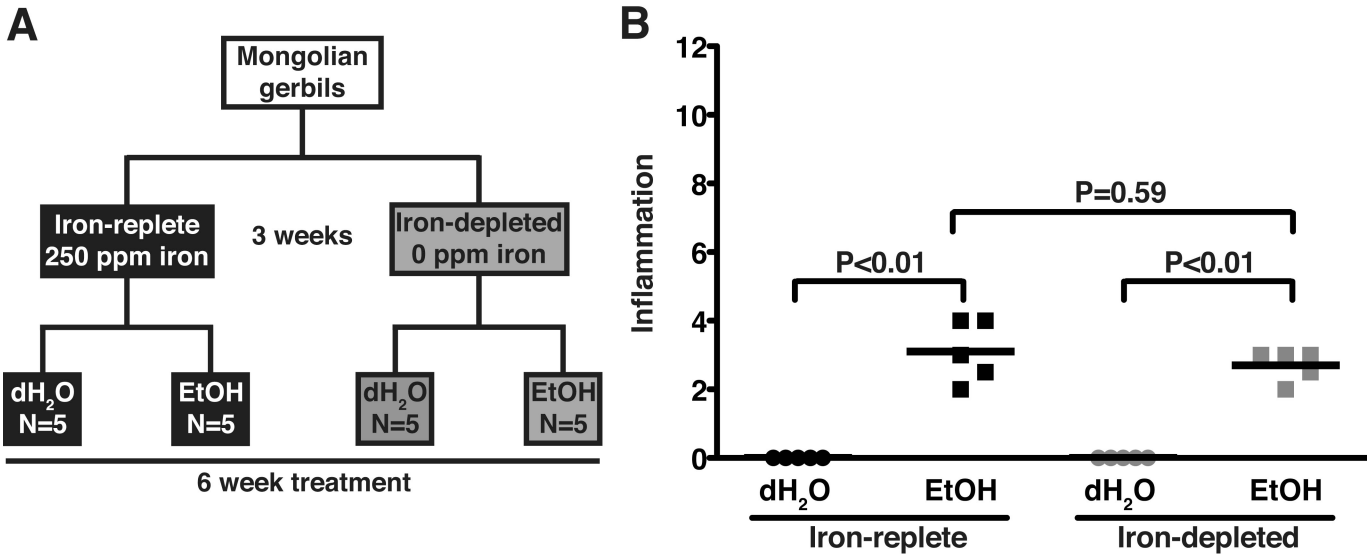
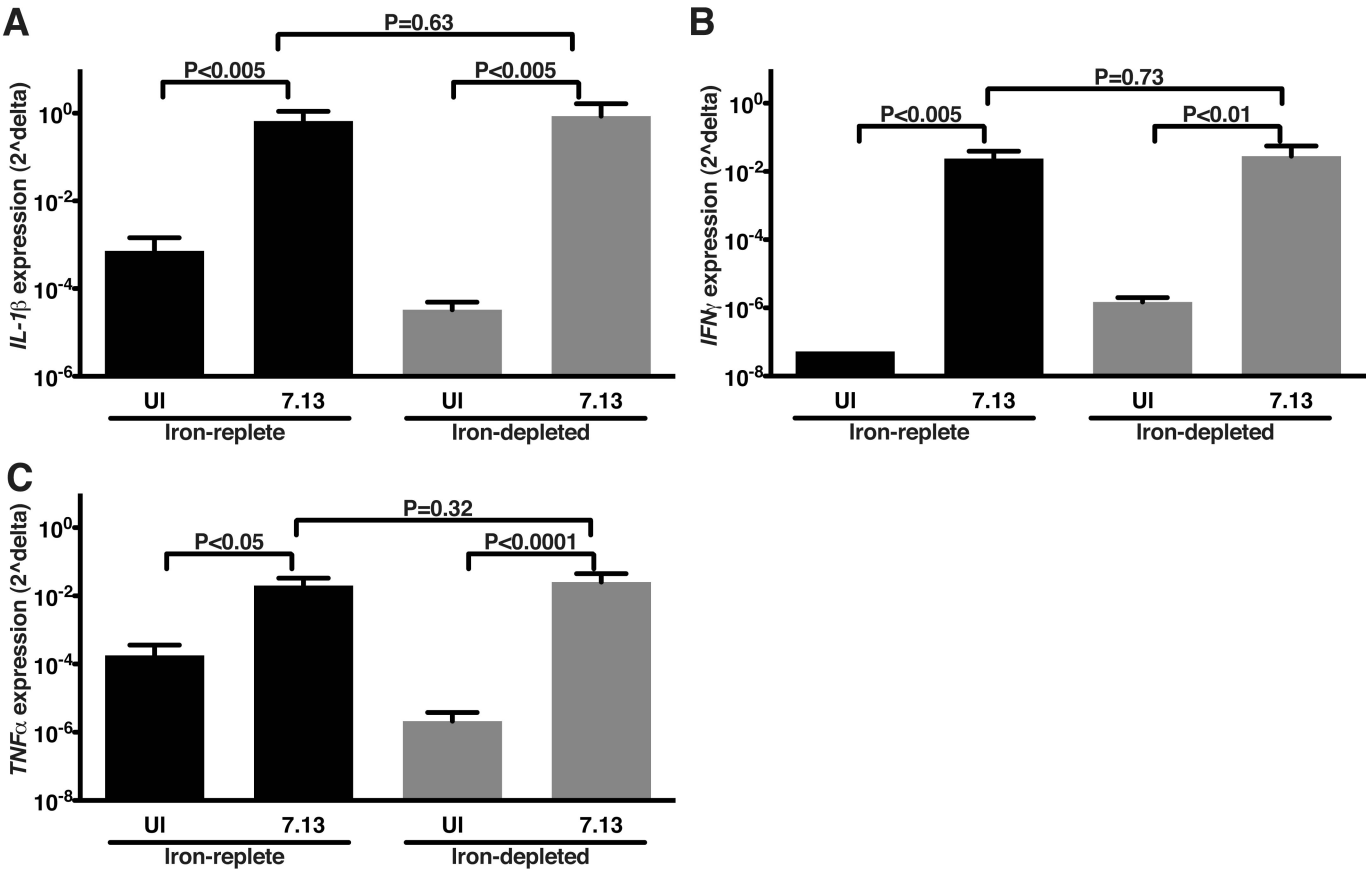


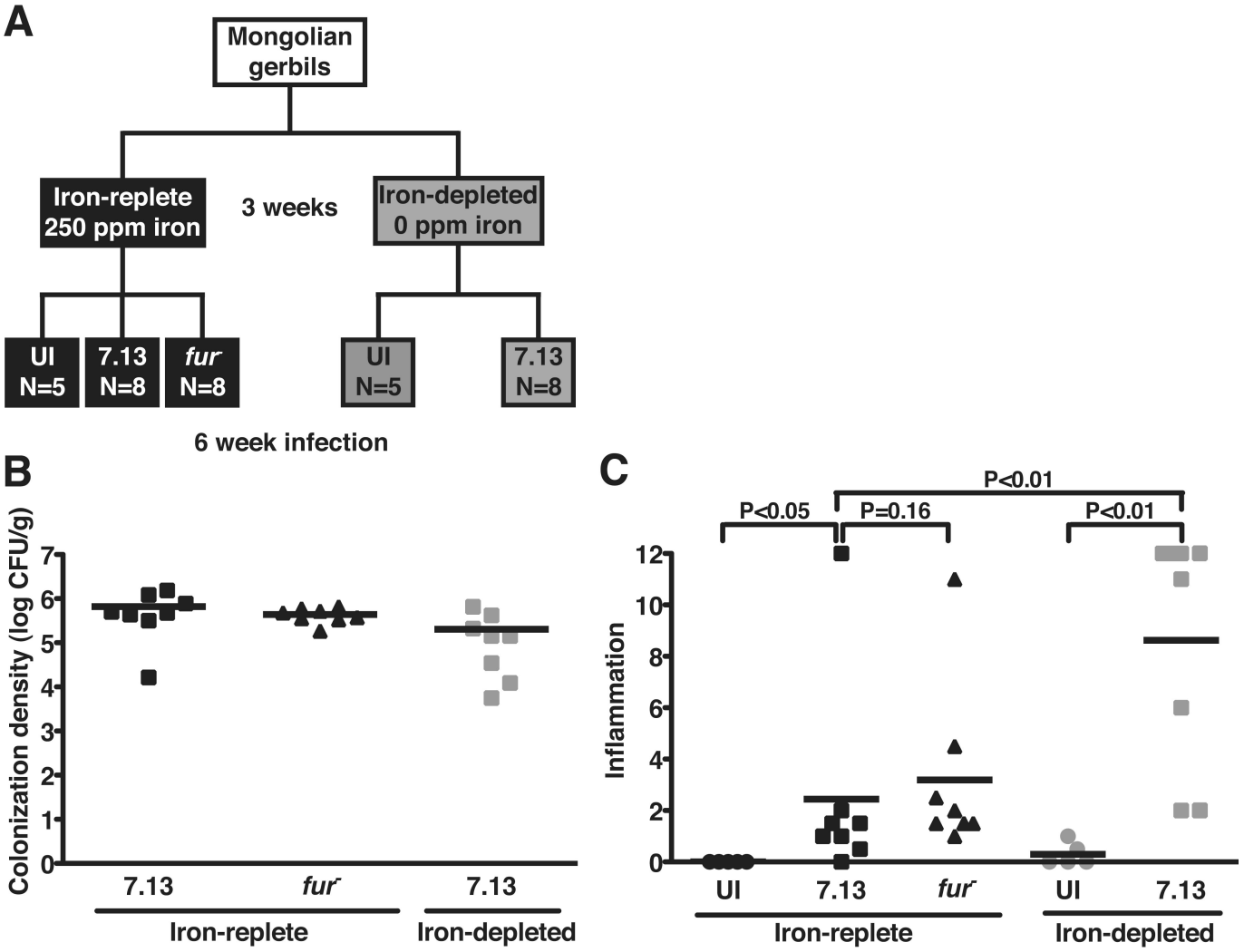
Supplemental Figure 1

Increased severity of *H. pylori*-induced gastric inflammation under conditions of iron depletion is not related to the effects of iron deficiency on the uninfected host. (**A**) Mongolian gerbils were maintained on iron-replete or iron-depleted diets for three weeks prior to treatment and throughout the duration of the experiment. Animals were then treated bi-weekly with deionized water (dH_20) as a vehicle control or 50% ethanol (EtOH) for a total of six weeks. Animals were euthanized following six weeks of treatment. (**B**) Inflammation was assessed and scored (0-12). Mean values are shown and Mann-Whitney U tests were used to determine statistical significance between groups.



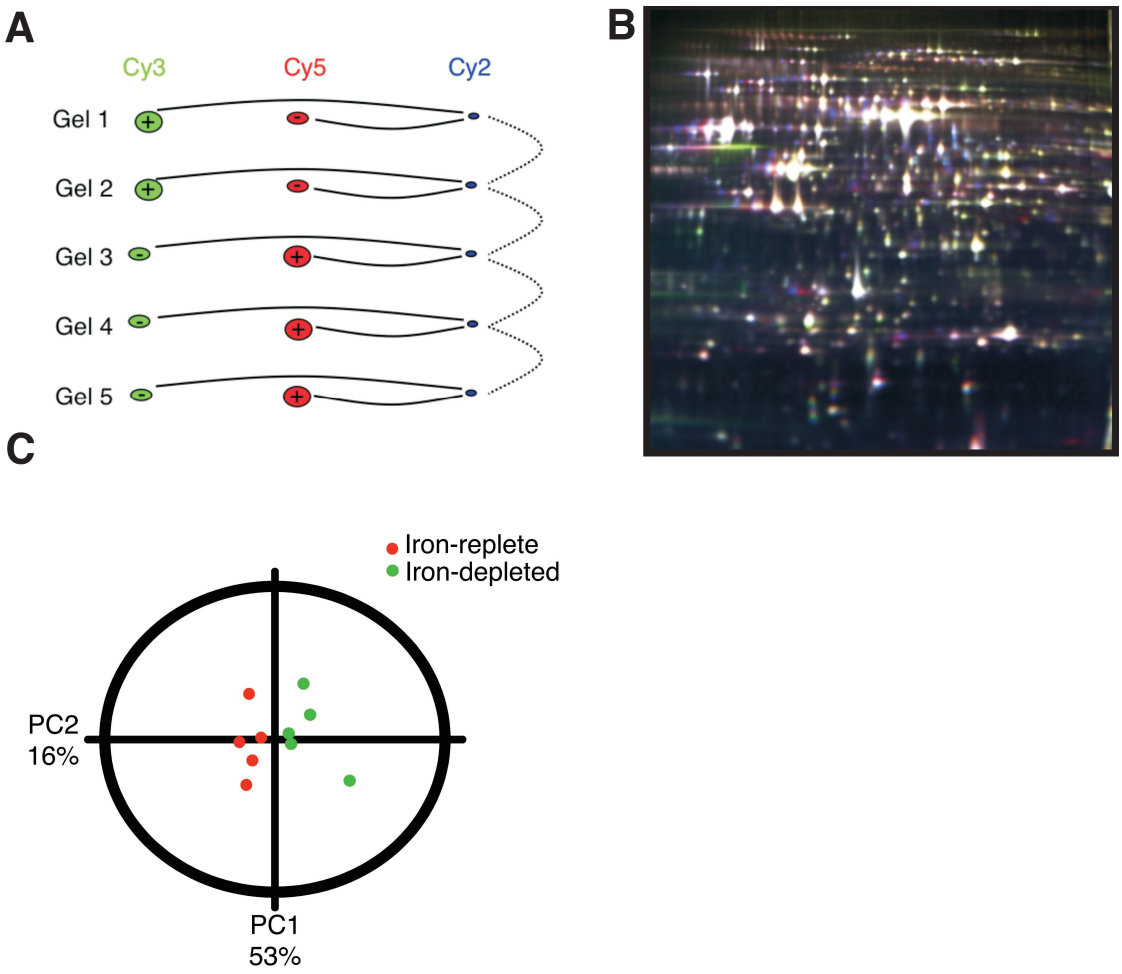
Supplemental Figure 2

Gastric mucosal cytokine expression levels under iron-replete and iron-depleted conditions in uninfected and *H. pylori*-infected gerbils, six weeks post-challenge. Gastric tissue was harvested from uninfected (UI) and *H. pylori* strain 7.13-infected gerbils maintained on either iron-replete or iron-depleted diets and then subjected to proinflammatory cytokine profiling by quantitative real-time RT-PCR. Expression levels of (**A**) *IL-1β*, (**B**) *IFNγ*, and (**C**) *TNFα* were normalized to expression levels of gerbil *18S*, as determined by the delta-delta CT methods (2^delta). Error bars indicate standard error of the mean from experiments performed on five independent tissue samples, and Mann-Whitney tests were used to determine statistical significance between groups.



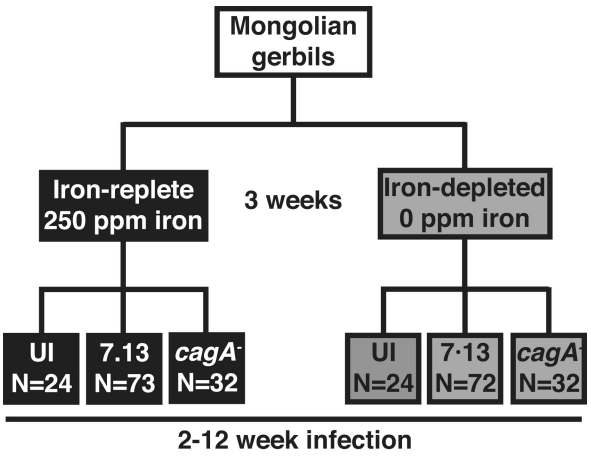
Supplemental Figure 3

Increased disease severity induced by wild-type *H. pylori* strain 7.13 under conditions of iron depletion occurs via a *fur*-independent mechanism. (**A**) Mongolian gerbils were maintained on iron-replete or iron-depleted diets for three weeks prior to challenge and throughout the duration of the experiment. Animals were challenged with Brucella broth as uninfected (UI) controls, wild-type carcinogenic *H. pylori* strain 7.13, or a 7.13 *fur*$^-$ isogenic mutant. Animals were euthanized six weeks post-challenge. (**B**) Colonization density (log colony forming units/gram gastric tissue, log CFU/g) was determined by quantitative culture. (**C**) Inflammation was assessed by histopathology and scored 0-12. Mean values are shown and Mann-Whitney U tests were used to determine statistical significance between groups.



Supplemental Figure 4

Proteomic analysis of ten minimally passaged *in vivo*-adapted *H. pylori* strains, harvested twelve weeks post-challenge, using two dimensional differential gel electrophoresis couples with mass spectrometry (2D-DIGE/MS). (**A**) A schematic representation of protein sample loading within a five gel DIGE experiment shows that each gel is coordinated by a Cy2-labeled pooled internal standard. *H. pylori* protein samples from strains harvested from iron-replete (+, N=5) and iron-depleted (-, N=5) gerbils were labeled with either Cy3 or Cy5. (**B**) The false-colored representative pH 4-7 gel contained three differentially labeled samples. The Cy2-labeled internal standard (blue), Cy3-labeled experimental samples (green), and Cy5-labeled experimental samples (red) are overlaid in the representative gel. (**C**) Principal component analysis (PCA) accurately segregated the ten individual DIGE expression maps by two principle components (PC1 and PC2) and demonstrated high reproducibility between biological replicates within each group. ANOVA and Student's t tests were used to determine statistical significance between groups.



Supplemental Figure 5

Experimental design. Mongolian gerbils were maintained on iron-replete or iron-depleted diets for three weeks prior to challenge and throughout the duration of the experiment. Animals were challenged with Brucella broth as an uninfected (UI) vehicle control, wild-type carcinogenic *H. pylori* strain 7.13, or a 7.13 *cagA*$^-$ isogenic mutant. Animals were euthanized at two, six, and twelve weeks post-challenge.

Supplemental Table 1

Composition of iron-replete and iron-depleted rodent diets.

	Iron-replete (250 ppm iron)	Iron-depleted (0 ppm iron)
Ingredients	**Amount**	
Corn Starch	46.4192%	46.5692%
Dextrin	15.5000%	15.5000%
Casein	14.0000%	14.0000%
Sucrose	10.0000%	10.0000%
Powdered Cellulose	5.0000%	5.0000%
Soybean Oil	4.0000%	4.0000%
AIN-93 Mineral Mix/No Iron	3.5000%	3.5000%
AIN-93 Vitamin Mix	1.0000%	1.0000%
Choline Bitartrate	0.2500%	0.2500%
L-Cystine	0.1800%	0.1800%
Ferric Citrate	0.1500%	0.0000%
t-Butylhydroquinone	0.0008%	0.0008%
Nutritional Profile[A]	**Amount**	
Protein	**12.5%**	**13.0%**
Arginine	0.48%	0.49%
Histidine	0.35%	0.36%
Isoleucine	0.80%	0.67%
Leucine	1.20%	1.21%
Lysine	0.99%	1.02%
Methionine	0.38%	0.36%
Cystine	0.22%	0.23%

Phenylalanine	0.64%	0.67%
Tyrosine	0.66%	0.71%
Threonine	0.53%	0.54%
Tryptophan	0.14%	0.15%
Valine	0.95%	0.8%
Alanine	0.40%	0.39%
Aspartic Acid	0.87%	0.90%
Glutamic Acid	2.40%	2.86%
Glycine	0.21%	0.27%
Proline	1.05%	1.65%
Serine	0.60%	0.77%
Fat	**4.2%**	**4.1%**
Linoleic Acid	2.05%	2.04%
Linolenic Acid	0.31%	0.31%
Omega-3 Fatty Acids	0.31%	0.31%
Total Saturated Fatty Acids	0.65%	0.59%
Total Monounsaturated Fatty Acids	0.89%	0.84%
Polyunsaturated Fatty Acids	2.36%	2.36%
Fiber	**5.1%**	**5.0%**
Carbohydrates	**73.5%**	**73.7%**
Energy (kcal/g)[B]	**3.81%**	**3.82%**
Protein, 0.500 kcal	13.1%	13.5%
Fat (ether extract), 0.378 kcal	9.9%	9.6%
Carbohydrates, 2.941 kcal	77.0%	76.9%

Minerals		
Calcium	0.76%	0.50%
Phosphorus	0.39%	0.31%
Phosphorus (available)	0.32%	0.31%
Potassium	0.36%	0.36%
Magnesium	0.05%	0.05%
Sodium	0.13%	0.13%
Chloride	0.20%	0.20%
Fluorine	1.0 ppm	1.0 ppm
Iron	250 ppm	0 ppm
Zinc	35 ppm	35 ppm
Manganese	11 ppm	11 ppm
Copper	6.6 ppm	6.0 ppm
Iodine	0.21 ppm	0.21 ppm
Chromium	1.0 ppm	1.0 ppm
Molybdenum	0.15 ppm	0.15 ppm
Selenium	0.15 ppm	0.19 ppm

Vitamins		
Vitamin A	4.0 IU/g	4.0 IU/g
Vitamin D-3	1.0 IU/g	1.0 IU/g
Vitamin E	78.2 IU/kg	78.2 IU/kg
Vitamin K	0.29 ppm	0.29 ppm
Thiamin Hydrochloride	6.1 ppm	6.0 ppm
Riboflavin	6.2 ppm	6.5 ppm
Niacin	30 ppm	30 ppm
Pantothenic Acid	15 ppm	16 ppm
Folic Acid	2.1 ppm	2.1 ppm
Pyridoxine	5.8 ppm	5.8 ppm

Biotin	0.2 ppm	0.2 ppm
Vitamin B-12	25 mcg/kg	28 mcg/kg
Choline Chloride	1,279 ppm	1,250 ppm

[A]Nutrients expressed as percent of ration on an as-fed basis except where otherwise indicated.

[B]Energy (kcal/g) – sum of decimal fractions of protein, fat, and carbohydrate.

Supplemental Table 2

Inductively coupled plasma dynamic reaction cell mass spectrometry (ICP-DRC-MS) operating conditions and parameters for trace iron analysis.

Parameter	Setting/Type
Nebulizer	Meinhard type A quartz
Radio frequency power	1400 W
Plasma argon flow	15 L/min
Nebulizer argon flow	0.9 L/min
Injector	2.0 mm i.d. quartz
Monitored ion (m/z)	$^{54}Fe^{+}$, $^{56}Fe^{+}$
Reaction gas	NH_3
NH_3 flow	0.8 mL/min
RPq	0.7