E08-02-0110 Hoflack

Figure S1. Electron microscopy profiles of AP-3-coated liposomes. Liposomes with (A) LIMP-II wt tails or (B) no cytoplasmic domains, obtained under standard conditions and purified on density gradient, were fixed and processed for electron microscopy. Magnification: 44k.

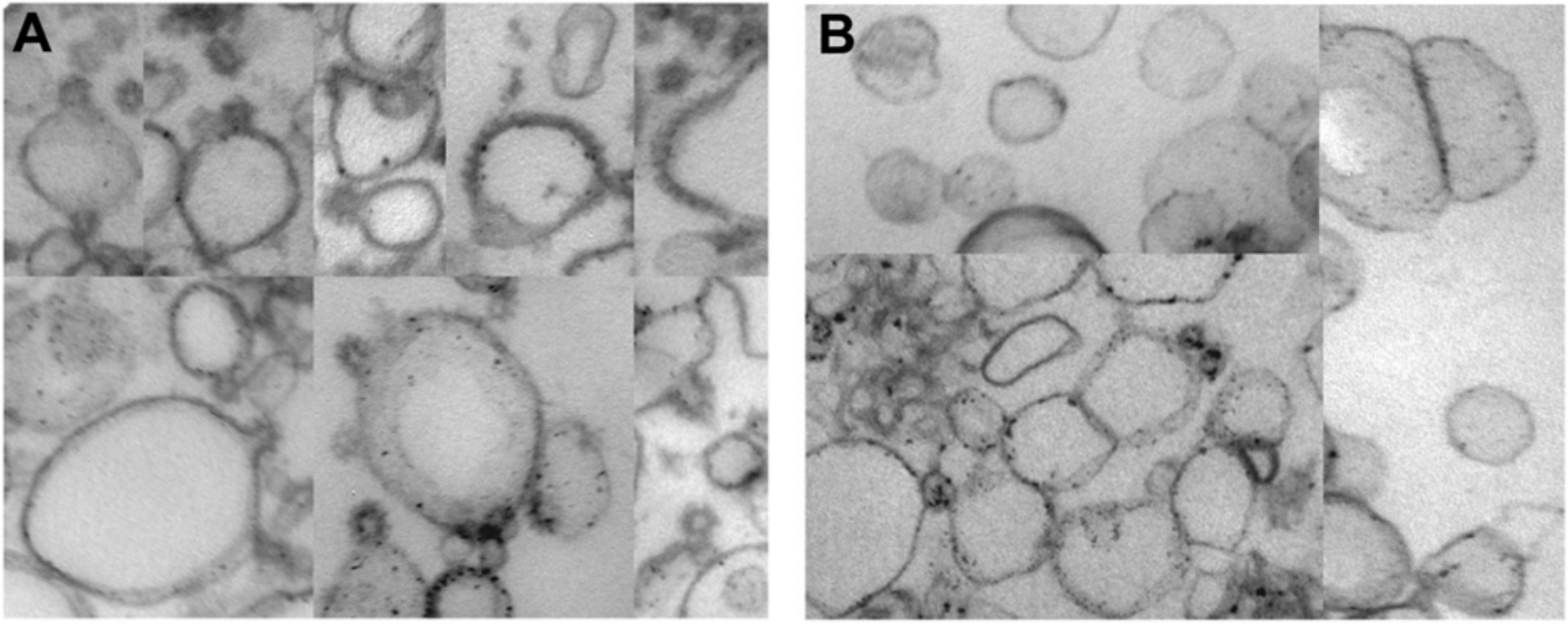
Figure S2. Protein profile of AP-3 coated liposomes. Liposomes containing LIMP II and PI-3P (LIMP IIwt/PI3P) or liposomes devoid of these components (-/-) were incubated with cytosol and GTP-γ-S, then purified by floatation on density gradient and analyzed on 7% and 15% SDS-PAGE. The different lanes stained by Coomassie brillant blue were scanned. The black line represents the reconstituted protein profile of liposomes with LIMP II and PI-3P whereas the gray line represents that of liposomes without these membrane components. The lanes were cut into 50 slices that were analyzed by mass spectrometry.

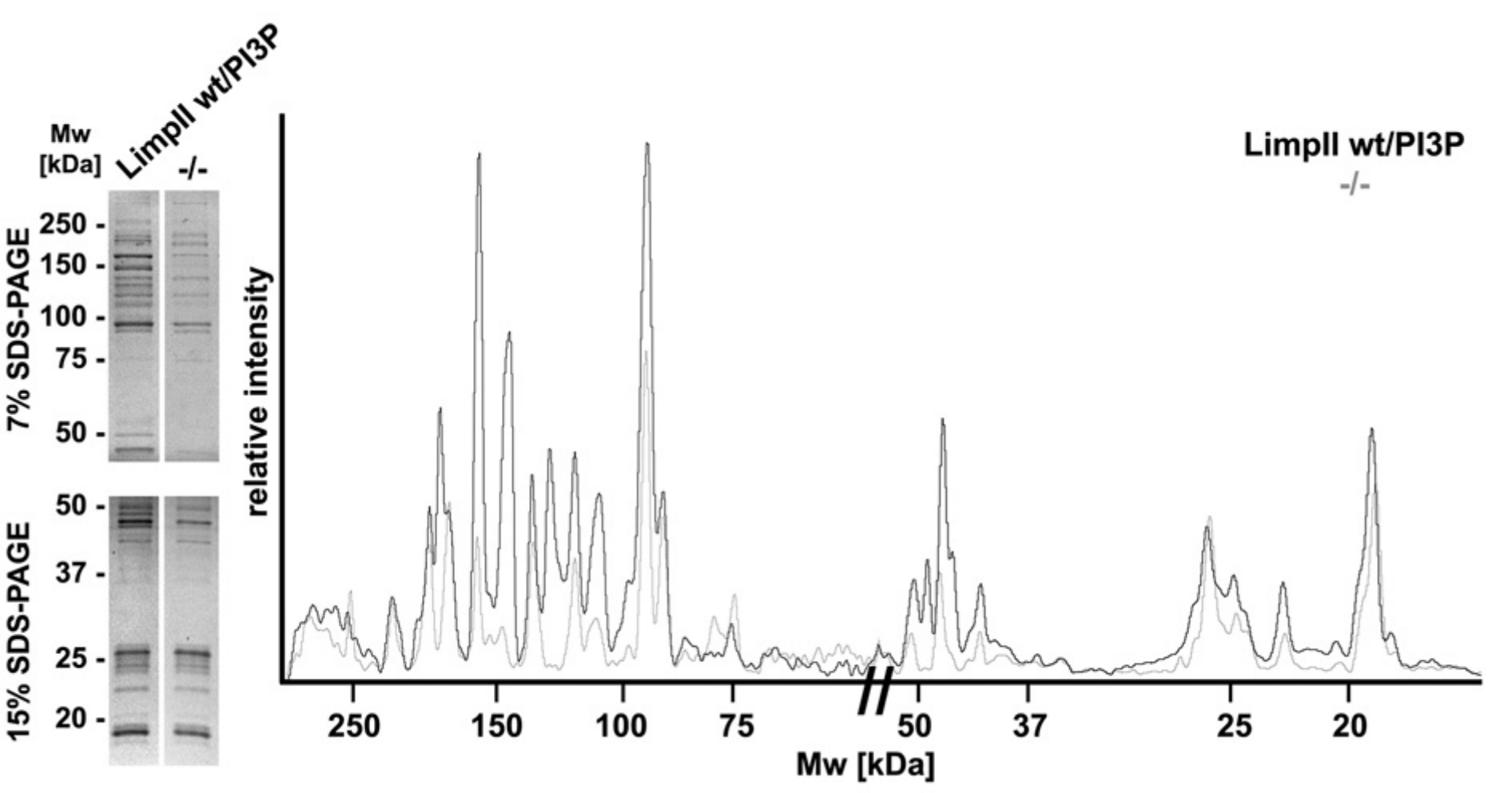
Figure S3. Efficiencies of siRNA-mediated interference with molecules associated with AP-3-coated liposomes. Cells were transfected for 72h with control non-targeting siRNAs (siNon) or with siRNAs against (A) AP-3δ, (B) PI-4KIIα, (C) β-PIX or (D) AP-1γ. Non-transfected cells were also included as a control C. Cells were further harvested and analyzed by Western blot using antibodies against the respective proteins, using tubulin as a loading control.

Figure S4. LAMP-1 sorting in siRNA-treated cells. Non-targeting (A) (siNon), (B) AP-3 (siAP3) or (C) Big1 (siBIG1)-treated HeLa cells expressing GFP-MPR were incubated with anti LAMP-1 antibodies for 4h, fixed, permeabilized and labeled with the secondary antibody (red) and with DAPI nuclear staining (blue), and then analyzed by confocal fluorescence microscopy. Scale bars, 20 μm.

Figure S5. GFP-MPR sorting in siRNA-treated cells. Cells expressing GFP-MPR transfected for 72h with siRNAs against the indicated molecules or with control nontargeting siRNAs (siNon) were incubated for 4h with anti-GFP, then fixed, permeabilized and labeled with secondary antibodies (red) and analyzed by fluorescence confocal microscopy. Scale bars, 10 μm.

Figure S6. AP-1 localization in siRNA-treated cells. Cells expressing GFP-MPR transfected for 72h with siRNAs against the indicated molecules or with control nontargeting siRNAs (siNon) were fixed, permeabilized and labeled with antibodies against the AP-1γ (red) and analyzed by fluorescence confocal microscopy. Scale bars, 10 μm.


A
B


Mw
[kDa]
LimpII wt/PI3P
-/-
250
150
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50
7% SDS-PAGE
50
37
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relative intensity
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-/-
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50
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Mw [kDa]

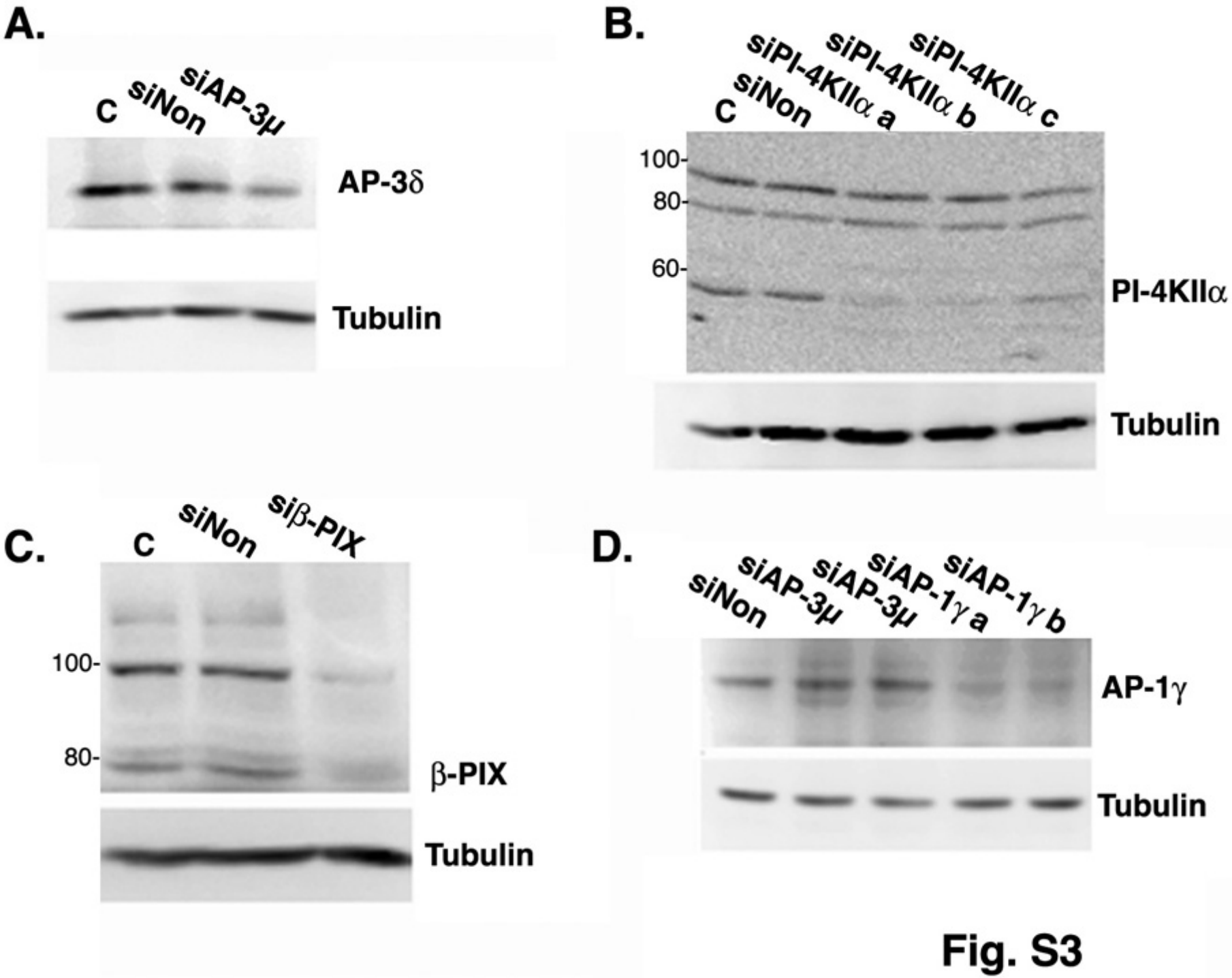
A.
C
siNon
siAP-3μ
AP-3δ
Tubulin
B.
siPI-4KIIα a
siPI-4KIIα b
siPI-4KIIα c
C
siNon
100-
80-
60-
PI-4KIIα
Tubulin
C.
C
siNon
siβ-PIX
100-
80-
β-PIX
Tubulin
D.
siNon
siAP-3μ
siAP-3μ
siAP-1γ a
siAP-1γ b
AP-1γ
Tubulin

Fig. S3

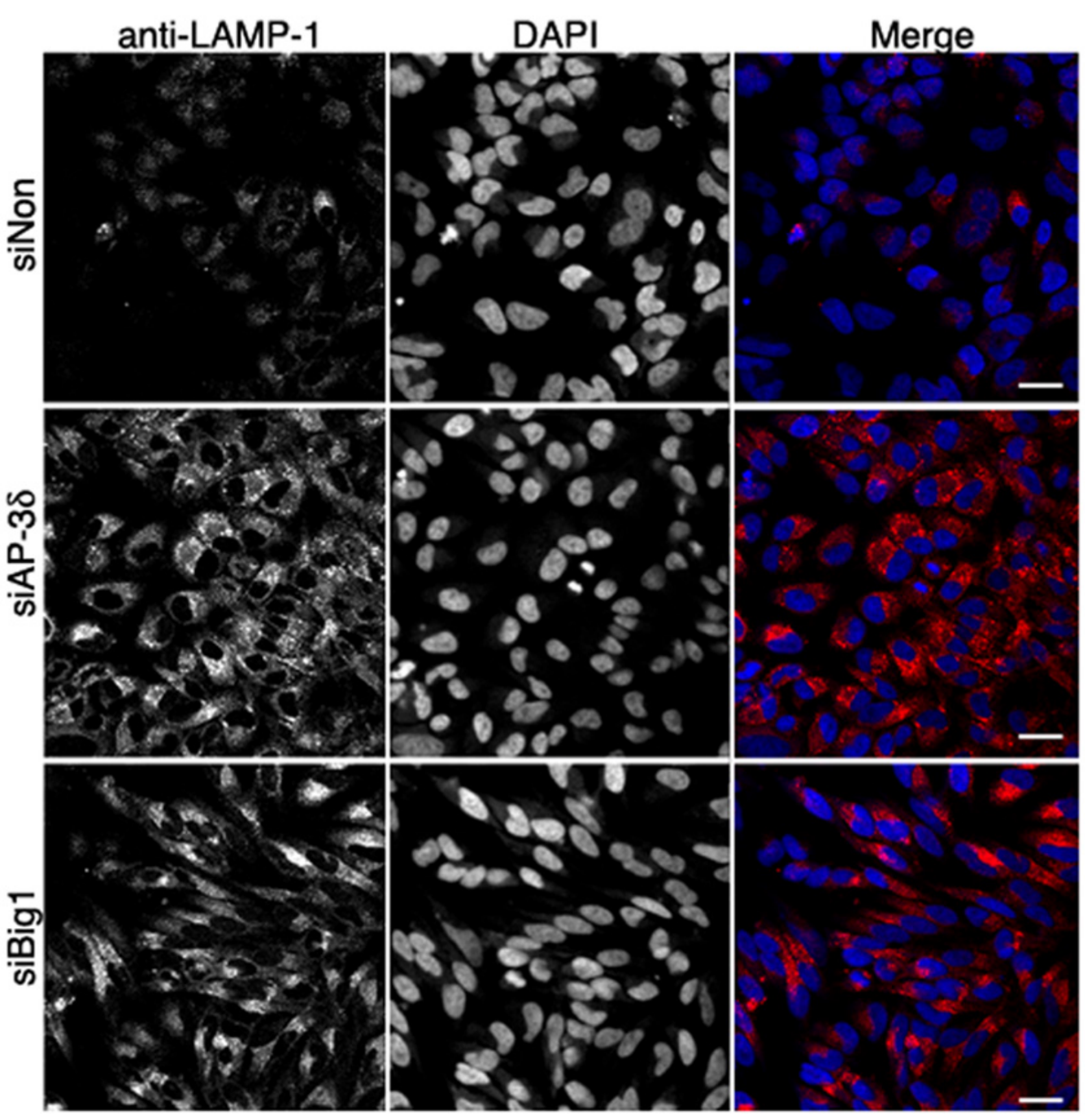

Fig. S4

anti-GFP 4h
GFP-MPR
Merge
siNon
siAP-1γ
siAP-3μ
siBIG1
siARAP1
siPI-3KIIIC3
siPI-4KIIα
siBorg4

Fig. S5

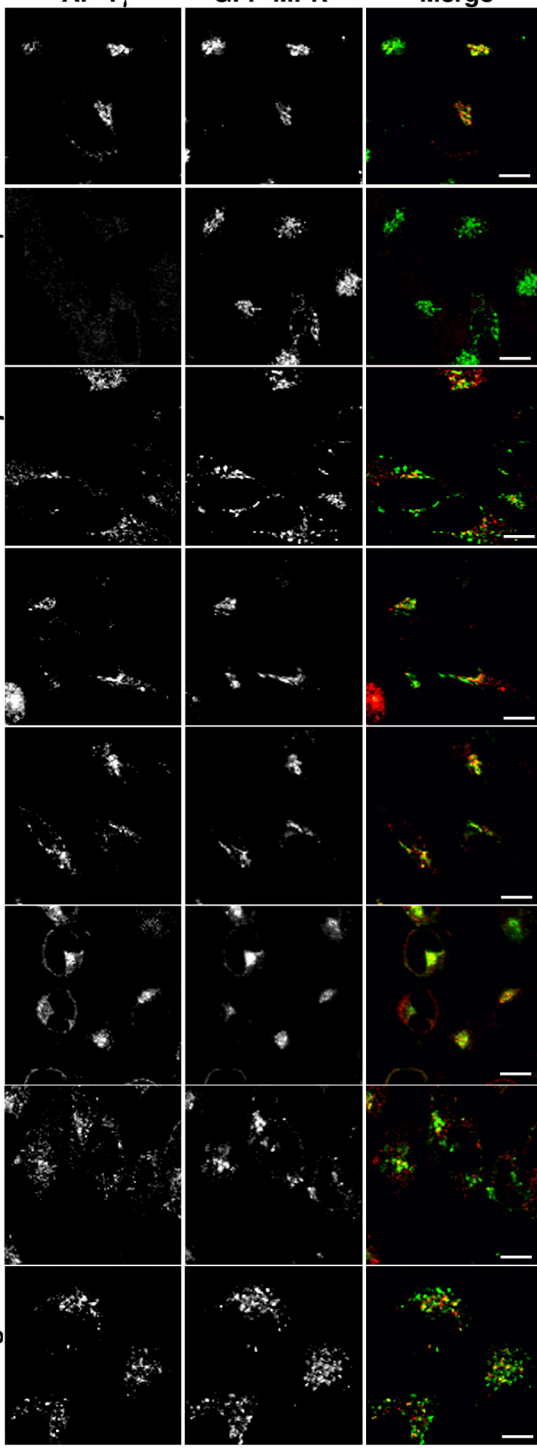
AP-1γ
GFP-MPR
Merge
siNon
siAP-1γ
siAP-3μ
siBIG1
siARAP1
siPI-3KIIIC3
siPI-4KIIα
siBorg4

Fig. S6