Knock-in reconstitution studies reveal an unexpected role of Cys65 in regulating APE1/Ref-1 subcellular trafficking and function

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SUPPLEMENTARY INFORMATION

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Inducible siRNA of APE1 and generation of APE1 knock-in cell lines

The oligonucleotides used for siRNA of APE1 were as follows: sense, 5'-CCTGCCACACTCAAGATCTGC-3'; antisense, 5'-GCAGATCTTGAGTGTGGCAGG-3'. These sequences were drawn following the empirical rules of Mittal (Mittal *et al.*, 2004) and were designed to recognize and bind to a 21 base sequence placed 175 nucleotides after the AUG initiation codon of APE1 gene. As a control, we used the scrambled (Scr-1) oligonucleotide sequences: sense, 5'- AGTCTAACTCGCCACCCCGTA-3'; antisense, 5'-TACGGGGTGGCGAGTTAGACT-3'. These sequences were checked with BLAST (http://www.ncbi.nlm.nih.gov/blast/) for their inability to pair any human cDNA sequence. Sequences were cloned into *BglII* and *HindIII* restriction sites of the pTer vector (van de Wetering *et al.*, 2003), which presents a tetracycline- (doxycycline-) responsive promoter to form the so called pTer/APE1 vector and pTer/Scr, respectively. In a first step, HeLa cells were transfected with pcDNA6/TR to generate stable Tet-repressor expressing cell clones, which were further selected for the acquired resistance by incubation with blasticidin (5 μg/ml) (Invitrogen, Milan, Italy) for 14 days. Individual clones were isolated by using cell cloning cylinders (Sigma, Milan, Italy), transferred and grown stepwise into 24-well, 12-well, 6-well plates for expansion to 10^7 cells. Clone TR5 was shown to express the Tet-repressor at higher levels and was therefore selected for transfection with pTer/APE1 vector previously linearized with Bst1107I (Fermentas, St.Leon Rot, UK) and subjected to selection with zeocine (200 μg/ml) (Invitrogen) for 14-21 days. Thirty single clones were isolated by using cell cloning cylinders transferred and grown stepwise for expansion to 10^7 cells. As a control, we used cell clones transfected with the empty pTer vector or with pTer/Scr vector. For inducible siRNA experiments, doxycycline (Sigma) was added to the culture medium at the final concentration of 1 μg/ml and cells were grown for 10 days. Total cellular extracts were analyzed for APE1 expression by immunoblotting.

For generation of APE1 knock-in cell lines, an APE1 expression vector was generated by cloning an *EcoRI-BamHI* fragment from pFLAG-CMV-5.1/APE1 (Sigma) into p3XFLAG-CMV-14 vector (Sigma). To avoid the degradation of the ectopic APE1 mRNA by the specific siRNA sequence described above, two nucleotides of the APE1-cDNA coding sequence were mutated by using the Site-Directed Mutagenesis Kit (Stratagene), leaving unaffected the APE1 amino acid sequence: siRNA: 5'-CCTGCCACACTCAAGATCTGC-3'; APE1: 5'-CCTGCAACGCTCAAGATCTGC-3'. The Site-Directed Mutagenesis Kit was used to generate the APE1 mutants K6K7/R6R7, 31-34A, C65S, H309N, C310S and H309N+C310S. All the mutants were confirmed by DNA sequencing (MWG, Ebersberg, Germany). Then, the APE1 siRNA clone was transfected with p3XFLAG-CMV/APE1 WT and mutants, previously digested with *ScaI* (Fermentas). Forty-eight h after transfection, the cells were subjected to selection with

geneticin (Invitrogen) for 14 days and selected for the acquired resistance. Individual clones were isolated by using cell cloning cylinders (Sigma), transferred and grown for expansion to 10^7 cells in the presence of selective antibiotics. As control, the siRNA control clone was transfected with the p3XFLAG-CMV-14 empty vector. After 10 days of doxycycline treatment at the final concentration of 1 μg/ml, total or nuclear and cytoplasmic cellular extracts were analyzed for APE1 expression by immunoblotting, thus revealing the silencing of the endogenous APE1 and the expression of the ectopic flagged WT and mutant forms of the protein.

Immunofluorescence analysis

HeLa or SF767 cells in the number of 10^5 per 24 X multiwall plate were seated on glass coverslips and left to attach overnight. Then cells were fixed in 4% (w/v) paraformaldehyde in PBS for 20 min, at room temperature, permeabilized for 5 min with PBS–0.25% (w/v) Triton X-100 and incubated with 5% normal fetal bovine serum in PBS–0.1% (v/v) Tween-20 (blocking solution) for 30 min, to block unspecific binding of the antibodies. Cells were then incubated with the mouse monoclonal anti-APE1 (IgG2b) antibody previously conjugated with Alexa Fluor-568 Zenon Labeling Kit (Molecular Probes, Invitrogen) following manufacturer instructions. A final anti-APE1 dilution of 1:30 was prepared in blocking solution and cells were incubated for 3 h, at 37°C, in a humid chamber. After washing three times with PBS-0.1% (v/v) Tween-20 (washing solution) for 5 min, a second blocking step of 15 min was performed in the dark, followed by three washes with washing solution for 5 min. Then, cells were mounted on microscope glass with Duolink Mounting Media (Olink Biosciences). In the case of colocalization experiments with nucleophosmin (NPM1), instead of mounting the coverslips on microscope glass, cells were incubated with anti-NPM1 polyclonal antibody (Abcam) diluted 1:200 in blocking solution, at 4°C, overnight. After washing, cells were incubated with secondary antibody anti-rabbit FITC conjugated (Jackson Immuno Laboratories, West Grove, PA, USA) for 2 h. The preparations were then washed three times with washing solution for 5 min, in the dark.

Cell growth assays and apoptosis studies

For proliferation assays, cells were harvested at indicated times, stained with Trypan blue (Sigma), plated in triplicate and counted. Colony survival assays were performed as already described (Plumb, 1999; Vascotto *et al.*, 2009a). Briefly, an equal number (500) of control (Scr-1), siRNA and APE1-knock-in cells were plated in petri dishes and grown with medium containing or not doxycycline (1 μg/ml). On day 10, the medium was removed and colonies were stained with 2

ml of cristal violet solution (10% w/v in 70% aqueous ethanol) for 2 min. Then, dye was poured off and plates were rinsed with tap water and allowed to dry. Colonies were counted by using ImageQuant TL software (GE Healthcare, Milan, Italy). For each experimental point, the mean, SD and statistical significance were calculated by performing three independent experiments of cell colony count.

Cell cycle studies were performed by flow cytometry by using a FACScan apparatus (Becton Dickinson, Franklin Lakes, NJ, USA). The number of apoptotic cells in control and APE1 siRNA cells after 0 and 10 days of treatment with 1 μg/ml of doxycycline was determined as previously reported (Nicoletti *et al.*, 1991), by evaluating the number of cells with subdiploid DNA content through flow cytometric determination. Briefly, 2 x 10^6 cells were harvested and washed once with cold PBS/0.1% sodium azide solution, resuspended in 1 ml of low-salt stain solution [4 mM sodium citrate, 3% polyethylene glycol 8000, 1 mg/ml propidium iodide solution (Invitrogen, Carlsbad, CA, USA), 180 U/ml RNAse A (Sigma) and 1% Triton X-100 in PBS/sodium azide solution] and then incubated, in the dark, at 37°C, for 20 min, with gentle mixing every 5 min. Then, 1 ml of high-salt stain solution (0.4 M sodium chloride, 3% polyethylene glycol 8000, 1 mg/ml propidium iodide solution and 1% Triton X-100 in PBS/sodium azide solution) was added by gentle pipetting and samples were stored at 4°C, overnight. Cells were centrifuged at 5000 x *g* for 5 min, at 4°C, the supernatant was removed, pellet resuspended in 500 μl of low-salt solution and then cells were analyzed on a Becton-Dickinson Canto using an Ar laser (excitation 488 nm). For each sample, 25,000 single events were detected and data analysis was performed by using both WinMDI 2.8 (written by Joseph Trotter, Scripps Research Institute, La Jolla, CA) and ModFitLT V3.0 software program.

Apoptosis was also assessed by staining of phosphatidylserines exposed on cell membranes with FITC labeled Annexin V, according to manufacturer instruction (Roche Diagnostic Italia, Monza, Italy). Samples were analyzed by flow cytometry using a FACScan apparatus. In addition, caspases activation was also measured; caspase-3/7 activation was measured by an immunofluorescent assay (Apo-ONE homogeneous caspase assay by Promega Corp., Madison, WI, USA) according to manufacturer's instructions.

Microarray analysis

Total RNA was extracted in triplicate by using TRIzol reagent kit (Invitrogen), and quantified. Total RNA quality was determined by using the Agilent Bioanalyser (Agilent Technologies, Stockport, Cheshire, UK). Human genome HG U133 PLUS2 microarrays

were run according to manufacturer's instructions (Affymetrix Inc., Santa Clara, CA, USA) and submitted in MIAME (Minimum Information About a Microarray Experiment)-compliant format to the ArrayExpress database (www.mged.org/Workgroups/MIAME/miame.html, accession number E-MEXP-1315). Array data were normalized and summarized using RMAExpress (Bolstad *et al.*, 2003). Differential expression in response to siRNA treatment was calculated using Cyber-T (Baldi and Long, 2001). A false-discovery correction was applied to *p*-values to produce a *q*-values (Storey and Tibshirani, 2003). Validation of gene expression analysis was performed through Q-PCR, as previously reported (Vascotto *et al.*, 2009b).

Quantitative real-time PCR

RNA expression levels of selected genes from microarray experiments were quantified by quantitative real-time PCR (Q-PCR) using an Applied Biosystem ABI 7300 (Applied Biosystems, Foster City, CA, USA) on independent biological replicas. Genes of interest were validated by TaqMan Gene Espression assays (Applied Biosystems) and specific sequences are available on request. The results were normalized to the relative amounts of β2-microglobulin (B_2M). PCR was carried out in a final volume of 25 μl with the Platinum Quantitative PCR SuperMix-UDG with Rox (Invitrogen) using 5 μl of cDNA and 1.25 μl each assay-on-demand, in triplicate. Relative gene expression levels were calculated according to the comparative C_T method of relative quantitation *vs* B_2M. To normalized data, $\Delta\Delta C_T$ was calculated for each sample by using the mean of its ΔC_T values subtracted of the mean ΔC_T value of HL60 cells, considered as a calibrator; relative quantization (RQ) value was expressed as $2^{-\Delta\Delta CT}$.

Visualization of the F-actin cytoskeleton

HeLa cells were grown on coverslips at a density of 80,000-100,000 cells per coverslip. One day after plating, cells were fixed in fresh 4% paraformaldehyde for 20 min, at room temperature, washed three times, and permeabilized in 0.1% (v/v) Triton X-100 for 5 min. Cells were then washed twice, blocked with 5% goat serum in PBS (blocking solution) for 15 min and incubated for 90 min in 0.25 U/coverslip of Alexa 488-phalloidin diluted in blocking solution. After three washings, the microscope slides were mounted onto slides in Mowiol® 4-88 supplemented with DABCO (1,4-diazabicyclo[2.2.2]octane) (4:1) to retard photobleaching. All incubation and washing steps were performed at room temperature in PBS, unless otherwise indicated. Coverslips were visualized through a Leica TCS SP laser-scanning confocal microscope (Leica Microsystems,

Wetzlar, Germany) equipped with a 488 nm Ar laser, a 543 nm HeNe laser and a 63x oil fluorescence objective.

Preparation of cell extracts and anti-Flag coimmunoprecipitation

For preparation of total cell lysates and coimmunoprecipitation, cells were harvested by trypsinization and centrifuged at 250 x *g* for 5 min, at 4°C. Supernatant was removed, and the pellet was washed once with ice-cold PBS and then centrifuged again as described before. Cell pellet was resuspended in lysis buffer containing 50 mM Tris HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, and 1% (w/v) Triton X-100, supplemented with protease inhibitor cocktail (Sigma), 0.5 mM PMSF, 1 mM NaF, and 1 mM Na_3VO_4, at a cell density of 10^7 cells/ml, and rotated for 30 min, at 4°C. After centrifugation at 12,000 x *g* for 10 min, at 4°C, the supernatant was collected as total cell lysate. Protein concentration was determined by using the Bio-Rad protein assay (Bio-Rad, Hercules, CA). Samples were then coimmunoprecipitated for 3 h using anti-Flag M2 affinity gel (Sigma) following the manufacturer's instructions. Proteins were eluted by incubation with 0.15 mg/ml 3X Flag peptide in Tris-buffered saline (TBS) and subjected to Western blotting analysis.

For cell nuclear and cytoplasmic extracts, cells were collected as previously reported and the pellet was resuspended in buffer A (10 mM Tris-HCl pH 7.5, 1.5 mM $MgCl_2$, and 10 mM KCl, supplemented with protease inhibitor cocktail, 0.5 mM PMSF, 1 mM NaF and 1 mM Na_3VO_4) at a cell density of 3 x 10^7 cells/ml, and incubated on ice for 10 min. Nuclei were collected by centrifugation at 2,000 x *g* for 10 min, at 4°C. The supernatant was considered as the cytoplasmic fraction. Nuclei were washed twice with the same volume of buffer A to minimize cytoplasmic contamination and then resuspended in buffer B (20 mM Tris-HCl pH 7.5, 0.42 M KCl, 1.5 mM $MgCl_2$, 20% v/v glycerol, supplemented with protease inhibitor cocktail, 0.5 mM PMSF, 1 mM NaF, and 1 mM Na_3VO_4) and incubated on ice for 30 min. The suspension was centrifuged at 15,000 x *g* for 30 min, at 4°C, and the supernatant was collected as nuclear protein extract. Nuclear extracts were then analyzed for protein content (Bradford, 1976), stored at –80°C in aliquots and used for endonuclease assay and Western blotting analysis.

Two-dimensional polyacrylamide gel electrophoresis of immunoprecipitated material

Fifteen-twenty μg of immunoprecipitated material were loaded onto 13 cm, pH 3-10 L IPG strips (GE Healthcare). IEF was conducted by using an IPGPhor II system (GE Healthcare) according to the manufacturer's instructions. Focused strips were equilibrated with 6 M urea, 26 mM DTT, 4% w/v SDS, 30% v/v glycerol in 0.1 M Tris-HCl (pH 6.8) for 15 min, followed by 6 M

urea, 0.38 M iodoacetamide, 4% w/v SDS, 30% v/v glycerol, and a dash of bromophenol blue in 0.1 M Tris-HCl, pH 6.8, for 10 min. The equilibrated strips were applied directly to 10% SDS-polyacrylamide gels and separated at 130 V. Gels were fixed and stained by ammoniacal silver (Shevchenko *et al.*, 1996). Gels were scanned with an Image Master 2-D apparatus and analyzed by the Melanie 5 software (GE Healthcare), which allowed estimation of relative differences in spot intensities for each represented protein.

Identification of the differentially-expressed spots by mass spectrometry analysis

Differential spots from 2-DE were excised from the gel, triturated and washed with water. Proteins were *in-gel* reduced, S-alkylated and digested with trypsin as previously reported (Vascotto *et al.*, 2009b). Digest aliquots were removed and subjected to a desalting/concentration step on μZipTipC_{18} (Millipore Corp., Bedford, MA, USA) by using acetonitrile as eluent before MALDI-TOF-MS analysis. Peptide mixtures were loaded on the MALDI target, using the dried droplet technique and α-cyano-4-hydroxycinnamic acid as matrix, and analyzed by using Voyager-DE PRO mass spectrometer (Applied Biosystems, Framingham, MA, USA). Spectra were elaborated using the DataExplorer 5.1 software (Applied Biosystems) and manually inspected to get the peak lists. Internal mass calibration was performed with peptides deriving from enzyme autoproteolysis. Mascot software package was used to identify spots unambiguously from updated all taxa NCBI non-redundant sequence database (NCBInr 20080610, containing 6573034 protein sequences) by using a mass tolerance value of 50-70 ppm, trypsin as proteolytic enzyme, a missed cleavages maximum value of 2 and Cys carbamidomethylation and Met oxidation as fixed and variable modification, respectively. Candidates with Mascot's scores greater than 81 ($P < 0.05$ for a significant identification) (Supplementary Table S2) were further evaluated by the comparison with their calculated mass and pI using the experimental values obtained from 2-DE.

Endonuclease assays

Determination of AP endonuclease activity of APE1 was performed as already reported (Vascotto *et al.*, 2009a). Same amounts of nuclear extract from $APE1^{WT}$ and $APE1^{C65S}$ expressing cells were incubated with a 5'-^{32}P-end-labeled 26-mer oligonucleotide containing a single tetrahydrofuranyl artificial AP site at position 14 (here called dsFDNA), which is cleaved to a 14-mer in the presence of AP endonuclease activity. Samples from reaction mixtures (10 μl) containing the protein of interest, 2.5 pmol of the 5'-^{32}P end-

labeled oligonucleotide dsFDNA, 50 mM HEPES, 50 mM KCl, 10 mM MgCl2, 1 μg/μl bovine serum albumin, and 0.05% (wt/vol) Triton X-100 (pH 7.5) were allowed to proceed for 15 min, at 37°C. Reactions were halted by adding 10 μl of 96% (v/v) formamide and 10 mM EDTA, with xylene cyanol and bromophenol blue as dyes. AP assay products (10 μl) were separated on a 20% polyacrylamide gel containing 7 M urea. Gels were then exposed to film for autoradiography and quantification was performed as described previously (Vascotto *et al.*, 2009a).

Supplementary Table 1. Comparative gene expression profile of APE1^{WT} and APE1^{C65S}-expressing cells reveals differential expression of cell cycle- and growth factor-related species.

Out of 19,235 genes in the database 2,924 contain disulphide bonds (15%). Interestingly, among the 38 significant genes found as differentially expressed between APE1^{WT} and APE1^{C65S} expressing cells, 13 contain disulphide bonds (34%).

Affymetrix ID	Symbol	Ref Seq no.	Gene Title	fold change	q-value
206897_at	PAGE1	NM_003785	P antigen family, member 1 (prostate associated)	-11.6	1.50E-05
207739_s_at	GAGE1	NM_001472	G antigen 1	-4.5	0.003351
209656_s_at	TMEM47	AL136550	transmembrane protein 47	-4.3	0.001047
216799_at	COR2B	AL442084	Coronin, actin binding protein, 2B	1.6	0.153627
206029_at	ANKRD1	NM_014391	ankyrin repeat domain 1 (cardiac muscle)	1.8	0.051766
205016_at	TGFA	NM_003236	transforming growth factor, alpha	1.9	0.029981
1560652_at	---	AL832136	MRNA; cDNA DKFZp686L0310 (from clone DKFZp686L0310)	2	0.163034
229868_s_at	GDF15	AA129612	Growth differentiation factor 15	2	0.163034
237273_at	KCNU1	AA758685	potassium channel, subfamily U, member 1	2	0.024942
213765_at	MFAP5	AW665892	microfibrillar associated protein 5	1.6	0.163034
224823_at	MYLK	AA526844	myosin, light chain kinase	2	0.017403
208892_s_at	DUSP6	BC003143	dual specificity phosphatase 6	2.1	0.123629
231867_at	ODZ2	AB032953	odz, odd Oz/ten-m homolog 2 (Drosophila)	2.2	0.000764
203889_at	SCG5	NM_003020	secretogranin V (7B2 protein)	2.2	0.028584
204011_at	SPRY2	NM_005842	sprouty homolog 2 (Drosophila)	2.2	0.175265
235122_at	---	AI800713	CDNA clone IMAGE:6254031	2.4	0.008069
235740_at	---	BG250585	Transcribed locus	2.5	0.051766
205239_at	AREG	NM_001657	amphiregulin (schwannoma-derived growth factor)	2.5	0.123629
215388_s_at	CFH /// CFHR1	X56210	complement factor H /// complement factor H-related 1	2.6	0.12467
213800_at	CFH	X04697	complement factor H	2.8	0.017403
202157_s_at	CUGBP2	U69546	CUG triplet repeat, RNA binding protein 2	2.6	0.015421
1569555_at	GDA	BC012859	guanine deaminase	2.6	0.051766
231411_at	LHFP	BE674089	Lipoma HMGIC fusion partner	2.6	0.051766
223340_at	SPG3A	AF131801	spastic paraplegia 3A (autosomal dominant)	2.6	0.001517
205302_at	IGFBP1	NM_000596	insulin-like growth factor binding protein 1	2.7	0.003946
232176_at	SLITRK6	R70320	SLIT and NTRK-like family, member 6	5.4	0.000155
202499_s_at	SLC2A3	NM_006931	solute carrier family 2 (facilitated glucose transporter), member 3	3.5	0.038307
204637_at	CGA	NM_000735	glycoprotein hormones, alpha polypeptide	3.7	0.166426
218847_at	IGF2BP2	NM_006548	insulin-like growth factor 2 mRNA binding protein 2	3.8	0.038426
201859_at	SRGN	NM_002727	serglycin	3.9	3.58E-06
223949_at	TMPRSS3	AB038160	transmembrane protease, serine 3	3.9	4.64E-05
230748_at	SLC16A6	AI873273	solute carrier family 16, member 6 (monocarboxylic acid transporter 7)	4.5	0.004289
204014_at	DUSP4	NM_001394	dual specificity phosphatase 4	4.6	0.166426
225927_at	MAP3K1	AA541479	mitogen-activated protein kinase kinase kinase 1	5	0.12467
203824_at	TSPAN8	NM_004616	tetraspanin 8	5	4.64E-05
204363_at	F3	NM_001993	coagulation factor III (thromboplastin, tissue factor)	5.2	0.004762
206343_s_at	NRG1	NM_013959	neuregulin 1	5.2	0.000223
223044_at	SLC40A1	AL136944	solute carrier family 40 (iron-regulated transporter), member 1	6	4.64E-05
229450_at	IFIT3	AI075407	interferon-induced protein with tetratricopeptide repeats 3	8.4	0.051766

Supplementary Table S2. Protein identification as ascertained by mass spectrometric analysis.

SpotUP matches to: sp|P30101|PDIA3_HUMAN
Score: 108 Expect: 8.2e-006
Protein disulfide-isomerase A3 OS=Homo sapiens GN=PDIA3 PE=1 SV=4
Nominal mass (M_r): **57146**; Calculated pI value: **5.98**
NCBI BLAST search of sp|P30101|PDIA3_HUMAN against nr
Unformatted sequence string for pasting into other applications
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Number of mass values searched: **9**
Number of mass values matched: **8**
Sequence Coverage: **23%**
Matched peptides shown in **Bold Red**

```
     1 MRLRRLALFP GVALLLAAAR LAAASDVLEL TDDNFESRIS DTGSAGLMLV
    51 EFFAPWCGHC KRLAPEYEAA ATRLKGIVPL AKVDCTANTN TCNKYGVSGY
   101 PTLKIFRDGE EAGAYDGPRT ADGIVSHLKK QAGPASVPLR TEEEFKKFIS
   151 DKDASIVGFF DDSFSEAHSE FLKAASNLRD NYRFAHTNVE SLVNEYDDNG
   201 EGIILFRPSH LTNKFEDKTV AYTEQKMTSG KIKKFIQENI FGICPHMTED
   251 NKDLIQGKDL LIAYYDVDYE KNAKGSNYWR NRVMMVAKKF LDAGHKLNFA
   301 VASRKTFSHE LSDFGLESTA GEIPVVAIRT AKGEKFVMQE EFSRDGKALE
   351 RFLQDYFDGN LKRYLKSEPI PESNDGPVKV VVAENFDEIV NNENKDVLIE
   401 FYAPWCGHCK NLEPKYKELG EKLSKDPNIV IAKMDATAND VPSPYEVRGF
   451 PTIYFSPANK KLNPKKYEGG RELSDFISYL QREATNPPVI QEEKPKKKKK
   501 AQEDL
```

Start - End	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Sequence
74 - 94	2317.0540	2316.0467	2316.1930	-63	2	R.LKGIVPLAKVDCTANTNTCNK.Y
105 - 119	1652.7550	1651.7477	1651.7590	-7	1	K.IFRDGEEAGAYDGPR.T
148 - 173	2938.3180	2937.3107	2937.3709	-20	1	K.FISDKDASIVGFFDDSFSEAHSEFLK.A
305 - 329	2703.2790	2702.2717	2702.3915	-44	1	R.KTFSHELSDFGLESTAGEIPVVAIR.T
306 - 329	2575.3430	2574.3357	2574.2966	15	0	K.TFSHELSDFGLESTAGEIPVVAIR.T
336 - 344	1172.5390	1171.5317	1171.5332	-1	0	K.FVMQEEFSR.D
352 - 363	1515.7540	1514.7467	1514.7518	-3	1	R.FLQDYFDGNLKR.Y
472 - 482	1370.7340	1369.7267	1369.6878	28	0	R.ELSDFISYLQR.E

No match to: 1164.6100

SpotDOWN matches to: sp|P30041|PRDX6_HUMAN
Score: 131 Expect: 4.1e-008
Peroxiredoxin-6 OS=Homo sapiens GN=PRDX6 PE=1 SV=3
Nominal mass (M_r): **25133**; Calculated pI value: **6.00**
NCBI BLAST search of sp|P30041|PRDX6_HUMAN against nr
Unformatted sequence string for pasting into other applications
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Number of mass values searched: **8**
Number of mass values matched: **7**
Sequence Coverage: **37%**
Matched peptides shown in **Bold Red**

```
     1 MPGGLLLGDV APNFEANTTV GRIRFHDFLG DSWGILFSHP RDFTPVCTTE
    51 LGRAAKLAPE FAKRNVKLIA LSIDSVEDHL AWSKDINAYN CEEPTEKLPF
   101 PIIDDRNREL AILLGMLDPA EKDEKGMPVT ARVVFVFGPD KKLKLSILYP
   151 ATTGRNFDEI LRVVISLQLT AEKRVATPVD WKDGDSVMVL PTIPEEEAKK
   201 LFPKGVFTKE LPSGKKYLRY TPQP
```

Start - End	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Sequence
2 - 22	2098.1060	2097.0987	2097.0855	6	0	M.PGGLLLGDVAPNFEANTTVGR.I
42 - 53	1395.6780	1394.6707	1394.6500	15	0	R.DFTPVCTTELGR.A
57 - 64	931.5490	930.5417	930.5287	14	1	K.LAPEFAKR.N
85 - 106	2649.3420	2648.3347	2648.2428	35	1	K.DINAYNCEEPTEKLPFPIIDDR.N
98 - 106	1085.6150	1084.6077	1084.5917	15	0	K.LPFPIIDDR.N
133 - 142	1135.6740	1134.6667	1134.6438	20	1	R.VVFVFGPDKK.L
145 - 155	1191.6620	1190.6547	1190.6659	-9	0	K.LSILYPATTGR.N

No match to: 2210.1060

Supplementary Figure Legends

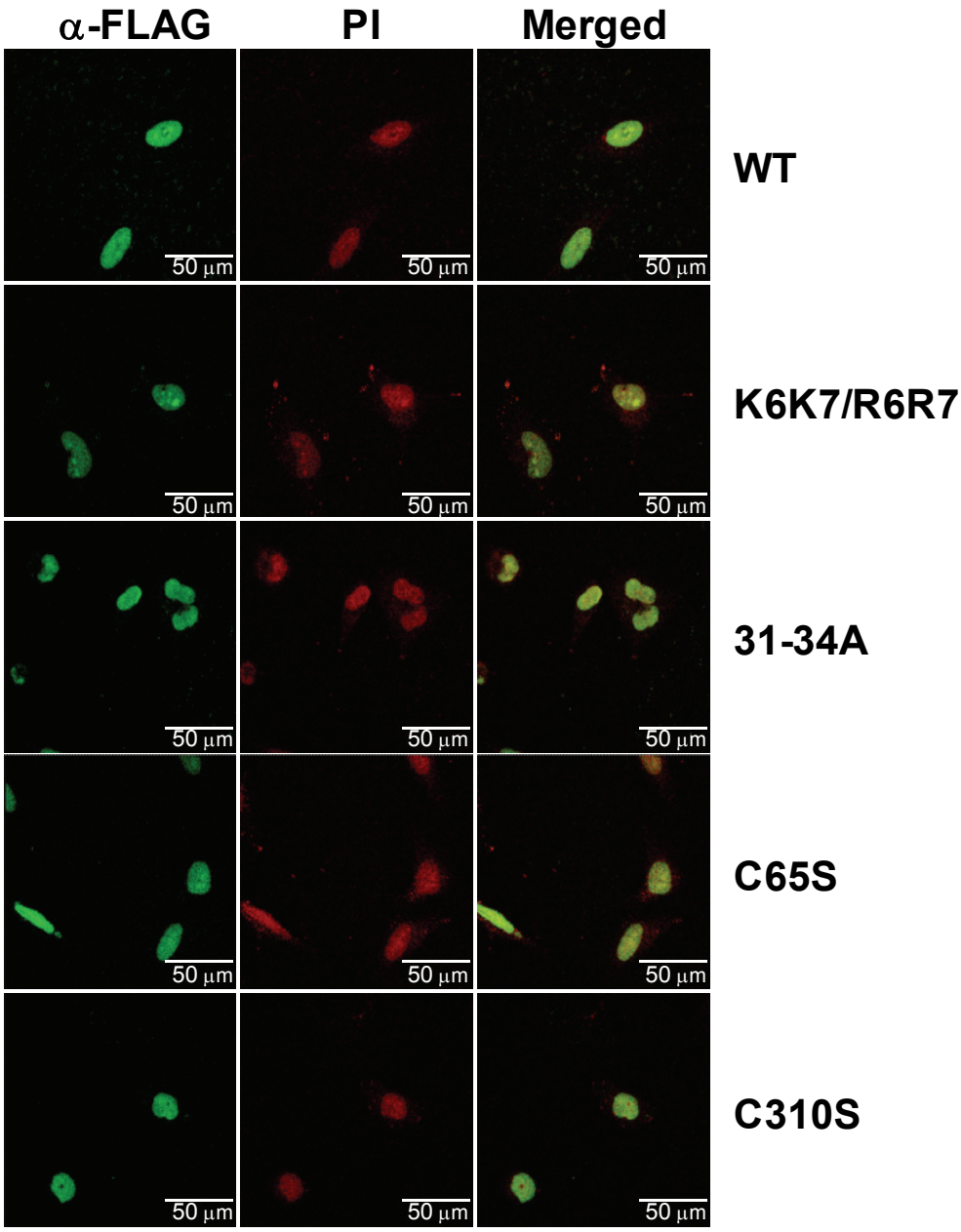
Supplementary Figure S1.

Immunofluorescence analysis to characterize the localization of the ectopic Flag-tagged APE1 proteins. HeLa cells stably transfected with the siRNA-resistant Flag-tagged APE1 cDNA encoding plasmids were fixed and stained for anti-Flag-APE1 (green). Nucleus was stained with propidium iodide (PI). Merged images (yellow) show the localization of APE1 within nucleus of transfected cells.

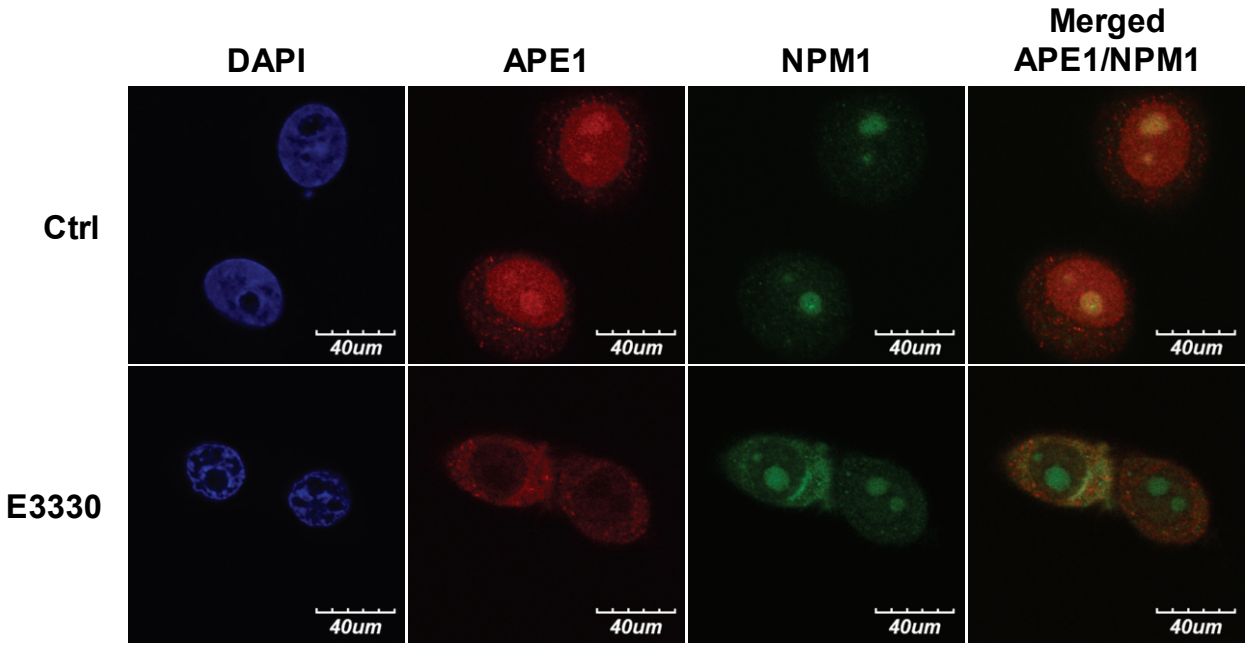
Supplementary Figure S2.

APE1 colocalizes with nucleophosmin and lose its nuclear localization after E3330 treatment. APE1 and NPM1 subcellular localization on SF767 cells was analyzed through confocal microscopy after treatment with 140 μM E3330 for 6 h. A specific APE1 monoclonal antibody derivatized with Alex Fluor 568 was used in combination with a polyclonal anti-NPM1, followed by incubation with an anti-rabbit FITC-conjugated antibody. Nuclear compartment was stained with DAPI. Control sample represents cells treated with DMSO. In control, APE1 and NPM1 colocalize within nucleoli, while E3330 treatment determinates APE1 cytoplasmic relocalization, but does not affect nucleolar structure.

Supplementary FIGURE 1



Supplementary FIGURE 2



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