

Supplementary Materials:

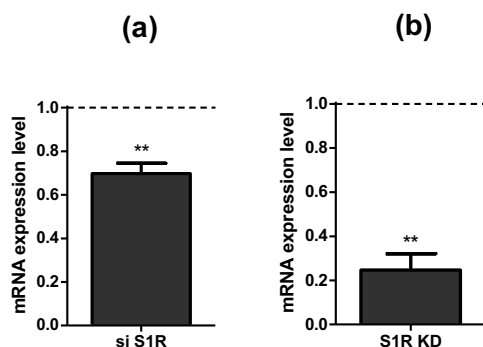


Figure S1. Knock-down efficiency of S1R siRNA in unsorted (a) and sorted (b) cells determined by qRT-PCR and normalized to housekeeping gene. Paired t-test, ** $p < 0.01$, $n = 5$ (a), $n = 3$ (b).

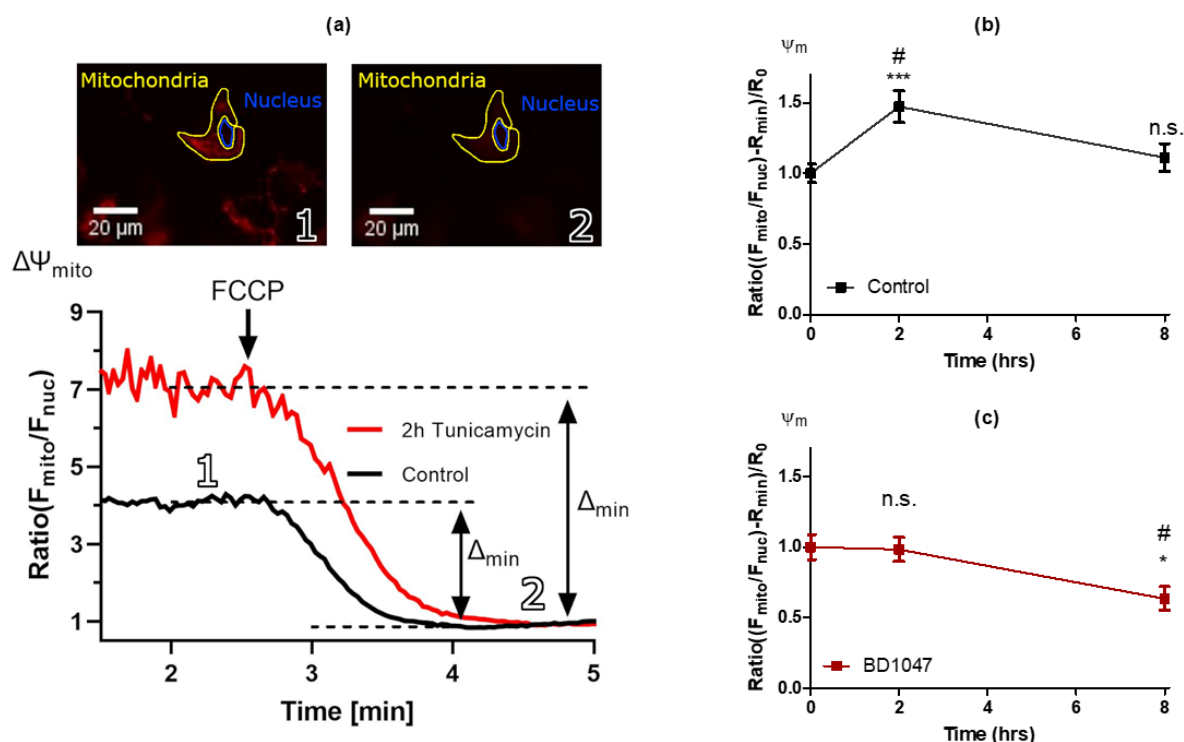


Figure S2. Protocol for Ψ_m measurements and the impact of BD1047 on Ψ_m . (a) Exemplary images of SH-SY5Y cells stained with TMRM (red) and selected regions of interest (ROI) for mitochondria and nucleus. Experimental protocol used to assess Ψ_m is shown below for 2 cells, one treated with DMSO (black line) and the other with tunicamycin for 2 hours (red line). Change in the TMRM fluorescence ratio of mitochondrial to nucleus ROI from basal to after FCCP treatment was used to estimate Ψ_m . Time course of Ψ_m after tunicamycin treatment, normalized to respective DMSO treated controls and presented as mean \pm SEM in control (b) and BD1047 treated (c) cells. One-way ANOVA with Tukey's multiple comparison test, *** $p < 0.001$, * $p < 0.05$, # $p < 0.05$ (2 h tunicamycin against 8 hr tunicamycin), n.s. – not significant; Control DMSO (62 cells/10 experiments), Control 2hr tunicamycin (37 cell/5 experiments), Control 8hr tunicamycin (42 cells/7 experiments), BD1047 DMSO (33 cells/5 experiments), S1R KD 2 h tunicamycin (26 cells/4 experiments), S1R KD 8 h tunicamycin (23 cells/4 experiments);

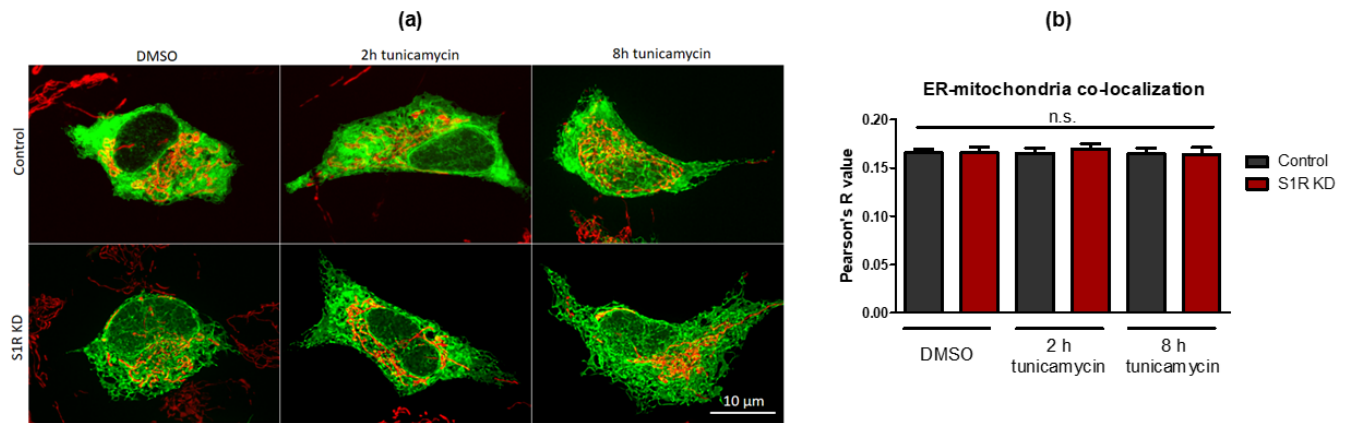


Figure S3. Tunicamycin treatment and S1R KD do not affect ER-mitochondria co-localization in SH-SY5Y neuroblastoma cells. **(a)** Representative z-projections of 3D-stacks of SH-SY5Y cells with 200 nm increments are shown; ER is labelled with D1ER (green) and mitochondria with TMRM (red). **(b)** Bar graphs represent MEAN \pm SEM of ER-mitochondrial co-localization represented by Pearson's R value. One-way ANOVA with Tukey's multiple comparison test, n.s. – not significant; Control DMSO (158 cells/16 experiments), Control 2hr tunicamycin (90 cell/9 experiments), Control 8hr tunicamycin (76 cells/8 experiments), S1R KD DMSO (119 cells/12 experiments), S1R KD 2hr tunicamycin (90 cells/9 experiments), S1R KD 8hr tunicamycin (74 cells/8 experiments).

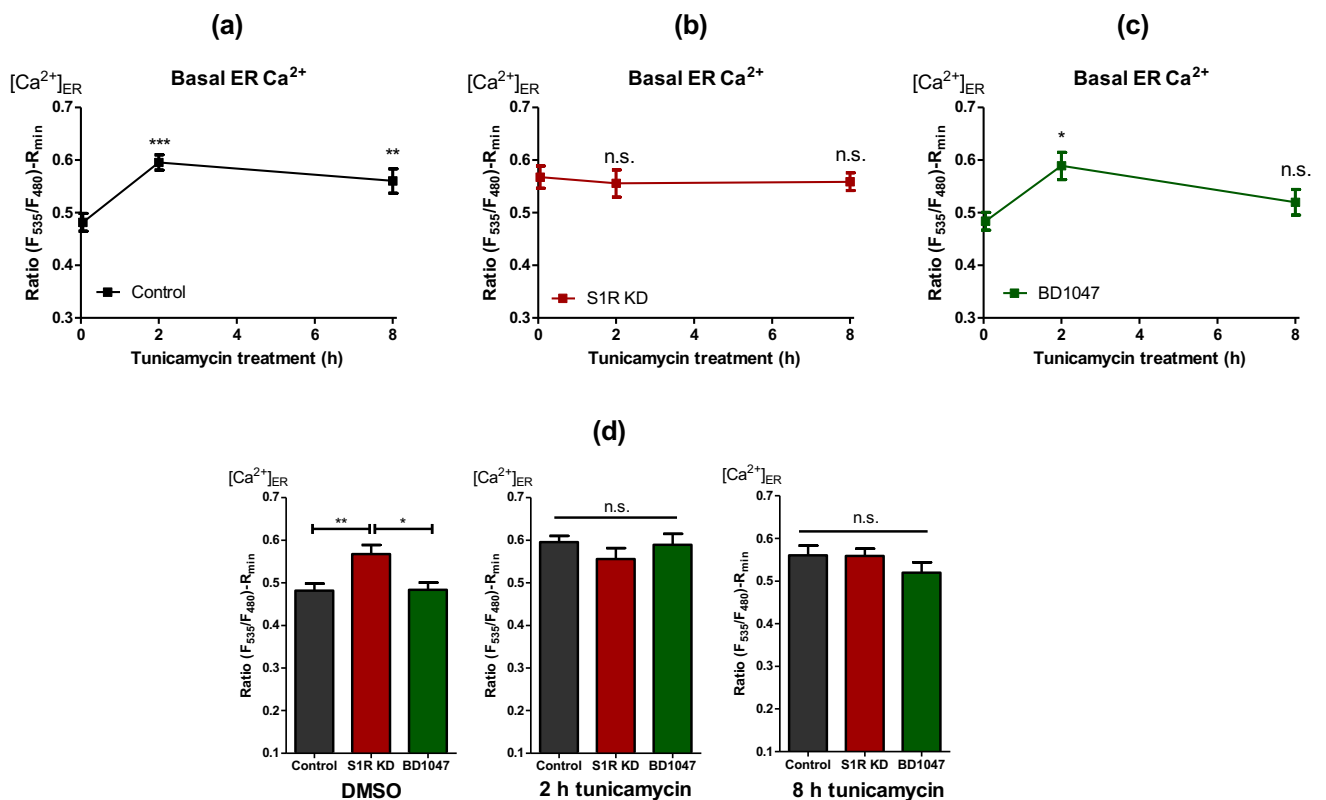


Figure S4. Tunicamycin treatment and S1R KD increase the basal ER Ca^{2+} level. Time course of ER Ca^{2+} level after tunicamycin treatment, presented as mean \pm SEM and assessed by normalized D1ER ratio in

control SH-SY5Y cells **(a)**, in cells with S1R KD **(b)** and BD1047 treatment **(c)**. Bar graphs represent MEAN \pm SEM of ER Ca^{2+} in control (black), S1R KD (red) and BD1047 treated (green) cells before tunicamycin treatment (left), after 2 h (middle) or 8 h (right) tunicamycin treatment **(d)**. One-way ANOVA with Tukey's multiple comparison test, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, n.s. – not significant; Control DMSO (43 cells/15 experiments), Control 2hr tunicamycin (44 cell/16 experiments), Control 8hr tunicamycin (38 cells/12 experiments), S1R KD DMSO (19 cells/9 experiments), S1R KD 2hr tunicamycin (27 cells/10 experiments), S1R KD 8hr tunicamycin (19 cells/6 experiments), BD1047 DMSO (23 cells/11 experiments), BD1047 2hr tunicamycin (9 cells/6 experiments), BD1047 8hr tunicamycin (18 cells/6 experiments).

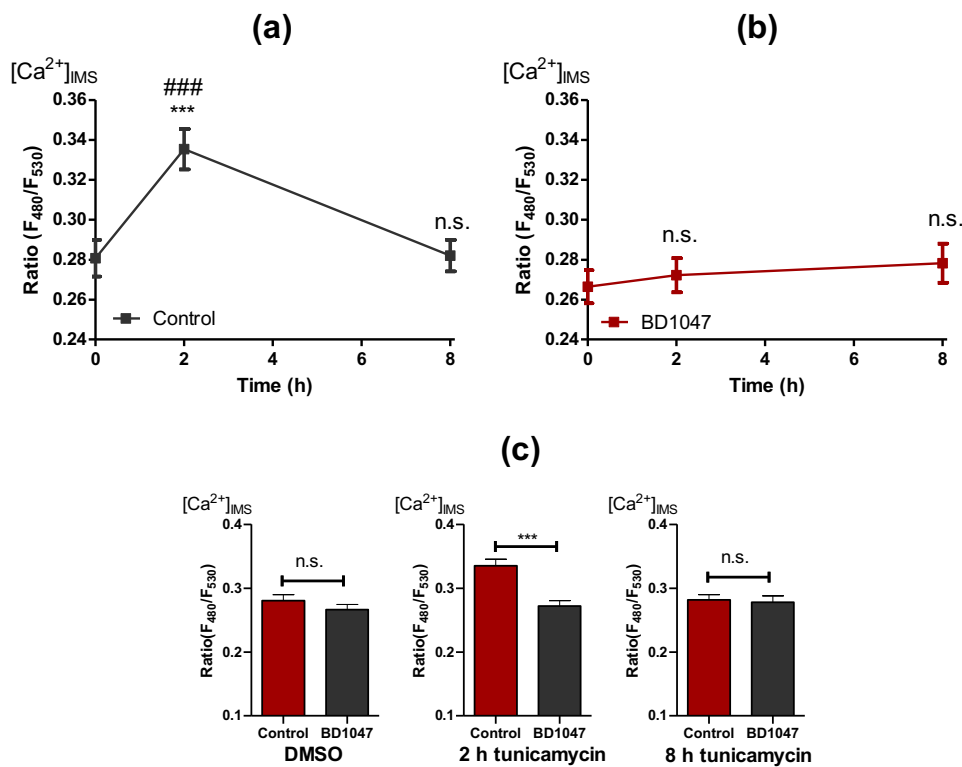


Figure S5. Time course of IMS Ca^{2+} level after tunicamycin treatment, presented as MEAN \pm SEM and assessed by IMS-GEM-GECCO ratio in control SH-SY5Y cells **(a)** and in cells treated with BD1047 **(b)**. Bar graphs represent MEAN \pm SEM of IMS Ca^{2+} levels in control (black) and BD1047 treated (red) cells before tunicamycin treatment (left), after 2 h (middle) or 8 h (right) tunicamycin treatment **(c)**. One-way ANOVA with Tukey's multiple comparison test **(a-b)**, *** $p < 0.001$, ### $p < 0.001$ (control 2 h tunicamycin against control 8 h tunicamycin), n.s. – not significant; unpaired t-test **(c)**, *** $p < 0.001$, n.s. – not significant; Control DMSO (86 cells/9 experiments), Control 2 h tunicamycin (72 cell/8 experiments), Control 8 h tunicamycin (71 cells/8 experiments), BD1047 DMSO (61 cells/7 experiments), BD1047 2 h tunicamycin (71 cells/7 experiments), BD1047 8hr tunicamycin (63 cells/7 experiments).