Supporting Information

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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 Δ 160p53 isoform. As size markers, cells were transfected with either fullength p53 or Δ 133p53 α 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 Δ 133p53 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 Δ 133p53 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.

Α	160
Human	MFCQLAKTCPVQLWVDSTPPPGTRVRAMAIYKQSQHMTEVVRRCPHHERCSDSDG
Gerbil Mouse	MAIYKNSQHMTEVVRRCPHHERCSENEASDPRG MAIYKKSOHMTEVVRRCPHHERCSDGDG
Human	LAPPQHLIRVEGNLRVEYLDDRNTFRHSVVVPYEPPEVGSDCTTIHYNYMCNSSCMGGMN
Gerbil	RAPPQHLIRVEGNLHAEYVDDRQTFRHSVLVPYESPEVGSDCTTIHYNYMCNSSCMGGMN
Mouse	LAPPQHLIRVEGNLYPEYLEDRQTFRHSVVVPYEPPEAGSEYTTIHYKYMCNSSCMGGMN
Human	RRPILTIITLEDSSGNLLGRNSFEVRVCACPGRDRRTEEENLRKKGEPHHELPPGSTKRA
Gerbil Mouse	RRPILTIITLEDPSGNLLGRNSFEVRVCACPGRDRRTEEENLRKKQR-CPELPQGSAKRA RRPILTITTLEDSSGNLLGRDSFEVRVCACPGRDRRTEEENFRKKEVLCPELPPGSAKRA
nouse	
Human	I. PNNTSSSPOPKKK PI. DGE Y FTI. O I RGRER FEMFRE I. NEA I. E I. KDAOAGKE PGGSRAHSS
Gerbil	LPTNTSSSPQSKRKPADGEYFTLKIRGRKRFEVFRELNEALELKDAQAAGESGDGRAQAS
Mouse	LPTCTSASPPQKKKPLDGEYFTLKIRGRKRFEMFRELNEALELKDAHATEESGDSRAHSS
Human	HLKSKKGQSTSRHKKLMFKTEGPDSD
Mouse	YLKTKKGQSTSRHKKTMVKKVGPDSD
В	C
В	Bethyl) D
B	C pylori $+$ $+$ $IP: $ $\stackrel{S}{\cong}$ $\stackrel{Contriment}{E} $ $\stackrel{IP: }{\longrightarrow} $ $\stackrel{V}{\longrightarrow} $ $\stackrel{Input}{-} $ $\stackrel{N/S}{-} $ $\frac{p73 (Bethyl)}{-}$
B Hp: <u>Control</u> <u>H</u>	C . pylori + + + ↓ L153p53 C C IP: ½ L HP: ½ L H
B Hp: +	C $\frac{pylori}{+}$ $+ \Delta 153p53$ $+ actin$ C $Hp: \qquad P63$ $WB: p53(DO-11)$ C $+ Ab$
B Hp: +	C $\frac{pylori}{+ +}$ $(P: \overset{\circ}{\Sigma} \overset{\circ}{E} \overset{\circ}{H})$ $(P: \overset{\circ}{\Sigma} \overset{\circ}{H})$ $(P: \overset{\circ}{\Sigma} \overset{\circ}{E} \overset{\circ}{H})$ $(P: \overset{\circ}{H})$
B Hp: <u>Control</u> <u>H</u>	C $\frac{pylori}{+ + +}$ $(P: \overset{S}{\geq} \overset{C}{\leftarrow} + p63$ $WB: p63(4A4)$ $(P: \overset{S}{\rightarrow} \overset{C}{\leftarrow} Ab$ $WB: p63(4A4)$
B Hp: $\frac{Control}{-}$ $\frac{H}{+}$	C $Pylori$ $+$ $+$ $+$ $+$ $+$ $+$ $+$ $+$ $+$ $+$
B Hp: $\frac{Control}{+}$	C $Pylori$ $+$ $+$ $+$ $+ \Delta 153p53$ $+ actin$ $IP: 2$ E $+ p63$ $WB: p53(DO-11)$ $WB: p63(4A4)$ $WB: p63(4A4)$ $KATO III$
B Hp: <u>Control</u> <u>H</u> + D (ploj), ejcy 2	C $Pylori$ $+ +$
B Hp:	C $Pylori$ $+$ $+$ $+$ $+$ $+$ $+$ $+$ $+$ $+$ $+$
B Hp:	C $Pylori$ $+ + +$ $+ - \Delta 153p53$ $+ - actin$ $IP: 2$ $P = P63$ $WB: p53(DO-11)$ $WB: p53(DO-11)$ $WB: p63(4A4)$ $WB: p63(4A4)$ $WB: p63(4A4)$
B Hp: <u>Control</u> <u>H</u> + D (log) (log) 3 0 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	C $Pylori$ $P: $ $P:$
B Hp: Control H + + Control 0 cell cócle	C $P = \frac{p p l o r i}{4} + \frac{1}{4} + \frac{1}{4$
B Hp: Control H Hp: Or Control H Control Of Collection of	C $P: 2$ $P:$

Fig. S3. (*A*) Sequence alignment of human, gerbil, and murine isoforms of p53. (*B*) Expression analysis of gerbil Δ 153p53 protein using Western blotting with p53 (DO-11) antibody. The Δ 153p53 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 Δ 133p53 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– Δ 133p53 protein complexes were analyzed by Western blotting (WB) with the p53 (DO-11) antibody, which recognizes the Δ 133p53 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 Δ 133p53 expression plasmid and co-cultured with *H. pylori* with *H. pylori* strain J166 for 24 h is shown.

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Fig. 54. Regulation of NF- κ B activity by Δ 133p53. (*A*) Gastric cancer cell line AGS was cotransfected with Δ 133p53 and either empty vector or NF- κ B inhibitor, lkB super repressor (SR), at a 1:1 ratio. NF- κ B activity was assessed using the pNF κ B-Luc reporter. (*B*) NF- κ B activity was assessed in p53-null Kato III cells transfected with either Δ 133p53 expression vector (Δ 133p53) or empty control vector (Vect) and co-cultured with *Helicobacter pylori* (Hp) (+) for 8 h or left uninfected (-). NF- κ B activity was assessed using the pNF κ B-Luc reporter. (*C*) Kato III cells stably transfected with Δ 133p53 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 l κ B protein (*Right*). (*D*) Kato III cells were transfected with either Δ 133p53 or empty control vector for 48 h and analyzed for mRNA expression of the indicated NF- κ B 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 Δ 133p53 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 *W H. pylori* strain J166 or its isogenic *cagA*-, *cagE*-, or *vacA*-null mutant for an additional 8 h. Expression of Δ 133p53 protein was assessed by Western blotting.

Gene	Forward	Reverse
Human genes		
p53	TAACAGTTCCTGCATGGGCGGC	AGGACAGGCACAAACACGCACC
∆133p53	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
∆153p53	TGGCCTTCCCCTGATCCTCCA	CGCGGATCGGACGCTTCGTT
GAPDH	TTCAACGGCACAGTCAAGGC	GCCTTCTCCATGGTGGTGAAG

Table S1.	Primer sequences	for quantitative	RT-PCR analyses
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