Netrin-1 interacts with amyloid precursor protein and regulates amyloid-β production

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SUPPLEMENTARY MATERIALS

Supplementary Figure legend:

Suppl. Figure 1. APLP1 interacts with the DCC/netrin-1 complex.

DCC immunoprecipitation from DCC/netrin-1 transfected HEK293T cells was analyzed by MALDI-TOF MS protein identification. A voyager Spec is shown together with the ProFound result regarding the better protein candidate.

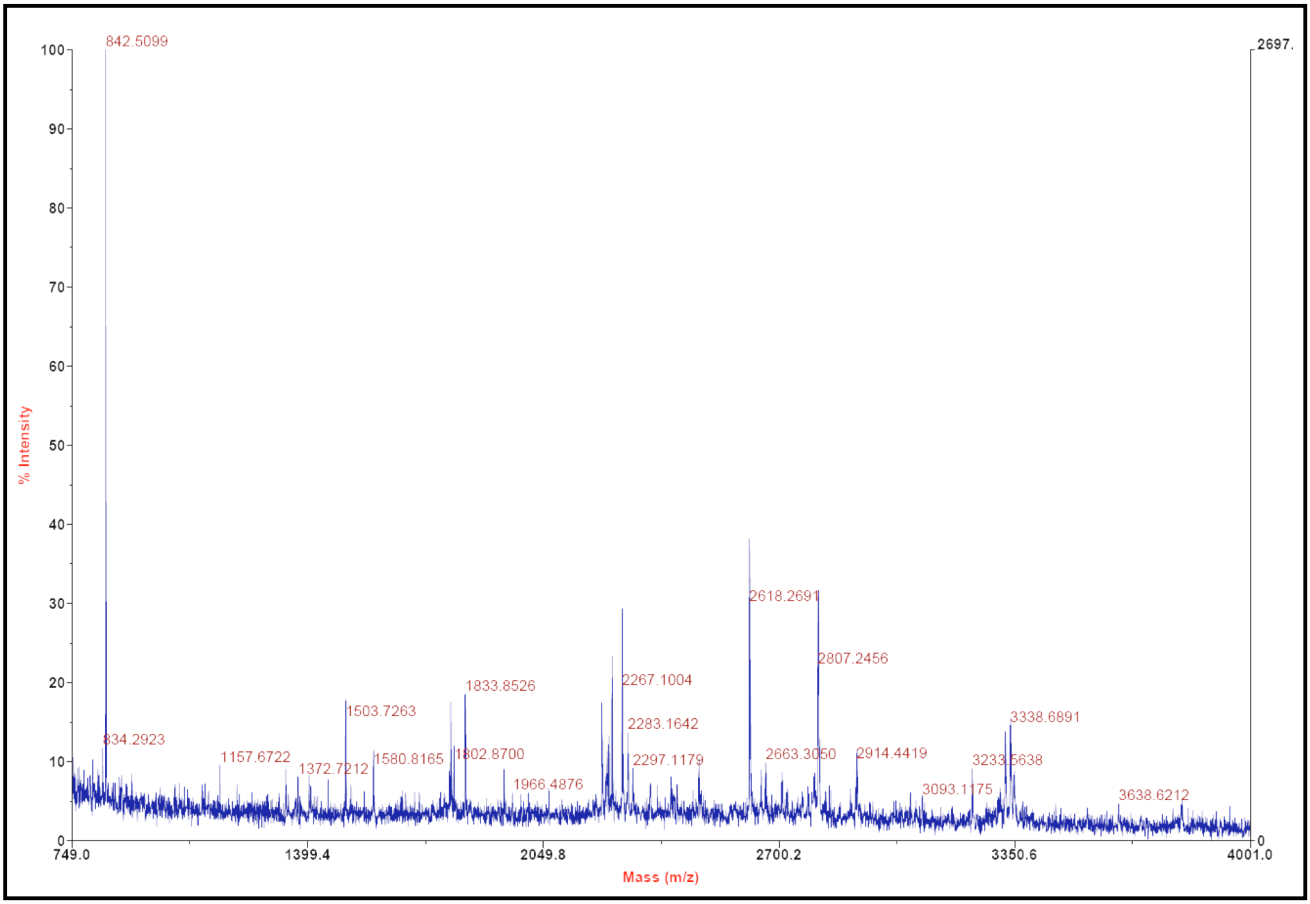
Suppl. Figure 2. Netrin-1 interacts with APP and APLP1.

A-B, HEK293T cells were transiently transfected with myc-tagged netrin-1 and/or APP and/or flagM2-tagged APLP1. Either conditioned medium (**B**) or cell lysate (**A**) was utilized for immunoprecipitation, using either an anti-c-myc antibody (for netrin) (**B**), an anti-FlagM2 antibody for APLP1 (**A**). Immunoprecipitations were subjected to polyacrylamide gel electrophoresis, transferred, and probed with antibodies raised against N-terminal APP (**B**) or c-myc (netrin-1) (**A**). In A-B, total proteins before pull-down are shown. **C**, HEK293T cells were transiently transfected with myc-tagged netrin-1, netrin-2 or ΔC netrin-1 (net (V,VI)), netrin G1, and APP. Cell lysate was utilized for immunoprecipitation, using an anti-c-myc antibody (for netrin). **D**, 150 ng of recombinant αAPPs was added to increasing concentrations of purified c-myc-tagged netrin-1 in 1ml reaction buffer. Netrin-1 pull-down was performed (with anti-c-myc), and the concentration of αAPPs pulled down with netrin-1 was quantified after Western blotting, using anti-APP antibody and NIH image software. A similar analysis was performed with DCC-EC. Bottom panels: input of αAPPs and DCC-EC shown by Western blot.

Suppl. Figure 3. Netrin-1 activates APP signaling.

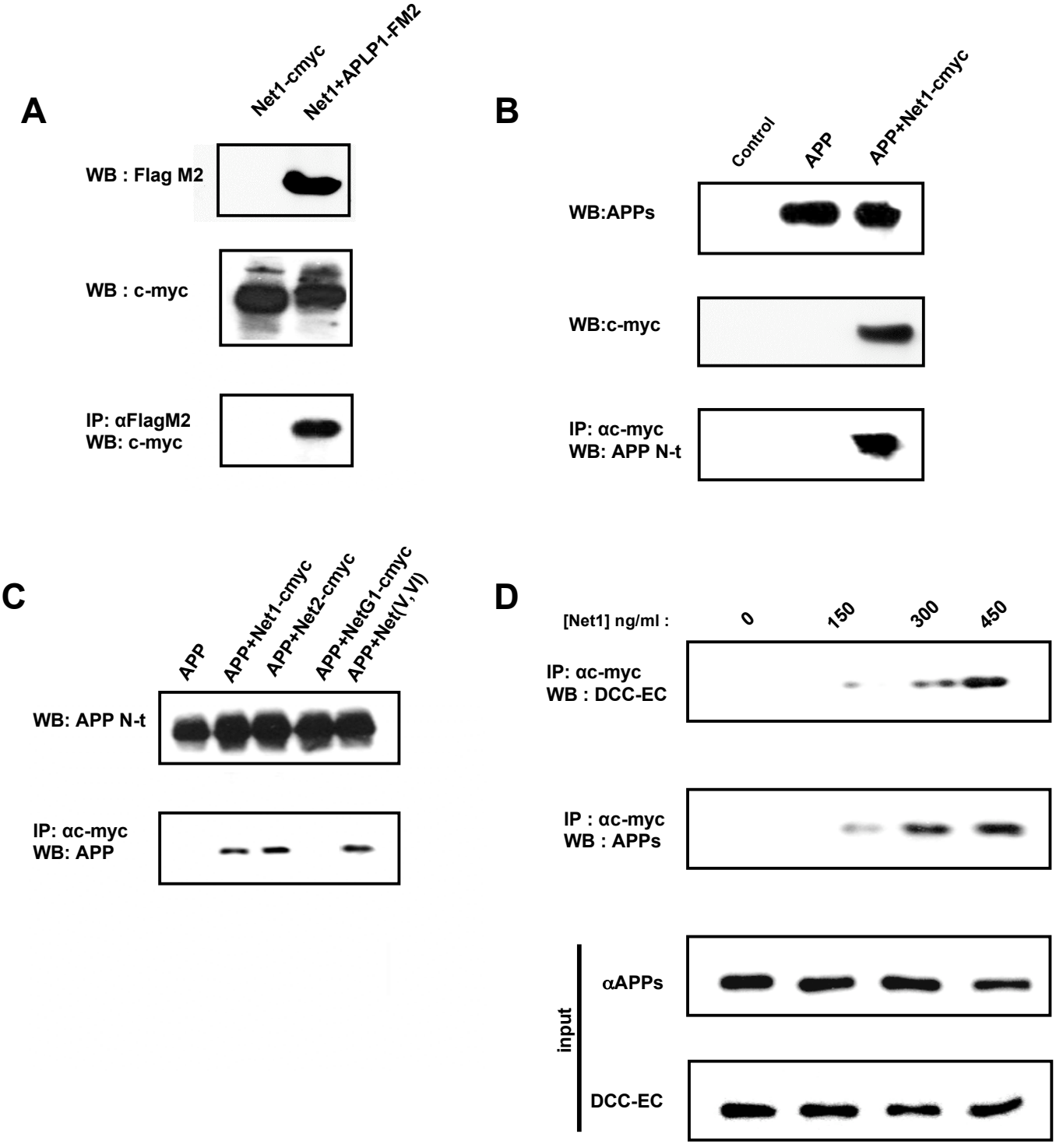
A, HEK293T cells were transiently co-transfected with APP and DAB-1-expressing constructs, and further incubated in the presence (or absence) of netrin-1. Pull-down was performed using an anti-DAB-1 antibody, and APP was detected in this pull-down using an N-terminal anti-APP antibody. The upper panels represent APP and DAB-1 before the pull-down, the lower panel being APP detected in the immunoprecipitation. **B-C**, Supplement data to Fig.3CD. Primary neuronal cultures from E16.5 hAPP transgenic embryos (PDAPP(J20) in C57BL/6J background) were treated with vehicle (PBS) or with 300 ng/ml netrin-1 added to the culture media every 24 h for 3 days, starting 1.5 day after plating. Cultures were fixed, treated with RNAse and stained with a 1:1000 dilution of an antibody specific for the C-terminal domain of APP (amino acids 649-664, antiserum I (R1155) (37)) followed by Alexa488-conjugated donkey anti-rabbit IgG (Invitrogen) and counterstained with TOTO-3 to visualize DNA. Stacks of images (z step = 250 nm) were acquired with a laser scanning confocal microscope (Nikon PCM-2000) at 600X magnification and collected with SimplePCI (Compix Inc., Sewickley, PA) software. For each condition, five separate fields were chosen in which individual cells were clearly distinguishable (avoiding clumps of neuronal bodies). **B**, A representative maximum intensity projection image of fields acquired for each condition is shown. **C,** Distribution of intensity of anti-I immunoreactivity across nuclei (Upper panel, green traces overlaid on images) representative of each condition were determined using the Histogram module of the Zeiss 510 LSM image analysis software. Lower panel, plots of intensity as a function of distance.

Suppl. Fig. 1



Rank	Probability	Est'd Z	Protein Information and Sequence Analyse Tools (T)	%	*pI*	kDa	®
+1	9.3e-001	0.99	T gi|2829415|sp|P51693|APP1_HUMAN AMYLOID-LIKE PROTEIN 1 PRECURSOR (APLP)	20	5.5	72.87	®

Suppl. Fig. 2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Net1-cmyc
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WB: c-myc
B
Control
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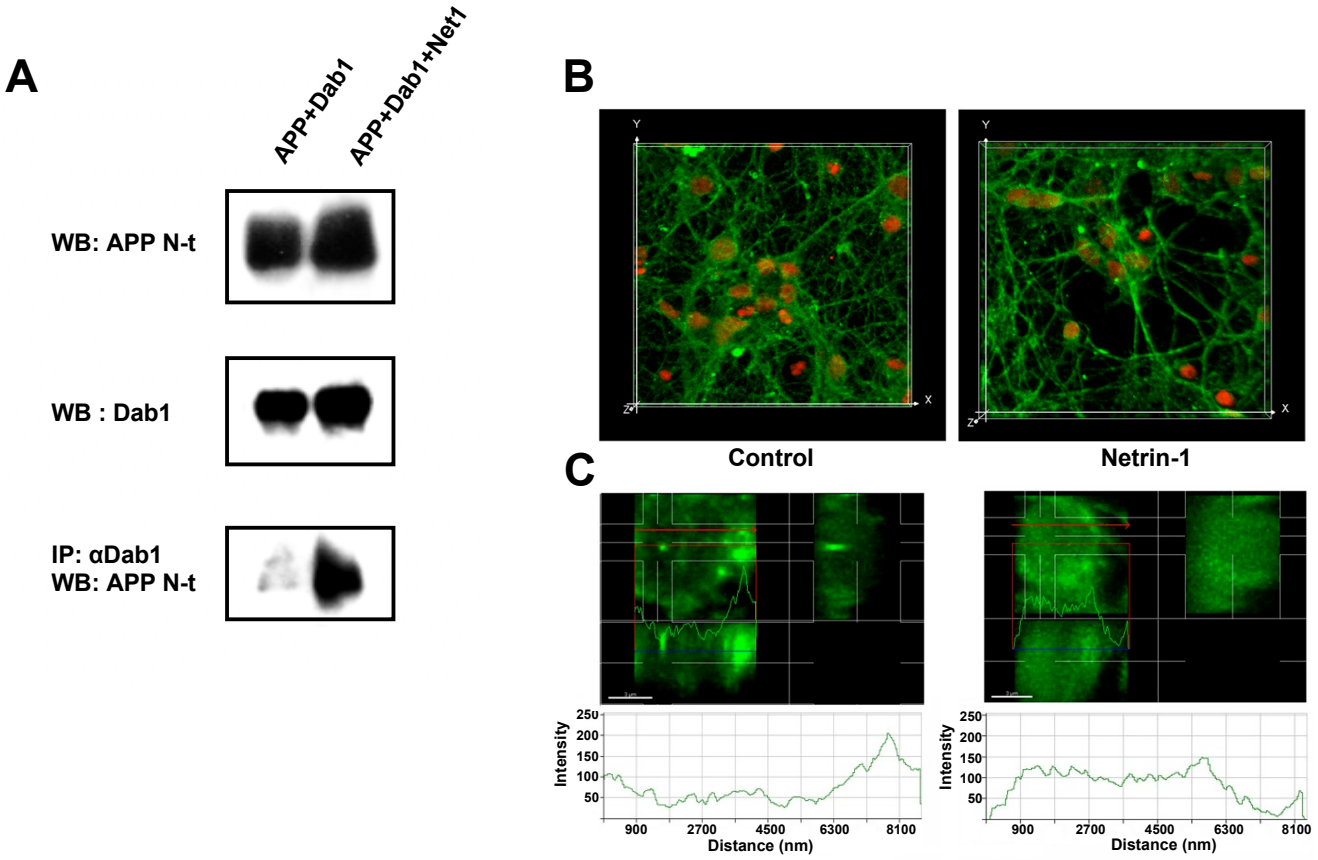
APP+Net1-cmyc
WB:APPs
WB:c-myc
IP: αc-myc
WB: APP N-t
C
APP
APP+Net1-cmyc
APP+Net2-cmyc
APP+NetG1-cmyc
APP+Net(V, VI)
WB: APP N-t
IP: αc-myc
WB: APP
D
[Net1] ng/ml :
0
150
300
450
IP: αc-myc
WB : DCC-EC
IP : αc-myc
WB : APPs
input
αAPPs
DCC-EC

Suppl. Fig.3


A
APP+Dab1
APP+Dab1+Net1
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WB : Dab1
IP: αDab1
WB: APP N-t
B
Control
Netrin-1
C
Intensity
250
200
150
100
50
900
2700
4500
6300
8100
Distance (nm)