Supporting Information

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SI Materials and Methods

Microbiological Techniques and Illumination Conditions. Cells of *Rhizobium leguminosarum* bv. viciae 3841 (1) were grown at 28 °C in TY (5 g/L tryptone, 3 g/L yeast extract, 0.87 g/L $CaCl_2.2H_2O$) or Y minimal medium containing 0.2% (wt/vol) mannitol as the carbon source and 6 mM sodium glutamate as the nitrogen source (2). *Escherichia coli* cultures were grown at 37 °C in LB medium (10 g/L Tryptone, 5 g/L NaCl, 5 g/L yeast extract). The antibiotics spectinomycin (Spc) (200 μg/mL), streptomycin (Str) (400 μg/mL), gentamycin (Gm) (20 μg/mL), and tetracycline (Tet) (6 μg/mL) were added when needed. Bacterial growth was monitored at OD_{600}, using a Beckman Coulter DU-530 spectrophotometer. Plasmids were mobilized into *Rle* by biparental mating from donor *E. coli* S17.1 in conjugation experiments (3). Continuous white light from fluorescent tubes (cool daylight) was used with a fluence of 15 $\mu mol \cdot m^{-2} \cdot s^{-1}$. For light vs. dark experiments, sample handling was carried out under dim red light-emitting diode light (<1 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Dark cultures were covered using two layers of aluminum foil. Light cultures were kept uncovered in plastic or glass containers that are transparent to visible light but filter out UV wavelengths. Photon flux was measured using a Quantum Meter (Apogee Instruments model QMSW-SS).

Generation of *lov* Mutant and Complemented Strains. A 1.1-kb *lov* fragment was amplified using specific primers (uLOV_F and uLOV_R) and cloned into pGEMT-easy (Promega). A 2-kb fragment containing the Spc^r cassette from pHP45Ω (4) digested with SmaI was ligated into blunt-ended pGEMT-*lov* (digested with XhoI and EcoRV and filled in with *Klenow* polymerase). The resulting *lov*::Spc^r construct was subcloned into the suicide vector pJQ200 (KS) (5) and transferred by biparental mating to *Rle*. Double recombinants $Spc^r/Gm^s/Suc^r$ were selected. Mutation of the *lov* gene was confirmed by PCR sequencing, using specific primers (5_flank_F and 3_flank_R) flanking the mutation site. To construct the *lov* complemented strain, a 1.4-kb fragment containing the complete *lov* ORF and its regulatory sequences was amplified using specific primers (5_flank_F and LOV_myc_R), cloned into the pGEMT-easy vector, and subcloned into the EcoRI restriction site of pLAFR3 (6). The resulting pLAFR3-*lov* plasmid (p*lov*) was transformed into the *lov* mutant. Generation of *lov* versions containing C75A or H163A point mutations was performed by overlapping PCR. For LOV_C75A and LOV_H163A two pairs of primers were used: 5_flank_F-C75A_R, C75A_R-LOV_myc_R and 5_flank_F-H163A_R, H163A_R-LOV_myc_R, respectively. The final PCR products of the Myc-tagged mutants and wild-type LOV versions were amplified using 5_flank_F and LOV_myc_R primers and the 1.4-kb products were cloned into pGEMT-easy, sequenced, subcloned into the EcoRI restriction site of the pLAFR3 vector, and mobilized into the *lov* mutant strain to generate *lov* + p*lov*, *lov* + pC75A, and *lov* + pH163A strains. The presence of all *lov* constructs was confirmed by PCR sequencing (using primers 5_flank_F and LOV_myc_R). Protein expression was confirmed by Western blot (Fig. S2). All primers are listed in Table S2.

LOV Domain Expression and Light-Induced Absorption Changes. A 0.4-kb fragment was amplified from *Rle* genomic DNA, using specific primers (LOV_dom_F and LOV_dom_R). The amplicon was cloned into BamHI and NdeI restriction sites of the pET11a vector (Novagen). The protein was expressed in *E. coli* BL21(DE3) pLysE and purified as described previously (7). Difference spectra were collected on a Hewlett Packard 8452A diode array spectrometer as previously described (8). Primers are listed in Table S2.

Quantification of Biofilm Formation by Crystal Violet Staining. *Rle* strains were cultured in TY medium for 1–4 d under shaking (200 rpm) in 96-well U-bottom culture polystyrene plates (Greiner Bio One) in light or dark conditions. Attached bacteria were quantified as described previously (9).

Exopolysaccharide Preparation and Sugar Quantification. *Rle* strains were cultured for 72 h in TY medium at 28 °C in light or dark conditions. Cells were centrifuged at 8,000 × *g* for 20 min and the supernatant was transferred to a clean tube. This supernatant was centrifuged again at 15,000 × *g* for 15 min and exopolysaccharide (EPS) from the extracellular milieu was precipitated, adding 3 vol of cold ethanol and NaCl (0.3 M final). Precipitated material was dissolved in water and the total monosaccharide equivalents were quantified by the anthrone assay as described previously (10).

SEM. *Rle* strains were cultured under light or dark conditions for 16 h and then 20 μL of the culture was seeded on glass coverslips and chemically fixed with 2.5% (vol/vol) glutaraldehyde in cacodylate buffer, pH 7.4. The samples were dehydrated in a graded series of ethanol and critical-point dried (CPD). CPD samples were mounted onto sticky carbon discs and coated with 15 nm of gold before analysis. Field emission scanning electron microscopy images were taken with a ZEISS SUPRA 40 microscope in the secondary electron mode, using an in-lens detector to improve resolution.

Flagellar Proteins Purification. For the isolation of flagellar proteins, 50 mL of *R. leguminosarum* culture was harvested by low-speed centrifugation (9,800 × *g*) and resuspended in a solution containing 0.5 mM $CaCl_2$, 0.1 mM EDTA, and 20 mM HEPES (pH 7.2). Flagella were detached from motile cells by vortexing at maximum power for 30 s and separated from cells by serial centrifugations (8,000 × *g* for 10 min and 15,000 × *g* for 15 min). Purified flagella were sedimented at 163,000 × *g* (45,000 rpm; Beckman 60Ti rotor) for 2 h and resuspended in 100 μL of SDS/PAGE loading buffer (21 mM Tris·Cl, pH 6.8, 2% (wt/vol) SDS, 10% (vol/vol) glycerol, 0.01% bromophenol blue). Preparations were separated on 12% SDS/PAGE and stained with Coomassie Brilliant Blue R-250.

Swimming Assay. Wild-type *R. leguminosarum* and *lov* mutant strains were inoculated onto soft TY agar plates (0.25% wt/vol) and incubated for 48 h under different illumination conditions: light (fluences of 15 or 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$) or covered with two layers of aluminum foil for darkness. Swimming halo was determined for each strain, in each condition.

Western Blot. Total extracts of bacteria cultured under different illumination conditions were separated by 12% SDS/PAGE. The same amount of protein was loaded into each lane of the gels. Proteins were transferred to Immobilon-NC Transfer Membranes (Millipore). Membranes were blocked with non-fat milk in PBS and incubated with Myc-Tag mouse mAb (Cell Signaling Technology) (1:1,000) and then with anti-mouse IgG (Fc specific)-peroxidase antibody produced in goat (Sigma) (1:5,000). Detection was achieved using the Chemiluminiscent ECL Plus Western Blotting Detection System (Amersham Biosciences) on High-Performance Chemiluminescence Film (Amersham).

Nodulation Assay. Nodulation tests were performed using peas (*Pisum sativum* variety Frisson) as previously described (11). Cultures were grown in TY medium for 48 h in darkness, and subcultures (1:200 dilution) were grown for 24 h in light or dark conditions. Bacteria were resuspended in Fahraeus (FP) medium (11) to a final OD_{600} of 0.1. In competence experiments 1:1 mix cultures (WT and *lov* mutant) were used to inoculate peas. One milliliter of bacterial suspension was placed into 0.5% (wt/vol) FP-agar medium in a 250-mL flask containing a 2-wk-old pea plant. After inoculation, only the aerial part of the plant was exposed to light. Plants were grown for 21 d at 21 °C (16 h light/8 h darkness) in an incubation chamber (Percival Scientific; I-30BLL) equipped with cool-white fluorescent tubes. In each independent experiment, five plants were inoculated for each rhizobial strain and treatment. Total, reddish, and white nodules for each plant were quantified. Nodules (at least 15 for each treatment) were harvested from plant roots at day 21. Individual nodules from each plant were placed in wells of a 96-well plate containing 100 μL of 10% (vol/vol) glycerol. Nodules were mechanically ruptured and cfu per nodule were determined using the appropriate antibiotics.

Sequence Analysis. The protein sequences of LOV-histidine kinase (LOV-HK) from *Brucella abortus* 2308 (BAB2_0652) and *Rle* (pRL110320) were aligned using the MUSCLE server (http://www.ebi.ac.uk/Tools/msa/muscle/) (12). The alignment was hand edited according to secondary structure prediction from the Phyre server (http://www.sbg.bio.ic.ac.uk/servers/phyre/) (13). Multiple amino acid sequence alignment was performed with the MAFFT program (14). Coloring scheme is according to ClustalX (http://www.clustal.org/). The phylogenetic tree was constructed by the maximum-likelihood method. The model of Whelan and Goldman was used to infer the tree. A discrete Gamma distribution was used to model evolutionary rate differences among sites (+*G*, parameter = 1.1136). Evolutionary analysis was conducted in MEGA5 software (15).

LOV Domain Construction Sequence. MHHHHHHAFERTRMPMVVTDGRKPDLPIVLANKAFLELTGYPAQEVLGRNCRFLQGPATSPIAVAEIRAAIAGEREVSVEILNYKKSGEQFWNRLHLSPVHGDDGKILYFFGSQIDMTEYRRIEAL

LOV WT and Mutant Myc-Tagged Sequences. >LOV_WT_myc
MTPHTKEKLHGDLPSASSKAASADRKELAAIAFERTRMPMVVTDGRKPDLPIVLANKAFLELTGYPAQEVLGRNCRFLQGPATSPIAVAEIRAAIAGEREVSVEILNYKKSGEQFWNRLHLSPVHGDDGKILYFFGSQIDMTEYRRIEALEASEHRLLMEVDHRSKNVLAIVDSIVRLSNADDPALYAAAIQHRVQALARAHTLLAARGWTNISLEELIRQQVTPFAATRAIFNGPDINMPAPVVQPLALVLHELAVNAAHHGALAVAQGRLSISWKPRPSGAGFYIRWQEVGAPTPPKLAKRGFGTVIVGAMVEKQLKGRLQKIWSDEGLLIDIEIPSAGPTCAEQKLISEEDL
>LOV_C75A_myc
MTPHTKEKLHGDLPSASSKAASADRKELAAIAFERTRMPMVVTDGRKPDLPIVLANKAFLELTGYPAQEVLGRNARFLQGPATSPIAVAEIRAAIAGEREVSVEILNYKKSGEQFWNRLHLSPVHGDDGKILYFFGSQIDMTEYRRIEALEASEHRLLMEVDHRSKNVLAIVDSIVRLSNADDPALYAAAIQHRVQALARAHTLLAARGWTNISLEELIRQQVTPFAATRAIFNGPDINMPAPVVQPLALVLHELAVNAAHHGALAVAQGRLSISWKPRPSGAGFYIRWQEVGAPTPPKLAKRGFGTVIVGAMVEKQLKGRLQKIWSDEGLLIDIEIPSAGPTCAEQKLISEEDL
>LOV_H163A_myc
MTPHTKEKLHGDLPSASSKAASADRKELAAIAFERTRMPMVVTDGRKPDLPIVLANKAFLELTGYPAQEVLGRNCRFLQGPATSPIAVAEIRAAIAGEREVSVEILNYKKSGEQFWNRLHLSPVHGDDGKILYFFGSQIDMTEYRRIEALEASEHRLLMEVDARSKNVLAIVDSIVRLSNADDPALYAAAIQHRVQALARAHTLLAARGWTNISLEELIRQQVTPFAATRAIFNGPDINMPAPVVQPLALVLHELAVNAAHHGALAVAQGRLSISWKPRPSGAGFYIRWQEVGAPTPPKLAKRGFGTVIVGAMVEKQLKGRLQKIWSDEGLLIDIEIPSAGPTCAEQKLISEEDL

Isolation of Total RNA from *R. leguminosarum* 3841. *R. leguminosarum* 3841 and *lov* mutant were cultured in Y minimal medium at 28 °C. Bacteria were cultured in light conditions ON until they reached early exponential phase, and half the culture was treated with 3 h 30 min of darkness. Then aliquots of 1×10^9 bacteria were harvested from light and dark treatments. Bacterial pellets were resuspended in 100 μL of a solution containing 84 μL of TE buffer, 15 μL of 10% SDS, and 1 μL of 10 μg/μL proteinase K. The samples were then incubated at 37 °C for 1 h and 600 μL of Qiagen RLT lysis buffer was added. Total RNA was isolated following the Qiagen RNeasy Mini Bacterial protocol. DNA was subsequently removed by digestion with DNase RNase-free according to the manufacturer's instructions (Promega). RNA was quantified using a NanoDrop spectrophotometer (ND-1000; Thermo Fisher Scientific).

Real-Time Quantitative RT-PCR Assay. Reverse transcription was performed with a transcriptor first-strand SuperScritp III cDNA kit (Invitrogen), using random decamer primers (Invitrogen) and RNasin ribonuclease inhibitor (Promega). cDNA samples were used as templates in real-time PCRs. The Primer3 program (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) was used to design primers for PCR products ranging from 90 to 110 bp (Table S3). Real-time PCRs containing SYBR Green were performed in 96-well plates, using a Mx3005P Stratagene instrument, and analyzed with MXPro and LinReg programs. Relative quantification using a standard curve method was performed for each set of primers. Results for each target mRNA were normalized to the expression levels of *R. leguminosarum initiation factor-1* (IF-1) mRNA.

Statistical Analysis. A two-sample Kolmogorov–Smirnov test was used to determine whether two datasets differ significantly. It has the advantage of making no assumption about the distribution of data (it can be non-normal). Two compatible samples are statistically identical. A two-tailed unpaired Student's *t* test was used to compare means.

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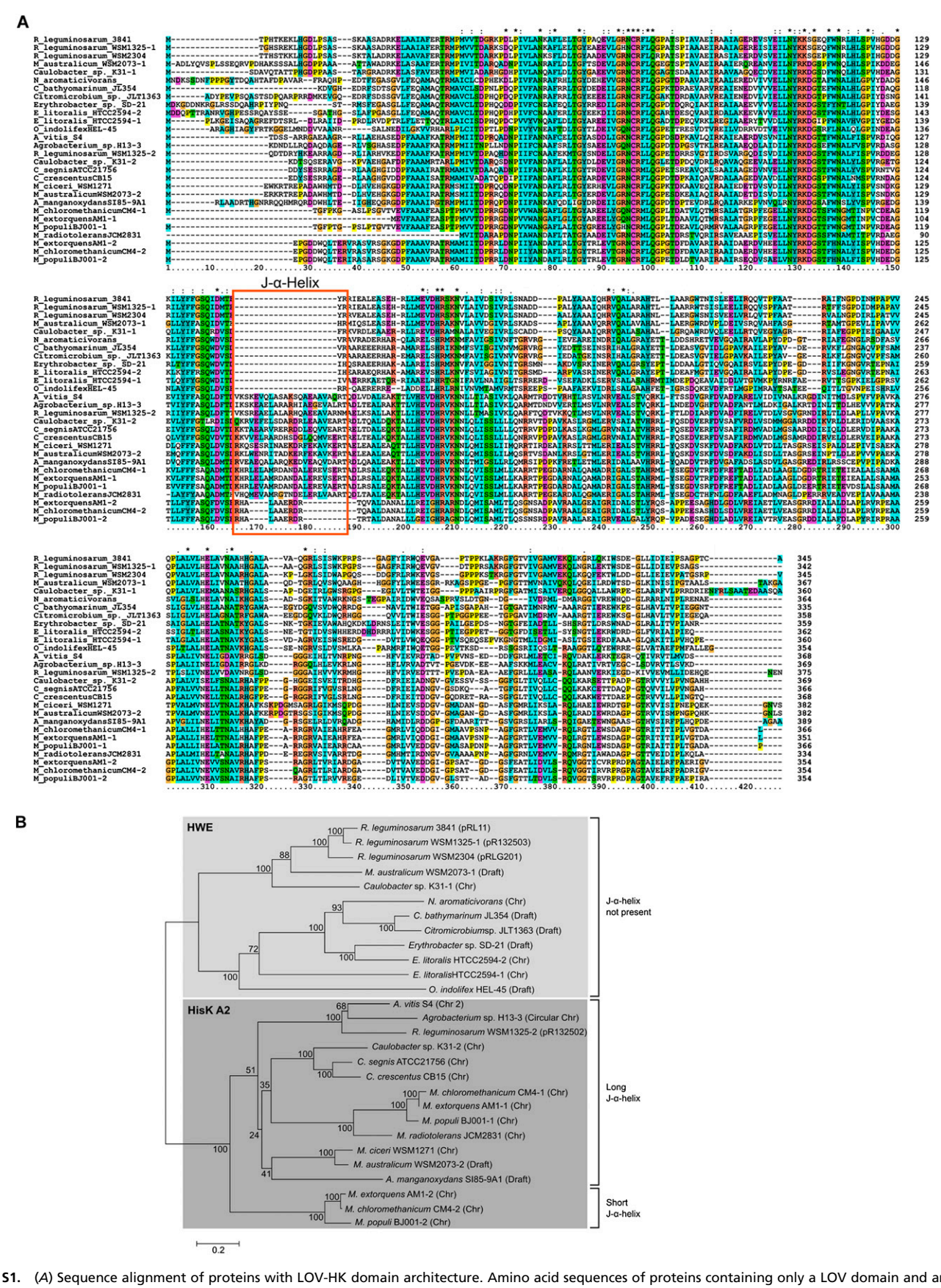

Fig. S1. (*A*) Sequence alignment of proteins with LOV-HK domain architecture. Amino acid sequences of proteins containing only a LOV domain and an HK domain were aligned using the MAFFT program. Coloring scheme is according to ClustalX. The red box indicates the region corresponding to the Jα-helix. (*B*) A phylogenetic tree of LOV-HK proteins was constructed by the maximum-likelihood method, using the Whelan and Goldman model. The tree is drawn to scale,

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with branch lengths measured in the number of substitutions per site. The bootstrap support values are shown in each branch. All positions containing gaps and missing data were eliminated. Evolutionary analysis was conducted in MEGA5 software. The shaded boxes indicate the proteins with histidine kinase domains belonging to the HWE or HisKA2 families. The genomic position of each sequence is indicated between parentheses (Chr, primary chromosome; Draft, unassembled genomic sequence and therefore the genomic position of the gene is uncertain). Sequences that have similar size of the Jα-helix are indicated with brackets. Sequences and IDs are as follows: *R. leguminosarum* bv. *viciae* 3841 [GenBank (GB) ID YP_771353], *R. leguminosarum* bv. *trifolii* WSM2304 (GB ID YP_002279192), *R. leguminosarum* bv. *trifolii* WSM1325 (R_leguminosarum_WSM1325-1, GB ID YP_002978926, and -2, YP_002984928), *Mesorhizobium australicum* WSM2073 (M. australicum_WSM2073-1, GB ID ZP_08989700, and -2, ZP_08989088), *Caulobacter* sp. K31 (Caulobacter_sp_K31-1, GB ID YP_001684922, and -2, YP_001686173), *Novosphingobium aromaticivorans* DSM 12444 (GB ID YP_497617), *Citromicrobium bathyomarinum* JL354 (GB ID ZP_06861302), *Citromicrobium* sp. JLT1363 (GB ID ZP_08700536), *Erythrobacter* sp. SD-21 (GB ID ZP_01864363), *Erythrobacter litoralis* HTCC2594 (E. litoralis_HTCC2594-1, GB ID YP_457485, and -2, YP_458419), *Oceanibulbus indolifex* HEL-45 (GB ID ZP_02152322), *Agrobacterium vitis* S4 (GB ID YP_00254736), *Agrobacterium* spp. H13-3 (GB ID YP_004278691), *Caulobacter segnis* ATCC 21756 (GB ID YP_003594909), *Caulobacter crescentus* CB15 (GB ID NP_419104), *Methylobacterium chloromethanicum* CM4 (M. chloromethanicum_CM4-1, GB ID YP_002423111, and -2, YP_002422352), *M. extorquens* AM1 (M_extorquens_AM1-1, GB ID YP_002965335, and -2, YP_002964506), *M. populi* BJ001 (M._populi_BJ001-1, GB ID YP_001927152, and -2, YP_001926163), *M. radiotolerans* JCM 2831 (GB ID YP_001753317), *Mesorhizobium ciceri* bv. biserrulae WSM1271 (GB ID YP_004142370), and *Aurantimonas manganoxydans* SI85-9A1 (GB ID ZP_01228325).

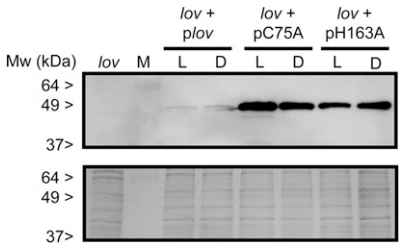

Fig. S2. Western blot of complemented *lov* strains. Protein expression in light (L) or darkness (D) of Myc-tagged LOV, LOV C75A, and LOV H163A in *lov*-complemented strains was confirmed by Western blot (*Upper*), using anti-Myc mAbs; Coomassie blue-stained SDS/PAGE (*Lower*) was used as a loading control.

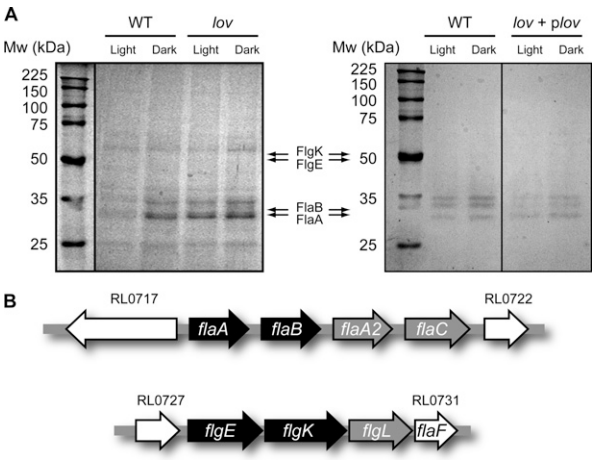

Fig. S3. Light modulation of flagellar proteins abundance. (*A*) Coomassie blue-stained 12% SDS/PAGE of purified flagellar proteins from *R. leguminosarum* WT and *lov* mutant strains cultured under light or dark conditions. (*B*) Genomic organization of two *R. leguminosarum* flagellar clusters. Solid arrows indicate ORFs identified by MALDI-TOF from SDS/PAGE bands.

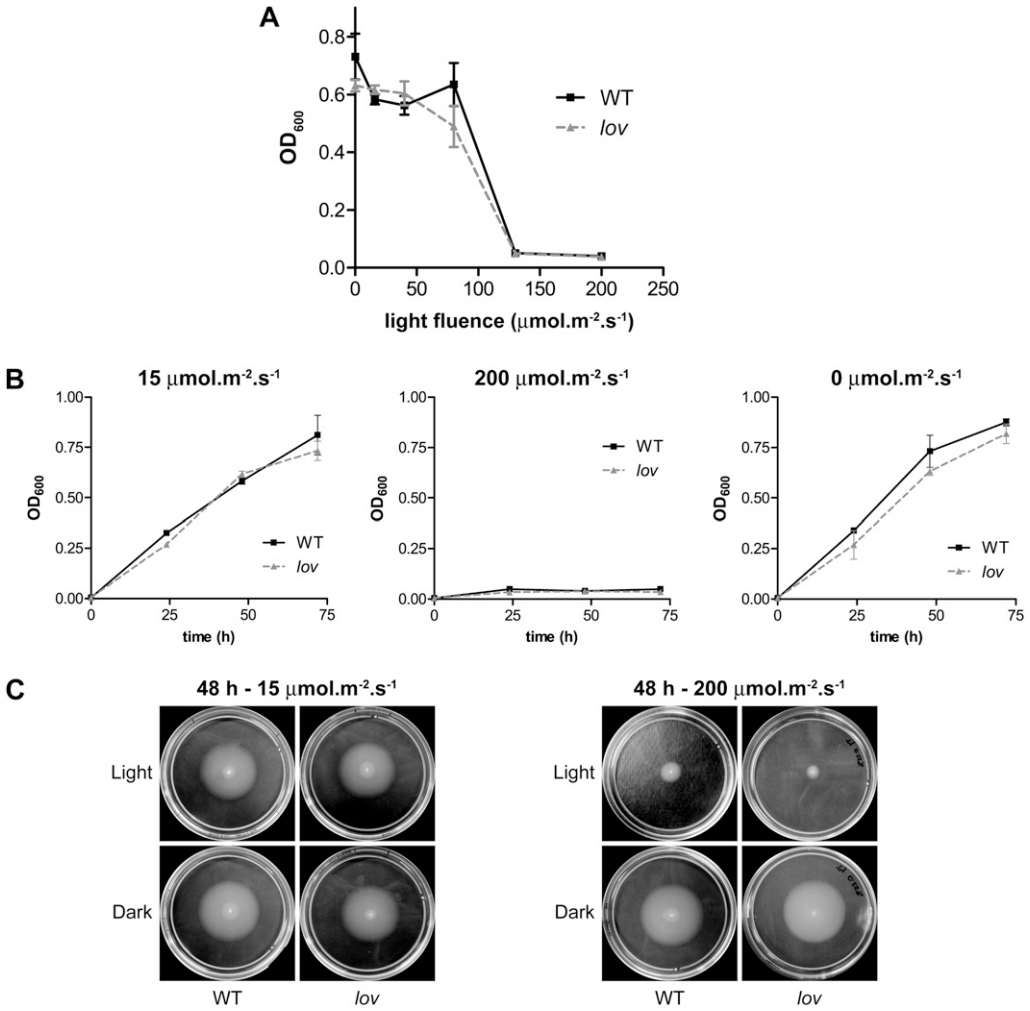

Fig. S4. Light influence on *R. leguminosarum* growth. (*A*) Optical density at 600 nm of static liquid cultures of *R. leguminosarum* WT and *lov* mutant strains illuminated with different white-light fluences in TY medium after 48 h (starting OD_{600} = 0.05). (*B*) *R. leguminosarum* WT and *lov* mutant strains growth curves under white light of 0, 15, or 200 μmol·m^{-2}·s^{-1} in TY liquid media. (*C*) Swimming assay of *R. leguminosarum* WT and *lov* strains in TY agar plates (0.25% wt/vol) under the same lighting conditions as in *B*. Light, uncovered plastic plate or glass tube; dark, covered with two layers of aluminum foil.

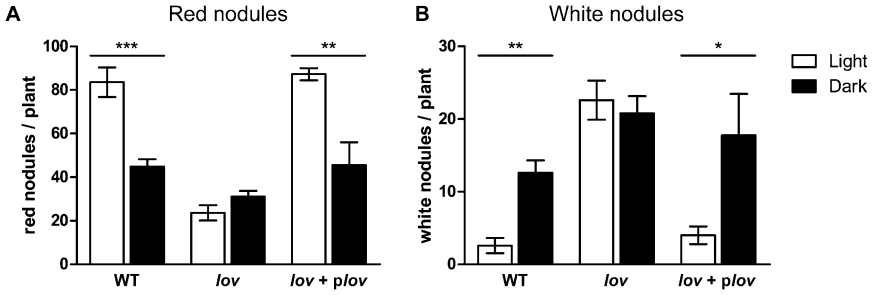

Fig. S5. Light modulation of symbiotic parameters in *Rle*. Pea plants were inoculated with white light- or dark-incubated cultures of *Rle* WT, *lov* mutant, or *lov*-complemented strains. After 21 d the number of (*A*) reddish nodules or (*B*) white nodules per plant was determined. Values are expressed as mean ± SD (n = 5), representative of three independent experiments performed. Statistical analysis was carried out by performing a two-tailed Student's t test (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

Table S1. Identity and similarity of LOV and HK domains from R-LOV-HK and B-LOV-HK

	LOV domain (%)	HK domain (%)	Overall (%)
Identity	62/103 (60)	60/203 (30)	125/313 (40)
Similarity	90/103 (87)	137/203 (68)	229/313 (73)

The Fig. 1 alignment was used to calculate the identity and similarity percentages.

Table S2. Primers used for cloning, screening, and mutagenesis

Name	Sequence	Genomic position in pRL11
uLOV_F	TTGTGAGCGTGGAAGCATGACAC	355,504–355,526
uLOV_R	TTATGCGCAGGTCGGGCCTGCCG	356,557–356,535
5_flank_F	CATAGAGATGATCGCATACTCGATCG	355,220–355,245
3_flank_R	GACCTCACTTTTCCTGCGCCGACGTGC	356,586–356,560
LOV_dom_F	TTCATATGCATCACCATCATCATCACGCATTTGAACGCACCAGGATGCC	355,613–355,635
LOV_dom_R	TCGGATCCTTACAGTGCCTCGATCCGCCGGTATTC	355,969–355,946
C75A_F	GTGCTAGGCCGCAACGCCCGTTTCCTGCAGGGC	355,727–355,759
C75A_R	GCCCTGCAGGAAACGGGCGTTGCGGCCTAGCAC	355,727–355,759
H163A_F	CTGATGGAAGTCGACGCCAGATCCAAGAATGTC	355,991–356,023
H163A_R	GACATTCTTGGATCTGGCGTCGACTTCCATCAG	355,991–356,023
LOV_myc_R	CATCGGCAGGCCCGACCTGCGCAGAGCAGAAACTGATCTCTGAGGAGGACCTCTAA	356,532–356,554

Table S3. Primers used for quantitative PCR

Name	Sequence	ORF
IF-1_RT_F	CGAAAACGAACACGAGATCA	RL0616
IF-1_ RT_R	GTAGGGCGTCATTTCCACAA	RL0616
FlaA_ RT_F	ACGCCAACATGGAAGAAGAG	RL0718
FlaA_ RT_R	GGATGTTCTGCGAAGAGGAG	RL0718
FlgE_ RT_F	GGCTTTCAGCTGATGGGTTA	RL0728
FlgE_ RT_R	CAGCCCTTCCTGATTGACAT	RL0728