Kleiblova et al., http://www.jcb.org/cgi/content/full/jcb.201210031/DC1

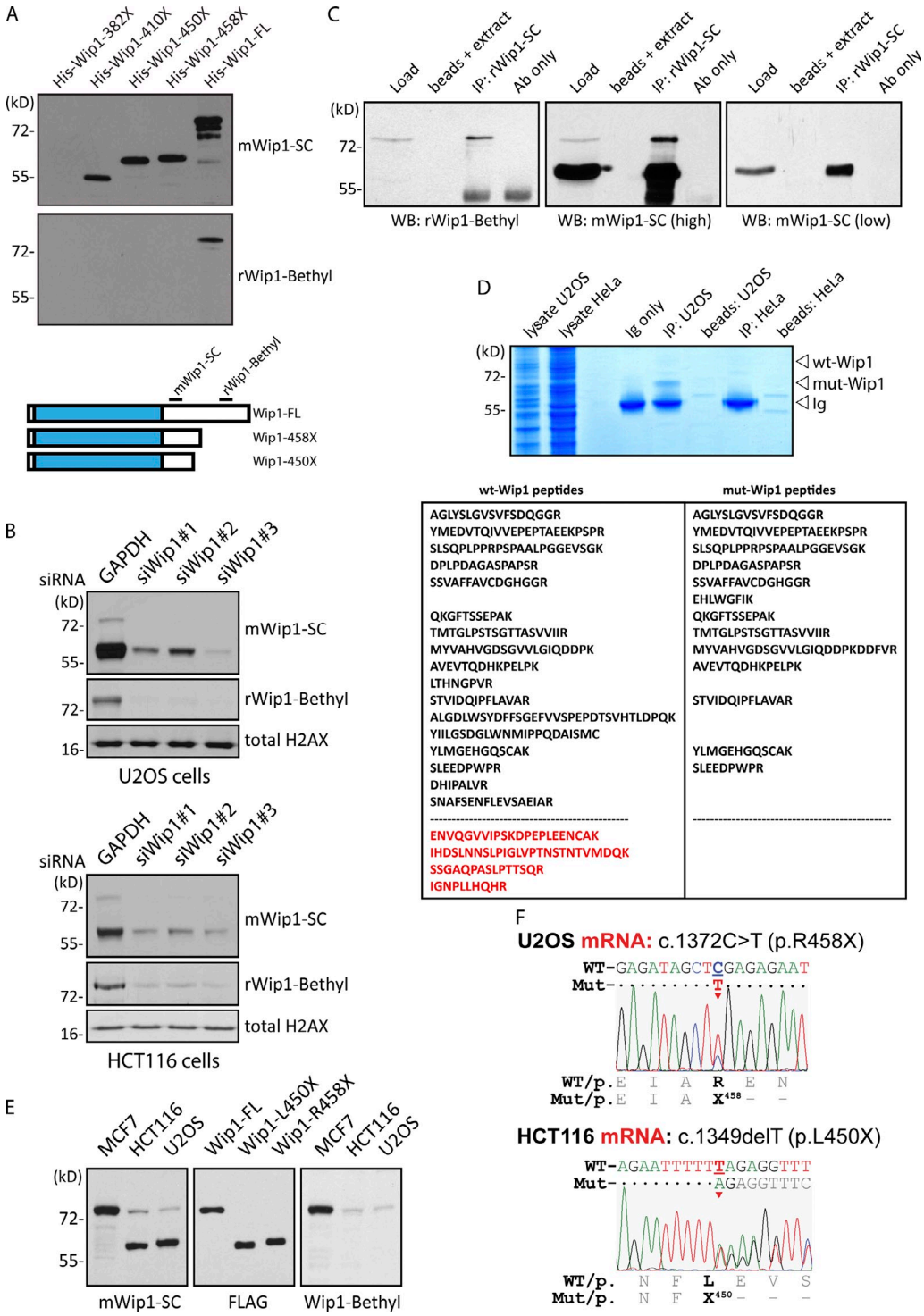

Figure S1. **Validation of reagents.** (A) Epitope mapping of anti-Wip1 antibodies using bacterially expressed and purified FL or truncated His-Wip1. Note that the anti-Wip1 (Santa Cruz Biotechnology, Inc. [SC]) recognizes an epitope between amino acid residues 380–410 of Wip1 and stains both truncated mutants of Wip1 (L450X and R458X), whereas anti-Wip1 (Bethyl Laboratories, Inc.) directed against residues 500–550 recognizes only FL-Wip1. (B) U2OS or HCT116 cells were transfected by the indicated siRNAs, and total cell lysates were probed for Wip1. (C) Wip1 was immunoprecipitated from U2OS cells by rabbit anti-Wip1 (Santa Cruz Biotechnology, Inc.) and probed by anti-Wip1 (Bethyl Laboratories, Inc.) and mouse anti-Wip1 (Santa Cruz Biotechnology, Inc.). WB, Western blot. (D) Wip1 was immunoprecipitated from U2OS or HeLa cells. Proteins were separated by SDS-PAGE, and bands corresponding to both forms of Wip1 were subjected to MS. Identified peptides are shown. Peptides from exon 6 found only in the FL-Wip1 are shown in red. (E) Cells were transfected with FLAG–Wip1-FL, FLAG–Wip1-L450X, or FLAG–Wip1-R458X, and the electrophoretic mobility of tagged proteins was compared with endogenous Wip1 signal in U2OS, HCT116, and MCF7 cells. (F) Total cellular RNA was isolated from U2OS and HCT116 cells and converted to cDNA by reverse transcription, and exon 6 was sequenced. Chromatograms of the affected regions are shown. Mutations are indicated by arrowheads. WT, wild-type Wip1; Mut, mutated Wip1; c., nucleotide sequence; p., peptide sequence. Note that both wild-type and mutated alleles are expressed. Ab, antibody; IP, immunoprecipitation.

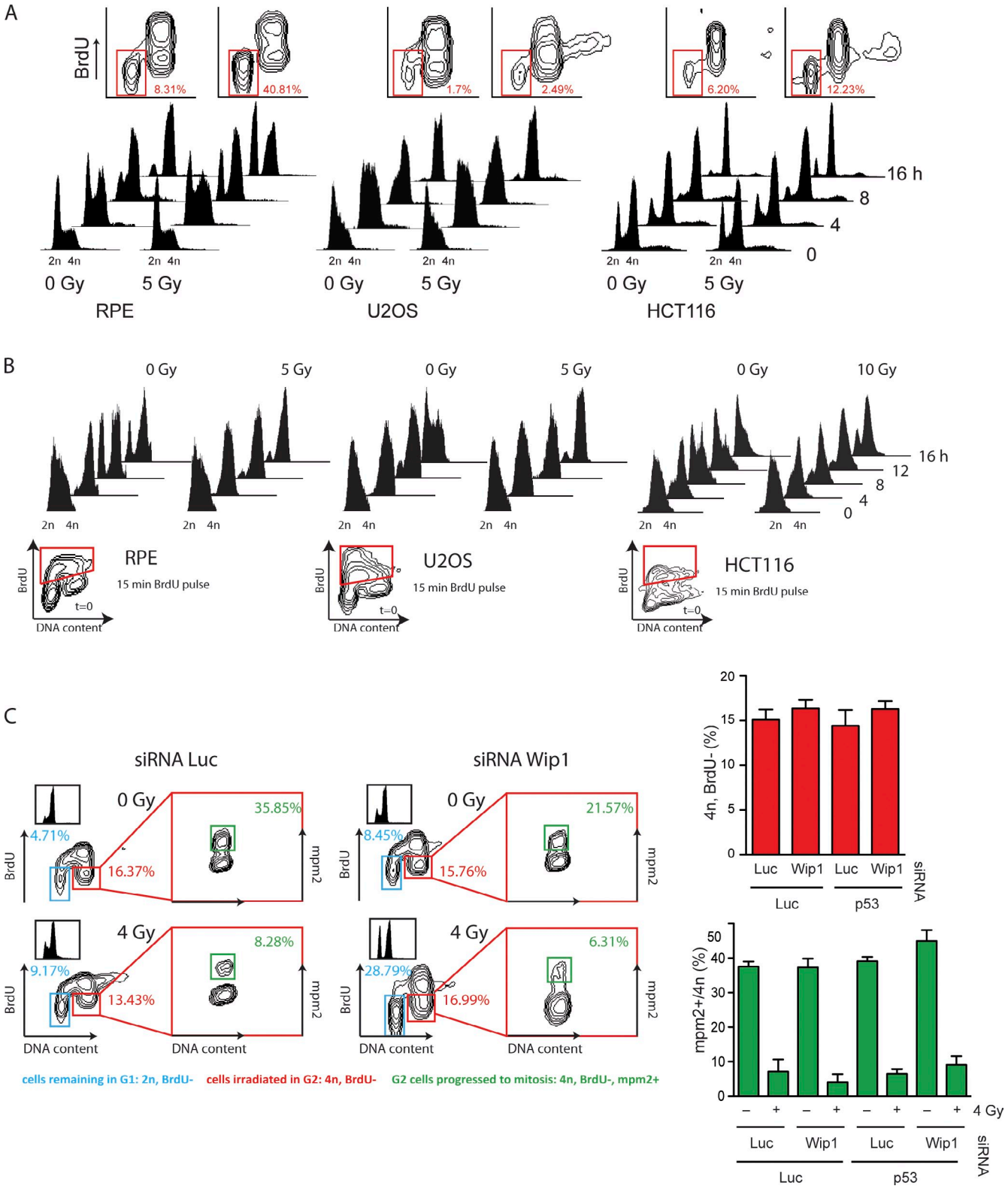

Figure S2. **G1 checkpoint is impaired in cells with truncated Wip1.** (A) Asynchronous cells were irradiated as indicated, cultured further for 16 h in the presence of 10 µM BrdU, and stained with propidium iodide. Population of 2n, BrdU-negative cells (G1 cells) was quantified by flow cytometry (red numbers). (B) Cells were pulsed with 10 µM BrdU (15 min), irradiated as indicated, and after fixation, at the indicated times stained for BrdU and DNA content. To assess intra-S and G2 checkpoint arrest, cell cycle progression of BrdU-positive cells was determined by flow cytometry. (C) An example of the experiment shown in Fig. 4 A. U2OS cells transfected by siRNA targeting luciferase (Luc) or Wip1 were treated with BrdU and STLC, irradiated or not irradiated with 4 Gy, and grown for a further 16 h. Cells were stained for BrdU, mdm2 (mitotic marker), and propidium iodide (DNA content) and analyzed by flow cytometry. Cells arrested in G1 (2n, BrdU negative), cells arrested in G2 (4n, BrdU negative), or mitotic cells (4n, mdm2 positive) are shown. Error bars indicate standard deviations. RPE, retinal pigment epithelium.

Table S1. **Mutation analysis of the *PPM1D* gene in cancer patients and noncancer controls**

WIP1 (exon [e]/ intron [i])	Nucleotide change	Protein change	rs number (dbSNP)	High-risk breast/ovarian cancer		Unselected colorectal cancer	Noncancer controls
				n = 330[a]	*n* = 398[b]	*n* = 304[a]	*n* = 450[a]
Truncating variants							
e6	c.1372C>T[c]	p.R458X	–	0	0	1[e]	0
e6	c.1451T>G[d]	p.L484X	–	0	1[f]	0	0
e6	c.1601_1615del	p.F534X	–	1[g]	0	0	0
e6	c.1602dupT	p.K535X	–	0	0	1[h]	0
Missense variants							
e1	c.29G>A	p.S10N	–	0	ND	0	1
e1	c.131C>G[d]	p.S44W	–	2	ND	0	0
e1	c.226C>T	p.R76C	–	0	ND	0	1
e1	c.307G>A	p.V103M	–	1	ND	0	0
e1	c.332A>G	p.E111G	rs75400620	0	ND	0	1
e6	c.1405A>G	p.K469E	rs61756416	1	1	1	1
e6	c.1486A>G[d]	p.I496V	rs35491690	1	2	1	0
e6	c.1670G>A	p.R557Q	–	0	1	0	0
e6	c.1715G>A	p.R572Q	–	0	0	1	0
Silent and intronic variants							
e1	c.90G>A[d]	p.E30	rs16944543	1	ND	0	2
e1	c.234T>A[d]	p.P78	–	0	ND	1	0
e1	c.456C>T[d]	p.A152	rs149400522	3	ND	2	2
i2	c.702-101T>C	unknown	–	1	ND	0	3
i2	c.702-38A>G	unknown	–	0	ND	1	0
i2	c.702-21A>G	unknown	rs138641521	8	ND	1	5
i4	c.1017+51A>G	unknown	rs144142345	3	ND	2	3
3′UTR	c.*37A>G	unknown	rs191317670	3	1	0	0
3′UTR	c.*44dupA	unknown	–	1	1	0	7

Numbering of the nucleotide sequence is based on the NCBI GenBank reference sequence NT_010783.15 (gene identifier: 224514953). Minus signs indicate novel mutations identified in this study. c., nucleotide sequence; p., peptide sequence; dbSNP, single nucleotide polymorphism database.
[a]Analysis set (mutation analysis of the entire *PPM1D* coding sequence).
[b]Validation set (mutation analysis of exon 6 only).
[c]Mutation identical to that found in U2OS human osteosarcoma cell line.
[d]Variants described in Ruark et al. (2013).
[e]Female patient with a poorly differentiated rectosigmoideal adenocarcinoma (72 yr); family history of cancer is unknown.
[f]Female patient with bilateral breast cancer diagnosed at the age of 55 and 67 yr; her mother suffered from breast cancer (57 yr); mother's mother suffered very probably from breast cancer (died at 85 yr, only anamnestic data); patient's brother suffered from lung cancer (smoker, died at 62 yr).
[g]Female patient with bilateral breast cancer diagnosed at the age of 40 and 42 yr; her mother suffered from cervical cancer (64 yr) and hematological malignancy (72 yr); mother's sister suffered from an unknown oncological diagnosis; patient's father developed gastric cancer (56 yr).
[h]Female patient with rectal cancer at the age of 51 and ovarian cancer in 61 yr; her mother died of breast cancer (aged 49), and father was diagnosed with a mediastinal tumor (at 79 yr).