TABLE S1. Primers

Primer	Sequence
KC19	ATATATATGAATTCACCATGGAAACCCGATTCCTCAG
KC30	CGGCAGAAAAGTCCACATTG
KC31	CACACTACCATCGGCGCTA
KC407	GACAACAAAGAAGTTCAACTCTTTATGTATTGATCTTCATCGCGATCTTTCTCTCGAAATTTACCAATCAATTG
KC408	CAAAGAAGTTCAACTCTTTATGTATTGATCTTCCTCGTGATCTTTCTCTCGAAATTTACCAATCAATTGCTTC
KC409	CTCTTTATGTATTGATCTTCCTCGCGATCTTTATCTCGAAATTTACCAATCAATTGCTTCTGTCG
KC410	CTCTTTATGTATTGATCTTCCTCGCGATCTTTGTCTCGAAATTTACCAATCAATTGCTTCTGTCG

Table S2. Single-base change alleles of *L*

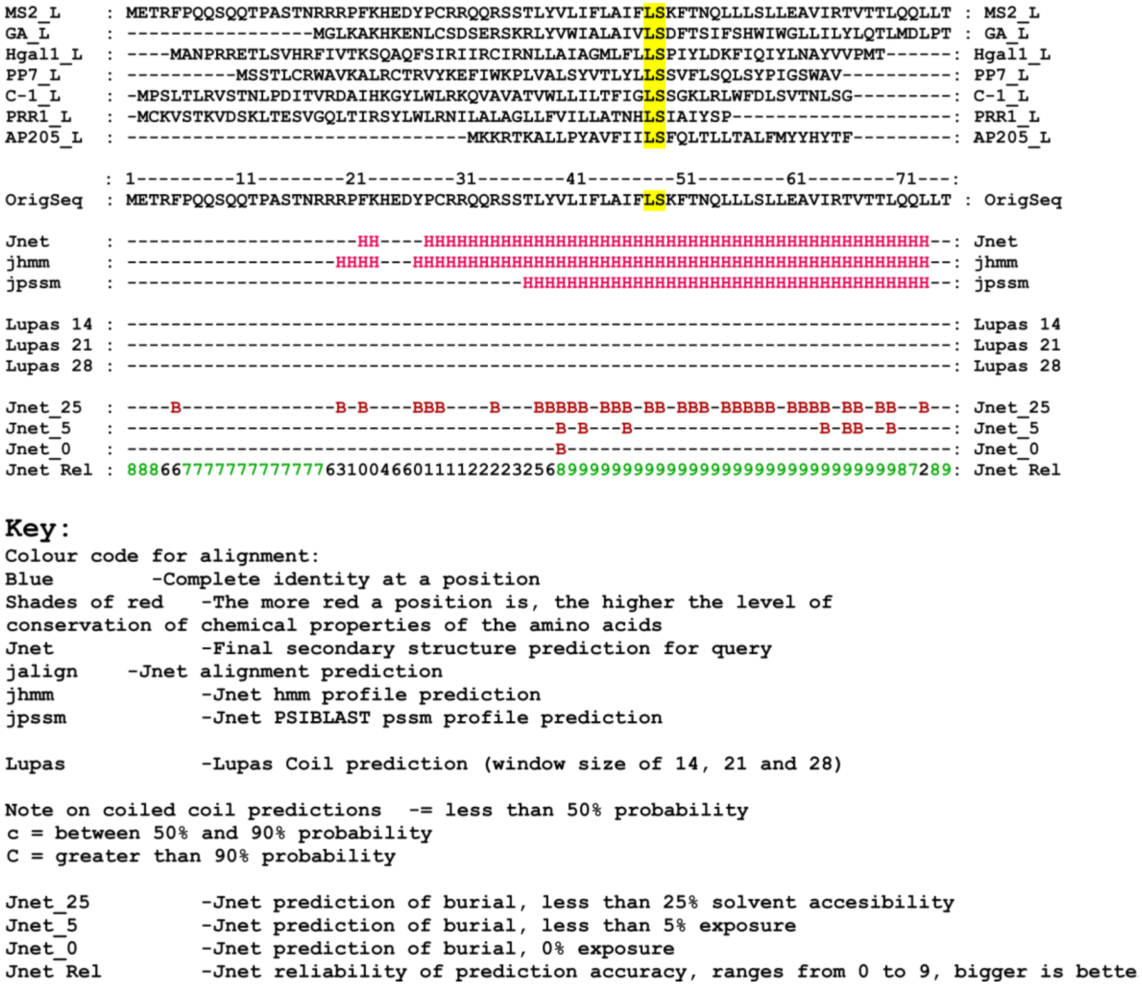
Serial	Mutant number[§]	Position of the mutation in *L*	Base pair(s) changed	Amino acid(s) position	Amino acid(s) changed	Lysis*	Protein levels[#]
1	90	3	G→T	1	M→I	-	-
2	185	3	G→A	1	M→I	-	-
3	205	2	T→C	1	M→T	-	-
4	261	4	G→T	2	E→Stop	-	N.D.
5	190	8	C→T	3	T→I	-	-
6	223	7	A→T	3	T→S	-	-
7	326	17	C→T	6	P→L	-	-
8	127	22	C→T	8	Q→Stop	-	N.D.
9	139	23	A→T	8	Q→L	-	-
10	288	23	A→T	8	Q→L	-	-
11	219	28	C→T	10	Q→Stop	-	N.D.
12	391	31	C→T	11	Q→Stop	-	N.D.
13	65	38	C→T	13	P→L	+	+
14	345	38	C→T	13	P→L	+	+
15	331	43	T→G	15	S→A	+	+
16	50	52	A→G	18	R→G	+	+
17	177	53	G→T	18	R→I	+	+
18	291	52	A→T	18	R→Stop	-	N.D.
19	48	55	C→A	19	R→S	+	-
20	67	56	G→A	19	R→H	+	-
21	142	58	C→T	20	R→W	+	-
22	170	58	C→T	20	R→W	+	-
23	313	59	G→T	20	R→L	+	-
24	183	67	A→G	23	K→E	+	-
25	319	67	A→G	23	K→E	+	-
26	321	67	A→T	23	K→Stop	-	N.D.
27	343	67	A→G	23	K→E	+	-
28	384	67	A→T	23	K→Stop	-	N.D.
29	46	74	A→T	25	E→V	+	-
30	172	74	A→G	25	E→G	+	-
31	184	75	G→T	25	E→D	+	-
32	300	74	A→G	25	E→G	+	-
33	301	74	A→G	25	E→G	+	-
34	302	74	A→G	25	E→G	+	-
35	307	74	A→G	25	E→G	+	-

36	308	74	A→G	25	E→G	+	-
37	309	74	A→G	25	E→G	+	-
38	317	74	A→G	25	E→G	+	-
39	322	74	A→G	25	E→G	+	-
40	323	74	A→G	25	E→G	+	-
41	324	74	A→G	25	E→G	+	-
42	327	74	A→G	25	E→G	+	-
43	192	77	A→G	26	D→G	+	-
44	157	81	C→G	27	Y→Stop	-	N.D.
45	200	87	T→A	29	C→Stop	-	N.D.
46	268	86	G→A	29	C→R	-	-
47	294	87	T→A	29	C→Stop	-	N.D.
48	77	89	G→A	30	R→Q	+	+
49	265	89	G→T	30	R→L	+	+
50	344	88	C→T	30	R→Stop	-	N.D.
51	365	88	C→T	30	R→Stop	-	N.D.
52	166	91	A→T	31	R→Stop	-	N.D.
53	253	91	A→T	31	R→Stop	-	N.D.
54	267	92	G→T	31	R→I	+	+
55	394	94	C→T	32	Q→Stop	-	N.D.
56	10	99	A→T	33	Q→H	-	+
57	57	99	A→T	33	Q→H	-	+
58	196	97	C→T	33	Q→Stop	-	N.D.
59	361	97	C→T	33	Q→Stop	-	N.D.
60	243	100	A→T	34	R→Stop	-	N.D.
61	348	100	A→T	34	R→Stop	-	N.D.
62	213	107	C→G	36	S→Stop	-	N.D.
63	358	107	C→A	36	S→Stop	-	N.D.
64	3	115	T→C	39	Y→H	-	-
65	252	117	T→A	39	Y→Stop	-	N.D.
66	356	117	T→A	39	Y→Stop	-	N.D.
67	375	119	T→A	40	V→E	-	-
68	276	122	T→A	41	L→Stop	-	N.D.
69	285	122	T→A	41	L→Stop	-	N.D.
70	290	122	T→A	41	L→Stop	-	N.D.
71	149	125	T→A	42	I→N	-	-
72	211	127	T→C	43	F→L	-	+
73	52	130	C→G	44	L→V	-	+
74	234	131	T→C	44	L→P	+	+
75	258	131	T→C	44	L→P	+	+
76	31	133	G→C	45	A→P	+	+

77	7	137	T→A	46	I→N	-	-
78	34	136	A→T	46	I→F	+	+
79	227	137	T→A	46	I→N	-	-
80	133	140	T→A	47	F→Y	-	+
81	203	140	T→A	47	F→Y	-	+
82	353	140	T→A	47	F→Y	-	+
83	221	143	T→C	48	L→P	-	+
84	95	146	C→T	49	S→L	-	+
85	155	146	C→A	49	S→Stop	-	N.D.
86	160	146	C→T	49	S→L	-	+
87	224	145	T→A	49	S→T	-	+
88	320	146	C→A	49	S→Stop	-	N.D.
89	389	145	T→A	49	S→T	-	+
90	2	148	A→G	50	K→E	-	+
91	106	150	A→T	50	K→N	-	+
92	118	150	A→T	50	K→N	-	+
93	187	148	A→T	50	K→Stop	-	N.D.
94	206	149	A→T	50	K→I	-	+
95	229	150	A→T	50	K→N	-	+
96	259	148	A→C	50	K→Q	-	-
97	293	149	A→T	50	K→I	-	+
98	318	148	A→G	50	K→E	-	+
99	342	150	A→T	50	K→N	-	+
100	378	148	A→T	50	K→Stop	-	N.D.
101	383	150	A→T	50	K→N	-	+
102	396	148	A→T	50	K→Stop	-	N.D.
103	41	152	T→C	51	F→S	-	+
104	83	152	T→C	51	F→S	-	+
105	314	155	C→A	52	T→N	-	-
106	19	158	A→G	53	N→S	-	+
107	59	158	A→G	53	N→S	-	+
108	114	157	A→G	53	N→D	-	+
109	161	157	A→C	53	N→H	-	+
110	165	158	A→G	53	N→S	-	+
111	250	158	A→T	53	N→I	-	-
112	334	159	T→A	53	N→Q	-	-
113	355	159	T→A	53	N→K	-	-
114	370	159	T→A	53	N→Q	-	-
115	85	160	C→T	54	Q→Stop	-	-
116	107	164	T→A	55	L→Stop	-	N.D.
117	306	164	T→A	55	L→Stop	-	N.D.

118	22	167	T→A	56	L→H	-	+
119	55	167	T→A	56	L→H	-	+
120	242	167	T→A	56	L→H	-	+
121	277	167	T→C	56	L→P	-	-
122	296	167	T→C	56	L→P	-	-
123	385	167	T→A	56	L→H	-	+
124	350	170	T→C	57	L→P	-	-
125	108	179	T→C	60	L→P	-	-
126	204	178	C→G	60	L→V	-	-
127	247	179	T→A	60	L→Q	-	-
128	304	179	T→C	60	L→P	-	-
129	372	179	T→A	60	L→Q	-	-
130	216	188	T→A	63	V→E	-	+
131	371	188	T→A	63	V→E	-	+
132	103	197	C→A	66	T→K	-	+
133	215	197	C→G	66	T→R	-	-
134	171	205	A→T	69	T→S	-	-
135	138	211	C→T	71	Q→Stop	-	N.D.
136	104	214	C→T	72	Q→Stop	-	N.D.
137	84	218	T→A	73	L→Stop	-	N.D.
138	191	218	T→A	73	L→Stop	-	N.D.
139	202	218	T→A	73	L→Stop	-	N.D.

§ The number refers to the mutant clone isolate from the selection plates.
* “+”, indicates lysis in liquid culture, “-”, indicates no lysis in liquid culture.
# “+”, indicates expression levels of the *L* allele comparable to WT, “-”, indicates less than WT expression levels.
N.D. Not determined.



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MS2_L    : METRFPQQSQQTPASTNRRRPFKHEDYPCRRQQRSSTLYVLIFLAIFLSKFTNQLLLSLLEAVIRTVTTLQQLLT : MS2_L
GA_L     : -----------------MGLKAKHKENLCSDSERSKRLYVWIALAIVLSDFTSIFSHWIWGLLILYLQTLMDLPT : GA_L
Hgal1_L  : ----MANPRRETLSVHRFIVTKSQAQFSIRIIRCIRNLLAIAGMLFLLSPIYLDKFIQIYLNAYVVPMT------: Hgal1_L
PP7_L    : ----------MSSTLCRWAVKALRCTRVYKEFIWKPLVALSYVTLYLLSSVFLSQLSYPIGSWAV----------: PP7_L
C-1_L    : -MPSLTLRVSTNLPDITVRDAIHKGYLWLRKQVAVATVWLLILTFIGLSSGKLRLWFDLSVTNLSG---------: C-1_L
PRR1_L   : -MCKVSTKVDSKLTESVGQLTIRSYLWLRNILALAGLLFVILLATNHLSIAIYSP--------------------: PRR1_L
AP205_L  : -------------------------------MKKRTKALLPYAVFIILSFQLTLLTALFMYYHYTF---------: AP205_L

         : 1---------11--------21--------31--------41--------51--------61--------71---:
OrigSeq  : METRFPQQSQQTPASTNRRRPFKHEDYPCRRQQRSSTLYVLIFLAIFLSKFTNQLLLSLLEAVIRTVTTLQQLLT : OrigSeq

Jnet     : ---------------------HH----HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH--: Jnet
jhmm     : -------------------HHHH---HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH--: jhmm
jpssm    : ------------------------------------HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH--: jpssm

Lupas 14 : ---------------------------------------------------------------------------: Lupas 14
Lupas 21 : ---------------------------------------------------------------------------: Lupas 21
Lupas 28 : ---------------------------------------------------------------------------: Lupas 28

Jnet_25  : ----B--------------B-B----BBB----B---BBBBB-BBB-BB-BBB-BBBBB-BBBB-BB-BB--B--: Jnet_25
Jnet_5   : ---------------------------------------B-B---B-----------------B-BB--B-----: Jnet_5
Jnet_0   : ---------------------------------------B-----------------------------------: Jnet_0
Jnet Rel : 888667777777777777631004660111122223256899999999999999999999999999999987289: Jnet Rel

Key:
Colour code for alignment:
Blue        -Complete identity at a position
Shades of red   -The more red a position is, the higher the level of
conservation of chemical properties of the amino acids
Jnet            -Final secondary structure prediction for query
jalign    -Jnet alignment prediction
jhmm            -Jnet hmm profile prediction
jpssm           -Jnet PSIBLAST pssm profile prediction

Lupas           -Lupas Coil prediction (window size of 14, 21 and 28)

Note on coiled coil predictions  -= less than 50% probability
c = between 50% and 90% probability
C = greater than 90% probability

Jnet_25         -Jnet prediction of burial, less than 25% solvent accesibility
Jnet_5          -Jnet prediction of burial, less than 5% exposure
Jnet_0          -Jnet prediction of burial, 0% exposure
Jnet Rel        -Jnet reliability of prediction accuracy, ranges from 0 to 9, bigger is bette
```

Fig. S1. Secondary structure analysis of L and heterologous L sequences.
A representative from each of the L sub-groups were chosen and aligned with respect to the LS motif (highlighted in yellow). The aligned sequences were then used as input for secondary structure prediction on Jpred 4 server [26].