## Supplementary Information

# Misassembly of full length Disrupted-in-schizophrenia 1 (DISC1) protein is linked to altered dopamine homeostasis and behavioral deficits

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Running title: tgDISC1 rat

**Supplementary Figure 1 Determination of the level of overexpression of transgenic human DISC1 in the tgDISC1 rat.** (**a**) Western blot of endogenous rat Disc1 and transgenic human DISC1 in tgDISC1 and negative control brain homogenates. Left panel shows endogenous Disc1, right panel transgenic DISC1 signal of P58 brain homogenates. Dilutions of brain homogenates ranging from 1 mg to 250 µg brain equivalents and 2 ng to 16 pg of recombinant DISC1 protein species (rat Disc1 (590-784), left panel; human DISC1 (598-785), right panel) were loaded. The amount of full length endogenous rat Disc1 and transgenic human DISC1 protein (arrows) were used to estimate level of overexpression in relation to recombinant DISC1. (**b**) Calculation of the amount of endogenous / transgenic DISC1. The approximated factor of overexpression is about 11-fold. The estimated increase in transgene expression has caveats: since there is no antibody that binds rat and human DISC with equal affinity, expression of rat and human transgenic DISC1 was compared to defined amounts of recombinant rat and human DISC1 species, respectively, using two different antibodies. Also, up to 60 different spliced transcripts of DISC1 in humans have been described[1] that may correspond to the variety of immunoreactive signals also in rats. Here, the uniform, non-spliced full length human DISC1 immunoreactive band was compared to the main full length endogenous Disc1 band (arrows), disregarding any other splice variants.

*Methods:*

*Determination of the level of overexpression of transgenic human DISC1 in the tgDISC1 rat.*

To determine the approximate range of overexpression of the transgenic human DISC1 in relation to endogenous rat Disc1 whole brain homogenates of P58 tgDISC1 and negative control rats were analyzed together with a serial dilution of recombinant DISC1 protein species.

There is no antibody available that equally recognizes endogenous rat and transgenic human DISC1 that would allow to easily assess the level of overexpression of the transgene in direct

comparison to endogenous Disc1. Therefore the amount of transgenic DISC1 was estimated in relation to recombinant human DISC1 (598-785)[2] with the 14F2 antibody, whereas the human-precleared C-term antibody[3] (see also Supplemental Figure S2d) was used to determine the amount of endogenous rat DISC1 by comparison with recombinant rat Disc1 (590-784)[4] species. Although the bias of antibody preference towards recombinant proteins at the expense of binding DISC1 protein in the brainsamples cannot be ruled out, a rough estimation regarding the overexpression level of transgenic DISC1 could be calculated. For that the accurate fluorescent Odyssey Western blot quantification method using the LI-COR Odyssey CLX (LI-COR Biosciences, Lincoln, NE, USA) and the corresponding Image Studio Version 2.1 software was used.

Brain homogenates of tgDISC1 and negative control animals were loaded onto SDS polyacrylamide gels. Additionally a range of recombinant proteins of either rat Disc1 (590-784) or human DISC1 (598-785) was loaded onto the same blot (2 ng diluted by factor 5 down to 16 pg). Blots were incubated with primary antibodies specific for human DISC1 (14F2) and rat Disc1 (hu-precleared C-term) and the corresponding secondary fluorescent antibodies (LI-COR, Biosciences, NE, USA). For calculation of the fluorescent signal of the recombinant protein species whole lane measurements were performed, taking also protein multimers into account. The fluorescent signals of the recombinant protein dilutions were plotted against the amount of recombinant protein loaded. Based on the line of best fit the fluorescent signal of the actual sample could be set in relation to the signal derived from the recombinant proteins. The “sample signal” was either the full-length huDISC1 band of the transgene or the full length band of endogenous Disc1.

The fluorescent signal of the transgenic DISC1 band in brain homogenate equalled approximately 490 pg of recombinant human DISC1 (598-785), whereas the fluorescent signal of endogenous Disc1 equalled approximately 43 and 44 pg of recombinant Disc1 (590-784) in tgDISC1 and negative control rats, respectively. By calculating the ratio of the 490 pg of transgenic DISC1 and the 44 pg of endogenous DISC1, an overexpression factor

of 490/44 = 11 was estimated. The approximated factor of overexpression of tgDISC1 compared to endogenous Disc1 levels represents a rough estimation as two separate antibodies were used, one for rat and one for human DISC1, and the affinity of each for recombinant compared to expressed DISC1 is not known.

**Supplementary Figure S2 Regional comparison of aggregated DISC1 in the tgDISC1 and negative control rat.** (**a**) Immunohistochemistry of cryosections of the tgDISC1 rat (left hemisphere) or Sprague Dawley negative control rat (right hemisphere) by DAB staining. Bar 1 mm. (**b**) Western blots comparing the insoluble fraction (upper two panels) and homogenates (lower panels) of medial prefrontal cortex (mPFC) and dorsal striatum (dStr) of the tgDISC1 rat. Although the promoter activity (see PrP signal) and expression level of the transgenic DISC1 is the same in both brain regions, less aggregated DISC1 is detectable in the mPFC (lower insoluble fraction panel; see also Figure 1b, c). High molecular weight bands in the insoluble fraction appear in both regions (upper insoluble fraction panel). (**c**) Western blot of insoluble fraction (upper panel) and homogenates (lower panel) from negative controls (-) or tgDISC1 rats (+), stained simultaneously with an antibody specific for human DISC1 (mAB 14F2; green) and rat DISC1 (hu-DISC1 precleared ratDisc1 C-term Ab; red). Recruitment of endogenous Disc1 by overexpression of huDISC1 into the insoluble fraction occurs in the mPFC and dStr. In the homogenate endogenous Disc1 expression itself is not changed in transgenic animals compared to negative controls. (**d**) No recognition of transgenic DISC1 by the human-precleared ratDisc1 specific C-term antibody. Whereas the original C-term antibody has some affinity towards huDISC1, the precleared C-term does not recognize transfected full length huDISC1, proving specificity of the ratDisc1 signal of (c). The blot depicted has the same samples loaded three times, was cut in pieces and probed with the original C-term, the hu-precleared C-term, and eluted hu-affinity C-term antibodies, respectively.

*Methods:*

*Immunohistochemistry of complete frontal sections.*

For the comparison of complete frontal sections of transgenic and negative control rats, animals were perfused with PBS pH 7.4 and brains were bisected along their midline. Prepared brains were stored at -40°C. Sections were cut, blocked and incubated with mAB 3D4 as described in main paper. As secondary antibody the premixed Histofine Simple stain Rat MAX PO (Nichirei, Japan) was incubated for 1 h at RT. After washing 3x in PBS pH 7.4, sections were incubated with DAB peroxidase substrate Kit using the manufacturer's instructions (Vector Laboratories, Burlingame, CA, USA). Finally, sections were dehydrated in an ascending ethanol series and mounted in Eukitt (Sigma-Aldrich, MO, USA). All images were collected with a Zeiss Axiovision Apotome.2 confocal microscope, complete frontal rat brain images were reconstructed with the Zeiss Zen 2011 software Panorama module (Zeiss, Germany).

*Preclearing of the C-term antibody raised against ratDISC1.*

The polyclonal ratDisc1 C-term antibody was raised against the C-terminal part (598-824) of ratDisc1.[3] Affinity purification of the antibody was performed by incubating serum with recombinant ratDisc1 (598-824) coupled to NHS-activated Sepharose 4 Fast Flow beads (GE Healthcare, Germany). For obtaining ratDISC1 specific antiserum that would not recognize human DISC1 ("hu-precleared"), rat affinity-purified antiserum was incubated with recombinant huDISC1 (598-854) coupled to NHS sepharose (GE Healthcare, Germany). Two mg of C-term antibody were incubated with the coupled beads for 2 h at room temperature. The hu-precleared C-term antibody was in the flow-through, whereas human DISC1 specific C-term was eluted from bead material.

**Supplemental Figure S3 Colocalization of γ-tubulin with DISC1 in perinuclear aggregates in the striatum of the tgDISC1 rat and tgDISC1 primary neurons.** (**a**) Immunohistochemical staining of the tgDISC1 rat with γ-tubulin (green) and α-DISC1 (FFD5; red) antibody.[5] In the displayed striatal neurons the DISC1 signal colocalizes with the centrosomal γ-tubulin signal (white arrows), a magnification thereof is shown in (b). Bar 10 µm. (**b**) To verify the colocalization of the two signals, a fluorescent intensity histogram was performed along the white arrow, showing a concordant increase in red and green channel at the cross-section of the DISC1 aggregate. (**c**) Immunohistochemical stainings of primary neurons derived from the tgDISC1 rat and negative controls. While the α-DISC1 antibody (FFD5; red) recognizes DISC1 aggregates in tgDISC1 neurons, DISC1 signal is absent in the neurons of negative controls, whereas the γ-tubulin signal can be detected in every cell. Thus, colocalization of DISC1 and γ-tubulin signal can only be detected in the tgDISC1 cortical neurons (white arrow). Bar 10 µm.

*Methods:*

*Immunohistochemistry of DISC1 and γ-tubulin in tgDISC1 rat brain.*

For the immunostaining of transgenic rats, animals were perfused with PBS pH 7.4 and sagittal cryo sections were cut on a Cryostat (Leica CM1900; Leica, Germany) and dried for 20 min at RT. The sections were post-fixed with prechilled methanol for 15 min at -20 °C and subsequently washed with PBS pH 7.4. After blocking with antibody diluent (Dako, Hamburg, Germany) sections were incubated with the polyclonal α-DISC1 FFD5 and γ-tubulin antibody for 16 h at 4°C in antibody diluent. After two PBS washes the secondary antibodies (anti-mouse IgG AlexaFluor594 1:300 and AlexaFluor488 1:300; Invitrogen, CA, USA) were applied for 1 h at RT. Sections were washed first in PBS and then in PBS plus 0.05% Tween-20 for 10 min. Subsequently sections were washed shortly in distilled water, 70% ethanol and incubated 2x 5 min in Sudan Black (Division Chroma, Münster, Germany) in 70% ethanol to block autofluorescence. After washing the sections in 70% ethanol and $H_2O$ they

were mounted with ProLong Gold with DAPI (Invitrogen, CA, USA). All images were collected with a Zeiss Axiovision Apotome.2 confocal microscope (Zeiss, Oberkochen, Germany). Fluorescence density histogram was done with the ZEN2012 software (Zeiss, Oberkochen, Germany).

*Preparation of primary neurons.*

Cortical neurons were prepared from DISC1 transgenic and negative control Sprague Dawley rats at embryonic day 18 (E18). In brief, cortices without meninges were collected and trypsinized in 15 mL of 0.05% trypsin, 0.02% EDTA in PBS (GIBCO, Carlsbad, CA, USA) for 15 min. The reaction was stopped by the addition of 35 mL Neurobasal medium (supplemented with 10% FBS, 1x B27 supplement, 1x penicillin / streptomycin, 1x GlutaMAX; all GIBCO, CA, USA) and cells were mechanically dissociated from the tissue and cleared by pipetting through a filter. The cell suspension was centrifuged at 800 x *g* for 5 min and the cell pellet resuspended in supplemented Neurobasal medium. Cells were plated onto 6-well plates freshly coated with poly-L-ornithine (100 µg/mL; Sigma-Aldrich, MO, UDA) at a density of 5 x $10^5$ cells per well. Next day the medium was replaced. After 14 days in culture (DIV 14) the primary neurons were used for the respective experiments. To induce DISC1 aggregation, primary neurons were incubated with 50 µM DA for 24 h.

*Immunohistochemistry of DISC1 and γ-tubulin in tgDISC1 and negative control primary neurons.*

Cortical primary neurons of tgDISC1 and negative control animals were seeded onto glass coverslips and incubated as described for 14 d. Fixation was performed by washing the neurons with PBS and incubation with pre-chilled methanol for 15 min at -20 °C. Neurons were permeabilized with PBS (plus 0.5% saponine, 5% milk powder, 1% BSA) and incubated with the polyclonal α-DISC1 "FFD5" and γ-tubulin antibody for 16 h at 4°C in PBS supplemented with 5% milk powder, 1% BSA and 0.5% saponine. Subsequently, neurons

were washed thrice with PBS and incubated with secondary antibody (anti-mouse IgG AlexaFluor594 1:300 and AlexaFluor488 1:300; Invitrogen, CA, USA) for 1 h in PBS plus 0.5% saponine and 1% BSA. Cells were mounted with ProLong Gold with DAPI (Invitrogen, CA, USA). Images were collected with a Zeiss Axiovision Apotome.2 confocal microscope (Zeiss, Germany).

**Supplementary Figure S4 Thioflavin S (ThS) mediated characterization of cellular striatal DISC1 aggregates in the tgDISC1 rat.** (**a**), (**b**) Costaining of ThS with α-DISC1 (3D4) antibody shows no overlap of DISC1 aggregates with Thioflavin S signals in the striatal sections of adult (a) and aged (b) tgDISC1 animals. (**c**) Costaining of ThS with stereotactically injected recombinant DISC1 fragment (598-785 labeled with Dylight598) reveals a strong overlap of both signals in cytoplasmatic perinuclear aggregates in cells in close proximity to the injection site. Bar 10 µm.

*Methods:*

*ThS staining and immunohistochemistry of tgDISC1 and control rat section.*

For the characterization of the neuronal, striatal intracellular DISC1 aggregates in sections of the transgenic rats, animals were perfused with PBS pH 7.4 and brains were bisected along their midline. Prepared brains were stored at -40°C. Sections were cut, blocked and incubated with mAB 3D4 as described in main paper with brief modifications. In short, frozen sagittal sections were dried for 20 min at RT. The sections were post-fixed with ice-cold 4% paraformaldehyde solution buffered with PBS pH 7.4 (PFA, Sigma-Aldrich, MO, USA). After blocking with antibody diluent (Dako, Hamburg, Germany) sections were incubated with the mAb 3D4 for 16 h at 4°C in antibody diluent. After two washes with PBS plus 0.05% Tween-20 (PBS-T) the secondary antibody (anti-mouse IgG AlexaFluor594 1:300; Invitrogen, CA, USA) was applied for 1 h at RT. Sections were washed 3x in PBS-T for 10 min.

Subsequently, sections were incubated for 8 min in 0.05% Thioflavin S in 50% ethanol at RT, washed shortly in distilled water and incubated 2x 5 min in Sudan Black (Division Chroma, Münster, Germany) in 70% ethanol to block autofluorescence. Finally, sections were washed briefly in 70% ethanol and $H_2O$ and mounted with ProLong Gold with DAPI (Invitrogen, CA, USA). For the Dylight598 labelled recombinant DISC1 (598-785) injected control animals[6] the same protocol lacking the immunohistochemical staining was applied. All images were collected with a Zeiss Axiovision Apotome.2 confocal microscope (Zeiss, Oberkochen, Germany).

**Supplementary Figure S5 Amphetamine supersensitivity and hyperexploration phenotypes of the tgDISC1 rats are stable during aging.** (**a**) Locomotor response to 0.2 mg/kg amphetamine in aged tgDISC1 rats. Compared to the saline trial, tgDISC1 rats showed increased locomotion at the periphery of the open field after amphetamine treatment (*p = 0.011), whereas negative controls did not. (**b**) Increased frequency of center entry in tgDISC1 rats after amphetamine treatment. TgDISC1 rats displayed enhanced transits into and out of the center of the open field after amphetamine treatment compared to controls ($^{\#}$p = 0.027). Compared to the saline trial only the tgDISC1 rats reacted with an increased frequency of center entry after amphetamine treatment (*p = 0.033). (**c**) Hyperexploration of the stationary object in OPR test trial in aged tgDISC1 rats. When exploration of the stationary and the displaced object in the test trial was compared, tgDISC1 rats explored the stationary object significantly longer ($^{\#}$p = 0.051), with a trend also for the displaced object (p = 0.068). (**d**) Discrimination index of object place recognition (OPR) task in aged rats. Both groups performed at chance level, meaning that spatial memory is not intact in both aged animal groups. All means ± SEM.

*Methods:*

*Amphetamine challenge of aged rats.*

Aged animals were tested at an age of 20-21 months. Rats underwent the same amphetamine challenge as described in the main paper with the difference that 0.2 mg/kg d-amphetamine were used (NC n = 9, TG n = 11).

Analysis of the total distance moved at the margin by a two-way ANOVA gave a main effect for treatment ($F_{1, 18}$ = 8.298, p = 0.010), but not for genotype and a subsequent paired t-test showed increased margin distance moved for the tgDISC1 rats (p = 0.011), but not the controls (p = 0.466) after amphetamine treatment.

Another two-way ANOVA was performed to analyze the frequency of center entries between the groups. Main effects for genotype ($F_{1, 18}$ = 4.619, p = 0.045) and a strong trend for treatment ($F_{1, 18}$ = 4.229, p = 0.055) were shown. A subsequent one-way ANOVA emphasized a genotype difference in center entries upon amphetamine treatment (p = 0.027). A paired t-test revealed that the tgDISC1 rats had an increased frequency of center entries after amphetamine treatment (p = 0.033), whereas the negative controls had not (p = 0.934).

*Hyperexploration in OPR task in aged rats.*

OPR in aged tgDISC1 and negative control rats was performed as described in [7] (data of "control" condition). The data of negative control animals described here was part of Trossbach et al.[7] and was for this publication compared to data of tgDISC1 rats, including statistical re-analysis with genotype as main factor (NC n = 11, TG n = 10).

For the comparison of exploration of the stationary and the displaced object in the test phase, a two-way ANOVA revealed main effects for genotype ($F_{1, 19}$ = 5.710, p = 0.027), but not object ($F_{1, 19}$ = 0.188, p = 0.669). Subsequent one-way ANOVAs showed a genotype effect for exploration of the stationary object (p = 0.051), and a trend for the displaced object (p = 0.068). One-sample t-test against chance level at 0.0 revealed deficiency in object

discrimination for both genotypes (NC p = 0.778, TG p = 0.949). These results indicate that hyperexploration in the tgDISC1 rat is a behavioral trait that is stable during aging. Exploring the less interesting object reflects deficient discrimination between objects of the aged tgDISC1 rats probably due to age-dependent cognitive decline.

**Supplementary Figure S6 Validation of behavioral phenotypes in an independent founder line of the tgDISC1 rat (founder line 1).** (**a**) Amphetamine hypersensitivity in the tgDISC1 rat shown by distance moved. Total distance moved in animals treated with saline (white bars) and d-amphetamine (0.5 mg/kg i.p.; grey bars) is presented. Whereas the d-amphetamine had no significant effect on locomotion in the negative control animals (p = 0.140; n = 12), it led to increased distance moved in the tgDISC1 rats of founder line 1 (Fo.1 / TG1) validating a hypersensitivity to d-amphetamine (*p = 0.033, n = 12). (**b**) Amphetamine hypersensitivity in the tgDISC1 rat shown by center distance. The effect of d-amphetamine on locomotion of tgDISC1 rats was even stronger when the distance moved in the center of the arena was analyzed (TG1 *p = 0.022, NC p = 0.500). (**c**) The Rotarod task as measure of motor learning ability and attention. Negative control animals learned to walk on the constantly rotating wheel, whereas tgDISC1 rats of founder line 1 did not (*p = 0.046, n = 12). (**d**) Discrimination indices of the object recognition (OR) and object place recognition task (OPR). Both, negative controls and tgDISC1 rats of founder line 1 were able to distinguish objects in the OR task (left panel) and the OPR task (right panel) as shown by discrimination indices above chance level at 0.0. (**e**) More exploration of tgDISC1 rats in OR task. In contrast to negative control rats, the tgDISC1 explored the new object more the old one in the test trial (NC p = 0.098, TG1 ***p < 0.001, n = 11), although no genotype difference in exploration of the new object could be detected. (**f**) More exploration of the tgDISC1 rats in the OPR task. Also in the spatial object memory task

OPR the tgDISC1 rats of founder line 1 explored the displaced object longer than the stationary one (NC p = 0.069, TG1 *p = 0.027, n = 12). All means ± SEM.

*Methods:*

*Amphetamine challenge of tgDISC1 rats of founder line 1.*

The Amphetamine challenge was performed as described in the main paper. A two-way ANOVA analysis of the total distance moved of negative controls and tgDISC1 rats of a different founder line (Fo.1 / TG1) revealed a main effect for treatment ($F_{1, 22}$ = 4.852, p = 0.038), but not for genotype. A subsequent one-tailed paired test showed differences for tgDISC1 rats (*p = 0.033), but not for negative controls (p = 0.140). When the center distance was analyzed the two-way ANOVA showed again a main effect for trial ($F_{1, 22}$ = 6.410, p = 0.019). The paired t-test displayed a difference of saline to amphetamine trial for tgDISC1 rats (*p = 0.022) and not for negative controls (p = 0.500).

*Rotarod*.

A two-way ANOVA comparing the seconds spent on the rotarod without falling off revealed main effects for trial ($F_{2, 36}$ = 12.588, p < 0.001) and genotype-trial interaction ($F_{2, 36}$ = 12.588, p =0.040). A U-test showed a genotype difference in trial 3 (p = 0.046), arguing that the tgDISC1 rats have a deficit in the rotarod task.

*Hyperexploratory behavior in tgDISC1 rats of founder line 1.*

*Discrimination indices.* The discrimination index for the novelty preference tasks is calculated as follows: OR [time spent exploring the new object – time spent exploring the old object]/[total time spent exploring the two objects]; OPR [time spent exploring the object at novel location – time spent exploring the object at old location]/[total time spent exploring the two objects]; ORTO [time spent exploring the old familiar object – time spent exploring the recent familiar object]/[total time spent exploring the two objects]. The mean of this index

value has to be significantly different from chance level at zero value 0.0, animals with a discrimination index >0 can distinguish objects, performance <0 shows a deficit in object discrimination.

Calculation of the discrimination indices and one-sample t-tests against chance level at zero value showed that negative control and tgDISC1 rats of founder line 1 were able to distinguish the respective objects in the OR task (NC $p = 0.058$, TG1 $p < 0.001$; $n = 11$ each) as well as the OPR task (NC $p = 0.033$, TG1 $p = 0.009$; $n = 12$ each). When duration of object exploration in the test trial was analyzed, the two-way ANOVA showed main effects for object in the OR ($F_{1, 20} = 22.934$, $p < 0.001$) and the OPR task ($F_{1, 22} = 6.410$, $p = 0.019$). One-way ANOVAs did not give genotype differences in the exploration of either object (old $p = 0.130$, new $p = 0.459$, stationary $p = 0.172$, displaced $p = 0.161$).

Subsequent paired t-tests comparing the duration of exploration of the new/displaced and the old/stationary object revealed increased exploration of the new/displaced object in tgDISC1 rats (OR: $p < 0.001$, OPR: $p = 0.027$), but not in negative control rats (OR: $p = 0.098$, OPR: $p = 0.069$), showing that tgDISC1 rats explored the interesting object more extensively as their respective controls.

**Supplementary Figure S7 Memory function in the tgDISC1 rat.** (**a**) Discrimination index of the object recognition (OR) task. Negative controls and tgDISC1 rats performed above chance level at 0.0, showing that both genotypes have intact object memory (NC *$p = 0.020$, TG ***$p < 0.001$). TgDISC1 rats explored the new object even more than negative controls (**$p = 0.005$), resulting in a higher index. (**b**) Discrimination index of the object place recognition (OPR) task. Both groups had intact spatial memory, as negative controls and tgDISC1 rats have a discrimination index above chance level (NC *$p = 0.020$, TG ***$p < 0.001$). (**c**) Discrimination index of the object recognition for temporal order (ORTO) task. Like in the other novelty preference tasks, both animal groups performed above chance level

(NC *p = 0.011, TG ***p < 0.001). (**d**) No hyperexploration in the ORTO test trial. In the test trial, negative controls and tgDISC1 rats both explored the old familiar object more than the recent familiar one (NC *p = 0.045, TG *p = 0.019). No differences between the genotypes emerged. All means ± SEM.

*Methods:*

*Novelty preference tasks*.

Object recognition (OR) and object place recognition (OPR) experiments were performed as described in the main paper. The object recognition for temporal order test (ORTO) consists of three trials of 5 min separated by 25 min inter trial intervals, sample trial 1, 2, and the final test trial. In the first sample trial animals were presented with two similar objects at distinct positions in the arena. In the second sample trial animals were allowed to explore two equal novel objects located at the same position as in the first sample trial. In the test trial animals were confronted with one object from the first (old familiar) and one from the second sample trial (recent familiar). Positions of objects were identical in all three trials. Memory formation is intact if the animals spent more time at the old familiar than the recent familiar object. Animals that failed to explore an object in one of the trials were excluded from the analysis (OR and ORTO: NC: n = 10, TG: n = 12; OPR: NC: n = 8, TG: n = 12).

Novelty preference tasks for memory function in tgDISC1 rats revealed no memory deficits for the tgDISC1 rat. TgDISC1 rats and negative controls performed above chance level in all three memory tasks, the OR task (distinguishing between two distinct objects old and new in the test trial), the OPR (recognition of spatial displacement of an object, the displaced object in contrast to the stationary one in the test trial) and the ORTO (distinguishing of an old familiar object of first sample trial from the recent familiar object of second sample trial in the third test trial). Animals were able to distinguish between the novel/repositioned/old familiar object and the old/stationary/recent familiar object as indicated by the discrimination index

calculated for each genotype in the three novelty preference tasks (one-sample t-test against chance level at 0.0; OR: NC p = 0.020, TG p < 0.001; OPR: NC p = 0.020, TG p < 0.001; ORTO: NC p = 0.011, TG p < 0.001). One-way ANOVAs detected genotype differences only in the OR task (p = 0.005), but not in OPR (p = 0.762) or ORTO (p = 0.189).

*Object recognition for temporal order*. In contrast to the OR and OPR task, the analysis of the test trial by a two-way ANOVA did not show main effects in genotype ($F_{1, 20}$ = 1.390, p = 0.252), but in object exploration ($F_{1, 20}$ = 11.212, p = 0.003). Paired t-tests revealed differences for both genotypes in exploration of the recent and old familiar object: NC p = 0.045, TG p = 0.019.

**Supplementary Figure S8 Dopamine homeostasis in the dStr of the tgDISC1 rat.** (**a**) Dopamine content of different brain regions determined by *post-mortem* neurochemistry. TgDISC1 rats had less total dopamine content in the dStr, the amygdala (AM), and the hippocampus (Hi), whereas no changes were detected in the mPFC, nucleus accumbens (NAc) and cerebellum (CE). Values are presented for tgDISC1 rats as percent of negative control rats. (**b**) Total dopamine values of (a) as pg per mg brain tissue. Depicted are total dopamine concentrations in the respective areas of (a). SEM = standard error of mean, SD = standard deviation, unpaired t-tests were conducted. (**c**) Receptor autoradiography data. No difference in receptor densities in the dStr of tgDISC1 rats or negative controls were found for receptor binding of [$^3$H]SCH23390, [$^3$H]AMPA, and [$^3$H]ketanserin specific for D1Rs, AMPARs, and 5-$HT_{2A}$-Rs, respectively. (**d**) No difference in substance P and encephalin mRNA levels in the dStr by *in-situ* hybridization. Neither in the dorsolateral (DLS, first panel) nor the dorsomedial (DMS; second panel) part of the dorsal striatum changes in Substance P mRNA, a correlate for activity of D1R-expressing medium spiny neurons (MSNs), could be detected in tgDISC1 rats and negative controls. Enkephalin

mRNA level as marker for activity of D2R-MSNs were also comparable in the DMS and DLS of tgDISC1 and negative control rats (third and fourth panel). (**e**) Peak dopamine release and clearance in the NAc of tgDISC1 rats. Cyclic voltammetry measurements of striatal slices revealed no difference in either the peak release (left panel) or clearance (right panel) of extracellular dopamine in tgDISC1 rats, standing in contrast to data obtained from the dStr (Figure 3d,e). (**f**) Reduced striatal DAT levels in tgDISC1 rats. Representative Western blot corresponding to Figure 3f. All means ± SEM.

*Methods:*

*Receptor autoradiography.*

Receptor autoradiography was performed as described in main paper. Additional information: For assessment of dopamine D1 receptors sections were incubated in 50 mM Tris-HCl (pH 7.4) containing NaCl (120 mM ), KCl (5 mM), $CaCl_2$ (2 mM), $MgCl_2$ (1 mM), and Mianserin (1 µM) for 20 min. Subsequent incubation with [$^3$H]SCH23390 (1.74 nM) or SKF83566 (1 µM) for 90 min at 22°C yielded total and non-specific binding. AMPA and serotonin 5-$HT_{2A}$ receptors were labelled with [$^3$H]AMPA (9.6 nM) and [$^3$H]ketanserin (1.18 nM) in 50 mM Tris-HCl (pH 7.4; 45 min at 22°C) containing 0.1% ascorbic acid and NaCl (150 mM), 50 mM Tris acetate (pH 7.2; 45 min at 4 °C) containing potassium thiocyanate (100 mM), or 170 mM Tris HCl (pH 7.7; 120 min at 22°C) using the displacers quisqualat (10 µM) and mianserin (10 µM), respectively.

Slices were washed at least twice in ice-cold buffer and rapidly rinsed in ice-cold distilled water, placed under a stream of dry air, and exposed to tritium-sensitive film sheets (Carestream, USA) for 15 weeks. Autoradiograms from film sheets were digitalized and processed with an image-analysis system (Zeiss, Germany). (NC, TG n = 10).

Unpaired t-tests revealed no significant differences. Dorsal striatum: [$^3$H]SCH23390 p = 0.900, [$^3$H]AMPA p = 0.114, [$^3$H]ketanserin p = 0.473. Nucleus accumbens: [$^3$H]raclopride p = 0.398, [$^3$H]SCH23390 p = 0.252, [$^3$H]AMPA p = 0.269, [$^3$H]ketanserin p = 0.129.

In-situ *hybridization of SP and ENK.*

The expression of substance P and enkephalin in the striatum was measured by *in situ* hybridization histochemistry, using oligonucleotide probes labeled with [$^{33}$P]-dATP.[8]

Unpaired t-tests did not show genotype differences for either region or peptide marker: DLS / substance P: p = 0.108, DMS / substance P: p = 0.299, DLS / enkephalin: p = 0.781, DMS / enkephalin: p = 0.308 (NC, TG n = 5).

**Supplementary Figure S9 Comparison of dopamine-induced DISC1 aggregation in SH-SY5Y cells expressing inducible full-length human DISC1 (S704, L607) or (C704, F607) variant.** (**a**) Characterization of dopamine-induced DISC1 (S704, L607) aggresomes. Split channels for DAPI (blue), vimentin (green), and DISC1 (red) are depicted for four treatment options (+ / - DISC1, + / - DA). Extended data correspond to Figure 4c. Bar 10 µm. (**b**) Characterization of dopamine-induced DISC1 (C704, F607) aggresomes. Double-staining shows that also DISC1 (C704, F607) aggresomes (red) are caged by vimentin (green). Bar 10 µm. (**c**) Appearance of DISC1 (C704, F607) HMW bands upon DA-treatment of cells. Western blot of the lysate (left panel) or insoluble fraction (right panel) of inducible SH-SY5Y cells expressing full-length DISC1 (C704, F607) are shown. Cells were treated with dopamine at indicated concentrations. HMW DISC1-immunoreactive bands appear (arrow) with increasing concentrations of DA.

**Supplementary Figure S10 Dopamine-mediated induction of cellular DISC1 (S704, L607) aggresomes.** (**a**) Quantitative determination of aggresome positive SH-SY5Y cells. Percentage of DISC1 aggresome positive cells increased over time upon incubation with 100 µM DA. Subsequent washout of the 100 µM DA led to disappearance of the

aggresomes within 24 h. (**b**) Colocalization of dopamine-mediated DISC1 aggregates with different aggresomal markers. The left panel shows a marked overlap in the perinuclear DISC1 signal (red) with γ-tubulin indicated by the yellow signals in the merged image in the lower left. There is no colocalization of DISC1 aggregates with the signals for HSP70 (middle left) and A11 (middle right) antibodies or ThT staining (right panel). (**c**) Colocalization of dopamine-mediated DISC1 aggregates with γ-tubulin. Upon dopamine treatment (lower panels), DISC1 forms aggresomes that colocalizes with γ-tubulin. Without dopamine treatment (upper panels) DISC1 is dispersed throughout the cell and γ-tubulin staining looks normal. Bar = 10 μm.

*Methods:*

*Immunocytochemistry with aggresomal markers.*

The following antibodies were used for ICC: HSP70 (rabbit, 1:100; Santa Cruz Biotechnology, Dallas, TX, USA), γ-tubulin (mouse, 1:1 000; Sigma-Aldrich, MO, USA), A11 (rabbit, 1:5 000; Millipore, Temecula, CA, USA), ThT (0.05%; Sigma-Aldrich, MO, USA).

**Supplemental Figure S11 DISC1 and DAT complex into a heterogenous protein assembly.** (**a**) Removal of *N*-glycosylation of DAT in cell lysates. In SH-SY5Y cells expressing DAT-V5 and induced for DISC1 (S704, L607) treatment of cell lysates with the de-*N*-glycosylating enzyme PNGase F led to a shift in molecular size of DAT. The glycosylated DAT immunoreactive band of ~80 kDa was condensed to a 50 kDa band[9]. (**b**) Co-immunoprecipitation of DISC1 by DAT in cell lysates after preclearing by ultracentrifugation in a sarkosyl-containing buffer (see below). In cell lysates of SH-SY5Y cells expressing DAT-V5 and DISC1 (S704, L607), DISC1 got immunoprecipitated by DAT after preclearing the input by ultracentrifugation to deplete putative traces of membranes. Treatment of the magnetic bead-bound proteins and the input lysate with PNGase F (+)

deglycosylated DAT and reduced its molecular size as depicted in (a). Upper two panels show DISC1 signal at two different exposure times, lower panel DAT signal. (**c**) Solubility of human DISC1 derived from striatal membrane preparations in RIPA buffer. Human DISC1 purified from striatal membrane preparations of tgDISC1 rats dissolved in RIPA buffer were pelleted upon ultracentrifugation (+) at 100 000 x *g*. DAT, as well as Na/K-ATPase (as a membrane marker protein) remained soluble in the post-spin supernatant with only a minor portion in the pellet. Nearly all DISC1 was pelleted after ultracentrifugation and hence not available for co-immunoprecipitation with DAT. Usage of sarkosyl-containing buffer in striatal membrane preparations as in (b) was unsuccessful due to too low concentrations of DISC1 available (data not shown). This indicates (see Figure 5) that primarily the RIPA-insoluble DISC1 *multimers* seem to interact with DAT. (**d**) No residual membrane fragments detectable after dissolving striatal membrane preparations in RIPA buffer. To rule out the possibility that the co-IP of DISC1 by DAT in striatal membrane preparations resulted from putative membrane fragments still present after solubilizing the membranes in RIPA buffer, a membrane floating experiment was performed to test for residual membrane traces. When striatal membrane preparations were suspended in RIPA based buffer *without* detergents (left side), membranes left the initial high sucrose fraction and were concentrated in the 1.3 M / 0.85 M sucrose interphase, as expected (positive control). One fiftieth of this interphase was loaded onto the gel and contained Na/K-ATPase, DISC1 and DAT. However, when striatal membrane preparations were dissolved in RIPA *with detergents* (right side) and underwent the same protocol, no residual membranes could be detected in the 1.3 M / 0.85 M sucrose interphase, and here the *complete* membrane interphase was loaded onto the gel. All DISC1, DAT and Na/K-ATPase stayed in the initial 1.3 M sucrose fraction. This demonstrates that preclearing by ultracentrifugation is not necessary to remove (residual) membranes from the immunoprecipitation material, and that the DISC1-DAT complex is not artifactual by containing residual membrane fractions with DAT not directly or indirectly complexed to DISC1.

*Methods:*

*Co-immunoprecipitation (co-IP) of DISC1 and DAT from cells.*

SH-SY5Y neuroblastoma cells stably transfected with inducible full-length DISC1 (S704, L607) and constitutive DAT-V5 (pLHCX-DAT-V5) were induced with 1 µg/mL doxycycline for 24 h. Cells were lyzed in RIPA buffer (50 mM Tris pH 7.6, 150 mM NaCl, 1% NP-40, 0.5% DOC, 0.1% SDS; supplemented with 30 mM $MgCl_2$, 5 mM $CaCl_2$, 40 U/mL DNaseI, 0.5 mM DTT, protease inhibitor) and DNA was digested for 30 min at 37°C. Co-IP was performed as described in main paper for striatal plasma membrane preparations.

*PNGase F treatment.*

The same SH-SY5Y neuroblastoma cells expressing DISC1 and DAT were lyzed in RIPA buffer supplemented with 30 mM $MgCl_2$, 5 mM $CaCl_2$, 40 U/mL DNaseI, 0.5 mM DTT. After DNA digestion for 30 min at 37°C 15 µg of total protein lysate were used for the PNGase F treatment according to manufacturer's instructions (New England Biolabs, Ipswich, MA, USA) to remove *N*-glycosylation of DAT[9] and obtain a dense band to verify the antibody signal. In short, 0.5x Denaturation Buffer, 1x G7 buffer, 1% NP-40, as well as 1 000 units PNGase F or water were added to the lysate in a final volume of 20 µL and incubated for 3 h at 37°C before Western blotting. The shift in DAT size was visualized with the MAB369 antibody (Merck Millipore; Darmstadt, Germany).

*Co-IP of DISC1 and DAT from cells with preclearing.*

The SH-SY5Y neuroblastoma cells expressing DISC1 and DAT were lyzed in VRL buffer (50 mM HEPES pH7.4, 250 mM sucrose, 100 mM KAc, supplemented with 0.2% sarcosyl, 30 mM $MgCl_2$, 5 mM $CaCl_2$, 40 U/mL DNaseI, 0.5 mM DTT, protease inhibitor / PI) were incubated for 30 min at 37°C. The samples were precleared by ultracentrifugation (100 000 x

*g*, 15 min) in the MLS-50 rotor in an Optima ultracentrifuge (Beckman Coulter, Krefeld, Germany). Afterwards the lysates were supplemented with 1% TX100 and used for the subsequent co-IP.

The cell lysates were equally divided into two samples. One sample did not receive an antibody for capturing, the other one was mixed with 3 µg of a polyclonal antibody raised against DAT (AB2231, Merck Millipore; Darmstadt, Germany) in duplicate and incubated for 2 h at 4°C, before addition of 15 µL of Protein A/G magnetic beads (Pierce, Rockford, IL, USA) and further incubation for 16 h at 4°C. The beads were washed 3x with 1 mL of VRL plus 0.5 mM DTT, 0.2% sarcosyl and 1% TX-100. One IP reaction as well as 15 µg of cell lysate were digested with PNGase F to remove *N*-linked glycosylation of DAT as described above.

Proteins were eluted by addition of loading buffer containing 2% β-mercaptoethanol for 10 min without boiling and were used for Western blot. Membranes were incubated with the antibodies 14F2 (detecting huDISC1) and MAB369 antibody (detecting DAT; Merck Millipore; Darmstadt, Germany). In PNGase F treated samples a shift in molecular weight of DAT can be detected.

*Solubility assay.*

Striatal membrane preparations were solubilized in RIPA buffer supplemented with 0.5 mM DTT and PI and were centrifuged at 100 000 x *g* for 15 min at 4°C. The pre-spin input as well as the post-spin supernatant and pellet underwent Western blotting and were incubated with antibodies against DISC1 (14F2; Korth Lab), DAT (MAB369; Merck Millipore, Germany) and Na/K-ATPase (Cell Signaling, Danvers, MA, USA).

*Floating membrane assay.*

To investigate whether residual membranes are present in RIPA dissolved striatal membrane preparations that could lead to false positive results in the co-IP experiments membranes

equalling one rat striatum were dissolved in either RIPA buffer with detergents (50 mM Tris pH 7.6, 150 mM NaCl, 1% NP-40, 0.5% DOC, 0.1% SDS plus 0.5 mM DTT and protease inhibitor) or 50 mM Tris pH 7.6, 150 mM NaCl, supplemented with 0.5 mM DTT and protease inhibitor (RIPA without detergents). Afterwards the same buffers containing 2 M sucrose were added to reach a final sucrose concentration of 1.3 M in a volume of 500 µL and put at the bottom of a discontinuous sucrose gradient. The other two layers contained 0.85 M sucrose (1 mL) and no sucrose (3 mL), all dissolved in either RIPA or Tris/NaCl as described above.

The gradient was centrifuged for 2 h at 100 000 x *g* and 4°C (Optima ultracentrifuge, Beckman, Germany). Samples from all layers, as well as the interphases between the 1.3 M / 0.85 M and 0.85 M / 0 M sucrose layers were collected. The membrane containing 1.3 M / 0.85 M interphase was diluted in 3 volumes of 50 mM Tris pH 7.6, 150 mM NaCl, 0.5 mM DTT and PI and pelleted by ultracentrifugation (100 000 x *g*, 4°C, 15 min). The resulting membrane pellet was dissolved in 10 µL of 2x loading buffer containing β-mercaptoethanol in the detergent condition and in 500 µL of the loading buffer in the non-detergent condition. Whereas the complete membrane fraction (10 µL) of the detergent condition was loaded, only 1/50 (10 µL) of the non-detergent condition was loaded onto the SDS gel. Samples from all layers, an input sample taken before the spin and the 10µL of the pellet dissolved in 50 µL of loading buffer were also loaded for both conditions.

Membranes were stained for DISC1 (14F2), Na/K-ATPase (Cell Signaling) and DAT (MAB369; Merck Millipore).

**Supplementary Figure S12 Evidence for an *in situ* DISC1 / DAT complex *in vitro* and *in vivo*.** (**a**) Co-localization of DAT with the periphery of DISC1 aggresomes by confocal microscopy. DISC1 (S704, L607) induced SH-SY5Y cells stably transfected with DAT-V5 were treated with 100 µM DA for 24 h. In the cytoplasm, peripheral non-aggregated DISC1

(FFD5; green) overlaps with DAT (V5-tag; red). The main perinuclear DISC1 aggresome weakly colocalizes with DAT exclusively at the outer limit of the protein aggregate, as expected, as shown by confocal microscopy. Additional, diffuse co-localization was detected throughout the cytoplasm. For colocalization, the Zeiss ZEN 2012 colocalization module software was used. The fluorescence intensity cut-off was set to a medium level in both channels, overlapping signals are colored in yellow, strong colocalization is pseudo-colored in blue (merge; lower right panel). Nuclei were stained with DAPI (blue). Bar 20 µm. (**b**) Immunofluorescence detection of DISC1 and DAT interaction by Proximity Ligation Assay (PLA) *in vitro*. PLA signals (green), DAPI staining (blue) and merged PLA/DAPI (right panel) of non-induced SH-SY5Y tet-on flDISC1 cells constitutively expressing DAT-V5 (lower panel) or of DISC1 (S704, L607) induced cells (upper panel) are shown. PLA signals were virtually absent in uninduced cells but were strongly increased after induction of DISC1 expression indicating an *in situ* interaction between DISC1 and DAT *in vitro*. Bar 10 µm. Note that the positive signals of the PLA are not necessarily an exact reflection of their cellular localization but rather a reflection of the likelihood of interaction.[10] (**c**) Detection of DISC1 and DAT interaction by PLA in striatal brain sections of tgDISC1 rats and negative controls. The figure shows merged signals of PLA signals (green), DAPI staining (blue) on striatal brain sections of tgDISC1 rats (top row) and control animals (bottom row). In contrast to negative control animals, strong PLA signals were only detected in tgDISC1 rats using primary antibodies against DISC1 and DAT (left panel, first column, yellow frame) indicating an *in situ* interaction between DISC1 and DAT *in vivo*. As a positive control, strong PLA signal were also present when detecting DISC1 protein with two different antibodies from mouse and rabbit origin (right panel, fifth column, purple frame). Only very few background signals were present in control experiments with only one of the primary antibodies being used (second and third column) or without any primary antibody (fourth column). As additional control an antibody against TRIOBP1,[11] a non-DISC1 interacting protein (right section, sixth column), was used. Note that the positive signals of the PLA are not necessarily an exact reflection of their

cellular localization but rather a reflection of the likelihood of interaction.[10] A very weak diffuse non-specific background PLA signal was detected in controls, unrelated to the presence of primary or secondary antibodies, and clearly minute compared to true interactions (yellow and purple framed slides). Bar 10 µm.

*Methods:*

*Proximity Ligation Assay.*

The Duolink *in situ* Proximity Ligation assay (PLA) is capable of detecting protein-protein interactions in cell and tissue samples.[10] In this method two primary antibodies, raised in different species that are binding the two interacting proteins, are recognized by oligonucleotide labelled secondary antibodies (PLA probes). Oligonucleotides of PLA probes that are in near proximity to each other are hybridized with two detection oligonucleotides, which are then ligated into a closed circle. Following amplification by rolling cycle amplification the concatemeric product is hybridized with fluorescent labelled probes to visualize the protein-protein interaction *in situ* by fluorescence microscopy.

PLAs were carried out using the Duolink PLA probe anti-mouse PLUS and anti-rabbit MINUS and the Duolink InSitu Detection Reagent Green kit (Sigma-Aldrich, MO, USA) according to manufacturer's recommendations. The following antibodies and concentrations were used:

α-DISC1 mAB "3D4" affinity purified 1:50, α-DISC1 "FFD5" polyclonal affinity purified 1:50, α-DAT 1:500 (AB2231, Merck Millipore, Germany), α-TRIOBP 1:500 (HPA019769, Sigma-Aldrich, MO, USA)

SH-SY5Y cells inducible for DISC1 (S704, L607) and constitutively expressing V5-DAT were used to demonstrate an interaction between DISC1 and DAT *in vitro*. DISC1 expression was induced with 1 µg/mL doxycycline for 24 h. Cells were fixed in 4% paraformaldehyde (PFA), permeabilized and fixed with PBS supplemented with 5% skimmed milk powder, 0.5% saponine and 1% BSA. Cryosections (8 µm) of striatum of tgDISC1 brains and negative control animals were dried for 30 min at 37°C, post fixed with 4% PFA for 10 min and

blocked with antibody diluent (Dako, Hamburg, Germany) for 1 h at RT. All samples were incubated with the respective primary antibodies diluted in antibody diluent for 16 h at 4°C. Afterwards the samples were washed with PBS followed by incubation with a 1:5 dilution of the PLA probes for 1 h at 37°C in a preheated humidity chamber. After washing with Duolink wash buffer A, samples were incubated with Duolink Ligation-Ligase solution for 30 min at 37°C, before washing again in buffer A and incubation with Duolink Amplification-Polymerase solution for 100 min at 37°C in the dark. Finally, samples were washed in Duolink wash buffer B, mounted with ProLong Gold with DAPI (Invitrogen, CA, USA) and analyzed with a Zeiss Apotome microscope and ZEN2012 software (Zeiss, Oberkochen, Germany).

**Supplementary Figure S13 Influence of DISC1 (S704, L607) and DA on DAT solubility.** (**a**) Recruitment of DAT by DISC1 aggregates in SH-SY5Y cells. Densitometric analysis of DAT Western blot band intensity was performed in lysates (left panel) or insoluble fractions (right panel) of DISC1 (S704, L607) induced SH-SY5Y cells treated with 100 µM DA. In the lysates no significant differences were detectable in DAT immunoreactivity (sc-14002, Santa Cruz, USA) with or without DISC1 expression or induction of DISC1 aggregates by prior peak dopamine exposure (100 µM; Kruskal-Wallis, $p = 0.200$). However, in the insoluble fraction DISC1 aggregation by dopamine treatment pulled down DAT indicating recruitment of DAT by insoluble DISC1 (Kruskal Wallis test with post-hoc Dunnett). For both panels, columns represent means ± SEM of three replicate experiments. (**b**) Representative Western blot corresponding to (a). GAPDH signal is shown as loading control of equal lysate input for the insoluble proteome preparation.

**Supplementary Figure S14 Dopamine-mediated induction of a distinct high molecular weight (HMW) DISC1 band.** (**a**) Western blot of the lysate (upper panel) and

insoluble fraction (lower panel) of negative control or tgDISC1 primary cortical rat neurons, treated with 0 or 50 µM DA. High molecular weight (HMW) DISC1-immunoreactive bands corresponding to aggregated DISC1 appear after DA treatment (arrow; lower panel). To show equal input of protein for the preparation of the insoluble fraction, actin was used as loading control in the lysate fraction. (**b**) Mass spectrometry based identification data of the high-molecular weight band of DISC1. NLF cells were transiently transfected with full length DISC1 (S704, L607) tagged with a C-terminal His-Tag and treated with 100 µM DA for 24 h. The insoluble Ni-NTA affinity purified fraction was separated by PAGE and the 180 kDa band was analyzed by tandem mass spectrometry. In two independent experiments DISC1 (Q9NRI5) was under the two most abundant (as revealed by ion intensity per MW) identified proteins. A total of 49 unique DISC1 peptides were found leading to a sequence coverage of 51.6% (28 unique peptides and 42.4% coverage in the second experiment). Two exemplified spectra of identified DISC1 peptides are shown in the left panel.

*Methods:*

*Analysis of DISC1 by liquid chromatography (LC)-electrospray ionization (ESI)-MS/MS*

DISC1 containing proteins lysates were separated by polyacrylamide gels. Relevant bands from silver stained gels were cut out, destained, reduced, alkylated with iodoacetamide as described[12] and digested in 50 mM $NH_4HCO_3$ with with 0.05 µg trypsin (Serva, Heidelberg, Germany) or 0.05 µg GluC (Promega Mannheim, Germany).

For LC-MS/MS analysis, peptides were extracted from the gel with 1:1 (v/v) 0.1% trifluoro acetic acid / acetonitrile. After removal of acetonitrile, digested peptides were subjected to liquid chromatography. For peptide separation, an Ultimate 3000 Rapid Separation liquid chromatography system (Thermo Scientific, Dreieich, Germany) was used. After a pre-concentration of peptides on an Acclaim PepMap100 trap column (3 µm C18 particle size, 100 Å pore size, 75 µm inner diameter, 2 cm length, Dionex / Thermo Scientific, Dreieich, Germany) at a flow rate of 6 µl / min using 0.1% (v/v) TFA as mobile phase for 10 min, the

peptides were separated on an analytical column (Acclaim PepMapRSLC, 2 µm C18 particle size, 100 Å pore size, 75 µm inner diameter, 25 cm length, Dionex / Thermo Scientific, Dreieich, Germany) at 60°C using a 54 gradient from 4 to 40% solvent B (solvent A: 0.1% (v/v) formic acid in water, solvent B: 0.1% (v/v) formic acid, 84 % (v/v) acetonitrile in water) at a flow rate of 300 nL / min.

For mass spectrometric measurements, peptides were injected via a nano electrospray ionization source into a LTQ-Orbitrap Elite high resolution mass spectrometer (Thermo Scientific, Bremen, Germany) operated in positive mode. Capillary temperature was set to 275°C and source voltage to 1.4 kV. The orbitrap analyzer of the instrument was used for survey scans over a mass range from 350-1 700 m/z at a resolution of 60 000 (at 400 m/z). The target value for the automatic gain control was 1 000 000 and the maximum fill time 200 ms. In a data dependent setting, the 15 most intense > 2+ charged peptide ions (minimal signal intensity 500) were isolated and fragmented using collision induced dissociation (CID) in the linear ion trap (LTQ) part of the instrument. For the analysis of peptide fragments the available mass range was 200-2 000 m/z at a resolution of 5 400 (at 400 m/z), the maximal fill time was set to 300 ms and the automatic gain control target value to 10 000. Already fragmented ions were excluded from fragmentation for 45 sec.

Searches within the homo sapiens entries of the SwissProt part of the UniProt KB (downloaded at 2.2.2015, 20 200 homo sapiens entries) were carried out using MaxQuant version 1.5.0.30 (MPI for Biochemistry, Martinsried, Germany). Following parameters were applied: mass tolerance precursor (Orbitrap): 20 ppm fist search, 4.5 ppm second search, mass tolerance fragment spectra (linear ion trap): 0.5 Da, fixed modification: carbamidomethyl at cysteines, variable modifications: methionine oxidation and protein n-terminal acetylation, cleavage specificity: trypsin with a maximum of two missed cleavage sites. The pre-set values were used for the other options, a false discovery rate of 1% was used on both peptide and protein level for peptide respectively protein identification.

**Supplementary Figure S15** No influence of dopamine receptor antagonists on DA-induced aggregation in SH-SY5Y cells. (**a**) Western blot of SH-SY5Y cell lysates (upper panels) with doxycycline-induced DISC1 (S704, L607) expression, incubated with 100 µM DA for 24 h to induce transient aggregates (lower panel) in the presence of 10 µM haloperidol (D2 receptor antagonist) or 10 µM SCH23390 (D1 receptor antagonist). Upon dopamine treatment, HMW DISC1 bands (arrow) appeared in the insoluble fraction. GAPDH was used as loading control for the lysate input (middle panel). (**b**) Quantitative determination of aggresome positive SH-SY5Y cells. Percentage of DISC1 aggresome positive cells increased over time upon incubation with 100 µM DA, the parallel incubation with specific D1R and D2R antagonists did not decrease the total number of cells with DISC1 aggregates. (**c**) D1R and D2R expression in SH-SY5Y cells as a control for the lack of DA antagonist effects. Western blot of DISC1-inducible SH-SY5Y cells, uninduced (-) or induced (+) and control striatum homogenate of a wild type rat brain showed weak receptor expression by a rabbit α-D1R (left panel; #sc14001, Santa Cruz Antibodies, USA) or rabbit α-D2R antibody (right panel; #AB1558, Abcam, UK). DA receptors exist in glycosylated and unglycosylated forms, as indicated. All means ± SEM.

**Supplementary Figure S16** **Influence of DAT inhibitor on HMW DISC1 (S704, L607).** (**a**) Representative Western Blot of the insoluble fraction of SH-SY5Y cells expressing full length DISC1. Treatment with the DAT inhibitor GBR12909 decreased the formation of HMW DISC1 in the insoluble fraction. (**b**) Densitometric quantification of the HMW DISC1 band of (a). The HMW DISC1 Western Blot signal can be induced by prior treatment of DISC1 induced SH-SY5Y cells with 100 µM dopamine for 24 h (one-way ANOVA with post-hoc Bonferroni $p < 0.001$). This effect is significantly decreased when the cells were coincubated with 20 nM GBR12909 ($^*p = 0.014$).

**Supplementary Figure S17 Amphetamine sensitization and its influence on endogenous DISC1 aggregation.** (**a**) Locomotor response of amphetamine-sensitized and control animals (saline treatment) after a low-dose amphetamine challenge. Amphetamine-sensitized animals reacted more profoundly to the 0.5 mg/kg amphetamine dose as measured by distance moved in the open field (***p < 0.001) (**b**) Inverse correlation between insoluble Disc1 levels in striatum and locomotor activity in an open-field test performed with d-amphetamine sensitized rats (open squares) or controls (black dots). Decreased striatal aggregated Disc1 levels are directly correlated to the behavioral phenotype of hyperlocomotion (Pearson correlation coefficient r = -0.654, **p = 0.002). (**c**) Representative Western blot of the purified, insoluble fraction of the mPFC and dStr of amphetamine-sensitized animals (n = 10) and vehicle-treated controls (dStr: n = 9; mPFC: n = 10). Rat brain homogenates from mPFC (top) and dorsal striatum (bottom) under baseline control conditions (saline; right side) and after d-amphetamine sensitization (left side) are shown. After d-amphetamine sensitization, endogenous HMW Disc1 species decreased in the insoluble fraction of the dStr, whereas no changes can be detected in the mPFC, concomitant with changes in dopamine content in the respective brain regions. (**d**) Densitometric quantitation of insoluble Disc1 HMW species in dStr and mPFC from d-amphetamine-sensitized rats or controls. In striatum depletion of dopamine during d-amphetamine sensitization lowers aggregated Disc1 (** p = 0.007), whereas sensitization tends to increase it in the mPFC (p = 0.288).

*Methods:*

*Amphetamine sensitization.*

To sensitize wild type Wister rats, animals were treated i.p. with 2 mg/kg d-amphetamine or its saline vehicle (1 mL/ kg) on five subsequent days. After 14 days during which the animals

were kept undisturbed, a test for behavioral sensitization of the locomotor response to amphetamine was conducted by measuring locomotor behavior induced by 0.5 mg/kg d-amphetamine an open field (as described in main paper). On the following day the animals in the sensitization group received an additional challenge of 2 mg/kg d-amphetamine while the control group was treated with saline. Brain dissection of mPFC and dStr was carried out 24 h following the last injection. Preparation of the insoluble fraction per performed as described in main paper. For the detection of endogenous ratDisc1, the #440 antibody was used.

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