

## Trimethylation of Elongation Factor-Tu by the Dual Thermoregulated Methyltransferase EftM Does Not Impact Its Canonical Function in Translation

Samantha M. Prezioso<sup>1,2</sup>, Duc M. Duong<sup>3</sup>, Emily G. Kuiper<sup>4,5</sup>, Qiudong Deng<sup>3</sup>, Sebastián Albertí<sup>6</sup>, Graeme L. Conn<sup>5,7</sup>, Joanna B. Goldberg<sup>2,7,8\*</sup>

\*Correspondence and requests for materials should be addressed to JBG (email: joanna.goldberg@emory.edu)

### **Affiliations**

<sup>1</sup>Microbiology and Molecular Genetics (MMG) Program, Graduate Division of Biological and Biomedical Sciences, Emory University, Atlanta, GA 30322, USA.

<sup>2</sup>Division of Pulmonology, Allergy/Immunology, Cystic Fibrosis and Sleep, Department of Pediatrics, Emory University School of Medicine, Atlanta, GA 30322, USA.

<sup>3</sup>Emory Integrated Proteomics Core, Emory University, Atlanta GA 30322

<sup>4</sup>Biochemistry, Cell and Developmental Biology (BCDB) Program, Graduate Division of Biological and Biomedical Sciences, Emory University, Atlanta, GA 30322, USA.

<sup>5</sup>Department of Biochemistry, Emory University School of Medicine, Atlanta, GA 30322, USA.

<sup>6</sup>Instituto Universitario de Investigación en Ciencias de la Salud, Universidad de las Islas Baleares, Palma de Mallorca, Spain.

<sup>7</sup>Emory Antibiotic Resistance Center, Atlanta, GA 30322, USA.

<sup>8</sup>Emory+Children's Center for Cystic Fibrosis and Airway Disease Research, Atlanta, GA 30322, USA.

## Supplemental Table 2

Significant Proteins from PA14					Corresponding Values from PAO1 Data Set 1				Corresponding Values from PAO1 Data Set 2				Corresponding Values from PAHM4				
		Fold change WT vs Δ	p-value WT vs Δ	Fold change Comp vs Δ	p-value Comp vs Δ	Fold change WT vs Δ	p-value WT vs Δ	Fold change Comp vs Δ	p-value Comp vs Δ	Fold change WT vs Δ	p-value WT vs Δ	Fold change Comp vs Δ	p-value Comp vs Δ	Fold change WT vs Δ	p-value WT vs Δ	Fold change Comp vs Δ	p-value Comp vs Δ
<b>PA14_24370</b>	*	1.3E+07	4.2E-10	1.1E+07	3.1E-11	N.D.	N/A	N.D.	N/A	-1.298	0.367	-1.094	0.870	-1.212	0.345	-1.288	0.190
<b>PA14_64050</b>	#	-1.3E+05	0.003	-1.283	0.019	1.066	0.985	1.037	0.991	-1.680	0.056	-1.467	0.151	N.D.	N/A	N.D.	N/A

## Supplemental Table 3

Significant Proteins from PAHM4					Corresponding Values from PAO1 Data Set 1				Corresponding Values from PAO1 Data Set 2				Corresponding Values from PA14				
		Fold change WT vs Δ	p-value WT vs Δ	Fold change Comp vs Δ	p-value Comp vs Δ	Fold change WT vs Δ	p-value WT vs Δ	Fold change Comp vs Δ	p-value Comp vs Δ	Fold change WT vs Δ	p-value WT vs Δ	Fold change Comp vs Δ	p-value Comp vs Δ	Fold change WT vs Δ	p-value WT vs Δ	Fold change Comp vs Δ	p-value Comp vs Δ
<b>PAHM4_RS15825</b>	N/A	-1.647	0.015	-1.668	0.014	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
<b>PAHM4_RS08095</b>	(PA5074)	-1.480	2.914E-04	-1.153	0.044	1.967	0.083	1.588	0.241	-1.164	0.343	-1.083	0.713	1.088	0.919	-1.072	0.949
<b>PAHM4_RS12930</b>	(PA3712)	1.457	0.012	1.476	0.010	1.179	0.642	1.066	0.932	-1.255	0.091	-1.085	0.645	1.242	0.497	-1.139	0.758
<b>PAHM4_RS25435</b>	(PA3106)	-1.394	0.030	-1.363	0.039	-1.191	0.705	-1.025	0.993	-1.106	0.430	1.077	0.609	-1.057	0.928	1.129	0.716
<b>PAHM4_RS17805</b>	(PA2912)	-1.348	4.795E-06	-1.085	0.007	N.D.	N/A	N.D.	N/A	N.D.	N/A	N.D.	N/A	N.D.	N/A	N.D.	N/A
<b>PAHM4_RS18780</b>	(PA1117)	-1.330	0.020	-1.336	0.018	1.344	0.640	1.651	0.395	-1.031	0.980	1.094	0.841	# -319.367	0.837	1.013	0.999
<b>PAHM4_RS16925</b>	(PA0354)	-1.242	0.001	-1.133	0.015	1.053	0.872	-1.250	0.152	1.000	1.000	1.010	0.979	-1.076	0.832	1.014	0.993
<b>PAHM4_RS10405</b>	(PA1880)	-1.235	0.021	-1.287	0.009	N.D.	N/A	N.D.	N/A	-1.070	0.926	-1.189	0.770	1.091	0.868	-1.097	0.851
<b>PAHM4_RS00050</b>	(PA0225)	-1.138	0.042	-1.137	0.044	N.D.	N/A	N.D.	N/A	-1.347	0.480	1.139	0.856	N.D.	N/A	N.D.	N/A
<b>PAHM4_RS05090</b>	(PA4329)	1.098	0.001	1.052	0.024	1.045	0.781	1.089	0.437	1.046	0.658	-1.058	0.535	1.142	0.461	-1.046	0.902

**Supplemental Table 2: Whole-cell proteomic analysis reveals that trimethylation of EF-Tu by EftM has little impact on the proteome.**

Proteins from PA14 significantly changing in  $\Delta$  (PA14 *eftM*::tn) compared to WT (PA14) and Comp (PA14 *eftM*::tn *attTn7*::P<sub>*eftM*</sub>-*eftM*-FLAG). The corresponding values from PAO1 and PAHM4 are listed to the right for comparison.

Bold; proteins detected in one data set only. Positive fold change; abundance is higher in strain with EftM. Negative fold change; abundance is higher in strain without EftM. ★ denotes that the protein was not detected in all three biological triplicates of  $\Delta$ ; ‡ denotes not detected in biological triplicates of WT. N.D; not detected in all three biological triplicates of all three strains (WT,  $\Delta$ , Comp) for that data set. N/A; not applicable.

**Supplemental Table 3: Whole-cell proteomic analysis reveals that trimethylation of EF-Tu by EftM has little impact on the proteome.**

Proteins from PAHM4 significantly changing in  $\Delta$  (PAHM4 $\Delta$ *eftM*) compared to WT (PAHM4) and Comp (PAHM4 $\Delta$ *eftM* *attTn7*::P<sub>*eftM-HM4*</sub>-*eftM*<sub>*HM4*</sub>-FLAG). The corresponding values from PAO1 and PA14 are listed to the right for comparison.

Bold; proteins detected in one data set only. Positive fold change; abundance is higher in strain with EftM. Negative fold change; abundance is higher in strain without EftM. ★ denotes that the protein was not detected in all three biological triplicates of  $\Delta$ ; ‡ denotes not detected in biological triplicates of WT. N.D; not detected in all three biological triplicates of all three strains (WT,  $\Delta$ , Comp) for that data set. N/A; not applicable.

**Supplemental Table 4. Strain List**

ID	Genotype	Reference
PAO1	<i>P. aeruginosa</i> PAO1	Barbier, Owings <i>et al</i> 2013
PAO1 $\Delta$ <i>eftM</i>	PAO1 <i>eftM</i> $\Delta$ 255-487	Barbier, Owings <i>et al</i> 2013
PASP09	PAO1 $\Delta$ <i>eftM</i> <i>attTn7</i> ::P <sub><i>eftM</i></sub> - <i>eftM</i> <sub>PAO1</sub> -FLAG	This Study
PASP106	PAO1 $\Delta$ <i>eftM</i> <i>attTn7</i> ::P <sub><i>eftM-10SCR</i></sub> - <i>eftM</i> <sub>PAO1</sub> -FLAG	This Study
PASP20	PAO1 $\Delta$ <i>eftM</i> <i>attTn7</i> ::P <sub><i>eftM</i></sub> - <i>eftM</i> <sub>HM4</sub> -FLAG	This Study
PASP76	PAO1 <i>attCTX</i> ::P <sub><i>eftM</i></sub> - <i>lacZ</i> <i>FRT</i>	This Study
PASP65	PAO1 <i>attCTX</i> ::P <sub><i>rpoD</i></sub> - <i>lacZ</i> <i>FRT</i>	This Study
PA14	<i>P. aeruginosa</i> PA14	Rahme, Stevens <i>et al.</i> 1995
PA14 <i>eftM</i> ::tn	PA14_08970::MAR2xT7	Liberati <i>et al.</i> 2006
PASP12	PA14 <i>eftM</i> ::tn <i>attTn7</i> ::P <sub><i>eftM</i></sub> - <i>eftM</i> <sub>PAO1</sub> -FLAG	This Study
PAHM4	<i>P. aeruginosa</i> PAHM4	Varga <i>et al.</i> 2015
PAHM4 $\Delta$ <i>eftM</i>	PAHM4 <i>eftM</i> $\Delta$ 255-487	This Study
PASP51	PAHM4 $\Delta$ <i>eftM</i> <i>attTn7</i> ::P <sub><i>eftM</i></sub> - <i>eftM</i> <sub>HM4</sub> -FLAG	This Study

**Supplemental Table 5. Oligonucleotides**

ID	Target	Sequence
SMP10	complementation of <i>eftM</i> (PAO1)	TGG ATC CCC AGA CCT TCC ACG GCA GTT G
SMP45	complementation of <i>eftM</i> (PAO1) with FLAG tag	TGA ATT CCT ACT TGT CAT CGT CAT CCT TGT AGT CGC GCT TCA CGC AGA C
SMP216	creation of scrambled -10 promoter	AAG GCC CGG GCC TGG GGA TTA CCG CCG CGC CCT TCG CCG CCG GTC C
SMP217	creation of scrambled -10 promoter	CAG GTG CTG CAG CTC GGC GAA
SMP17	complementation of <i>eftM</i> (PAHM4)	TGG ATC CAA CCA GAT CCT CCA CGG CAG TTG
SMP47	complementation of <i>eftM</i> (PAHM4) with FLAG tag	TGA ATT CCT ACT TGT CAT CGT CAT CCT TGT AGT CGC GCT TGA TGC AGG C
SMP189	P <sub><i>eftM</i></sub> - <i>lacZ</i> reporter	TGG ATC CCC CGG GCT GCA GGA GTC CGC CCG CGA TCC GCC GGC CGG T
SMP196	P <sub><i>eftM</i></sub> - <i>lacZ</i> reporter	TCA TGG TCA TGG GCG GCA AGG ACC GGC GGC GAA GGG CGC
SMP184	P <sub><i>eftM</i></sub> - <i>lacZ</i> reporter	TCC TTG CCG CCC ATG ACC ATG ATT ACG GAT TC
SMP179	P <sub><i>eftM</i></sub> - <i>lacZ</i> and P <sub><i>rpoD</i></sub> - <i>lacZ</i> reporter	CGA GGT CGA CGG TAT CGA TAA GCT TTA TTT TTG ACA CCA GAC C
SMP176	P <sub><i>rpoD</i></sub> - <i>lacZ</i> reporter	TGG ATC CCC CGG GCT GCA GGC CTT GAA AAG CAG TTC TTC GAC
SMP177	P <sub><i>rpoD</i></sub> - <i>lacZ</i> reporter	TCA TGG TCA TAA CAC CCT ATC CAC TGA AGG T
SMP178	P <sub><i>rpoD</i></sub> - <i>lacZ</i> reporter	GGA TAG GGT GTT ATG ACC ATG ATT ACG GAT TC
SMP155	<i>eftM</i> qPCR	GAC ATC AAC CAG CCG ATG CTC
SMP156	<i>eftM</i> qPCR	CAG CCC TGC GTT GTA GTG GA
<i>rpoD</i> F	<i>rpoD</i> qPCR	GGG CGA AGA AGG AAA TGG TC
<i>rpoD</i> R	<i>rpoD</i> qPCR	CAG GTG GCG TAG GTG GAG AA
oJV1040	<i>omlA</i> qPCR	AAA ATC GAC ATC CAG CAA GG
oJV1041	<i>omlA</i> qPCR	GGT CGC TGT CGT TGA AGA AC
SMP22	<i>eftM</i> 5' RACE GSP1	TGC GTT GTA GTG GAT CGA ATA G
SMP49	<i>eftM</i> 5' RACE GSP2	GAG CAT CGG CTG GTT GAT GTC