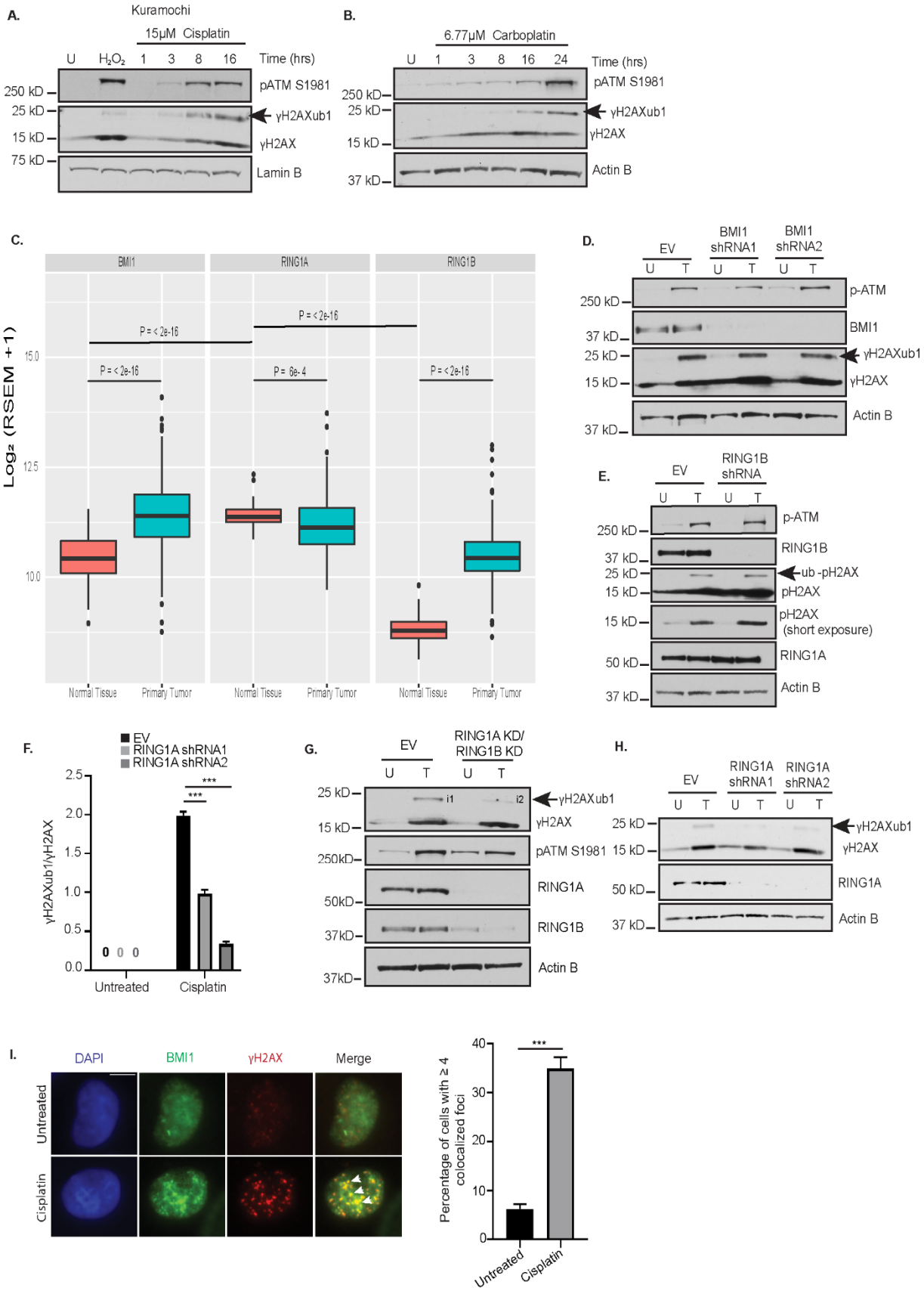
A.
Kuramochi
15μM Cisplatin
U
H2O2
1
3
8
16
Time (hrs)
250 kD
25 kD
15 kD
75 kD
pATM S1981
γH2AXub1
γH2AX
Lamin B
B.
6.77μM Carboplatin
U
1
3
8
16
24
Time (hrs)
250 kD
25 kD
15 kD
37 kD
pATM S1981
γH2AXub1
γH2AX
Actin B
C.
BMI1
RING1A
RING1B
Log2 (RSEM +1)
15.0
12.5
10.0
P = < 2e-16
P = < 2e-16
P = < 2e-16
P = 6e-4
P = < 2e-16
Normal Tissue
Primary Tumor
Normal Tissue
Primary Tumor
Normal Tissue
Primary Tumor
D.
EV
BMI1 shRNA1
BMI1 shRNA2
U
T
U
T
U
T
250 kD
37 kD
25 kD
15 kD
37 kD
p-ATM
BMI1
γH2AXub1
γH2AX
Actin B
E.
EV
RING1B shRNA
U
T
U
T
250 kD
37 kD
25 kD
15 kD
15 kD
50 kD
37 kD
p-ATM
RING1B
ub -pH2AX
pH2AX
pH2AX (short exposure)
RING1A
Actin B
F.
EV
RING1A shRNA1
RING1A shRNA2
γH2AXub1/γH2AX
2.5
2.0
1.5
1.0
0.5
0.0
***
***
0
0
0
Untreated
Cisplatin
G.
EV
RING1A KD/ RING1B KD
U
T
U
T
25 kD
15 kD
250kD
50kD
37kD
37kD
i1
i2
γH2AXub1
γH2AX
pATM S1981
RING1A
RING1B
Actin B
H.
EV
RING1A shRNA1
RING1A shRNA2
U
T
U
T
U
T
25 kD
15 kD
50 kD
37 kD
γH2AXub1
γH2AX
RING1A
Actin B
I.
DAPI
BMI1
γH2AX
Merge
Untreated
Cisplatin
Percentage of cells with ≥ 4 colocalized foci
40
30
20
10
0
***
Untreated
Cisplatin

Supplementary Figure S1: RING1A mediates monoubiquitination of γH2AX in response to carboplatin

(A) Kuramochi cells were untreated (U), treated with 2 mM H_2O_2 for 30 minutes or 15 μM cisplatin (IC50 dose) for the indicated time points. Lysates were analyzed by WB (N=3). **(B)** OVCAR5 cells were untreated (U) or treated with 6.77 μM carboplatin (IC50 dose) for the indicated time points. Lysates were analyzed by WB (N=3). **(C)** Box plots of BMI1, RING1A and RING1B expression in normal ovary from GTEX dataset and primary tumor samples from TCGA. OVCAR5 cells were infected with empty vector (EV) or 2 independent BMI1 viral shRNAs **(D)** or RING1B viral shRNA **(E)** and then untreated (U) or treated with 12 μM cisplatin (T) for 8 hours. Lysates were analyzed by WB (N=3). **(F)** Graph depicts mean ± SEM of densitometric analysis of γH2AXub1 relative to γH2AX in EV and RING1A KD cells from Figure 1B (N=3). **(G)** OVCAR5 cells infected with EV viral shRNA or both RING1A and RING1B viral shRNA were untreated (U) or treated with 12μM cisplatin (T) for 8 hours. Intensity 1 (i1) is 2.40 and intensity 2 (i2) is 1.49 relative to respective loading control. **(H)** OVCAR5 cells were infected with EV or 2 independent RING1A viral shRNAs and then untreated (U) or treated with 6.77 μM carboplatin (IC50 dose) for 24 hours. Cell lysates were analyzed by WB. **(I)** Kuramochi cells were treated with 15 μM cisplatin for 8 hours and then immunofluorescence analysis was performed for BMI1 (green) and the damage marker γH2AX (red). Merge image shows overlap of γH2AX and BMI1. White arrows indicate examples of BMI1 foci that co-localize with γH2AX. Graph displays mean percentage of cells with ≥ 4 γH2AX and BMI1 co-localized foci ± SEM (N=3). Scale bar = 5 μm.