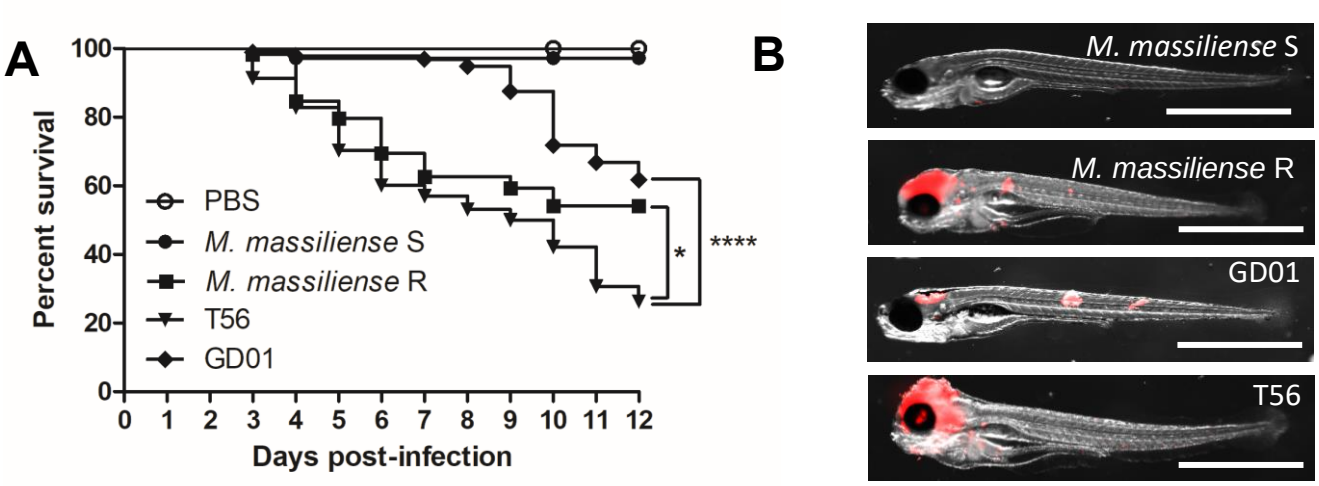


**Fig. S1. Comparative virulence of various *M. massiliense* strains in zebrafish embryos. (A)** Zebrafish embryos at 30 hpf were infected *via* caudal vein injection with approximately 200-250 CFU of each corresponding strain. Embryonic survival was monitored daily over a 12-day period. Represented data is the merged of 4 independent experiments, with approximately 20 embryos per group. * $P < 0.05$, **** $P < 0.001$. **(B)** Representative images taken at 5 dpi, highlighting the heterogeneity in disease phenotypes between the different isolates. Scale bars represent 1 mm. Red areas highlight the bacterial localisation using fluorescence microscopy. Data shown is the merge of three independent experiments (n=30/group for each replicate).

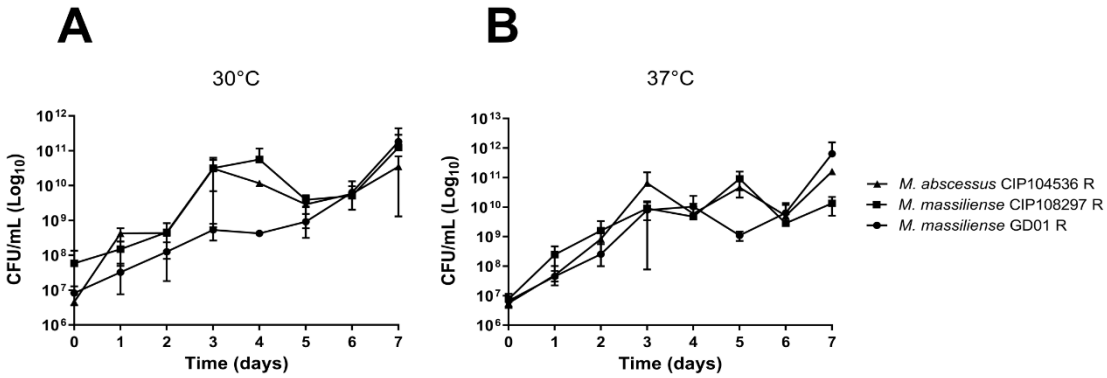


**Fig. S2. Growth curves of rough strains carrying pTEC27 at 30°C and 37°C.** *M. abscessus* CIP104536$^{T}$, *M. massiliense* CIP108297 and GD01 were grown for 7 days in Middlebrook 7H9 supplemented with OADC, 0.025% tyloxapol and 500 µg/ml hygromycin. CFU counts determined by plating serial dilutions onto in Middlebrook 7H10 and enumeration of the colonies after 4 days of incubation at 30°C **(A)** or 37°C **(B)**. Data shown is the mean ± standard deviation of two independent experiments run in triplicate.

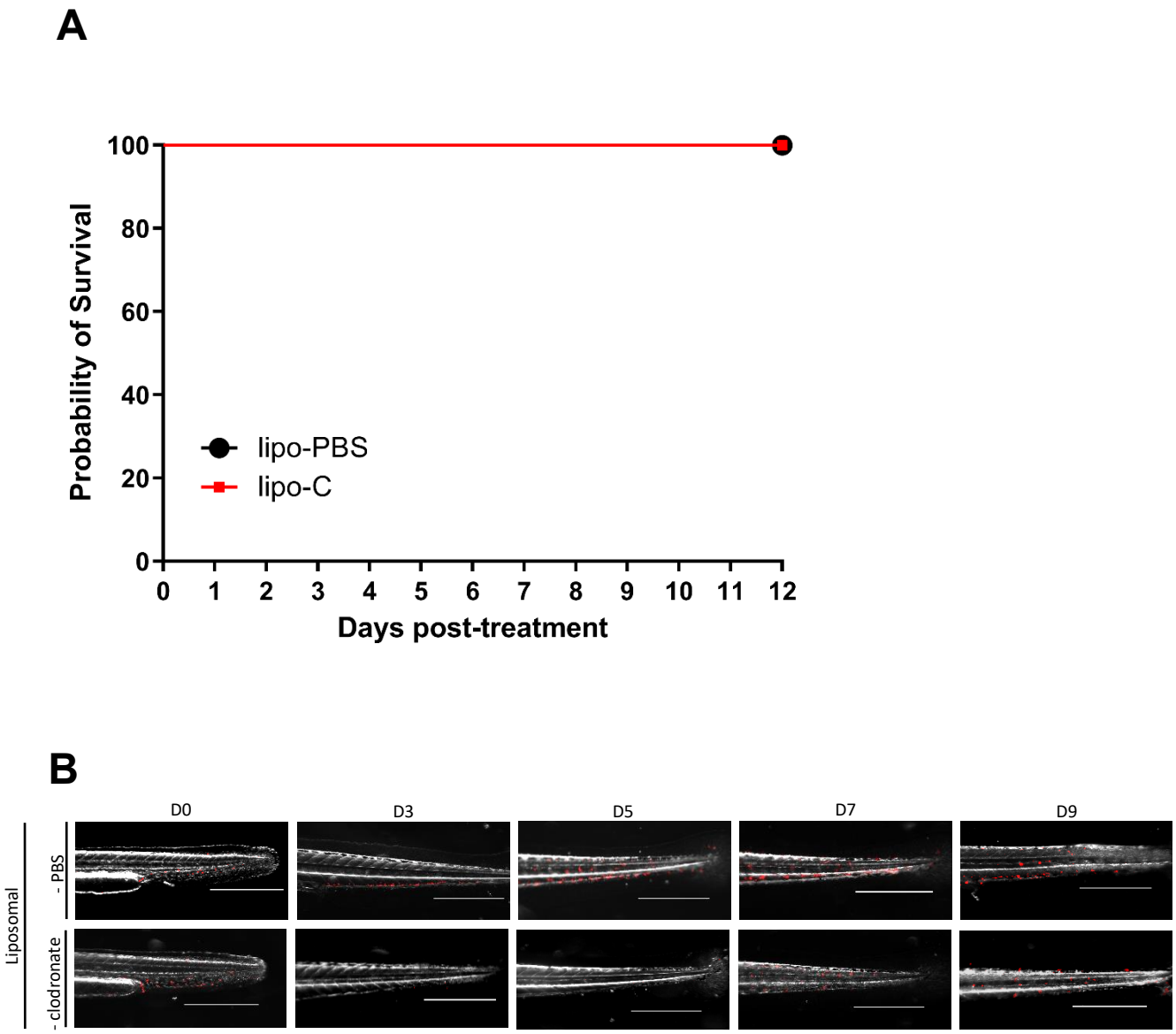


**Fig. S3. Impact of liposomal-clodronate on zebrafish survival and macrophage depletion.** Tg(*mpeg1:mCherry)* embryos harbouring red fluorescent macrophages were injected with 3 nL of either liposomal-PBS (lipo-PBS) or liposomal-clodronate (lipo-C) in the caudal vein at 24 hpf. **(A)** Embryo survival was monitored daily over a 12 day period (n=30/group). **(B)** Representative images of zebrafish tails showing transient macrophage depletion with lipo-clodronate. Macrophages are labelled in red. Scale bars represent 500 µm.

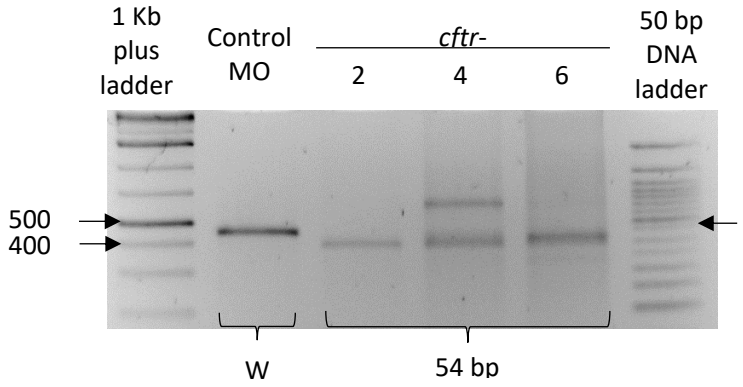


**Fig. S4. Targeted knockdown of cftr using splicing morpholino in embryos.** *cftr* specific products were amplified from RNA isolated from whole embryos at 2, 4 and 6 dpf. The *cftr*-MO blocks normal splicing resulting a deletion in exon 3, resulting in a 54 bp deletion in exon 3, and leading to the knockdown of CFTR expression (Bernut et al., 2019; Johansen and Kremer, 2020a).

**Table S1.** Drug susceptibility/resistance profile of *M. massiliense* GD01. Rough *M. abscessus* CIP104536$^{T}$ and *M. massiliense* CIP108297$^{T}$ were included for comparison. MIC (µg/mL) were determined after 4 days of incubation, following the CLSI guidelines.

| Strain (morphotype) | MIC (µg/mL) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **IPM** | **CFX** | **CLR[1]** | **TGC** | **CFZ** | **AMK** | **CIP** | **ZEO** | **KAN** | **BDQ** | **LNZ** | **GEN** | **RFB** | **Cpd12** | **E JMCh-6** |
| *M. abscessus* (R) | 16 | 64 | 4 | 1 | 1 | 64 | 128 | 32 | 16 | 0.03 | 64 | 64 | 12.5 | 0.06 | 0.125 |
| *M. massiliense* (R) | 32 | 64 | 0.5 | 2 | 0.5 | 32 | 64 | >128 | 16 | 0.06 | 64 | 64 | 25 | 0.06 | 0.125 |
| GD01 (R) | 32 | 64 | >128 | 2 | 0.5 | >128 | 64 | >128 | >128 | 0.015 | 64 | > 128 | 25-50 | 0.25 | 0.125-0.25 |

IPM, imipenem; CFX, cefoxitin; CLR, clarithromycin; TGC, tigecycline; CFZ, clofazimine; AMK, amikacin; CIP, ciprofloxacin; ZEO, zeocin; KAN, kanamycin; BDQ, bedaquiline; LNZ, linezolid; GEN, gentamycin; RFB, rifabutin; Cpd12, indole-2 carboxamide; EJMCh-6, benzimidazole.