Supplementary Figure 1. Time-dependent induction of the AGS cell elongation phenotype by *H. pylori*.

AGS cells were infected for the indicated times with *H. pylori* strain 26695. The number of elongated cells in each experiment was quantitated in triplicate in 10 different 0.25 mm² fields.

Supplementary Figure 2. Nature of the three CagA protein species detected by 2-DE.

(A) MKN-28 cells were infected for the indicated times with *H. pylori* strains 26695 or TN2-GF4. Cell lysates were separated by 2-DE and phosphorylation of injected CagA was examined using α -PY-99 and α -CagA antibodies. (B) AGS cells were infected for the indicated times with strain 26695 or isogenic $\Delta virB5$, $\Delta virB10$ or $\Delta cagA$ mutants. Separation of CagA protein species revealed either one full-length CagA^{PY} spot (spot 1, red arrows, pl=7.0) or two spots (spots 1 and 2; spot 2, green arrows, pl=7.5) as indicated. The α -CagA antibody probe revealed a third spot of non-phosphorylated CagA (spot 3, blue arrows, pl=7.5). Overlay of both exposures yielded two or three spots as shown. As another control, treatment of wt-infected cell lysates with the protein tyrosine phosphatase PTP-N1 led to significant attenuation of spots 1 and 2, and concomitant intensification of non-phospho CagA at spot 3.

Supplementary Figure 3. Time-dependent activation of c-Src and c-Abl kinases by *H. pylori*. (A) AGS cell Sc-rc or c-Abl phosphorylation during infection with *H. pylori* 26695 for the indicated time intervals was assessed by Western blotting using α -c-Src-

PY-418 and α -c-Abl-PY-412 antibodies, respectively. α -GAPDH immunoblot intensities served as loading control. (**B**) AGS cell c-Src or c-Abl phosphorylation shown in *panel* A was quantified by densitometric measurement of immunoblot band intensities using a luminescence image analyzer.

Supplementary Figure 4. Pharmacological inhibition of c-Src and c-Abl blocks *H. pylori*-induced cell scattering and elongation. (A) Phase contrast micrographs of AGS cells infected with *H. pylori* strain 26695 in the absence or presence of the c-Src inhibitor PP2 (10 μ M) or the c-Abl inhibitor SKI- DV2-43 (1 μ M). Both inhibitors block *H. pylori*-induced cell scattering and elongation. (B) The effect of the inhibitors on c-Src and c-Abl kinase activity was assessed by immunoblotting (arrows). α -Src and α -Abl immunoblot intensities served as loading control. (C) AGS cell c-Src or c-Abl phosphorylation shown in *panel* B was quantified by densitometric measurement of immunoblot band intensities using a luminescence image analyzer. (D) The number of elongated cells was quantitated in triplicate in 10 different 0.25 mm² fields.

Supplementary Figure 5. *In vitro* phosphorylation of CagA from the East Asian strain TN2-GF4 by c-Abl or c-Src kinases. Lysates of *H. pylori* expressing the EPIYA-ABD CagA version were subjected to *in vitro* phosphorylation assays using recombinant c-Src kinase or c-Abl kinase as indicated. Immunoblotting using a phospho-specific α -PY-CagA-EPIYA-C/D and α -CagA antibodies (arrows). This antibody does not cross-react with phosphorylated EPIYA-A or –B motifs (data not shown), indicating that both c-Src and c-Abl phosphorylate CagA at the EPIYA-D motif in TN2-GF4.

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Supplementary Figure 6. Role of EPIYA-motifs in CagA phosphorylation during infection of AGS cells with *H. pylori*.

(A) AGS cells were infected for 4 hours with CagA-expressing *H. pylori* strains as indicated. Phosphorylation of wt CagA and Y>F substitution mutants was assessed by immunoblots using α -PY-99 and α -CagA antibodies (arrows). (B) Relative CagA phosphorylation levels were quantified by densitometric measurement of band intensities using a luminescence image analyzer.

Supplementary Figure 7. Interaction of wt CagA and phospho-mimetic CagA mutants with host signalling factors during *H. pylori* infection. AGS cells were infected for 4 hours with CagA-expressing *H. pylori* strains as indicated, and cell lysates were subjected to immunoprecipitation (IP) with α -CagA antibodies. Western blotting using α -Csk (C), α -PI3-K (D) or α -SHP-2 (E) indicate that each of these factors formed a complex with wt CagA and with some but not all selected phospho-mimetic CagA EPIYA^{Y>D} mutants.

Supplementary Figure 8. Sequence comparison of the EPIYA-motifs in CagA proteins from different clinical *H. pylori* strains used in Figure 7 and Figure S9.

The respective sections in the corresponding *cagA* genes were amplified by PCR and sequenced. Protein sequence alignment was done using the ClustalW2 program (http://www.ebi.ac.uk/Tools/msa/clustalw2/). Western CagA sequences (blue) and East

Asian CagA sequences (black) are shown. The EPIYA-motifs in the regions A, B C and/or D are shaded with yellow.

Supplementary Figure 9. Clinical East Asian *H. pylori* strains expressing CagA EPIYA-motifs AD or BD, but not AB, induce moderate AGS cell elongation. (A) AGS cells were infected for 4 hours with indicated strains expressing combinations of two EPIYA-motifs, specifically AB, BD or AD, and two strains expressing all three ABD EPIYA-motifs as control (specific EPIYA-motifs and flanking sequences are shown in Figure S8). CagA phosphorylation was examined using α -PY-CagA and α -CagA antibodies (arrows). The asterisk in the lower panel indicates antibody cross-reactivity with an unknown phosphorylated host cell protein. (B) Quantification of CagA phosphorylation signals using the luminescence image analyzer. (C) The number of elongated cells in each experiment was quantitated in triplicate in 10 different 0.25 mm² fields.

Supplementary Figure 10. Dual infection of *H. pylori* strains expressing different single phosphorylatable or phospho-mimetic EPIYA-motifs induces AGS cell elongation.

AGS cells were infected for 4 hours with CagA EPIYA^{Y>F} (**A**) or EPIYA^{Y>D} (**B**) mutant *H. pylori* strains as indicated. Representative phase contrast micrographs of infected AGS cells from the experiments in Figure 8A/B are shown.







Α









Western st	rains NH ₋ CagA (TIGR 26695)		A B C/	D -COOH
1 885 960 1186 East-Asian strains 1 <th1< th=""> <th1< th=""> 1 1</th1<></th1<>					
	Δ		в		
26695					
20095	NNNNNGLKNRAFPT	AKVNKKKTGOVAS	PEEPTYTO	VAKKVTPKID	OLNOTASGLGG
NCTC11638	NNNNNNGLKN~~FPT	AKVNKKKAGOAAS	LEEPIYAO	VAKKANAKID.	RINOTASCI.GV
Но77	NNNNNGLKN~~EPTY	AKVNKKKAGOVAS	PEPTYAO	VAKKVNAKID	RLNOTASGLGG
J99	NNNNNGLENST~~~~	~~~~~~~~~~~~	~~ EPTYTO	VAKKVKAKID	RLDOTASGLGD
P344	NNNNN~GLENST~~~~	. ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	~~EPIYTO	VAKKVKAKID	RLDOIASGLGD
USA2964	NNNNNGLKN~~EPIY	AKVNKKKAGOAA~	~~~~~~~	~~~~~~~~	~~~~~~~~~~
Hp51	NNNNNGLKNSTEPIY	AKVNKKKAGQAAA	~~~~~~~	~~~~~~~~~	~~~~~~~~~
Oki105	NNNNNNGLKN~~ <mark>EPIY</mark>	AEVNKKKTGQVAS	PEEPIYTQ	VAKKVKAKID:	RLDQIASGLGG
Oki149	NNNNNNGLKN~~ <mark>EPIY</mark>	AKVNKEKAGQATS	PEESIYT	VAKKVKAKID	QLDQIASGFGN
Cal9	SNNNNNGLKNNT~~~~		~~ <mark>EPIYA</mark> Q	VAKKVSAKID	QLNEATSAINR
3461	SNNNNNGLKNNT~~~~	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	~~ <mark>EPIYA</mark> Q	VAKKVSAKID	QLNEATSAINR
Oki326	SNNNNNGLKNNT~~~~	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	~~ <mark>EPIYA</mark> R	VAKKVSAKID	QLNEATSAINR
Oki388	SNNNNNGLKNNT~~~~	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	~~ <mark>EPIYA</mark> Q	VAKKVSAKID	QLNEATSAINR
TN2-GF4	SNNNNNGLKNNT <mark>EPIY</mark>	<mark>A</mark> QVNKKKAGQATS	PE <mark>EPIYA</mark> Q	VAKKVSAKID	QLNEATSAINR
35A	SNNNNNGLKNST <mark>EPIY</mark>	<mark>a</mark> kvnkkktgqaas:	PE <mark>EPIYA</mark> Q	VAKKVSAKID	QLNEATLAINR
			•		
			C		
26695	VGQAAG~FPLKRHDKV	/DDLSKVGLSASP~	EPIYATID	DLGGP~~~FP	LKRHDKVDDLS
2003-370	VGQVAG~FPLKRHDKV	/DDLSKVGRSVSP~	<mark>epiya</mark> tid	DLGGP~~~FP	LKRHDKVDDLS
NCTC11638	VGQAAG~~~~~~~	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	~~~~~~~	~~~~FP	LKRHDKVDDLS
Hp77	VEQAVG~~~~~~~	~~~~~~~~~	~~~~~~~	~~~~FP	LKRHDKVDDLS
J99	VGQAAS~FLLKRHDKV	/DDLSKVGLSANH~	EPIYATID	DLGGP~~~FP	LKRHDKVDDLS
P344	VGQAAS~FLLKRHDKV	/DDLSKVGLSASP~	EPIYATID	DLGGP~~~FP	LKRHDKVDDLS
USA2964	~~~~G~FPLKKHGK\	/DDLSKVGLSASP~	EPIYATID	DLGGP~~~FP	LKRHDKVEDLS
Hp51	~~~~G~F'PLKRHDK\	/DDLSKVGRSVSP~	EPIYA <mark>T</mark> ID	DLGGP~~~FP	LKRHDKVDDLS
Oki105	VG~QAG~~~~~~~	\sim	~~~~~~~	~~~~FS	LKGHTKVGDLS
Oki149	VG~QAG~~~~~~~			~~~~F'P.	LKRHTKVDDLS
Caly	KIDRINKIASAGKGVG	GFSGA~GRSASP~	EPIYATID	FDEANQAGFP	LKKSTAVNDLS
3461	KIDRINKIASAGKGVG	JGFSGA~GRSASPE	EPIYATID	f deanqagf'P.	LKKSAAVNDLS
UK1326	KIDRINKIASAGKGVO	JGESGA~GRSASP~	EPIYATID	e deanqage P.	LKKSAAVNDLS
UKIJUK	KIDKINKIASAGKGV(GESGA~GRSASP~	EPIYATID	e detinqage'P.	LKKSAAVNDLS
INZ-GE4	NIDKINKIASAGKGV(JGESGA~GRSASP~	EFIIATID	e delanqage P.	LKKHAAVNULS
ACC	AIDKINKIASAGKGVO	bgr SGA~GKSASP~ <mark>.</mark>	EPIIA <mark>FID</mark>	e de tinqage P.	LKKSAAVNDLS

Supplementary Figure 9



