Table S1. Growth and nodulation phenotype of WT plants and wildtype homozygous sibblings (M5 seeds) from mutant plants

Plant length is expressed in centimeters and the number of ITs per centimeter of root. Values are means \pm SE (n= 60 for plant length and nodule number; n=10-20 for ITs). Means denoted by the same letter do not significantly differ (P=0.05) based on the Duncan's multiple range test.

WT	A102V w/w	E127K w/w
14.3 ± 0.5 a	12.8 ± 0.8 a	13.5 ± 0.4 a
6.8 ± 0.2 a	6.6 ± 0.2 a	6.3 ± 0.3 a
2.9 ± 0.1 a	2.5 ± 0.2 a	2.5 ± 0.2 a
32.0 ± 1.4 a	28.4 ± 1.0 a	28.8 ± 1.3 a
35.0 ± 1.4 a	30.9 ± 1.1 a	31.3 ± 1.4 a
	WT $14.3 \pm 0.5 \text{ a}$ $6.8 \pm 0.2 \text{ a}$ $2.9 \pm 0.1 \text{ a}$ $32.0 \pm 1.4 \text{ a}$ $35.0 \pm 1.4 \text{ a}$	WTA102V w/w 14.3 ± 0.5 a 12.8 ± 0.8 a 6.8 ± 0.2 a 6.6 ± 0.2 a 2.9 ± 0.1 a 2.5 ± 0.2 a 32.0 ± 1.4 a 28.4 ± 1.0 a 35.0 ± 1.4 a 30.9 ± 1.1 a

Table S2. Growth parameters of non-nodulated LjGlb1-1 mutant plants, and derived wild-type homozygous siblings, supplied with combined nitrogen

Plants were grown on Fåhraeus medium supplemented with 1.5 mM NH_4NO_3 for three weeks. Lengths are expressed in centimeters and weights in grams. Means (± SE, *n*= 9-12) denoted by the same letter do not significantly differ (*P*=0.05) based on the Duncan's multiple range test.

	WT	A102V	E127K	96642	A102V (w/w)	E127K (w/w)
Shoot length	4.68 ± 0.09 a	4.33 ± 0.20 ab	3.29 ± 0.24 c	3.86 ± 0.15 b	4.69 ± 0.17 a	4.56 ± 0.12 a
Shoot weight	29.83 ± 1.19 a	27.00 ± 1.65 a	$20.67\pm1.73~\mathrm{b}$	$26.31\pm0.87~\text{a}$	30.11± 1.67 a	29.89 ± 1.11 a
Root length	5.37 ± 0.21 a	$4.13\pm0.23~\text{b}$	$2.00\pm0.33~\mathrm{c}$	$3.73\pm0.22~\mathrm{b}$	5.40 ± 0.18 a	5.09 ± 0.14 a
Root weight	29.25 ± 1.04 a	$23.89\pm2.21~\mathrm{b}$	12.67 ± 0.87 c	$24.54\pm1.53~\mathrm{b}$	29.78 ± 1.19 a	27.56 ± 1.31 a
Leaf number	4.92 ± 0.15 a	4.11 ± 0.31 b	$2.56\pm0.45~\text{c}$	$3.92\pm0.14~\text{b}$	4.89 ± 0.11 a	4.78 ± 0.15 a

Table S3. Effect of SNP application to roots onnodulation of WT plants

Seedlings were inoculated with *M. loti* MAFF303099 DsRed and grown on nitrogen-free Fåhraeus medium for four weeks. The numbers of ITs are expressed per centimeter of root. Means (\pm SE, *n*= 9-11) denoted by different letters significantly differ (*P*=0.05) based on the Student's *t*-test.

	Control	SNP
Incipient ITs	3.6 ± 0.4 a	$10.5\pm0.7~\text{b}$
Long ITs	25.5 ± 2.1 a	$6.6\pm0.7\;b$
Total ITs	29.1 ± 2.1 a	17.2 ± 1.2 b

Ljap	1	MSTLGSTCHTEEQEALVVKSWSVMKKNSAELGLKLELKIFEIAPSAQKLFSFLRD	55
Msat	1	MGTLDTKGETEEQEALVVKSWNAMKKNSAELGLKLELKIFEIAPSAQKLESFLKD	55
Gmax	1	MTTTLERGESEEQEALVVKSWNVMKKNSGELGLKFELKIFEIAPSAQKLFSFLRD	55
Afir	1	MNTLEGRGETEEQEAVVVKSWNAMKPNAGELGLKFELKIFEIAPSAQKLFSFLRD	55
Pand	1	MSSSEVNKVFTEEQEALVVKAWAVMKKNSAELGLQFFLKIFEIAPSAKNLFSYLKD	56
Tori	1	MSSSEVDKVFTEEQEALVVKSWAVMKKNSAELGLKFFLKIFEIAPSANNLFSYLKD	56
Atha	1	MESEGKIVETEEQEALVVKSWSVMKKNSAELGLKLFIKIFEIAPTTKKMFSFLRD	55
Osat	1	MALVEDNNAVAVSFSEEQEALVLKSWAILKKDSANIALRFELKIFEVAPSASQMESFLRN	60
Taes	1	MSAAER-AVVESEEKDALVLKSWAIMKKDSANLGLRFELKIFEIAPSARQMFPFLRD	56
Hvul	1	MSAAEG-AVVESEEKEALVLKSWAIMKKDSANLGLRFELKIFEIAPSARQMFPFLRD	56
Zmay	1	MALAEADDG-AVVEGEEQEALVLKSWAVMKKDAANLGLRFELKVFEIAPSAEQMESFLRD	59
		*	
Ljap	56	SKVPLEENPKLKPHAMSVFVMTCESAAQLRKAGKVTVRESTLKKL(APHYKYGVVNEHFE	115
Msat	56	SKVPLEQNTKLKPHAMSVFLMTCESAVQLRKSGKVTVRESSLKKLCA NHFKYGVVDEHFE	115
Gmax	56	STVPLEQNPKLKPHAVSVFVMTCDSAVQLRKAGKVTVRESNLKKLCATHFRTGVANEHFE	115
Afir	56	SNVSLERNPKLKSHAMSVFLMTCESAVQLRKAGKVTVRESSLKKLCA/HFKHGVVDEHYE	115
Pand	57	SPVPLEQNPKLKPHATTVFVMTCESAVQLRKAGKVTVKESDLKRICAIHFKTGVVNEHFE	116
Tori	57	SPIPLEQNPKLKPHAMTVFVMTCESAVQFRKAGKVTVRESNLKRICAIHFKNGVVHEHFE	116
Atha	56	SPIPAEQNPKLKPHAMSVFVMCCESAVQLRKTGKVTVRETTLKRLCASHSKYGVVDEHFE	115
Osat	61	SDVPLEKNPKLKTHAMSVFVMTCEAAAQLRKAGKVTVRDTTLKRLCA THLKYGVGDAHFE	120
Taes	57	SDVPLETNPKLKTHAVSVFVMTCEAAAQLRKAGKITVRETTLKRLCGTHLKYGVADGHFE	116
Hvul	57	SDVPLETNPKLKTHAVSVFVMTCEAAAQLRKAGKITVRETTLKRLCGTHLKYGVADGHFE	116
Zmay	60	SDVP15KNPKLKTHAMSVFVMTCEAAAQLRKAGKVTVRETT15KRLCATHLRYGVADGHFE	119
	110		1 (1
Ljap	116	VTKFALLDTIKE AVP-EMWSPEMKNAWTQAYDQLVGAIKSEMKPSSS	161
Msat	110	VTKFALLETIKEAVP-EMWSPAMKNAWGEAYDQLVNAIKSEMKPSS-	160
Gmax	110	VTKFALLETIKEAVP-EMWSPAMKNAWGEAYDQLVDAIKSEMKPPSS	161
Afir	117	VTKFALLETIKEAVP-EMWSPEMKIAWGEAYDQLVAAIKSAMKPSS-	160
Pand	117		162
Tori	116		162
Atha	121	VARIADDITISTY E AMA TAMA SAMA SAMA SAMA SAMA SAMA SAMA	166
Usat	117	VYN DENTER TER TER TER TER TER TER DANNA WCER AND DE TAN AN TROEMER A DE T	162
Taes	117		162
HVUL	120	VTACENTIETER TO DA DIMINE EN KAMA EN YSATAN DA TKREMKODA -	165
zmay	ΤζΟ		TOJ

Figure S1: Alignment of some class 1 Hbs showing conservation of A102 and E127. Both residues are within red boxes and histidine residues that are important for heme assembly are marked with red asterisks. *Abbreviations* (accession numbers in parentheses): Afir, *Alnus firma* (BAE75956); Atha, *Arabidopsis thaliana* (AEC06463); Gmax, *Glycine max* (AAA97887); Hvul, *Hordeum vulgare* (AAB70097); Ljap, *Lotus japonicus* (BAE46739); Msat, *Medicago sativa* (AAG29748); Osat, *Oryza sativa* (AAM19125); Pand, *Parasponia andersonii* (AAB86653); Taes, *Triticum aestivum* (AAN85432); Tori, *Trema orientalis* (AAC28426); Zmay, *Zea mays* (AAG01375).



Figure S2. Inhibition of NO-associated fluorescence by cPTIO in roots of WT and mutant plants. The figure shows representative epifluorescence and bright-field images of roots after 1-h incubation with 20 μM DAF-FM DA combined or not with 3 mM cPTIO. Bars, 200 μm.



Figure S3: Purification of wild-type recombinant LjGlb1-1. SDS-gels (12.5%) stained with Coomassie blue. Lanes (5 µg protein): 1, preinduced culture; 2, induced with 0.25 mM IPTG; 3, after ammonium sulfate (30-75%) fractionation; 4, after Ni-affinity chromatography. Molecular mass markers (kDa) are shown on the left. Similar results were obtained for the mutated versions A102V and E127K.



