

# **PML-Nuclear Bodies Regulate the Stability of the Fusion Protein Dendra2-Nrf2 in the Nucleus**

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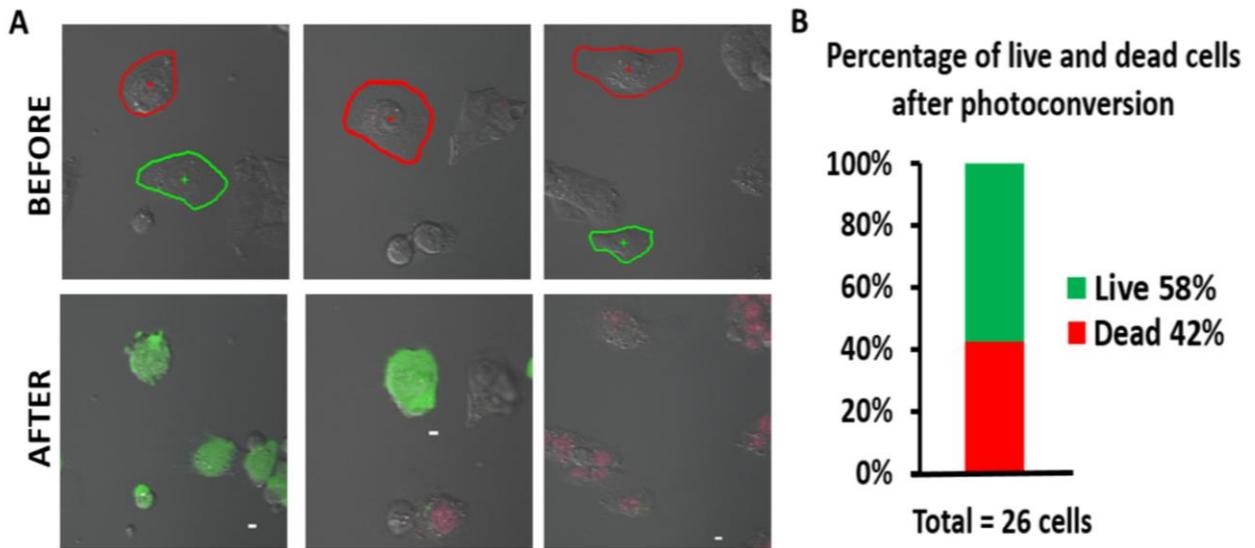
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## **Supplementary Material**

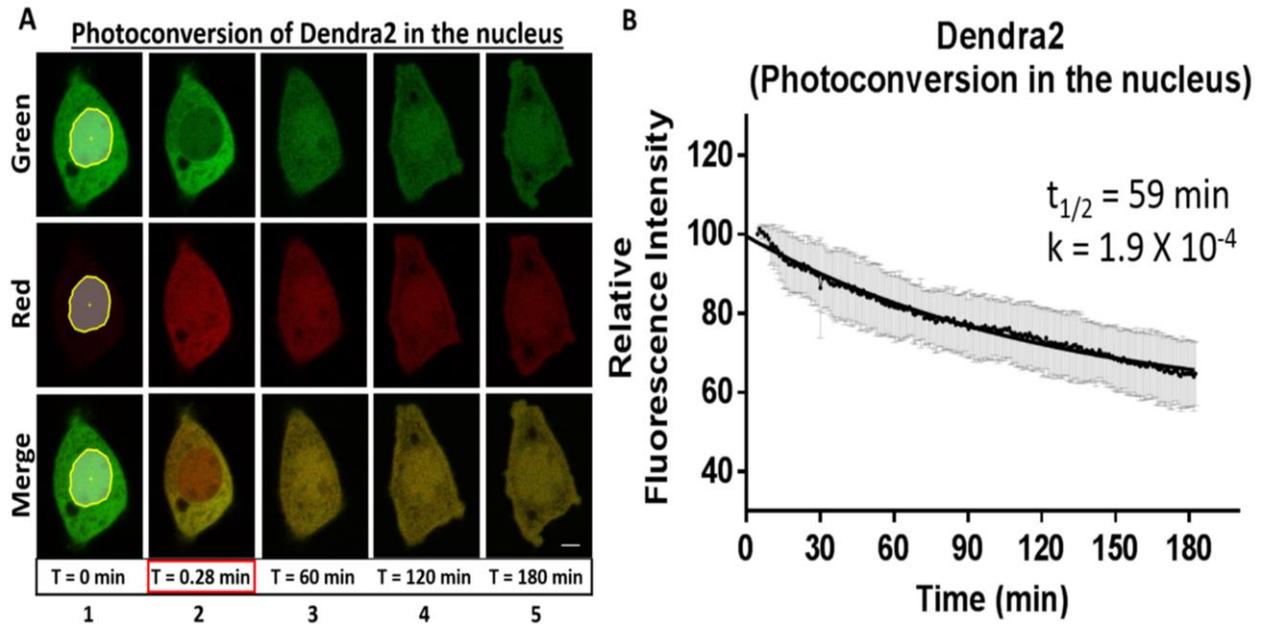
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Amos M. Sakwe

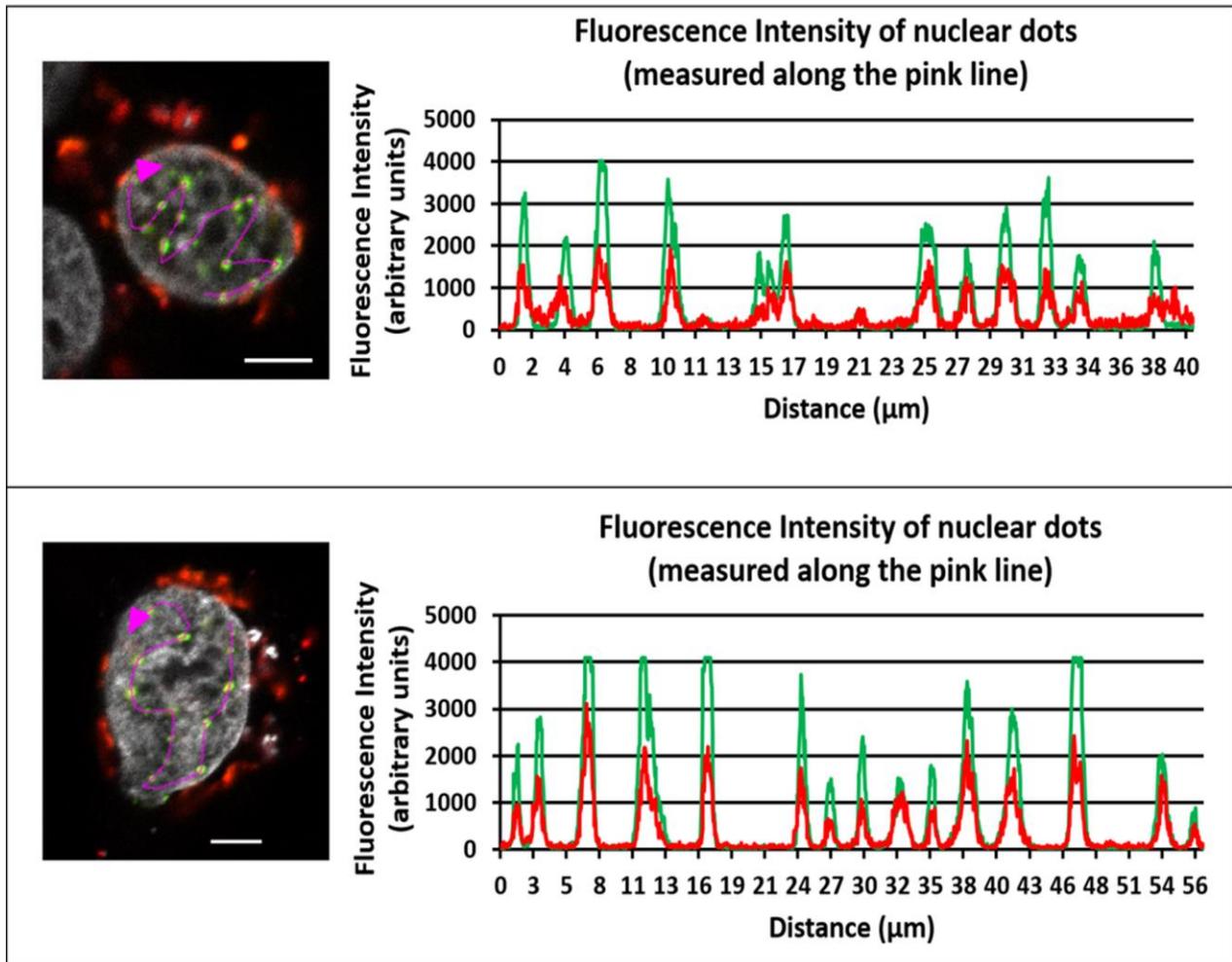
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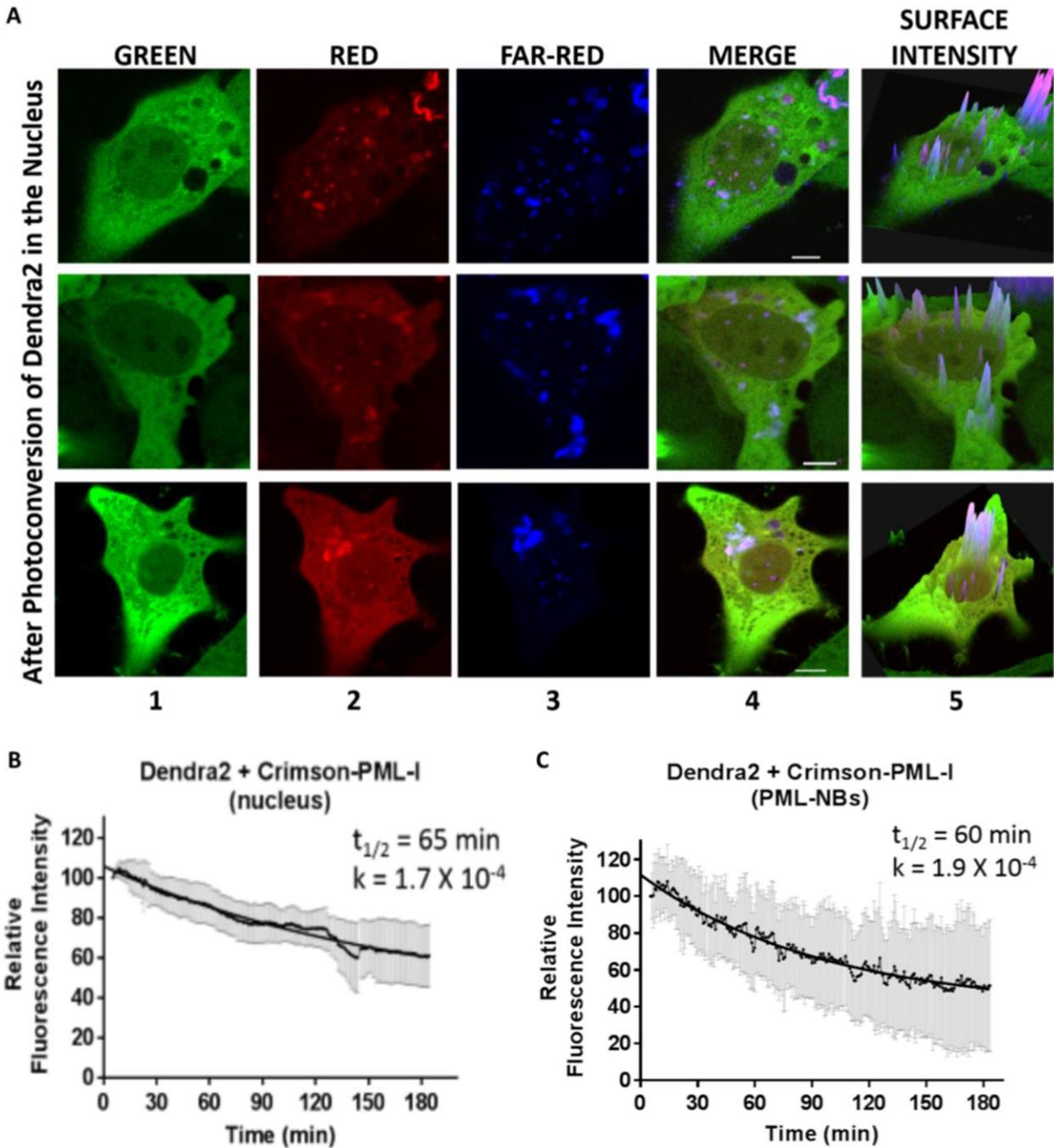
**Figure S1. Cell viability of photoconverted cells using fluorescent microscopy analysis.** Non-transfected HepG2 cells ( $5 \times 10^5$ ) were plated in glass bottom MatTek dishes and visualized 48 h later. Some cells were demarcated around the cells margin and a 405 nm laser pulse was applied for 2.0 sec to the indicated area, simulating photoconversion. After 3 h, the cells were labeled with 5X concentration of live and dead cell dye for 10 minutes and images were captured in the green and red channels before and after application of the laser (A). The live (green) and dead (red) cells, after application of the laser, were quantified and plotted as a percentage of total number of cells (B).



**Figure S2. Monitoring degradation of Dendra2 in the nucleus of single live cells.** HepG2 cells ( $5 \times 10^5$ ) stably expressing Dendra2 were seeded on glass bottom MatTek dishes and visualized 48 h later. *A*, the nucleus was outlined and used as the point of application for the 405 nm laser pulse for 0.5 sec. Time-lapse images were captured every 45 seconds in the green and red channels after photoconversion. The images in column 2 were captured immediately after photoconversion ( $T = 0.28$  min). *B*, the average fluorescence decay after photoconversion in the red channel was quantified in the nucleus under basal conditions and plotted over time as described in fig. 3 ( $n = 6$  cells). Scale bars, 5  $\mu$ m.



**Figure S3. Co-localization of GFP-PML-I and Crimson-PML-I measured in PML-containing nuclear bodies.** HepG2 cells ( $5 \times 10^5$ ) were co-transfected with  $0.25 \mu\text{g}$  of pGFP-PML-I and  $0.5 \mu\text{g}$  of pCrimson-PML-I, fixed, and visualized by confocal microscopy. Fluorescence intensities measured along the pink lines in the nuclei from Fig. 6B (column 4- top and bottom rows, respectively) demonstrate co-localization of GFP-PML-I and Crimson-PML-I in the green and far-red channels. Scale bars,  $5 \mu\text{m}$



**Figure S4. Monitoring degradation of Dendra2 in PML-NBs.** *A*, HepG2 cells ( $5 \times 10^5$ ) stably expressing Dendra2 were transfected with pCrimson-PML-I ( $1 \mu\text{g}$ ) in glass bottom MatTek dishes and visualized 48 h later. Prior to photoconversion of Dendra2, the nucleus was outlined and photoconverted using a 405 nm laser for 0.5 sec. Time-lapse images were captured in the green (*column 1*), red (*column 2*) and far-red (*column 3*) channels after photoconversion. Merged images are shown in *columns 4* and *5* to illustrate the cellular distribution of fluorescence within the live intact cells. *B* and *C*, the average fluorescence decay, after photoconversion, in the red channel was quantified in the nucleus (*B*) or in PML-NBs (*C*) (indicated by the pink dots in the nuclei of *A-column 4*) and plotted over time ( $n = 8$  cells, 26 PML-NBs). Scale bars, 5  $\mu\text{m}$ .

**TABLE S1**

<b>Table S1. Details for constructing expression plasmids</b>							
<b>Expression plasmid</b>	<b>Gene template</b>	<b>Vector</b>	<b>Restriction Enzymes</b>	<b>Primers 5'→3'</b>	<b>Linker size (nucleotides)</b>	<b>Linker sequence</b>	<b># of amino acids in fusion protein</b>
Dendra2-Nrf2	GFP-Nrf2	pDendra2-C	HindIII BamHI-HF	Forward: ACTCGAGCTCAAGCTTGTGATTGATTGACATCCTTTGGAGGC Reverse: TAGATCCGGTGGATCCCTAGTTTTCTTTGTATCTGGCTTCTTGCTTTTGGG	24	SGDSGVYK	831
Crimson-PML-I	GFP-PML-I	pE2-Crimson-C1	EcoRI-HF BamHI-HF	Forward: CTCAAGCTTCGAATTCATGCCTCCCCCGAGACCCCTCTGAAG Reverse: TAGATCCGGTGGATCCTCAGCTCTGCTGGGAGGCCCTCTGCCAAG	27	RSRAQASNS	1,095

Vectors were purchased from Clontech Laboratories Inc., Mountain View, CA. Restriction enzymes were purchased from New England Bio labs Inc., Ipswich, MA. Primers were purchased from Life Technologies.

TABLE S2

<b>Table S2. Average Decay Parameters of Photoconverted Nuclear Dendra2 Measured in the Nucleus and Whole Cell</b>				
	<b>Nucleus</b>		<b>Whole</b>	
	Half-life ( $t_{1/2}$ ) min	Avg. Decay Rate (k)	Half-life ( $t_{1/2}$ ) min	Avg. Decay Rate (k)
-	59	$1.9 \times 10^{-4}$	92	$1.2 \times 10^{-4}$
<b>Crimson-PML-I</b>	65	$1.7 \times 10^{-4}$	50	$2.3 \times 10^{-4}$
<b>Crimson-PML-I-2KR</b>	71	$1.6 \times 10^{-4}$	111	$1.0 \times 10^{-4}$
The average half-life and decay rate values of Dendra2 were calculated using PyFDAP v1.1.2 software.				