

Figure S1.

HCT116 cells were treated with 2 μ g/ml of ADR for 2 h. At 36 h after treatment, cells were harvested. Whole cell extracts were subjected to immunoblotting with anti-modified citrulline (MC) or anti-p53 antibody.

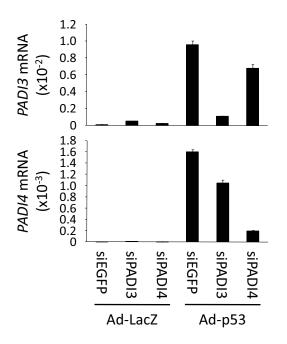


Figure S2.

At 6 h after transfection with each siRNA, U373MG cells were infected with Ad-p53 or Ad-LacZ. At 36 h after infection, mRNA levels of PADI3 (upper) and PADI4 (lower) were analyzed by qPCR analysis. Relative expression was shown with standard deviations (n=2).

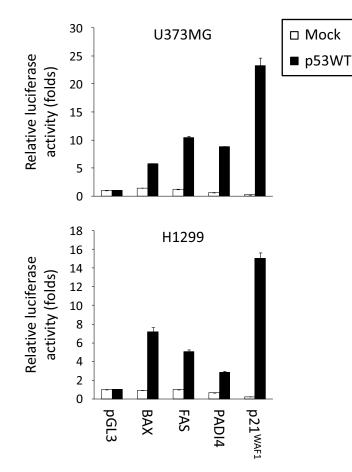


Figure S3.

Results of luciferase assay of p53BS-AB (PADI4) are shown. Luciferase activity is indicated relative to the activity of mock vector with standard deviations (n=2). Reporter plasmids which contain p53-responsive elements of BAX, FAS, and p21^{WAF1} were used as positive controls.

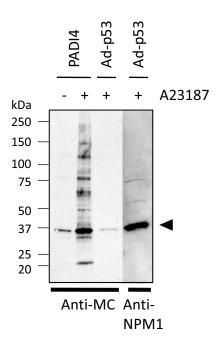


Figure S4.

HEK293T cells were transfected with plasmid expressing HA-PADI4 (lane 1 and 2). U373MG cells were infected with Ad-p53 at 20 MOI (lane 3 and 4). At 48 h after treatment, cells were cultured with or without 5 μ M of A23187 for 1 h, followed by immunoblotting with anti-MC or anti-NPM1 antibody. Arrowhead on the right indicates the band corresponding to NPM1 protein.

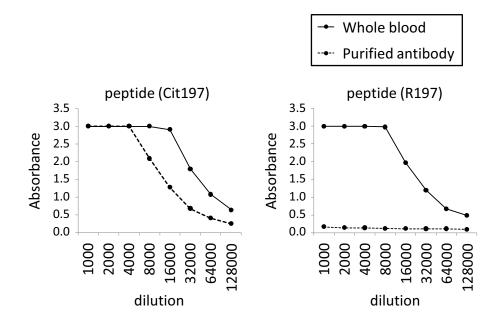


Figure S5.

Generation of antibody against citrulinated NPM1 at arginine 197 residue. Antibody titers and specificity were determined by enzyme-linked immunosorbent assay (ELISA) using peptide Cit197 that was used for immunization and peptide Arg 197 that was used for adsorption.



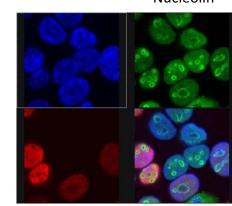
С

Myc-NPM

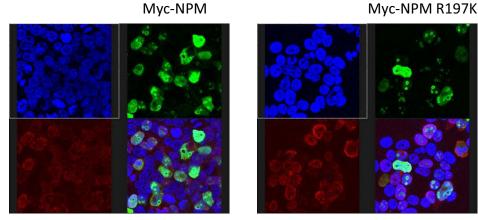
p53

В

Nucleolin



HA-PADI4



HA-PADI4

HA-PADI4

Figure S6.

A, Subcellular distribution of NPM1 proteins was examined by immunocytochemistry. At 36 h after cotransfection of plasmid expressing myc-NPM1 with p53, H1299 cells were fixed and stained with anit-myc antibody (Alexa fluor 488) and anti-p53 antibody (Alexa fluor 594). B, Subcellular distribution of Nucleolin proteins was examined by immunocytochemistry. At 36 h after transfection with plasmid expressing HA-PADI4, HEK293T cells were fixed and stained with anit-nucleolin antibody (Alexa fluor 488) and anti-HA antibody (Alexa fluor 594). C, Subcellular distribution of NPM1 proteins was examined by immunocytochemistry. At 36 h after co-transfection of plasmid expressing myc-NPM1 or myc-NPM R197K with HA-PADI4, HEK293T cells were fixed and stained with anit-myc antibody (Alexa fluor 488) and anti-HA antibody (Alexa fluor 594).

Supplementary Table S1

Sequences of primers and RNA nucleotides

Site-directed mutagenesis	Forward	Reverse
PADI4D350A	GACCAGTGGATGCAGGCTGAAATGGAGATCGGC	GCCGATCTCCATTTCAGCCTGCATCCACTGGTC
PADI4D473A	TCCGTGGGCCACGTGGCCGAGTTCCTGAGCTTT	AAAGCTCAGGAACTCGGCCACGTGGCCCACGGA
siRNA oligonucleotides	Sense	Antisense
siNPM1	GGACAAGAAUCCUUCAAGATT	UCUUGAAGGAUUCUUGUCCTT
siPADI3	CCACAAACUUGUCCUCCAUTT	AUGGAGGACAAGUUUGUGGTT
siPADI4	GGUCCUGCUACAAACUGUUTT	AACAGUUUGUAGCAGGACCTT
sip53	GACUCCAGUGGUAAUCUACTT	GUAGAUUACCACUGGAGUCTT
siEGFP	GCAGCACGACUUCUUCAAGTT	CUUGAAGAAGUCGUGCUGCTT
Quantitative real-time PCR	Forward	Reverse
PADI1	CATGACGCCCAACACTCA	GAGCCATGAGTGTCCATCAC
PADI2	AGAGCCTTGTGCAGGAGAAC	AGGATGTCACGGTTCCAGTC
PADI3	GCCTTCTTCCCTGACTTGGT	AGCAGCAGCCATTGATGA
PADI4	TTCTCTAAGGCGGAAGCTTTT	AGCAGGGAACACACCTTCTC
PADI6	CAGGTGCCTCTGGAGGTTTA	TCGTCACTGTGTCCACAAAAC
B2M	TTCTGGCCTGGAGGCTATC	TCAGGAAATTTGACTTTCCATTC
ChIP assay / Gene reporter assays	Forward	Reverse
PAD4BS-A	TGGGTGTGAAGGAAATGACA	CCAAGGCCATTGAATGAGTT
PAD4BS-B	CACCTCAAGCCACACAGCTA	AAGTGATTTGCCCATCTTGG
PAD4BS-AB	TGGGTGTGAAGGAAATGACA	AAGTGATTTGCCCATCTTGG
BAX	AGGCTGAGACGGGGTTATCT	AGGCTGGGCCTGTATCCTAC
FAS	GAATTGAAGCGGAAGTCTGG	TTAACCACTGCTTCGGTGC
p21 ^{WAF1}	ACCTTTCACCATTCCCCTAC	GCCCAAGGACAAAATAGCCA