The membrane-topogenic vectorial behaviour of Nrf1 controls its post-translational modification and transactivation activity

Yiguo Zhang[1,2,*], John D. Hayes[2]

[1]The NSFC-funded Laboratory of Cell Biochemistry and Gene Regulation, College of Medical Bioengineering and Faculty of Life Sciences, University of Chongqing, No. 174 Shazheng Street, Shapingba District, Chongqing, 400044, China
[2]Jacqui Wood Cancer Centre, James Arrott Drive, Division of Cancer Research, Medical Research Institute, Ninewells Hospital & Medical School, University of Dundee, DD1 9SY, Scotland, UK
*Correspondence: yiguozhang@cqu.edu.cn, or y.z.zhang@dundee.ac.uk

SUPPLEMENTAL RESULTS

It should be noted that supplemental results obtained from experimental methods and bioinformatic analysis are described in their figure legends. Some of these results are also mentioned in the text.

Figure S1. Comparison of possible membrane-associated regions from Nrf1 with other transmembrane proteins.

(**A**) **An alignment of the hydrophobic, semihydrophobic and/or amphipathic amino acid sequences from Nrf1 with other conserved membrane-associated segments**. Bioinformatic analysis revealed that:

i) Nrf1-TM1 (aa 7-26) is conserved with Na^+/H^+ antiporter NhaA2 (aa 404-423) and the electron transport complex protein RnfE (aa 20-39).

ii) Nrf1-TMc (aa 705-725) is represented by other transmembrane helical regions folded by aa 348-368 from plastidic glucose transporter 1 (PLST1), aa 62-82 from sodium-coupled neutral amino acid transporter 11 (SNAT11).

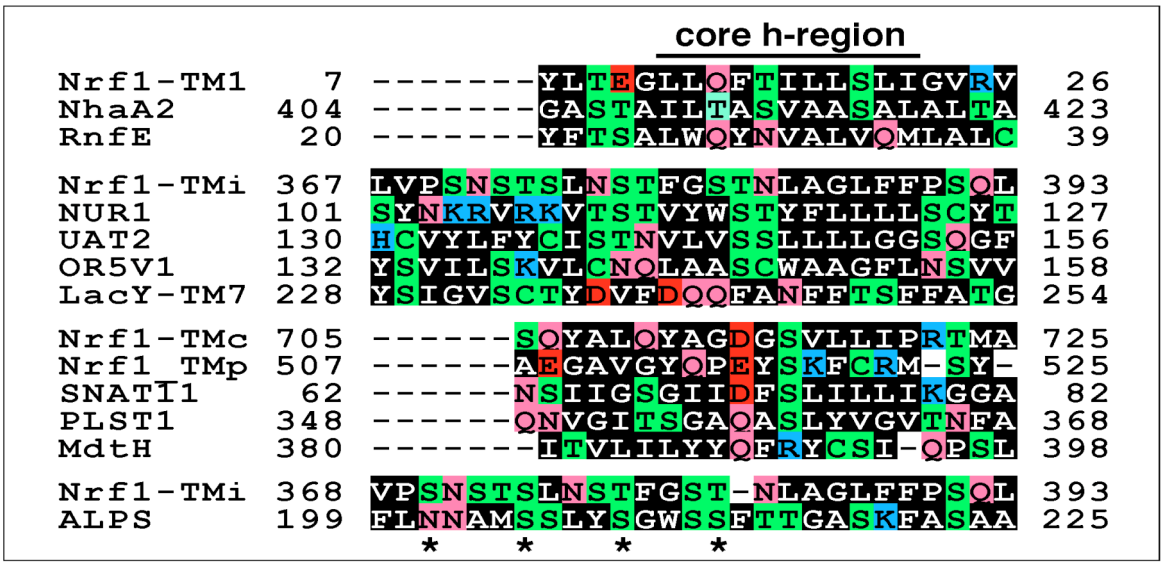
iii) Nrf1-TMp (aa 507-525) has 70% sequence similarity with TMc (705-725), and it is possible that their interactions occur through the putative salt bridge formed between Lys^{519} (in TMp) and Asp^{714} (in TMc) within the membrane lipid leaflet close to the cytoplamic interface. Additional two or three salt bridges may also be formed between Glu^{508} (in TMp) and either Arg^{25} (in TM1) or Arg^{722} (in TMc), and between Glu^{10} (in TM1) and Arg^{523} (in TMp), respectively, which are located within the luminal and cytoplasmic interfaces of the membrane lipid bilayer. Within and around TMp, Cys^{521} and Cys^{533} are likely to be involved in response to redox stress. Furthermore, TMp is conserved with known transmembrane helices wheeled by the indicated amino acids from membrane proteins, including MdtH (a multidrug resistance protein).

iv) Nrf1-TMi (aa 367-393) is homologous with other transmembrane regions formed by aa 101-127 from nuclear rim protein 1 (NUR1), aa 130-156) from urea active transporter 2 (UAT2) or aa 132-158 from olfactory receptor OR5V1. Importantly, comparison of TMi with the ArfGAP1 lipid-packing sensor (ALPS, aa 199-223) reveals that both are highly conserved, particularly within their N-terminal three-fifth portions that contain a GSK-3 phosphorylation site (*). However, **TMi is distinguishable from ALPS because its hydrophobic heptapeptide ^{384}LAGLFFP^{390} is not present in ALPS**. Notably, the core TMi sequence (aa 375-393) has considerable aliphaticity and hydropathicity, that are 1.95 and 3.45 times greater than the respective values of ALPS, and also are 3.75 and 2.00 times higher than those of the flexible TM7 helix folded by aa 228-254 from LacY[1]. Based on these biophysico-chemical properties, we predict that this Phe/Leu-rich TMi is able to fold into a glycine-kinked amphipathic semihydrophobic helical hinge, with its N-terminal amphipathic portion lying flat on the plane of one leaflet of the membrane lipid bilayer and its C-terminal hydrophobic portion partially spanning across the hydrophobic interior reaching towards the other leaflet. Furthermore, the topological orientation of TMi is also controlled by its flanking glycopeptides.

(B) Comparison of the TM1, TMi, TMp and TMc helices within Nrf1 with other known transmembrane regions. These potential helices were wheeled using the HeliQuest programme. Some of the polar and charged amino acids in the membrane-spanning helices enable an intramolecular interaction between TM1 and other semihydrophobic and/or amphipathic helices folded by certain regions within the NHB1-CNC factor, as other transmembrane proteins was described[2-4]. Three physico-chemical parameters related with the helical folding (i.e. aliphaticity, hydropathicity and instability indexes) were also calculated using the ProParam tool (http://web.expasy.org/ protparam/).

Figure S1

A, Comparison of Nrf1 and known membrane-spanning proteins



B, Comparison of helical regions in Nrf1 with other transmembrane regions

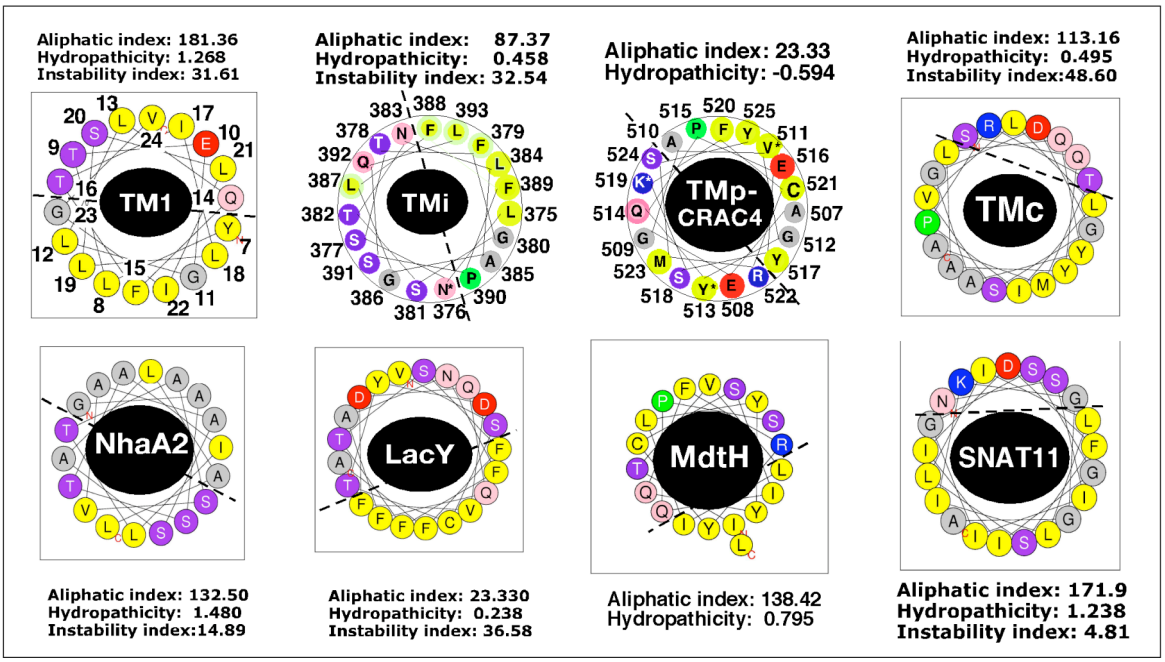


Figure S2

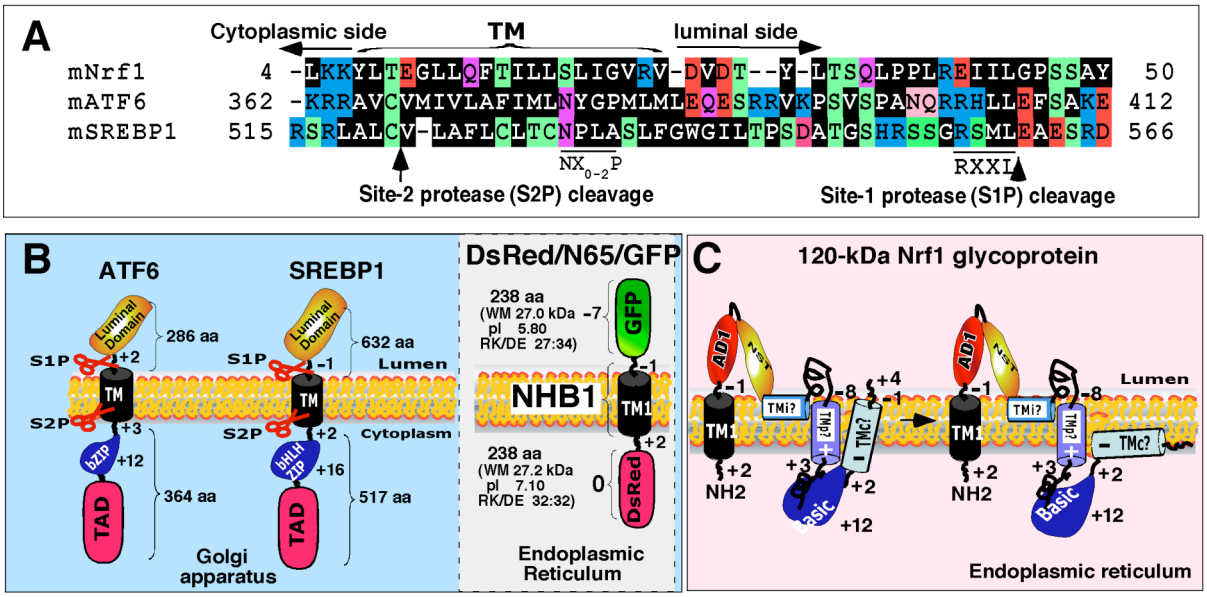


Figure S2. Comparison of the TM1 region of Nrf1 with the transmembrane domains of ATF6 and SREBP1.

(A) An alignment of the NHB1-associated TM1 region in Nrf1 with the transmembrane domains of ATF6 and SREBP1. The TM1-adjoining signal peptide of Nrf1 appears to share certain similarity with the transmembrane sequences of mouse ATF6 and SREBP1. Importantly, the TM1-associated NHB1 sequence of Nrf1 lacks either RxxL or $Nx_{0\text{-}2}P$ motifs recognized by Site-1 protease (S1P) and Site-2 protease (S2P), respectively, but these two cleavage sites are present in ATF6 and SREBP1. It is clear that both S1P and S2P can enable proteolytic processing of ATF6 and SREBP1 to be consecutively cleaved in the two arrow-indicated peptide bonds immediately following the Leu residue within the RxxL motif and the Cys residue within their $Nx_{0\text{-}2}P$-containing transmembrane domains, respectively[5], so that either the cytoplasmic 364 aa of ATF6 (covering its TAD and DNA-binding bZIP domain) or equivalent 517 aa of SREBP1 (covering its TAD and bHLH-ZIP domain) is allowed to be released from Golgi membranes before their translocation into the nucleus whereupon they are able to function as an active transcription factor.

(B,C) The TM1-associated NHB1 sequence in Nrf1 is not processed through a mechanism similar to RIP of ATF6 and SREBP1. According to the positive-inside and charge difference rules[1-3, 6-9], the local membrane-topology of the TM1-associated NHB1 sequence in Nrf1 appears to resemble those of the transmembrane domains of ATF6 and SREBP1 that are orientated in an N_{cyt}/C_{lum} fashion within membranes (**B**), but the overall topology of Nrf1 is obviously different from those of ATF6 and SREBP1(**C**). Indeed, our previous work[10-12], along with the present study, has revealed that Nrf1 is anchored in the ER membrane through its TM1 in an N_{cyt}/C_{lum} orientation, and both its NST glycodomain and its flanking TADs are transiently translocated into the lumen whereupon the NST domain is glycosylated. However, our previous electron and confocal microscopy data showed that Nrf1 is neither transported into the Golgi apparatus nor the mitochondria [11]. Therefore, Nrf1 is unlikely to be processed in the ER through a mechanism similar to RIP of ATF6 and SREBP1 by S1P and S2P; both enzymes are located in the Golgi apparatus.

(B, *left*) The membrane-topological folding of Nrf1, in particular TM1, is unaffected by its N-terminal and/or C-terminal tags. Green fluorescence protein (GFP comprising 238 aa with a mass of ~27 kDa) shares ~50% sequence similarity with DsRed. The net negatively charged (-7) GFP (KR/ED = 27:34, pI 5.8) is attached to the C-terminus of wild-type Nrf1 or its mutants, and thus should facilitate translocation of Nrf1 into the lumen of ER along the electrochemical potential differences of the ER membrane. The GFP-fused proteins were subjected to membrane protease protection reactions, called fluorescence protease protection (FPP) assay as described by other authors[13,14], who has employed the FPP to determine the topology of membrane proteins (which terminus is facing either the luminal or the cytoplasmic sides of membranes). It is to note that some expression constructs for Nrf1, its mutants or its fusion proteins may produce different isoforms that can also exhibit distinct subcellular locations and topologies relatively to membranes. The superimposed images exhibited from these distinct isoforms are hard to be determined by immunocytochemistry and microscopy following FPP *in vivo*. Thus, we have investigated differences in the electrophoretic migration of distinct Nrf1 isoforms and/or its degraded peptide species, that resulted from FPP, in order to determine whether its TM1 and/or TMc regions in Nrf1-GFP and N65-GFP (Fig. 5, c & e) are protected by membranes against proteinase attack.

The observation that some of the GFP-fused protein signal, but not the luminal CRT signal, are gradually reduced during PK digestion in membrane protection assays of Nrf1/GFP (Fig. 5e) suggests that some regions of Nrf1 could direct the luminal GFP-containing peptides to be partially repositioned across membranes into the cyto/nucleoplasmic side where they are not protected by membranes so as to be proteolytically digested. However, this phenomenon was not apparently observed in the membrane PK protection assays of either DsRed/N65/GFP (Fig. 5b) or N65/GFP fusion proteins (Fig. 5c).

On the base of FPP, we employed double fluorescence (DsRed and GFP) sandwiched proteins in membrane proteinase protection reactions (called dFPP, Fig. 5, b & d),). Theoretically, attachment of the DsRed (comprising 238 aa with 27.2 kDa) immediately to the N-terminus of TM1-associated NHB1 signal sequence in Nrf1 should not affect the topology of TM1 within membranes. This is due to the fact that the net charge of DsRed (KR/ED = 32:32; pI 7.10) is zero such that the resulting sandwiched proteins can also abide by the positive-inside and charge difference rules between the TM1-flanking sequences that determine its topological orientation of N_{cyt}/C_{lum} within the prototypic Nrf1 protein within membranes. Furthermore, DsRed is also much smaller than the cytoplasmic domains [comprising 364 aa and 517 aa) of ATF6 and SREBP1, with net positively charged DNA-binding domains (+12 and +16) that do not alter their N_{cyt}/C_{lum} orientation spanning membranes (Fig. S2B). In fact, our data that have been presented (Fig. 5) demonstrate that after TM1 anchors relevant fusion proteins within membranes, the DsRed portion is positioned on the cyto/nucleoplasmic side of membranes, where it is almost completely digested by PK, whereas the GFP epitope is partitioned into the luminal side, where it is protected by membranes, but if the luminal GFP-fused peptides would be partially repartitioned out of membranes into the cytoplasm, they could also be gradually digested.

Figure S3

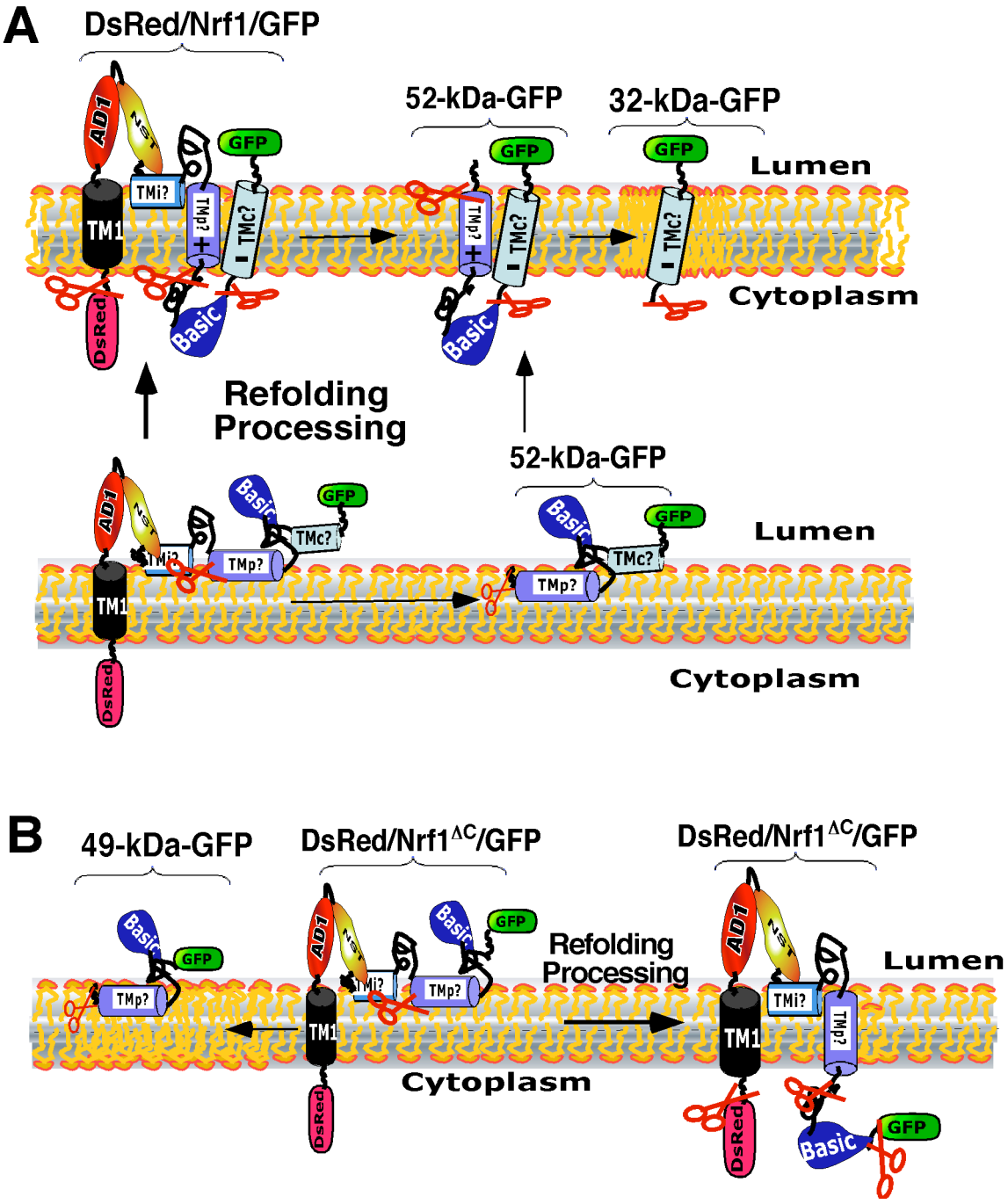


Figure S3. Two different models proposed to depict the possible membrane-topological folding of Nrf1.

Based on the data shown in Figures 5 and 6, together with other data obtained from the glycosylation mapping, mutagenesis mapping and bioinformatics (unpublished)[15], we propose two different model that the possible membrane-topological folding of DsRed/Nrf1/GFP (A) or DsRed/Nrf1$^{\Delta C}$/GFP (B), which is determined by its TM1, which adopts an N_{cyt}/C_{lum} orientation within membranes. Besides TM1, the putative TMc region may be also required for proper folding of Nrf1 with a similar orientation to that of TM1 within membranes, because both the full-length DsRed/Nrf1/GFP and its 52-kDa GFP-containing fragment appeared more sensitive to PK digestion and thus gave rise to a 32-kDa GFP-fused peptide with the putative TMc region of 4~5 kDa facing the ER lumen. By contrast, the full-length DsRed/Nrf1$^{\Delta C}$/GFP appeared to be rapidly degraded to produce a 49-kDa GFP-fused polypeptide, but the 49-kDa peptide lacking TMc was more resistant to PK so that it was not further digested. These results have led us to predict that their large C-terminal domains of a fraction of Nrf1 (or Nrf1$^{\Delta C}$) that connect with GFP are initially translocated into the lumen of ER, and that subsequently, some regions of the luminal portions are reintegrated into membranes and/or repartitioned out of membranes through a possible cooperation of TMc with TMp, before they were exposed to proteases in extra-luminal subcellular compartments. An additional possibility that cannot be ruled out is that some of the luminal portions of Nrf1 were proteolytically processed by an unidentified luminal-resident protease. The scissors indicate the position of potential cleavage sites for PK or other proteases that are not protected by membranes.

Figure S4

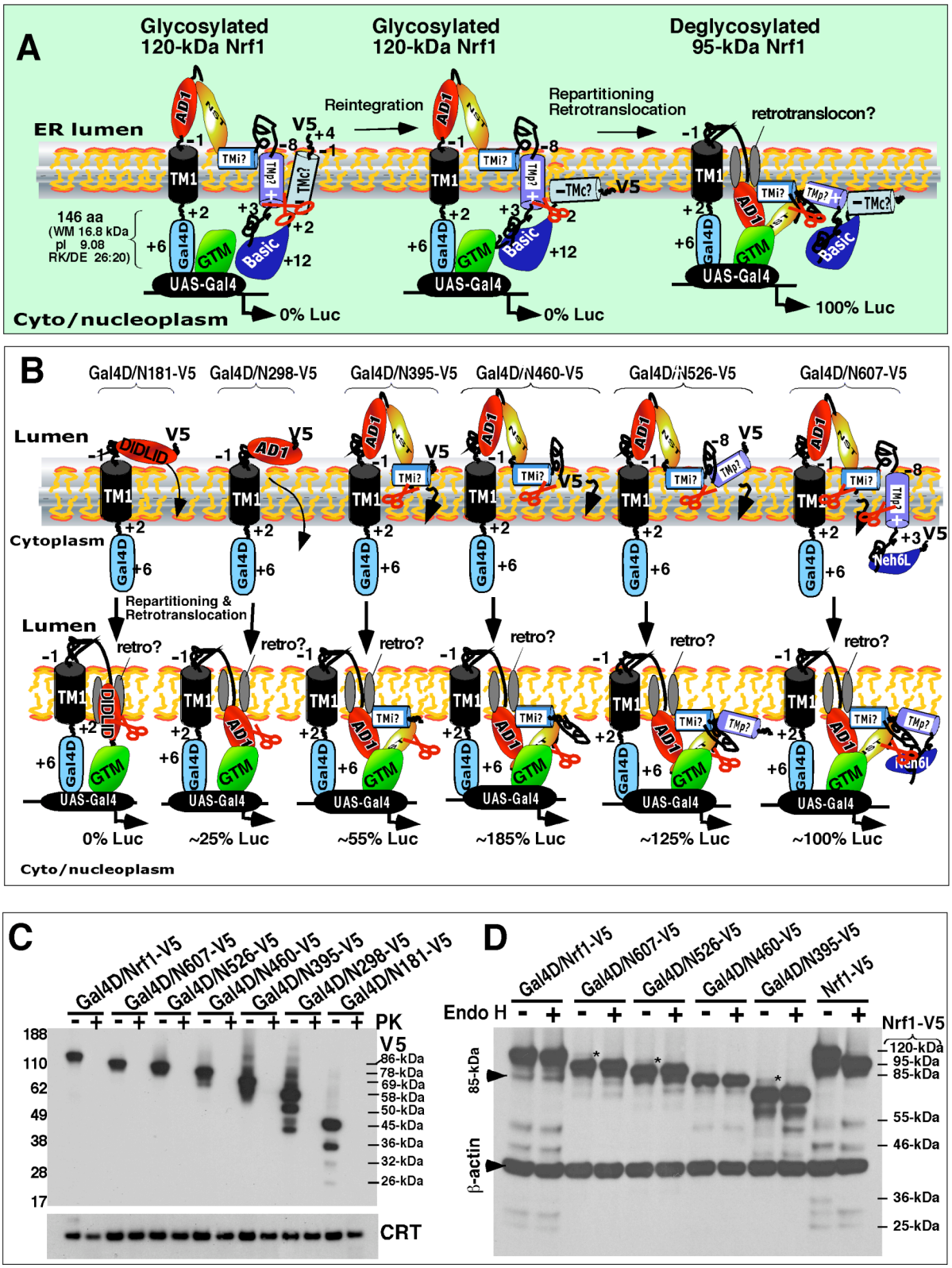


Figure S4. Vectorial behaviour of Nrf1 controls the ability of its TADs to mediate target gene expression.

(A) A model for the explanation of Gal4-based reporter assay linked with protease protection assays (called GRAPPA) to measure whether the vectorial behaviour of Nrf1 has an effect on its TADs-mediated transactivation activity of gene expression. According to the positive-inside rule, attachment of the net positively charged Gal4 DNA-binding domain (Gal4D, KR/ED = 26:20, with a mass of 16.8 kDa) immediately to the N-terminal NHB1 signal anchor sequence of Nrf1 should cause an enhancement in the folding of TM1 in the N_{cyt}/C_{lum} orientation within membranes. This notion is also consistent with the fact that the membrane-topologies of ATF6 and SREBP1 (Fig. S2B) are unaffected by the large positively-charged cytoplasmic domains of these two factors, of which their DNA-binding (bZIP and bHLH-ZIP) domains are much more basic than Gal4D. However, an additional possibility cannot be ruled out that the basic Gal4D portion of fusion

factors positioned in the cytoplasmic side of membranes could promote its partial repartitioning of the net negatively charged domains from the luminal side across membranes into the cyto/nuleoplasmic side before functioning as a *bona fide* TAD (also see Fig. S2C). It is important to note that the cyto/nucleoplasmic Gal4D portion of various fusion proteins may be tempo-spatially separated by membranes from the luminal TADs in Nrf1. Only after some of these TADs are partially repartitioned across membranes into the cyto/nulcoplasm, they could be allowed to gain access to general transcriptional machinery in order to transactivate gene expression. Therefore, the vectorial behaviour of Nrf1 appears to dictate its membrane-topogenesis and target gene transactivation.

(B) The GRAPPA was employed to examine whether the membrane-topogenic vectorial behaviour of Nrf1 controls its ability of the various TADs to transactivate gene expression. The GRAPPA was used to examine:

i) whether the TM1-associated NHB1 sequences in Gal4D/Nrf1 fusion proteins were proteolytically processed to disassociate from TADs of Nrf1. If doing so, the Gal4D portion would be released from membranes, Gal4-UAS-driven reporter gene is unable to be activated by TADs of Nrf1. However, the fact that this reporter gene activity is activated by Gal4D/Nrf1 fusion factor suggests that NHB1 is not cleaved off *via* a mechanism similar to RIP accounting for ATF6 and SREBP-1, as consistent with our previous work[10,11].

ii) Whether the TADs of Nrf1, along with the Gal4D portion, are positioned in the cyto/nucleoplasmic side of membranes. If doing so, the Gal4-based reporter gene should be directly activated by AD1 (an essential TAD of Nrf1). However, the finding that the reporter gene expression is not sufficiently activated by either a portion of AD1 in Gal4D/N181-V5 or by the entire AD1 in Gal4D/N298-V5 suggests that AD1 within some fractions of Nrf1 may be translocated in the lumen; this notion is also supported by glycosylation mapping data (unpublished)[15].

iii) Whether the translocated TADs of Nrf1 is retained in the lumen. If doing so, the extra-luminal Gal4-reporter gene is unable to be activated by TADs in Gal4D/Nrf1 fusion factors. However, the evidence for N-linked glycosylation of wild-type Nrf1 and its fusion factors, together with glycosylation mapping data (unpublished)[15], demonstrates that the NST-adjoining TADs are initially translocated in the lumen. The fact that target reporter genes driven by ARE and UAS are, to varying extents, transactivated by Nrf1 and Gal4D/Nrf1 fusion factors (i.e. Gal4D/N395-V5, Gal4D/N460-V5, Gal4D/N526-V5, Gal4D/N607-V5 and Gal4D/Nrf1-V5), respectively, demonstrates that some regions of TADs are partially repartitioned across membranes into the cyto/nucleoplasmic side, allowing interaction of the TADs with general transcriptional machinery (GTM) to transactivate reporter gene expression.

The fact that the first N-terminal 50 aa of Gal4D are essential for its cognate DNA-binding to the reporter gene suggests that only the full-length proteins of Gal4/Nrf1 fusion factors (i.e. Gal4D/N298-V5) enable transactivation of the Gal4-target reporter gene mediated by TADs of Nrf1. Therefore, it is postulated that those degraded isoforms arising from the Gal4D portion of various Gal4/Nrf1 fusion factors should be non-functional. As such, these smaller isoforms were also recovered in the ER membrane factions expressing either Gal4D/N298-V5 or Gal4D/N181-V5 with various lengths of Gal4D (Fig. 6, C & D), suggesting that some regions of NTD may direct Nrf1 to target for a degradation pathway, which remains to be further identified. Nonetheless, the production of these degraded isoforms is prevented in the cases of Gal4D/N395-V5, Gal4D/N460-V5, Gal4D/N526-V5 and Gal4D/N607-V5 (Fig. 6, G & H), suggesting a requirement of the NST glycodomain together with its flanking domains for the correct folding of Nrf1 in the ER. Overall, the ability of these fusion proteins to transactivate the Gal4D-UAS reporter gene is considered as a measure of their membrane-topogenic behaviours in distinct vectorial processes. The luciferase reporter activity is recalculated after normalization to the value obtained from Gal4D/Nrf1-V5 factor as shown by the percentage (100%). In addition, the arrows represent the luminal domains that may be partially repartitioned and retrotranslocated through an unclear mechanism (marked by 'retro?') across membranes into the cytoplasmic side. This process may be monitored through Hrd1- and VCP/p97-mediated extraction pathway[16,17], but the detailed mechanisms remain to be further elucidated.

(C) The residual amounts of the luminal-resident polypeptides and its fragments are completely digested following prolonged incubation with PK. Prolongation of the ER membrane PK protection reactions of the indicated Gal4D/Nrf1 fusion proteins over 60 min. These seven fusion proteins and their PK-digested polypeptides migrated with masses of between 135 kDa and 26 kDa.

(D) A fraction of the luminal-resident polypeptides is glycosylated in the ER, and some of other fractions may be deglycosylated in the extra-luminal subcellular compartments. Deglycosylation digestion was performed on the indicated total cell lysates (30 μg protein) using 500 units of endoglycosidase (Endo) H for 60 min. The digests were analyzed by western blotting with the V5 antibody. The star (*) indicates some of the glycosylated proteins that migrated slowly during electrophoresis in the 4-12% LDS/NuPAGE gel. Several isoforms of Nrf1 with distinct molecular weights, and β-actin are indicated.

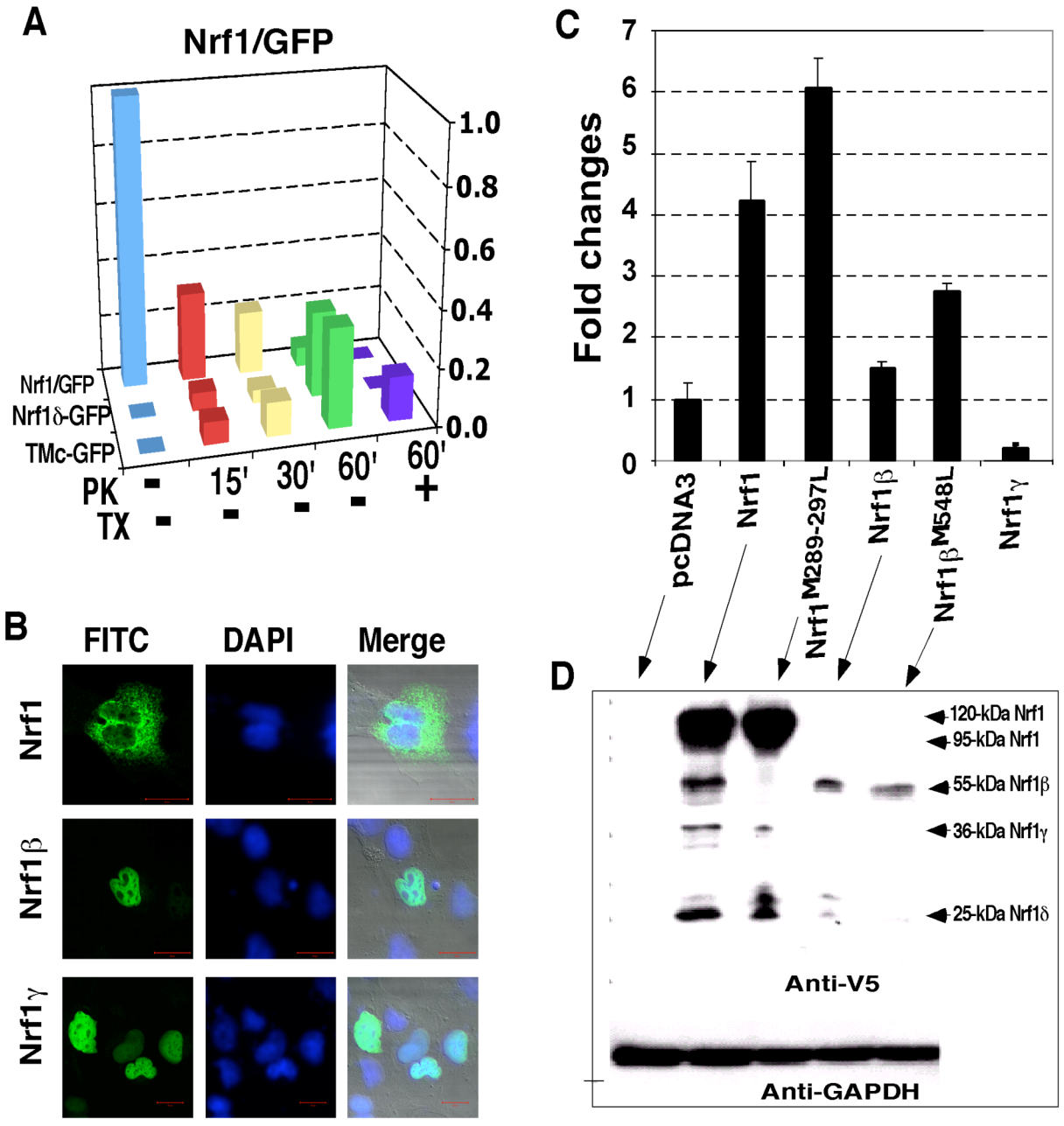
Figure S5. Newly-translated short Nrf1 isoforms cannot be tethered to ER membranes.

(A) A residual amount of GFP-tagged Nrf1 proteins is digested to yield two peptides of 52-kDa and 32-kDa with its TMc region facing the ER lumen. Membrane PK protection assays of ER-rich fractions expressing the wild-type Nrf1/GFP fusion proteins were performed as described (Fig. 5d), followed by immunoblotting with GFP antibodies. The intensity of immunoblots was calculated after normalization to the value of CRT as shown graphically. The results showed that ~78% of the GFP-tagged Nrf1 proteins was digested following 15-min incubation with PK, whereas the continuous incubation for 60 min could allow the remaining 22% of this protein to be further digested by PK to yield two smaller peptides of 52-kDa and 32-kDa (both include Nrf1γ and its TMc region fused with GFP, respectively). The data suggest that the membrane-topology of Nrf1 is dynamic with its TMc regions being folded into two different location fashions; this region may be either positioned on the cytoplasmic side of membranes, where it is not protected from PK digestion, or be partitioned into the luminal side in close proximity to membranes so that it can be partially protected against PK, even in the presence of TX.

(B) The full-length Nrf1 is localized in the ER whereas its short isoforms Nrf1β and Nrf1γ in the nuclear compartments. COS-1 cells were transfected with expression constructs for V5-tagged Nrf1, Nrf1β and Nrf1γ (1.3 μg DNA of each) and allowed to recover for 24 h. Subcellular location of these proteins was examined by immuocytochemistry followed by confocal imaging. FITC-labelled second antibody was used to locate V5-tagged proteins. Nuclear DNA was stained by DAPI. The merge signal represents the results obtained when the two images were superimposed with DIC from normal light microscopy. Bar = 20 μm. In addition, immuno-electronic microscopy along with subcellular fractionation revealed that the full-length Nrf1, but neither Nrf1β nor Nrf1γ, is also localized in the nuclear membranes[10, 11]. These findings, together with protection assay data, indicate that TM1 determines the folding of Nrf1 within the ER and nuclear membranes, and that upon the absence of TM1, the TMc or TMp regions alone cannot serve as a stable integral membrane-spanning structure that tether the new-translated Nrf1β and Nrf1γ proteins to membranes.

(C, D) Prevention of internal translation of Nrf1 to yield short isoforms Nrf1β and Nrf1δ causes an increase in its transactivation activity. The V5-tagged Nrf1, Nrf1β and Nrf1γ and their point mutant (Met into Leu) proteins are indicated. Expression constructs for Nrf1, Nrf1β, Nrf1γ or their mutants (1.2 μg of DNA each), together with both P_{SV40}*nqo1-ARE*-Luc (0.6 μg) and β-gal (0.2 μg) reporters were cotransfected into COS-1 cells. At 24-h following transfection, luciferase activity was measured and normalized to that of β-gal. The data are shown graphically as fold changes (mean ± S.D. in plane **C**) that were calculated from three different independent experiments performed in triplicate. The ectopic expression of these proteins in COS-1 cells was also visualized by western blotting with V5 antibody. GAPDH is an internal protein loading control. Taken together with our previous data[10] and others[18, 19], these findings indicate that, when compared with the full-length Nrf1 factor, **Nrf1β** (also LCR-F1) is a weak activator of ARE-driven luciferase reporter gene transcription, whilst **Nrf1γ** and (possibly **Nrf1δ**) functions as a transcriptional repressor that has an capability to inhibit the endogenous transactivation activity.

Figure S5



ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (NSFC, key program 91129703 and project 31270879) awarded to Prof. Yiguo Zhang (University of Chongqing, China), and the Fundamental Research Funds for the Central Universities in China (CDJRC11230003) awarded to Prof. Yiguo Zhang, and by the Association for International Cancer Research (grant 09-0254) awarded to Prof. John D. Hayes and Prof. Ronald Hay (University of Dundee, UK).

Author contributions: Y.Z. designed this study, performed the experiments, analyzed the data, prepared figures and wrote the paper. J.D.H. helped analysis of the data and wrote this paper.
Competing interests: The authors declare no competing financial interests.

SUPPLEMENTAL REFERENCES

1. Dowhan, W. & Bogdanov, M. Lipid-dependent membrane protein topogenesis. *Annu. Rev. Biochem*. **78**, 515-40 (2009).
2. von Heijne, G. Membrane-protein topology. *Nat. Rev. Mol. Cell Biol*. **7**, 909-18 (2006).
3. Meindl-Beinker, N. M., Lundin, C., Nilsson, I., White, S. H. & von Heijne, G. Asn- and Asp-mediated interactions between transmembrane helices during translocon-mediated membrane protein assembly. *EMBO Rep* **7**, 1111-6 (2006).
4. Langosch, D. & Arkin, I. T. Interaction and conformational dynamics of membrane-spanning protein helices. *Protein Sci*. **18,** 1343-58 (2009).
5. Ye, J. et al. ER stress induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs. *Mol. Cell* **6**, 1355-64 (2000).
6. Bowie, J. U. Flip-flopping membrane proteins. *Nat. Struct. Mol. Biol*. **13**, 94-6 (2006).
7. Holthuis, J. C. & Levine, T. P. Lipid traffic: floppy drives and a superhighway. *Nat. Rev. Mol. Cell Biol*. **6**, 209-20 (2005).
8. Tong, K. I. et al. Keap1 recruits Neh2 through binding to ETGE and DLG motifs: characterization of the two-site molecular recognition model. *Mol. Cell Biol*. ***26*,** 2887-900 (2006).
9. Bogdanov, M., Xie, J. & Dowhan, W. Lipid-protein interactions drive membrane protein topogenesis in accordance with the positive inside rule. *J. Biol. Chem*. **284**, 9637-41 (2009).
10. Zhang, Y., Lucocq, J. M. & Hayes, J. D. The Nrf1 CNC/bZIP protein is a nuclear envelope-bound transcription factor that is activated by t-butyl hydroquinone but not by endoplasmic reticulum stressors. *Biochem J.* **418**, 293-310 (2009).
11. Zhang, Y., Lucocq, J. M., Yamamoto, M. & Hayes, J. D. The NHB1 (N-terminal homology box 1) sequence in transcription factor Nrf1 is required to anchor it to the endoplasmic reticulum and also to enable its asparagine-glycosylation. *Biochem J.* **408**, 161-72 (2007).
12. Zhang, Y. & Hayes, J. D. Identification of topological determinants in the N-terminal domain of transcription factor Nrf1 that control its orientation in the endoplasmic reticulum membrane. *Biochem J*. **430**, 497-510 (2010).
13. Lorenz, H., Hailey, D. W., Wunder, C. & Lippincott-Schwartz, J. The fluorescence protease protection (FPP) assay to determine protein localization and membrane topology. *Nat. Protoc*. **1**, 276-279 (2006).
14. Lorenz, H., Hailey, D. W. & Lippincott-Schwartz, J. Fluorescence protease protection of GFP chimeras to reveal protein topology and subcellular localization. *Nat. Methods* **3**, 205-210 (2006).
15. Zhang, Y., Li S., Chen J., & Hayes, J. Topological reparttioning of Nrf1 across membranes to transactivate cytoprotective genes through its acidic glucose-responsive domains. *Submitted to Sci Rep* (2013).
16. Steffen, J., Seeger, M., Koch, A. & Kruger, E. Proteasomal degradation is transcriptionally controlled by TCF11 via an ERAD-dependent feedback loop. *Mol. Cell* **40,** 147-58 (2010).
17. Tsuchiya, Y. et al. Dual Regulation of the Transcriptional Activity of Nrf1 by beta-TrCP- and Hrd1-Dependent Degradation Mechanisms. *Mol. Cell Biol*. **31**, 4500-12 (2011).
18. Caterina, J. J., Donze, D., Sun, C. W., Ciavatta, D. J. & Townes, T. M. Cloning and functional characterization of LCR-F1: a bZIP transcription factor that activates erythroid-specific, human globin gene expression. *Nucleic Acids Res*. **22**, 2383-91 (1994).
19. Novotny, V., Prieschl, E. E., Csonga, R., Fabjani, G. & Baumruker, T. Nrf1 in a complex with fosB, c-jun, junD and ATF2 forms the AP1 component at the TNFα promoter in stimulated mast cells. *Nucleic Acids Res*. **26**, 5480-5 (1998).