Supporting Information Appendix:

α-Difluoromethylornithine Reduces Gastric Carcinogenesis by Causing Mutations in *Helicobacter pylori cagY*

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Figs. S1 to S9

Table S1 to S3

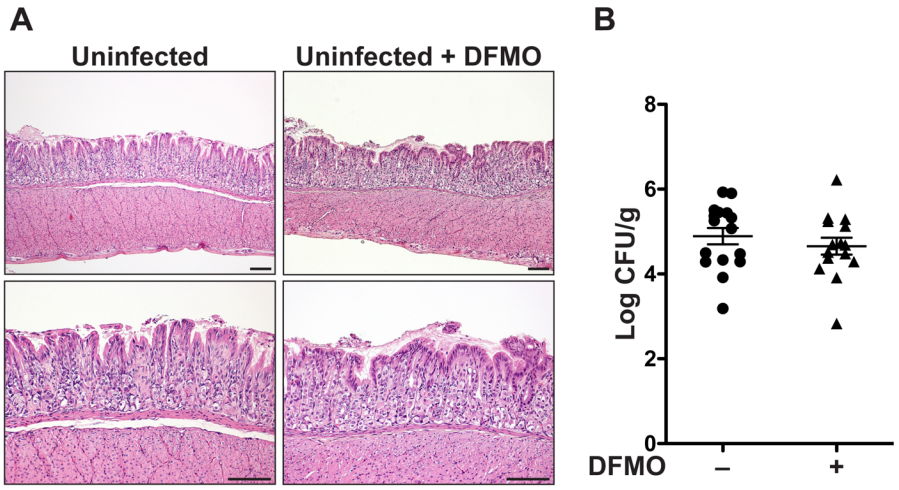

Figure S1. (A) H&E staining of gastric tissues from uninfected gerbils, showing normal gastric histology. 7 gerbils per group in control and DFMO-treated. Scale bars = 100 μm. (B) Colonization density in gastric tissues of gerbils infected with *H. pylori* 7.13 for 12 weeks and treated or not with DFMO.

Parental 7.13 from 1 to 5466

Alignment to:

Control-5 Matches:5466; Mismatches:0; Gaps:0; Unattempted:0
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Parental 7.13 from 1 to 5466

Alignment to:

DFMO-1 Matches:5466; Mismatches:0; Gaps:0; Unattempted:0
DFMO-2 Matches:5145; Mismatches:0; Gaps:321; Unattempted:0
DFMO-3 Matches:5466; Mismatches:0; Gaps:0; Unattempted:0
DFMO-4 Matches:5142; Mismatches:0; Gaps:324; Unattempted:0
DFMO-5 Matches:5313; Mismatches:21; Gaps:150; Unattempted:0
DFMO-6 Matches:5466; Mismatches:0; Gaps:0; Unattempted:0
DFMO-8 Matches:4047; Mismatches:0; Gaps:1419; Unattempted:0
DFMO-9 Matches:5466; Mismatches:0; Gaps:0; Unattempted:0
DFMO-10 Matches:5466; Mismatches:0; Gaps:0; Unattempted:0
DFMO-11 Matches:5466; Mismatches:0; Gaps:0; Unattempted:0
DFMO-12 Matches:5466; Mismatches:0; Gaps:0; Unattempted:0
DFMO-14 Matches:5466; Mismatches:0; Gaps:207; Unattempted:0
DFMO-15 Matches:5466; Mismatches:0; Gaps:0; Unattempted:0
DFMO-16 Matches:5466; Mismatches:0; Gaps:0; Unattempted:0

1>ATGAATGAAGAAAACGATAAACTTGAAACTTCTAAAAAAATCCAACAACATTCACCCCAAGATTTATCCAATGAAGAAGCAACAGAAGCCAATCATTTTG>100
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2460>CAC----------->2462
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4976>CTGATGGTGTGATAATACCTCTAGCAAACGCTCAAGCAGCAGGCATGTTGGGTGAAGCAGGGGTAGATGGCTATGTGAATAATCACTTTATGAAGCGCAT>5075

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3657>AGGCTTTGCTGTGATAGCAAGCGTGGTTAATAGCTTCTTGCAAACTGCGCCTATCATAGCTCTAGATAAACTCATAGGTCTTGGCAAAGGTAGAAGTGAA>3756
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5376>ATCTGTGGTAGATGAAATTATCAAACAAAGCACTAAAACTTTGTCTAGAGAACATGAAGAAATCACCACAAGCCCCAAAGGTGGCAATTAA>5466
5055>ATCTGTGGTAGATGAAATTATCAAACAAAGCACTAAAACTTTGTCTAGAGAACATGAAGAAATCACCACAAGCCCCAAAGGTGGCAATTAA>5145
5376>ATCTGTGGTAGATGAAATTATCAAACAAAGCACTAAAACTTTGTCTAGAGAACATGAAGAAATCACCACAAGCCCCAAAGGTGGCAATTAA>5466
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5262>ATCTGTGGTAGATGAAATTATCAAACAAAGCACTAAAACTTTGTCTAGAGAACATGAAGAAATCACCACAAGCCCCAAAGGTGGCAATTAA>5352
5376>ATCTGTGGTAGATGAAATTATCAAACAAAGCACTAAAACTTTGTCTAGAGAACATGAAGAAATCACCACAAGCCCCAAAGGTGGCAATTAA>5466
3957>ATCTGTGGTAGATGAAATTATCAAACAAAGCACTAAAACTTTGTCTAGAGAACATGAAGAAATCACCACAAGCCCCAAAGGTGGCAATTAA>4047
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5376>ATCTGTGGTAGATGAAATTATCAAACAAAGCACTAAAACTTTGTCTAGAGAACATGAAGAAATCACCACAAGCCCCAAAGGTGGCAATTAA>5466

Figure S2. *cagY* sequence alignments for parental *H. pylori* 7.13, 6 output strains from untreated gerbils, and 14 DFMO output strains. Control = Output strains isolated from untreated gerbils. DFMO = Output strains isolated from DFMO-treated gerbils.

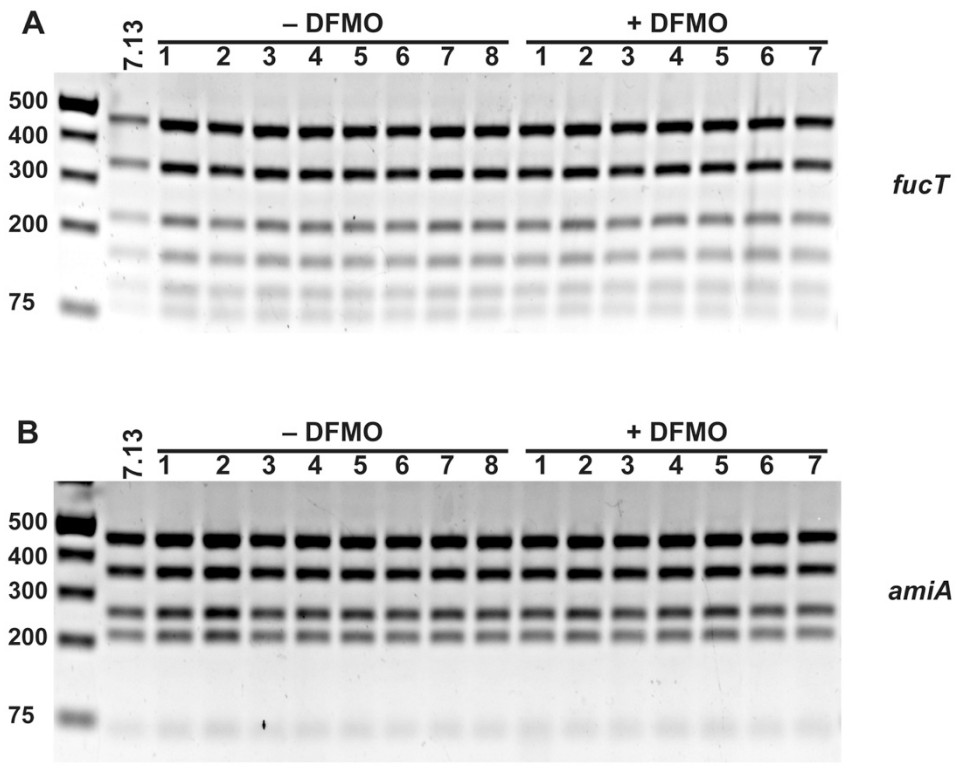

Figure S3. (A) Fucosyltransferase (*fucT*) and (B) N-acetylmuramoyl-L-alanine amidase (*amiA*) RFLP profiles of *H. pylori* gerbil output strains.

Parental 7.13 from 1 to 3655

Alignment to:

Control-3 Matches:3655; Mismatches:0; Gaps:0; Unattempted:0
Control-4 Matches:3655; Mismatches:0; Gaps:0; Unattempted:0
Control-11 Matches:3655; Mismatches:0; Gaps:0; Unattempted:0
Control-12 Matches:3655; Mismatches:0; Gaps:0; Unattempted:0
Control-13 Matches:3655; Mismatches:0; Gaps:0; Unattempted:0
Control-14 Matches:3655; Mismatches:0; Gaps:0; Unattempted:0
DFMO-1 Matches:3655; Mismatches:0; Gaps:0; Unattempted:0
DFMO-2 Matches:3655; Mismatches:0; Gaps:0; Unattempted:0
DFMO-3 Matches:3655; Mismatches:0; Gaps:0; Unattempted:0
DFMO-4 Matches:3655; Mismatches:0; Gaps:0; Unattempted:0
DFMO-5 Matches:3655; Mismatches:0; Gaps:0; Unattempted:0
DFMO-6 Matches:3655; Mismatches:0; Gaps:0; Unattempted:0
DFMO-8 Matches:3655; Mismatches:0; Gaps:0; Unattempted:0
DFMO-9 Matches:3655; Mismatches:0; Gaps:0; Unattempted:0
DFMO-10 Matches:3655; Mismatches:0; Gaps:0; Unattempted:0
DFMO-11 Matches:3655; Mismatches:0; Gaps:0; Unattempted:0
DFMO-12 Matches:3655; Mismatches:0; Gaps:0; Unattempted:0
DFMO-14 Matches:3655; Mismatches:0; Gaps:0; Unattempted:0
DFMO-15 Matches:3655; Mismatches:0; Gaps:0; Unattempted:0
DFMO-16 Matches:3655; Mismatches:0; Gaps:0; Unattempted:0

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Figure S4. *cagA* sequence alignments for parental *H. pylori* 7.13, 6 control output strains, and 14 DFMO output strains. Control = Output strains isolated from untreated gerbils. DFMO = Output strains isolated from DFMO treated gerbils.

Parental 7.13 from 1 to 5466

Alignment to:

DFMO-4 Matches:5142; Mismatches:0; Gaps:324; Unattempted:0

7.13 [DFMO-4] Matches:5140; Mismatches:2; Gaps:324; Unattempted:0

DFMO-8 Matches:4047; Mismatches:0; Gaps:1419; Unattempted:0

7.13 [DFMO-8] Matches:4045; Mismatches:2; Gaps:1419; Unattempted:0

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   1>ATGAATGAAGAAAACGATAAACTTGAAACTTCTAAAAAAATCCAACAACATTCACCCCAAGATTTATCCAATGAAGAAGCAACAGAAGCCAATCATTTTG>100
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Figure S5. *cagY* sequence alignments for parental *H. pylori* 7.13, DFMO-4, 7.13 [DFMO-4], DFMO-8 and 7.13 [DFMO-8].

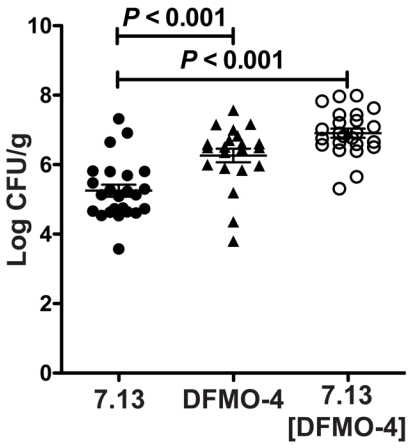

Figure S6. Colonization density in gastric tissues of gerbils infected with *H. pylori* 7.13, DFMO-4, or 7.13 [DFMO-4] for 12 weeks. ANOVA with Newman-Keuls multiple comparisons test was used.

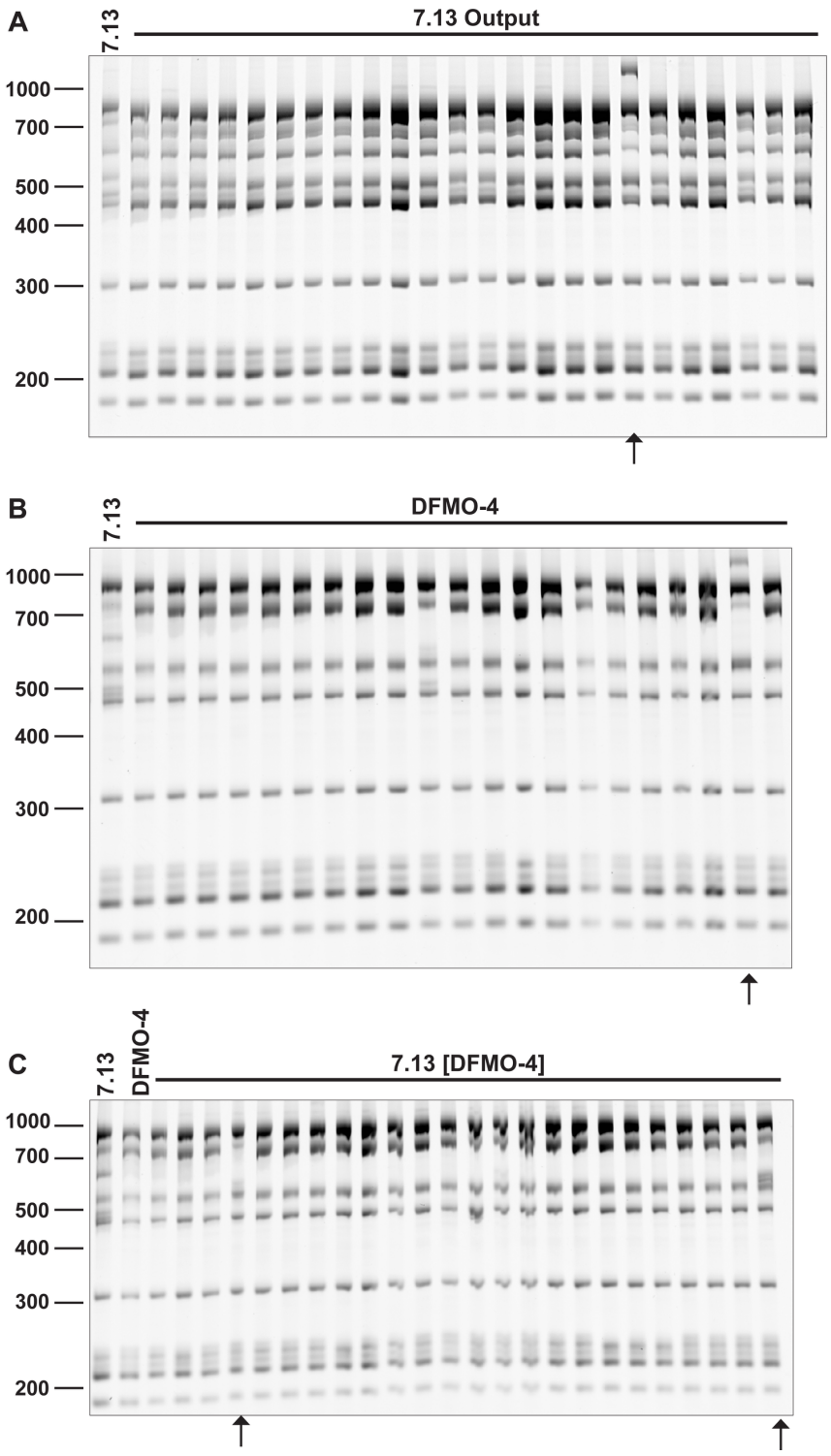

Figure S7. *cagY* RFLP profile of output strains from gerbils infected with (A) *H. pylori* 7.13, (B) DFMO-4 or (C) 7.13 [DFMO-4]. Arrows indicate strains with a different RFLP profile compared to the strain used for infection.

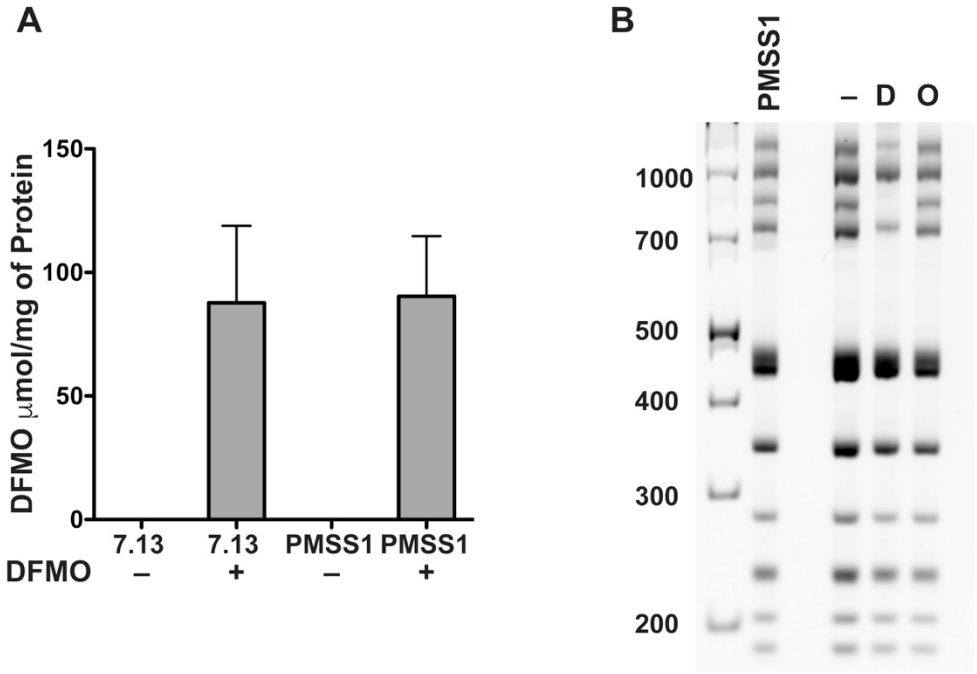

Figure S8. (A) Quantification of DFMO levels by mass spectrometry in *H. pylori* strains 7.13 and PMSS1 after 24 h of culture with DFMO. Error bars represent SEM. (B) *cagY* RFLP profile of *H. pylori* PMSS1 serially passaged on regular plates or plates supplemented with DFMO (D) or ornithine (O). RFLP profile is shown for passage 20.

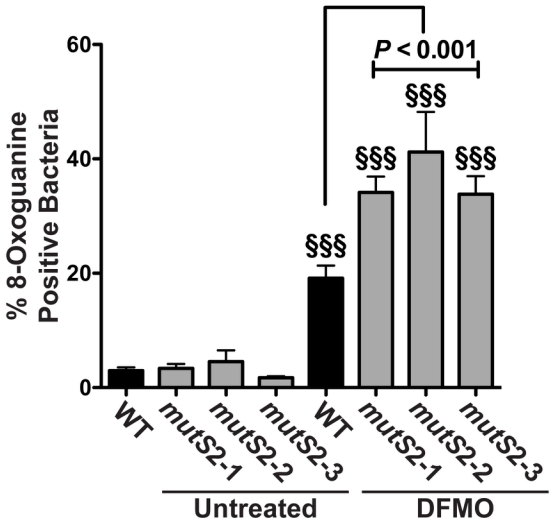

Figure S9. Oxidative DNA damage in 3 *H. pylori* Δ*mutS2* clones. Percentage of 8-oxoguanine-positive bacteria assessed by flow cytometry in *H. pylori* WT and Δ*mutS2* after DFMO treatment for 24 h. §§§P<0.001 versus untreated bacteria.

Table S1. Frequency of rifampicin-resistant colonies in control and DFMO gerbil output strains.

	Frequency of rifampicin resistance	SE
Parental	7.152×10^{-8}	1.277×10^{-8}
Control Output	6.547×10^{-8}	2.577×10^{-8}
DFMO Output	1.154×10^{-7}	4.915×10^{-8}

Table S2. Frequency of rifampicin-resistant colonies in strains serially passaged on regular or DFMO-containing plates.

	Frequency of rifampicin resistance	SE
Control in vitro	1.282×10^{-6}	2.693×10^{-7}
DFMO in vitro	7.256×10^{-7}	6.873×10^{-7}

Table S3. List of primers

Species	Target gene	Sequence (5'–3')
Human	*CXCL8*	F: TCTTGGCAGCCTTCCTGATT
		R: TCTTTAGCACTCCTTGGCAAAAC
Human	*GAPDH*	F: AGCCTCAAGATCATCAGCAATG
		R: GGGTGCTAAGCAGTTGGTGG
H. pylori	*sodB*	F: GGGCATATCAATTGGCATTTTG
		R: CCACTGAGCCTAAGCCTTCTTTT
H. pylori	*katA*	F: CAGTGCGGATGCGGTAAGA
		R: TGTTCCCTACTAAATCCCAGTTACCT
H. pylori	*mutS2*	F: CCATCATTCCATTATCCCCTATTT
		R: GCCGGCAAAAGTAGAGATATTGTT
H. pylori	*16S*	F: TGCAAGTGGAGCCAATCTT
		R: GGAACGTATTCACCGCAACA
H. pylori	*fucT*	F: AAAGGATAACCATGTTCCAACCCC
		R: ACGCTCAAAAAAGAAGCGTTAGCG
H. pylori	*amiA*	F: GTGGATAAGATTATCAGCGTGCAT
		R: CATCTACAACCTAATCATTGCCTAA
H. pylori	*cagA* Seq1	F: ATTTTTAGCAGTCTTTGACACC
H. pylori	*cagA* Seq2	F: TGGAAATGTTAGATGTTGAGGGAGTCGC
H. pylori	*cagA* Seq3	F: TCTTCAAGGTAGCCTAAAACATGATGGC
H. pylori	*cagA* Seq4	R: ACAGCAACAAGGAATTGGTTG
H. pylori	*cagA* Seq5	F: ACAGCAACAAGGAATTGGTTG
H. pylori	*cagA* Seq6	R: ATATGATACCATGAATTGGTAGC