Supplementary Material For:

Reports

Combining RNAi and in vivo confocal microscopy analysis of the photoconvertible fluorescent protein Dendra2 to study a DNA repair protein

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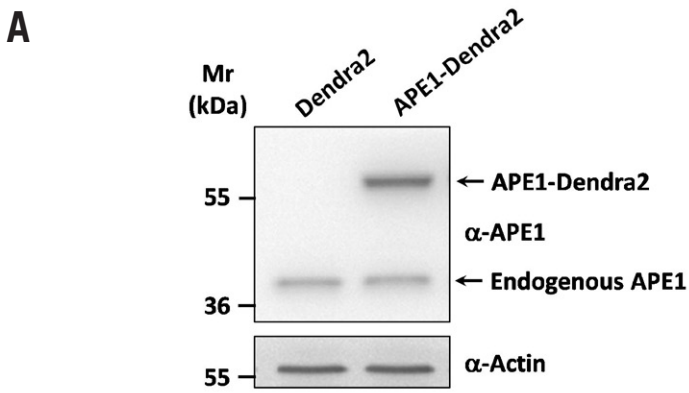
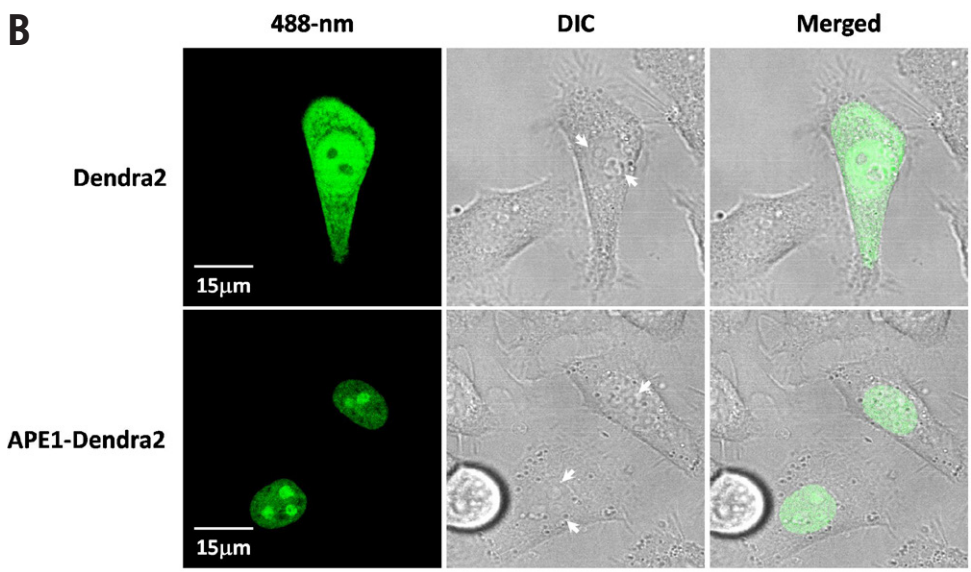
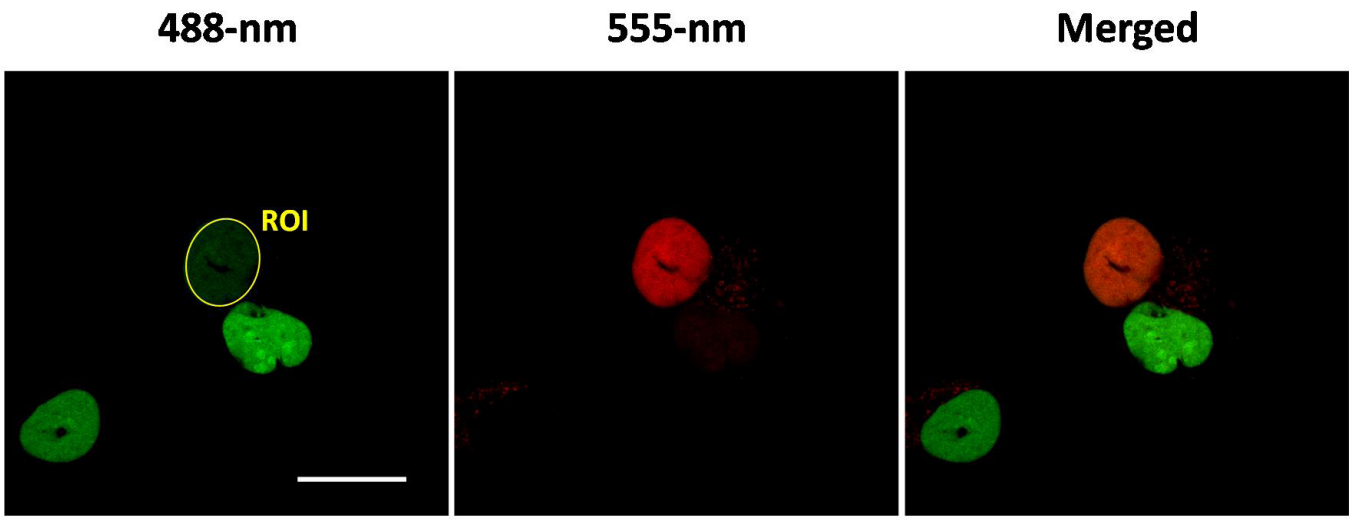
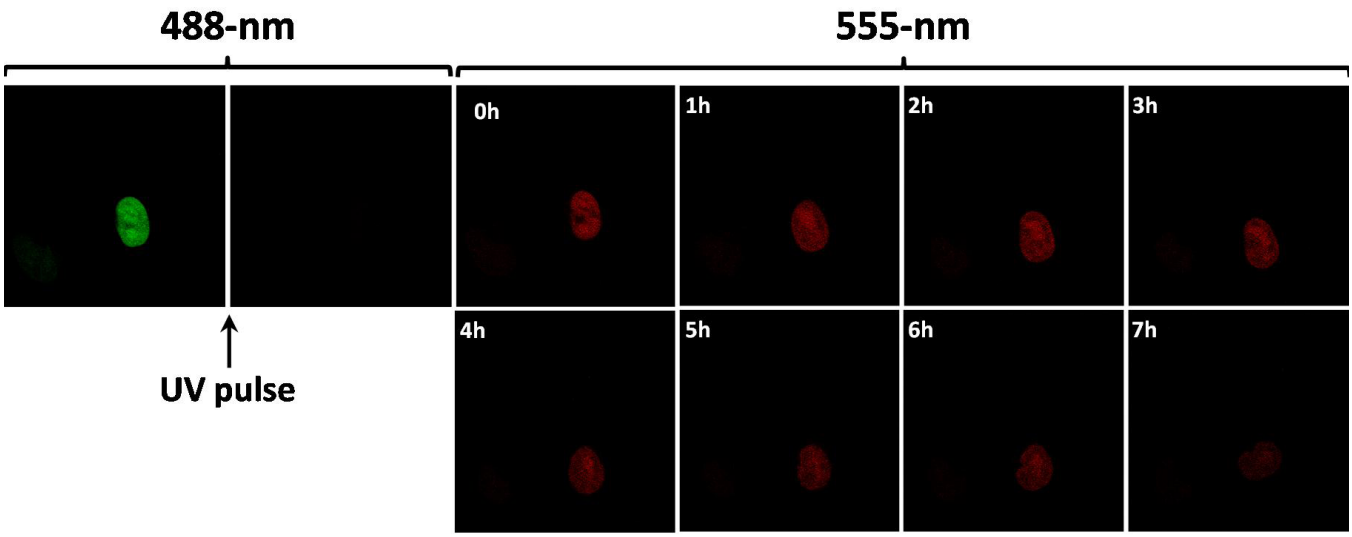


Supplementary Figure S1. Panel A: SF767 cells were transiently transfected with pDendra2-N and pDendra2-N-APE1 vectors. 48 h after transfection, cells were collected, lysed, and 10 µg of total cell extract was separated on a 12% SDS-PAGE and transferred to a nitrocellulose membrane for immunodetection with anti-APE1 and anti-actin antibodies. In the second lane, an upper band of approximately 60 kDa is visible, corresponding to APE1-Dendra2 recombinant fusion protein. Panel B: SF767 cells were transiently transfected with pDendra2-N and pDendra2-N-APE1 vectors. 4 x 10^5 cells were collected 24 h after transfection, seeded onto a glass bottom Petri dish, grown with DMEM without phenol red for an additional 24 h, and then visualized through a confocal microscope. As for HeLa stable clones, Dendra2 was present within the cytoplasm and nucleus but was completely excluded from nucleoli. Expression of Dendra2 in fusion with APE1 resulted in relocalization of the green signal within the nuclear compartment and the accumulation of the recombinant fusion protein in the nucleoli.





Supplementary Figure S2. The region of interest (ROI) was irradiated with a 405-nm diode laser regulated at 100% of power for a single scan with a pixel dwell time of 1.27 µs to determine the photoconversion of Dendra2 only in the selected region (white bar = 15 µm).



Supplementary Figure S3. SF767 cells were transiently transfected with pDendra2-N and pDendra2-N-APE1 vectors. 4 x 10^5 cells were collected 24 h after transfection, seeded onto a glass bottom Petri dish, grown with DMEM without phenol red for an additional 24 h, and visualized through a confocal microscope. Dendra2 was efficiently photoconverted from green to red with a UV pulse and then cell images were acquired each hour for seven hours. Image analysis confirmed the half-life of APE1 after seven hours.

Supplementary Movie S1. HeLa cells stably expressing APE1-Dendra2 recombinant fusion protein were monitored for 14 hours after photoconversion by acquiring images every hour. The field shows four out of nine photoconverted cells. Stack images of 488-nm and 555-nm were sequentially mounted to create the movie.