A Cell-based Biosensor to Report DNA Damage in Micro- and Nano-systems

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SUPPORTING INFORMATION TO METHODS

Engineering the DNA damage cell-based biosensor

We engineered a reporter gene construct encoding the fluorescent TurboRFP protein, the expression of which is under the control of the p53-responsive p21 promoter. The sequence of the p53-responsive p21 promoter region was selected 667 base-pairs upstream of the transcription start site of the murine p21 gene:

[TCGAGCTCAAGCCAACATGTTGGGACATGTTCAGGGCGCTGGCAAGTGGCAACATCGTG
GAACAACATGTTGGGACATGTTCGATCACGCTGCGCGCGGCCAAATGGCCGTACAACATGT
TGGGACATGTTCACACACCCGCCGCGCTGCGTTTTCTGGATACAACATGTTGGGACATGTTC
AGCCGCTACAGGGCGCGTCAGATTCGCAGCCAACATGTTGGGACATGTTCACGTGGCACTG
TTCGCGCAACATCGCCTTACAACATGTTGGGACATGTTCATGTGCGCGGAACCCCTATTCTA
TCGCCTACAACATGTTGGGACATGTTCGCCATACCACATTTGGCAAGACCGACCAAACAAC
ATGTTGGGACATGTTCAGTCCACACCTCCCCCGTCAGCGACGCCCACAACATGTTGGGACA
TGTTCACCGTCAACAGCGCGTCATCCATTCCACCGCAACATGTTGGGACATGTTCAATTCGC
GTTAAATTTTTGTGTACAGGTTGCAACATGTTGGGACATGTTCGCGCTCAGCTCATTTTTGC
GGTCATCGATACAACATGTTGGGACATGTTCAGGAGTATCCTCCAGCGCGGGGATCTGTCA
CAACATGTTGGGACATGTTCACAACTGGGTATATAAGGTACGGGCCCGGGATCC] (Blue Heron, WA, USA). The p21 promoter was cloned into the multiple cloning site (MCS) of pTurboPRL-RFP backbone vector (Evrogen), containing neomycin resistance gene NeoR that confers resistance against G418 antibiotic for selection of stably transfected clones (Figure S1). The plasmid was deposited to Addgene (the plasmid number is 52432) and will be made available upon manuscript acceptance. The NIH-3T3 cells were transfected with the plasmid using Superfect (Qiagen). After overnight incubation,

selection medium containing 1 mg/ml genetecin (G418, Invitrogen) was introduced to select the stably transfected clones. The selection process was carried out for two weeks, after which single-cell clones were isolated using limiting dilution cloning into 96-well plates. The isogenic clones were expanded and then assayed, using DNA-damaging agent methyl methanesulfonate, to identify the clone with the highest fluorescent response to DNA damage.

Comet assay

Biosensor cells were treated with MMS diluted in cell culture medium not supplemented with bovine calf serum at concentrations of 0.05-1.5 mM. The control cells were exposed to the same medium without MMS and to the same number of washes. The comet assay was done as previously described.[1] The cells were incubated with MMS for 30 min, washed with PBS, trypsinized, loaded into the multiwell comet array plate made using molten 1% normal melting point agarose (Invitrogen) and microwell molds, kindly provided by Prof. Engelward's lab, covered with low melting point agarose (Invirtogen), lysed and electrophoresed using the alkaline comet protocol. The electrophoresis was followed by series of washes with 0.4 M Tris, pH 7.5 to neutralize the gels. Slides were stained with SYBR Gold (Invitrogen), imaged, automatically capturing the images using an epifluorescent microscope and analyzed automatically using custom software written in MATLAB (The Mathworks) provided by Prof. Engelward's lab.[1]

SUPPLEMENTARY FIGURES

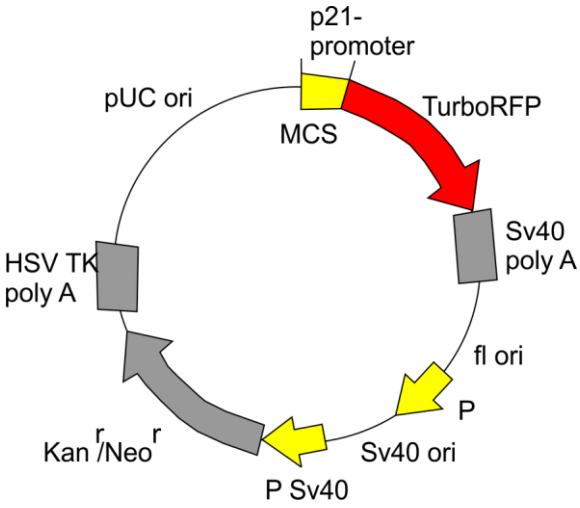

Figure S1. The pTurboPRL-RFP backbone vector (Evrogen) used to engineer the reporter gene construct encoding the fluorescent TurboRFP protein, the expression of which is under the control of the p53-responsive p21 promoter. The p21 promoter was cloned into the multiple cloning site (MCS) of pTurboPRL-RFP backbone vector, which also contains a neomycin resistance gene NeoR that confers resistance against G418 antibiotic for selection of stably transfected clones.

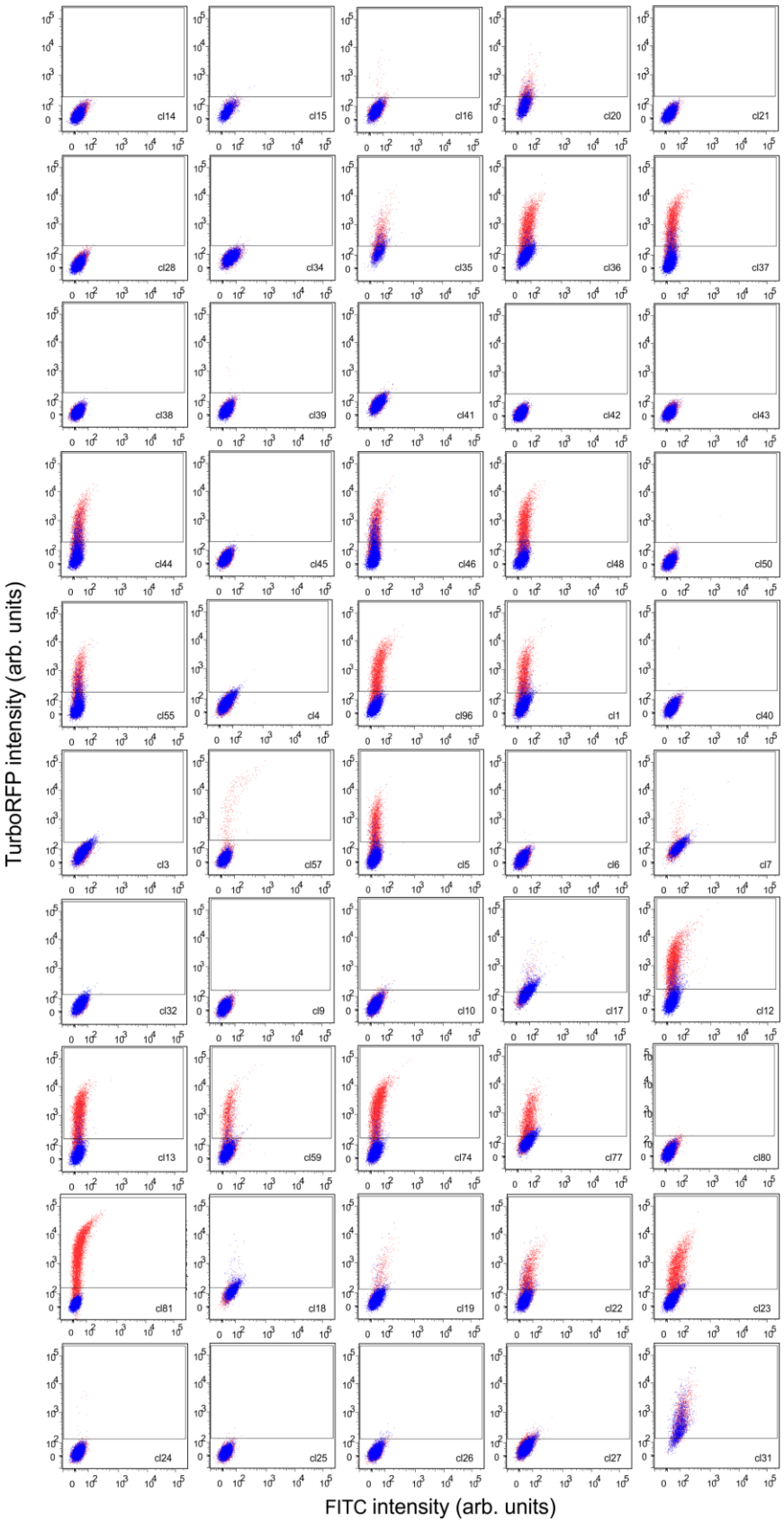
TurboRFP intensity (arb. units)
FITC intensity (arb. units)
cl14
cl15
cl16
cl20
cl21
cl28
cl34
cl35
cl36
cl37
cl38
cl39
cl41
cl42
cl43
cl44
cl45
cl46
cl48
cl50
cl55
cl4
cl96
cl1
cl40
cl3
cl57
cl5
cl6
cl7
cl32
cl9
cl10
cl17
cl12
cl13
cl59
cl74
cl77
cl80
cl81
cl18
cl19
cl22
cl23
cl24
cl25
cl26
cl27
cl31

Figure S2. Flow cytometry scatter plots of TurboRFP fluorescence intensity (FITC channel was used as a reference channel) of 50 clones exposed to 1mM MMS. Red - fluorescence values of the cells exposed to MMS; blue - fluorescence values of unexposed control cells of the same clone.

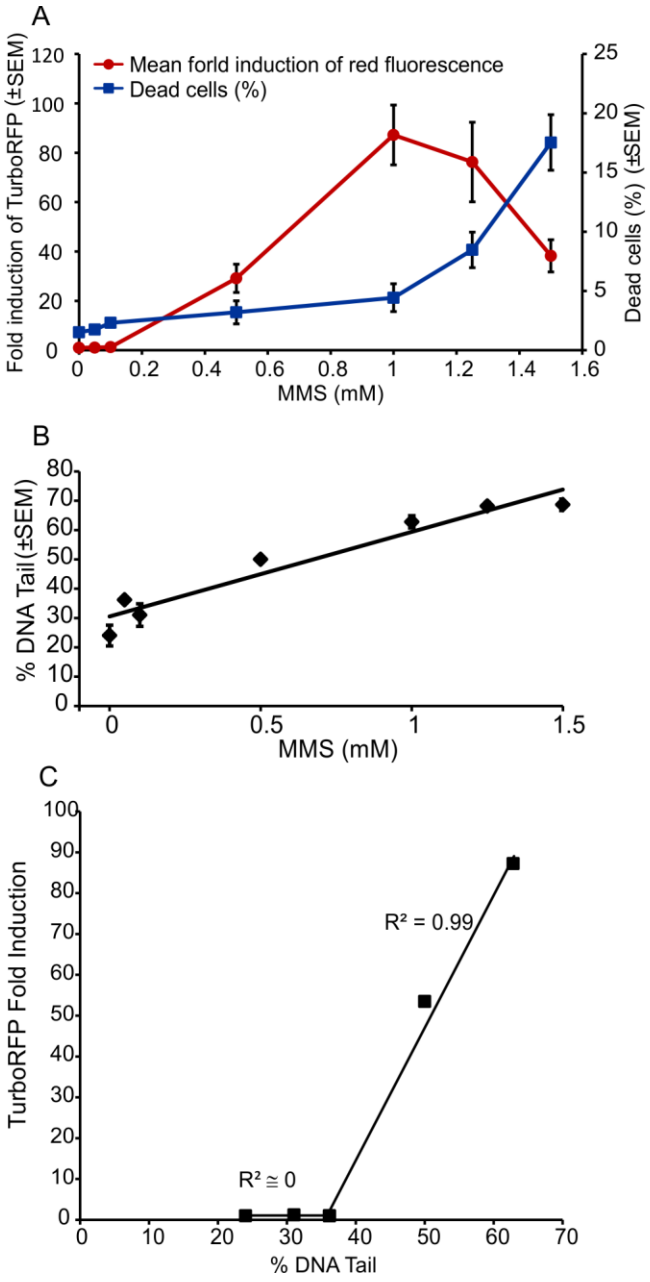

Figure S3. A) Mean fold induction of RFP fluorescence 24 hrs after exposure to MMS and % dead cells (determined by Sytox-Blue positive cells) across varying concentrations of MMS. The analysis was done using FC. **B)** % tail DNA (average of median % DNA) versus MMS concentration of biosensor cells following comet assay (R^2=0.93). (n=2 experiments, error bars: SEM). **C)** Mean fold induction of RFP fluorescence 24 hrs after exposure to MMS plotted against % DNA in tail caused by the same

MMS concentration (0.05 - 1 mM) after 30 min exposure. At MMS concentrations of 0.05-0.1 mM there was no apparent linear relationship between the red fluorescence fold induction and % DNA tail measured by comet assay ($R^2 \cong 0$), whereas above 36% DNA in tail we observed a linear relationship between the red fluorescence fold induction and % DNA tail ($R^2 = 0.99$).

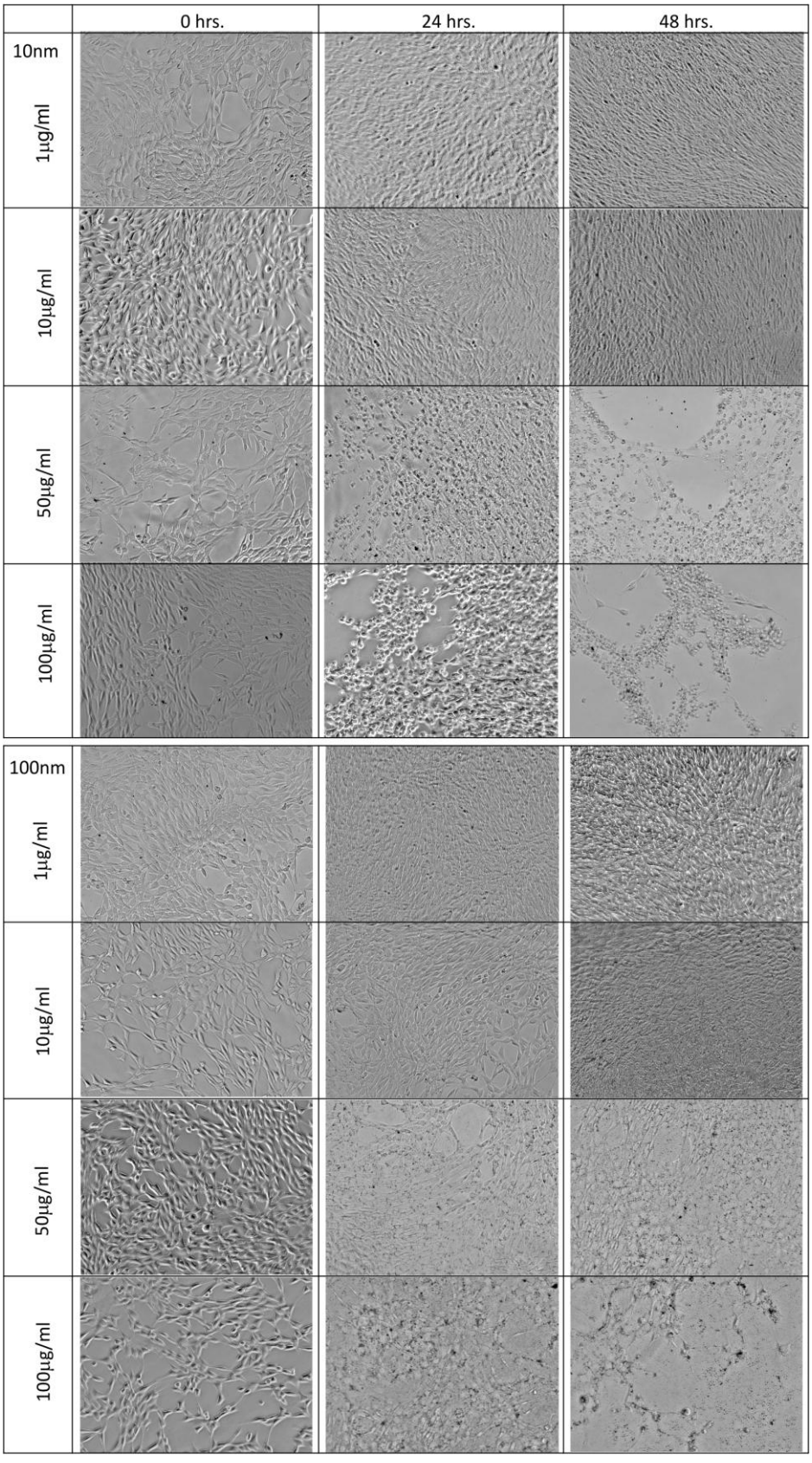
0 hrs.
24 hrs.
48 hrs.
10nm
1µg/ml
10µg/ml
50µg/ml
100µg/ml
100nm
1µg/ml
10µg/ml
50µg/ml
100µg/ml

Figure S4. Phase images of biosensor cells exposed to various concentrations of 10 and 100 nm Ag-NPs, taken at three time points of exposure: 0 hrs, 24 hrs and 48 hrs. For the 10 nm Ag-NPs, the concentrations of 50 and 100 μg/ml resulted in significant cell loss after exposure of 24 hrs. Exposure of cells to 100 μg/ml of the 100 nm Ag-NPs showed a decrease in cell viability after 48 hrs.

REFERENCES

(1) Wood, D. K.; Weingeist, D. M.; Bhatia, S. N.; Engelward, B. P. *Proc Natl Acad Sci U S A* **2010**, 107, (22), 10008-13.