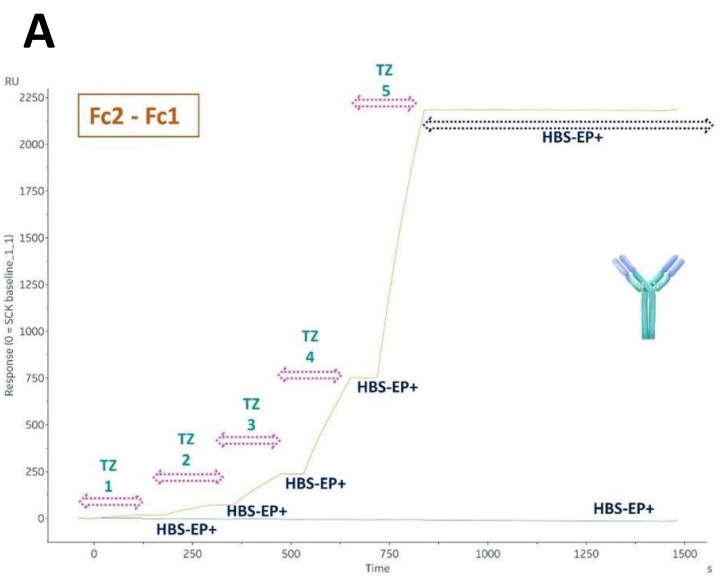
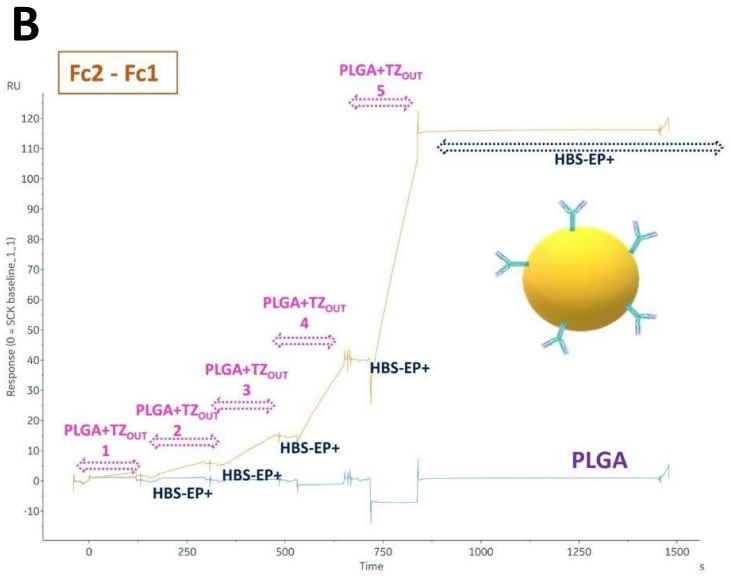
Supplementary material





TZ free		
	TZ [µg/ml]	ΔRU
1	0,026	18
2	0,08	70
3	0,24	238
4	0,72	752
5	2,16	2183
	Dissociation	2181

PLGA+TZ$_{OUT}$			
	NPs [µg/ml]	TZ [µg/ml]	ΔRU
1	1	0,026	1,7
2	3	0,08	5,2
3	9	0,24	14,2
4	27	0,72	39,7
5	81	2,16	115,6
	Dissociation		115,8

PLGANaked			
	NPs [µg/ml]	TZ [µg/ml]	ΔRU
1	1	0	0,1
2	3	0	0,3
3	9	0	0,3
4	27	0	0,7
5	81	0	0,8
	Dissociation		0,7

Fig. S1 Specific HER2-TZ binding documented in SPR sensorgrams and relative RU. (A) Sensorgram for free TZ and (B) for PLGA+TZ$_{OUT}$ nanoparticles at different concentrations compared with PLGANake.

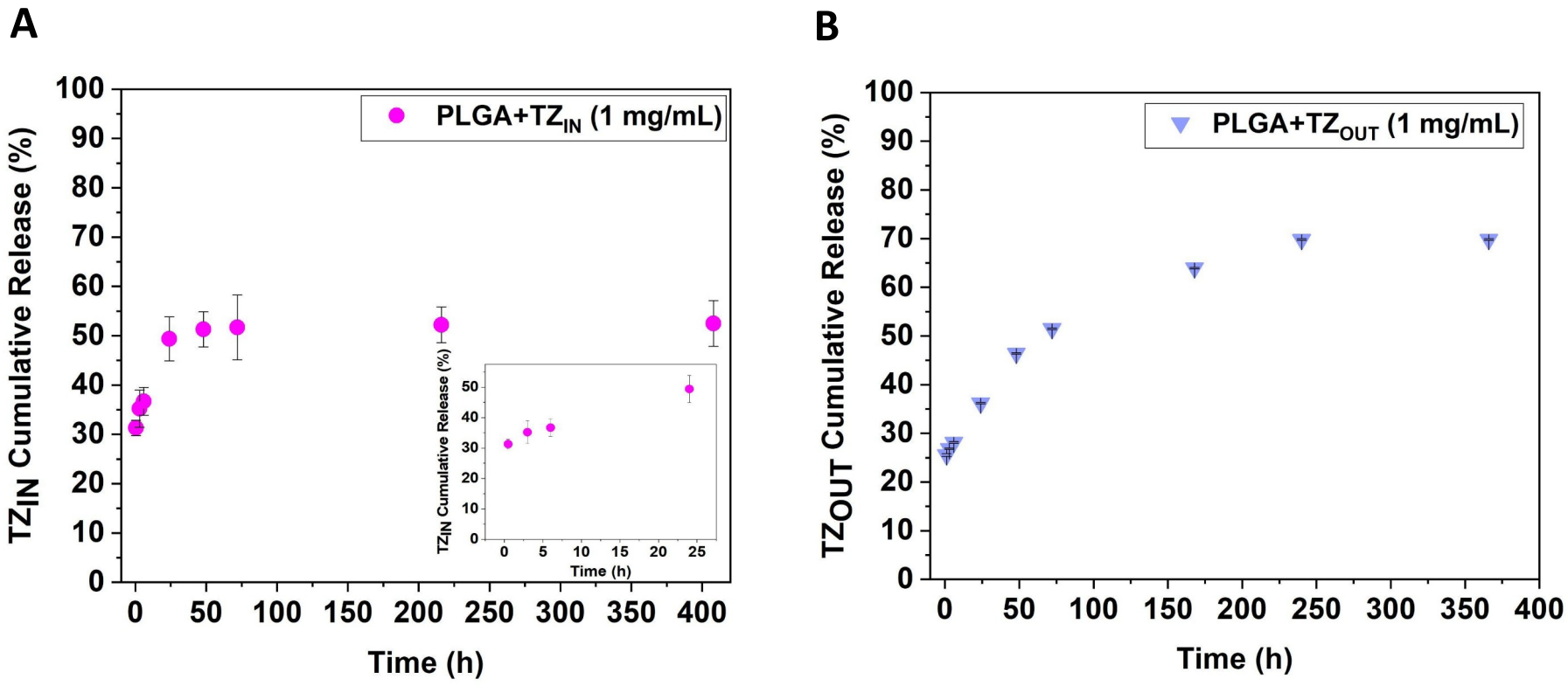


Fig. S2 Cumulative release profile of trastuzumab. (A) TZ released from PLGA nanoparticles, which encapsulate the mAB inside its aqueous core (PLGA+TZ_{IN}); (B) TZ released from PLGA with empty core and subsequently functionalized on its surface with the TZ (PLGA+TZ_{OUT}). The experiments were performed three times in PBS buffer at 37°C for 14 days. The inset of figure a shows the percentage of drug release during the first 24 hours of incubation. The data are expressed as percentage of TZ released (amount of released over the total encapsulated TZ) versus time.

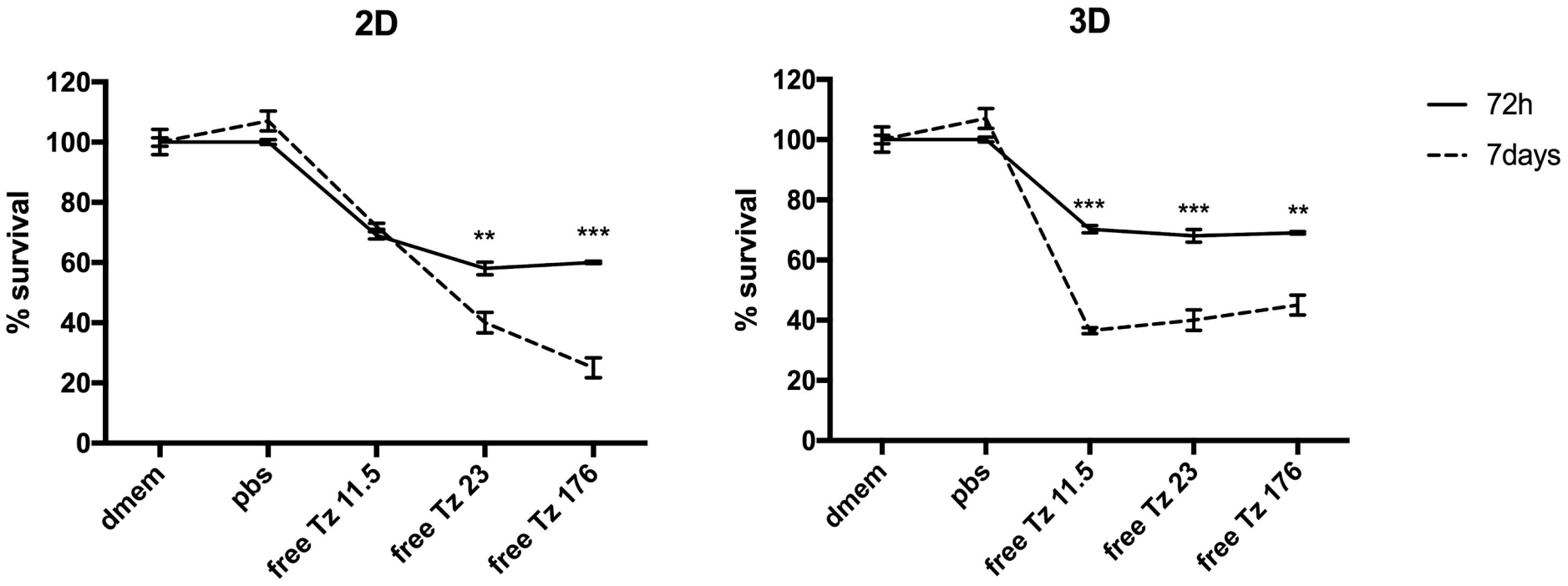


Fig. S3 Efficacy of free TZ on cell proliferation inhibition in BT-474 cell line after 72 hours or 7 days of treatment in 2D and 3D culture models.

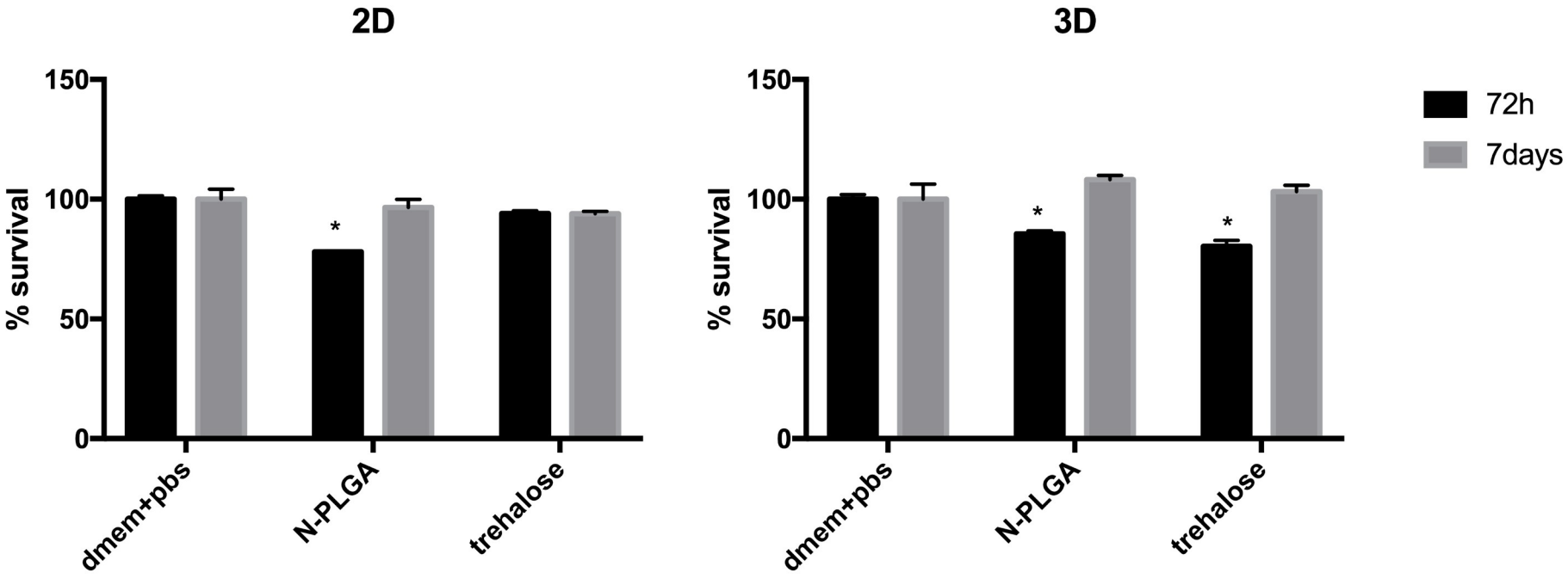
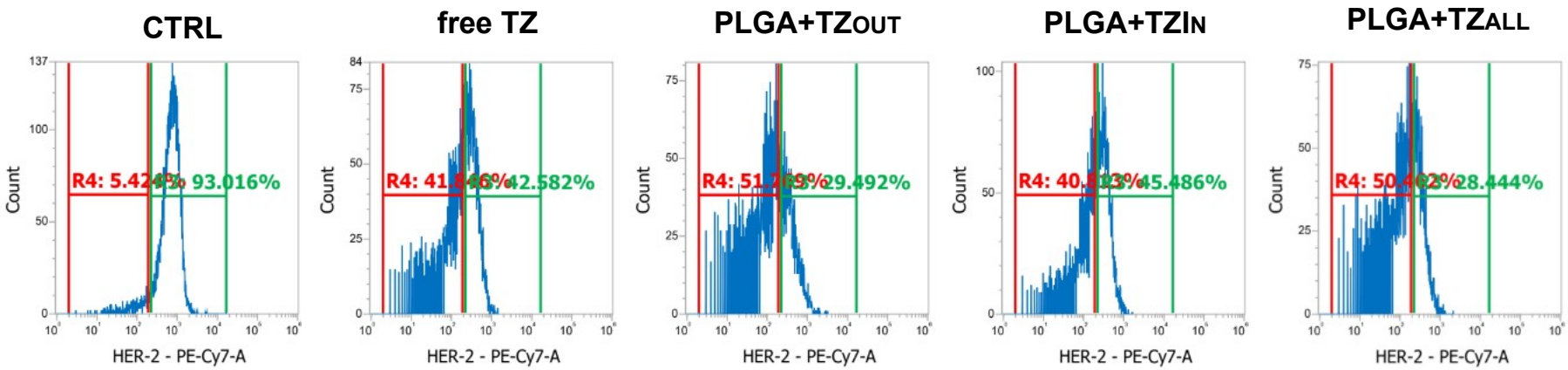


Fig. S4 Effect of naked PLGA (N-PLGA) and trehalose on cell proliferation inhibition in BT-474 cell line after 72 hours or 7 days of treatment in 2D and 3D culture models. *p<0.05, compared to untreated controls.

72 hours



7 days

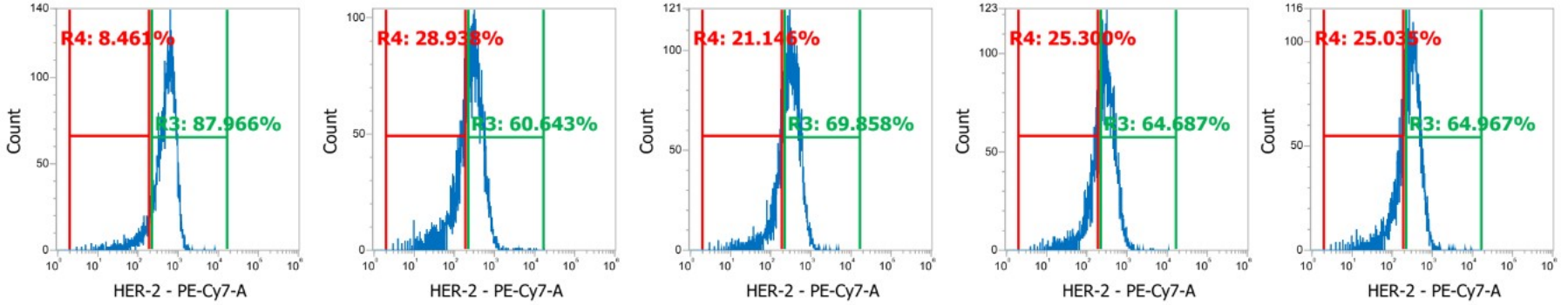


Fig. S5 Flow cytometry analysis of HER2 expression in BT-474 cell line untreated (CTRL), treated with different PLGA+TZ nanoparticles or treated with free TZ after 72 hours or 7 days.