SUPPLEMENTAL MATERIAL

Baron et al., https://doi.org/10.1084/jem.20160662

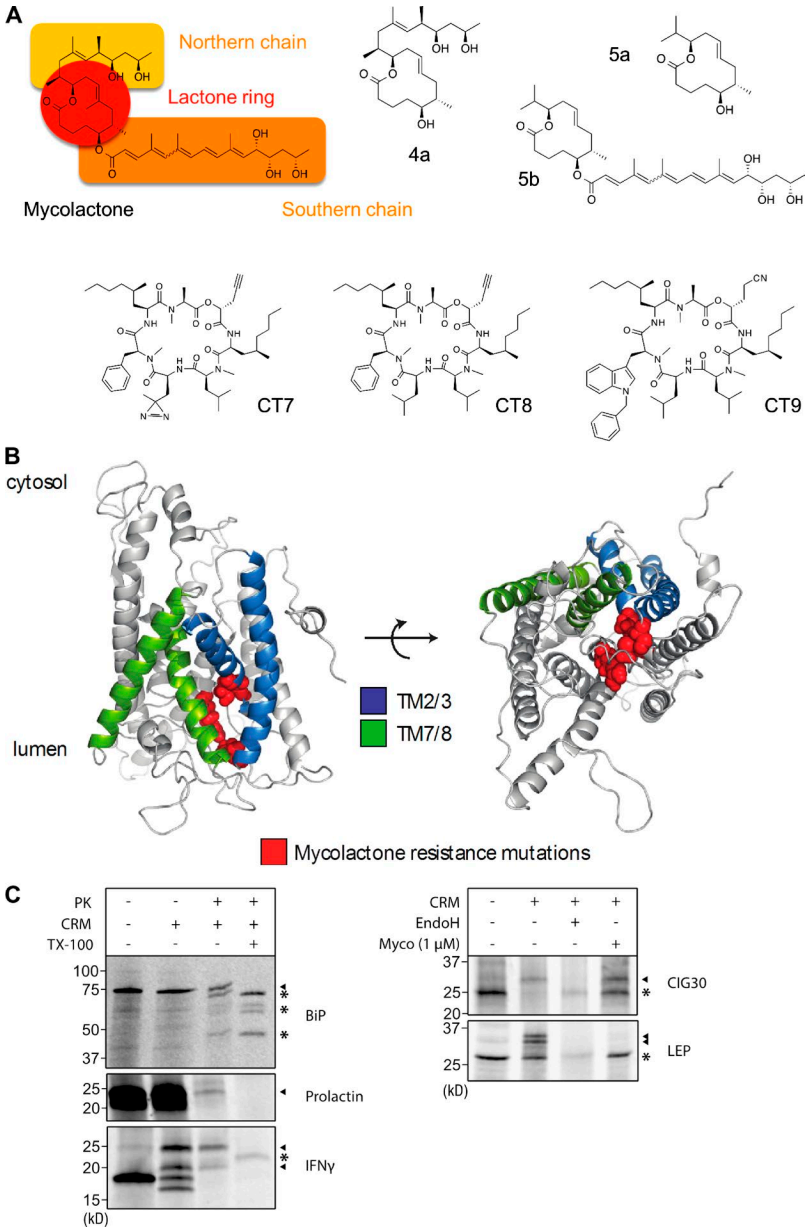

Figure S1. **Mycolactone and cotransin analogs used in this study, localization of mycolactone-resistance mutations in Sec61α and IVT controls.** (A) Chemical structures of natural mycolactone (A/B form; from *M. ulcerans* strain 1615), synthetic subunits of mycolactone, and cotransin variants used in this study. (B) Mutations associated with mycolactone resistance are located near the lumenal plug of Sec61α. A homology model of human Sec61α showing the location of mycolactone-resistance mutations (red) is shown. Lateral gate helices and hydrophobic transmembrane domains TM2/3 and TM7/8 are colored in blue and green, respectively. (C, left) ER translocation of BiP, prolactin, and IFN-γ were confirmed by proteinase K (PK) treatment in the presence and absence of detergent (TX-100). Correctly translocated protein species are indicated with arrowheads. Protease-resistant fragments are indicated with asterisks. ER translocation of these nonglycosylated proteins does not cause a change in band mobility, and resistance to proteinase K was used to indicate the fraction of ER-translocated polypeptide. Treating microsomes with proteinase K and detergent abolished the protected species, indicating correct translocation to the lumen of ER microsomes. (Right) Glycosylation of CIG30 and LEP was assessed by EndoH treatment. Membrane-integrated, glycosylated protein species are indicated with arrowheads and nonintegrated species are indicated with an asterisk. Difference in band mobility indicates that these proteins become glycosylated within the ER lumen upon correct membrane integration. EndoH treatment demonstrates that the altered mobility is caused by protein glycosylation. Myco, mycolactone.

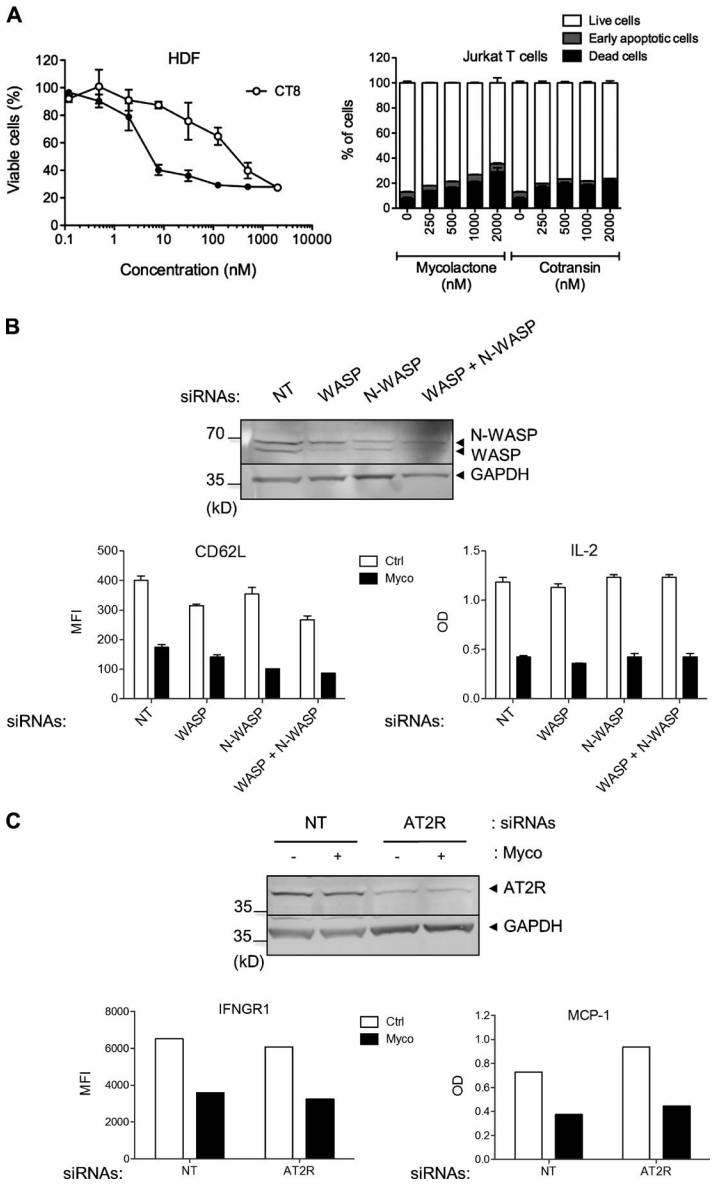

Figure S2. **Cytotoxicity of mycolactone and CT8 and contribution of WASP/N-WASP and AT2R to mycolactone effects on secretory protein production.** (A) Differential cytotoxicity of mycolactone and CT8. (Left) Cell viability, as assessed by methyl-thiazolyl-tetrazolium reduction of human primary dermal fibroblasts (HDF) incubated with mycolactone, CT8, or solvent for 72 h. (Right) Induction of apoptosis in Jurkat T cells incubated with mycolactone, CT8, or solvent for 48 h. Annexin V^+/propidium iodide $(PI)^-$ cells were identified as early apoptotic cells, annexin V^+/PI^+ cells as late apoptotic (dead) cells, and annexin V^-/PI^- cells as live cells. Data are mean percentages ± SD of duplicates relative to solvent and are representative of three independent experiments. (B) Mycolactone (Myco)-mediated inhibition of secreted and membrane protein production is WASP/N-WASP independent. Western blot analysis of total WASP, N-WASP, and GAPDH as loading control in Jurkat T cells transfected with siRNAs targeting WASP, N-WASP, or both proteins or nontargeting (NT) siRNAs as controls for 48 h is shown. (Left) Flow cytometric analysis of CD62L surface expression in siRNA-transfected Jurkat T cells exposed to 25 nM mycolactone or solvent (Ctrl) for 16 h. Mean fluorescent intensities (MFI) ± SD of duplicates are shown. (Right) IL-2 production by siRNA-transfected Jurkat T cells treated with 25 nM mycolactone or solvent for 1 h before activation with PMA/IO for 16 h. Mean OD ± SD of duplicates is shown. Data are representative of two independent experiments. (C) Mycolactone-mediated inhibition of secretory protein production is AT2R independent. Western blot analysis of total AT2R and GAPDH as loading control in HeLa cells transfected with siRNAs targeting AT2R or nontargeting siRNAs as controls for 60 h and then incubated with 50 nM mycolactone or solvent for 16 h is shown. (Left) Flow cytometric analysis of IFNGR1 surface expression in siRNA-transfected HeLa cells exposed to 50 nM mycolactone or solvent for 16 h. Mean fluorescent intensities are shown. (Right) MCP-1 production by siRNA-transfected HeLa cells treated with 50 nM mycolactone or solvent for 16 h. Mean OD ± SD of duplicates is shown. Data are representative of two independent experiments.

Table S1. **Proteomic profiling of mycolactone-exposed Jurkat T cells**

Accession[a]	Gene[a]	Full protein name[a]	Mean[b]	SD[b]	GO_CC[a]	SS or TMD	Protein type	IFN-γ induced[c]
Down-regulated								
Q96PP8	**GBP5**	**Guanylate-binding protein 5**	**−2.76**	**0.75**	**Cytoplasm, membrane**			**+**
Q07108	CD69	Early activation antigen CD69	−2.90	0.47	Plasma membrane	TMD	SP II	–
P13746	**HLA-A**	**HLA class I histocompatibility antigen, A-11 α chain**	**−1.91**	**0.84**	**ER, golgi, plasma membrane**	**SS**	**SP I**	**+**
P32456	**GBP2**	**Interferon-induced guanylate-binding protein 2**	**−2.29**	**0.06**	**Cytoplasm, nucleus, golgi**			**+**
P10321	**HLA-C**	**HLA class I histocompatibility antigen, Cw-7 α chain**	**−2.12**	**0.10**	**ER, golgi, plasma membrane**	**SS**	**SP I**	**+**
O95727	CRTAM	Cytotoxic and regulatory T cell molecule	−2.79	1.35	Plasma membrane	SS	SP I	–
Q92854	SEMA4D	Semaphorin-4D	−1.68	0.19	Plasma membrane	SS	SP I	–
P42224	**STAT1**	**HUMAN signal transducer and activator of transcription 1-α/β**	**−1.70**	**0.03**	**Cytoplasm, nucleus**			**+**
P01850	TRBC1	T cell receptor β-1 chain C region	−1.47	0.34	Plasma membrane	TMD	SP	–
P61769	**B2M**	**β-2-microglobulin**	**−1.55**	**0.14**	**Secreted**	**SS**		**+**
O43736	ITM2A	Integral membrane protein 2A	−1.56	0.05	Membrane	TMD	SP II	–
P09693	CD3G	T cell surface glycoprotein CD3 γ chain	−1.30	0.36	Plasma membrane	SS	SP I	–
P01737	TCRA	T cell receptor α chain V region PY14	−1.39	0.17	Plasma membrane	SS		–
P04234	CD3D	T cell surface glycoprotein CD3 δ chain	−1.29	0.02	Plasma membrane	SS	SP I	–
P32455	**GBP1**	**Interferon-induced guanylate-binding protein 1**	**−1.37**	**0.21**	**Cytoplasm, golgi, secreted**			**+**
P27701	CD82	CD82 antigen	−0.90	0.35	Plasma membrane	TMD	MP	–
O94901	SUN1	SUN domain–containing protein 1	−1.02	0.18	Nucleus membrane	TMD	SP II	–
Q8TDB6	**DTX3L**	**E3 ubiquitin–protein ligase DTX3L**	**−1.06**	**0.08**	**Cytoplasm, nucleus**			**+**
P04439	**HLA-A**	**HLA class I histocompatibility antigen, A-3 α chain**	**−1.12**	**0.11**	**Plasma membrane**	**SS**	**SP I**	**+**
Q8IXQ6	**PARP9**	**Poly [ADP-ribose] polymerase 9**	**−1.17**	**0.20**	**Cytoplasm, nucleus**			**+**
Q03518	TAP1	Antigen peptide transporter 1	−1.20	0.25	ER, membrane	TMD	MP	–
P42892	ECE1	Endothelin-converting enzyme 1	−1.09	0.16	Plasma membrane	TMD	SP II	–
O75787	ATP6AP2	Renin receptor	−0.99	0.03	Plasma membrane	SS	SP I	–
P13598	ICAM2	Intercellular adhesion molecule 2	−0.92	0.06	Plasma membrane	SS	SP I	–
P43489	TNFRSF4	Tumor necrosis factor receptor superfamily member 4	−1.35	0.65	Plasma membrane	SS	SP I	–
P30533	LRPAP1	α-2-macroglobulin receptor-associated protein	−0.89	0.00	ER, cytoplasm	SS		–
Q9BQE5	APOL2	Apolipoprotein L2	−0.88	0.00	Cytoplasm			–
Q15904	ATP6AP1	V-type proton ATPase subunit S1	−0.75	0.13	Membrane, vacuole	SS	SP	–
O14672	ADAM10	Disintegrin and metalloproteinase domain– containing protein 10	−0.87	0.04	Plasma membrane	SS	SP I	–
P11021	HSPA5 (BiP)	78-kD glucose-regulated protein	−0.76	0.07	ER lumen	SS		–
O75976	CPD	Carboxypeptidase D	−0.84	0.10	Membrane	SS	SP I	–
P48723	HSPA13	Heat shock 70-kD protein 13	−0.90	0.19	ER	SS		–
Q460N5	PARP14	Poly [ADP-ribose] polymerase 14	−0.67	0.08	Nucleus, cytoplasm			–
O95399	UTS2	Urotensin 2	−0.74	0.04	Secreted	SS		–
Q96JJ7	TMX3	Protein disulfide-isomerase TMX3	−0.74	0.04	ER	SS	SP	–
P07766	CD3E	T cell surface glycoprotein CD3 ε chain	−0.68	0.02	Plasma membrane	SS	SP I	–
P06127	CD5	T cell surface glycoprotein CD5	−0.71	0.05	Plasma membrane	SS	SP I	–
Q13217	DNAJC3	DnaJ homolog subfamily C member 3	−0.59	0.08	ER	SS		–
Q8NHV1	GIMAP7	GTPase IMAP family member 7	−0.58	0.04	Lipid droplet, cytoplasm			–
Q6PIU2	NCEH1	Neutral cholesterol ester hydrolase 1	−0.64	0.05	Membrane	TMD	SP II	–
Q9UBV2	SEL1L	Protein sel-1 homolog 1	−0.59	0.02	ER	SS	SP I	–
P20645	M6PR	Cation-dependent mannose-6–phosphate receptor	−0.60	0.00	Membrane	SS		–
Q4G148	GXYLT1	Glucoside xylosyltransferase 1	−0.62	0.07	Membrane	TMD	SP II	–
Q99805	TM9SF2	Transmembrane 9 superfamily member 2	−0.57	0.01	Endosome, membrane	SS	MP	–
Q99519	NEU1	Sialidase 1	−0.74	0.26	Membrane	SS	MP	–
Q8NFQ8	TOR1AIP2	Torsin-1A–interacting protein 2	−0.69	0.18	ER	TMD	SP	–
P09326	CD48	CD48 antigen	−0.81	0.36	Plasma membrane	SS		–
P19474	**TRIM21**	**E3 ubiquitin–protein ligase TRIM21**	**−0.62**	**0.10**	**Cytoplasm, nucleus**			**+**
P80303	NUCB2	Nucleobindin 2	−0.54	0.00	Golgi, membrane, ER, nucleus	SS	MP	–
Q13308	PTK7	Inactive tyrosine-protein kinase 7	−0.59	0.07	Membrane	SS	SP I	–
Q03519	TAP2	Antigen peptide transporter 2	−0.58	0.10	ER, membrane	TMD	MP	–
P05107	ITGB2	Integrin β-2	−0.51	0.00	Plasma membrane	SS	SP I	–
Up-regulated								
Q96CX6	LRRC58	Leucine-rich repeat–containing protein 58	1.28	1.04	Unknown			–

Table S1. **Proteomic profiling of mycolactone-exposed Jurkat T cells** *(Continued)*

Accession[a]	Gene[a]	Full protein name[a]	Mean[b]	SD[b]	GO_CC[a]	SS or TMD	Protein type	IFN-γ induced[c]
P08107	HSPA1A	Heat shock 70-kD protein 1A/1B	0.91	0.11	Cytoplasm			–

Proteins that were downregulated or upregulated by mycolactone treatment are shown. Those induced by IFN-γ are bold. GO_CC, gene ontology cellular component; MP, multipass; SP I, single-pass type I; SP II, single-pass type II; SS, signal sequence; TMS, transmembrane domain.

[a]According to the database UniProt.

[b]Mean and SD of $\log_2$ mycolactone/control ratios from two SILAC experiments.

[c]Induced by IFN-γ (+) or not (–), according to the database Interferome.