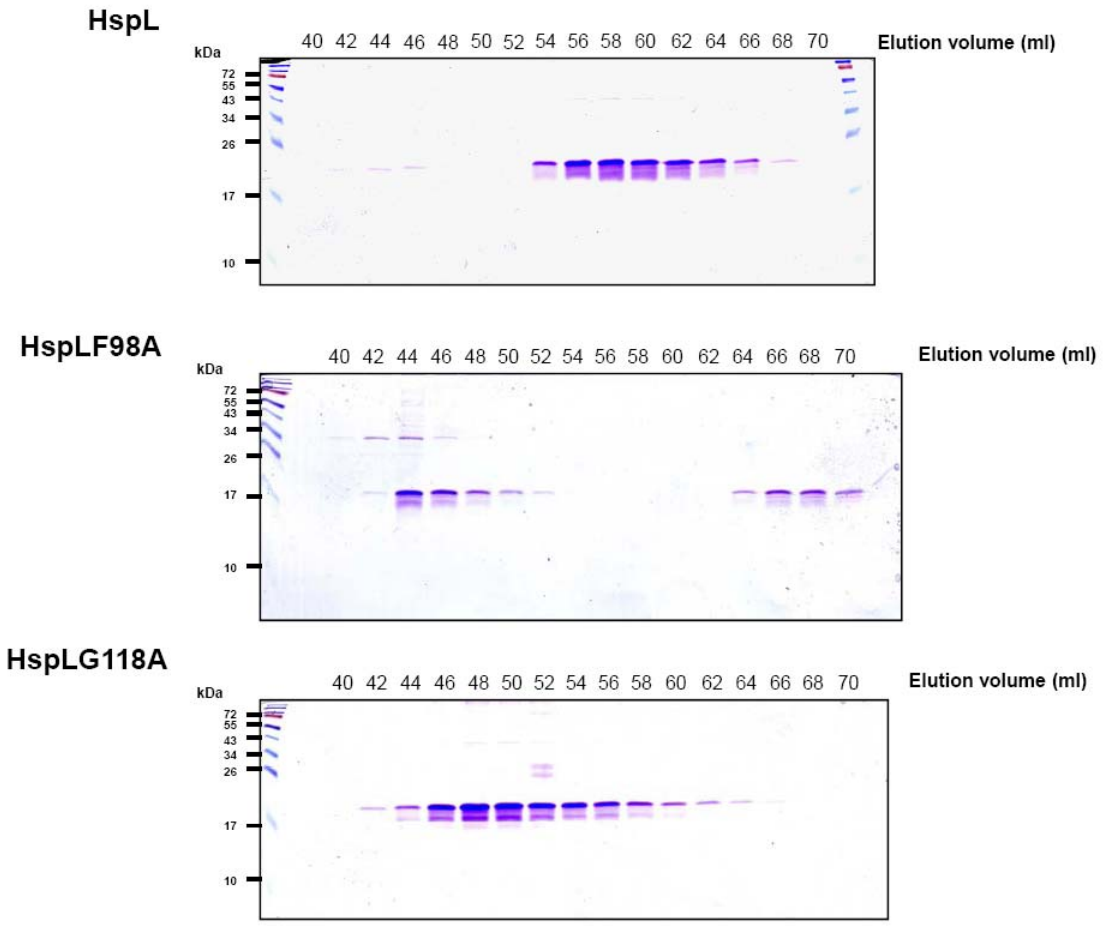
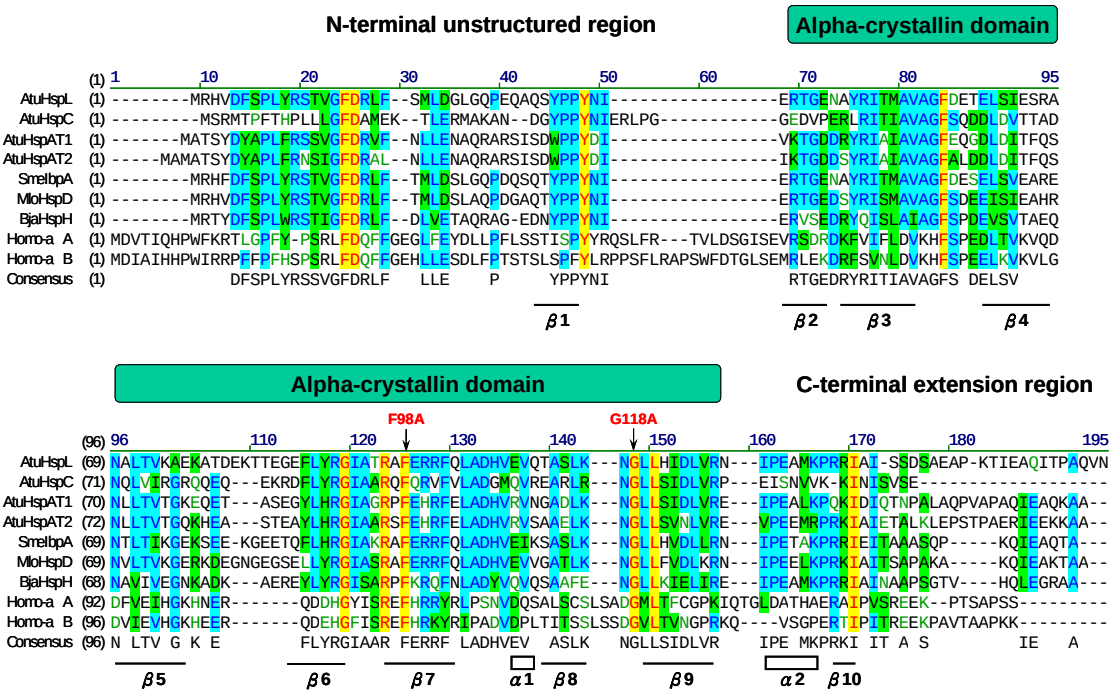
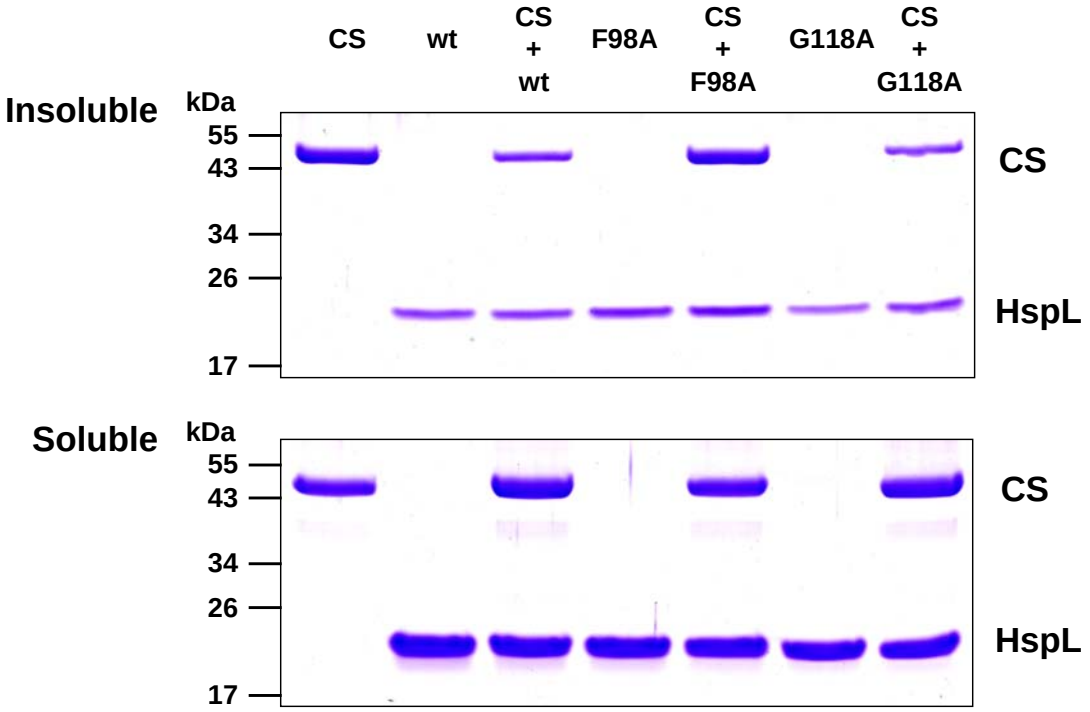


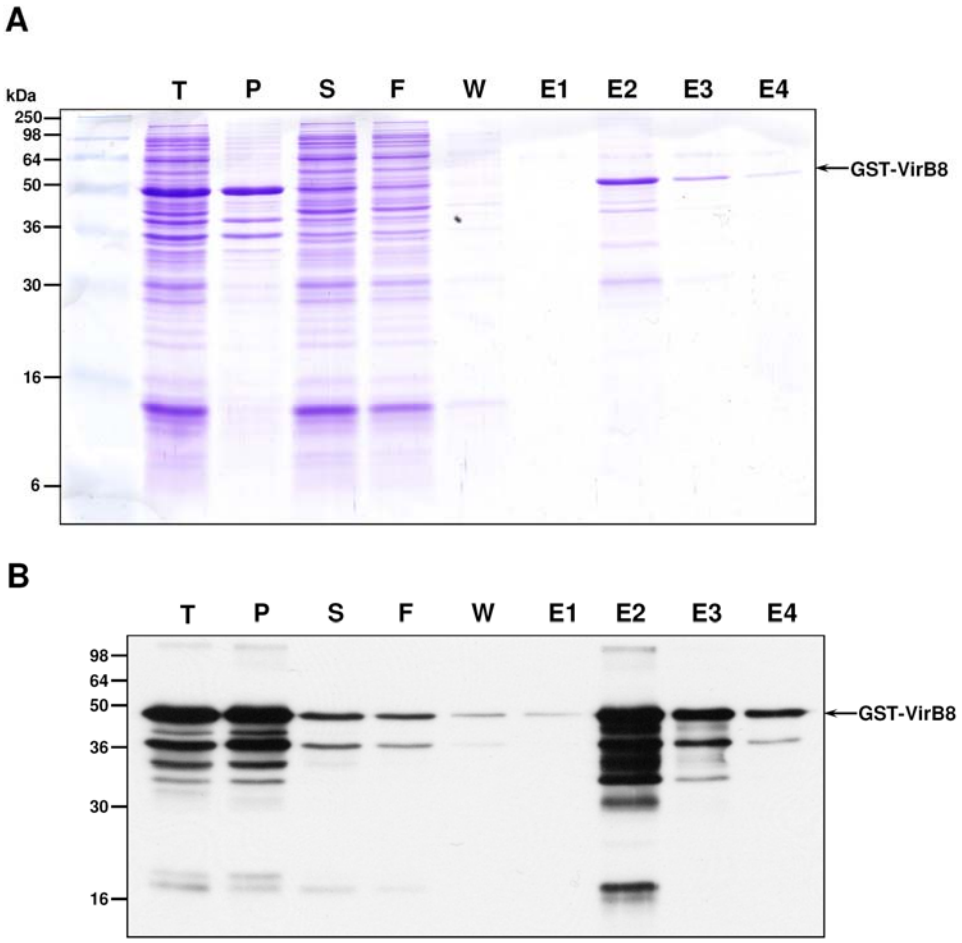
Supplemental Fig. S1. Full SDS-PAGE of Fig. 2B. 10 µl of each 2-ml interval eluted from 1.0 mg/ml of purified HspL-His, HspLF98A, and HspLG118A was resolved in 15% Glycine SDS-PAGE followed by coomassie blue staining. The molecular weight markers (kDa) are indicated on the left.



Supplemental Figure S2. Multiple sequence alignments and domain prediction of HspL and selected homologous proteins encoded by Rhizobiaceae and *Homo sapiens*. A multiple alignment of the Alpha-crystallin domain, bordered by an N-terminal unstructured region and a C-terminal extension region in HspL and selected homologous proteins encoded in Rhizobiaceae and *Homo sapiens*, is presented based on BLASTP analysis conducted using the NCBI WebSite (http://www.ncbi.nlm.nih.gov/). The conserved amino acids in all analyzed proteins are highlighted in yellow blocks and the blue and green blocks indicate the identical and similar amino acids in most but not all proteins, respectively. The conserved amino acid residues F98A and G118 selected for Ala substitution are indicated by arrows. AtuHspL, AtuHspC, AtuHspAT1, AtuHspAT2 are the *A. tumefaciens* α-Hsp proteins HspL, HspC, HspAT1, and HspAT2; SmeIpbA: *Sinorhizobium meliloti* IbpA; MloHspD: *Mesorhizobium loti* HspD; BjaHspH: *Bradyrhizobium japonicum* HspH; Homo-aA and Homo-aB: *Homo sapiens* alpha-crystallin A and B.



Supplemental Figure S3. The effect of HspL and its variants (HspLF98A and HspLG118A) on heat-induced precipitation of CS. 600 nM CS was incubated at 43°C for 1 h in the absence or presence of 1.2 μM HspL or its variants. The heated proteins were centrifuged at 16,000 x g for 15 min to separate the insoluble pellet and soluble supernatant. The pellet was directly resuspended in 20 μl 2X SDS sample buffer. The pellet (insoluble) or supernatant (soluble) concentrated by trichloroacetic acid were resolved by 12% Tricine-SDS-PAGE followed by coomassie blue staining. The molecular weight markers (kDa) are indicated on the left.



Supplemental Figure S4. GST-VirB8 purification. *E. coli* BL21(DE3) strain containing pETGSTB8 was induced by 0.2 mM IPTG at 28^{o}C for 2 h. The extracts of soluble proteins were applied to a Glutathione-Agarose column (Sigma-Aldrich) to purify GST-VirB8 protein. The total protein (T), insoluble pellet (P), soluble supernatant (S), flow through (F), wash fraction (W), and eluted fractions (E1~E4) were resolved by 15% Glycine-SDS-PAGE followed by (A) coomassie blue staining or (B) western blotting with VirB8 specific antiserum. The molecular weight markers (kDa) are indicated on the left.

Supplemental Table 1. Bacterial strains and plasmids

Strains/plasmids	Relevant characteristics	References/ sources
A. tumefaciens		
NT1RE-Sp	Rm^R, Em^R, Sp^R, NT1RE containing spectinomycin resistant gene (*aadA*)	(1)
NT1RE(pJK270)	Rm^R, Em^R, Km^R/Nm^R, pJK270 is pTiC58Tra^C with *Tn*5 insertion in T-DNA region without affecting virulence	(2)
EML1057	Rm^R, Em^R, Km^R/Nm^R, markerless *hspL* deletion mutant in NT1RE(pJK270)	(1)
EML1280	Rm^R, Em^R, Km^R/Nm^R, Tc^R, pHspL in EML1057	(1)
EML1682	Rm^R, Em^R, Km^R/Nm^R, Tc^R, pHspLF98A in EML1057	This study
EML1683	Rm^R, Em^R, Km^R/Nm^R, Tc^R, pHspLG118A in EML1057	This study
EML2211	Rm^R, Em^R, Km^R/Nm^R, Tc^R, pHspL-His in EML1057	This study
E. coli		
DH10B	Host for DNA cloning	Invitrogen
BL21(DE3)	Host for overexpressing proteins driven by T7 promoter	(3)
S. cerevisiae		
AH109	Host for yeast two-hybrid analysis	Clontech
pETHspL	Ap^R, overexpression of HspL-His in *E. coli*	(1)
pETHspLF98A	Ap^R, overexpression of HspLF98A-His in *E. coli*	This study
pETHspLG118A	Ap^R, overexpression of HspLG118A-His in *E. coli*	This study
pETGSTB8	Km^R, overexpression of GST-VirB8 in *E. coli*	This study
pEML652	Ap^R, Tc^R, pRU1064 was digested with *Pst*I to remove reporter gene (*gfpUV* and *gusA*)	(4)
pHspL	Ap^R, Tc^R, expression of *hspL* gene containing its promoter and ORF on pEML652	(1)
pHspLF98A	Ap^R, Tc^R, expression of *hspLF98A* gene containing its promoter and ORF on pEML652	This study
pHspLG118A	Ap^R, Tc^R, expression of *hspLG118A* gene containing its promoter and ORF on pEML652	This study

pHspL-His	Ap^R, Tc^R, expression of *hspL-his* gene containing its promoter and ORF on pEML652	This study
pML122ΔKm	The IncQ plasmid RSF1010 derivative pML122 with removal of *nptII* gene	(5)
pGADT7	The DNA-AD vector, for yeast two-hybrid assay	Clontech
pGBKT7	The DNA-BD vector, for yeast two-hybrid assay	Clontech
pGADB8	Ap^R, the pGADT7 containing full-length genes of *virB8* for Y2H assay	This study
pGBKB8	Km^R, the pGBKT7 containing full-length genes of *virB8* for Y2H assay	This study
pGADHspL	Ap^R, the pGADT7 containing full-length genes of *hspL* for Y2H assay	This study
pGBKHspL	Km^R, the pGBKT7 containing full-length genes of *hspL* for Y2H assay	This study

REFERENCES

1. Tsai, Y. L., Wang, M. H., Gao, C., Klusener, S., Baron, C., Narberhaus, F., and Lai, E. M. (2009) *Microbiology* 155(Pt 10), 3270-3280
2. Kao, J. C., Perry, K. L., and Kado, C. I. (1982) *Mol Gen Genet* 188(3), 425-432
3. Studier, F. W., Rosenberg, A. H., Dunn, J. J., and Dubendorff, J. W. (1990) *Methods Enzymol* 185, 60-89
4. Liu, A. C., Shih, H. W., Hsu, T., and Lai, E. M. (2008) *J Appl Microbiol* 105(5), 1372-1383
5. Labes, M., Puhler, A., and Simon, R. (1990) *Gene* 89(1), 37-46

Supplemental Table 2. Primer information

Name and size of PCR product	Primers	Sequence[a]
hspL promoter＋*hspLF98A*-L	HspL-F-p-HindIII	5’-AC**AAGCTT**TGTCTTCACTGGCGC-3’
	HspL-F98A-R	5’-AAGCGGCGCTCGGCAGCGCGCGT-3’
hspLF98A-R	HspL-F98A-F	5’-ACGCGCGCTGCCGAGCGCCGCTT-3’
	HspL-PM-R	5’- CCGATGCCGTCATCGAGGGTATCT-3’
hspL promoter＋*hspLG118A*-L	HspL-F-p-HindIII	5’-AC**AAGCTT**TGTCTTCACTGGCGC-3’
	HspL-G118A-R	5’-TCGATGTGAAGCAGGGCGTTCTTCA-3’
hspLG118A-R	HspL-G118A-F	5’-TGAAGAACGCCCTGCTTCACATCGA-3’
	HspL-PM-R	5’- CCGATGCCGTCATCGAGGGTATCT-3’
hspL promoter＋ORF -His(928bp)	HspL-F-p-HindIII	5’-AC**AAGCTT**TGTCTTCACTGGCGC-3’
	HspL-His-R	5’-TT**AAGCTT**AGTGGTGGTGGTGGTGGTGGTTGACCT GAGCCGGGGTAATCTGGGCTTC-3’
HspLF98A or HspLG118A ORF without stop codon (using pHspLF98A or pHspLG118A as template)	HspL-NdeI-F	5’-**CATATG**CGTCACGTTGATTTTTCCC-3’
	HspL-XhoI-R	5’-**CTCGAG**GTTGACCTGAGCCGGG-3’
VirB8 ORF for pET42b(+)	virB8N-SpeI-F	5’-**ACTAGT**ATGAAGGGGTCTGAATACGCCTTGC-3’
	virB8C-PstI-R	5’-**CTGCAG**TCATGGTTCGCTGTGGCCTG-3’
VirB8-Y2H (721bp)	B8-NdeI-F	5’-**CATATG**AAGGGGTCTGAATACGC-3’
	B8-EcoRI-R	5’-**GAATTC**ATGGTTCGCTGTGGCCT-3’
HspL-Y2H (492bp)	HspL-NdeI-F	5’-**CATATG**CGTCACGTTGATTTTTCCC-3’
	HspL-BamHI-R	5’-**GGATCC**TTAGTTGACCTGAGCCG-3’

[a]The sequences of designed restriction enzyme sites were shown in bold and the sequences complementary to specific region in *A. tumefaciens* genome is shown in Italian

Supplemental Table 3. Tumor assays of various *Agrobacterium* strains on potato tuber discs.

Infection strains	**Tumorigenesis efficiency** [1]		
	Experiment 1	**Experiment 2**	**Experiment 3**
Wild type	11.7 ±1.1 [a]	9.2 ± 0.8 [a]	11.3 ± 0.8 [ac]
ΔhspL	8.1 ±0.9 [b]	5.9 ± 0.8 [b]	7.9 ± 0.7 [b]
ΔhspL (pHspL)	12.1 ± 0.9 [a]	10.2 ± 1.0 [a]	13.2 ± 0.9 [c]
ΔhspL (pHspLF98A)	8.0 ±0.7 [b]	6.0 ± 0.6 [b]	7.8 ± 0.7 [b]
ΔhspL (pHspLG118A)	11.3 ± 0.7 [ab]	9.0 ± 0.7 [a]	9.6 ± 0.7 [ab]

[1] Potato tuber discs were inoculated with 10 µl (10^8 cfu/ml) of bacterial cells. Tumorigenesis efficiency is presented as number of tumors per disc, with standard errors averaged from results of 60 potato tuber discs. Means annotated with the same letter (a-d) are not significantly different; those with different letters are significantly different ($P < 0.05$) according to Duncan's multiple range test.

Supplemental Table 4. Mobilization of RSF1010-derivative pML122ΔKm between agrobacteria.

Donor strain	**Transfer frequency** [a]		
	Experiment 1	**Experiment 2**	**Experiment 3**
NT1RE (pJK270)	2.4×10^{-5}	7.0×10^{-6}	6.9×10^{-6}
ΔhspL	2.0×10^{-5}	5.2×10^{-6}	5.9×10^{-6}
ΔhspL (pHspL)	8.0×10^{-5}	1.0×10^{-5}	1.3×10^{-5}
ΔhspL (pHspLF98A)	1.9×10^{-5}	5.7×10^{-6}	6.1×10^{-6}
ΔhspL (pHspLG118A)	4.2×10^{-5}	8.0×10^{-6}	9.4×10^{-6}

[a] expressed as number of transconjugants per input donor