Electronic Supplementary Information (ESI)

Enhanced targeting of triple-negative breast carcinoma and malignant melanoma by photochemical internalization of CSPG4-targeting immunotoxins

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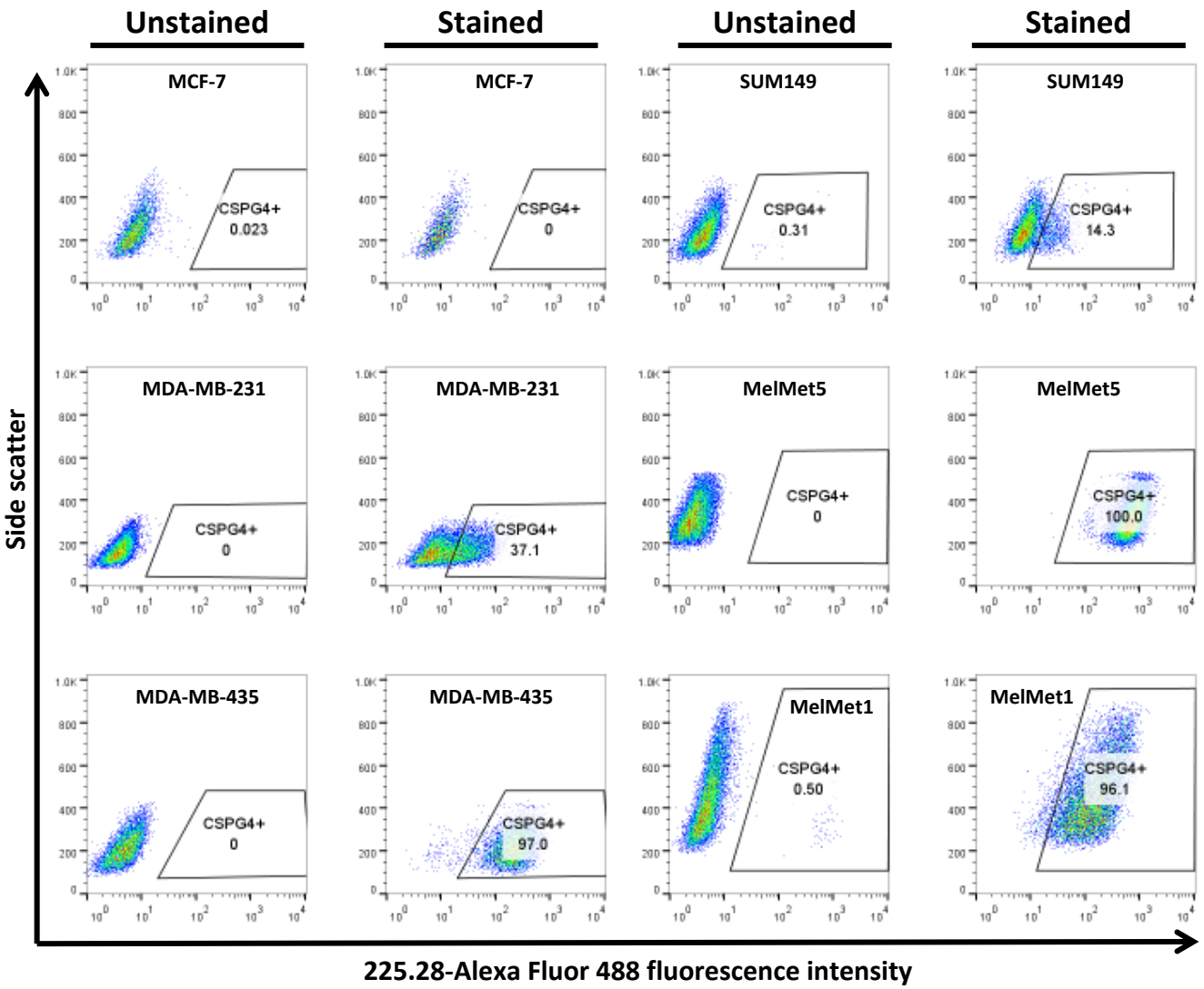
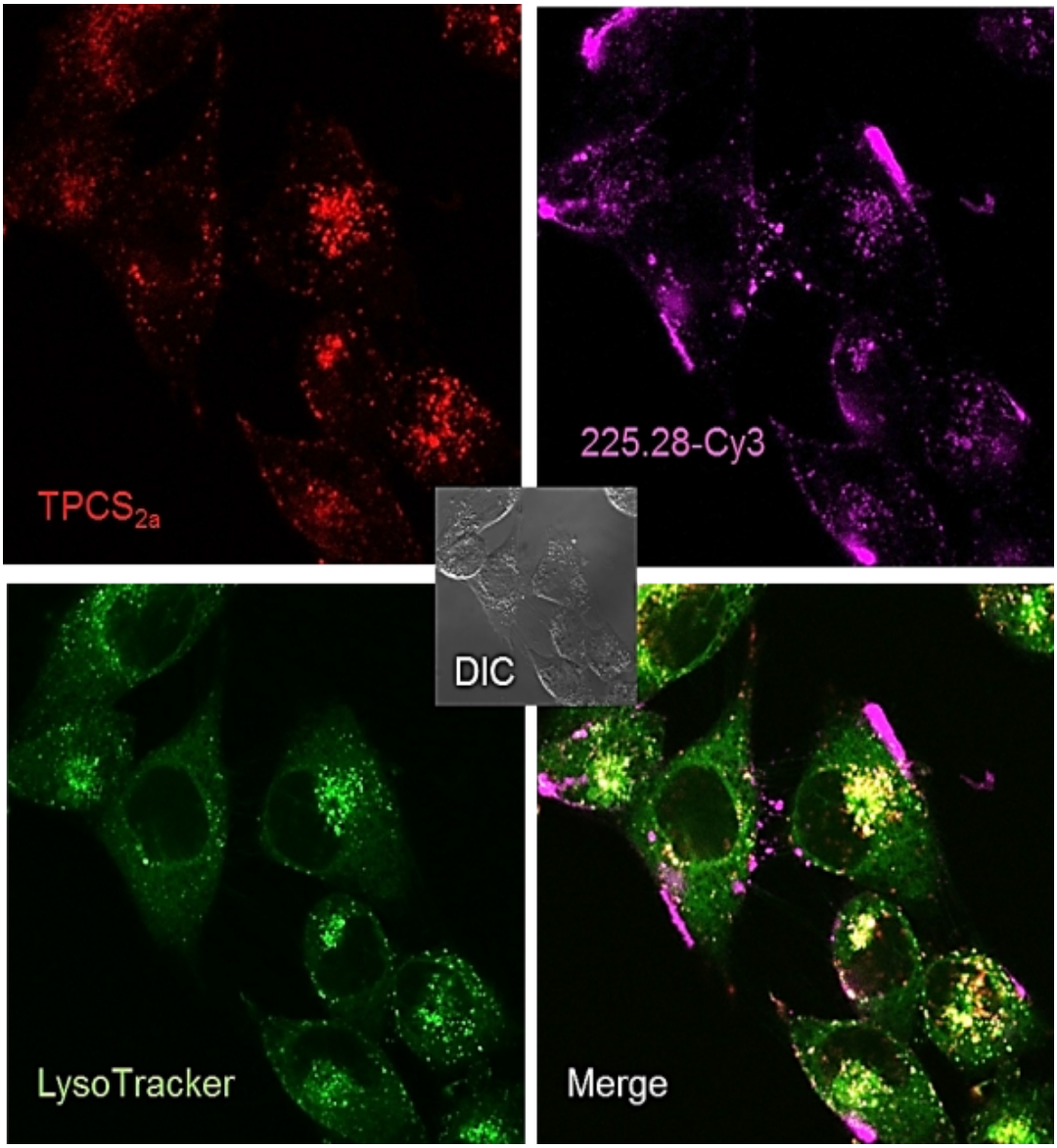
Supplementary Fig. 1



Supplementary Fig. S1. Surface expression of CSPG4 evaluated by flow cytometry and presented as a dot plot of side scatter versus 225.28-Alexa Fluor 488 staining (same data as Figure 1). Number in each gate represents percentage of cells defined as CSPG4^{+}. Figure shows a single representative experiment out of three independent experiments.

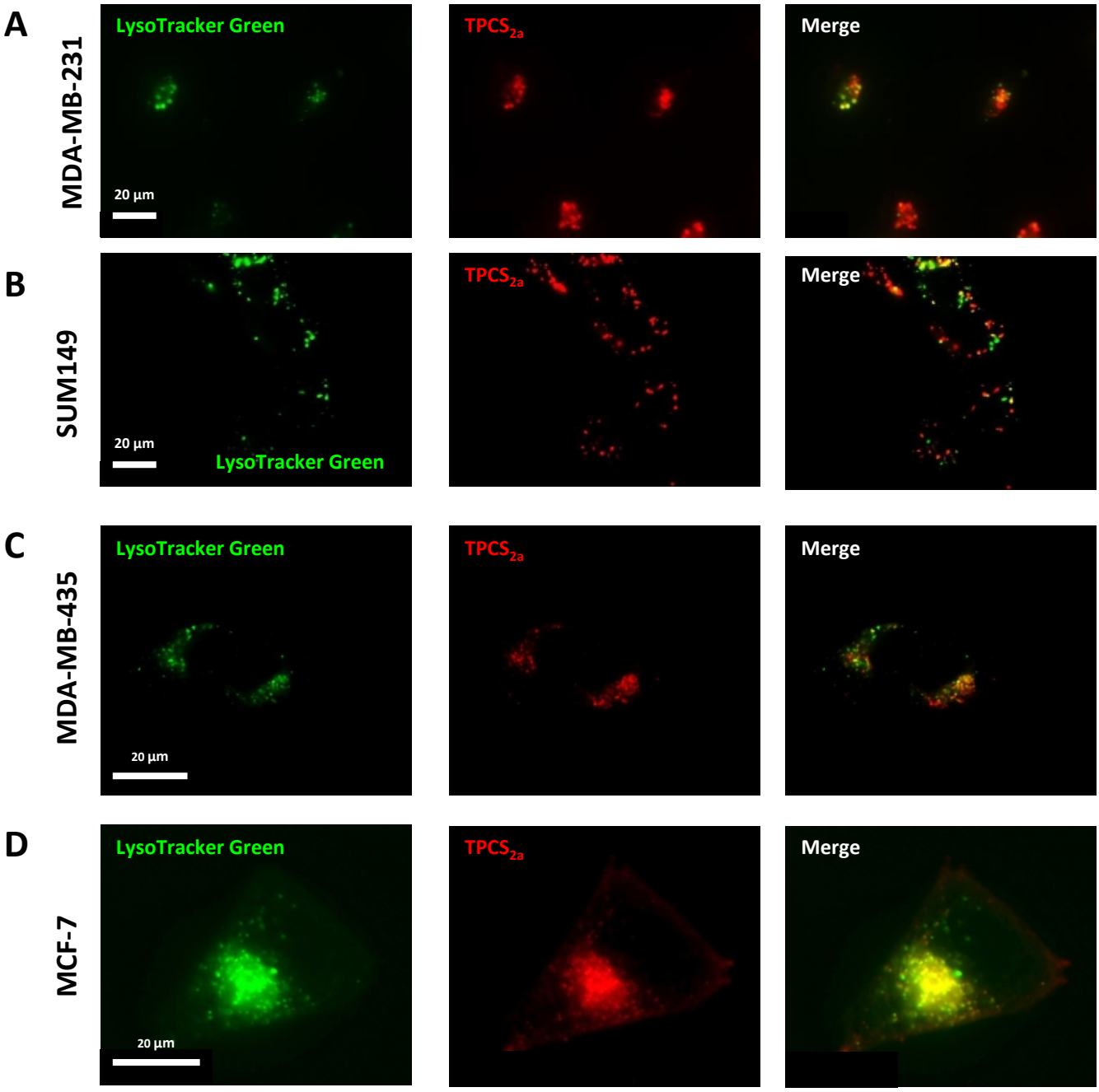
Supplementary Fig. 2



Supplementary Figure S2. The CSPG4-targeting mAb 225.28-mAb co-localizes with the PCI photosensitizer TPCS2a and LysoTracker. Confocal microscopy of MDA-MB-435 cells reveals partly co-localization of TPCS2a, 225.28-Cy3 ®. 1 µg/ml $TPCS_{2a}$ was co-incubated with Cy3-labelled, CSPG4-recognizing mAbs (30 nM), 225.28 for 18 h. directly on coverslips , washed and chased for 4 h. prior to investigation. LysoTracker® Green, a dye accumulating in endo-lysosomal compartments was added 1-2 h. prior examination.

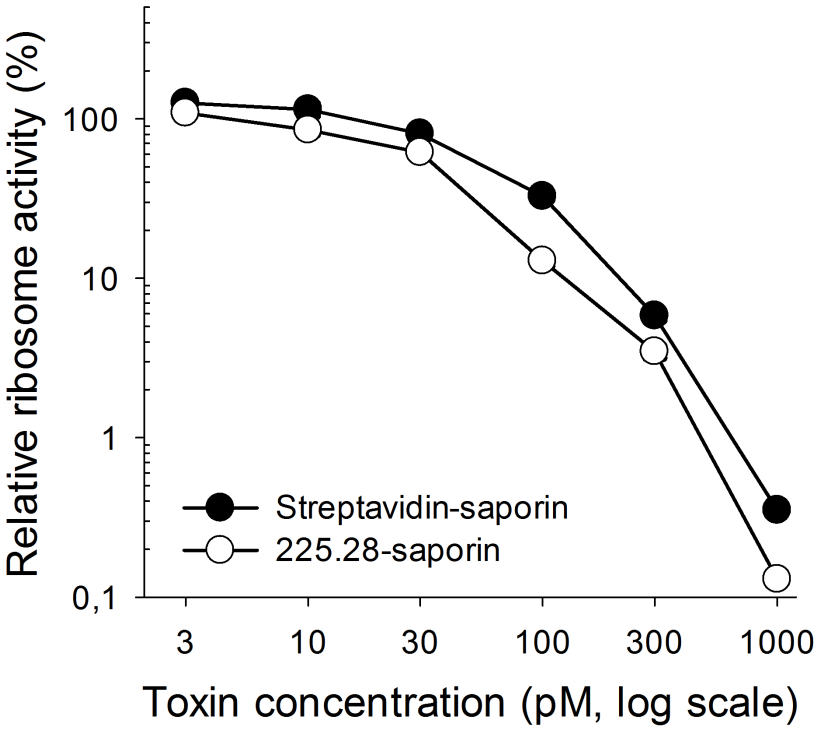
Degree of co-localization in a set of four different micrographs with 5-10 cells/picture. For 225.28-Cy3 there is around 63% co-localization (Manders) with TPCS2a, and of this population the majority (78%) is co-localized with LysoTracker Green. Hence, co-localization measurements indicate that 49% of TPCS2a, 225.28-Cy3 and Lysotracker green is co-localised, which is in line with a previous work where we studied co-localization of the same photosensitizer and LysoTracker Green with an antibody targeting CD133 (Bostad et al. 2013: https://doi.org/10.1016/j.jconrel.2013.03.023).

Supplementary Fig. 3



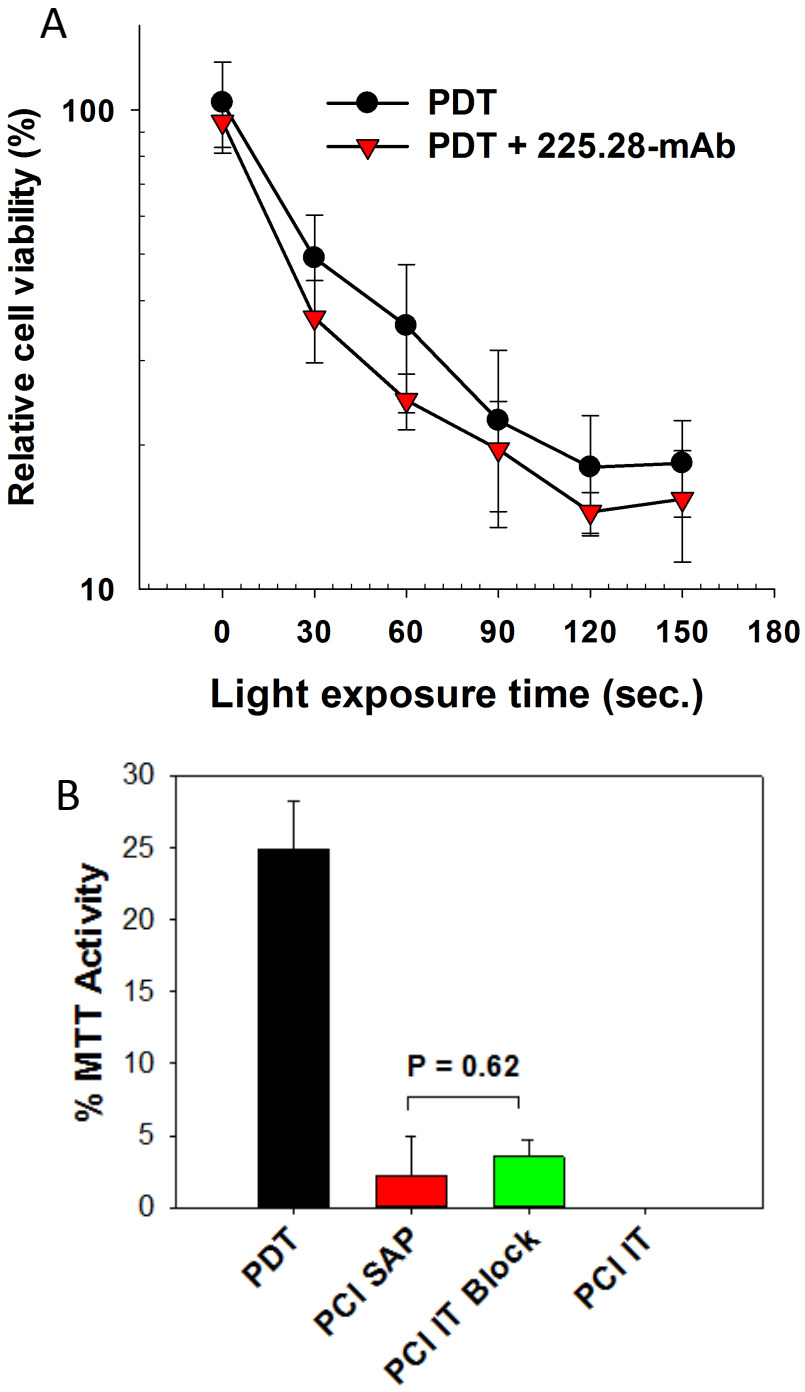
Supplementary Figure S3. Co-localization of $TPCS_{2a}$ and LysoTracker Green (LTG) in the human breast cancer cell panel. Subcellular localization of the PCI-photosensitizer $TPCS_{2a}$ and LTG in **A)** MDA-MB-231, **B)** SUM149, **C)** MDA-MB-435 and **D)** MCF-7. Cells were incubated for 18 hrs in medium containing $TPCS_{2a}$ (1 µg/ml) and chased for 4 hrs in fresh medium prior to examination by epi-fluorescence microscopy. LTG (1 µM) was added to the medium 1 hrs prior to microscopy. Micrographs shows reproduced and representative cells with fluorescence of the dyes.

Supplementary Fig. 4



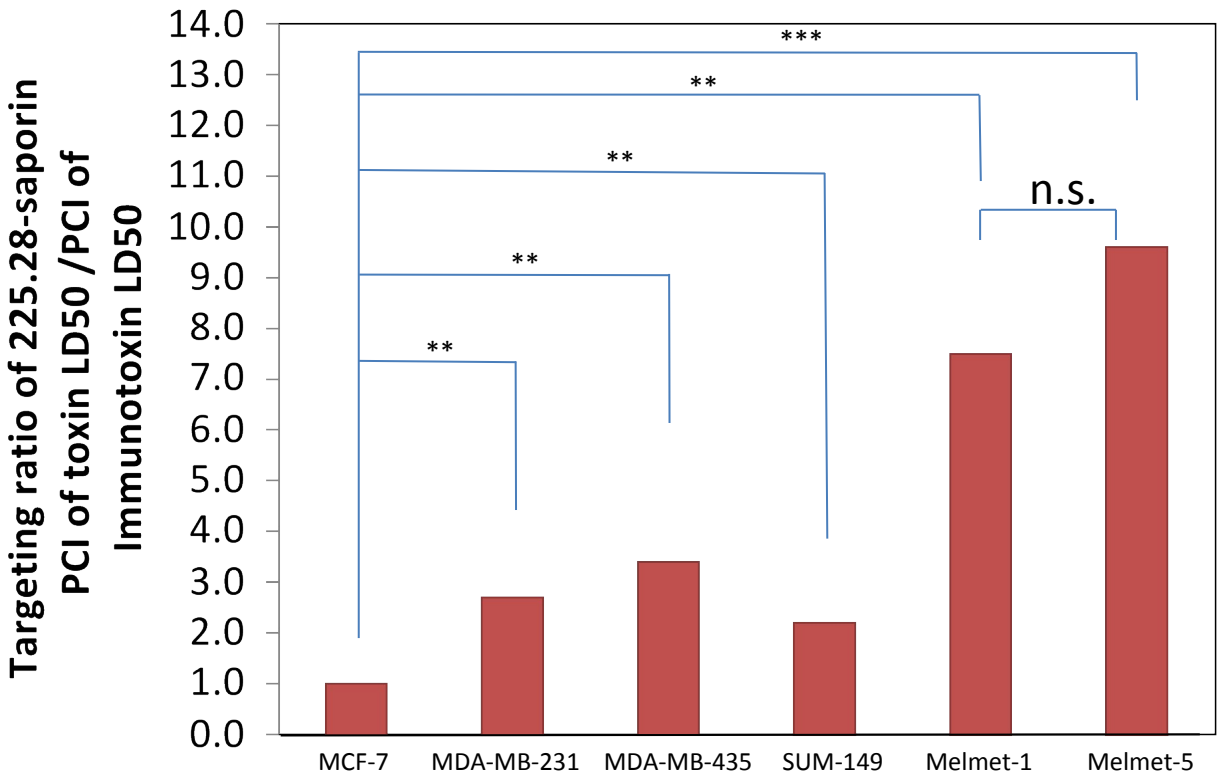
Supplementary Figure S4. Ribosome-inhibiting activity of 225.28-saporin compared to streptavidin-saporin in a cell-free system. The ribosome activity was evaluated by the transcription and translation of the luciferase enzyme and subsequent luminescent quantification by the addition of luciferin. Relative values calculated from a control sample with no added toxin. Figure shows a single representative experiment out of three independent experiments. Bars = SD of three technical replicates (bars smaller than the symbols).

Supplementary Fig. 5



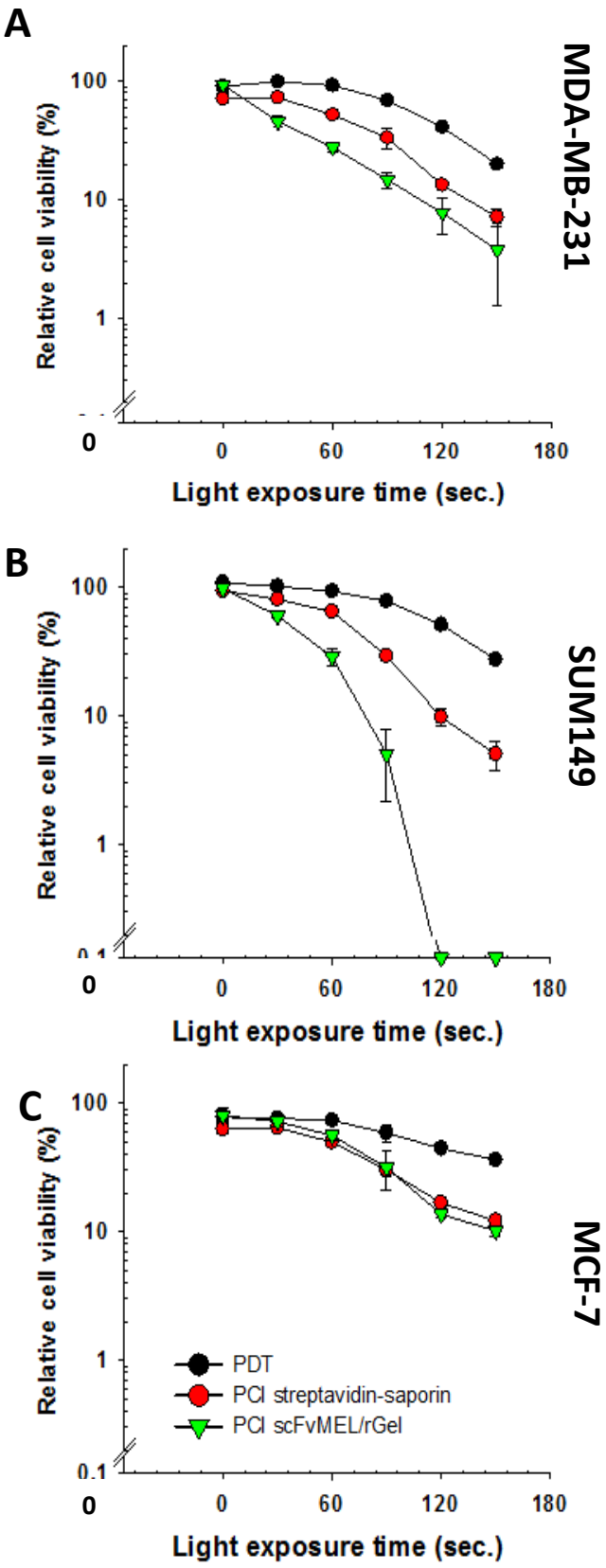
Supplementary Figure S5. Effect of combining PDT and 225.28 mAb and blocking CSPG4 prior to PCI. A) MDA-MB-435 cells was incubated with 0.2 µg/ml $TPCS_{2a}$ (PDT), or $TPCS_{2a}$ in combination with 1 nM 225.28-mAb for 18 hrs, chased in drug-free medium and illuminated after 4 hrs. B) Blocking CSPG4 with 100-fold excess, 500 nM 225.28 mAb prior to PCI of 5 nM 225.28-saporin results in abolishment of the enhanced targeting effect of the immunotoxin (IT) 225.28-saporin in the MDA-MB-435 cells. While the viability of the cells were reduced by 100% in the PCI IT group, there were no significant differences between PCI SAP (PCI of saporin) and PCI IT Block (pre-block and block with 500 nM 225.28 mAb + PCI of 5 nM 225.28-saporin). Relative cell viability was examined using the MTT assay 48 hrs post illumination. Figures shows single representative experiments out of three independent experiments. Bars = SD of three technical replicates.

Supplementary Fig. 6



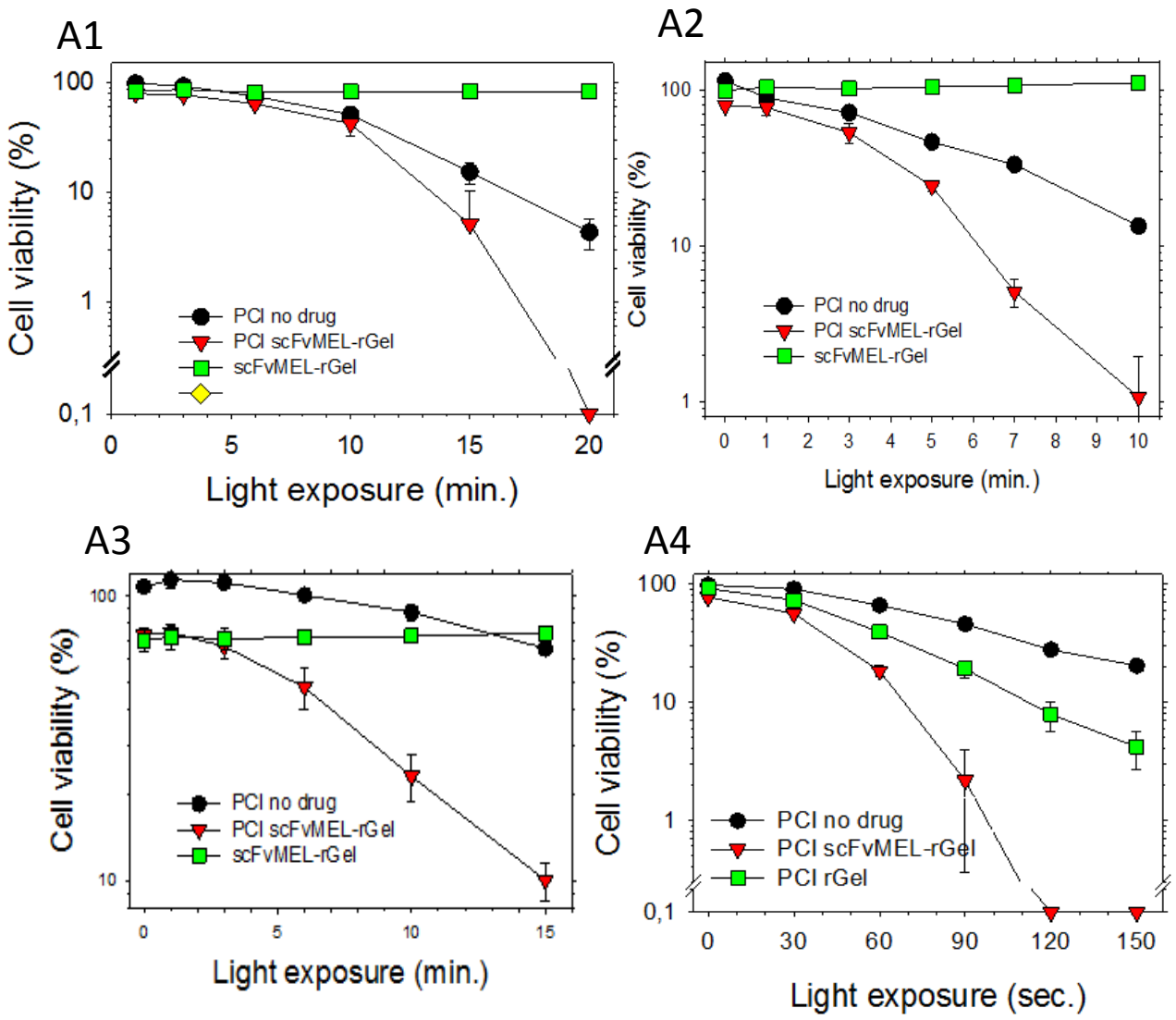
Supplementary Figure S6. Fold increase targeting capacity of 225.28-saporin versus Saporin. For each experiment (n = 3-9 independent experiments per cell line, MTT with technical triplicates and clonal cell survival with duplicates) with PCI of 225-28-saporin and PCI of saporin, the light exposure time (min) needed to obtain 50% reduction in cell viability or clonal cell survival after PCI of saporin was divided on the light exposure time (min) needed to obtain 50% reduction in cell viability or clonal cell survival after PCI of 225.28-saporin. The bars shows the average ratio with standard deviation bars. ** = P<0.01 and **** = P<0.0001

Supplementary Fig. 7



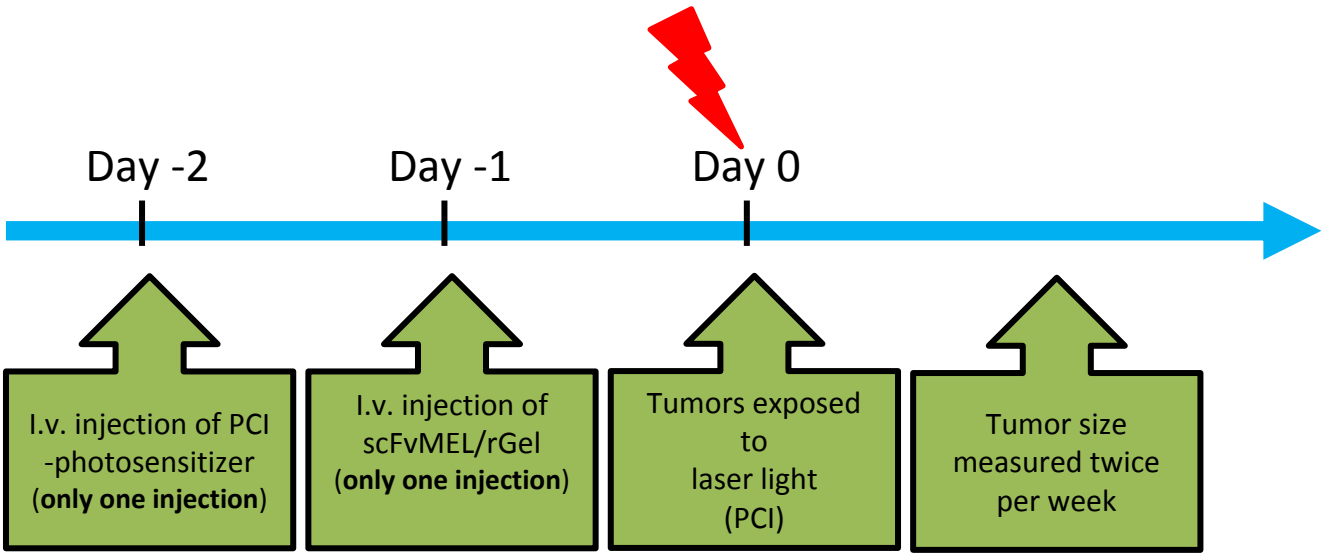
Supplementary Figure S7. Specific and cytotoxic effects of PCI of scFvMEL/rGel in CSPG4-expressing TNBC cells. MDA-MB-231 cells incubated with 0.2 µg/ml TPCS2a with or without 1 nM scFvMEL/rGel or rGel. **B)** SUM149 cells incubated with 0.05 µg/ml TPCS2a with or without 1 nM scFvMEL/rGel or rGel. **C)** MCF-7 cells incubated with 0.6 µg/ml $TPCS_{2a}$ with or without 5 nM scFvMEL/rGel or rGel. 18 hrs post incubation start, cells were washed, chased and examined by the MTT assay 48-72 hrs post light exposure. Figures shows a Single representative experiment out of three independent experiments. Bars = SD of three technical replicates.

Supplementary Fig. 8



Supplementary Figure S8. Specific and cytotoxic effects of PCI of scFvMEL-rGel in the CSPG4-expressing A-375/A-375-GFP cells (Repetition of experiments based on fig 5a). A1-3: The cells were incubated with 5 µg/ml AlPc2a +/- 100 nM scFvMEL-rGel or rGel. Cells were also incubated with the scFvMEL-rGel alone. **A4:** Cells incubated with 0.2 µg/ml TPPS2a +/- 16.5 nM scFvMEL-rGel or rGel. 18 hrs post incubation start, cells were washed and then chased in drug-free medium for 4 hrs. Finally, cells were examined by the MTT assay 48-72 hrs post light exposure. Figure shows single experiments. Bars = SD of three technical replicates.

Supplementary Fig. 9



Supplementary Figure S9. Experimental design of *in vivo* PCI of scFvMEL-rGel . PCI Photosensitizer was intravenously (i.v.) injected 48h prior to PCI (laser-activation) of Immunotoxin, which was injected i.v. 24h prior to light. Tumor growth or reduction was measured by a digital caliper twice per week.

Supplementary Table 1.

	MDA-MB-231	MDA-MB-435	Sum149	Melmet 1	Melmet 5	Melmet 1	Melmet 5
Avarage lethality PCI (no drug)	50%	50%	50%	50%	50%	70%	80%
Avarage lethality IT	12%	41%	20%	72%	69%	72%	69%
DL	1.78	0.73	3.52	-0.25	-0.14	2.03	1.54
Se(DL)	0.22	0.28	1.31	0.23	0.44	0.91	0.50
T-value	8.21	2.59	2.68	-1.06	-0.33	2.23	3.08
df	8	8	8	8	8	8	8
p-value	< 0.001	0.0330	0.0280	0.4760	0.7520	0.0284	0.0075
Combined effect	Synergistic	Synergistic	Synergistic	Additive	Additive	Synergistic	Synergistic

Supplementary Table 1. Statistical analysis of the combined effect of $PCI_{no\ drug}$ (= photosensitizer + light) and and Immunotoxin (IT). A synergistic combination of the two treatment was defined as a significant positive DL (difference in logarithm) value (t-test; $p \leq 0.05$). se(DL): standard error of DL, df: degrees of freedom.