

Supplementary Table 1. The mass, yield and retention time for 27 lysine mutants of kalata B1.

Twenty-three mutants with single point mutation are sorted by loops. Three mutants with double lysine and one mutant with triple lysine mutation are listed at the bottom. The yield and retention time of the P24K mutant in loop 5 was not determined as the linear precursor of this mutant was insoluble in the standard folding buffer.

	Mutant	Mass (Da)	% Yield	Retention time^a (min)
Wild-type	Kalata B1	2892.3	N/A	43.7
Loop 1	G6K	2963.4	9.1	40.6
	E7K	2891.4	8.6	41.9
	T8K	2919.4	14.8	33.5
Loop 2	V10K	2921.3	22.3	35.9
	G11K	2963.4	27.3	40.8
	G12K	2963.4	14.9	40.9
	T13K	2919.4	16.7	42.3
Loop 3	N15K	2906.4	35.7	42.1
	T16K	2919.4	27.4	41.7
	P17K	2923.4	15.2	42.7
	G18K	2963.4	22.2	42.9
Loop 4	T20K	2919.4	38.4	42.7
Loop 5	S22K	2933.4	27.2	40.5
	W23K	2834.3	22.9	34.1
	P24K	2923.4	N/D	N/D
	V25K	2921.3	28.8	30.8
Loop 6	T27K	2919.4	31.2	37.8
	R28K	2864.3	32.9	43.5
	N29K	2906.4	29.2	42.3
	G1K	2963.4	23.1	41.6
	L2K	2907.3	35.4	37.9
	P3K	2923.4	20.5	38.1
	V4K	2921.3	10.9	39.2
Double-lysine	T20K/S22K	2960.5	28.7	39.5
	T20K/N29K	2933.4	21.2	41.7
	T20K/G1K	2990.5	20.8	40.8
Triple-lysine	T20K/N29K/G1K	3004.6	15.9	39.7

^aThe retention time of peptides was assessed using analytical HPLC with a 1%/min gradient of solvent B against solvent A starting from 5% solvent B.

Supplementary Table 2. Chemical shifts of α -proton signal for the 26 lysine mutants of kalata B1.

Residue	kalata B1	G6K	E7K	T8K	V10K	G11K	G12K	T13K	N15K	T16K	P17K	G18K	T20K	S22K	W23K	V25K	T27K	R28K	N29K	G1K	L2K	P3K	V4K	T20K/S22K	T20K/N29K	T20K/G1K	T20K/N29K/G1K
C5	4.45	4.33	4.48	4.40	4.37	4.39	4.38	4.40	4.40	4.33	4.41	4.38	4.40	4.40	4.39	4.41	4.40	4.43	4.39	4.41	4.42	4.46	4.49	4.39	4.38	4.41	4.38
G6	3.77	3.70	3.81	3.72	3.69	3.72	3.72	3.73	3.70	3.71	3.73	3.70	3.67	3.75	3.72	3.74	3.75	3.73	3.72	3.73	3.73	3.76	3.73	3.72	3.72	3.73	3.72
E7	4.75	4.61	4.68	4.60	4.69	4.72	4.71	4.73	4.72	4.74	4.71	4.70	4.71	4.73	4.73	4.71	4.74	4.73	4.71	4.72	4.72	4.69	4.70	4.70	4.70	4.72	4.68
T8	4.55	4.52	4.54	4.47	4.48	4.51	4.51	4.50	4.51	4.51	4.51	4.49	4.51	4.53	4.64	4.51	4.54	4.51	4.50	4.51	4.51	4.51	4.49	4.52	4.50	4.51	4.50
C9	5.03	4.86	4.70	4.67	4.76	4.86	4.72	4.69	4.84	4.86	4.94	4.89	4.90	4.97	4.92	4.76	4.86	4.89	4.92	4.93	4.88	4.92	4.89	4.87	4.86	4.88	4.88
V10	3.81	3.83	3.96	3.97	4.18	3.81	4.12	3.96	3.89	3.86	3.81	3.79	3.82	3.81	3.87	3.96	3.88	3.81	3.81	3.82	3.84	3.86	3.80	3.85	3.83	3.85	3.83
G11	4.07	3.98	3.97	3.94	3.95	4.35	3.87	3.94	3.97	3.99	4.02	3.99	4.00	4.03	3.91	3.96	3.99	4.00	4.01	4.02	4.00	4.01	3.98	3.98	3.99	3.99	3.98
G12	4.26	4.17	4.13	4.11	4.13	4.11	4.65	4.06	4.14	4.16	4.21	4.18	4.18	4.20	4.03	4.12	4.16	4.18	4.19	4.20	4.19	4.20	4.18	4.16	4.16	4.18	4.17
T13	4.71	4.67	4.72	4.69	4.65	4.63	4.52	4.67	4.68	4.68	4.67	4.65	4.65	4.69	4.58	4.67	4.69	4.66	4.67	4.67	4.67	4.69	4.65	4.66	4.64	4.66	4.64
C14	4.70	4.67	4.69	4.74	4.66	4.63	4.56	4.67	4.68	4.64	4.66	4.64	4.65	4.64	4.64	4.69	4.68	4.66	4.66	4.67	4.68	4.68	4.65	4.67	4.67	4.68	4.67
N15	4.72	4.64	4.66	4.64	4.64	4.66	4.67	4.62	4.18	4.76	4.65	4.64	4.66	4.66	4.58	4.63	4.64	4.66	4.66	4.67	4.65	4.68	4.64	4.61	4.63	4.64	4.63
T16	4.30	4.40	4.63	4.38	4.31	4.41	4.40	4.43	4.21	4.51	4.18	4.37	4.34	4.28	4.35	4.43	4.47	4.39	4.32	4.33	4.42	4.33	4.33	4.37	4.37	4.39	4.34
P17	4.26	4.20	4.22	4.20	4.18	4.20	4.19	4.21	4.19	4.22	3.97	4.18	4.21	4.20	4.20	4.21	4.22	4.20	4.21	4.21	4.22	4.22	4.20	4.20	4.20	4.20	4.19
G18	3.94	3.88	3.90	3.87	3.86	3.88	3.87	3.90	3.89	3.87	3.91	3.78	3.88	3.90	3.87	3.88	3.90	3.87	3.90	3.90	3.89	3.91	3.88	3.90	3.88	3.90	3.88
C19	5.37	5.23	5.33	5.27	5.27	5.29	5.29	5.26	5.26	5.22	5.31	5.27	5.23	5.35	5.24	5.22	5.09	5.26	5.31	5.29	5.28	5.25	5.31	5.23	5.23	5.19	5.18
T20	4.53	4.47	4.51	4.47	4.44	4.48	4.24	4.22	4.44	4.49	4.48	4.46	4.52	4.50	4.42	4.47	4.51	4.47	4.48	4.49	4.48	4.51	4.45	4.52	4.51	4.52	4.51
C21	4.59	4.50	4.58	4.41	4.45	4.52	4.51	4.46	4.50	4.50	4.54	4.52	4.52	4.53	4.55	4.57	4.45	4.54	4.52	4.52	4.53	4.57	4.52	4.50	4.52	4.51	4.50
S22	4.79	4.69	4.71	4.69	4.68	4.71	4.73	4.69	4.69	4.72	4.74	4.71	4.71	4.58	4.64	4.69	4.72	4.71	4.72	4.74	4.72	4.73	4.68	4.54	4.69	4.71	4.69
W23	4.08	4.04	4.06	4.06	4.05	4.00	4.12	4.05	4.04	4.02	4.05	4.02	4.04	4.00	3.96	4.08	3.97	4.04	4.04	4.04	4.04	4.08	4.07	4.08	4.03	4.04	4.03
P24	3.46	3.40	3.34	3.31	3.39	3.35	3.24	3.27	3.39	3.37	3.42	3.38	3.41	3.44	4.52	3.39	3.45	3.40	3.41	3.41	3.40	3.42	3.39	3.43	3.39	3.40	3.39
V25	4.21	4.16	4.19	4.29	4.15	4.17	4.19	4.19	4.18	4.15	4.17	4.15	4.17	4.21	4.34	4.42	4.14	4.17	4.17	4.18	4.18	4.26	4.14	4.19	4.17	4.17	4.18
C26	5.07	5.05	5.07	5.00	5.01	5.05	5.10	5.05	5.03	5.07	5.03	5.01	5.07	4.98	5.06	5.15	4.88	5.04	5.02	5.02	5.05	5.11	5.02	4.98	5.06	5.05	5.02
T27	5.08	4.98	4.98	4.99	5.02	5.01	4.99	5.01	5.00	4.99	5.03	5.01	5.01	5.09	5.01	4.99	4.98	5.01	5.01	5.03	4.99	5.04	4.93	5.05	5.01	5.01	4.99
R28	4.75	4.69	4.76	4.69	4.74	4.69	4.69	4.69	4.69	4.73	4.70	4.71	4.70	4.70	4.69	4.71	4.75	4.67	4.72	4.68	4.71	4.79	4.70	4.71	4.69	4.69	4.67
N29	4.41	4.44	4.46	4.44	4.52	4.44	4.42	4.43	4.34	4.35	4.36	4.34	4.33	4.38	4.32	4.36	4.34	4.34	3.87	4.34	4.37	4.38	4.36	4.33	3.84	4.31	3.84
G1	3.92	3.85	3.87	3.85	3.77	3.85	3.85	3.85	3.84	3.86	3.87	3.85	3.87	3.88	3.87	3.87	3.85	3.86	3.86	3.57	3.89	3.88	3.86	3.86	3.84	3.58	3.59
L2	5.08	4.99	4.98	4.99	4.91	5.01	4.99	5.00	5.00	5.01	5.03	5.01	5.02	5.02	5.01	4.99	5.09	5.01	5.01	5.01	4.99	4.61	4.94	5.00	4.99	4.98	4.95
P3	5.09	4.98	4.97	4.99	4.99	5.01	5.00	5.00	5.01	5.01	5.04	5.01	5.03	5.08	5.04	5.11	5.11	5.04	5.01	5.01	5.03	4.52	4.94	5.04	4.99	4.99	4.95
V4	4.68	4.55	4.59	4.59	4.58	4.61	4.60	4.61	4.61	4.61	4.63	4.58	4.62	4.64	4.63	4.62	4.64	4.63	4.61	4.62	4.63	4.55	4.65	4.63	4.61	4.62	4.58

Supplementary Figure 1. Effects of the 22 correctly folded lysine mutants of kalata B1 on the development of eggs to third instar (L3) larvae of *T. columbriformis*. Each dose was conducted in triplicate and the error bars represent the standard deviation per treatment.

