Crystal structure of the ATP-gated P2X4 ion channel in the closed state

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Supplementary Table 1. Data collection statistics

Dataset	Native (A[a])	Native (B[b])	Native (A -low energy-)	Gd[c] (A)
Space group	R3	R32	R3	R3
Wavelength (Å)	1.0000	1.0000	1.6000	1.5629
Cell (a x c) (Å)[d]	234.9 x 138.4	100.8 x 431.3	235.0 x 138.9	236.3 x 138.7
Resolution (Å)	50-3.46 (3.52-3.46)	50-3.09 (3.20-3.09)[e]	50-5.0 (5.09-5.00)	50-4.2 (4.27-4.20)
Completeness (%)	99.9 (99.8)	90.1 (69.9)	98.8 (88.0)	99.6 (98.3)
Redundancy	5.0 (5.0)	3.3 (2.8)	3.7 (2.9)	3.6 (3.0)
Rmerge (%)	5.3 (79.1)	8.0 (48.4)	2.9 (31.2)	4.0 (39.8)
I/σI	18.1 (1.4)	18.2 (1.8)	20.1 (2.1)	17.0 (1.8)

Dataset	Se[f] (A)	Se-C51M[g] (A)	Se-L349M[h] (A)
Space group	R3	R3	R3
Wavelength (Å)	0.9787	0.9787	0.9786
Cell (a x c) (Å)	234.6 x 138.4	235.4 x 138.7	236.6 x 139.6
Resolution (Å)	50-4.6 (4.68-4.60)	50-3.36 (3.42-3.36)	50-4.2 (4.27-4.20)
Completeness (%)	99.6 (95.3)	97.1 (83.1)	99.8 (99.7)
Redundancy	6.8 (5.1)	7.1 (4.9)	6.3 (5.6)
Rmerge (%)	2.5 (44.0)	6.0 (57.7)	4.9 (53.7)
I/σI	19.3 (1.8)	19.5 (1.4)	19.9 (1.9)

[a] △zfP2X4-A (△N27/△C8).
[b] △zfP2X4-B (△N27/△C8/C51F/N78K/N187R).
[c] Derivative data set from a crystal grown in the presence of $GdCl_3$.
[d] Hexagonal setting.
[e] Values for the highest resolution shell are shown in parentheses.
[f] Data set from a SeMet derivative crystal of the parent △zfP2X4-A construct.
[g] Data set from a SeMet derivative crystal of the △zfP2X4-A (C51M) mutant.
[h] Data set from a SeMet derivative crystal of the △zfP2X4-A (L349M) mutant.

Supplementary Table 2. Crystallographic refinement statistics

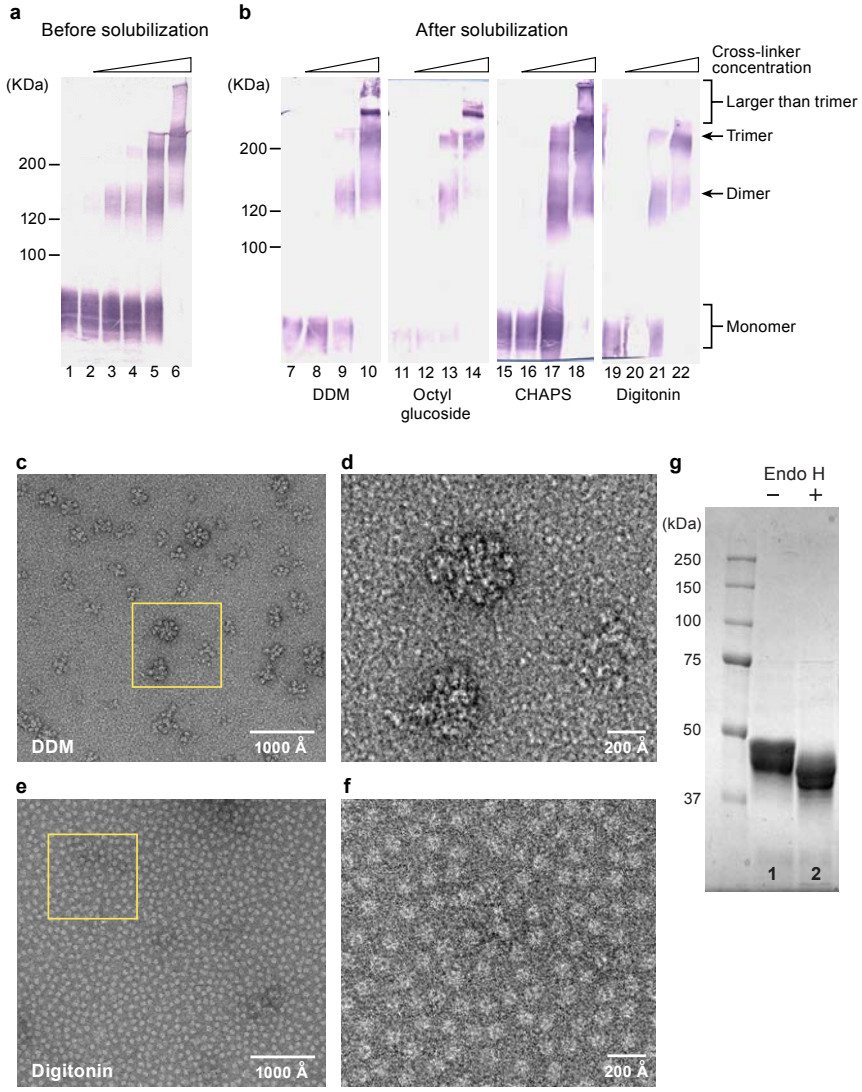
Phasing

Figure of merit	0.34
NCS correlation (%)[a]	78.4

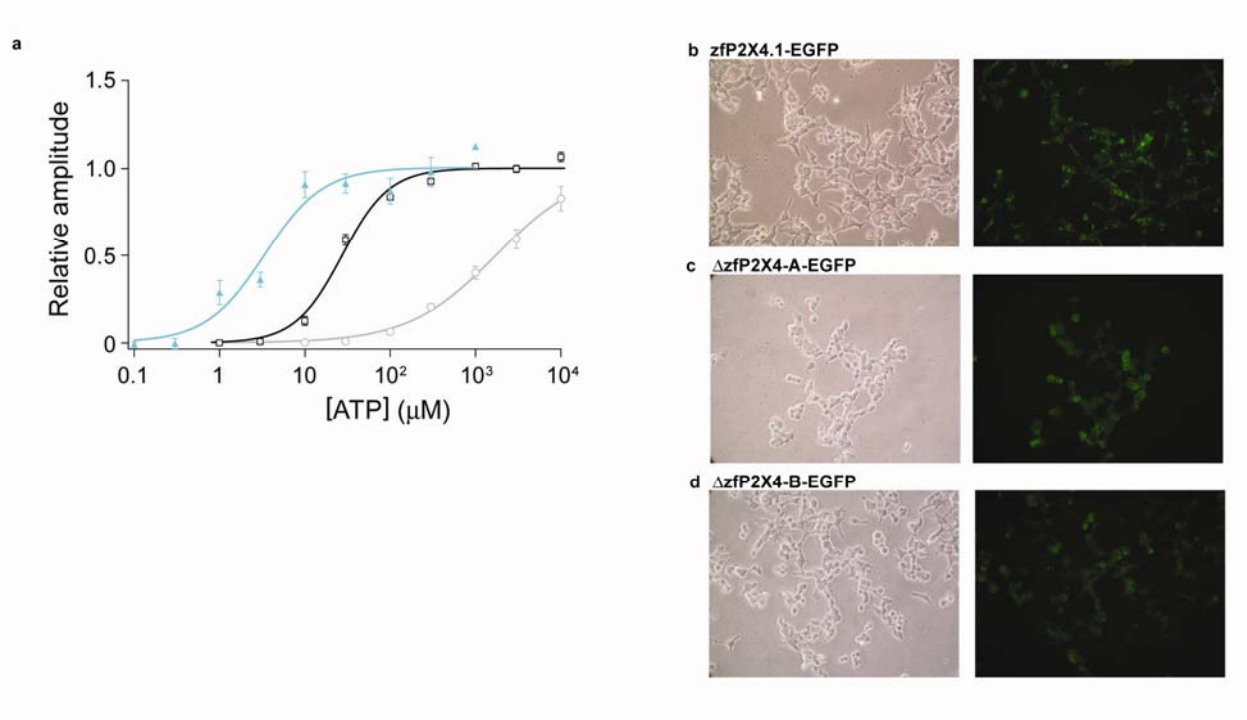
Refinement

Data	Native (A[b])	Native (B[c])
Resolution range (Å)	47.74-3.46	29.11-3.10
R_{work}/R_{free}	0.277/ 0.289	0.248/ 0.278
Number of reflections	32647	14372
Completeness (%)	87.8[d]	90.7[e]
Number of atoms	7294	2500
RMSD bond (Å)	0.007	0.008
RMSD angle (º)	1.272	1.483
Average B-factor ($Å^2$)	126.1	105.4
Ramachandran plot		
Favoured (%)	85.4	86.3
Allowed (%)	14.6	13.7
Generously allowed (%)	0	0
Disallowed (%)	0	0

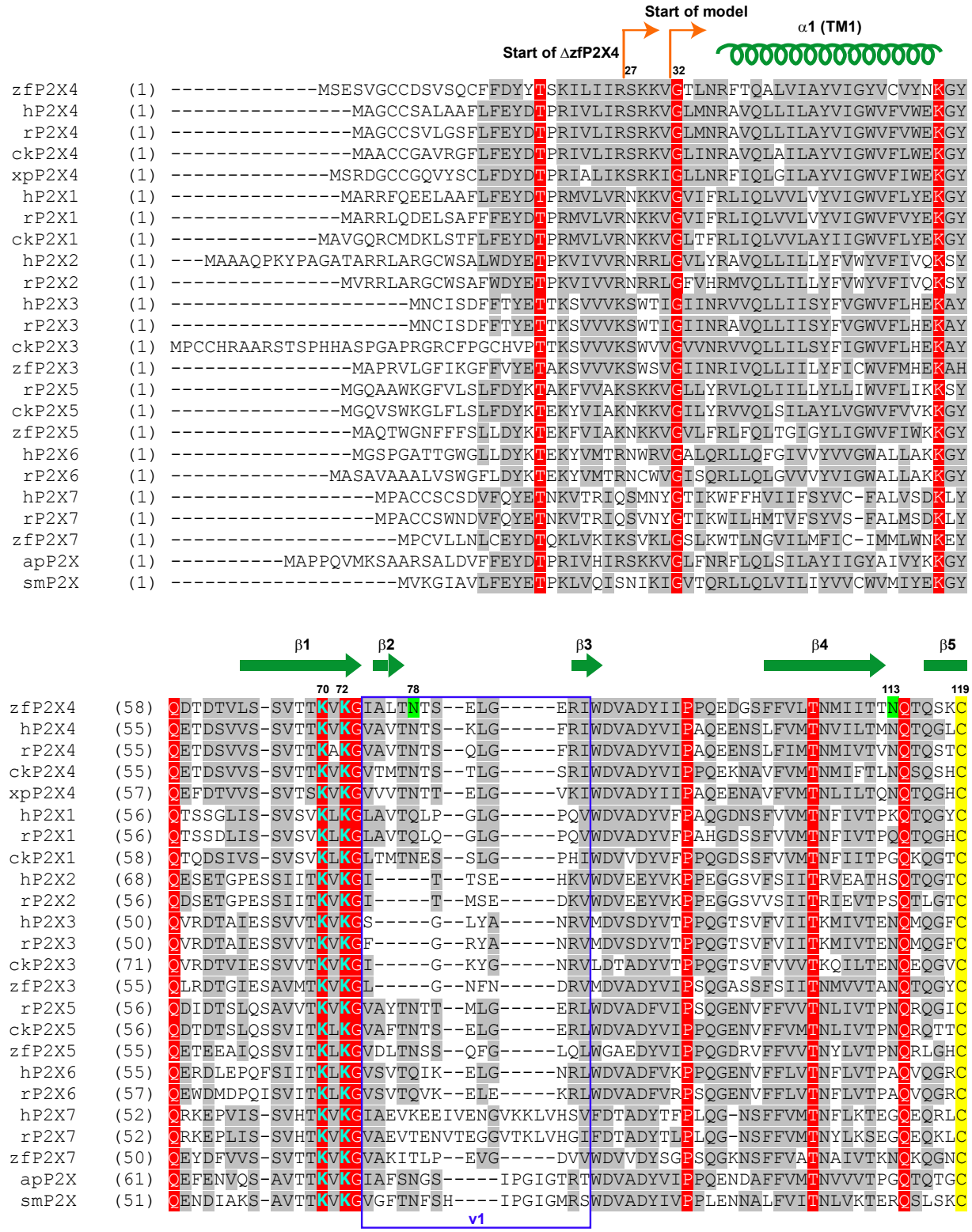
[a]Correlation between the electron density areas related by non-crystallographic symmetry.
[b]△zfP2X4-A (△N27/△C8).
[c]△zfP2X4-B (△N27/△C8/C51F/N78K/N187R).
[d]Fobs ≤ 0 were excluded from the refinement (6073 out of 37125 reflections in 47.73-3.30 Å). 4.8% of the reflections were excluded from the refinement for R_{free} calculation.
[e]5.1% of the reflections were excluded from the refinement for R_{free} calculation.



Supplementary Fig. 1. P2X receptors are glycosylated and tend to aggregate. a,b, Western blot analysis showing that rat P2X4 (rP2X4) is a trimer before solubilization (**a**) and after solubilization only in digitonin but not in dodecyl-maltoside (DDM), β-octyl-glucoside, or CHAPS (**b**). The membrane fraction from T-REx293 cells expressing rP2X4 was isolated by homogenization followed by centrifugation at 66,000 x g for 40 min. The resulting pellet was cross-linked with glutaraldehyde (GA) at room temperature for 30 min, either directly or after solubilization in different detergents. Cross-linked products were analyzed by colorimetric Western blot using anti-His primary antibody. Lanes 1-6 indicate cross-linking with increasing concentrations of GA (0, 50 μM, 250 μM, 2.5 mM, and 25 mM, respectively). After solubilization with DDM (lanes 7-10), n-octyl-β-D-glucoside (lanes 11-14), CHAPS (lanes 15-18), or digitonin (lanes 19-22), rP2X4 was cross-linked with increasing concentrations of GA (0, 50 μM, 500 μM, and 5 mM, respectively). **c-f,** Negatively stained electron micrograph. rP2X4 was purified either in 1 mM DDM (**c**, **d**) or 0.5 % digitonin (**e**, **f**), applied to carbon-coated copper grids, stained with 2 % uranyl acetate, cooled with liquid nitrogen, and observed using a Tecnai G2 F20 cryo-electron microscope at 50,000 x magnification. **d** and **f** are magnified images of the yellow squares in **c** and **e**, respectively. **g,** Affinity purified ΔzfP2X4 was treated with (+) and without (-) endoglycosidase H (Endo H), cleaved with thrombin, further purified by size exclusion chromatography, resolved by SDS-PAGE and stained with Coomassie Brilliant Blue.



Supplementary Fig. 2. ATP dose-dependent activation and expression levels of zfP2X4 constructs. a, ATP dose-dependent activation of zfP2X4.1-EGFP (light grey, EC_{50}= 1730μM, Hill slope (n) = 0.9), ΔzfP2X4-A-EGFP (black, EC_{50}= 27.4μM, n=1.6) and ΔzfP2X4-B-EGFP (blue, EC_{50}= 3.4μM, n=1.3) determined from whole cell, patch-clamp recordings. Peak ATP evoked current amplitudes were measured for a range of ATP concentrations and fit using the Hill equation. Data are displayed normalized to the maximum current (I_{max}) estimated by the best fit of the Hill equation. Error bars indicate s.e.m. for 3-6 cells. **b-d,** Equal numbers of tsA201 cells (250,000) were transfected with 1ug of the zfP2X4.1-EGFP construct (**b**), the ΔzfP2X4-A construct (**c**), or the ΔzfP2X4-B construct (**d**). Bright field (left panels) and epifluorescent (right panels) images were photographed 36 hours post-transfection.


Start of model
α1 (TM1)
Start of ΔzfP2X4
27
32
zfP2X4 (1) ----------MSESVGCCDSVSQCFFDYYTSKILIIRSKKVGTLNRFTQALVIAYVIGYVCVYNKGY
hP2X4 (1) ----------MAGCCSALAAFLFEYDTPRIVLIRSRKVGLMNRAVQLLILAYVIGWVFVWEKGY
rP2X4 (1) ----------MAGCCSVLGSFLFEYDTPRIVLIRSRKVGLMNRAVQLLILAYVIGWVFVWEKGY
ckP2X4 (1) ----------MAACCGAVRGFLFEYDTPRIVLIRSRKVGLINRAVQLAILAYVIGWVFLWEKGY
xpP2X4 (1) ----------MSRDGCCGQVYSCLFDYDTPRIALIKSRKIGLLNRFIQLGILAYVIGWVFIWEKGY
hP2X1 (1) ----------MARRFQEELAAFLFEYDTPRMVLVRNKKVGVIFRLIQLVVLVYVIGWVFLYEKGY
rP2X1 (1) ----------MARRLQDELSAFFFEYDTPRMVLVRNKKVGVIFRLIQLVVLVYVIGWVFVYEKGY
ckP2X1 (1) ----------MAVGQRCMDKLSTFLFEYDTPRMVLVRNKKVGLTFRLIQLVVLAYIIGWVFLYEKGY
hP2X2 (1) ---MAAAQPKYPAGATARRLARGCWSALWDYETPKVIVVRNRRLGVLYRAVQLLILLYFVWYVFIVQKSY
rP2X2 (1) ----------MVRRLARGCWSAFWDYETPKVIVVRNRRLGFVHRMVQLLILLYFVWYVFIVQKSY
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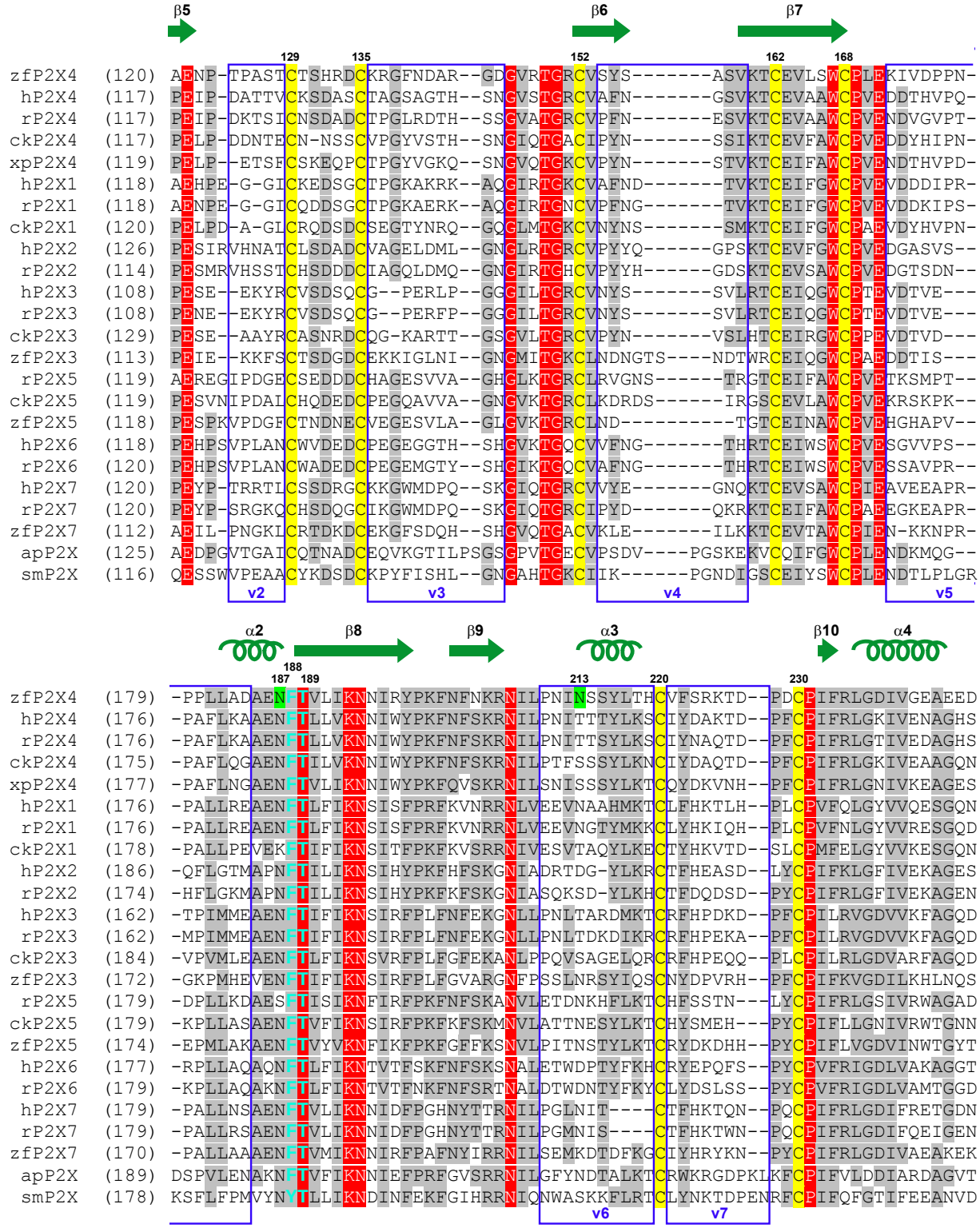
rP2X3 (1) ----------MNCISDFFTYETTKSVVVKSWTIGIINRAVQLLIISYFVGWVFLHEKAY
ckP2X3 (1) MPCCHRAARSTSPHHASPGAPRGRCFPGCHVPTTKSVVVKSWVVGVVNRVVQLLILSYFIGWVFLHEKAY
zfP2X3 (1) ----------MAPRVLGFIKGFFVYETAKSVVVKSWSVGIINRIVQLLIILYFICWVFMHEKAH
rP2X5 (1) ----------MGQAAWKGFVLSLFDYKTAKFVVAKSKKVGLLYRVLQLIILLYLLIWVFLIKKSY
ckP2X5 (1) ----------MGQVSWKGLFLSLFDYKTEKYVIAKNKKVGILYRVVQLSILAYLVGWVFVVKKGY
zfP2X5 (1) ----------MAQTWGNFFFSLLDYKTEKFVIAKNKKVGVLFRLFQLTGIGYLIGWVFIWKKGY
hP2X6 (1) ----------MGSPGATTGWGLLDYKTEKYVMTRNWRVGALQRLLQFGIVVYVVGWALLAKKGY
rP2X6 (1) ----------MASAVAAALVSWGFLDYKTEKYVMTRNCWVGISQRLLQLGVVVYVIGWALLAKKGY
hP2X7 (1) ----------MPACCSCSDVFQYETNKVTRIQSMNYGTIKWFFHVIIFSYVC-FALVSDKLY
rP2X7 (1) ----------MPACCSWNDVFQYETNKVTRIQSVNYGTIKWILHMTVFSYVS-FALMSDKLY
zfP2X7 (1) ----------MPCVLLNLCEYDTQKLVKIKSVKLGSLKWTLNGVILMFIC-IMMLWNKEY
apP2X (1) ----------MAPPQVMKSAARSALDVFFEYDTPRIVHIRSKKVGLFNRFLQLSILAYIIGYAIVYKKGY
smP2X (1) ----------MVKGIAVLFEYETPKLVQISNIKIGVTQRLLQLVILIYVVCWVMIYEKGY
β1
β2
β3
β4
β5
70 72
78
113
119
zfP2X4 (58) QDTDTVLS-SVTTKVKGIALTNTS--ELG-----ERIWDVADYIIPPQEDGSFFVLTNMIITTNQTQSKC
hP2X4 (55) QETDSVVS-SVTTKVKGVAVTNTS--KLG-----FRIWDVADYVIPAQEENSLFVMTNVILTMNQTQGLC
rP2X4 (55) QETDSVVS-SVTTKAKGVAVTNTS--QLG-----FRIWDVADYVIPAQEENSLFIMTNMIVTVNQTQSTC
ckP2X4 (55) QETDSVVS-SVTTKVKGVTMTNTS--TLG-----SRIWDVADYVIPPQEKNAVFVMTNMIFTLNQSQSHC
xpP2X4 (57) QEFDTVVS-SVTSKVKGVVVTNTT--ELG-----VKIWDVADYIIPAQEENAVFVMTNLILTQNQTQGHC
hP2X1 (56) QTSSGLIS-SVSVKLKGLAVTQLP--GLG-----PQVWDVADYVFPAQGDNSFVVMTNFIVTPKQTQGYC
rP2X1 (56) QTSSDLIS-SVSVKLKGLAVTQLQ--GLG-----PQVWDVADYVFPAHGDSSFVVMTNFIVTPQQTQGHC
ckP2X1 (58) QTQDSIVS-SVSVKLKGLTMTNES--SLG-----PHIWDVVDYVFPPQGDSSFVVMTNFIITPGQKQGTC
hP2X2 (68) QESETGPESSIITKVKGI-----T--TSE-----HKVWDVEEYVKPPEGGSVFSIITRVEATHSQTQGTC
rP2X2 (56) QDSETGPESSIITKVKGI-----T--MSE-----DKVWDVEEYVKPPEGGSVVSIITRIEVTPSQTLGTC
hP2X3 (50) QVRDTAIESSVVTKVKGS-----G--LYA-----NRVMDVSDYVTPPQGTSVFVIITKMIVTENQMQGFC
rP2X3 (50) QVRDTAIESSVVTKVKGF-----G--RYA-----NRVMDVSDYVTPPQGTSVFVIITKMIVTENQMQGFC
ckP2X3 (71) QVRDTVIESSVVTKVKGI-----G--KYG-----NRVLDTADYVTPPQGTSVFVVVTKQILTENQEQGVC
zfP2X3 (55) QLRDTGIESAVMTKVKGL-----G--NFN-----DRVMDVADYVIPSQGASSFSIITNMVVTANQTQGYC
rP2X5 (56) QDIDTSLQSAVVTKVKGVAYTNTT--MLG-----ERLWDVADFVIPSQGENVFFVVTNLIVTPNQRQGIC
ckP2X5 (56) QDTDTSLQSSVITKLKGVAFTNTS--ELG-----ERLWDVADYVIPPQGENVFFVMTNLIVTPNQRQTTC
zfP2X5 (55) QETEEAIQSSVITKLKGVDLTNSS--QFG-----LQLWGAEDYVIPPQGDRVFFVVTNYLVTPNQRLGHC
hP2X6 (55) QERDLEPQFSIITKLKGVSVTQIK--ELG-----NRLWDVADFVKPPQGENVFFLVTNFLVTPAQVQGRC
rP2X6 (57) QEWDMDPQISVITKLKGVSVTQVK--ELE-----KRLWDVADFVRPSQGENVFFLVTNFLVTPAQVQGRC
hP2X7 (52) QRKEPVIS-SVHTKVKGIAEVKEEIVENGVKKLVHSVFDTADYTFPLQG-NSFFVMTNFLKTEGQEQRLC
rP2X7 (52) QRKEPLIS-SVHTKVKGVAEVTENVTEGGVTKLVHGIFDTADYTLPLQG-NSFFVMTNYLKSEGQEQKLC
zfP2X7 (50) QEYDFVVS-SVTTKVKGVAKITLP--EVG-----DVWDVVDYSGPSQGKNSFFVATNAIVTKNQKQGNC
apP2X (61) QEFENVQS-AVTTKVKGIAFSNGS-----IPGIGTRTWDVADYVIPPQENDAFFVMTNVVVTPGQTQTGC
smP2X (51) QENDIAKS-AVTTKVKGVGFTNFSH----IPGIGMRSWDVADYIVPPLENNALFVITNLVKTERQSLSKC
v1


β5
β6
β7
129
135
152
162
168
zfP2X4 (120) AENP-TPASTCTSHRDCKRGFNDAR--GDGVRTGRCVSYS-------ASVKTCEVLSWCPLEKIVDPPN-
hP2X4 (117) PEIP-DATTVCKSDASCTAGSAGTH--SNGVSTGRCVAFN-------GSVKTCEVAAWCPVEDDTHVPQ-
rP2X4 (117) PEIP-DKTSICNSDADCTPGLRDTH--SSGVATGRCVPFN-------ESVKTCEVAAWCPVENDVGVPT-
ckP2X4 (117) PELP-DDNTECN-NSSCVPGYVSTH--SNGIQTGACIPYN-------SSIKTCEVFAWCPVEDDYHIPN-
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rP2X1 (118) AENPE-G-GICQDDSGCTPGKAERK--AQGIRTGNCVPFNG-------TVKTCEIFGWCPVEVDDKIPS-
ckP2X1 (120) PELPD-A-GLCRQDSDCSEGTYNRQ--GQGLMTGKCVNYNS-------SMKTCEIFGWCPAEVDYHVPN-
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rP2X2 (114) PESMRVHSSTCHSDDDCIAGQLDMQ--GNGIRTGHCVPYYH------GDSKTCEVSAWCPVEDGTSDN--
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rP2X3 (108) PENE--EKYRCVSDSQCG--PERFP--GGGILTGRCVNYS-------SVLRTCEIQGWCPTEVDTVE---
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ckP2X5 (119) PESVNIPDALCHQDEDCPEGQAVVA--GNGVKTGRCLKDRDS------IRGSCEVLAWCPVEKRSKPK--
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smP2X (116) QESSWVPEAACYKDSDCKPYFISHL--GNGAHTGKCIIK------PGNDIGSCEIYSWCPLENDTLPLGR
v2
v3
v4
v5
α2
β8
β9
α3
β10
α4
187
188
189
213
220
230
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ckP2X1 (178) -PALLPEVEKFTIFIKNSITFPKFKVSRRNIVESVTAQYLKECTYHKVTD--SLCPMFELGYVVKESGQN
hP2X2 (186) -QFLGTMAPNFTILIKNSIHYPKFHFSKGNIADRTDG-YLKRCTFHEASD--LYCPIFKLGFIVEKAGES
rP2X2 (174) -HFLGKMAPNFTILIKNSIHYPKFKFSKGNIASQKSD-YLKHCTFDQDSD--PYCPIFRLGFIVEKAGEN
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rP2X3 (162) -MPIMMEAENFTIFIKNSIRFPLFNFEKGNLLPNLTDKDIKRCRFHPEKA--PFCPILRVGDVVKFAGQD
ckP2X3 (184) -VPVMLEAENFTLFIKNSVRFPLFGFEKANLPPQVSAGELQRCRFHPEQQ--PLCPILRLGDVARFAGQD
zfP2X3 (172) -GKPMHEVENFTIFIKNSIRFPLFGVARGNFPSSLNRSYIQSCNYDPVRH--PFCPIFKVGDILKHLNQS
rP2X5 (179) -DPLLKDAESFTISIKNFIRFPKFNFSKANVLETDNKHFLKTCHFSSTN---LYCPIFRLGSIVRWAGAD
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apP2X (189) DSPVLENAKNFTVFIKNNIEFPRFGVSRRNILGFYNDTALKTCRWKRGDPKLKFCPIFVLDDIARDAGVT
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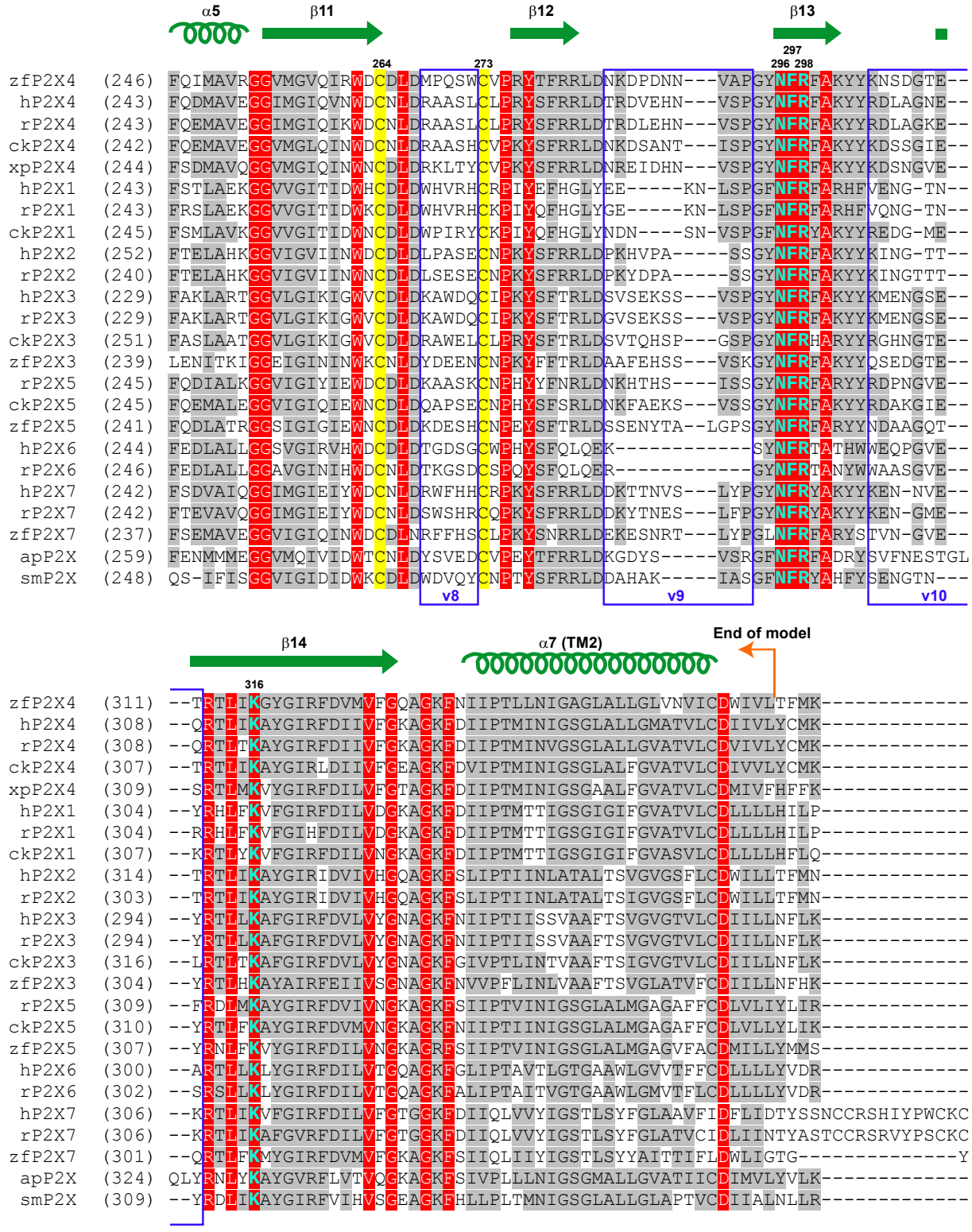
v6
v7


α5
β11
β12
β13
264
273
297
296 298
v8
v9
v10
β14
α7 (TM2)
End of model
316
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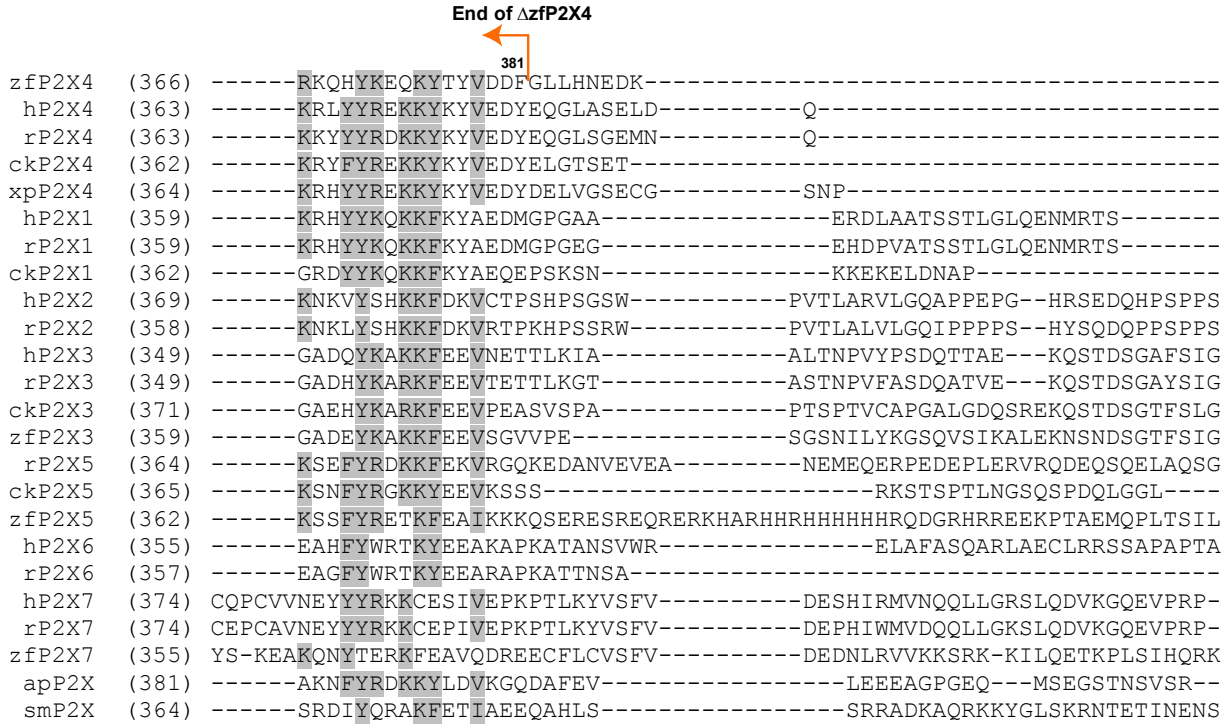
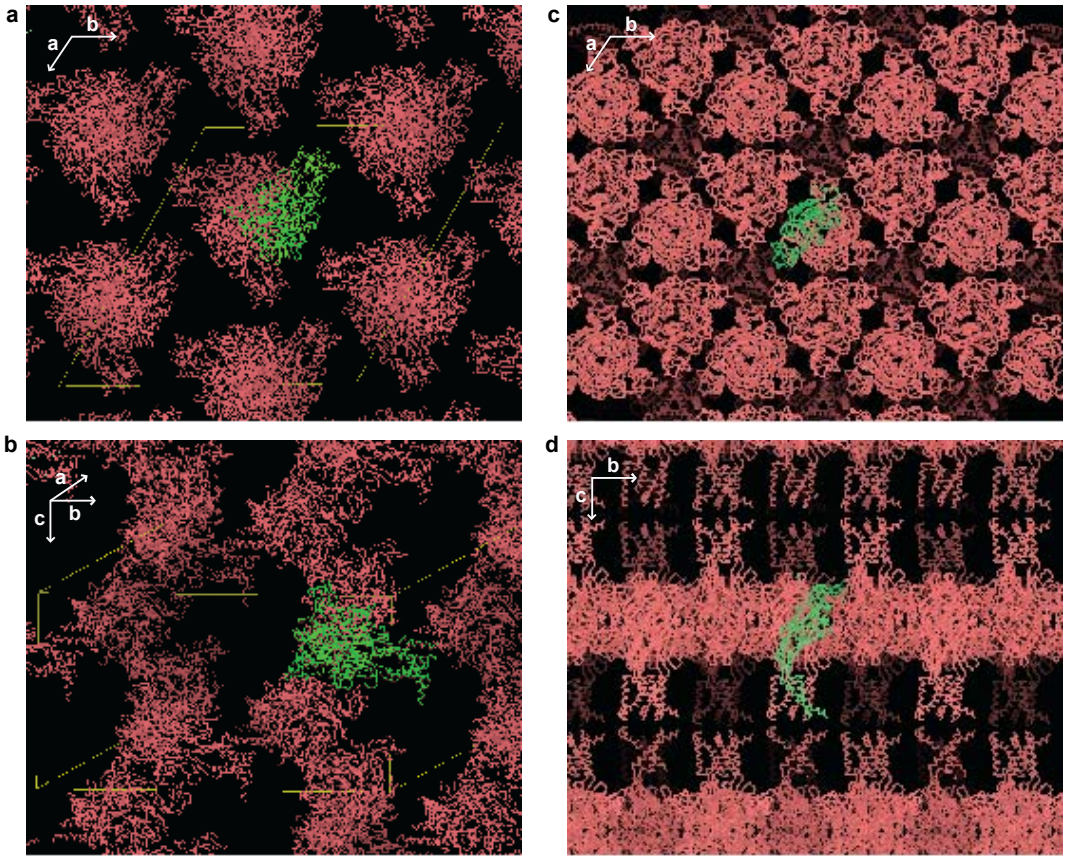
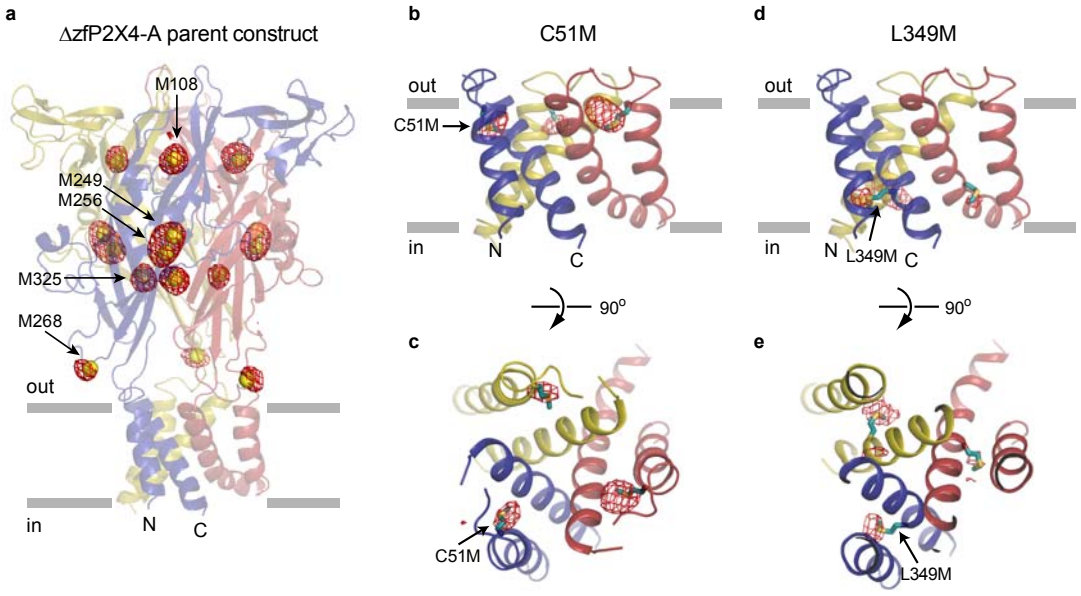
rP2X4 (243) FQEMAVEGGIMGIQIKWDCNLDRAASLCLPRYSFRRLDTRDLEHN---VSPGYNFRFAKYYRDLAGKE--
ckP2X4 (242) FQEMAVEGGVMGLQINWDCNLDRAASHCVPKYSFRRLDNKDSANT---ISPGYNFRFAKYYKDSSGIE--
xpP2X4 (244) FSDMAVQGGVMGIQINWNCDLDRKLTYCVPKYSFRRLDNREIDHN---VSPGYNFRFAKYYKDSNGVE--
hP2X1 (243) FSTLAEKGGVVGITIDWHCDLDWHVRHCRPIYEFHGLYEE-----KN-LSPGFNFRFARHFVENG-TN--
rP2X1 (243) FRSLAEKGGVVGITIDWKCDLDWHVRHCKPIYQFHGLYGE-----KN-LSPGFNFRFARHFVQNG-TN--
ckP2X1 (245) FSMLAVKGGVVGITIDWNCDLDWPIRYCKPIYQFHGLYNDN----SN-VSPGFNFRYAKYYREDG-ME--
hP2X2 (252) FTELAHKGGVIGVIINWDCDLDLPASECNPKYSFRRLDPKHVPA-----SSGYNFRFAKYYKING-TT--
rP2X2 (240) FTELAHKGGVIGVIINWNCDLDLSESECNPKYSFRRLDPKYDPA-----SSGYNFRFAKYYKINGTTT--
hP2X3 (229) FAKLARTGGVLGIKIGWVCDLDKAWDQCIPKYSFTRLDSVSEKSS---VSPGYNFRFAKYYKMENGSE--
rP2X3 (229) FAKLARTGGVLGIKIGWVCDLDKAWDQCIPKYSFTRLDGVSEKSS---VSPGYNFRFAKYYKMENGSE--
ckP2X3 (251) FASLAATGGVLGIKIGWVCDLDRAWELCLPRYSFTRLDSVTQHSP---GSPGYNFRHARYYRGHNGTE--
zfP2X3 (239) LENITKIGGEIGININWKCNLDYDEENCNPKYFFTRLDAAFEHSS---VSKGYNFRFAKYYQSEDGTE--
rP2X5 (245) FQDIALKGGVIGIYIEWDCDLDKAASKCNPHYYFNRLDNKHTHS----ISSGYNFRFARYYRDPNGVE--
ckP2X5 (245) FQEMALEGGVIGIQIEWNCDLDQAPSECNPHYSFSRLDNKFAEKS---VSSGYNFRFAKYYRDAKGIE--
zfP2X5 (241) FQDLATRGGSIGIGIEWNCDLDKDESHCNPEYSFTRLDSSENYTA--LGPSGYNFRFARYYNDAAGQT--
hP2X6 (244) FEDLALLGGSVGIRVHWDCDLDTGDSGCWFHYSFQLQEK----------SYNFRTATHWWEQPGVE--
rP2X6 (246) FEDLALLGGAVGINIHWDCNLDTKGSDCSFQYSFQLQER----------GYNFRTANYWWAASGVE--
hP2X7 (242) FSDVAIQGGIMGIEIYWDCNLDRWFHHCRPKYSFRRLDDKTTNVS---LYPGYNFRYAKYYKEN-NVE--
rP2X7 (242) FTEVAVQGGIMGIEIYWDCNLDSWSHRCQPKYSFRRLDDKYTNES---LFPGYNFRYAKYYKEN-GME--
zfP2X7 (237) FSEMAVEGGVIGIQINWDCDLNRFFHSCLPKYSNRRLDEKESNRT---LYPGLNFRFARYSTVN-GVE--
apP2X (259) FENMMMEGGVMQIVIDWTCNLDYSVEDCVPEYTFRRLDKGDYS-----VSRGFNFRFADRYSVFNESTGL
smP2X (248) QS-IFISGGVIGIDIDWKCDLDWDVQYCNPTYSFRRLDDAHAK-----IASGFNFRYAHFYSENGTN---
zfP2X4 (311) --TRTLIKGYGIRFDVMVFGQAGKFNIIPTLLNIGAGLALLGLVNVICDWIVLTFMK----------
hP2X4 (308) --QRTLIKAYGIRFDIIVFGKAGKFDIIPTMINIGSGLALLGMATVLCDIIVLYCMK----------
rP2X4 (308) --QRTLTKAYGIRFDIIVFGKAGKFDIIPTMINVGSGLALLGVATVLCDVIVLYCMK----------
ckP2X4 (307) --TRTLIKAYGIRLDIIVFGEAGKFDVIPTMINIGSGLALFGVATVLCDIVVLYCMK----------
xpP2X4 (309) --SRTLMKVYGIRFDILVFGTAGKFDIIPTMINIGSGAALFGVATVLCDMIVFHFFK----------
hP2X1 (304) --YRHLFKVFGIRFDILVDGKAGKFDIIPTMTTIGSGIGIFGVATVLCDLLLLHILP----------
rP2X1 (304) --RRHLFKVFGIHFDILVDGKAGKFDIIPTMTTIGSGIGIFGVATVLCDLLLLHILP----------
ckP2X1 (307) --KRTLYKVFGIRFDILVNGKAGKFDIIPTMTTIGSGIGIFGVASVLCDLLLLHFLQ----------
hP2X2 (314) --TRTLIKAYGIRIDVIVHGQAGKFSLIPTIINLATALTSVGVGSFLCDWILLTFMN----------
rP2X2 (303) --TRTLIKAYGIRIDVIVHGQAGKFSLIPTIINLATALTSIGVGSFLCDWILLTFMN----------
hP2X3 (294) --YRTLLKAFGIRFDVLVYGNAGKFNIIPTIISSVAAFTSVGVGTVLCDIILLNFLK----------
rP2X3 (294) --YRTLLKAFGIRFDVLVYGNAGKFNIIPTIISSVAAFTSVGVGTVLCDIILLNFLK----------
ckP2X3 (316) --LRTLTKAFGIRFDVLVYGNAGKFGIVPTLINTVAAFTSIGVGTVLCDIILLNFLK----------
zfP2X3 (304) --YRTLHKAYAIRFEIIVSGNAGKFNVVPFLINLVAAFTSVGLATVFCDIILLNFHK----------
rP2X5 (309) --FRDLMKAYGIRFDVIVNGKAGKFSIIPTVINIGSGLALMGAGAFFCDLVLIYLIR----------
ckP2X5 (310) --YRTLFKAYGIRFDVMVNGKAGKFNIIPTIINIGSGLALMGAGAFFCDLVLLYLIK----------
zfP2X5 (307) --YRNLFKVYGIRFDILVNGKAGRFSIIPTVINIGSGLALMGAGVFACDMILLYMMS----------
hP2X6 (300) --ARTLLKLYGIRFDILVTGQAGKFGLIPTAVTLGTGAAWLGVVTFFCDLLLLYVDR----------
rP2X6 (302) --SRSLLKLYGIRFDILVTGQAGKFALIPTAITVGTGAAWLGMVTFLCDLLLLYVDR----------
hP2X7 (306) --KRTLIKVFGIRFDILVFGTGGKFDIIQLVVYIGSTLSYFGLAAVFIDFLIDTYSSNCCRSHIYPWCKC
rP2X7 (306) --KRTLIKAFGVRFDILVFGTGGKFDIIQLVVYIGSTLSYFGLATVCIDLIINTYASTCCRSRVYPSCKC
zfP2X7 (301) --QRTLFKMYGIRFDVMVFGKAGKFSIIQLIIYIGSTLSYYAITTIFLDWLIGTG----------Y
apP2X (324) QLYRNLYKAYGVRFLVTVQGKAGKFSIVPLLLNIGSGMALLGVATIICDIMVLYVLK----------
smP2X (309) --YRDLIKAYGIRFVIHVSGEAGKFHLLPLTMNIGSGLALLGLAPTVCDIIALNLLR----------



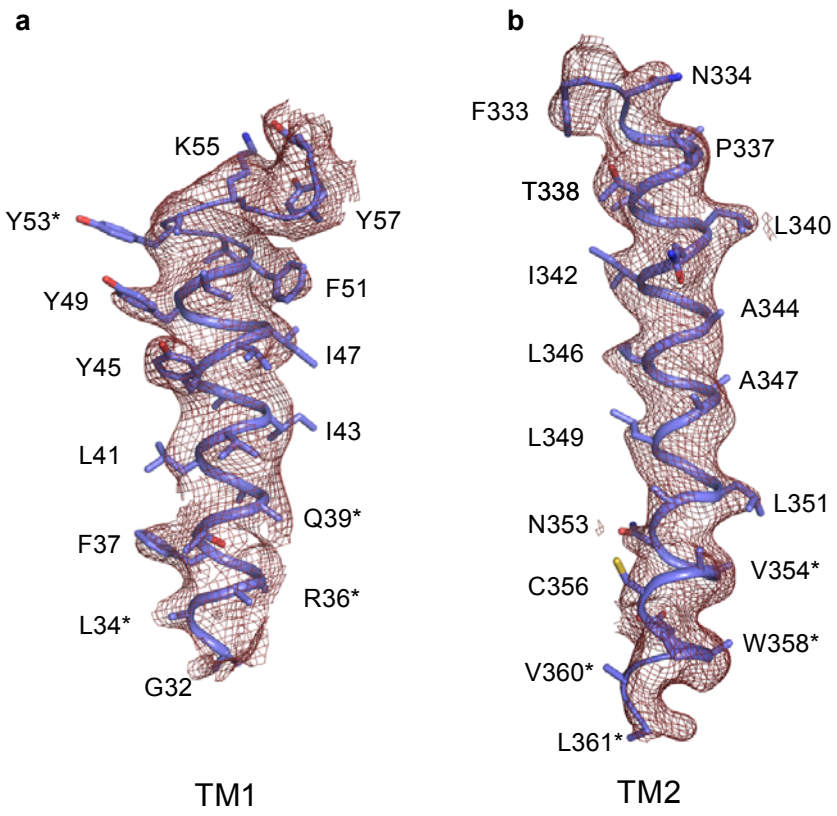
Supplementary Fig. 3. Sequence alignment. Sequence alignment of P2X receptors from zebrafish (zfP2X4.1, GI: 12656589; zfP2X4.2, 33520721; zfP2X3, 7542523; zfP2X5, 21667663; and zfP2X7, 33520711), human (hP2X1, 4505545; hP2X2, 25092719; hP2X3, 28416925; hP2X4, 116242696; hP2X6, 6469324; and hP2X7, 29294631), rat (rP2X1, 1352689; rP2X2, 18093098; rP2X3, 1030065; rP2X4, 1161345; rP2X5, 1279659; rP2X6, 1279661; and rP2X7, 1322005), chicken (ckP2X1, 45383135; ckP2X3, 118091425; ckP2X4, 82223288; and ckP2X5, 82217039), African clawed frog (xpP2X4, 12007266), sea slug (apP2X, 39578339), and blood fluke (smP2X, 51988420). Starting and ending residues of ΔzfP2X4 construct and the current model are indicated with orange arrows. Secondary structure elements are depicted in cartoon representation and are labeled as in Figure **3a**. The sequence alignment was made using Vector NTI. Conserved cysteine residues are highlighted in yellow, other conserved residues are in red, and less conserved residues are in grey. The four glycosylated asparagines are labeled as in Figure **3a** and are highlighted in green.



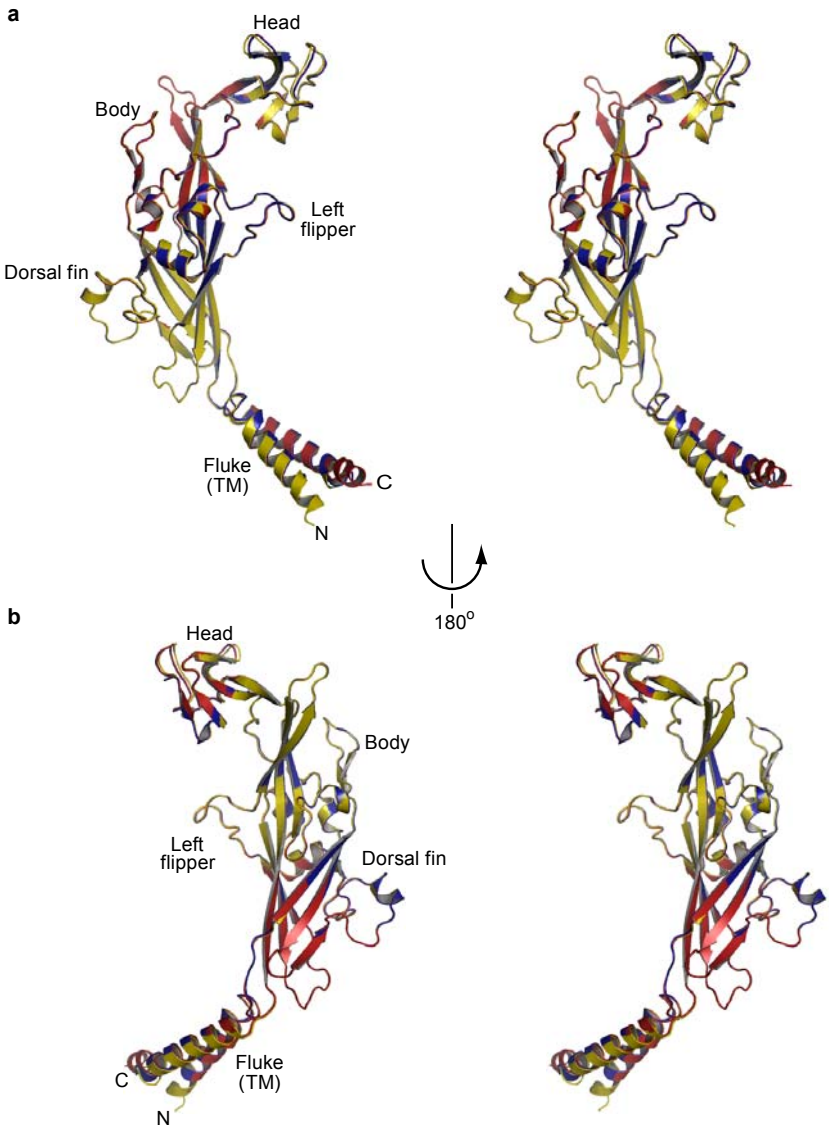
Supplementary Fig. 4. Crystal packing of ΔzfP2X4 constructs. a, b, Packing of receptors in the R3 space group. ΔzfP2X4-A crystals viewed perpendicular to the a-b plane (**a**) and perpendicular to the b-c plane (**b**). The unit cell (hexagonal setting) is shown in yellow. **c, d,** Packing of receptors in the R32 space group. ΔzfP2X4-B crystals viewed perpendicular to the a-b plane (**c**) and perpendicular to the b-c plane (**d**). Symmetrically-related molecules are represented as Cα traces in red, with one crystallographic unique molecule or subunit shown in green.



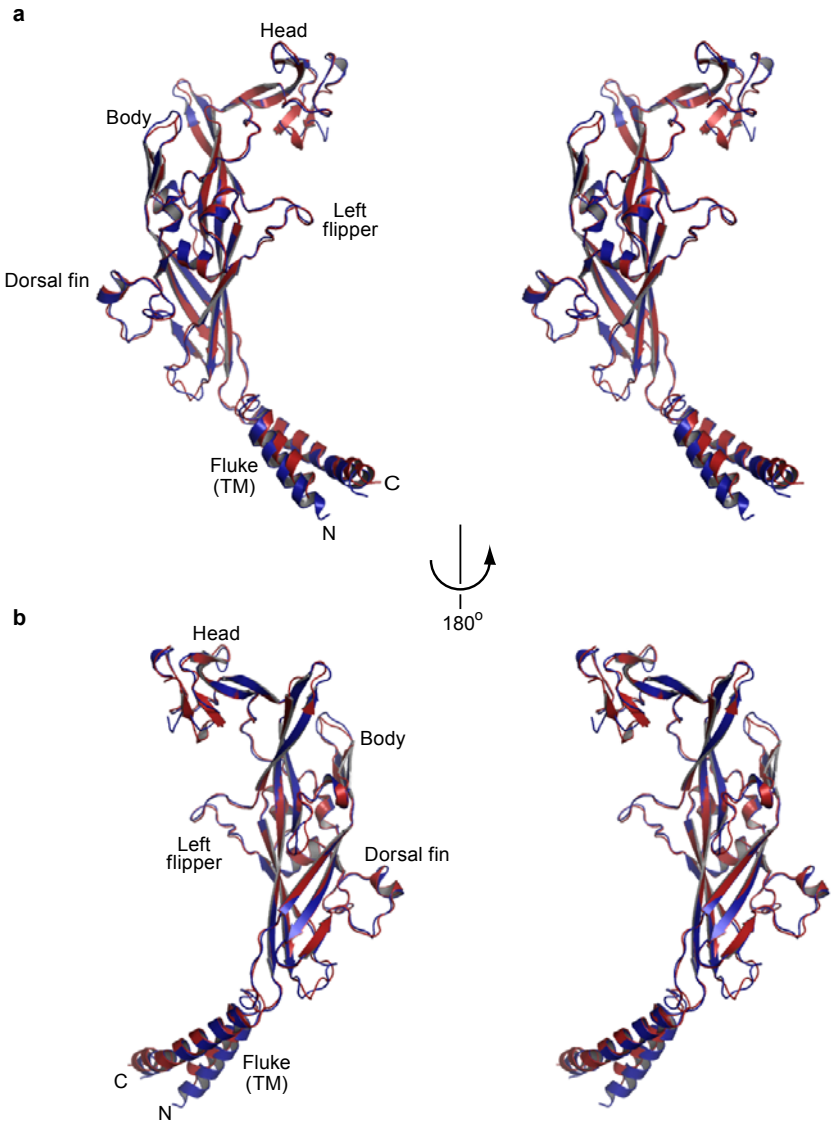
Supplementary Fig. 5. Anomalous difference Fourier maps from selenomethionine derivatives of ΔzfP2X4-A. **a,** An anomalous difference map from a selenomethionine derivative of the parent ΔzfP2X4-A contoured at 3.6 σ (red) confirms the location of methionine residues. The methionine sulfur atoms are yellow spheres. **b-e,** Anomalous difference maps from selenomethionine derivatives of the C51M (**b**, **c**) and L349M constructs (**d**, **e**) contoured at 3.0 σ (red) confirm the assignment of residues on the transmembrane helices. Predicted locations of the mutated methionine residues are shown in stick representation.



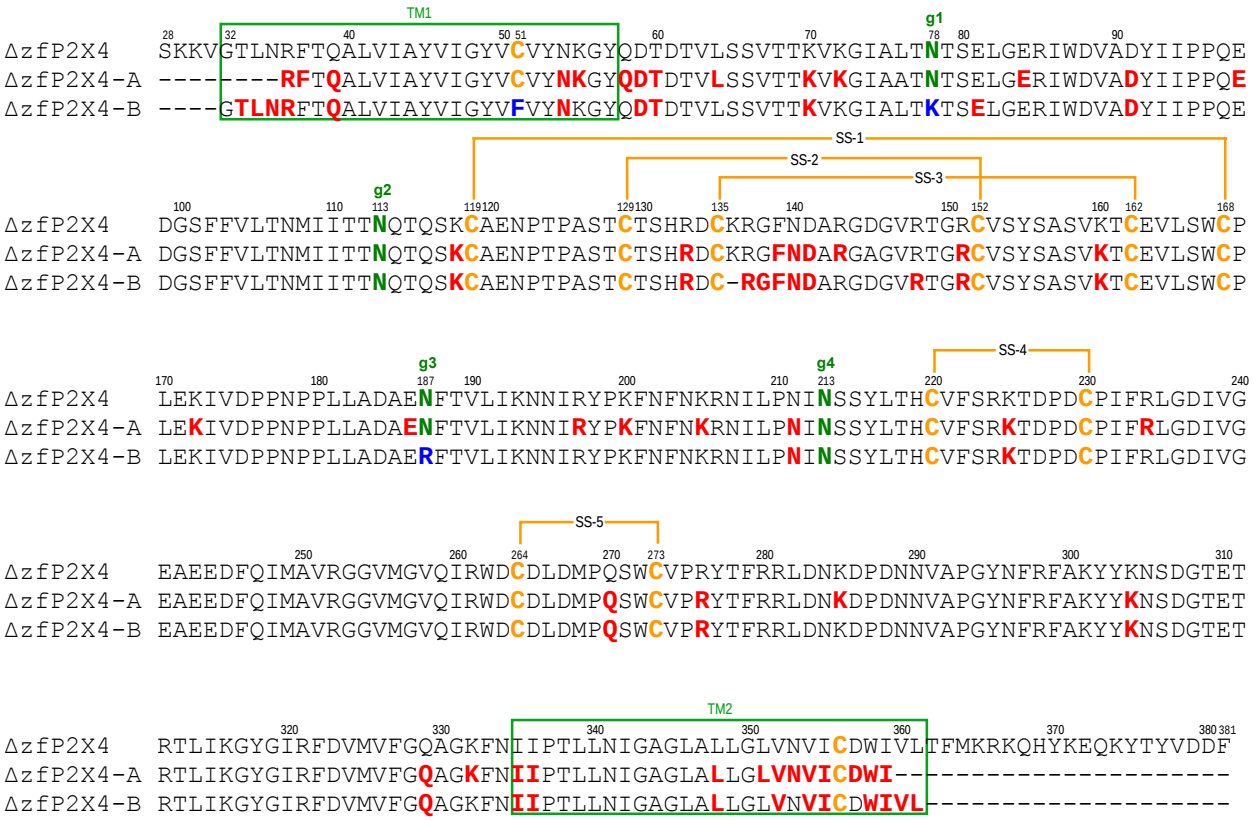
Supplementary Fig. 6. Electron densities in the transmembrane regions from the ΔzfP2X4-B crystal structure. Shown are 2Fo-Fc maps contoured at 0.8 for TM1 (**a**) and TM2 (**b**). The residues with weak side chain densities (asterisked), except for Y53, are shown as alanine.



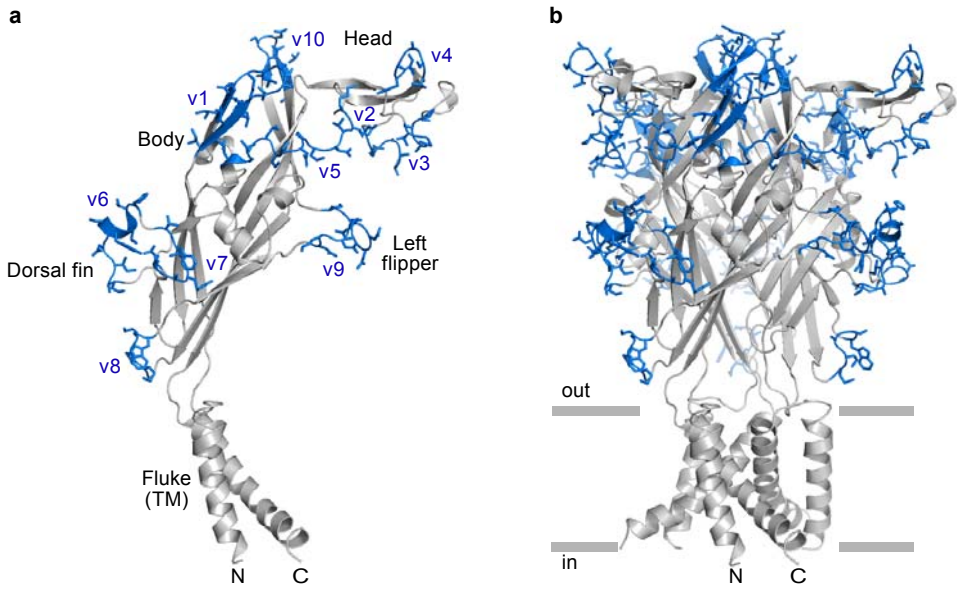
Supplementary Fig. 7. Stereoview of a superposition of the three crystallographically independent subunits from the ΔzfP2X4-A (R3) crystal form. a, Subunit B (red) and C (yellow) are superimposed onto subunit A (blue) using Cα positions in the extracellular region. **b,** Superposition as viewed along the non-crystallographic three-fold axis rotated 180º from **a**.



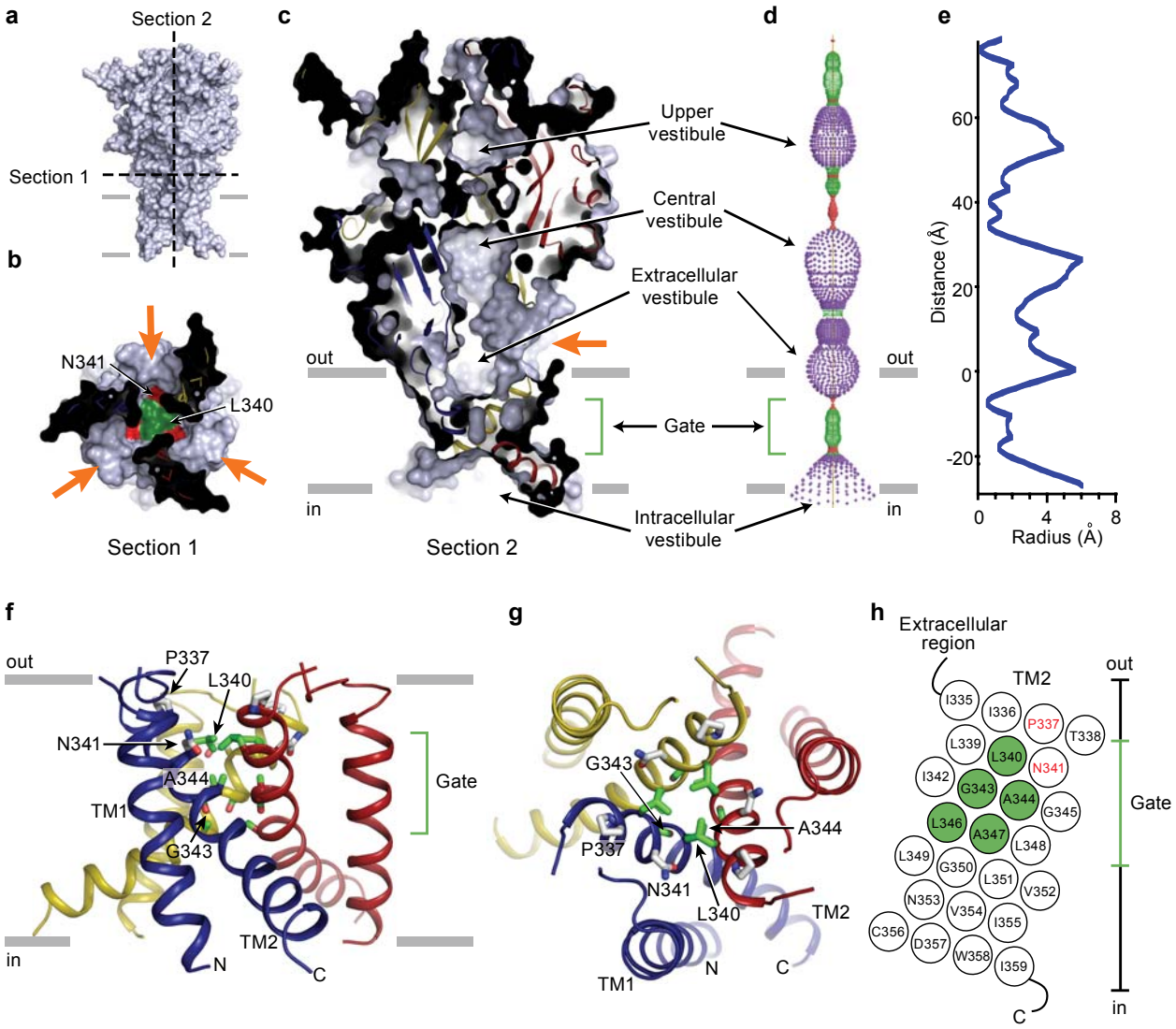
Supplementary Fig. 8. Stereoview of the subunit superposition between the ΔzfP2X4-A and ΔzfP2X4-B crystal structures. a, ΔzfP2X4-A Subunit A (red) is superimposed onto a ΔzfP2X4-B protomer (blue) using Cα positions in the extracellular region. **b,** Superposition as viewed along the non-crystallographic three-fold axis rotated 180º from **a**.



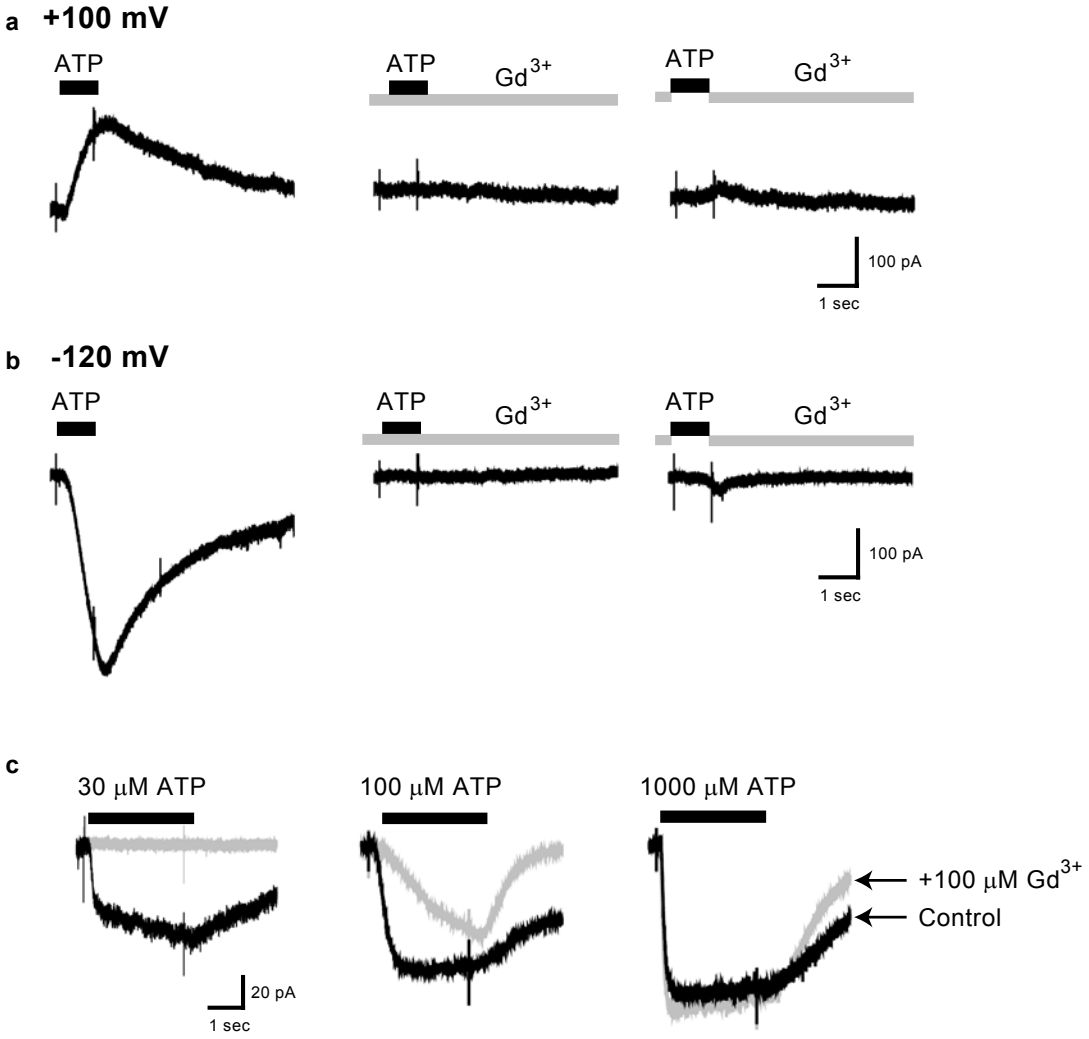
Supplementary Fig. 9. Sequence and features of the constructs used in the current structural study. The ΔzfP2X4 construct was used to form the ΔzfP2X4-A (R3 space group) crystals. The sequence labeled as ΔzfP2X4-A defines the residues from one of the three crystallographically independent subunits (A chain) in the ΔzfP2X4-A crystal structure. The residues without clear main chain electron densities are not included in the crystal structure and are shown here as dashed lines; the residues with weak side chain densities are built as alanines in the structure and are shown in red letters. To produce the ΔzfP2X4-B construct we made the following mutations: C51F, N78K, and N187R; the changes in amino acid residues are shown in blue. Cysteine residues are depicted in orange and disulfide bonded pairs are indicated. The green boxes labeled TM1 and TM2 are the α-helical transmembrane regions based on the crystal structures.



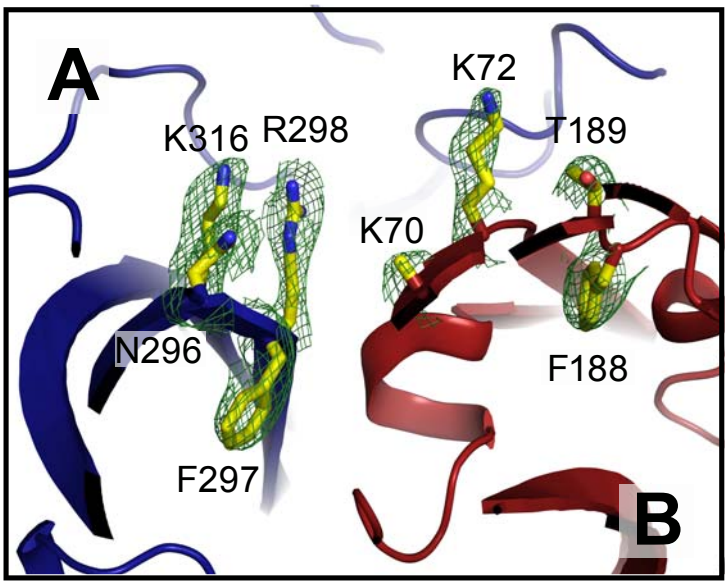
Supplementary Fig. 10. The locations of variable residues. Stretches of five or more non-conserved residues are highlighted in blue. **a,** A single protomer. **b,** The assembled trimer.



Supplementary Fig. 11. Extracellular vestibules and the ion channel domain. a, Sections of the receptor parallel (section 1) and perpendicular (section 2) to the membrane. **b,** Possible ion pathways are highlighted with orange arrows. Solvent facing residues in TM2 (L340, green and N341, red) are coloured differently. **c,** Section 2 reveals a closed conformation of the pore and shows that the gate is located about halfway across the membrane bilayer. Three vestibules (upper, central and extracellular vestibules) are located on the molecular 3-fold axis, with the extracellular vestibule connected to the bulk solution through a fenestration (orange arrow). **d,** Pore lining surface calculated by the Hole[49] program. Each colour represents a different radius range measured from the receptor centre (red: <1.15 Å, green: 1.15-2.3 Å, and purple: >2.3 Å). **e,** Pore radius plotted against the distance from the extracellular membrane boundary. **f,** Cartoon representations of the transmembrane domain viewed parallel to the membrane plane. P337 and N341 are shown in grey and potential gate residues (L340, G343, A344, and A347) are shown in green. **g,** Transmembrane domain viewed perpendicular to the membrane plane. **h,** Helical net diagram of TM2. Residues that occlude the transmembrane pore are depicted in green. Residues analogous to gating residues in cASICmfc are in red font.



Supplementary Fig. 12. Gadolinium inhibits ATP-evoked currents in a concentration dependent, voltage independent manner and accelerates the rate of deactivation. a, b, ATP (30μM, 1 sec) was applied at positive (+100mV, **a**) and negative (-120mV, **b**) membrane potentials. ATP-evoked currents were completely blocked when 100 μM $GdCl_3$ was present in both control and ATP-containing test solutions (middle traces), indicating that inhibition of currents was voltage independent. Furthermore, the presence of 100 μM $GdCl_3$ only in the control solution and not in the ATP-containing test solution (right traces) was sufficient to largely abolish the ATP evoked currents, suggesting that sequestration of ATP by gadolinium was not responsible for the inhibition. Scale: 100 pA, 1 sec. **c**, ATP (3 sec) was applied at varying concentrations in the absence (black) or presence (grey) of 100 μM $GdCl_3$. Gadolinium was present in both the bath and ATP-containing solutions when applied. Gadolinium completely abolished currents evoked by 30 μM ATP and partially inhibited currents evoked by 100 μM ATP while currents evoked by 1mM ATP were seemingly unaffected. Also note the increased rate of deactivation in the presence of gadolinium. Scale: 20 pA, 1 sec.



Supplementary Fig. 13. Side chain density in the ATP-binding pocket viewed ~90º from Figure 6b. Conserved residues previously proposed to be involved in ATP binding are labeled, and side chains are presented in stick representation in yellow. 2Fo-Fc electron density maps around the side chains are depicted in green. K70 is shown as an alanine due to an ambiguous side chain density.