Table S1. Primers used in this study.

Primers	Sequence*	Application
5' codY Arm 1	5' – CAAAAACAGGCATGCGGTAATC – 3'	*codY* deletion
3' codY Arm 1	5' – GCAAAAGCTCCATGGCTTGACGTG – 3'	*codY* deletion
5' codY Arm 2	5' – GAATCACGTTCCCATGGTATGAAAG – 3'	*codY* deletion
3' codY Arm 2	5' – GTCCGTCATCCTGCAGACATTCAGC – 3'	*codY* deletion
CodY Rev	5' – TCTAGAAGCAATTGCTTATTTTTCCAACTC – 3'	codY comp
CodY For	5' – CTGCAGAAGGCTGGAGGAGAAAAGATG – 3'	codY compl
opp F	5' – CCAGGCAGACGATGTTATTT – 3'	qRT-PCR
opp R	5' – GCTGGTTCGTGCTTCTTT – 3'	qRT-PCR
efaA L	5' – TGCCGCTTATATTTGGGAAA – 3'	qRT-PCR
efaA R	5' – CGCCTTCTGTTCCTTCTTTG – 3'	qRT-PCR
efaB L	5' – TGATGGGTGATGCGATTTC – 3'	qRT-PCR
efaB R	5' – AAATCGGTGGAACTTTTTGC – 3'	qRT-PCR
efaC L	5' – TTTTGAAACGGTCTTGCTAGG – 3'	qRT-PCR
efaC R	5' – ATATCAATGCCGACGAAAGG – 3'	qRT-PCR
mntH1 L	5' – GAGAAAGCCAAAGCAATTCG – 3'	qRT-PCR
mntH1 R	5' – TTGACCCGAAGCCAGTAAAG – 3'	qRT-PCR
mntH2 L	5' – CCGTGTTGAAATGGGTGAAC – 3'	qRT-PCR
mntH2 R	5' – AATTCCACAACCGTCCAAAC – 3'	qRT-PCR
sodA L	5' – CAGCGATTGAAAAACATCCA – 3'	qRT-PCR
sodA R	5' – TTCATCAAAGCTGCCAAATG – 3'	qRT-PCR
feoB L	5' – GGAGATCCAGCACCATTCAT – 3'	qRT-PCR
feoB R	5' – CGCCAACCCTTTACCTAGAA – 3'	qRT-PCR
fhuG L	5' – CTTGGCTAGTCATCGGGTTC – 3'	qRT-PCR
fhuG R	5' – CATTTGCGAGCAATATGTGG – 3'	qRT-PCR
rel L	5' – TCGACCAAAACATATTTATTCAATCTATCG – 3'	qRT-PCR
rel R	5' – TAGATTGATACATATTCGCTTTTGGC – 3'	qRT-PCR

*Underlined bases correspond to restriction sites included to aid in the cloning of PCR products.

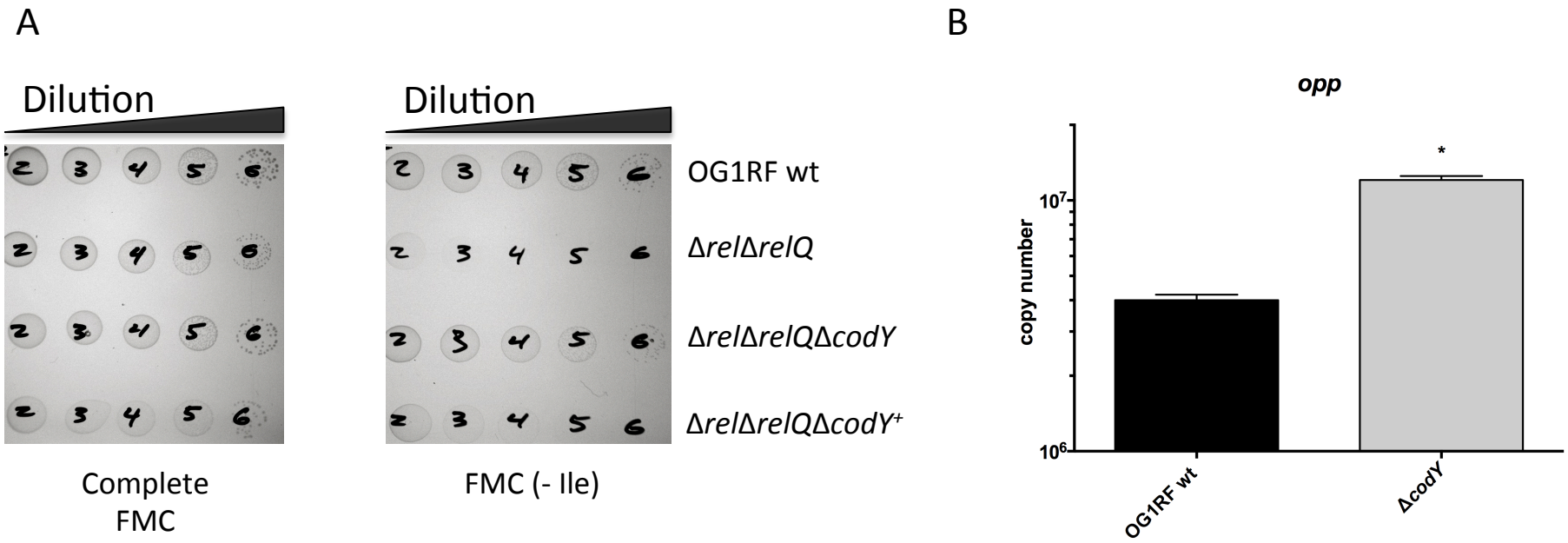

Figure S1. CodY inactivation in a (p)ppGpp^0 background restores isoleucine prototrophy. (A) Plate titrations of *E. faecalis* OG1RF, (p)ppGpp^0 , (p)ppGpp^{0}Δ*codY* and the complemented (p)ppGpp^{0}Δ*codY*$^+$ strains on FMC plates depleted of isoleucine. Plates were supplemented with 1% rhamnose to induce *codY* expression *in trans*. Strains were grown in triplicate to OD_{600} ~ 0.5, serially diluted, spotted on FMC (-Ile) plates and incubated for 24h. (B) The *E. faecalis* OG1RF and Δ*codY* strains were grown in complete FMC to mid-exponential growth phase and transcript levels of *opp* determined by qRT-PCR. The bar graphs show the average and standard deviations of three independent experiments performed in triplicates. Gene expression of mutant strain was compared to that of the parent strain via Student's *t* test (*P< 0.05).

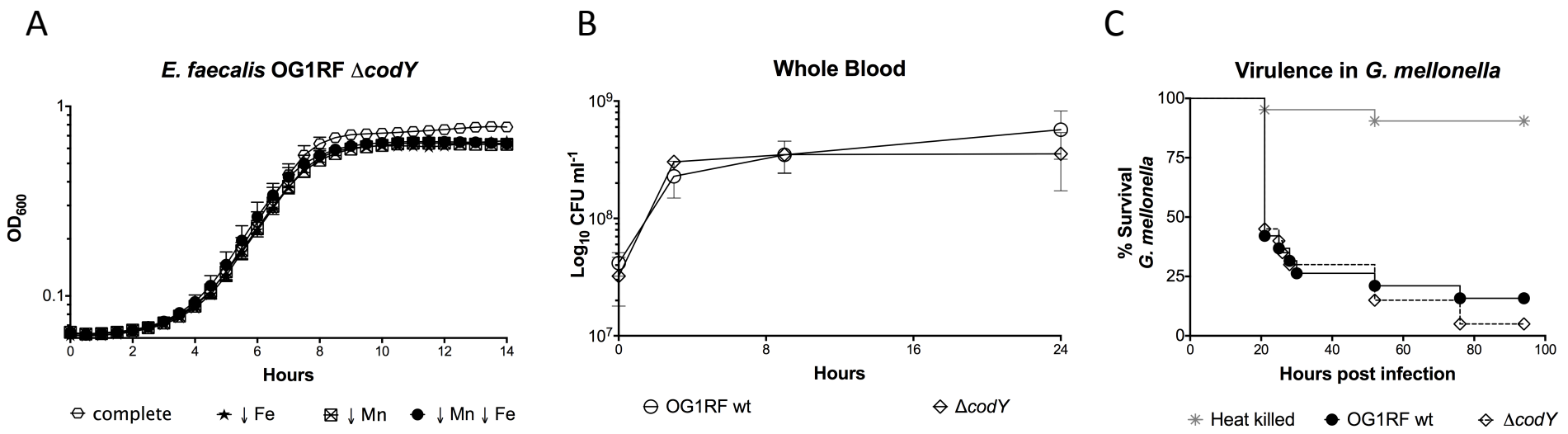

Figure S2. Phenotypic characterization of Δ*codY*. (A) Growth of the OG1RF Δ*codY* strain in metal-depleted FMC. (B) Growth of OG1RF and Δ*codY* strains in whole blood. For each time point, aliquots were serially diluted and plated on TSA plates for CFU enumeration. (C) Kaplan-Meier plots of *G. mellonella* larvae injected with OG1RF Δ*codY* strains. Graphs A-B show the average and standard deviations of at least three independent experiments. The Kaplan-Meyer plot (C) is a representative of an experiment repeated three independent times (*$p < 0.05$).

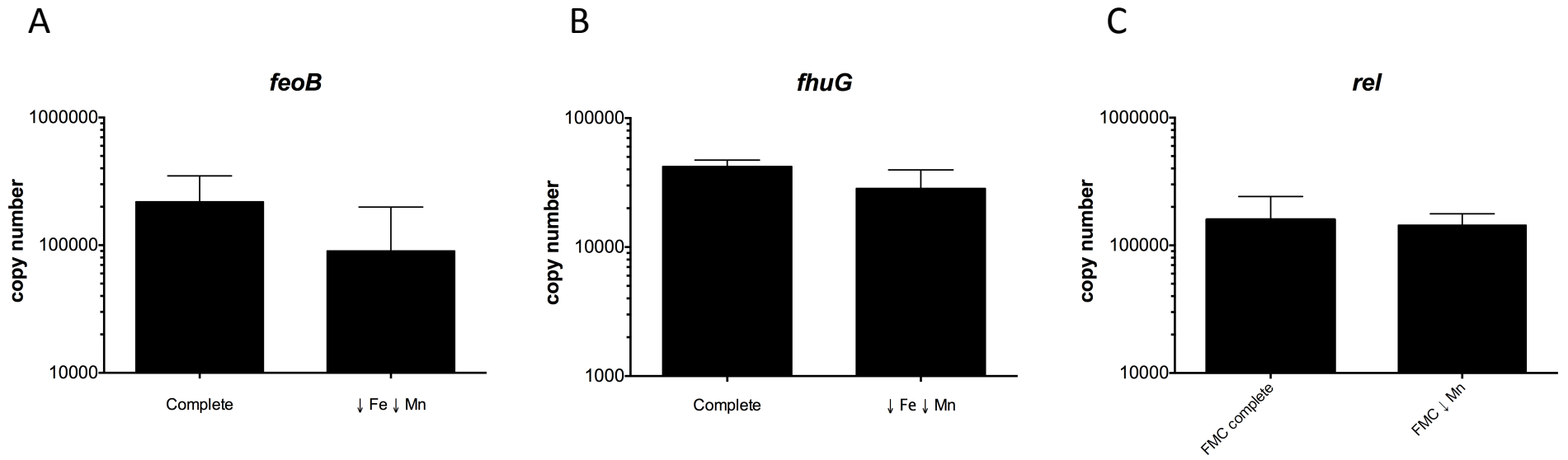

Figure S3. Transcriptional expression of *rel* and of Fe transporters. The *E. faecalis* OG1RF wild-type strain was grown in FMC complete, Fe and Mn-depleted (A-B), or Mn-depleted FMC (C) to mid-exponential growth phase and transcript levels of *feoB, fhuG* and *rel* determined by qRT-PCR. The bar graphs show the average and standard deviations of three independent experiments performed in triplicates. Differences were compared via Students *t* test (*$P \leq 0.05$).

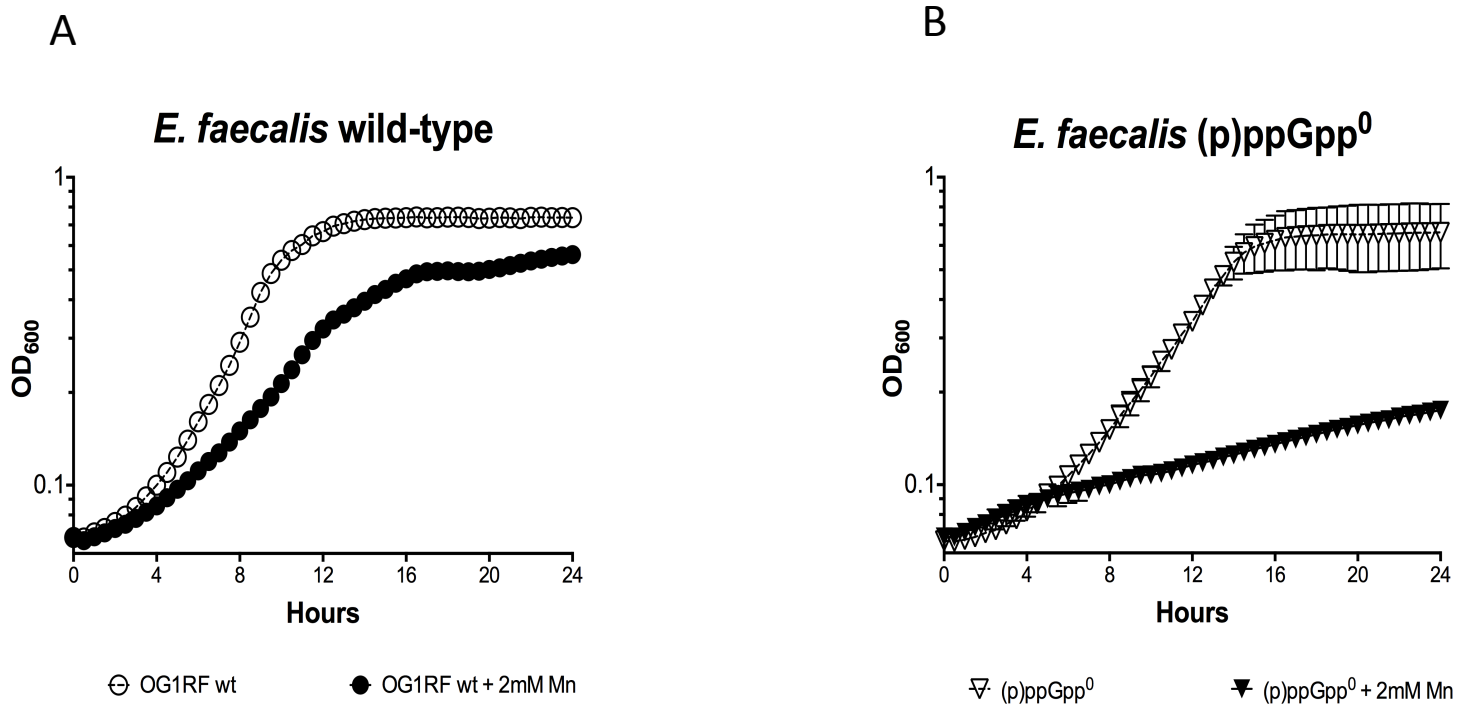

Figure S4. The (p)ppGpp^0 strain is more sensitive to Mn toxicity. Growth of *E. faecalis* OG1RF wild-type (A) and (p)ppGpp^0 (B) strains in complete FMC in the presence or absence of 2 mM $MnSO_4$. Graphs show the average and standard deviations of at least three independent experiments.

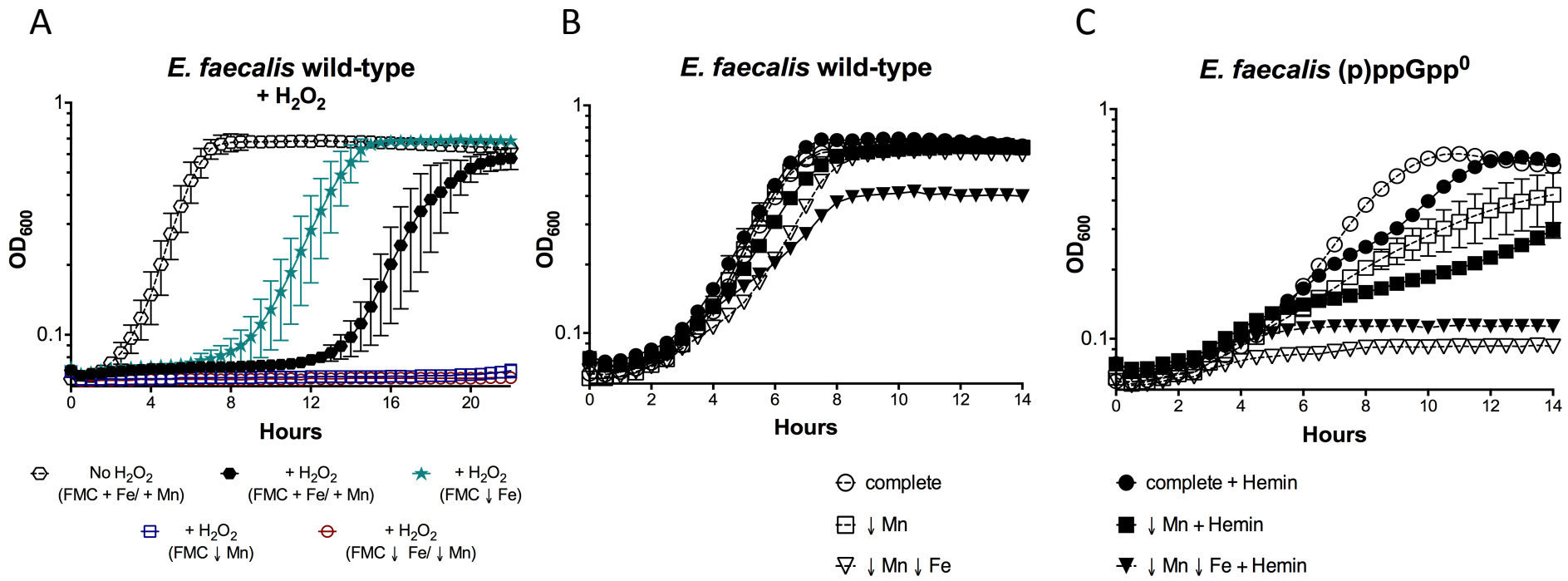

Figure S5. Relevance of Mn to ROS stress. (A) Mn is essential for *E. faecalis* protection from toxic H_2O_2. While Fe depletion reduces Fenton chemistry-derived damage to the cell, Mn depletion in the presence of H_2O_2 completely abolishes growth. (B-C) Heme supplementation does not restore the growth defect of the (p)ppGpp^0 strain. Growth of *E. faecalis* wild-type (A-B) and OG1RF (p)ppGpp^0 (C) strains in complete, Mn-depleted, or Fe/Mn-depleted FMC in the presence or absence of 2 mM H_2O_2 (A) or 5 μM hemin (B-C). Graphs show the average and standard deviations of at least three independent experiments.