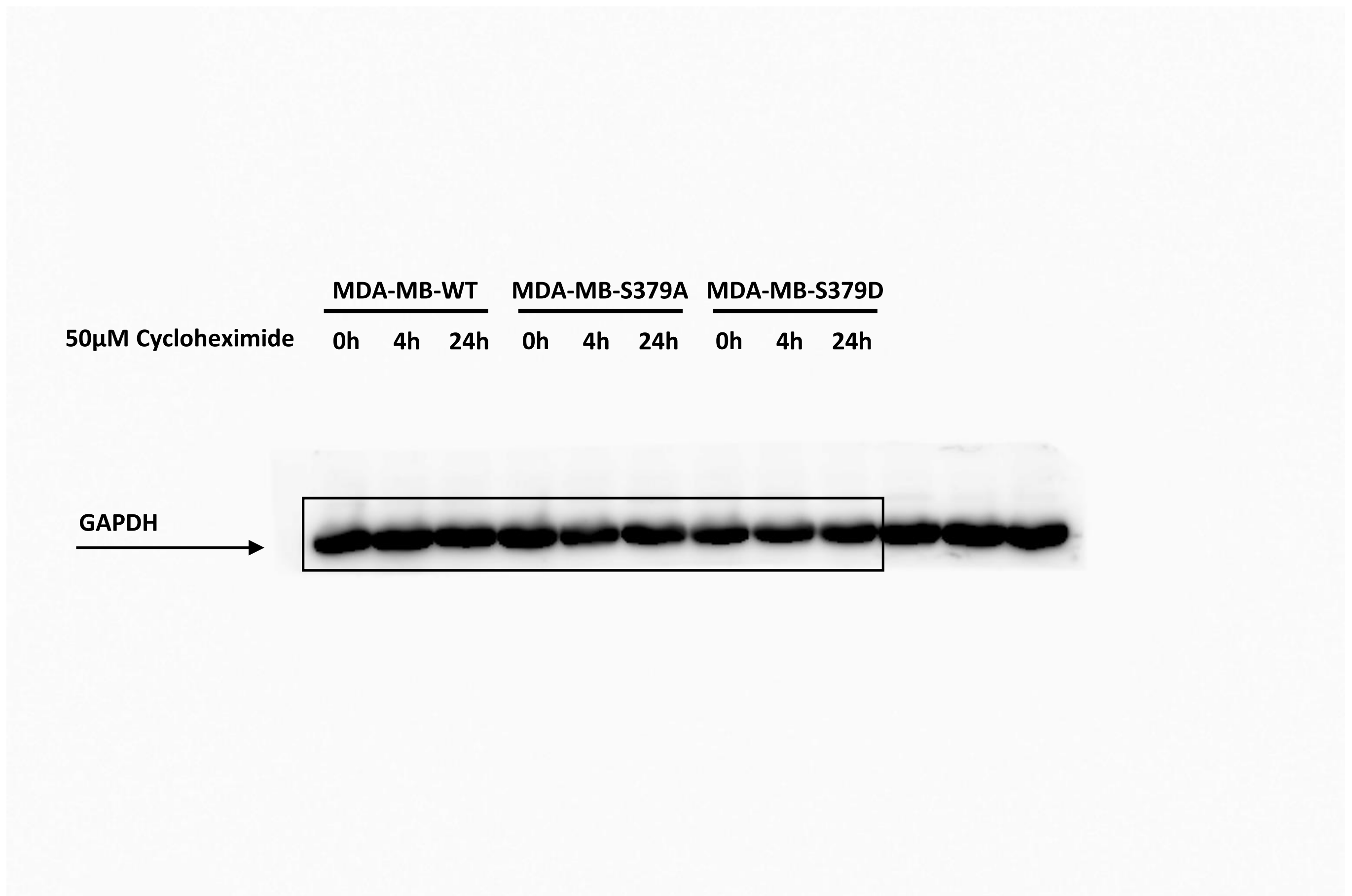
For figure 5f





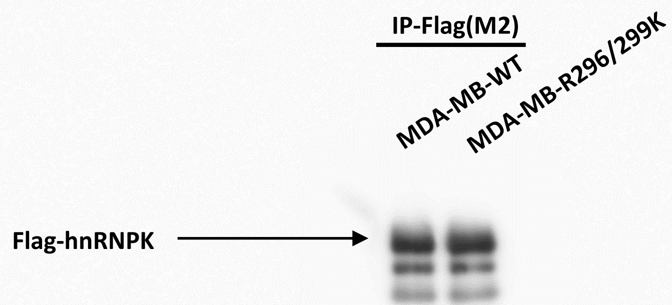
Supplementary figure 8. Continued.

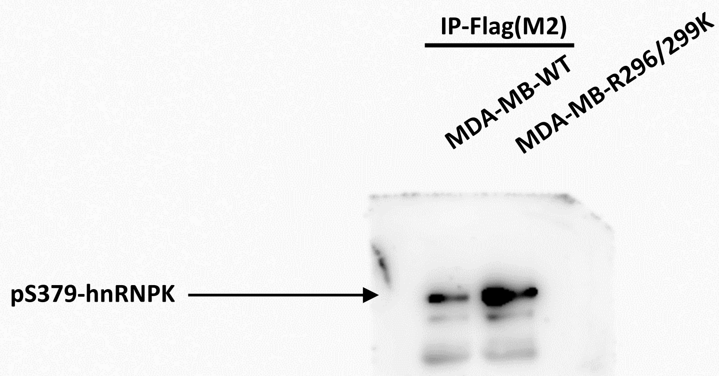
For figure 5f



Supplementary figure 8. Continued.

For figure 6c





Supplementary figure 9. Raw images of the cropped blots presented in the main figure 6.

For figure 6c

IP-Flag(M2)

MDA-MB-WT

MDA-MB-R296/299K

Flag-hnRNPK

IP-Flag(M2)

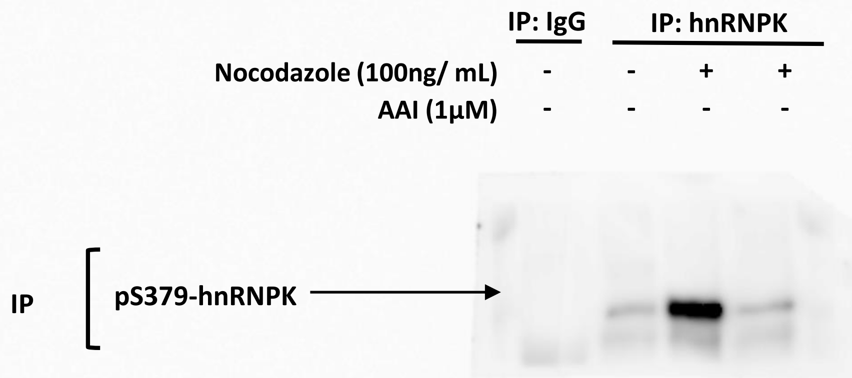
MDA-MB-WT

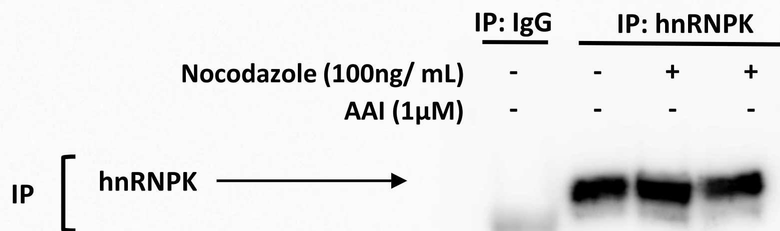
MDA-MB-R296/299K

GAPDH

Supplementary figure 9. Continued.

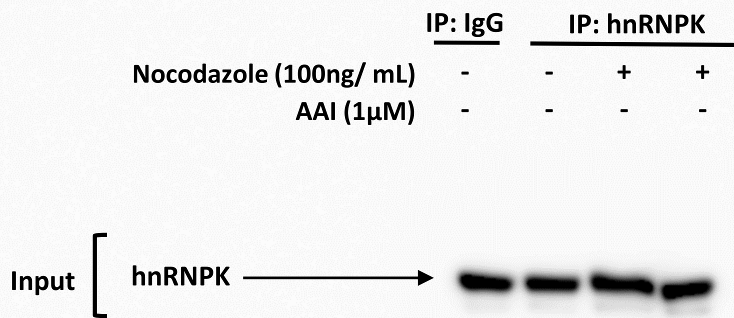
For supplementary figure 1b

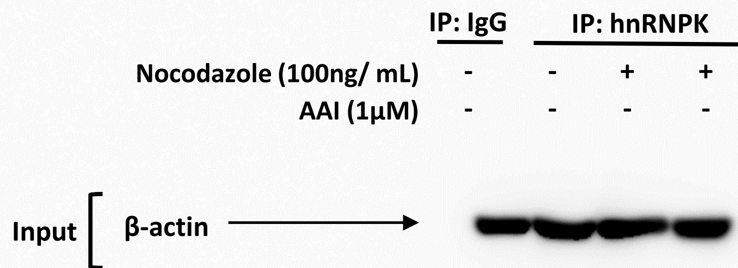




Supplementary figure 10. Raw images of the cropped blots presented in the supplementary figure 1.

For supplementary figure 1b





Supplementary figure 10. Continued.

For supplementary figure 3

IP [Flag-hnRNPK

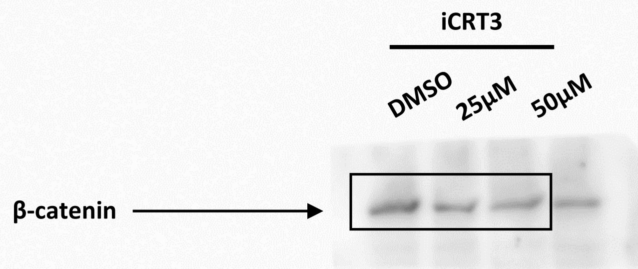
IP [Ab412 (methylation)

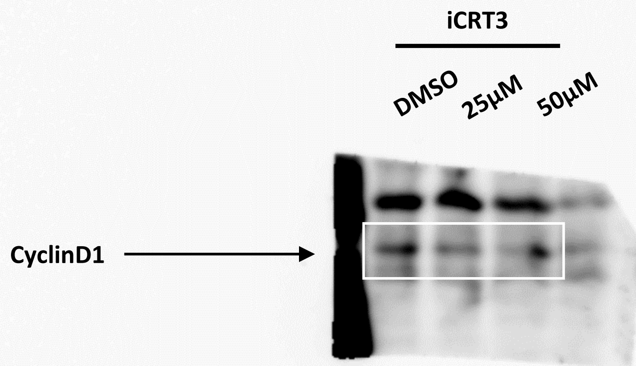
Supplementary figure 11. Raw images of the cropped blots presented in the supplementary figure 3.

For supplementary figure 3

Supplementary figure 11. Continued.

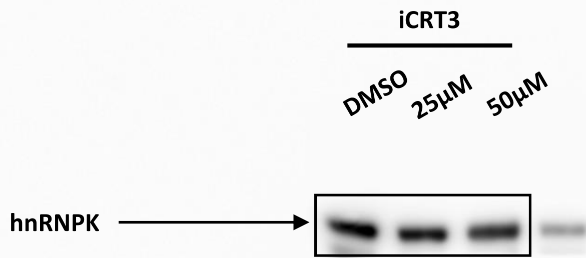
For supplementary figure 4b



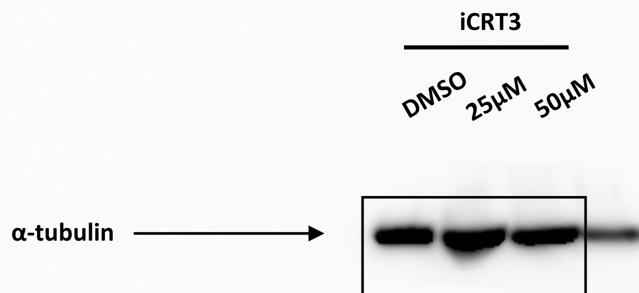


Supplementary figure 12. Raw images of the cropped blots presented in the supplementary figure 4.

For supplementary figure 4b



For supplementary figure S2b



Supplementary figure 12. Continued.