

## Supplementary Materials

Bioengineering of bacterial pathogens for noninvasive imaging and *in vivo* evaluation of therapeutics

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**Supplementary Information Data 1. DNA sequences of Promoter – thymidine kinase fusion in *P. aeruginosa***

***P. aeruginosa* Promoter\_lasR (428bp, GenBank: M59425.1) + *E. coli* TK (GenBank: DQ384607.1) fusion for pUC18T-mini-Tn7T-Gm (Genbank: AY599232.2) cloning for *P. aeruginosa* chromosomal integration (BamHI and HindIII)**

**GGATCC**cgctgtgcctttgcgcgtgcgccgcgcacagacgtctgcgcctcggatcgcccggccgagagcggcgcacgcgtggc  
gatggccgacagtgaacccggggaccaggtgtgactgggtattcagttcgataaaatgtgatctagatcacattaaacgtttgcttaccct  
ctaggacgggtatcgtactaggtgcatcaaacgctgcggtctattgtaagtgggactgaaatgtgcctttccggcacaacccaactctata  
gagtgggctgactggacatctcaggggctgcgggcacgggcgcatgcgcctcggcaggaagccgggattctcggactgccgtacaac  
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ggcagaaattgatgatcgctttggtgccgggaaagtgcggtatagtttgcacgcctgcaaaattattaacaaaattcatcattatt  
gatgagattcgtgcggaacatgaacagcaggcaattcattgcgactggttgatgaatgccagttttaaccagacaacaagtatatgaattat  
cggaggtgtcgaactcgaatacccgtactttgttatggtttacgtaccgattttcaggtgaattattttatggcagccaataactactggc  
atggtccgacaaactggtgaattaaaacctctgtttttgtggccgtaaagcaagcatggtgctgcgcttgcgactcaagcaggcagacctat  
aacgaaggtgagcaggtggttaattggtggtaatgaacgatacgtttctgtatgccgtaaacactataaagaggcggttacaagtcgactcatta  
acggtattcaggaaaggcatgccacgattaa**AAGCTT**

**Sequentially cloned into pBBR1MCS5 following PCR with below primers and cloning with desired restriction sites.**

5'ProLasR w/KpnI  
GAAACGGTACCCGCTGTGCCTTTGCGCGTG

3' ProLasR w/ClaI  
GAAACATCGATAGCGCTACGTTCTTCTTAAACTATTAACCAATCAGCC

5'E.coli\_TK w/ClaI  
CAAACATCGATATGGCACAGCTATATTTCTACTATTCCGCAATGAATGC

3'E.coli\_TK w/XbaI  
CAAACTCTAGATTAATCGTGGCGATGCCTTTCCTGAATAGC

**Primers for PCR amplification and subcloning of PromoterLasR-TK into pUC18T-mini-Tn7T-Gm vector using pBBR1MCS5-PlasR-TK plasmid template**

5'ProLasR w/BamHI  
GAAACGGATCCCGCTGTGCCTTTGCGCGTG

3'E.coli\_TK w/HindIII  
CAAACAAGCTTTTAATCGTGGCGATGCCTTTCCTGAATAGC

**Supplementary Information Data 2. DNA sequences of Promoter – thymidine kinase fusion in *A. baumannii***

***A. baumannii* Promoter\_16srDNA (A1S\_r01) promoter (327bp, GenBank: CP000521.1) + pantoate--beta-alanine ligase (vitB5 pathway) Ribosome Binding Site and *E. coli* TK**

GGATCC**gattagattggttgcttaagtgatgaattgatgatgagatggttagagactaagtttaaaaatcgaattacctgttgactt  
taagagattaagagtaatatagccgacctagctgctggtgacgaaccagtaagaagatcattaagagaattgaagaacaacttgtgtggatt  
tttactgattgattaatcgaataatcttcattgattgattggttaaattactcgaagttatttgagcgaatttaagtcagtaattgatgagccaga  
attggcacctgtctataaataaggtgcaaaatgatttaactgacacactttccaagttgagctgtaattc**ATCGATatggcacagctatatt  
tctactattccgcaatgaatcgggtaagtctacagcattgttcaatcttcatacaattaccaggaacgcggcatgcgactgctgtatatac  
gcagaaattgatgatcgcttgggtccgggaaagtcagttcgcgtataggtttgcatcgcctgcaaaattatttaacaaaattcatcattattg  
atgagattcgtgcggaaatgaacagcaggcaattcattgcgtactggttgatgaatgccagttttaaccagacaacaagtatatgaattac  
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cgaaggtgagcaggtggaattggtggaatgaacgatacgtttctgtatgccgtaaacactataaagagcgttacaagtcgactcattaac  
ggctattcaggaaaggcatcgccacgattaa**AAGCTT**

**Primers for Prrn\_RBS-TK cloning into pUC18T-mini-Tn7T-Gm vector using pUC57-  
Prrn\_RBS-TK**

5'Pro\_16S\_AB w/BamHI

GAAACGGATCCGATTAGATTGGTTGCTTTAAGTGATGAATTTGATGATG

3'E.coli\_TK w/HindIII

CAAACAAAGCTTTTAATCGTGGCGATGCCTTTCCTGAATAGC

PTn7R (Chromosomal integration verification)

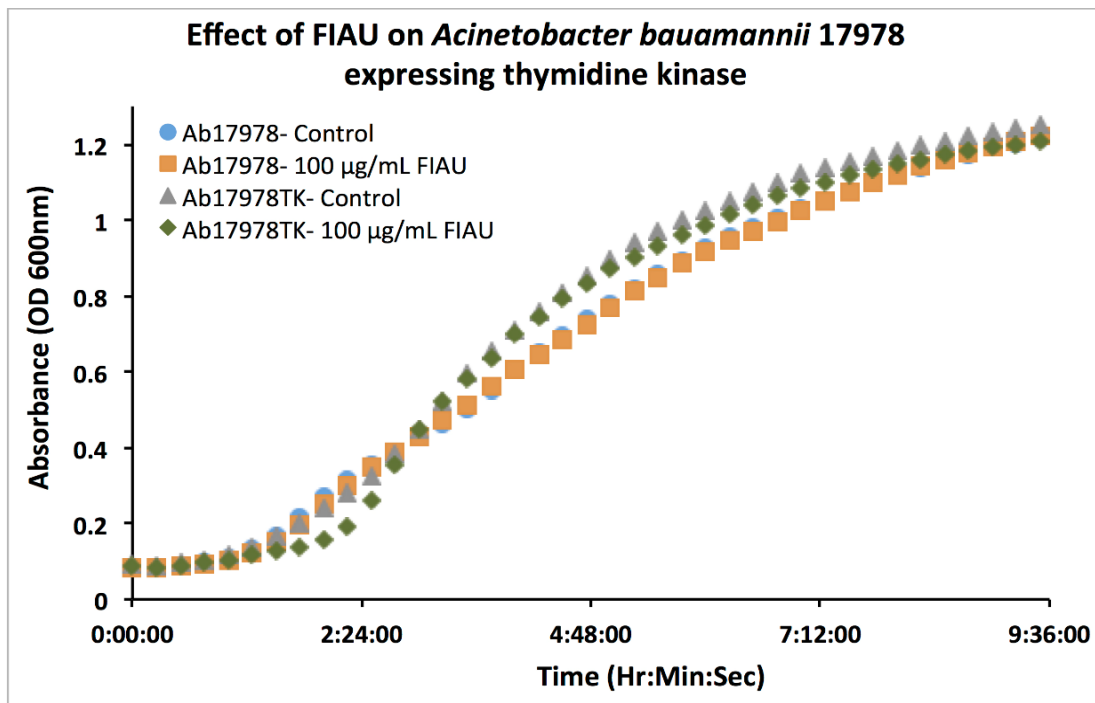
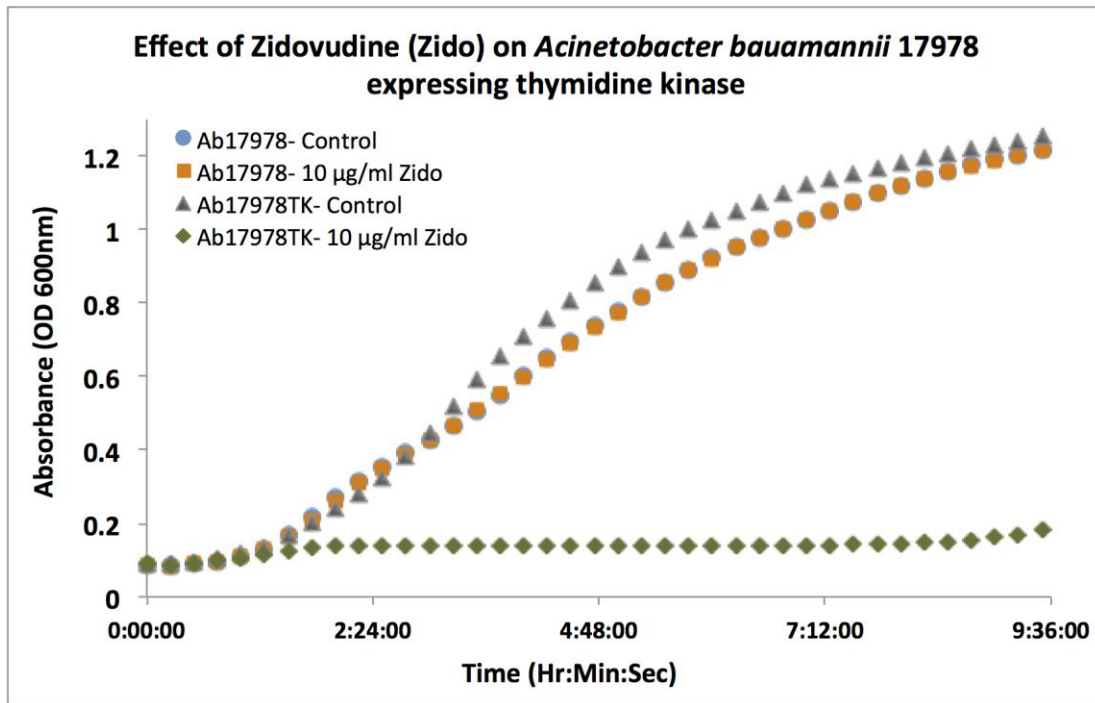
GTAAACTGAAATCAGTCCAGTTATGCTGTGA

PglmSF1 (Chromosomal integration verification)

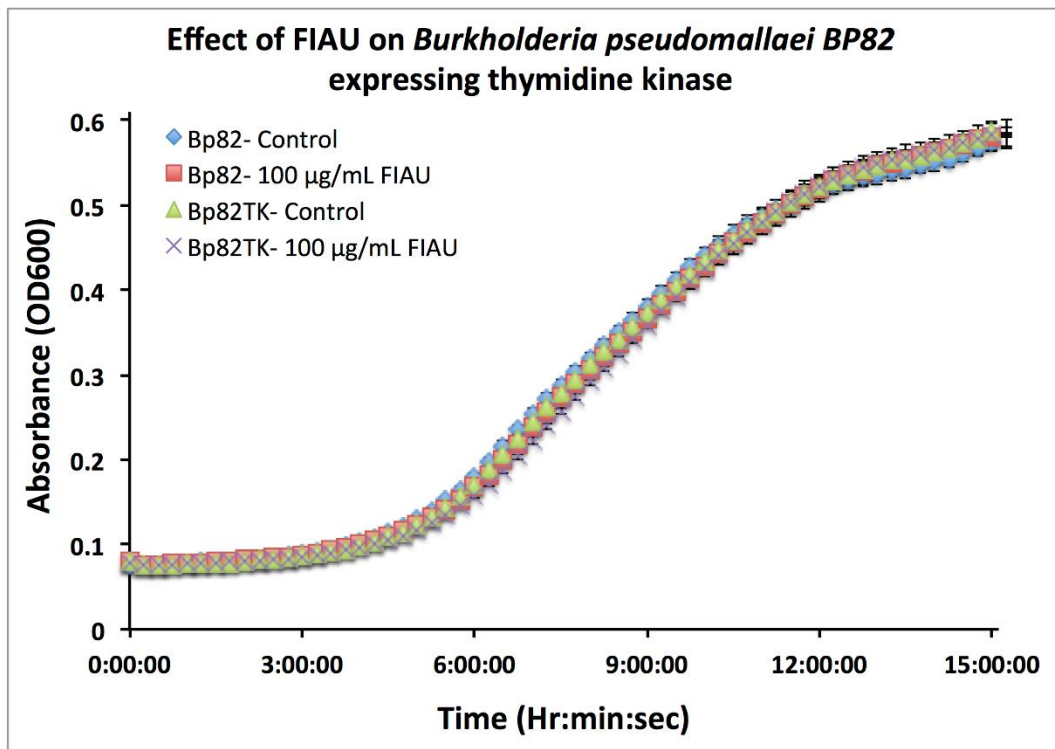
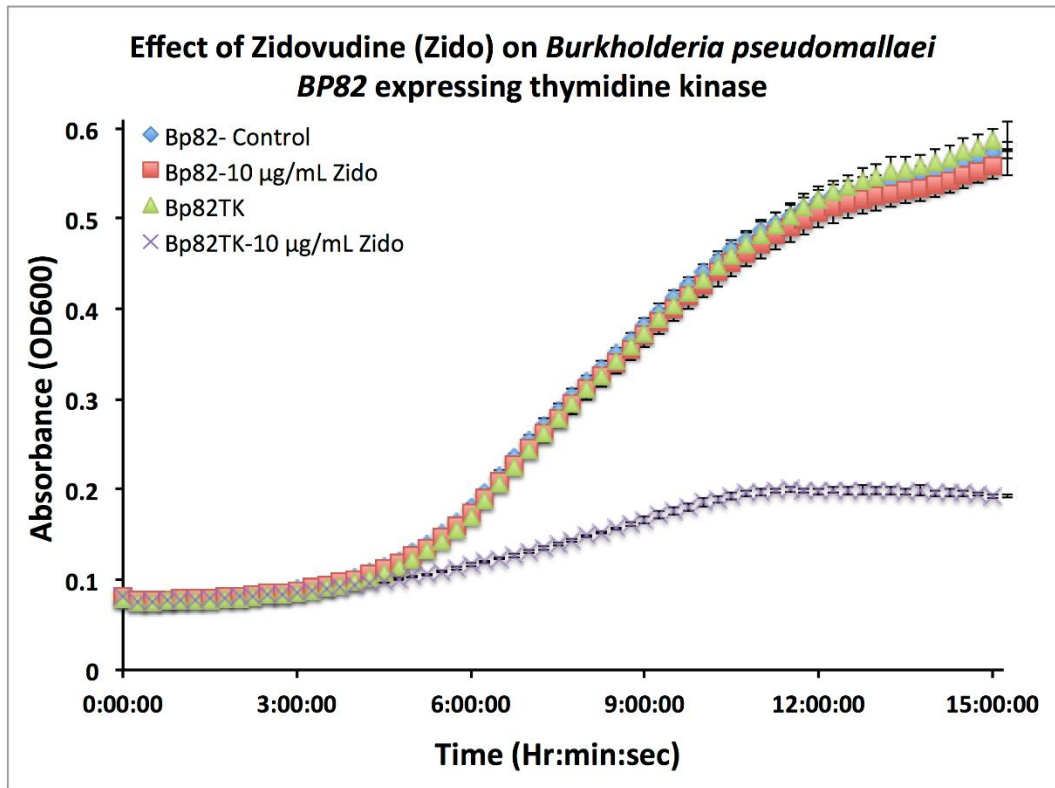
TTCGTTTTTGCTGATGAAAATAGCGGTG



Supplementary Information Figure 1. Growth inhibition studies with WT and *tk* engineered *Acinetobacter baumannii* ATCC 17978 in the presence of Zidovudine and FIAU



Supplementary Information Figure 2. Growth inhibition studies with WT and *tk* engineered *B. pseudomallei* Bp82 in the presence of Zidovudine and FIAU



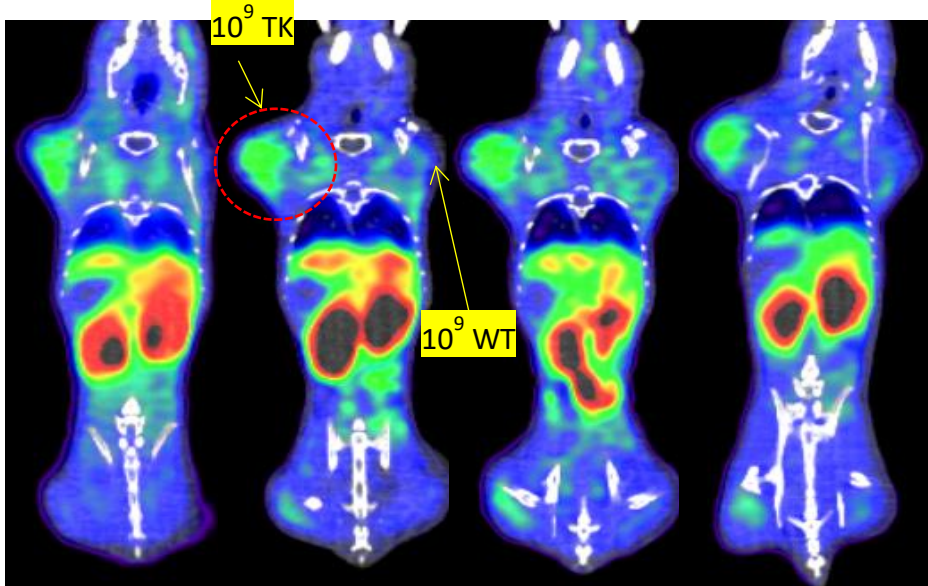
### **Supplementary Information Figure 3. Dynamic PET/CT scans with PAO1 and PAO1TK infected mice**

*In vivo* images of mice (m03, upper panel, and m04, lower panel) injected with  $10^9$  CFU PAO1TK (left upper and lower quadrants) and PAO1 (right upper and lower quadrants) and imaged at different time points after [ $^{18}\text{F}$ ]FIAU injection. Mouse m03 images are threshold adjusted to focus on the upper limbs injected with bacteria, while m04 is threshold adjusted to show lower limbs with bacteria. Table below shows the volume-of-interest (VOI) and signal intensities (UL-upper right, LL-lower right UR-upper right, LR- lower right, and BG-background). The signal intensities were used to tabulate the corresponding signal-to-noise ratios.

To quantify the PET data, a sphere equivalent to a 50  $\mu\text{l}$  volume was used so that the sample size was the same for each treatment. The 50  $\mu\text{l}$  sphere or VOI was applied to the images and the amount of radioactivity quantified. The background signal was defined as the amount of PET signal present in the VOI applied to the muscle in the non-infected limb, e.g., in mouse 03, background (BG) equals 4.61 %ID/g at 1 h10min. For the determination of the bacterial-tk signal, the 50  $\mu\text{l}$  VOI was applied to the limb containing the bacteria expressing thymidine kinase, e.g., mouse 03 signal in the upper right limb (UL) was 6.06 %ID/gm. The signal-to-noise was the ratio of the activity in the UL divided by BG or  $6.06/4.61$  which equals 1.32.

**In vivo images of mouse (m03) with  $10^9$  PAO1 (WT) and PAO1TK (TK)**

~1h10m    ~2h30m    ~3h50m    ~4h55m



~50 $\mu$ L VOIs used for analysis

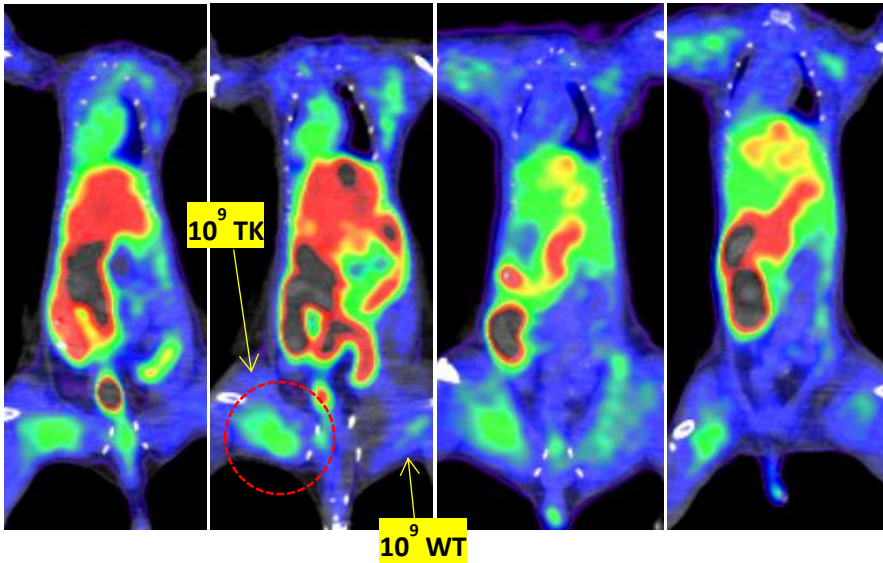
m03	Mean (nCi/cc)			Sig to Noise	
	UL	LL	BG	UL-BG	LL-BG
Scan @ ~1h10m	12219.90	11734.20	9287.46	1.32	1.26
~2h30m	7558.64	7490.90	5017.08	1.51	1.49
~3h50m	4569.47	4656.05	2934.45	1.56	1.59
~4h55m	3157.26	2922.62	2134.91	1.48	1.37

m03	Decay Corrected Mean (%ID/g)			Sig to Noise	
	UL	LL	BG	UL-BG	LL-BG
Scan @ ~1h10m	6.06	5.82	4.61	1.32	1.26
~2h30m	6.23	6.18	4.14	1.51	1.49
~3h50m	6.36	6.48	4.08	1.56	1.59
~4h55m	6.49	6.01	4.39	1.48	1.37



**In vivo images of mouse (m04) with  $10^9$  PAO1 (WT) and PAO1TK (TK)**

~1h10m    ~2h30m    ~3h50m    ~4h55m



~50 $\mu$ L VOIs used for analysis

m04	Mean (nCi/cc)			Sig to Noise	
	UL	LL	BG	UL-BG	LL-BG
~1h10m	11556.00	11203.40	7802.61	1.48	1.44
~2h30m	7567.50	7022.72	3951.73	1.91	1.78
~3h50m	4683.13	4512.75	2399.43	1.95	1.88
~4h55m	3064.61	3006.66	2024.68	1.51	1.49

m04	Decay Corrected Mean (%ID/g)			Sig to Noise	
	UL	LL	BG	UL-BG	LL-BG
~1h10m	5.72	5.55	3.86	1.48	1.44
~2h30m	6.23	5.78	3.25	1.91	1.78
~3h50m	6.50	6.26	3.33	1.95	1.88
~4h55m	6.29	6.17	4.15	1.51	1.49

**Note: Animals m03 and m04 both died just prior to scanning at ~4h55m**

**Supplementary Information Figure 4: Bacterial imaging in BALB/c after infection with  $10^7$  CFU PAO1 (WT) or PAO1TK (TK) detected with [ $^{18}\text{F}$ ]FIAU**

