Supplemental Data

ALPHA-HELICAL DOMAINS PROMOTE TRANSLOCATION OF INTRINSICALLY DISORDERED POLYPEPTIDES INTO THE ENDOPLASMIC RETICULUM

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Running head: unstructured domains and ER import

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FIGURE LEGENDS

Supplementary Figure 1

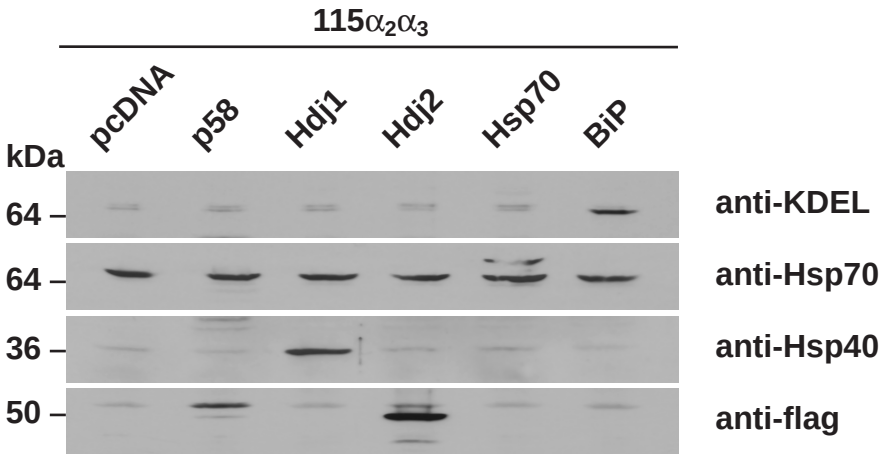
Expressions levels of PrP constructs have no impact on ER import efficiency. (A) PrP-A115X/31^{CHO} is not modified with N-linked glycans. Transiently transfected N2a cells were labeled with [^{35}S] methionine for 15 min (pulse, p) and then chased (c) for 15 and 30 min in fresh medium. PrP-A115X/31^{CHO} transfected cells were analyzed in the presence of MG132 (50 µM). The proteins were immunoprecipitated using the mAb 3F4 and analyzed on SDS-PAGE. Open arrowhead represents unglycosylated PrP, closed arrowhead the glycosylated form (high mannose glycans), double closed arrowhead the complex glycosylated fraction. (B) Overexpression of PrP does not saturate the translocation machinery. N2a cells were transfected with different amounts (0,01 µg - 1 µg) of plasmid DNA. Expression of PrP was analyzed by immunoblotting using the mAb 3F4. Shown are several exposure times. (C) N2a cells transiently transfected with the constructs indicated were pulse labeled with [^{35}S] methionine for 15 min and then chased for 5, 10 and 15 min in fresh medium (chase). The proteins were immunoprecipitated using the mAb 3F4 and analyzed on SDS-PAGE. Right panel: quantification was performed using the phosphoimager Storm840 (Molecular dynamics)

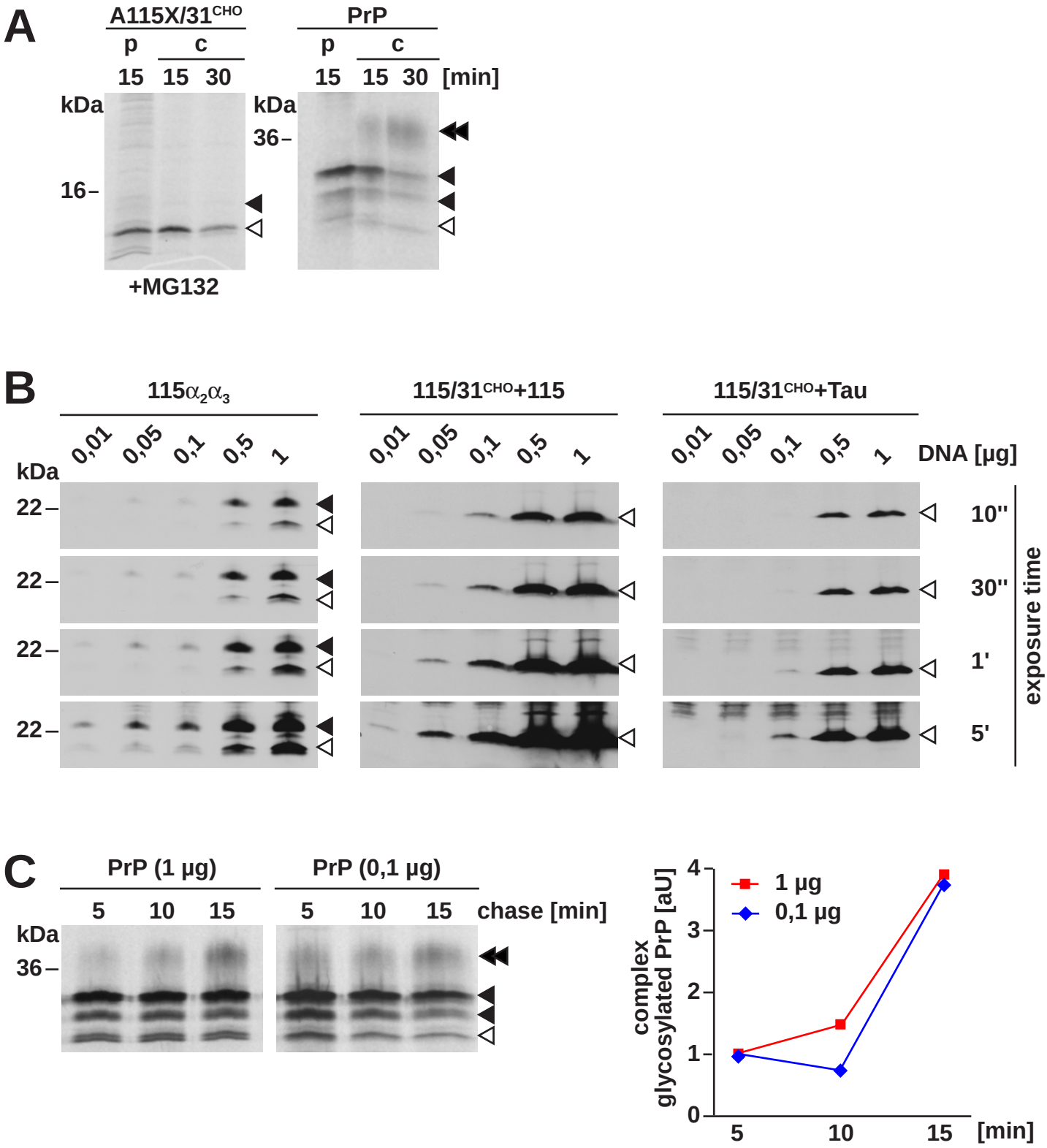
and the ImageQuaNT^{TM} software (version 4.2). Data represent the relative amount of complex glycosylated PrP after 5, 10 and 15 min chase. Blue: 0,1 µg transfected PrP; red: 1 µg transfected PrP.

Supplementary Figure 2

Expression controls of the experiments shown in figure 5D. N2a cells were co-transfected with 115$\alpha_2\alpha_3$ and the chaperones indicated or a vector control (pcDNA). Proteins present in the cell lysate were analyzed by Western blot. The expression of FLAG tagged p58^{IPK} (p58) and FLAG tagged Hdj2 was detected with the mAb M2 flag (anti-flag), Hdj1 with anti-Hsp40 antiserum (anti-Hsp40) and BiP over-expression with anti-KDEL antibody. The anti-Hsp70 antibody recognizes both, endogenous Hsp70 (lower band, anti-Hsp70 panel) and over-expressed EYFP-Hsp70 (upper band, anti-Hsp70 panel).

Supplementary figure 2, Miesbauer et al., 2009




A
A115X/31CHO
PrP
p
c
15 15 30 [min]
kDa
36
16
+MG132
B
115α2α3
115/31CHO+115
115/31CHO+Tau
0,01 0,05 0,1 0,5 1
DNA [µg]
kDa
22
10''
30''
1'
5'
exposure time
C
PrP (1 µg)
PrP (0,1 µg)
5 10 15 chase [min]
kDa
36
complex glycosylated PrP [aU]
1 µg
0,1 µg
[min]