Supporting information

Nanoparticle-based fluoroionophore for analysis of potassium ion dynamics in 3D tissue models and *in vivo*

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Supplementary figures S1-S10

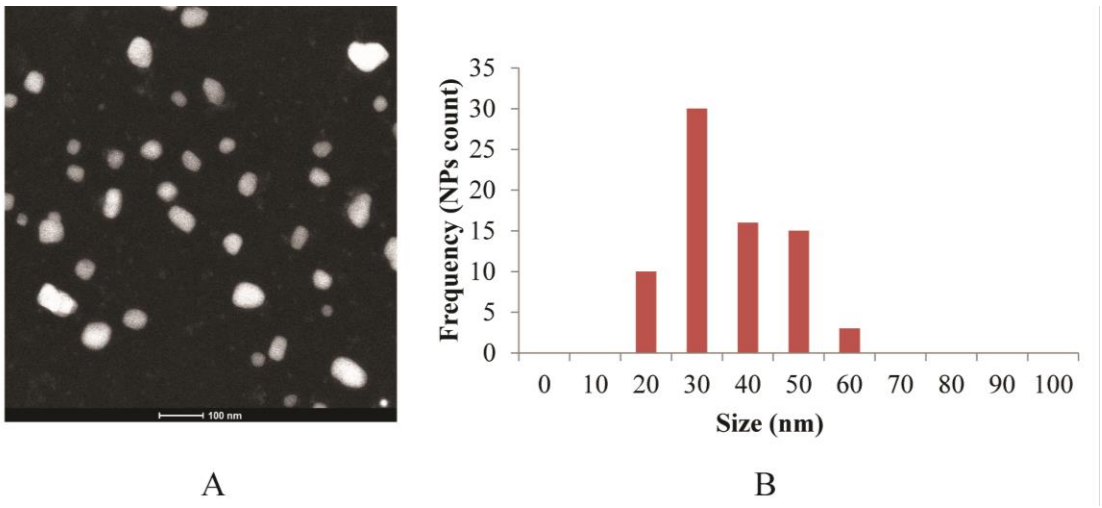

Figure S1. Analysis of size of FI3 nanoparticles by electron microscopy. STEM images of FI3 nanoparticles (scale bar 100 nm) Size distribution histogram is shown on the right.

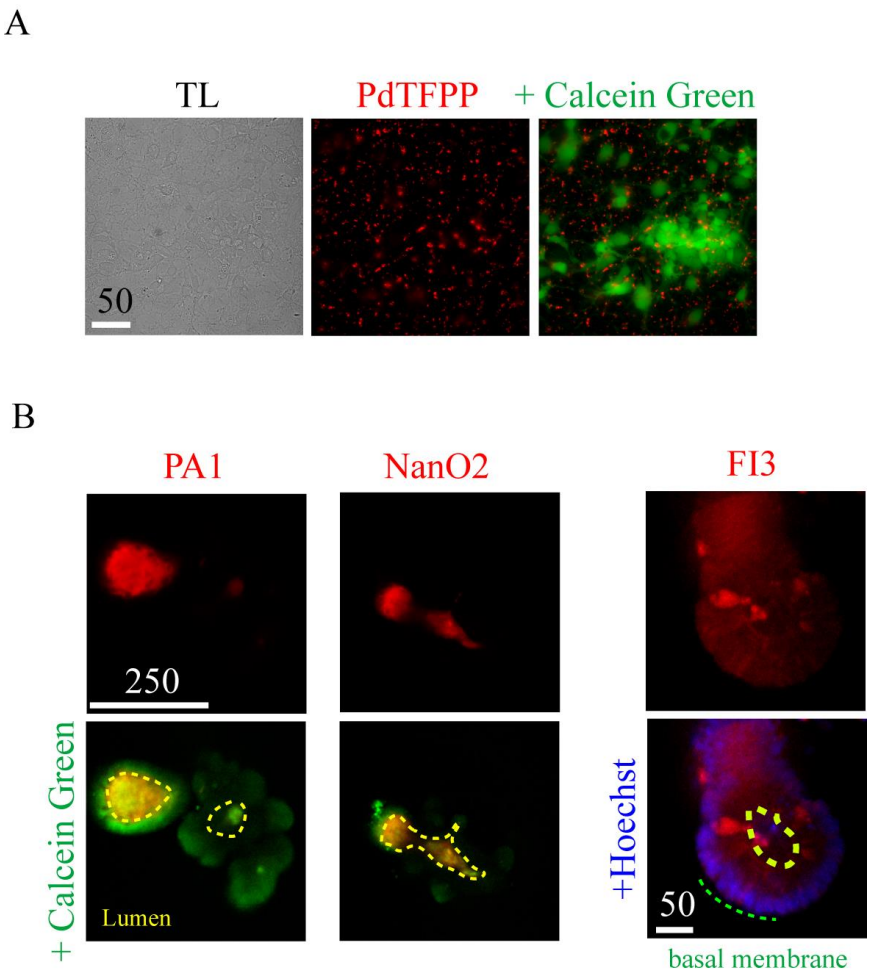

Figure S2. Poor efficiency of staining of primary neurons and intestinal organoids with conventional RL100-based nanosensors. A: staining of primary neurons with PdTFPP/ RL100 nanoparticles (10 μg/ml, 16 h), counter-stained with Calcein Green. B: Staining of mouse intestinal organoids with PA1 and NanO2 (10 μg/ml, 16 h), counter-stained with Calcein Green or Hoechst 33342 and FI3, for comparison. Scale bar is in μm.

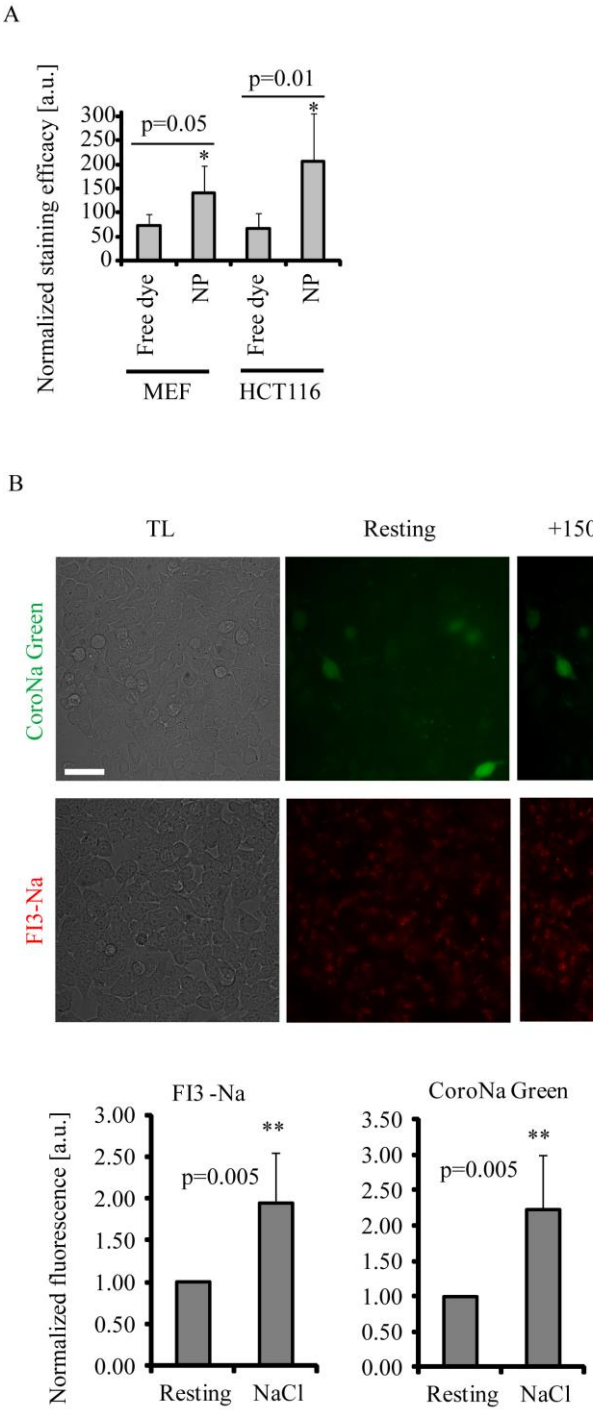

Figure S3. Comparison of staining efficacy with different cell lines and Na^+-sensitivity of FI3-Na in cells. A: Average staining efficacy of HCT116 and MEF cells with free (0.1 μM) and encapsulated in nanoparticles (10 μg/ml) FI3. Cells were incubated with dye and dye/NP at indicated concentrations (16 h), washed, imaged and quantified. B: Comparison of FI3-Na with another Na^+ indicator CoroNa Green in HCT116 cells. Cells were stained either with CoroNa Green-AM (4 μM, 0.5 h) or FI3 nanoparticles (10 μg/ml) and measured live at rest or after adding 150 mM NaCl (total ~0.3 M final concentration in medium). Normalized responses in fluorescence are shown below. N=3. Scale bar is 50 μm.

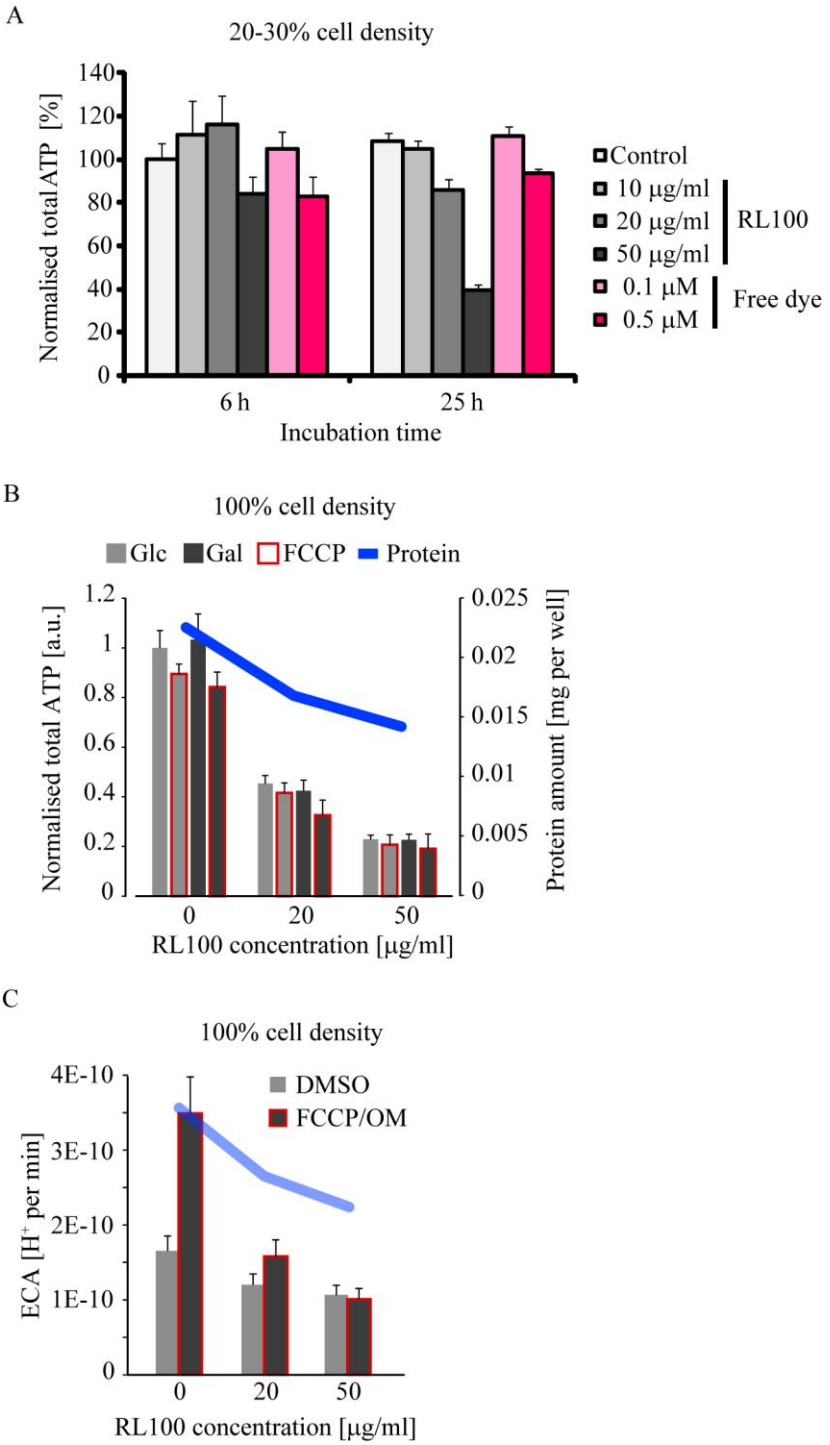

Figure S4. Evaluation of toxicity of FI3 nanoparticles with primary neural cells. Cells were grown on microplates, stained with FI3 at indicated concentration and times and then proceeded to analysis of cell energy budget by measuring total ATP and extracellular acidification. A: Total cellular ATP in cells cultured at regular 20-30% density. ATP data are normalized to total protein. B, C: Cells growing at high density and exposed to higher concentrations of FI3 and incubation in glucose- or galactose-containing media display stronger decrease of viability after treatment. B: Total cellular ATP. Blue line indicate total protein amount in the samples. C: Extracellular acidification (glycolytic flux). To achieve maximal uncoupling, cells were treated with 1 µM FCCP and 10 µM oligomycin (FCCP/OM). Error bars indicate standard deviation (N=4).

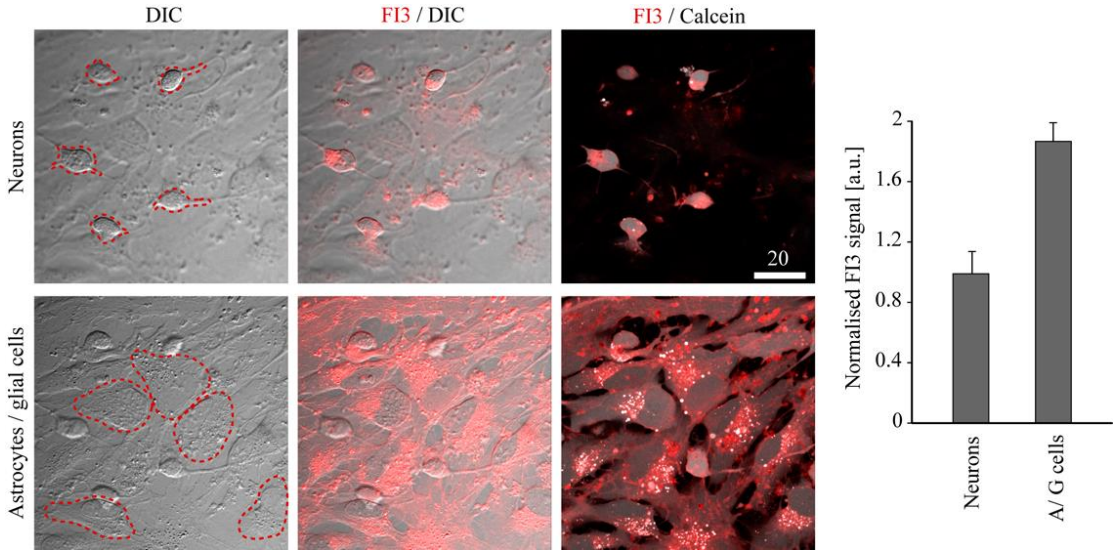

Figure S5. Comparison of cell staining with FI3 nanoparticles between neurons and astro-glial cells. The live cells were stained with FI3 (10 μg/ml, 16 h), counter-stained with Calcein Green and imaged. Representative neurons (top) and astro-glial cell (bottom) are highlighted on transmission light (DIC) and combined fluorescence images. FI3 is shown in red, Calcein Green is shown in grayscale. For calculations, 4 confocal planes (0.5 μm each) were stacked together. N = 8 (neurons) and 21 astro-glial cells. In the right panel, the calculated data are shown as m ± SEM. Scale bar is in μm.

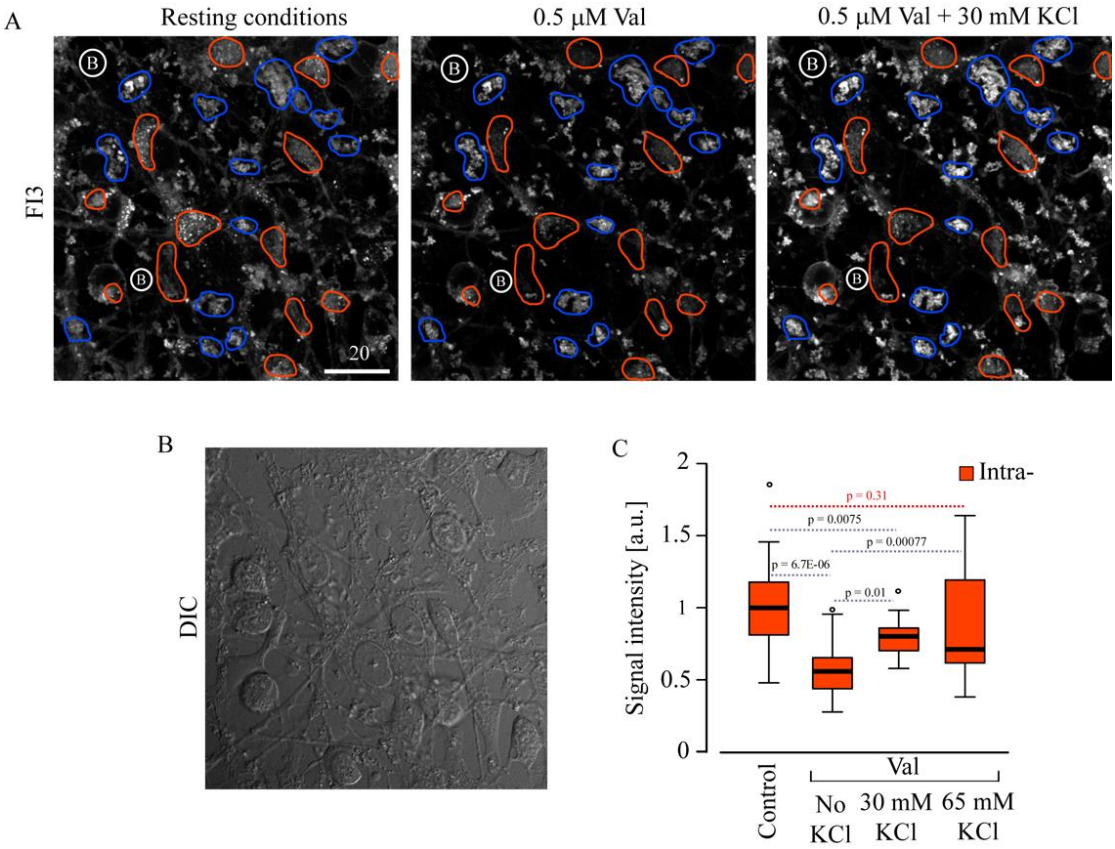

Figure S6. Stimulation of rat primary neural cells with Valinomycin and KCl. The live cells were stained with FI3 nanoparticles (10 μg/ml, 16 h) and treated sequentially with valinomycin (Val) and KCl (at different concentrations). A: Fluorescence microscopy images of resting and treated cells (8 confocal planes taken with 0.5 μm step). ROI are highlighted by red (intracellular) and blue (extracellular)

colour. B: DIC image (a single plane). C. Calculated responses of intracellular FI3 nanoparticles to the treatment; p values are shown (U test); red-colored p value shows non-significant difference in FI3 fluorescence between cells at rest and upon treatment with high KCl in the presence of Val. N= 3. Scale bar is in μm.

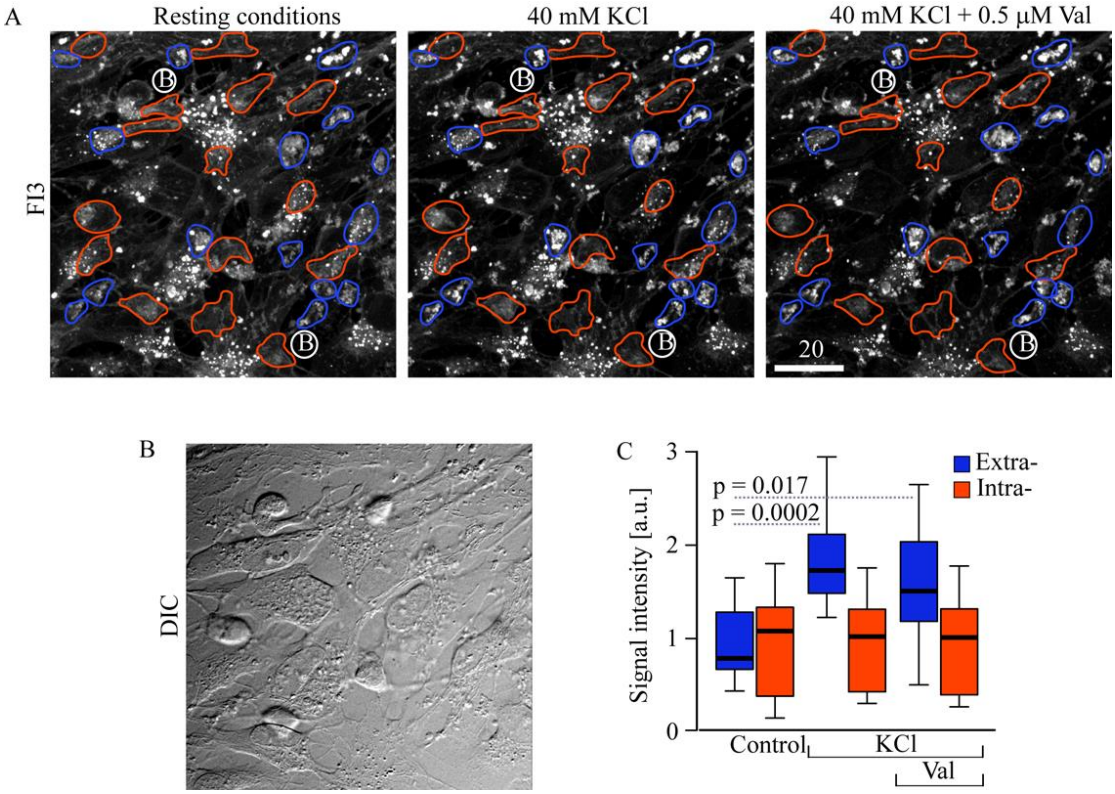

Figure S7. Stimulation of rat primary neural cells with KCl and Valinomycin. The live cells were stained with FI3 nanoparticles (10 μg/ml, 16 h) and treated sequentially with KCl and valinomycin (Val). A: Fluorescence microscopy images of resting and treated cells (8 confocal planes taken with 0.5 μm step). ROI are highlighted by red (intracellular) and blue (extracellular) color. B: DIC image (a single plane). C. Calculated responses of intra- and extracellularly located FI3 nanoparticles to the treatment; p values are shown (U test). N= 3. Scale bar is in μm.

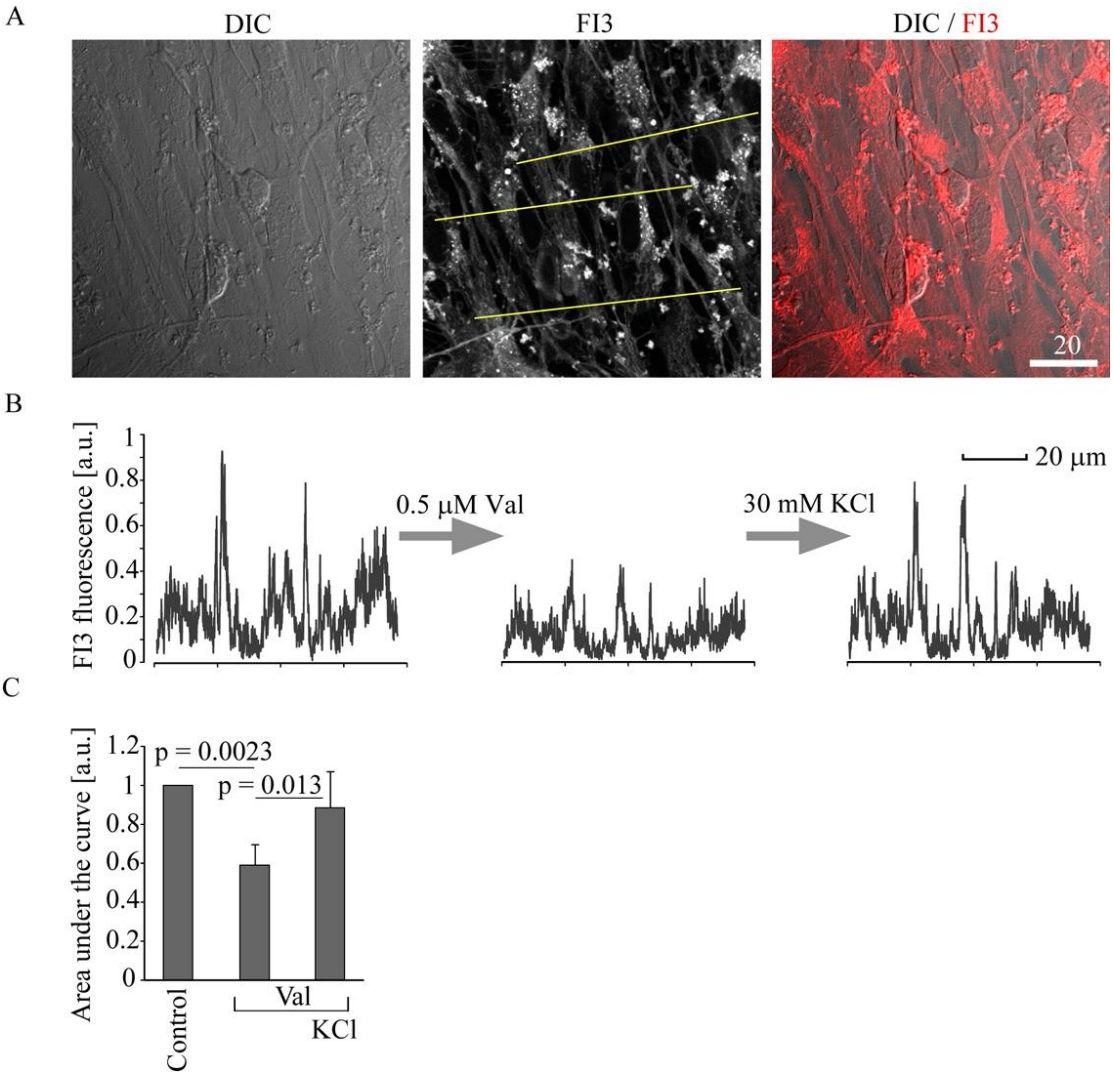

Figure S8. Stimulation of rat primary neural cells with Valinomycin and KCl. The live cells were stained with FI3 nanoparticles (10 μg/ml, 16 h) and treated with valinomycin (Val) and KCl as indicated. A: DIC (a single plane) and fluorescence microscopy images (8 confocal planes taken with 0.5 μm step). B: Line profile analysis of the changes in FI3 fluorescence, induced by treatments and (C) calculated responses in intracellular K^+. Error bars show SEM. Asterisks demonstrate significant difference ($p < 0.01$, U test). $N = 3$. Scale bar is in μm.

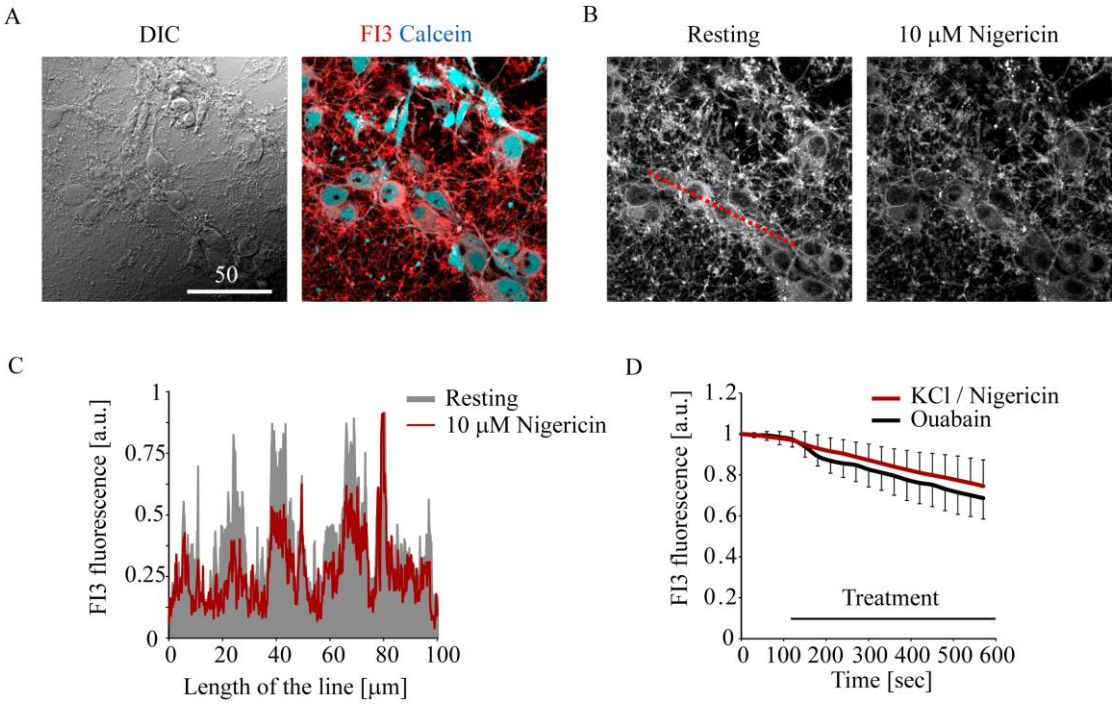

Figure S9. Stimulation of rat primary neural cells with ouabain and nigericin. Live cells were stained with FI3 (10 μg/ml, 16 h), counter-stained with Calcein Green and imaged. A: Representative image of cells shown as transmission light (DIC) and fluorescence (FI3 in red, Calcein Green in blue). Images represent stacks of 2 (DIC) or 3 (fluorescence) focal planes (0.5 μm). B. Examples of decrease in intracellular FI3 fluorescence upon addition of nigericin (10 μM, 7 min) in the presence of 20 mM KCl. C. Representative line profile analysis of the response to nigericin (dotted line is shown in B). D. Calculated reduction of intracellular FI3 fluorescence upon treatment of cells with nigericin (10 μM) and ouabain (50 μM). Scale bar is in μm.

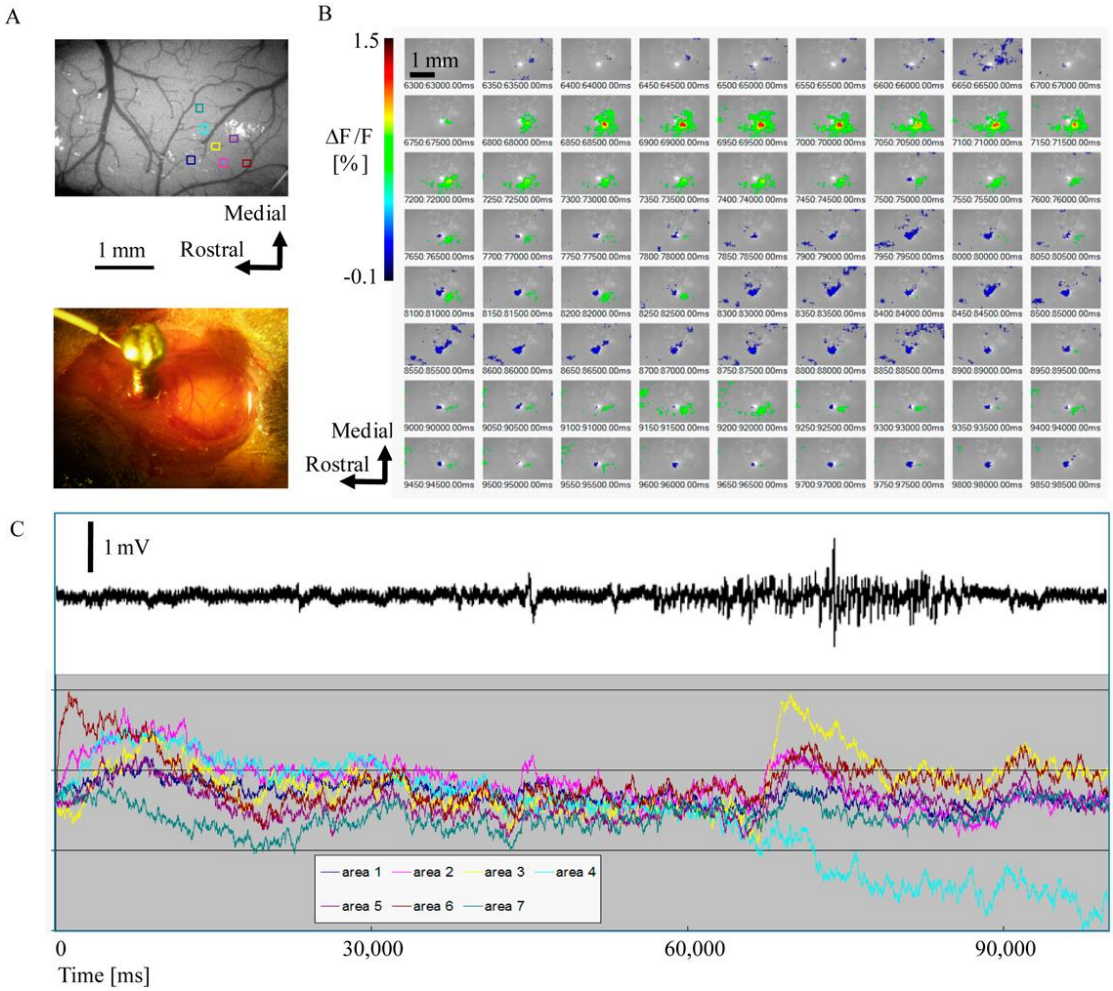

Figure S10. *In vivo* brain imaging of responses to epileptic seizures with FI3 nanoparticles. A: Region of imaging, with locations of ROI, where fluorescence was calculated. Bottom: photo of operated area (cranial window) with the screw-type electrode connected for the EEG. B: Pseudocolor images of the cortex before and after stimulus onset. C: calculated the epileptic seizures on the EEG (top) and the integrated fluorescence signal in the indicated ROI (bottom).