Alzheimer's Protection Effect of A673T Mutation May Be Driven by Lower Aβ Oligomer Binding Affinity

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Running title: *Mutant oligomers have lower synaptic binding affinity than wt*

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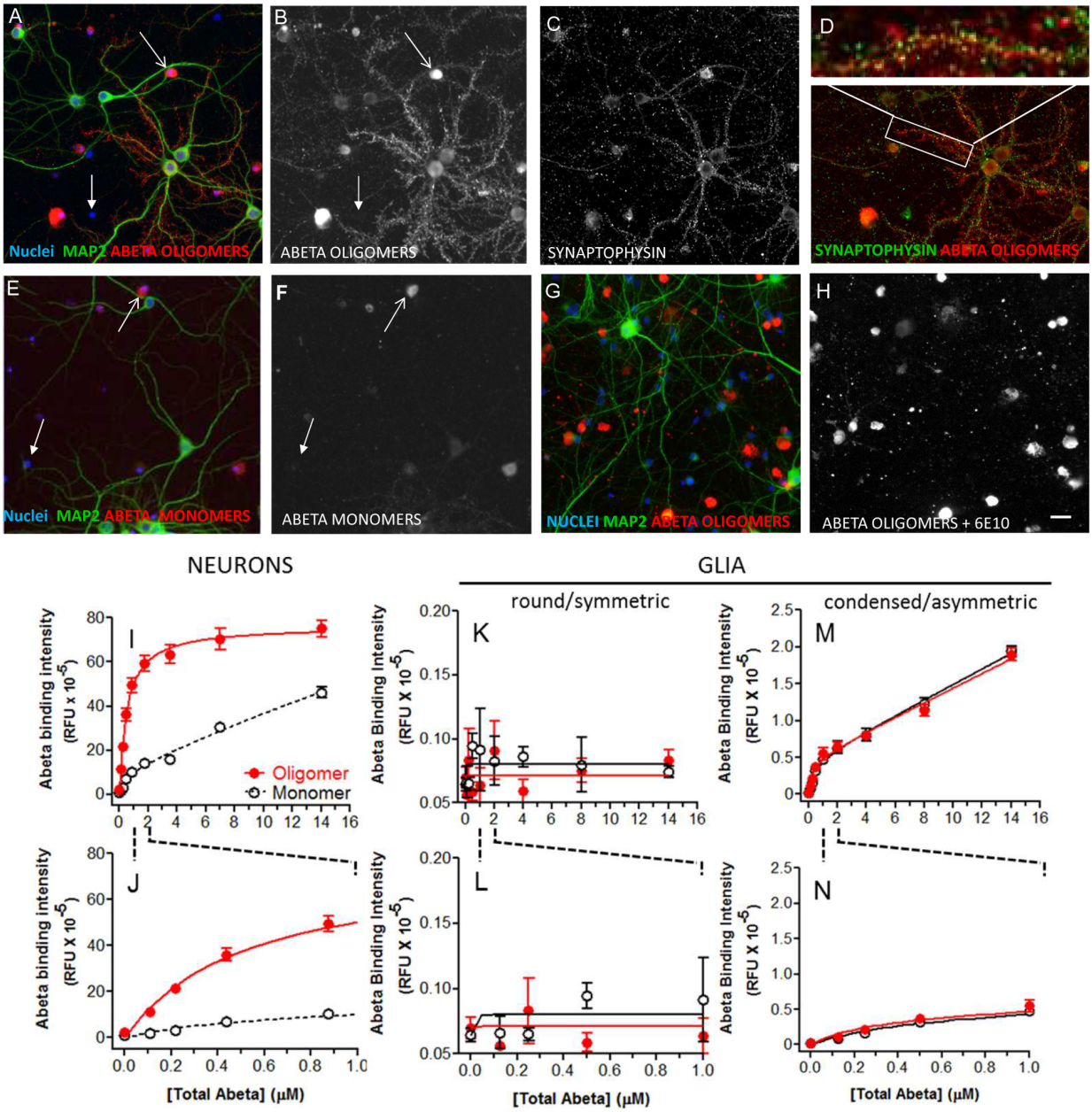

Supplemental Figure 1 (Figure 6 from Izzo et al. 2014a). Aβ oligomers bind to a single saturable receptor site on neuronal synaptic puncta. A,B. Wild-type Aβ 1-42 oligomers bind to some but not all neurons when added to cultures for 60 minutes (total Aβ concentration = 440 nM, visualized with 6E10 immunolabeling). A subset of neurons (immunopositive for MAP2,

green) exhibit punctate postsynaptic oligomer binding (red) along their neurites; 37% ± 3% of these puncta colocalize with presynaptic terminals immunolabeled for synaptophysin (**C,D**). Nuclei (DAPI +, blue) of several non-neuronal cells (glia, MAP2-negative) exhibit Aβ binding to the cell body. **B,F,H.** Immunolabeling of Aβ, alone, is shown for clarity. **E,F.** Fresh Aβ 1-42 monomers, added to cells with identical concentrations and conditions, is characterized by very low intensity punctate labeling on neurites and labeling of neuron and glia cell bodies. **G,H.** 6E10 (monoclonal antibody to Abeta 3-8) added to cultures prior to oligomers blocks binding of Aβ oligomers to neurite puncta but not to cell bodies of neurons or glia. **I.** Binding isotherms for Aβ oligomers (red closed circles) and fresh Aβ monomer (black open circles; treatment for 60 minutes with 44 nM- 14 μM total Aβ concentration) indicate that oligomer binding to neuronal puncta fits a single-site, saturable model (Kd = 518 ± 41 nM). Binding of fresh monomer fits a two-site model with a high affinity site (412 ±48 nM) and a second non-saturable binding site. **J.** Same data as **I**, expanded to show concentrations used for competition binding studies (440 nM total Aβ concentration); at these low concentrations binding intensity of oligomers to neuronal synaptic puncta is five-fold higher than with monomer. **K,L**. Binding of synthetic Aβ oligomers and fresh monomer to glial cells with round/symmetric nuclear morphology (closed arrowheads in **B**, **F**) is not above background levels (zero Aβ concentration in all graphs). **M,N**. Binding of synthetic Aβ oligomers and fresh monomer to glia with condensed/asymmetric nuclear morphology (open arrowheads in **B**,**F**) fits a two site model with a high affinity site (Kd = 289 ± 150 nM) and a second non-saturating site (Kd > 1 M). Scale bars = 20μm.

Supplemental References:

Izzo N. J., Staniszewski A., To L., Fa M., Teich A. F., Saeed F., Wostein H., et al. (2014a) Alzheimer's therapeutics targeting amyloid beta 1-42 oligomers I: Abeta 42 oligomer binding to specific neuronal receptors is displaced by drug candidates that improve cognitive deficits. *PLoS One* **9**, e111898.