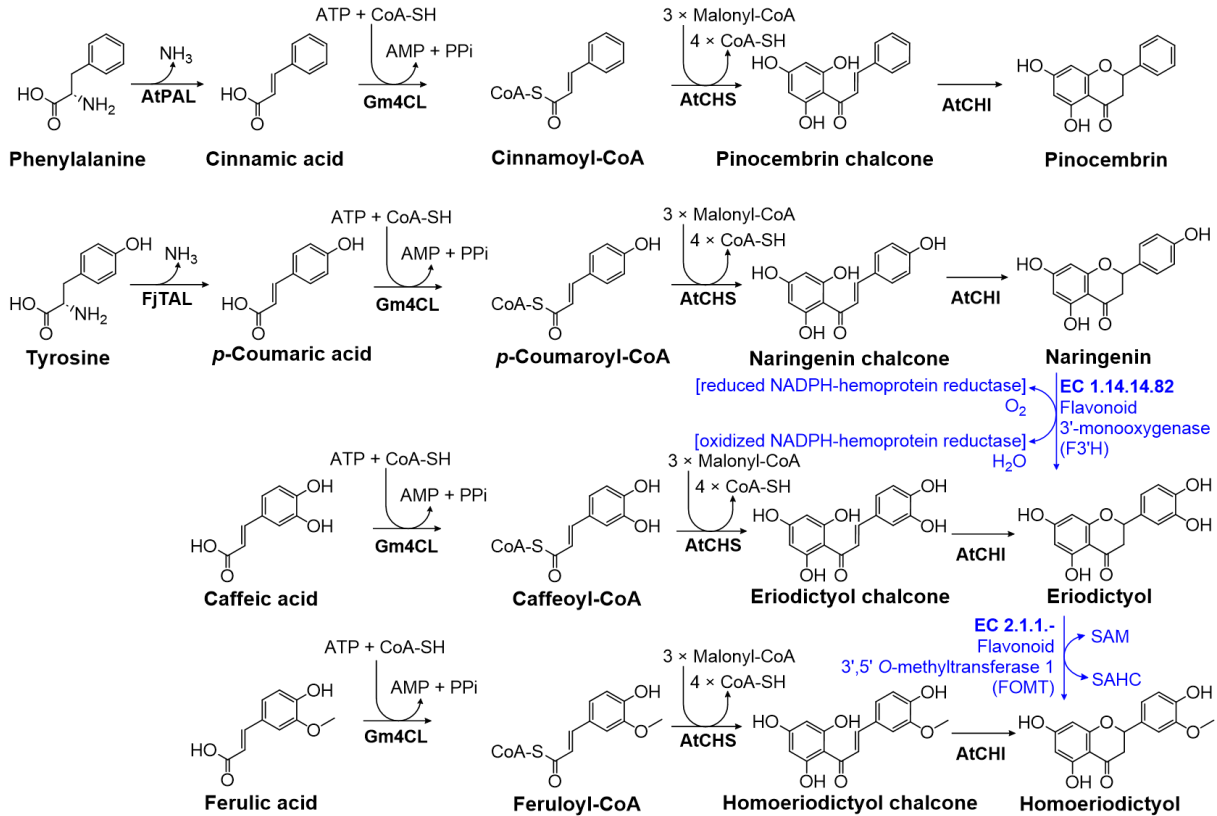


Supplementary Material

1 Supplementary Figures and Tables

1.1 Supplementary Figures



Supplementary Figure S1: Biosynthetic routes to gatekeeper flavonoids employed by Dunstan *et al.* (Dunstan *et al.*, 2020). AtPAL: phenylalanine ammonia lyase from *Arabidopsis thaliana*; FjTAL: tyrosine ammonia lyase from *Flavobacterium johnsoniae*; Gm4CL: 4-coumarate-CoA ligase from *Glycine max*; AtCHS: chalcone synthase from *Arabidopsis thaliana*; AtCHI: chalcone isomerase from *Arabidopsis thaliana*. In blue: Alternative enzymatic routes to eriodictyol and homoeriodictyol more commonly cited as being the natural routes to these compounds.

1.2 Supplementary Tables

Supplementary Table S1: Plasmids used in this study that had previously been constructed by Dunstan *et al.* (Dunstan *et al.*, 2020).

Plasmid SBC number	Antibiotic marker	Description
SBC005753 (pTyr+)	Km ^R	Upregulation of tyrosine
SBC006456	Cm ^R	Formation of naringenin from tyrosine
SBC006524	Km ^R	Formation of pinocembrin from phenylalanine
SBC006845	Km ^R	Formation of eriodictyol from caffeic acid

Supplementary Table S2: Sequences of the synthesized DNA parts. Gene coding sequences are shown in uppercase letters. EcoRI and BamHI restriction enzyme recognition sites are italicized.

Gene	Sequence
GmF3H	<i>gaattcaaaaagatctgagctcttgtaaggcaagggctcaattgagccccattttgtcgtatatacagtttcga</i> <i>accgcgaattgaatacagaggaggaaagacgtacaATGGCTCCAACCGCTAAGACCCTGACATATTTAGCCC</i> <i>AGGAGAAAACATTAGAATCAAGTTTTGTACGAGATGAAGAGGAACGCCCGAAGGTGGCTTATAACGAATTTT</i> <i>CAGATGAGATCCCGGTCATTTCCCTGGCCGGCATTGACGAAGTGGATGGCCGTCGGCGCGAAAATTTGTGAGA</i> <i>AAATCGTTGAGGCCTGCGAGAATTGGGGTATCTTCCAAGTAGTGGATCACGGCGTGGATCAACAACCTGGTCG</i> <i>CAGAAATGACCGCTTAGCCAAAGAATTTTCGCGCTTCCGCCGATGAAAAATTACGATTCGATATGTCCG</i> <i>GTGCAAAGAAAGGCGGCTTATTGTGTCTTCACATCTGCAGGGCGAATCAGTTCAAGACTGGCGTGAAATCG</i> <i>TTACGTACTTCTCGTATCCTAAACGTGAGCGTGATTACTCACGCTGGCCCGATACACCCGAAGGATGGCGTA</i> <i>GCGTGACCGAGGAATATAGCGACAAAGTTATGGGGCTGGCCTGTAATTAATGGAAGTACTGTTCGGAGGCCA</i> <i>TGGGCCCTGGAGAAAAGGCCCTGAGCAAAGCGTGTGTGGACATGGATCAGAAAGTAGTGGTCAATTATTACC</i> <i>CCAAATGTCTCAGCCGGATTTAACGCTAGGTCTGAAACGCCACACCGATCCGGGCACCATTACGTTGTCTGC</i> <i>TTCAGGACCAGGTTGGGGGATTGCAGGCGACACGCGATAACGGTAAAACCTGGATAACGGTCCAGCCGGTAG</i> <i>AAGCGGCTTTGTGGTTAACCTGGGCGACCACGCGCACTATCTGTCAAATGGTTCGCTTTAAGAACGCAGATC</i> <i>ATCAAGCTGTTGTTAACTCAAATCATAGCCCGCTTTCGATTGCCACTTTTCAGAATCCGGCGCTTAACGCGA</i> <i>CCGTTTATCCGCTTAAAATCCGCGAGGGCGAAAAGCCGGTGTGGAAGAACCATCACGTTTCGCAGAAATGT</i> <i>ATCGCCGTAAAGATGAGTAAAGATATTGAAATGCCCCGATGAAGAAATTAGCCAAAGAAAAGCATCTGCAGG</i> <i>ATTTAGAGAAATGAAAACATCTGCAGGAACTGGATCAGAAAGCGAAGTTAGAAAGCAAACCCGTTGAAAGAAA</i> <i>TTCTTGCCTAAttgtagactcggatcc</i>
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Supplementary Table S3: Plasmids constructed in this study.

Plasmid SBC ID	Plasmid backbone	Genetic construct
SBC015646	p15A	[<i>P_{trc}</i> —RBS—PcFNS-I—T]
SBC015647	pSC101	[<i>P_{trc}</i> —RBS—PcFNS-I—T]
SBC015648	p15A	[<i>P_{lacUV5}</i> —RBS—PcFNS-I—T]
SBC015649	pSC101	[<i>P_{lacUV5}</i> —RBS—PcFNS-I—T]
SBC015650	p15A	[<i>P_{trc}</i> —RBS—PaFNS-I—T]
SBC015651	pSC101	[<i>P_{trc}</i> —RBS—PaFNS-I—T]
SBC015652	p15A	[<i>P_{lacUV5}</i> —RBS—PaFNS-I—T]
SBC015653	pSC101	[<i>P_{lacUV5}</i> —RBS—PaFNS-I—T]
SBC015673	p15A	[<i>P_{trc}</i> —RBS—SbFNS-II—RBS—AtCPR—T]
SBC015674	p15A	[<i>P_{trc}</i> —RBS—ObFNS-II—RBS—AtCPR—T]
SBC015675	p15A	[<i>P_{trc}</i> —RBS—GmFNS-II—RBS—AtCPR—T]
SBC015676	p15A	[<i>P_{trc}</i> —RBS—GhFNS-II—RBS—AtCPR—T]
SBC015677	p15A	[<i>P_{trc}</i> —RBS—LjFNS-II—RBS—AtCPR—T]
SBC015883	p15A	[<i>P_{trc}</i> —RBS—GmF3H—RBS—CuFLS—T]
SBC015884	p15A	[<i>P_{trc}</i> —RBS—GmF3H—T]—[<i>P_{trc}</i> —RBS—CuFLS—T]
SBC015885	p15A	[<i>P_{trc}</i> —RBS—GmF3H—T]—[<i>P_{lacUV5}</i> —RBS—CuFLS—T]
SBC015886	p15A	[<i>P_{lacUV5}</i> —RBS—GmF3H—RBS—CuFLS—T]
SBC015887	p15A	[<i>P_{lacUV5}</i> —RBS—GmF3H—T]—[<i>P_{trc}</i> —RBS—CuFLS—T]
SBC015888	p15A	[<i>P_{lacUV5}</i> —RBS—GmF3H—T]—[<i>P_{lacUV5}</i> —RBS—CuFLS—T]
SBC015889	p15A	[<i>P_{trc}</i> —RBS—CuFLS—RBS—GmF3H—T]
SBC015890	p15A	[<i>P_{trc}</i> —RBS—CuFLS—T]—[<i>P_{trc}</i> —RBS—GmF3H—T]
SBC015891	p15A	[<i>P_{trc}</i> —RBS—CuFLS—T]—[<i>P_{lacUV5}</i> —RBS—GmF3H—T]
SBC015892	p15A	[<i>P_{lacUV5}</i> —RBS—CuFLS—RBS—GmF3H—T]
SBC015893	p15A	[<i>P_{lacUV5}</i> —RBS—CuFLS—T]—[<i>P_{trc}</i> —RBS—GmF3H—T]
SBC015894	p15A	[<i>P_{lacUV5}</i> —RBS—CuFLS—T]—[<i>P_{lacUV5}</i> —RBS—GmF3H—T]

Supplementary Table S4: MS parameters and sample dilutions for LC-MS/MS analysis.

Compound	Ionization mode	Parent m/z	Daughter m/z	Dwell time [s]	Cone voltage [V]	Collision energy [V]	Further dilution from quenched samples
Apigenin	Positive	271.0209	152.0209	0.015	94	30	2000
Naringenin	Negative	271.0381	151.0249	0.015	64	18	500 or 2000
<i>p</i> -Coumaric acid	Negative	163.0181	119.0673	0.015	4	14	500 or 2000
Chrysin	Positive	255.0260	152.9334	0.015	4	34	500
Pinocembrin	Negative	255.1543	213.1080	0.015	4	20	500
Cinnamic acid	Negative	147.0005	103.0428	0.015	20	10	500
Luteolin	Positive	287.0819	134.9831	0.015	50	30	100
Eriodictyol	Negative	287.0949	151.0526	0.015	2	14	100
Caffeic acid	Negative	179.0719	135.0888	0.015	32	14	500
Kaempferol	Positive	287.0796	152.9927	0.015	84	30	100 or 500
Dihydrokaempferol	Negative	287.0953	259.1015	0.015	22	14	100 or 500
Quercetin	Negative	301.0746	150.9966	0.015	10	22	8
Dihydroquercetin	Negative	303.0902	285.1047	0.015	2	12	8

Supplementary Table S5: LC-program for LC-MS/MS analysis. LCMS.FLAV-1 was used as default, while LCMS.FLAV-2 was used when experiments included luteolin as an expected analyte.

Program	Time [min]	Flowrate [mL min ⁻¹]	%A (water with 0.1% formic acid)	%B (acetonitrile with 0.1% formic acid)
LCMS.FLAV-1	0	0.5	60	40
	1.5	0.5	5	95
	1.9	0.5	5	95
	2	0.5	60	40
	3	0.5	60	40
LCMS.FLAV-2	0	0.5	60	40
	0.8	0.5	60	40
	1.5	0.5	5	95
	1.9	0.5	5	95
	2	0.5	60	40
	3	0.5	60	40

Supplementary References

- Dunstan, M. S., Robinson, C. J., Jervis, A. J., Yan, C., Carbonell, P., Hollywood, K. A., et al. (2020). Engineering *Escherichia coli* towards *de novo* production of gatekeeper (2*S*)-flavanones: naringenin, pinocembrin, eriodictyol and homoeriodictyol. *Synth. Biol.* 5, ysaa012. doi:10.1093/synbio/ysaa012