Supporting Information

IMAGING OXYGEN IN NEURAL CELL AND TISSUE MODELS BY MEANS OF ANIONIC CELL-PERMEABLE PHOSPHORESCENT NANOPARTICLES

Ruslan I. Dmitriev, Sergey M. Borisov, Alina V. Kondrashina, Janelle M.P. Pakan, Ujval Anilkumar, Jochen H.M. Prehn, Alexander V. Zhdanov, Kieran W. McDermott, Ingo Klimant, Dmitri B. Papkovsky

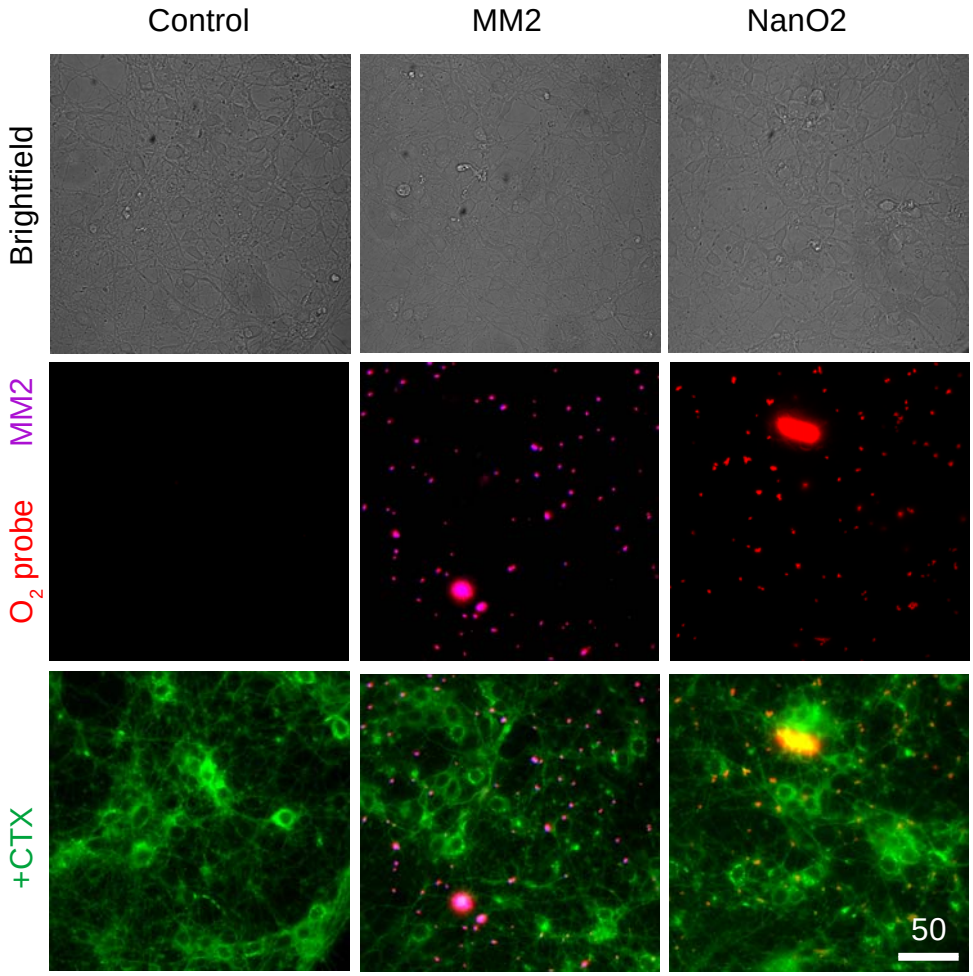

Figure S1. Fluorescence microscopy of cultured neural cells (E18, 6 DIV), stained with NanO2 (red) or MM2 (violet) at 10 μg ml^{-1} for 16 h and counter-stained with CTX-Alexa 488 (5 ng ml^{-1}, 0.5 h, green). Control: unstained cells. Scale bar in μm.

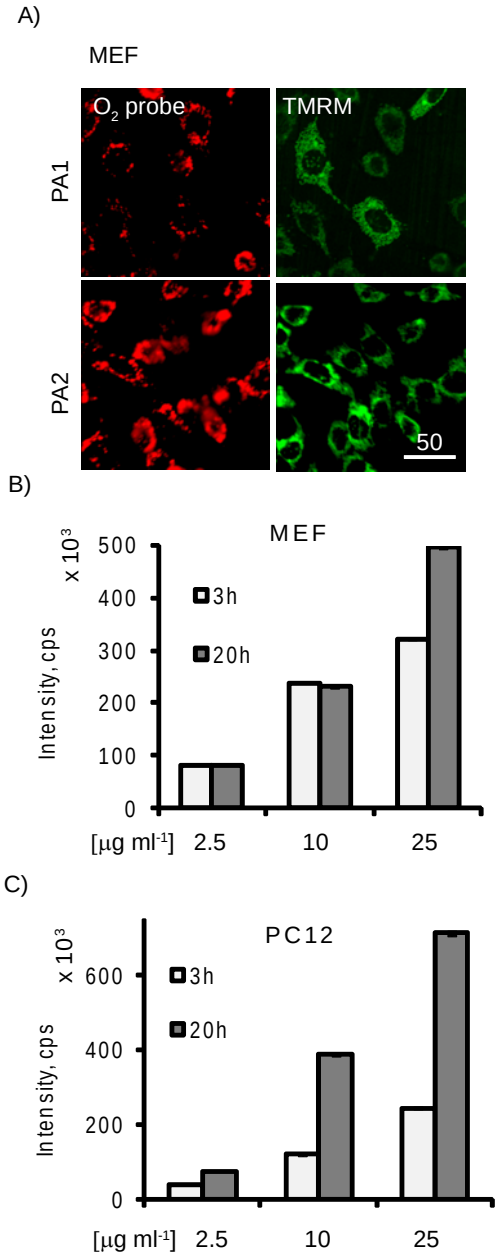

Figure S2. Staining of MEF and PC12 cells with PA2 and PA1 probes. A: confocal microscopy images of cultured MEF cells, stained with PA2 and PA1 (MEF: 10 µg ml^{-1}; 16 h, red) and counter-stained with TMRM (20 nM, green) B, C: average cell staining efficiency of PA2 probe, at different probe concentration and loading time, measured on TR-F plate reader and expressed in counts per second (cps, phosphorescence). Scale bar in µm.

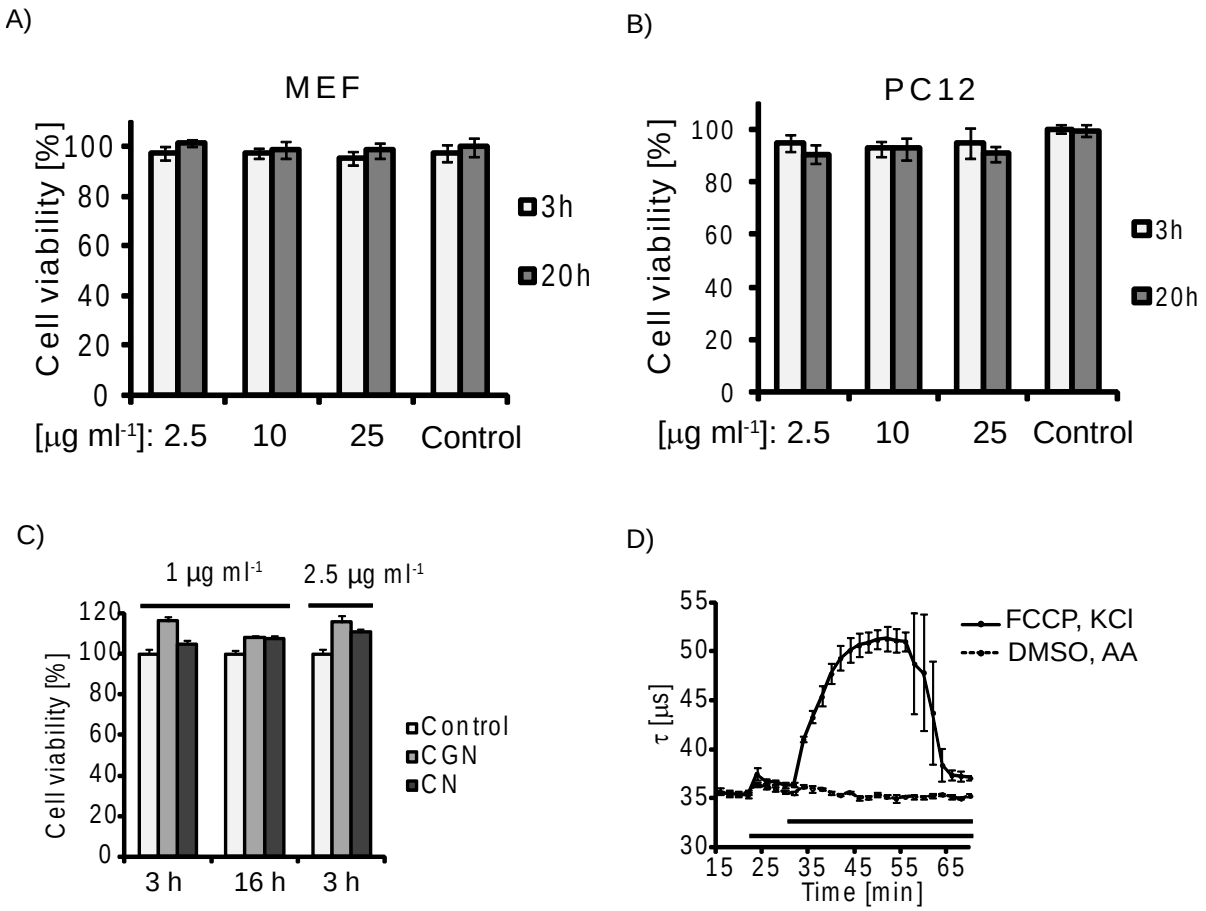

Figure S3. A-C: Assessment of cell viability effects with PA2 probe with MEF (A), PC12 (B) and primary mouse neural cells (C). Cells were stained at different probe concentration and time intervals (indicated), then lysed and analysed with CellTiter-Glo viability assay kit. CGN - cerebellar granule neurons; CN - cortical neurons. D: Monitoring of cell oxygenation (phosphorescence lifetime scale) of mouse cortical neurons (7 DIV), stained with PA2 (2.5 μg ml^{-1}; 16 h) on TRF microplate reader. After stabilisation of baseline, cells were treated with FCCP (500 nM) or DMSO (mock), then with KCl (50 mM) or AntA (10 μM), as indicated by horizontal bars (below).

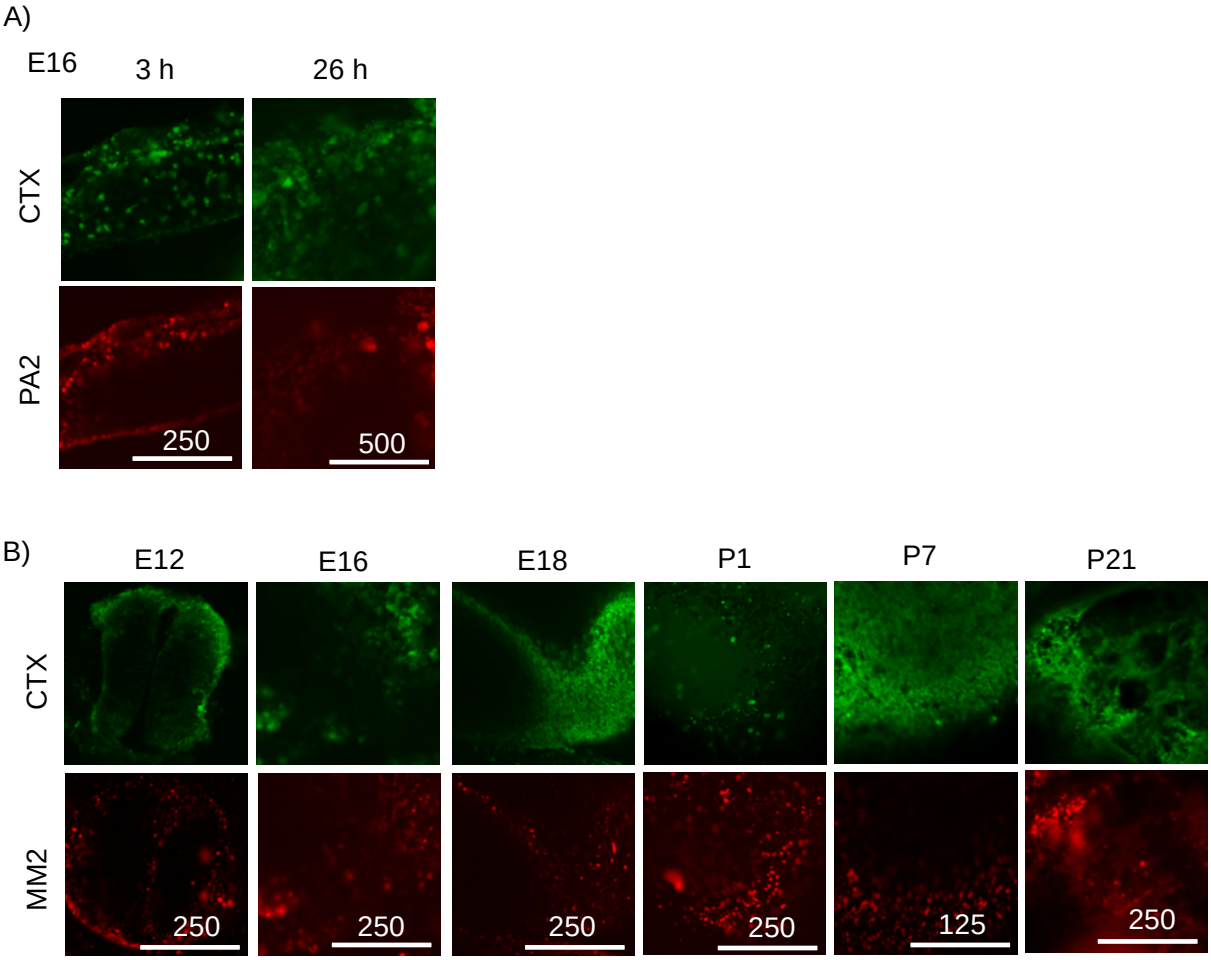

Figure S4. A: staining of cultured brain slices (E16) with PA2 for different time intervals (3, 26 h). B: comparison of staining of brain slices prepared from animals of various ages. Single optical confocal sections co-stained with CTX and MM2 (3 h). Scale bar in μm.

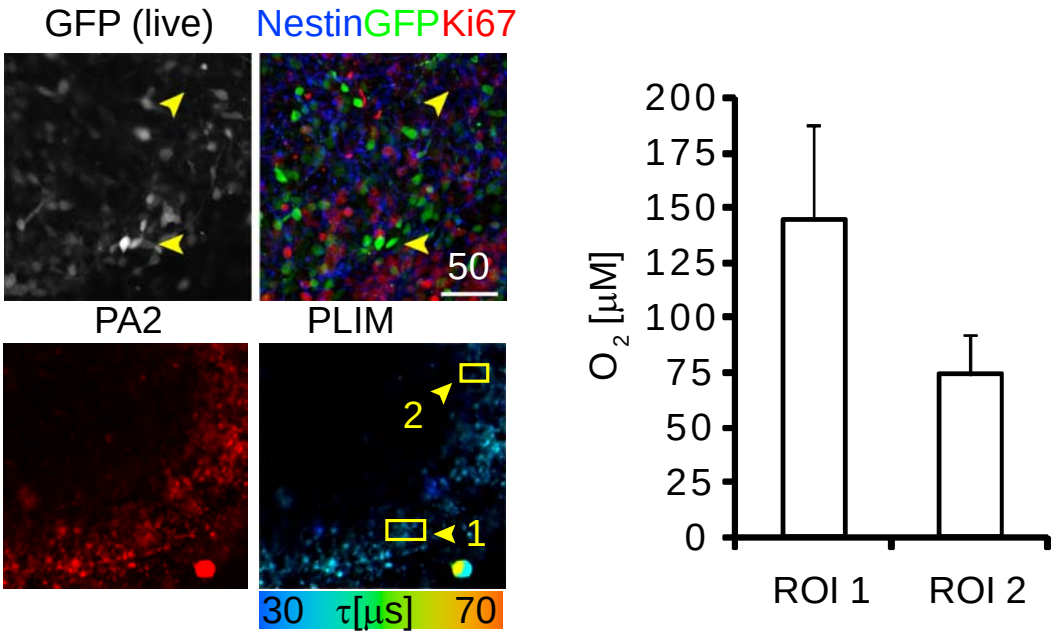

Figure S5. Correlation of spatial heterogeneity of O_2 distribution (revealed with PA2 probe staining) with BLBP-GFP marker (live and fixed samples) and immunofluorescent staining

(Nestin, Ki67) for two ROIs (yellow rectangles) within the same optical section (right). Scale bar in μm.