Functional integration of two CYP450 genes involved in biosynthesis of tanshinones for improved diterpenoid production by synthetic biology

Yaping Mao[a,†], Ying Ma[a,†],Tong Chen[a], Xiaohui Ma[b], Yanqin Xu[c], Junling Bu[a], Qishuang Li[a,d], Baolong Jin[a], Yanan Wang[a], Yong Li[a,e], Guanghong Cui[a], Yujun Zhao[a], Jinfu Tang[a], Ye Shen[a], Changjiangsheng Lai[a], Wen Zeng[a] , Min Chen[a], Juan Guo [a,*], and Luqi Huang [a,*]

[a] State Key Laboratory Breeding Base of Dao-di Herbs, National Resource Center for Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing 100700, China;

[b] Yunnan University of Traditional Chinese Medicine,Kunming 650500,China;

[c] College of Pharmacy, Jiangxi University of Traditional Chinese Medicine, Nanchang 330004, China;

[d] School of Pharmacy, Jiangsu University, Zhenjiang 212013,China;

[e] Shandong University of Traditional Chinese Medicine, Jinan 250355, China.

[†] These authors have equally contributed to this manuscript.

[*] Authors to whom correspondence should be addressed:

E-mail: guojuanzy@163.com; ORCID: 0000-0002-1327-4428 (J.G.)

E-mail: huangluqi01@126.com; ORCID: 0000-0002-2070-4318 (L-Q.H)

Tel.: +86-010-64087469 (J.G.); Fax: +86-010-84027175 (J.G.).

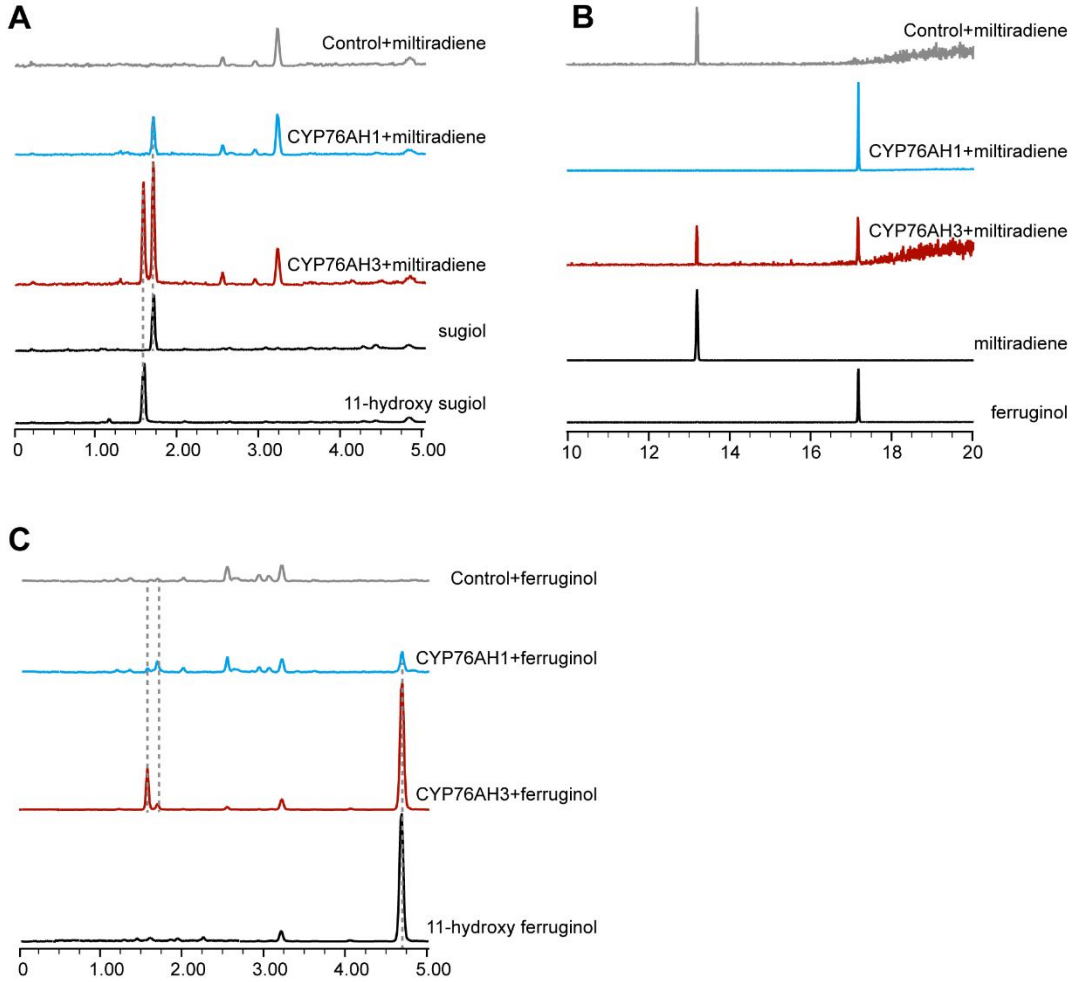

Figure S1. *In vitro* enzyme assay of CYP76AH1 and CYP76AH3 using miltiradiene (**A**, **B**) and ferruginol (**C**) as substrate. **A** and **C** are enzyme reaction analysis in UPLC-Qtof-MS, **B** is the GC-MS result of miltiradiene enzyme reaction.

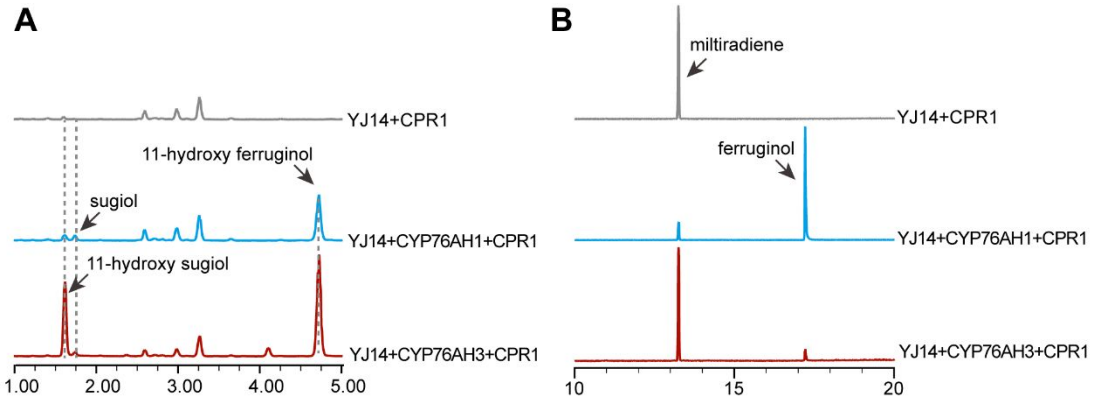

Figure S2. UPLC-Qtof-MS result (**A**) and GC-MS result (**B**) of diterpnoid production in the yeast strain harboring miltiradiene production module (YJ14) plus *CPR1* from *Salvia miltiorrhiza* (grey), miltiradiene production module (YJ14) plus *CYP76AH1* and *CPR1* (blue), and miltiradiene production module (YJ14) plus *CYP76AH3* and *CPR1* (red).

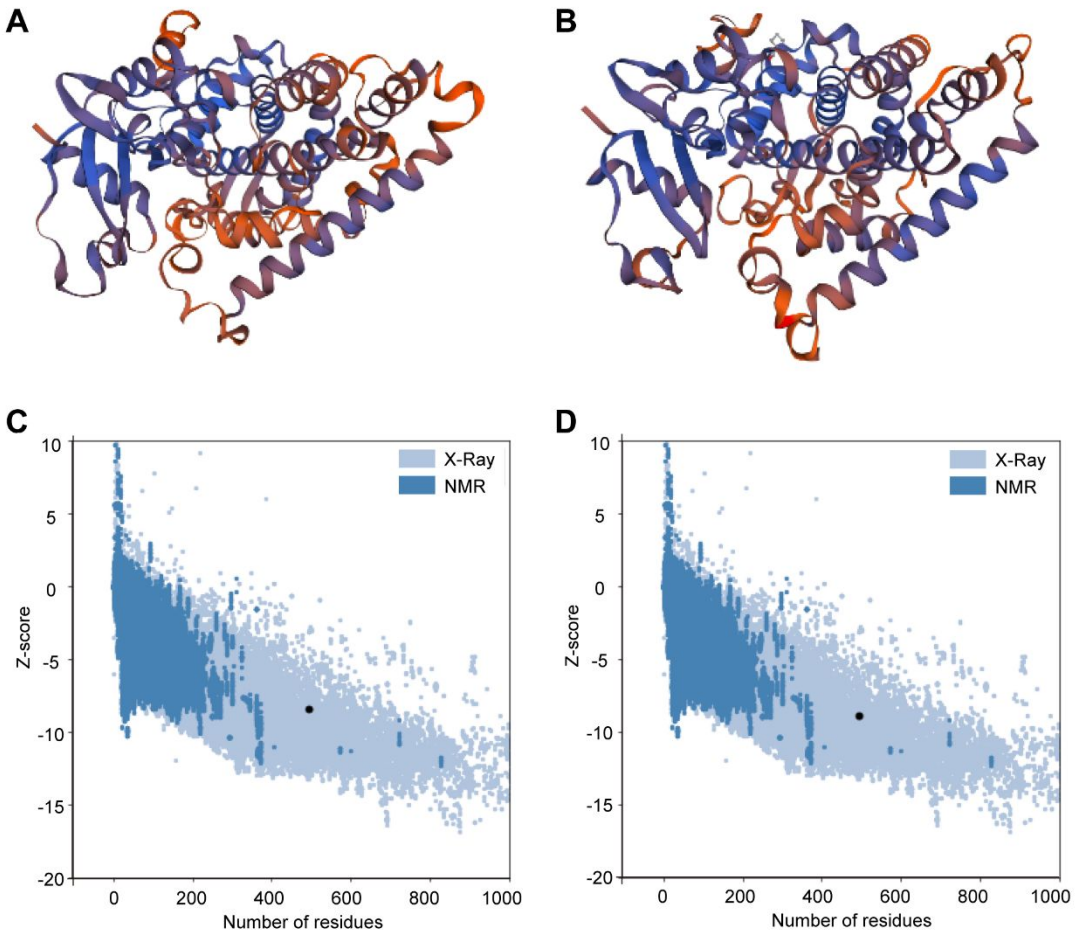

Figure S3. Homology modeling of CYP76AH1 (**A**) using the crystal structure of CYP1A2 from human as template, and homology modeling of CYP76AH3 (**B**) using the crystal structure of CYP17A1 from zebra fish as template. The ProSA energy graphs for CYP76AH1= −8.4 with 495 amino acid residues in the model (**C**) and for CYP76AH3= −8.84 with 494 amino acid residues in the model (**D**) were all in a negative range indicating the reasonable model for model.

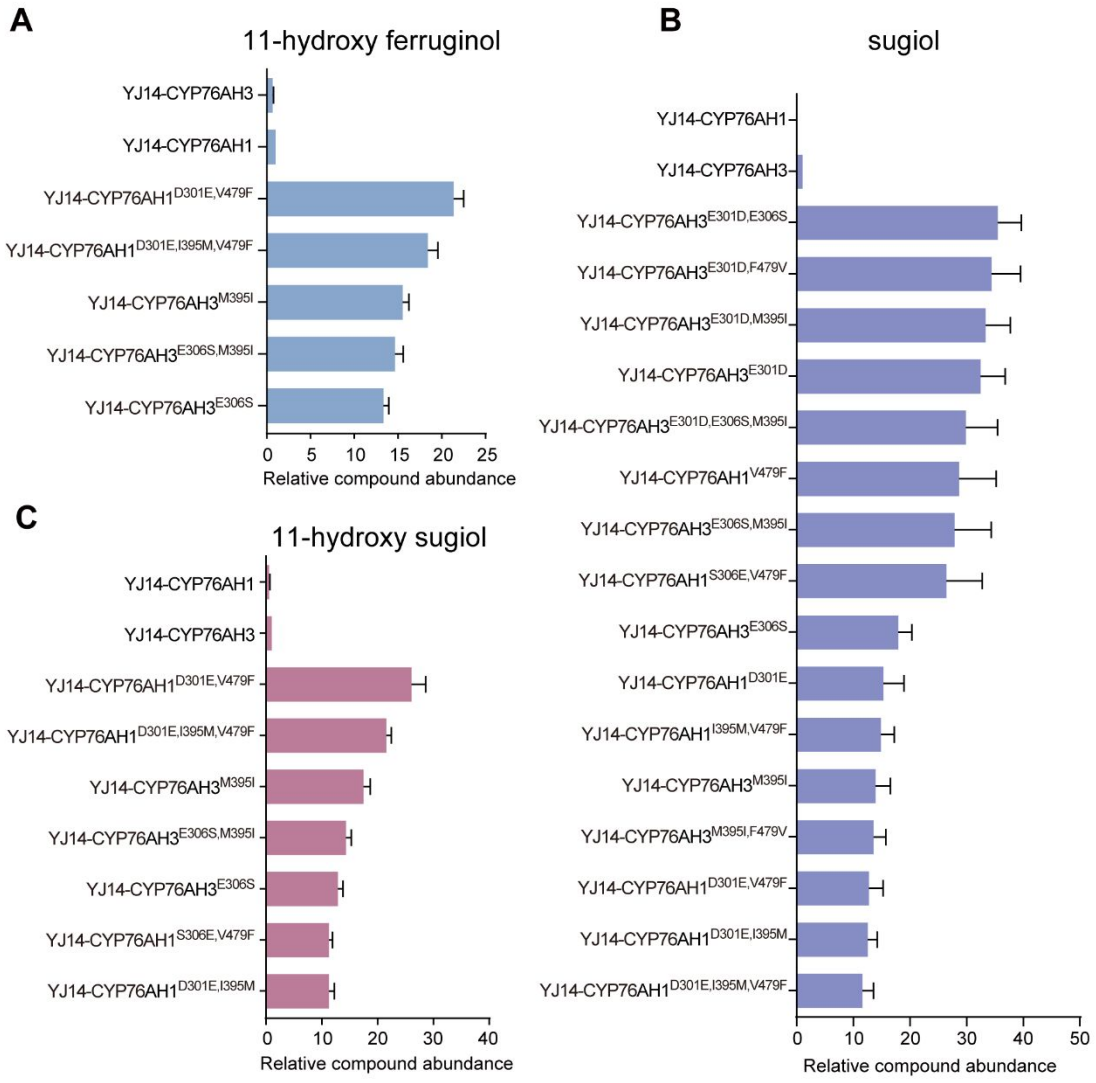

Figure S4. Relative yields of 11-hydroxy ferruginol(**A**), sugiol(**B**), and 11-hydroxy sugiol(**C**) from miltiradiene in yeast strains harboring SmCPR1 plus either CYP76AH1, CYP76AH3, and their mutated substitutes after fermentation. Yields higher than YJ14-CYP76AH1/CYP76AH3 were showed.

Table S1. *S. cerevisiae* strains used in this study.

Strains	**Genotype or characteristic**	**Resource**
BY4741	*MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0*	ATCC
YJ14	BY4741/pYX212-(*BTS1-ERG20*)+(*SmKSL-SmCPS*)	***15***
YJ14-CYP76AH1	YJ14/pESC-HIS-*CYP76AH1*/pESC-LEU-*SmCPR1*	This study
YJ14-CYP76AH3	YJ14/pESC-HIS-*CYP76AH3*/pESC-LEU-*SmCPR1*	This study
YJ14-CYP76AH1^{D301E,V479F}	YJ14/pESC-HIS-*CYP76AH1*^{D301E,V479F}/pESC-LEU-*SmCPR1*	This study
YJ51	BY4741/*LEU2*/*MET15*/pYX212-(*BTS1-ERG20*)+(*SmKSL1-SmCPS1*)+*CYP76AH1*+*SmCPR1*/pESC-HIS-*CYP76AH3*	***10***

Table S2. Primers used in this study.

Primer	Sequence(5'to3')
76AH1-HisBamHI-F1	CTTTAACGTCAAGGAGAAAAAACCCCGATGGATTCTTTTCCTCTCCTCGCCGCGCTCT
76AH1-D301E-R1	CATGGCCCACTCGATCGAGGTCGTGTTCGTCTCCGATCCTCCCACGAACAAGTCCAGC
76AH1-D301E-F2	GCTGGACTTGTTCGTGGGAGGATCGGAGACGAACACGACCTCGATCGAGTGGGCCATG
76AH1-HisBamHI-R2	GACGCCCGGGCCCTATAGTGAGTCGTATTACGGCTAAGACTTAACTATTGGGATAATC
76AH1-HisBamHI-F1	CTTTAACGTCAAGGAGAAAAAACCCCGATGGATTCTTTTCCTCTCCTCGCCGCGCTCT
76AH1-S306E-R1	CATGGCCCACTCGATCTCGGTCGTGTTCGTGTCCGATCCTCCCACGAACAAGTCCAGC
76AH1-S306E-F2	GCTGGACTTGTTCGTGGGAGGATCGGACACGAACACGACCGAGATCGAGTGGGCCATG
76AH1-HisBamHI-R2	GACGCCCGGGCCCTATAGTGAGTCGTATTACGGCTAAGACTTAACTATTGGGATAATC
76AH1-HisBamHI-F1	CTTTAACGTCAAGGAGAAAAAACCCCGATGGATTCTTTTCCTCTCCTCGCCGCGCTCT
76AH1-I395M-R1	GGTCCAGATACTCGGATCCCTCCCCATCGCATACGCGTTGATGAGTATCTGAGTTCCC
76AH1-I395M-F2	GGGAACTCAGATACTCATCAACGCGTATGCGATGGGGAGGGATCCGAGTATCTGGACC
76AH1-HisBamHI-R2	GACGCCCGGGCCCTATAGTGAGTCGTATTACGGCTAAGACTTAACTATTGGGATAATC
76AH1-HisBamHI-F1	CTTTAACGTCAAGGAGAAAAAACCCCGATGGATTCTTTTCCTCTCCTCGCCGCGCTCT
76AH1-V479F-R1	GAACCCAAACAACTCGCCGGCGTGATCGGCGGCCGCCGTGGAGTCATCTTCCAGTTTC
76AH1-V479F-F2	GAAACTGGAAGATGACTCCACGGCGGCCGCCGATCACGCCGGCGAGTTGTTTGGGTTC
76AH1-HisBamHI-R2	GACGCCCGGGCCCTATAGTGAGTCGTATTACGGCTAAGACTTAACTATTGGGATAATC
76AH3-HisBamHI-F1	CTTTAACGTCAAGGAGAAAAAACCCCGATGGATTCTTTCTCTCTTCTGGCTGCACTCT
76AH3-E301D-R1	CATGATCCACTCGATCTCGGTGGTGCTCGTGTCCGAACCCCCGACGAAGAGGTCGAGC
76AH3-E301D-F2	GCTCGACCTCTTCGTCGGGGGTTCGGACACGAGCACCACCGAGATCGAGTGGATCATG
76AH3-HisBamHI-R2	GTGCCGCTCAGGATCGTTCCGTATAAGGCATGAGCTAAGACTTAACTATTGGGATAAT
76AH3-HisBamHI-F1	CTTTAACGTCAAGGAGAAAAAACCCCGATGGATTCTTTCTCTCTTCTGGCTGCACTCT
76AH3-E306S-R1	CATGATCCACTCGATCGAGGTGGTGCTCGTCTCCGAACCCCCGACGAAGAGGTCGAGC
76AH3-E306S-F2	GCTCGACCTCTTCGTCGGGGGTTCGGAGACGAGCACCACCTCGATCGAGTGGATCATG
76AH3-HisBamHI-R4	GTGCCGCTCAGGATCGTTCCGTATAAGGCATGAGCTAAGACTTAACTATTGGGATAAT
76AH3-HisBamHI-F1	TCAAGGAGAAAAAACCCCGATGGATTCTTTCTCTCTTCTGGCT
76AH3-M395I-R1	GTGGAGTCTCTGCCTATGGCCCACGCGTTGATGAGCACCTGAGT
76AH3-M395I-F2	GACTCAGGTGCTCATCAACGCGTGGGCCATAGGCAGAGACTCCA
76AH3-HisBamHI-R2	CTATAGTGAGTCGTATTACGGTGCCTTATACGGAACGATCC
76AH3-HisBamHI-F1	CTTTAACGTCAAGGAGAAAAAACCCCGATGGATTCTTTCTCTCTTCTGGCTGCACTCT
76AH3-F479V-R1	CACGCCAAACAACACGCCCTGGTGCTCGGCGAGGGGCGCATCGGGGCGTTCGAGCTTC
76AH3-F479V-F2	GAAGCTCGAACGCCCCGATGCGCCCCTCGCCGAGCACCAGGGCGTGTTGTTTGGCGTG
76AH3-HisBamHI-R2	GTGCCGCTCAGGATCGTTCCGTATAAGGCATGAGCTAAGACTTAACTATTGGGATAAT
76AH1-HisBamHI-F1	CTTTAACGTCAAGGAGAAAAAACCCCGATGGATTCTTTTCCTCTCCTCGCCGCGCTCT
76AH1-D301E,S306E-R1	CATGGCCCACTCGATCTCGGTCGTGTTCGTCTCCGATCCTCCCACGAACAAGTCCAGC
76AH1-D301E,S306E-F2	GCTGGACTTGTTCGTGGGAGGATCGGAGACGAACACGACCGAGATCGAGTGGGCCATG
76AH1-HisBamHI-R2	GACGCCCGGGCCCTATAGTGAGTCGTATTACGGCTAAGACTTAACTATTGGGATAATC
76AH1-HisBamHI-F1	CTTTAACGTCAAGGAGAAAAAACCCCGATGGATTCTTTTCCTCTCCTCGCCGCGCTCT
76AH1-D301E-R1	CATGGCCCACTCGATCGAGGTCGTGTTCGTCTCCGATCCTCCCACGAACAAGTCCAGC
76AH1-D301E-F2	GCTGGACTTGTTCGTGGGAGGATCGGAGACGAACACGACCTCGATCGAGTGGGCCATG
76AH1-I395M-R2	GGTCCAGATACTCGGATCCCTCCCCATCGCATACGCGTTGATGAGTATCTGAGTTCCC

76AH1-I395M-F3	GGGAACTCAGATACTCATCAACGCGTATGCGATGGGGAGGGATCCGAGTATCTGGACC
76AH1-HisBamHI-R3	GACGCCCGGGCCCTATAGTGAGTCGTATTACGGCTAAGACTTAACTATTGGGATAATC
76AH1-HisBamHI-F1	CTTTAACGTCAAGGAGAAAAAACCCCGATGGATTCTTTTCCTCTCCTCGCCGCGCTCT
76AH1-D301E-R1	CATGGCCCACTCGATCGAGGTCGTGTTCGTCTCCGATCCTCCCACGAACAAGTCCAGC
76AH1-D301E-F2	GCTGGACTTGTTCGTGGGAGGATCGGAGACGAACACGACCTCGATCGAGTGGGCCATG
76AH1-V479F-R2	GAACCCAAACAACTCGCCGGCGTGATCGGCGGCCGCCGTGGAGTCATCTTCCAGTTTC
76AH1-V479F-F3	GAAACTGGAAGATGACTCCACGGCGGCCGCCGATCACGCCGGCGAGTTGTTTGGGTTC
76AH1-HisBamHI-R3	GACGCCCGGGCCCTATAGTGAGTCGTATTACGGCTAAGACTTAACTATTGGGATAATC
76AH1-HisBamHI-F1	CTTTAACGTCAAGGAGAAAAAACCCCGATGGATTCTTTTCCTCTCCTCGCCGCGCTCT
76AH1-S306E-R1	CATGGCCCACTCGATCTCGGTCGTGTTCGTGTCCGATCCTCCCACGAACAAGTCCAGC
76AH1-S306E-F2	GCTGGACTTGTTCGTGGGAGGATCGGACACGAACACGACCGAGATCGAGTGGGCCATG
76AH1-I395M-R2	GGTCCAGATACTCGGATCCCTCCCCATCGCATACGCGTTGATGAGTATCTGAGTTCCC
76AH1-I395M-F3	GGGAACTCAGATACTCATCAACGCGTATGCGATGGGGAGGGATCCGAGTATCTGGACC
76AH1-HisBamHI-R3	GACGCCCGGGCCCTATAGTGAGTCGTATTACGGCTAAGACTTAACTATTGGGATAATC
76AH1-HisBamHI-F1	CTTTAACGTCAAGGAGAAAAAACCCCGATGGATTCTTTTCCTCTCCTCGCCGCGCTCT
76AH1-S306E-R1	CATGGCCCACTCGATCTCGGTCGTGTTCGTGTCCGATCCTCCCACGAACAAGTCCAGC
76AH1-S306E-F2	GCTGGACTTGTTCGTGGGAGGATCGGACACGAACACGACCGAGATCGAGTGGGCCATG
76AH1-V479F-R2	GAACCCAAACAACTCGCCGGCGTGATCGGCGGCCGCCGTGGAGTCATCTTCCAGTTTC
76AH1-V479F-F3	GAAACTGGAAGATGACTCCACGGCGGCCGCCGATCACGCCGGCGAGTTGTTTGGGTTC
76AH1-HisBamHI-R3	GACGCCCGGGCCCTATAGTGAGTCGTATTACGGCTAAGACTTAACTATTGGGATAATC
76AH1-HisBamHI-F1	CTTTAACGTCAAGGAGAAAAAACCCCGATGGATTCTTTTCCTCTCCTCGCCGCGCTCT
76AH1-I395M-R1	GGTCCAGATACTCGGATCCCTCCCCATCGCATACGCGTTGATGAGTATCTGAGTTCCC
76AH1-I395M-F2	GGGAACTCAGATACTCATCAACGCGTATGCGATGGGGAGGGATCCGAGTATCTGGACC
76AH1-V479F-R2	GAACCCAAACAACTCGCCGGCGTGATCGGCGGCCGCCGTGGAGTCATCTTCCAGTTTC
76AH1-V479F-F3	GAAACTGGAAGATGACTCCACGGCGGCCGCCGATCACGCCGGCGAGTTGTTTGGGTTC
76AH1-HisBamHI-R3	GACGCCCGGGCCCTATAGTGAGTCGTATTACGGCTAAGACTTAACTATTGGGATAATC
76AH3-HisBamHI-F1	CTTTAACGTCAAGGAGAAAAAACCCCGATGGATTCTTTCTCTCTTCTGGCTGCACTCT
76AH3-E301D,E306S-R1	CATGATCCACTCGATCGAGGTGGTGCTCGTGTCCGAACCCCCGACGAAGAGGTCGAGC
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76AH3-E301D-R1	CATGATCCACTCGATCTCGGTGGTGCTCGTGTCCGAACCCCCGACGAAGAGGTCGAGC
76AH3-E301D-F2	GCTCGACCTCTTCGTCGGGGGTTCGGACACGAGCACCACCGAGATCGAGTGGATCATG
76AH3-M395I-R2	CCAGATGGTGGAGTCTCTGCCTATGGCCCACGCGTTGATGAGCACCTGAGTCCCTTTC
76AH3-M395I-F3	GAAAGGGACTCAGGTGCTCATCAACGCGTGGGCCATAGGCAGAGACTCCACCATCTGG
76AH3-HisBamHI-R3	GTGCCGCTCAGGATCGTTCCGTATAAGGCATGAGCTAAGACTTAACTATTGGGATAAT
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76AH3-E306S-R1	CATGATCCACTCGATCGAGGTGGTGCTCGTCTCCGAACCCCCGACGAAGAGGTCGAGC
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76AH3-M395I-R2	CCAGATGGTGGAGTCTCTGCCTATGGCCCACGCGTTGATGAGCACCTGAGTCCCTTTC
76AH3-M395I-F3	GAAAGGGACTCAGGTGCTCATCAACGCGTGGGCCATAGGCAGAGACTCCACCATCTGG
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76AH1-V479F-R3	GAACCCAAACAACTCGCCGGCGTGATCGGCGGCCGCCGTGGAGTCATCTTCCAGTTTC
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76AH1-HisBamHI-R4	GACGCCCGGGCCCTATAGTGAGTCGTATTACGGCTAAGACTTAACTATTGGGATAATC
76AH1-HisBamHI-F1	CTTTAACGTCAAGGAGAAAAAACCCCGATGGATTCTTTTCCTCTCCTCGCCGCGCTCT
76AH1-S306E-R1	CATGGCCCACTCGATCTCGGTCGTGTTCGTGTCCGATCCTCCCACGAACAAGTCCAGC

76AH1-S306E-F2	GCTGGACTTGTTCGTGGGAGGATCGGACACGAACACGACCGAGATCGAGTGGGCCATG
76AH1-I395M-R2	GGTCCAGATACTCGGATCCCTCCCCATCGCATACGCGTTGATGAGTATCTGAGTTCCC
76AH1-I395M-F3	GGGAACTCAGATACTCATCAACGCGTATGCGATGGGGAGGGATCCGAGTATCTGGACC
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76AH1-V479F-F4	GAAACTGGAAGATGACTCCACGGCGGCCGCCGATCACGCCGGCGAGTTGTTTGGGTTC
76AH1-HisBamHI-R4	GACGCCCGGGCCCTATAGTGAGTCGTATTACGGCTAAGACTTAACTATTGGGATAATC
76AH3-HisBamHI-F1	CTTTAACGTCAAGGAGAAAAAACCCCGATGGATTCTTTCTCTCTTCTGGCTGCACTCT
76AH3-E301D,E306S-R1	CATGATCCACTCGATCGAGGTGGTGCTCGTGTCCGAACCCCCGACGAAGAGGTCGAGC
76AH3-E301D,E306S-F2	GCTCGACCTCTTCGTCGGGGGTTCGGACACGAGCACCACCTCGATCGAGTGGATCATG
76AH3-M395I-R2	CCAGATGGTGGAGTCTCTGCCTATGGCCCACGCGTTGATGAGCACCTGAGTCCCTTTC
76AH3-M395I-F3	GAAAGGGACTCAGGTGCTCATCAACGCGTGGGCCATAGGCAGAGACTCCACCATCTGG
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76AH3-E301D,E306S-R1	CATGATCCACTCGATCGAGGTGGTGCTCGTGTCCGAACCCCCGACGAAGAGGTCGAGC
76AH3-E301D,E306S-F2	GCTCGACCTCTTCGTCGGGGGTTCGGACACGAGCACCACCTCGATCGAGTGGATCATG
76AH3-F479V-R3	CACGCCAAACAACACGCCCTGGTGCTCGGCGAGGGGCGCATCGGGGCGTTCGAGCTTC
76AH3-F479V-F3	GAAGCTCGAACGCCCCGATGCGCCCCTCGCCGAGCACCAGGGCGTGTTGTTTGGCGTG
76AH3-HisBamHI-R3	GTGCCGCTCAGGATCGTTCCGTATAAGGCATGAGCTAAGACTTAACTATTGGGATAAT
76AH3-HisBamHI-F1	CTTTAACGTCAAGGAGAAAAAACCCCGATGGATTCTTTCTCTCTTCTGGCTGCACTCT
76AH3-E301D-R1	CATGATCCACTCGATCTCGGTGGTGCTCGTGTCCGAACCCCCGACGAAGAGGTCGAGC
76AH3-E301D-F2	GCTCGACCTCTTCGTCGGGGGTTCGGACACGAGCACCACCGAGATCGAGTGGATCATG
76AH3-M395I-R2	CCAGATGGTGGAGTCTCTGCCTATGGCCCACGCGTTGATGAGCACCTGAGTCCCTTTC
76AH3-M395I-F3	GAAAGGGACTCAGGTGCTCATCAACGCGTGGGCCATAGGCAGAGACTCCACCATCTGG
76AH3-F479V-R3	CACGCCAAACAACACGCCCTGGTGCTCGGCGAGGGGCGCATCGGGGCGTTCGAGCTTC
76AH3-F479V-F3	GAAGCTCGAACGCCCCGATGCGCCCCTCGCCGAGCACCAGGGCGTGTTGTTTGGCGTG
76AH3-HisBamHI-R4	GTGCCGCTCAGGATCGTTCCGTATAAGGCATGAGCTAAGACTTAACTATTGGGATAAT
76AH3-HisBamHI-F1	CTTTAACGTCAAGGAGAAAAAACCCCGATGGATTCTTTCTCTCTTCTGGCTGCACTCT
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76AH1-D301E,S306E-F2	GCTGGACTTGTTCGTGGGAGGATCGGAGACGAACACGACCGAGATCGAGTGGGCCATG
76AH1-I395M-R2	GGTCCAGATACTCGGATCCCTCCCCATCGCATACGCGTTGATGAGTATCTGAGTTCCC
76AH1-I395M-F3	GGGAACTCAGATACTCATCAACGCGTATGCGATGGGGAGGGATCCGAGTATCTGGACC
76AH1-V479F-R3	GAACCCAAACAACTCGCCGGCGTGATCGGCGGCCGCCGTGGAGTCATCTTCCAGTTTC
76AH1-V479F-F4	GAAACTGGAAGATGACTCCACGGCGGCCGCCGATCACGCCGGCGAGTTGTTTGGGTTC

76AH1-HisBamHI-R4	GACGCCCGGGCCCTATAGTGAGTCGTATTACGGCTAAGACTTAACTATTGGGATAATC
76AH3-HisBamHI-F1	CTTTAACGTCAAGGAGAAAAAACCCCGATGGATTCTTTCTCTCTTCTGGCTGCACTCT
76AH3-E301D,E306S-R1	CATGATCCACTCGATCGAGGTGGTGCTCGTGTCCGAACCCCCGACGAAGAGGTCGAGC
76AH3-E301D,E306S-F2	GCTCGACCTCTTCGTCGGGGGTTCGGACACGAGCACCACCTCGATCGAGTGGATCATG
76AH3-M395I-R2	CCAGATGGTGGAGTCTCTGCCTATGGCCCACGCGTTGATGAGCACCTGAGTCCCTTTC
76AH3-M395I-F3	GAAAGGGACTCAGGTGCTCATCAACGCGTGGGCCATAGGCAGAGACTCCACCATCTGG
76AH3-F479V-R3	CACGCCAAACAACACGCCCTGGTGCTCGGCGAGGGGCGCATCGGGGCGTTCGAGCTTC
76AH3-F479V-F3	GAAGCTCGAACGCCCCGATGCGCCCCTCGCCGAGCACCAGGGCGTGTTGTTTGGCGTG
76AH3-HisBamHI-R4	GTGCCGCTCAGGATCGTTCCGTATAAGGCATGAGCTAAGACTTAACTATTGGGATAAT