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Supplemental Material to:

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Real-time dynamics of methyl-CpG-binding domain protein 3 and its role in DNA demethylation by fluorescence correlation spectroscopy

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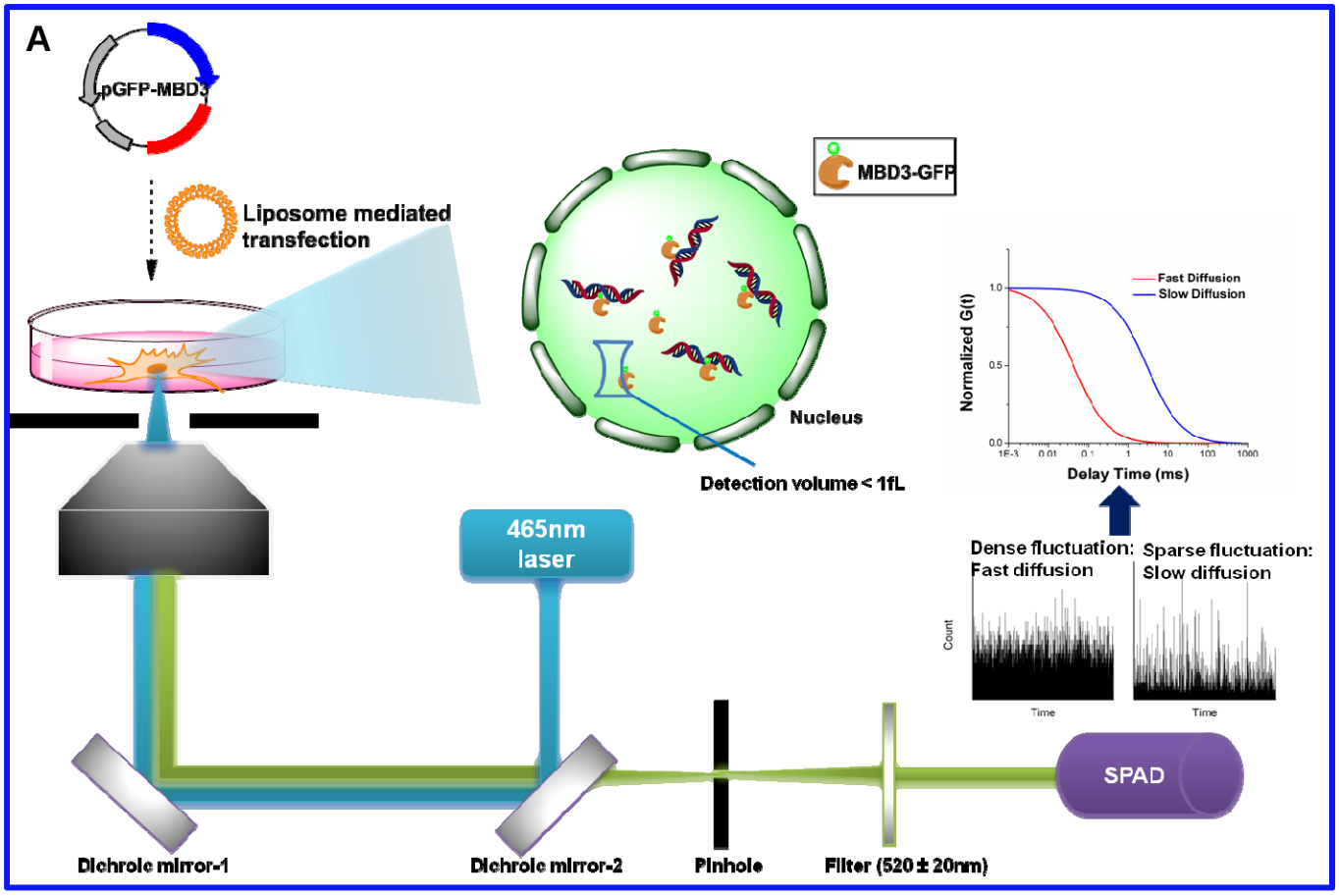
SUPPORTING FIGURES



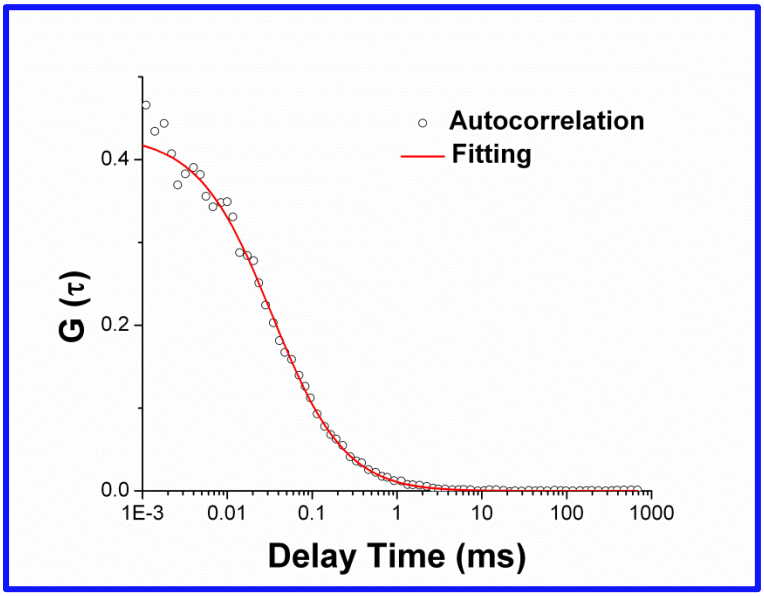


Figure S1. (A) FCS experimental procedure and schematic of the instrumentation. The Microtime200 time-resolved fluorescence microscope (Picoquant, GmbH, Berlin, Germany) is fitted with a picosecond pulsing diode laser (LDH470, 465 nm Exitation) for monitoring GFP

and Alexa488 and is controlled by a laser driver (Sepia PDL 808 Driver) with a repetition rate of 40 MHz. The laser beam is focused onto the sample by an apochromatic 60 X water immersion objective with 1.2 N.A. The excitation and emission are separated by a dual band dichroic mirror (z467/638rpc, Chroma) between the objective and a 50μm pinhole. A band-pass filter (500-540 nm, Chroma Corp, VT) was used to reject the off-focus photons with a pinhole. Fluorescence signal is collected by single photon avalanche photodiode (SPAD, SPCM-AQR-14, Perkin-Elmer Inc.) in the Time Tagged Time Resolved Single Photon (TTTR) mode (Time Harp200 PicoQuant). The raw fluorescent signals are analyzed and exported by SymPhoTime200 software (Picoquant, GmbH). Based on the time correlated fluorescence intensity trace and correlation fitting functions the diffusion information can be extracted. The signal intensity and fluctuation frequency determines the calculated diffusion properties. **(B)** The system was calibrated with 5 nM Rhodamine 123.

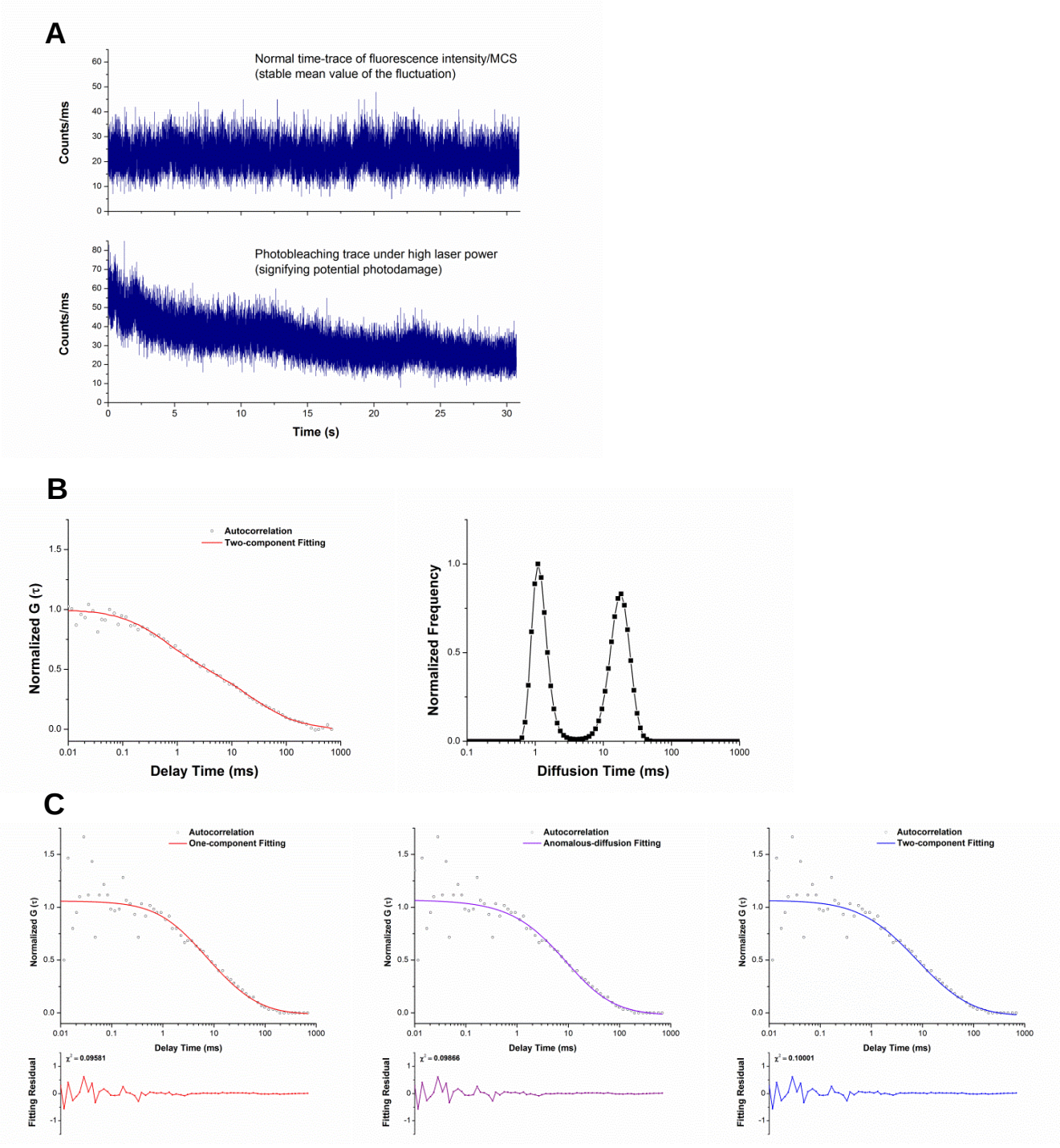
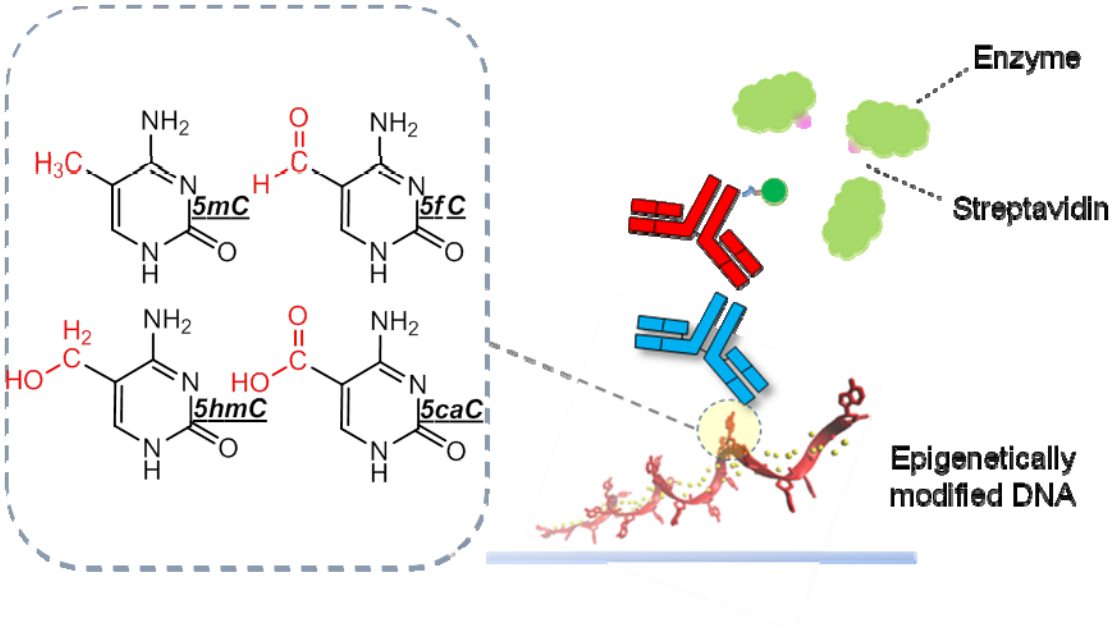


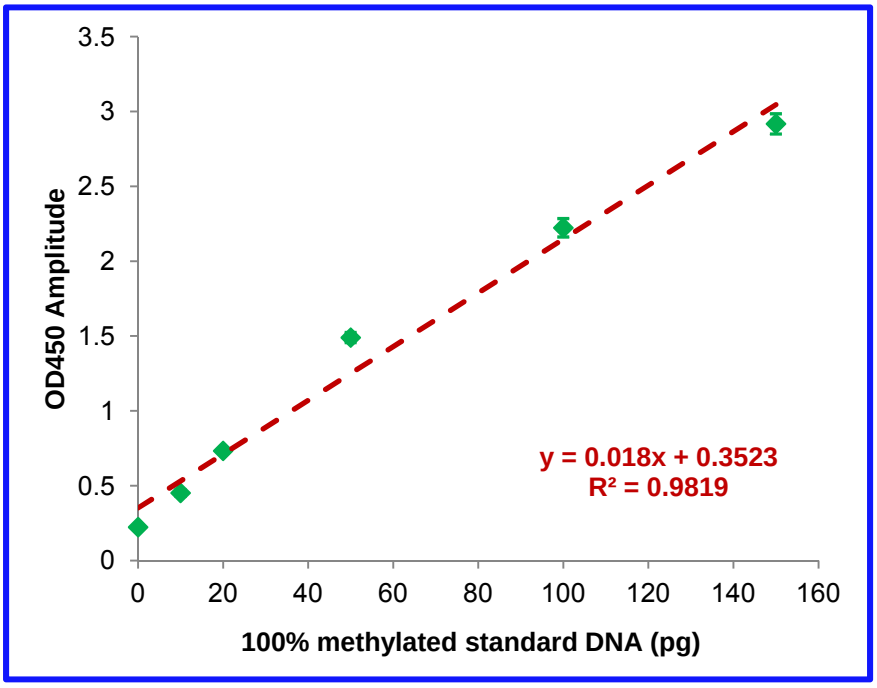
Figure S2. (A) Optimization for excitation power. In order to produce detectable FCS signal and avoid photodamage/photobleaching, the laser power was precisely controlled to be within 0.2-0.4 μW. Here the multi-channel scaling (MCS)/fluorescence intensity trace was used to monitor potential photodamage. **(B)** In the first 16 hours of transfection, the MBD3 diffusion can be fitted by a two-component model since part of these were in the process of locating targets. **(C)** Considering the low transfection of MBD3-GFP, the one-component diffusion

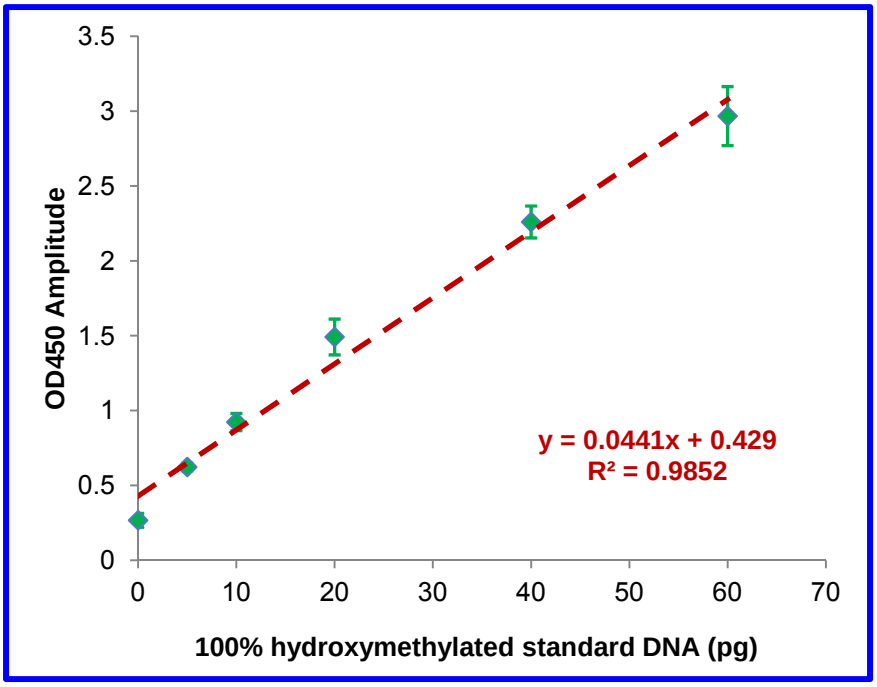
model or an anomalous diffusion model with “α” greater than 0.8 was found to yield a better fit compared to other models after 24 hours of transfection.

A


5mC
5fC
5hmC
5caC
Enzyme
Streptavidin
Epigenetically
modified DNA

B


OD450 Amplitude
100% methylated standard DNA (pg)
y = 0.018x + 0.3523
R² = 0.9819
0
0.5
1
1.5
2
2.5
3
3.5
0
20
40
60
80
100
120
140
160



C

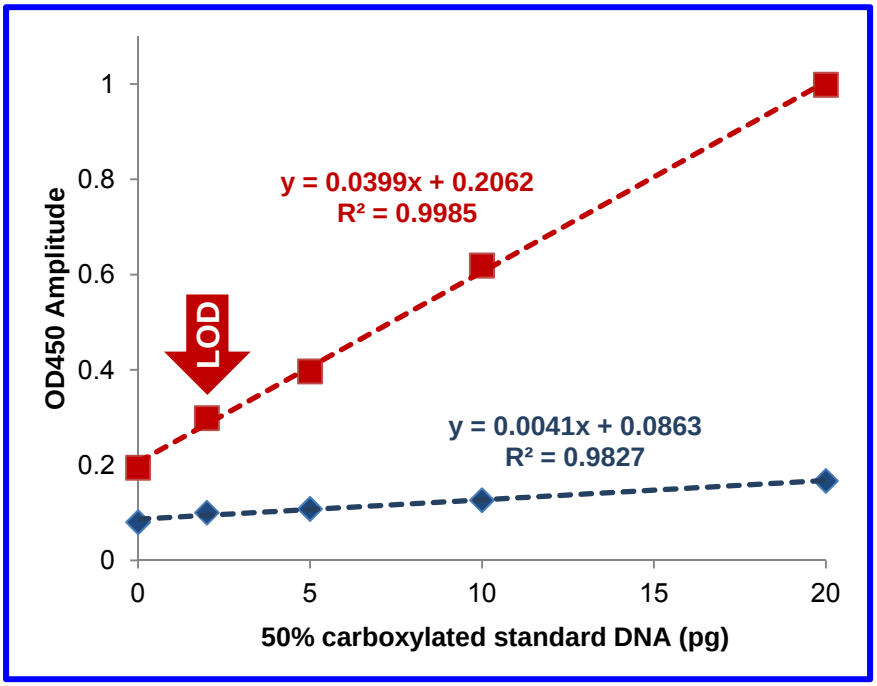
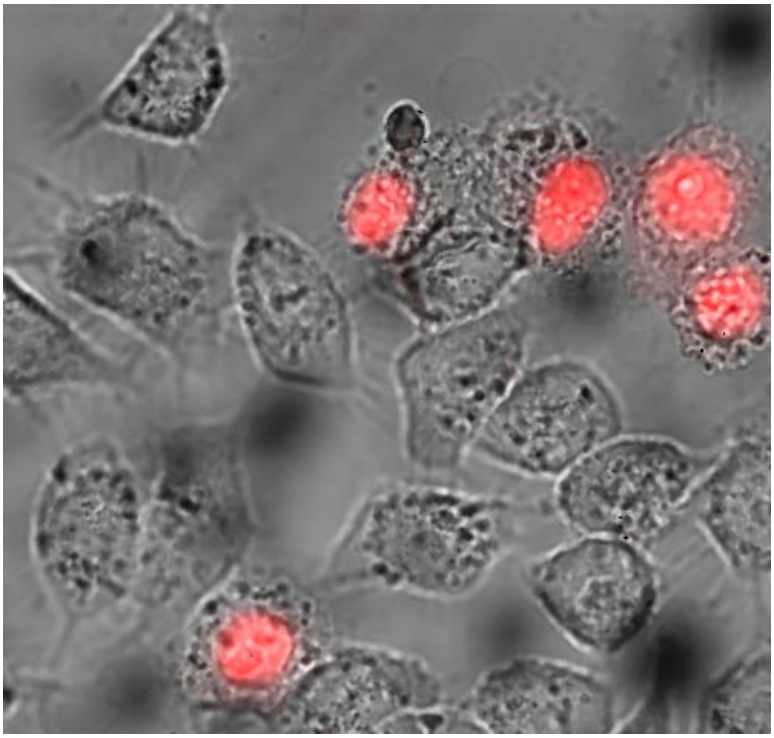


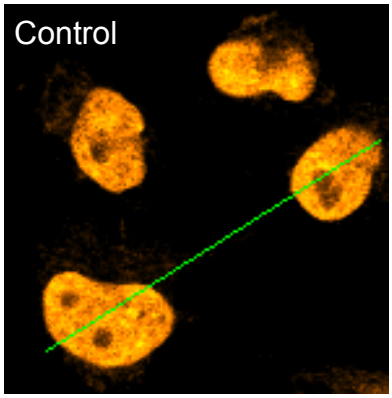
Figure S3. Schematic representation of the monitoring of cytosine derivatives based on modified ELISA. **(A)** Double strand genomic DNA can be used directly on the microtiter well. DNA bearing the target epigenetic marks including 5mC, 5hmC, 5fC and 5hmC on the well can be recognized by the corresponding primary antibodies, followed by secondary antibody stain specific to the primary antibodies. For signal amplification, the secondary antibodies were modified with biotin so that it is highly reactive with the streptavidin coupled to Horseradish

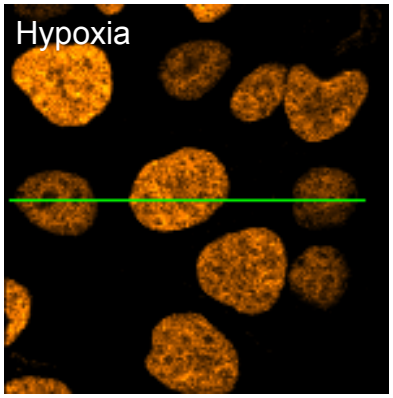
Peroxidase (HRP) enzyme, for amplified colorimetric signal generation. The final signal is directly proportional to the number of enzyme molecules. **(B)** 5mC and 5hmC standard curves were obtained by this method using the fully methylated or hydroxymethylated sequence which contains 22.4% cytosine derivatives. Limit of detection (LOD) limit can reach up to 5 pg in the DNA standards. **(C)** Signal comparison between modified and conventional ELISA for 5caC. Signal amplification can provide a LOD of 2.5 pg, while the standard ELISA was not able to produce a detectable signal even at a LOD of 20 pg.

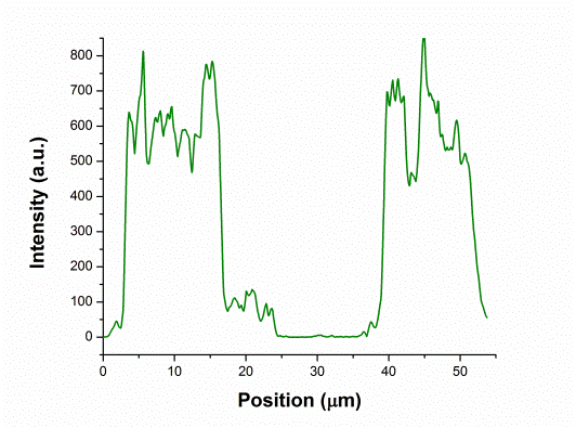
A



B


Control


Hypoxia


Intensity (a.u.)
0
100
200
300
400
500
600
700
800
0
10
20
30
40
50
Position (μm)

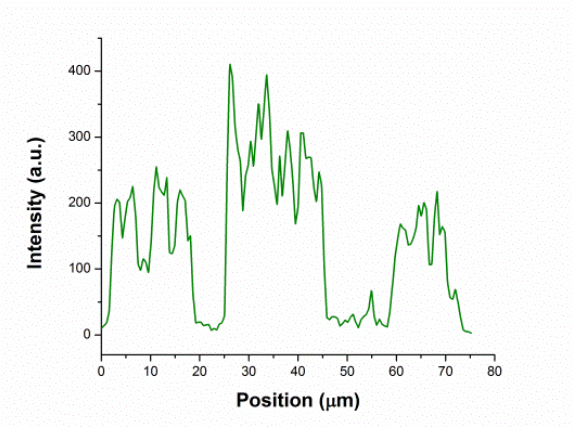

Intensity (a.u.)
0
100
200
300
400
0
10
20
30
40
50
60
70
80
Position (μm)

Figure S4. (A) Representative image of PI uptake experiment after 4 hours of hypoxia showed similar proportion (~ 28%, n > 200 cells) of hypoxia-sensitive cells as observed by MBD3 FCS detection. **(B)** Further, the cells displayed a variable response in terms of 5mC levels (by immunofluorescence profile) towards hypoxic stress.