

Supplementary information (SI)

***In Vitro* Evaluation of Immunogenic Potential of Gramicidin S and its Photocontrolled Analogues**

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Table S1. Cytotoxic activity of LMB033-closed, LMB033-open and gramicidin S monitored in two-dimensional assay. IC₅₀ values are presented as absolute value $\pm$ SEM in μ M (n=4).

	LMB033-closed				LMB033-open			
Cell line	10 mins	1 h	24 hrs	72 hrs	10 mins	1 h	24 hrs	72 hrs
4T1	134.2 $\pm$ 1.5	111.5 $\pm$ 1.8	66.5 $\pm$ 0.8	61.6 $\pm$ 1.7	18.8 $\pm$ 0.3	14.3 $\pm$ 0.1	9.2 $\pm$ 0.2	11.6 $\pm$ 0.3
HEK-293	193.3 $\pm$ 2.2	158.3 $\pm$ 1.8	113.8 $\pm$ 1.8	116.8 $\pm$ 2.3	21.4 $\pm$ 0.3	16.9 $\pm$ 0.3	13.0 $\pm$ 0.1	13.5 $\pm$ 0.7
HeLa	N/D	138.3 $\pm$ 3.5	83.0 $\pm$ 3.0	92.0 $\pm$ 5.8	24.1 $\pm$ 0.5	14.3 $\pm$ 0.3	9.4 $\pm$ 0.2	9.4 $\pm$ 1.6
HepG2	N/D	209.7 $\pm$ 14.1	126.8*	121.4*	27.3 $\pm$ 0.4	21.4 $\pm$ 0.7	13.8 $\pm$ 0.5	14.5*
LLC	176.7 $\pm$ 6.8	136.5 $\pm$ 4.2	73.8 $\pm$ 1.2	70.5 $\pm$ 2.7	24.5 $\pm$ 1.1	17.8 $\pm$ 2.0	7.6 $\pm$ 0.2	6.0 $\pm$ 0.2
MDA-MB-231	161.3 $\pm$ 1.8	114.1 $\pm$ 1.0	84.6 $\pm$ 1.0	101.3 $\pm$ 0.7	19.2 $\pm$ 0.2	15.1 $\pm$ 0.2	9.9 $\pm$ 0.3	11.5 $\pm$ 0.1
	Gramicidin S							
Cell line	10 mins	1 h	24 hrs	72 hrs				
4T1	11.3 $\pm$ 0.2	9.7 $\pm$ 0.2	6.4 $\pm$ 0.4	6.9 $\pm$ 0.1				
HEK-293	12.9 $\pm$ 0.4	11.6 $\pm$ 0.1	8.3 $\pm$ 0.2	8.4 $\pm$ 0.7				
HeLa	19.3 $\pm$ 2.9	10.3 $\pm$ 0.6	8.9 $\pm$ 0.5	4.7 $\pm$ 3.5				
HepG2	22.0 $\pm$ 0.4	19.3 $\pm$ 0.5	12.6 $\pm$ 0.2	14.1*				
LLC	14.2*	9.9 $\pm$ 1.0	4.9 $\pm$ 0.3	5.2 $\pm$ 0.7				
MDA-MB-231	12.3 $\pm$ 0.3	10.8 $\pm$ 0.2	9.7 $\pm$ 0.4	10.4 $\pm$ 0.2				

N/D (non-defined) marks cases where IC₅₀ activity falls out of tested concentration range.

* SEM was not defined due to specifics of data analysis methodology.

Table S2. Cytotoxic activity of LMB002-closed, LMB002-open and gramicidin S monitored in two-dimensional assay. IC₅₀ values are presented as absolute value $\pm$ SEM in μ M (n=4).

	LMB002-closed				LMB002-open			
Cell line	10 mins	1 h	24 hrs	72 hrs	10 mins	1 h	24 hrs	72 hrs
4T1	58.7 $\pm$ 0.5	59.1 $\pm$ 0.7	37.1 $\pm$ 0.2	44.6 $\pm$ 1.4	23.3 $\pm$ 0.3	16.1 $\pm$ 0.1	12.3 $\pm$ 0.2	10.8 $\pm$ 0.4
HEK-293	134.5 $\pm$ 1.3	105.1 $\pm$ 1.7	83.0 $\pm$ 1.5	87.3 $\pm$ 1.8	25.5 $\pm$ 0.8	18.6 $\pm$ 0.5	13.6 $\pm$ 0.3	12.9 $\pm$ 0.1
HeLa	79.3 $\pm$ 1.8	84.9 $\pm$ 1.5	48.4 $\pm$ 1.0	52.6 $\pm$ 4.6	12.6 $\pm$ 0.5	11.4 $\pm$ 0.3	7.7 $\pm$ 0.7	13.6 $\pm$ 1.1
HepG2	241.9*	150.0 $\pm$ 3.3	106.0 $\pm$ 2.0	92.6 $\pm$ 1.2	34.3 $\pm$ 0.8	26.7 $\pm$ 0.6	17.6 $\pm$ 2.3	16.9*
LLC	97.6 $\pm$ 3.5	98.5 $\pm$ 1.7	70.3 $\pm$ 1.9	43.3 $\pm$ 2.7	20.5 $\pm$ 0.4	22.6 $\pm$ 0.3	11.5 $\pm$ 0.2	8.8 $\pm$ 0.4
MDA-MB-231	84.6 $\pm$ 1.1	83.5 $\pm$ 1.3	64.3 $\pm$ 0.3	69.5*	16.4 $\pm$ 0.5	21.0 $\pm$ 0.3	15.7 $\pm$ 0.1	21.1 $\pm$ 0.2
	Gramicidin S							
Cell line	10 mins	1 h	24 hrs	72 hrs				
4T1	10.3 $\pm$ 0.2	8.3 $\pm$ 0.1	6.4 $\pm$ 0.2	6.2 $\pm$ 0.5				
HEK-293	15.4 $\pm$ 0.1	12.7 $\pm$ 0.2	10.4 $\pm$ 0.1	11.1 $\pm$ 0.1				
HeLa	10.9 $\pm$ 0.3	9.8 $\pm$ 0.2	6.8 $\pm$ 0.7	8.3 $\pm$ 0.4				
HepG2	24.0 $\pm$ 0.9	19.6 $\pm$ 1.5	13.4 $\pm$ 0.3	15.0*				
LLC	13.1 $\pm$ 0.8	12.7 $\pm$ 0.7	6.0 $\pm$ 0.2	4.4 $\pm$ 0.2				
MDA-MB-231	13.9 $\pm$ 0.2	11.0 $\pm$ 0.1	9.1 $\pm$ 0.3	10.4 $\pm$ 0.1				

* SEM was not defined due to specifics of data analysis methodology.

Table S3. Cytotoxic activity of LMB033-closed, LMB033-open and gramicidin S monitored in three-dimensional (spheroid) assay. IC₅₀ values are presented as absolute value $\pm$ SEM in μ M (n=5).

Cell line	LMB033-closed			LMB033-open			Gramicidin S		
	10 mins	24 hrs	72 hrs	10 mins	24 hrs	72 hrs	10 mins	24 hrs	72 hrs
4T1	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
HEK-293	81.0 $\pm$ 22.9	92.1*	69.8 $\pm$ 2.2	6.6 $\pm$ 2.6	9.6 $\pm$ 2.3	11.9 $\pm$ 1.0	14.3 $\pm$ 11.6	12.9 $\pm$ 1.1	9.0 $\pm$ 1.1
HeLa	50.1 $\pm$ 8.0	39.0 $\pm$ 4.4	N/D	3.3 $\pm$ 1.7	8.5 $\pm$ 5.9	N/D	2.9 $\pm$ 1.5	2.9 $\pm$ 1.6	N/D
HepG2	79.1 $\pm$ 2.9	106.8 $\pm$ 1.6	176.2 $\pm$ 12.2	7.6 $\pm$ 0.9	22.5 $\pm$ 1.1	31.9*	4.8 $\pm$ 0.8	5.5 $\pm$ 0.8	7.2*
LLC	117.8 $\pm$ 13.4	67.0 $\pm$ 3.8	26.6 $\pm$ 11.3	27.4 $\pm$ 7.5	24.6 $\pm$ 2.9	6.7 $\pm$ 2.2	27.4 $\pm$ 5.2	6.9 $\pm$ 1.8	4.1 $\pm$ 2.1
MDA-MB-231	212.6 $\pm$ 36.6	62.6 $\pm$ 18.7	73.1 $\pm$ 2.9	40.4 $\pm$ 2.5	36.9 $\pm$ 3.1	8.8 $\pm$ 2.5	32.2 $\pm$ 9.5	5.5 $\pm$ 2.3	9.2 $\pm$ 0.5

N/D (non-defined) marks cases where IC₅₀ activity falls out of tested concentration range.

* SEM was not defined due to specifics of data analysis methodology.

Table S4. Cytotoxic activity of LMB002-closed, LMB002-open and gramicidin S monitored in three-dimensional (spheroid) assay. IC₅₀ values are presented as absolute value $\pm$ SEM in μ M (n=5).

	LMB002-closed			LMB002-open			Gramicidin S		
Cell line	10 mins	24 hrs	72 hrs	10 mins	24 hrs	72 hrs	10 mins	24 hrs	72 hrs
4T1	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
HEK-293	24.8 $\pm$ 7.6	49.8 $\pm$ 3.3	58.4 $\pm$ 3.5	8.5 $\pm$ 0.8	18.2 $\pm$ 3.1	20.8 $\pm$ 0.9	10.9 $\pm$ 10.9	12.9 $\pm$ 1.1	9.0 $\pm$ 1.1
HeLa	22.8 $\pm$ 4.0	27.4 $\pm$ 2.6	N/D	2.2 $\pm$ 2.0	2.8 $\pm$ 3.9	N/D	2.9 $\pm$ 1.5	2.9 $\pm$ 1.6	N/D
HepG2	35.7 $\pm$ 3.6	66.0 $\pm$ 2.3	67.3 $\pm$ 2.0	11.8 $\pm$ 0.1	20.4 $\pm$ 1.1	22.8 $\pm$ 2.8	6.7*	5.5 $\pm$ 0.8	7.2*
LLC	26.2 $\pm$ 5.4	49.4 $\pm$ 2.3	46.8 $\pm$ 3.6	7.1 $\pm$ 6.6	22.3 $\pm$ 2.6	3.5 $\pm$ 1.9	27.4 $\pm$ 5.2	6.9 $\pm$ 1.8	4.1 $\pm$ 2.1
MDA-MB-231	101.3 $\pm$ 6.3	45.2 $\pm$ 11.0	22.3 $\pm$ 4.4	37.0 $\pm$ 2.3	41.0 $\pm$ 3.9	6.9 $\pm$ 2.8	26.1 $\pm$ 5.3	5.5 $\pm$ 2.3	8.5 $\pm$ 0.5

N/D (non-defined) marks cases where IC₅₀ activity falls out of tested concentration range.

* SEM was not defined due to specifics of data analysis methodology.

Figure S1. Dose-dependent curves for gramicidin S and its photocontrolled analogues tested on 4T1 cell line in two-dimensional format. Each point represents average value (n=4) with error bars depicting SEM.

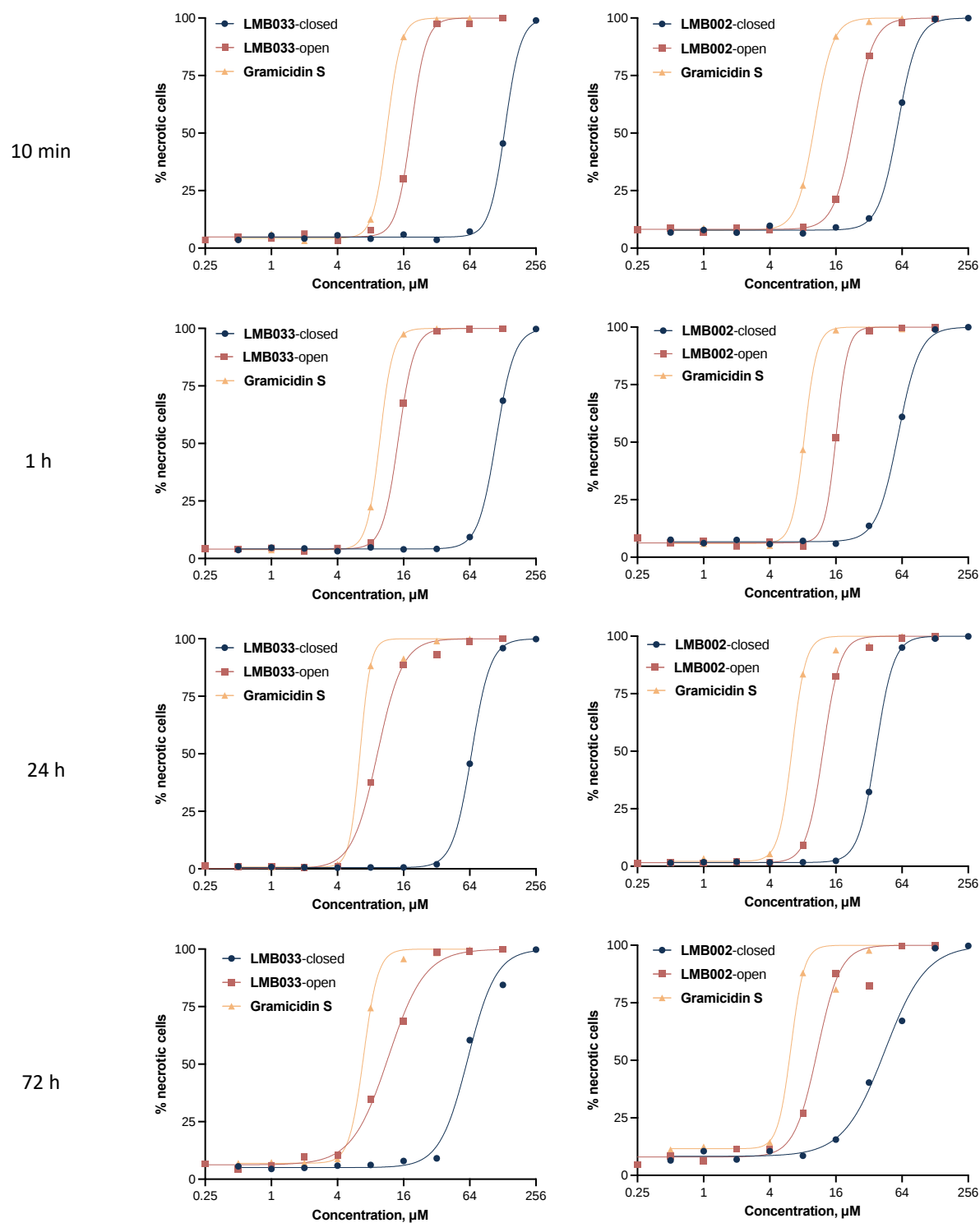


Figure S2. Dose-dependent curves for gramicidin S and its photocontrolled analogues tested on HEK-293 cell line in two-dimensional format. Each point represents average value (n=4) with error bars depicting SEM.

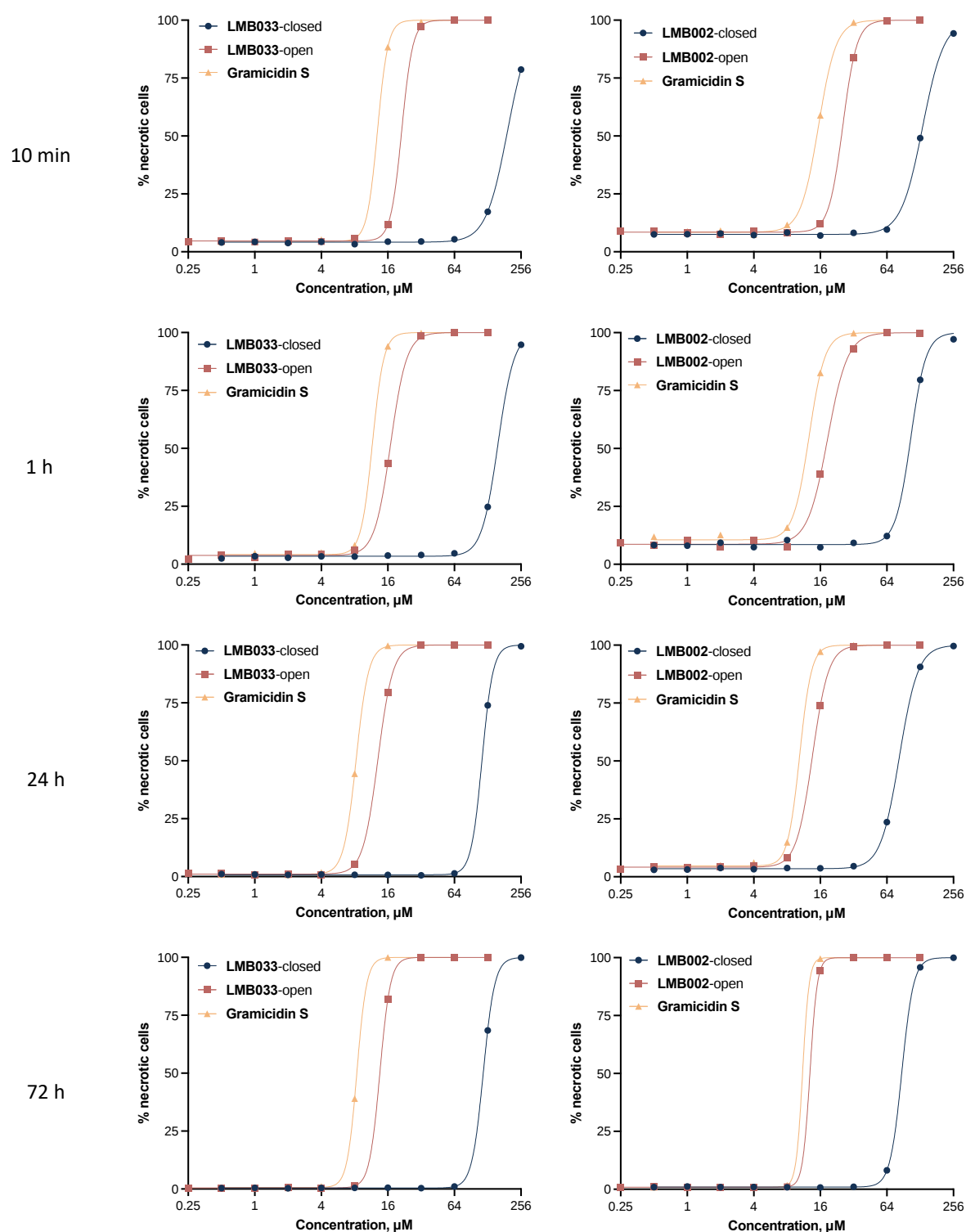


Figure S3. Dose-dependent curves for gramicidin S and its photocontrolled analogues tested on HeLa cell line in two-dimensional format. Each point represents average value (n=4) with error bars depicting SEM.

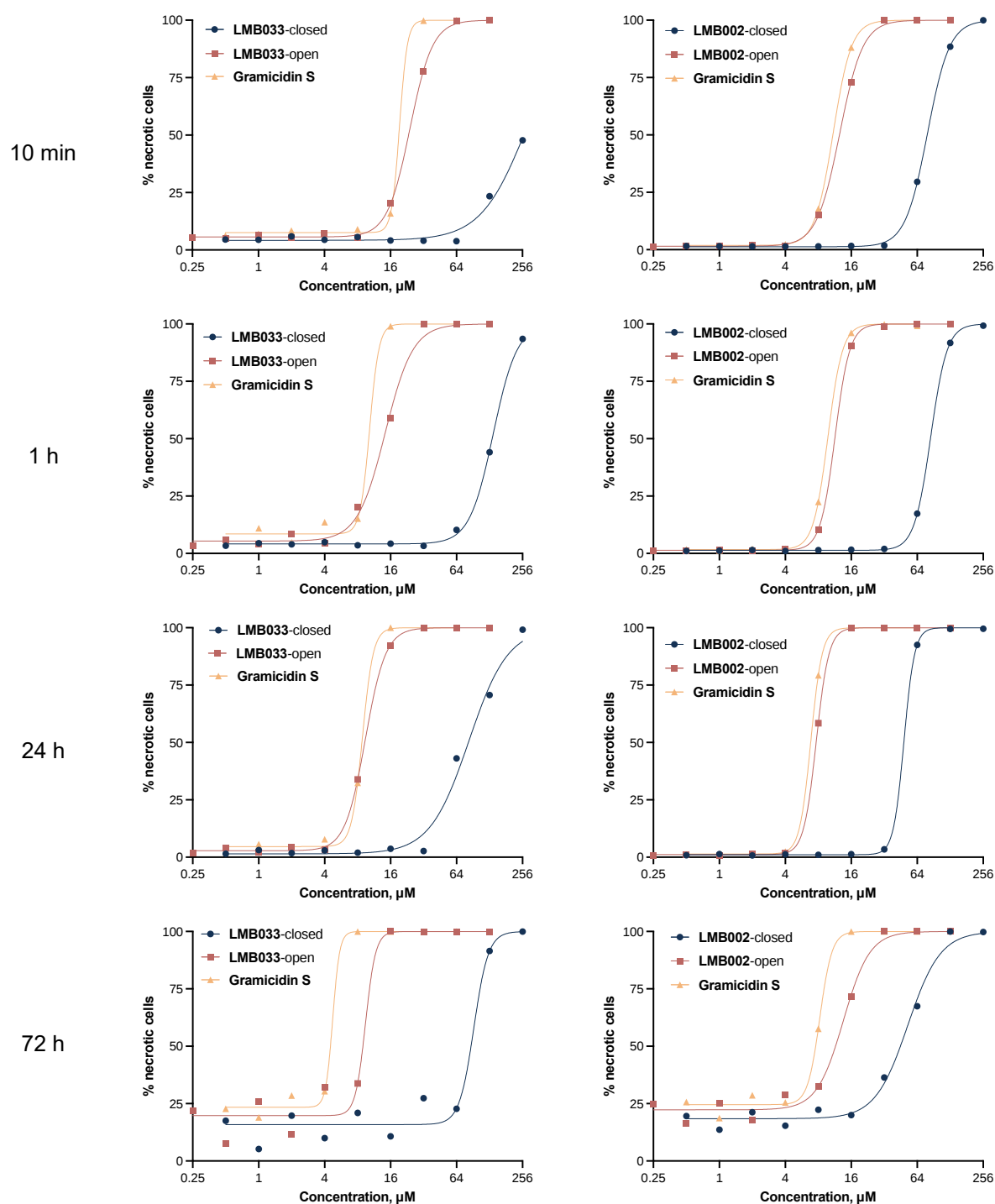


Figure S4. Dose-dependent curves for gramicidin S and its photocontrolled analogues tested on HepG2 cell line in two-dimensional format. Each point represents average value (n=4) with error bars depicting SEM.

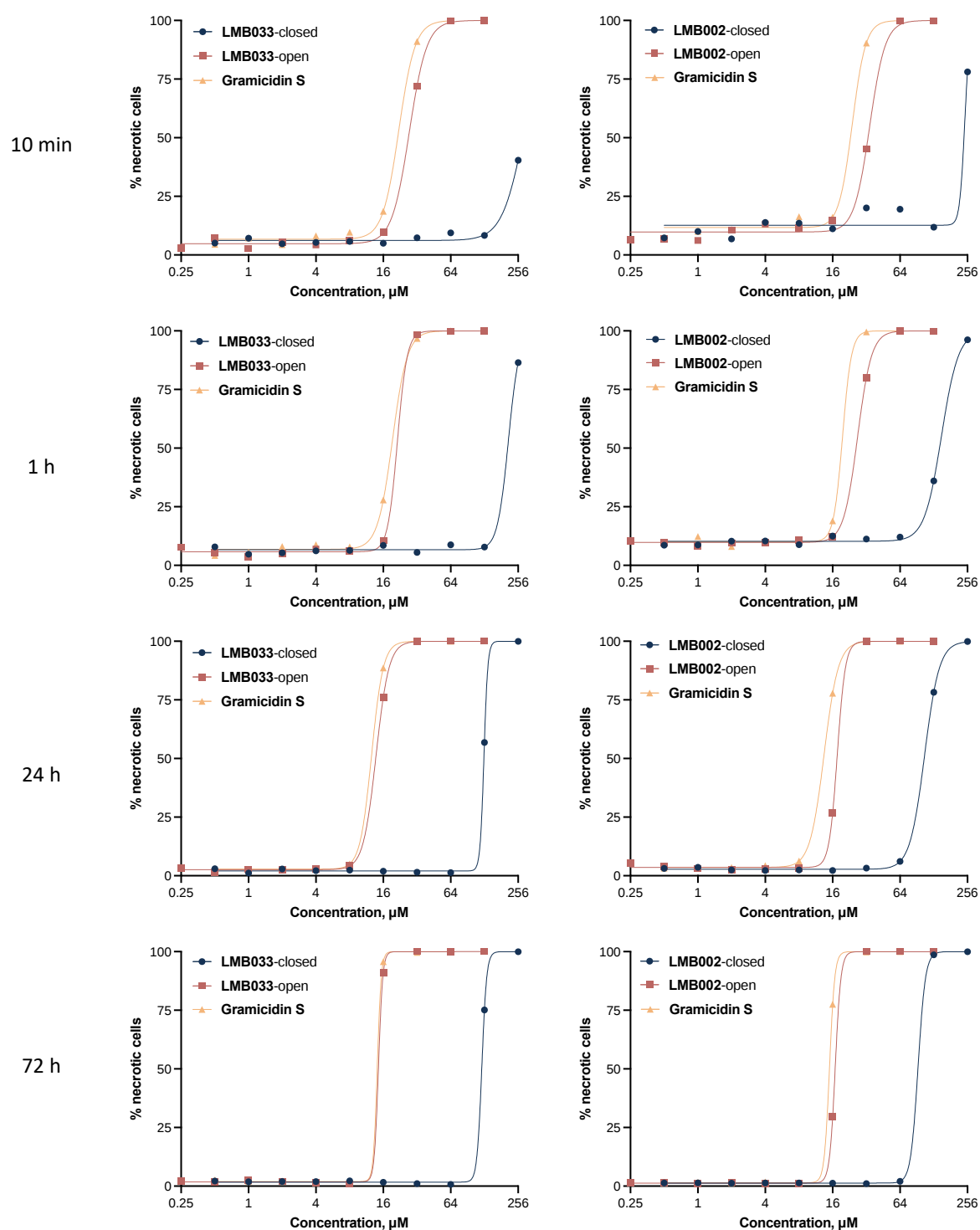


Figure S5. Dose-dependent curves for gramicidin S and its photocontrolled analogues tested on LLC cell line in two-dimensional format. Each point represents average value (n=4) with error bars depicting SEM.

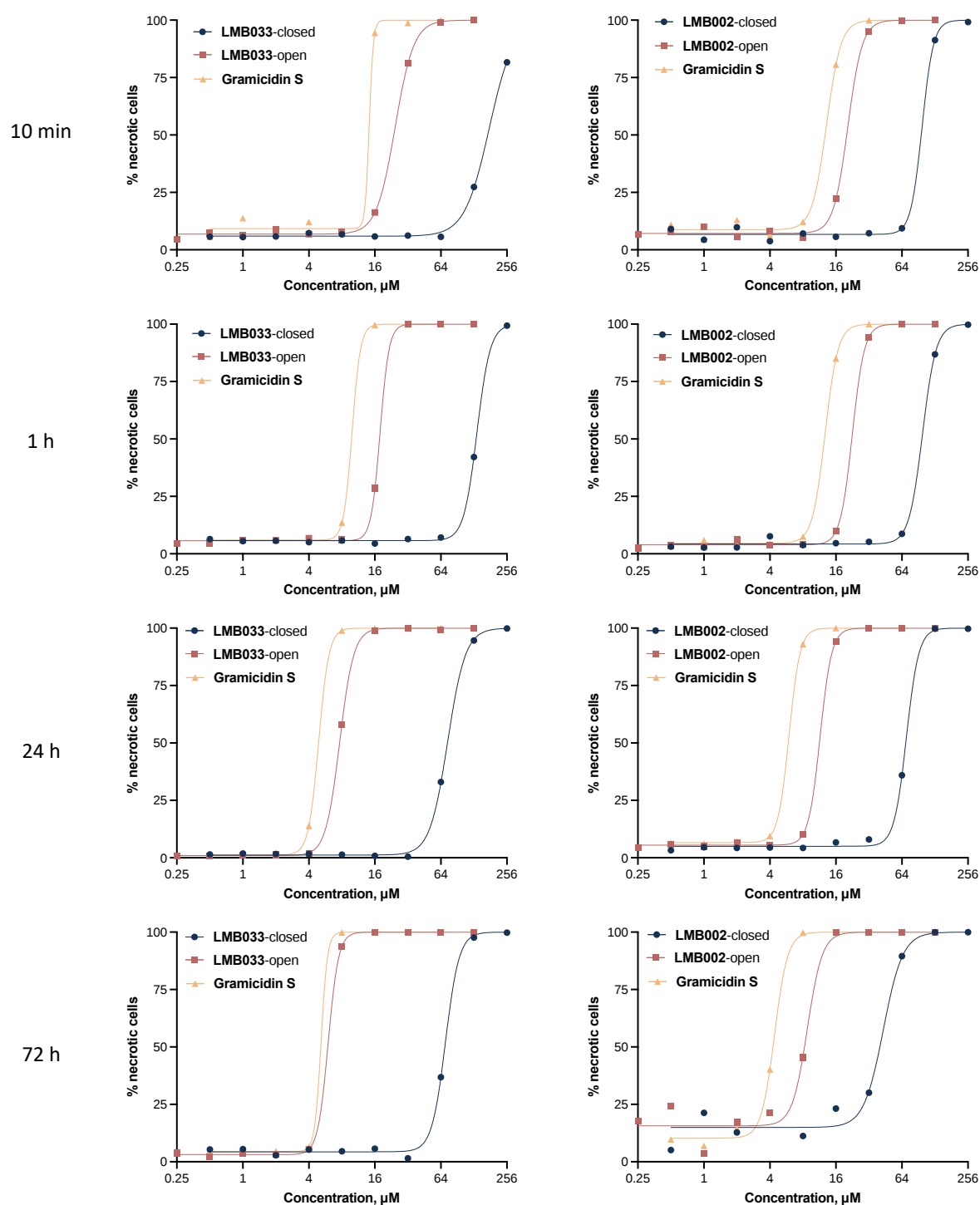


Figure S6. Dose-dependent curves for gramicidin S and its photocontrolled analogues tested on MDA-MB-231 cell line in two-dimensional format. Each point represents average value (n=4) with error bars depicting SEM.

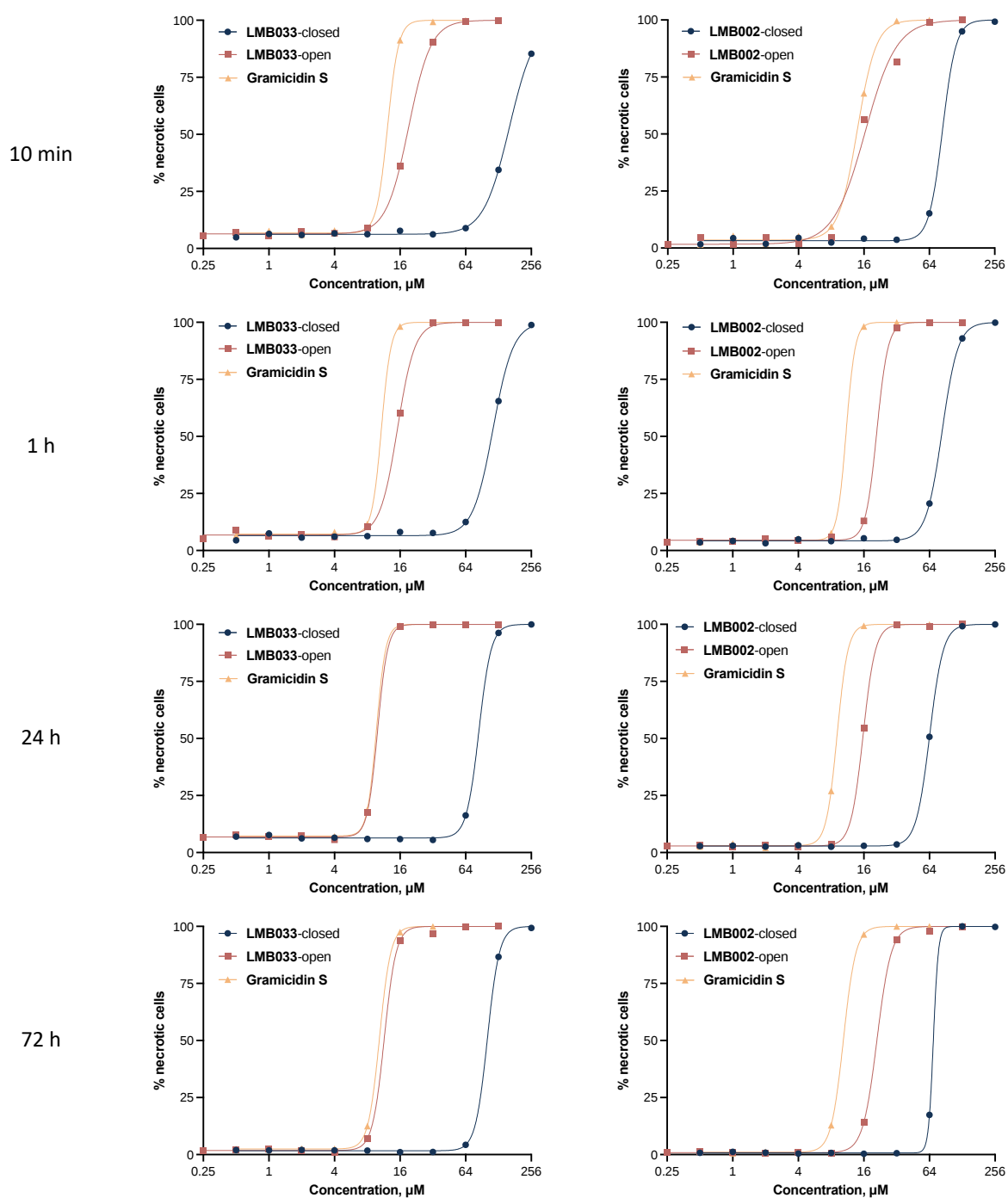


Figure S7. Dose-dependent curves for gramicidin S and its photocontrolled analogues tested on HEK-293 cell line in three-dimensional format. Each point represents average value (n=5) with error bars depicting SEM.

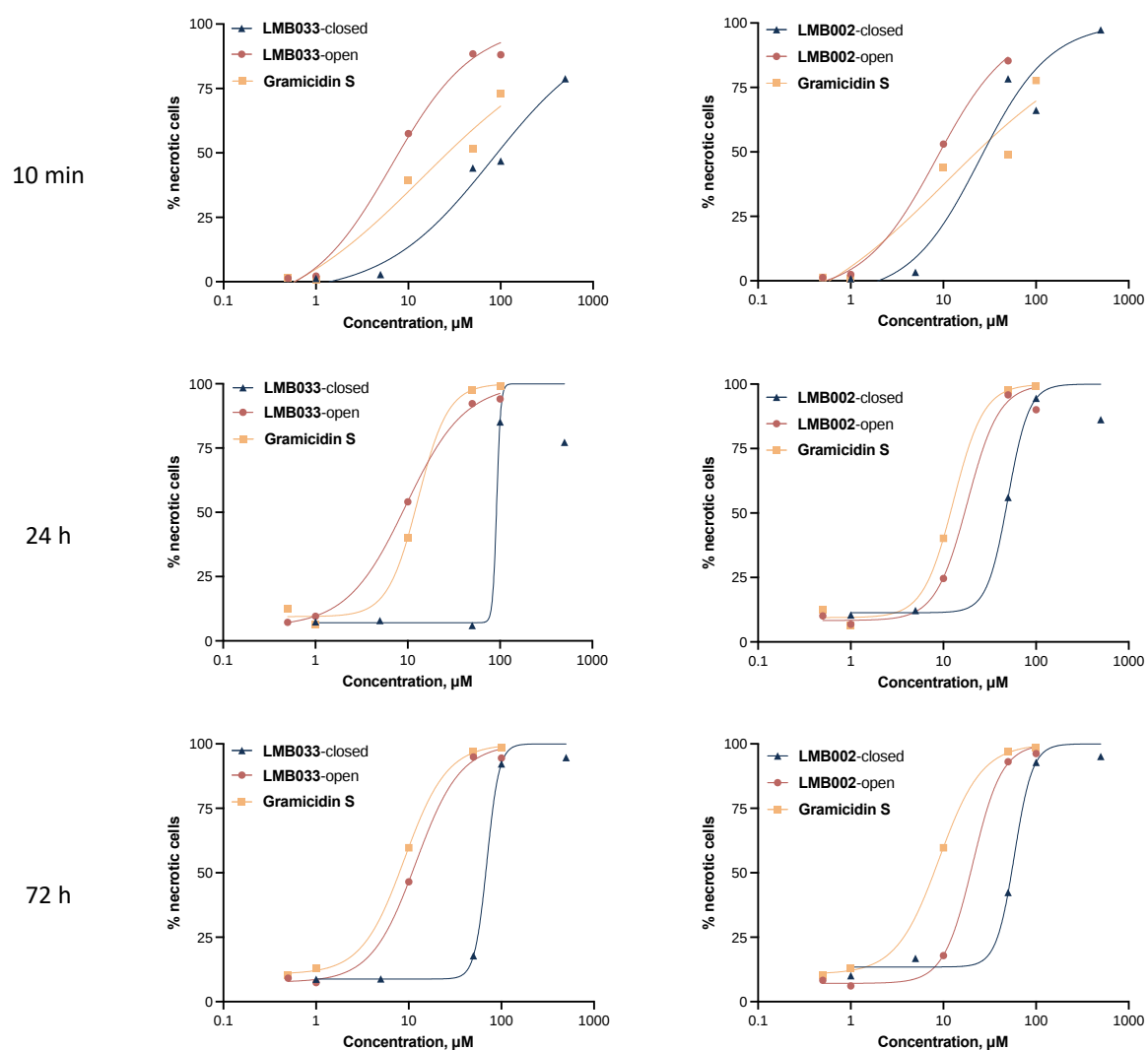


Figure S8. Dose-dependent curves for gramicidin S and its photocontrolled analogues tested on HeLa cell line in three-dimensional format. Each point represents average value (n=5) with error bars depicting SEM.

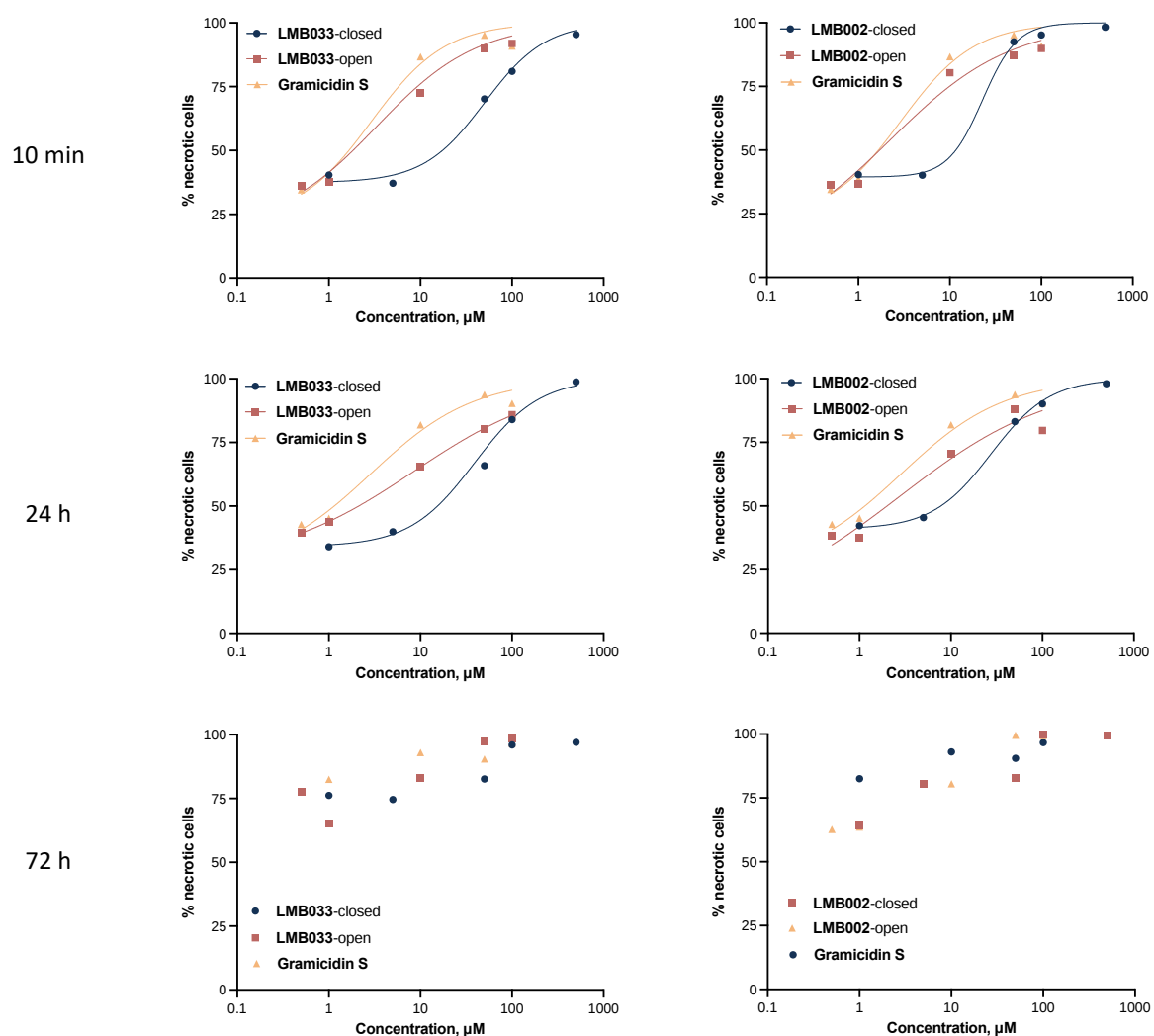


Figure S9. Dose-dependent curves for gramicidin S and its photocontrolled analogues tested on HepG2 cell line in three-dimensional format. Each point represents average value (n=5) with error bars depicting SEM.

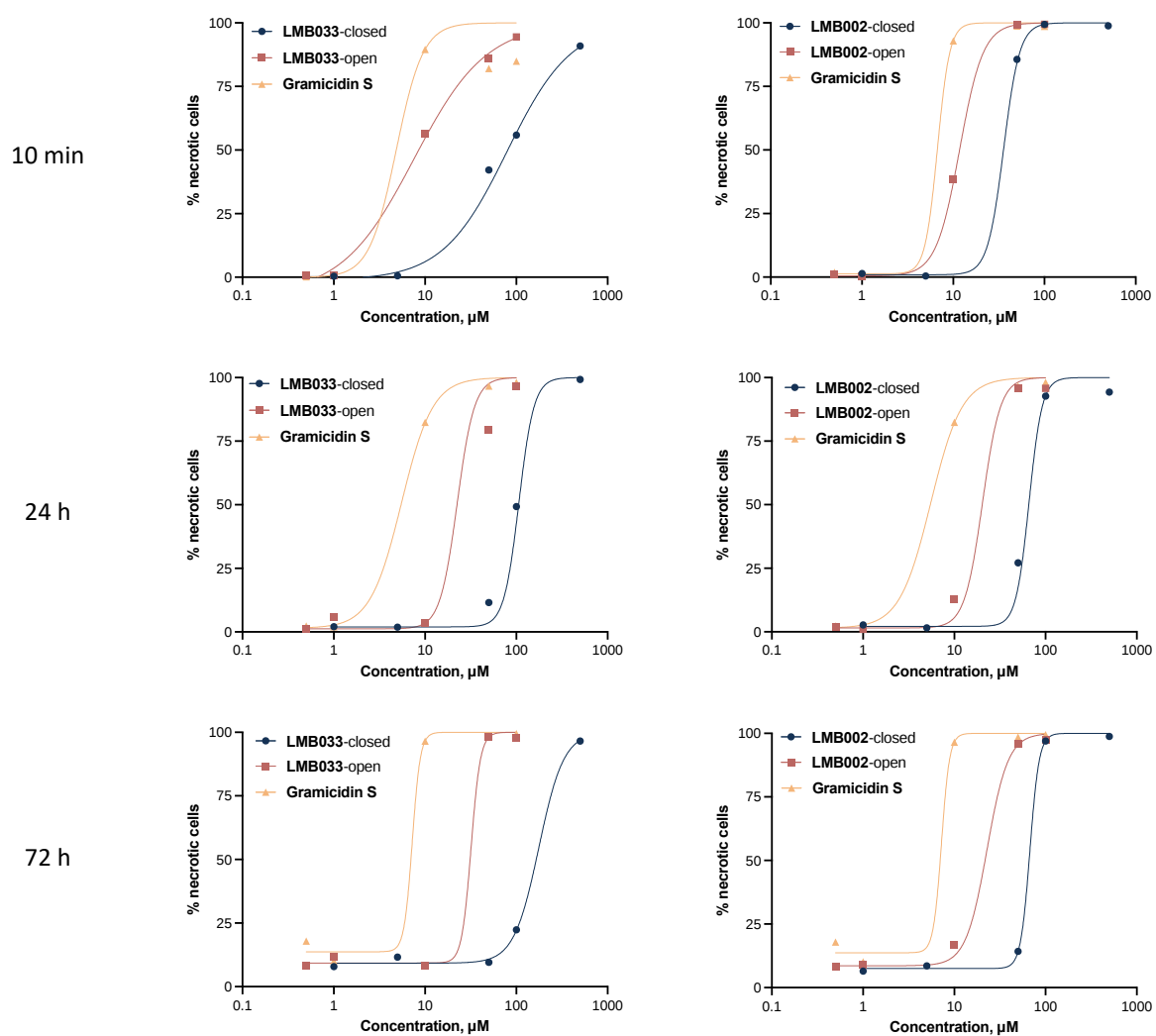


Figure S10. Dose-dependent curves for gramicidin S and its photocontrolled analogues tested on LLC cell line in three-dimensional format. Each point represents average value (n=5) with error bars depicting SEM.

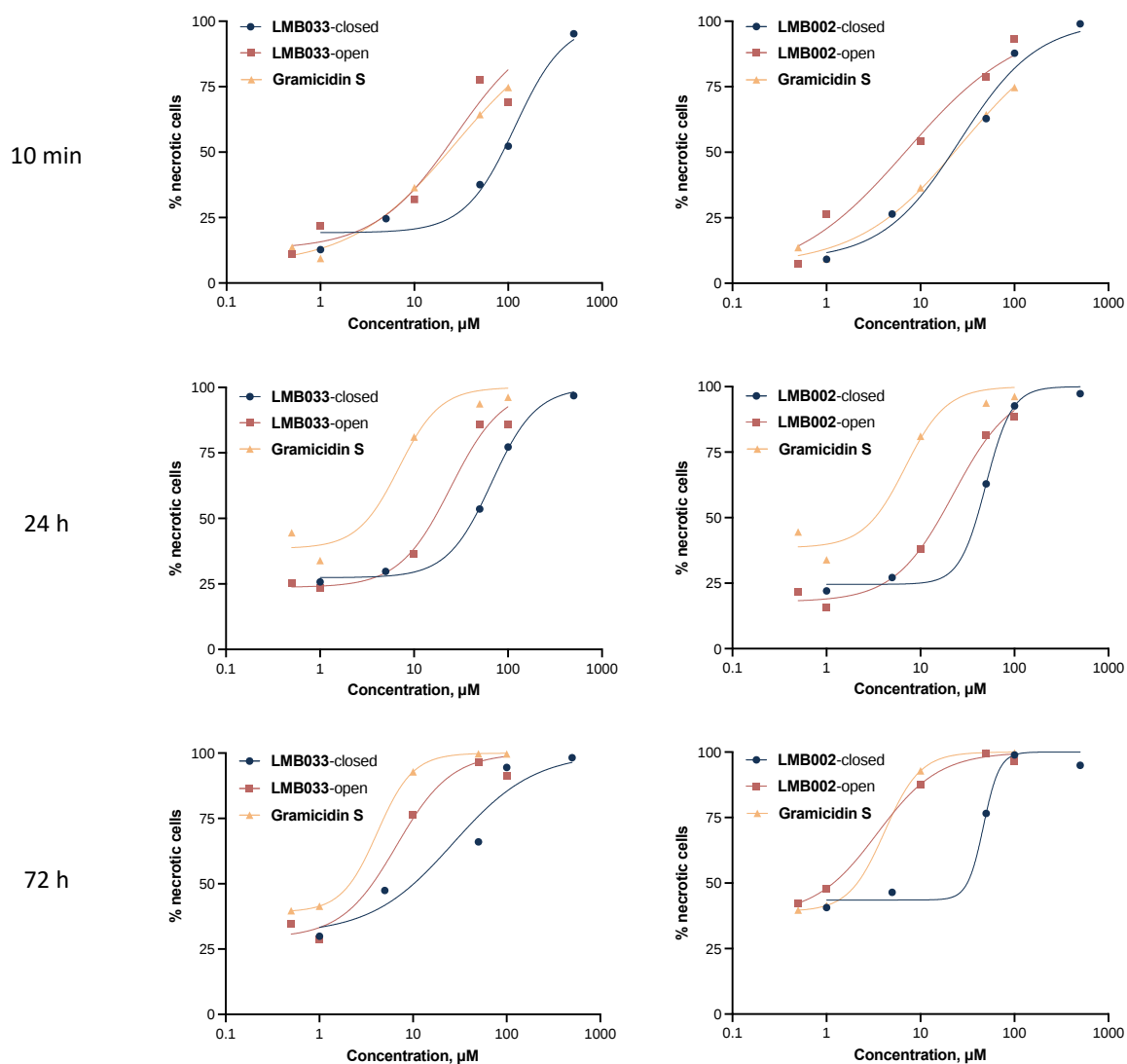


Figure S11. Dose-dependent curves for gramicidin S and its photocontrolled analogues tested on MDA-MB-231 cell line in three-dimensional format. Each point represents average value (n=5) with error bars depicting SEM.

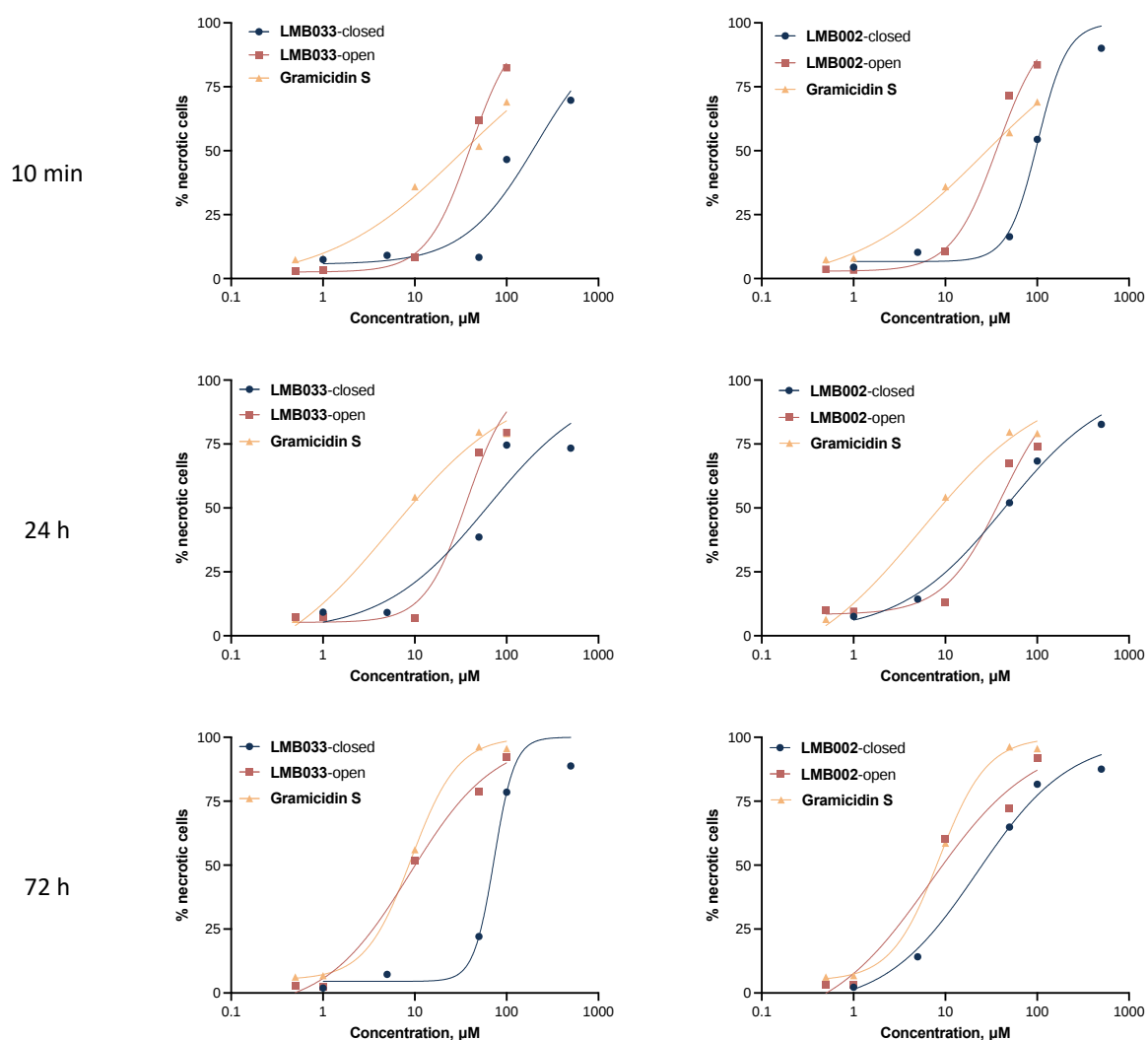


Figure S12. Kinetic curves of ATP release monitored for 1000 minutes after addition of gramicidin S and its photocontrolled analogues (run 1). Each point represents average value (n=3) with error bars depicting SEM.

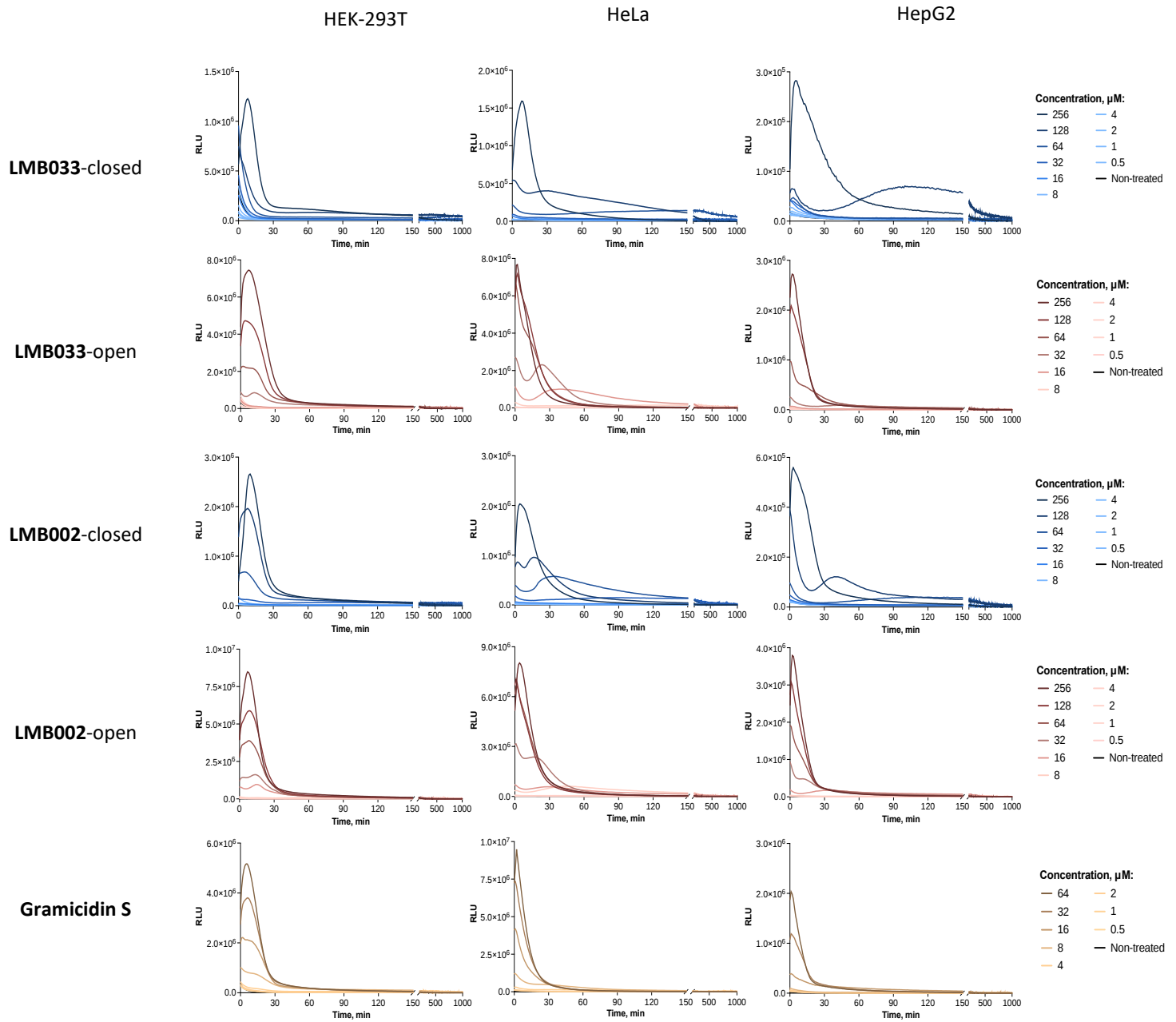


Figure S13. First derivatives of kinetic curves of ATP release monitored for 1000 minutes after addition of gramicidin S and its photocontrolled analogues (run 1). Each point represents average value (n=3) with error bars depicting SEM.

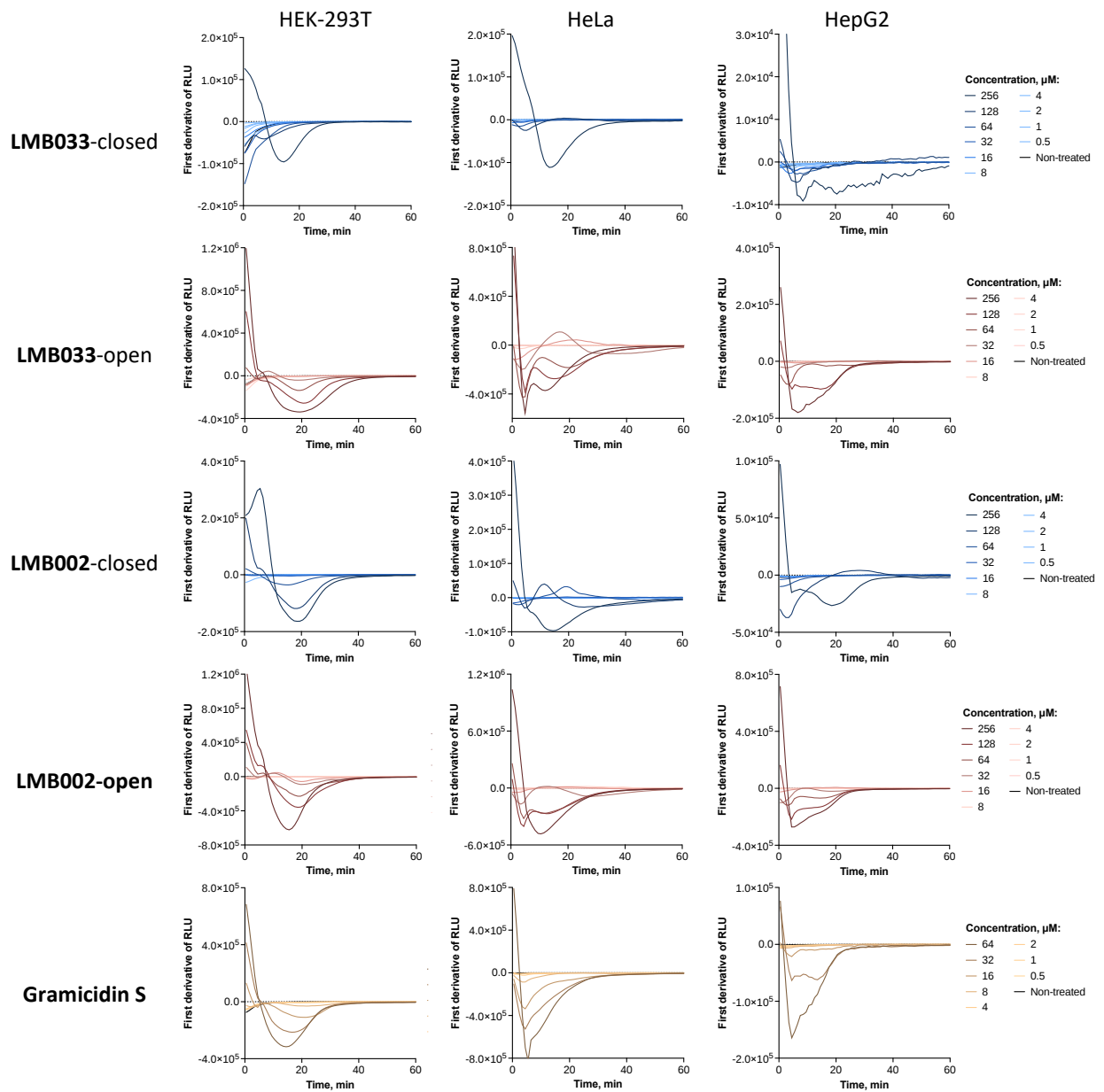


Figure S14. Kinetic curves of ATP release monitored for 1000 minutes after addition of gramicidin S and its photocontrolled analogues (run 2). Each point represents average value (n=3) with error bars depicting SEM.

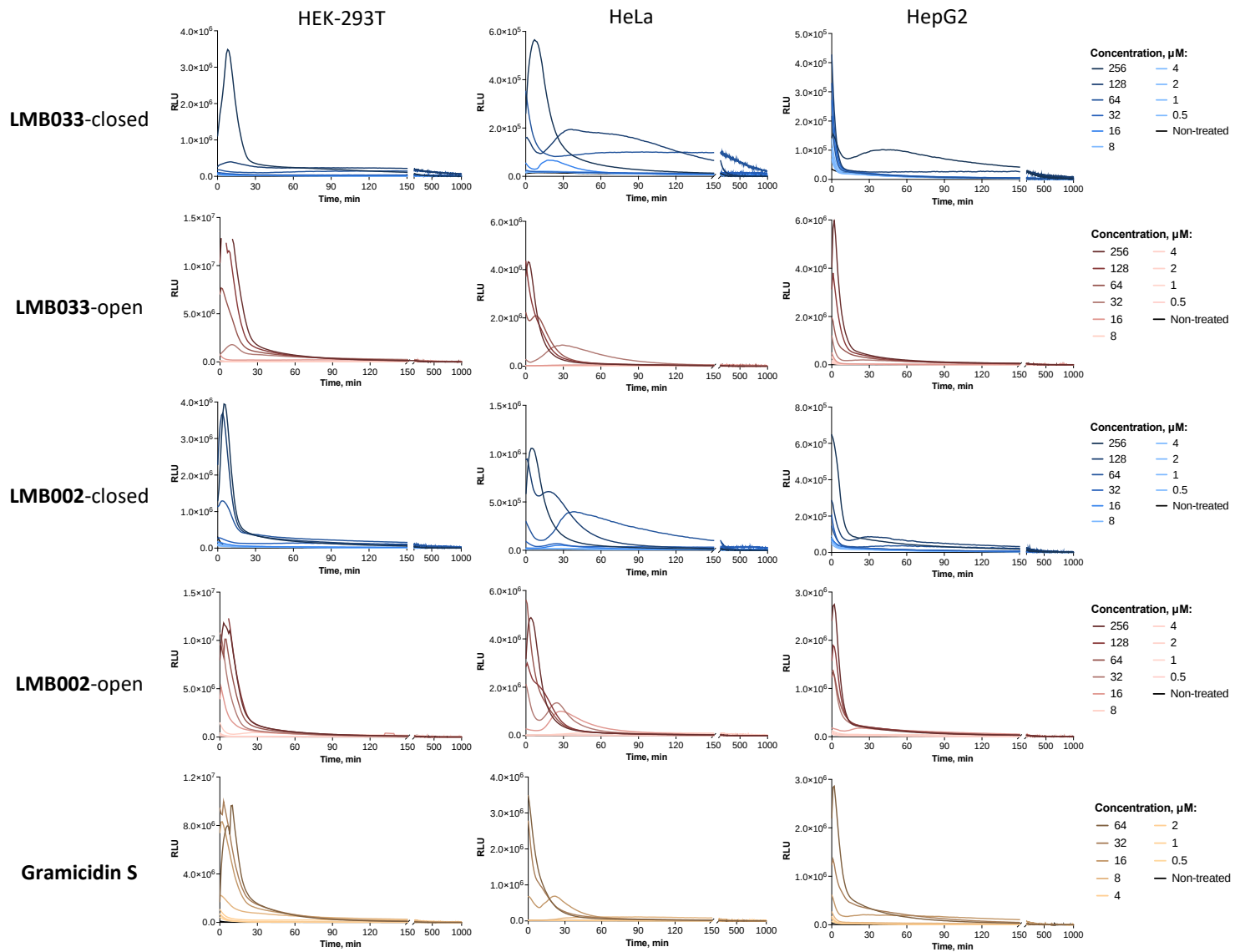


Figure S15. Area-under-curve (AUC) histograms of ATP release monitored for 1000 minutes after addition of gramicidin S and its photocontrolled analogues (run 2). Each point represents average value (n=3) with error bars depicting SEM.

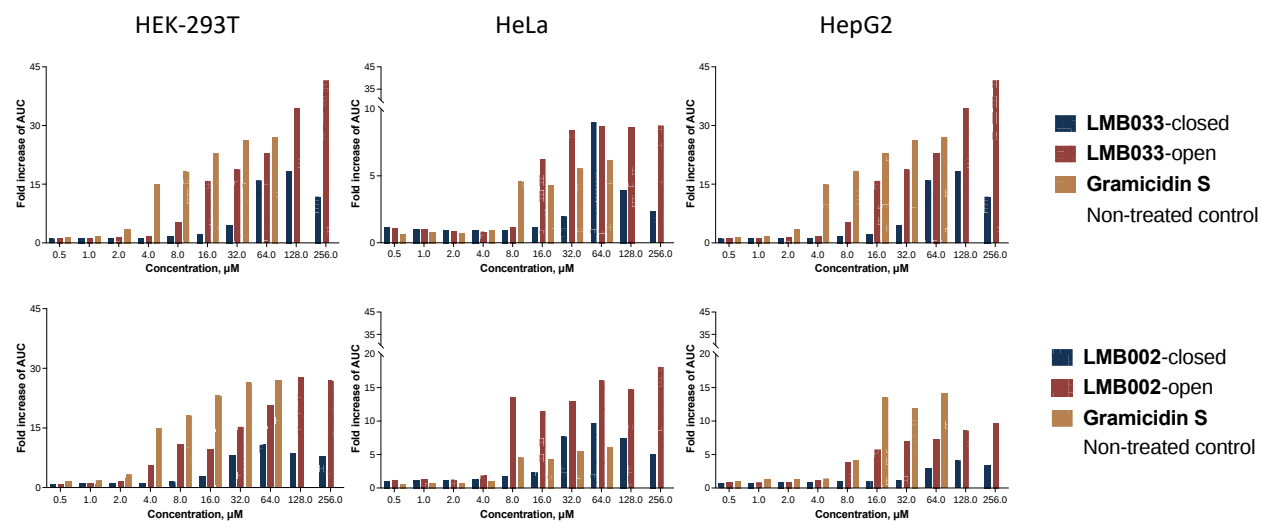


Figure S16. First derivatives of kinetic curves of ATP release monitored for 1000 minutes after addition of gramicidin S and its photocontrolled analogues (run 2). Each point represents average value (n=3) with error bars depicting SEM.

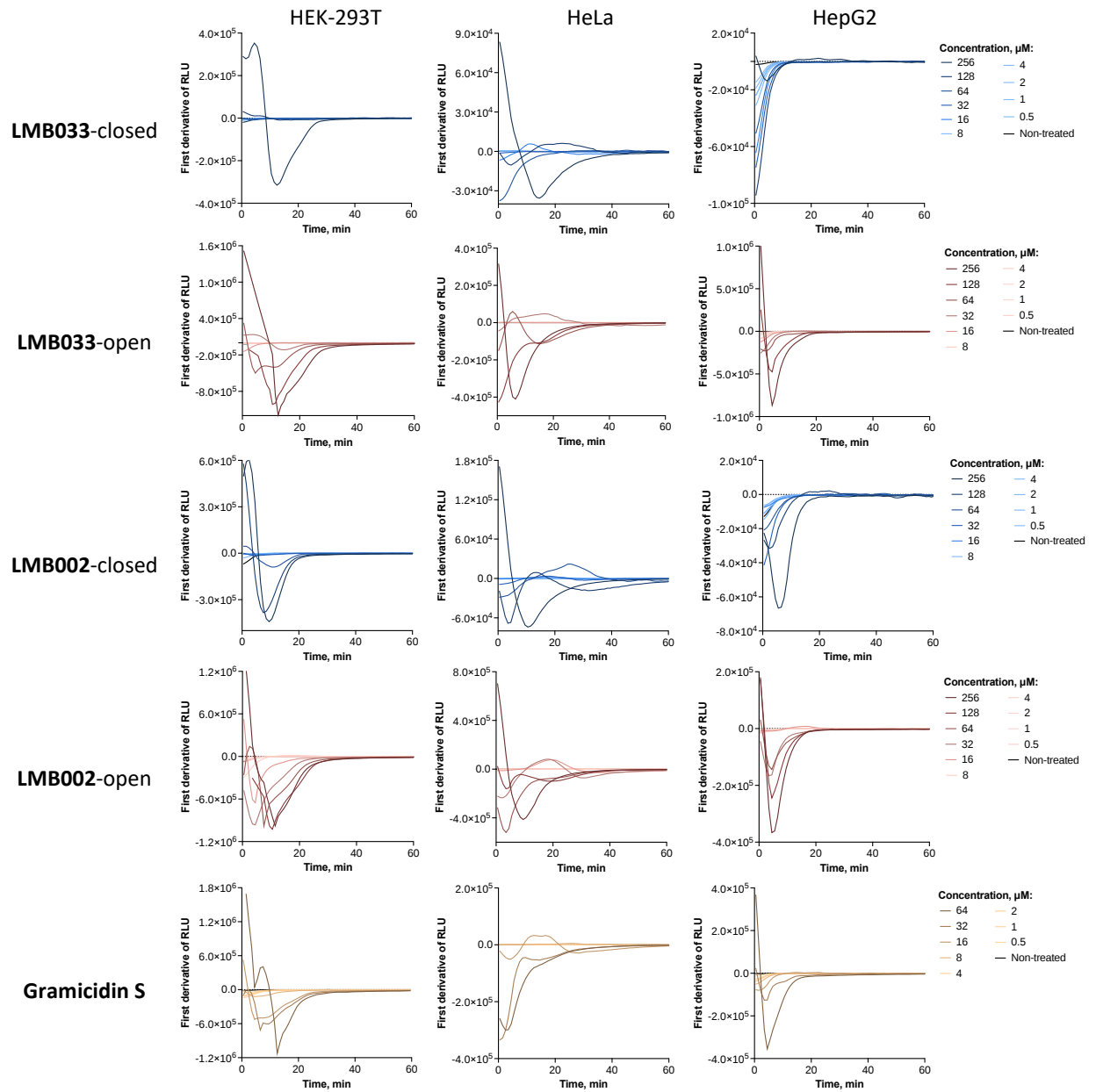


Figure S17. Scatterplots of MDA-MB-231 cells treated with LMB033-open at concentration of 4, 8, 16 and 32 μM for 30 mins, 3 hours, 6 hours and 24 hours with antibody-staining for membrane-bound CALR. The percentages on each graph illustrate fraction of cells gated as CALR-positive.

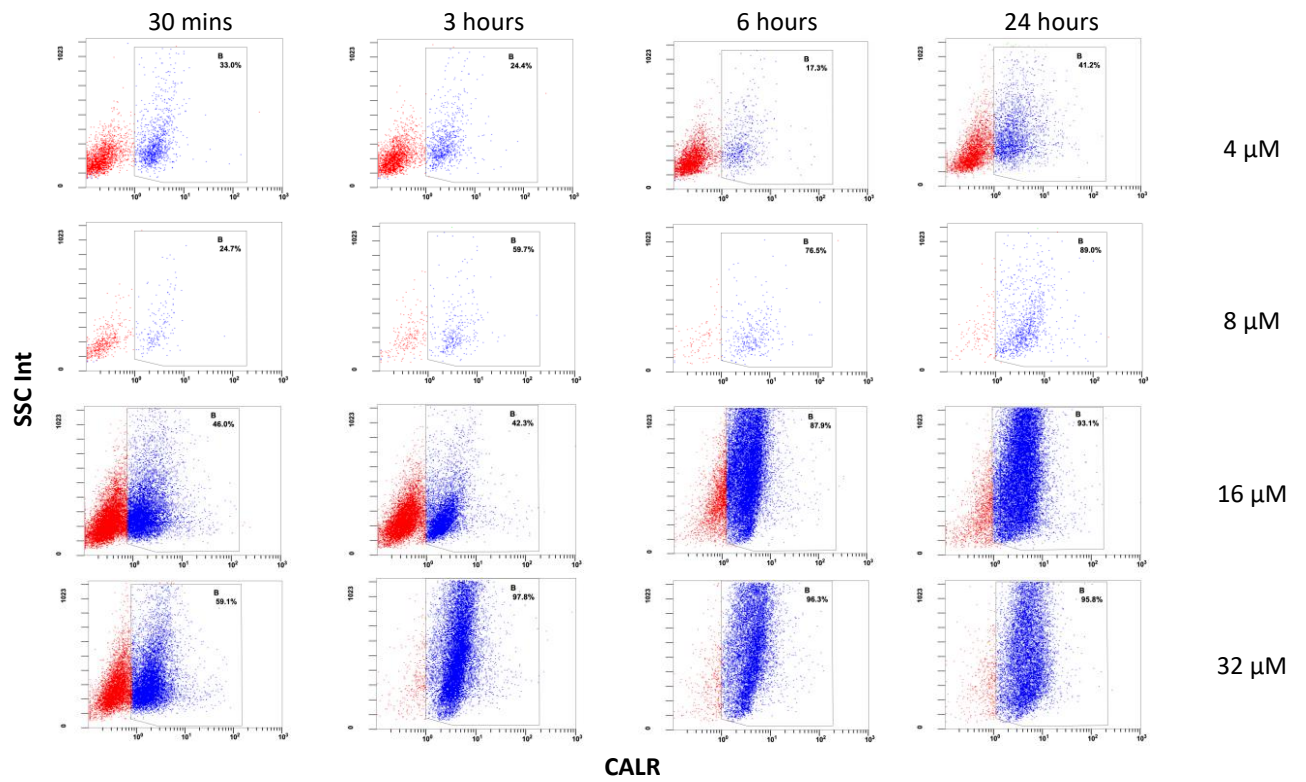


Figure S18. Scatterplots of MDA-MB-231 cells treated with LMB002-open at concentration of 4, 8, 16 and 32 μM for 30 mins, 3 hours, 6 hours and 24 hours with antibody-staining for membrane-bound CALR. The percentages on each graph illustrate fraction of cells gated as CALR-positive.

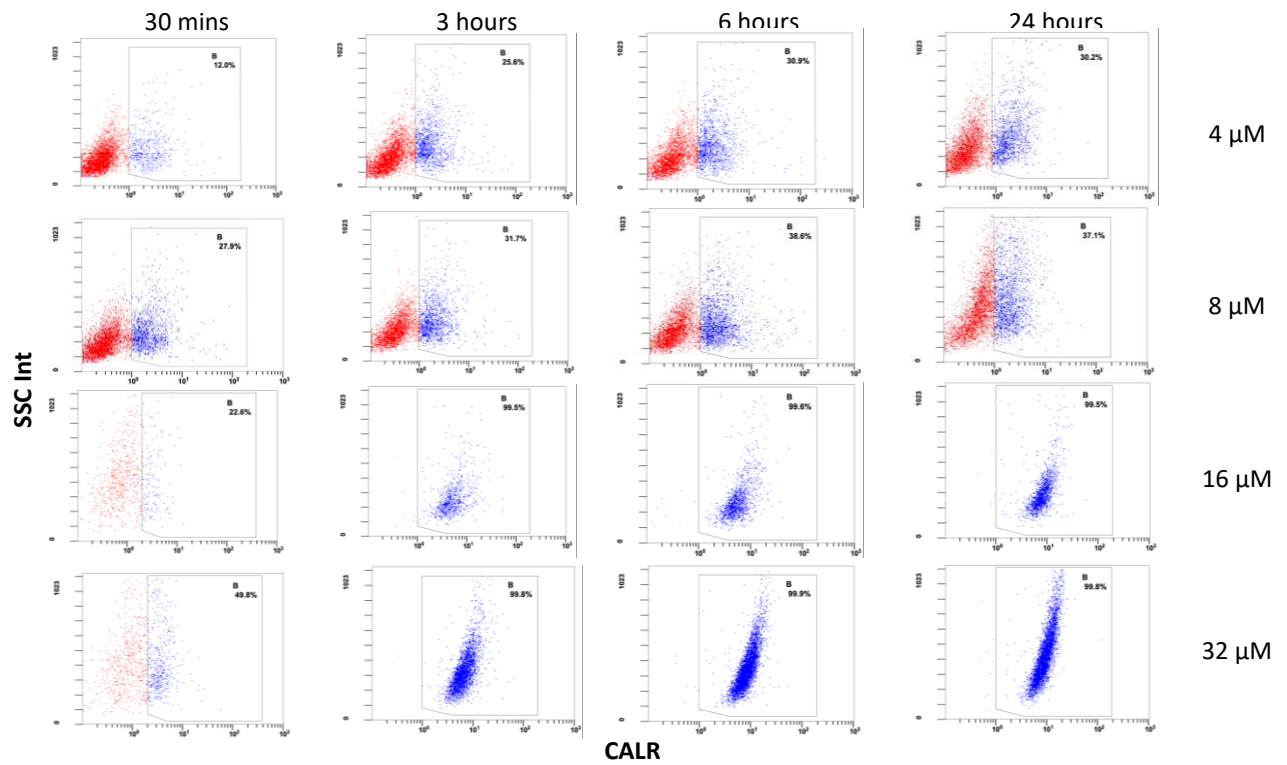


Figure S19. Scatterplots of MDA-MB-231 cells treated with gramicidin S at concentration of 4, 8, 16 and 32 μM for 30 mins, 3 hours, 6 hours and 24 hours with antibody-staining for membrane-bound CALR. The percentages on each graph illustrate fraction of cells gated as CALR-positive.

