Supplementary Figure Legends

Supplementary Figure S1. shRNA-mediated knockdown and ectopic expression of *LKB1* in indicated cell lines

(**A**) Indicated cell lines stably expressing a non-specific (NS) shRNA or *LKB1* shRNAs were analyzed for the expression of *LKB1* transcript by RT-qPCR (Top) and protein by immunoblot (Bottom) respectively. Actin was used as an internal control for both RT-qPCR and immunoblot analyses **(B)** Indicated cell lines stably expressing an empty vector (V), Flag-LKB1 wild-type (WT) or Flag-LKB1 kinase dead mutant (KD) were analyzed by FLAG-immunoblot. Actin was used as a loading control. Error Bars shows Standard Error Mean (SEM).

Supplementary Figure S2. LKB1 loss sensitizes human cells to gamma irradiation. (A) Representative wells of clonogenic assays for indicated cell lines expressing a non-specific (NS) shRNA or *LKB1* shRNAs and gamma irradiated (2G). (B) Representative images of A549 and H460 cells expressing an empty vector (V), Flag-LKB1 wild-type (WT) or Flag-LKB1 kinase dead mutant (KD) and gamma irradiated (2G).

Supplementary Figure S3. LKB1 loss sensitizes cells to DNA damaging chemotherapeutic agents. Indicated cell lines expressing a non-specific (NS) shRNA or *LKB1* shRNAs were treated with either adriamycin or etoposide at the indicated drug concentrations. 48 hrs after drug treatment cell viability was measured by trypan blue exclusion assay. Percentage cell viability relative to cells expressing NS shRNA are plotted. Error Bars shows Standard Error Mean (SEM). (*, $p<0.01$; **, $p<0.001$).

Supplementary Figure S4. LKB1 loss leads to increased DNA damage. (**A**) HCT116 cells expressing the indicated shRNAs or (**B**) A549 cells ectopically expressing LKB1 WT, LKB1 KD, or an empty vector were gamma-irradiated (2G) and stained for γH2AX. Representative images for unirradiated (0) and 6 hrs post-irradiated cells are shown.

Supplementary Figure S5. LKB1 protects genome from genotoxic stress

(**A**) H1299 cells expressing indicated shRNAs were gamma irradiated (2G) and 48 hrs post-irradiation was stained for γH2AX. Percentage γH2AX positive cells are plotted at the indicated time points. (**B**) H460 cells stably expressing empty vector, wild-type LKB1 (LKB1 WT) or kinase dead mutant of LKB1 (LKB1 KD) were gamma irradiated (2G). γH2AX staining at indicated time points after gamma-irradiation (2G) was performed. Percentage γH2AX positive cells are plotted at the indicated time points. (**C**) Spontaneous mutagenesis rate was measured via *HPRT* gene mutagenesis assay in H1299 cells expressing either *LKB1* shRNAs or a non-specific shRNA. Mutation rates under indicated conditions are plotted. (**D**) Spontaneous mutagenesis rate was measured via *HPRT* gene mutagenesis assay in H460 cells stably expressing an empty vector, wild type LKB1 or kinase dead LKB1 mutant. Mutation rates under indicated conditions are plotted. Error Bar shows Standard Error Mean (SEM). (*, $p<0.01$; **, $p<0.001$).

Supplementary Figure S6. Analyses of Homology-Directed DNA repair pathway genes

HCT116 cells expressing *LKB1* or a non-specific (NS) shRNAs were analyzed for the indicated genes by RT-qPCR analyses. Relative gene expression compared to the NS shRNA is presented. (*, $p<0.01$).

Supplementary Figure S7. LKB1 regulates the mRNA stability of BRCA1 mRNA

The half-life of BRCA1 mRNA was measured at indicated time points after treatment with the transcription blocker, actinomycin D (5 μM), in cell lines that either expressed *LKB1* shRNA or the non-specific shRNA. The relative BRCA1 mRNA abundance was normalized to actin at the indicated time points. The differences in the half-lives between non-specific and *LKB1* shRNA expressing cells were significant ($p<0.01$).

Supplementary Figure S8. LKB1 regulates BRCA1 expression by a posttranscriptional mechanism

(**A**) Luciferase assay was performed by transiently transfecting BRCA1 promoter-luciferase reporter construct in HCT116 cells that either expressed *LKB1* shRNAs or a non-specific shRNA and were either left unirradiated or gamma irradiation (20G). Relative firefly luciferase activity normalized to renilla luciferase activity is plotted. (**B**) Luciferase assay using BRCA1 promoter-

luciferase reporter was performed in A549 cells that either expressed an empty vector, wild type LKB1 (LKB1 WT) or a kinase dead LKB1 (LKB1 KD) cDNA. Relative firefly luciferase activity normalized to renilla luciferase activity is plotted. (**C**) Nuclear and cytoplasmic HuR levels were analyzed by immunoblot analyses in H1299 cells expressing either *LKB1* shRNAs or as a control non-specific shRNA. Actin and Histone H1 was used as controls (**D**) (Left) BRCA1 mRNA levels were measured via RT-qPCR in H1299 cells expressing either *LKB1* shRNAs or as a control non-specific shRNA after 24 hrs of AICAR treatment (1.5 mM). (Right) Indicated proteins were analyzed in H1299 cells that either express a non-specific shRNA or shRNAs against *LKB1* after 24 hrs of treatment with AICAR. Cytoplasmic HuR and actin were measured in cytoplasmic fraction (Cyto) and additional indicated proteins in whole cell extracts (WCE) via immunoblot analysis. Error Bars shows Standard Error Mean (SEM). (*, $p<0.01$).

Supplementary Figure S9. LKB1 regulates BRCA1 stability by controlling cytoplasmic localization of HuR

(**A**) A549 cells expressing indicated constructs were analyzed for BRCA1 mRNA by qRT-PCR (left) or protein by immunoblot (right). Relative BRCA1 mRNA (left) and protein (right) levels are shown under indicated conditions. (**B**) The half-life of BRCA1 mRNA was measured at indicated time points after treatment with the transcription blocker, actinomycin D (5 μM), in A549 cell line expressing the indicated constructs. The relative BRCA1 mRNA abundance was normalized to actin at the indicated time points. The differences in the half-lives between vector expressing cells and LKB1 wild-type construct expressing A549 cells were significant ($p<0.01$).
(**C**) A549 cells expressing indicated construct were fractionated into cytoplasmic and nuclear fractions. HuR expression was analyzed by immunoblot analysis. Actin and Histone H1 was used as fractionation and loading controls. Error Bars shows Standard Error Mean (SEM). (*, $p<0.01$).

Supplementary Figure S10. LKB1 regulate cytoplasmic localization of HuR

HCT116 cells expressing indicated shRNAs were analyzed for HuR expression by immunoflorescence after gamma irradiation. Representative images are presented.

Supplementary Figure S11. **Role of HuR in LKB1-mediated regulation of sensitivity to genotoxic stress**

(**A**) HCT116, SKMEL-28 and H1299 cells expressing indicated shRNAs were analyzed for *HuR* mRNA levels by RT-qPCR analysis. Actin was used as an internal control. (**B**) H1299 cells (left) and SKMEL-28 cells (right) were infected with indicated shRNAs and irradiated at indicated doses of gamma irradiation. Cell viability was measured by trypan blue exclusion assay 48 hrs post-irradiation. Relative cell viability in reference to unirradiated cells is plotted. (**C**) H1299 cells or SKMEL-28 cells expressing indicated shRNAs were gamma irradiated (2G) and 6 hrs post-irradiation was stained for γH2AX. Percentage γH2AX positive cells are plotted at indicated time points. (**D**) Spontaneous mutation of *HPRT* gene was measured in H1299 or SKMEL-28 cells expressing indicated shRNAs. Mutagenesis rate at indicated conditions are plotted. Error Bar shows Standard Error Mean (SEM). (*, $p<0.01$; **, $p<0.001$).

Supplementary Figure S12. Role of HuR in regulating the mRNA stability of BRCA1

The half-life of BRCA1 mRNA was measured at indicated time points after the treatment with the transcription blocker, actinomycin D (5 μM) in HCT116 cell line expressing the indicated shRNAs. The relative BRCA1 mRNA abundance was normalized to actin at the indicated time points and plotted. The differences in the half-lives between non-specific and LKB1 shRNA expressing cells were significant ($p<0.01$). Also, HuR shRNA was able to significantly rescue the BRCA1 mRNA half-life ($p<0.01$).

Supplementary Figure S13. Simultaneous shRNA-mediated knockdown of LKB1 and HuR partly restores genome integrity.

HCT116 cell lines expressing the indicated shRNAs were gamma-irradiated (2G). At 6 hrs post-irradiation, cells were stained for γH2AX. Representative γH2AX-positive images for unirradiated (0) and 6 hr post-irradiation are shown.

Supplementary Figure S14. BRCA1 knockdown promotes genome instability

(**A**) Indicated cell lines expressing a non-specific (NS) shRNA or *BRCA1* shRNAs were analyzed for *BRCA1* mRNA levels by RT-qPCR. Actin was used as an internal control. (**B**) Indicated cell lines expressing *BRCA1* shRNAs or non-specific (NS) shRNA were gamma

irradiated at indicated doses and analyzed for cell viability 48 hrs post irradiation. Relative cell viability in reference to irradiated cells is plotted. (**C**) Indicated cell lines expressing a non-specific (NS) shRNA or *BRCA1* shRNAs were gamma irradiated (2G) and 6 hr post-irradiation stained for γH2AX. Percentage γH2AX positive cells are plotted at different time points. (**D**) Spontaneous mutation of *HPRT* gene was determined in indicated cell lines that express either a non-specific (NS) shRNA or *BRCA1* shRNA's. Mutagenesis rate at indicated conditions are shown. Error Bars shows Standard Error Mean (SEM). (*, $p<0.01$; **, $p<0.001$).

Supplementary Figure S15. Role of BRCA1 in LKB1-mediated tumor suppression

HCT116 cells either expressing LKB1 or non-specific (NS) shRNAs expressing an empty vector or Flag-BRCA1 were analyzed for the expression of Flag-BRCA1 and Actin by immunoblot analyses.

Supplementary Figure S16. Ectopic expression of *BRCA1* counteracts the LKB1 loss-mediated sensitivity to genotoxic stress

HCT116 cell lines expressing indicated shRNAs, with or without BRCA1 were gamma irradiated (2G) and plated on 6 well plates. Representative wells for the indicated conditions are presented.

Supplementary Figure S17. Ectopic expression of *BRCA1* protect cells lacking LKB1 from DNA damage

HCT116 cells expressing the indicated shRNAs with an empty vector or with BRCA1 were gamma-irradiated (2G) and stained for γH2AX 6 hrs post-irradiation. Representative images under indicated condition 6 hrs post-irradiation are shown.

Supplementary Figure S18. BRCA1 protects LKB1 deficient cells from gamma irradiation induced sensitization. (**A**) LKB1 wild-type (LKB1 WT) or knockout (LKB1 KO) mouse embryonic fibroblasts (MEFs) with or without BRCA1 expression were analyzed for sensitivity to gamma irradiation. At 48 hrs post-irradiation, cell viability was measured by the trypan blue exclusion assay. The percentages of cell viability relative to corresponding unirradiated cells are plotted. (**B**) LKB1 wild-type (LKB1 WT) or knockout (LKB1 KO) mouse embryonic fibroblasts

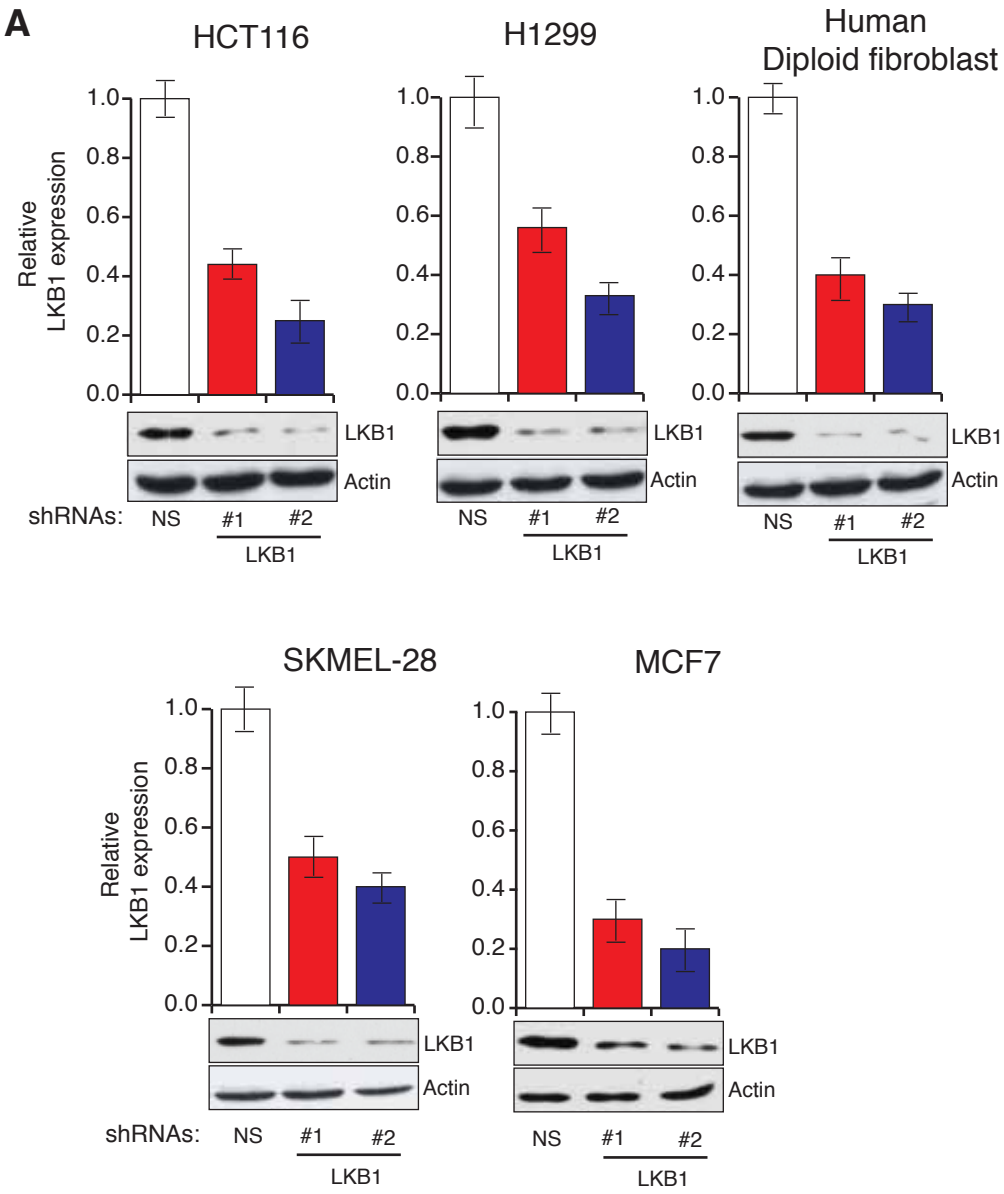
(MEFs) with or without BRCA1 expression were analyzed for indicated proteins by immunoblot analyses. Error Bars shows Standard Error Mean (SEM). (*, $p<0.01$).

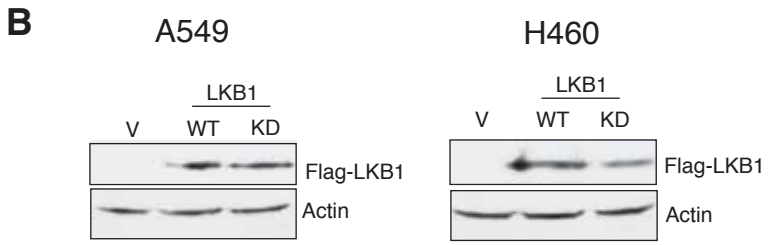
Supplementary Table S1. Primer sequences for RT-qPCR analysis; clone ID and catalog numbers for shRNAs (Open Biosystems); antibodies used; source and concentration of chemical inhibitors used.

Application	**Gene symbol**	**Forward primer (5′-3′)**	**Reverse primer (5′-3′)**
RT-qPCR	*LKB1*	GCAGACCAGCATGACAGATTT	GGATTAGGGCTTCCTCTTGGA
	HuR	AGACCACATGGCCGAAGACT	TTCAGCGTGTTGATCGCTCT
	ACTIN	GCATGGAGTCCTGTGGCATC	TTCTGCATCCTGTCGGCAAT
	HDR genes		
	BRCA1	CCCTCAAGGAACCAGGGATG	GCTGCACGCTTCTCAGTGGT
	RAD52	TGTCTGGGACTGAGGAAGCA	GAGTGTGCCCAGCCATTGTA
	XRCC2	TGGGCAAAGGACATGAACAG	CCCCACACTGTCTTCCACAA
	XRCC3	GCAAAGAGGATCCGAGGTTG	GCTCTGCTTTCCAGCCAGAT
	BRCA2	AAACAAAGGCAACGCGTCTT	CTTGGCCTCCTACTGCTGCT
	UBC13	GCAACGCCCGTTATTTTCAT	TGCTCCGCTACATCATTTGC
	RAD51B	ACTGACAATGGGCACACAGG	CTGGGAGGAGGGGGAAATAG
	RAD51C	TATTCTTGGGGGTGGAGTGC	TCGGTGTTCCTCTCCCTTGT
	RAD51D	TCTCAGGAGCCACAGGATCA	GAGCATTCCTGACCCCACTC
	MRE11	TCTTTCCCCAGGAGAAGCTG	AGAGGCTTCTCTGGCTGGTG
	RAD9B	CCCACTTAACCGCAACCAAT	AAGCTTCGCGATCTCGTCTC
	RAD1	GTTGCAGTGAGCCGAGATTG	GAGGGAGCACTCCCCCTAGT
	RAD17	GCTCCCTCTGAAGCGACACT	TGGGTTCACCCAGAGATTCC
	RAD51	CGACTCGCTGATGAGTTTGG	GGGCGATGATATTTCCTCCA
	RAD51AP1	AAAGCTGCAGCACAGCAGAG	GGAGCAGAGTCCACCGAAGT
	Gene symbol	**Clone ID**	**Catalog number**
shRNAs	*LKB1*	TRCN0000000409	RHS3979-9568779
		TRCN0000000411	RHS3979-9568781
	BRCA1	TRCN0000039835	RHS3979-9607224
		TRCN0000039837	RHS3979-9607226
	HuR	V3LHS_645063	RHS4430-99889189
		V3LHS_331822	RHS4430-101068641
	Protein symbol	**Antibody source**	
Immunoblot	LKB1	Cell signaling	
	BRCA1	Santa Cruz Biotechnology,Inc.	
	HuR	Santa Cruz Biotechnology, nc.	
	γH2AX	Thermo Scientific	
	M2 FLAG	Sigma-Aldrich	
	Inhibitor	**Concentration**	**Source**
	AICAR	1.5 mM	Cell signaling
	Actinomycin D	5 μM	Sigma-Aldrich
	6TG	1.5 μg/ml	Sigma-Aldrich
	Nocodazole	200 ng/ml	Sigma-Aldrich
	Adriamycin	As indicated	Sigma-Aldrich

	Etoposide	As indicated	Sigma-Aldrich

Supplementary Figure S1





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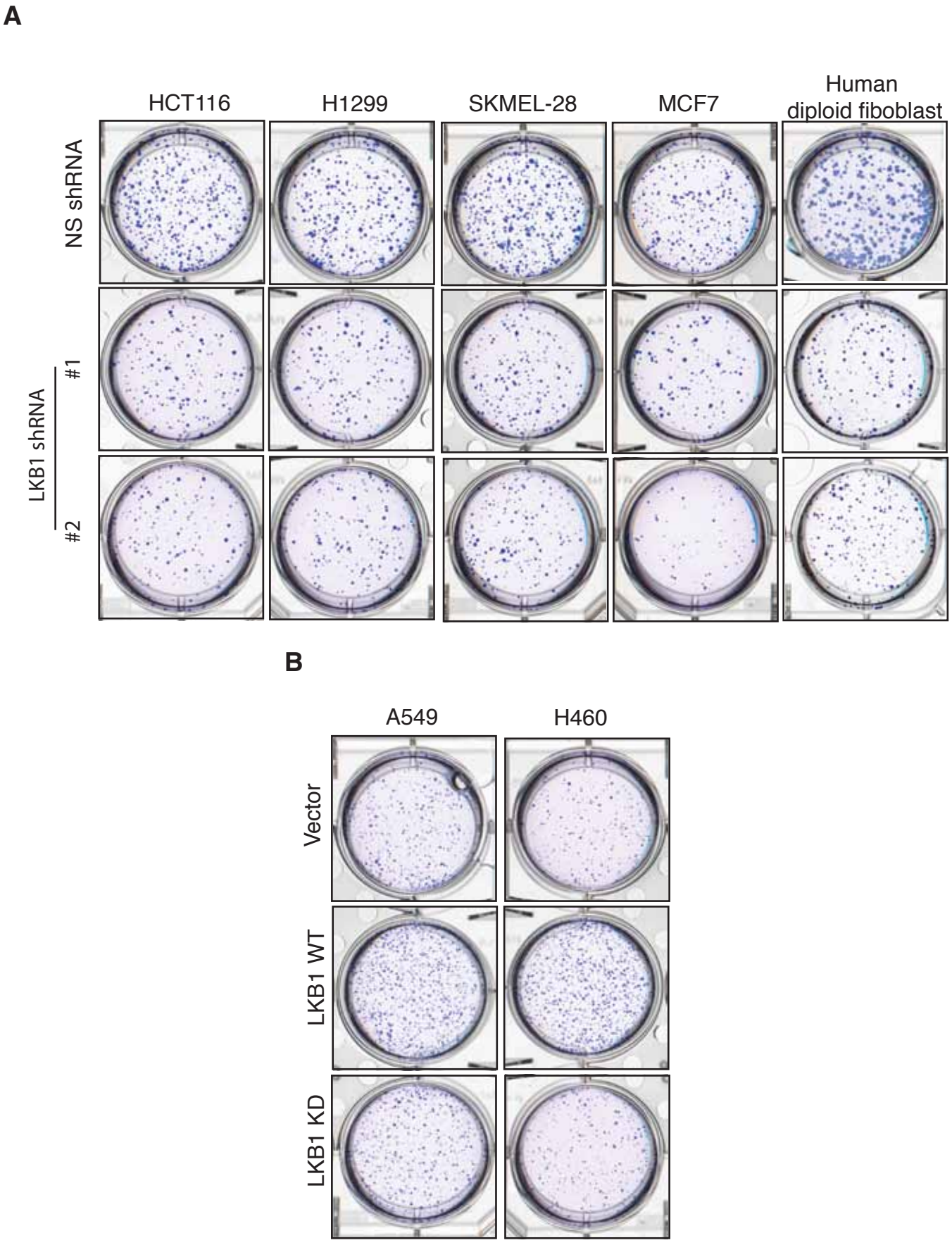

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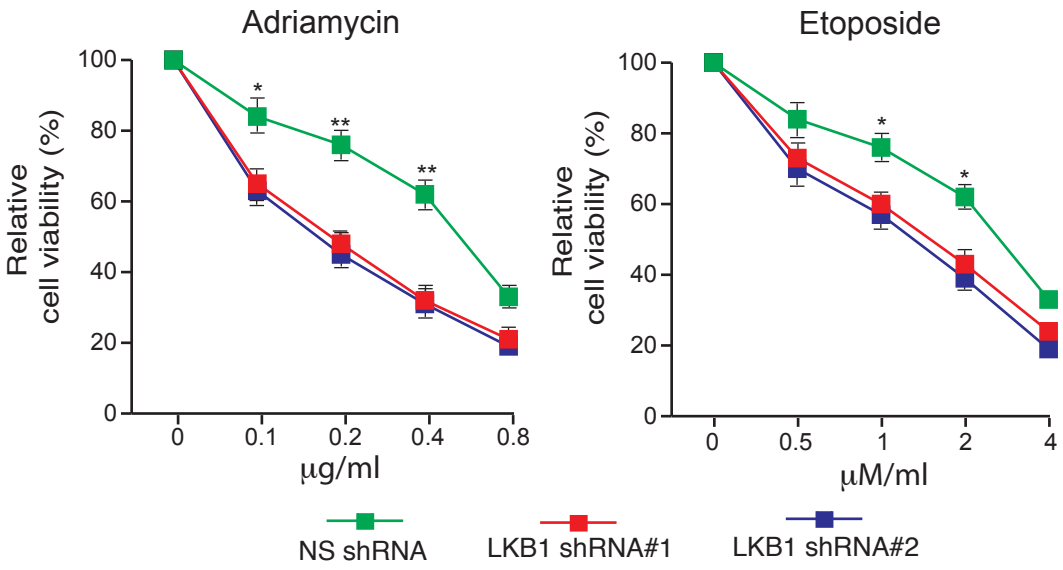

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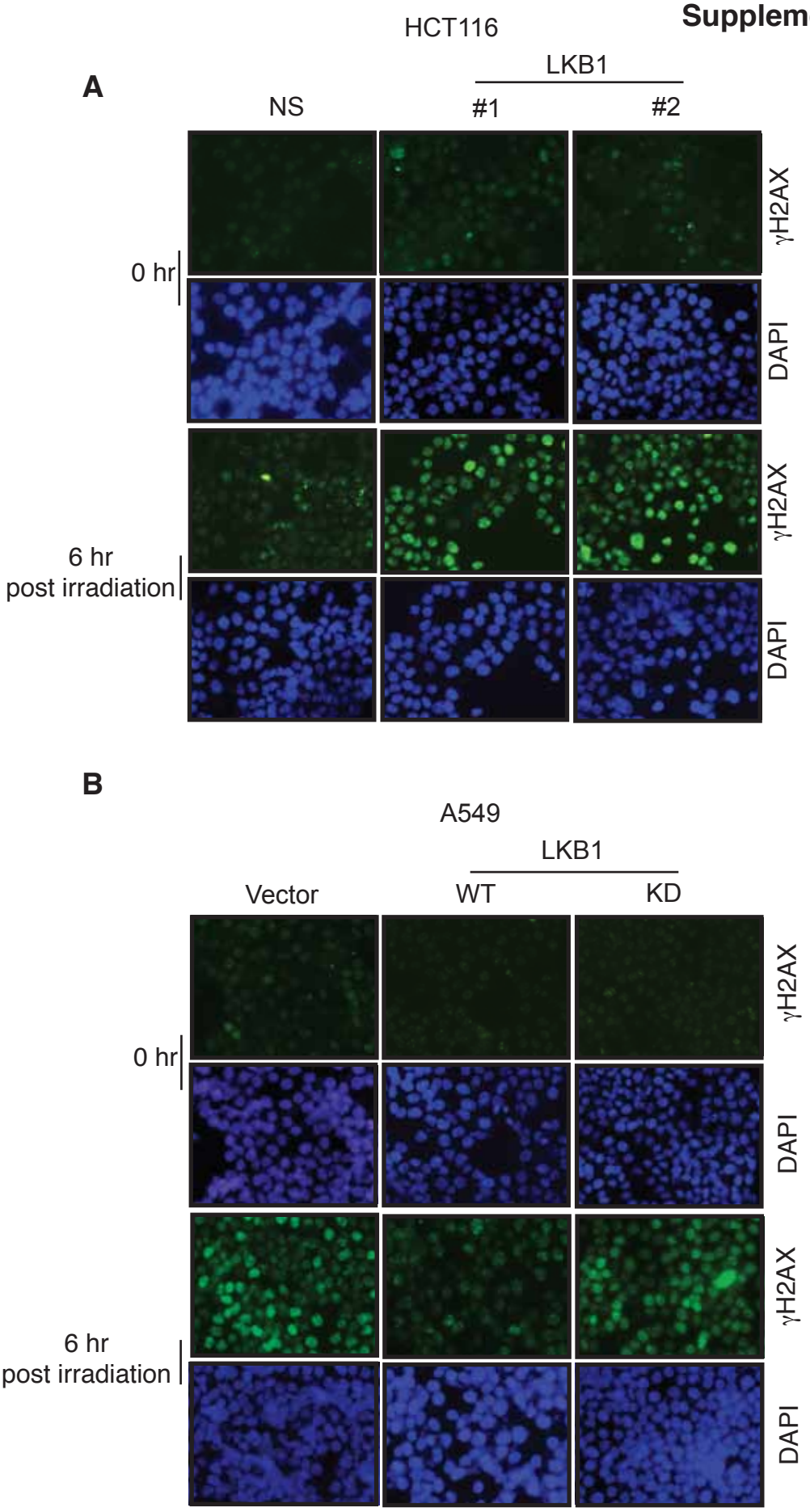

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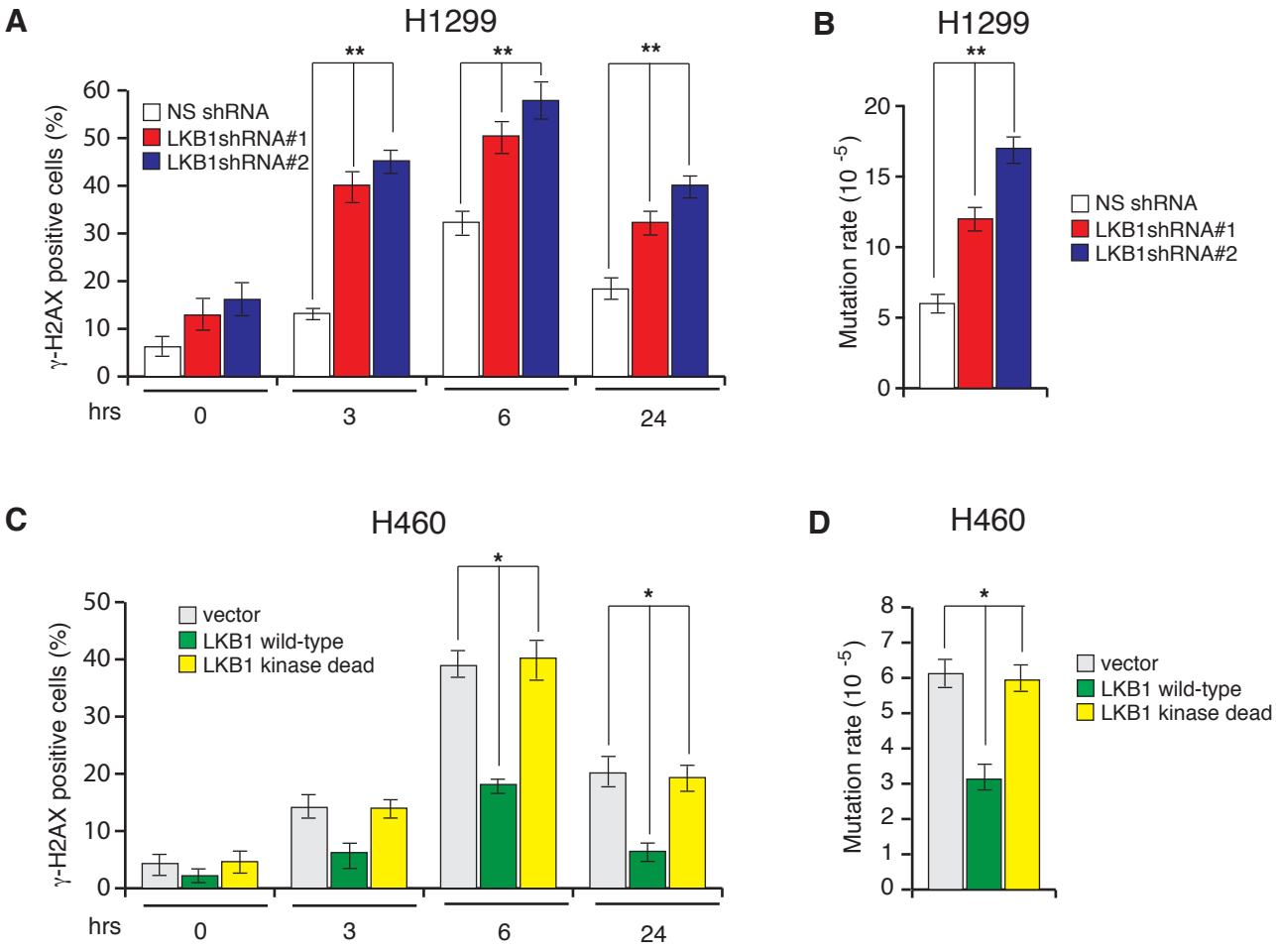

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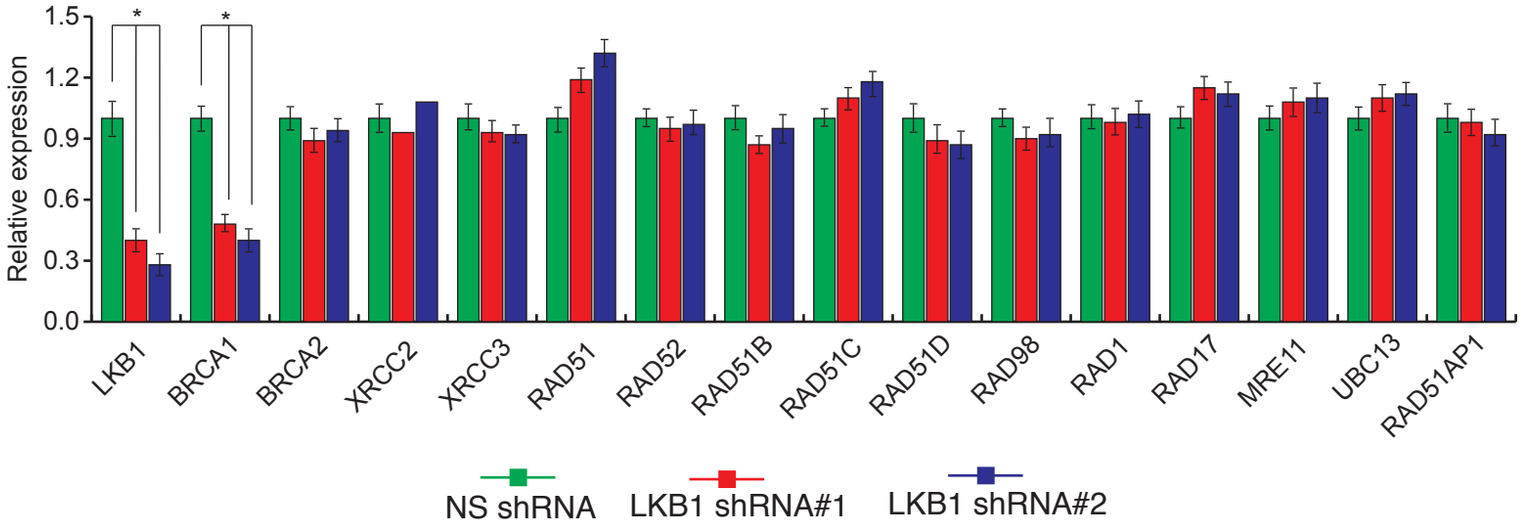

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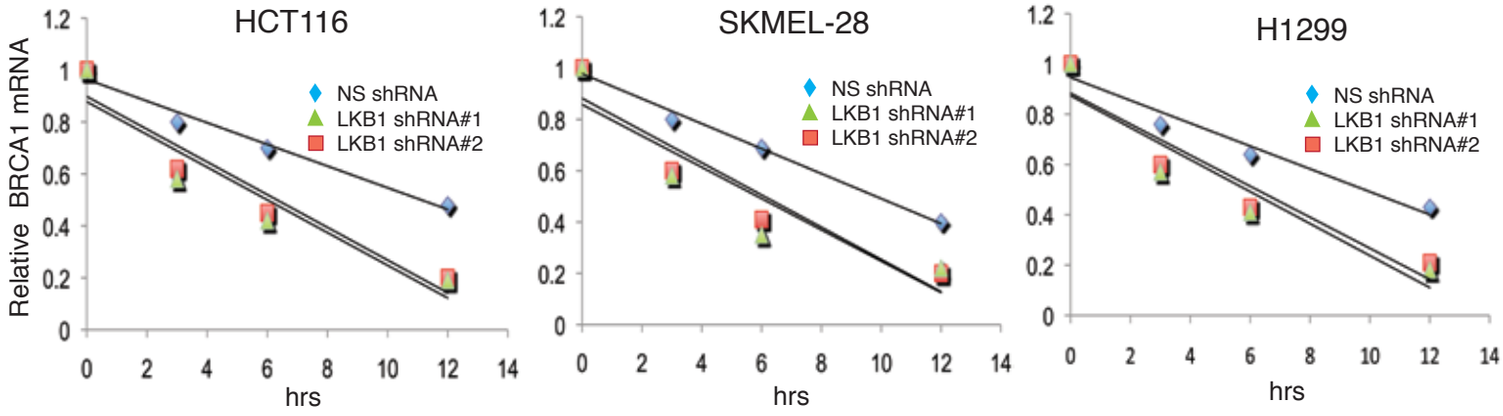

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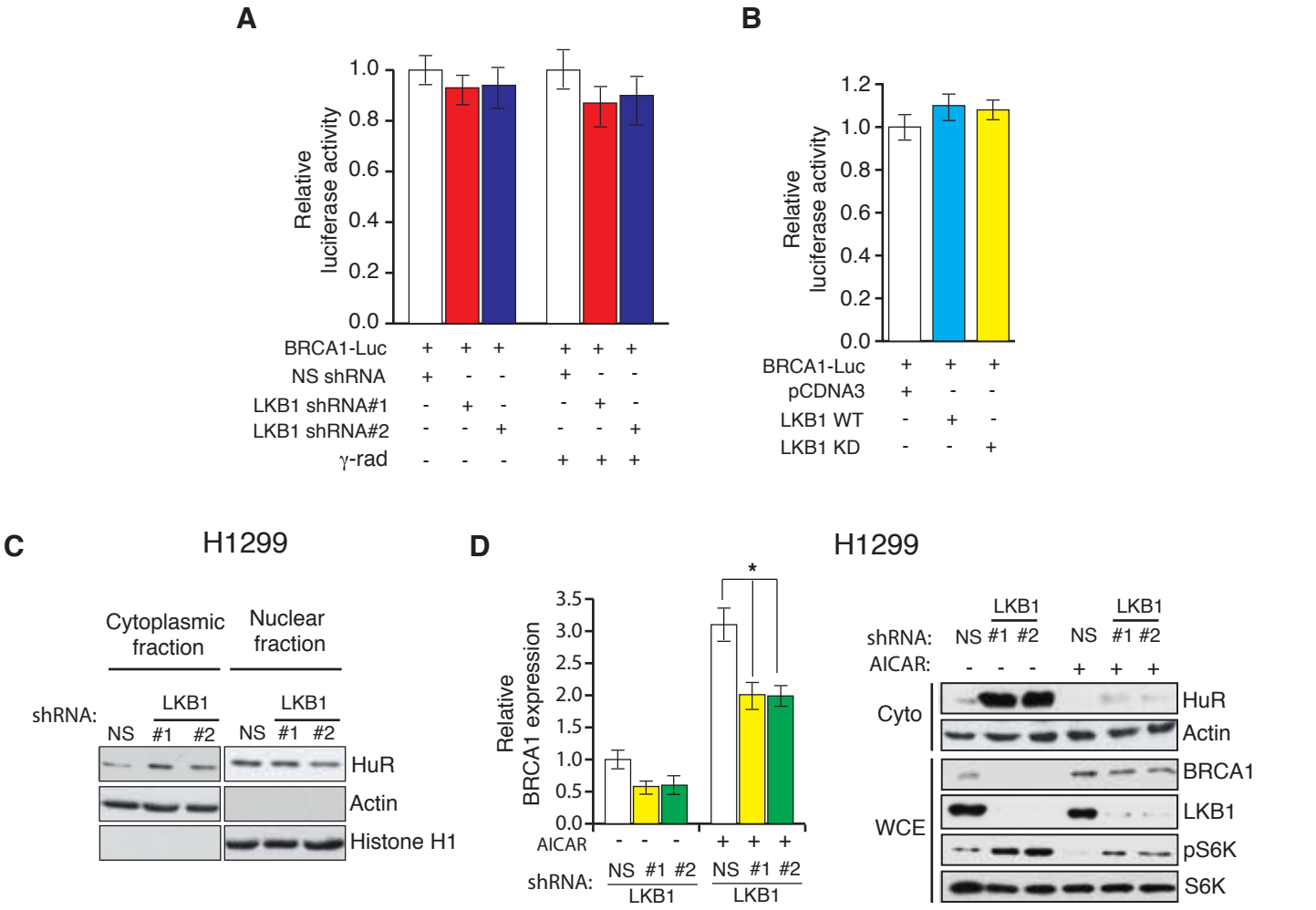

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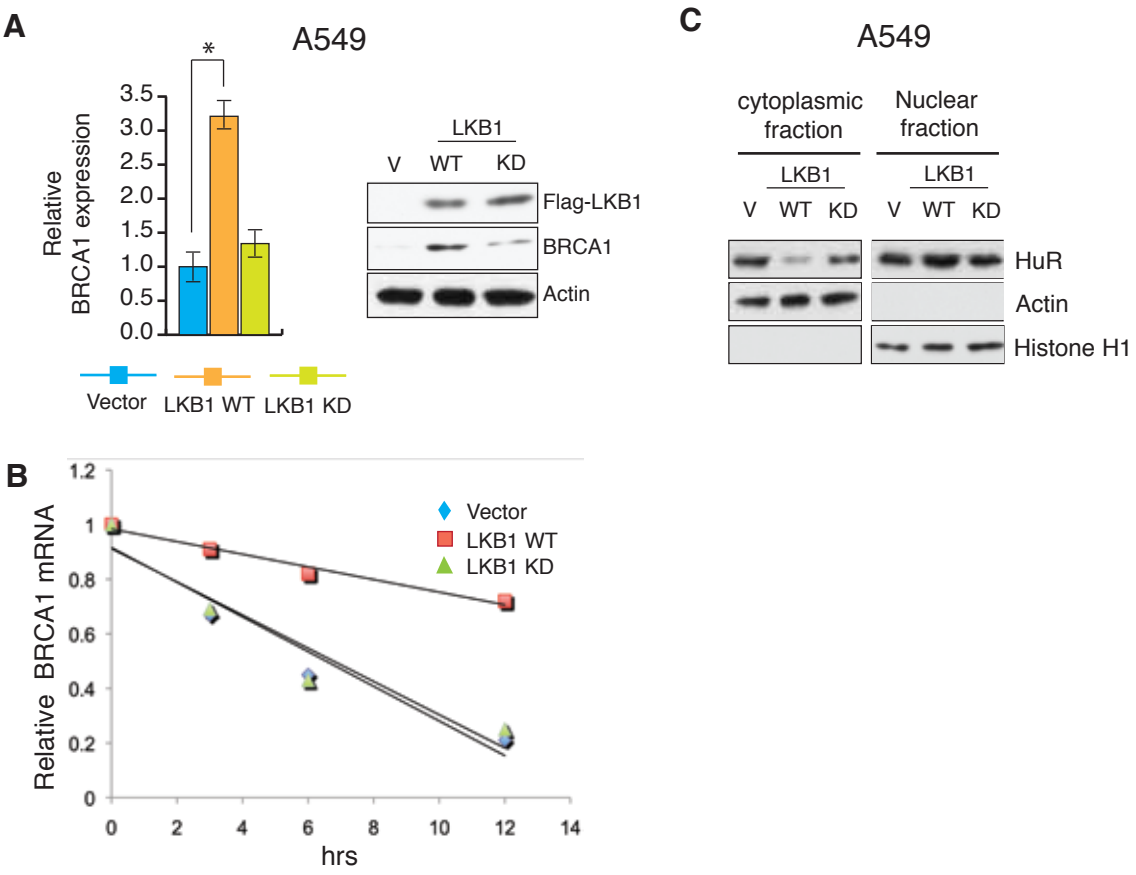

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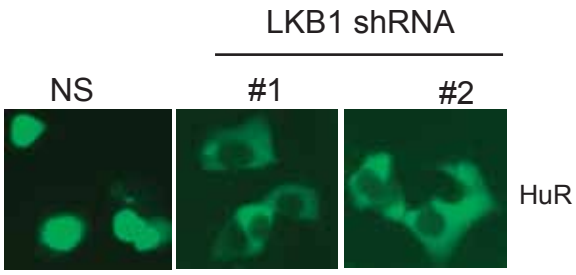

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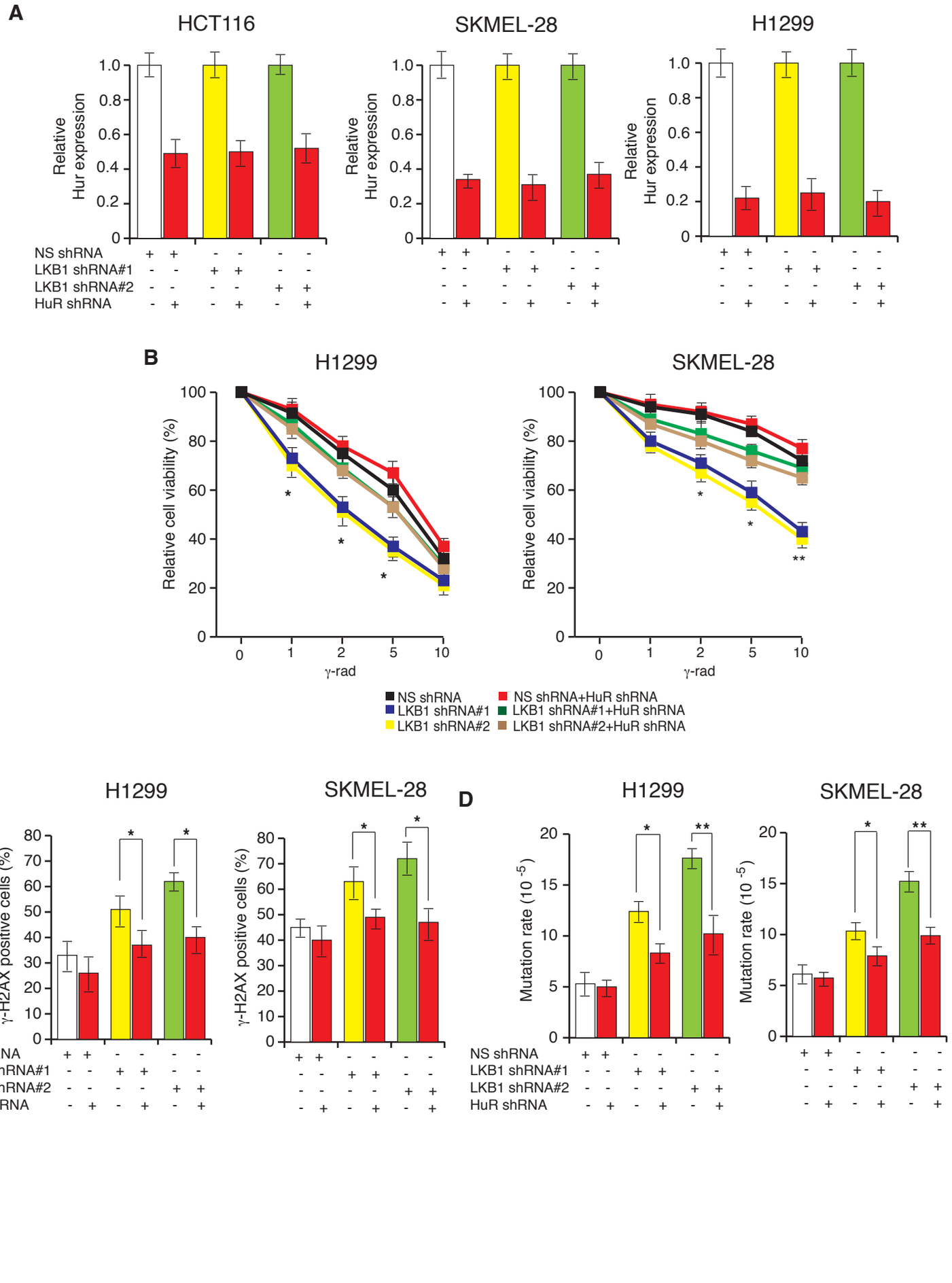

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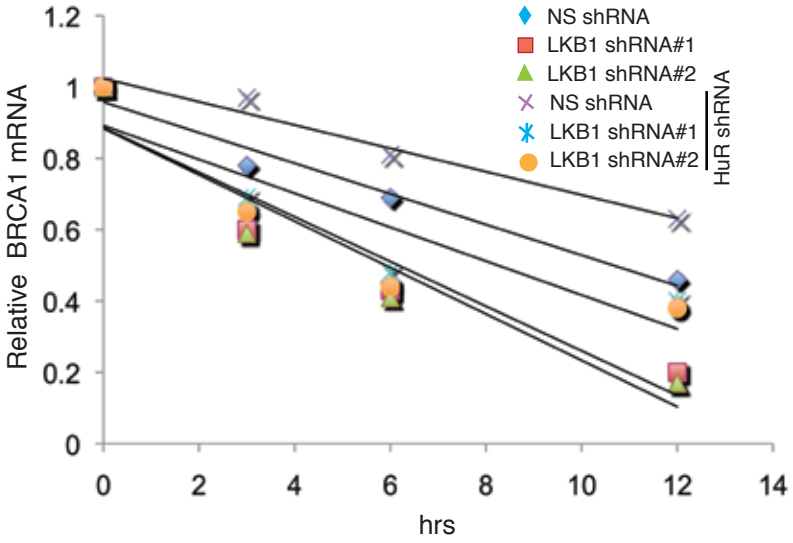

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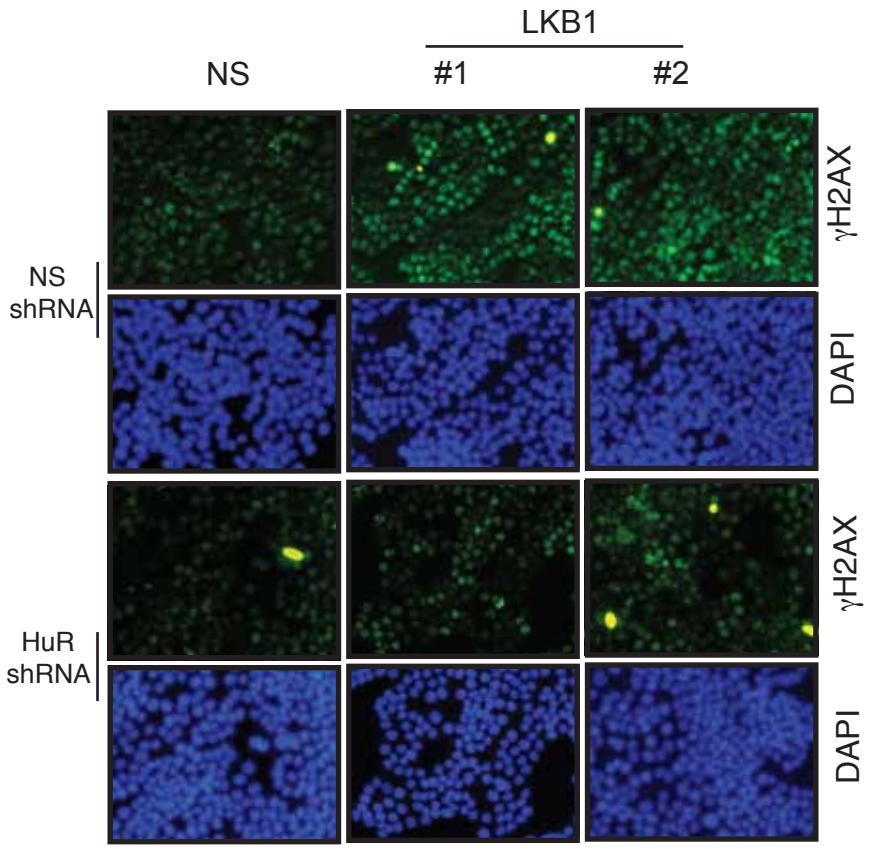

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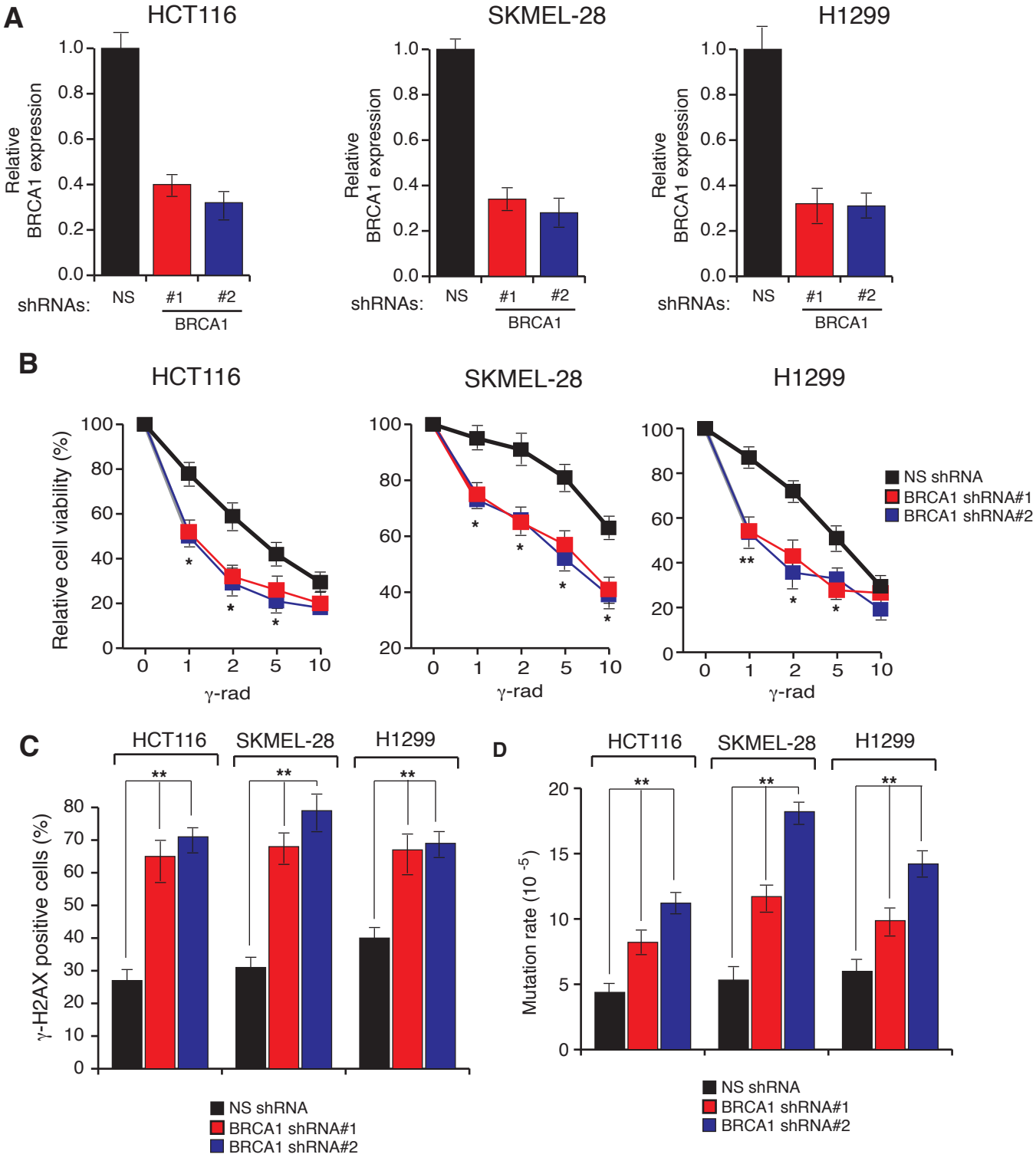

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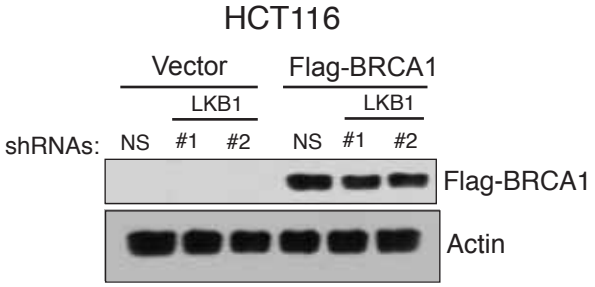

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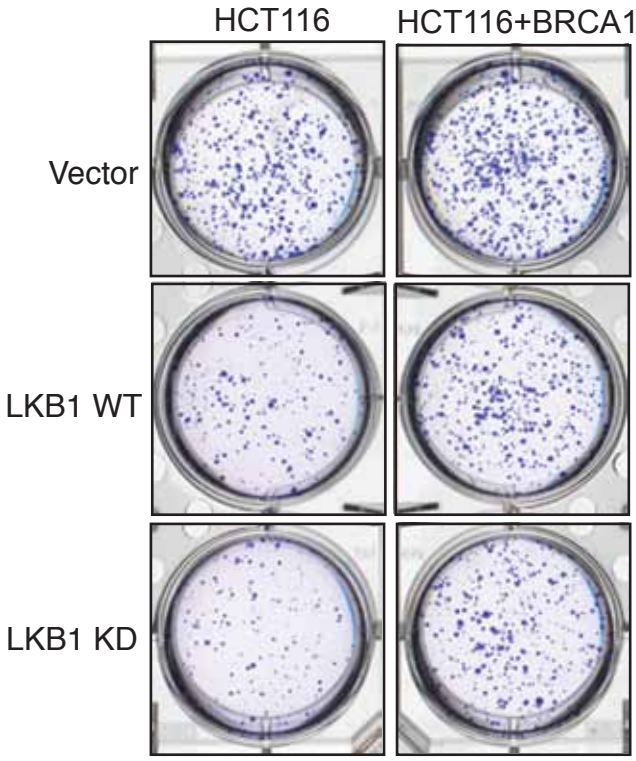

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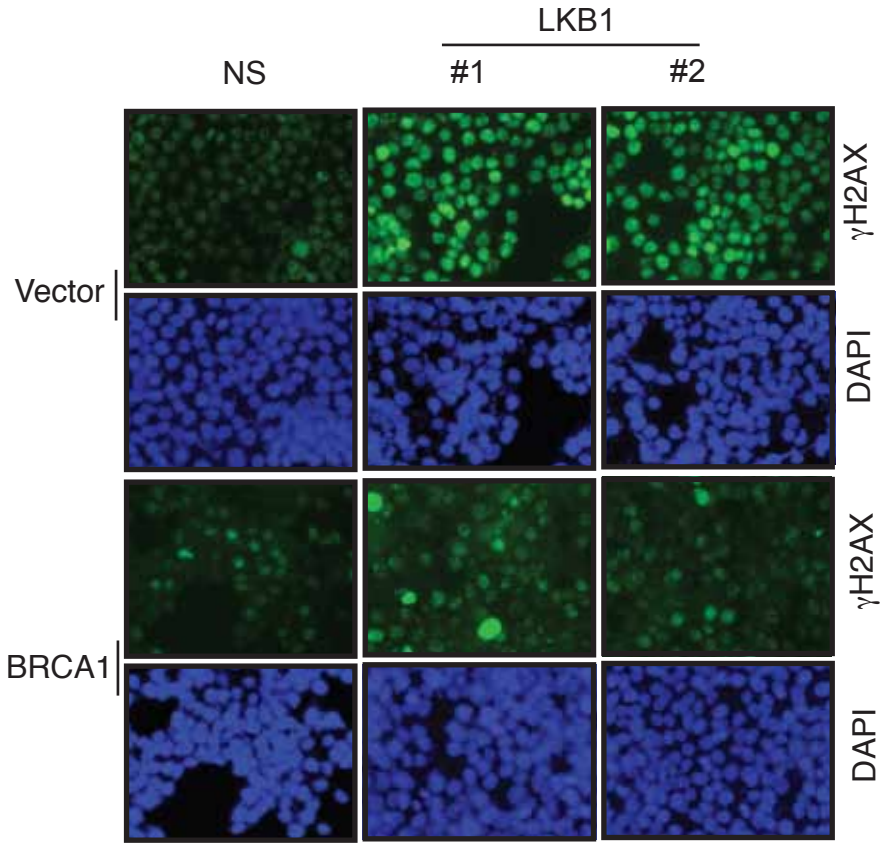

Supplementary Figure S18