Supporting Information

Wei et al. 10.1073/pnas.1205664109

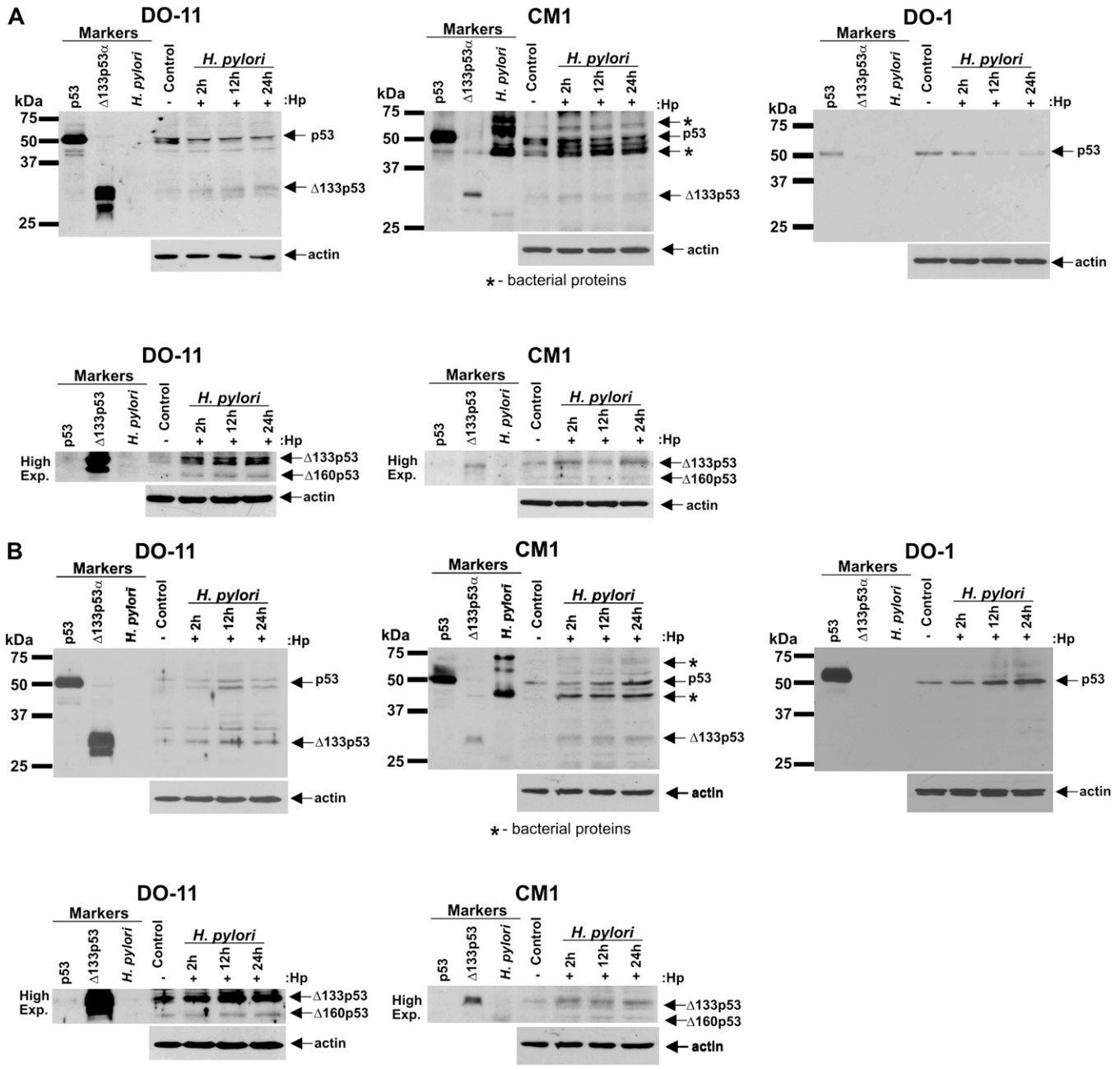

Fig. S1. Truncated p53 isoforms are up-regulated by *Helicobacter pylori*. (*A*) Protein lysates were prepared from control AGS gastric cancer cells (–) or those co-cultured with *H. pylori* strain J166 (+) for the indicated time and analyzed for expression of p53 isoforms by Western blotting using DO-11, CM1, and DO-1 antibodies. (*Lower*) Higher exposure images are required to facilitate detection of Δ160p53 isoform. As size markers, cells were transfected with either full-length p53 or Δ133p53α expression plasmids. *H. pylori* bacterial lysates were used for detection of antibody cross-reactivity. Although CM1 antibody cross-reacts with bacterial proteins, it can be used for detection of Δ133p53 isoform. The asterisk (*) indicates bacterial proteins that cross-react with CM1 antibody. (*B*) Same as in *A*, but SNU-1 gastric cancer cell line was analyzed.

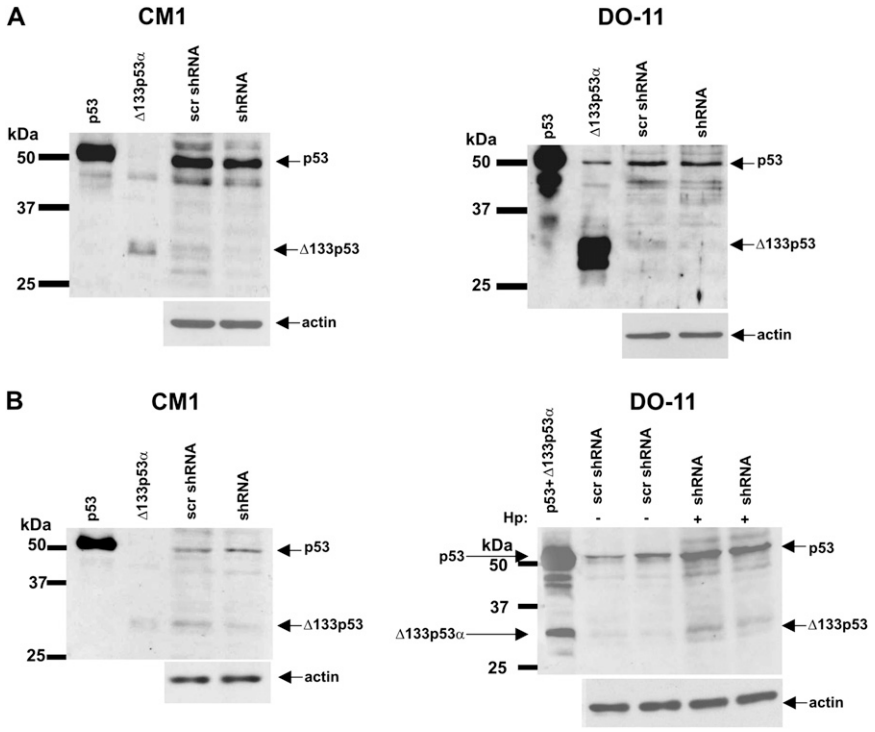

Fig. S2. Characterization of p53 isoforms. (*A*) Gastric cancer cell line AGS was stably transfected with either shRNA against Δ133p53 or scrambled control vector and were analyzed for expression of p53 isoforms by Western blotting. (*B*) Same as in *A*, but gastric cancer cell line SNU-1 was used. Hp, *Helicobacter pylori*.

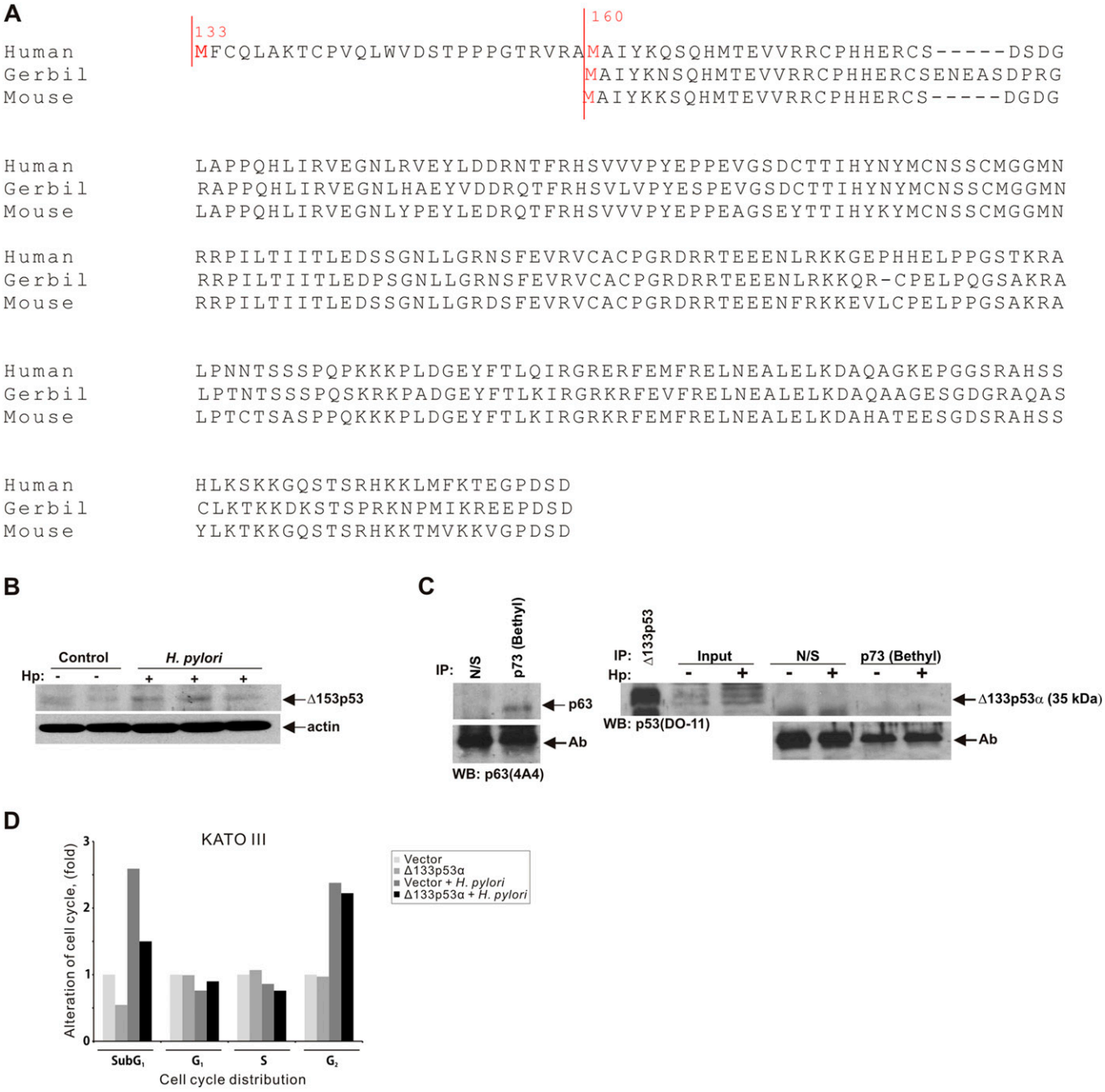

Fig. S3. (*A*) Sequence alignment of human, gerbil, and murine isoforms of p53. (*B*) Expression analysis of gerbil Δ153p53 protein using Western blotting with p53 (DO-11) antibody. The Δ153p53 protein migrated as a band with molecular mass of 32 kDa. Tissue extracts were prepared from animals infected with *Helicobacter pylori* strain 7.13 for 3 d. (*C*) Interaction of TAp73 and Δ133p53 proteins. Protein lysates from SNU-1 gastric cancer cells co-cultured with *H. pylori* (+) for 8 h or left uninfected (–) were immunoprecipitated with either p73 antibody or nonspecific rabbit IgG (N/S). The TAp73–Δ133p53 protein complexes were analyzed by Western blotting (WB) with the p53 (DO-11) antibody, which recognizes the Δ133p53 isoform. As a positive control, p73 immunoprecipitates were also analyzed for interaction with p63 using p63 (4A4) antibody. Equal antibody loading was verified by detecting light or heavy chains of antibodies. (*D*) Analysis of cell cycle by flow cytometry in Kato III cells that were stably transfected with either empty vector or Δ133p53 expression plasmid and co-cultured with *H. pylori* strain J166 for 24 h is shown.

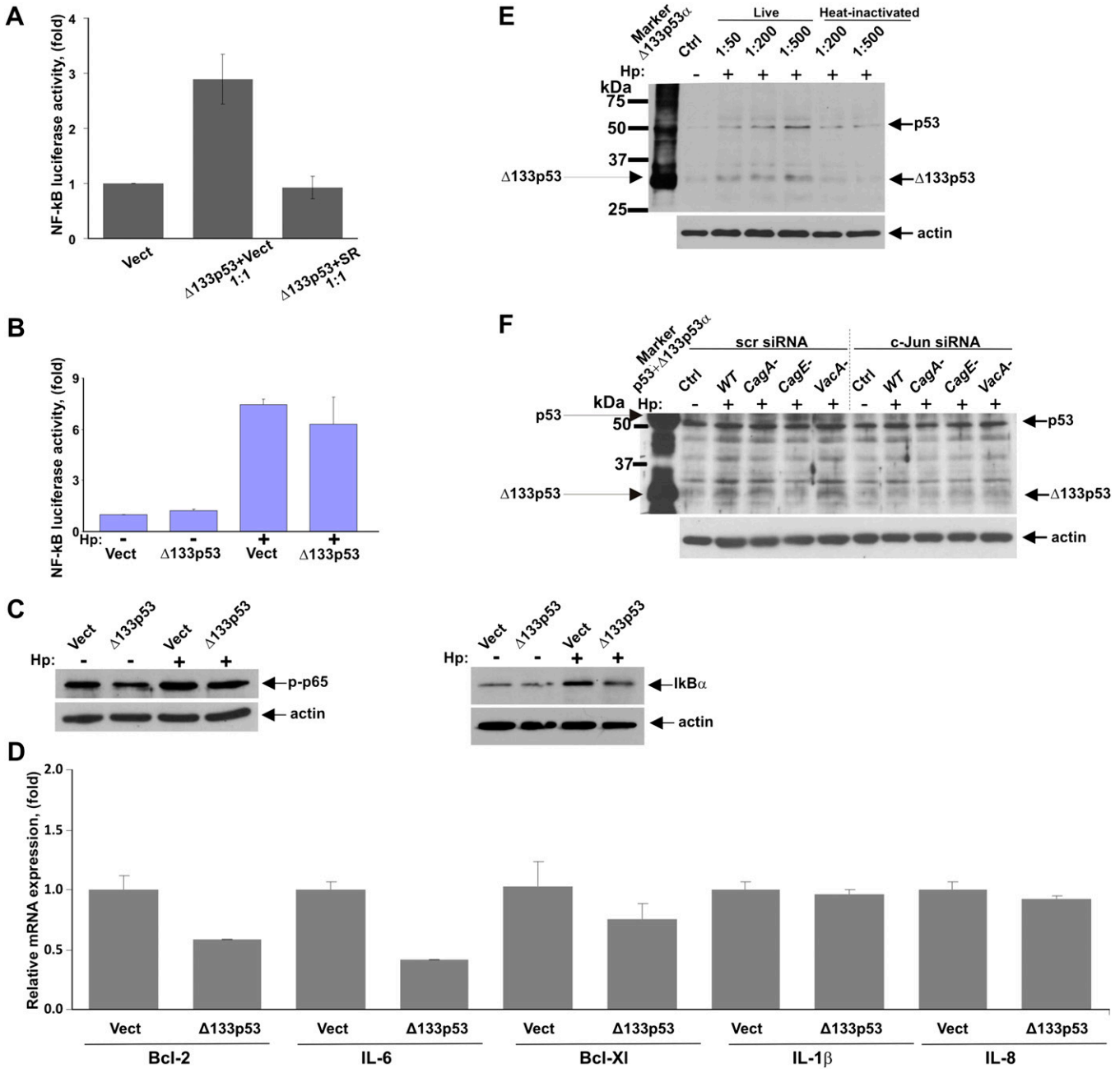

Fig. S4. Regulation of NF-κB activity by Δ133p53. (*A*) Gastric cancer cell line AGS was cotransfected with Δ133p53 and either empty vector or NF-κB inhibitor, IkB super repressor (SR), at a 1:1 ratio. NF-κB activity was assessed using the pNFκB-Luc reporter. (*B*) NF-κB activity was assessed in p53-null Kato III cells transfected with either Δ133p53 expression vector (Δ133p53) or empty control vector (Vect) and co-cultured with *Helicobacter pylori* (Hp) (+) for 8 h or left uninfected (–). NF-κB activity was assessed using the pNFκB-Luc reporter. (*C*) Kato III cells stably transfected with Δ133p53 or empty control vector were co-cultured with *H. pylori* for 3 h and analyzed for phosphorylation of p65 (RelA) protein at serine 536 (*Left*) and IκBα protein (*Right*). (*D*) Kato III cells were transfected with either Δ133p53 or empty control vector for 48 h and analyzed for mRNA expression of the indicated NF-κB target genes by quantitative PCR. (*E*) Protein lysates were prepared from control SNU-1 cells (–) or those co-cultured with *H. pylori* (+) strain J166 at the indicated cell/bacteria ratios or with heat-inactivated bacteria and were analyzed for expression of Δ133p53 protein by Western blotting. (*F*) SNU-1 cells were transfected with either siRNA against c-Jun or scrambled siRNA for 48 h and then co-cultured with WT *H. pylori* strain J166 or its isogenic *cagA*-, *cagE*-, or *vacA*-null mutant for an additional 8 h. Expression of Δ133p53 protein was assessed by Western blotting.

Table S1. Primer sequences for quantitative RT-PCR analyses

Gene	Forward	Reverse
Human genes		
p53	TAACAGTTCCTGCATGGGCGGC	AGGACAGGCACAAACACGCACC
Δ133p53	TGCCCTGACTTTCAACTCTGT	GGCCAGACCATCGCTATCT
IL-6	CGCCCCACACAGACAGCCAC	TCACCAGGCAAGTCTCCTCATTGAA
IL-1β	GATGAAGTGCTCCTTCCAGGACCT	TGCTGTGAGTCCCGGAGCGT
IL-8	TGTGAAGGTGCAGTTTTGCCAAGG	GTTGGCGCAGTGTGGTCCACTC
Bcl-2	GGATAACGGAGGCTGGGATGCCT	CAAGCTCCCACCAGGGCCAAA
Bcl-xL	GGTATTGGTGAGTCGGATCG	TGCTGCATTGTTCCCATAGA
Gerbil genes		
p53	CCTGTCATCCTTTGTCCCTT	GGAGTACGTGCATGTGACAG
Δ153p53	TGGCCTTCCCCTGATCCTCCA	CGCGGATCGGACGCTTCGTT
GAPDH	TTCAACGGCACAGTCAAGGC	GCCTTCTCCATGGTGGTGAAG