List of supplemental materials

Table S1.

PCR primers for cloning. Megaprime PCR reactions were carried out with the primers indicated and a pair of vector (pcDNA) primers: T7 (upstream) and ZeoSeq (downstream). All sequences are the direction from 5' to 3'. Top: sense strands, Bot: anti-sense strands. Additionally, primers to introduce single K to R mutations in APE1 were designed accordingly. The vector pcDNA3.1Zeo(+) was used for cloning unless noted.

Fig. S1.

Interaction of APE1 with the wild-type MDM2 by immunoprecipitation assay. The FLAG-enriched fractions were examined with anti-MDM2 antibody N-20 (top). Cells expressing MDM2 and the full-length APE1-FLAG (lane 1), ND42 APE1-FLAG lacking 42 a.a. of the N-terminus (lane 2), the full-length APE without FLAG fusion (lane 3), and the CD20 APE1-FLAG lacking the 20 a.a. of the C-terminus. The input cell lysates (before FLAG precipitation) were analyzed with anti-MDM2 (middle) and anti-FLAG (bottom). Asterisk in the top panel denotes a non-specific signal.

Fig. S2.

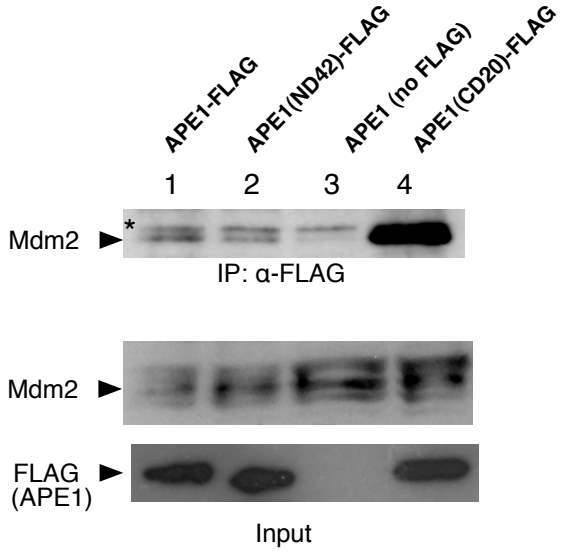
Sequence alignment of the N-terminal 60 amino acid residues in APE1. The protein sequences in Swiss-prot, from top to bottom (accession number): human (P27695), gorilla (A1YES6), orangutan (A2T7I6), pigmy chimpanzee (A1YFZ3), chimpanzee (A2T6Y4), bovine (P23196), rat (P43138), and mouse (P28352). The amino acid sequences were aligned using Clustalw (http://www.ebi.ac.uk/clustalw/). The description of the amino acid-coloring is in:

http://www.ebi.ac.uk/clustalw/clustalw_help.html#results

The tandem ubiquitin acceptor Lys residues (K24, K25, K27) are indicated with the magenta arrows.

purpose	sequence	RE enzymes used
MDM2 cloning	Top: AGGGGATCCACCATGTGCAATACCAACATGTCT Bot: ACACTCGAGCTAGGGGAAATAAGTTAGCAC	BamHI+XhoI
K[24/25/27]R	Top: AGCCAGAGGCCAGGAGGAGTAGGACGGCCGCAAA Bot: TTTGCGGCCGTCCTACTCCTCCTGGCCTCTGGCT	Megaprime with T7&ZeoSeq, BamHI+XhoI
K[31/32/35]R	Top: AAGACGGCCGCAAGGAGAAATGACAGAGAGGCAGCAGGAGAG Bot: ZeoSeq	EagI+XhoI
His-tagged SUMO cloning	Top: TTTTGGATCCACCATGGGCAGCAGCCACCACCACCACCACCACAGCAGCGGC TCTGACCAGGAGGCAAAACCTTCAACT Bot: AAAACTCGAGCTAAACTGTTGAATGACCCCCCGT	BamHI+XhoI
His-tagged Nedd8 cloning	Top1: CATCATCATCATGGTCTAATTAAAGTGAAGACGCTGACC Top2: TTTGGATCCACCATGGTGTCTCATCATCATCATCATCATGGTCTA Bot: CTGCCTCTCGAGTCATCCTCCTCTCAGAGCCAACACCAG	cascaded PCR (Top1 to Top2), BamHI+XhoI
G76A mutation in ubiquitin	Top: T7 Bot: CCCTCTAGACTCGAGTCAGGCACCTCTGAGACG	BamHI+XhoI
T7	TAATACGACTCACTATAGGG	
ZeoSeq	TGGCTGGCAACTAGAAGG	

Table S1


APE1-FLAG
APE1(ND42)-FLAG
APE1 (no FLAG)
APE1(CD20)-FLAG
1
2
3
4
Mdm2
*
IP: α-FLAG
Mdm2
FLAG
(APE1)
Input

Busso_S1


P27695|APEX1_HUMAN MPKRGKKGAVAEDGDELRTEPEAKKSKTAAKKNDKEAAGEGPALYEDPPDQKTSPSGKPA 60
A1YES6|APEX1_GORGO MPKRGKKGAVAEDGDELKTEPEAKKSKTAAKKNDKEAAGEGPALYEDPPDQKTSPSGKPA 60
A2T7I6|APEX1_PONPY MPKRGKKGAVAEDGDELKTEPEAKKSKTTAKKNDKEAAGEGPALYEDPPDQKTSPSGKPA 60
A1YFZ3|APEX1_PANPA MPKRGKKGAVAEDGDELRTEPEAKKSKTAAKKNDKEAAGEGPALYEDPPDQKTSPSGKPA 60
A2T6Y4|APEX1_PANTR MPKRGKKGAVAEDGDELRTEPEAKKSKTAAKKNDKEAAGEGPALYEDPPDQKTSPSGKPA 60
P23196|APEX1_BOVIN MPKRGKKGAVVEDAEEPKTEPEAKKSKAGAKKNEKEAVGEGAVLYEDPPDQKTSPSGKSA 60
P43138|APEX1_RAT MPKRGKR-AAAEDGEEPKSEPETKKSKGAAKKTEKEAAGEGPVLYEDPPDQKTSASGKSA 59
P28352|APEX1_MOUSE MPKRGKK-AAADDGEEPKSEPETKKSKGAAKKTEKEAAGEGPVLYEDPPDQKTSPSGKSA 59

Busso_Fig. S2