

Laminar shear stress inhibits inflammation by activating autophagy in human aortic endothelial cells through HMGB1 nuclear translocation

Supplementary Information

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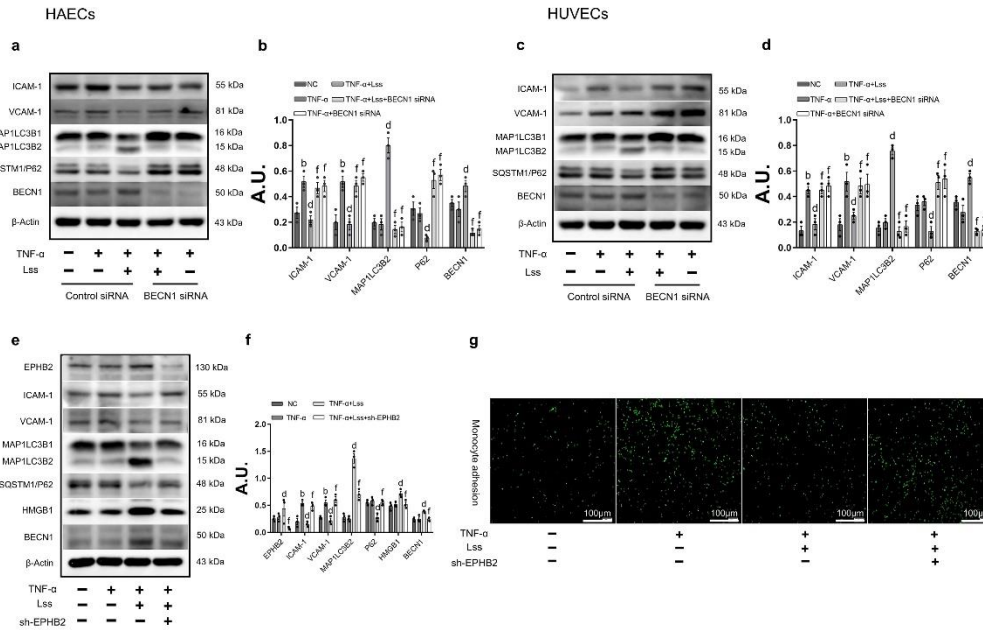


Fig. S1. LSS inhibits the inflammatory response in endothelial cells by activating autophagy. a-b. Western Blot shows the effect of BECN1 siRNA on anti-inflammatory effect of LSS in HAECs. c-d. Western Blot shows the effect of BECN1 siRNA on anti-inflammatory effect of LSS in HUVECs. e-g. Western Blot and confocal microscopy shows the effect of sh-EPHB2 on anti-inflammatory effect of LSS. Data are presented as mean $\pm$ SEM of three independent experiments. ^a $P < 0.05$, ^b $P < 0.01$ vs. NC group; ^c $P < 0.05$, ^d $P < 0.01$ vs. TNF- α group. ^e $P < 0.05$, ^f $P < 0.01$ vs. TNF- α +LSS group. AU, arbitrary units.

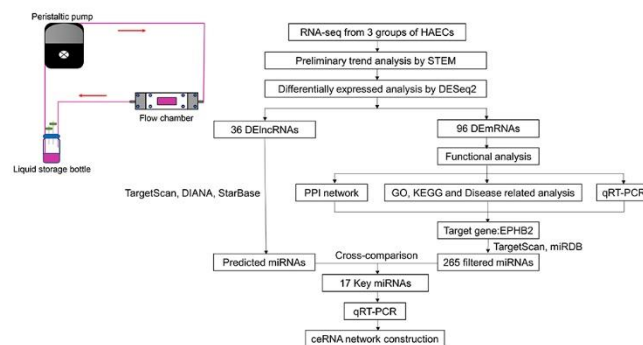


Fig. S2. Flowchart of the research showing the steps involved in construction of the LSS-related LOC107986345/miR-128-3p/EPHB2 regulatory axis.

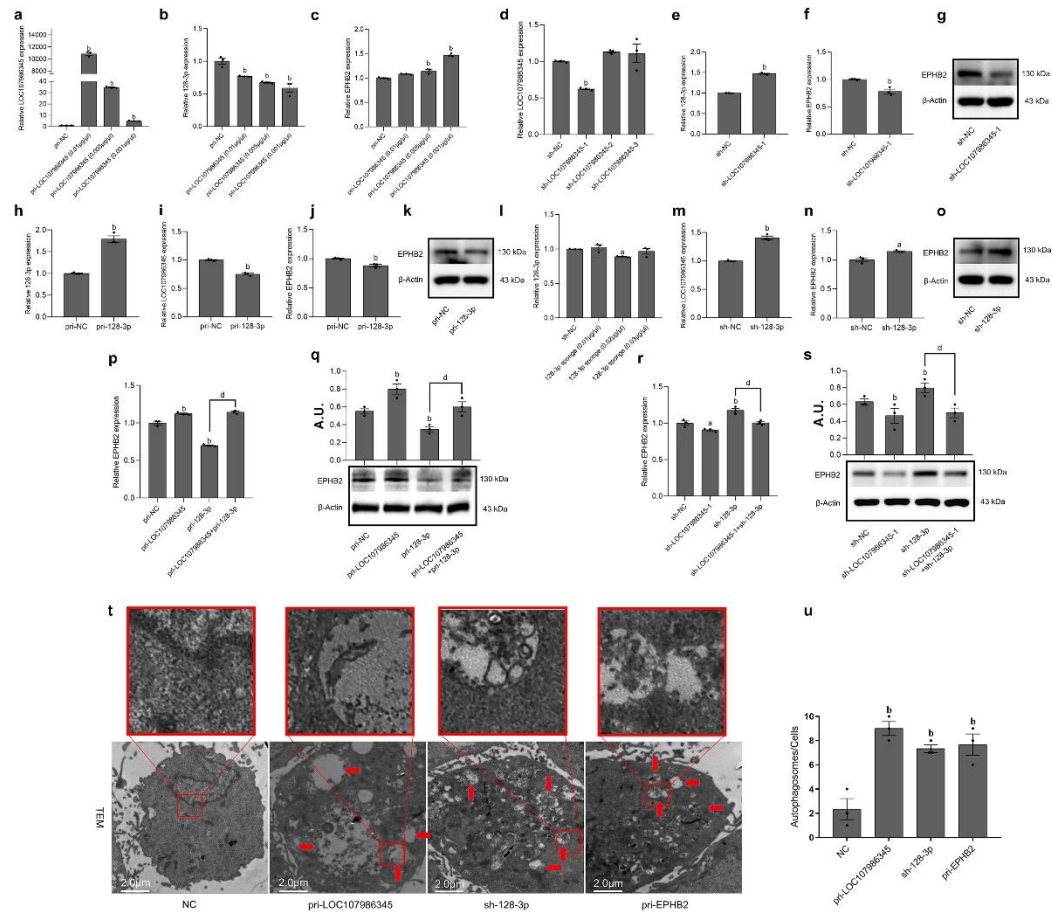


Fig. S3. Construction of the LSS-related LOC107986345/miR-128-3p/EPHB2 network.

a-c. qRT-PCR shows the expression of LOC107986345, miR-128-3p and EPHB2 after pri-LOC107986345. d-g. qRT-PCR and Western Blot shows the expression of LOC107986345, miR-128-3p and EPHB2 after sh-LOC107986345. h-k. qRT-PCR and Western Blot shows the expression of LOC107986345, miR-128-3p and EPHB2 after pri-miR-128-3p. l-o. qRT-PCR and Western Blot shows the expression of LOC107986345, miR-128-3p and EPHB2 after sh-miR-128-3p. p-q. qRT-PCR and Western Blot shows the expression of EPHB2 in endothelial cells co-transfected with LOC107986345 and miR-128-3p overexpression plasmids. r-s. qRT-PCR and Western Blot shows the expression of EPHB2 in endothelial cells co-transfected with LOC107986345 and miR-128-3p knockdown plasmids. t-u. TEM shows autophagosomes and autophagolysosomes in HAECs. Arrows indicate autophagosomes. Data are presented as mean $\pm$ SEM of three independent experiments. ^a $P < 0.05$, ^b $P < 0.01$ vs. NC group; ^c $P < 0.05$, ^d $P < 0.01$ vs. pri-miR-128-3p or sh-miR-128-3p group. AU, arbitrary units.

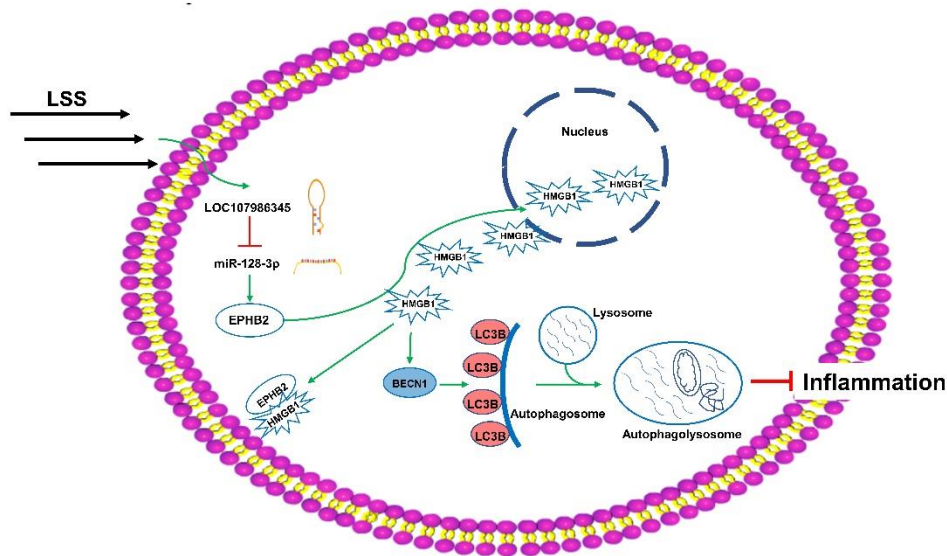


Fig. S4. Schematic diagram of anti-inflammatory effect of LSS through LOC107986345/miR-128-3p/EPHB2 axis in an autophagy-dependent pathway which induced by nuclear translocation of HMGB1 in endothelial cells.

When endothelial cells were treated with LSS, LSS-sensitive lncRNA, LOC107986345, combines with miR-128-3p to increase the expression of EPHB2. Up-regulation of EPHB2 expression can promote nuclear translocation of HMGB1, which can activate the autophagy pathway of endothelial cells. Autophagy activation significantly inhibited the increased expression of ICAM-1, VCAM-1, COX-2, and MMP-9 caused by TNF- α stimulation. The arrows represent promotion, whereas the inverted T means inhibition.

Table S1. 17 key miRNAs after the intersection of miRNAs that potentially bind to EPHB2 (Table 3) and miRNAs predicted by 36 DE lncRNAs (Table 4).

miRNA	lncRNA
hsa-miR-4306	LOC105374005, AC008505.1
hsa-miR-216a-3p	LOC107986196, LOC107986345
hsa-miR-128-3p	LOC107986196, LOC107986345
hsa-miR-27a-3p	LOC107986196, LOC107986345
hsa-miR-30c-5p	LOC107986345, MALAT1
hsa-miR-3681-3p	LOC107986345
hsa-miR-495-3p	LOC101930665, SNHG20
hsa-miR-9-5p	LOC101930275
hsa-miR-185-5p	AC008505.1
hsa-miR-4644	AC008505.1
hsa-miR-23c	AC008505.1, MALAT1
hsa-miR-23a-3p	AC008505.1, MALAT1
hsa-miR-150-5p	MALAT1
hsa-miR-204-5p	MALAT1
hsa-miR-211-5p	MALAT1
hsa-miR-23b-3p	MALAT1
hsa-miR-5688	SNHG20

Table S2. Sequence information for LOC107986345

LncRNA	FASTA
LOC107986345, XR_001742403.1	>XR_001742403.1 PREDICTED: Homo sapiens uncharacterized LOC107986345 (LOC107986345), ncRNA AAATTTTGCTTTCAGCAATTTTCCATTTTTTTCAGCATACTTATCACAATGAGGCAACCAATATGAA TCCTGAGAATGAAGATGGAAAATGAATATTTTAAATCCAATATCGAGTTGTTCTTTCAACATTTTGTAGTTT TAGTTTTAACTGAAAATATAAAGTTACATAATTGTGTGAGGAATAATTTTGCAGAGAAAATTTTAAAAA TTCACAAAAGGAAAAAGAAACCTTTACCATGAGTCTATAACATTGAAAGACTGTGTTAAGTGATTTTAA TAAAGCCAAACCAACACTTTTCAGCAGGAACTAAATGGCAGCATCTGATTTTCACGCTGATACATATGATT AGTGTGGTGCTCACTTTTACCCCTTCATTTTACCTTGGGTCTTGCCATTTTCTTCTATTTTTACGTTTTT TTTTTTTTTTTTCTTTTGGAAAGAGTAATTTTAGGGAGAAAAAACACTTTTTCTCATAGGTCGATTT AAAATGTTGGCCTTACCTTAATCTCCTCTCTCAAACTCAATCCACTATGTAATGGGTC AACATACATCTTTC TGTGAAGGACCAGATAGTAAATATTTAGTCTTTGCAGGCCATATGGTCTCTGTTGCCAGCTCTTCCATT GTGGTGTGAAAGCAGCCATAGACAACACAGAAATGAATAAGTGTAACGTGCCAATAAAAAACAGATGATA TGTTGAATTTAGCTCACAGAGTTTAGCTTGCTGCCCTGCAGGAGGCCCTTGGAGTAAAAGCTTCCTGAG AGGAGGAATTTTGTCTTTTTGTCTCAAGTTCTAGCTCCAGTACCTAAATAGTGCCTGTCCAGCTAGGTA TTGATGAATATTTGAACCTGTTGAACATACACCTAAAATAAACATTTGGCAAGATACAGTACTACACAA TTTGGAAAACACTTGGCTCCCATAGAAATCAAAGCCTTCTGAGTAATTAATTATTTGGCCTGATGATGA ATTACTGTGCCCTGAGATGATAGAGCTAATTTATTTTCAATTCAGTGGGACACACGTTATTTTCACT GTGAATTTGGTTAAAATGAAAAGATTTCTGCTCTAAGTCTGGATAGACCTTTATGTAATAGCATACTC TTCCTCTTTTTGAATCGCATGCAGTTGTCACTGCGATGATTTCCAGACAGAGGTTCCAAGTCTTTCAT CATGTTTGGGTAAAAGGCCCTCATTAACATACTAGTCTGCCATTTGAGTCTGTTCTCTTACGGAATATT TTCACCTGAATCAGTGGGTATAATTATCAGTGTCTGGTTGCTTCAAGTTATTTTCTTAATGCTGATGT TAATGCATGCCATCTTTATGCCTCAACACATTGCACAATGAAAACAAAAATATTTTGAAGACACAG CTGACATCTATATGAAATATTCAGTATCAGTGATTGAACCTTCAGCAAGCTCCTGTGGCCAGCAGGGTTTT ACGGAGGTGCAACTGCTCTCCCATGATCACTTATCATAAAGCCAGAGACAATTGGGCCAACGTAGCATCT TGCCTGCTTTTGTGTACAAAATATAAATAGAAATAGAGCAAAACAGGAAAAATAGTCCCGCAGCTGGAAGG CAACTTTAAAGAAAAATGATGTTTATAGCTATCTTGCCAGTAGCTGGCAAAGCAGTTTTTATAAGACATA TTTAATGTTTACAACCACTTGGAGGCTGGGGAAGGAGTAATACTTTGCAAAATATGTTAATGTTGTGAAC CACTCTGTGGCCGAGAAGCAACTCCGGCTAAAATATATTTAATGCTTAGAGTCATTTGGTGGTTTCGAAA AAAAAGAAACTTCTTGTAAATTTTAAACATTTATTGCTGATCAGTGGCTGCTAAAGTAACTATCAGTACA GCATGGTTTATGTTTAGGGACACGCTGTGCTGCTACTAACACAATTCATACTCTTCTCTCAAGGCTACAC AAAAAGTGTCAATAGCATACCTTTGCAATTGCACCACTAATAAAAAACACAGGAATCAAACAGACGAATA AAATGCTCCCTGCCACACAGCCATAACATCTATGGCCCTGAAAAATAGTCTGCCAAACTGCGTGCAGT GGACACTTCACGGCTGGCAGAGGAGATCACAAGGCCTTGCTCAATTTTCATCAAAAGTGTTAAAGCGAC TCAGCAGATTGTGAAGCACAAAGTGGAAGCTGATAATTGGTGTTCCTTACAAATCAACGGCTTGTCTCCAC ACATCAAGGTGAGAAAATCAATGTAAGAGGAGGAGAAATAACCCACACAACACATACATACTAGGAAATGA TAGTTGGATCTGAACAAAAAGGATATGCACTCTTAACAAGTTCAACATCCAAGTACTATTGCAAGTACAA CTGTATTTTCATGGAAAAA

Table S3. **shRNA/ siRNA target sequences**

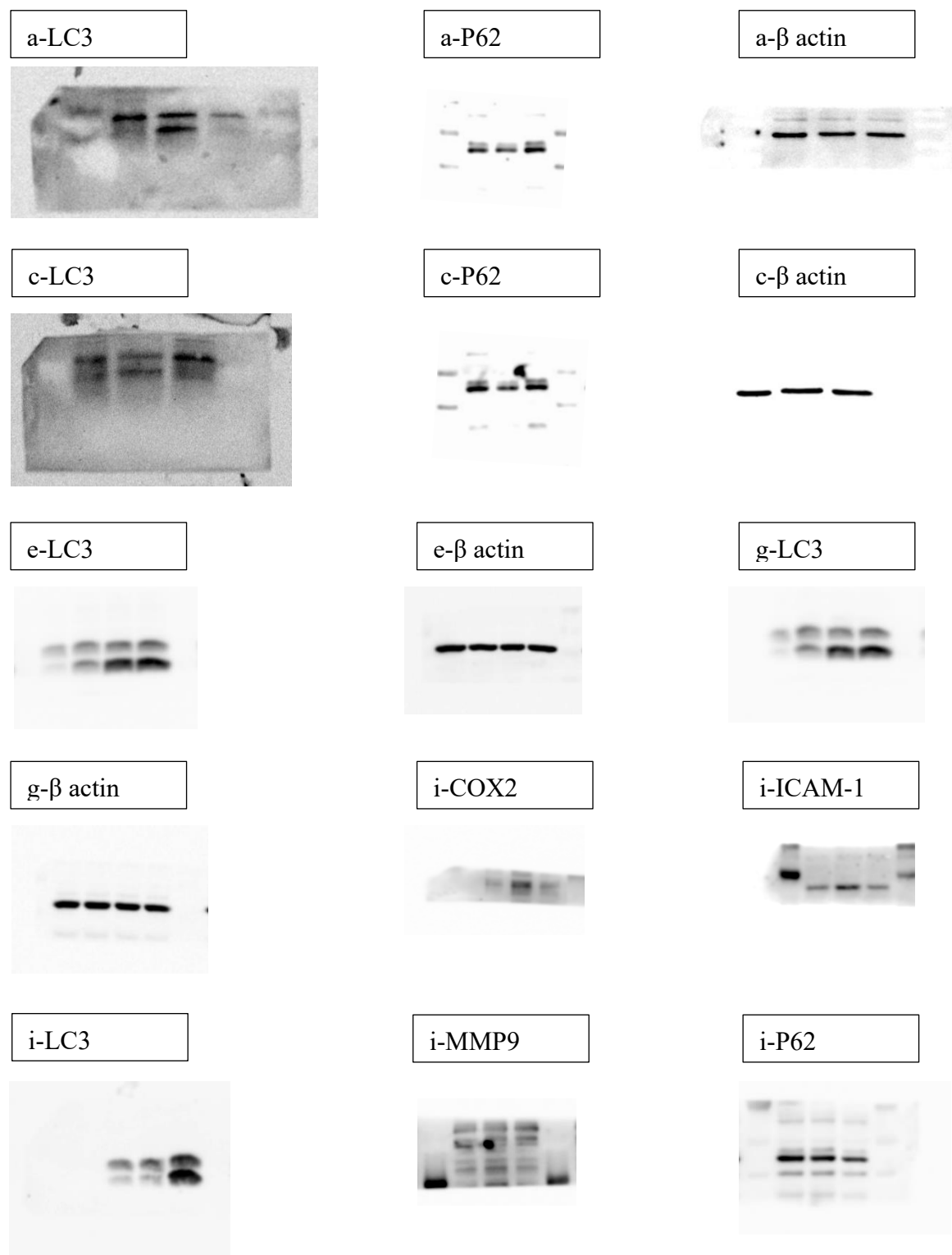
Name	Target Sequenece (5'-3')
sh-LOC107986345-1	GGGTAAAGGCCTCATTAACA
sh-LOC107986345-2	GCTCTCCCATGATCACTTATC
sh-LOC107986345-3	GCAACTGCTCTCCCATGATCA
Negative control (sh-LOC107986345)	CCTAAGGTTAAGTCGCCCTCG
miR-128-3p sponge (sh-128-3p)	aaagagaccaacactgtga
sh-EPHB2-1	ACGAGAACATGAACACTAT
sh-EPHB2-2	TGAACAGTATCCAGGTGAT
sh-EPHB2-3	ACCCGACTACACCAGCTTTAA
Negative control (sh-EPHB2)	GTTCTCCGAACGTGTCACGTT
HMGB1 siRNA	GGACAAGGCCCGTTATGAA
BECN1 siRNA	GGAGACATTATGGAGAGATT
Control siRNA	CCTAAGGTTAAGTCGCCCTCG

Table S4. Primer sequences

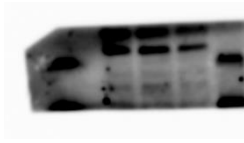
Gene Name	Forward Primer	Reverse Primer
EPHB2	GTACCTGGCAGACATGAACTAT	GTATCGTCCTCTAGAAAGCGTG
LOC107986345	CCTGCCCACACAGCCATAACATC	GTGCCAGCCGTGAAGTGTCC
LOC107986196	AGGATGTCCTTGAGAGGCTAACCC	GGTGCTCCTCATGCTGTCGTTC
has-miR-128-3p	CGCCGTCACAGTGAACCGGTCTCTTT	
hsa-miR-4306	CGCGCGTGGAGAGAAAGGCAGTA	
hsa-miR-216a-3p	CGCGCTCACAGTGGTCTCTGGGATTAT	
hsa-miR-27a-3p	CGCGTTCACAGTGGCTAAGTTCCGC	
hsa-miR-3681-3p	CGCGCACACAGTGCTTCATCCACTACT	
hsa-miR-495-3p	CGCGCGAAACAAACATGGTGCACTTCT	
hsa-miR-9-5p	GGCGCGCGTCTTTGGTTATCTAGCTGT	
hsa-miR-185-5p	CGCGTGGAGAGAAAGGCAGTTCCTGA	
hsa-miR-4644	CGCGCGTGGAGAGAGAAAAGAGACAGA	
hsa-miR-23c	CGCGCATCACATTGCCAGTGATTACCC	
hsa-miR-23a-3p	CGCCGATCACATTGCCAGGGATTTC	
hsa-miR-150-5p	CGCTCTCCCAACCCTTGTAACAGTG	
hsa-miR-30c-5p	CGCCGCGCTGTAAACATCCTACACTCT	
hsa-miR-204-5p	CGCCGTTCCCTTTGTCATCCTATGCCT	
hsa-miR-211-5p	CGCTTCCCTTTGTCATCCTTCGCCT	
hsa-miR-23b-3p	CGCGATCACATTGCCAGGGATTACCAC	
hsa-miR-5688	CGCGCGCGCTAACAAACACCTGTAAA	
β -actin	GGCCAACCGCGAGAAGATGAC	GGATAGCACAGCCTGGATAGCAAC
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT

Fig. S5. All uncropped western blots in this study.

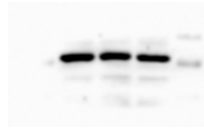
Fig.1



i-VCAM-1



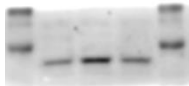
i- β actin



k-COX2



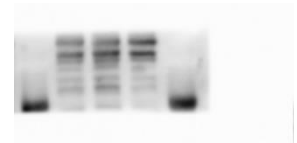
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k-LC3



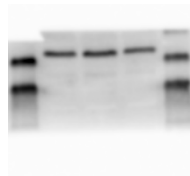
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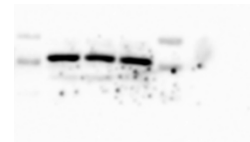
k-P62



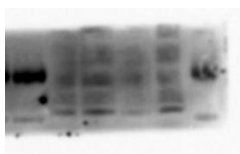
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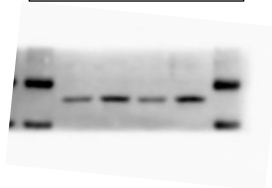
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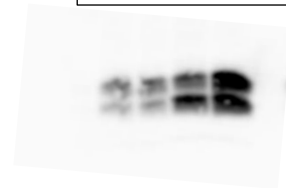
m-COX2



m-ICAM



m-LC3



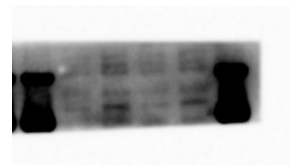
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m-P62



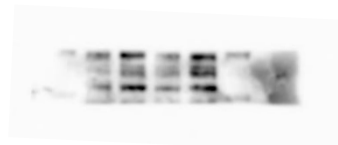
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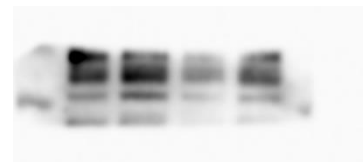
m- β actin



o-COX2



o-ICAM



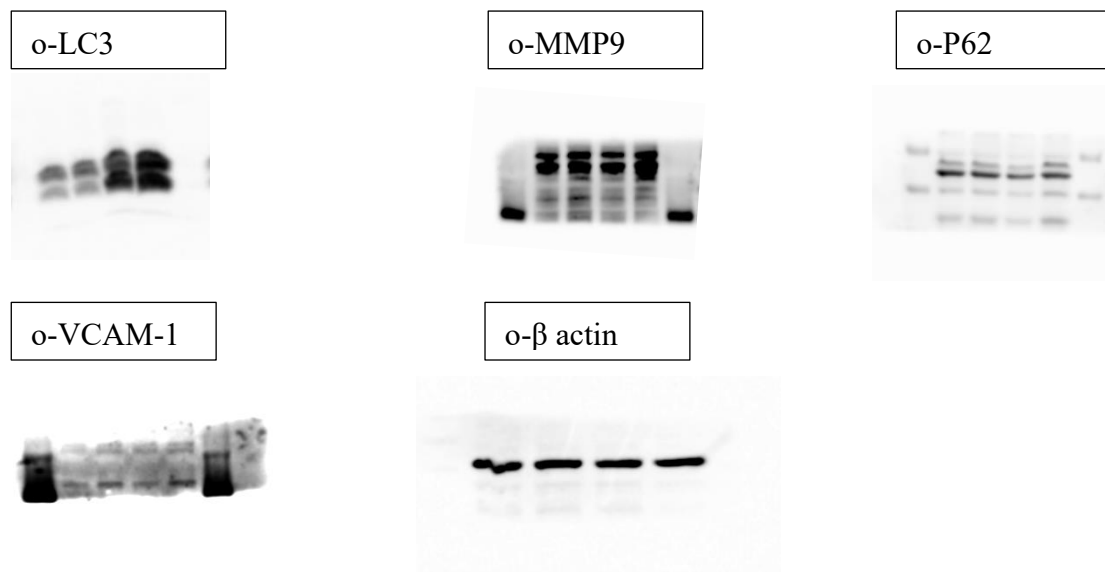
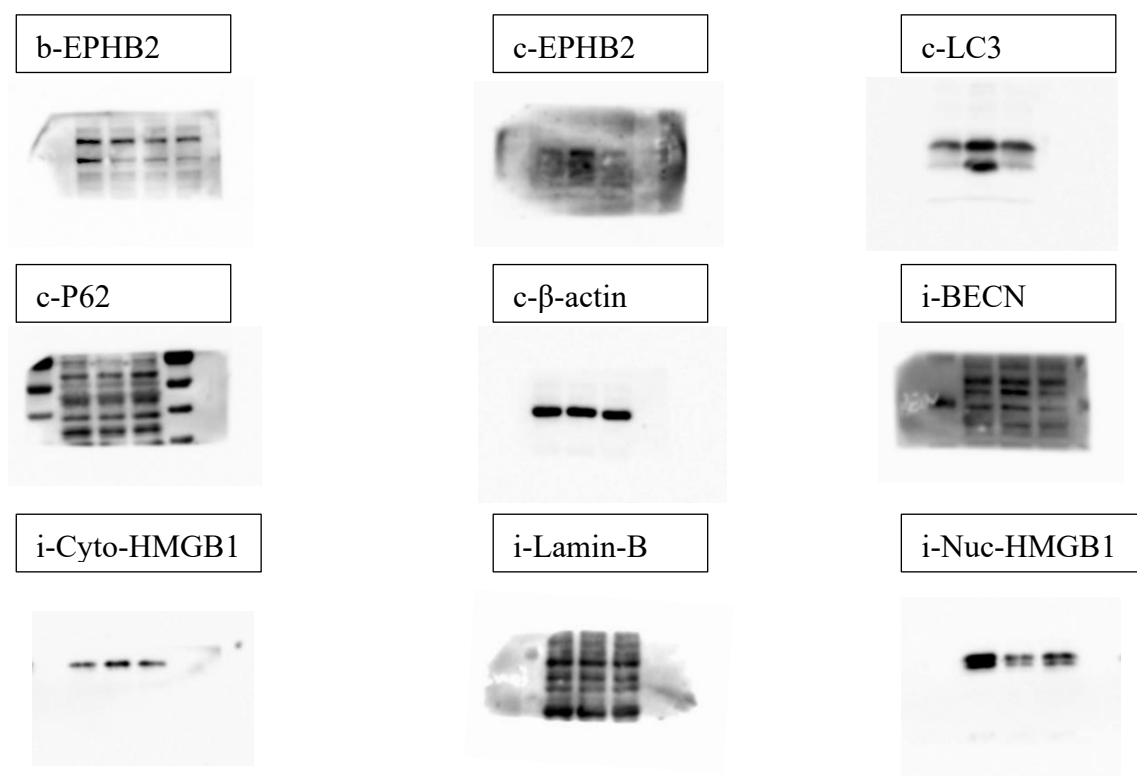


Fig.2



Fig.3



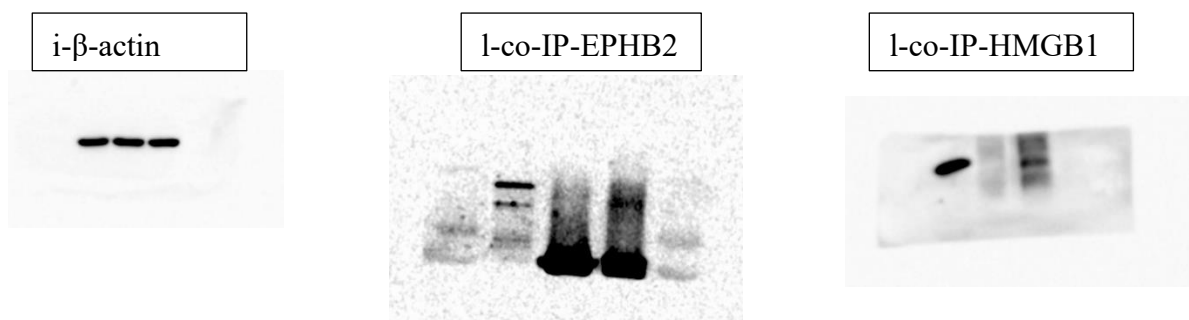
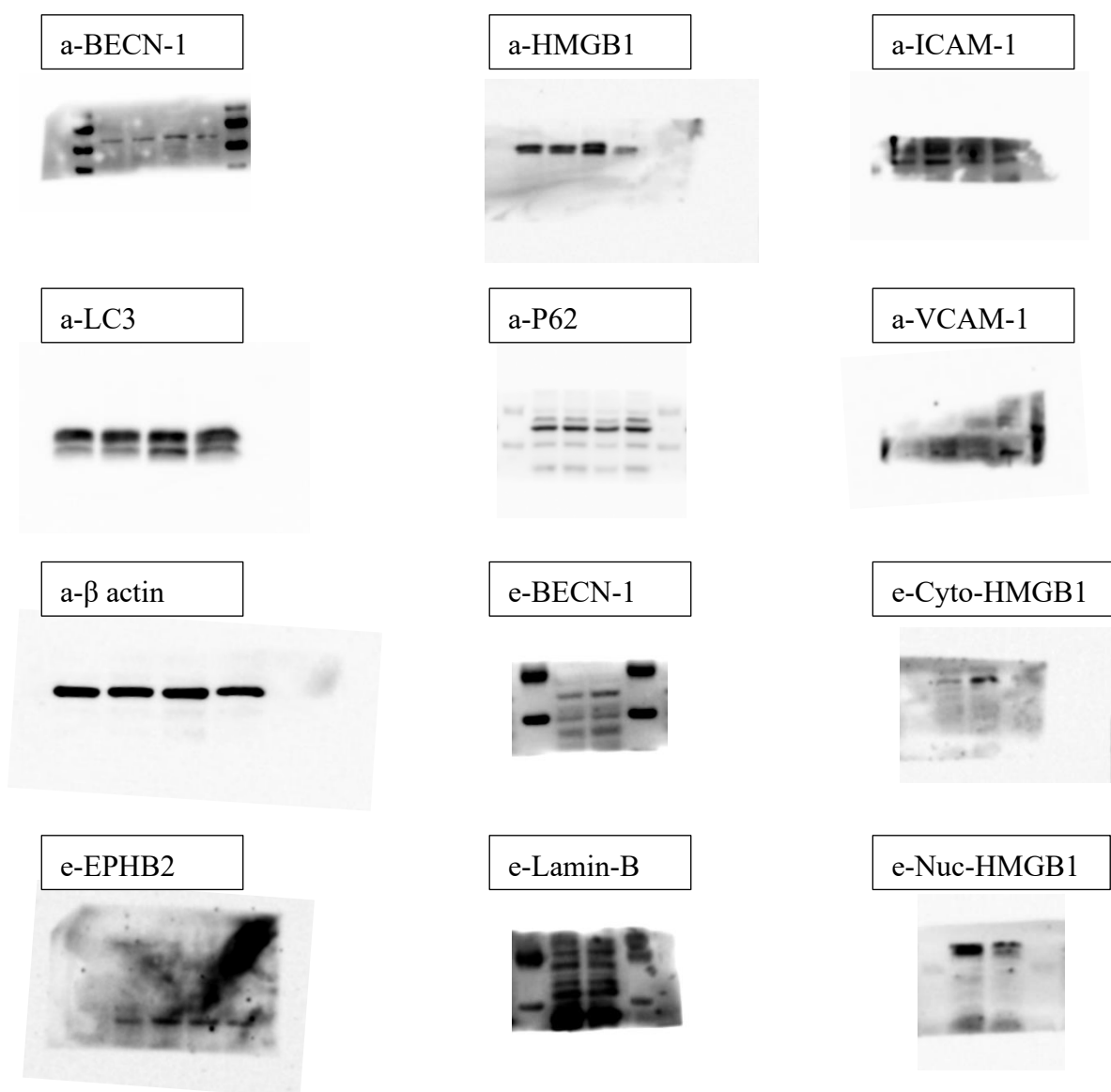
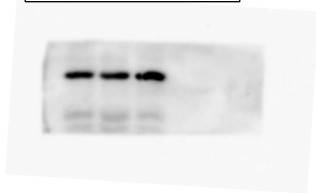


Fig.4



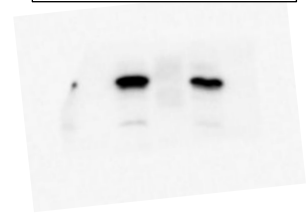
e- β actin



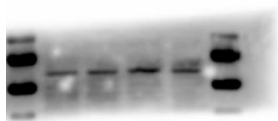
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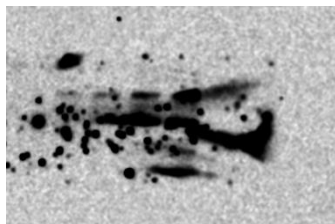
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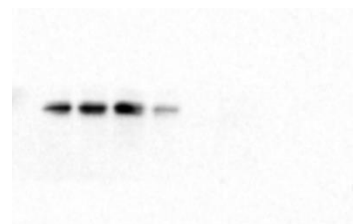
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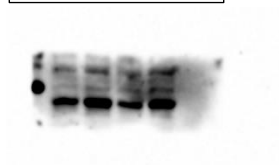
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h-HMGB1



h-ICAM-1



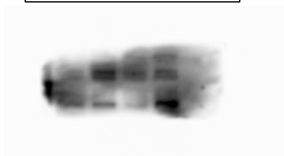
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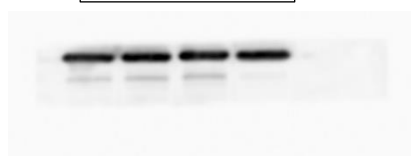
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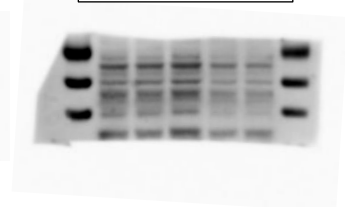
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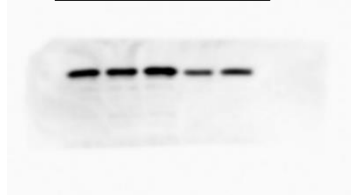
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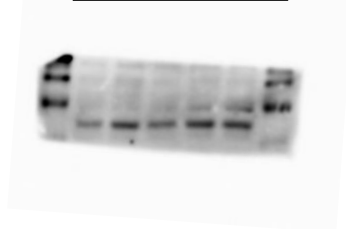
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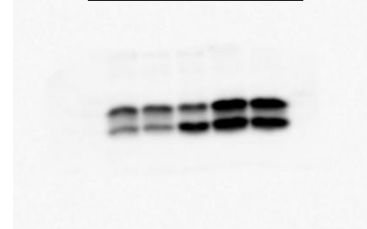
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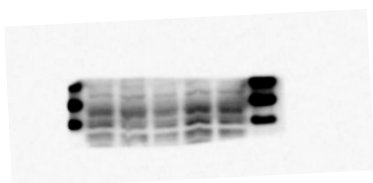
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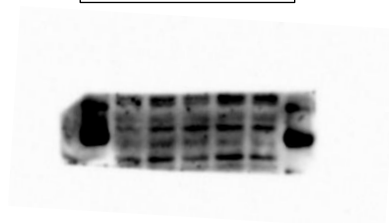
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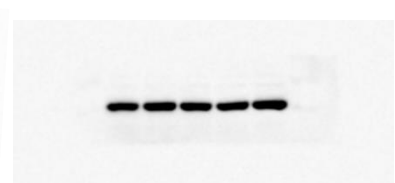
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k- β actin



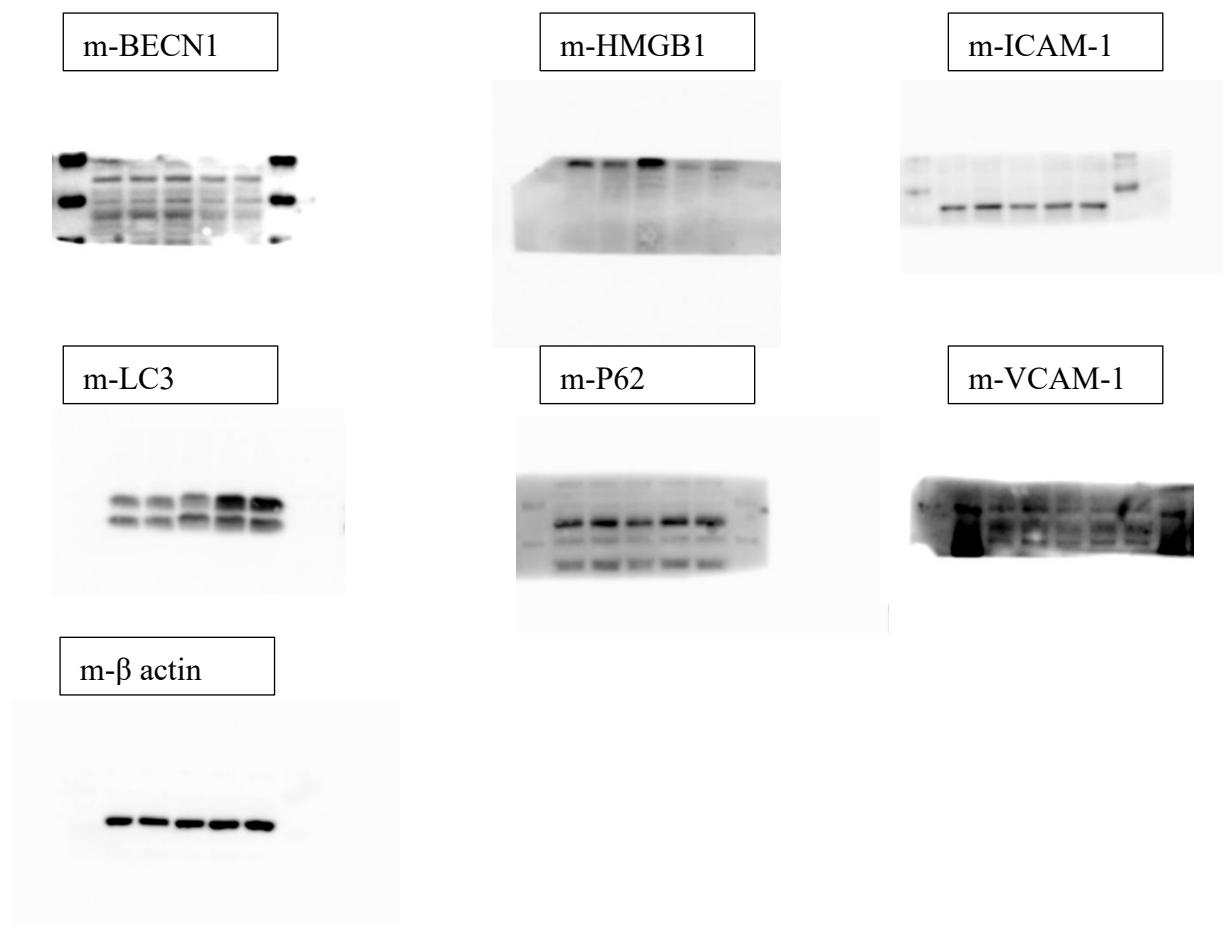


Fig.5

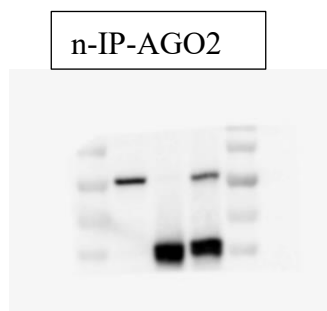
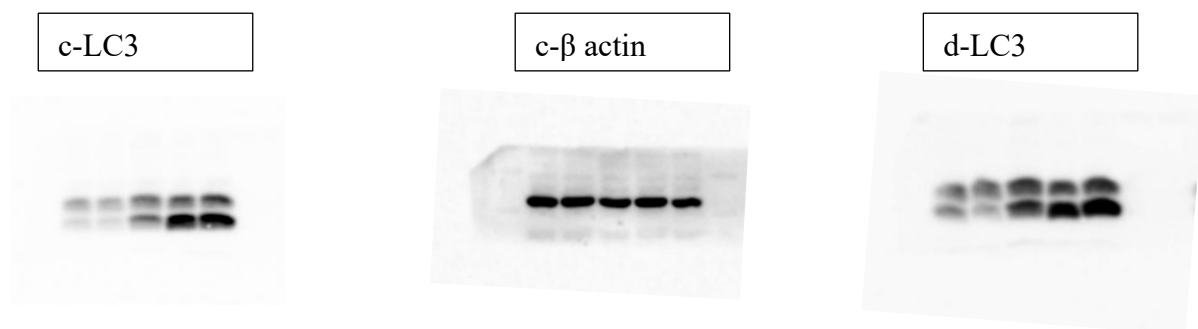
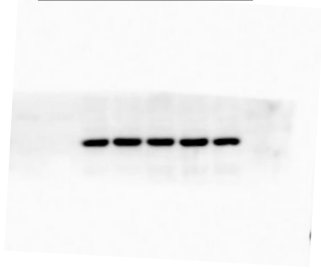


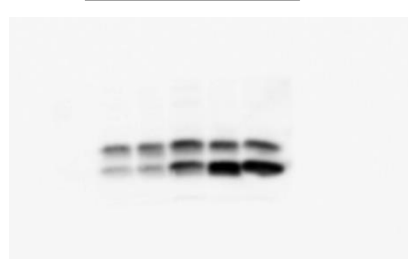
Fig.6



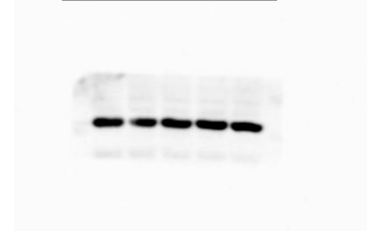
d- β actin



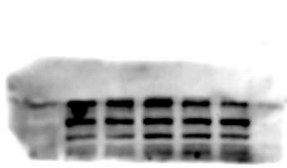
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e- β actin



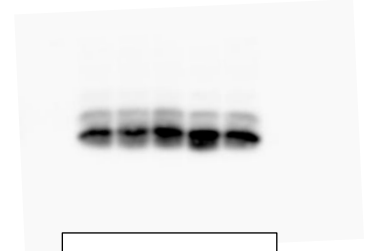
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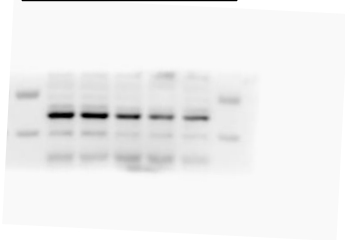
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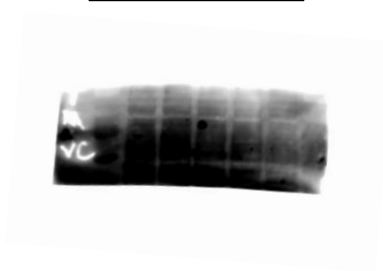
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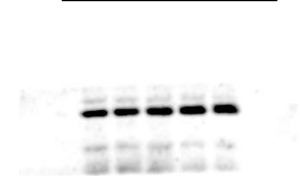
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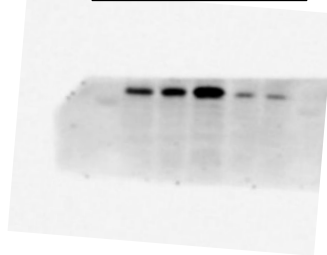
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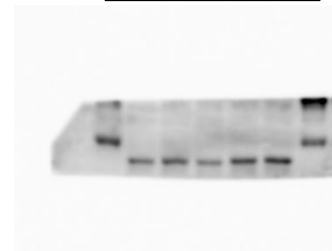
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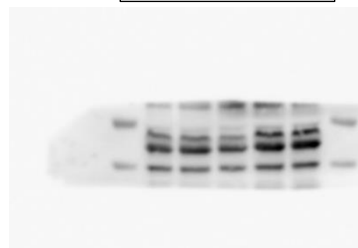
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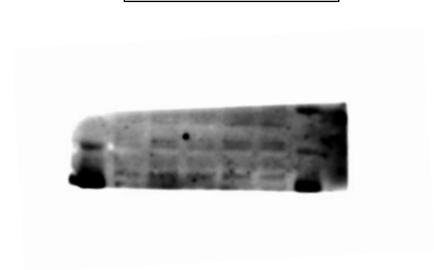
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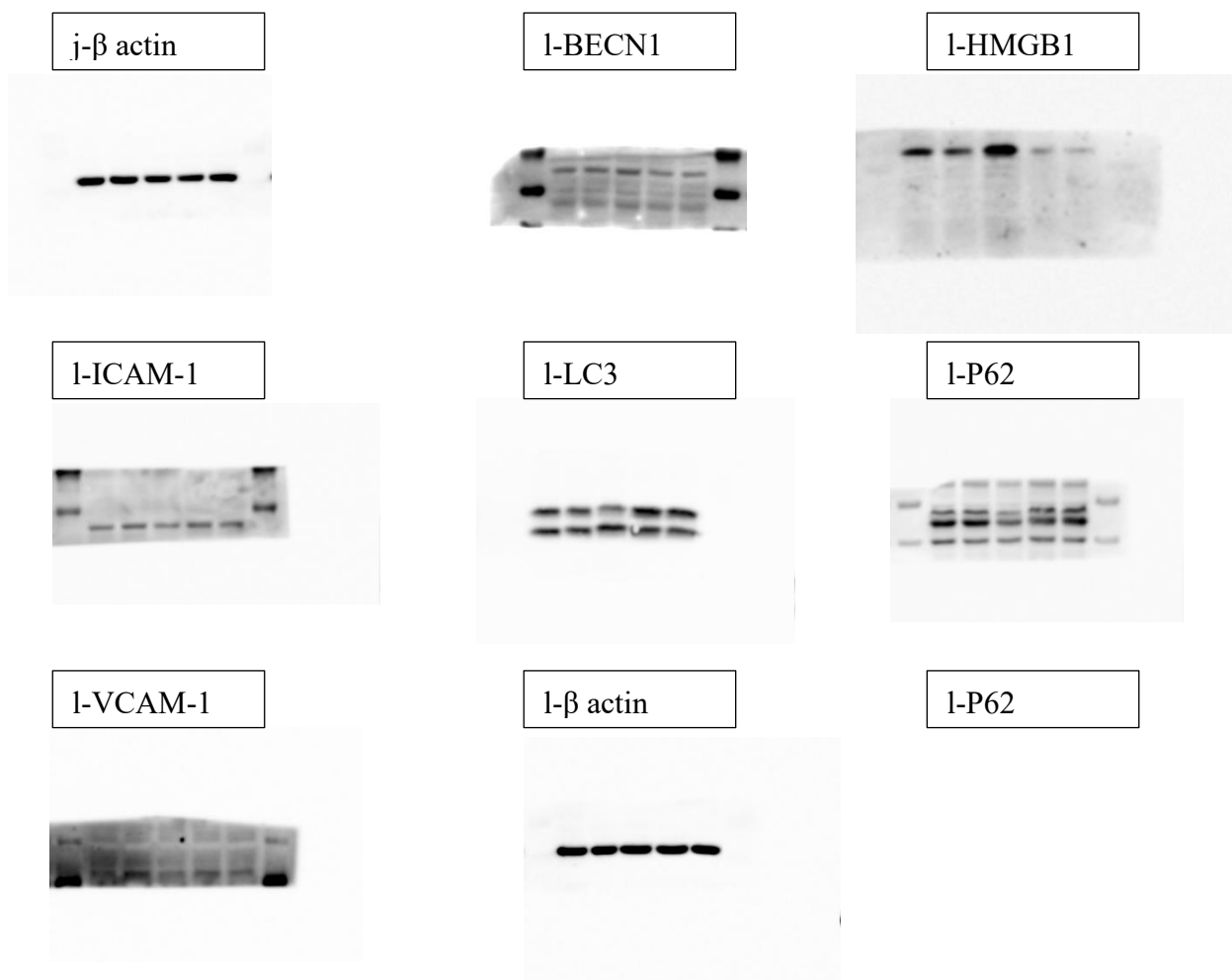
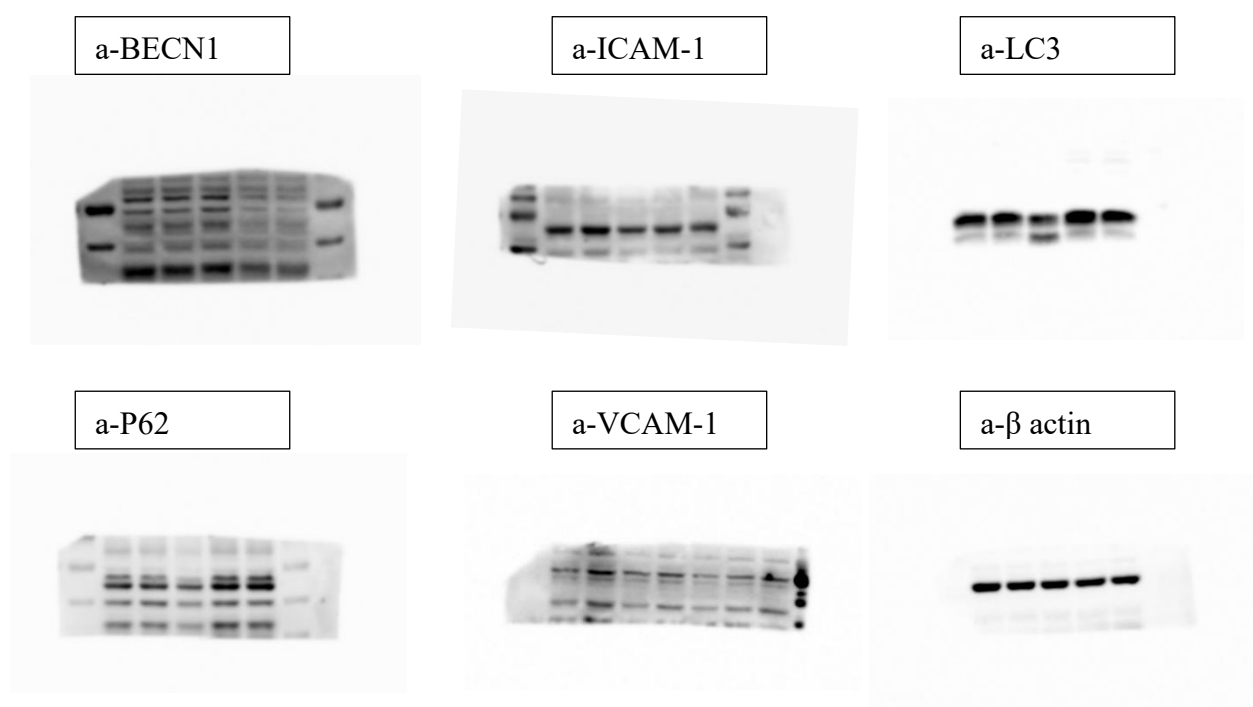
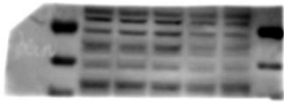


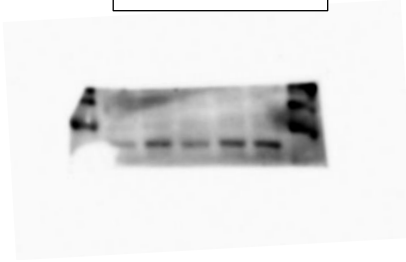
Fig.S1



c-BECN1



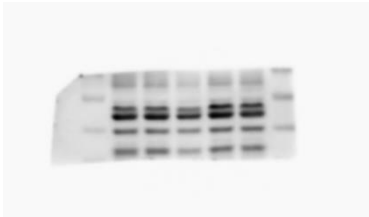
c-ICAM-1



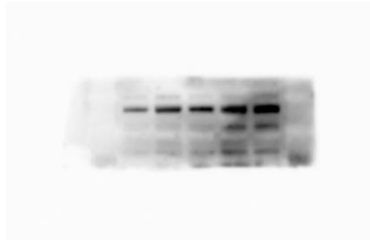
c-LC3



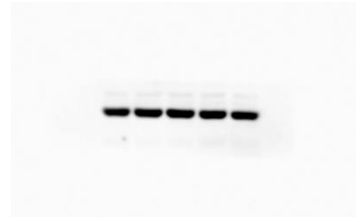
c-P62



c-VCAM-1



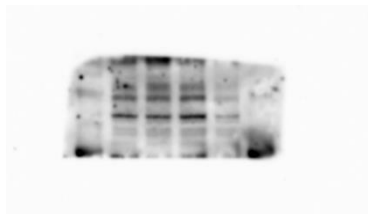
c-β actin



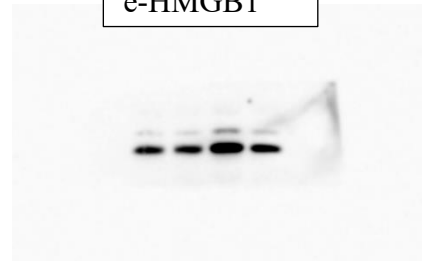
e-BECN1



e-EPHB2



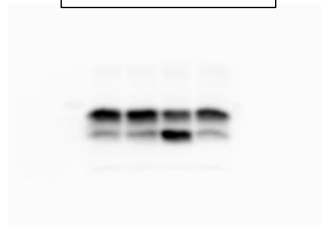
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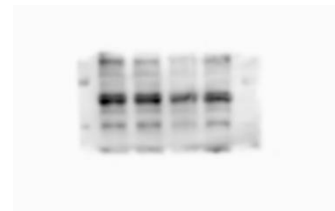
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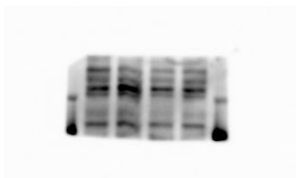
e-LC3



e-P62



e-VCAM-1



e-β actin



e-P62

Fig.S3

