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| **Supplementary File 1 – supplementary file 1a. *D. discoideum* material used in this study.** |
| **Strain** | **Characteristics** | **Source / Reference** |
| Ax2(Ka) | wt, parental strain of the *trafE* KO, *atg1* KO and GFP-trafE KI |  |
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| **Plasmids** |  |  |
| GFP-TrafA | pDM317-trafA, G418r | This study |
| GFP-TrafB | pDM317-trafB, G418r | This study |
| GFP-TrafC | pDM317-trafC, G418r | This study |
| GFP-TrafD | pDM317-trafD, G418r | This study |
| GFP-TrafE | pDM317-trafE, G418r | This study |
| dsRed-TrafE | pDM318-trafE, G418r | This study |
| ALIX-GFP | pDM1515-ALIX, Hygr | This study |
| GFP-Vps32 | pDM1513-Vps32, Hygr | This study |
| GFP-Vps4 | pDM1513-Vps4, Hygr | This study |
| GFP-Atg8a | pDM1513-Atg8a, Hygr | This study |
| mCherry-Plin | pDM1042-plin, Hygr | This study |
| pKOSG-trafE\_KO | *trafE* knock-out plasmid, Blasticidinr | This study |
| pPI183-trafE | *trafE* GFP knock-in plasmid, G418r | This study |

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| **Supplementary File 1 – supplementary file 1b. *M. marinum* material used in this study.** |
| **Strain** | **Characteristics** | **Source / Reference** |
| M | wt, parental strain | L. Ramakrishnan (University of Cambridge) |
| ∆RD1 | RD1 locus ablation mutant | L. Ramakrishnan (University of Cambridge) (Volkman et al., 2004) |
|  |  |  |
| **Plasmids** |  |  |
| pMSP12::DsRed/GFP | DsRed/GFP under control of the msp12 promoter, KanR | Addgene #30171 and #30167 (Cosma et al., 2004) |
| pCherry10 | mCherry under control of the G13 promoter, Hygr | Addgene #24664 (Carroll et al., 2010) |
| pMV306hsp+LuxG13 | Luciferase under control of theG13promoter, KanR | Addgene # 26161 (Andreu et al., 2010) |

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| **Supplementary File 1 – supplementary file 1c. Primers used in this study.** |
| **Primers** | **Sequence (5’-3’)** | **Purpose** |
| LR7F | CAGGATCCAAAATGGATATTTCTCAAATCC | DDB\_G0272454 / *trafA* 5' BamHI  |
| LR7R | CAACTAGTATGTTTATCACATTGAGAC | DDB\_G0272454 / *trafA* 3’ SpeI |
| LR8F | CAGGATCCAAAATGACAGAGTTTAAAATTAG | DDB\_G0285149 / *trafB* 5' BamHI |
| LR8R | CAACTAGTTTTAGTAGTTAAAGGATC | DDB\_G0285149 / *trafB* 3' SpeI |
| LR9F | CAGGATCCAAAATGTCAATTGATATAAAATTTAC | DDB\_G0290883 / *trafC* 5' BamHI  |
| LR9R | CAACTAGTAGACTCCAATGGTTCATATTC | DDB\_G0290883 / *trafC* 3' SpeI |
| LR9/10F | CAGGATCCAAAATGTCAATTGATATAAAATTTAC | DDB\_G0290883 / *trafD* 5' BamHI |
| LR10R | CAACTAGTAGACTCCAATGGTTCATATTC | DDB\_G0290961 / *trafD* 3' SpeI |
| LR32F | CAGGATCCAAAATGACAGTAAAATATTCAATTAATG | DDB\_G0290971 / *trafE* 5’ BamHI |
| LR32R | CAACTAGTTGGTAAAACTTGAATTCTAAG | DDB\_G0290971 / *trafE* 3’ SpeI |
| LR60F | AGCGCGTCTCCAATG CTGCAG GAATTATTATCAATTTATTTAGGTTGGATTTGGTC | *trafE* KO left arm forward; CS1, PstI |
| LR60R | AGCGCGTCTCCGTTG GTTCATTAATTGAATATTTTACTGTCATTTTATG | *trafE* KO left arm reverse; CS2 |
| LR61F | AGCGCGTCTCCCTTC GTTATTGGTCTTAGAATTCAAGTTTTAC | *trafE* KO right arm forward; CS3 |
| LR61R | AGCGCGTCTCCTCCC CTGCAG CAATTTTATATTGGTGGTGTATTTGATATTC | *trafE* KO right arm reverse; CS4, PstI |
| LR62F | GTAATAATAATCAACAAGAACAAAAACAAGAATATC | *trafE* gDNA upstream of KO Left Arm |
| LR62R | CTGCTGATGCTGTTGAGTAAG | *trafE* gDNA downstream of KO Right Arm |
| LR63F | GAGTCTTGTAAAAAATCATTCCCAAG | *trafE* qPCR & wt-positive screen |
| LR63R | GTTGGTTATTTATAACTTTGTCCATC | *trafE* qPCR & wt-positive screen |
| LR68F | GAAGGTATTCAAATCCTAAAGGATAATATG | 3' of DDB\_G0290973, upstream of *trafE*, use with BSR-R and qPCR primer LR63R |
| LR69F | CATGTGGTGTATCAAACTTTGTTTC | 3' of DDB\_G0290957, downstream of *trafE*, use with BSR-F and qPCR primer LR63F |
| LR70F | CAAATGGTTGGTTAACTGATGAAG | wt-positive combine with LR62R |
| LR70R | GTAAAATTATCTGATAGTGATTCATGATCTAC | wt-positive combine with LR62F |
| LR71F | CAGGTACCGAGTCTTGTAAAAAATCATTCCCAAG | *trafE* 3' KpnI on 5' |
| LR71R | CAAGATCTTGGTAAAACTTGAATTCTAAGACCAATAAC | *trafE* 3' BglII on 3' |
| LR72F | CAGTCGACAATAAAAAAAAAAAATAAAAAAAACAAATCCAATTATTTTTATATTAAATAATTAAAATAAATGTAAC | *trafE* UTR 5' SalI |
| LR72R | CAGAGCTCCTGCTGATGCTGTTGAGTAAG | *trafE* UTR 3' SacI |
| LR99F | CAAGATCTATGGTTCATGTATCAAGCTTTAAAAAC | *Atg8a* 5' BamHI |
| LR99R | CTACTAGTTAAATCACTACCAAAAGTATTTTCACCAC | *Atg8a* 3' SpeI |
| LR105F | CA AGATCTAAA ATGAAACTCTTTGGTAAACCAAAACCAAAAC | *Vps32* 5' BamHI |
| LR105R | CTACTAGTCATTGCTAAAGATTCTTCCAAAGCTCTAATC | *Vps32* 3' SpeI |

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