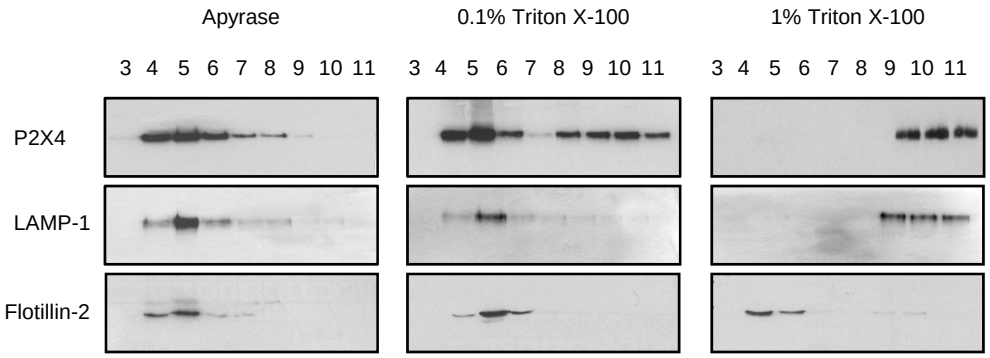
Supplementary figure 1. Localisation of the P2X4 receptor and the lysosome marker LAMP-1 within lipid rafts. HEK 293 cells stably expressing P2X4 receptors were apyrase treated (3.2 U/ml, 1 hr) and then lysed in either Na_2CO_3 (500 mM, pH 11) or MBS in the presence of triton X-100. After separation of lysates on density sucrose gradients, fractions 3-11 were immunoblotted for LAMP-1 lysosomal marker, P2X4 and the lipid raft marker flotillin-2. Predominant expression of P2X4 and lysosomes was within the lipid raft fractions as indicated by flotillin-2. However, increasing the concentration of triton X-100 to 1% relocated both P2X4 and lysosomes to outside of the lipid rafts. All bands were of the appropriate size (LAMP-1 ~ 120 kDa, P2X4 ~50 kDa, flotillin-2 ~ 42 kDa). Blots are representative of those from 3 separate experiments.

Supplementary figure 2. Chimera P2X1-2aβ receptor association with lipid rafts. (a.) Schematic representing the structure of chimera P2X1-2aβ. P2X1 receptor amino acids 16-30 have been replaced for those of the P2X2 receptor. (b.) HEK 293 cells stably expressing chimera P2X1-2aβ were lysed in either Na_2CO_3 (500 mM, pH 11) after ATP treatment (100 μM, 10 min), or lysed in the presence of Triton X-100. Lysates were separated by sucrose density gradients and fractions 3-11 immunoblotted for P2X1, the lipid raft marker flotillin-2 and the non-raft marker β-adaptin. Chimera P2X1-2aβ expression was similar to that of P2X1 - expressed in the raft fractions following treatment with ATP (corresponding with the location of flottillin-2) and redistributed predominantly to the non raft fractions (as indicated by the non-raft marker β-arrestin) in the presence of triton X-100. (c.) HEK 293 cells expressing P2X1 or chimera P2X1-2aβ were incubated for 24 hrs in lipofectamine 2000 and then lysed (Na_2CO_3 500 mM, pH 11), separated by sucrose density gradients and fractions immunoblotted for P2X1, flotillin-2 and β-adaptin. Lipofectamine treatment (30 μl in a T75 flask for 24 hours) had no effect on the distribution of the P2X receptors following density centrifugation. All bands were of the appropriate size (P2X1 ~ 55 kDa, flotillin-2 ~ 42 kDa and β-adaptin ~ 106 kDa). Blots are representative of those from 3 separate experiments.

Supplementary figure 3. Lipid raft disruption by mβ-CD and filipin has no effect on currents evoked by EC_{50} or maximal concentration of ATP for P2X2, P2X3 and P2X4 receptors. Summary data of mβ-CD (10 mM for 1h) on P2X2&3 and filipin (10 μM for 20 min) on P2X4 receptor–mediated currents evoked by application of different concentrations of ATP ($n > 6$ for each). Data shown are normalised to currents evoked under control conditions for either EC_{50} or maximal ATP concentrations.

Supplementary figure 4. Lipid raft disruption sensitivity is independent of receptor desensitization time-course. Summary of the effects of mβ-CD treatment compared to their time-course of desensitzation for currents mediated by P2X1 (star), P2X1-2a, P2X1-2b, P2X1-2c, P2X1-2d, P2X1-2eα P2X1-2eβ chimeric receptors (solid circles) and P2X1 receptors with point mutations (open diamonds) compared to the response to ATP in non-treated cells. Three P2X1 receptors with point mutations (M21C, L23C and K28C, within grey shadow) have time courses of desensitization similar to P2X1 but different sensitivities to mβ-CD treatment.



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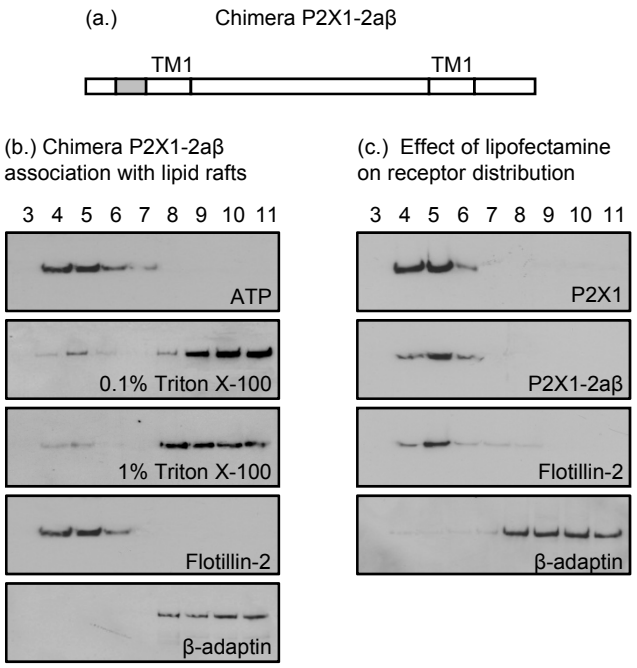

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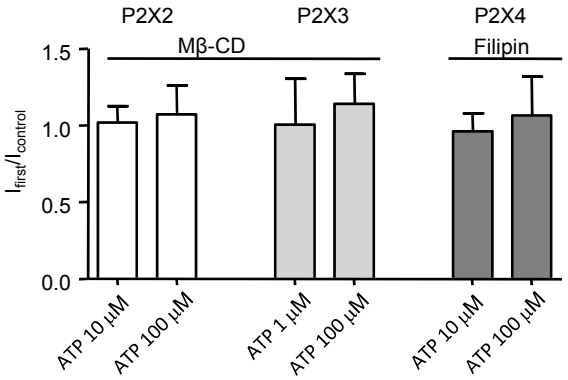

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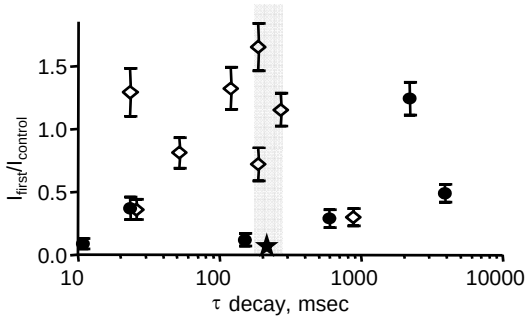

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