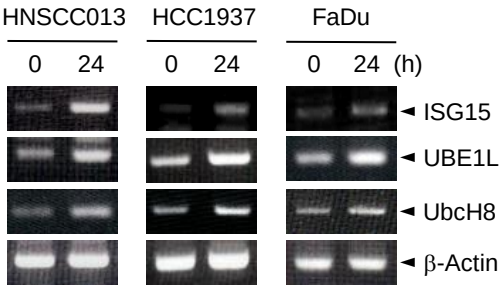
SUPPLEMENTAL INFORMATION
(Jeon et al.)

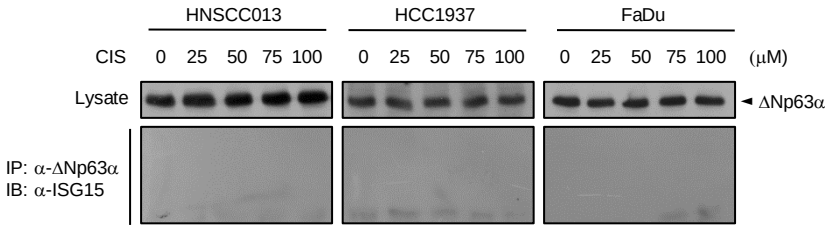
Supplemental Table 1
List of candidate proteins that can be ISGylated upon doxorubicin treatment

Proteins		Swiss-Prot
ANKH1	Ankyrin repeat and KH domain-containing protein 1	Q8IWZ3
CLIC2*	Chloride intracellular channel protein 2	O15247
CYTD	Cystatin-D	P28325
DKFZp586J1624.1	Hypothetical protein alternatively spliced product, similar to (AF266508) NELF protein	Q6X4W1
EZRI*	Ezrin	P15311
HYLS1	Hydrolethalus syndrome protein 1	Q96M11
IP6K1	Inositol hexakisphosphate kinase 1	Q92551
PEX6	Peroxisome assembly factor 2	Q13608
PRGC1	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha	Q9UBK2
Putative p150	Putative p150	O00362
SLK	STE20-like kinase (yeast)	Q9H2G2
SPEC1	Sperm antigen with calponin homology and coiled-coil domains 1	Q5M775
ST32A	Serine/threonine-protein kinase 32A	Q8WU08
SYAM	Probable alanyl-tRNA synthetase, mitochondrial precursor	Q5JTZ9
TMC5	Transmembrane channel-like protein 5	Q6UXY8
TP63	Tumor protein p63	Q9H3D4
hCG1997775	hCG1997775	
hCG2026748	hCG2026748	
hCG2023467	hCG2023467	

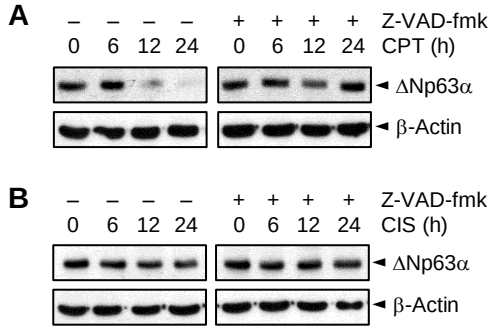
MCF10A cells were treated as in Figure 1C. Protein bound to protein A-conjugated Sepharose resins were subjected to LC/MS/MS analysis. The asterisks indicate the proteins that are known to be the targets for ISGylation.



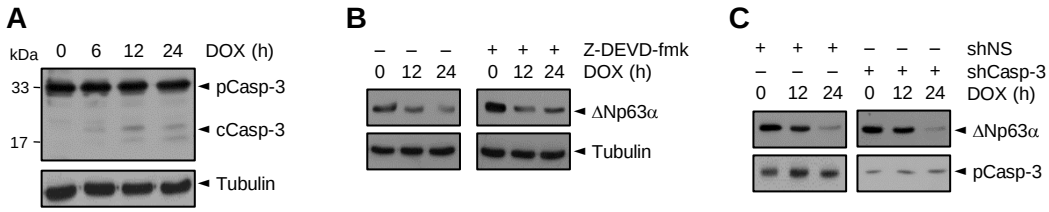
Supplemental Figure 1 Doxorubicin increases the transcript levels of ISG15-conjugating system in cancer cells. HNSCC013, HCC1937, and FaDu cells were incubated 0.1 μM doxorubicin for 24 h. Total RNAs were prepared and subjected to RT-PCR using the probes for ISG15, UbcH8, UBE1L, and β-actin.



Supplemental Figure 2 Cisplatin does not induce ISGylation of ΔNp63α or its cleavage. HNSCC013, HCC1937, and FaDu cells were incubated with increasing concentrations of cisplatin for 24 h. Cell lysates were subjected to immunoprecipitation with anti-ΔNp63α antibody followed by immunoblot with anti-ISG15 antibody. They were also directly probed with anti-ΔNp63α antibody.



Supplemental Figure 3 Camptothecin induces caspase-mediated cleavage of ΔNp63α. MCF10A cells were incubated with camptothecin (**A**) or cisplatin (**B**) in the absence or presence of Z-VAD-fmk followed by immunoblot with anti-ΔNp63α antibody.



Supplemental Figure 4 Caspase-3 is not involved in doxorubicin-mediated cleavage of ΔNp63α. (**A**) FaDu cells were incubated with doxorubicin followed by immunoblot with anti-caspase-3 antibody. (**B**) Cells were incubated with doxorubicin in the absence or presence of Z-DEVD-fmk followed by immunoblot with anti-ΔNp63α antibody. (**C**) Cells transfected with shNS or shCasp-3 were incubated with doxorubicin, followed by immunoblot with anti-ΔNp63α or anti-caspase-3 antibody.

MLYLENNAQT QFSEPQYTNL GLLNSMDQQ IQNGSSSTSPY NTDHAQNSVT **50**

APSPYAQPSS TFDALSPSPA IPSNTDYPGP HSFDVSFQQS STAKSATWTY **100**

STELKKLYCQ IAKTCPIQIK VMTPPPQGAV IRAMPVYK**K**A EHVTEVVKRC **150**
(K: 139)

PNHELSREFN EGQIAPPSHL IRVEGNSHAQ YVEDPITGRQ SVLVPYEPPQ **200**

VGTEFTTVL YNFMCNSSCVG GMNRRPILII VTLETRDGQV LGRRCFEARI **250**

CACPGRDRKA DEDSIRKQQV SDSTKNGDGT KRPFRQNTHG IQMTSIKKRR **300**

SPDDELLYLP VRGRETYEML LKI**K**ESLELM QYLPQHTIET YRQQQQQQHQ **350**
(K: 324)

HLLQKQTSIQ SPSSYGNSSP PLNKMNSMNK LPSVSQLINP QQRNALTPTT **400**

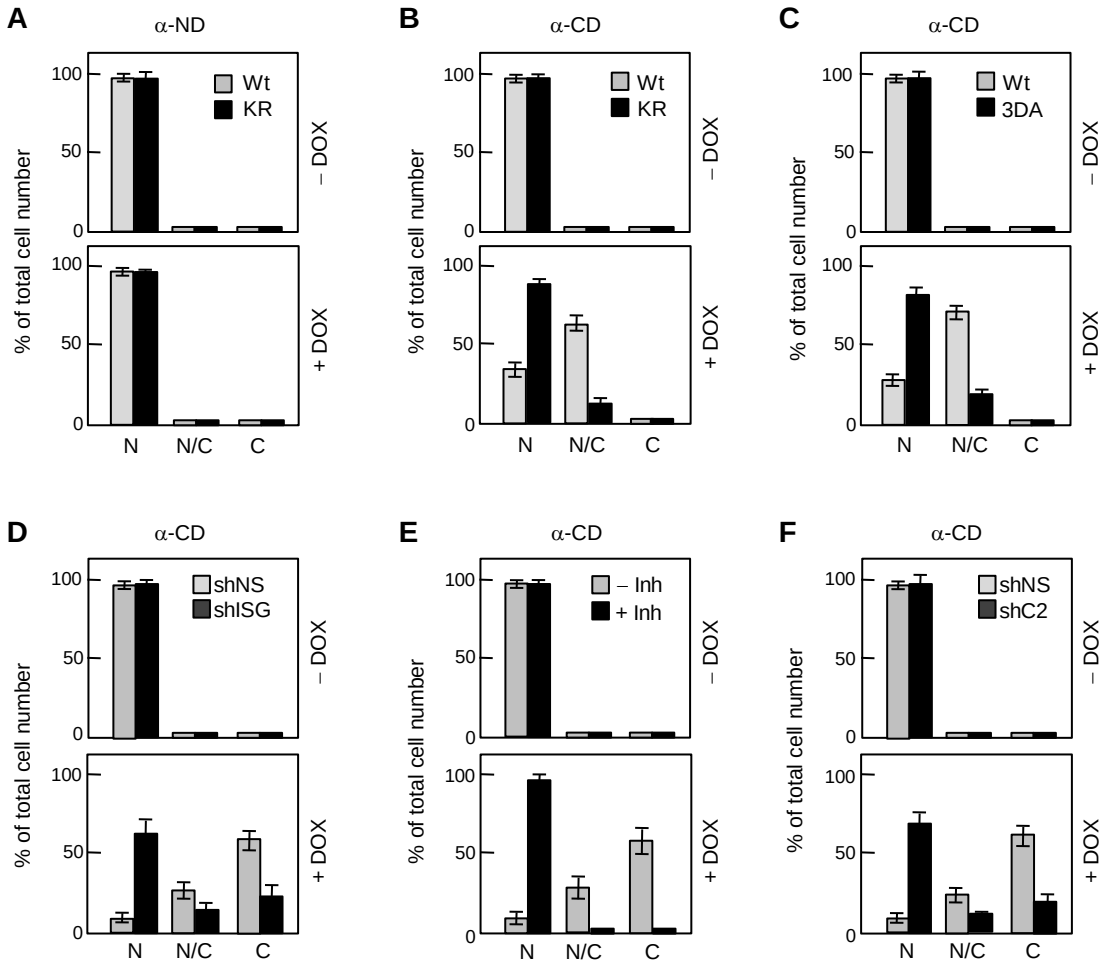
IP**D**GMGANIP MMGTHMPMAG DMNGLSPTQA LPPPLSMPST SHCTPPPPYP **450**
(D: 403)

T**D**CSIVSFLA RLGCSSCL**D**Y FTTQGLTTIY QIEHYSMD**D**L ASLKIPEQFR **500**
(D: 452; D: 469; D: 489)

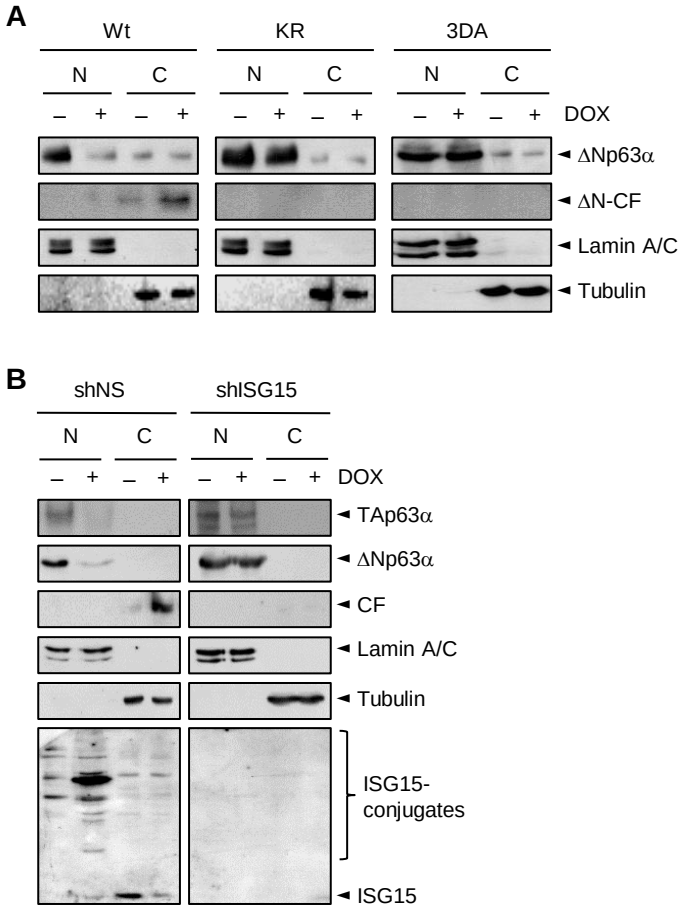
HAIWKGILDH RQLHEFSSPS HLLRTPSSAS TVSVGSSETR GERVIDAVRF **550**

TLRQTISFPP RDEWNDFNFD MDARRNKQQR IKEEGE **586**

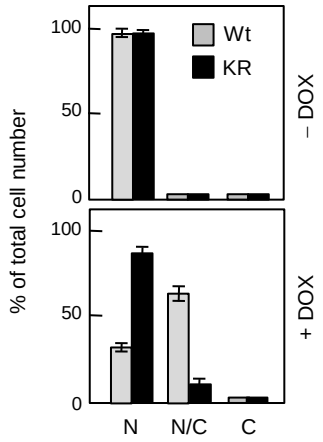
Supplemental Figure 5 The amino acid sequence of ΔNp63α. The ISGylation sites (blue) and the cleavage sites by caspase-3 (orange) as well as by caspase-2 (red) in ΔNp63α are indicated as the bold characters.



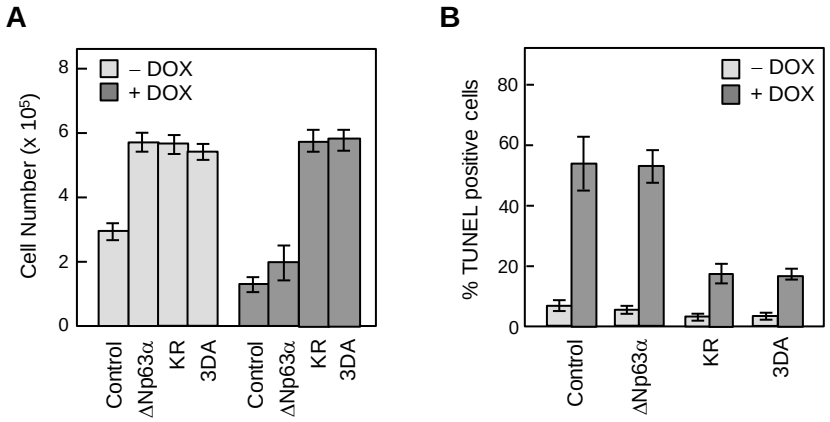
Supplemental Figure 6 Statistic analysis for subcellular localization of ΔNp63α and its mutant forms. Cells were cultured as in (**A-F**) of **Figure 5**, and the numbers of cells having ΔNp63α in the nucleus only (N), in the cytoplasm only (C), and in both (N/C) were counted and shown as % of total cell numbers. Error bars indicate the mean ± s.d. of three experiments.



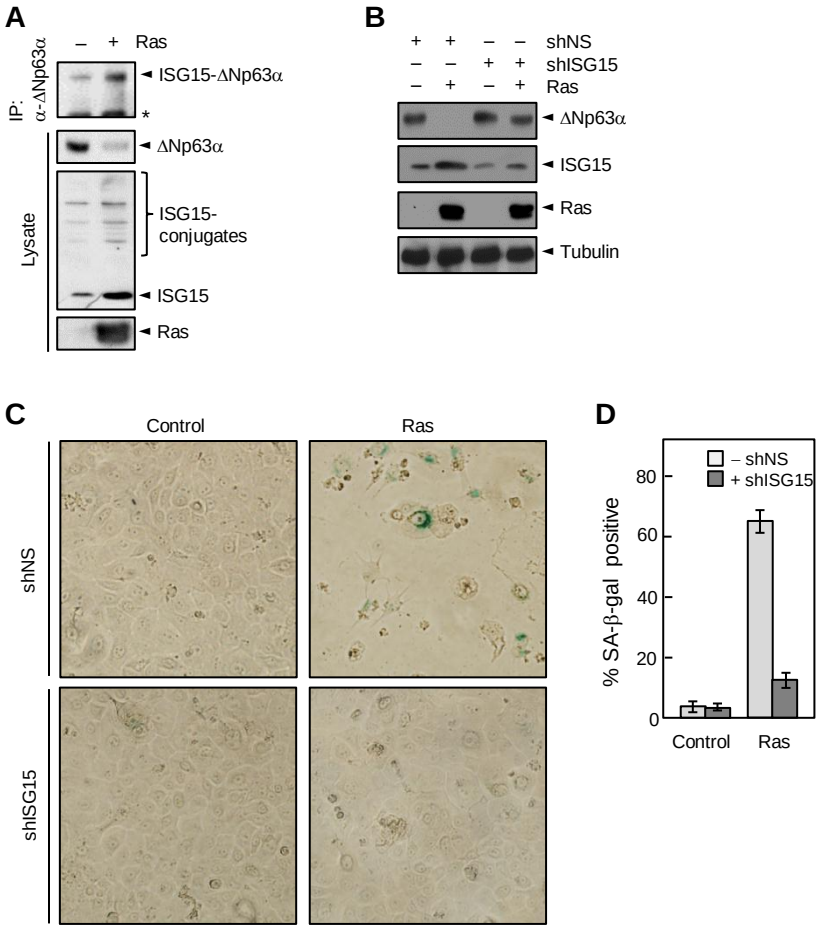
Supplemental Figure 7 Subcellular fractionation of ΔNp63α and its mutant forms. H1299 cells complemented with HisMax-tagged ΔNp63α, KR, and 3DA were incubated with doxorubicin. They were then harvested and fractionated into the nuclear (N) and cytoplasmic (C) fractions as described under "Methods." The fractions were then subjected to immunoblot with anti-N-domain or anti-C-domain antibody. (**B**) HNSCC013 cells incubated with doxorubicin for 24 h were fractionated into the nuclear (N) and cytoplasmic (C) fractions. The fractions were then subjected to immunoblot with antibodies directed to the N- and C-domains of ΔNp63α or with antibody specific to the N-domain of TAp63α. Note that the antibody directed to the C-domain of ΔNp63α also interacts with that of TAp63α.



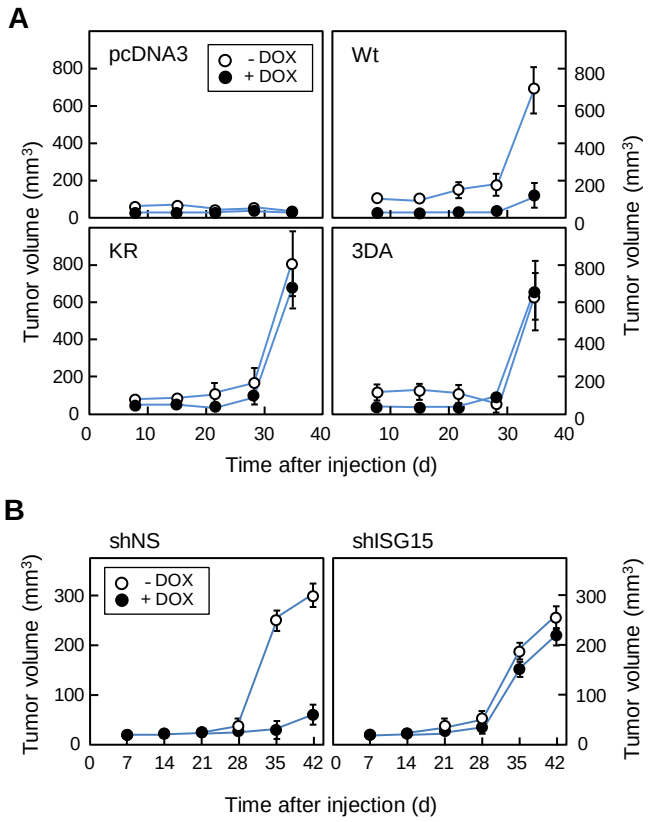
Supplemental Figure 8 Statistic analysis for subcellular localization of TAp63α and KR. Cells were cultured as in (**A**) of **Figure 6**, and the numbers of cells having ΔNp63α in the nucleus only (N), in the cytoplasm only (C), and in both (N/C) were counted and shown as % of total cell numbers. Error bars indicate the mean ± s.d. of two experiments.



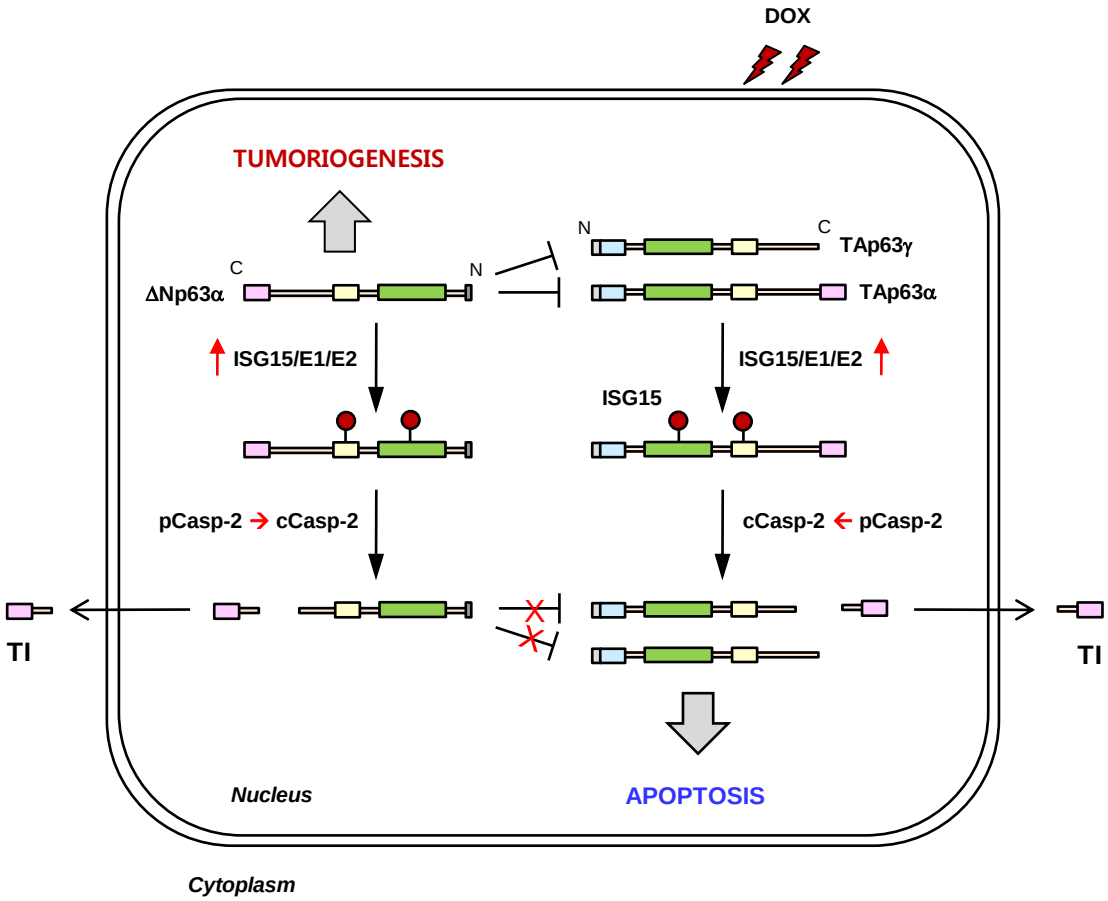
Supplemental Figure 9 Doxorubicin-induced ISGylation and caspase-2-mediated cleavage of ΔNp63α ablate its mitotic and anti-apoptotic functions. (**A**) Hep3B cells stably expressing ΔNp63α, KR, or 3DA were incubated for 24 h with and without doxorubicin, and their numbers were counted. (**B**) Cells incubated as in (A) were subjected to TUNEL assay. TUNEL positive cells were expressed as % of total cell numbers. Error bars indicate the mean ± s.d. of four experiments.



Supplemental Figure 10 ISGylation of ΔNp63α promotes oncogenic Ras-induced senescence. (**A**) Oncogenic H-Ras-V12 (Ras) was expressed in human primary keratinocytes. Cell lysates were subjected to immunoprecipitation with anti- ΔNp63α antibody followed by immunoblot with anti-ISG15 antibody. The asterisk indicates IgG heavy chain. (**B**) Ras was expressed in keratinocytes that had been transfected with shNS or shISG15. They were then subjected to immunoblot analysis. (**C**) Cells cultured as in (B) were subjected to assays for senescence-associated-β-galactosidase (SA-β-Gal) activity. (**D**) SA-β-Gal positive cells in (C) were quantified and expressed as % of total cell numbers. Error bars indicate the mean ± s.d. of three independent staining.



Supplemental Figure 11 ISGylation of ΔNp63α ablates its oncogenic function. (**A**) Tumors were developed as in Figure 9D, and their volumes (mm^3) were estimated from caliper measurements twice a week. Data points represent the mean ± s.e.m. of 10 mice per group. (**B**) Tumors were developed as in Figure 9F, and their volumes were estimated every week. Data points represent the mean ± s.e.m. of 15 mice per group.



Supplemental Figure 12 A model for the mechanism by which ISGylation of ΔNp63α abrogates its oncogenic function in response to doxorubicin.