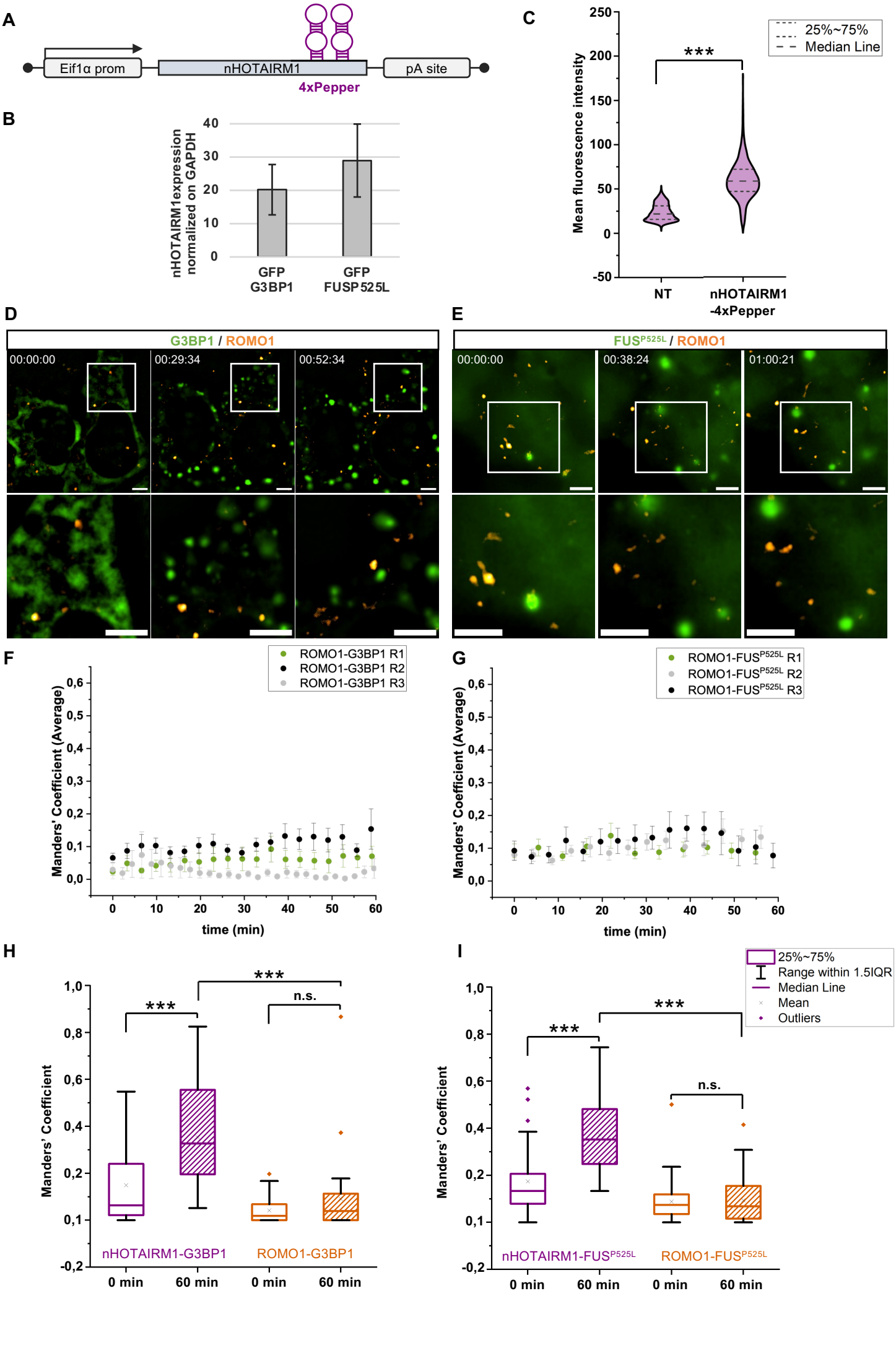
A
Eif1α prom
nHOTAIRM1
pA site
4xPepper
B
nHOTAIRM1expression normalized on GAPDH
GFP G3BP1
GFP FUSP525L
C
Mean fluorescence intensity
25%~75%
Median Line
***
NT
nHOTAIRM1 -4xPepper
D
G3BP1 / ROMO1
00:00:00
00:29:34
00:52:34
E
FUSP525L / ROMO1
00:00:00
00:38:24
01:00:21
F
ROMO1-G3BP1 R1
ROMO1-G3BP1 R2
ROMO1-G3BP1 R3
Manders' Coefficient (Average)
time (min)
G
ROMO1-FUSP525L R1
ROMO1-FUSP525L R2
ROMO1-FUSP525L R3
Manders' Coefficient (Average)
time (min)
H
Manders' Coefficient
***
n.s.
nHOTAIRM1-G3BP1
ROMO1-G3BP1
0 min
60 min
I
25%~75%
Range within 1.5IQR
Median Line
Mean
Outliers
Manders' Coefficient
nHOTAIRM1-FUSP525L
ROMO1-FUSP525L

Supplementary Fig 5 - Pepper allows following specific lncRNAs dynamics upon oxidative stress induction.

A Schematic representation of the engineered construct for the overexpression of nHOTAIRM1 tagged with an array of 4xPepper at its 3' end.

B Expression levels of nHOTAIRM1-4xPepper normalized on the one of GAPDH, quantified by qPCR in GFP-G3BP1 stable HEK 293T and in GFP-FUS^{P525L} stable HEK 293T. Error bars represent ± SEM (N=3).

C Violin plot comparing mean fluorescence intensity of non-transfected HEK 293T cells treated only with HBC with cells transfected with nHOTAIRM1-4xPepper and treated with HBC dye. ***p ≤ 0,001 corresponds to two tails unpaired Student's t-test (N=3) calculated on fluorescent intensity measurements of >5000 cells imaged from 3 independent biological replicates.

D Representative cells expressing both ROMO1 labelled with HBC620 (orange) and GFP-G3BP1 (green). In first panel, time 00:00:00 corresponds to 0.5 mM $NaAsO_2$ administration, while in following time points formation of SG can be followed (duration 1h; interval 1-5 min). Lower row shows a magnification view of the white boxes in the upper row. Scale bars correspond to 5 μm.

E Representative cell expressing both ROMO1 labelled with HBC620 (orange) and GFP-FUS^{P525L} (green). In first panel, time 00:00:00 corresponds to 0.5 mM $NaAsO_2$ administration, while in following time points formation of FUS^{P525L} condensates can be followed (duration 1h; interval 1-5 min). Lower row shows a magnification view of the white boxes in the upper row. Scale bars correspond to 5 μm.

F Average Manders' Coefficient between ROMO1 and G3BP1 over time upon $NaAsO_2$ administration. Dots represent average values of Manders' Coefficient. Error bars represent ± SEM. Average and SEM values were calculated from 5 to 10 time-lapse measurements (duration 1 hour; interval 1-5 min) collected from 3 independent biological replicates. Each colour (green, black and grey) corresponds to a single biological replicate.

G Average Manders' Coefficient between ROMO1 and FUS^{P525L} over time upon $NaAsO_2$ administration. Dots represent average values of Manders' Coefficient. Error bars represent ± SEM. Average and SEM values were calculated from 5 to 10 time-lapse measurements (duration 1 hour; interval 1-5 min) collected from 3 independent biological replicates. Each colour (green, grey and black) corresponds to a single biological replicate.

H Boxplots representing the value distribution of Manders' Overlap Coefficient between nHOTAIRM1 and G3BP1 (magenta) and between ROMO1 and G3BP1 (orange) at time 0 and time 60 upon $NaAsO_2$ administration. Horizontal lines in boxplots indicate median values; boxes extend from the 25th to the 75th percentile of each group's distribution of values; diamonds represent outliers. ***p ≤ 0,001 corresponds to two tails paired Student's t-test (N=3); ***p ≤ 0,001 corresponds to two tails unpaired Student's t-test (N=3).

I Boxplots representing the value distribution of Manders' Overlap Coefficient between nHOTAIRM1 and FUS^{P525L} (magenta) and between ROMO1 and FUS^{P525L} (orange) at time 0 and time 60 upon $NaAsO_2$ administration. Horizontal lines in boxplots indicate median values; boxes extend from the 25th to the 75th percentile of each group's distribution of values; diamonds represent outliers. ***p ≤ 0,001 corresponds to two tails paired Student's t-test (N=3); ***p ≤ 0,001 corresponds to two tails unpaired Student's t-test (N=3).