

Supplementary:

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CLUSTAL O(1.2.4) multiple sequence alignment
oprF          -----QGQNSVEIEAFGKRYFTDSVRNMKNADLYGGSIGYF 36
PAO1          MKLKNTLGVVIGSLVAASAMNAFAQQGNSVEIEAFGKRYFTDSVRNMKNADLYGGSIGYF 60
B136-33      MKLKNTLGVVIGSLVAASAMNAFAQQGNSVEIEAFGKRYFTDSVRNMKNADLYGGSIGYF 60
LESB58       MKLKNTLGVVIGSLVAASAMNAFAQQGNSVEIEAFGKRYFTDSVRNMKNADLYGGSIGYF 60
M18          MKLKNTLGVVIGSLVAASAMNAFAQQGNSVEIEAFGKRYFTDSVRNMKNADLYGGSIGYF 60
NCGM2.S1     MKLKNTLGVVIGSLVAASAMNAFAQQGNSVEIEAFGKRYFTDSVRNMKNADLYGGSIGYF 60
PA7          MKLKNTLGVVIGSLVAASAMNAFAQQGNSVEIEAFGKRYFTDSVRNMKNADLYGGSIGYF 60
PA14         MKLKNTLGVVIGSLVAASAMNAFAQQGNSVEIEAFGKRYFTDSVRNMKNADLYGGSIGYF 60
DK2          MKLKNTLGVVIGSLVAASAMNAFAQQGNSVEIEAFGKRYFTDSVRNMKNADLYGGSIGYF 60
SCV20265     MKLKNTLGVVIGSLVAASAMNAFAQQGNSVEIEAFGKRYFTDSVRNMKNADLYGGSIGYF 60
PAK          MKLKNTLGVVIGSLVAASAMNAFAQQGNSVEIEAFGKRYFTDSVRNMKNADLYGGSIGYF 60
*****
oprF          LTDDVELALSYPEYHDVVRTYETGNKKVHGNLTS LDAIYHFGT PGVGLRPYVSAGLAHQN 96
PAO1          LTDDVELALSYPEYHDVVRTYETGNKKVHGNLTS LDAIYHFGT PGVGLRPYVSAGLAHQN 120
B136-33      LTDDVELALSYPEYHDVVRTYETGNKKVHGNLTS LDAIYHFGT PGVGLRPYVSAGLAHQN 120
LESB58       LTDDVELALSYPEYHDVVRTYETGNKKVHGNLTS LDAIYHFGT PGVGLRPYVSAGLAHQN 120
M18          LTDDVELALSYPEYHDVVRTYETGNKKVHGNLTS LDAIYHFGT PGVGLRPYVSAGLAHQN 120
NCGM2.S1     LTDDVELALSYPEYHDVVRTYETGNKKVHGNLTS LDAIYHFGT PGVGLRPYVSAGLAHQN 120
PA7          LTDDVELALSYPEYHDVVRTYETGNKKVHGNLTS LDAIYHFGT PGVGLRPYVSAGLAHQN 120
PA14         LTDDVELALSYPEYHDVVRTYETGNKKVHGNLTS LDAIYHFGT PGVGLRPYVSAGLAHQN 120
DK2          LTDDVELALSYPEYHDVVRTYETGNKKVHGNLTS LDAIYHFGT PGVGLRPYVSAGLAHQN 120
SCV20265     LTDDVELALSYPEYHDVVRTYETGNKKVHGNLTS LDAIYHFGT PGVGLRPYVSAGLAHQN 120
PAK          LTDDVELALSYPEYHDVVRTYETGNKKVHGNLTS LDAIYHFGT PGVGLRPYVSAGLAHQN 120
*****
oprF          ITNINSDSQGRQOMTMANIGAGLKYYFTENFFAKASLDGQYGLEKRDNGHQGEWMAGLVG 156
PAO1          ITNINSDSQGRQOMTMANIGAGLKYYFTENFFAKASLDGQYGLEKRDNGHQGEWMAGLVG 180
B136-33      ITNINSDSQGRQOMTMANIGAGLKYYFTENFFAKASLDGQYGLEKRDNGHQGEWMAGLVG 180
LESB58       ITNINSDSQGRQOMTMANIGAGLKYYFTENFFAKASLDGQYGLEKRDNGHQGEWMAGLVG 180
M18          ITNINSDSQGRQOMTMANIGAGLKYYFTENFFAKASLDGQYGLEKRDNGHQGEWMAGLVG 180
NCGM2.S1     ITNINSDSQGRQOMTMANIGAGLKYYFTENFFAKASLDGQYGLEKRDNGHQGEWMAGLVG 180
PA7          ITNINSDSQGRQOMTMANIGAGLKYYFTENFFAKASLDGQYGLEKRDNGHQGEWMAGLVG 180
PA14         ITNINSDSQGRQOMTMANIGAGLKYYFTENFFAKASLDGQYGLEKRDNGHQGEWMAGLVG 180
DK2          ITNINSDSQGRQOMTMANIGAGLKYYFTENFFAKASLDGQYGLEKRDNGHQGEWMAGLVG 180
SCV20265     ITNINSDSQGRQOMTMANIGAGLKYYFTENFFAKASLDGQYGLEKRDNGHQGEWMAGLVG 180
PAK          ITNINSDSQGRQOMTMANIGAGLKYYFTENFFAKASLDGQYGLEKRDNGHQGEWMAGLVG 180
*****
oprF          GFNFGGSKAAPAPEPVADVCSDS DNDGVC DNVDKCPDT PANVTVDANGCPVAEVRVQL 216
PAO1          GFNFGGSKAAPAPEPVADVCSDS DNDGVC DNVDKCPDT PANVTVDANGCPVAEVRVQL 240
B136-33      GFNFGGSKAAPAPEPVADVCSDS DNDGVC DNVDKCPDT PANVTVDANGCPVAEVRVQL 240
LESB58       GFNFGGSKAAPAPEPVADVCSDS DNDGVC DNVDKCPDT PANVTVDANGCPVAEVRVQL 240
M18          GFNFGGSKAAPAPEPVADVCSDS DNDGVC DNVDKCPDT PANVTVDANGCPVAEVRVQL 240
NCGM2.S1     GFNFGGSKAAPAPEPVADVCSDS DNDGVC DNVDKCPDT PANVTVDANGCPVAEVRVQL 240
PA7          GFNFGGSKAAPAPEPVADVCSDS DNDGVC DNVDKCPDT PANVTVDANGCPVAEVRVQL 240
PA14         GFNFGGSKAAPAPEPVADVCSDS DNDGVC DNVDKCPDT PANVTVDANGCPVAEVRVQL 240
DK2          GFNFGGSKAAPAPEPVADVCSDS DNDGVC DNVDKCPDT PANVTVDANGCPVAEVRVQL 240
SCV20265     GFNFGGSKAAPAPEPVADVCSDS DNDGVC DNVDKCPDT PANVTVDANGCPVAEVRVQL 240
PAK          GFNFGGSKAAPAPEPVADVCSDS DNDGVC DNVDKCPDT PANVTVDANGCPVAEVRVQL 240
*****
oprF          DVKFD FDKSKVKENSYADIKNLADFMKQY P STTTVEGHTDSVGT DAYNQKLSERRANAV 276
PAO1          DVKFD FDKSKVKENSYADIKNLADFMKQY P STTTVEGHTDSVGT DAYNQKLSERRANAV 300
B136-33      DVKFD FDKSKVKENSYADIKNLADFMKQY P STTTVEGHTDSVGT DAYNQKLSERRANAV 300
LESB58       DVKFD FDKSKVKENSYADIKNLADFMKQY P STTTVEGHTDSVGT DAYNQKLSERRANAV 300
M18          DVKFD FDKSKVKENSYADIKNLADFMKQY P STTTVEGHTDSVGT DAYNQKLSERRANAV 300
NCGM2.S1     DVKFD FDKSKVKENSYADIKNLADFMKQY P STTTVEGHTDSVGT DAYNQKLSERRANAV 300
PA7          DVKFD FDKSKVKENSYADIKNLADFMKQY P STTTVEGHTDSVGT DAYNQKLSERRANAV 300
PA14         DVKFD FDKSKVKENSYADIKNLADFMKQY P STTTVEGHTDSVGT DAYNQKLSERRANAV 300
DK2          DVKFD FDKSKVKENSYADIKNLADFMKQY P STTTVEGHTDSVGT DAYNQKLSERRANAV 300
SCV20265     DVKFD FDKSKVKENSYADIKNLADFMKQY P STTTVEGHTDSVGT DAYNQKLSERRANAV 300
PAK          DVKFD FDKSKVKENSYADIKNLADFMKQY P STTTVEGHTDSVGT DAYNQKLSERRANAV 300
*****
oprF          RDVLVNEYGVGGRVNAVGYGESRPVADNATAEGRAINRRVEAEVEAEAK--- 325
PAO1          RDVLVNEYGVGGRVNAVGYGESRPVADNATAEGRAINRRVEAEVEAEAKTAA 353
B136-33      RDVLVNEYGVGGRVNAVGYGESRPVADNATAEGRAINRRVEAEVEAEAK--- 350
LESB58       RDVLVNEYGVGGRVNAVGYGESRPVADNATAEGRAINRRVEAEVEAEAK--- 350
M18          RDVLVNEYGVGGRVNAVGYGESRPVADNATAEGRAINRRVEAEVEAEAK--- 350
NCGM2.S1     RDVLVNEYGVGGRVNAVGYGESRPVADNATAEGRAINRRVEAEVEAEAK--- 350
PA7          RDVLVNEYGVGGRVNAVGYGESRPVADNATAEGRAINRRVEAEVEAEAK--- 350
PA14         RDVLVNEYGVGGRVNAVGYGESRPVADNATAEGRAINRRVEAEVEAEAK--- 350
DK2          RDVLVNEYGVGGRVNAVGYGESRPVADNATAEGRAINRRVEAEVEAEAK--- 350
SCV20265     RDVLVNEYGVGGRVNAVGYGESRPVADNATAEGRAINRRVEAEVEAEAK--- 350
PAK          RDVLVNEYGVGGRVNAVGYGESRPVADNATAEGRAINRRVEAEVEAEAK--- 350
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Figure 1. S1. The multiple sequence alignment of OprF among *P. aeruginosa* strains including PAO1, B136-33, LESB58, M18, NCGM2, PA7, PA14, DK2, SCV20265, and Pak. These alignments have revealed that the conserved sequences were visualized between these predominant strains of *Pseudomonas aeruginosa*

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CLUSTAL O(1.2.4) multiple sequence alignment
oprI      -----CSSHSKETEARTATEDAAARAQARADEAYRKADEALGAAQ 41
PAO1      MNNVLKFSALALAAVLTATGSSHSKETEARTATEDAAARAQARADEAYRKADEALGAAQ 60
B136-33   MNNVLKFSALALAAVLTATGSSHSKETEARTATEDAAARAQARADEAYRKADEALGAAQ 60
LESB58    MNNVLKFSALALAAVLTATGSSHSKETEARTATEDAAARAQARADEAYRKADEALGAAQ 60
M18       MNNVLKFSALALAAVLTATGSSHSKETEARTATEDAAARAQARADEAYRKADEALGAAQ 60
NCGM2.S1  MNNVLKFSALALAAVLTATGSSHSKETEARTATEDAAARAQARADEAYRKADEALGAAQ 60
PA7       MNNVLKFSALALAAVLTATGSSHSKETEARTATEDAAARAQARADEAYRKADEALGAAQ 60
PA14      MNNVLKFSALALAAVLTATGSSHSKETEARTATEDAAARAQARADEAYRKADEALGAAQ 60
DK2       MNNVLKFSALALAAVLTATGSSHSKETEARTATEDAAARAQARADEAYRKADEALGAAQ 60
PAK       MNNVLKFSALALAAVLTATGSSHSKETEARTATEDAAARAQARADEAYRKADEALGAAQ 60
          *****
oprI      KAQQTAEANERALRMLEKASRK 64
PAO1      KAQQTAEANERALRMLEKASRK 83
B136-33   KAQQTAEANERALRMLEKASRK 83
LESB58    KAQQTAEANERALRMLEKASRK 83
M18       KAQQTAEANERALRMLEKASRK 83
NCGM2.S1  KAQQTAEANERALRMLEKASRK 83
PA7       KAQQTAEANERALRMLEKASRK 83
PA14      KAQQTAEANERALRMLEKASRK 83
DK2       KAQQTAEANERALRMLEKASRK 83
PAK       KAQQTAEANERALRMLEKASRK 83
          *****

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Figure 1. S2. The multiple sequence alignment of OprI among *P. aeruginosa* strains including PAO1, B136-33, LESB58, M18, NCGM2.S2, PA7, PA14, DK2, and Pak. All the regions of this protein were conserved between these kinds of isolates

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CLUSTAL O(1.2.4) multiple sequence alignment
-----MERLAAPAAASASGTGVALTPPSA 24
DK2 MNPITLERAGLPYGVADAGDIPALGRPVARDVESLRVERLAAPAAASASGTGVALTPPSA 60
NCGM2.S1 MNPITLERAGLPYGVADAGDIPALGRPVARDVESLRVERLAAPAAASASGTGVALTPPSA 60
PA14 MNPITLERAGLPYGVADAGDIPALGRPVARDVESLRVERLAAPAAASASGTGVALTPPSA 60
PAK MNPITLERAGLPYGVADAGDIPALGRPVARDVESLRVERLAAPAAASASGTGVALTPPSA 60
popB MNPITLERAGLPYGVADAGDIPALGRPVARDVESLRVERLAAPAAASASGTGVALTPPSA 60
PAO1 MNPITLERAGLPYGVADAGDIPALGRPVARDVESLRVERLAAPAAASASGTGVALTPPSA 60
LESB58 MNPITLERAGLPYGVADAGDIPALGRPVARDVESLRVERLAAPAAASASGTGVALTPPSA 60
M18 MNPITLERAGLPYGVADAGDIPALGRPVARDVESLRVERLAAPAAASASGTGVALTPPSA 60
*****

DK2 ASQQRLEVANRAEIASLVQAVGEDAGLARQVVLVAGASTLLSAGLMSPOAFEIELAKITGE 84
NCGM2.S1 ASQQRLEVANRAEIASLVQAVGEDAGLARQVVLVAGASTLLSAGLMSPOAFEIELAKITGE 120
PA14 ASQQRLEVANRAEIASLVQAVGEDAGLARQVVLVAGASTLLSAGLMSPOAFEIELAKITGE 120
PAK ASQQRLEVANRAEIASLVQAVGEDVGLARQVVLVAGASTLLSAGLMSPOAFEIELAKITGE 120
popB ASQQRLEVANRAEIASLVQAVGEDVGLARQVVLVAGASTLLSAGLMSPOAFEIELAKITGE 120
PAO1 ASQQRLEVANRAEIASLVQAVGEDVGLARQVVLVAGASTLLSAGLMSPOAFEIELAKITGE 120
LESB58 ASQQRLEVANRAEIASLVQAVGEDVGLARQVVLVAGASTLLSAGLMSPOAFEIELAKITGE 120
M18 ASQQRLEVANRAEIASLVQAVGEDVGLARQVVLVAGASTLLSAGLMSPOAFEIELAKITGE 120
*****

DK2 VENQOKKLLKLTIEIQARKONLQKMEGNQOKIRESEEAAKEAKSGLAAKIFGWISAIASI 144
NCGM2.S1 VENQOKKLLKLTIEIQARKONLQKMEDNQOKIRESEEAAKEAKSGLAAKIFGWISAIASI 180
PA14 VENQOKKLLKLTIEIQARKONLQKMEDNQOKIRESEEAAKEAKSGLAAKIFGWISAIASI 180
PAK VENQOKKLLKLTIEIQARKONLQKMEGNQOKIRESEEAAKEAKSGLAAKIFGWISAIASI 180
popB VENQOKKLLKLTIEIQARKONLQKMEGNQOKIRESEEAAKEAKSGLAAKIFGWISAIASI 180
PAO1 VENQOKKLLKLTIEIQARKONLQKMEGNQOKIRESEEAAKEAKSGLAAKIFGWISAIASI 180
LESB58 VENQOKKLLKLTIEIQARKONLQKMEGNQOKIRESEEAAKEAKSGLAAKIFGWISAIASI 180
M18 VENQOKKLLKLTIEIQARKONLQKMEGNQOKIRESEEAAKEAKSGLAAKIFGWISAIASI 180
*****

DK2 IVGAIMVATGVGAAAGALMIAGGVMGVVVSQSVQQAADGLISKEVMEKLGFPALMGIEIAV 204
NCGM2.S1 IVGAIMVATGVGAAAGALMIAGGVMGVVVSQSVQQAADGLISKEVMEKLGFPALMGIEIAV 240
PA14 IVGAIMVATGVGAAAGALMIAGGVMGVVVSQSVQQAADGLISKEVMEKLGFPALMGIEIAV 240
PAK IVGAIMVATGVGAAAGALMIAGGVMGVVVSQSVQQAADGLISKEVMEKLGFPALMGIEIAV 240
popB IVGAIMVATGVGAAAGALMIAGGVMGVVVSQSVQQAADGLISKEVMEKLGFPALMGIEIAV 240
PAO1 IVGAIMVATGVGAAAGALMIAGGVMGVVVSQSVQQAADGLISKEVMEKLGFPALMGIEIAV 240
LESB58 IVGAIMVATGVGAAAGALMIAGGVMGVVVSQSVQQAADGLISKEVMEKLGFPALMGIEIAV 240
M18 IVGAIMVATGVGAAAGALMIAGGVMGVVVSQSVQQAADGLISKEVMEKLGFPALMGIEIAV 240
*****

DK2 ALLAAVVSFGGSAVGGGLAKLGAKIGGKAAEMTASLASKVADLGGKFGSLAGQSLSHSLKL 264
NCGM2.S1 ALLAAVVSFGGSAVGGGLAKLGAKIGGKAAEMTASLASKVADLGGKFGSLAGQSLSHSLKL 300
PA14 ALLAAVVSFGGSAVGGGLAKLGAKIGGKAAEMTASLASKVADLGGKFGSLAGQSLSHSLKL 300
PAK ALLAAVVSFGGSAVGGGLARLGAKIGGKAAEMTASLASKVADLGGKFGSLAGQSLSHSLKL 300
popB ALLAAVVSFGGSAVGGGLARLGAKIGGKAAEMTASLASKVADLGGKFGSLAGQSLSHSLKL 300
PAO1 ALLAAVVSFGGSAVGGGLARLGAKIGGKAAEMTASLASKVADLGGKFGSLAGQSLSHSLKL 300
LESB58 ALLAAVVSFGGSAVGGGLARLGAKIGGKAAEMTASLASKVADLGGKFGSLAGQSLSHSLKL 300
M18 ALLAAVVSFGGSAVGGGLARLGAKIGGKAAEMTASLASKVADLGGKFGSLAGQSLSHSLKL 300
*****

DK2 GVQVSDLTLDVANGAAQATHSGFQAKAANROADVQESRADLTTLQGVIERLKEELSRMLE 324
NCGM2.S1 GVQVSDLTLDVANGAAQATHSGFQAKAANROADVQESRADLTTLQGVIERLKEELSRMLE 360
PA14 GVQVSDLTLDVANGAAQATHSGFQAKAANROADVQESRADLTTLQGVIERLKEELSRMLE 360
PAK GVQVSDLTLDVANGAAQATHSGFQAKAANROADVQESRADLTTLQGVIERLKEELSRMLE 360
popB GVQVSDLTLDVANGAAQATHSGFQAKAANROADVQESRADLTTLQGVIERLKEELSRMLE 360
PAO1 GVQVSDLTLDVANGAAQATHSGFQAKAANROADVQESRADLTTLQGVIERLKEELSRMLE 360
LESB58 GVQVSDLTLDVANGAAQATHSGFQAKAANROADVQESRADLTTLQGVIERLKEELSRMLE 360
M18 GVQVSDLTLDVANGAAQATHSGFQAKAANROADVQESRADLTTLQGVIERLKEELSRMLE 360
*****

DK2 AFQEIIMERIFAMLOAKGETLHNLSSRPAAI 354
NCGM2.S1 AFQEIIMERIFAMLOAKGETLHNLSSRPAAI 390
PA14 AFQEIIMERIFAMLOAKGETLHNLSSRPAAI 390
PAK AFQEIIMERIFAMLOAKGETLHNLSSRPAAI 390
popB AFQEIIMERIFAMLOAKGETLHNLSSRPAAI 390
PAO1 AFQEIIMERIFAMLOAKGETLHNLSSRPAAI 390
LESB58 AFQEIIMERIFAMLOAKGETLHNLSSRPAAI 390
M18 AFQEIIMERIFAMLOAKGETLHNLSSRPAAI 390
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Figure 1. S3. The multiple sequence alignment of PopB among *P. aeruginosa* strains including DK2, NCGM2.S1, PA14, Pak, PAO1, LESB58, and M18. These alignments have revealed that the conserved sequences were conceived between the most strains of *Pseudomonas aeruginosa*

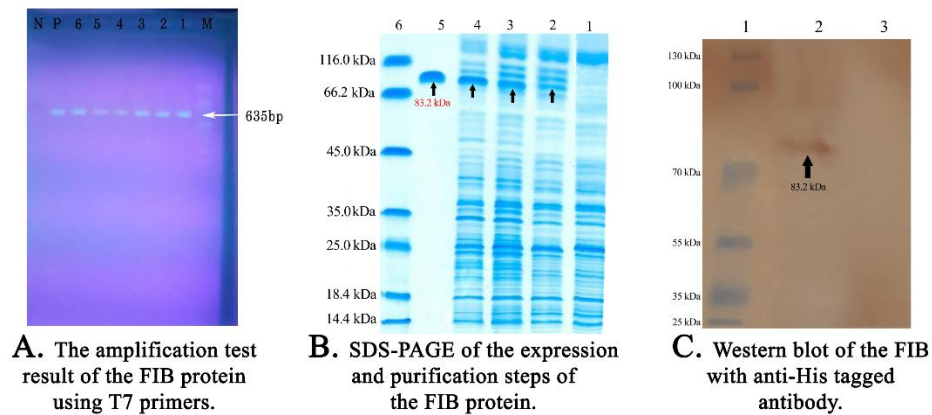


Figure 2. S. The expression and purification of the FIB protein via molecular methods. (A) The amplification test results were performed to confirm the *oprF-oprI-popB* fusion gene by colony-PCR. The extraction results were displayed in samples 1 to 6 via kit (wells 2-4) and boiling (wells 5-7) methods. The *Enterococcus faecalis* ATCC 51299 and *E.coli* BL21 utilized as the positive control (well 8) and negative controls (well 9). (B) The expression and purification steps of chimeric FIB fusion protein were carried out using the SDS-PAGE method. Lane 1: Non-induced *E. coli* BL21 clone, Lane 2: Induced *E. coli* BL21 clone for 4 h, Lane 3: Induced *E. coli* BL21 clone for 6 h, Lane 4: Induced *E. coli* BL21 clone for 12 h, Lane 5: Purified pr. FIB (83.2 kDa), and Lane 6: Protein marker. (C) The western blot of FIB protein with anti-His tag antibody was done via the Ni-NTA-purification, electroporation of SDS-PAGE, transferred by PVDF membrane, and immunoblotted with rabbit anti-6x His-tagged antibody. Lane 1, 2, and 3 were protein markers, western blot of the purified protein, and western blotting via the normal rabbit serum (as the negative control)

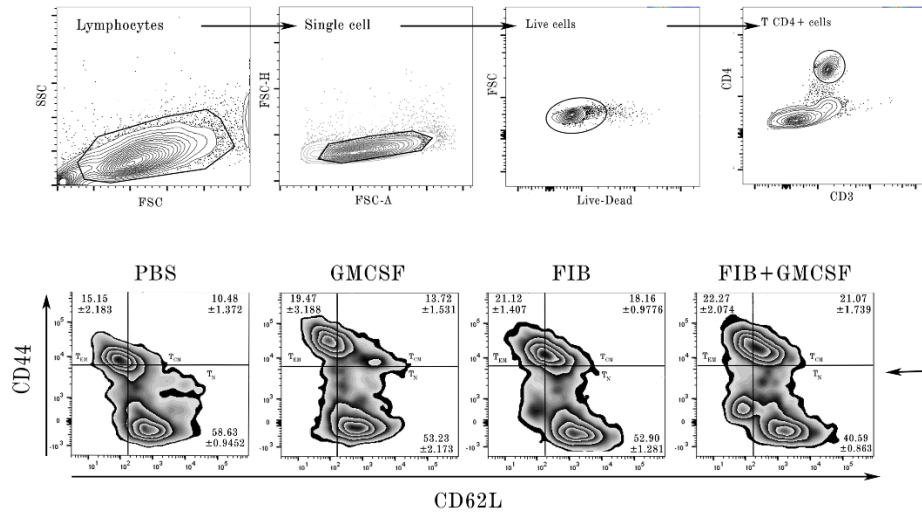


Figure 3.S. The gating strategy for (left to right) lymphocytes carried out following the singlet's recognition and live cells based on FSC. Furthermore, the contour plots of naïve (n=3), effector memory (n=3), and central memory (n=3) of splenic CD4⁺T cells were performed based on monoclonal antibodies recognition. Numbers inside plot regions represent means ± SD according to the users of FMO and iso-type control stained samples

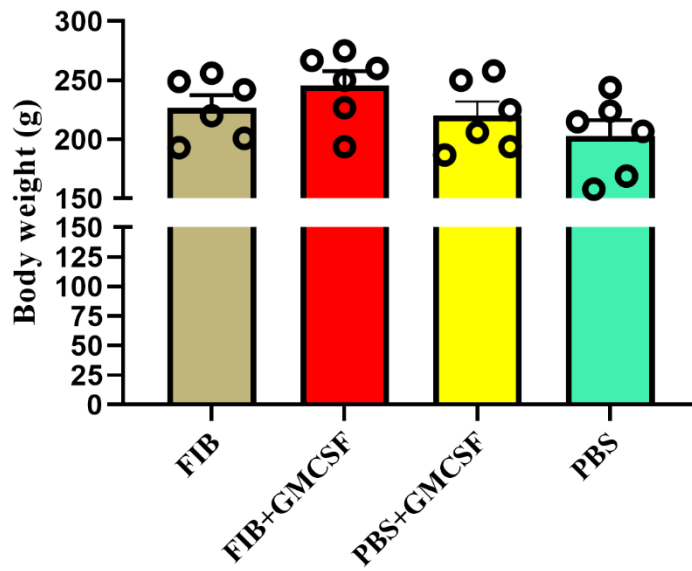


Figure 4-S: Total body weight at 24 hours post-challenge with *P. aeruginosa* strain PAO1. Total bodyweight of FIB, FIB+GMCSF, PBS+GMCSF and PBS (as a control group) immunized and challenged rats. Each circle describes the data of one rat. Error bars are mean \pm SEM values (n=6)