SUPPLEMENT

Bezrukova A.I., Basharova K.S., Galkina E.S., Epifanivskaya O.S., Baydakova G.V., Zakharova E.Yu., Pchelina S.N., Usenko T.S. (2026) Simultaneous inhibition of mTOR and STING as an approach to reduce alpha-synuclein and lysosphingolipid levels in peripheral blood monocytederived macrophages and the SH-SY5Y cell line: implications for therapy of Parkinson's disease. Biomeditsinskaya Khimiya, **72**(1), 42-61.

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In this study, the dose-dependent effects of the mTOR kinase activity inhibitor Torin1 (25, 50, 100, and 200 nM) and the STING protein inhibitor H-151 (0.25, 0.5, 1, and 2 μM) were evaluated on mTOR signaling activity (protein levels of p-mTOR (Ser2448) and p-RPS6 (Ser235/236)) and STING signaling activity (protein levels of STING and p-TBK1 (Ser172)), as well as on autophagy flux assessed by the levels of BECN1, p62, and LC3B-II, lysosomal degradation (CTSD protein level), activity of lysosomal hydrolases (GALC, GCase, ASMase, GLA), concentrations of lysosphingolipids (HexSph, LysoSM, LysoGb3), levels of different forms of alpha-synuclein, and the degree of apoptosis in primary cultures of peripheral blood macrophages from neurologically healthy individuals, as well as in the SH-SY5Y neuroblastoma cell line.

Also, the synergistic effect of combined inhibition of mTOR (Torin1) and STING (H-151) was evaluated on mTOR signaling activity (protein levels of p-mTOR (Ser2448) and p-RPS6 (Ser235/236)) and STING signaling activity (protein levels of STING and p-TBK1 (Ser172)), as well as on autophagy processes (protein levels of BECN1, p62, LC3B-II), lysosomal degradation (CTSD protein level), activity of lysosomal hydrolases (GALC, GCase, ASMase, GLA), concentrations of lysosphingolipids (HexSph, LysoSM, LysoGb3), levels of different forms of alpha-synuclein and the degree of apoptosis in primary peripheral blood macrophage cultures from neurologically healthy individuals and in the SH-SY5Y neuroblastoma cell line. Synergism for each parameter was analyzed using the HSA method.

Table 1. Synergistic effect of the Torin1 + H-151 combination in peripheral blood macrophages

Protein / Marker	mean_HSA	p_value	Synergy Index
Autophagy Proteins			
BECN1	-0.0065	9.42e-01	Additivity
GCase	-0.12	6.77e-02	Additivity
LC3B-II	-1.68	3.31e-08	Additivity
p62	0.21	4.32e-04	Additivity
p-RPS6	0.019	7.78e-01	Additivity
p-TBK1	0.18	2.40e-13	Additivity
i-CTSD	0.085	7.98e-05	Additivity
m-CTSD	1.51	6.41e-74	Additivity
mTOR	0.24	2.76e-04	Additivity
STING	-0.34	1.16e-03	Additivity
p-CTSD	-0.03	1.14e-03	Additivity
Lysosomal Hydrolase Activity and Lysosphingolipid Concentration			
ASMase	0.33	5.33e-01	Additivity
GCase	2.12	1.87e-01	Additivity
GLA	-0.31	7.83e-01	Additivity
GALC	0.59	2.67e-01	Additivity
LysoGb3	-1.41	3.81e-07	Additivity
HexSph	-1.06	3.20e-15	Additivity
LysoSM	0.023	2.78e-01	Additivity
Apoptosis			
Early apoptosis	-0.029	9.82e-01	Additivity
Late apoptosis	4.95	2.01e-01	Additivity
Necrosis	0.94	4.12e-01	Additivity

Table 2. Synergistic effect of the combination Torin1 + H-151 in the SH-SY5Y neuroblastoma cell line

Marker	HSA	p-value	Effect
Autophagy proteins			
BECN1	0.533	2.01e-47	Additive
GCase	-0.58	1.61e-28	Additive
LC3-II	0.533	2.01e-47	Additive
p62	0.44	2.32e-03	Additivity
p-RPS6	-0.30	4.40e-11	Additive
p-TBK1	0.052	7.17e-16	Additive
i.CTSD	0.533	2.01e-47	Additive
m.CTSD	1.48	6.04e-18	Additive
mTOR	0.21	1.60e-04	Additive
STING	-0.36	2.88e-08	Additive
p.CTSD	0.533	2.01e-47	Additive
α-synuclein	-0.38	7.89e-07	Additive
p-syn	-0.29	2.84e-02	Additive
α-synuclein tetramer	-0.24	2.59e-16	Additive
TH	-0.29	8.01e-15	Additive
Lysosomal hydrolase activity and lysosphingolipid concentration			
ASMase	3.56	3.61e-01	Additive
GCase	2.29	3.66e-01	Additive
GLA	3.01	3.10e-02	Additive
GALC	0.57	2.16e-03	Additive
LysoGb3	-0.28	8.46e-02	Additive
HexSph	-0.051	5.68e-02	Additive
LysoSM	-0.12	1.45e-03	Additive

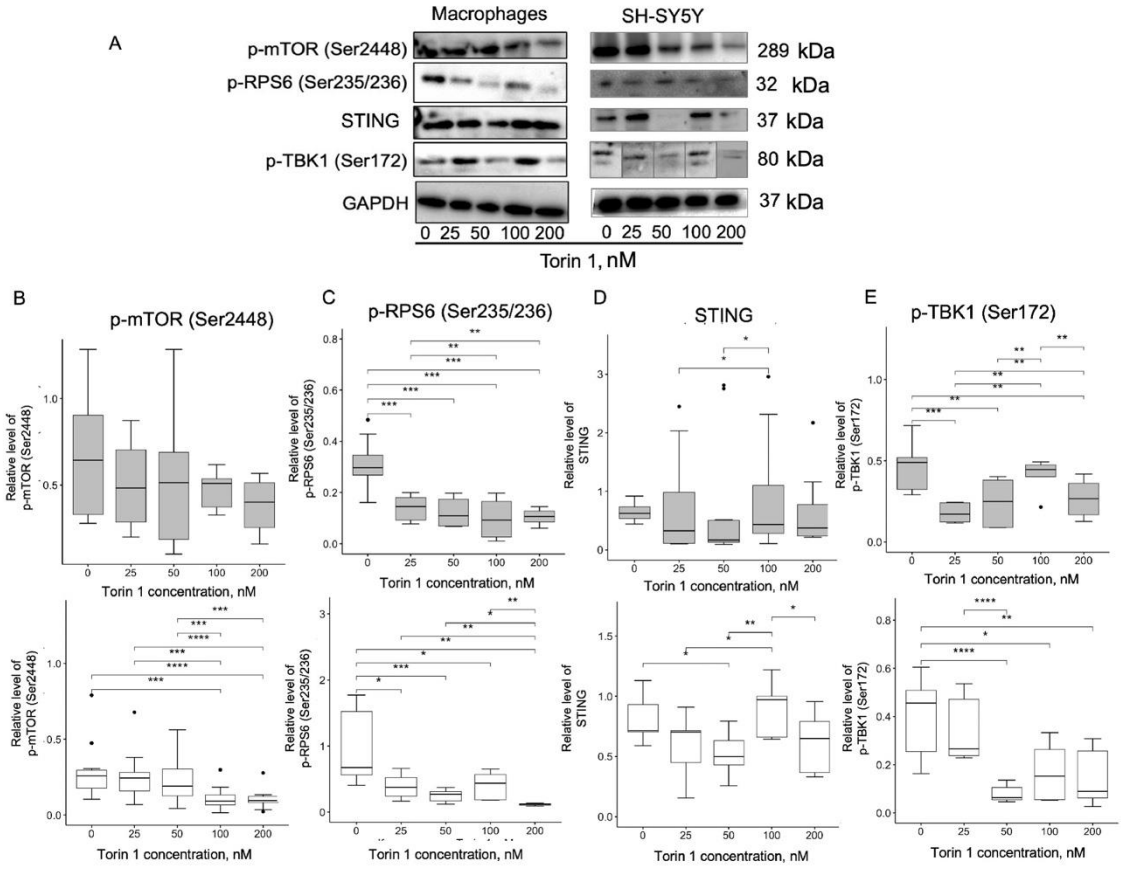

Figure S1. Evaluation of the efficacy of dose-dependent inhibition of the mTOR protein kinase and the STING protein by the small molecule Torin 1 in primary cultures of peripheral blood–derived macrophages (macrophages; gray bars) and the SH-SY5Y neuroblastoma cell line (SH-SY5Y; white bars). A – Western blot analysis; B – relative p-mTOR (Ser2448) levels; C – relative p-RPS6 (Ser235/236) levels; D – relative STING levels; E. relative p-TBK1 (Ser172) levels. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

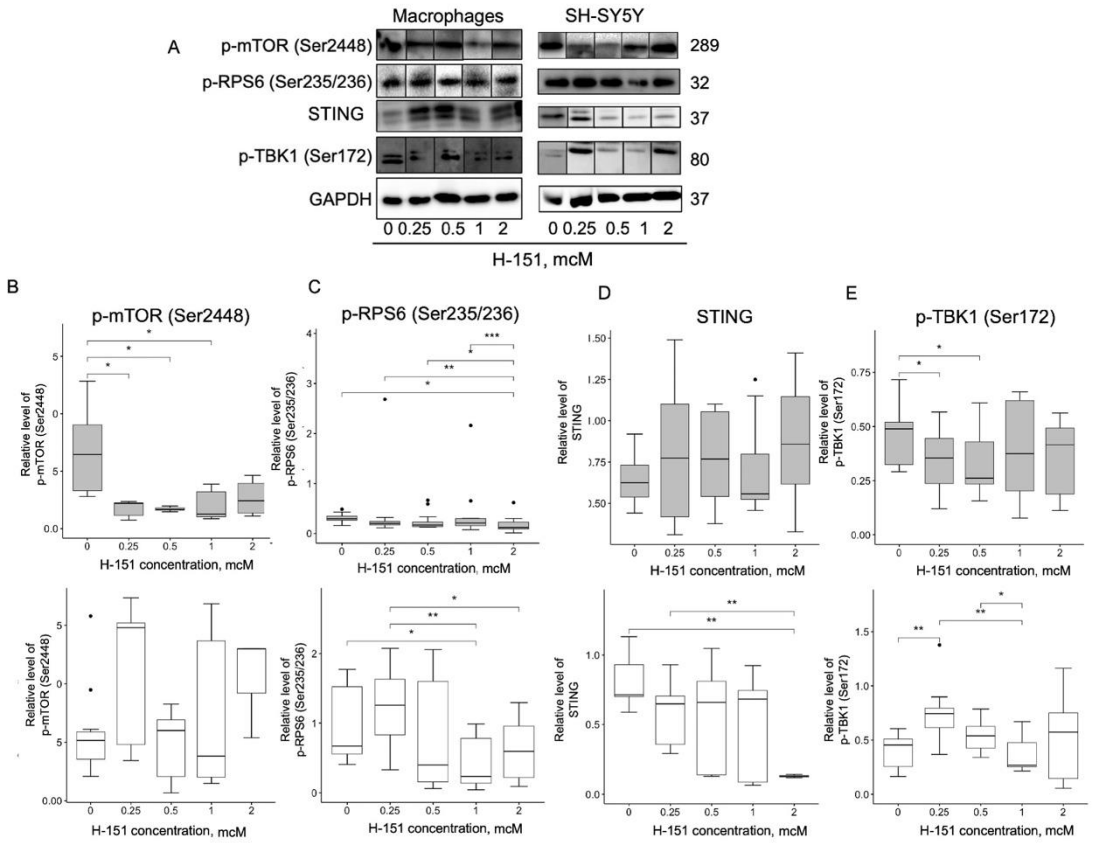

Figure S2. Evaluation of the efficacy of dose-dependent inhibition of the mTOR protein kinase and the STING protein by the small molecule H-151 in primary cultures of peripheral blood–derived macrophages (macrophages; gray bars) and the SH-SY5Y neuroblastoma cell line (SH-SY5Y; white bars). A – Western blot analysis; B – relative p-mTOR (Ser2448) levels; C – relative p-RPS6 (Ser235/236) levels; D. relative STING levels; E. relative p-TBK1 (Ser172) levels. *p < 0.05, **p < 0.01, ***p < 0.001.

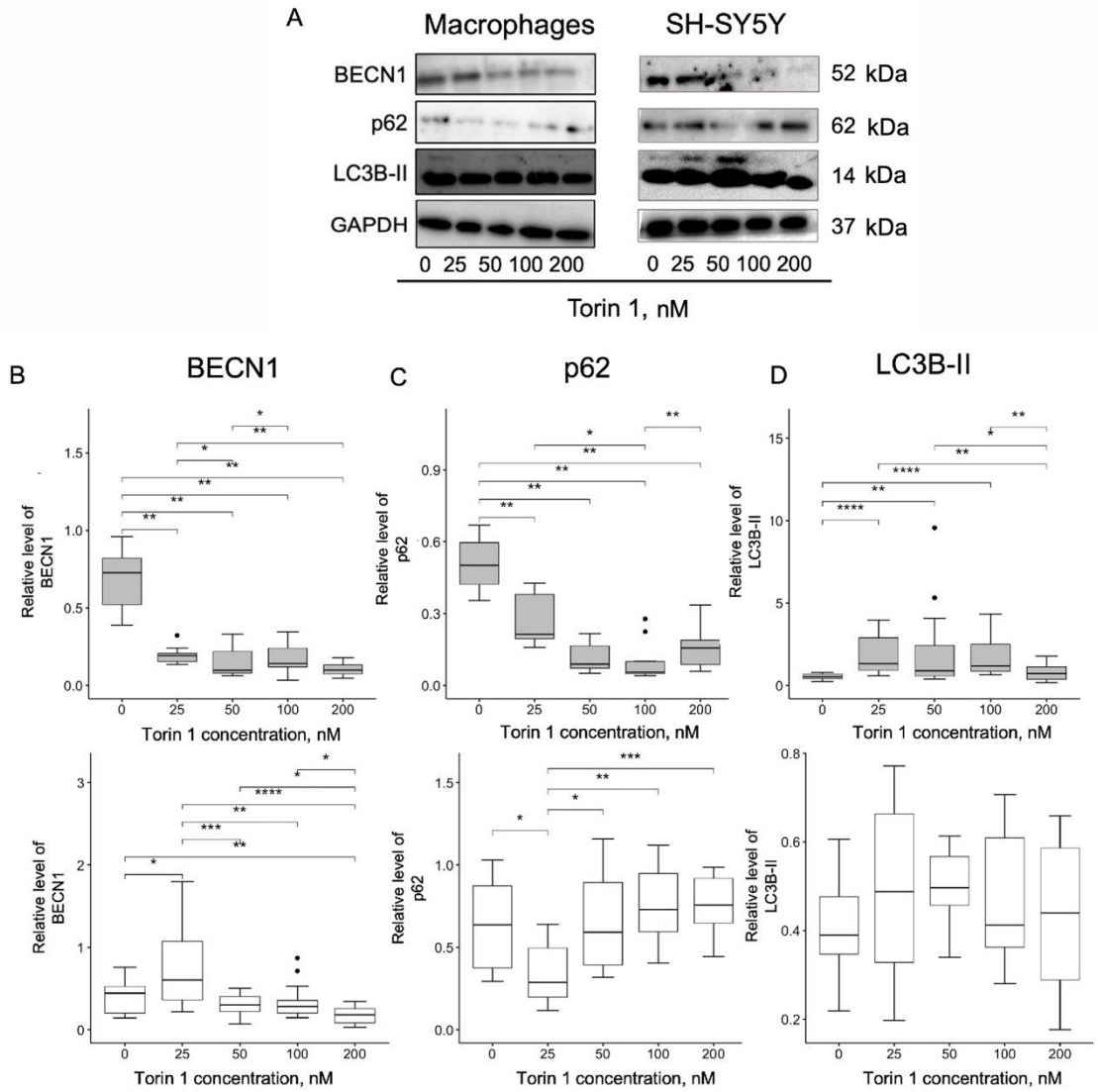

Figure S3. Evaluation of the dose-dependent effects of mTOR and STING inhibition by the small molecule Torin 1 in primary cultures of peripheral blood–derived macrophages (macrophages; gray bars) and the SH-SY5Y neuroblastoma cell line (SH-SY5Y; white bars) on key stages of autophagy. A – Western blot analysis; B – relative BECN1 levels; C – relative p62 levels; D – relative LC3B-II levels. T, Torin 1; H, H-151. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

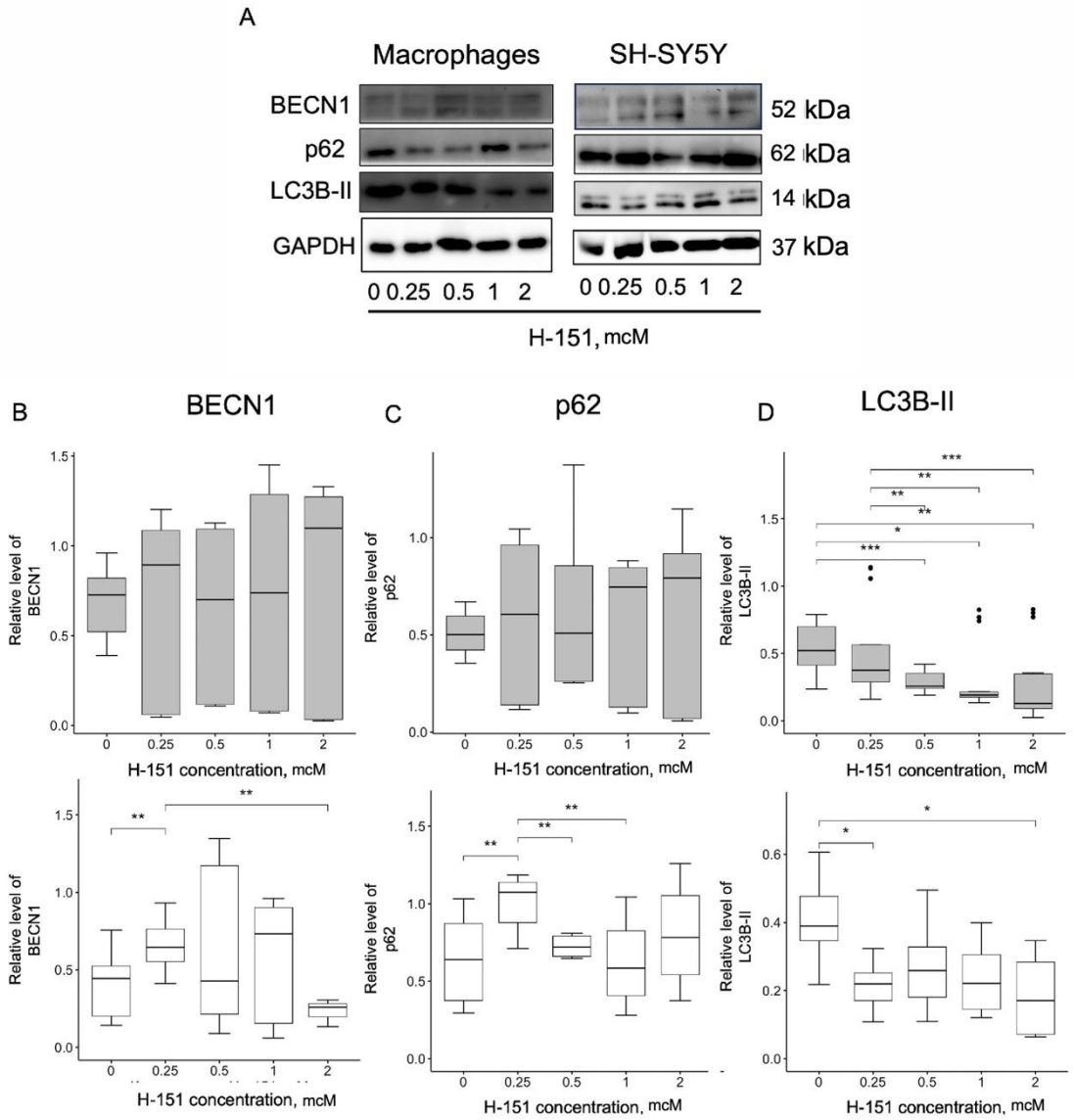

Figure S4. Evaluation of the dose-dependent effects of mTOR and STING inhibition by the small molecule H-151 in primary cultures of peripheral blood–derived macrophages (macrophages; gray bars) and the SH-SY5Y neuroblastoma cell line (SH-SY5Y; white bars) on key stages of autophagy. A – Western blot analysis; B – relative BECN1 levels; C – relative p62 levels; D – relative LC3B-II levels. T, Torin 1; H, H-151. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

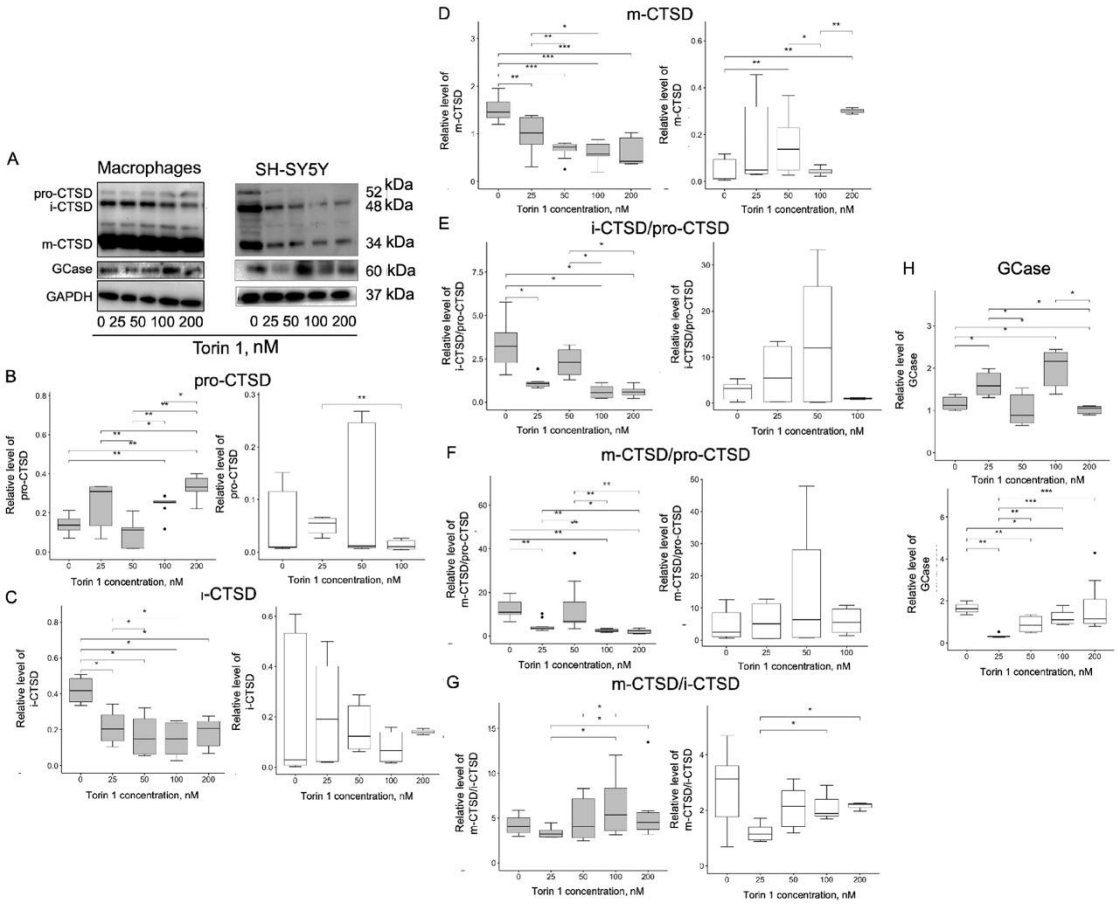

Figure S5. Evaluation of the dose-dependent effects of mTOR and STING inhibition by the small molecule Torin 1 in primary cultures of peripheral blood–derived macrophages (macrophages; gray bars) and the SH-SY5Y neuroblastoma cell line (SH-SY5Y; white bars) on lysosomal degradation. A – Western blot analysis; B – relative pro-CTSD levels; C – relative i-CTSD levels; D – relative m-CTSD levels; E – relative i-CTSD/pro-CTSD ratio; F – relative m-CTSD/pro-CTSD ratio; G – relative m-CTSD/i-CTSD ratio; H – relative GCase levels. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

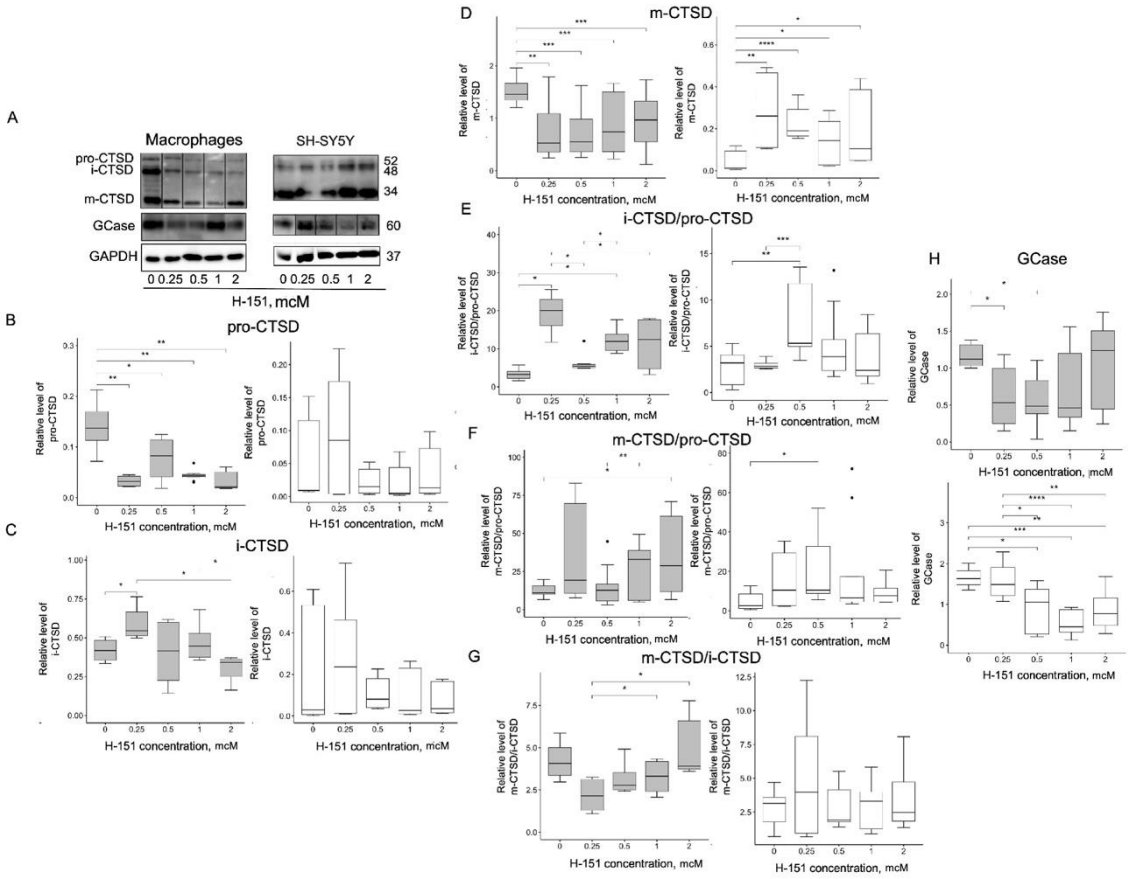

Figure S6. Evaluation of the dose-dependent effects of mTOR and STING inhibition by the small molecule H-151 in primary cultures of peripheral blood–derived macrophages (macrophages; gray bars) and the SH-SY5Y neuroblastoma cell line (SH-SY5Y; white bars) on lysosomal degradation. A – Western blot analysis; B – relative pro-CTSD levels; C – relative i-CTSD levels; D – relative m-CTSD levels; E – relative i-CTSD/pro-CTSD ratio; F – relative m-CTSD/pro-CTSD ratio; G –relative m-CTSD/i-CTSD ratio; H – relative GCase levels. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

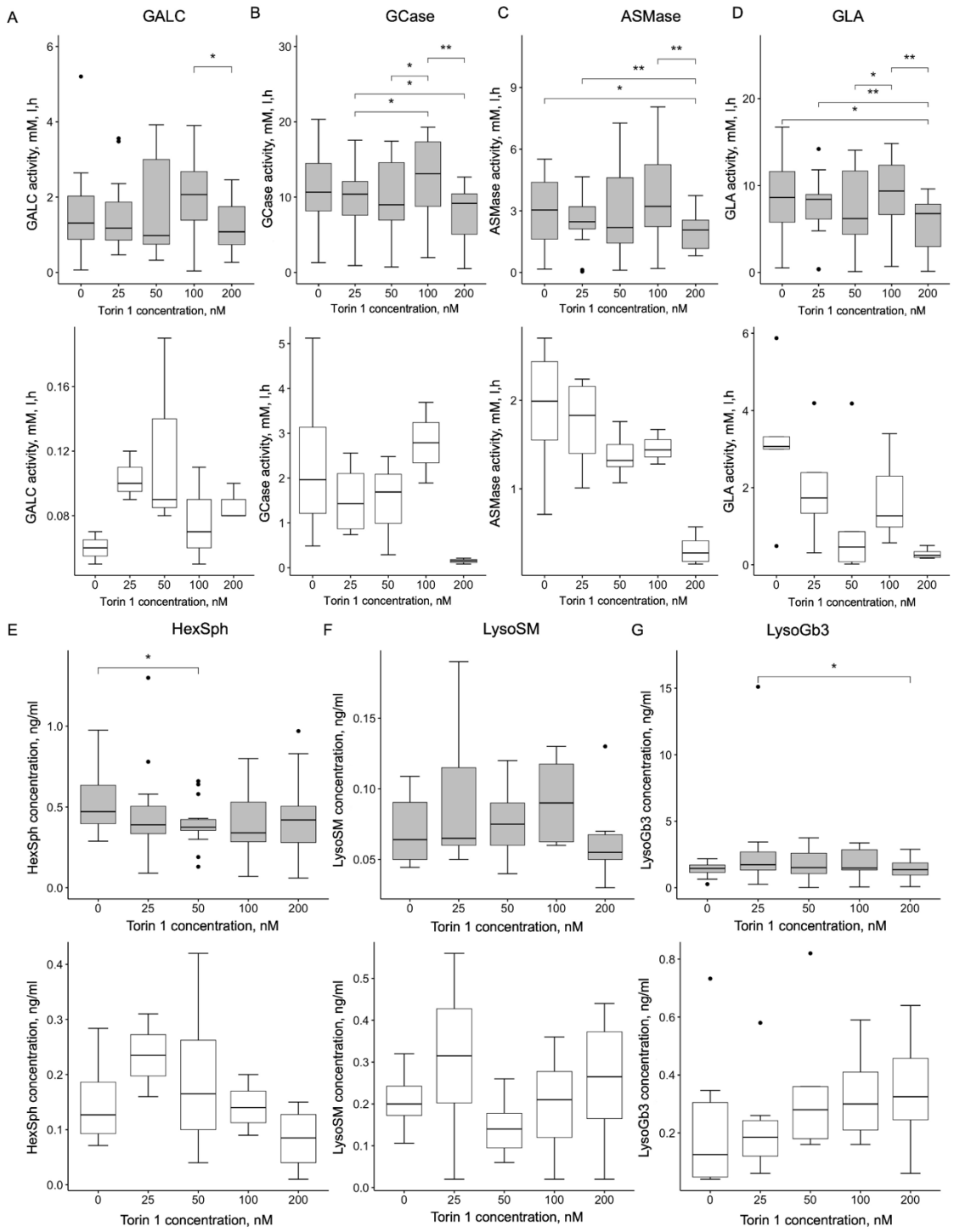

Figure S7. Evaluation of the dose-dependent effects of mTOR and STING inhibition by the small molecule Torin 1 in primary cultures of peripheral blood–derived macrophages (macrophages; gray bars) and the SH-SY5Y neuroblastoma cell line (SH-SY5Y; white bars) on lysosomal enzyme activity and lysosphingolipid levels. A – GALC; B – GCase; C – ASMase; D – GLA; E – HexSph; F –LysoSM; G – LysoGb3. *$p < 0.05$, **$p < 0.01$.

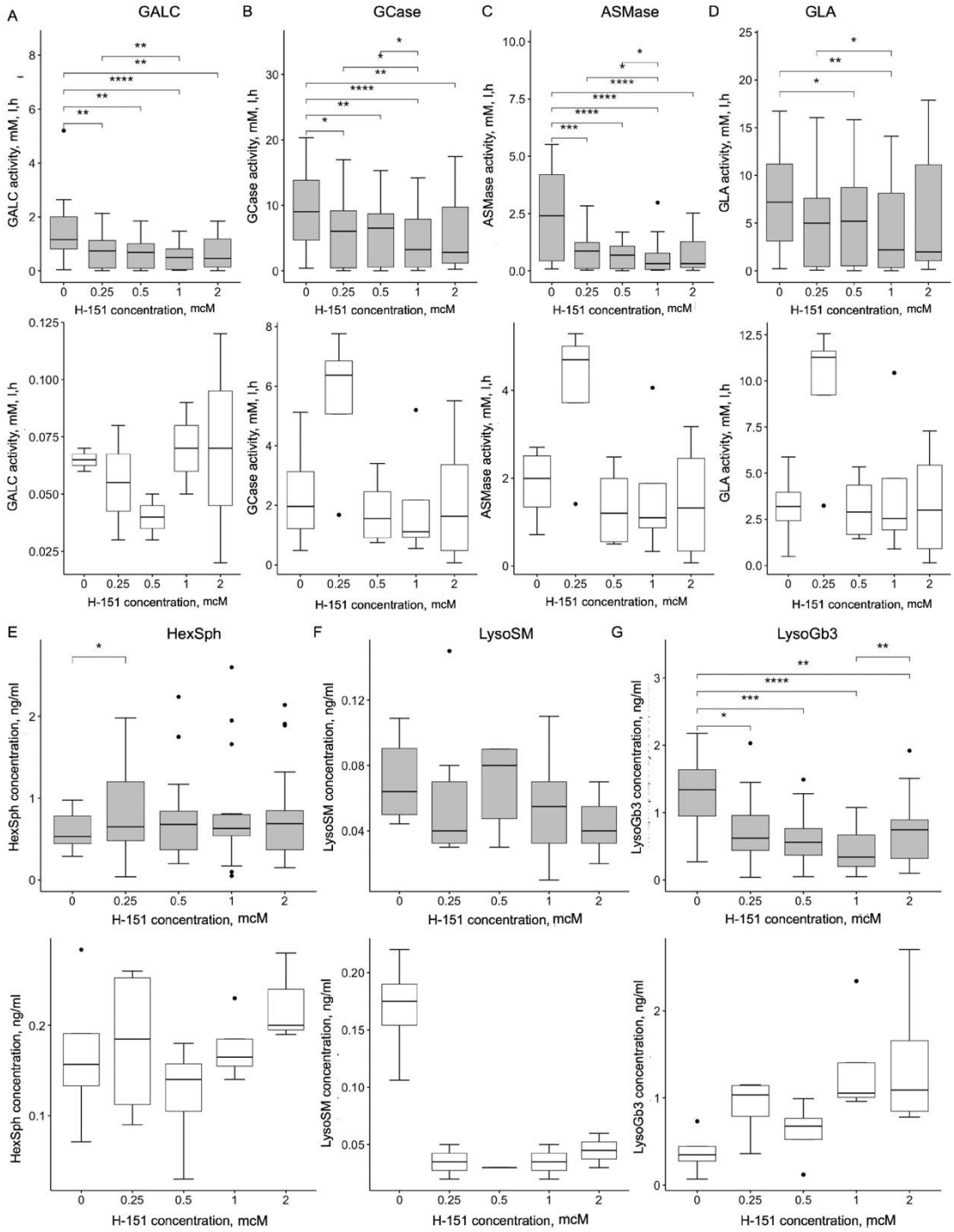

Figure S8. Evaluation of the dose-dependent effects of mTOR and STING inhibition by the small molecule H-151 in primary cultures of peripheral blood–derived macrophages (macrophages; gray bars) and the SH-SY5Y neuroblastoma cell line (SH-SY5Y; white bars) on lysosomal enzyme activity and lysosphingolipid levels. A – GALC; B – GCase; C – ASMase; D – GLA; E – HexSph; F – LysoSM; G – LysoGb3. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

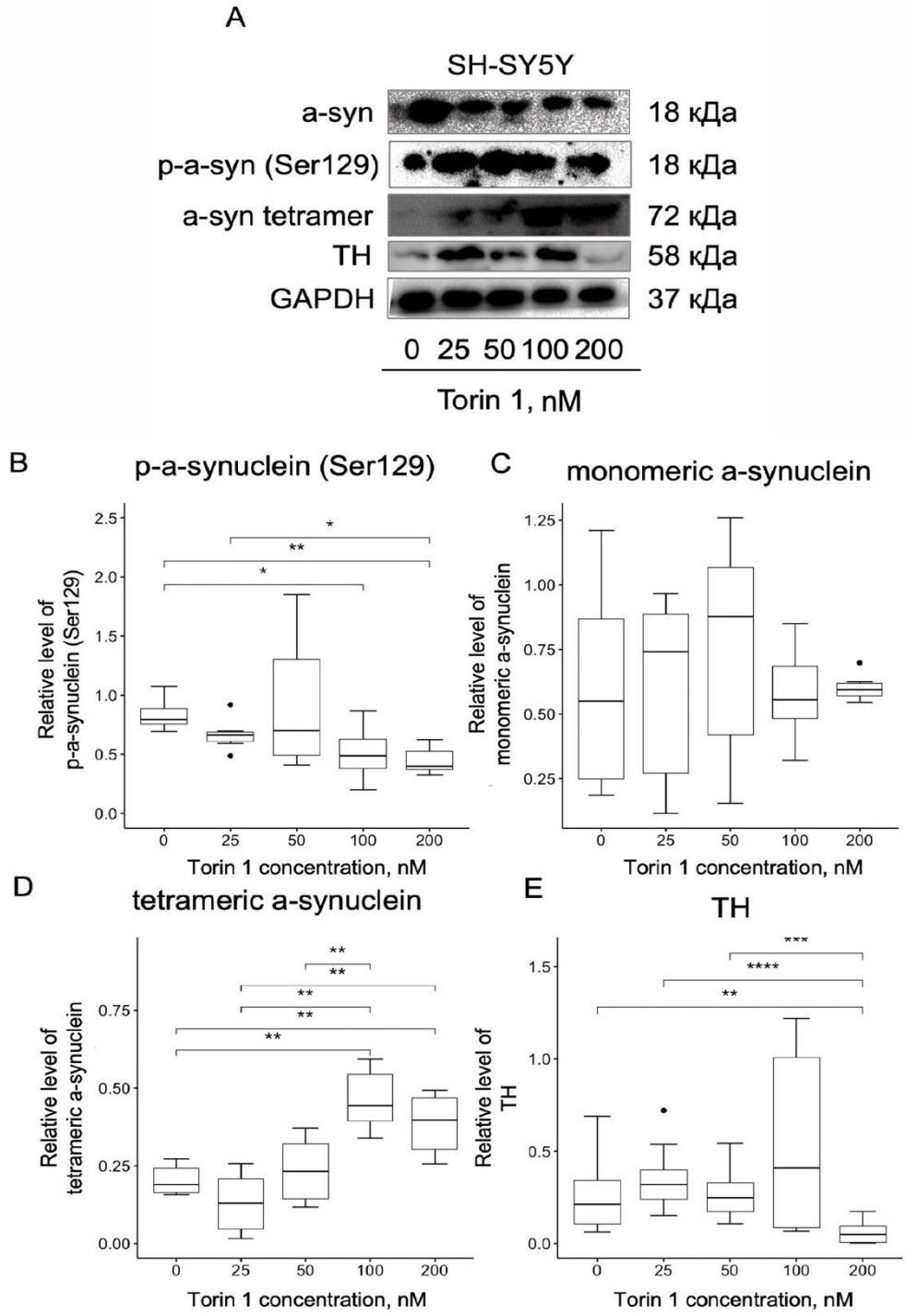

Figure S9. Evaluation of the dose-dependent effects of mTOR and STING inhibition by the small molecule Torin 1 in the SH-SY5Y neuroblastoma cell line (SH-SY5Y) on the levels of different α-synuclein species and tyrosine hydroxylase (TH). A – Western blot analysis; B – relative phosphorylated α-synuclein (Ser129) levels; C – relative monomeric α-synuclein levels; D – relative tetrameric α-synuclein levels; E – relative TH levels. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

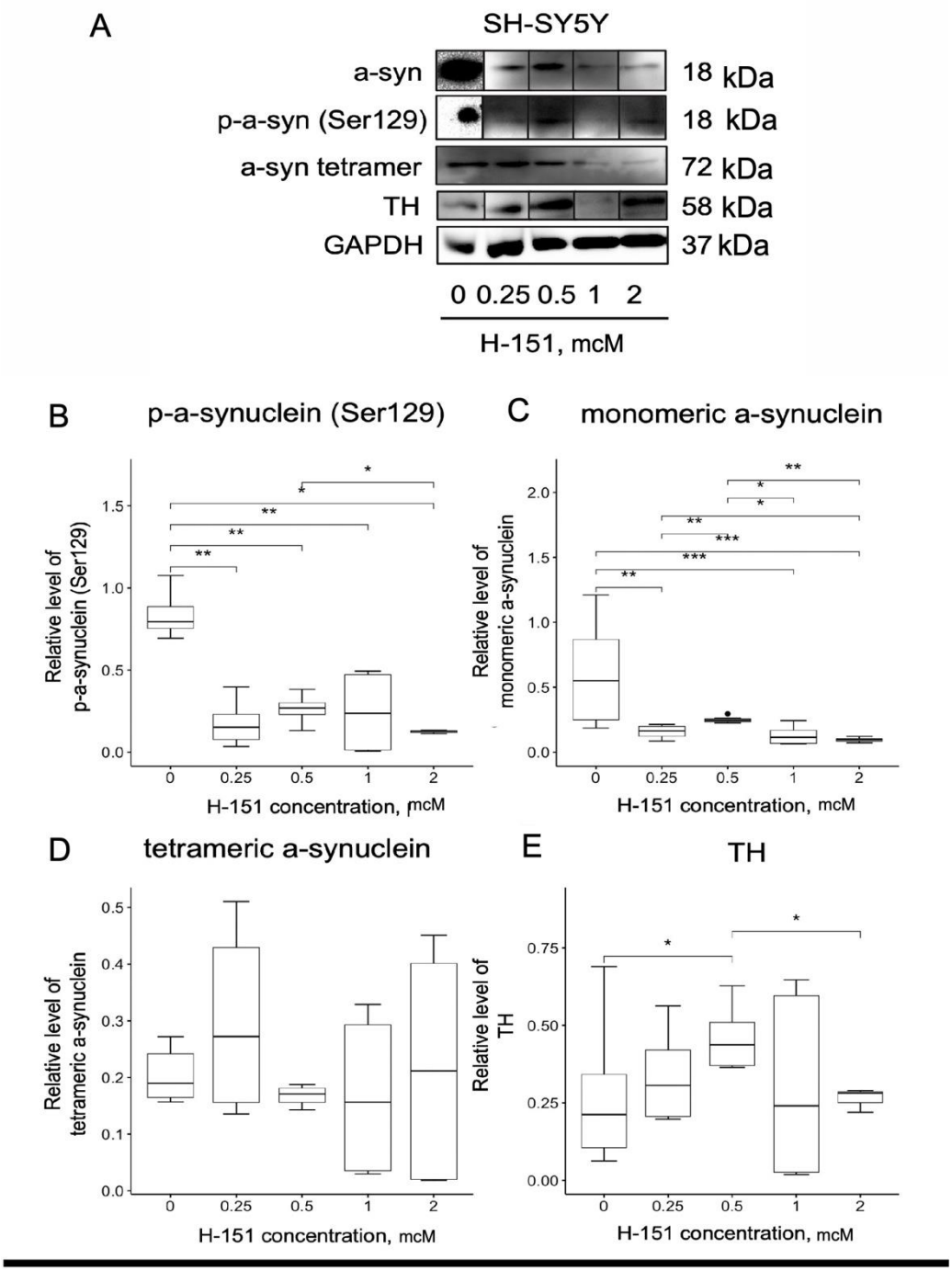

Figure S10. Evaluation of the dose-dependent effects of mTOR and STING inhibition by the small molecule H-151 in the SH-SY5Y neuroblastoma cell line (SH-SY5Y) on the levels of different α-synuclein species and tyrosine hydroxylase (TH). A – Western blot analysis; B – relative phosphorylated α-synuclein (Ser129) levels; C – relative monomeric α-synuclein levels; D – relative tetrameric α-synuclein levels; E – relative TH levels. *p < 0.05, **p < 0.01, ***p < 0.001.