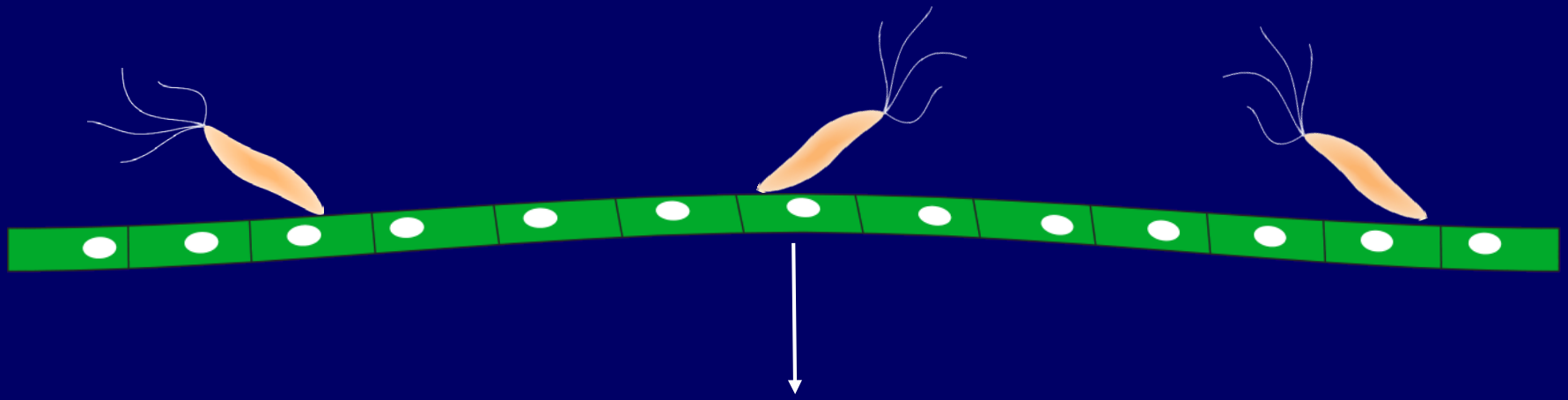
Helicobacter pylori-Induced Gastric Cancer: Mechanisms

Rick Peek

Vanderbilt University Medical Center

Host responses to *H. pylori* strain-specific virulence constituents influence carcinogenesis



Gastric inflammation

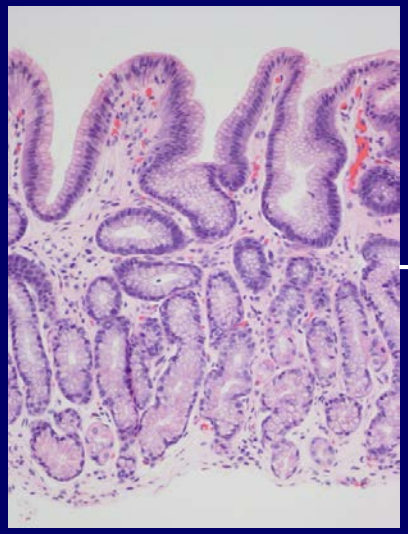
Decades

Gastric adenocarcinoma

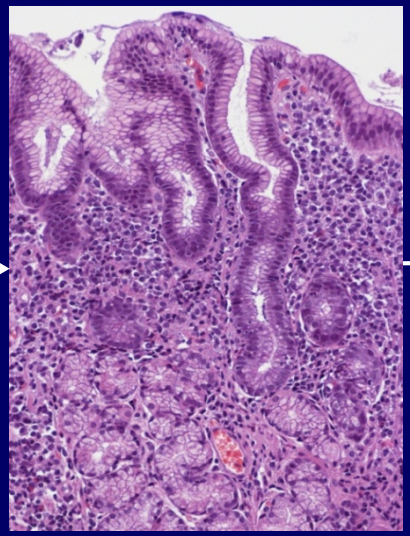
Adenocarcinoma of the Stomach

A leading cause of cancer-related death worldwide

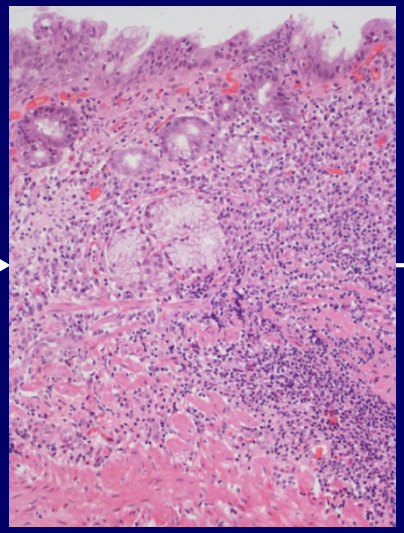
>800,000 deaths/year



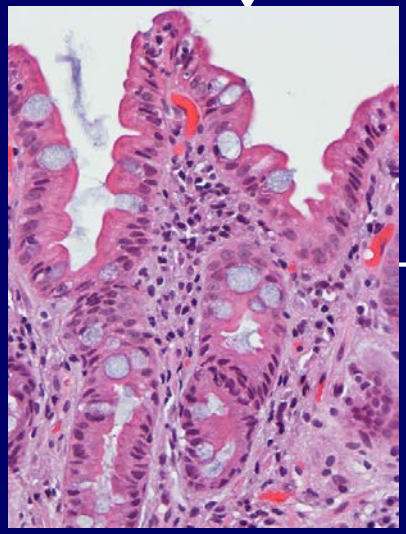
Normal



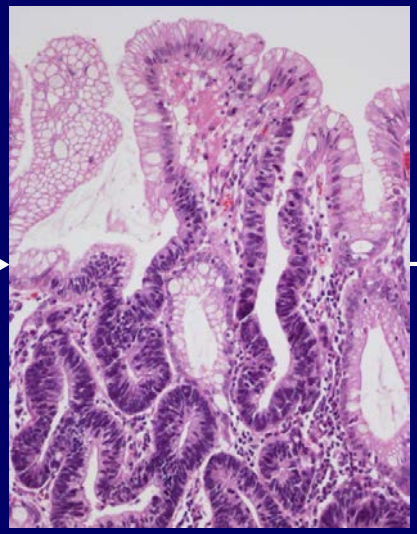
Non-atrophic gastritis



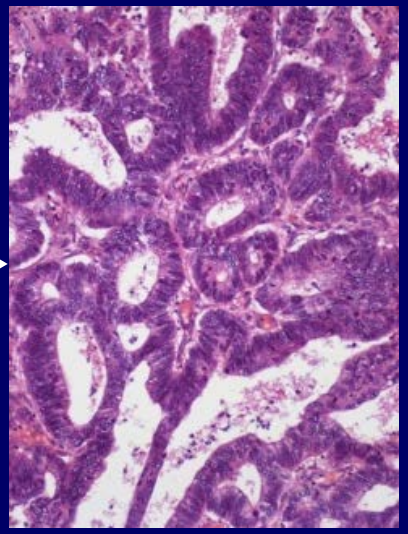
Atrophic gastritis



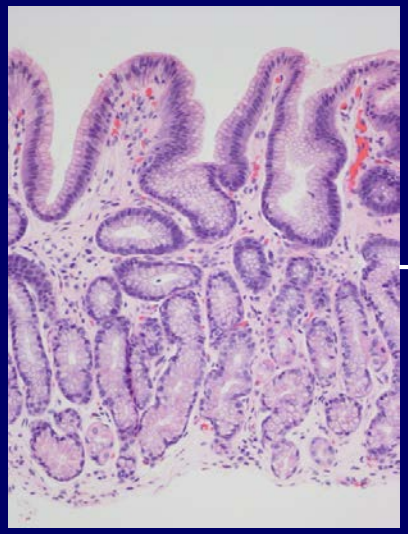
SPEM/Intestinal metaplasia



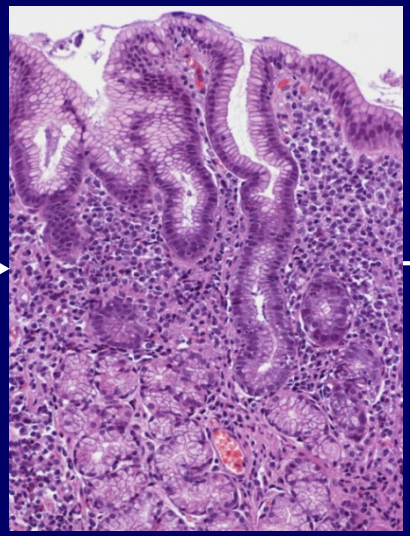
Dysplasia



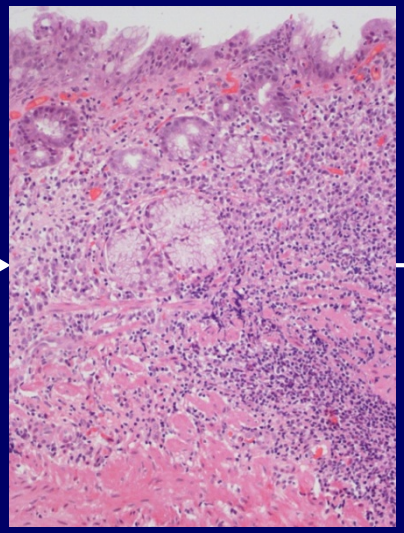
Cancer



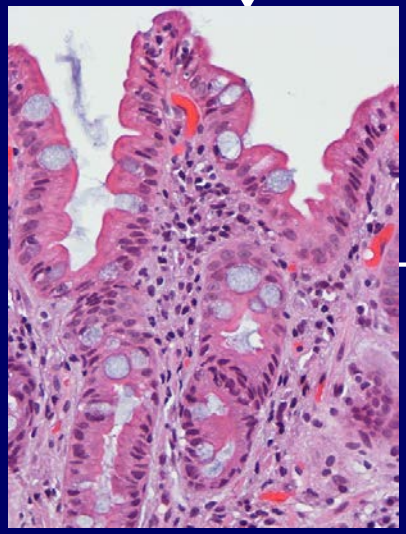
Normal



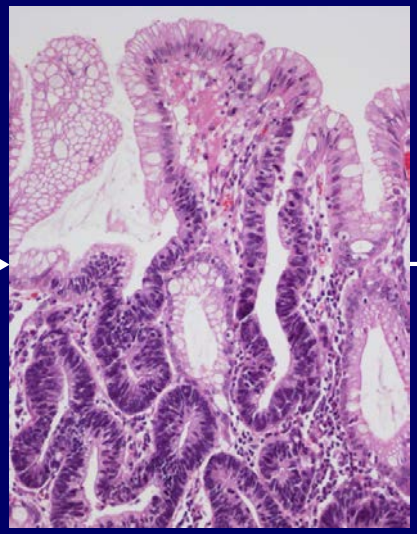
Non-atrophic gastritis



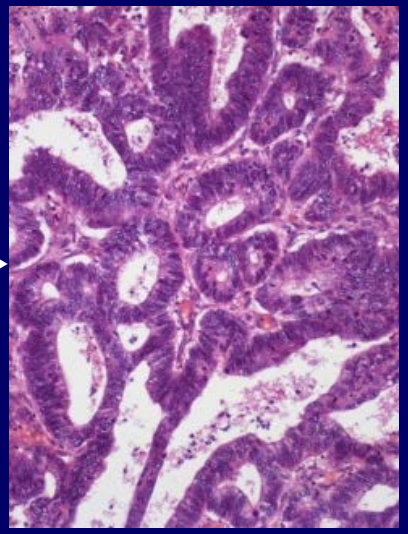
Atrophic gastritis



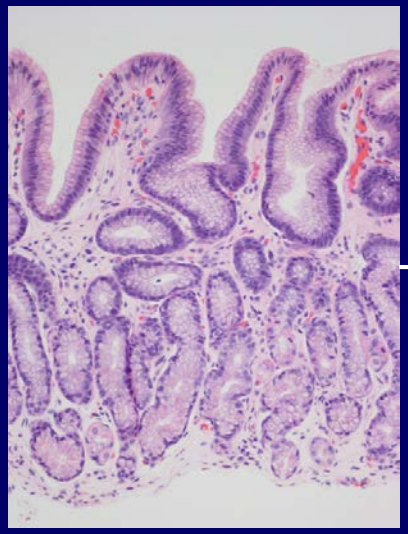
SPEM/Intestinal metaplasia



Dysplasia

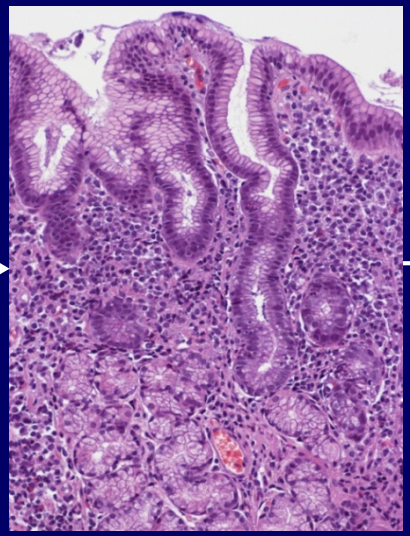


Cancer

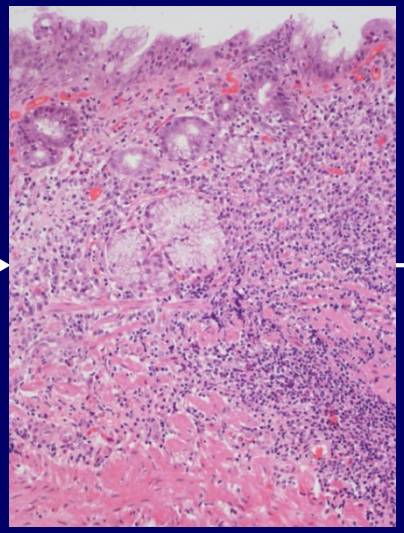


Normal

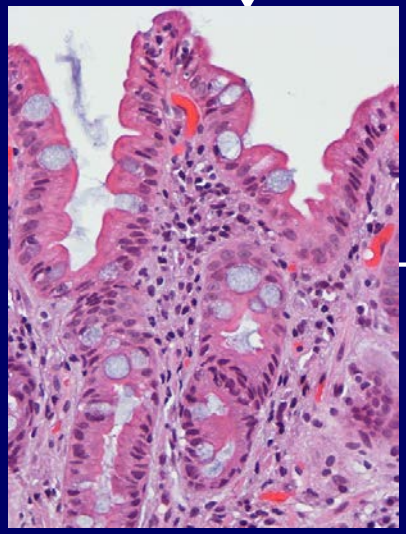
H. pylori



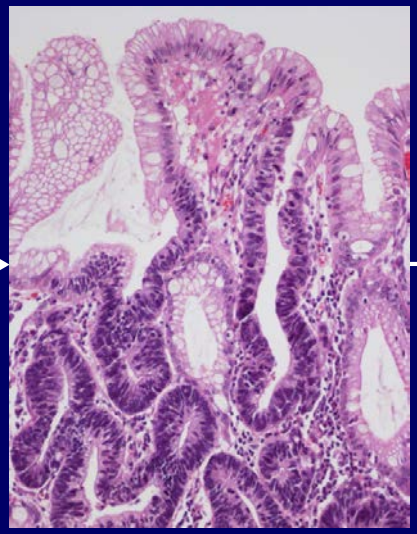
Non-atrophic gastritis



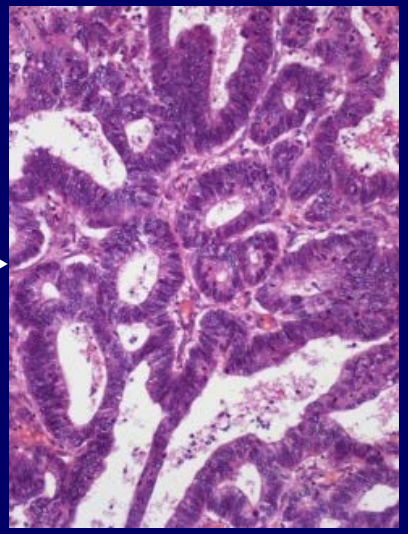
Atrophic gastritis



SPEM/Intestinal metaplasia

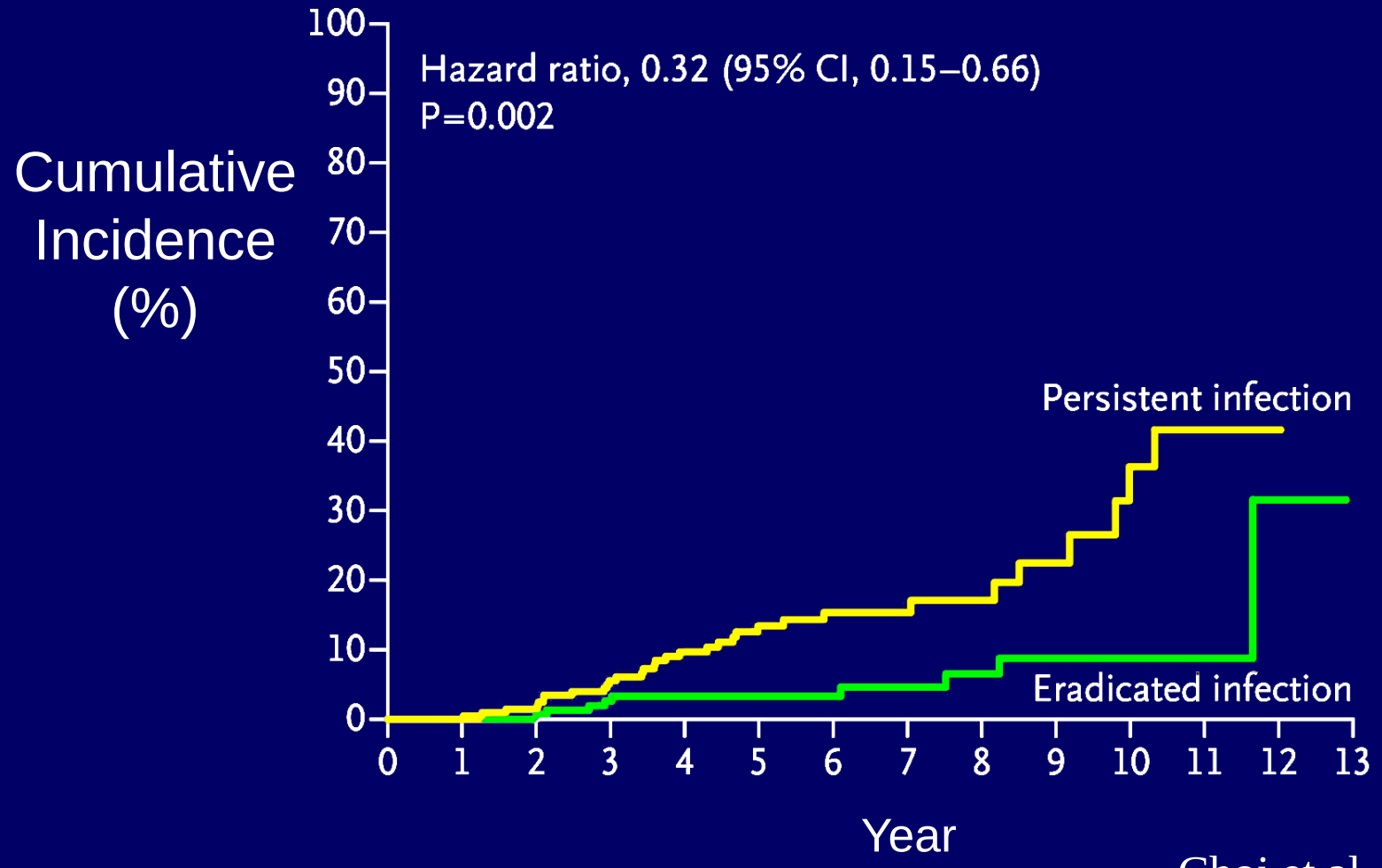


Dysplasia



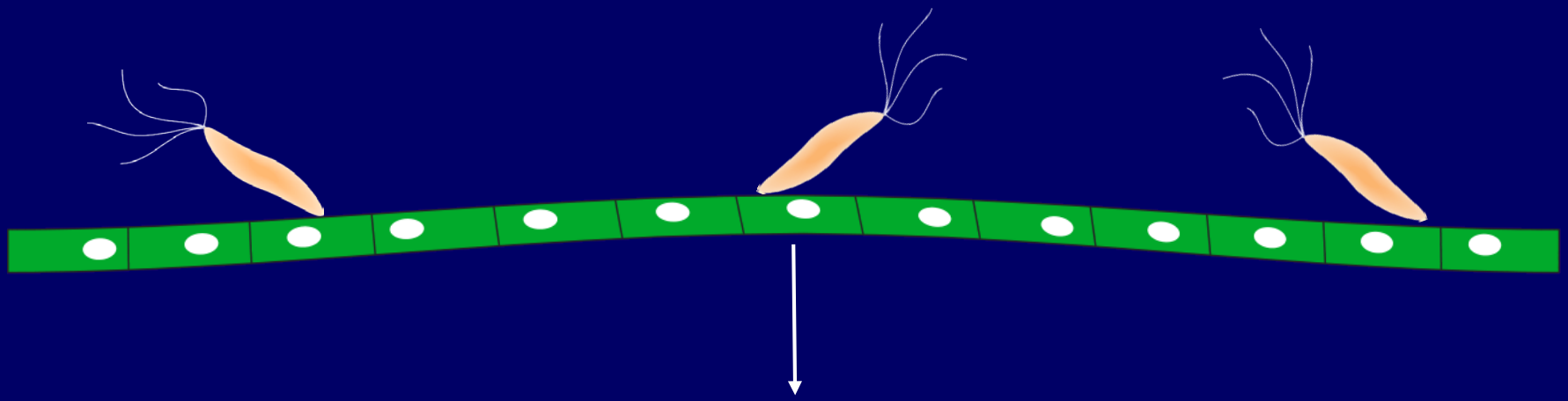
Cancer

Analysis of the incidence of metachronous gastric cancer, according to *H. pylori* status after trial medication



Choi et al., *NEJM*, 2018

Host responses to *H. pylori* strain-specific virulence constituents influence carcinogenesis

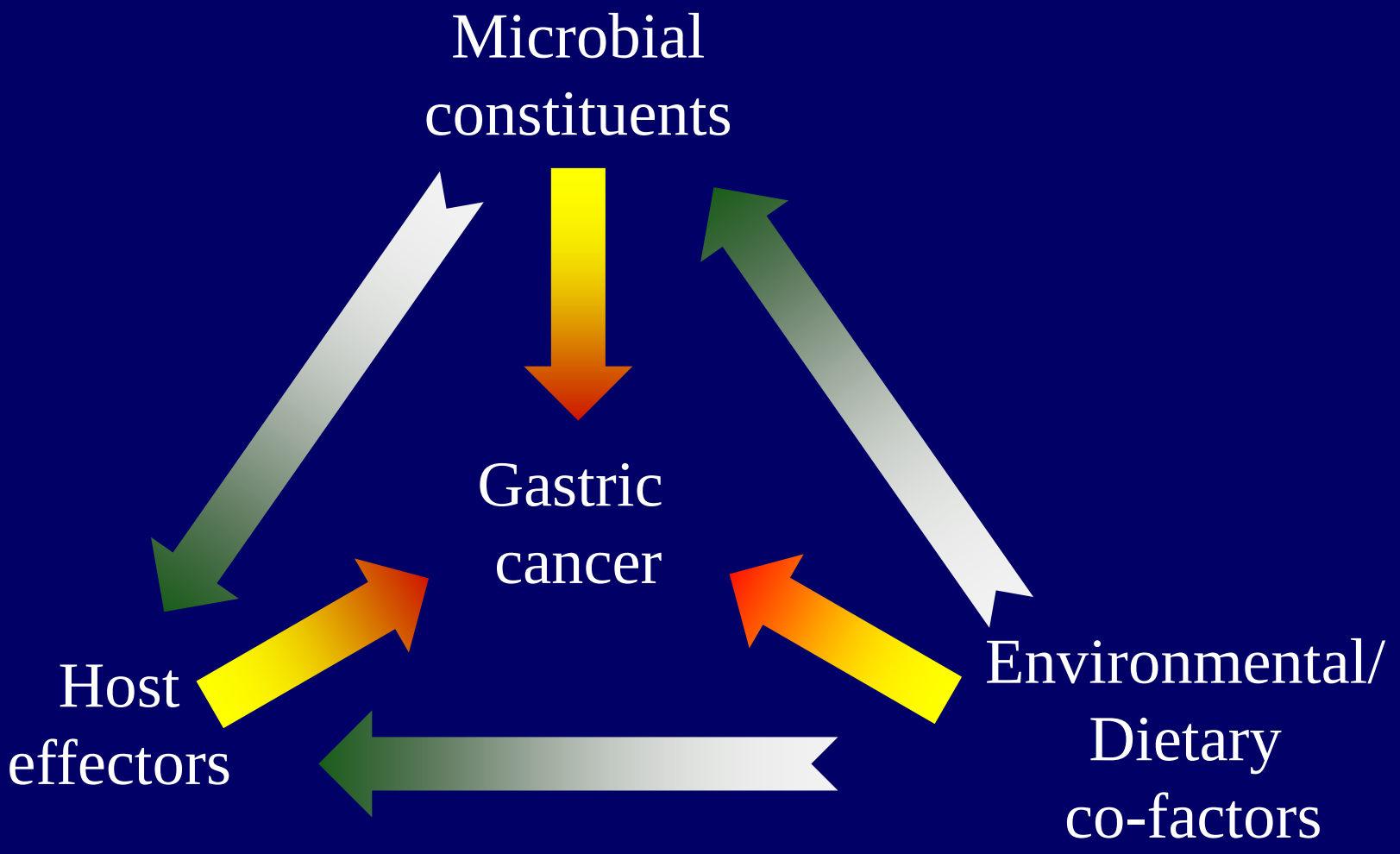


Gastric inflammation

Decades

Gastric adenocarcinoma (1-3%)

Pathologic interactions that mediate *H. pylori*-induced gastric cancer



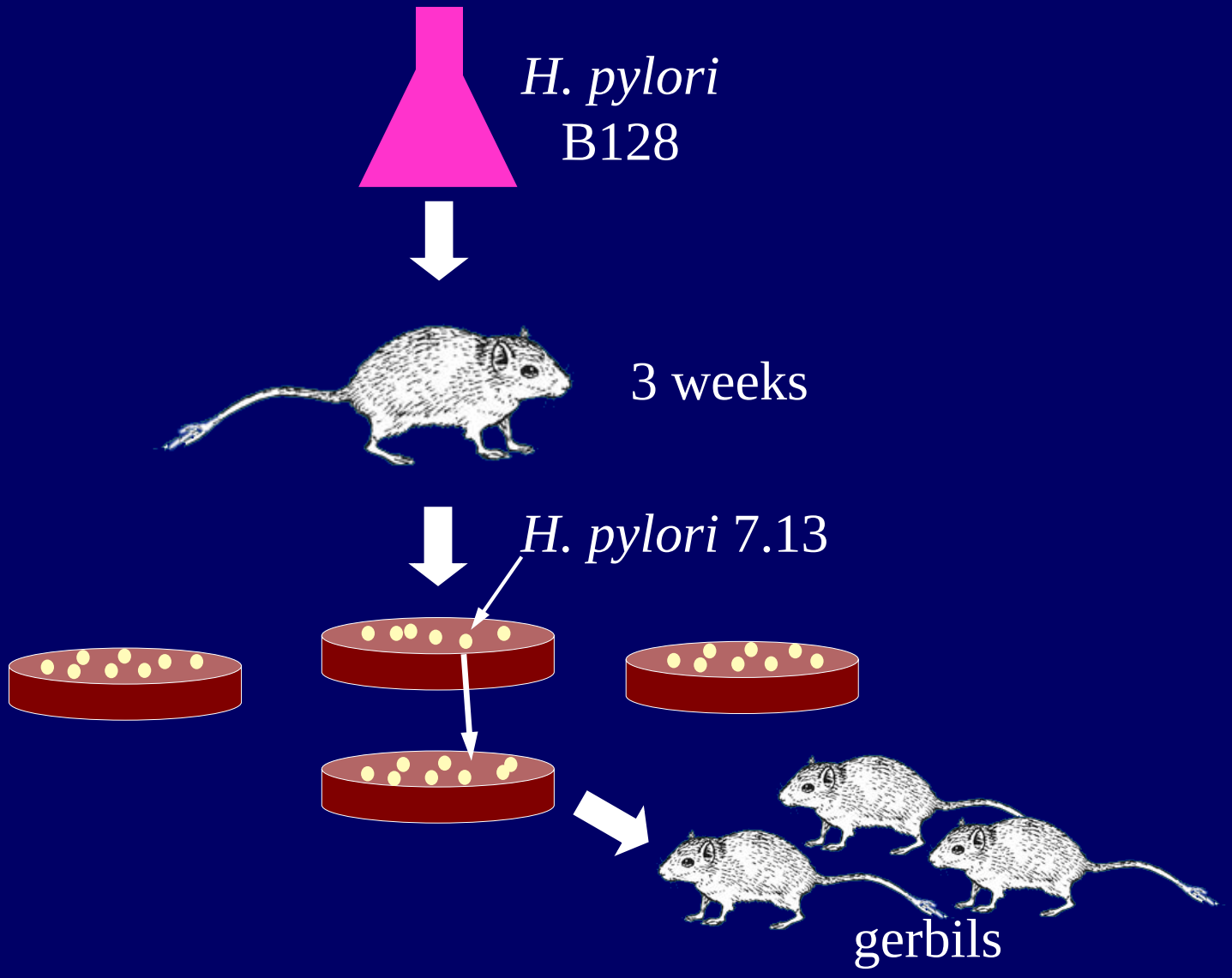
Iron deficiency increases the risk for gastric cancer

H. pylori infection is associated with iron deficiency, which affects 30% of the world's population

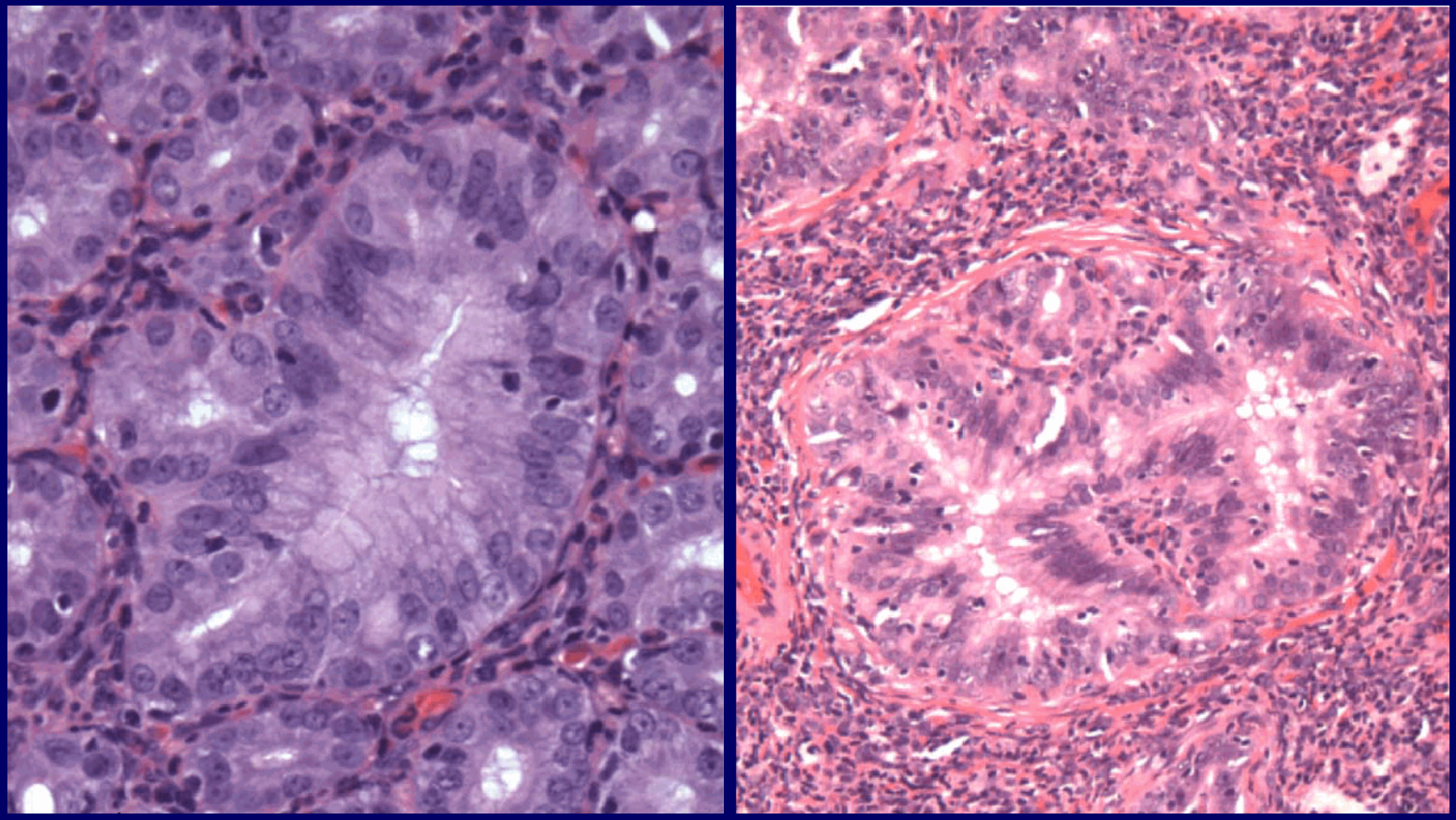
Iron deficiency is associated with a high incidence of

- Preneoplastic gastric lesions
- Gastric adenocarcinoma

In vivo derivation of *H. pylori* strain 7.13 from its human progenitor B128

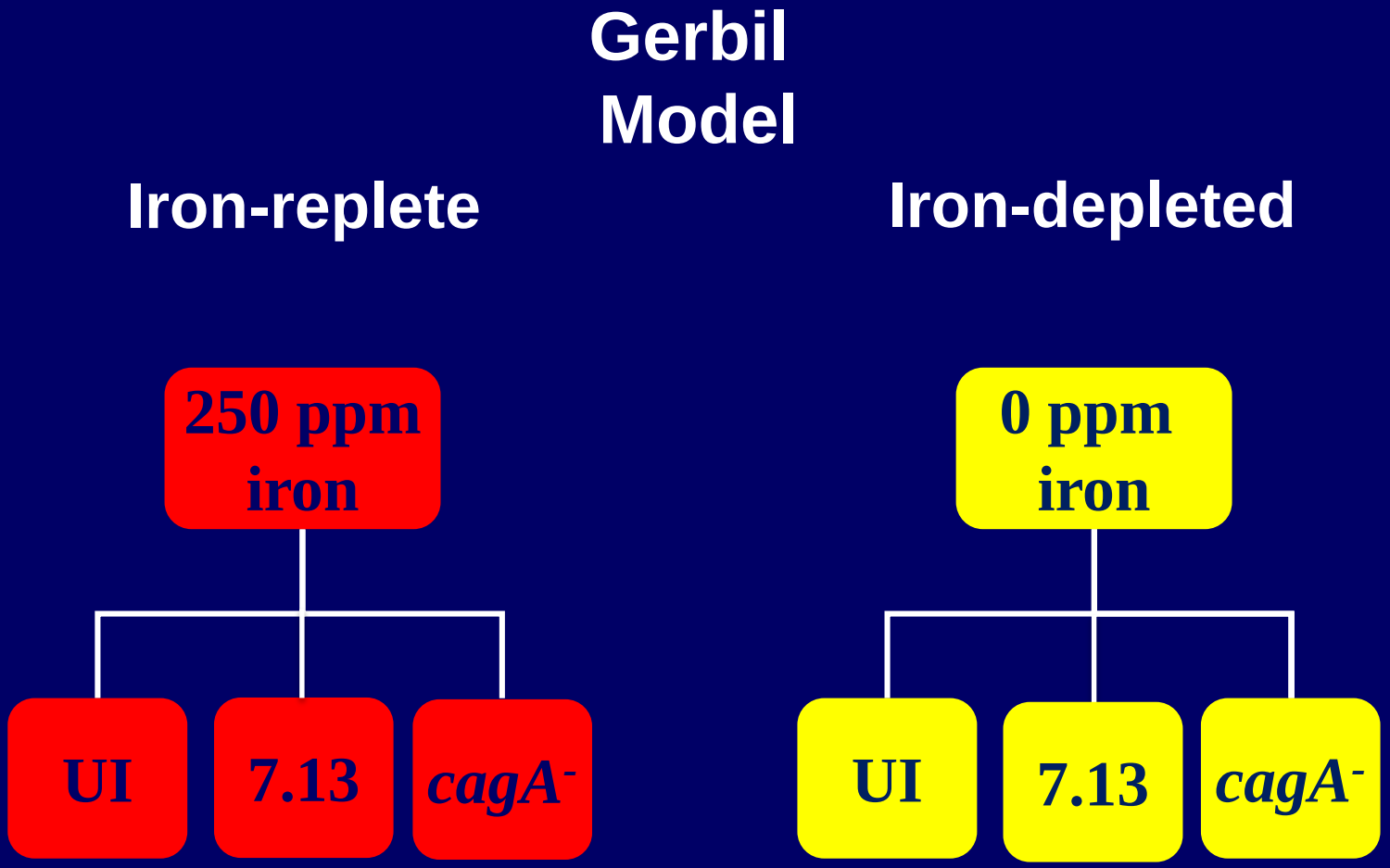


Development of premalignant and malignant lesions following infection with *H. pylori* 7.13

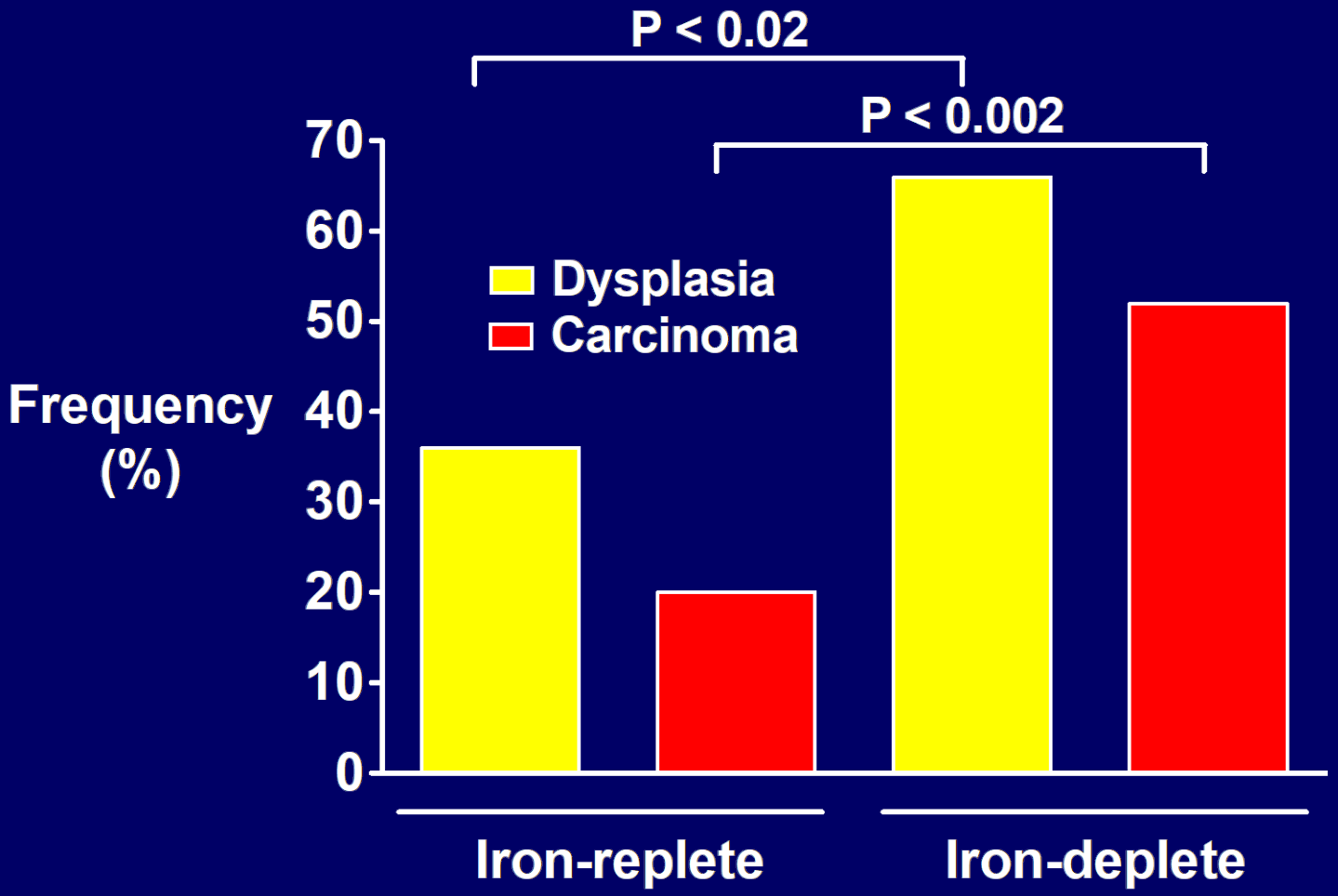


Franco et al., *PNAS*

Experimental design

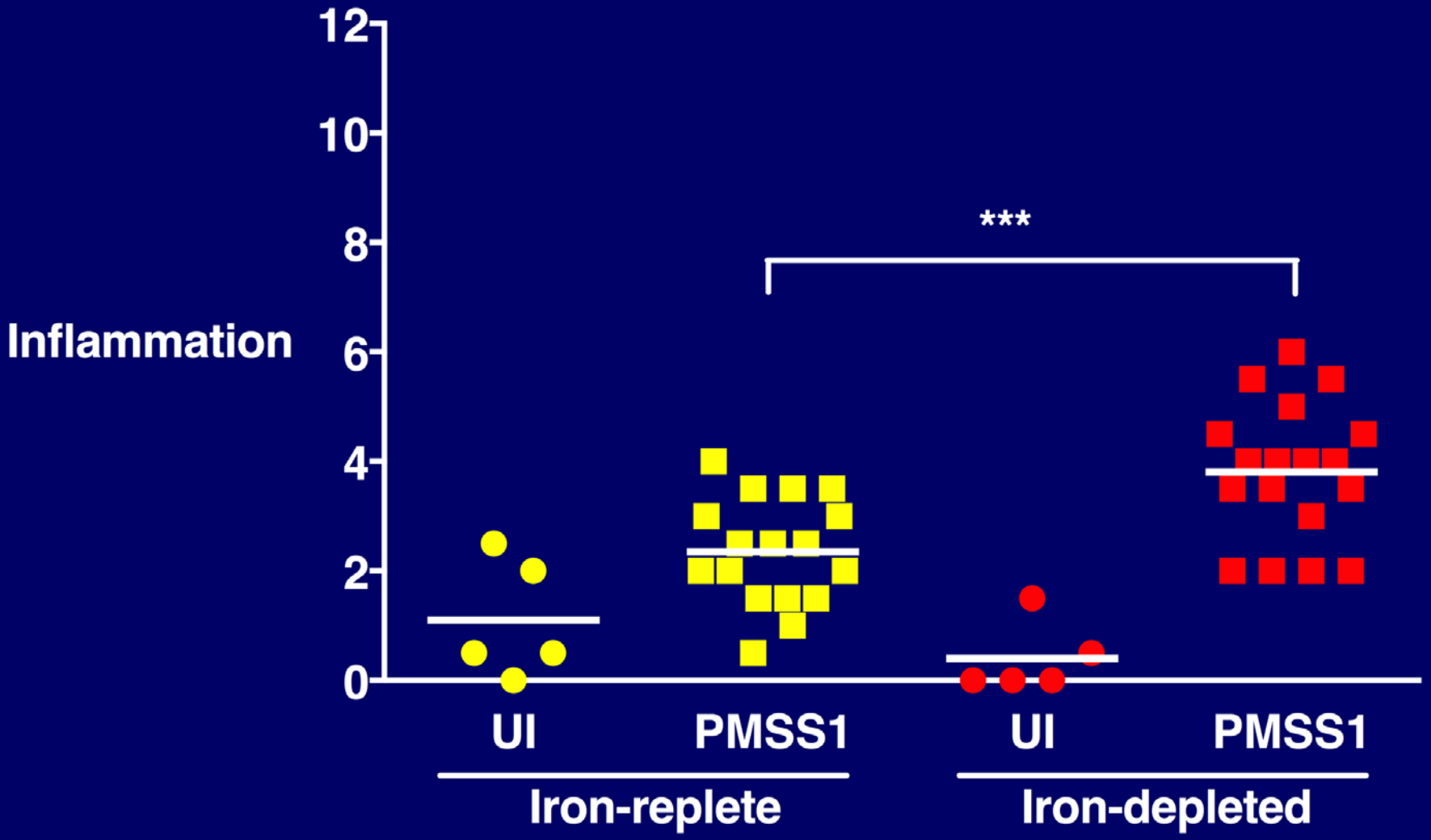


Dietary iron depletion increases the frequency of dysplasia and carcinoma

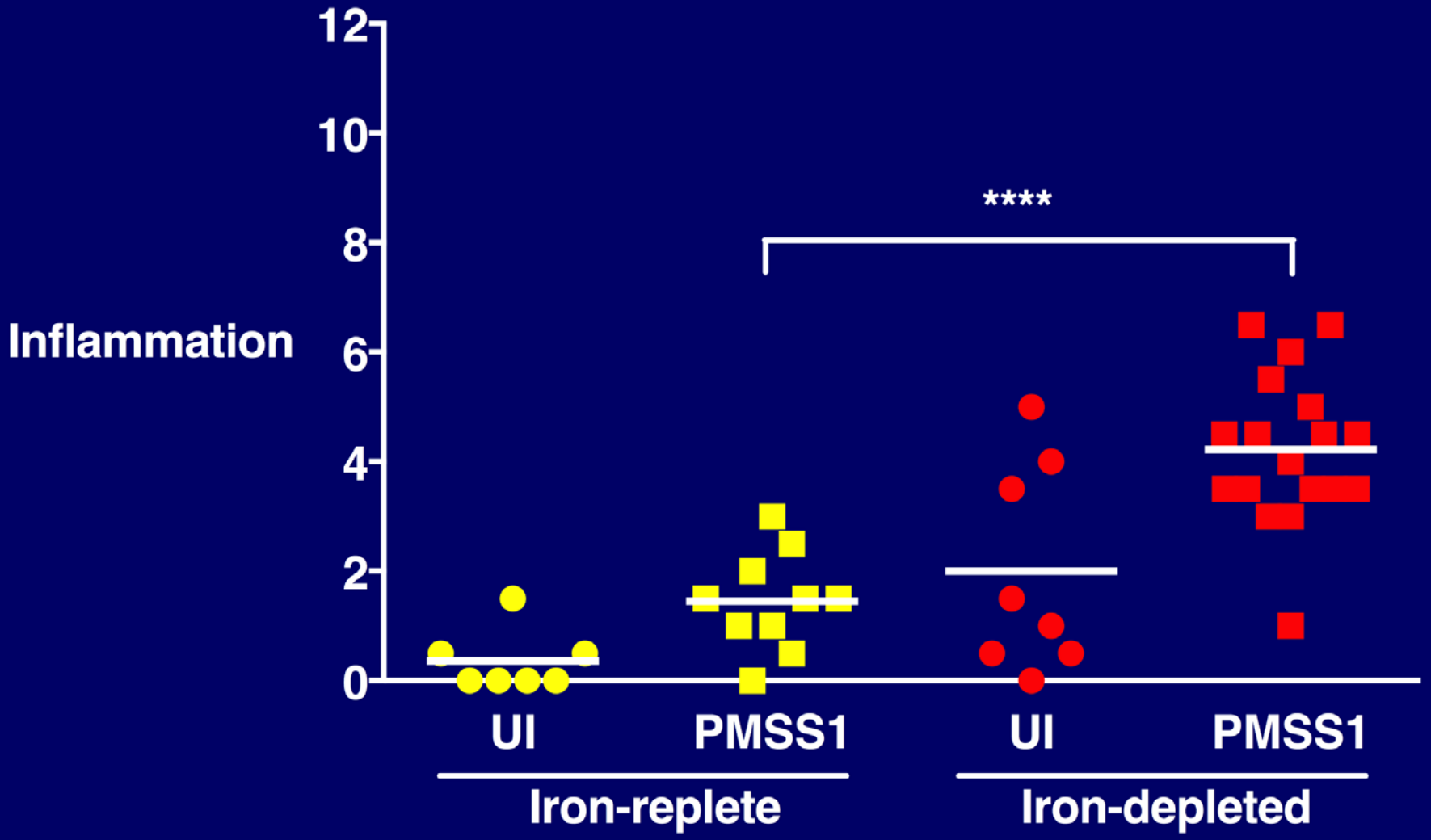


Noto et al., *JCI*

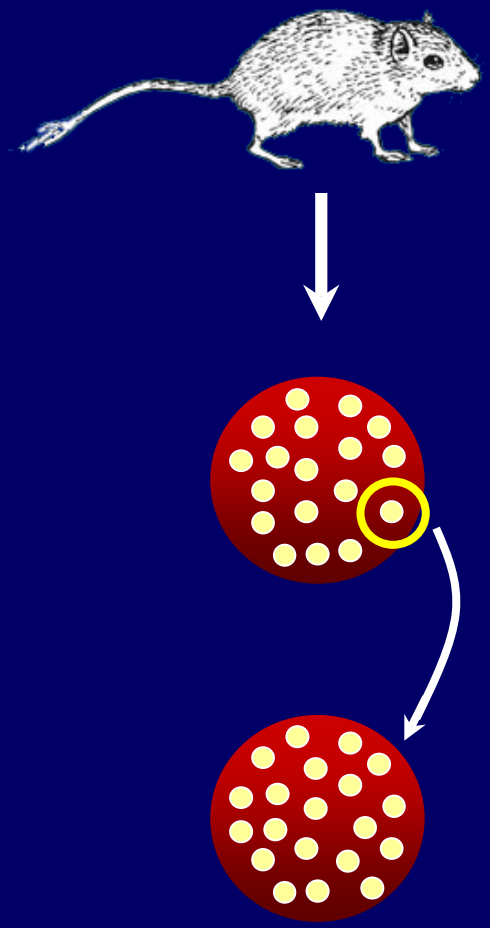
Iron deficiency heightens *H. pylori*-induced inflammation in C57Bl/6 mice



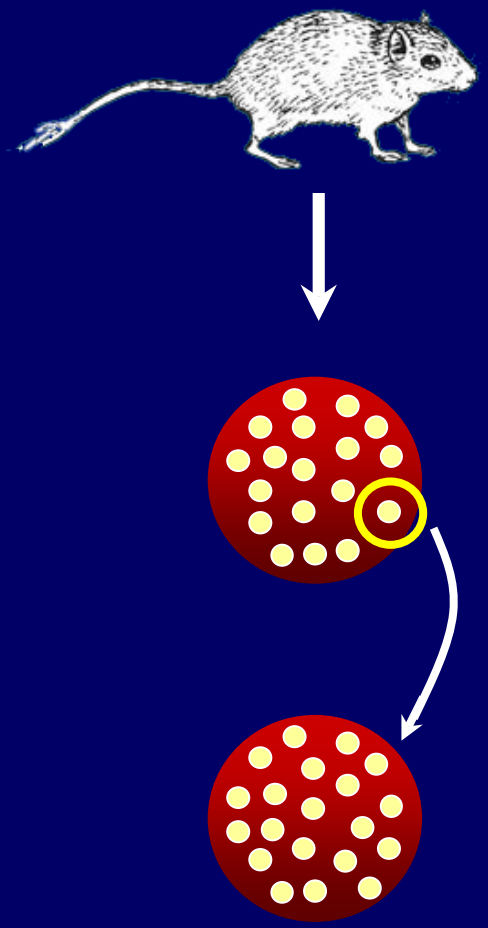
Iron deficiency heightens *H. pylori*-induced inflammation in INS-GAS mice



Isolation of *in vivo*-adapted *H. pylori* strains

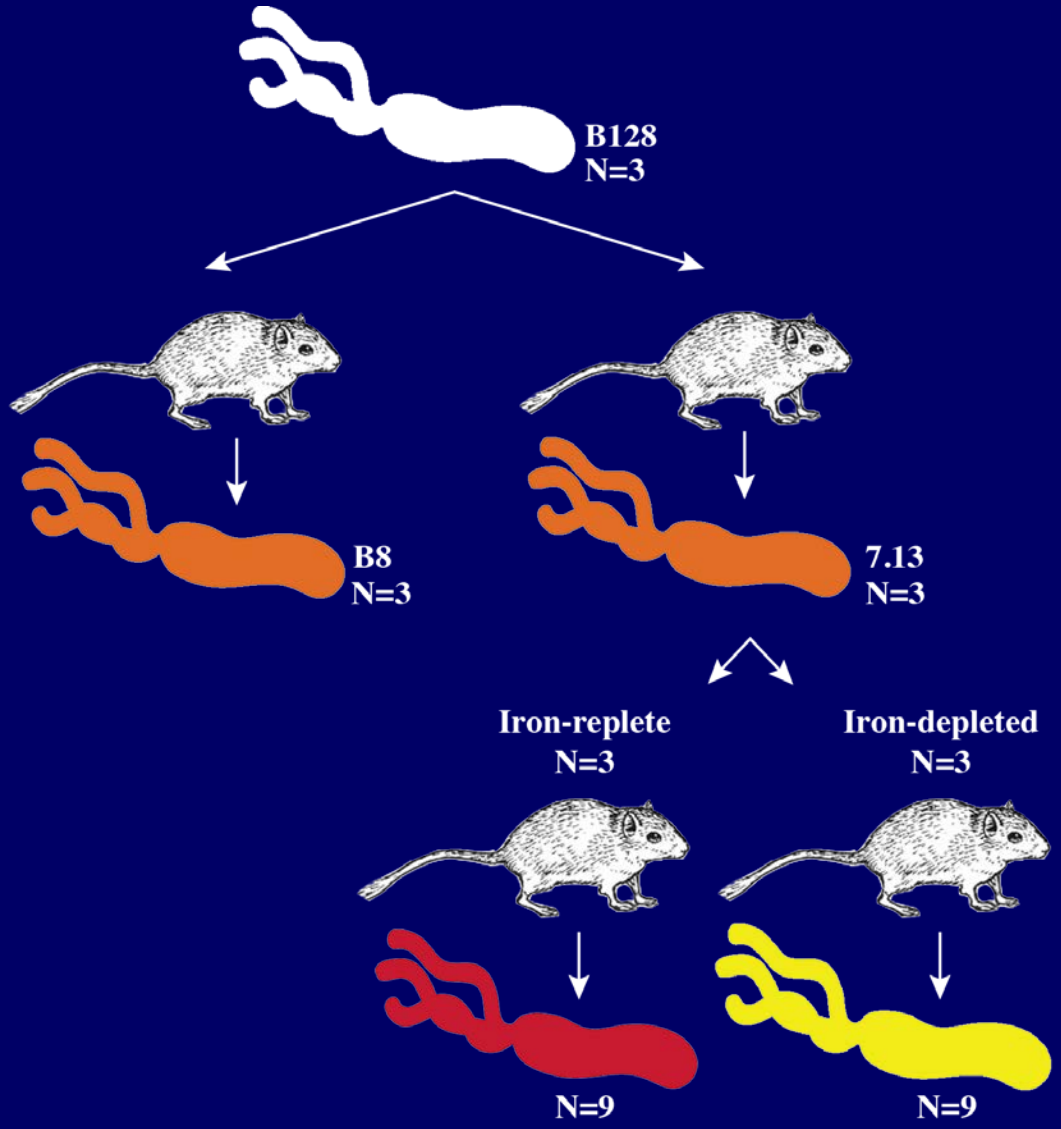


In vivo-adapted strains
Iron-replete gerbils



In vivo-adapted strains
Iron-deplete gerbils

In vivo-adapted *H. pylori* strains subjected to whole genome sequencing



Noto et al., *Gut*

SNPs identified following *in vivo*-adaptation to conditions of iron deficiency

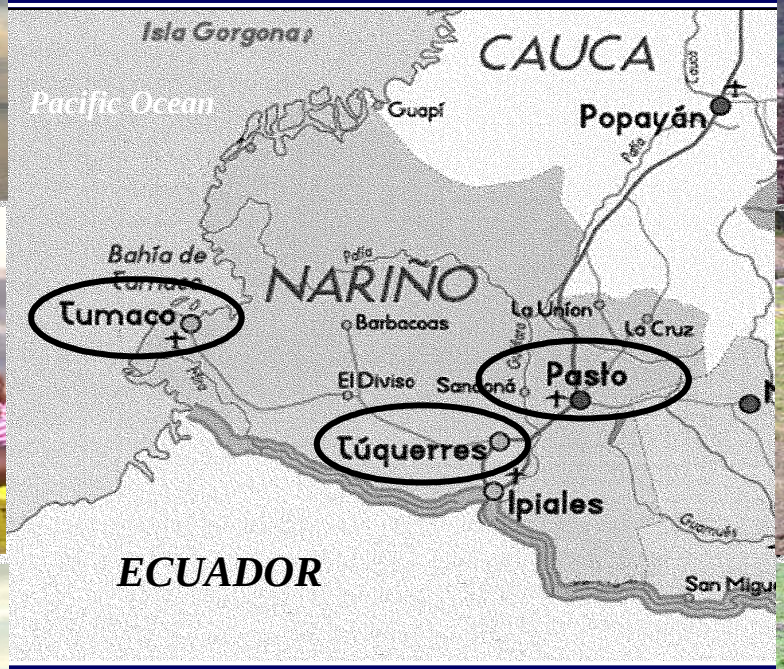
Gene	Function	HPB8 locus
Putative OMP	Outer membrane protein	593
Putative OMP	Outer membrane protein	626
cagY	Type IV secretion system	716
oipA	Outer inflammatory protein	838
Putative OMP	Outer membrane protein	1104
Putative OMP	Outer membrane protein	1139
fur	Ferric uptake regulator	1145

Noto et al., *Gut*

Nariño, Colombia

Low-risk area

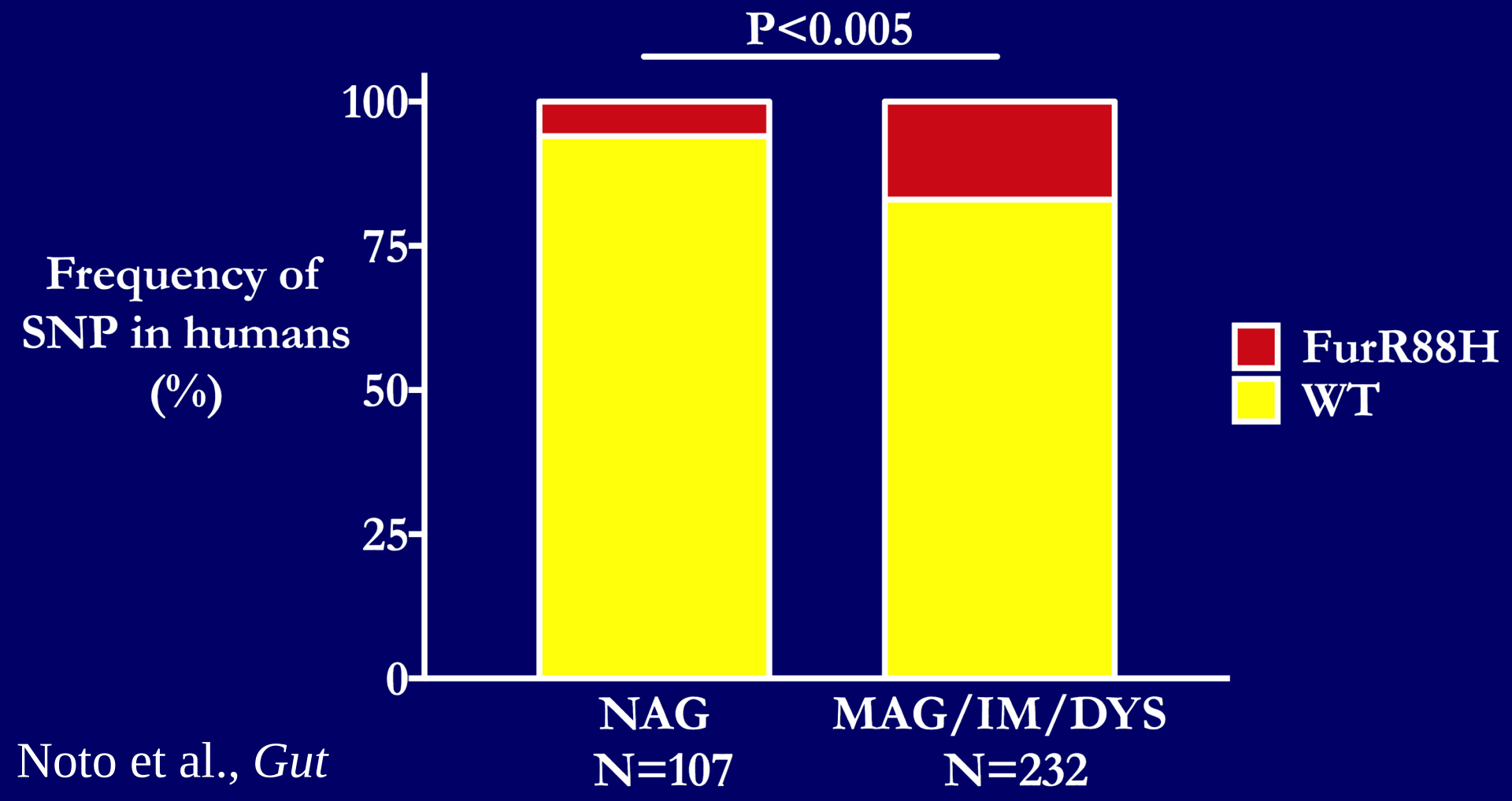
(6/100,000)



High-risk area

(150/100,000)

H. pylori isolates from patients with premalignant lesions more frequently harbor the FurR88H variant



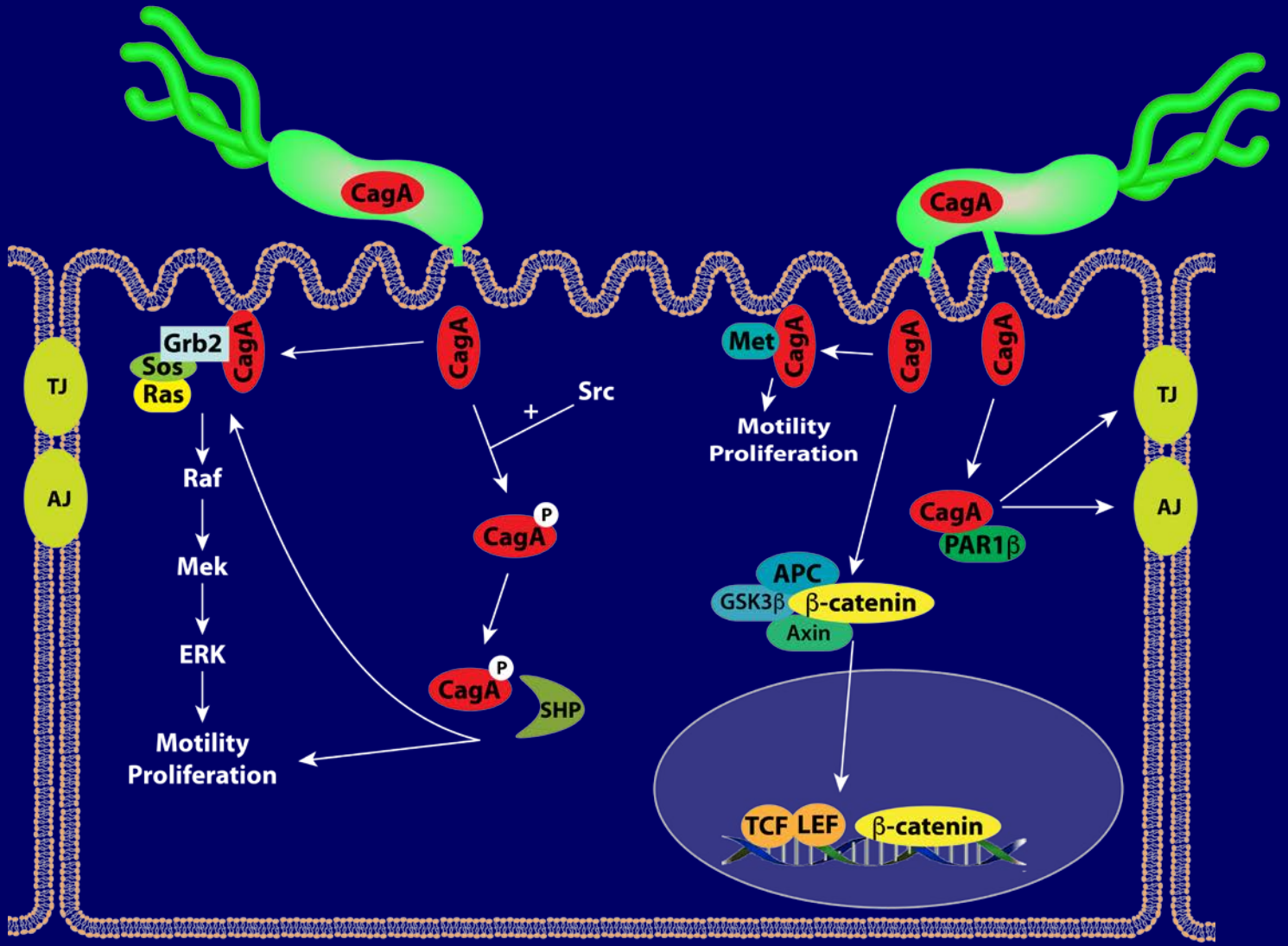
Noto et al., *Gut*

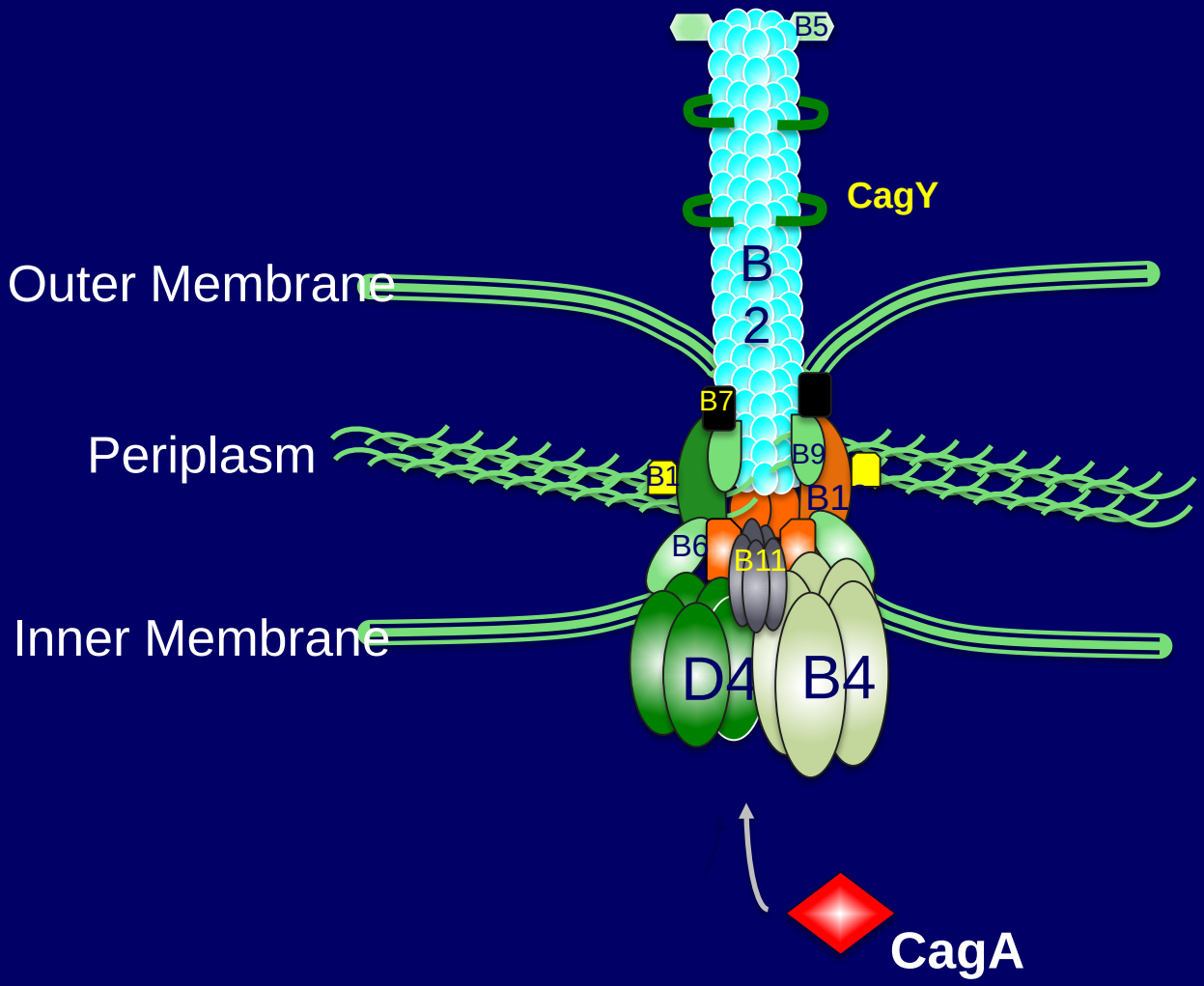
SNPs identified following *in vivo*-adaptation to conditions of iron deficiency

Gene	Function	HPB8 locus
Putative OMP	Outer membrane protein	593
Putative OMP	Outer membrane protein	626
cagY	Type IV secretion system	716
oipA	Outer inflammatory protein	838
Putative OMP	Outer membrane protein	1104
Putative OMP	Outer membrane protein	1139
fur	Ferric uptake regulator	1145

Noto et al., *Gut*

Molecular signaling alterations induced by *cag* T4SS-mediated translocation of CagA




B5
CagY
B
2
Outer Membrane
B7
Periplasm
B9
B1
B1
B6
B11
Inner Membrane
D4
B4
CagA

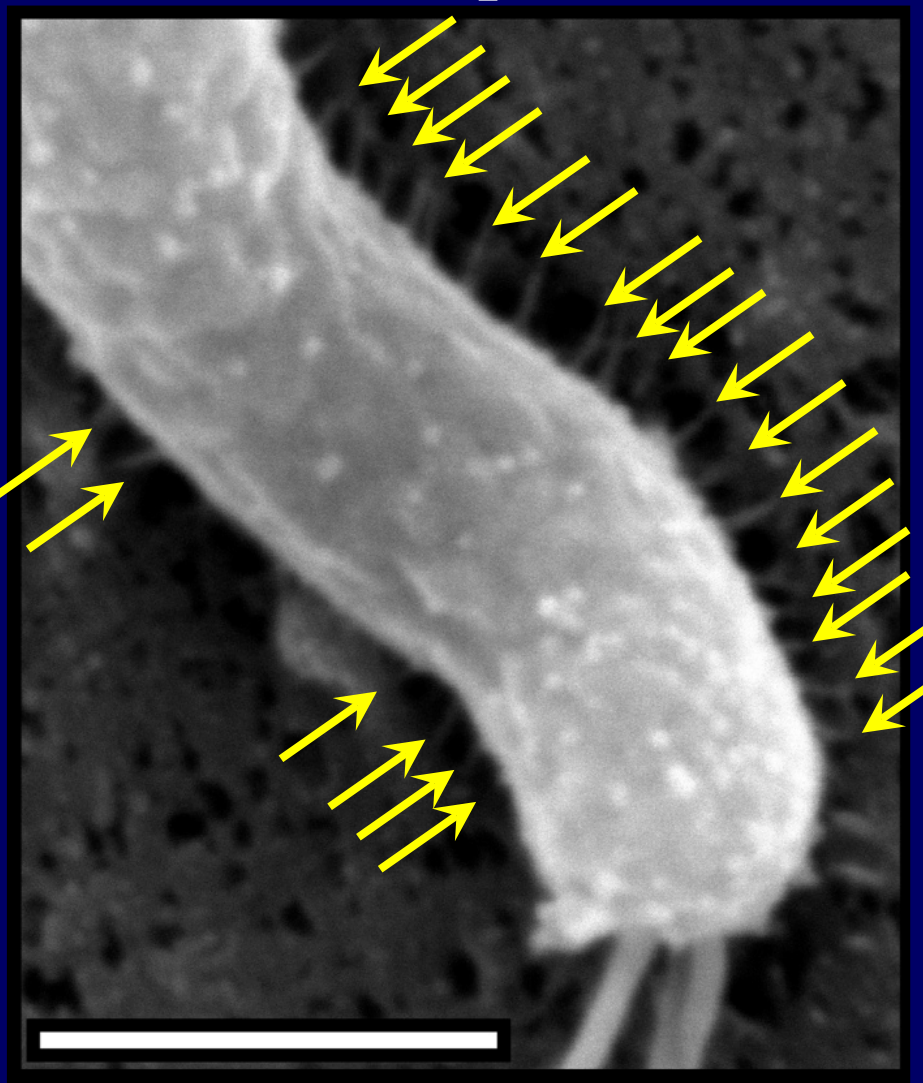
Iron depletion and assembly of the *cag* type IV secretion system

Iron-replete

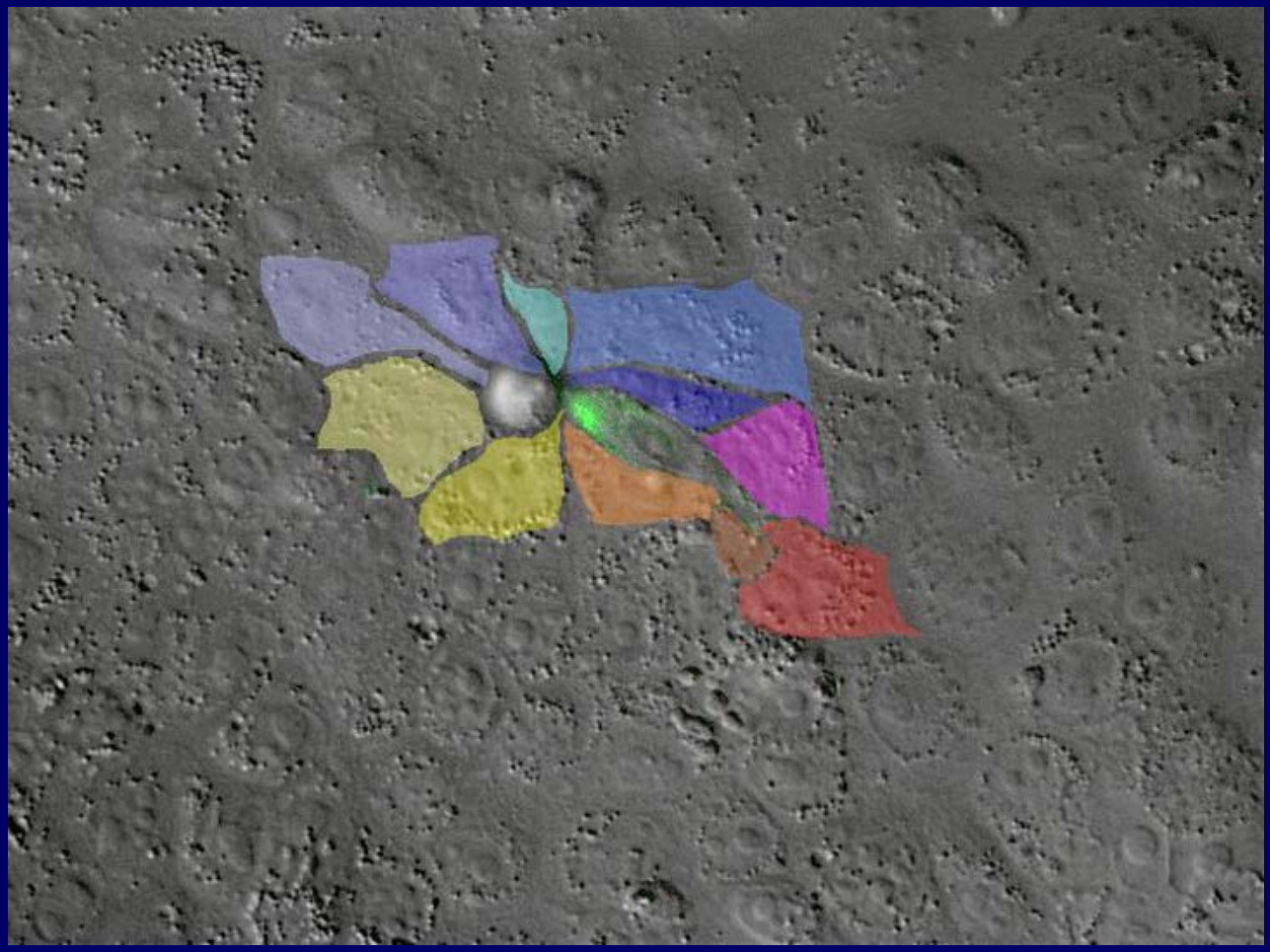
Iron depletion augments assembly of the *cag* type IV secretion system

Iron-replete

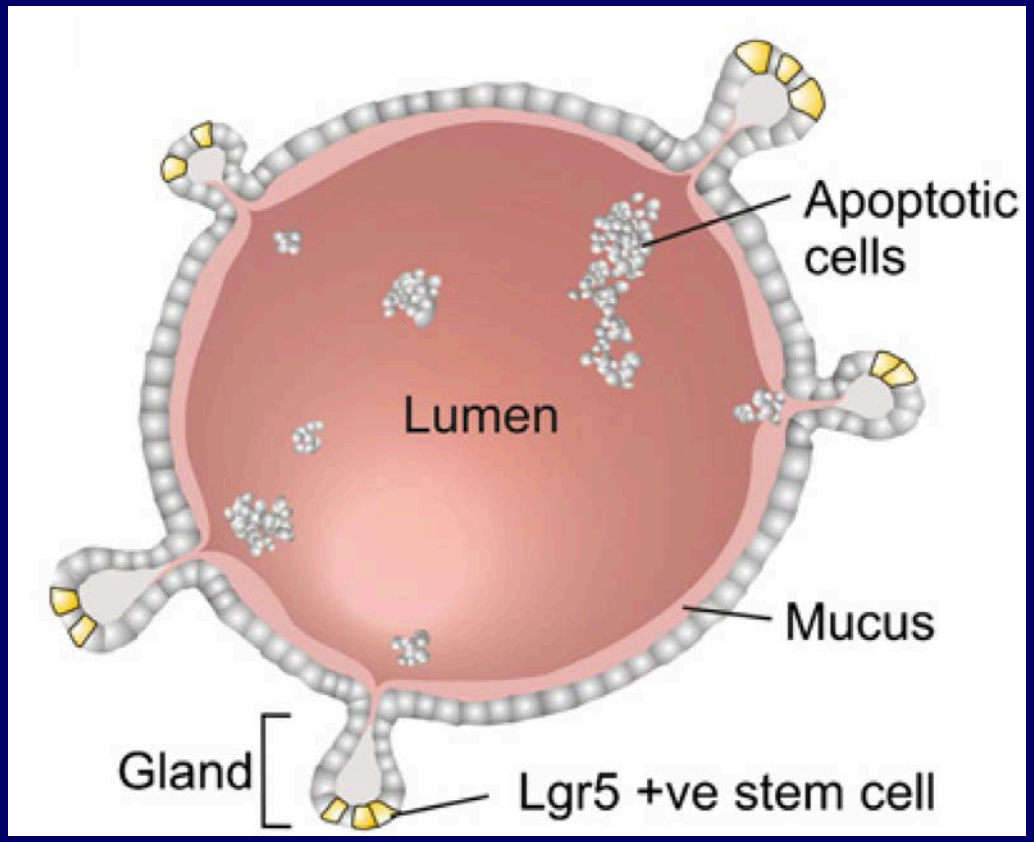
Iron-depleted



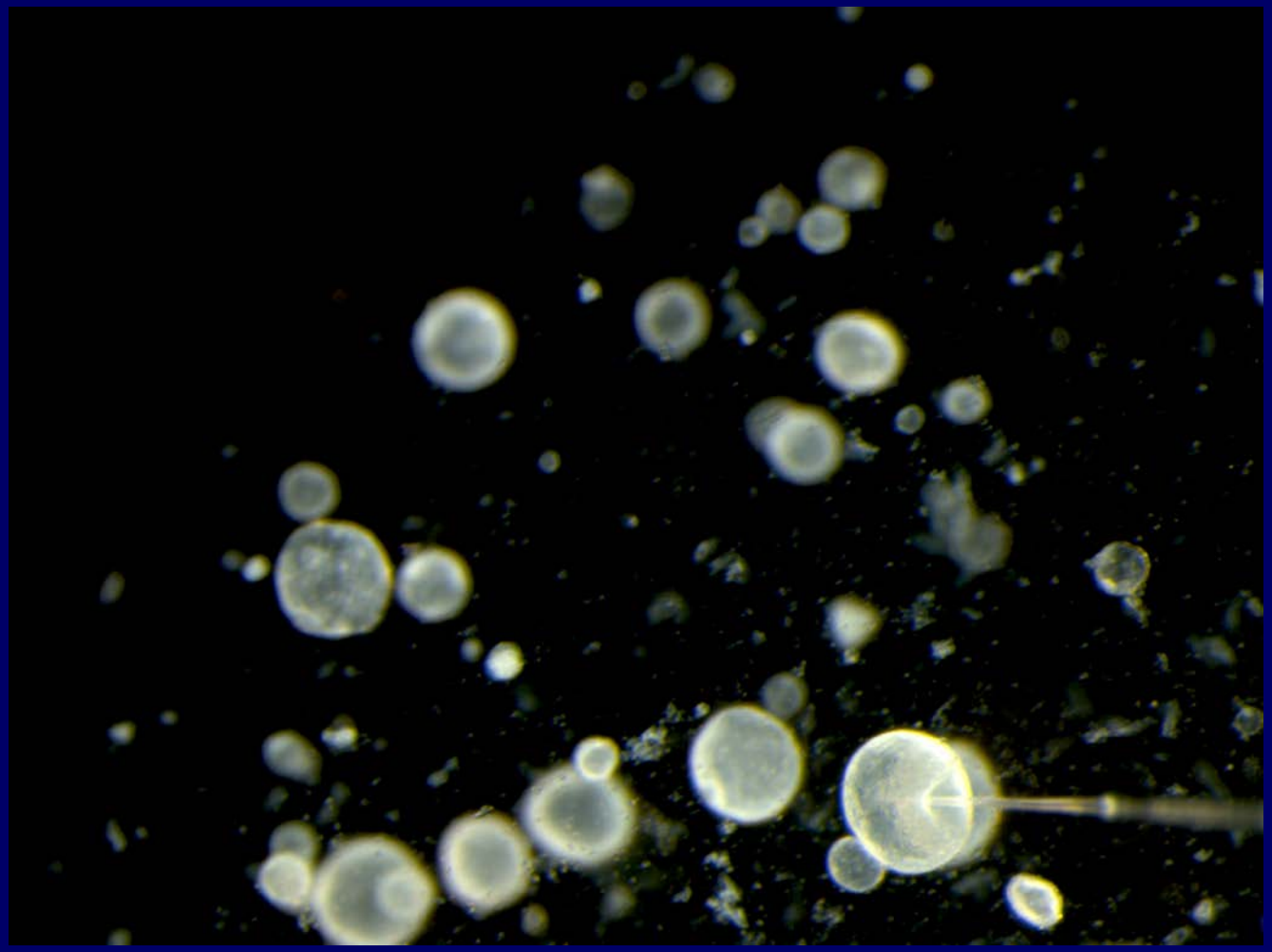
CagA-expressing cells acquire carcinogenic phenotypes



Self-renewing gastric organoid



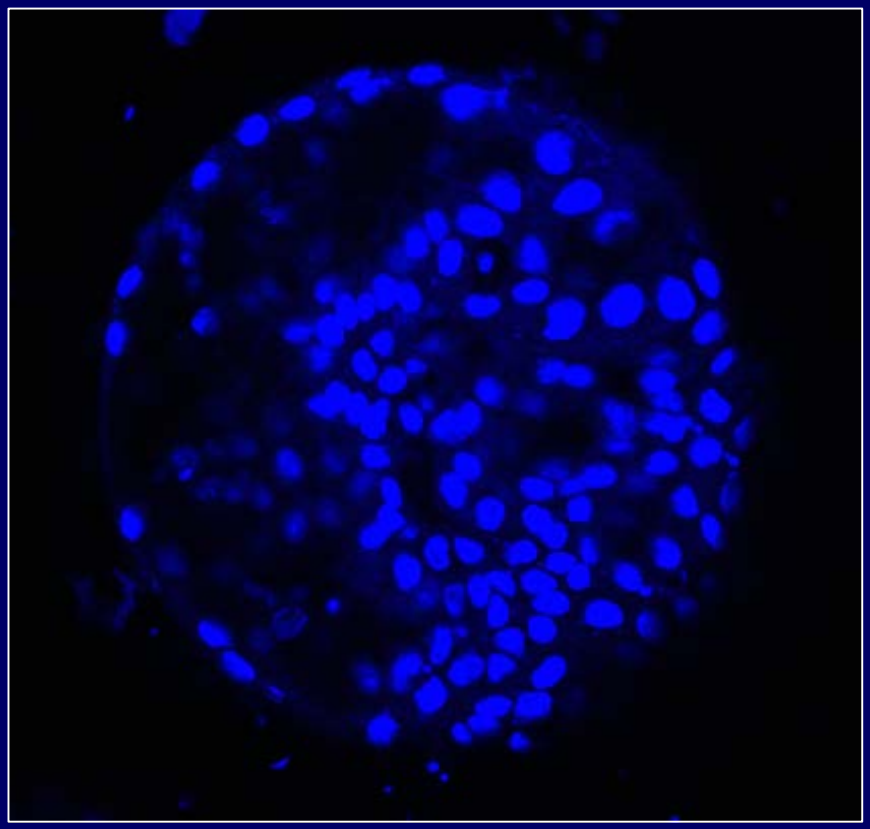
Microinjection of organoids

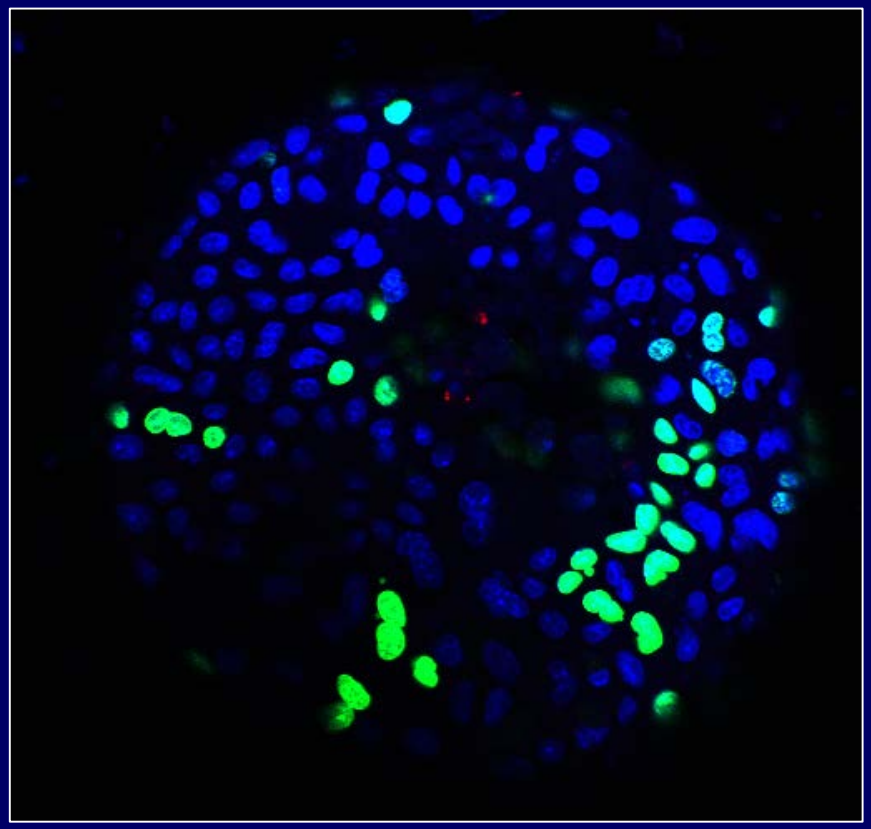


H. pylori increases proliferation

Uninfected

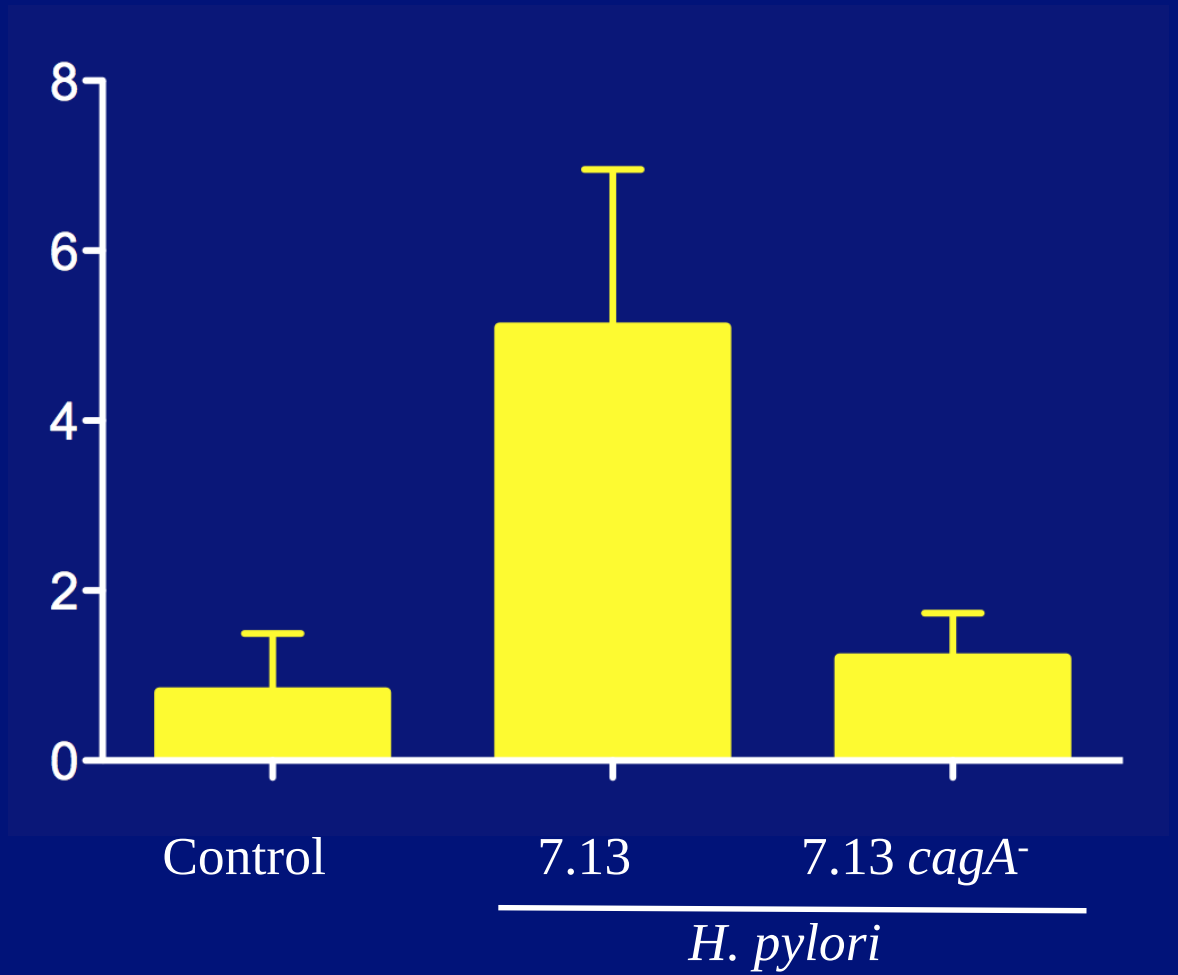
H. pylori cag^{+} strain 7.13





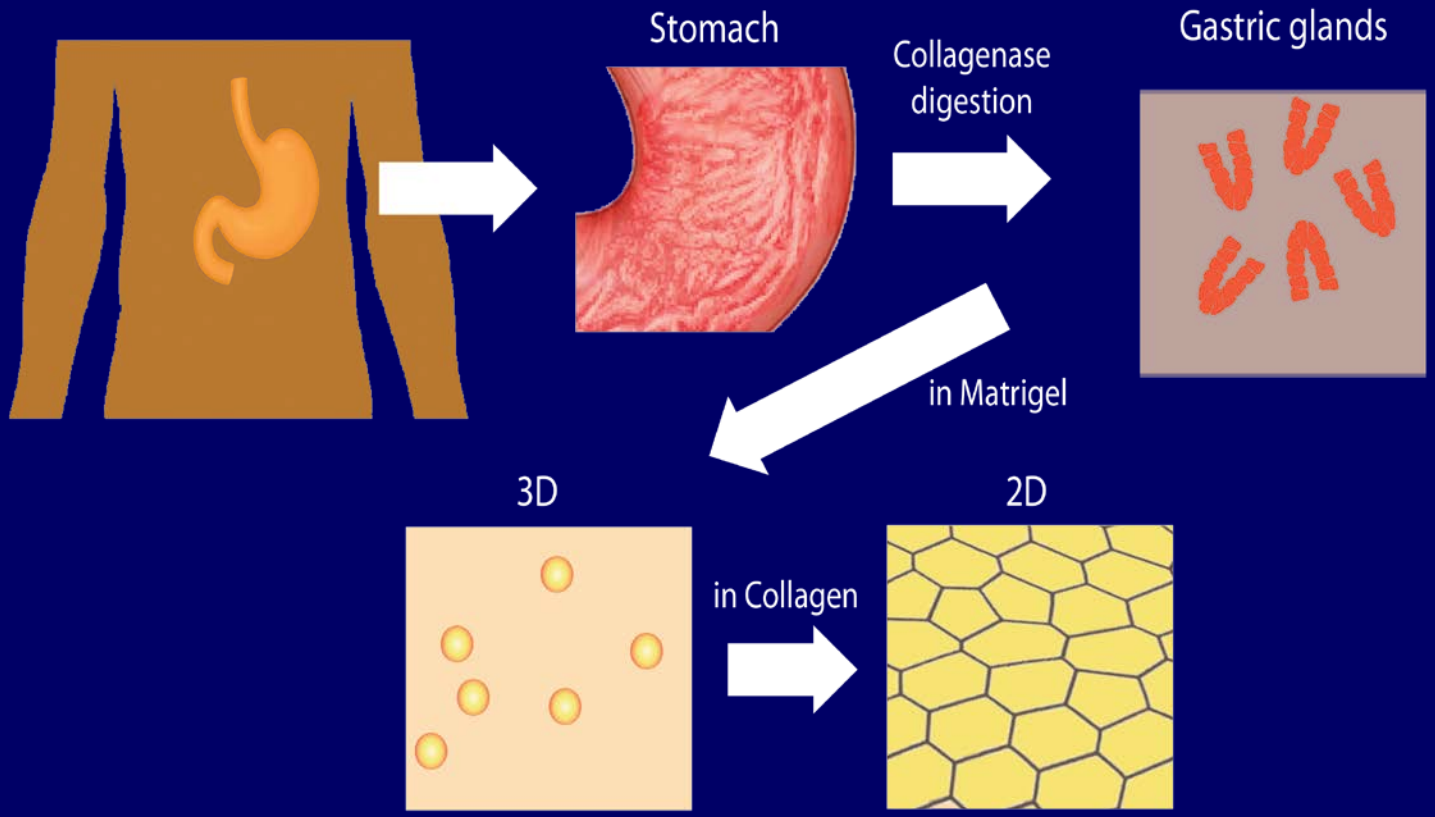
Wroblewski et al., *Gut*

Proliferation in gastric organoids infected by wild-type and isogenic mutant *cagA*$^{-}$ *H. pylori*

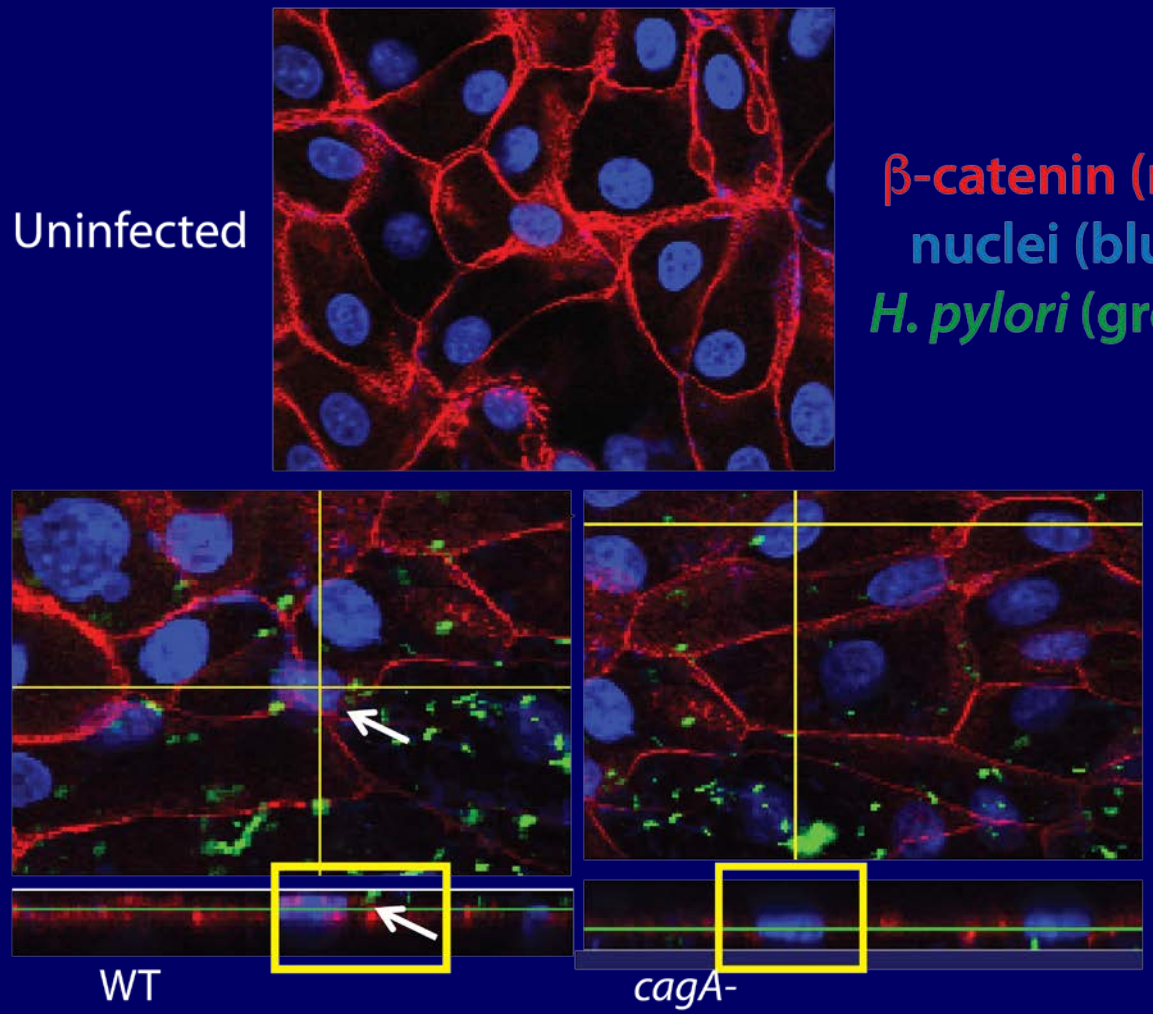


Wroblewski et al., *Gut*

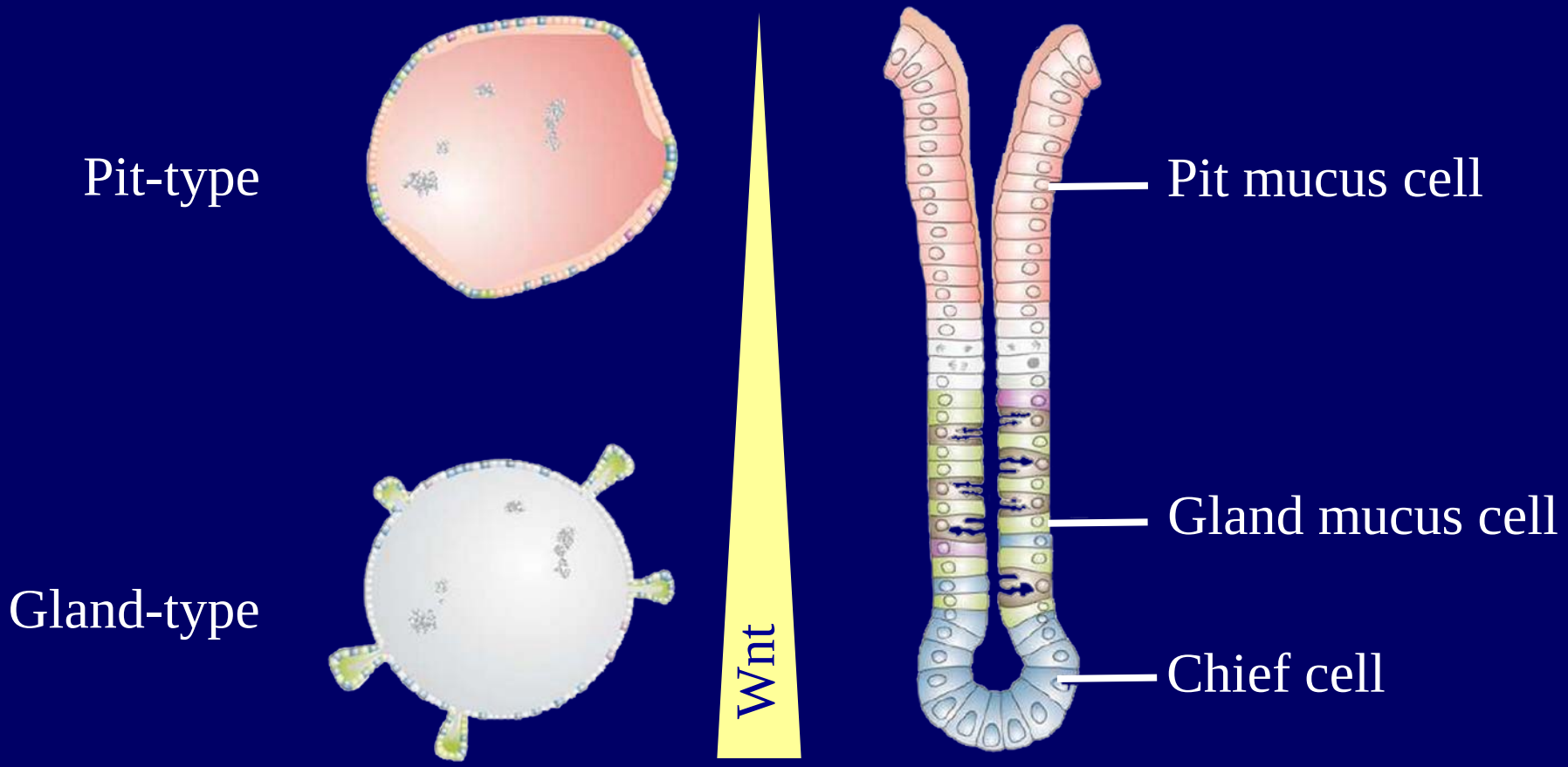
Development of human gastroid monolayers



Nuclear localization of β-catenin is selectively induced by *cag*$^{+}$ *H. pylori*



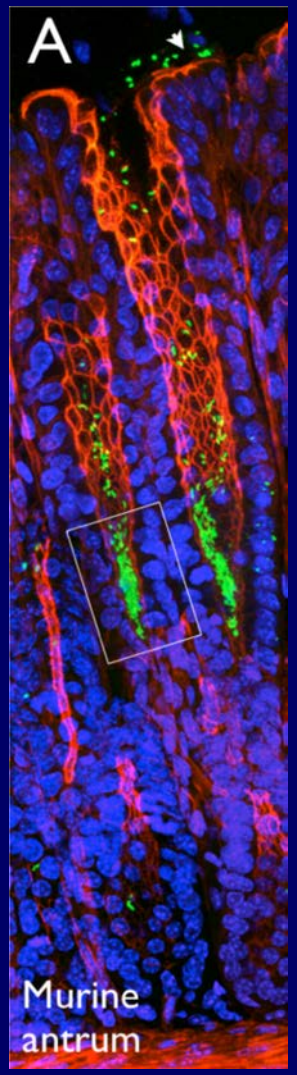
Differentiation of human gastroids

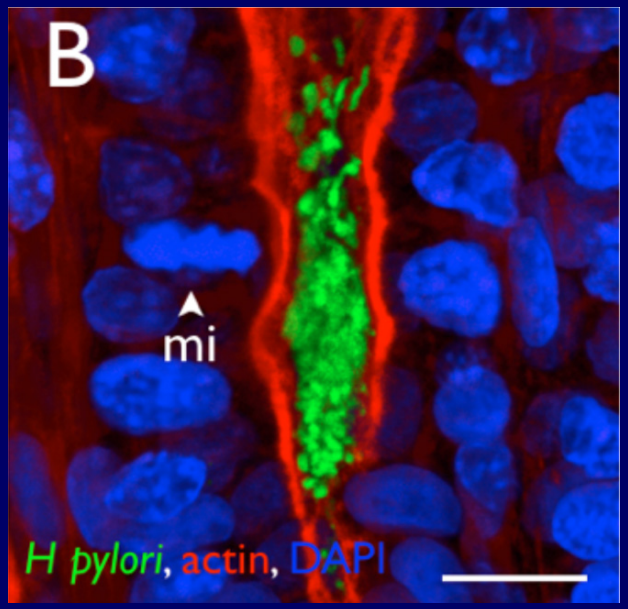


Bartfeld et al., *Gastroenterology*

How do *H. pylori cag*$^{+}$ strains interact with gastric progenitor/stem cells?

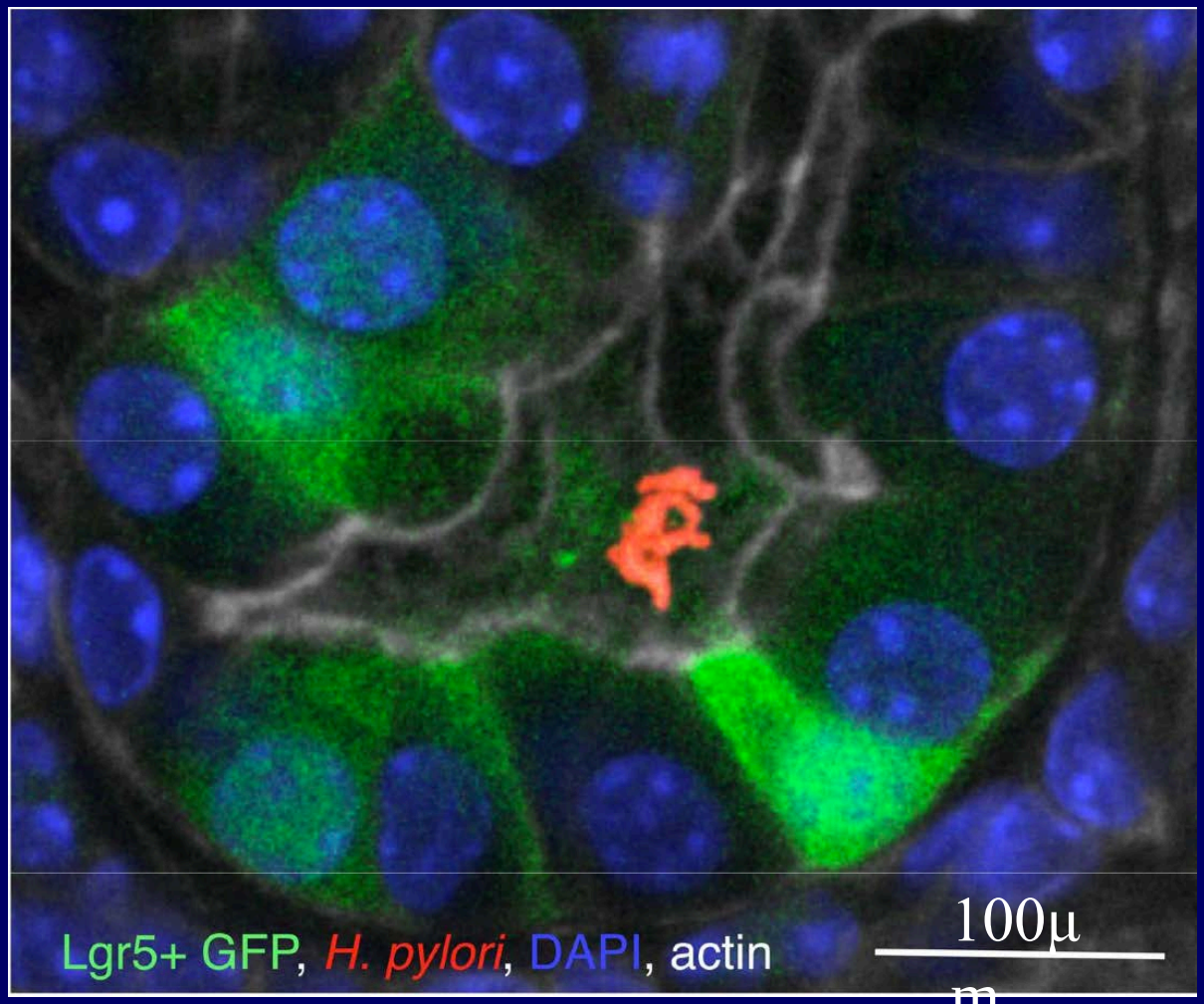
H. pylori colonize the progenitor cell compartments of murine gastric glands





Sigel et al., *Gastroenterology*

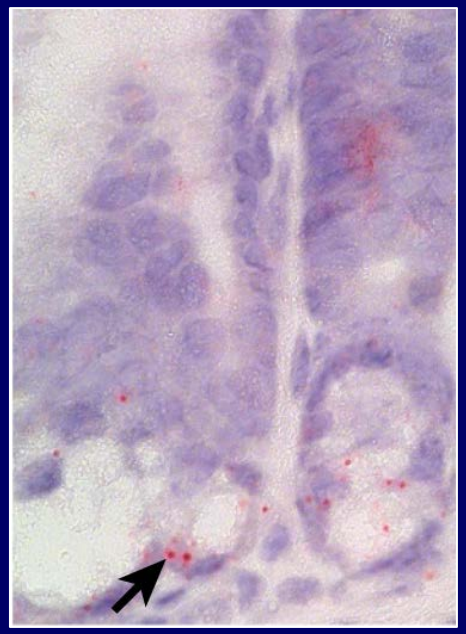
H. pylori colonizes the Lgr5 stem cell compartment in the antrum



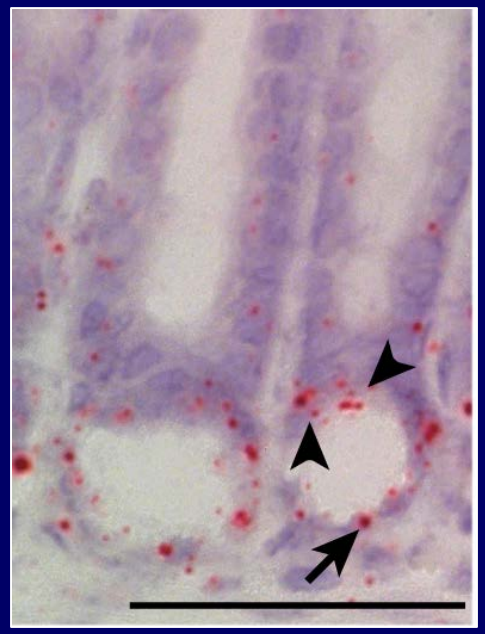
Sigal et al., *Gastroenterology*

Axin2 is expressed in $Lgr5^{+}$ cells and a distinct population of $Lgr5^{-}$ stem cells

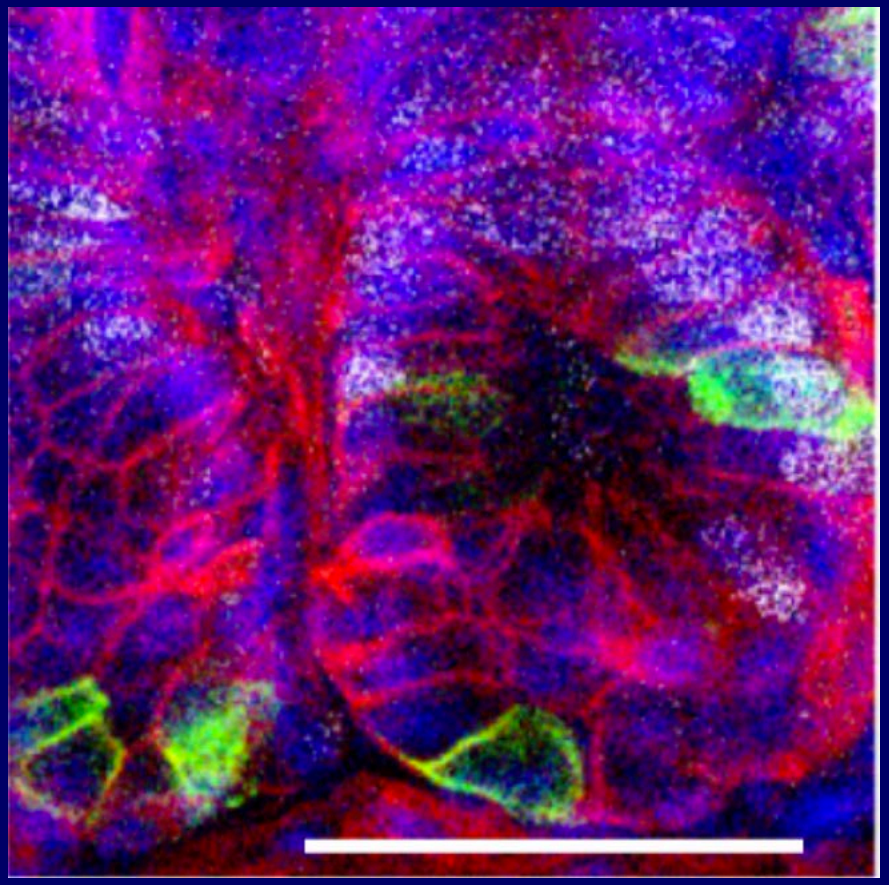
Lgr5



Axin2



ki67 **Axin2**



Sigal et al., *Nature*

Lrig1^{+} stem cells and *H. pylori*

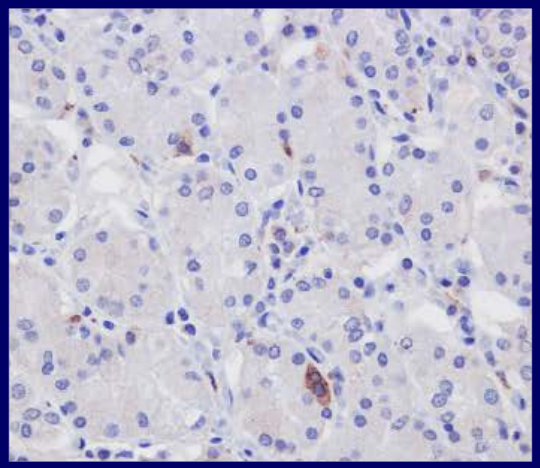
Leucine-rich repeats and immunoglobulin-like domains 1 (Lrig1) marks a population of quiescent stem cells.

Lrig1 functions as an inducible, negative feedback inhibitor of pan EGFR signaling.

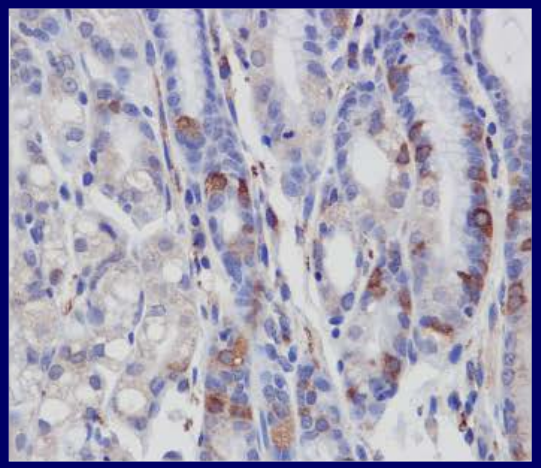
Present in both antral and corpus epithelium and expression is increased in infected mice (Noto et al.).

Loss of one *Apc* allele in Lrig1^{+} cells, thereby increasing β-catenin activation, uniformly leads to gastric hyperproliferation, hyperplasia, and dysplasia.

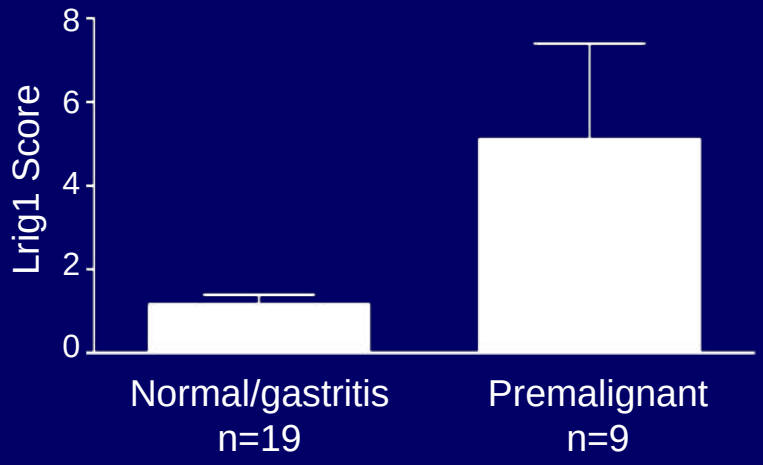
Lrig1 expression increases in gastric premalignant lesions in humans



Gastritis alone

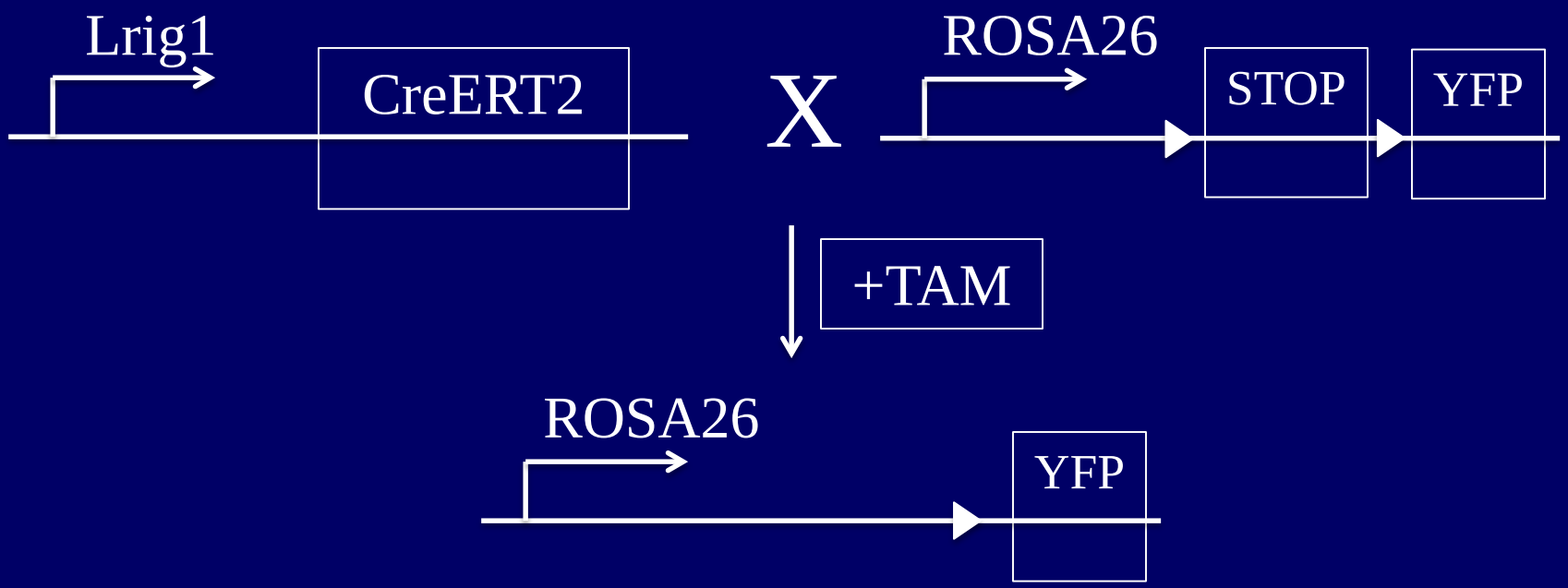


Atrophic gastritis



Wroblewski et al., *PNAS*

Lrig1 lineage tracing mouse model



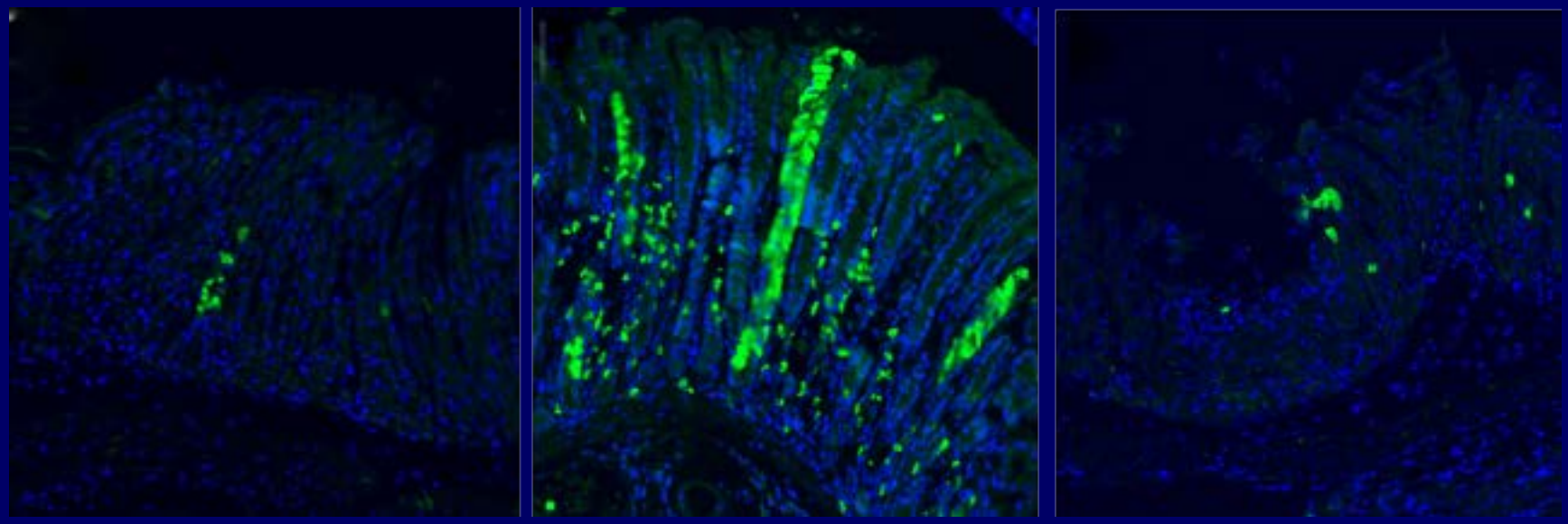
2mg tamoxifen i.p.

Infect with *H. pylori* wild-type *cag*$^{+}$ strain or

H. pylori isogenic *cag*$^{-}$ mutant

2 week and 8 week infection

H. pylori increases Lrig1 progenitor activity in a *cag*-dependent manner



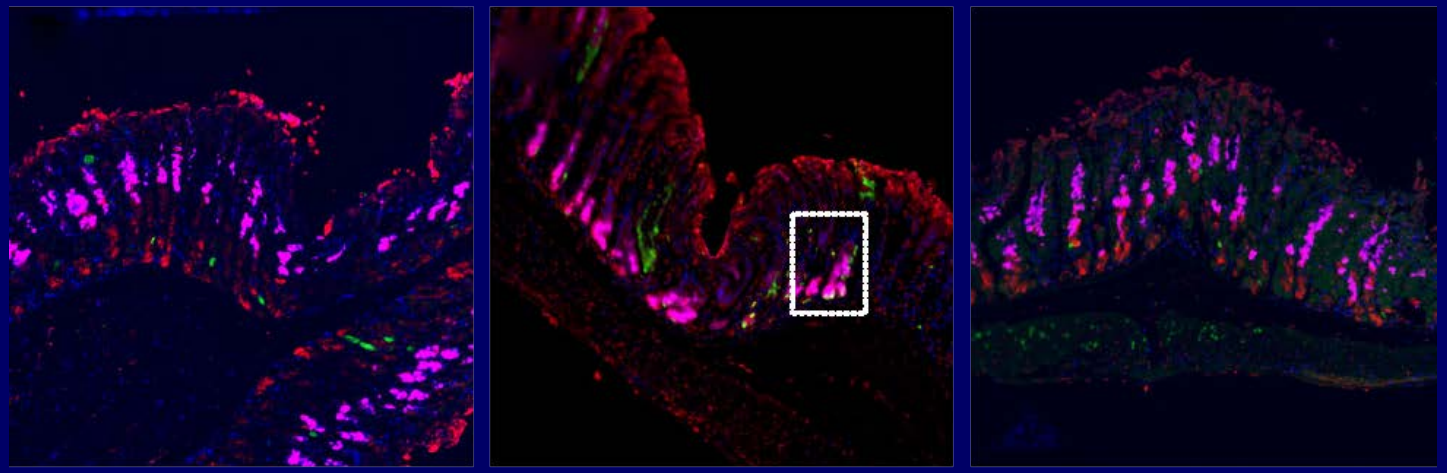
Uninfected | Wild type | *cag*$^{-}$

H. pylori

Wroblewski et al., *PNAS*

Lrig1 lineage traced cells co-localize with chief cells and SPEM cells in response to *cag*$^{+}$ *H. pylori*

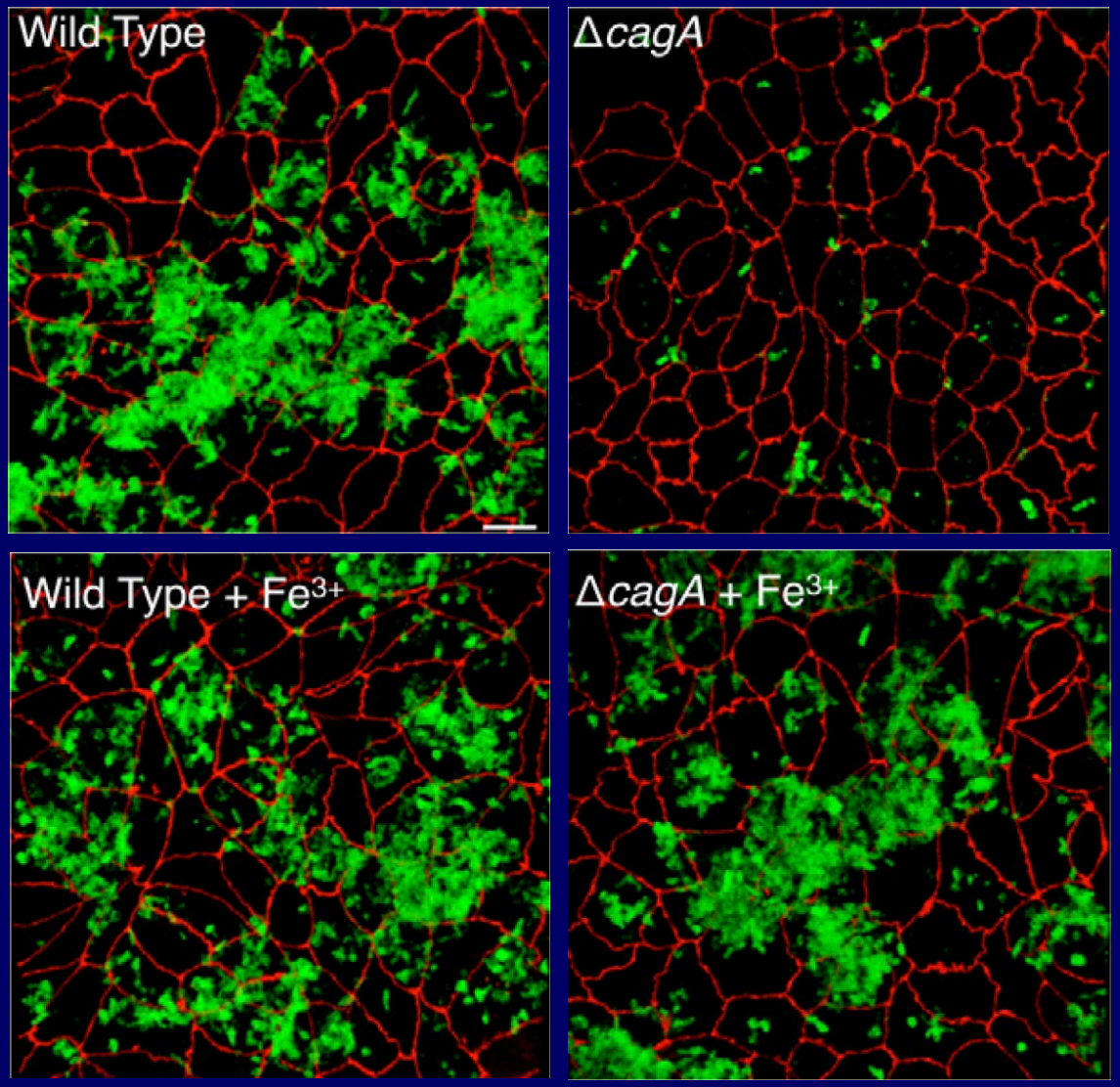
Uninfected | *H. pylori* WT | *H. pylori cag*$^{-}$



YFP (Lrig1)
GSII lectin (SPEM)
Intrinsic factor

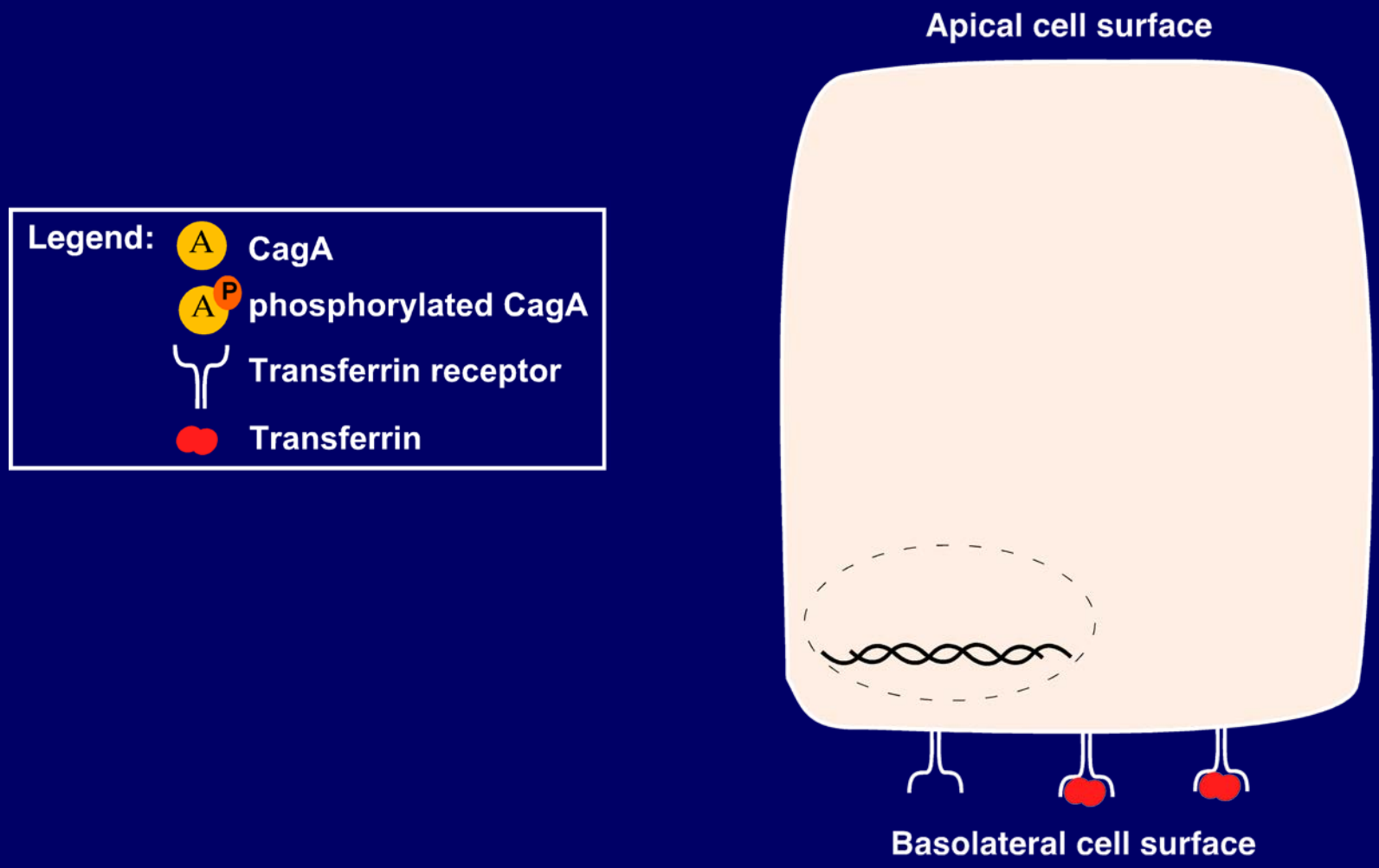
Wroblewski et al., *PNAS*

H. pylori colonization of polarized epithelium

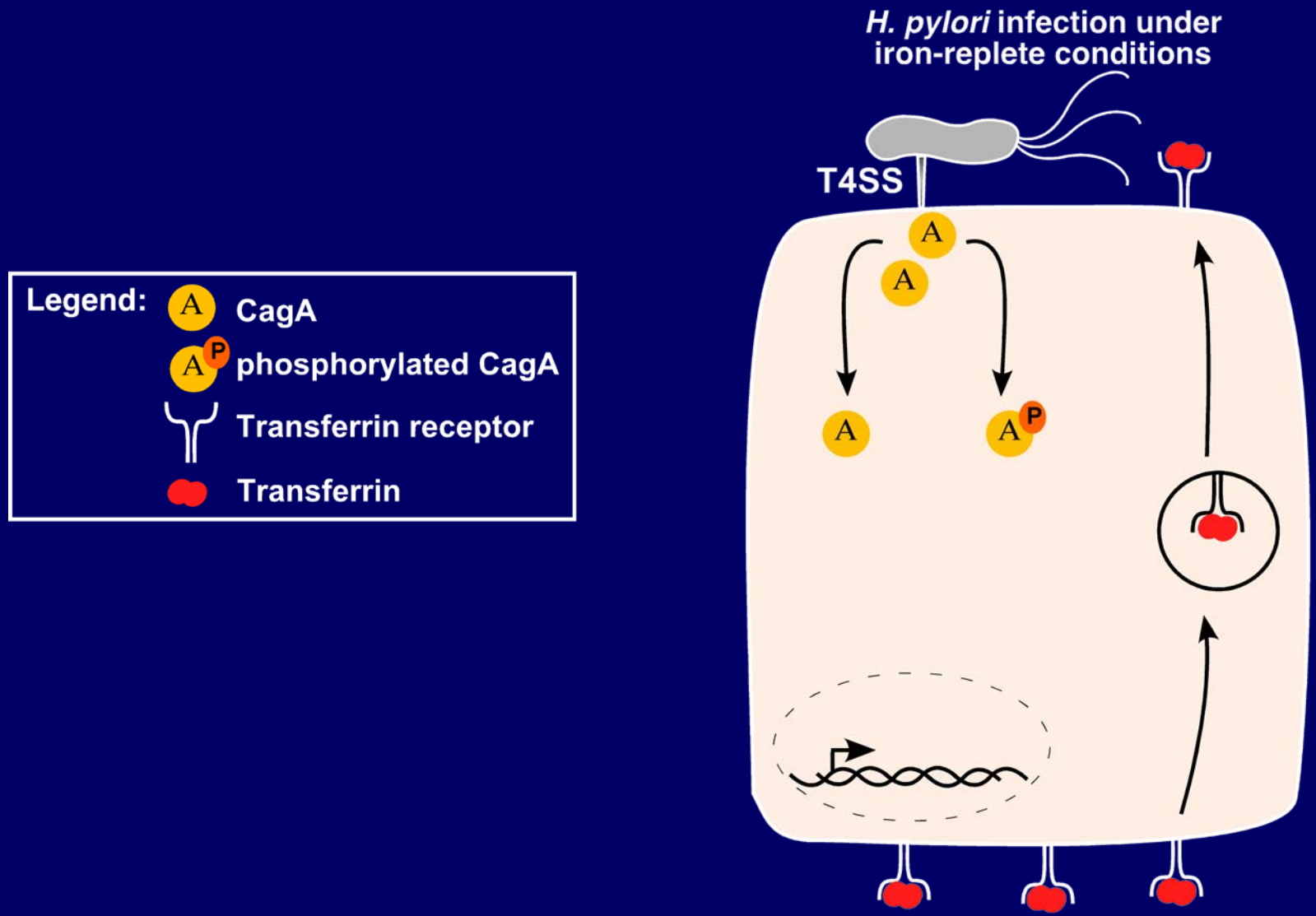


Tan et al.,
PLoS Pathogens

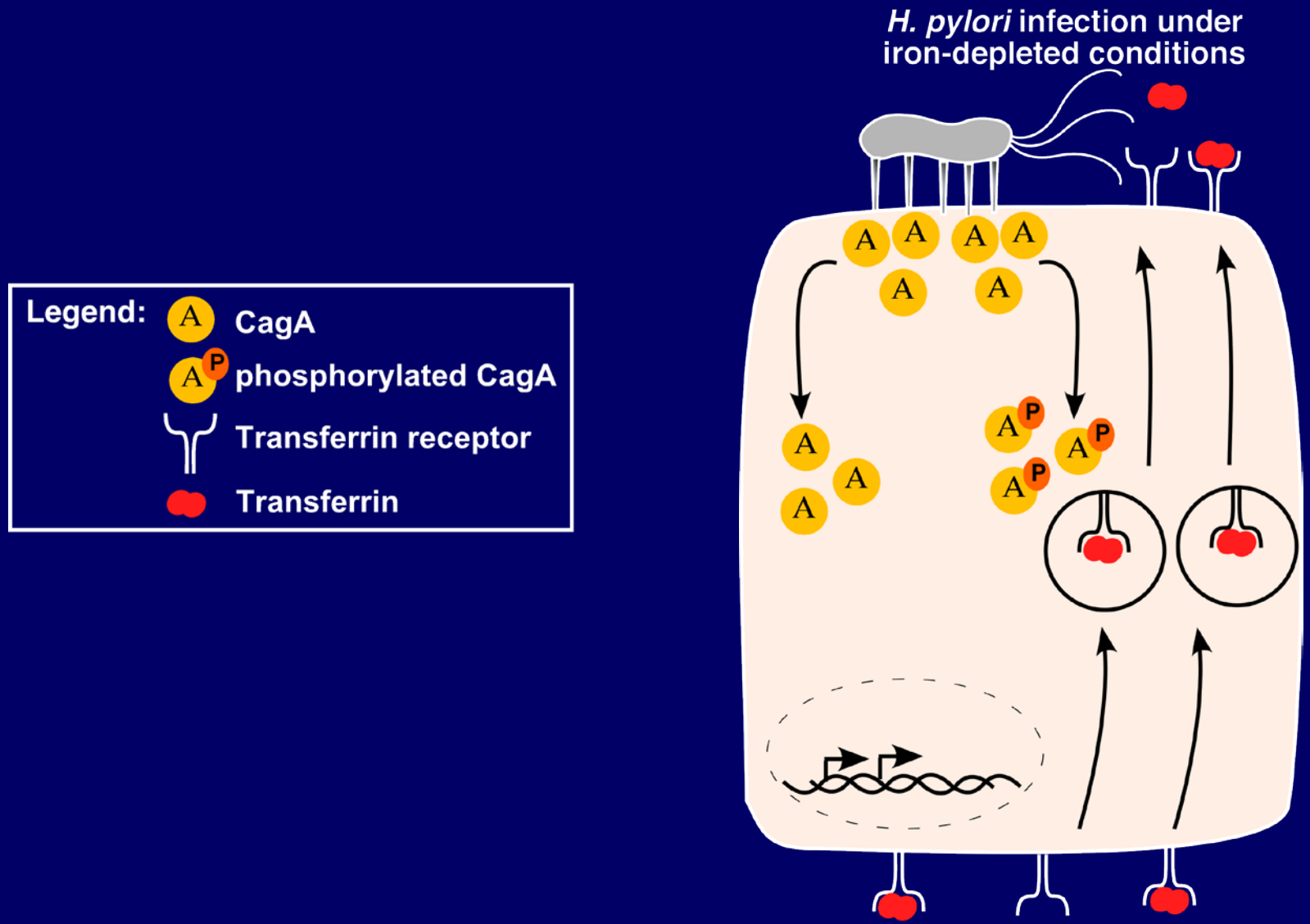
Model of *H. pylori*-induced transferrin recycling under conditions of iron depletion



Model of *H. pylori*-induced transferrin recycling under conditions of iron depletion



Model of *H. pylori*-induced transferrin recycling under conditions of iron depletion



Estimated odds ratios for gastric cancer incidence for *H. pylori*-, garlic-, or vitamin-treatment

	Fully adjusted	
Treatment	OR (95% CI)	*P*
H. pylori	0.61 (0.38-0.96)	0.032
Garlic	0.80 (0.53-1.20)	0.28
Vitamin	0.81 (0.54-1.22)	0.32

Ma et al., *JNCI*

Estimated odds ratios for gastric cancer incidence for *H. pylori*-, garlic-, or vitamin-treatment

	Fully adjusted	
Treatment	OR (95% CI)	*P*
H. pylori	0.61 (0.38-0.96)	0.032
Garlic	0.80 (0.53-1.20)	0.28
Vitamin	0.81 (0.54-1.22)	0.32

Ma et al., *JNCI*

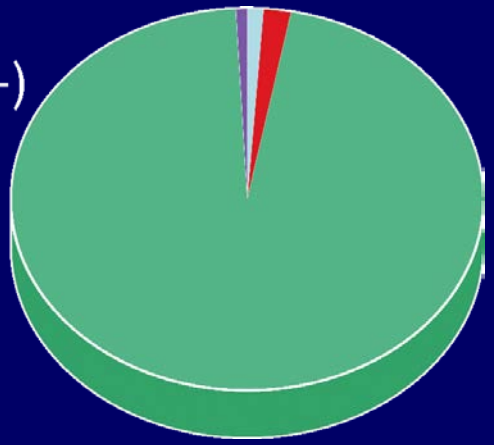
Shandong Intervention Trial

Efficacy of *H. pylori* eradication therapy at 15 years of follow-up: 47%

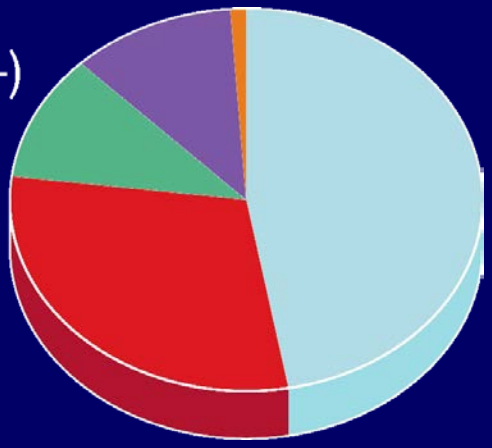
What are the roles of non-*H. pylori* constituents of the gastric microbiota in disease outcomes enhanced by iron deficiency?

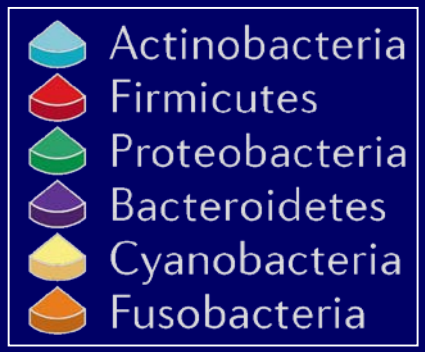
Compositional differences in the gastric microbiome with and without *H. pylori*

H. pylori (+)
stomach



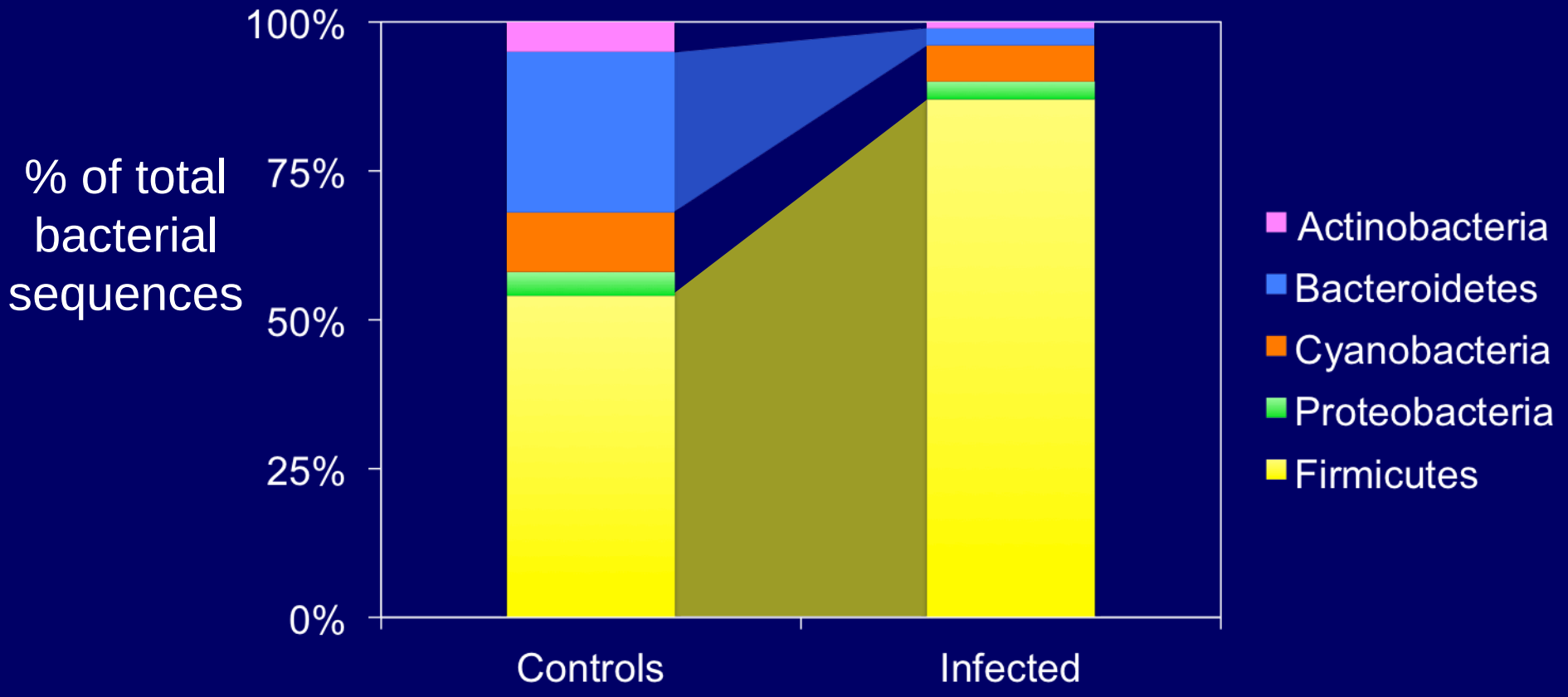
H. pylori (–)
stomach





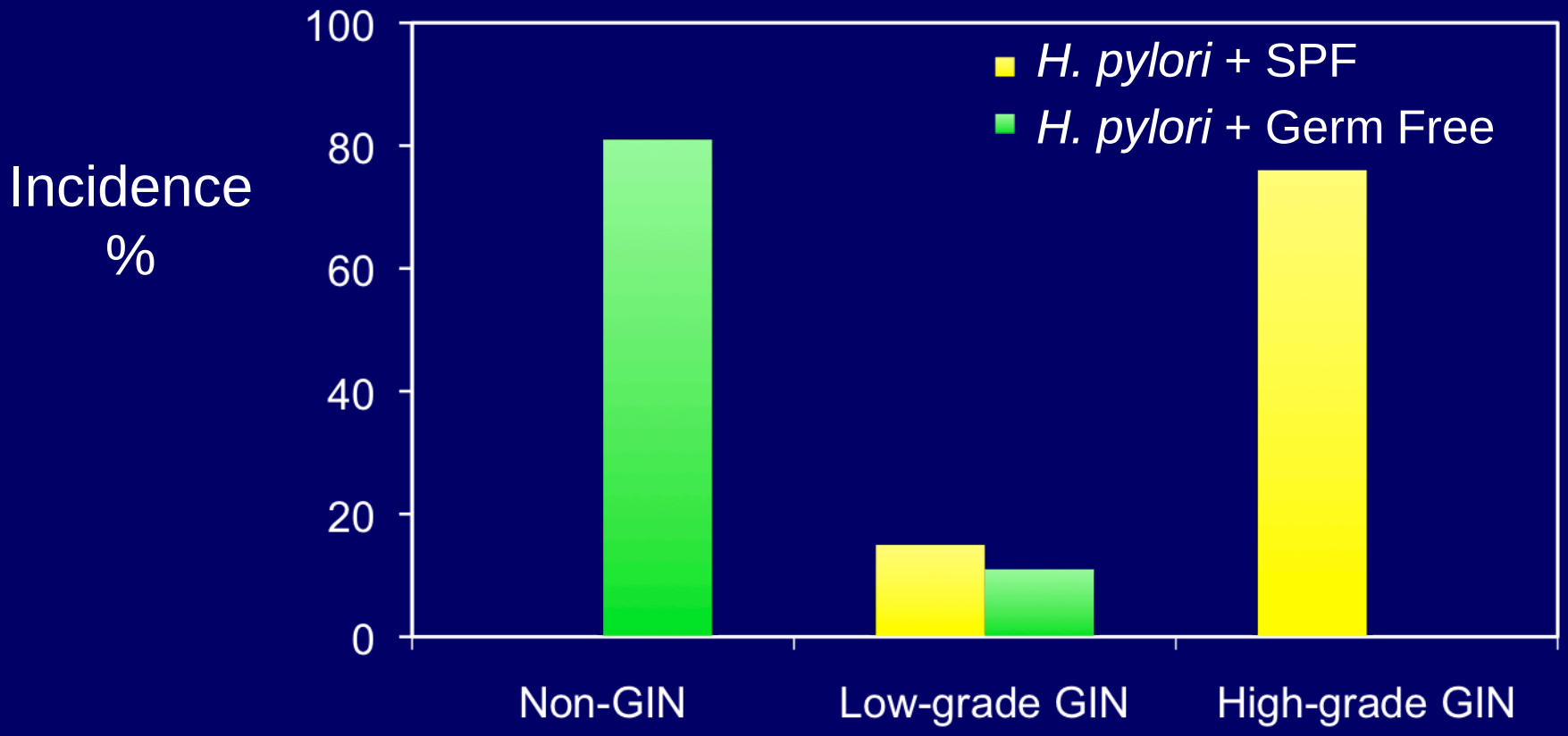
Cho and Blaser, *Nat Rev Gen*, 2012
Abreu and Peek, *Gastroenterology*, 2014

Gastric microbiota in *H. pylori*-infected and uninfected mice



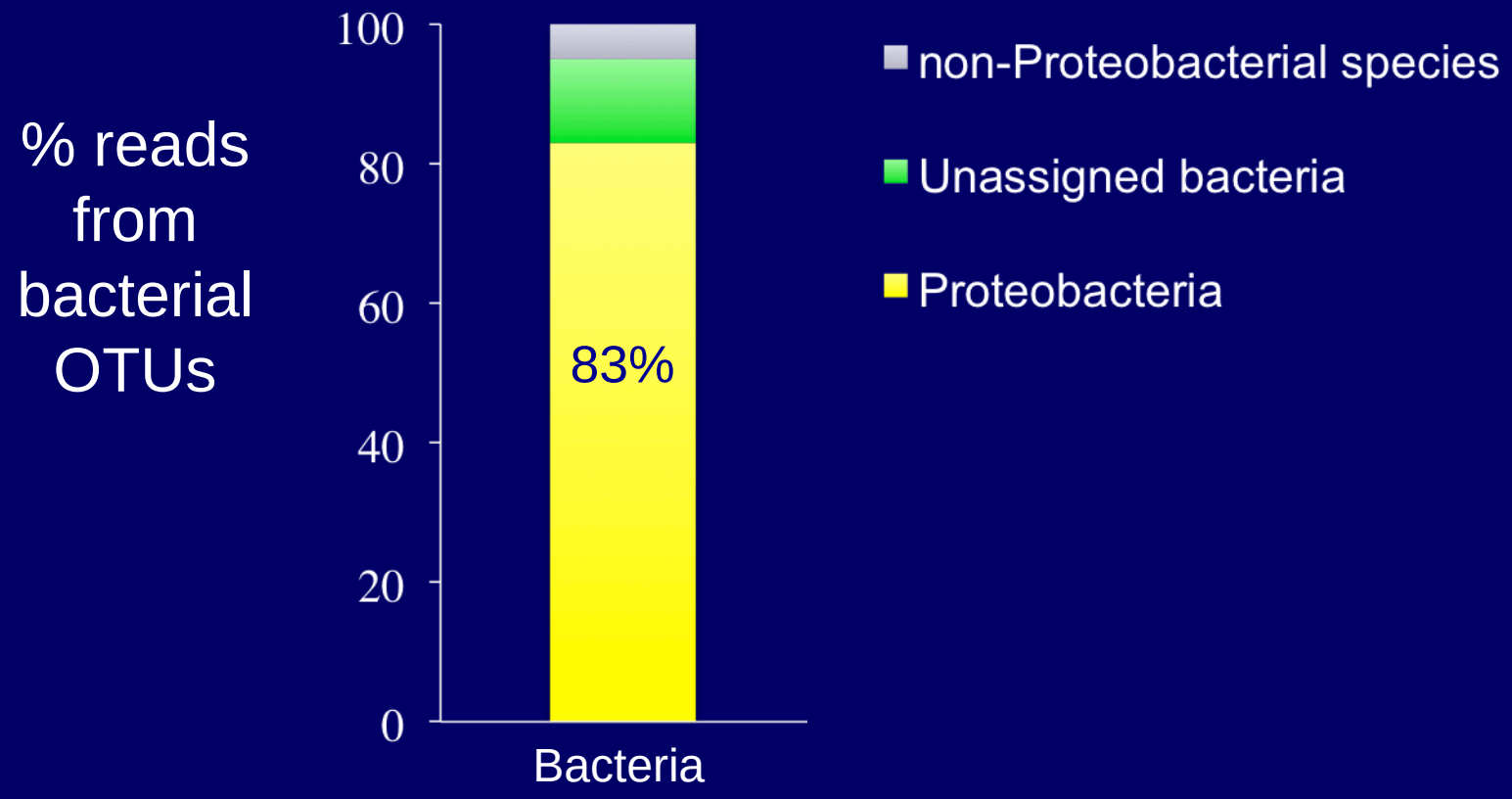
Lofgren et al., *Gastroenterology*

Gastric intraepithelial neoplasia (GIN) in conventionally housed (SPF) versus germ-free mice



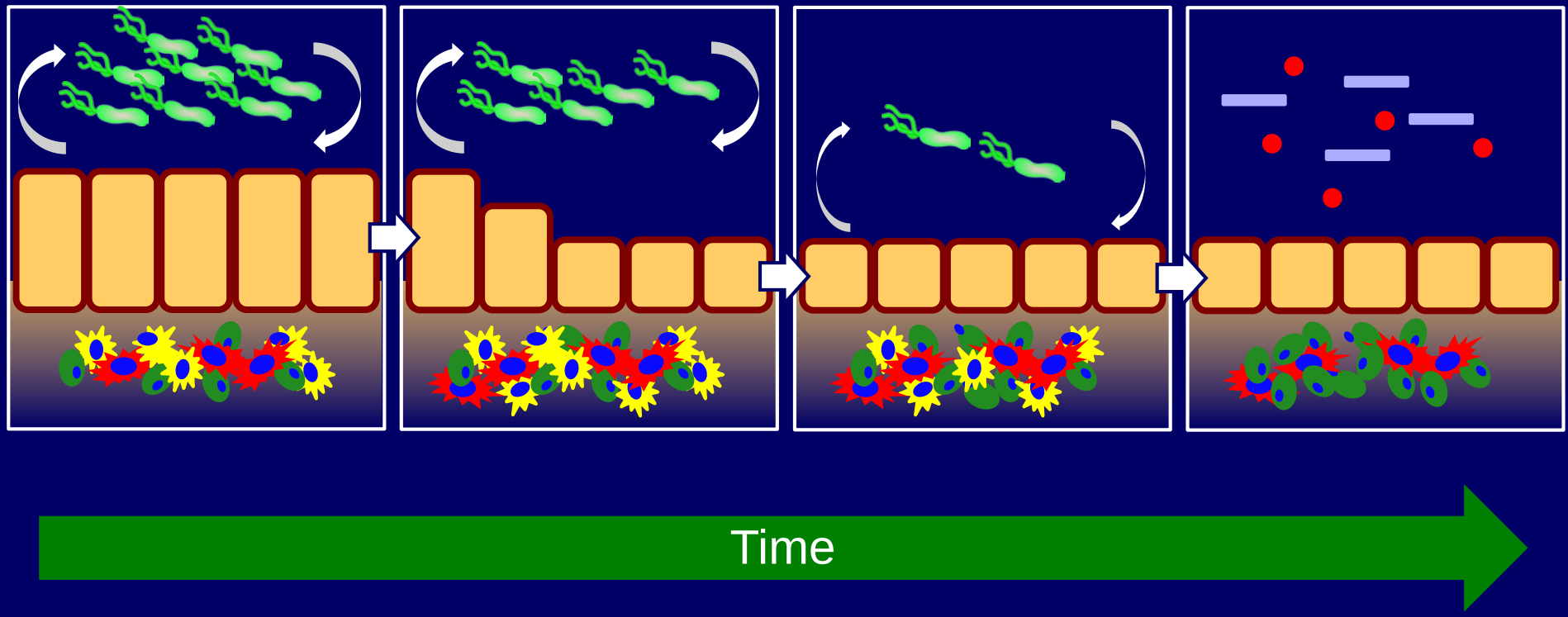
Lofgren et al., *Gastroenterology*

Distribution of operational taxonomic units (OTUs) from bacterial DNA integrations in gastric adenocarcinoma



Riley *et al*., *PLoS Comp Bio*, 2013

Multi-decade development of gastric adenocarcinoma initiated by *H. pylori*

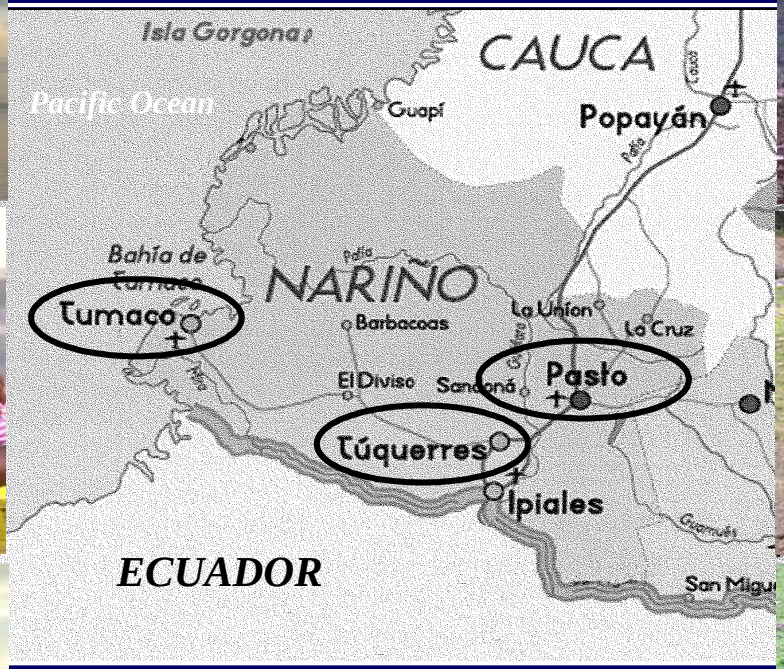


Plottel and Blaser, *Cell Host and Microbe*, 2013

Nariño, Colombia

Low-risk area

(6/100,000)



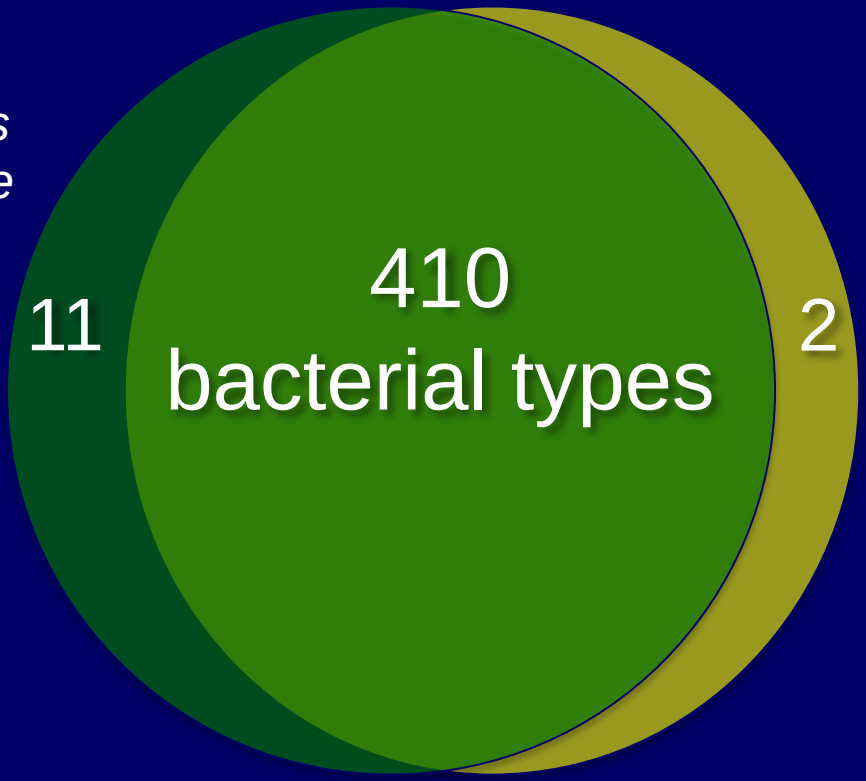
High-risk area

(150/100,000)

Cancer risk region-specific known bacteria

Low Risk

Staphylococcus sp.
Streptococcus oralis
Neisseria flavenscens
Porphyromonadaceae
Flavobacterium sp.
TM7
Rothia sp.
Prevotella oris
Capnocytophaga sp.
Actinomhyces sp
Haematobacter sp



High Risk

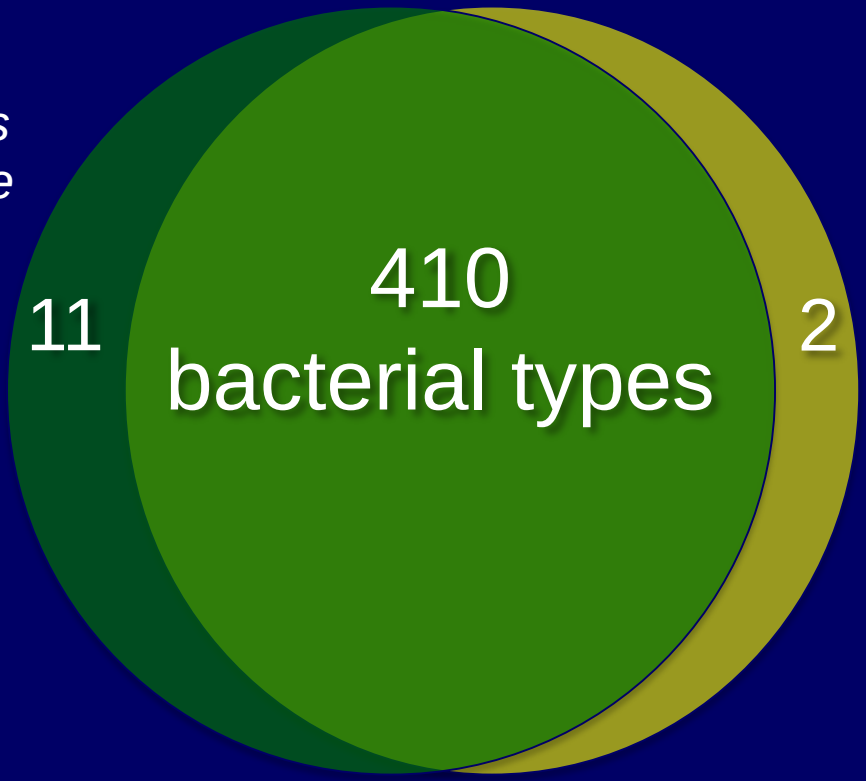
Leptotrichia wadei
Veillonella sp

Yang et al., *Science Reports*

Cancer risk region-specific known bacteria

Low Risk

Staphylococcus sp.
Streptococcus oralis
Neisseria flavenscens
Porphyromonadaceae
Flavobacterium sp.
TM7
Rothia sp.
Prevotella oris
Capnocytophaga sp.
Actinomhyces sp
Haematobacter sp

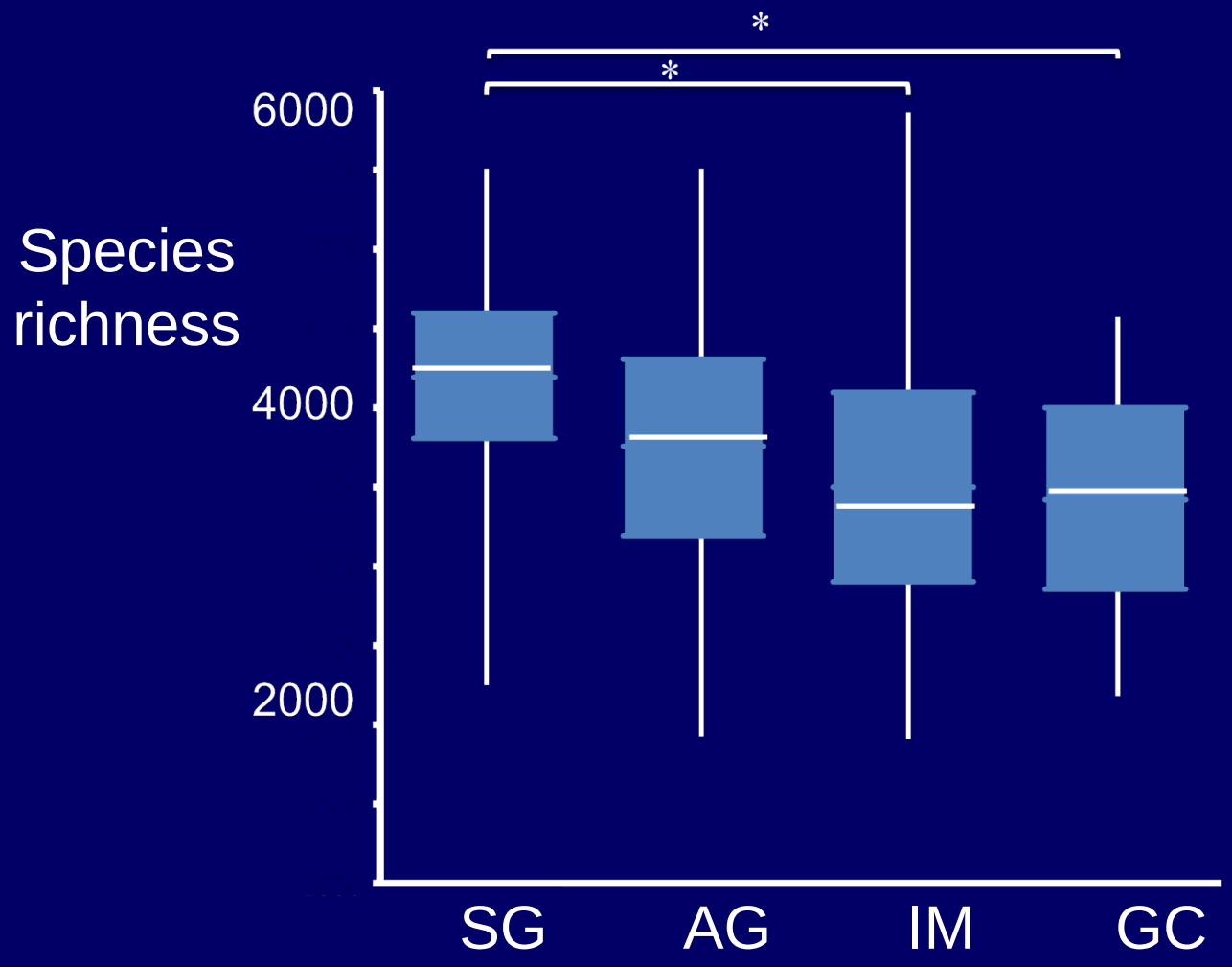


High Risk

Leptotrichia wadei
Veillonella sp

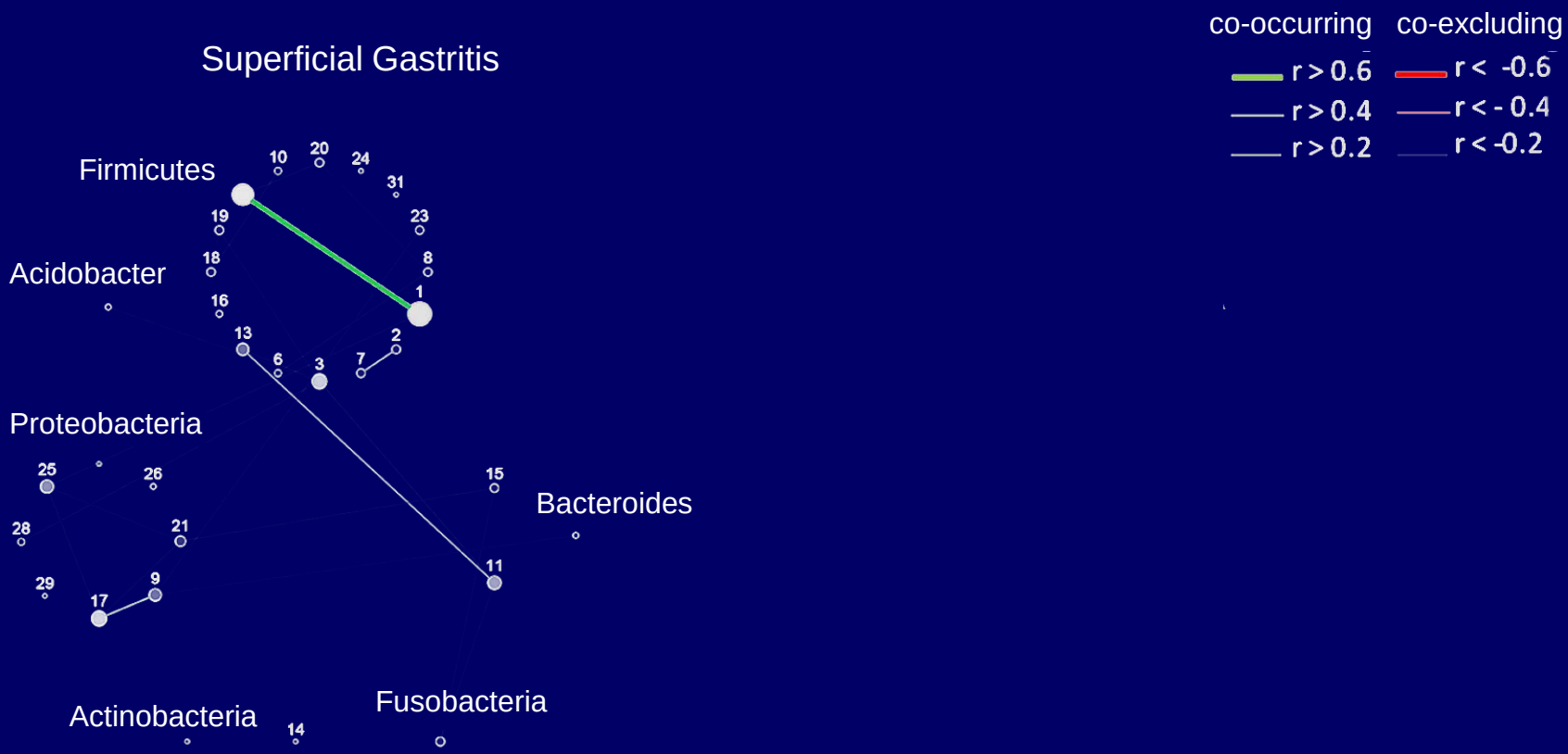
Yang et al., *Science Reports*

Gastric microbiome dysbiosis across stages of gastric carcinogenesis



Coker et al., *Gut*, 2018

Correlation strengths of gastric cancer enriched and depleted bacteria with disease progression



Coker et al., *Gut*, 2018

Gastric cancer-enriched oral bacterial taxa with significant interactions among ecological networks

Peptostreptococcus

Streptococcus

Parvimonas

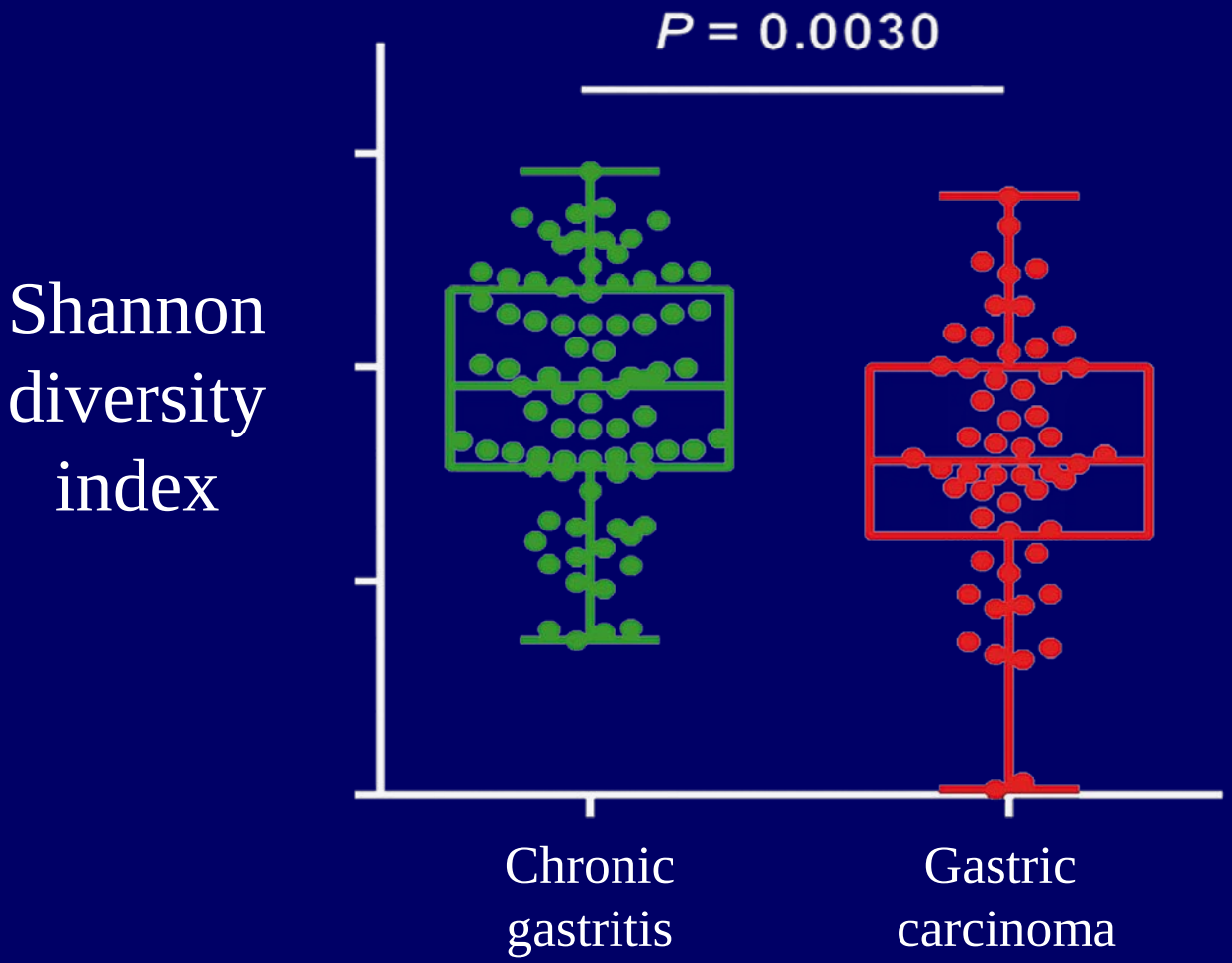
Slackia

Dialister

Coker et al., *Gut*, 2018

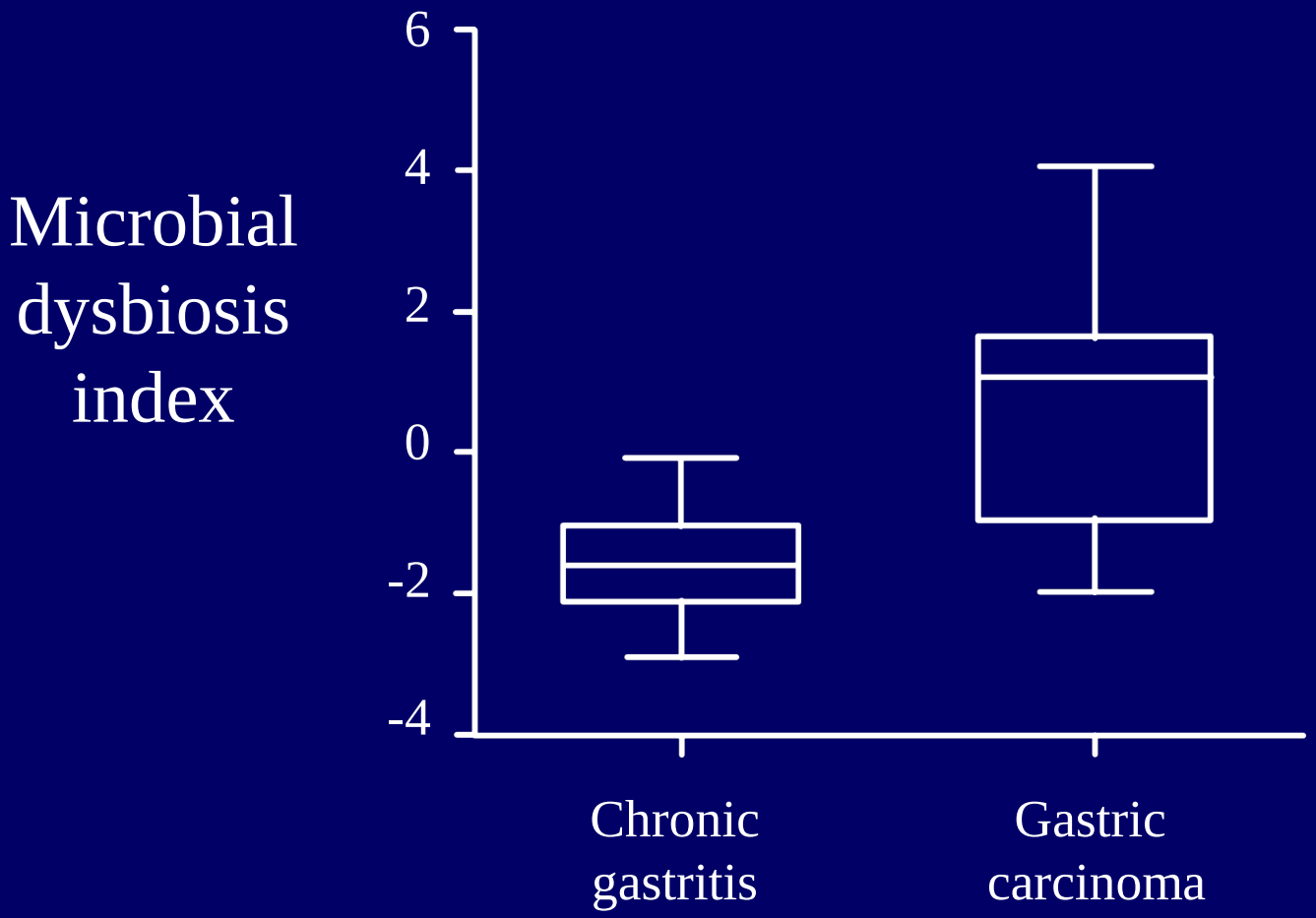
Can results in China be extrapolated to other populations?

Gastric microbiota profiles differ in chronic gastritis and gastric carcinoma



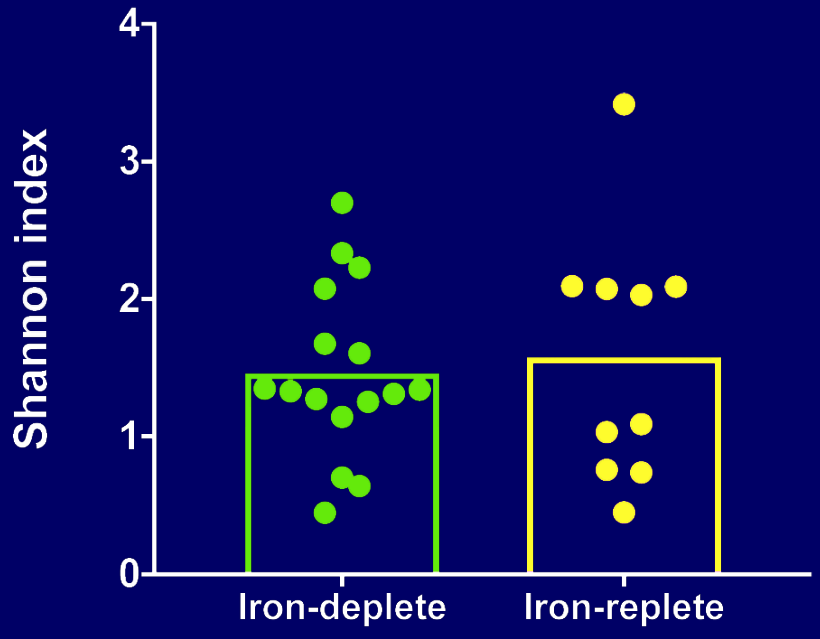
Ferreira et al., *Gut*, 2017

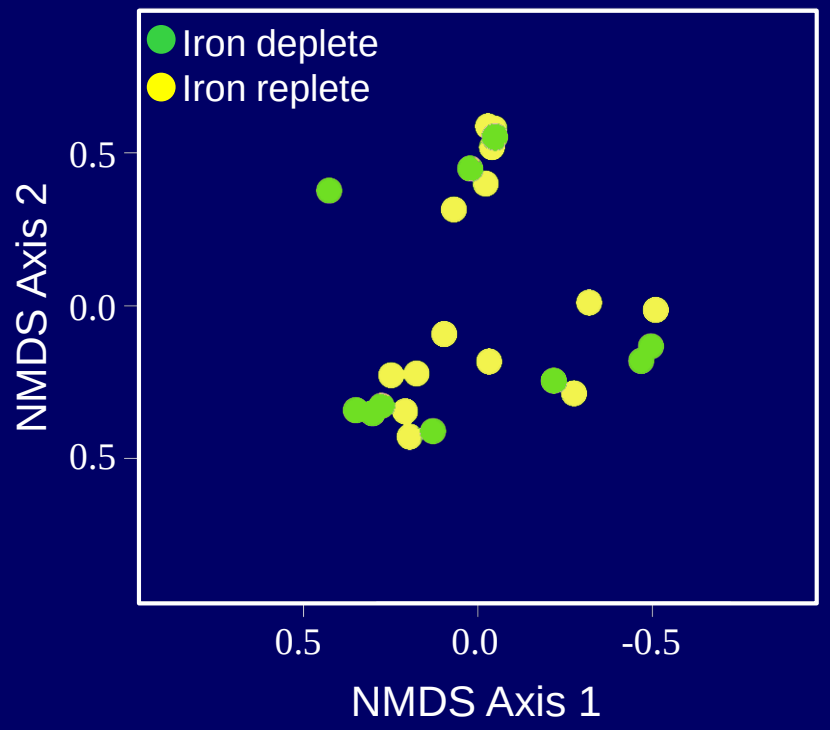
Microbial dysbiosis is associated with gastric carcinoma



Ferreira et al., *Gut*, 2017

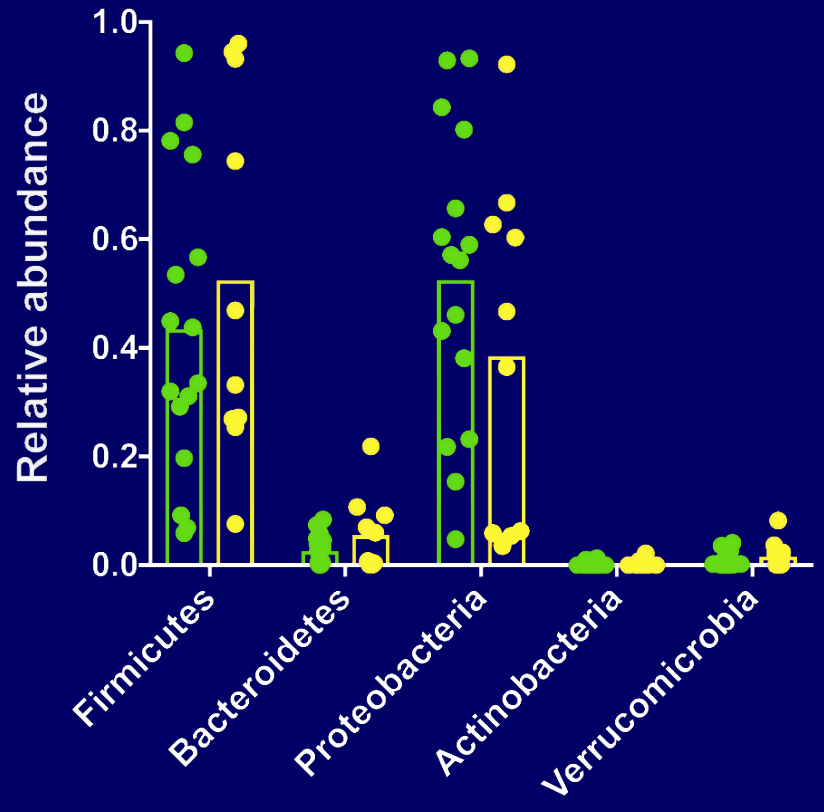
Dietary iron levels do not alter gastric microbial communities in *H. pylori*-infected gerbils





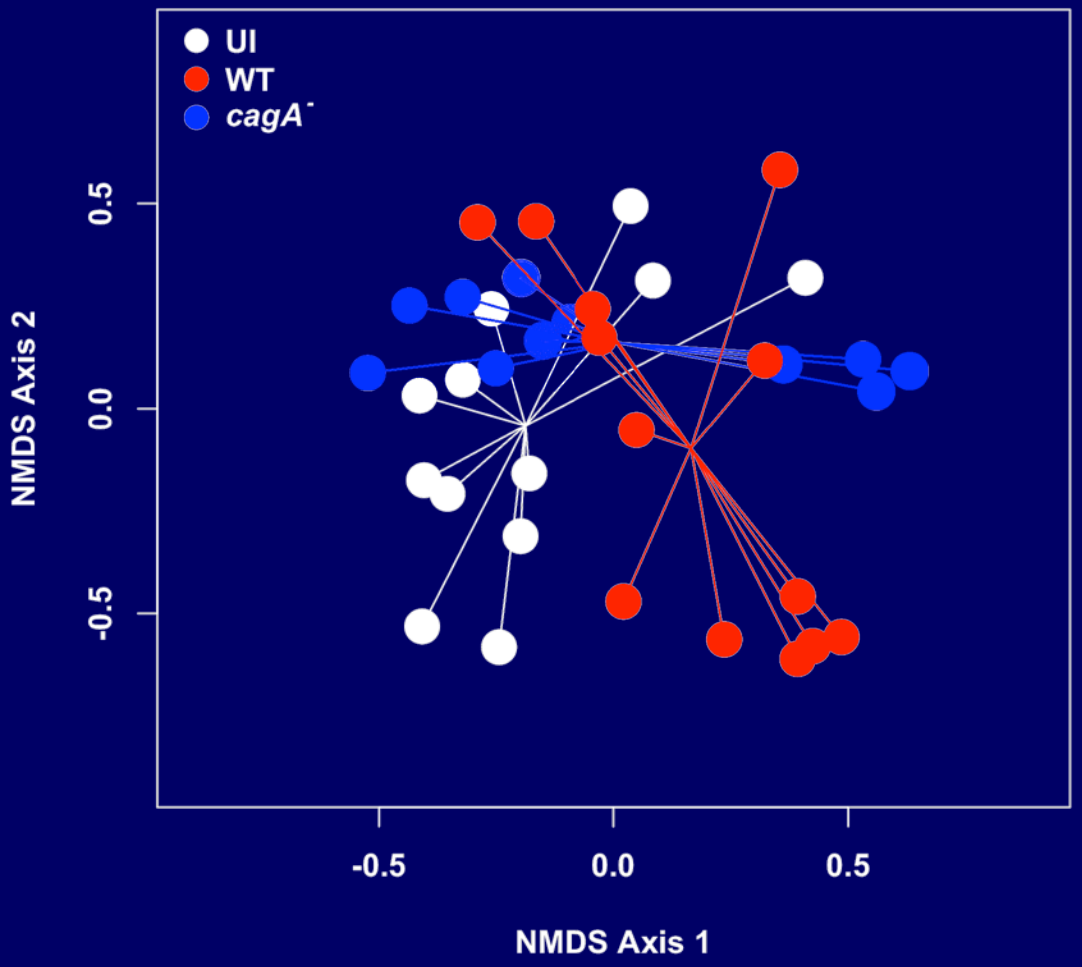
Noto et al., *mBio*

Iron levels in the diet do not alter composition of the gastric microbiota, when stratified by phyla levels, in *H. pylori*-infected gerbils



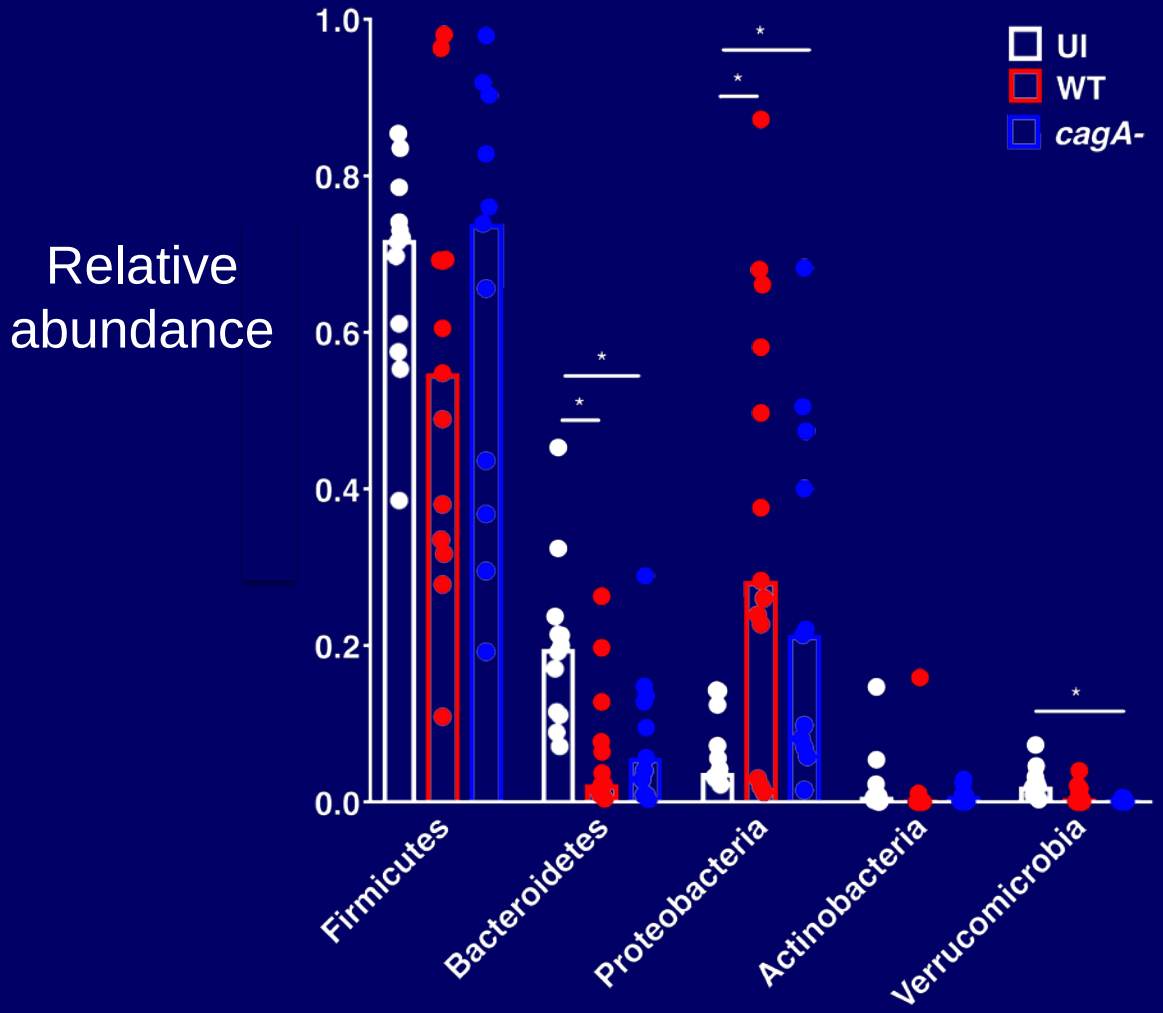
Noto et al., *mBio*

H. pylori infection alters the gastric mucosal microbiota in a *cagA*-dependent manner

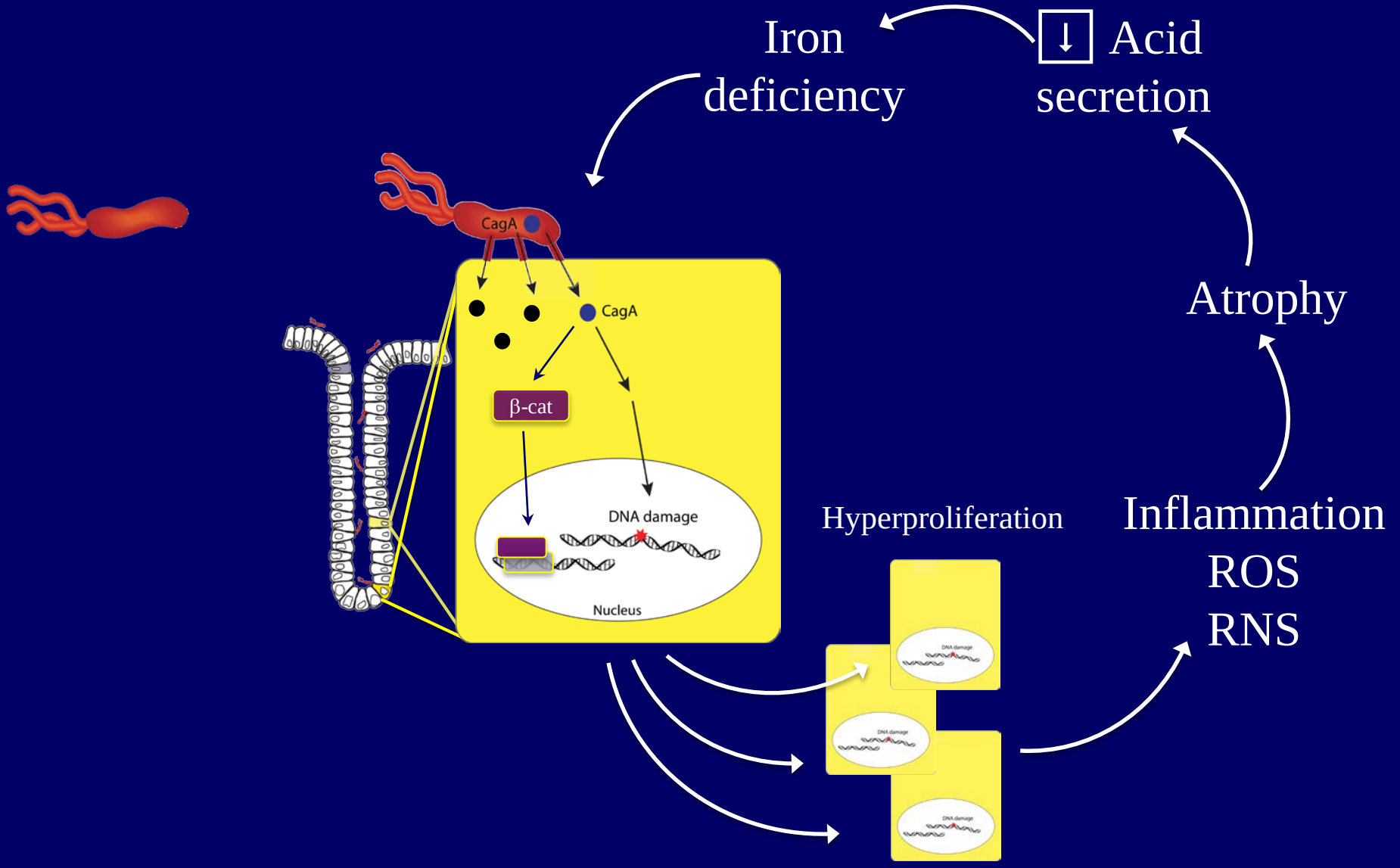


Noto et al., *mBio*

Presence of CagA alters composition of the gastric microbiota, when stratified by phyla levels, in *H. pylori*-infected gerbils

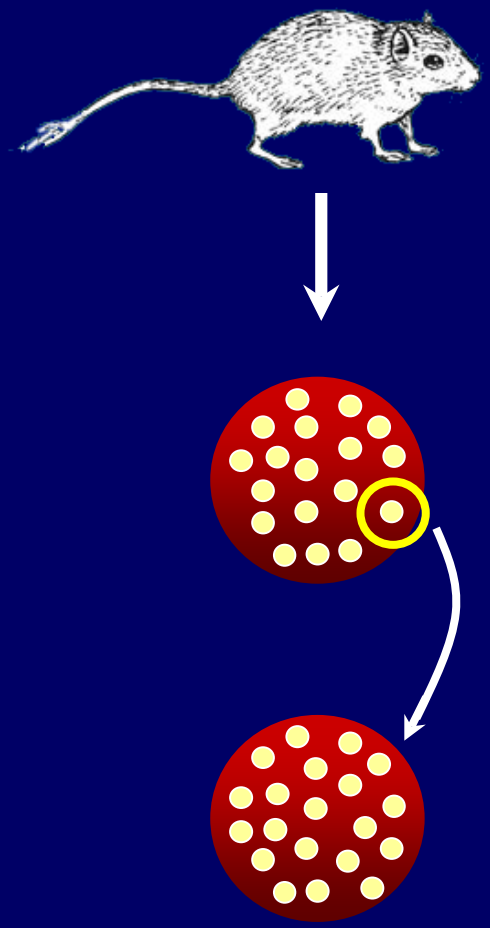


Noto et al., *mBio*

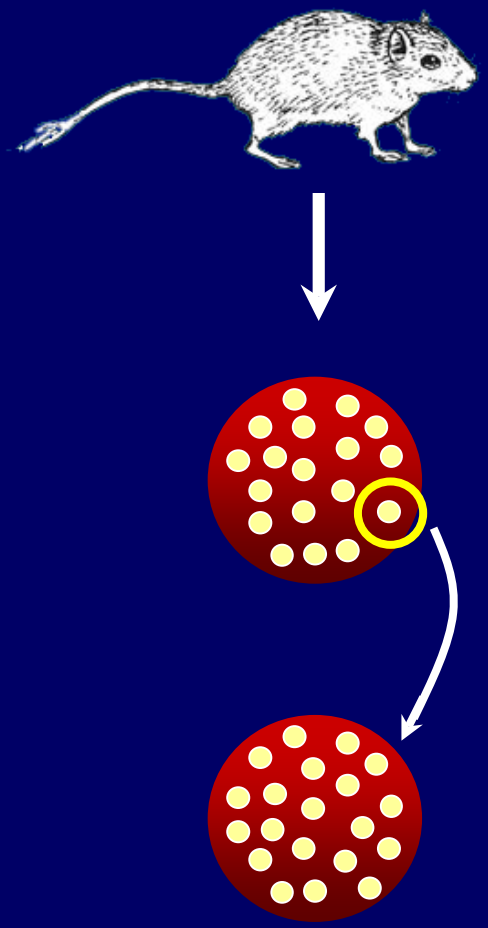

Iron deficiency
↓ Acid secretion
Atrophy
Inflammation
ROS
RNS
Hyperproliferation
CagA
CagA
β-cat
DNA damage
Nucleus

Can we use novel technologies to identify new virulence factors and comprehensively establish their role in our chronic infection models of iron deficiency?

Isolation of *in vivo*-adapted *H. pylori* strains



In vivo-adapted strains
Iron-replete gerbils



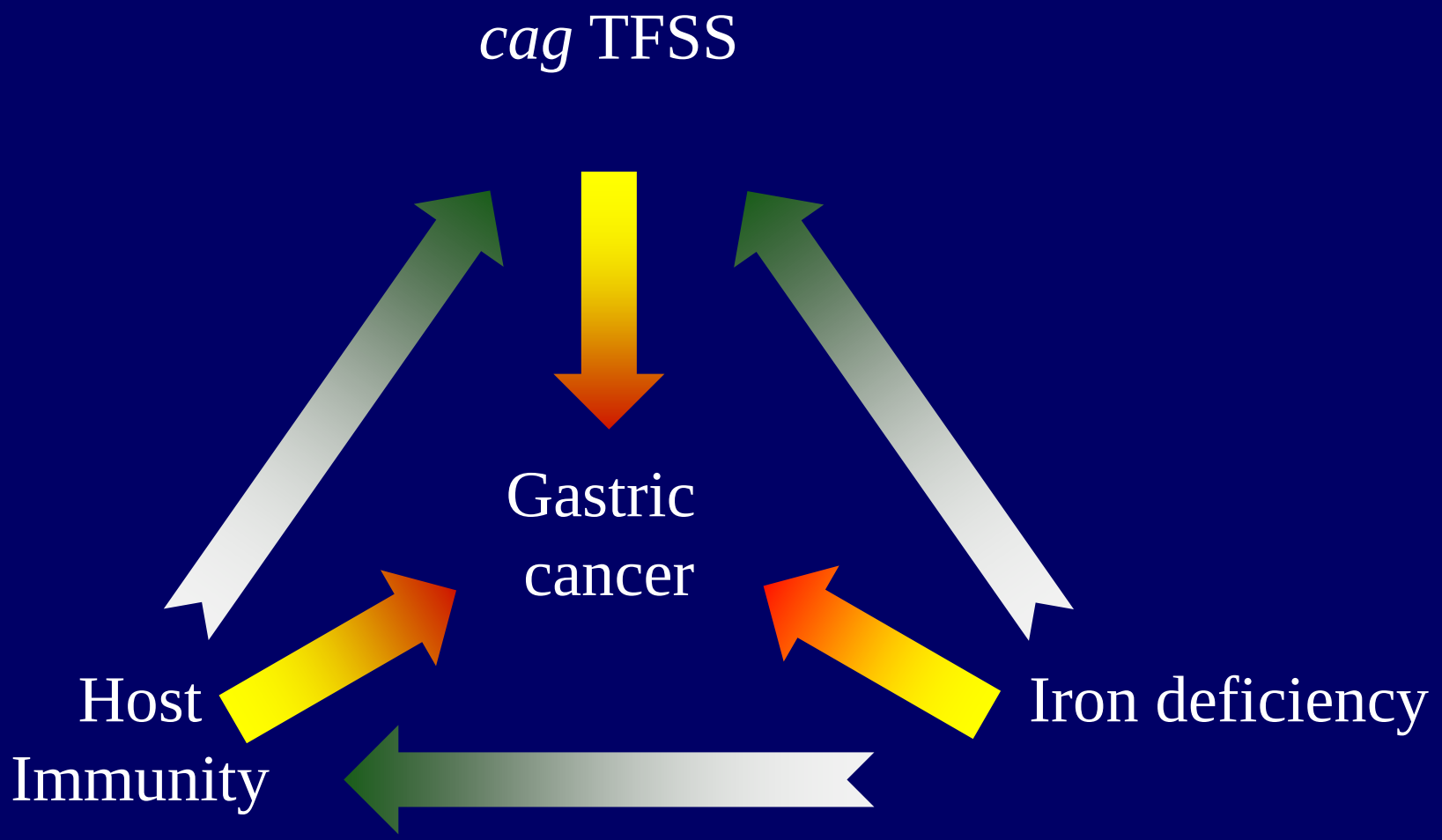
In vivo-adapted strains
Iron-deplete gerbils

Metabolomics pathways altered in *H. pylori* strains isolated from iron-depleted gerbils

Metabolic Pathways
Alanine and aspartate metabolism
Biopterin metabolism
Butanoate metabolism
Cysteine and methionine metabolism
Drug metabolism
Glycerophospholipid metabolism
Glycine and serine metabolism
Histidine metabolism
Linoleate metabolism
Pyrimidine metabolism
Taurine and hypotaurine metabolism
Tryptophan metabolism
Tyrosine Metabolism

Upper endoscopy in gerbils

Helicobacter pylori-induced gastric cancer: an axis of evil



Acknowledgements

Peek Lab
Seth Ogden
Daniel O'Brien
Aime Franco
Lydia Wroblewski
Shannon Allen
Uma Krishna
Judith Romero
Toni Nagy
Dawn Israel
Jennifer Noto
Giovanni Suarez
Matt Varga
Sam Dooyema
Shailja Shah

Jay Solnick
Steffen Backert
Barbara Schneider
Keith Wilson
Doug Morgan
Manuel Amieva
Carolina Sierra

Kay Washington
Blanca Piazuelo
Tim Cover
Jen Gaddy
Jim Fox
Constanza Camargo
Holly Algood