A General Method to Regulate Protein Stability Using Light

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Supporting Information

B-LID fusion antibodies. The following antibodies were used for immunoblotting: anti-HA (3F10, Roche Diagnostics), anti-YFP (JL-8, Clontech), anti-α-tubulin (Ab6046, Abcam), anti-rabbit IgG HRP (Molecular Probes), anti-mouse IgG HRP (ZYMED), anti-rat IgG HRP (Chemicon).

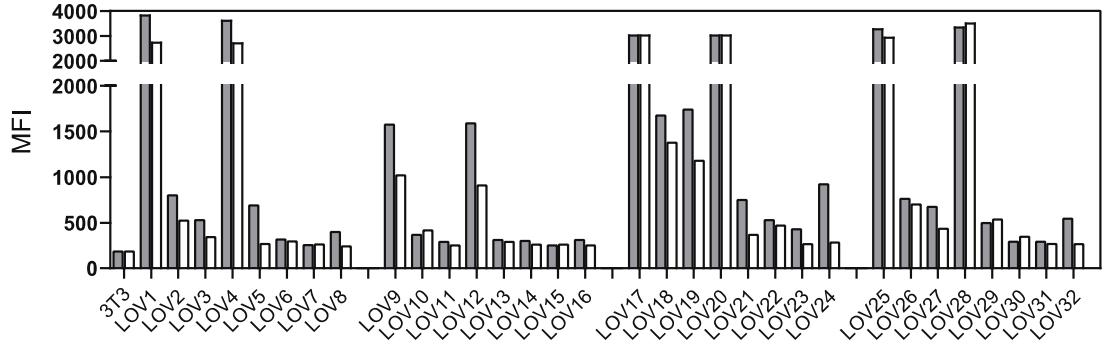
Microscopy. NIH3T3 cells expressing fluorescent proteins were imaged with a 10x, 20x or 40x objective on a Zeiss Axioskop 2 epifluorescence microscope equipped with a QICAM FAST 1394 digital CCD camera.

Cloning. Human β-actin fused to mCherry (Clontech) was cloned in the pBMN vector with i-Blasticidin. A 6-amino acid linker (GlyGlySerGlyGlySer) was introduced between β-actin and the fluorescent protein. The B-LID domain was fused to the C-terminus of mCherry or Actin-mCherry and an HA tag was introduced on the N-terminus of the mCherry fluorescent protein.

a

Photoreceptor	Degron	Photoreceptor Mutant: none	V416I	I532A	V416I/I532A
AsLOV2(404-546)	RRRGN	LOV1	LOV9	LOV17	LOV25
	RRRG	LOV5	LOV13	LOV21	LOV29
AsLOV2(404-545)	RRRGN	LOV2	LOV10	LOV18	LOV26
	RRRG	LOV6	LOV14	LOV23	LOV30
AsLOV2(404-544)	RRRGN	LOV3	LOV11	LOV19	LOV27
	RRRG	LOV7	LOV15	LOV23	LOV31
AsLOV2(404-543)	RRRGN	LOV4	LOV12	LOV20	LOV28
	RRRG	LOV8	LOV16	LOV24	LOV32

b

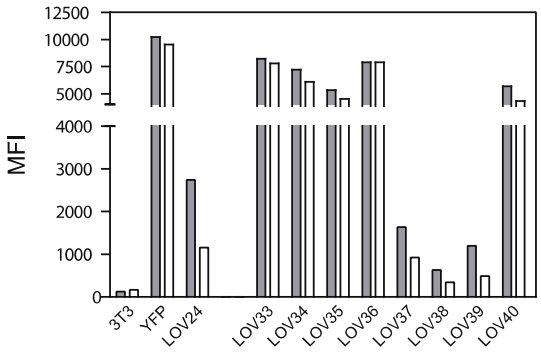


Supporting Figure S1. Various LOV2 domains possessing a C-terminal degron were evaluated for light-dependent stability. (a) Overview of prepared constructs. AsLOV2 domain (404-546) was used in full or truncated versions from the C-terminus. Degrons RRRGN or RRRG were fused to the C-termini of the LOV2 domains. Two additional mutations were tested to assess the effects of flavin adduct stability (V416I) or helix binding strength to the core domain (I532A). Combinations of both mutations were also examined. (b) The indicated LOV2 domains were fused to the C-terminus of eYFP, and these constructs were stably transduced into NIH3T3 cells and treated either as normal cultured cells (grey bars) or illuminated with 465-nm light for 2 hours. Median fluorescence intensity (MFI) was measured by analytical flow cytometry.

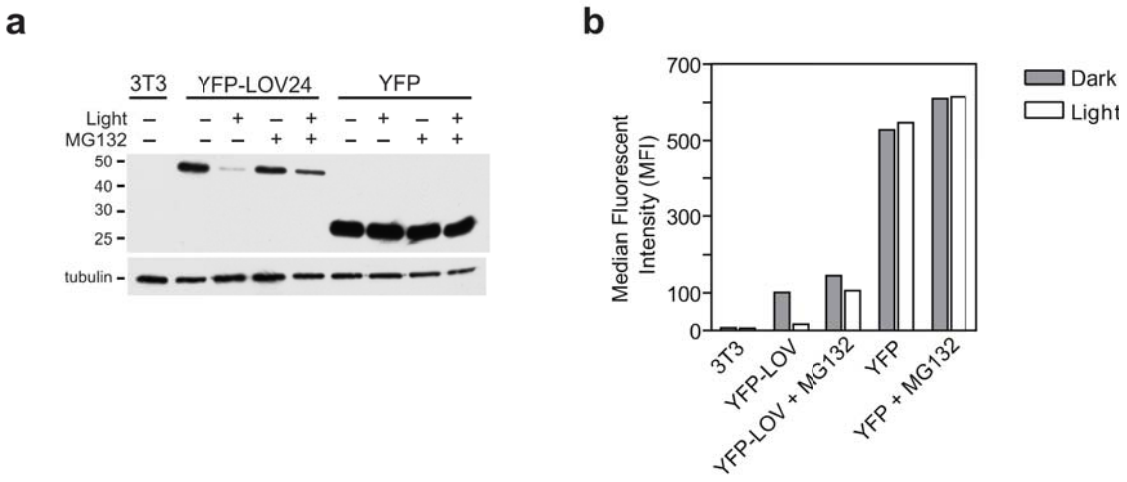
a

Photoreceptor	Degron	Mutant G528A/N538E
AsLOV2(404-546)	RRRGN	LOV33
	RRRG	LOV37
AsLOV2(404-545)	RRRGN	LOV34
	RRRG	LOV38
AsLOV2(404-544)	RRRGN	LOV35
	RRRG	LOV39
AsLOV2(404-543)	RRRGN	LOV36
	RRRG	LOV40

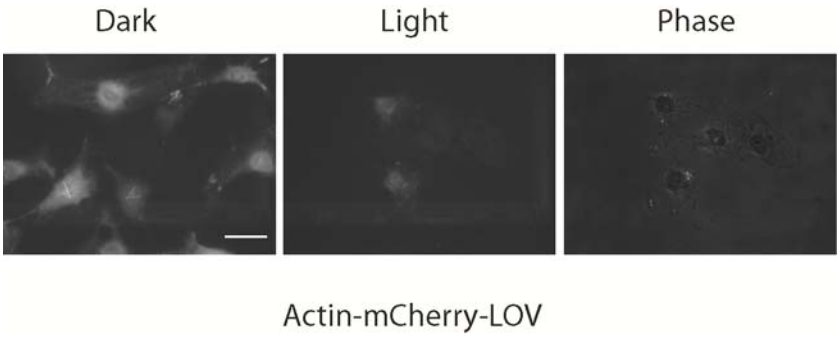
b



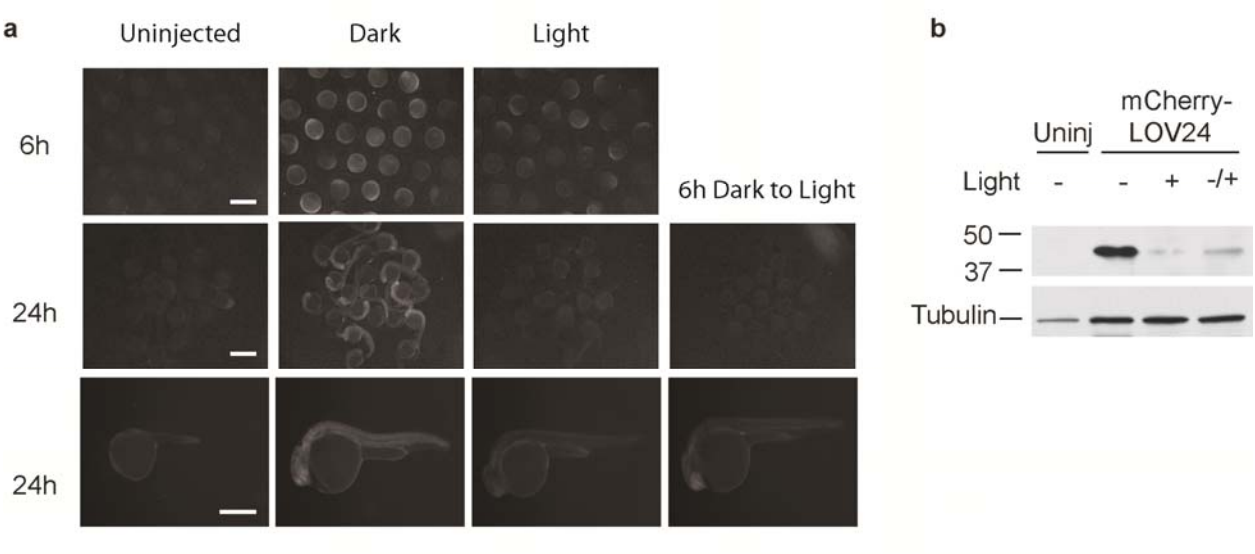
Supporting Figure S2. Additional mutations to modulate the interaction between the LOV2 core domain and the degron-containing helix were tested. (a) Nomenclature for the eight mutants tested. (b) Domains were fused to the C-terminus of eYFP and the constructs were evaluated using analytical flow cytometry as decribed in Supplementary Figure 1.



Supporting Figure S3. Illumination with blue light for 2 hours does not affect the levels of YFP as evidenced by immunoblot and flow cytometry. Cells stably expressing the YFP-LOV24 fusion protein or YFP alone were treated with either vehicle or 10 μM MG132 and were kept in the dark or illuminated with blue light for 2 hours followed by analysis of YFP stability by immunoblot of cell lysates (a) and analytical flow cytometry of living cells (b). Tubulin is the loading control.



Supporting Figure S4. β-Actin fused to mCherry-LOV24 is incorporated into microfilaments. NIH3T3 cells were stably transduced with β-actin-mCherry-LOV24 and kept in dark or illuminated with blue light for 2 hours. Phase contrast is shown for the cells that are exposed to light. Scalebar represents 25 μm.



Supporting Figure S5. Zebrafish embryos were microinjected with mRNA encoding HA-mCherry-LOV24. Embryos were raised in the dark or exposed to blue light for the indicated times and degradation was observed by fluorescence microscopy. Zebrafish embryos that were grown in the dark for 6 hours to allow protein expression were then illuminated with the light source to evaluate the degradation of mature protein (two right-most panels). Embryos that were illuminated with blue light showed reduced fluorescence compared to embryos that were kept in the dark. Scale bars represent 1 mm for images of multiple embryos and 250 μm for images of single embryos.

Supporting Table S1. Sequence of AsLOV2 and overview of the mutations used. The relevant point mutations as well as the RRRG degron are highlighted in red.

AsLOV2 (404-546):

FLATTLERIEKNFVITDPRLPDNPIIFASDSFLQLTEYSREEILGRNCRFLQGPETDRATVRKIRDAIDN
QTEVTVQLINYTKSGKKFWNLFHLQPMRDQKGDVQYFIGVQLDGTEHVRDAAEREGVMLIKKTAENIDEA
AKEL

AsLOV2 (404-546): V416I mutation

FLATTLERIEKNF**I**ITDPRLPDNPIIFASDSFLQLTEYSREEILGRNCRFLQGPETDRATVRKIRDAIDN
QTEVTVQLINYTKSGKKFWNLFHLQPMRDQKGDVQYFIGVQLDGTEHVRDAAEREGVMLIKKTAENIDEA
AKEL

AsLOV2 (404-546): I532A mutation

FLATTLERIEKNFVITDPRLPDNPIIFASDSFLQLTEYSREEILGRNCRFLQGPETDRATVRKIRDAIDN
QTEVTVQLINYTKSGKKFWNLFHLQPMRDQKGDVQYFIGVQLDGTEHVRDAAEREGVML**A**KKTAENIDEA
AKEL

AsLOV2 (404-546): V416I/I532A mutation

FLATTLERIEKNF**I**ITDPRLPDNPIIFASDSFLQLTEYSREEILGRNCRFLQGPETDRATVRKIRDAIDN
QTEVTVQLINYTKSGKKFWNLFHLQPMRDQKGDVQYFIGVQLDGTEHVRDAAEREGVML**A**KKTAENIDEA
AKEL

AsLOV2 (404-546): G528A/N538E mutation

FLATTLERIEKNFVITDPRLPDNPIIFASDSFLQLTEYSREEILGRNCRFLQGPETDRATVRKIRDAIDN
QTEVTVQLINYTKSGKKFWNLFHLQPMRDQKGDVQYFIGVQLDGTEHVRDAAERE**A**VMLIKKTAE**E**IDEA
AKEL

LOV24

FLATTLERIEKNFVITDPRLPDNPIIFASDSFLQLTEYSREEILGRNCRFLQGPETDRATVRKIRDAIDN
QTEVTVQLINYTKSGKKFWNLFHLQPMRDQKGDVQYFIGVQLDGTEHVRDAAEREGVML**A**KKTAENIDEA
A**RRRG**