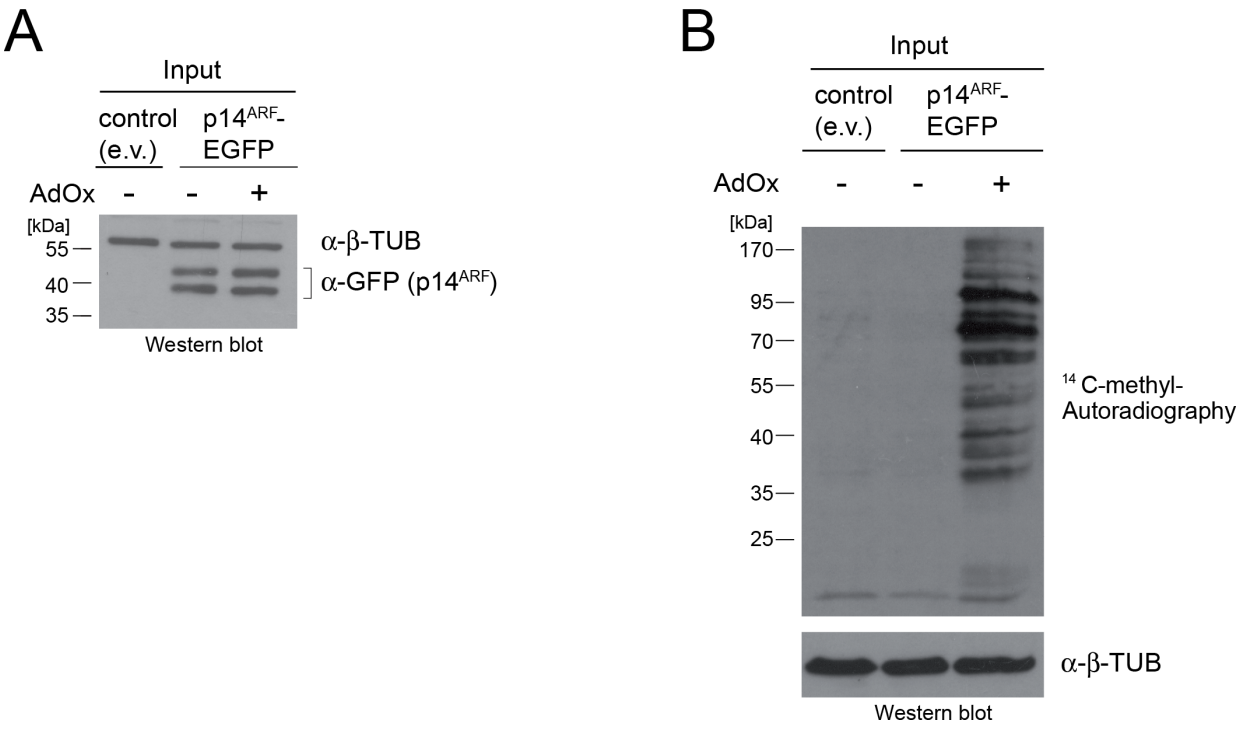
Appendix

Table of content

Appendix Supplementary Figures (S1-S16) and Figure Legends

Appendix Figure S1



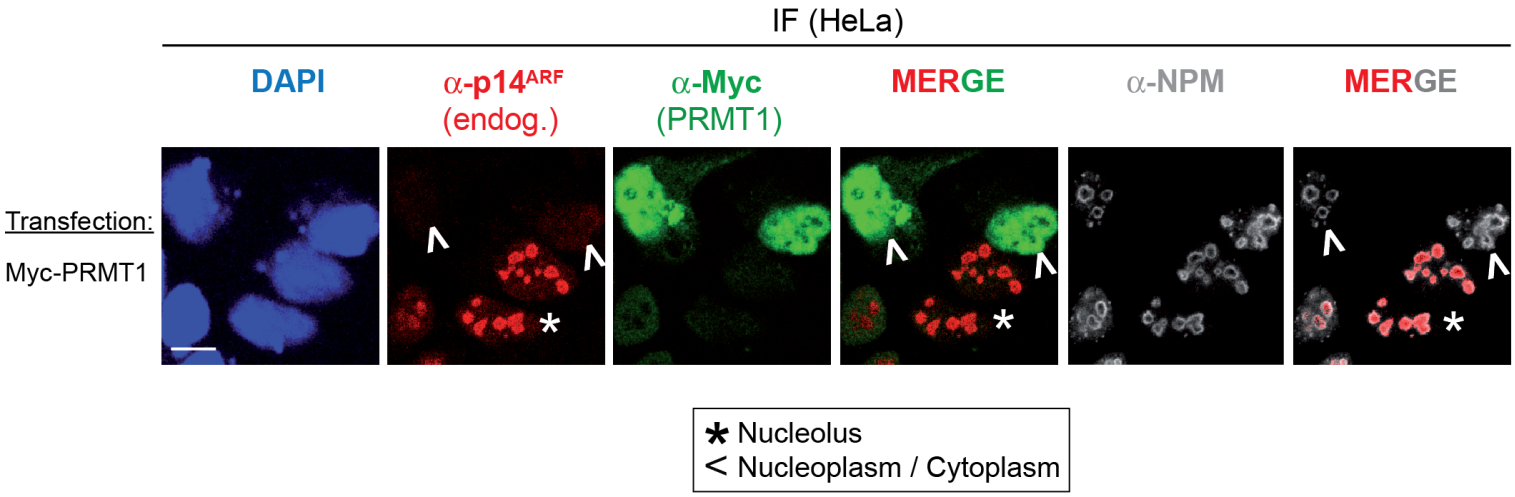
Appendix Figure S1

Metabolic labelling of HEK293 cells.

A: HEK293 cells were transfected with either empty vector (e.v., control) or C-terminally EGFP-tagged $p14^{ARF}$-containing plasmid. Subsequently, cells were treated with the global methyltransferase inhibitor adenosine dialdehyde, AdOx (+) or left untreated (-) for 72 h and then cultured in the presence of L-[^{3}H-methyl]-methionine. Overexpression of EGFP-tagged $p14^{ARF}$ (which typically occurred in a doublet band) in HEK293 cells was confirmed by immunoblotting using α-GFP and α-β-TUBULIN (loading control) antibodies. Size markers (in kDa) are shown on the left.

B: Hypomethylation caused by AdOx treatment (cell extracts from A) was verified by *in vitro* methyltransferase (MT) assay incubating 50µg lysate in the presence of [^{14}C-methyl]-SAM. Subsequently, reactions were blotted and analyzed by autoradiography or immunostaining with α-β-TUBULIN (loading control) antibody. Size markers (in kDa) are shown on the left.

Appendix Figure S2

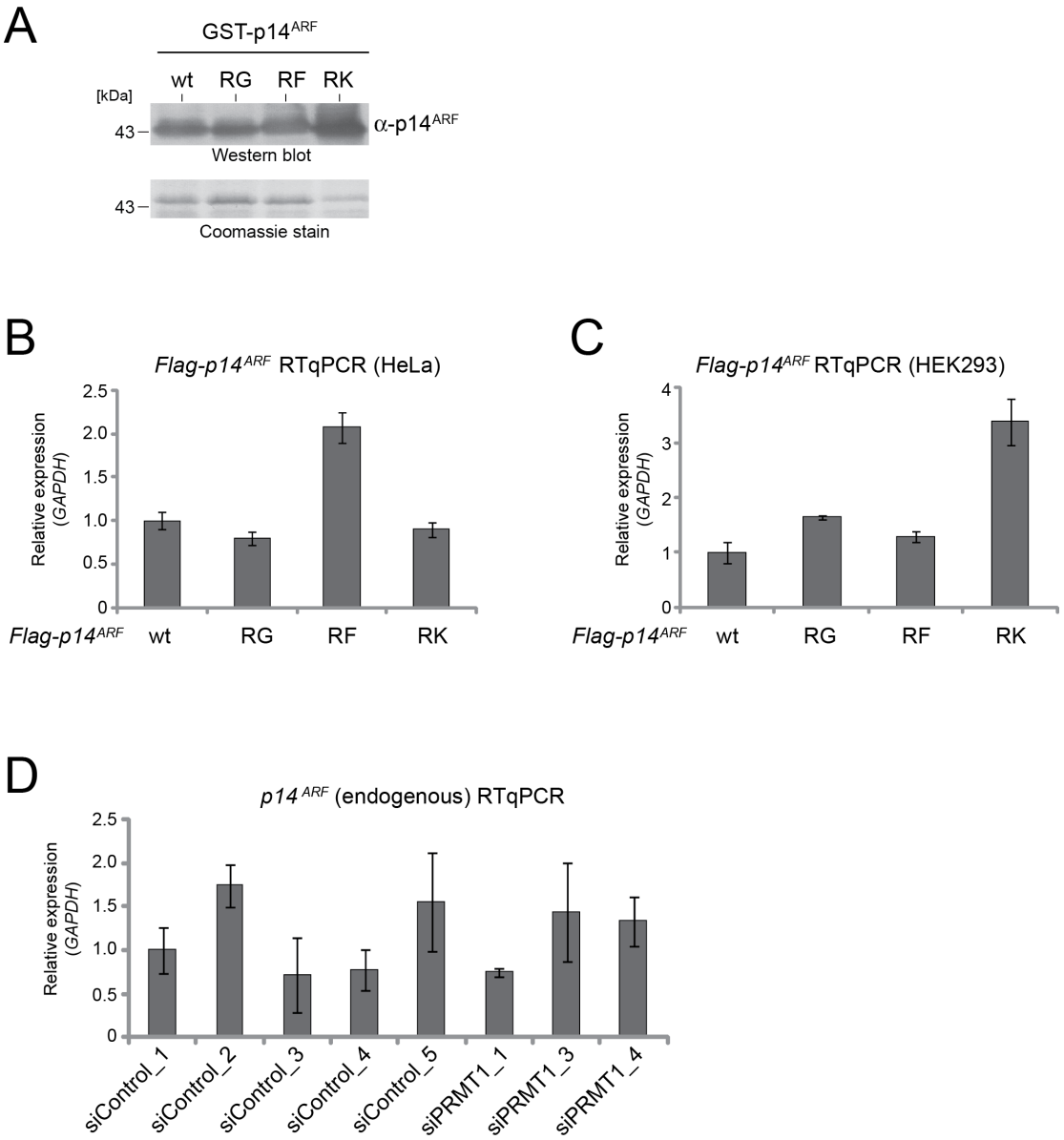


Appendix Figure S2

Nucleolar integrity in PRMT1-overexpressing HeLa cells.

HeLa cells were transfected with Myc-tagged wild type PRMT1-containing plasmid. Immunofluorescence (IF) staining was performed using α-p14^{ARF} (red, endogenous p14^{ARF}), α-Myc (green, exogenous PRMT1), α-NPM (grey, endogenous NPM as a nucleolar marker protein) antibodies and DAPI (blue, nuclei/DNA). Representative IF results are shown, with asterisks indicating cells with exclusive nucleolar and arrowheads indicating cells with predominantly nucleo-/cytoplasmic p14^{ARF} localization. Merge displays the combination of the p14^{ARF} and PRMT1 stainings as well as p14^{ARF} and NPM stainings. Scale bar: 10 μm.

Appendix Figure S3



Appendix Figure S3

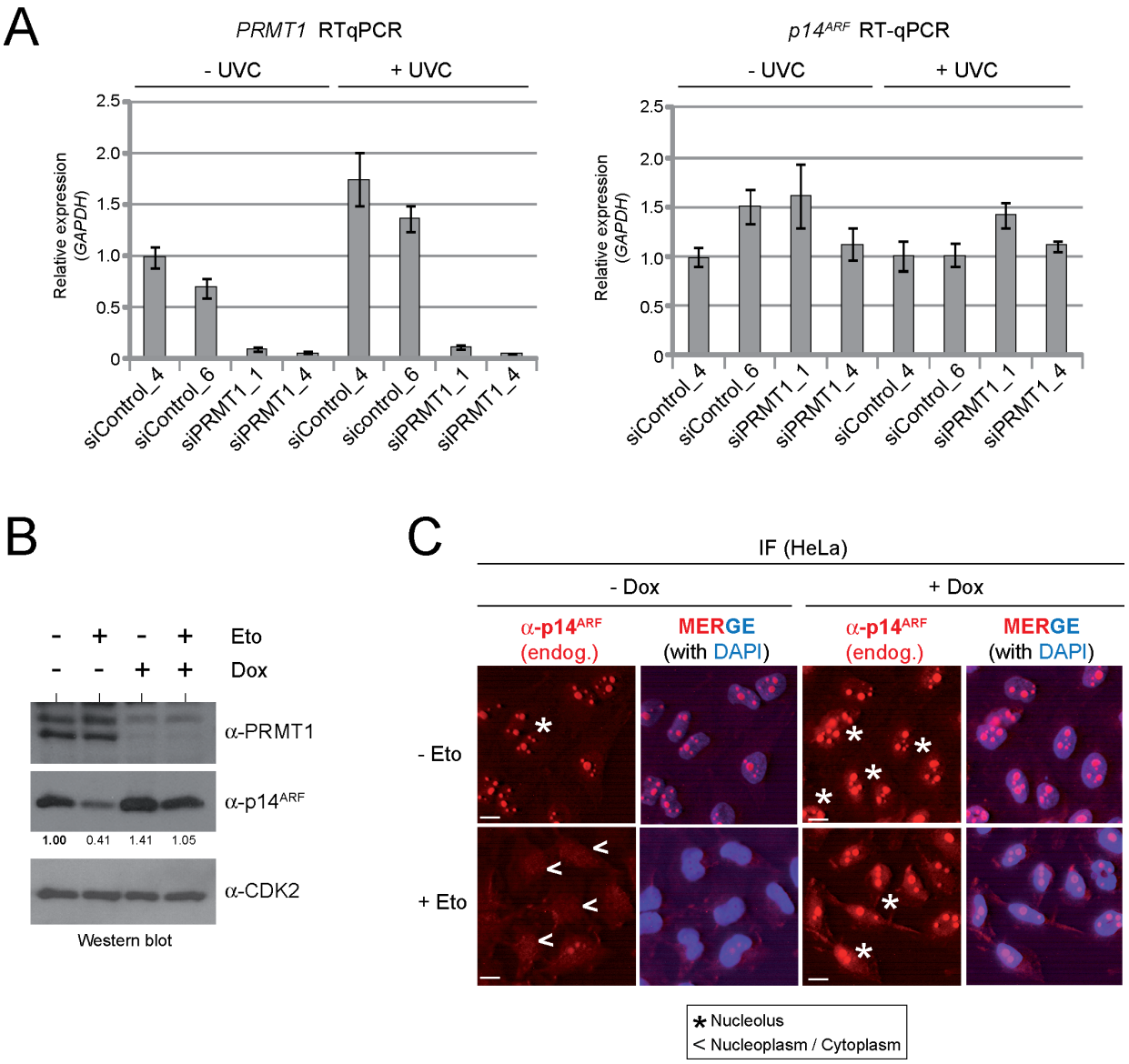
Transcript levels of exogenous wild type and mutant *p14ARF* as well as endogenous *p14ARF* upon PRMT1 depletion.

A: Full-length GST-tagged p14ARF wild type (wt) and mutant (RG, RF, RK) proteins were expressed in bacteria. Purified proteins were separated by SDS-PAGE and subsequently visualized either by Western blot analysis using α-p14ARF antibody (upper panel) or by Coomassie Blue staining (lower panel). The size marker (in kDa) is shown on the left.

B, C: HeLa and HEK293 cells were transfected with Flag-tagged wild type (wt) or mutant (RG, RF, RK) p14ARF-containing plasmids. Transcript levels of wt and mutant *p14ARF* in HeLa (**A**) and HEK293 cells (**B**) were determined by RT-qPCR. Values were normalized to *GAPDH* expression and presented relative to wt *p14ARF*, mean ± SD of triplicates.

D: HeLa cells were transfected with the indicated siRNAs. Transcript levels of endogenous wt *p14ARF* were determined by RT-qPCR. Values were normalized to *GAPDH* expression and presented relative to the siControl_1 condition, mean ± SD of triplicates.

Appendix Figure S4



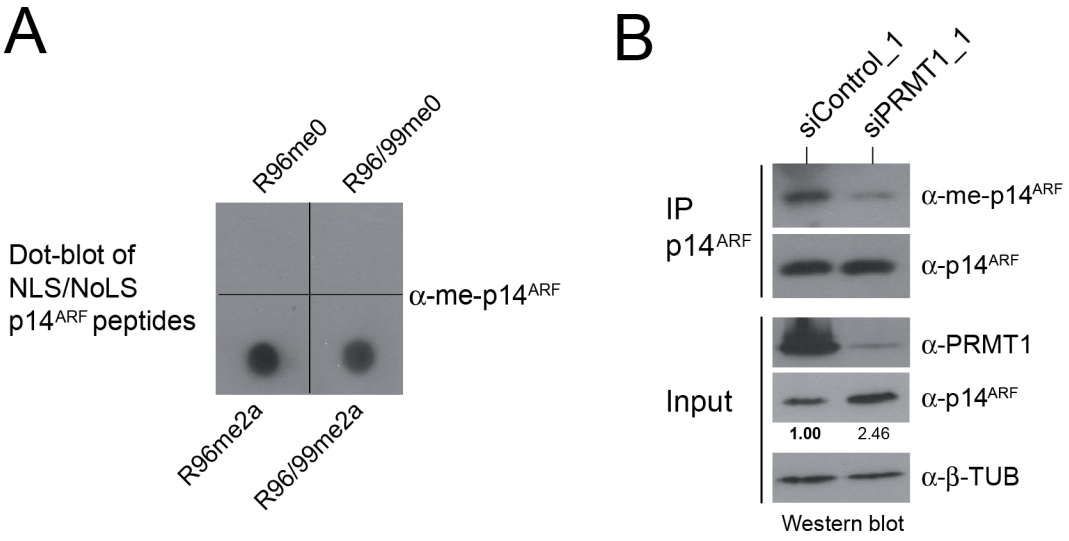
Appendix Figure S4

Impact of PRMT1 on stress-induced redistribution of p14^{ARF}.

A: HeLa cells were transfected with the indicated siRNAs (two control/non-targeting siRNAs and two PRMT1-specific siRNAs) and irradiated at 150 J/cm^2 UVC. After 24 hours, transcript levels of *PRMT1* (left panel) and *p14^{ARF}* (right panel) were determined by RT-qPCR. Values were normalized to *GAPDH* expression and presented relative to the siControl_4 condition, mean ± SD of triplicates.

B, C: HeLa cells expressing a doxycycline-inducible shRNA targeting PRMT1 were treated or not with doxycycline (Dox) for 6 days. Subsequently, cells were further treated with 20 µM etoposide (Eto) for 16 hours or left untreated. PRMT1 depletion and p14^{ARF} protein levels were analyzed by immunoblotting using the indicated antibodies (**B**). The cellular p14^{ARF} distribution was determined by immunofluorescence (IF) staining using α-p14^{ARF} (in red, endogenous p14^{ARF}) and in the merge additionally DAPI (in blue, nuclei/DNA). A representative IF result is shown (**C**), with asterisks indicating nucleolar and arrowheads indicating nucleo-/cytoplasmic p14^{ARF} localization. Scale bars: 15 µm.

Appendix Figure S5

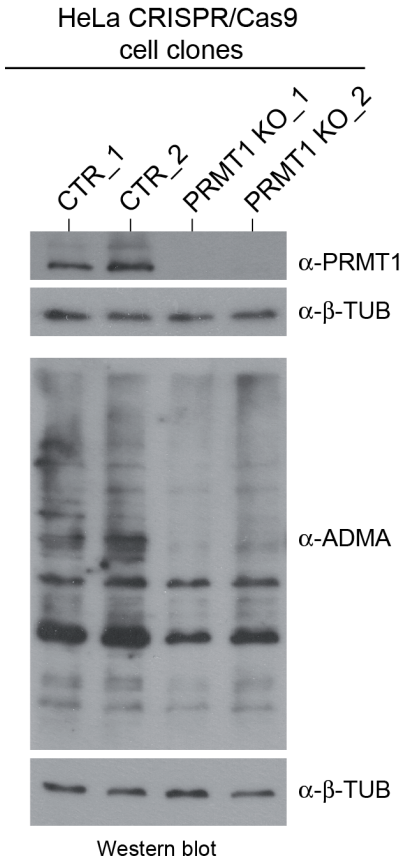


Appendix Figure S5

Characterization of the α-me-p14^{ARF} rat monoclonal antibody.

A: p14^{ARF} NLS/NoLS peptides encompassing aa 91-99 (unmodified=R96me0 or asymmetrically dimethylated=R96me2a) and aa 92-103 (unmodified=R96/99me0 or asymmetrically dimethylated=R96/99me2a) were spotted on nitrocellulose and stained with rat monoclonal α-me-p14^{ARF} antibodies.
B: H1299 cells were transfected with non-targeting siRNA (siControl_1) or PRMT1-specific siRNA (siPRMT1_1). Immunoprecipitation (IP) of endogenous p14^{ARF} was performed from cell lysates. IP reactions and input lysates were analyzed by immunoblotting using the indicated antibodies.

Appendix Figure S6

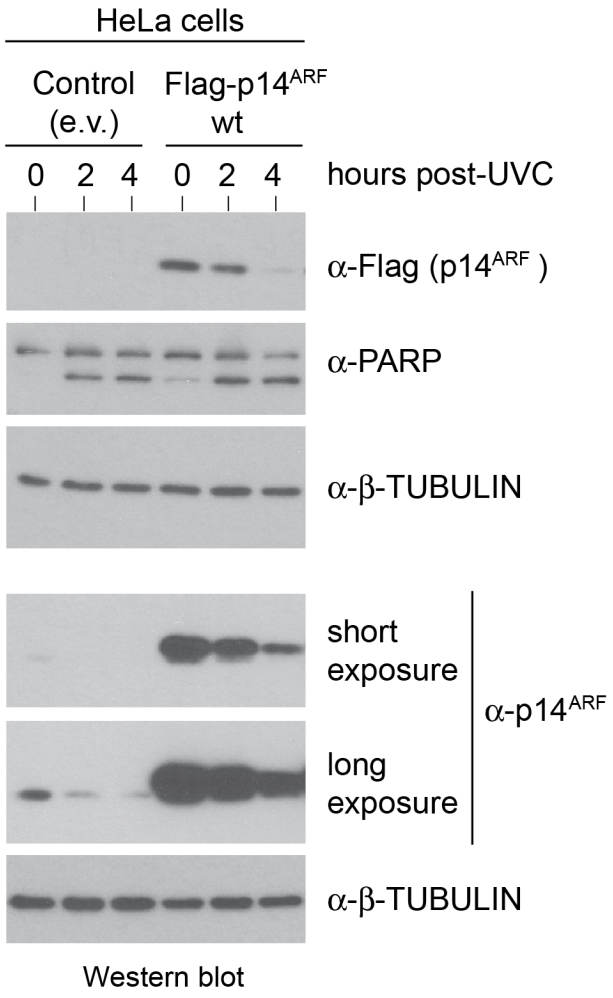


Appendix Figure S6

Characterization of the HeLa CRISPR/Cas9 cell clones.

CRISPR/Cas9-mediated control (gRNA directed against GFP) and PRMT1 deletion was established in HeLa cells. Protein extracts of the resulting CTR_1 and CTR_2 (CRISPR/Cas9 control) as well as PRMT1 KO_1 and KO_2 (CRISPR/Cas9-mediated PRMT1 deletion) cell clones were analyzed by immunoblotting for PRMT1 protein levels (α-PRMT1) and levels of asymmetric dimethylation of arginines in cellular proteins (α-ADMA). β-TUBULIN staining served as loading control.

Appendix Figure S7

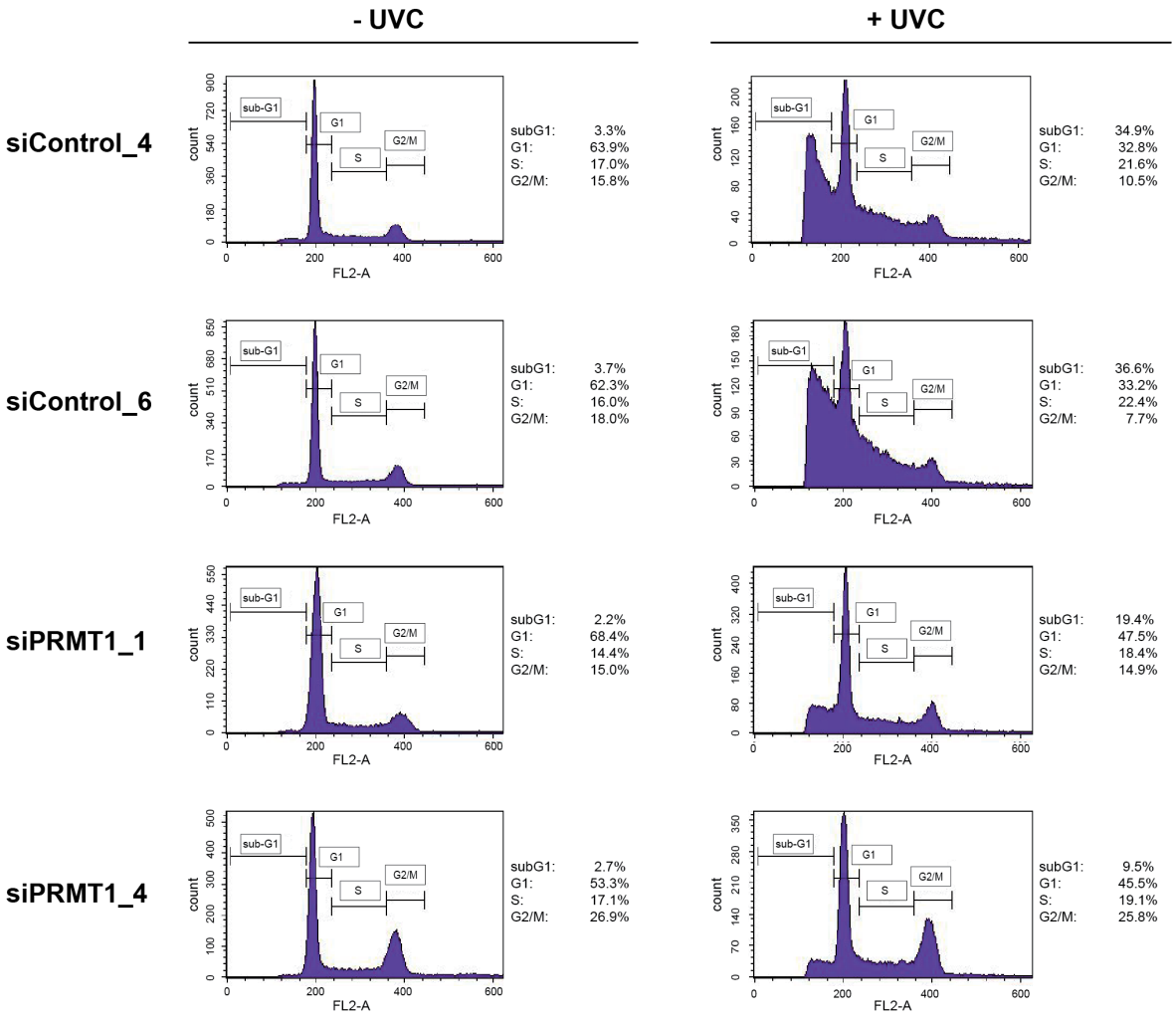


Appendix Figure S7

UVC-mediated reduction of the protein levels of exogenous Flag-tagged p14^{ARF}.

HeLa cells were transfected with empty vector (e.v., control) or Flag-tagged wild type (wt) p14^{ARF}-containing plasmids. Subsequently, cells were irradiated at 150 J/cm^2 UVC. After 0, 2 and 4 hours post-UVC, p14^{ARF} overexpression as well as endogenous p14^{ARF} levels were analyzed by immunoblotting using α-Flag (exogenous p14^{ARF} protein), α-p14^{ARF} (endogenous and exogenous p14^{ARF} protein), α-PARP (apoptosis marker) and α-β-TUBULIN (loading control) antibodies. For the α-p14^{ARF} stain, two exposure times are displayed.

Appendix Figure S8

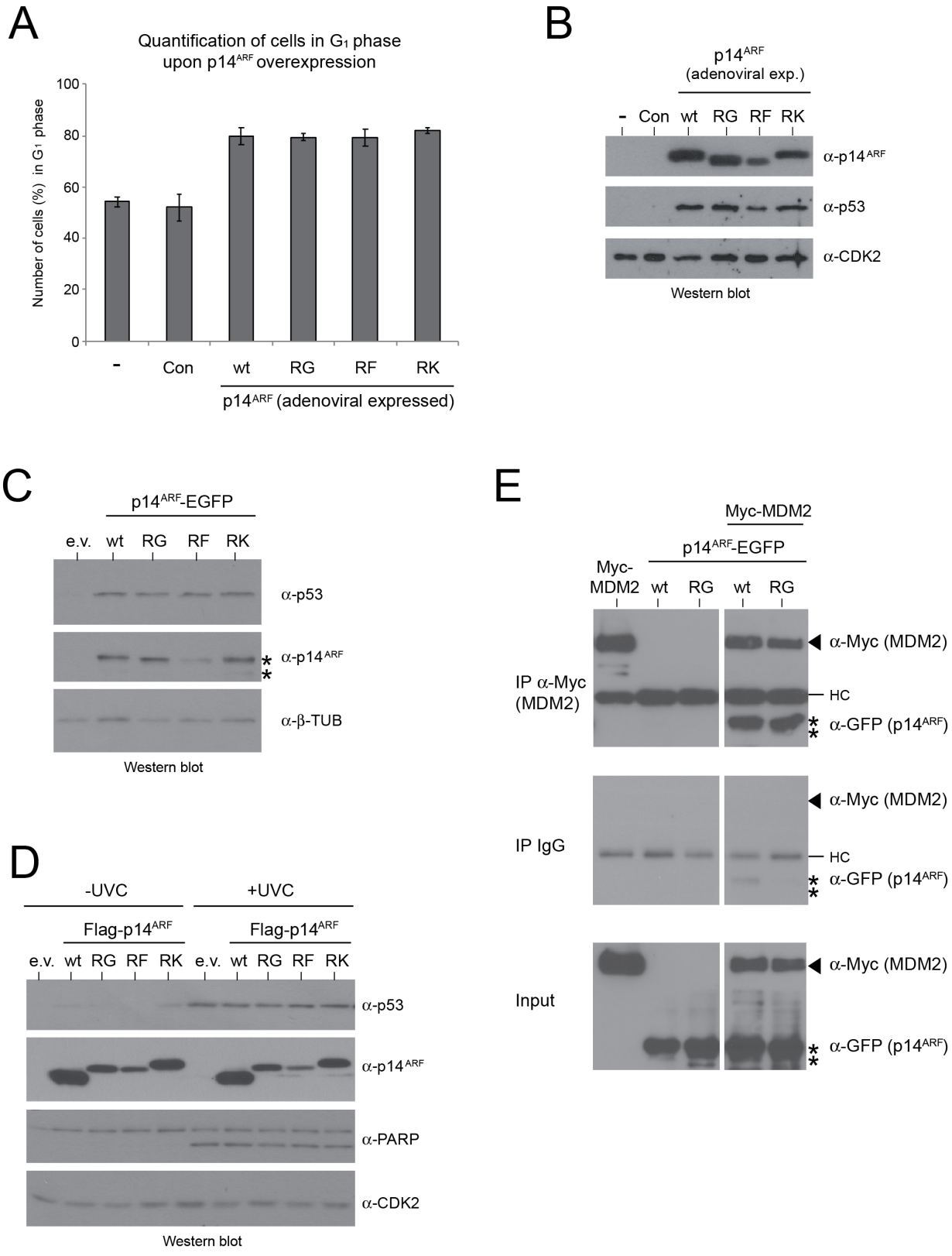


Appendix Figure S8

Representative cell cycle profile of HeLa cells upon PRMT1 knockdown and UVC irradiation.

HeLa cells were transfected with the indicated siRNAs (two control/non-targeting siRNAs and two PRMT1-specific siRNAs) and irradiated at 150 J/cm^2 UVC or not irradiated. After 24 hours, the distribution of cells undergoing apoptosis (subG1) and in various phases (G1, S, G2/M) of the cell cycle was determined by flow cytometry using propidium iodide (PI) DNA staining. A representative experiment is displayed here by histograms (primary data of Fig. 6A).

Appendix Figure S9

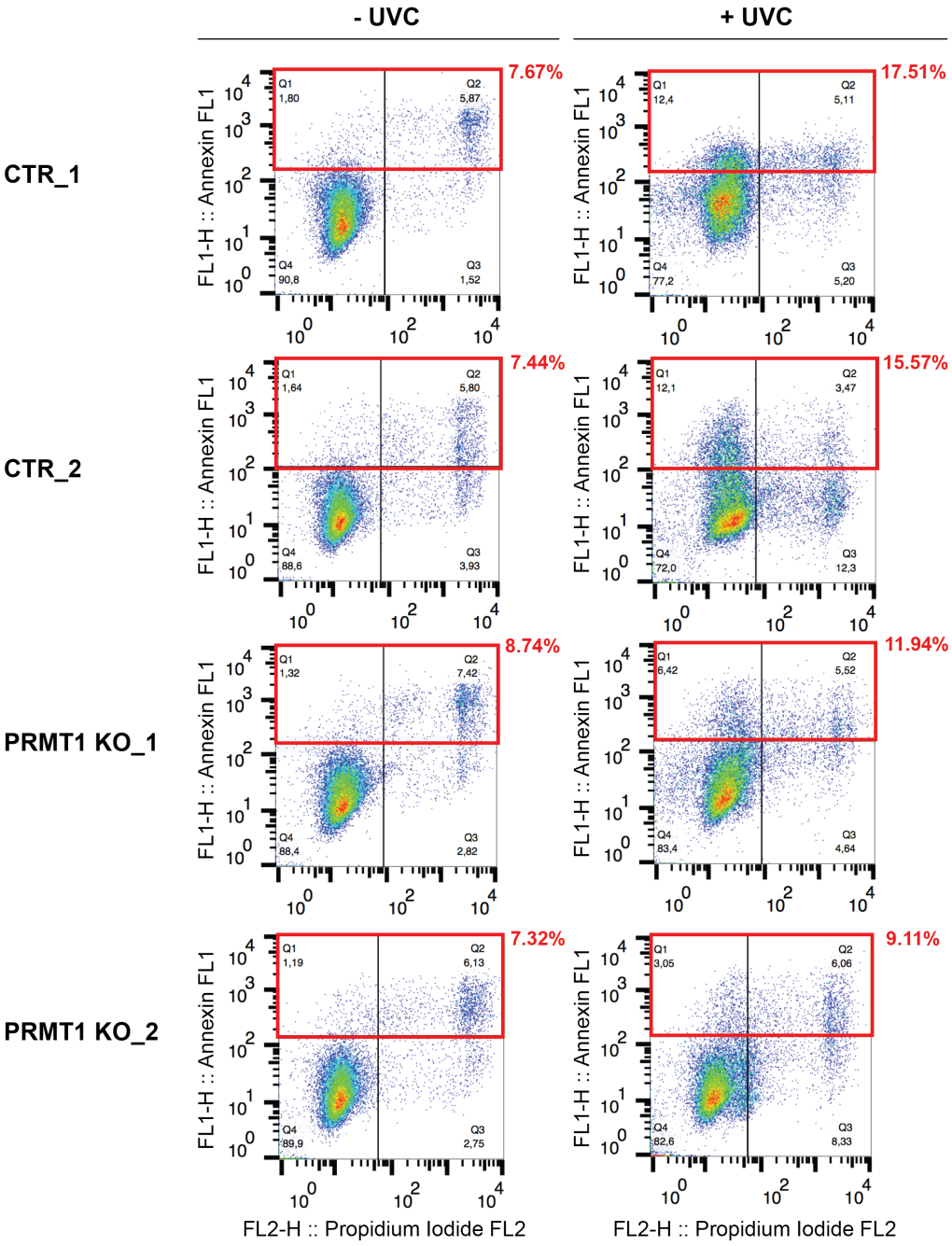


Appendix Figure S9

Influence of wild type and mutant p14^{ARF} proteins on cell cycle and p53 pathway.

A, B: U2OS cells were not infected (-) or infected with recombinant adenovirus encoding GFP (=Con, control), wild type (wt) and mutant p14^{ARF} proteins. Cell cycle distribution was analyzed by flow cytometry using propidium iodide (PI) DNA staining. The fraction of cells in G_1 phase was quantified for three independent experiments (**A)**. Overexpression of p14^{ARF} and endogenous p53 protein levels were monitored by immunoblotting using the indicated antibodies. CDK2 staining served as loading control (**B**).
C: MCF7 cells were transfected with empty vector (e.v.) or plasmids encoding C-terminally EGFP-tagged wild type (wt) and mutants p14^{ARF}. Overexpression of p14^{ARF} and endogenous p53 protein levels were monitored by immunoblotting using the indicated antibodies. The C-terminally EGFP-tagged p14^{ARF} proteins typically occurred in a doublet band, as indicated by the asterisks. β-TUBULIN staining served as loading control.
D: U2OS cells were transfected with plasmids encoding Flag-tagged wild type (wt) and mutant p14^{ARF} proteins. Cells were irradiated at 150 J/cm^2 UVC or not irradiated. After 7 hours, cell lysates were analyzed by immunoblotting for p14^{ARF} overexpression, p53 protein levels and PARP cleavage using the indicated antibodies. CDK2 staining served as loading control.
E: U2OS cells were transfected with plasmids encoding Myc-tagged MDM2 and EGFP-tagged wild type (wt) and mutant (6RG) p14^{ARF} proteins in the depicted combinations. Immunoprecipitations (IP) were performed from cell lysates using α-Myc antibody or IgG as negative control. IP reactions and input lysates were analyzed by immunoblotting using the indicated antibodies. Both left and right panels derive from the same blots and exposure times with the white line indicating where the blots were cut. Arrowheads highlight the expected location of MDM2; asterisks highlight the expected location of C-terminally EGFP-tagged p14^{ARF} (doublet band). HC indicates the signal derived from the antibody heavy chains.

Appendix Figure S10

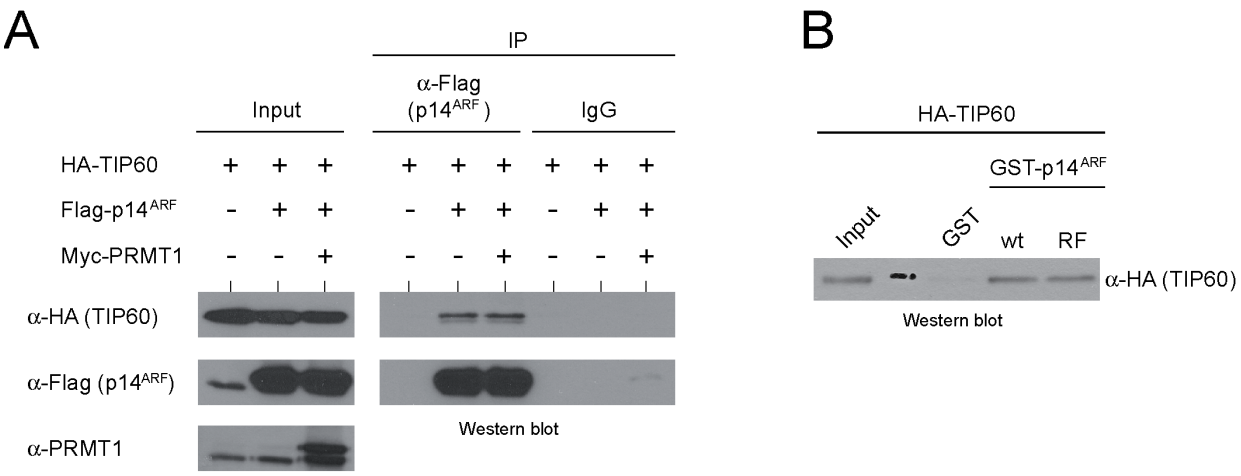


Appendix Figure S10

Representative FACS experiment of UVC-induced apoptosis in PRMT1 knockout in HeLa cells.

CRISPR/Cas9 control (CTR_1 and CTR_2) and PRMT1-deleted (KO_1 and KO_2) HeLa cell lines were irradiated at 150 J/cm^2 UVC or not irradiated. After 4 hours, the apoptotic cell fraction was determined by flow cytometry using FITC-labeled AnnexinV (AV) and propidium iodide (PI). A representative experiment is displayed here, which was integrated together with two further independent experiments in Fig. 6E. The viable cells are AV/PI double-negative (AV-/PI-, lower left quadrant, Q4), early apoptotic cells are AV single-positive (AV+/PI-, upper left quadrant, Q1), late apoptotic cells are AV/PI double-positive (AV+/PI+, upper right quadrant, Q2) and necrotic cells are PI single-positive (AV-/PI+, lower right quadrant, Q3). The sum of early and late apoptotic cell numbers is indicated in percentage (%).

Appendix Figure S11



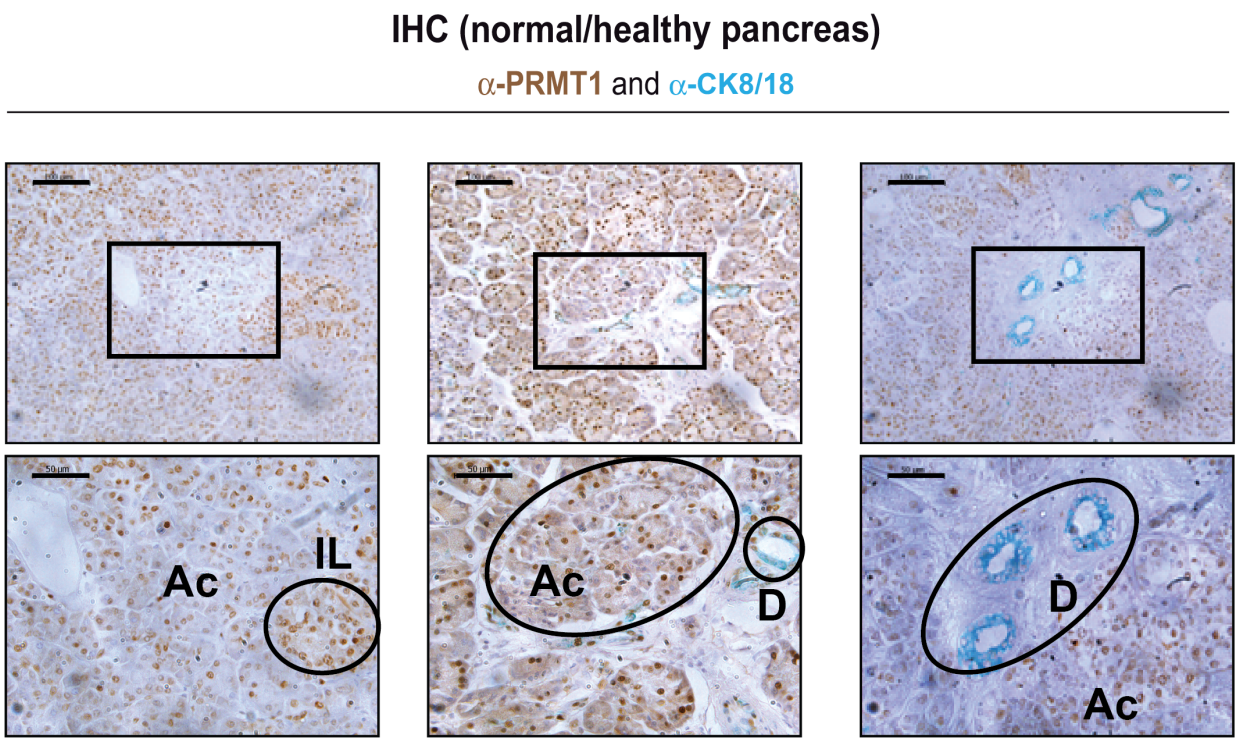
Appendix Figure S11

Influence of PRMT1 on p14^{ARF} and TIP60 interaction.

A: H1299 cells were transfected with plasmids encoding Myc-tagged PRMT1, Flag-tagged p14^{ARF} and HA-tagged TIP60 in the depicted combinations. Immunoprecipitations (IP) were performed from cell lysates using α-Flag antibody or IgG as negative control. IP reactions and input lysates were analyzed by immunoblotting using the indicated antibodies.

B: H1299 cells were transfected with a plasmid encoding HA-tagged TIP60. Cell lysates were incubated with recombinant GST alone or GST-tagged wild type (wt) as well as RF mutant p14^{ARF} proteins coupled to Glutathione beads. Pulldown reactions and input of HA-TIP60-overexpressing lysate were resolved by SDS-PAGE and analyzed by α-HA immunoblotting. Black line with dot indicates the 72 kDa marker band.

Appendix Figure S12

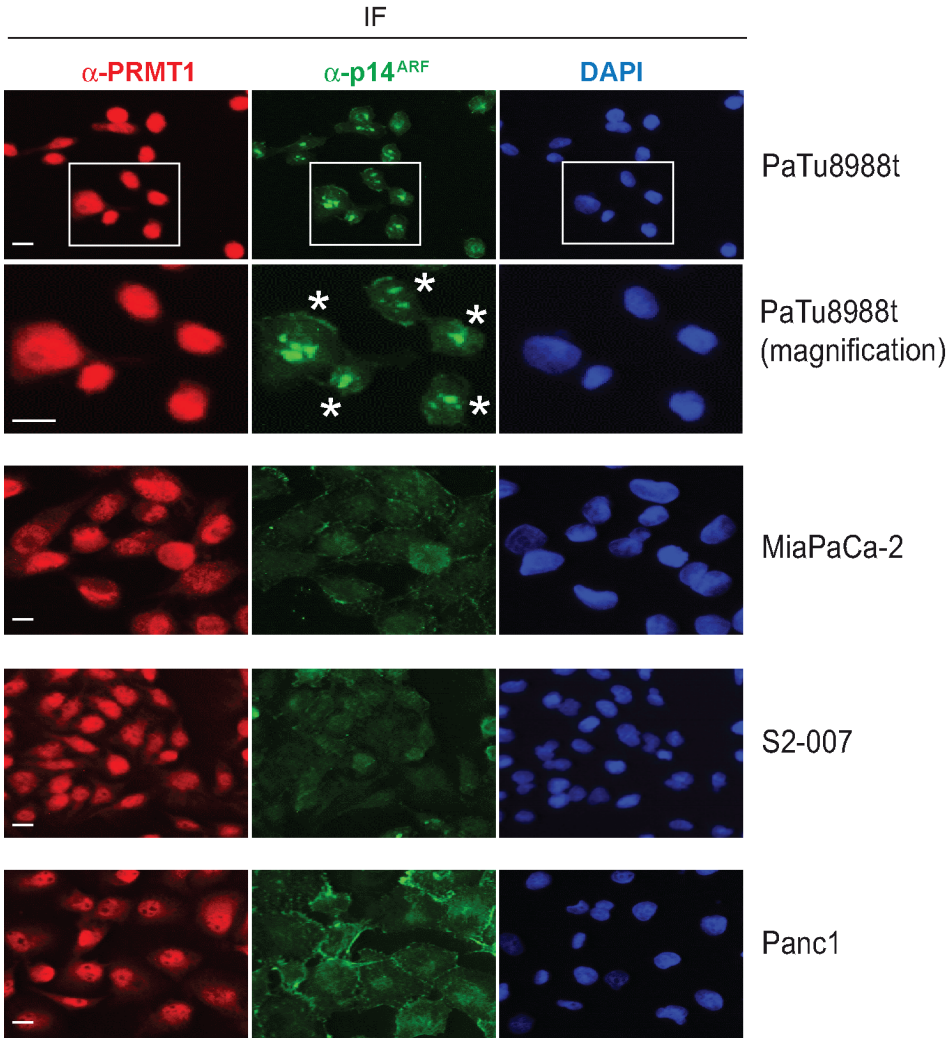


Appendix Figure S12

IHC stainings of PRMT1 in normal human pancreas.

Representative immunohistochemistry (IHC) stainings of PRMT1 (brown) and CK8/18 (blue, staining ductal cells of healthy pancreas) are shown for three cases of normal human pancreatic tissue. Upper and corresponding lower images show the same tissue section, with the lower images displaying a magnification as indicated in the upper images by the rectangles. Scale bars in the upper images: 100 µm. Scale bars in the lower images: 50 µm. Ac= acinar cells; D= ductal cells, IL= islet of Langerhans.

Appendix Figure S13

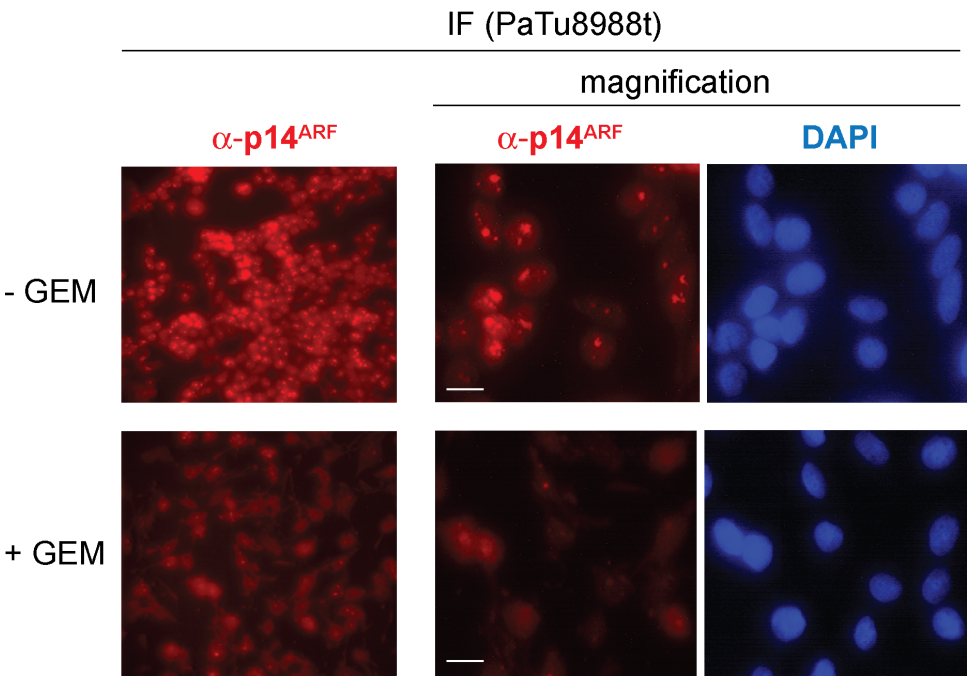


Appendix Figure S13

Cellular localization of p14^{ARF} in human pancreatic tumor cell lines.

Immunofluorescence (IF) staining of PaTu8988t (p14^{ARF}-proficient) as well as MiaPaCa-2, S2-007 and Panc1 (p14^{ARF}-deficient) cells was performed using α-PRMT1 (red, endogenous PRMT1), α-p14^{ARF} (green, endogenous p14^{ARF}) and DAPI (blue, nuclei/DNA). To visualize the predominant nucleolar localization of p14^{ARF} in PaTu8988t cells, a magnification (illustrated by the rectangles) is depicted, with the asterisks indicating the nucleolar p14^{ARF} localization. Representative IF images are shown. Scale bars: 15 µm.

Appendix Figure S14

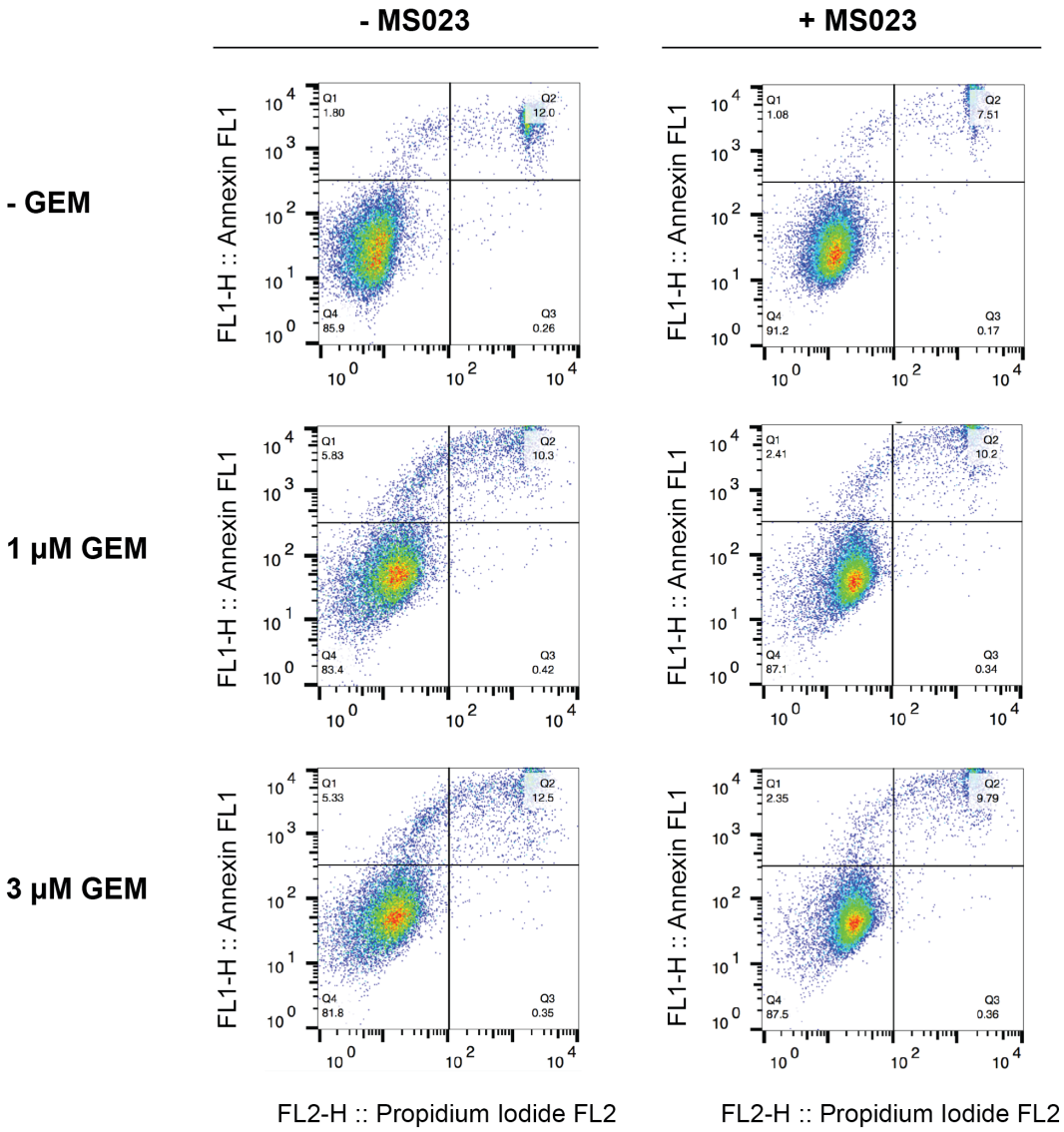


Appendix Figure S14

Gemcitabine-induced redistribution of p14^{ARF} out of the nucleoli in PaTu8988t cells.

PaTu8988t cells were treated with 3 μM gemcitabine (GEM) or left untreated. After 48 hours, cells were analyzed by immunofluorescence (IF) staining using α-p14^{ARF} (red, endogenous p14^{ARF}) and DAPI (blue, nuclei/DNA). Representative IF images are shown. Scale bars: 15 μm.

Appendix Figure S15

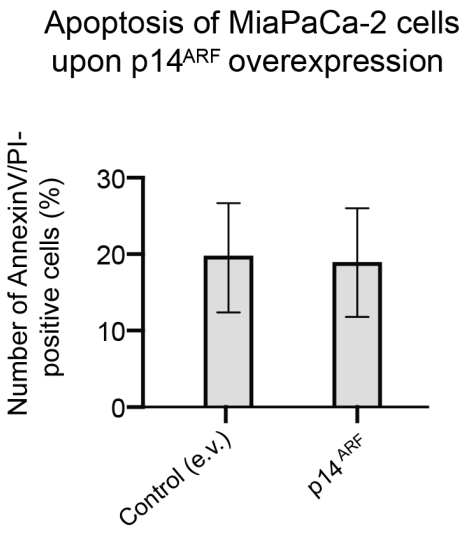


Appendix Figure S15

Representative FACS experiment of gemcitabine-induced cell death and PRMT inhibition in PaTu8988t cells.

PaTu8988t cells were treated (+) with 20 µM MS023 or left untreated (-) for 3 days. For the last 2 days, the cells were additionally exposed to 0, 1, or 3 µM gemcitabine (GEM). Subsequently, cell death was quantified by flow cytometry using FITC-labeled AnnexinV (AV) and propidium iodide (PI). A representative experiment is displayed here, which was integrated together with three further independent experiments in Fig. 8D. The viable cells are AV/PI double-negative (AV-/PI-, lower left quadrant, Q4), early apoptotic cells are AV single-positive (AV+/PI-, upper left quadrant, Q1), late apoptotic cells are AV/PI double-positive (AV+/PI+, upper right quadrant, Q2) and necrotic cells are PI single-positive (AV-/PI+, lower right quadrant, Q3).

Appendix Figure S16



Appendix Figure S16

Apoptosis of MiaPaCa-2 cells upon $p14^{ARF}$ overexpression.

MiaPaCa-2 cells were transfected with empty vector (e.v., control) or Flag-tagged wild type (wt) $p14^{ARF}$-containing plasmid. $p14^{ARF}$ overexpression was monitored by immunoblotting as depicted in the - GEM conditions of Fig. 8H. Apoptosis of unstressed cells was quantified by flow cytometry using FITC-labeled AnnexinV and propidium iodide (PI) for the same four independent experiments as in Fig. 8I (mean ± SD).

Appendix Supplementary Methods

Cell culture reagents

Human cell lines (HeLa, HEK293, HEK293T, U2OS, MCF7, H1299, PaTu8988t, MiaPaCa, S2-007, Panc1) were obtained from ATCC (American Type Culture Collection) and cultured according to ATCC's instructions. Insect cells (Sf9) were cultured in Sf-900™ II SFM medium (Gibco) and maintained in suspension at 26°C. For global methyltransferase inhibition, cells were treated with 20 µM adenosine dialdehyde (AdOx; Sigma-Aldrich). In order to induce DNA damage, cells were either irradiated at 150 J/m^2 UVC using the StrataLinker crosslinker (Stratagene) or treated with 20 µM etoposide (Sigma-Aldrich) or 1-10 µM gemcitabine (LC Laboratories). For PRMT inhibition, cells were treated with 20 µM of type I PRMT inhibitor MS023 hydrochloride (Cayman). For inducible shRNA expression, cells were treated with 1 µg/ml doxycycline (Sigma-Aldrich) for 6 days. For measurement of protein half-lives, cells were treated with 10 µM cycloheximide (AppliChem).

Plasmids

The following plasmids were used: pEGFP-N1-p14^{ARF}, which conveys a C-terminal EGFP-tag (Llanos et al., 2001), pcDNA-Flag-p14^{ARF} , which conveys a N-terminal Flag-tag (Addgene), pcDNA3.1-hPRMT1 wild type and mutant (Balint et al., 2005), pcDNA3.1-hPRMT6 (Hyllus et al., 2007), pcDNA3-HA-hPRMT5 (NM006109 was PCR-cloned via BamHI/XhoI into pcDNA3 with N-terminal double HA-tag), CMV-Myc-MEP50 (Licciardo et al., 2003), pcDNA3-Myc-MDM2 (Blattner et al., 2002), pcDNA3-HA-hTIP60 (Eymin et al., 2006), pGEX-GAR (Tang et al., 1998), pGEX-hPRMT1 (Scott et al., 1998), pGEX-mPRMT4 (Streubel et al., 2013), pFASTBAC-rPRMT1 and pFASTBAC-mPRMT4 (Naeem et al., 2007).

The complete ORF of p14^{ARF} (aa 1-132) and deletions (aa 1-64, aa 65-132, aa 31-132) were cloned via XhoI/HindIII sites into pGEX-2TK-P. For generation of p14^{ARF} mutants, site-directed mutagenesis PCR was performed using Ultra *Pfu* DNA polymerase (Stratagene/Agilent Technologies), pEGFP-N1-p14^{ARF} plasmid as template and primers, which harbor the specific mutation(s) and lead to alteration of a SmaI site. After PCR, the template was digested using DpnI (NEB) and the PCR products were transformed into E.coli DH5alpha. The complete ORFs of hPRMT5 (via BamHI/XhoI), hNPM (via EcoRI/PstI) and hp32 (via BamHI/XhoI) were inserted into the pFASTBAC HT-3xFlag B vector (Invitrogen).

For cloning of the doxycycline-inducible shPRMT1 lentiviral expression plasmid (pInducer10-shPRMT1), shRNA-mir sequence including shPRMT1 (5'-TGGAAGCAGACGGTGTTCT-3') was cloned via EcoRI/XhoI sites from pGIPZ vectors (Open Biosystems) into pInducer10 vector (Meerbrey et al., 2011).

For design of gRNA (guide RNA), a web-based tool (http://crispr.mit.edu) was used and the following target sites in human *PRMT1* or GFP (as control) were chosen:

gPRMT1_1.3 (minus strand): 5'-CACCTCCATGATGCAGTTCG-3'
gPRMT1_1.4 (plus strand): 5'-GGCCGCGAACTGCATCATGG-3'
gPRMT1_3.1 (minus strand): 5'-GGATGTCATGTCCTCAGCGT-3'
gPRMT1_3.2 (minus strand): 5'-GTGGATGCCAAAGTGTGCGT-3'
Control_gRNA (GFP): 5'-GGAGCGCACCATCTTCTTCA-3'

Pairs of oligos for these targeting sites (including the PAM sequence) were annealed and cloned into BsmBI-restricted lentiCRISPRv1 plasmid (Addgene), which allows bicistronic expression of Cas9 nuclease and sgRNA (single guide RNA)(Shalem et al., 2014).

Transfections and infections

Transient transfection of plasmids was performed using the calcium phosphate method or Fugene HD (Roche Diagnostics) according to manufacturer´s instructions. Short interfering RNA (siRNA) oligonucleotide duplexes were obtained from Eurogentec or Dharmacon. For transient transfection of siRNAs, Lipofectamine RNAiMax (Invitrogen) was used according to manufacturer´s instructions. The sense strands of the siRNA sequences are indicated below:

Name	Target gene	Species	Sequence (5'-3')
siControl_1	*GFP*	jellyfish	GCAAGCUGACCCUGAAGUU
siControl_2	*Luciferase*	firefly	GAUUAUGUCCGGUUAUGUA
siControl_3	non-targeting		CAUAAGCUGAGAUACUUCA
siControl_4	non-targeting		UAAGGCUAUGAAGAGAUAC

siControl_5	non-targeting		AUGAACGUGAAUUGCUCAA
siControl_6	non-targeting		UGGUUUACAUGUCGACUAA
siPRMT1_1	*PRMT1*	human	CGUGUAUGGCUUCGACAUG
siPRMT1_2	*PRMT1*	human	UCAAAGAUGUGGCCAUUAA
siPRMT1_3	*PRMT1*	human	GCAACUCCAUGUUUCAUAA
siPRMT1_4	*PRMT1*	human	GGUCAUCGGGAUCGAGUGU
siARF_1	$p14^{ARF}$	human	AAUCCGGAGGGUCACCAAGAACCUG
siARF_2	$p14^{ARF}$	human	AUCAGCACGAGGGCCACAGCGGCGG

For production of recombinant lentivirus, HEK293T cells were transfected with the lentiviral expression plasmids and the two packaging plasmids pMD2.G and psPAX2 using XtremeGene HD (Roche Diagnostics). Supernatants containing viral particles were harvested 2 days after transfection and filtered. HeLa cells were infected with virus upon addition of 8 µg/ml polybrene (Sigma-Aldrich) and subsequently selected with 2 µg/ml puromycin (Invivogen). For CRISPR/Cas9 infections, HeLa cells were infected with viruses encoding either the combination of four *PRMT1* gRNAs or the GFP control gRNA (for CTR). Cell pools were selected using 1µg/ml puromycin and subjected to single cell cloning to generate PRMT1 KO cell clones (KO_1, KO_2) and control cell clones (CTR_1, CTR_2).
For production of recombinant adenovirus, the AdEasy™ adenoviral vector system (Stratagene) was employed according to the manufacturer's instructions. ORFs of $p14^{ARF}$ (wt and mutant forms) and hPRMT1 were cloned into the pAdTrack CMV shuttle vector and recombined into pAdEasy-1 plasmid using E.coli BJ5183. Recombinant pAdEsay-1 plasmids were transfected in HEK293 cells for virus production. Recombinant adenovirus was harvested upon cell lysis and used for infection of U2OS cells.
Recombinant baculovirus (bacmid) was generated according to the Bac-to-Bac baculovirus system (Invitrogen). Sf9 cells were then transfected with bacmid DNA using X-tremeGENE HD transfection reagent (Sigma-Aldrich) according to the manufacturer´s instructions. After 3-5 days, supernatants were collected, filtered and used to infect Sf9 cells to amplify the virus. After two rounds of virus amplification and estimation of the final virus titer, 20 x 10^6 Sf9 insect cells per 15 cm dish were infected with 7.5 µl virus/10^6 cells.

Antibodies
The following primary antibodies were used:

Target	Antibody ID	Species	Source
ADMA	13522	rabbit	Cell Signaling Technology
β-TUBULIN	MAB3408	mouse	Millipore
CDK2	sc-163	rabbit	Santa Cruz
CK8/18	sc-52325	mouse	Santa Cruz
F-actin, Acti-stain ™ 670	PHDN1	-	Cytoskeleton
Flag	M1	mouse	Sigma-Aldrich
GAPDH	FL-335	rabbit	Santa Cruz
GFP	G6539	mouse	Sigma-Aldrich
GST	sc-138	mouse	Santa Cruz
IgG	I5381	mouse	Sigma-Aldrich
IgG	I5006	rabbit	Sigma-Aldrich
Myc	9E10	mouse	hybridoma supernatant
Myc	9B11	mouse	Cell Signaling Technology
Myc-FITC	SH1-26E7.1.3	mouse	Miltenyi Biotec
NPM	ab10530	mouse	Abcam
$p14^{ARF}$	NB200-111	rabbit	Novus
$p14^{ARF}$	A300-342A	rabbit	Bethyl
$p14^{ARF}$	P2610	mouse	Sigma-Aldrich
p32/C1QBP	sc-23884	mouse	Santa Cruz
p32/C1QBP	sc-271200	mouse	Santa Cruz
p53 (human)	DOI-1	mouse	hybridoma supernatant
p53 (murine/human)	FL-393	rabbit	Santa Cruz
PARP	9542	rabbit	Cell Signaling
PRMT1	07-404	rabbit	Millipore
PRMT1	ab3768	rabbit	Abcam

The following secondary antibodies for Western blot, immunofluorescence and immunohistochemistry stainings were used:

Target	Antibody ID	Species	Source
rabbit IgG, HRP-coupled	NA934	donkey	Amersham Biosciences
mouse IgG, HRP-coupled	NXA931	sheep	Amersham Biosciences
rabbit IgG, IRDey680	926-68071	goat	LI-COR
mouse IgG, IRDey800	926-32210	goat	LI-COR
mouse IgG, Alexa 568-coupled	A10037	donkey	Invitrogen
rabbit IgG, Cy3-coupled	111-165-045	goat	Jackson Immuno Research
mouse IgG, DyLight 488-coupled	115-545-062	goat	Jackson Immuno Research

For the generation of α-me-p14^{ARF} antibodies, a peptide encompassing aa 92-103 of p14^{ARF} with asymmetrically dimethylated arginines R96 and R99 (NH3-SHPTR(me2)ARR(me2)CPGG-COOH) was coupled to ovalbumin for immunization. Lou/c rats were immunized subcutaneously (s.c.) and intraperitoneally (i.p.) with a mixture of 40 µg peptide, 5 nmol CpG (TIB Molbiol) and an equal volume of incomplete Freund's adjuvant (Sigma-Aldrich). After 6 weeks, a boost without adjuvant was given i.p. and s.c. 3 days before fusion. Fusion of the myeloma cell line P3X63-Ag8.653 with the immune splenic cells was performed according to the standard procedure described by Koehler and Milstein (Köhler and Milstein, 1975). Hybridoma supernatants were screened in an enzyme-linked immunoassay on biotinylated dimethylated (R96/99me2a) and unmethylated/unmodified p14^{ARF} peptides. Supernatants positively tested on dimethylated peptides were further validated by dot blot analysis using p14^{ARF} peptides either unmodified (aa 91-99 or aa 92-103) or asymmetrically dimethylated at R96 alone (aa 91-99) or at both R96/R99 (aa 92-103). The hybridoma cells producing p14^{ARF} R96/R99me2a-reactive supernatants were cloned twice by limiting dilution. Experiments in this study were performed with the α-me-p14^{ARF} (=α-p14^{ARF} R96me2a and potentially R99me2a) hybridoma culture supernatant clone 3G11 (IgG_{G1}/κ).

Protein extraction, SDS-PAGE and immunoblotting

For protein extraction, human cells were lysed in IPH buffer (50 mM Tris/HCl pH 8.0; 5 mM EDTA; 0.5% (w/v) NP-40; 150-400 mM NaCl) or RIPA buffer (50 mM Tris/HCl pH 8.0; 0.1% (w/v) SDS; 0.5% (w/v) sodium deoxycholate; 1% (w/v) NP-40; 150 mM NaCl) containing protease inhibitors (leupeptin, aprotinin, PMSF; Sigma-Aldrich) and centrifuged. Protein concentration of the supernatant was measured using Bradford reagent and BSA (Sigma-Aldrich) as a standard. For immunoblotting, usually 10 – 50 µg protein was loaded onto a 7.5 – 15% SDS-polyacrylamide gel, separated via electrophoresis and transferred onto a PVDF membrane (Amersham). Membranes were blocked in TBS/0.1% Tween/4% milk powder, incubated with primary antibody for several hours to overnight, washed and incubated with HRP-coupled secondary antibodies. Antibody binding was detected using chemiluminescence reaction (Millipore) and X-ray films (Kodak). Quantification of protein bands was performed using the software program Fiji ImageJ. We observed the following migration peculiarities of the different p14^{ARF} proteins in SDS-PAGE: EGFP epitope-tagged p14^{ARF} typically migrated as a doublet band (of approx. 40 and 42 kDa molecular weight). Endogenous and Flag epitope-tagged p14^{ARF} migrated as single protein bands. Flag epitope-tagged p14^{ARF} always showed a clear migration difference between wild type and mutant p14^{ARF} proteins, with the mutants (especially the RK mutant) migrating slower than the wild type protein.

Immunoprecipitation

Proteins were extracted using IPH buffer with 150 mM NaCl. Usually, 1-2 mg protein were incubated with 1-6 µg antibody in 1 ml IPH buffer overnight at 4°C. Then precipitates were bound to BSA-blocked Protein A- or Protein G-Sepharose (GE Healthcare) and analyzed together with 1-5% input lysate by SDS-PAGE followed by immunoblotting.

Protein stability analysis

The protein stability of p14^{ARF} was determined by cycloheximide chase assay. HeLa cells expressing doxycycline inducible shPRMT1 were cultured in the absence or presence of doxycycline (1 µg/ml) for 6 days. Subsequently, cells were treated with the protein biosynthesis inhibitor cycloheximide (10 µM) and harvested within a time course of up to 6 hours chase. Protein lysates were prepared in IPH buffer and analyzed by SDS-PAGE followed by

immunoblotting. For quantitative Western blot analysis, membranes were stained with secondary antibodies linked to fluorescence dyes and then scanned in the LI-COR Odyssey Classic infrared imager to enable normalization of the $p14^{ARF}$ protein levels to the housekeeping protein β-TUBULIN.

RNA isolation and reverse transcription quantitative PCR (RT-qPCR)
Total RNA was isolated using the RNA-Mini kit (Seqlab) according to the manufacturer´s instructions including a DNAse I (PeqLab) digestion step. For cDNA synthesis, 0.1-1 µg of total RNA was reversed transcribed using random hexamer oligonucleotides and M-MLV reverse transcriptase (Thermo Scientific) according to the manufacturer's instruction. cDNA was subjected to PCR amplification using the listed primers, ABsolute ™QPCR SYBR®Green Mix (Life Technologies) and the MX-3000 PCR machine (Stratagene/Agilent). Data was analyzed using the ΔΔCT method comparing the gene of interest to the housekeeping gene *GAPDH*.

Forward (fwd) and reverse (rev) primers used for RT-qPCR of indicated gene transcripts:

Name	Sequence (5'-3')
*p14^{ARF}*_RT_fwd	CTACTGAGGAGCCAGCGTCT
*p14^{ARF}*_RT_rev	CTGCCCATCATCATGACCT
*hPRMT1*_RT_fwd	CCCCAAACAGCTGGTCAC
*hPRMT1*_RT_rev	GGTCTTCCACCTTGACGGTA
*hGAPDH*_RT_fwd	AGCCACATCGCTCAGACAC
*hGAPDH*_RT_rev	GCCCAATACGACCAAATCC

Recombinant proteins from bacteria or Sf9 cells
Recombinant GST-fusion proteins encoded by pGEX-2TK plasmids were expressed in E.coli BL21. Expression was induced with 1 mM IPTG for 3 hours. Cells were harvested, lysed in PBS containing 1% Triton X-100 and protease inhibitors (aprotinin, leupeptin; PMSF; Sigma-Aldrich). Lysates were sonicated and centrifuged. The supernatant was incubated with glutathione-agarose beads (Machery and Nagel) overnight at 4°C. After washing, bead-bound proteins were stored in PBS with 10% glycerol. Alternatively, proteins were eluted (50 mM Tris/HCl pH 8; 25 mM glutathione) in and dialyzed (Slide-A-Lyzer Mini Dialysis Units; Thermo Fisher) in PBS containing 10% glycerol.
For recombinant protein expression (Flag-PRMT1, Flag-PRMT4, Flag-PRMT5, Flag-NPM, Flag-p32) in Sf9 insect cells, cells infected for 3-5 days with recombinant baculovirus were harvested and lysed in BC buffer (20 mM HEPES pH 7.9; 150 mM NaCl; 10% glycerol; 0.4 mM EDTA). After centrifugation, the supernatant (whole cell protein extract) was incubated with α-Flag affinity gel (Sigma-Aldrich). Flag-tagged proteins were eluted from beads using 3xFlag peptides (0.25 mg/ml; Sigma-Aldrich). Expression, purification, elution and concentration of the respective proteins were estimated by SDS-PAGE followed by Coomassie staining (Coomassie Brilliant Blue G250; Fluka) or immunoblotting.

GST-pulldown assay
Approximately 0.2-0.5 µg of bead-bound GST-fusion protein or GST alone purified from bacteria was incubated with 0.1 µg of eluted Flag-tagged protein purified from Sf9 cells. Pulldown reactions were performed in BC buffer (20 mM HEPES pH 7.9; 150 mM NaCl; 10% glycerol; 0.4 mM EDTA) including protease inhibitors for 4 hours at 4°C under rotation. After extensive washes (six times) in BC buffer containing 200 mM NaCl, bound proteins were analyzed by SDS-PAGE followed by immunoblotting.

Peptide pulldown assay and dot blot analysis
The following unmodified and modified $p14^{ARF}$ NLS/LoLS peptides were used:

Name	Length (aa – aa)	C-terminus	Source
NLS/NoLS unmodified	91-99	Cysteine	Peptide Specialty Laboratories
NLS/NoLS R96me2a	91-99	Cysteine	Peptide Specialty Laboratories
NLS/NoLS unmodified	92-103	Cysteine	Peptide Specialty Laboratories
NLS/NoLS R96/R99me2a	92-103	Cysteine	Peptide Specialty Laboratories

Peptides were coupled via their C-terminal cysteine residue to SulfoLink resin (Pierce) according to the manufacturer´s instructions. In brief, 100 µl SulfoLink resin was washed with 1 ml coupling buffer (50 mM Tris pH 8.5; 5 mM EDTA) and incubated with 50 µg peptide in 1 ml coupling buffer for 30 min at RT under rotation and subsequently for 1 hour without rotation. Resin was washed four times in coupling buffer, blocked with 50 mM L-cysteine in 1 ml coupling buffer for 30 min under rotation and 45 min without rotation. After extensive washes in 1 M NaCl (six times) and subsequently A. bidest. (four times), the resin-coupled peptides were stored as a 50% slurry in 20% ethanol. For peptide pulldowns, 10 µg of coupled peptides were incubated with 0.5 µg recombinant Flag-tagged protein (purified from Sf9 cells) in BC buffer additionally containing 0.5% (w/v) NP-40 and protease inhibitors for 2 hours at 4°C under rotation. After extensive washes (six times) in BC buffer containing 200 mM NaCl and 0.5% (w/v) NP-40, bound proteins were analyzed by SDS-PAGE followed by immunoblotting.

Mass Spectrometry

Subsequent to *in vitro*-MT assay of $p14^{ARF}$ by PRMT1, assay reactions were separated by SDS-PAGE and peptides were in-gel reduced and alkylated with DTT and vinylpyridine, respectively. Enzymatic digestions with either trypsin, chymotrypsin or pepsin were performed as previously described (Lange et al., 2010; Schlosser et al., 2005; Thaa et al., 2017)

Peptides were analyzed by a reversed-phase capillary liquid chromatography system (Ultimate 3000 nanoLC system, Thermo Scientific) connected to an Orbitrap Elite mass spectrometer (Thermo Scientific). LC separations were performed on a capillary column (Acclaim Pep- Map100, C18, 3 µm, 100 Å, 75 µm i.d. × 25 cm, Thermo Scientific) at an eluent flow rate of 200 nL/min using a linear gradient of 3–50% B in 85 min. Mobile phase A contained 0.1% formic acid in water, and mobile phase B contained 0.1% formic acid in acetonitrile. Mass spectra were acquired in a data-dependent mode with one MS survey scan with a resolution of 60,000 (Orbitrap Elite) and MS/MS scans of the 15 most intense precursor ions in the linear trap quadrupole.

The processed MS data were analyzed on a MASCOT server (version 2.2.2, Matrix Science Ltd., London) and searched in-house against the SwissProt database (version 2010_10; 521,016 sequences). The mass tolerance of precursor and sequence ions was set to 10 ppm and 0.35 Da, respectively. A maximum of two and five missed cleavages was allowed for trypsin and chymotrypsin, respectively. Mono- and dimethyl modification of lysine and arginine, methionine oxidation, propionamide and pyridylethyl modifications of cysteine were used as variable modifications. MS/MS spectra of methylated peptides were manually verified and compared with the theoretical fragment ion spectra.

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