Supplementary Figure S5

**Monoclonal antibodies to human butyrylcholinesterase reactive with butyrylcholinesterase in animal plasma**

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Signal peptide 1 N-term Asn17 Arg42

Human MHSKVT-IICIRFLFWFLLLCMLIGKSHT**E**DDIIIATKNGKVRGM**N**LTVFGGTVTAFLGIPYAQPPLGRL**R**FKKPQSLTK  Rhesus MHSKVT-IICIRLLFWFLLLCMLIGKSHTEDDIVIATKNGKVRGMNLTVLGGTVTAFLGIPYAQPPLGRLRFKKPQSLTK  Horse MQSWGT-IICIRILLRFLLLWVLIGNSHTEEDIIITTKNGKVRGMNLPVLGGTVTAFLGIPYAQPPLGRLRFKKPQSLTK

Cat MQSKGT-IISIQFLLRFLLLWVLIGKSHTEEDIIITTKNGKVRGMNLPVLDGTVTAFLGIPYAQPPLGRLRFKKPQFLTK  Tiger MQSKGT-IISIQFLLRFLLLWVLIGKSHTEEDIIITTKNGKVRGMNLPVLDGTVTAFLGIPYAQPPLGRLRFKKPQFLTK  Rabbit MQDKDT-IISIRFLFWFLLLCMVIRKSHTE-DVIITTKNGRIRGINLPVLGGTVTAFLGIPYAQPPLGRLRFKKPQSLTK

Pig MQRKGT-IMYIRYFLWFLLLWMLVGKSYAEEDIIVTTKNGKVRGMNLPVLGGTVTAFLGIPYAQPPLGRLRFKKPQSMTK  Guinea pig MQTKHA-VTSTPFTLWFLLFCMLVEKCHAEEDIIVTTKTGKVRGMNLPVLGGTVTAFLGIPYAQPPVGRLRFKKPQPLDK  Mouse MQTQHTkVTQTHFLLWILLLCMPFGKSHTEEDFIITTKTGRVRGLSMPVLGGTVTAFLGIPYAQPPLGSLRFKKPQPLNK  Rat M------VTEIHFLLWILLLCMLFGKSHTEEDVIITTKTGRVRGLSMPILGGTVTAFLGIPYAQPPLGSLRFKKPQPLNK  Chicken MVWANGmSICARFLMWLLLLFMFIRKV-VPEDNVITTEKGRVRGTNLQVLGGTVTAFLGIPYGKPPIGRLRFQKPEPFEK  Cow MQSRST-VIYIRFVLWFLLLWVLFEKSHTEEDIIITTKNGKVRGMHLPVLGGTVTAFLGIPYAQPPLGRLRFKKPQSLTK

Glu90 Asn106 Gly116

Asn57 Cys65 Asp70 Trp82 Cys92 Trp112 Gly117 Tyr128

Human WSDIW**N**ATKYANS**C**CQNI**D**QSFPGFHGSEM**W**NPNTDLS**E**D**C**LYLNVWIPAPKPK**N**ATVLI**W**IYG**GG**FQTGTSSLHV**Y**DGK Rhesus WSDIWNATKYANSCYQNIDQSFPGFHGSEMWNPNTDLSEDCLYLNVWIPAPKPKNATVMIWIYGGGFQTGTSSLHVYDGK Horse WSNIWNATKYANSCYQNTDQSFPGFLGSEMWNPNTELSEDCLYLNVWIPAPKPKNATVMIWIYGGGFQTGTSSLPVYDGK Cat WSDIWNATKYANSCYQNADQSFPGFPGSEMWNPNTDLSEDCLYLNVWIPTPKPKNATVMIWIYGGGFQTGTSSLPVYDGK Tiger WSDIWNATKHANSCYQNADQSFPGFPGSEMWNPNTDLSEDCLYLNVWSPTPKPKNATVMIWIYGGGFQTGTSSLPVYDGK Rabbit WSDIWNATKYANSCYQNIDQSFPGFHGSEMWNPNTDLSEDCLYLNVWIPTPKPKNATVMIWIYGGGFQTGTSSLQVYDGK Pig WPDIWNATKYANSCYQNTDQSFPGFVGSEMWNPNTELSEDCLYLNVWIPAPKPKNATVMIWIYGGGFQTGTSSLHVYDGK Guinea pig WSEIWNATKYANSCHQNIDESFPGFHGSEMWNPNTDLSEDCLYLNVWIPAPKPKNATVMIWIYGGGFQTGTSSLHVYDGK Mouse WPDIHNATQYANSCYQNIDQAFPGFQGSEMWNPNTDLSEDCLYLNVWIPVPKPKNATVMVWIYGGGFQTGTSSLPVYDGK Rat WPDVYNATKYANSCYQNIDQAFPGFQGSEMWNPNTNLSEDCLYLNVWIPVPKPKNATVMVWVYGGGFQTGTSSLPVYDGK Chicken WSGIWKATKHANSCYQLIDTTYPGFPGTEMWNPKTNLSEDCLYLNVWIPSPKPKNATVMVWIYGGSFETGSTSLPVYDGK Cow WPDIWNATKYANSCYQNTDQSFPGFLGSEMWNPNTDLSEDCLYLNVWIPTPKPKNATVMIWIYGGSFQTGTSSLHVYDGK

Ala199

Arg147 Glu161 Asp170 Ser198

Human FLARVERVIVVSMNY**R**VGALGFLALPGNP**E**APGNMGLF**D**QQLALQWVQKNIAAFGGNPKSVTLFGE**SA**GAASVSLHLLSP Rhesus FLARVERVIVVSMNYRVGALGFLALPGNPEAPGNMGLFDQQLALQWVQKNIAAFGGNPKSVTLFGESAGAASVSLHLLSP Horse FLARVERVIVVSMNYRVGALGFLALSENPEAPGNMGLFDQQLALQWVQKNIAAFGGNPRSVTLFGESAGAASVSLHLLSP Cat FLARVERVIVVSMNYRVGALGFLALPGNPEVPGNMGLFDQQLALQWVQKNIAAFGGNPKSVTLFGESAGAGSVSLHLLSP Tiger FLARVERVIVVSMNYRVGALGFLALPGNPEIPGNMGLFDQQLALQWVQKNIAAFGGNPKSVTLFGESAGAGSVSLHLLSP Rabbit FLTRVERVIVVSMNYRVGALGFLALPGNPEAPGNMGLFDQQLALQWVQKNIAAFGGNPKSVTLFGESAGAASVSLHLLSP Pig FLSRVERVIVVSMNYRVGALGFLALPGNPEAPGNMGLFDQQLALQWVQKNIAAFGGNPKSVTLFGESAGAVSVSLHLLSP Guinea pig FLAQVERVIVVSMNYRVGALGFLALPGNSEASGNMGLFDQQLALQWVQNNIAAFGGNPASVTLFGESAGAASVGLHLFSP Mouse FLARVERVIVVSMNYRVGALGFLAFPGNPDAPGNMGLFDQQLALQWVQRNIAAFGGNPKSITIFGESAGAASVSLHLLCP Rat FLTRVERVIVVSMNYRVGALGFLAFPGNSEAPGNMGLFDQQLALQWIQRNIAAFGGNPKSVTLFGESAGAASVSLHLLCP Chicken FLARVERVIVVSMNYRTGALGFLALPGNKEVPGNAGLFDQRLALQWVQENIASFGGNPKSVTIFGESAGSASVSYHILSP Cow FLARVERVIVVSMNYRVGALGFLALPGNPEAPGNVGLFDQQLALQWVQKNIAAFGGNPKSVTLFGESAGAASVSLHLLSP

Asn256 Arg265

Trp231 Asn241 Cys252 Cys263

Human GSHSLFTRAILQSGSFNAP**W**AVTSLYEAR**N**RTLNLAKLTG**C**SRE**N**ETEIIK**C**L**R**NKDPQEILLNEAFVVPYGTPLSVNFG Rhesus GSHPLFTRAILQSGSSNAPWAVTSLYEARNRTLTLAKLTGCSRDNETEIVKCLRNKDPHEILLNEAFVVPYGTLLSVNFG Horse RSQPLFTRAILQSGSSNAPWAVTSLYEARNRTLTLAKRMGCSRDNETEMIKCLRDKDPQEILLNEVFVVPYDTLLSVNFG Cat RSQPLFTRAILQSGSSNAPWAVMSLDEAKNRTLTLAKFIGCSKENDTEIIKCLRNKDPQEILLNELLVVPSDTLLSVNFG Tiger RSQPLFTRAILQSGSSNAPWAVMSLDEAKNRTLTLAKFIGCSKENDTEIIKCLRNKDPQEILLNELLVVPSDTLLSVNFG Rabbit RSHPLFTRAILQSGSSNAPWAVMSLHEARNRTLTLAKFVGCSTENETEIIKCLRNKDAQEILLNEVFVVPFDSLLSVNFG Pig RSHPLFARAILQSGSSNAPWAVTSLYEARNRTLTLAKFIGCSRENETEIIKCLRNKDPQEILQNEVFVVPNHMLLSVNFG Guinea pig KSHPLFTRAILQSGSPSASWAVMSPYEAKNRTLSLAKFTGCFKDNETEMIKCLQNKDPQEILRNELFVLPYDTLLSVIFG Mouse QSYPLFTRAILESGSSNAPWAVKHPEEARNRTLTLAKFTGCPKENEMEMIKCLRSKDPQEILRNERFVLPSDSILSINFG Rat QSYPLFTRAILESGSSNAPWAVKHPEEARNRTLTLAKFIGCSKENEKEIITCLRSKDPQEILLNEKLVLPSDSIRSINFG Chicken KSHPLFTRAIMQSGSANAPWAAITASEARRRTVALAKQLKCPTSDETELILCLQDKDPKDILENEVYVVKYFSLLHIYFC Cow ESHPLFTRAILQSGSSNAPWAVTSRYEARNRTLTLAKFIGCSRENDTEIIKCLRNKDPQEILRHEVFVVPYGTLLSVNFG

Phe329

Glu325 Tyr332 Asn341

Human PTVDGDFLTDMPDILLELGQFKKTQILVGVNKD**E**GTA**F**LV**Y**GAPGFSKD**N**NSIITRKEFQEGLKIFFPGVSEFGKESILF Rhesus PTMDGDFLTEMPDILLELGQFKKTQILVGVNKDEGTAFLVYGAPGFSKDNDSIITRNEFQEGLKIFFPGVSEFGKESILF Horse PTVDGDFLTDMPDTLLQLGQFKRTQILVGVNKDEGTAFLVYGAPGFSKDNNSIITRKEFQEGLKIFFPRVSEFGRESILF Cat PVVDGDFLTDMPDTLLQLGQFKKTQILVGVNKDEGTAFLVYGAPGFSKDNDSIITRKEFQEGLKIYFPGVSEFGREAILF Tiger PVVDGDFLTDMPDTLLQLGQFKKTQILVGVNKDEGTAFLVYGAPGFSKDNDSIITRKEFQEGLKIYFPGVSEFGREAILF Rabbit PTVDGDFLTDMPDTLLQLGQLKKTQILVGVNKDEGTAFLVYGAPGFSKDNNSIITRKEFQDGLKIFFPRVSEFGKESILF Pig PTVDGDFLTDLPDTLLQLGQFKKTQILVGVNKDEGTAFLVYGAPGFSKDNNSIITRKEFEEGLKIFFPGVSEFGKESILF Guinea pig PTVDGDFLTDMPETLLQRGQVKKTQILVGVNKDEGTAFLVYRVPGFSKDNNSIINRSQFQQGLKICFPGASEFGRESILF Mouse PTVDGDFLTDMPHTLLQLGKVKKAQILVGVNKDEGTAFLVYGAPGFSKDNDSLITRKEFQEGLNMYFPGVSRLGKEAVLF Rat PTVDGDFLTDMPHTLLQLGKVKTAQILVGVNKDEGTAFLVYGAPGFSKDNDSLITRREFQEGLNMYFPGVSSLGKEAILF Chicken PTVDGDFLADMPEALIKNGIFKQTQVLVGVNKDEGTSFLVYGVPGFSKDSDSLINKTQFEVALTLSFPQVSKLAIESIIF Cow PTVDGDFLTDMPDTLLQLGQFKKTQILVGVNKDEGTAFLVYGAPGFSKDNNSIITRKEFQEGLKIFFPGVSEFGKESILF

Asp395 Tyr440

Cys400 Trp430 His438

Human HYTDWVDDQRPENYREALGDVVG**D**YNFI**C**PALEFTKKFSEWGNNAFFYYFEHRSSKLP**W**PEWMGVM**H**G**Y**EIEFVFGLPLE Rhesus HYTDWVDDQRPENYREALDDVVGDYNIICPALEFTKKFSEWGNNAFFYYFEHRSSKLPWPEWMGVMHGYEIEFVFGLPLE Horse HYMDWLDDQRAENYREALDDVVGDYNIICPALEFTKKFSELGNDAFFYYFEHRSTKLPWPEWMGVMHGYEIEFVFGLPLE Cat YYVDLLDDQRAEKYREALDDVLGDYNIICPALEFTTKFSELGNNAFFYYFEHRSSQLPWPEWMGVMHGYEIEFVFGLPLE Tiger YYVDLLDDQRAEKYREALDDVLGDYNIICPALEFTTKFSELGNNAFFYYFEHRSSQLPWPEWMGVMHGYEIEFVFGLPLE Rabbit HYADWLDDERPENYREALDDVVGDYNIICPALEFAKKCSEMGNNAYFYYFEHRSSKLPWPEWMGVMHGYEIEFVFGLPLE Pig HYMDWTDDQRAENYRDALDDVVGDYDIICPALEFTKKFSEMGNNAFFYYFEHRSSKLPWPEWMGVMHGYEIEFVFGLPLE Guinea pig HYADWLDDQRPENYREALDDVVGDYNFICPSLEFGKRFSDLGSPAFFYYFEHRSSKLPWPEWMGVMHGYEIEFVFGLPLE Mouse YYVDWLGEQPPEVYRDALDDVIGDYNIICPALEFTKKFAELENNAFFYFFEHRSSKLPWPEWMGVMHGYEIEFVFGLPLG Rat YYVDWLGDQTPEVYREAFDDIIGDYNIICPALEFTKKFAELEINAFFYYFEHRSSKLPWPEWMGVMHGYEIEFVFGLPLE Chicken QYTDWENEQKPEHYRDAMDDVIGDYHIICPAVEFAKTIAEVGNNVFFYFFEHRSSKLPWPEWMGVMHGYEIEFVFGLPLE Cow HYMDWLDDQRAEKYREALDDVVGDYNIICPALEFTKKFSDMGNNAFFYYFEHRSSKLPWPEWMGVMHGYEIEFVFGLPLE

Asn486 Arg515

Asn455 Asn481 Cys519

Human RRD**N**YTKAEEILSRSIVKRWANFAKYGNP**N**ETQN**N**STSWPVFKSTEQKYLTLNTESTRIMTKL**R**AQQ**C**RFWTSFFPKVLE Rhesus RRVNYTKAEEILSRSIVKRWANFAKYGNPNGTHNNSTKWPVFKSTEQKYLTLNTESSRILTKLRAQQCRFWTSFFPKVLE Horse RRVNYTKAEEILSRSIMKRWANFAKYGNPNGTQSNSTRWPVFKSTEQKYLTLNTESPKVYTKLRAQQCRFWTLFFPKVLE Cat RRVNYTRAEEILSRSIMNYWANFAKYGNPNGTQNNSTRWPAFRSTDQKYLTLNAESPKVYTKLRAQQCRFWTLFFPKVLE Tiger RRVNYTRAEEILSRSIMNYWANFAKYGNPNGTQNNSTRWPAFRSTDQKYLTLNAESPKVYTKLRAQQCRFWTLFFPKVLE Rabbit RRVNYTKAEEILSRSIMKRWANFAKYGNPNGTQNNSTRWPVFKSTEQKYLTLNTESPRIYTKLRAQQCRFWTLFFPKVLE Pig RRANYTKAEEILSRSIMKRWANFAKYGNPNGTQNNSTRWPVFKSNEQKYLTLNAESPRVYTKLRAQQCRFWTLFFPKVLE Guinea pig RRVNYTKAEEILSRSIMKYWANFAKYGHPNGTQSNSSGWPVFKSIEQKYLTLNTESPRIYTKLRAQQCRFWTLFFPKVLE Mouse RRVNYTRAEEIFSRSIMKTWANFAKYGHPNGTQGNSTMWPVFTSTEQKYLTLNTEKSKIYSKLRAPQCQFWRLFFPKVLE Rat RRVNYTRAEEIFSRSIMKTWANFAKYGHPNGTQGNSTVWPVFTSTEQKYLTLNTEKSKINSKLRAPQCQFWRLFFPKVLE Chicken RRVNYTKAEEILSRSMLRYWASFAKTGNPNGTLINGTRWPVFTSTEQKYLTLNTDASEILTKLRAQQCRFWNMFFPKVLE Cow RRVNYTKAEEIFSRSIMKRWANFAKYGNPNGTQNNSTRWPVFKSNEQKYFTLNTESPKVNTKLRAQQCRFWTLFFPKVLE

Phe547 Tyr553 Phe561

Trp543 Trp550 Trp557 Tyr564 Cys571

Human MTGNIDEAEWE**W**KAG**F**HR**W**NN**Y**MMD**W**KNQ**F**ND**Y**TSKKES**C**VGL

Rhesus MTGNIDEAEWEWKAGFHRWSNYMMDWKNQFNDYTSKKESCVGL

Horse LTGNIDEAEREWKAGFHRWNNYMMDWKNQFNDYTSKKESCSDF

Cat MTGNIDEAEREWRAGFYRWNNYMMDWKNQFNDYTSKKESCAGL

Tiger MTGNIDEAEREWRAGFYRWNNYMMDWKNQFNDYTSKKESCAGL

Rabbit MTGNIDEAEQEWKAGFHRWNNYMMDWKNQFNDYTSKKESCAGF

Pig MTGNIDEAEREWKAGFHRWNNYMMDWKNQFNDYTSKKESCADL

Guinea pig MAGNLDEVEQKWKAGFHLWNNYMTDWKNQFNDYISKKESCVDL

Mouse MTGDIDETEQEWKAGFHRWSNYMMDWQNQFNDYTSKKESCTAL

Rat ITGDIDEREQEWKAGFHRWSNYMMDWKNQFNDYTSKKETCTDL

Chicken MTGNIDEAEREWKAGFHRWNNYMMDWKNQFNDYTSKKERCAGSN

Cow ITGNIDEVEREWKAGFHRWNNYMMDWKNQFNDYTSKKESCAGL

**Figure S5**. Sequence alignment of BChE from 12 species. Numbering of residues starts with Glu1 at the N-terminus of the mature, secreted human BChE protein ([Lockridge et al., 1987b](#_ENREF_7)); numbers in parentheses use Met at the start of the signal peptide as residue number 1. BChE in all species has the catalytic triad residues Ser198 (226), Glu325 (353) and His438 (466). All have the peripheral anionic site residues Asp70 (98) and Tyr332 (360) ([Nicolet et al., 2003](#_ENREF_9)). The active site gorge in all species is lined by 8 aromatic residues Trp82 (110), Trp112 (140), Tyr128 (156), Trp231 (259), Phe329 (357), Tyr332 (360), Trp430 (458) and Tyr440 (468). All have Trp82 (110) at the choline-binding site at the bottom of the active site gorge. The residues in the oxyanion hole Gly116 (144), Gly117(145) and Ala199 (227) that stabilize the tetrahedral intermediate during catalysis, are present in all species, except chicken and bovine. Chicken and bovine BChE have Ser117 (145) in place of Gly117 (145). BChE in all species has 6 cysteine residues that form 3 intrachain disulfide bonds, C65-C92, C252-C263, C400-C519 (93-120, 280-291, 428-547) and one interchain disulfide bond Cys 571 (599) near the C-terminus for covalent binding to a second subunit ([Lockridge et al., 1987a](#_ENREF_6)). The 40 residues at the C-terminus constitute the tetramerization domain containing 7 aromatic residues Trp543 (571), Phe547 (575), Trp550 (578), Tyr553 (581), Trp557(585), Phe561(589), Tyr564 (592) that intercalate with polyproline rich peptides to form BChE tetramers ([Altamirano and Lockridge, 1999](#_ENREF_1); [Li et al., 2008](#_ENREF_5); [Biberoglu et al., 2012](#_ENREF_3); [Biberoglu et al., 2013](#_ENREF_2); [Larson et al., 2014](#_ENREF_4)). Three salt bridges are conserved Arg42-Glu90, Arg147-Asp170, Asp395-Arg515 (70-118, 175-198, 423-543), but a fourth salt bridge Glu161-Arg265 (189-293) is not conserved in guinea pig, mouse, and chicken BChE ([Nicolet et al., 2003](#_ENREF_9); [Nachon et al., 2005](#_ENREF_8)). Human and monkey BChE have 9 N-linked glycans Asn17, Asn57, Asn106, Asn241, Asn256, Asn341, Asn455, Asn481, Asn486 ([Lockridge et al., 1987b](#_ENREF_7)), whereas horse, cat, tiger, rabbit, pig, guinea pig, and cow have 8, mouse and rat have 7, and chicken has 6 N-linked glycans. Two of the N-glycans in chicken are at different positions compared to the other species.

Accession numbers: P06276 human BChE, AFE79500 rhesus monkey BChE, Q9N1N9 horse BChE, O62760 domestic cat BChE, O62761 tiger BChE, XP\_002716414 rabbit BChE, XP\_003358712 pig BChE, XP\_003469406 guinea pig BChE, Q499C7 mouse BChE, Q9JKC1 rat BChE, Q90ZK8 chicken BChE, P32749 cow BChE.

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