SUPPORTING INFORMATION

Figure S1

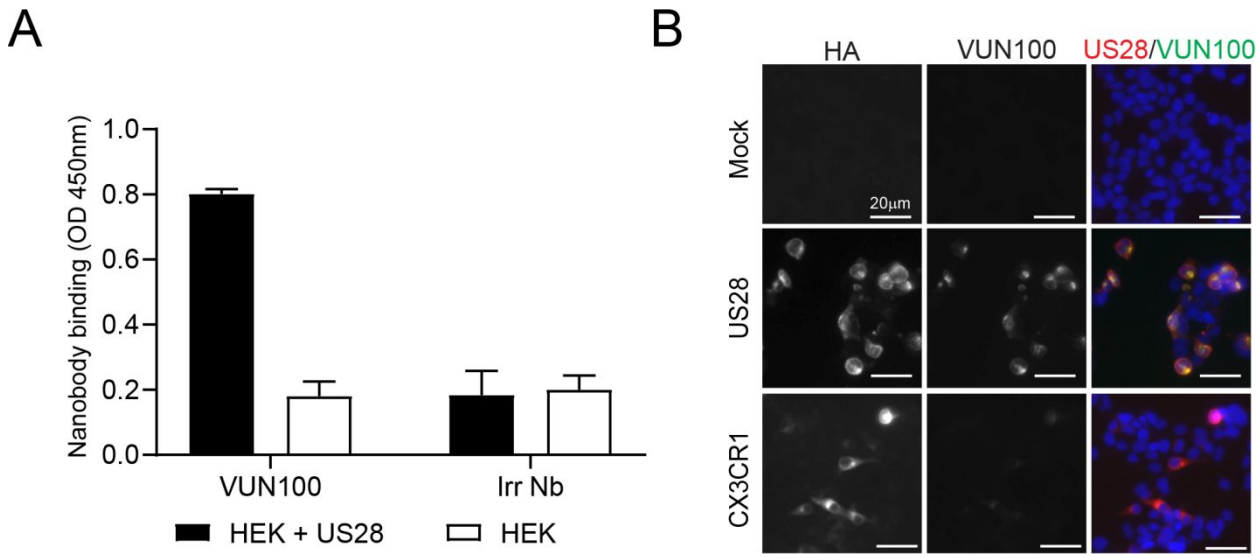

Figure S1. VUN100 binds selectively to US28. A) *Binding of VUN100 and irrelevant nanobody (binding to the azodye RR6; Irr Nb) to US28-expressing HEK293T membranes (HEK+US28) and mock transfected HEK293T membranes (HEK), as determined by ELISA.* ***B)*** *Immunofluorescence microscopy of the binding of VUN100 to US28 and CX3CR1. Receptor expression was detected using an N-terminal HA-tag and anti-HA antibody (HA). VUN100 binding was detected. VUN100 binding was detected using the Myc-tag and an anti-Myc antibody (VUN100).*

Figure S2

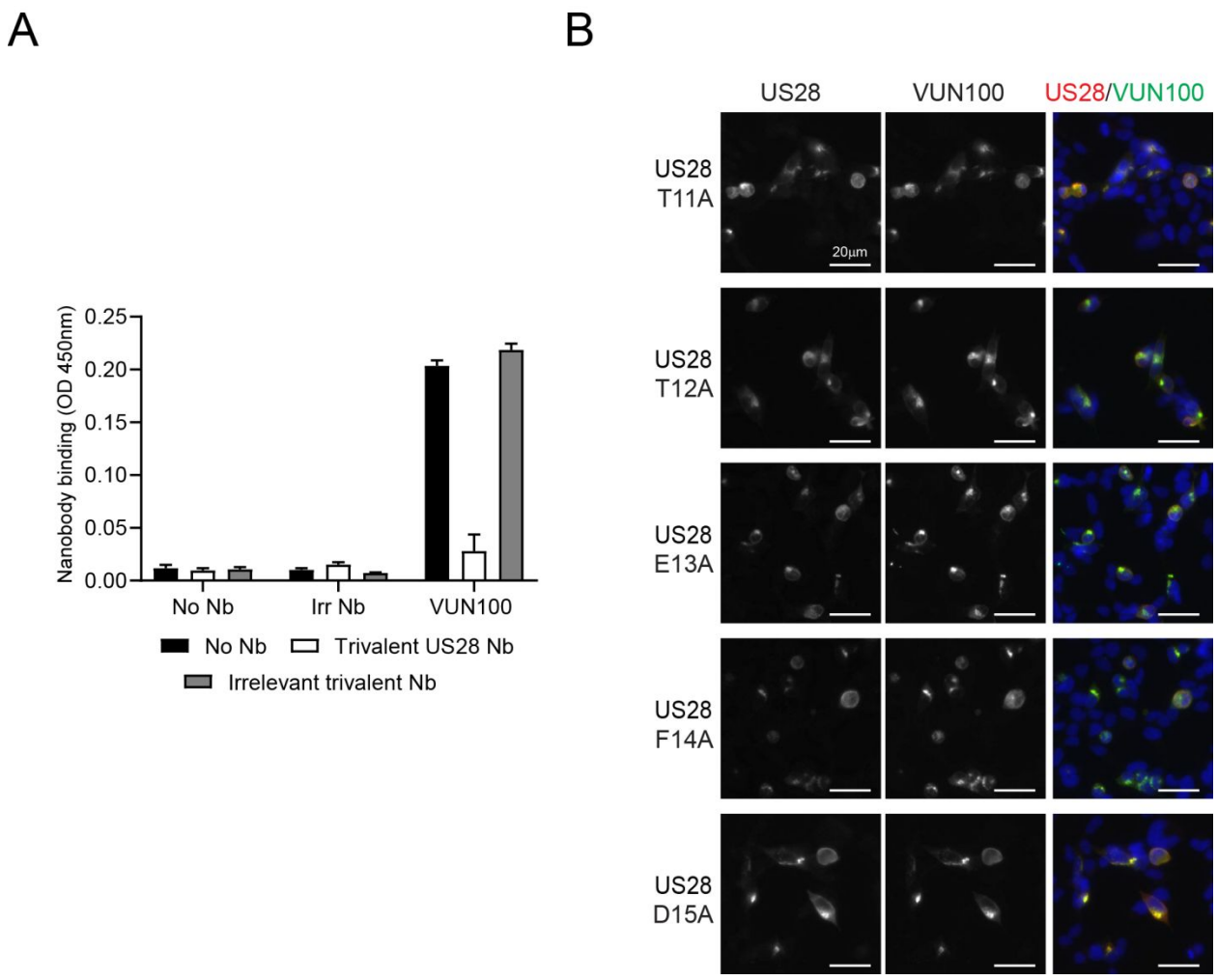

Figure S2. VUN100 binds the same epitope as the US28 Nb. A) *Competition binding ELISA of VUN100 and irrelevant nanobody (binding to the azodye RR6; Irr Nb) to US28-expressing HEK293T membranes (HEK+US28). Binding of the nanobodies was detected using the Myc-tag and an anti-Myc antibody. Nanobodies were displaced by untagged trivalent US28 nanobody (US28 Nb) or untagged trivalent irrelevant nanobody.* ***B)*** *Immunofluorescence microscopy of the binding of VUN100 to N-terminus US28 mutants with the amino acids at positions 11-15 being substituted by alanines.*

Figure S3

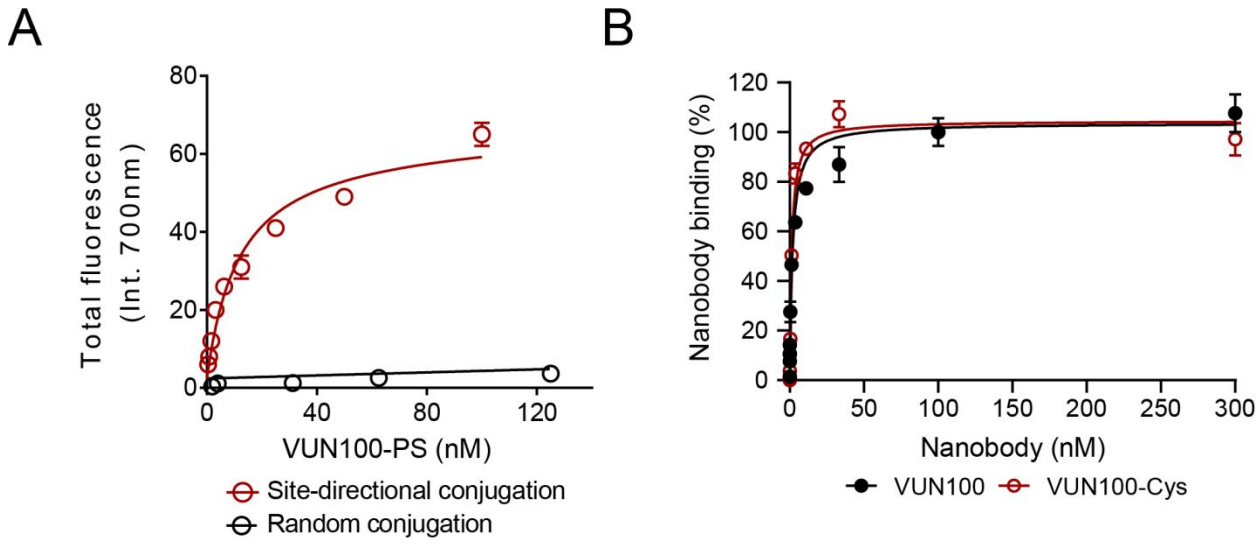

Figure S3. Binding of randomly conjugated VUN100-PS and VUN100-Cys to US28. ***A)*** *Binding of different concentrations of randomly or site-directionally conjugated VUN100-PS to US28 positive cells on ice. Fluorescence of VUN100-PS bound to cells was detected using an Odyssey infrared scanner at 700 nm.* ***B)*** *Binding of different concentrations of VUN100 and VUN100-Cys to US28-expressing membranes. Specific binding is shown after subtraction of aspecific binding to US28-negative membranes.*

Figure S4

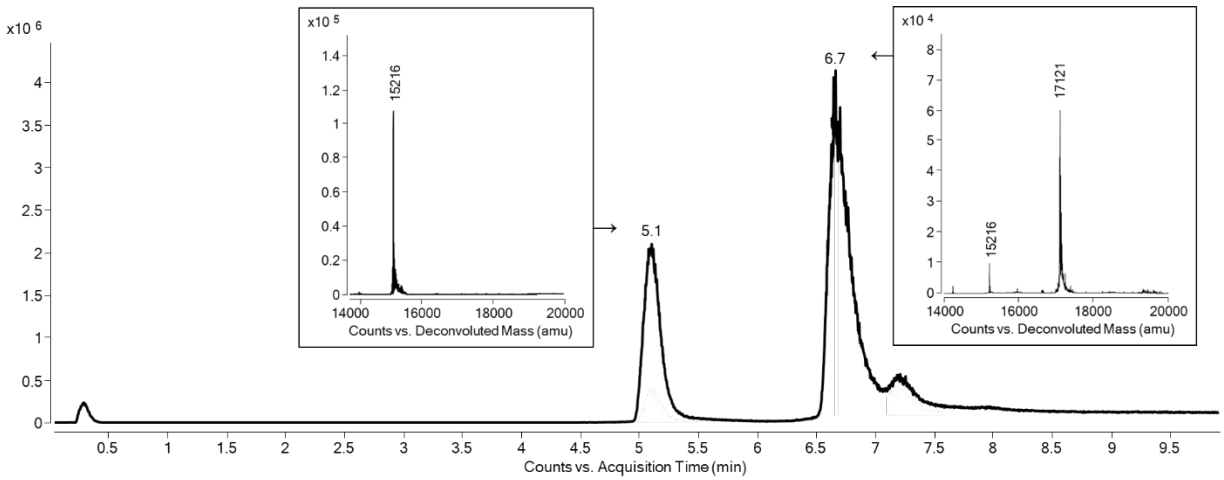

Figure S4. Extracted biomolecule LC-MS chromatogram of VUN100-PS. *Photosensitizer conjugated (6.7 min) and unconjugated nanobodies (5.1 min) are separated and identified according to their deconvoluted mass spectra (inserts).*

Figure S5

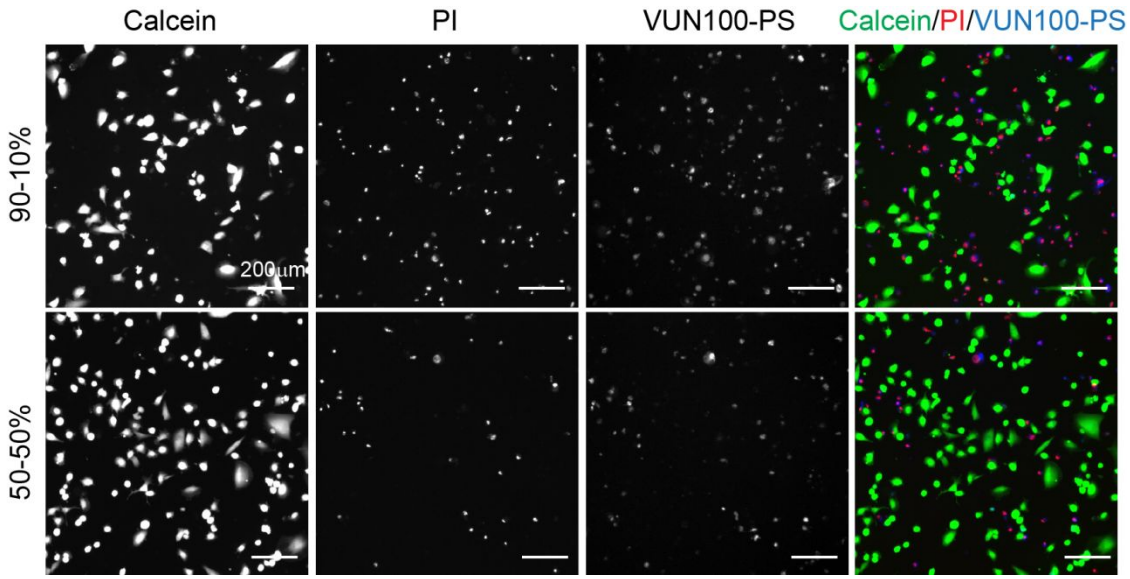

Figure S5. Nanobody-targeted PDT treatment on different ratios of co-cultures of US28 positive and US28 negative cells. *Different ratios of US28 positive and US28 negative cells were co-cultured (90-10% and 50-50% of US28 positive and US28 negative cells). Cells were stained with propidium iodide (PI) and calcein) 24h after nanobody-targeted PDT to determine cell viability and the selectivity of the nanobody-targeted PDT.*