

Supplemental Material to:

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**Nuclear localization signal deletion mutants of lamin A
and progerin reveal insights into lamin A processing and
emerin targeting**

Nucleus 2013; 5(1)

<http://dx.doi.org/10.4161/nucl.28068>

<http://www.landesbioscience.com/journals/nucleus/article/28068/>

Figure S1

AGACCCCGTCCCAGCGGGCGGCCACCCGCAGCGGGGCGCAGGCCAGCTCCACTCCGCTGTCGCCCACCCGCATCAC
LMNA 5F (→)
CCGGCTGCAGGAGAAGGAGGACCTGCAGGAGCTCAATGATCGCTTGGCGGTCTACATCGACCGTGTGCGCTCGCTGG
AAACGGAGAACGCAGGGCTGCGCCTTCGCATCACCGAGTCTGAAGAGGTGGTCAGCCGCGAGGTGTCCGGCATCAAG
GCCGCCTACGAGGCCGAGCTCGGGGATGCCCGCAAGACCCTTGACTCAGTAGCCAAGGAGCGCGCCCGCCTGCAGC
TGGAGCTGAGCAAAGTGCGTGAGGAGTTTAAGGAGCTGAAAGCGCGCAATACCAAGAAGGAGGGTGACCTGATAGCT
GCTCAGGCTCGGCTGAAGGACCTGGAGGCTCTGCTGAACTCCAAGGAGGCCGCACTGAGCACTGCTCTCAGTGAGAA
GCGCACGCTGGAGGGCGAGCTGCATGATCTGCGGGGCCAGGTGGCCAAGCTTGAGGCAGCCCTAGGTGAGGCCAAG
AAGCAACTTCAGGATGAGATGCTGCGGCGGGTGGATGCTGAGAACAGGCTGCAGACCATGAAGGAGGAACTGGACTT
CCAGAAGAACATCTACAGTGAGGAGCTGCGTGAGACCAAGCGCCGTCATGAGACCCGACTGGTGGAGATTGACAATG
GGAAGCAGCGTGAGTTTGAGAGCCGGCTGGCGGATGCGCTGCAGGAACTGCGGGGCCAGCATGAGGACCAGGTGGA
GCAGTATAAGAAGGAGCTGGAGAAGACTTATTCTGCCAAGCTGGACAATGCCAGGCAGTCTGCTGAGAGGAACAGCAA
CCTGGTGGGGGCTGCCACGAGGAGCTGCAGCAGTCGCGCATCCGCATCGACAGCCTCTCTGCCAGCTCAGCCAGC
TCCAGAAGCAGCTGGCAGCCAAGGAGGCGAAGCTTCGAGACCTGGAGGACTCACTGGCCCGTGAGCGGGACACCAG
CCGGCGGCTGCTGGCGGAAAAGGAGCGGGAGATGGCCGAGATGCGGGCAAGGATGCAGCAGCAGCTGGACGAGTAC
CAGGAGCTTCTGGACATCAAGCTGGCCCTGGACATGGAGATCCACGCCTACCGCAAGCTCTTGAGGGGCGAGGAGGA
GAGGCTACGCCTGTCCCCCAGCCCTACCTCGCAGCGCAGCCGTTGGCCGTGCTTCCTCTCACTCATCCCAGACACAGG
GTGGGGGCAGCGTCACC AAAAAGCGCAAACCTGGAG TCCACTGAGAGCCGCA GCAGCTTCTCACAGCACGCACGCAC
LMNA 3F (→) NLS LMNA 5R (←)
TAGCGGGCGCGTGGCCGTGGAGGAGGTGGATGAGGAGGGCAAGTTTGTCCGGCTGCGCAACAAGTCCAATGAGGAC
CAGTCCATGGGCAATTGGCAGATCAAGCGCCAGAATGGAGATGATCCCTTGCTGACTTACCGGTTCCCACCAAAGTTCA
CCCTGAAGGCTGGGCAGGTGGTGACGATCTGGGCTGCAGGAGCTGGGGCCACCCACAGCCCCCTACCGACCTGGT
GTGGAAGGCACAGAACACCTGGGGCTGCGGGAACAGCCTGCGTACGGCTCTCATCAACTCCACTGGGGAAGAAGTGG
CCATGCGCAAGCTGGTGCCTCAGTACTGTGGTTGAGGACGACGAGGATGAGGATGGAGATGACCTGCTCCATCAC
CACCACGGCTCCCACTGCAGCAGCTCGGGGGACCCCGCTGAGTACAACCTGCGCTCGCGCACCGTGCTGTGCGGGAC
CTGCGGGCAGCCTGCCGACAAGGCATCTGCCAGCGGCTCAGGAGCCCAGGTGGGGCGGACCCATCTCCTCTGGCTCTT
CTGCCTCCAGTGTACGGTCACTCGCAGCTACCGCAGTGTGGGGGGCAGTGGGGGTGGCAGCTTCGGGGACAATCTG
GTCACCCGCTCCTACCTCCTGGGCAACTCCAGCCCCGAACCCAGAGCCCCCAGA ACTGCAGCATCATGTAATCTAGA
150 bp deletion in progerin LMNA 3R (←)
GTCGAC

Fig S1: Sequences and positions of the primers used for generation of the NLS mutants. The 150bp deletion in progerin cDNA is highlighted in orange. Primers used for NLS deletion by PCR mutagenesis are shown in blue. The sequences underlined in blue indicate that the primer sequences are consecutive. The red box indicates the position of the NLS deletion.

Figure S2

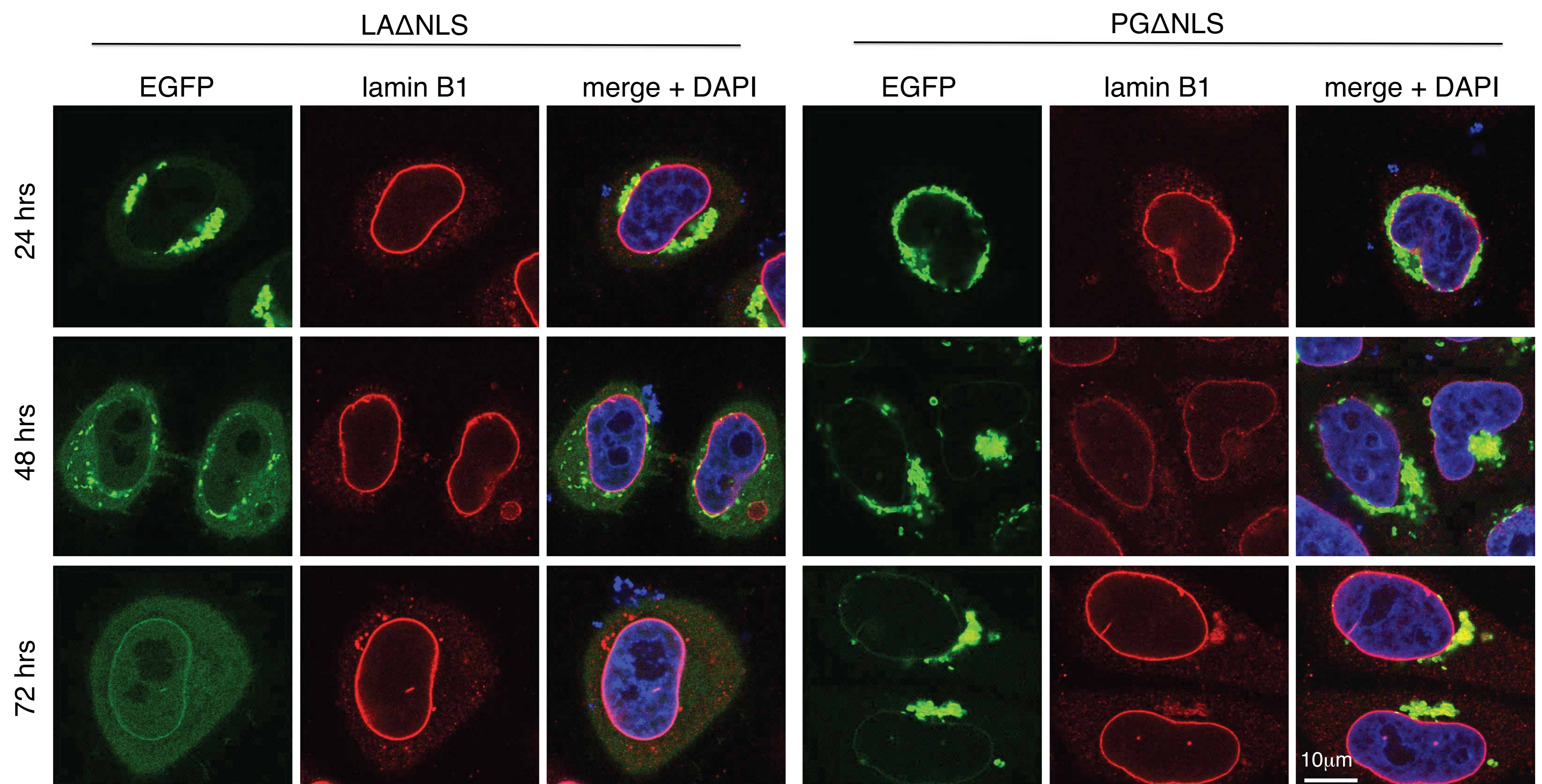


Fig S2: The time course experiment. The signals of EGFP-LA Δ NLS and EGFP-PG Δ NLS were fixed and photographed at 24, 48 and 72 hours post-transfection. We found that the aggregates of LA Δ NLS gradually solubilized with the passage of time. In contrast, the PG Δ NLS aggregates remained associated with the ER throughout the 72 hour experiment. Scale Bar: 10 μ m.

Figure S3

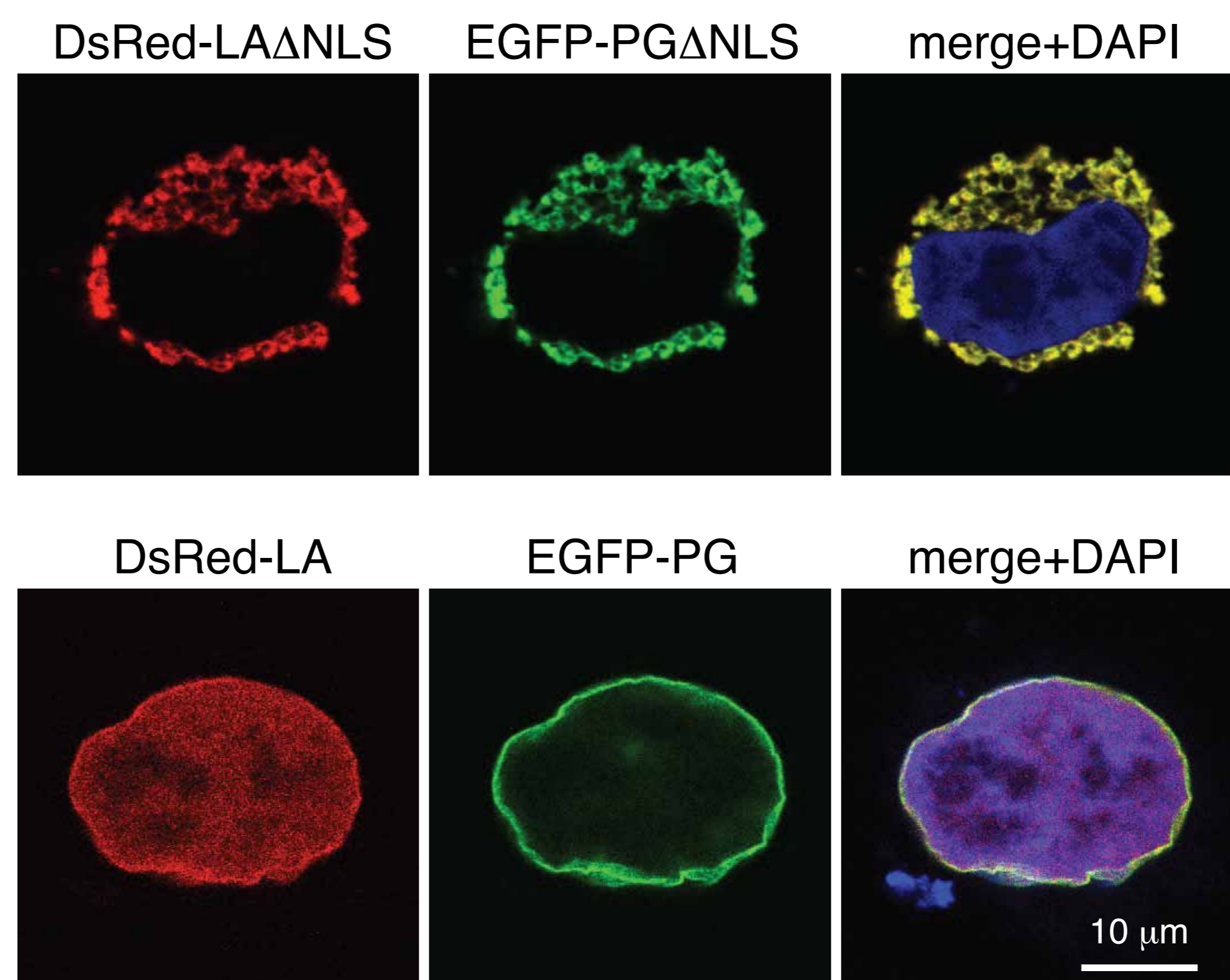


Fig S3: Confocal images show that LA Δ NLS and PG Δ NLS aggregates are co-localized in a sub-domain of the ER. HeLa cells were co-transfected with either LA Δ NLS and PG Δ NLS or lamin A and progerin. Lamin A and LA Δ NLS are in red and progerin and PG Δ NLS are in green. The distribution of DNA was detected with DAPI in blue. In merged images, yellow indicates overlapping between red and green. Scale bar: 10 μ m.

Figure S4

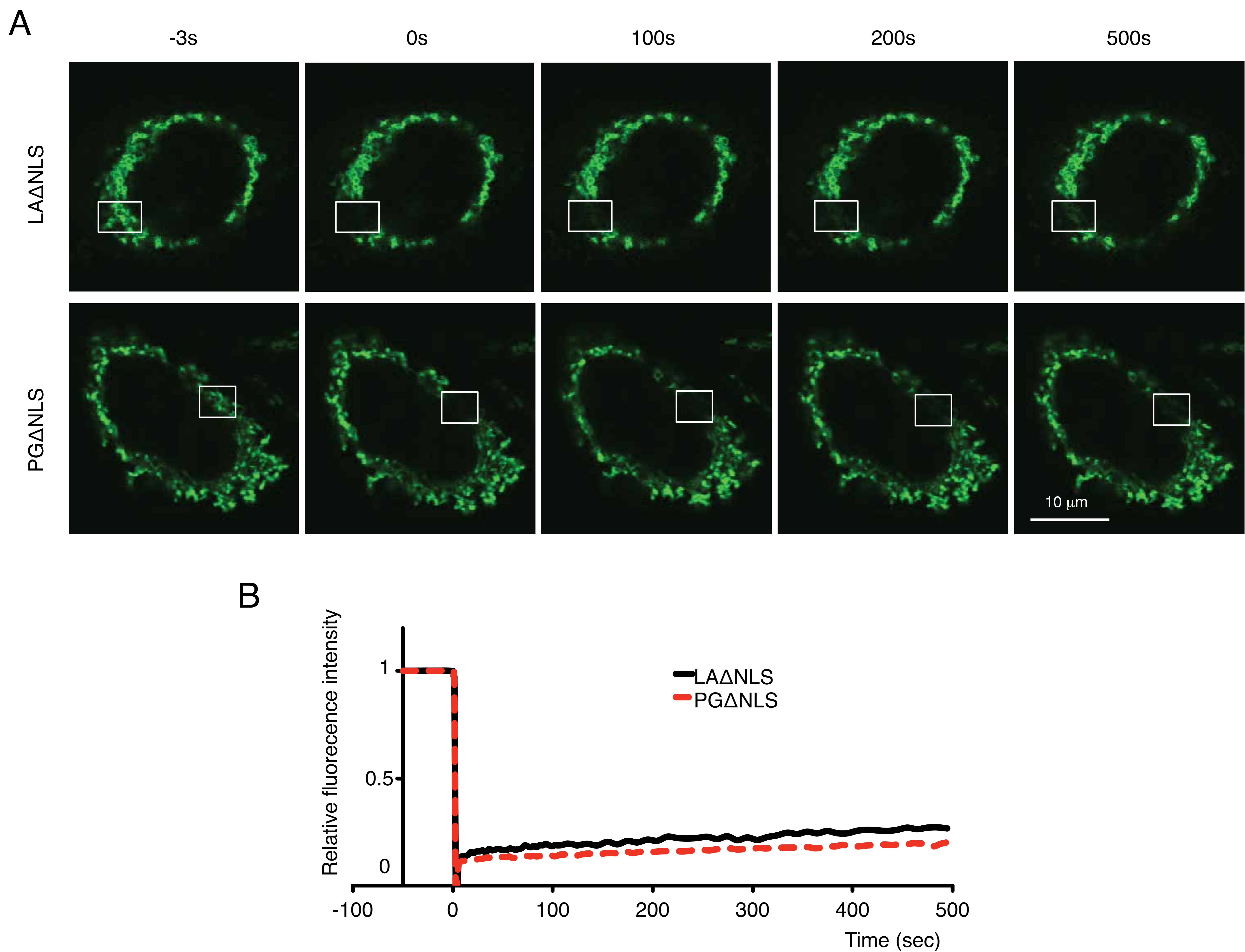


Fig S4: Fluorescence Recovery After Photo bleaching (FRAP) analysis of LA Δ NLS and PG Δ NLS aggregates. (A) Confocal images of the representative pictures at the denoted timepoints during the FRAP experiment. Squares indicate the photobleached areas. The Δ NLS mutants are shown in green. Bars, 10 μ m. (B) Quantification of (A).