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Genetically Encoded Photosensitizers Targeted to Methylated DNA

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pharmaceuticals MDPI

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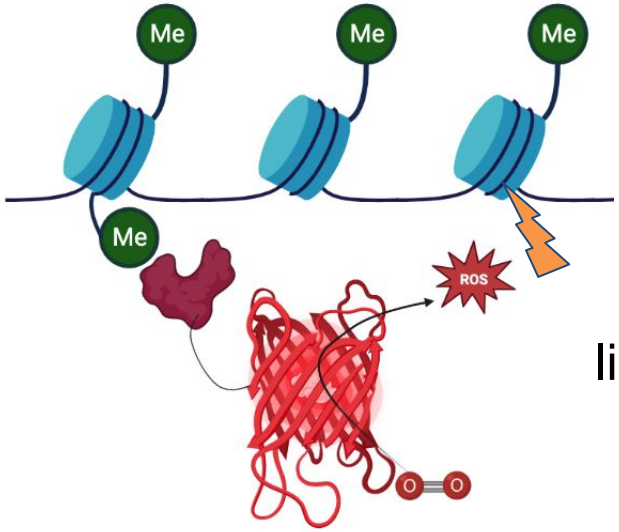
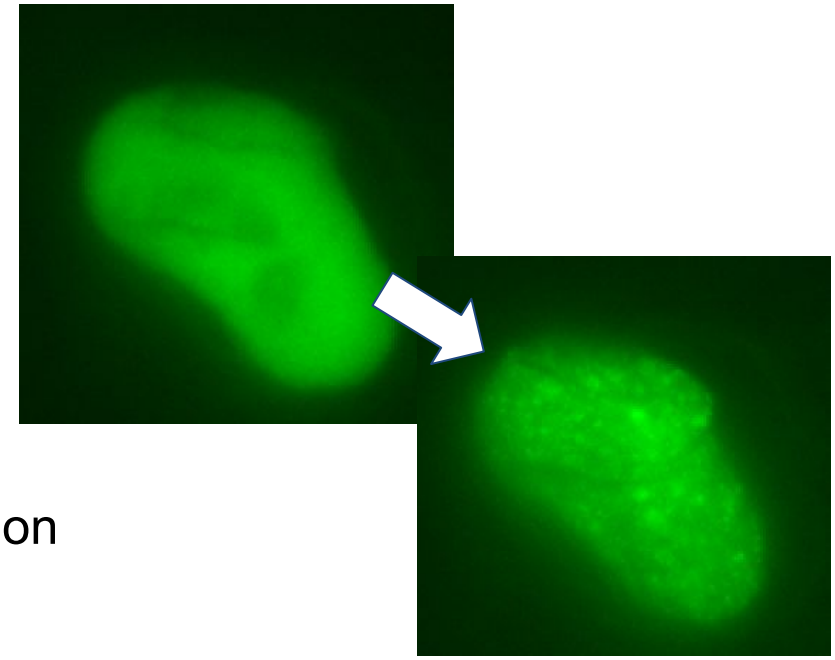
Genetically Encoded Photosensitizers Targeted to Methylated DNA



MECP2-KillerRed2

light illumination



DNA reparation complex protein XRCC1 re-distribution indicating the presence of the DNA breaks

Genetically encoded photosensibilizers are widely used in fundamental research and translational medicine due to their ability to generate reactive oxygen species (ROS) after photosensitizing. Previously, it was shown in mice that red dimeric fluorescent protein KillerRed is a potential photosensitizer that can be used for photodynamic therapy of cancer.

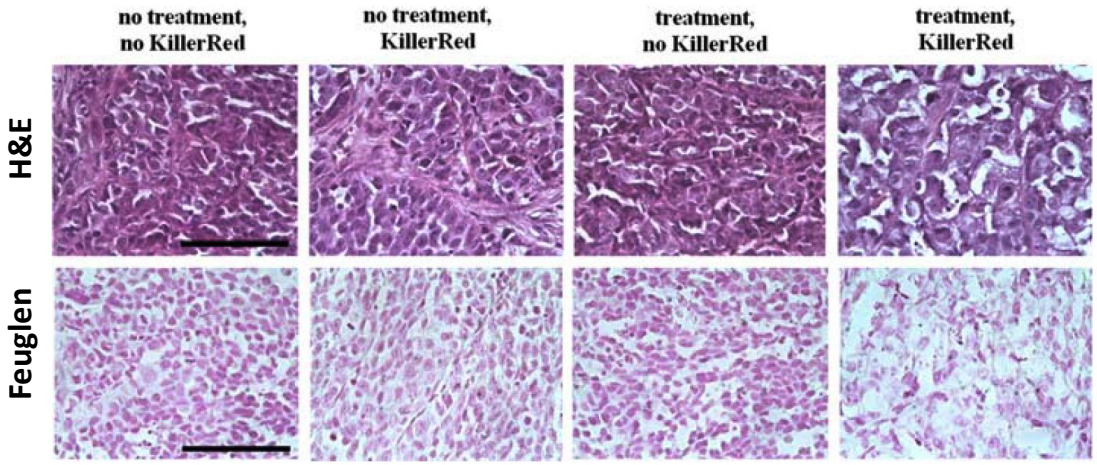
Now we have constructed and tested a new genetically encoded photosensibilizer molecule which introduces DNA breaks and activates the repair system in cancer-derived and embryonic cell lines. The molecule consists of two parts: KillerRed2 (mutant of KillerRed with enhanced phototoxicity) and methyl-CpG binding protein MECP2. The complex activates redistribution of DNA repair protein after illumination with lower power compared to the previously used constructs.

The new genetically encoded construct has shown the improved ability to generate DNA breaks in the cancer cell lines.

Keywords: genetically encoded photosensibilizers; KillerRed.

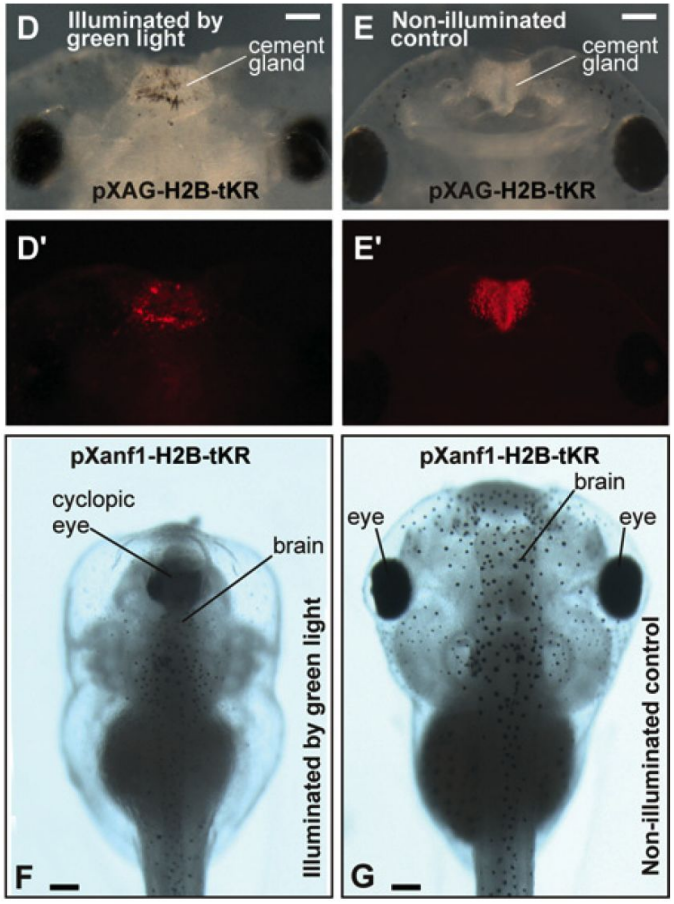
Introduction

Genetically encoded photosensibilizers are widely used in fundamental research and translational medicine due to their ability to generate reactive oxygen species after photosensitizing. Previously, it was shown in mice that red dimeric fluorescent protein KillerRed is a potential photosensitizer that can be used for photodynamic therapy of cancer [1].



Histological sections of HeLa tumors before and after KillerRed-mediated laser irradiation. Scale bar 100 μm.

Introduction

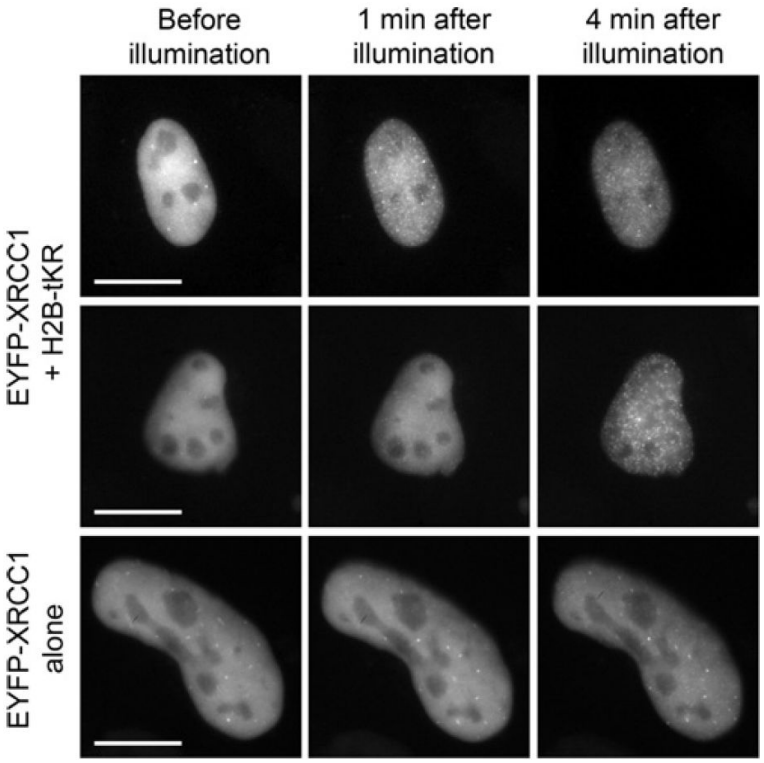


Also it was demonstrated that HeLa cells expressing KillerRed fused to histone H2B cease proliferation upon illumination.

Transgenic tadpoles expressing H2B-tandem KillerRed construct under the control of forebrain-specific Xanf1 promoter either illuminated (F) or not (G) by the green light. [2]

Scale bar 100 μm.

Introduction



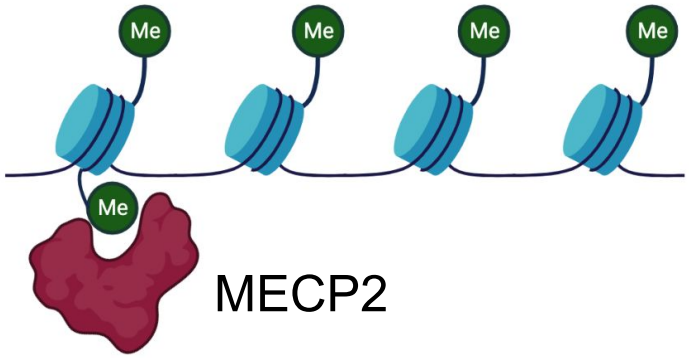
DNA repair protein X-ray repair cross-complementing protein 1 (XRCC1) redistributed in the cell nuclei indicating that the mechanism of phototoxic action of the construct involved DNA breaks generation. [2]

Representative HEK293 cells co-expressing EYPF-XRCC1 and H2B-tdKillerRed or EYPF-XRCC1 only. Scale bar 10 μm.

Aim

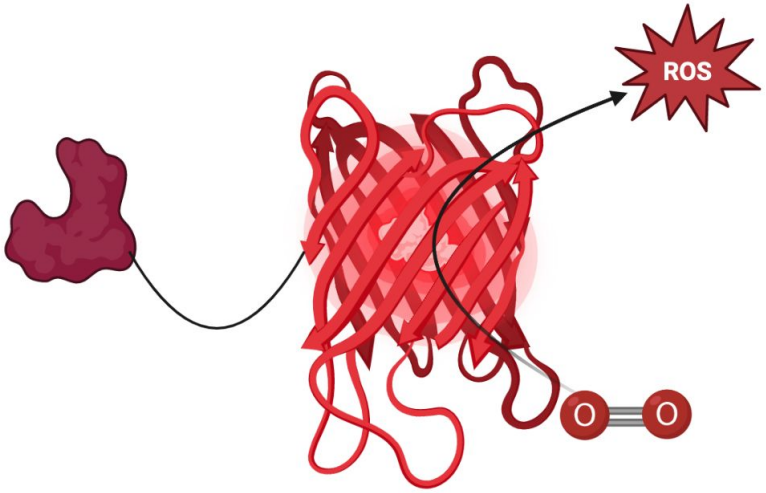
It is impossible to use the previously described H2B-tdKillerRed construct for *in vivo* application due to the construct largeness and recombination of tandem sequences.

Thus we aimed to test another candidate target, methylation-reader protein MECP2, with monomeric and dimeric photosensitizers with enhanced ROS generation ability.



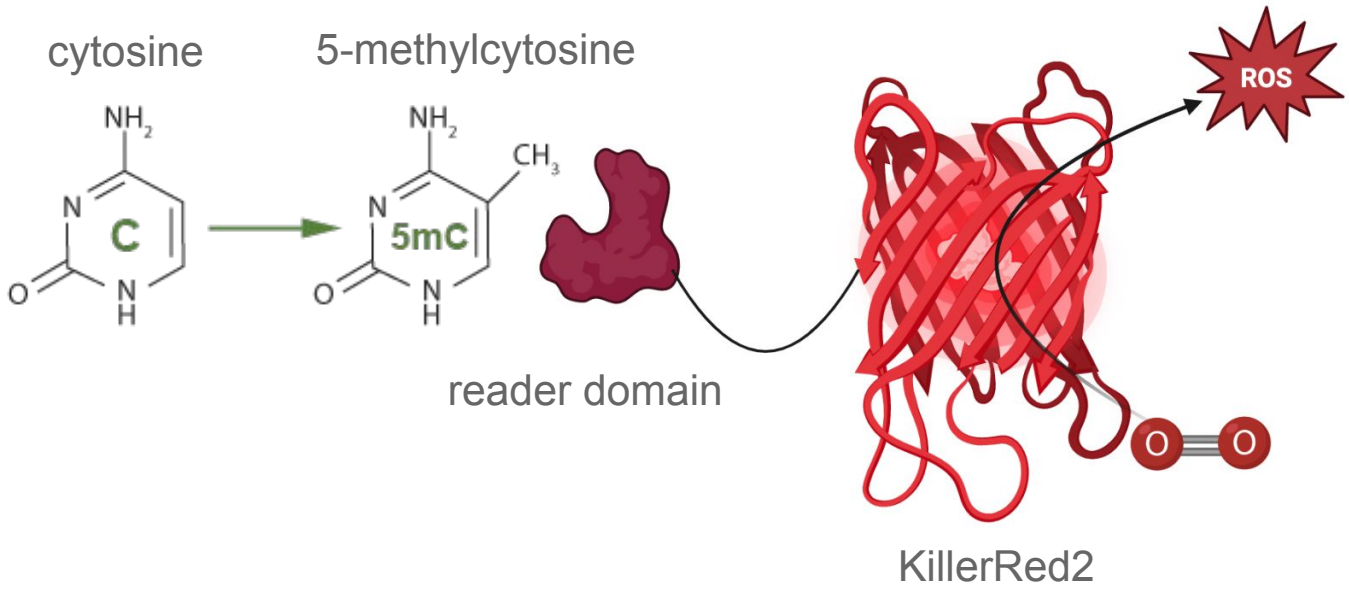
Results

Now we have constructed and tested a new genetically encoded photosensitizer which introduces DNA breaks and activates the repair system in cancer-derived and embryonic cell lines more efficiently then previously described.



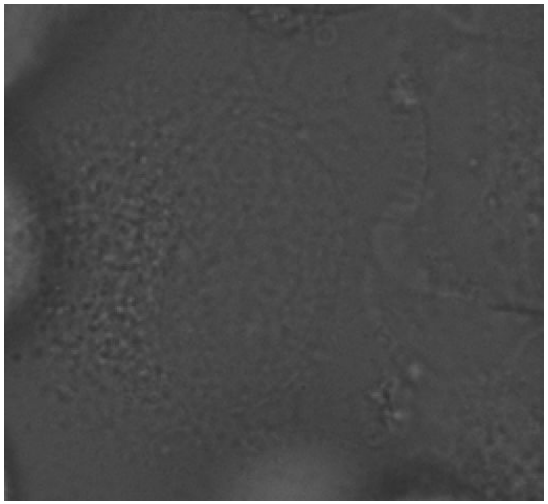
Results

The molecule consists of two parts: KillerRed2 (mutant of KillerRed with enhanced phototoxicity [3]) and methyl-CpG binding protein MECP2.

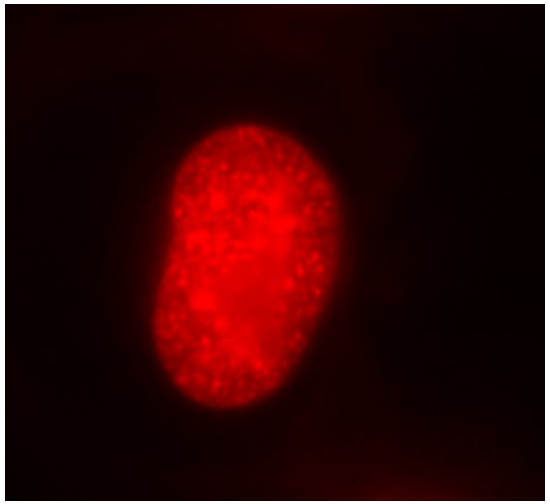


Results

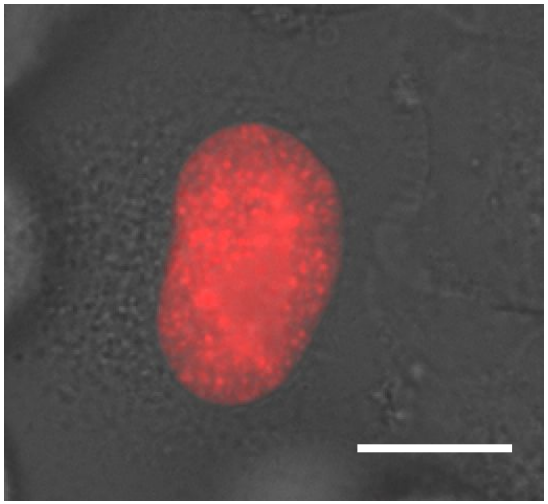
Transient transfection of HEK293 cells with plasmid encoding the construct MECP2-KillerRed2 under constitutive promoter. A representative cell imaging was performed with Keyence BZ-9000 with brightfield and TxRed filter sets.



HEK293 cell, brightfield



HEK293 cell, MECP2-KillerRed2

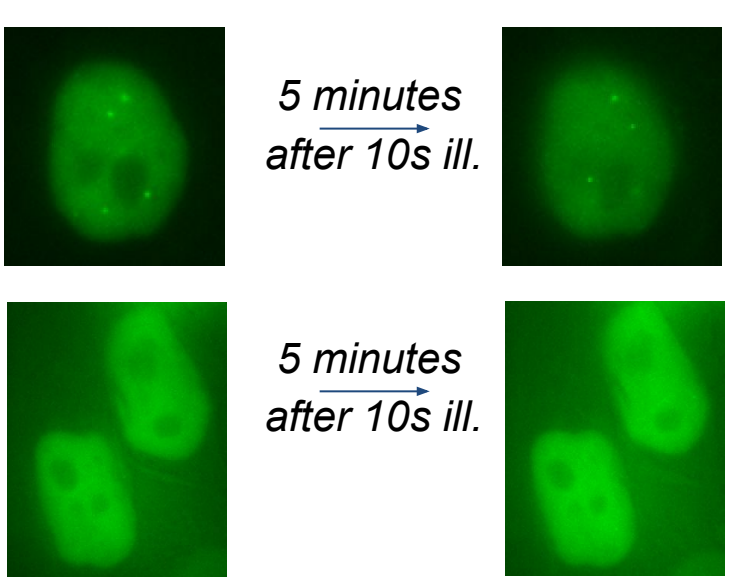


HEK293 cell, merged

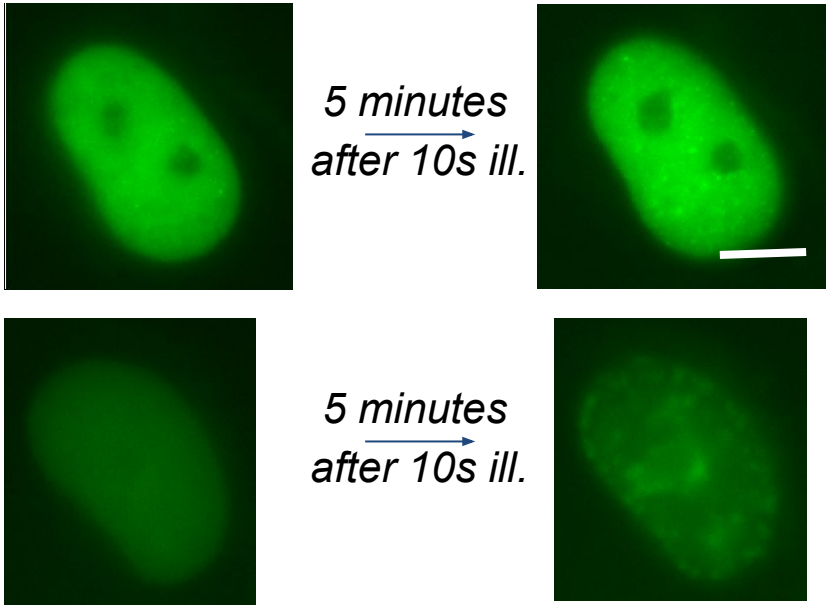
Scale bar 10 μm.

Results

The MECP2-KillerRed2 complex activates XRCC1 redistribution (granules formation) after illumination with lower power compared to the MECP2-SuperNova2.



MECP2-SuperNova2

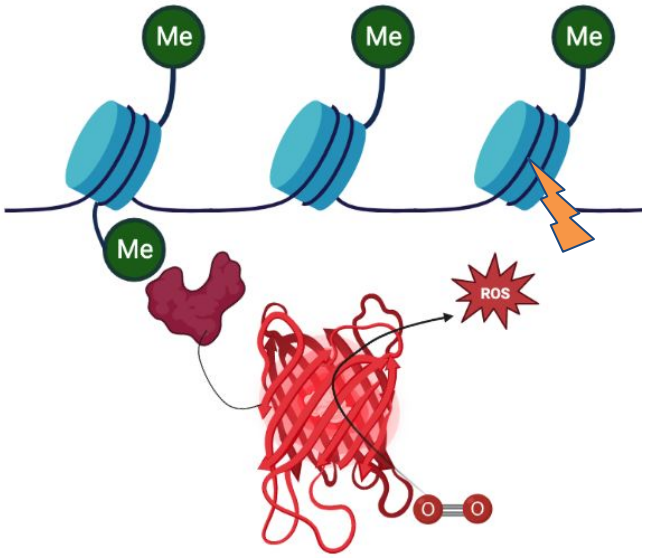


MECP2-KillerRed2

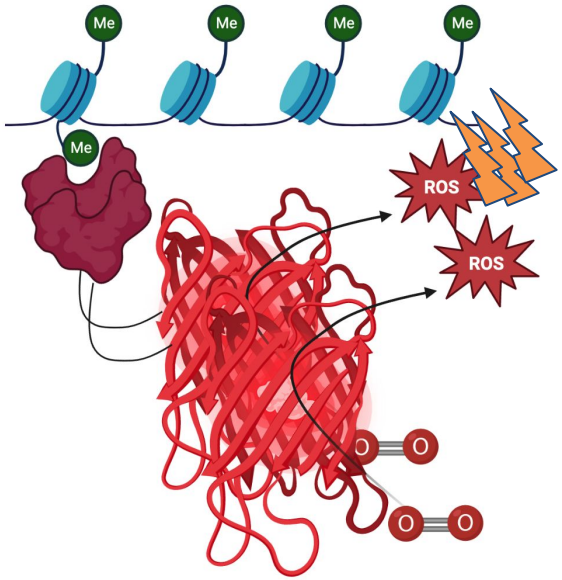
Scale bar 10 μm.

Results

It is known that methyl-reader domain of MECP2 interacts with DNA after dimer formation [4]. Thus the dimeric KillerRed2 fused to MECP2 increases the affinity of the methyl-reader, which increases the concentration of produced ROS in the nucleus.



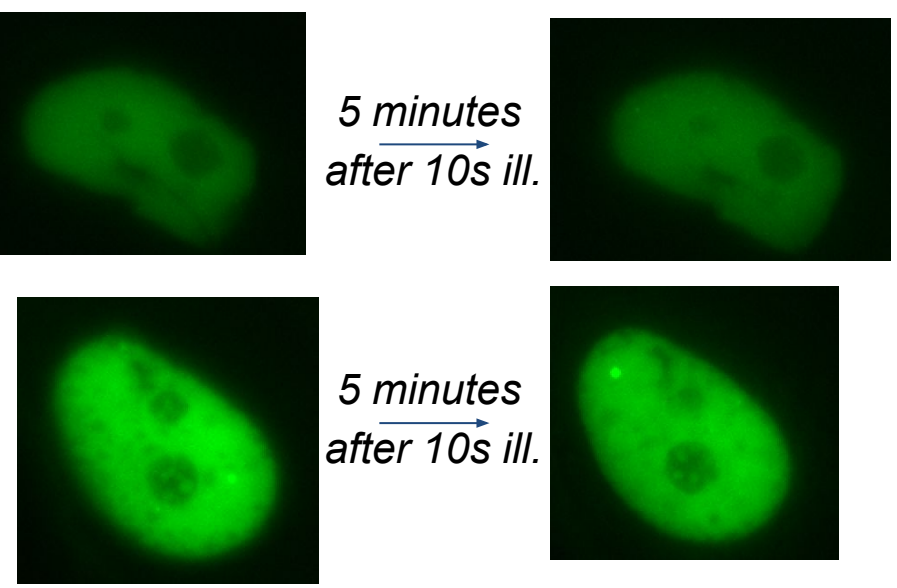
MECP2-SuperNova2



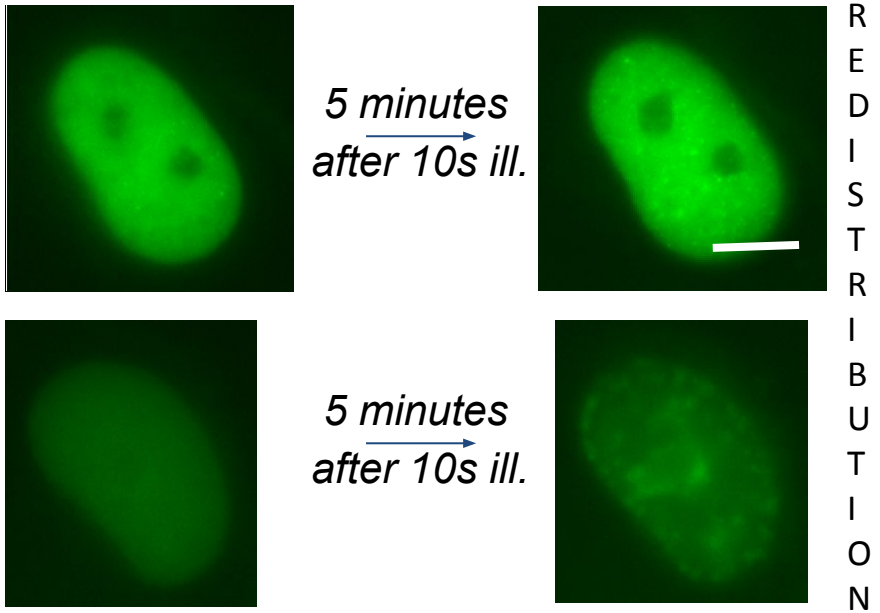
MECP2-KillerRed2

Results

The complex activates XRCC1 redistribution after illumination with lower power compared to the H2B-fuze with the same length, H2B-SuperNova2.



H2B-SuperNova2

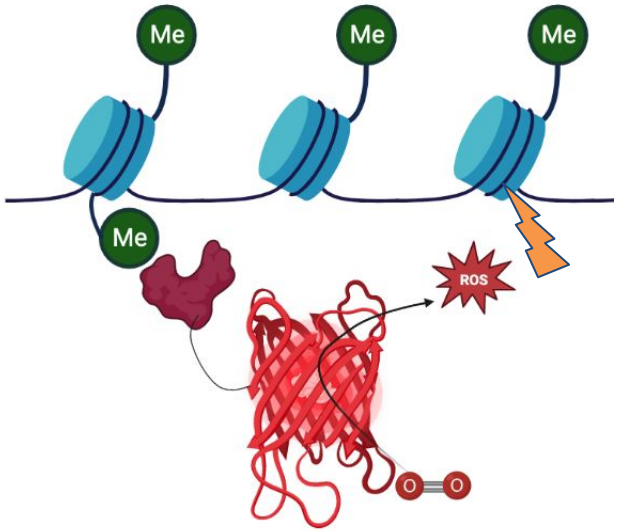


MECP2-KillerRed2

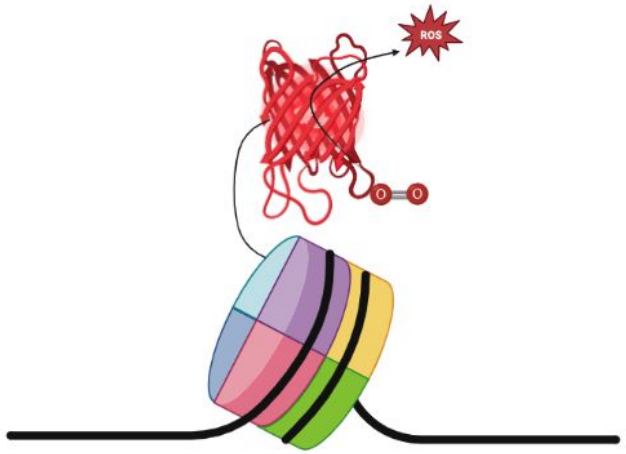
Scale bar 10 μm.

Results

The complex activates XRCC1 redistribution after illumination with lower power compared to the H2B-SuperNova2. We suppose it can be explained by the lower distance between induced ROS and DNA compared to the H2B-fuzed construct.



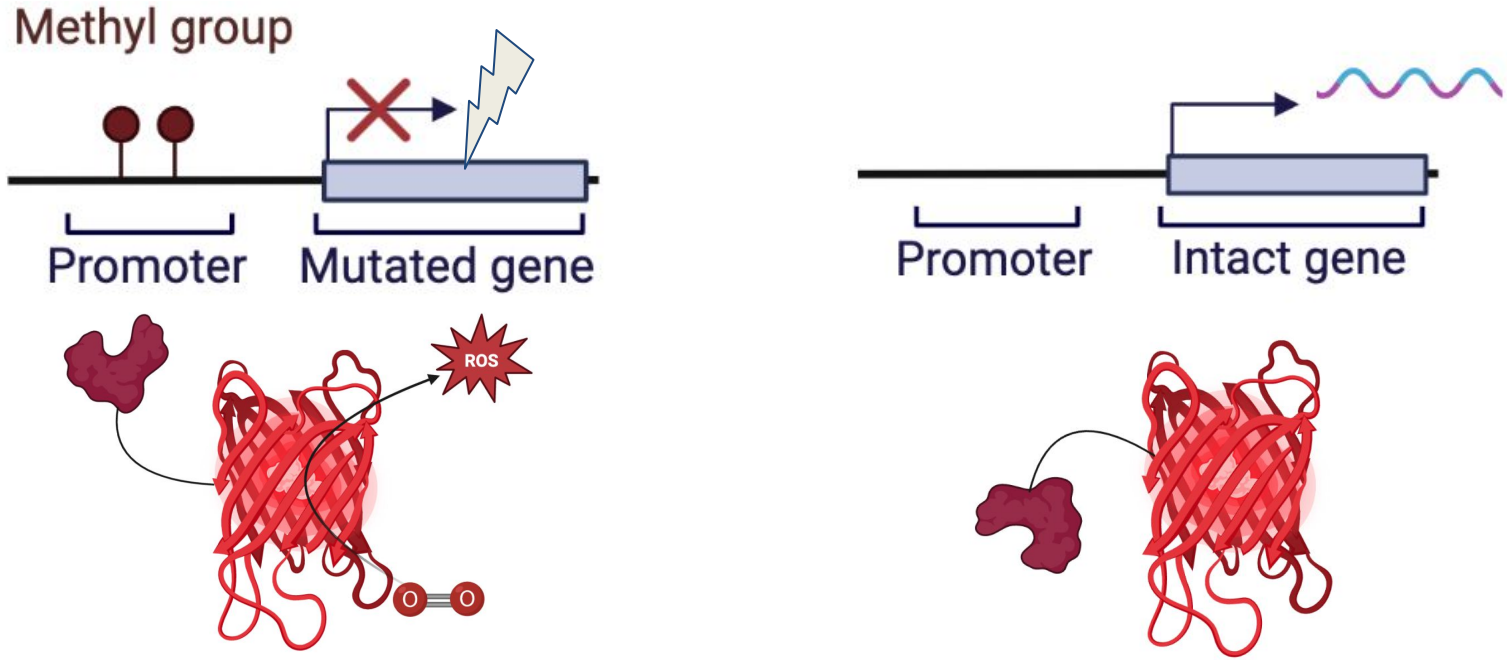
MECP2-KillerRed2



H2B-SuperNova2

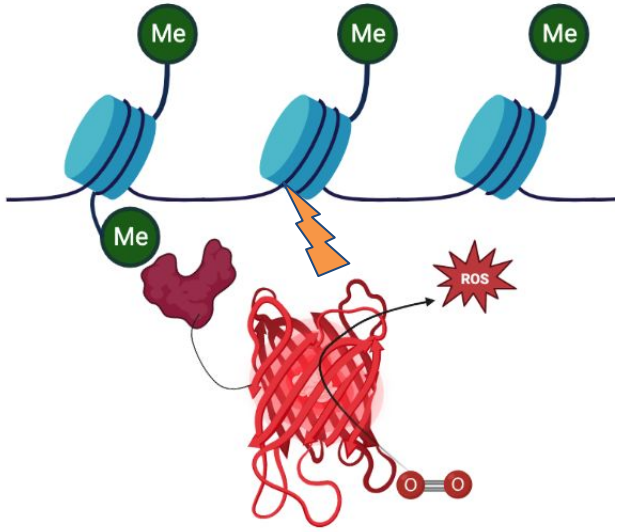
Discussion

We hypothesize that the system should be error-prone for the expressed genes as it is targeted to the DNA which is silenced by methylation. Thus it should not affect the cell transcriptome after the DNA reparation.



Conclusions

Taking everything into consideration, the new genetically encoded construct has shown the improved ability to generate DNA breaks in the cancer cell lines. We suppose that MECP2-KillerRed2 is the better candidate for *in-vivo* test therapy, in comparison with MECP2-SuperNova2 and H2B-SuperNova2, because 1) the use of the new photosensitizer with enhanced phototoxicity; 2) tighter contact between the photosensitizer and the DNA.



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Acknowledgments

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