Supplementary Information

Stress-protective signaling of prion protein is corrupted by scrapie-prions

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Material and Methods

EndoH and PNGaseF digestion

For EndoH or PNGaseF digestion, protein lysates were adjusted to 0.5% SDS, boiled for 10 min and treated with EndoH for 1 h. For PNGaseF digestion the boiled lysates were diluted to 0.1% SDS and incubated with for 16 h with PNGaseF at 37 °C.

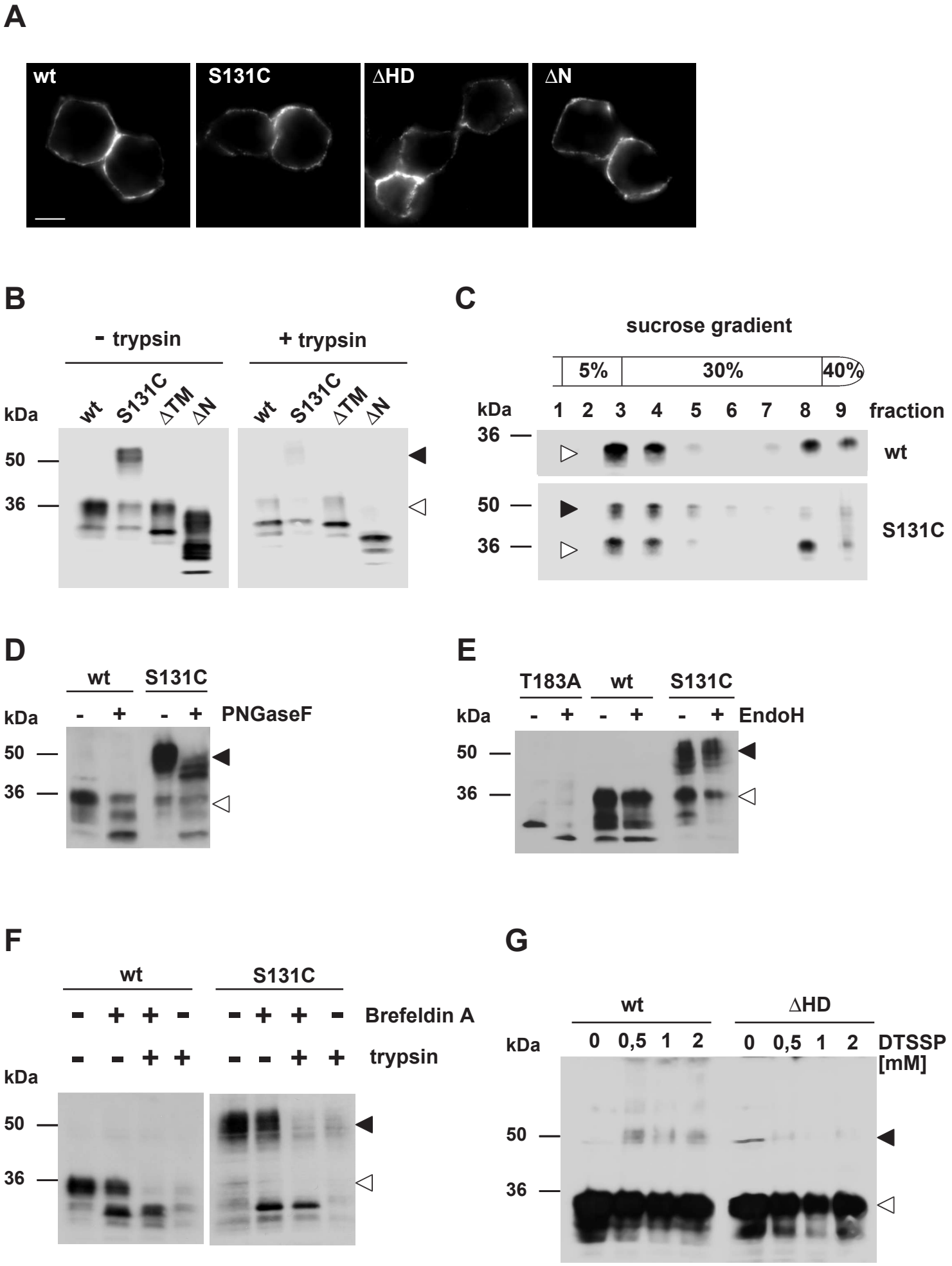
Trypsination of cells and Brefeldin A treatment

To analyze cell surface localization of PrP intact cells were incubated with 200 µl trypsin for 2 min at 37°C; the reaction was stopped by addition of 2 ml of DMEM and cells were collected form the cell culture dish. To study the cellular compartment in which PrP dimerizes, the ER to Golgi transport was inhibited by incubating cells with Brefeldin A

(10μg/ml) for 2 h. Subsequently, cells were harvested either by scraping the cells off the plate or by trypsination and PrP was analyzed by Western blotting.

Figure Legend

Supplementary Figure 1. Wt PrP and mutants with an impaired stress-protective capacity are complex glycosylated and located at the plasma membrane (A, B, C) Dimeric PrP is localized at the cell surface. **(A)** SH-SY5Y cells were transfected with the indicated PrP constructs, fixed and analyzed by indirect immunofluorescence using the mAb 3F4. Size bars: 25 μm. **(B)** SH-SY5Y cells were transfected with wt PrP, S131C, PrPΔN or PrPΔHD, as indicated. Cells were scraped off the plate (- trypsin) or trypsinated (+ trypsin), lysed in buffer A and PrP was detected by Western blotting. **(C)** Dimeric PrP is localized in detergent-insoluble microdomains. N2a cells were transfected with wt PrP or S131C, lysed in cold buffer C, placed beneath a discontinuous sucrose gradient and centrifuged at 140.000 g for 18 h at 4°C. Fractions were collected from top to bottom of the gradient, precipitated by TCA and PrP was detected by immunoblotting using the mAb 3F4. **(D, F)** Dimeric PrP is complex glycosylated. Transfected SH-SY5Y cells were lysed and treated with PNGaseF or EndoH as indicated and PrP was detected by immunoblotting. As a positive control for an EndoH sensitive PrP mutant PrP-T183A {Kiachopoulos, 2005 #945} was used. **(F)** Dimerization of PrP occurs in a post-ER compartment. Transfected SH-SY5Y cells were incubated with (+) or without (-) Brefeldin A (10 μg/ml) for 2 h at 37°C and harvested either by trypsination (+ trypsin) or scraped off the plate (- trypsin). PrP was detected by Western blotting under non-reducing conditions. **(G)** wt PrP can be cross-linked in N2a cells. Crude membranes from wtPrP transfected N2a cells were incubated with DTSSP at the concentrations indicated and PrP was detected by the pAb A7. Closed arrowheads indicate dimeric forms of PrP, open arrowheads indicate PrP monomers.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