

Genetically encoded epigenetic probes for visualization of enhancers landscapes in live-cell fluorescence microscopy

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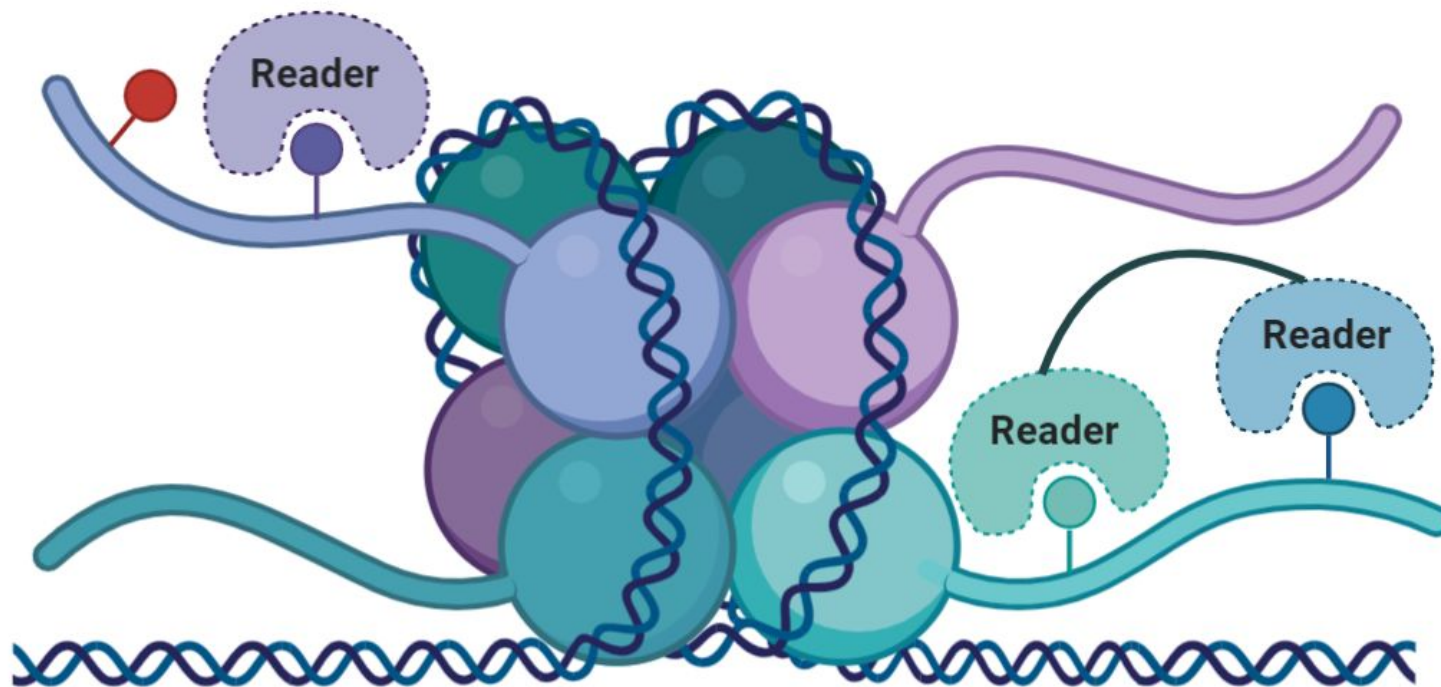
❖ **Idea&design**

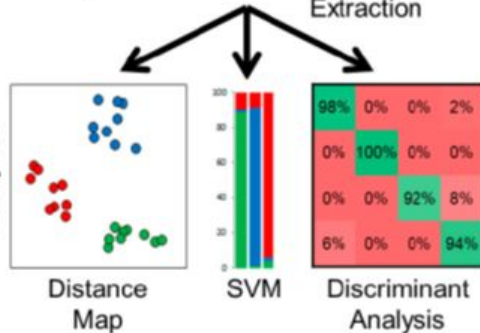
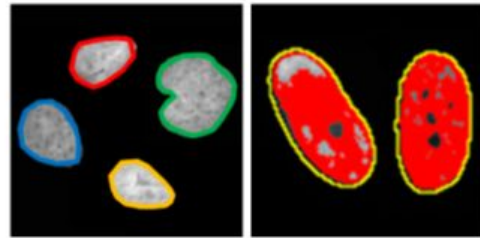
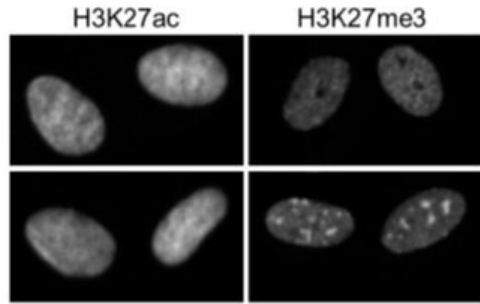
❖ **Results**

❖ **Future aim**

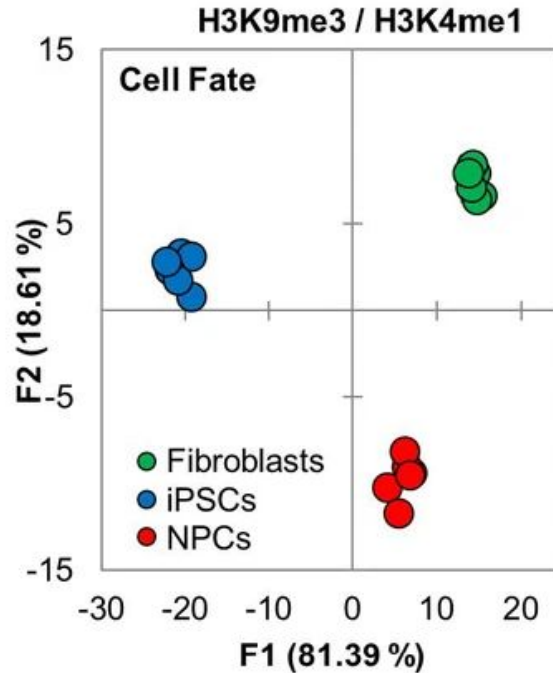
❖ **Conclusion**

Histone modification reader domains (HMRD)





Microscopic Imaging of the Epigenetic Landscape (MIEL)



	Fibroblasts	NPCs	iPSCs
Fibroblasts	100	0	0
NPCs	0	100	0
iPSCs	0	0	100

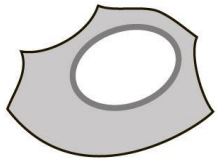
Mean Accuracy: 100%

Farhy C, Hariharan S, Ylanko J, Tersikh A, et al. Improving drug discovery using image-based multiparametric analysis of the epigenetic landscape. *Elife*. 2019

LiveMIEL

MIEL

Cell propagation
Treatment



Cell fixation
Staining with antibodies
Microscopy

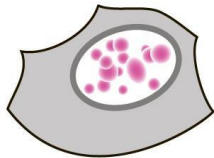
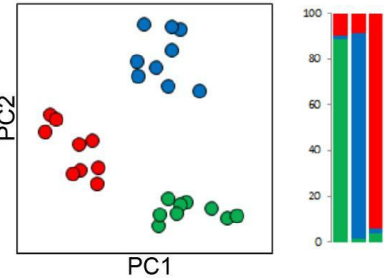


Image analysis:
Segmentation
Feature extraction

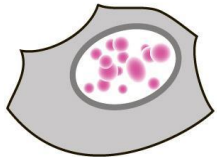
WellIndex	Treatment_Sum	Ch1_TXT_NiCh1_TXT_NiCh1_TXT_Ni
7006000	WT101-Fibro_H3K27me3_H3K4me3	0.028874 0.175719 0.0779014
7006000	WT101-Fibro_H3K27me3_H3K4me3	0.028874 0.175719 0.0779014
7006000	WT101-Fibro_H3K27me3_H3K4me3	0.0092725 0.139706 0.0577746
7006000	WT101-Fibro_H3K27me3_H3K4me3	0.0120932 0.152563 0.0501089
7006000	WT101-Fibro_H3K27me3_H3K4me3	0.006556 0.160766 0.0411373
7006000	WT101-Fibro_H3K27me3_H3K4me3	0.0415059 0.187935 0.0746055
7006000	WT101-Fibro_H3K27me3_H3K4me3	0.012987 0.139706 0.074026
7006000	WT101-Fibro_H3K27me3_H3K4me3	0.0051993 0.125436 0.0970537
7006000	WT101-Fibro_H3K27me3_H3K4me3	0.025523 0.225352 0.102055
7006000	WT101-Fibro_H3K27me3_H3K4me3	0.0188007 0.229292 0.0715706
7006000	WT101-Fibro_H3K27me3_H3K4me3	0.0079536 0.179435 0.0659841
7006000	WT101-Fibro_H3K27me3_H3K4me3	0.0204888 0.119617 0.0765504
7006000	WT101-Fibro_H3K27me3_H3K4me3	0.0284091 0.136092 0.0497139
7006000	WT101-Fibro_H3K27me3_H3K4me3	0.0204118 0.144309 0.0647059
7006000	WT101-Fibro_H3K27me3_H3K4me3	0.0099857 0.126597 0.0477889
7006000	WT101-Fibro_H3K27me3_H3K4me3	0.0191972 0.151568 0.0706806
7006000	WT101-Fibro_H3K27me3_H3K4me3	0.0155559 0.167331 0.0530973

Distance map
Classification

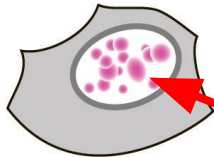


LiveMIEL

Cells expressing
HMRD-FP

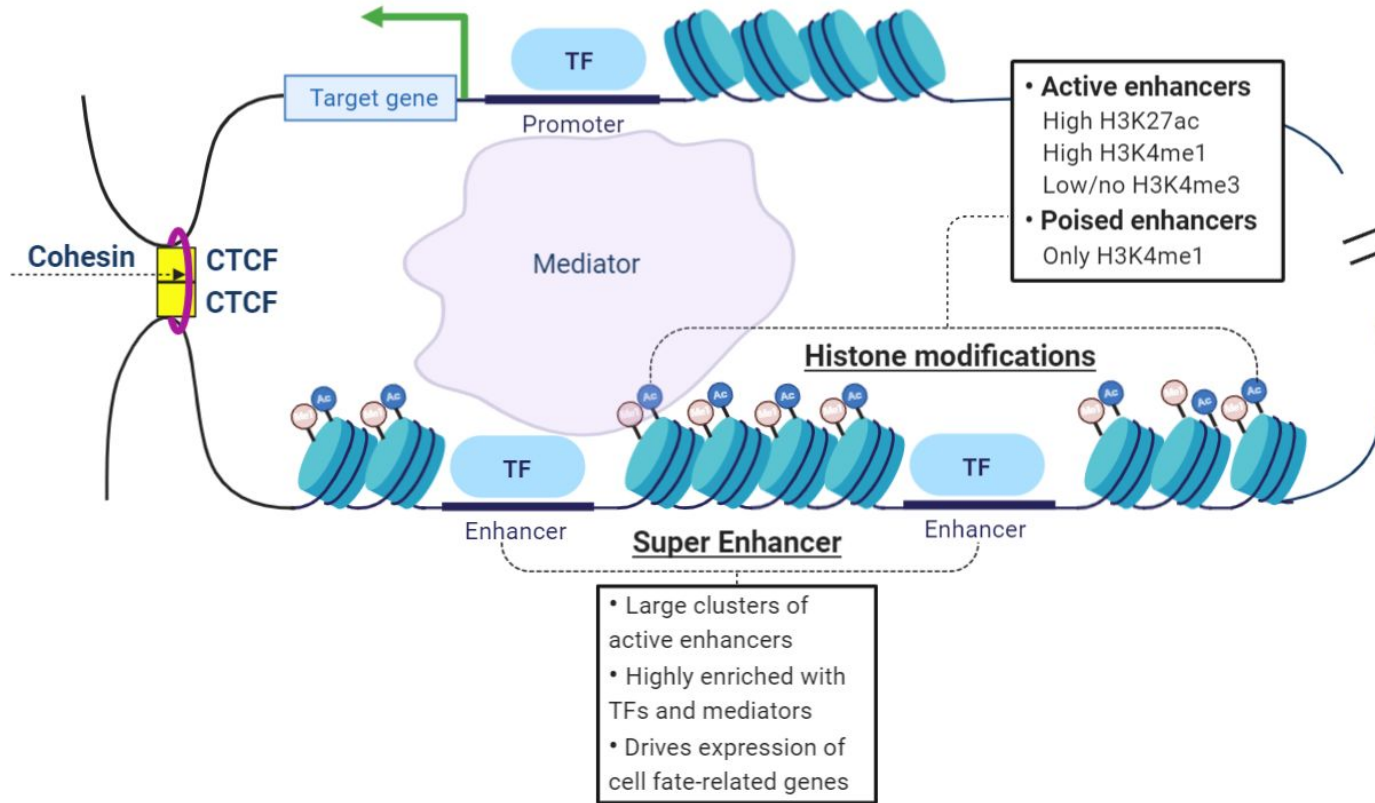


Cell propagation
Treatment
Live cell microscopy

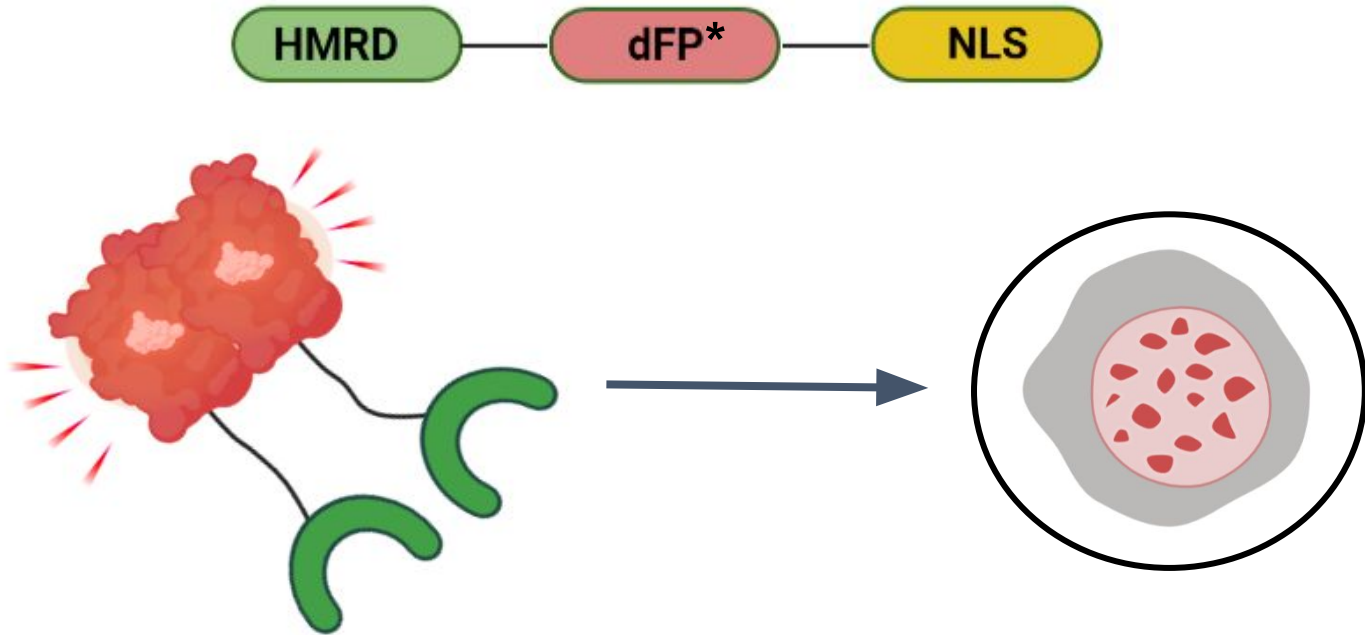


Genetically encoded epigenetic probes (GEEPs)

Enhancers and super-enhancers



GEEPs design



**Due to the low affinity of HMRDs, a dimerizing fluorescent protein is used.*

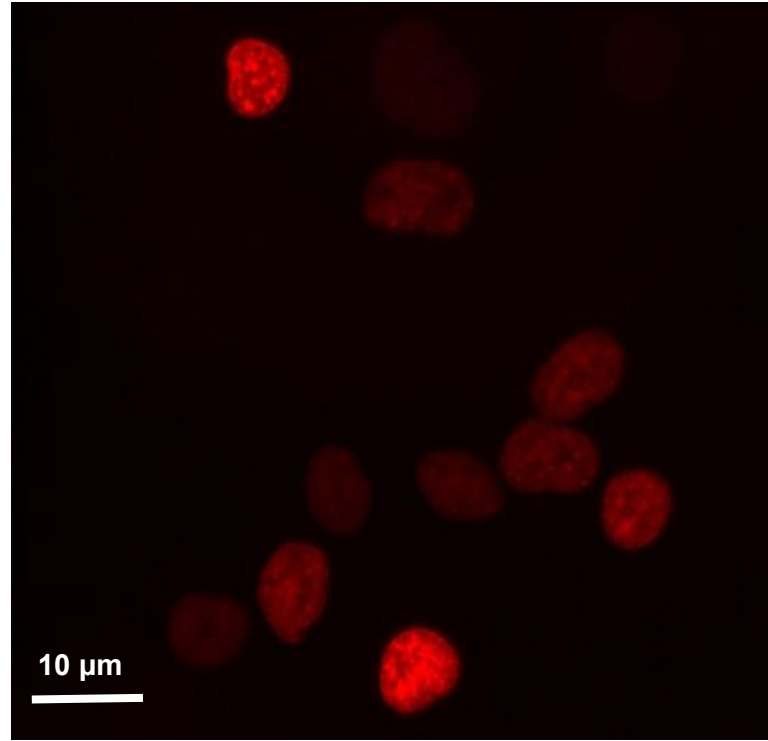
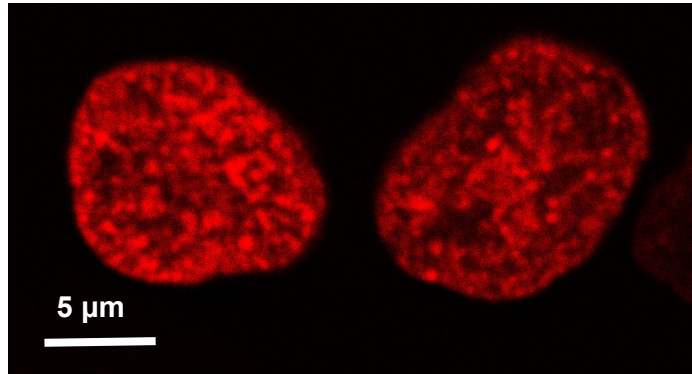
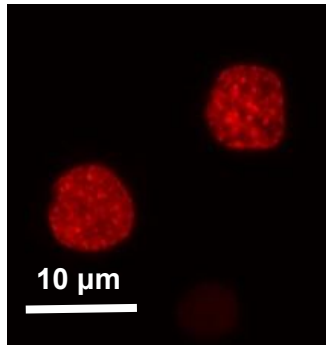
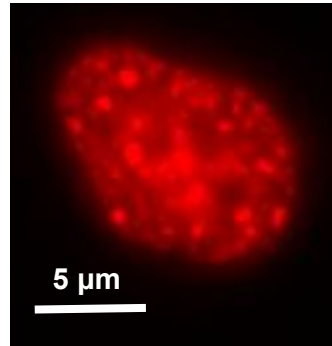
❖ **Idea&design**

❖ **Results**

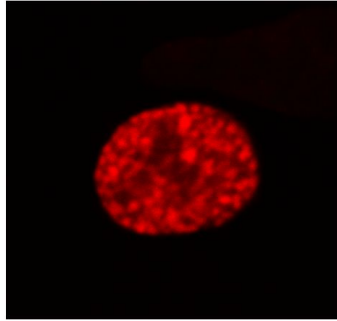
❖ **Future aim**

❖ **Conclusion**

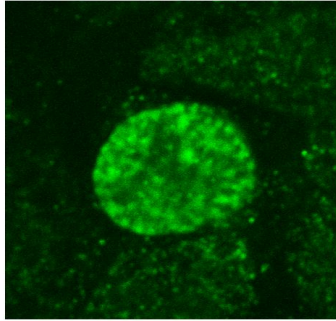
HEK 293T cells transfected with DPF3 - Katushka



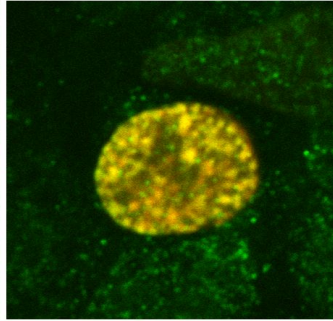
H3K4me1 antibody staining



HEK cells transfected with DPF3_Katushka

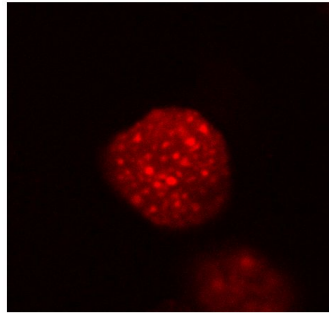


HEK cells stained with H3K4me1 AB

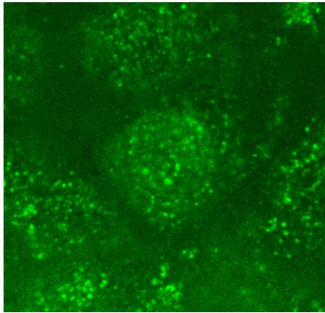


Merge

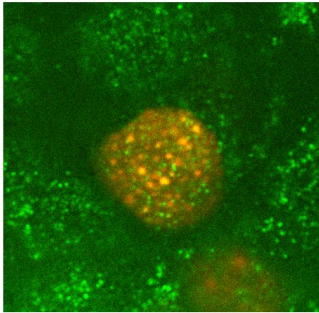
Experiment: DPF3_Katushka
Pearson's R value (no threshold): 0.78*



HEK cells transfected with MPP8_Katushka



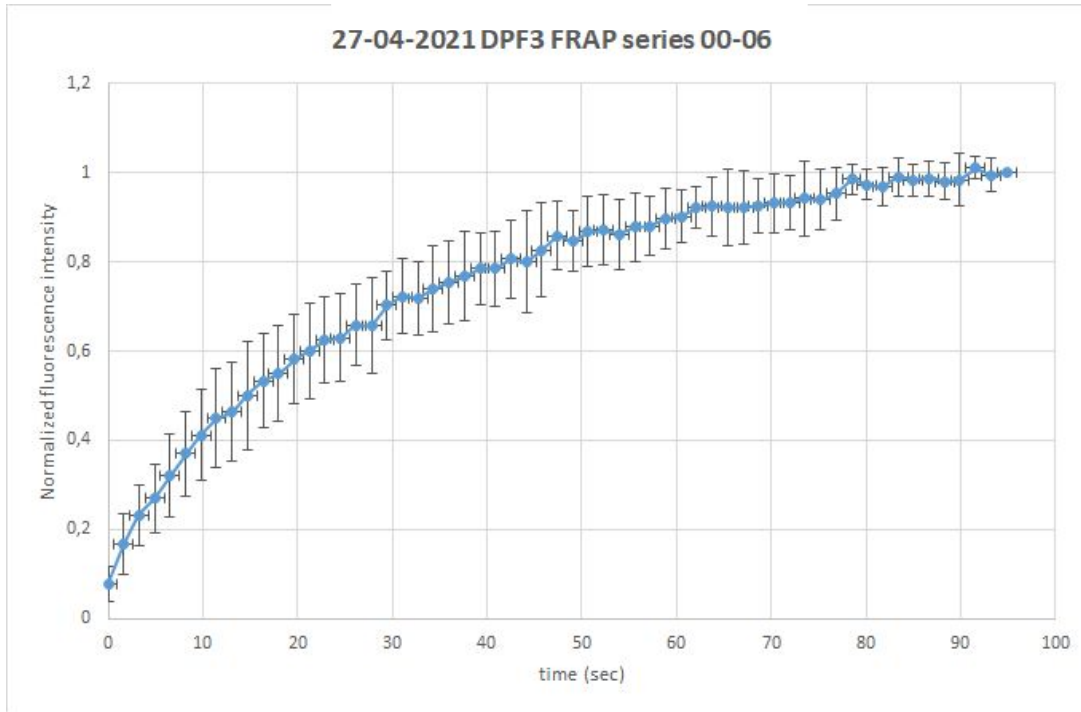
HEK cells stained with H3K4me1 AB



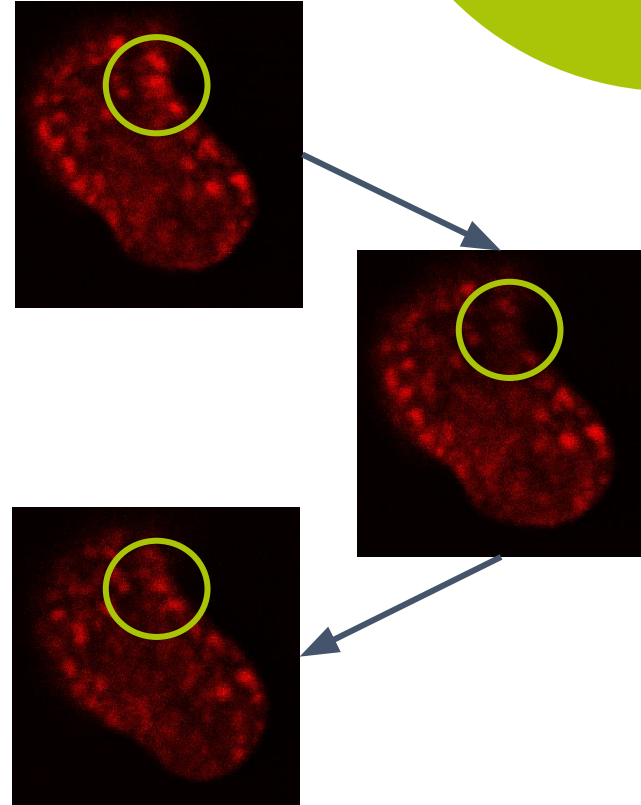
Merge

Negative control: MPP8_Katushka
Pearson's R value (no threshold): 0.19*

DPF3-Katushka FRAP* (Fluorescence recovery after photobleaching)



*Averaged by 7 cells



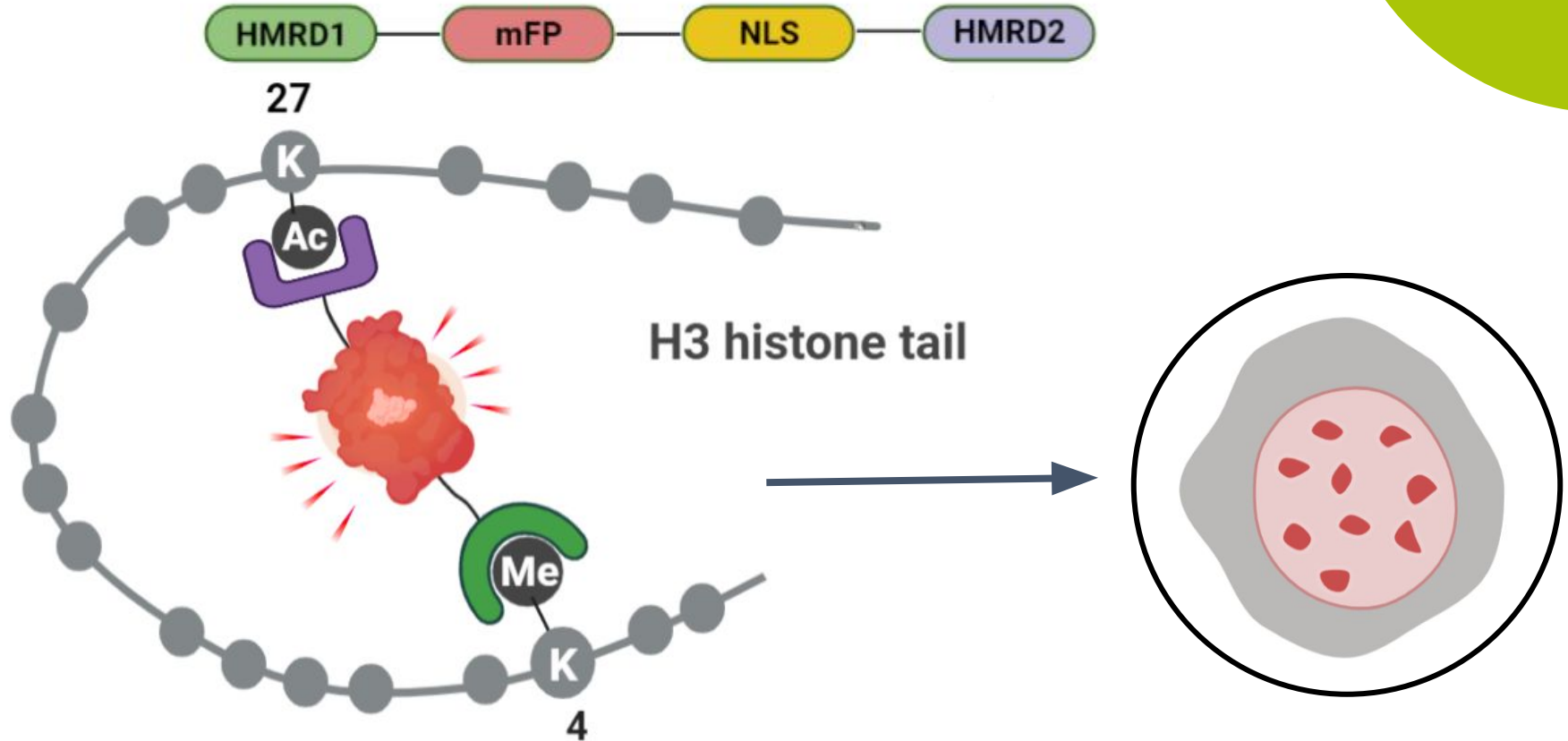
❖ **Idea&design**

❖ **Results**

❖ **Future aim**

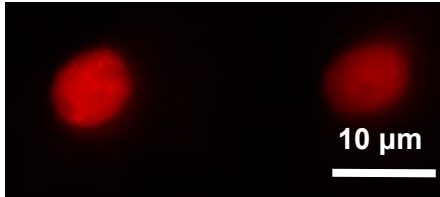
❖ **Conclusion**

Bi-GEEPs for super-enhancers

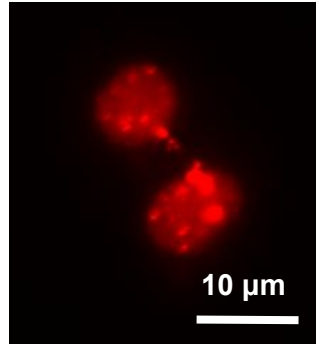


HEK 293T cells transfected with H3K27ac-binding domains

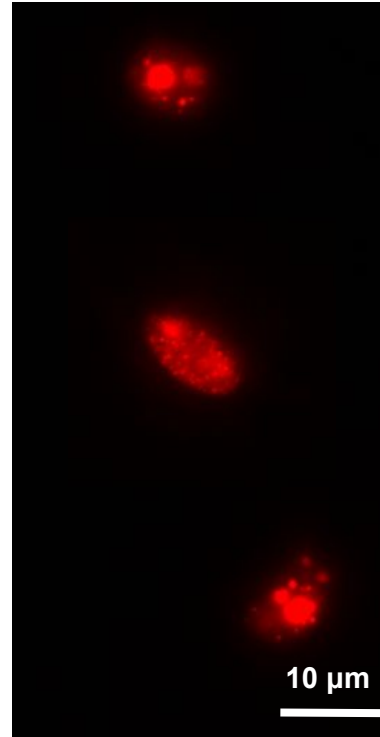
YAF9 - Katushka
(H3K27ac)



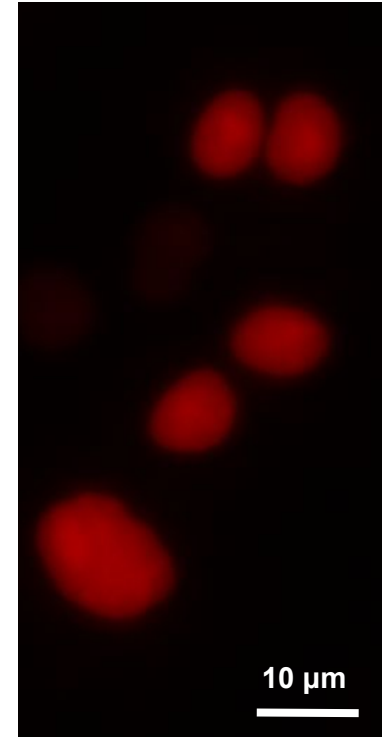
BRD9 - Katushka
(H3K27ac)



YEATS2 - Katushka
(H3K27ac)



GAS41 - Katushka
(H3K27ac)



❖ **Idea&design**

❖ **Results**

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Conclusions

1. **GEEPs based on DPF3 reader domain provide fluorescent patterns representing H3K4me1;**
2. **GEEPs for H3K27ac epigenetic landscape has proven to be more tricky and are yet to be developed;**
3. **These GEEPs can be successfully used alongside with other probes in various research applications in live cells;**
4. **Fluorescent GEEPs based on reader domains provide a fine alternative for antibody-based research, including the MIEL method allowing to assess epigenetic landscape dynamics.**

Thanks to:

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Afanasii Stepanov, Moscow, Skoltech

Skoltech



thx.

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