SUPPLEMENTARY DATA EMBOR-2010-34157V1 Revision

Mediator is a Transducer of Amyloid Precursor Protein-Dependent Nuclear Signaling

Xuan Xu, Haiying Zhou, and Thomas G. Boyer

SUPPEMENTARY FIGURES

Figure S1

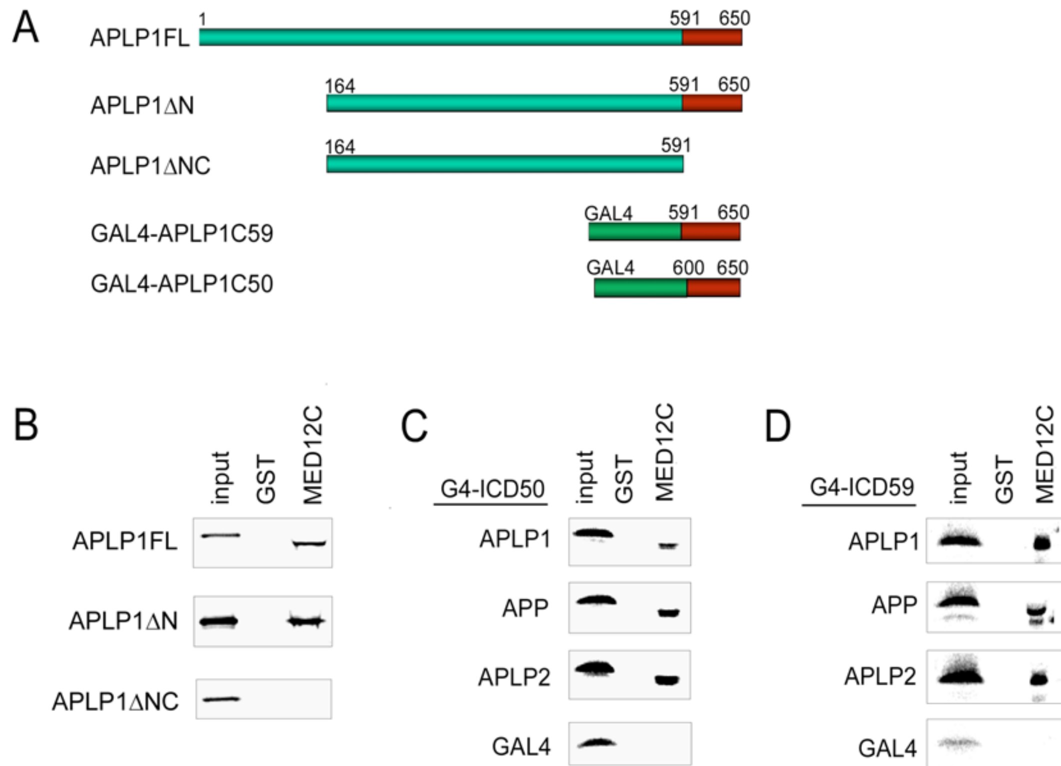

Figure S1. APP/APLP ICDs interact with MED12. (**A**) Schematic diagram of APLP1 derivatives used for in vitro binding assays. The APLP1 59 amino acid C-terminal intracellular domain (ICD) is highlighted. Numbers refer to amino acid coordinates. (**B-D**) Recombinant full-length

APLP1 and its specified truncation derivatives were expressed and radiolabeled with [^{35}S]methionine by translation in vitro prior to incubation with glutathione-Sepharose-immobilized GST or GST-MED12C (aa 1616-2177) as indicated. Bound proteins were resolved by SDS-PAGE, and visualized by phosphorimager analysis. Input, 10% of in vitro translation product used for binding. GAL4 corresponds to the DNA binding domain (aa 1-147) of the yeast GAL4 protein, to which the APP/APLP ICDs were appended. Note that γ-secretase-mediated cleavage of APP/APLP family members can occur at two positions generating ICDs of 50 and 59 amino acids, both of which were tested for MED12 interaction (**C**,**D**); proteomics analyses have revealed that the ICD50 is the predominant intracellular species produced (Gu et al., 2001; Kimberly et al., 2001).

Figure S2

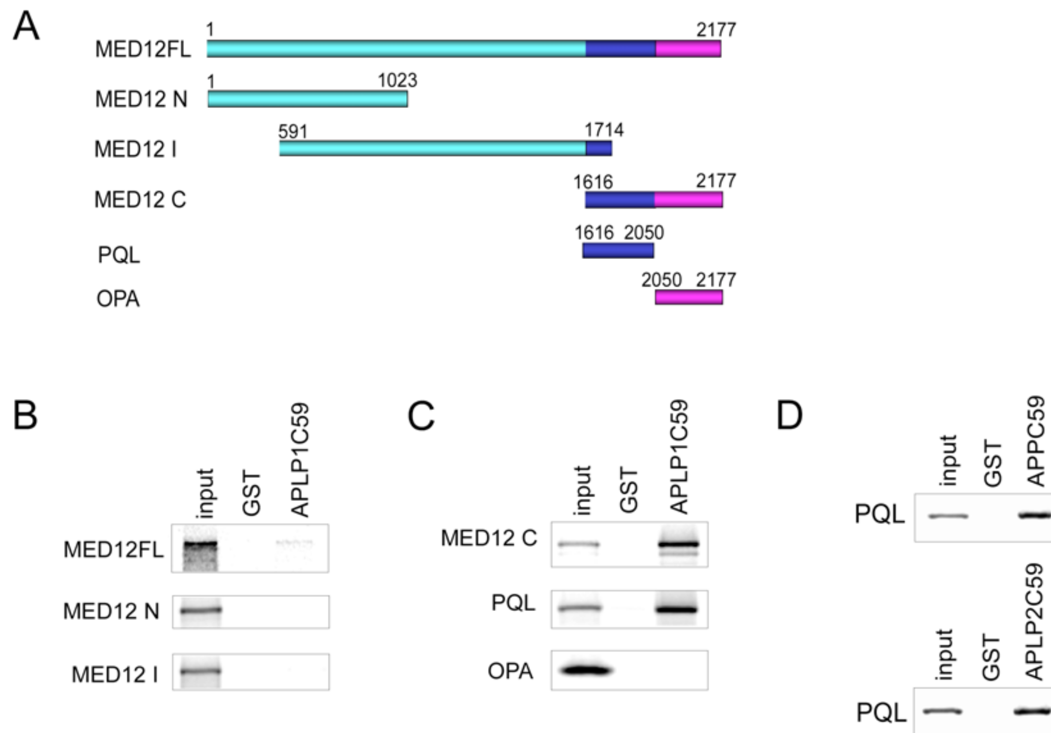

Figure S2. The MED12 PQL domain interacts with the APP/APLP ICDs. (**A**) Schematic diagram of MED12 derivatives used for in vitro binding assays. The MED12 PQL and OPA domains are highlighted. Numbers refer to amino acid coordinates. (**B-D**) Recombinant full-length MED12 and its specified truncation derivatives were expressed and radiolabeled with [^{35}S]methionine by translation in vitro prior to incubation with glutathione-Sepharose-immobilized GST or GST-APP/APLP ICD59 (C59) as indicated. Bound proteins were resolved by SDS-PAGE, and visualized by phosphorimager analysis. Input, 10% of in vitro translation product used for binding.

SUPPLEMENTARY MATERIALS AND METHODS

Plasmids. Full-length MED12 and truncation fragments used in glutathione S-transferase (GST) pull-down experiments have been described previously (Kim et al., 2006). pCS2+H_6T_7-APLP1 (encoding full-length APLP1), pET28C-APLP1$_{\Delta}$N (encoding APLP1 aa 164-650), pCS2+H_6T_7-APLP1ΔNC (aa 164-591), pBind-APLP1C50/59, pBind-APPC50/59 and pBind-APLP2C50/59 were constructed by PCR-based methods using human fetal brain cDNA as template DNA. Human APP/APLP ICD59 cDNA sequences were subcloned into the *E.coli* expression plasmid pGEX-6p-1 for production of GST-fusion proteins used in GST pull-down experiments.

Glutathione S-Trasferase (GST) pull-down assays. GST-MED12c or GST-APP/APLP ICDs were expressed in E.coli strain BL21 (DE3), and soluble lysates were prepared as described previously (Ding et al., 2008; Zhou et al., 2006) Recombinant MED12 and APP/APLPs derivatives were expressed and radiolabeled with [^{35}S]methionine by coupled in vitro transcription-translation reactions (TNT sp6/T7 Quickcoupled transcription-translation system; Promega). Radiolabeled recombinant proteins were incubated with GST derivatives immobilized on glutathione-sepharose beads (GE Healthcare Life Sciences), washed, eluted, and bound proteins resolved by 10% or gradient SDS-PAGE followed by Phosporimager analysis as described previously (Ding et al., 2008; Zhou et al., 2006).

SUPPLEMENTARY REFERENCES

Ding, N., Zhou, H., Esteve, P.O., Chin, H.G., Kim, S., Xu, X., Joseph, S.M., Friez, M.J., Schwartz, C.E., Pradhan, S. and Boyer, T.G. (2008) Mediator links epigenetic silencing of neuronal gene expression with x-linked mental retardation. *Mol Cell*, **31**, 347-359.

Gu, Y., Misonou, H., Sato, T., Dohmae, N., Takio, K. and Ihara, Y. (2001) Distinct intramembrane cleavage of the beta-amyloid precursor protein family resembling gamma-secretase-like cleavage of Notch. *J Biol Chem*, **276**, 35235-35238.

Kim, S., Xu, X., Hecht, A. and Boyer, T.G. (2006) Mediator is a transducer of Wnt/beta-catenin signaling. *J Biol Chem.*, **281**, 14066-14075. Epub 12006 Mar 14024.

Kimberly, W.T., Zheng, J.B., Guenette, S.Y. and Selkoe, D.J. (2001) The intracellular domain of the beta-amyloid precursor protein is stabilized by Fe65 and translocates to the nucleus in a notch-like manner. *J Biol Chem*, **276**, 40288-40292.

Zhou, H., Kim, S., Ishii, S. and Boyer, T.G. (2006) Mediator modulates Gli3-dependent Sonic hedgehog signaling. *Mol Cell Biol*, **26**, 8667-8682.