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 4**. *PrP23-89 does not change cellular ATP levels or lysosomal acidity during serum starvation*. Cells were serum starved without and with 1  $\mu$ M PrP23-89 alone, 1  $\mu$ M PrP23-89 with 4  $\mu$ M CuCl<sub>2</sub> or with 4  $\mu$ M CuCl<sub>2</sub> alone and incubated under normal culture conditions for 24 hours. After 24 hours cells were either (**A**) lysed for ATP assay or (**B**) stained with Lysosensor Green. (**A**). Cells were lysed in RIPA buffer (see methods) and assayed for ATP content using Molecular Probes' luminescent ATP determination kit (Invitrogen) as per the product protocol. Protein content of lysates was also determined in parallel by BCA assay. Hollow bars show serum deprived ATP concentrations with the addition of PrP23-89 making no difference to cellular ATP levels in response to serum deprivation. (**B**) Cells were incubated for 1 h in normal growth media containing 1  $\mu$ M Lysosensor-green (Invitrogen) as per the manufacturer's instructions. Images were collected using a Nikon epi-fluorescence microscope. No changes in lysosomal acidity (fluorescence intensity) or morphology were observed for any treatment.

