# Mycolactone enhances the $Ca^{2+}$ leak from endoplasmic reticulum by trapping Sec61 translocons in a $Ca^{2+}$ permeable state

Pratiti Bhadra, Scott Dos Santos, Igor Gamayun, Tillman Pick, Clarissa Neumann, Joy Ogbechi, Belinda S. Hall, Richard Zimmermann, Volkhard Helms, Rachel E. Simmonds, Adolfo Cavalié

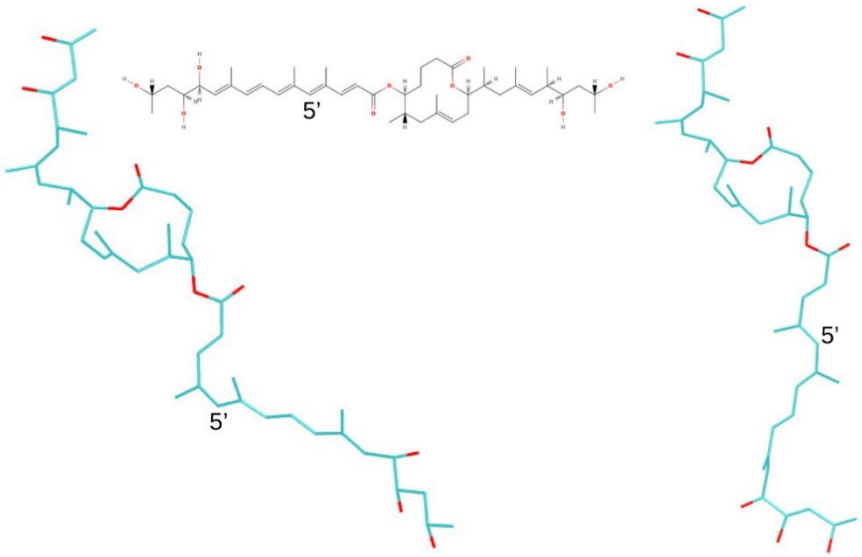
## Supplementary Figure

**Supplementary Figure S1.** Conformations of (a) mycolactone A (Z isomer) and (b) mycolactone B (E isomer).

**Supplementary Figure S2.** Docking regions in and around human Sec61α for (a) *idle* and *inhibited* states, and (b) for the *open* state.

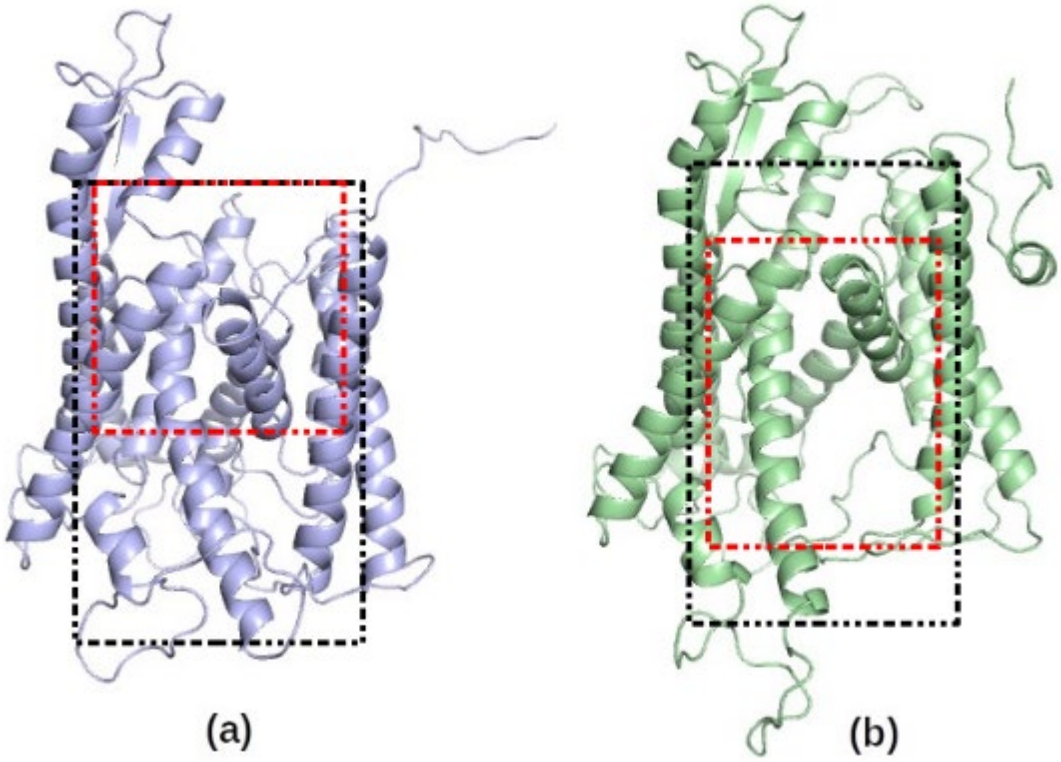
**Supplementary Figure S3.** Effects of mycolactone on cytosolic $Ca^{2+}$ transients of RAW 264.7 cells.

**Supplementary Figure S4.** Effects of the $Ca^{2+}$ leak enhancer trifluoperazine on the $Ca^{2+}$ mobilisation of HCT116 cells.

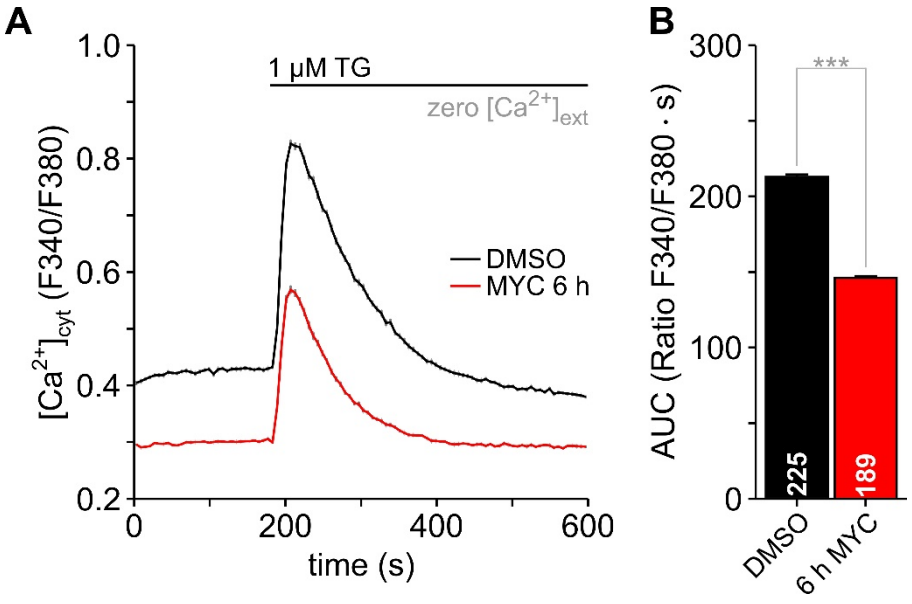



(a) Mycolactone A (Z isomer) (b) Mycolactone B (E isomer)

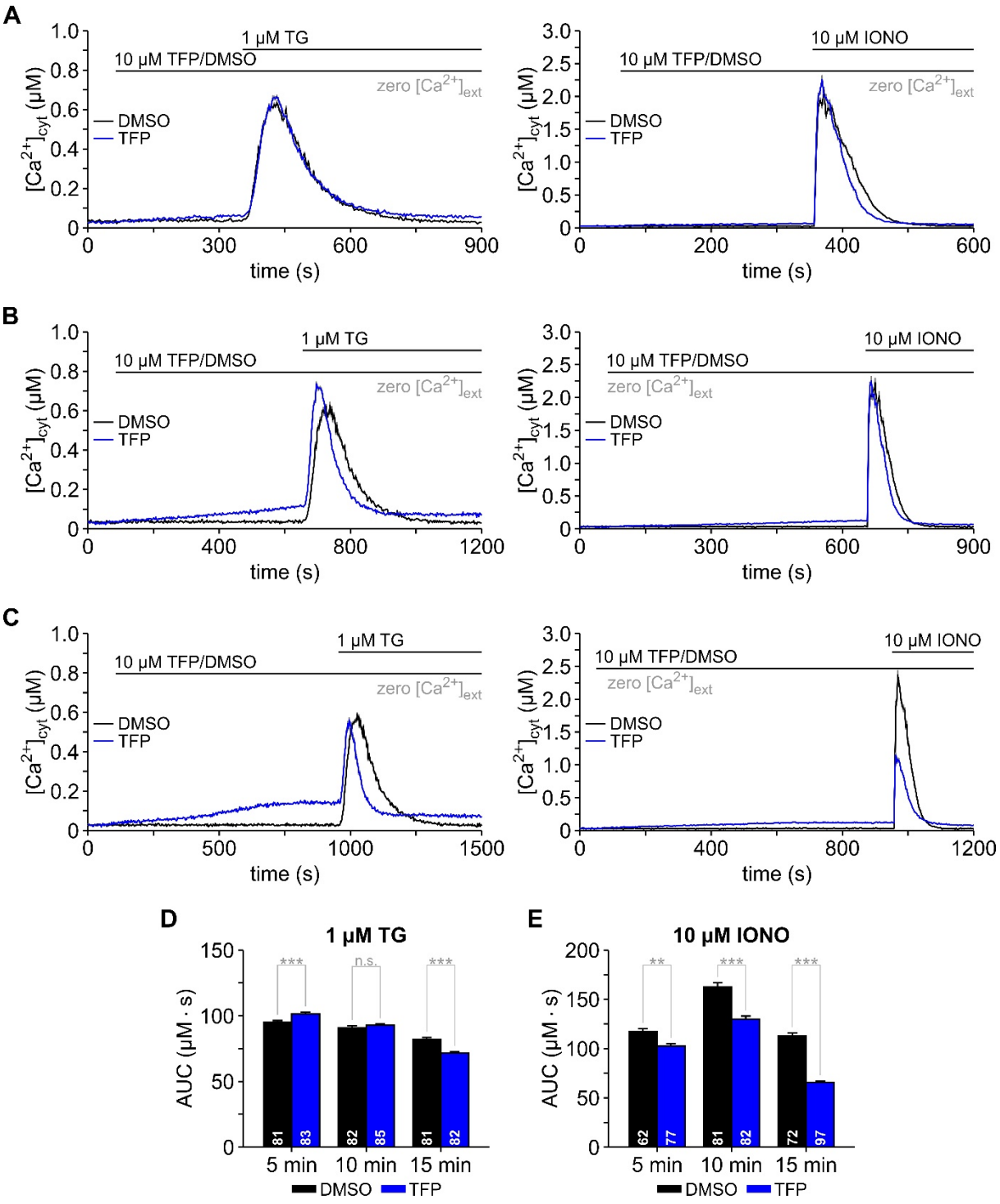
**Supplementary Figure S1. Conformations of (a) mycolactone A (Z isomer) and (b) mycolactone B (E isomer).** The images were generated using VMD-1.9.4 and online PubChem Sketcher V2.4.




**Supplementary Figure S2. Docking regions in and around human Sec61α for (a) *idle* and *inhibited* states, and (b) for the *open* state.** The black-dashed boxes represent the grid box (100 Å × 100 Å × 126 Å) used for the first stage of docking and the red-dashed boxes show the grid box used in the second (finer) docking stage (90 Å × 80 Å × 80 Å), (80 Å × 90 Å × 80 Å) and (80 Å × 80 Å × 90 Å) for *idle*, *intermediate,* and *open* state, respectively. The conformations in (a) and (b) are homology models of *idle* and *open* states, respectively.




**Supplementary Figure S3. Effects of mycolactone on cytosolic $Ca^{2+}$ transients of RAW 264.7 cells.** Cytosolic $Ca^{2+}$ was imaged with FURA-2 and changes in $[Ca^{2+}]_{cyt}$ are given as F340/F380 ratios. Cells were treated with 0.05 % DMSO or with 125 ng/ml mycolactone (MYC) for 6 h before FURA-2 loading and $Ca^{2+}$ imaging. $Ca^{2+}$ mobilisation was induced by applying thapsigargin (**A**, 1µM TG). The statistical analysis of the corresponding area under the curve (AUC) for DMSO and MYC treated RAW 264.7 cells is shown in **B**. Data is presented as means ± SEM; ***, $p<0.001$. The number of cells is given within the graph bars in **B**.




**Supplementary Figure S4. Effects of the $Ca^{2+}$ leak enhancer trifluoperazine on the $Ca^{2+}$ mobilisation of HCT116 cells**. Trifluoperazine (TFP) was used as a reference substance for comparison with the effects of mycolactone on ER $Ca^{2+}$ leak. $[Ca^{2+}]_{cyt}$ was imaged with FURA-2 in HCT116 cells that were exposed "online" to 10 µM TFP (TFP) or 0.05 % DMSO (DMSO) for 5 min (**A**), 10 min (**B**) and 15 min (**C**). At the end of the mycolactone treatment, $Ca^{2+}$

mobilisation was induced with thapsigargin (1 µM TG, *left panels*) or with ionomycin (10 µM IONO, *right panels*) to estimate the $Ca^{2+}$ leak from ER and the total $Ca^{2+}$ content in the cells, respectively. TFP effects on TG- and IONO-induced $Ca^{2+}$ transients were quantified as area under the curve (AUC) and are shown in **D** and **E**, respectively. The number of cells is given within the graph bars (**D**, **E**). Data is presented as means ± SEM; n.s., non-significant; **, $p<0.01$; ***, $p<0.001$.