Table S1. Oligonucleotides used in this study [#].

Primer name	Sequence (5´-to-3')*	Restriction site	Use
hasR mut_UP_FW	GCTCTAGAGCCTGCGCTACAGCG	*Xba*I	Generation of the pDM4Δ*hasR* construct
hasR mut_UP_RV	CGGAATTCCGCCCCGTCTTGCGC	*Eco*RI	Generation of the pDM4Δ*hasR* construct
hasR mut_DOWN_FW	CGGAATTCTGTTCGAAGACCGCCTG	*Eco*RI	Generation of the pDM4Δ*hasR* construct
hasR mut_DOWN_RV	CCGCTCGAGTGGTGAATGCCGGAGC	*Xho*I	Generation of the pDM4Δ*hasR* construct
phuR mut_UP_FW	GCTCTAGAAAGGCTGGGAGTGCTGC	*Xba*I	Generation of the pDM4Δ*phuR* construct
phuR mut_UP_RV	CGGAATTCGACCCCGTCGATCTGC	*Eco*RI	Generation of the pDM4Δ*phuR* construct
phuR mut_DOWN_FW	CGGAATTCGGCGCGCCGCAGGG	*Eco*RI	Generation of the pDM4Δ*phuR* construct
phuR mut_DOWN_RV	CCGCTCGAGAGGGACACAGGTGGATC	*Xho*I	Generation of the pDM4Δ*phuR* construct
feoB mut_UP_FW	CGGAATTCTCGGTCACCGGCCAG	*Eco*RI	Generation of the pEXΔ*feoB* construct
feoB mut_UP_RV	CGGGATCCACCACGTTGACCAGC	*Bam*HI	Generation of the pEXΔ*feoB* construct
feoB mut_DOWN_FW	CGGGATCCTGATGACCTGCCTG	*Bam*HI	Generation of the pEXΔ*feoB* construct
feoB mut_DOWN_RV	GGGAAGCTTCGGCGGACCCTACATC	*Hind*III	Generation of the pEXΔ*feoB* construct
tonB1 mut_UP_FW	GCTCTAGAACCGTCTGTCCGGACTG	*Xba*I	Generation of the pDM4Δ*tonB* construct
tonB1 mut_UP_RV	CGGAATTCGGGATCGCTGCCGTG	*Eco*RI	Generation of the pDM4Δ*tonB* construct
tonB1 mut_DOWN_FW	CGGAATTCTTCTTCAAGATCGAGAAGC	*Eco*RI	Generation of the pDM4Δ*tonB* construct
tonB1 mut_DOWN_RV	CCGCTCGAGCGTCGGCCTGGAGAG	*Xho*I	Generation of the pDM4Δ*tonB* construct
fpvR mut_UP_FW	GCTCTAGAAGGAACTGCGGCAGATG	*Xba*I	Generation of the pDM4Δ*fpvR* construct
fpvR mut_UP_RV	CGGAATTCGGCGTGCAGGCGCATG	*Eco*RI	Generation of the pDM4Δ*fpvR* construct
fpvR mut_DOWN_FW	CGGAATTCCGCAGCCTGGTCGACG	*Eco*RI	Generation of the pDM4Δ*fpvR* construct
fpvR mut_DOWN_RV	CCGCTCGAGGGTGTACTGGGCAC	*Xho*I	Generation of the pDM4Δ*fpvR* construct
pvdA compl FW	CCGGAATTCGATTTCGCTATTCGTGCTCG	*Eco*RI	Generation of the pUCP*pvdA* construct
pvdA compl RV	CCCAAGCTTCTGGCGGGGAAACGGGC	*Hind*III	Generation of the pUCP*pvdA* construct
pchD compl FW	CCGGAATTCGATGGTCATCAGGTTTTCCT	*Eco*RI	Generation of the pUCP*pchD* construct
pchD compl RV	CCCAAGCTTCGGCAGGCGTTCGCTCC	*Hind*III	Generation of the pUCP*pchD* construct
tonB1 compl FW	CCGGAATTCGGACGGCCAGTTCGCC	*Eco*RI	Generation of the pUCP*tonB1* construct
tonB1 compl RV	CCCAAGCTTCGCTCGAAGGCGCGGC	*Hind*III	Generation of the pUCP*tonB1* construct
phuR compl FW	CCGGAATTCGAAAGGCTGGGAGTGCTG	*Eco*RI	Generation of the pUCP*phuRhasR* construct
phuR compl RV	CGGGGTACCACCTGTGGCATGGAAAGC	*Kpn*I	Generation of the pUCP*phuRhasR* construct

hasR compl FW	CGGGGTACCGGCGGGAGTGACGCTGC	*Kpn*I	Generation of the pUCP*phuRhasR* construct
hasR compl RV	GAAGATCTCCTTCACTGGGCAAAACGG	*Bgl*II	Generation of the pUCP*phuRhasR* construct
mCherry_FW	TATAAGCTTTGGTGAGCAAGGGCGAGG	*Hind*III	Generation of the miniCTX P*pvdA'*-'mCherry construct
mCherry_RV	TATGTCGACTCACTTGTACAGCTCGTCCATG	*Sal*I	Generation of the miniCTX P*pvdA'*-'mCherry construct
P*pvdA*_FW	CGGAATTCCTTCCTGTTGCGCAAGC	*Eco*RI	Generation of the miniCTX P*pvdA*'-'mCherry construct
P*pvdA*_RV	CCCAAGCTTCCTGAGTCATTTCCAGTTCC	*Hind*III	Generation of the miniCTX P*pvdA*'-'mCherry construct

[#] Unless otherwise stated, PCRs were performed using the genomic DNA of *P. aeruginosa* PAO1 as the template.
*Restriction sites are underlined.

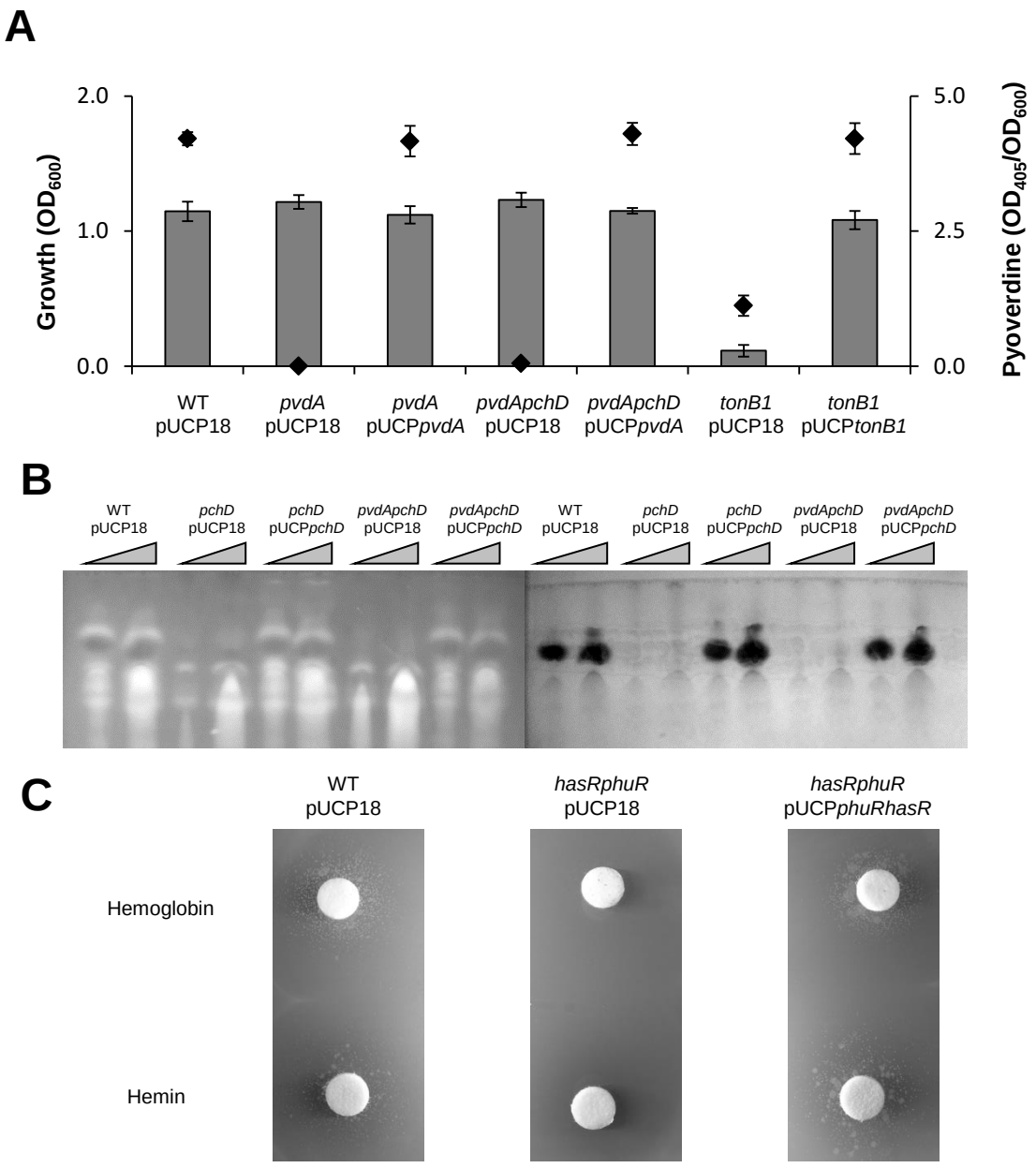

Figure S1. Genetic complementation of different *P. aeruginosa* iron-uptake mutants. (A) Growth (bars, left y axis) and pyoverdine production (diamonds, right y axis) of the wild type (WT) strain and of *pvdA*, *pvdApchD* and *tonB1* mutants carrying plasmids pUCP18 (empty vector), pUCP*pvdA* (containing a wild-type copy of *pvdA* with its own promoter region), or pUCP*tonB1* (containing a wild-type copy of *tonB1* with its own promoter region), as indicated. Growth was measured after 14 h in the iron-poor medium DCAA. (B) Pyochelin extracted from culture supernatants of WT, *pchD* and *pvdApchD* strains carrying plasmids pUCP18 or pUCP*pchD* (containing a wild-type copy of *pchD* with its own promoter region), after 36-h growth in GGP, and separated (2.5 or 5.0 µl of extract) by thin layer chromatography (TLC). Chromatograms are visualized by exposure to UV light (left panel) and by spraying with 100 µM $FeCl_3$ (right panel). (C) Growth of WT and *hasRphuR* strains carrying plasmids pUCP18 or pUCP*phuRhasR* (containing a wild-type copy of both *phuR* and *hasR* genes with their own promoter regions), in the presence of hemoglobin or hemin on M9 agarose plates containing 0.2% glucose and 1 mM DIP. Pictures in (B) and (C) are representative of three independent experiments giving similar results.

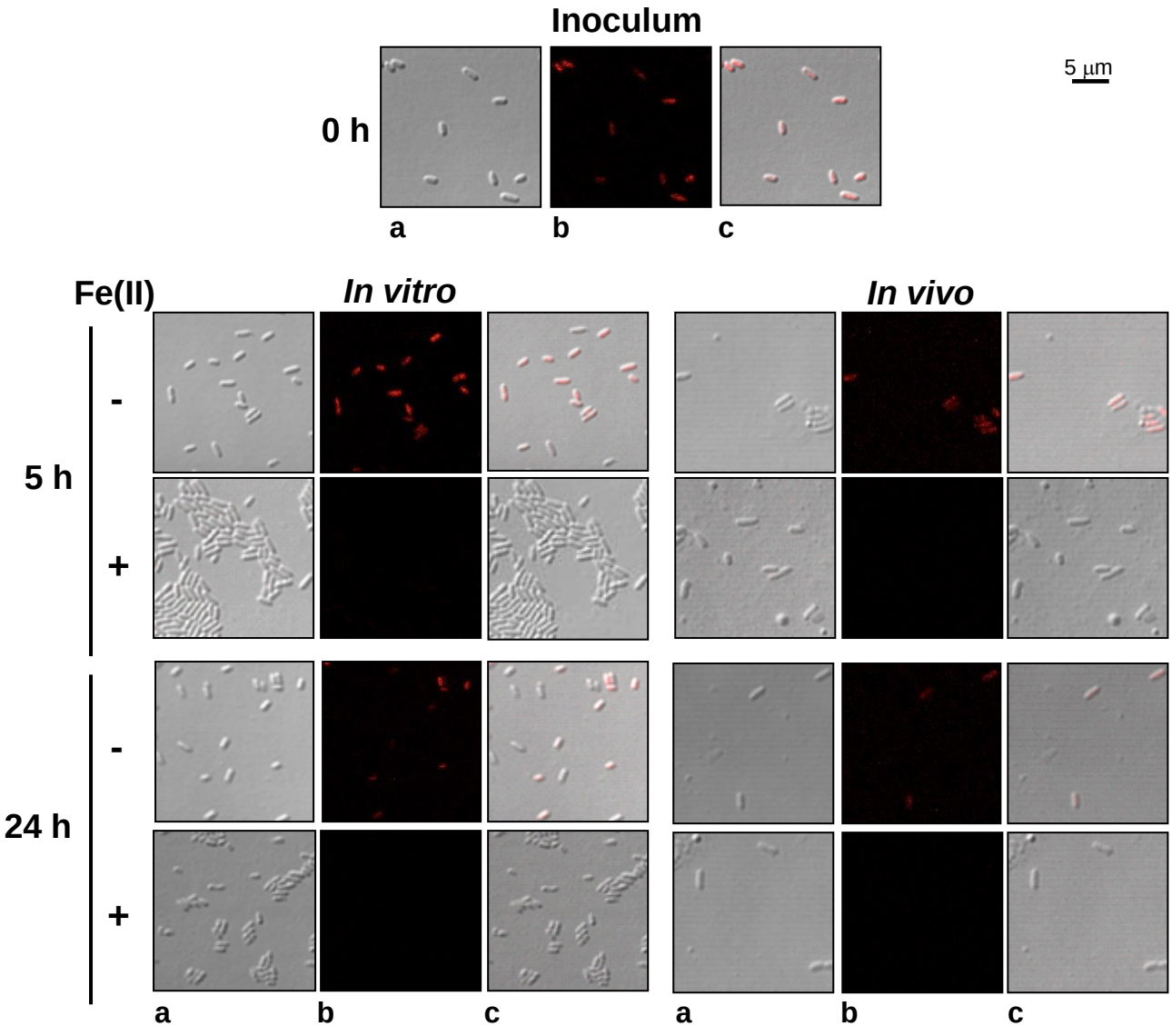

Figure S2. PAO1 wild type perceives the mouse lung as an iron poor environment. Confocal microscopy images showing the *in vitro* and *in vivo* fluorescence of the PAO1 strain carrying the P*pvdA*'-'mCherry fusion. Bacterial cultures were pre-grown in the iron-poor medium DCAA, observed by confocal microscopy (0 h) and diluted to obtain $6x10^7$ CFU/ml. For the *in vitro* experiment, bacteria were grown in DCAA supplemented or not with 10 µM $FeSO_4$ and samples were analysed after 5 h and 24 h. For the *in vivo* experiment, mice were inoculated with bacterial suspension in PBS supplemented or not with 10 µM $FeSO_4$. After 5 h and 24 h from the infection, bronco-alveolar lavages (BAL) were performed and analysed by confocal microscopy. Panels show pictures taken under visible light at differential interference contrast (DIC) (a), the fluorescence emission upon excitation at 585 nm (b), and the overlay of visible and fluorescence images (c). Representative images are shown. A Leica SP5 Confocal Laser Scanning Microscope equipped with a 63X oil immersion objective was used.