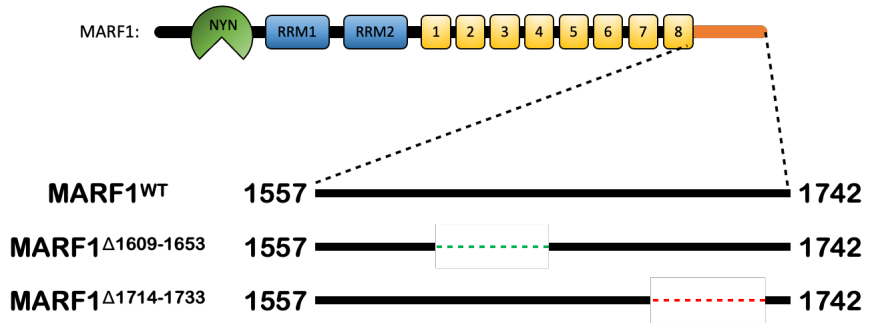
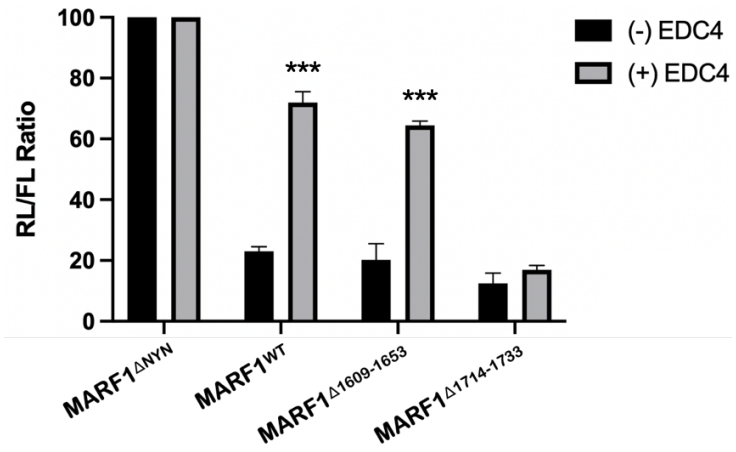
A

```
   H. sapiens   ILEVPESHTASELKLGADGSGPSHTEQELLRLTDDSPVDLLCAPVPSCLPSPQLRPDPVI 1644
X. tropicalis   ------------------------MDKEL--LCLTSPMDLLCGPVPSCLPSPQLHPDPVV 1588
     D. rerio   A-----------------TTSPASGELELLCLSSSSAVDLLCAPVPSCLPSPALRPDPVQ 1408
                                       : **  *   * :****.********* *:****

   H. sapiens   LQSADLIQFEERPQEPSEIMILNQEEKMEIPIPGKSKT--LTSDSSSSCISAAVPVPPCP 1702
X. tropicalis   TQPADLIQFEEHFHFSDLVYP---EETQYNPLCNGTQPCNFPTSADLDTFSEPSTQNICP 1645
     D. rerio   --PPDLMHFQPPVNH---------------------------------------QPRP 1425
                    **::*:   :                                          *

   H. sapiens   SSETSESLLSKDPVESPAKKQPKNRVKLAANFSLAPITKL    1742
X. tropicalis   Q----ESKSTKELPESPVKRQHRNRVKLAANFSFAPVTKL    1681
     D. rerio   ANGSDPAQPSAGVWDSPARRSARSRVRLAANFSFTAAQPV    1465
                      :  :    :**.::. :.**:******::    :
```

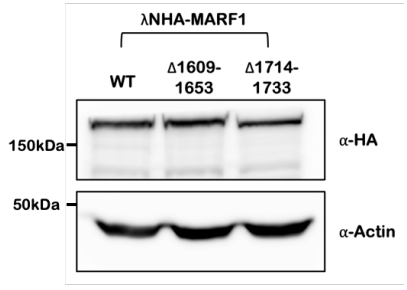
B



C



D



E

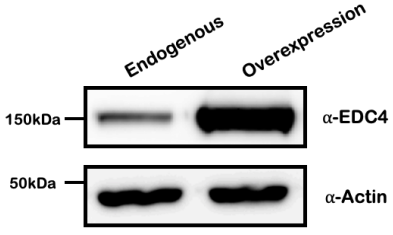


Figure S1. Brothers et al., 2022

Figure S1. (A) Phylogenetic alignment of the MARF1 C-terminal regions found within the annotated species. Two conserved regions were identified 1609-1653 (green) and 1714-1733 (red). Conserved residues are marked with (*), while conservative substitutions are marked with (:) and semi-conservative substitutions are marked with (.). (B) Schematic diagram of the MARF1 C-terminal deletion mutants generated. (C) Western blot analysis of MARF1 C-terminal deletion mutants. (D) RL-MAML1 activity detected in extracts of transfected HeLa cells expressing the annotated proteins and FL as a transfection normalization control. Histograms represent the mean RL activity detected from three experiments, normalized to FL activity and the RL/FL ratio of a catalytically inactive MARF1 ($MARF1^{\Delta NYN}$) set to 100. Error bars represent the SEM and statistical significance was calculated using a two-tailed T-test comparing RL activity in the (+) to (-) EDC4 overexpression samples (*** = $p < 0.001$). (E) Western blot analysis of EDC4-overexpression in $MARF1^{WT}$ expressing cells.

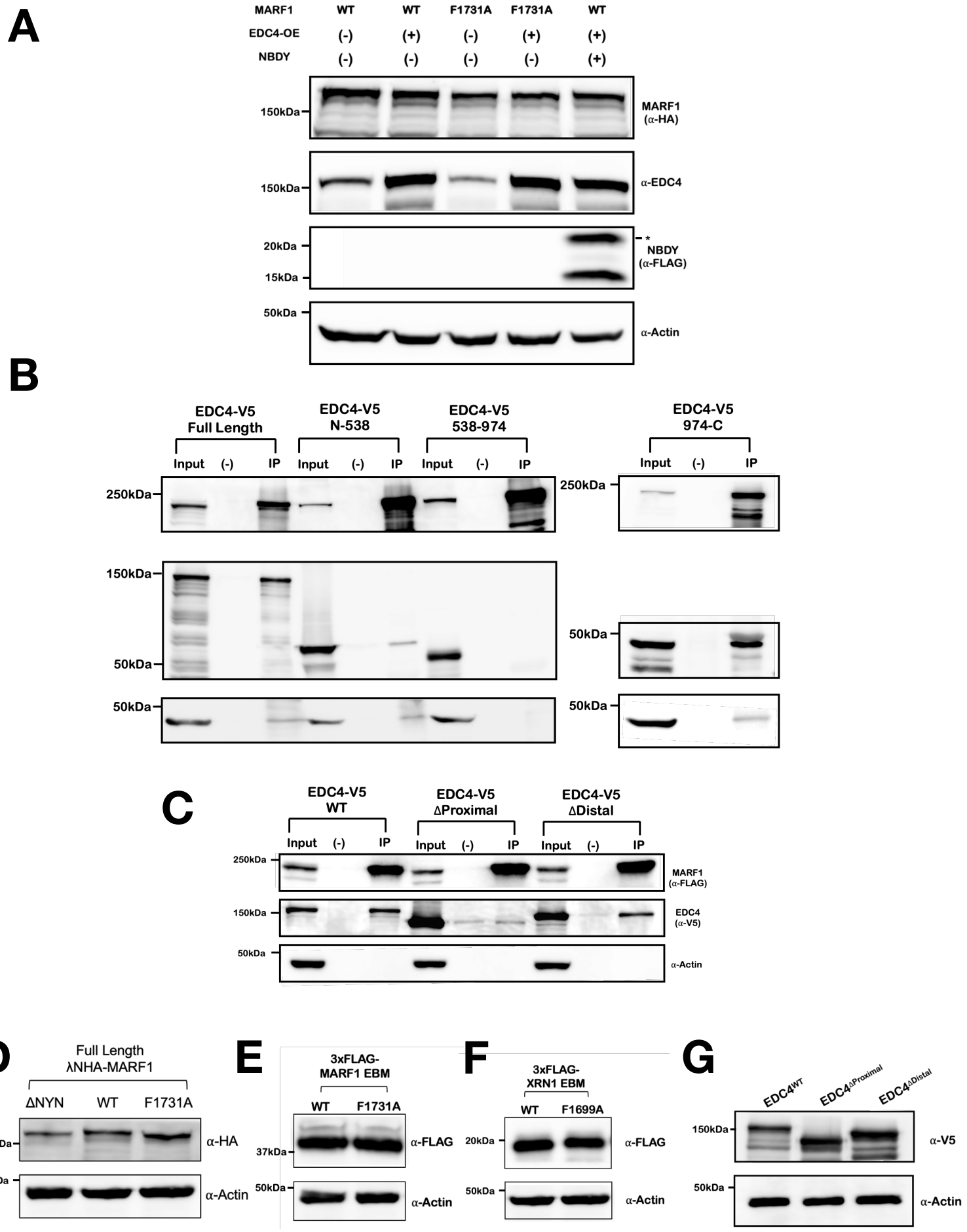


Figure S2. Brothers et al., 2022

Figure S2. (A) Western blots of HeLa lysates obtained from actinomycin D treatment experiments in figures 1F and 6C. The (*) denotes NBDY harboring a possible PTM that is consistent with the doublet observed by D'Lima *et al.* (2017). (B&C) Additional images of western blots from co-immunoprecipitation experiments corresponding to Figure 2B. (D-F) Western blots from HeLa cell extracts from the corresponding luciferase experiments (Figures 3B & 6D-G) expressing the indicated proteins. (E) Western blots from HeLa cell extracts expressing $EDC4^{WT}$ or α-Helical deletion mutants.

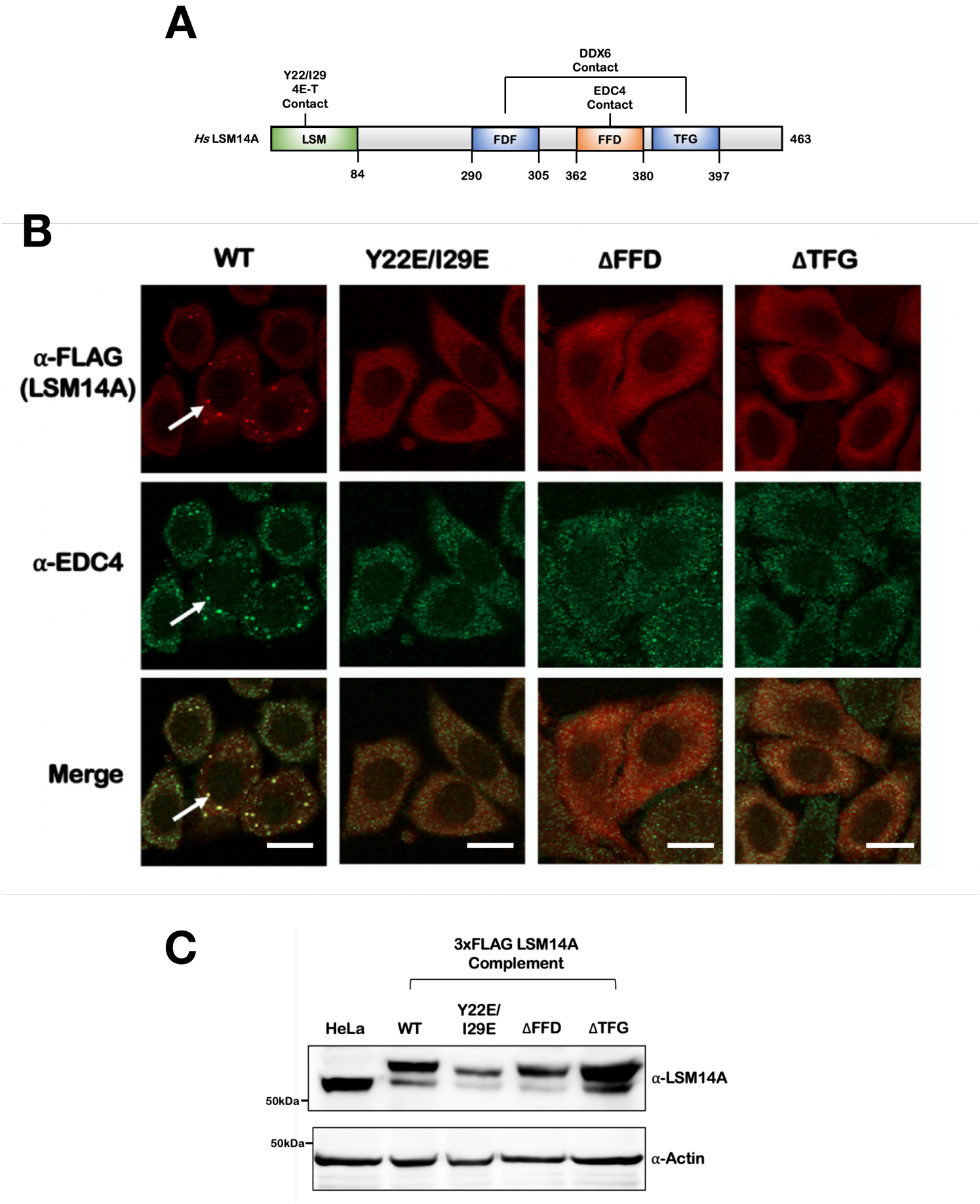


Figure S3. Brothers et al., 2022

Figure S3. (A) Schematic diagram of the LSM14A protein structure, highlighted at positions which maintain key protein interactions that are required to support P-body formation. (B) Images generated through confocal microscopy of immunofluorescent staining of non-transfected HeLa cells stably expressing FLAG-tagged LSM14A. The merged image represents the overlap of the α-FLAG (red) and α-EDC4 (green) signals and scale bars represent 20μm. White arrows mark visible P-bodies as defined by the concentrated signal overlap of FLAG-LSM14A and EDC4. (C) Western blots of complemented FLAG-LSM14A cell lines.

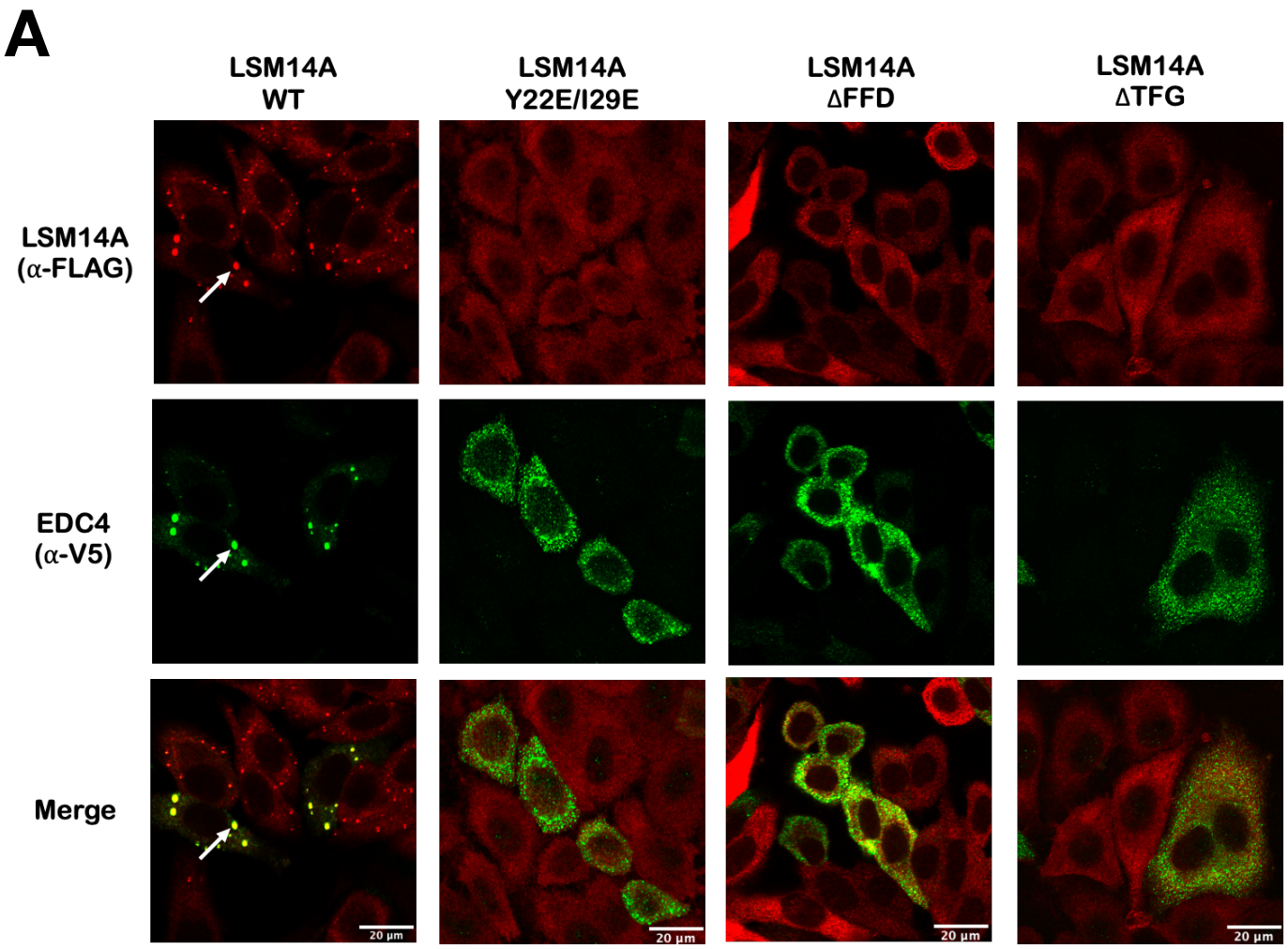


Figure S4. Brothers et al., 2022

Figure S4. (A) Additional fields of view corresponding to cells in Figure 5A. Images were generated through confocal microscopy of immunofluorescent staining of HeLa cells stably expressing FLAG-tagged LSM14A variants as indicated. Cells were transfected with V5-tagged EDC4 prior to staining. The merged image represents the overlap of the α-FLAG (red) and α-V5 (green) signals and scale bars represent 20μm. White arrows indicate P-bodies as defined by intense signal overlap between red and green staining.

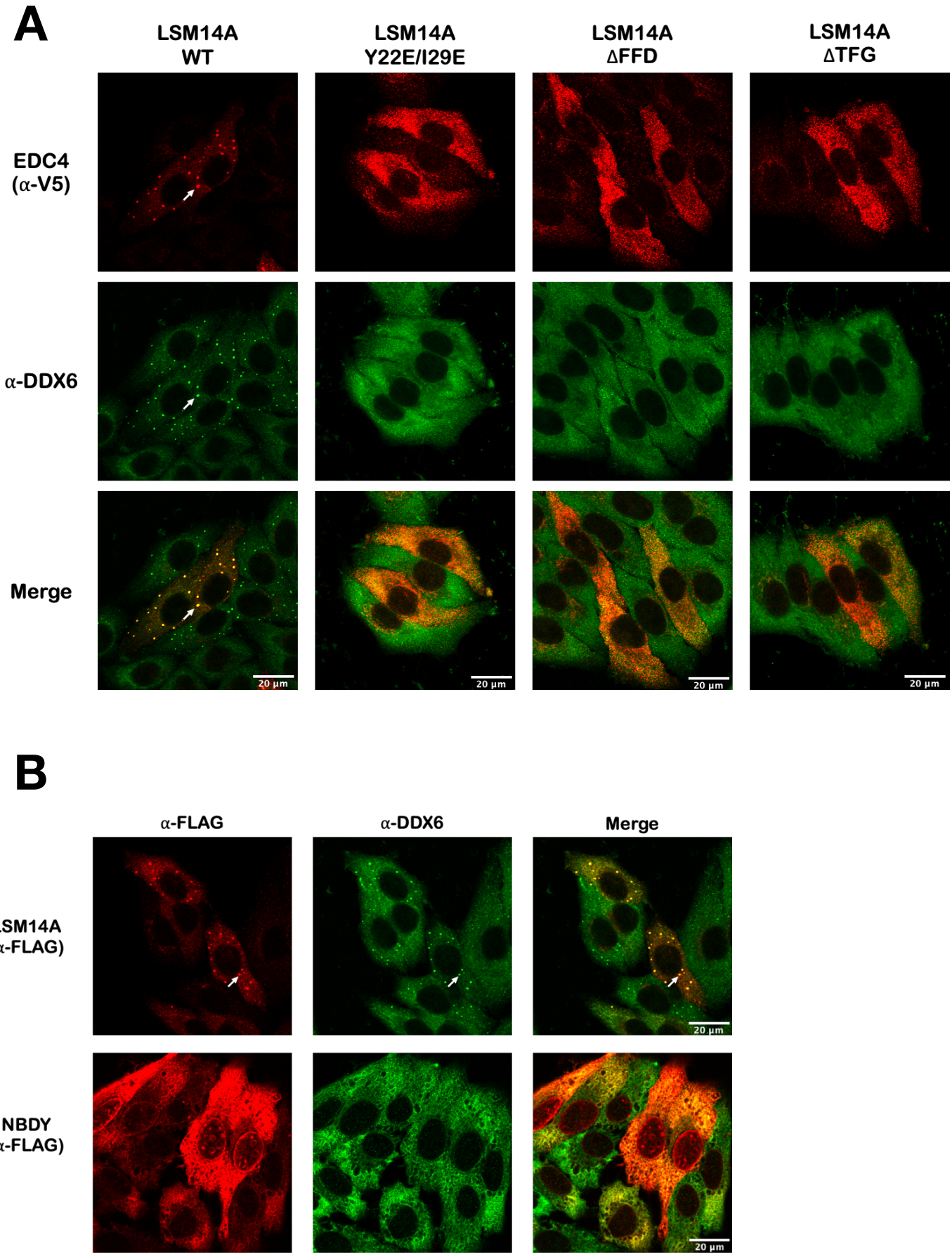


Figure S5. Brothers et al., 2022

Figure S5. (A) Images generated through confocal microscopy of immunofluorescent staining of HeLa cells stably expressing FLAG-tagged LSM14A and overexpressing V5-EDC4. The merged image represents the overlap of the α-V5 (red) and α-DDX6 (green) signals and scale bars represent 20μm. White arrows mark visible P-bodies as defined by the concentrated signal overlap of V5-EDC4 and DDX6. (B) Images generated through confocal microscopy of immunofluorescent staining WT HeLa cell. Cells were transfected with either FLAG-LSM14A (top) or FLAG-NBDY (bottom) prior to staining. The merged image represents the overlap of the α-FLAG (red) and α-DDX6 (green) signals and scale bars represent 20μm. White arrows represent P-bodies as defined by foci with strong signal overlap between red and green staining.

Supplemental Materials and Methods:

qPCR Information:

Gene	Oligos	Location	Accession Number	Amplicon Length	Specificity Screen
MAML1	Fwd: GACTCTCTCAACAAAAAGCGTCT Rev: AGGAAATGACTCACTGGGGTTA	Exon 2	NM_014757.5	78bp	Primer BLAST
GAPDH	Fwd: GTGGAGATTGTTGCCATCAACGA Rev: CCCATTCTCGGCCTTGACTGT	Exon 2-3	NM_002046	104bp	Primer BLAST
Renilla Luciferase	Fwd: GAGTTCGCTGCCTACCTGGAGCCAT Rev: GGATCTCGCGAGGCCAGGAGAG	N/A	MN030571	79bp	Primer BLAST

Site-directed mutagenesis primer list:

Deletion/Mutation	Primers
MARF1$^{\Delta 1609\text{-}1653}$	Fwd: CCCAGTCACACAGAGCAGCCTCAAGAGCCTTCTGAAATTATG Rev: TTCAGAAGGCTCTTGAGGCTCCTGCTCTGTGTGACTGGGCCC
MARF1$^{\Delta 1714\text{-}1733}$	Fwd: CTCAGCAAGGACCCCGTGTTAGCACCTATAACCAAGCTTTAA Rev: TTAAAGCTTGGTTATAGGCACGGGGTCCTTGCTGAGCAGTGA
MARF1^{F1731A}	Fwd: GAGTCAAATTGGCAGCCAACgccTCCTTAG Rev: AGGTGCTAAGGAggcGTTGGCTGCCAATTT
XRN1-EBM^{F1699A}	Fwd: GAAAACTGGCTGTTAAgccTGGTGTTTCTAAACCTTCTGAG Rev: TTTAGAAACACCAggcTTAACAGCCAGTTTTCTTGATTTTCT
EDC4$^{\Delta Proximal}$	Fwd: gccaggaagagctgctgcagcatatcctgcagctgctgcagcagg Rev: tgcagcagctgcaggatatgctgcagcagctcttcctggctgtgc
EDC4$^{\Delta Distal}$	Fwd: actgccaggcccagcaagccggcctcgtgacccccagcctccct Rev: agggaggctgggggtcacgagggcttgctgggcctggcagtcaag