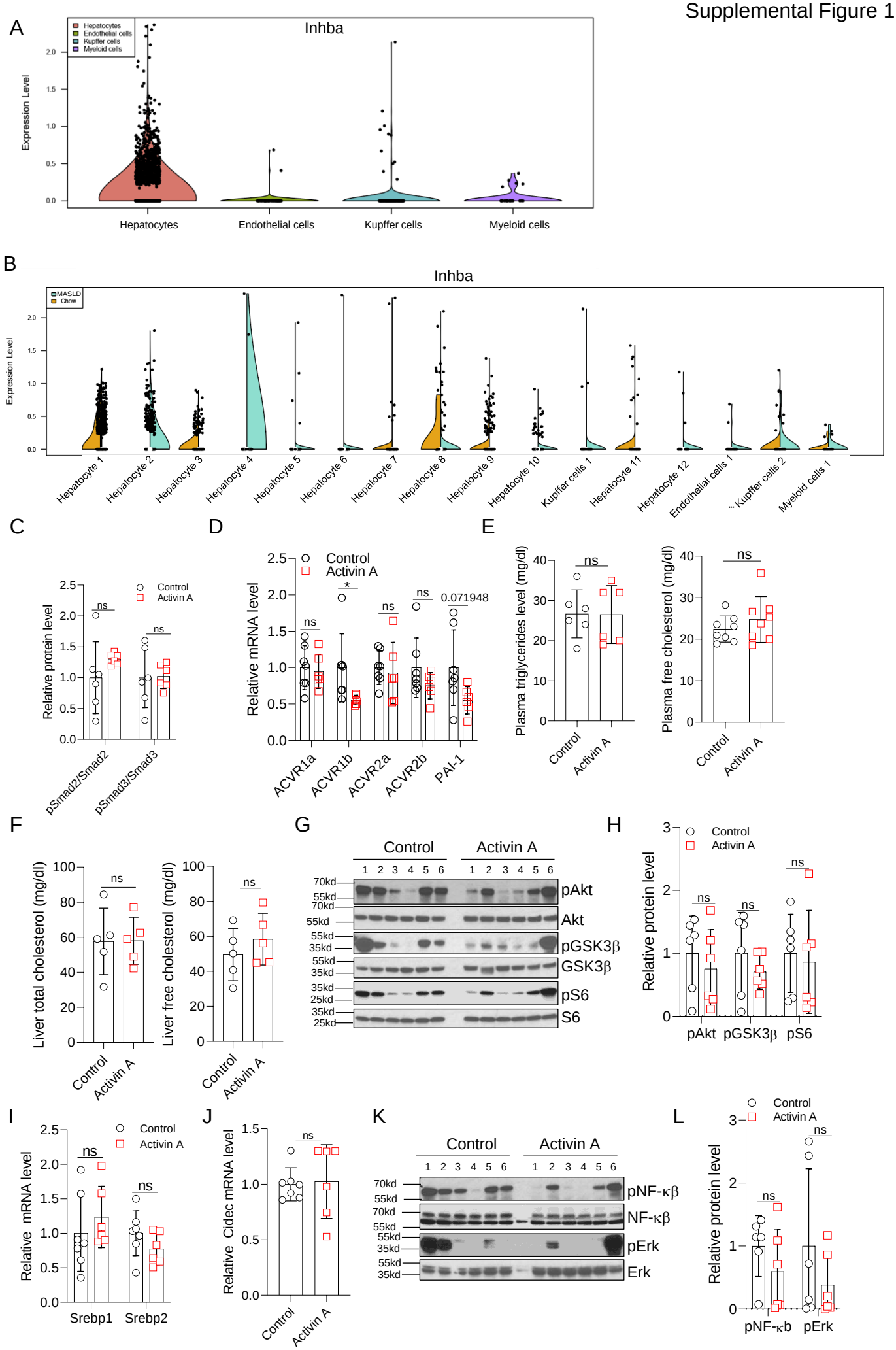
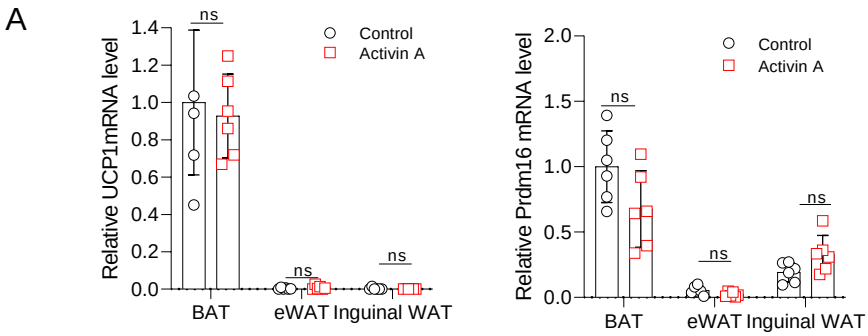
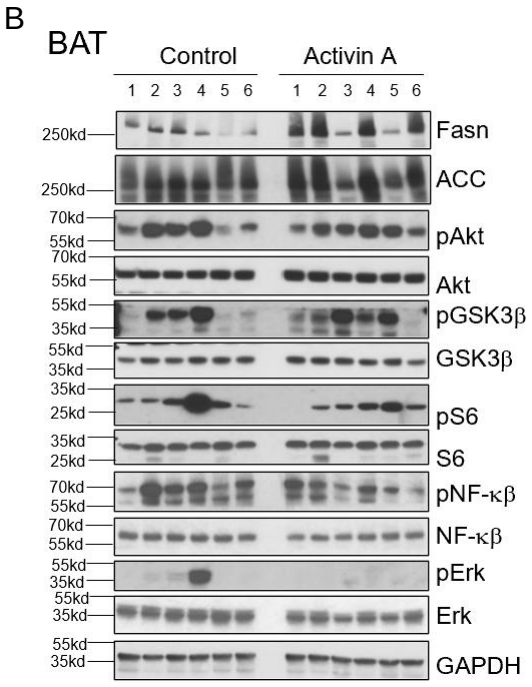
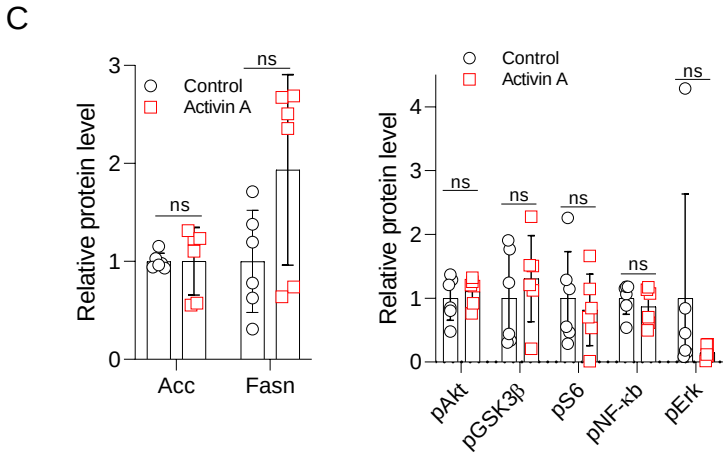
Supplemental Figure 1

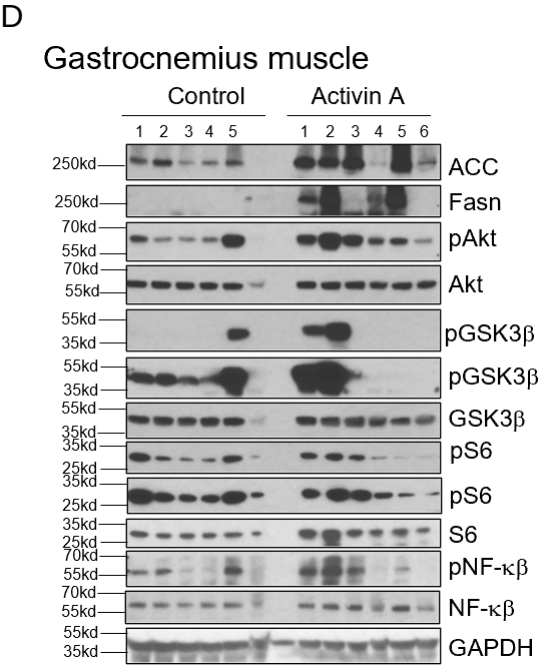


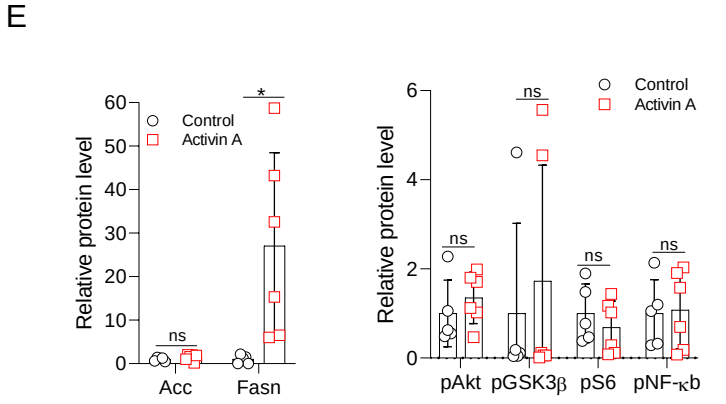
Supplemental Figure 1, related to Figure 1. A. Violin plots show that Activin A (inhba) is predominantly expressed in hepatocytes in mouse livers by single-cell RNA sequencing. Overlay of livers with chow and 6 week-MASLD diet is represented. Expression level values are log2 transformed. **B.** Violin plots show that Activin A (inhba) expression in different cell clusters. Expression level values are log2 transformed. **C**. Quantification of pSmad2/Smad2 and pSmad3/Smad3 ratios in Figure 1J. **D**. Relative mRNA levels of Activin receptors in control and Activin A-overexpressing MASLD livers. N=6-7 mice. **E**. Activin A overexpression did not change plasma triglyceride and free cholesterol levels in MASLD mice. N=6 mice. **F**. Activin A overexpression did not change liver total cholesterol and free cholesterol in MASLD mice. N=5-6 mice. **G-H**. Western blotting for Akt/S6 pathway activity in livers after 12 weeks of sugar water-FPC diet followed by overnight fasting and sacrifice. The data were generated using the same samples shown in Figure 1R and normalized to GAPDH immunoreactivity shown in 1R. N=6 mice. **I**. Activin A overexpression did not change mRNA levels of Srebp1 or Srebp2 in MASLD livers. N=6-7 mice. **J**. Cidec mRNA levels in Activin A-expressing and control MASLD livers. N=6-7 mice. **K-L**. Western blotting for pNF-κβ and pErk in livers after 12 weeks of sugar water-FPC diet followed by overnight fasting and sacrifice. GAPDH as loading control. The data were generated using the same samples shown in Figure 1R and normalized to GAPDH immunoreactivity shown in 1R. N=6 mice. N=6 mice. Data are presented as means ± SEM. * $p<0.05$ by unpaired Student *t*-test or nonparametric tests.





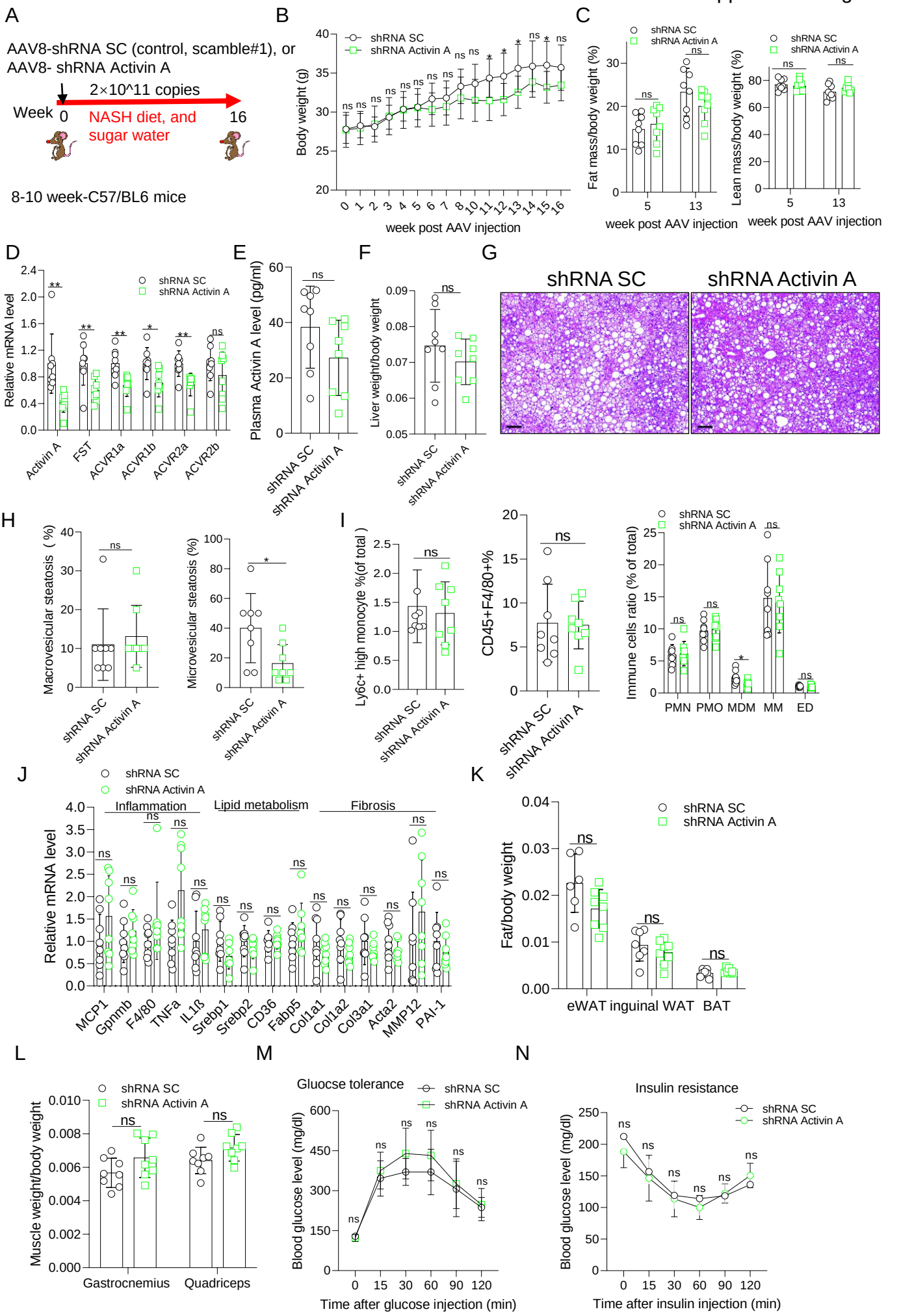






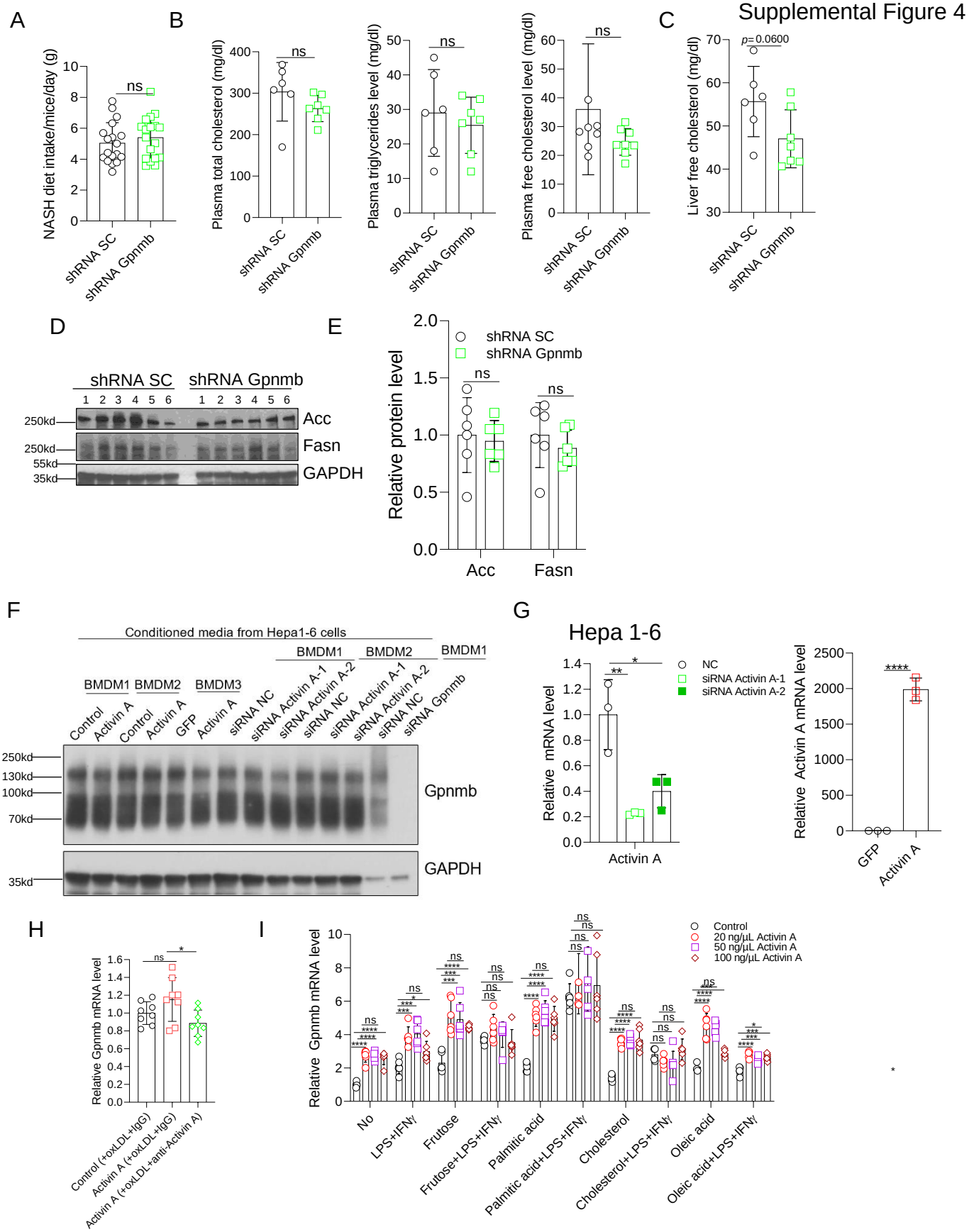
Supplemental Figure 2, related to Figure 2. **A**. Relative mRNA levels of thermogenic factors UCP1 and Prdm16 in fat tissues in MASLD mice. N=6 mice. **B**. Western blotting shows protein levels of Acc, Fasn, and markers of Akt/S6 and Erk pathway activity in BAT after 12 weeks of sugar water-FPC diet followed by overnight fasting prior to sacrifice. GAPDH as loading control. N=6 mice. **C**. Quantification of Acc, Fasn, pAkt, pGsk3β, pS6, pNF-κβ and pErk protein levels in B. N=6. **D**. Western blotting shows protein levels of Acc and Fasn, and markers for Akt/S6 and Erk pathway activity in gastrocnemius muscle after 12 weeks of sugar water-FPC diet followed by overnight fasting prior to sacrifice. GAPDH as the loading control. N=5-6 mice. **E**. Quantification of protein levels of Acc and Fasn, pAkt, pGsk3β, pS6, and pNF-κβ in D. N=5-6 mice. Data are presented as means ± SEM. * p<0.05 by unpaired Student t-test or nonparametric test.

Supplemental Figure 3

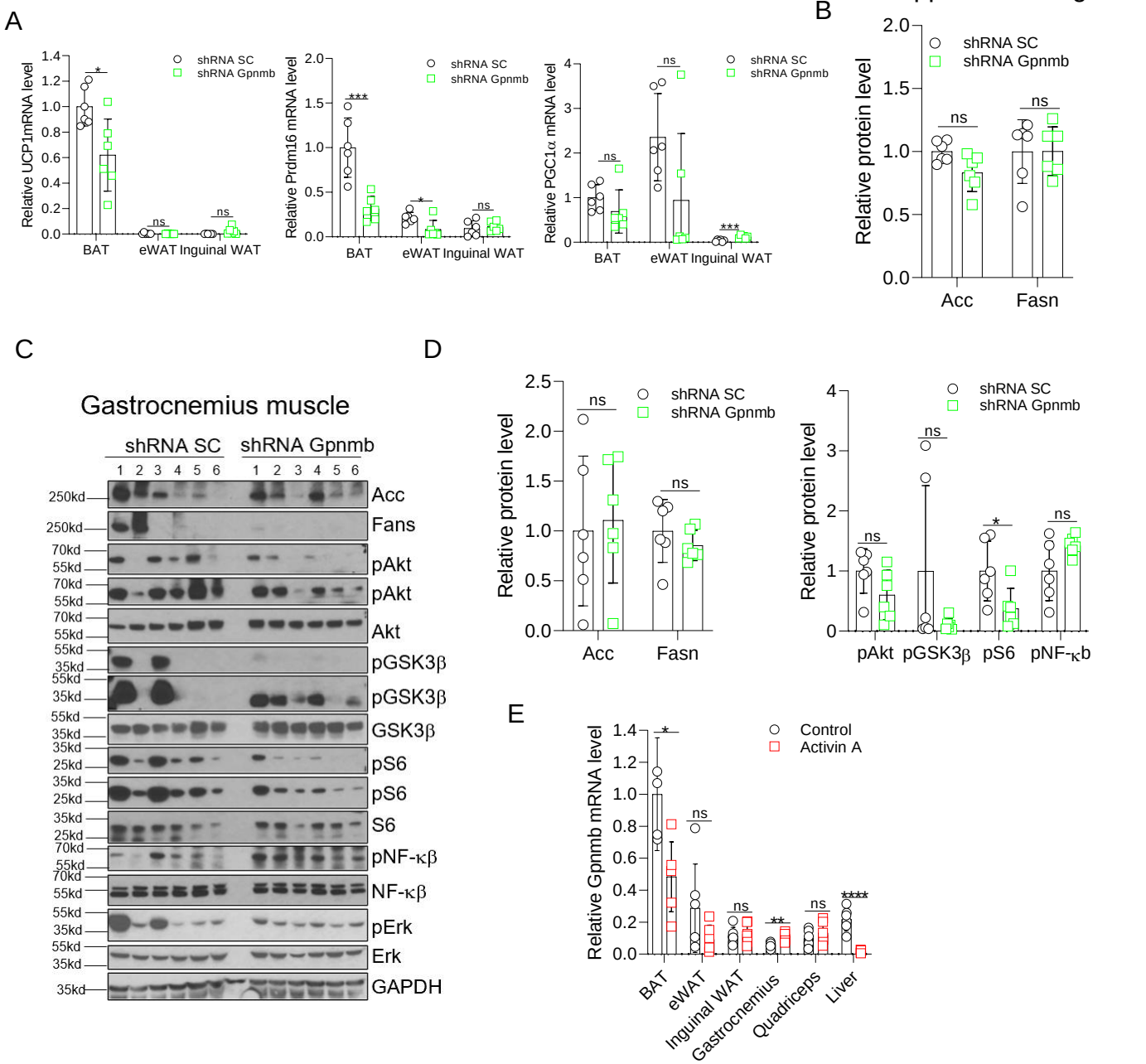


Supplemental Figure 3. Activin A knockdown does not change liver steatosis, inflammation, or metabolic syndrome in MASLD mice. A. Schematic of the experimental design for Activin A knockdown studies. **B.** Body weight changes with time after AAV injection. N=8 mice. **C.** Fat mass and lean mass by MRI at 5 and 13 weeks after AAV injection and initiation of sugar water-FPC diet. N=8 mice. **D**. Q-PCR shows mRNA levels of Activin A, FST and Activin receptors in control and Activin A knockdown MASLD livers. N=8 mice. **E**. Plasma Activin A levels by ELISA. N=8 mice. **F**. Liver/body weight ratio. N=8 mice. **G.** Representative Hematoxylin and Eosin (H&E) staining in control and Activin A knockdown livers. N=6 mice. Scale bar, 50 μm. N=6 mice. **H**. Quantification of macrovesicular and microvesicular steatosis in G. N=6 mice. **I**. Quantification of immune cells by flow cytometry using similar gating strategies as in Figure 3H, 3J. **J**. Relative mRNA levels of inflammatory markers MCP1, Gpnmb, F4/80 and IL1-β, lipid metabolic markers Srebp1, Srebp2, CD36 and Fabp5, and fibrosis markers Col1a1, Col1a2, Col3a1, Acta2, MMP12 and PAI-1 in MASLD livers. N=8 mice. **K**. BAT, eWAT and inguinal WAT weight normalized to body weight of in control and Activin A knockdown MASLD mice. N=8 mice. **L**. Gastrocnemius and quadricep weight normalized to body weight in control and Activin A-knockdown MASLD mice. N=8 mice. **M-N**. glucose tolerance and insulin resistance were tested on week 14 and week 15 post AAV injection, respectively. N=8 mice. Data are presented as means ± SEM. * $p<0.05$, **, $p<0.01$, ***, $p<0.001$, ****, $p<0.0001$ by unpaired Student *t*-tests or nonparametric tests.

Supplemental Figure 4

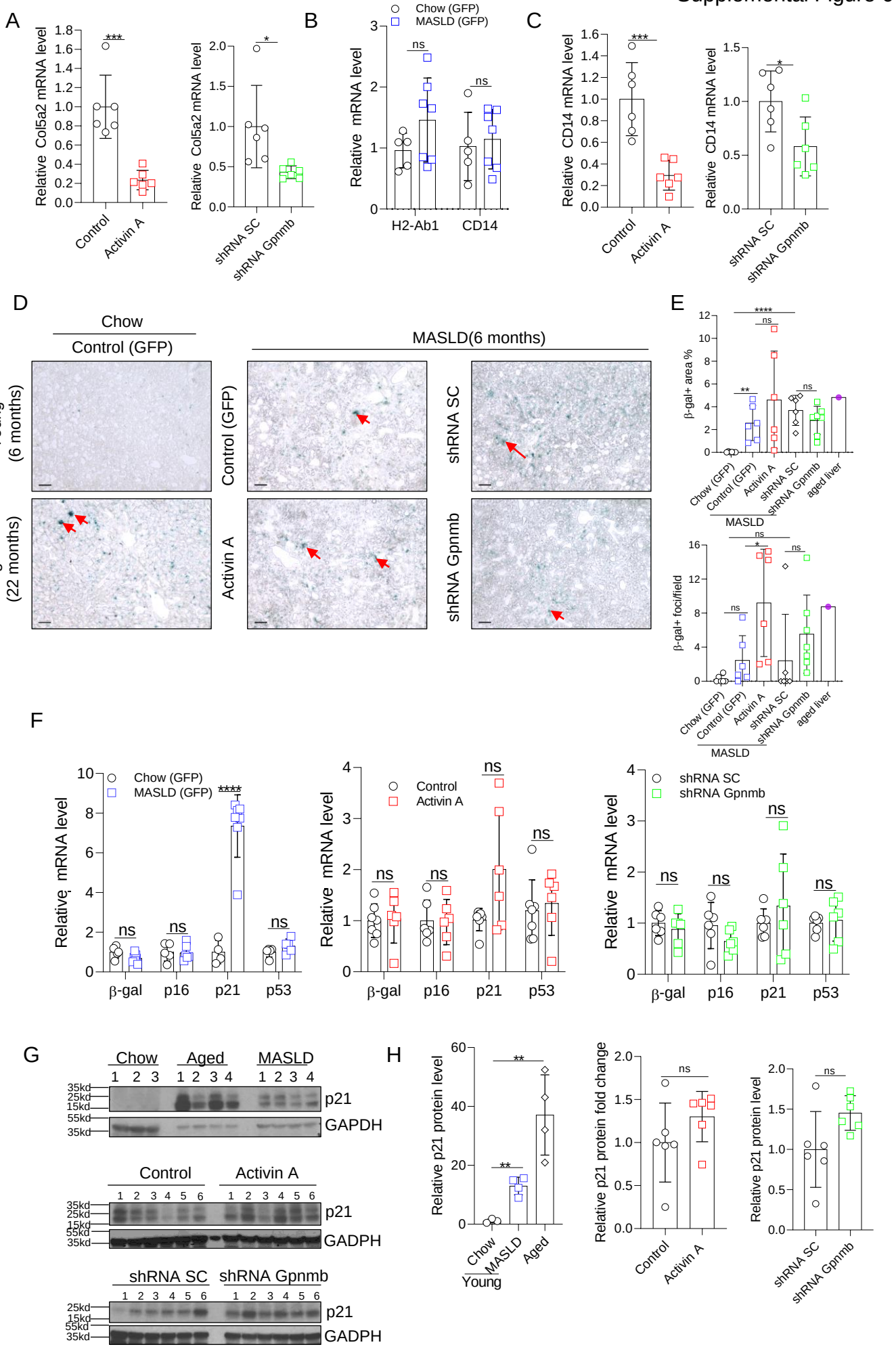


Supplemental 4, related to Figure 4. A. Average food intake in control and Gpnmb-knockdown MASLD mice. Fresh FPC diet was provided twice weekly, and diet consumption was measured. N=8 mice. **B**. Plasma total cholesterol, triglycerides, and free cholesterol levels in control and Gpnmb-knockdown MASLD mice. N=6 mice. **C**. Gpnmb-knockdown did not change liver free cholesterol in MASLD mice. N=6 mice. **D**. Western blotting shows protein levels of Acc and Fasn in livers after 12 weeks of sugar water-FPC diet followed by overnight fasting prior to sacrifice. GAPDH as loading control. N=6 mice. **E**. Quantification of Acc and Fasn protein levels in D. N=6. **F.** Gpnmb protein levels of BMDM cells treated for 48 hours with conditioned media from Hepa 1-6 cells expressing Activin A, GFP control, Activin A siRNA or siRNA negative control (NC). BMDMs were treated with Gpnmb siRNA as a control. **G**. Activin A mRNA levels from Hepa 1-6 cells in F. N=3. **H**. QPCR shows conditioned media from Activin A overexpressing Hepa 1-6 cells did not decrease Gpnmb mRNA expression in BMDM with oxLDL treatment, with or without a neutralizing Activin A antibody. N =4 mice. **I**. Recombinant Activin A protein did not decrease Gpnmb mRNA expression in BMDMs in vitro with or without lipids and/or inflammatory factors LPS and IFNγ. BMDMs were treated with or without 20ng/ml, 50ng/ml or 100ng/ml Activin A protein, with or without 100 ng/ml LPS and 50ng/ml IFNγ, with or without 200 μM Palmitic acid, 100 μM cholesterol, or 30 μM Oleic acid for 24 hours. N=3 mice. Data are presented as means ± SEM. * p<0.05 by unpaired Student t-test or nonparametric test.



Supplemental Figure 5, related to Figure 5. A. mRNA levels of thermogenic factors UCP1, Prdm16 and PGC1α in fat tissues in Gpnmb-knockdown mice and scrambled controls after 16 weeks of sugar water-FPC diet followed by overnight fasting prior to sacrifice. N=6 mice. **B.** Quantification of protein levels of Acc and Fasn in BAT in Figure 5J. **C.** Western blotting shows protein levels of Acc and Fasn, and markers for Akt/S6 and Erk signaling pathway activity in gastrocnemius muscle in Gpnmb-knockdown mice and controls after 16 weeks of sugar water-FPC diet followed by overnight fasting prior to sacrifice. N=5-6 mice. **D**. Quantification of protein levels of Acc and Fasn, pAkt, pGsk3β, pS6, and pNF-κβ protein levels in 5C. N=6 mice. **E**. Q-PCR shows Gpnmb mRNA levels in BAT, eWAT, inguinal WAT, gastrocnemius and quadriceps in Activin A-expressing and control MASLD mice. N=6 mice. Data are presented as means ± SEM. * p<0.05, **, p<0.01, ***, p<0.001, ****, p<0.0001 by unpaired Student t-tests (and nonparametric test).

Supplemental Figure 6



Supplemental Figure 6, related to Figure 7. A. Relative mRNA levels of Col5a2 in liver in each group. N=6 mice. **B**. Relative mRNA levels of H2-Ab1 and CD14 in MASLD livers compared with chow controls. N=5-7 mice. **C**. Relative mRNA levels of CD14 in each group. N=6 mice. **D.** β-gal staining of senescent cells in liver samples from each group. Aged liver (22 month) as the positive control. Scale bar, 50 μm. **E.** Quantification of β-gal+ area ratio and number of β-gal+ foci (integrated density>=8000 and area>=40 μm2) in each group. Four random images were quantified for each mouse. N=6-7 mice. **F**. Relative mRNA levels of senescence markers (β-gal, p16, p21 and p53) in liver in each group. N=5-7 mice. **G**. Western blotting shows protein levels of p21 in the livers in each group. Aged livers as the positive control. N=3-6 mice. **H**. Quantification of p21 protein levels in G. N=3-6 mice. Data are presented as means ± SEM. * $p<0.05$, **, $p<0.01$, ***, $p<0.001$, ****, $p<0.0001$ by unpaired Student *t*-tests or nonparametric tests.

A

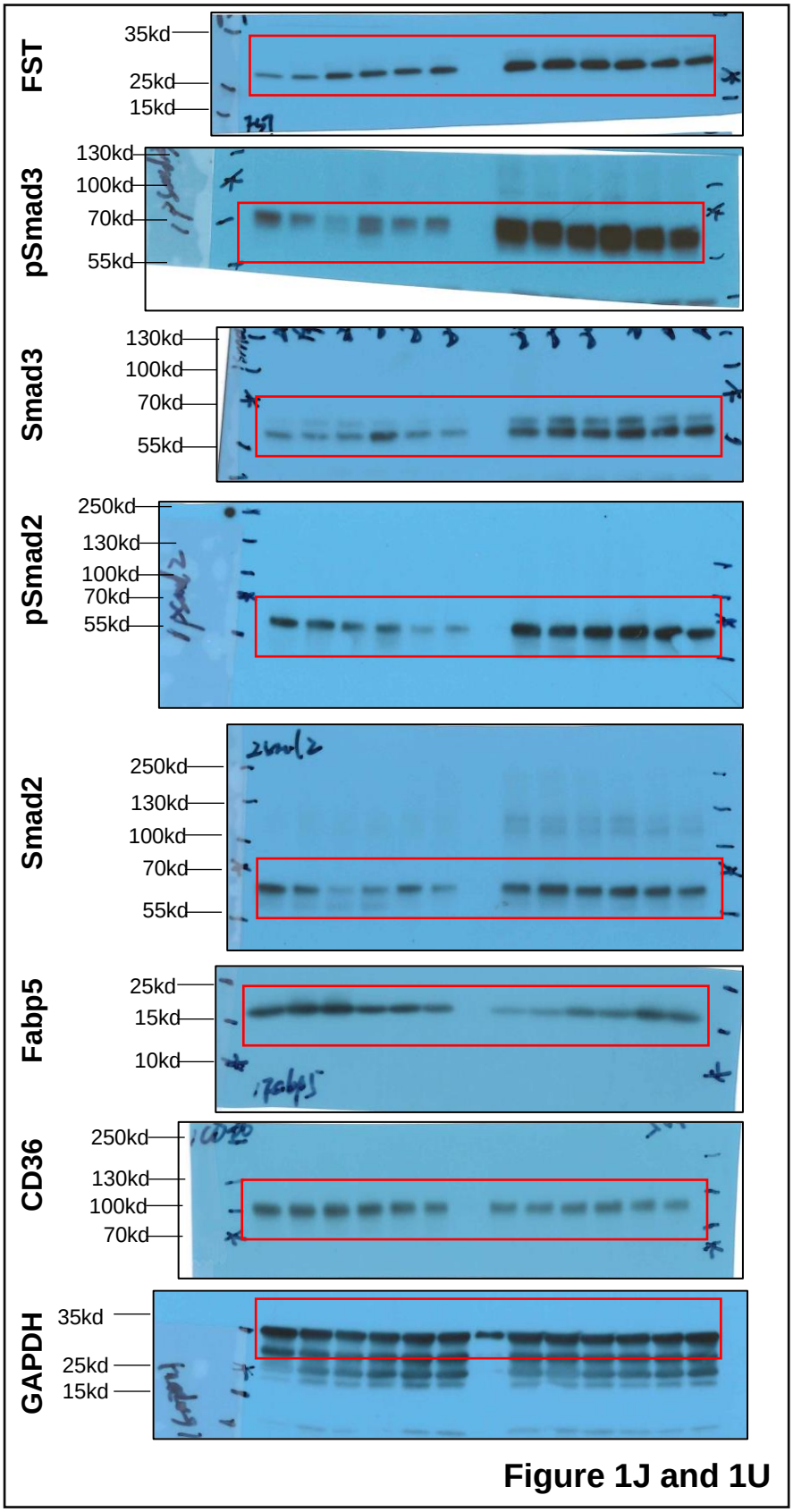


Figure 1J and 1U

B

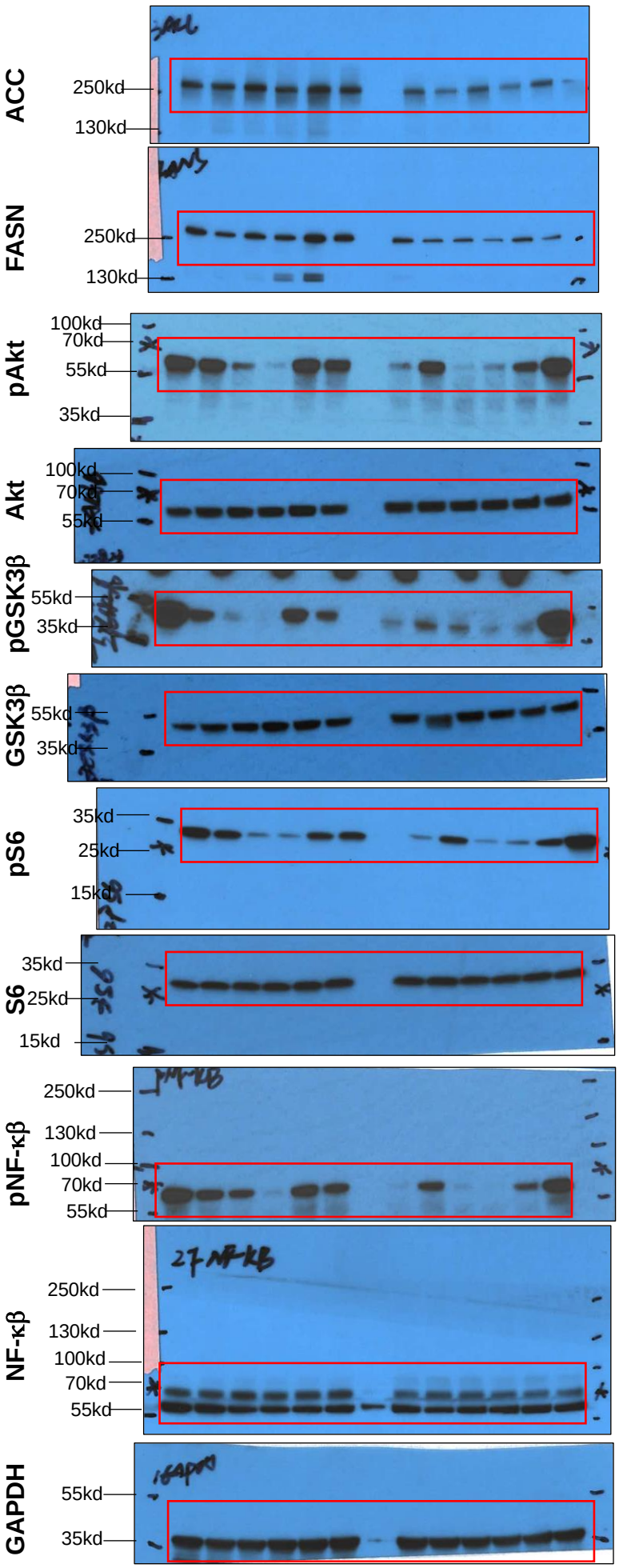


Figure 1R, and supplemental 1G, and 1K, continued

B

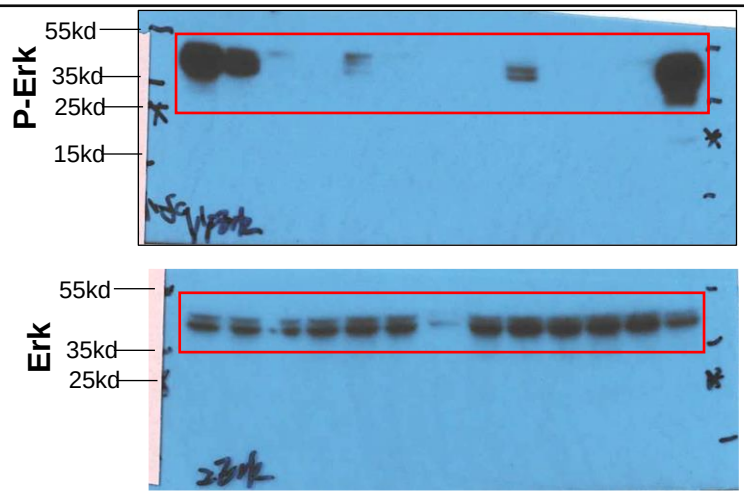


Figure 1R, Supplemental Figure 1G and 1K, continued

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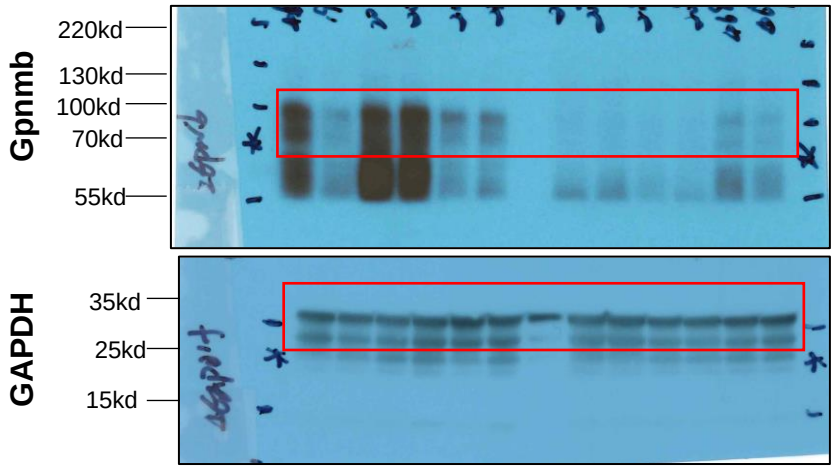

Figure 3F

D

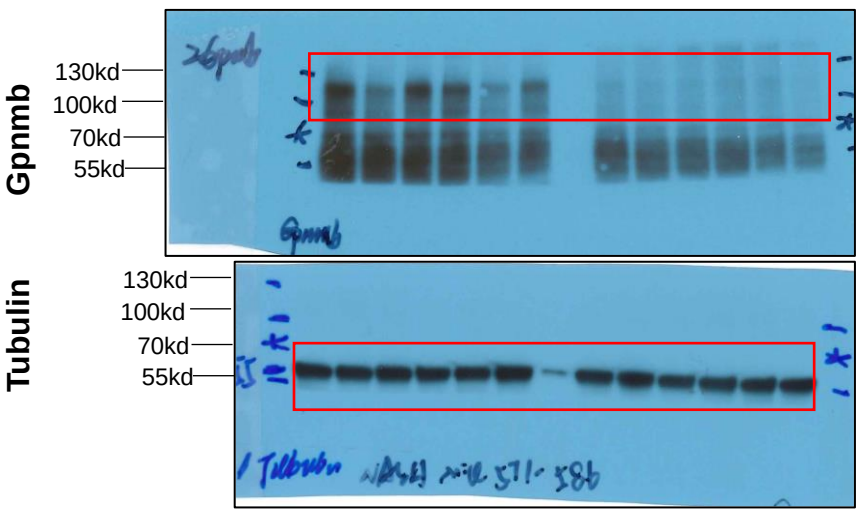

Figure 4F

E

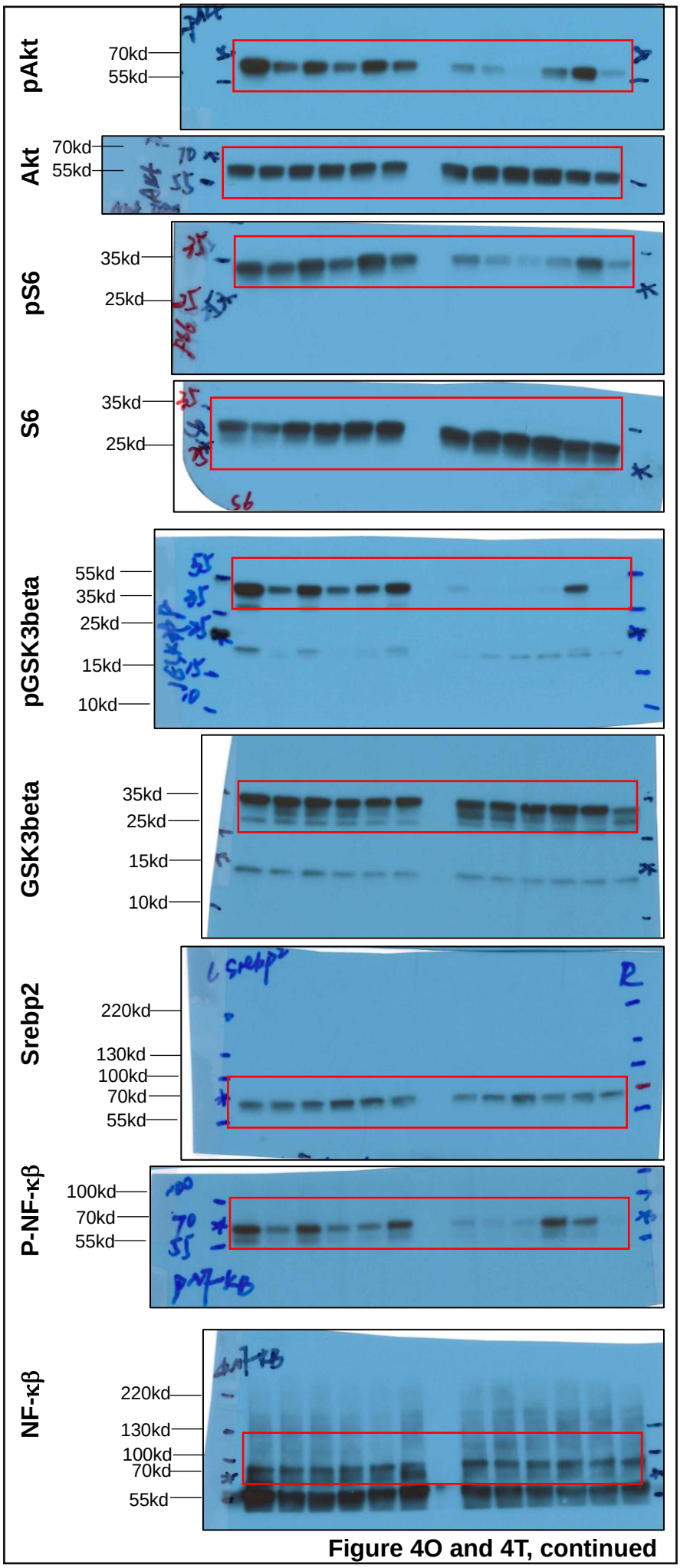

Figure 4O and 4T, continued

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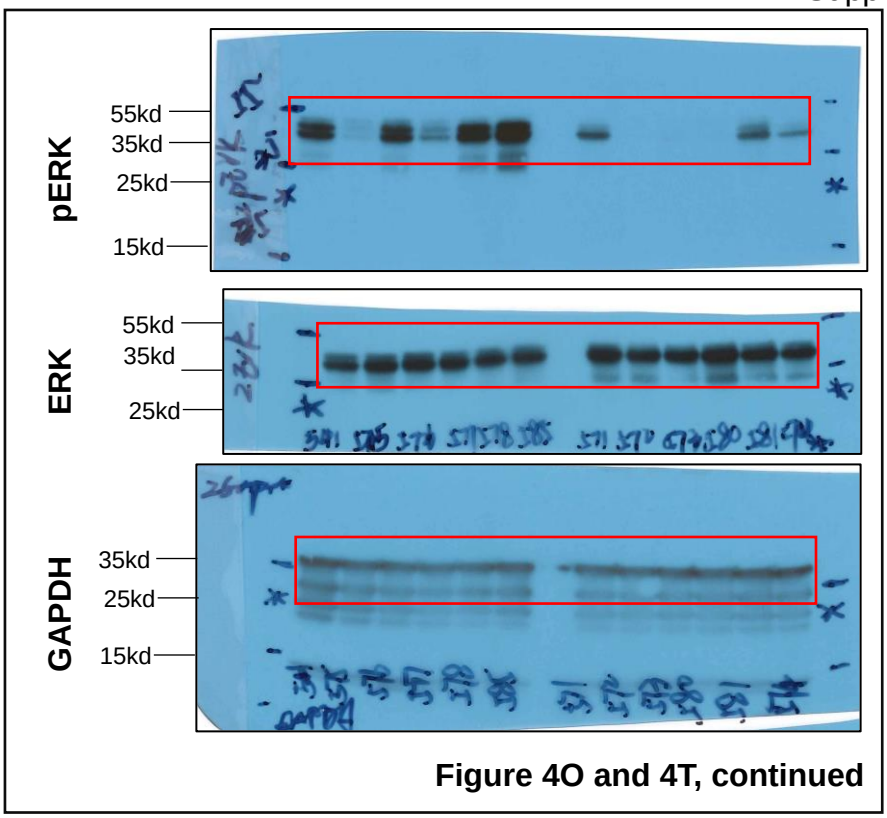


Figure 4O and 4T, continued

F

Supplemental Figure 7

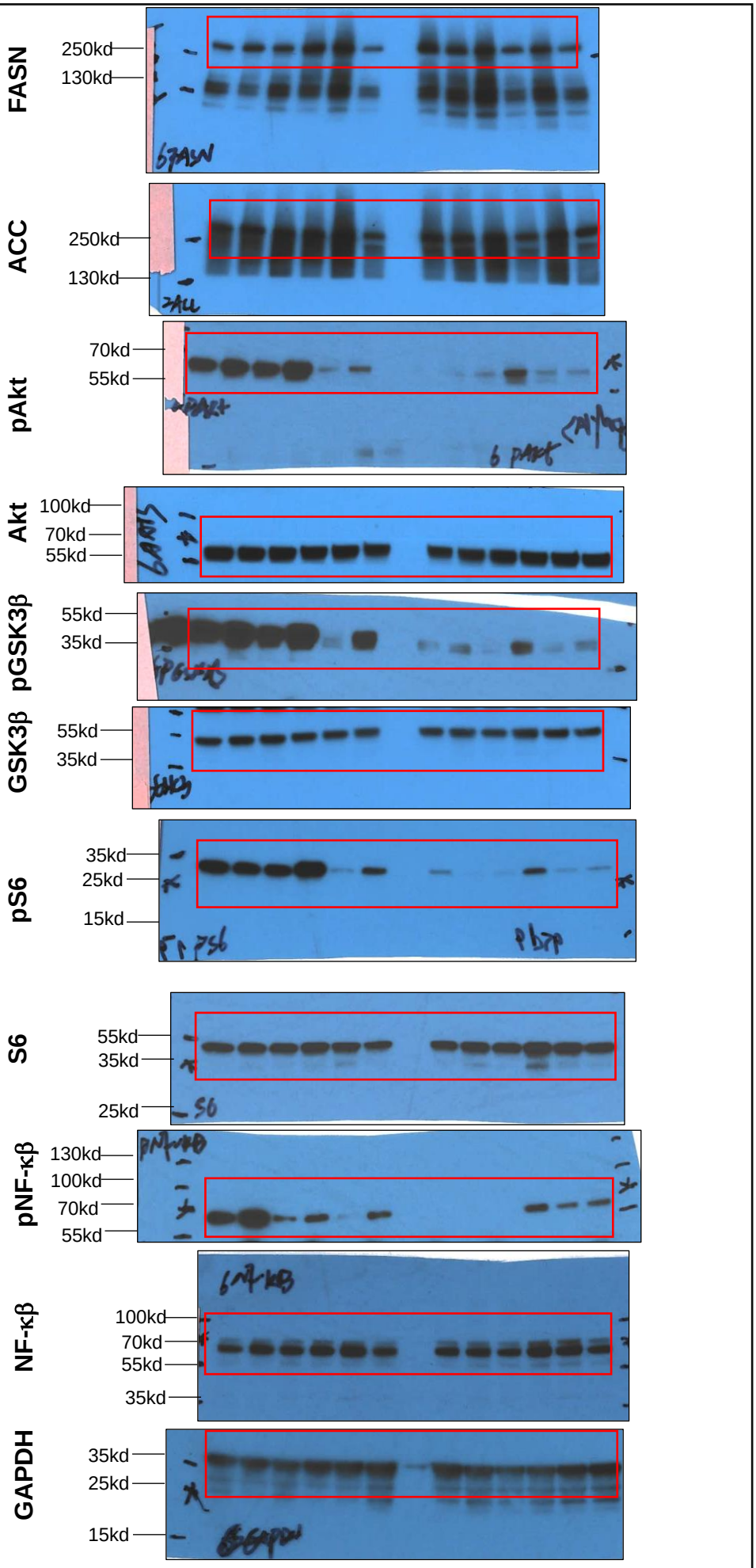
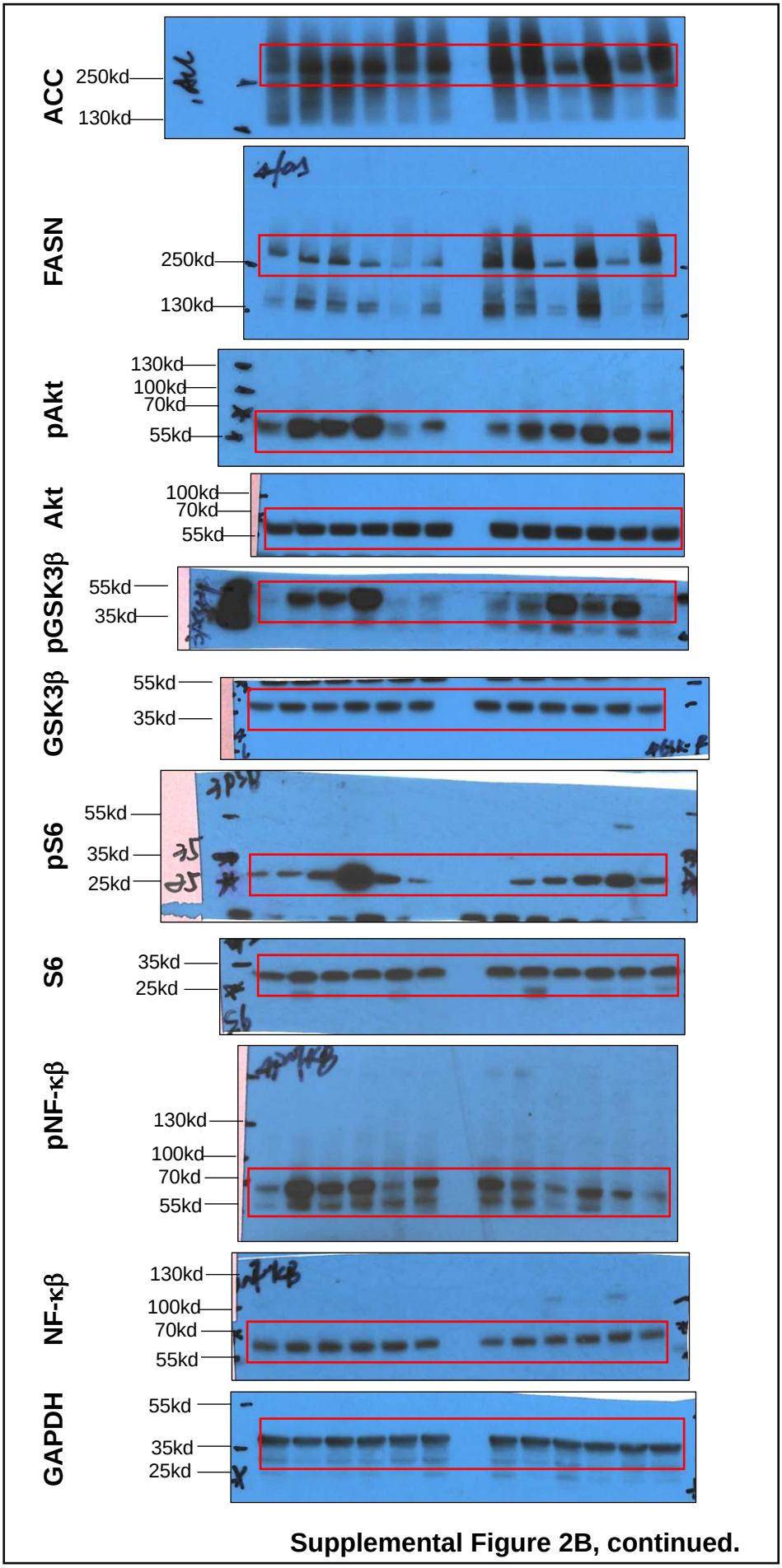
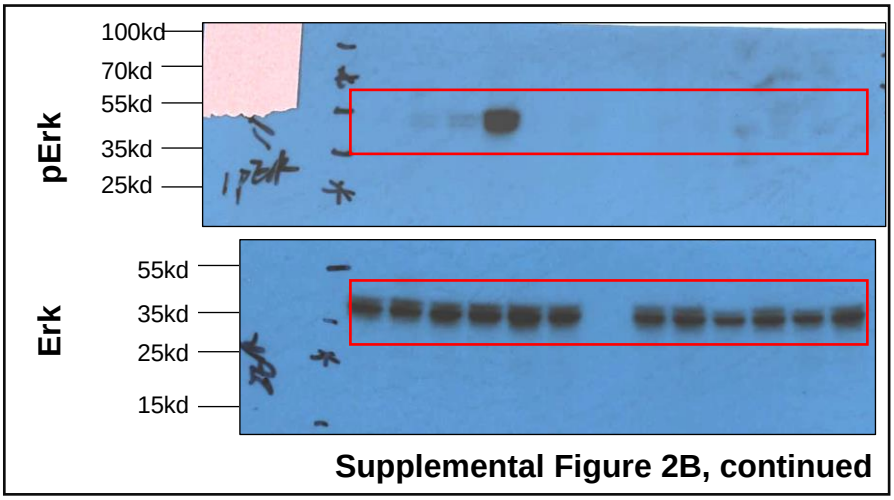


Figure 5J



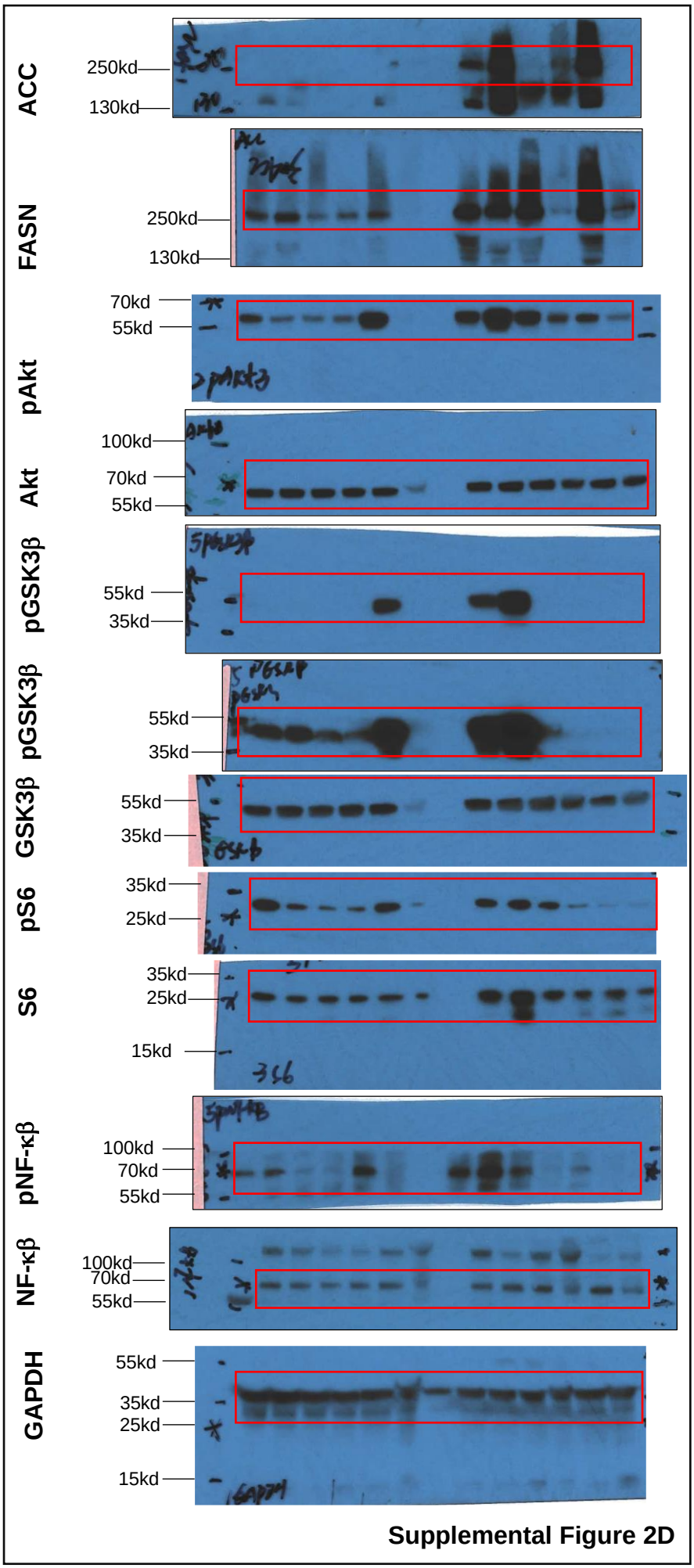
Supplemental Figure 2B, continued.

G



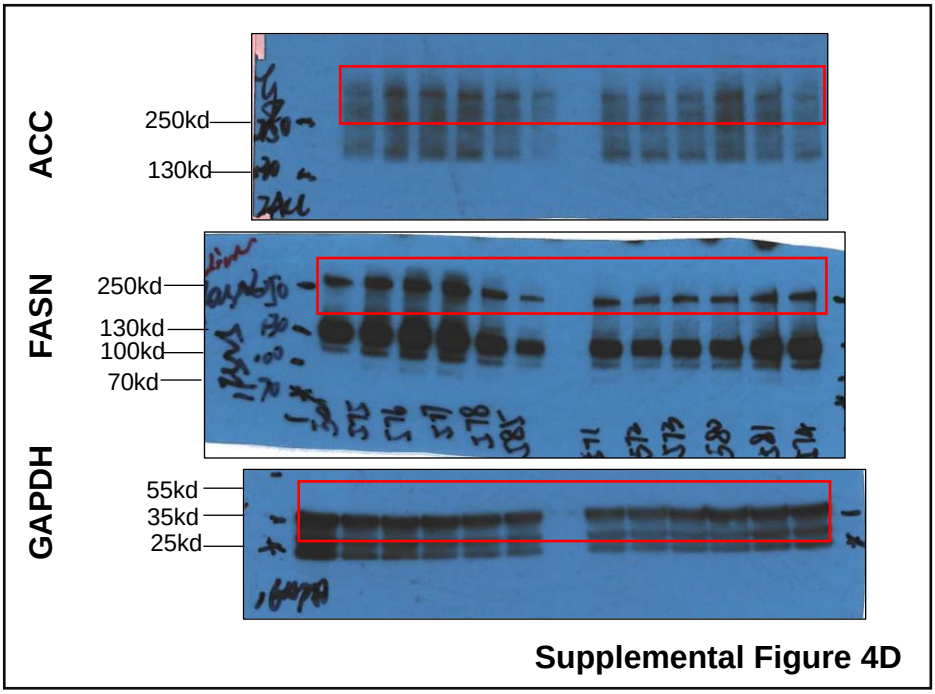
Supplemental Figure 2B, continued

H



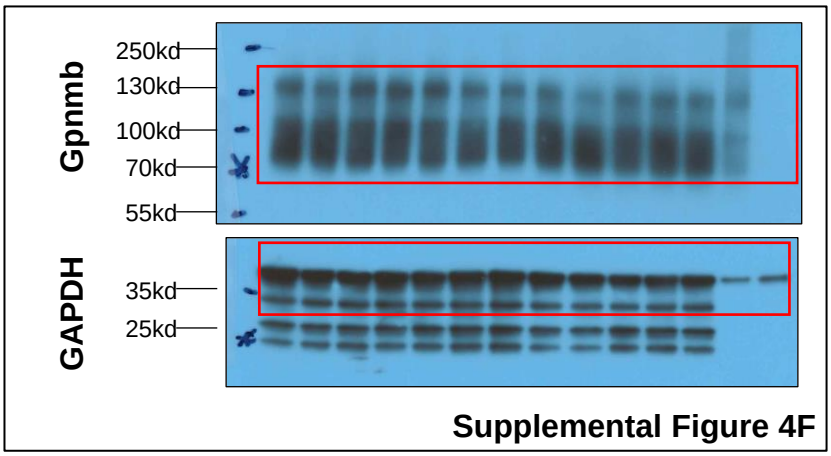
Supplemental Figure 2D

I

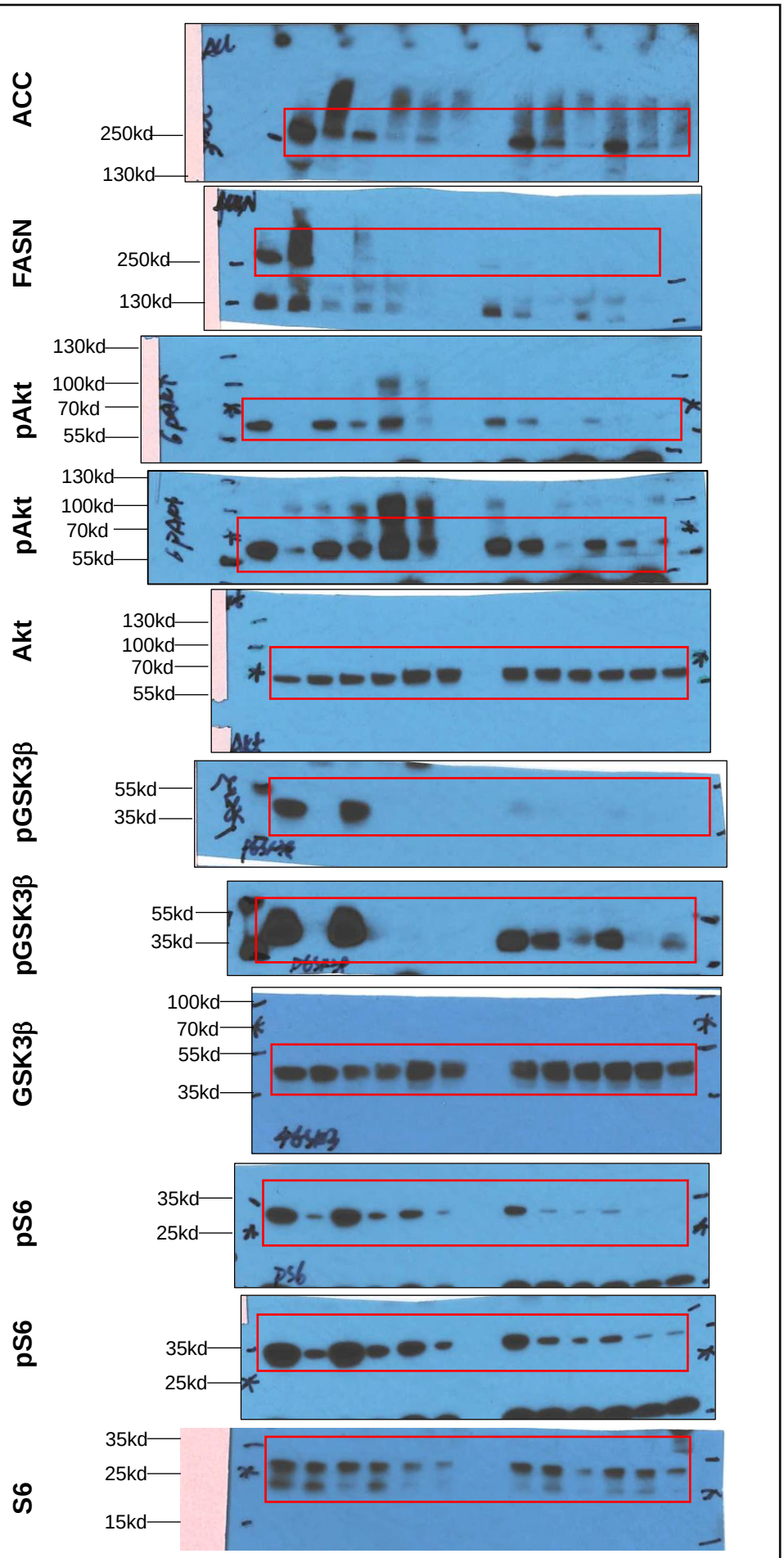


Supplemental Figure 4D

J

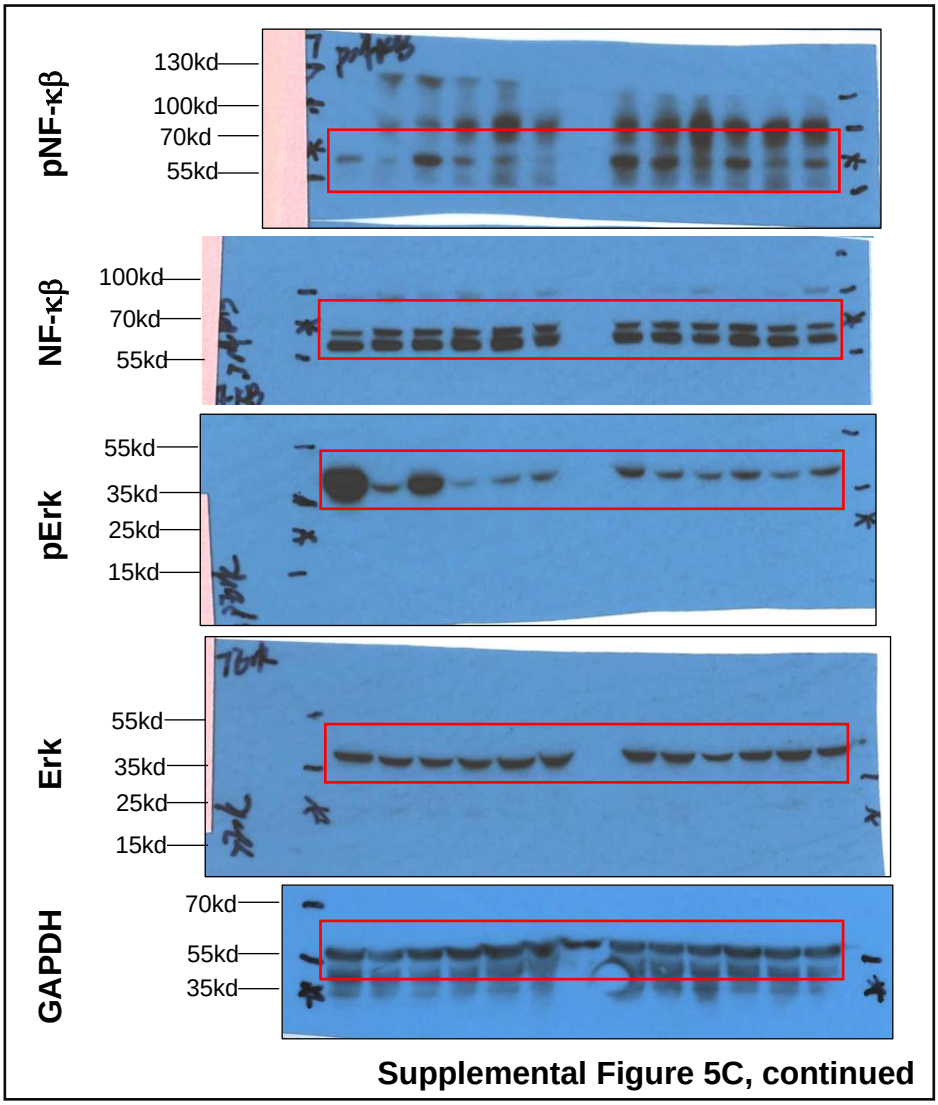


Supplemental Figure 4F



Supplemental Figure 5C, continued

K



Supplemental Figure 5C, continued

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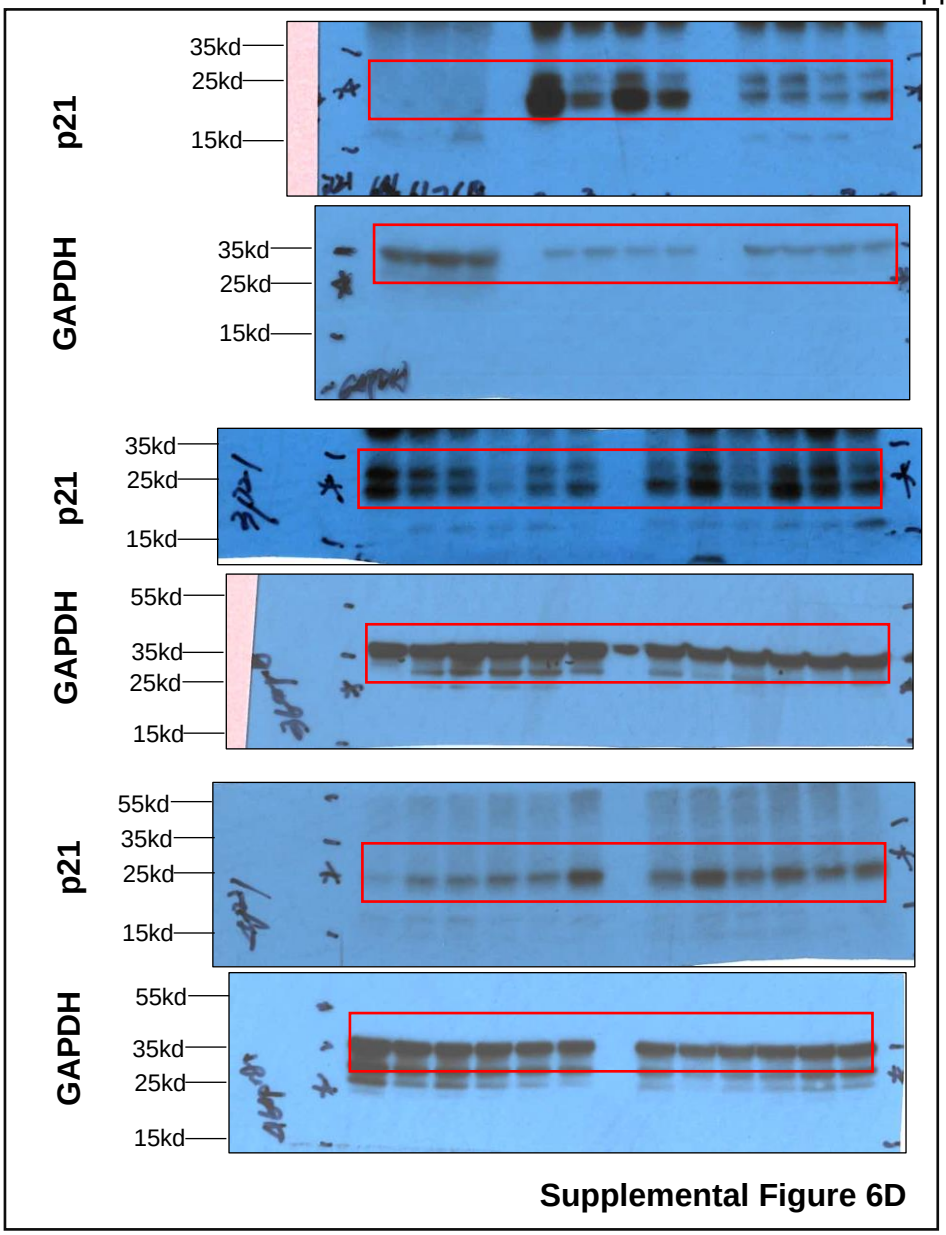

Supplemental Figure 7. Unedited blots. A. Related to **Figure 1J and 1U**. **B.** Related to **Figure 1R, Supplemental Figure 1G and Supplemental 1K**. **C**. Related to **Figure 3F**. **D**. Related to **Figure 4F**, **E.** Related to **Figure 4O and 4T**. **F.** Related to **Figure 5J**. **G**. Related to **Supplemental Figure 2B**. **H.** Related to Supplemental **Figure 2D**. **I.** Related to **Supplemental Figure 4D**. **J.** Related to Supplemental **Figure 4F**. **K.** Related to **Supplemental Figure 5C. L.** Related to **Supplemental Figure 6D**. The bands used in the figures were highlighted in the red rectangle.