The prion protein N2 fragment mitigates stress-induced intra-cellular ROS production by modulating endocytosis-dependant MEK1 signalling; **Cellular and Molecular Life Sciences**; Haigh, CL*. McGlade, AR. Collins, SJ. *Department of Pathology, The University of Melbourne, Australia, 3010, chaigh@unimelb.edu.au

Supplementary Figure 1. CF10 cell responses to serum deprivation. A. Trypan blue exclusion was used to determine the proportion of cells that retained membrane integrity over four days after serum withdrawal. Counts were performed using a Countess (Invitrogen) cell counter. No change in viability was observed between the serum deprived cells and cells incubated with 10% (v/v) serum (Two-way ANOVA, F = 2.36, p = 0.138, n = 4). B. Cytotoxicity, as determined by LDH release from cells, was assayed 24 hours post serum deprivation. No significant increase in LDH release was seen following serum withdrawal (students t-test, t = 0.149, p = 0.889, n = 3). C. Metabolism of PrestoBlue, a formazan based dye, shows a significant increase in signal over normal serum control cells (students t-test, t = 3.17, p = 0.043, n = 3). **D** & E. TMRM mitochondrial membrane potential stain. **D**. Cells were loaded with mitotracker green (which accumulates in polarised mitochondria and becomes covalently linked) under normal culture conditions then transferred into serum +/media for 30 minutes. Media was then changed to include the TMRM membrane potential dye (Invitrogen) and FRET between the two fluorophores measured. A change in FRET efficiency would be indicative of mitochondrial membrane permeabilisation, which results in functional impairment of the organelle [1,2]. No change in FRET efficiency is seen (students t-test, t = 0.408, p = 0.698, n = 4) and the relative amounts of TMRM taken up by the serum deprived cells (E) are the same as that measured for cells incubated under normal serum conditions (students t-test, t = 0.637, p = 0.548, n = 4). F. Proxyl-Fluorescamine spin trapping shows a significant increase in the rate of free radical binding (and therefore fluorescence production) in the serum deprived cells (students t-test, t = 2.473, p = 0.045, n =4).

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