## Krzysztof Kucharz SUPPLEMENTARY VIDEOS:

## PAPER 1:

NMDA receptor stimulation induces reversible fission of the neuronal endoplasmic reticulum.

Kucharz K, Krogh M, Ng AN, Toresson H PLoS ONE 4:e5250. :2009



<u>Video S1</u>: Reversibility of ER fission. Time-lapse recording of a neuron transfected to express RedER exposed to 20  $\mu$ M glutamate at t = 5 min resulting in rapid ER fission. 25  $\mu$ M MK-801 was added at t = 11 min and within 20 min the ER vesicles fused. Pixel dimensions: 151×303, frame rate: 25 fps, images collected at 1 Hz.



<u>Video S2:</u> FRAP experiment on untreated CA1 neuron. Time-lapse recording of a neuron in an organotypic slice expressing RedER under the Thy1 promoter (line 18). Bleaching was performed after the 5th frame (t = 24 s) in the ROI marked with a white rectangle. After bleaching fluorescence recovered rapidly indicating that the ER was continuous. This neuron was not included in the analysis. Pixel dimensions: 366×341, frame rate: 8 fps, images collected at 0.2 Hz.



<u>Video S3:</u> FRAP experiment on glutamate treated CA1 neuron. Time-lapse recording of a neuron in an organotypic slice expressing RedER under the Thy1 promoter (line 18). 100  $\mu$ M glutamate was added 5 min prior to recording. Bleaching was performed after the 5th frame (t = 24 s) in the ROI marked with a white rectangle and a clear reduction in FRAP was noted. Pixel dimensions: 365×348, frame rate: 8 fps, images collected at 0.2 Hz.

## PAPER 2:

Reversible Endoplasmic Reticulum fission in murine hippocampal pyramidal neurons in organotypic hippocampal slices is dependent on extracellular Ca<sup>2+</sup>, NMDA receptor activation and is augmented by hypothermia.

Kucharz K, Wieloch T and Toresson H. Submitted to European Journal of Neuroscience.



Supplementary Video 1: Potassium-induced reversible ER fission.

The video shows the reversible ER fission in CA2 hippocampal neuron in slice expressing RedER. The neuron was treated as described in results section. The video starts exactly at the moment where K<sup>+</sup>ACSF is introduced to imaging chamber. After 20 seconds, the first sign of fragmentation can be detected. After 1.5 minute of exposure to K<sup>+</sup>, the slice was washed with ACSF and the subsequent recovery of ER continuity could be observed. The video was recorded with frequency 0.75 Hz and is being displayed in 25 fps in 2 frame step. The video has been exported in .mov format. Scale bar = 10  $\mu$ m.

Rapid Endoplasmic Reticulum fragmentation in cortical neurons of the mouse brain following cardiac arrest. An in vivo study. Kucharz K, Wieloch T and Toresson H. Manuscript.



<u>Supplementary Video 1. 3D reconstruction of continuous ER *in vivo*.</u> The animation shows the 3D reconstruction of continuous ER structure in dendrite in pyramidal neurons of superficial cortex layer before the cardiac arrest.



Supplementary Video 2. Timelapse recording of ER fragmentation *in vivo*. The timelapse recording shows the rapid ER fission occurring after the first minute of cardiac arrest. The timer starts at the beginning of the video, 60 seconds after cardiac arrest. The ER fragmented rapidly, and the first signs of ER fission can be observed at 18th second of recording while at 31st second ER displays no detectable signs of continuity. The images were collected at 0.22 Hz, the movie is encoded to 20 frames per second. Scale bar =  $20 \mu m$ .



<u>Supplementary Video 3. 3D reconstruction of fragmented ER *in vivo*.</u> The animation shows the 3D reconstruction of fragmented ER morphology in dendrite in pyramidal neurons of superficial cortex layer 2 minutes after the cardiac arrest.