Supplementary Material

to

Identification of a Chemoreceptor in *Pseudomonas aeruginosa* that specifically mediates Chemotaxis towards α-Ketoglutarate

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Fig. S1) Composition of Biolog Screens used for the high-throughput ligand screening of McpK-LBD.
Screen PM 1.

A1 Negative Control	A2 L-Arabinose	A3 N-Acetyl-D-Glucosamine	A4 D-Saccharic Acid	A5 Succinic Acid	A6 D-Galactose	A7 L-Aspartic Acid	A8 L-Proline	A9 D-Alanine	A10 D-Trehalose	A11 D-Mannose	A12 Dulcitol
B1 D-Serine	B2 D-Sorbitol	B3 Glycerol	B4 L-Fucose	B5 D-Glucuronic Acid	B6 D-Gluconic Acid	B7 D,L-α-Glycerol-Phosphate	B8 D-Xylose	B9 L-Lactic Acid	B10 Formic Acid	B11 D-Mannitol	B12 L-Glutamic Acid
C1 D-Glucose-6-Phosphate	C2 D-Galactonic Acid-γ-Lactone	C3 D,L-Malic Acid	C4 D-Ribose	C5 Tween 20	C6 L-Rhamnose	C7 D-Fructose	C8 Acetic Acid	C9 α-D-Glucose	C10 Maltose	C11 D-Melibiose	C12 Thymidine
D-1 L-Asparagine	D2 D-Aspartic Acid	D3 D-Glucosaminic Acid	D4 1,2-Propanediol	D5 Tween 40	D6 α-Keto-Glutaric Acid	D7 α-Keto-Butyric Acid	D8 α-Methyl-D-Galactoside	D9 α-D-Lactose	D10 Lactulose	D11 Sucrose	D12 Uridine
E1 L-Glutamine	E2 m-Tartaric Acid	E3 D-Glucose-1-Phosphate	E4 D-Fructose-6-Phosphate	E5 Tween 80	E6 α-Hydroxy Glutaric Acid-γ-Lactone	E7 α-Hydroxy Butyric Acid	E8 β-Methyl-D-Glucoside	E9 Adonitol	E10 Maltotriose	E11 2-Deoxy Adenosine	E12 Adenosine
F1 Glycyl-L-Aspartic Acid	F2 Citric Acid	F3 m-Inositol	F4 D-Threonine	F5 Fumaric Acid	F6 Bromo Succinic Acid	F7 Propionic Acid	F8 Mucic Acid	F9 Glycolic Acid	F10 Glyoxylic Acid	F11 D-Cellobiose	F12 Inosine
G1 Glycyl-L-Glutamic Acid	G2 Tricarballylic Acid	G3 L-Serine	G4 L-Threonine	G5 L-Alanine	G6 L-Alanyl-Glycine	G7 Acetoacetic Acid	G8 N-Acetyl-β-D-Mannosamine	G9 Mono Methyl Succinate	G10 Methyl Pyruvate	G11 D-Malic Acid	G12 L-Malic Acid
H1 Glycyl-L-Proline	H2 p-Hydroxy Phenyl Acetic Acid	H3 m-Hydroxy Phenyl Acetic Acid	H4 Tyramine	H5 D-Psicose	H6 L-Lyxose	H7 Glucuronamide	H8 Pyruvic Acid	H9 L-Galactonic Acid-γ-Lactone	H10 D-Galacturonic Acid	H11 Phenylethyl-amine	H12 2-Aminoethanol

Screen PM2A.

A1 Negative Control	A2 Chondroitin Sulfate C	A3 α-Cyclodextrin	A4 β-Cyclodextrin	A5 γ-Cyclodextrin	A6 Dextrin	A7 Gelatin	A8 Glycogen	A9 Inulin	A10 Laminarin	A11 Mannan	A12 Pectin
B1 N-Acetyl-D-Galactosamine	B2 N-Acetyl-Neuraminic Acid	B3 β-D-Allose	B4 Amygdalin	B5 D-Arabinose	B6 D-Arabitol	B7 L-Arabitol	B8 Arbutin	B9 2-Deoxy-D-Ribose	B10 i-Erythritol	B11 D-Fucose	B12 3-0-β-D-Galacto-pyranosyl-D-Arabinose
C1 Gentiobiose	C2 L-Glucose	C3 Lactitol	C4 D-Melezitose	C5 Maltitol	C6 a-Methyl-D-Glucoside	C7 β-Methyl-D-Galactoside	C8 3-Methyl Glucose	C9 β-Methyl-D-Glucuronic Acid	C10 α-Methyl-D-Mannoside	C11 β-Methyl-D-Xyloside	C12 Palatinose
D1 D-Raffinose	D2 Salicin	D3 Sedoheptulosan	D4 L-Sorbose	D5 Stachyose	D6 D-Tagatose	D7 Turanose	D8 Xylitol	D9 N-Acetyl-D-Glucosaminitol	D10 γ-Amino Butyric Acid	D11 δ-Amino Valeric Acid	D12 Butyric Acid
E1 Capric Acid	E2 Caproic Acid	E3 Citraconic Acid	E4 Citramalic Acid	E5 D-Glucosamine	E6 2-Hydroxy Benzoic Acid	E7 4-Hydroxy Benzoic Acid	E8 β-Hydroxy Butyric Acid	E9 γ-Hydroxy Butyric Acid	E10 a-Keto-Valeric Acid	E11 Itaconic Acid	E12 5-Keto-D-Gluconic Acid
F1 D-Lactic Acid Methyl Ester	F2 Malonic Acid	F3 Melibionic Acid	F4 Oxalic Acid	F5 Oxalomalic Acid	F6 Quinic Acid	F7 D-Ribono-1,4-Lactone	F8 Sebacic Acid	F9 Sorbic Acid	F10 Succinamic Acid	F11 D-Tartaric Acid	F12 L-Tartaric Acid
G1 Acetamide	G2 L-Alaninamide	G3 N-Acetyl-L-Glutamic Acid	G4 L-Arginine	G5 Glycine	G6 L-Histidine	G7 L-Homoserine	G8 Hydroxy-L-Proline	G9 L-Isoleucine	G10 L-Leucine	G11 L-Lysine	G12 L-Methionine
H1 L-Ornithine	H2 L-Phenylalanine	H3 L-Pyroglutamic Acid	H4 L-Valine	H5 D,L-Carnitine	H6 Sec-Butylamine	H7 D.L-Octopamine	H8 Putrescine	H9 Dihydroxy Acetone	H10 2,3-Butanediol	H11 2,3-Butanone	H12 3-Hydroxy 2-Butanone

Screen PM3B

A1 Negative Control	A2 Ammonia	A3 Nitrite	A4 Nitrate	A5 Urea	A6 Biuret	A7 L-Alanine	A8 L-Arginine	A9 L-Asparagine	A10 L-Aspartic Acid	A11 L-Cysteine	A12 L-Glutamic Acid
B1 L-Glutamine	B2 Glycine	B3 L-Histidine	B4 L-Isoleucine	B5 L-Leucine	B6 L-Lysine	B7 L-Methionine	B8 L-Phenylalanine	B9 L-Proline	B10 L-Serine	B11 L-Threonine	B12 L-Tryptophan
C1 L-Tyrosine	C2 L-Valine	C3 D-Alanine	C4 D-Asparagine	C5 D-Aspartic Acid	C6 D-Glutamic Acid	C7 D-Lysine	C8 D-Serine	C9 D-Valine	C10 L-Citrulline	C11 L-Homoserine	C12 L-Ornithine
D-1 N-Acetyl-L-Glutamic Acid	D2 N-Phthaloyl-L-Glutamic Acid	D3 L-Pyroglutamic Acid	D4 Hydroxylamine	D5 Methylamine	D6 N-Amylamine	D7 N-Butylamine	D8 Ethylamine	D9 Ethanolamine	D10 Ethylenediamine	D11 Putrescine	D12 Agmatine
E1 Histamine	E2 β-Phenylethyl-amine	E3 Tyramine	E4 Acetamide	E5 Formamide	E6 Glucuronamide	E7 D,L-Lactamide	E8 D-Glucosamine	E9 D-Galactosamine	E10 D-Mannosamine	E11 N-Acetyl-D-Glucosamine	E12 N-Acetyl-D-Galactosamine
F1 N-Acetyl-D-Mannosamine	F2 Adenine	F3 Adenosine	F4 Cytidine	F5 Cytosine	F6 Guanine	F7 Guanosine	F8 Thymine	F9 Thymidine	F10 Uracil	F11 Uridine	F12 Inosine
G1 Xanthine	G2 Xanthosine	G3 Uric Acid	G4 Alloxan	G5 Allantoin	G6 Parabanic Acid	G7 D,L-α-Amino-N-Butyric Acid	G8 γ-Amino-N-Butyric Acid	G9 ε-Amino-N-Caproic Acid	G10 D,L-α-Amino-Caprylic Acid	G11 δ-Amino-N-Valeric Acid	G12 α-Amino-N-Valeric Acid
H1 Ala-Asp	H2 Ala-Gln	H3 Ala-Glu	H4 Ala-Gly	H5 Ala-His	H6 Ala-Leu	H7 Ala-Thr	H8 Gly-Asn	H9 Gly-Gln	H10 Gly-Glu	H11 Gly-Met	H12 Met-Ala

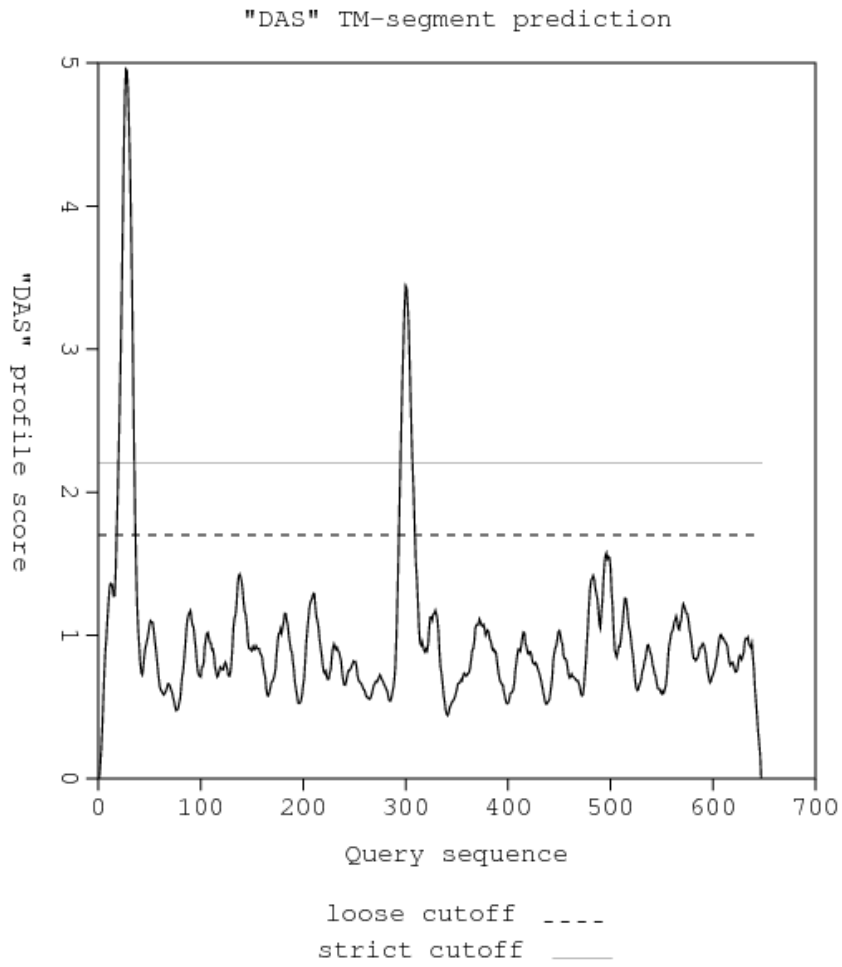
Screen PM4A

A1 Negative Control	A2 Phosphate	A3 Pyrophosphate	A4 Trimeta-phosphate	A5 Tripoly-phosphate	A6 Triethyl Phosphate	A7 Hypophosphite	A8 Adenosine- 2'-monophosphate	A9 Adenosine- 3'-monophosphate	A10 Adenosine- 5'-monophosphate	A11 Adenosine- 2',3'-cyclic monophosphate	A12 Adenosine- 3',5'-cyclic monophosphate
B1 Thiophosphate	B2 Dithiophosphate	B3 D,L-α-Glycerol Phosphate	B4 β-Glycerol Phosphate	B5 Carbamyl Phosphate	B6 D-2-Phospho-Glyceric Acid	B7 D-3-Phospho-Glyceric Acid	B8 Guanosine- 2'-monophosphate	B9 Guanosine- 3'-monophosphate	B10 Guanosine- 5'-monophosphate	B11 Guanosine- 2',3'-cyclic monophosphate	B12 Guanosine- 3',5'-cyclic monophosphate
C1 Phosphoenol Pyruvate	C2 Phospho-Glycolic Acid	C3 D-Glucose-1-Phosphate	C4 D-Glucose-6-Phosphate	C5 2-Deoxy-D-Glucose 6-Phosphate	C6 D-Glucosamine-6-Phosphate	C7 6-Phospho-Gluconic Acid	C8 Cytidine- 2'-monophosphate	C9 Cytidine- 3'-monophosphate	C10 Cytidine- 5'-monophosphate	C11 Cytidine- 2',3'-cyclic monophosphate	C12 Cytidine- 3',5'-cyclic monophosphate
D1 D-Mannose-1-Phosphate	D2 D-Mannose-6-Phosphate	D3 Cysteamine-S-Phosphate	D4 Phospho-L-Arginine	D5 O-Phospho-D-Serine	D6 O-Phospho-L-Serine	D7 O-Phospho-L-Threonine	D8 Uridine- 2'-monophosphate	D9 Uridine- 3'-monophosphate	D10 Uridine- 5'-monophosphate	D11 Uridine- 2',3'-cyclic monophosphate	D12 Uridine- 3',5'-cyclic monophosphate
E1 O-Phospho-D-Tyrosine	E2 O-Phospho-L-Tyrosine	E3 Phosphocreatine	E4 Phosphoryl Choline	E5 O-Phosphoryl-Ethanolamine	E6 Phosphono Acetic Acid	E7 2-Aminoethyl Phosphonic Acid	E8 Methylene Diphosphonic Acid	E9 Thymidine- 3'-monophosphate	E10 Thymidine- 5'-monophosphate	E11 Inositol Hexaphosphate	E12 Thymidine 3',5'-cyclic monophosphate
F1 Negative Control	F2 Sulfate	F3 Thiosulfate	F4 Tetrathionate	F5 Thiophosphate	F6 Dithiophosphate	F7 L-Cysteine	F8 D-Cysteine	F9 L-Cysteinyl-Glycine	F10 L-Cysteic Acid	F11 Cysteamine	F12 L-Cysteine Sulfinic Acid
G1 N-Acetyl-L-Cysteine	G2 S-Methyl-L-Cysteine	G3 Cystathionine	G4 Lanthionine	G5 Glutathione	G6 D,L-Ethionine	G7 L-Methionine	G8 D-Methionine	G9 Glycyl-L-Methionine	G10 N-Acetyl-D,L-Methionine	G11 L- Methionine Sulfoxide	G12 L-Methionine Sulfone
H1 L-Djenkolic Acid	H2 Thiourea	H3 1-Thio-β-D-Glucose	H4 D,L-Lipoamide	H5 Taurocholic Acid	H6 Taurine	H7 Hypotaurine	H8 p-Amino Benzene Sulfonic Acid	H9 Butane Sulfonic Acid	H10 2-Hydroxyethane Sulfonic Acid	H11 Methane Sulfonic Acid	H12 Tetramethylene Sulfone

Screen PM5

A1 Negative Control	A2 Positive Control	A3 L-Alanine	A4 L-Arginine	A5 L-Asparagine	A6 L-Aspartic Acid	A7 L-Cysteine	A8 L-Glutamic Acid	A9 Adenosine-3',5'-cyclic monophosphate	A10 Adenine	A11 Adenosine	A12 2'-Deoxy Adenosine
B1 L-Glutamine	B2 Glycine	B3 L-Histidine	B4 L-Isoleucine	B5 L-Leucine	B6 L-Lysine	B7 L-Methionine	B8 L-Phenylalanine	B9 Guanosine-3',5'-cyclic monophosphate	B10 Guanine	B11 Guanosine	B12 2'-Deoxy Guanosine
C1 L-Proline	C2 L-Serine	C3 L-Threonine	C4 L-Tryptophan	C5 L-Tyrosine	C6 L-Valine	C7 L-isoleucine + L-Valine	C8 trans-4-Hydroxy L-Proline	C9 (5) 4-Amino-Imidazole-4(5)-Carboxamide	C10 Hypoxanthine	C11 Inosine	C12 2'-Deoxy Inosine
D1 L-Ornithine	D2 L-Citrulline	D3 Chorismic Acid	D4 (-)Shikimic Acid	D5 L-Homoserine Lactone	D6 D-Alanine	D7 D-Aspartic Acid	D8 D-Glutamic Acid	D9 D,L-α,ε-Diamino-pimelic Acid	D10 Cytosine	D11 Cytidine	D12 2'-Deoxy Cytidine
E1 Putrescine	E2 Spermidine	E3 Spermine	E4 Pyridoxine	E5 Pyridoxal	E6 Pyridoxamine	E7 β-Alanine	E8 D-Pantothenic Acid	E9 Orotic Acid	E10 Uracil	E11 Uridine	E12 2'-Deoxy Uridine
F1 Quinolinic Acid	F2 Nicotinic Acid	F3 Nicotinamide	F4 β-Nicotinamide Adenine Dinucleotide	F5 δ-Amino-Levulinic Acid	F6 Hematin	F7 Deferoxamine Mesylate	F8 D-(+)-Glucose	F9 N-Acetyl D-Glucosamine	F10 Thymine	F11 Glutathione (reduced form)	F12 Thymidine
G1 Oxaloacetic Acid	G2 D-Biotin	G3 Cyano-Cobalamine	G4 p-Amino-Benzoic Acid	G5 Folic Acid	G6 Inosine + Thiamine	G7 Thiamine	G8 Thiamine Pyrophosphate	G9 Riboflavin	G10 Pyrrolo-Quinoline Quinone	G11 Menadione	G12 m-Inositol
H1 Butyric Acid	H2 D,L-α-Hydroxy-Butyric Acid	H3 α-Keto-Butyric Acid	H4 Caprylic Acid	H5 D,L-α-Lipoic Acid (oxidized form)	H6 D,L-Mevalonic Acid	H7 D,L-Carnitine	H8 Choline	H9 Tween 20	H10 Tween 40	H11 Tween 60	H12 Tween 80

A



B

>PA5072/McpK.
MYDWWVLQLAKLSVSRKLMVGFGVLLALLLLVVISSNRTLTHQTALSEQLAEVASLMEQT
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DDLAAADQQRSAARGVLVGTAQQALDSFARLEELMDASLAQQAGDPQALQRSRAVADLHQ
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RGGIEQFRAGVIRTRQAQQAMQSSTQDMARAGRTLTEAGRQLRESTASRDRASLWLIAAL
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MGDSLRELVGRISDGVSQLASSAEELSAVTEQTRAGVNSQKVETDQVATAMHEMAATVQD
VARNAELASQAARQADEEARQGDAVVDQAVTRIERLASEMDVSSEAMARLKNESEQIGSV
LDVIKSVAEQTNLLALNAAIEAARAGDAGRGFAVVADEVRGLAQRTQQSTAEIEGLIQRL
QQGAGEAAERLENSRSLTASTVELARRAGAALDSITRTVSDIQNMNLQIATAAEQQSTVA
EEINRSVLSVRDVAEQSAAASEQTAASSGELARLGTQLQAQVGRFRL

Fig. S2) Prediction of transmembrane regions of chemoreceptor PA5072 from *P. aeruginosa* PAO1. A) Output from the DAS transmembrane (Cserzo et al. (1997) *Protein Engineering* **10**, 673-676) prediction server. B) Sequence of the PA5072/McpK chemoreceptor with the two transmembrane regions predicted highlighted in red.

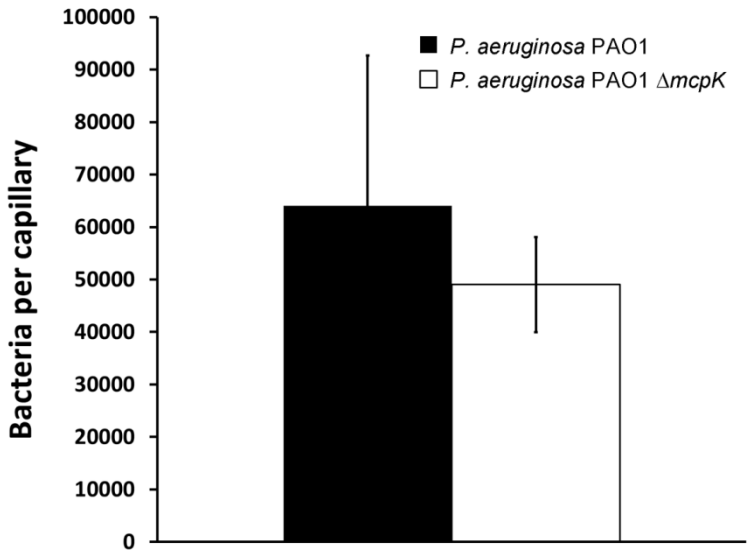

Fig. S3) Quantitative capillary chemotaxis assays of *P. aeruginosa* towards 0.1 % (w/v) casamino acids. Shown are means and standard deviations from three independent experiments conducted in triplicate.

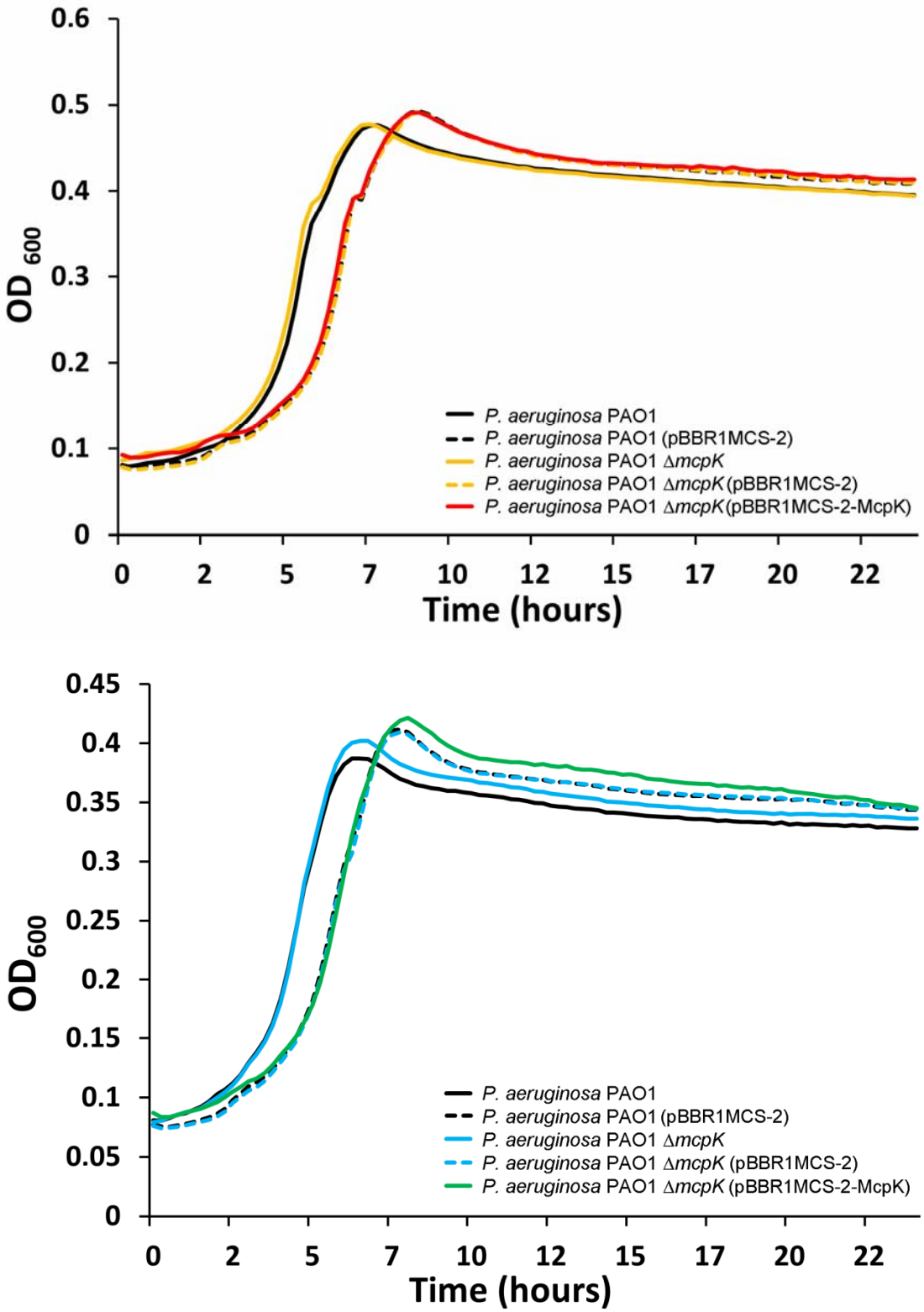

Fig. S4) Growth curve of different strains in M9 minimal medium supplemented with 10 mM α-ketoglutarate (upper graph) or succinate (lower graph).

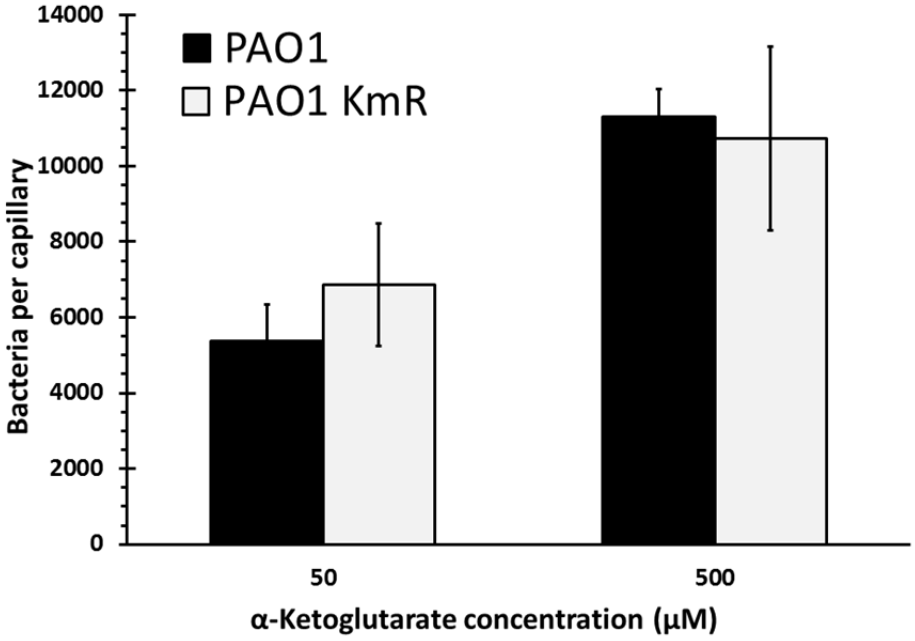

Fig. S5) Chemotaxis of *P. aeruginosa* PAO1 and *P. aeruginosa* PAO1-Km to alpha-ketoglutarate. Shown are means and standard deviations from two experiments conducted in triplicates.

Name	Sequence (5'-3')	Purpose
McpK-LBD_fw	CATATGAGCAACCGCACCCTCACG	Cloning of McpK-LBD into expression vector
McpK-LBD_rv	GGATCCTTAGCTGGCGCGGTCG	Cloning of McpK-LBD into expression vector
PA5072UpF-HindIII	ATAAAGCTTAAAAACTCCGGTAAAAGACTGAAGGT	Generation of Δ*mcpK* mutant
PA5072UpR-XbaI	CTAGTCTAGACTAGCGTCATTACTCCAGGCAGTGCG	Generation of Δ*mcpK* mutant
PA5072DownF-XbaI	CTAGTCTAGACTAGGACATCCAGAACATGAACCTGCA	Generation of Δ*mcpK* mutant
PA5072DownR-EcoRI	AAGAATTCTGGCGTCATCTCCCAAAAGGC	Generation of Δ*mcpK* mutant
glmS-EcoRI_fw	TAATGAATTCGTGCGCGAATCCGACCTGAC	Generation of PAO1-Km strain
glmS-BamHI_rv	TAATGGATCCCGGCGTTACTCGACGGTGAC	Generation of PAO1-Km strain
glmS-BamHI_fw	TAATGGATCCGCTCGCTGTCAATCGCGCAAC	Generation of PAO1-Km strain
glmS-HindIII_rv	TAATAAGCTTGCTGGTCTTCCTGATGGCACG	Generation of PAO1-Km strain
McpK-comp_fw	TAATGGTACCGTGCTGCTGAACAGCTACCG	Generation of complementation plasmid
McpK-comp_rv	TAATTCTAGACCCAAAAGGCGAAAGCCTGA	Generation of complementation plasmid
mcpK_fw	TGCTTCTGCTGGTGGTGATC	qRT-PCR of the *mcpK* gene
mcpK_rv	TTGCTGGGTCTGTTCCATCAG	qRT-PCR of the *mcpK* gene
rpoD_fw	AGAAGAAAGCGACGACAGCA	qRT-PCR of the *rpoD* gene
rpoD_rv	CTTCTTGGCCTTGTCGAGCT	qRT-PCR of the *rpoD* gene
gyrB_fw	CTGAACACCAACAAGACCGC	qRT-PCR of the *gyrB* gene
gyrB_rv	TCGTTGAAGCTGTCGTTCCA	qRT-PCR of the *gyrB* gene
tlpQ_fw	TGAAAAGCGCCAGTACACAG	qRT-PCR of the *tlpQ* gene
tlpQ_rv	CCATGAAATAGCGCTGGATGC	qRT-PCR of the *tlpQ* gene
ctpH_fw	CGAAGACGTGATGGAAGAAACG	qRT-PCR of the *ctpH* gene
ctpH_rv	TTTCCAATTGGCGGATGACC	qRT-PCR of the *ctpH* gene
pctA_fw	TTCGCACTGTTCACCCTCTAC	qRT-PCR of the *pctA* gene
pctA_rv	ATGTTGCTGGAAGTCACGTC	qRT-PCR of the *pctA* gene
pctC_fw	TTTTCGCCTTCAGCTGCTTC	qRT-PCR of the *pctC* gene
pctC_rv	TTTCCCCGAGGTAGTTTTCCG	qRT-PCR of the *pctC* gene

Table S1) Oligonucleotides used in this study

Ligand	Tm Shift (°C)	ITC binding to recombinant McpK-LBD
Compounds that caused Tm shifts of at least 2 °C		
α-ketoglutarate	+5.2	Yes
Uracil	+4.2	No
γ-Aminobutyric acid	+4.1	No
5-Aminovaleric acid	+3.9	No
Carbamyl phosphate	+3.5	No
Phenylethylamine	+3.5	No
D-Glucosaminic acid	+3.5	No
Methyl pyruvate	+3.3	No
L-Glutamic acid	+2.3	No
D-Galacturonic acid	+2.3	No
L-Glutamine	+2.2	No
D-Galactonic acid-γ-lactone	+2	No
Itaconate	-2.2	No
Structurally related compounds		
Citrate	+0.8	No
Butyrate	+0.7	No
Malate	+0.5	No
Fumarate	+0.3	No
Tricarballylate	+0.3	No
Lactate	+0.2	No
Pyruvate	+0.1	No
Oxaloacetate	+0	No
Acetate	-0.2	No
Succinate	-0.3	No
cis-Aconitate	Not tested	No
Isocitrate	Not tested	No
Valerate	Not tested	No
Glutarate	Not tested	No
2-Aminoadipate	Not tested	No

Table S2) Binding studies of different ligands to recombinant McpK-LBD. The upper part shows compounds that altered the McpK-LBD Tm by at least 2 °C in a thermal shift assays screen of compounds listed in Supp. Fig. 1. The last column indicates the outcome of ITC studies of these compounds to McpK-LBD. The lower part shows compounds with structural similarity to αKG that were analyzed for binding to McpK-LBD by ITC.