

Supplementary

***Helicobacter pylori* CagA Induces Cortactin Y-470 Phosphorylation-Dependent Gastric Epithelial Cell Scattering via Abl, Vav2 and Rac1 Activation**

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Table S1. Key resources of the present study.

Reagent	Source	Identifier
Antibodies		
Mouse monoclonal anti-GAPDH	Santa Cruz Biotechnology, Dallas, TX, USA	Cat# sc-47724; RRID:AB_627678
Mouse monoclonal anti-phosphotyrosine PY99	Santa Cruz Biotechnology, Dallas, TX, USA	Cat# sc-7020; RRID:AB_628123
Rabbit polyclonal anti-CagA	Austral Biologicals, San Ramon, CA, USA	Cat# HPP-5003-9; RRID: AB_10920428
Mouse monoclonal anti- β -Actin	Sigma-Aldrich, St. Louis, MO, USA	Cat# A5441, RRID:AB_476744
Mouse monoclonal anti-AIIB2 integrin- β 1	Developmental Studies Hybridoma Bank, Iowa City, IA, USA	Cat#AB_528306; RRID:AB_528306
Rabbit polyclonal anti-Vav2	Santa Cruz Biotechnology, Dallas, TX, USA	Cat# sc-20803; RRID:AB_793922
Mouse monoclonal anti-Rac1	Cytoskeleton, Denver, CO, USA	Cat# ARC03; RRID:AB_2721173
Mouse monoclonal anti-Cortactin	Merck-Millipore, Burlington, MA, USA	Cat# 05-180; RRID:AB_309647
Rabbit polyclonal anti-Cortactin, phospho-specific (Tyr421)	Merck-Millipore, Burlington, MA, USA	Cat# AB3852; RRID:AB_177443
Rabbit polyclonal anti-Cortactin, phospho-specific (Tyr466)	Merck-Millipore, Burlington, MA, USA	Cat# AB3795; RRID:AB_569553
Rabbit polyclonal anti-Cortactin, phospho-specific (Tyr486) antibody	Merck-Millipore, Burlington, MA, USA	Cat# AB3853; RRID:AB_177444
Rabbit polyclonal anti-Abl, phospho-specific (Y412)	Sigma-Aldrich, St. Louis, MO, USA	Cat# 07-788; RRID:AB_612042
Rabbit polyclonal anti-GFP	Thermo Fisher Scientific, Waltham, MA, USA	Cat# A-11122; RRID:AB_221569
Goat polyclonal anti-Rabbit IgG (H + L) Cross-Adsorbed, FITC	Thermo Fisher Scientific, Waltham, MA, USA	Cat# F-2765, RRID:AB_253652
Rabbit polyclonal anti-Vav2	Proteintech, Rosemont, IL, USA	Cat# 21924-1-AP, RRID:AB_11182283
Goat anti-Rabbit IgG (H + L), HRP	Thermo Fisher Scientific, Waltham, MA, USA	Cat# 31462; RRID:AB_228338
Goat anti-Mouse IgG, IgM (H + L), HRP	Thermo Fisher Scientific, Waltham, MA, USA	Cat# 31446; RRID:AB_228318
Bacterial Strains		
<i>Helicobacter pylori</i> strain P1	[1]	not available

<i>Helicobacter pylori</i> strain P12	[2]	not available
Inhibitors		
Imatinib mesylate	Sigma-Aldrich, St. Louis, MO, USA	Cat# SML1027
SKI-DV2-43	[3]	not available
Commercial Kits		
Bradford assay kit	Bio-Rad, Hercules, CA, USA	Cat# 5000002
Rac1 activation assay kit	Cytoskeleton, Denver, CO, USA	Cat# BK035
Cell Lines		
AGS	ATCC, Manassas, VA, USA	Cat# CRL-1739; RRID:CVCL_0139
siRNA Oligonucleotides		
Cortactin siRNA (h)	Santa Cruz Biotechnology, Dallas, TX, USA	Cat# sc-35093
Control siRNA-A	Santa Cruz Biotechnology, Dallas, TX, USA	Cat# sc-37007
Vav2 siRNA (h)	Santa Cruz Biotechnology, Dallas, TX, USA	Cat# sc-41738
Rac1 siRNA (h)	Santa Cruz Biotechnology, Dallas, TX, USA	Cat# sc-36351
Recombinant DNA Plasmids		
Mouse Cortactin wt-EGFP	[4]	not available
Mouse Cortactin Y421/466/482F-EGFP	[4]	not available
Mouse Cortactin Y421/466/482D-EGFP	[4]	not available
Mouse Cortactin Y466F-EGFP	[4]	not available
Mouse Cortactin Y466D-EGFP	[4]	not available
Human Vav2 wt (HA-tagged)	S. Moores (Harvard Medical School, Boston, USA)	not available
Human Vav2 wt-EGFPC2	[5]	not available
Human Vav2 Y159F/Y172F-EGFPC2	[5]	not available
Human Vav2 R425C-EGFPC2	[5]	not available
Human Vav2 W673R-EGFPC2	[5]	not available
Human Vav2 G693R-EGFPC2	[5]	not available
Software and Algorithms		
GraphPad Prism v8.0	GraphPad Software, San Diego, CA, USA	www.graphpad.com (accessed on: 20 August 2021)
ImageJ-win64 v2.0	[6]	https://imagej.nih.gov/ij/ (accessed on: 20 August 2021)
LAS AF v4.0	Leica Microsystems, Wetzlar, Germany	https://www.leica-microsystems.com (accessed on: 20 August 2021)
Image Lab software v6.1	Bio-Rad, Hercules, CA, USA	https://www.bio-rad.com/de-de/product/image-lab-software?ID=KRE6P5E8Z (accessed on: 20 August 2021)

References

1. Backert, S.S.; Ziska, E.E.; Brinkmann, V.V.; Zimny-Arndt, U.U.; Fauconnier, A.; Jungblut, P.P.; Naumann, M.M.; Meyer, T.F. Translocation of the *Helicobacter pylori* CagA protein in gastric epithelial cells by a type IV secretion apparatus. *Cell. Microbiol.* **2000**, *2*, 155–164, doi:10.1046/j.1462-5822.2000.00043.x
2. Fischer, W.; Windhager, L.; Rohrer, S.; Zeiller, M.; Karnholz, A.; Hoffmann, R.; Zimmer, R.; Haas, R. Strain-specific genes of *Helicobacter pylori*: genome evolution driven by a novel type IV secretion system and genomic island transfer. *Nucleic Acids Res.* **2010**, *38*, 6089–6101, doi:10.1093/nar/gkq378.
3. von Bubnoff, N.; Veach, D.R.; Miller, W.T.; Li, W.; Sanger, J.; Peschel, C.; Bornmann, W.G.; Clarkson, B.; Duyster, J. Inhibition of wild-type and mutant Bcr-Abl by pyrido-pyrimidine-type small molecule kinase inhibitors. *Cancer Res.* **2003**, *63*, 6395–6404.
4. Tegtmeyer, N.; Wittelsberger, R.; Hartig, R.; Wessler, S.; Martinez-Quiles, N.; Backert, S. Serine phosphorylation of cortactin controls focal adhesion kinase activity and cell scattering induced by *Helicobacter pylori*. *Cell Host Microbe* **2011**, *9*, 520–531.
5. Tamas, P.; Solti, Z.; Bauer, P.; Illes, A.; Sipeki, S.; Bauer, A.; Downward, J.; Buday, L. Mechanism of epidermal growth factor regulation of Vav2, a guanine nucleotide exchange factor for Rac. *J. Biol. Chem.* **2003**, *278*, 5163–5171.
6. Schneider, C.A.; Rasband, W.S.; Eliceiri, K.W. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* **2012**, *9*, 671–675, doi:10.1038/nmeth.2089.