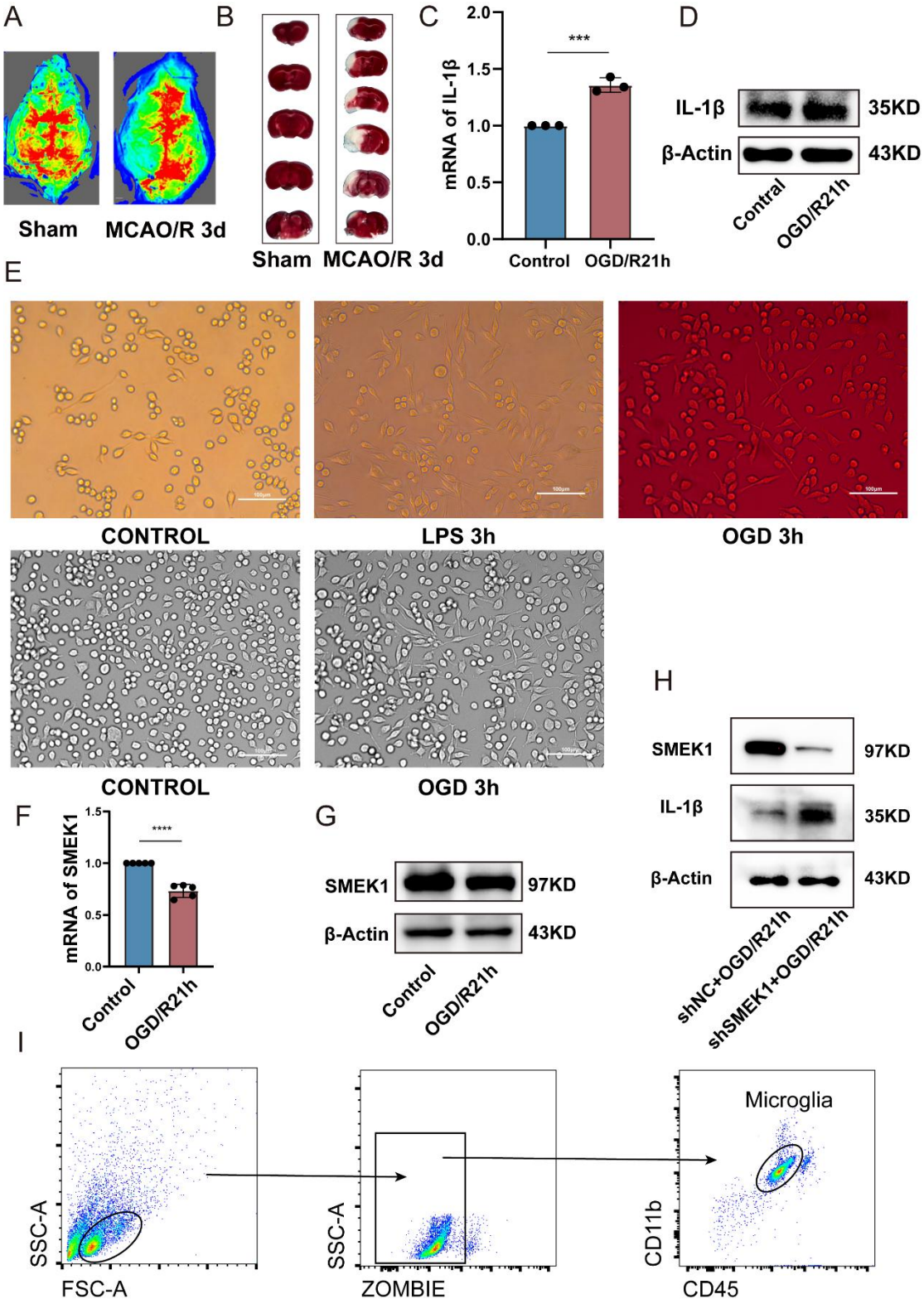
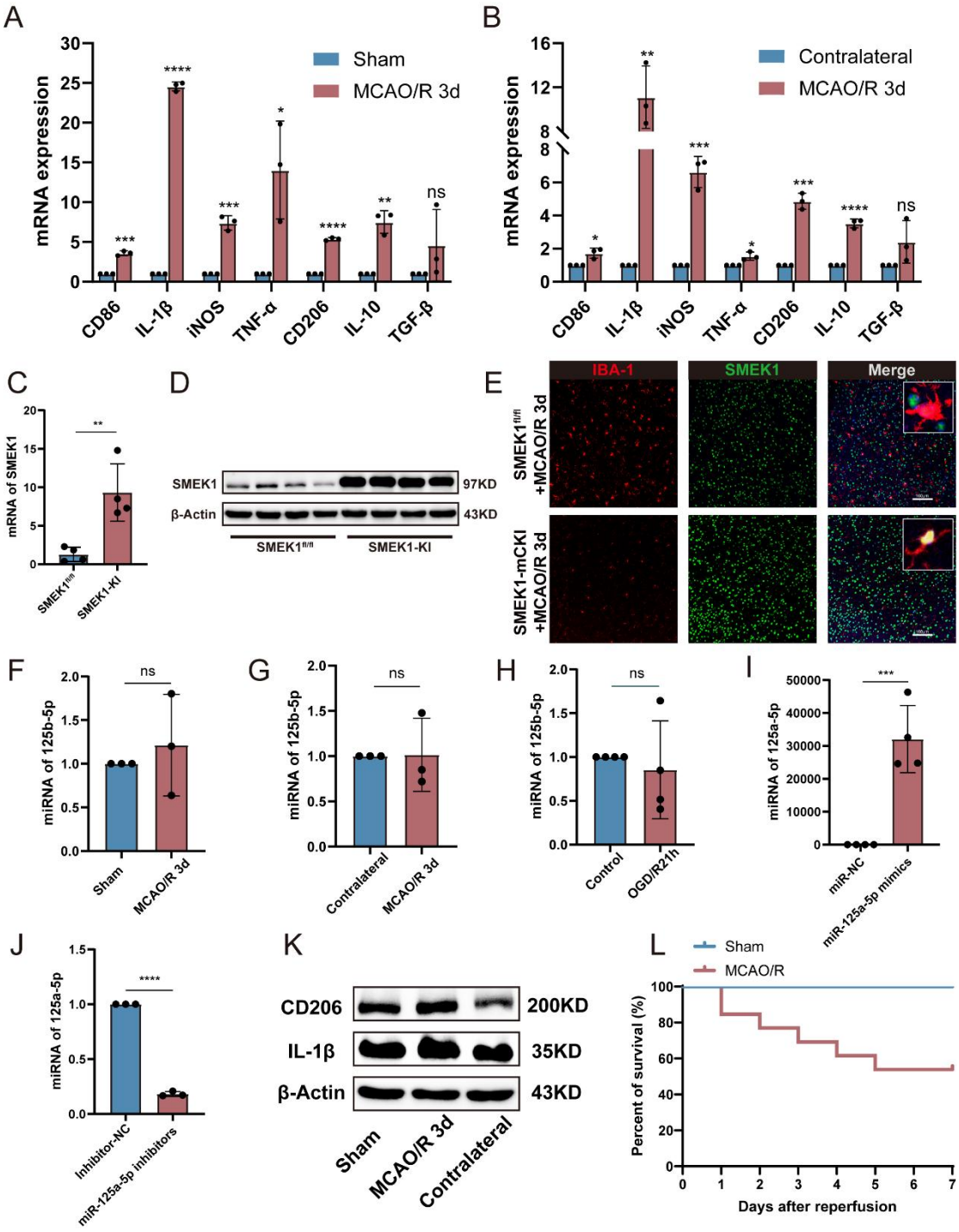


Supplementary figure 1. Validation of MCAO/R and OGD/R models and the gating strategy for microglia.

(A) Representative photographs of laser speckle contrast images of cortical CBF. (B)

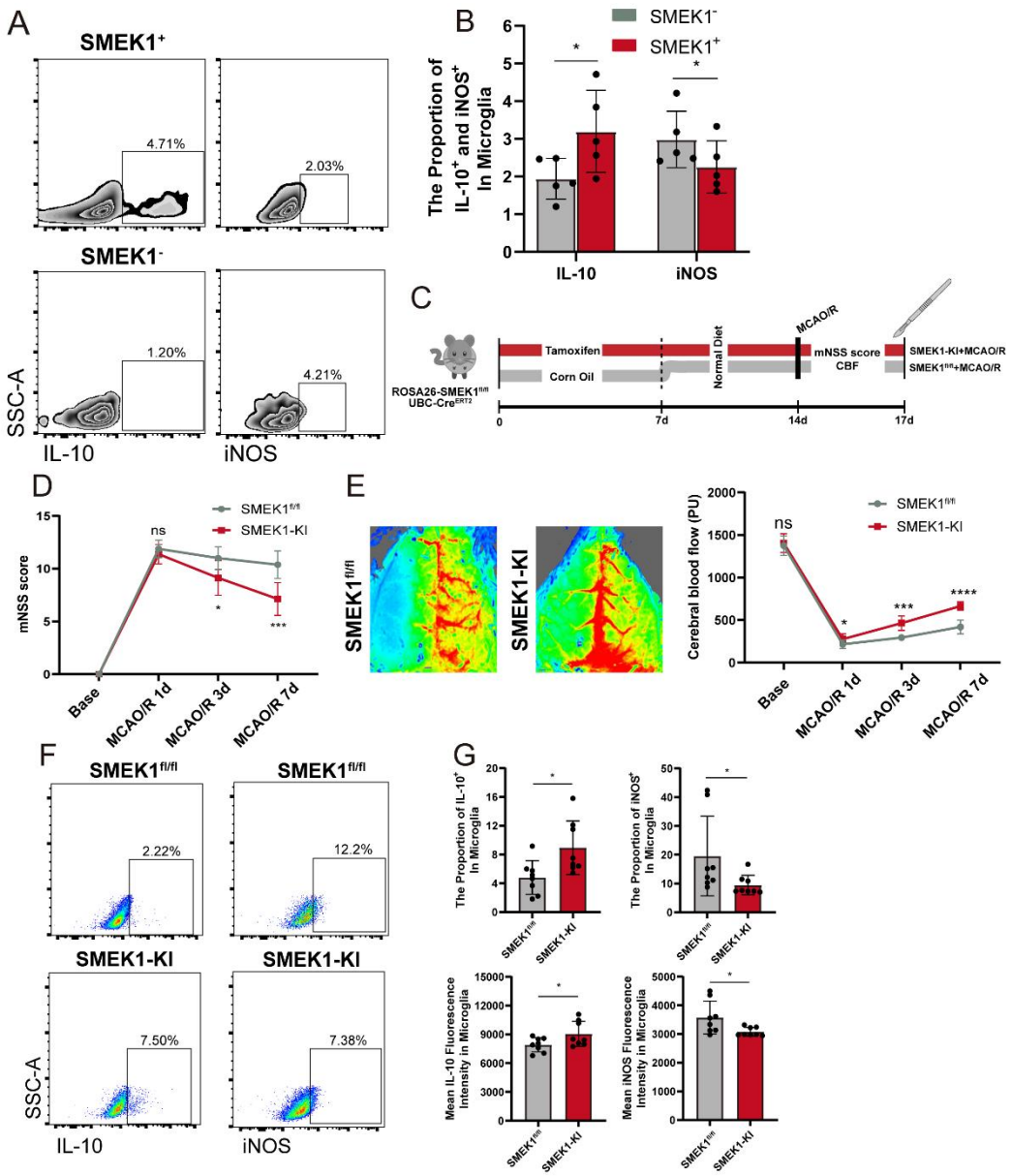
Infarct size was determined by TTC staining. Determination of IL-1β mRNA (C) and protein (D) expression in BV2 cells subjected to OGD. n = 3 per group. (E) Representative images showing the morphology of BV2 cells in the control, LPS 3 h and OGD groups. Determination of SMEK1 mRNA (F) and protein (G) expression in BV2 cells subjected to OGD/R21h. n = 5 per group. (H) The protein expression of SMEK1 and IL-1β in shNC and shSMEK1 BV2 cells subjected to OGD/R21h was assessed using WB. (I) Gating strategy for microglia isolated from the brains after MCAO/R 3d mice. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$ vs. Control group. ns, not significant.



Supplementary figure 2. Validation of SMEK1-KI mice and survival of MCAO/R mice.

(A, B) The expression of CD86, IL-1β, iNOS, TNF-α, CD206, IL-10 and TGF-β in the brains of mice in the Sham, MCAO/R 3d and Contralateral groups was assessed using qPCR. n = 3 per group. Determination of SMEK1 mRNA (C) and protein (D)

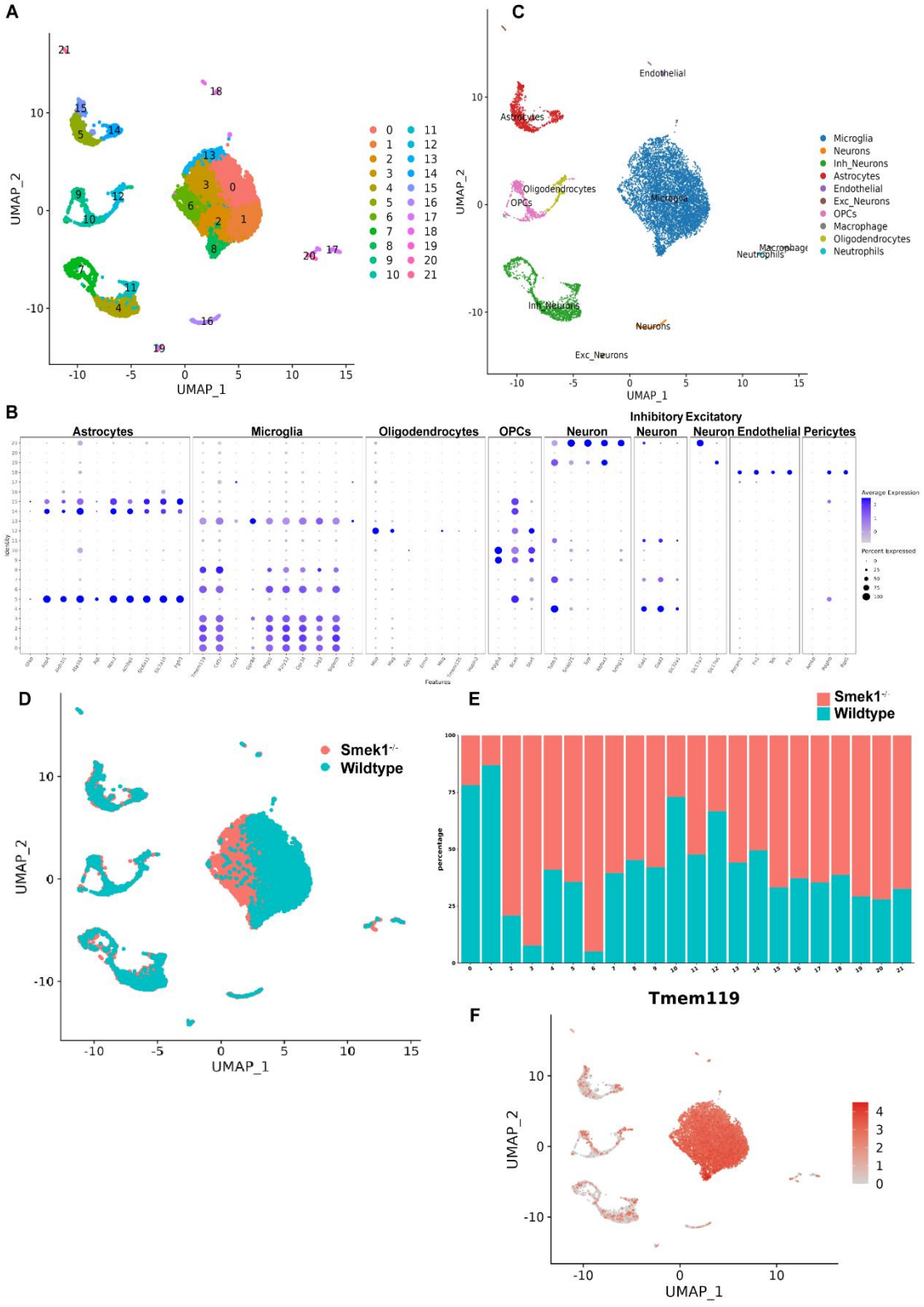
expression in SMEK1$^{fl/fl}$ and SMEK1-KI mice. n = 4 per group. (E) Representative photos of SMEK1^{+} microglia in SMEK1$^{fl/fl}$ and SMEK1-mCKI mice, scale bar: 100μm. (F-H) The expression of miR-125b-5p in the brains of mice in the Sham, MCAO/R 3d, and Contralateral groups and in BV2 cells subjected to OGD/R21h. n = 3 per group. (I, J) The expression of miR-125a-5p in BV2 cells transfected with miR-125a-5p mimics or inhibitors. n = 3 per group. (K) Western blot analysis of CD206 and IL-1β in the Sham, MCAO/R 3d and Contralateral groups. (L) The percent survival of Sham and MCAO/R mice. n = 15 per group. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001 vs. Sham or Contralateral or SMEK1$^{fl/fl}$ or Control or miR-NC or Inhibitor-NC group. ns, not significant.



Supplementary figure 3. Neuroinflammation was reduced after MCAO/R in SMEK1-KI mice.

(A) Gating strategy for IL-10$^+$ and iNOS$^+$ cells in SMEK1$^+$ or SMEK1$^-$ microglia of MCAO/R mice. (B) Flow cytometry analysis of IL-10 and iNOS expression in SMEK1$^+$ or SMEK1$^-$ microglia from the brains of MCAO/R 3d mice. n = 5 per group.

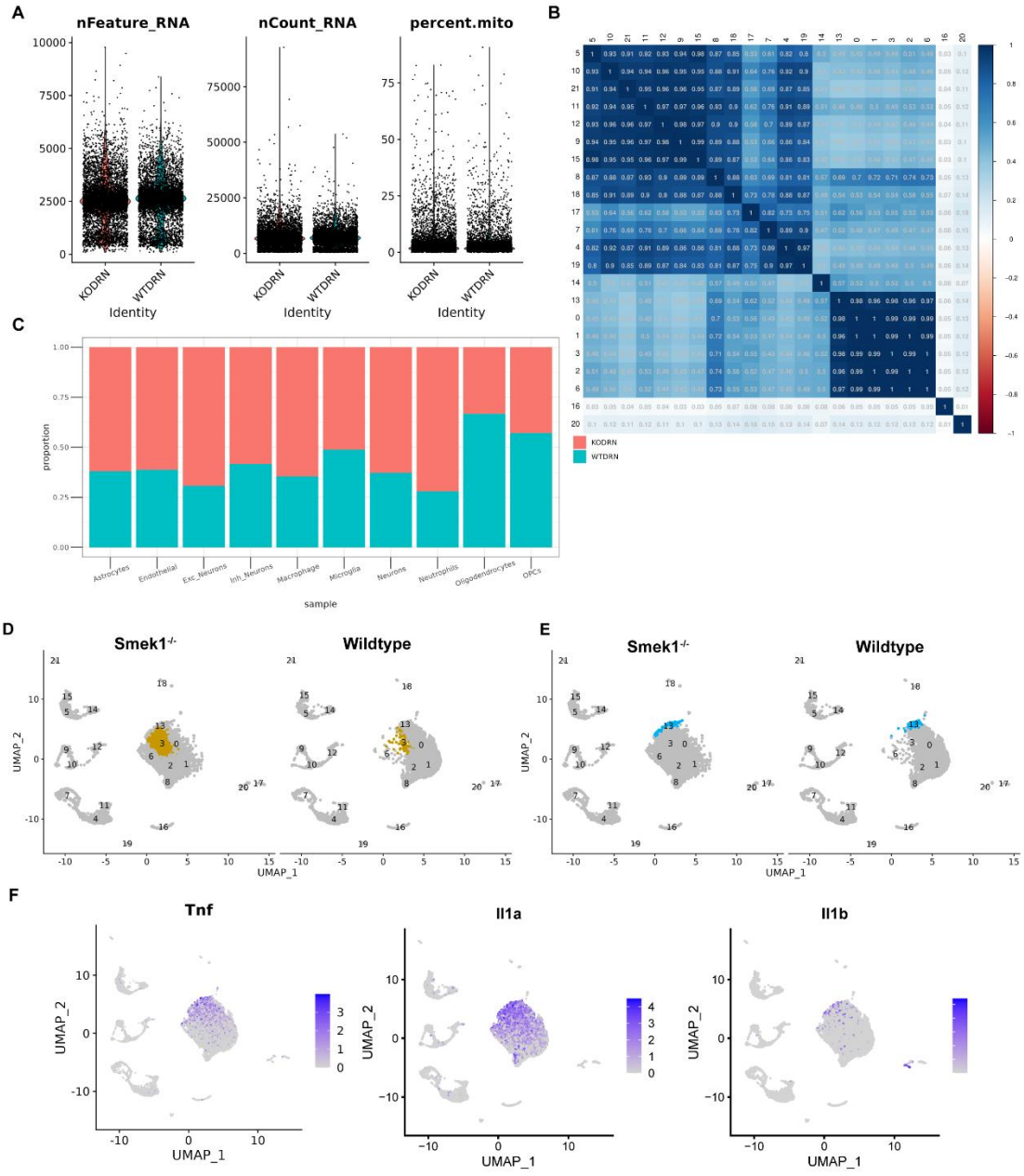
(C) Schematic diagram of the experimental design for SMEK1-KI mice. (D) Neurological function was evaluated by the mNSS test. (E) Representative photographs of laser speckle contrast images and quantitative analyses of cortical CBF. (F) Gating strategy for IL-10^{+}, iNOS^{+} microglia in the brains of control and SMEK1-KI mice after MCAO/R. (G) Flow cytometry analysis of IL-10, iNOS expression in microglia from the brains of control and SMEK1-KI mice after MCAO/R. D-G, n = 8 for each group. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001 vs. SMEK1^{-} or Base or SMEK1$^{fl/fl}$ group. ns, not significant.



Supplementary figure 4. scRNA-seq analysis strategy.

(A) scRNA-seq profiling of SMEK1$^{-/-}$ and wild-type mouse cells obtained from the hippocampus and cortex (GSE171986). (B) Dot plots of the expression values of

enriched cell type-specific markers for the cell types. (C) Uniform manifold approximation and projection (UMAP) plot showing the cell distribution of different cell types. (D) UMAP plot showing the cell distribution among different SMEK1$^{-/-}$ and wild-type mice. (E) Sequenced cell distribution represented in each cluster. (F) UMAP plot showing Tmem119 as a microglia-specific marker gene.



Supplementary figure 5. scRNA-seq analysis features.

(A) The genes (features), counts, and mitochondrial gene percentages of each sample.

(B) Correlation heatmaps showing hierarchical clustering of Pearson correlation scores calculated between averaged cell types. (C) Sequenced cell distribution represented in each cell type. (D) UMAP plot showing the distribution of cluster 3 among SMEK1$^{-/-}$ and wild-type mice. (E) UMAP plot showing the distribution of cluster 13 among SMEK1$^{-/-}$ and wild-type mice. (F) UMAP plot showing the distribution of proinflammatory cytokines in microglia.

Supplementary table 1. The primers used for amplification

Species	Primer name	Primer sequences(5'to3')	Base number
Mouse	TGFβ Forward	CACCGGAGAGCCCTGGATA	19
Mouse	TGFβ Reverse	TGTACAGCTGCCGCACACA	19
Mouse	SMEK1 F	AACACTGCATACCAGAAACAACA	23
Mouse	SMEK1 R	CAGGTCCTGAGTGATGTCCA	20
Mouse	IL-1β F	GCAACTGTTCCTGAACTCAACT	22
Mouse	IL-1β R	ATCTTTTGGGGTCCGTCAACT	21
Mouse	IL-10 F	GCTCTTACTGACTGGCATGAG	21
Mouse	IL-10 R	CGCAGCTCTAGGAGCATGTG	20
Mouse	iNOS F	GTTCTCAGCCCAACAATACAAGA	23
Mouse	iNOS R	GTGGACGGGTCGATGTCAC	19
Mouse	CD86 F	CGGCATGGAATAGTCATCACCC	22
Mouse	CD86 R	AGCGGAATAGTCTATAGCCCTC	22
Mouse	CD206 F	GCTTCTCTGGAATGCCTTCG	20
Mouse	CD206 R	CAACATTTCGGAACATGCCTA	21
Mouse	TNF-α F	CCCTCACACTCAGATCATCTTCT	23
Mouse	TNF-α R	GCTACGACGTGGGCTACAG	19
Mouse	β-ACTIN F	CGTTGACATCCGTAAAGACCTC	22
Mouse	β-ACTIN R	CCACCGATCCACACAGAGTAC	21
Mouse	PDK3 F	TCCTGGACTTCGGAAGGGATA	21
Mouse	PDK3 R	GAAGGGCGGTTCAACAAGTTA	21
Mouse	HIF-1α F	ACCTTCATCGGAAACTCCAAAG	22
Mouse	HIF-1α R	CTGTTAGGCTGGGAAAAGTTAGG	23
Mouse	LDHA F	TGTCTCCAGCAAAGACTACTGT	22
Mouse	LDHA R	GACTGTACTTGACAATGTTGGGA	23
Mouse	U6 F	CAGCACATATACTAAAATTGGAACG	25
Mouse	U6 R	ACGAATTTGCGTGTCATCC	19
Mouse	miR-125a-5p F	ACTACATCCCTGAGACCCTTTAAC	24
Mouse	miR-125a-5p R	TATGGTTTTGACGACTGTGTGAT	23
Mouse	miR-125b-5p F	ACTGATAAATCCCTGAGACCCTAAC	25
Mouse	miR-125b-5p R	TATGGTTTTGACGACTGTGTGAT	23

Supplementary Table 1.

The primers used for amplification.

Supplementary table 2. The sequences used for microRNA synthesis

Species	MicroRNA name	MicroRNA sequences(5'to3')	Base number
Mouse	miR-NC sense	UUCUCCGAACGUGUCACGUTT	21
Mouse	miR-NC antisense	ACGUGACACGUUCGGAGAATT	21
Mouse	miR-125a-5p mimics sense	UCCCUGAGACCCUUUAACCUGUGA	24
Mouse	miR-125a-5p mimics antisense	ACAGGUUAAAGGGUCUCAGGGAUU	24
Mouse	inhibitor-NC	CAGUACUUUUGUGUAGUACAA	21
Mouse	miR-125a-5p inhibitors	UCACAGGUUAAAGGGUCUCAGGGA	24

Supplementary Table 2.

The sequences used for miRNA synthesis.

Supplementary table 3. The primers used for amplification in CHIP-qPCR			
Species	Primer name	Primer sequences(5'to3')	Base number
Mouse	HIF-1α Forward	AAAAGTGAGCCAGCATGTACC	21
Mouse	HIF-1α Reverse	AGACTACCGCATTGAGATCACC	22
Mouse	LDHA F	TCGATGCATTTCGGGCTCCT	20
Mouse	LDHA R	CACGATGTCCCTGCAAGAGT	20

Supplementary Table 3.

The primers used for amplification via ChIP–qPCR.

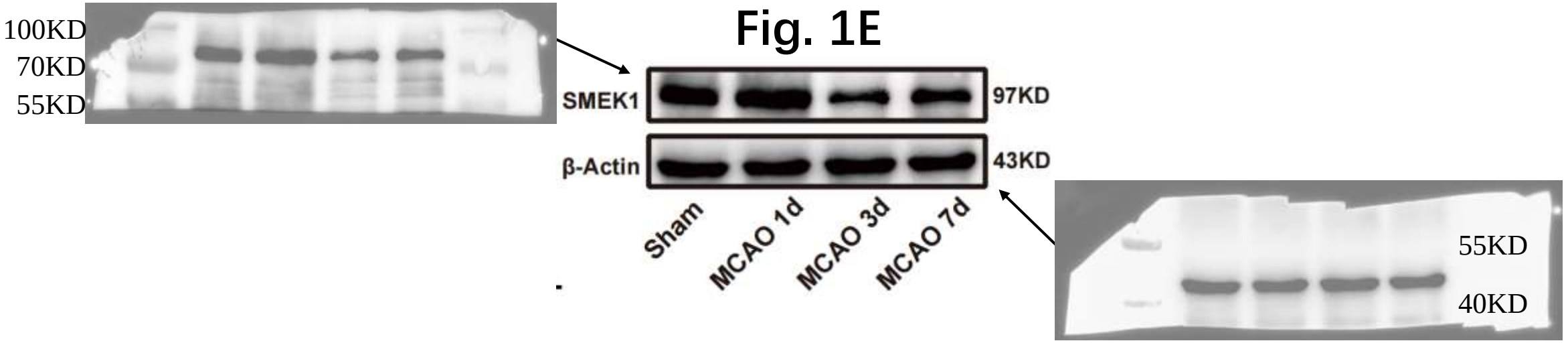
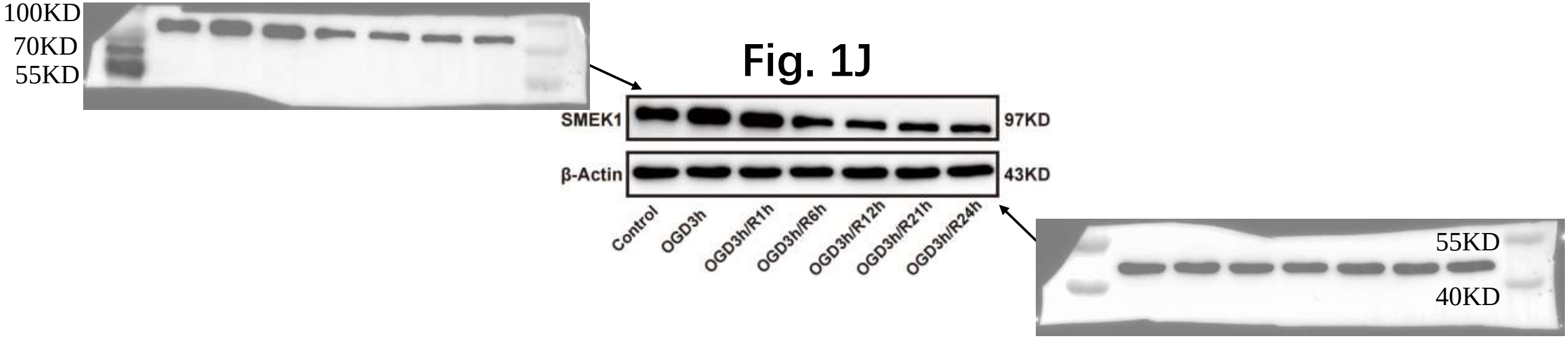

100KD
70KD
55KD
SMEK1
97KD
β-Actin
43KD
Sham
MCAO 1d
MCAO 3d
MCAO 7d
55KD
40KD

Fig. 1E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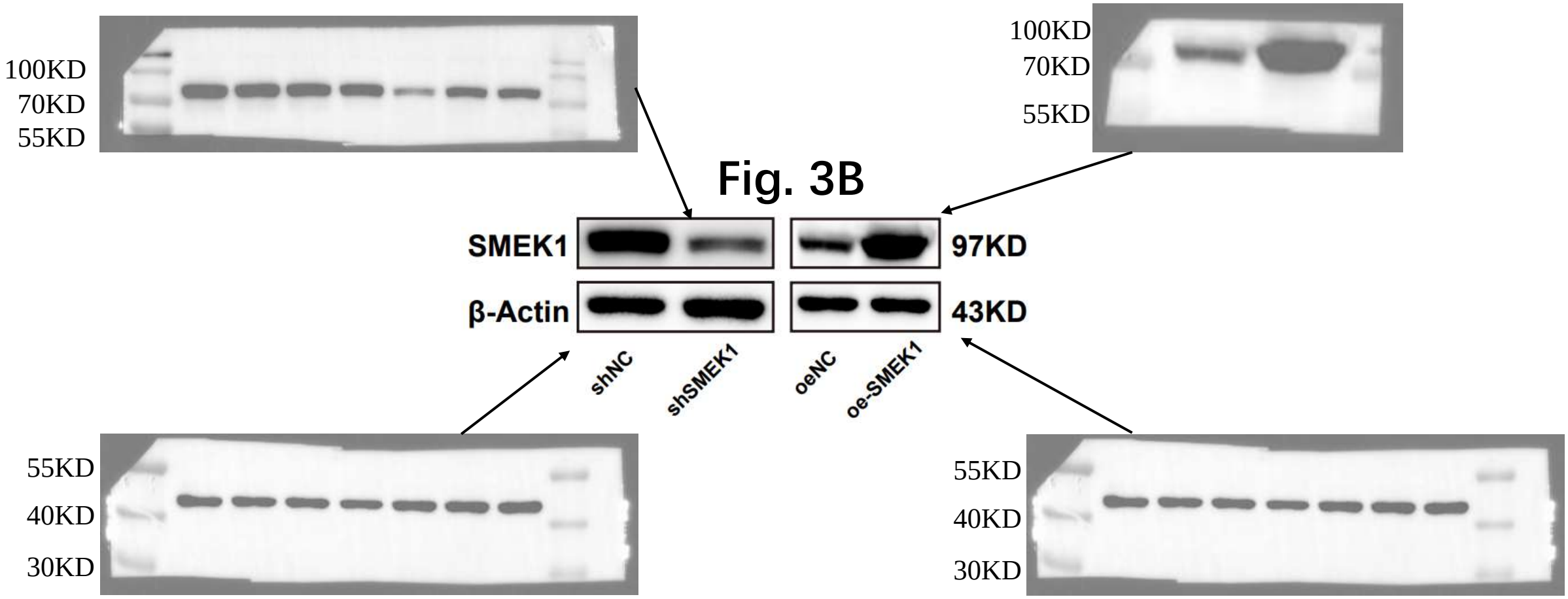
70KD
55KD
SMEK1
97KD
β-Actin
43KD
Control
OGD3h
OGD3h/R1h
OGD3h/R6h
OGD3h/R12h
OGD3h/R21h
OGD3h/R24h
55KD
40KD

Fig. 1J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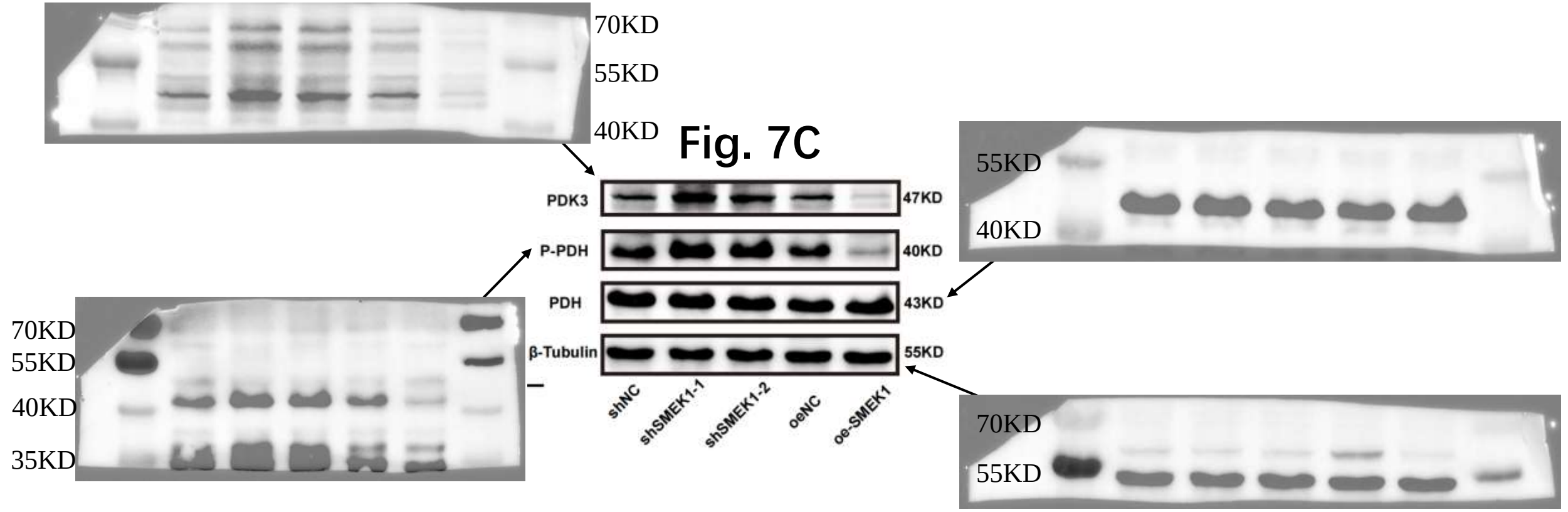
55KD
100KD
70KD
55KD
SMEK1
97KD
β-Actin
43KD
shNC
shSMEK1
oeNC
oe-SMEK1
55KD
40KD
30KD
55KD
40KD
30KD

Fig. 3B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P-PDH
40KD
PDH
43KD
β-Tubulin
55KD
shNC
shSMEK1-1
shSMEK1-2
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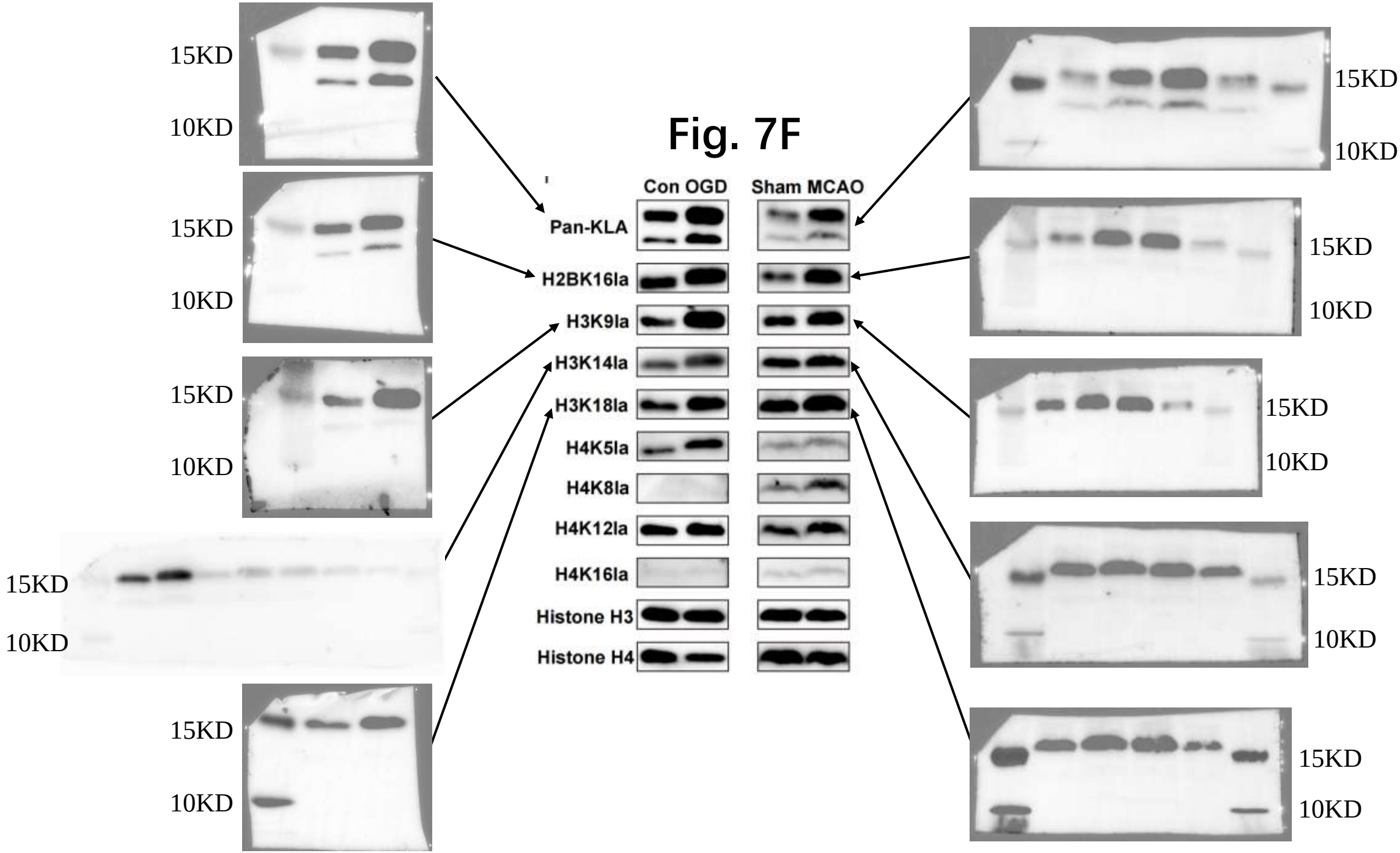
35KD
55KD
40KD
70KD
55KD

Fig. 7C

Fig. 7F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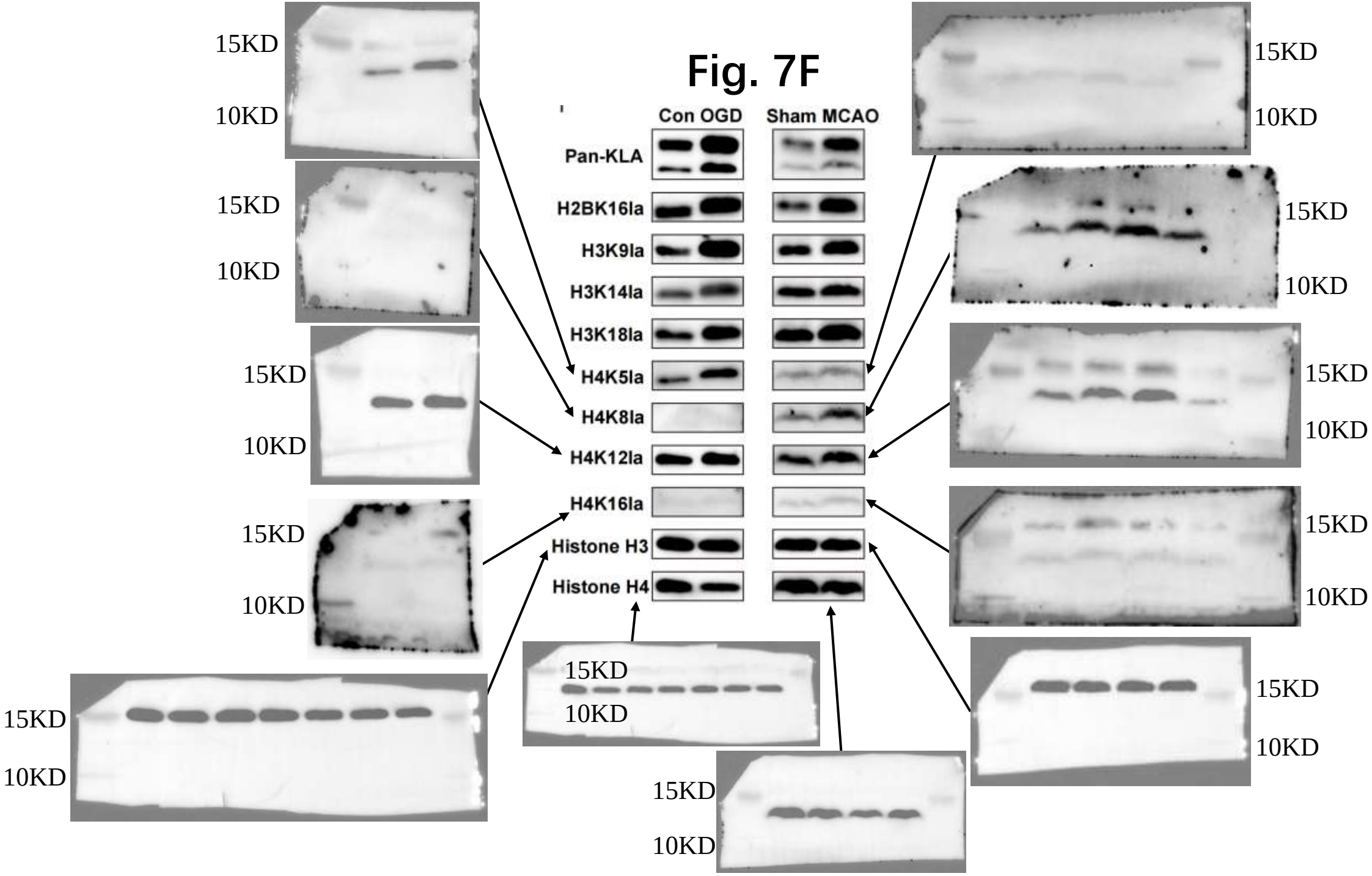
Sham MCAO
Pan-KLA
H2BK16la
H3K9la
H3K14la
H3K18la
H4K5la
H4K8la
H4K12la
H4K16la
Histone H3
Histone H4
15KD
10KD

Fig. 7F


Con OGD
Sham MCAO
Pan-KLA
H2BK16la
H3K9la
H3K14la
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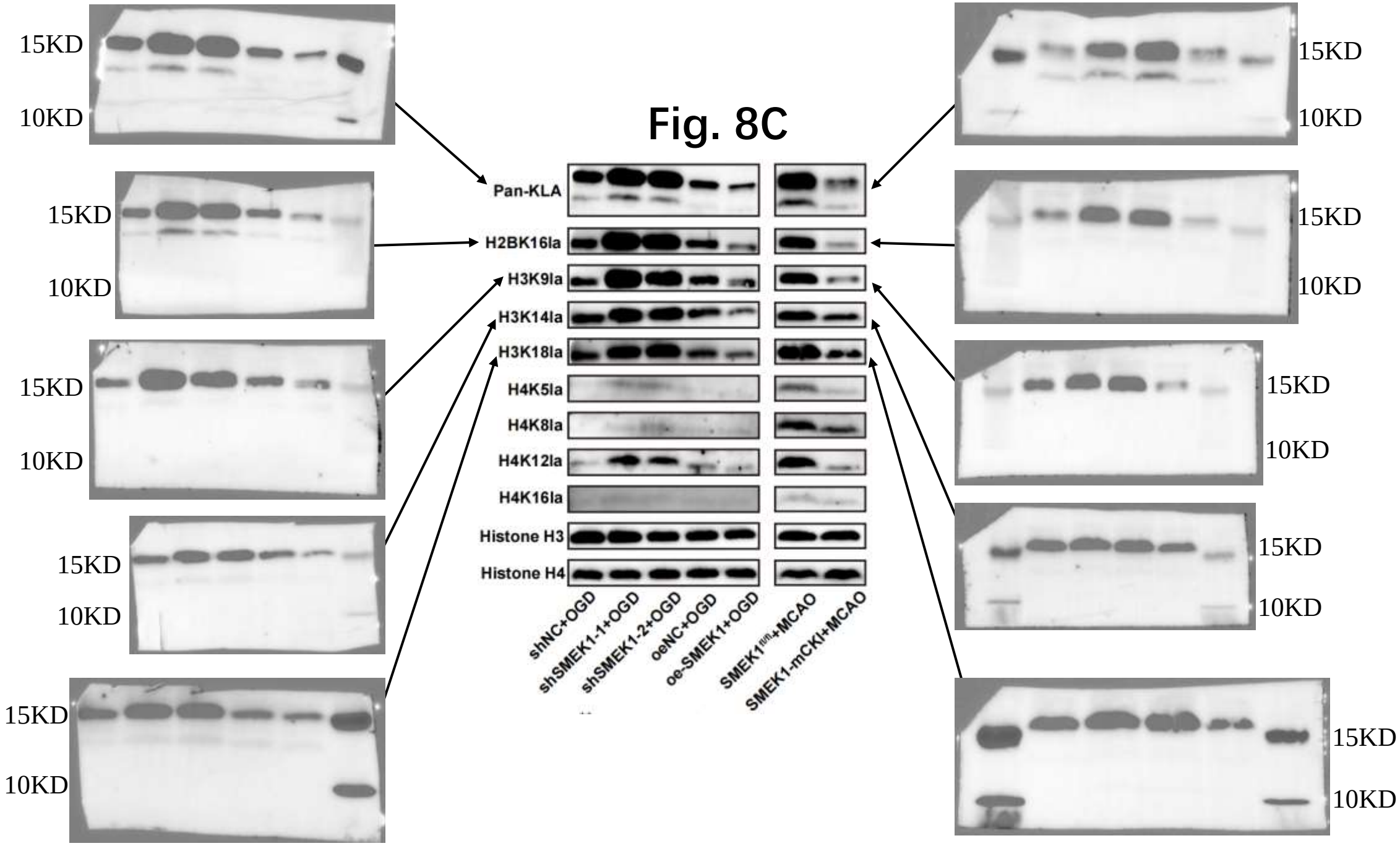
H4K16la
Histone H3
Histone H4
15KD
10KD

Fig. 8C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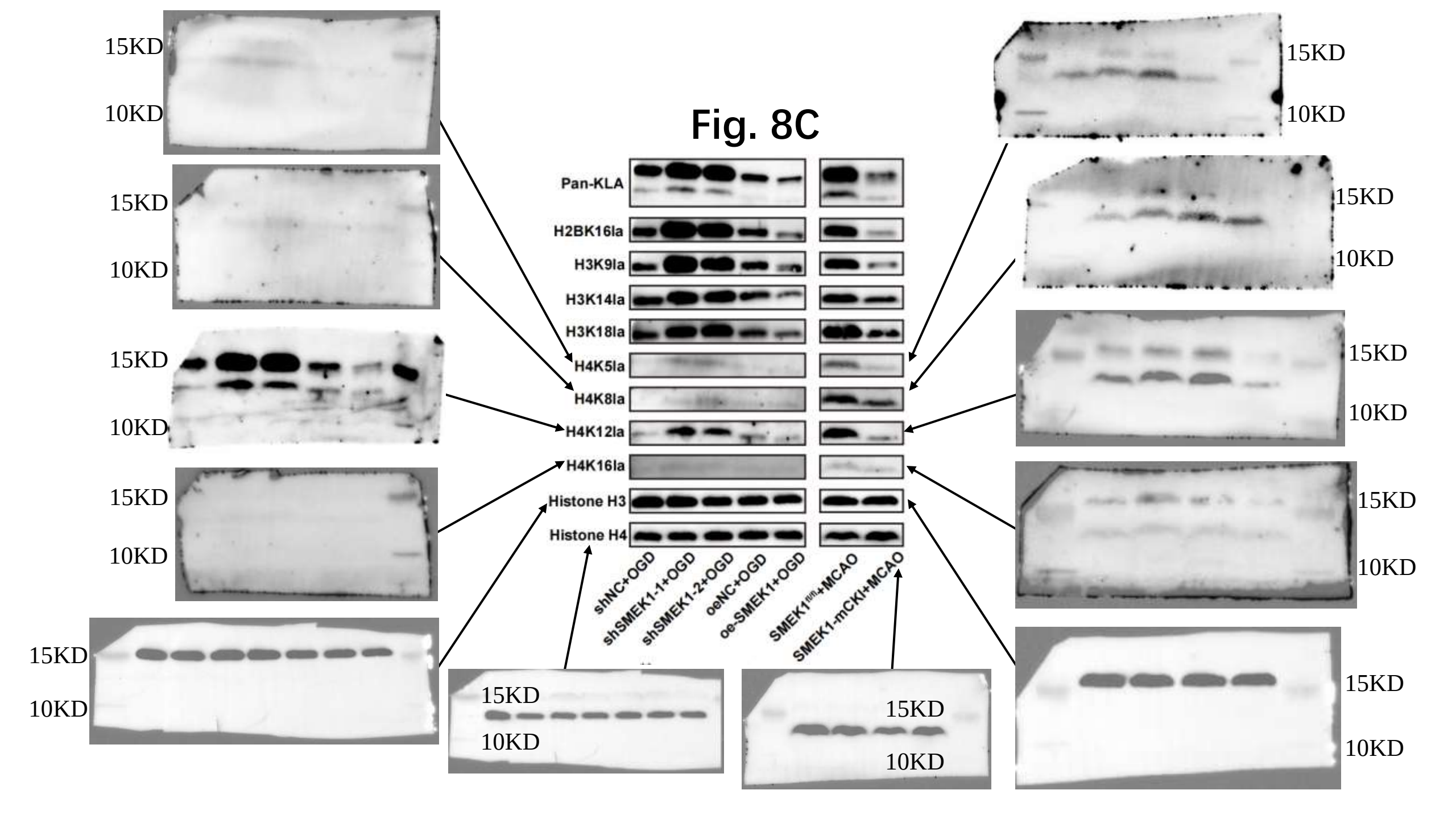
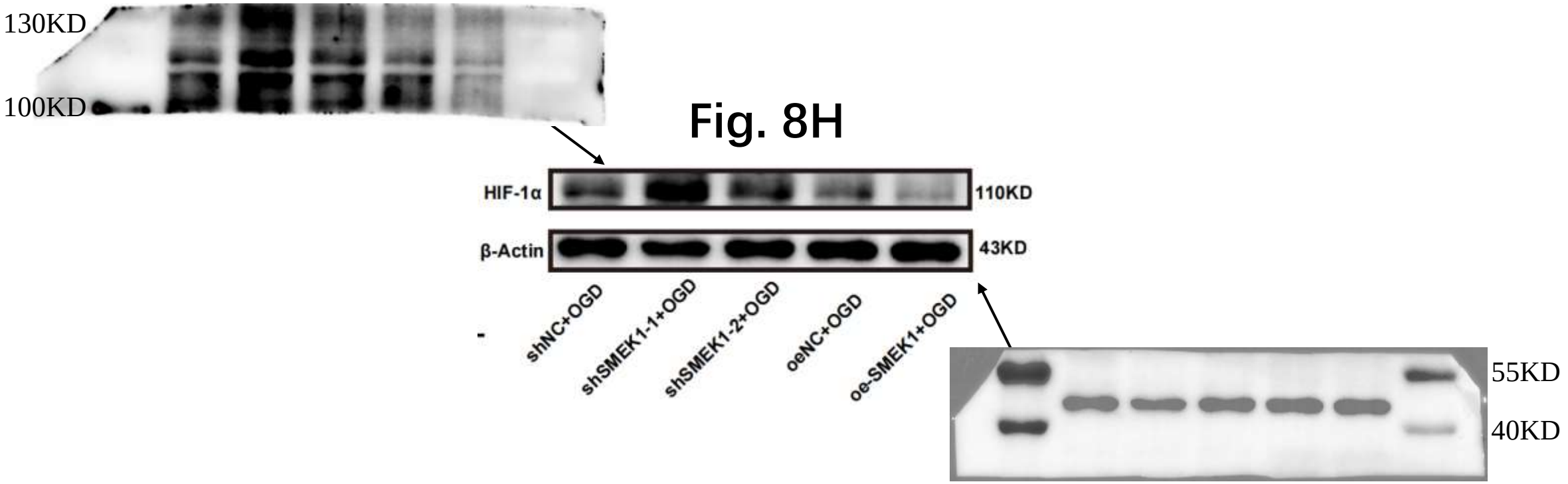
10KD
Pan-KLA
H2BK16la
H3K9la
H3K14la
H3K18la
H4K5la
H4K8la
H4K12la
H4K16la
Histone H3
Histone H4
shNC+OGD
shSMEK1-1+OGD
shSMEK1-2+OGD
oeNC+OGD
oe-SMEK1+OGD
SMEK1^dm+MCAO
SMEK1-mCKI+MCAO

Fig. 8C


Pan-KLA
H2BK16la
H3K9la
H3K14la
H3K18la
H4K5la
H4K8la
H4K12la
H4K16la
Histone H3
Histone H4
shNC+OGD
shSMEK1-1+OGD
shSMEK1-2+OGD
oeNC+OGD
oe-SMEK1+OGD
SMEK1^dm+MCAO
SMEK1-mCKI+MCAO
15KD
10KD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100KD
HIF-1α
110KD
β-Actin
43KD
-
shNC+OGD
shSMEK1-1+OGD
shSMEK1-2+OGD
oeNC+OGD
oe-SMEK1+OGD
55KD
40KD

Fig. 8H

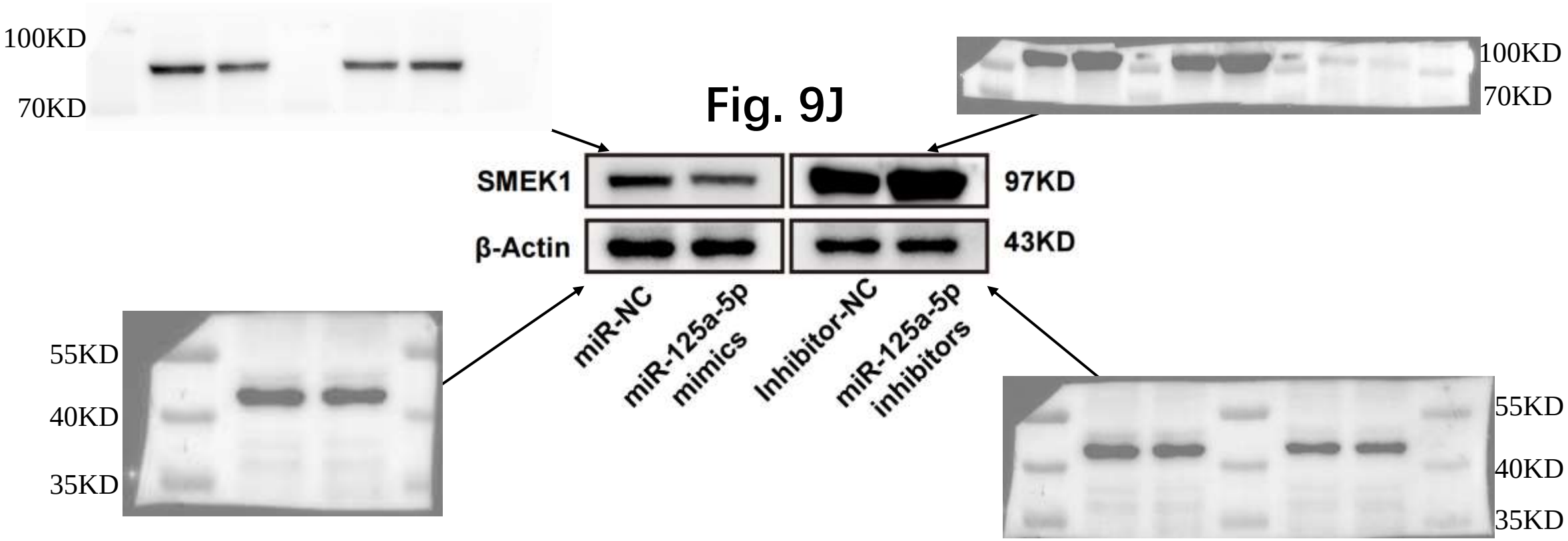
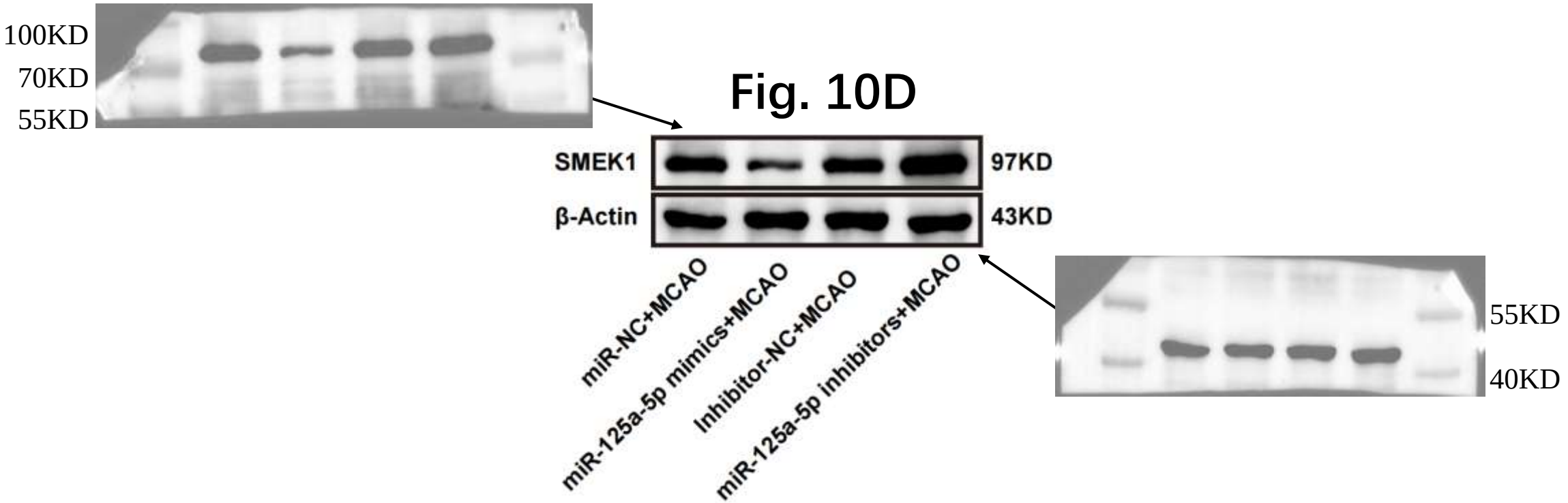

100KD
70KD
100KD
70KD
SMEK1
97KD
β-Actin
43KD
miR-NC
miR-125a-5p mimics
Inhibitor-NC
miR-125a-5p inhibitors
55KD
40KD
35KD
55KD
40KD
35KD

Fig. 9J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55KD
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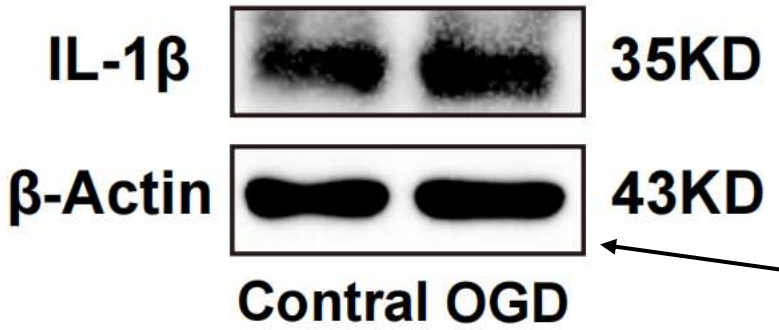
97KD
β-Actin
43KD
miR-NC+MCAO
miR-125a-5p mimics+MCAO
Inhibitor-NC+MCAO
miR-125a-5p inhibitors+MCAO
55KD
40KD

Fig. 10D

Fig. S1D



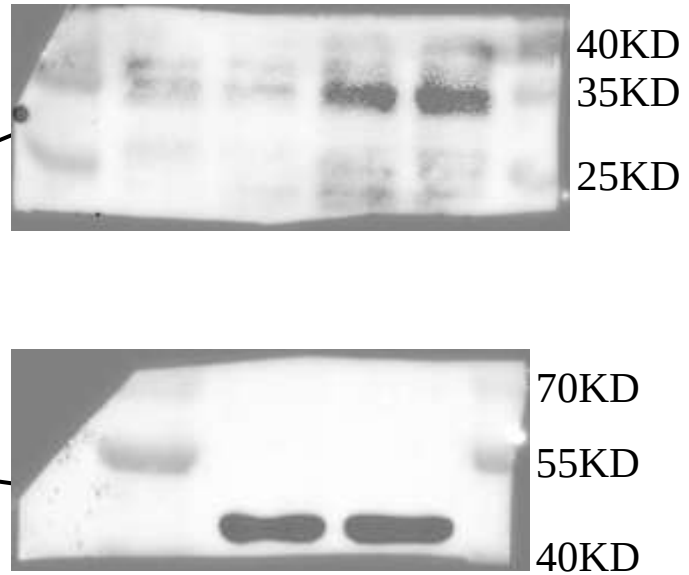


Fig. S1G

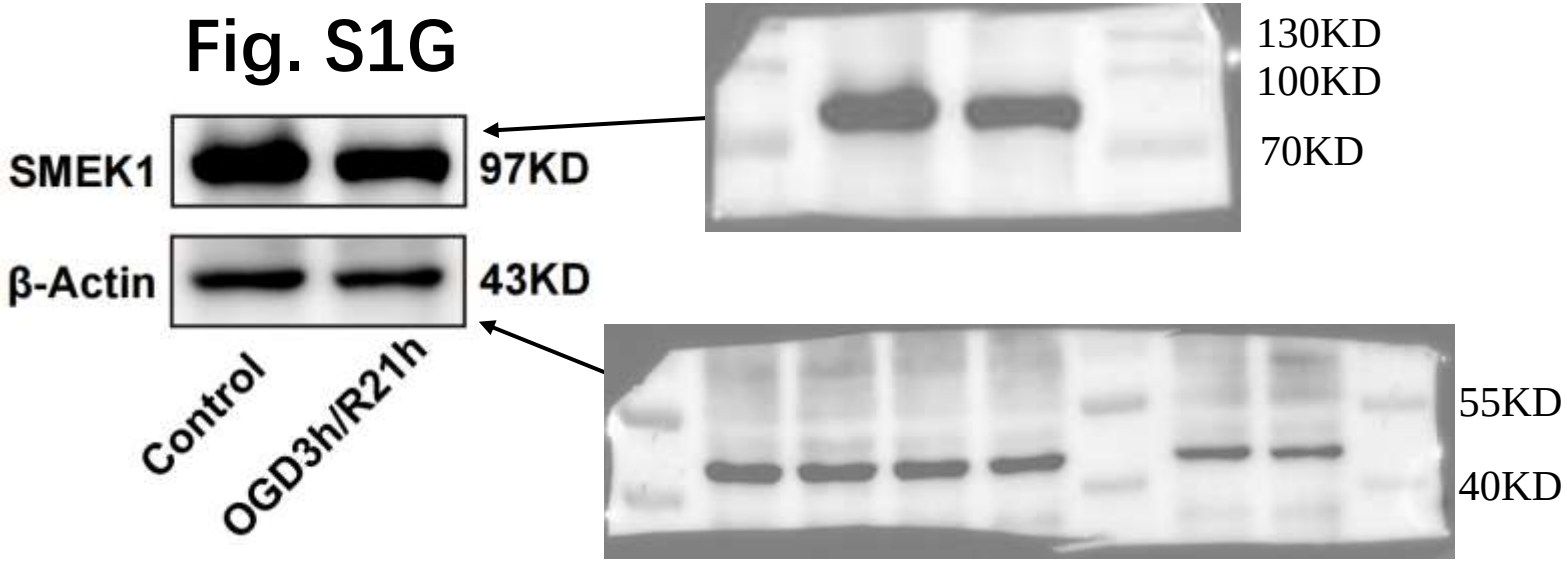
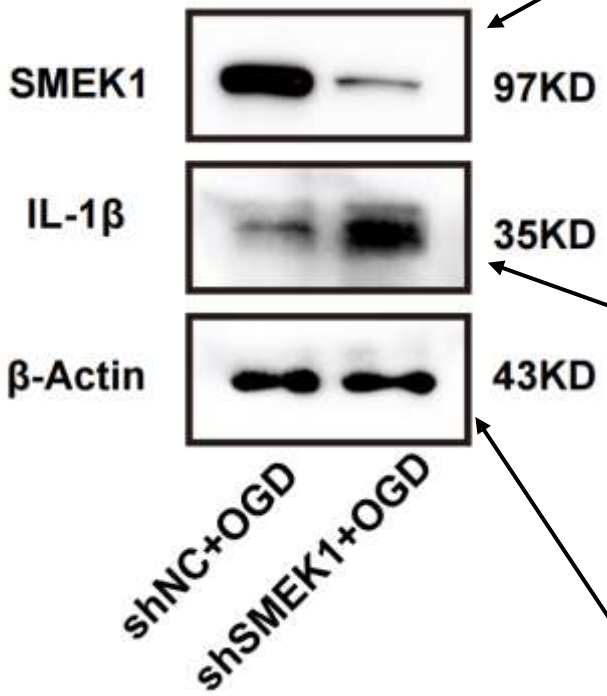


Fig. S1H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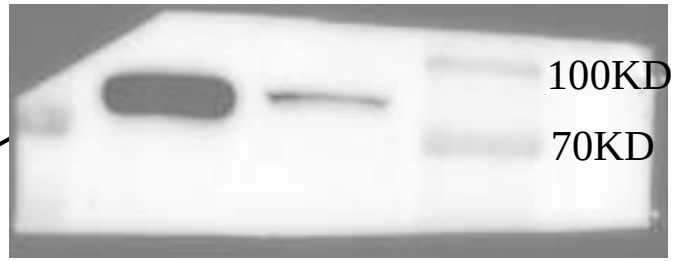
IL-1β
35KD
β-Actin
43KD
shNC+OGD
shSMEK1+OGD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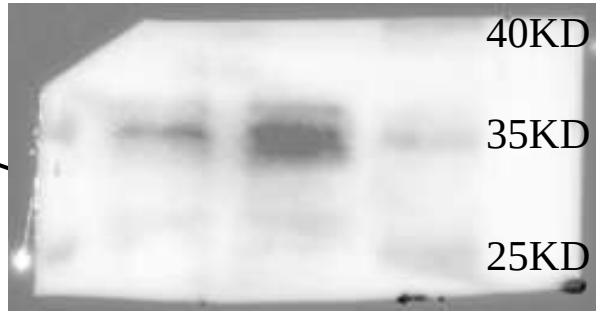
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25KD

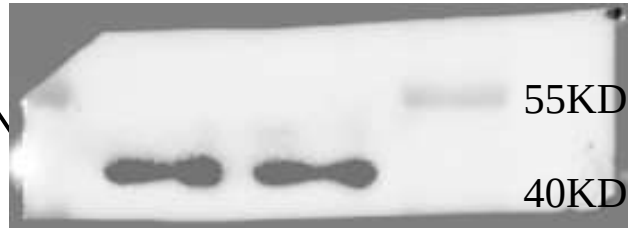
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Fig. S2D

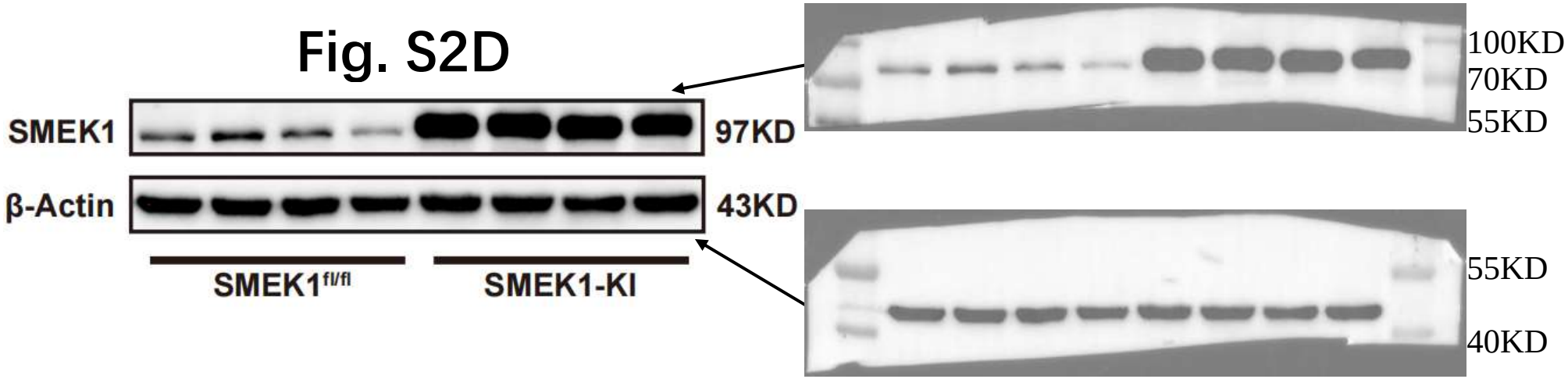

Fig. S2K