

## **Appendix**

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**Appendix Figure S1. Netrin-1 depletion induces dopamine neuronal cell death and upregulates DCC and UNC5B receptors (Related to Figure 1 and 2)**

**A.** Immunostainings of DCC or UNC5B in the rat SN region. DCC and UNC5B (red), Tyrosine hydroxylase (TH) (green), DAPI (blue).

**B.** Immunofluorescent staining of netrin-1 and DCC on nigral sections from netrin-1<sup>fl/fl</sup> mice. Netrin-1 (Cy5), DCC (red), DAPI (blue) (Scale bar: 500  $\mu$ m).

**C.** Immunofluorescent staining of netrin-1 and UNC5B on nigral sections from netrin-1<sup>fl/fl</sup> mice. Netrin-1 (Cy5), UNC5B (red), DAPI (blue) (Scale bar: 500  $\mu$ m).

**D.** Immunofluorescent staining of TH and VMAT2 on netrin-1<sup>fl/fl</sup> mice brain sections injected with control adenovirus vector. TH (red) and VMAT2 (Cy5) (Scale bar: 500  $\mu$ m). Quantification of TH and VMAT2 fluorescent intensity in SN and striatum (Str). N = 3 each group. Error bars represent the mean  $\pm$  SEM. Statistical significance was determined using an unpaired t-test.

**E.** Immunofluorescent staining of TH and VMAT2 on netrin-1<sup>fl/fl</sup> mice brain sections injected with Cre adenovirus vector. TH (red) and VMAT2 (Cy5) (Scale bar: 500  $\mu$ m). Quantification of TH and VMAT2 fluorescent intensity in SN and striatum (Str). N = 3 each group. Error bars represent the mean  $\pm$  SEM. Statistical significance was determined using an unpaired t-test.

## **Appendix Figure S2. Netrin-1 titration induces primary neuron death and DCC/UNC5B cleavage by caspases**

**A-B.** Primary cortical **(A)** and dopamine **(B)** neurons (DIV 10) were treated with 100 – 200 ng of human recombinant netrin-1 protein, or a titrating netrin-1 mAb (2F5) 0.5- 5  $\mu$ g and a recombinant netrin-1 binding protein (4Fbn, the 4th fibronectin domain of the extracellular domain of DCC) 0.5-5  $\mu$ g for 24 hr. Immunoblot showed the cleaved UNC5B, DCC and caspase 3 after 2F5 and 4Fbn incubation.

**C.** LDH assay on primary cortical neurons and dopamine neurons (DIV 10). Data are shown as mean  $\pm$  SEM. Statistical significance was determined by an unpaired t-test. N = 3 each group. \*P< 0.05, \*\*P< 0.01.

**D.** Immunoblot from primary midbrain dopamine neurons treated with 3 caspase 3 or pan-caspase inhibitor. (E-F) TUNEL assay in primary neurons with knockdown of DCC or UNC5B.

**E-F.** TUNEL assay on primary cortical neurons (DIV 10) treated with shDCC or shUNC5B and control adenovirus vector for 24 hr. **(E)** Representative images. **(F)** Quantification bar graph. Data are shown as mean  $\pm$  SEM. Statistical significance was determined by an unpaired t-test. N = 3 each group. \*P< 0.05, \*\*P< 0.01. All experiments were repeated 3 times.

**Appendix Figure S3. Netrin-1 is neuroprotective in a progressive and an acute mouse model of PD**

**A.** Validation of tamoxifen-induced netrin-1 overexpression (O/E) in netrin-1 inducible transgenic mice, by western blot (bottom panel). The spinal cord (sc) of E11.5 mice embryo was used as a positive control. N=4 brains/condition.

**B.** Representative images by immunohistochemistry of S129-phosphorylated alpha synuclein in wild type (WT) and SNCA transgenic (SNCA Tg) mice (Scale bar, 100  $\mu$ m). Quantification bar graph of S129-phosphorylated alpha synuclein-positive cell count (right panel). Data are shown as mean  $\pm$  SEM. Statistical significance was determined by an unpaired t-test. N = 3 each group. \*P< 0.05, \*\*P< 0.01.

#### **Appendix Figure S4. Characterization of netrin-1 distribution and effects after intrastriatal injection**

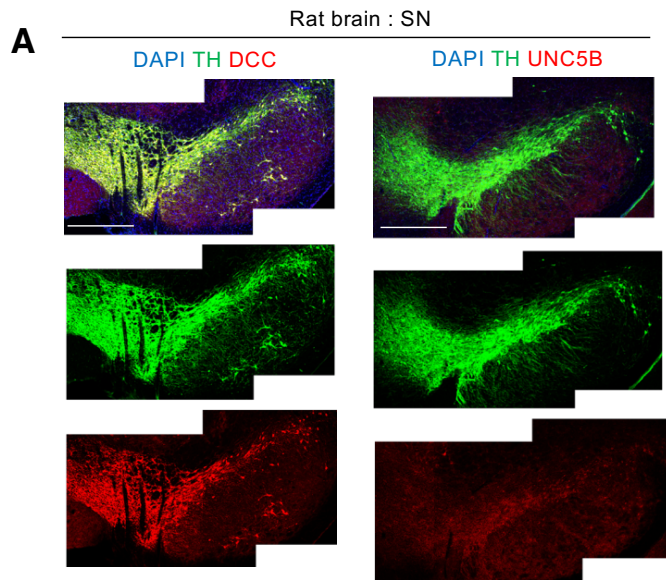
**A-B.** Distribution of 125I-netrin-1 and 125I-GDNF after intrastriatal injection in rats. **(A)** Radioactivity of 125I-labelled proteins in different rat brain areas. Means +SEM are shown, N=10 animals in each group. **(B)** Photomicrograph of an autoradiographic film after intrastriatal injections of 125I-netrin-1 and 125I-GDNF in rats. Brain slice figures modified from the rat brain atlas of Paxinos and Watson (1997). N=10 animals in each 4 group.

**C.** Diffusion of netrin-1 24h after intrastriatal injection in rats. Fluorescence microscopy representative images of netrin-1 His-tagged (Red) in cortex (CTX), striatum (STR), hippocampus (HIPPO) and substantia nigra (SN); “C”: control side, “L” : Lesioned side, Green : TH staining for dopamine (and catecholaminergic neurons) (Scale bar, 2000  $\mu$ m). N=4 animals/group.

**D.** Quantification of TH positive neurons from mouse (E13) ventral midbrain primary culture treated with recombinant human Netrin-1, GDNF (positive control) or no factor (negative control). Means + SEM are shown, N = 4. Unpaired t-test, \*P < 0.05, \*\*P < 0.01 compared to control group.

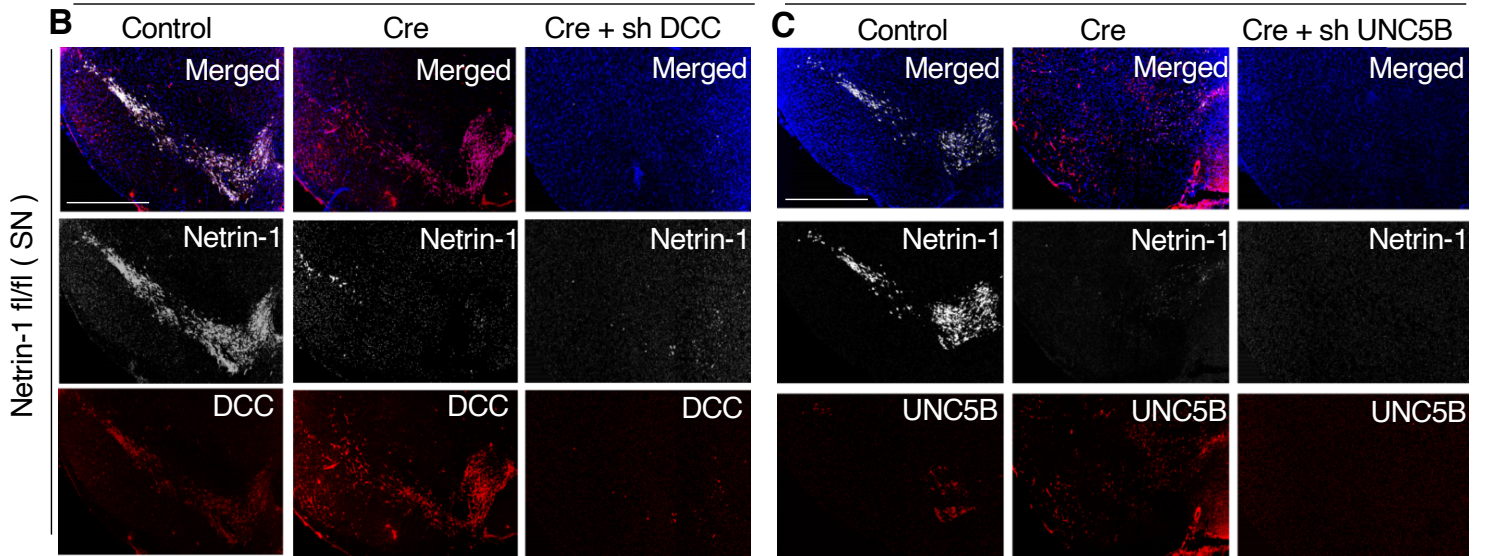
**E.** Measurement of TH+ surface area normalised by the number of total TH+ cell from mouse (E13) ventral midbrain primary culture. Unpaired t-test \*P < 0.05, N=3 independent experiments, Means + SEM are shown

**F.** Immunoblot on striatal rat lysates showing the modulation of DCC, PS6 (Ser235/236), PTEN levels 4h after intrastriatal injection of recombinant human Netrin-1. N=3 animal/condition.



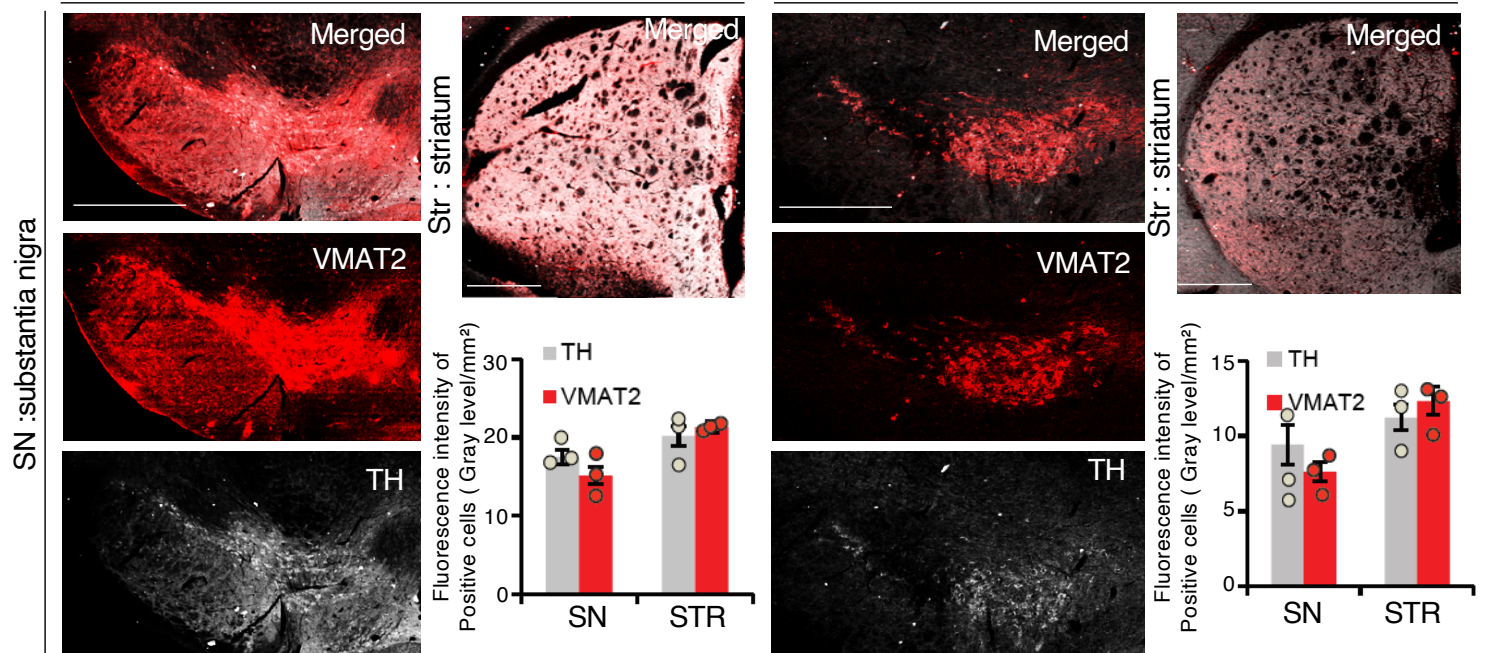
Mouse brain : SN

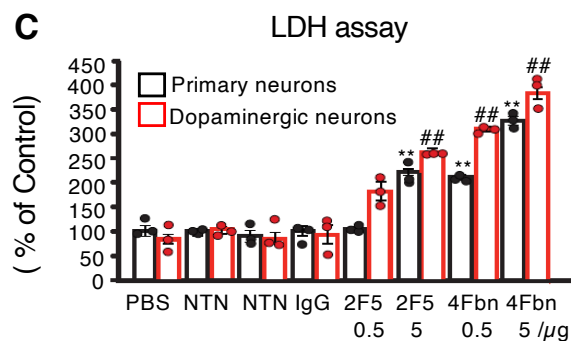
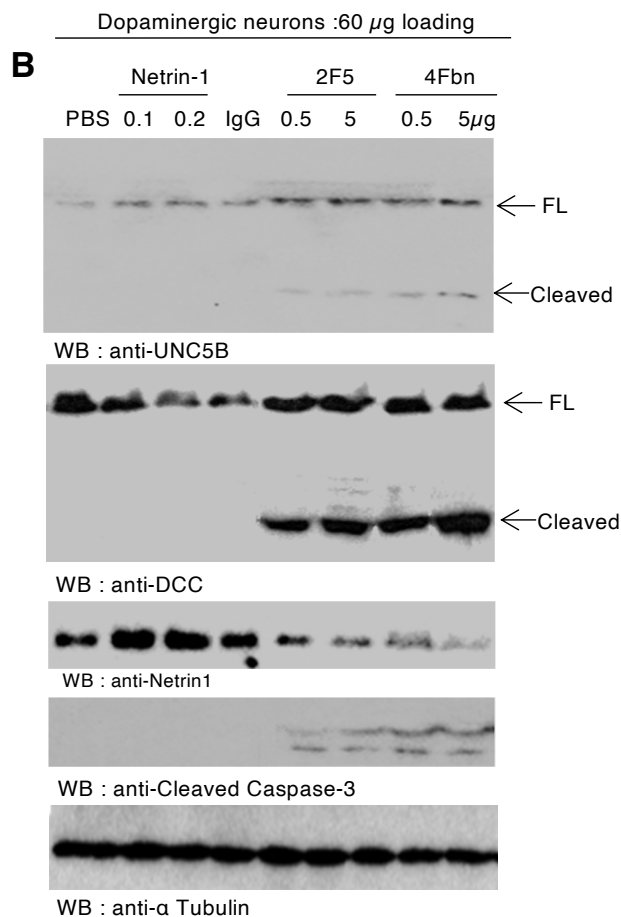
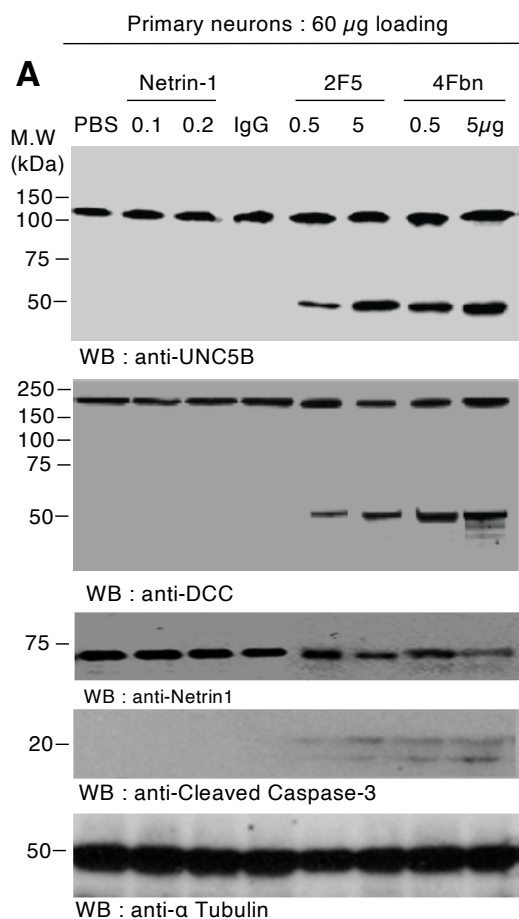
Mouse brain : SN



**D** Netrin-1 fl/fl with CTL virus

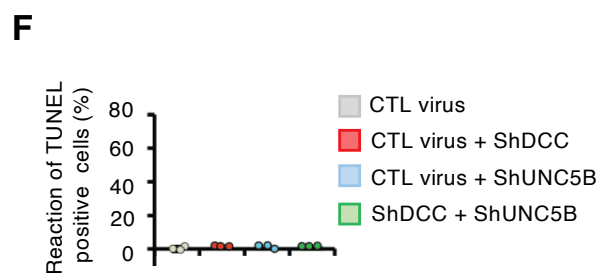
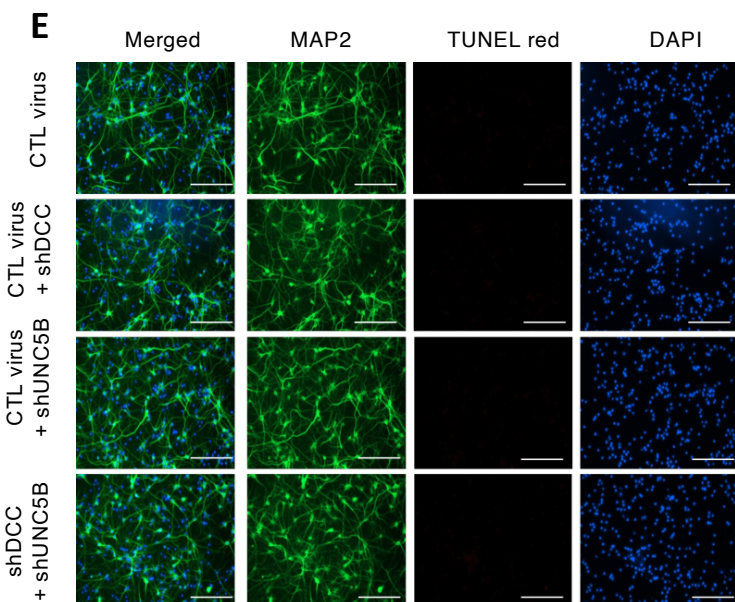
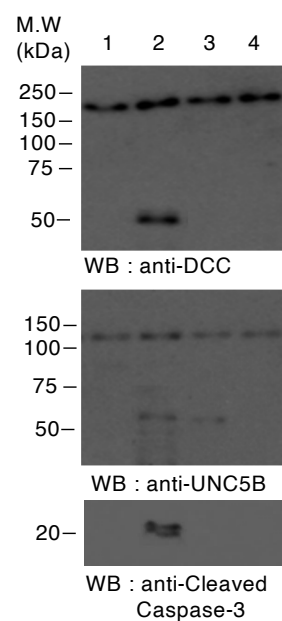
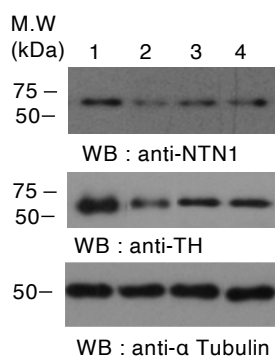
**E** Netrin-1 fl/fl with Cre virus





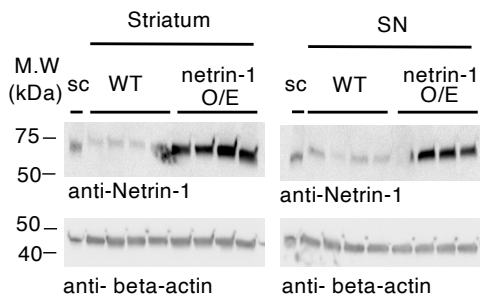
**D**

1: CTL  
2: 4Fbn  
3: 4Fbn + caspase 3 inhibitor  
4: 4Fbn + Pan-caspase inhibitor



**A**

One week after tamoxifen injection (i-p)



**B**

