Supplementary information for Scientific Reports

Characterization of the polyphenol oxidase gene family reveals a novel microRNA involved in posttranscriptional regulation of *PPOs* in *Salvia miltiorrhiza*

Caili Li#, Dongqiao Li#, Jiang Li, Fenjuan Shao, Shanfa Lu*

Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, No.151, Malianwa North Road, Haidian District, Beijing 100193, China.

Supplementary Table S1 Primers used for 5'-RACE of *SmPPOs*.

Gene name	Primer sequence (5'-3')
SmPPO13	Nesting: TGGATTCCGGGTCATGCCGAGATCC
	Nested: GTCAGACATCGGTGGGCAGCAGTTCATG
SmPPO14	Nesting: AGATCCGTGGGAGCAGGCGGGAGAT
	Nested: TTGCATGAAACTGCGCGGGTCATCG
SmPPO15	Nesting: CGTACGTATTCCGGTGTCAGCCTGTGCG
	Nested: TGCGATTGAGTGGGTTGCGGTTGCAG
SmPPO16	Nesting: CCTGTTCGTAGCGGCCGGTACAGTAAGC
	Nested: AGCCGCCCTCCGACAATCCTCCCTT
SmPPO17	Nesting: TCTTCTACGCACATCACGCCAACG
	Nested: TCTTTCCTTACCCAGGGCCTCAATC
SmPPO18	Nesting: CCCGACGACTTCAACAACGCCTCC
	Nested: GAGGATTGTCGGAGGGCGGCTGTCT
SmPPO19	Nesting: ATCTCTAGACGTCAACTGCTGCCCG
	Nested: CGCTTCCGATTTTGCCTCCTGACCT

Supplementary Table S2 Primers used for 3'-RACE of *SmPPOs*.

Gene name	Primer sequence (5'-3')
SmPPO17	Nesting: CACGGAGTTTCGCTTTCTTCATAC
	Nested: ACTTCGTCGGCTTTGCCTTTGGTTG
SmPPO18	Nesting: ACTTCGTCGGCTTTGCCTTTGGT
	Nested: CGCTTTCCGCAGTCCTAACCTTAT
SmPPO19	Nesting: TGGCGAGAACTTTGGCATTGACC
	Nested: CGTCGGCTTTGCCTTTGGTTGGT

Supplementary Table S3 Primers used for the PCR-amplification of coding sequences of *SmPPOs*

Gene name	Primer sequence (5'-3')
SmPPO1	Forward: ATGGCTTCGTCGATTCTCTCACAT
	Reverse: GTCATCAAGCTCGATCTTGATATCATG
SmPPO2	Forward: ATGTCATCTGTTCCTCTTTCCACCG
	Reverse: CCACCAATGGTAATATCTTCTCCTCCA
SmPPO3	Forward: ATGGCATTTCCTCCACTTTCCTC
	Reverse: TGACACCACCAATGGTAATGTCTTC
SmPPO4	Forward: ATGGCCTCTCTTCAATCTTCATGC
	Reverse: CGCGACTTAACAATTTTGATACCA
SmPPO5	Forward: ATGGCTTCCCTTCAATCTTCTTTCTC
	Reverse: GCGGGATGATCTTAATACCACCAAT
SmPPO6	Forward: ATGGCTTCCCTCTACCTTCATTGCA
	Reverse: AGCTTTAGGTGGATTCTCAATGATCTTGA
SmPPO7	Forward: ATGGCTTCTCTTCCATCTTCACTCA
	Reverse: AGCAGCAACACGCGGGATAATCT
SmPPO8	Forward: ATGGCTTCCCTTCAATCTTCATTC
	Reverse: ACACGCGGGATGATCTTAATACC
SmPPO9	Forward: ATGGCTTCTTCCCTTCAGTCCTTAAG
	Reverse: TTTACGCTGCGCTGGGATGA
SmPPO10	Forward: ATGGCTTCCCTTCAATCTTCATTCAC
	Reverse: TCAAGCGGTAACACGCGGGAT
SmPPO11	Forward: ATGGCTTCTTCCCTTATTGCTTCATG
	Reverse: ACGCTGCGCTGGGACGATCTT
SmPPO12	Forward: ATGGCTTCCCTTCAATCTTCATTCA
	Reverse: AGCGGTAACACGCGGGATGATC
SmPPO13	Forward: ATGGCTTCCCTCTACCTTCATTGC
	Reverse: TCAAGATCTTCGGGGTGGAGAAG
SmPPO14	Forward: ATGGCATCACTTCCGTTTTCCAC
	Reverse: TTAAACGTAGATGATCTTGACCCCAC
SmPPO15	Forward: ATGGCTTCCCTTCAATCTTCATTC
	Reverse: CACGCGGGATGATCTTAATACCA
SmPPO16	Forward: ATGGGGCACCAAACCATGGC
	Reverse: TCAAGCGGTAACACGAGGAATGATC
SmPPO17	Forward: ATGCTCCTCGGTCTTGGAGGGC
	Reverse: TCAAACGGGAACACGCGGGATG
SmPPO18	Forward: ATGGCGAAGATGAGGGAGCTG
	Reverse: TCAAGCGGTAACACGCGGGATG
SmPPO19	Forward: ATGGCTTCCCTTCAATCTTC
	Reverse: TCAAGCAGTAACACGCGGGATG

Supplementary Table S4 Gene names of *PPOs*.

Gene name	Accession no.
Populus trichocarpa	
PtPPO1	Potri.011G108300
PtPPO2	Potri.001G387900
PtPPO3	Potri.011G047300
PtPPO4	Potri.T062100
PtPPO5	Potri.001G388600
PtPPO6	Potri.001G388400
PtPPO7	Potri.001G388300
PtPPO8	Potri.T061900
PtPPO9	Potri.001G388100
PtPPO10	Potri.011G108200
PtPPO11	Potri.001G388900
PtPPO12	Potri.001G388800
PtPPO13	Potri.004G156500
PtPPO14	Potri.001G388200
PtPPO15	Potri.T062200
PtPPO16	Potri.001G388000
Glycine max	
GmPPO1	Glyma06g42170.1
GmPPO2	Glyma07g31270.1
GmPPO3	Glyma07g31280.1
GmPPO4	Glyma07g31300.1
GmPPO5	Glyma07g31310.1
GmPPO6	Glyma13g25150.1
GmPPO7	Glyma13g25180.1
GmPPO8	Glyma13g25260.1
GmPPO9	Glyma13g31590.1
GmPPO10	Glyma15g07710.1
GmPPO11	Glyma18g45900.1
Zea mays	
ZmPPO1	GRMZM2G108103_T01
ZmPPO2	GRMZM2G121605_T01
ZmPPO3	AC233851.1_FGT017
ZmPPO4	GRMZM2G319062_T01
ZmPPO5	GRMZM2G137909_T01
ZmPPO6	AC209206.3_FGT014
Oryza sativa	
OsaPPO1	LOC_Os01g58100.1
OsaPPO2	LOC_Os04g53300.1
Phycomitrella patens	
PpPPO1	AAX69084.1
PpPPO2	XP_001770109.1

PpPPO3	XP_001785624.1
PpPPO4	XP_001752158.1
PpPPO5	XP_001773770.1
PpPPO6	XP_001785949.1
PpPPO7	XP_001767174.1
PpPPO8	XP_001766337.1
PpPPO9	XP_001766259.1
PpPPO10	XP_001766553.1
PpPPO11	XP_001755077.1
PpPPO12	XP_001785245.1

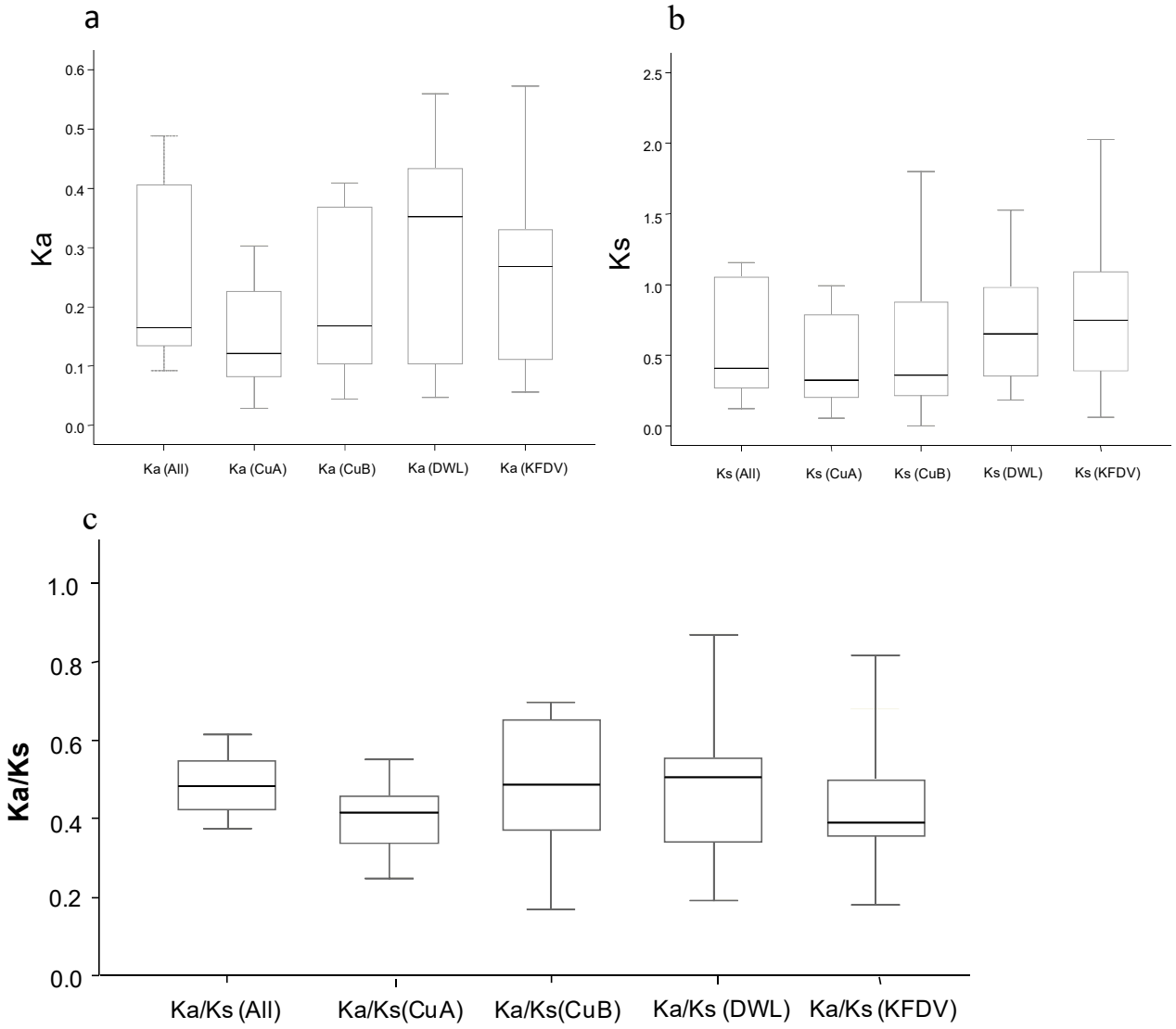
Supplementary Table S5 Primers used for quantitative real-time RT-PCR of *SmPPOs*

Gene name	Primer sequence (5'-3')
SmPPO1	Forward: GAAGAGGAGCAAGAAGGAGAAG
	Reverse: TCGTCCTCGTCGTTGATGTA
SmPPO2	Forward: TCGTGAGGTTTGAGGTGTTC
	Reverse: AGCCTGATCTTGCTCTTCAC
SmPPO3	Forward: GGTAAGGGTGTTGGTGGATAG
	Reverse: TATTTGGCGGTGTCGATCTC
SmPPO4	Forward: GCCTTCTACTCCCATCACTCTA
	Reverse: CGTTGAGGTAGTCAGGTTTGTT
SmPPO5	Forward: CGGTCAATCTCAAGGACATCT
	Reverse: CCTTTGGTTGGTTTGGACAC
SmPPO6	Forward: GCATGTGGACTCTATGGAAGG
	Reverse: GAAAGAGGCGTTGAGGTAGTC
SmPPO7	Forward: CACAGTGTTCCCTCTCAAACT
	Reverse: CCTCACGAGCTTGCTGTTAT
SmPPO8	Forward: CAAAGTGTTCCCTCTCACTCTC
	Reverse: AACTCACCAGCTTGGAACTATC
SmPPO9	Forward: CTGAAGAAGGCGGTTAGAGTTT
	Reverse: TTGGTGCAATCGGTCTCTATG
SmPPO10	Forward: GGTGCATATCGAAGAGTGGAT
	Reverse: GAGAGTGAGAGGGAACAGATTG
SmPPO11	Forward: GGAGCTCTACGAGAACATTGAC
	Reverse: GACGATCTTGACACCACCAA
SmPPO12	Forward: CAATCTGTTCCCTCTCACTCTC
	Reverse: ACCTCACGACCTTCAAACTATC
SmPPO13	Forward: TGTGGACTCTGTGGCAATC
	Reverse: GCGTCCTCGTCGTAGAATAAG
SmPPO14	Forward: CGACTACGGCTAAGGCAATTA
	Reverse: CTCCTTCTCCTTCTTGCTTCTC

SmPPO15	Forward: AACTCCATGGATGGCTTACAG Reverse: ACAGTGTTGGTGAGAGTGATG
SmPPO16	Forward: CGTGTATGAACACAGCGAAAC Reverse: GAGAGTGAGAGGGAACAGATTG
SmPPO17	Forward: CAATGTGTTCCCTCTCACTCTC Reverse: AACTCACCAGCTTGGAACTATC
SmPPO18	Forward: TCGTGAGGTTCGATGTGTTC Reverse: CTTTCCGCAGTCCTAACCTTAT
SmPPO19	Forward: CCTCTCACTCTCACCAAGACTA Reverse: CCTCACGATCTTGGACCTTTC

Supplementary Table S6 Primers used for analysis of Smi-miR12112-directed cleavage of *SmPPOs*

Gene name	Primer sequence (5'-3')
SmPPO3	Nesting: GCAGCAAGCAGTACAAGTAGTG Nested: GTGTAGACGATGATTCACAGAAG
SmPPO5	Nesting: GTCGACAAATCTCAAGCAACATGGAG Nested: GAAACATTGGCGATGGATGCAG
SmPPO9	Nesting: CGGTTAACCGAACCATAAACCGG Nested: GATATGTAGATTTTATTGTGGAGC
SmPPO11	Nesting: GCTTCCCCTCTCTCACACACACAC Nested: CATTGGAGGAATTGGAACATGCG
SmPPO13	Nesting: GGTCTGCCTCCATGGAACGATTGTC Nested: GGAGAAATGCTAGCGTAGGGC



Supplementary Figure S1 Divergence of different classes in SmPPOs. (a) the Ka values of different classes in SmPPOs; (b) the Ks values of different classes in SmPPOs; (c) the Ka/Ks ratios of different classes in SmPPOs.