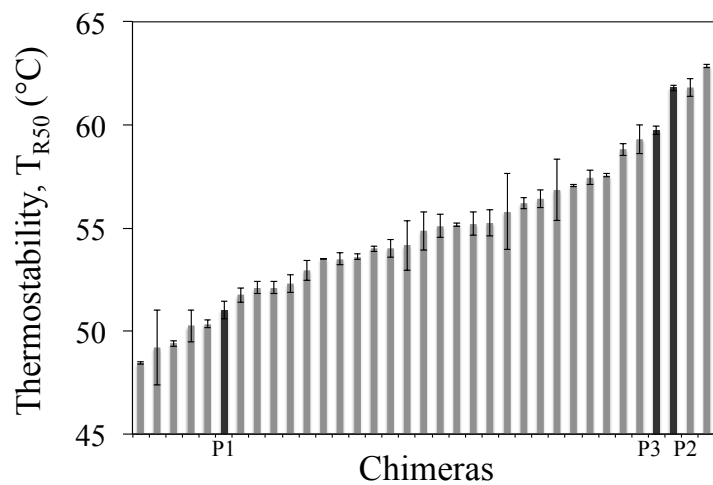


## **Supplementary information**

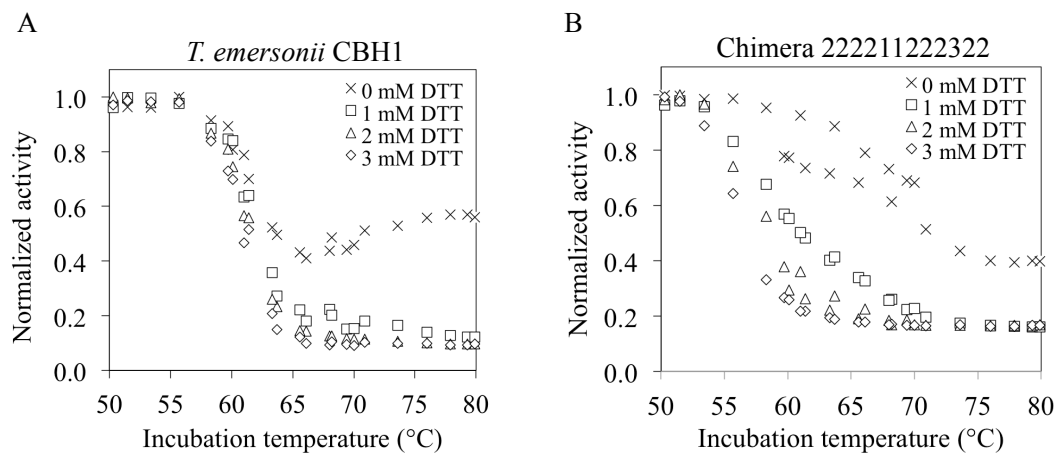
### ***H. jecorina* cellobiohydrolase I stabilizing mutations identified using non-contiguous recombination.**

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**Supplementary Figure 1.** Thermostabilities of a maximally informative subset (32 chimeras) of the library. The  $T_{R50}$  is the incubation temperature at which a chimera's residual activity after 10 minutes is half its activity before incubation. Measurements were performed in duplicate. The parental enzymes are highlighted.



**Supplementary Figure 2**  $T_{R50}$  measurements for a range of DTT concentrations. A) Parental cellulase *T. emersonii* CBH1 recovers some activity after incubation if there is no DTT present. The enzyme does not recover activity when incubated with 1 mM DTT. B) Chimeric cellulase 222211222322 is able to recover some activity after incubation with 1 mM DTT.

**Supplementary Table 1** Amino acid sequences of the maximally informative subset of 35 chimeric cellulases. The *H. jecorina* CBHI linker and cellulose binding domain are attached to the C-terminus. The chimera nomenclature is a series of numbers, each representing a parent for the blocks A-L. For example, chimera 123113322331 has parent 1's sequence for block A, parent 2 for block B, etc. The three chimeras with no detectable activity are highlighted with an asterisk.

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211213233112

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322321222212

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131232232321

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311211331111

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122122133333 \*

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233122223223

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GKIIDTNKPFVTVVTOFLTDDGTDGTGLSEIKRFYIQNSNVI PNAESKIPGNPGNSITQEYCD AQKVAFG  
DTDDFSQHGGMAKMGAAQQGMVLVMSLWDDYANMLWLSDYPTDADPTTPGIARGACPTTSGVPAQV  
ESQVPNSYVTYSNIKFGPINSTFTASPPGGNRGTTTTTRRPATTTGSSPGPTQSHYGQCGGIGYSGPTVC  
ASGTTCCQVLNPPYYSQCL

333222211111

QQACTATAENHPPLTWQKCTAPGSCTQQTGAVVLDANNRWVHTVTVSGSTNCYTGNQWDTSLCTDGKSCAQ  
TCCLDGADYEGTYGVTSSGNSLKLNFTVGSNVGSRLYLLQDDSTYQIFKLLGNEFSFDVDVSNLPCGLN  
GALYFVAMDADGGMSKYSGNKAGAKYGTGYCDSQCPRDLKFINGEANVGNWTPSTNDANAGFGRYGSCC  
AEMDIWEANSISEALTPHPCDTPGQSRCEADTCGGTYSNDRYAGTCDPDGCDWNPYRLGNTSFYGPSS  
FTLDTTKKLTVVTOFETSGAINRYVQNGVTFQQPNAELGSYSGNELNDDYCTAEAEFGGSSFSHDGG  
LAKMGAAQQGMVLVMSLWDDHYANMLWLSDYPTDQAGAPGAERGSCTTSGVPSDVESQSPNSYVTY  
SNIKFGPIGSTGNPSSGNPPGGNRGTTTTTRRPATTTGSSPGPTQSHYGQCGGIGYSGPTVCASGTTCCQV  
LNPPYYSQCL

231333111132

QSACSLTAENHPSLWQKCTSGGSCTQQTGAVTIDANNRWHTATNSSTNCYTGNWTSSTLCPDGETCAK  
NCCVDGADYSSTYGITTSGNLSLNLKLVTKHQYGTNVGSRVYLMENDTKYQEFLLGNEFSFDVDVSNLPC  
GCLNGALYFVMSDADGGMSKYSKYPTNTAGAKYGTGYCDSQCPRDLKFINGEANVGNWTPSTNNANTGIGRY  
GCCSEMEDIWEANSISNALTPHPCDTPGQSRCEADTCGGTYSNDRYAGTCDPDGCDWNPYRLGNTSFYG  
PGMTVDTTKKMTVVTOFETSGAINRFYVQDGKI IAQPNALGSYSGNELNDDYCTAEAEFGGSSFSK  
GGLTQFKKATSGGMVLVMSVWDDYANMLWLSDYPTDADPTTPGIARGTCPTTSGVPAEIEAQSPNSN  
VIFSNIKFGPIGSTVPPSSGNPPGGNRGTTTTTRRPATTTGSSPGPTQSHYGQCGGIGYSGPTVCASGTT  
CQVLNPPYYSQCL

233232323133

QQACSLTAENHPSLWQKCTAPGSCTQQTGAVVLDANNRWVHTVTVSGSTNCYTGNWTWDTSLCTDGKSCAQ  
TCCLDGADYEGTYGVTSSGNSLKLNFTVGSNVGSRLYLMENDTKYQMFELLNREFSFDVDVSNLGCGLN  
GALYFVMSDADGGMSKYSGNKAGAKYGTGYCDAQCPRDLKFINGEANVGNWTPSTNNANTGIGRYGSCC  
SEMDVWEANNMATAFTPHPCDTPGQSRCEADTCGGTYSNDRYAGVCDPDGCDNFAYRQGDKTFYGKGMT  
VDTNKKMTVVTOFLTDDGTDGTGLSEIKRFYVQDGKI IANAESKIPGNPGNSITQEYCD AQEVAFGSS  
FSDKGGMAQMSKALAGPMVLVMSLWDDYANMLWLSDYPTDADPTTPGIARGACPTTSGVPSDVESQV  
PNSYVTYSNIKFGPIGSTVPPSSGNPPGGNRGTTTTTRRPATTTGSSPGPTQSHYGQCGGIGYSGPTVCA  
SGTTCQVLNPPYYSQCL

333112122312 \*

QQACTLQSETHPPLTWKRCSSGGTCTVNGSVVLDANNRWHTVTVSGSTNCYTGNQWDTSLCTDGKSCAQ  
TCCLDGADYEGTYGVTSSGDSLKLNFTVQSAQKNVGSRLYLMASDTTYQEFLLNREFSFDVDVSNLPC  
GLNGALYFVMSDADGGMSKYSGNKAGAKYGTGYCDSQCPRDLKFINGEANVGNWTPSTNDANAGFGRYG  
SCCSEMEDIWEANSISNAVTPHPCDTPGQSRCEADTCGGTYSNDRYGGTCDPDGCDNFYRGMNTSFYGP  
GSSFTLDTTKKLTVVTOFLTDDGTDGTGLSEIKRYVQNGVTFQQPNSDISGVTGNSITTEFCTAQKQA  
FSNTDDFNKGGGLTQFKKATSGGMVLVMSLWDDHYANMLWLSDYPTDQAGAPGAERGTCTTSGVPAQ  
VESQSPNSYVTYSNIRFGPIGSTGNPSSGNPPGGNRGTTTTTRRPATTTGSSPGPTQSHYGQCGGIGYSGPTVC  
ASGTTCCQVLNPPYYSQCL

133331223212

QQACSLTAENHPSLTWQECTSGGSCCTQNGAVVIDANWRWTHTVSGSTNCYDGNWTWDTSLCTDGKSCAQ  
TCCVDGAAAYASTYGVTTSGSSLSIGFVTKHQYGTNVGARLYLMENDTKYQIFKLLNREFSFDVDVSQLP  
CGLNGALYFVAMDADGGMSKYSGNKAGAKYGTGYCDSQCPRDLKFINGEANVGNWTPSTNNANTGIGRY  
GSCCAEMDVWEANSISNAFTPHPCDTPGQSRCEADTCGGTYSSDRYAGTCDPDGCDFNYPYRQNTSFYG  
PGSSFTLDTTKTLTVVTQFLTDGTDGTGLSEIKRYVQNGVTFQOPNSKIPGNPGNSITQEYCDAQKV  
AFGDTDDFSQHGGMAKMGAAAMQGMVLMVMSLWDDYYANMLWLDSTYPTNETSSTPGAVRGTCPTTSGVP  
AEIEAQSPNAKVTFISNIFKFGPIGSTGNASPPGGNRGTTTTTRRPATTTGSSPGPTQSHYGQCGGIGYSGP  
TVCASGTTTCQVLNPYYSQCL

131112222121

QSACTLQSETHPPLTWQKCSSGGTCTQQTGSVVLNANWRWTHATNSSTNCYDGNWTWSSSTLCPDGETCAK  
NCCLDGADYEGTYGVTTSGNSLKNFVTSQAQKNVGSRLYLMASDTTYQIFKLLNREFSFDVDVSNLPC  
GLNGALYFVAMDADGGVSKYPTNTAGAKYGTGYCDSQCPRDLKFINGEANVGNWTPSTNNANTGIGRYG  
SCCAEMDVWEANSISEAVTPHPCDTPGQSRCEADTCGGTYSDNRYGGTCDPDGCDFNPYRMGNTSFYGP  
GKIIDTTKPFVTVVTQFLTDGTDGTGLSEIKRFYIQNSNVIQPNSDISGVTGNSITTEFCTAQEQAFG  
GSSFSHDHGLAKMGAAAMQGMVLMVMSLWDDYYANMLWLDSTYPTNETSSTPGAVRGSCPTTSGVPAQVE  
SQSPNSYVTVSNIFKFGPINSTFTPSGGNPPGGNRGTTTTTRRPATTTGSSPGPTQSHYGQCGGIGYSGPT  
VCASGTTTCQVLNPYYSQCL

322211223233

QQACTLQSETHPPLTWQECTAPGSCCTQNGSVVIDANWRWVHDVNGYTNCYTGNQWDPTYCPDDETCQAQ  
NCCLDGAAAYASTYGVTTSGSSLSIGFVTGSNVGARLYLMASDTTYQIFKLLNREFSFDVDVSQLGCGLN  
GALYFVAMDADGGVSKYPNNKAGAKYGTGYCDSQCPRDLKFIDGEANVEGWQPPSSNDANAGFDHGSCC  
AEMDVWEANMATAFTPHPCDTPGQTMCSGDDCGGTYSDNRYGGTCDPDGCDFNAYRQGDKTFYKGMT  
VDTNKKMTVVVTQFLTDGTDGTGLSEIKRFYVQDGKIIANAESKIPGNPGNSITQEYCDAQKVAFGDTD  
DFSQHGGMAKMGAAAMQGMVLMVMSLWDDHYAQLMLWLDSTYPIDQAGAPGAERGACPTDSGVPDVESQV  
PNAKVTFISNIFKFGPIGSTVPASPPGGNRGTTTTTRRPATTTGSSPGPTQSHYGQCGGIGYSGPTVCASGT  
TCQVLNPYYSQCL

223311222322

QQACTLQSETHPPLTWKRCCTSGGSCSTVNGSVVIDANWRWTHTVSGSTNCYTGNWTWDTSLCTDDKSCAQ  
TCCVDGAAAYASTYGVTTSGDSLKLFVTVGSNVGARLYLMASDTTYQIFKLLNREFTFDVDVSQLP  
CGLNGALYFVAMDADGGMSKYSGNKAGAKYGTGYCDSQCPRDLKFIDGEANVEGWQPPSSNNANTGIGDH  
GSCCAEMDVWEANSISNAVTPHPCDTPGQTMCSGDDCGGTYSDNRYGGTCDPDGCDFNYPYRMGNTSFYG  
PGKIIDTTKPFVTVVTQFLTDGTDGTGLSEIKRFYIQNSNVIQPNSDISGVTGNSITTEFCTAQKQAF  
SNTDDFNHRHGLAKMGAAAMQGMVLMVMSLWDDYAAQMLWLDSDYPTDADPTTPIARGTCPTDSGVP  
IEAQSPNAKVTFISNIRFGPINSTFTGPPGGNRGTTTTTRRPATTTGSSPGPTQSHYGQCGGIGYSGPTVC  
ASGTTTCQVLNPYYSQCL

121213333323

QSACTLQSETHPPLTWKRCCTAPGSCSTVNGSVVIDANWRWVHATNSSTNCYDGNWTWSSSTLCPDDETCAK  
NCCLDGADYSSTYGITTSGDSLNLKLFVTVGSNVSRYLMASDTTYQMFELGNEFTFDVDVSNLGCGLN  
GALYFVMSMDADGGVSKYPTNTAGAKYGTGYCDAQCPDLKFIDGEANVEGWQPPSSNNANTGIGDHGSCC  
SEMDVWEANMATAFTPHPCCTTVGQTMCSGDDCGGTYSDNRYGGVCDPDGCDFNAYRQGDKTFYKGGKI  
IDTNKPFVTVVTQFHKNSAGVLSEIKRFYIQNSNVIQPNNAESKIPGNPGNSITQEYCDAQKVAFSNTDDFN  
RKGGMQMSKALAGPMVLMVMSVWDDYYAQLMLWLDSTYPTNETSSTPGAVRGACPTDSGVPDVESQV  
SNVIFSNIRFGPINSTFTGPPGGNRGTTTTTRRPATTTGSSPGPTQSHYGQCGGIGYSGPTVCASGTTTCQ  
VLNPYYSQCL

123333232233

QQACSLTAENHPSLWQECTSGGSC TTQNGAVTIDANWRWTHTVSGSTNCYDGN TWDTSLCTDDKSCAQ  
TCCVDGADYSSTYGITTS GSSLNLKFVTKHQYGTNVGSRVYLMENDTKYQIFKLLGNEFSFDVDVSNLG  
CGLNGALYFVAMDADGGMSKYSGNKAGAKYGTGYCDSQCPRDLKFIDGEANVEGWQPSSNNANTGIGDH  
GSCCAEMDVWEANNMATAVTPHPCDTPGQTMCSGDDCGGTYSSTRYAGTCDPDGCDFNAYRMGDKTFYG  
KGMTVDTNKKMTVVVTFHKN SAGVLSEIKRFYVQDGKI IANAESDISGVTGNSITTEFCTAQKQAFGDT  
DDFSQHGLAKMGAAMQQGMVLVMSVWDDYYAQLWLDSTYPTNETSSTPGAVRGACP TDSGVP AEIEA  
QVPNSNVI FSNIKFGPIGSTVPASPPGNGRGT TTRRPATTTGSSPGPTQSHYGQCGGIGYSGPTVCAS  
GTTCQVLNPYYSQCL

**Supplementary Table 2** Amino acid sequences of 7 chimeric cellulases predicted to be stable. The *H. jecorina* CBHI linker and cellulose binding domain are attached to the C-terminus. The chimera nomenclature is a series of numbers, each representing a parent for the blocks A-L. For example, chimera 123113322331 has parent 1's sequence for block A, parent 2 for block B, etc.

222233221322

QQACSLTAENHPSLTWKRCTAPGSCSTVNGAVTIDANWRWVHDVNGYTNCYTGNWTWDPPTYCPDDETCQAQ  
NCCLDGADYSSTYGITTSGLSLNLFVVTGNSVGSRVYLMENDTKYQIFKLLNREFTFDVDVSNLPCGLN  
GALYFVAMDADGGVSKYPNNKAGAKYGTGYCDSQCPRDLKFIDGEANVEGWQPSSNNANTGIGDHGSCC  
AEMDIWEANSISNALTPHPCDTPGQTMCSGDDCGGTYSSDRYAGTCDPDGCDWNPYRLGNTSFYGPVKI  
IDTTKPFVVTQFLTDGDTGTGLSEIKRFYIQNSNVIPQPNALGSYSGNSLNDYCTAEKAEFSNTD  
DFNRHGGLAKMGAAMQQGMVLVMSVWDDYAAQMLWLDSDYPTDADPTTPGIARGTCPTDSGVPDVESSQ  
SPNSNVIFSNIRFGPINSTFTGPPGGNRGTTTTTRRPATTTGSSPGPTQSHYGQCGGIGYSGPTVCASGT  
TCQVLNPPYYSQCL

232233222322

QQACSLTAENHPSLTWKRCTAPGSCSTVNGAVTIDANWRWVHDVNGYTNCYTGNWTWDPPTYCPDGETCAQ  
NCCLDGADYSSTYGITTSGLSLNLFVVTGNSVGSRVYLMENDTKYQIFKLLNREFTFDVDVSNLPCGLN  
GALYFVAMDADGGVSKYPNNKAGAKYGTGYCDSQCPRDLKFINGEANVGNWTPSTNNANTGIGRYGSCC  
AEMDVWEANSISNAVTPHPCDTPGQSRCEADTCGGTYSSDRYAGTCDPDGCDFNPYRMGNTSFYGPVKI  
IDTTKPFVVTQFLTDGDTGTGLSEIKRFYIQNSNVIPQPNDSISGVTGNSITTEFCTAQKQAFSNTD  
DFNRHGGLAKMGAAMQQGMVLVMSVWDDYAAQMLWLDSDYPTDADPTTPGIARGTCPTTSGVPSDVESSQ  
SPNSNVIFSNIRFGPINSTFTGPPGGNRGTTTTTRRPATTTGSSPGPTQSHYGQCGGIGYSGPTVCASGT  
TCQVLNPPYYSQCL

222211222322

QQACTLQSETHPPLTWKRCTAPGSCSTVNGSVVIDANWRWVHDVNGYTNCYTGNWTWDPPTYCPDDETCQAQ  
NCCLDGAAAYASTYGVTTSGDSLISIGFVTGNSVGARLYLMASDTTYQIFKLLNREFTFDVDVSNLPCGLN  
GALYFVAMDADGGVSKYPNNKAGAKYGTGYCDSQCPRDLKFIDGEANVEGWQPSSNNANTGIGDHGSCC  
AEMDVWEANSISNAVTPHPCDTPGQTMCSGDDCGGTYSDNRYGGTCDPDGCDFNPYRMGNTSFYGPVKI  
IDTTKPFVVTQFLTDGDTGTGLSEIKRFYIQNSNVIPQPNDSISGVTGNSITTEFCTAQKQAFSNTD  
DFNRHGGLAKMGAAMQQGMVLVMSLWDDYAAQMLWLDSDYPTDADPTTPGIARGTCPTDSGVPDVESSQ  
SPNAKVTFSNIRFGPINSTFTGPPGGNRGTTTTTRRPATTTGSSPGPTQSHYGQCGGIGYSGPTVCASGT  
TCQVLNPPYYSQCL

222223222322

QQACTATAENHPPLTWKRCTAPGSCSTVNGAVTIDANWRWVHDVNGYTNCYTGNWTWDPPTYCPDDETCQAQ  
NCCLDGADYSSTYGITTSGLSLNLFVVTGNSVGSRVYLLQDDSTYQIFKLLNREFTFDVDVSNLPCGLN  
GALYFVAMDADGGVSKYPNNKAGAKYGTGYCDSQCPRDLKFIDGEANVEGWQPSSNNANTGIGDHGSCC  
AEMDVWEANSISNAVTPHPCDTPGQTMCSGDDCGGTYSDRYAGTCDPDGCDFNPYRMGNTSFYGPVKI  
IDTTKPFVVTQFLTDGDTGTGLSEIKRFYIQNSNVIPQPNDSISGVTGNSITTEFCTAQKQAFSNTD  
DFNRHGGLAKMGAAMQQGMVLVMSVWDDYAAQMLWLDSDYPTDADPTTPGIARGTCPTDSGVPDVESSQ  
SPNSNVIFSNIRFGPINSTFTGPPGGNRGTTTTTRRPATTTGSSPGPTQSHYGQCGGIGYSGPTVCASGT  
TCQVLNPPYYSQCL

222211221322

QQACTLQSETHPPLTWKRCTAPGSCSTVNGSVVIDANWRWVHDVNGYTNCYTGNWTWDPPTYCPDDETCQAQ  
NCCLDGAAAYASTYGVTTSGDSLISIGFVTGNSVGARLYLMASDTTYQIFKLLNREFTFDVDVSNLPCGLN  
GALYFVAMDADGGVSKYPNNKAGAKYGTGYCDSQCPRDLKFIDGEANVEGWQPSSNNANTGIGDHGSCC  
AEMDIWEANSISNALTPHPCDTPGQTMCSGDDCGGTYSDNRYGGTCDPDGCDWNPYRLGNTSFYGPVKI  
IDTTKPFVVTQFLTDGDTGTGLSEIKRFYIQNSNVIPQPNALGSYSGNSLNDYCTAEKAEFSNTD

DFNRHGGLAKMGAAMQQGMVLVMSLWDDYAAQMLWLDSDYPTDADPTTPGIARGTCPTDSGVPSDVESQ  
SPNAKVTFNSIRFGPINSTFTGPPGGNRGTTTTTRRPATTTGSSPGPTQSHYGQCGGIGYSGPTVCASGT  
TCQVLNPPYYSQCL

322211222322

QOACTLQSETHPPLTWKRCTAPGSCSTVNGSVVIDANWRWVHDVNGYTNCYTGNWTDPTYCPDDETCAQ  
NCCLDGAAAYASTYGVTTSGDSL SIGFVTGSNVGARLYLMASDTTYQIFKLLNREFTFDVDVSQLPCGLN  
GALYFVAMDADGGVSKYPNNKAGAKYGTGYCDSQCPRDLKFIDGEANVEGWQPSSNDANAGFGDHGSCC  
AEMDVWEANSISNAVTTPHPCDTPGQTMCSGDDCGGTYSDNRYGGTCDPDGCDNFNRYMGNTSFYGPCKI  
IDTTKPFVVTQFLTDGDTGTLSEIKRFYIQNSNVIPQPNSDISGVTGNSITTEFCTAQKQAFSNTD  
DFNRHGGLAKMGAAMQQGMVLVMSLWDDHYAQMLWLDSTYPIDQAGAPGIARGTCPTDSGVPSDVESQS  
PNAKVTFNSIRFGPINSTFTGPPGGNRGTTTTTRRPATTTGSSPGPTQSHYGQCGGIGYSGPTVCASGTT  
CQVLNPPYYSQCL

122211222322

QOACTLQSETHPPLTWKRCTAPGSCSTVNGSVVIDANWRWVHDVNGYTNCYDGNWTDPTYCPDDETCAQ  
NCCLDGAAAYASTYGVTTSGDSL SIGFVTGSNVGARLYLMASDTTYQIFKLLNREFTFDVDVSQLPCGLN  
GALYFVAMDADGGVSKYPNNKAGAKYGTGYCDSQCPRDLKFIDGEANVEGWQPSSNNANTGIGDHGSCC  
AEMDVWEANSISNAVTTPHPCDTPGQTMCSGDDCGGTYSDNRYGGTCDPDGCDNFNRYMGNTSFYGPCKI  
IDTTKPFVVTQFLTDGDTGTLSEIKRFYIQNSNVIPQPNSDISGVTGNSITTEFCTAQKQAFSNTD  
DFNRHGGLAKMGAAMQQGMVLVMSLWDDYAAQMLWLDSTYPNETPTTPGIARGTCPTDSGVPSDVESQ  
SPNAKVTFNSIRFGPINSTFTGPPGGNRGTTTTTRRPATTTGSSPGPTQSHYGQCGGIGYSGPTVCASGT  
TCQVLNPPYYSQCL

**Supplementary Table 3.** T<sub>A50</sub> measurements of *H. jecorina* CBHI with block G from *T. emersonii* CBHI and *C. thermophilum* CBHI.

Chimera	T <sub>A50</sub> (°C)
111111211111	48.3 (± 0.5)
111111311111	47.8 (± 0.1)

**Supplementary Table 4.** Stability of *H. jecorina* CBHI with the single mutation F362M and other stabilizing point mutations.

Mutations	T <sub>A50</sub> (°C)
wild-type	46.9 (± 0.1)
F362M	49.6 (± 0.2)
E120I, T122K	47.4 (± 0.5)
E120M, T122K	46.6 (± 0.1)
E120M, F362M	48.8 (± 0.1)
S175A, K356H	48.4 (± 0.1)
S175A, F362M	48.3 (± 0.2)
K356H, F362M	49.3 (± 0.1)
S175A, K356H, F362M	49.1 (± 0.4)