Supplementary Figures, Methods and References.

Prion protein facilitates uptake of zinc into neuronal cells

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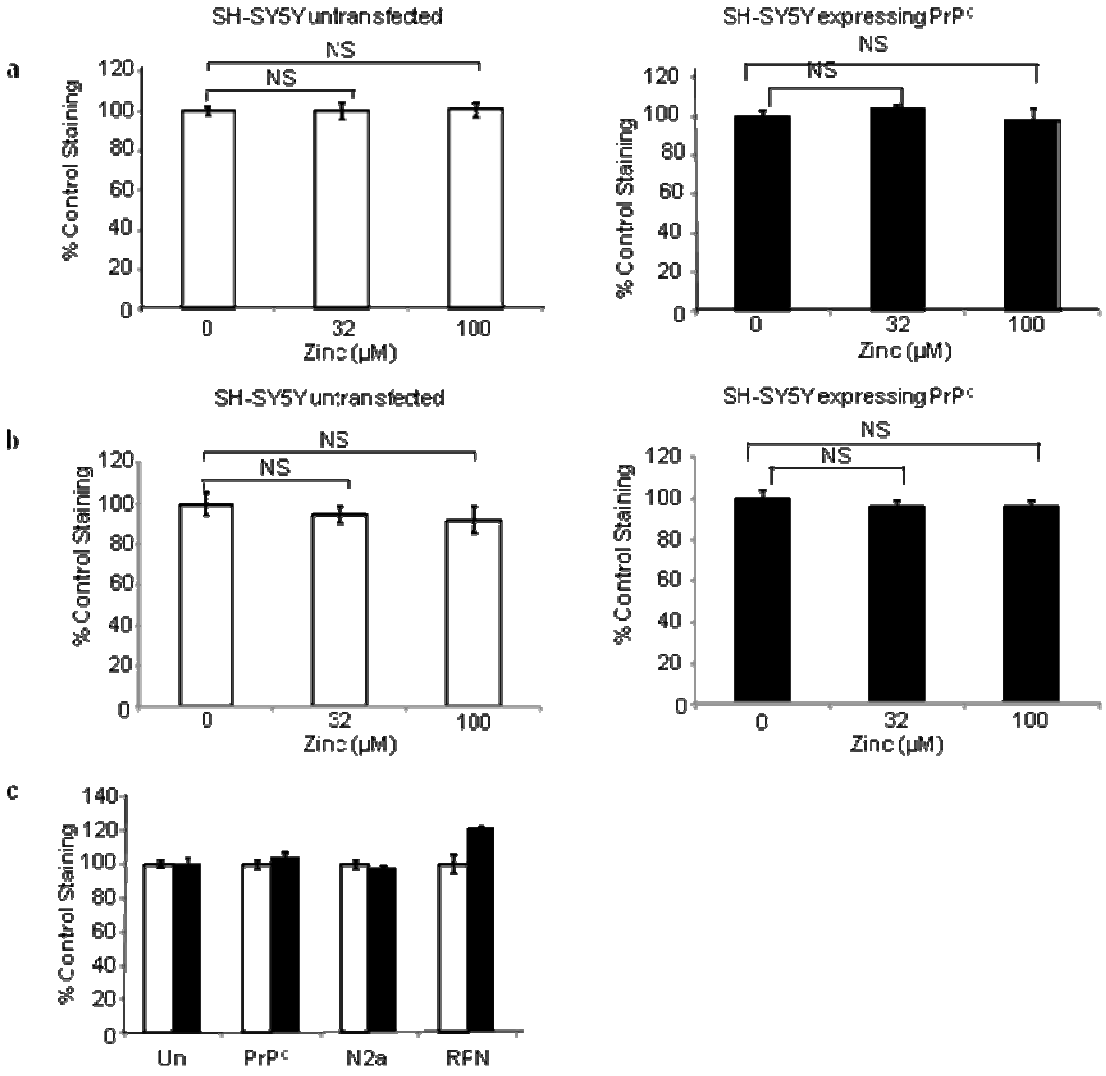
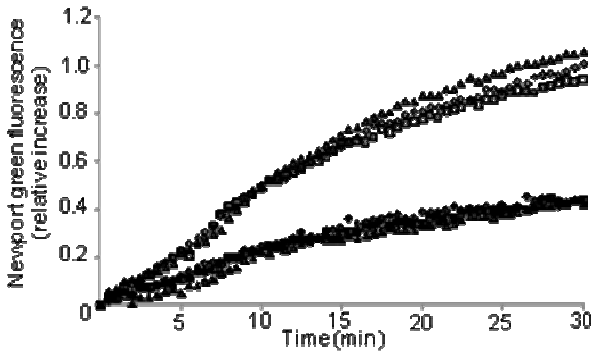


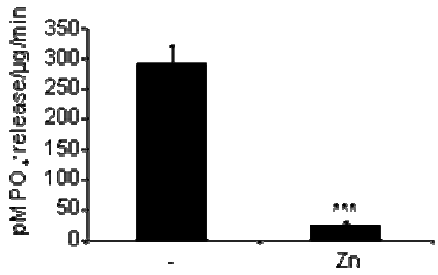
Supplementary Figure S1. Exposure to zinc does not affect the viability of the cells

To determine whether zinc exposure affected cell viability, either untransfected SH-SY5Y cells or those expressing PrP^C were stained with the DNA-binding fluorochrome Hoescht 33342 (8.8 μM) and their fluorescence measured. (a) Viability after 30 min exposure to the indicated Zn concentrations. (b) Viability after 6 h of treatment. (c) Comparison of viability by Hoescht 33342 staining in all the cell types used in this study following exposure to Zn for 30 min. White bars; 0 μM Zn. Black bars; 32 μM Zn. (RPN = rat primary neurons). Data plotted as mean ± SEM (n=8). Independent T-test, N.S., not significant.



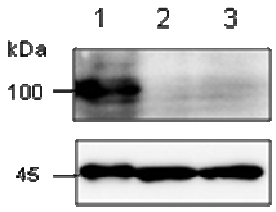
Supplementary Figure S2. siRNA knockdown of PrP^C reduces zinc uptake

Zinc uptake measured using Newport green in rat primary hippocampal neurons exposed for up to 30 min to 32 µM Zn^{2+} (open symbols) or to 32 µM Zn^{2+} following knockdown of PrP^C expression with siRNA (closed symbols). Data shown as the relative Newport green fluorescence corrected against DNA content and plotted as all values in the triplicate measurement.



Supplementary Figure S3. Zinc inhibits protein tyrosine phosphatase activity

Protein tyrosine phosphatase activity was measured in untransfected SH-SY5Y cell lysate incubated with 0 or 1000 µM $ZnCl_2$. Data shown as mean (± SEM) (n=3). Independent t-test, ***, $P<0.001$.



Supplementary Figure S4. Reduced GluA1 in cells expressing PrP^C mutants

Lysates (1 mg) from SH-SY5Y cells expressing wild type PrP^C (lane 1), A116V (lane 2) or PG14 (lane 3) were immunoprecipitated with an antibody against GluA1, subjected to SDS PAGE and western blot analysis with an antibody against GluA1 (upper panel). Western blot for actin (lower panel) shows equal protein loading going into the immunoprecipitation.

Supplementary Methods

Cell culture and construction of prion protein mutants

Human SH-SY5Y cells, murine N2a and prion-infected ScN2a cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 50 U/ml penicillin and 0.1 mg/ml streptomycin. Cells were maintained in a humidified incubator at 37^{o}C with 5% CO_2. SH-SY5Y cells were transfected by electroporation with the various cDNAs encoding wild type murine PrP^{C} with the epitope for antibody 3F4 (Met108 and Met111) or mutant constructs. P101L, D177N/M128 and D177N/V128 were generated from wild type murine PrP^{C} encoding the epitope for antibody 3F4 (Met108 and Met111) using the Quikchange II XL Site-Directed Mutagenesis Kit (Stratagene) with the following primers: P101L forward 5′- AAT CAG TGG AAC AAG CTC AGC AAA CCA AAA ACC -3′; reverse 5′- GGT TTT TGG TTT GCT GAG CTT GTT CCA CTG ATT -3′; D177N forward 5′- AAC AAC TTC GTG CAC AAC TGC GTC AAT ATC ACC -3′; reverse 5′- GGT GAT ATT GAC GCA GTT GTG CAC GAA GTT GTT -3′; M128V forward 5′- GGC CTT GGT GGC TAC GTG CTG GGG AGC GCC ATG -3′; reverse 5′- CAT GGC GCT CCC CAG CAC GTA GCC ACC AAG GCC -3′ and the resulting constructs verified by DNA sequencing. Other constructs have been described previously [20,31, 59]. Each stable cell line was obtained by antibiotic selection as described previously [59].

Preparation of rat primary hippocampal neurons

The hippocampus of 6-8 day old Wistar rats was removed and incubated with 0.25 µg/ml trypsin for 15 min at 37ºC in PBS. Trypsin digestion was terminated by the addition of equal amounts of PBS, supplemented with 16 µg/ml soybean trypsin inhibitor (type I-S; Sigma), 0.5 µg/ml DNAse1 (type II from bovine pancreas; 125 kU/ml; Sigma) and 1.5 mM $MgSO_4$. The tissue was then pelleted by centrifugation at 3000g for 5 min, resuspended in 2 ml PBS with

100 µg/ml soybean trypsin inhibitor, 0.5 µg/ml DNAseI and 1.5 mM $MgSO_4$ and then centrifuged at 3000g for 5 min to pellet the hippocampal neurones. The pellet was resuspended in 5 ml of minimal Earle's medium supplemented with 10% FBS, 13 mM glucose, 50 IU/ml penicillin and 50 µg/ml streptomycin and added to poly-L-lysine (1.5 mg/ml) coated plates. After 24 h, the media was topped up and then after a further 24 h, the culture medium was replaced with one containing 10% heat inactivated horse serum and 80 µM fluorodexoyuridine (FUDR) to prevent proliferation of non-neuronal cells. After a further 48 h, the media was replaced with serum-free Neurobasal media, supplemented with 2% B27, 50 IU/ml penicillin and 50 µg/ml streptomycin, 80 µM FUDR, 25 mM glutamic acid and 0.5 mM glutamine. The culture media was replaced with fresh media every 3-4 days, and cells were maintained in a humidified incubator at 37ºC, 95% air, 5% CO_2. All experiments were conducted on cells after 10 days in culture.

Supplementary Reference

59 Walmsley, A. R., Zeng, F. & Hooper, N. M. Membrane topology influences N-glycosylation of the prion protein. *EMBO J.* **20**, 703-712 (2001).