

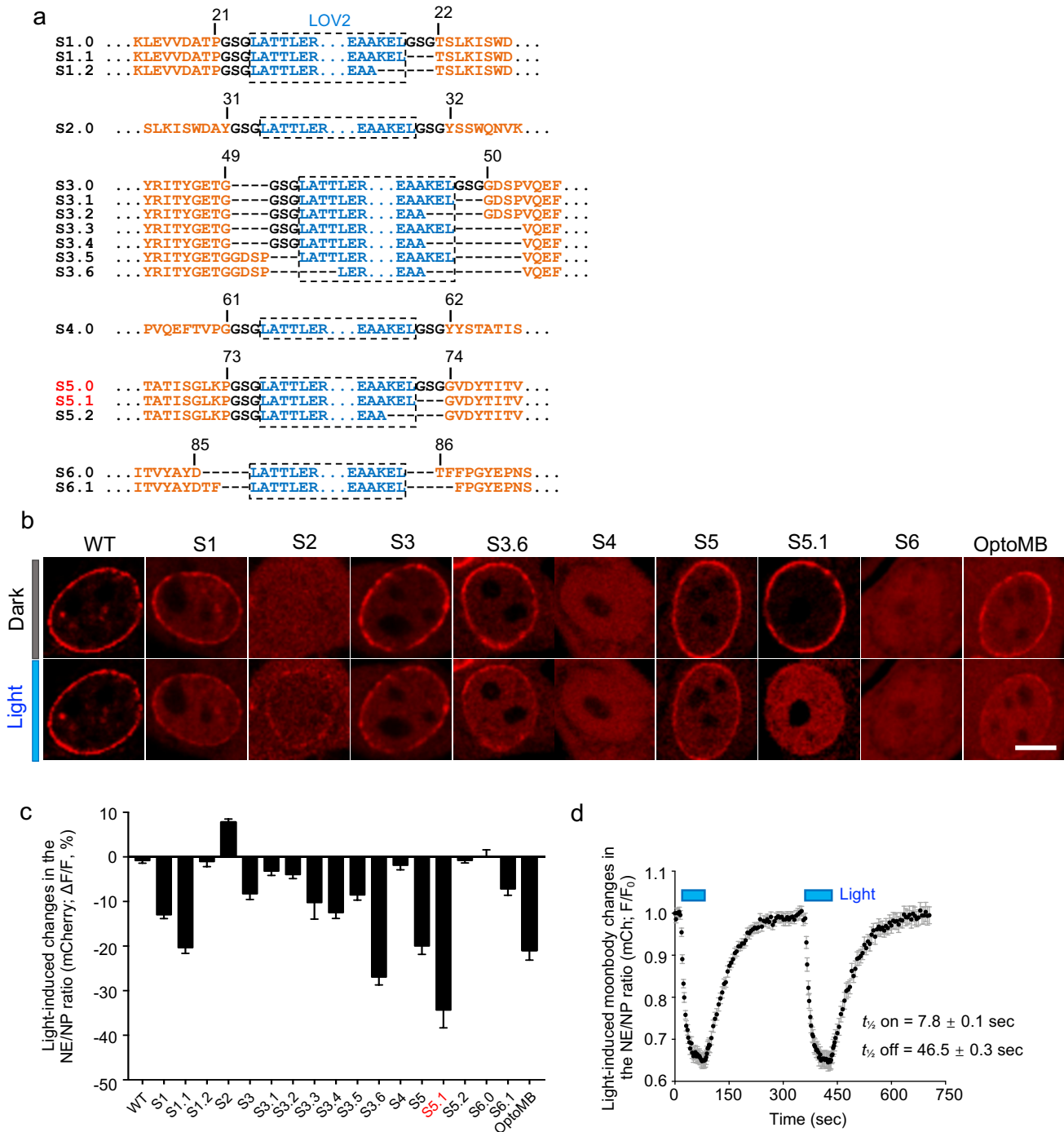
Supporting information for

## **Design of smart antibody mimetics with photosensitive switches**

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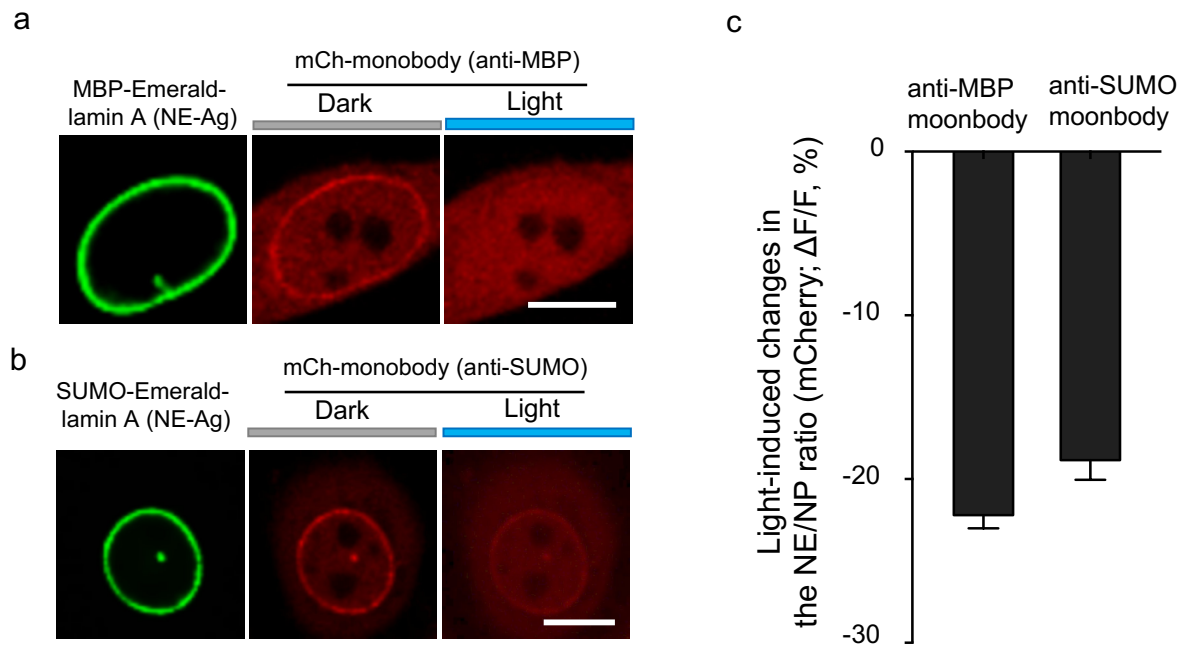
### **Contents:**

Supplementary Figures 1-4



**Supplementary Figure 1 | The design and optimization of moonbody variants.**

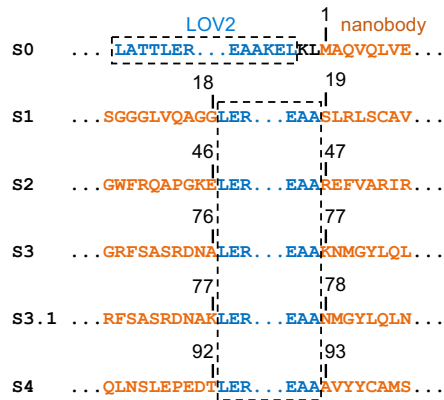
- Sequence information for moonbody variants tested in the study. The monobody-LOV2 junction regions were shown for each construct.
- Representative confocal images of HeLa cells co-expressing the antigen (NE-SH2; not shown) and the indicated mCh-moonbody variants (red) before and after photostimulation at 470 nm for 120 sec. Scale bar, 10  $\mu$ m.
- Summary of the degrees of dissociation (quantified by the NE/NP ratio of mCh signals) from the target (NE-SH2) for the indicated moonbody variants. Selected data were also presented in **Fig. 1d**. n = 6-25 cells (mean  $\pm$  sem).
- Quantification of reversible changes in the NE/NP ratio. HeLa cells co-expressing the anti-SH2 moonbody and NE-tethered SH2 were subjected to two light-dark cycles of stimulation. n = 27 cells (mean  $\pm$  sem).



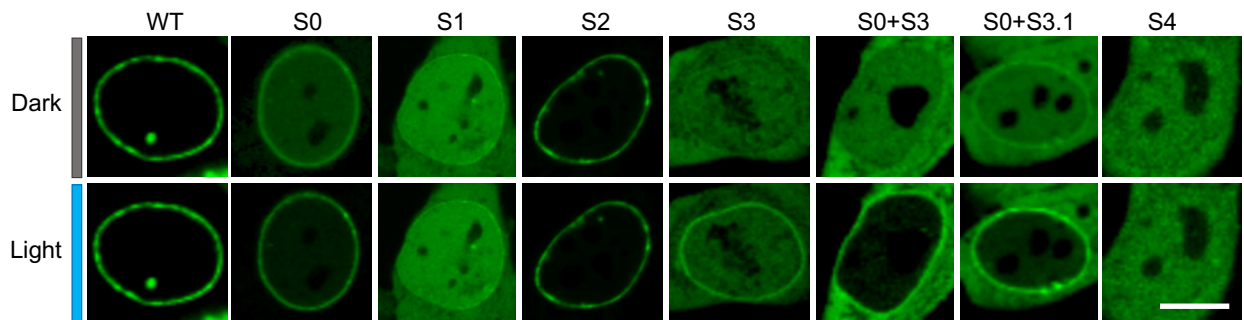
**Supplementary Figure 2 | Characterization of moonbodies against SUMO and MBP.**

- Confocal images of a HeLa cell co-expressing an anti-MBP moonbody (mCh-tagged; red) and NE-tethered GFP-MBP in the dark or after blue light illumination for 120 sec at 470 nm. Scale bar, 10  $\mu$ m.
- Confocal images of a HeLa cell co-expressing an anti-SUMO moonbody (mCh-tagged; red) and NE-tethered GFP-SUMO (green) before (dark) and after blue light illumination for 120 sec at 470 nm. Scale bar, 10  $\mu$ m.
- Quantification of light-dependent response (as the NE/NP ratio of mCh signals) of the MBP or SUMO-specific moonbodies.  $n = 33-67$  cells from three independent assays (mean  $\pm$  sem).

a



b



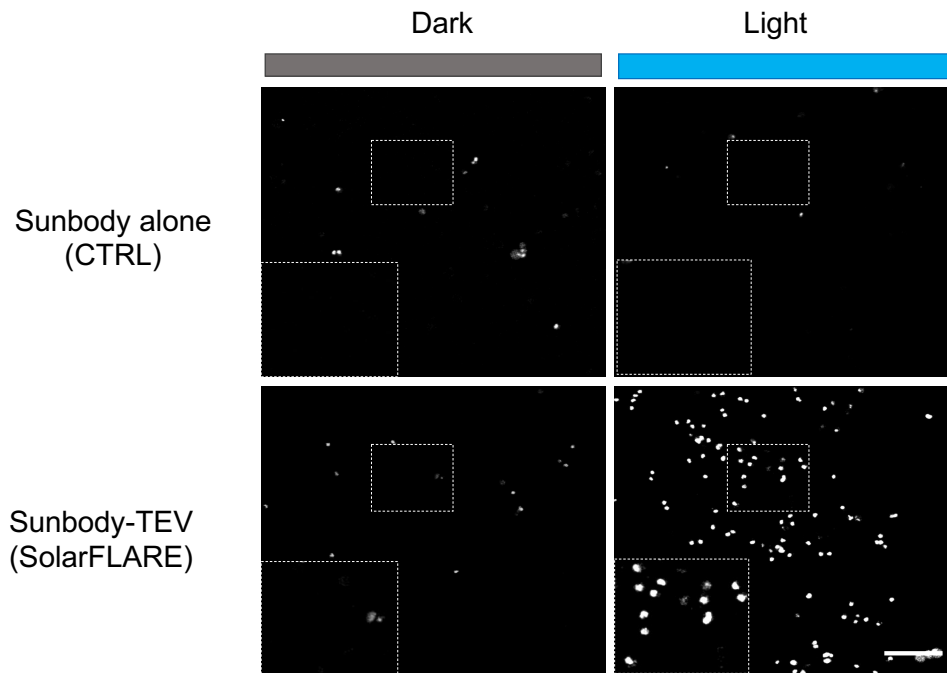
c

Sunbody variants	Target binding (dark)	Light-induced change
WT	Strong	-
S0	Strong	++
S1	Weak	-
S2	Strong	-
S3	Weak	+++
S0 + S3	No binding	+++++
S0 + S3.1	Weak	++++
S4	No binding	-

### Supplementary Figure 3 | The design and optimization of sunbody variants.

- Sequence information of sunbody variants tested in the study. The sunbody-LOV2 junction regions were shown for each construct.
- Representative confocal images of HeLa cells co-expressing the NE-tethered mCherry (as antigen; not shown) and the indicated GFP-sunbody variants (green). Scale bar, 10  $\mu$ m.
- Summary of the degrees of antigen (mCh) binding for the indicated GFP-sunbody variants before and after light stimulation (see panel c). -, <5%; +, 5-10%; ++, 10-20%; +++, 20%-40%; +++++, 40%-60%; +++++>60% increase in the NE/NP ratio.

SYTOX blue staining of nuclei (dying/dead cells)



**Supplementary Figure 4 | A SolarFLARE system tailored for light-inducible MLKL-NT expression to induce necroptosis.** Representative confocal images of HeLa cells transfected with the SolarFLARE system (sunbody-TEV + FLARE; bottom), or the sunbody alone + FLARE combination as control (top), before and after light stimulation. SYTOX Blue staining was used to report dying cells. The quantification results were shown in **Fig. 6c**. Scale bar, 100  $\mu\text{m}$ .