

**Supplementary information for “Genome-wide identification of essential genes in *Mycobacterium intracellulare* by transposon sequencing — Implication for metabolic remodeling”**

**Authors**

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## Legends of supplementary data

**Additional file 1: Figure S1.** Pellicle formation in *M. intracellulare* ATCC13950 under hypoxic conditions. (A) Photographs of aerobically-cultured planktonic bacteria and hypoxically-cultured pellicle bacteria. Pellicles were specifically formed in hypoxic conditions (5% oxygen). (B) Time-course change of pellicle thickness cultured under hypoxic conditions. The data were obtained from three independent assays (mean  $\pm$  SD).

**Additional file 2: Figure S2.** Overview of the transposon (Tn) insertion data in aerobically-cultured planktonic bacteria (PLK) and hypoxically-cultured pellicle bacteria (PEL). (A) Distribution and density of Tn insertion on the ATCC13950 genome. Tn insertion reads (vertical black bars) are shown in the upper row. Coding sequence (CDS) (red), %GC plot (black, upward = above average, downward = below average) and GC skew (purple) are shown in the lower row. (B) Sequencing reproducibility. The graph compares the number of insertions per gene between batches of PLK and PEL, respectively.

**Additional file 3: Table S1.** Essential genes of *M. intracellulare* ATCC13950 Tn mutant library bacteria.

**Additional file 4: Table S2.** Number of Tn insertion sites hit per gene in the *M. intracellulare* ATCC13950 Tn mutant library bacteria.

**Additional file 5: Table S3.** List of essential genes of *M. intracellulare* ATCC13950 shared with those of *M. tuberculosis* H37Rv and *M. marinum* E11.

**Additional file 6: Table S4.** Homologs of essential genes of *M. intracellulare* ATCC13950 in *M. tuberculosis* H37Rv and *M. marinum* E11 corresponding to existing antituberculous drug targets.

**Additional file 7: Table S5.** Result of next-generation sequencing and mapping data of aerobically-cultured planktonic bacteria (PLK) and hypoxically-cultured pellicle bacteria (PEL).

**Additional file 8: Table S6.** Specific essential genes of *M. intracellulare* ATCC13950 under hypoxia.

**Additional file 9: Table S7.** Essential genes specific to hypoxic conditions that showed significant decrease of fitness compared to aerobic conditions.

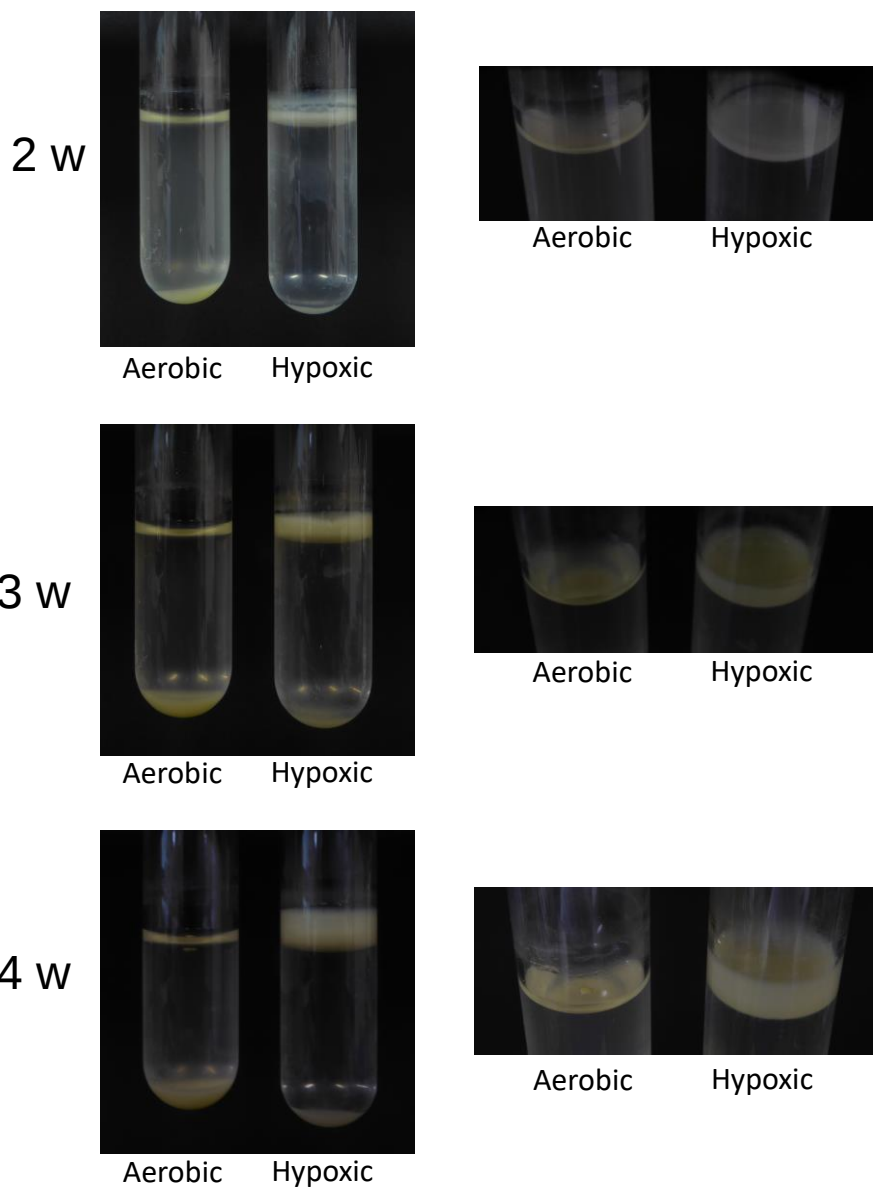
**Additional file 10: Table S8.** Genes showing fitness change under hypoxia compared to aerobic conditions.

**Additional file 11: Table S9.** Representative virulence-associated genes showing decreased fitness for hypoxic survival to form pellicles.

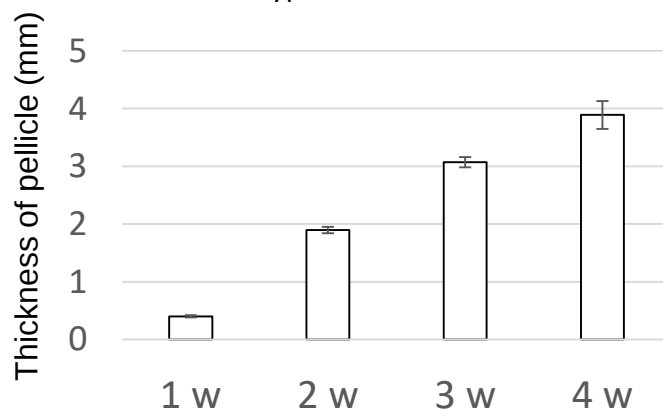
**Additional file 12: Figure S3.** Interpretation of MIC data in PLK bacteria and PEL bacteria. The PLK bacteria sink on the bottom of the round-bottom plate. The PLK bacteria can be observed as a circular dot in the center of the well. The PEL bacteria form a membranous pellicle floating on the air-liquid interface. The PEL bacteria can be observed as covering all over the well.

**Additional file 13: Figure S4.** Effect of metabolic inhibitors of the TnSeq-hit pathways on pellicle formation under hypoxia. (A) View of the culture in glass tubes in aerobic and hypoxic conditions. (B) Quantification of pellicle thickness cultured under hypoxia. The significant difference compared to no drug control was shown by an asterisk. Pellicles were not formed under aerobic conditions.

(A)

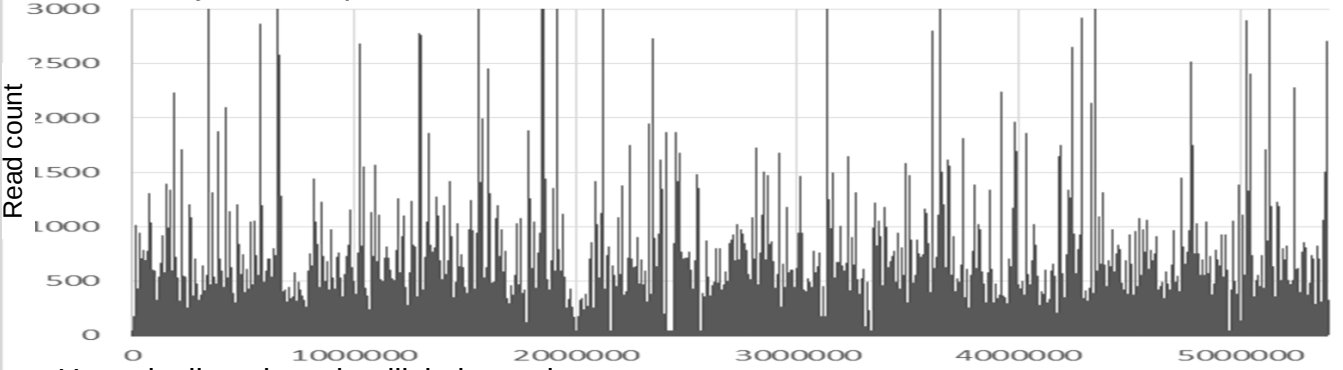


(B)



**Fig S1**

# A Aerobically-cultured planktonic bacteria



# Hypoxically-cultured pellicle bacteria

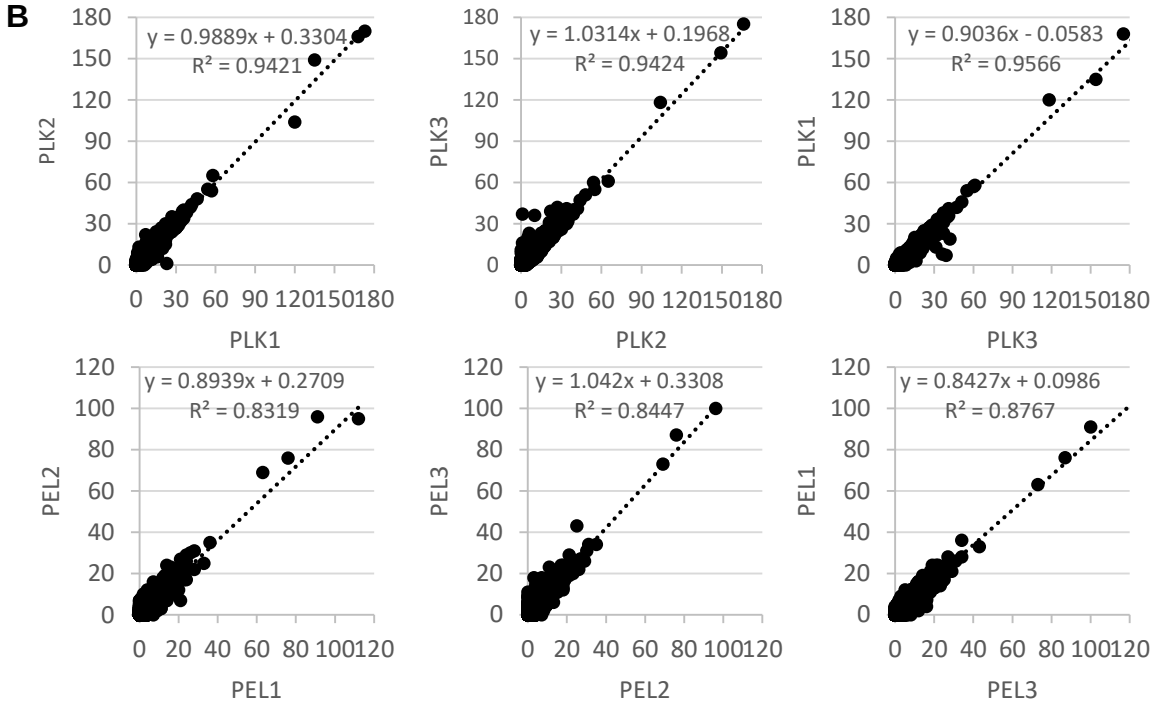
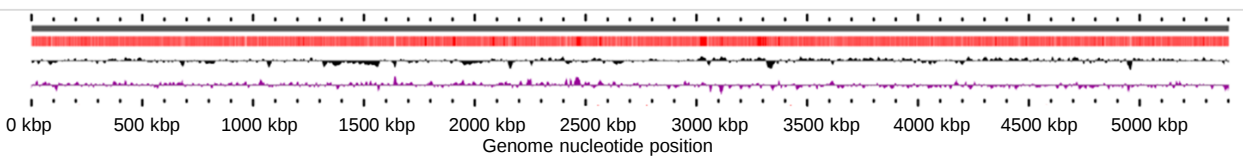
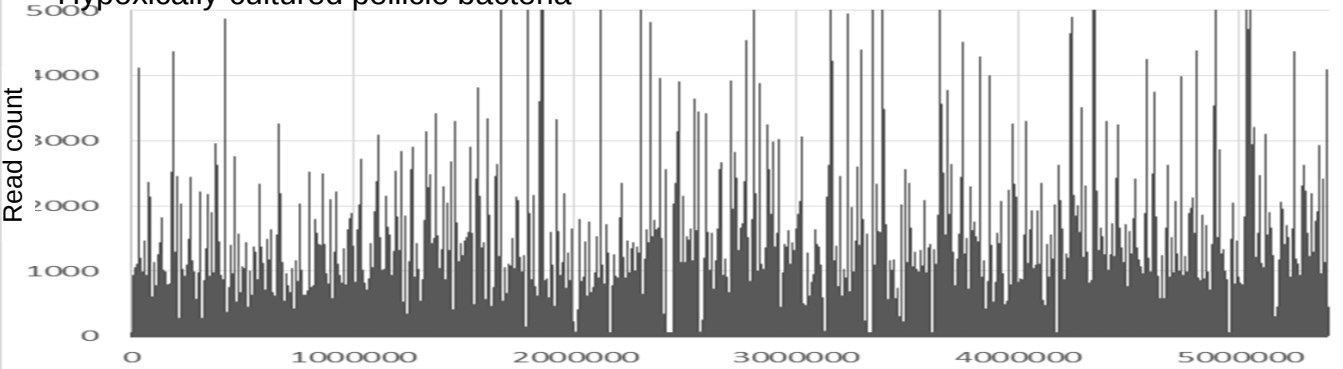


Fig S2

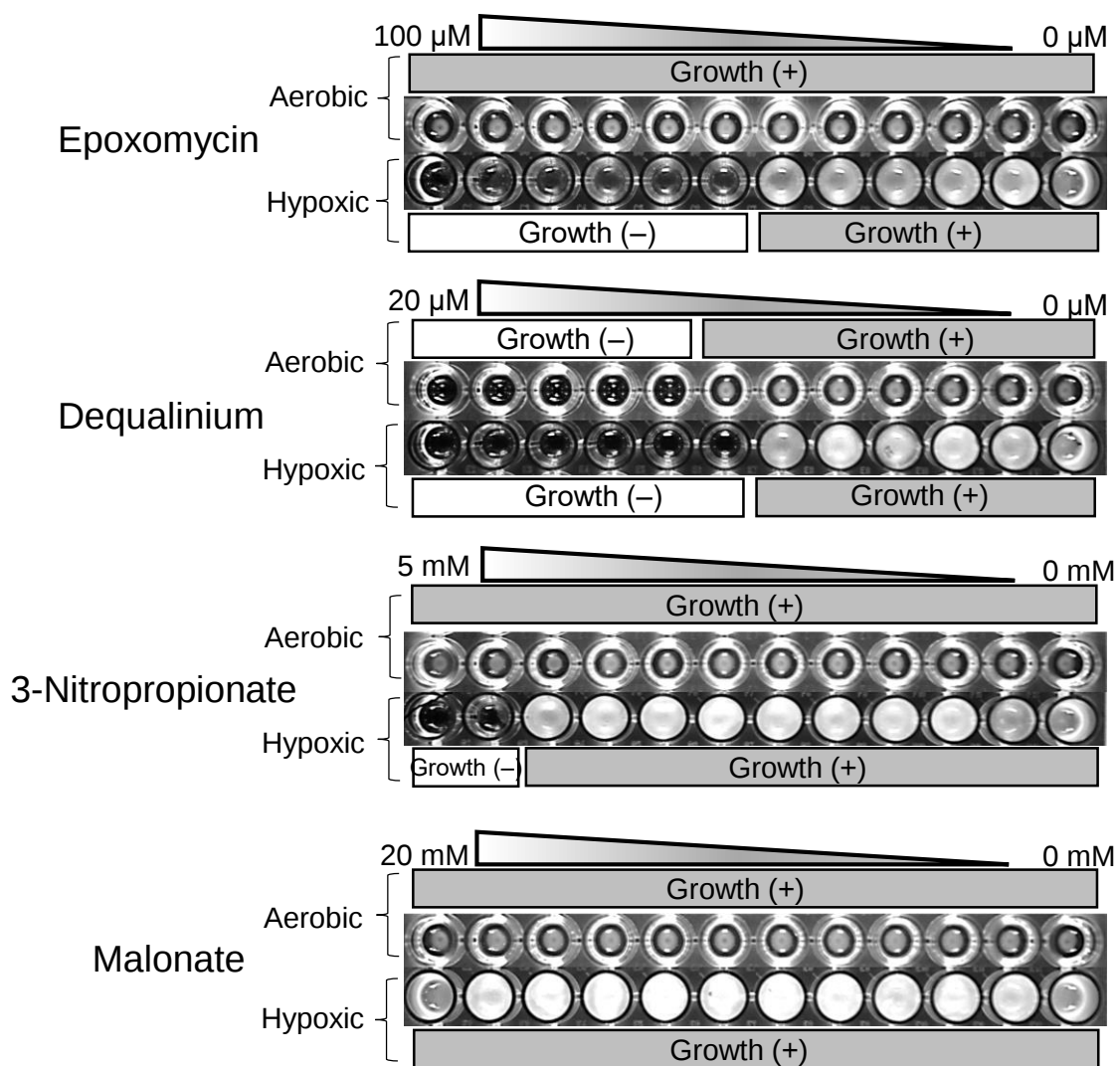
**Table S4.** Homologs of essential genes of *M. intracellulare* ATCC13950 in *M. tuberculosis* H37Rv and *M. marinum* E11 corresponding to existing antituberculous drug targets.

| Gene locus  | Gene         | Function                                      | Homolog in <i>M. tuberculosis</i> H37Rv | Homolog in <i>M. marinum</i> E11 (designated as locus tag) | Target drug              |
|-------------|--------------|-----------------------------------------------|-----------------------------------------|------------------------------------------------------------|--------------------------|
| OCU_RS25020 | <i>gyrB</i>  | DNA topoisomerase (ATP-hydrolyzing) subunit B | <i>rv0005</i>                           | MMARE11_00050                                              | fluoroquinolone          |
| OCU_RS25025 | <i>gyrA</i>  | intein-containing DNA gyrase subunit A        | <i>rv0006</i>                           | MMARE11_00060                                              | fluoroquinolone          |
| OCU_RS26150 | <i>embB</i>  | arabinosyltransferase                         | <i>rv3795</i>                           | MMARE11_51780                                              | ethambutol               |
| OCU_RS26155 | <i>embA</i>  | arabinosyltransferase                         | <i>rv3794</i>                           | MMARE11_51770                                              | ethambutol               |
| OCU_RS26175 | <i>embC</i>  | arabinosyltransferase                         | <i>rv3793</i>                           | MMARE11_51760                                              | ethambutol               |
| OCU_RS40185 | <i>inhA</i>  | enoyl-[acyl-carrier-protein] reductase FabI   | <i>rv1484</i>                           | MMARE11_22120                                              | isoniazid/ethionamide    |
| OCU_RS41875 | <i>dfrA</i>  | dihydrofolate reductase                       | <i>rv2763c</i>                          | NH                                                         | para aminosalicylic acid |
| OCU_RS45570 | <i>alr</i>   | alanine racemase                              | <i>rv3423c</i>                          | MMARE11_10770                                              | cycloserine              |
| OCU_RS46230 | <i>rpoB</i>  | DNA-directed RNA polymerase subunit $\beta$   | <i>rv0667</i>                           | MMARE11_09460                                              | rifampin                 |
| OCU_RS48685 | <i>mmpL3</i> | membrane protein mmpL3                        | <i>rv0206c</i>                          | MMARE11_04100                                              | SQ109                    |

NH: no homology more than 80% of percent identity of nucleotide sequence

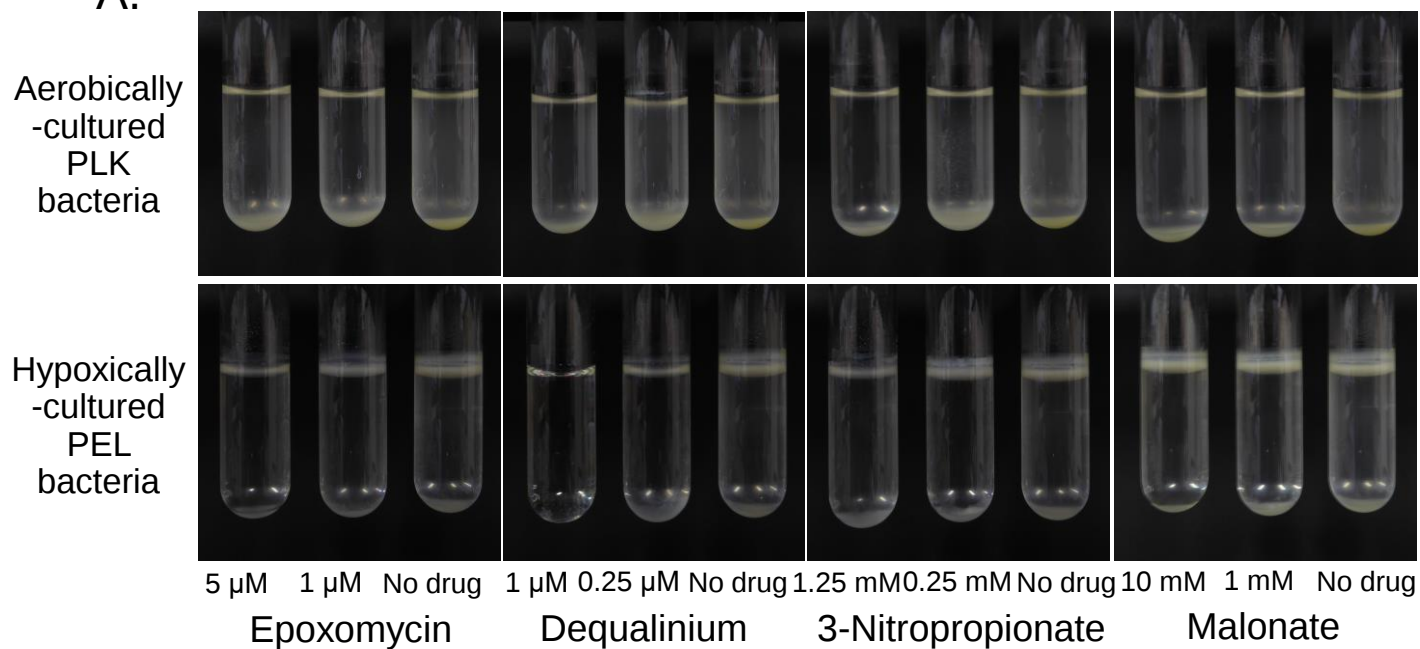
**Table S5.** Result of next-generation sequencing and mapping data of planktonic bacteria (PLK) and pellicle bacteria (PEL)

| Library | No. of sequence reads | No. (%) of aligned reads | No. (%) of insertion site flanking sequences hit in library | Average no. of reads/flanking sequence |
|---------|-----------------------|--------------------------|-------------------------------------------------------------|----------------------------------------|
| PLK1    | 10,932,182            | 5,539,472 (54.9%)        | 38,430 (59.8%)                                              | 201                                    |
| PLK2    | 8,579,984             | 5,175,328 (64.3%)        | 34,356 (53.4%)                                              | 234                                    |
| PLK3    | 7,817,364             | 4,700,645 (64.2%)        | 36,225 (56.3%)                                              | 217                                    |
| PEL1    | 9,271,142             | 5,355,509 (61.7%)        | 18,270 (28.4%)                                              | 491                                    |
| PEL2    | 9,634,505             | 5,570,149 (61.4%)        | 18,142 (28.2%)                                              | 532                                    |
| PEL3    | 9,914,886             | 5,542,869 (59.8%)        | 16,595 (25.8%)                                              | 506                                    |

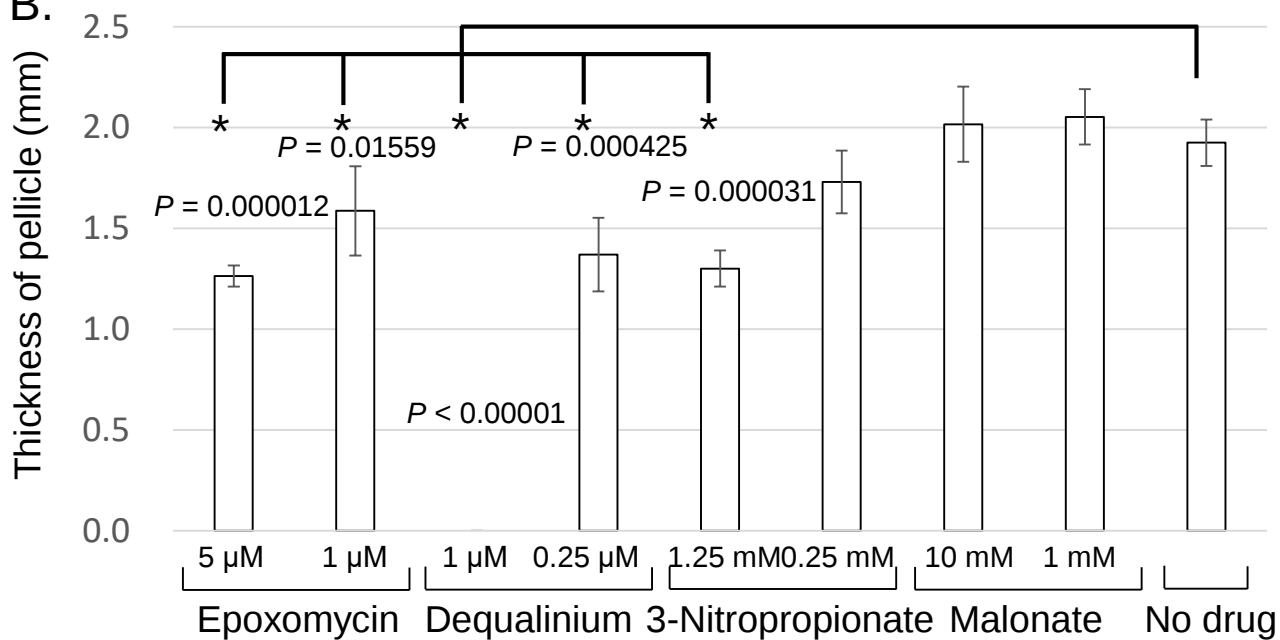


**Fig S3**

A.



B.



**Fig S4**