Fig. S1. (A) Clustal Omega alignment of B′ family

```
PPP2R5A      ----------------------------------------------------MSSSSPP   7
PPP2R5B      ---------------------------------METK-------------LPPASTPTSP   14
PPP2R5C      MPNKNKKEKESPKAGKSGKS----SKEG--QDTVES--------------E-QISVRKNS   39
PPP2R5D      MPYKLKKEKEPPKVAKCTAKPSSSGKDGGGENTEEAQPQPQPQPQPQAQSQPPSSNKRPS   60
PPP2R5E      ------------------------------------------------------------   0
                                    L                            S


PPP2R5A      AGAASAAISASEKVDGFTRKSVRKAQRQKRSQGSSQFRSQGSQAELHPLPQLKDATSNEQ   67
PPP2R5B      SSPGLSPVPPPDKVDGFSRRSLRRA-RPRRSHSSSQFRYQSNQQELTPLPLLKDVPASEL   73
PPP2R5C      LVAVPSTVSAKIKVPV-----SQPIVKKDKRQNSSRFS-ASNNRELQKLPSLKDVPPADQ   93
PPP2R5D      NSTPPPTQLSKIKYSG-----GPQIVKKERRQSSSRFN-LSKNRELQKLPALKDSPTQER   114
PPP2R5E      MSSAPTTPPSVDKVDGFSRKSVRKA-RQKRSQSSSQFRSQGKPIELTPLPLLKDVPSSEQ   59
                         *             :  : :.**:*   ..  **  ** ***    :

PPP2R5A      QELFCQKLQQCCILFDFM-DSVSDLKSKEIKRATLNELVEYVSTNRGVIVESAYSDIVKM   126
PPP2R5B      HELLSRKLAQCGVMFDFL-DCVADLKGKEVKRAALNELVECVGSTRGVLIEPVYPDIIRM   132
PPP2R5C      EKLFIQKLRQCCVLFDFVSDPLSDLKWKEVKRAALSEMVEYITHNRNVITEPIYPEVVHM   153
PPP2R5D      EELFIQKLRQCCVLFDFVSDPLSDLKFKEVKRAGLNEMVEYITHSRDVVTEAIYPEAVTM   174
PPP2R5E      PELFLKKLQQCCVIFDFM-DTLSDLKMKEYKRSTLNELVDYITISRGCLTEQTYPEVVRM   118
              :*: :** ** ::***: * ::*** ** **: *.*:*: :  .*. : *  * : : *


PPP2R5A      ISANIFRTLPPSDNP---DFDPEEDEPTLEASWPHIQLVYEFFLRFLESPDFQPSIAKRY   183
PPP2R5B      ISVNIFRTLPPSENP---EFDPEEDEPNLEPSWPHLQLVYEFFLRFLESPDFQPSVAKRY   189
PPP2R5C      FAVNMFRTLPPSSNPTGAEFDPEEDEPTLEAAWPHLQLVYEFFLRFLESPDFQPNIAKKY   213
PPP2R5D      FSVNLFRTLPPSSNPTGAFDPEEDEPTLEAAWPHLQLVYEFFLRFLESPDFQPNIAKKY   234
PPP2R5E      VSCNIFRTLPPSDSN---EFDPEEDEPTLEASWPHLQLVYEFFIRFLESQEFQPSIAKKY   175
             .: *:*******..    :********.** :***:*******:***** :***.:**:*
                    M             RKK KR    LR   P       H
                                   G  GH     C
                                             L

PPP2R5A      IDQKFVQQLLELFDSEDPRERDFLKTVLHRIYGKFLGLRAFIRKQINNIFLRFIYETEHF   243
PPP2R5B      VDQKFVLMLLELFDSEDPREREYLKTILHRVYGKFLGLRAYIRKQCNHIFLRFIYEFEHF   249
PPP2R5C      IDQKFVLQLLELFDSEDPRERDFLKTTLHRIYGKFLGLRAYIRKQINNIFYRFIYETEHH   273
PPP2R5D      IDQKFVLALLDLFDSEDPRERDFLKTILHRIYGKFLGLRAYIRRQINHIFYRFIYETEHH   294




                             H
                             Y
PPP2R5A      NGVAELLEILGSIINGFALPLKAEHKQFLMKVLIPMHTAKGLALFHAQLAYCVVQFLEKD   303
PPP2R5B      NGVAELLEILGSIINGFALPLKTEHKQFLVRVLIPLHSVKSLSVFHAQLAYCVVQFLEKD   309
PPP2R5C      NGIAELLEILGSIINGFALPLKEEHKIFLLKVLLPLHKVKSLSVYHPQLAYCVVQFLEKD   333
PPP2R5D      NGIAELLEILGSIINGFALPLKEEHKMFLIRVLLPLHKVKSLSVYHPQLAYCVVQFLEKE   354

                                 V

PPP2R5A      TTLTEPVIRGLLKFWPKTCSQKEVMFLGEIEEILDVIEPTQFKKIEEPLFKQISKCVSSS   363
PPP2R5B      ATLTEHVIRGLLKYWPKTCTQKEVMFLGEMEEILDVIEPSQFVKIQEPLFKQVARCVSSP   369
PPP2R5C      STLTEPVVMALLKYWPKTHSPKEVMFLNELEEILDVIEPSEFVKIMEPLFRQLAKCVSSP   393
PPP2R5D      SSLTEPVIVGLLKFWPKTHSPKEVMFLNELEEILDVIEPSEFSKVMEPLFRQLAKCVSSP   414
PPP2R5E      PSLTEPVIRGLMKFWPKTCSQKEVMFLGELEEILDVIEPSQFVKIQEPLFKQIAKCVSSP   355
              :*** *: .*:*:**** : ******.*:*********::* *: ****:*::****
```

```
PPP2R5A      HFQVAERALYFWNNEYILSLIEENIDKILPIMFASLYKISKEHWNPTIVALVYNVLKTLM   423
PPP2R5B      HFQVAERALYFWNNEYILSLIEDNCHTVLPAVFGTLYQVSKEHWNQTIVSLIYNVLKTFM   429
PPP2R5C      HFQVAERALYYWNNEYIMSLISDNAAKILPIMFPSLYRNSKTHWNKTIHGLIYNALKLFM   453
PPP2R5D      HFQVAERALYYWNNEYIMSLISDNAARVLPIMFPALYRNSKSHWNKTIHGLIYNALKLFM   474
PPP2R5E      HFQVAERALYYWNNEYIMSLIEENSNVILPIMFSSLYRISKEHWNPAIVALVYNVLKAFM   415
             **********:******:***.:*   :** :* :**: ** *** :* .*:**.** :*
                  K                   K

PPP2R5A      EMNGKLFDDLTSSYKAERQREKKKELEREELWKKLEELKLKKALEKQNSAYNMHS-----   478
PPP2R5B      EMNGKLFDELTASYKLEKQQEQQKAQERQELWQGLEELRLRRLQGTQGAKEAPLQ-----   484
PPP2R5C      EMNQKLFDDCTQQFKAEKLKEKLKMKEREEAWVKIENLAKANPQYTVYSQASTMSIPVAM   513
PPP2R5D      EMNQKLFDDCTQQYKAEKQKGRFRMKEREEMWQKIEELARLNPQYPMFRAPPPLPPVYSM   534
PPP2R5E      EMNSTMFDELTATYKSDRQREKKKEKEREELWKKLEDLELKRGLRRDGIIPT--------   467
             *** .:**: *  :* :: : : :  **:* *  :*:*   .
                                                          S    L

PPP2R5A      ------------ILSNTSAE----------------------------------------   486
PPP2R5B      ------------RLTPQVAASGGQS-----------------------------------   497
PPP2R5C      ETDGPLFEDVQMLRKTVKDEAHQAQKDPKKDRPLARRKSELPQDPHTKKALEAHCRADEL   573
PPP2R5D      ETETPTAEDIQLLKRTVETEAVQMLKDIKKEKVLLRRKSELPQDVYTIKALEAHKRAEEF   594
PPP2R5E      ------------------------------------------------------------   467
                                    V                            V


PPP2R5A      --------  486
PPP2R5B      --------  497
PPP2R5C      ASQDGR--  579
PPP2R5D      LTASQEAL  602
PPP2R5E      --------  467
```

Fig. S1 (B). Clustal Omega alignment of B″ family

```
PPP2R3A      DFAQELVECKSSRGSLSQEKEMMQI----LQETLTTSSQANLSVCRSPVGDKAKDTTSAV     656
PPP2R3B      ----------MPPGKVLQPVLKMKVDELFLYWLSEASTQRMLQDCLRRIKAPGRDQPTPG     50
PPP2R3C      ------------------------------------------------------------     0


PPP2R3A      LIQQ-----TPEVIKIQNKPEKKPGTPLPPPATSPSSPRPLSPVPHVNN-----------     700
PPP2R3B      DGEQPGAWPTAPLAAPRPSGLEPPGTPGPGPALPLGAASSPRNAPHVRGTRRSAGTRVV-     109
PPP2R3C      -----MDWKEV--------LRRRLATPNTCPNKKKS-------EQELKDEEMDLFTKYYS     40
                                  .  .**   *    .         .:..

PPP2R3A      ------VVNAPLSINIPRFYFPEGLPDTCSNHEQTLSRIETAFMDIEEQKAD--------     746
PPP2R3B      QTRKEEPLPPATSQSIPTFYFPRGRPQDSVNVDAVISKIESTFARFPHERAT--------     161
PPP2R3C      EWKGGRKNTNEFYKTIPRFYYRLPAEDEVLLQK-LREESRAVFLQRKSRELLDNEELQNL     99
                           .** **:     :     .   .. .:.*     ..

PPP2R3A      -----------------------IYEMGKIAKVCGCPLYWKAPMFRAA-GGEKTGFVTAQ     782
PPP2R3B      -----------------------MDDMGLVAKACGCPLYWKGPLFYGA-GGERTGSVSVH     197
PPP2R3C      WFLLDKHQTPPMIGEEAMINYENFLKVG-EKAGAKCKQFFTAKVFAKLLHTDSYGRISIM     158
                                    : .:*     . *  ::.. :*      :  * ::
                Q

PPP2R3A      SFIA-MWRKLLNNHHDDASKFICLLAKPNCSSLEQEDFIPLLQDVVDTHPGLTFLKDAPE     841
PPP2R3B      KFVA-MWRKILQNCHDDAAKFVHLLMSPGCNYLVQEDFVPFLQDVVNTHPGLSFLKEASE     256
PPP2R3C      QFFNYVMRKVWLH---QTRIGLSLYDVAGQGYLRESDLENYILELIPTLPQLDGLE--KS     213
             .*.  : **:  :   ::   : *    . . * :.*:   : ::: * * *  *:   .
                                                  S

PPP2R3A      FHSRYITTVIQRIFYTVNRSWSGKITSTEIRKSNFLQTLALLEEEE--DINQITDYFSYE     899
PPP2R3B      FHSRYITTVIQRIFYAVNRSWSGRITCAELRRSSFLQNVALLEEEA--DINQLTEFFSYE     314
PPP2R3C      FYSFYVCTAVRKFFFFLDPLRTGKIKIQDILACSFLDDLLELRDEELSKESQETNWFSAP     273
             *:* *: *.::::*: ::   :*:*.  ::  ..**: :  *.:*   . .* *::**

PPP2R3A      HFYVIYCKFWELDTDHDLYISQADLSRYNDQASSSRIIERIFSGAVTRGKTIQKEGRMSY     959
PPP2R3B      HFYVIYCKFWELDTDHDLLIDADDLARHNDHALSTKMIDRIFSGAVTRGRKVQKEGKISY     374
PPP2R3C      SALRVYGQYLNLDKDHNGMLSKEELSRYGTATMTNVFLDRVFQECLT------YDGEMDY     327
                 :* :: :**.**:  :.  :*:*:.  : :. :::*:*. .:*       :*.:.*

PPP2R3A      ADFVWFLISEEDKRNPTSIEYWFRCMDVDGDGVLSMYELEYFYEEQCERMEAMGIEPLPF     1019
PPP2R3B      ADFVWFLISEEDKKTPTSIEYWFRCMDLDGDGALSMFELEYFYEEQCRRLDSMAIEALPF     434
PPP2R3C      KTYLDFVLALENRKEPAALQYIFKLLDIENKGYLNVFSLNYFFRAIQELMKIHGQDPVSF     387
               :: *::: *::: *::::* *: :*::..* *.::.*:**:.   . :.  . : : *
                                   S

PPP2R3A      HDLLCQMLDLVKPAVDGKITLRDLKRCRMAHIFYDTFFNLEKYLDHEQRDPFAVQKDVEN     1079
PPP2R3B      QDCLCQMLDLVKPRTEGKITLQDLKRCKLANVFFDTFFNIEKYLDHEQKEQISLLRDGDS     494
PPP2R3C      QDVKDEIFDMVKPKDPLKISLQDLINSNQGDTVTTILIDLNGFWTYENREALVANDSENS     447
             :*   :::*:***    **:*:** ... .. .   ::::: :  :*::: :    . :.

PPP2R3A      DGPEPSDWDRFAAEEYETLVAEESAQAQFQEGFEDYETDEPASPSEFGNKSNKIL-----     1134
PPP2R3B      GGPELSDWEKYAAEEYDILVAEETAGEPWEDGFEAELSPVEQKLSALRSPLAQRPFFEAP     554
PPP2R3C      A--DLDDT----------------------------------------------------     453
                : .*

PPP2R3A      -----SASLPEKCGKLQSVDEE 1151
PPP2R3B      SPLGAVDLYEYACGDEDLEPL- 575
PPP2R3C      ---------------------- 453
```

Fig. S1. Sequence alignment showing the similarity within isoforms of the B′ subunit family (A) and B″ subunit family (B).

(A) Sequence alignment of human PP2A-B′ subunits showing the conservation and diversity in protein sequence within B’ family members where residues highlighted in yellow are mutations implicated in NDD identified from published studies and publicly available clinical databases. Mutated residues are color coded where residues in red are pathogenic, green are benign/ likely benign, and blue are of uncertain significance. Underlined GH mutation is an insertion/deletion mutation, underlined T mutation is deletion mutation, boxed residues are nonsense mutations, upward arrow shows the frame shift mutations leading to termination. Residues within the grey box make up the acidic loop in PP2A-B′. Accession number for protein sequences are Q15172, NP_006235, NP_001339842, NP_006236 and NP_001269108 for PPP2R5A, PPP2R5B, PPP2R5C, PPP2R5D, and PPP2R5E, respectively.

(B) Sequence alignment of human PP2A-B″ subunits showing the conservation and diversity in protein sequence within B″ family members. Color coding of the residues follows the same style as S2 (A). Accession number for protein sequences are NP_002709; NP_037371and NP_060387 for PPP2R3A, PPP2R3B and PPP2R3C, respectively

Table S1

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