Supplemental Material

Helicobacter pylori AddAB helicase-nuclease and RecA promote recombination-based DNA repair and survival during stomach colonization

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Figure S1. ATP concentration-dependence of ds DNA exonuclease activity in cell-free extracts of *H. pylori* and *E. coli*. Extracts of *H. pylori* strain NSH57 (●) or *E. coli* strain V66 (○) were assayed for ATP-dependent solubilization of [^{3}H] T7 DNA as described in Experimental Procedures. The ATP concentration of the reaction is indicated. Data are the mean specific activity determined with two amounts of extract (Eichler and Lehman, 1977) at each ATP concentration.

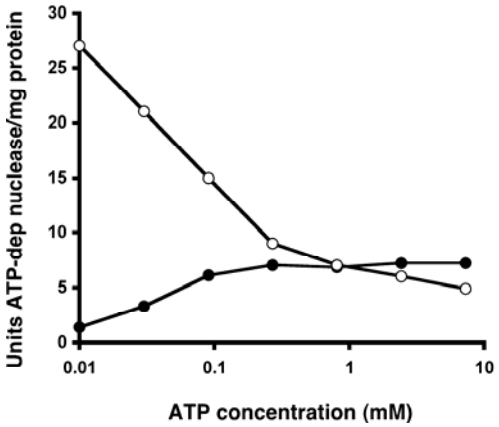

Figure S2. Growth of *recA*, *addA* and *addB* mutant strains is comparable to wild-type bacteria *in vitro*. Bacteria were grown in Brucella Broth supplemented with 10% fetal bovine serum at 37 ºC in parallel 96 well plate cultures in a microaerobic atmosphere. At the indicated times, dilutions of duplicate wells were plated to determine colony forming units. Experiment shown is representative of two independent biological replicates.

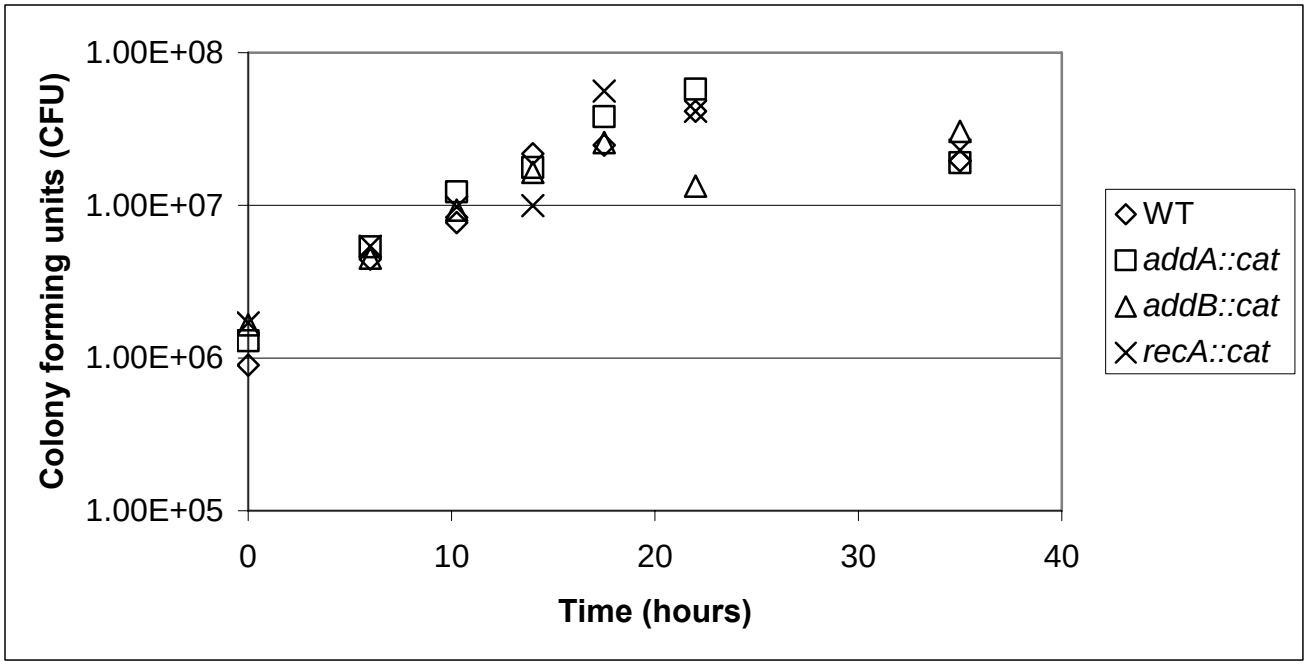

Table S1. Bacterial Strains

Strain	Genotype	Reference or source
E. coli		
V66	*argA21 hisG4 met recF143 rpsL31 galK2 xyl-5* λ^- F^-	(Schultz *et al.*, 1983)
V2381	*ΔrecBCD2731<kan> hisG4 met recF143 rpsL31 galK2 xyl-5* λ^- F^-	(Amundsen and Smith, 2007)
V3060	*ΔrecBCD2731<kan> hisG4 met recF143 rpsL31 galK2 xyl-5* F^- (λDE3; imm^{21} *Δnin5 Sam7 lacUV5* T7 gene *1* [RNA polymerase])	This work
H. pylori		
NSH57	wild type	(Baldwin *et al.*, 2007)
NSH74	*ΔaddB::cat*	this work
NSH58	*ΔaddA::cat*	this work
NSH92	*ΔrecA::cat*	this work
NSH66	*ΔruvC::cat*	this work
NSH94	*ΔaddB::cat rdx::addB*^{S771P}	this work
NSH95	*ΔaddA::cat rdxA::addA*^{6His}	this work
J166	wild type	(Solnick *et al.*, 2001)
J166△recA	*ΔrecA::km*	this work
J166△addA	*ΔaddA::km*	this work
J166△ruvC	*ΔruvC::km*	this work

Table S2. Primers used for cloning and sequencing	
Name	Sequence
1553-1	GCTCTAGATAGCATGTGTGAATTTGACGC
1553-2	ACGCGTCGACGCCTAGTGCAAAATAACTTTC
1553-3	ATCCACTTTTCAATCTATATCGTCTAAAATGCGCTCTTTCAT
1553-4	CCCAGTTTGTCGCACTGATAAACCAGCAAAGCCATAAAGCGC
addAbp1100	ATGCTCTGCTTGACATCG
addAbp1800	CCATAAAGCTCAAATTGC
addAbp700	CAAATAAATAGAGCTTG
AddA-C1 (SalI)	ACGCGTCGACTCAGACCCATAATTTTTCAAG
AddA-C1(SalI)	ACGCGTCGACTCAGACCCATAATTTTTCAAG
AddA-N1(NcoI)	CATGCCATGGATACCAAAAGACAATGC
AddA-Ntag(EcoRI)	GGAATTCGGATACCAAAAGACAATGCAT
AddB C1 (AvrII)	CCCCTAGGTCATCGGTTGCACATGTCTTT
AddB N1 (NdeI)	GGAATTCCATATGAACTTAGAAAAACTTTTTG
addB-1	GCTCTAGAGCGCCATGCTTTGACTTGTTG
addB-2	ACGCGTCGACGATAAAATGCCTAATAGATGC
addB-3	ATCCACTTTTCAATCTATATCCCTTCGCCTTGCTCTAAATAG
addB-4	CCCAGTTTGTCGCACTGATAACCAAGCTCAAACAAGAAATTG
HP0153-1	GCTCTAGACGTTCGCAATTTTAGGGTATA
HP0153-2	ACGCGTCGACAGCCCTTAACCTCTCATCTAC
HP0153-3	ATCCACTTTTCAATCTATATCGTCTTCATCTATTGCC
HP0153-4	CCCAGTTTGTCGCACTGATAAGAGCCTTTAGAAGAAATGGAG

HP0877-1	GCTCTAGAGTAACGATCACATCTAAAGCG
HP0877-2	ACGCGTCGACCGATTTAGCCAAATGCGGATC
HP0877-3	ATCCACTTTTCAATCTATATCGCCGTGATTAAAGAAAGCTTG
HP0877-4	CCCAGTTTGTCGCACTGATAAACGCATGCGCAACGCTTAAAG
pDuet 5362	GTCCGGCGTAGAGGATCG
pDuet153REV	GCCGCAAGCTTGTCGACCTG
RecApEt-1	GGAATTCCATATGGCAATAGATGAACACAAA
RecApEt-2	CCGCTCGAGTTCCATTTCTTCTAAAGGCTC
addAup1 (NotI)	AAT GCGGCCGC GGAGCCACGATAGGGATATGGAG
addAup2 (PstI)	AAT CTGCAG CGCACCAAACTAGGGTCTAAATGG
addAdown1 (HincII)	AAT GTCGAC GCTCAAGTGTCTCATTACGCTGAG
addAdown2 (XhoI)	AAT CTCGAG CATAGCGTCCTTATGCTCGCTCG
recAup1 (NotI)	AAT GCGGCCGC TCGTTACTGCCCTTAATGAGCTC
recAup2 (PstI)	AAT CTGCAG CAATTTGTTTGATCGCTAAAGAAATCGC
recAdown1 (HincII)	AAT GTCGAC AATGAAGAGATCATGCCCTTACCC
recAdown2 (XhoI)	AAT CTCGAG AAAAGACAATCAGGGAGCTATGGC
ruvCup1 (NotI)	AAT GCGGCCGC GATGGAGTGGGCTTGCATTGAAAC
ruvCup2 (PstI)	AAT CTGCAG CTTGTTGGAAGCATGAGAAATGATAGC
ruvCdown1 (HincII)	AAT GTCGAC TGCTATCACGCATGCGCAACGC

Table S3. Plasmids

Plasmid	Genotype	Source or reference
pETDuet-1	Inducible expression vector	Novagen
pJF22	*addB*^{S771P} in pETDuet-1	This study
pJF23	*addA*^{6His} in pETDuet-1	This study
pJF25	*addA* in pETDuet-1	This study
pJF30	*addA addB*^{S771P} in pETDuet-1	This study
pJF31	*addA*^{6His} *addB*^{S771P} in pETDuet-1	This study
pSA405	*addA addB* in pETDuet-1	This study
pRdxA	wild type	(Smeets *et al.*, 2000)
pJF27	*addB*^{S771P} in pRdxA	This study
pJF29	*addA*^{6His} in pRdxA	This study
pBR322	Cloning vector	(Bolivar *et al.*, 1977)
pMR3	*recBCD – argA* in pBR322	(Amundsen and Smith, 2007)
pJ150	*babA* in pGEM-T Easy	This study
pJ151	*babB* in pGEM-T Easy	This study

Supplemental Table 4. ID_{50} determination for wild type, Δ*recA::cat* and Δ*addA::cat*									
			Accumulated[a]						
Number of cells in inoculum	**Number Infected**	**Number Uninfected**	**Infected**	**Uninfected**	**Total**	**Accumulated % Infected**	**Proportionate Distance[b]**	**log ID50[c]**	**ID50**
wild-type NSH57									
3.34E+06	4	1	12	1	13	92			
3.34E+05	4	1	8	2	10	80			
3.34E+04	3	1	4	3	7	57	0.84	4.4	2.3E+04
3.34E+03	1	4	1	7	8	13			
recA::cat									
2.40E+10	0	5	1	5	6	20			>2.4E+10
2.40E+09	1	4	1	9	10	10			
2.40E+08	0	5	0	14	14	0			
2.40E+07	0	5	0	19	19	0			
2.40E+06	0	5	0	24	24	0			
addA::cat									
2.90E+08	5	0	8	0	8	100			
2.90E+07	3	2	3	2	5	60	0.83	7.3	2.0E+07
2.90E+06	0	5	0	7	7	0			
2.90E+05	0	5	0	12	12	0			
2.90E+04	0	5	0	17	17	0			
[a]Accumulated infected are obtained by adding successfive entries in column 2 from bottom to top; accumulated uninfected are obtained by adding successfive entries in column 3 from the top to the bottom; accumulated totals are obtained by adding the values for column 4 and 5 at each dose.									
[b]Proportionate distance = (50% - next lowest %)/(next highest % - next lowest %)									
[c]Log ID50 = (proportionate distance * log dose increment) + log(dose of the next lowest percent)									

References

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