

Disruption of *PTPRO* Causes Childhood-Onset

Nephrotic Syndrome

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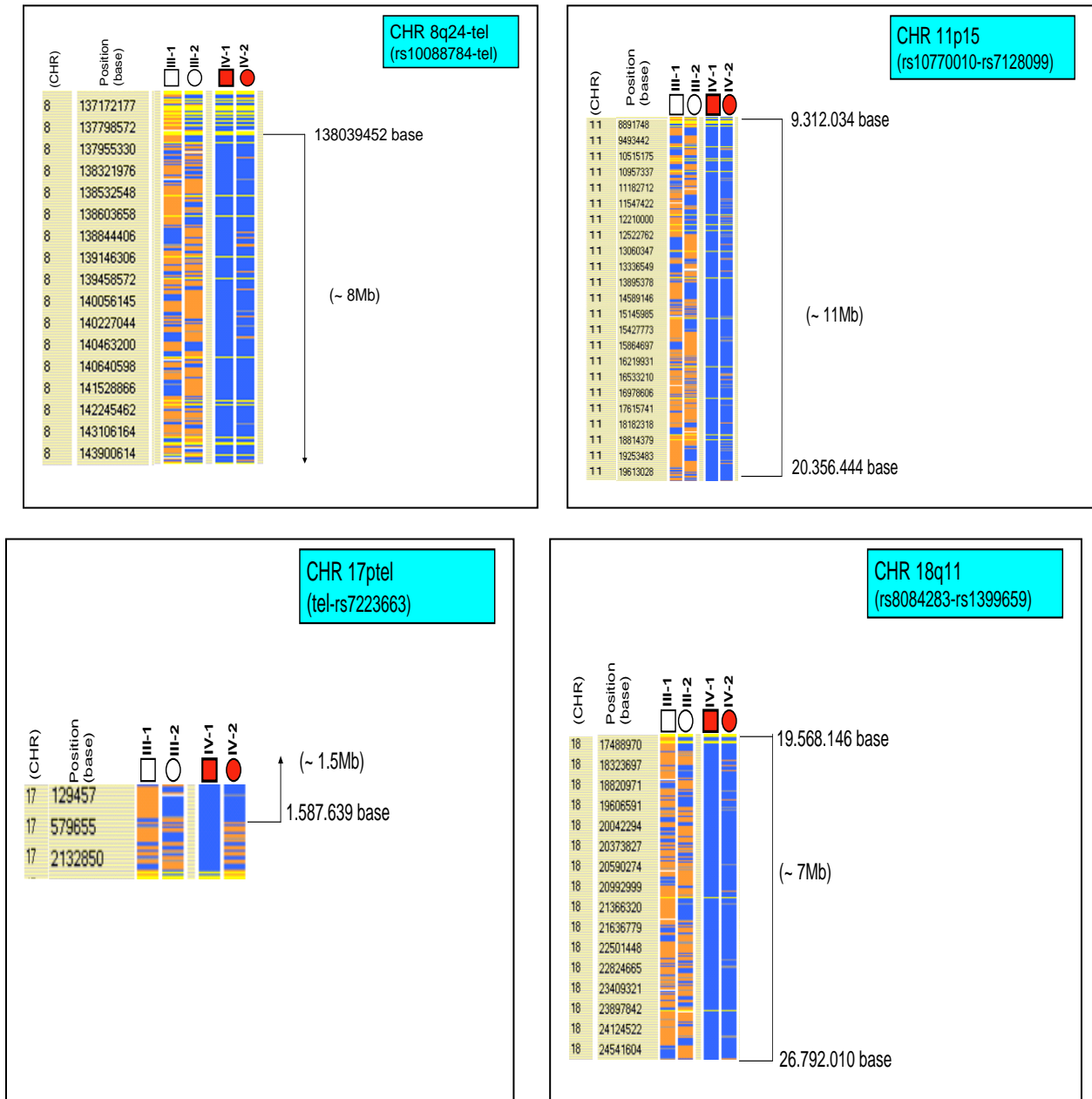


Figure S1. Critical Regions of Homozygosity Obtained via 250K SNP Analysis in Family A

In all, 5 large homozygous segments on chromosome 12p12, 8qtel, 11p15, 17ptel, and 18q11 regions were observed in the genome-wide analysis of family A. Details of the chromosome 12p12 region are presented in the text (Figure 1a-b) and the remaining 4 localizations are included in this supplemental figure. SNP numbers (i.e. rs10770010 and rs7128099) located in the proximal and distal boundaries of each critical region are shown upper right.

Ex1 Ex2
1 MGHLPTGIHGARRLLPLLWLFVLFKNATAFHVTVQDDNNIVVSLEASDVI 50
51 SPASVYVVKITGESKNYFFEFEEFNSTLPPPVIKASYHGLYYIITLVVV 100
Ex3
101 NGNVVTKPSRSITVLTKPLPVTSVSIYDYKPSPETGVLFEIHYPEKYNVF 150
Ex4
151 TRVNISYWEGKDFRTMLYKDFFKGKTVFNHWLPGMCYSNITFQLVSEATF 200
Ex5
201 NKSTLVEYSGV SHEPKQHRTAPYPPQNISVRIVNLNKNWEEQSGNFPEE 250
251 SFMRSQDTIGKEKLFHFTEETPEIPSGNISSGWPDFNSSDYETTSQPYWW 300
301 DSASAAPESEDEFVSVLPMEYENNSTLSETEKSTSGSFSFFPVQMILTWL 350
Ex6
351 PPKPPTAFDGFHIIHEREENFTEYLMVDEEAHEFVAELKEPGKYKLSVT 400
Ex7
401 FSSSGSCETRKSQSAKSLSFYISPSGEWIEELTEKPQHVSVHVLSTTAL 450
Ex8
451 MSWTSSQENYNSTIVSVSLTCQKQKESQRLEKQYCTQVNSSKPIIENLV 500
Ex9
501 PGAQYQVV IYLRKGPLIGPPSDPVTFAIVPTGIKDLMLYPLGPTAVVLSW 550
Ex10
551 TRPYLGVFRKYVEMFYFNPATMTSEWTTYEIAATVSLTASVRIANLLP 600
Ex11
601 AWYYNFRV TMVTWGDPELSCCDSSTISFITAPVAPEITSVEYFNLLYIS 650
Ex12
651 WTYGDDTTDLSHSRMLHWMVVAEGKKIKKSVTRNVMTAILSLPPGDIYN 700
Ex13
701 LSV TACTERGSNTSMLRLVKLE PAPPKSLFAVNKTQTSVTLWVEEGVAD 750
Ex14
751 FFEVFCQQVGSSQKTKLQEPVAVSSHVVTISSLLPATAYNCSVTSFSHDS 800

801 PSVPTFIAVSTMVTEMNPNVVV **ISVLAAILSTLLIGLLLVTLII** LRKKHLQ 850

851 MAR **ECGAGTFVNFASLERDGLPYN** WSKNGLKKRKLTN **PVQLDDFDAYIK** 900

901 **DMAKDSYKFSLQFE** ELKLIIGLDIPHFAADLPLNRCKNRYTNILPYDFSR 950

951 VRLVSMNEEEGADYINANYIPGYNSPQEYIATQGPLPETRNDFWKMVLQQ 1000

1001 **KSQIIVMLTQCNEKRRVKCDHYWPFTEEPIAYGDITVEMISEEEQDDWAC** 1050

1051 RHFRIINYADEMQDVMHFNNTAWPDHGVPTANAAESILQFVHMVRQQATKS 1100

1101 **KGPMIIHCSAGVGR** TGTFFIALDRLLQHIRDHEFVDILGLVSEMRSYRMSM 1150

1151 **VQTEEQYIFIHQCVQLMWMKKKQQFCISDVIYENVSKS**

Figure S2. Protein Domains of Human PTPRO

Protein domains of human PTPRO (Q16827) transcript variant 2 (NM_002848.2), as predicted using UniProt (<http://www.uniprot.org/blast/?about=Q16827|876-903>). Note that exon 17 is lacking variant 2 (see text).

The extent of putative domains is highlighted as follows: yellow: **signal peptide**; light blue: **fibronectin type-III domain**; green: **transmembrane domain**; pink: **substrate-binding region**; gray: **tyrosine protein phosphatase domain**.

Skipping exons related to splice donor mutations described herein are shown as red.

Encoding exons are shown at their start positions.

Signal peptide

H._sapiens	MGHLPTGIHGARRLLPLLWLVFLVLFKNAFAFHVTVQDDNNIVVSLLEASDVISPASVYVVKI	60
P._troglodytes	MGHLPTGIHGARRLLPLLWLVFLVLFKNAFAFHVTVQDDNNIVVSLLEASDVISPASVYVVKI	60
C._familiaris	-----LQNAATTFHVTVQDDNNIVVSLLEASDVMSPPSSVYVVKI	38
M._musculus	MGHLPRGTLGGRLLPLLGLFVLLKIVTTFHVAVQDDNNIVVSLLEASDIVSPASVYVVRV	60
R._norvegicus	MGHLPRERRGGRLLPLLGLFVLLKIVSTFHVTVRDDNNIVVSLLEASDVVSPASVYVVRV	60
X._tropicalis	-----	
D._rerio	-----MSSARARFFFIFIFIQSAAGFRVQLQDGVRLMSLDDGDIGPVGSEYAVRV	52
F._rubripes	-----LTALPVPVQVSWAFRAWVNEDGRIMAALHESDLQENATAYAAQL	44

Fibronectin Type-3

H._sapiens	TGE--SKNYFFEFEEFNSTLPPPVIFKASYHGLYIIITLVVVNGNVVTKPSRSITVLTKE	118
P._troglodytes	TGE--SKNYFFEFEEFNSTLPPPVIFKASYHGLYIIITLVVVNGNVVTKPSRSITVLTKE	118
C._familiaris	TGE--SKNYFFEFEEFNSTLPPPVTFKANYHGLYIIITLVVVNGNVVTKPSRSITVLTKE	96
M._musculus	AGE--SKNYFFEFEEFNSTLPPPVVFKATYHGLYIIITLVVVNGNVVTKPSRSITVLTKE	118
R._norvegicus	AGE--SKNYFFEFEEFNSTLPPPVVFKATYHGLYIIITLVVVSGNVVTKPSRSITVLTKE	118
X._tropicalis	-----	
D._rerio	SGEPLTHTLLFQQADAHTLPEPLLFNASYHGLLISLMTDG----RTHTTTSITIQP	107
F._rubripes	SGDAQTTVALFAN-----VSKALSFNTSHHGLCYSGLLIKVGHWTWSPVSSVPLTKE	98

Fibronectin Type-3

H._sapiens	LPVTSVSIYDYKPSPETGVLFEEIHYPEKYNVFTRVNISYEWEGKDFRTMLYKDFFKGKTVF	178
P._troglodytes	LPVTSVSIYDYKPSPETGVLFEEIHYPEKYNVFTRVNISYEWEGKDFRTMLYKDFFKGKTVF	178
C._familiaris	LPVTSVSIYDYKPSPETGVLFEEIHYPEKYNVFTRVNISYEWEGKDFRTMLYKDFFKGKTVF	156
M._musculus	LPVTSVSIYDYKPSPETGVLFEEIHYPEKYNVFSRVNISYEWGRDFRTMLYKDFFKGKTVF	178
R._norvegicus	LPVTSVSIYDYKPSPETGVLFEEIHYPEKYNVFSRVNISYEWEGKDFRTMLYKDFLKGKTVF	178
X._tropicalis	-----	
D._rerio	LPLDSVEMWDYAPSPETAVVFQIRSPDR-NIFTRVNISYTEGHQRRYMLYKDFLHGKTVF	166
F._rubripes	LPVHSAHILDYKESPETGVVFDIEPPAG-TIFSRVNITFTEGEESSMLYKDFYQGRKTVF	157

H._sapiens	NHWLPGMCYSNITFQLVSEATFNKSTLVEYSGVSHPEKQHRRTAPYPPQNISVRIVNLN--	236
P._troglodytes	NHWLPGMCYSNITFQLVSEATFNKSTLVEYSGVSHKPKQHRRTAPYPPQNISVRIVNLN--	236
C._familiaris	NHWLPGICYSNITFQLVSEATFNKSTLVEYSGISHPEKQHRRTAPYPPRNISVRIVNLN--	214
M._musculus	NHWLPGLCYSNITFQLVSEATFNKSTLVEYSGVSHPEKQHRRTAPYPPRNISVRIVNLN--	236
R._norvegicus	NHWLPGICYSNITFQLVSEATFNKSTLVECSGVSHPEKQHRRTAPYPPRNISVRFVNLN--	236
X._tropicalis	-----	
D._rerio	KHWLPGVYSNITFQLISEASVNRSALLSRSDITHNPQQHRTVPPNPLNVSLKILHLSGR	226
F._rubripes	KHWLPGVCYRDITFQLVSEARVFRNSLVAHSDVTHKPLNHRTPVPPNLSHSIVHLS--	215

Fibronectin Type-3

H._sapiens	-----KNNWEEQSGNFPEESFMRS-QDTIGKEKLFHFTTEETPEIPSGNI	279
P._troglodytes	-----KNNWEEQSGNFPEESFMRS-QDTIGKEKLFHFTTEETPEILSGNI	279
C._familiaris	-----KNNWEEQSGNFPEESFMRS-QDTVQKDKLFHFTDETPEIPSGNI	257
M._musculus	-----KNNWEEPSGSPFEDSFIKPPQDSIGRDRRFHFPETPEIPPSNV	280
R._norvegicus	-----KNNWEEPSGSPFEDSFIKIP-QDAIGRDRPFHFPETPEIPASNA	279
X._tropicalis	-----	
D._rerio	GPPGPVLTGAILKNSHNASVVRREDDLPEYEEQEPSTDLNTESNLTHTQLNQSESENES	286
F._rubripes	-----QRVEQVSPESHEDSHTFPPQQSARRS-----	242

H._sapiens	SSG-----WPDFNSSDYETTSQPYWWSASAAPESEDE--FVSVLPMEYENNS--	325
P._troglodytes	SSG-----WPDFNSSDYETTSQPYWWSASAAPESEDE--FVSVLPMEYENNS--	325
C._familiaris	SSG-----WPDFNSSDYETTSQPYWWSASATPESEDD--FVSVLPMEYENNS--	303
M._musculus	SSGSPSNVSSAWDPNSTDYESTSQPFWWSASAAPENED--FVSALPADYDTET--	335
R._norvegicus	SSG-----WPDFNGTEYESTSQPYWWSASAAPENED--FVSVLPADYDTEA--	325
X._tropicalis	-----	
D._rerio	ESE-----PVTAEP TLNSSTQSLWAWQTVSPAPTEEEEEGFVNALVPEYEDSNEPEG	337
F._rubripes	-----	

Fibronectin Type-3

H._sapiens	-----TLSETEKSTSGSFSFFPVQMLTFLWLPKPPPTAFDGFHIIEREENFTEYLMVDEE	380
P._troglodytes	-----TLSETEKSTSGSFSFFPVQMLTFLWLPKPPPTAFDGFHIIEREENFTEYLMVDEE	380
C._familiaris	-----TLSETEKSTAGSLSFPPVQMLNWLWLPKPPPTAFDGFHIIEREENFTEYLTVDEE	358
M._musculus	-----TLDRTEKPTADPFSAFPVQMTLSWLWLPKPPPTAFDGFNIIEREENFTDYLTVDDE	390
R._norvegicus	-----TLGRTEKPTADPFSAFPVQMTLSWLWLPKPPPTAFDGFNIIEREENFTDYLTVDDE	380
X._tropicalis	-----	
D._rerio	SALGIPLEPAVMPAVMPTPLAPVLLQLRWSPAPHTAYDAFNIIYIRNGNSTEATVDEN	397
F._rubripes	-----FGRRGTPQRPPELP-AKVPLNTGTSQRGTDAVNQT	277

H._sapiens	AHEFVAELKEPGKYKLSVTFSSSGSCETRKSQSAKLSFYISPSGEWIEELTEKPOHVS	440
P._troglodytes	AHEFVAELKEPGKYKLSVTFSSSGSCETRKSQSAKLSFYISPSGEWIEELTEKPOHVS	440
C._familiaris	AHEFVAELKEPGKYKLSVTFSSAGSCDTRKSQSAKLSFYISPLGEWIEELTEKPOHVS	418
M._musculus	AHEFVAELKEPGKYKLSVTFSSSGACETRKSQSAKLSFYISPTGEWIEELTEKPOHVS	450
R._norvegicus	AHEFVAELKEPGKYRLSVTFSSSGACETRKSQSAKLSFYISPTGEWIEELTEKPOHVS	440
X._tropicalis	-----	
D._rerio	THEFLAELSESGETYRIHVTFLLSAGDCEARESSANTAFYFYSPLSGEWMQPPERPQAVS	457
F._rubripes	TRDNWADPTEP-----VLRDADEEFVN	299

Fibronectin Type-3

H._sapiens	VHVLSSTTALMSWTSSQENYNSTIVSVVSLTCQKQKESORLEKQYCTQVNSSKPIIENLV	500
P._troglodytes	VHVLSSTTALMSWTSSQENYNSTIVSVVSLTCQKQKESORLEKQYCTQVNSSKPIIENLV	500
C._familiaris	VHVLSSTTALMSWTSSQENYNGTIVSVVSLTCQKQKESORLEKQYCTQVNSSKSIENLV	478
M._musculus	VHVLSSTTALMSWTSSQENYNSTIVSVVSLTCQKQKESORLEKQYCTQVNSSKPIIENLV	510
R._norvegicus	VHVLSSTTALLSWTSSQENYNSTIVSVVSLTCQKQKESORLEKQYCTQVNSSKPIIENLV	500
X._tropicalis	-----	
D._rerio	VKMLDSSSTAASVWAPSTHTYNGSLISVQSLTCLRPSISQRMELNYCSEENITSDIISSLT	517
F._rubripes	AVVEDWASGGLGFIKLRQNRSLMPLLLPS-----QENSTSDIITRLT	344

Fibronectin Type-3

H._sapiens	PGAQYQVVVYLRKGPLIGPPSDPVTFAIVPTGKIDLMLYPLGPTAVVLSWTRPYLGVFRK	560
P._troglodytes	PGAQYQVVVYLRKGPLIGPPSDPVTFAI-----DLMLYPLGSTAVVLSWTRPYLGVFRK	554
C._familiaris	PGAQYQVVVYLRKGPLIGPPSDPVTFAIVPTGKIDLMLYPLGPTAVVLSWTRPYLGVFRK	538
M._musculus	PGAQYQVVVYLRKGPLIGPPSDPVTFAIVPTGKIDLMLYPLGPTAVVLSWTRPYLGVFRK	570
R._norvegicus	PGAQYQVVVYLRKGPLIGPPSDPVTFAIVPTGKIDLMLYPLGPTAVVLSWTRPYLGVFRK	560
X._tropicalis	-----	
D._rerio	PGAQYRVVVYHTNGPLVSPASEPVIIDIEPTGVRDLVVYPLSPSAVILSWQRPYNVAFRK	577
F._rubripes	PGAQYRVVVYHTNGPLISPPSEPVIIDIEPTGVHELAVYPLSPTAVILSWRRPYHVAFRK	404

H._sapiens	YVVEFMFYFNPMATMTSEWTTYEIAATVSLTASVRIANLLPAWYVNFVMTVMTWGDPELSC	620
P._troglodytes	YVVEFMFYFNPMATMTSEWTTYEIAATVSLTASVRIANLLPAWYVNFVMTVMTWGDPELSC	614
C._familiaris	YVVEFMFYFNPMATMTSEWTTYEIAATVSLTASVRIANLLPAWYVNFVMTVMTWGDPELSC	598
M._musculus	YVVEFMFYFNPTTMTSEWTTYEIAATVSLTASVRIASLLPAWYVNFVMTVMTWGDPELSC	630
R._norvegicus	YVVEFMFYFNPTTMTSEWTTYEIAATVSLTSSVRIANLLPAWYVNFVMTVMTWGDPELSC	620
X._tropicalis	-----	
D._rerio	YVLQTFNNSATQTAQWSTYYEIAATASVIASVRVTDLLPAWFYVNFVMTVMTWGDPELSC	637
F._rubripes	YVLETFNPNVTLTSEWTTYEIAATESVIASVRVTDLLPAWYVNFVSMVMTWGEPELSC	464

Fibronectin Type-3

H._sapiens	CDSSTISFITAPVAPEITSVEYFNLSLLYISWTYGDGTTDLSHSRMLHWMVVAEGKK----	676
P._troglodytes	CDSSTISFITAPVAPEITSVEYFNLSLLYISWTYGDGTTDLSHSRMLHWMVVAEGKK----	670
C._familiaris	CDSSTISFITAPVAPEITSVEYFNLSLLYISWTYGDGTTDLSHSRMLHWMVVAEGKK----	654
M._musculus	CDSSTISFITAPVAPEITSVEYFNLSLLYISWTYGDATTDLSHSRMLHWMVVAEGRR----	686
R._norvegicus	CDSSTISFITAPVAPEITSVEYFNLSLLYISWTYGDATTDLSHSRMLHWMVVAEGRR----	676
X._tropicalis	-----LTAPVAPQITSVEYSNGLLHITWAYGDGAFDLSHSRILHWQVIAEGKN----	48
D._rerio	CDTSTVSVFITAPEAPHISSVESHGVSFVFWTYGDLFTDLTHSRMLHWQVVAEGKKSARR	697
F._rubripes	CDSSAVSFVTAPEAPHISSVHYSRGLVFCWYTGVEFVVDLSHSRILHWQVLAIGKKGPE	524

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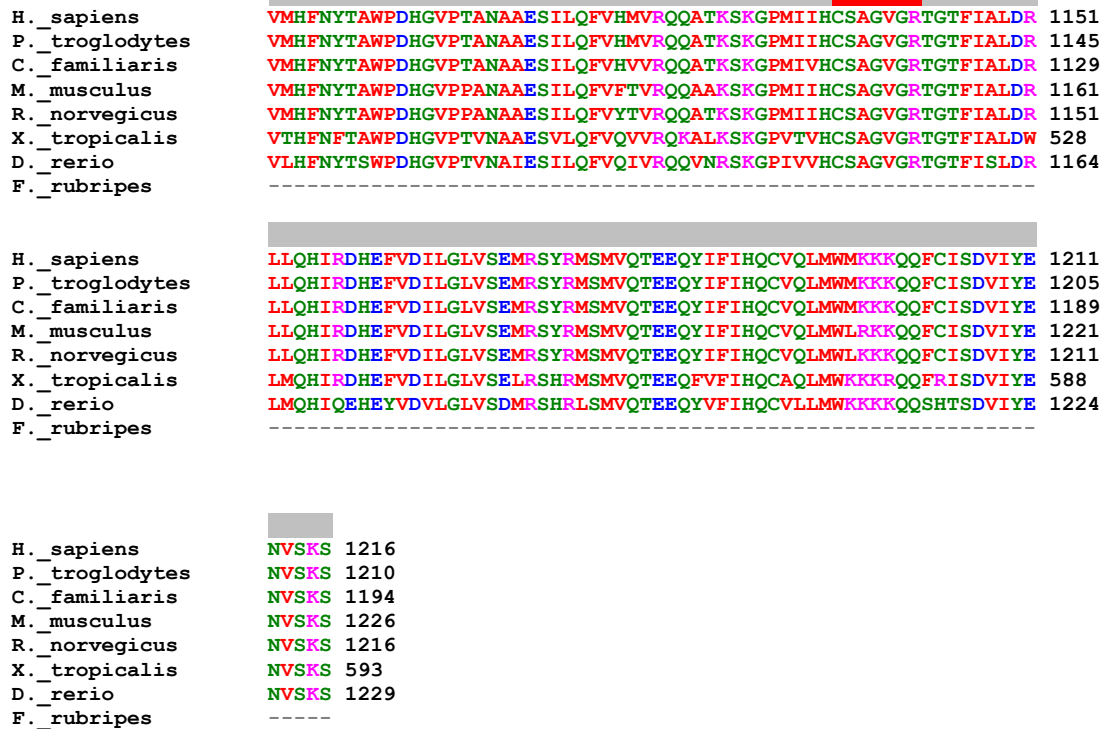


Figure S3. CLUSTAL v.2.0.12 Amino Acid Multiple Sequence Alignment of the Full Length of PTPRO throughout Evolution

Amino acid residues are given as single letter codes with the following color for residues: red: small hydrophobic or aromatic (AVFPMILWY); blue: acidic (DE); magenta: basic (RHK); green: hydroxyl, amine, basic, and glutamine (STYHCNGQ). The lowest row indicates conservation among the group of sequences. Periods indicate semi-conserved substitutions. Colons indicate conserved substitutions. Representations of putative protein domains predicted using UniProt are drawn above the sequence group, and are marked and color coded as follows: yellow: signal peptide; light blue: fibronectin type-III domain; green: transmembrane domain; gray: tyrosine protein phosphatase domain. A red horizontal bar represents the substrate-binding region. Skipped exon 16 in family A is indicated within box together with their evolutionary conservation, which includes *F. rubripes*. A blue horizontal

Table S1. Primer Sequences

Forward primer (5'→3')	Reverse primer (5'→3')	Product size (bp)
GL_Ex1F ggcacgttcttgaggac	GL_Ex1R ggaaaaggggtggaggag	298
GL_Ex2F gcataactttattgccaattatctg	GL_Ex2R gggaagaaacaggggaaca	397
GL_Ex3F atcggcctttctctctc	GL_Ex3R gagaacatcatttgggaaaagc	289
GL_Ex4F tgcccataacagtagtgaagg	GL_Ex4R Tttcttgcctctggatcagaat	383
GL_Ex5F tctgctgtgtacctgactcatt	GL_Ex5R Tttcaattccccttctcttg	592
GL_Ex6F tctatacccagggcctttcc	GL_Ex6R Cccaccagtccattgtct	285
GL_Ex7F gaccgcaatctttcttga	GL_Ex7R Gcattgccctgtaagggtgt	388
GL_Ex8F ccagcctctgtgaacattctg	GL_Ex8R Acccctcctcaataccta	247
GL_Ex9F cattgctaagacattccttctaact	GL_Ex9R cctgttccataggaagactg	298
GL_Ex10F gaaacacaaaaagacaatataaagga	GL_Ex10R tgctctccttgggaactg	280
GL_Ex11F ggattcatgctctgctggt	GL_Ex11R tgcttagtggtttcaatagtca	300
GL_Ex12F ttgggtggtgcaactgat	GL_Ex12R gctgaatcgaatgatgcaaa	246
GL_Ex13F gaaaacagaagtgtctgaaaagag	GL_Ex13R tttgccagataattagcctta	446
GL_Ex14F aattggtcagatggggaatg	GL_Ex14R Cttgggatttctggtggaa	396
GL_Ex15F gcccttaagactagatgaaaagca	GL_Ex15R caacttaggtgtgcaaaaaca	298
GL_Ex16F gctttgcaagcagtaacctg	GL_Ex16R ctttgagcacacacagcat	174
GL_Ex17F ttgcaacttttcatctttccat	GL_Ex17R tccttttgttacttcttgaagc	201
GL_Ex18F acctgatgtaattatgaaagctgtc	GL_Ex18R Aagcgtcccttctcttga	228
GL_Ex19F tttccaagaagattaagaagtatga	GL_Ex19R caggcccttccattacaga	227
GL_Ex20F gggcttgtagcaataaacc	GL_Ex20R gcaatgacagtcctcaacc	236
GL_Ex21F ttgatagaaaggccaaaa	GL_Ex21R Gttagcttgggatgcctga	189
GL_Ex22F ataggcgtgaagcagcattt	GL_Ex22R Ggcaggttttgcctgaagt	249
GL_Ex23F tccttctagcctgtttgtt	GL_Ex23R tcagcaggtgcagcatagg	223
GL_Ex24F gcatgcttaactagcagagctc	GL_Ex24R tccccttacaacaaactcc	293
GL_Ex25F gcaacagagcaagactccatc	GL_Ex25R gaataatgtaagttttcagtcca	300
GL_Ex26F ttgacatgtctgctctgaa	GL_Ex26R TCCATCACATCCCCCTCTAA	229
D12S1303F cctcagccagaaagactga	D12S1303R Cttcagttatggccatgcaa	376-392
GL_cDNA1F CCAGTTCGCCATTGTGAG	GL_cDNA1R TGCCTCAGATACCAGCTGAA	670
GL_cDNA2F AAAGACTTCCGGACAATGCTA	GL_cDNA2R CCAGGTTCCCTTCAGTTCTGC	695
GL_cDNA3F CCACTGCTTTTGTGATGGGTTC	GL_cDNA3R AGGTCTGGTCCAGCTCAGAA	596
GL_cDNA4F TTGGACCACCTTCAGATCCT	GL_cDNA4R GCAGTCATGACATTGCGTGT	517

GL_cDNA5F	GL_cDNA5R	579
GGTGGTTGCAGAAGGAAAAA	TTGACAAATGTACCAGCTCCA	
GL_cDNA6F	GL_cDNA6R	482
TGGTAGTGATCTCCGTGCTG	TCTCACACGGCTGAAGTCAT	
GL_cDNA7F	GL_cDNA7R	382
CCGTTACACAAACATCCTACCA	CATCACATCCTGCATCTCGT	
GL_cDNA8F	GL_cDNA8R	488
ACGACTGGGCCTGTAGACAC	ATCTGGAAGCAAGGGAGGAT	
hBactin-F	hBactin-R	164
CGCAAAGACCTGTACGCCAAC	GAGCCGCCGATCCACACG	
qWT_F_	qWT_R	202
ACTGACAAACCCGGTTCA A	TCTCACACGGCTGAAGTCAT	
qM_F	qM_R	120
ACT GAC AAA GAG TTG AAA	TCTCACACGGCTGAAGTCAT	
TTG		

PTPRO exon-flanking primers, as shown by GL abbreviation, D12S1303 microsatellite primers, cDNA primers, and qRT-PCR primers. hBactin indicates the beta actin housekeeping gene. qWT indicates qRT-PCR primers for amplifying wild-type allele. qM indicates qRT-PCR primers for amplifying mutant allele.