

Appendix files

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Summary and legends of the Appendix figures and tables:

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Appendix Table S2: List of antibodies used in this study

Appendix Table S3: Number of tomograms collected and total number of particles cropped from the fibrils within the pathological TDP-43 immunopositive aggregates localized by CLEM, or observed neurofilaments, for each patient.

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Appendix Figure S1: Seeding properties of pathological aggregates extracted from post-mortem brain tissue

Representative images of seeded cells 3 days after transfection. Left panels show the overview picture, white box indicates the area shown in the right panels. White arrows correspond to the detected phosphorylated neoaggregates labelled in Fig 1A.

Appendix Figure S2: Seeding properties of pathological aggregates extracted from post-mortem brain tissue

Representative images of seeded cells 6 days after transfection. Left panels show the overview picture, white box indicates the area shown in the right panels. White arrows correspond to the detected phosphorylated neoaggregates labelled in Fig 1A.

Appendix Figure S3: Surface creation using Imaris

“Surface” tool in Imaris was used to quantify seeding of TDP-43 over time. Surface for DAPI, condensed TDP-43-HA and diffused TDP-43-HA, and phosphorylated TDP-43 (pTDP-43) were created using the confocal images. Surface colocalization tool was then used to generate nuclear TDP-43 (colocalization between condensed TDP-43-HA and DAPI) and neoaggregates (diffused TDP-43-HA and pTDP-43) surfaces.

Appendix Figure S4: Correlative light and electron microscopy of neoaggregates

A) Light microscopy image (LM) showing an HA-immunoreactive inclusion (green color, black arrowhead) identified in ultrathin sections from resin-embedded cell monolayers. The LM images were overlaid with low magnification EM images (EM/LM overlay) to localize the same region within the cells (dotted black square) for EM imaging at higher magnification (green outline, EM). Accumulated TDP-43-HA fibrils (green outline) are disordered surrounded by linear and non-immunopositive filaments. **B)** EM images taken in non-seeded controls of similar non-HA immuno-positive filaments as shown in A. Scale bars LM and EM/LM overlay, 10 μ m; EM as shown.

Appendix table S1: List of patient samples used

Patient ID	Brain Bank	Postmortem delay (hours:mins)	Age at onset (years)	Age at death (years)	Duration of disease	Gender	Clinical Diagnosis	Pathological Diagnosis	Mutations	Used in:			
										Confocal	Biochemistry	STORM	EM
TDP-case1	UCL	68:55:00	83	87	4	F	PSP	FTLD-TDP-A				x	
TDP-case2	UCL	99:45:00	62	68	6	F	Picks (bvFTD)	FTLD-TDP-A	GRN Q130fs	x	x		
TDP-case3	UCL	33:40:00	47	53	6	M	MND	FTLD-TDP-A			x	x	
TDP-case4	UCL	85:20:00	57	63	6	F	FAD (actually bvFTD)	FTLD-TDP-A	GRN C31fs	x	x	x	
TDP-case5	UCL	51:52:00	66	71	5	M	FTD/MND	FTLD-TDP-A	C9orf72		x	x	x
TDP-case6	UCL	29:20:00	49	55	6	M	FAD (bvFTD)	FTLD-TDP-A	GRN C31fs	x		x	
TDP-case7	UCL	86:10:00	66	72	6	M	Picks disease/PNFA	FTLD-TDP-A		x	x		
TDP-case8	UCL	85:35:00	56	67	11	F	HD Early onset (actually PNFA)	FTLD-TDP-A	C9orf72		x		
TDP-case9	UCL	90:05:00	57	71	14	M	FTLD	FTLD-TDP-A	GRN R493X		x		
TDP-case10	UCL	40:25:00	57	60	3	M	FTLD	FTLD-TDP-A			x		
TDP-case11	NBB	72:00:00	75	76	1	F	PiD	FTLD-TDP-A	GRN		x	x	
TDP-case12	UCL	60:00:00	43	45	2	M	Dementia bvFTD	FTLD-TDP-A	C9ORF72			x	
TDP-case13	UCL	92:55:00	57	62	5	M	FTD	FTLD-TDPA					x

TDP-case14	UCL	7:50:00	70	83	13	F	FTD	FTLD-TDPA					x
TDP-case15	UCL	72:35:00	53	61	8	M	FTLD (bvFTD)	FTLD-TDPA	GRN C31fs				x
TDP-case16	NBB	05:00	61	66	5	F	PiD	FTLD-TDP-A	GRN	x	x		
TDP-case17	UCL	83:40:00	59	73	14	F	Picks (semantic dementia)	FTLD-TDP-C		x	x	x	
TDP-case18	UCL	51:50:00	50	65	15	M	SD	FTLD-TDP-C				x	
TDP-case19	UCL	70:45:00	61	66	5	M	FTD	FTLD-TDP-C			x	x	
TDP-case20	UCL	26:15:00	77	80	3	F	FTLD (PNFA)	FTLD-TDP-C		x	x	x	
TDP-case21	UCL	31:10:00	58	72	14	F	SD	FTLD-TDP-C			x	x	
TDP-case22	UCL	19:00	64	74	10	M	FTD/SD	FTLD-TDP-C			x		x
TDP-case23	UCL	943:12:00	67	76	9	M	SD	FTLD-TDP-C			x		
TDP-case24	UCL	54:05:00	53	71	18	M	SD	FTLD-TDP-C			x		
TDP-case25	NBB	10:00	40	63	23	M	PiD	FTLD-TDP-C			x	x	
TDP-case26	NBB	04:00	63	74	11	F	PiD FTD	FTLD-TDP-C			x	x	
TDP-case27	UCL	39:00:00	71	76	5	M	PiD	FTLD-TDP-C			x		
TDP-case28	UCL	24:40:00	52	65	13	F	FTLD	FTLD-TDPC					x
TDP-case29	UCL	76:00:00	44	67	23	M	FTD (semantic dementia)	FTLD-TDPC					x

TDP-case30	UCL	70:45:00	61	66	5	M	FTD	FTLD-TDPC					x
TDP-case31	NBB	07:00	N/A	79	N/A	M	N/A	non-neurodegenerative control		x	x	x	
TDP-case32	NBB	06:00	N/A	79	N/A	M	N/A	non-neurodegenerative control			x	x	
TDP-case33	NBB	04:00	N/A	78	N/A	F	N/A	non-neurodegenerative control			x	x	
TDP-case34	NBB	05:00	N/A	79	N/A	M	N/A	non-neurodegenerative control			x	x	
TDP-case35	NBB	08:00	N/A	73	N/A	M	N/A	non-neurodegenerative control		x	x		
TDP-case36	NBB	06:00	N/A	72	N/A	F	N/A	non-neurodegenerative control		x			

Appendix table S2: List of antibodies used in this study

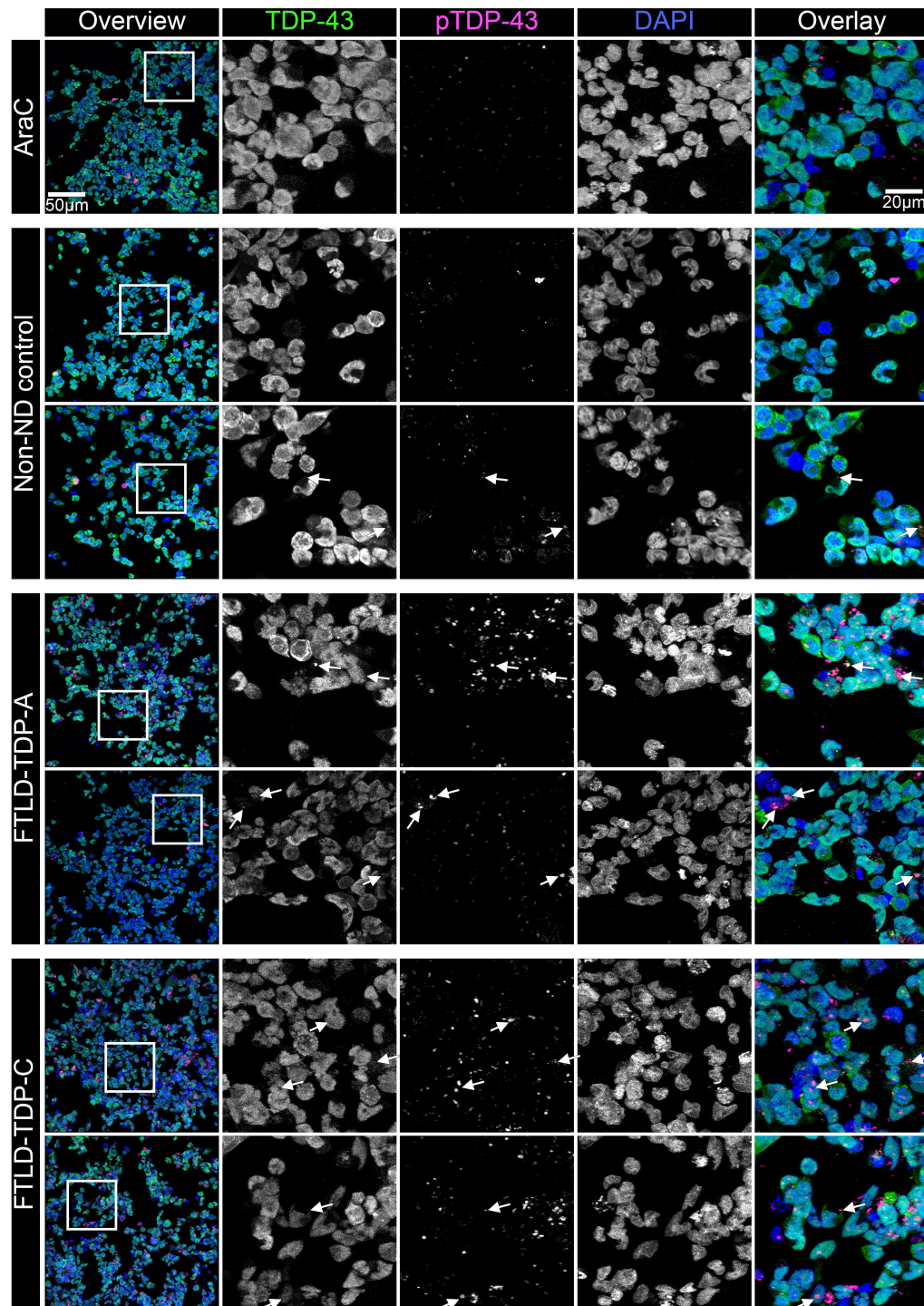
Antibody	Type	Source	Western Blot	IF Confocal	IF STORM	IHC	RRID
HA	Rabbit, mAb	Cell Signaling, C29F4-3724S	1:1000	1:1000	1:1000	1:1000	AB_1549585
pTDP-43 ^{pS403/404}	Human, mAb	custom made	1:1000	1:1000	1:2000	1:500	
pTDP-43 ^{pS404/404}	Rabbit, pAb	CosmoBio, CAC-TIP-PTD-P05	1:2500				AB_1961902
pTDP-43 ^{pS409/410}	Mouse, mAb	CosmoBio, CAC-TIP-PTD-M01	1:2500				AB_1961900
pTDP-43 ^{pS409/410}	Mous, mAb	ProteinTech, 66318-1-Ig		1:500			AB_2881699
TDP-43 6H6	Mouse, mAb	Proteintech, 60019-2-Ig	1:1000	1:1000			AB_2200520
TDP-43 Nterm	Rabbit pAb	Proteintech, 10782-2-AP	1:1000	1:1000			AB_615042
TDP-43 (3H8)	Mouse mAb	Novus Biologicals, NBP1-92695				1:1000	AB_11005586
Lamin B1	Mouse, mAb	Proteintech, 66095-1-Ig		1:1000	1:500	1:200	AB_11232208
MAP2	Ck polycl	AbCAm, ab5392				1:500	AB_2138153
Nucleolus	Mouse mAb	SCBT, anti-RPA40			1:200		AB_10988062
Nucleopore complexes	Mouse, mAb	AbCam, ab24609			1:200		AB_448181
PNF	Mouse, mAb	Biolegend, SMI 31P				1:200	AB_2028812
SOD1	Rabbit mAb	Enzo Life Sciences, ADI-SOD-100-F	1:5000				AB_10616253
Tubulin	Mouse, mAb	ProteinTech, 10068-1-AP			1:200		AB_2303998

Appendix table S3: Number of tomograms collected and total number of particles cropped from the fibrils within the pathological TDP-43 immunopositive aggregates localized by CLEM, or observed neurofilaments, for each patient.

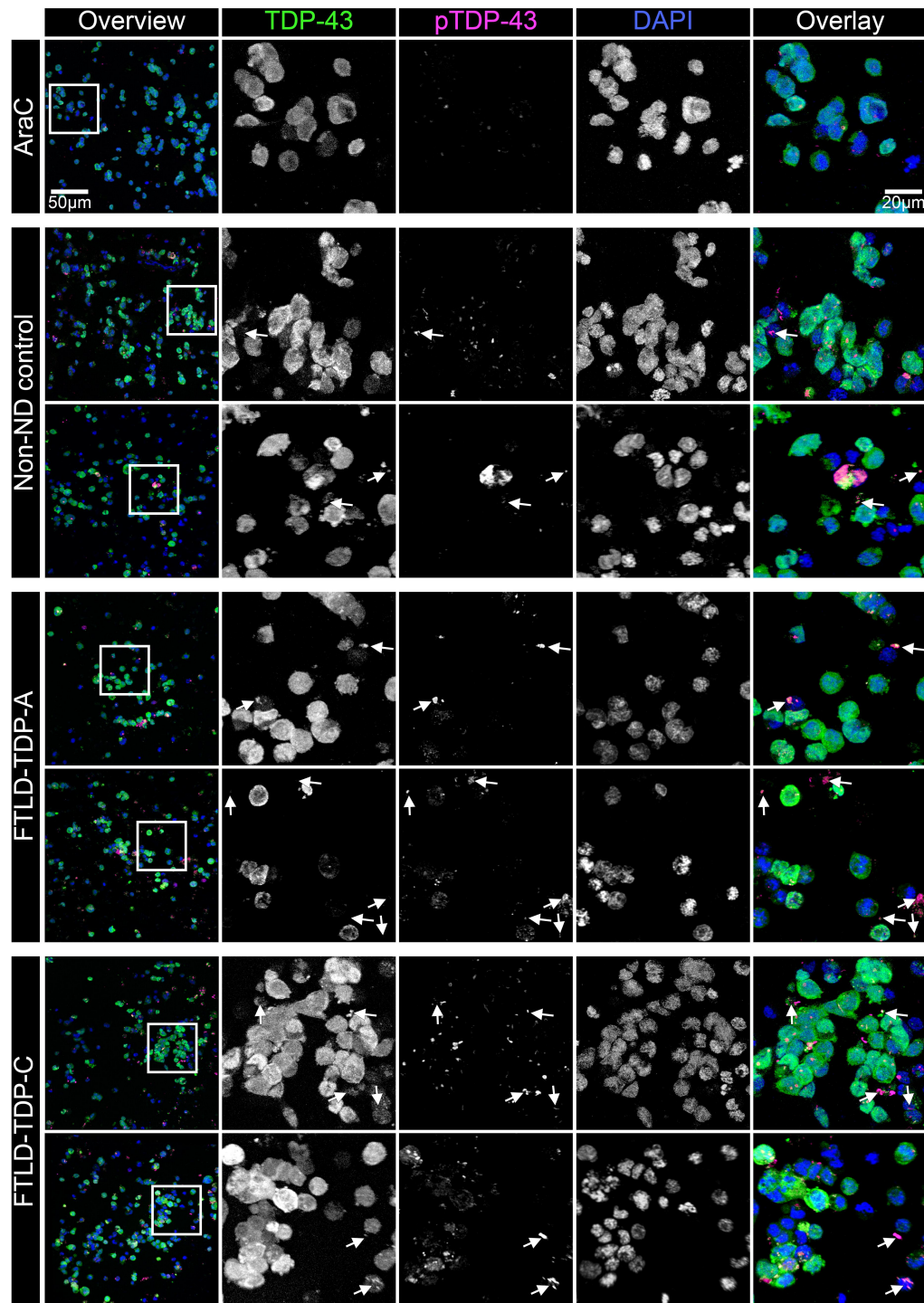
FTLD subtype	Patient	Fixative used	Number of Tomograms	Total number of cropped particles	Associated Figures
A	TDP-case14	4% form, 0.1% glut	4	2643	SFig. 1A
	TDP-case5	4% form, 0.1% glut	4	3298	SFig. 1B
	TDP-case15	4% form, 0.1% glut	5	5926	SFig. 1C
	TDP-case13 Aggregate 1 Aggregate 2	4% formalin	2	1887	Fig. 1A, SFig. 1D
			3	2322	SFig. 1E
C	TDP-case28 Aggregate 1 Aggregate 2	4% form, 0.1% glut	5	1986	SFig. 2A
			4	2519	SFig. 2B
	TDP-case22	4% form, 0.1% glut	5	1802	SFig. 2C
	TDP-case30 Aggregate 1 Aggregate 2 Aggregate 3	4% formalin	4	2185	Fig. 1B, SFig. 2D
		4% form, 0.1% glut	2	809	SFig. 2E
		4% formalin	5	2243	Fig. 1C SFig. 2G
	TDP-case21 Aggregate Neurofilaments	4% form, 0.1% glut	5	2835	SFig. 2F
			2	965	data not shown

Appendix table S4: Numerical parameters used for the alignment projects of the fibrils from all samples during sub-tomogram averaging.

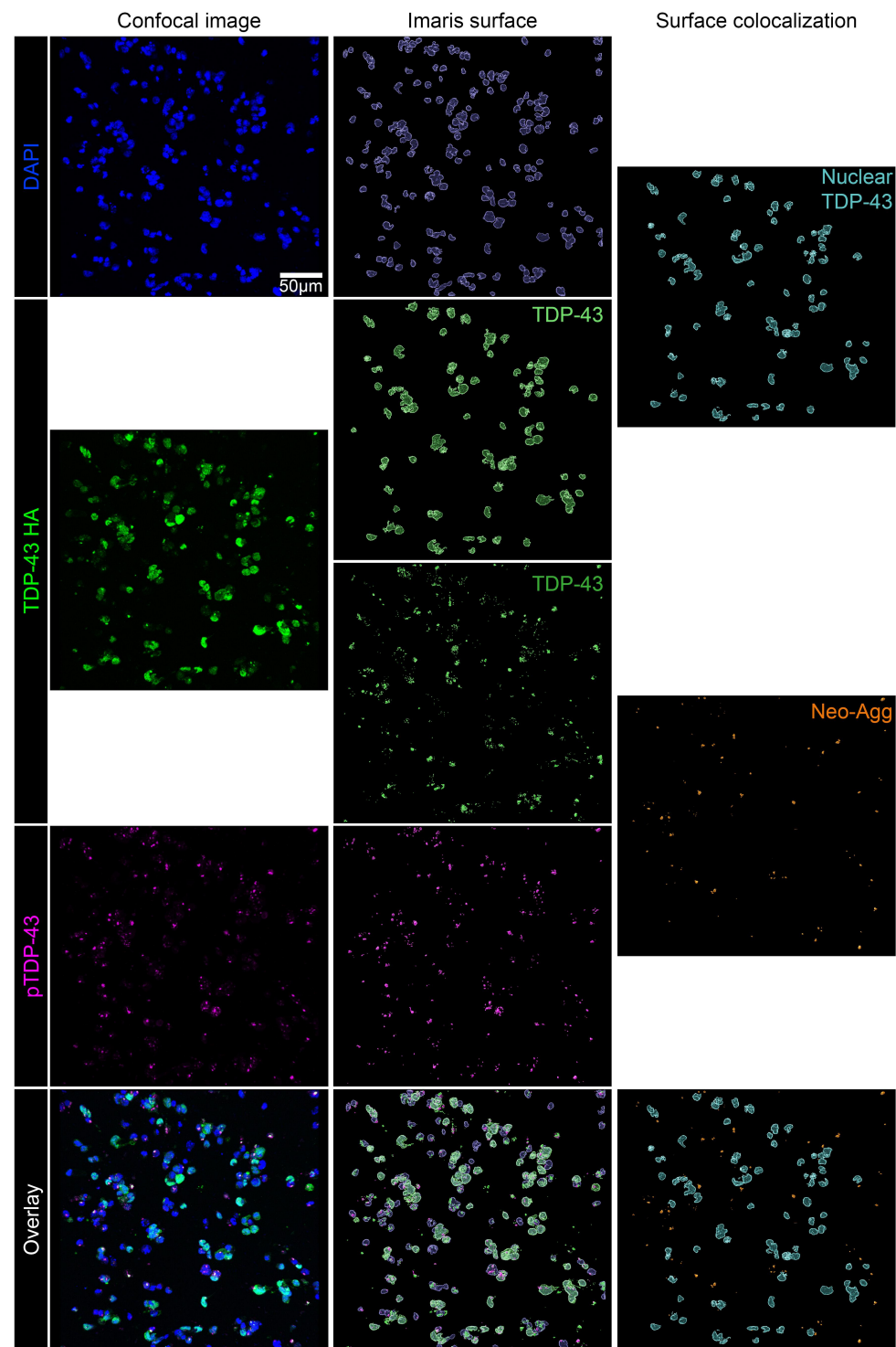
	Round 1	Round 2	Round 3
iterations	3	3	2
references	1	1	1
cone aperture	30	10	10
cone sampling	10	5	5
azimuth rotation range	90	360	10
azimuth rotation sampling	10	45	5
refine	5	5	5
refine factor	2	2	2
high pass	2	2	2
low	10	18	32
symmetry	c1	c1	c1
Particle dimensions	64	64	128
shift limits	30 30 10	10 10 90	4 4 4
shift limiting way	1	2	2
separation in tomogram	0	0	0
basick MRA	0	0	0
threshold parameter	0.2	0.2	0.2
threshold modus	0	0	0

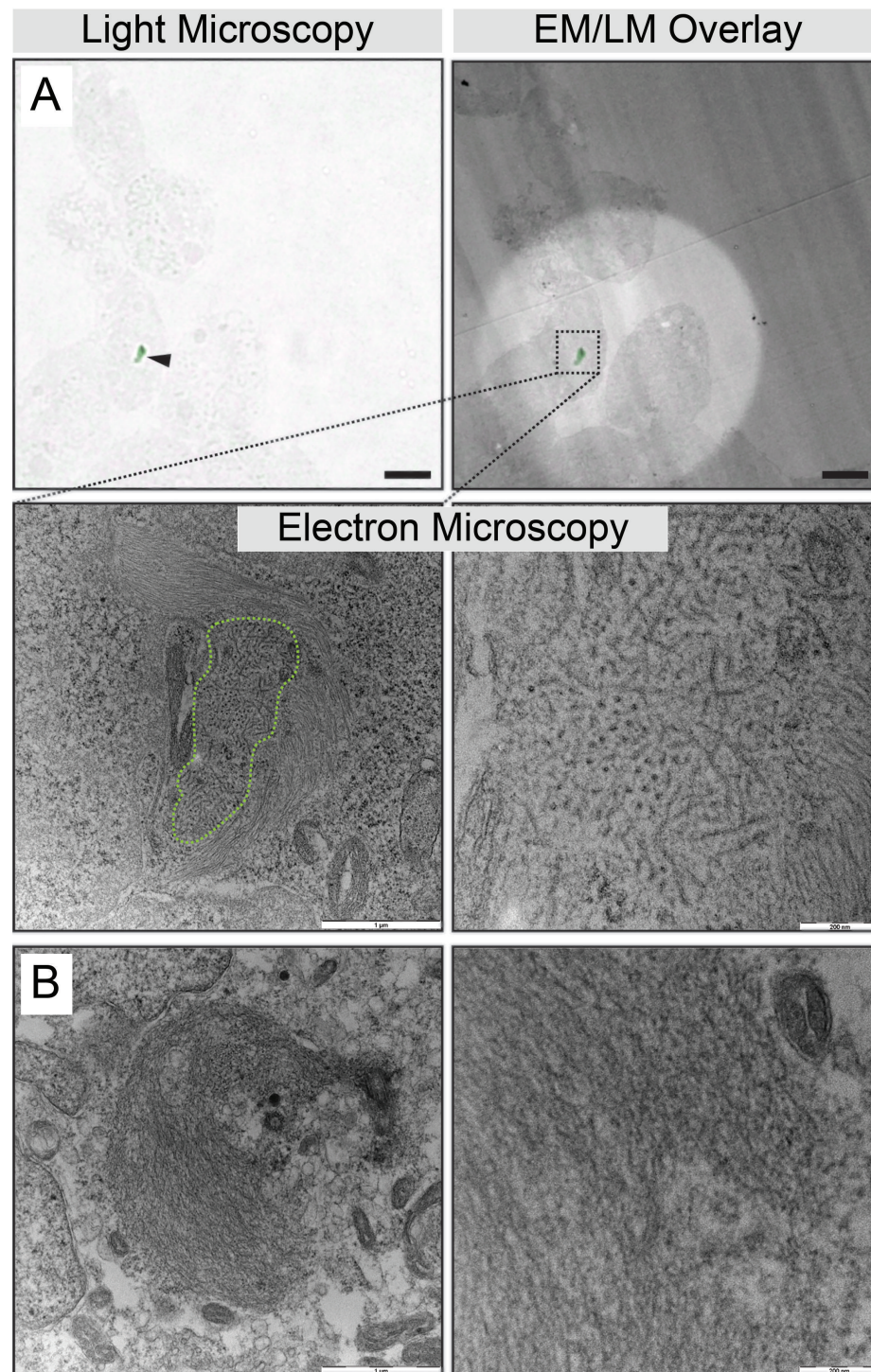


De Rossi, Lewis and al, Appendix Figure S1



De Rossi, Lewis and al, Appendix Figure S2





De Rossi, Lewis et al, Appendix Figure S4