High-level production of sesquiterpene patchoulol in *Saccharomyces cerevisiae*

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31 **Table S1 Primers used in this study**

Primers	Primer sequence
PT-F	TATTTTGAAAATTCGAATTCATGGAATTGTATGCTCAATC
PT-R	ATTATTAAGAGCTCAGATCTTAATATGGAACTGGATGCAGGT
Gal10 F	AACTTCTTTGCGTCCATCCA
TADH R	GCAAGGTAGACAAGCCGACAAC
FPTS -F	AGGAGAAAAAACCCCGGATCCATGGCTTCAGAAAAAGAAATTAGG
FPTS -R	GCGGTACCAAGCTTACTCGAGTTAATATGGAACTGGATGCAGGT
PTS-F	ATTTTTGAAAATTCGAATTCATGGAATTGTATGCTCAATCAG
PTS-R	TAATTAAGAGCTCAGATCTTTAATATGGAACTGGATGCAGGT
ERG19-F	TCACTAAAGGGCGGCCGCATGACCGTTTACACAGCATC
ERG19-R	CTTGTAATCCATCGATACTAGTTTATTCCTTTGGTAGACCAGTCT
IDI-F	AGGAGAAAAAACCCCGGATCCATGACTGCCGACAACAATAGTA
IDI-R	GATCTTAGCTAGCCGCGGTACCTTATAGCATTCTATGAATTTGCCTG
ACS1-F	TCACTAAAGGGCGGCCGCATGACCGTTTACACAGCATC
ACS1-R	CTTGTAATCCATCGATACTAGTTTATTCCTTTGGTAGACCAGTCT
ERG10-F	CCTCACTAAAGGGCGGCCGCATGTCTCAGAACGTTTACATTG
ERG10-R	CTTGTAATCCATCGATACTAGTTCATATCTTTTCAATGACAATAGAG
GAL1-F	TCGAATTCATGACTGCCGACAACAATAGTATGCC
CYC-R	TAAGGCTGCTGCTGAACGTGGTTTAAGAGCTATGCTGGAAACAG
gre3 gF	TGGTTGTAGAATCAAGCCCGGTTTAAGAGCTATGCTGGAAACAG
gre3 gR	CGGGCTTGATTCTACAACCAGATCATTTATCTTTCACTGCGGAGA
HO gF	GCGAGTACTGGACCAAATCTGATCATTTATCTTTCACTGCGG
HO gR	AGATTTGGTCCAGTACTCGCGTTTAAGAGCTATGCTGGAAAC
1622 gF	GCATTCGGCGGATATGGAAGGATCATTTATCTTTCACTGCGG
1622 gR	CTTCCATATCCGCCGAATGCGTTTAAGAGCTATGCTGGAAACAG
1014 gF	CCTAGTATCCCGTGAATATGGATCATTTATCTTTCACTGCGG
1014 gR	CATATTCACGGGATACTAGGGTTTAAGAGCTATGCTGGAAACAG
911 gF	GGGAAACAAGACAATATTACGATCATTTATCTTTCACTGCGG
911 gR	GTAATATTGTCTTGTTTCCCGTTTAAGAGCTATGCTGGAAACAG
1021 gF	GCCTCTGTGTGGTGGTAATTGATCATTTATCTTTCACTGCGGAG
1021 gR	AATTACCACCACACAGAGGCGTTTAAGAGCTATGCTGGAAAC
pERG20 gF	ATTCGGTTTGGAAGGTGACCGATCATTTATCTTTCACTGCGG
pERG20 gR	GGTCACCTTCCAAACCGAATGTTTAAGAGCTATGCTGGAAAC
pHXT1-F	GCGTGTCCCGGTGGGTTCTGGGATGCAGGTCTCATCTGGAATATAATTCC
pHXT1-R	ATGCCAATTGTAATAGCTTTCCCATGATTTTACGTATATCAACTAGTTGACGATTATGA
gre3 UF	TGGGAATATTACCGCTCGAAGA
gre3 UR	AGAAGGTTTTGGGACGCTCGAAGGCACTGACAACTATTTGCTTCT
gre3 U CYC R	GGATATAGAAGCAAATAGTTGTCAGTGCCTTCGAGCGTCCCAAAACC
gre3 D TADH R	ACACATATACAGCATCGGAATGAGGCTGAATTGGAGCGACCTCAT
gre3 DF	AGCATGAGGTCGCTCCAATTCAGCCTCATTCCGATGCTGTATATG
gre3 DR	GACTATGGTGCAATCGCTGA
HO UF	CGGACAGCATCAAACTGTAAG

HO UR	AGCATGAGGTCGCTCCAATTCAGGACGACCAGGTCAGCTAGGGAG
HO U CYC R	AGCGGATCACGAAAAGTGATCTCGACTTCGAGCGTCCCAAAACCTTC
HO D TADH R	TTCCTCCCTAGCTGACCTGGTCGTCCTGAATTGGAGCGACCTCATGC
HO DF	TTGAGAAGGTTTTGGGACGCTCGAAGTCGAGATCACTTTTCGTGATCC
HO DR	CCAGCATGCTATAATGCTGG
1622 UF	CCACCAATTGCAGAGGGTAG
1622 UR	TGAGAAGGTTTTGGGACGCTCGAAGCCGTAAGTTCTTGGAAATCACT
1622 U CYC R	AAACTCTCCGAATCATTGTTCGGGACTGAATTGGAGCGACCTCAT
1622 D TADH R	TCCTAGTGATTTCCAAGAACTTACGGCTTCGAGCGTCCCAAAACC
1622 DF	ATAGCATGAGGTCGCTCCAATTCAGTCCCGAACAATGATTCGGAG
1622 DR	CACGTGCGGTCTAAGAAGAT
1014 UF	GGGATTGGAAAAAACTAATTAAGGAG
1014 UR	ATAGCATGAGGTCGCTCCAATTCAGCCTTTATGTGCGTATTGCTTTC
1014 U TADH R	CTACCATGAAAGCAATACGCACATAAAGGCTGAATTGGAGCGACCTCATG
1014 D CYC R	TTTCTTAATATTCGGCTATTTCTGACTTCGAGCGTCCCAAAACCT
1014 DF	AAGGTTTTGGGACGCTCGAAGTCAGAAATAGCCGAATATTAAGAAAATTG
1014 DR	TAACTTGACGTTTGTATATGTAAGG
911 UF	CTTTCCGTCTAAGGCTTCTAC
911 UR	ATAGCATGAGGTCGCTCCAATTCAGACATTTATATTTATGCCCATTCAAC
911 U CYC R	TATTTCATTTTTCATTTACTTCTCCACTTCGAGCGTCCCAAAACCTT
911 D TADH R	GTTGAATGGGCATAAATATAAATGTCTGAATTGGAGCGACCTCATGCTAT
911 DF	GAAGGTTTTGGGACGCTCGAAGTGGAGAAGTAAATGAAAAATGAAATAGC
911 DR	ACACCAGAGCATATTCAGTC
1021 UF	CTCCAAGGTAGGAAAGTCGA
1021 UR	ATGAGGTCGCTCCAATTCAGGGTTTAACTGCTAAGAGACACTG
1021 U CYC R	ATGAATTTCATCACGTGCGTCTTCGAGCGTCCCAAAACCT
1021 D TADH R	TGTCTCTTAGCAGTTAAACCCTGAATTGGAGCGACCTCAT
1021 DF	AGGTTTTGGGACGCTCGAAGACGCACGTGATGAAATTCATT
1021 DR	CTATGAATTTGTCAGCCGCA
TADH F	TCAAATCGTTGGTAGATACGTTGTT
gre3 yzR	AAAAGCCTTCAAGTTGGGTT
HO yzR	TGGCGTATTTCTACTCCAGCA
1622 yzR	GCTCTTGGACGTCCAACCTG
1014 yzR	GTGGGAATTTACGGCAAAAT
911 yzR	CGTTCGATAGGATTAGGGC
1021 yzR	TTTGAAGGCAATACGCTACTG
1021U ERG9 F	TGTCTCTTAGCAGTTAAACCCCCATCTTCAACAACAATACCGA
ERG9 1021U R	GTATTGTTGTTGAAGATGGGGGTTTAACTGCTAAGAGACACTG
1021D ERG9 R	ATGAATTTCATCACGTGCGTGTACTTAGTTATTGTTCGGAGTTGT
ERG9 1021D F	TCCGAACAATAACTAAGTACACGCACGTGATGAAATTCAT
ROX1 U F	ATTTTTGAAAATTCGAATTCATGGAATTGTATGCTCAATC
ROX1 U R	TTAATTAAGAGCTCAGATCTTTAATATGGAACTGGATGCAGGT
ROX1 D F	AACTTCTTTGCGTCCATCCA
ROX1 D R	GCAAGGTAGACAAGCCGACAAC

MOT3 U F	AGGAGAAAAAACCCCGGATCCATGGCTTCAGAAAAAGAAATTAGG
MOT3 U R	GCGGTACCAAGCTTACTCGAGTTAATATGGAACTGGATGCAGGT
MOT3 D F	AACCTCTGGCGAAGAATTACTAGTTTAATATGGAACTGGATGCAGGTACAATTG
MOT3 D R	AGTAAGAATTTTTGAAAATTCGAATTCATGGAATTGTATGCTCAATCAGTTGGTG
62 U F	TTGAGCATACAATTCCATGAATTCGAATTTTCAAAAATTCTTACTTTTTTTTTGGATGGA CGCAAA
62 U R	CATCCAGTTCCATATTAAACTAGTAATTCTTCGCCAGAGGTTTGGTCAAG
62 D F	TTCTTTTTCTGAAGCCATGAATTCGAATTTTCAAAAATTCTTACTTTTTTTTTGGATGGA CGCAAA
62 D R	AGTAAGAATTTTTGAAAATTCGAATTCATGGCTTCAGAAAAAGAAATTAGGAGAGAGA G
64 U F	TTGAGCATACAATTCCATGAATTCGAATTTTCAAAAATTCTTACTTTTTTTTTGGATGGA CGCAAA
64 U R	CATCCAGTTCCATATTAAACTAGTAATTCTTCGCCAGAGGTTTGGTCAAG
64 D F	TTCTTTTTCTGAAGCCATGAATTCGAATTTTCAAAAATTCTTACTTTTTTTTTGGATGGA CGCAAA
64 D R	AGTAAGAATTTTTGAAAATTCGAATTCATGGCTTCAGAAAAAGAAATTAGGAGAGAGA G
ROX1 GF	TCTTCCTCGGTGTCAAGCTCGTTTAAGAGCTATGCTGGAAACAG
ROX1 GR	GAGCTTGACACCGAGGAAGAGATCATTTATCTTTCACTGCGGAG
MOT3 GF	CTCTGCATCTACCTCCTGGTGTTTAAGAGCTATGCTGGAAACAG
MOT3 GR	ACCAGGAGGTAGATGCAGAGGATCATTTATCTTTCACTGCGGAG
62 GF	GCCTCATCCCCACGGGAATAGTTTAAGAGCTATGCTGGAAACAG
62 GR	TATTCCCGTGGGGATGAGGCGATCATTTATCTTTCACTGCGGAG
64 GF	AGCGTGAGTTCATCTGGGAGGTTTAAGAGCTATGCTGGAAACAG
64 GR	CTCCCAGATGAACTCACGCTGATCATTTATCTTTCACTGCGGAG
ERG11 F	GGAGAAAAAACCCCGGATCCATGTCTGCTACCAAGTCAATCG
ERG11 R	CGGTACCAAGCTTACTCGAGTTAGATCTTTTGTTCTGGATTTCTCTTTT
ERG24 F	GGAGAAAAAACCCCGGATCCATGGTATCAGCTTTGAATCCC
ERG24 R	CGGTACCAAGCTTACTCGAGTTAATAAACATATGGAATGATCTTGTAAGGAACT
POS5 F	GGAGAAAAAACCCCGGATCCATGTTTGTCAGGGTTAAATTGAAT
POS5 R	CGGTACCAAGCTTACTCGAGTTAATCATTATCAGTCTGTCTCTTGGTC
ROX1 F	GGAGAAAAAACCCCGGATCCATGAATCCTAAATCCTCTACACC
ROX1 R	CTAGCCGCGGTACCAAGCTTTCATTTCGGAGAAACTAGGCT
MOT3 F	GGAGAAAAAACCCCGGATCCATGAATGCGGACCATCACCT
MOT3 R	CTAGCCGCGGTACCAAGCTTCTATTTGTTGTGACTAACAATAAGG
ROX1 U F	ATTTTTGAAAATTCGAATTCATGGAATTGTATGCTCAATC
ROX1 U R	TTAATTAAGAGCTCAGATCTTTAATATGGAACTGGATGCAGGT
ROX1 D F	AACTTCTTTGCGTCCATCCA
ROX1 D R	GCAAGGTAGACAAGCCGACAAC
MOT3 U F	AGGAGAAAAAACCCCGGATCCATGGCTTCAGAAAAAGAAATTAGG
MOT3 U R	GCGGTACCAAGCTTACTCGAGTTAATATGGAACTGGATGCAGGT
MOT3 D F	AACCTCTGGCGAAGAATTACTAGTTTAATATGGAACTGGATGCAGGTACAATTG
MOT3 D R	AGTAAGAATTTTTGAAAATTCGAATTCATGGAATTGTATGCTCAATCAGTTGGTG

62 U F	TTGAGCATACAATTCCATGAATTCGAATTTTCAAAAATTCTTACTTTTTTTTTGGATGGACGCAAA
62 U R	CATCCAGTTCCATATTAAACTAGTAATTCTTCGCCAGAGGTTTGGTCAAG
62 D F	TTCTTTTTCTGAAGCCATGAATTCGAATTTTCAAAAATTCTTACTTTTTTTTTGGATGGACGCAAA
62 D R	AGTAAGAATTTTTGAAAATTCGAATTCATGGCTTCAGAAAAAGAAATTAGGAGAGAGAG
64 U F	TTGAGCATACAATTCCATGAATTCGAATTTTCAAAAATTCTTACTTTTTTTTTGGATGGACGCAAA
64 U R	CATCCAGTTCCATATTAAACTAGTAATTCTTCGCCAGAGGTTTGGTCAAG
64 D F	TTCTTTTTCTGAAGCCATGAATTCGAATTTTCAAAAATTCTTACTTTTTTTTTGGATGGACGCAAA
64 D R	AGTAAGAATTTTTGAAAATTCGAATTCATGGCTTCAGAAAAAGAAATTAGGAGAGAGAG
ROX1 GF	TCTTCCTCGGTGTCAAGCTCGTTTAAGAGCTATGCTGGAAACAG
ROX1 GR	GAGCTTGACACCGAGGAAGAGATCATTTATCTTTCACTGCGGAG
MOT3 GF	CTCTGCATCTACCTCCTGGTGTTTAAGAGCTATGCTGGAAACAG
MOT3 GR	ACCAGGAGGTAGATGCAGAGGATCATTTATCTTTCACTGCGGAG
62 GF	GCCTCATCCCCACGGGAATAGTTTAAGAGCTATGCTGGAAACAG
62 GR	TATTCCCGTGGGGATGAGGCGATCATTTATCTTTCACTGCGGAG
64 GF	AGCGTGAGTTCATCTGGGAGGTTTAAGAGCTATGCTGGAAACAG
64 GR	CTCCCAGATGAACTCACGCTGATCATTTATCTTTCACTGCGGAG
ERG11 F	GGAGAAAAAACCCCGGATCCATGTCTGCTACCAAGTCAATCG
ERG11 R	CGGTACCAAGCTTACTCGAGTTAGATCTTTTGTTCTGGATTTCTCTTTT
ERG24 F	GGAGAAAAAACCCCGGATCCATGGTATCAGCTTTGAATCCC
ERG24 R	CGGTACCAAGCTTACTCGAGTTAATAAACATATGGAATGATCTTGTAAGGAACT
POS5 F	GGAGAAAAAACCCCGGATCCATGTTTGTCAGGGTTAAATTGAAT
POS5 R	CGGTACCAAGCTTACTCGAGTTAATCATTATCAGTCTGTCTCTTGGTC
ROX1 F	GGAGAAAAAACCCCGGATCCATGAATCCTAAATCCTCTACACC
ROX1 R	CTAGCCGCGGTACCAAGCTTTCATTTCGGAGAAACTAGGCT
MOT3 F	GGAGAAAAAACCCCGGATCCATGAATGCGGACCATCACCT
MOT3 R	CTAGCCGCGGTACCAAGCTTCTATTTGTTGTGACTAACAATAAGG

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49 **Figure captions**

50 **Table S1** Primers used in this study

51 **Table S2** The DEGs data for RNA-seq of HPT08 and PT07 strains

52 **Figure S1** Volcano map for RNA-seq of HPT08 and PT07 strains. On the left is Case

53 compared to Control down-regulated genes, on the right is Case compared to Control

54 up-regulated genes. The blue dot on the left side of the figure indicates a total of 260

55 down-regulated genes, while the red dot on the right indicates a total of 158 up-

56 regulated genes.

57 **Figure S2** Effect of weakening the endogenous ERG20 on patchoulol production (A)

58 and cell growth (B). The value is the mean of three independent experiments. (*

59 represents P value < 0.05; * *represents P value < 0.01. O represents original control,

60 + represents overexpression of genes, -- represents weakening of genes.)

61 **Figure S3** Effects of medium composition changes on the synthesis of patchoulol (A)

62 Patchoulol production with changes in medium composition (B) Effects of 3%/5%

63 glucose, 3%/5% yeast extract and 10% glycerin on the cell growth. (C) Effects of pH

64 4.96/7.13, addition of phosphate and Ca^{2+} on the cell growth. The value is the mean of

65 three independent experiments.

66 **Figure S4** Effect of adding dodecane at different times on patchoulol production at

67 shake flask level.

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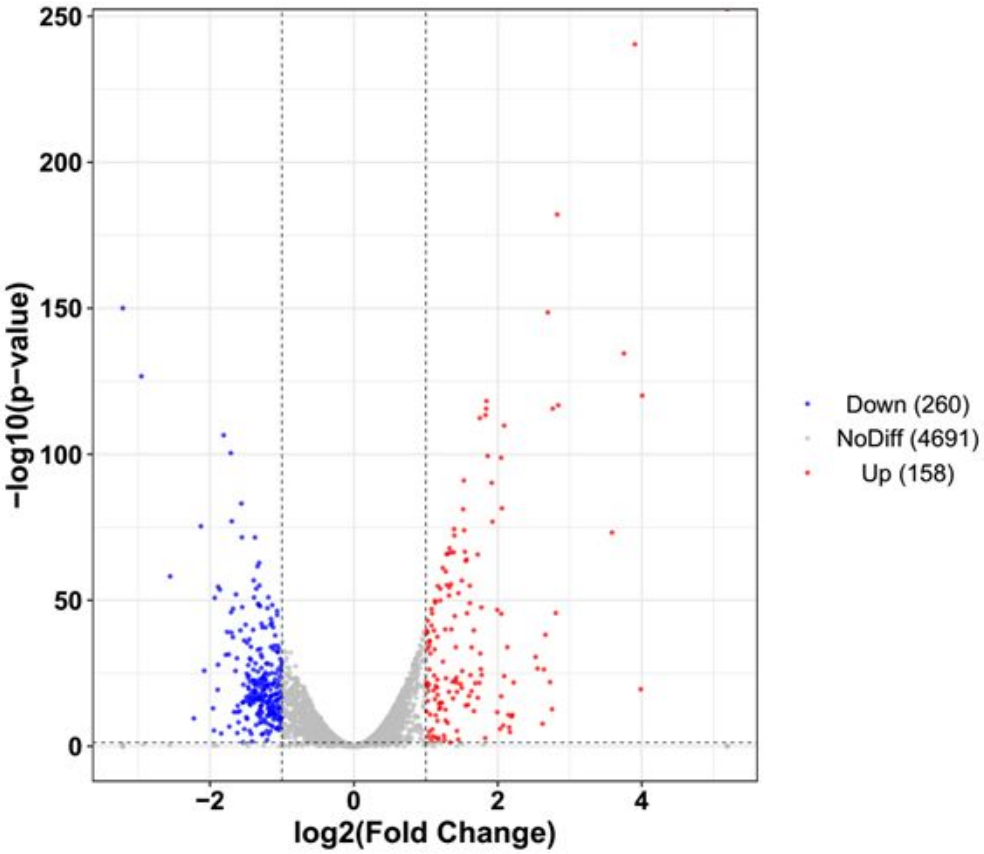
72 **Figure S1**


250
200
150
100
50
0
−log10(p−value)
−2
0
2
4
log2(Fold Change)
Down (260)
NoDiff (4691)
Up (158)

85 **Figure S2**

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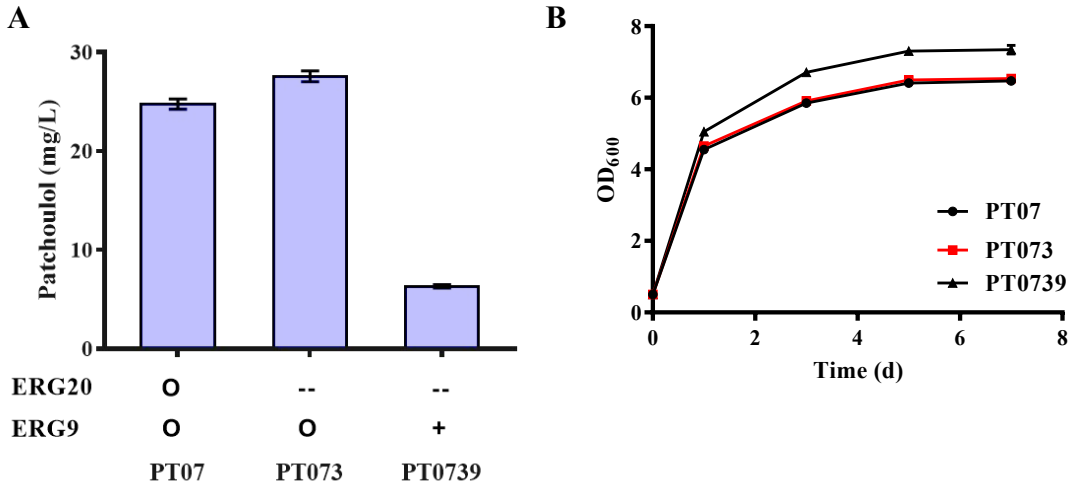
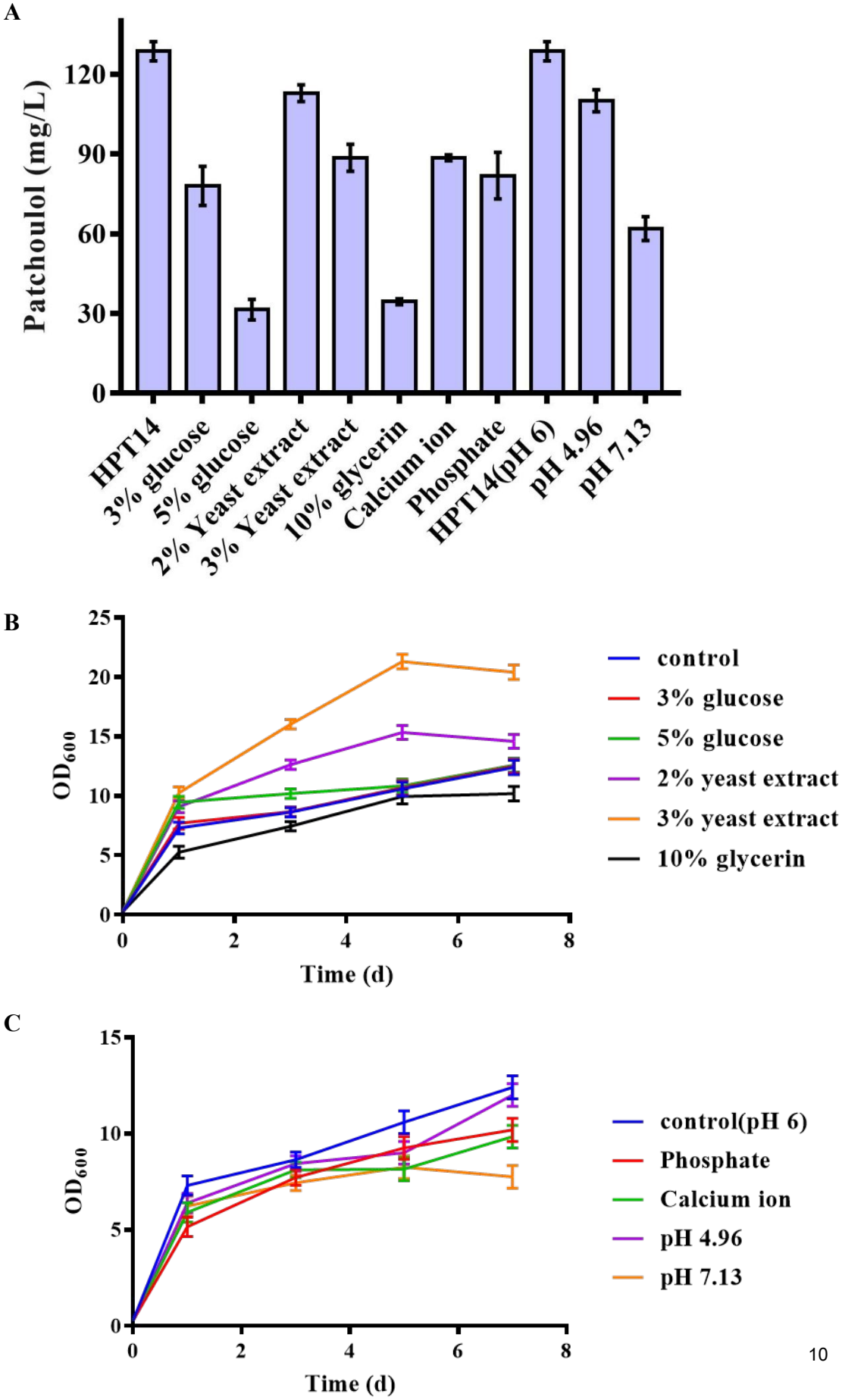


107 **Figure S3**



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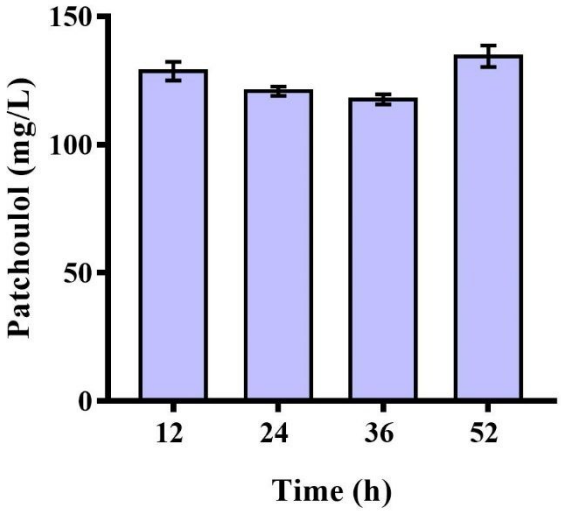
121 **Figure S4**



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