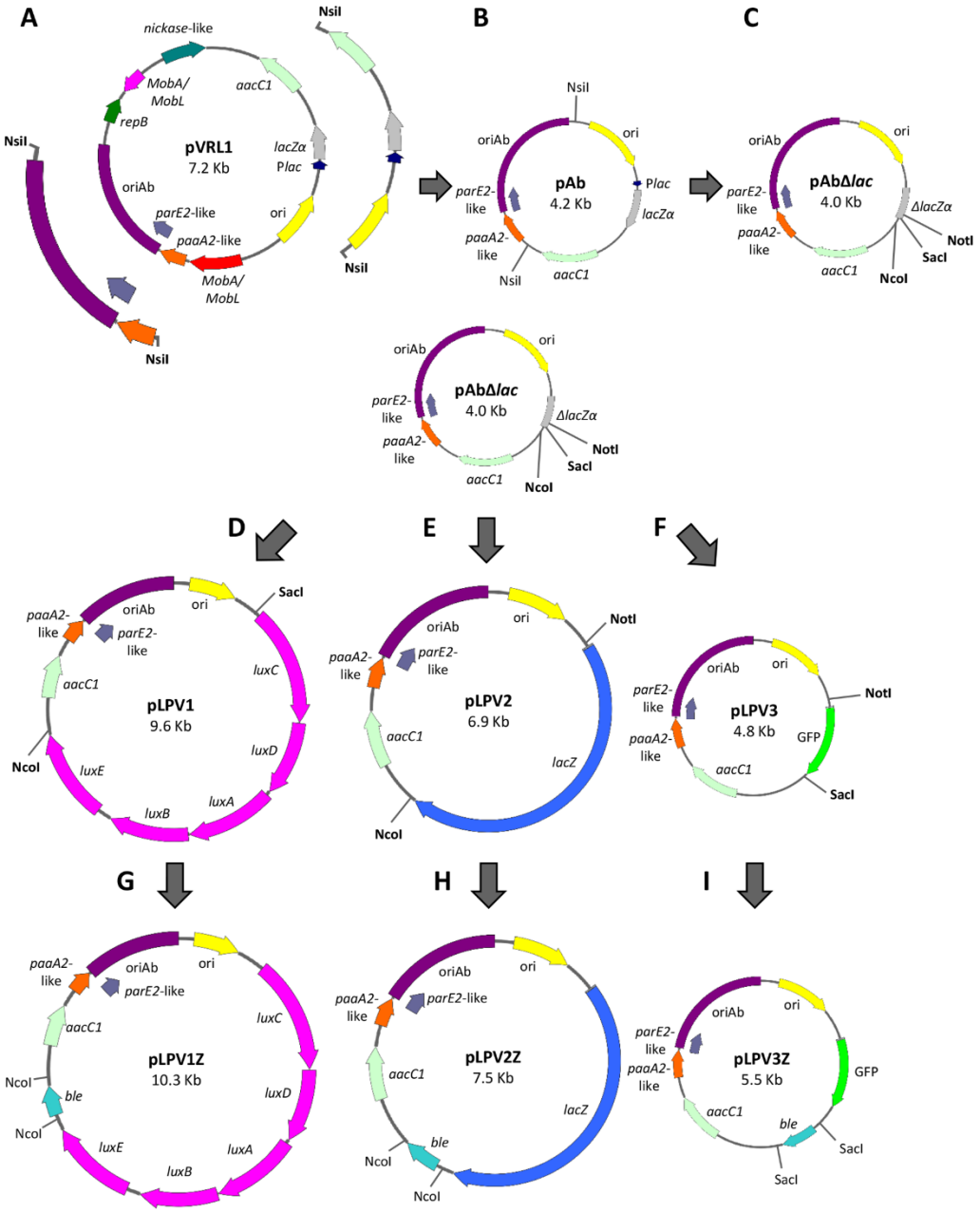

FIG S1 Schematic representation of cloning steps in pLPV1Z, pLPV2Z, and pLPV3Z construction. (A) pVRL1 (1) was employed as template to amplify: *i*) the 1,634 bp-region containing the oriAb and the TA module, and *ii*) the 2,643 bp-region encompassing the ColE1-like origin of replication for *E. coli* (ori), the *aacC1* gene responsible for Gm resistance, and the MCS within *lacZα* gene. Amplicons with artificially-introduced restriction sites (Table S1) are shown as external arcs of circle. Both PCR products were digested with NsiI and ligated to obtain the pAb plasmid (B). The pAb region encompassing the *lac* promoter (P*lac*), CAP binding site and the first 24 codons of the *lacZα* ORF was deleted to obtain pAbΔ*lac* (C). Three different reporter systems were cloned in pAbΔlac : *i*) the *luxCDABE* operon amplified from miniCTX1-lux vector, cloned into SacI/NcoI unique restriction sites to generate pLPV1 (D); *ii*) the *lacZ* gene amplified from pVRL2*lacZ*, cloned into NotI/NcoI unique restriction sites to generate pLPV2 (E); *iii*) gene coding for GFP amplified from pVRL2*gfp*, cloned into NotI/SacI unique restriction sites to generate pLPV3 (F). Finally the *ble* gene encoding Zeo resistance was cloned into the NcoI (pLPV1 and pLPV2) or SacI (pLPV3) unique restriction sites to obtain pLPV1Z, pLPV2Z, and pLPV3Z promoter-probe vectors (G, H and I, respectively). Abbreviations: *paaA2*-like, antitoxin component of the TA system; *parE2*-like, the toxin component of the TA system. Unique cutter restrictions enzymes are in bold. All the genes are reported in scale over the total length of the vectors. Image obtained by the Snapgene software (from GSL Biotech; available at snapgene.com).

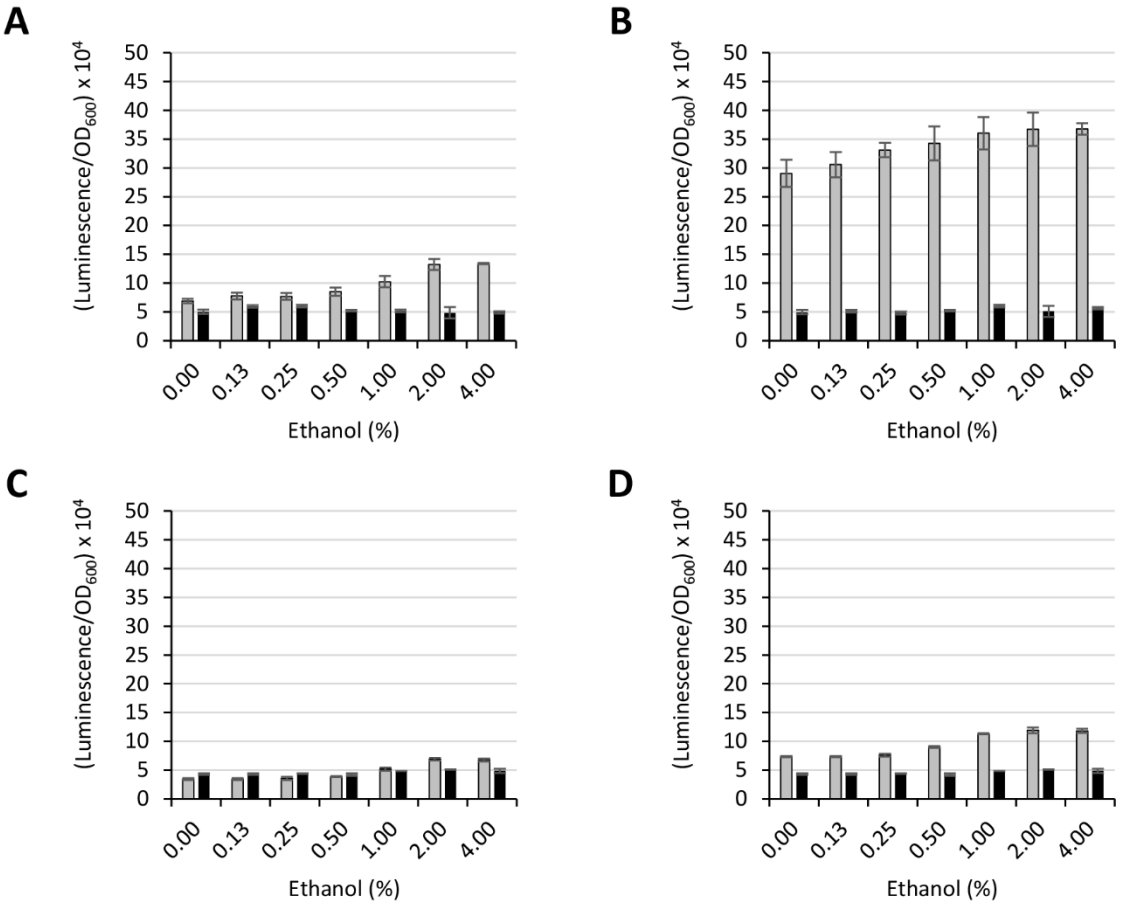

Fig S2 Regulation of the *A. baumannii* ethanol-inducible *adhP* and *yahK* genes. (A) *A. baumannii* ATCC17978(pLPV1Z::P*adhP*), (B) ATCC17978(pLPV1Z::P*yahK*), (C) AYE(pLPV1Z::P*adhP*) and (D) AYE(pLPV1Z::P*yahK*) were grown overnight in LB, then diluted at OD_{600}=0.1 in LB and challenged with different ethanol concentrations, as indicated. The luminescent emission was recorded 15 min post-induction. Grey and black histograms indicate pLPV1Z::P*adhP* (A,C) or pLPV1Z::P*yahK* (B,D) fusions and the promoterless pLPV1Z vector, respectively. Data are the means ± SDs from three independent experiments.

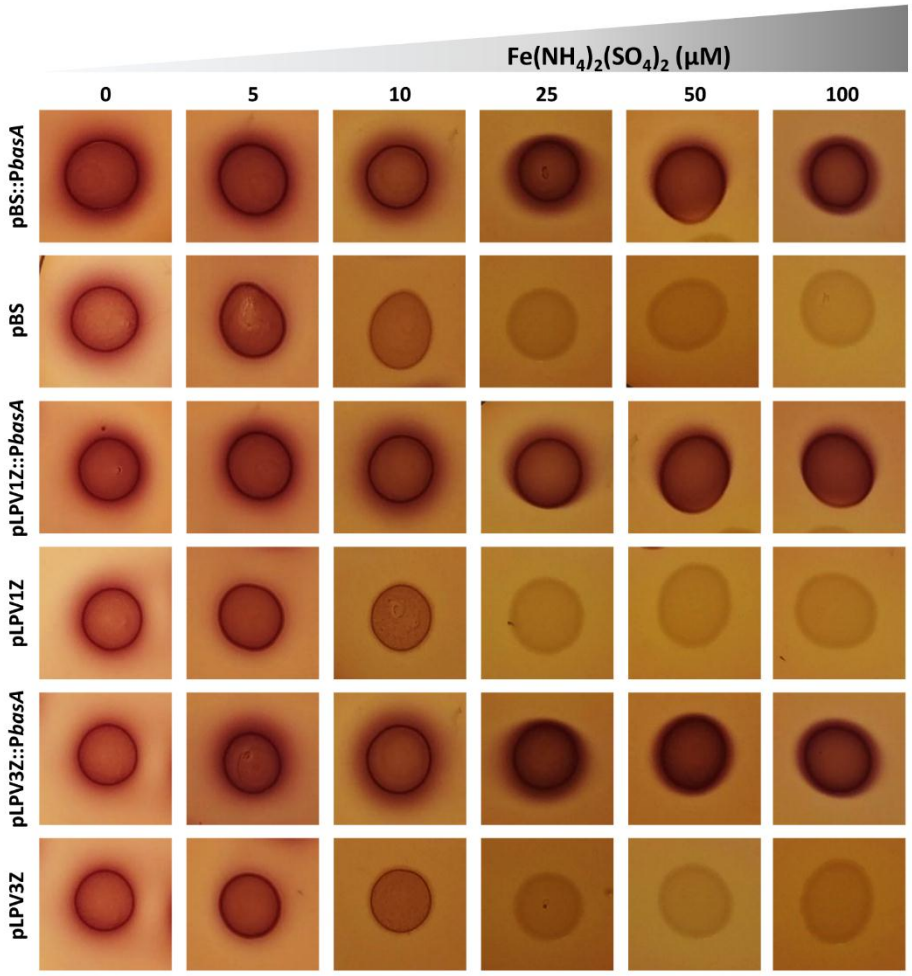

FIG S3 Fur titration assay (FURTA; 2) of P*basA* cloned in pLPV1Z and pLPV2Z. A suspension of *E. coli* H1717 carrying the indicated plasmids (10 µl, OD_{600}=1, corresponding to ≈10^6 cells), were grown on MacConkey agar supplemented with increasing $Fe(NH_4)_2(SO_4)_2$ concentrations for 24 hours. The presence of a Fur box in P*basA* is evidenced by lactose fermentation (red patches) in the presence of ≥10 µM $Fe(NH_4)_2(SO_4)_2$. Images are representative of three independent experiments giving similar results.

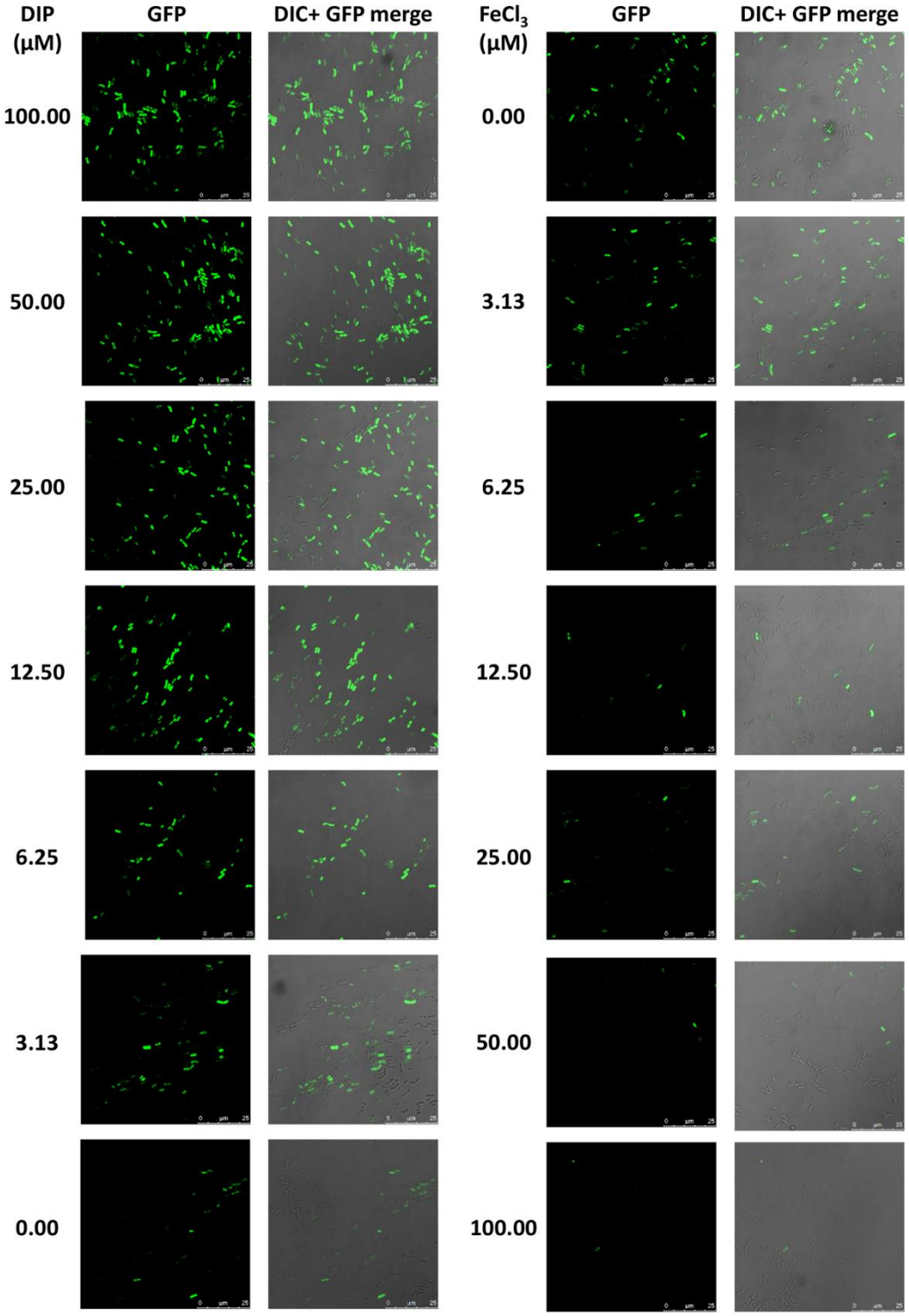

FIG S4 Confocal microscopy images of *A. baumannii* ATCC19606^T(pLPV3Z::P*basA*) and ATCC19606^T(pLPV3Z). Bacteria were grown for 18 h in LB, then washed and resuspended at OD_{600}=0.1 in M9-S supplemented with different concentrations of DIP or $FeCl_3$, as indicated. After 6-h incubation at 37°C, bacterial cultures were harvested and 15 µl were spotted on glass slide covered with 0.5% agarose. The GFP emitting-cells were visualized using Leica SP5 confocal laser scanning microscope equipped with a 63× oil immersion objective. Representative images of GFP and GFP + Differential Interference Contrast (DIC) merged channels are shown. Scale bar, 25 µm.

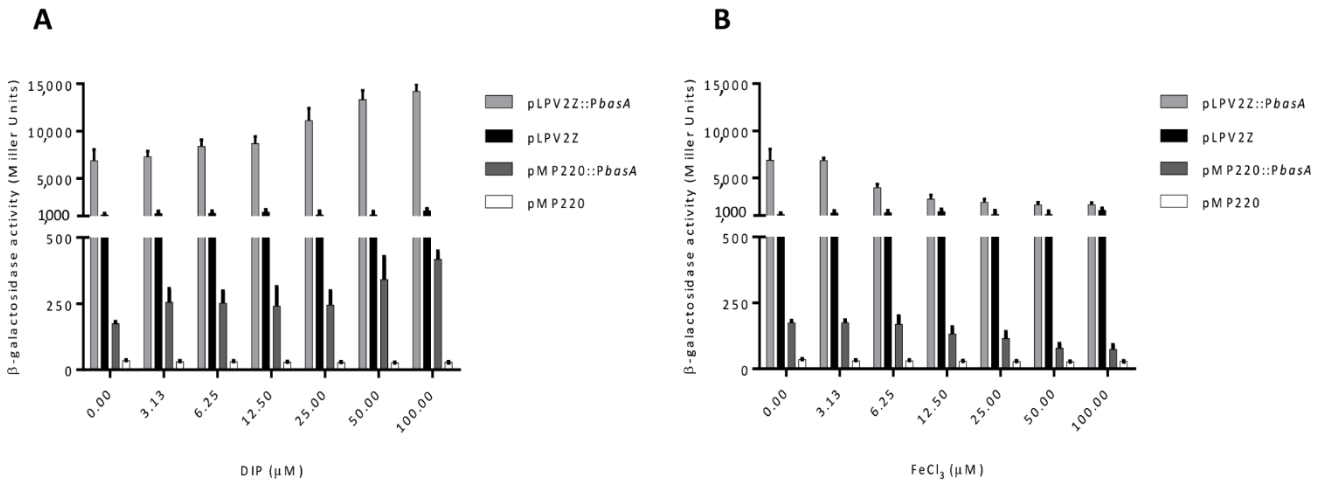

FIG S5 Regulation of *A. baumannii basA* gene cloned in the two different pLPV2Z and pMP220 (3) promoter-probe vectors. *A. baumannii* ATCC19606^T strains carrying pLPV2Z::P*basA* and pMP220::P*basA* vectors or the corresponding promoterless vectors were grown overnight in LB, washed with saline and inoculated (OD_{600}=0.1) in M9-S supplemented with either DIP (A) or $FeCl_3$ (B), and incubated for 6 h at 37°C before β-galactosidase activity determination. Data are the means ± SDs from three independent experiments.

Table S1. Extraction yields and copy number of pLPV plasmids

Strain(plasmid)	Plasmid yield (μg DNA/ml culture OD_{600} = 1)	PCN (plasmid copies/ chromosome)
E. coli DH5α(pLPV1Z)	1.65 ± 0.13	ND
E. coli DH5α(pLPV2Z)	1.49 ± 0.33	75 ± 10
E. coli DH5α(pLPV3Z)	1.26 ± 0.53	ND
E. coli DH5α(pVRL1)	1.04 ± 0.03	72 ± 8
A. baumannii ATCC19606^T(pLPV1Z)	0.26 ± 0.02	ND
A. baumannii ATCC19606^T(pLPV2Z)	0.44 ± 0.07	57 ± 12
A. baumannii ATCC19606^T(pLPV3Z)	0.31 ± 0.07	ND
A. baumannii ATCC19606^T(pVRL1)	0.45 ± 0.09	56 ± 5

ND, not determined.

References

1. Lucidi M, Runci F, Rampioni G, Frangipani E, Leoni L, Visca P. 2018. New shuttle vectors for gene cloning and expression in multidrug-resistant *Acinetobacter* species. Antimicrobial Agents and Chemotherapy 62:e02480-17. doi:10.1128/AAC.02480-17.

2. Stojiljkovic I, Bäumler AJ, Hantke K. 1994. Fur regulon in gram-negative bacteria. Identification and characterization of new iron-regulated *Escherichia coli* genes by a *fur* titration assay. J Mol Biol 236:531–545.

3. Spaink HP, Okker RJ, Wijffelman CA, Pees E, Lugtenberg BJ. 1987. Promoters in the nodulation region of the *Rhizobium leguminosarum* Sym plasmid pRL1JI. Plant Mol Biol 9:27–39.