

Supplementary Information

Helicobacter pylori-induced gastric cancer is orchestrated by MRCK β -mediated Siah2 phosphorylation

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>SIAH2

MSRPSSTGPSANKPCSKQPPQPQHTPSAAPPAAATISAAGPGSSAVPAAAAVISGPG
GGGGAGPVSPQHHELTSLFECPVCFDYVLPPIQCQAGHLVCNQCRCQLSCCPTCRGA
LTPSIRNLAMEKVASAVLFPCKYATTGCSLTLHHTEKPEHEDICEYRPYSCPCPGASCKW
QGSLEAVMSHLMHAHKSITTLQGEDIVFLATDINLPGAVDWMMQSCFGHHFMLVLEKQ
EKYEGHQFFAIVLLIGTRKQAEFAYRLELNGNRRRLTWEATPRSIHDGVAAAIMNSDC
LVFDTAIAHLFADNGNLGINVTISTCCP

>MRCKβ

MSAKVRLKKLEQLLLDGPWRNESALSVETLLDVLVCLYTECSHSALRRDKYVAEFLEWA
KPFTQLVKEMQLHREDFEIIKVIKRGAFGEVAVVKMKNTERIYAMKILNKWEMLKRAETA
CFREERDVLVNGDCQWITALHYAFQDENHLYLVMDYYVGGDLLTLLSKFEDKLPEDMAR
FYIGEMVLAIDSIHQLHYVHRDIKPDNVLLDVNGHIRLADFGSCLKMNDGTVQSSVAVG
TPDYISPEILQAMEDGMGKYGPECDWWSLGVCMYEMLYGETPFYAESLVETYGKIMNH
EERFQFPSHVTVDVSEEAKDLIQRILCSRRRLGQNGIEDFKKHAFFEGLNWNENIRNLEAP
YIPDVSSPSDTSNFDVDDDDVLRNTEILPPGSHTGFSGLHLPFIGFTFTTESCFSDRGSLK
SIMQSNTLTKDEDVQRDLEHSLQMEAYERRIRLEQEKLELSRKLQESTQTVQSLHGSS
RALSNSNRDKEIKLNEEIERLKNKIADSNRLERQLEDTVALRQEREDSTQRLRGLEKQH
RVVRQEKEELHKQLVEASERLKSQAKELKDAHQQRKLALQEFSELNERMAELRAQKQK
VSRQLRDKEEEMEVATQKVDAMRQEMRRAEKLKRELEAQLDDAVAEASKERKLREHSE
NFCKQMESELEALKVKQGGRGAGATLEHQQEISKIKSELEKKVLFYEEELVRREASHVL
EVKNVKKEVHDESHQLALQKEILMLKDKLEKSKRERHNEMEEAVGTIKDKYERERAML
FDENKKLTAENEKLCFVDKLTQAQRQLEDELQDLAAKESVAHWEAQIAEIIQWVSDEK
DARGYLQALASKMTEELEALRSSSLGSRRLDPLWKVRRSQKLDMSARLELQSALEAEIR
AKQLVQEELRKVKDANLTLESKLDSEAKNRELLEEMEILKKKMEEKFRADTGLKLPDFQ
DSIFEYFNTAPLAHDLTFRSSASEQETQAPKPEASPSMSVAASEQQEDMARPPQRPSA
VPLPTTQALALAGPKPKAHQFSIKSFSSPTQCSHCTSLMVGLIRQGYACEVCSFACHVS
CKDGAPQVCPIPPEQSKRPLGVDVQRGIGTAYKGHVKVPKPTGVKKGWQRAYAVVDCD
KLFLYDLPEGKSTQPGVIASQVLDLRDDEFSSVSLASDVIAHATRRDIPCIFRVTASLLGA
PSKTSSLLILTENENEKRKWWGILEGLQSILHKNRLRNQVVHVPLEAYDSSLPLIKAILTAAI
VDADRIAVGLEEGLYVIEVTRDVIVRAADCKKVHQIELAPREKIVILLCGRNHHVHLYPWS
SLDGAEGSFDIKLPETKGCQLMATATLKRNSGTCLFVAVKRLILCYEIQRTPFHRKFNEI
VAPGSVQCLAVLRDRLCVGYPSGFCLLSIQGDGQPLNLVNPNDPSLAFLSQQSFDALCA
VELESEEYLLCFSHMGLYVDPQGRRARAEQELMWPAAPVACSCSPTHVTYSEYGVDFV
DVRTMWWQTIGLRRIRPLNSEGLNLLNCEPPRLIYFKSKFSGAVLNVPDTSNKKQML
RTRSKRRFVFKVPEEERLQQRREMLRDPPELRSKMISNPTNFNHVAHMGPGDGMQVLM
DLPLSAVPPSQEERPGPAPTNLARQPPSRNKPYISWPSSGGSEPSVTVPLRSMSDPDQ
DFDKEPDSSTKHSTPSNSSNPSGPPSPNSPHRSQPLEGLEQPACDT

-----Predicted interaction result of the 1st two proteins:-----

The probability for interacting of the 1st two proteins: **0.9943**

The probability for non-interacting of the 1st two proteins: **0.0057**

Decision: **SIAH2 and MRCKB can interact**

Fig. S1. Result of LR_PPI analysis showing the probability of Siah2-MRCKβ interaction.

A

Siah2 sites	Predicted score
<u>S</u> ⁶	0.996
<u>T</u> ²⁷⁵	0.986
<u>T</u> ²⁷⁹	0.931
<u>S</u> ²⁸²	0.992

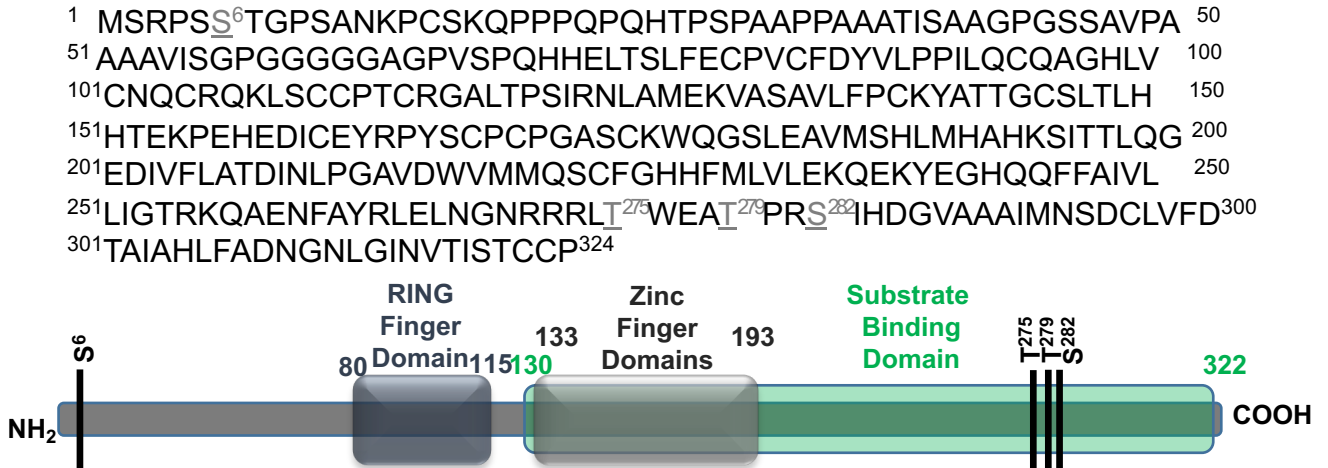
B

Fig. S2. Identification of novel phosphorylatable residues. (A) Novel Siah2 phosphorylatable residues along with their predicted scores as identified using NetPhos 3.0. (B) Sequence and multi-domain schematic depiction of human Siah2 protein. Identified highly probable phosphorylatable amino acids are underlined in the sequence and represented by vertical lines in the domain structure.

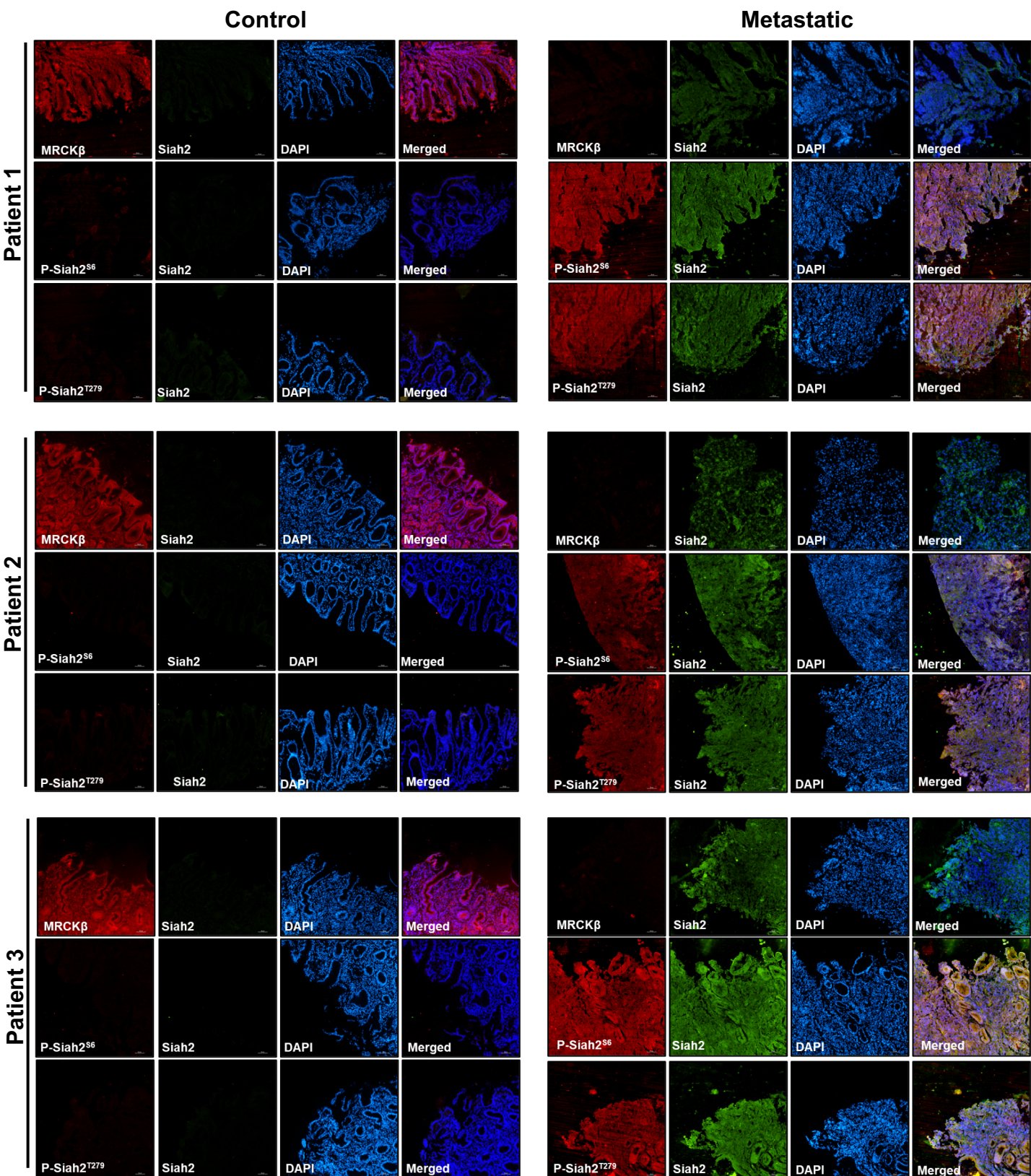


Fig. S3i. Human gastric biopsy tissues exhibit enhanced Siah2 phosphorylation and MRCKβ downregulation. Immunofluorescence microscopy images showing the status of Siah2, P-S⁶-Siah2, P-T²⁷⁹-Siah2 and MRCKβ in Patient #1-3. Objective: 20X and scale bar represents 50 μm.

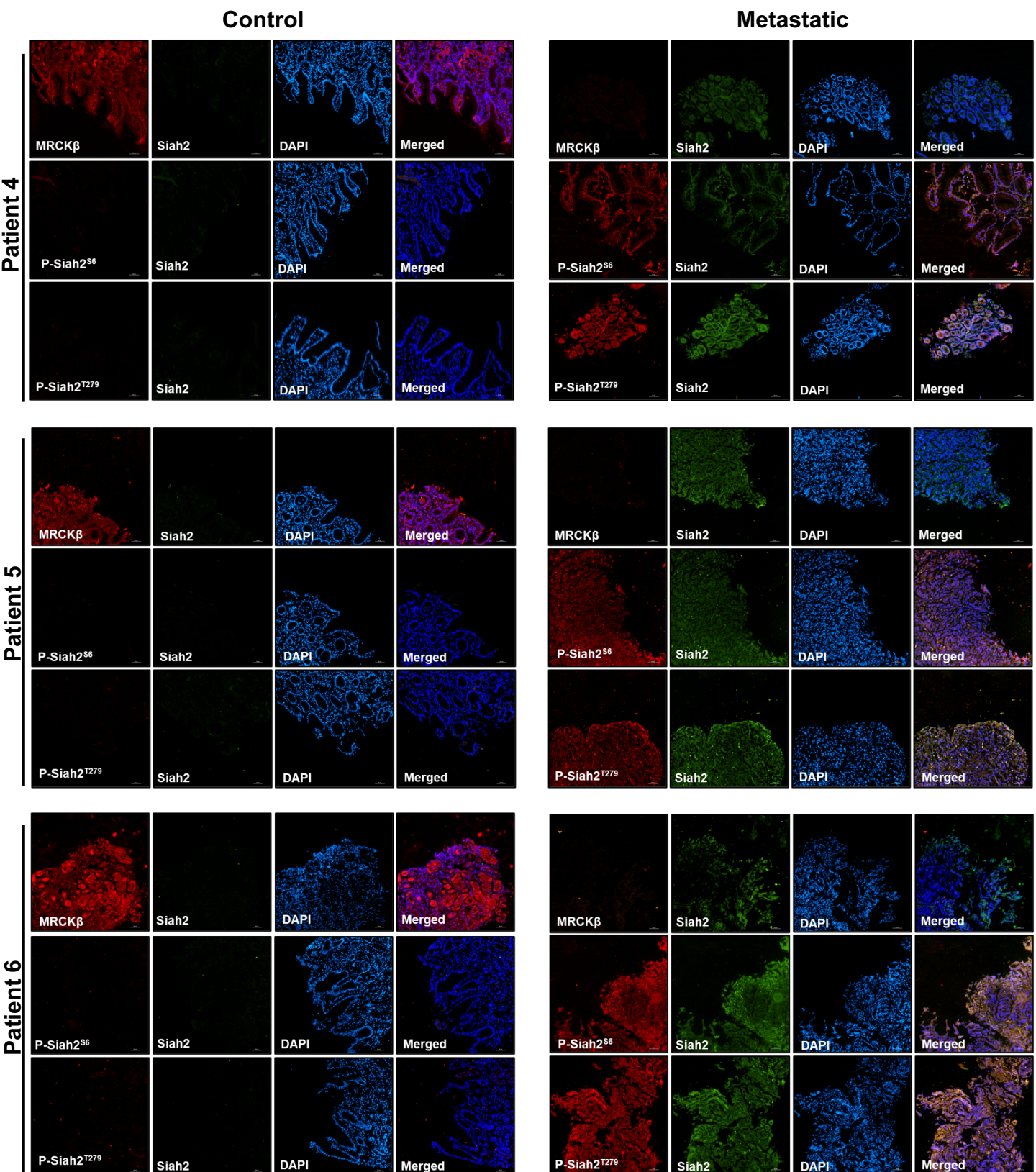


Fig. S3ii. Human gastric biopsy tissues exhibit enhanced Siah2 phosphorylation and MRCK β downregulation. Immunofluorescence microscopy images showing the status of Siah2, P-S⁶-Siah2, P-T²⁷⁹-Siah2 and MRCK β in Patient #4-6. Objective: 20X and scale bar represents 50 μ m.

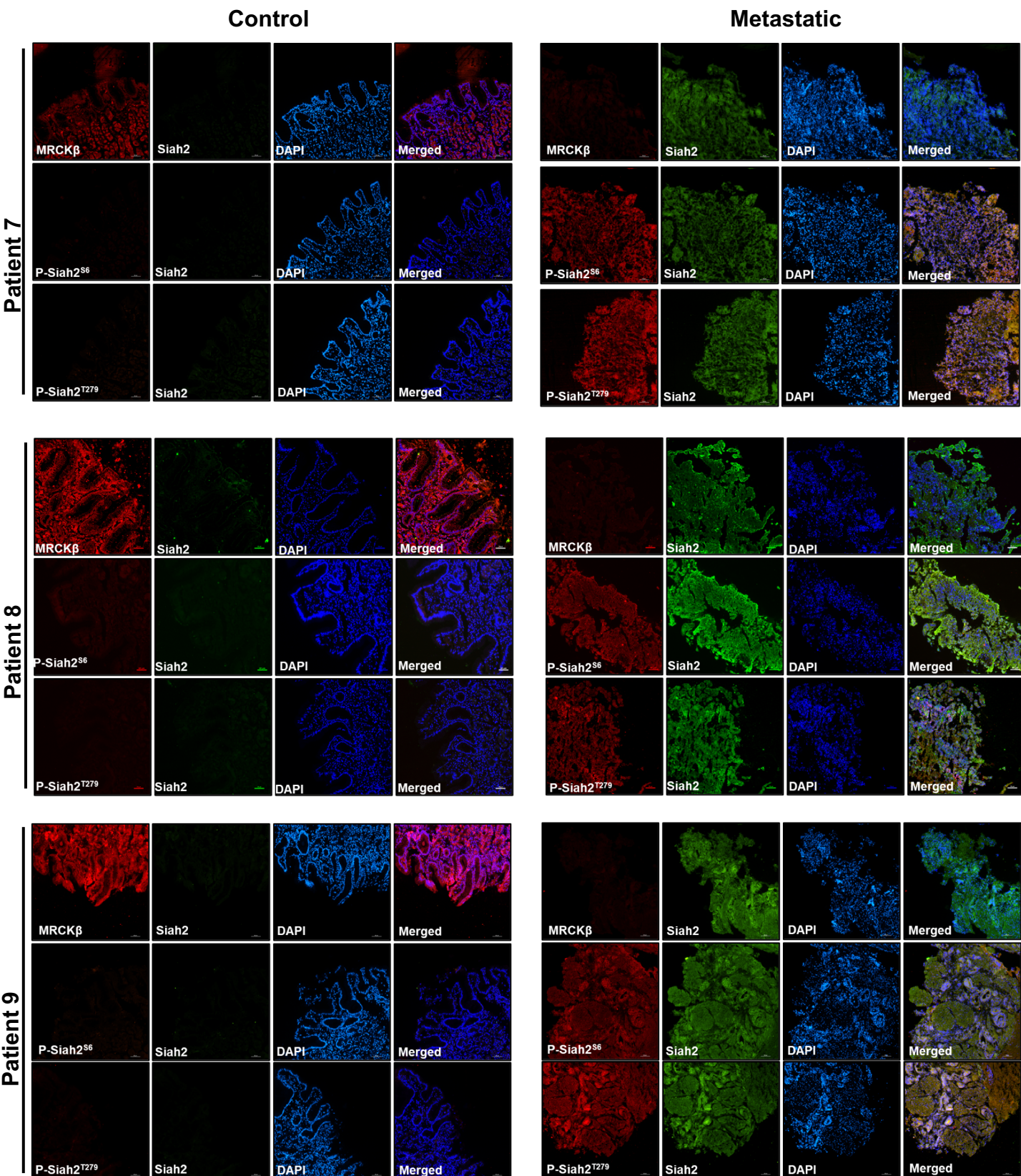


Fig. S3iii. Human gastric biopsy tissues exhibit enhanced Siah2 phosphorylation and MRCK β downregulation. Immunofluorescence microscopy images showing the status of Siah2, P-S⁶-Siah2, P-T²⁷⁹-Siah2 and MRCK β in Patient #7-9. Objective: 20X and scale bar represents 50 μ m.

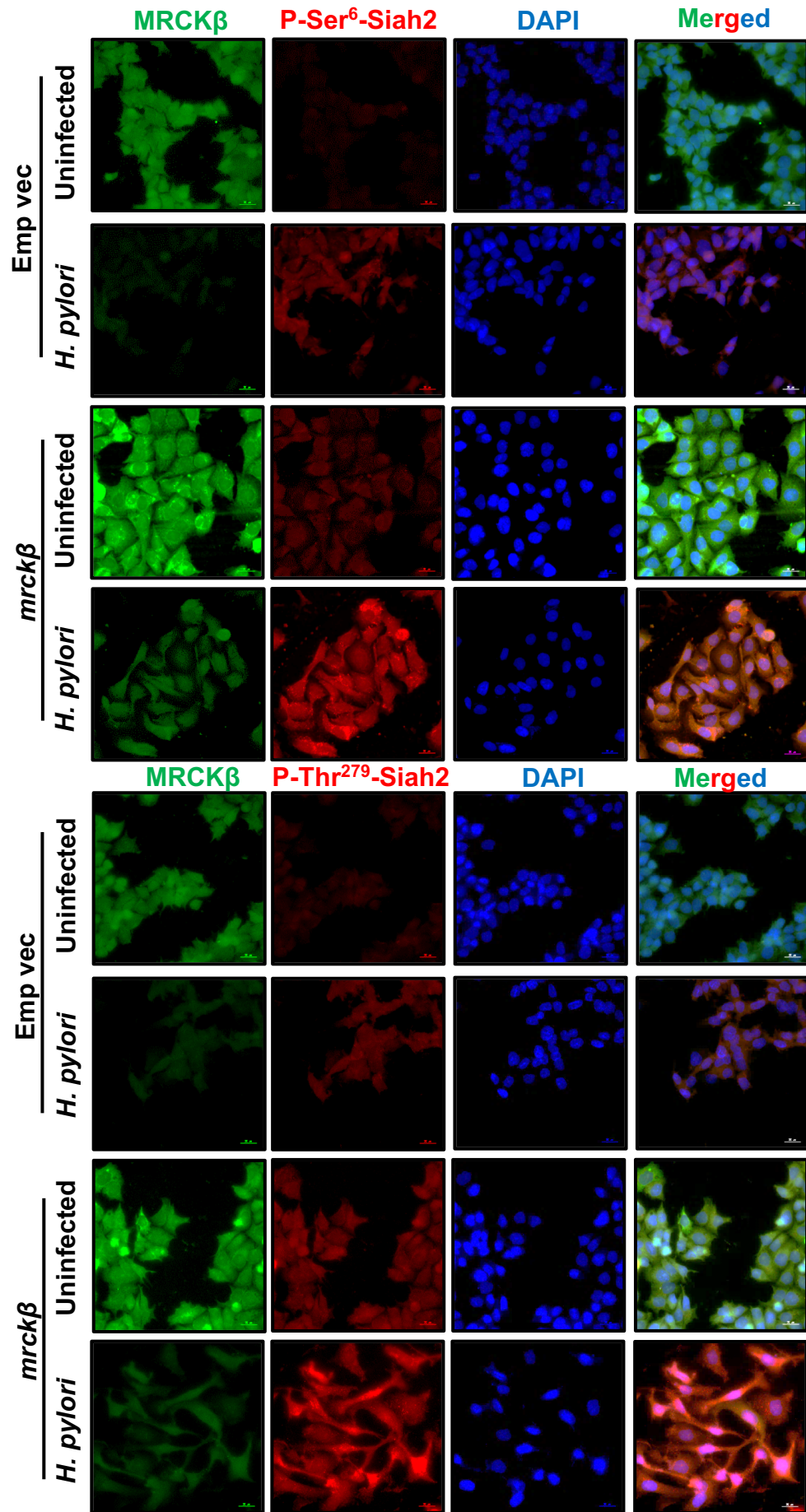


Fig. S4. Phosphorylated Siah2 is enhanced upon *mrckβ* overexpression. Immunofluorescence microscopy images of AGS cells overexpressing the empty vector or *mrckβ* and infected with 200 MOI *H. pylori* for 12 h showing enhanced P-S⁶-Siah2 and P-T²⁷⁹-Siah2. Objective: 60X and scale bar represents 20 μ m.