Supporting Information

Correlative 3D cryo X-ray imaging reveals intracellular location and effect of designed antifibrotic protein-nanomaterial hybrids

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Supplementary Figure S1: Single slice from a cryo-3D-SIM stack of different NIH-3T3 cells comparing the blue (405 nm excitation, top) and green (488 nm excitation, bottom) channels to show the levels of autofluorescence and the intracellular concentration of TPR-AuNC compared to TPR-Hsp90-AuNC signal. From left to right it shows control (A and E), TGF-β (B and F), TPR-Hsp90-AuNC (C and G) and TPR-AuNC (D and H). Scale bars 20 µm

Supplementary Figure S2: Single slice from a CLXT correlated volume showing control and TPR-AuNC NIH-3T3 cell samples. Red signal coming from the mitotracker CMXRos and green one from the Alexa488 channel. In the control all the green signal is auto-fluorescence while in the TPR-AuNC there is in addition to the auto-fluorescence, some signal coming from internalized TPR-AuNC. M = Mitochondria, MVB = multivesicular body, N = Nucleus, ER = endoplasmic reticulum, LD = lipid droplet. Scale bars 2 µm

Supplementary Figure S3: Comparison of the histogram of the cryo-3D-SIM signal for each treatment in NIH-3T3 cells. The red circle shows the measured area. Control, TGF-β and TPR-AuNC are contrast corrected, TPR-Hsp90-AuNC has its upper limit increased for visualization purposes. Scale bars 20 µm.

Supplementary Figure S4: Resolution estimation of one of the cryo-SXT volumes shown in Figure 1 following the $FSC_{e/o}$ criterion (0.25 $FSC_{e/o}$ cutoff frequency).[1]

Supplementary Figure S5: Correlation accuracy map of the CLXT figure shown at the bottom (from Figure 1D in main text). The map was calculated with the build in feature in the ecCLEM plugin of ICY. The color-scale bar varies from 63.25 nm to 175.66 nm; the squares show the location of the tomographic reconstructions. Scale bars 10 µm

Supplementary Figure S6: Comparison of MVB morphology for NIH-3T3 (A, B, C & D) and primary Fibroblasts (E, F, G & H) collected by cryo-SXT. M = Mitochondria and the arrows point at the MVBs. Scale bars 1 µm

Supplementary Figure S7: TPR-AuNC co-localization study of anti-Alix (A & B), anti-Flotillin (C & D) and anti-CD9 (E and F) using confocal microscopy. The inset show the merged (‘), green only (Alexa488, ’’) and red (from the respective antibody, ’’’) signal. Scale bars: A – F main figure 5 µm, inset A – D 1 µm, inset E – F 2 µm

Supplementary Figure S8: Co-localization study of LC3 with TPR-AuNC (A & B) and TPR-Hsp90-AuNC (C & D) using confocal microscopy showing some foci however no co-localization. The inset show the merged (‘), green only (Alexa488, ’’) and red (from the respective antibody, ’’’) signal. Scale bars: A – D 5 µm, insets 1 µm

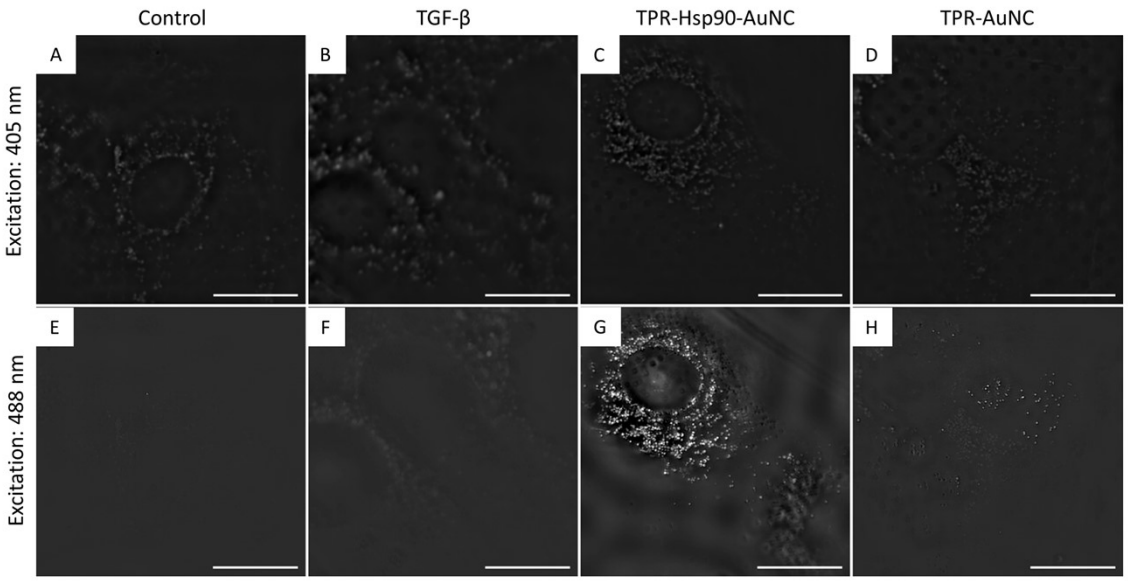
Supplementary Figure S9: Testing the four biomarkers in the control cells using confocal microscopy. The images are 488 nm and 561 nm emission channels merged together and show, apart from some individual foci, no increased biomarker signal. The figures show anti-Alix (A & B), anti-Flotillin (C & D) and anti-CD9 (E and F). No signal in the 488 nm channel is observed, which is in accordance with the lack of protein hybrid nanomaterial. Scale bars: A – G 5 µm; H 2 µm

Supplementary Figure S10. A single slice of the 3D reconstructed absorbance by cryo-SXT showing a comparison of NIH-3T3 cells treated with TGF-β (left) and TPR-Hsp90-AuNC (right) after 48 h incubation showing collagen on top of the quantifoil support. TGF-β treated cells show structured collagen bundles, while cells treated with TPR-Hsp90-AuNC show some signs of unstructured collagen, although in most cases none can be found. Bottom: TEM thin

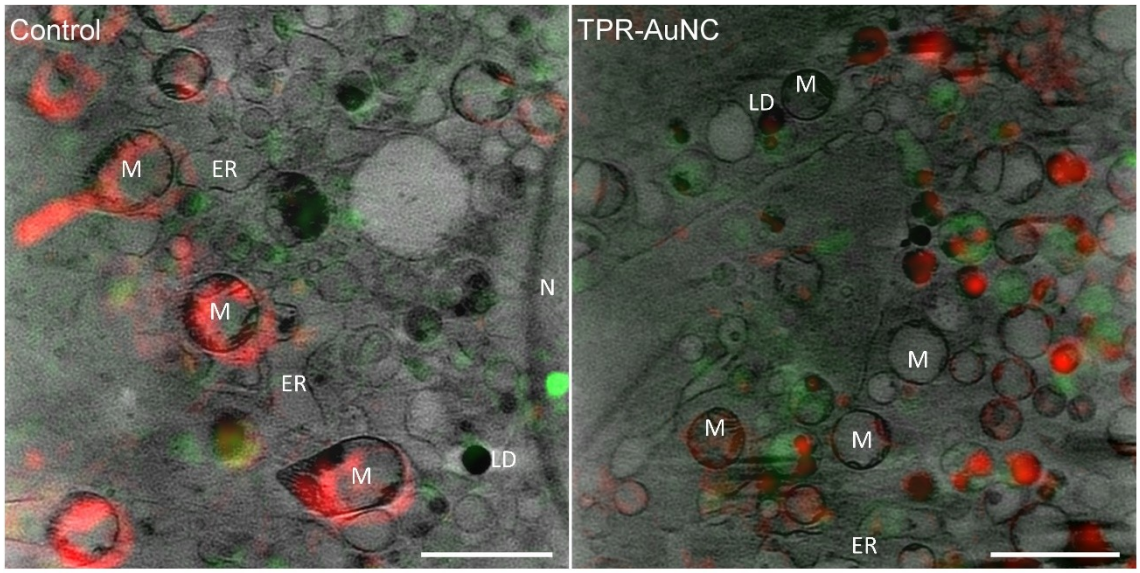
sections data of the same sample-types showing some structured collagen in the gap between 2 cells for the TGF-β cells (area indicated by red contour) and nothing between the cells for the TPR-Hsp90-AuNC cells suggesting the successful inhibition of Hsp90 and therefore collagen deposition. Scale bars top: 2 µm, bottom: 500 nm. Q = hole in quantifoil support, SC = Structured collagen, USC = Unstructured collagen, Gap = space between 2 cells.

1. Cardone G, Grünewald K, Steven AC. A resolution criterion for electron tomography based on cross-validation. Journal of Structural Biology. 2005 Aug;151(2):117–29.

S1


Control
TGF-β
TPR-Hsp90-AuNC
TPR-AuNC
A
B
C
D
E
F
G
H
Excitation: 405 nm
Excitation: 488 nm

S2



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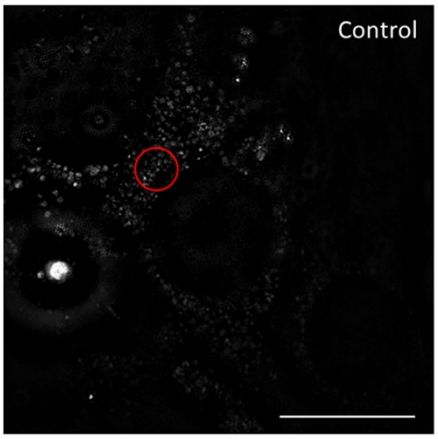
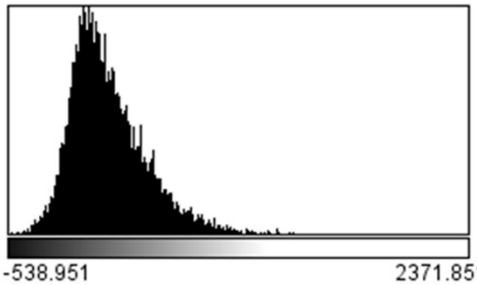

Control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2371.851
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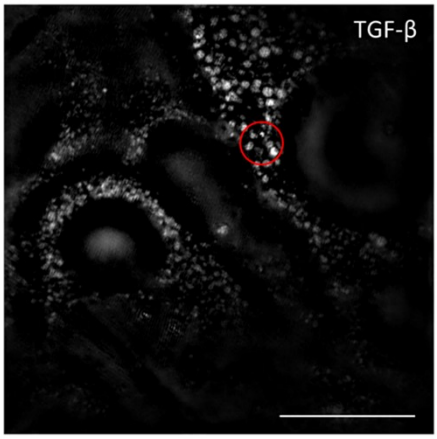
Bin Width: 11.370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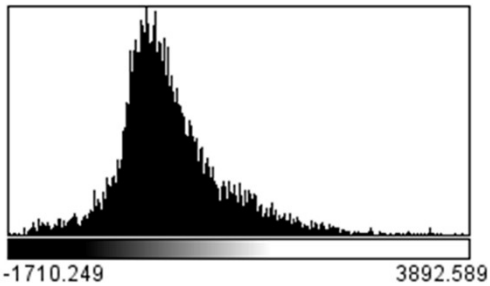
-1710.249
3892.589
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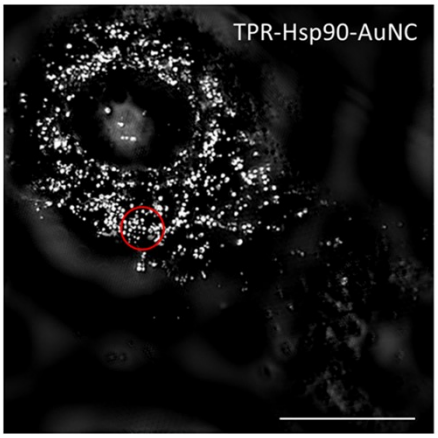
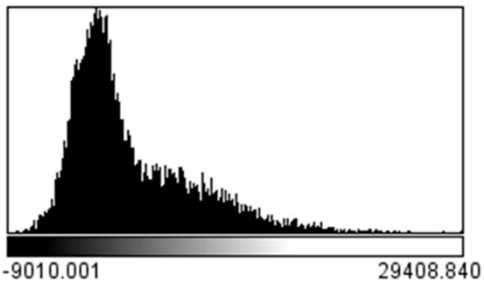
Bin Width: 21.886


TPR-Hsp90-AuNC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29408.840
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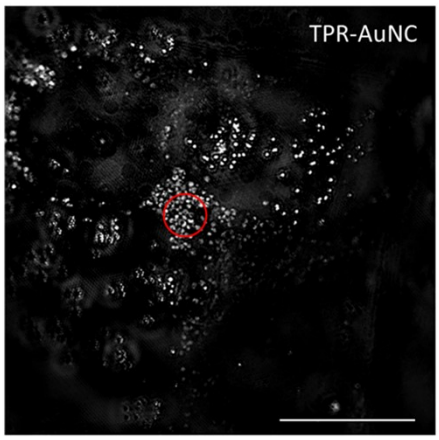
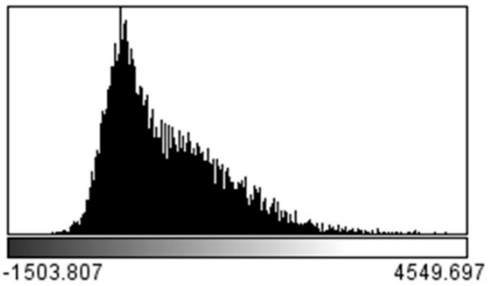

TPR-AuNC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4549.697
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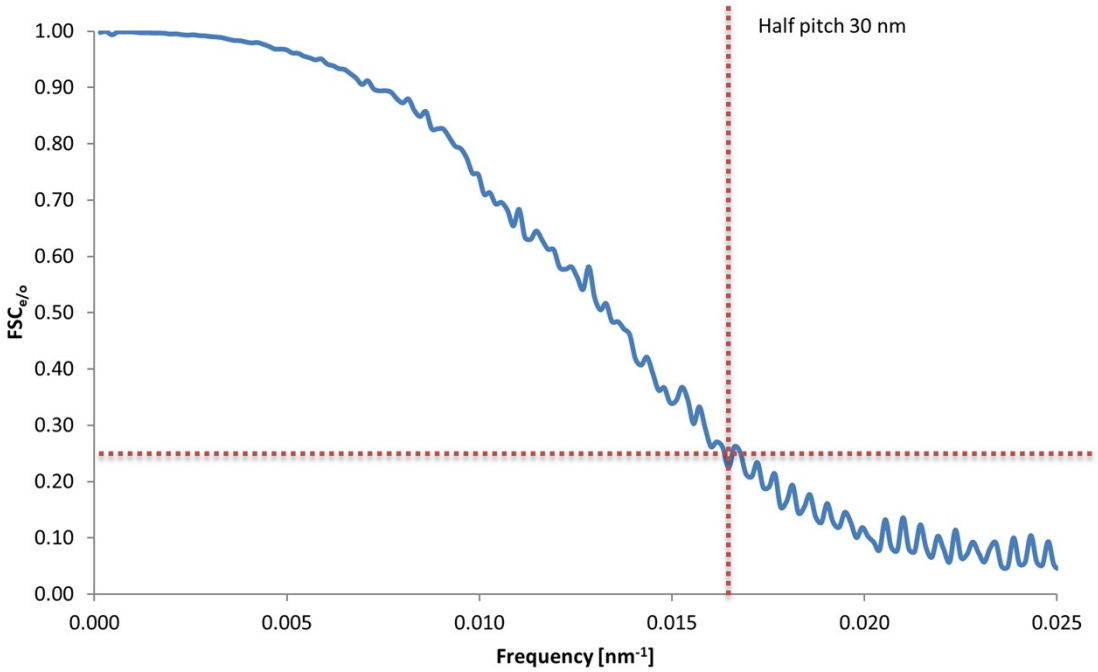
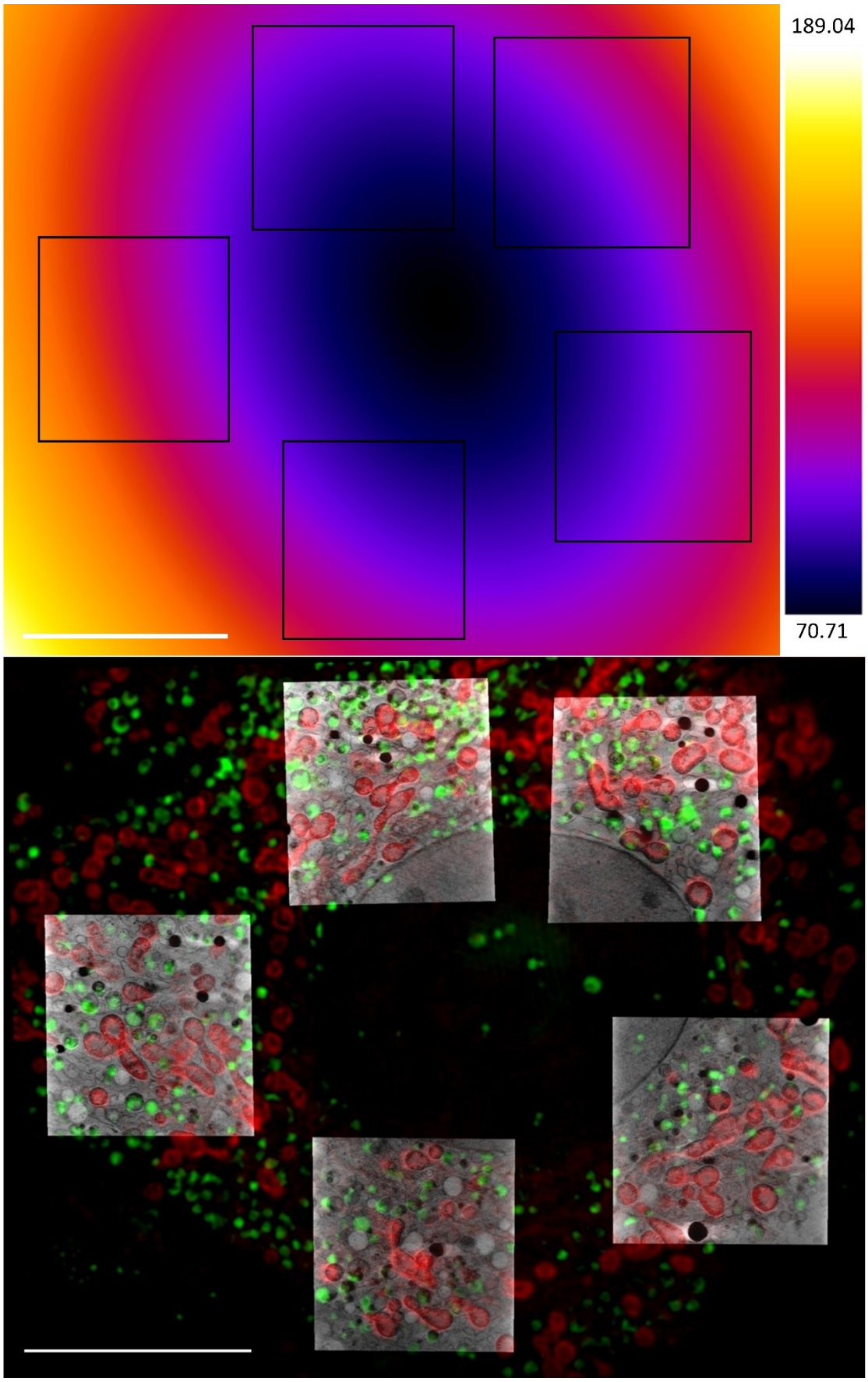
Bin Width: 23.646

S4


Half pitch 30 nm
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
FSC$_{e/o}$
0.000
0.005
0.010
0.015
0.020
0.025
Frequency [nm^{-1}]

S5



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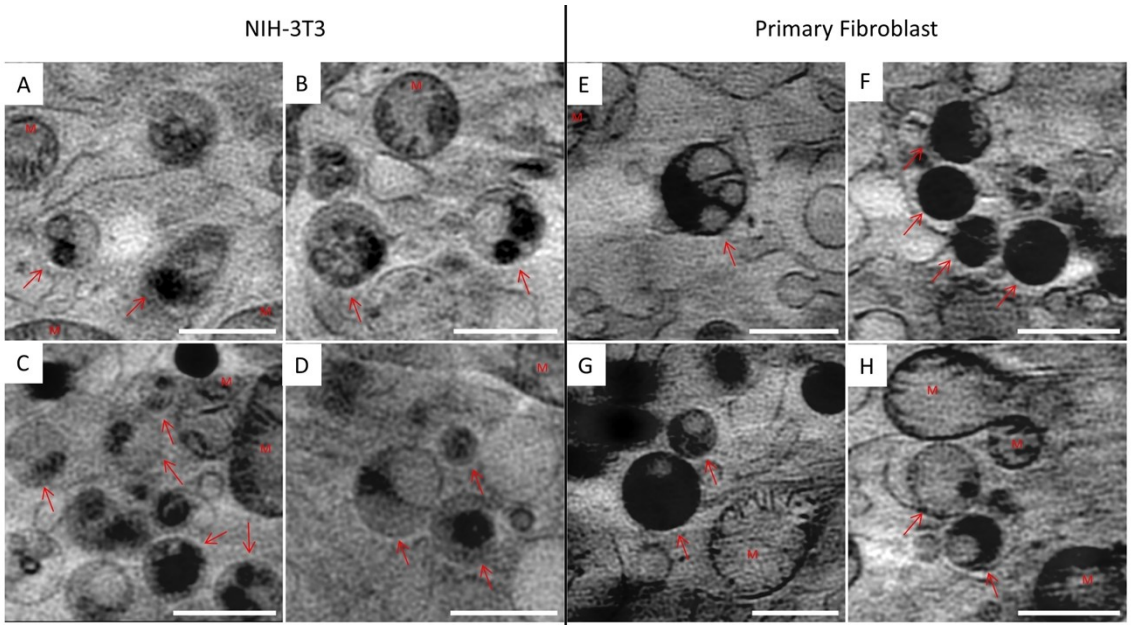
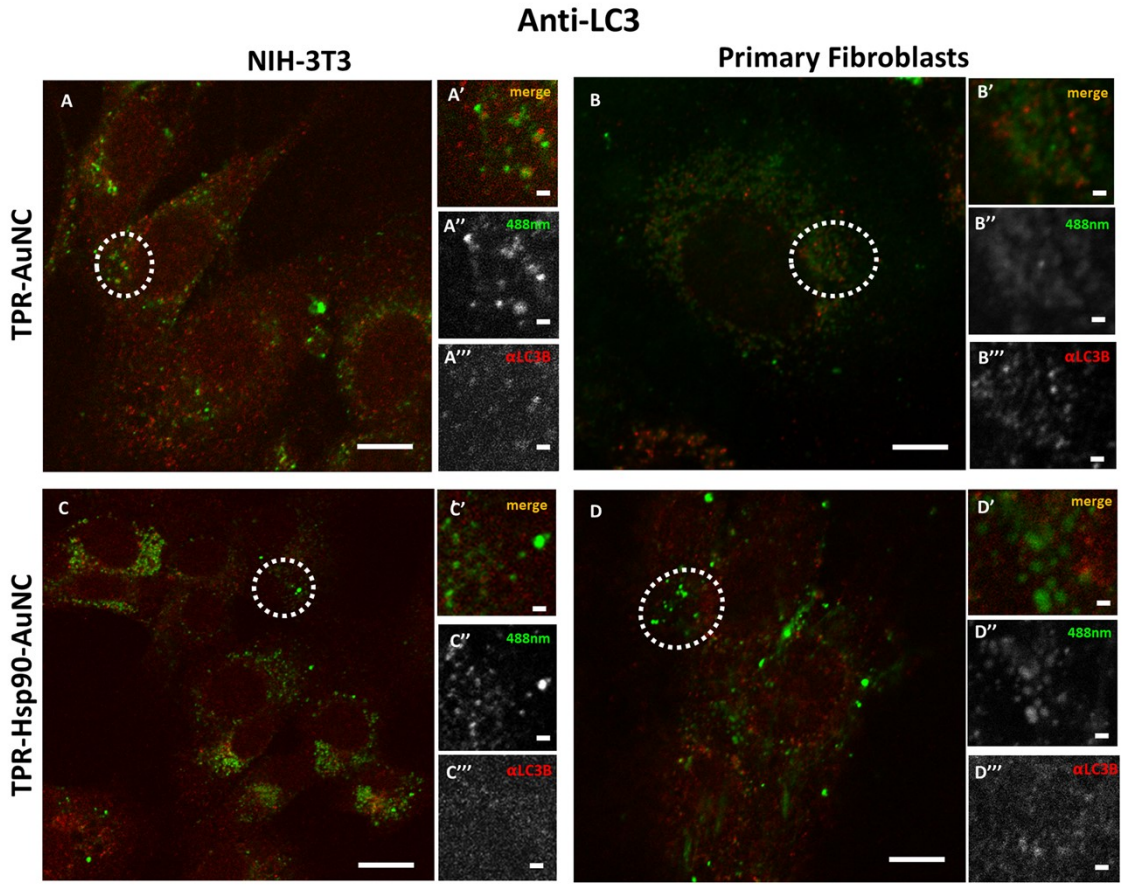
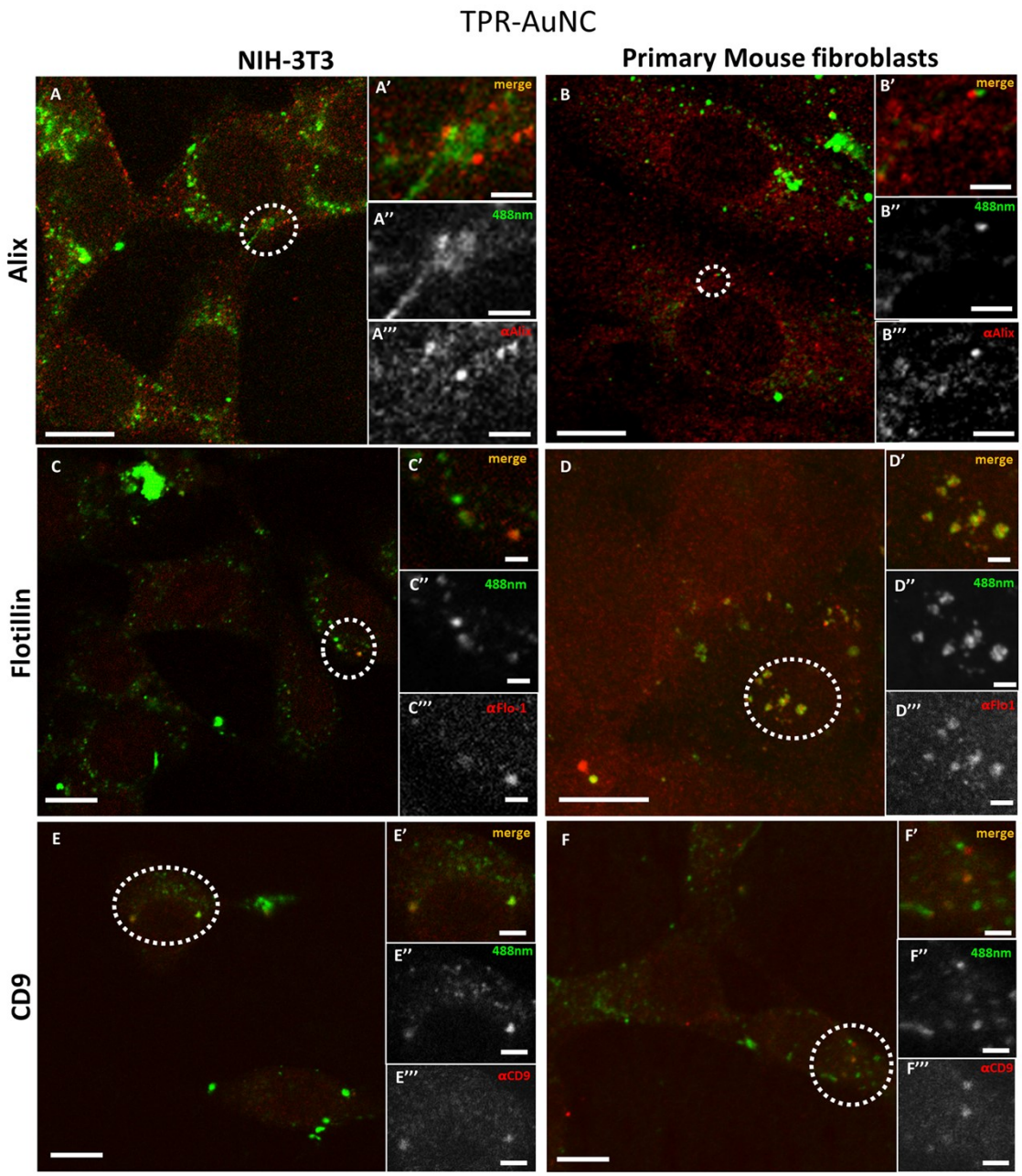
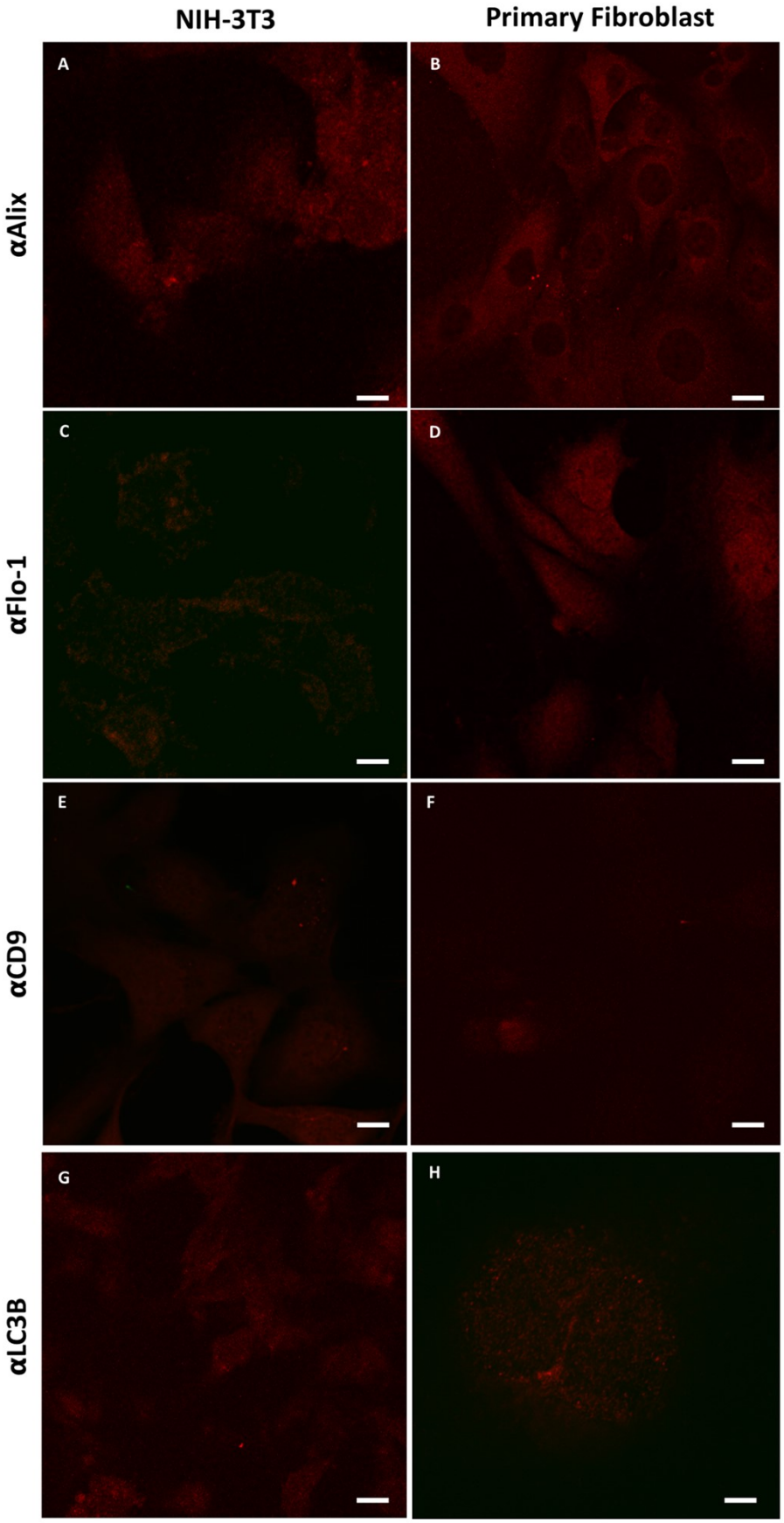
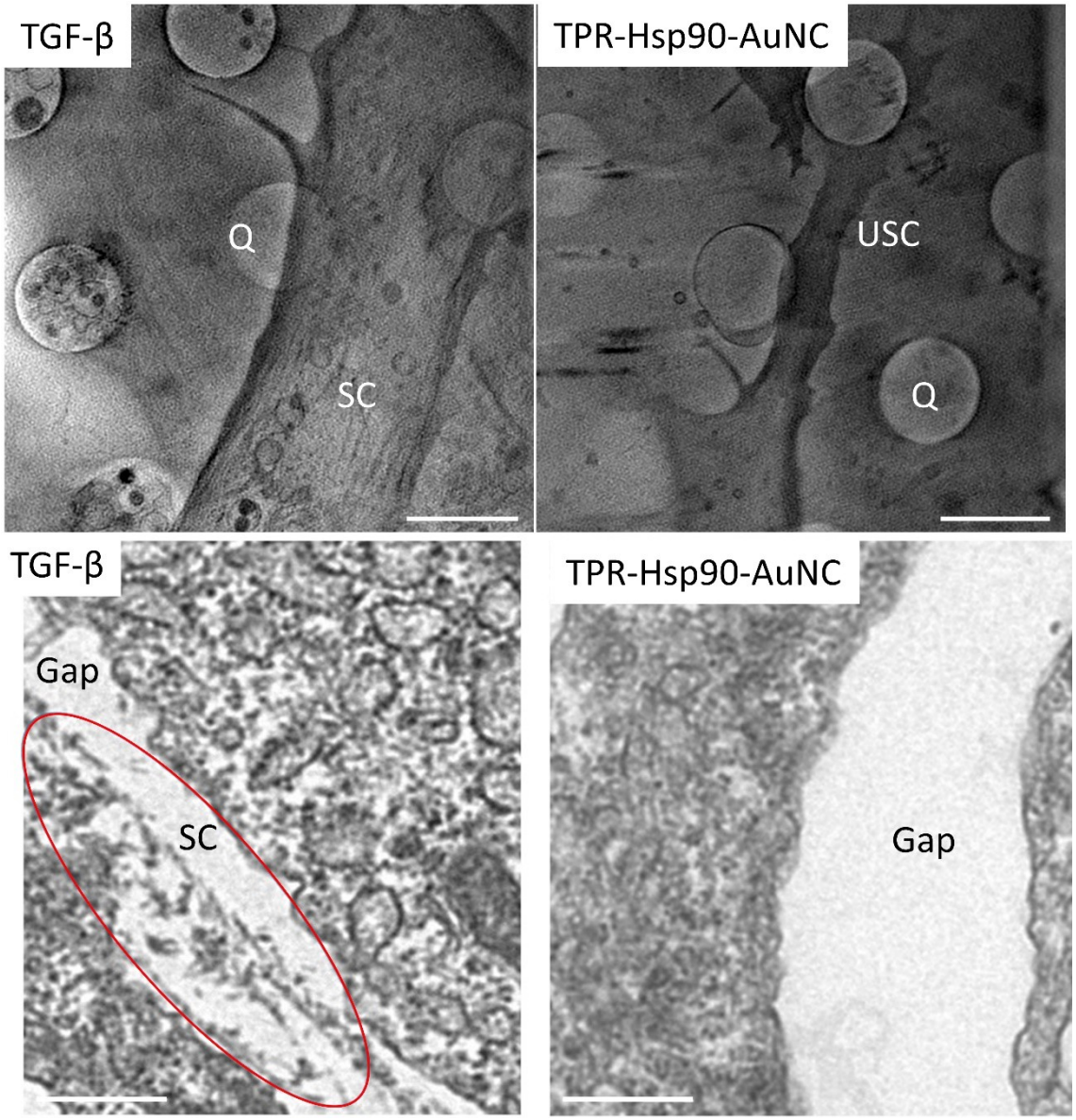



Anti-LC3
NIH-3T3
Primary Fibroblasts
TPR-AuNC
TPR-Hsp90-AuNC
A
A'
merge
A''
488nm
A'''
αLC3B
B
B'
merge
B''
488nm
B'''
αLC3B
C
C'
merge
C''
488nm
C'''
αLC3B
D
D'
merge
D''
488nm
D'''
αLC3B


TPR-AuNC
NIH-3T3
Primary Mouse fibroblasts
Alix
Flotillin
CD9
A
A'
merge
A''
488nm
A'''
αAlix
B
B'
merge
B''
488nm
B'''
αAlix
C
C'
merge
C''
488nm
C'''
αFlo-1
D
D'
merge
D''
488nm
D'''
αFlo1
E
E'
merge
E''
488nm
E'''
αCD9
F
F'
merge
F''
488nm
F'''
αCD9


NIH-3T3
Primary Fibroblast
αAlix
αFlo-1
αCD9
αLC3B
A
B
C
D
E
F
G
H


TGF-β
Q
SC
TPR-Hsp90-AuNC
USC
Q
TGF-β
Gap
SC
TPR-Hsp90-AuNC
Gap