Supplementary Material

Revisiting the idea that amyloid beta peptide acts as an agonist for P2X7

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Figs. S1 to S3

**A**

**B**



**Figure S1.** **Aβ25-35 induced [Ca2+]i responses in microglial BV-2 cells** **when the Aβ25-35 peptides are dissolved in water.** (A) BV-2 cells and (B) P2X7-deficient BV-2 cells were incubated in Ca2+ containing buffer (see Methods) and challenged with the Aβ25-35 peptide, dissolved in water, and in the concentration range 30µM -100µM. AZ10606120 is a commercially available antagonist of hP2X7, and BzATP is a specific agonist for P2X7 receptors. Data points represent the mean ± SD of 5 replicated experiments with triplicates on each plate.



**Figure S2.** **Aβ25-35 and YO-PRO-1 dye uptake in HEK293-hP2X7 cells when the Aβ25-35 peptides are dissolved in water.** HEK293-hP2X7 cells were incubated with 2 µM YO-PRO-1 dye in low-divalent buffer (see Methods) and challenged with Aβ25-35 peptides dissolved in water over the concentration range 30µM -100µM. AZ10606120 is a commercially available antagonist of hP2X7, and BzATP is a specific agonist for P2X7 receptors. Apyrase was used to metabolise any released ATP in the media. Data points represent the mean ± SD of 5 replicated experiments with triplicates on each plate.



**Figure S3. Aβ1-42 does not potentiate ATP-induced YO-PRO-1 uptake in HEK293-hP2X7 cells** Cells were incubated with YO-PRO-1 dye in the low-divalent buffer (see Methods) and were pre-incubated with the Aβ1-42 peptide in the concentration range 30µM – 30nM. AZ10606120 and JNJ (JNJ47965567) are the commercially available antagonists of hP2X7, and ATP is an agonist for P2X7 receptors. Data points represent the mean ± SD of 5 replicated experiments with triplicates on each plate. One-way ANOVA was performed with Dunnett’s multiple comparisons test using 1 mM ATP as the control, \* represent P<0.05.