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 mm^2 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 Δ*virB5*, Δ*virB10* or Δ*cagA* mutants. Separation of CagA protein species revealed either one full-length $CagA^{PY}$ spot (spot 1, red arrows, pI=7.0) or two spots (spots 1 and 2; spot 2, green arrows, pI=7.5) as indicated. The α-CagA antibody probe revealed a third spot of non-phosphorylated CagA (spot 3, blue arrows, pI=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 μM) or the c-Abl inhibitor SKI- DV2-43 (1 μM). 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 mm^2 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.

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 assesed 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

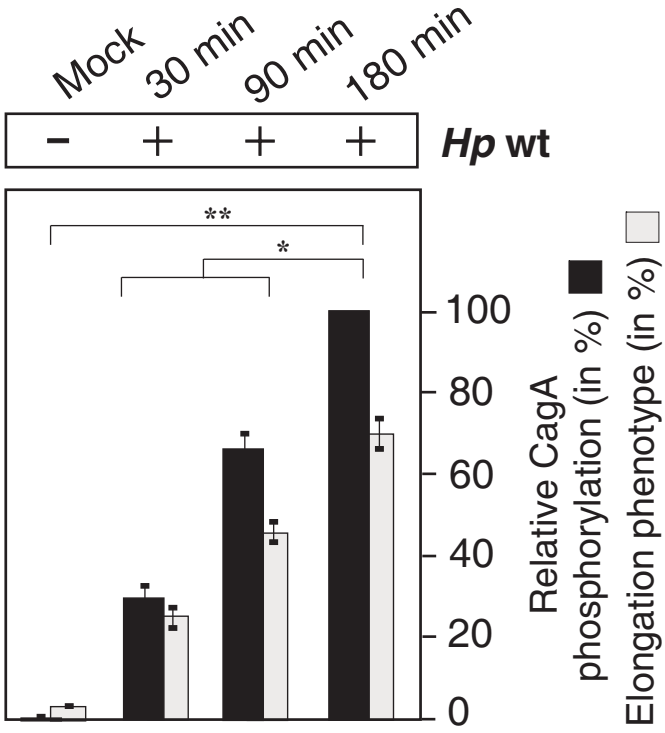
Asian CagA sequences (black) are shown. The EPIYA-motifs in the regions A, B C and/or D are shaded with yellow.

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 mm^2 fields.

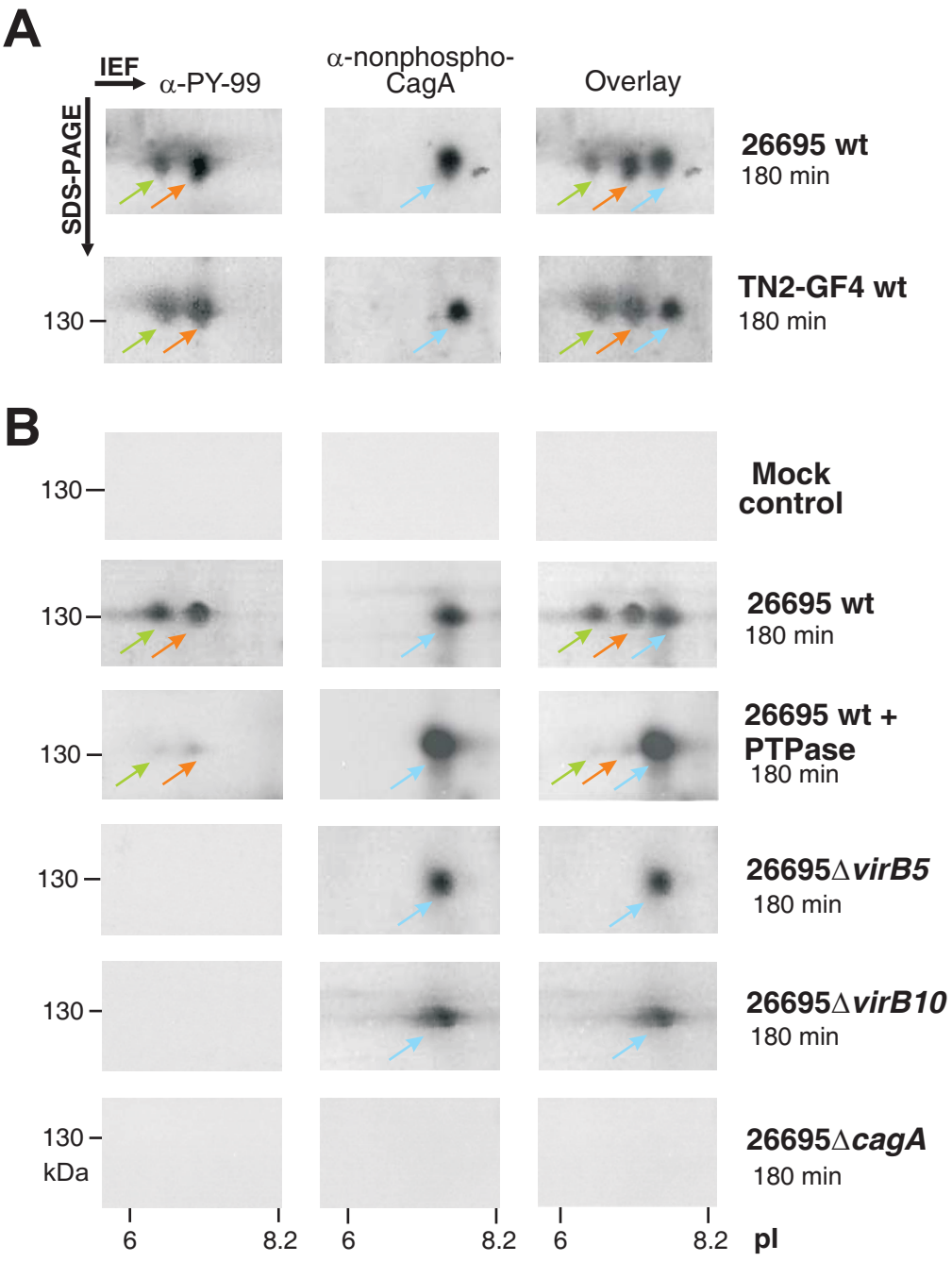
Supplementary Figure 10. Dual infection of *H. pylori* strains expressing different single phosphorylatable or phospho-mimetic EPIYA-motifs induces AGS cell elongation.

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.

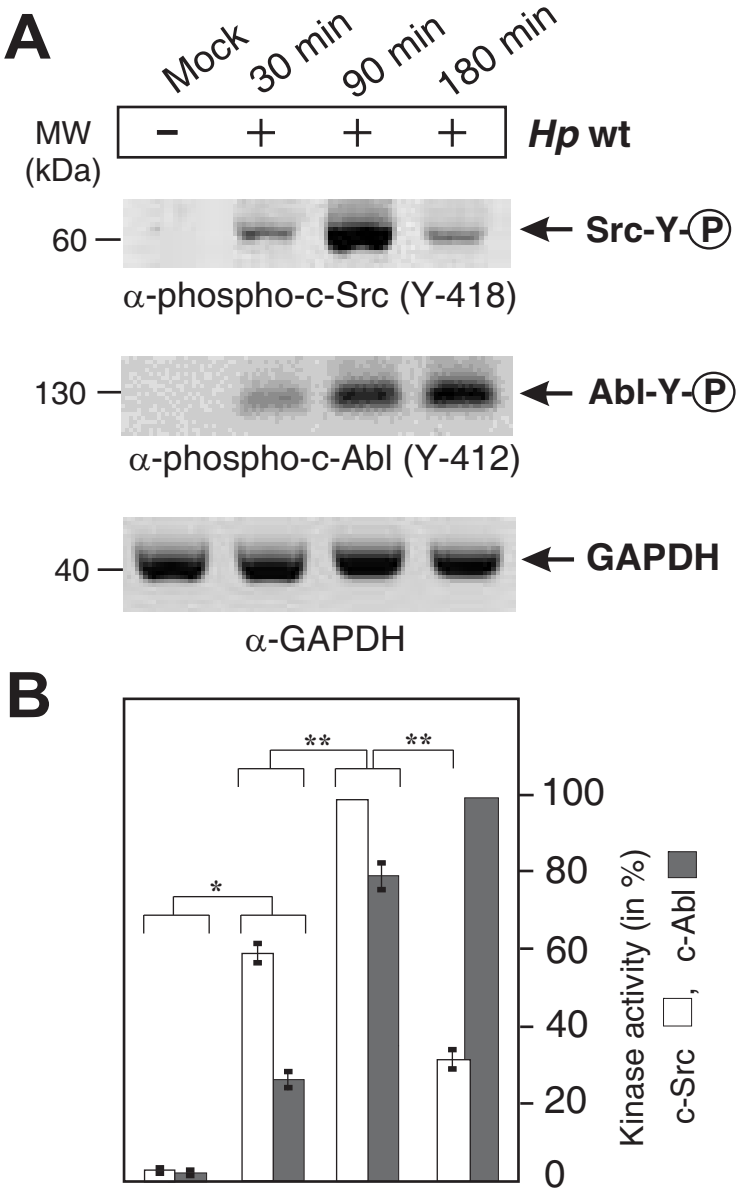
Supplementary Figure 1



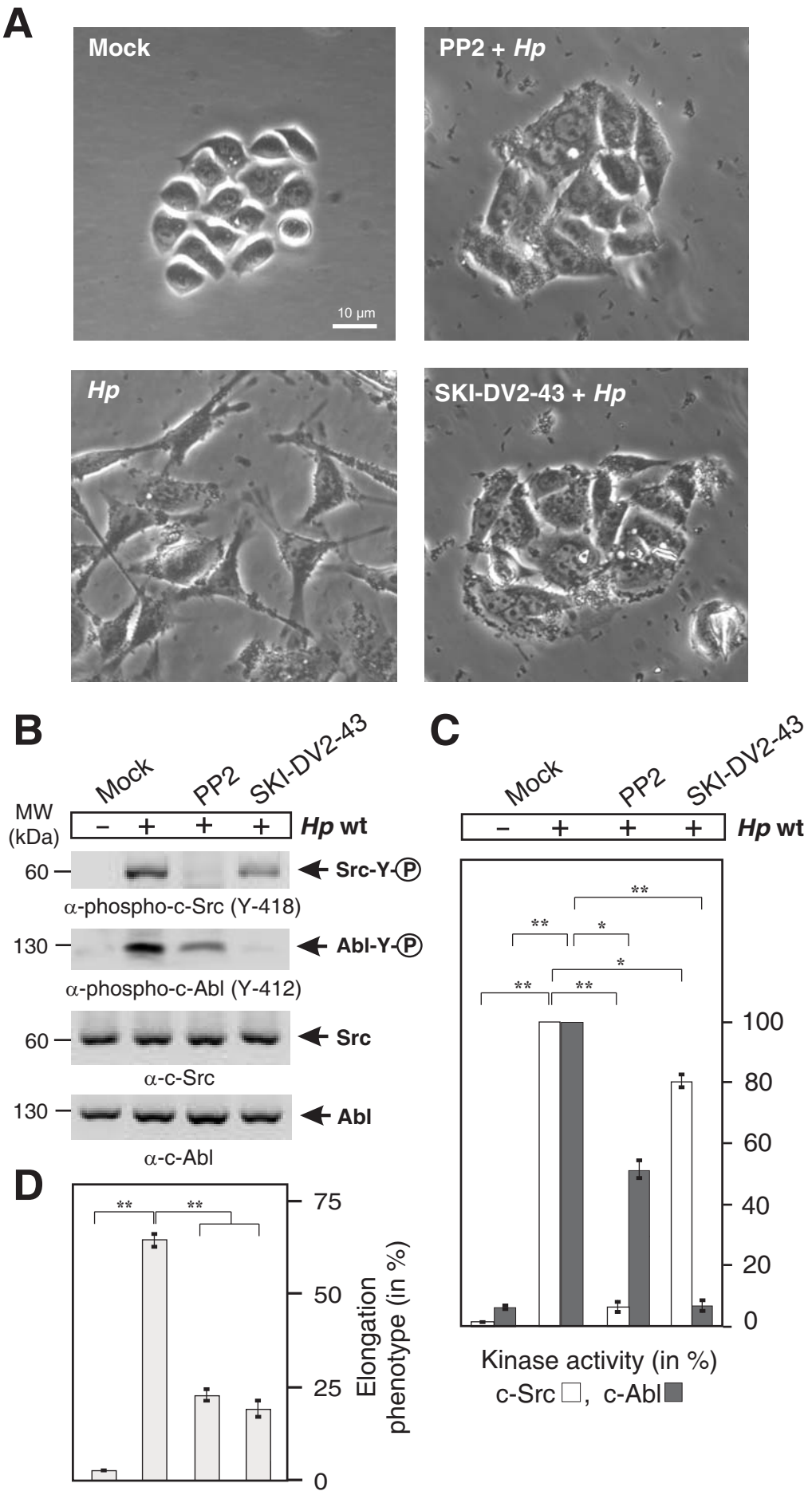
Supplementary Figure 2



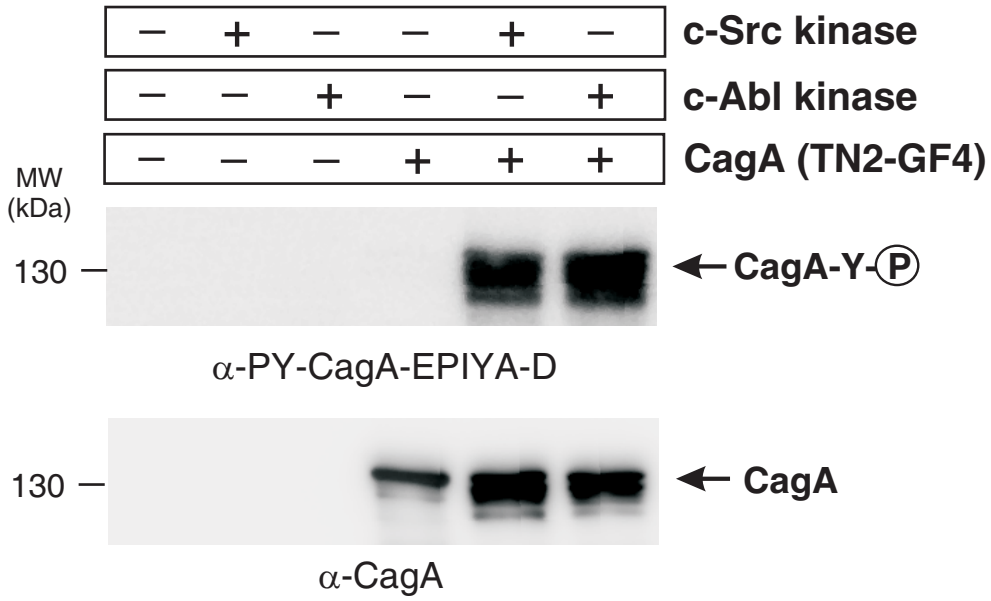
Supplementary Figure 3



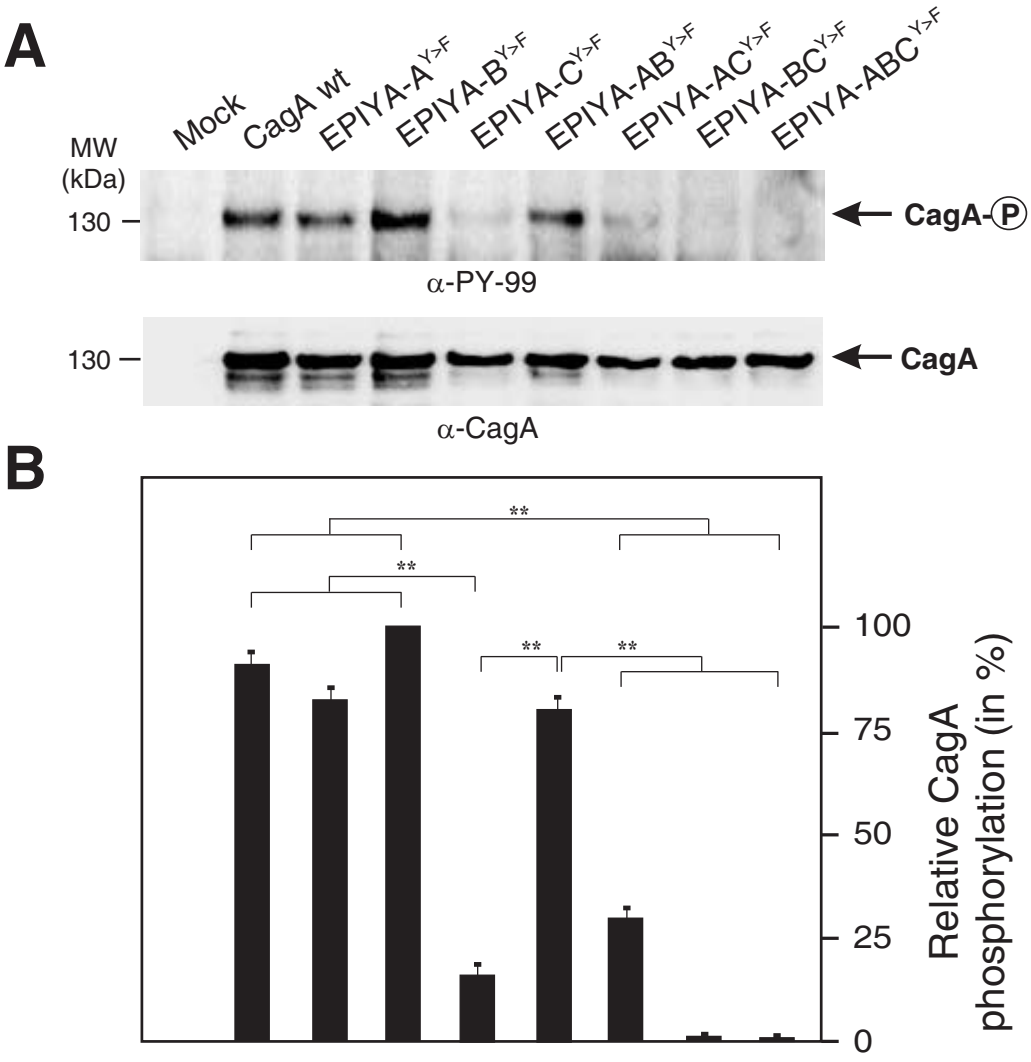
Supplementary Figure 4



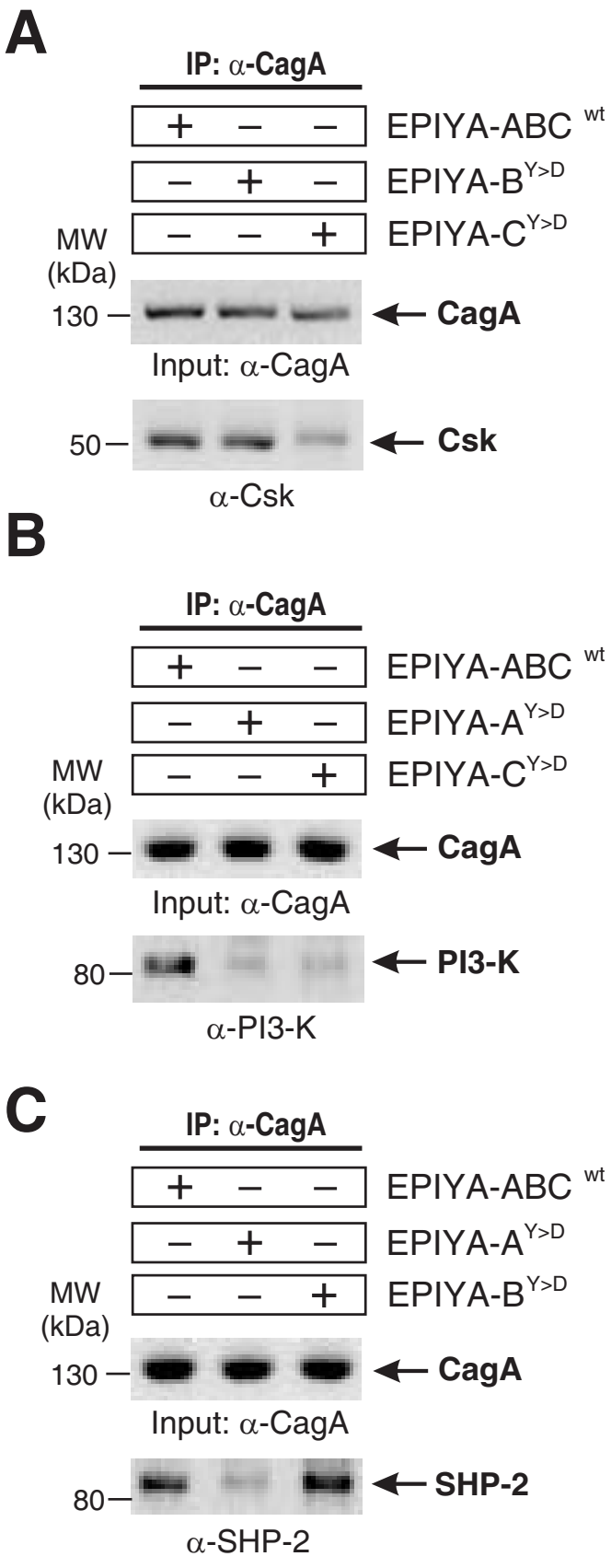
Supplementary Figure 5



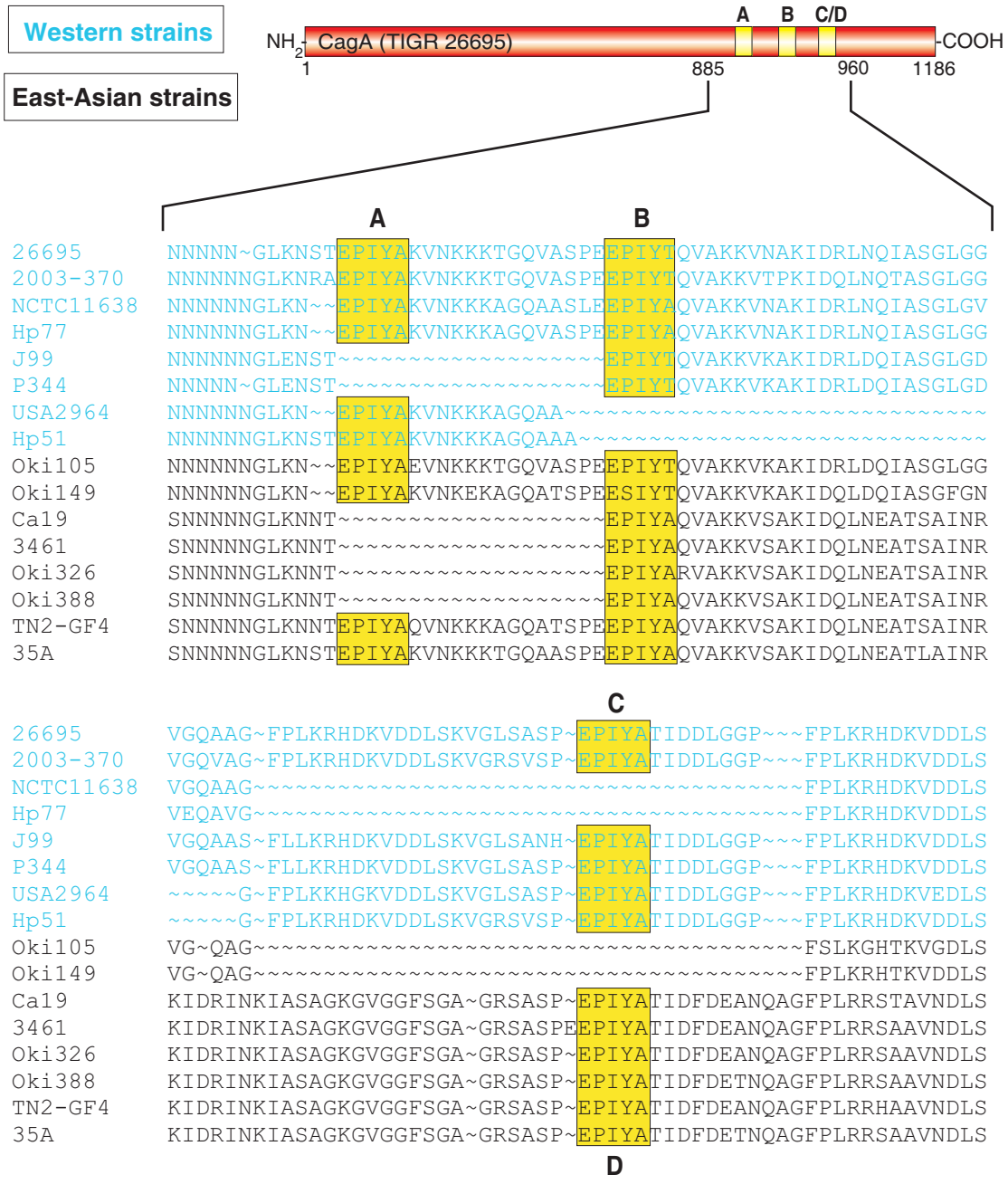
Supplementary Figure 6



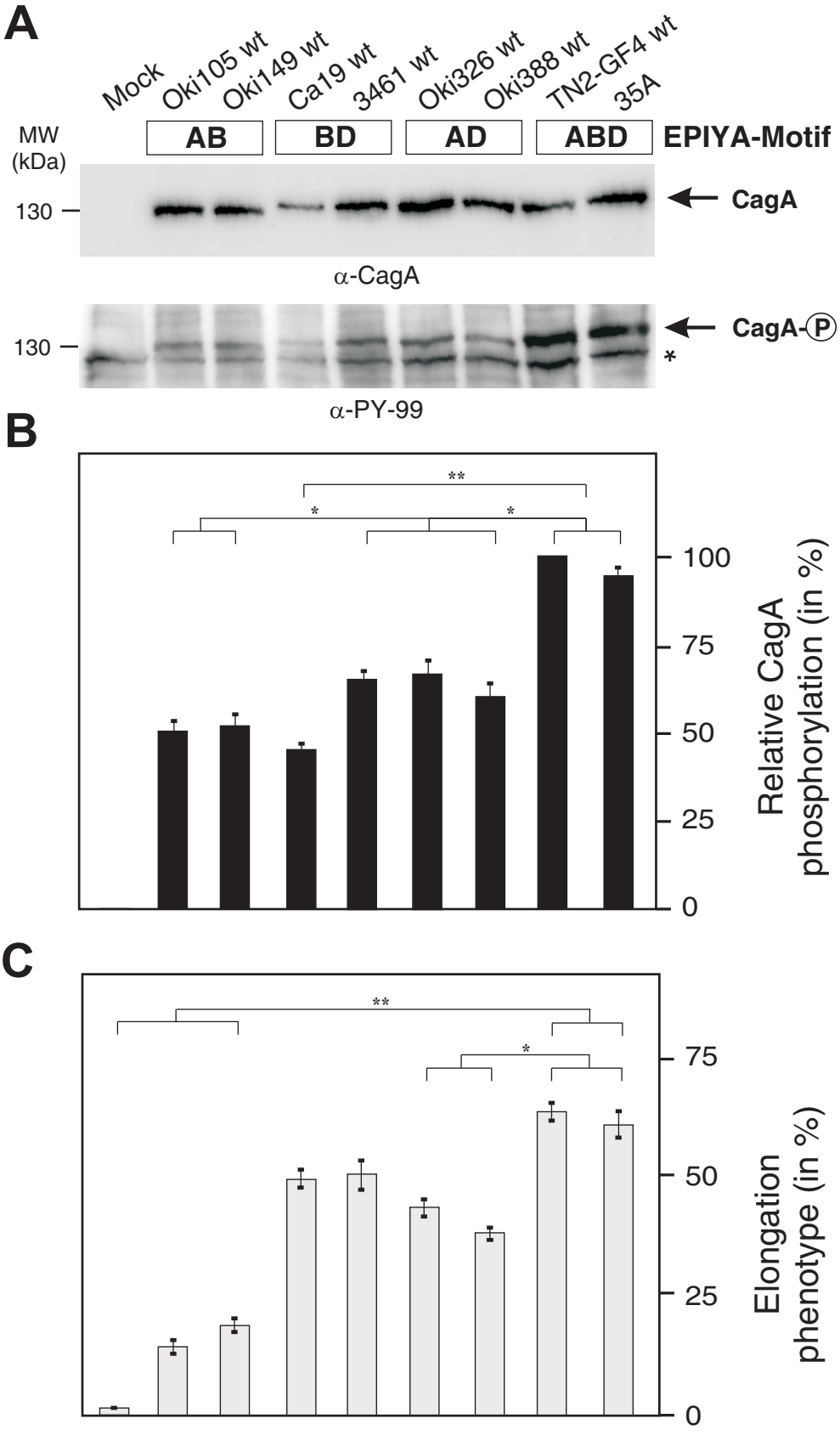
Supplementary Figure 7



Supplementary Figure 8



Supplementary Figure 9



Supplementary Figure 10