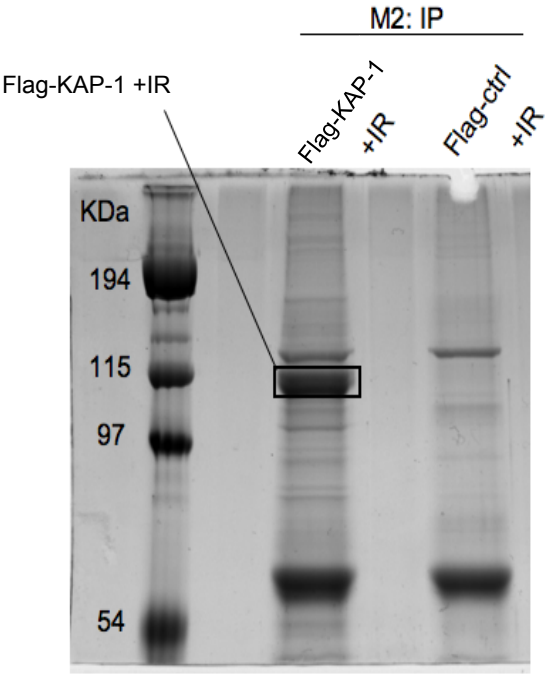
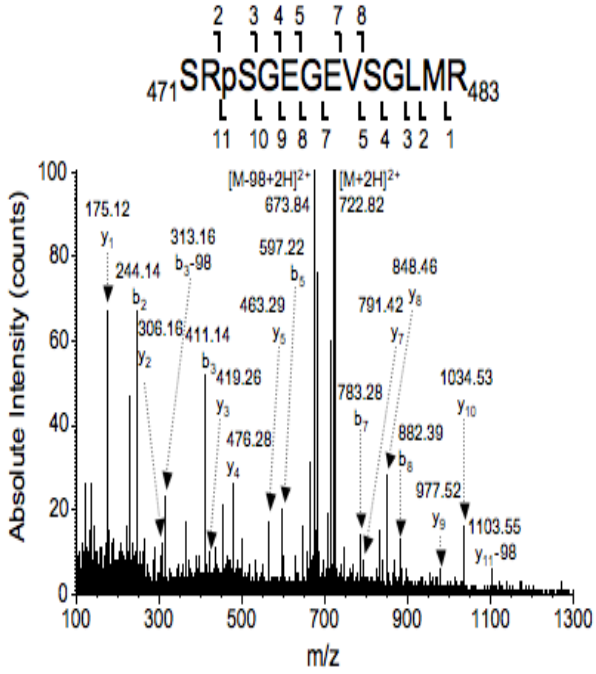
A



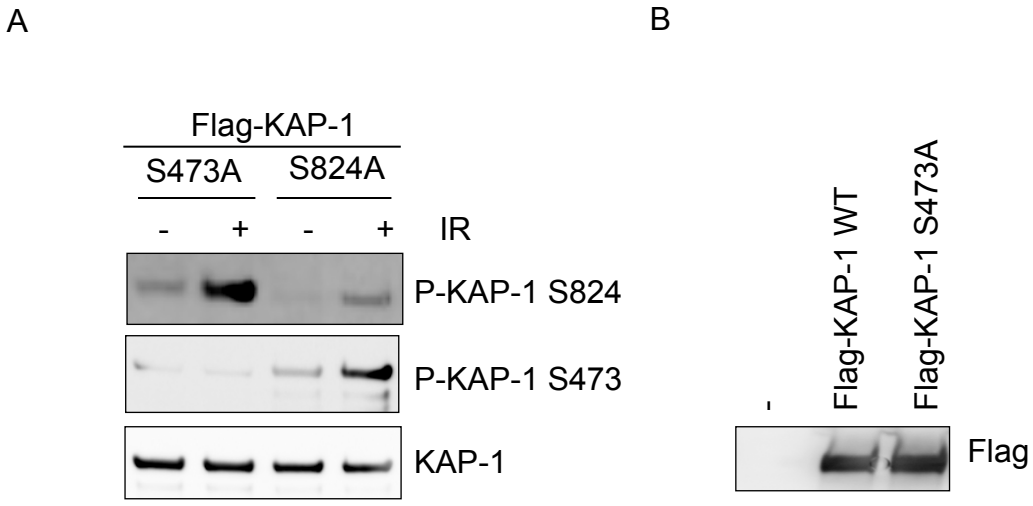
B



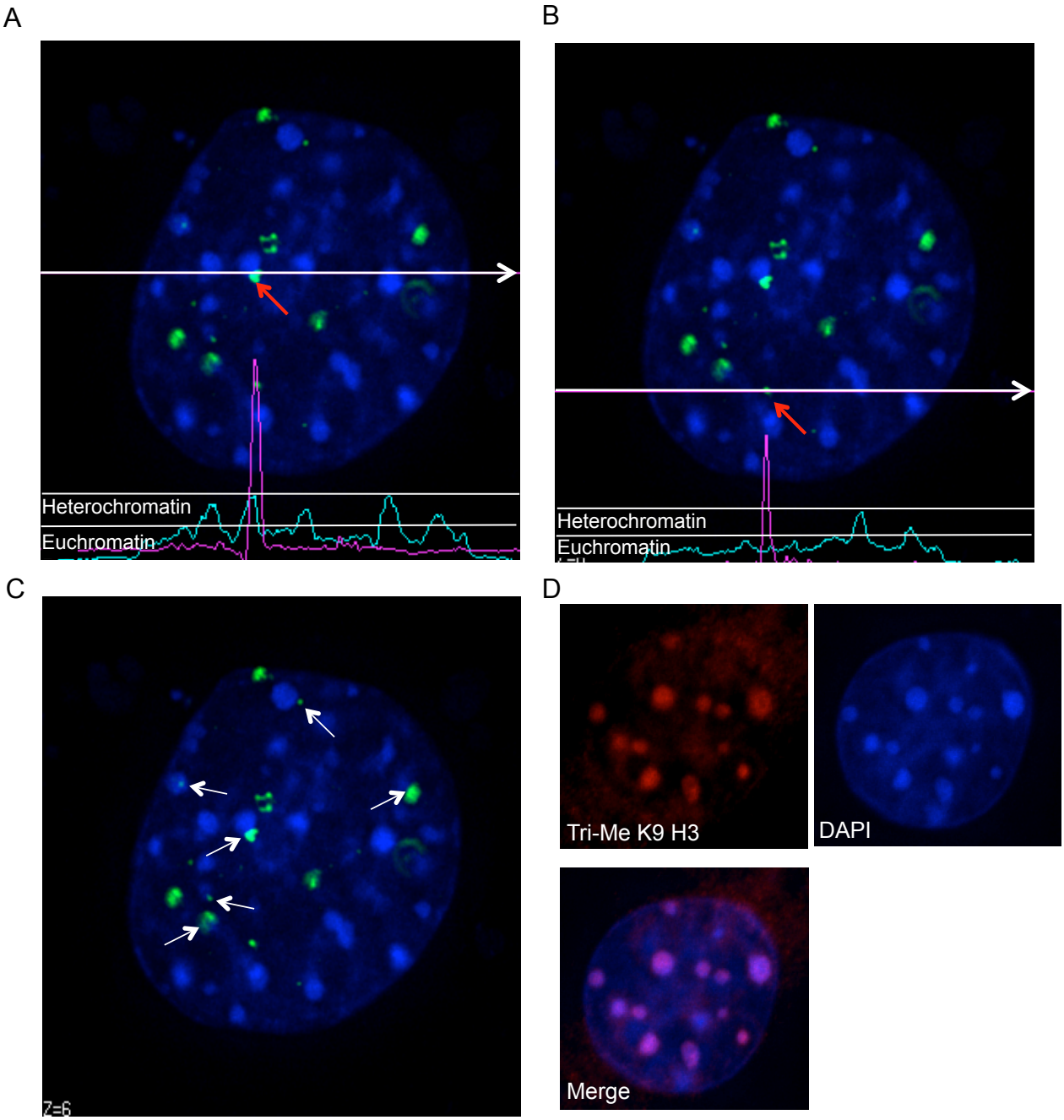
C

Sequence	Mascot Ions Score	Expectation Value	Site	No. Occasions Identified[§]
Ac-AASAAAASAAAASAASG**pS**PGPGEGSAGGEKR/S	101	4.2×10^{-8}	S19	16
STAPSAAASASASAAAS**S**PAGGGAEALELLEH*C*GV*C*R	56	6.8×10^{-3}	S50	17
K/LLA**S**LVKR/L	58	2.4×10^{-4}	S258	2
K/QG**pS**GSSQPMEVQEGYGFGSGDDPYSSAEPHVSGVKR/S	73	6.5×10^{-5}	S437	4
K/QGSGS**pS**QPMEVQEGYGFGSGDDPYSSAEPHVSGVKR/S	70	1.3×10^{-4}	S440	4
R/**pS**GEGEVSGLMR/K	68	3.8×10^{-5}	S473	>25
R/V**pS**LERLDLDLTADSQPPVFK/V	52	2.8×10^{-3}	S489	8
R/LA**p**(**S**P**S**)GSTSSGLEVVAPEGTSAPGGGPGTLDDSATICR/V	57	2.4×10^{-3}	S594 or S596	17 5
R/LQEKL**pS**PPYSSPQEFAQDVGR/M	79	6.0×10^{-6}	S752	17
K/LSPPY**p**(**SS**)PQEFAQDVGR/M	68	8.6×10^{-5}	S757 or S756	14 8
K/FSAVLVEPPPMSLPGAGL**p**(**SS**)QELSGGPGDGP/-	41	7.1×10^{-2}	S824 or S823	10 4

Supplementary Figure 1. A. 293T cells were transfected with Flag alone or Flag-KAP-1 WT and harvested one hour after treatment with IR (10Gy). Flag-KAP-1 was immunoprecipitated, resolved on an SDS PAGE gel and stained with Colloidal Coomassie G-250. The band corresponding to Flag-KAP-1 was excised and used for mass spectrometric phospho-peptide and phospho-residue mapping. B. The doubly charged precursor peptide at m/z 722.82 which represents KAP-$1_{471-483}$ (doubly charged 1444.67 peptide) was selected for fragmentation in a nanoESI-MSMS analysis. The fragmented ions matched the sequence of the KAP-$1_{471-483}$ peptide where serine-473 is phosphorylated, the phospho-serine was detected between ions b_2 and b_3 and also redundantly between ions y_{10} and y_{11}-98. C. Detected KAP-1 phosphopeptides and phosphosites. § The number of times the phosphosite has been reported according to www.phosphosite.org.

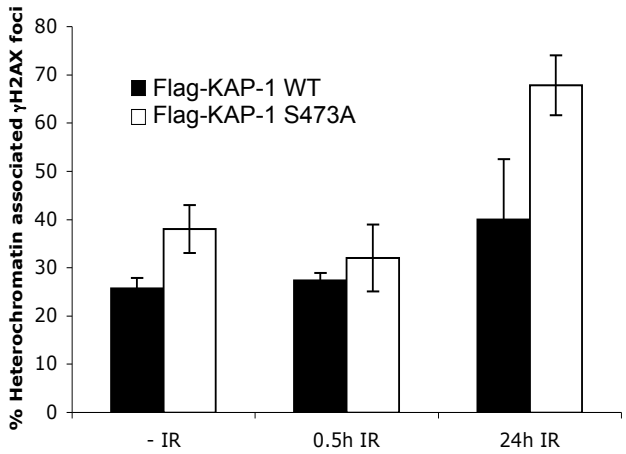


Supplementary Figure 2. A. HeLa cells were transfected with the indicated constructs and mock-treated or treated with 6 Gy IR. Cell extracts were taken 1 h post-IR and immunoblotting performed with the indicated antibodies. B. HeLa cells were treated as in A. (in the absence of IR) and immunoblotted with Flag antibodies.



Supplementary Figure 3. Identifying γH2AX foci associated with regions of heterochromatin. A-C. NIH3T3 cells were transfected with Flag-KAP-1 S473A. After 24 hours cells were treated with 2 Gy IR and harvested 24 hours post-IR. Cells were stained with γH2AX (green) and DADI (blue). High resolution images were catpured using a Delta Vision PDV microscope, images were deconvolved using softWoRx Suite software. Signal intensity chromatographs are shown at the base of A. and B. Pink lines indicate γH2AX signal and light blue lines indicate DAPI signal. A. Shows the chromatograph of a γH2AX foci (indicated by a red arrow) associated with heterochromatin. B. Shows the chromatograph of a γH2AX foci (indicated by a red arrow) not associated with heterochromatin. C. Shows an image with the heterochromatin-associated γH2AX foci indicated by white arrows. D. NIH3T3 cells were fixed and stained with trimethylated K9 histone H3 and DAPI.

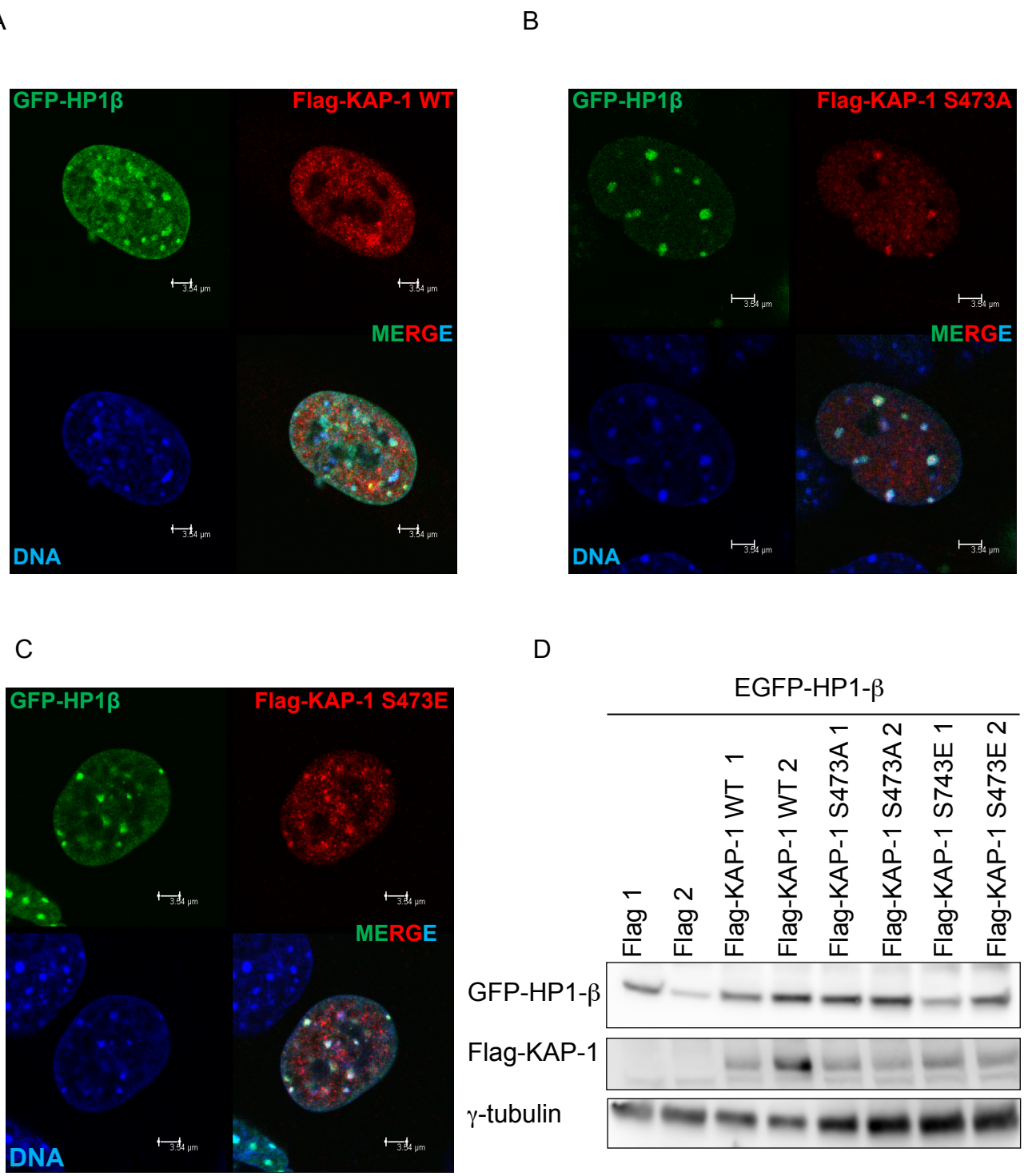
A



B

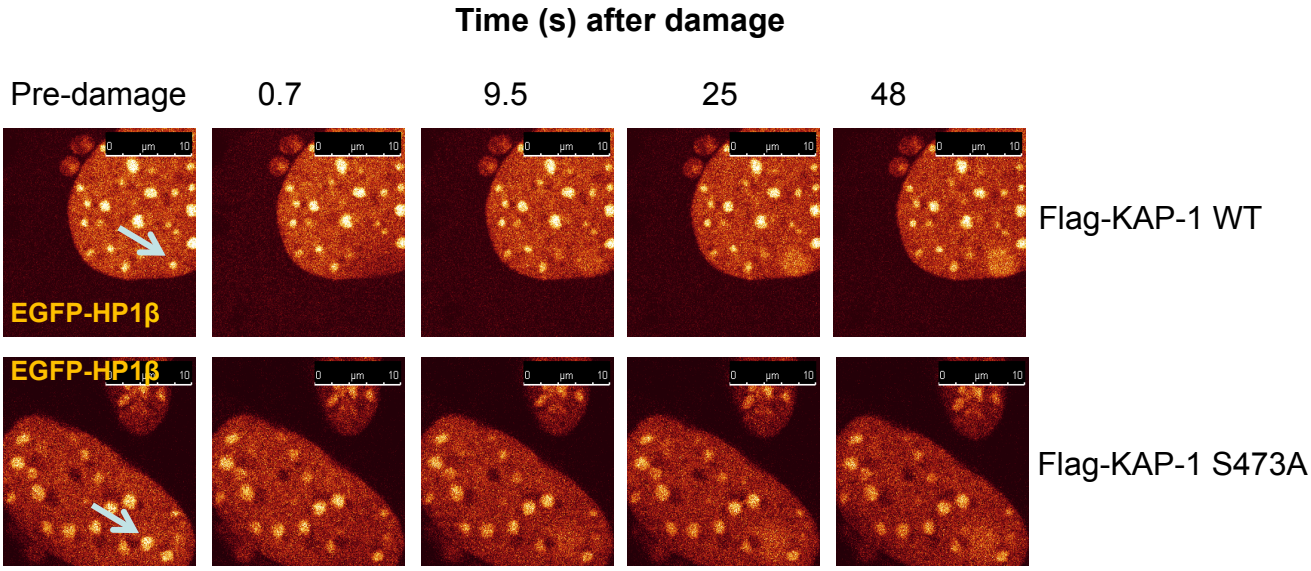
	KAP-1 WT - IR	KAP-1 WT 0.5hIR	KAP-1 WT 24h IR	KAP-1 S473A - IR	KAP-1 S473A 0.5h IR	KAP-1 S473A 24h IR
Total γH2Ax foci/cell	0.38	29	1.82	0.82	29	4.5
γH2Ax foci/cell associated with HC	0.10	7.83	0.69	0.31	9.67	3.03
% HC associated γH2Ax foci	25.67	27.33	40	38	32	67.8

Supplementary Figure 4. > 67% of unrepaired γH2AX foci are associated with heterochromatin in cells expressing S473A. Growth-arrested confluent NIH3T3 cells were transfected with Flag-KAP-1 WT or S473A. After 24 hours cells were exposed to 2 Gy IR and harvested at either 0.5 or 24 hour/s post-IR. Cells were immunostained for γH2AX and DAPI and were analysed as described in Supplementary Figure 3. Briefly, captured images were examined and the total number of γH2AX foci and γH2AX foci associated with heterochromatin were counted. From this the percentage of heterochromatin associated γH2AX foci was calculated. A. Graphical representation of the percentage of heterochromatin associated γH2AX foci. B. A table showing the data values collected.



Supplementary Figure 5. Stable expression of EGFP-HP1-β and Flag-KAP-1 or, S473A in MEF cells. Immunofluorescence images of fixed MEFs stably expressing EGFP-HP1β and FLAG-KAP1 WT (A), S473A (B) or S473E (C). KAP-1 is visualised using anti-FLAG staining and DNA using DAPI. D. Expression of EGFP-HP1-β, Flag-KAP-1 WT, S473A and S473E in MEFs as shown by immunoblotting with the indicated antibodies.

A

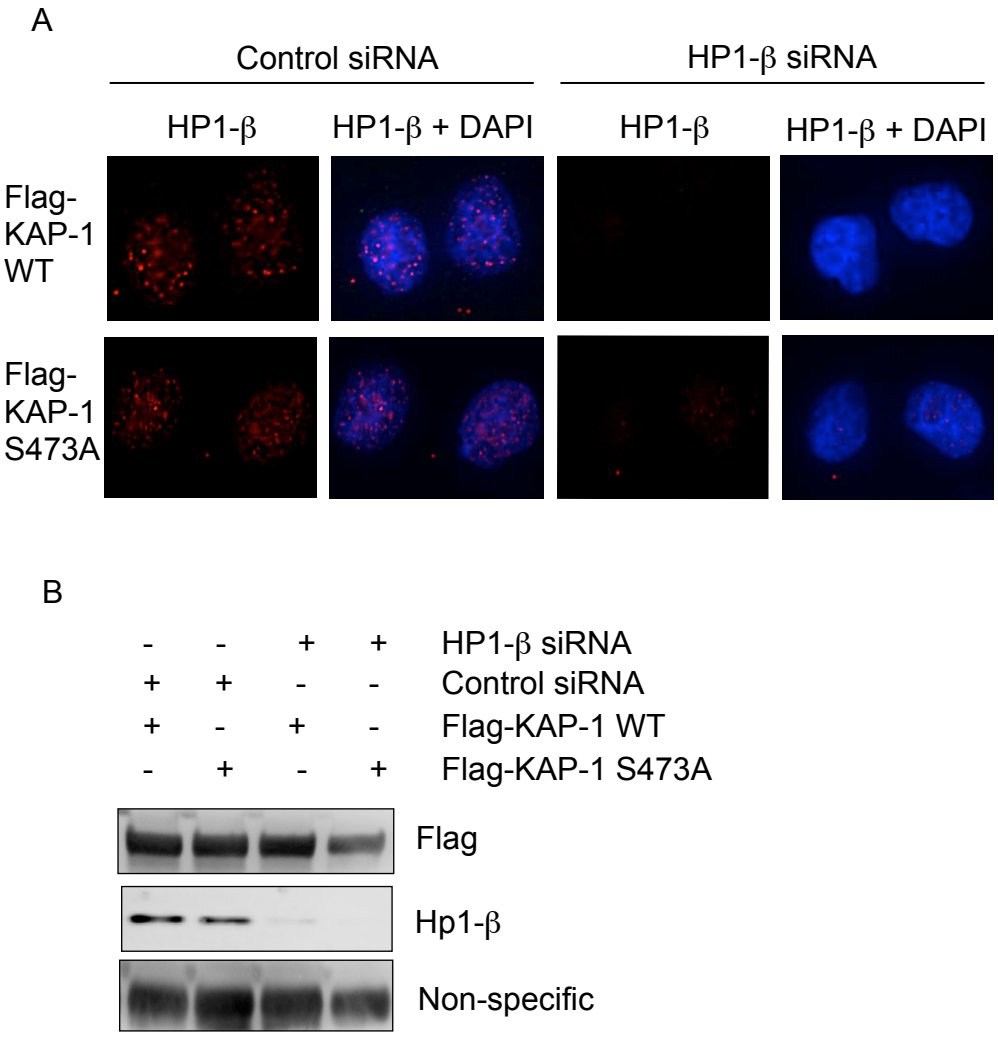


B

Results of FRAP one phase association curve fits

		Undamaged	Damaged	95 % confidence intervals	
Flag-KAP-1 WT	Half time of recovery	5.5	4.5	5.1 to 6.1	4.3 to 4.8
	Plateau of recovery	68.6	80.5	67.7 to 69.5	79.9 to 81.1
Flag-KAP-1 S473A	Half time of recovery	5.06	4.4	4.7 to 5.5	4.2 to 4.7
	Plateau of recovery	74.6	79.4	73.8 to 75.5	78.8 to 80.0

Supplementary Figure 6. EGFP-HP1-β fluorescence recovery. A. Time-lapse imaging of EGFP-HP1β following 405nm laser activated DNA damage in living cells transiently expressing Flag-KAP1 WT or S473A. B. Results of the fits to the EGFP-HP1β FRAP recovery curves shown in Figure 5.



Supplementary Figure 7. A, B. HeLa cells were transfected with control or HP1-β siRNA 24 h before transfection with Flag-KAP-1 WT or S473A. Cells were fixed or extracted 48 h post-siRNA transfection and immunofluorescence (A) or immunoblotting (B) was carried out with the indicated antibodies.