Supplementary information

Parkin is transcriptionally regulated by ATF4:

evidence for an interconnection between mitochondrial stress and ER stress

Lena Bouman[1], Anita Schlierf[1], A. Kathrin Lutz[1], Jixiu Shan[2], Alexandra Deinlein[1], Jessica Kast[1], Zohreh Galehdar[3], Vincenza Palmisano[1], Nadja Patenge[5], Daniela Berg[5], Thomas Gasser[5], Regina Augustin[6], Dietrich Trümbach[6], Isabella Irrcher[3], David S. Park[3,4], Wolfgang Wurst[6,7], Michael S. Kilberg[2], Jörg Tatzelt[1] and Konstanze F. Winklhofer[1*]

[1]Adolf Butenandt Institute, Neurobiochemistry, Ludwig Maximilians University, and German Center for Neurodegenerative Diseases, Schillerstr. 44, 80336 Munich, Germany; [2]Department of Biochemistry and Molecular Biology, Genetics Institute, Shands Cancer Center and Center for Nutritional Sciences, University of Florida College of Medicine, Gainesville, FL 32610, USA; [3]Cellular and Molecular Medicine, University of Ottawa, Ottawa, Canada; [4]Department of Cogno-Mechatronics Engineering, Pusan National University, South Korea; [5]Department of Neurodegeneration, Hertie Institute for Clinical Brain Research, University of Tübingen, and German Center for Neurodegenerative Diseases Hoppe-Seyler-Str. 3, 72076 Tübingen, Germany; [6]Helmholtz Centre Munich, German Research Centre for Environmental Health, Technical University Munich, Institute of Developmental Genetics, and German Center for Neurodegenerative Diseases, Ingolstaedter Landstr. 1, 85764 Munich/Neuherberg, Germany. [7]Max Planck Institute of Psychiatry, Kraepelinstr. 2, 80804 Munich, Germany.

*To whom correspondence should be addressed:

Konstanze F. Winklhofer, Schillerstr. 44, D-80336 Munich, telephone: +49 89 2180 75483, fax: +49 89 2180 75415, E-mail: Konstanze.Winklhofer@med.uni-muenchen.de

Running title: Parkin, ER stress and mitochondria

Key words: ATF4/ c-Jun/ER stress/JNK/parkin/Parkinson's disease/UPR

Supplemental Figure 1:

Mitochondrial stress induced by CCCP activates ATF4-dependent transcription. HEK293T cells were transfected with the ATF4RE-luc reporter contruct containing a confirmed ATF4-binding site (A), or with park-luc (B), containing the putative ATF4-binding site of the parkin promoter. One day after transfection the cells were exposed to CCCP (10 μM for 24 h). Shown is the fold induction of luciferase activity in CCCP-treated cells in comparison to non-treated control cells.

Supplemental Figure 2:

c-Jun knockdown efficiency corresponding to the experiment shown in Fig. 5 D. mRNA levels were determined by quantitative RT-PCR using c-Jun-specific primers.

Supplemental Figure 3:

Parkin-deficiency decreases cell viability in response to ER stress. HEK293T cells (A) or SH-SY5Y cells (B) were transfected with parkin-specific or control siRNA duplexes. Three days later the cells were stressed with TG (10 μM) or TM (2 μg/ml) for 16 h. Cellular viability was determined by the MTT assay. Shown is the relative viability of cells transfected with parkin siRNA in comparison to control siRNA-transfected cells treated with ER stressors based on triplicates of at least 3 independent experiments. The parkin knockdown efficiency was verified by Western blotting using the anti-parkin mAb PRK8 (A, lower panel) or by quantitative RT-PCR using parkin-specific primers (B, lower panel).

Supplemental Figure 4:

CHOP, phospho-c-Jun and phospho-JNK are sensitive to the severity of ER stress and are increaed in parkin-deficient cells. A, B. Levels of CHOP, phospho-c-Jun and phospho-JNK increase with the severity of ER stress in SH-SY5Y cells. SH-SY5Y cells were treated

with thapsigargin (TG) at the indicated concentrations for 16 h to analyze CHOP levels (A) or 1 h to determine the levels of phospho-c-Jun and phospho-JNK (B) by Western blotting. Loading was controlled by re-probing the blots for β-actin. **C, D.** CHOP, phospho-c-Jun and phospho-JNK levels are increased in parkin-deficient patient fibroblasts. Skin fibroblasts from a patient with pathogenic mutations in the parkin gene leading to a loss of parkin expression, were incubated with TG at the indicated concentrations for 16 h to analyze CHOP levels (C) or 1 h to determine the levels of phospho-c-Jun and phospho-JNK (D) by Western blotting. As a control, age- and gender-matched skin fibroblasts were analyzed in parallel. Loading was controlled by re-probing the blots for β-actin. The two JNK-immunoreactive bands represent phosphorylated JNK1 (46 kDa) and JNK2/3 (54 kDa), respectively.

Supplemental Figure 5:

ER stress luciferase reporter constructs cloned to quantify the ER stress response. Binding of ER stress-specific transcription factors to specific sites and UPR transducers involved are schematically shown. ERSE: ER stress response element, UPRE: unfolded protein response element, ERSE II: ER stress response element II, ATF4RE: ATF4 response element.

Supplemental Figure 6:

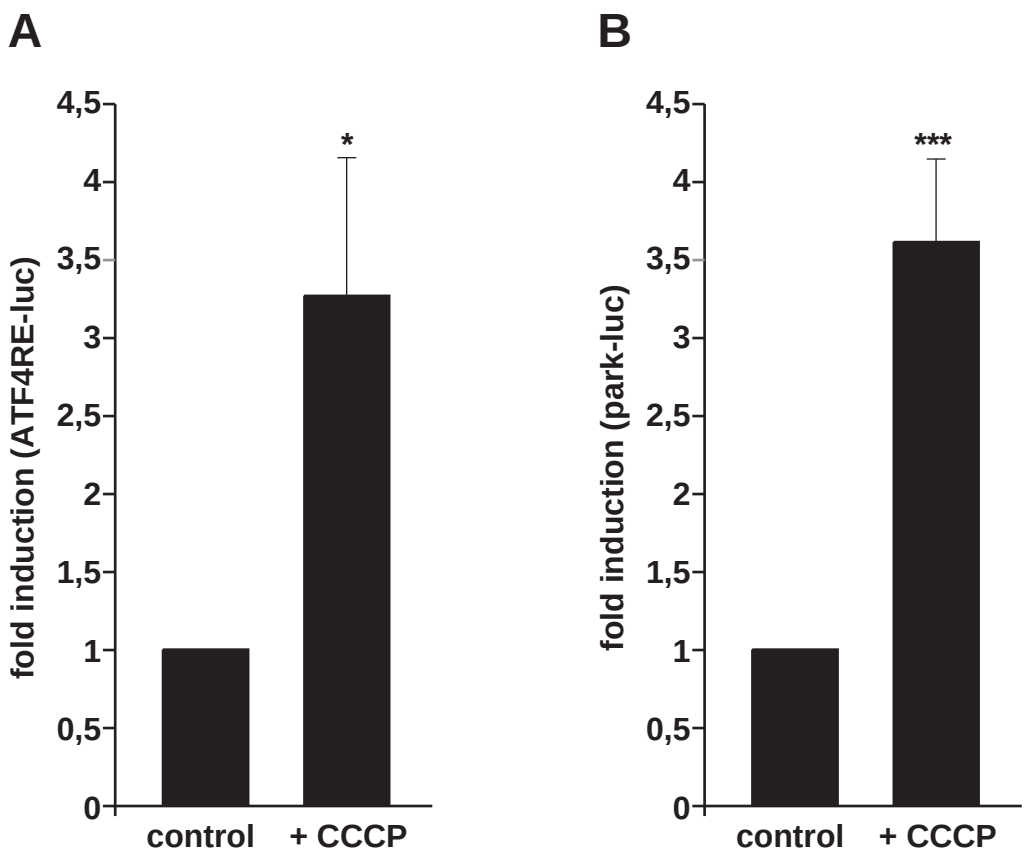
CHOP suppresses the activation of the park-luc reporter construct in response to ER stress. HEK293T cells were co-transfected with the park-luc reporter plasmid and ATF4, CHOP (1), or GFP (as a control). 8 h after transfection cells were incubated with 1 μM TG for 14 h. Shown is the fold induction of luciferase activity in comparison to GFP-expressing control cells based on triplicates of at least 3 independent experiments (left panel). Expression levels of ATF4 and CHOP were analyzed by immunoblotting using the anti-ATF4 pAb C-20

or the anti-CHOP sc-7351 mAb (Santa Cruz Biotechnology, Santa Cruz, CA, USA) (right panel). Loading was controlled by re-probing the blots for β-actin. *** $p < 0.001$.

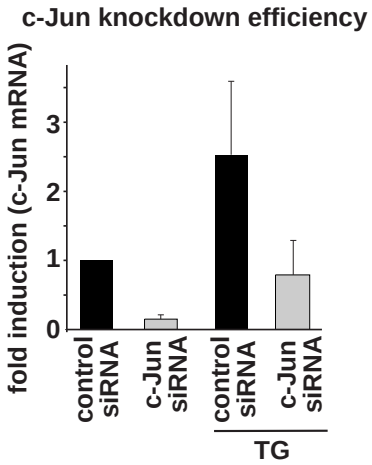
Reference:

1. Al Sarraj J, Vinson C, Thiel G. Regulation of asparagine synthetase gene transcription by the basic region leucine zipper transcription factors ATF5 and CHOP. Biol Chem. 2005 Sep;386(9):873-879.

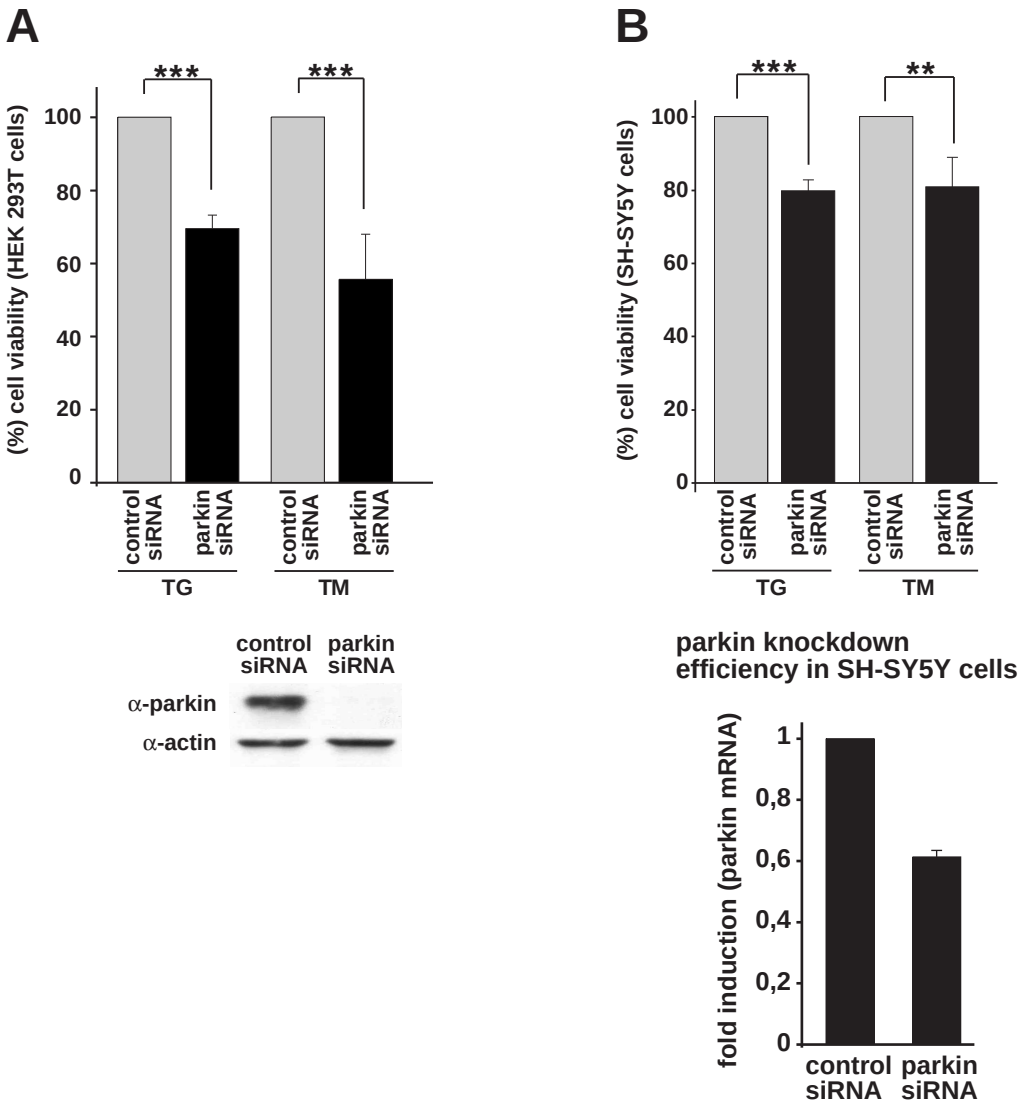
Supplemental Figure 1, Bouman et al.

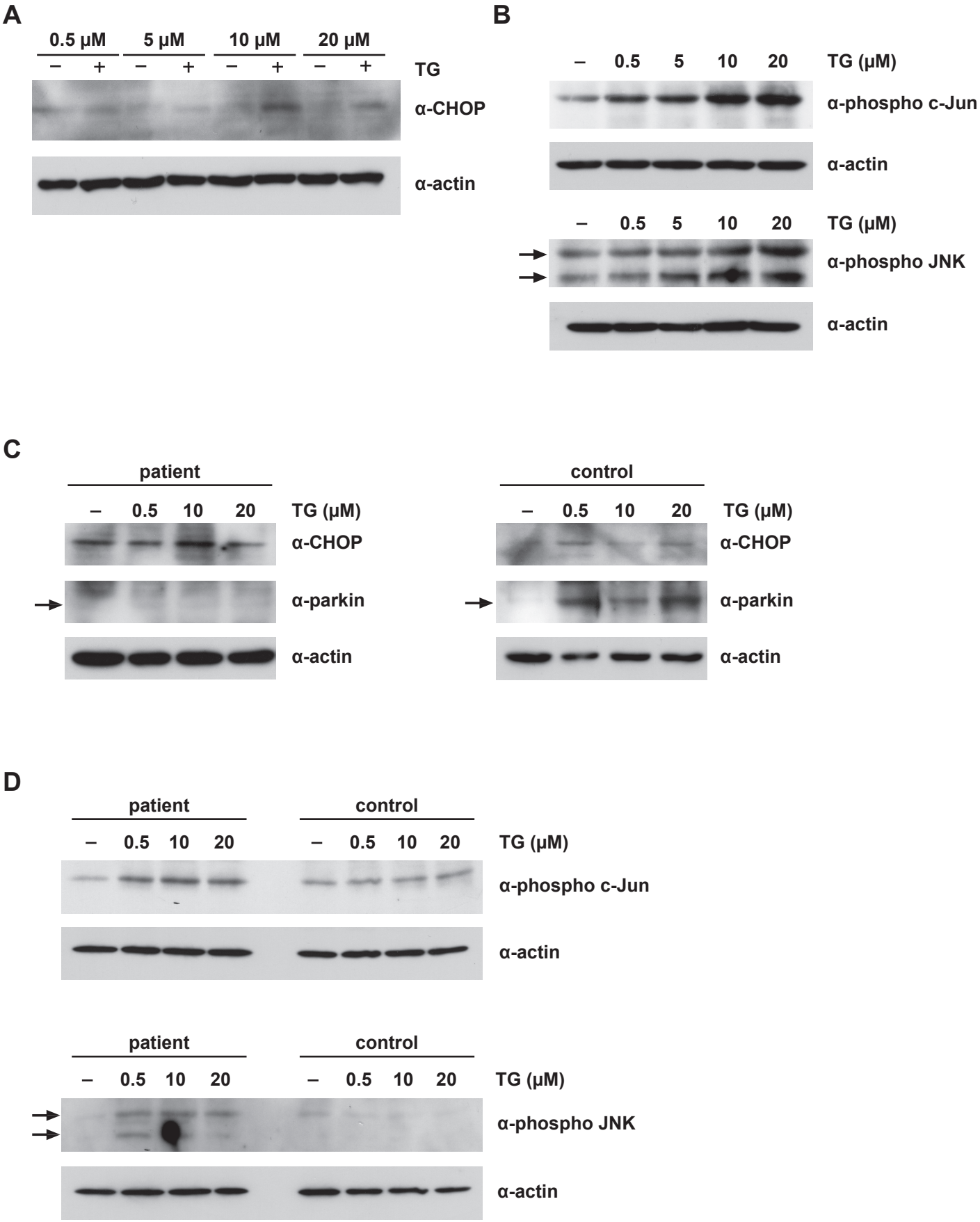


Supplemental Figure 2, Bouman et al.

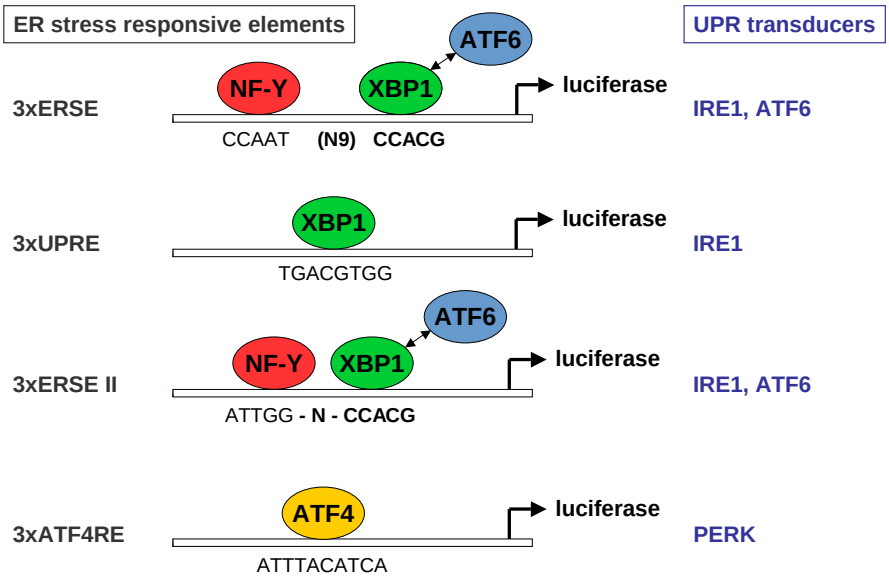


Supplemental Figure 3, Bouman et al.




A
0.5 µM
5 µM
10 µM
20 µM
– + – + – + – +
TG
α-CHOP
α-actin
B
– 0.5 5 10 20 TG (µM)
α-phospho c-Jun
α-actin
– 0.5 5 10 20 TG (µM)
α-phospho JNK
α-actin
C
patient
– 0.5 10 20 TG (µM)
α-CHOP
α-parkin
α-actin
control
– 0.5 10 20 TG (µM)
α-CHOP
α-parkin
α-actin
D
patient
control
– 0.5 10 20 – 0.5 10 20 TG (µM)
α-phospho c-Jun
α-actin
patient
control
– 0.5 10 20 – 0.5 10 20 TG (µM)
α-phospho JNK
α-actin

Supplemental Figure 5, Bouman et al.



Supplemental Figure 6, Bouman et al.