The subcellular localization of bHLH transcription factor TCF4 is

mediated by multiple nuclear localization and nuclear export signals

Beata Greb-Markiewicz, Wioletta Kazana, Mirosław Zarębski, Andrzej Ożyhar

Supporting Information:

FIGURE S1. Western blot analysis of expression of YFP tagged TCF4 and TCF4 derivatives in transfected COS-7 cells. Detection with anti-GFP antibodies. On the bottom of images presented theoretical molecular mass of proteins.

FIGURE S2. Subcellular distribution of selected TCF4 derivatives. Subcellular localization of the expressed proteins were analysed by confocal microscopy in COS-7 or N2a cells 24 h after transfection. Draq5 was added to the cells for DNA visualization. Not typical (rare events) images of subcellular distribution are presented for YFP-TCF4, YFP-TCF4/147-300, YFP-TCF4/550-670, YFP-TCF4/550-670/K606A, YFP-TCF4/1-549.

FIGURE S3. (A) Prosite prediction of domains in TCF4 protein. (B) Scansite prediction of surface accessibility. (C) PONDR, METADISORDER and DYNAMINE *in silico* analyses of the disorder character of TCF4.

FIGURE S4. Subcellular distribution of the YFP-TCF4/1-601 derivative. (A) Schematic representation of TCF4 protein. Region of the studied bHLH domain of TCF4 is shown by the red rectangle. Expressed deletion mutants of TCF4 are depicted as arrows. (B) Subcellular localization of the expressed proteins were analysed by confocal microscopy in COS-7 cells 24 h after transfection. Not typical (rare events) images of subcellular distribution are presented. (C) Subcellular localization of the expressed proteins were analysed by fluorescence microscopy in COS-7 and N2a cells 24 h after transfection in the absence or presence of additional factors such as LMB or methanol. Representative images of subcellular distribution are presented.

FIGURE S5. (A) Phosphorylation sites of the bHLH domain predicted by NetPhos and DEPP servers. (B) Results of *in silico* analyses of the bHLH domain of TCF4 by PONDR, METADISORDER and DYNAMINE servers.





Figure S2

YFP-TCF4 in COS-7 cells (Supplement to Fig.1)



YFP-TCF4/147-300 in COS-7 cells (Supplement to Fig. 3)



YFP-TCF4/550-670 after 24h (Supplement to Fig.8)





YFP-TCF4/550-670/K606A (Supplement to Fig.8)





YFP-TCF4/1-549 (Supplement to Fig. 9)





Figure S4









YFP-TCF4/1-549 + Methanol





YFP-TCF4/1-549 + LMB





Figure S5

