

Additional file 6. Functionality and substrate specificity of VvCCD1, VvCCD4a and VvCCD4b in a heterologous *in vivo* bacterial system. CCDs were expressed in *Escherichia coli* engineered to accumulate specific carotenoids. Carotenoids produced before cleavage were determined using UPLC. Volatile apocarotenoids produced after cleavage were determined using GC-MS.

Carotenoid accumulating plasmid (pAC-)	Carotenoid → Apocarotenoid	Cleavage position	pTWIN1 (vector control)	VvCCD1	VvCCD4a	VvCCD4b
			Average ng.L ⁻¹ apocarotenoid produced ± standard deviation (n=3)			
pAC-ZETA	ζ-carotene (80%) ¹ → Geranylacetone	9,10(9',10')	803.61±75.20	ND ²	ND	2255.04±74.57
pAC-NEUR	Neurosporene (100%) ¹ → Geranylacetone	(9',10')	388.61±12.73	184.18±53.08	671.43±32.15	646.39±37.01
pAC-LYC	Lycopene (100%) ¹ → 6-MHO	5,6(5',6')	393.19±49.42	660.70±44.36	756.00±59.27	782.00±15.45
pAC-EPSILON	ε-carotene (70%) ¹ → α-ionone	9,10(9',10')	40.24±8.35	336.15±16.32	190.91±3.57	262.08±1.88
pAC-BETA	β-carotene (100%) ¹ → β-ionone	9,10(9',10')	76.28±8.47	349.61±20.46	< LOQ ³	< LOQ

¹ The percentage of the specific carotenoid substrate present in the strain before VvCCD induction as determined by UPLC analysis

² “ND” Not detected

³ “< LOQ” Below level of quantification