Supporting Information

Table S1. Primers for cloning into the USER version of the vector pEAQ.

	Forward and reverse primers
***Tg*TPS2**	F: 5'-GGCTTAAUATGGCTGTGTATGTTAACTCTACAACAG-3' R: 5'-GGTTTAAUTTATGCTGGAATGGGATTTATGAGAACCGAGGT-3'
***Tg*CYP76AE1**	F: 5'-GGCTTAAUATGGACTGGAACGAGAACTATGTCATTT-3' R: 5'-GGTTTAAUTTATCATGTCGACTTTGGTTTTGGAATTGCTTG-3'
***Tg*CYP76AE2**	F: 5'-GGCTTAAUATGGAGTGGAACTGGAACTATGTTACGT-3' R: 5'-GGTTTAAUTTATGGCAAATTCTTTCTTCGTGTTGGAACAGC-3'
***Tg*CYP76AE8**	F: 5'-GGCTTAAUATGGAGTGGATGTGGAACTATGTTGTTT-3' R: 5'-GGTTTAAUTTATGCAGCCATTCGTTTTGGAACTGCTCTCAA-3'
***Tg*CYP76AF7**	F: 5'-GGCTTAAUATGGAATATTACCATGCTTTAGTTACAT-3' R: 5'-GGTTTAAUTTAGTTATTTCTAGGTGTTGGAACAAGAAGCAG-3'
***Tg*CYP76B79**	F: 5'-GGCTTAAUATGGATTTAAATATAGTTATGATCGGTG-3' R: 5'-GGTTTAAUTTAAGGAACAACTGAAGTTGCTACAACACGAAG-3'
***Tg*CYP71AH8**	F: 5'-GGCTTAAUATGGGTTTTTCAATTTTCTTGACAACCT-3' R: 5'-GGTTTAAUTTACTAACTATACACGTACGGTGAAGCAACAAG-3'
***Tg*CYP71AJ5**	F: 5'-GGCTTAAUATGATGATGGACCAGCAAACCCTGTTTC-3' R: 5'-GGTTTAAUTTAGACTCGTGATGTCGCAATCACCAAAAGAGG-3'
***Tg*CYP71AJ14**	F: 5'-GGCTTAAUATGATACTAGATCAACAATTCTTGTTTC-3' R: 5'-GGTTTAAUTTAGATACGTGGCGTTGCAATCACCAACAGAGG-3'
***Tg*CYP71AT12**	F: 5'-GGCTTAAUATGGCTCCATTTTATTTCTATCTTCTTC-3' R: 5'-GGTTTAAUTTATCAGTTATACTTCTTGGCTACTAGACGTAG-3'
***Tg*CYP71AU8**	F: 5'-GGCTTAAUATGCCTGCTCATTTGGGGCAACAAGCAA-3' R: 5'-GGTTTAAUTTAGATAGAATATGGAATTGCGACAGCGAGAAG-3'
***Tg*CYP71AU90**	F: 5'-GGCTTAAUATGGTTTTCATTACAGGAGACGCAAGAT-3' R: 5'-GGTTTAAUTTAGCAGCAACACGAAGTTGCAACAGCAAGGAG-3'

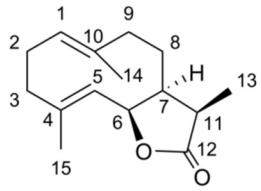
***Tg*CYP71BK1**	F: 5'-GGCTTAAUATGGGGTACCAGTCTGTAACATTAACAC-3' R: 5'-GGTTTAAUTTATGGTTGGTATTTGGTAGGCACAAGGTAGAG-3'
***Tg*CYP71BK6**	F: 5'-GGCTTAAUATGGAAATGTTCTCTGAATCTTTAAGCT-3' R: 5'-GGTTTAAUTTACTCTGTTTGGATTTCATGCTTGATCGGTAC-3'
***Tg*CYP71D183**	F: 5'-GGCTTAAUATGGCTCTCCAGTTGGTGCCCTTATTTC-3' R: 5'-GGTTTAAUTTAATTTTCGAGGGTAGAAATATAAGGATTTGC-3'
***Tg*CYP71D311**	F: 5'-GGCTTAAUATGGAGTTTCAATATCCAACTCATTTCG-3' R: 5'-GGTTTAAUTTAAGGCTTCTAGGTAGGGCGGGAACCAGAACC-3'
***Tg*CYP71319 ortholog**	F: 5'-GGCTTAAUATGGAGATTTACTCACCTATTCTTGCCC-3' R: 5'-GGTTTAAUTTAAGCCTTCTGAGCTGCATATTCAGCGGTGGG-3'
***Tg*CYP71AS14**	F: 5'-GGCTTAAUATGGAAATCTTTTCGGAATCTCTGAACT-3' R: 5'-GGTTTAAUTTACTCTGTTTGGATTTCATGCTTGATCGGTAC-3'
***Tg*CYP706C30 ortholog**	F: 5'-GGCTTAAUATGTTTAAGGATATACAAGCCACTGTCT-3' R: 5'-GGTTTAAUTTACTGGTAGAGCTCAGGATTAGATAATCTCGC-3'

Table S2. In tube *in situ* primers. Primers are specific for *Tg*TPS2 and *Tg*CYP76AE2.

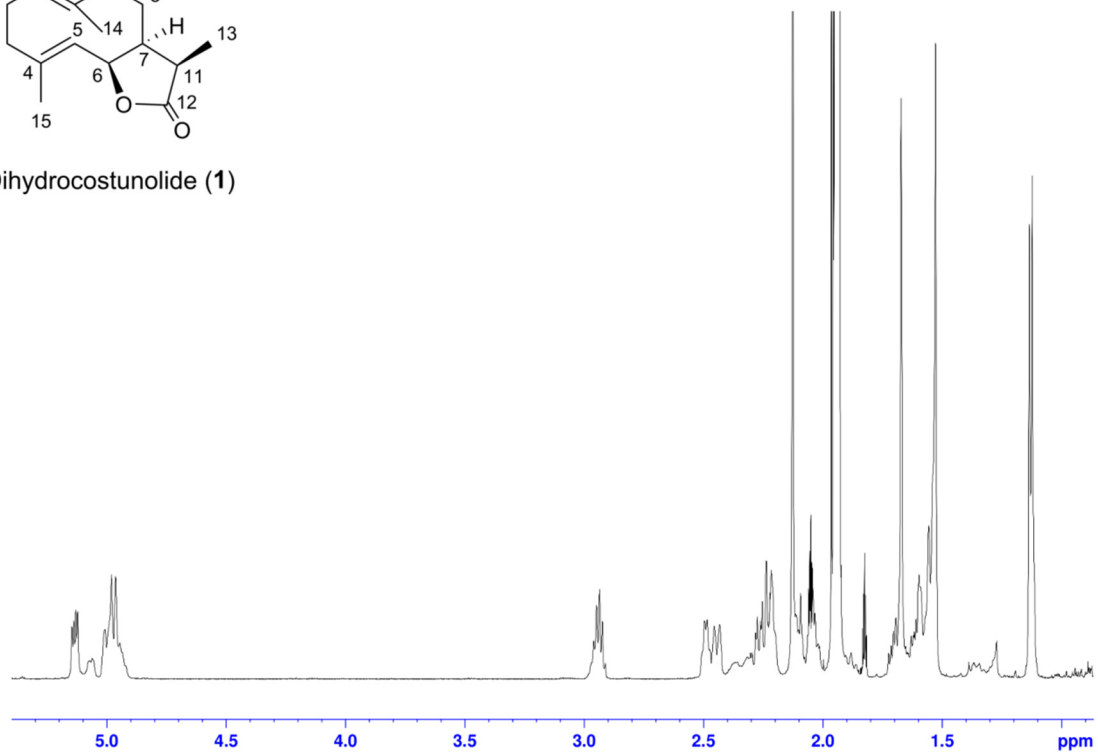
Gene	Primer sequence
18S	F: 5'-CGGGGAAACTTACCAGGTCC-3' R: 5'-TAAGAAGCTGGCCGTGAAGG-3'
*Tg*TPS2	F: 5'-GGTGATTTGCAGACTCAGTGAC-3' R: 5'-TTATGCTGGAATGGGATTTATGAG-3'
*Tg*CYP76AE2	F: 5'-CAGACGTTTCACGCTCCCTC-3' R: 5'-CCATGACATCATCTCATCTGCAC-3'

Figure S1. ^{13}C and ^{1}H NMR spectra of product **1**, **2** and **3**.

1D Proton Spectrum of dihydrocostunolide (1) in MeCN-d_3

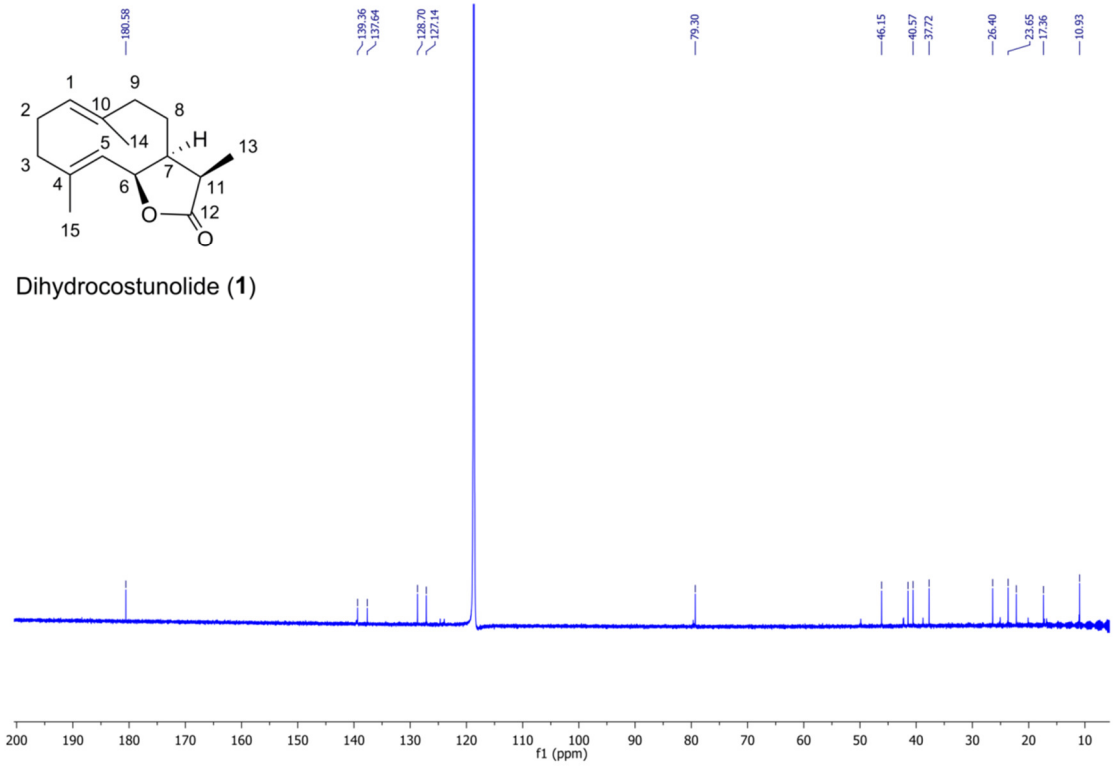


Dihydrocostunolide (**1**)

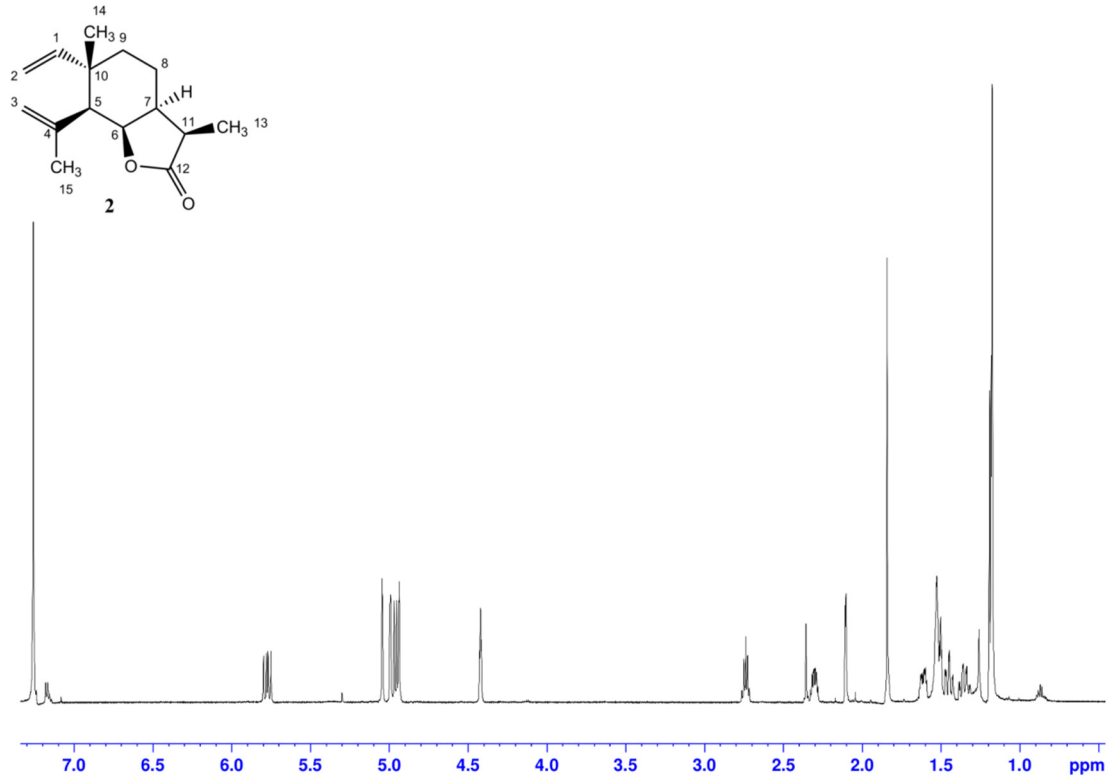


1D Carbon Spectrum of dihydrocostunolide (1) in MeCN-d_3

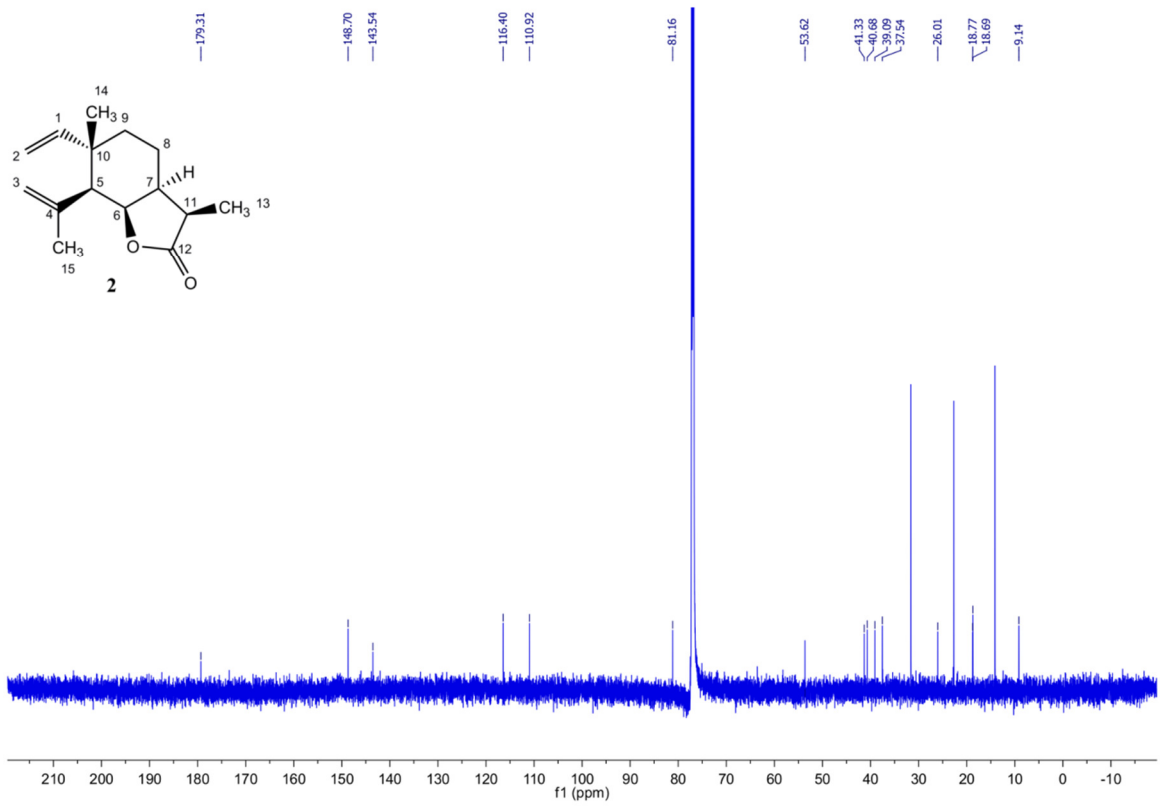
Dihydrocostunolide (**1**)



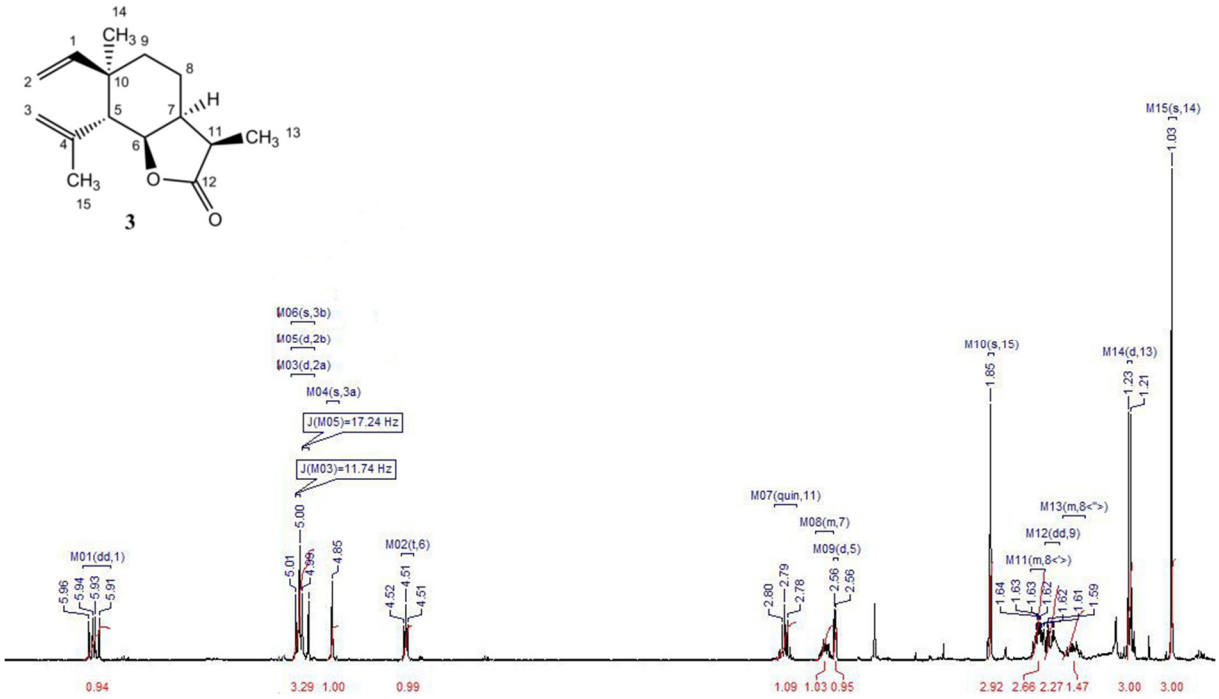
1D Proton Spectrum of 2 in Chloroform-d_1



1D Carbon Spectrum of 2 in Chloroform-d_1



1D Proton Spectrum of 3 in Chloroform-d_1



1D Carbon Spectrum of 3 in Chloroform-d_1

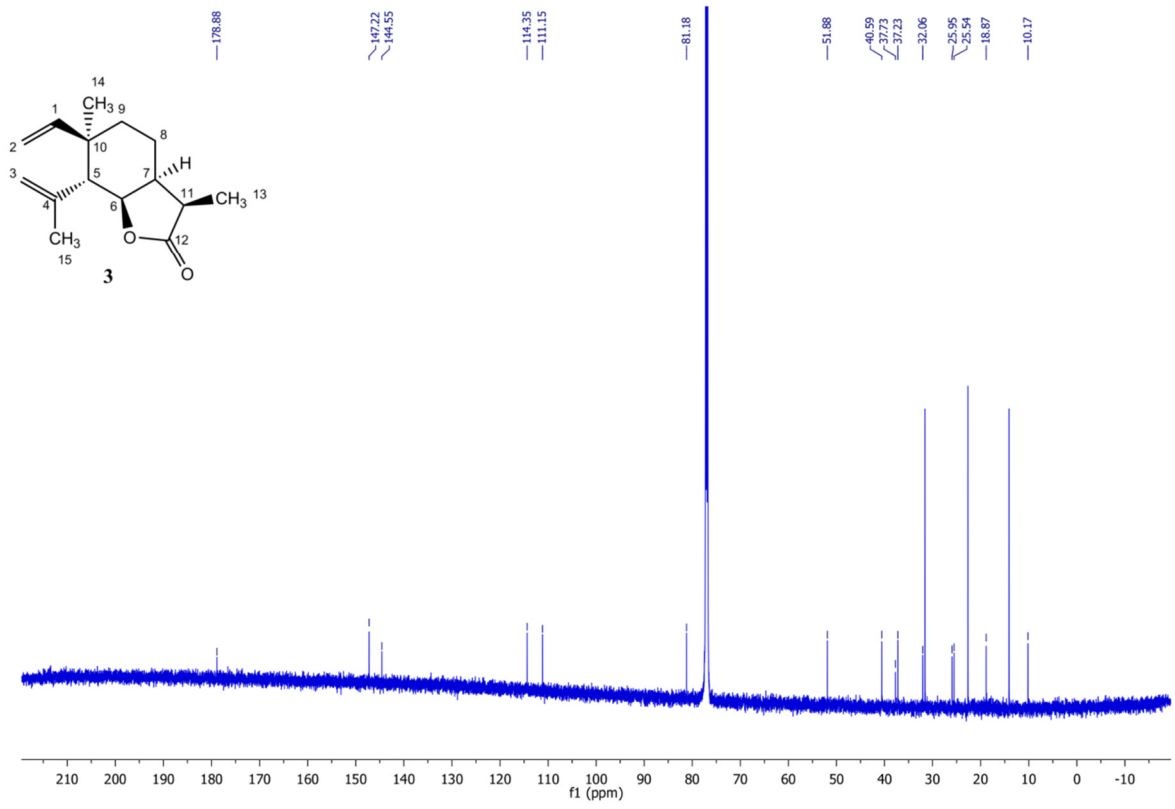


Figure S2. Cope-rearrangement of **1** upon GC-MS analysis. *denotes column contamination.

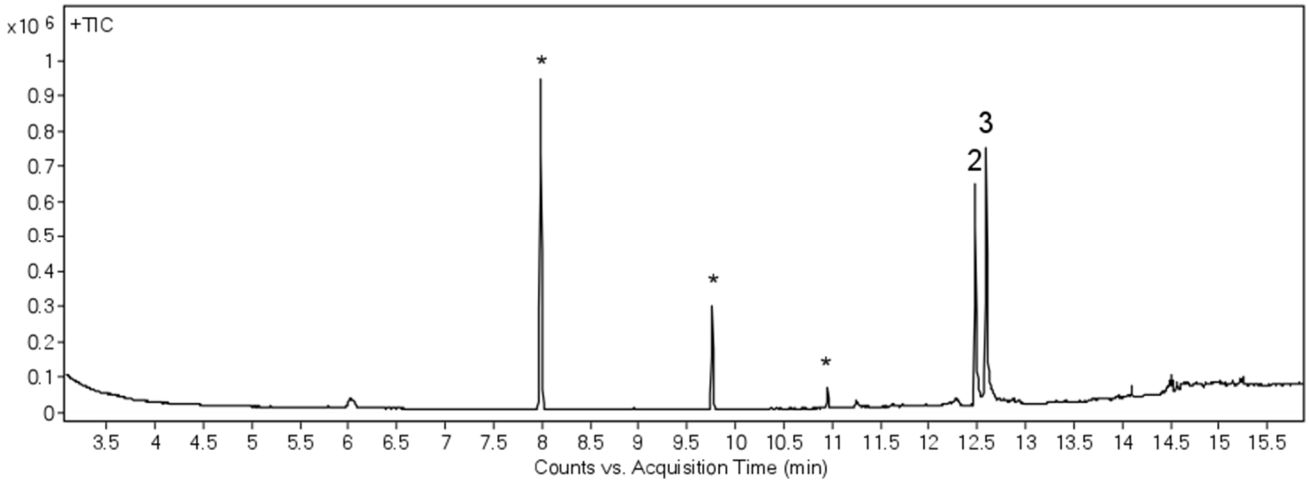


Figure S3. MALDI-MSI analysis of *T. garganica* taproot section and structures of metabolites. **A:** optical image of taproot section with DHB matrix applied by spray deposition, **B:** distribution of thapsigargicin K adduct, $[M+K]^+$ *m/z* 661.26040 (calc. 661.26209, 0.01 ppm error), **C:** distribution of nortrilobolide K adduct, $[M+K]^+$ *m/z* 547.19380 (calc. 547.19401, 0.38 ppm error), **D:** distribution of trilobolide K adduct, $[M+K]^+$ *m/z* 561.20730 (calc. 561.20966, 0.01 ppm error), **E:** distribution of thapsivillosin I K adduct, $[M+K]^+$ *m/z* 645.2327 (calc. 645.23079, 2.97 ppm error), **F:** distribution of thapsivillosin L K adduct, $[M+K]^+$ *m/z* 633.23240 (calc. 633.23079, 2.55 ppm error),. Images normalized to RMS and scaled to 0-60 % of maximum signal intensity using FlexImaging 4.1 to enhance visualization. Results demonstrate thapsigargicin and related metabolites to be localized to concentric circles of secretory ducts.

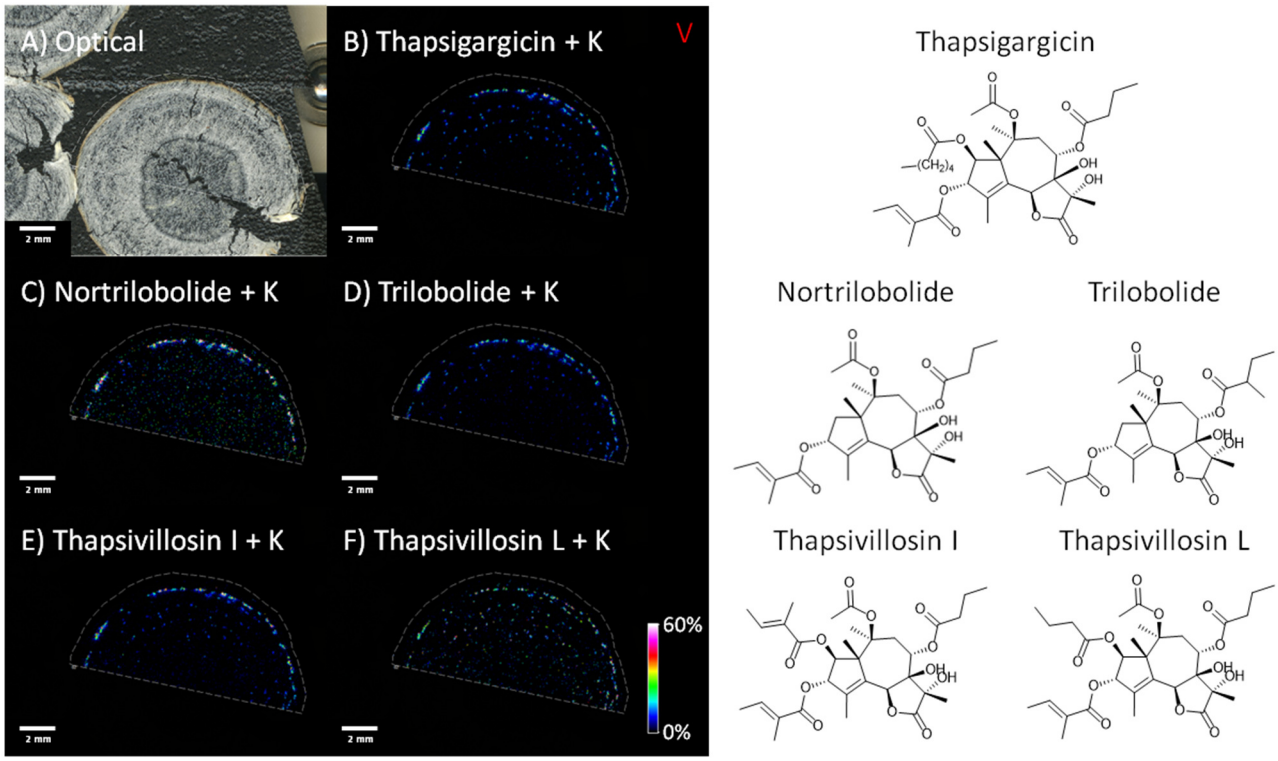


Figure S3-2: MALDI-MSI analysis of *T. garganica* taproot section. Images normalized to RMS and scaled between 0-100% of maximum signal intensity using FlexImaging 4.1 to enhance visualization. Metabolites and lipids were tentatively annotated using accurate mass match to LipidMaps database (www.lipidmaps.org), searching for M+H, M+Na and M+K using a mass tolerance of <0.01 m/z. Results demonstrate different ion signatures for different tissue types including epidermis, parenchyma, stele and concentric circles of secretory ducts. **A:** optical image of taproot section with sublimed DHB matrix, **B)** distribution of disaccharide K adduct localized to parenchyma, $[M+K]^+$ *m/z* 381.0779 (calc. 381.07937, 3.8 ppm error), **C)** distribution of thapsigargin K adduct, $[M+K]^+$ *m/z* 689.2924 (calc. 689. 29339, 1.43 ppm error), **D)** distribution of unknown ion m/z 483.0226 localised to epidermis, **E)** distribution of tentative PC(34:2) H adduct, $[M+H]^+$ *m/z* 758.5637 (calc. 758.5695, 7.6 ppm error), **F)** distribution of tentative GlcCer(d36:1) K adduct, $[M+K]^+$ *m/z* 780.5421, (calc. 780.5387, 4.3 ppm), **G)** distribution of tentative SHexCer(t34:1) H adduct, $[M+H]^+$ *m/z* 796.5202, (calc. 796.5240, 4.7 ppm error), **H)** distribution of tentative PS(46:10) K adduct, $[M+K]^+$ *m/z* 950.5342, (calc. 950.5367, 2.6 ppm error), **I)** distribution of tentative DGDG(54:12) K adduct, $[M+K]^+$ *m/z* 1215.7350 (calc. 1215.7320, 2.5 ppm error).

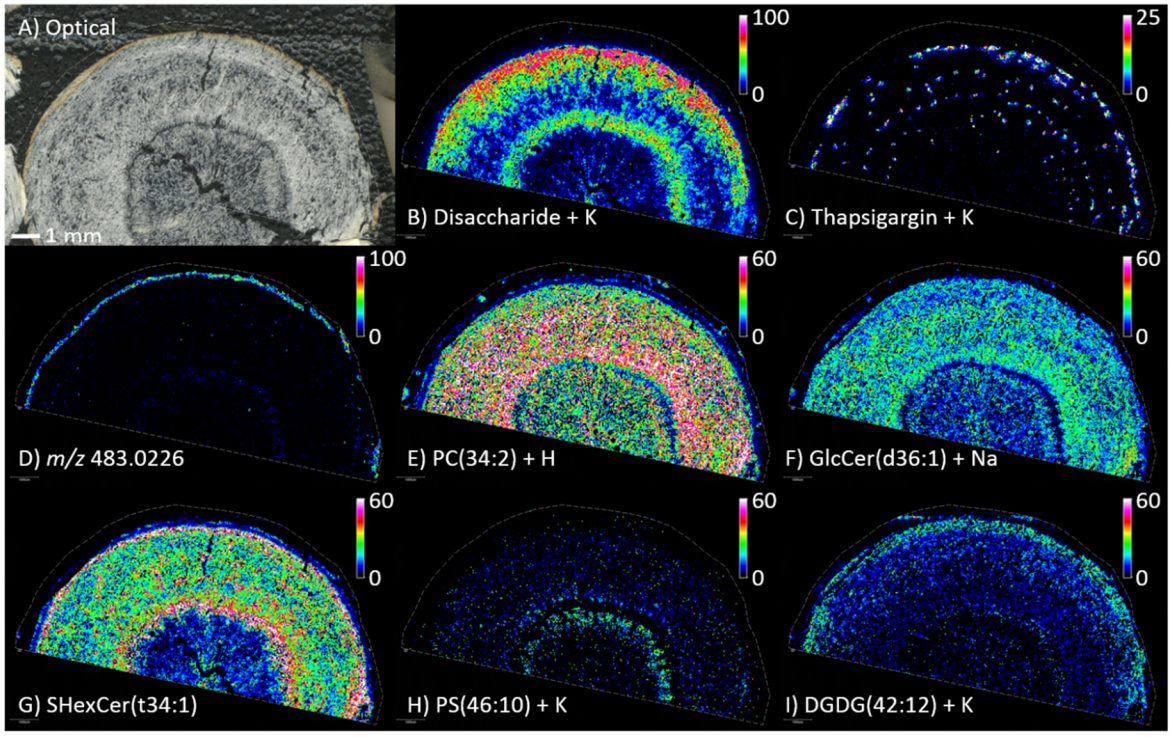
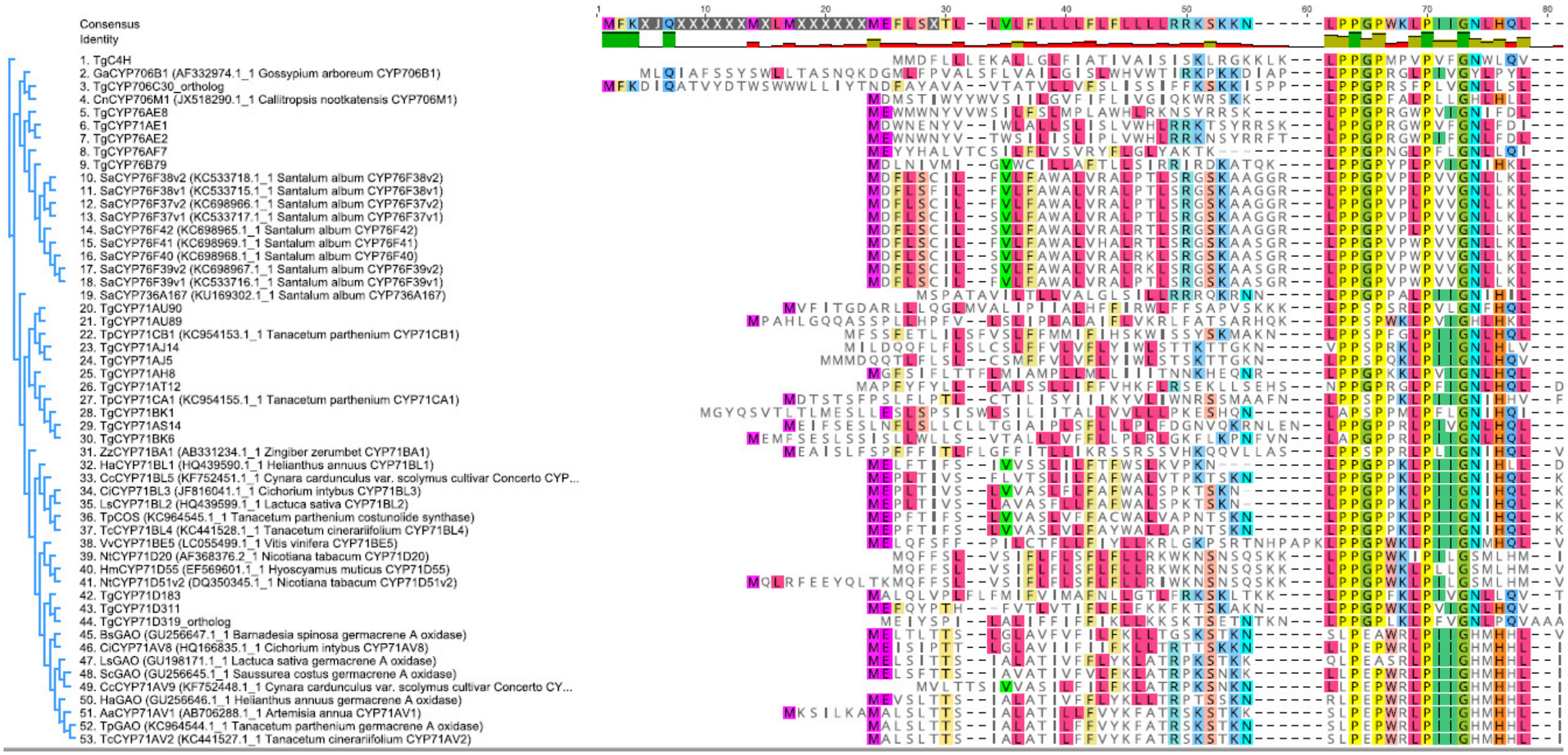
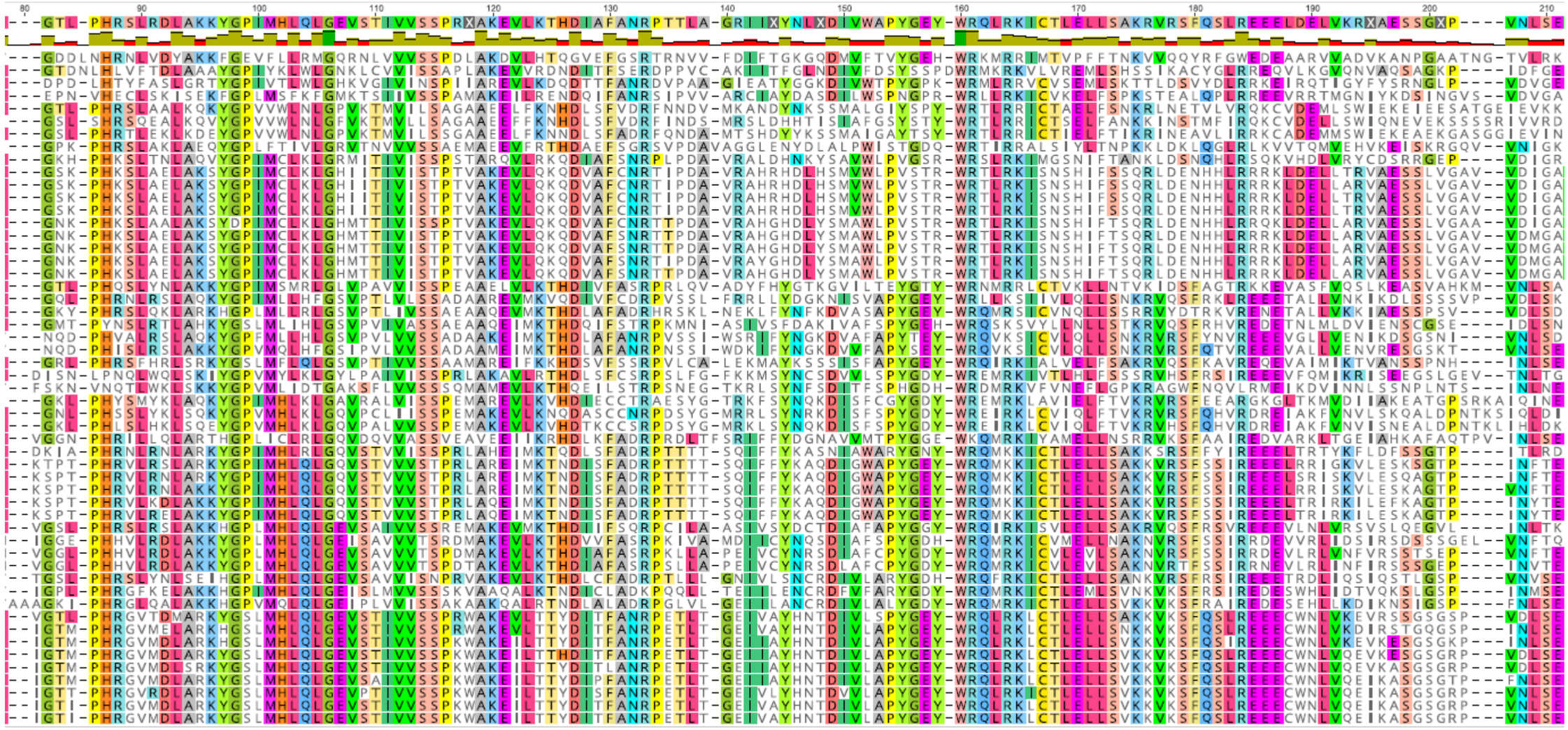
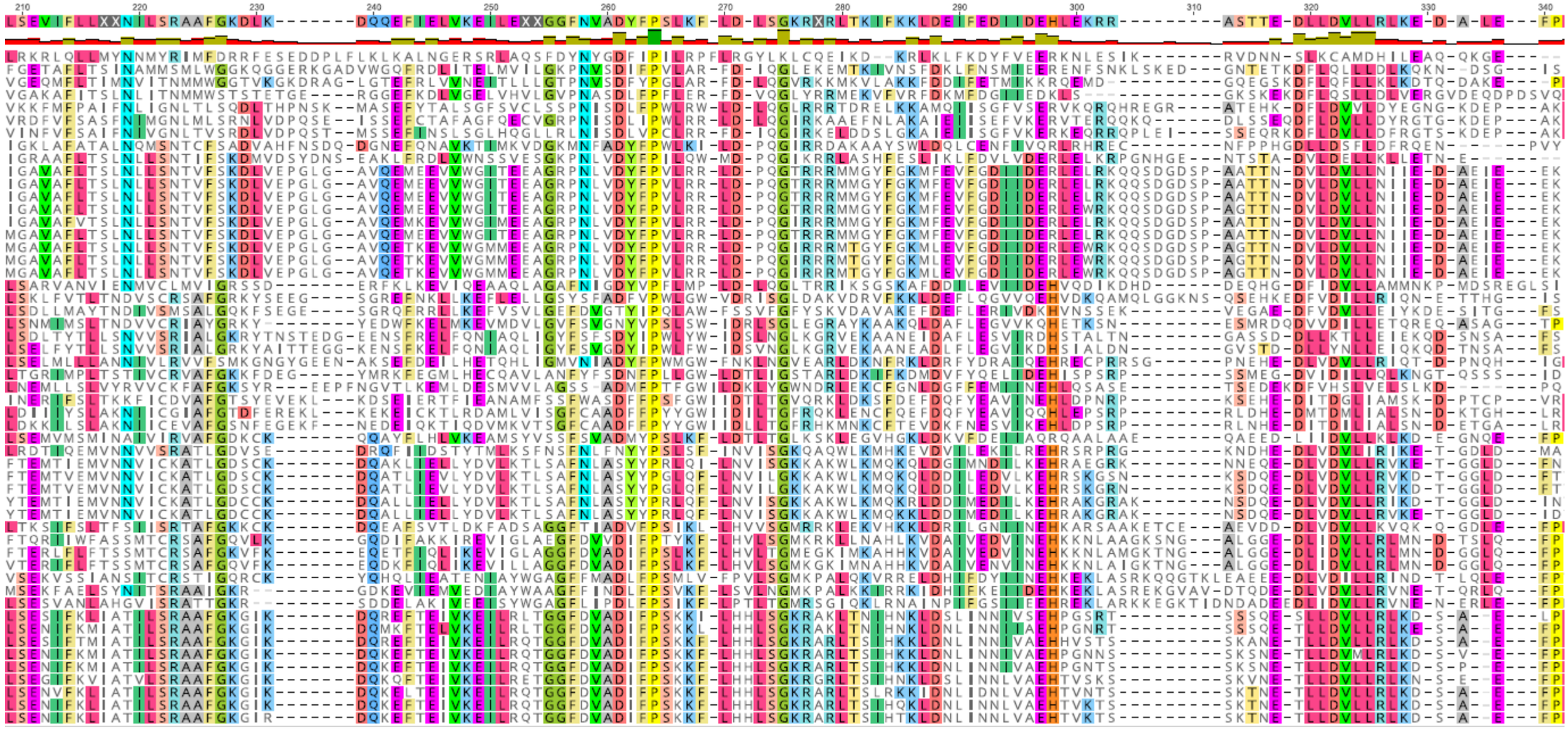
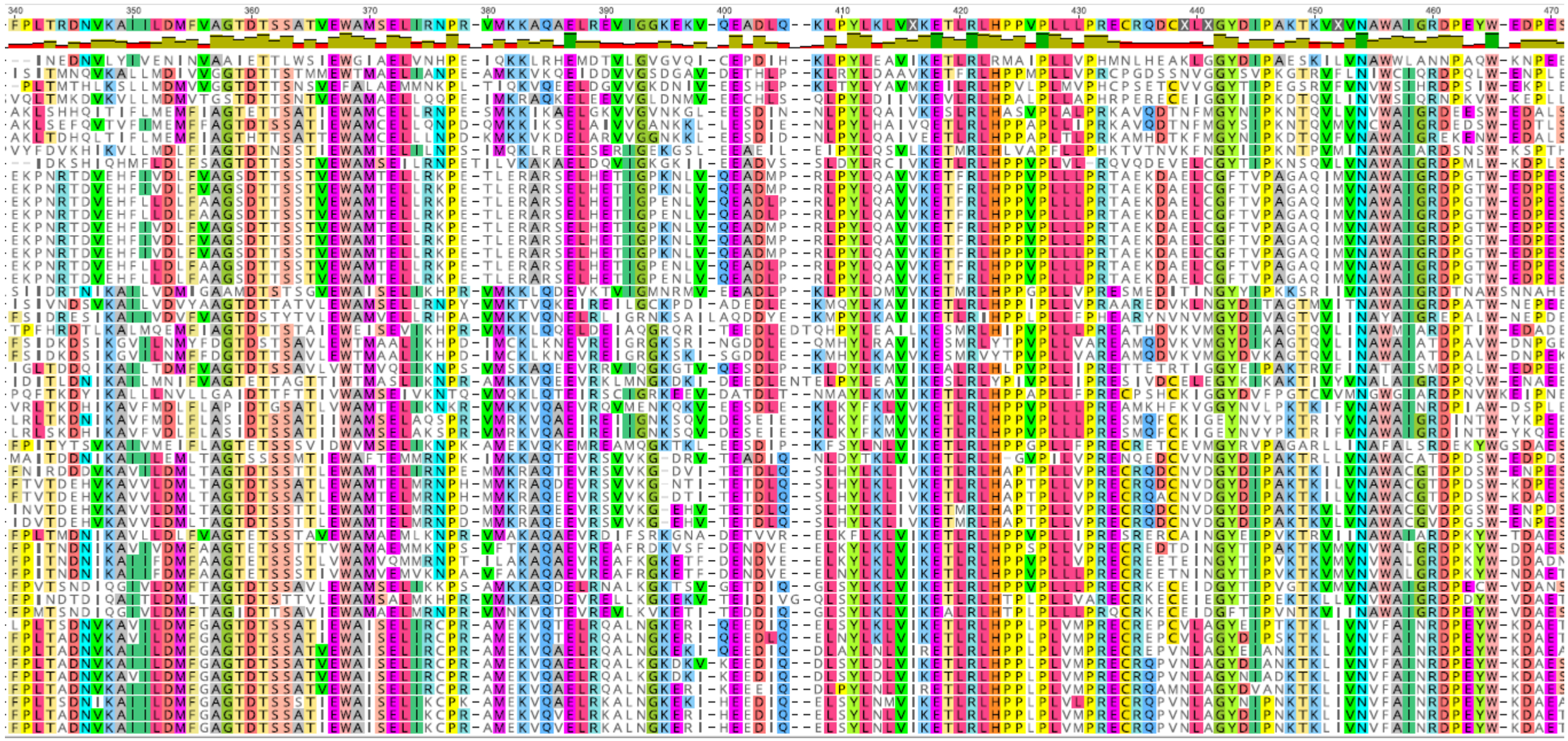


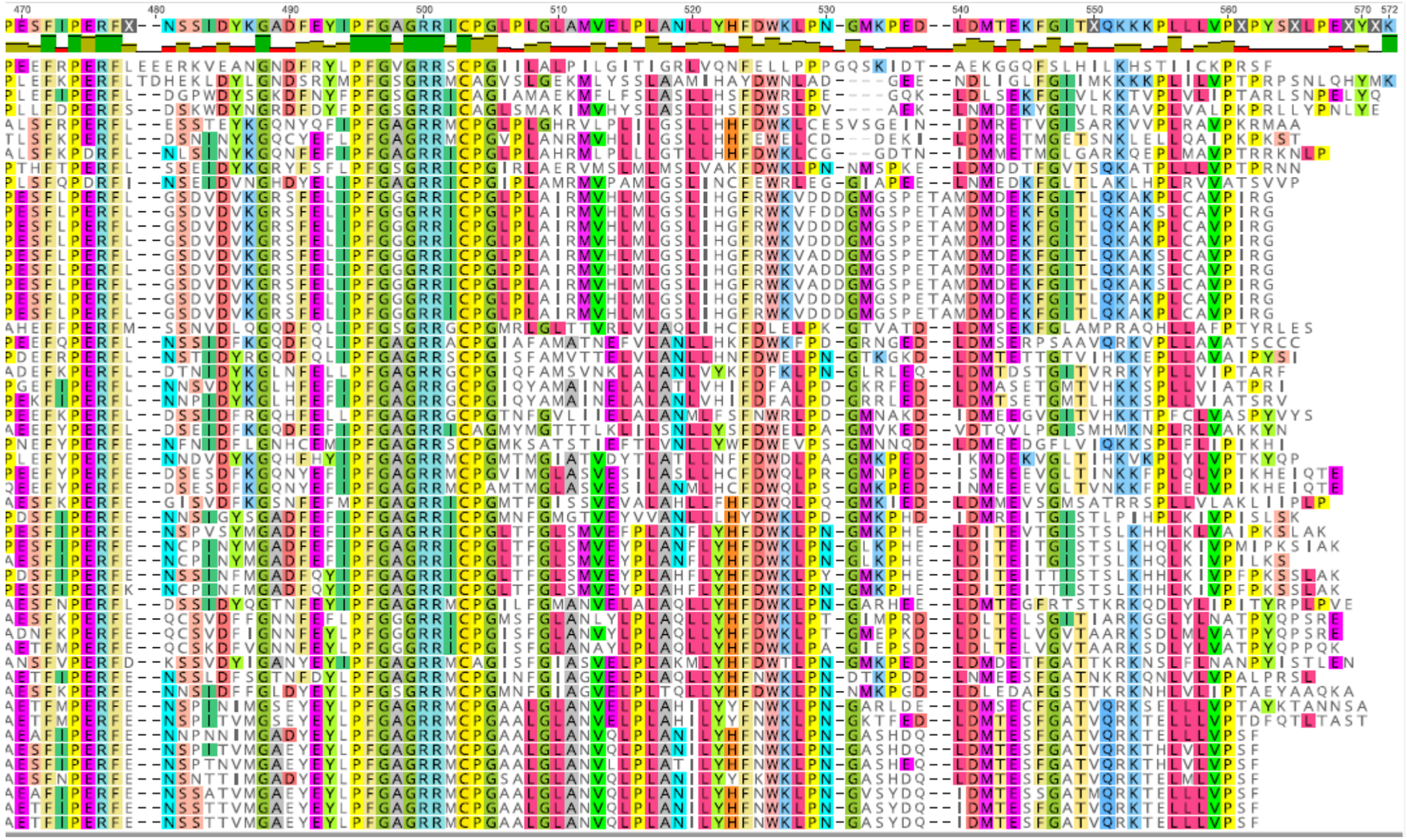
Figure S4. Alignment for the figure 2 tree. The figure also include numbers for the additional gene sequences.








340
350
360
370
380
390
400
410
420
430
440
450
460
470


470
480
490
500
510
520
530
540
550
560
570
572