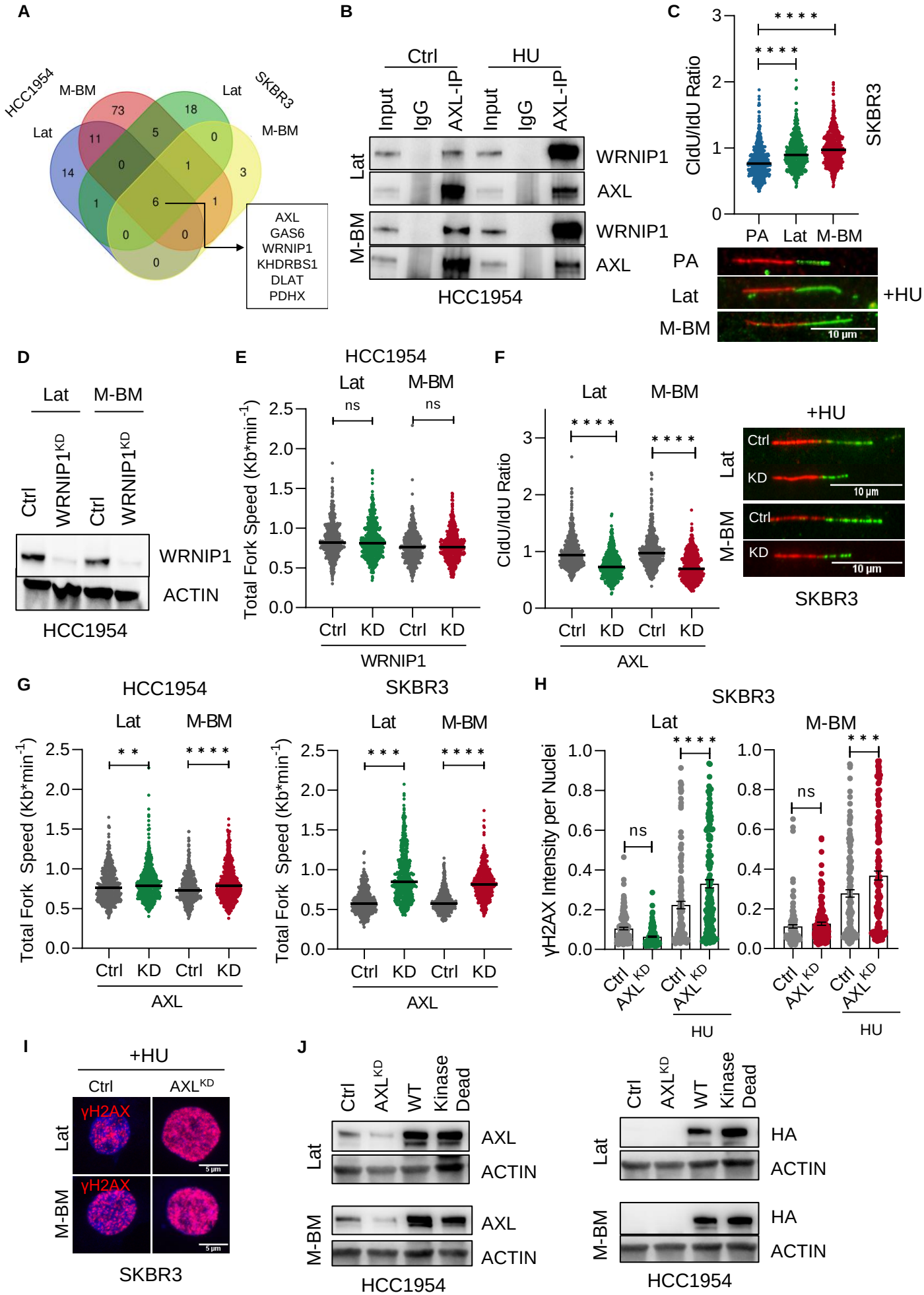
A
HCC1954
M-BM
Lat
SKBR3
M-BM
Lat
73
18
11
5
0
14
0
1
3
1
6
1
0
0
0
AXL
GAS6
WRNIP1
KHDRBS1
DLAT
PDHX
B
Ctrl
HU
Input
IgG
AXL-IP
Input
IgG
AXL-IP
Lat
M-BM
WRNIP1
AXL
WRNIP1
AXL
HCC1954
C
CldU/IdU Ratio
SKBR3
PA
Lat
M-BM
+HU
10 µm
D
Lat
M-BM
Ctrl
WRNIP1KD
Ctrl
WRNIP1KD
WRNIP1
ACTIN
HCC1954
E
HCC1954
Lat
M-BM
ns
ns
Total Fork Speed (Kb*min-1)
Ctrl
KD
Ctrl
KD
WRNIP1
SKBR3
F
Lat
M-BM
CldU/IdU Ratio
+HU
Ctrl
KD
Lat
M-BM
10 µm
SKBR3
AXL
G
HCC1954
SKBR3
Lat
M-BM
Total Fork Speed (Kb*min-1)
AXL
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SKBR3
Lat
M-BM
γH2AX Intensity per Nuclei
ns
ns
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AXL KD
HU
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+HU
Ctrl
AXLKD
Lat
M-BM
γH2AX
5 µm
SKBR3
J
Ctrl
AXLKD
WT
Kinase Dead
Lat
M-BM
AXL
ACTIN
HA
HCC1954

Supplementary Figure 4. AXL-WRNIP1 depletion attenuates DNA replication stress response in Lat and M-BM cells.

A, Venn diagram highlighting number of AXL interacting proteins identified by mass spectrometry analysis in HCC1954 and SKBR3 Lat and M-BM cells. Protein candidates were filtered by normalizing to IgG control in Lat and M-BM cells. 2 ≥ PSM, 2 ≥ fold change and 1% FDR. **B,** Immunoblot showing AXL-WRNIP1 interaction in HCC1954 Lat and M-BM cells under normal (Ctrl) and replication stress induced condition (in presence of 4mM HU for 5hrs). **C,** Relative fork degradation measured by the CldU/IdU ratio in presence of 4mM HU. Representative images of DNA fibers from SKBR3 PA, Lat and M-BM cells are also shown. IdU (red color) and CldU (green color). N = 3 for all experiments. Data are presented as mean ± SEM. Mann–Whitney U test, ****, P < 0.0001. **D,** Immunoblotting of WRNIP1 in HCC1954 WRNIP1 knockdown ($WRNIP1^{KD}$) Lat and M-BM cells. **E**, Total fork speed measured by the length sum of IdU and CldU and such values were converted into kilobases using the conversion factor 1 μm = 2.59 kb of HCC1954 Lat and M-BM cells under WRNIP1 depletion. N = 3 for all experiments. Data are presented as mean ± SEM. Mann–Whitney U test, ns: no significant. **F,** Relative fork degradation measured by the CldU/IdU ratio in presence of 4mM HU upon SKBR3 AXL knockdown (AXL^{KD}) in Lat and M-BM cells. N = 3 for all experiments. Data are presented as mean ± SEM. Mann–Whitney U test, ****, P < 0.0001. **G,** Total fork speed measured by the length sum of IdU and CldU and such values were converted into kilobases using the conversion factor 1 μm = 2.59 kb of HCC1954 and SKBR3 Lat and M-BM cells under AXL depletion. N = 3 for all experiments. Data are presented as mean ± SEM. Mann–Whitney U test, ****, P < 0.0001, ***, P < 0.001 **, P < 0.01. **H-I,** γH2AX intensity quantification of SKBR3 Lat and M-BM cells treated with 4mM HU for 5hrs. Representative image of γH2AX intensity under HU. Data are presented as mean ± SEM. One-way ANOVA was used, followed by the Dunnett test. ***, P < 0.001, ns: not significant. **J,** Immunoblotting of AXL and HA-tag of control (Ctrl), AXL^{KD}, and reintroduced wildtype (WT) and kinase dead AXL full length in HCC1954 AXL^{KD} Lat and M-BM cells.