Inventory of Supplementary Material

Ovarian carcinoma *CDK12* mutations misregulate expression of DNA repair genes via deficient formation and function of the Cdk12/CycK complex

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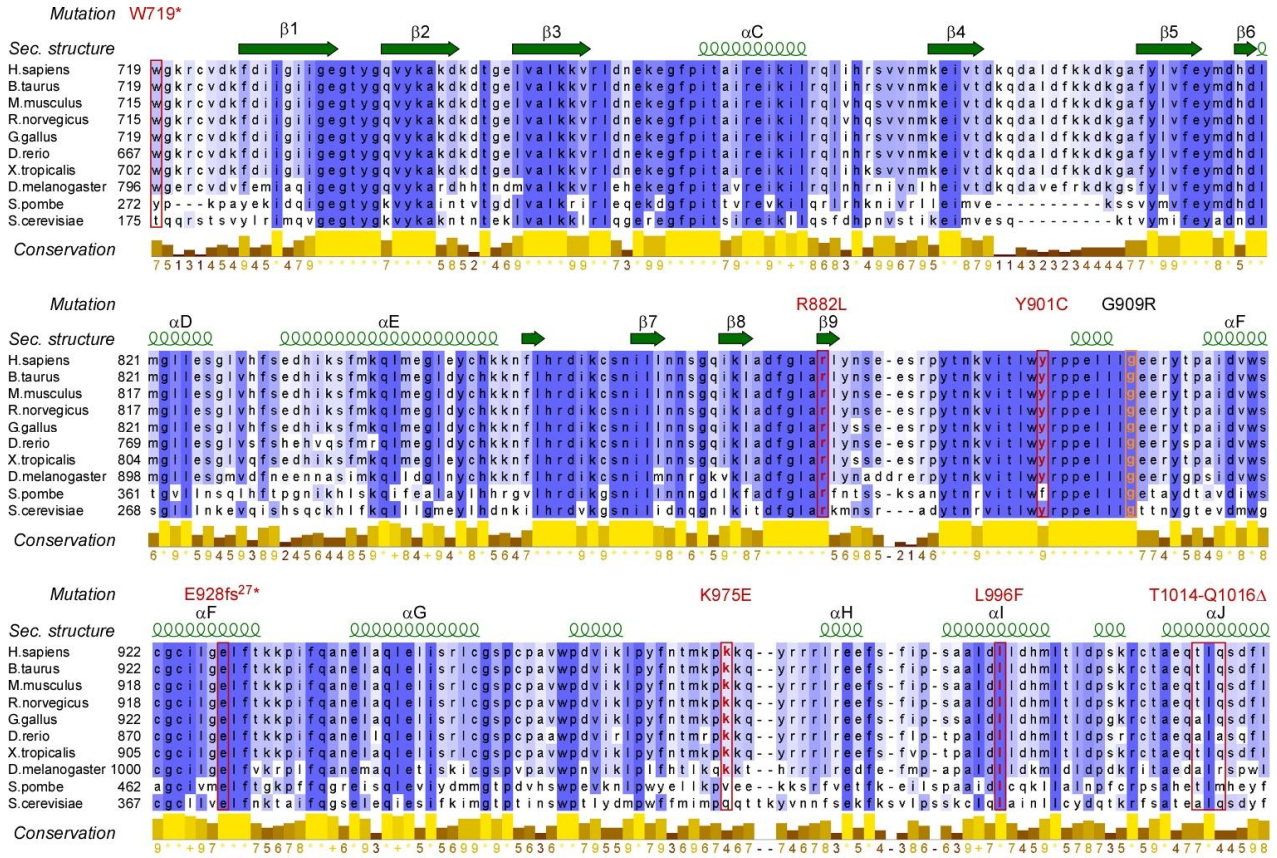
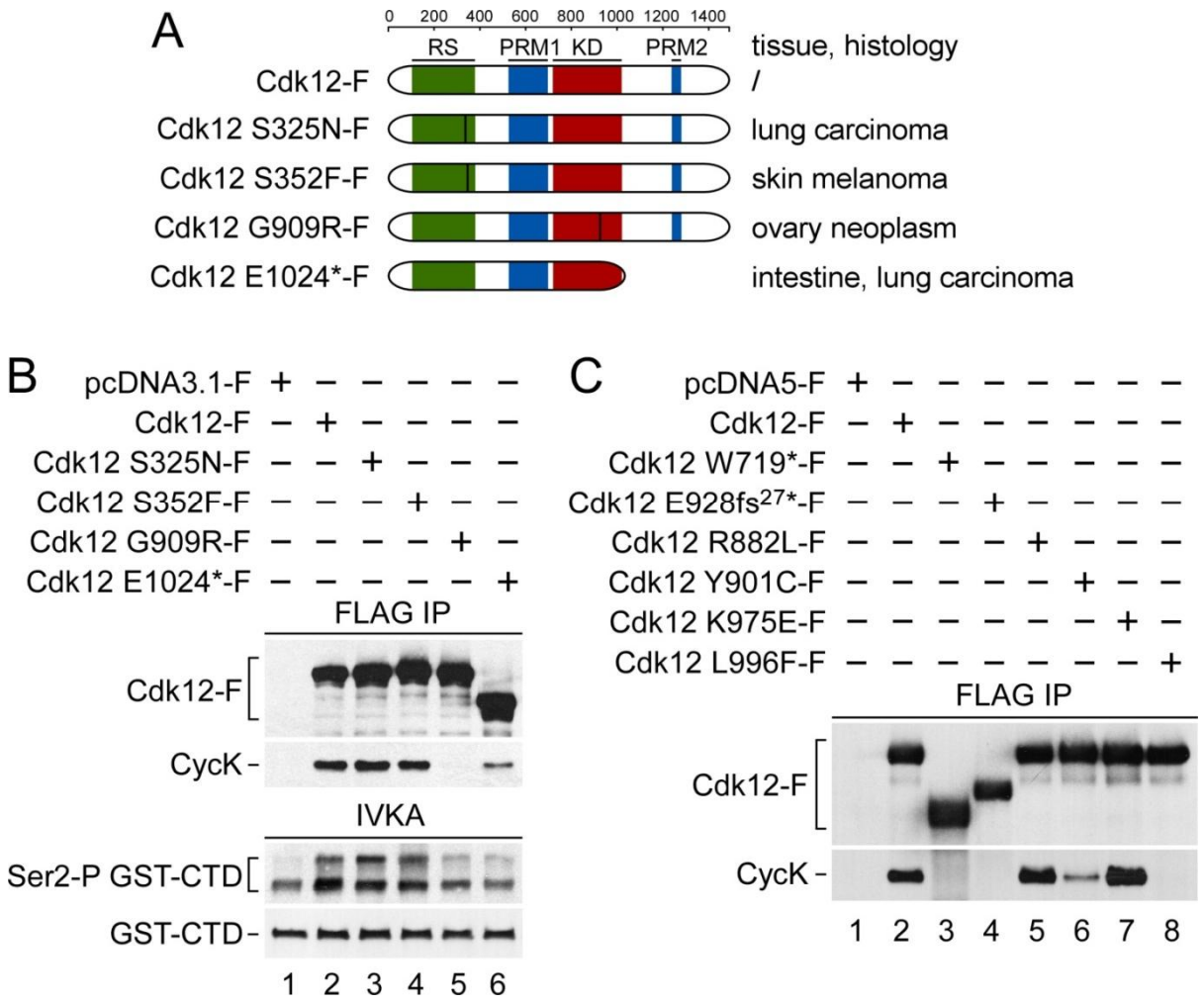
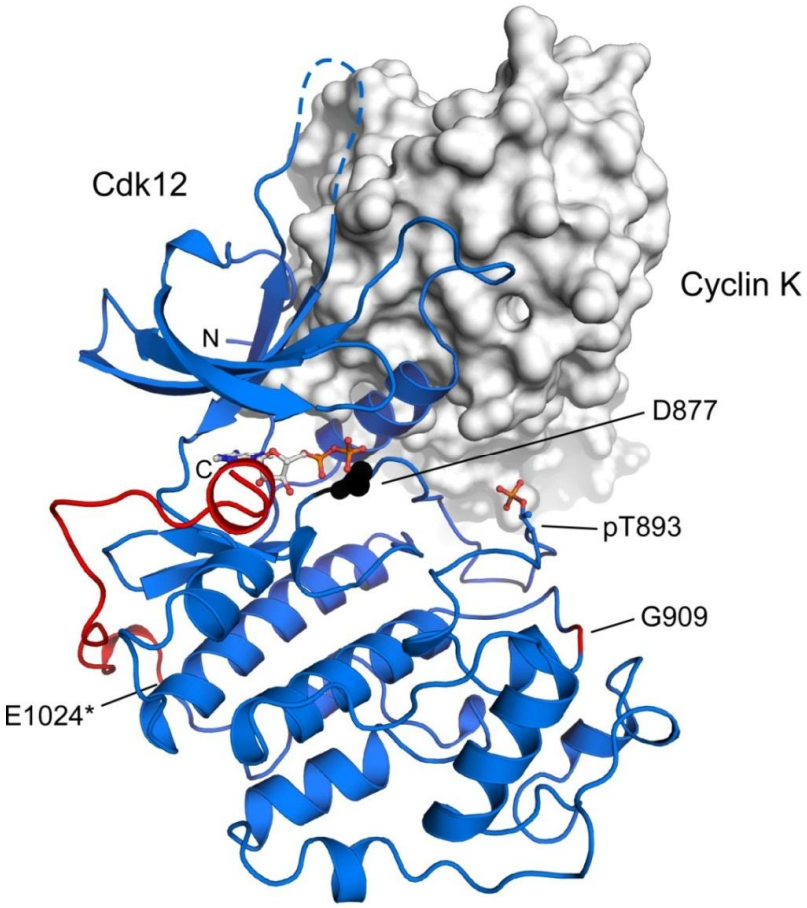
SUPPLEMENTARY FIGURES AND FIGURE LEGENDS



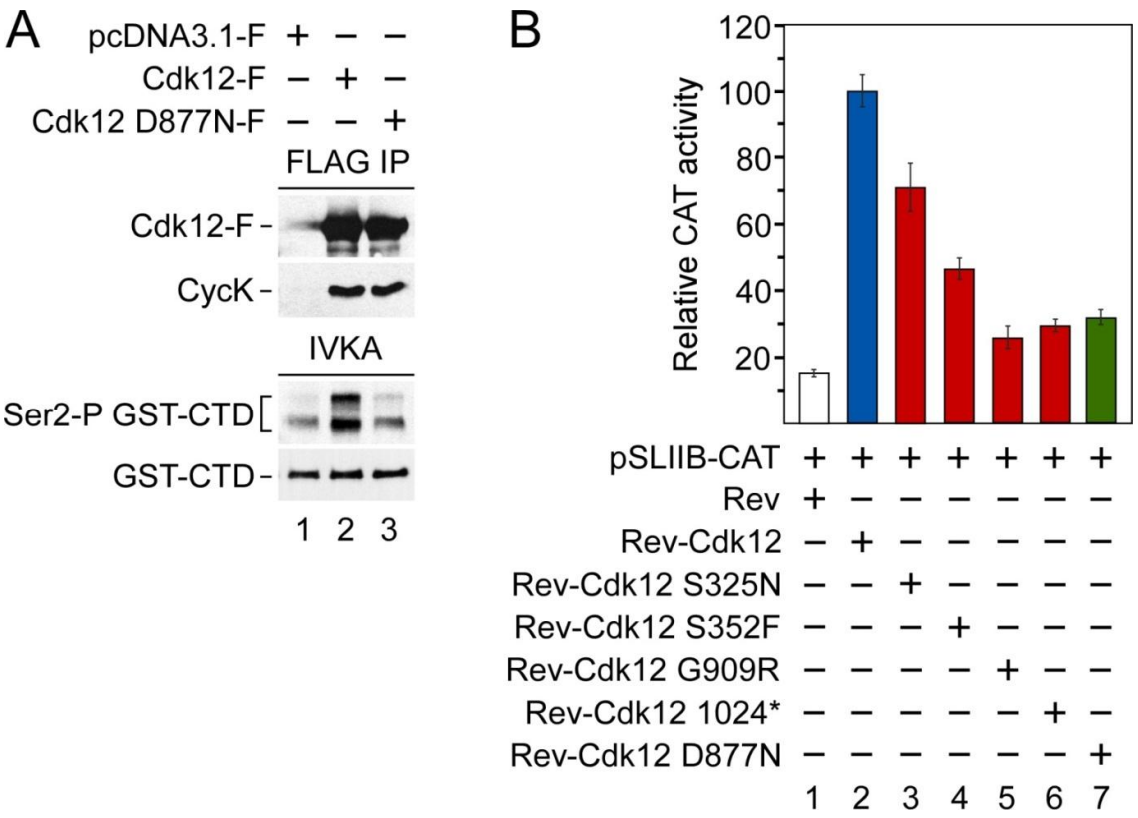
Supplementary Figure S1. Evolutionary conservation of the mutated amino acid residues within the KD of Cdk12. Alignment of Cdk12 kinase domains from different species indicated on the left is presented. Secondary structure elements are indicated on top. Due to the space constraints, the alignment is split into three portions. Increased level of dark blue color indicates higher evolutionary conservation. The conservation is also represented in different shades of gold with numbers below each amino acid indicating the degree of identity from the lowest (1) to the highest (*). Whereas the *CDK12* mutations identified in HGS-OvCa are labeled in red, the residue mutated in another type of cancer is marked in black and ochre. Numbers before sequences on the left indicate positions of first amino acids within the corresponding full-length Cdk12 proteins. NCBI reference sequence identifiers and lengths of Cdk12 proteins in amino acids are: NP_057591 (H.sapiens Cdk12; 1490 aa), NP_001192630 (B.taurus; 1490 aa), NP_001103096 (M.musculus; 1484 aa), NP_001029039 (R.norvegicus; 1484 aa), XP_425866 (G.gallus; 1477 aa), XP_003200579 (D.rerio; 1293 aa), NP_001128283 (X.tropicalis; 1239 aa), NP_649325 (D.melanogaster; 1157 aa), CAB16269 (S.pombe; 593 aa), CAA81980 (S.cerevisiae; 528 aa). The sequence alignment was prepared using Clustal Omega multiple sequence alignment tool from EMBL-EBI.



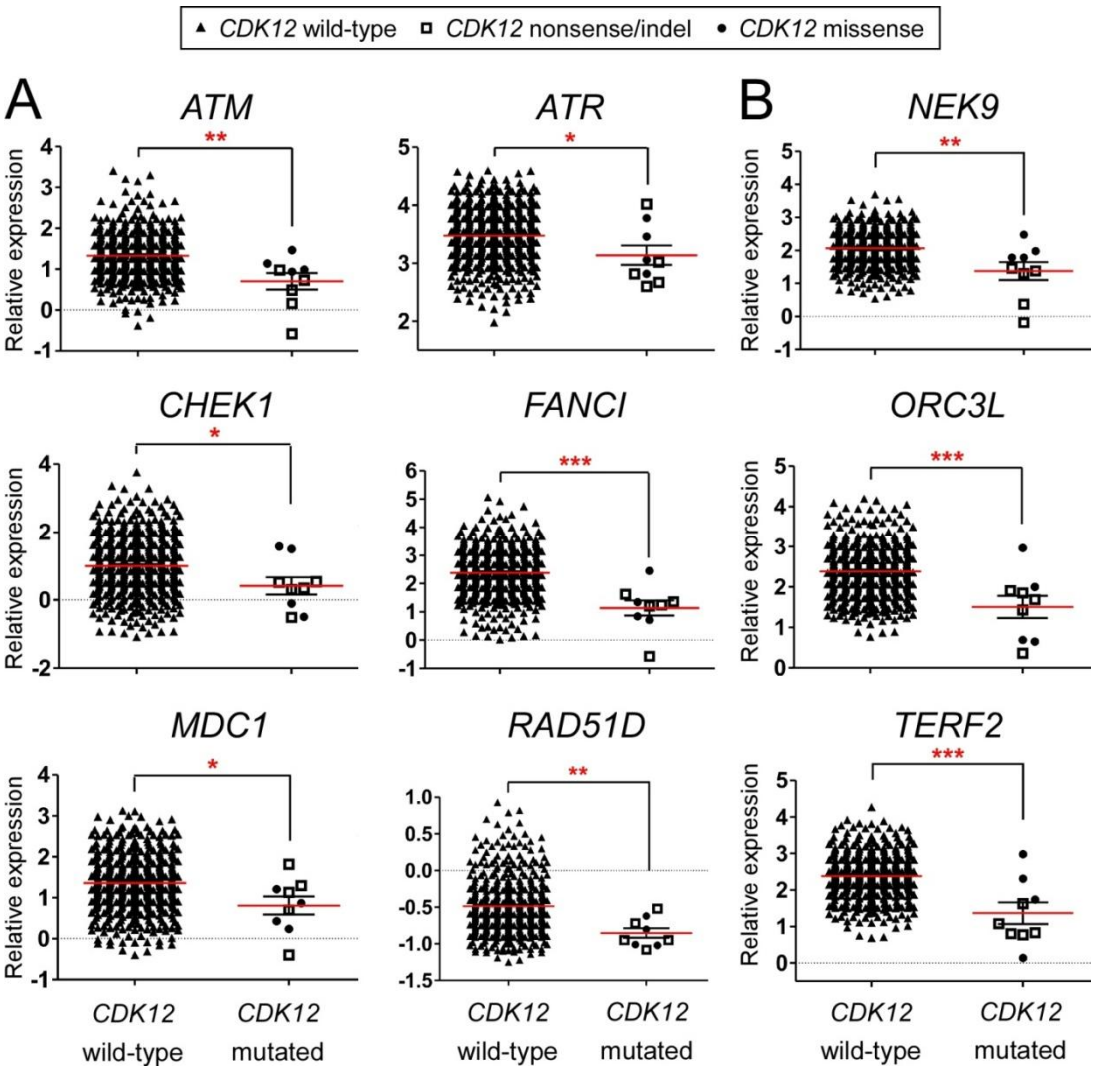
Supplementary Figure S2. *CDK12* mutations from other cancers that impact the KD of Cdk12 have detrimental effects on the formation and activity of the Cdk12/CycK complex. (**A**) Schematic depiction of wild-type and mutant Cdk12 proteins containing individual *CDK12* mutations. Highly structured kinase domain (KD; red), arginine/serine rich region (RS; green) and two regions with proline-rich motifs (PRM1 and PRM2; blue) are depicted. The ruler on top indicates the length of Cdk12 protein in amino acids. Vertical lines denote sites of individual missense mutations. Cancer tissue/histology with a particular *CDK12* mutation is listed on the right. (**B**) Effects of the *CDK12* mutations on the interaction between Cdk12 and CycK. The indicated wild-type and mutant Cdk12-F proteins were expressed transiently in HEK 293 cells, immuno-purified from WCEs using FLAG-M2 agarose (FLAG IP) and examined for their interaction with endogenous CycK. The complexes were examined for their interaction with endogenous CycK (top) and for their kinase activity by *in vitro* kinase assay (IVKA) towards recombinant GST-CTD as a substrate (bottom). Levels of Cdk12-F, CycK, Ser2-P GST-CTD isoforms, and input GST-CTD (30%) were detected by Western blotting. (**C**) Amounts of the purified Cdk12-F complexes used for *in vitro* kinase assays. The indicated wild-type and mutant FLAG epitope-tagged Cdk12 proteins (Cdk12-F) were immuno-purified from whole cell extracts WCEs of the individual HEK 293 Flp-In T-Rex cell lines using FLAG-M2 agarose (FLAG IP) and used for the *in vitro* kinase assays as in Figure 1D. Levels of Cdk12-F and CycK proteins in the IPs were detected by Western blotting using FLAG and CycK antibodies.



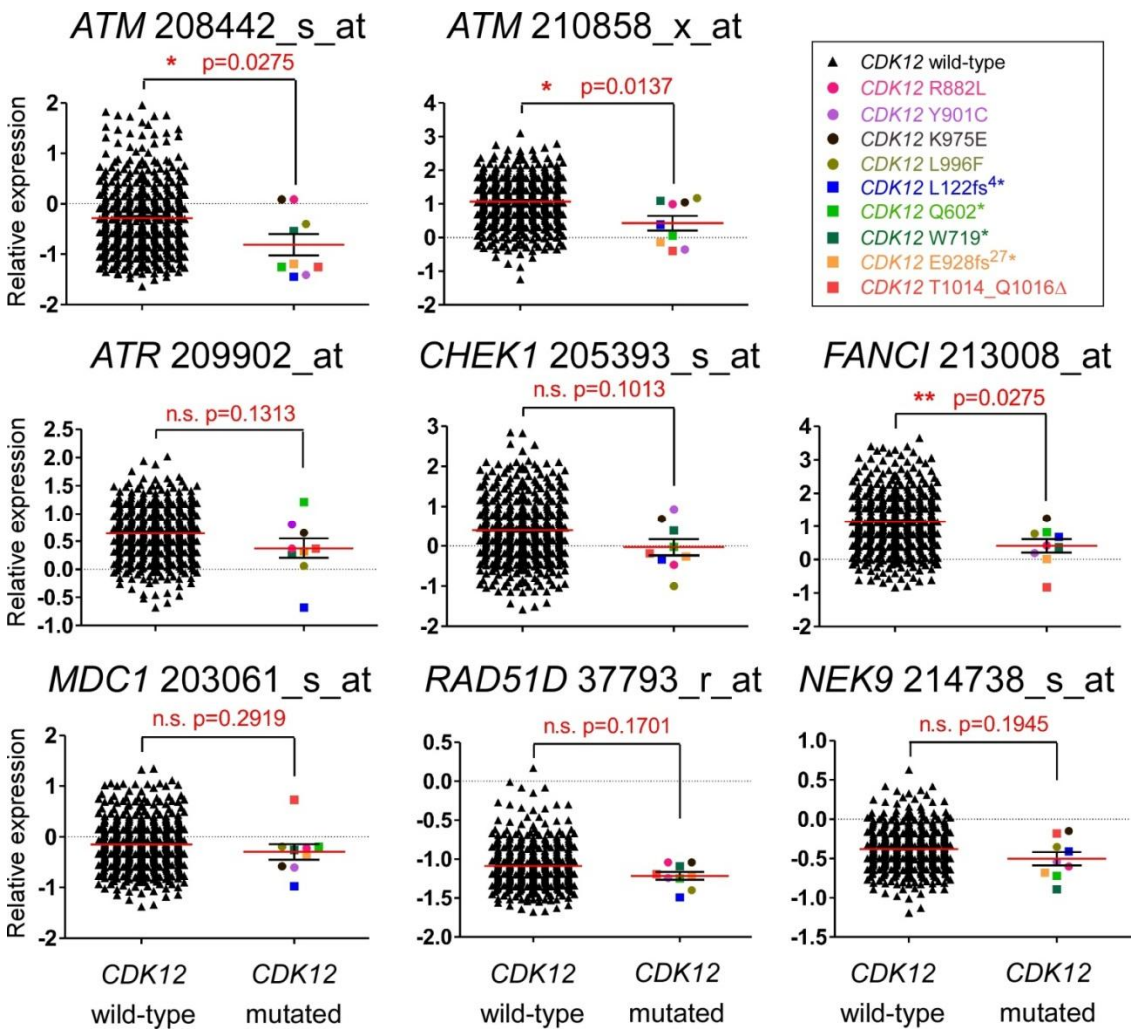
Supplementary Figure S3. Location of the G909R and E1024* *CDK12* KD mutations. Overall structure of Cdk12/CycK is presented. Cdk12 is shown as cartoon representation in blue and CycK as surface representation in grey. The *CDK12* missense G909R mutation and the truncated C-terminal extension of the canonical KD that is absent in the *CDK12* nonsense E1024* mutation are depicted in red. pT893 is highlighted in orange and D877 is shown in black.



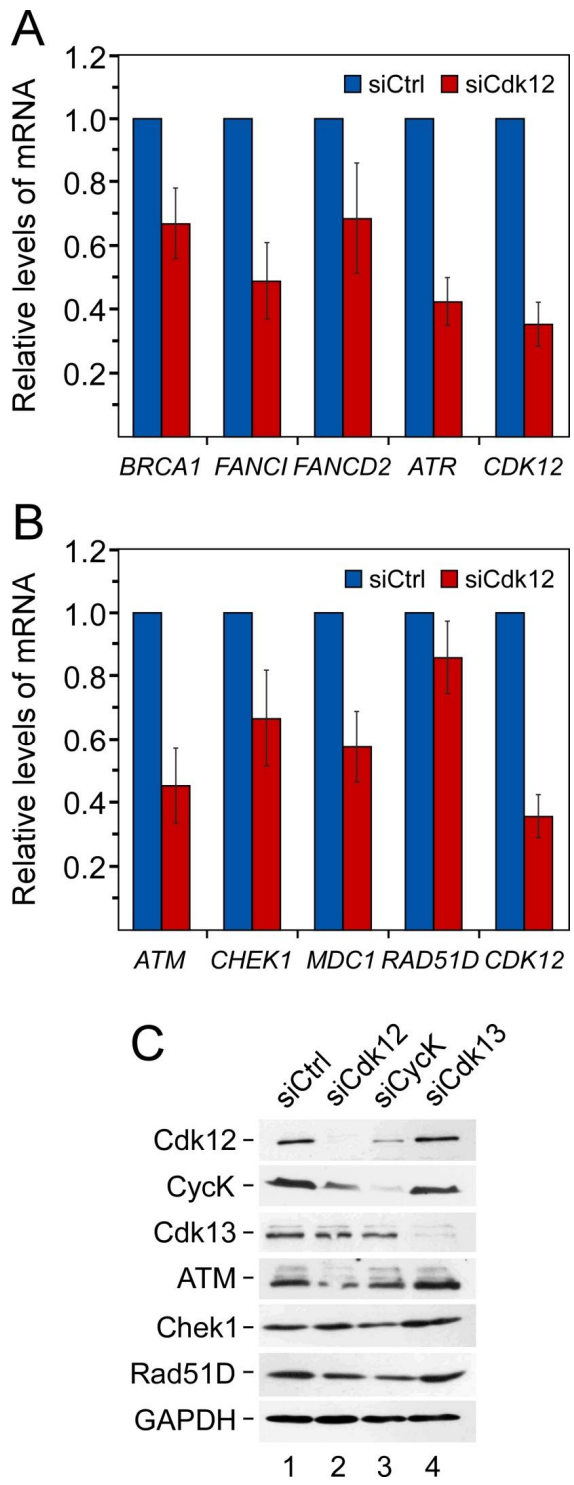
Supplementary Figure S4. Generation of the kinase-dead form of Cdk12 and impact of a set of *CDK12* mutations from other cancers on transcriptional activation by Cdk12. (**A**) Substitution of aspartic acid residue at position 877 of the activation segment DGF motif with asparagine residue produces a kinase-dead form of Cdk12, which still binds CycK. The indicated wild-type and mutant Cdk12-F proteins were expressed transiently in HEK 293 cells and immuno-purified from WCEs using FLAG-M2 agarose (FLAG IP). The complexes were examined for their interaction with endogenous CycK (top) and for their kinase activity by *in vitro* kinase assay (IVKA) towards recombinant GST-CTD as a substrate (bottom). Levels of Cdk12-F, CycK, Ser2-P GST-CTD isoforms, and input GST-CTD (30%) were detected by Western blotting. (**B**) A set of *CDK12* mutations from different cancers decrease transcriptional activation by Cdk12. HEK 293T cells were co-transfected with pSLIIB-CAT reporter gene and plasmids encoding the proteins indicated below the graph. Transcriptional activities of Rev (white bar), the mutant Rev-Cdk12 chimeras (red bars) and catalytically dead Rev-Cdk12 D887N control chimera (green bar) are represented as CAT activities relative to the activity of wild-type Rev-Cdk12 chimera (blue bar), which was set to 100%. Error bars represent the mean ± SD.



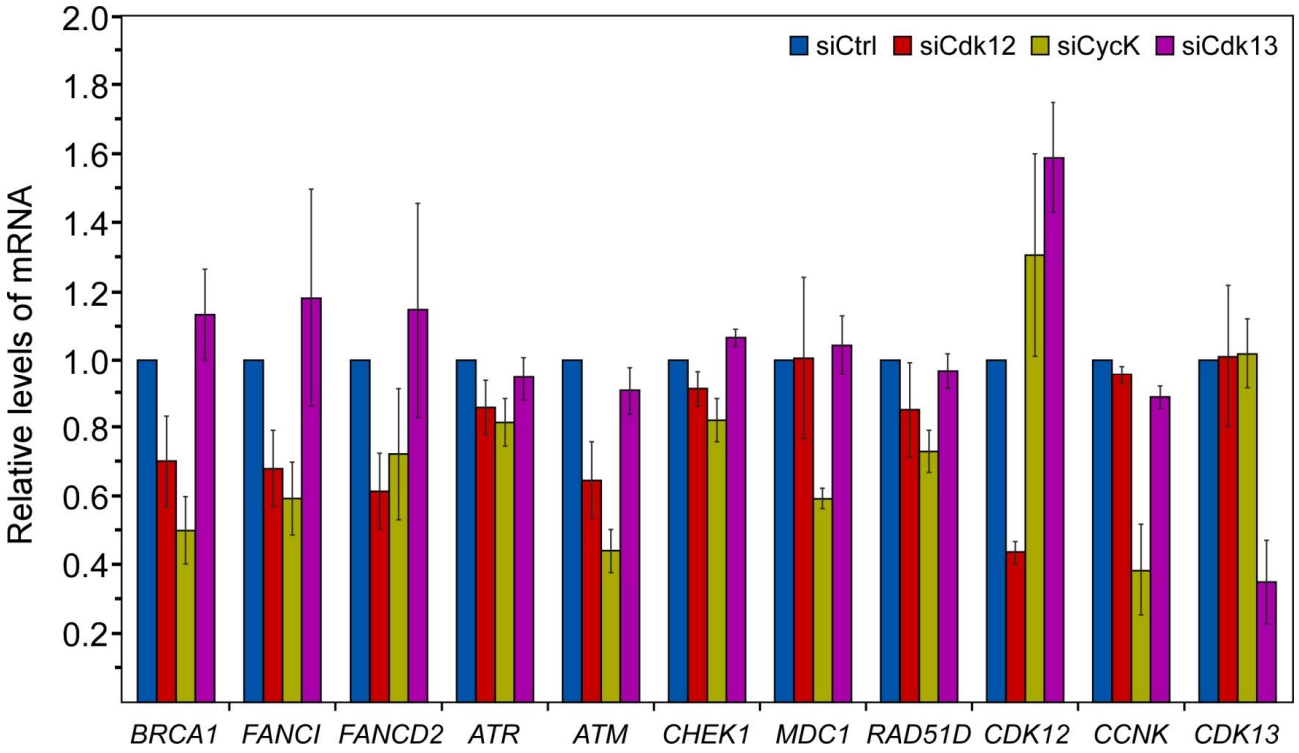
Supplementary Figure S5. Alternative representation of the down-regulation of the crucial DDR genes in HGS-OvCa patient samples with mutations in *CDK12*. (**A** and **B**) Graphs show comparisons of relative expression levels between the HGS-OvCa samples with the wild-type or mutated *CDK12*. The identity of genes is indicated on top of each graph. The data were generated using the same probes as in Figure 4. Whereas samples with the wild-type *CDK12* are plotted as black triangles, those containing the nonsense and internal deletion (indel) or missense *CDK12* mutations are depicted as open squares or black circles, respectively, as indicated by the legend above the graphs. Results are expressed as mean (red line) with standard error of the mean (SEM) (black whiskers). The number of asterisks (*) indicates the degree of significance as follows: * = P ≤ 0.05; ** = P ≤ 0.01; *** = P ≤ 0.001. P values are not shown and are the same as in Figure 4. Panel **A** shows genes related to the HR pathway, while other affected DDR genes are shown in panel **B**.



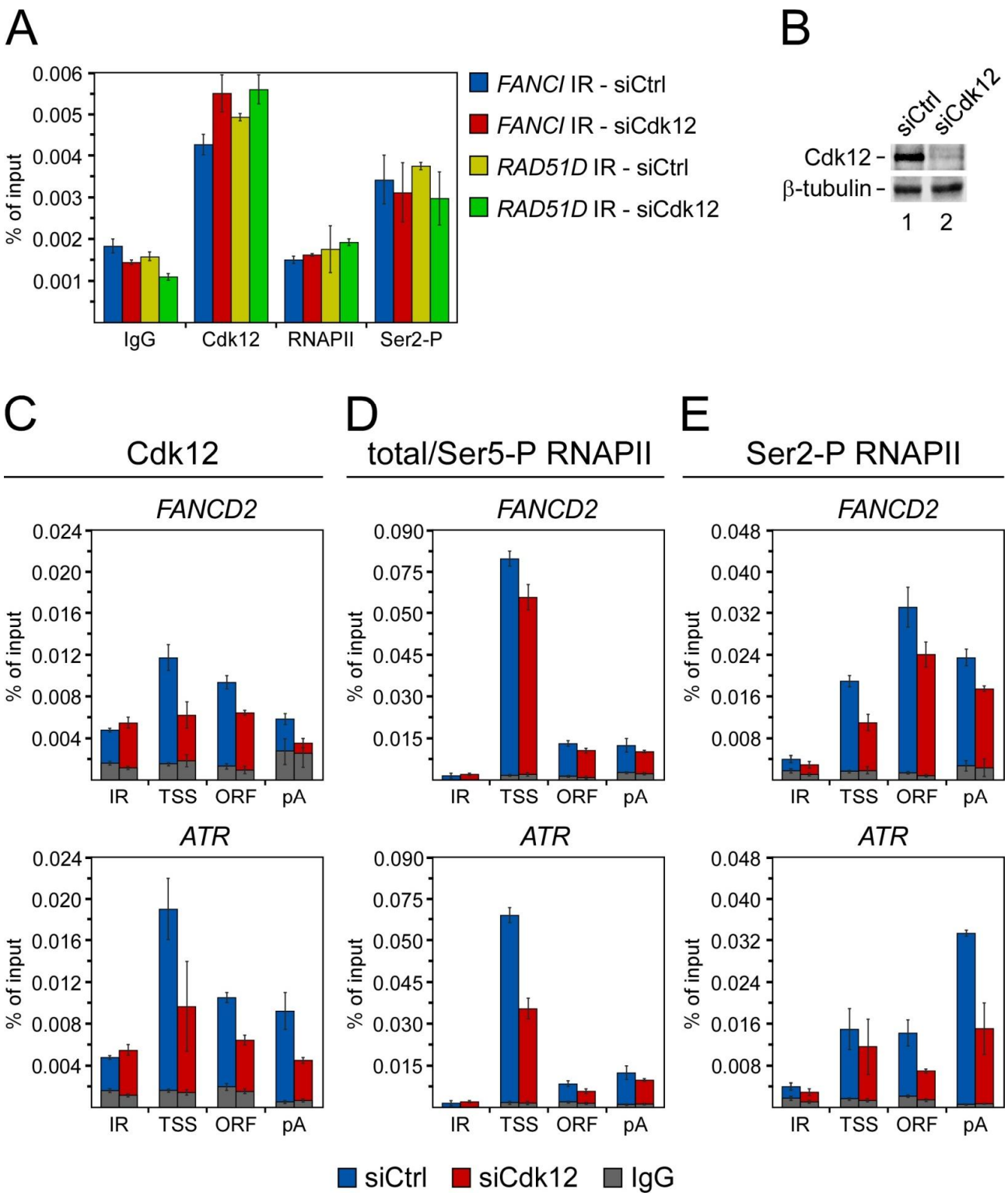
Supplementary Figure S6. The crucial DDR genes are down-regulated in HGS-OvCa patient samples with mutations in *CDK12* as determined by other microarray probes. Graphs show comparisons of relative expression levels between the HGS-OvCa samples with the wild-type or mutated *CDK12*. The identity of genes together with the microarray probe is indicated on top of each graph. Whereas samples with the wild-type *CDK12* are plotted as black triangles, those containing individual missense or nonsense/indel *CDK12* mutations are depicted as colored circles or squares, respectively, as indicated by the legend in the top right corner. Results are expressed as mean (red line) with standard error of the mean (SEM) (black whiskers). P values are given next to red asterisks (*) and the number of asterisks indicates the degree of significance as follows: * = P ≤ 0.05; ** = P ≤ 0.01; *** = P ≤ 0.001. 'n.s.' stands for non-significant; P value (P > 0.05).



Supplementary Figure S7. Depletion of Cdk12 decreases the mRNA and protein levels of critical HR genes in HCT116 cells. (**A** and **B**) Relative mRNA levels of genes indicated below the bars were determined by RT-qPCR using total RNA samples isolated from HCT116 cells treated with the control (blue bars) or Cdk12 #1 siRNA (red bars). The error bars represent the mean ± SD. (**C**) Levels of proteins indicated on left were determined by Western blotting using WCEs of HCT116 cells treated with the control, Cdk12 #1, CycK or Cdk13 siRNA.



Supplementary Figure S8. Depletion of CycK and Cdk12 decreases the mRNA levels of critical HR genes in Caov-3 cells. Relative mRNA levels of genes indicated below the bars were determined by RT-qPCR using total RNA samples isolated from Caov-3 cells treated with the control (blue bars), Cdk12 #2 (red bars), CycK (olive green bars) or Cdk13 siRNA (purple bars). The error bars represent the mean ± SD.



Supplementary Figure S9. (**A**) Two independent intergenic regions display comparable levels of IgG and specific proteins. Levels of IgG, Cdk12, total/Ser5-P RNAPII, and Ser2-P RNAPII occupancy at *FANCI* (blue and red bars) and *RAD51D* (yellow and green bars) intergenic regions were determined by ChIP-qPCR analysis in control or Cdk12 knockdown HeLa DR-GFP cells. Results are presented as percent of input DNA and plotted as the mean ± SD. (**B**) Efficient depletion of Cdk12 in ChIP-qPCR experiments. Levels of proteins indicated on left were determined by Western blotting using WCEs of HeLa DR-GFP cells treated with the control or Cdk12 #2 siRNA. (**C, D, E**) Cdk12/CycK is present at the established HR genes to promote phosphorylation of the CTD of RNAPII at

Ser2. Control (blue bars) or Cdk12 knockdown HeLa DR-GFP cells (red bars) were subjected to ChIP-qPCR analysis to determine the levels of Cdk12 (panel **C**), total/Ser5-P RNAPII (panel **D**) and Ser2-P RNAPII (panel **E**) occupancy at *RAD51D* intergenic region (IR) and three gene-specific regions as indicated below the graphs. The identity of genes analyzed is indicated on top of each graph. Levels of IgG signals at IR and gene regions are presented as gray bars. Results are presented as percent of input DNA and plotted as the mean ± SD.

SUPPLEMENTARY TABLES

Supplementary Table S1. *CDK12* mutations in HGS-OvCa.

Mutation (aa)	Mutation (nt)	Mutation ID	Mutation Type	Validation
L122fs^{4*}	363_364delCT	COSM69050	Deletion - Frameshift	yes
S363*	1088C>G	COSM1324005	Nonsense	no
Q602*	1804C>T	COSM78919	Nonsense	yes
P653L	1958C>T	COSM1324004	Missense	no
W719*	2156G>A	COSM118018	Nonsense	yes
S785fs^{4*}	2351_2352insA	COSM1324003	Insertion - Frameshift	no
R882L	2645G>T	COSM70301	Missense	yes
Y901C	2702A>G	COSM74101	Missense	yes
E928fs^{27*}	2778_2779insG	COSM69187	Insertion - Frameshift	yes
K975E	2923A>G	COSM70302	Missense	yes
R983_E984>Q	2948_2950delGAG	COSM1324002	Deletion - In frame	no
L996F	2988A>T	COSM70303	Missense	yes
T1014_Q1016del	3036_3044delACAGACCCT	COSM69117	Deletion - In frame	yes
A1174G	3521C>G	COSM94100	Missense	yes
/	1028_1046+14del 33	COSM1324006	Intronic deletion	no

Information on *CDK12* mutations was obtained from the COSMIC database on April 20, 2014. Validated *CDK12* mutations identified in the HGS-OvCa TCGA study are marked in green.

Supplementary Table S2. *CDK12* mutations in human cancers.

Position	Mutation	Region	Count	Primary Tissue	Mutation Type
46	K46fs*1	/	1	Lung	Insertion - Frameshift
55	L55L	/	1	Endometrium	Substitution - coding silent
55	L55F	/	1	Cervix	Substitution - Missense
86	D86N	/	1	Breast	Substitution - Missense
104	R104R	RS	1	Breast	Substitution - coding silent
122	L122fs*4	RS	1	Ovary	Deletion - Frameshift
148	S148L	RS	1	Urinary tract	Substitution - Missense
172	K172*	RS	1	NS	Substitution - Nonsense
189	E189K	RS	1	Urinary tract	Substitution - Missense
192	S192Y	RS	1	Large intestine	Substitution - Missense
208	K208T	RS	1	Endometrium	Substitution - Missense
213	V213M	RS	1	Endometrium	Substitution - Missense
238	S238L	RS	1	Lung	Substitution - Missense
265	S265R	RS	1	Lung	Substitution - Missense
266	P266fs*72	RS	1	Prostate	Deletion - Frameshift
276	S276T	RS	1	Prostate	Substitution - Missense
283	S283L	RS	1	Lung	Substitution - Missense
316	S316N	RS	1	Large intestine	Substitution - Missense
323	S323L	RS	1	Lung	Substitution - Missense
325	S325N	RS	1	Lung	Substitution - Missense
327	Y327C	RS	1	Lung	Substitution - Missense
334	S334C	RS	1	Liver	Substitution - Missense
352	S352F	RS	1	Skin	Substitution - Missense
363	S363*	RS	1	Ovary	Substitution - Nonsense
406	R406fs*29	/	1	Prostate	Deletion - Frameshift
421	K421N	/	1	Large intestine	Substitution - Missense
425	V425A	/	1	Lung	Substitution - Missense
434	S434S	/	1	Breast	Substitution - coding silent
438	K438R	/	1	Lung	Substitution - Missense
446	K446K	/	1	Lung	Substitution - coding silent
449	R449T	/	1	Lung	Substitution - Missense
466	T466A	/	1	Oesophagus	Substitution - Missense
471	E471Q	/	1	Lung	Substitution - Missense
475	S475Y	/	1	Endometrium	Substitution - Missense
485	E485E	/	1	Lung	Substitution - coding silent
487	S487S	/	1	Endometrium	Substitution - coding silent
488	E488*	/	1	Breast	Substitution - Nonsense
489	K489E	/	1	Lung	Substitution - Missense
502	D502Y	/	1	Large intestine	Substitution - Missense
535	P535S	PRM1	1	Endometrium	Substitution - Missense
536	P536fs*74	PRM1	1	Large intestine	Deletion - Frameshift
536	P536P	PRM1	1	Endometrium	Substitution - coding silent
538	P538fs*72	PRM1	1	Large intestine	Deletion - Frameshift
544	P544fs*31	PRM1	1	NS	Insertion - Frameshift
545	P545fs*65	PRM1	1	NS	Deletion - Frameshift
554	L554_P556del LPP	PRM1	1	Endometrium	Deletion - In frame
566	L566L	PRM1	1	Urinary tract	Substitution - coding silent
569	S569C	PRM1	1	Breast	Substitution - Missense
578	A578V	PRM1	1	Lung	Substitution - Missense
583	T583P	PRM1	1	Oesophagus	Substitution - Missense
602	Q602*	PRM1	1	Ovary	Substitution - Nonsense
602	Q602R	PRM1	1	Large intestine	Substitution - Missense
631	P631P	PRM1	1	Lung	Substitution - coding silent
649	L649R	PRM1	1	Kidney	Substitution - Missense
653	P653H	PRM1	1	Endometrium	Substitution - Missense
653	P653L	PRM1	1	Ovary	Substitution - Missense

663	R663C	PRM1	1	Large intestine	Substitution - Missense
677	G677Y	PRM1	1	Lung	Substitution - Missense
677	G677D	PRM1	1	Endometrium	Substitution - Missense
683	P683fs*70	PRM1	1	Large intestine	Deletion - Frameshift
683	P683T	PRM1	1	Endometrium	Substitution - Missense
691	I691V	PRM1	1	Pancreas	Substitution - Missense
708	R708C	/	1	Large intestine	Substitution - Missense
718	D718N	KD N-lobe	1	Large intestine	Substitution - Missense
719	W719*	KD N-lobe	1	Ovary	Substitution - Nonsense
722	R722C	KD N-lobe	1	Large intestine	Substitution - Missense
723	C723Y	KD N-lobe	1	Large intestine	Substitution - Missense
732	I732fs*21	KD N-lobe	1	Kidney	Deletion - Frameshift
740	Q740E	KD N-lobe	1	Lung	Substitution - Missense
758	V758V	KD N-lobe	1	Lung	Substitution - coding silent
765	E765G	KD N-lobe	1	Urinary tract	Substitution - Missense
779	R779C	KD N-lobe	1	Large intestine	Substitution - Missense
785	S785fs*4	KD N-lobe	1	Ovary	Insertion - Frameshift
802	F802V	KD N-lobe	1	Large intestine	Substitution - Missense
813	F813V	KD N-lobe	1	Lung	Substitution - Missense
824	L824P	KD C-lobe	1	Stomach	Substitution - Missense
845	E845D	KD C-lobe	1	Lung	Substitution - Missense
850	C850C	KD C-lobe	1	Kidney	Substitution - coding silent
852	K852E	KD C-lobe	1	Lung	Substitution - Missense
853	K853N	KD C-lobe	1	Endometrium	Substitution - Missense
870	S870C	KD C-lobe	1	Lung	Substitution - Missense
872	Q872*	KD C-lobe	1	Large intestine	Substitution - Nonsense
882	R882R	KD C-lobe	1	Large intestine	Substitution - coding silent
882	R882Q	KD C-lobe	1	Large intestine	Substitution - Missense
882	R882L	KD C-lobe	1	Ovary	Substitution - Missense
887	E887K	KD C-lobe	1	Oesophagus	Substitution - Missense
890	R890H	KD C-lobe	2	Endometrium/ Large intestine	Substitution - Missense
890	R890R	KD C-lobe	1	Large intestine	Substitution - coding silent
901	Y901C	KD C-lobe	3	Large intestine/ Ovary	Substitution - Missense
901	Y901*	KD C-lobe	1	Breast	Substitution - Nonsense
901	Y901Y	KD C-lobe	1	large intestine	Substitution - coding silent
902	R902P	KD C-lobe	1	Breast	Substitution - Missense
909	G909R	KD C-lobe	1	Ovary	Substitution - Missense
912	R912C	KD C-lobe	2	Large intestine	Substitution - Missense
912	R912H	KD C-lobe	1	Large intestine	Substitution - Missense
914	T914T	KD C-lobe	1	Breast	Substitution - coding silent
928	E928fs*27	KD C-lobe	1	Ovary	Insertion - Frameshift
932	K932N	KD C-lobe	1	Large intestine	Substitution - Missense
937	Q937fs*9	KD C-lobe	1	Large intestine	Deletion - Frameshift
939	N939S	KD C-lobe	1	Breast	Substitution - Missense
947	L947L	KD C-lobe	1	Lung	Substitution - coding silent
952	C952fs*21	KD C-lobe	1	Prostate	Deletion - Frameshift
959	V959G	KD C-lobe	1	Skin	Substitution - Missense
964	I964M	KD C-lobe	1	Breast	Substitution - Missense
974	P974L	KD C-lobe	1	Large intestine	Substitution - Missense
975	K975E	KD C-lobe	1	Ovary	Substitution - Missense
975	K975R	KD C-lobe	1	Large intestine	Substitution - Missense
981	R981C	KD C-lobe	1	Lung	Substitution - Missense
983	R983_E984>Q	KD C-lobe	1	Ovary	Complex - deletion inframe
986	F986L	KD C-lobe	1	Endometrium	Substitution - Missense
987	S987C	KD C-lobe	1	Lung	Substitution - Missense
993	A993T	KD C-lobe	1	Lung	Substitution - Missense
996	L996F	KD C-lobe	1	Ovary	Substitution - Missense
1009	C1009R	KD C-lobe	1	Large intestine	Substitution - Missense

1012	E1012E	KD C-lobe	1	Lung	Substitution - coding silent
1014	T1014_Q1016 del	KD C-lobe	1	Ovary	Deletion - In frame
1024	E1024*	KD extension	2	Large intestine/ Lung	Substitution - Nonsense
1024	E1024G	KD extension	1	Large intestine	Substitution - Missense
1024	E1024E	KD extension	2	Large intestine	Substitution - coding silent
1061	P1061P	KD extension	1	Urinary tract	Substitution - coding silent
1066	S1066Y	/	1	Large intestine	Substitution - Missense
1067	R1067*	/	1	Endometrium	Substitution - Nonsense
1088	Q1088*	/	1	Kidney	Substitution - Nonsense
1094	V1094L	/	1	Breast	Substitution - Missense
1100	D1100N	/	1	Kidney	Substitution - Missense
1144	P1144S	/	1	Breast	Substitution - Missense
1153	L1153L	/	1	NS	Substitution - coding silent
1171	E1171K	/	1	Large intestine	Substitution - Missense
1171	E1171Q	/	1	NS	Substitution - Missense
1174	A1174G	/	2	Ovary	Substitution - Missense
1179	L1179F	/	1	NS	Substitution - Missense
1187	V1187A	/	1	Large intestine	Substitution - Missense
1198	E1198*	/	1	NS	Substitution - Nonsense
1210	L1210fs*23	/	1	Endometrium	Deletion - Frameshift
1271	G1271fs*23	PRM2	1	Large intestine	Deletion - Frameshift
1293	L1293F	/	1	Lung	Substitution - Missense
1299	A1299A	/	1	Large intestine	Substitution - coding silent
1300	A1300T	/	1	Large intestine	Substitution - Missense
1325	P1325S	/	1	Skin	Substitution - Missense
1329	S1329F	/	1	Urinary tract	Substitution - Missense
1331	R1331*	/	1	Large intestine	Substitution - Nonsense
1331	R1331Q	/	1	Oesophagus	Substitution - Missense
1367	V1367I	/	1	Lung	Substitution - Missense
1367	V1367L	/	1	Lung	Substitution - Missense
1370	L1370L	/	1	Lung	Substitution - coding silent
1403	L1403L	/	1	Lung	Substitution - coding silent
1422	K1422N	/	1	Large intestine	Substitution - Missense
1427	S1427R	/	1	Kidney	Substitution - Missense
1446	G1446E	/	1	Prostate	Substitution - Missense
1463	T1463fs*>29	/	1	Large intestine	Insertion - Frameshift
1483	G1483R	/	1	Endometrium	Substitution - Missense
	/		1	Ovary	intronic
	/		1	Kidney	intronic
	/		1	Breast	intronic

Information on *CDK12* mutations was obtained from the COSMIC database on April 20, 2014. Mutations within the RS (light green), PRM1/2 (light blue), KD N-terminal lobe (light magenta), KD C-terminal lobe (darker magenta), and KD C-terminal extension (dark magenta) regions are highlighted.

Supplementary Table S3. List of genes of which mRNA expression was statistically analyzed using HGS-OvCa patient sample data sets from the TCGA study (1).

Gene	Gene related to HR	Significant down-regulation with at least one probe (p<0.05)	Identified in (2).
ATM	yes	yes	no
ATR	yes	yes	yes
CHEK1	yes	yes	no
MDC1	yes	yes	yes
FANCI	yes	yes	yes
RAD51D	yes	yes	no
CHEK2	yes	no	no
BRCA1	yes	no	yes
BRCA2	yes	no	no
BRCC3	yes	no	yes
PALB2	yes	no	no
ATAD5	yes	no	no
FANCA	yes	no	no
FANCC	yes	no	no
FANCE	yes	no (p=0.14)	no
MRE11A	yes	no	no
NBN	yes	no	no
RAD50	yes	no	no
DSS1	yes	no	no
RAD51	yes	no	no
RAD51B	yes	no	no
RAD51C	yes	no	no
RAD52	yes	no	no
RAD54L	yes	no	no
XRCC2	yes	no	no
XRCC3	yes	no	no
DMC1	yes	no	no
FEN1	yes	no	no
RBBP8	yes	no	no
RNF8	yes	no	no
RPA1	yes	no	no
SHFM1	yes	no	no
TP53BP1	yes	no	no
USP1	yes	no	no
CTIP	yes	no	no
FANCD2	yes	probes not available	yes
MMS22L	yes	probes not available	yes
RNF168	yes	probes not available	no
NEK9	no	yes	yes
ORC3L	no	yes	yes
TERF2	no	yes	yes
ERCC1	no	no (p=0.07)	yes
ERCC4	no	no (p=0.11)	no
CDK10	no	no	yes
DDB2	no	no	yes
ITPA	no	no (p=0.07)	yes
KNTC1	no	no	yes
MUS81	no	no	yes
NCAPD2	no	no	yes
OGG1	no	no	yes

PNKP	no	no	yes
RCF4	no	no	yes
RCF5	no	no	no
RUVBL1	no	no	yes
SMARCC2	no	no	yes
SMUG1	no	no	yes
TAP1	no	no	no
TAP2	no	no	yes
TDP1	no	no	yes
TSG101	no	no	yes
ZWINT	no	no	yes

Genes related to HR are depicted in light magenta, while other DDR genes are depicted in light blue. Green color depicts the significantly down-regulated genes

SUPPLEMENTARY MATERIALS AND METHODS

Supplementary Table S4. Plasmids used in this study.

Gene	Vector	Restriction sites	Figure
/	pcDNA5/FRT/TO	/	/
3XFLAG	pcDNA5/FRT/TO/3XFLAG	/	1, 6, S2C
CDK12	pcDNA5/FRT/TO/3XFLAG	BamHI-NotI	1, 6, S2C
CDK12 W719*	pcDNA5/FRT/TO/3XFLAG	BamHI-NotI	1, 6, S2C
CDK12 E928fs^{27}*	pcDNA5/FRT/TO/3XFLAG	BamHI-NotI	1, 6, S2C
CDK12 R882L	pcDNA5/FRT/TO/3XFLAG	BamHI-NotI	1, 6, S2C
CDK12 Y901C	pcDNA5/FRT/TO/3XFLAG	BamHI-NotI	1, 6, S2C
CDK12 K975E	pcDNA5/FRT/TO/3XFLAG	BamHI-NotI	1, 6, S2C
CDK12 L996F	pcDNA5/FRT/TO/3XFLAG	BamHI-NotI	1, 6, S2C
3XFLAG	pcDNA3.1/3XFLAG	NheI-AflII	S2B, S4
CDK12	pcDNA3.1/3XFLAG	NheI-AflII	S2B, S4
CDK12 S325N	pcDNA3.1/3XFLAG	NheI-AflII	S2B
CDK12 S352F	pcDNA3.1/3XFLAG	NheI-AflII	S2B
CDK12 G929R	pcDNA3.1/3XFLAG	NheI-AflII	S2B
CDK12 1024*	pcDNA3.1/3XFLAG	NheI-AflII	S2B
CDK12 D877N	pcDNA3.1/3XFLAG	NheI-AflII	S4
REV	pREV	-	3
REV, CDK12	pREV	NheI-SalI	3, S4
REV, CDK12 W719*	pREV	NheI-SalI	3
REV, CDK12 E928fs^{27}*	pREV	NheI-SalI	3
REV, CDK12 R882L	pREV	NheI-SalI	3
REV, CDK12 Y901C	pREV	NheI-SalI	3
REV, CDK12 K975E	pREV	NheI-SalI	3
REV, CDK12 L996F	pREV	NheI-SalI	3
REV, CDK12 S325N	pREV	NheI-SalI	S4
REV, CDK12 S352F	pREV	NheI-SalI	S4
REV, CDK12 G909R	pREV	NheI-SalI	S4
REV, CDK12 1024*	pREV	NheI-SalI	S4
REV, CDK12 D877N	pREV	NheI-SalI	3, S4
CAT	pSLIIB-CAT	/	3, S4
I-SCEI	pCβASce	XhoI	6

Supplementary Table S5. DNA oligonucleotides used in RT-qPCR assay.

Gene	Primer Sequence (5'–3')	Figure
ATM	GCTCAGGAAGGAATGAGAGAAA CCACAGCTATCAACGTCAGTAA	4, S7, S8
ATR	TGGCTCACCAGAGAGAAATG GCAGCAAGATCAGGTAGTAGAA	4, S7, S8
BRCA1	TGAGGCATCAGTCTGAAAGCC AAAATGTCACTCTGAGAGGAT	4, S7, S8
CDK12	CCTGGAGATGATGACATGGATAG GAGGAAGGTCTGTGAGTAAGTG	4, S7, S8
CDK13	GCAGTGTGGCCTGATGTAATC CTGCTTCTGTCTTCTTCGC	S8
CHEK1	GGTTGACTTCCGGCTTTCTAA TCTTCTGGCTGCTCACAATATC	4, S7, S8
CCNK	TCACAGCCGTCTCAAAGCTC CTTCTTTGGGAGAAACAA	S8
FANCD2	CATCTCTAATGACCAGCTCC AGTTCAGCCATCATCACACG	4, S7, S8
FANCI	ACAAGTGAGCCAAGAAACC CAAGGGCTGTAAGTGTGGT	4, S7, S8
HPRT	CCAGACAAGTTTGTTGTAGGATATGCCCTTGAC ACTCCAGATGTTTCCAAACTCAACTTGAACTCTC	4, S7, S8
MDC1	ACAGGAAGGGAGAGAGAACA AAAGCACTGGGTAGCTTGTAG	4, S7, S8
RAD51D	CAGGTGGTGCATGCATTTG CACCTTCACAGTTCCTGAAGA	4, S7, S8

Supplementary Table S6. DNA oligonucleotides used in ChIP-qPCR assay.

Primer	Primer Sequence (5'–3')	Figure
ATM-TSS	CCTGACTTTCCTTCCGAATC GAGGTTCTGGGCAGTTTAAG	5
ATM-ORF	AGGACACGAAGGGAGATT CCTTCCTGAGCTTTCAAGTAT	5
ATM-pA	CCAAGGACAAATGAGGAGTAG CCAGCAGTGTATTGGGATAG	5
ATR-TSS	GTTGGCGTGGTTGACTA AATCAGCGGAGGAGGAT	S9
ATR-ORF	GTAGCCTGTGATGCTGTTT AGGTGACAACGAAGGATTTAG	S9
ATR-pA	TGTGCTTCAAAGGGTACTTC CTACATCACCACAGAGCTATTAC	S9
CHEK1-TSS	GGAGTTCCTCCCATTTCTTC GAAGCAGAGTGCTTTGTAAAC	5
CHEK1-ORF	GTAGGGAGCTGGAATCATTATC TCAAGGGAGATGGGAGAAA	5
CHEK1-pA	GGCAATAGGTAGCATGGATA GAGGGTCAGAGATGAAGAAG	5
FANCD2-TSS	CCGAAGCGGCGATAAATAA GCTCTTCACCACCTATACTTAC	S9
FANCD2-ORF	CCTAGTGTGTAGTAGTGGAAATG TTGAGGGCAGAGGATCTAATA	S9
FANCD2-pA	GTATTGCCTGTAAACTCAACC GTGCTCTCCTGGGAATTT	S9
MDC1-TSS	GACAGCCTTTAAGACAGAGAG ATCTTTCTTCCTGGGAATACG	5
MDC1-ORF	GCAAGTCCTGAAAGAGATAGG GCTACTGGTCTCTCTACTTCT	5
MDC1-pA	TCCCTCATTGCTCCATTC GAGTTCTCAGGTGGATGAC	5
RAD51D-TSS	AGCCACAGGATCAAGACA GGCCCTCTAGGAATGGAG	5
RAD51D-ORF	TGGCCTGAGTTTCTCTCT TGGCATGCACCTGTAATC	5
RAD51D-pA	CCAGGAAGATGCCAGTAATC CCAAGTGGGTAGCTTCTTTAG	5
FANCI-IR	GATCTAGGATGACAGCAGAATAG GTGGACCTCTCTGGTCTATAA	S9
RAD51-IR	GAAGCTCCAAGAGGGACAATAG GCGTAGATGCTCCCATCTTATC	S9

SUPPLEMENTARY REFERENCES

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2. Blazek D*, et al.* (2011) The Cyclin K/Cdk12 complex maintains genomic stability via regulation of expression of DNA damage response genes. *Genes & development* 25(20):2158-2172.