

Supplementary Information

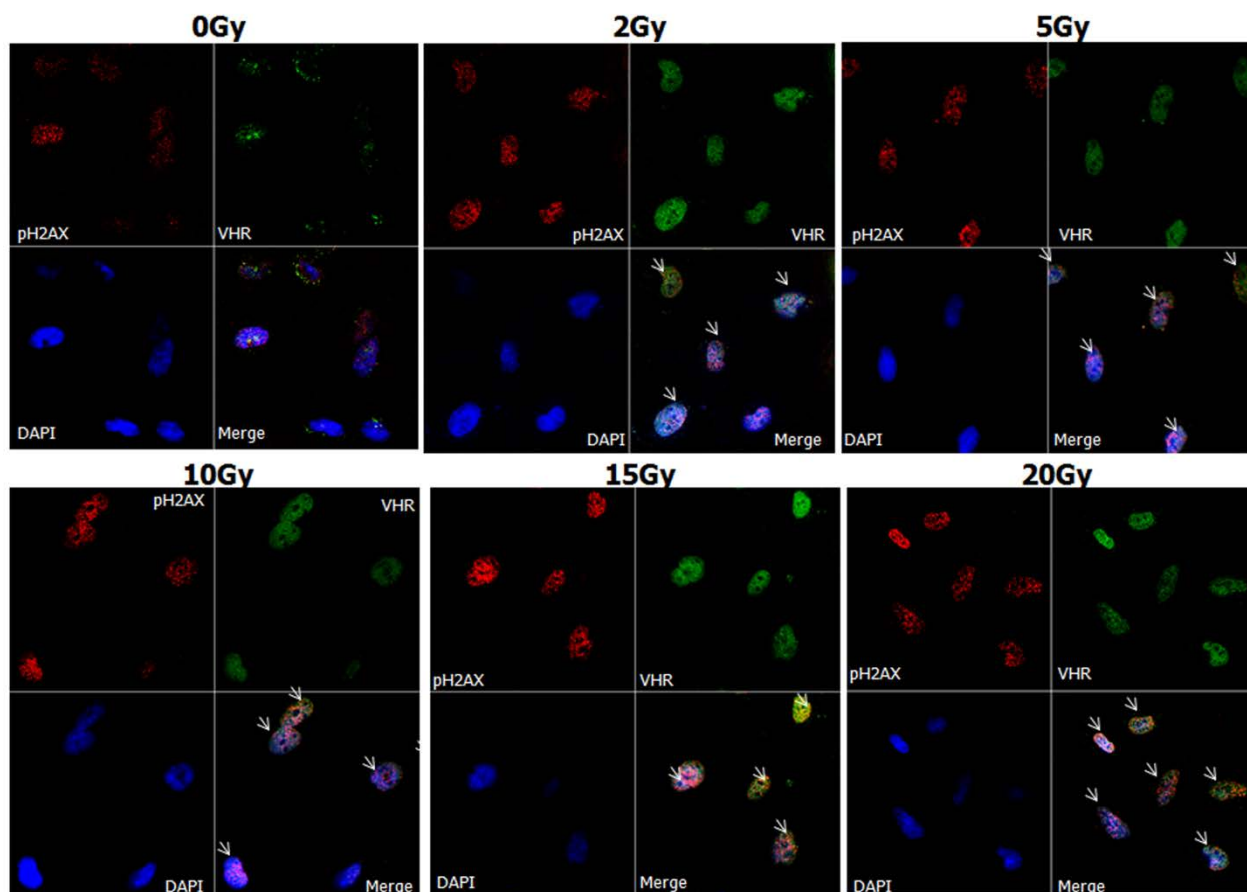


Figure S1. Expression and co-localisation of VHR (green) and pH2AX (red) proteins at the IRIFs of unsynchronised HeLa cells exposed to different doses of gamma radiation (from 0.5 to 20 Gy) and collected 1 h later.

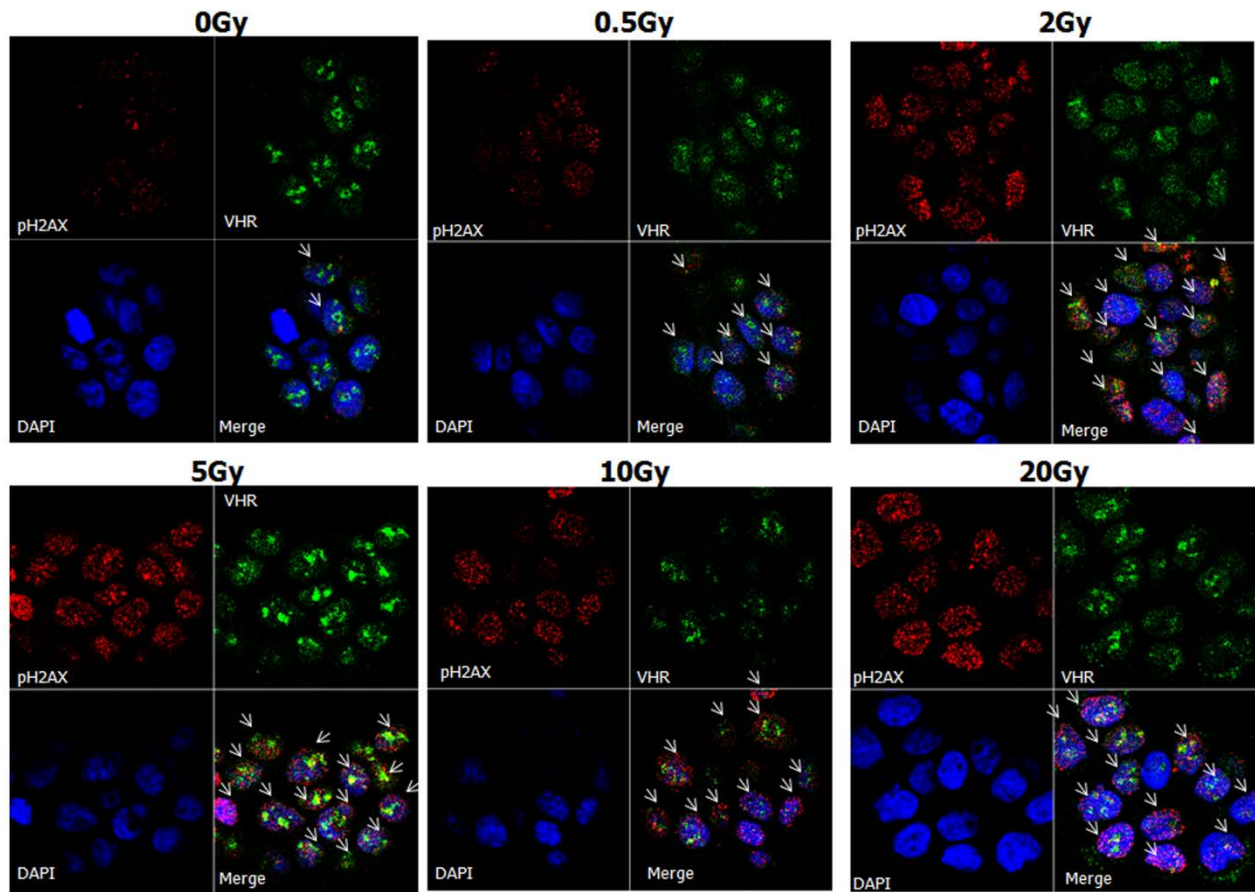


Figure S2. Expression and co-localisation of VHR (green) and pH2AX (red) proteins at the IRIFs of unsynchronised HaCaT cells exposed to different doses of gamma radiation (from 0.5 to 20 Gy) and collected 1 h later.

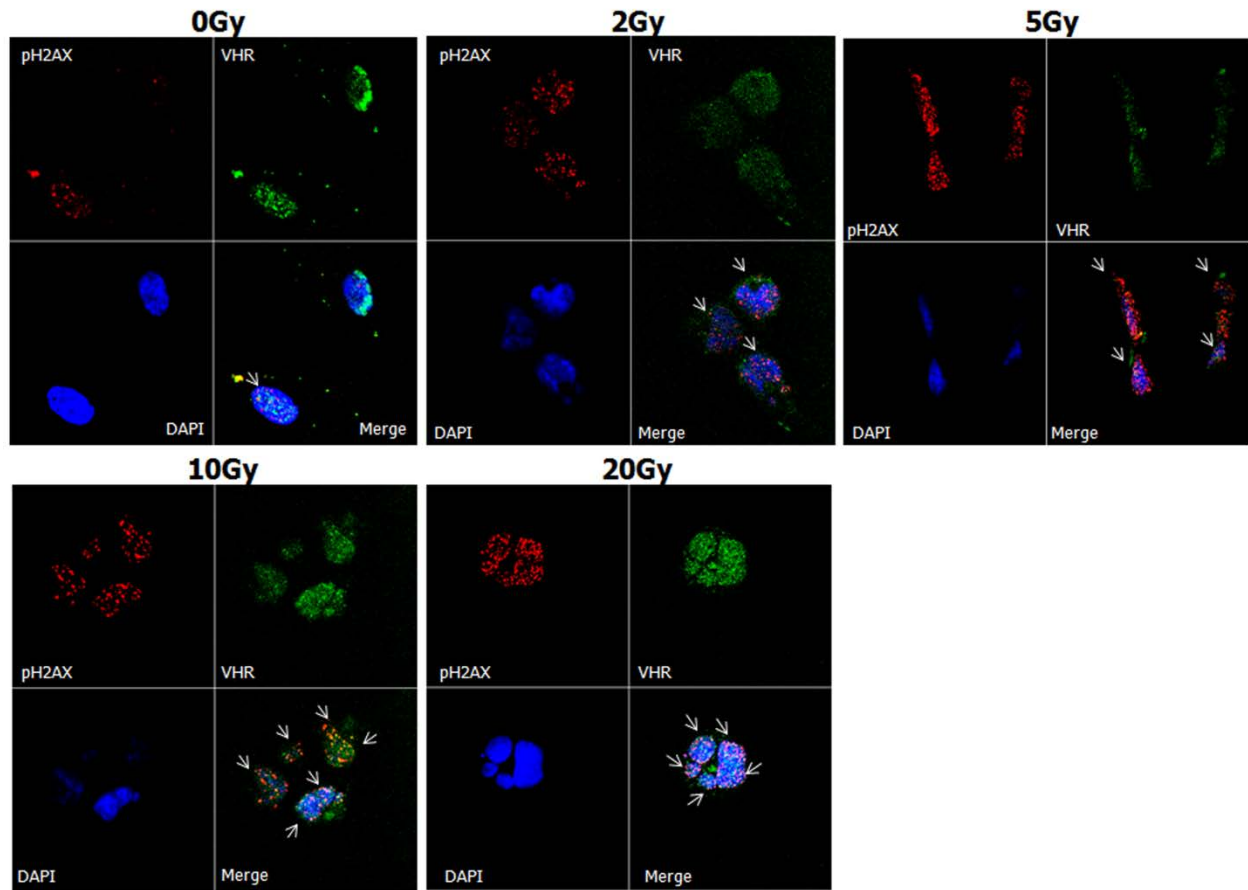


Figure S3. Expression and co-localisation of VHR (green) and pH2AX (red) proteins at the IRIFs of unsynchronised HEK293 cells exposed to different doses of gamma radiation (from 0.5 to 20 Gy) and collected 1 h later.

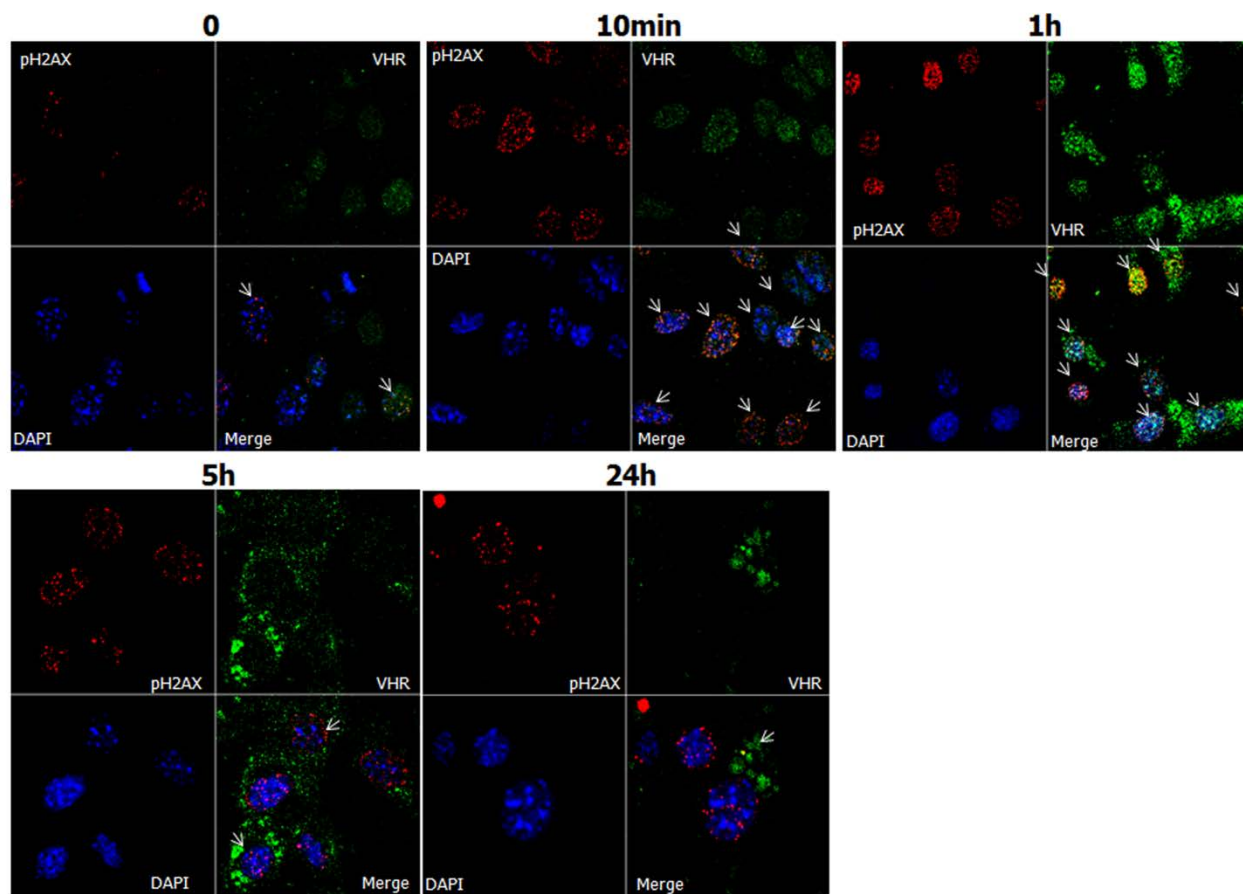


Figure S4. Expression and co-localisation of VHR (green) and pH2AX (red) proteins at the IRIFs of unsynchronised MeWo cells exposed to gamma radiation with 10 Gy and collected up to 24 h after damage.

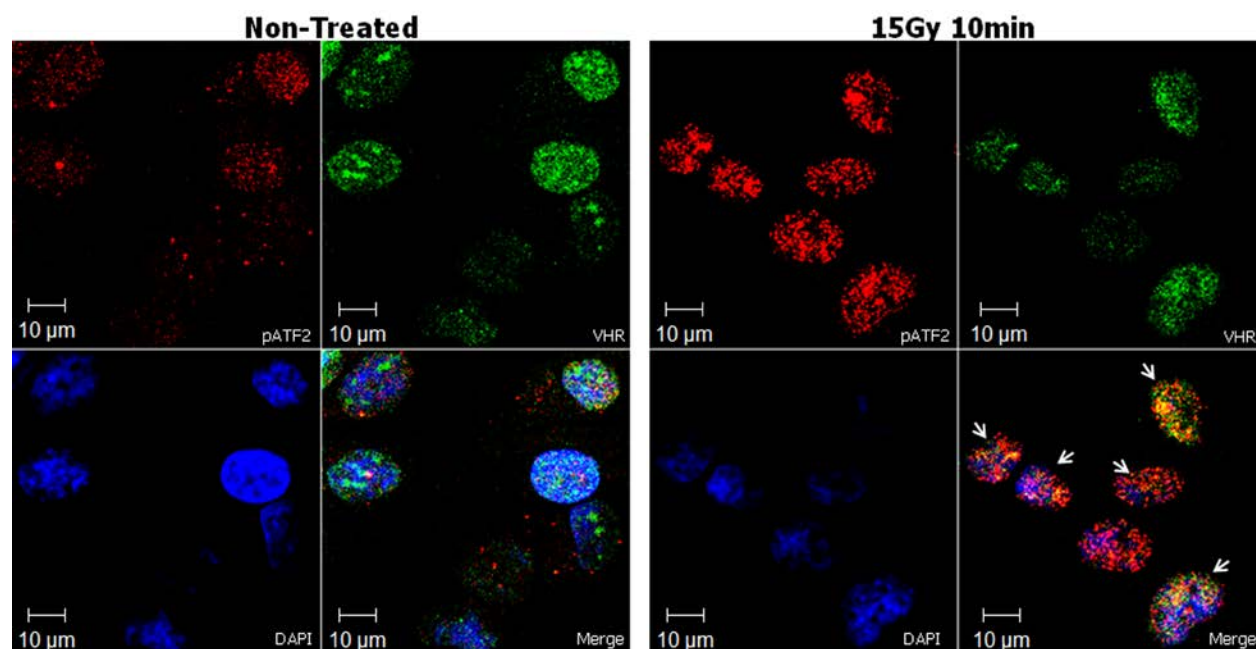


Figure S5. Laser scanning confocal microscopy shows that co-localisation between VHR and ATF2 (Ser 490/498) proteins affects a majority of cells after DNA damage by gamma radiation. Additionally, co-localisation is strong, specific and mainly localized at IRIFs.

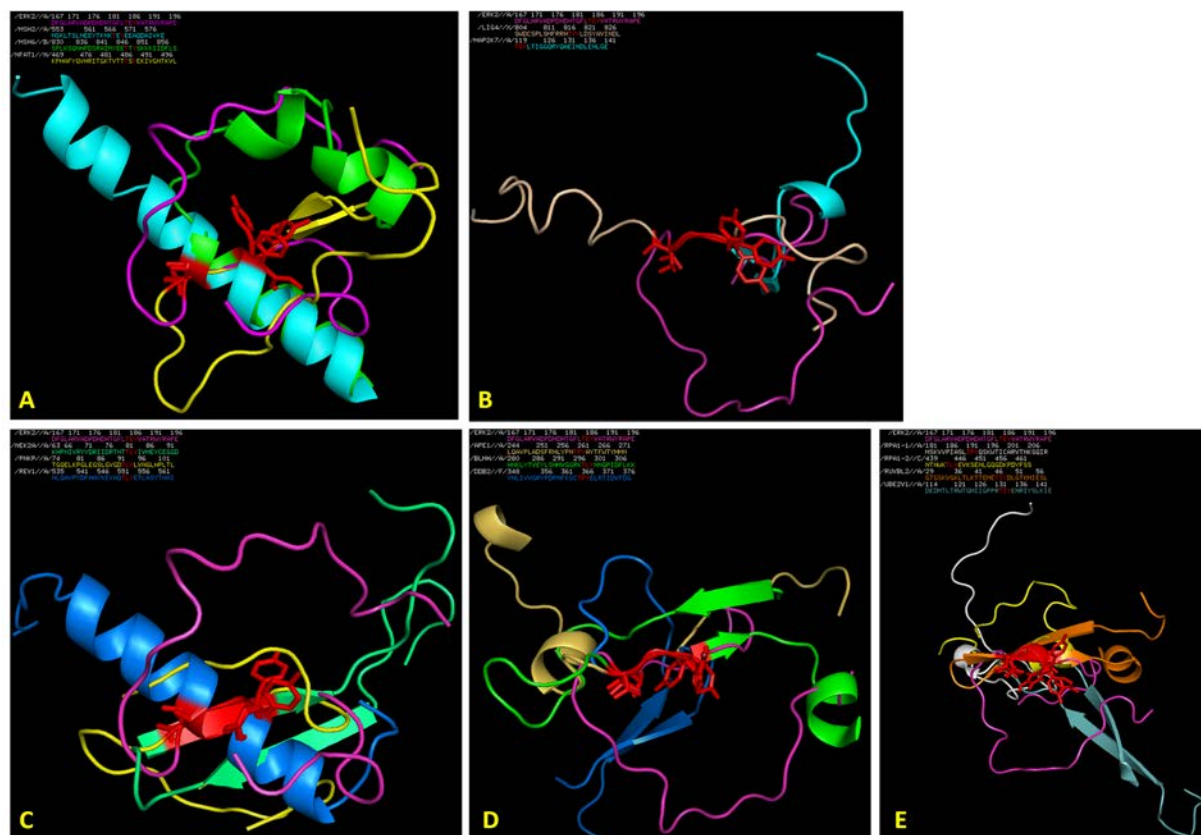


Figure S6. Structural alignment of the ERK2 A-loop (magenta) with the corresponding sequences of 15 protein hits identified in this work (Table S2), highlighting the TXY motif (in red). A. MSH2 (cyan), MSH6 (green) and NFAT1 (yellow). B. LIG4 (pink) and MAP2K7 (cyan). C. NEK2A (green), PNKP (yellow) and REV1 (blue). D. APE1 (golden), BLMH (green) and DDB2 (blue). E. RPA1-1 (silver), RPA1-2 (yellow), RUVBL2 (copper) and UBE2V1 (cyan).

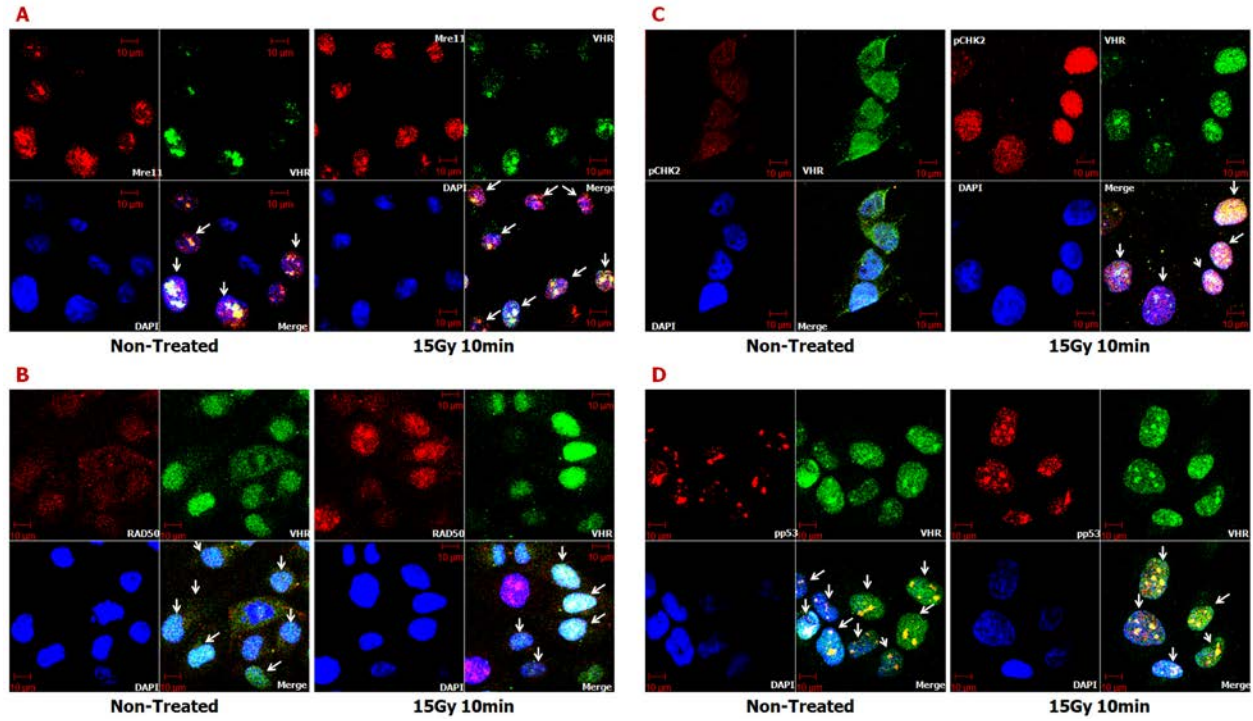


Figure S7. Laser scanning confocal microscopy showing co-localisation of VHR with proteins not found in the bioinformatics mining but well known to be key proteins of relevant DNA repair signalling pathways and directly correlated with the experimental proteins identified in the pull-downs of Figure 7: A) MRE11, B) RAD50, C) pCHK2 (Thr68), and D) p-p53 (Ser15).

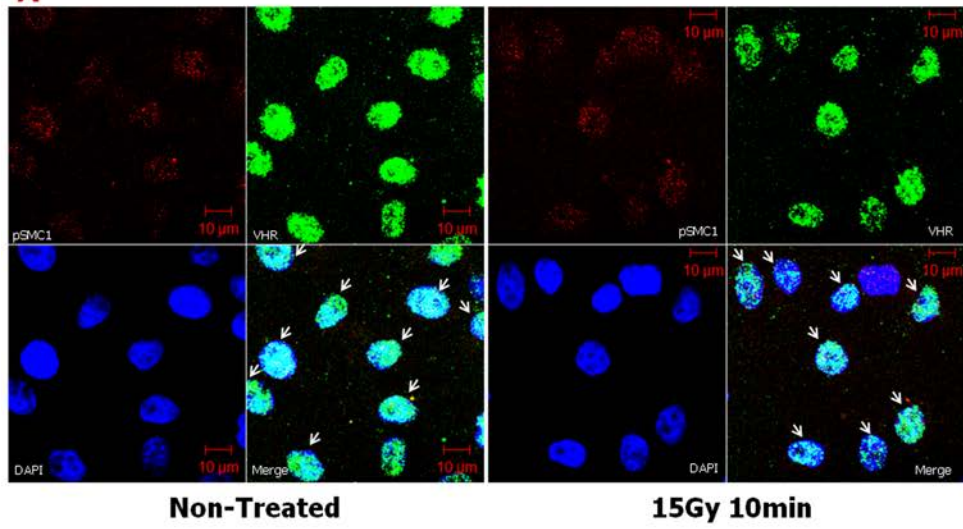
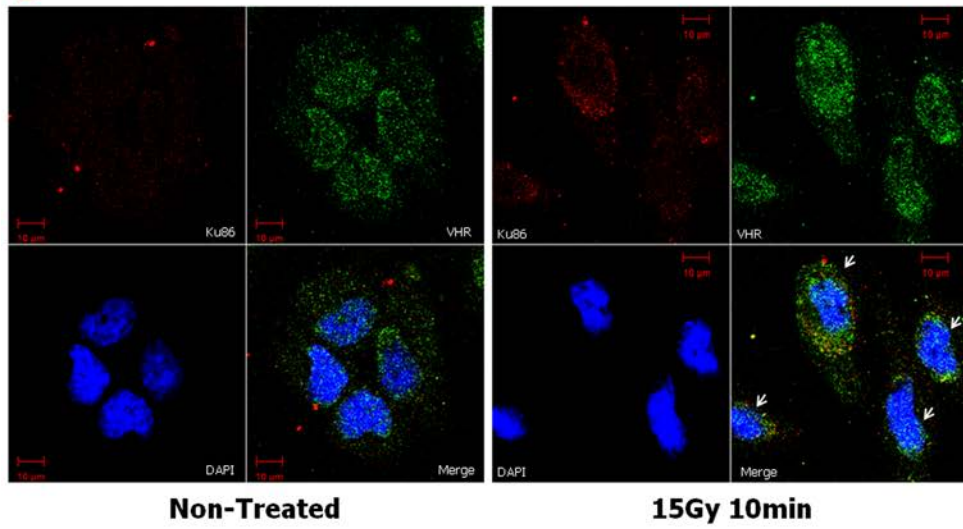
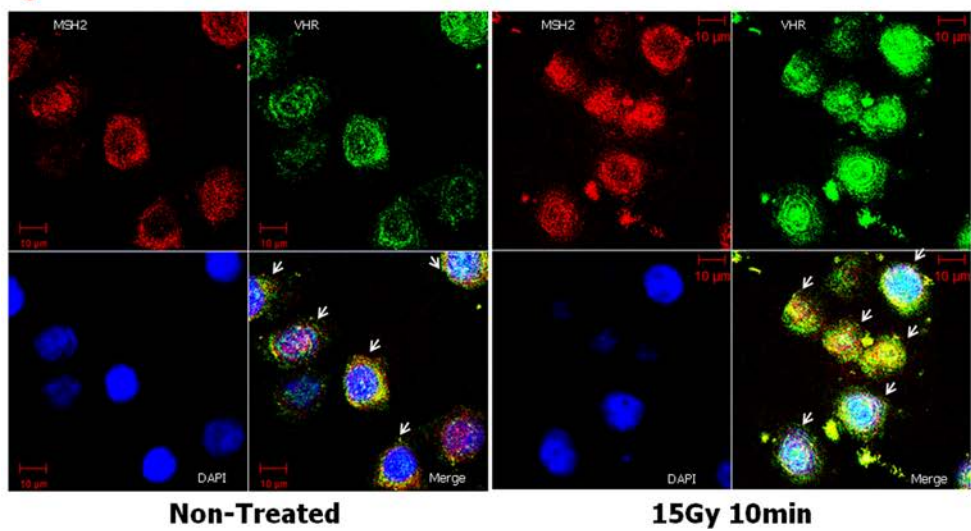
A**B****C**

Figure S8. Laser scanning confocal microscopy showing co-localisation of VHR with protein targets found in the bioinformatics mining and representing three well-known DNA repair pathways activated by gamma radiation: A) pSMC1-Ser360, B) KU86, C) MSH2.

Table S1. Quantification of protein co-localisation in laser confocal microscopy experiments through measurements of overlap coefficients and R and R² correlations using the LSM-Meta software (Zeiss) and the individual fluorescence of fluorophores from each channel.

Protein-Treatment	Overlap Coefficient	Correlation R	Correlation R x R
APE-IR	0.8	0.24	0.06
APE-N	0.8	0.13	0.02
BRCA2-IR	0.8	0.20	0.04
BRCA2-N	0.8	0.10	0.01
CENP-F-IR	0.8	0.27	0.07
CENP-F-N	0.8	0.01	0.00
CYCA-IR	0.8	0.18	0.03
CYCA-N	0.8	0.23	0.05
pATM-IR	0.8	0.23	0.05
pATM-N	0.9	0.38	0.15
pATR-IR	0.8	0.25	0.06
pATR-N	0.9	0.25	0.06
pBRCA1-IR	0.8	0.23	0.05
pBRCA1-N	0.7	0.06	0.00
NBS1-IR	0.8	0.29	0.04
NBS1-N	0.6	0.19	0.01
pCHK2-IR	0.9	0.43	0.18
pCHK2-N	0.8	0.16	0.02
pP53-IR	0.9	0.36	0.13
pP53-N	0.9	0.40	0.16
RAD50-IR	0.8	0.21	0.04
RAD50-N	0.8	0.06	0.00
MRE11-IR	0.8	0.07	0.00
MRE11-N	0.8	0.10	0.01
pERK-IR	0.9	0.56	0.31
pERK-N	0.1	0.20	0.04
pP38-IR	0.6	0.41	0.17
pP38-N	0.3	0.33	0.11
pJNK-IR	0.8	0.24	0.06
pJNK-N	0.2	0.15	0.02
pSMC1-IR	0.8	0.26	0.05
pSMC1-N	0.7	0.06	0.03
Ku86-IR	0.5	0.49	0.18
Ku86-N	0.4	0.39	0.01
MSH2-IR	0.8	0.24	0.08
MSH2-N	0.8	0.16	0.03

Table S2. FASTA format of 121 sequences from 101 proteins corresponding to the A-loop of MAPK (31 amino acids maximum) and including the seven VHR substrates (blue sequences) shown in Figure 3A.

Protein	Uniprot ID	TXV-Containing Sequence
AEN	Q8WTP8	DFQALKYVHPRSQTRDTTYVPNFLSEPL
AP-1	P05412	MTAKMETTFYDDALNASFLP
APE1	P27695	ELLQAVPLADSRHLYPNTPYAYTFWTYMMN
ASPM	Q8IZT6	AFNECHGATCLPLSVRRSTTYSSHLASENRE
ATM-1	Q13315	PLLMILSQLLPQQRHGERTPYVLRCLTEVAL
ATM-2	Q13315	WRNMQWDHCTSVSKEVEGTSYHESLYNALQS
ATM-3	Q13315	YHESLYNALQSLRDEFSTFYESLKYARVKE
ATM-4	Q13315	ELRNGKMKAFLLSARFSDTYQRIENYMKSS
ATRX-1	P46100	EKSKSSGSSRSKRKPSIVTKYVESDDEKPLD
ATRX-2	P46100	RFNIPTGTNLPPVSFNSQTPYIPFNLGALSA
BLMH	Q13867	YNKLYTVEYLSNMVGGKRLYNNQPIDFLKK
BRCA1-2	P38398	GPHDLTETSYLPRQDLEGTPYLESIGISLFS
BRCA1-3	P38398	VDVEEQLEESGPHDLTETSYLPRQDLEGTP
BRCA2	P51587	ISTLKKTKNKFIYAIHDETSYKGGKIPKDQK
BubR1	O60566	PRGNTASLIAPPAVLPSFTPYVEETARQPVM
CCNA1	P78396	ILVDWLVEVGEEYKLRAETLYLAVNFLDRFL
CCNE1	P24864	ILLDWMVEVCEVYKLHRETFYLAQDFDRYM
CDK14-2	O94921	EDTWPGVHSLPHFKPERFTLYSSKNLRQAWN
CDK19	Q9BWU1	EDIRKMPEYPTLQKDFRRTTYANSSLIKME
CDK5RAP1	Q96SZ6	SEVQFN SAVPTNLSRGFTTNYKTKQGGLRFA
CENPE	Q02224	DELTALQFASTAKYMKNTPYVNEVSTDEAL
CENPF-1	P49454	NKCRDELVRTTAQYDQASTKYTALEQLKKL
CENPF-2	P49454	ETGAVKPTGECSGEQSPDTNYEPPGEDKTQG
CHAF1B	Q13112	SLLLTPAGCVESGENVMNTTYVFSRKNLKR
CLSPN	Q9HAW4	VLQDSDSETEDTNASPEKTTYDSAEENKEN
CTC1	Q2NKJ3	IIVQVPAQLVWHRALRPGTAYVLTELRSKI
DAP6	Q9UER7	DCSSLTGRVIEQRIPYRGTRYPEVNRRIERL
DDB2	Q92466	RYNLIVVGRYPDPNFKSCTPYELRTIDVFDG
DTL	Q9NZJ0	YPLQSLLTGYQCSGNDHTSYGETGVPVPPF
E2F2	Q14209	IRVDGLPSPKTPKSPGEKTRYDTSGLLTKK
E2F8	A0AVK6	IESVNVAPENAGTQQGRATNYDSPVPGSQSP
EMSY	Q7Z589	SPRPASPASNVVVLPSGSTVYVKSVCSD
EPC2	Q52LR7	NTGHNNINGVVQPSGTSKTLYSTNMALSSSP
ERCC2	P18074	FDAMRHAAQCVGRAIRGKTDYGLMVFADKRF
ERCC3	P19447	RFGMTSSMSGADDTVYMEYHSSRSKA
ERCC6-1	Q03468	EEEDDEVEGAADLSGDGTDYELKPLPKGGK
ERCC8	Q13216	KVWDNTNTLQTADVNFEEETVYSHHMSPVSTK
EYA2	O00167	TAMAAYGQTQYSAGIQATPYTAYPPPAQAY
FANCE	Q9HB96	GLAATTSMAYAKMLTMVTKYQANITETQRL
FANCL	Q9NW38	EASLLRQCPLLLPQNRSKTVYEGFISAQGRD
FSD1	Q9BTV5	PPRLKEDQPMVIEGIRQTEYTLTGLKFDK
HERC2	Q95714	LKWKFISDGSVNGWGWRFTVYPIMPAAGPKE
HIPK2	Q9H2X6	AFCSVKKLIKIEPSSNWDMTGYGSHSKVYSQS
IFI16-1	Q16666	NIVYGVFMLHKKTVNQKTTIYEIQDDRGMKD
IFI16-2	Q16666	SFVNGVFEVHKKNVRGEFTYIEIQDNTGKME
KAP1-2	Q13263	KDHTVRSTGPAKSRDGERTVYCNVHKHEPLV
KAT5	Q92993	LDHKTLYYDTPFLFYVMTEYDCKGFHIVGY
KIF20B	Q96Q89	SALLRQIKEVTVHNSDDTLYGSLTNSLNIS
KIF23-1	Q02241	QLHTPEGYRLNRNGDYKETQYSFKQVFGTHT
KIF23-2	Q02241	EKKNKTLEYKIEILEKTTTIYEEDKRNLQQE
KNTC1-1	P50748	SLEVSSVSSLVQTGISTDTIYLLEGVCKNDP
KNTC1-2	P50748	ESFPTLLLISKLMKFSLDTLVSTAKHVFEK
L3MBTL1	Q9Y468	NMAGVEQPPSPELRQEGVTEYEDGGAPAGDG
LIGASE-4	P49917	LEYRYSWDCSPLSMFRRHTVYLD SYAVINDL
MAP2K7	O14733	RSMESIEIDQKLQEIIMKTGYLTIGGQRYQA
MAPK7-1	Q13164	DFGMARGLCTSPAETHQYFMTEYVATRWYRAPE
MAPK7-2	Q13164	RVRAYIQSLPPRPQVPWETVYPGADRQALS
MASTL-1	Q96GX5	SQLSQGLVCPMSVDQKDTTPYSSKLLKSCLE
MASTL-2	Q96GX5	KRRSCMPHQQTTPNQIKSGTPYRTPKSVRRGV
MASTL-3	Q96GX5	NLQHQTMPFIPQPDDETDSYFEARNTAQHL
MEF2A	Q02078	VEALNKKEHRGCDSPDPDTSYVLTTPHTEEKY
MEKK2	Q9Y2U5	DFGASKRLQTIICLSGTGMKSVTGTYPWMSPE
MITF	O75030	DSGIVPDFEVGEEFHEEPKTYEELKSQPLKS
MLTK	Q9NYL2	NIIQFYGVILEPPNYGIVTEYASLGSLYDYI
MNAT1	P51948	MDDQGCPRCKTTKYRNPSLKLNVN
MNDA	P41218	TMVYGLFMLQKKSVMKNTIYEIQDNTGSMD

MSH2|P43246|KFTNSKLTSLNEEYTKNKTEYEEAQDAIVKE
MSH6-1|P52701|MSRQSTLYSFFPKSPALS
MSH6-2|P52701|AVCDEPSEPEEEEEMEVGTYYVTDKSEEDNE
MSH6-3|P52701|SPLKSQNHPDSRAIMYEETTSKKKIIDFLS
NAP57-1|060832|TSQWPLLLKNFDKLNVRTTHYTPLACGSNPL
NAP57-2|060832|LFQRPPLIAAVKRQLRVRTIYESKMIEYDPE
NBS1|060934|SQGVSVDEKLMPSAPVNTTTYVADTESEQAD
NEDD9|Q14511|FGQQKLYQVPNPQAAPRDTIYQVPPSYQNQG
NEK2A|P51955|KHPNIVRYYDRIIDRTNTTLYIVMEYCEGGD
NEK9|Q8TD19|ELHYIPIRVLGRGAFGEATLYRRTEDDSLTV
NFAT1|Q13469|KPHAFYQVHRITGKTVTTTSYEKIVGNTKVL
NIPBL2|Q6KC79|AMTRKILNITDVVAACRDTGYDWEQLLQNL
p97-ERK3|Q16659|RKYLDGDREKYLEDPAFDNYSTEPQWQYSD
PA2G4|Q9UQ80|DVLVSSGEGKAKDAGQRTTIYKRDPKQYGL
PTAS1|Q75925|LPHQASPVSRTPSLPAVDTSYINTSLIQDYR
PKN1|Q16512|AAASSAAFSTRLAGPFPATHYSTLCCKPAPLT
PNKP|Q96T60|TGTQELKPGLEGSLGVGDTLYLVNGLHPLTL
POLK|Q9UBT6|SSSGVQKAVTRTKRPLMTKYSTSKKIKPNN
Po1r2b|P30876|IINGSEKVLIAQEKMATNTVYVFAKDSKYA
PSMB2|P49721|TAAANFTRRNADCLRSRTPYHVNLLLAGYD
RAG|P15918|LMLELGGILRTFKFIFRGTYDEKLVREVEG
REV1|Q9UBZ9|PNLQAVPYDFHAYKEVAQTLYETLASYNHI
REV3L|Q60673|KKLAPLKDFWPKVPDSPATKYPIYPLTPKKS
RFWD3|Q6PCD5|APLDAYFQVSRTQPDLPATTYDSETRNPVSE
RIF1-3|Q5UIP0|VEMSTEEGIIDANKTETNTEYSKSEEKLDNN
RPA1-1|P27694|LSHTSGGTQSKVVPISLTPYQSKWTICARV
RPA1-2|P27694|SISDLKSGGVGGSNTNWKTLYEKSENLGQG
RUVBL2-1|Q9Y230|TGTGSKVGKLTLLKTTMETIYDLGTMIESL
RUVBL2-2|Q9Y230|MAPVLIMATNRGITRIRGTSYQSPHGIPIDL
RUVBL2-3|Q9Y230|SPHGIPIDLLDRLLIVSTTPYSEKDTKQILR
RUVBL2-4|Q9Y230|QVDDIKRVYSLFLDESSTQYMKYQDAFLF
S6K- α 2|Q15349|IGVGSYSVCKRCVHKATDTEYAVKIIDKSKR
Separase|Q14674|IKEYGASPVLSQGVDPSTFYVLNPHNNLSS
SES1|Q9Y6P5|SDDEEVTPARAVSRHFEDTSYGYKDFSRHGM
SETDB2|Q96T68|TEKCPPKFSNNKELTVETKYDNISRIQYHS
S1x1|Q15475|YRVRRKFPLPRTIWDGEETS YCFKEKSRGVL
SMC1A|Q14683|VYPEQGDCVISKVLTFDLTKYPDANPNPNEQ
SMC3|Q9UQE7|NLPGEVTFPLNKLVDVTDAYPETNDAIPMI
SPT6|Q7KZ85|CRTSDLMDRNNEWKLPKDTYYDFDAEAADHK
SPY1-1|Q5MJ70|MRHNQMCCEPPTVTYVYKSGSNRSHQ
SPY1-2|Q5MJ70|NFFIALYLANTVEEDEEETKYEIFPWALGKN
TCAB1|Q9BUR4|DTIYDYCWYSLMSSAQPDTSYVASSSRENPI
TNIK|Q9UKE5|QLKDHIDRTKKKRGEKDETEYEYSGSEEEEE
TPX2|Q9ULW0|RKANLQQAIVTPLKPVNTYYKEAEKENLVE
UBE2V1|Q13404|DEDMTLTRWTGMIIGPPRTIYENRIYSLKIE
UNG1-1|P13051|MIGQKTLYSFFSPSPARK
Whsc1L1-2|Q9BZ95|LPPLTNGYPSSISVYETQTKYQSYNQYPNGS
ZBTB32|Q9Y2Y4|PYACSVCGKRFSLKHQMETHYRVHTGEKPF5
[ERBB2|P04626|DFGLARLLDIDEYHADGGKVPKWMMALE](#)
[ERK1|P27361|DFGLARIADPEHDHTGFLTEYVATRWYRAPE](#)
[ERK2|P28482|DFGLARVADPDHDHTGFLTEYVATRWYRAPE](#)
[JNK1|Q13263|DFGLARTAGTSFMMTPYVVTRYRAPE](#)
[JNK2|Q13263|DFGLARTACTNFMMPYVVTRYRAPE](#)
[JNK3|Q13263|DFGLARTAGTSFMMTPYVVTRYRAPE](#)
[P38|Q16539|DFGLARHTDEMTGYVATRWYRAPE](#)

Table S3. Proteins with solved structures deposited in the PDB that were among the 64 hits obtained at the end of bioinformatics analyses. The 15 sequences below were used for the structural alignments shown in Fig. S3 and correspond to the structured region comprising the 31 amino acids and containing the TXY motifs.

Protein	TXY-Containing Sequence	#PDB
APE1	ELLQAVPLADSFRLYPNT TPY AYTFWTYMMN	1E9N
BLMH	YNKLYTVEYLSNMVGGRK TL YNNQPIDFLKK	2CB5
DDB2	RYNLIVVGRYPDPNFKSC TPY ELRTIDVFDG	3EI4
LIG4	LEYRYSWDCSPLSMFRRH TVY LDSYAVINDL	3II6
MAP2K7	RSMESIEIDQKLQEIMKQ TGY LTIGGQRYQA	2DYL
MSH2	KFTNSKLTSLNEEYTKNK TEY EEAQDAIVKE	2O8B
MSH6 - 3	SPLKSQNHPDSRAIMYEE TTY SKKKIIDFLS	2O8B
NEK2A	KHPNIVRYDRIIDRTNT TL YIVMEYCEGGD	2W5H
NFAT1	KPHAFYQVHRITGKTVTT TSY EKIVGNTKVL	1A02
PNKP	TGTQELKPGLESLGVGD TL YLVNGLHPLTL	2BRF
REV1	PNLQAVPYDFHAYKEVAQ TL YETLASYTHNI	3GQC
RPA1 - 1	LSHTSGGTQSKVVPIASL TPY QSKWTICARV	1FGU
RPA1 - 2	SISDLKSGGVGGSNTNWK TL YEVKSENLGQG	1L10
RUVBL2 - 1	TGTGSKVGKLTCLKTTEME TIY DLGTMIESL	2CQA
UBE2V1	DEDMTLTRWTGMIIGPPR TIY ENRIYSLKIE	2A4D

Table S4. Statistical and topological analyses of the VHR networks from Figure 8 showing the simplest network parameters for comparison (data obtained using the Network Analyzer Cytoscape plugin).

SIMPLE NETWORK PARAMETERS	57 PROTEINS NETWORK	CLUSTER 1	CLUSTER 9
Clustering coefficient	0.347	0.875	0.608
Network diameter	6	2	7
Network centralization	0.156	0.372	0.201
Shortest paths	1541322	20022	552
Characteristic path lenght	2.775	1.367	3.684
Avg. number of neighbors	29.96	89.17	4.75
Number of nodes	1242	142	24
Network density	0.024	0.632	0.206
Network heterogeneity	1.237	0.266	0.590
Number of self-loops	444	34	14
Analysis time (sec)	452622	53743	0.025