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Supplemental information

Chloroquine and cytosolic galectins affect endosomal escape of antisense oligonucleotides after Stabilin-mediated endocytosis

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Figure S1

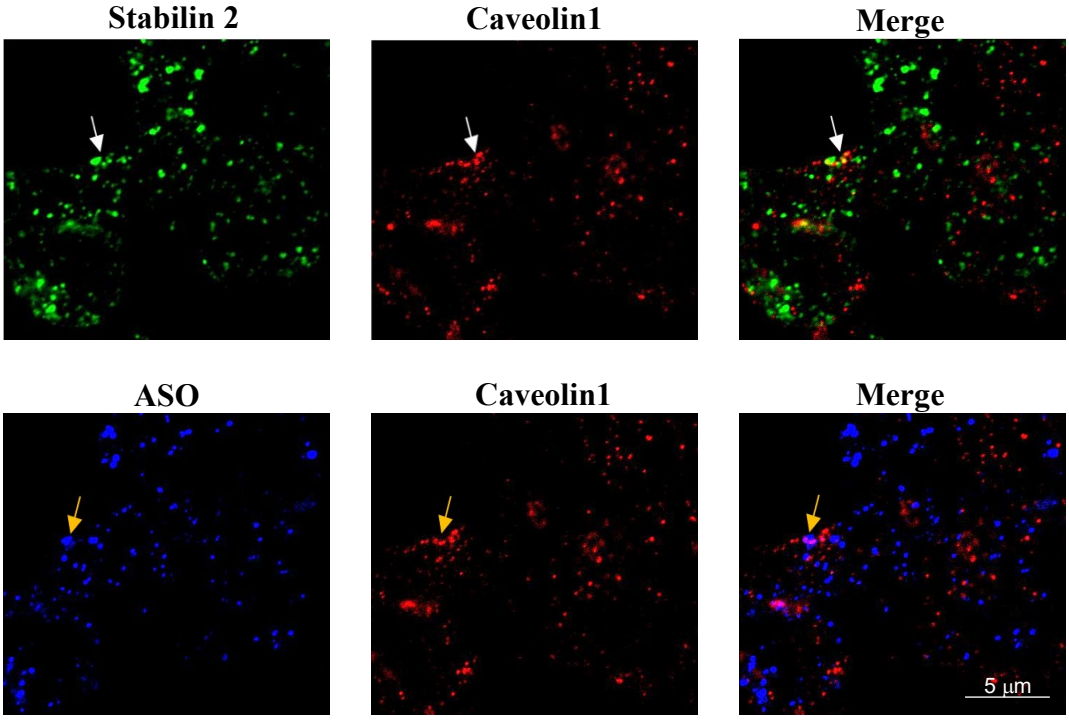
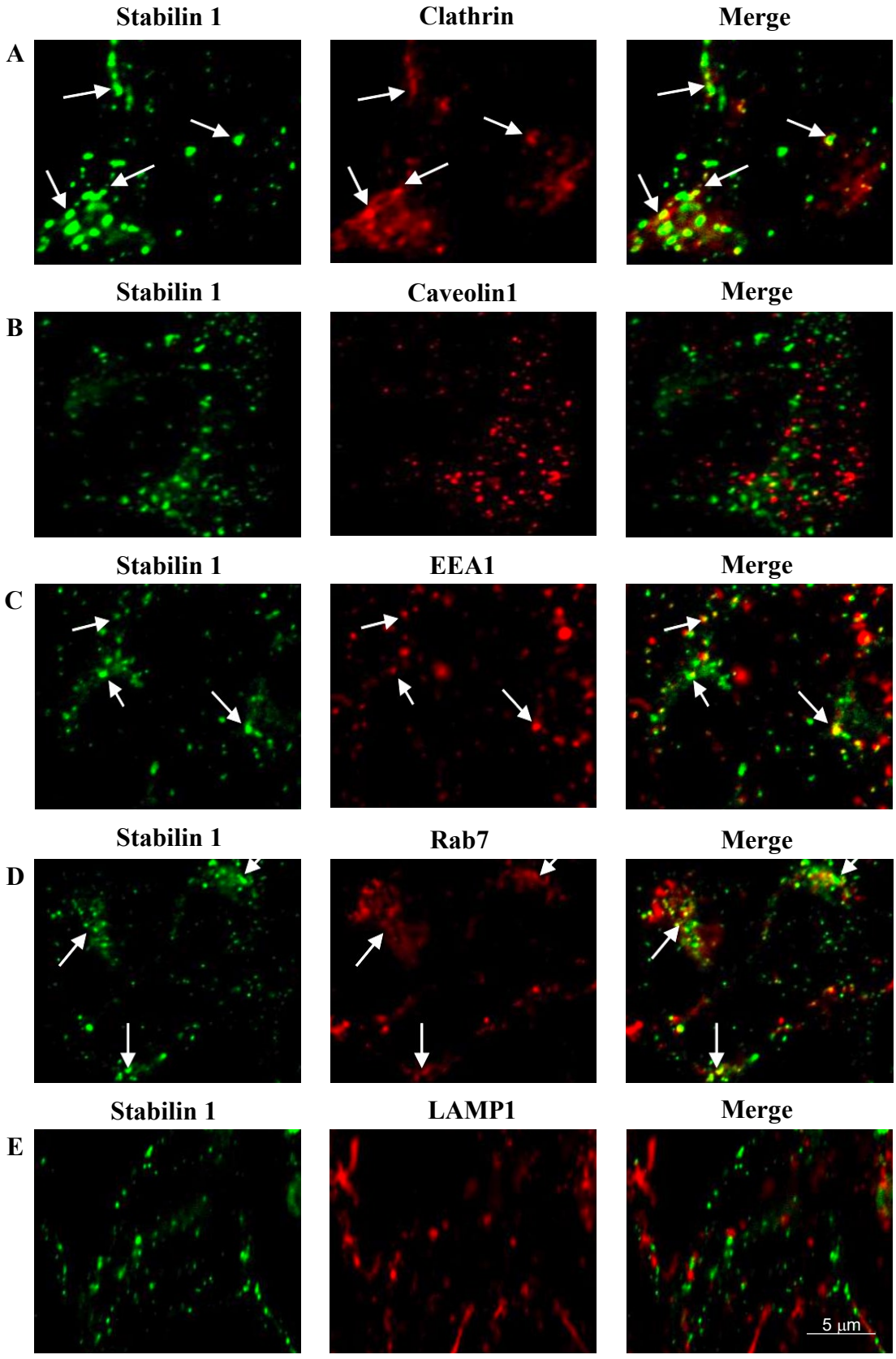
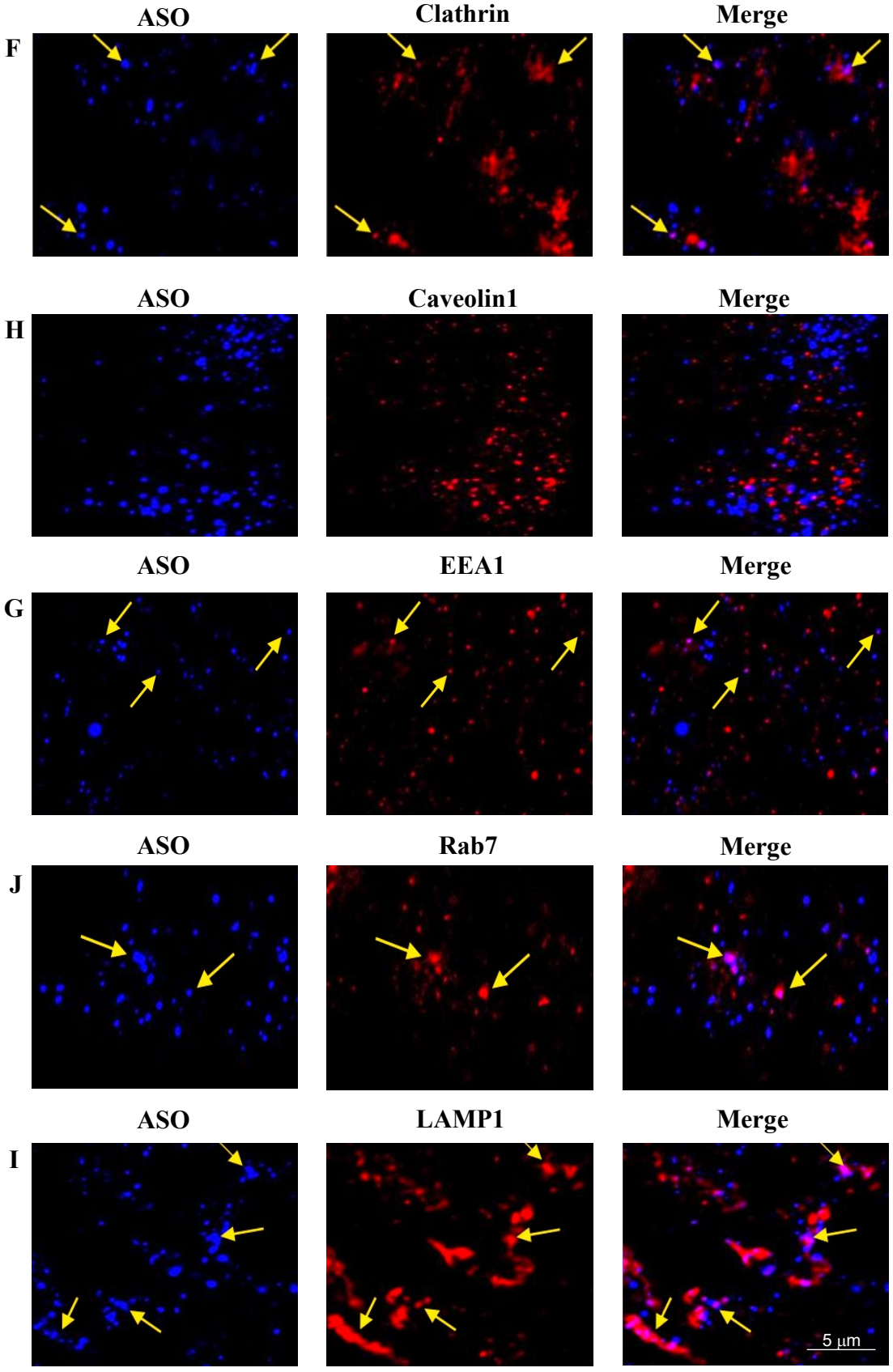


Fig. S1: No colocalization was found between Caveolin and Stabilin 2 receptor nor with PS-ASO (n=6 images)

Figure S2


Stabilin 1
Clathrin
Merge
A
Stabilin 1
Caveolin1
Merge
B
Stabilin 1
EEA1
Merge
C
Stabilin 1
Rab7
Merge
D
Stabilin 1
LAMP1
Merge
E
5 µm


F
ASO
Clathrin
Merge
H
ASO
Caveolin1
Merge
G
ASO
EEA1
Merge
J
ASO
Rab7
Merge
I
ASO
LAMP1
Merge
5 μm

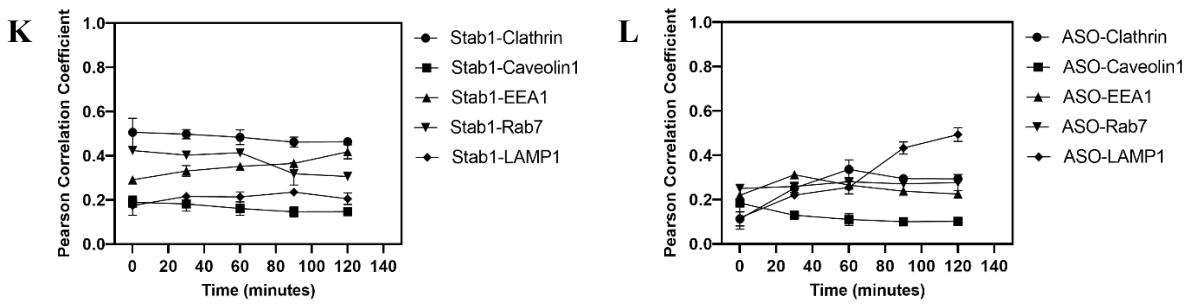


Fig. S2: Trafficking of ASO and Stabilin 1 receptor in HEK293 cell stably expressing stabilin 1 receptor. Experiment was performed as explained in **Figure 1**. Stabilin 1 receptor was found to co-localize in **(A)** Clathrin vesicle (Clathrin), (C) Early endosome (EEA1), **(D)** Late endosome (Rab7), and small amount in **(E)** Lysosome (LAMP1). However, ASO differed in trafficking in Stab1 cell line. ASO was found to colocalize in **(F)** Clathrin (Clathrin), whereas not much colocalization was observed with **(H)** Early endosome (EEA1), or **(I)** Late endosome (Rab7). ASO was finally degraded in **(J)** Lysosome (LAMP1)**. (K)** and **(L)** shows correlation quantification **(Pearson Correlation Coefficient)** between Stabilin1-endolysosomal system, and ASO-endolysosomal system respectively, at different time intervals (0,30,60,90 and120 min).
No colocalization was found between Caveolin and Stabilin 1 receptor (B) or (G) ASO (n=6 images)

Figure S3

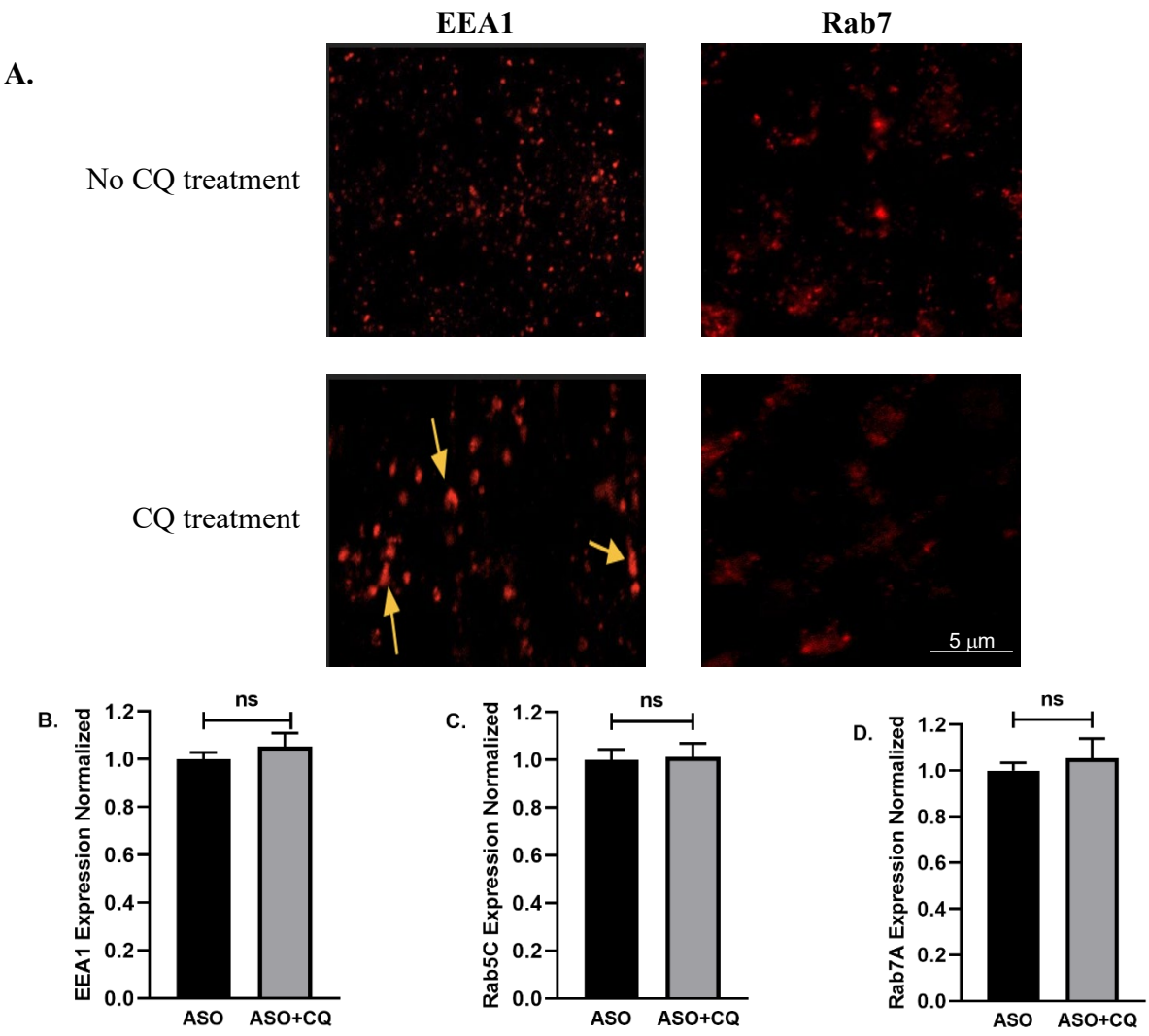


Fig. S3: Chloroquine treatment causes leakiness and enlarges the endosomal vesicle. (A) Cells were first treated with chloroquine for 1 hour followed with 30 min ASO+CQ (pulse) and 1 hour Chase. After treatment, cells were fixed, immunocytochemistry was performed, and image analysis was done to identify changes in early and late endosomal vesicle with and without chloroquine treatment

Chloroquine does not modulate EEA1/Rab5c/Rab7a mRNA expression. 190 HARE cells were treated with 0.1 µM ASO (24 hrs.), followed by 60 µM Chloroquine after 6 hours (18 hrs.). 24 hrs. after, cells were assessed for (B) EEA1 expression (C) Rab5c expression (D) Rab7a expression by qPCR.

Figure S4

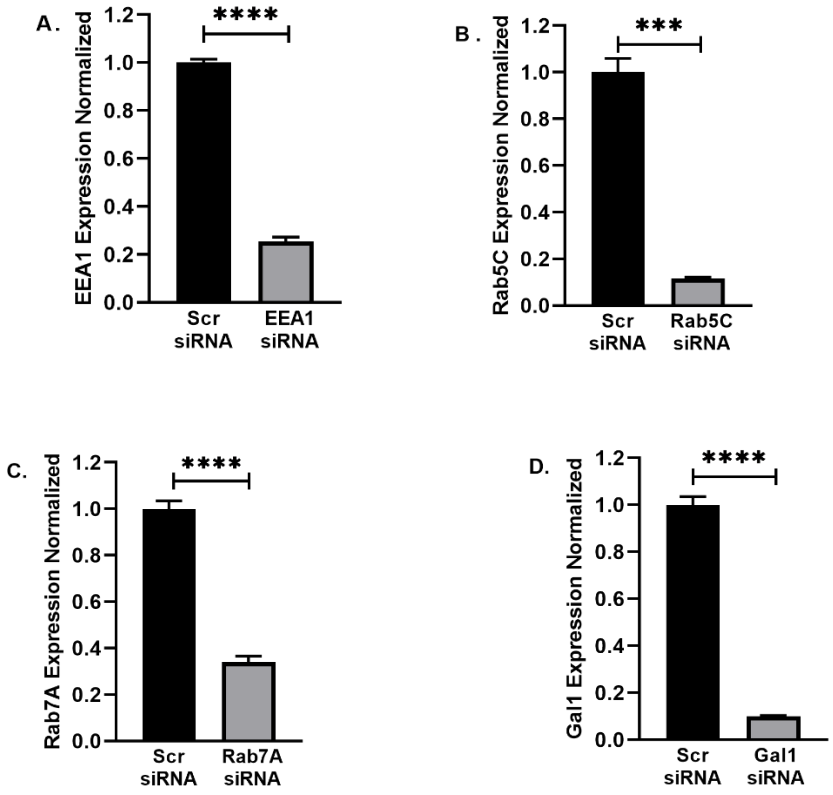


Fig. S4: Assessing the knockdown efficiency of siRNAs. 190 HARE cells were treated with **(A)** EEA1 siRNA, **(B)** Rab5c siRNA **(C)** Rab7a siRNA (**4D**) Gal1 respectively for 48 hrs. After 48 hours, cells were assessed for mRNA expression with qRT-PCR. Statistical analysis was performed using Student's t-test. Data presented as mean ± sem. * indicates $p \leq 0.05$, ** indicates $p \leq 0.01$, *** indicates $p \leq 0.001$, **** indicates $p \leq 0.0001$. $n \geq 3$ in triplicate.

Figure S5

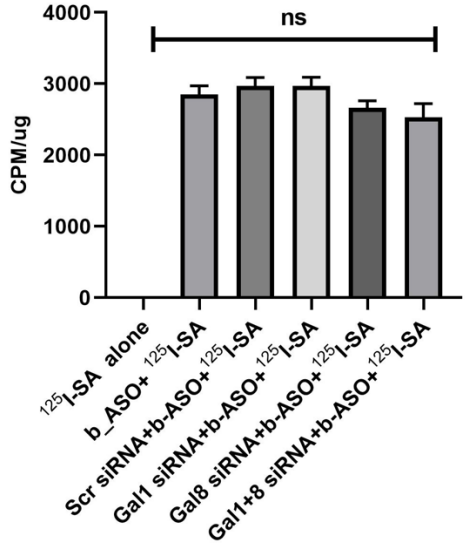
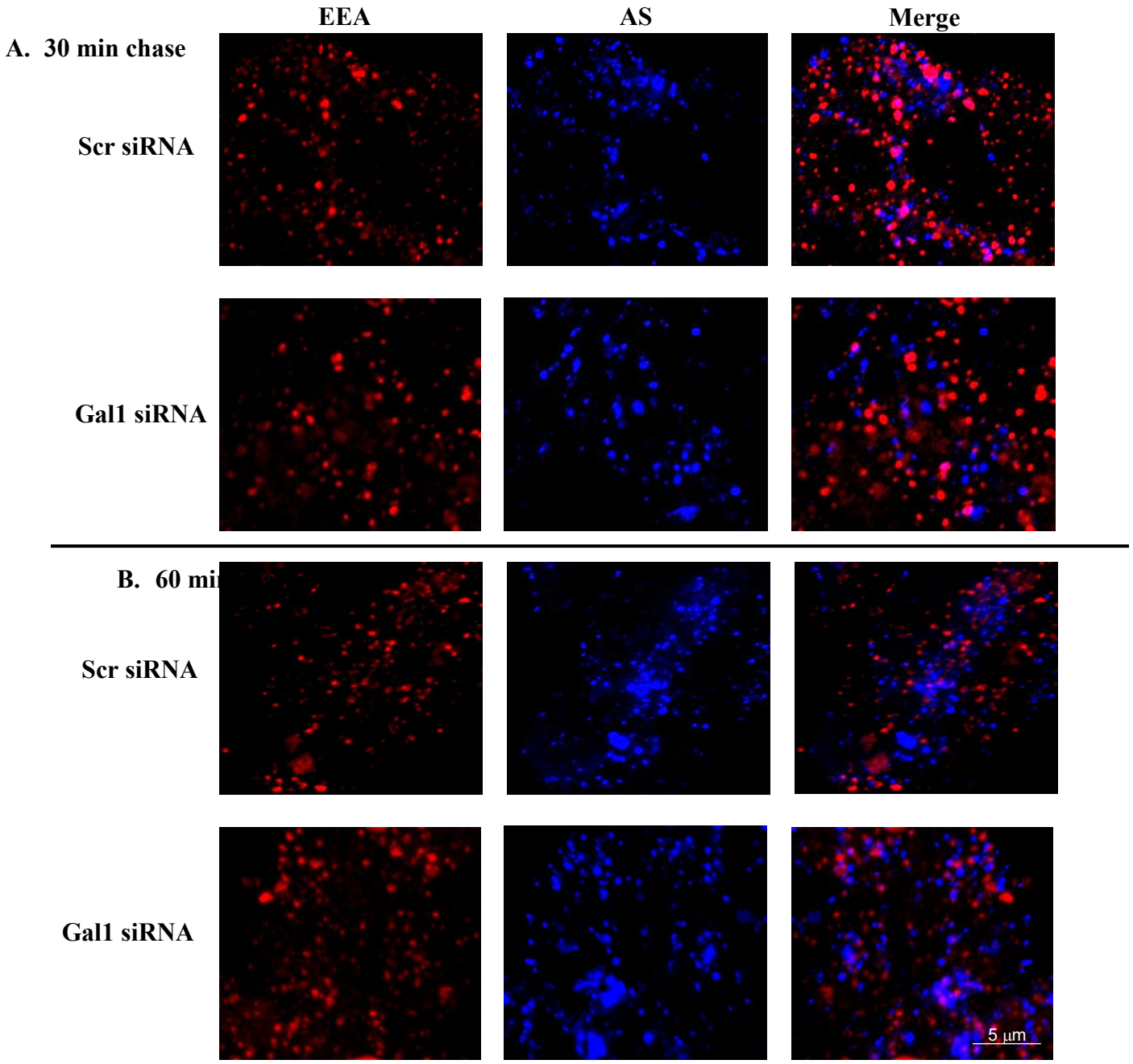


Fig. S5: Galectin knockdown does not inhibit endocytic uptake of PS-ASOs. 190 HARE cells were cultured in 24-well plates and treated with specified siRNAs for 48 hrs. After 48 hrs., cells were exposed to 0.1 μM ^{125}I-ASO (b-ASO attached with ^{125}I-SA) for 3 hrs. (first bar didn't receive b-ASO treatment). After 3 hrs., cells were washed with 3x with 1.0 ml HBSS, lysed in 0.3 ml 0.3 N NaOH and cell lysates were measured for radioactivity and protein content. The data shown represent total binding of each sample in triplicate as mean±SD.

Figure S6


EEA
AS
Merge
A. 30 min chase
Scr siRNA
Gal1 siRNA
B. 60 mi
Scr siRNA
Gal1 siRNA
5 μm

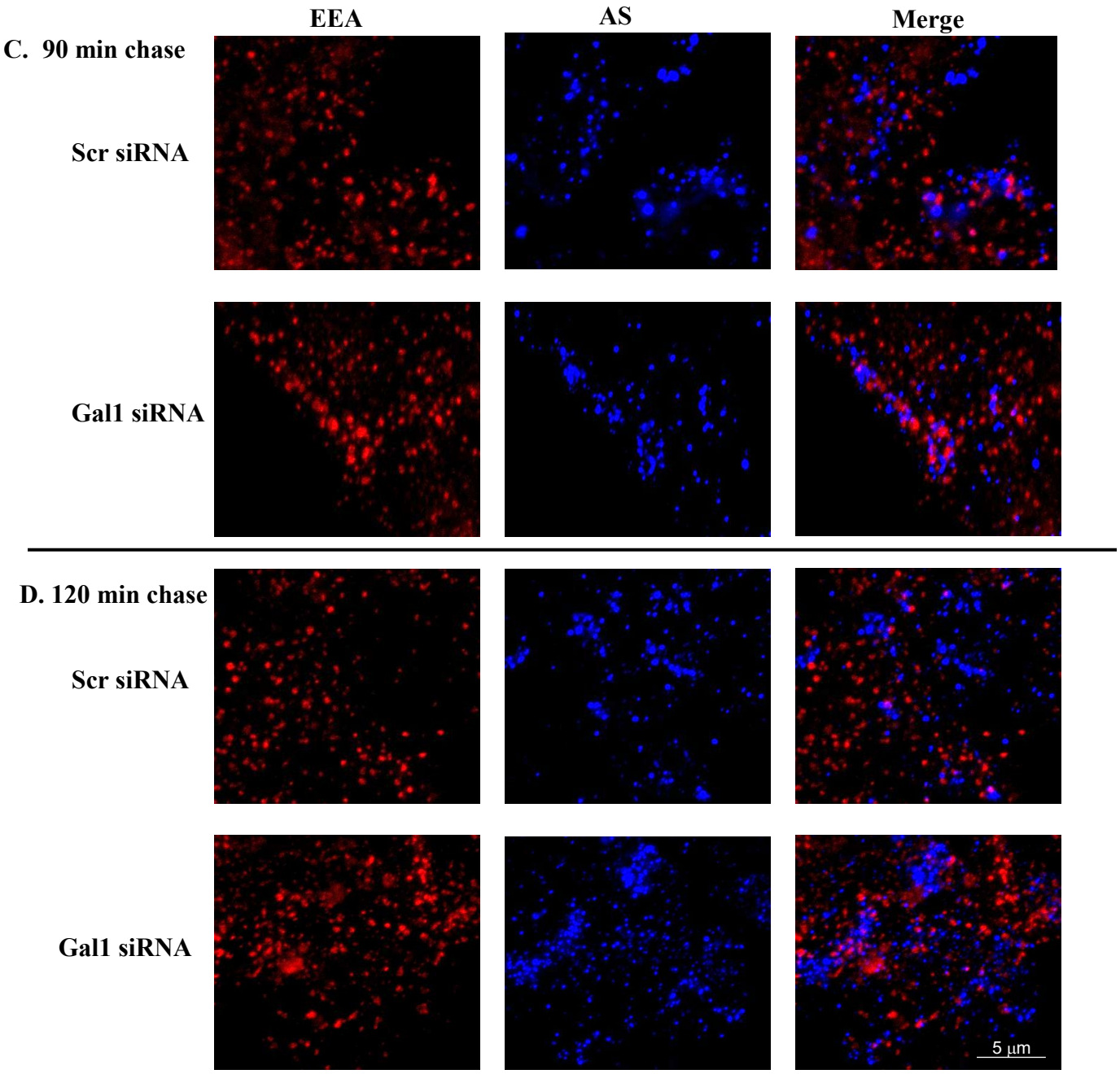
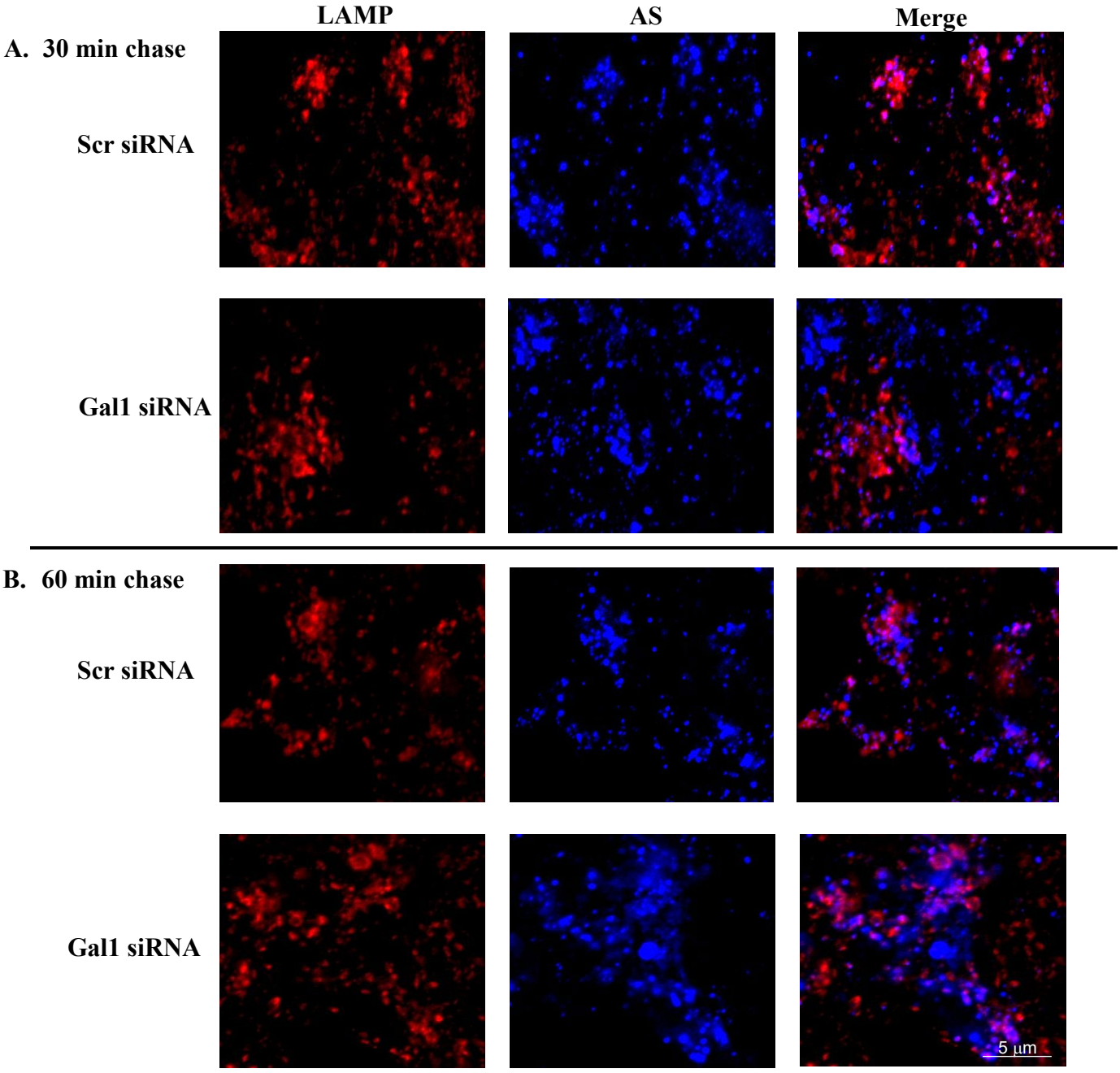


Fig. S6: 190 HARE cell lines were treated with Scr siRNA or Gal1 siRNA for 48 hrs. After siRNA treatment, cells were incubated with 0.1µM ASO for 30 minutes **(Pulse**), followed by incubation with no ASO media for variable time **(A)** 30 min, **(B)** 60 min, **(C)** 90 min, and **(D)** 120 min **(Chase).** Immunocytochemistry was performed and mounted slides were imaged by confocal microscopy. (n=6 images).

Figure S7


LAMP
AS
Merge
A. 30 min chase
Scr siRNA
Gal1 siRNA
B. 60 min chase
Scr siRNA
Gal1 siRNA
5 µm

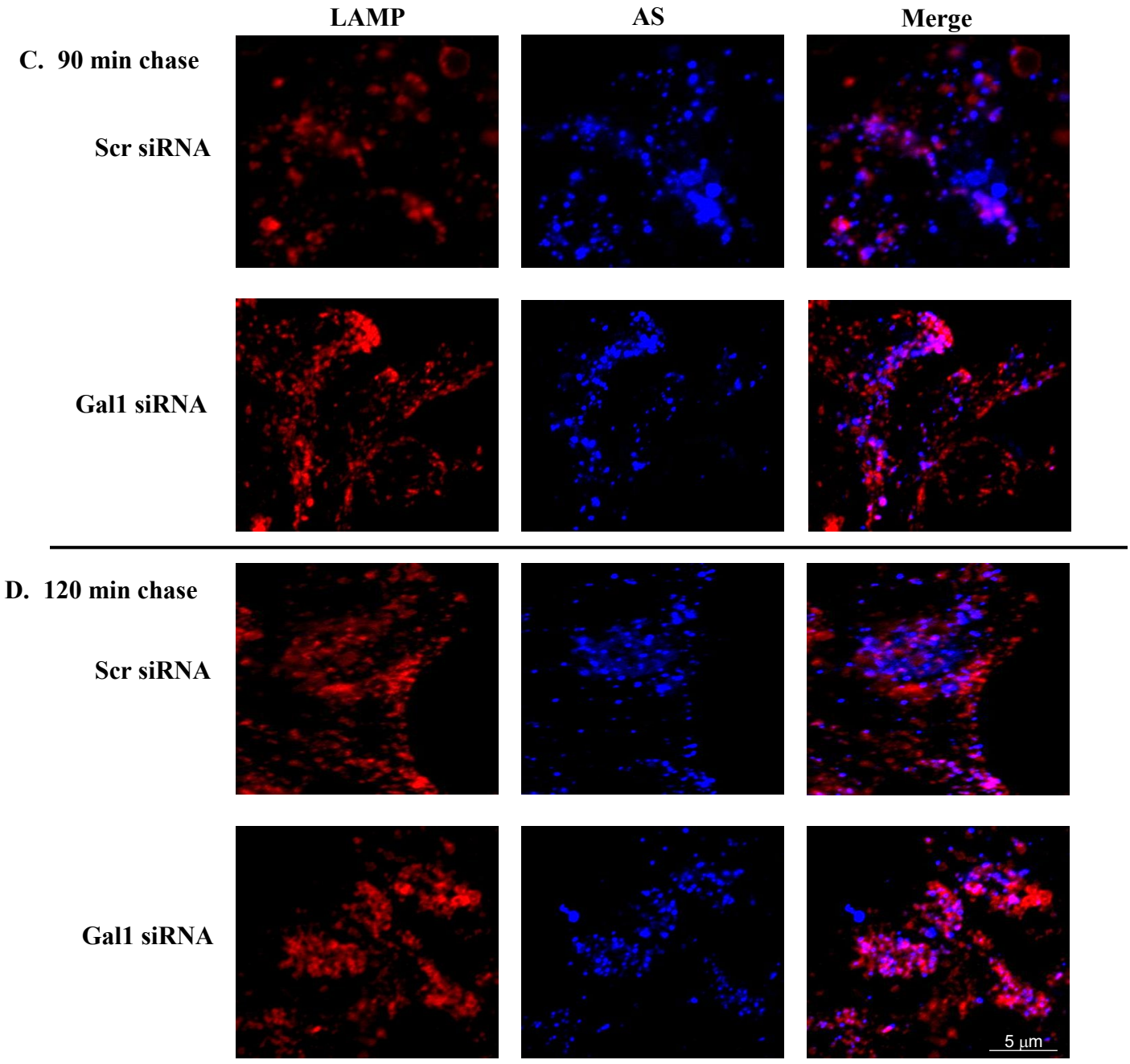


Fig. S7: 190 HARE cell lines were treated with Scr siRNA or Gal1 siRNA for 48 hrs. After siRNA treatment, cells were incubated with 0.1µM ASO for 30 minutes **(Pulse**), followed by incubation with media without ASO for variable time intervals **(A)** 30 min, **(B)** 60 min, **(C)** 90 min, and **(D)** 120 min **(Chase).** Immunocytochemistry was performed and mounted slides were imaged by confocal microscopy. (n=6 images).

Table S1

Primers used to screen Galectin expression in HEK293 cells and mouse primary cells. These are all listed in the 5' to 3' direction.

Human	Forward	Reverse
Gal1	GCTTGTGGTCTGGTCGCCAGC	GTCAAAGGCCACACATTTGATC
Gal2	CTTGAGGTTAAGAACATGGAC	CTTTTAACTTGAAAGAGGACATG
Gal3	GCTCCATGATGCGTTATCTGGG	CAGGTTATAAGGCACAATCAGTG
Gal4	GATGGTTCTTCGAGCTGTGAGCC	GATGGTTCTTCGAGCTGTGAGCC
Gal7	GGCACGGTGCTGAGAATTCGC	GAAGATCCTCACGGAGTCCAGC
Gal8	CTACAGAATATCATCTATAACCC	GGGTACTTTGTAAGTCCGAGCTG
Gal8	GGTCTCCAGGACGGACTTCAGATC	CACGCCGGGAGGTTTTTGTCTGC
Mouse		
Gal1	GGTCGCCAGCAACCTGAATCTCAAAC	CGCACTTAATCTTGAAGTCTCCATC
Gal2	GGTCGCCAGCAACCTGAATCTCAAAC	GAAATTTGAGGTCAAAGACCTGAAC
Gal3	CGCTTAACGATGCCTTAGCTGGCTC	CTGCAGTAGGTGAGCATCGTTGACCG
Gal4	CCACCTACAATCCGACTCTGCCC	GAAGTCGAAAAGGTGCTGGCCATTGG
Gal6	GGTGGCCTCAGTGTCGGGATGTCC	CAAGGTGAGGTCACCATTGATCTCC
Gal7	TGCTACCCAGCACAAGACCTCCCTG	GAAGATCTTCACTGAATGCAGCTGC
Gal8	CCTACAAAATATCATCTATAACCCG	GTCTTAAATCTGTGTTTGTACTCC
Gal9	CATACATTAACCCGATCATCCCCTTTAC	GTCTGCACGTGGGTCAGCTGGATATC