**Supplementary Figure 6**. *RedoxSensor Red analysis of PrP23-50 influence on intracellular ROS production*. CF10 cells were labelled with RedoxSensor Red fluorescent indicator, and MitoTracker green to indicate compartmental partitioning, then incubated in normal (10% v/v serum), serum-free or serum-free with 1 μM PrP23-50 phenol-red free OptiMEM. At 90 minutes images were collected and cellular fluorescence quantified with MitoTracker co-localisation used to determine the cytosolic and lysosomal fractions. Shown below are representative plates of each condition (**A**) and quantification of total fluorescence (**B**), cytosolic fractions (**C**) and lysosomal fractions (**D**). Serum deprivation significantly increases RedoxSensor Red fluorescence (**B**; $F = 19.26$, $p < 0.001$, $n = 3$) and this is restored to the levels of ROS produced under normal culture conditions by PrP23-50. The cytosolic component (fluorescence co-localised with MitoTracker) of ROS produced was not significantly increased in the serum deprived cells, however PrP23-50 still reduced this significantly (**C**; $F = 5.668$, $p = 0.006$, $n = 3$). The main increase in ROS seen in response to serum deprivation was in the lysosomes and this was significantly reduced by PrP23-50 (**D**; $F = 65.09$, $p < 0.001$, $n = 3$). Scale bars = 20 μm.

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