nature portfolio

https://doi.org/10.1038/s41586-023-06090-9

Supplementary information

Heteromeric clusters of ubiquitinated ER-shaping proteins drive ER-phagy

In the format provided by the authors and unedited

FIGURE 1 - C

n1

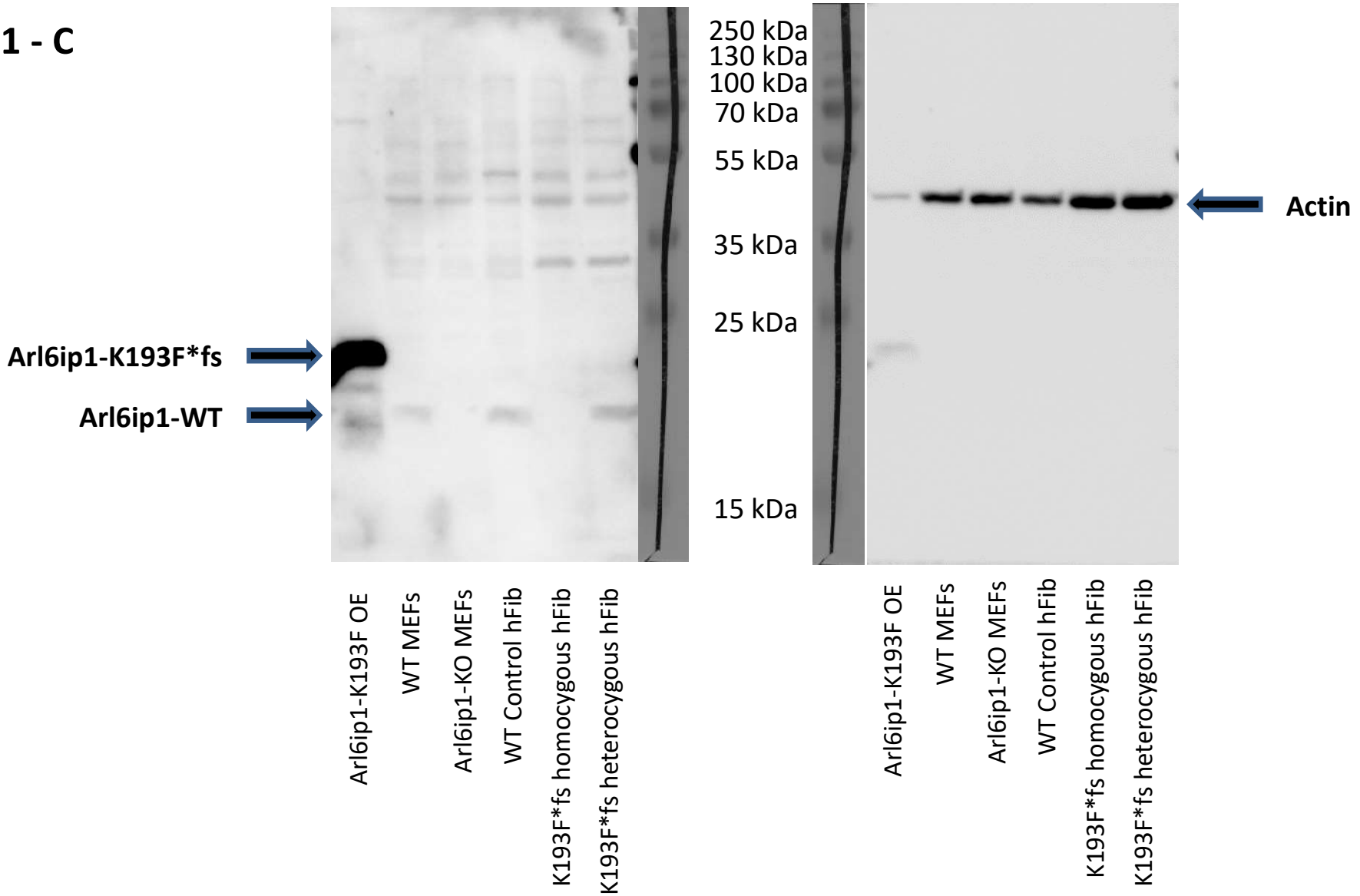

Arl6ip1-K193F*fs
Arl6ip1-WT
250 kDa
130 kDa
100 kDa
70 kDa
55 kDa
35 kDa
25 kDa
15 kDa
Actin
Arl6ip1-K193F OE
WT MEFs
Arl6ip1-KO MEFs
WT Control hFib
K193F*fs homocygous hFib
K193F*fs heterocygous hFib
Arl6ip1-K193F OE
WT MEFs
Arl6ip1-KO MEFs
WT Control hFib
K193F*fs homocygous hFib
K193F*fs heterocygous hFib

FIGURE 1 - C

n2

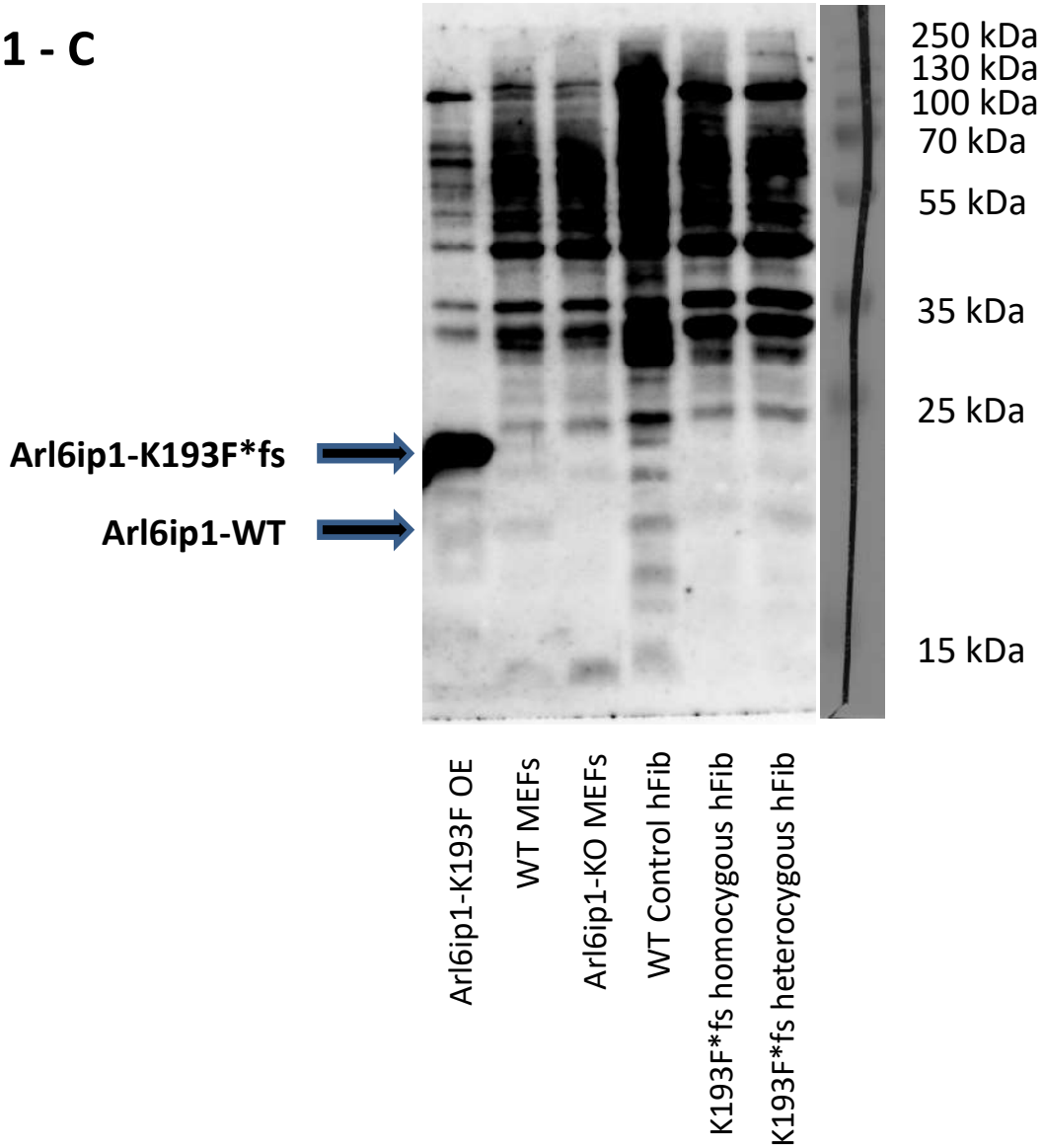

Arl6ip1-K193F*fs
Arl6ip1-WT
250 kDa
130 kDa
100 kDa
70 kDa
55 kDa
35 kDa
25 kDa
15 kDa
Arl6ip1-K193F OE
WT MEFs
Arl6ip1-KO MEFs
WT Control hFib
K193F*fs homocygous hFib
K193F*fs heterocygous hFib

FIGURE 1 - F

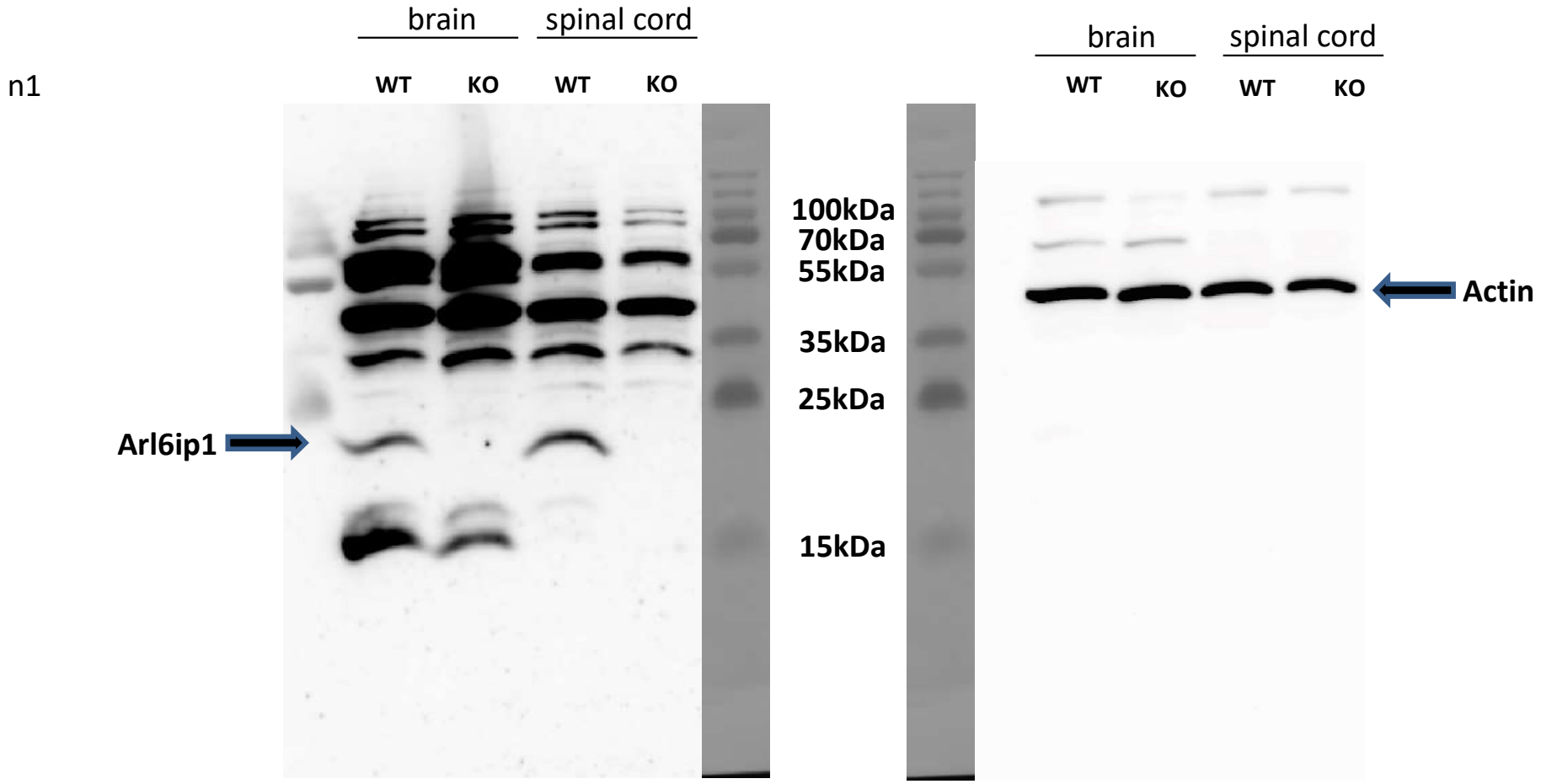

brain
spinal cord
n1
WT
KO
WT
KO
Arl6ip1
100kDa
70kDa
55kDa
35kDa
25kDa
15kDa
brain
spinal cord
WT
KO
WT
KO
Actin

FIGURE 1 - F

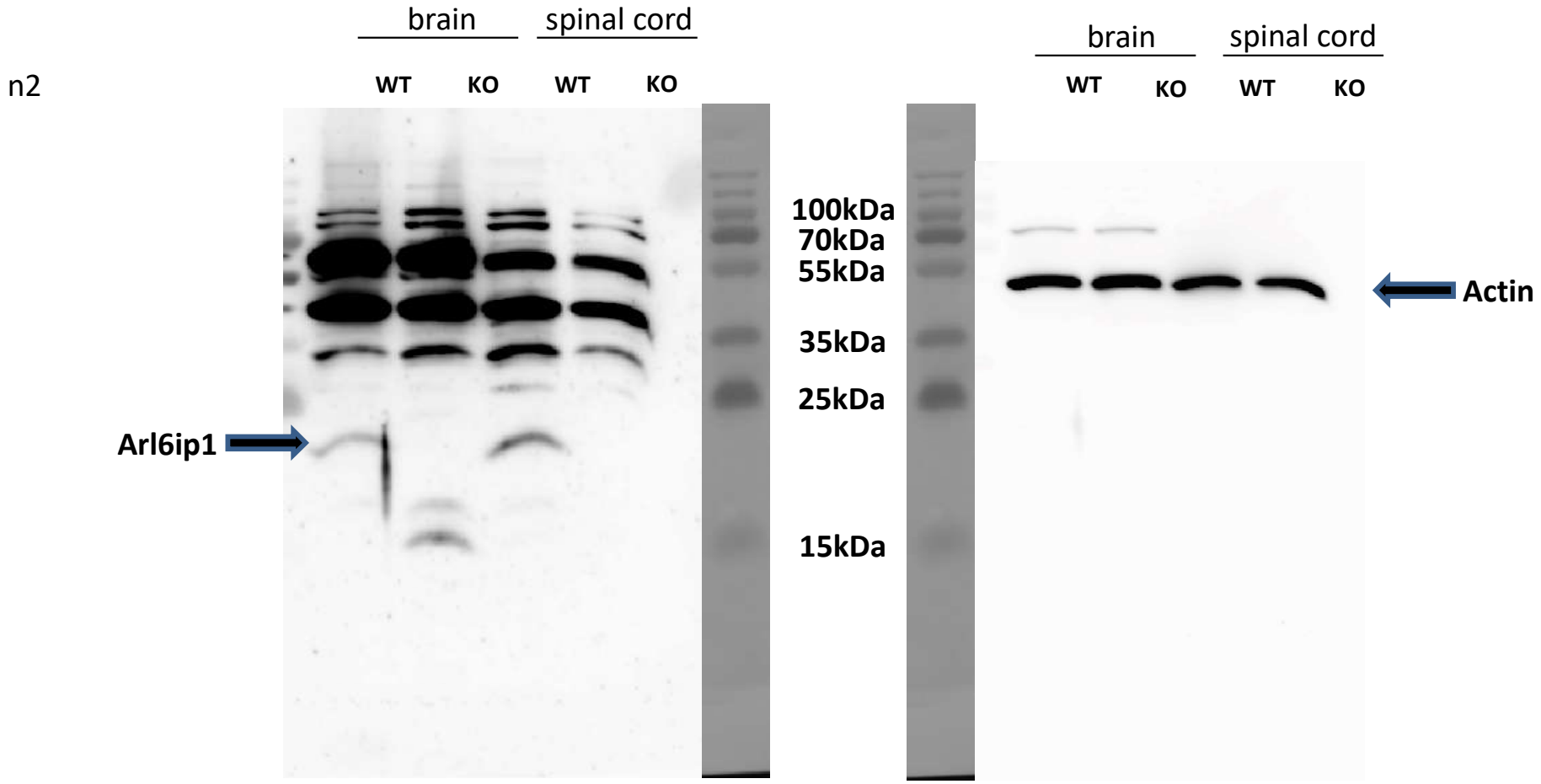
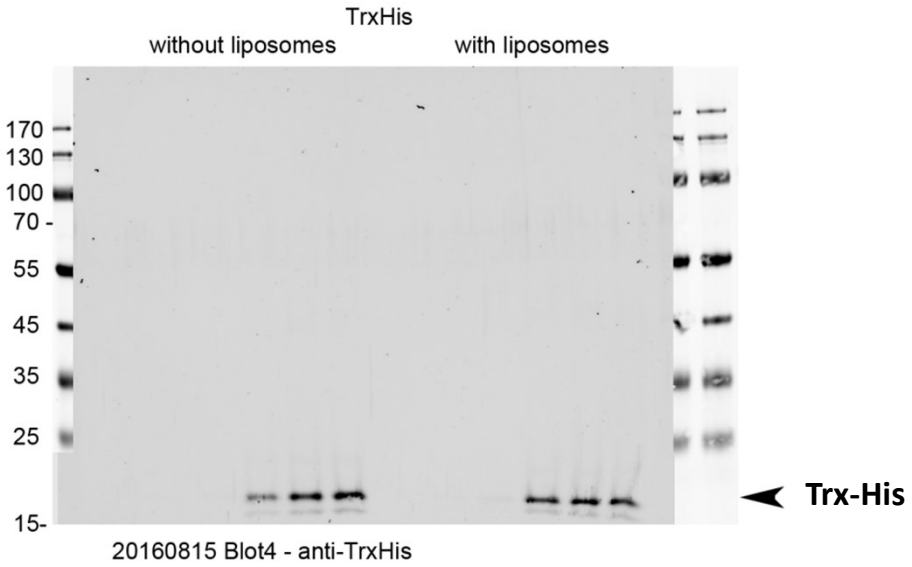
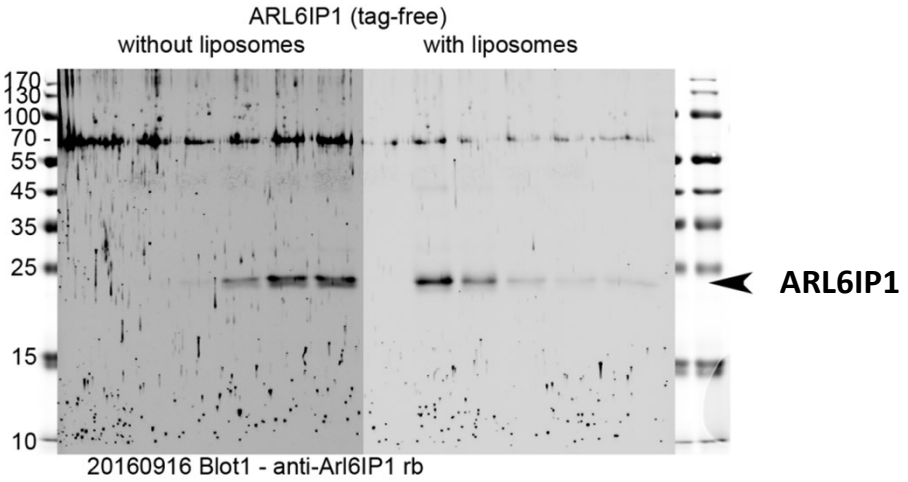

n2
brain
spinal cord
WT
KO
WT
KO
Arl6ip1
100kDa
70kDa
55kDa
35kDa
25kDa
15kDa
brain
spinal cord
WT
KO
WT
KO
Actin

FIG 2. C


TrxHis
without liposomes
with liposomes
170
130
100
70 -
55
45
35
25
15-
20160815 Blot4 - anti-TrxHis
Trx-His


ARL6IP1 (tag-free)
without liposomes
with liposomes
170
130
100
70 -
55
45
35
25
15
10
20160916 Blot1 - anti-Arl6IP1 rb
ARL6IP1

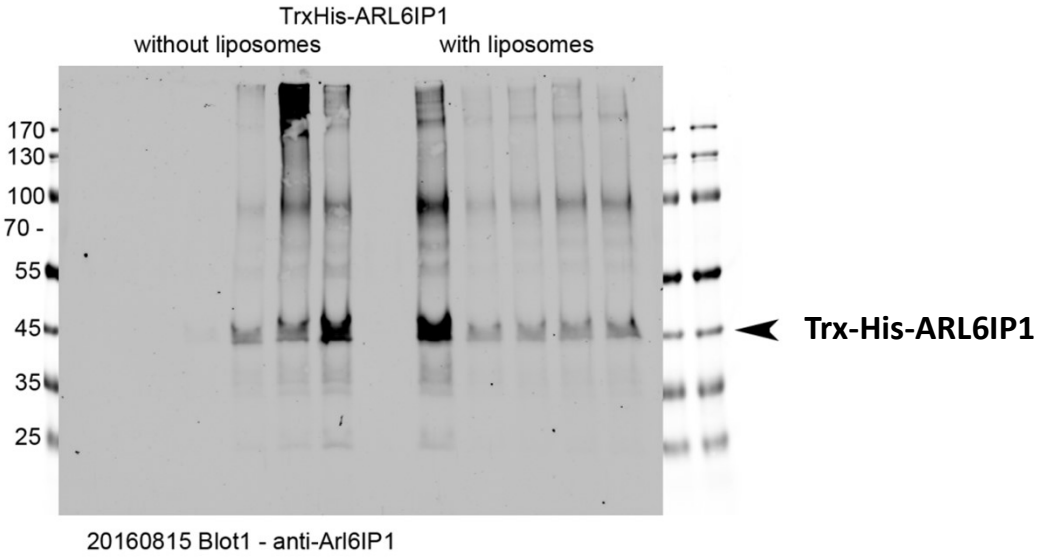

TrxHis-ARL6IP1
without liposomes
with liposomes
170
130
100
70 -
55
45
35
25
20160815 Blot1 - anti-Arl6IP1
Trx-His-ARL6IP1

FIG 2. E

INPUT

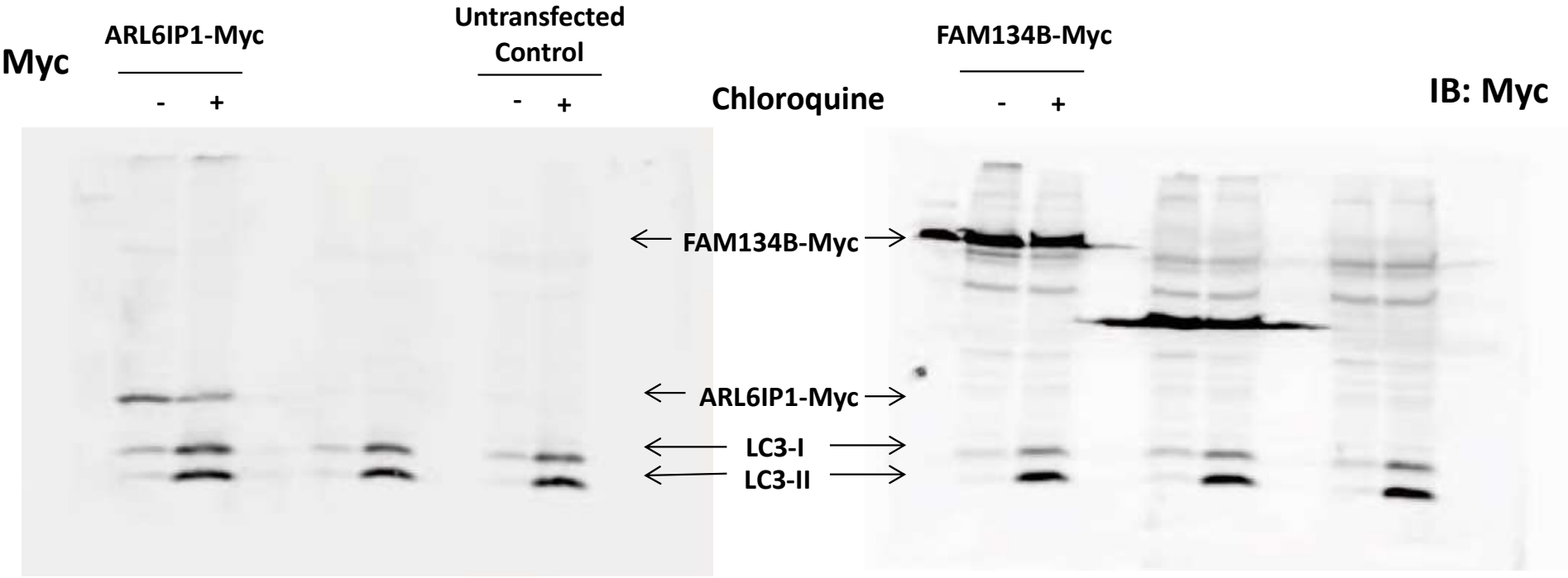

IB: Myc
ARL6IP1-Myc
- +
Untransfected Control
- +
Chloroquine
FAM134B-Myc
- +
IB: Myc
FAM134B-Myc
ARL6IP1-Myc
LC3-I
LC3-II

FIG 2. E

IP Myc

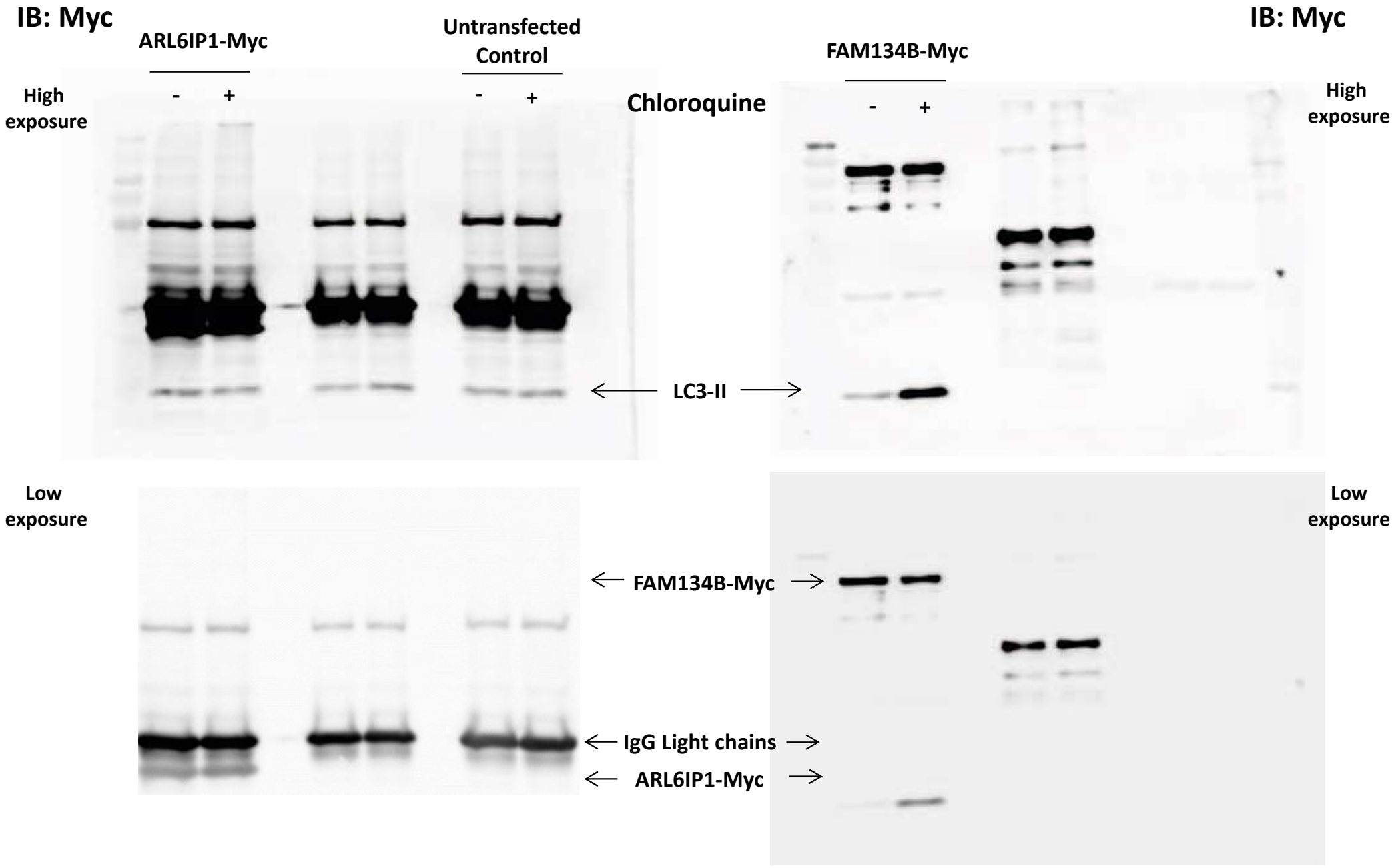

IB: Myc
ARL6IP1-Myc
Untransfected Control
High exposure
- + - +
Chloroquine
IB: Myc
FAM134B-Myc
High exposure
- +
LC3-II
Low exposure
FAM134B-Myc
IgG Light chains
ARL6IP1-Myc
Low exposure

FIG 2. G

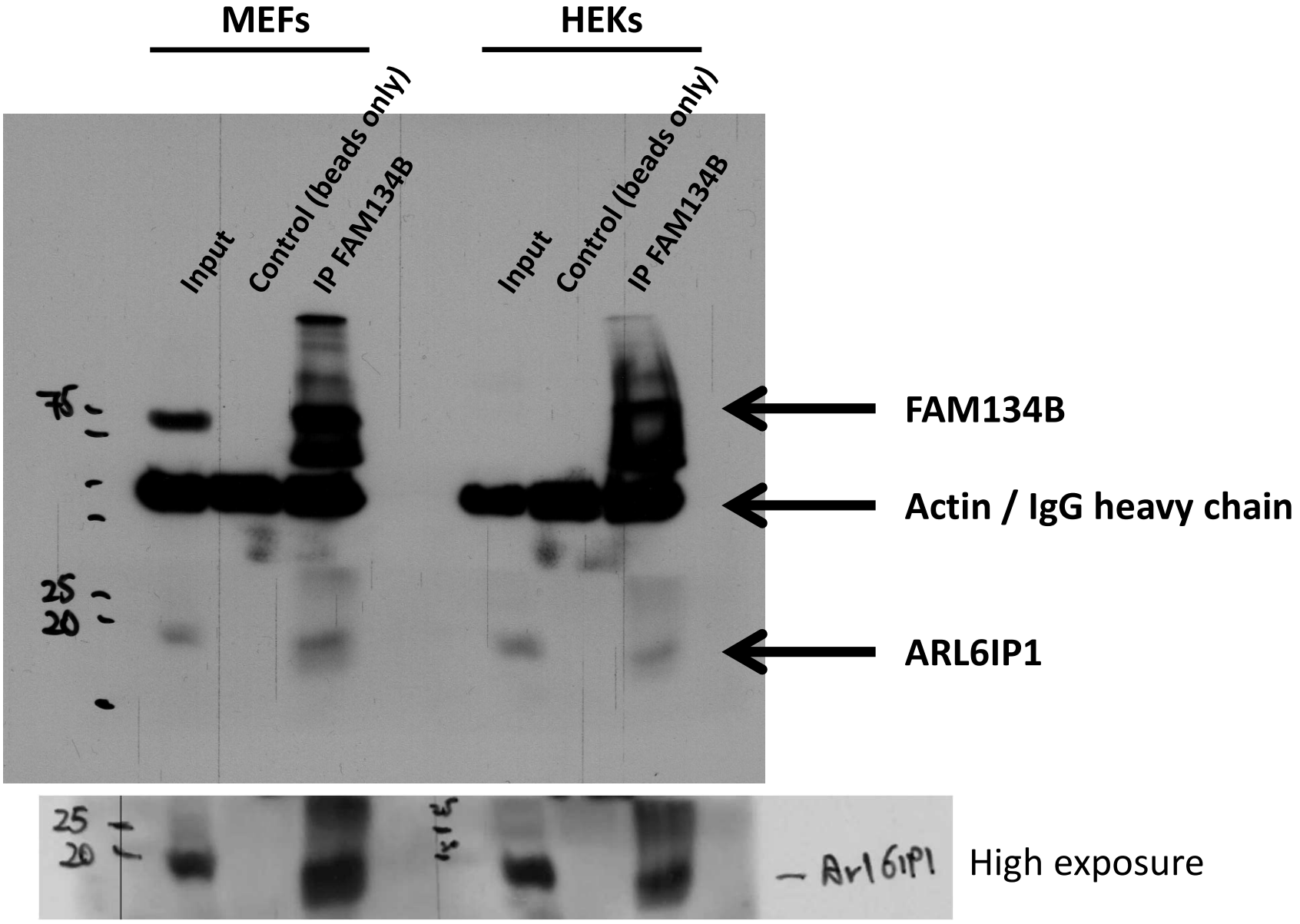
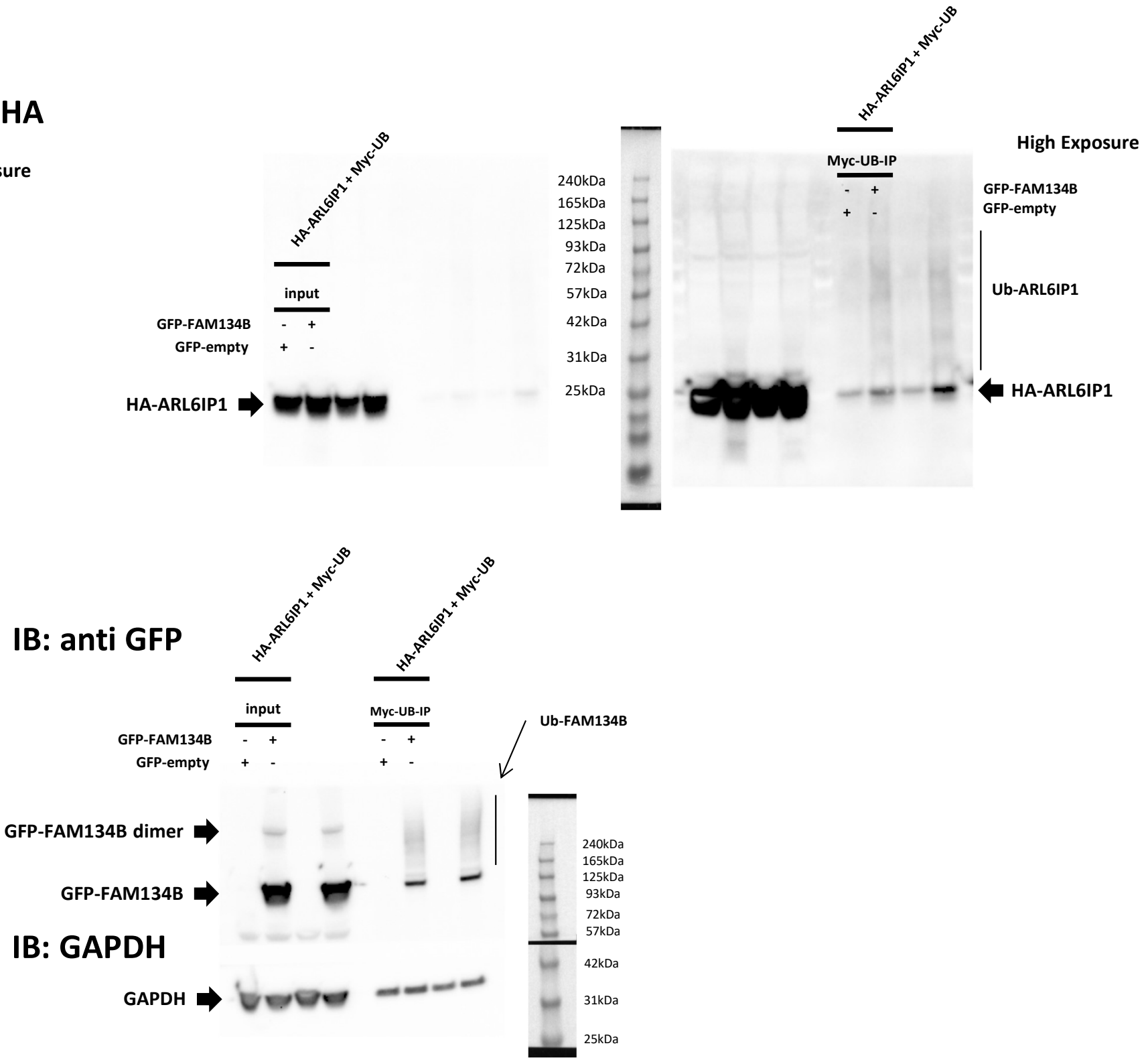
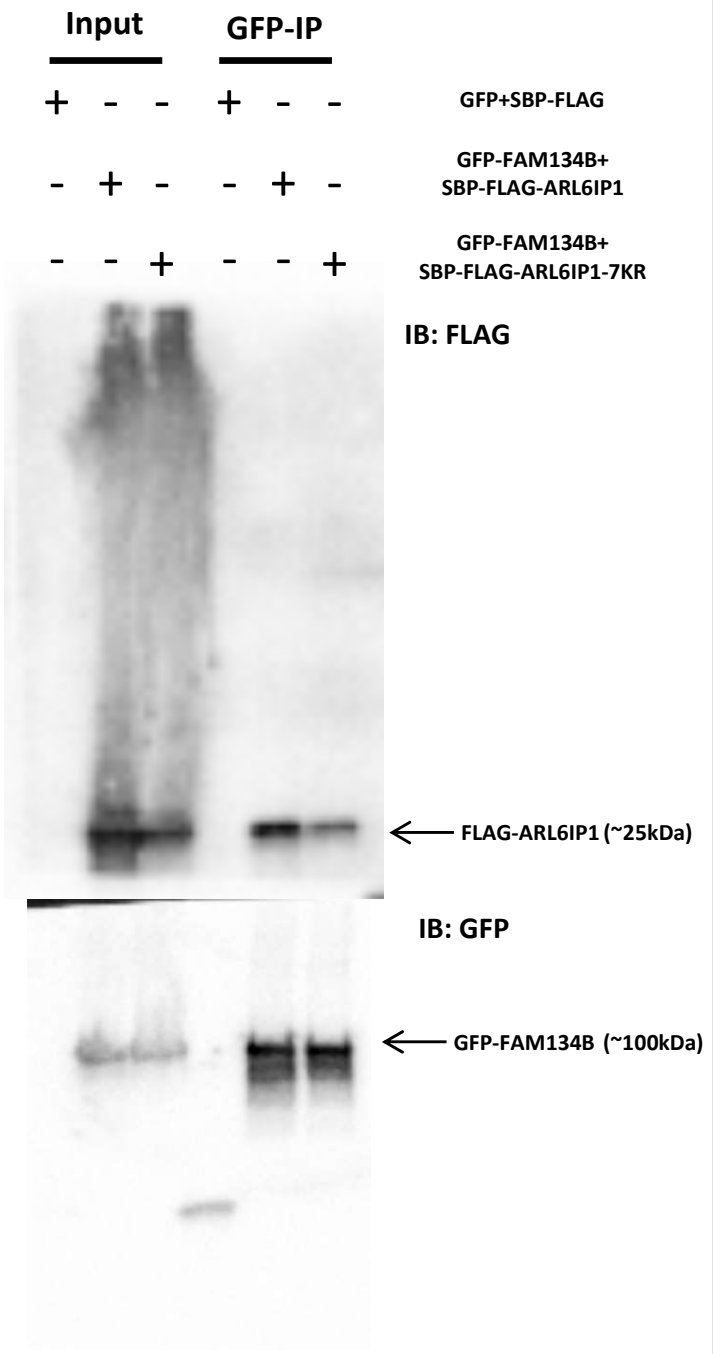
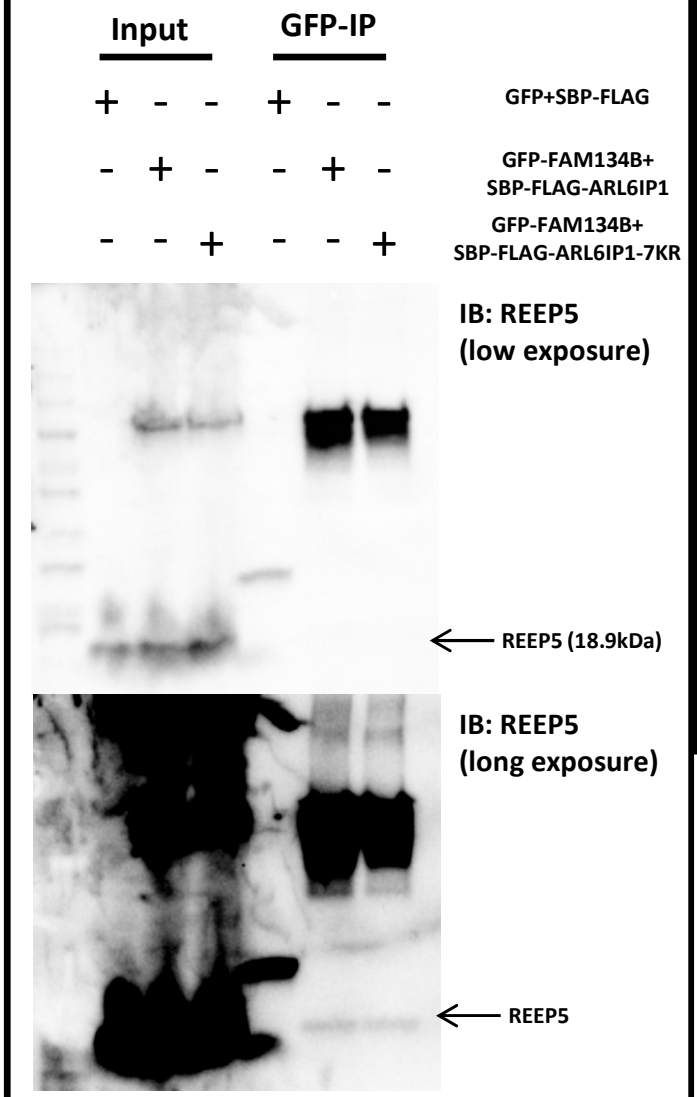

MEFs
HEKs
Input
Control (beads only)
IP FAM134B
Input
Control (beads only)
IP FAM134B
75
25
20
FAM134B
Actin / IgG heavy chain
ARL6IP1
25
20
Arl6IP1
High exposure

FIG 4. C


IB: anti HA
Low Exposure
HA-ARL6IP1 + Myc-UB
input
GFP-FAM134B - +
GFP-empty + -
HA-ARL6IP1
240kDa
165kDa
125kDa
93kDa
72kDa
57kDa
42kDa
31kDa
25kDa
HA-ARL6IP1 + Myc-UB
High Exposure
Myc-UB-IP
GFP-FAM134B
GFP-empty
Ub-ARL6IP1
HA-ARL6IP1
IB: anti GFP
HA-ARL6IP1 + Myc-UB
HA-ARL6IP1 + Myc-UB
input
Myc-UB-IP
Ub-FAM134B
GFP-FAM134B - + - +
GFP-empty + - + -
GFP-FAM134B dimer
GFP-FAM134B
IB: GAPDH
GAPDH
240kDa
165kDa
125kDa
93kDa
72kDa
57kDa
42kDa
31kDa
25kDa


Input
GFP-IP
+ - - + - -
GFP+SBP-FLAG
- + - - + -
GFP-FAM134B+
SBP-FLAG-ARL6IP1
- - + - - +
GFP-FAM134B+
SBP-FLAG-ARL6IP1-7KR
IB: FLAG
FLAG-ARL6IP1 (~25kDa)
IB: GFP
GFP-FAM134B (~100kDa)


Input
GFP-IP
+ - - + - -
GFP+SBP-FLAG
- + - - + -
GFP-FAM134B+
SBP-FLAG-ARL6IP1
- - + - - +
GFP-FAM134B+
SBP-FLAG-ARL6IP1-7KR
IB: REEP5
(low exposure)
REEP5 (18.9kDa)
IB: REEP5
(long exposure)
REEP5

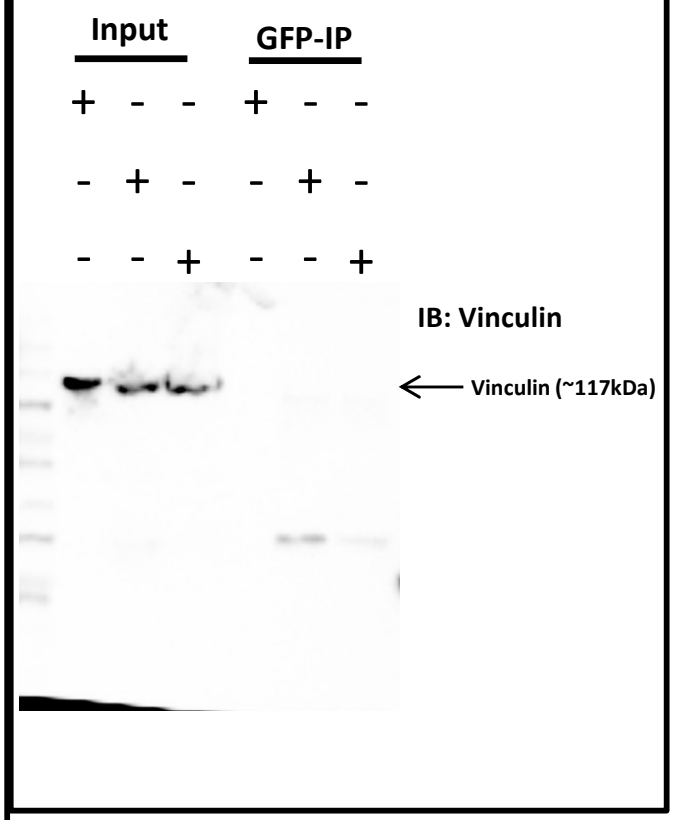
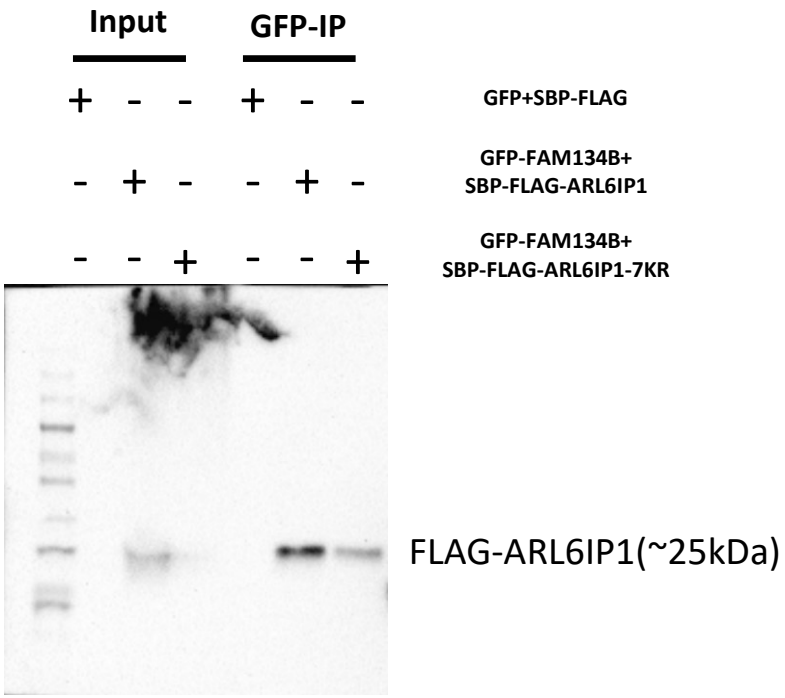

Input
GFP-IP
+ - - + - -
- + - - + -
- - + - - +
IB: Vinculin
Vinculin (~117kDa)

FIG 4. E

n=1

FIG 4. E

n=2


Input
GFP-IP
+ - - + - -
GFP+SBP-FLAG
- + - - + -
GFP-FAM134B+
SBP-FLAG-ARL6IP1
- - + - - +
GFP-FAM134B+
SBP-FLAG-ARL6IP1-7KR
FLAG-ARL6IP1(~25kDa)

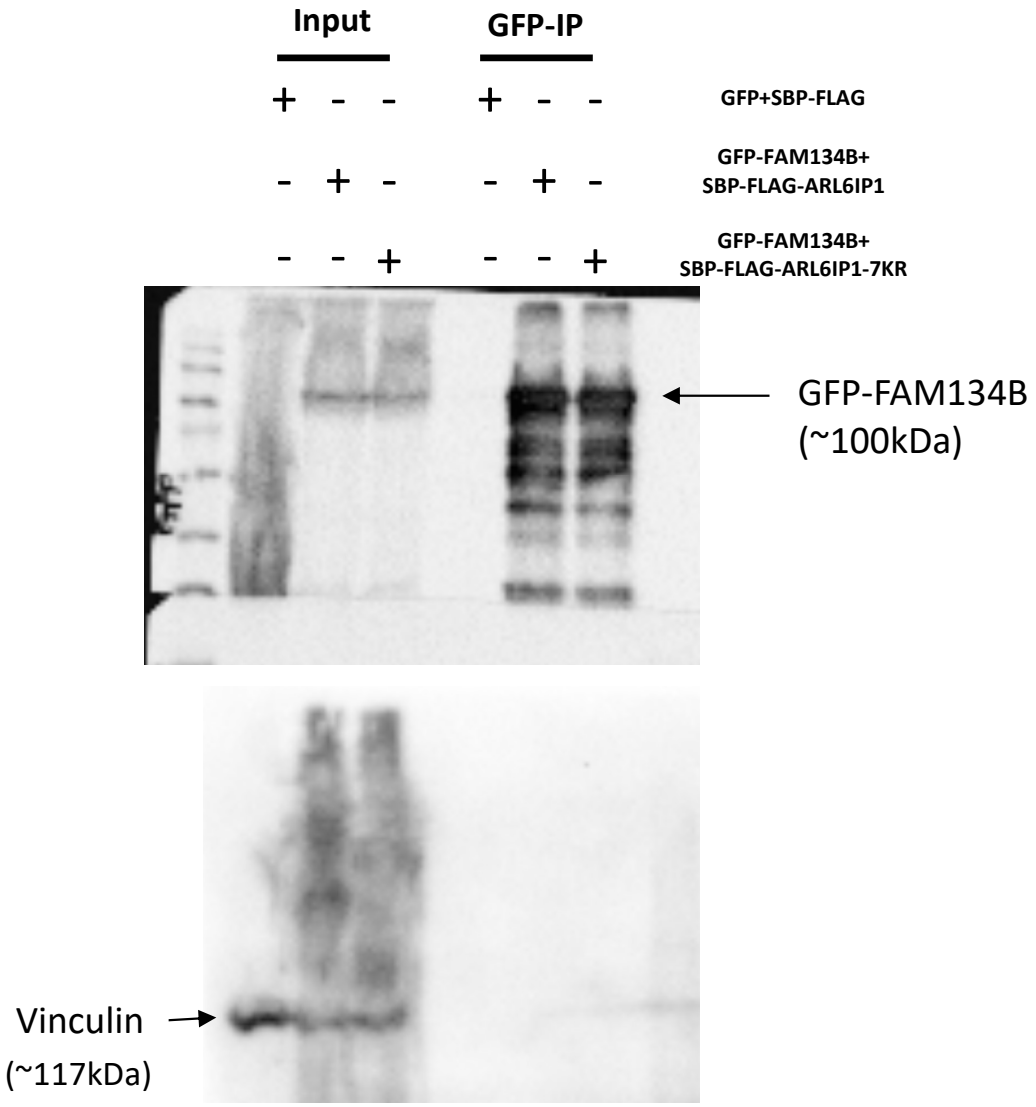

Input
GFP-IP
+ - - + - -
GFP+SBP-FLAG
- + - - + -
GFP-FAM134B+
SBP-FLAG-ARL6IP1
- - + - - +
GFP-FAM134B+
SBP-FLAG-ARL6IP1-7KR
GFP-FAM134B
(~100kDa)
Vinculin
(~117kDa)

FIG 4. G

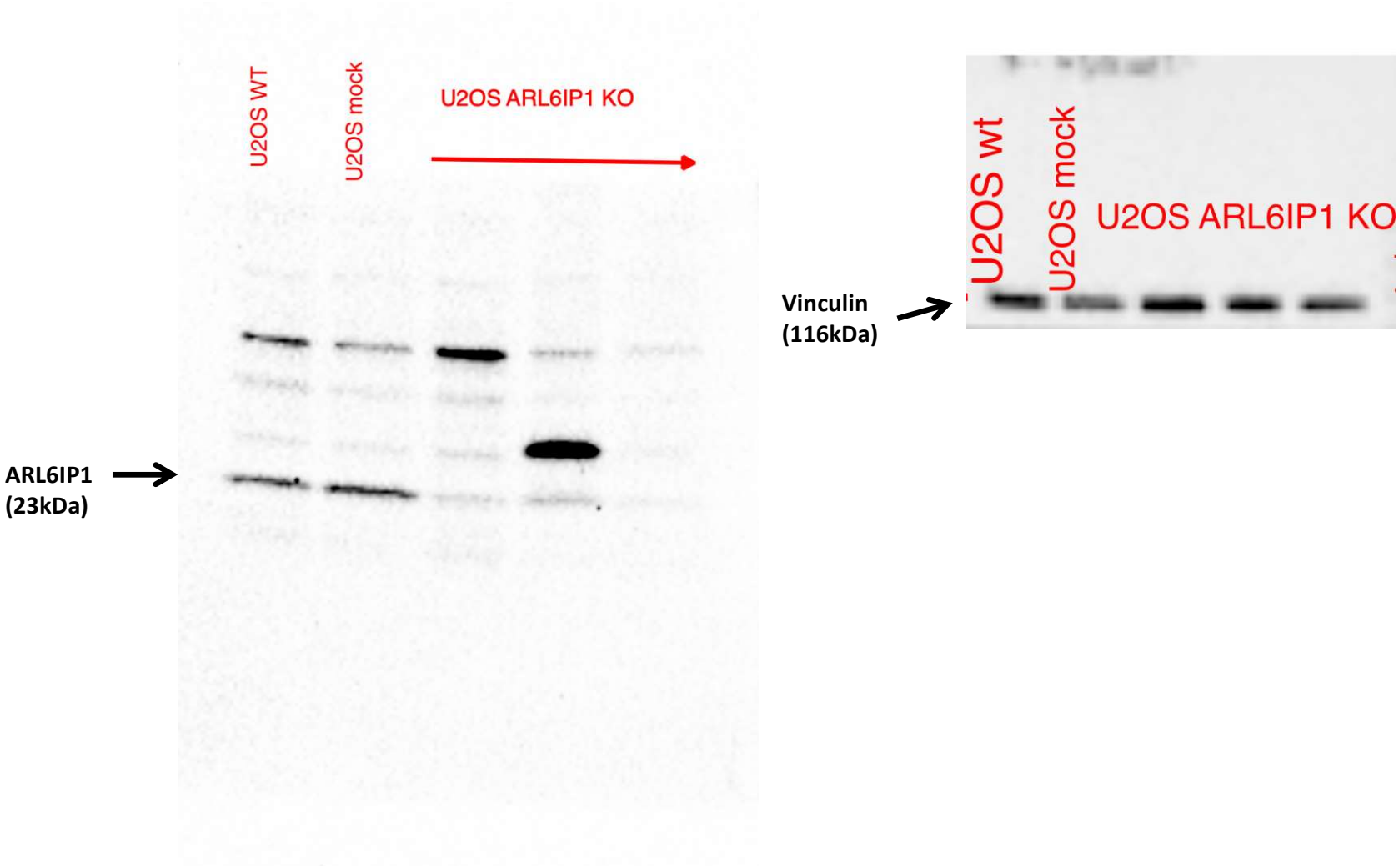
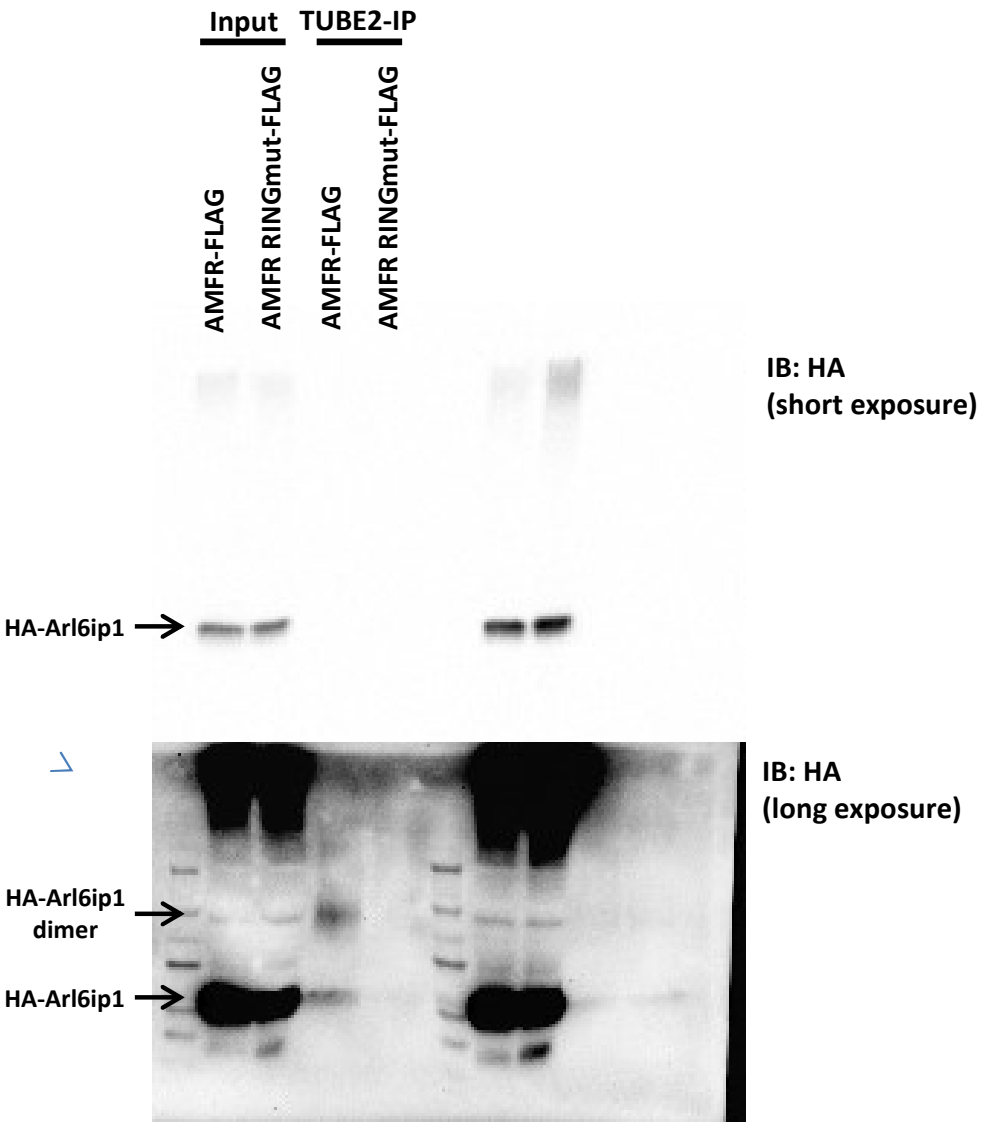

U2OS WT
U2OS mock
U2OS ARL6IP1 KO
ARL6IP1
(23kDa)
Vinculin
(116kDa)
U2OS wt
U2OS mock
U2OS ARL6IP1 KO

FIG 4. H


Input
TUBE2-IP
AMFR-FLAG
AMFR RINGmut-FLAG
AMFR-FLAG
AMFR RINGmut-FLAG
IB: HA
(short exposure)
HA-Arl6ip1
IB: HA
(long exposure)
HA-Arl6ip1
dimer
HA-Arl6ip1

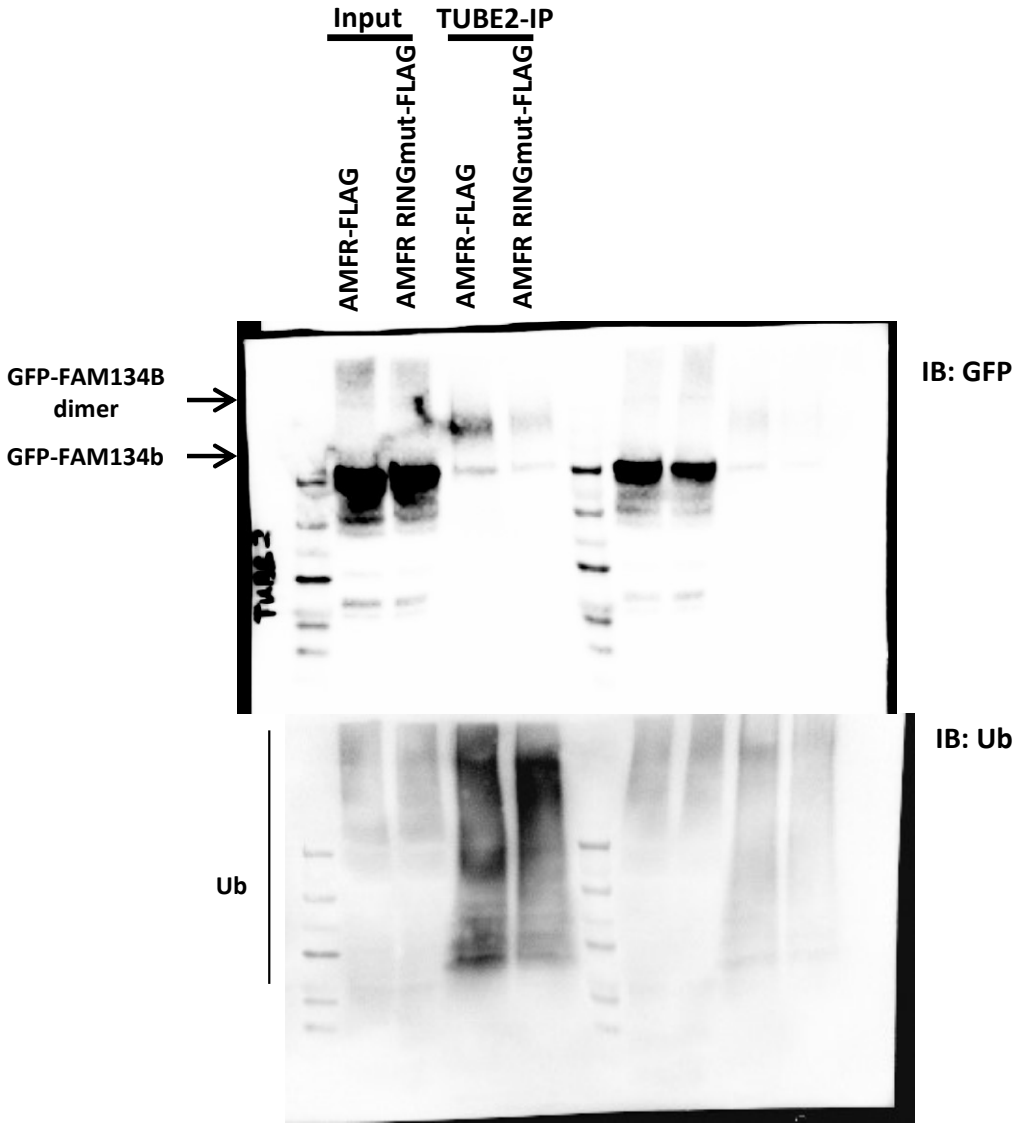

Input
TUBE2-IP
AMFR-FLAG
AMFR RINGmut-FLAG
AMFR-FLAG
AMFR RINGmut-FLAG
GFP-FAM134B
dimer
GFP-FAM134b
IB: GFP
IB: Ub
Ub

FIG 4. H

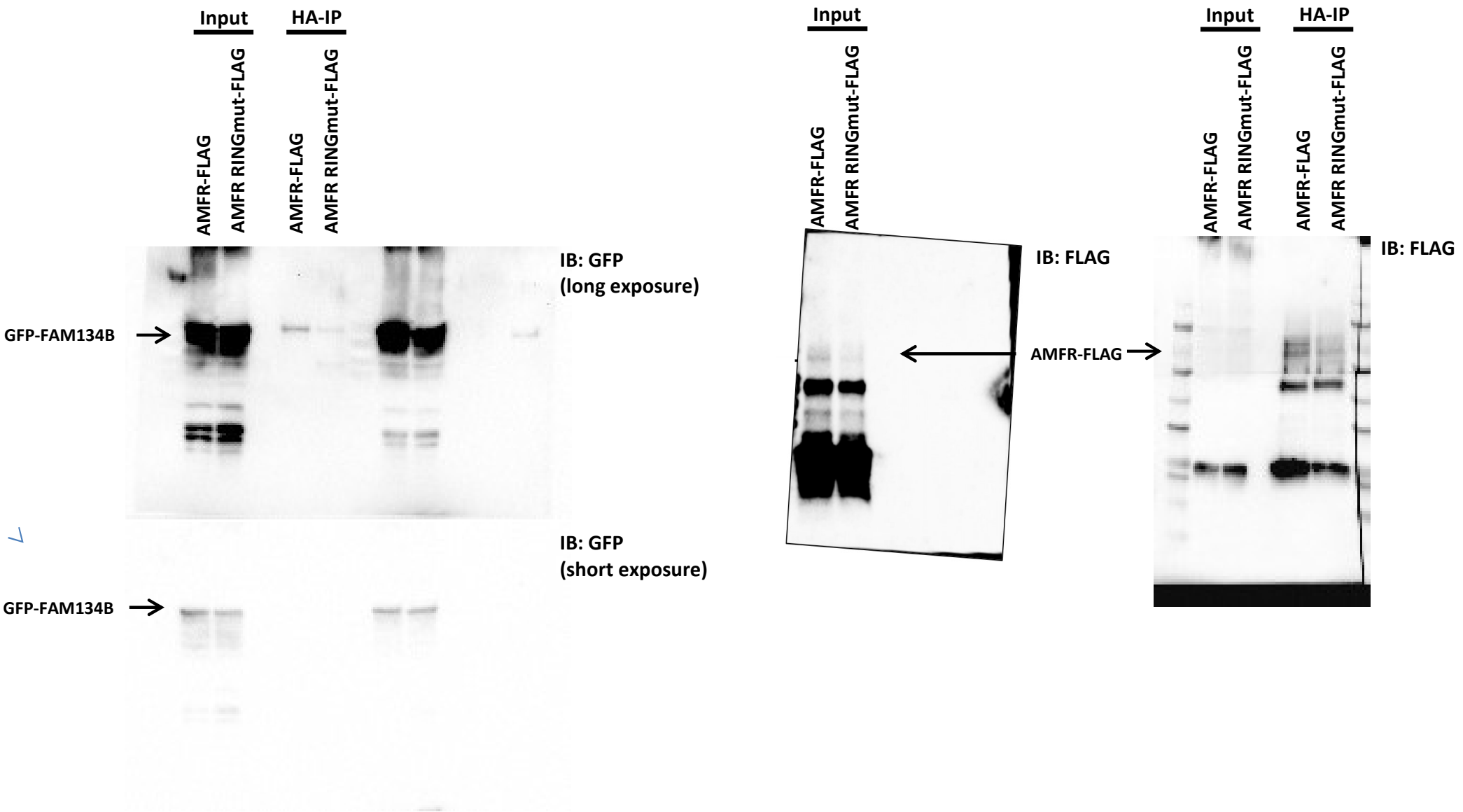
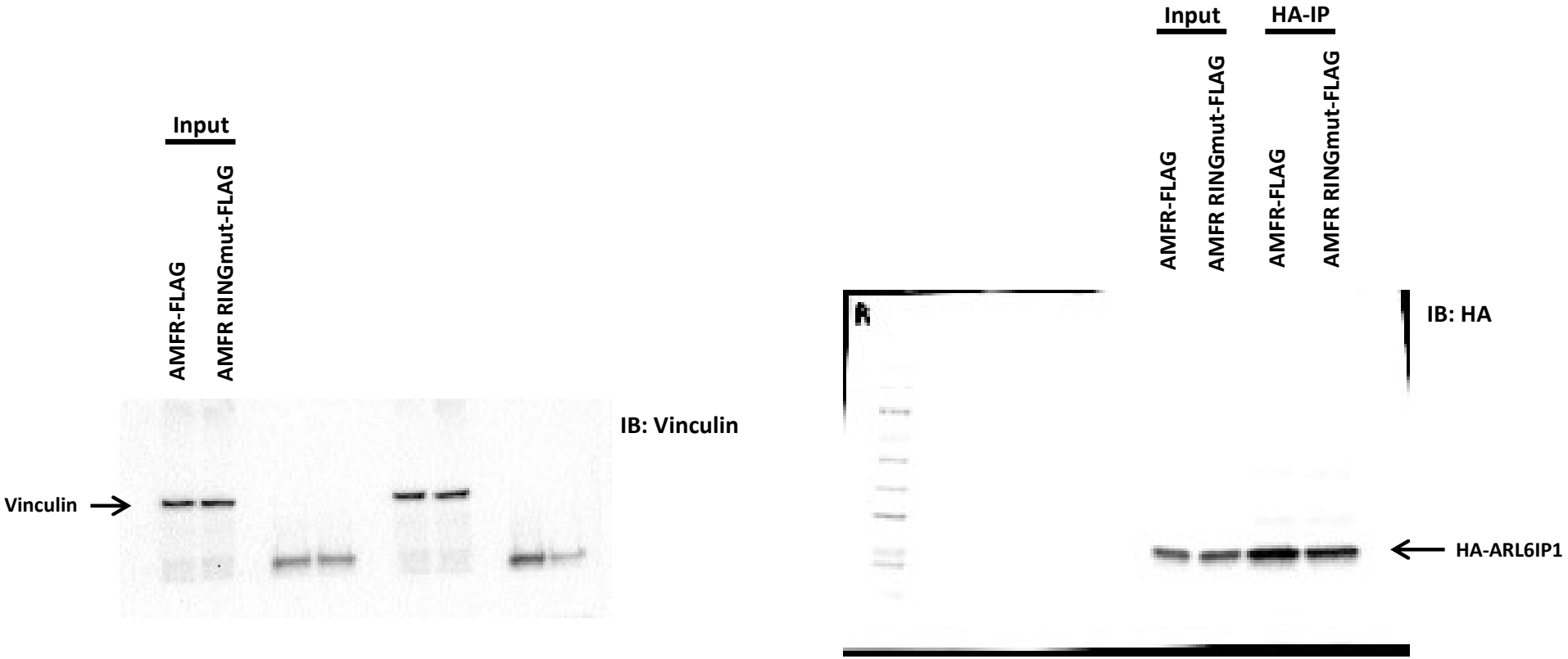

Input
HA-IP
AMFR-FLAG
AMFR RINGmut-FLAG
AMFR-FLAG
AMFR RINGmut-FLAG
IB: GFP
(long exposure)
GFP-FAM134B
IB: GFP
(short exposure)
GFP-FAM134B
Input
AMFR-FLAG
AMFR RINGmut-FLAG
IB: FLAG
AMFR-FLAG
Input
HA-IP
AMFR-FLAG
AMFR RINGmut-FLAG
AMFR-FLAG
AMFR RINGmut-FLAG
IB: FLAG

FIG 4. H


Input
AMFR-FLAG
AMFR RINGmut-FLAG
IB: Vinculin
Vinculin
Input
HA-IP
AMFR-FLAG
AMFR RINGmut-FLAG
AMFR-FLAG
AMFR RINGmut-FLAG
IB: HA
HA-ARL6IP1

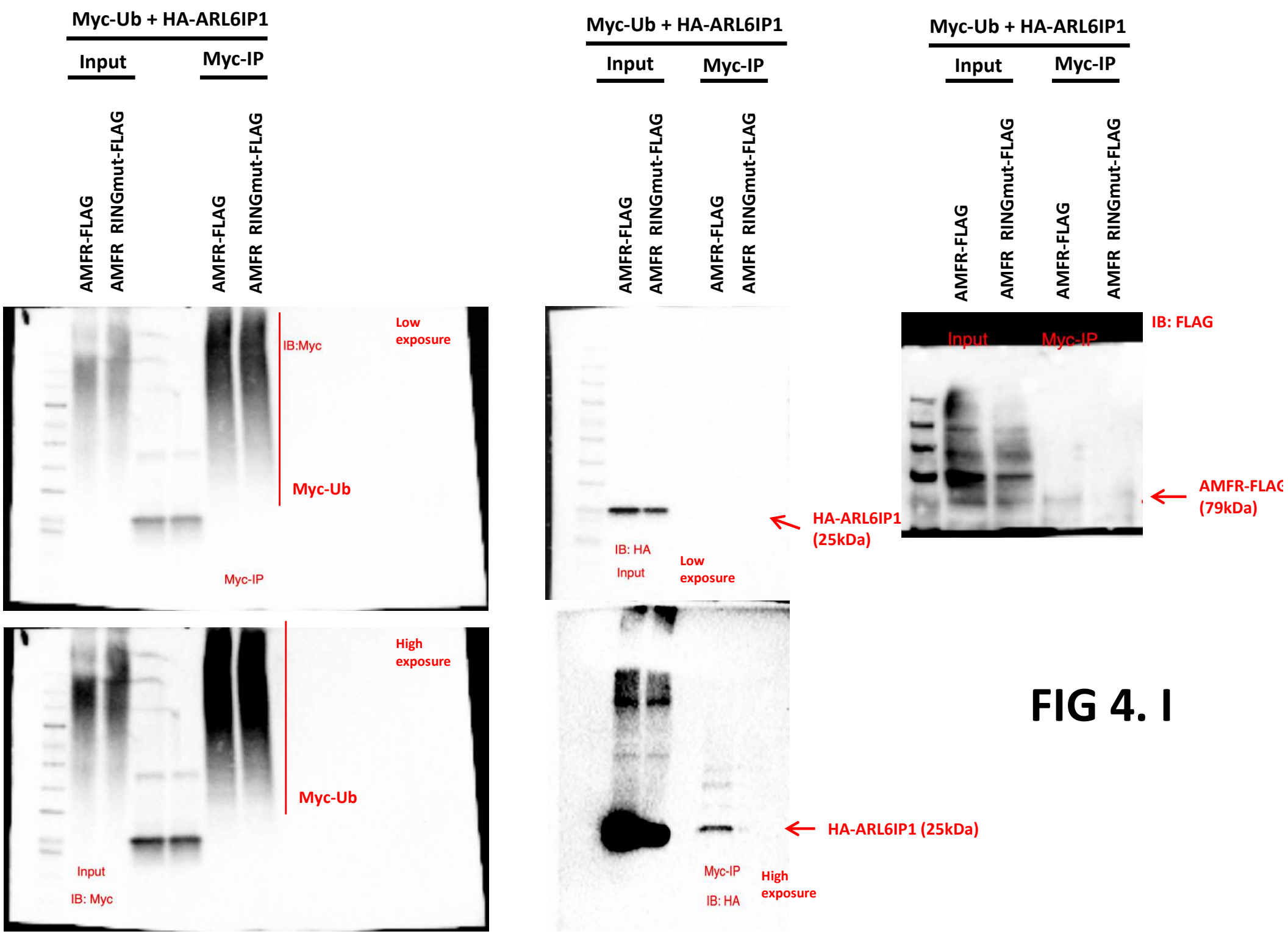
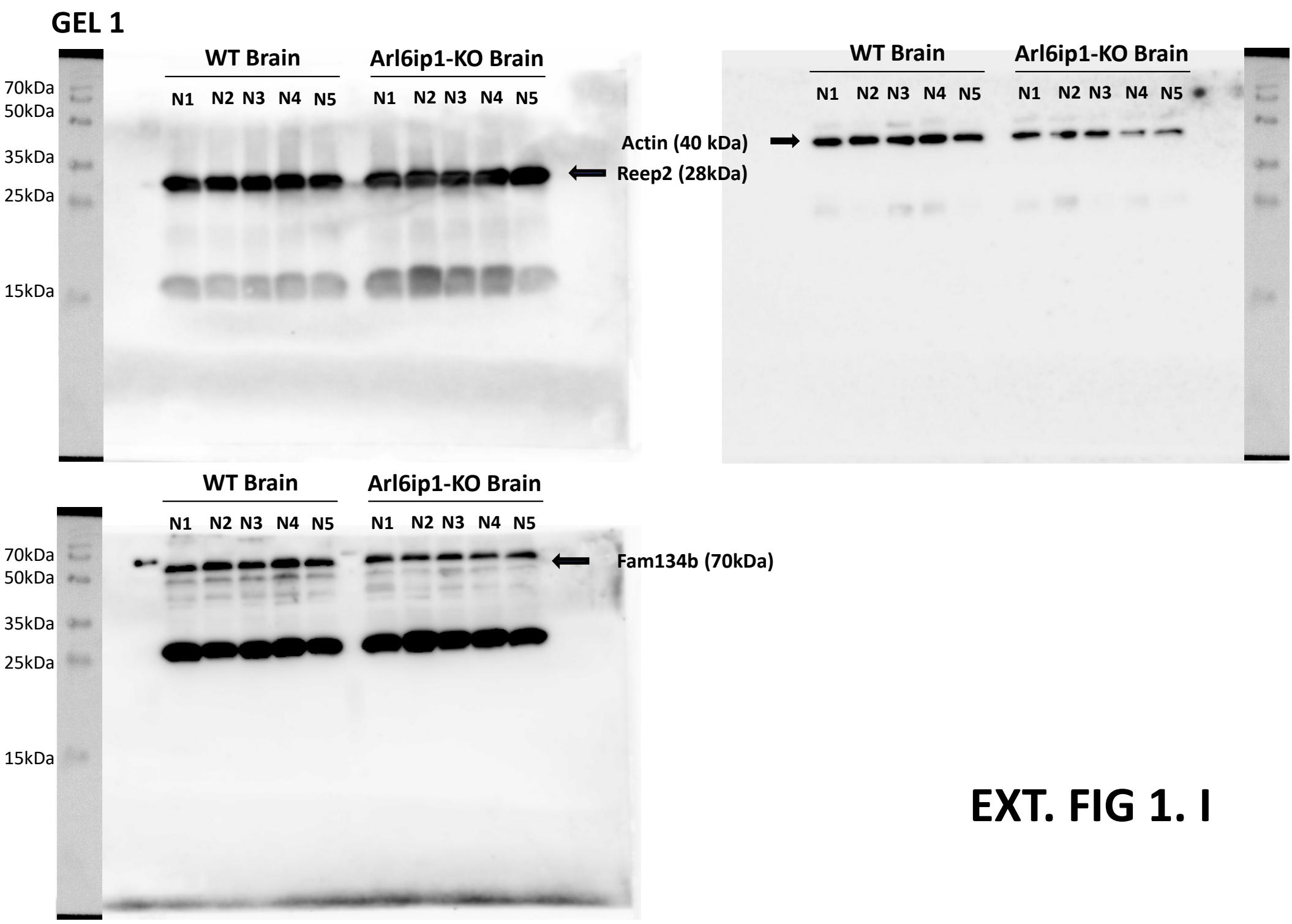
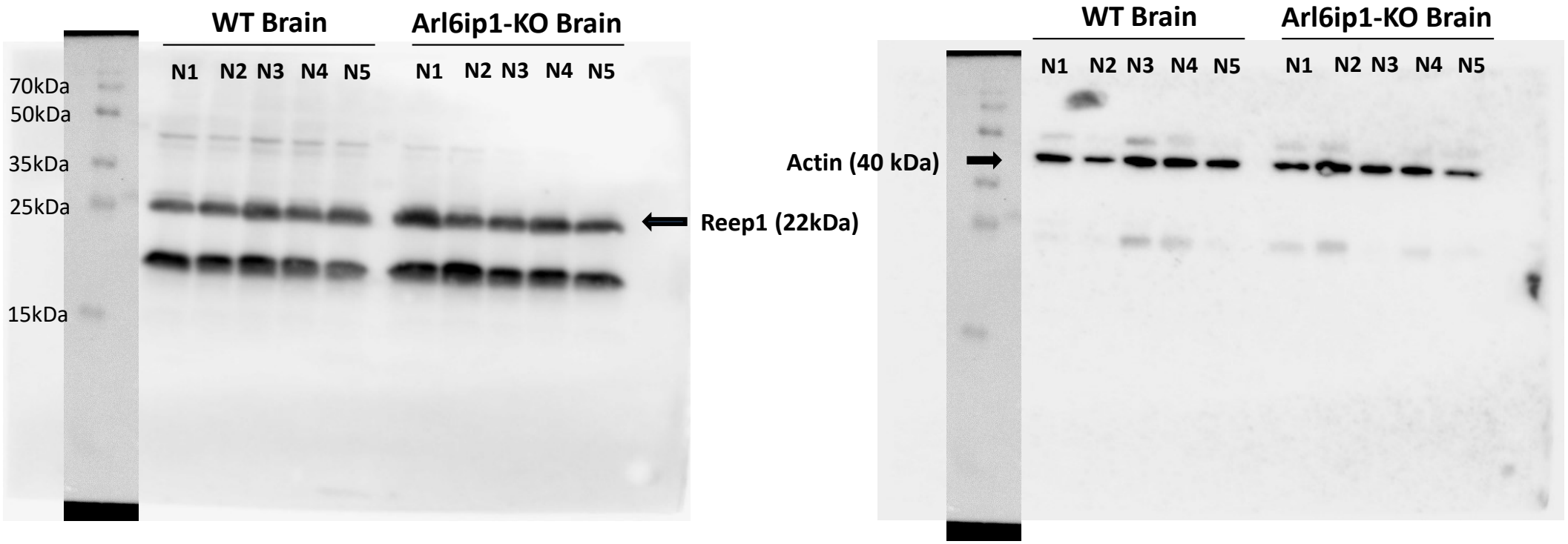

Myc-Ub + HA-ARL6IP1
Input
Myc-IP
AMFR-FLAG
AMFR RINGmut-FLAG
IB:Myc
Low exposure
Myc-Ub
Myc-IP
High exposure
Input
IB: Myc
Myc-Ub + HA-ARL6IP1
IB: HA
Low exposure
HA-ARL6IP1 (25kDa)
Myc-IP
High exposure
Myc-Ub + HA-ARL6IP1
IB: FLAG
AMFR-FLAG (79kDa)

FIG 4. I


GEL 1
WT Brain
Arl6ip1-KO Brain
N1 N2 N3 N4 N5
N1 N2 N3 N4 N5
70kDa
50kDa
35kDa
25kDa
15kDa
Reep2 (28kDa)
Actin (40 kDa)
Fam134b (70kDa)

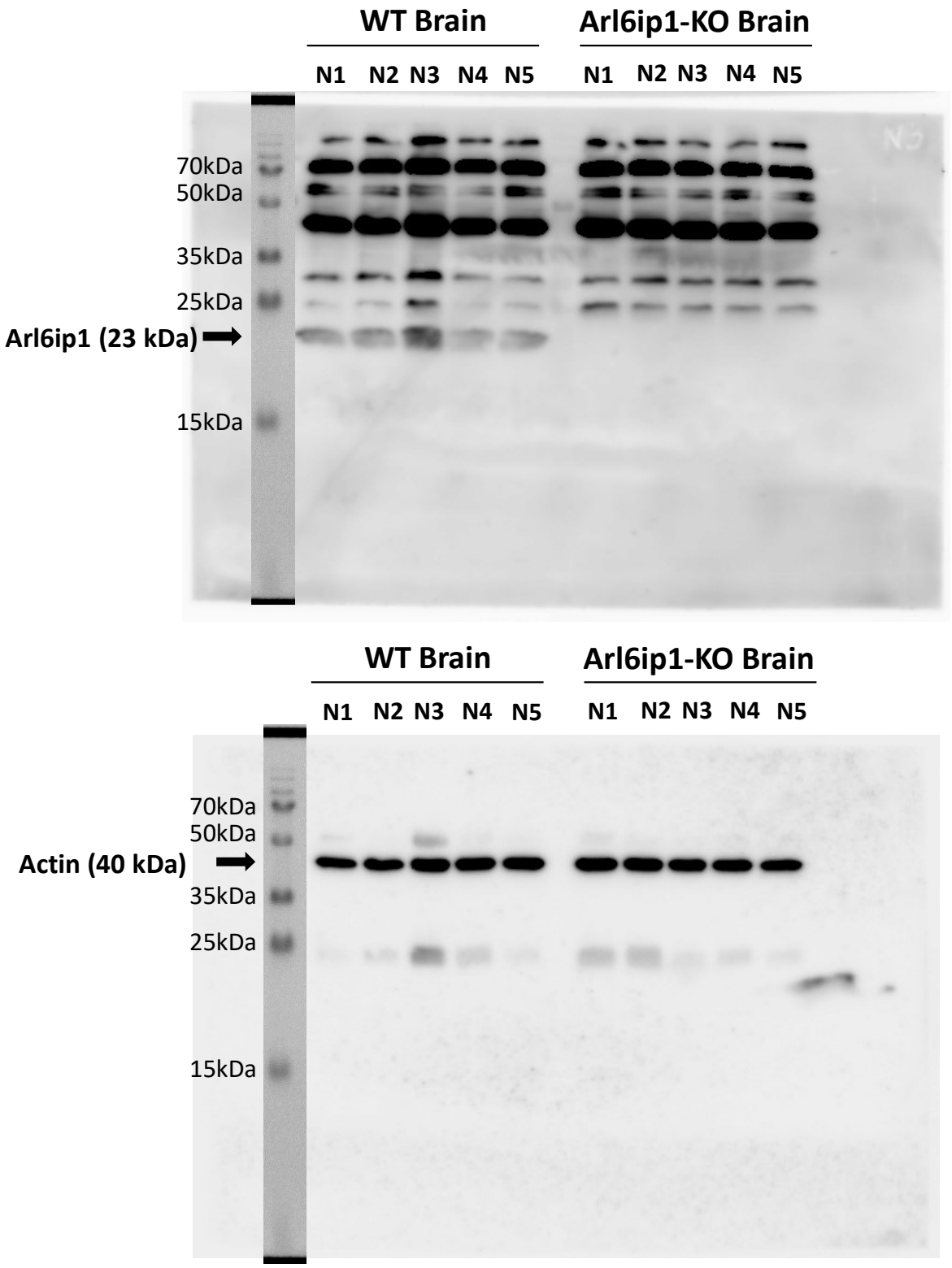
EXT. FIG 1. I

GEL 2

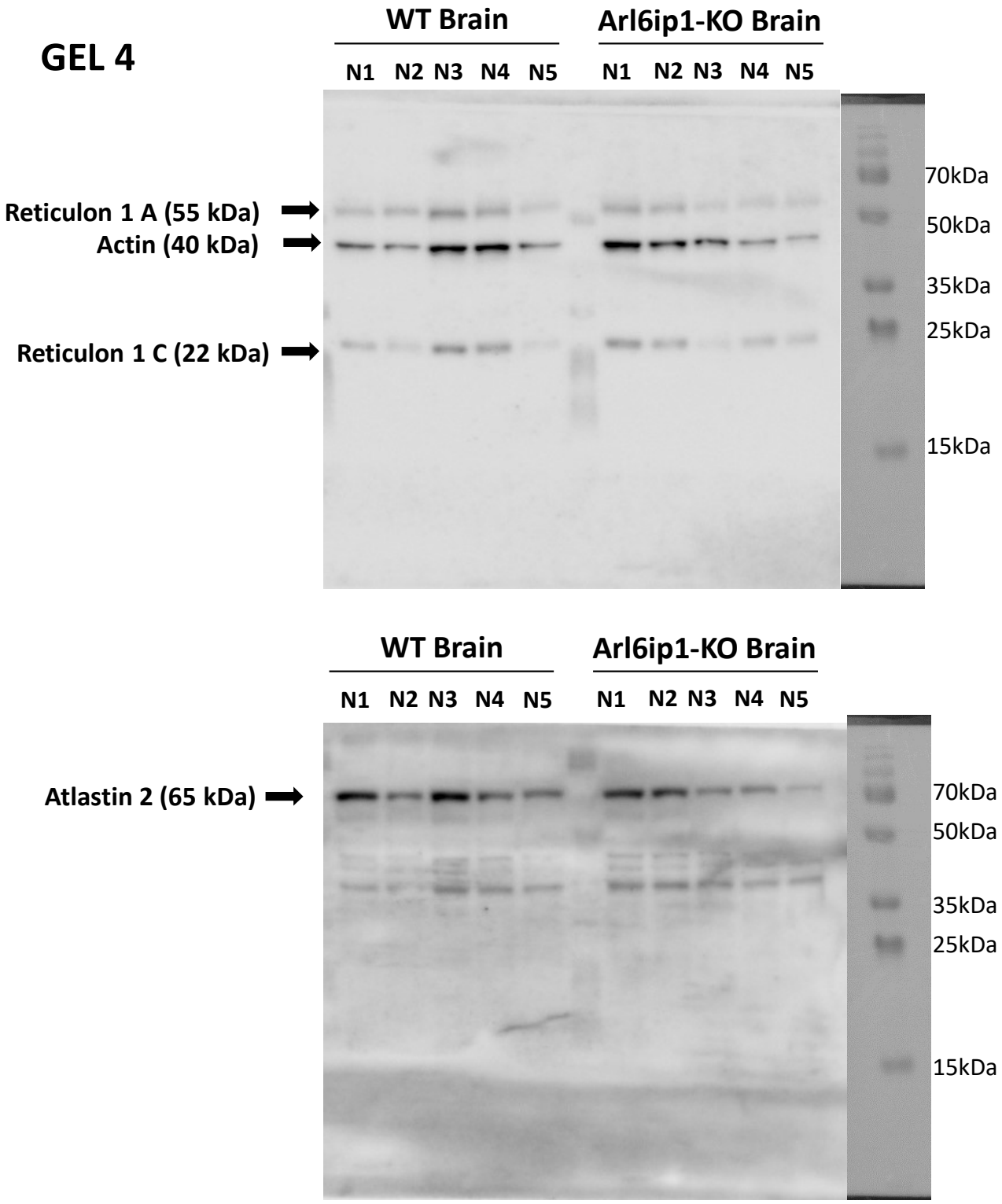


EXT. FIG 1. I

GEL 3

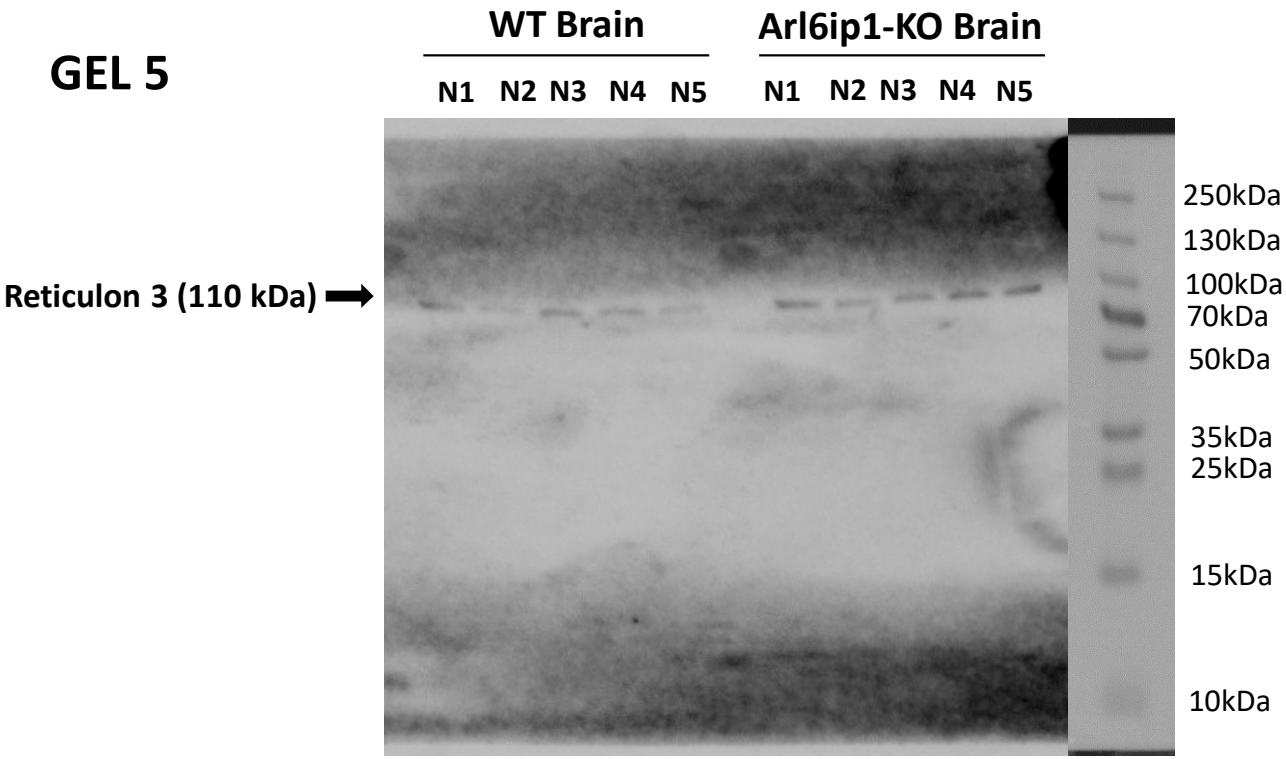


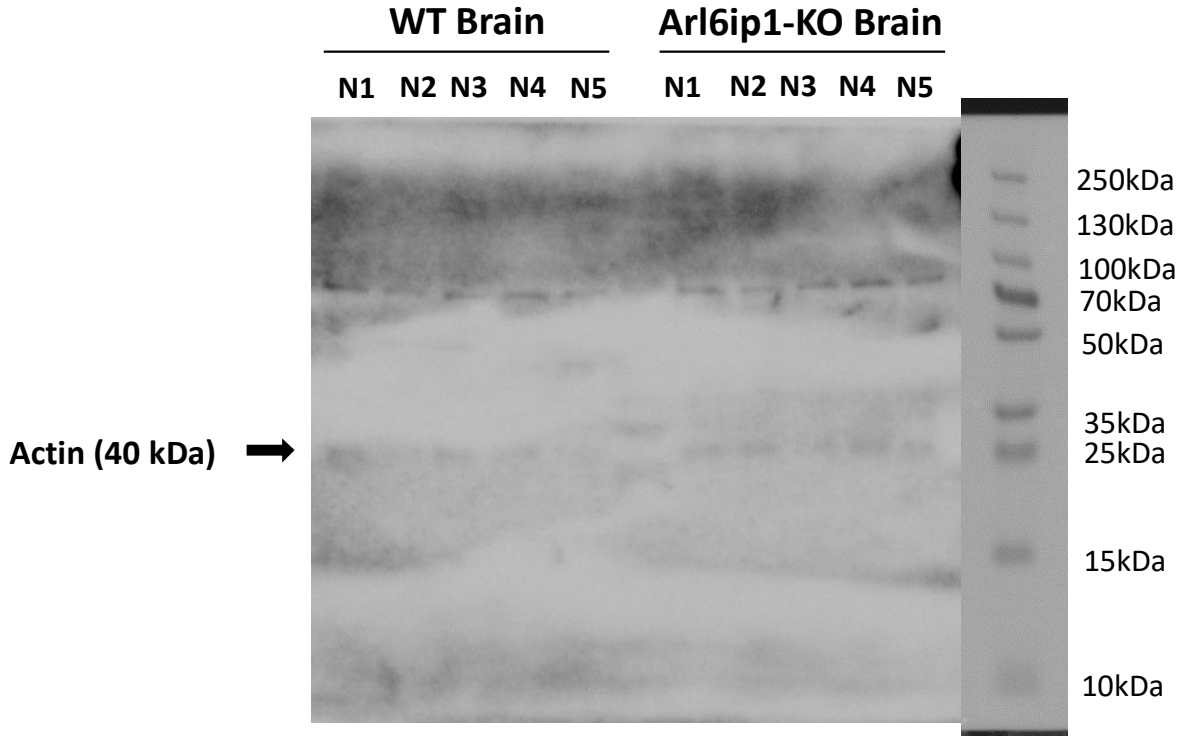
EXT. FIG 1. I


GEL 4
WT Brain
Arl6ip1-KO Brain
N1 N2 N3 N4 N5
N1 N2 N3 N4 N5
Reticulon 1 A (55 kDa)
Actin (40 kDa)
Reticulon 1 C (22 kDa)
70kDa
50kDa
35kDa
25kDa
15kDa
WT Brain
Arl6ip1-KO Brain
N1 N2 N3 N4 N5
N1 N2 N3 N4 N5
Atlastin 2 (65 kDa)
70kDa
50kDa
35kDa
25kDa
15kDa

EXT. FIG 1. I

GEL 5

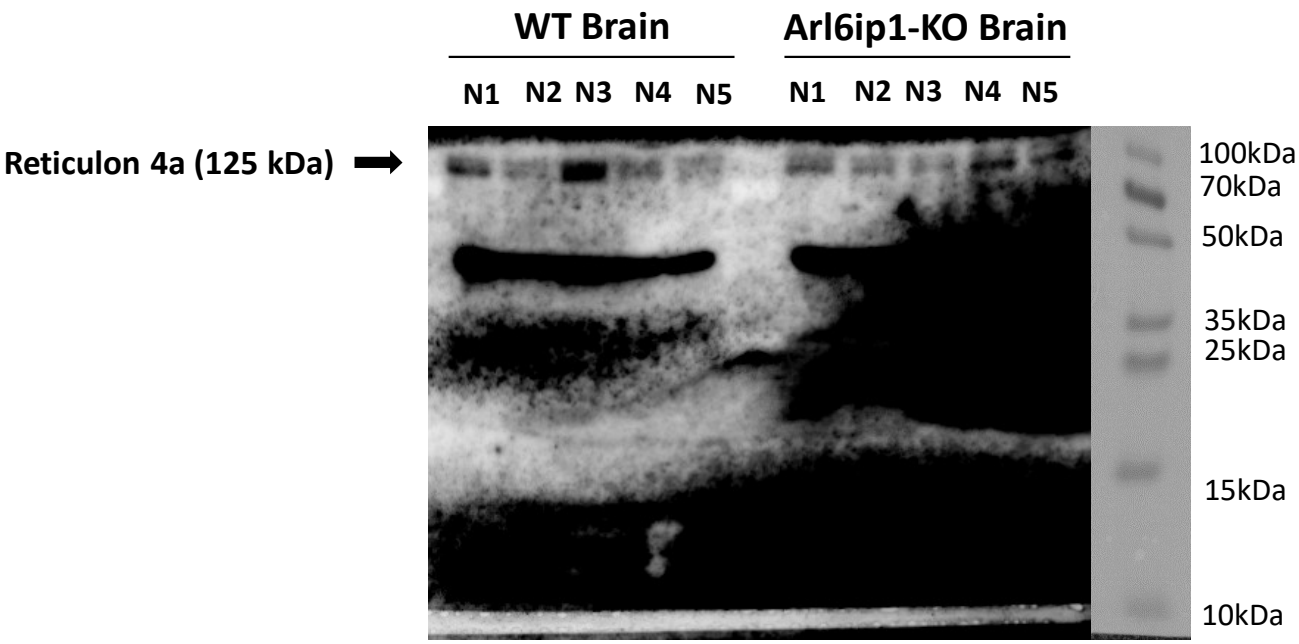




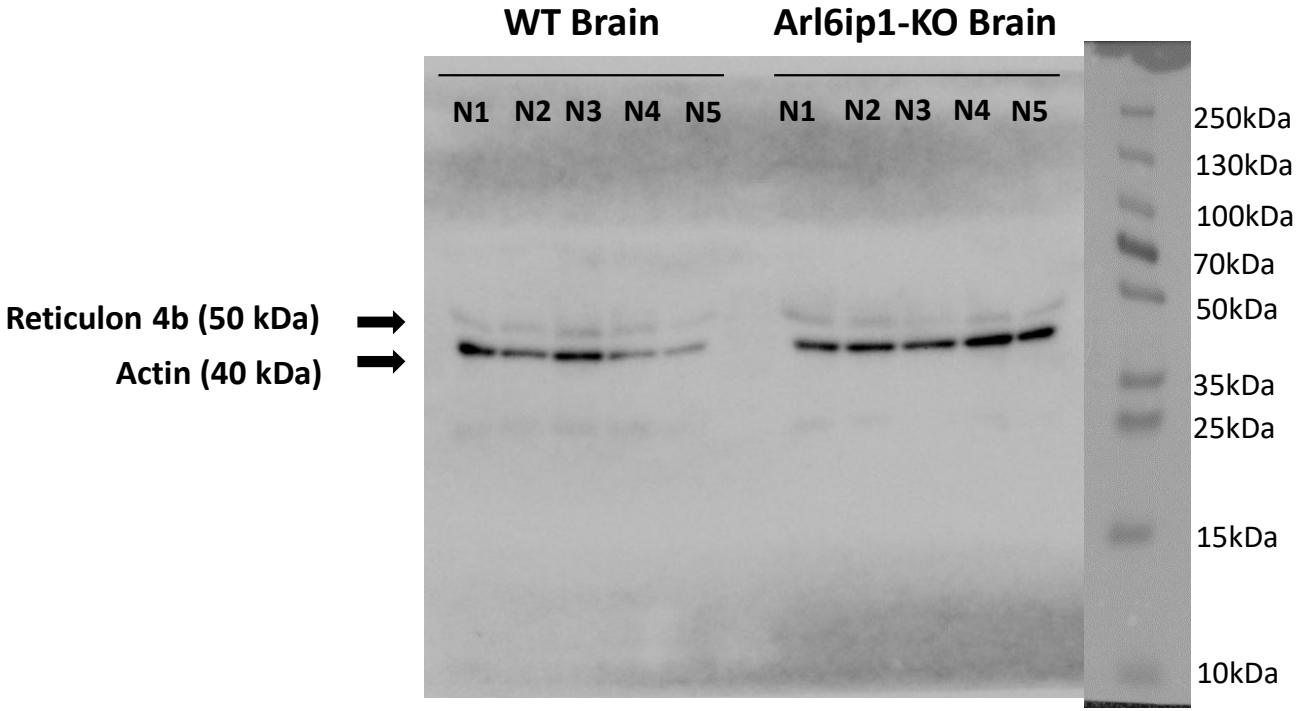
EXT. FIG 1. I

GEL 6

High exposure

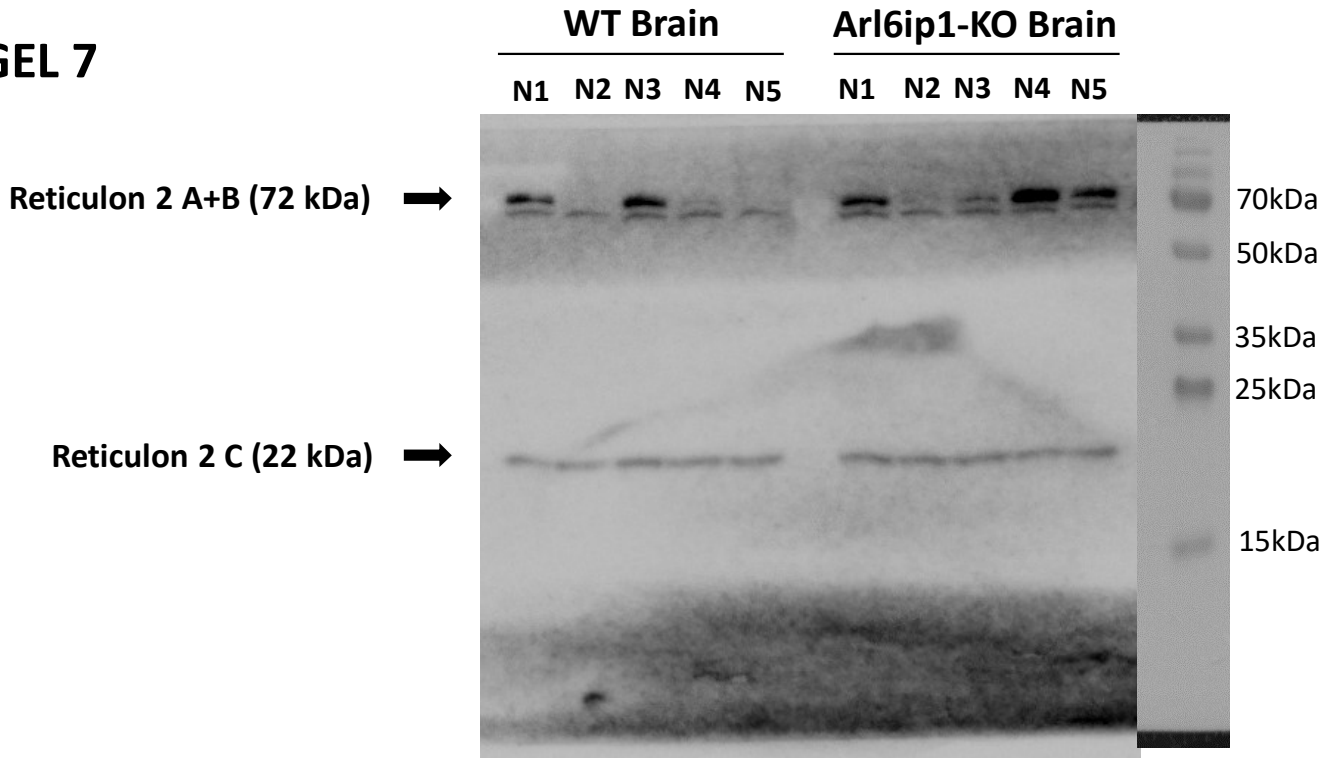


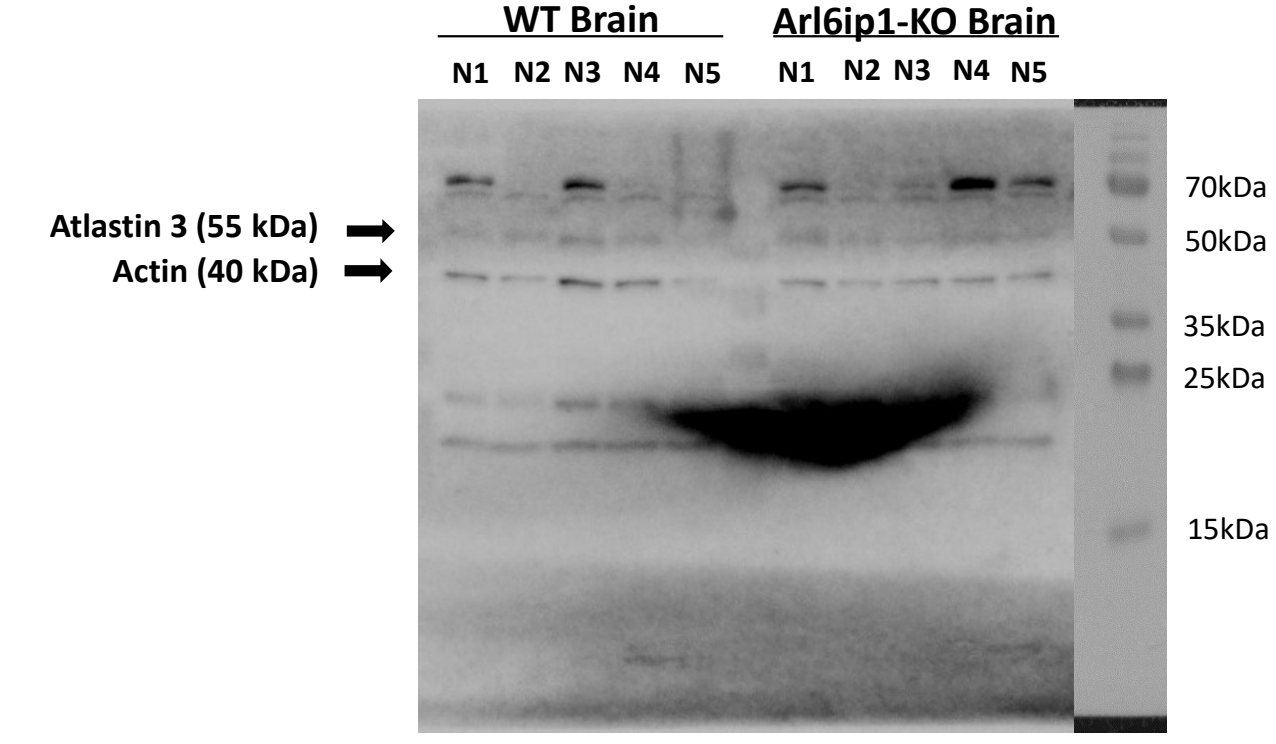
Low exposure



EXT. FIG 1. I

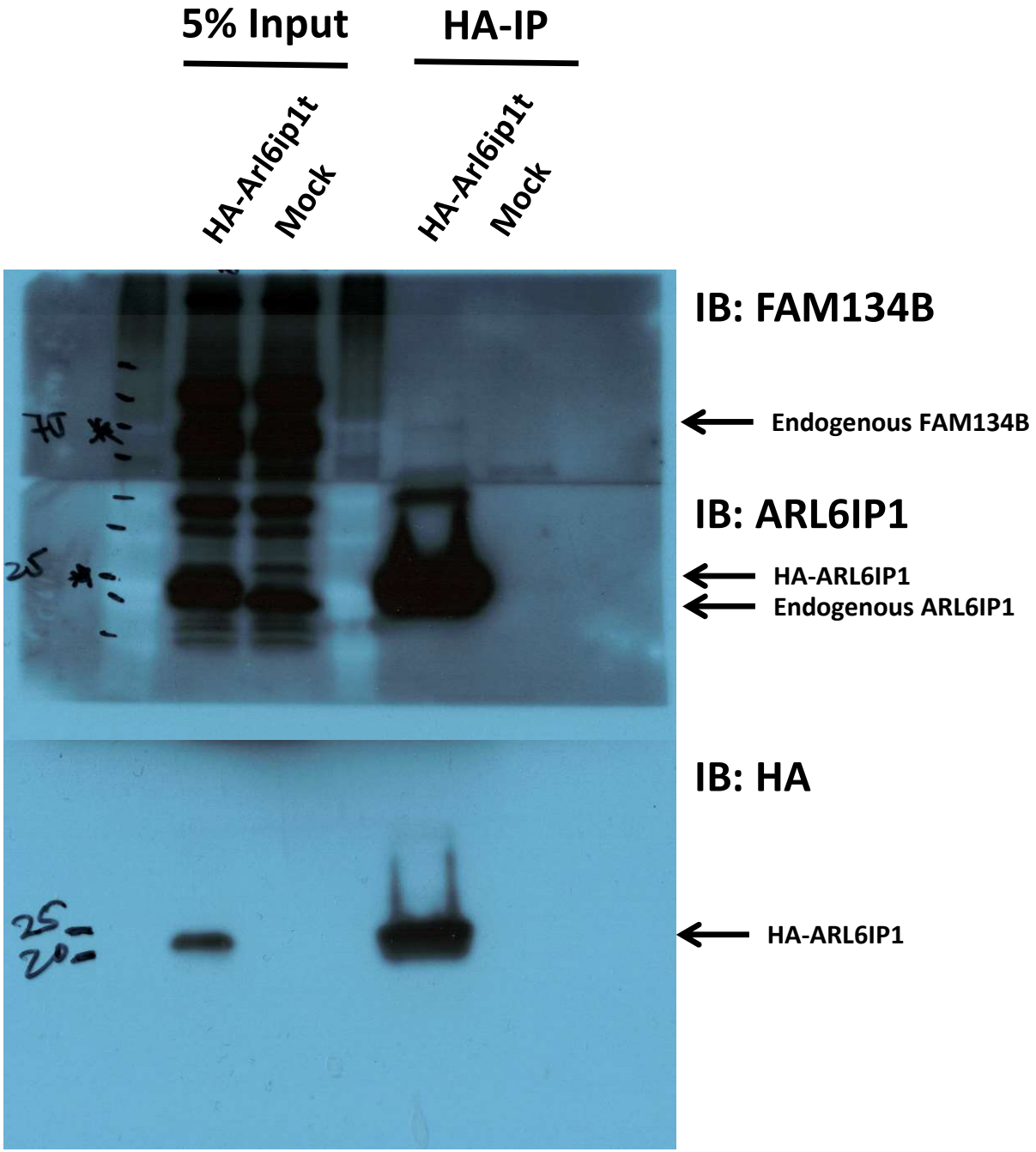
GEL 7



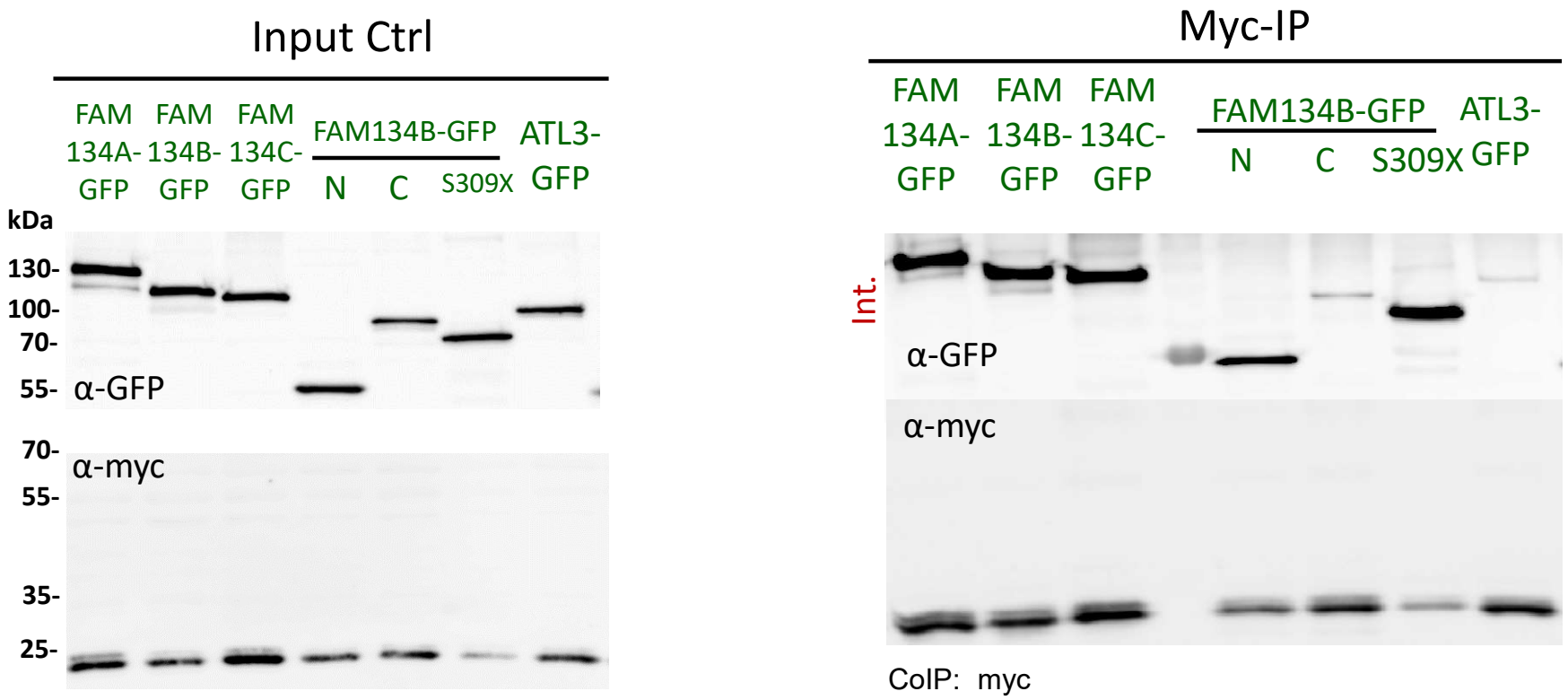


EXT. FIG 1. I

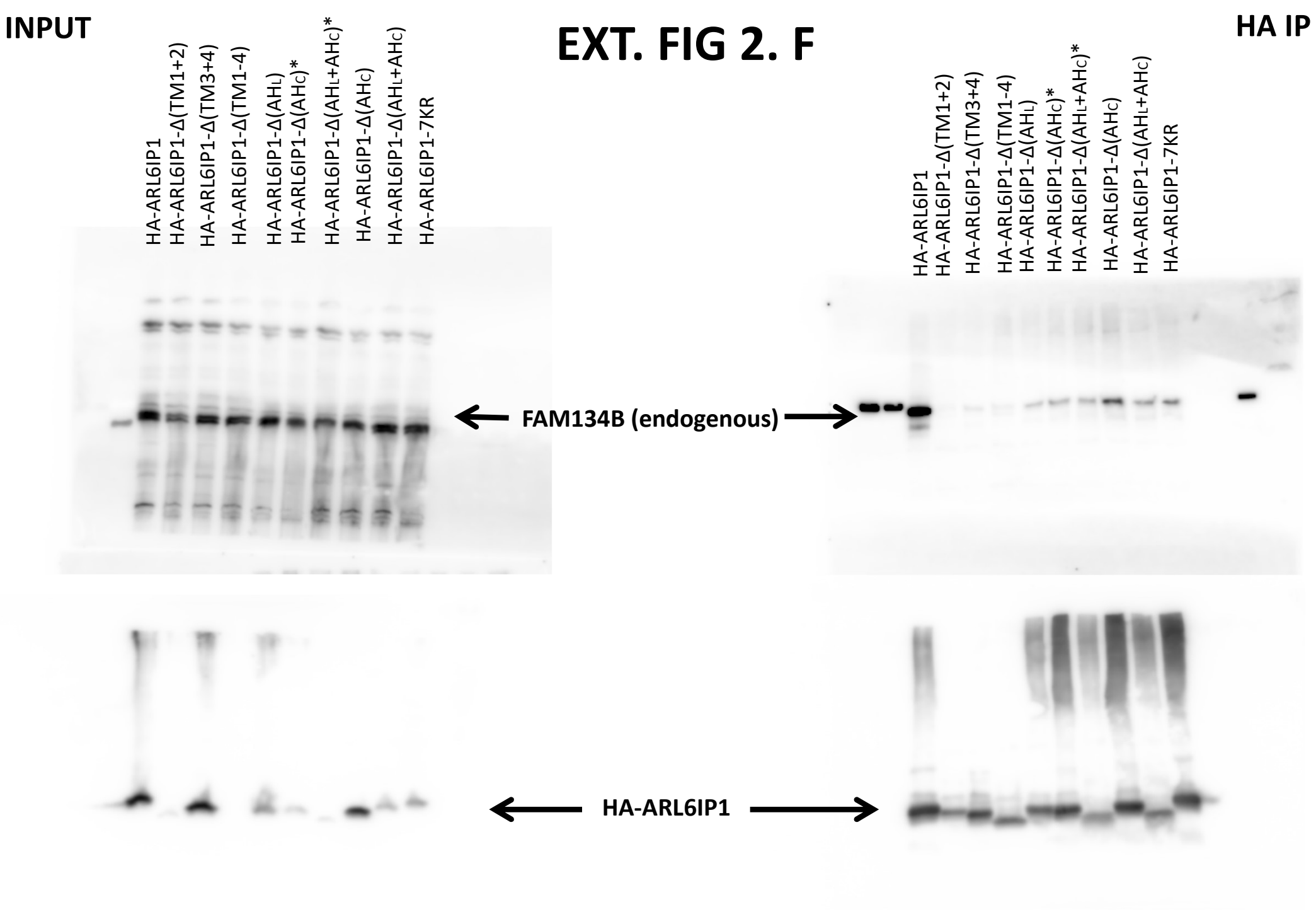
EXT. FIG 2. D


5% Input
HA-IP
HA-Arl6ip1t
Mock
HA-Arl6ip1t
Mock
70
25
25
20
IB: FAM134B
Endogenous FAM134B
IB: ARL6IP1
HA-ARL6IP1
Endogenous ARL6IP1
IB: HA
HA-ARL6IP1

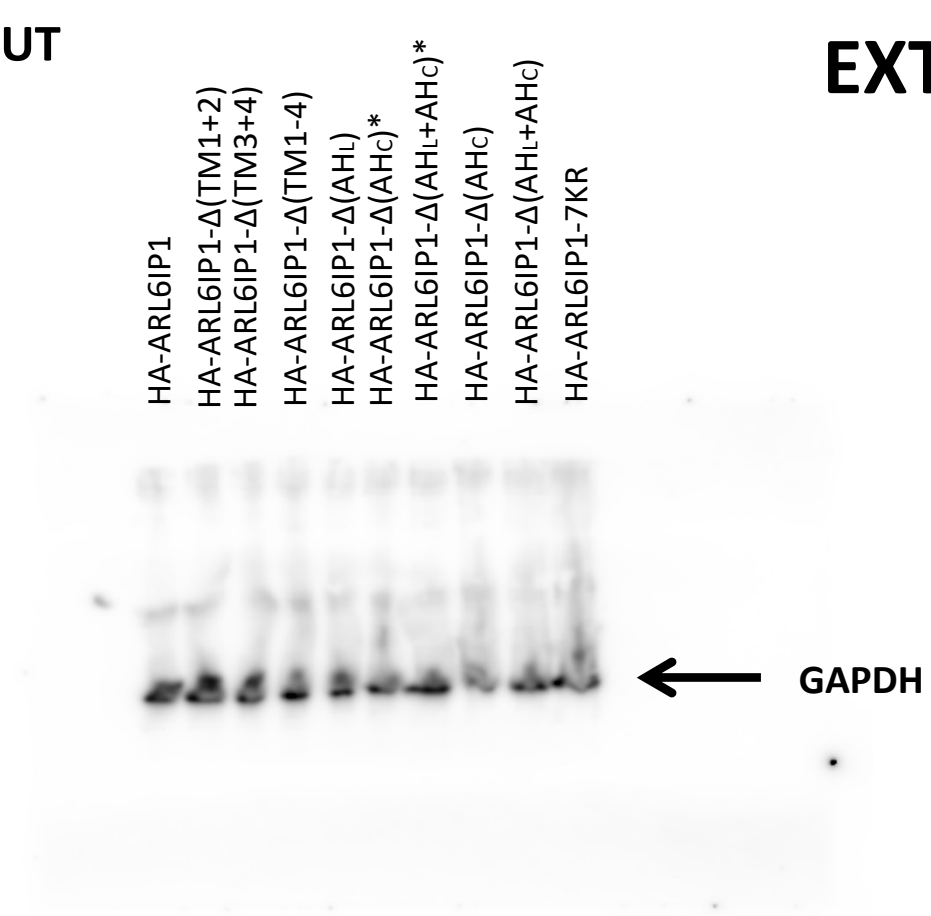
EXT. FIG 2. E


Input Ctrl
FAM 134A-GFP
FAM 134B-GFP
FAM 134C-GFP
FAM134B-GFP
N
C
S309X
ATL3-GFP
kDa
130-
100-
70-
55-
α-GFP
70-
55-
35-
25-
α-myc
Myc-IP
FAM 134A-GFP
FAM 134B-GFP
FAM 134C-GFP
FAM134B-GFP
N
C
S309X
ATL3-GFP
Int.
α-GFP
α-myc
CoIP: myc

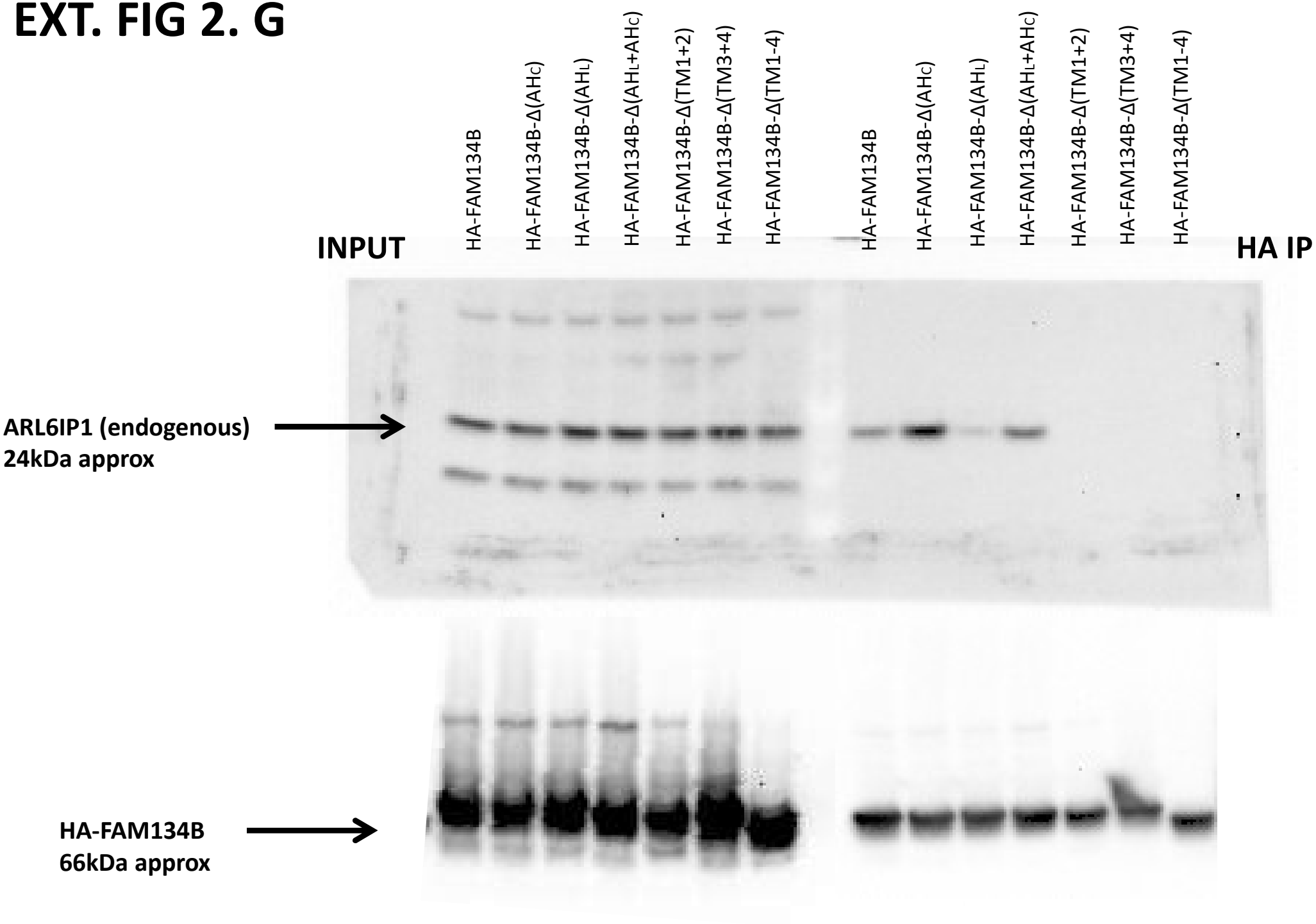
EXT. FIG 2. F


INPUT
HA IP
HA-ARL6IP1
HA-ARL6IP1-Δ(TM1+2)
HA-ARL6IP1-Δ(TM3+4)
HA-ARL6IP1-Δ(TM1-4)
HA-ARL6IP1-Δ(AHL)
HA-ARL6IP1-Δ(AHC)*
HA-ARL6IP1-Δ(AHL+AHC)*
HA-ARL6IP1-Δ(AHC)
HA-ARL6IP1-Δ(AHL+AHC)
HA-ARL6IP1-7KR
FAM134B (endogenous)
HA-ARL6IP1

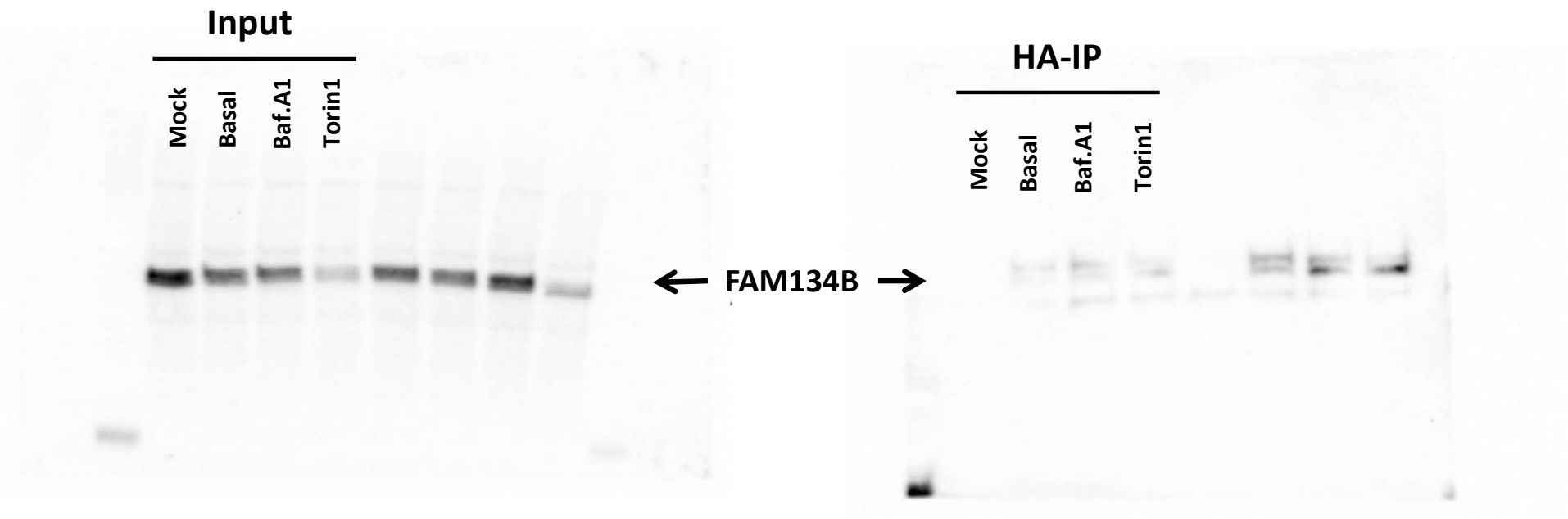
EXT. FIG 2. F


INPUT
HA-ARL6IP1
HA-ARL6IP1-Δ(TM1+2)
HA-ARL6IP1-Δ(TM3+4)
HA-ARL6IP1-Δ(TM1-4)
HA-ARL6IP1-Δ(AHL)
HA-ARL6IP1-Δ(AHC)*
HA-ARL6IP1-Δ(AHL+AHC)*
HA-ARL6IP1-Δ(AHC)
HA-ARL6IP1-Δ(AHL+AHC)
HA-ARL6IP1-7KR
GAPDH

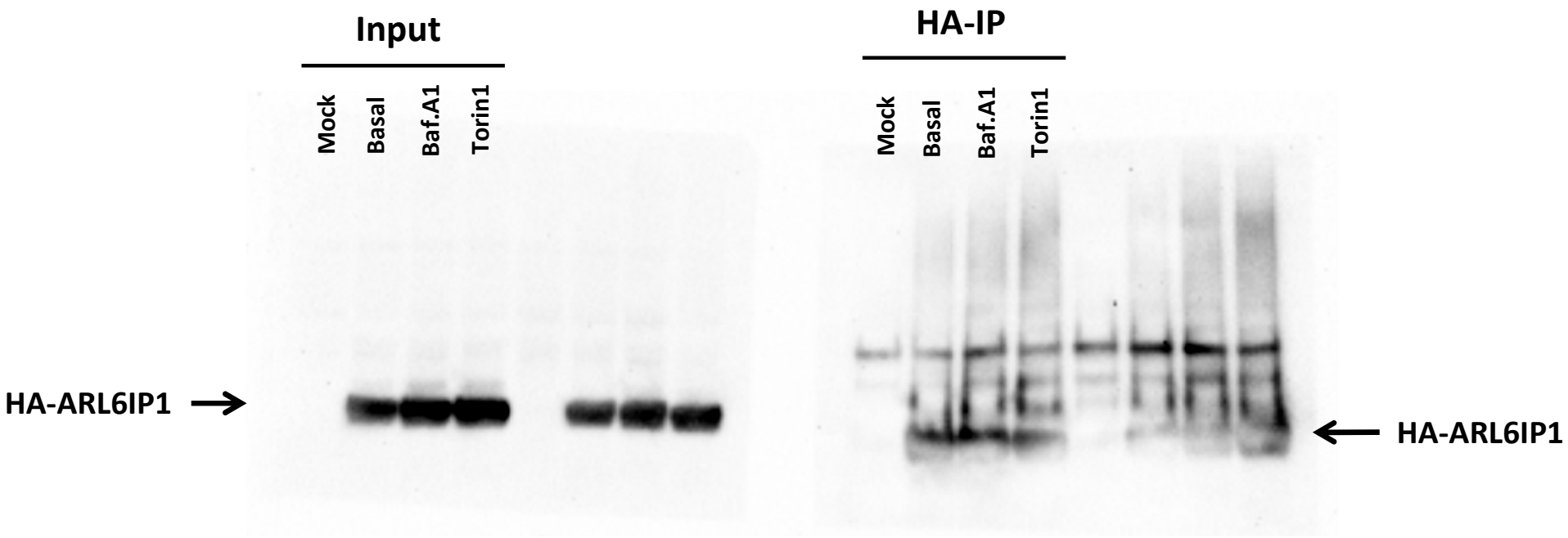
EXT. FIG 2. G


INPUT
HA IP
HA-FAM134B
HA-FAM134B-Δ(AHC)
HA-FAM134B-Δ(AHL)
HA-FAM134B-Δ(AHL+AHC)
HA-FAM134B-Δ(TM1+2)
HA-FAM134B-Δ(TM3+4)
HA-FAM134B-Δ(TM1-4)
HA-FAM134B
HA-FAM134B-Δ(AHC)
HA-FAM134B-Δ(AHL)
HA-FAM134B-Δ(AHL+AHC)
HA-FAM134B-Δ(TM1+2)
HA-FAM134B-Δ(TM3+4)
HA-FAM134B-Δ(TM1-4)
ARL6IP1 (endogenous)
24kDa approx
HA-FAM134B
66kDa approx

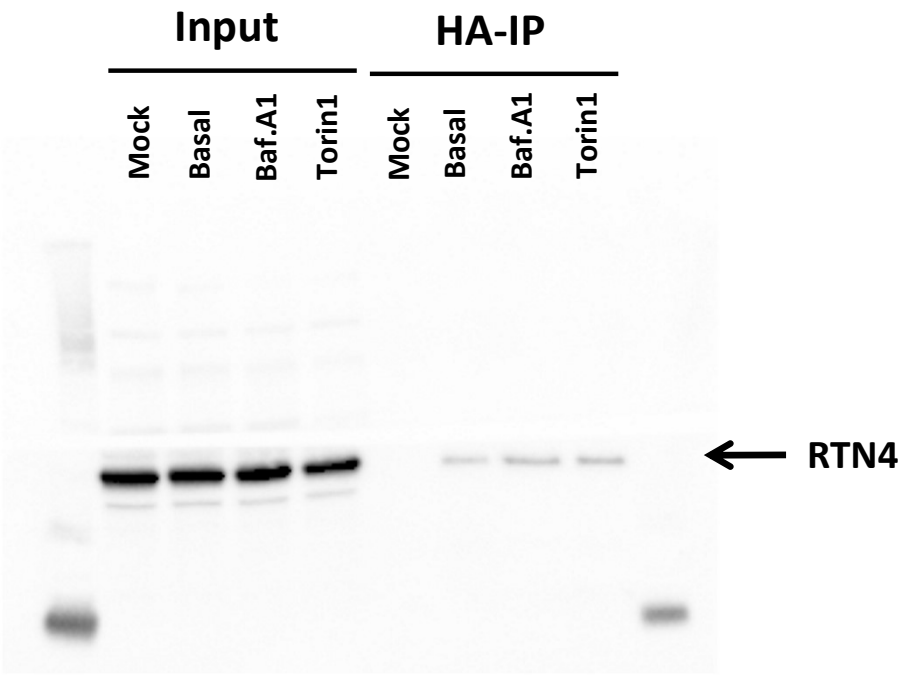
EXT. FIG 3. E

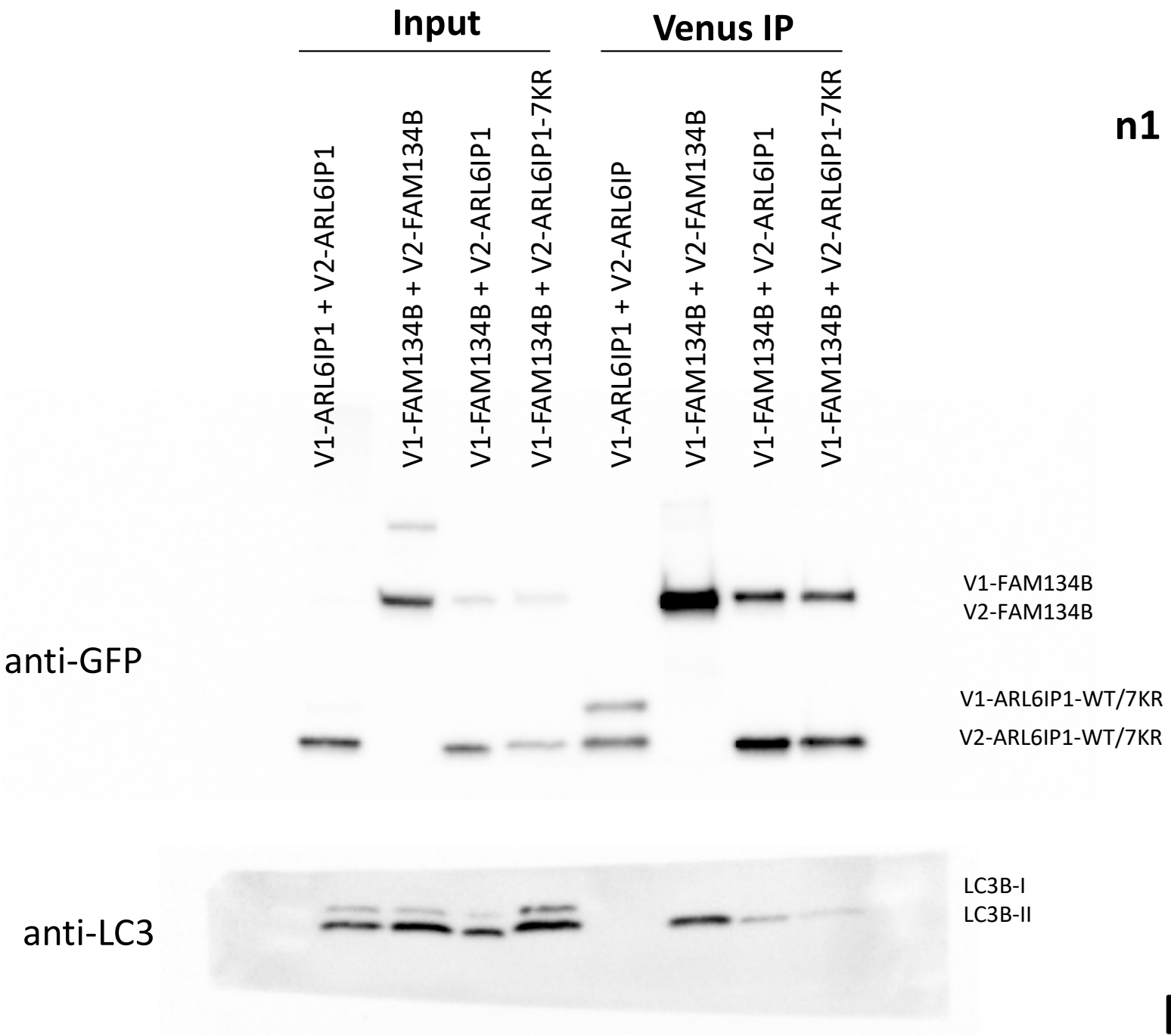

Input
Mock
Basal
Baf.A1
Torin1
HA-IP
Mock
Basal
Baf.A1
Torin1
FAM134B

EXT. FIG 3. E

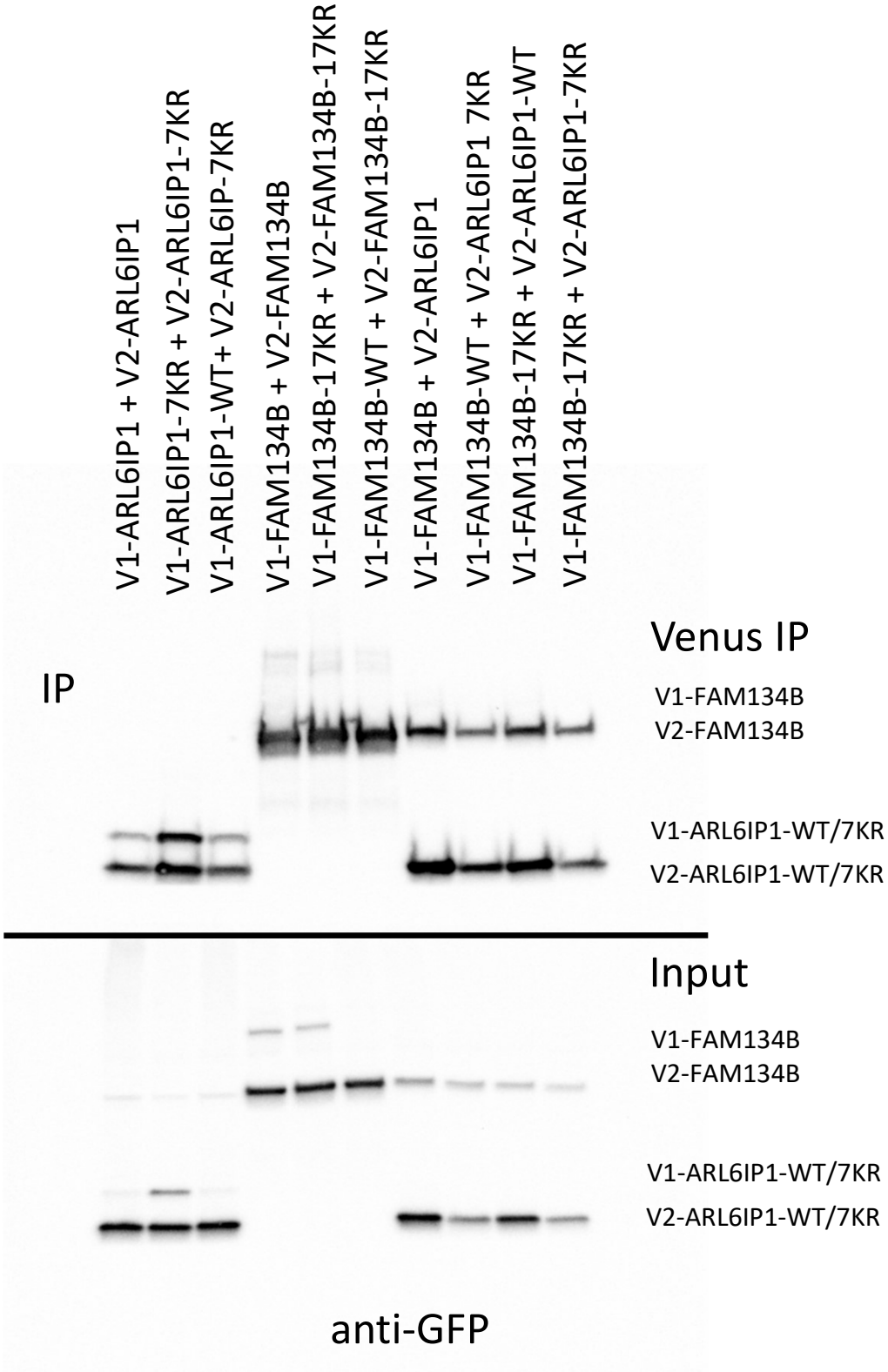

Input
HA-IP
Mock
Basal
Baf.A1
Torin1
Mock
Basal
Baf.A1
Torin1
HA-ARL6IP1
HA-ARL6IP1

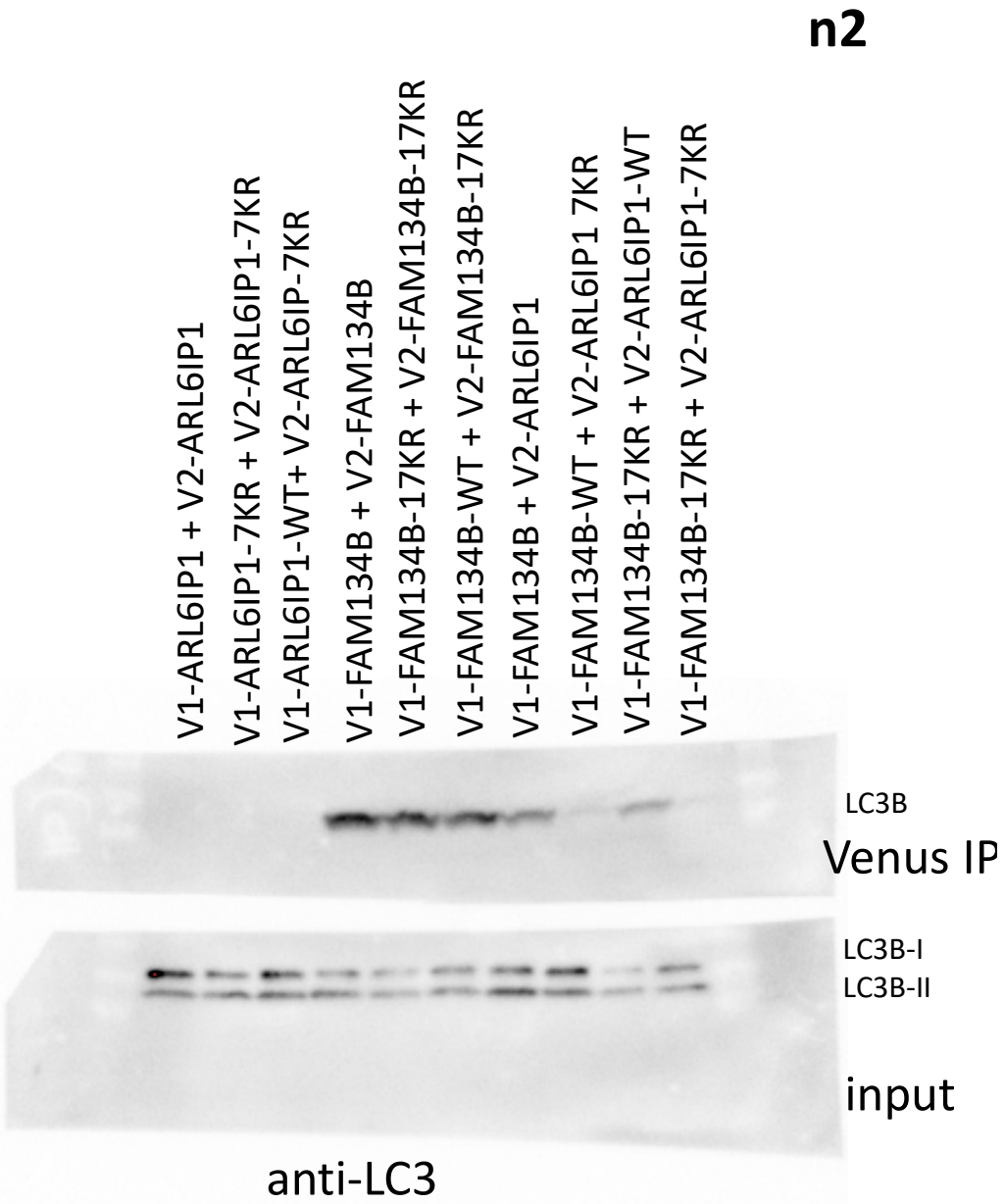
EXT. FIG 3. E


Input
HA-IP
Mock
Basal
Baf.A1
Torin1
Mock
Basal
Baf.A1
Torin1
RTN4


Input
Venus IP
V1-ARL6IP1 + V2-ARL6IP1
V1-FAM134B + V2-FAM134B
V1-FAM134B + V2-ARL6IP1
V1-FAM134B + V2-ARL6IP1-7KR
V1-ARL6IP1 + V2-ARL6IP
V1-FAM134B + V2-FAM134B
V1-FAM134B + V2-ARL6IP1
V1-FAM134B + V2-ARL6IP1-7KR
n1
anti-GFP
V1-FAM134B
V2-FAM134B
V1-ARL6IP1-WT/7KR
V2-ARL6IP1-WT/7KR
anti-LC3
LC3B-I
LC3B-II

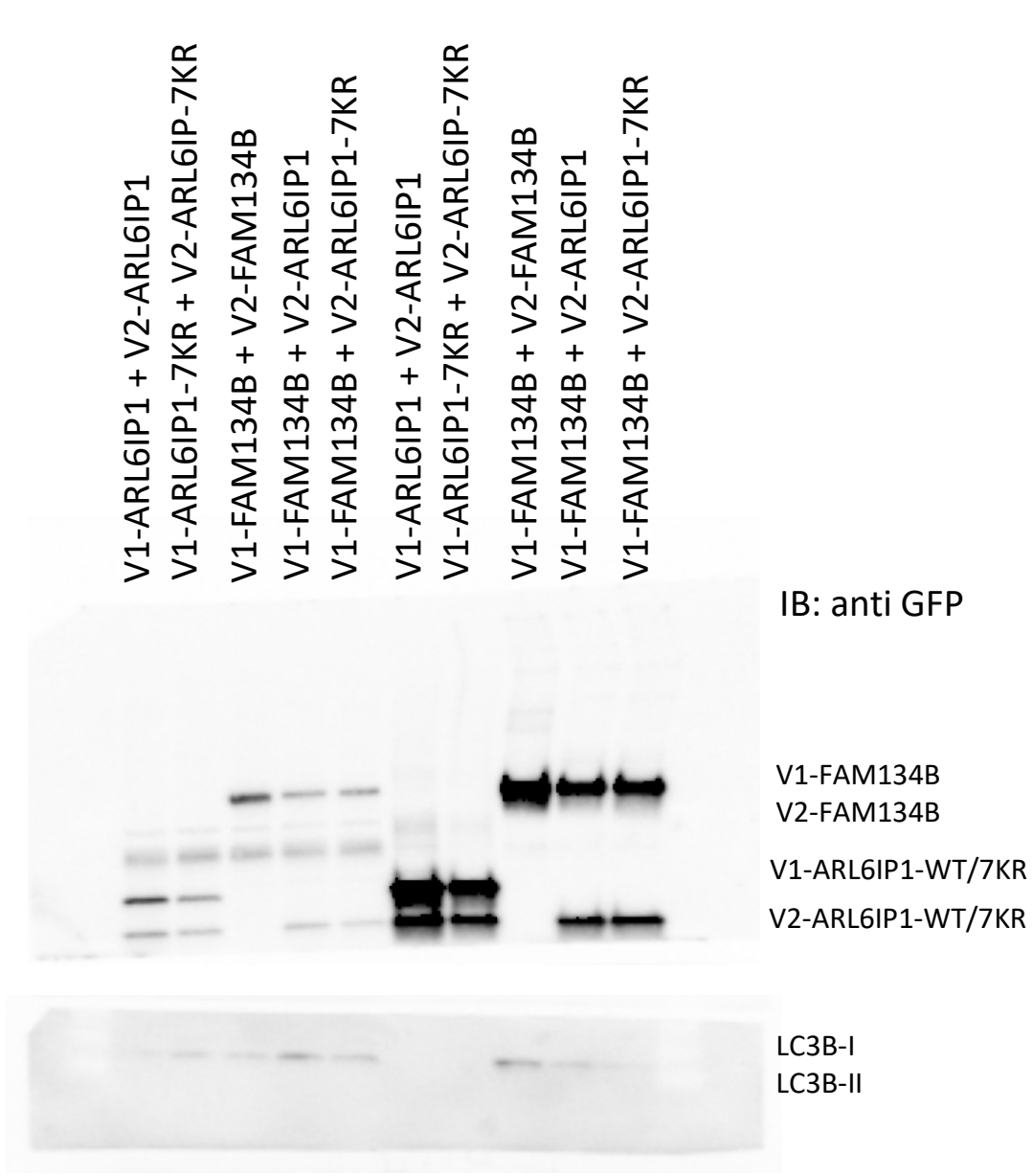
EXT. FIG 5. D


V1-ARL6IP1 + V2-ARL6IP1
V1-ARL6IP1-7KR + V2-ARL6IP1-7KR
V1-ARL6IP1-WT+ V2-ARL6IP-7KR
V1-FAM134B + V2-FAM134B
V1-FAM134B-17KR + V2-FAM134B-17KR
V1-FAM134B-WT + V2-FAM134B-17KR
V1-FAM134B + V2-ARL6IP1
V1-FAM134B-WT + V2-ARL6IP1 7KR
V1-FAM134B-17KR + V2-ARL6IP1-WT
V1-FAM134B-17KR + V2-ARL6IP1-7KR
IP
Venus IP
V1-FAM134B
V2-FAM134B
V1-ARL6IP1-WT/7KR
V2-ARL6IP1-WT/7KR
Input
V1-FAM134B
V2-FAM134B
V1-ARL6IP1-WT/7KR
V2-ARL6IP1-WT/7KR
anti-GFP


n2
V1-ARL6IP1 + V2-ARL6IP1
V1-ARL6IP1-7KR + V2-ARL6IP1-7KR
V1-ARL6IP1-WT+ V2-ARL6IP-7KR
V1-FAM134B + V2-FAM134B
V1-FAM134B-17KR + V2-FAM134B-17KR
V1-FAM134B-WT + V2-FAM134B-17KR
V1-FAM134B + V2-ARL6IP1
V1-FAM134B-WT + V2-ARL6IP1 7KR
V1-FAM134B-17KR + V2-ARL6IP1-WT
V1-FAM134B-17KR + V2-ARL6IP1-7KR
LC3B
Venus IP
LC3B-I
LC3B-II
input
anti-LC3

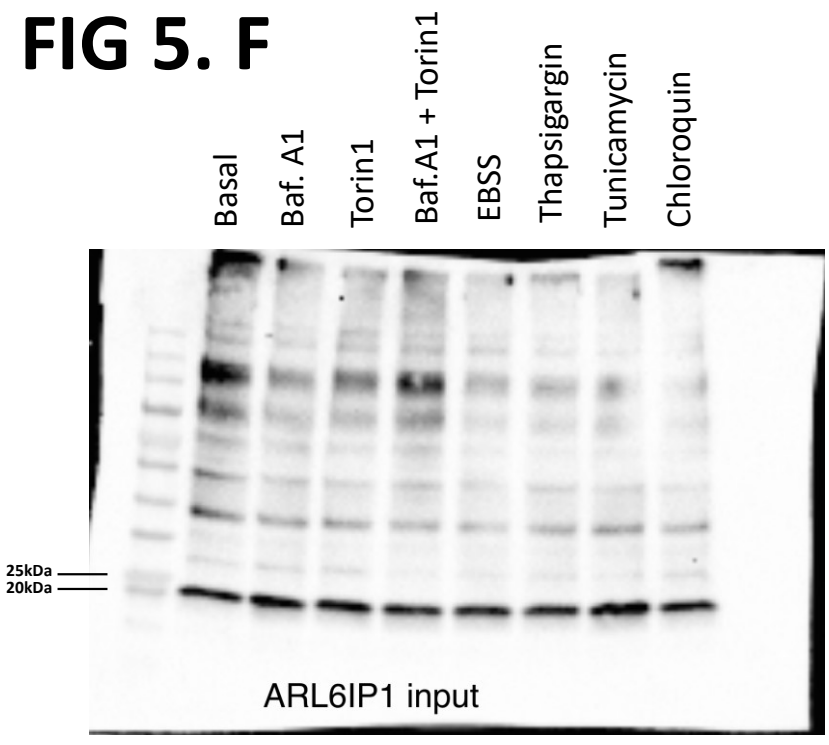
EXT. FIG 5. D

n3 shown in the figure.

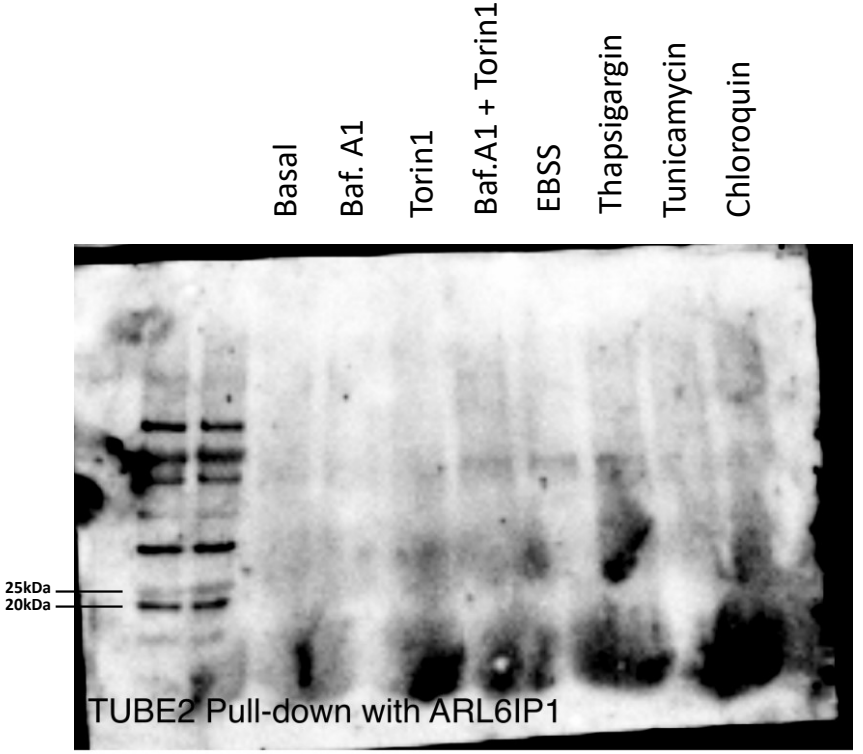


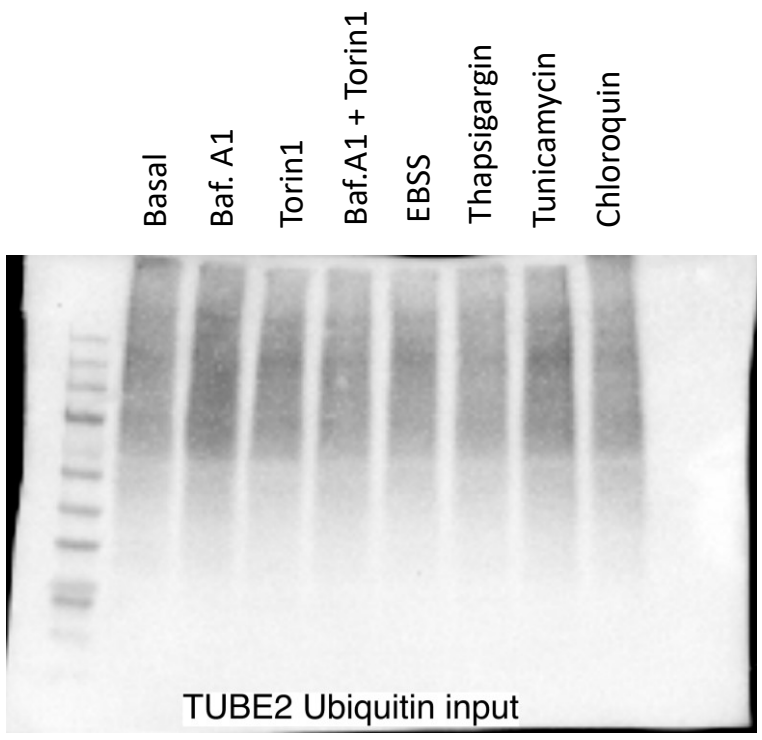
EXT. FIG 5. D

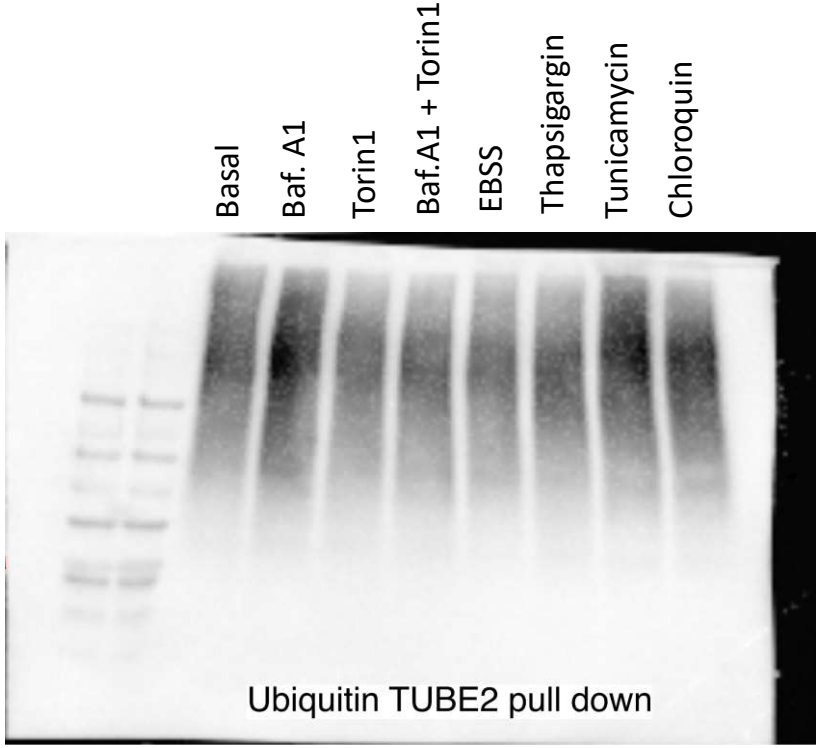
EXT. FIG 5. F



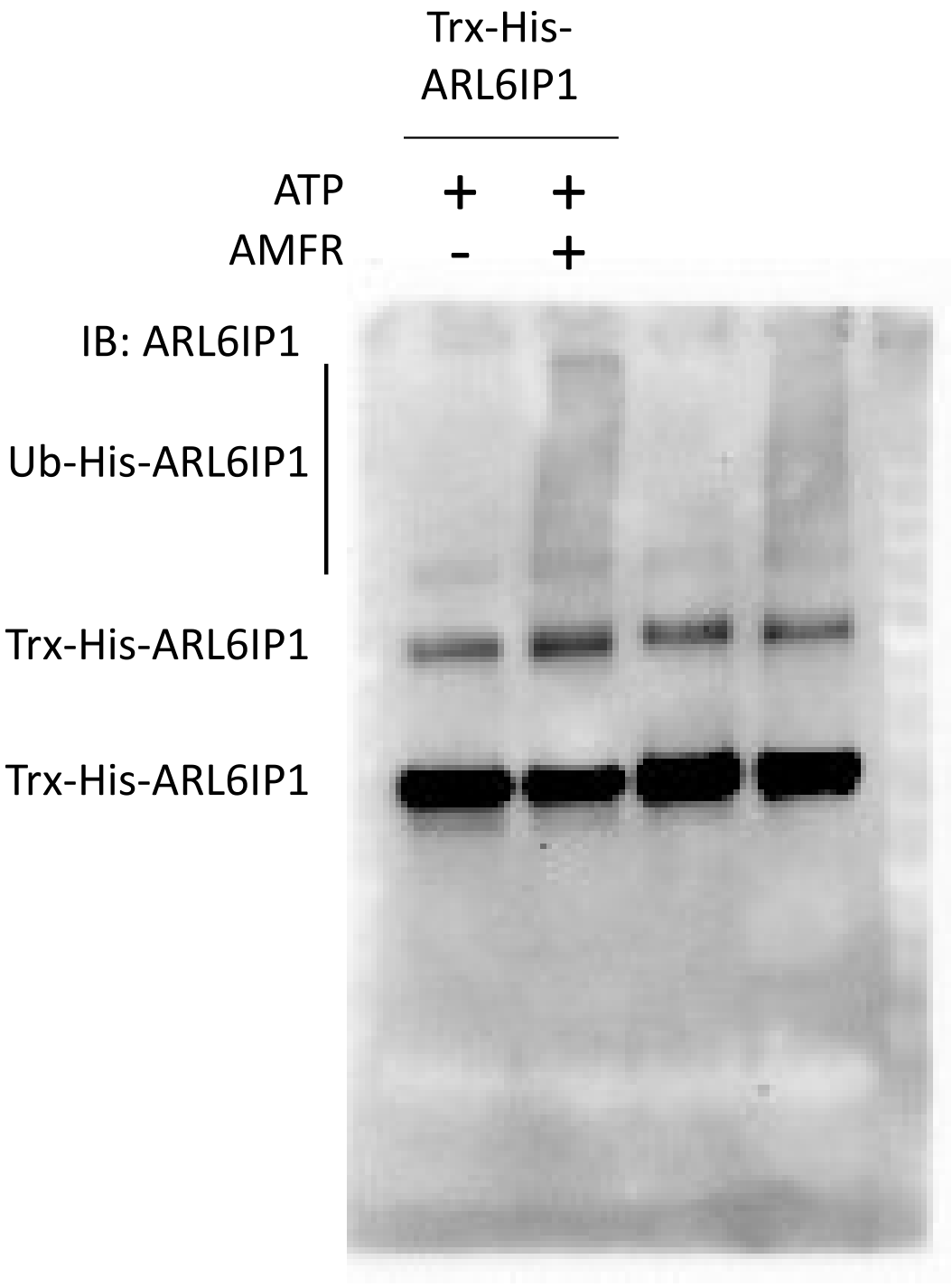
IB: Arl6ip1 (24kDa)

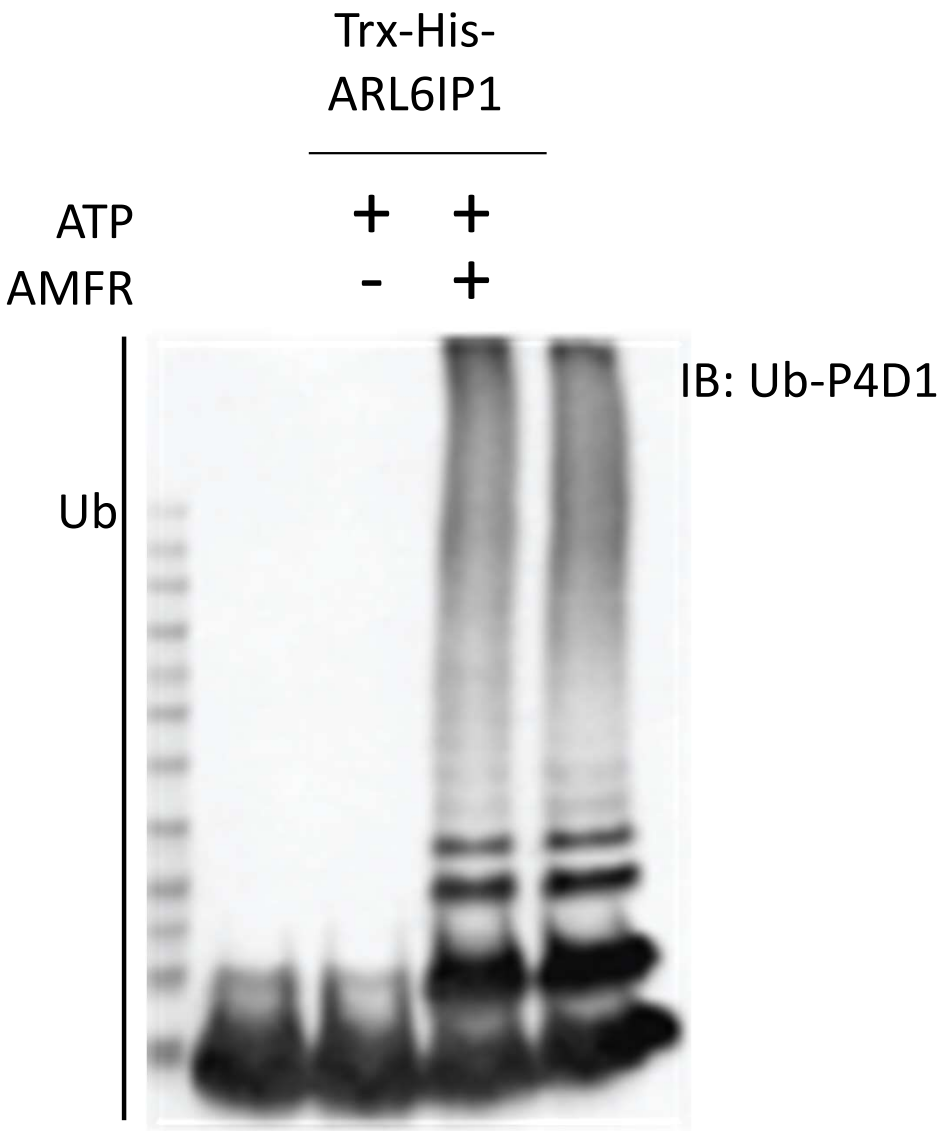




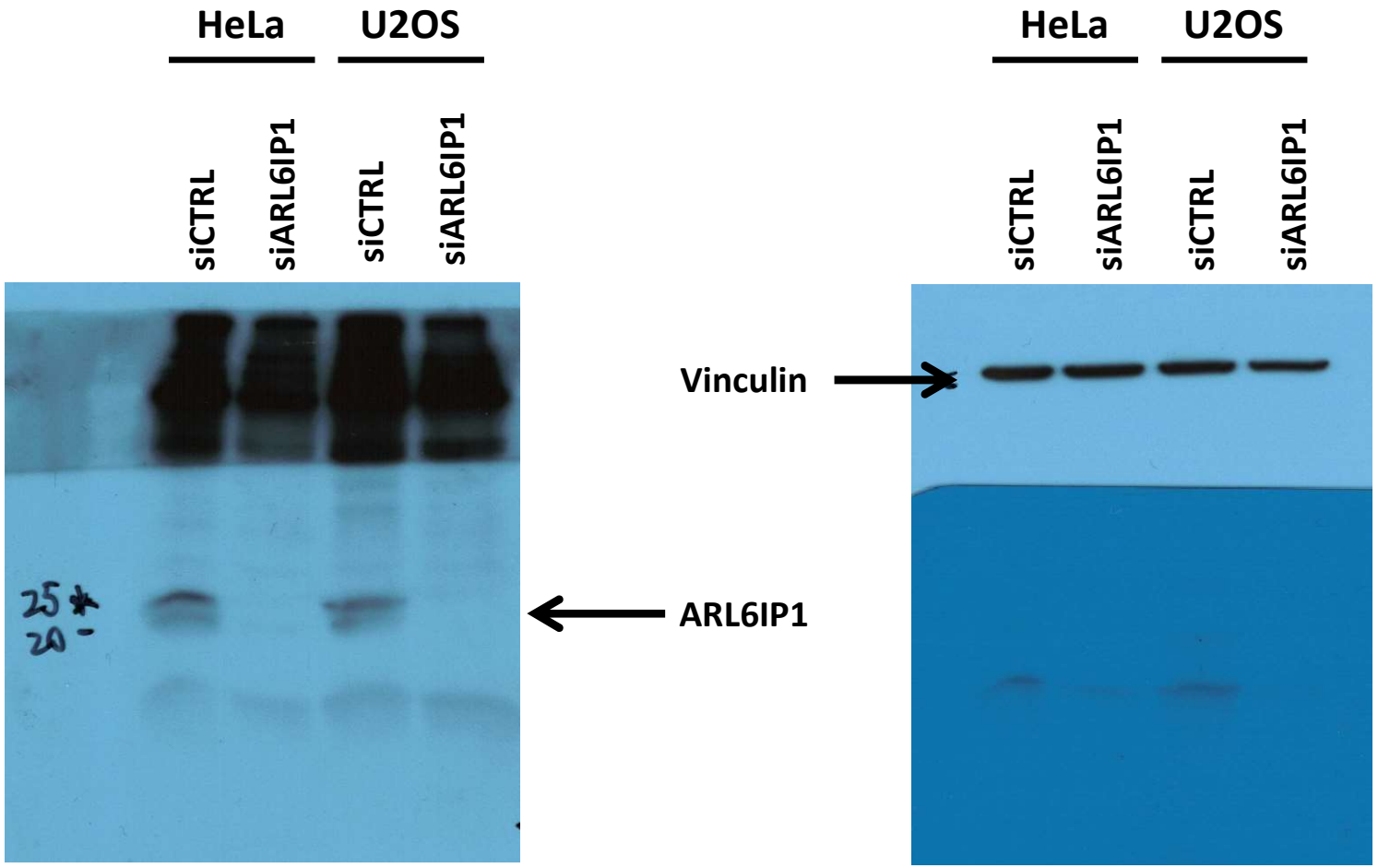


EXT. FIG 5. G

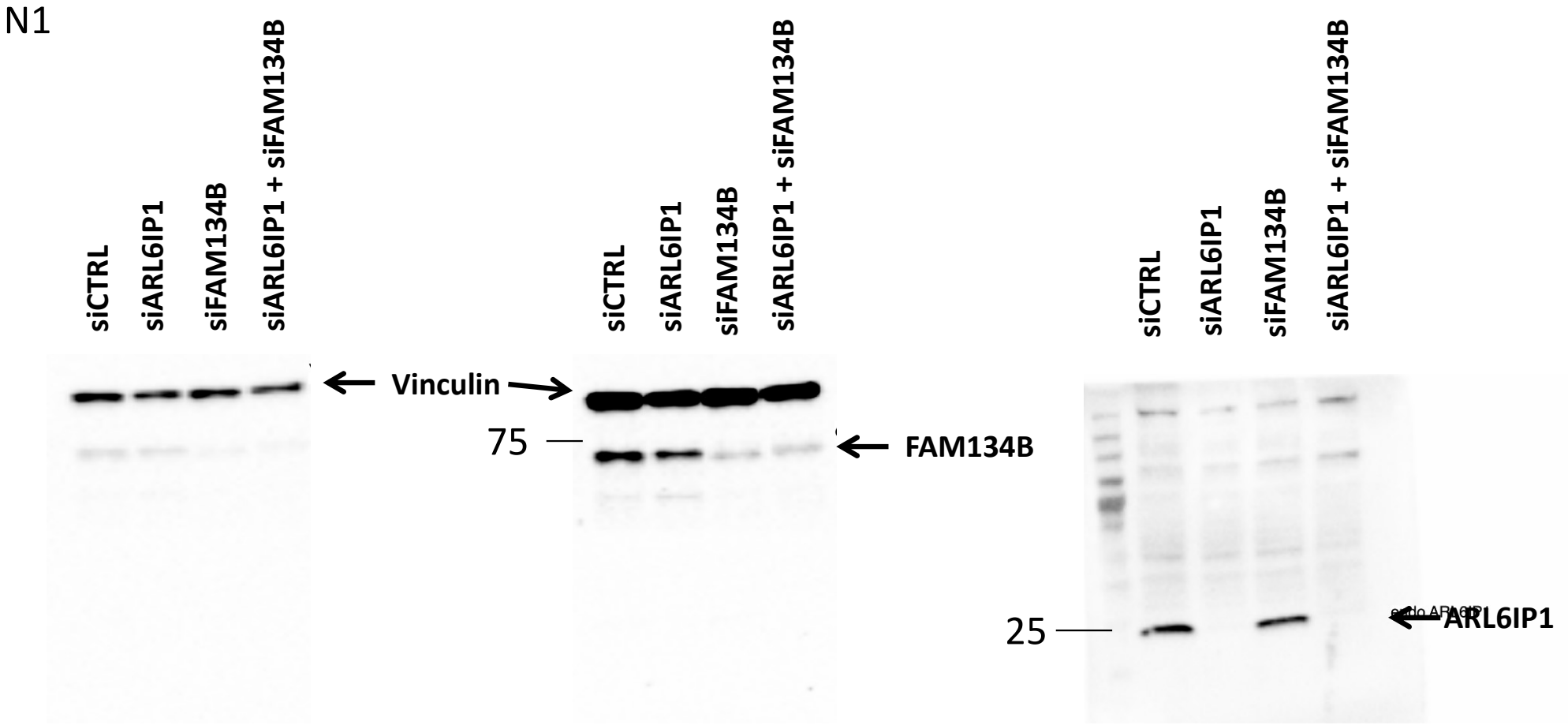

Trx-His-ARL6IP1
ATP + +
AMFR - +
IB: ARL6IP1
Ub-His-ARL6IP1
Trx-His-ARL6IP1
Trx-His-ARL6IP1


Trx-His-ARL6IP1
ATP + +
AMFR - +
Ub
IB: Ub-P4D1

EXT. FIG 6. B

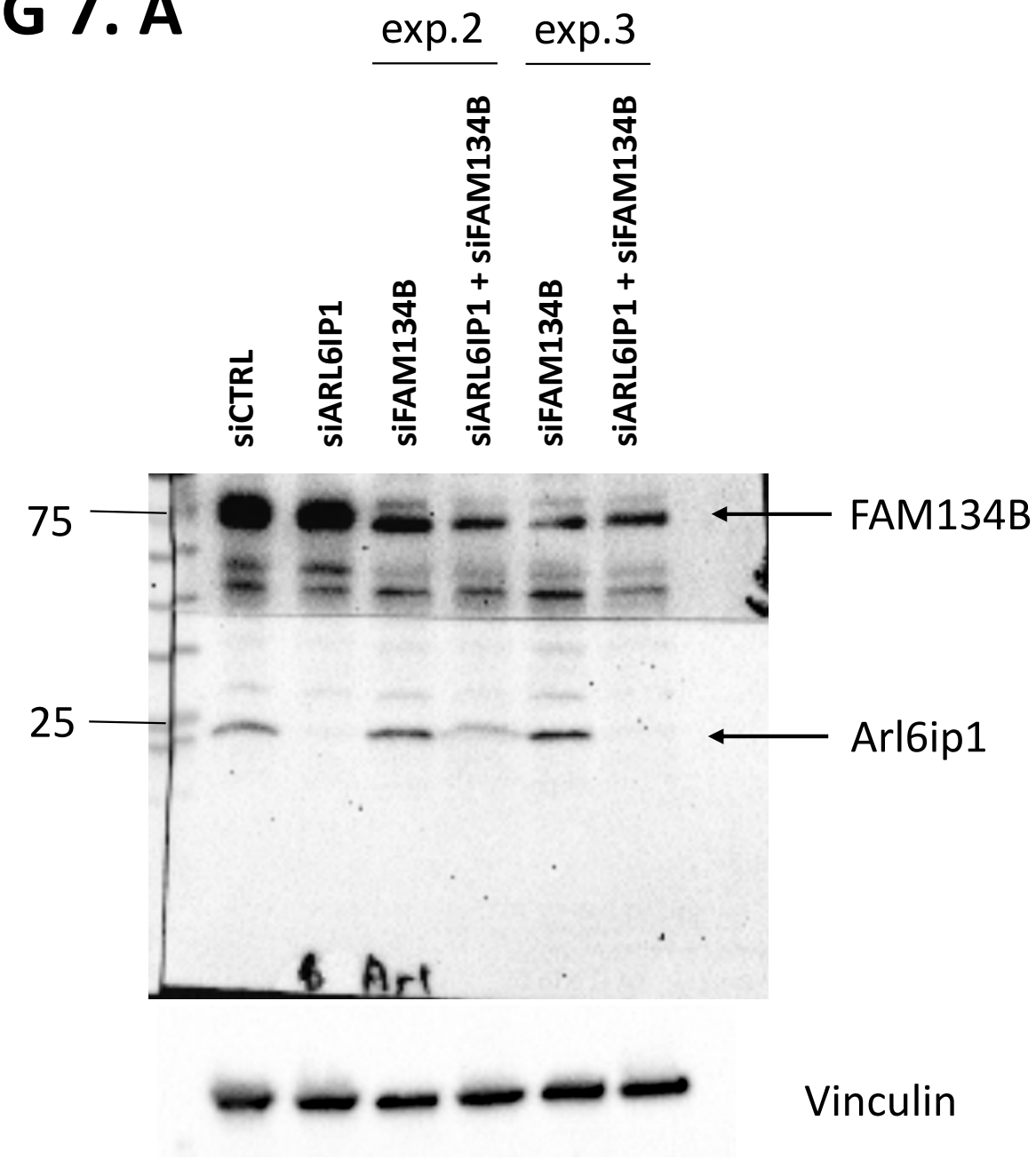

HeLa
U2OS
siCTRL
siARL6IP1
siCTRL
siARL6IP1
25
20
ARL6IP1
HeLa
U2OS
siCTRL
siARL6IP1
siCTRL
siARL6IP1
Vinculin

EXT. FIG 7. A


N1
siCTRL
siARL6IP1
siFAM134B
siARL6IP1 + siFAM134B
Vinculin
75
FAM134B
25
ARL6IP1

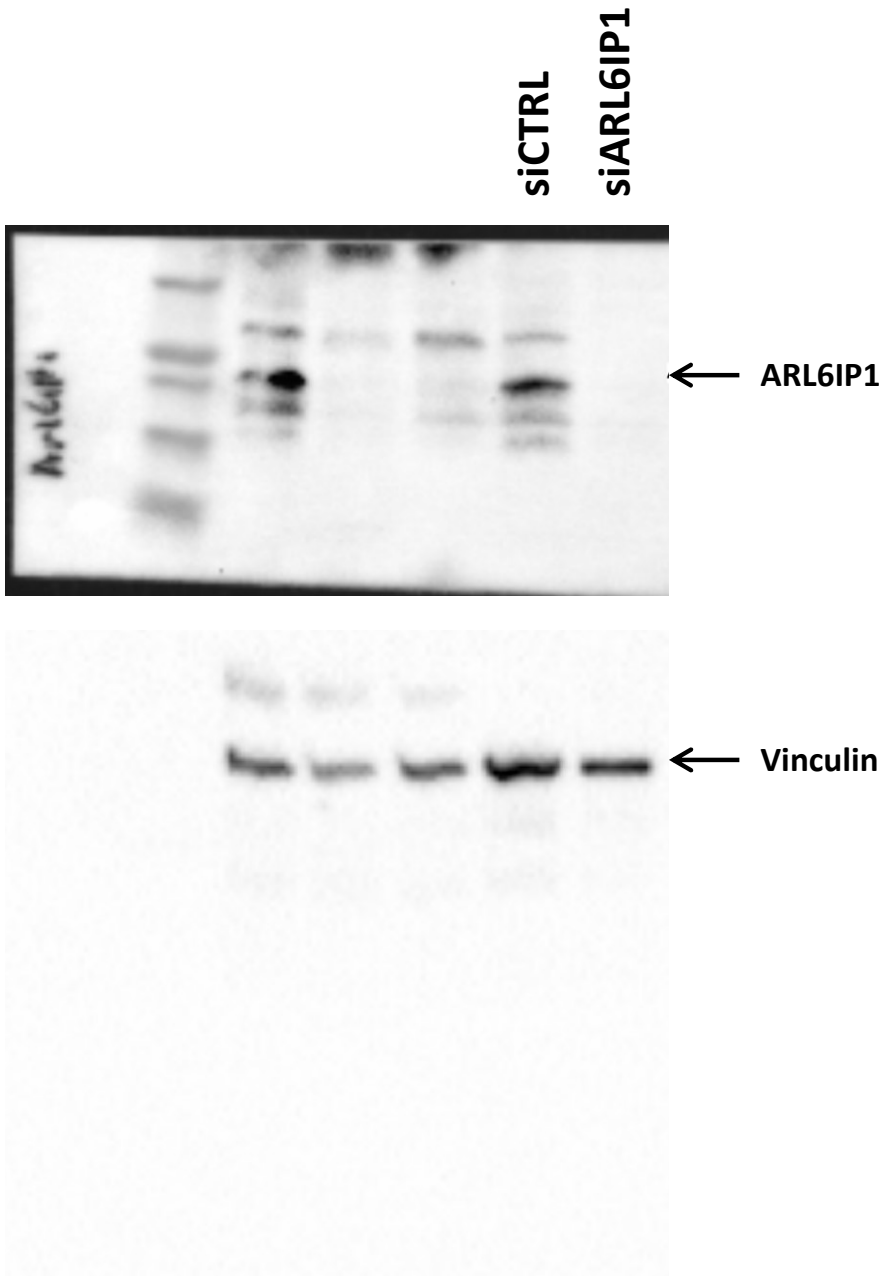
EXT. FIG 7. A

N2 & N3

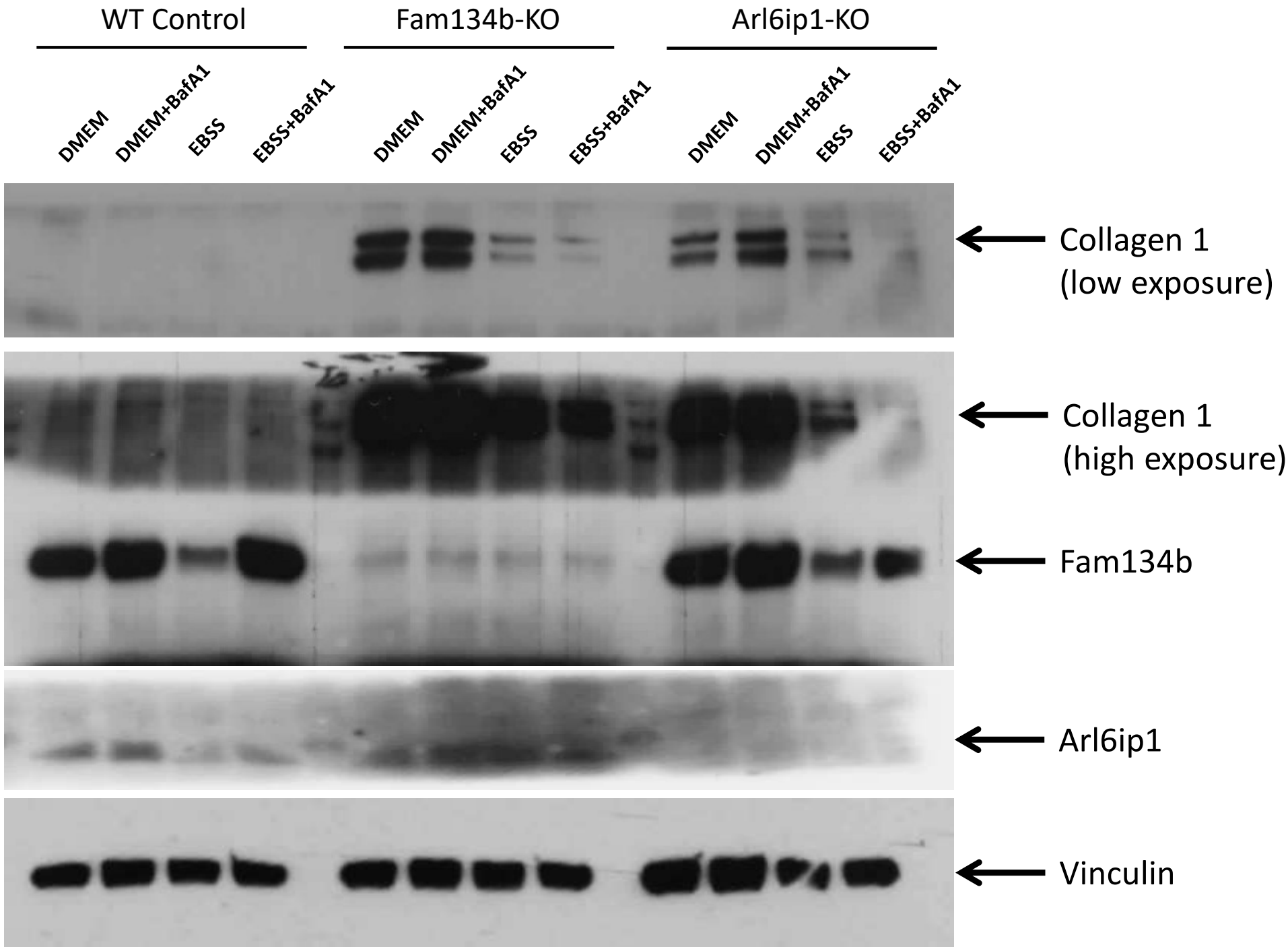


EXT. FIG 7. D

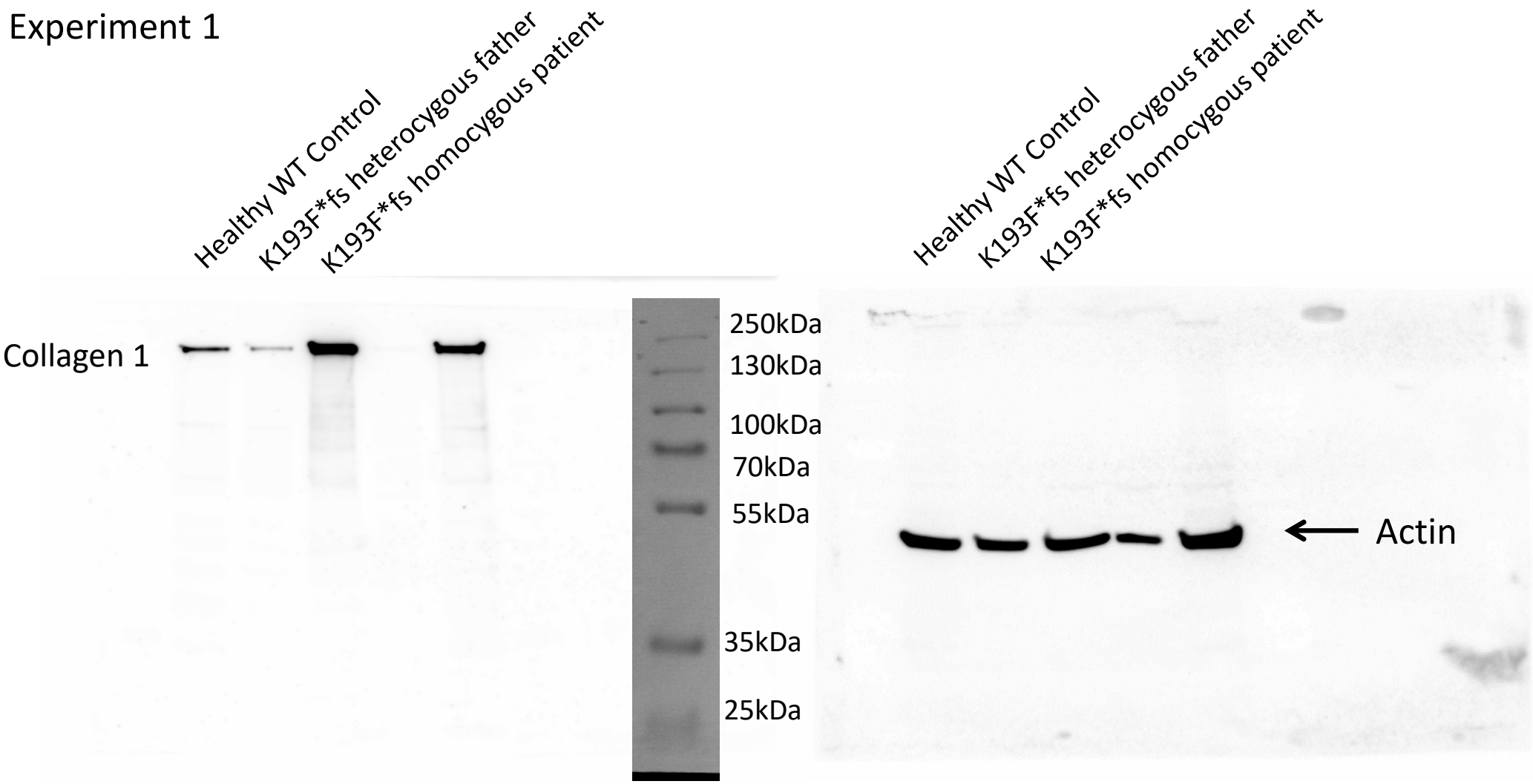
U2OS
mCherry-GFP-LC3



EXT. FIG 9. E

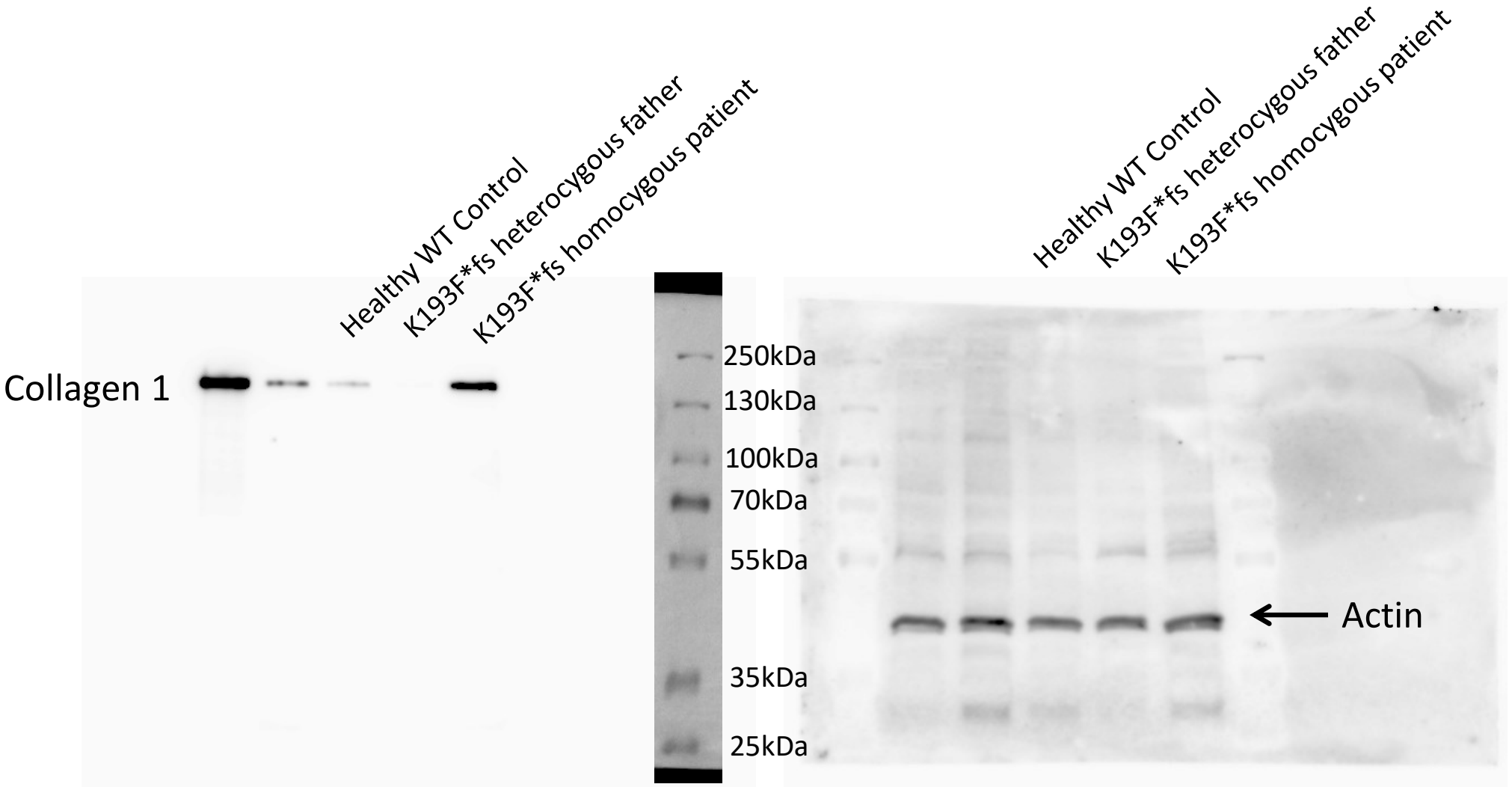

WT Control
Fam134b-KO
Arl6ip1-KO
DMEM
DMEM+BafA1
EBSS
EBSS+BafA1
DMEM
DMEM+BafA1
EBSS
EBSS+BafA1
DMEM
DMEM+BafA1
EBSS
EBSS+BafA1
Collagen 1
(low exposure)
Collagen 1
(high exposure)
Fam134b
Arl6ip1
Vinculin

Experiment 1



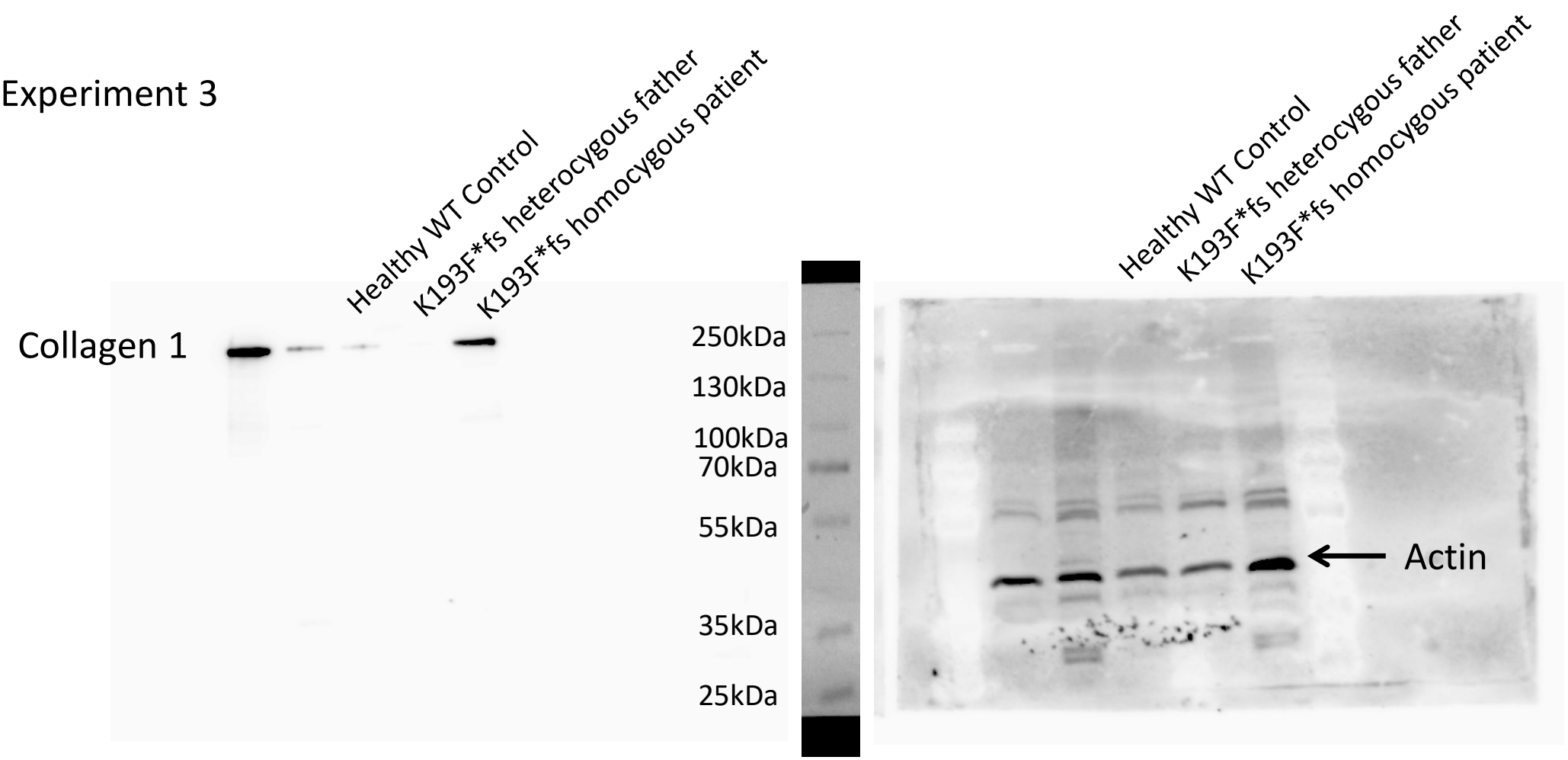
EXT. FIG 9. F

Experiment 2



EXT. FIG 9. F

Experiment 3



EXT. FIG 9. F