

ATG7 and ATG14 restrict cytosolic and phagosomal *Mycobacterium tuberculosis* replication in human macrophages

In the format provided by the
authors and unedited

ATG7 and ATG14 restrict cytosolic and phagosomal *M. tuberculosis* replication in human macrophages

Beren Aylan^{1*}, Elliott M. Bernard^{1*#}, Enrica Pellegrino^{1*} et al.,

Supplementary Legends

Supplementary Movie 1- Live imaging of Mtb replication in WT and ATG14 KO iPSDM

Imaging was performed using the OPERA Phenix microscope with 40× 1.1NA water-immersion objective with a 10% overlap between adjacent fields. Maximum projection of 5 planes with 1µm distance from a single field of view were monitored every 1.5 h for 96 h. For imaging on the Opera Phenix, Brightfield was detected using $\lambda_{ex} = \text{Transmission}/\lambda_{em} = 650\text{-}760\text{nm}$, PI (Red) was detected using $\lambda_{ex} = 561\text{nm}/\lambda_{em} = 570\text{-}630\text{nm}$ and E2-Crimson Mtb (Green) was detected using $\lambda_{ex} = 640\text{ nm}/\lambda_{em} = 650\text{-}760\text{ nm}$ using a 16 bits sCMOS camera.

Supplementary Table 1. Primers and primer sequences used in this study.

Primer Name	Sequence 5'→3'
Oligo_orbit_cpsA	GCGCGCCAGTTGGTTCGACGGCGCCTGGCCGAGGCATATGATGGC GCGTTCTGAGGGCAATCGCCACGCGGTTTGTCTGGTCAACCACC GCGGTCTCAGTGGTGTACGGTACAAACCATCGATGGCGGCGGCGT GCCCTGCGTGAAGTAGGTAAGTTATCCGACCACTCCACGCAGCCC GTCGGCGC
Oligo_orbit_esxBA	ACATTTTGGCGAGGAAGGTAAAGAGAGAAAGTAGTCCAGCATGG CAGAGATGAAGACCGATGCCGCTACCGGTTTGTCTGGTCAACCAC CGCGGTCTCAGTGGTGTACGGTACAAACCACCGAAGGCAACGTCA CTGGGATGTTTCGCATAGGGCAACGCCGAGTTCGCGTAGAATAGCG AAACACGGG
cpsA_delver_1	CGTCTTGCACCGTCACCAG
cpsA_delver_2	GCGGTTTCGTTTCGTCGGCATC
cpsA_delver_4	GAAAGAGCTCGAGACCCGGG
RD1_ver_1	GCTAAATACCGCACGGCTGA
RD1_ver_2	GATCAGCTGAGACATCAGCG
oriE-up	ACGCCTGGTATCTTTATAGTCC
ATG7_GF2	AGGATGGTTTTCAAAGTTGTGTCTT
ATG7_GR2	ACAGTGGCTTGTATACAGAAGTAGA
ATG14_GF1	TCTGTCTGAGAGTTTACAGCTTGTT
ATG14_GR1	TGCCTCACAACACACAATAAAA

Supplementary Table 2. Monocyte and macrophages medium.

XVIVO-Factory medium	OXM-Factory medium
X-VIVO 15 (Lonza, BE02-060F)	DMEM/F-12 (Gibco, 12634010)
2mM GlutaMAX (Gibco, 35050-038)	2mM GlutaMAX (Gibco, 35050-038)
50 μ M β -mercaptoethanol (Gibco, 21985023)	50 μ M β -mercaptoethanol (Gibco, 21985023)
100 ng/ml hM-CSF (Peprotech, 300-25)	15mM HEPES (Gibco, 15630080)
25 ng/ml hIL-3 (Peprotech, 200-03)	5 μ g/mL human insulin (Sigma, T89702-1G)
	100 ng/ml hM-CSF (Peprotech, 300-25)
	25 ng/ml hIL-3 (Peprotech, 200-03)
XVIVO-differentiation medium	OXM-differentiation medium
X-VIVO 15 (Lonza, BE02-060F)	DMEM/F-12 (Gibco, 12634010)
2mM GlutaMAX (Gibco, 35050-038)	2mM GlutaMAX (Gibco, 35050-038)
50 μ M β -mercaptoethanol (Gibco, 21985023)	50 μ M β -mercaptoethanol (Gibco, 21985023)
100 ng/ml hM-CSF (Peprotech, 300-25)	15mM HEPES (Gibco, 15630080)
	5 μ g/mL human insulin (Sigma, T89702-1G)
	100 ng/ml hM-CSF (Peprotech, 300-25)

Supplementary Table 3. Guide RNAs and their sequences used in this study.

Name sgRNAs	ID (WGE)	Sequence 5'->3'
CRISPR1-ATG7	950233638	TTATTTTGAGGTAAACAGTT
CRISPR2-ATG7	950233647	TAACATATCCATGCAAAACC
CRISPR3-ATG7	950233717	CTCCCTCATAGGTGGACCAC
CRISPR4-ATG7	950233744	ATCAGAGTCAATTAGGTGCC
CRISPR1_ATG14	1117404230	ATCGAGTTTAAGTTTAGCAC
CRISPR2_ATG14	1117404227	AACCATGATTAAAATGACTC
CRISPR3_ATG14	1117404194	TAAGCAAGTCTTTTGACTCC
CRISPR4_ATG14	1117404186	GGGTCTTATTCTAGGGTCAT
ATG5_ex1_sg1	1013029311	GTGCTTCGAGATGTGTGGTT
ATG5_ex2_sg1	1013028724	AAGAGTAAGTTATTTGACGT
ATG5_ex1_sg2	1013029309	AACTTGTTTCACGCTATATC
ATG5_ex2_sg2	1013028721	GAGATATGGTTTGAATATGA
CRISPR_1_ATG14_pool	1117404214	TGAAGGCCTTCTCAAACCA

CRISPR_2_ATG14_pool	1117404210	ACAACGGCACCAAGAGAAAA
CRISPR_3_ATG14_pool	1117404200	CTCGATTGGAAAAATGACAG
CRISPR_1_ATG7_pool	950233651	AGAAATAATGGCGGCAGCTA
CRISPR_2_ATG7_pool	950233657	TGCCCCTTTTAGTAGTGCCT
CRISPR_3_ATG7_pool	950233668	GAAGCTGAACGAGTATCGGC

Supplementary Table 4. FACS Antibodies used in this study.

Antibody	Clone	Cat#
CD14-Alexa488	MΦP9	562689
CD119-PE	GIR-208	558934
CD86-BV421	2331	562433
CD11b-bv421	ICRF44	562632
CD163-FITC	GHI/61	563697
CD169-PE	7-239	565248
CD206-APC	19.2	561763
CD16-Alexa647	3G8	557710
Alexa488 isotype		557703
Alexa647 isotype		557714
PE isotype		12-4015-82
BV421 isotype		562438