Supplemental material

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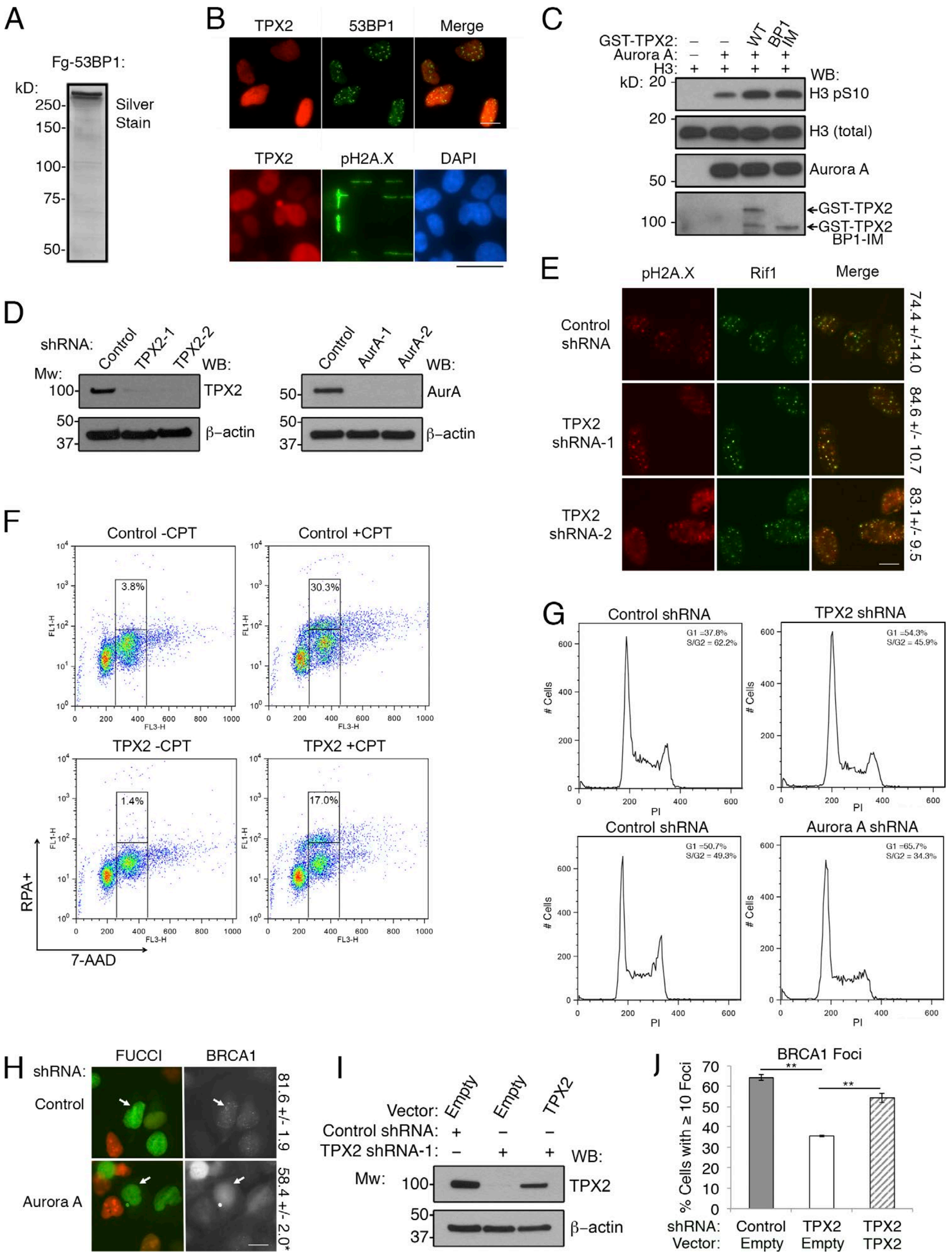

Figure S1. **TPX2/Aurora A impair BRCA1 foci formation and DNA end resection in S/G2 but do not localize to DSBs. (A)** Flag-53BP1 from Fig. 1 was purified from Sf9 cells infected with pDEST-BB-Flag-53BP1 recombinant baculovirus and verified by silver stain. **(B)** U2OS cells were γ-irradiated (5 Gy) and analyzed for TPX2 and 53BP1 localization (top). Scale bar, 10 µm. U2OS cells were subjected to laser microirradiation and stained for TPX2 and pH2A.X localization (bottom). Scale bar, 50 µm. **(C)** GST-TPX2 and GST-TPX2-BP1-IM both activate Aurora A in vitro. Recombinant His-Aurora A and the indicated GST proteins were incubated with histone H3.1 and ATP. Phosphorylated H3 (serine 10) was analyzed by Western blot. **(D)** Western blot of U2OS cells following shRNA-mediated knockdown of TPX2 (left) or Aurora A (right). **(E)** U2OS cells expressing the indicated shRNAs were γ-irradiated (5 Gy) and analyzed for Rif1 and pH2A.X foci formation. Quantification of Rif1 foci (percentage of cells with ≥10 foci ± SD) is shown on the right. Scale bar, 10 µm. Foci were quantitated from three biological replicate experiments with *n* ≥ 180 cells per experiment. Statistics: Student's two-tailed *t* test. P > 0.3 (not significant) for both shRNA-treated cells compared with control. **(F)** Representative flow cytometry plots and gating for quantitation shown in Fig. 2 B. Total S/G2 phase cells were gated according to 7-AAD staining, and RPA-positive cells were quantified as a percentage of this population. Percentages indicate the average of four biological replicates. **(G)** Representative cell cycle profiles of WT U2OS and TPX2 knockdown cells (top) and WT U2OS and Aurora A knockdown cells (bottom) as determined by propidium iodide DNA staining. G1 and S/G2 populations were determined using the Dean/Jett/Fox algorithm in Flow Jo software. Percentages represent the average of three biological replicate experiments. **(H)** U2OS-FUCCI cells were treated with a control or Aurora A shRNA, and S/G2 phase cells (yellow or green cells; indicated by white arrows) were specifically analyzed for BRCA1 foci formation. Quantification of BRCA1 foci in S/G2 phase cells (percentage of cells with ≥10 foci ± SD) is shown on the right. Scale bar, 10 µm. Foci were quantitated from three biological replicate experiments with *n* ≥ 170 cells per experiment. Statistics: Student's two-tailed *t* test; *, P < 0.05. **(I)** Western blot of U2OS cells expressing the indicated combination of shRNA and TPX2 expression vector as described for Fig. 3 C. **(J)** Cells expressing the indicated combination of shRNA and TPX2 expression vector were quantified for BRCA1 foci. Graph depicts the average of two biological replicate experiments with *n* ≥ 180 cells per experiment. Error bars represent ± SD. Statistics: Student's two-tailed *t* test; **, P < 0.01.

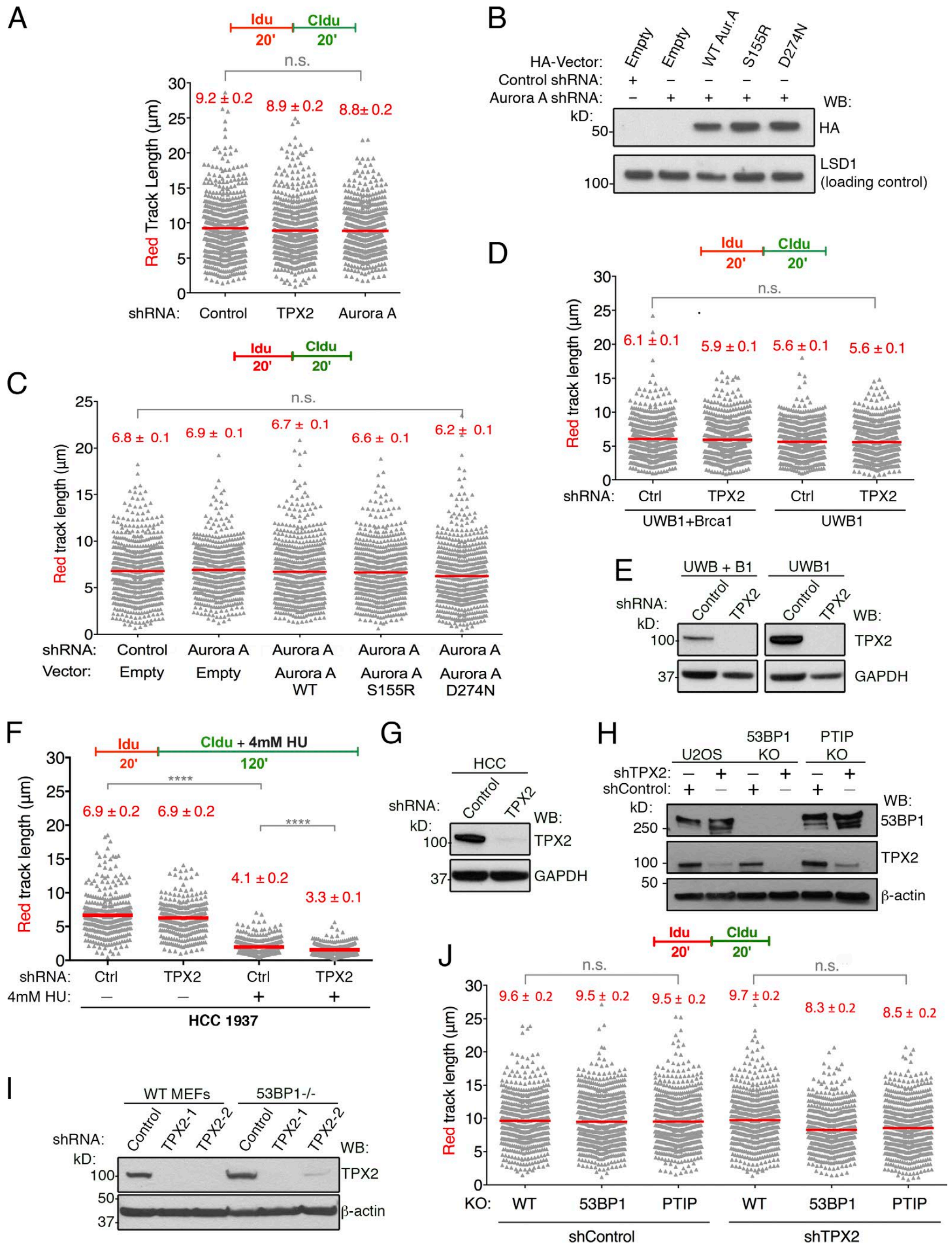

Figure S2. **Loss of TPX2/Aurora A results in replication fork deprotection but does not affect replication fork speed. (A)** Schematic of the single-molecule DNA fiber track analysis (top) and size distribution of IdU track length in TPX2- and Aurora A–depleted cells in the absence of HU (bottom). Cells were infected with control, TPX2, or Aurora A shRNA before IdU and CldU labeling, as indicated. Out of two biological replicates; *n* ≥ 300 tracks scored for each dataset. Bars represent median. Statistics: Mann–Whitney; n.s., not significant. **(B)** Western blot (WB) of HA-Aurora A expression for rescue experiment shown in Fig. 3 D. **(C)** Schematic of the single-molecule DNA fiber track analysis (top) and size distribution of IdU track length in U2OS cells expressing the indicated combination of shRNA and Aurora A expression vector in the absence of HU, as performed in A. Out of two biological replicate experiments; *n* ≥ 300 tracks were scored for each dataset. Bars represent median. Statistics: Mann–Whitney; n.s., not significant. **(D)** Schematic of the single-molecule DNA fiber track analysis (top) and size distribution of IdU track length in BRCA1-deficient (UWB1) and -proficient (UWB1 + BRCA1) cells in the absence of HU (bottom). Cells were treated with control or TPX2 shRNA before IdU and CldU labeling, as indicated. Out of two biological replicate experiments; *n* ≥ 300 tracks scored for each dataset. Bars represent median. Statistics: Mann–Whitney; n.s., not significant. **(E)** Western blot of BRCA1-deficient (UWB1) and -proficient (UWB1 + BRCA1) cells following treatment with either control or TPX2 shRNA. **(F)** Schematic of the single-molecule DNA fiber track analysis (top) and size distribution of IdU track length in BRCA-deficient HCC 1937 cells expressing the indicated shRNAs in the presence or absence of HU. *n* ≥ 300 tracks were scored in one biological experiment. Statistics: Mann–Whitney; ****, P < 0.0001. **(G)** Western blot of HCC 1937 cells following treatment with either control or TPX2 shRNA. **(H)** Western blot of WT U2OS, 53BP1 KO, and PTIP KO cells following treatment with the indicated shRNA. **(I)** Western blot of WT and 53BP1$^{-/-}$ MEFs following treatment with the indicated shRNA. **(J)** Schematic of the single-molecule DNA fiber track analysis (top) and size distribution of IdU track length in WT U2OS, 53BP1 KO, and PTIP KO cells following treatment with control or TPX2 shRNA in the absence of HU (bottom). Out of two biological repeats; *n* ≥ 300 tracks scored for each dataset. Bars represent median. Statistics: Mann–Whitney; n.s., not significant.

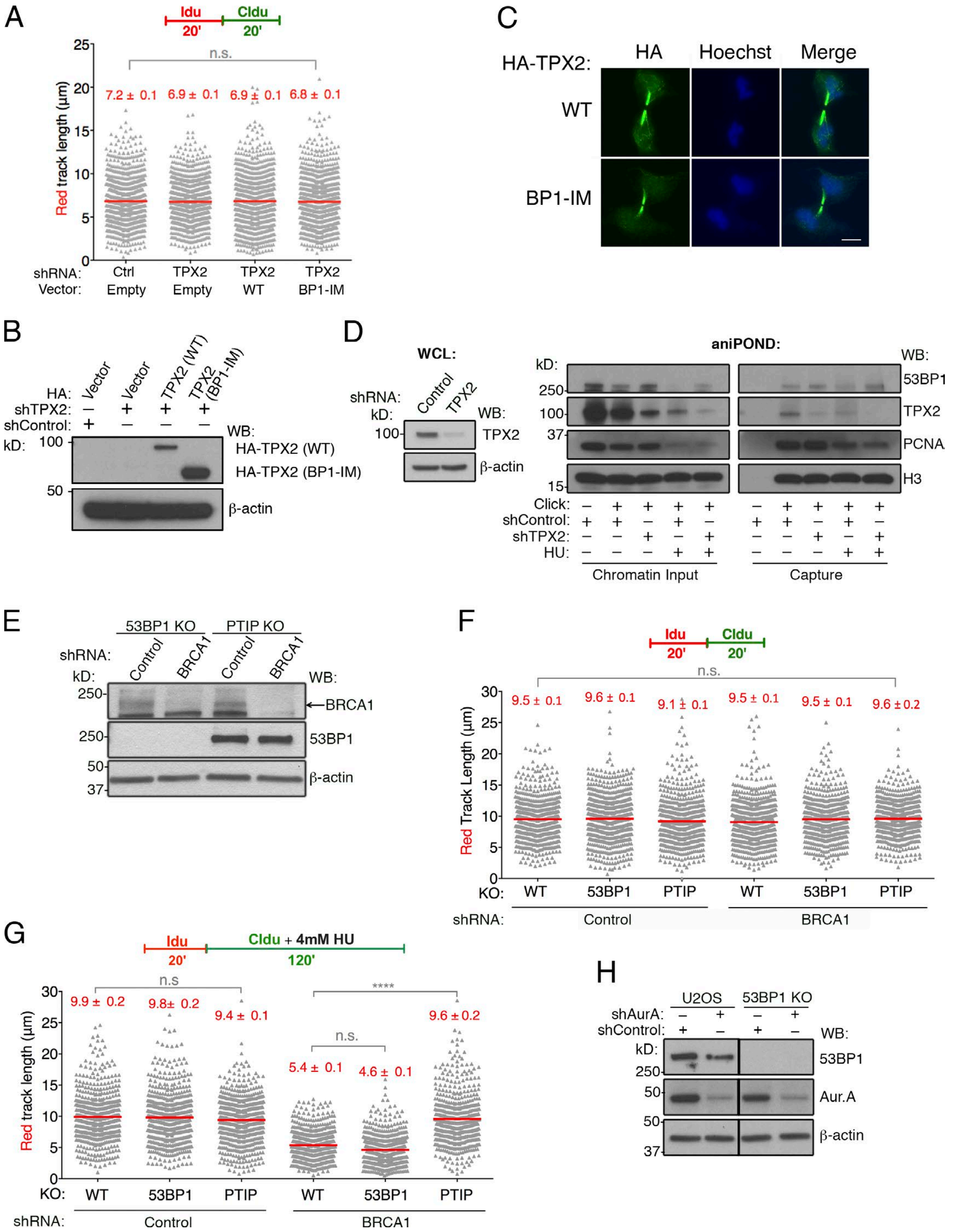

Figure S3. **TPX2/Aurora A protect stalled replication forks in a manner that is independent of its mitotic functions, but through a direct interaction with 53BP1. (A)** Schematic of the single-molecule DNA fiber track analysis (top) and size distribution of IdU track length in U2OS cells expressing the indicated shRNA and TPX2 expression vector in the absence of HU (bottom). Out of three biological repeats; *n* ≥ 300 tracks scored for each dataset. Bars represent median. Statistics: Mann–Whitney; n.s., not significant. **(B)** Western blot of U2OS cells treated with the indicated shRNA and TPX2 rescue vectors that were used for fiber analysis in A and Fig. 4 G. **(C)** TPX2 WT and TPX2-BP1-IM both localize to spindles during mitosis. U2OS cells expressing the indicated HA-tagged proteins were analyzed by immunofluorescence without nuclear extraction, as described in Materials and methods. Scale bar, 10 µm. **(D)** U2OS cells infected with the indicated shRNAs were pulse labeled with EdU and subsequently treated with 4 mM HU for 2 h. Proteins bound to nascent DNA were purified by aniPOND as described in Materials and methods and analyzed by Western blot (right). Shown is a representative of two biological replicates. Western blot of whole cell lysates for the control and TPX2 knockdown cells is shown on the left. **(E)** Western blot of 53BP1 KO and PTIP KO cells following treatment with control or BRCA1 shRNA. Arrow indicates BRCA1 band. **(F and G)** Loss of PTIP but not 53BP1 rescues replication fork degradation due to BRCA1 depletion. Size distribution of IdU track length in WT U2OS, 53BP1 KO, and PTIP KO cells following treatment with control or BRCA1 shRNA in the absence (F) or presence (G) of HU. Out of two biological repeats; *n* ≥ 300 tracks scored for each dataset. Bars represent median. Statistics: Mann–Whitney; ****, P < 0.0001, n.s., not significant. **(H)** Western blot of WT U2OS and 53BP1 KO cells following treatment with control or Aurora A shRNA, as used in Fig. 4 H.

Table S1. **MS analysis of Flag-HA-53BP1**

Z-Score	% TAPs	Ratio	ASF	S-Score	D-Score	UniPep	Tot Pep	IPI	GeneID	Symbol
MS Run 1										
10.79	3	7/270	170.3	124.06	770.46	113	399	IPI00029778	7158	*TP53BP1*
9.41	36	97/270	13.8	18.43	30.74	99	122	IPI00296337	5591	*PRKDC*
10.61	1	4/270	58.8	84.59	694.96	1,43	106	IPI00103951, IPI00045496	57646	*USP28*
11.99	2	6/270	26	57.31	384.48	43	73	IPI00470805	9656	*MDC1*
12.34	6	17/270	8.3	30.61	121.99	22	59	IPI00031650	84309	*NUDT16L1*
11.55	1	2/270	55	86.17	1001.19	30	55	IPI00032958	54443	*ANLN*
12.45	2	6/270	20.3	49.75	333.73	37	55	IPI00305289	3832	*KIF11*
1.48	99	266/270	29.8	7.33	7.39	34	53	IPI00003362	3309	*HSPA5*
10.43	1	4/270	27.8	57.51	472.5	39	49	IPI00293845	55183	*RIF1*
11.66	1	3/270	31	63.64	603.74	38	45	IPI00293921	11073	*TOPBP1*
11.39	1	2/270	35.5	68.74	798.67	25	35	IPI00008477	22974	*TPX2*
−0.92	99	266/270	55.1	5.61	5.65	19	31	IPI00003865	3312	*HSPA8*
8.47	39	104/270	4.5	8.97	14.45	16	31	IPI00011200	26227	*PHGDH*
6.62	4	12/270	13.6	25.1	119.06	23	28	IPI00021405	4000	*LMNA*
6.87	6	16/270	10.8	21.74	89.29	3,20	28	IPI00305282, IPI00107531	10111	*RAD50*
10.73	1	2/270	29	60.37	701.48	21	27	IPI00306400	3833	*KIFC1*
3.22	60	163/270	5.9	6.31	8.11	16	24	IPI00002214	3838	*KPNA2*
10.18	18	48/270	3.8	11.37	26.98	14	23	IPI00024661	9632	*SEC24C*
−0.34	96	260/270	27.2	4.67	4.76	15	21	IPI00007752	10383	*TUBB2C*
1.06	93	250/270	10.3	4.76	4.95	9	21	IPI00014424	1917	*EEF1A2*
9.26	2	6/270	9.3	29.24	196.15	1,15	19	IPI00745518, IPI00220113	4134	*MAP4*
0.68	7	18/270	36.4	16.88	65.38	1,14	19	IPI00641743, IPI00019848	3054	*HCFC1*
11.87	1	3/270	12	40.25	381.84	14	18	IPI00295502	58525	*WIZ*
1.23	91	245/270	10	4.33	4.54	10	17	IPI00220740	4869	*NPM1*
2.39	91	246/270	7.7	4.32	4.53	12	17	IPI00000874	5052	*PRDX1*
6.86	19	51/270	4.4	9.2	21.18	13	16	IPI00465044	55920	*RCC2*
8.01	6	15/270	6.6	16.97	72	13	16	IPI00001159	10985	*GCN1L1*
1.58	36	96/270	9	6.5	10.89	13	15	IPI00220834	7520	*XRCC5*
4.54	44	118/270	4.1	5.86	8.86	6	15	IPI00419585	5478	*PPIA*
11.05	3	8/270	5.1	21.74	126.28	9	14	IPI00021248	5347	*PLK1*
5.28	4	12/270	8.3	17.1	81.12	12	13	IPI00004233	4288	*MKI67*
12.01	1	2/270	12.5	41.89	486.75	13	13	IPI00465022	23347	*SMCHD1*
11.7	1	3/270	9	32.86	311.77	10	12	IPI00299463	4683	*NBN*
12.68	2	6/270	5.3	23.24	155.88	12	12	IPI00007927	10592	*SMC2*
12.03	1	3/270	8	32.86	311.77	9	12	IPI00298940	6790	*AURKA*
9.06	3	9/270	5.9	18.97	103.92	5	12	IPI00019329	8655	*DYNLL1*
8.84	9	23/270	3.5	11.87	40.67	10	12	IPI00328298	10051	*SMC4*
1.83	74	201/270	4.5	3.84	4.46	5	11	IPI00003935	8349	*HIST2H2BE*
7.08	3	8/270	7.5	19.27	111.94	10	11	IPI00029159	4361	*MRE11A*
−0.44	96	260/270	17.2	3.38	3.44	8	11	IPI00007750	7277	*TUBA4A*
1.15	17	46/270	9	8.04	19.47	11	11	IPI00003965	7874	*USP7*

Table S1. **MS analysis of Flag-HA-53BP1 *(Continued)***

Z-Score	% TAPs	Ratio	ASF	S-Score	D-Score	UniPep	Tot Pep	IPI	GeneID	Symbol
9.4	4	11/270	4.3	16.43	81.41	8	11	IPI00604402	65263	*PYCRL*
−0.1	96	259/270	12.4	3.39	3.46	6	11	IPI00011654	203068	*TUBB*
11.53	1	3/270	7.7	31.46	298.5	10	11	IPI00182798	22976	*PAXIP1*
2.34	83	225/270	4.4	3.63	3.98	7	11	IPI00025447	1915	*EEF1A1*
−1.41	99	266/270	33.7	3.34	3.37	8	11	IPI00304925	3304	*HSPA1B*
5.75	12	32/270	3.5	9.19	26.68	9	10	IPI00017376	10483	*SEC23B*
0.11	79	214/270	11.3	3.55	3.99	9	10	IPI00186290	1938	*EEF2*
−0.57	89	239/270	28.2	3.36	3.57	8	10	IPI00019359	3857	*KRT9*
14.96	1	2/270	6.5	34.86	405	8	9	IPI00096972	10919	*EHMT2*
12.91	1	2/270	8	34.86	405	9	9	IPI00028275	9793	*CKAP5*
−1.09	99	266/270	42.6	2.85	2.87	8	8	IPI00220327	3848	*KRT1*
4.49	3	9/270	7.8	15.49	84.85	8	8	IPI00026970	11198	*SUPT16H*
1.28	31	85/270	5.8	5.04	8.98	6	8	IPI00453473	8294	*HIST1H4I*
0.38	97	261/270	6.5	2.88	2.93	4	8	IPI00166768	84790	*TUBA1C*
9.9	1	4/270	6.5	23.24	190.92	3	8	IPI00294632	0	no symbol
1.88	10	28/270	6.4	8.22	25.51	3,3	7	IPI00216230, IPI00030131	7112	*TMPO*
5.59	6	15/270	4.7	11.22	47.62	7	7	IPI00009790	5214	*PFKP*
1.4	25	68/270	6	5.27	10.51	5	7	IPI00412259	389901	*LOC389901*
3.19	3	7/270	8.4	15.21	94.48	6	6	IPI00339381	6596	*HLTF*
8.08	1	4/270	5	20.12	165.34	6	6	IPI00216113	11004	*KIF2C*
16.37	0	1/270	6	40.25	40.25	6	6	IPI00006196	4926	*NUMA1*
2.84	13	35/270	4.7	6.8	18.9	6	6	IPI00064328	10419	*PRMT5*
0.08	64	172/270	8.6	3.07	3.85	6	6	IPI00220644	5315	PKM2
9.79	1	2/270	7	28.46	330.68	6	6	IPI00159969	5978	*REST*
1.23	61	166/270	4.1	3.12	3.98	6	6	IPI00017375	10484	*SEC23A*
1.37	25	67/270	4.5	4.92	9.87	5	6	IPI00465430	2547	*XRCC6*
−1.04	96	260/270	12.5	2.5	2.54	6	6	IPI00216049	3190	*HNRPK*
10.63	1	2/270	6.5	28.46	330.68	6	6	IPI00289986	472	*ATM*
−1.31	98	264/270	21.9	2.26	2.29	5	5	IPI00301277	3305	*HSPA1L*
3.75	10	27/270	3.7	7.07	22.36	5	5	IPI00025512	3315	*HSPB1*
12.77	1	2/270	4.5	25.98	301.87	5	5	IPI00304895	55034	*MOCOS*
5.63	2	5/270	5.6	16.43	120.75	4	5	IPI00020021	7913	*DEK*
5.9	4	11/270	3.1	11.08	54.89	5	5	IPI00000846	1108	*CHD4*
0.62	95	256/270	4.1	2.3	2.36	2	5	IPI00011134	3311	*HSPA7*
10.39	3	8/270	2.5	12.99	75.47	5	5	IPI00010368	3796	*KIF2A*
12.77	1	2/270	4.5	25.98	301.87	4	5	IPI00180781	197259	*MLKL*
12.77	1	2/270	4.5	25.98	301.87	5	5	IPI00747787	23310	*NCAPD3*
6.5	9	23/270	2.2	7.66	26.25	4	5	IPI00012578	3840	*KPNA4*
MS Run 2										
10.51	3	7/270	170.3	122.49	760.75	113	389	IPI00029778	7158	*TP53BP1*
12.42	1	4/270	58.8	91.49	751.65	1,51	124	IPI00103951, IPI00045496	57646	*USP28*
9.41	36	97/270	13.8	18.43	30.74	96	122	IPI00296337	5591	*PRKDC*
11	2	6/270	26	54.91	368.34	40	67	IPI00470805	9656	*MDC1*
12.57	1	4/270	27.8	63.11	518.48	47	59	IPI00293845	55183	*RIF1*

Table S1. **MS analysis of Flag-HA-53BP1 *(Continued)***

Z-Score	% TAPs	Ratio	ASF	S-Score	D-Score	UniPep	Tot Pep	IPI	GeneID	Symbol
11.55	1	2/270	55	86.17	1001.19	30	55	IPI00032958	54443	*ANLN*
10.44	6	17/270	8.3	28.18	112.31	22	50	IPI00031650	84309	*NUDT16L1*
10.17	2	6/270	20.3	45	301.87	34	45	IPI00305289	3832	*KIF11*
11.4	1	3/270	31	62.93	596.99	36	44	IPI00293921	11073	*TOPBP1*
0.85	99	266/270	29.8	6.61	6.66	27	43	IPI00003362	3309	*HSPA5*
9.63	6	16/270	10.8	25.65	105.38	3,27	39	IPI00305282, IPI00107531	10111	*RAD50*
11.72	1	2/270	35.5	69.71	810	29	36	IPI00008477	22974	*TPX2*
−0.88	99	266/270	55.1	5.7	5.74	23	32	IPI00003865	3312	*HSPA8*
12.33	1	2/270	29	64.69	751.65	21	31	IPI00306400	3833	*KIFC1*
7.6	39	104/270	4.5	8.53	13.74	17	28	IPI00011200	26227	*PHGDH*
13.2	2	6/270	9.3	34.86	233.83	2,15	27	IPI00396171, IPI00220113	4134	*MAP4*
5.89	4	12/270	13.6	23.72	112.5	21	25	IPI00021405	4000	*LMNA*
3.06	60	163/270	5.9	6.17	7.94	15	23	IPI00002214	3838	*KPNA2*
−0.34	96	260/270	27.2	4.67	4.76	15	21	IPI00007752	10383	*TUBB2C*
8.36	18	48/270	3.8	10.34	24.52	15	19	IPI00024661	9632	*SEC24C*
0.63	7	18/270	36.4	16.43	63.64	1,12	18	IPI00641743, IPI00019848	3054	*HCFC1*
−0.96	99	266/270	33.7	4.27	4.31	13	18	IPI00304925	3304	*HSPA1B*
1.23	91	245/270	10	4.33	4.54	9	17	IPI00220740	4869	*NPM1*
11.21	1	3/270	12	39.12	371.08	14	17	IPI00295502	58525	*WIZ*
8.52	6	15/270	6.6	17.49	74.22	13	17	IPI00001159	10985	*GCN1L1*
0.6	93	250/270	10.3	4.16	4.32	7	16	IPI00014424	1917	*EEF1A2*
2.15	91	246/270	7.7	4.19	4.39	11	16	IPI00000874	5052	*PRDX1*
1.58	36	96/270	9	6.5	10.89	10	15	IPI00220834	7520	*XRCC5*
11.05	3	8/270	5.1	21.74	126.28	9	14	IPI00021248	5347	*PLK1*
−0.9	99	266/270	42.6	3.77	3.8	13	14	IPI00220327	3848	*KRT1*
−0.42	89	239/270	28.2	3.98	4.23	13	14	IPI00019359	3857	*KRT9*
−0.28	96	260/270	17.2	3.67	3.74	8	13	IPI00007750	7277	*TUBA4A*
11.14	4	11/270	4.3	17.86	88.5	9	13	IPI00604402	65263	*PYCRL*
9.6	9	23/270	3.5	12.35	42.33	2,10	13	IPI00411559, IPI00328298	10051	*SMC4*
7.74	3	8/270	7.5	20.12	116.91	12	12	IPI00029159	4361	*MRE11A*
2.11	31	85/270	5.8	6.17	11	7	12	IPI00453473	8294	*HIST1H4I*
2.66	83	225/270	4.4	3.79	4.16	7	12	IPI00025447	1915	*EEF1A1*
11.08	1	2/270	12.5	40.25	467.65	12	12	IPI00465022	23347	*SMCHD1*
1.83	74	201/270	4.5	3.84	4.46	6	11	IPI00003935	8349	*HIST2H2BE*
10.72	1	3/270	9	31.46	298.5	11	11	IPI00299463	4683	*NBN*
11.02	1	3/270	8	31.46	298.5	8	11	IPI00298940	6790	*AURKA*
11.53	1	3/270	7.7	31.46	298.5	10	11	IPI00182798	22976	*PAXIP1*
3.16	44	118/270	4.1	5.02	7.59	6	11	IPI00419585	5478	*PPIA*
4.6	19	51/270	4.4	7.63	17.56	9	11	IPI00465044	55920	*RCC2*
13.54	1	4/270	5	25.98	213.45	9	10	IPI00216113	11004	*KIF2C*
0.5	17	45/270	21.5	7.75	7.75	10	10	IPI00302592	2316	*FLNA*
−0.22	96	259/270	12.4	3.23	3.3	6	10	IPI00011654	203068	*TUBB*

Table S1. **MS analysis of Flag-HA-53BP1 *(Continued)***

Z-Score	% TAPs	Ratio	ASF	S-Score	D-Score	UniPep	Tot Pep	IPI	GeneID	Symbol
3.61	4	12/270	8.3	14.23	67.5	1,8	9	IPI00301293, IPI00004233	4288	*MKI67*
7.25	6	15/270	4.7	12.73	54	8	9	IPI00009790	5214	*PFKP*
−0.52	96	260/270	12.5	3.06	3.12	8	9	IPI00216049	3190	*HNRPK*
−1.07	98	264/270	21.9	2.86	2.89	6	8	IPI00301277	3305	*HSPA1L*
0.36	64	172/270	8.6	3.54	4.44	1,5	8	IPI00479186, IPI00220644	5315	*PKM2*
3.1	25	68/270	4.3	5.64	11.23	7	8	IPI00021290	47	*ACLY*
5.99	3	9/270	5.9	15.49	84.85	4	8	IPI00019329	8655	*DYNLL1*
1.65	25	68/270	6	5.64	11.23	7	8	IPI00412259	389901	*LOC389901*
13.08	1	2/270	7	32.86	381.84	8	8	IPI00159969	5978	*REST*
4.47	6	16/270	4.6	10.87	44.65	7	7	IPI00054042	2969	*GTF2I*
7.34	2	6/270	5.3	17.75	119.06	7	7	IPI00007927	10592	*SMC2*
-0.21	79	214/270	11.3	2.97	3.34	7	7	IPI00186290	1938	*EEF2*
3.37	13	35/270	4.7	7.35	20.41	6	7	IPI00064328	10419	*PRMT5*
0.67	17	46/270	9	6.41	15.53	7	7	IPI00003965	7874	*USP7*
3.91	3	9/270	7.8	14.49	79.37	7	7	IPI00026970	11198	*SUPT16H*
10.02	1	2/270	8	30.74	357.18	7	7	IPI00028275	9793	*CKAP5*
−1.08	95	257/270	22.1	2.71	2.78	7	7	IPI00007765	3313	*HSPA9*
12.41	1	2/270	6.5	30.74	357.18	7	7	IPI00289986	472	*ATM*
8.65	1	4/270	6.5	21.74	178.59	4	7	IPI00294632	0	[no symbol]
4.56	10	27/270	3.7	7.75	24.49	6	6	IPI00025512	3315	*HSPB1*
16.37	0	1/270	6	40.25	40.25	4,2	6	IPI00383594, IPI00011840	84939	*MUM1*
3.35	12	32/270	3.5	7.12	20.67	6	6	IPI00017376	10483	*SEC23B*
16.37	0	1/270	6	40.25	40.25	5	6	IPI00412298	545	*ATR*
1.17	95	256/270	4.1	2.52	2.58	3	6	IPI00011134	3311	*HSPA7*
0.29	84	226/270	6	2.68	2.68	5	6	IPI00217465	3006	*HIST1H1C*
13.48	1	3/270	3.7	23.24	220.45	6	6	IPI00160901	51512	*GTSE1*
−0.05	97	261/270	6.5	2.49	2.53	4	6	IPI00166768	84790	*TUBA1C*
1.37	2	6/270	19.8	16.43	110.23	6	6	IPI00329605	4437	*MSH3*
16.37	0	1/270	5	36.74	36.74	5	5	IPI00293963	9425	*CDYL*
6.21	9	24/270	2.3	7.5	25.16	4	5	IPI00013774	3065	*HDAC1*
16.37	0	1/270	5	36.74	36.74	5	5	IPI00465045	57609	*DIP2B*
0.87	61	166/270	4.1	2.85	3.64	5	5	IPI00017375	10484	*SEC23A*
0.47	43	115/270	5.7	3.43	5.25	5	5	IPI00021187	8607	*RUVBL1*
8.31	7	18/270	1.9	8.66	33.54	4	5	IPI00106698	3672	*ITGA1*
14.04	1	4/270	2.5	18.37	150.93	4	5	IPI00299214	7083	*TK1*
−0.46	84	227/270	9.4	2.44	2.66	3	5	IPI00006510	81027	*TUBB1*
1.49	61	166/270	2.8	2.85	3.64	2	5	IPI00026272	3012	*HIST1H2AE*
6.5	9	23/270	2.2	7.66	26.25	5	5	IPI00012578	3840	*KPNA4*
8.95	2	6/270	3.5	15	100.62	3	5	IPI00062037	140735	*DYNLL2*
0.06	39	106/270	10.5	3.57	3.57	5	5	IPI00293464	1642	*DDB1*

Identified proteins with four or more unique peptides are shown. The sample was analyzed in duplicate, and the results of each run are indicated (MS Run 1 and MS Run 2).

Table S2. **Antibodies used in this study**

Antibody	Method	Source	Identifier
Flag (mouse)	WB and IP	Sigma	F3165
HA (mouse)	WB, IF, and IP	BioLegend	901501
β-Actin HRP (mouse)	WB	Sigma	A3854
Alexa Fluor 488 (mouse)	IF	Invitrogen	A11029
Alexa Fluor 488 (rabbit)	IF	Invitrogen	A11034
Alexa Fluor 488 (rat)	Flow cytometry and DNA fiber	Invitrogen	A11006
Alexa Fluor 594 (mouse)	IF	Invitrogen	A11032
Alexa Fluor 594 (rabbit)	IF	Invitrogen	A11037
Pacific Blue (mouse)	IF (FUCCI)	Invitrogen	P31582
Aurora A (mouse)	WB and IF	Abcam	ab13824
TPX2 (rabbit)	WB	Bethyl	A300-430A
TPX2 (rabbit)	IF and IP	Bethyl	A300-429A
53BP1 (mouse)	WB and IF	BD Biosciences	612522
GAPDH	WB	Abcam	ab181602
Histone H3 (rabbit)	WB	Abcam	ab1791
GST (rabbit)	WB	Santa Cruz	SC-459
Histone H3 pS10 (rabbit)	WB	Cell Signaling	9701S
LSD1 (mouse)	WB	Santa Cruz	SC-53875
BRCA1 (mouse)	IF	Santa Cruz	SC-6954
BRCA1 (mouse)	WB	Abcam	Ab16781
pH2AX (mouse)	IF	Abcam	Ab26350
pH2AX (rabbit)	IF	Active Motif	39117
Rad51 (rabbit)	IF	Santa Cruz	SC-8349
Rif1 (rabbit)	IF	Bethyl	A300-569A
RPA32 (rat)	Flow cytometry	Cell Signaling	2208S
HRP (mouse)	WB	Cell Signaling	7076S
HRP (rabbit)	WB	Cell Signaling	7074S
BrdU (rat)	DNA fiber	Abcam	ab6326
BrdU (mouse)	DNA fiber	BD Biosciences	347580

IF, immunofluorescence; IP, immunoprecipitation; WB, Western blot.