Supplementary Information

ATAD5 restricts R-loop formation through PCNA unloading and RNA helicase maintenance at the replication fork

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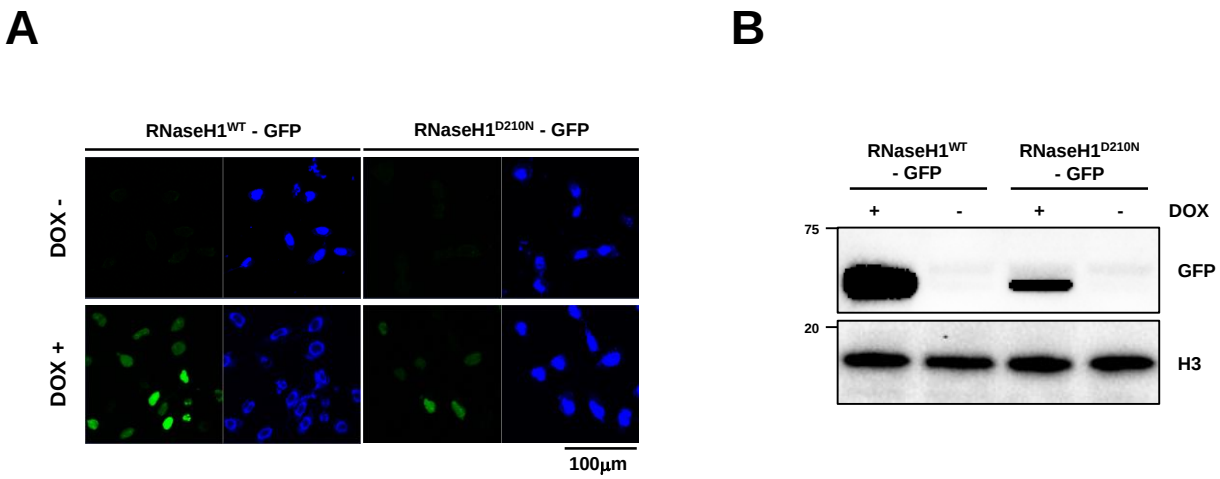
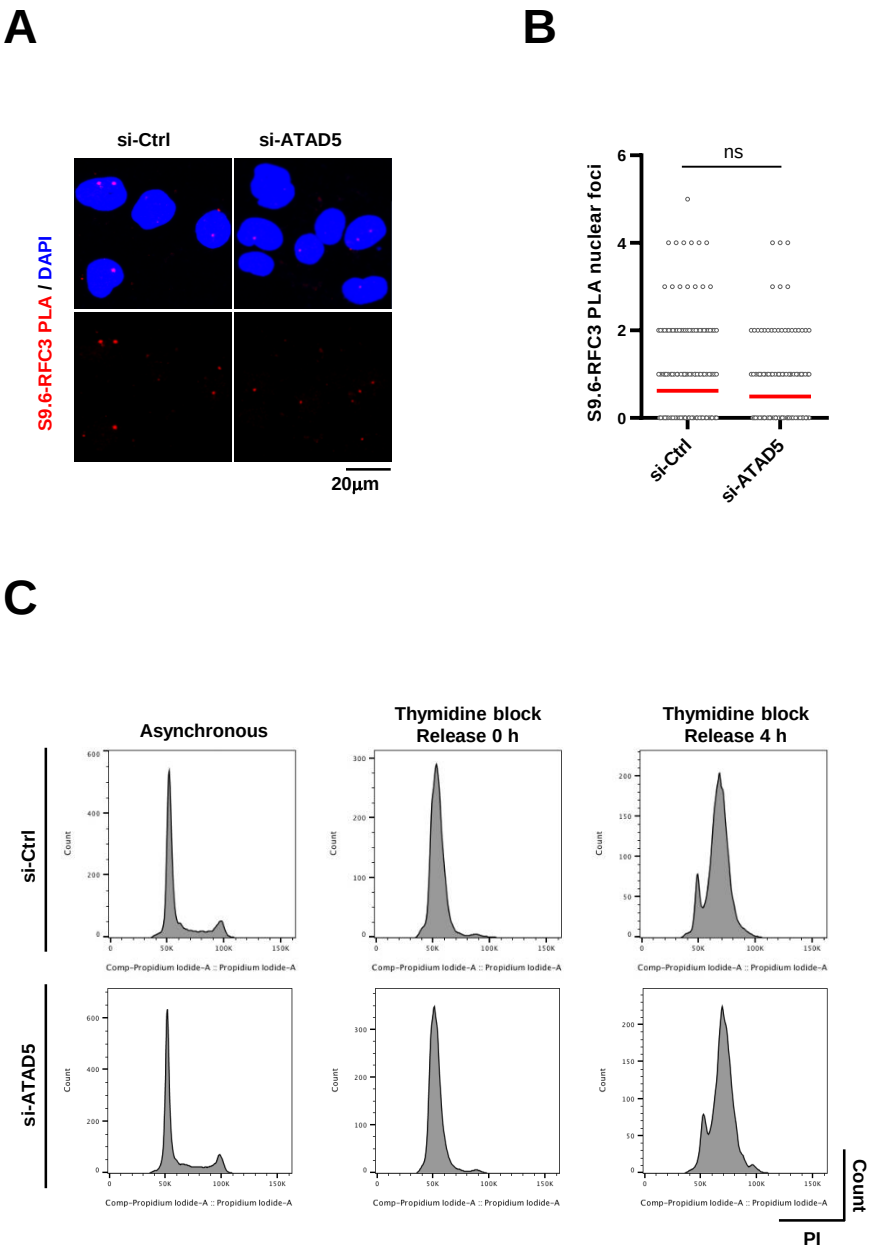
Supplementary Figures

Supplementary Figure 1



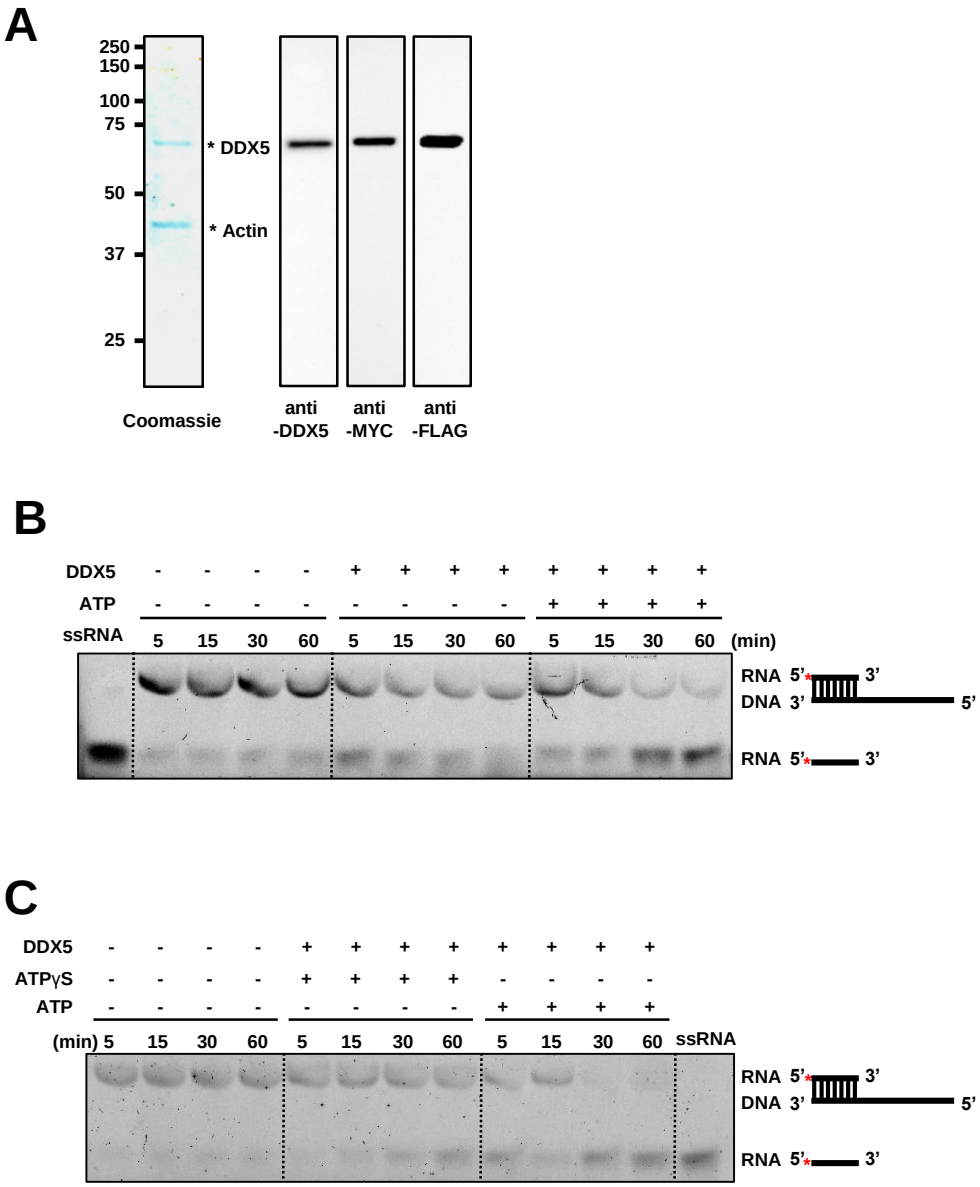
Supplementary Figure 1. HeLa-TetOn-RNaseH1-GFP cells expressing wild-type (WT) or nuclease deficient (D210N) RNaseH1 in a doxycycline-inducible manner are generated

(A, B) After doxycycline (DOX) treatment for 48 h, cells were fixed for immunostaining (A) or chromatin bound fractions were prepared for immunoblotting (B).


A
si-Ctrl
si-ATAD5
S9.6-RFC3 PLA / DAPI
20µm
B
ns
S9.6-RFC3 PLA nuclear foci
si-Ctrl
si-ATAD5
C
Asynchronous
Thymidine block Release 0 h
Thymidine block Release 4 h
si-Ctrl
si-ATAD5
Count
PI

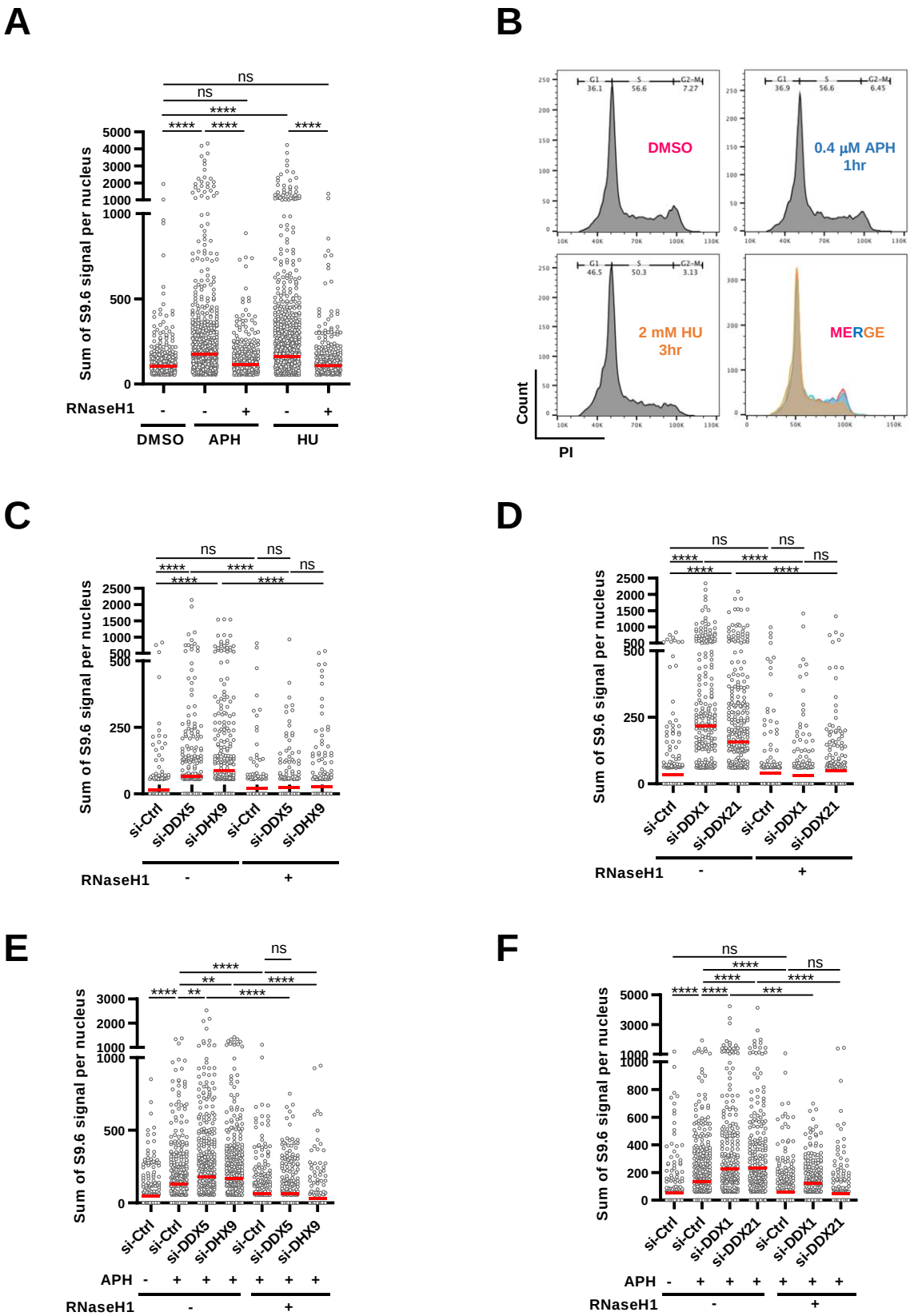
Supplementary Figure 2. The number of S9.6-RFC3 PLA foci is not increased in ATAD5-depleted cells

(A, B) U2OS cells were transfected with *Ctrl* or *ATAD5* siRNA for 48 h and fixed for a PLA between S9.6 and RFC3. (A) Representative PLA images. (B) Number of PLA nuclear foci was counted and plotted. (A, B) Three independent experiments were performed and one representative result was displayed. Red bar indicates mean value. Statistical analysis: two-tailed Student's *t*-test; ns, not significant. (C) HeLa cells were transfected with *Ctrl* or *ATAD5* siRNA for 48 h. After 30 h from transfection, cells were treated with 2.5 mM thymidine for 18 h and released into fresh media for 4 h before harvesting for FACS analysis with propidium iodide (PI) staining.


A
250
150
100
75
50
37
25
* DDX5
* Actin
Coomassie
anti -DDX5
anti -MYC
anti -FLAG
B
DDX5
ATP
ssRNA
5 15 30 60 5 15 30 60 5 15 30 60 (min)
RNA 5'* 3'
DNA 3' 5'
RNA 5'* 3'
C
DDX5
ATPγS
ATP
(min) 5 15 30 60 5 15 30 60 5 15 30 60 ssRNA
RNA 5'* 3'
DNA 3' 5'
RNA 5'* 3'

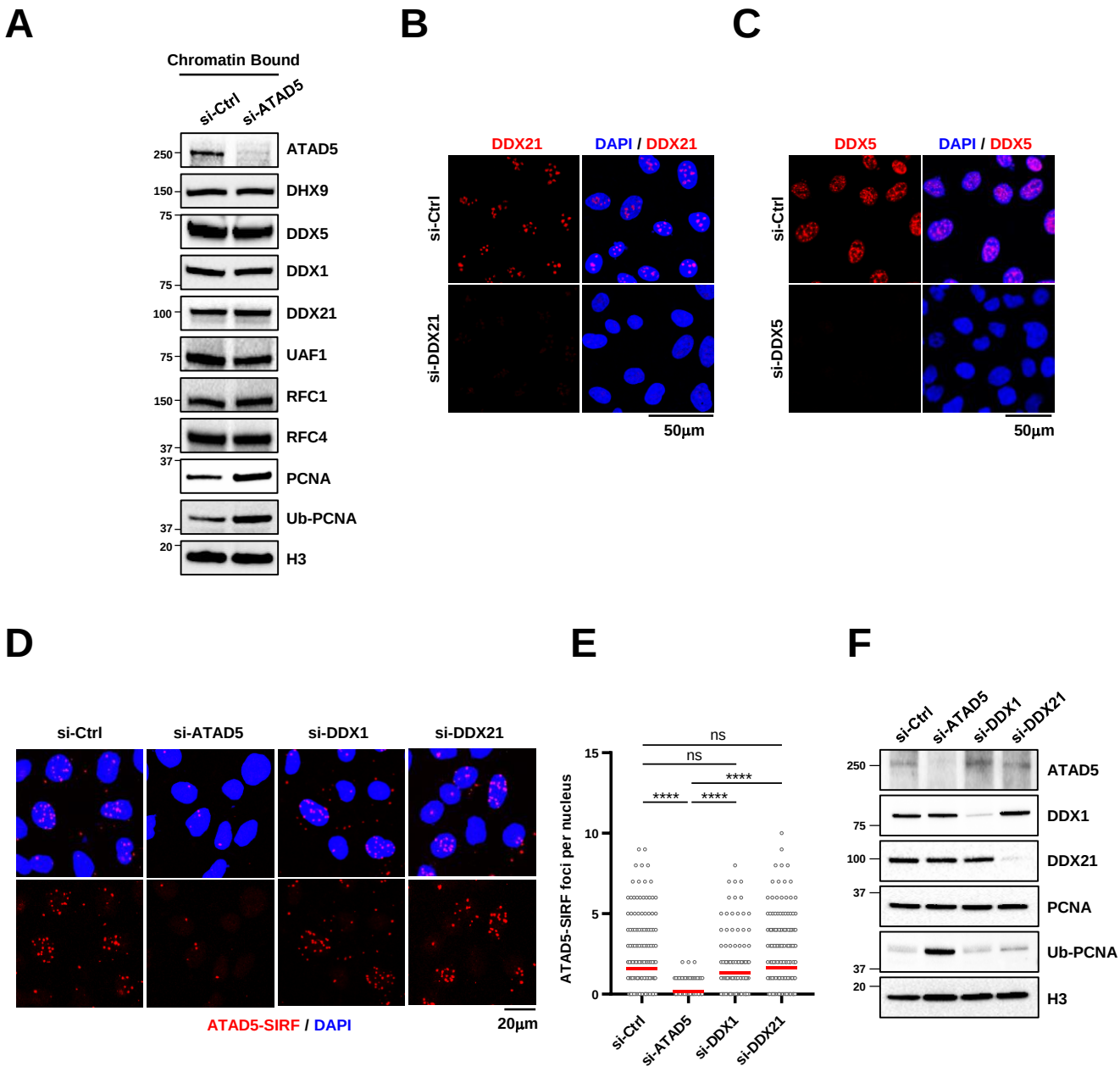
Supplementary Figure 3. Purified DDX5 protein has ATP hydrolysis-dependent DNA-RNA hybrid unwinding activity

(A) The purified human recombinant C-Myc/FLAG-tagged DDX5 protein was subjected to coomassie blue staining and immunoblotting with indicated antibodies. (B, C) The *in vitro* DNA-RNA hybrid unwinding assay. DDX5 protein was incubated with 6-carboxyfluorescein (FAM)-tagged DNA-RNA hybrid oligomers with or without ATP (B) or with ATPγS (C). ssRNA = FAM-tagged single strand RNA; * = FAM dye.


A
B
C
D
E
F
Sum of S9.6 signal per nucleus
RNaseH1
DMSO
APH
HU
0.4 µM APH 1hr
2 mM HU 3hr
MERGE
Count
PI
si-Ctrl
si-DDX5
si-DHX9
si-DDX1
si-DDX21
ns

Supplementary Figure 4. RNaseH1 expression reduces S9.6 signal increased by depletion of ATAD5/UAF1-interacting RNA helicases under normal and replication stress conditions

(A, C-F) After transfection and doxycycline treatment for 48 h, HeLa-TetOn-RNaseH1-GFP cells were subjected to immunostaining with S9.6 antibody. The intensity of S9.6 staining was quantified and plotted. Statistical analysis: two-tailed Student's *t*-test; **** $p < 0.001$, *** $p < 0.005$, ** $P < 0.01$, * $p < 0.05$ and ns, not significant. (A, B) 2 mM HU was treated 3 h before fixation. (A, B, E, F) 0.4 μM aphidicolin was treated 1 h before fixation. (B) After treated with indicated chemicals, HeLa cells were fixed for FACS analysis.


A
Chromatin Bound
si-Ctrl
si-ATAD5
250
150
75
75
100
75
150
37
37
37
20
ATAD5
DHX9
DDX5
DDX1
DDX21
UAF1
RFC1
RFC4
PCNA
Ub-PCNA
H3
B
DDX21
DAPI / DDX21
si-Ctrl
si-DDX21
50μm
C
DDX5
DAPI / DDX5
si-Ctrl
si-DDX5
50μm
D
si-Ctrl
si-ATAD5
si-DDX1
si-DDX21
ATAD5-SIRF / DAPI
20μm
E
ATAD5-SIRF foci per nucleus
15
10
5
0
ns
ns
****
****
****
si-Ctrl
si-ATAD5
si-DDX1
si-DDX21
F
si-Ctrl
si-ATAD5
si-DDX1
si-DDX21
250
75
100
37
37
20
ATAD5
DDX1
DDX21
PCNA
Ub-PCNA
H3

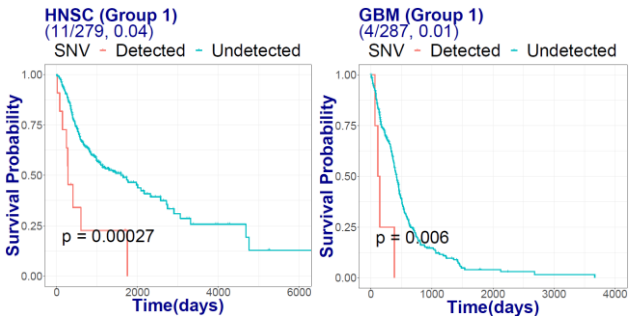
Supplementary Figure 5. Depletion of DDX1 or DDX21 does not affect an ATAD5 SIRF signal

(A) HEK293T cells were transfected with *Ctrl* or *ATAD5* siRNAs for 48 h. Chromatin fractions were prepared for immunoblotting. (B-F) U2OS cells were transfected with indicated siRNAs for 48 h. Then, cells were fixed for immunostaining (B, C) or ATAD5-SIRF assay (D, E) or whole cell extracts were prepared for immunoblotting (F). (D) Representative SIRF images. (E) SIRF signal foci were counted and plotted. Three independent experiments were performed and one representative result was displayed. Red bar indicates mean value. Statistical analysis: two-tailed Student's *t*-test; **** $p < 0.001$ and ns, not significant.

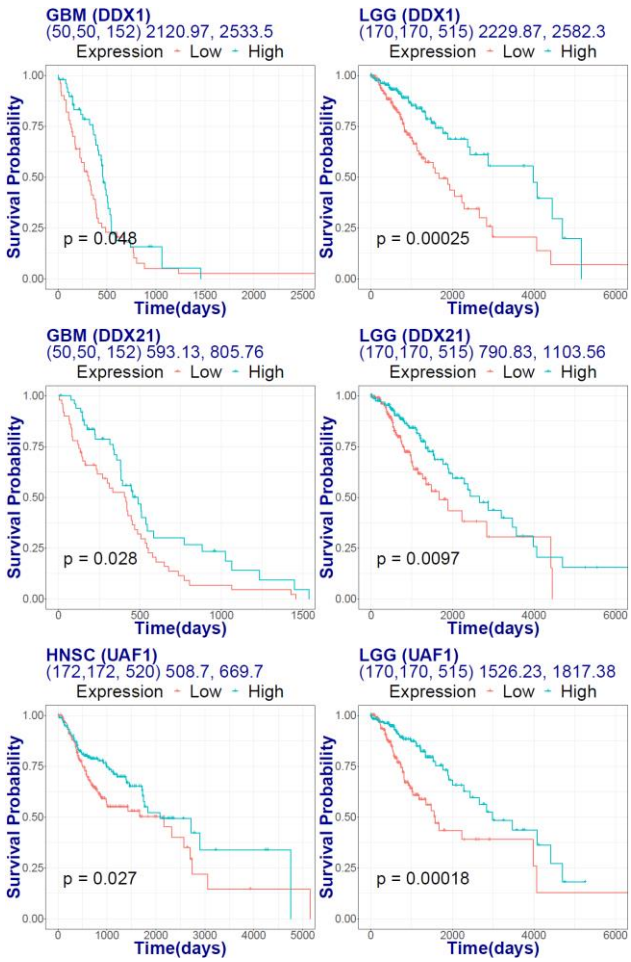
A

Group	Genes
GROUP I	DDX1, DDX5, DDX21, DHX9

B



C



Supplementary Figure 6. Genes of ATAD5/UAF1-interacting RNA helicases are mutated or downregulated in several human tumors

(A) A table describing a category used for group analysis. (B) Kaplan-Meier overall survival analysis for patients with head-neck squamous cell carcinoma (HNSC) and glioblastoma (GBM) by mutation status of Group 1 genes. (C) Kaplan-Meier curve analysis of overall patient survival for patients with HNSC, GBM, or low-grade glioma (LGG) by expression levels of DDX1, DDX21, and UAF1 (high, 33rd percentile cutoffs; low, 66th percentile cutoffs).