Electronic Supplementary Material (ESI) for Nanoscale.

Electronic Supplementary Information

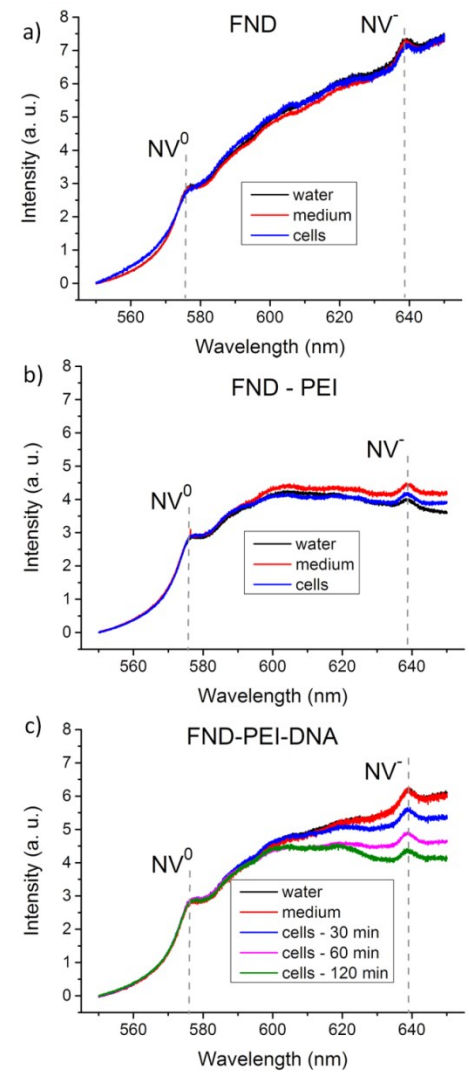


Fig. S1. The influence of media and the intracellular environment on photoluminescence spectra obtained from a Raman Renishaw inVia microscope, 514 nm excitation wavelength. Spectra show NV PL of a) oxidized FND particles in water, medium and cells; b) FND-PEI complex in water, medium and cells; c) FND-PEI-DNA complex in water, medium and cells after 30, 60 and 120 min incubation.

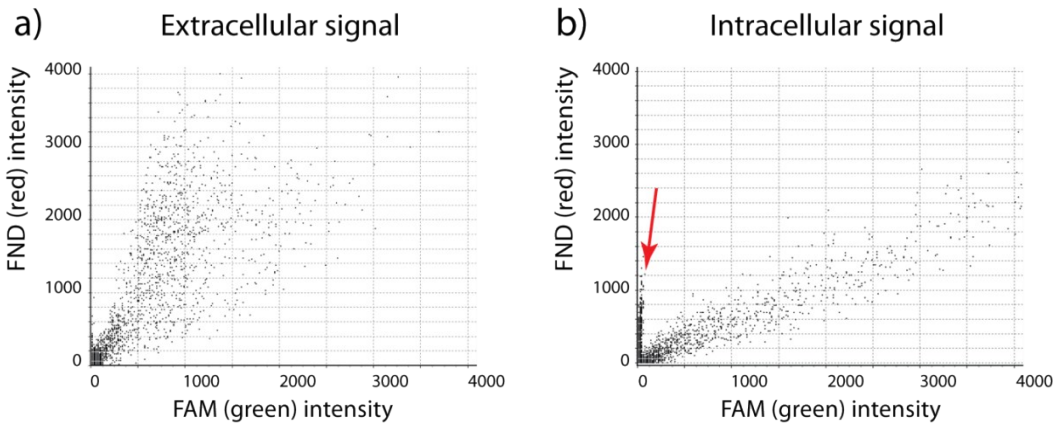


Fig. S2. Visualization of red (FND) and green (FAM-labelled DNA) signal colocalization derived by Olympus software at the end of the experimental period. Representative images of a) extracellular and b) intracellular signal distribution in IC-21 cells incubated with FND-PEI-DNA complexes for 2 h. Prominent separation of the red signal derived from DNA-free FNDs is marked with an arrow.

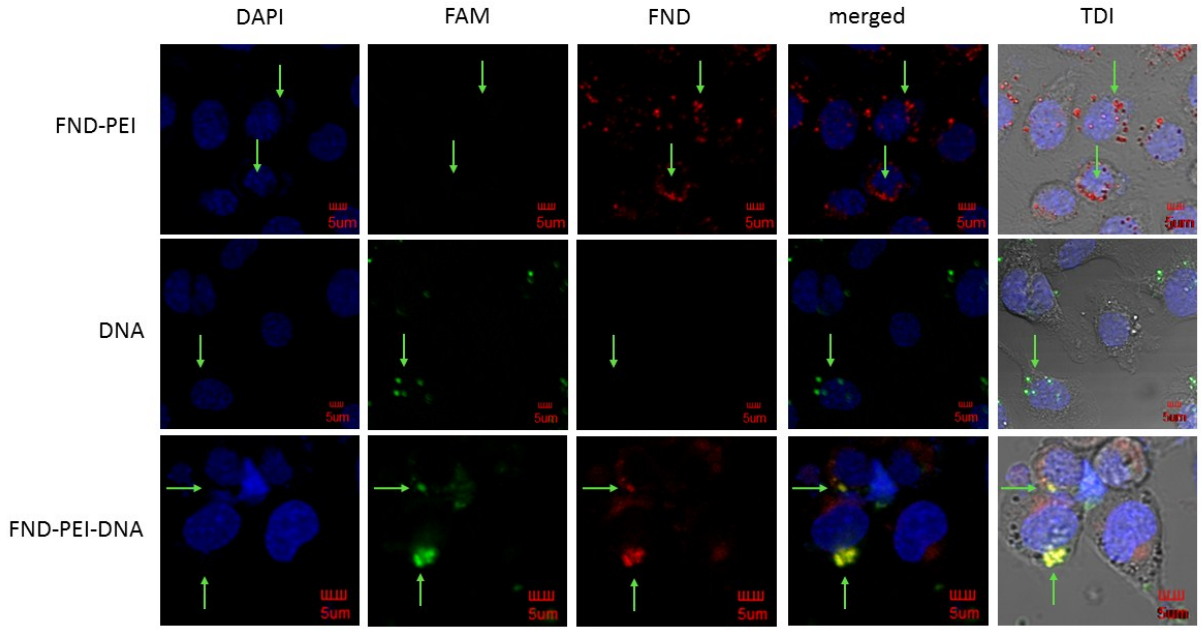
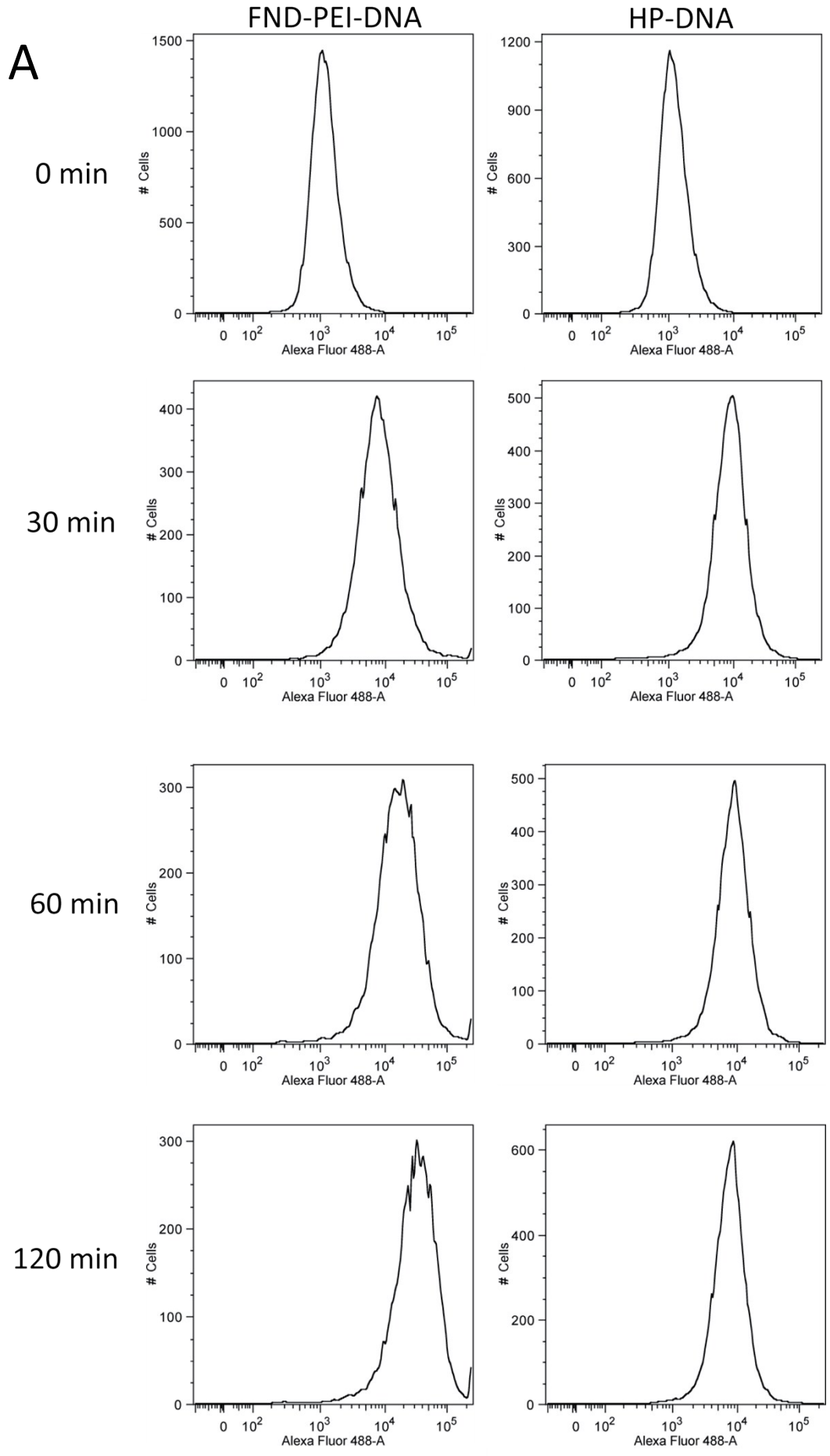


Fig. S3. Confocal analysis of FND and FAM signal colocalization (detailed view). IC-21 macrophages incubated with FND complexes for 30 min. FND-PEI: cells incubated with 25 µg/ml FND-PEI, DNA: cells transfected with X-tremeGENE HP transfection reagent and 165 ng of the 137-kb DNA fragment labelled with FAM, FND-PEI-DNA: cells transfected with 25 µg/ml FND-PEI and 165 ng of the DNA. From the left: DAPI staining of nuclei; FAM signal; fluorescence of NV centers; merged fluorescence. Arrows point at cells that have internalized or are about to engulf nanodiamond particles and/or DNA oligonucleotide.

A


FND-PEI-DNA
HP-DNA
0 min
30 min
60 min
120 min
Cells
Alexa Fluor 488-A

B

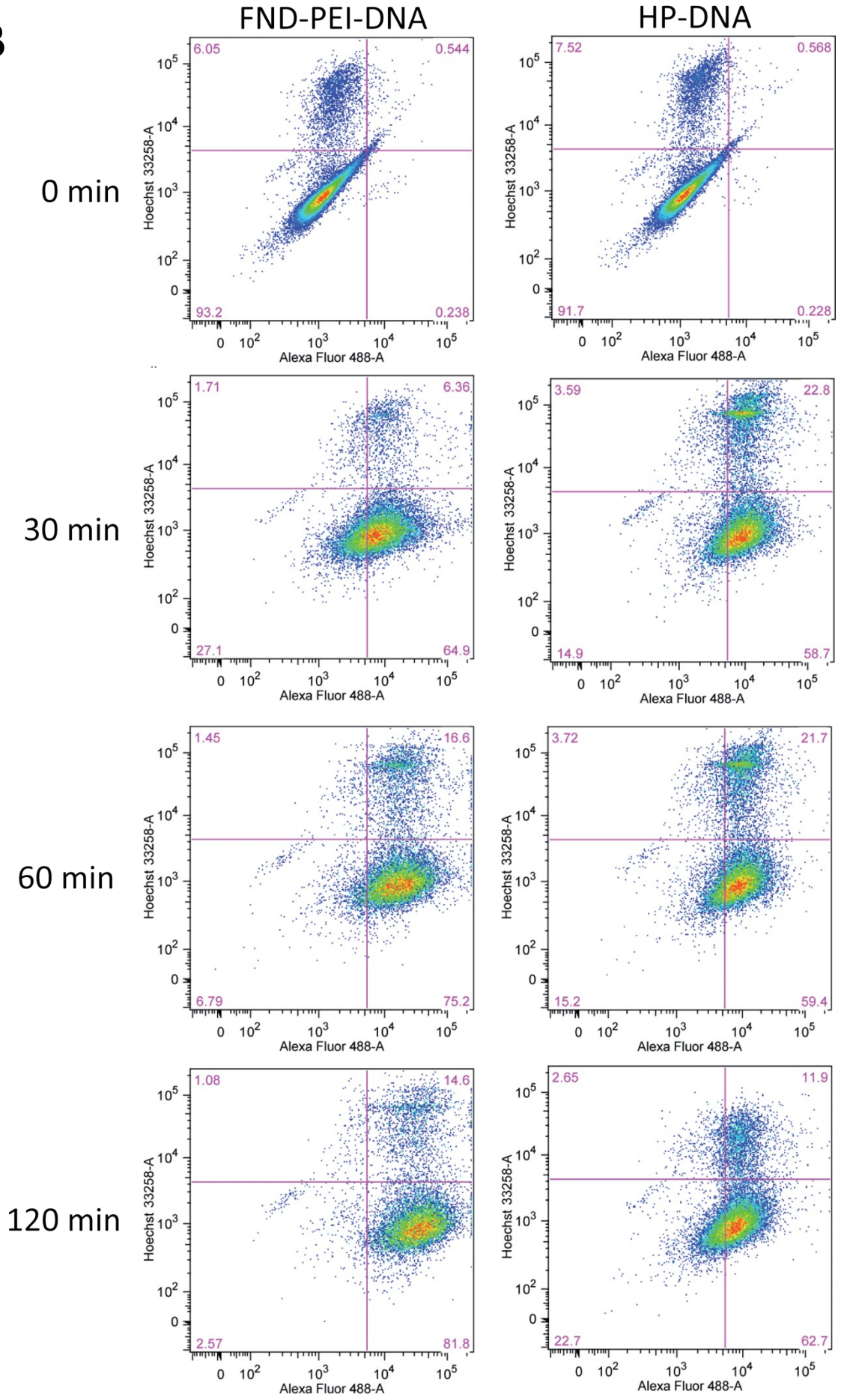

FND-PEI-DNA
HP-DNA
0 min
30 min
60 min
120 min
Hoechst 33258-A
Alexa Fluor 488-A
6.05
0.544
93.2
0.238
7.52
0.568
91.7
0.228
1.71
6.36
27.1
64.9
3.59
22.8
14.9
58.7
1.45
16.6
6.79
75.2
3.72
21.7
15.2
59.4
1.08
14.6
2.57
81.8
2.65
11.9
22.7
62.7

Fig. S4. Flow cytometry analysis of transfection efficiency of AlexaFluor 488-labeled oligonucleotide in IC-21 cells using FND-PEI-DNA complex or a commercial transfection system X-tremeGENE HP. These data are source data for Fig. 3. a) Representative histograms of Alexa Fluor 488 intensity measured using flow cytometer in IC-21 cells incubated with transfection mixture for 30, 60, and 120 minutes. AlexaFluor 488-labeled oligonucleotide was used to form the transfection complex with FND-PEI (FND-PEI-DNA) or X-tremeGENE (HP-DNA). The histograms show intensity of Alexa Fluor 488 signal (x-axis) and cell counts (y-axis). We observed a time-dependent increase of cells exhibiting AlexaFluor 488-positive signal in both FND-PEI-DNA and HP-DNA alternatives. However, this percentage was always higher for FND-PEI-DNA. b) Representative dot plots of Alexa Fluor 488 and Hoechst 33258 signals and appropriate percentage of events in each quadrant (Q I-IV). The dot plot analyses show distribution of cells positive or negative on Alexa Fluor 488 and/or on Hoechst 33258. The intensity of Alexa Fluor 488 signal is shown on x-axis and the intensity of Hoechst 33258 signal on y-axis. The appropriate percentage of cells in each quadrant (Q I-IV, counted clockwise, starting at the top left corner) is revealed. Here, Q I represent Hoechst 33258 positive cells; dead cells with membrane permeable for Hoechst dye without Alexa Fluor 488 signal. Q II represent double positive cells; cells positively transfected with Alexa Fluor 488-oligo but possessing permeable membrane (high Hoechst 33258 signal). Q III represent cells transfected with Alexa Fluor 488-oligo which are alive without Hoechst 33258 signal. Q IV represents double negative cells, with no signal either from Alexa Fluor 488 or Hoechst 33258.

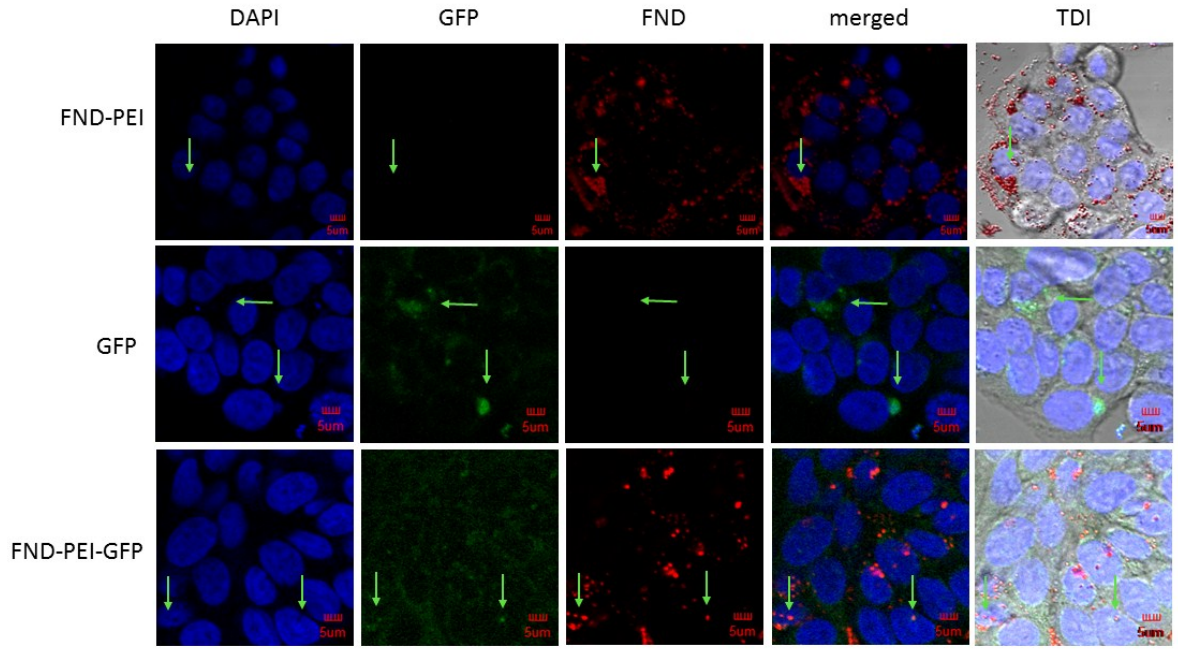


Fig. S5. Detection of green fluorescent protein (GPF) signal 48 h after transfection of HT-29 cells with FND-PEI and X-tremeGENE HP transfection reagent, both complexed with the pGFP expression plasmid. FND-PEI: cells incubated with 25 µg/ml FND-PEI, GFP: cells transfected with X-tremeGENE HP transfection reagent and 2 µg pGFP, FND-PEI-GFP: cells transfected with 25 µg/ml FND-PEI and 2 µg pGFP. From the left: GFP signal; fluorescence of NV centers; merged fluorescence with bright field. Arrows point out cells that internalized nanodiamonds and/or plasmid DNA.