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Supplementary Material for

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Optical control of nanobody-mediated protein activity

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modulation with photocleavable fluorescent protein

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M. Endo ^a, S. Tomizawa ^a, K. Qiaoyue ^a, T. Ozawa ^{a,1}

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Figure S1. Photocleavage of optoNb60 candidates.

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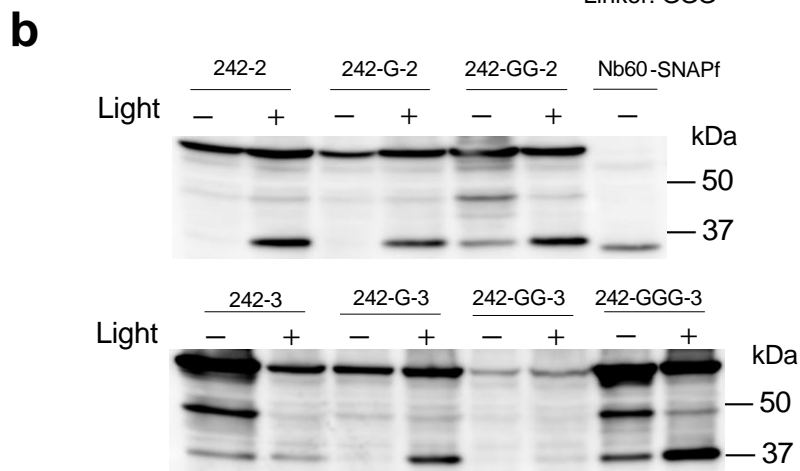
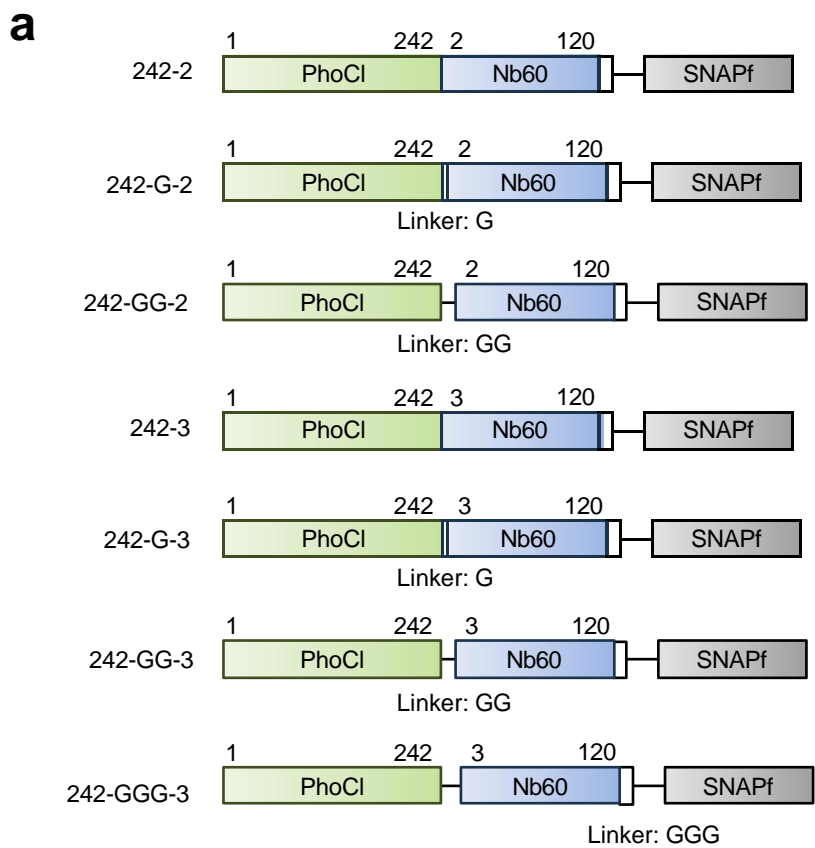
Figure S2. Light-dependent interaction between β 2AR and optoNb60.

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Figure S3. Investigation of inhibitory effect on ligand-induced intracellular cAMP increase by optoNb60.

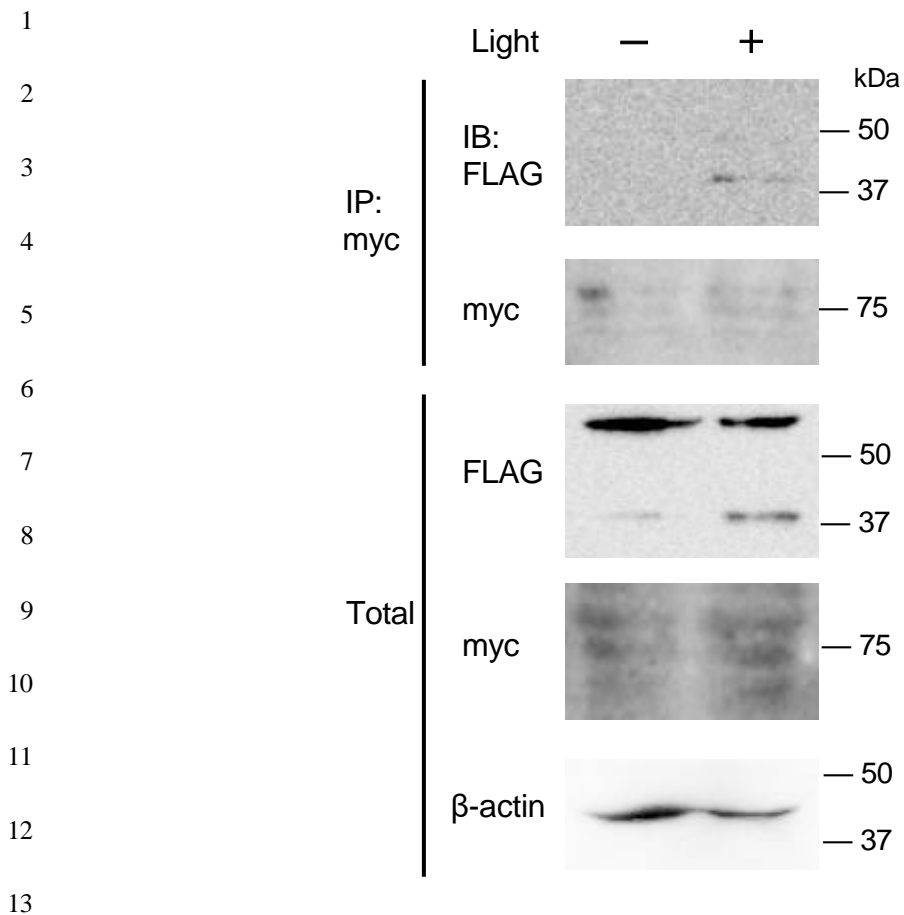
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Supplementary Figure 1.

Photocleavage of optoNb60 candidates. (a) Box structures of the candidate molecules. (b) The photocleavage of the candidate molecules. The membrane was immunoblotted with anti-FLAG antibody.

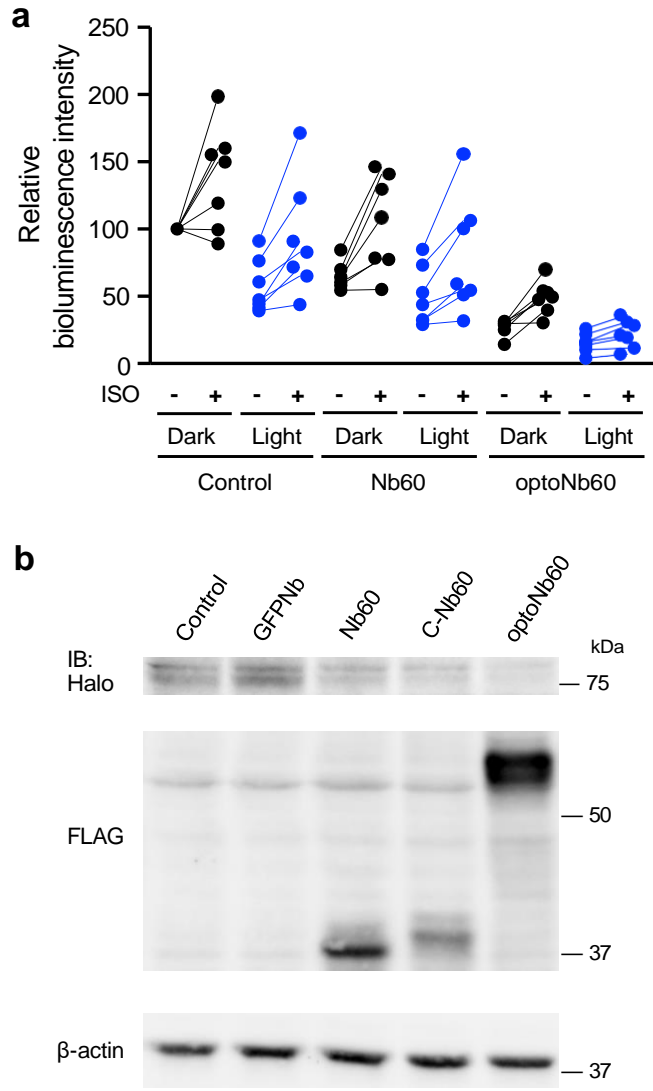


14 **Supplementary Figure 2.**

15 Light-dependent interaction between β 2AR and optoNb60. Cells expressing both optoNb60
 16 and Halo- β 2AR-myc were immunoprecipitated (IP) with anti-myc antibody. The membrane
 17 was immunoblotted (IB) with indicated antibodies.

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Supplementary Figure 3.

Investigation of inhibitory effect on ligand-induced intracellular cAMP increase by optoNb60.

(a) Ligand-induced intracellular cAMP changes. Control: no expression of any nanobodies. Each Glosensor-22F bioluminescence value was normalized to the value obtained in control cells without ligand or light stimulation in each independent experiment (n = 7). The statistical difference was analyzed by paired t-test. N.S., P > 0.05, *P < 0.05. (b) Expression levels of Halo-β2AR and Nanobodies. The membrane was immunoblotted (IB) with indicated antibodies. C-Nb60: The C-terminal part of PhoCl (HYGNRVFTKYPR) was added to the N-terminus of optoNb60.