Supplementary figure 1

Supplementary data

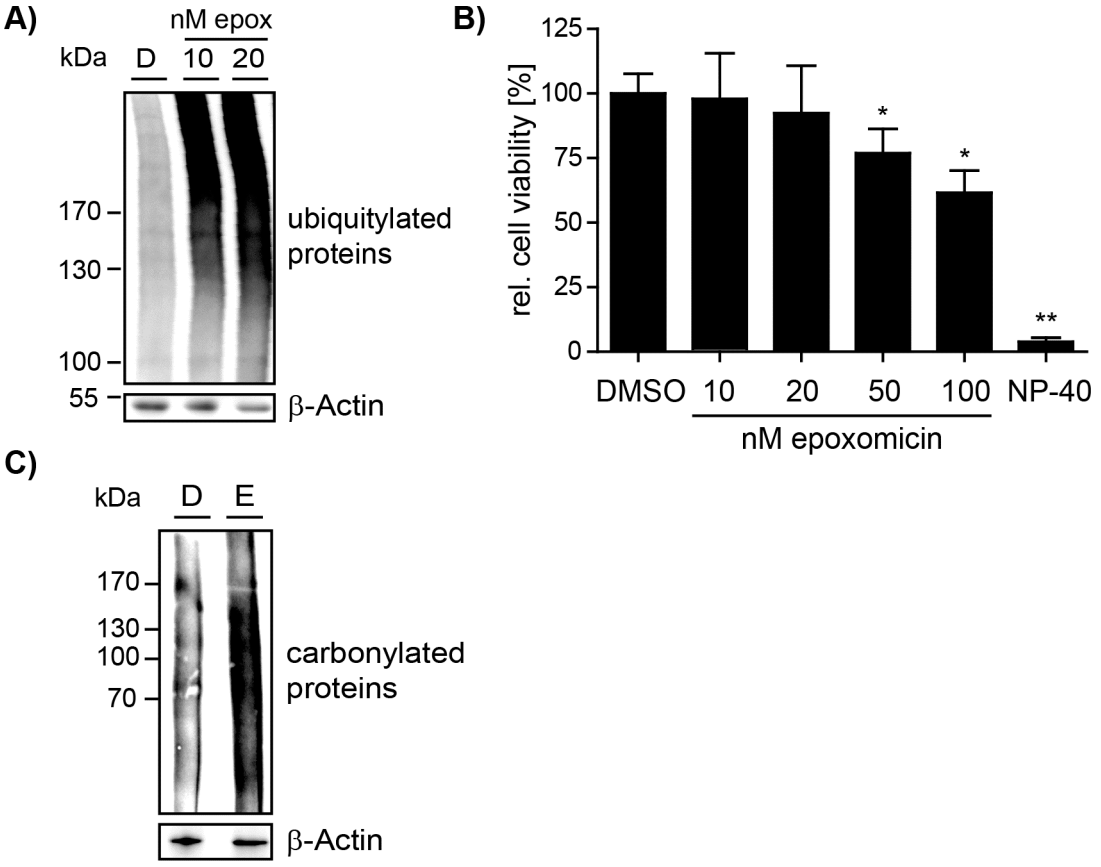
Supplemental material and methods

Cell culture

EA.hy926 human umbilical vein cells were cultured in Basal Iscove, SK-N-BE(2) human neuroblastoma cells in RPMI, and Cos-7 african green monkey fibroblasts, HEK-293 human embryonic kidney cells and LN-18 human glioblastoma cells were cultured in DMEM. All media contained 10 % FCS, 2 mM L-glutamine (Biochrom) and 100 U/ml penicillin and 100 µg/ml streptomycin (Pan-Biotech). All cells were kept at 37 °C and 5 % CO_2 in a humidified atmosphere.

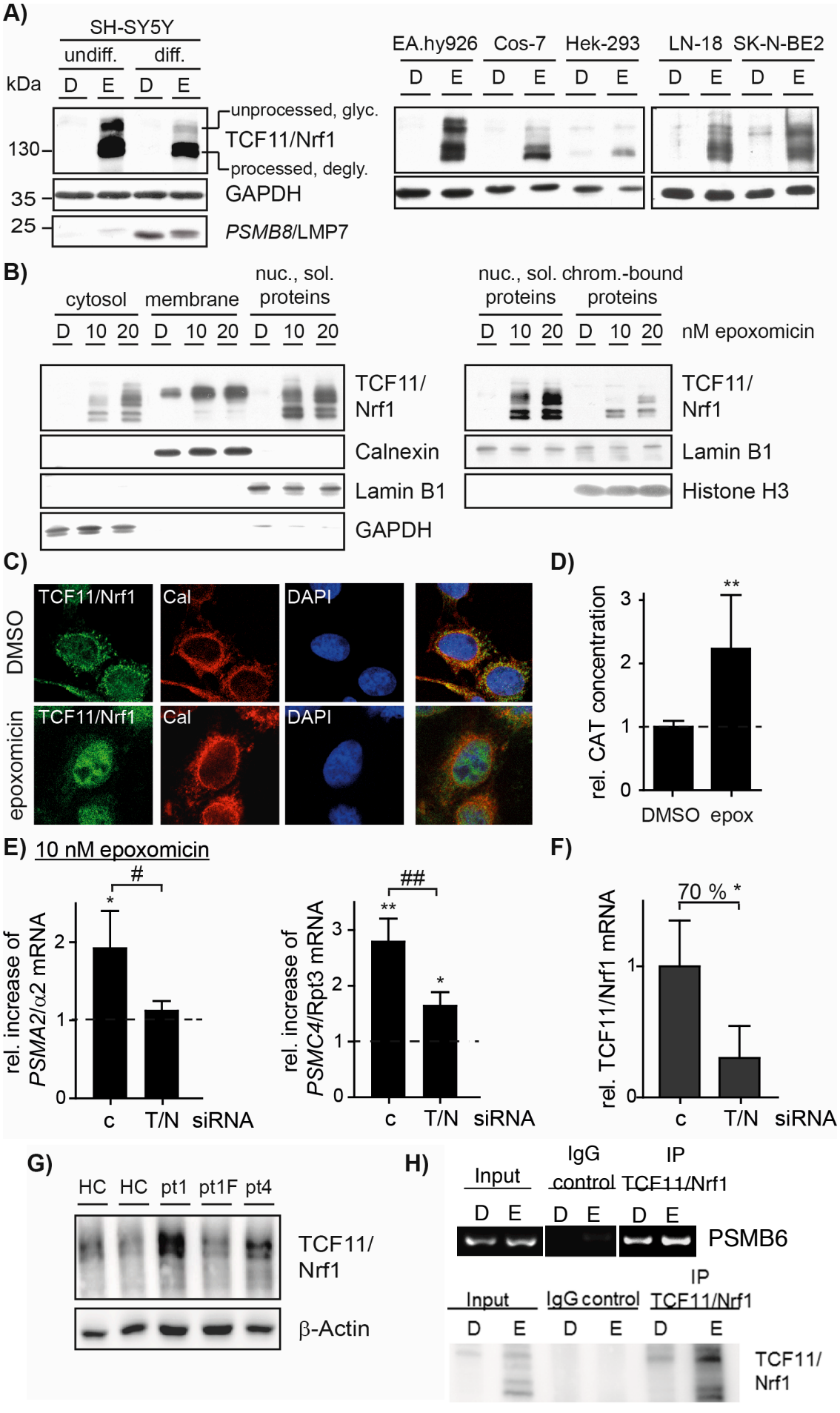
For RT-PCR, the primers against XBP-1 were as follows; forward CCTTGTAGTTGAGAACCAGG and reverse GGGGCTTGGTATATATGTGG.

Supplementary figure 1



Supplementary figure 1: Proteasome inhibition with epoxomicin leads to accumulation of polyubiquitylated proteins, protein carbonyls, and reduces cell viability. (A) Cultured SH-SY5Y cells were treated for 18 hrs with epoxomicin (epox) or 0.1 % DMSO (D) and analysed by immunoblotting against ubiquitylated proteins and β-actin. **(B)** Relative cell viability of cells exposed to the indicated concentrations of epoxomicin or 0.1 % DMSO for 20 hrs was determined by XTT assay. To act as a control, cells were treated for 30 min before the assay procedure with 0.5 % NP-40-containing culture media. The mean values of the relative viability ± S.D. from three independent experiments are shown. **(C)** Cultured SH-SY5Y cells were exposed to 10 nM epoxomicin (E) or 0.1 % DMSO (D) for 24 hr. Levels of carbonylated proteins and β-actin in cell lysates was analysed by immunoblotting.

Supplementary figure 2

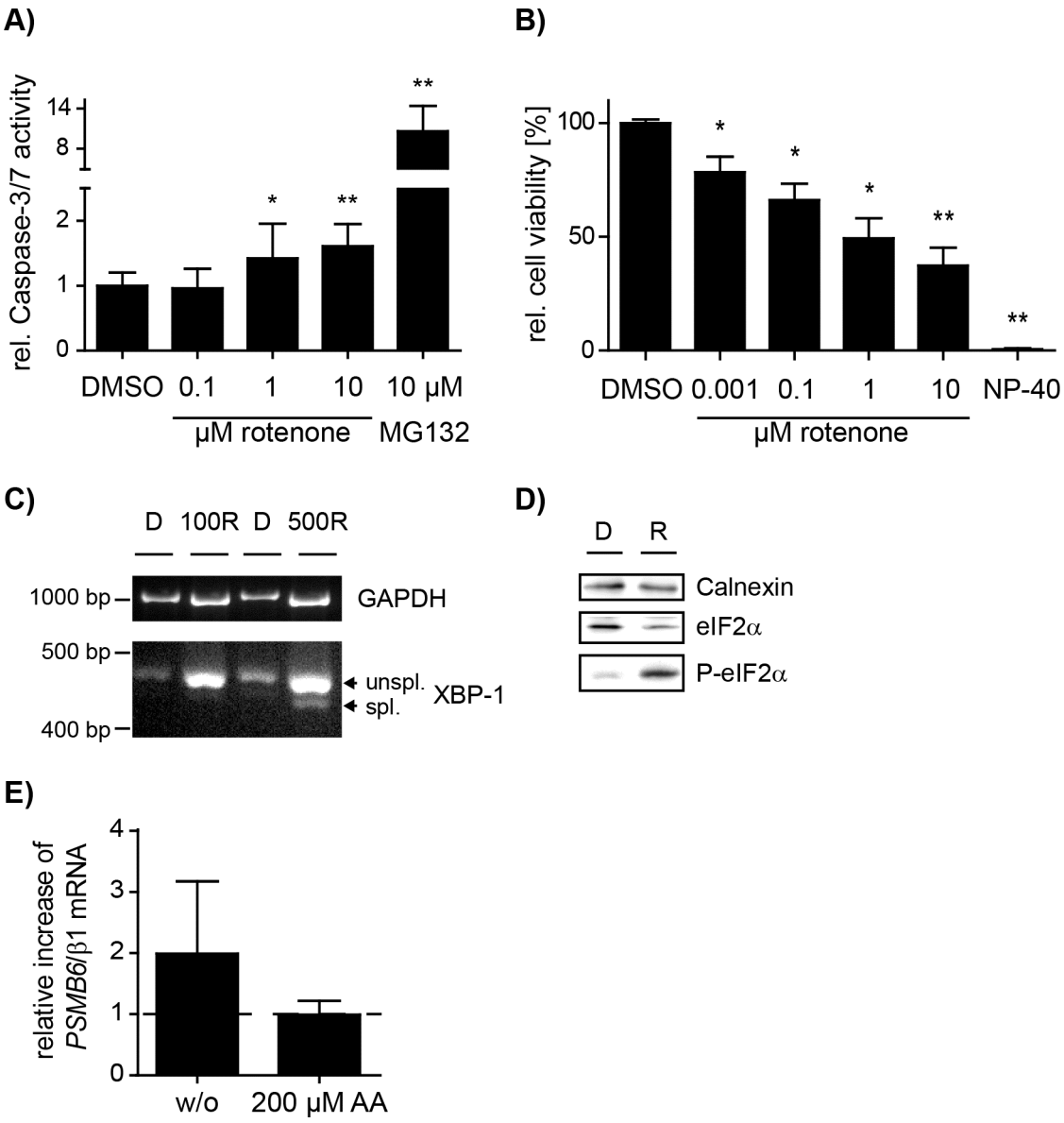


Supplementary figure 2

Supplementary figure 2: Proteasome impairment leads to activation of TCF11/Nrf1 and its transactivation of proteasome gene expression.

Several cell types including undifferentiated (undiff.) and differentiated (diff.) SH-SY5Y were incubated with 10 nM epoxomicin (E) or 0.1 % DMSO (D) for 18 hr. The TCF11/Nrf1 protein amount was examined by immunoblotting. *PSMB8*/ß5i/LMP7 expression is shown for immunoproteasome expression, GAPDH is shown as a loading control. **(B)** Cultured SH-SY5Y cells were exposed to the indicated concentrations of epoxomicin or the vehicle control 0.1 % DMSO (D) for 3 hr and afterwards separated in cytosolic, membrane, soluble nuclear and chromatin-bound fraction. Levels of TCF11/Nrf1 and loading controls Lamin B1, Histon H3, Calnexin and GAPDH were analysed by immunoblotting. **(C)** Immunocytochemistry of cells treated for 18 hrs with epoxomicin or 0.1 % DMSO. Cells were stained for calnexin (red) and TCF11/Nrf1 (green). Nuclei were stained by DAPI (blue). **(D)** SH-SY5Y cells were transfected with the *PSMB6*/β1 promoter containing the CAT reporter gene construct and co-transfected with pcDNA3.1-lacZ. For the last 18 hrs of transfection, cells were exposed to 10 nM epoxomicin (epox). The CAT concentration of cell lysates was determined by ELISA and normalized to β-galactosidase activity. Shown are the mean values of the relative CAT concentration ± S.D. from 12 replicates of six independent experiments. **(E)** For the last 16-18 hrs of a 48 hrs TCF11 knockdown, SH-SY5Y cells were exposed to 10 nM epoxomicin. The relative mRNA level of *PSMC4*/Rpt3, *PSMA2*/α2, *PSMB6*/β1 and TCF11/Nrf1 was analyzed by quantitative real time PCR. Shown are the mean values normalised to the DMSO control ± S.D. from four independent experiments. (*/#: $p<0.05$; **/##:$p< 0.005$). **(F)** Knock-down control for TCF11/Nrf1 gene expression by quantitative real time PCR revealed a mean depletion of 70% TCF11/Nrf1 gene expression. Shown are the mean values normalised to the DMSO control ± S.D. from four independent experiments. (**G**) Human primary keratinocytes from healthy control subjects, two PRAAS patients with mutations in PSMB4/ß7, and one healthy heterozygous carrier (pt1F) were examined for TCF11/Nrf1 expression in immunoblots. Only the patients show significant activation of TCF11/Nrf1. Actin served as loading control. **(H)** Cultured SH-SY5Y cells were exposed to 20 nM epoxomicin for 16 hrs (E) or not (DMSO solvent control, D). Transcription factor DNA complexes were chromatin immunoprecipitated by an antibody against TCF11/Nrf1 or an unspecific IgG antibody used as the control. The binding of TCF11/ Nrf1 to the *PSMB6*/β1 promoter was analysed by PCR. The samples were used in parallel for immunoblot analysis of TCF11/Nrf1 in the precipitates as an antibody specificity control.

Supplementary figure 3

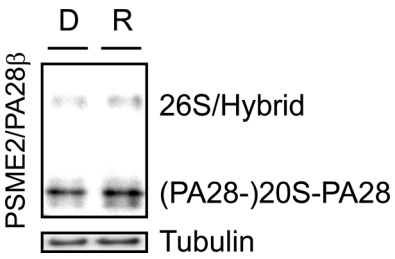


Supplementary figure 3: Rotenone exposure induces cell death and activates the unfolded protein response. (A) Caspase-3/7 activity was determined from SH-SY5Y cells treated with the indicated concentrations of rotenone, 10 µM MG132 or 0.1 % DMSO for 24 hr. The mean value of the relative activity ± S.D. from 11 replicates of five independent experiments is shown. **(B)** Relative cell viability of SH-SY5Y cells exposed to rotenone (20 hrs; 0.001-10 µM) was determined by XTT assay. As a control, cells were treated 30 min before the assay with 0.5 % NP-40-containing culture media. The mean values of the relative viability ± S.D. from three independent experiments are shown. **(C)** SH-SY5Y cells were treated with 100 or 500 nM rotenone (R) or 0.1 % DMSO (D) for 48 hr. The XBP-1 mRNA

Supplementary figure 3

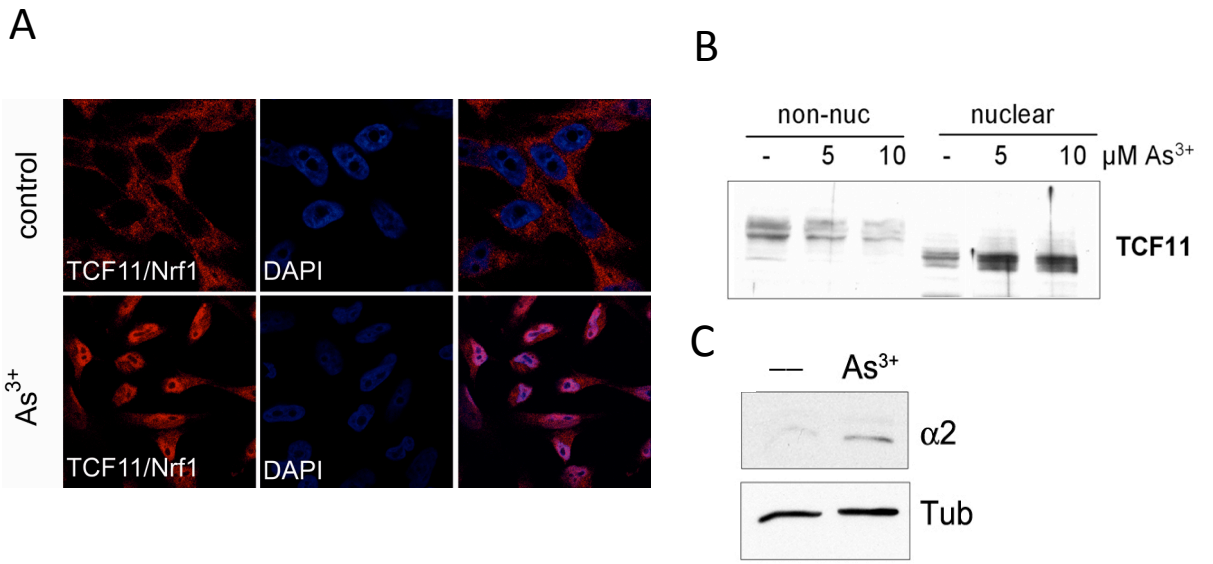
was analysed by PCR using specific primers targeting the unspliced as well as spliced version. A PCR using specific primers against GAPDH was performed as control. **(D)** SH-SY5Y cells were treated with 100 nM rotenone (R) or 0.1 % DMSO (D) for 48 hrs. The protein amount was examined by immunoblotting against eIF2α and P-eIF2α. GAPDH is shown as a loading control. **(E)** Cultured SH-SY5Y cells were treated with 200 µM ascorbic acid (AA) for 20 hrs. For the last 10 hrs cells were treated or co-treated with 100 nM rotenone or 0.1 % DMSO. The relative mRNA levels of PSMB6/β1 were analysed by quantitative real time PCR. Shown are the mean values normalised to the corresponding DMSO control ± S.D. from three independent experiments.

Supplementary figure 4



Supplementary figure 4: Proteasome complexes in SH-SY5Y cells contain the PA28 regulator. Cultured SH-SY5Y cells were exposed to 100 nM rotenone (R) or 0.1 % DMSO (D) for 8 hrs. Proteasome complexes were separated in native gels and immunoblotted against *PSME2*/PA28β.

Supplementary figure 5



Supplementary figure 5: TCF11/Nrf1 translocates into the nucleus upon arsenic exposure. (A) For immunocytochemistry, EA.hy926 cells were incubated with 10 µM As^{3+} for 6 hr and then stained for TCF11/Nrf1 (red) and nuclei by DAPI (blue). (**B**) EA.hy926 cells were exposed to the indicated concentrations of As^{3+} for 6 hrs, fractionated into nuclear and non-nuclear fraction and then stained for TCF11/Nrf1 in immunoblots. **(C)** EA.hy926 cells were exposed to 10µM As^{3+} for 6 hrs and analysed in immunoblots for proteasome subunit induction (exemplified by α2) and tubulin (Tub) as loading control.