

## Supplementary Information

**Title: Ligand-induced BIK1 monoubiquitination regulates plant immunity**

**Authors:**

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**#Corresponding authors:**

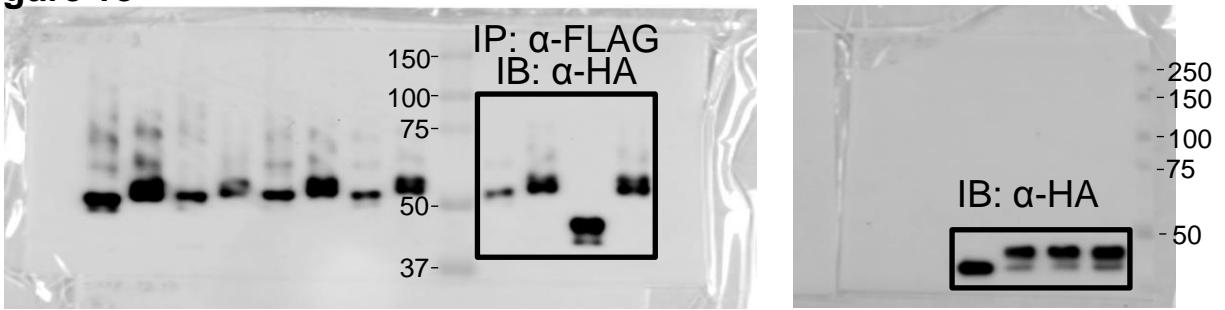
Ping He, email: pinghe@tamu.edu  
Libo Shan, email: lshan@tamu.edu

## Content

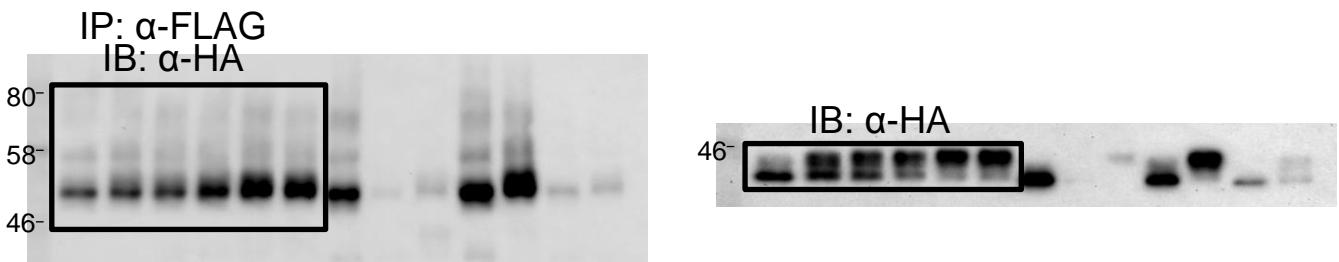
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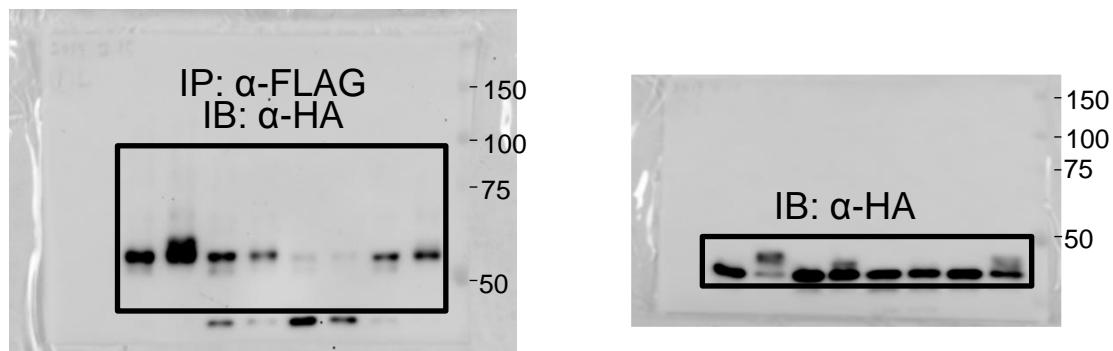
**Figure 1e**



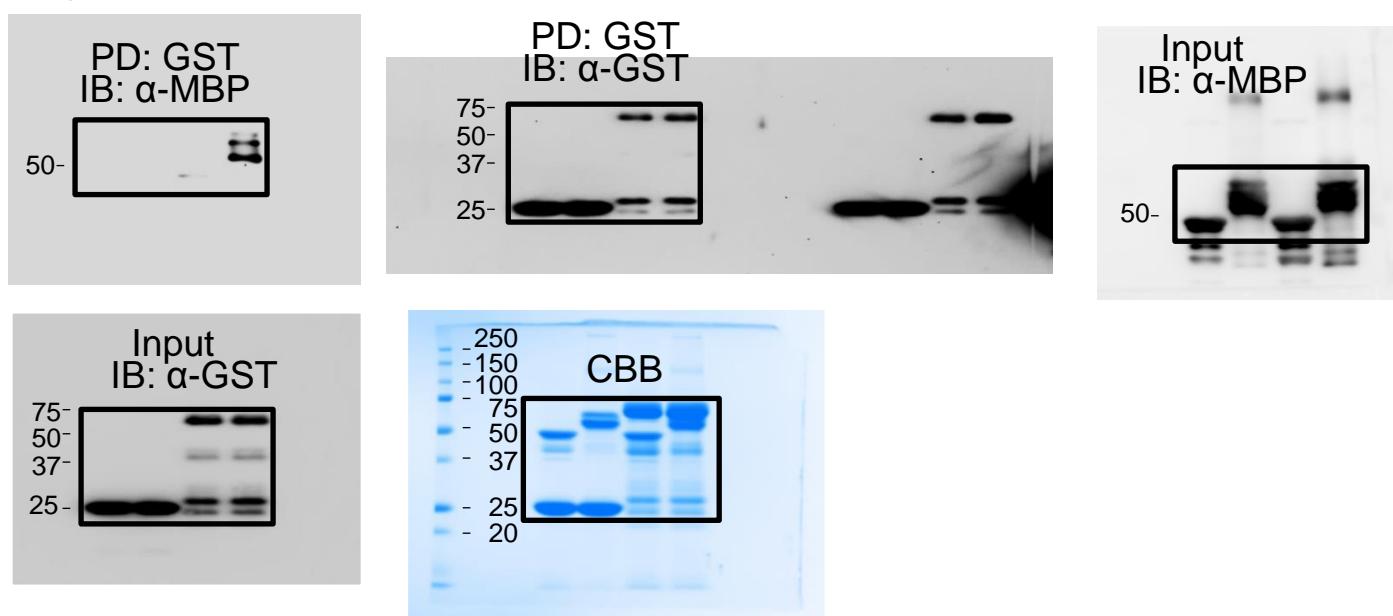
**Figure 1f**



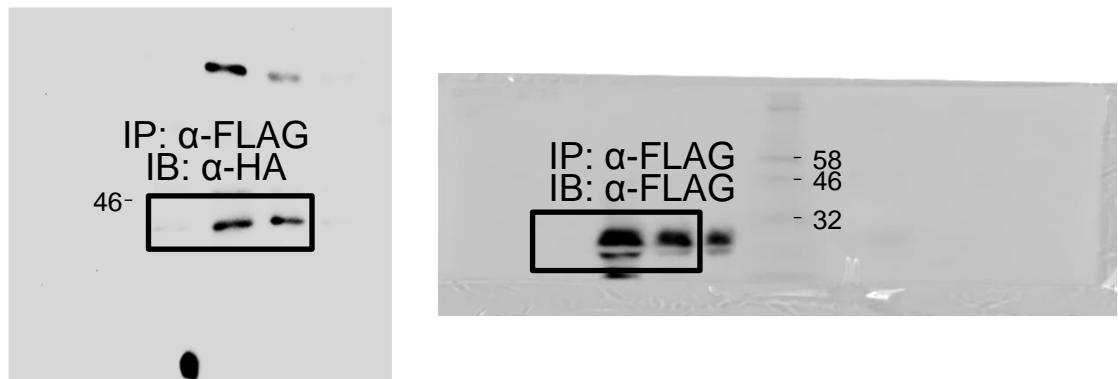
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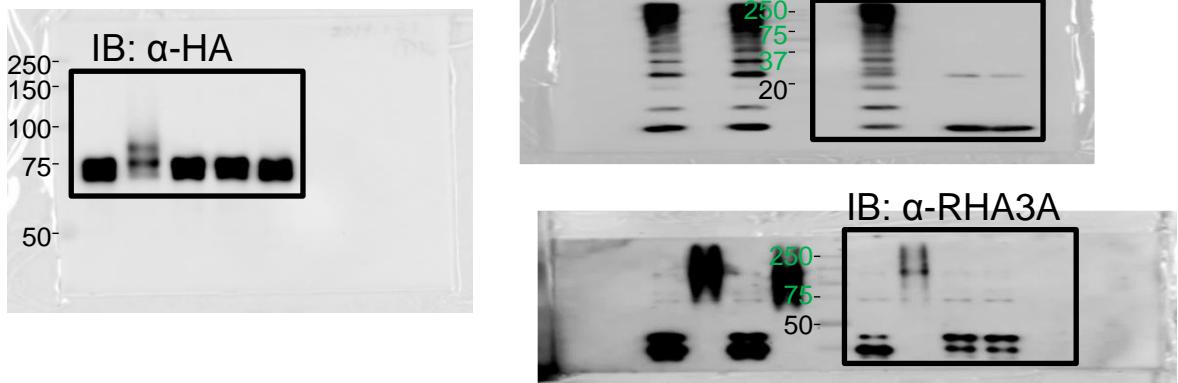
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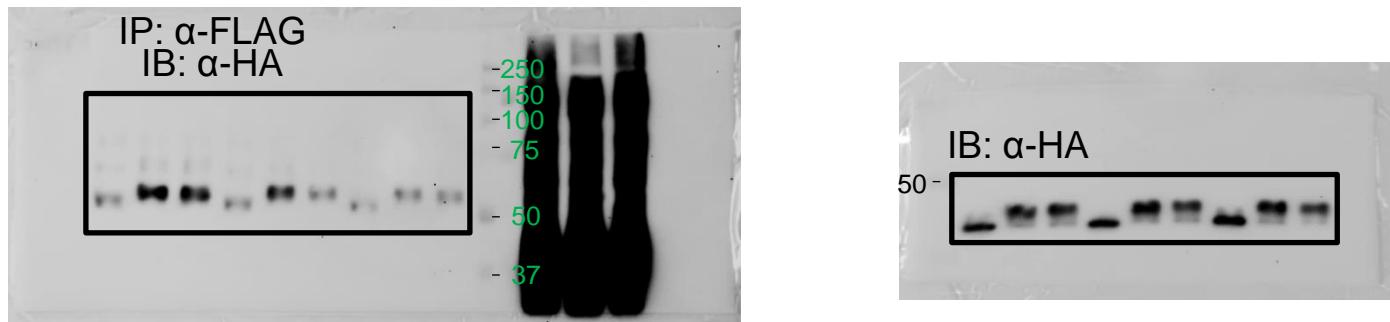
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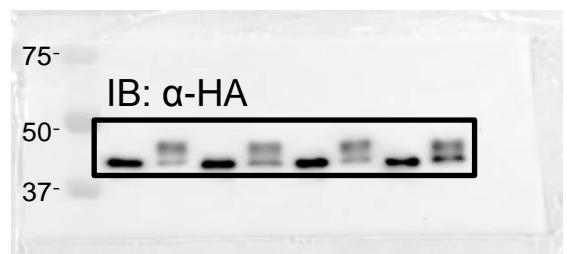
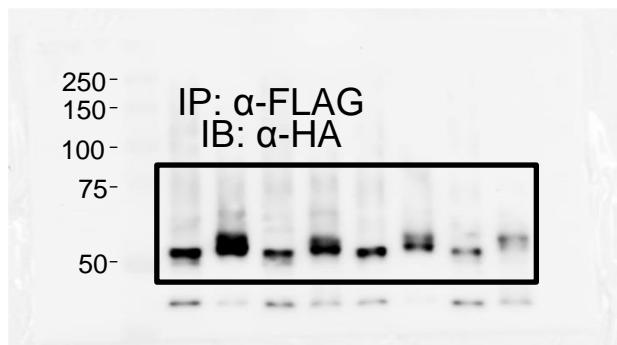
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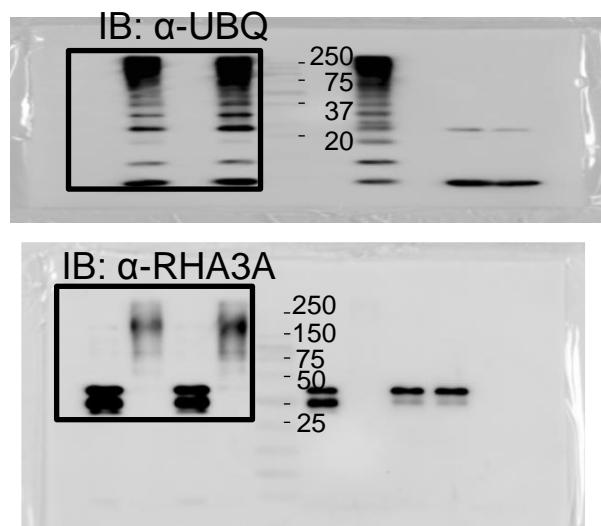
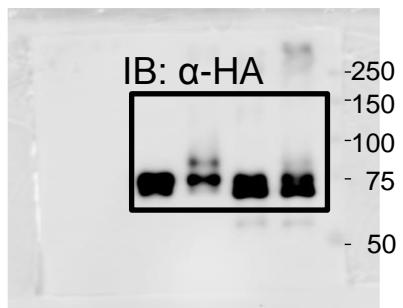
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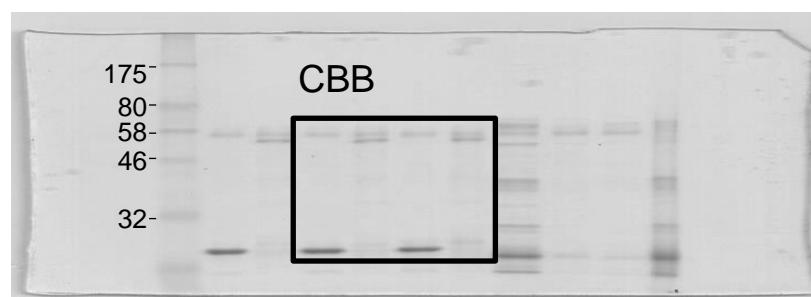
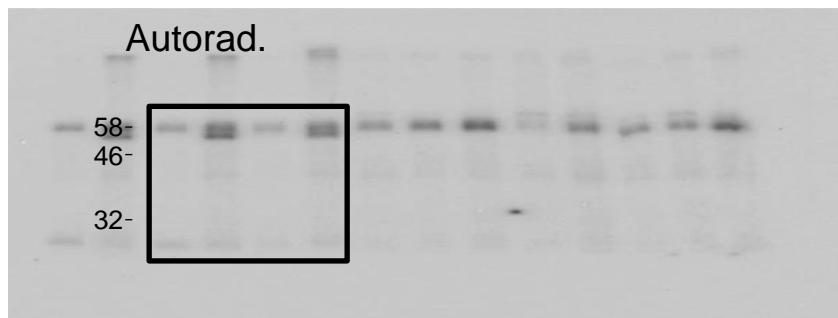
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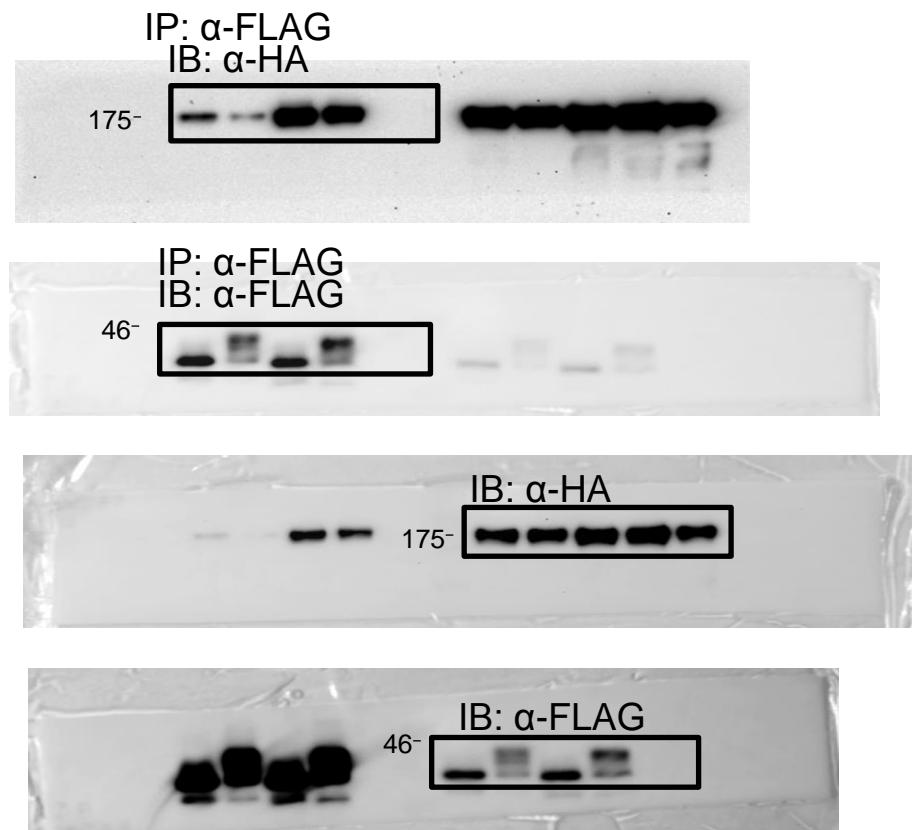
**Figure 3e**



**Figure 3f**

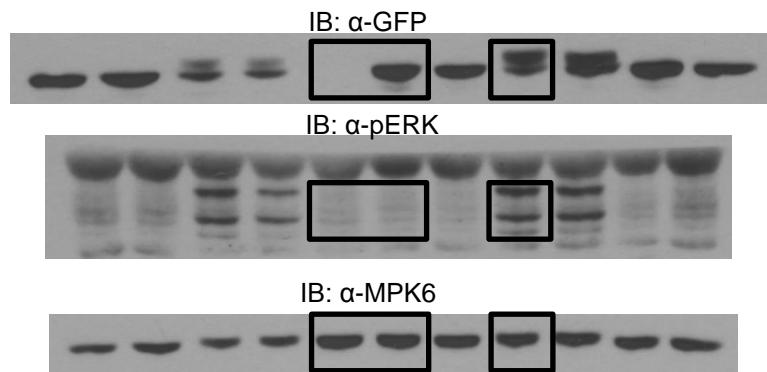


**Figure 4g**

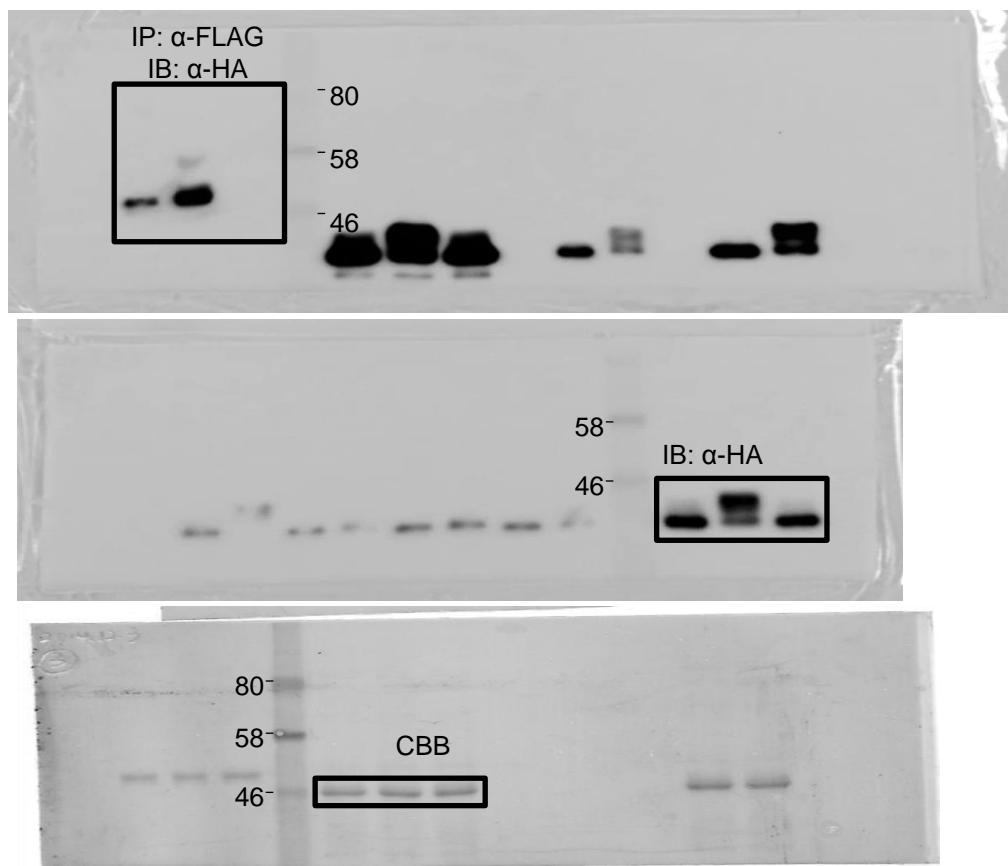


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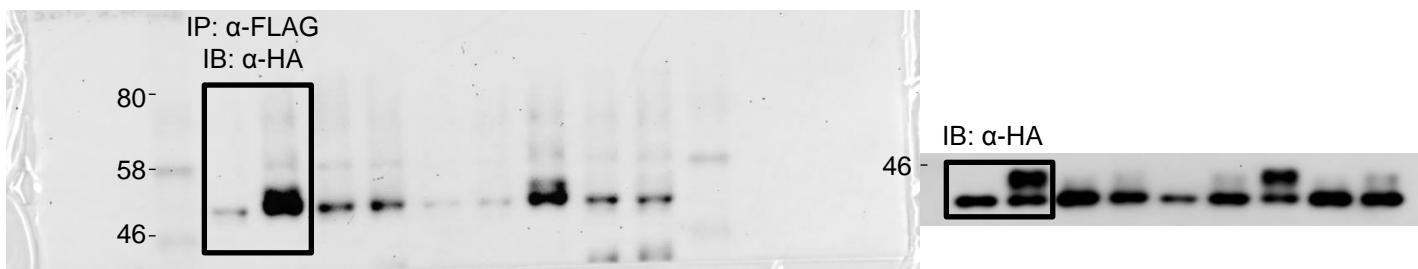
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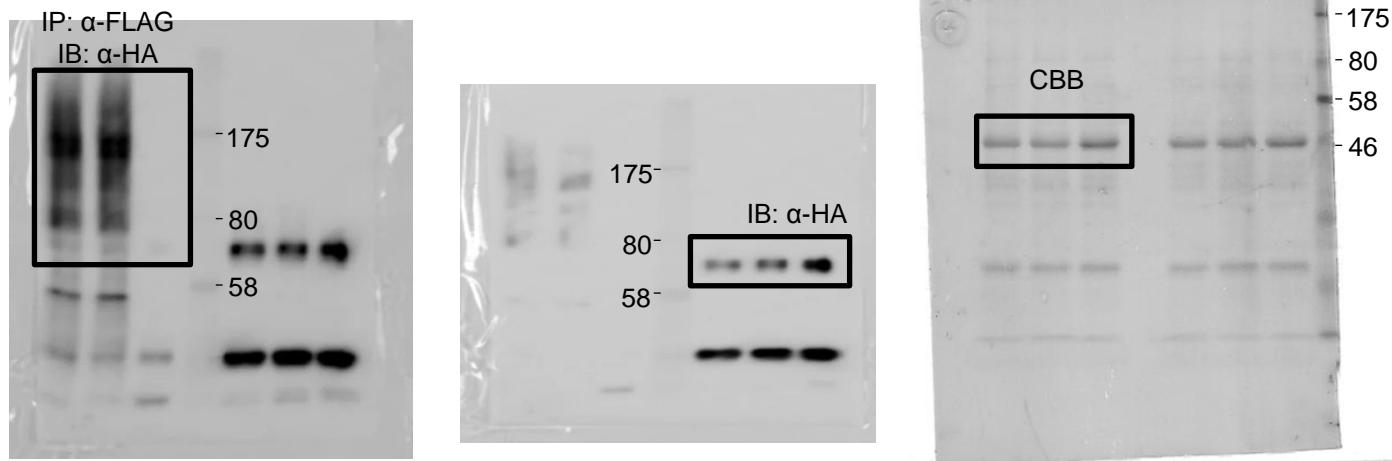
## Extended Data Figure 2a



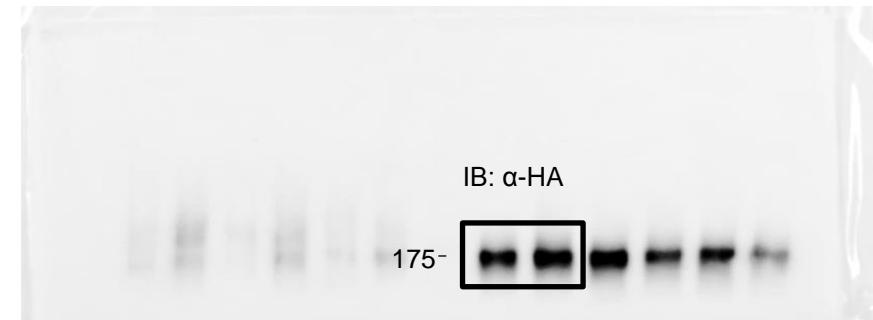
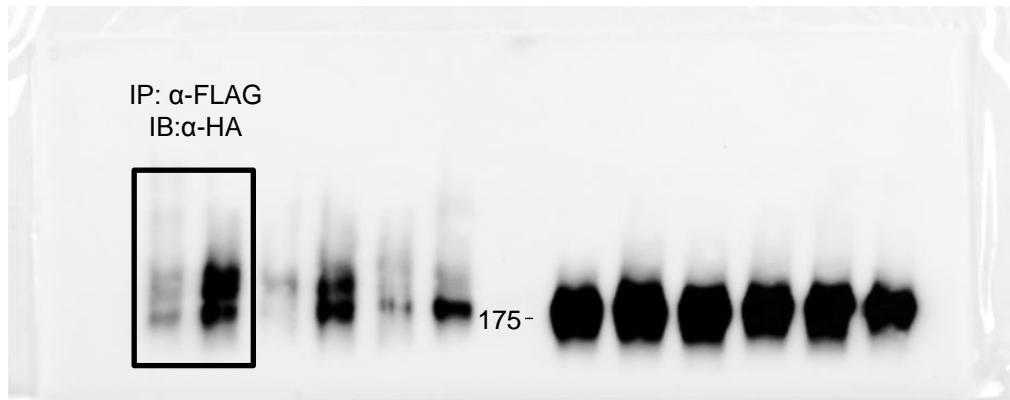
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## Extended Data Figure 2c

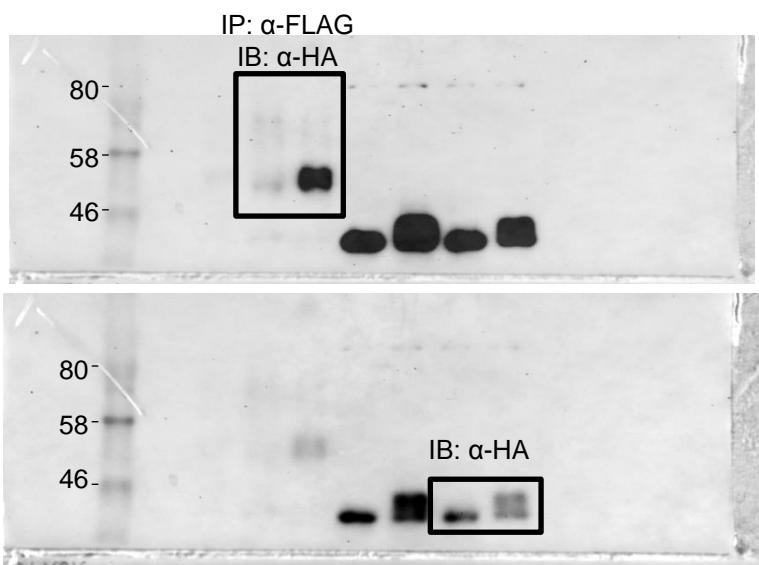


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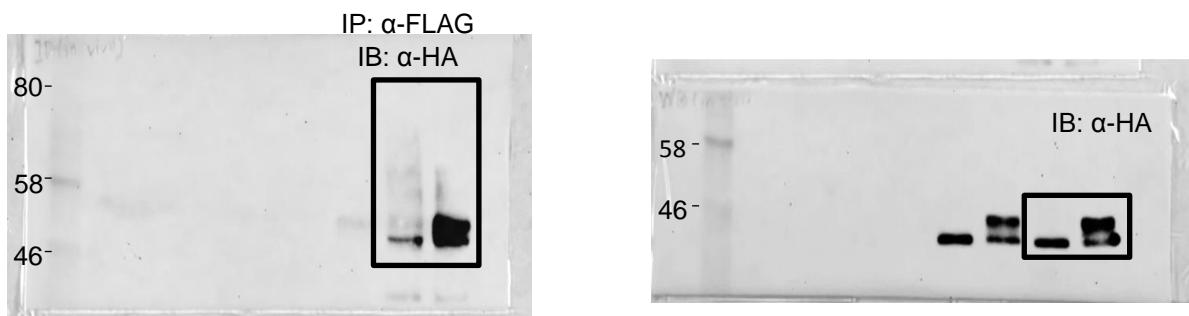
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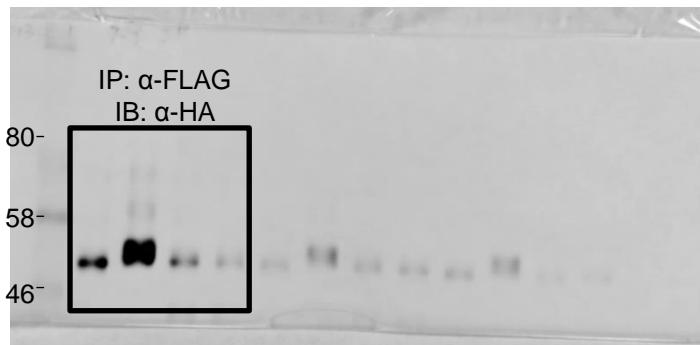


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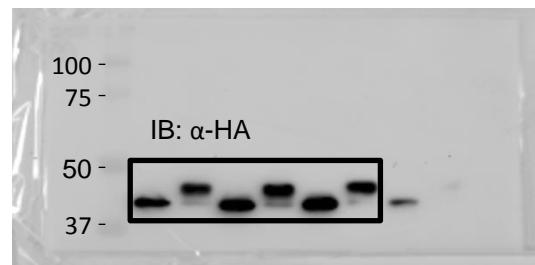
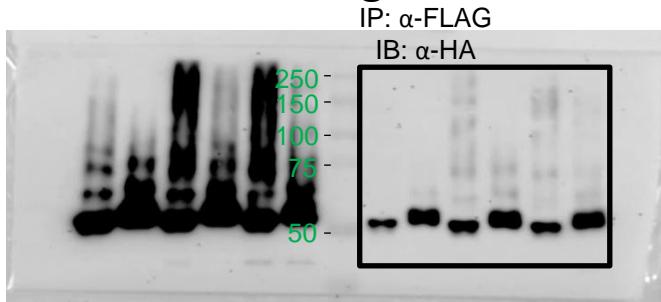
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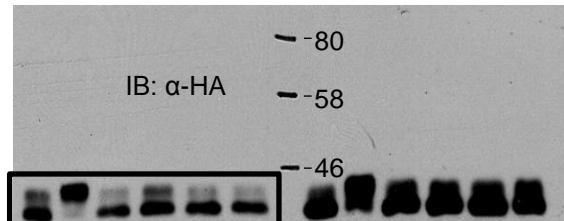
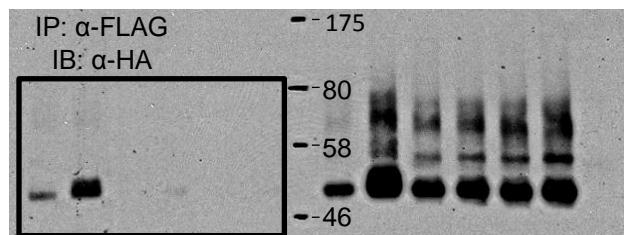
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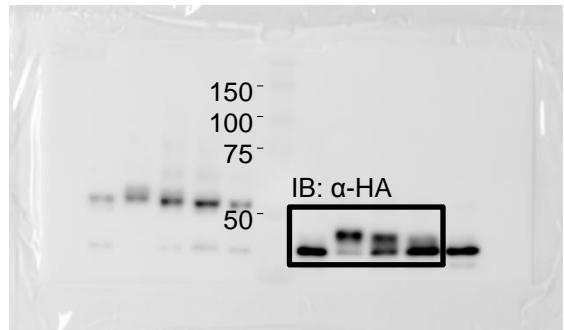
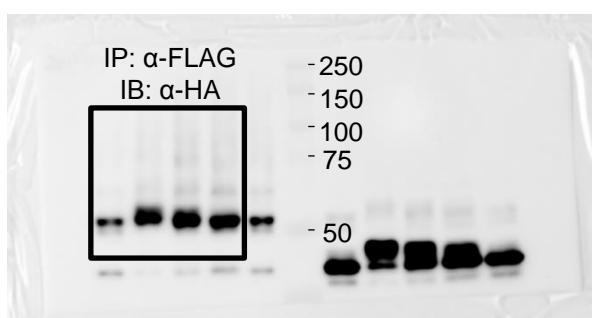
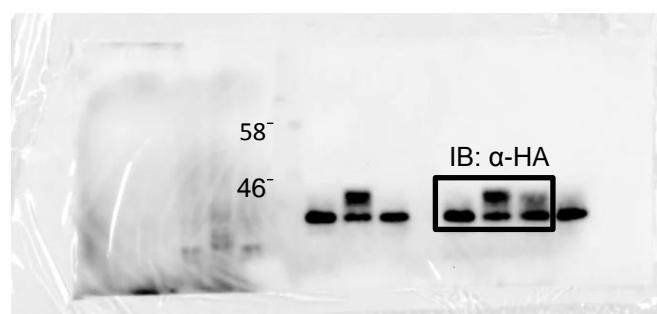
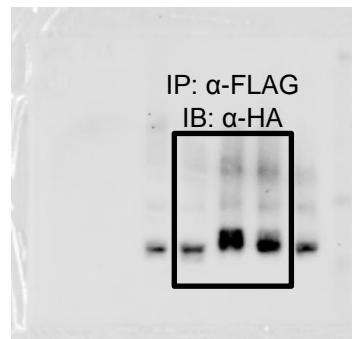
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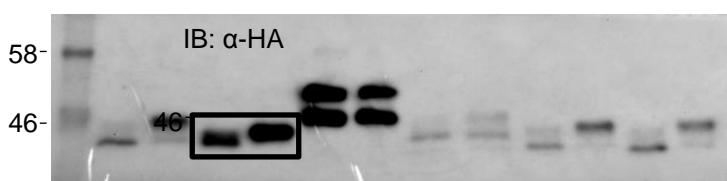
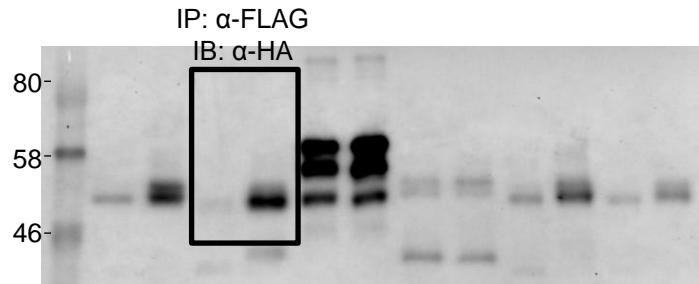
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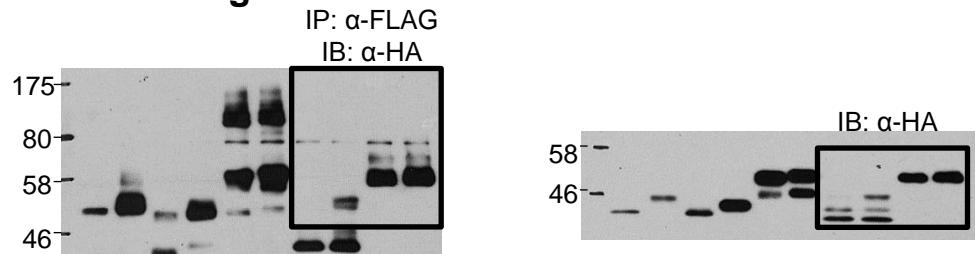
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## Extended Data Figure 2k



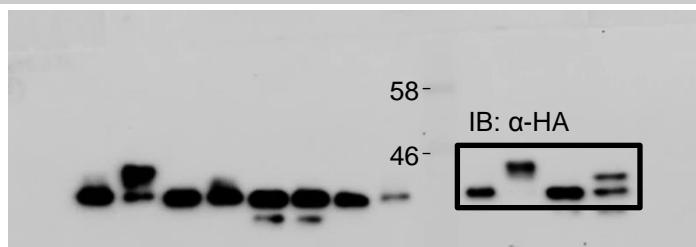
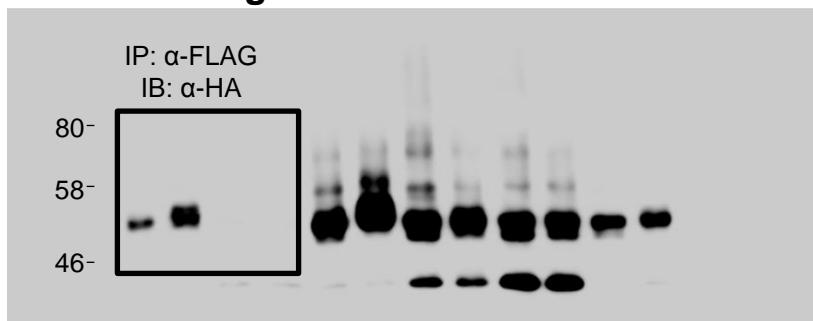
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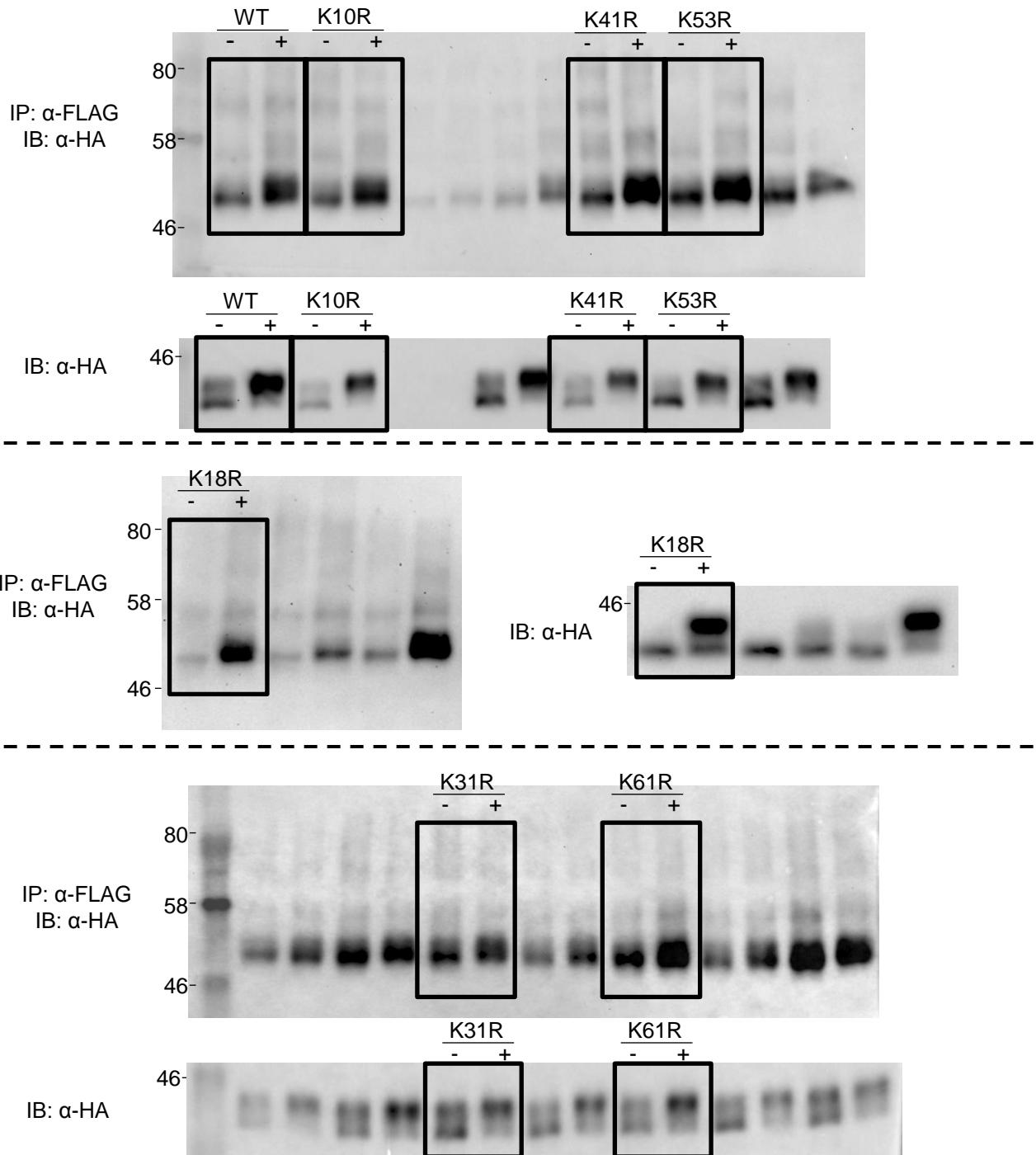
## Extended Data Figure 3a



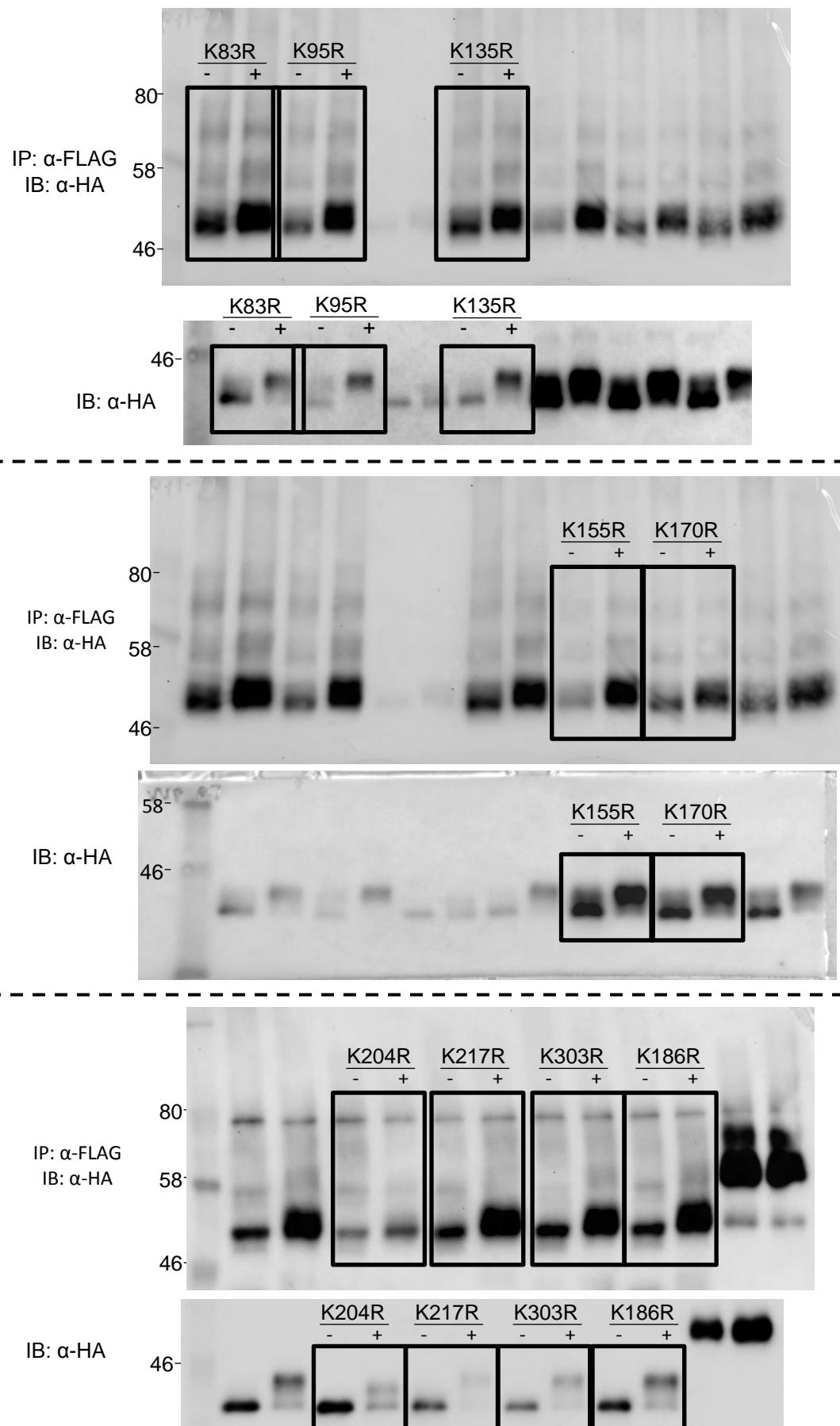
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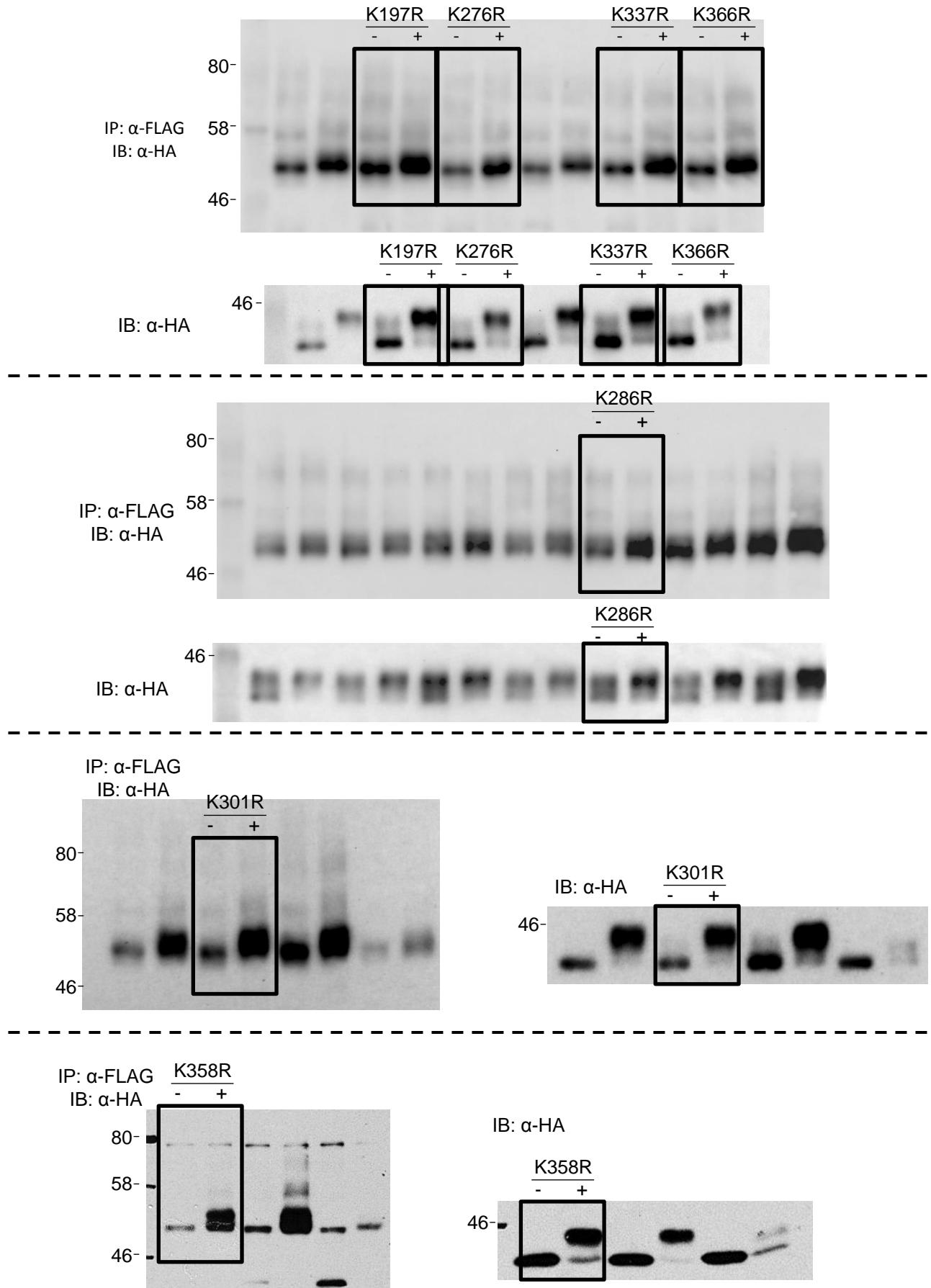
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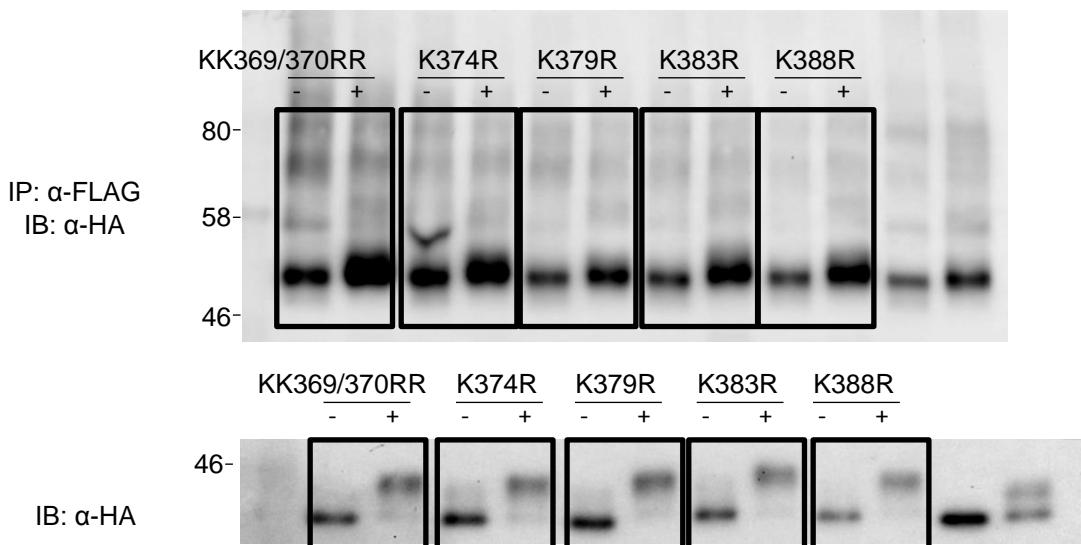
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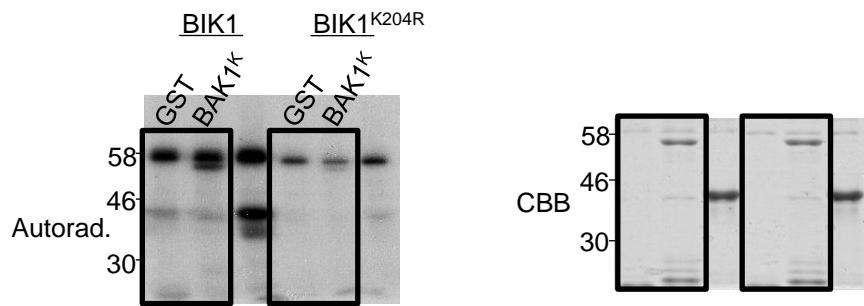
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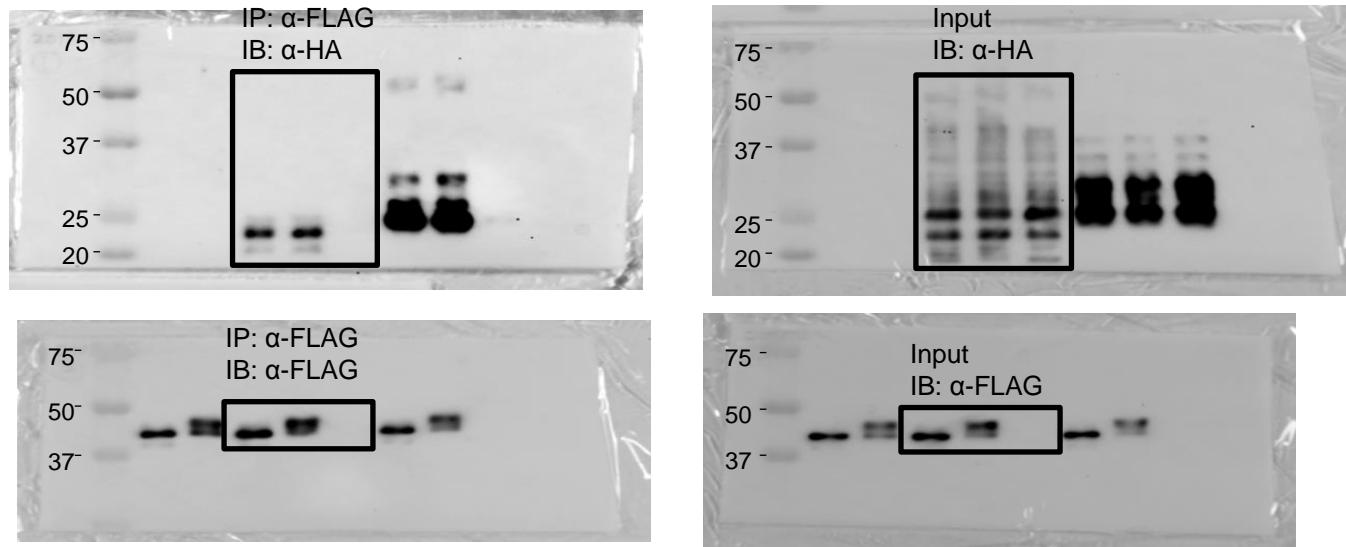
## Extended Data Figure 3d continued



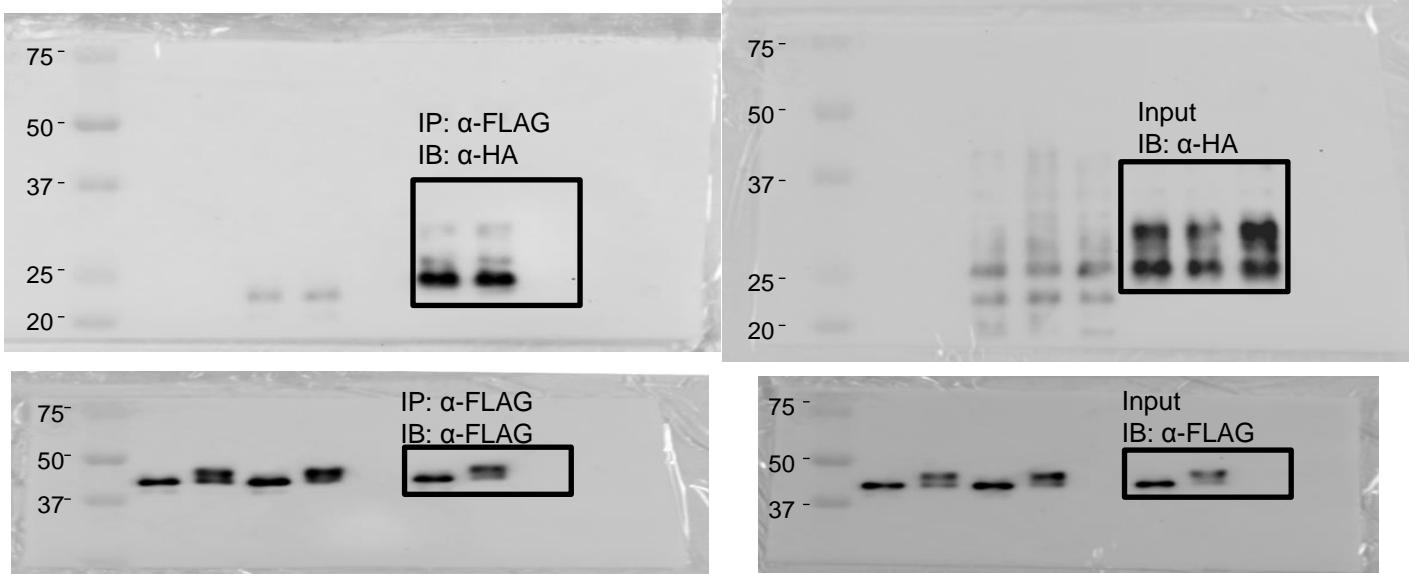
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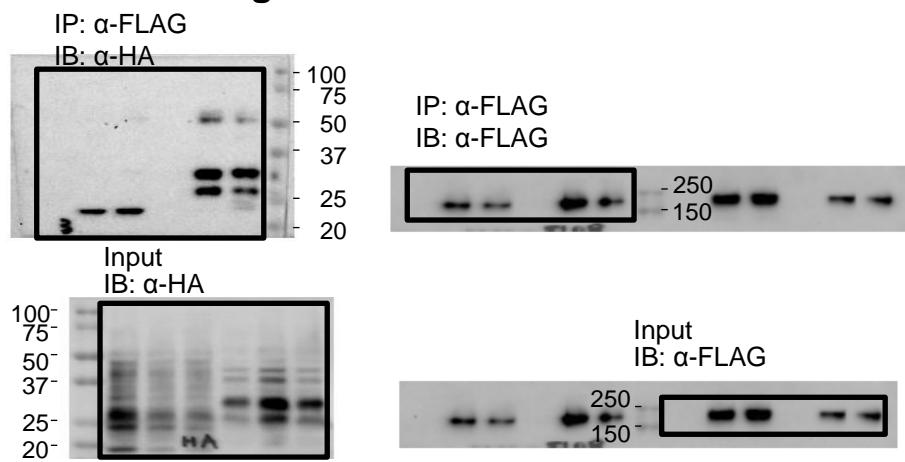
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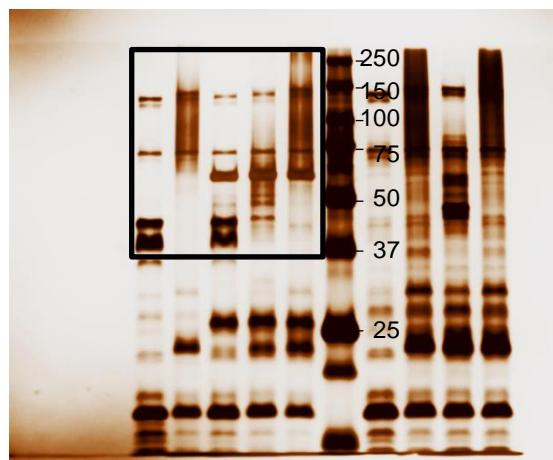
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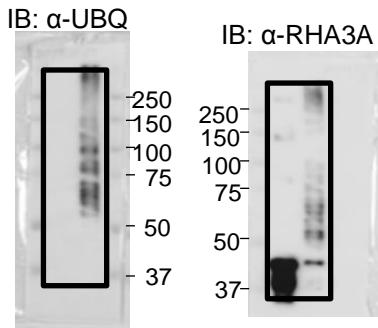
## Extended Data Figure 4d



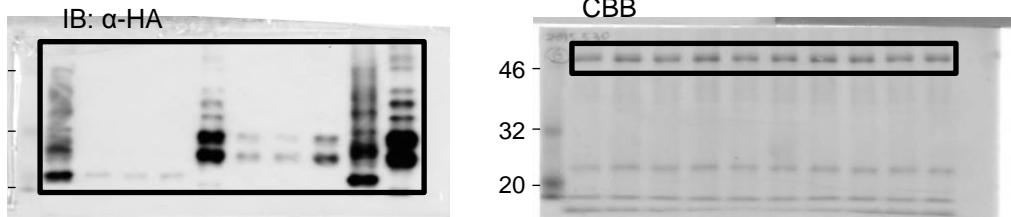
## Extended Data Figure 5a



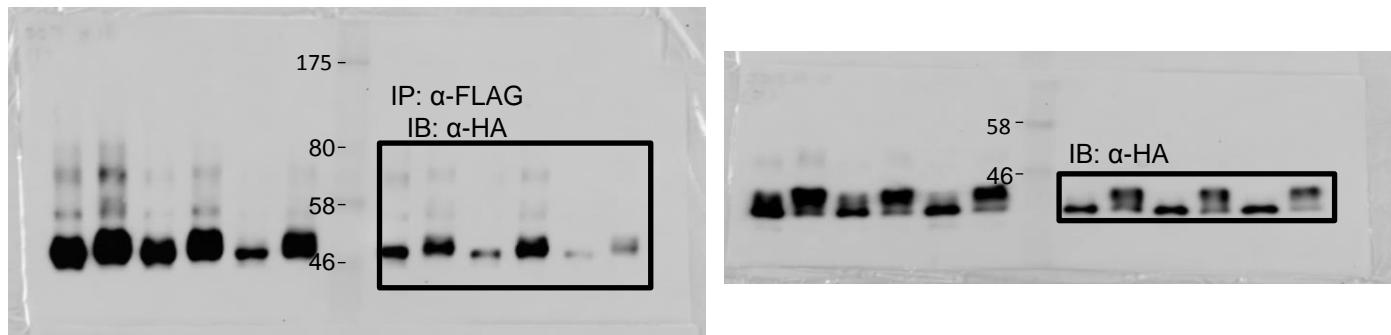
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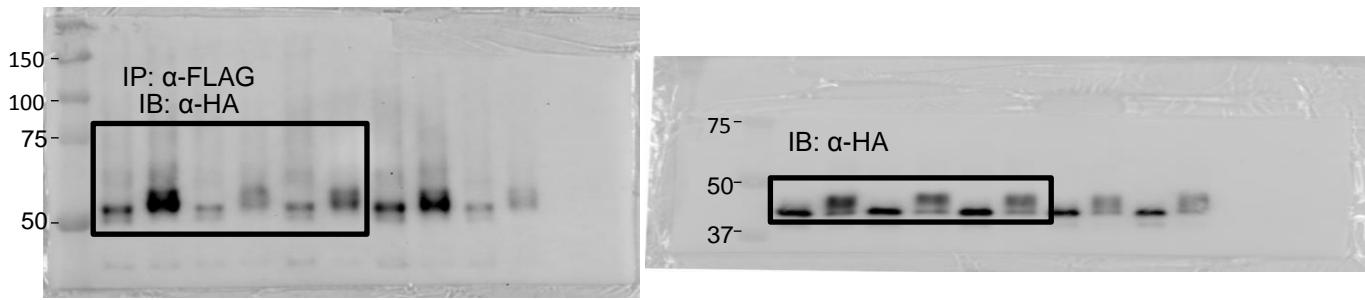
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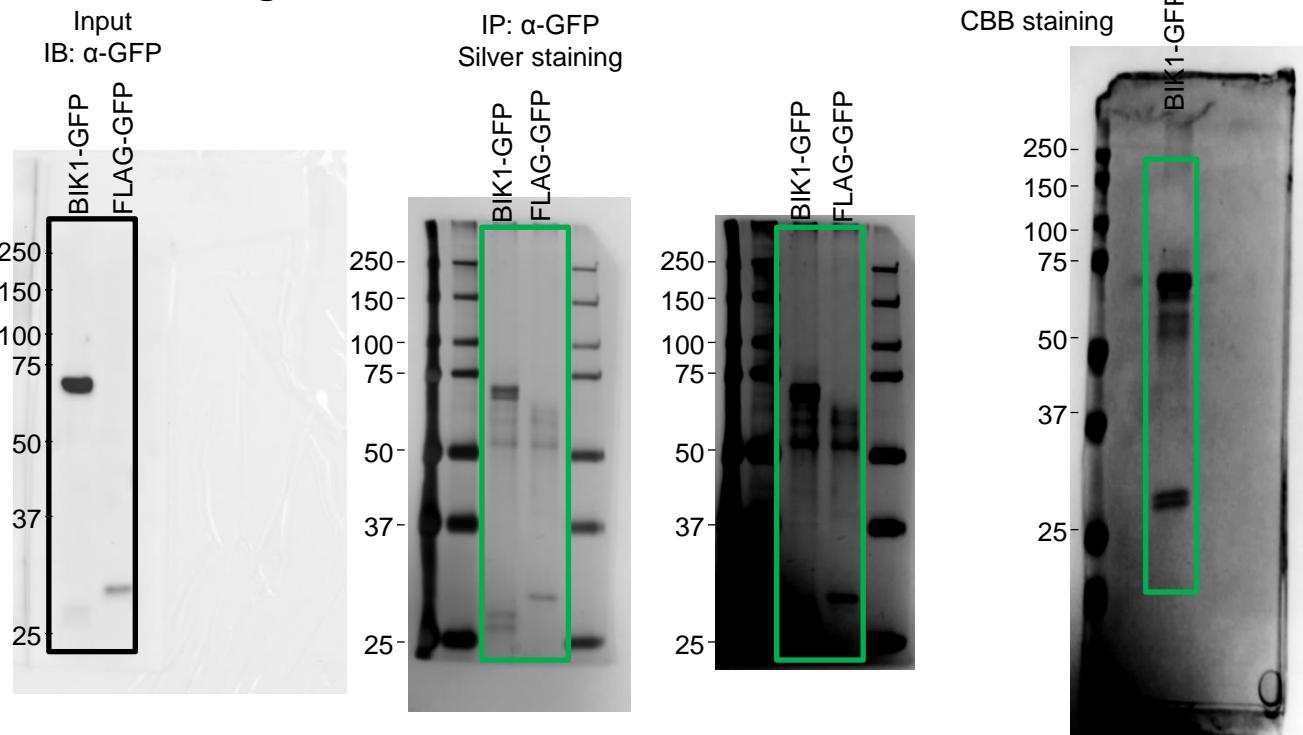
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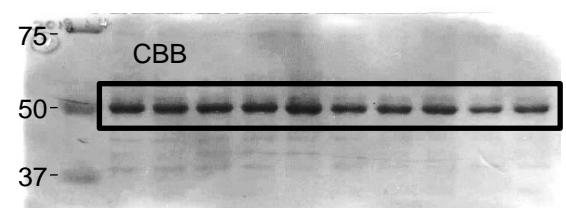
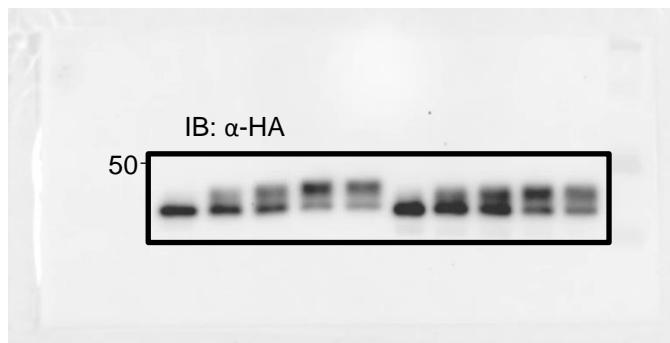
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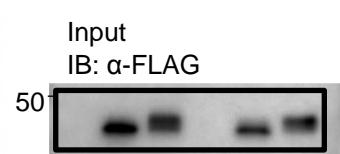
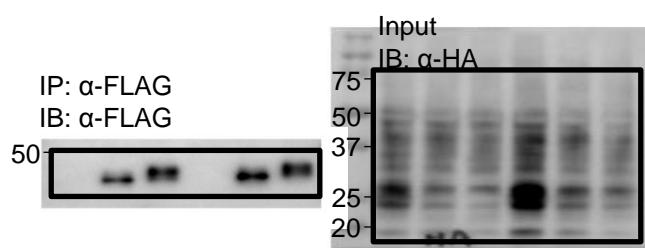
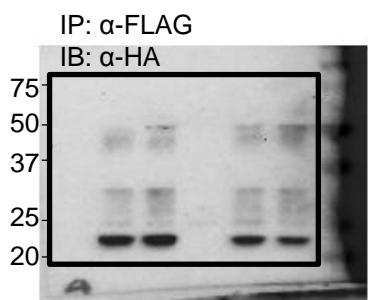
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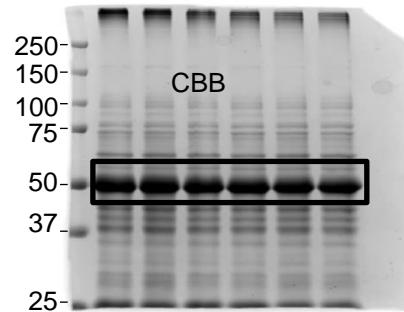
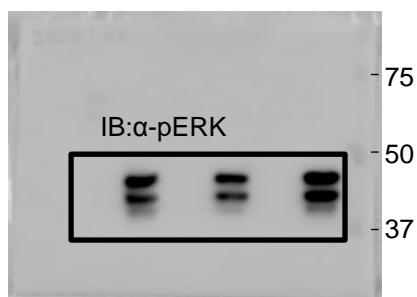
## Extended Data Figure 8a



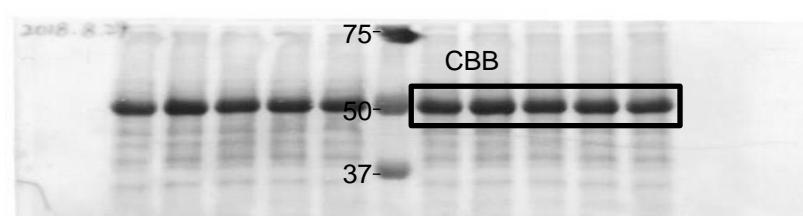
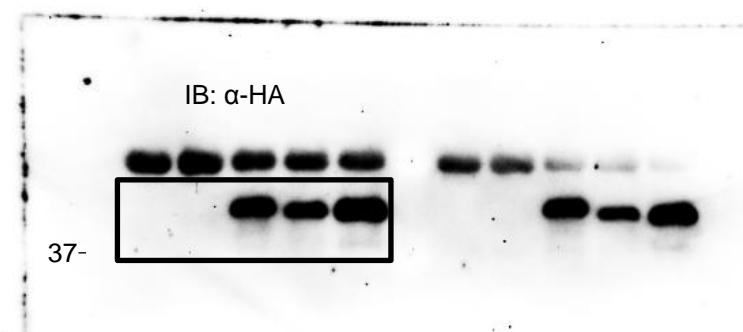
## Extended Data Figure 8b



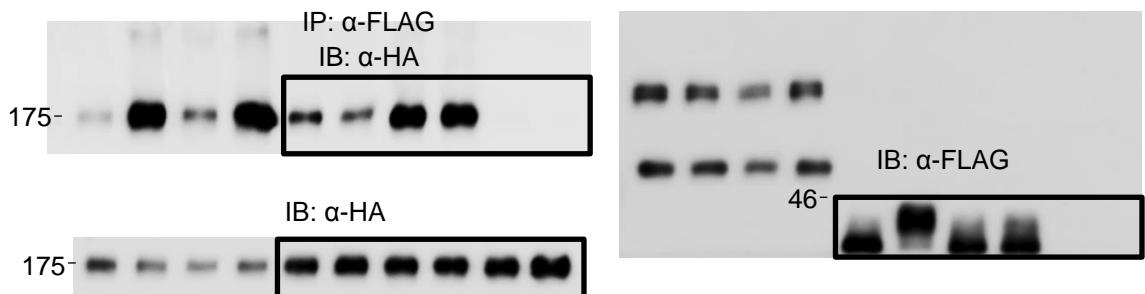
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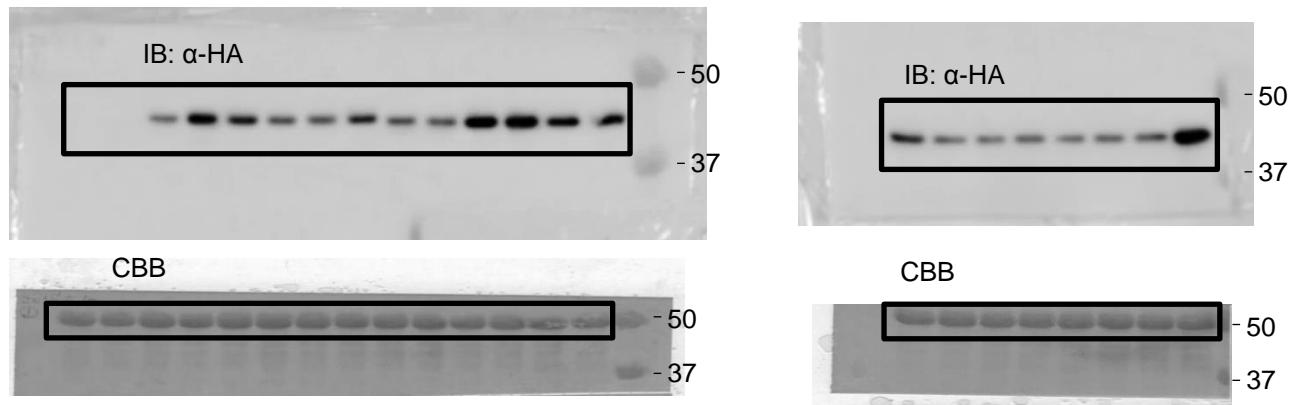
## Extended Data Figure 8f



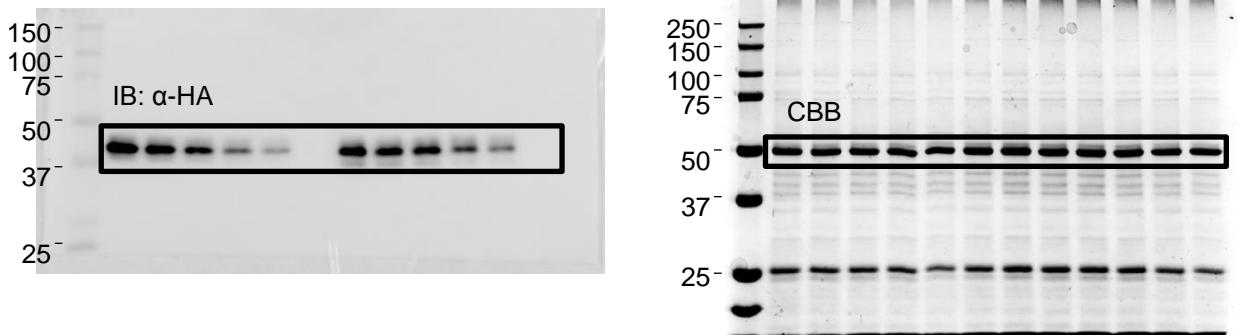
## Extended Data Figure 10a



## Extended Data Figure 10c



## Extended Data Figure 10d



Supplementary Figure 1. Uncropped images.

**Supplementary Table 1. Primers used in this study**

**Cloning primers**

Primer	Sequence
RHA3A-BamHI-F	<u>CGGGATCC</u> CATGACTCGACCGTCAAGATTAC
RHA3A-StuI-R	<u>GAAGGCCT</u> AGGAAGAACGTAGGAATGGTAG
RHA3B-BamHI-F	<u>CGGGATCC</u> CATGACTCGCTCCTCTAGATTC
RHA3B-SmaI-R	TCCCCCGGGAGGAAGAAAAGCAGGAATAATG
RHA3A-CD-BamHI-F	<u>CGGGATCCC</u> GATGCGCTTGGCTCCGGCG
RHA3B-CD-BamHI-F	<u>CGGGATCC</u> GCTCGCTGCGCTTGGCTCCGC
pRHA3A-SacI-F	CCGGAG <u>CTC</u> CTCAATTGATAGAATTCTCCG
pRHA3A-BamHI-R	CGGGAT <u>CCT</u> GCTTTTGAGCTTGGAGAC
pRHA3B-SacI-F	TCCGAG <u>CTC</u> GCTTCCGTTCTCAATTATTG
pRHA3B-BamHI-R	<u>CGGGATCC</u> TTGTTGGTTGAGTTC
NOS-SacI-R	CCGGAG <u>CTC</u> GAATACTCATGTTGACAGC
Usp2-cc-BamHI-F	<u>CGGGATCC</u> AATTCAAAGAGTGCCAGGGTC
Usp2-cc-SmaI-R	TCCCCGGGCATACGGGAGGGTGGACTGGC

The restriction enzyme sites are underlined.

**Point mutation primers**

Primer	Sequence
BIK1 K10R-F	CTTCAGTTCTCGAGTC <u>A</u> GAGCAGACATCTTCCAC
BIK1 K10R-R	GTGGAAGATGTCTG <u>C</u> T <u>G</u> ACTCGAGAACTGAAG
BIK1 K18R-F	CATCTTCCACAATGG <u>T</u> <u>G</u> GAGCAGCGATCTTATG
BIK1 K18R-R	CATAAAGATCG <u>T</u> GCT <u>C</u> <u>C</u> TACCATTGTGGAAAGATG
BIK1 K31R-F	GTCTCTCAAGTC <u>GG</u> <u>A</u> T <u>C</u> GTCTTCGACTGTAG
BIK1 K31R-R	CTACAGTCGAAGACG <u>A</u> <u>C</u> <u>T</u> CCGACTTGAGAGAC
BIK1 K41R-F	CGGCGG <u>C</u> T <u>A</u> <u>G</u> GGACGGAAGGGGAG
BIK1 K41R-R	CTCCCC <u>T</u> CCG <u>C</u> <u>T</u> CTGAGCCGCC
BIK1 K53R-F	GAGTC <u>G</u> ACCCCTGT <u>C</u> <u>A</u> <u>G</u> CTTCACCTTAACG
BIK1 K53R-R	CGTTAAAGGTGAAG <u>C</u> <u>T</u> GACAGGGGTCGA <u>A</u> CTC
BIK1 K61R-F	CACCTTAACGA <u>A</u> <u>C</u> <u>T</u> CGCCACAAGAAAC
BIK1 K61R-R	GTTTCTTGTGG <u>C</u> <u>G</u> AGT <u>C</u> <u>T</u> GAGTT <u>A</u> AGGTG
BIK1 K83R-F	CTTTGGTTGT <u>G</u> T <u>C</u> <u>T</u> <u>A</u> <u>G</u> GGCTGGTTAGATGAG
BIK1 K83R-R	CTCAT <u>T</u> AA <u>C</u> <u>C</u> <u>G</u> <u>C</u> <u>T</u> <u>A</u> <u>A</u> <u>G</u> ACACAACCAAAG
BIK1 K95R-F	CACT <u>T</u> CA <u>T</u> CC <u>G</u> <u>A</u> <u>C</u> <u>T</u> <u>A</u> <u>G</u> ACCTGGAACCGGTTG
BIK1 K95R-R	CAAACCGG <u>T</u> CC <u>A</u> <u>G</u> <u>G</u> <u>T</u> <u>C</u> <u>A</u> <u>G</u> TCGGAGTGAGAGTG
BIK1 K135R-F	CACCCAA <u>A</u> <u>T</u> CTAG <u>T</u> <u>A</u> <u>G</u> ACTGATCGGTTATTGC
BIK1 K135R-R	GCAATAACCG <u>A</u> <u>C</u> <u>G</u> <u>T</u> <u>C</u> <u>A</u> <u>T</u> AGATTGGGTG
BIK1 K155R-F	CTACGAGTTAT <u>G</u> <u>C</u> <u>A</u> <u>A</u> <u>G</u> AGGAAGTCTTGAGAATC

BIK1 K155R-R	GATTCTCAAGACTTC <u>C</u> TTTGATAA <u>A</u> CTCGTAG
BIK1 K170R-F	GAGGTGCATATTT <u>A</u> G <u>C</u> CCACTCCCATGGTTTC
BIK1 K170R-R	GAAACC <u>A</u> TGGAGTGG <u>C</u> TA <u>A</u> ATATGCACCTC
BIK1 K186R-F	GCG <u>C</u> TTGATGCAG <u>C</u> A <u>G</u> GGGGCTGCTTCTTC
BIK1 K186R-R	GAAGAAA <u>A</u> GCAAG <u>C</u> CCC <u>T</u> GCTGCATCAAGCGC
BIK1 K197R-F	CACAG <u>C</u> GAT <u>C</u> CGTC <u>A</u> G <u>G</u> GTGATATACCGAGAC
BIK1 K197R-R	GT <u>C</u> TCGGTATATCA <u>T</u> GACGGGATCGCTGTG
BIK1 K204R-F	GATATAC <u>C</u> CGAGACATT <u>A</u> G <u>G</u> CCTCGA <u>A</u> ACATCTTAC
BIK1 K204R-R	GTAAGATGTC <u>G</u> AGG <u>C</u> T <u>A</u> ATGTCTCGGTATATC
BIK1 K217R-F	GC <u>G</u> GA <u>C</u> TAAC <u>C</u> GA <u>A</u> <u>G</u> ACTTCTGACTTGGAC
BIK1 K217R-R	GT <u>C</u> CAA <u>A</u> GT <u>C</u> AGAA <u>A</u> GT <u>C</u> TTGC <u>G</u> TTGTAG <u>C</u> CGC
BIK1 K276R-F	GAG <u>A</u> TTTATCTGG <u>T</u> <u>A</u> G <u>G</u> CGAGCG <u>G</u> TTGGATC
BIK1 K276R-R	GAT <u>C</u> CAAC <u>C</u> GCT <u>C</u> <u>T</u> <u>A</u> CC <u>A</u> GATAAA <u>A</u> CTC
BIK1 K286R-F	CATAAC <u>A</u> GA <u>C</u> GG <u>C</u> <u>T</u> <u>A</u> <u>G</u> AGAA <u>A</u> AC <u>C</u> TTGTG
BIK1 K286R-R	CACAAG <u>G</u> TTT <u>C</u> TT <u>C</u> <u>T</u> <u>A</u> <u>G</u> CCGGTCTGTTATG
BIK1 K301R-F	CGTAC <u>C</u> CT <u>C</u> ACA <u>A</u> GC <u>A</u> <u>G</u> GT <u>A</u> AG <u>G</u> TT <u>C</u> CT <u>C</u> TA <u>A</u> TC
BIK1 K301R-R	GATTAG <u>G</u> AG <u>A</u> AC <u>C</u> TT <u>A</u> CG <u>T</u> <u>C</u> <u>T</u> <u>G</u> CT <u>T</u> <u>G</u> AG <u>G</u> TAC <u>G</u>
BIK1 K303R-F	C <u>C</u> TC <u>A</u> AG <u>C</u> AA <u>A</u> CG <u>T</u> <u>A</u> <u>G</u> GG <u>T</u> <u>T</u> <u>C</u> CT <u>A</u> AT <u>G</u> TGG
BIK1 K303R-R	CCACGATTAG <u>G</u> AG <u>A</u> AC <u>C</u> <u>C</u> <u>T</u> <u>A</u> CG <u>T</u> <u>T</u> <u>G</u> CT <u>T</u> <u>G</u> GAGG
BIK1 K337R-F	CT <u>C</u> TC <u>A</u> TT <u>G</u> A <u>A</u> CC <u>C</u> <u>A</u> <u>G</u> TC <u>G</u> CG <u>CC</u> CG <u>A</u> CC <u>A</u> T <u>G</u>
BIK1 K337R-R	CAT <u>G</u> GT <u>C</u> GG <u>GG</u> CG <u>G</u> CG <u>A</u> <u>C</u> <u>T</u> <u>G</u> GG <u>T</u> <u>T</u> CAA <u>A</u> T <u>G</u> AG <u>A</u> G
BIK1 K358R-F	GG <u>A</u> CA <u>A</u> CT <u>T</u> GG <u>G</u> <u>A</u> <u>C</u> <u>G</u> CG <u>A</u> GT <u>C</u> AG <u>A</u> CC
BIK1 K358R-R	GG <u>T</u> CT <u>G</u> ACT <u>CG</u> <u>G</u> <u>C</u> <u>G</u> T <u>CC</u> CA <u>A</u> GT <u>T</u> GT <u>C</u> CC
BIK1 K366R-F	CAG <u>A</u> CCA <u>A</u> TC <u>CG</u> GT <u>T</u> <u>A</u> <u>G</u> GG <u>A</u> T <u>AC</u> CA <u>A</u> GA <u>A</u> ACT <u>T</u> G
BIK1 K366R-R	CA <u>A</u> G <u>T</u> TT <u>C</u> T <u>G</u> GT <u>T</u> <u>A</u> <u>C</u> <u>C</u> <u>T</u> <u>A</u> AC <u>CG</u> G <u>T</u> <u>T</u> GG <u>T</u> CT <u>G</u>
BIK1 KK369/370RR-F	CG <u>G</u> TT <u>A</u> AG <u>G</u> AT <u>AC</u> CA <u>A</u> <u>G</u> <u>G</u> AG <u>A</u> CT <u>T</u> GG <u>T</u> <u>T</u> <u>T</u> <u>A</u> AA <u>A</u> CT <u>G</u>
BIK1 KK369/370RR-R	CAG <u>T</u> TT <u>A</u> AA <u>A</u> CC <u>A</u> AG <u>T</u> <u>C</u> <u>T</u> <u>C</u> <u>T</u> <u>G</u> GT <u>T</u> <u>A</u> CC <u>T</u> <u>T</u> <u>A</u> AC <u>CG</u>
BIK1 K374R-F	CA <u>A</u> G <u>A</u> AA <u>A</u> CT <u>T</u> GG <u>T</u> <u>T</u> <u>T</u> <u>A</u> <u>G</u> AA <u>T</u> GG <u>T</u> <u>A</u> CT <u>A</u> CT <u>A</u> AG
BIK1 K374R-R	CT <u>T</u> TA <u>G</u> GT <u>A</u> CC <u>A</u> GT <u>T</u> <u>C</u> <u>T</u> <u>A</u> AA <u>A</u> CC <u>A</u> AG <u>T</u> <u>T</u> CT <u>T</u> G
BIK1 K379R-F	GT <u>T</u> TT <u>A</u> AA <u>A</u> CT <u>T</u> GG <u>T</u> <u>A</u> CT <u>A</u> CT <u>A</u> <u>G</u> GT <u>C</u> AT <u>CC</u> GA <u>AA</u> AC <u>CG</u>
BIK1 K379R-R	CG <u>T</u> TT <u>T</u> CG <u>G</u> AT <u>G</u> AC <u>C</u> <u>T</u> <u>A</u> GT <u>T</u> <u>A</u> CC <u>A</u> GT <u>T</u> <u>T</u> <u>A</u> AA <u>A</u> C
BIK1 K383R-F	CT <u>A</u> AG <u>T</u> CAT <u>CC</u> GA <u>A</u> <u>A</u> <u>G</u> AC <u>GG</u> TT <u>A</u> CA <u>A</u> AA <u>A</u> AC
BIK1 K383R-R	GT <u>T</u> TT <u>T</u> GT <u>A</u> AA <u>A</u> CC <u>G</u> T <u>C</u> <u>T</u> <u>T</u> CG <u>G</u> AT <u>G</u> ACT <u>T</u> AG
BIK1 K388R-F	GAAAA <u>A</u> CG <u>T</u> <u>T</u> <u>T</u> AC <u>A</u> AA <u>A</u> <u>G</u> AC <u>C</u> TT <u>T</u> GG <u>C</u> AG <u>G</u> C
BIK1 K388R-R	GC <u>C</u> TC <u>G</u> CA <u>AA</u> AG <u>G</u> T <u>C</u> <u>T</u> <u>T</u> GT <u>T</u> <u>G</u> TA <u>A</u> AC <u>CG</u> TT <u>T</u> T <u>C</u>
RHA3A I104A-F	CG <u>A</u> CG <u>G</u> GA <u>A</u> GT <u>T</u> GT <u>C</u> <u>T</u> <u>G</u> CT <u>C</u> ACT <u>G</u> ACT <u>T</u> C
RHA3A I104A-R	GA <u>A</u> GT <u>C</u> AG <u>T</u> GA <u>G</u> GA <u>C</u> <u>A</u> <u>G</u> CA <u>C</u> AT <u>CC</u> GT <u>CG</u>

The codon of targeted amino acids is underlined. Mutated nucleotides are marked as red.

### amiRNA primers

Primer	Sequence
RHA3A amiRNA480-1	GATTTGTCAATACTCCACGGTCTCTTTGTATTCC
RHA3A amiRNA480-2	GACCGTGGAGTGTATTGACAAAATCAAAGAGAACATGA
RHA3A amiRNA480-3	GACCAGGGAGTGTATAGACAAATTCACAGGTCGTGATATG
RHA3A amiRNA480-4	GAATTGTCTATACTCCATGGTCTACATATATATTCC
RHA3A amiRNA109-1	GATCAAGTAATCTTGACGGTCGTTCTCTTTGTATTCC
RHA3A amiRNA109-2	GAACGACCGTCAAGATTACTGATCAAAGAGAACATGA
RHA3A amiRNA109-3	GAACAACCGTCAAGAATACTTGTTCACAGGTCGTGATATG
RHA3A amiRNA109-4	GAACAAGTATTCTTGACGGTTGTTCTACATATATATTCC
RHA3A amiRNA211-1	GATCAACGCAGATAAGAGCGCTATCTCTTTGTATTCC
RHA3A amiRNA211-2	GATAGCGCTTTATCTCGTTGATCAAAGAGAACATGA
RHA3A amiRNA211-3	GATAACGCTTTATCAGCGTTGTTCACAGGTCGTGATATG
RHA3A amiRNA211-4	GAACAAACGCTGATAAGAGCGTTATCTACATATATATTCC
RHA3B amiRNA444-1	GATTATGCATATTGCACACTCCGTCTCTTTGTATTCC
RHA3B amiRNA444-2	GACGGAGTGTGCAATATGCATAATCAAAGAGAACATGA
RHA3B amiRNA444-3	GACGAAGTGTGCAATTGCATATTCACAGGTCGTGATATG
RHA3B amiRNA444-4	GAATATGCAAATTGCACACTTCGTCTACATATATATTCC
RHA3B amiRNA113-1	GATAATCTAGAGGAGCGAGTCAGTCTCTTTGTATTCC
RHA3B amiRNA113-2	GACTGACTCGCTCCTCTAGATTATCAAAGAGAACATGA
RHA3B amiRNA113-3	GACTAACTCGCTCCTGTAGATTTCACAGGTCGTGATATG
RHA3B amiRNA113-4	GAAAATCTACAGGAGCGAGTTAGTCTACATATATATTCC
RHA3B amiRNA214-1	GATCTACGCATACGAGAGCGCATTCTCTTTGTATTCC
RHA3B amiRNA214-2	GAATGCGCTCTCGTATCGTAGATCAAAGAGAACATGA
RHA3B amiRNA214-3	GAATACGCTCTCGTAAGCGTAGTTCACAGGTCGTGATATG
RHA3B amiRNA214-4	GAACATACGCTTACGAGAGCGTATTCTACATATATATTCC

### CRISPR cloning primers

Primer	Sequence
L1L2T2-BsF	ATATATGGTCTCGATTGAGCCAAGCGCATCGTACGAGTT
L1L2T2-F	TGAGCCAAGCGCATCGTACGAGTTAGAGCTAGAAATAGC
L1L2T4-R	AACGAGTTAACCGGCCGTCAATCTCTAGTCGACTCTAC
L1L2T4-BsR	ATTATTGGTCTCGAACGAGTTAACCGGCCGTCAATCTCTAGTCGACT

### **qRT-PCR primers**

Primer	Sequence
RHA3A-F	CAGGATTCTTACGCCGGTGA
RHA3A-R	TTCATCTCCGCCGTAGAAC
RHA3B-F	CGGAGGGCGAAGAAATCAGA
RHA3B-R	CACTTCACCAGGCACCAGTAT
ACTIN2-F	GCACCCCTGTTCTTCTTACGGA
ACTIN2-R	GTGAGACACACCATCACCAGA