Supplemental data

Residues 155 and 348 contribute to determination of $P2X_7$ receptor function via distinct mechanisms revealed by single nucleotide polymorphisms

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Short title: Expression and function of $P2X_7$ receptors

Fig.S1 Effects of Y155H and T348A mutations on ATP-induced currents mediated by the $rP2X_7$ receptor

A. Representative ATP-induced current densities in cells expressing the $rP2X_7$ WT, Y155H or T348A mutant receptors in normal extracellular solution. The dotted line denotes the maximal current for the WT $rP2X_7$ receptor. B. ATP concentration-current density curves from recordings shown in A. The smooth lines represent the best fits to the Hill equation. C. Mean maximal current density in parallel experiments. The cell number in each case is indicated. ***, $p<0.001$, the maximal current density compared to WT receptor (*t*-test).

Fig.S2 Effects of reciprocal mutations of residues 155 and 348 on BzATP-induced currents mediated by the human and rat $P2X_7$ receptors

A. Representative BzATP-induced current densities in cells expressing the $hP2X_7$ WT, H155Y or A348T mutant receptors in low divalent cation extracellular solution (top), and BzATP concentration-current density curves (bottom). B. Representative BzATP-induced current densities in cells expressing the $rP2X_7$ WT, Y155H or T348A mutant receptors in low divalent cation extracellular solution (top), and BzATP concentration-current density curves (bottom). In these experiments, the normal extracellular solution was used to wash off BzATP, and then extracellular was returned to the low divalent cation solution, before applications of the following concentrations of BzATP. The dotted line denotes the currents for the WT $P2X_7$ receptors. The smooth lines represent the best fits to the Hill equation. The cell number in each case is indicated in brackets. ***, $p<0.001$, **, $p<0.01$, *, $p<0.05$, the maximal current density compared to the WT receptors (*t*-test).

Fig.S3 Representative ATP-induced current densities in cells expressing $hP2X_7$ mutant receptors

Mutations were introduced into positions surrounding residue 155 (A-B) and residue 348 (C). The dotted line denotes the maximal current for the WT $hP2X_7$ receptor.

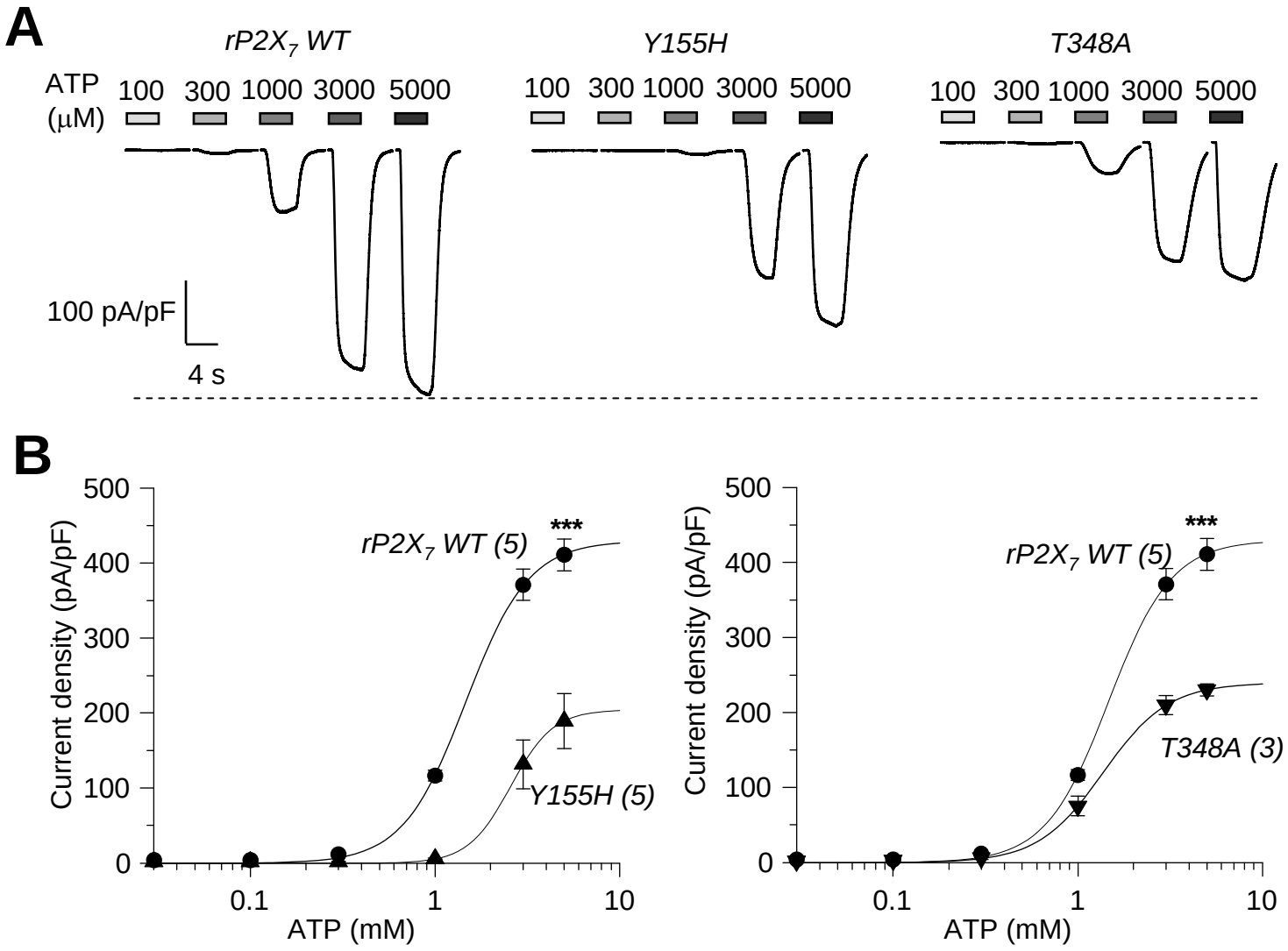
Fig.S4 Effect of mutation of residues at position 155 on cell surface and total expression level of $hP2X_7$ receptors

Representative Western blots showing biotin-labelling or surface expression of the indicated WT and mutant $hP2X_7$ receptors (top), and total protein expression for $P2X_7$ receptor and GFP in whole cell lysate (bottom). -ve denotes the cells that were transfected with the empty vector.

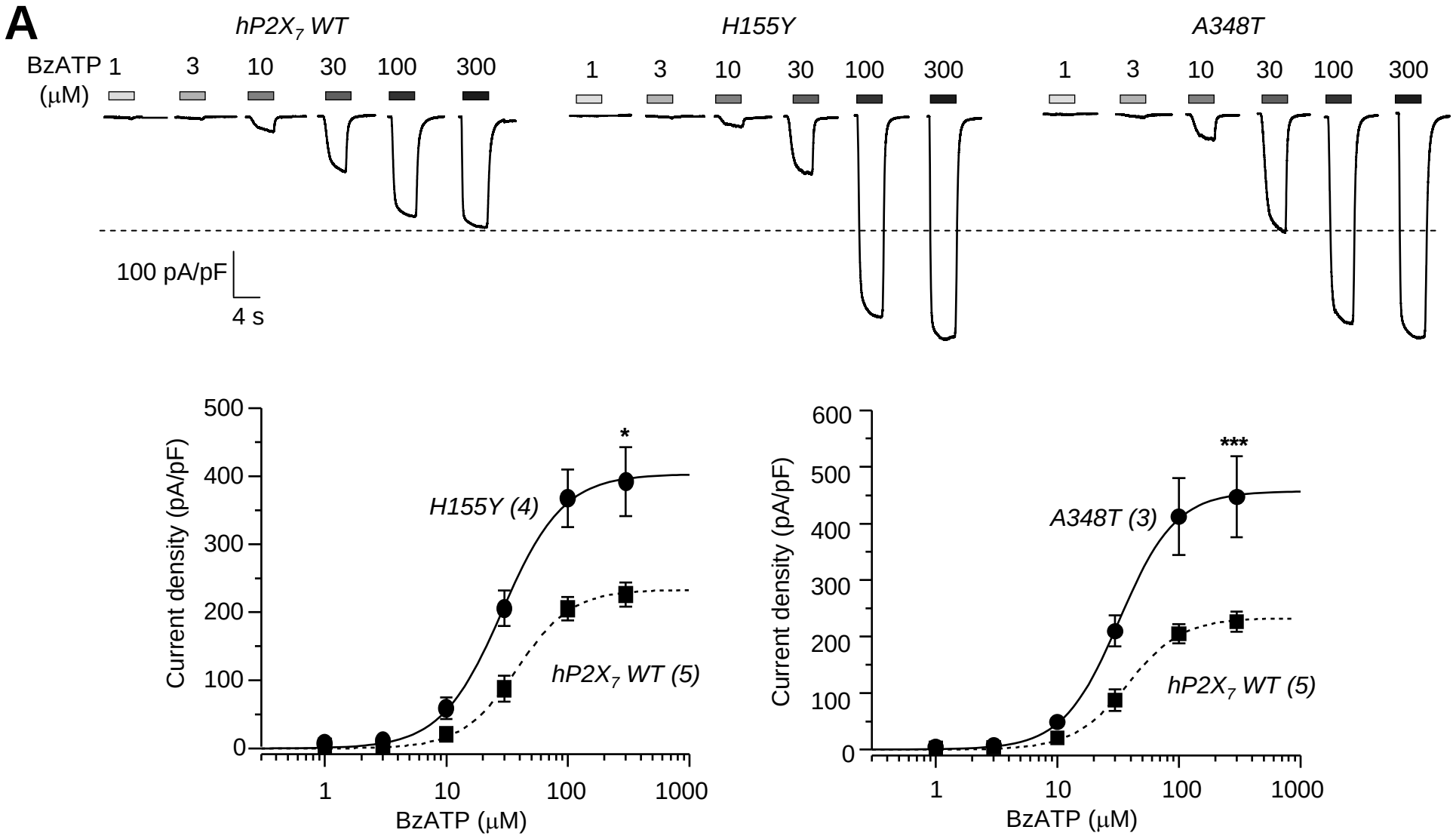
Fig.S5 Inhibition of hP2X$_7$ WT and H155Y mutant receptors by antagonist KN-62
ATP concentration-current density curves before (control) and after 4 min exposure to 100 nM KN-62 for the hP2X$_7$ WT (A) and H155Y mutant receptors (B) (n = 6 for each case). The dotted line denotes the currents for the receptors before applications of KN-62.

Fig.6S Location of intracellular hP2X$_7$ proteins and GFP-LAMP1
Representative confocal images of HEK293 cells co-expressing hP2X$_7$ WT receptor (red) and GFP-tagged LAMP1 (green), a protein located to the late endocytic organelles. Scale bar is 10 μm.

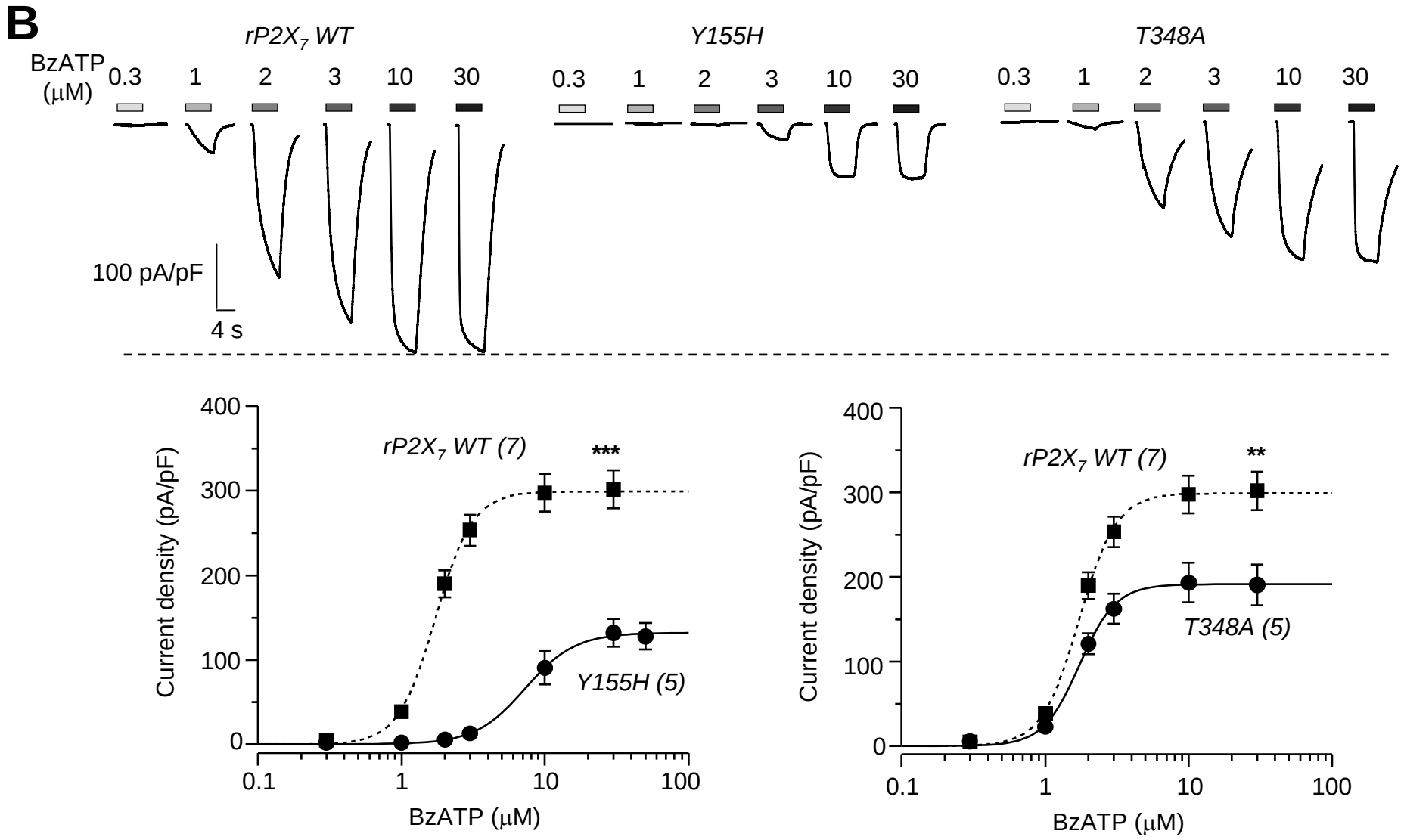
Supplemental Fig.1



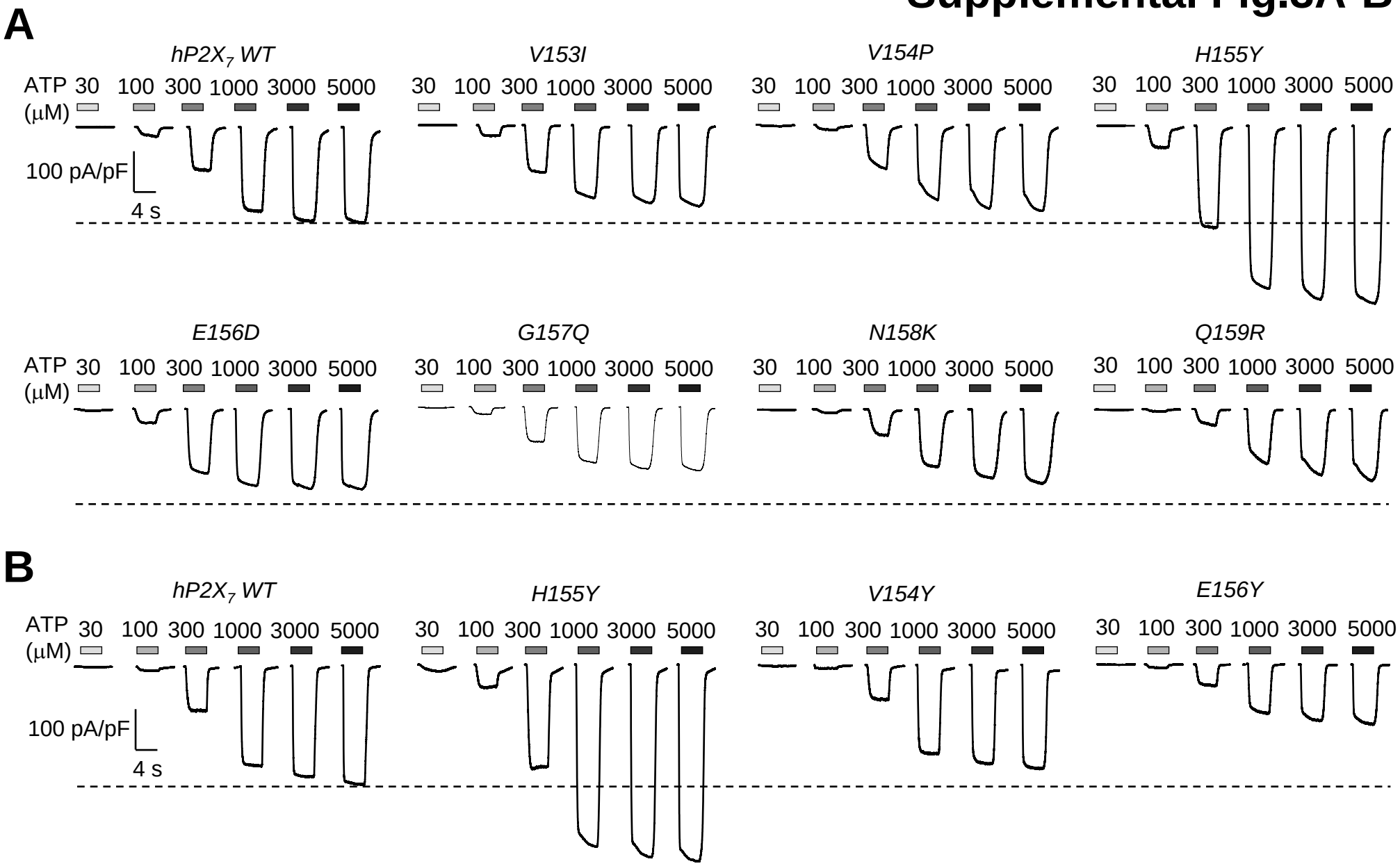
Supplemental Fig.2A



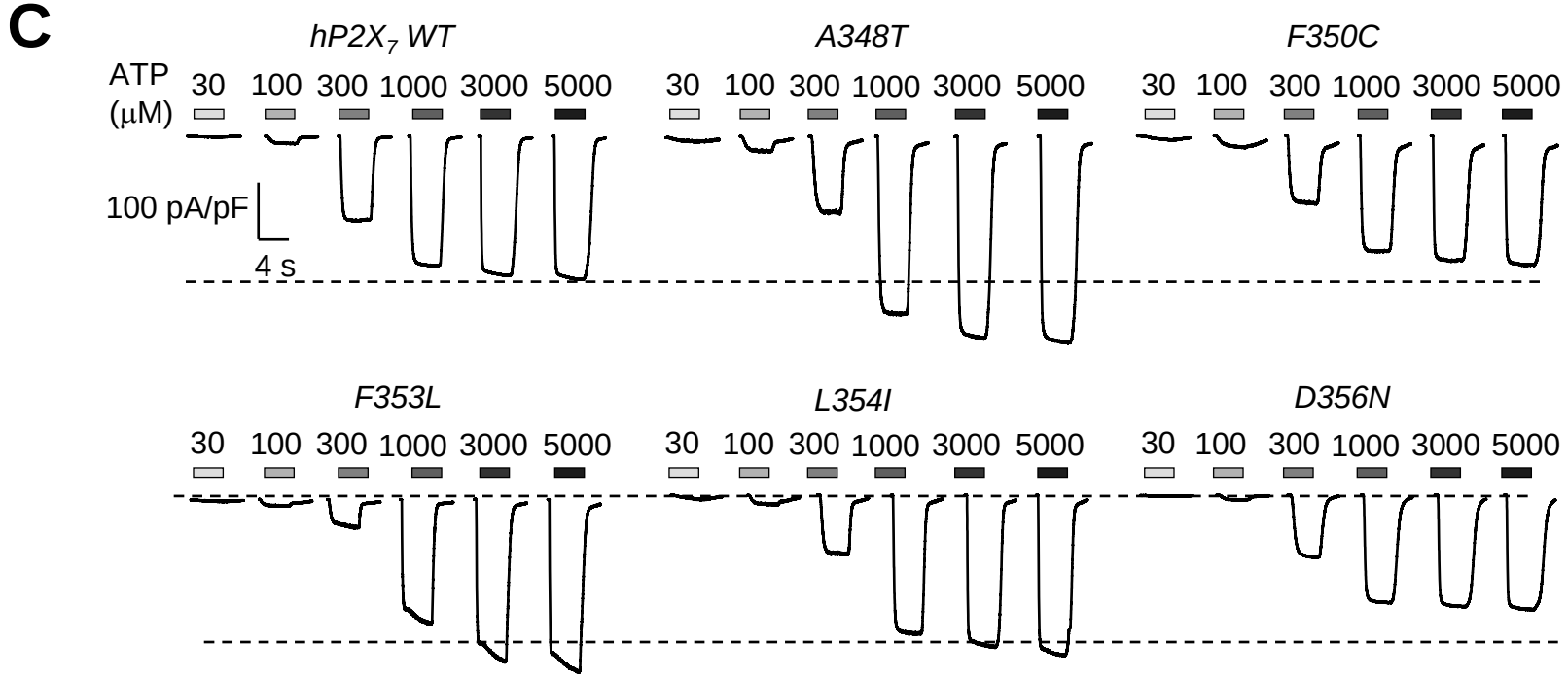
Supplemental Fig.2B



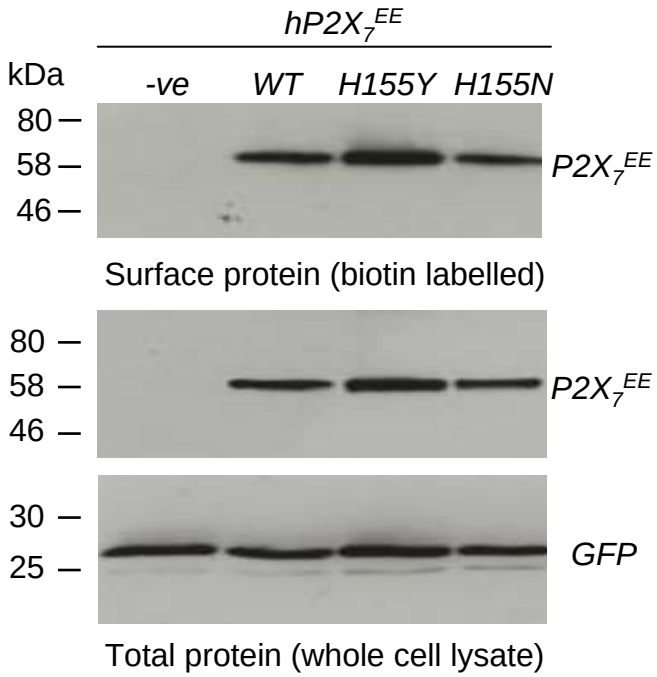
Supplemental Fig.3A-B



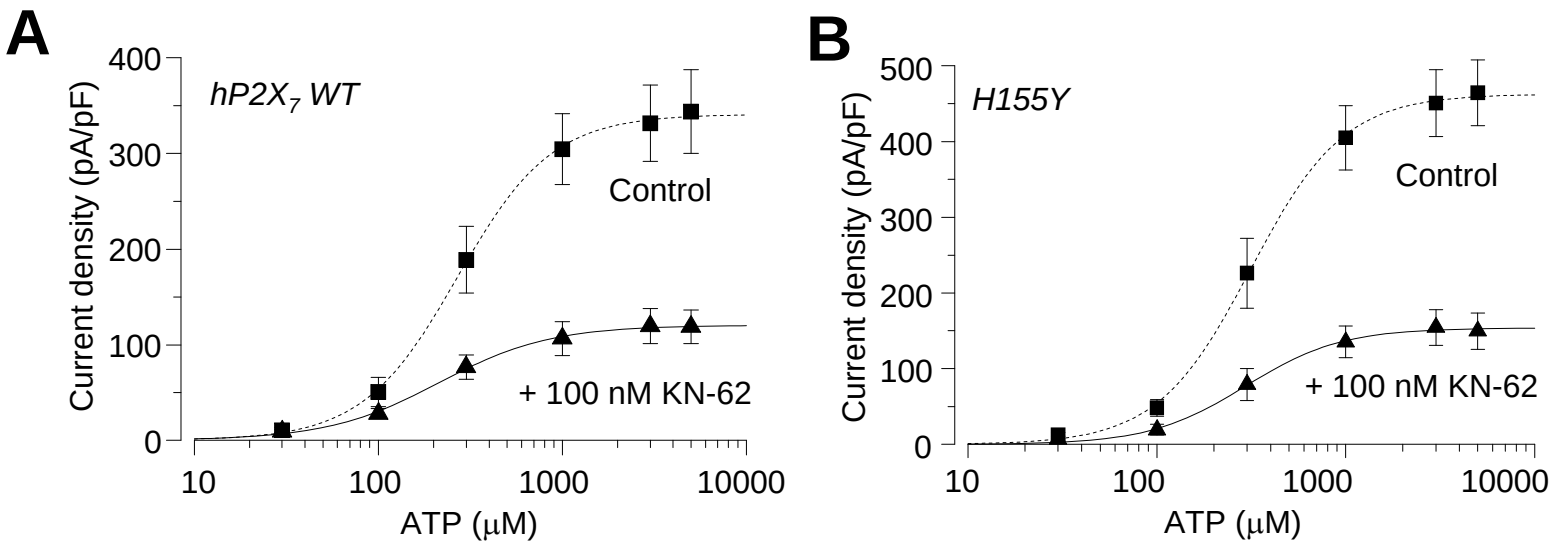
Supplemental Fig.3C



Supplemental Fig.4



Supplemental Fig.5



Supplemental Fig.6