Supporting Information

The progesterone 5β-reductase/iridoid synthase family is a catalytic reservoir for specialized metabolism across land plants

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Table S1. PRISE homologues described in this report.

Species, family, order (lineage)	Names: sequence ID*	Iridoids/cardenolides occurrence
Antirrhinum majus, Plantaginaceae, Lamiales (core eudicot)	**Antirrhinum_majus_1**: MF281392 **Antirrhinum_majus_2**: MF281393 **Antirrhinum_majus_3**: MF281394 **Antirrhinum_majus_4**: MF281395	Iridoids[1]
Arabidopsis thaliana, Brassicaceae, Brassicales (core eudicot)	**Arabidopsis_thaliana_1** (At4g24220): BT008479 **Arabidopsis_thaliana_2** (At5g58750): BT029538	
Amborella trichopoda, Amborellaceae, Amborellales (basal angiosperm)	**Amborella_trichopoda_1**: XM_006841525 **Amborella_trichopoda_2**: XM_006848150 **Amborella_trichopoda_3**: XM_006847811	
Bupleurum falcatum, Apiaceae, Apiales (core eudicot)	**Bupleurum_falcatum**: JX673780	
Beta vulgaris, Amaranthaceae, Caryophyllales (core eudicot)	**Beta_vulgaris_1**: XM_010675329 **Beta_vulgaris_2**: XM_010689225 **Beta_vulgaris_3**: XM_010673358 **Beta_vulgaris_4**: XM_010687262 **Beta_vulgaris_5**: XM_010673632	
Camptotheca acuminata, Nyssaceae, Cornales (core eudicot)	**Camptotheca_acuminata_1:** KU842378 **Camptotheca_acuminata_2:** KU842379	Iridoids[2]
Citrus clementina, Rutaceae, Sapindales (core eudicot)	**Citrus_clementina_1**: XM_006433044 **Citrus_clementina_2**: XM_006433538 **Citrus_clementina_3**: XM_006432463 **Citrus_clementina_4**: XM_006432455 **Citrus_clementina_5**: XM_006442983 **Citrus_clementina_6**: XM_006442984 **Citrus_clementina_7**: XM_006424996	
Cryptomeria japonica, Cupressaceae, Pinales (gymnosperm)	**Cryptomeria_japonica_1**: AK406828 **Cryptomeria_japonica_2**: AK406861 **Cryptomeria_japonica_3**: AK415075 **Cryptomeria_japonica_4**: AK416036	
Cucumis melo, Cucurbitaceae, Cucurbitales (core eudicot)	**Cucumis_melo_1**: XM_008459154 **Cucumis_melo_2**: XM_008449152	
Catharanthus roseus, Apocynaceae, Gentianales (core eudicot)	**Catharanthus_roseus_1**: KJ873882 **Catharanthus_roseus_2**: KJ873883 **Catharanthus_roseus_3**: KJ873884 **Catharanthus_roseus_4**: KJ873885 **Catharanthus_roseus_5**: KJ873886 (ISY) **Catharanthus_roseus_6**: KJ873887	Iridoids[3]
Coccomyxa subellipsoidea, Coccomyxaceae (green alga)	**Coccomyxa_subellipsoidea**: XM_005645604	
Dendrobium catenatum, Orchidaceae, Asparagales (monocot)	**Dendrobium_catenatum_1**: XM_020838092 **Dendrobium_catenatum_2**: XM_020848973	
Diphasiastrum digitatum, Lycopodiaceae, Lycopodiales (lycophyte)	**Diphasiastrum_digitatum_1**: WAFT-2005045[1] **Diphasiastrum_digitatum_2**: WAFT-2014713[1] **Diphasiastrum_digitatum_3**: WAFT-2013184[1]	
Digitalis lanata, Plantaginaceae, Lamiales (core eudicot)	**Digitalis_lanata_1**: AY585867 **Digitalis_lanata_2**: HM210089	Cardenolides[4]
Digitalis purpurea, Plantaginaceae, Lamiales (core eudicot)	**Digitalis_purpurea_1**: AJ310673 **Digitalis_purpurea_2**: GU062787	
Erysimum crepidifolium, Brassicaceae, Brassicales (core eudicot)	**Erysimum_crepidifolium_1**: ADG56544 (GU354236) **Erysimum_crepidifolium_2**: KF234078	Cardenolides[5]
Gossypium raimondii, Malvaceae, Malvales (core eudicot)	**Gossypium_raimondii_1**: XM_012589862 **Gossypium_raimondii_2**: XM_012589863 **Gossypium_raimondii_3**: XM_012627262 **Gossypium_raimondii_4**: XM_012624714 **Gossypium_raimondii_5**: XM_012583385	
Isoetes tegetiformans, Isoetaceae, Isoetales (lycophyte)	**Isoetes_tegetiformans_1**: PKOX-2097590[1] **Isoetes_tegetiformans_2**: PKOX-2098237[1]	
Lactuca sativa, Asteraceae, Asterales (core eudicot)	**Lactuca_sativa_1:** XM_023873729 **Lactuca_sativa_2:** XM_023881031 **Lactuca_sativa_3:** XM_023890552 **Lactuca_sativa_4:** XM_023900544 **Lactuca_sativa_5:** XM_023900546 **Lactuca_sativa_6:** XM_023902925 **Lactuca_sativa_7:** XM_023876481	
Musa acuminata, Musaceae, Zingiberales (monocot)	**Musa_acuminata_1**: XM_009407733 **Musa_acuminata_2**: XM_009398620 **Musa_acuminata_3**: XM_009404020	
Mentha x *piperita*, Lamiaceae, Lamiales (core eudicot)	**Mentha_piperita**: GU451677	

Marchantia polymorpha, Marchantiaceae, Marchantiales (bryophyte)	**Marchantia_polymorpha**: Mapoly0190s0002[2]	
Medicago truncatula, Fabaceae, Fabales (core eudicot)	**Medicago_truncatula_1**: XM_013606299 **Medicago_truncatula_2**: XM_013606297 **Medicago_truncatula_3**: XM_003609687 **Medicago_truncatula_4**: XM_003609689	
Nelumbo nucifera, Nelumbonaceae, Proteales (eudicot)	**Nelumbo_nucifera_1**: XM_010273442 **Nelumbo_nucifera_2**: XM_010278835 **Nelumbo_nucifera_3**: XM_010261051 **Nelumbo_nucifera_4**: XM_010274509 **Nelumbo_nucifera_5**: XR_737103	
Nepeta cataria, Lamiaceae, Lamiales (core eudicot)	**Nepeta_cateria_1:** KY882233 **Nepeta_cateria_2:** KY882234	Iridoids[6]
Olea europaea, Oleaceae, Lamiales (core eudicot)	**Olea_europaea_1**: KT954038 (ISY) **Olea_europaea_2**: KT954040	Iridoids[7]
Phaeoceros carolinianus, Notothyladaceae, Notothyladales (bryophyte)	**Phaeoceros_carolinianus_1**: RXRQ-2018832[1] **Phaeoceros_carolinianus_2**: RXRQ-2138560[1]	
Pellia epiphylla, Pelliaceae, Metzgeriales (bryophyte)	**Pellia_epiphylla_1**: PIUF-2092008[1] **Pellia_epiphylla_2**: PIUF-2012430[1]	
Picea glauca, Pinaceae, Pinales (gymnosperm)	**Picea_glauca_1**: BT118472 **Picea_glauca_2**: BT111670 **Picea_glauca_3**: BT111257	
Physcomitrella patens, Funariaceae, Funariales (bryophyte)	**Physcomitrella_patens**: XM_001754153	
Ricinus communis, Euphorbiaceae, Malpighiales (core eudicot)	**Ricinus_communis_1**: XM_002510077 **Ricinus_communis_2**: KP282668 **Ricinus_communis_3**: XM_002518611 **Ricinus_communis_4**: XM_002525592	
Sesamum indicum, Pedaliaceae, Lamiales (core eudicot)	**Sesamum_indicum_1**: XM_011071474 **Sesamum_indicum_2**: XM_011094221 **Sesamum_indicum_3**: XM_011071475 **Sesamum_indicum_4**: XM_011089881 **Sesamum_indicum_5**: XM_011082559	Iridoids[8]
Selaginella moellendorfii, Selaginellaceae, Selaginalles (lycophyte)	**Selaginella_moellendorfii_1**: XM_002967475 **Selaginella_moellendorfii_2**: XM_002960044 **Selaginella_moellendorfii_3**: XM_002991948 **Selaginella_moellendorfii_4**: XM_002989781 **Selaginella_moellendorfii_5**: XM_002960137 **Selaginella_moellendorfii_6**: XM_002969631 **Selaginella_moellendorfii_7**: XM_002960138 **Selaginella_moellendorfii_8**: XM_002981242 **Selaginella_moellendorfii_9**: XM_002989782 **Selaginella_moellendorfii_10**: XM_002984001	
Sorghum bicolor, Poaceae, Poales (monocot)	**Sorghum_bicolor_1**: XM_002465047 **Sorghum_bicolor_2**: XM_002449218 **Sorghum_bicolor_3**: XM_002467624	
Vitis vinifera, Vitaceae, Vitales (core eudicot)	**Vitis_vinifera_1**: XM_002272235 **Vitis_vinifera_2**: FQ381232 **Vitis_vinifera_3**: XM_002276123 **Vitis_vinifera_4**: XM_002277929	

* All sequence accession (ID) numbers are from GenBank unless otherwise indicated.
[1] Sequences from the 1KP Project[9]
[2] Sequence from the *Marchantia polymorpha* genome database[10]

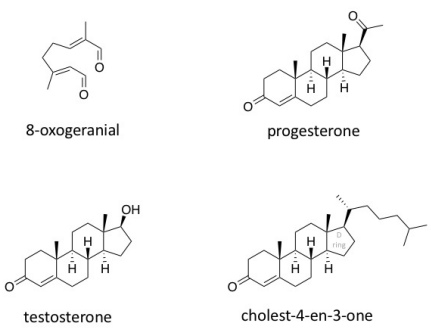

Figure S1. Substrates used in enzyme assays with PRISEs.

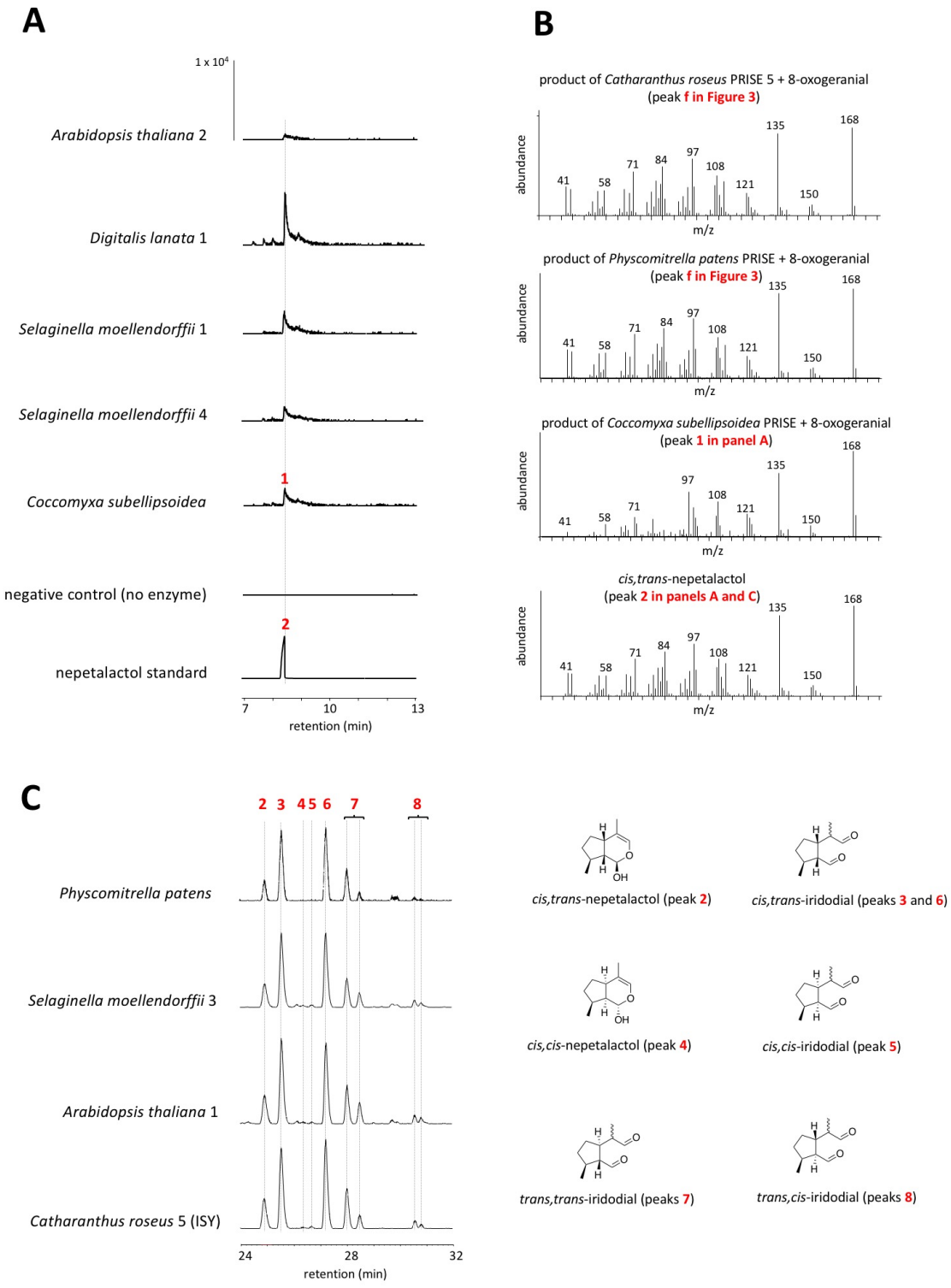

Figure S2. GC-MS analysis of activities on 8-oxogeranial by selected PRISEs.
A: Extracted ion chromatograms (*m/z* 168) from GC-MS analyses of assays with product levels too low to be observed on the normalized scale used in Figure 3.

B: EI mass spectrum comparison between authentic standard of nepetalactol, major product from the previously-characterized iridoid synthase from *Catharanthus roseus*,[11] and products from selected PRISEs in this study.
C: GC-MS chromatogram using the chiral column (Supelco, β-DEX 225, 30 m x 250 µm x 0.25 µm) and method as described by Kries and co-workers[12] to compare product profiles of 8-oxogeranial reduction by selected PRISEs across land plants.

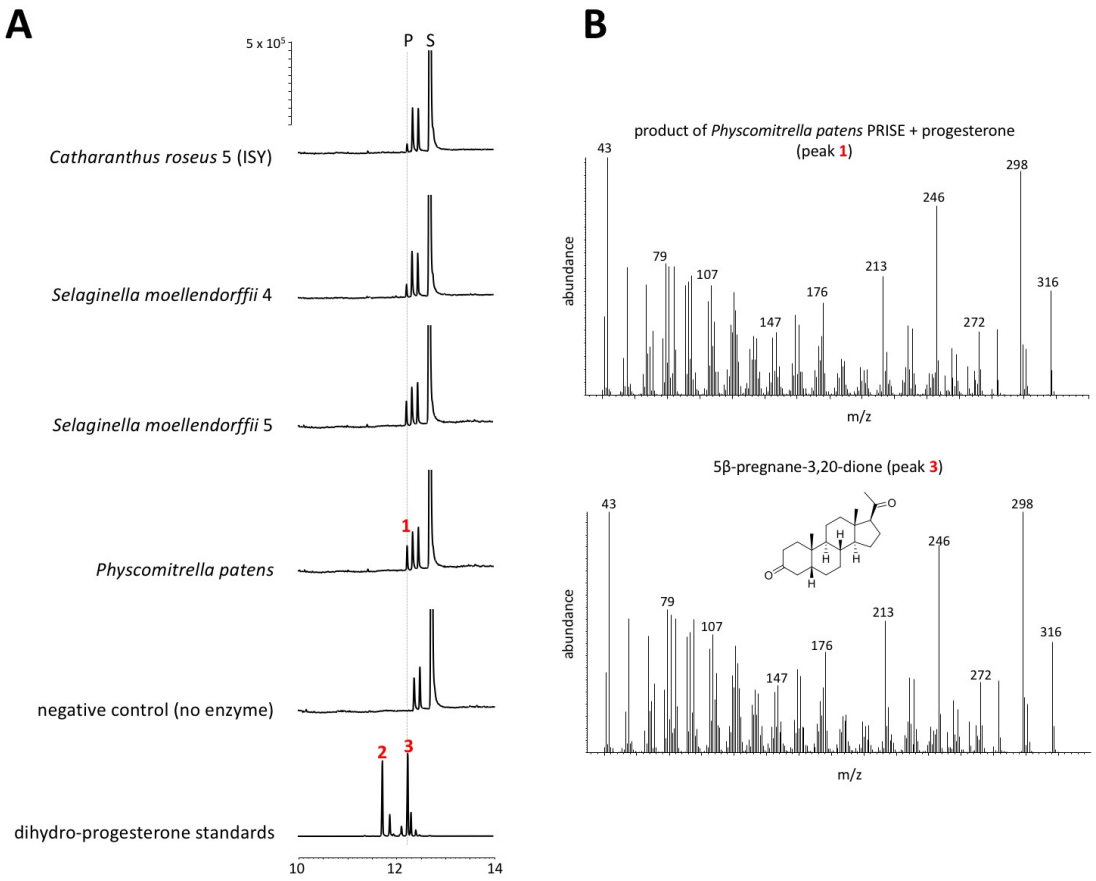

Figure S3. GC-MS analysis of activities on progesterone by selected PRISEs
A: GC-MS chromatograms of assays with product levels too low be observed on the normalized scale used in Figure 3. S: substrate. P: expected product.
B: EI mass spectrum comparison between authentic standard of 5β-pregnane-3,20-dione (5β-dihydroprogesterone) and product by PRISE from the moss *Physcomitrella patens*.

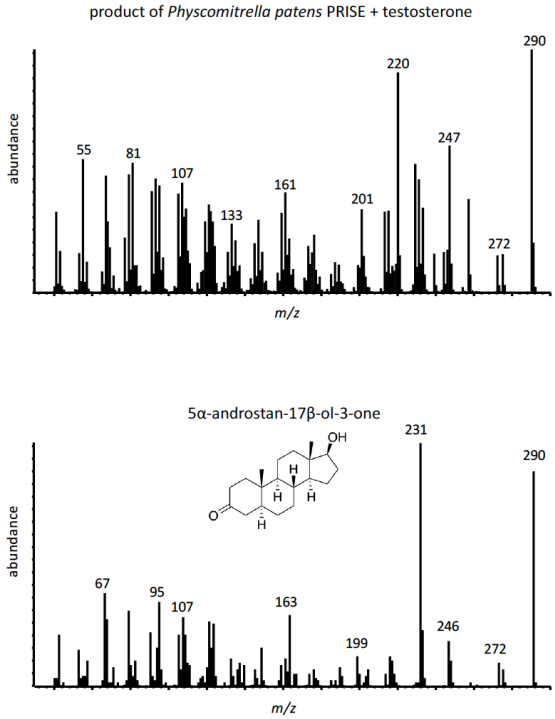

Figure S4. EI mass spectrum comparison between authentic standard of 5α-androstan-17β-ol-3-one (5α-dihydrotestosterone) (bottom) with the product from the assay of *Physcomitrella patens* (moss) PRISE with testosterone (top). Results indicated that reduction occurred but the product was not 5α-dihydrotestosterone**.**

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