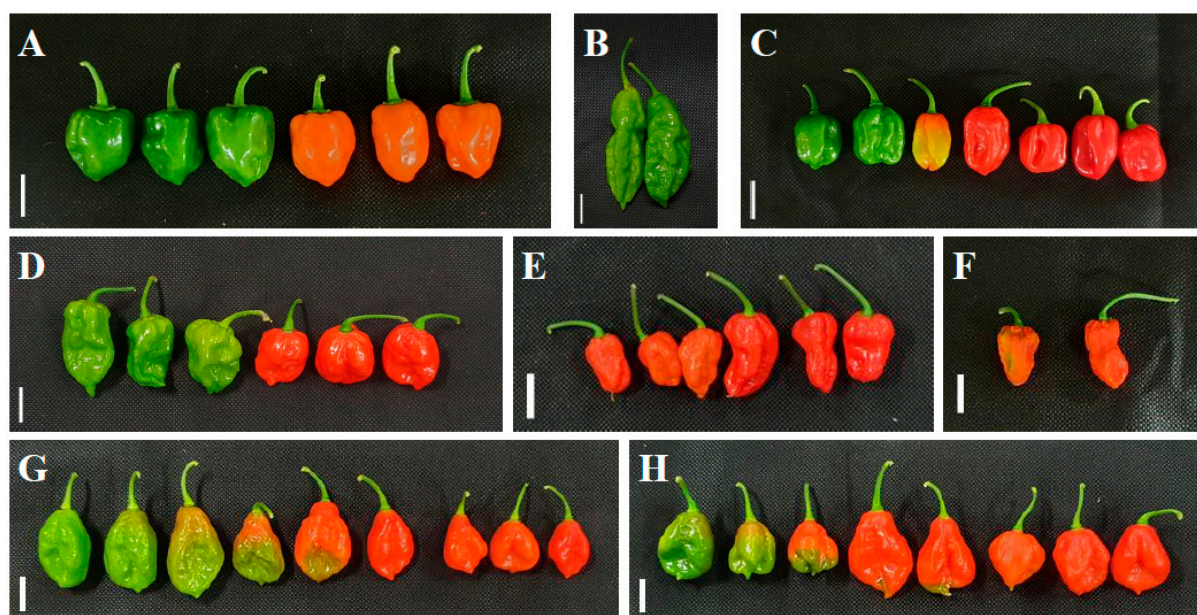
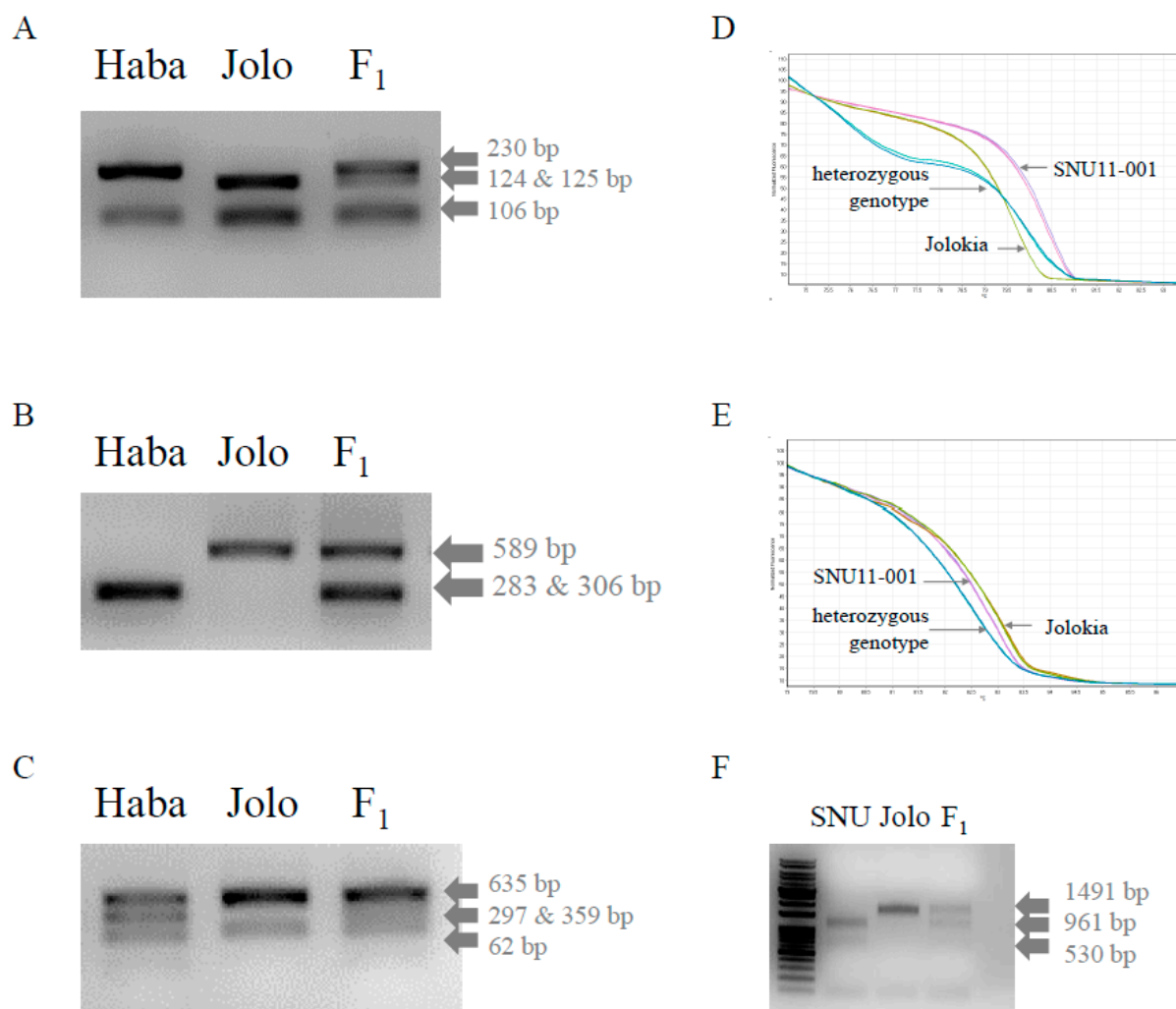


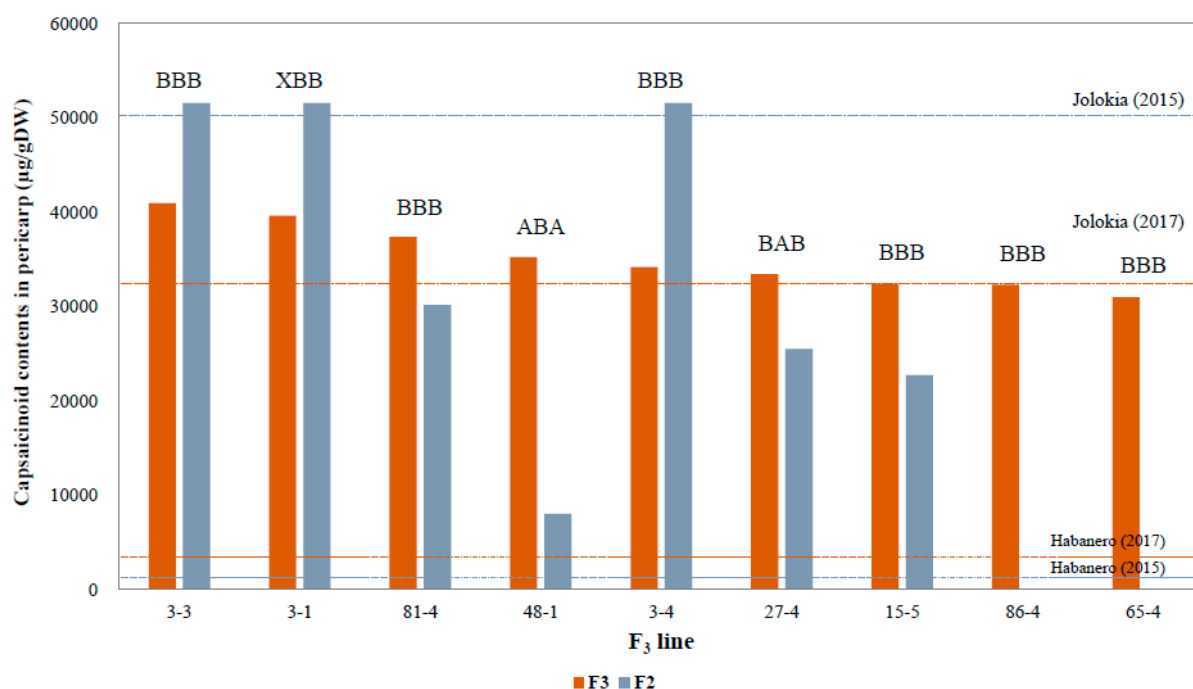
Supplementary Data



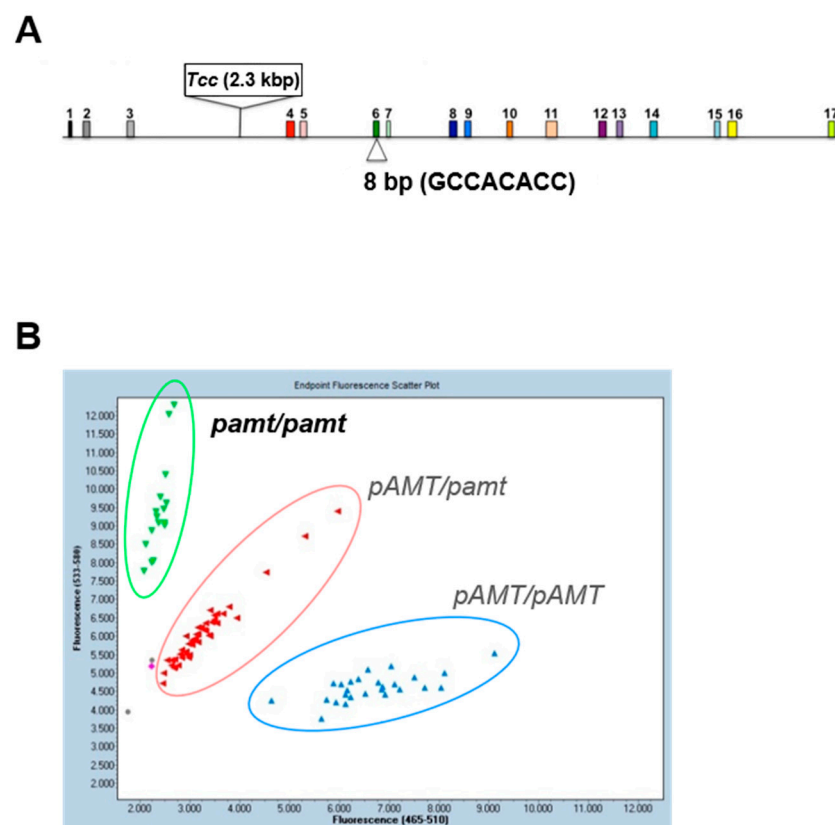
Supplementary Figure S1. Fruits of parental lines and selected lines. Parental lines were A. 'HB', B. 'BJ', and C. 'SNU11-001'. Three pungent lines, D. HJ-3-4-1-3-1, E. HJ-3-1-3-1-2, and F. HJ-3-1-3-1-1, were derived from 'HB' and 'BJ'. Two lines containing high level of capsinoids, G. SJ-103-4-3-3-4 and H. SJ-103-4-3-3-5, were derived from 'SNU11-001' and 'BJ'. White bar is 2 cm.



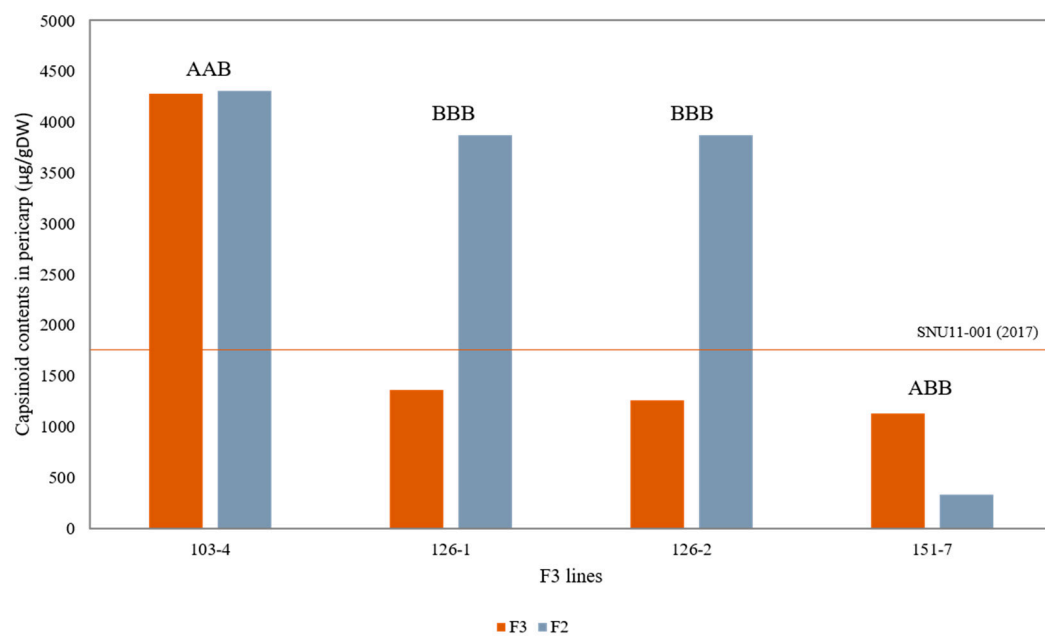
Supplementary Figure S2. Development of QTL markers to select pungent peppers with high capsaicinoid contents in the pericarp. Three CAPS markers, A. HJ16_CAP6.3_qtlseq_caps, B. HJ16_CAP6.4_caps and C. HJ17_CAP11_caps could distinguish 'HB' and 'BJ' genotypes. Two HRM markers, D. SJ_TCP4.1_hrm and E. SJ_TCP6.2_hrm, and a CAPS marker F. SJ_TCP_6.1_caps could distinguish 'SNU11-001' and 'BJ' genotype.



Supplementary Figure S3. Capsaicinoid contents of the pericarp in ‘HJ’ F₂ and F₃ lines. The genotypes of the F₃ lines at three capsaicinoid QTLs are indicated above each bar; the order of the three markers is HJ16_CAP6.3_qtlseq_caps, HJ17_CAP11_caps, and HJ16_CAP6.4_caps, with ‘A’ and ‘B’ indicating ‘HB’ and ‘BJ’ alleles, respectively. QTL genotype of HJ F₂ 3 was XBB while HJ F₃ 3-1’s genotype was not detected. ‘X’ means not determined. The dotted lines indicate the ‘HB’ and ‘BJ’ capsaicinoid contents in two years (2015 and 2017).



Supplementary Figure S4. *pAMT* marker for distinguishing the *pamt* mutant from the wild-type allele. A. *pamt* mutant gene of ‘SNU11-001’ has a 2.3 kbp transposon of *C. chinense* (*Tcc*) in the third intron and an eight bp insertion in sixth exon. B. *pAMT* genotype was analyzed using KASP based on the eight bp insertion. *pAMT* mutant allele, *pAMT* normal allele and *pAMT* heterozygous genotype were plotted no y-axes, x-axes and diagonal position, respectively.



Supplementary Figure S5. Capsinoid contents of the pericarp in selected 'SJ' F₂ and F₃ plants. The genotypes of the F₂ lines at three capsinoid QTLs are indicated above each bar; the order of the three markers is SJ_TCP4.1_hrm, SJ_TCP6.2_hrm, and SJ_TCP_6.1_caps, with 'A' and 'B' indicating 'SNU11-001' and 'BJ' alleles, respectively. The line indicates the 'SNU11-001' capsinoid contents in 2017.

Table S1. Capsaicinoid contents and QTL genotypes of the 'HJ' F₂ and F₃ lines.

line no.	capsaicinoid content in pericarp (µg/gDW)		QTL marker genotype		
	F ₂	F ₃	HJ6.3_qtlseq_3	HJ11_1	peri_chr6_4
3-3	51,563	40,941	B	B	B
3-1	51,563	39,630	n.d.	n.d.	n.d.
81-4	30,153	37,385	B	B	B
48-1	8,026	35,247	A	B	A
3-4	51,563	34,185	B	B	B
27-4	25,511	33,449	B	A	B
15-5	22,731	32,425	B	B	B
86-4	n.d.	32,314	B	B	B
65-4	n.d.	30,986	B	B	B

Table S2. Capsaicinoid contents per fruit in 'HJ' F₅ lines.

line no.	capsaicinoid contents per fruit (µg/gDW)
F ₅ 3-1-3-1	70,526
F ₅ 3-3-2-2	n.d.
F ₅ 3-3-2-3	46,608
F ₅ 3-4-1-1	57,299
F ₅ 3-4-1-2	48,186
F ₅ 3-4-1-3	53,702
F ₅ 3-4-2-1	55,339
F ₅ 3-4-2-2	35,652
F ₅ 3-4-2-3	36,858
F ₅ 3-4-4-1	48,531
F ₅ 3-4-4-2	39,738

Table S3. Capsinoid contents of the placenta, pericarp and fruit in the 'HJ' F₆ and F₇ lines.

line no.	Capsinoids(ug/gDW)		PL/PE contents ratio	avg. amounts of fruit (ug)
	Placenta	Pericarp		
F ₆ 3-4-1-3-1	155,818	51,880	3	57,369
F ₆ 3-1-3-1-1	141,113	57,648	2	64,127
F ₆ 3-3-2-2-3	130,766	45,476	3	51,598
F ₆ 3-3-2-3-5	147,180	55,208	3	60,627
F ₆ 3-1-3-1-3	155,805	42,853	4	54,665
F ₆ 3-1-3-1-2	179,812	45,790	4	59,177
F ₇ 3-1-3-1-2	171,635	38,844	4	23,123
F ₇ 3-4-1-3-1	155,352	27,956		25,533
F ₇ 3-1-3-1-1	142,245	36,135	4	19,654
F ₇ 3-4-2-1-3	122,163	40,084	3	31,443
F ₇ 3-3-2-2-1	117,099	21,844	5	16,635
F ₇ 3-3-2-3-1	106,031	25,445	4	18,473

Table S4. Capsinoid contents and QTL genotypes of the 'SJ' F₂ *pAMT* mutant lines.

F ₂ plant	Capsinoids(μg/gDW)		PL/PE contents ratio	avg. amounts of fruit (μg)	QTL marker genotype		
	Placenta	Pericarp			SJ_TCP4.1_hrm	SJ_TCP6.2_hrm	SJ_TCP6.1_caps
131	n.a.	355	n.a.	n.a.	A	B	B
47	64,492	3,880	17	4,227	A	B	B
2	60,730	4,268	14	3,732	B	B	B
70	53,288	11,744	5	6,445	A	B	B
126	50,404	3,868	13	3,164	B	B	B
22	49,966	871	57	2,359	A	B	B
148	45,842	1,149	40	1,882	A	A	B
145	42,287	1,877	23	1,431	A	B	B
149	39,963	977	41	2,056	B	A	B
104	37,542	1,364	28	1,198	A	B	A
117	36,395	1,703	21	2,289	A	B	A
74	35,420	781	45	981	A	A	B
33	34,971	2,011	17	2,229	A	A	B
169	34,020	581	59	285	A	A	B
12	30,884	1,276	24	1,489	A	B	B
24	29,071	4,681	6	2,077	A	B	B
151	26,797	327	82	1,003	A	B	B
38	26,557	834	32	732	B	B	B
103	25,805	4,309	6	1,981	A	A	B
216	25,061	423	59	1,361	A	B	B
96	24,454	1,938	13	1,754	A	B	B
119	23,887	1,042	23	1,047	A	A	B
144	23,080	4,365	5	1,780	B	B	A
43	22,571	1,842	12	1,206	A	A	B
158	19,992	1,601	12	1,285	A	B	B
110	19,597	1,253	16	680	A	B	B
4	19,268	5,630	3	1,583	A	B	B
10	18,360	9,985	2	3,175	B	B	B
176	16,327	325	50	329	A	A	B
121	15,905	1,618	10	2,565	B	A	B
41	14,319	7,621	2	2,536	B	B	B
94	14,275	11,056	1	2,185	B	B	B
81	13,345	2,478	5	488	A	B	B
184	12,944	2,400	5	1,168	A	A	A
69	12,063	985	12	620	A	B	B
165	11,901	738	16	516	A	B	B
220	11,626	635	18	n.a.	B	B	B
174	8,822	391	23	384	A	B	B
226	8,798	171	52	271	B	A	A
224	8,738	179	49	1,300	A	B	B
212	8,078	200	40	364	A	B	B
182	6,259	260	24	157	A	A	A
50	5,239	1,105	5	491	A	B	B
183	5,232	286	18	161	A	B	B
210	5,138	698	7	300	A	B	A
199	3,704	435	9	59	B	B	B
218	688	623	1	234	A	A	B

Table S5. Capsinoid contents of the 'SJ' F₂ and F₃ lines.

F ₃ line	capsinoid contents in pericarp (µg/gDW)	Capsinoid contents of F ₂ (µg/gDW)	
		pericarp	placenta
103-4	4,279	4,309	25,805
126-1	1,366	3,868	50,404
126-2	1,262	3,868	50,404
151-7	1,134	327	26,797

Table S6. Capsinoid contents of the placenta, pericarp and fruit in the 'SJ' F₆ and F₇ lines.

line no.	Capsinoids(µg/gDW)		PL/PE contents ratio	avg. amounts of fruit (ug)
	Placenta	Pericarp		
F ₆ 151-1-4-3-3	10,827	2,392	5	2,166
F ₆ 103-4-3-3-4	10,382	3,443	3	1,606
F ₆ 103-4-3-3-5	6,697	3,532	2	3,639
F ₇ 103-4-3-3-4	84,325	14,405	6	8,307
F ₇ 103-4-3-3-5	82,197	18,503	4	10,666
F ₇ 151-1-4-3-3	33,179	14,338	2	6,066

Table S7. SNP information for the 'HJ' and 'SJ' QTL markers.

Population	Marker	Chr.	Physical location in <i>C. chinense</i> v. 1.2	SNP (‘Habanero’ or ‘SNU11-001’/‘Jolokia’)
‘Habanero’ × ‘Jolokia’	HJ16_CAP6.3_qtlseq_caps	6	223,429,166	C/T
	HJ16_CAP6.4_caps	6	226,585,896	A/G
	HJ17_CAP11_caps	11	38,833,824	A/G
‘SNU11-001’ × ‘Jolokia’	SJ_TCP4.1_hrm	4	210,030,398	G/A
	SJ_TCP6.2_hrm	6	233,547,059	A/G
	SJ_TCP6.1_caps	6	231,953,335	A/G

Table S8. Segregation of *pAMT* alleles in the 'SJ' F₂ population.

Generation	Number of plants				Expected ratio	χ^2	P-value
	Total	<i>pAMT/pAMT</i>	<i>pAMT/pamt</i>	<i>pamt/pamt</i>			
F ₂	173	41	85	47	1:2:1	0.468	0.791

Table S9. KASP mixture components for the *pAMT* genotype analysis.

Components	Quantity (µL)
DNA (10 ng/µL)	5.0
KASP 2X Master mix	5.0
KASP Assay mix (primer mix)	0.14
MgCl ₂	0.06
Total amount	10.20

Table S10. KASP PCR conditions for the *pAMT* genotype analysis.

Step	Temperature	Time	Number of cycles
Hot-start activation	94°C	15 minutes	1
Denaturing	94°C	20 seconds	10
Annealing / Elongation	57–55°C (dropping 0.2°C per cycle)	1 minute	
Denaturing	94°C	20 seconds	
Annealing / Elongation	55°C	1 minute	26
Denaturing	94°C	20 seconds	3
Further cycling Annealing / Elongation	57°C	1 minute	

