Supplementary Online Materials for

Rapid and Specific No-wash Activation of a Far-red Fluorogen in Subcellular Compartments by Targeted Fluorogen Activating Proteins.

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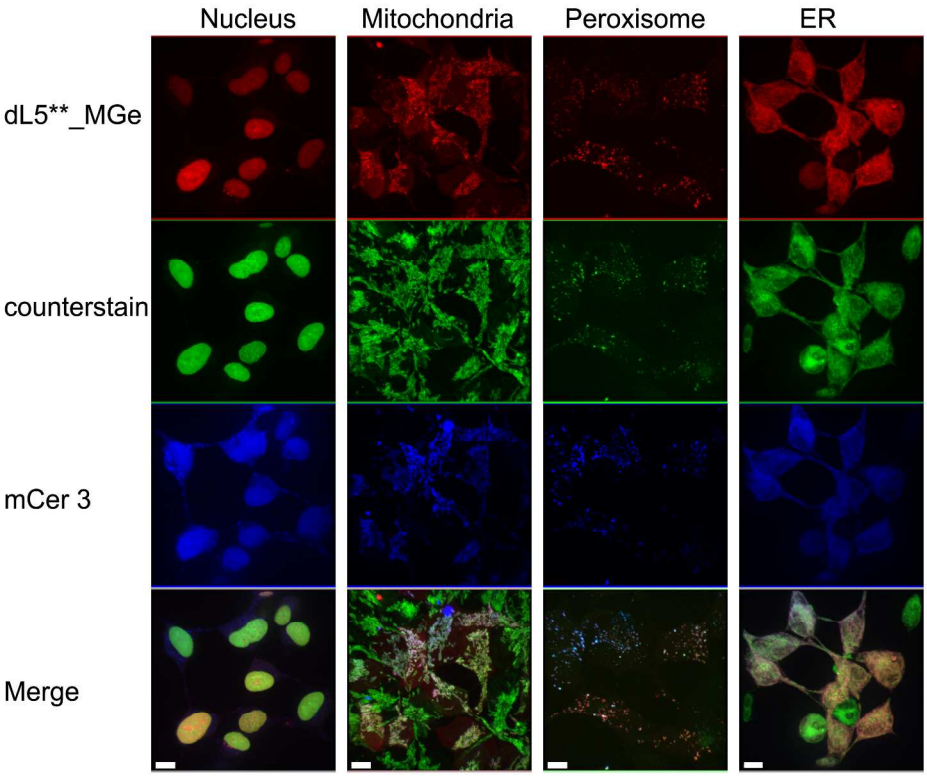
Supplementary Table 1. Primers used for constructing the plasmids for FAP, mCerulean3 expression in HEK cells.

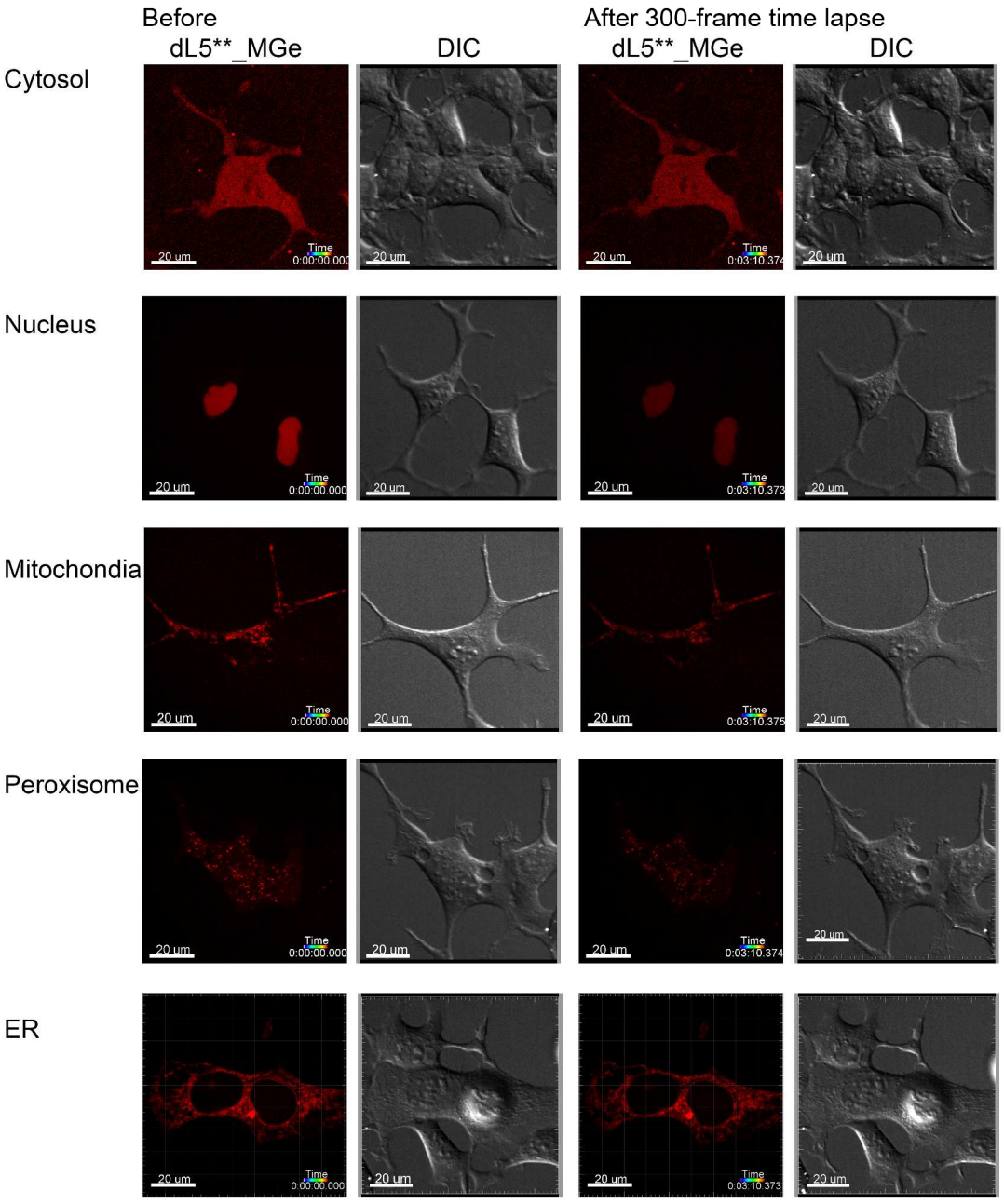
BABEmodF	GATCCCTTGAATTCGGAGGTTCTCATATGGGCGGGTCAACCGGTATCTTACTCGAGG
BABEmodR	TCGACCTCGAGTAAGATACCGGTTGACCCGCCCATATGAGAACCTCCGAATTCAAGG
BamKappaF	TATAGGATCCGGCTTGGGGATATCCACCATGG
EcoKappaR	TATAGAATTCGTCACCAGTGGAACCTGGAACC
EcomycSfidL5F	TATAGAATTCGAGCAGAAGCTGATCAGCGAGGAGGACCTGGGCCTTTCTGGCCTGCAGGCCGTCGTTACCCAAGA
EcoSfidL5F	TATAGAATTCGGCCTTTCTGGCCTGCAGGCCGTCGTTACCCAAGA
NdeSfidL5R	TATATACATATGGGCCAGACCGGCCGCGGAGAGGACGGTCAGCTGG
BamKozATGF	GATCCGGCTTGGGGATATCCACCATGG
EcoKozATGR	AATTCCATGGTGGATATCCCCAAGCCG
BamNLSfor	GATCCGCCACCATGAACAAGAACTCTGCTAAGCGTCGTAAGAAGGGTACTTCTGCTAAGACTAAACGTCCTAAAGTTG
EcoNLSrev	AATTCAACTTTAGGACGTTTAGTCTTAGCAGAAGTACCCTTCTTACGACGCTTAGCAGAGTTCTTGTTCATGGTGGCG
BamMitoF	TATATAGGATCCGCCACCATGCTCGCTACAAG
EcoMitoR	TATATAGAATTCTCTAGATTCTTCAGCCCTTAC
EcoCOXF	TATATAGAATTCATGTCCGTCCTGACGCCG
COXovF	ATGTCCGTCCTGACGCCGCTGCTGCTGCGGGGCTTGACAGGCTCGGCCCGGCGGCTCCCAGTGCC
COXovR	AAGCTTCCCCTCCGGCGGCAACGAATGGATCTTGGCGCGCGGCACTGGGAGCCGCC
EcoCOXR	TATATAGAATTCAAGCTTCCCCTCCGGCG
dH6SfiF	TATATAGGCCTTTCTGGCCTGCAAGTCCAGTTGCAAGAATCTGGAC
dH6SfiR	TATATAGGCCAGACCGGCCGCGGAGACAGTGACCAGGGTACC
CharlieSfiF	TATATAGGCCTTTCTGGCCTGCAGGTGCAGCTGGTGGAGT
CharlieSfiR	TATATAGGCCAGACCGGCCGCTTTGATATCCACTTTGGTCCCTCCG
NdeG4SF	TATGGGAGGCGGTGGGTCTGGTGGCGGTGGCAGCA
AgeG4SR	CCGGTGCTGCCACCGCCACCAGACCCACCGCCTCCCA
AgeCFPFor	TATATAACCGGTATGGTGAGCAAGGGCGAGG
XhoStCFPRev	TATATACTCGAGTTACTTGTACAGCTCCTCCATGCC
XhoCFPRev	TATATACTCGAGCTTGTACAGCTCGTCCATGCC
XhoStSKLCFPR	TATATACTCGAGTTACAGCTTGCTCTTGTACAGCTCGTCCATGCC
XhoKDELSTOPF	TCGAGAAGGATGAACTGTAATGATTATCACAACTCGTCTTTC
XhoKDELSTOPR	TCGAGAAAGACGAGTTGTGATAATCATTACAGTTCATCCTTC



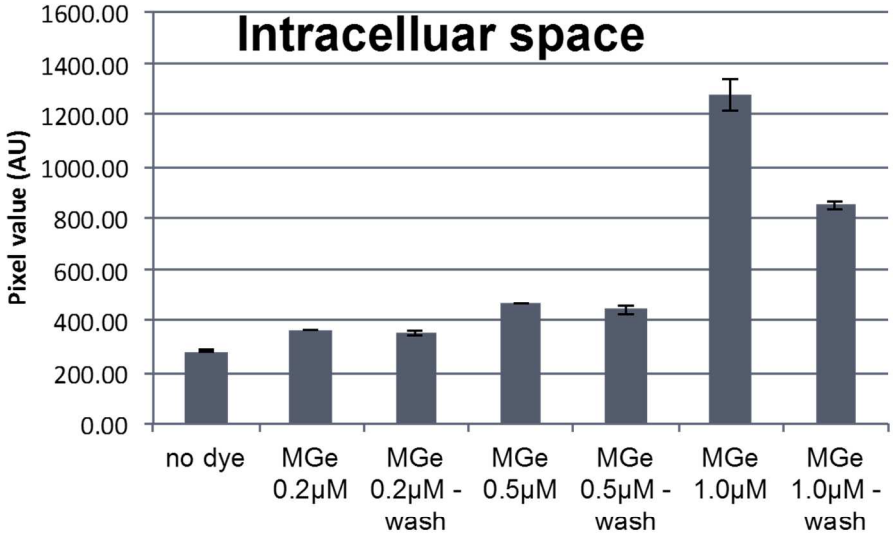
Nucleus	*Pearson's coefficient*
dL5**-MGe vs. SYTO24	**0.79 ± 0.08**
dL5**-MGe vs. mCer 3	**0.62 ± 0.05**
SYTO24 vs. mCer 3	**0.60 ± 0.04**
Mitochondria	
dL5**-MGe vs. MitoTracker	**0.59 ± 0.04**
dL5**-MGe vs. mCer 3	**0.57 ± 0.12**
mCer 3 vs. MitoTracker	**0.42 ± 0.08**

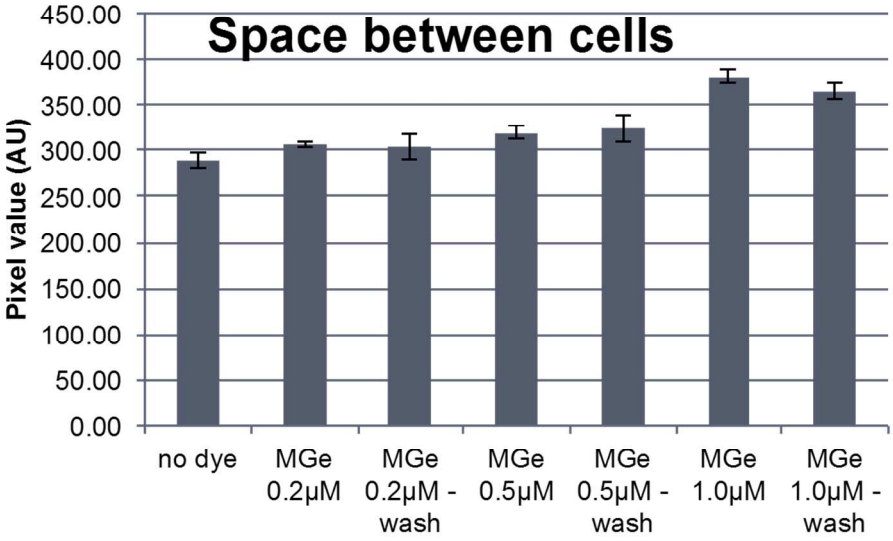
Peroxisome	*Pearson's coefficient*
dL5**-MGe vs. Bodipy-AKL	**0.51 ± 0.06**
dL5**-MGe vs. mCer 3	**0.55 ± 0.04**
mCer 3 vs. Bodipy-AKL	**0.57 ± 0.02**
ER	
dL5**-MGe vs. ER-Tracker	**0.69 ± 0.08**
dL5**-MGe vs. mCer 3	**0.75 ± 0.07**
mCer 3 vs. ER-Tracker	**0.75 ± 0.11**

Supplementary Figure S1. Colocalization analysis of constructs with cell tracker dyes. Each construct was stained with a green "organelle tracker dye" and the colocalization coefficients were determined using Pearson's R analysis (range from -1 for anticorrelated to +1 for noiseless correlation). The values show that each of these constructs is well colocalized with the tracker dyes.

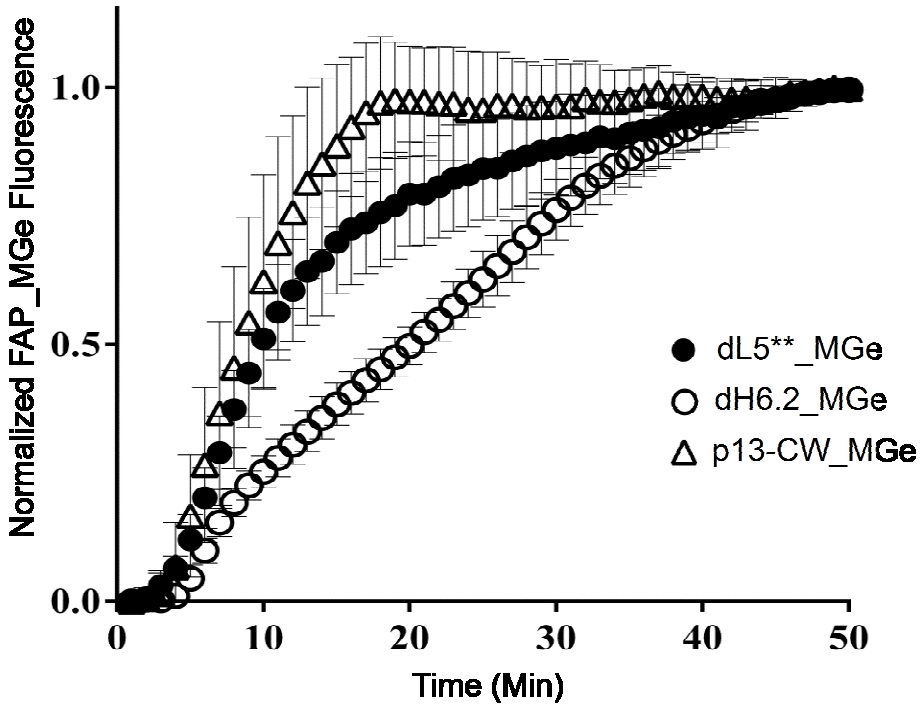


Supplementary Figure S2. Photobleaching of the FAP-fluorogen pair does not induce changes in cell morphology. After 300s of continuous illumination, the cell morphology was not markedly altered as determined by DIC images, regardless of compartment.

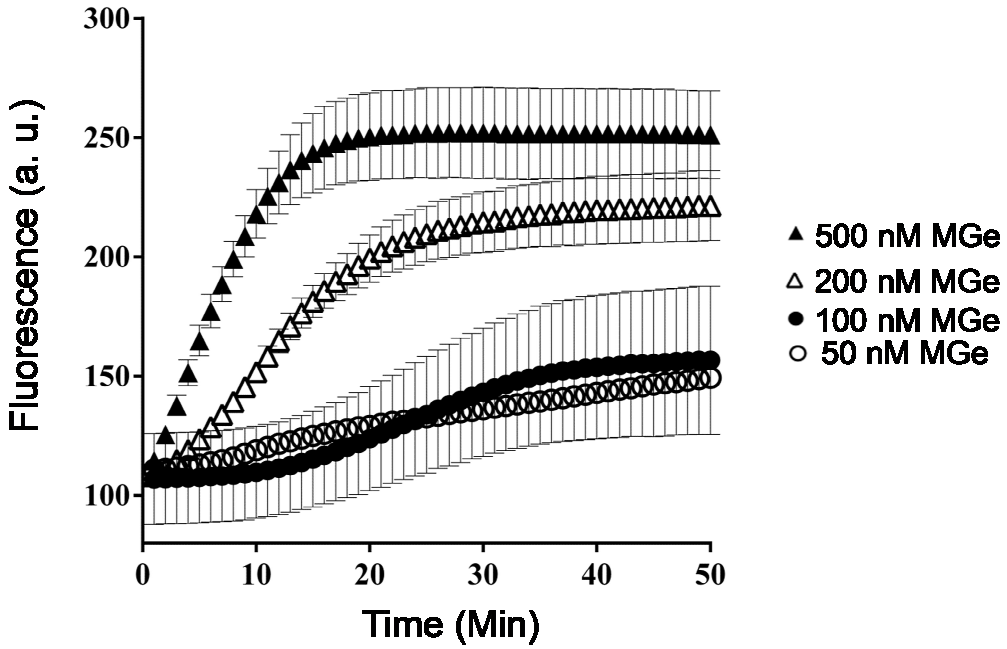




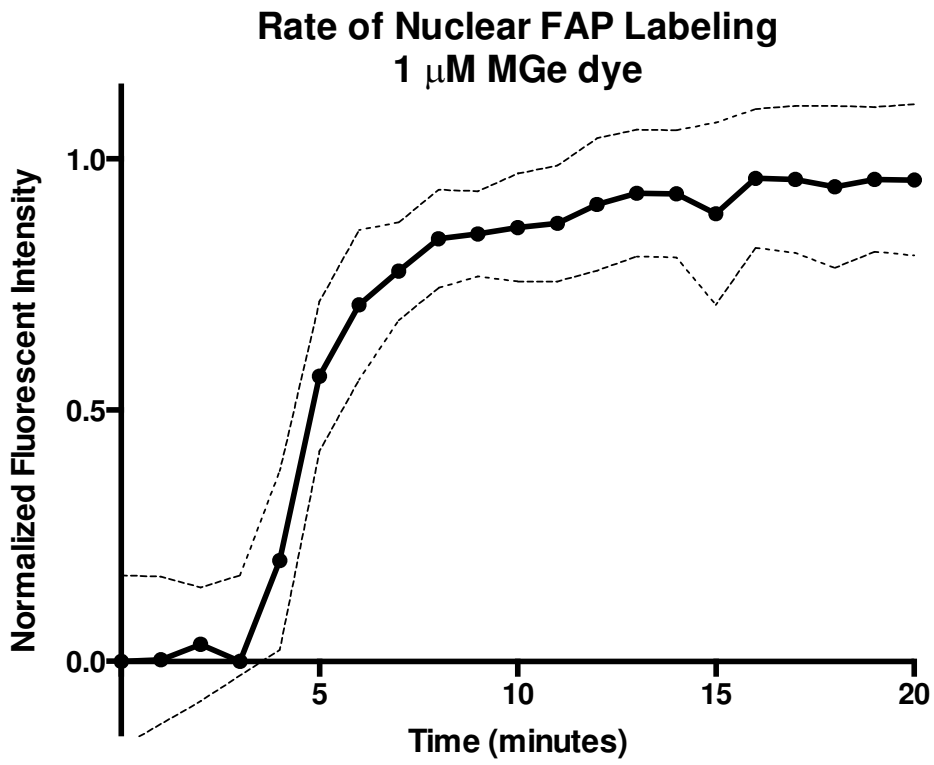
Supplementary Figure S3. Nonspecific activation of MGe fluorogen. Non-transfected HEK-293 cells analyzed for nonspecific dye activation on cells (above) and in inter-cellular regions (bottom).



Supplementary Figure S4. Clone-dependent activation rates. Activation of dye with dl5**, dH6.2, p13-CW targeted to the nucleus of HEK cells upon addition of 200 nM MGe.

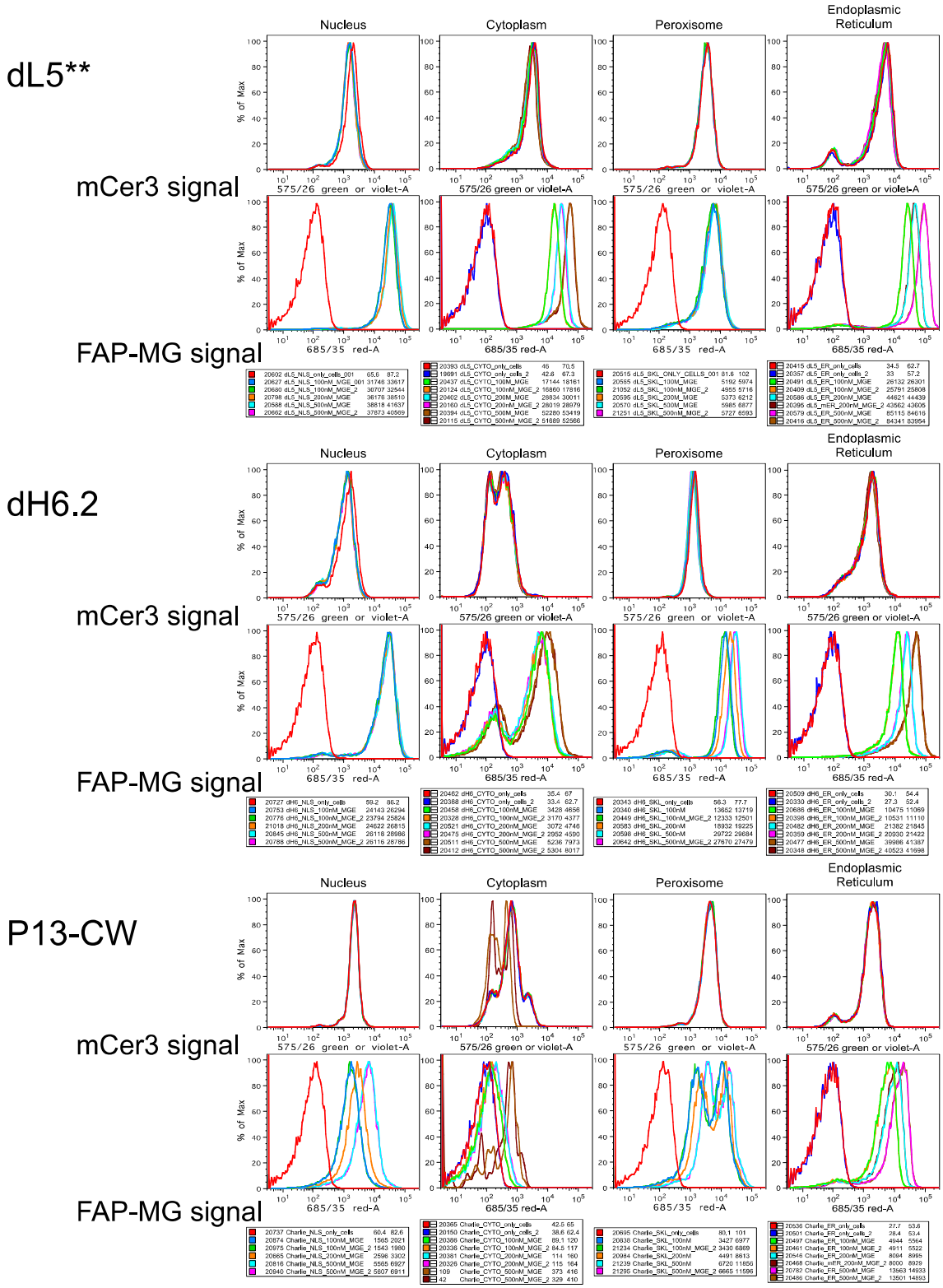


Supplementary Figure S5. Dose-dependent fluorescence activation of FAP dL5**_MGe in cytosol.



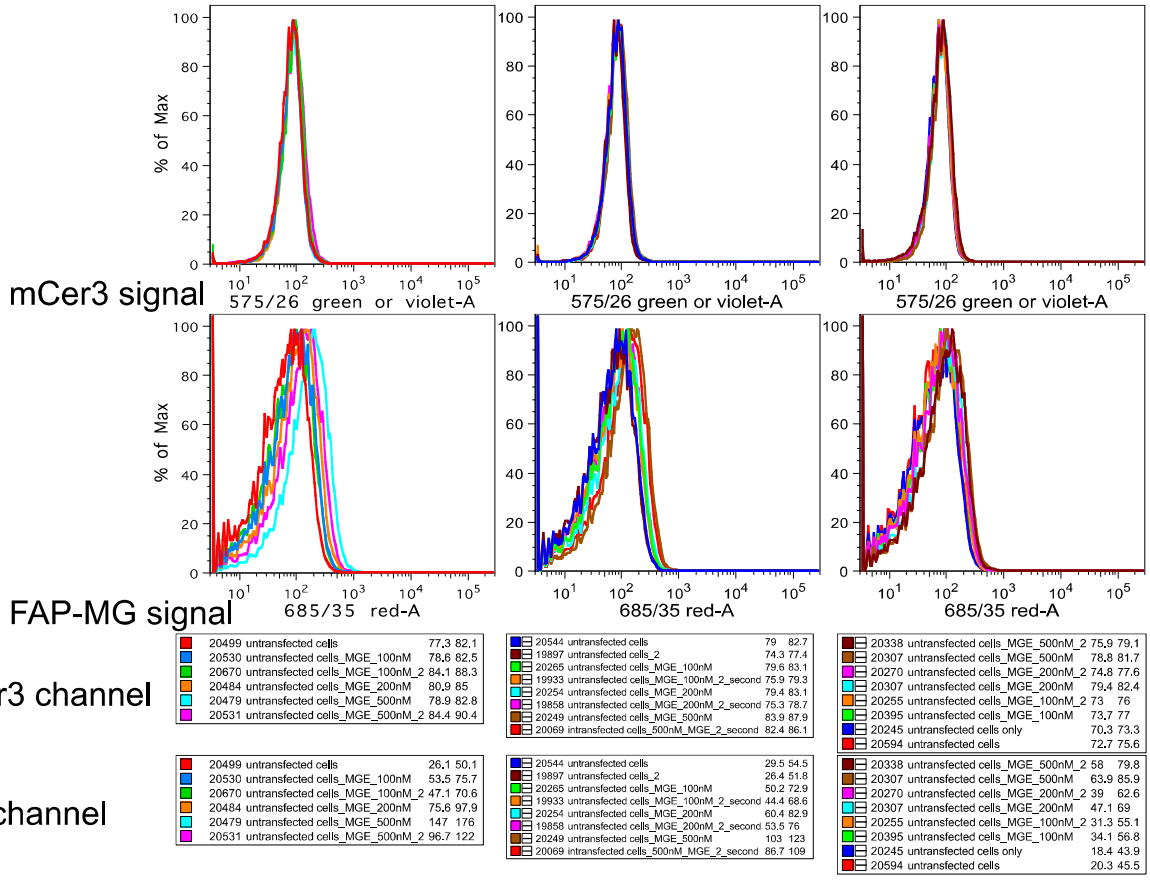
Supplementary Figure S6. Rate of fluorogen activation on NLS-dL5**-mCer3 expressing cells with 1 μM added MGe dye. At time t=0 min, image acquisition was started and the media was brought to 1 μM MGe dye, with image acquisition set for 1 frame per minute. The labeling plateau was reached within 10 minutes, typically in 7-8 minutes. The standard deviation of three independent measurements in three separate dishes is plotted in the dotted-line error band.

Supplementary Movie S1. Loading of nuclear, NLS-dL5**-mCer3, with 1 μM MGe dye.



Supplementary Figure S7. Flow cytometry histograms showing concentration-dependent FAP activation in stable expressing cell populations. In data tables, left value is number of cells analyzed, right values are mean and median fluorescent intensity values of cells respectively.

Untransfected HEK cells



Supplementary Figure S8. Flow cytometry histograms showing nonspecific activation of MGe dye on untransfected HEK cells. In data tables below histograms, left value is number of cells analyzed and right values are mean and median fluorescent intensity values of cells respectively.