Supplemental Materials for:

Mouse Hepatocyte Overexpression of NF-κB-inducing Kinase (NIK) Triggers Fatal Macrophage-dependent Liver Injury and Fibrosis

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Materials and Methods

Human liver biopsies. Surgical specimen were obtained from subjects without liver pathology: 2 Caucasian males (20 and 53 years of age); alcoholic cirrhosis: 3 Caucasian males (53, 53, and 58 years), 1 Hispanic male (57 years) and 2 Caucasian females (59 and 60 years); and PBC: 1 Hispanic female (53 years) and 2 Caucasian females (47 and 50 years). Samples were obtained without additional identifiers under an exempt protocol that was approved by the Human Subjects Review Committee at the University of Michigan.

Generation of *albumin*-Cre adenoviruses. The mouse *albumin* promoter and poly-A sequences were inserted in a pAdtrack vector to prepare a green fluorescent protein (GFP) pShuttle vector. *Cre* cDNA was inserted between the *albumin* promoter and the poly-A sequences of GFP pShuttle vector. GFP or Cre pShuttle vectors were co-transformed with pAdeasy1 into BJ5183 cells to generate GFP or Cre adenoviral vectors. GFP or *albumin*-Cre adenoviruses were produced in Q293A cells and purified using CsCl.

Adenoviral and AAV infection. Mice (7-9 weeks) were infected with *albumin*-Cre or GFP adenoviruses (1-3x10^{11} viral particles per mouse), or with adeno-associated virus (AAV)-GFP or AAV-Cre (1.5x10^{11} genome copies per mouse), via tail vein injection. AAV-Cre was described previously (1). *STOP-tomato* reporter male mice (8-weeks) were infected with Cre adenoviruses (1x10^{11} viral particles per mouse) for 3 days.

CCl_4, alcohol, and $GdCl_3$ treatments. Adult male mice (8-9 weeks) were treated with 2 µl/g CCl_4 (10% in olive oil) or olive oil three times per week for 3-4 weeks. Male mice (7-8 weeks) were fed a Lieber-DeCarli regular ethanol (6%) liquid diet (Dyets, Inc., Bethlehem, PA, cat#710260) for 1 or 3 weeks, and a maltose dextrin diet (equal calories) was used as control. *STOP-NIK* male mice (8-9 weeks) were intraperitoneally injected with 10 mg/kg $GdCl_3$ (Sigma-Aldrich, St. Louis, MO, G7532) or H_2O twice 1 and 4 days prior to Cre or GFP adenoviral infection ($1x10^{11}$ viral particles per mouse). Mice were treated with $GdCl_3$ or H_2O for additional two times 6 and 12 days after adenoviral infection.

Blood analysis. Blood samples were collected from tail veins. Blood glucose levels were determined using glucometers (Bayer Corp., Pittsburgh, PA). Plasma ALT and ALP activity and bilirubin levels were measured using an ALT reagent set, an ALP reagent set, and a total bilirubin reagent set (Pointe Scientific Inc., Canton, MI).

Immunoblotting. Tissue samples were homogenized in lysis buffer (50 mm Tris, pH 7.5, 1% Nonidet P-40, 150 mM NaCl, 2 mM EGTA, 1 mM Na_3VO_4, 100 mM NaF, 10 mM $Na_4P_2O_7$, 1 mM benzamidine, 10 µg/ml aprotinin, 10 µg/ml leupeptin; 1 mM phenylmethylsulfonyl fluoride). Proteins were separated by SDS-PAGE, immunoblotted with the indicated antibodies, and visualized using the Odyssey infrared imaging system (Li-Cor Biosciences, Lincoln, NE) or ECL (Perkinelmer, Waltham, MA, NEL101001EA). Keratin-18 (K18) analysis was performed as described previously (2). Briefly, liver tissues were homogenized and subjected to centrifugation (14000 rpm, 10 min, 4^0C). Pellets were resuspended in a buffer (10 mM Tris-HCl, pH 7.6, 140 mM NaCl, 1.5 M KCl, 5 mM EDTA, 0.5% Triton X-100, 10 µg/ml aprotinin, 10 µg/ml leupeptin; 1 mM phenylmethylsulfonyl fluoride) by rotating at 4^0C for 30 min, and then subjected to centrifugation (14000 rpm, 20 min, 4^0C). Pellets were resuspended in a Novex Tris-Glycine SDS Sample Buffer (Life Technologies, Grand Island, NY, LC2676), heated at 99^0C for 5 min, and subjected to centrifugation (14000 rpm, 2 min, room temperatures). K18 in supernatants was separated by SDS-PAGE and immunoblotted with anti-K18 antibody (3). Antibody information was described in Table A.

Hydroxyproline assays. Liver samples were homogenized in 6 N HCl, hydrolyzed at 100^0C for 18 h, and centrifuged at 10000 rpm for 5 min. Supernatant was dried in speed-vacuum, dissolved

in H_2O, and neutralized with 10 N NaOH. Samples were incubated in a chloramine-T solution (60 mM chloramines-T (Sigma, 857319), 20 mM citrate, 50 mM acetate, pH 6.5) for 25 min at room temperatures, and then in Ehrlich's solution (Sigma, 038910) at 65^0C for additional 20 min. Hydroxyproline content was measured using a Beckman Coulter AD 340 Plate Reader (570 nm) and normalized to liver weight.

ROS assays. Liver samples were homogenized in a lysis buffer, mixed with a dichlorofluorescein diacetate fluorescent probe (DCF, Sigma, D6883) to a final probe concentration of 5 μM for 1 h at 37^0C. DCF fluorescence was measured using a BioTek Synergy 2 Multi-Mode Microplate Reader (485 nm excitation and 527 nm emission).

Real-time quantitative PCR (qPCR). Total RNAs were extracted using TRIzol reagents (Life technologies). The first-strand cDNAs were synthesized using random primers (Life Technologies, 48190-011) and M-MLV reverse transcriptase (Promega, Madison, WI). Relative mRNA abundance of different genes was measured using SYBR Green PCR Master Mix (Life Technologies, 4367659) and Mx3000P real-time PCR system (Stratagene, LA Jolla, CA), and normalized to 18S levels. Primers were listed in Table B.

Immunostaining. Liver frozen sections (7 μM) were prepared using a Leica cryostat (Leica Biosystems Nussloch GmbH, Nussloch, Germany). Primary hepatocytes were grown on collagen-coated glass slides. Liver sections and primary hepatocytes were fixed in 4% paraformaldehyde for 30 min, blocked for 3 h with 5% normal goat serum (Life Technologies) supplemented with 1% BSA, and incubated with the indicated antibodies at 4^0C overnight. The sections were incubated with Cy2 or Cy3-conjugated secondary antibodies and visualized using a BX51 microscope equipped with a DP72 Digital Camera (Olympus, Tokyo, Japan). Antibody dilutions were: α-SMA (Sigma, A5228):1:5,000; K18 (lab made): 1:1,000; K19 (lab made): 1:100; F4/80 (eBiosience, San Diego, CA, 14-4801-82): 1:500; CD-3 (eBiosience, 14-0032-81): 1:100; CD-45 (eBiosience, 47-0451-80): 1:1,000.

Liver paraffin sections (5 μM) were stained with hematoxylin and eosin (H & E) or with 0.1% Sirius-red (Sigma, 365548) and 0.1% Fast-green (Sigma, F7252) (dissolved in saturated picric acid).

TUNEL assays. Liver frozen sections (7 μm) or primary hepatocytes were fixed with 4% paraformaldehyde and subjected to TUNEL assays using an In Situ Cell Death Detection Kit (Roche Diagnostics, Indianapolis, IN, 11684817910) following the manufacturer's recommended procedure. The sections were also stained with DAPI to estimate total cell number. TUNEL-positive cells were counted and normalized to total cell number.

Primary hepatocyte cultures. Primary hepatocytes were prepared from adult mice by liver perfusion with type II collagenase (Worthington Biochem, Lakewood, NJ) as described previously (4). Hepatocytes were grown on collagen-coated plates in William's medium E (Sigma, W4125) supplemented with 2% FBS, 100 units ml^{-1} penicillin, and 100 μg ml^{-1} streptomycin, and infected with adenoviruses as described previously (4).

Preparation of conditioned medium. Primary hepatocytes were infected with β-Gal, NIK, G855R, KA or IKKβ(SE) adenoviruses for 18 h and then grown in fresh growth medium. Hepatocyte conditioned medium was collected 24 h later. To prepare L929-conditioned medium, 0.24 x 10^6 L929 cells were seeded on a 150 mm-plate in 150 ml DMEM supplemented with 100 units/ml penicillin and 100 μg/ml streptomycin, 1% L-glutamine, and 10% heat-inactivated (56^0C for 30 min) FBS (Life Technologies, 10082). L929 conditioned medium was collected every 7-day for two times, filtered (0.22μM), and stored in -20^0C.

Bone marrow derived macrophage (BMDM) differentiation. Primary bone marrow cells were isolated from the femur and tibia of wild type mice (8-9 weeks) and cultured in DMEM supplemented with 20% L929 conditioned medium, 10% heat-inactivated FBS, and 100 units/ml penicillin and 100 μg/ml streptomycin. Medium was changed every 2-day. BMDMs were fully differentiated 6 days later, and then treated with 50% hepatocyte conditioned medium for 18 h. Gene expression was measured by qPCR.

Hepatocyte-BMDM co-cultures. BMDMs were differentiated for 4 days, and 1x10^5 cells were seeded in an insert (Corning Incorporated, Corning, NY, 3413). WT primary hepatocytes were infected with β-Gal, NIK, G885R or KA adenoviruses. Adenoviruses were removed 18 h later, and infected hepatocytes were subsequently co-cultured with or without BMDMs for 2 days. Culture medium was collected for ALT assays, and hepatocytes were subjected to TUNEL

assays. Similarly, primary hepatocytes were isolated from *STOP-NIK* mice and infected with GFP or *albumin*-Cre adenoviruses for 18 h, and then were co-cultured with or without BMDMs for additional 3 days prior to TUNEL assays.

Electrophoretic Mobility Shift Assays. After viral infection (36 h), primary hepatocytes were scraped in hypotonic buffer (10 mM Hepes, pH 7.9; 10 mM KC1, 1 mM EDTA; 0.6% NP-40; 1 mM DTT; 10 µg/ml aprotinin, 10 µg/ml leupeptin; 1 mM phenylmethylsulfonyl fluoride), incubated on ice for 15 min, and subjected to centrifugation at 2,000xg. Pellets were resuspended in nuclear extraction buffer (20 mM Hepes pH 7.9; 0.4 M NaCl; 1 mM EDTA; 10% glycerol; 1 mM DTT; 10 µg/ml aprotinin, 10 µg/ml leupeptin; 1 mM phenylmethylsulfonyl fluoride) and incubated (with rotation) for 30 min at 4°C. Nuclear extracts were obtained after centrifugation at 10,000xg Nuclear protein (8 µg) was incubated at room temperature for 15 min with [^{32}P]-labeled NF-□ [illegible]-agttgaggggactttcccagg-3'; Reverse: 5'-gcctgggaaagtcccctc-3') in binding buffer (10 mM Tris-Cl, pH 7.5; 50 mM NaCl; 1 mM EDTA; 1 mM DTT; 0.1 µg/µl polydIdC; 20% glycerol). Nuclear protein was pre-incubated with unlabeled NF-□ [illegible]gonucleotides (100-fold) for 10 min as a negative control for non-specific interaction. Reaction mixtures were resolved on a non-denaturing gel (5% acrylamide; 2.5% glycerol; 0.25 x TBE), immobilized in a blotting paper using a gel dryer (Bio-Rad Laboratories, Inc, Hercules, CA), and visualized by exposure to auto-rad film (BioExpress, Kaysville, UT).

Viability assays. Hepatocyte viability was measured by the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays. Hepatocytes were incubated with MTT (75 µg/ml) for 4 h. After extensive PBS washes, cells were solubilized in DMSO. Absorbance (570 nm) of cell extracts was measured using a Beckman Coulter AD 340 Plate Reader. The viability of hepatocytes infected with β-Gal adenoviruses for 2 days was defined as 100%.

Statistical Analysis. Data were presented as means ± s.e.m. Differences between groups were analyzed with two-tailed Student's t test. $P < 0.05$ was considered statistically significant.

References

1. Sun Z, Miller RA, Patel RT, Chen J, Dhir R, Wang H, Zhang D, et al. Hepatic Hdac3 promotes gluconeogenesis by repressing lipid synthesis and sequestration. Nat Med 2012;18:934-942.

2. Ku NO, Toivola DM, Zhou Q, Tao GZ, Zhong B, Omary MB. Studying simple epithelial keratins in cells and tissues. Methods Cell Biol 2004;78:489-517.
3. Ku NO, Liao J, Omary MB. Phosphorylation of human keratin 18 serine 33 regulates binding to 14-3-3 proteins. EMBO J 1998;17:1892-1906.
4. Zhou Y, Jiang L, Rui L. Identification of MUP1 as a regulator for glucose and lipid metabolism in mice. J Biol Chem 2009;284:11152-11159.

Table A

Antibody	Company	Cat#	Species raised in	Mono/polyclonal	Dilution
α-SMA	Sigma	A5228	mouse	monoclonal	1:1000
Caspase-3	cell signaling technology	9661	rabbit	polyclonal	1:2000
Flag	Sigma	F1804	mouse	monoclonal	1:10000
Keratin-18 (D4668)	Bisher Lab	none	rabbit	polyclonal	1: 1000
NF-κB2	Santacruz	sc7386	mouse	monoclonal	1:3000
NF-κB2	cell signaling technology	4882	rabbit	polyclonal	1:3000
p85	Rui lab	none	rabbit	polyclonal	1:10000
phospho-MAPK	cell signaling technology	9101	rabbit	polyclonal	1:1000
phospho-JNK	Santacruz	sc6254	mouse	monoclonal	1:1000
ERK	cell signaling technology	4695	rabbit	monoclonal	1:2000
JNK	Santacruz	sc571	rabbit	polyclonal	1:1000
Tubulin	Santacruz	sc5286	mouse	monoclonal	1:8000

Table B

Genes	Forward	Reverse
CCL2	5'-ACTGAAGCCAGCTCTCTCTTCCTC-3'	5'-TTCCTTCTTGGGGTCAGCACAGAC-3'
CCL5	5'-CCACTTCTTCTCTGGGTTGG-3'	5'-GTGCCCACGTCAAGGAGTAT-3'
CXCL5	5'-TGCATTCCGCTTAGCTTTCT-3'	5'-CAGAAGGAGGTCTGTCTGGA-3'
TNFα	5'-CATCTTCTCAAAATTCGAGTGACAA-3'	5'-TGGGAGTAGACAAGGTACAACCC-3'
IL-1β	5'-GCCTTGGGCCTCAAAGGAAAGAATC-3'	5'-GGAAGACACAGATTCCATGGTGAAG-3'
IL-6	5'-AGCCAGAGTCCTTCAGA-3'	5'-GGTCCTTAGCCACTCCT-3'
IL-10	5'-CTGGACAACATACTGCTAACCG-3'	5'-GGGCATCACTTCTACCAGGTAA-3'
iNOS	5'-CAGGGCCACCTCTACATTTG-3'	5'-TGCCCCATAGGAAAAGACTG-3'
TGFβ1	5'-TTGCTTCAGCTCCACAGAGA-3'	5'-TGGTTGTAGAGGGCAAGGAC-3'
α-SMA	5'-GGAGAAGCCCAGCCAGTCGC-3'	5'-AGCCGGCCTTACAGAGCCCA-3'
vimentin	5'-GACCTCACTGCTGCCCTGCG-3'	5'-GACTCCTGCTTGGCCTGGCG-3'
collagen1a1	5'-TCACCTACAGCACCCTTGTG-3'	5'-GGTGGAGGGAGTTTACACGA-3'
TIMP	5'-GCTAAATTCATGGGTTCCCCAG-3'	5'-GAGAAAGCTCTTTGCTGAGCAG-3'
Bim	5'-CGGTCCTCCAGTGGGTATTT-3'	TATGGAAGCCATTGCACTGAGA-3'
PUMA	5'-TACGAGCGGCGGAGACAAG-3'	GTGTAGGCACCTAGTTGGGC-3'
Noxa	5'-TGGAGTGCACCGGACATAAC-3'	TCGTCCTTCAAGTCTGCTGG-3'
FAS	5'-CTCCGAGTTTAAAGCTGAGG-3'	5'-TGTACTCCTTCCCTTCTGTGC-3'
DR5	5'-TGACGGGGAAGAGGAACTGA-3'	5'-GGCTTTGACCATTTGGATCT-3'
18S	5'-CGCTTCCTTACCTGGTTGAT-3'	5'-GAGCGACCAAAGGAACCATA-3'
NIK (h)	5'-CCCACCTTTTCAGAAGCATT-3'	5'-CATTTTGCCCTCTGTAGCATGG-3'
GAPDH (h)	5'-CGACCACTTTGTCAAGCTCA-3'	5'-AGGGGTCTACATGGCAACTG-3'

Supplemental Figure Legends

Figure S1. Hepatocyte-specific activation of the *STOP-Tomato* reporter by *albumin*-Cre adenoviral infection. *STOP-Tomato* reporter mice were infected with *albumin*-Cre adenoviruses via tail vein injection, and mice were euthanized 3 days later. (A) Liver sections were immunostained with the indicated antibodies. (B) Primary hepatocytes were purified and visualized in a bright field or by immunostaining with antibodies to albumin or K18. (C) Primary hepatocytes in culture dishes were visualized using GFP (a marker of *albumin*-Cre adenoviral infection) and Tomato-red (reporter) fluorescence.

Figure S2. *Albumin*-Cre adenoviral infection does not cause liver injury and inflammation in WT mice. WT mice were infected with GFP (Con) or *albumin*-Cre adenoviruses via tail-vein injection and euthanized 27 days later. (A) Body weight, nonfasting blood glucose, plasma ALT activity, and total blood bilirubin levels. Con: n=3; Cre: n=3. (B) H & E and Sirius-red/fast green staining of liver paraffin sections. (C) Liver weight, hydroxyproline content, and ROS levels. Con: n=3; Cre: n=3. (D-E) Liver sections were stained with anti-F4/80 antibody (D) or TUNEL reagents (E). Inserts: enlarged, merged images.

Figure S3. Hepatocyte-specific overexpression of NIK causes liver inflammation and fibrosis. *STOP-NIK* mice were infected with AAV-GFP or AAV-Cre via tail vein injection and sacrificed 9 days after infection. (**A**) Tissue extracts were immunoblotted with antibodies against Flag or the p85 regulatory subunit of PI 3-kinase. (B) Liver weight and hydroxyproline levels. AAV-GFP: n=3; AAV-Cre: n=3. (C) H & E and Sirius-red/fast green staining of liver paraffin sections. Sirius-red areas were quantified and normalized to the total areas. (D-F**)** Liver cryostat sections were immunostained with anti-F4/80 (D-E) or anti-α-SMA antibody (F). F4/80-positive and α-SMA-positive cells were quantified and normalized to total cell number. *$*p<0.05$*.

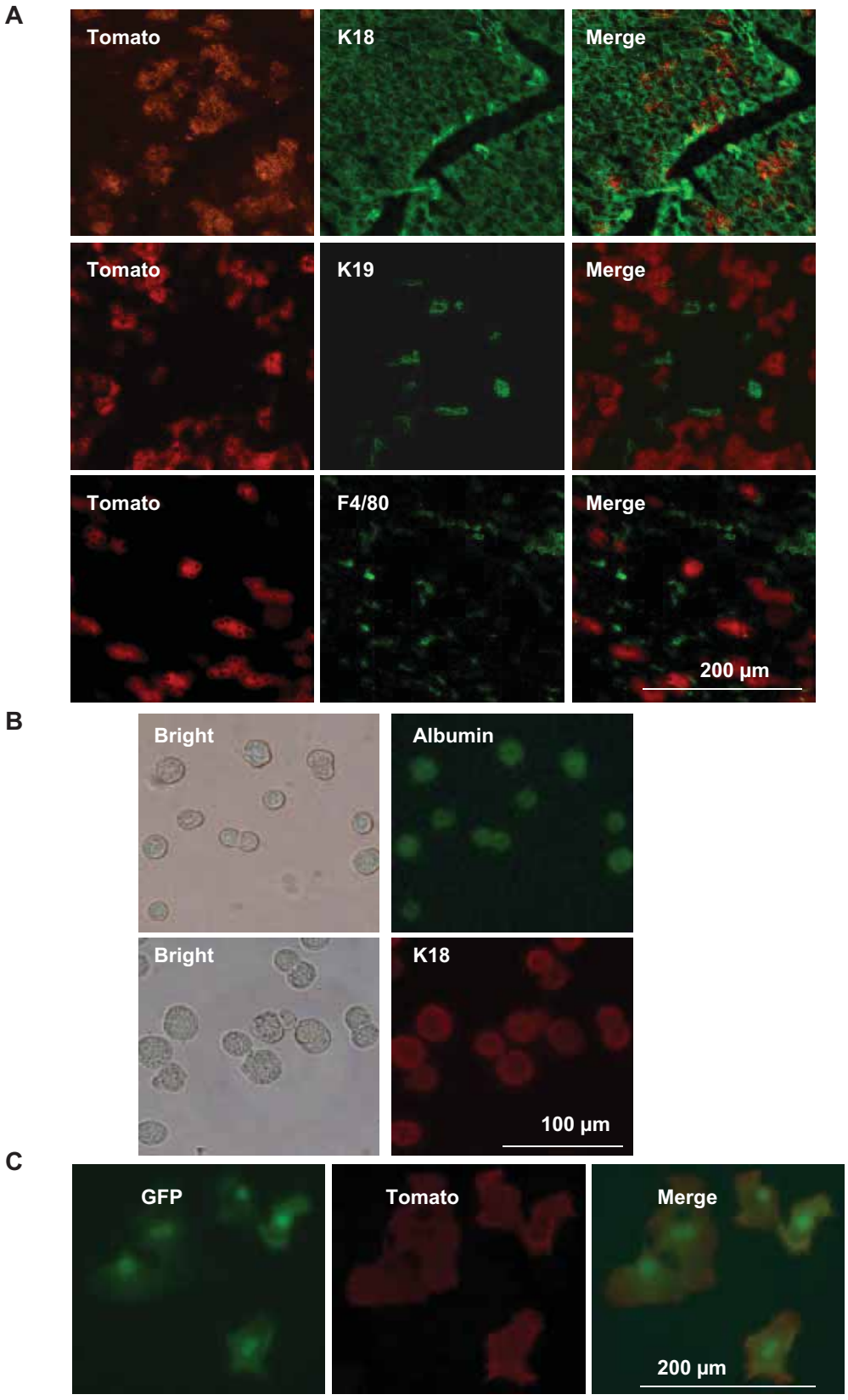
Figure S4. Hepatocyte-specific overexpression of NIK promotes immune response in the liver. *STOP-NIK* mice were infected with GFP (Con) or *albumin*-Cre adenoviruses via tail vein injection and euthanized 18 days later. Liver cryostat sections were immunostained with anti-CD3 (A) or anti-CD45 (B) antibody. CD3-positive and CD45-positive cells were counted and normalized to DAPI-positive cells. Inserts: enlarged, merged images. Con: n=4; Cre: n=4-5. *$*p<0.05$*.

Figure S5. Transgenic overexpression of NIK in hepatocytes promotes liver inflammation. Con, $Tg^{+/-}$ and $Tg^{+/+}$ male mice were sacrificed at 13 weeks of age. (A) Liver NIK mRNA levels were measured by qPCR and normalized to 18S levels. Con: n=5; $Tg^{+/-}$: n=3; $Tg^{+/+}$: n=5. (B) Liver extracts were immunoblotted with antibodies against NF-kB2 or p85. (C) Liver cryostat sections were immunostained with anti-CD3 or anti-CD45 antibody. Inserts: enlarged, merged images. (D) CD3-positive and CD45-positive cells were counted and normalized to DAPI-positive cells. Con: n=4; Tg+/+: n=4. *$p<0.05$.

Figure S6. Depletion of Kupffer cells/macrophages attenuates NIK-induced liver injury, inflammation, and fibrosis. *STOP-NIK* male mice were pretreated with $GdCl_3$ prior to *albumin*-Cre adenoviral infection and sacrificed 18 days after infection. (A) H & E staining of liver paraffin sections. (B) Liver sections were stained with TUNEL, Sirius-red/fast green, DAPI, or antibodies to F4/80 or α-SMA anti-α-SMA as indicated. (C) Liver hydroxyproline contents were measured and normalized to liver weight. Con: n=6; Cre+H_2O: n=6; Cre+$GdCl_3$: n=6. (D) The mRNA levels of the indicated genes in the liver were measured by qPCR and normalized to 18S levels. Con: n=5-6; Cre+H_2O: n=4; Cre+$GdCl_3$: n=4-5. (E) Liver extracts were immunoblotted with antibodies against α-SMA or p85. *$p<0.05$.

Figure S7. NIK cell-autonomously activates proinflammatory in primary hepatocytes. (A) Primary hepatocytes were infected with β-Gal (n=3) or NIK (n=3) adenoviruses. ROS levels were measured 48 h after infection and normalized to total protein levels. (B) Conditioned medium was prepared from primary hepatocytes infected with β-Gal or NIK adenoviruses. An aliquot of conditioned medium was heated at 100^0C for 5 min. BMDMs were stimulated with the indicated conditioned medium for 18 h, and the expression of the indicated genes was measured by qPCR and normalized to 18S levels. Gal: n=3; NIK: n=3; NIK+heat: n=3. (C) Primary hepatocytes were infected with β-Gal (n=3) or NIK (n=3) adenoviruses and co-cultured with or without BMDMs. ALT activity in culture medium was measured 48 h after BMDM co-culture. (D-E) Primary hepatocytes were isolated from *STOP-NIK* mice and infected with GFP (Con) or *albumin*-Cre adenoviruses. (D) The expression of the indicated genes in hepatocytes was measured 42 h after infection (normalized to 18S levels). Con: n=3; Cre: n=3. (E) Infected hepatocytes were subsequently co-cultured with or without BMDMs for 3 days and subjected to TUNEL assays. Insert: enlarged, merged images. *$p<0.05$.

Figure S8. NIK and NIK(G855R), but not NIK(KA), promote BMDM-mediated hepatocyte apoptosis. (A) Primary hepatocytes were infected with β-Gal, NIK, G855R or KA adenoviruses. NIK, G855R, and KA contain an N-terminal Flag tag. Cell extracts were prepared 42 h after infection and immunoblotted with anti-Flag or anti-p85 antibody. (B) Primary hepatocytes were co-infected with NF-κB2 (p100 form) adenoviruses and β-Gal, NIK, NIK(G855R) or KA adenoviruses. Cell lysates (36 h after infection) were immunoblotted with antibodies to the indicated antigens. (C-D) Primary hepatocytes were infected with with β-Gal, NIK or NIK(G855R) adenoviruses. (C) Nuclear protein were prepared 36 h later and used for EMSA assays using [^{32}P]-DNA probes containing a NF-κB binding site. An excessive amount of non-labelled NF-κB probes was added to compete for p52 NF-κB2 (non-specific). (D) Cell lysates were immunoblotted with anti-Flag or anti-p85 antibodies. (E) Primary hepatocytes were infected with β-Gal, NIK, G885R or KA adenoviruses and co-cultured with or without BMDMs for 2 days. Hepatocytes were stained with TUNEL reagents or DAPI. Insert: enlarged, merged images. (F) Conditioned medium was prepared from primary hepatocytes infected with β-Gal (n=3), NIK (n=3), G855R (n=3), KA (n=3) or IKKβ(SE) (n=3) adenoviruses for 18 h. BMDMs were treated with the indicated conditioned medium for 18 h, and the expression of the indicated genes was measured by qPCR and normalized to 18S levels. *$p<0.05$.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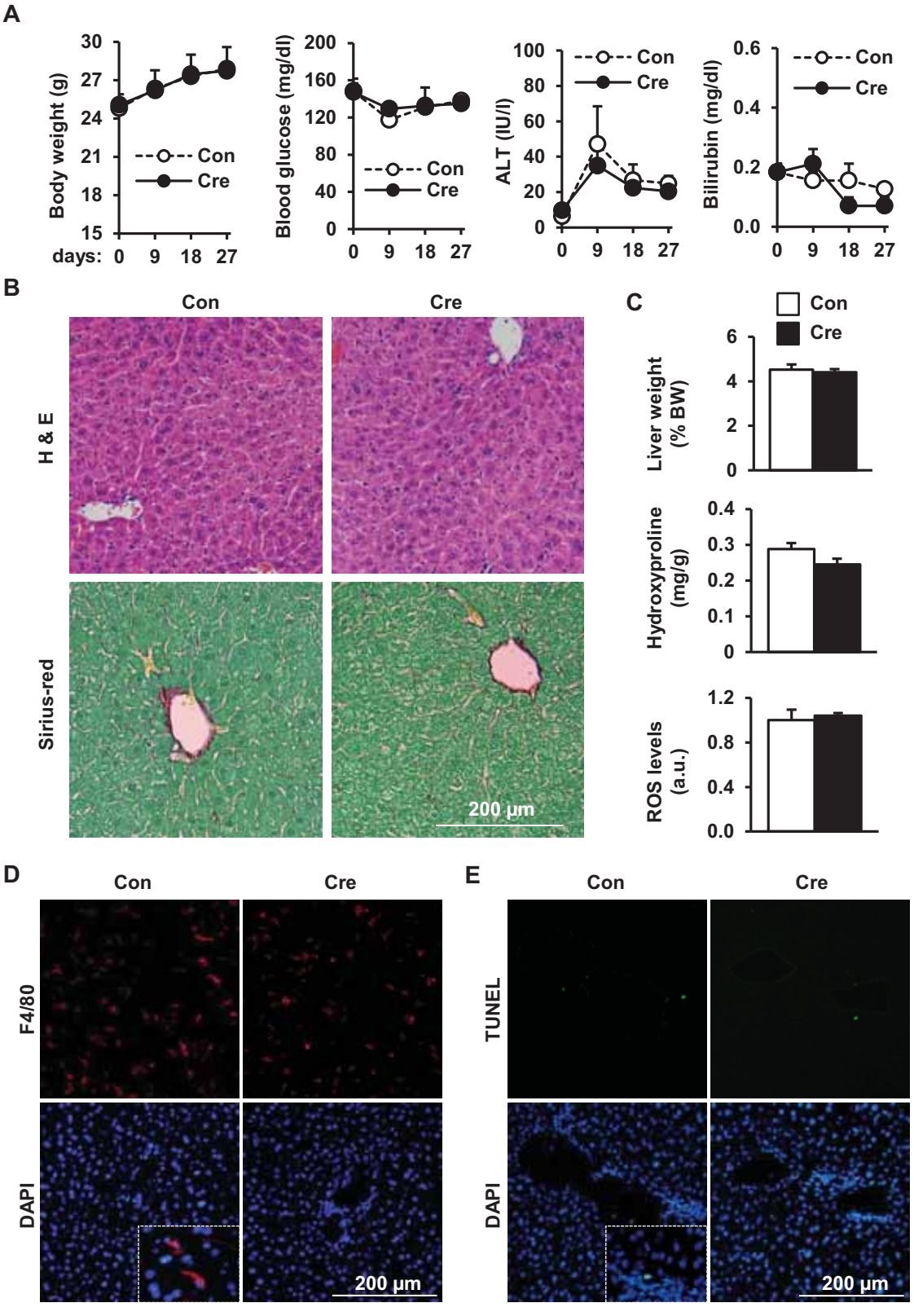
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40
0
ALT (IU/l)
100
80
60
40
20
0
Bilirubin (mg/dl)
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0.4
0.2
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B
Con
Cre
H & E
Sirius-red
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Liver weight (% BW)
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2
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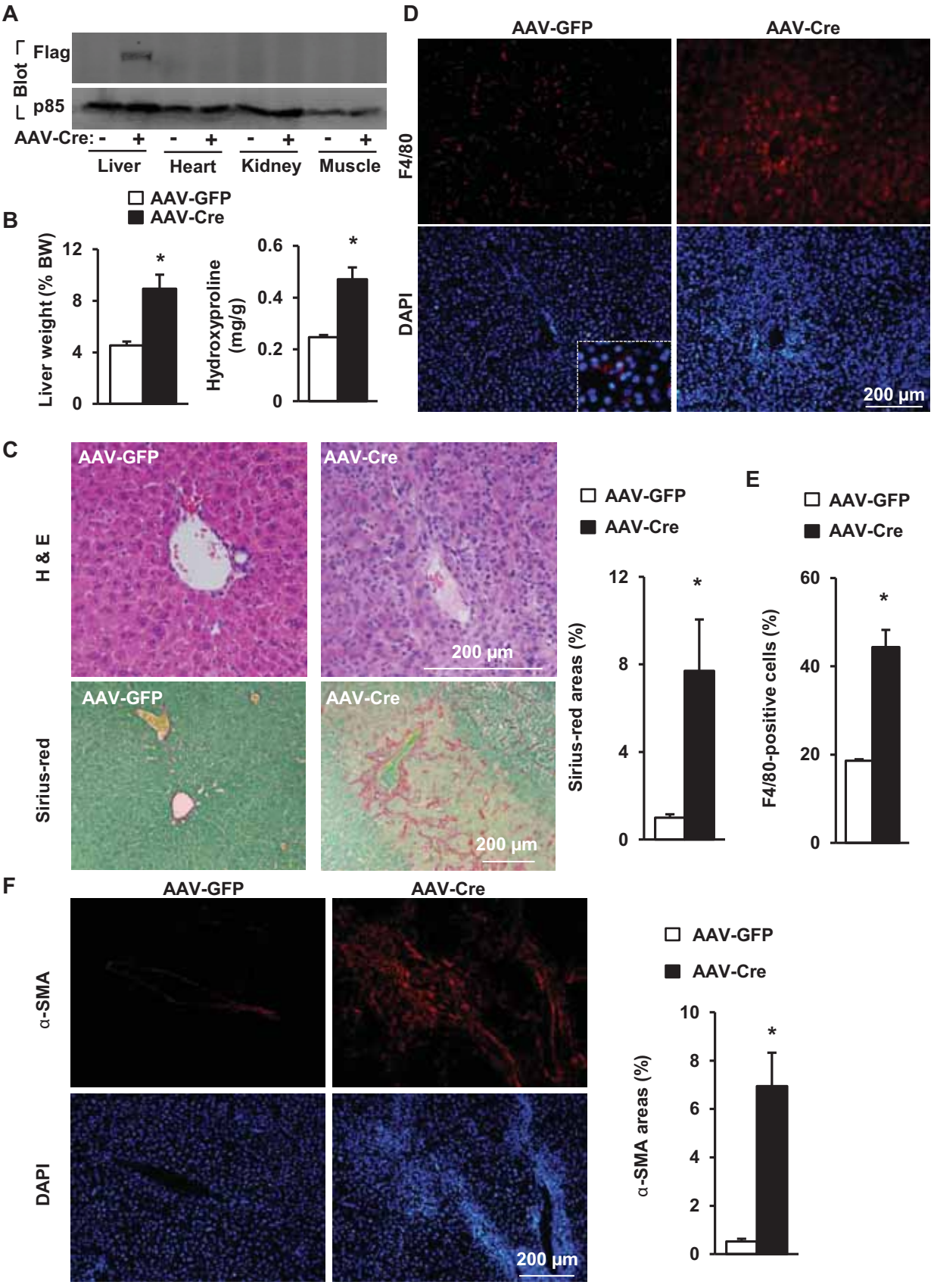
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Hydroxyproline (mg/g)
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F4/80-positive cells (%)
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40
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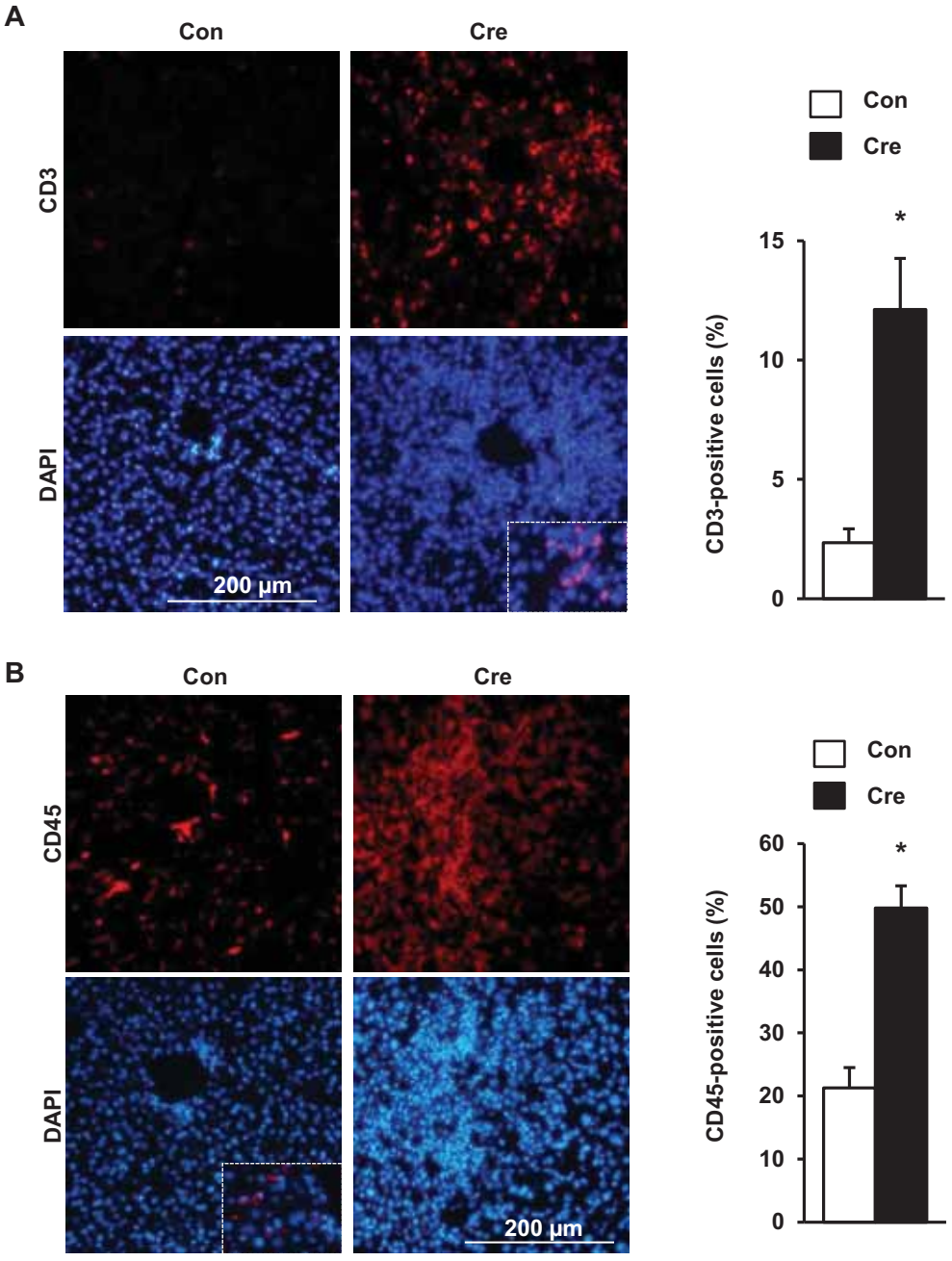
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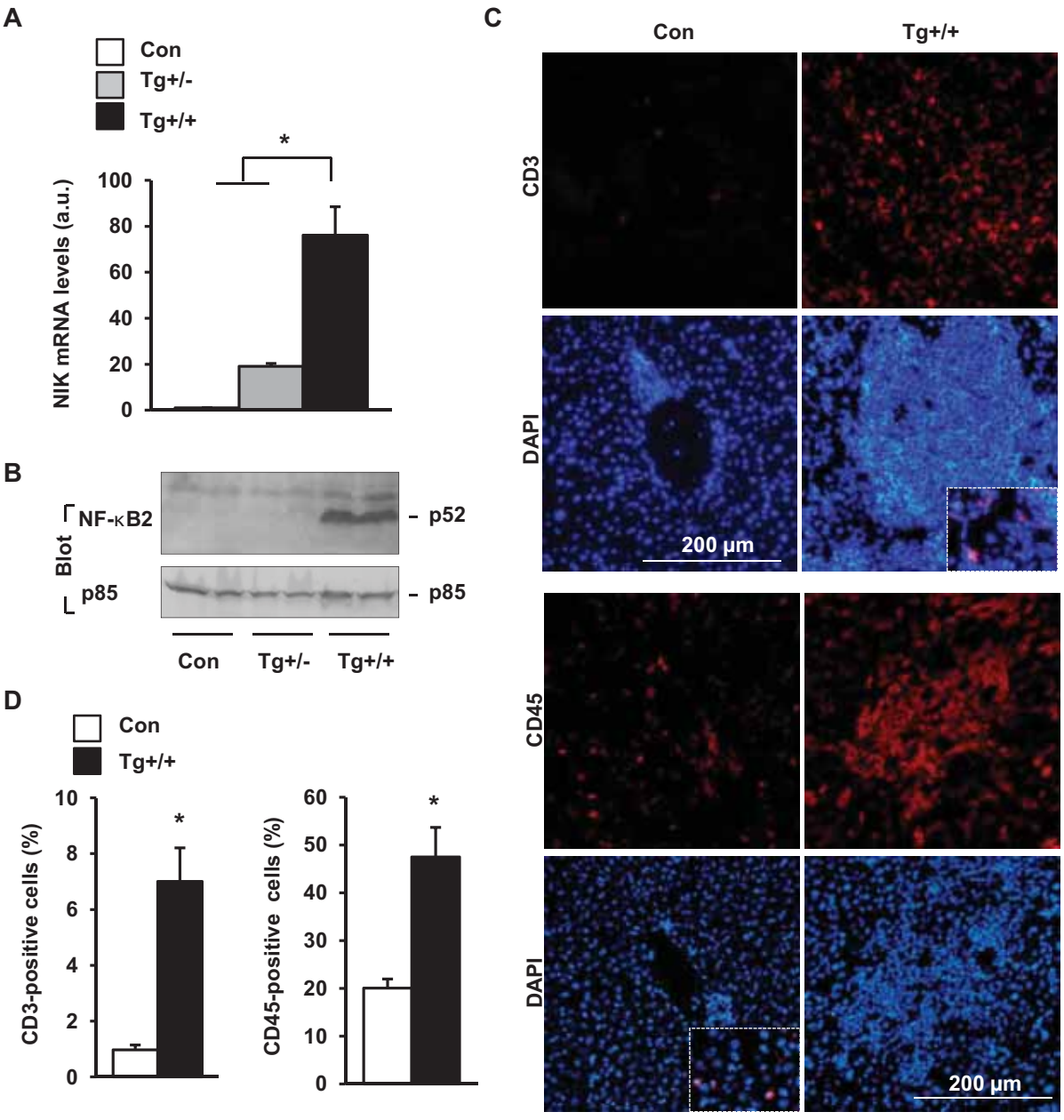
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40
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p85
p52
p85
Con
Tg+/-
Tg+/+
C
Con
Tg+/+
CD3
DAPI
200 μm
CD45
DAPI
200 μm
D
Con
Tg+/+
CD3-positive cells (%)
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0
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CD45-positive cells (%)
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40
30
20
10
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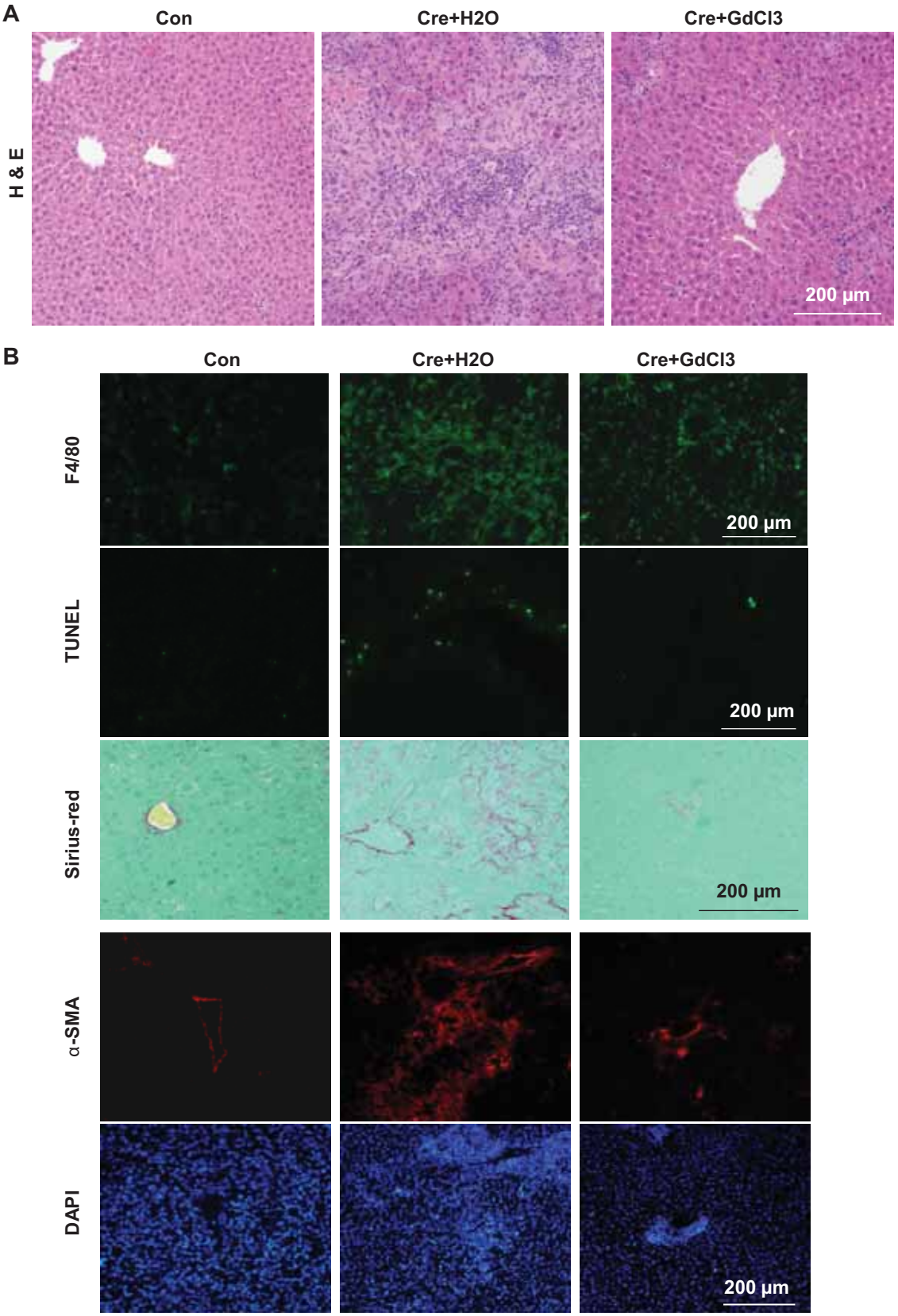
Figure S6A-B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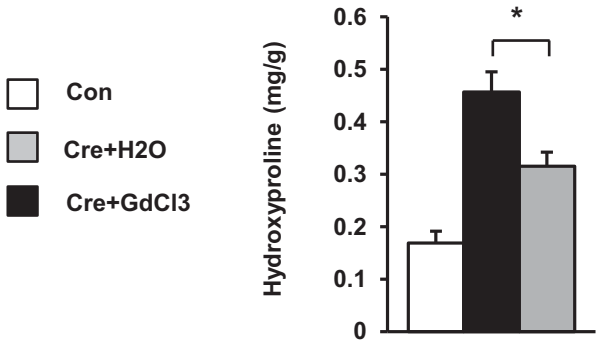
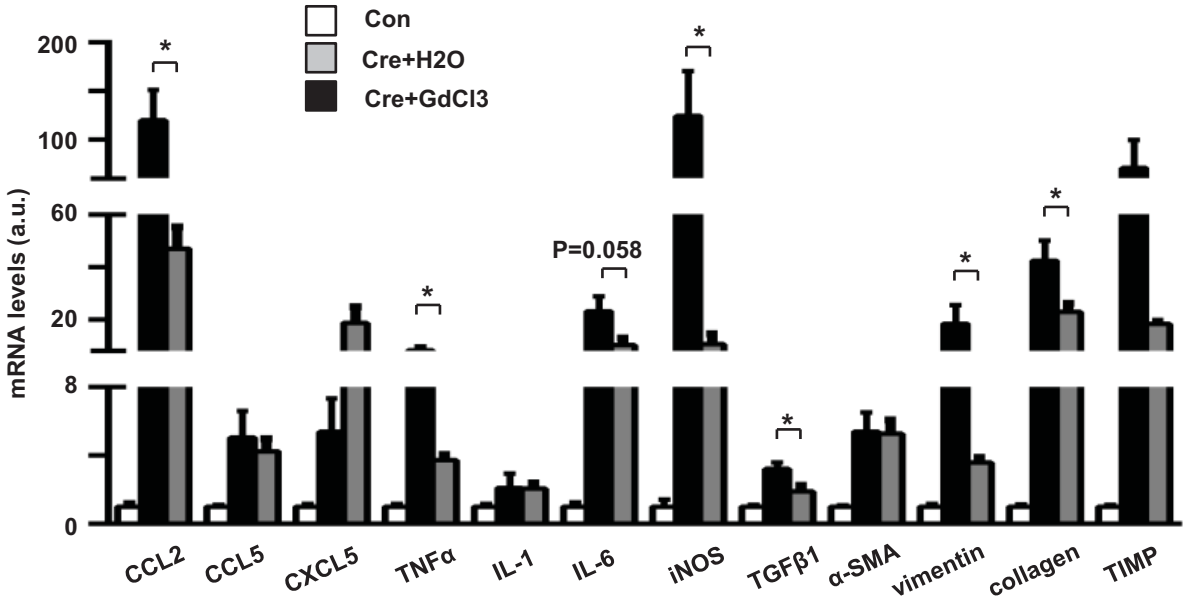
Sirius-red
α-SMA
DAPI
200 μm

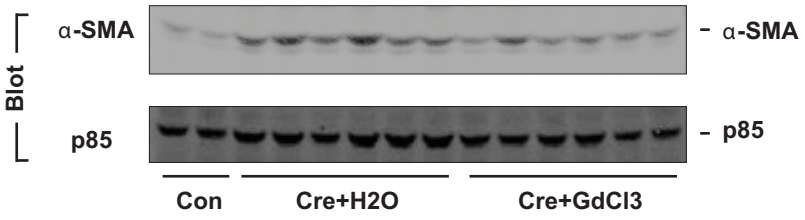
C

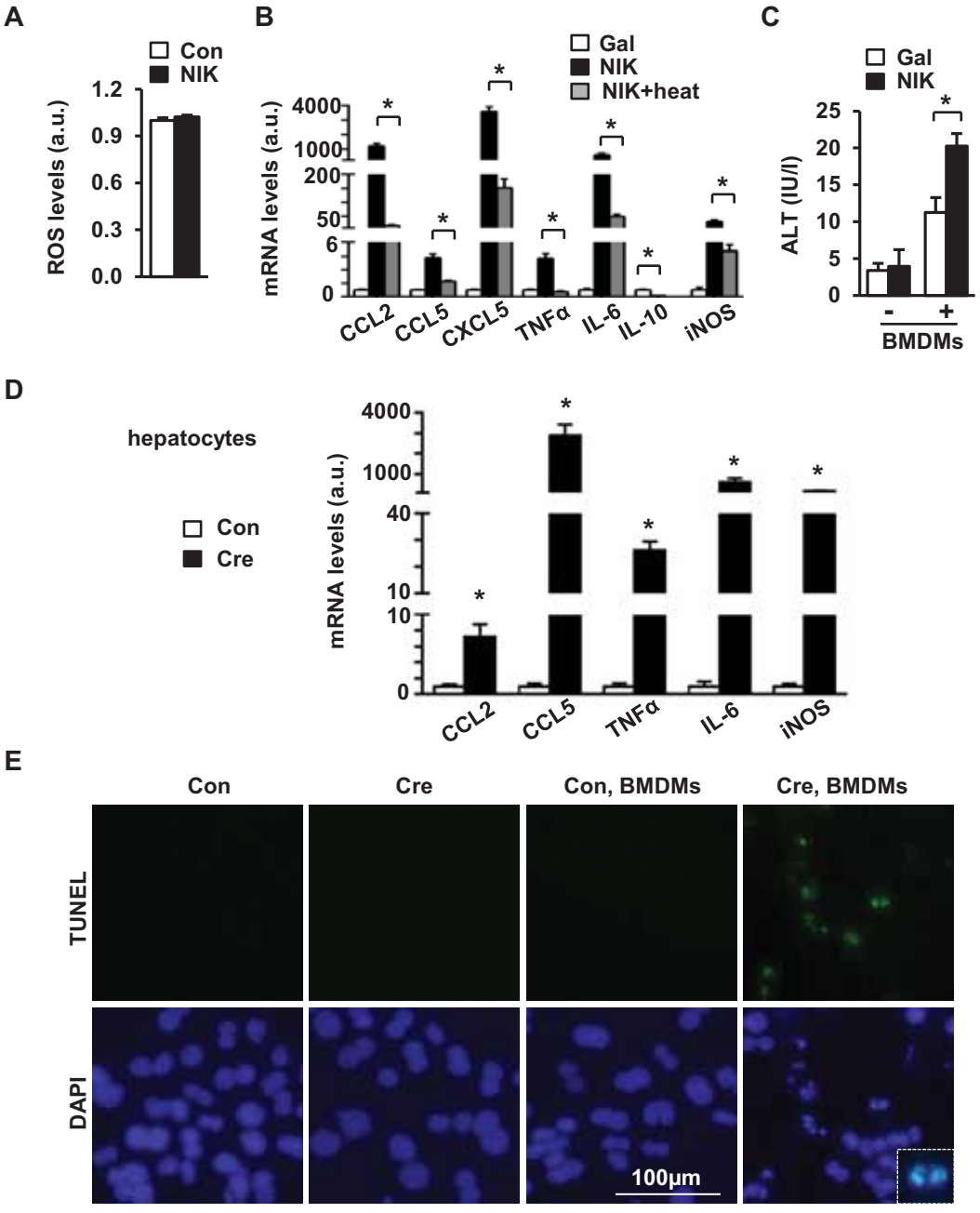

Con
Cre+H2O
Cre+GdCl3
Hydroxyproline (mg/g)
0.6
0.5
0.4
0.3
0.2
0.1
0
*

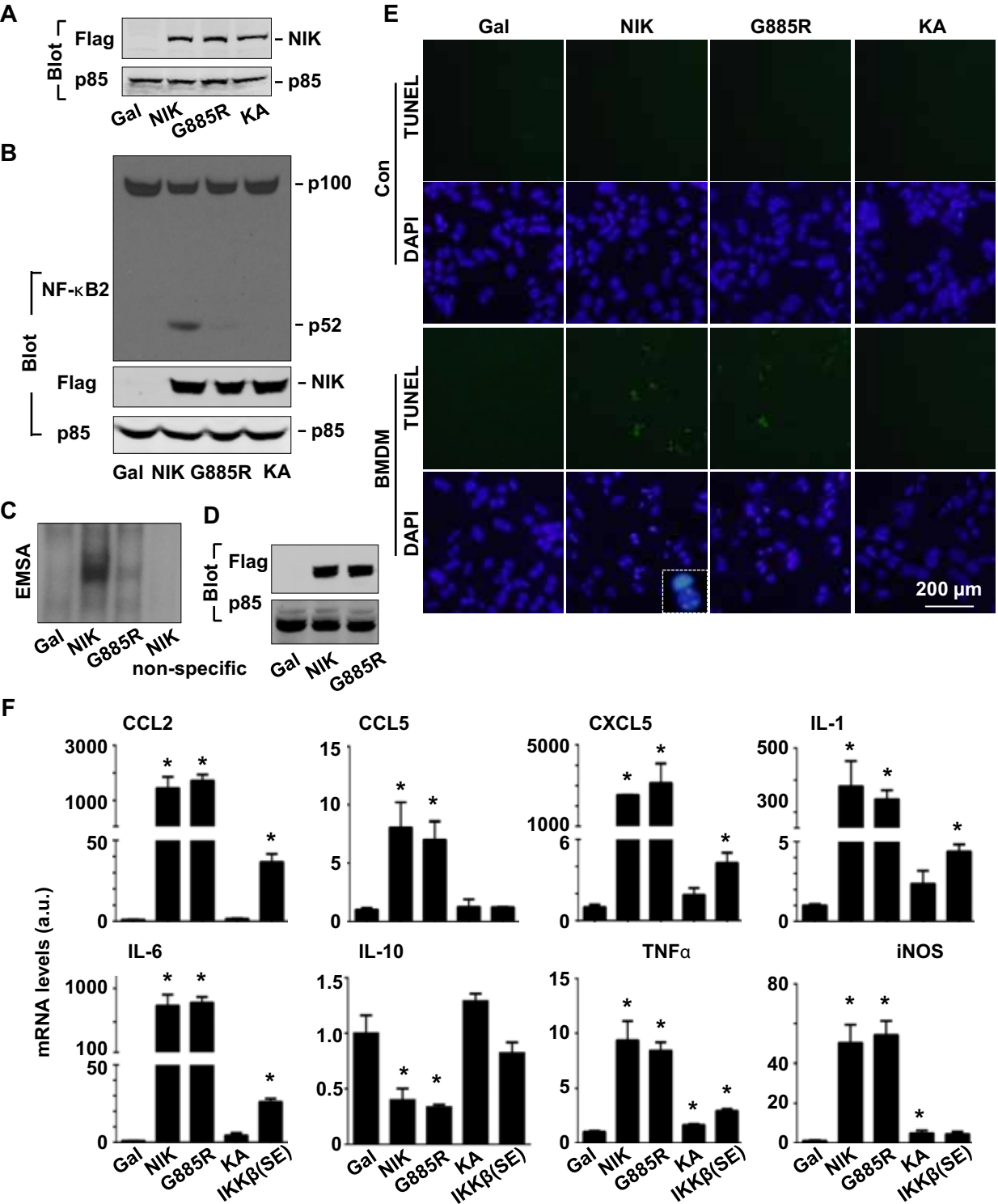
D


Con
Cre+H2O
Cre+GdCl3
mRNA levels (a.u.)
200
100
60
20
8
0
P=0.058
*
CCL2
CCL5
CXCL5
TNFα
IL-1
IL-6
iNOS
TGFβ1
α-SMA
vimentin
collagen
TIMP

E


Blot
α-SMA
p85
α-SMA
p85
Con
Cre+H2O
Cre+GdCl3


A
Con
NIK
ROS levels (a.u.)
1.2
0.9
0.6
0.3
0.0
B
Gal
NIK
NIK+heat
mRNA levels (a.u.)
4000
1000
200
50
6
0
CCL2
CCL5
CXCL5
TNFα
IL-6
IL-10
iNOS
C
Gal
NIK
ALT (IU/l)
25
20
15
10
5
0
-
+
BMDMs
D
hepatocytes
Con
Cre
mRNA levels (a.u.)
4000
1000
40
10
10
0
CCL2
CCL5
TNFα
IL-6
iNOS
E
Con
Cre
Con, BMDMs
Cre, BMDMs
TUNEL
DAPI
100μm


A
Blot
Flag
p85
NIK
p85
Gal
NIK
G885R
KA
B
NF-κB2
p100
p52
Blot
Flag
NIK
p85
p85
Gal NIK G885R KA
C
EMSA
Gal
NIK
G885R
NIK
non-specific
D
Blot
Flag
p85
Gal
NIK
G885R
E
Gal
NIK
G885R
KA
Con
TUNEL
DAPI
BMDM
TUNEL
DAPI
200 μm
F
CCL2
CCL5
CXCL5
IL-1
IL-6
IL-10
TNFα
iNOS
mRNA levels (a.u.)
Gal
NIK
G885R
KA
IKKβ(SE)