

Title: Dynamic phenotypic reprogramming and chemoresistance induced by lung fibroblasts in small cell lung cancer

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1 Supplementary materials and methods

1.1 Cell treatments

For JAK2/STAT3 inhibitor treatment, H69 and H2227 were pre-treated with 0.8 μ M AZD-1480 (MedChemExpress, NJ, USA) for 24 h, then co-cultured with fibroblasts or separately cultured with complete medium containing 0.8 μ M AZD-1480, then expression of NE genes or c-MYC, HES1 or cell viability were determined.

1.2 Small interfering RNA (siRNA) transfection

MRC-5 or HFL1 were seeded in transwell inserts (Labselect, Hefei, Anhui, China) which put into 6 or 24-well plate for 24 h, then 80 nM (6 well plate) or 20 nM (24 well plate) siRNA (GenePharma, Suzhou, Jiangsu, China) diluted with Opti-MEM medium (Gibco, Carlsbad, CA, USA) was transfected to MRC-5 or HFL1 cells by Lipofectamine RNAiMAX reagent (Invitrogen, Carlsbad, CA, USA) for 6 h. Another 24 h later, MRC-5 or HFL1 transfected with siRNA were co-cultured with H69 or H2227 cells for another 6 days. Sequences of siRNA targeted to IL6, IGFBP1, CCL20 and negative control were listed in Supplementary Table S4.

1.3 Lentivirus packaging and infection

Well grown HEK-293T cells were seeded in 10-cm dish for 24 h before plasmids transfection, then 10 μ g expression plasmid GV248 carried MYC RNAi fragment or Scramble fragment (Genechem, Shanghai, China) and 10 μ g lentivirus packaging plasmids (7.5 μ g psPAX+ 2.5 μ g pMD2G) (Fenghui Biotechnology, Changsha, Hunan, China) were diluted with opti-MEM medium and transfected in HEK-293T cells by Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA) for 6 h. Transfected cell cultured for another 48 h, then supernatant was collected and centrifuged to obtain the lentivirus. Virus was subpackaged and stored at -80°C for specified experiments. The shRNA sequence targeted to MYC was listed in Supplementary Table S4.

1.4 Cell proliferation analysis

SCLC cell line H69 and H2227 which been treated with AZD-1480 or lentivirus or not were seeded in 24-well plates at 2×10^4 cells /well and co-cultured with MRC-5 or HFL1 which were untreated or transfected with siRNA, or cultured separately. 5 days later, cell viability was determined by Cell Counting Kit-8 (CCK-8) reagent (Beyotime, Beijing, China), the optical absorbance was measured at 450 nm by CLARIOstar microplate reader (BMG Labtech, Offenburg, Germany).

For clone formation test, SCLC cell line H2227 were seeded in 24-well plated at 500 cells/well and co-cultured with MRC-5 and HFL1 respectively or separately cultured for 7 days. The cell clones were washed by PBS and fixed by 4% paraformaldehyde, and then stained by 0.1 % (m/v) crystal violet for 15 min. Cell clones of every well were photographed and counted by Image J software.

1.5 Cell viability assay

SCLC cells co-cultured with fibroblasts for 7 days and then seeded in 96- well plates and exposed to Cisplatin or Etoposide for another 48 h. Cell viability was measured by CCK-8 reagent. The inhibition rate was calculated with the formula as: inhibition rate= $1 - \text{OD}_{450}(\text{drug treatment group}) / \text{OD}_{450}(\text{control group}) \times 100\%$.

1.6 Western blot analysis

To extract the total protein of cells, RIPA reagent supplemented with protease inhibitor and phosphatase inhibitors (Beyotime, Beijing, China) was added to SCLC cells and lysed on ice for 30 min. The concentrations of supernatant were determined using BCA protein assay kit (Beyotime, Beijing, China). The same amount of protein was separated by SDS-PAGE and transferred onto PVDF membranes (Millipore, Darmstadt, Germany). The blots were blocked and cut prior to hybridisation with antibodies overnight at 4 °C. The blots incubated with HRP-conjugated secondary antibodies and then chemiluminescence of blots were stimulated by ECL reagent (Beyotime, Beijing, China) and detected by GeneGnome XRQ NPC imaging system. Quantitative analysis of blots was performed using Image J software. The details of antibodies were listed in Supplementary Table S5.

1.7 RNA extraction and quantitative real-time PCR (qRT-PCR)

Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). ReverTra Ace qPCR RT Master Mix (TOBOYO, Osaka, Japan) was used to reverse transcribe 1 µg RNA to cDNA. Then cDNA was used to perform qRT-PCR with SYBR Green qPCR Mix (Beyotime, Beijing, China) in an Agilent Mx3000P qPCR System (Palo Alto, CA, USA). The relative mRNA expressions were calculated using the $2^{-\Delta\Delta C_t}$ method. GAPDH was used as a loading control. Primers were generated by Beijing Dingguo Changsheng Biotechnology (Beijing, china) and sequences were listed in Supplementary Table S2.

2 Supplementary Figures and Tables

2.1 Supplementary Tables

Supplementary Table S2: Sequences of primers for qRT-PCR and ChIP-PCR

Genes	Sequences
<i>GAPDH</i>	F: 5'-GCCGCACCTCAGCTTATTATG-3'
	R: 5'-AAGTGGTCGTTGAGGGCAATG-3'
<i>IL6</i>	F: 5'-ACTCACCTCTTCAGAACGAATTG-3'
	R: 5'-CCATCTTTGGAAGGTTTCAGGTTG-3'
<i>CCL20</i>	F: 5'-GCGAATCAGAAGCAGCAAGCAA-3'
	R: 5'-TGTTTTGGATTTGCGCACACAG-3'
Genes	Sequences

<i>IGFBP1</i>	F: 5'-TTGGGACGCCATCAGTACCTA-3'
	R: 5'-TTGGCTAAACTCTCTACGACTCT-3'
<i>ASCL1</i>	F: 5'-CGCGGCCAACAAGAAGATG-3'
	R: 5'-CGACGAGTAGGATGAGACCG-3'
<i>SYP</i>	F: 5'-TTAGTTGGGGACTACTCCTCG-3'
	R: 5'-GGCCCTTTGTTATTCTCTCGGTA-3'
<i>NCAM1</i>	F: 5'-GGCATTTACAAGTGTGTGGTTAC-3'
	R: 5'-TTGGCGCATTCTTGAACATGA-3'
<i>IL6R</i>	F: 5'-CATGTGCGTCGCCAGTAGT-3'
	R: 5'-AGCTCAAACCGTAGTCTGTAGA-3'
<i>MYC</i> (ChIP)	F: 5'-CACAAGGGTCTCTGCTGACTC-3'
	R: 5'-TCAAAGGTGCTAGACGGGAG-3'

Supplementary Table S3: Gene signatures

Signatures	Genes
NE genes	BEX1, ASCL1, INSM1, CHGA, TAGLN3, KIF5C, CRMP1, SCG3, SYT4, RTN1, MYT1, SYP, KIF1A, TMSB15A, SYN1, SYT11, RUNDC3A, TFF3, CHGB, FAM57B, SH3GL2, BSN, SEZ6, TMSB15B, CELF3.
Non-NE genes	RAB27B, TGFB2, SLC16A5, S100A10, ITGB4, YAP1, LGALS3, EPHA2, S100A16, PLA2, ABCC3, ARHGDIB, CYR61, PTGES, CCND1, IFITM2, IFITM3, AHNK, CAV2, TACSTD2, TGFBI, EMP1, CAV1, ANXA1, MYOF.
Signatures	Genes

Cytolytic activity (CYT)	GZMA, PRF1.
Antigen presentation machinery (APM) signature	HLA-A, HLA-B, HLA-C, B2M, TAP1, TAP2.
T-cell-inflamed gene expression profile (GEP)	PSMB10, HLA-DQA1, HLA-DRB1, CMKLR1, HLA-E, NKG7, CD8A, CCL5, CXCL9, CD27, CXCR6, IDO1, STAT1, TIGIT, LAG3, CD274, PDCD1LG2, CD276.

Supplementary Table S4: Sequences of siRNAs and shRNA.

Targets	Sequences
siIL6-1	5'-CUGUGCAGAUGAGUACAAATT-3'
siIL6-1	5'-GACCCAACCACAAAUGCCATT-3'
siCCL20	5'-CCGUAUUCUUCAUCCUAAATT-3'
siIGFBP1	5'-CCAGAGAGCACGGAGAUAATT-3'
Negative control	5'-UUCUCCGAACGUGUCACGUTT-3'
shMYC	5'-CCCAAGGTAGTTATCCTTAAA-3'
Scramble	5'- TTCTCCGAACGTGTCACGT -3'

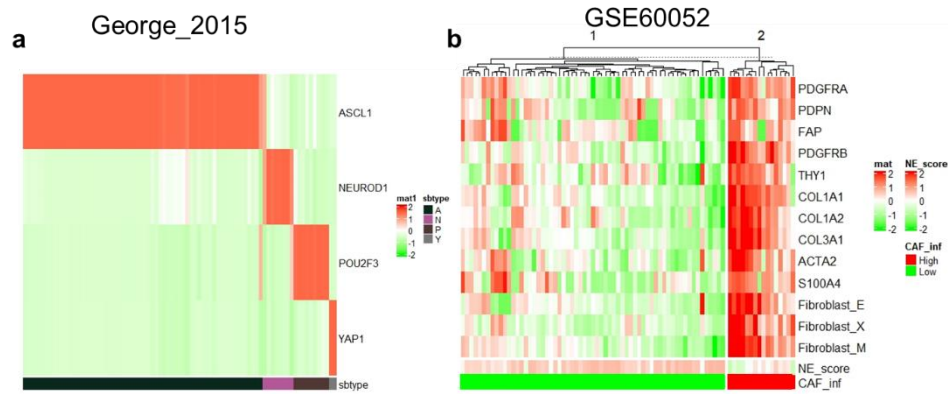
Supplementary Table S5: Information of antibodies

Antibodies	Sources
GAPDH	Wanleibio (Shengyang, Liaoning, china)
β -actin	Wanleibio (Shengyang, Liaoning, china)
Antibodies	Sources

ASCL1	CST (Danvers, Mass, USA)
NEUROD1	CST (Danvers, Mass, USA)
c-MYC	CST (Danvers, Mass, USA)
HES1	CST (Danvers, Mass, USA)
SYP	CST (Danvers, Mass, USA)
pSTAT3 (Ser727)	CST (Danvers, Mass, USA)
STAT3	CST (Danvers, Mass, USA)
pJAK2 (Tyr1007)	CST (Danvers, Mass, USA)
JAK2	CST (Danvers, Mass, USA)
REST	Santa Cruz (Santa Cruz, CA, USA)
INSM1	Abclonal (Wuhan, Hubei, China)
HRP-conjugated goat anti-Rabbit IgG (H+L)	Wanleibio (Shengyang, Liaoning, china)
HRP-conjugated goat anti-Mouse IgG (H+L)	Beyotime (Beijing, China)

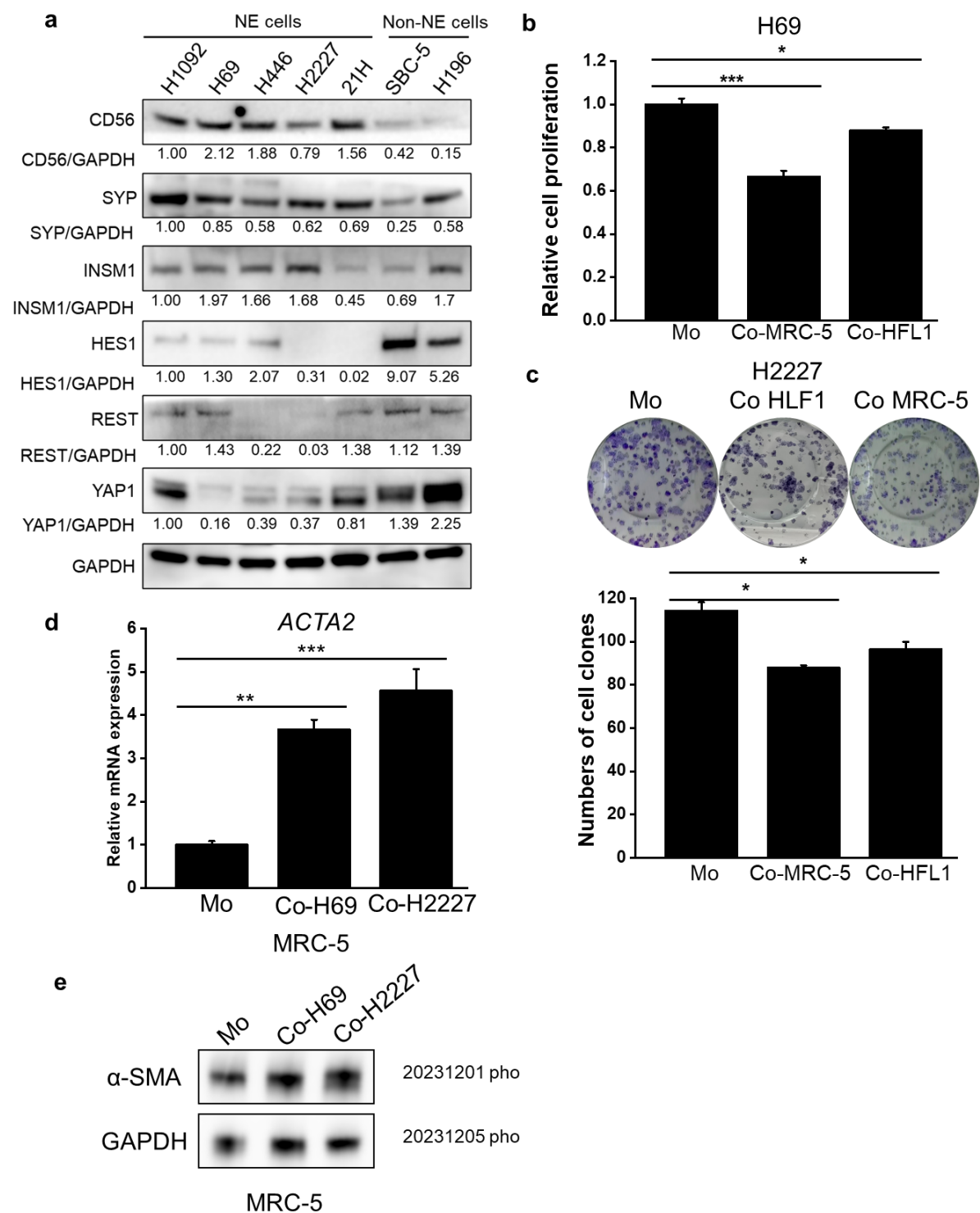
2.2 Supplementary Figures

Supplementary Figure S1



Subtyping SCLC cases and clustering CAF infiltration. (a) Heatmap displayed expression of ASCL1, NEUROD1, POU2F3 and YAP1 in George's cohort (n=81); (b) The expression of CAF markers and abundance of CAFs and the clustering results of GSE60052 dataset (n=79) were displayed in heatmap, NE scores were shown under the heatmap.

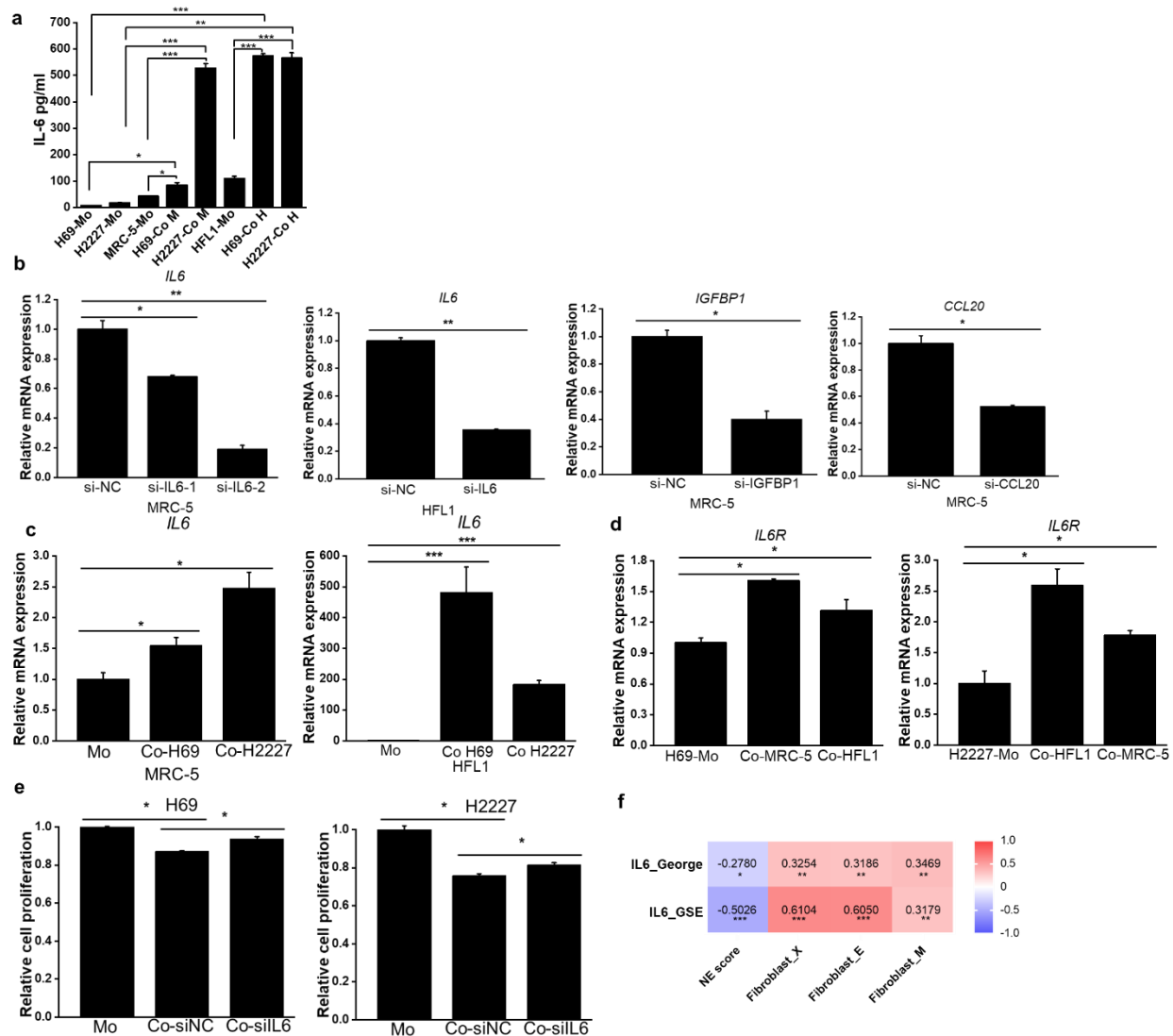
Supplementary Figure S2



Identification of NE and Non-NE cells and evaluation the effect of fibroblasts on proliferation of SCLC cells. (a) Western blot evaluated the expression of NE markers (CD56, SYP, INSM1) and Non-NE markers (HES1, REST, YAP1) in SCLC cells, the blots were cropped prior to hybridisation with antibodies, original images were displayed in Supplementary information; (b) Cell proliferation was

determined by CCK-8 reagent in H69 cells which separately cultured or co-cultured with fibroblasts (n=3); (c) Colony formation assay was performed to evaluate the proliferation of H2227 cells upon co-culture with fibroblasts or cultured individually (n=3). (d-e) The mRNA expression of ACTA2 determined by qRT-PCR (n=3) and protein expression of α -SMA evaluated by western blot of mono- and co-cultured MRC-5 cells. The blots displayed in (e) were cropped prior to hybridisation with antibodies, original images were displayed in Supplementary information. * $P < 0.05$, *** $P < 0.001$.

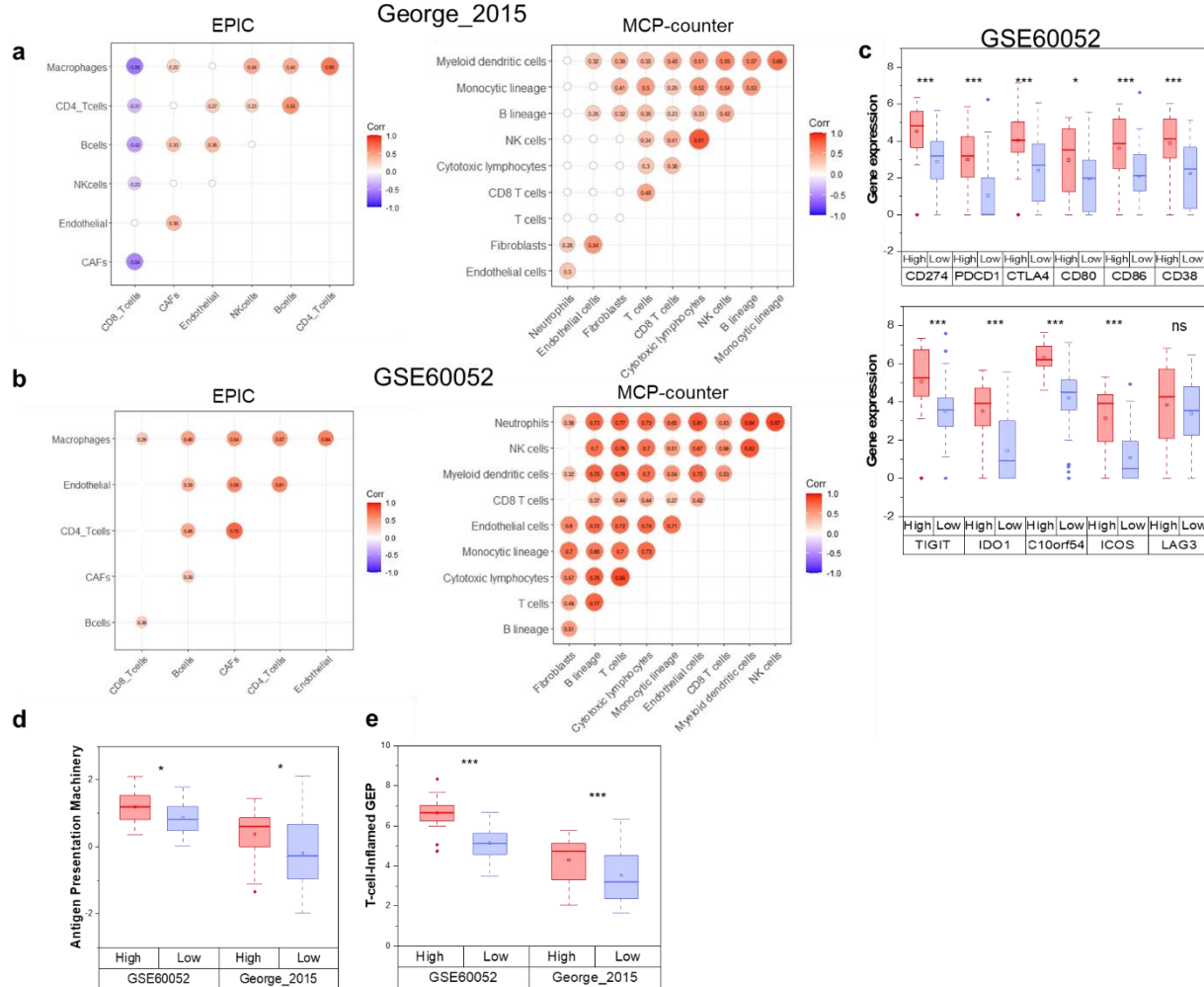
Supplementary Figure S3



Exploration of IL-6 and IL-6R in CAF-induced NE phenotypic reprogramming. (a) Secreted IL-6 from separately cultured MRC-5, HFL1, H69 and H2227 cells or co-cultured cells were determined by ELISA (n=4); (b) Expression of IL6, IGFBP1 and CCL20 was determined by qRT-PCR in MRC-5 and HFL1 cells upon transfection with indicated siRNA (n=3). (c, d) Expression of IL6 (c) and IL6R (d) was tested by qRT-PCR in MRC-5 and HFL1 cells or H69 and H2227 cells respectively upon co-culture with fibroblasts or cultured individually (n=3); (e) Cell proliferation was determined by CCK-

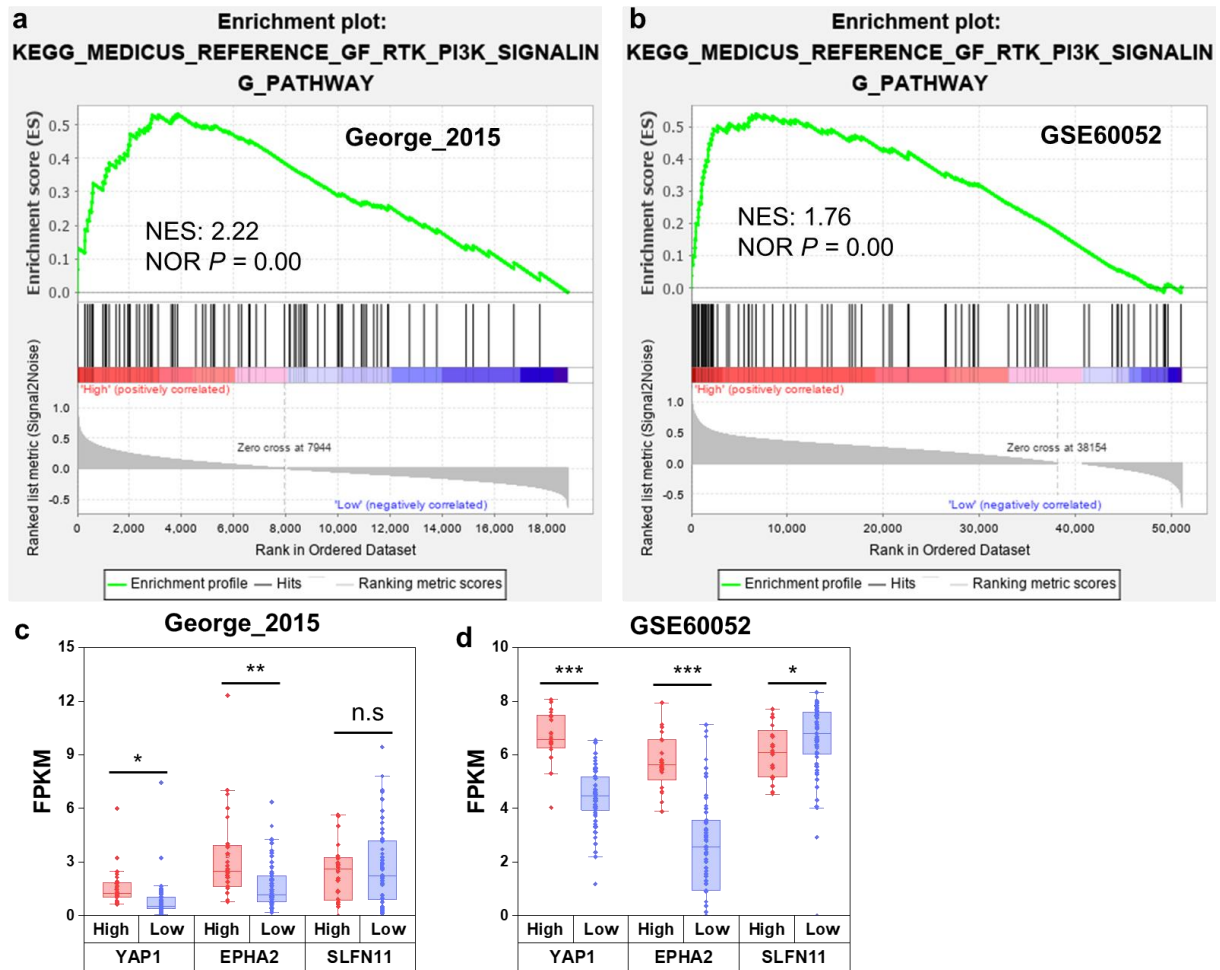
8 assay after transfection with siRNA started to IL6 or negative control (NC); (f) Pearson's correlation of expression of IL6 with NE score and CAF abundance in George's cohort and GSE60052 dataset. * $P < 0.05$, ** $P < 0.01$.

Supplementary Figure S4



Association of CAFs with immune features in SCLC cohorts. (a, b) Pearson's correlation of CAF abundance with immune cell abundance in George's cohort (a) and GSE60052 dataset (b); (c) Expression of checkpoint molecules of high- and low-infiltration groups in GSE60052 dataset; (D-E) APM signature (d) and T-cell-inflamed GEP (e) of high- and low-infiltration groups in George's cohort and GSE60052 dataset. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Supplementary Figure S5



Chemoresistance related pathway and genes in SCLC cohorts. (a-b) GSEA with normalized enrichment score (NES) and nominal P -values for KEGG pathway in CAF high- and low-infiltration group in George's cohort (a) and GSE60052 dataset (b); (c-d) Expression of Chemoresistance related genes of CAF high- and low-infiltration groups in George's cohort and GSE60052 dataset. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Original images

Figure 2b

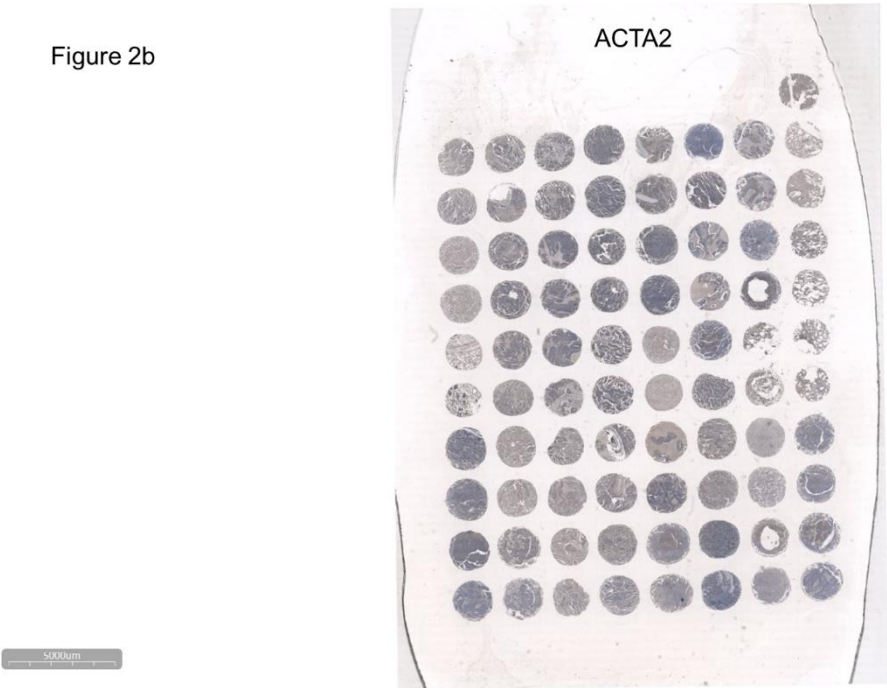


Figure 2b

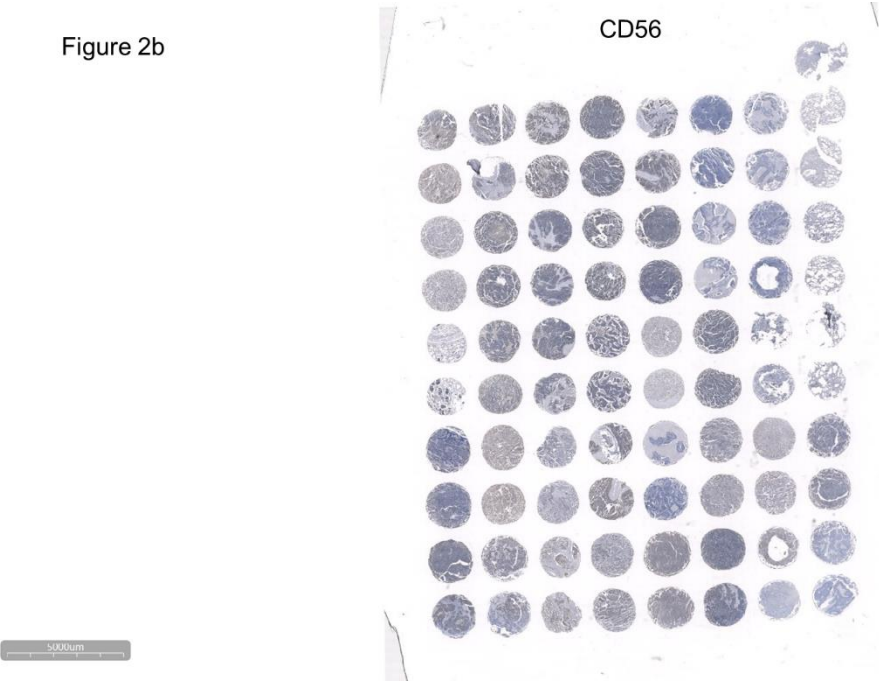


Figure 2b

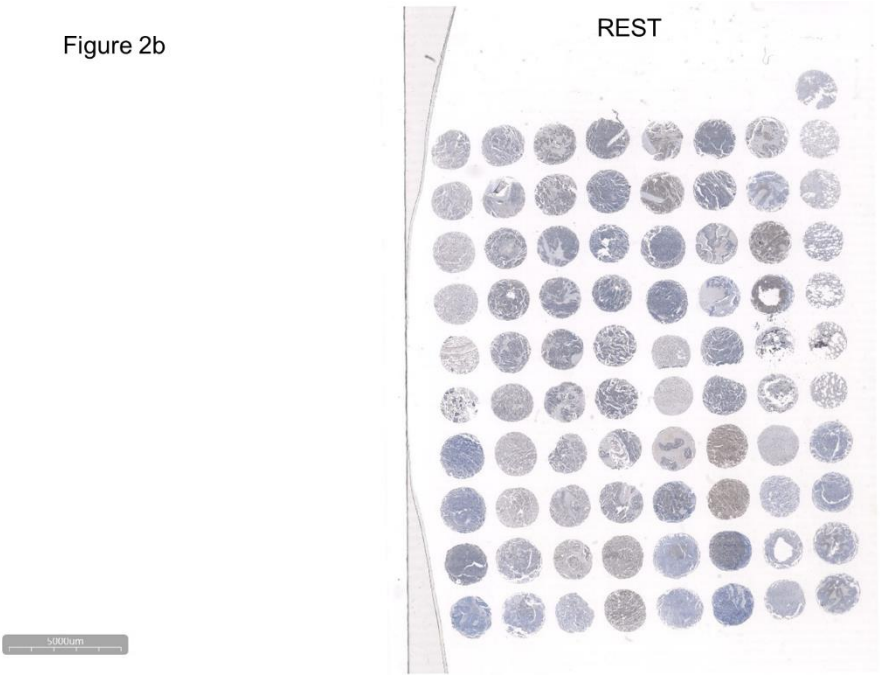


Figure 3c

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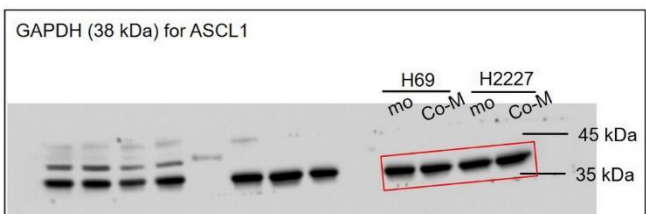
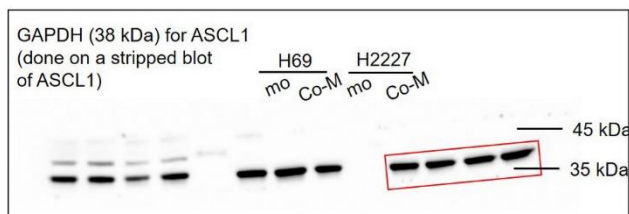
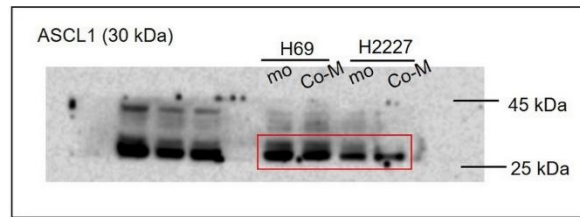
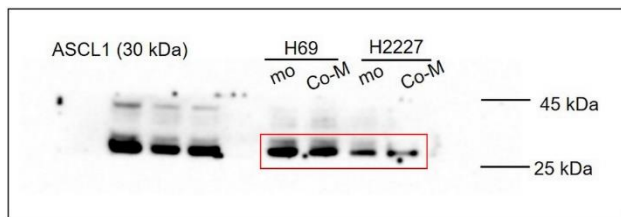
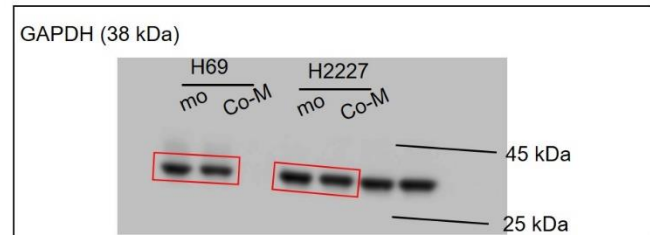
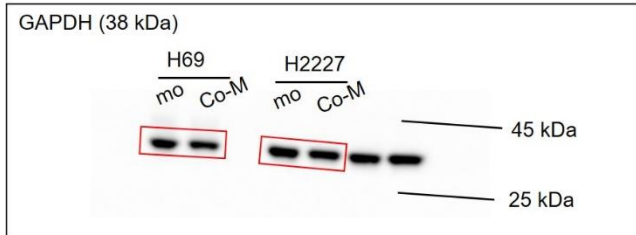
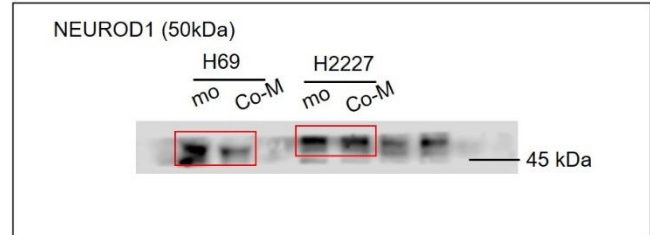
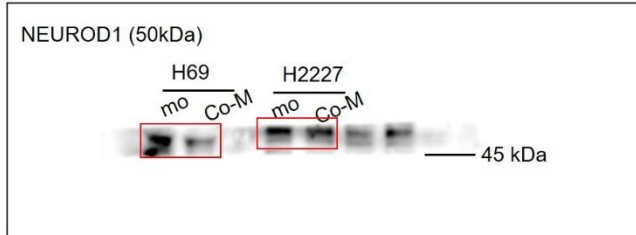
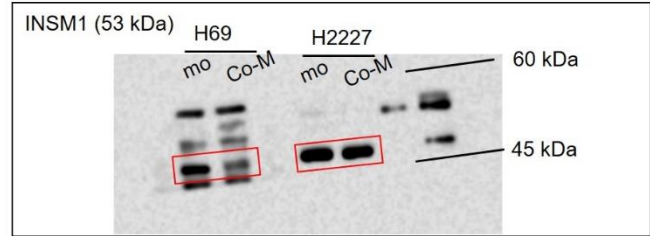
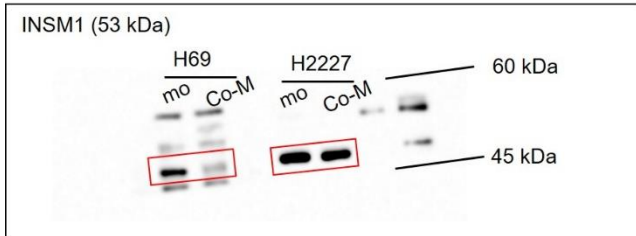
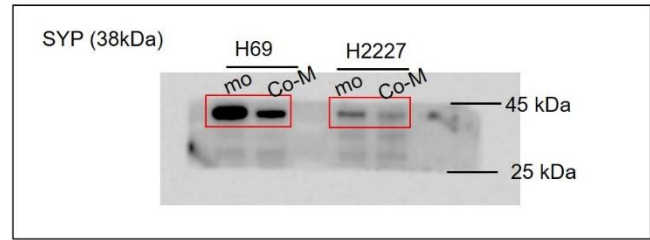
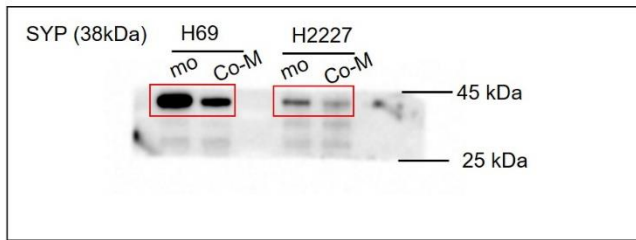


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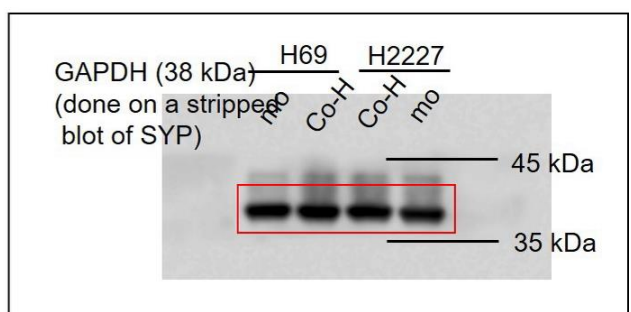
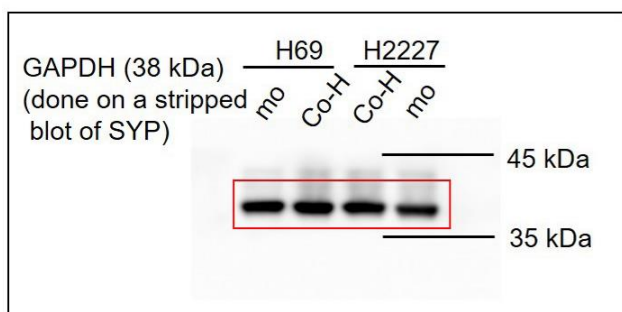
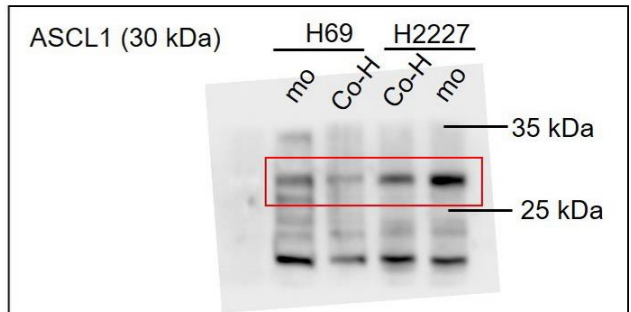
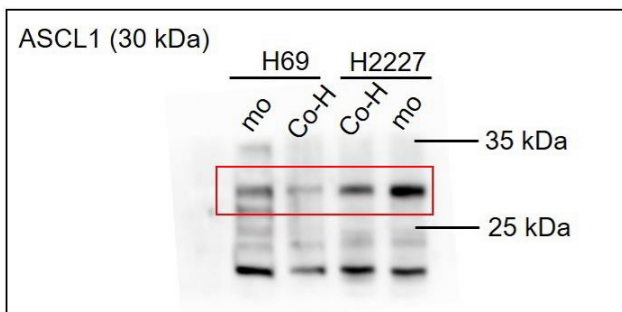
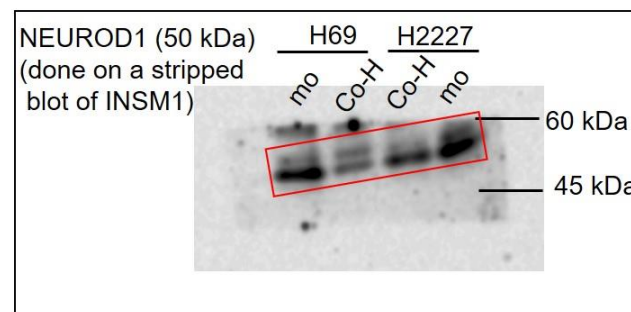
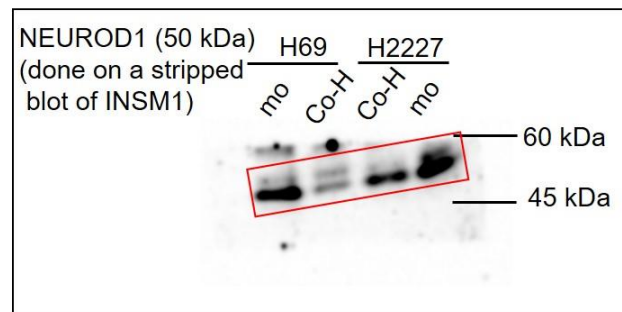
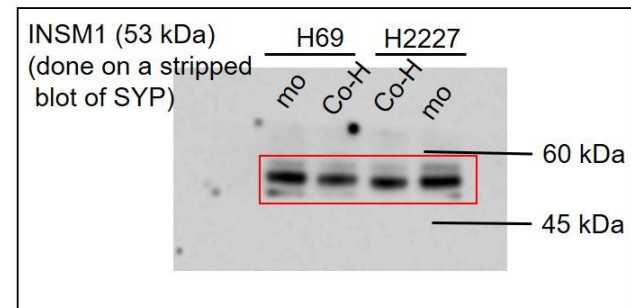
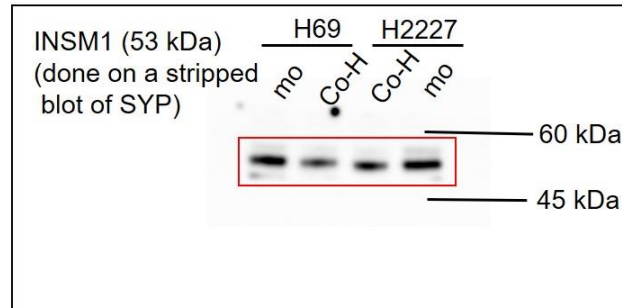
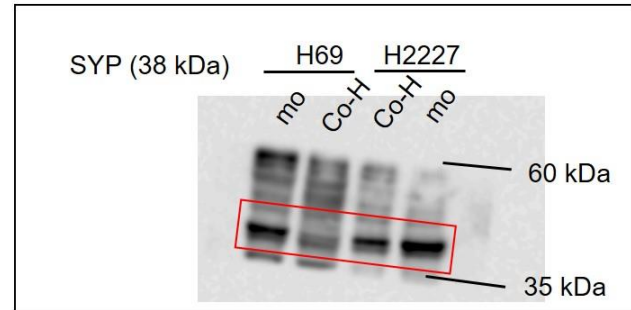
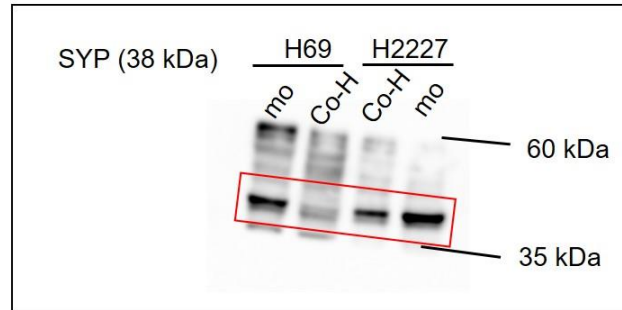
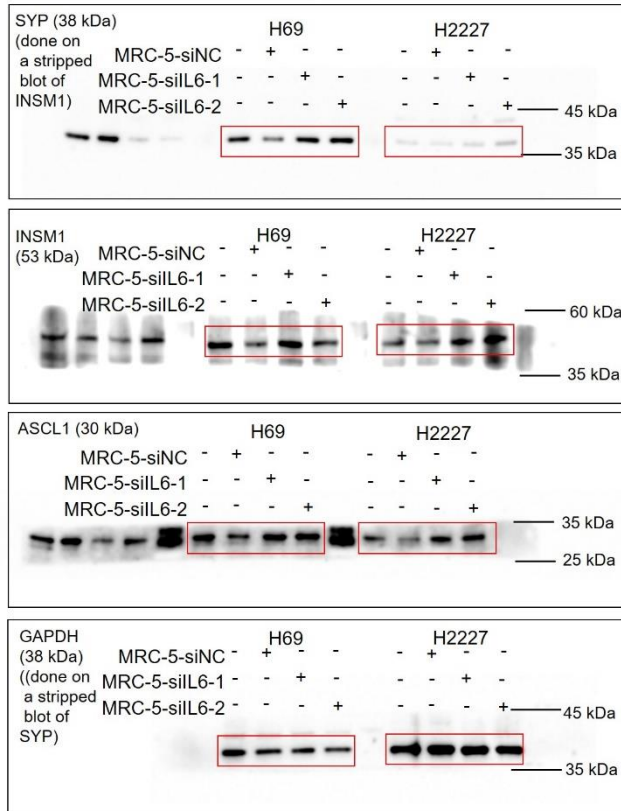


Figure 3g



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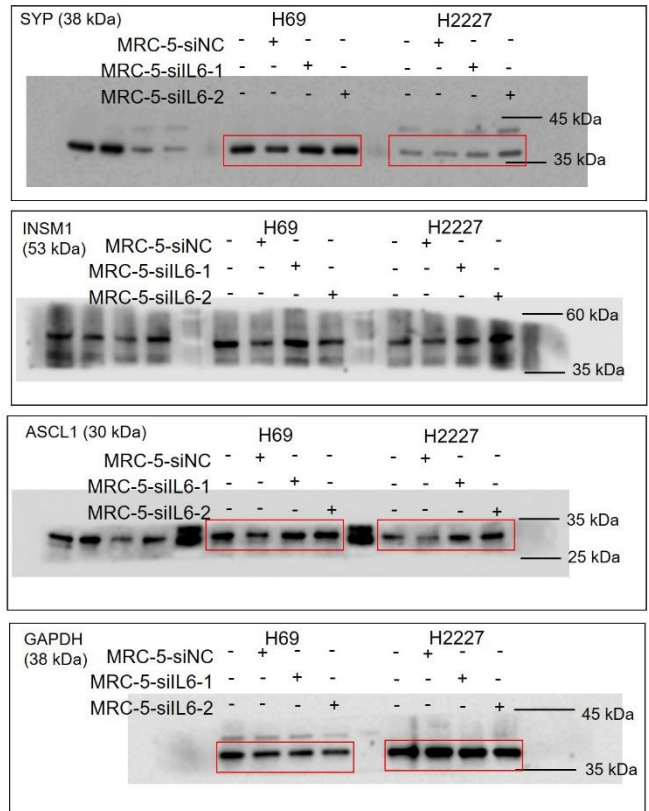
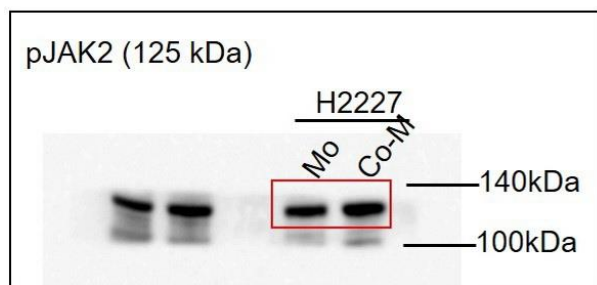
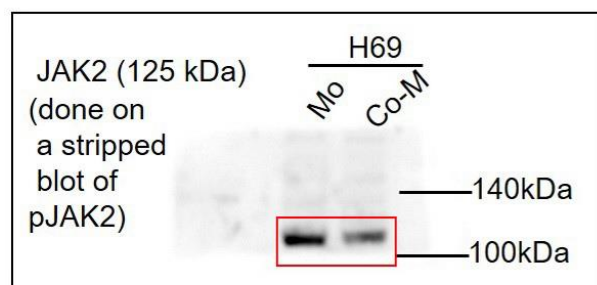
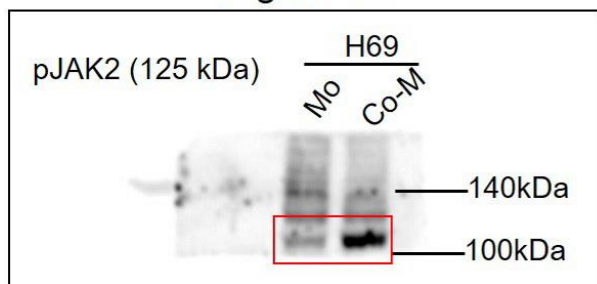


Figure 4c



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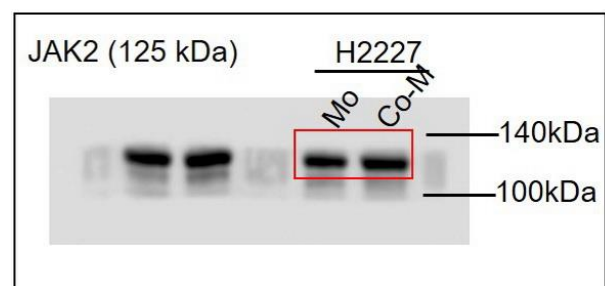
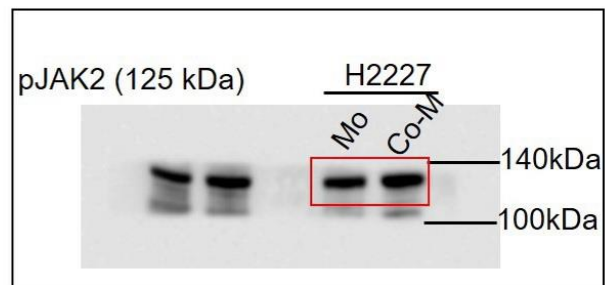
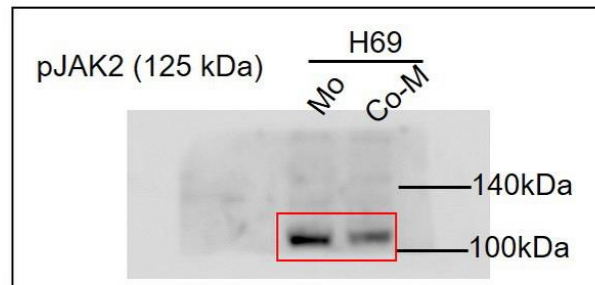
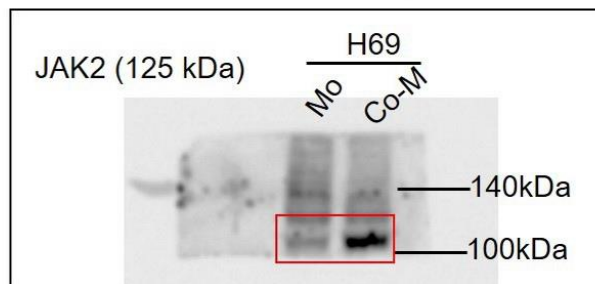
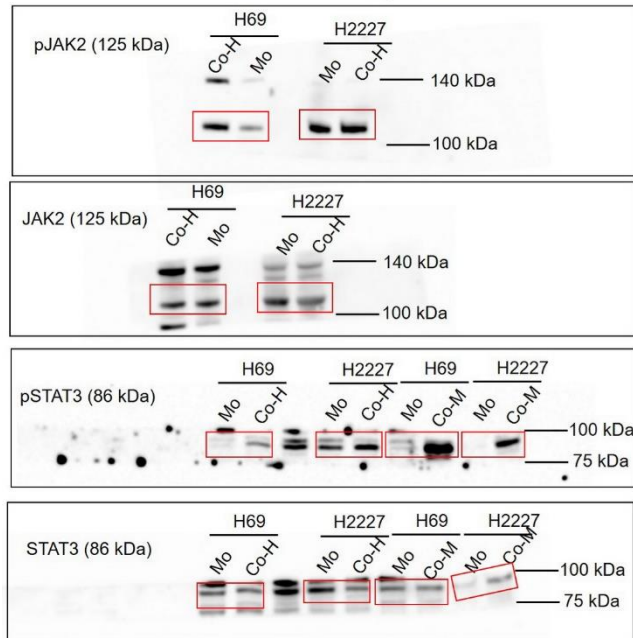


Figure 4c



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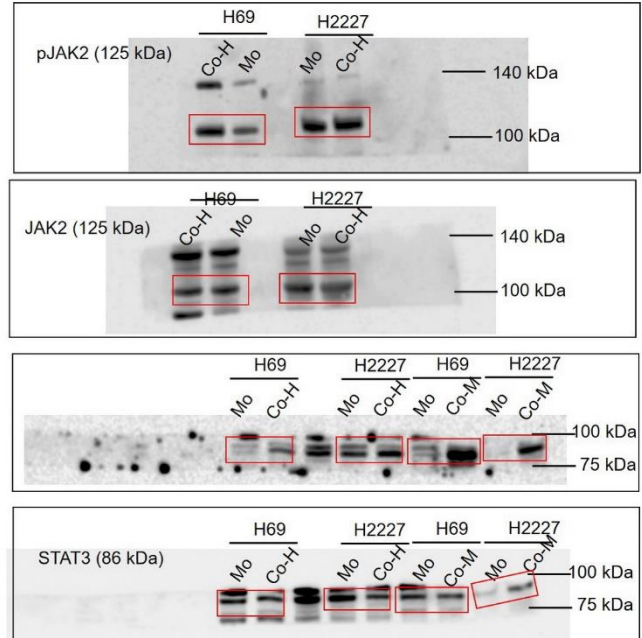
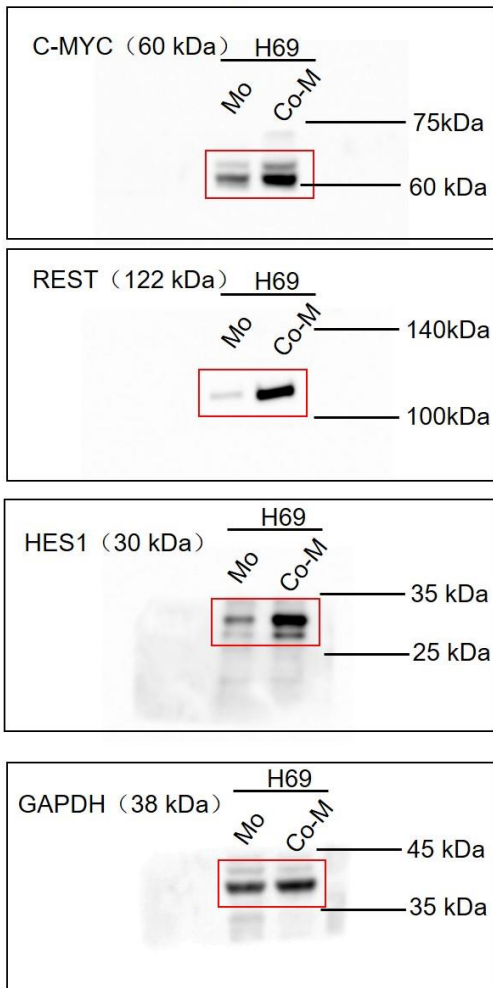


Figure 4d



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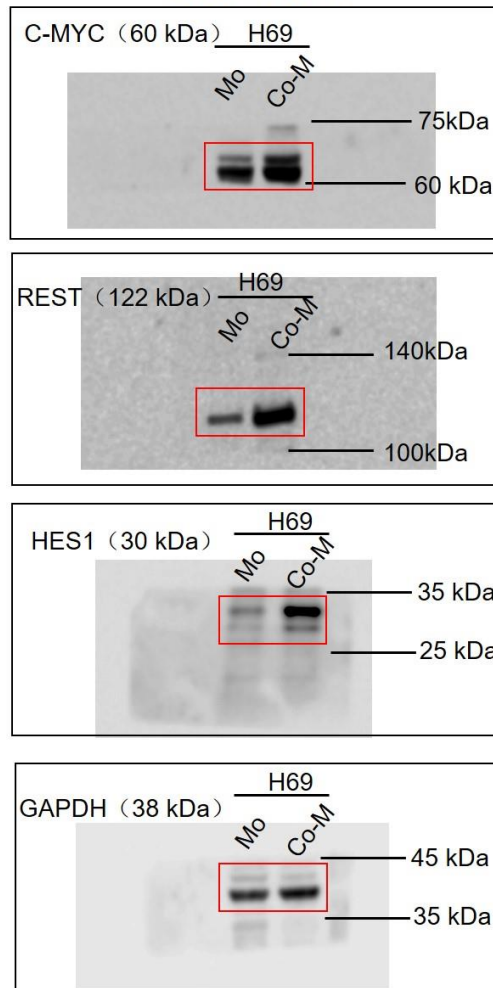
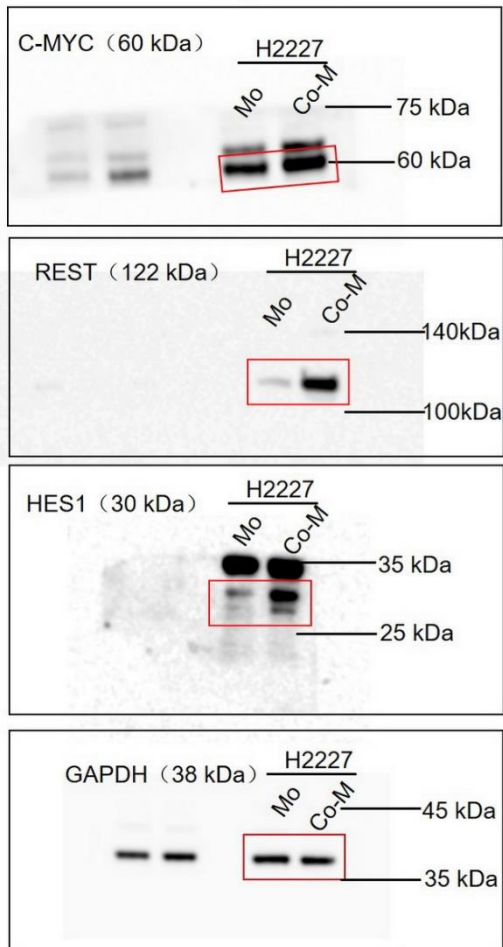


Figure 4d



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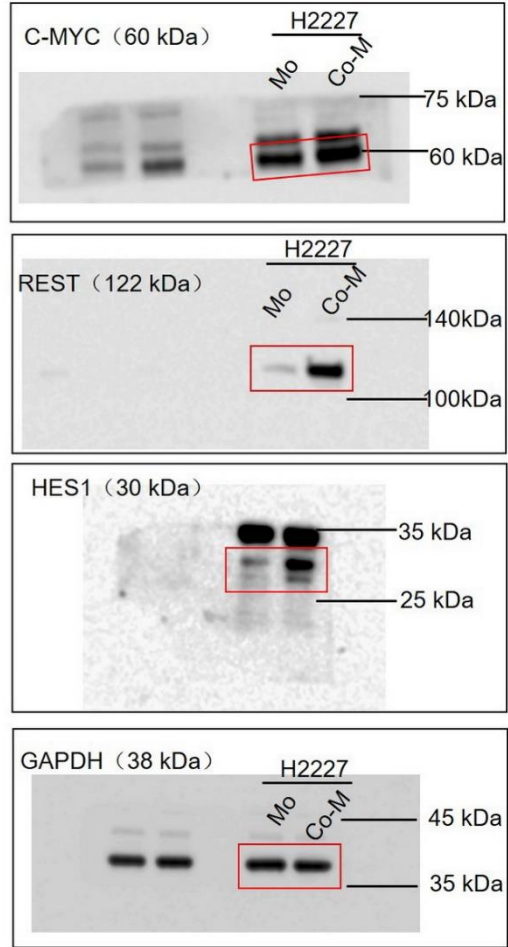
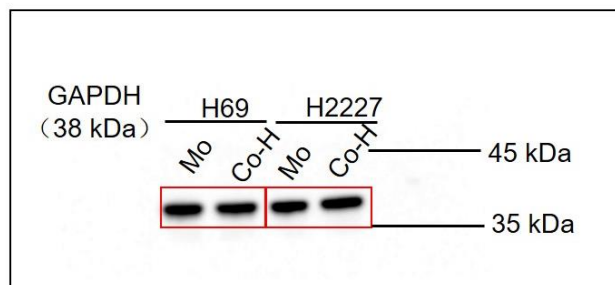
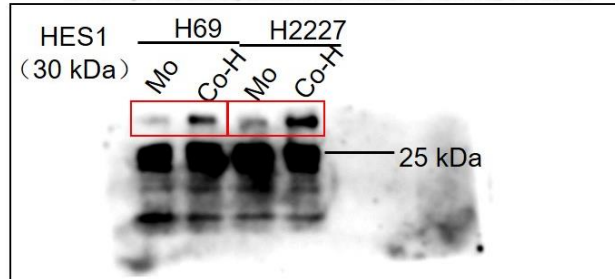
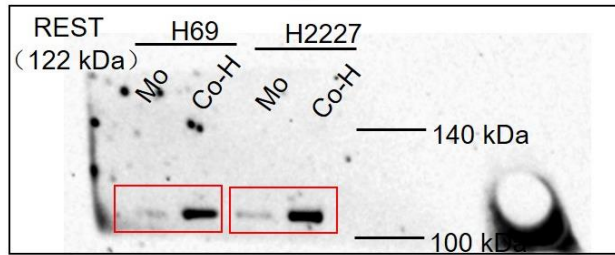
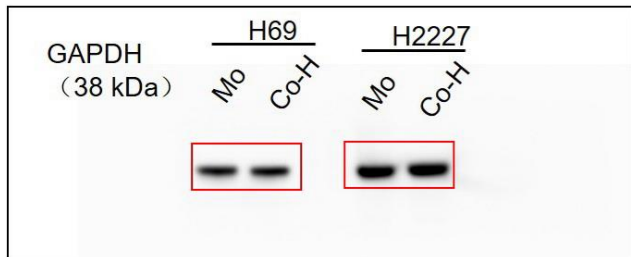
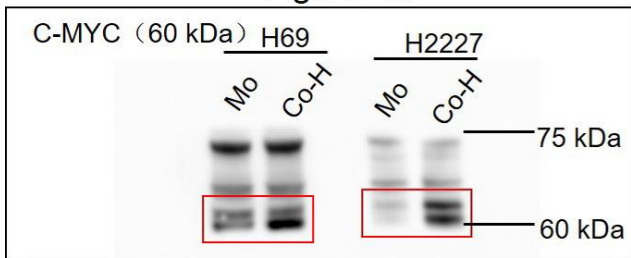


Figure 4d



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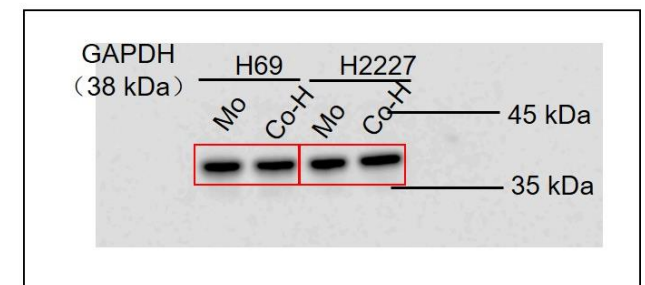
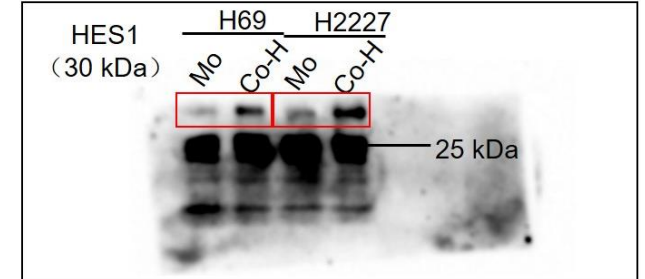
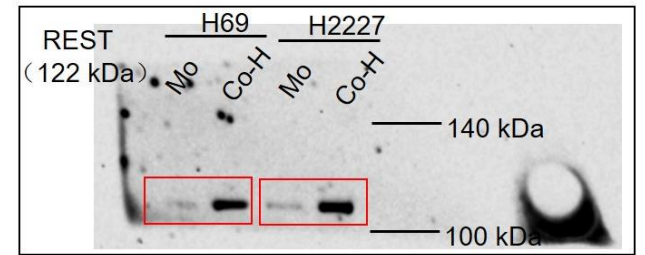
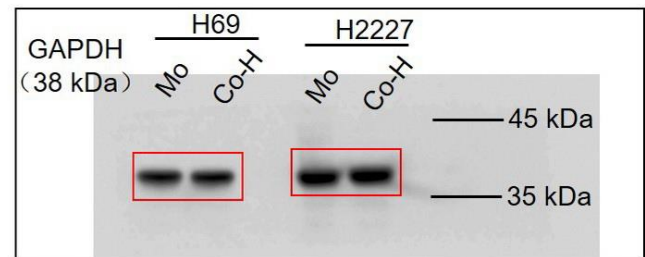
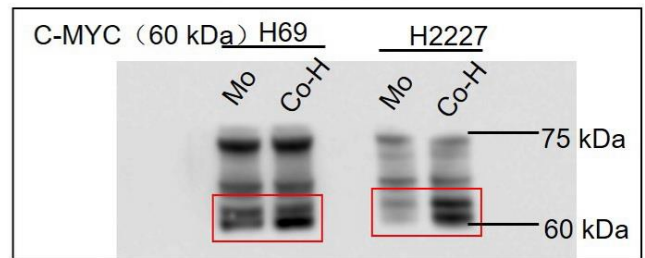
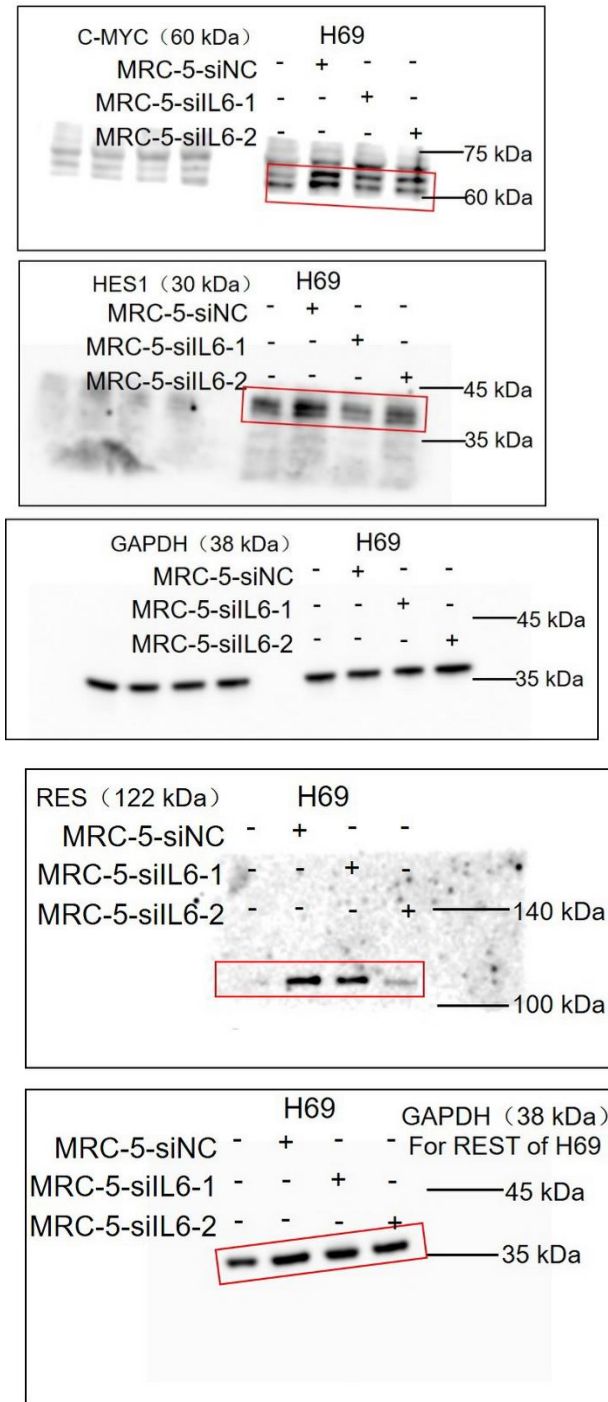


Figure 6a



Low contrast

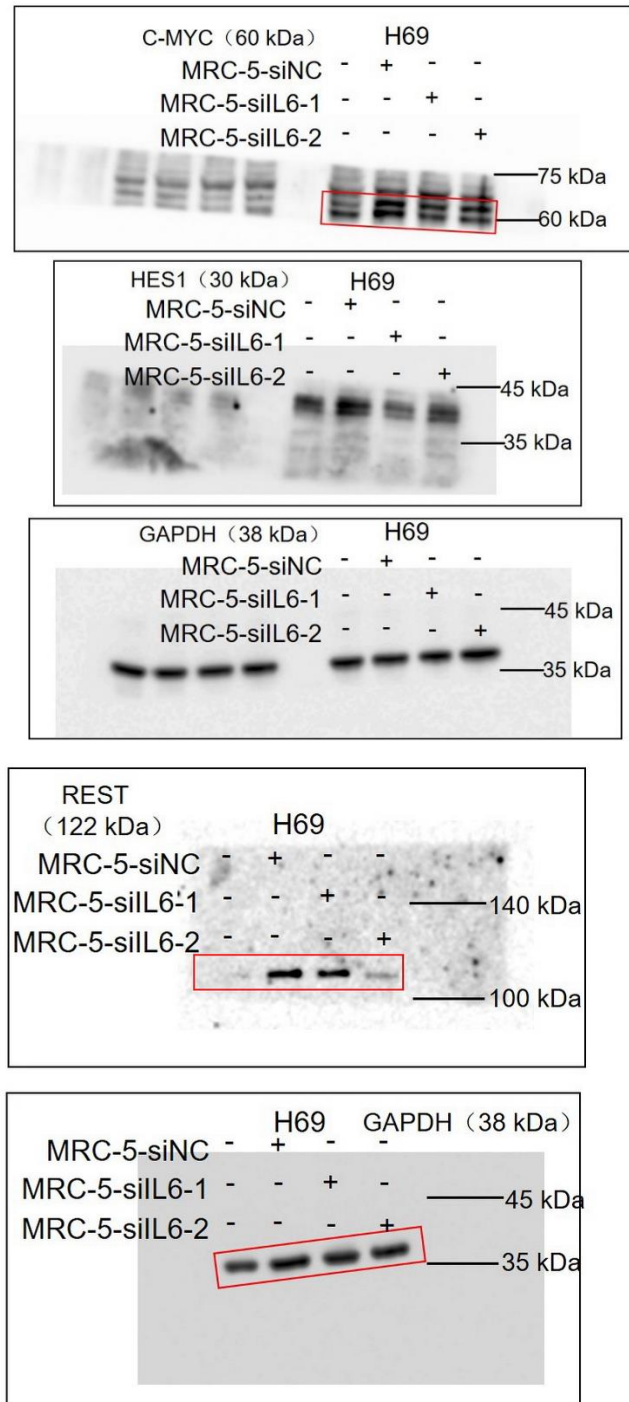
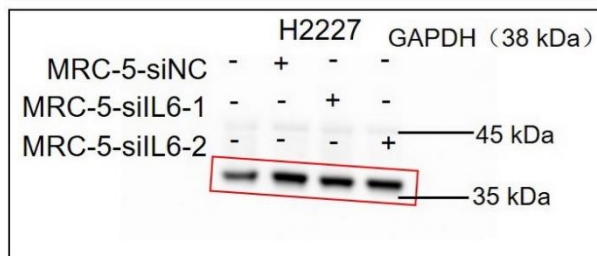
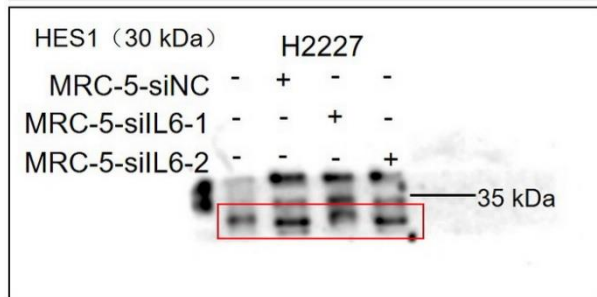
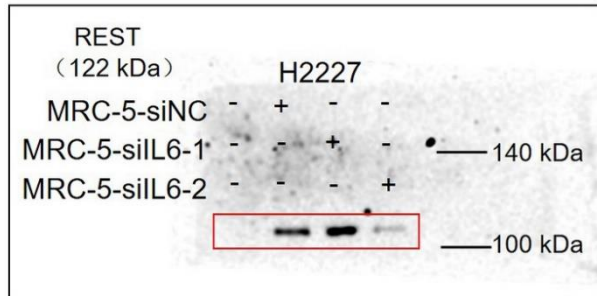
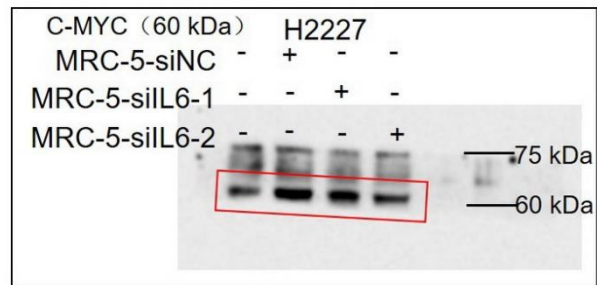


Figure 6a



Low contrast

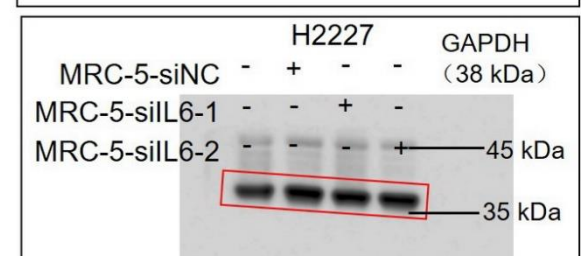
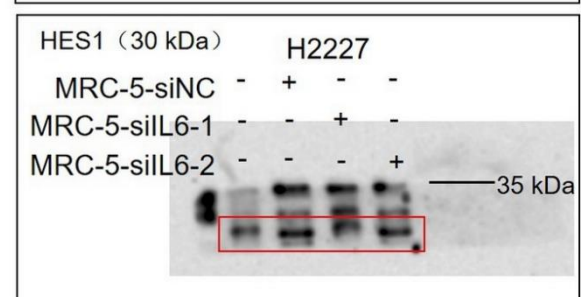
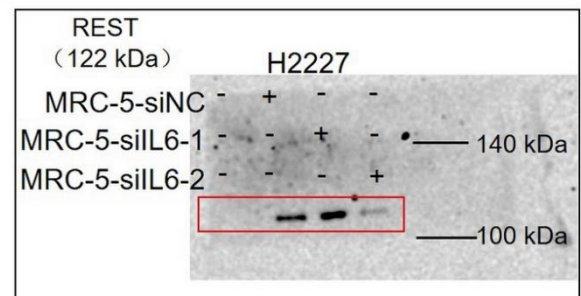
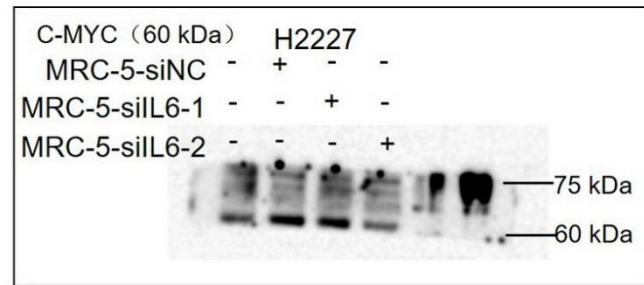


Figure 6b

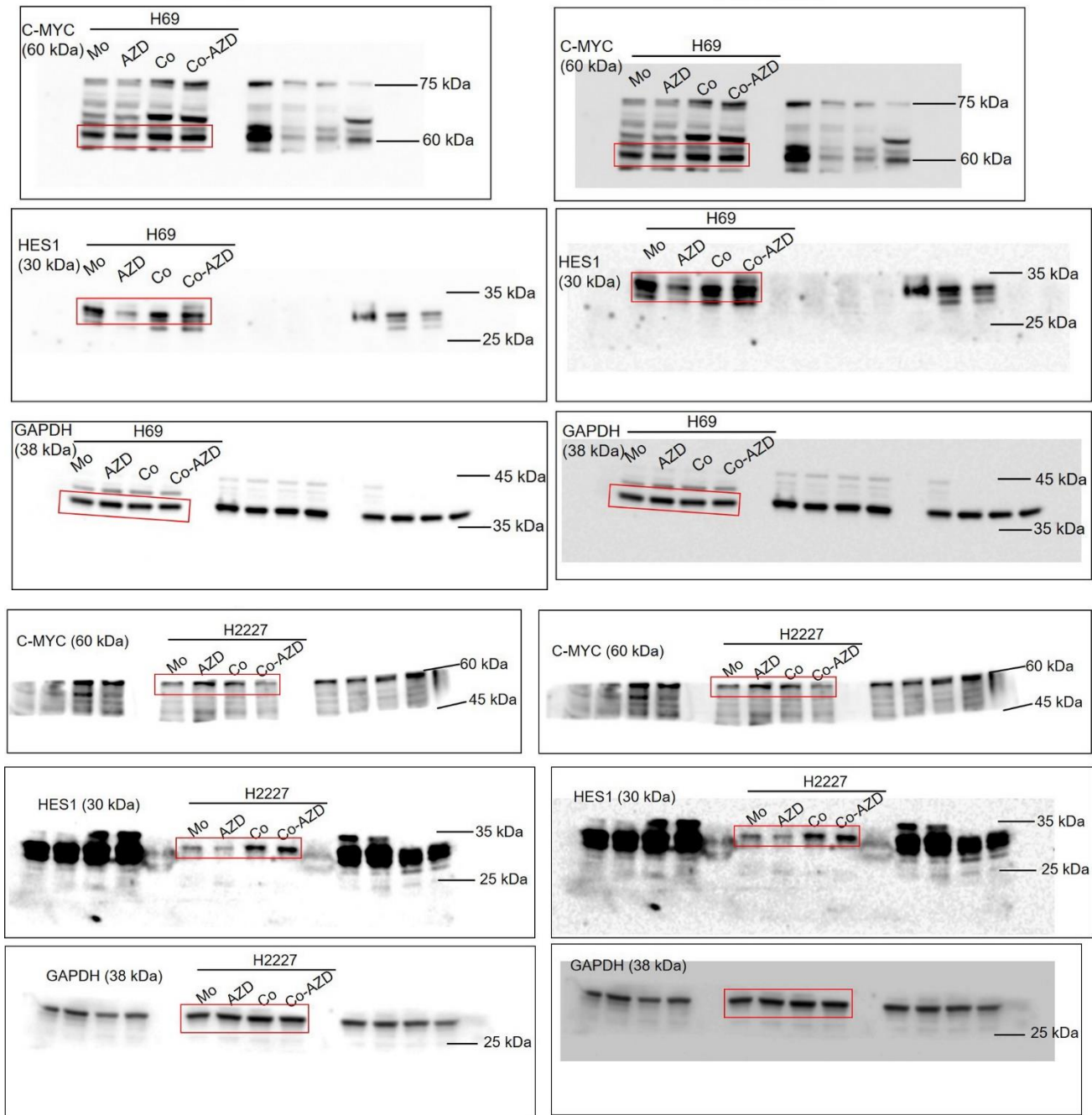


Figure 6e

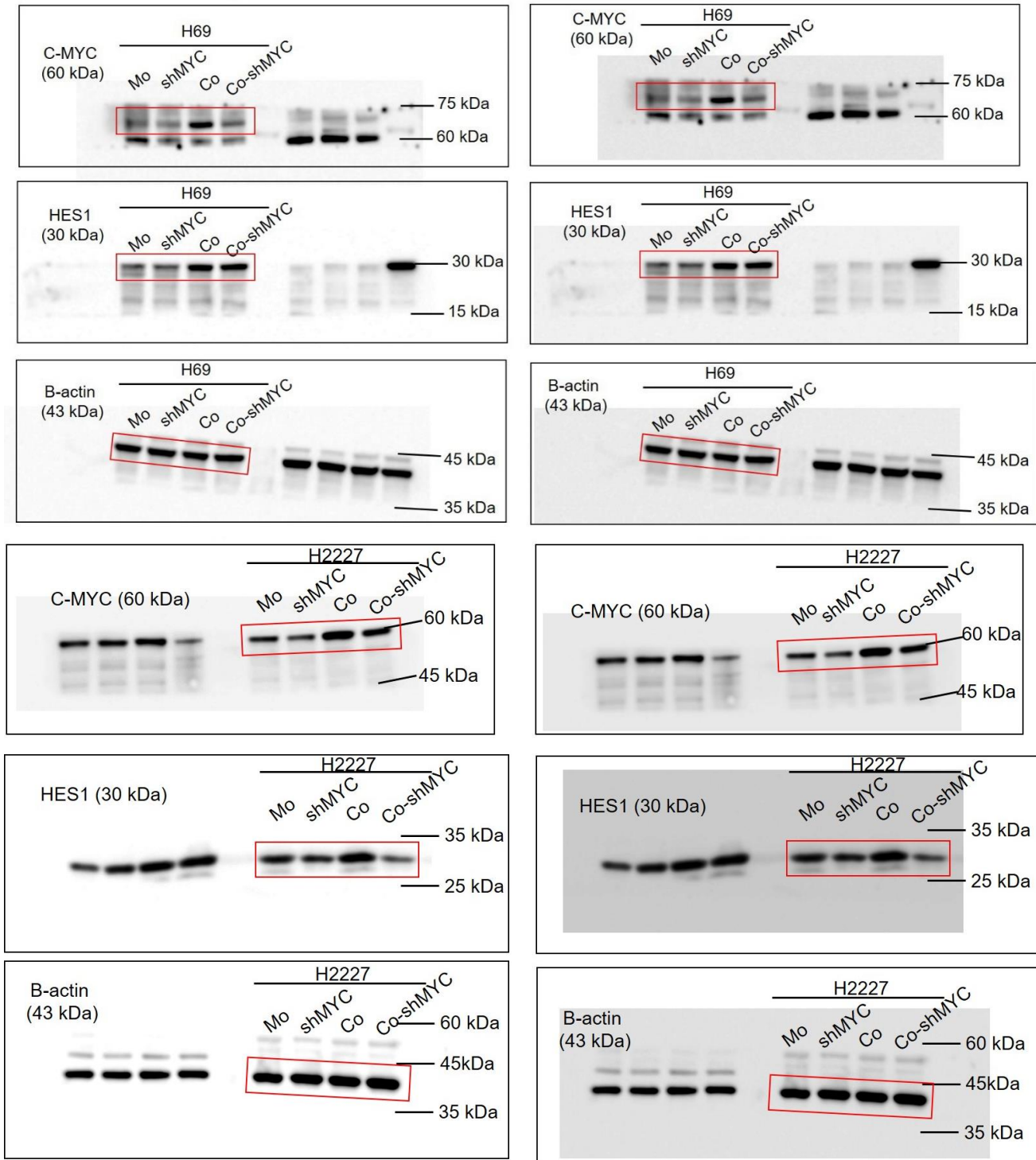


Figure S2a

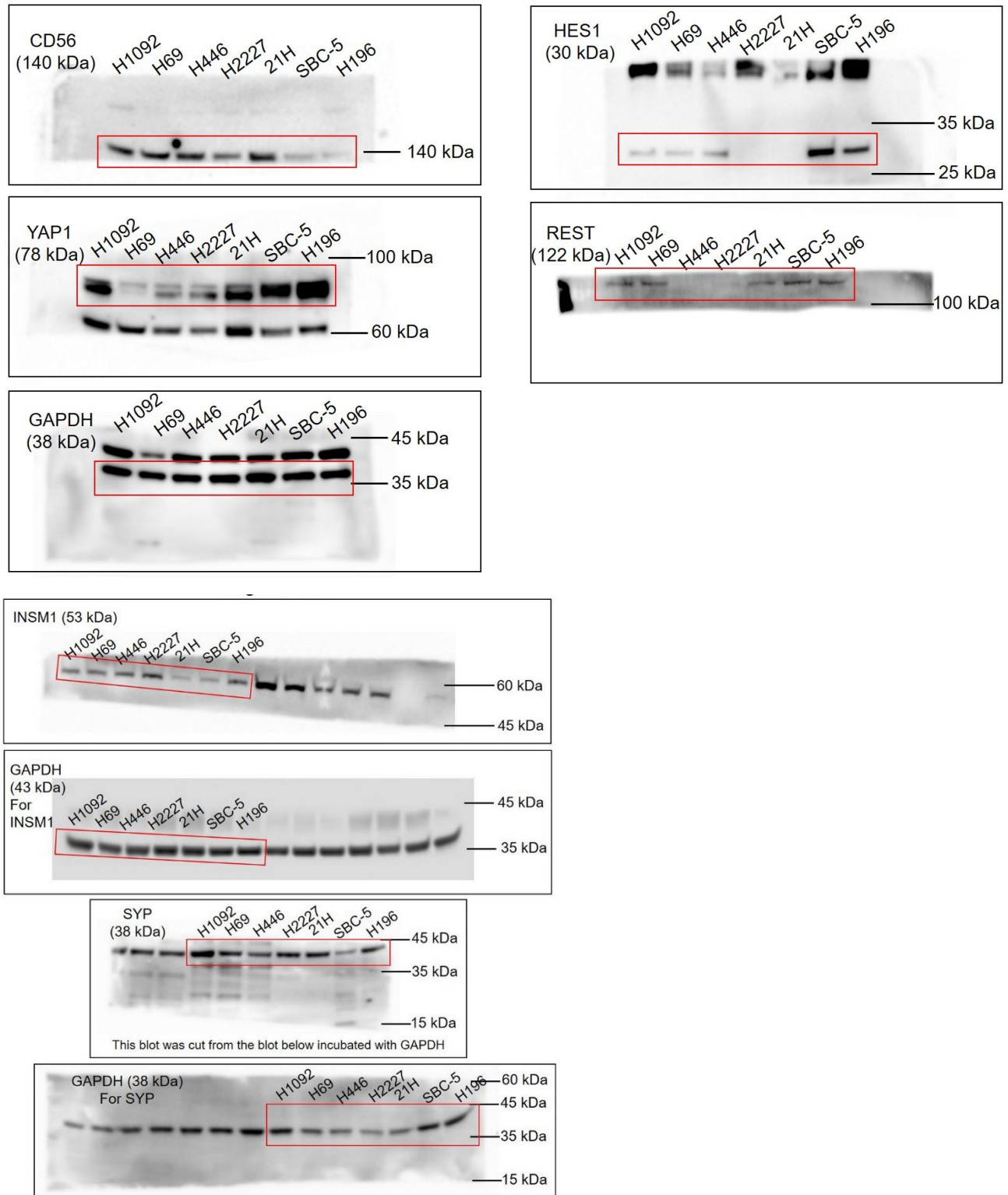
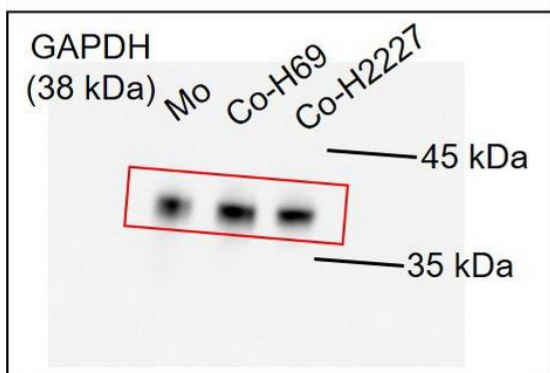
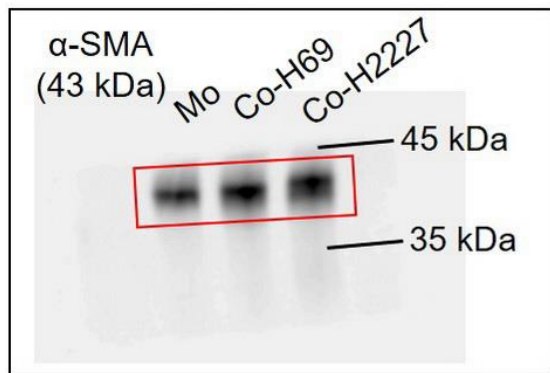
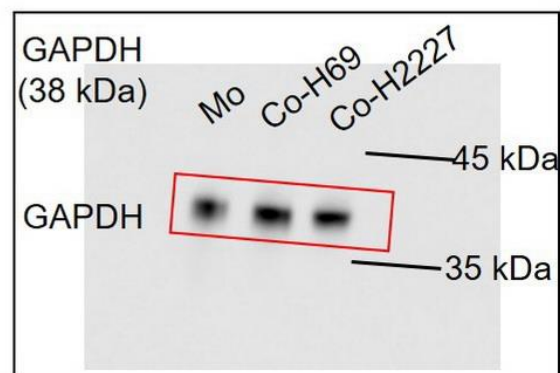
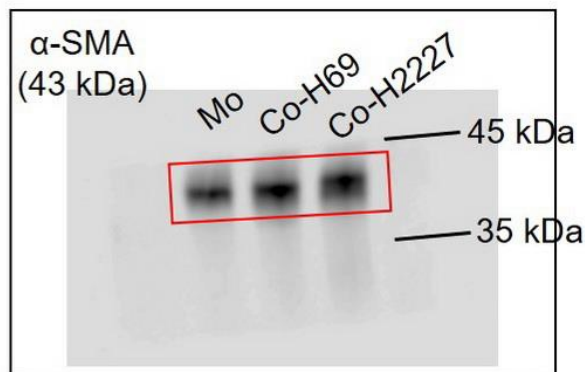


Figure S2e

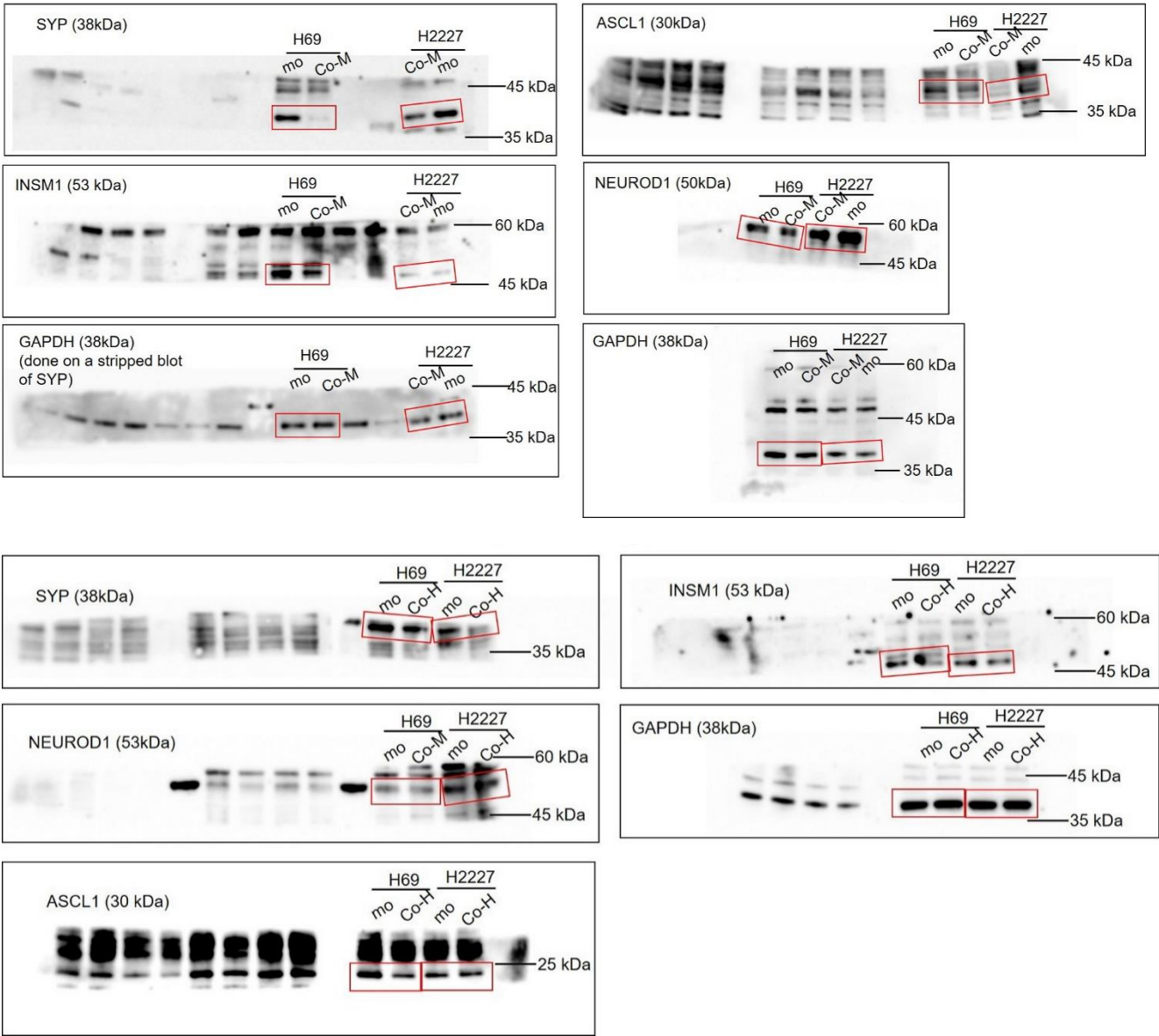


Low contrast

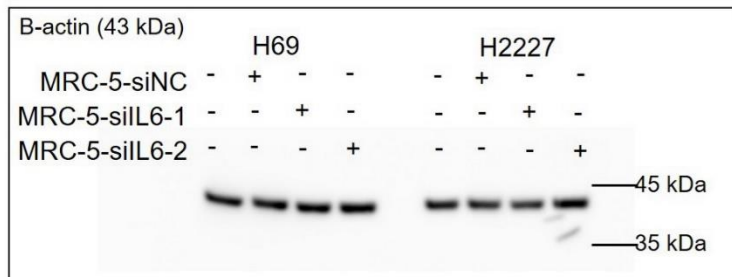
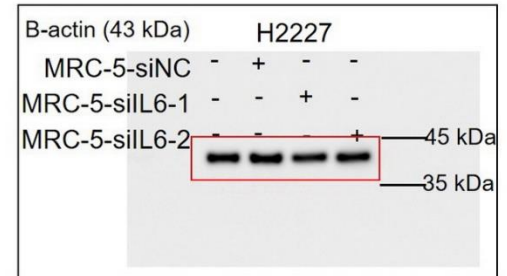
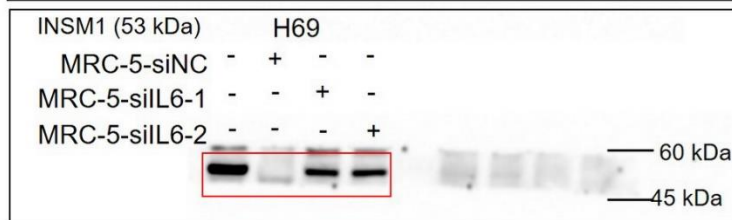
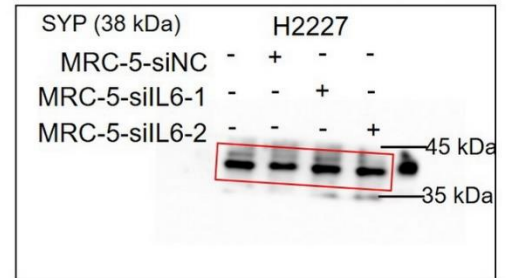
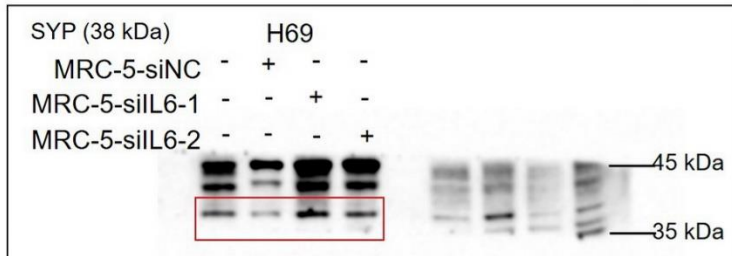
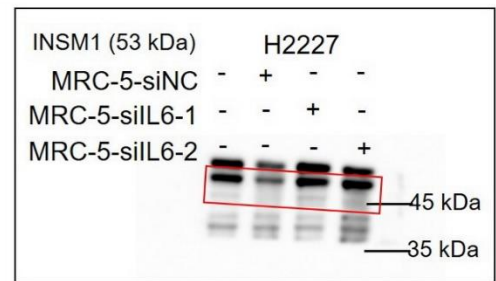
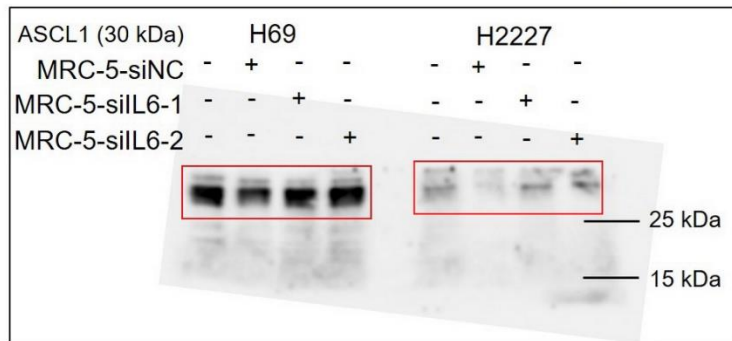


Replicates for western blots

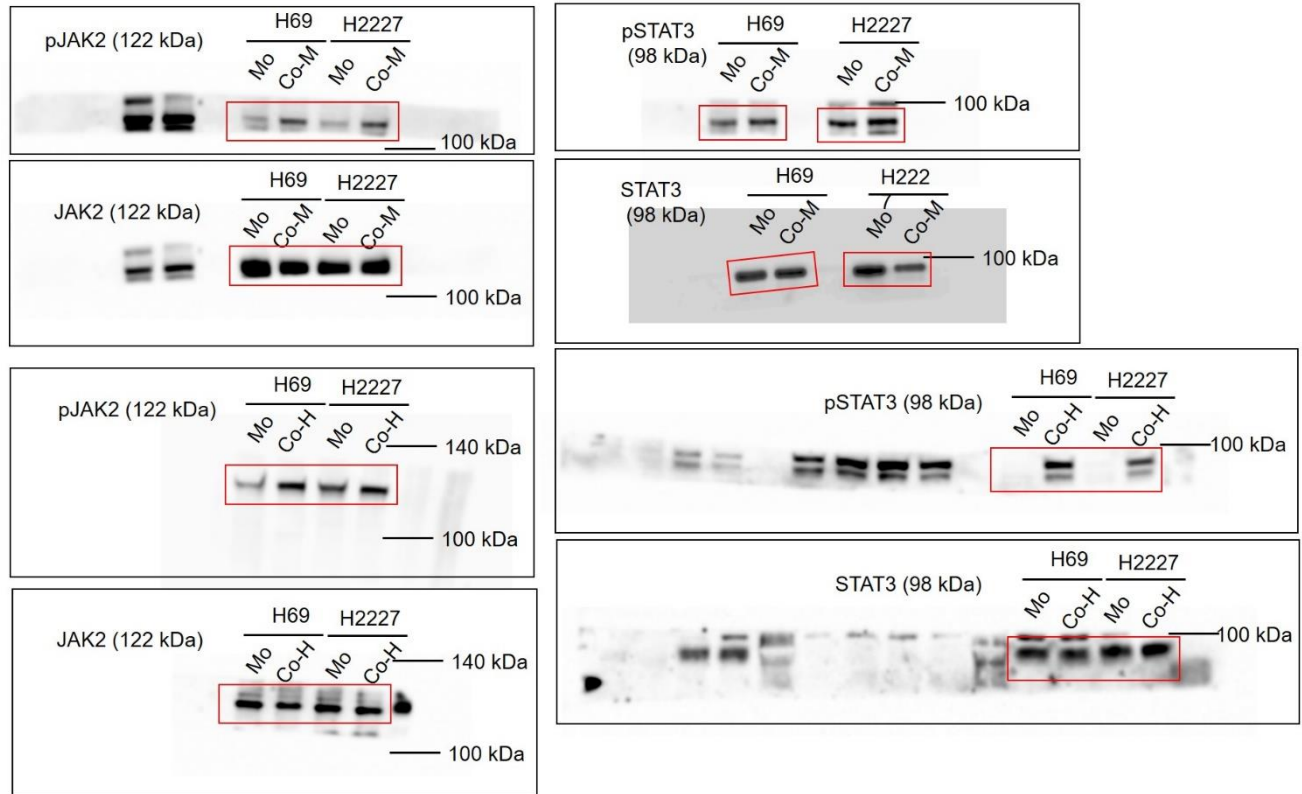
Replicates for Figure 3c



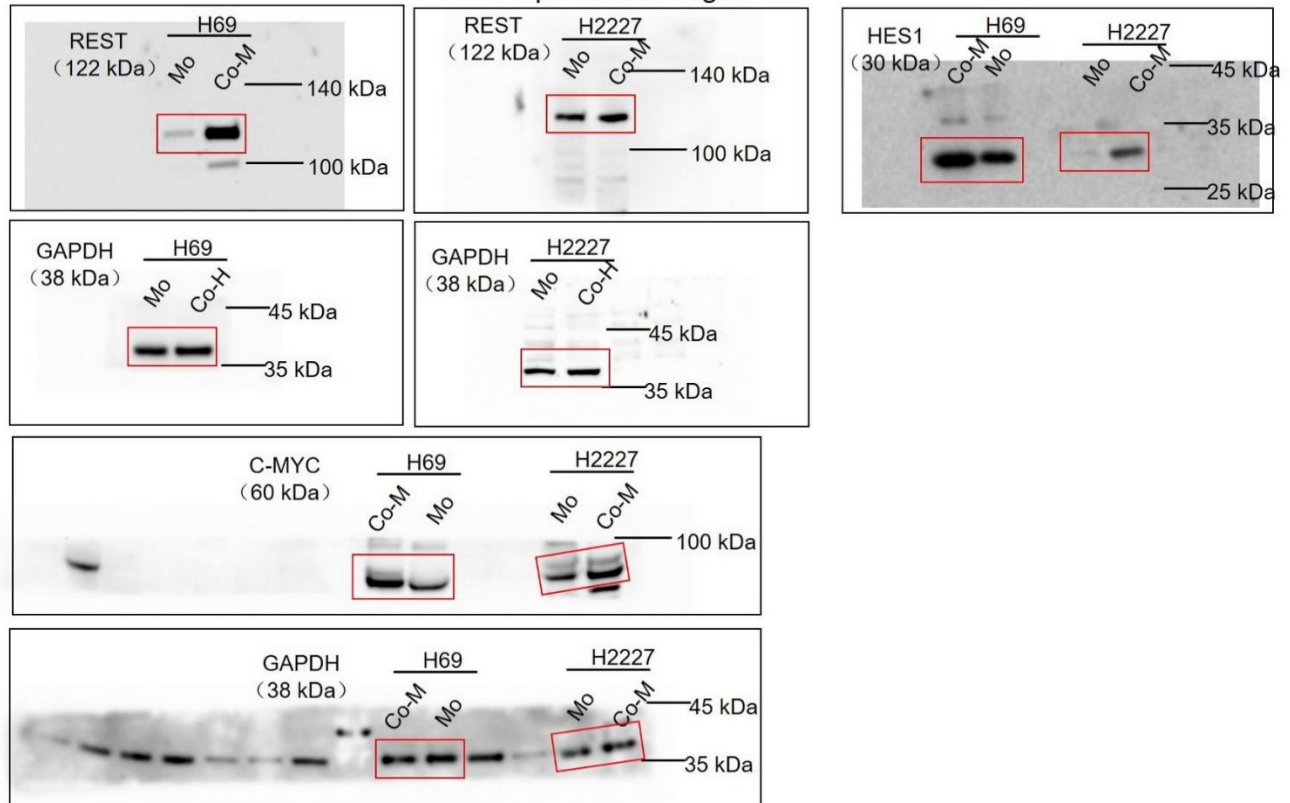
Replicates for Figure 3g



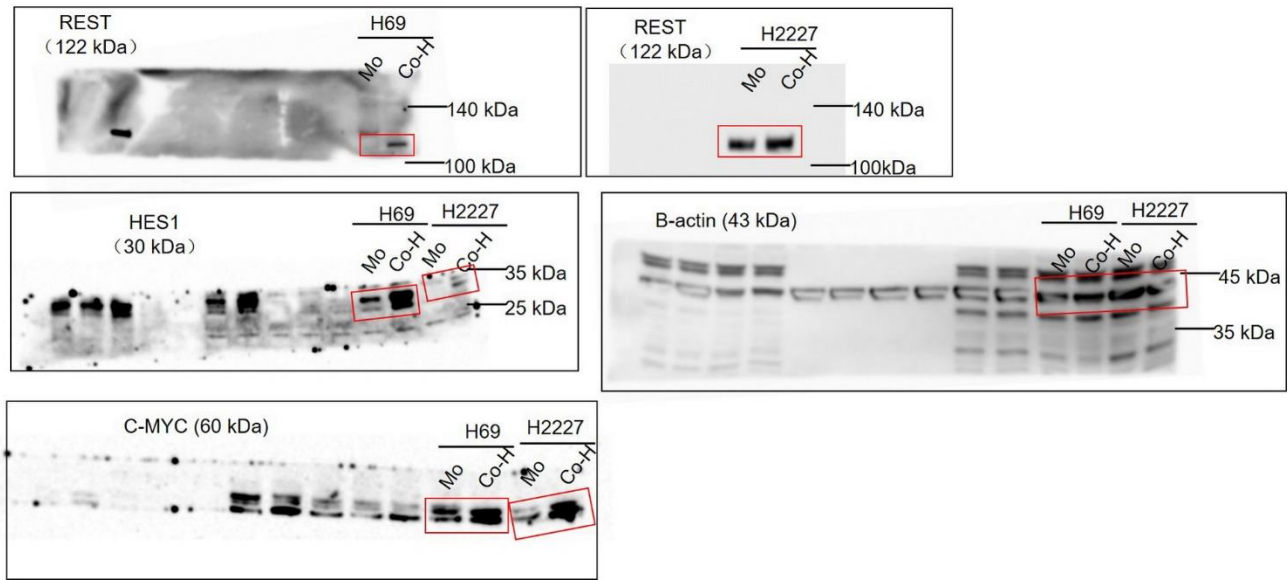
Replicates for Figure 4c



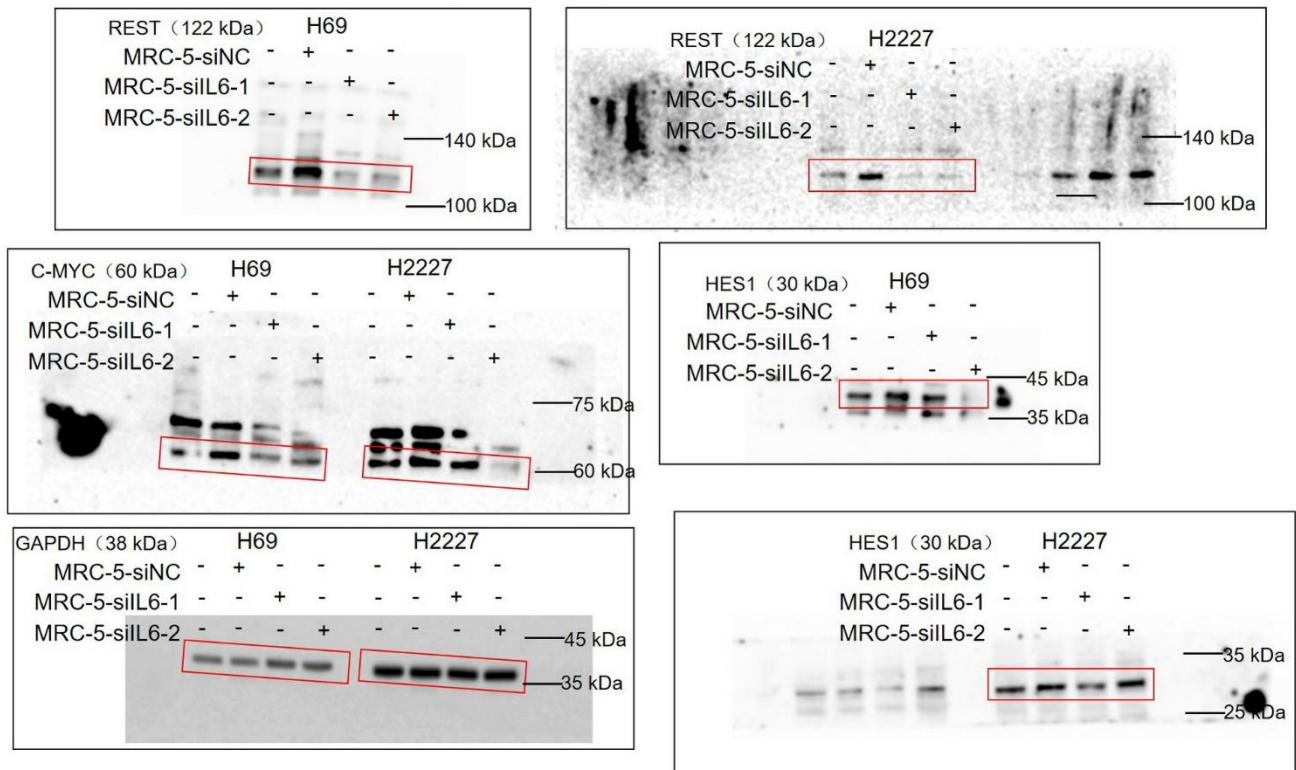
Replicates for Figure 4d



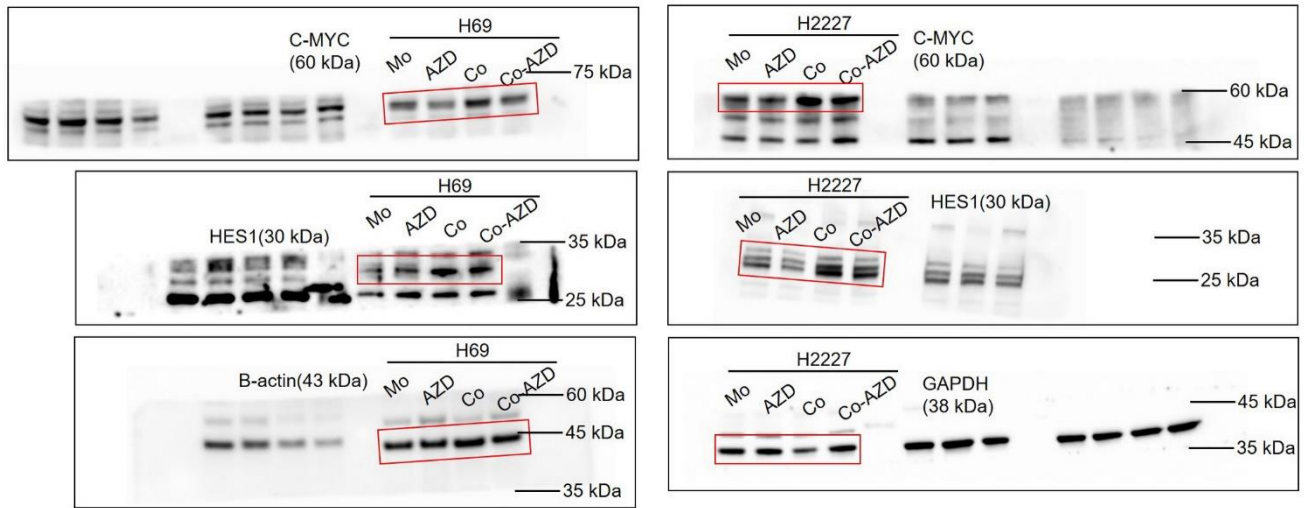
Replicates for Figure 4d



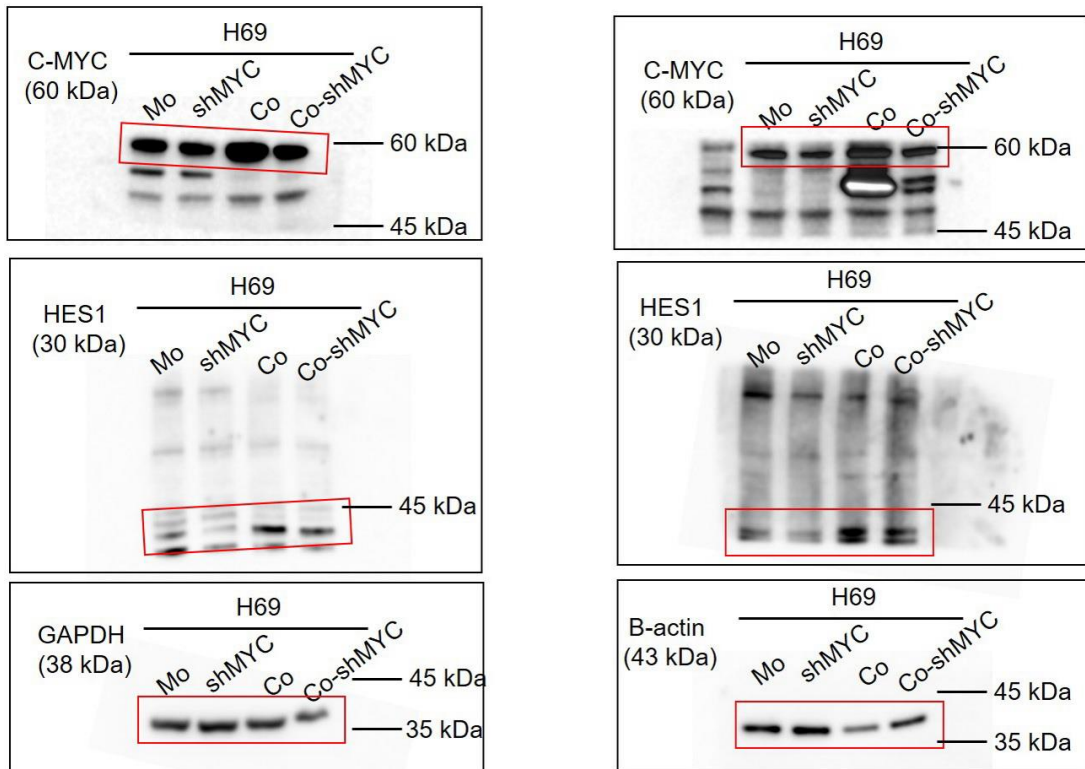
Replicates for Figure 6a



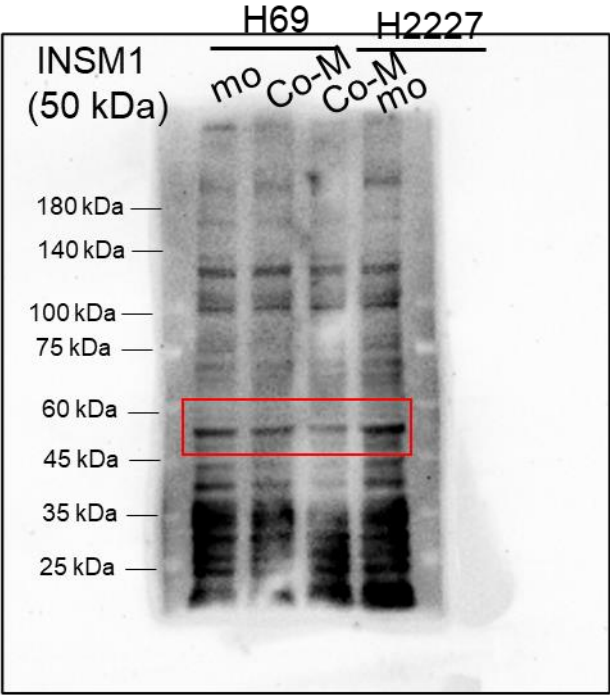
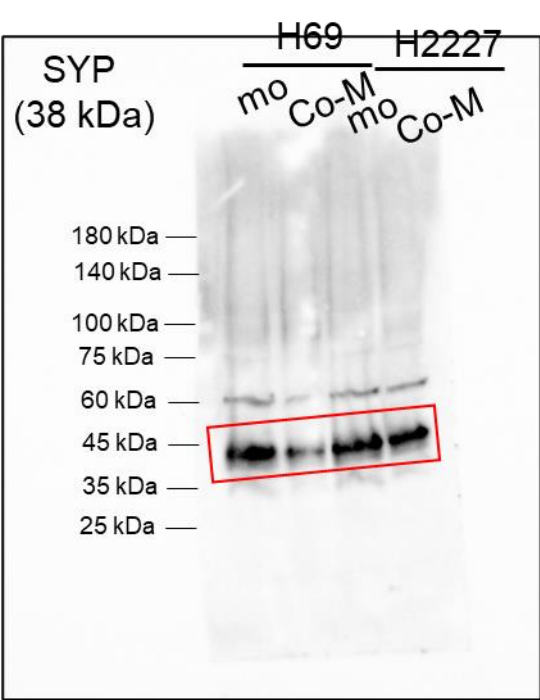
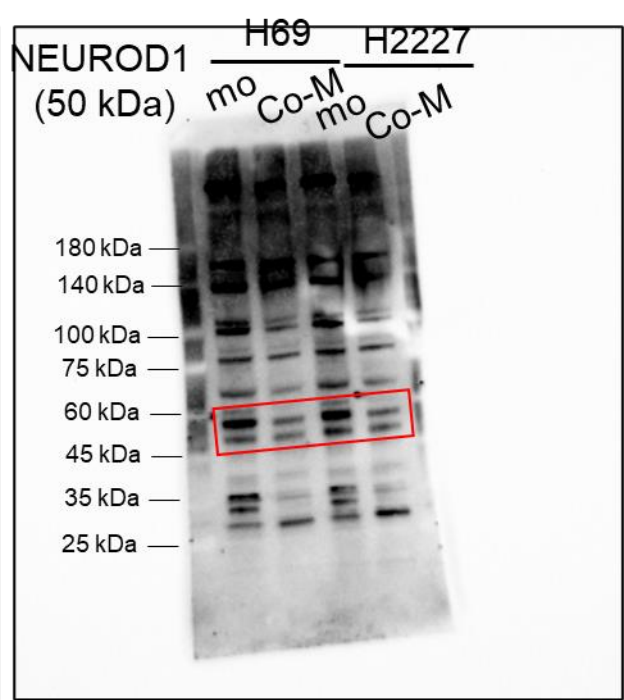
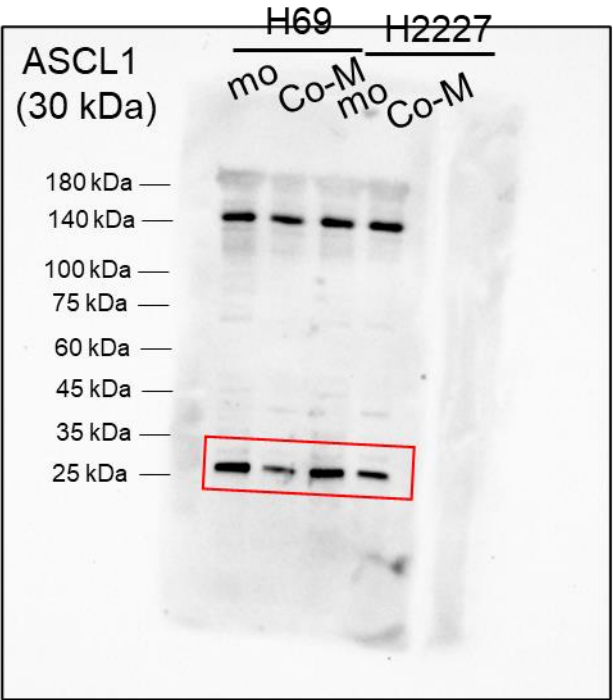
Replicates for Figure 6b



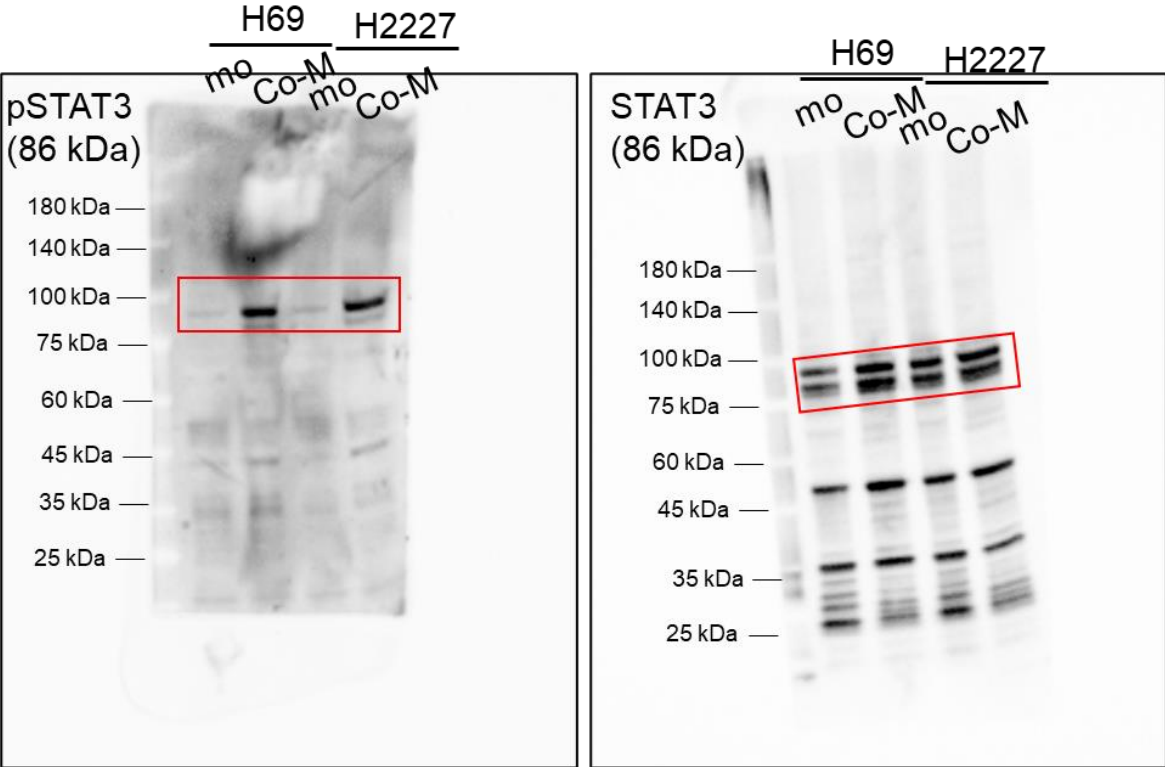
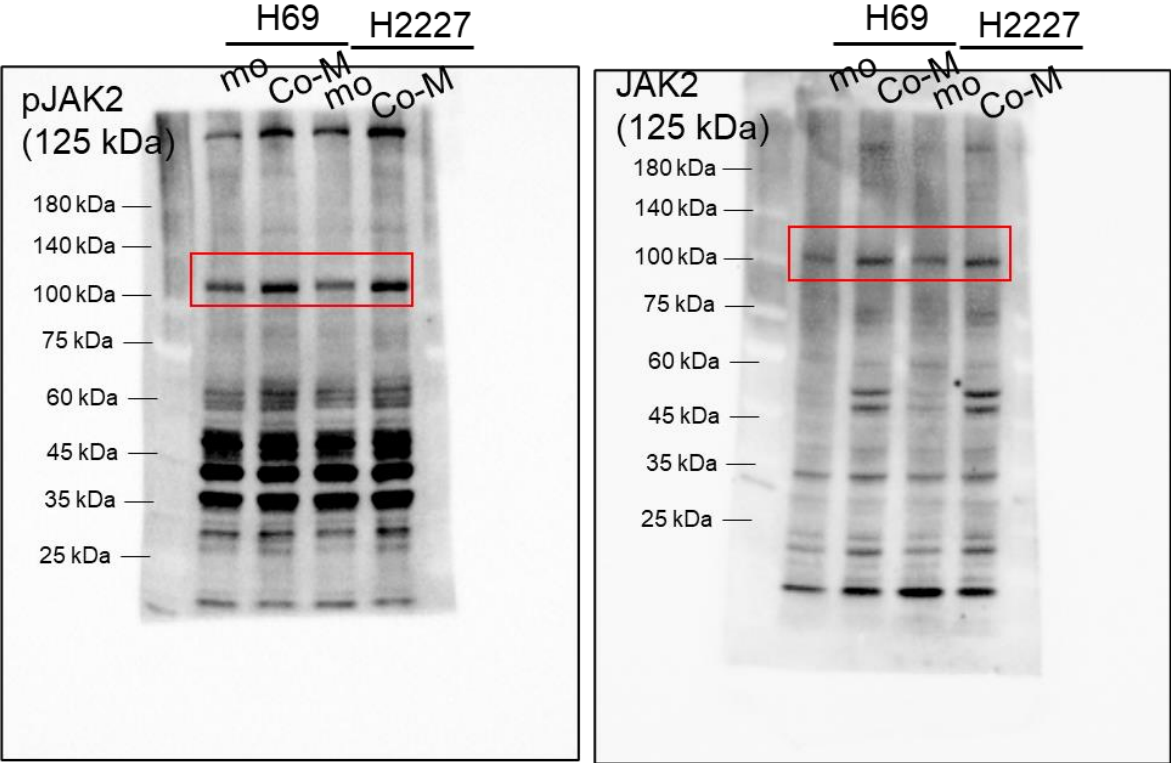
Replicates for Figure 6e



Replicates (fuller-length, original, unprocessed blots)



Replicates (fuller-length, original, unprocessed blots)



Replicates (fuller-length, original, unprocessed blots)

