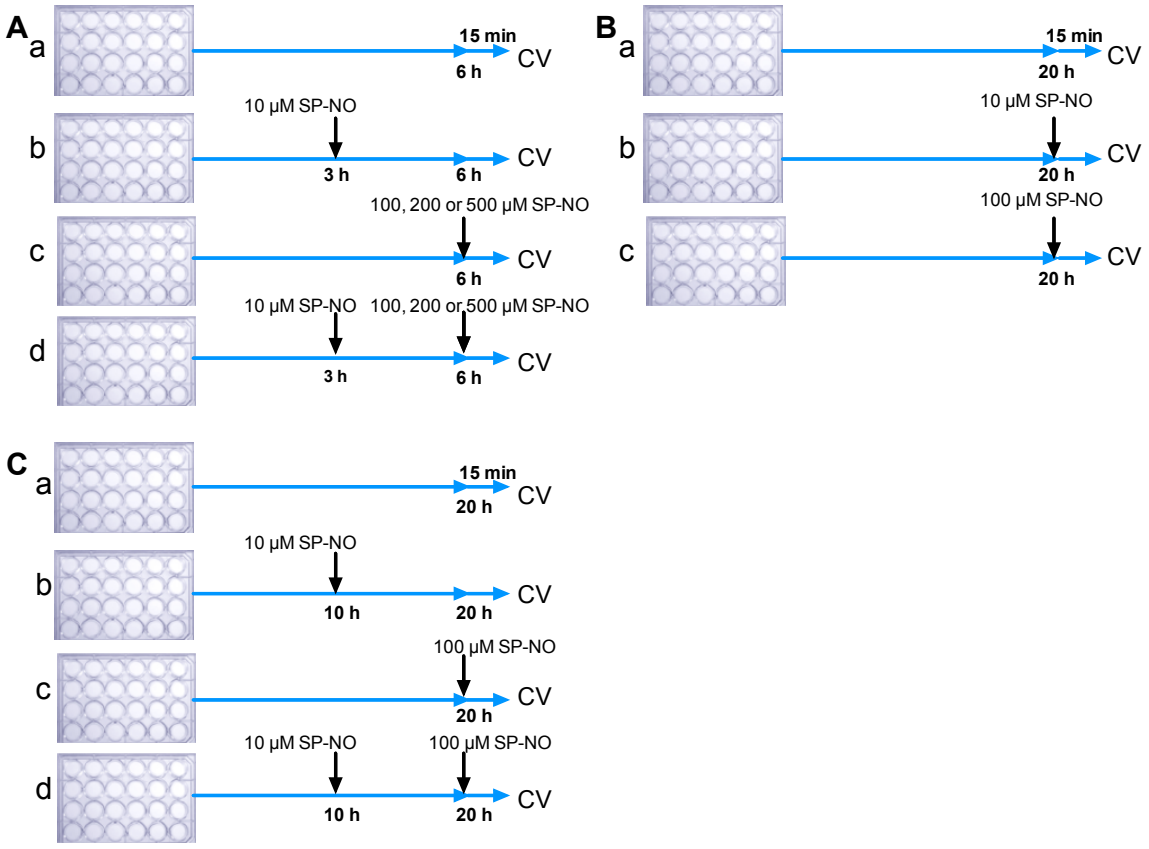


FIG S1. Schematics of experiments shown in Fig. 1 (A), Fig. 6A (B) and Fig. 6B (C). The arrows indicate the time (after inoculation) and concentration of NO addition for different experiments. For all NO treatments at 6 or 20 h, biofilms were incubated for 15 min before being assessed for biofilm biomass by crystal violet (CV) staining.

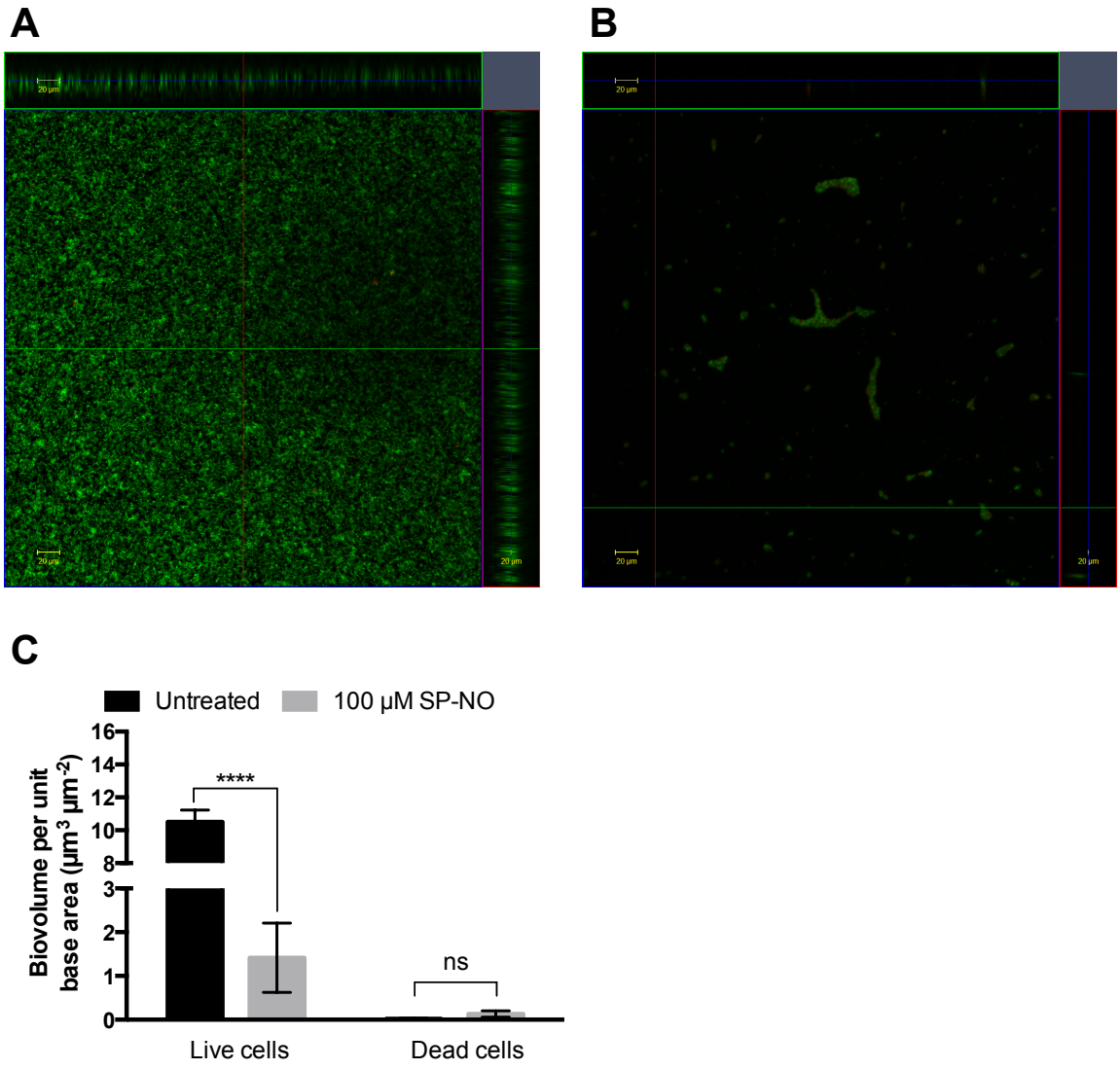


FIG S2. Treatment with 100 µM SP-NO disperses biofilms and does not impact viability of biofilm cells. *P. aeruginosa* biofilms grown in multiwell plate batch cultures for 6 h and subsequently left untreated (A) or treated with 100 µM SP-NO (B) for 15 min were stained with the LIVE/ DEAD BacLight Bacterial Viability Kit where live cells appear green and dead cells stain red. Images of untreated (A) and NO-treated (B) biofilms were acquired by CLSM. The main central images show horizontal optical sections (x-y) of the biofilms, and the side and top panels show vertical optical sections (x-z and y-z, respectively). Scale bars are 20 µm. Biofilm quantification (C) was performed

using IMARIS. Error bars indicate standard deviation (n = 6). Asterisks indicate statistically significant differences compared to untreated control samples (****, $P < 0.0001$). ns = No significant difference.

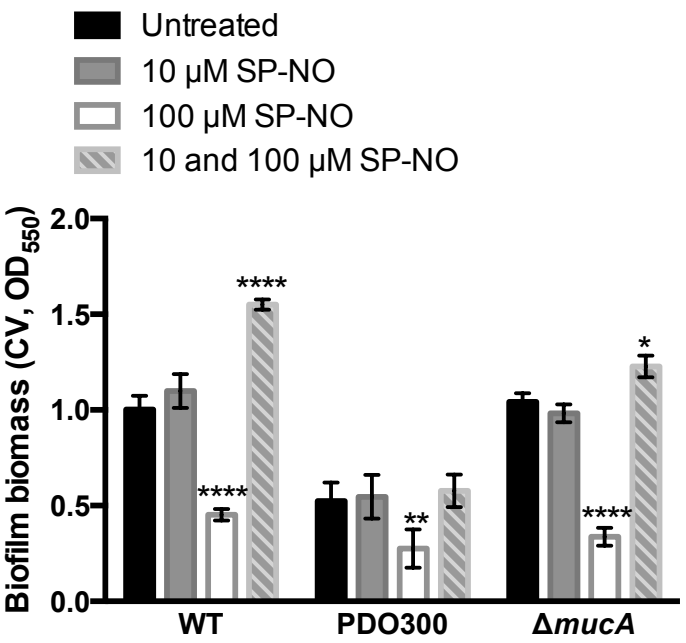


FIG S3. Biofilms of alginate-overproducing *P. aeruginosa* strains were also impaired in their dispersal response after a pre-treatment with NO. Biofilms of *P. aeruginosa* wild-type (WT), PDO300 and Δ*mucA* were grown in M9 minimal medium supplemented with 0.4% glucose and 2% (w/v) casamino acid in multiwell plate batch cultures for a total of 6 h 15, including or not a pre-treatment with 10 µM SP-NO at t = 3 h (thus 3 h 15 exposure time), and a dispersal treatment with 100 µM SP-NO at t = 6 h (thus 15 min exposure time). At the end of the incubation, biofilm biomass was analyzed by CV staining. Error bars indicate standard deviation (n = 3). Asterisks indicate statistically significant differences compared to untreated control samples (*, $P < 0.1$; **, $P < 0.01$; ****, $P < 0.0001$).

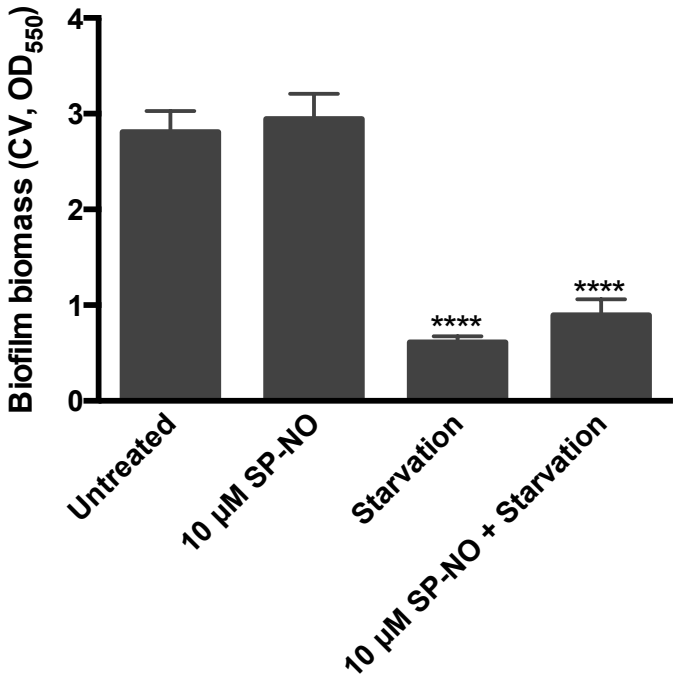


FIG S4. NO-pre-treated biofilms are dispersed by starvation. *P. aeruginosa* biofilms were grown in multiwell plate batch cultures for a total of 6 h 30 including or not a pre-treatment with 10 µM SP-NO at t = 3 h and a dispersal treatment with fresh M9 medium in the presence (non-starvation) or absence (starvation) of 0.4% glucose at t = 6 h for 30 min. Error bars indicate standard deviation (n = 3). At the end of the incubation, biofilm biomass was analyzed by CV staining. Asterisks indicate statistically significant differences compared to untreated samples (****, $P < 0.0001$).

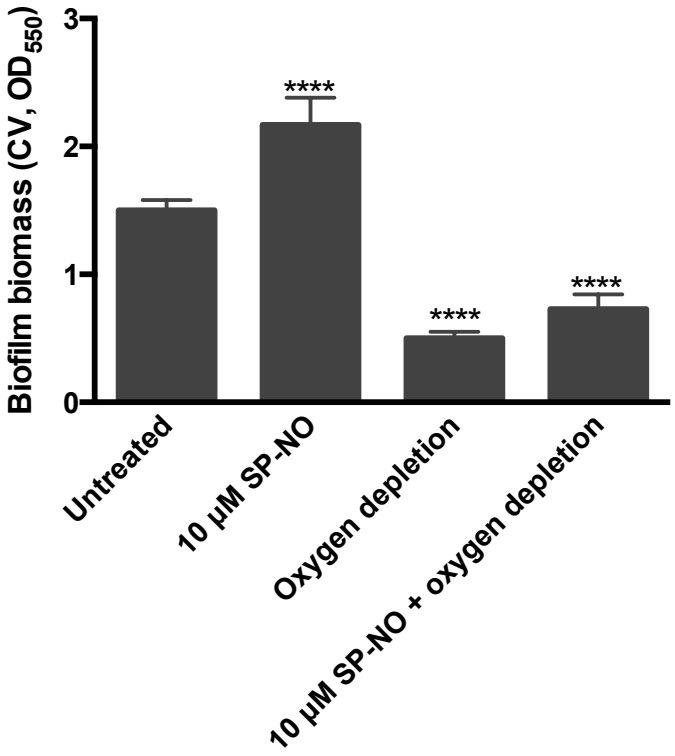


FIG S5. NO-pre-treated biofilms are dispersed by oxygen depletion. *P. aeruginosa* biofilms were grown in multiwell plate batch cultures for a total of 6 h 30 including or not a pre-treatment with 10 µM SP-NO at t = 3 h and a dispersal treatment with oxygen depletion at t = 6 h for 30 min. After 6 h of incubation with fast agitation at 180 rpm, the shaking speed was maintained at 180 rpm or reduced to 60 rpm to reduce oxygen tension and the biofilms were incubated for a further 30 min before CV staining. Error bars indicate standard deviation (n = 3). Asterisks indicate statistically significant differences compared to untreated samples (****, $P < 0.0001$).

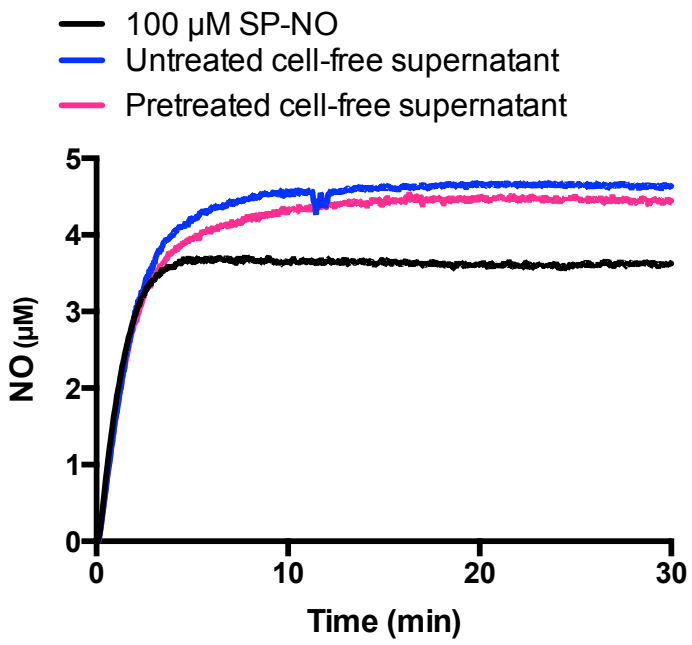


FIG S6. NO-pre-treated *P. aeruginosa* cell-free supernatant does not scavenge NO. NO levels in solution liberated from 100 µM SP-NO were measured amperometrically, in the absence or presence of *P. aeruginosa* cell-free supernatant. The cell-free supernatant was grown in multiwell plates for 3 h and exposed to 10 µM SP-NO, incubated for a further 3 h (6 h total) before collection.

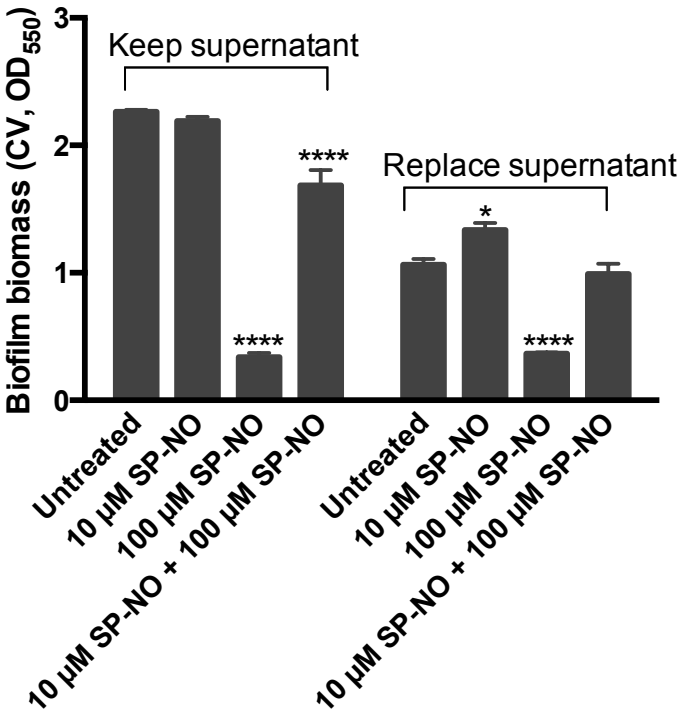


FIG S7. Replacing the supernatant does not impact on NO pre-treatment-induced impaired dispersal. Biofilms were grown in multiwell plate batch cultures for 6 h. After 6 h, the biofilm culture supernatants were either replaced or not with fresh M9 medium in the presence or absence of 100 µM SP-NO and the plates were incubated for a further 15 min, followed by assessment of total biofilm biomass by CV staining. For the pretreatment experiments, biofilms were grown for 3 h, exposed to 10 µM SP-NO, incubated for a further 3 h incubation (6 h total) before replacing the supernatant and being exposed to 100 µM SP-NO. Error bars indicate standard deviation (n = 3). Asterisks indicate statistically significant differences compared to untreated samples (*, $P < 0.1$; ****, $P < 0.0001$).

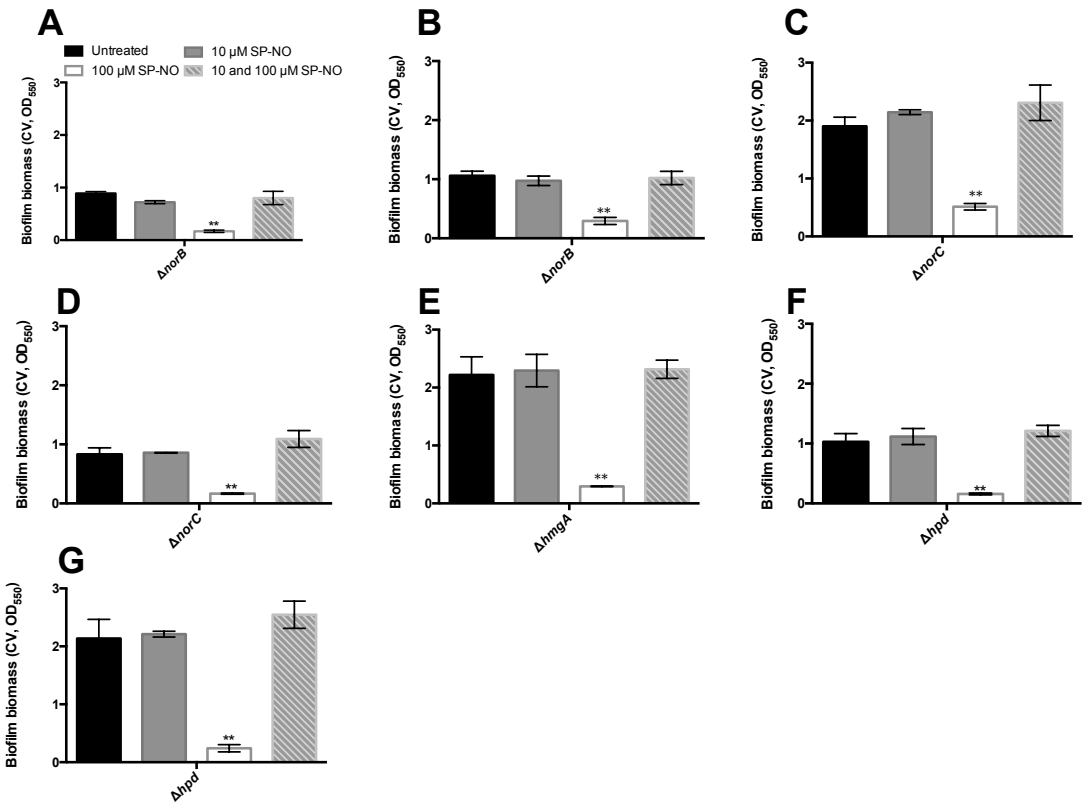


FIG S8. Biofilms of transposon mutant strains Δ*norB* PW1962 *norB*-A04::IS*phoA*/hah (A) and PW1961 *norB*-A11::IS*phoA*/hah (B), Δ*norC* PW1959 *norC*-B02::IS*lacZ*/hah (C), and PW1960 *norC*-H04::IS*phoA*/hah (D), Δ*hmgA* PW4489 *hmgA*-C03::IS*phoA*/hah (E), Δ*hpd* PW2577 *hpd*-H01::IS*lacZ*/hah (F), and PW2578 *hpd*-H02::IS*lacZ*/hah (G) were grown in multiwell plate batch cultures for a total of 6 h 15, including or not a pre-treatment with 10 µM SP-NO at t = 3 h (thus 3 h 15 exposure time), and a dispersal treatment with 100 µM SP-NO at t = 6 h (thus 15 min exposure time). At the end of the incubation, biofilm biomass was analyzed by CV staining. Error bars indicate standard deviation (n = 3). Asterisks indicate statistically significant differences compared to untreated samples (**, $P < 0.01$).

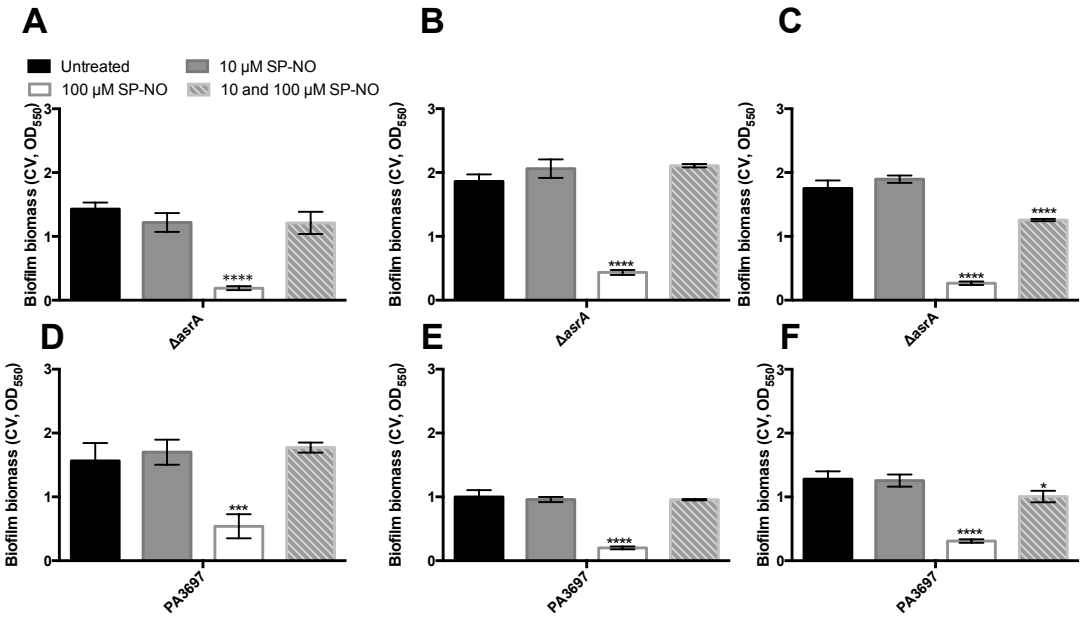
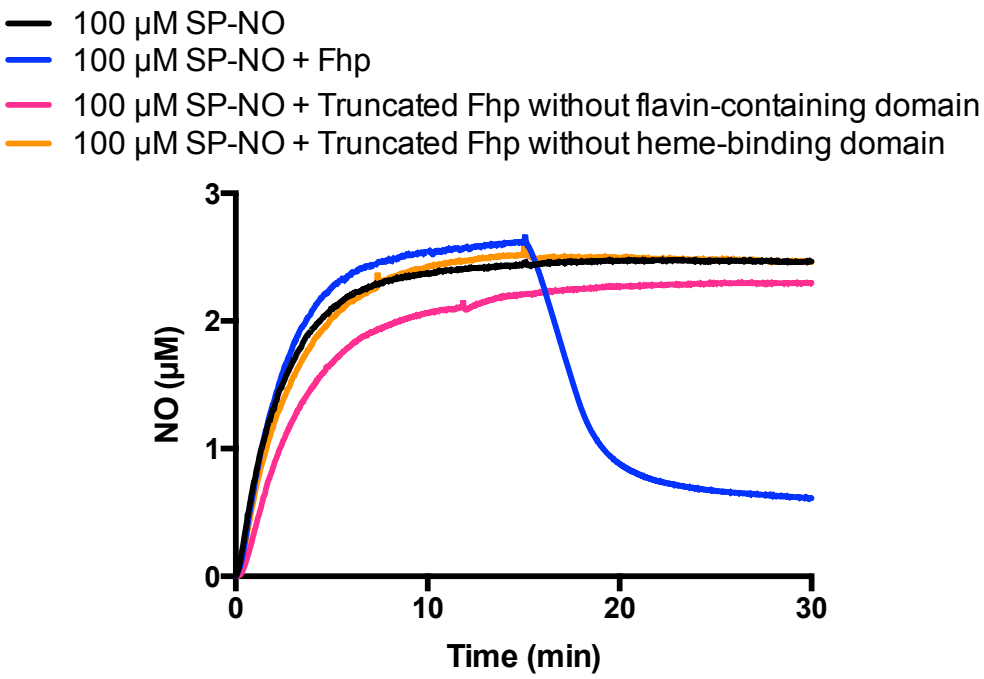


FIG S9. Biofilms of transposon mutants Δ*asrA* PW2411 *asrA*-A07::IS*lacZ*/hah (A), PW2413 *asrA*-G11::IS*lacZ*/hah (B), and PW2414 *asrA*-C03::IS*lacZ*/hah (C), ΔPA3697 PW7256 PA3697-H04::IS*lacZ*/hah (D), PW7257 PA3697-C03::IS*lacZ*/hah (E) and PW7258 PA3697-D09::IS*phoA*/hah (F) were grown in multiwell plate batch cultures for a total of 6 h 15, including or not a pre-treatment with 10 µM SP-NO at t = 3 h (thus 3 h 15 exposure time), and a dispersal treatment with 100 µM SP-NO at t = 6 h (thus 15 min exposure time). At the end of the incubation, biofilm biomass was analyzed by CV staining. Error bars indicate standard deviation (n = 3). Asterisks indicate statistically significant differences compared to untreated samples (*, $P < 0.1$; ***, $P < 0.001$; ****, $P < 0.0001$).



88

89 **FIG S10. NOD activity of purified Fhp and truncated Fhp.** NO released from 100 µM

90 SP-NO was measured amperometrically in 100 mM sodium phosphate buffer, pH 7.0,

91 containing 0.3 mM EDTA, 100 µM NADPH, and 1 µM FAD at room temperature. Fhp

92 and truncated Fhp were added 15 min after adding SP-NO.

93 **Table S1. Primers used in this work**

Primer name	Sequence (5' – 3')
*fhp*_for	ATTTCGACCTCTTTGCAGTC
*fhp*_rev	CTCCTTTTTCCGAAAAAGGG
*fhp*_RT_for	AATTACCTGCATGACCGGGTC
*fhp*_RT_rev	CGGAAACAGATCCAGCTCGT
*rpoD*_RT_for	CCTGCGCCTGGTGATTT
*rpoD*_RT_rev	GTGGCGTAGGTGGAGAACTT
Fhp-f7852	TACTTCCAATCCATGTTGTCCAATGCCCAACGTGC
Fhp-r7876	TATCCACCTTTACTGTCAGGCGTCCAGCGCGGCGG
Fhp-r7880	TATCCACCTTTACTGTCAATACACCGACTCCTCGGCC
Fhp-f7854	TACTTCCAATCCATGGATGGCGGCTGGCGCGG
Fhp-r7877	TATCCACCTTTACTGTCACGGACCGAAGAACTCGTAG

94