Protocol

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## Photoimmunotechnology as a powerful biological tool for molecular-based elimination of target cells and microbes, including bacteria, fungi and viruses

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## Supplementary Figure 1 | Examples of test and control group designs for

photoimmunotechonology. Examples are shown for PIAS using SA–IR700 on the target bacteria Staphylococcus aureus (SA) and methicillin-resistant SA (MRSA) and non-target bacteria, including related and unrelated staphylococci. We investigated whether PIAS eliminated drug-resistant strains of SA. **c** Example for Box 2 using (iii). The target-specific effect of PIAS was investigated using non-target bacteria, including unrelated and clorsly-related bacteria. a-c SA–IR700, 1 µg/test; NIR illumination, 30 J/cm<sup>2</sup>. SA–IR700 was used in both the PIAS (-) and (+) groups. d Example for Box 2 (iv). To investigate the target-selective bactericidal effects of PIAS; PIAS using SA-targeting conjugate was subjected to a mixed sample of MRSA and Staphylococcus epidermidis. MRSA and S. epidermidis formed yellow and pink colonies, respectively, on MSA. Scale bars indicate 3 cm. MSA, mannitol salt agar with egg yolk; NIR, near-infrared; OPAII, OPAII Staphylococcus agar; c.f.u., colony-forming units; n.d., not detected; n.s., not significant (p > 0.05). Mean and s.d. values are shown from triplicate samples; data and images are representative of at least three independent experiments. For statistical analyses, ANOVA (a) and ANOVA with the Sidak test (d) were performed. \*\*\*\*p < p0.0001. All panels shown in this figure have been previously published<sup>10</sup> and rearranged for the protocol. The source data are available in Supplementary Data 1 at the following web address: https://www.nature.com/articles/s42003-022-03586-4#Sec24.



## Supplementary Figure 2 | Examples of test and control group design for PIAS target

**specificity**. As microbes, we used the fungi *Candida albicans* (CA) and *Candida stellata* and the bacteria methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus epidermidis*. **b** Example for Box 2 (vi). Targeted elimination of PIAS using CA–IR700 conjugate(s) and/or SA–IR700 conjugate(s) was investigated on a mixed sample of CA, MRSA, and the non-target/commensal bacterium *S. epidermidis*. CA–IR700, 20 µg/test; SA–IR700, 1 µg/test; NIR, near-infrared light. c.f.u., colony-forming units; n.d., not detected. Mean and s.d. values are shown for triplicate samples; data are representative of at least three independent experiments. For statistical analyses, ANOVA with Dunnett's test was performed. \*\*\*\**p* < 0.0001. All panels shown in this figure have been previously published<sup>10</sup> and rearranged for the protocol. The source data are available in Supplementary Data 1 at the following web address: https://www.nature.com/articles/s42003-022-03586-4#Sec24.