Supporting information

Goulet et al. 10.1073/pnas.1216515109

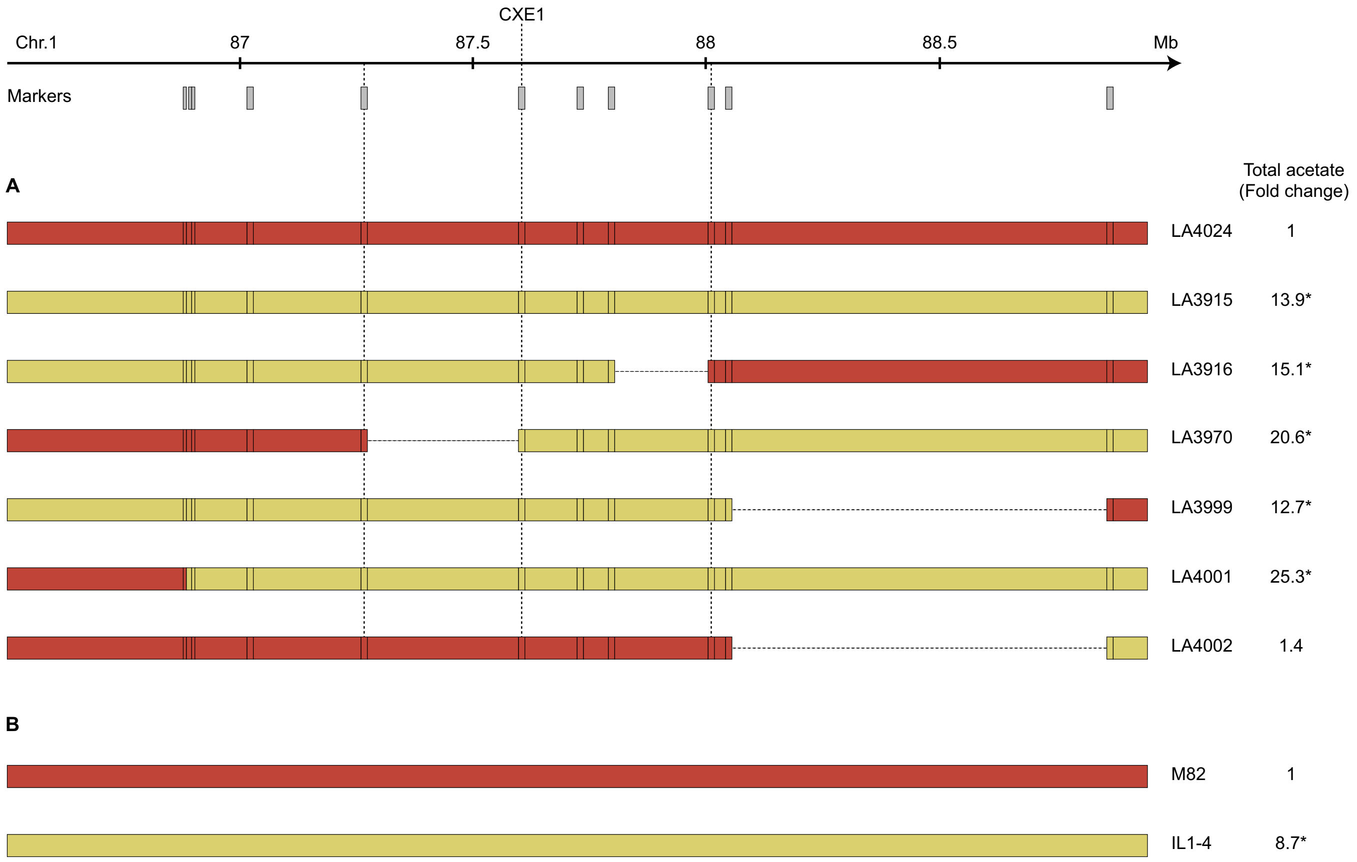

Fig. S1. Mapping of the quantitative trait loci on chromosome 1 for acetate esters. Genotypes of several introgression lines derived from the green-fruited parent *Solanum habrochaites* (*A*) or *S. pennellii* (*B*). Regions from the tomato parent are in red and those from the green-fruited species are in green. All of the lines that have the genotype of a green-fruited species at the *CXE1* position accumulate a high amount of acetate esters in the fruit. In addition to the illustrated lines, LA3929 (16.9*-fold more acetate esters), LA3995 (9.3*-fold more), and LA3998 (11.1*-fold more) from the *S. habrochaites* population also have the green-fruited genotype in the *CXE1* region. *$P < 0.05$.

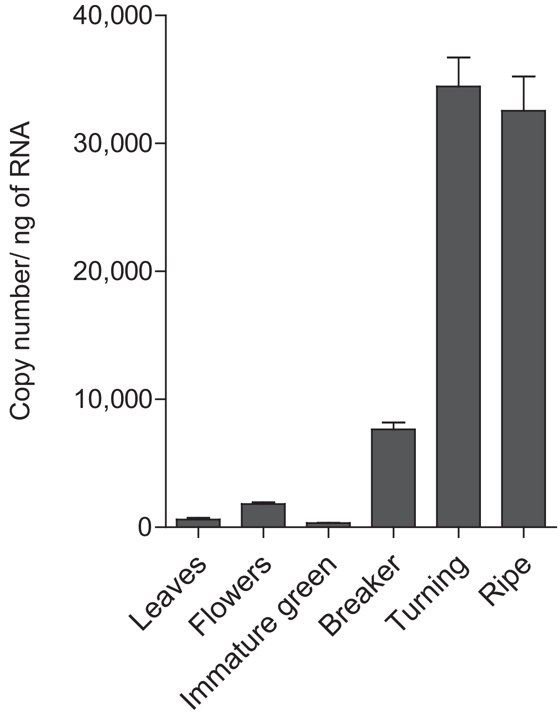

Fig. S2. Expression of *SlCXE1* in *Solanum lycopersicum* cv. M82. Transcript levels (±SE) were measured in leaves, flowers, and four stages of fruit development (immature green, breaker, turning, and ripe).

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SlCXE1/1-339    MAEIVHDFFPLMRVYKDGRIERLAGEGFVPPESDPETGVQIKDVQIDPQINLSARLYLPK
SlCXE2/1-309    MAEIVHDFFPLMRVYKDGRIERLAGEGFVPPESDPETGLQIKDIEIDPQINLSARLYLPK
SlCXE3/1-312    MAEIEHDFFPLMRVHKDGRIERLAGEVFVPPESDPETGVQIKDVQIDPQINLSARLYLPK
SlCXE4/1-310    MAEIVYDFFPFMRVYKDGRIERLMGEGFVPPESDPETGVQIKDVQIDPQINLSARLYLPK
SlCXE5/1-312    MAEIEHDLSPLIRVYRDGRIERMMGEGFVPPESDPETGVQIKDIEIDPQINLSARLYLPK

SlCXE1/1-339    NVDPVQKIPLFVYFHGGGFVIESAFSPTYHKYLSLVAAEAKVAIVSVNYRLAPEYPLPIA
SlCXE2/1-309    N---VQKIPLFVYFHGGGFVIESASSPSYHKHLSTVAAEAKVVIVSVNYRLAPEYPLPIA
SlCXE3/1-312    NVDPVQKIPLFVYFHGGGFVIESAFSPTYHKYLSLVAAKTKVAIISVNYRLAPEYPLPIA
SlCXE4/1-310    HVDTVEKIPLFVYFHGGGFLIESAYSPSYHKHISKVAAEAKVVIVSVNYRLAPEYLLPIA
SlCXE5/1-312    NVDPVQKIPLFVYFHGGAFVIESASSPTYHKHLSMVAAEAKVIIVSINYRLAPEYPLPIA

SlCXE1/1-339    YEDSWLALKWVTSHANG-DGREPWLKDYADFNRVFLGGDSAGGNIAHHIGIRLGLEKFEG
SlCXE2/1-309    YEDSWLALKWIASHANG-DGHEPWLKEHADFSRVYFGGDSAGGNIAHHIAIRVGLEKLDG
SlCXE3/1-312    YEDSWLALKWVTSHANG-DGREPWLKDNADFNRVYLGGDSAGGNIAHHIAIRVGLEKLDR
SlCXE4/1-310    YEDSWLALKWVASHANGDDGHEPWLKDHADFNRLFLGGDSAGGNIAHHIAIRVGLEKLDG
SlCXE5/1-312    YEDSWIALKWIASHANG-DGDEPWLKDHANFNRVYFGGDSAGGNIAHHMAIRVGLEKLEG

SlCXE1/1-339    VKIDGIFLACPYFWGKDRIEGEGENLLAKDFVEDLVLIGNPNSTGLDKDPIDLGSKDLFE
SlCXE2/1-309    VKLEGIFLACPFFWGKDPIDGEGENLGAKDFV---------------------------E
SlCXE3/1-312    VKIDGIFLACPSFWGKDPIDGEGEIVGAKDFV---------------------------E
SlCXE4/1-310    MKLKGIFLACPFFWGKDPIDGEGEKLVVI------------------------------E
SlCXE5/1-312    VKLDGIFLACPYFWGKDLIDGEGENLFVKDFI---------------------------D

SlCXE1/1-339    KLWLFVNPTSSGLDDPLINPEKDPKLSGLGCDKLVVYVAGKDPLRFRGFYYKEVLEKSGW
SlCXE2/1-309    KLWLFAYPNSLGLDDPLINPEKDPNLSSLGCDKVVVYVAGKDPLRFRGFYYKEALEKSGW
SlCXE3/1-312    KLWLFANPNSSGLDDPLINPEKDPNLSRLGCDKVVVYVAGKDLLRFRGLYYKEVLEKSGW
SlCXE4/1-310    KLWLFVNPNSSGLDDPLINPVKDPNLSSLGCDKVVVYVAGKDVLRFRGLYYKEVLEKSGW
SlCXE5/1-312    KLWLFANPNGSGLDDPMINPEKDPKLCSLGCEKVVVYVAGKDALRLRGLYYKEALEKSGW

SlCXE1/1-339    PGTVEVVEVKGKGHVFHLFVPEAEEAIAMLKKLASFLNQS
SlCXE2/1-309    PGTVEVVEVKDEEHVFHLFATETENAMSMMKKLVSFLNQS
SlCXE3/1-312    PGTVEVVEVKDEEHVFHLTATETENAMAMMKKLVSFLNQS
SlCXE4/1-310    PGTVEVVEVKDEAHVFYLSATETENAMAMMKKLASFLNQS
SlCXE5/1-312    PGTVEVVEVKDEEHVFHLFAPEAENAMAMLKKLVSFLNQS
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Fig. S3. Protein alignment of the five tandem esterases in *S. lycopersicum*. The unique sequence within SlCXE1 is underlined.

S. tuberosum MAEIVHDFFPLMRVYKDGRIERLAGEVFVPPESDPETGVQIKDVEIDPQINLSARLYLPK
S. pennellii MAEILHDFFPLMRVNKDGRIERLAGEGFVPSESDPETGVQIKDVQIDPQINLSARLYLPK
S. habrochaites MAEIIHDFFPLLRVYKDGRIERLAGEGFVPPESDPETGVQIKDVQIDPQINLSARLYLPK
S. pimpinellifolium MAEIVHDFFPLMRVYKDGRIERLAGEGFVPPESDPETGVQIKDVQIDPQINLSARLYLPK
S. lycopersicum MAEIVHDFFPLMRVYKDGRIERLAGEGFVPPESDPETGVQIKDVQIDPQINLSARLYLPK
S. cheesmaniae MAEIVHDFFPLMRVYKDGRIERLAGEGFVPPESDPETGVQIKDVQIDPQINLSARLYLPK
S. galapagense MAEIVHDFFPLMRVYKDGRIERLAGEGFVPPESDPETGVQIKDVQIDPQINLSARLYLPK
S. chmielewskii MAEIIHDFFPLMRVYKDGRIERLAGEGFVPTESDPETGVQIKDVQIDPQINLSARLYLPK
S. neorickii MAEIIHDFFPLMRVYKDGRIERLAGEGFVPPESDPETGVQIKDVQIDPQINLSARLYLPK

S. tuberosum NVDPVQKIPLFVYFHGGGFVIESAFSPTYHKYLSLVAAEAKVAIVSVNYRLAPEYPLPIA
S. pennellii NVDPVQKIPLFVYFHGGGFVIESAFSPTYHKYLSLVAAEAKVAIVSANYRLAPEYPLPIA
S. habrochaites NVDPVQKIPLFVYFHGGGFVIESAFSPTYHKYLSLVAAEAKVAIVSVNYRLAPEYPLPIA
S. pimpinellifolium NVDPVQKIPLFVYFHGGGFVIESAFSPTYHKYLGLVAAEAKVAIVSVNYRLAPEYPLPIA
S. lycopersicum NVDPVQKIPLFVYFHGGGFVIESAFSPTYHKYLSLVAAEAKVAIVSVNYRLAPEYPLPIA
S. cheesmaniae NVDPVQKIPLFVYFHGGGFVIESAFSPTYHKYLSLVAAEAKVAIVSVNYRLAPEYPLPIA
S. galapagense NVDPVQKIPLFVYFHGGGFVIESAFSPTYHKYLSLVAAEAKVAIVSVNYRLAPEYPLPIA
S. chmielewskii NVDPVQKIPLFVYFHGGGFVIESAFSPTYHKYLSLVAAEAKVAIVSVNYRLAPEYPLPIA
S. neorickii NVDPVQKIPLFVYFHGGGFVIESAFSPTYHKYLSLVAAEAKVAIVSVTTG----------

S. tuberosum HEDSWLALKWVTSHANGDGREPWLKDNADFNLVYLGGDSAGGNIAHHIAIRFGLEKLEGV
S. pennellii YEDSWLALKWVTSHANGDGREPWLKDYADFNRVFLGGDSAGGNIAHHIGIRLGLEKFEGV
S. habrochaites YEDSWLALKWVTSHANGDGREPWLKDYADFNRVFLGGDSAGGNIAHHIGIRLGLEKFEGV
S. pimpinellifolium YEDSWLALKWVTSHANGDGREPWLKDYADFNRVFLGGDSAGGNIAHHIGIRLGLEKFEGV
S. lycopersicum YEDSWLALKWVTSHANGDGREPWLKDYADFNRVFLGGDSAGGNIAHHIGIRLGLEKFEGV
S. cheesmaniae YEDSWLALKWVTSHANGDGREPWLKDYADFNRVFLGGDSAGGNIAHHIGIRLGLEKFEGV
S. galapagense YEDSWLALKWVTSHANGDGREPWLKDYADFNRVFLGGDSAGGNIAHHIGIRLGLEKFEGV
S. chmielewskii YEDSWLALKWVTSHANGDGREPWLKDYADFNRVFLGGDSAGGNVAHHIGIRLGLEKFEGV
S. neorickii --

S. tuberosum KIDGIFLACPFFWGKDPIDGEGENLLAKDFV**EDLVLSGNPNSTGLDKDPIDGEGENLGAK**
S. pennellii KIDGIFLACPYFWGKDRIEGEGENLLAKDFV**EDLVLVGNPNSTGLDKDPID**-----**LGSK**
S. habrochaites KIDGIFLACPYFWGKDRIEGEGENLLAKDFV**EDLVLIGNPNSTGLDKDPID**-----**LGSK**
S. pimpinellifolium KIDGIFLACPYFWGKDRIEGEGENLLAKDFG**EDHVLIGNPNSTGLDKDPID**-----**LGSK**
S. lycopersicum KIDGIFLACPYFWGKDRIEGEGENLLAKDFV**EDLVLIGNPNSTGLDKDPID**-----**LGSK**
S. cheesmaniae KIDGIFLACPYFWGKDRIEGEGENLLAKDFV**EDLVLIGNPNSTGLDKDPID**-----**LGSK**
S. galapagense KIDGIFLACPYFWGKDRIEGEGENLLAKDFV**EDLVLIGNPNSTGLDKDPID**-----**LGSK**
S. chmielewskii KIDGIFLACPYFWGKDRIEGEGENLLAKDLV**EDLVLVGNPNSTGLDKDPID**-----**LGSK**
S. neorickii --

S. tuberosum **DLF**EKLWLFVNPNSSGLDDPLINPEKDPKLSGLGCEKVLMYVAGKDPLRFRGLYYKEALE
S. pennellii **NLF**EKLWLFVNPTSSGFDDPLINPEKDPKLSGLGCDKVVVYVAGKDPLRFRGFYYKEVLE
S. habrochaites **DLF**EKLWLFVNPTSSGLDDPLINPEKDPKLPGLGCDKLVVYVAGKDPLRFRGFYYKELLE
S. pimpinellifolium **DLF**EKLWLFVNPTSSGLDDPLINPEKDPKLYGLGCDKLVVYVAGKDPLRFRGFYYKEVLE
S. lycopersicum **DLF**EKLWLFVNPTSSGLDDPLINPEKDPKLSGLGCDKLVVYVAGKDPLRFRGFYYKEVLE
S. cheesmaniae **DLF**EKLWLFVNPTSSGLDDPLINPEKDPKLSGLGCDKLVVYVAGKDPLRFRGFYYKEVLE
S. galapagense **DLF**EKLWLFVNPTSSGLDDPLINPEKDPKLSGLGCDKLVVYVAGKDPLRFRGFYYKEVLE
S. chmielewskii **DLF**EKLWLFVNPTSSGLDDPLINPEKDPELSGLGCAKLVVYVAGKDPLRFRGFYYKELFE
S. neorickii --

S. tuberosum KSGWLGTVEVVEVKDKAHVFHLFVPEAEEAMAMLKKLASFLNQS----------------
S. pennellii KSGWPGTVEVVEVKGKGHVFHLFVPEAEEAIAMLKKLASFLNQS----------------
S. habrochaites KSGWPGTVEIVEVKGKGHVFHLFVPEAEEAIAMLKKLASFLNQS----------------
S. pimpinellifolium KSGWPGTVEVVEVKGKGHVFHLFVPEAEEAIAMLKKLASFLNQS----------------
S. lycopersicum KSGWPGTVEVVEVKGKGHVFHLFVPEAEEAIAMLKKLASFLNQS----------------
S. cheesmaniae KSGWPGTVEVVEVKGKGHVFHLFVPEAEEAIAMLKKLASFLNQS----------------
S. galapagense KSGWPGTVEVVEVKGKGHVFHLFVPEAEEAIAMLKKLASFLNQS----------------
S. chmielewskii KSGWPGTVEVVEVKGKGHVFHLFVPEAEEAIAMLKKLASFLNQSQDPLIFNLVCHGNSTN
S. neorickii --

S. tuberosum --------------
S. pennellii --------------
S. habrochaites --------------
S. pimpinellifolium --------------
S. lycopersicum --------------
S. cheesmaniae --------------
S. galapagense --------------
S. chmielewskii KAACAKANESSTIF
S. neorickii --------------

Fig. S4. Protein alignment of CXE1 orthologs in the *Solanum* genus. The unique sequence within CXE1 is underlined. *S. neorickii* has a deletion that creates an early stop codon. *S. chmielewskii* has a mutation in the stop codon that results in a longer protein. That same mutation is also present in the DNA sequence of *S. neorickii*.

Table S1. Correlation of acetate esters with consumer appreciation of tomato

Volatile	Liking: Spearman's correlation coefficient*	Liking: *P* value
Total acetate esters	−0.511	1.52*E*-06
Propyl acetate	−0.459	1.88*E*-05
Isobutyl acetate	−0.450	2.84*E*-05
Butyl acetate	−0.435	5.54*E*-05
2-Methylbutyl acetate	−0.399	2.47*E*-04
sec-butyl acetate	−0.393	3.14*E*-04
Hexyl acetate	−0.348	1.54*E*-03
cis-3-hexenyl acetate	−0.299	0.007
3-Methylbutyl acetate	−0.228	0.042

*Consumer preference and composition data were originally published in Tieman et al. (1). The correlation between consumer liking and volatile concentrations in 68 tomato varieties was performed on the 50 most abundant volatiles. Volatiles were expressed as a percentage of total volatile content.

1. Tieman D, et al. (2012) The chemical interactions underlying tomato flavor preferences. *Curr Biol* 22(11):1035–1039.

Table S2. Content of several acetate esters relative to the total amount of volatiles in wild accessions of the tomato clade

Species/ accession no.	Propyl acetate content (%) (±SE)	Isobutyl acetate content (%) (±SE)	3-Methylbutyl acetate content (%) (±SE)	2-Methylbutyl acetate content (%) (±SE)	*cis*-3-Hexenyl acetate content (%) (±SE)
S. lycopersicum					
LA1207	0.011 ± 0.003	0.094 ± 0.026	0.004 ± 0.002	0.037 ± 0.008	0.119 ± 0.021
LA1287	0.034 ± 0.007	0.097 ± 0.012	0.024 ± 0.010	0.093 ± 0.027	0.298 ± 0.0480
LA1456	0.027 ± 0.006	0.103 ± 0.009	0.015 ± 0.006	0.088 ± 0.0270	0.248 ± 0.036
LA1621	0.036 ± 0.012	0.132 ± 0.020	0.009 ± 0.004	0.144 ± 0.040	0.202 ± 0.043
LA1703	0.007 ± 0.001	0.156 ± 0.017	0.004 ± 0.002	0.047 ± 0.006	0.055 ± 0.005
LA1705	0.026 ± 0.011	0.086 ± 0.019	0.027 ± 0.006	0.049 ± 0.016	0.257 ± 0.0384
S. pimpinellifolium					
LA0373	0.010 ± 0.001	0.135 ± 0.011	0.001 ± 0.001	0.013 ± 0.001	0.162 ± 0.0248
LA3158	0.003 ± 0.001	0.067 ± 0.013	0.004 ± 0.004	0.009 ± 0.001	0.098 ± 0.012
LA3159	0.004 ± 0.000	0.049 ± 0.015	0.000 ± 0.000	0.018 ± 0.002	0.173 ± 0.0279
LA3160	0.004 ± 0.001	0.072 ± 0.012	0.000 ± 0.000	0.013 ± 0.004	0.111 ± 0.017
LA3161	0.003 ± 0.000	0.066 ± 0.008	0.000 ± 0.000	0.009 ± 0.002	0.107 ± 0.013
S. cheesmaniae					
LA0422	0.936 ± 0.092	0.243 ± 0.026	0.019 ± 0.002	0.202 ± 0.030	0.481 ± 0.039
LA0428	0.006 ± 0.001	0.059 ± 0.005	0.002 ± 0.001	0.061 ± 0.023	0.370 ± 0.086
LA1412	0.689 ± 0.192	0.368 ± 0.037	0.480 ± 0.165	0.274 ± 0.076	0.456 ± 0.121
S. galapagense					
LA0483	1.416 ± 0.080	0.807 ± 0.107	0.399 ± 0.090	0.612 ± 0.062	0.769 ± 0.215
S. neorickii					
LA0247	1.872 ± 0.714	0.948 ± 0.280	0.144 ± 0.057	0.781 ± 0.241	0.434 ± 0.081
LA2200	0.333 ± 0.061	0.190 ± 0.019	0.032 ± 0.009	0.154 ± 0.020	0.285 ± 0.072
S. chmielewskii					
LA1028	2.197 ± 0.658	1.620 ± 0.360	0.050 ± 0.008	0.783 ± 0.199	0.401 ± 0.067
LA1306	1.306 ± 0.614	1.930 ± 0.351	0.034 ± 0.034	0.448 ± 0.197	2.386 ± 1.304
S. pennellii					
LA0716	0.996 ± 0.069	2.750 ± 0.351	7.525 ± 1.568*		0.912 ± 0.046
LA2560	0.242 ± 0.050	0.848 ± 0.066	52.119 ± 5.992*		0.924 ± 0.147

*High abundance and close retention time prevent an efficient separation of 3-Methylbutyl acetate and 2-Methylbutyl acetate.

Table S3. Transcript abundance of the different carboxylesterases in *S. lycopersicum*, *S. pennellii*, and IL 1–4

	CXE1 (copy no./µg of RNA) (±SE)	CXE2 (copy no./µg of RNA) (±SE)	CXE3 (copy no./µg of RNA) (±SE)	CXE4 (copy no./µg of RNA) (±SE)	CXE5 (copy no./µg of RNA) (±SE)
S. lycopersicum (M82)	6,547,392 ± 660,852	993 ± 22	16 ± 6	64 ± 9	18,788 ± 760
IL 1–4	732,017 ± 112,662	2,904 ± 21	5,741 ± 654	882 ± 80	11,384 ± 999
S. pennellii (LA0716)	15,602 ± 6,242	1,518 ± 536	2,697 ± 947	2,909 ± 1095	4,140 ± 1,374

Table S4. Transcript abundance of the different carboxylesterases in the transgenics lines

	CXE1 (copy no./µg of RNA) (±SE)	CXE2 (copy no./µg of RNA) (±SE)	CXE3 (copy no./µg of RNA) (±SE)	CXE4 (copy no./µg of RNA) (±SE)	CXE5 (copy no./µg of RNA) (±SE)
Flora-Dade (control)	5,984,902 ± 660,094	1,620 ± 337	55 ± 20	365 ± 72	43,013 ± 7071
Line #3	92,300 ± 22,835	230 ± 20	18 ± 4	53 ± 10	4,655 ± 546
Line #6	360,762 ± 52,380	649 ± 109	52 ± 17	246 ± 33	9,803 ± 843
Line #180	9,302 ± 1,305	118 ± 8	6 ± 1	13 ± 5	1,713 ± 285

Table S5. Enzymatic activity of SlCXE1, SlCXE5, and SlCXE1del

	K_m (mM) (±SE)			k_{cat} (s^{-1}) (±SE)			k_{cat}/K_m		
Volatile	SlCXE1	SlCXE1del	SlCXE5	SlCXE1	SlCXE1del	SlCXE5	SlCXE1	SlCXE1del	SlCXE5
2-Methylbutyl acetate	0.65 ± 0.05	2.12 ± 0.20	1.82 ± 0.65	84.89 ± 1.77	1.19 ± 0.01	1.34 ± 0.11	131.45	0.56	0.74
3-Methylbutyl acetate	0.62 ± 0.03	3.22 ± 0.36	3.89 ± 0.29	88.71 ± 0.68	1.17 ± 0.07	1.82 ± 0.20	144.24	0.36	0.47
cis-3-hexenyl acetate	0.47 ± 0.03	1.57 ± 0.15	3.38 ± 0.46	68.97 ± 0.56	3.05 ± 0.18	3.35 ± 0.22	145.60	1.95	0.99
Hexyl acetate	0.26 ± 0.04	1.45 ± 0.23	1.54 ± 0.63	65.05 ± 1.84	1.94 ± 0.12	2.94 ± 0.14	250.87	1.34	1.91
Isobutyl acetate	0.95 ± 0.08	2.14 ± 0.18	6.69 ± 0.95	34.48 ± 0.40	0.40 ± 0.01	0.32 ± 0.03	36.19	0.19	0.05
Butyl acetate	1.29 ± 0.10	1.18 ± 0.39	4.79 ± 0.43	58.60 ± 0.48	0.73 ± 0.03	0.97 ± 0.07	45.57	0.62	0.20
Pentyl acetate	0.40 ± 0.05	1.79 ± 0.16	3.02 ± 0.49	104.10 ± 0.55	1.92 ± 0.33	2.69 ± 0.09	261.75	1.08	0.89
Propyl acetate	4.04 ± 0.27	6.75 ± 1.43	10.50 ± 4.34	17.59 ± 0.06	0.42 ± 0.04	0.66 ± 0.05	4.36	0.06	0.06
sec-butyl acetate	3.87 ± 0.64	12.75 ± 5.52	8.80 ± 3.47	10.54 ± 0.49	0.52 ± 0.07	0.17 ± 0.01	2.72	0.04	0.02
Phenyl ethyl acetate	0.22 ± 0.01	1.33 ± 0.07	3.48 ± 0.74	99.79 ± 1.86	10.67 ± 0.27	4.57 ± 0.12	453.58	8.05	1.31
Benzyl acetate	0.42 ± 0.02	1.73 ± 0.09	3.04 ± 0.30	173.28 ± 3.36	19.70 ± 0.36	5.95 ± 0.13	413.76	11.40	1.96