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ORIGINAL RESEARCH—BASIC

Targeting Host Sulphonyl Urea Receptor 2 Can Reduce Severity of *Helicobacter pylori* Associated Gastritis

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BACKGROUND AND AIMS: While most *Helicobacter pylori*-infected individuals remain asymptomatic throughout their lifetime, in a significant proportion, the resulting severe chronic gastritis drives the development of gastric cancer. In this study, we examine a new therapeutic target, a host potassium channel regulatory subunit, SUR2 (encoded by *ABCC9*), with potential to protect against *H pylori*-associated diseases. **METHODS:** SUR2 gene (ABCC9) expression in human gastric biopsies was analyzed by quantitative polymerase chain reactions. *Helicobacter*-infected mice were administered the SUR2-channel agonists, pinacidil and nicorandil, then gastric tissues analyzed by histology, immunohistochemistry and quantitative polymerase chain reaction, and splenic tissues by enzyme-linked immunosorbent assays. *In vitro* studies were performed on human and mouse macrophages, human gastric epithelial cells and mouse splenocytes. **RESULTS:** *ABCC9* expression in human and mouse stomachs is downregulated with *H pylori* infection. Treatment of *Helicobacter*-infected mice with SUR2 channel modulators, pinacidil or nicorandil, significantly reduced gastritis severity. In gastric epithelial cells, nicorandil-induced opening of the SUR2 channel increased intracellular K^+ and prevented *H pylori*-mediated Ca^{2+} influx and downstream pro-inflammatory signaling. **CONCLUSION:** SUR2 is a novel host factor that regulates *Helicobacter* pathogenesis. Pharmacological targeting of SUR2 provides a potential approach for reducing the severity of *H pylori*-associated gastritis, without eradicating infection.

Keywords: SUR2; Gastritis; *Helicobacter pylori*; Therapeutic; Potassium channel

Introduction

While most individuals infected with *Helicobacter pylori* remain asymptomatic throughout their lifetime, in some, the resulting chronic inflammation (gastritis) over decades drives development of peptic ulcers, gastric MALT (mucosa-associated lymphoid tissue) lymphoma, or gastric adenocarcinoma (gastric cancer).[1] Gastric cancer is the most serious consequence of *H pylori* infection, being the fifth most prevalent cancer and fourth leading cause of cancer-related deaths globally.[2] Progression to malignancy is a multistep process[1,3] and largely preventable, provided *H pylori* is eradicated early enough, thereby removing the inflammatory drive toward cancer.

Despite increasing antimicrobial resistance, therapies based on antibiotics currently remain the frontline method for treating *H pylori* infection.[4] However, an expert World Health Organization panel recently listed *H pylori* among the top 10 pathogenic bacteria for which new therapies are urgently needed.[5] There is therefore a high priority to identify novel antibiotics, or alternative treatments, for either eradicating *H pylori* infection or preventing associated diseases.[5]

Elements that modify the severity of *H pylori*-induced gastritis, including environmental, bacterial virulence and host factors, influence the susceptibility of a host to associated diseases. Potassium channels comprise a heterogenous family of host factors for which little is known regarding *H pylori* pathogenesis. These transmembrane proteins, by the selective transportation of potassium ions (K^+) across cell membranes, are involved in an array of biological processes in diverse cell types. They consist of several subsets, including voltage-gated potassium channels which open and close in response to transmembrane electrical potential, and inwardly rectifying potassium channels (Kir) that transfer positive charge into a cell via the inward transportation of ions, in particular K^+. While not well studied, some of these channels are emerging as pro-malignant factors that contribute to gastric cancer progression.[6,7]

ATP-sensitive Kir (K_{ATP}) channels typically comprise a core of 4 pore-forming Kir subunits, surrounded by 4 sulphonylurea receptors (SURs), forming a functional hetero-octomeric complex.[8] SUR subunits serve as regulatory clamps around the channel pore, controlling the flow of K^+ ions into the cell. K_{ATP} channels in the heart, brain, pancreas, and vascular smooth muscles are well studied, as their aberrant expression is associated with pathologies including

Abbreviations used in this paper: **MALT, mucosa- associated lymphoid tissue; PBS, phosphate buffered saline; SURs, sulphonylurea receptors.**

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coronary artery disease, neurological diseases, hypertension, cardiac ischemia, and diabetes mellitus.[9] Moreover, several K_{ATP} channel modulating drugs, including pinacidil and nicorandil, have been approved for clinical practice and used in the treatment of these conditions. However, there are only sparse reports of K_{ATP} channels in the stomach, which are neither investigated in detail nor in the context of *Helicobacter*-induced gastritis and associated diseases.

This study demonstrates that K_{ATP} channels containing SUR2 (encoded by the *ABCC9* gene) play an important role in *H pylori*-induced gastritis. Notably, treatment with either of 2 K_{ATP} channel agonists, pinacidil and nicorandil, ameliorated gastritis in a murine model of *Helicobacter* infection.

Materials and methods

Human gastric biopsies

Gastric mucosal tissues were obtained from *H pylori* positive individuals with clinically significant chronic atrophic gastritis and disease-free controls as previously described (approval number 097.1998, Melbourne Health Research Directorate Human Ethics Committee).[10]

Bacterial culture and infection of mice

H pylori Sydney strain 1 (SS1),[11] *H pylori* clinical isolate 251,[12] and *H felis* (ATCC 49179, CS1)[13] were cultured as previously described.[14] Mice were infected with a single orogastric dose of 10^7 *H pylori* SS1 or *H felis* CS1 in 100 μL brain heart infusion broth (Oxoid).

Mouse infection studies

All animal procedures were approved by the Animal Ethics Committee, University of Melbourne (AEC1714170 and AEC1814406) and carried out in adherence to the Animal research: reporting of *in vivo* experiments (ARRIVE) guidelines. Adult female C57BL/6 mice (6–7 weeks old; Walter and Eliza Hall Institute, Kew, Australia) were housed in filter-top cages (5 mice per cage) with *ad libitum* access to feed and sterile water. Randomization was carried out as follows: upon arrival, mice were arbitrarily assigned to cages. After one week of acclimatization, mice were ear-clipped and assigned a number within the cage. Cages were then randomly taken from the rack and assigned a group number (2 cages or 10 mice per group). Sample size calculation was performed a priori (based on our previous experience with the Helicobacter infection models) with the power of the experiment set to 80% and a 0.05 significance threshold. Each mouse (or tissues collected from each mouse, such as stomach or spleen, as indicated in Results and graphs) is considered an experimental unit for the studies reported here.

For the drug treatment study (N = 60), there were 3 control groups (no treatment, pinacidil treatment, nicorandil treatment) and 3 *H felis* infection groups (no treatment, pinacidil treatment, nicorandil treatment). For the treatment groups, mice were maintained on drinking water containing nicorandil or pinacidil (Sigma-Aldrich; 1mg/kg/day). Mice were infected as described above (uninfected mice received media only) and drug treatments (via drinking water) were started at after 4 weeks of infection. At study conclusion (twelve weeks post infection), the following parameters were measured: stomach histology for grading of atrophy and immune cell infiltration, food and water consumption, qPCR analysis for *Helicobacter* colonization, gastric cytokines and parietal cell marker expression (qPCR), spleen cytokine levels (enzyme-linked immunosorbent assay [ELISA]), and SUR2 expression (immunohistochemistry). No exclusion criteria were set, and all experimental units were included in analysis.

Quantification of gene expression by qPCR

Primers used in this study are listed in Table. RNA extracted from stomach tissues using TRIzol reagent (Invitrogen) was converted to cDNA with GoScript Reverse Transcription System (Promega). For qPCR, duplicate reactions of 15μL containing 7.5μL QuantiTect SYBR Green PCR Master Mix (Qiagen), 300 nM primers (Table) and 2μL of cDNA (1:10 dilution), were performed in an Mx3000P cycler (Agilent Technologies). Primer efficiencies were calculated with LinRegPCR[13] and gene expression quantified relative to *Rpl32*.

To quantify bacteria, longitudinally halved mouse stomachs were homogenized in 1mL of TRIzol reagent and genomic DNA extracted according to the manufacturer's protocol and used to quantify *H felis* genome copies (with primers targeting *flaB*) relative to mouse *Gapdh* copies by qPCR as above.

Assessment of gastritis

Longitudinally halved stomachs were fixed in 10% neutral buffered formalin, processed, embedded in paraffin and 5μm sections stained with hematoxylin and eosin. Stained sections were blinded and assessed histologically for infiltrating inflammatory immune cells and atrophy as previously described.[15]

Mammalian cell culture and stimulation

RAW264.7 (mouse macrophage, ATCC TIB71) and AGS (human gastric epithelial, ATCC CRL-1739) cell lines,[16] red-cell depleted primary splenocytes and intraperitoneal macrophages from naïve C57BL/6 mice, were cultured in Roswell Park Memorial Institute medium 1640 supplemented with 10% heat-inactivated fetal calf serum (Gibco). Cells ($2x10^5$ per ml) were stimulated with live *H pylori* SS1 or 251 (multiplicity of infection = 10), or SS1 lysate (10μg/ml) with or without nicorandil (100μM). Supernatants were collected 24 hours poststimulation and cytokines analyzed by ELISA. SUR2/Kir6.1 expression in AGS cells was visualized by immunofluorescence assay.

Immunofluorescence assay with AGS cells

AGS cells grown on Nunc Lab-Tek 8-chamber slides were fixed with 4% paraformaldehyde for 20 minutes at ambient temperature. Cells were then blocked with 2% goat (SUR2) or donkey (Kir6.1) serum in 0.3M glycine-phosphate buffered saline (PBS) buffer, followed by overnight incubation at 4 °C with mouse antihuman SUR2 (1:300; Abcam #ab174631) or rabbit anti-human Kir6.1 (1:100; Abcam #ab251809) primary antibodies. Cells were then incubated with goat anti-mouse IgG Alexa Fluor 488 (SUR2; Thermo Fisher Scientific #A11001) or donkey anti-rabbit Alexa Fluor 488 (Kir6.1; Thermo Fisher Scientific #A21206) diluted to 1:500. Actin cytoskeletons were

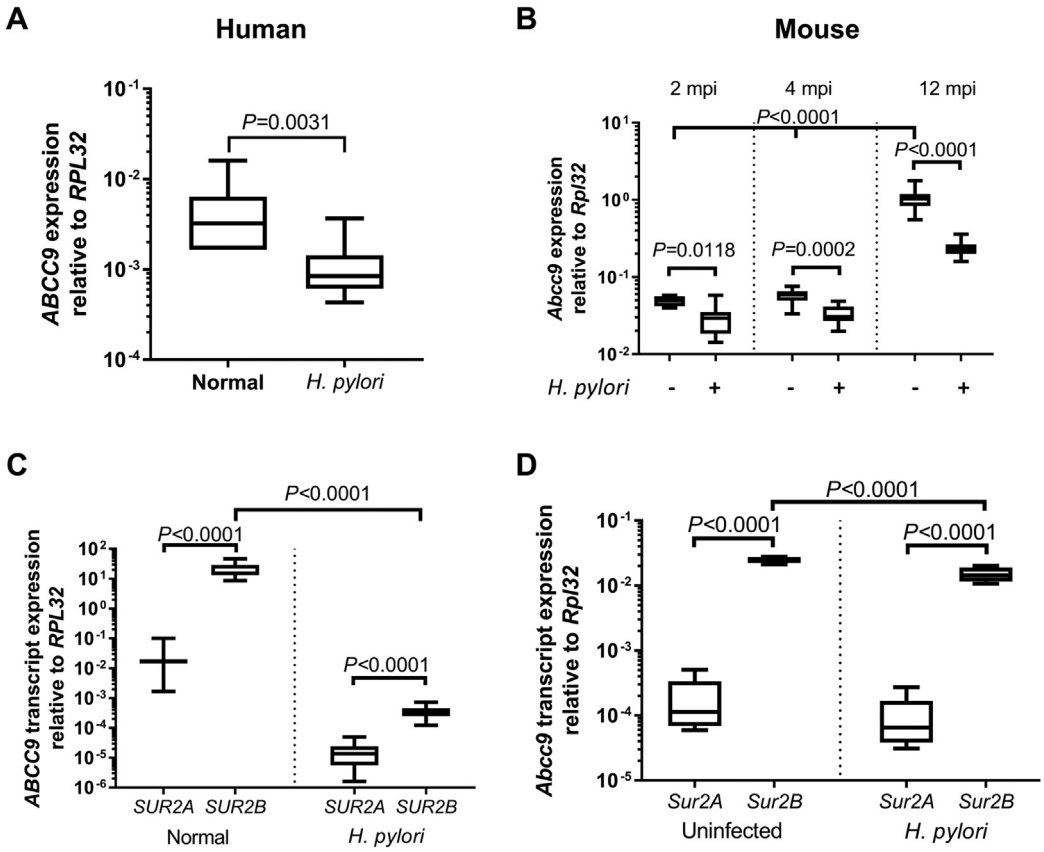

Figure 1. Reduced *ABCC9* expression in stomachs infected with *H pylori* SS1. *H pylori* infection significantly reduced overall *ABCC9* gene expression (all transcripts) in (A) human gastric mucosa (normal = 16, *H pylori* = 15) and (B) mouse stomachs at 2-, 4-, and 12-month postinfection (mpi) as determined by quantitative polymerase chain reaction (Mann-Whitney). *SUR2B* splice variant expression was significantly higher than *SUR2A* in both (C) humans and (D) mice at 4 mpi (Wilcoxon matched-pairs test).

stained with phalloidin-iFluor 647 (Abcam #176759) and cells mounted in ProLong Gold Antifade mountant with 4′,6-diamidino-2-phenylindole counterstain (Invitrogen). Fluorescence images were captured using a Zeiss LSM 780 laser scanning confocal microscope and processed using ZEN software (Zeiss).

Quantification of cytokines by ELISA

Cell culture supernatants and spleen homogenates (in PBS) were centrifuged to remove cell/tissue debris prior to quantification of cytokines by ELISA as previously described (2). DuoSet ELISA kits (R&D Systems) were used to quantify mouse interleukins IL-6, IL-17A, macrophage inflammatory protein 2 (MIP-2), interferon gamma (IFN-γ), and human IL-8 (using the manufacturer's protocol). Plates were read at 450nm in a Multiskan GO microplate reader (Thermo Fisher Scientific) and sample concentration of each cytokine was determined against a standard curve of relevant recombinant cytokine.

Immunohistochemistry with mouse stomach tissues

Immunohistochemistry was performed on formalin-fixed paraffin embedded stomach cross-sections (5μm thick). Tissue sections were de-waxed and rehydrated followed by antigen retrieval in boiling sodium citrate buffer (10mM sodium citrate, 0.05% Tween 20, pH 6.0) for 40 minutes. Primary antibodies used were as follows: mouse antihuman SUR2 (Abcam #ab174631, in combination with Vector M.O.M immunodetection kit) and mouse IgG1 isotype control (Abcam), each diluted 1:200 and rabbit anti-mouse CK19 (ab15463, Abcam #ab15463) diluted 1:50. Immunofluorescent detection of bound primary antibodies was achieved using the following secondary antibody conjugates: donkey anti-rabbit IgG-Alexa Fluor 594 and goat anti-mouse IgG Alexa Fluor 488 (Thermo Fisher Scientific). Samples were then mounted in Pro-Long Gold medium containing 4′,6-diamidino-2-phenylindole counterstain (Invitrogen). Fluorescence images were captured using a Zeiss LSM 780 laser scanning confocal microscope and processed using ZEN Blue software (Zeiss).

Ca^{2+}/K^{+} ion indicator dye assay

Black, optical-bottom, 96-well plates (Thermo Scientific) seeded with AGS cells ($2x10^5$ cells/ml) in serum-free Roswell Park Memorial Institute 1640 media were loaded with 2μM of either intracellular Ca^{2+} indicator Cal-520 AM or K^{+} indicator ION Potassium Green-2 AM (Abcam) fluorescent dyes in 0.02% Pluronic F-127 and 0.04% dimethyl sulfoxide and incubated in the dark for 90 minutes. Cells were washed twice with PBS and

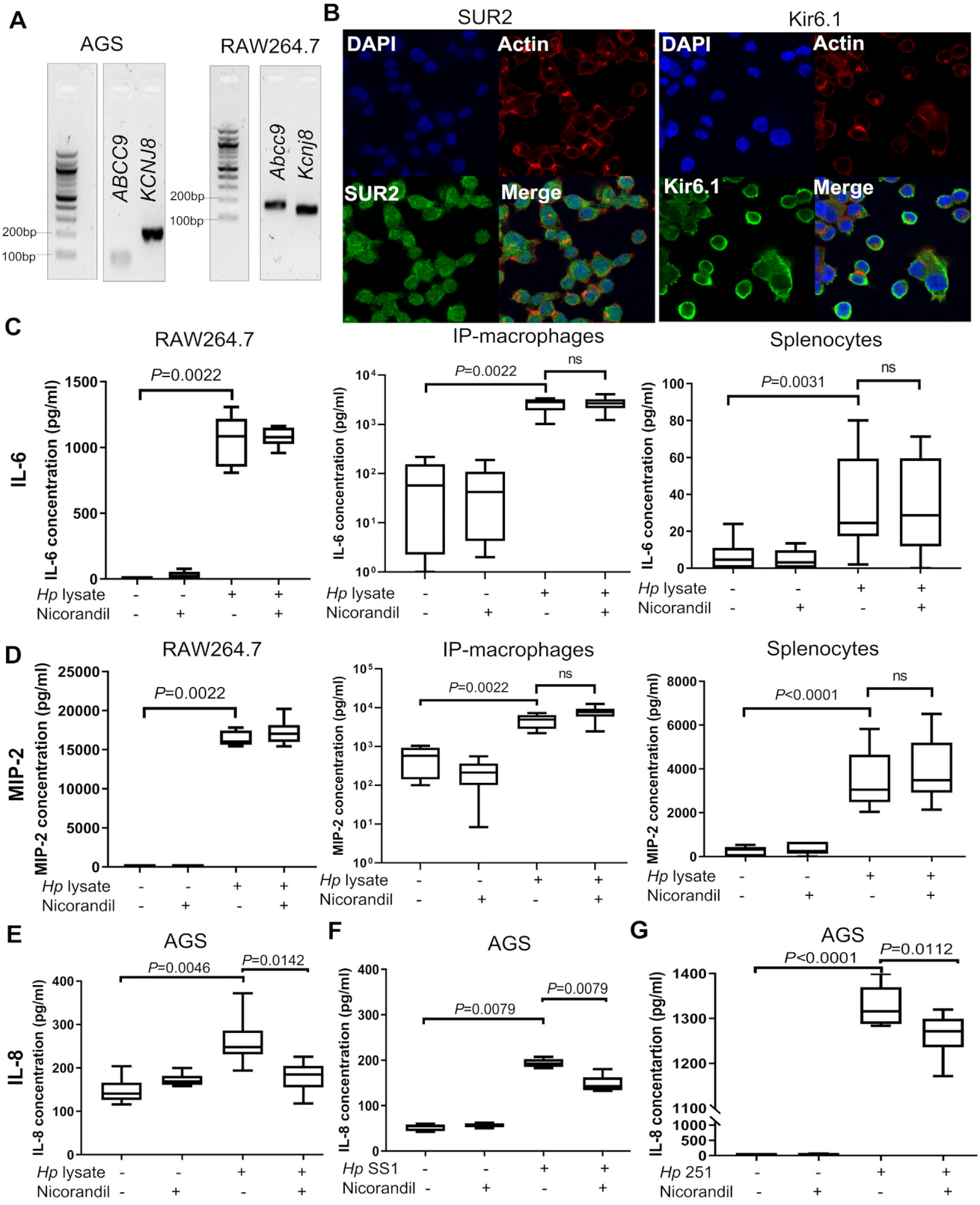

Figure 2. Nicorandil inhibits the inflammatory effect of *H pylori* on epithelial cells. A) K_{ATP} channel subunit transcript expression in human AGS gastric epithelial and murine RAW 264.7 macrophage cell lines. B) Immunofluorescence images showing expression of SUR2 and Kir6.1 proteins in AGS cells. Blue, 4′,6-diamidino-2-phenylindole; red, actin; green, SUR2/Kir6.1 as labeled. C, D) Nicorandil (100 μM) had no effect on (C) IL-6 and (D) MIP-2 secretion by RAW264.7 macrophages, primary mouse intraperitoneal (IP) macrophages or splenocytes (as annotated) stimulated for 24 hours with *H pylori* SS1 lysate. E–G) Nicorandil treatment (100 μM) significantly reduced the IL-8 response of AGS cells to 24 hours stimulation with (E) *H pylori* SS1 lysate, (F) live *H pylori* SS1 and (G) live Cag-positive *H pylori* 251(MOI = 10). Graphs show group medians (horizontal bar), interquartile range (box), 10th and 90th percentiles (bars). *P* values were calculated using Mann-Whitney tests (ns, $P > .05$).

serum-free media (no pH indicator) added to the wells. Reads were taken in an FLUOstar OPTIMA (BMG Labtech) fluorescent microplate reader (excitation/emission: Ca^{2+} 485/520, K^+ 520-10/560-10) at baseline (F_0) and at indicated time-points after addition of *H pylori* (MOI = 10) and/or nicorandil (100μM). Intracellular Ca^{2+} and K^+ levels are expressed as percentage baseline corrected fluorescence intensities (F/F_0*100).

Statistical analyses

Data were analyzed using GraphPad Prism version 7.0 (GraphPad Software Inc; San Diego, USA) with the Mann-Whitney *U* test, two-way ANOVA or Wilcoxon matched-pairs test, as specified. Area under curve analysis was performed for mice water intake and body weight measurements. *P*-values $<.05$ were considered statistically significant.

Results

Reduced ABCC9 expression in human and mouse stomachs upon H pylori infection

To examine the potential role of SUR2 channels in *H pylori*-associated diseases, we first assessed the effect of *H pylori* on *ABCC9* gene expression in infected gastric tissues. This identified *ABCC9* expression to be significantly lower in individuals with *H pylori* infection/chronic atrophic gastritis, as compared to uninfected controls (Figure 1A). A similar observation was made in mice, where gastric *Abcc9* expression was reduced in C57BL/6 mice infected with *H pylori* for 2, 4, and 12 months (Figure 1B).

The *ABCC9* gene undergoes alternative splicing, generating variants with tissue-specific expression; for example, cardiac and skeletal muscles predominantly express *SUR2A*, while *SUR2B* is more common in vascular smooth muscles. Analysis of these variants identified *SUR2B* as the predominant isoform in both human (Figure 1C) and mouse gastric tissues (Figure 1D), irrespective of *H pylori* infection status.

Nicorandil suppresses the pro-inflammatory response to H pylori by epithelial cells

In vitro stimulation assays were performed to determine if SUR2 can modify the inflammatory response of gastric epithelial and immune cells to *H pylori*. RAW264.7 murine macrophage and human AGS gastric epithelial cell lines were selected as a prescreen demonstrated their concomitant expression of both the *Abcc9*/SUR2 and *Kcnj8*/KIR6.1 subunits that are required for a functional K_{ATP} channel (Figure 2A and B). These cells were then stimulated with *H pylori*, with or without addition of the SUR2 channel agonist, nicorandil. Nicorandil treatment had no effect on the pro-inflammatory IL-6 and MIP-2 response of RAW264.7 macrophages to *H pylori* stimulation (Figure 2C and D). A similar lack of effect was also observed using non-induced mouse primary splenocytes and intraperitoneal macrophages. AGS cells were then stimulated with lysed or live *H pylori* and culture supernatants examined for IL-8 secretion. IL-8 is known to be the most up-regulated cytokine in *H pylori* exposed gastric epithelial cells.[17] In contrast to the immune cells, nicorandil treatment significantly reduced the IL-8 response of AGS cells to stimulation with either lysed or live *H pylori* SS1 (Figure 2E and F).

As *H pylori* SS1 has a dysfunctional cag pathogenicity island (cagPAI),[12] we further evaluated *H pylori* strain 251 which possesses a functional cagPAI and is therefore considered more pathogenic. Nicorandil significantly reduced the IL-8 response of AGS cells to *H pylori* 251 (Figure 2G), demonstrating that SUR2 activation also exerted an anti-inflammatory response on epithelial cells exposed to cagPAI positive *H pylori*.

Nicorandil-induced increases in intracellular K^+ levels counteract intracellular Ca^{2+} influx in response to pro-inflammatory stimuli

The effect of K_{ATP} channel activators including pinacidil and nicorandil on smooth muscle tissues is well studied, revealing these compounds cause muscle relaxation via membrane hyperpolarization and subsequent closure of voltage-gated calcium channels.[18,19] The effect of these activators on other cell types is less well-known. Nicorandil treatment reduced IL-8 secretion by *H pylori*-stimulated AGS cells (Figure 2E–G), which has been shown to be regulated by Ca^{2+} signaling.[20,21] We therefore hypothesized that the inhibitory effects of nicorandil treatment on cytokine secretion could be due to SUR2-mediated regulatory effects on intracellular Ca^{2+}. Nicorandil treatment of unstimulated AGS cells preloaded with fluorogenic Ca^{2+}- or K^+-sensitive dyes produced an immediate increase in intracellular K^+, which was sustained for at least 24 hours (Figure 3A), and a modest early increase in intracellular Ca^{2+} which gradually normalized relative to untreated cells (Figure 3B). Intracellular Ca^{2+} levels were, as expected, increased by *H pylori* stimulation from one to 24 hours postinfection (Figure 3C and D). This increase was completely prevented by nicorandil treatment thus demonstrating the direct effect of this channel modulating drug on intracellular Ca^{2+}.

Intracellular K^+ levels spiked significantly upon nicorandil treatment in uninfected cells at 24 hours postdrug treatment (Figure 3F) but were comparable between untreated, infected cells and uninfected controls (Figure 3G) thus showing that K^+ level within the cell was not greatly affected by *H pylori* stimulation. Given the previously demonstrated role of Ca^{2+}-signaling on regulating cytokine secretion by gastric epithelial cells,[21] the nicorandil-mediated inhibition of pro-inflammatory IL-8 secretion by *H pylori*-stimulated AGS cells is therefore potentially explained by the suppression of Ca^{2+}-dependent pro-inflammatory signaling (Figure 3F).

Treatment with SUR2 channel agonists reduce gastritis severity in Helicobacter-infected mice

To examine the effect of modulating SUR2 function on *Helicobacter*-induced gastritis, groups of mice were infected

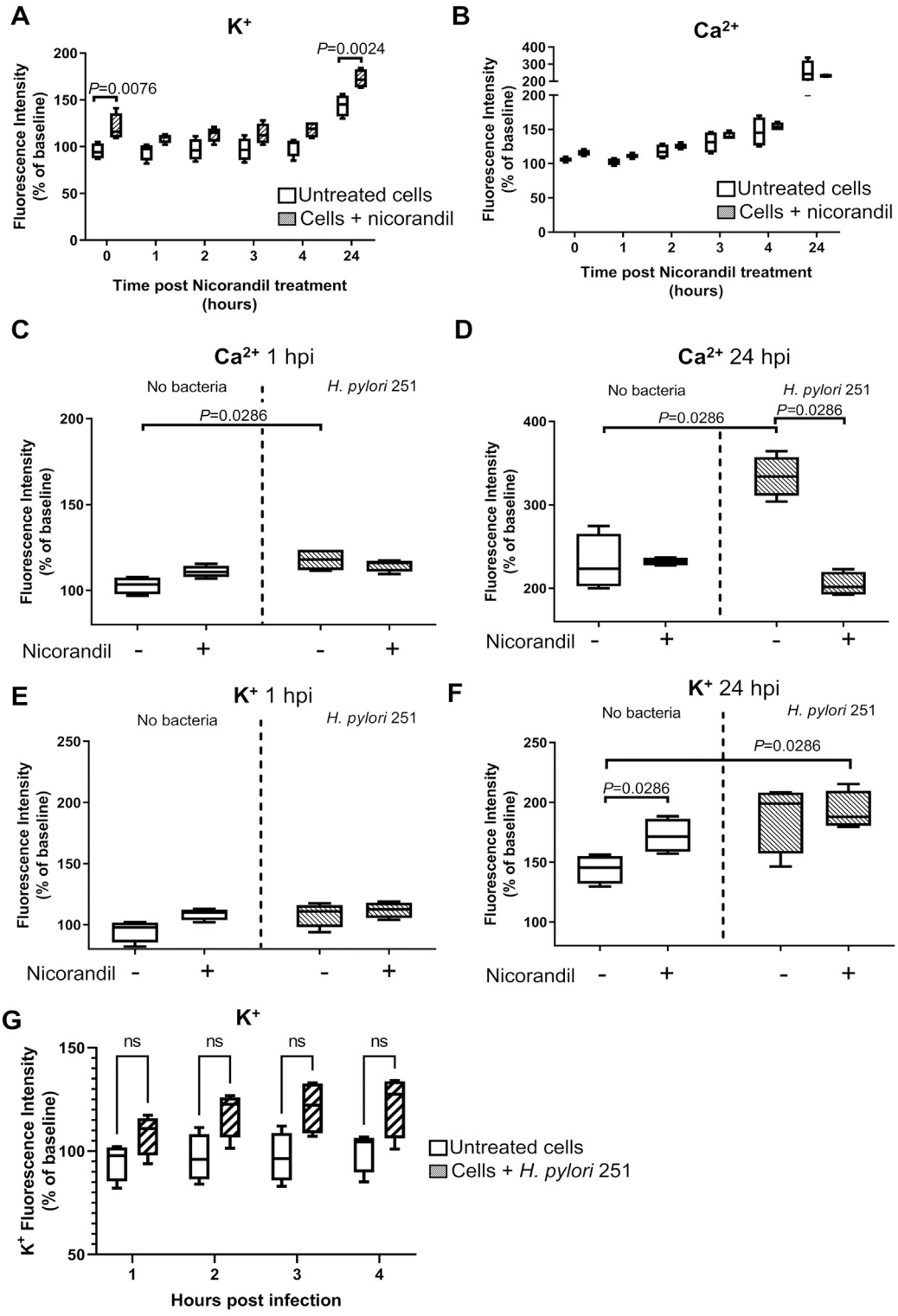

Figure 3. Nicorandil treatment prevents *H pylori*-induced Ca^{2+} influx in AGS cells. Intracellular potassium K^{+} (A) and Ca^{2+} (B) in untreated (clear box) or nicorandil-treated (100 μM; shaded box) AGS cells, expressed as a percentage of initial baseline reads from each well. Intracellular K^{+} spiked immediately after nicorandil addition and stayed elevated at 24 hours post-treatment. Ca^{2+} increased slightly but this effect was not significant. C) Intracellular Ca^{2+} levels increased upon *H pylori* 251 stimulation (MOI = 10) at 1 hour post infection (hpi) and D) 24 hpi. Nicorandil treatment significantly reduced *H pylori* induced Ca^{2+} influx, comparable to uninfected cells. Intracellular K^{+} at 1 hpi (E) and 24 hpi (F). *P* values were calculated by Mann-Whitney tests. G) Intracellular K^{+} in uninfected and *H pylori*-stimulated cells (without nicorandil treatment). All differences among infected cells and uninfected controls were nonsignificant (ns, $P > .05$) as calculated with two-way ANOVA followed by Sidak's multiple comparisons test.

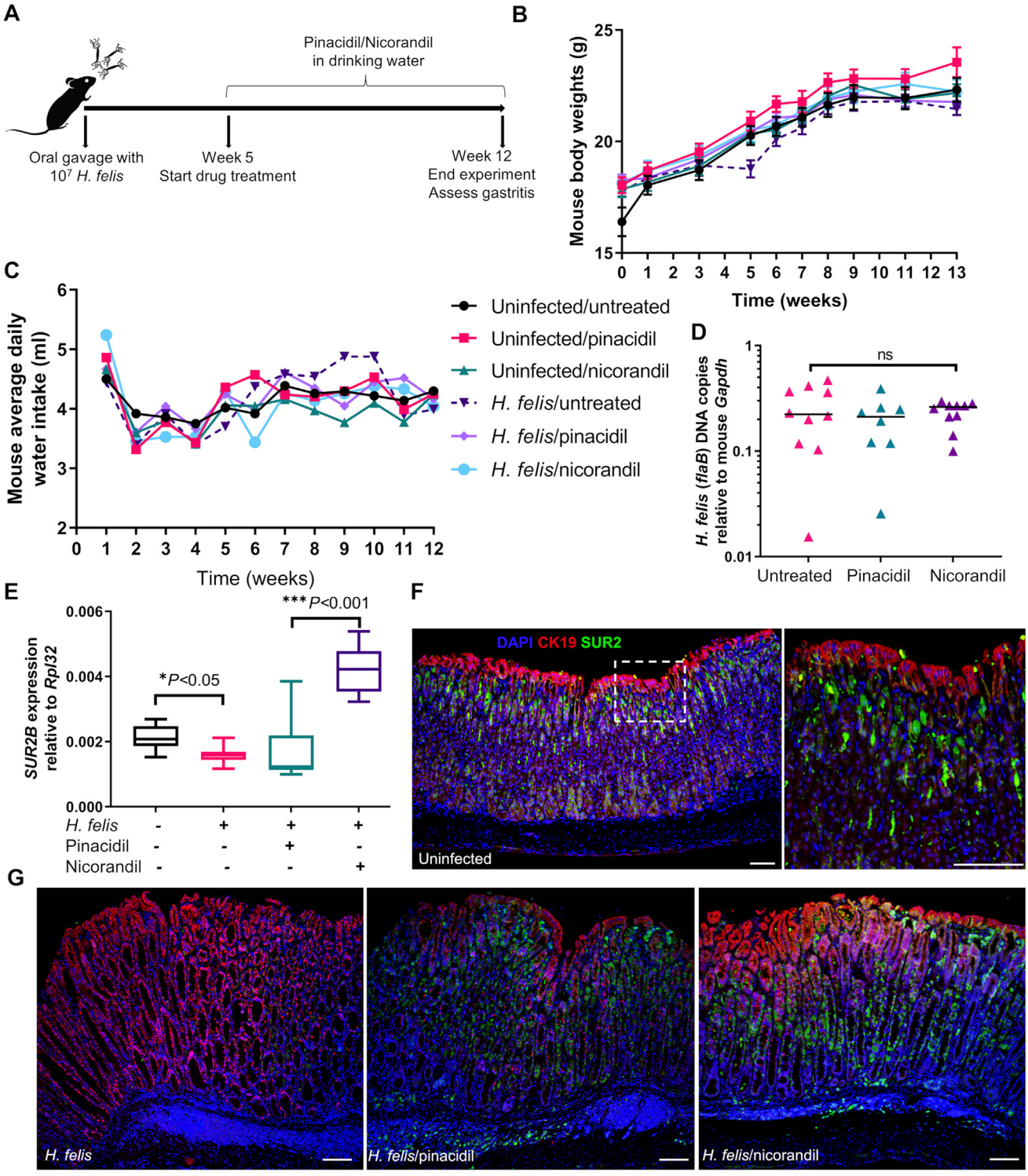

Figure 4. SUR2-K_{ATP} channel agonists do not impact *H felis* CS1 colonization but counteract infection-related SUR2 downregulation in a mouse gastritis model. A) Mice were infected with *H felis* CS1 for 4 weeks, then treated for another 8 weeks with either pinacidil or nicorandil (via drinking water). Age-matched uninfected controls (with or without drug treatment) were analyzed in parallel. Drug treatment had no effect on (B) mouse body weights, or (C) daily water intake as measured by 'area under curve' analysis. D) Gastric colonization with *H felis*, measured by qPCR, was not affected by drug treatment. E) *Sur2B* expression in stomach halves, measured by qPCR. F) SUR2 expression in the stomach mucosa as detected by immunofluorescence staining in a 5μm cross-section through the corpus epithelium; SUR2 (green), cytokeratin 19 (CK19, an epithelial cell marker; red) and cell nuclei (4′,6-diamidino-2-phenylindole, blue). G) SUR2 expression was significantly reduced upon *H felis* infection (3 mpi) and restored upon pinacidil and nicorandil treatment. Infection groups as labeled. Scale bars, 100μm. qPCR data analyzed by Mann-Whitney tests: ns, not-significant $P > .05$; other P values as annotated.

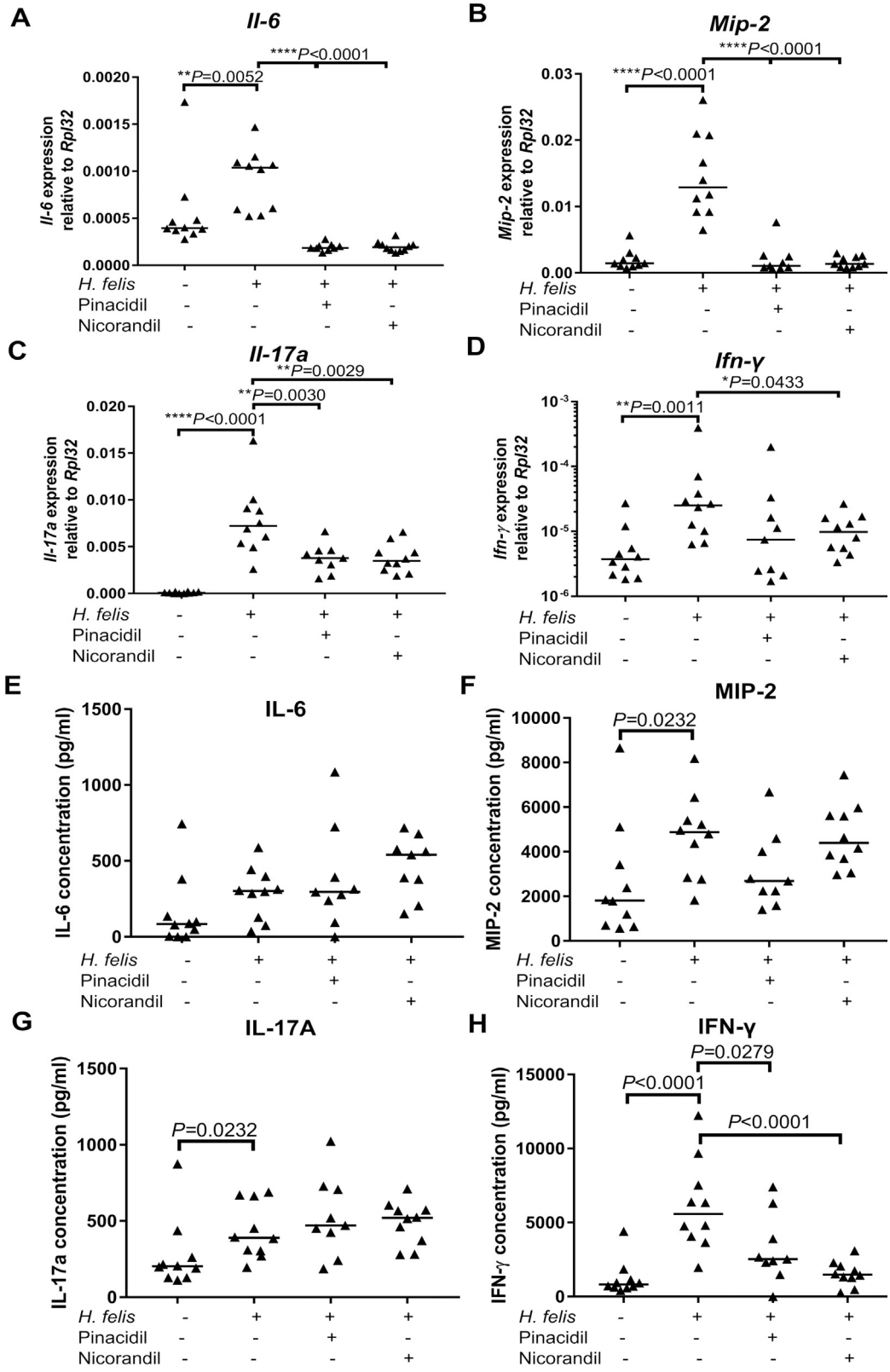

Figure 5. Anti-inflammatory effects of pinacidil and nicorandil are associated with a reduced local gastric cytokine response. (A–D) mRNA levels of cytokines in stomach halves from *H felis* CS1 infected, drug-treated mice (as described in Figure 4A) were quantified by qPCR. (E–H) Cytokines in splenic homogenates were quantified by enzyme-linked immunosorbent assay. Graphs show each mouse as an individual marker and group medians are depicted by horizontal lines. *P* values were calculated using Mann-Whitney tests.

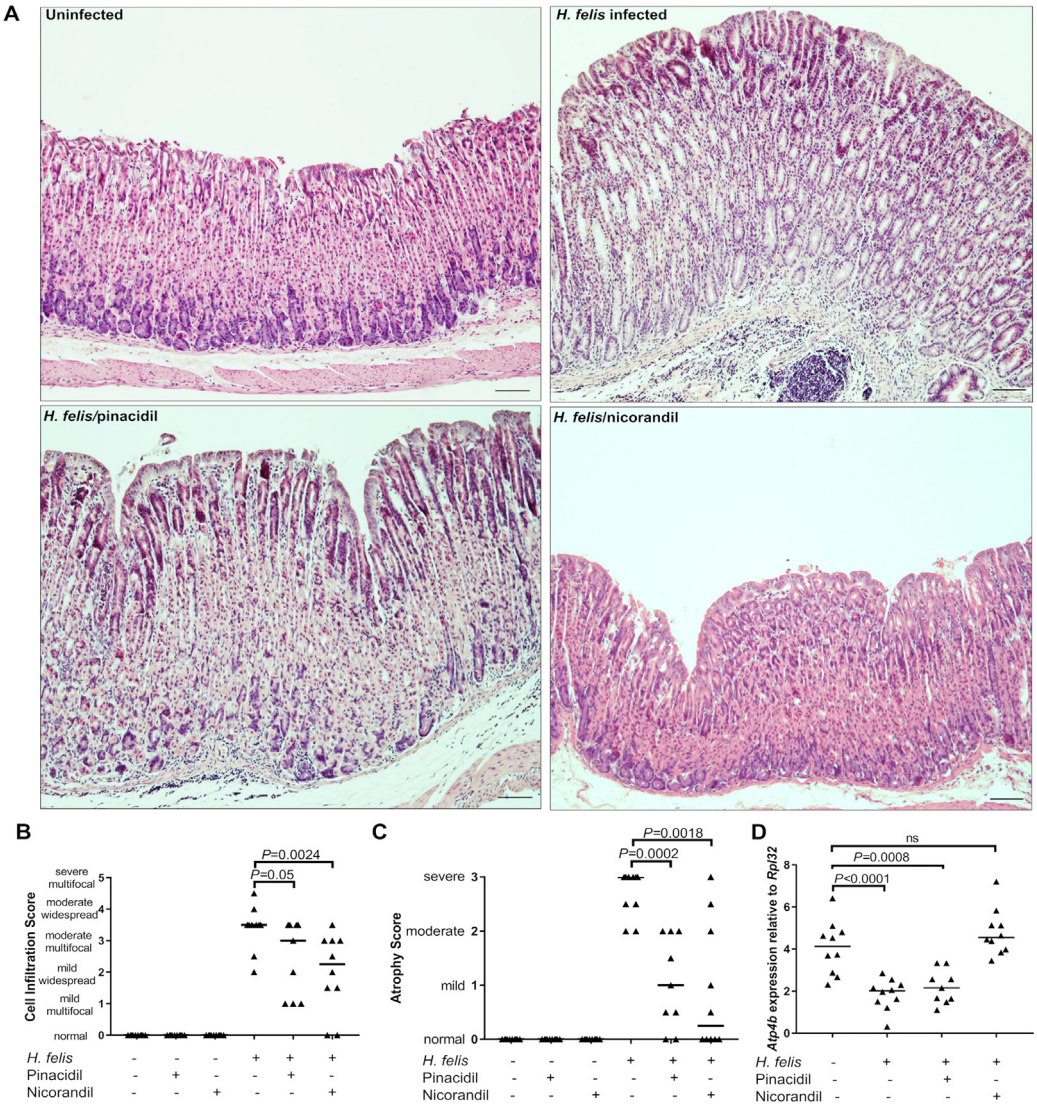

Figure 6. Histopathological analysis of mouse stomachs. A) Representative gastric histopathology (H&E-stained sections) from *H felis* CS1 infected mice and uninfected control at 12 weeks postinfection (as annotated). B–D) Analysis of mouse gastric sections at 12 weeks postinfection showed that both pinacidil and nicorandil significantly reduced immune cell infiltration (B) and atrophy (C) compared to untreated/infected controls. D) Loss of *Atp4b* parietal cell marker expression in *H felis* infected gastric tissues was restored by nicorandil treatment. Data points on each graph show individual mice with group medians depicted by horizontal lines. *P* values were calculated using Mann-Whitney tests.

with *H felis* for 4 weeks, then treated with pinacidil or nicorandil for an additional 8 weeks, before assessment of gastritis severity at 12 weeks postinfection (Figure 4A). These drugs were selected to provide an analysis of the effect of differential agonism of SUR2 channels: pinacidil activates both SUR2A/B channels while nicorandil has a greater specificity for SUR2B.[21]

Long-term administration of both pinacidil and nicorandil was well tolerated with no impact on water intake or body weights, either with or without *H felis* infection (Figure 4B and C) and no effect on *H felis* colonization (Figure 4D). Gene expression studies on stomach halves showed that *SUR2B* expression was downregulated upon infection (Figure 4E). Nicorandil, but not pinacidil, treatment was successful in increasing *SUR2B* expression in infected stomach tissues. Immunohistochemical staining showed gastric SUR2 expression to be predominantly located in the crypts of the corpus epithelium (Figure 4F). The expected reduction in SUR2 tissue expression resulting from *H felis* infection was ameliorated by treatment with either pinacidil or nicorandil (Figure 4G).

To further characterize the anti-inflammatory effects of pinacidil and nicorandil in *H felis*-infected mouse stomach tissues, the expression of key pro-inflammatory cytokines was analyzed. While gastric expression of *Il-6, Mip-2, Il-17A, and Ifnγ* genes were all significantly increased upon *H felis* infection, both pinacidil and nicorandil treatments reduced expression of all 4 cytokines in infected mice, in some cases

to baseline levels (Figure 5A–D). This appeared to be predominantly a localized effect, as cytokine levels in splenic tissues from the same mice showed drug-induced reduction of only IFN-γ (Figure 5E–H). Histological analysis (Figure 6A) showed that while infected control mice typically presented with severe atrophic gastritis characterized by moderate immune cell infiltration and severe atrophy with associated parietal cell loss, groups treated with either pinacidil or nicorandil had significantly reduced levels of atrophy and immune cell infiltrates (Figure 6B and C). There was a trend toward greater effectiveness with nicorandil treatment, which reduced the atrophy score to zero (similar to normal uninfected controls) in 50% (5/10) of infected mice (Figure 6C). This was supported by qPCR analysis of the parietal cell marker *Atp4b*, which encodes the β subunit of H^+/K^+ ATPase; parietal cell loss (marked by a reduction in *Atp4b* levels) in infected mice was restored to baseline by nicorandil, but not pinacidil treatment (Figure 6D).

Table. Primers Used in This Study

Primer name	Primer sequence (5′->3′)
Human qPCR primers	
ABCC9 all transcripts F	ATGGTGTACTACAAAATTCCTGC
ABCC9 all transcripts R	TCACAGACATGCACAAACAGG
SUR2A F	TTCTATTATGGATGCAGGCC
SUR2B R	ACCAAAGTGGAAAAGAGGCC
SUR2B F	GTTATTGTGATGAAGCGAGG
SUR2B R	TTACAGAGGTCAAGCTGATG
RPL32 F	CATCTCCTTCTCGGCATCA
RPL32 R	ACCCTGTTGTCAATGCCTC
Mouse qPCR primers	
Abcc9 all transcripts F	CTTTGTGGACGCACTCAACC
Abcc9 all transcripts R	TGTGTCCGGGAAAATGAAGC
Rpl32 F	GAGGTGCTGCTGATGTGC
Rpl32 R	GGCGTTGGGATTGGTGACT
Sur2A F	GTAACCATAGCTCACCGTGTCTC
Sur2A R	CATTCTTGTGCTGGAGCAGG
Sur2B F	CCATAGCTCATCGGGTTCACAC
Sur2B R	ACACTCCATCTTCCTGGGCC
Ifn-g F	CAGCAACAGCAAGGCGAAA
Ifn-g R	CTGGACCTGTGGGTTGTTGAC
Il-6 F	GAAAATTTCCTCTGGTCTTCTGG
Il-6 R	TGGAAATTGGGGTAGGAAGG
Il-17a F	tccagaaggccctcagacta
Il-17a R	agcatcttctcgaccctgaa
Mip-2 F	AGTGAACTGCGCTGTCAATG
Mip-2 R	TTCAGGGTCAAGGCAAACTT
Genomic DNA qPCR primers	
Mouse Gapdh F	TGCACCACCAACTGCTTAG
Mouse Gapdh R	GGATGCAGGGATGATGTTC
H felis flaB F	TTCGATTGGTCCTACAGGCTCAGA
H felis flaB R	TTCTTGTTGATGACATTGACCAACGCA

F, forward primer; R, reverse primer.

Discussion

This study identifies SUR2 as a new host factor of importance in *H pylori* pathogenesis that can be pharmacologically targeted to protect against the pathological consequences of this infection. Most diseases associated with chronic *H pylori* infection, including gastric cancer and peptic ulcer disease, arise from a prolonged and exaggerated gastritis. It is now well recognized that this continuous *H pylori*-induced inflammatory assault on the gastric tissue is the key driver for metaplastic changes and the development of gastric cancer. Factors that modulate the severity of *H pylori*-induced gastritis therefore influence the susceptibility or resistance of an individual regarding progression to malignancy. Despite their considerable potential to modify inflammation, potassium channels have been poorly studied in the context of *H pylori* infection and pathogenesis. There are more than 75 different potassium channels, some of which have been described as gastric oncogenic factors and may serve as potential prognostic biomarkers.[6,7] However, their functions in the stomach have not been well investigated apart from a role for a few potassium channels in assisting acid secretion.[23,24] This study of SUR2 provides the first direct evidence that a potassium channel might play an important role in *Helicobacter* pathogenesis.

The important functionality of SUR2 was demonstrated by the ability of 2 K_{ATP} channel modulators, pinacidil and nicorandil, to reduce the severity of gastritis caused by *Helicobacter* infection in mice. Notably, half of the infected nicorandil-treated mice presented with zero gastric atrophy, in comparison with infected controls, all of which had moderate to severe atrophic gastritis. This was associated with a reduction in pro-inflammatory cytokines in the stomach, consistent with a marked reduction in gastritis. To our knowledge, this is the first report of a drug treatment targeting a host factor and producing a therapeutic reduction of *Helicobacter*-induced gastritis, without impacting bacterial colonization levels thereby demonstrating a direct effect on inflammation. Given the different selectiveness of these 2 drugs, the observation that nicorandil had better efficacy than pinacidil indicates these drug-mediated reductions in *Helicobacter*-induced gastritis involved the targeting of SUR2B rather than SUR2A.

The distribution of SUR2B expression suggested these drug-mediated effects would likely act via immune and/or epithelial cells. Studying murine macrophages and splenocytes revealed no obvious effect of nicorandil treatment on *H pylori*-induced secretion of pro-inflammatory cytokines by immune cells. In contrast, nicorandil treatment of human gastric epithelial cells significantly reduced *H pylori*-induced production of IL-8, an important chemokine in *H pylori* pathogenesis involved in the recruitment of inflammatory immune cells. In fact, *IL-8* has been shown to be the most upregulated gene in genome-wide profiling of gastric epithelial cells exposed to *H pylori*.[16] Hence, these data support the anti-gastritis effects of nicorandil being due to its action on gastric epithelial cells. Importantly, this anti-inflammatory effect was also induced in response to the more pathogenic cagPAI positive strain of *H pylori*.

A key part of the *H pylori*-epithelial cell interaction involves the activation of host cell pattern recognition receptors, leading to PI3K activation,[25] then an increase in

intracellular Ca^{2+}[21] which promotes activation of a mitogen-activated protein kinase/nuclear factor kappa B driven pro-inflammatory responses.[26] Here, analyses showed that opening the SUR2 potassium channel leads to K^+ influx in gastric epithelial cells, which prevents the rise in intracellular Ca^{2+} triggered by *H pylori*. Previous studies in pancreatic islet cells and cardiomyocytes have also shown that opening K_{ATP} channels can attenuate Ca^{2+} accumulation by preventing membrane depolarization and the closing of voltage-gated calcium channels.[18,19,27] Data presented in this study therefore suggest that pinacidil and nicorandil suppressed gastritis in infected mice via K_{ATP} channel mediated attenuation of *Helicobacter*-induced Ca^{2+} signaling in gastric epithelial cells.

The identification of new drug treatments for *H pylori* infection to prevent associated diseases has been identified by a World Health Organization expert panel as highly important due to the level of antimicrobial resistance of *H pylori* to current treatment regimens.[5] This study raises the possibility of a different approach than treating the infection, instead by pharmacologically targeting SUR2 to treat the disease-causing gastritis. Such an approach could potentially confer an advantage over conventional eradication therapies, as modifying a host factor to protect against gastritis will not apply selective pressure to the bacteria, and so is unlikely to generate the drug resistance that occurs with antibiotics. Moreover, there is evidence that *H pylori* infection might provide some protection against other disease states including asthma and inflammatory bowel disease.[28,29] Hence, targeting SUR2 to control gastritis might retain any beneficial effects associated with *H pylori* colonization.

In this study, pinacidil and nicorandil were used as model drugs to demonstrate the effect of modifying SUR2 activation. However, while these drugs have been used clinically, their effects on the cardiovascular system and pancreatic cells could be contraindicative for the treatment of *H pylori* gastritis in some individuals. Pinacidil can cause fluid retention,[30] and nicorandil has been associated with an increased risk for gastrointestinal ulceration, although this has not been measured in the context of *H pylori* infection[31] and has been shown to be protective in chemically induced gastric ulcer animal models.[32] Hence translating these findings to a treatment for *H pylori*-induced gastritis might require the development of a more specific pharmacotherapy approach for targeting gastric SUR2B.

Conclusion

In conclusion, we have identified a novel host factor, SUR2, which is of significance in *H pylori* infection. This is the first potassium channel identified to play an important role in *H pylori* pathogenesis, influencing the severity of *Helicobacter*-induced gastritis. Importantly, SUR2 is shown to be a druggable target, allowing for the treatment of gastritis without eradicating *Helicobacter* infection. By using 2 different model drugs, we have shown that SUR2B is the primary gastric SUR2 subunit that can be targeted to modify gastritis, and the anti-inflammatory effects of the SUR2-channel agonists are most likely mediated via gastric epithelial cells where drug-induced K^+ influx prevents the rise in intracellular Ca^{2+} that can otherwise trigger downstream pro-inflammatory signaling. Overall, targeting SUR2 offers a promising avenue for treating *H pylori*-induced gastritis and peptic ulcer disease as well as preventing the development of gastric cancer, while avoiding issues relating to antimicrobial resistance.

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Sohinee Sarkar performed the experiments, analyzed the data, compiled the figures, and wrote the manuscript. Ghazal Alipour Talesh performed immune analysis experiments. Trevelyan R. Menheniott acquired human patient tissues and obtained funding. Philip Sutton conceived the study, performed data analysis, participated in manuscript writing, obtained funding, and supervised the research.

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The authors disclose no conflicts.

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Ethical Statement:
The corresponding author, on behalf of all authors, jointly and severally, certifies that their institution has approved the protocol for any investigation involving humans or animals and that all experimentation was conducted in conformity with ethical and humane principles of research.

Data Transparency Statement:
All data generated or analyzed during this study are included in this published article (or available from the corresponding author upon reasonable request).

Reporting Guidelines:
ARRIVE/Care and Use of Laboratory Animals.