## **Supplementary Information**



Supplementary Figure S1. Baseline levels of dopamine and DOPAC in 14-3-3 $\zeta$  KO mice. Baseline DA and DOPAC levels were measured in the cortex (a-c) and hippocampus (d) by HPLC/EC. (a) 14-3-3 $\zeta$  KO mice (white bar; n=6; 4 male and 2 female) have slightly increased DA compared to 14-3-3 $\zeta$  WT mice (closed bar; n=6; 3 male and 3 female) in the cortex. (b) DOPAC levels are similar between 14-3-3 $\zeta$  KO mice (white bar) and 14-3-3 $\zeta$  WT mice (closed bar) in the cortex. Dopamine turnover (DOPAC / DA ratio) is reduced in 14-3-3 $\zeta$  KO mice (white bar) compared to 14-3-3 $\zeta$  WT mice (closed bar). (d) 14-3-3 $\zeta$  KO mice (white bar; n=6; 4 male and 2 female) have slightly increased DA compared to 14-3-3 $\zeta$  WT mice (closed bar; n=6; 3 male and 3 female) in the hippocampus. No DOPAC was detected in the hippocampus using HPLC/EC.



Supplementary Figure S2. Isoform specificity of 14-3-3 $\zeta$  monoclonal antibodies. His tagged 14-3-3 isoforms were expressed in bacteria. Monoclonal 14-3-3 $\zeta$  antibodies (M-6, N-4, H-5, D1-4, G1-7, B-1, P-1 and E-8) were used for western blot analysis in comparison to the anti-14-3-3 $\zeta$  C-16 antibody (Santa Cruz). Anti-6XHis was used as a loading control.



Supplementary Figure S3. Normal expression of TH in 14-3-3 $\zeta$  KO mice. (a) Quantitation of anti-TH western blot normalised to anti-Alpha tubulin confirms that 14-3-3 $\zeta$  KO (open bar) mice have similar levels of TH compared to WT mice (closed bar). (b) Quantitation of anti-phospho Ser-31 TH western blot normalised to anti-Alpha tubulin confirms that 14-3-3 $\zeta$  KO (open bar) mice have similar levels of phospho Ser-31 TH compared to WT mice (closed bar). (c) Quantitation of anti-phospho Ser-40 TH western blot normalised to anti-Alpha tubulin confirms that 14-3-3 $\zeta$  KO (open bar) mice (closed bar). (c) Quantitation of anti-phospho Ser-40 TH western blot normalised to anti-Alpha tubulin confirms that 14-3-3 $\zeta$  KO (open bar) mice have similar levels of phospho Ser-40 TH compared to WT mice (closed bar).



Supplementary Figure S4. Normal expression of DA receptors in 14-3-3 $\zeta$  KO mice. Quantitation of anti-DA receptors D1-D5 western blot normalised to anti-Alpha tubulin confirms that 14-3-3 $\zeta$  KO (open bars) mice have similar levels of DA receptors compared to WT mice (closed bars).

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![](_page_4_Figure_1.jpeg)

Supplementary Figure S5. Expression of 14-3-3 $\zeta$  in dopaminergic neurons. (a) Sagittal brain sections stained with anti-TH (red) and anti-14-3-3 $\zeta$  M-6 (green) show 14-3-3 $\zeta$  expression in the VTA / SN of 14-3-3 $\zeta$  WT mice. Staining was not observed in 14-3-3 $\zeta$  KO sections. Scale bar = 100um. (b) 14-3-3 $\zeta$  is also expressed in dopaminergic terminals in the striatum. Scale bar = 200um.

![](_page_5_Figure_1.jpeg)

Supplementary Figure S6. 14-3-3 $\zeta$  associates with DAT. SN4741 cells were transiently transfected with Flag-His-DAT and Myc-14-3-3 $\zeta$ . DAT was precipitated from protein lysates with anti-Flag antibody and anti mouse IgG control antibodies. Immunoprecipitates were probed with anti-Myc monoclonal antibody to recognise 14-3-3 $\zeta$  and anti-Flag to recognise DAT. Note that 14-3-3 $\zeta$  and DAT are present in the input and Flag (DAT) immunoprecipitation (IP; arrow) but not in the control IgG IP.