Supplementary methods

EMSA

Double-stranded oligonucleotides containing the NF-κB /p65 binding site (DICER κB probe) were labeled with biotin (Biotin 3' end DNA labeling Kit, Pierce). The sequences of the oligonucleotides used in these studies were listed in Supplementary Table S1. EMSA was performed according to manufacturer's instructions (Lightshift EMSA kit, Pierce). Binding reactions were carried out at room temperature for 20 min and then a 50–100-fold molar excess of cold competitor oligonucleotides was added, and the reactions were incubated for an additional 15 min. DNA-protein complexes were analyzed by 6% polyacrylamide gel electrophoresis in 0.5×TBE.

Supplementary Tables

Table S1. List of primers, probes and siRNAs used in this study.		
PCR Primers used to amplify the DICER promoter.		
Name	Forward primer (5'-3')	Reverse primer (5'-3')
pGL3-(-897/+65)	TCCGGTACCAGGGGCGCATAGTAGGTTCT	GGCAAGCTTCCGCTGTCAGGTTACTCCATC
pGL3-(-646/+65)	GGCGGTACCGGGCCACCATCTATTTCTCA	
pGL3-(-450/+65)	TCCGGTACCGTTACGGTCTGTGGGTGC	
pGL3-(-314/+65)	GTGGGTACCACGCCAAGGTCCAGTCCA	
pGL3-(-450/-314)	TAAGGTACCCCACGTTACGGTCTG	CGCAAGCTTTTTGAGGCCCGTT
pGL3-(-646/-180)	TGTGGTACCTACAAACAGAGGGCCACCAT	CGCAAGCTTATGAGAGCGAGCCTGTGATT
pGL3-(-450/-180)	TCCGGTACCGTTACGGTCTGTGGGTGC	
EMSA probes		
NF-κB consensus oligo	AGTTGAGGG GACTTTCCCAGG C	TCAACTCCCCTGAAAGGGTCCG
DICER promoter oligo	GGGGCCGGGATTAACATTTCA	TGAAAGGTTAATCCCGGCCCC
PCR Primers used to amplify the DICER promoter in ChIP assay.		
DICER promoter	GTTACGGTCTGTGGGTGC	ATGAGAGCGAGCCTGTGATT
Control	TGTGGGCTGGTACAACTTCA	GGCAGAAGAATCGCTTGAAC
siRNAs		
p65 si1	UAUAGCCUCAGGGUACUCCAUCAGCTT	GCUGAUGGAGUACCCUGAGGCUAUATT
p65 si2	GCCCUAUCCCUUUACGUCATT	UGACGUAAAGGGAUAGGGCTT
scramble p65 si1	CGACGGAACGCGGCACUCGTT	CGAGUGCCGCGUUCCGUCGTT
siNS	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT
DICER si1	ACUGCUUGAAGCAGCUCUGGATT	UCCAGAGCUGCUUCAAGCAGUTT
DICER si2	GGAACAUAUCAGAUUUAUATT	UAUAAAUCUGAUAUGUUCCTGTT
p50 si1	CACAGAUGUUCAUAGACAATT	UUGUCUAUGAACAUCUGUGGG
p50 si2	CCAUGGACACUGAAUCUAATT	UUAGAUUCAGUGUCCAUGGTT
RT Primers		
miR-125b	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTCACAA	

stem loop		
miR-130a stem loop	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACATGCCCT	
Let-7a stem loop	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAACTATAC	
qPCR Primers		
GAPDH	TGCACCACCAACTGCTTAG	GACGCAGGGATGATGTTC
actin(hsa)	CCTCGCCTTTGCCGATCCG	ATGCCGGAGCCGTTGTCG
actin(mmu)	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT
p65	CACCTCAATGGCTACACAGGACCA	ATCTTGAGCTCGGCAGTGTT
DICER(hsa)	TTAACCTTTTGGTGTTTGATGAGTGT	GCGAGGACATGATGGACAATT
DICER(mmu)	GGTCCTTTCTTTGGACTGCCA	GCGATGAACGTCTTCCCTGA
TNF-α(hsa)	GGACACCATGAGCACTGAAAGC	TGCCACGATCAGGAAGGAGAAG
TNF-α(mmu)	CTATGGCCCAGACCCTCACA	TTGAGATCCATGCCGTTGG
IL-6(hsa)	TTCGGTCCAGTTGCCTTCTC	GAGGTGAGTGGCTGTCTGTG
IL-8(hsa)	CGCCTTTACAATAATTTCTGTGTTGGCG	CTTGGCAGCCTTCCTGATTTCT
miR-125b	GCCCTCCCTGAGACCTCAA	GTGCAGGGTCGGAGGT
Let-7a	TCGGCAGGTGAGGTAGTAGG	
miR-130a	GCCGCCAGTGCAATGTTAAA	
pri-miR-125b	GAAGAATTCTACCGCATCAAACCA	CTGCAGACAATCAATAAGGTCCAA
Pri-miR-130a	TCACTATTAGGTACAGAGTAG	CCTCAAGCAGCATTACCATCA
pri-let7a-1	GCGCGAGGAAACCAGGAT	GCACCACAGGAAGGCTTTTTT
pri-let7a-2	GATTCCTTTTCACCATTCACCCTGGATGTT	TTTCTATCAGACCGCCTGGATGCAGACTTT

Table S2. List of antibodies used in this study.		
Antibody	Catalog number	Company
p65	Sc-7151	Santa Cruz
p50	Sc-7178	Santa Cruz
IκB-α	Sc-371	Santa Cruz
	9242	Cell Signaling
Rabbit IgG	Sc-2027	Santa Cruz
Drosha	Sc-33748	Santa Cruz
DICER	Sc-30226	Santa Cruz
	13D6	Clonegene
α-tubulin	T6074	Sigma
anti-rabbit secondary antibodies	NA934	Amersham Biosciences
anti-mouse secondary antibodies	NA931	Amersham Biosciences

Supplementary Figure Legends

Figure S1 Identification of *DICER* promoter

(A) Annotation of the putative *DICER* promoter sequence. The predicted consensus κB site was shadowed. (B) HEK293 cells were transfected with the proximal promoter construct pGL3 (-450/-180) (C) and subsequently treated with APDC for 8h, or transfected with mκB-pGL3 (-450/-180) (MUT) and luciferase activity was determined. *P<0.05, **P<0.01 vs C. (C) Verification of the specificity of p65 siRNA by Western blotting. HEK293 cells were transfected for 72h with N.S., Scr, and p65-si1 or cotransfected with p65-si1 and p65-GFP or mutant form of p65 (p65 (m)) that was not recognized by p65-si1 and harvested for immunoblot analysis using the indicated antibodies. One representative blot of three independent experiments was shown. Decrease in IκB-α protein levels by p65-si1 was rescued by reconstitution with p65 (m). (D) Overexpression of p50 did not affect *DICER* promoter activity. HEK293 cells were cotransfected with pGL3 (-450/-180) and empty vectors (Ctrl) or p50 constructs (p50) for 48h and then subjected to the luciferase assay. (E) EMSAs were performed with HeLa cell nuclear extracts using DICER κB probe. Competition was done with the NF-κB consensus probe at 50- or 100-fold excess. (F) HeLa cells treated with TNF-α (10 ng/ml) for the indicated times were analyzed by EMSAs using the labeled DICER κB probe. (G) Chromatin complexes from Huh7 cells treated with saline or LPS at the indicated times precipitated by IgG, protein A beads (Ctrl), anti-p65 or anti-p50 antibodies, were assayed by PCR using the negative control primers spanning the region upstream of the DICER promoter. Data were representative of three independent experiments and normalized with the input DNA.

Figure S2 NF-κB/p65 regulation of DICER expression

(A) DICER mRNA levels in HEK293 cells transfected with GFP or p65-GFP for 48h were determined by qRT-PCR and normalized to GAPDH. Fold changes were shown relative to GFP-transfected cells, where DICER mRNA levels were set to 1. **P<0.01 vs GFP. (B) DICER and p65 mRNA levels were determined in HEK293 cells transfected with either N.S. or p65-si1 for 72h. Fold changes were shown relative to N.S., where DICER and p65 mRNA levels were set to 1. *P<0.05, **P<0.01 vs N.S. The dose (C) and time (D) effect of p65 expression on DICER expression assayed by immunoblot of the extracts isolated from HEK293 cells transfected with GFP or indicated amounts of p65-GFP for 48h or 0.1 μg p65-GFP for the indicated times using the

indicated antibodies. The effect of downregulation of p65 (E) and time-dependent downregulation of p65 (F) on DICER expression assayed by immunoblot of the extracts isolated from HEK293 cells transfected with N.S., scrambled (Scr), p65-si1 and p65-si2 for 72h or p65-si1 for the indicated times using the indicated antibodies. (G) HEK293 cells were transfected with empty vector (Ctrl) or p50 for 48h, or nonsense siRNA (N.S.) or p50-specific siRNAs (p50 si1 and p50 si2) for 72h (H) and harvested for immunoblot analysis using the indicated antibodies. For immunoblots, data were representative of three independent experiments, tubulin served as a loading control and quantitation normalized with the GFP- or N.S.-transfected cells was presented as means ± SEM of three independent experiments.

Figure S3 LPS did not affect the expression of Dicer in RAW264.7 cells. (A) RAW264.7 cells were stimulated with LPS (100 ng/ml) for the indicated times. Dicer and TNF-α mRNA levels were determined using qRT-PCR and normalized to actin mRNA levels. Data were means ± SEM of three independent experiments in triplicates. **P<0.01 vs 0h. (B) RAW264.7 cells were treated with LPS (100 ng/ml) for the indicated times and harvested for immunoblot using the indicated antibodies. Knockdown of DICER did not affect the NF-κB pathway. (C) Huh7 cells were transfected with N.S. or DICER-specific siRNAs (DICER si1 and DICER si2) for 72h and harvested for immunoblot analysis using the indicated antibodies. (D) Knockdown of DICER did not affect the activation of NF-κB signaling. Huh7 cells transfected with DICER RNAi treated with LPS (1 μg/ml) for the indicated times and harvested for immunoblot using the indicated antibodies. Tubulin served as a loading control.

Figure S4 Putative miRNAs targeting TNF-α.

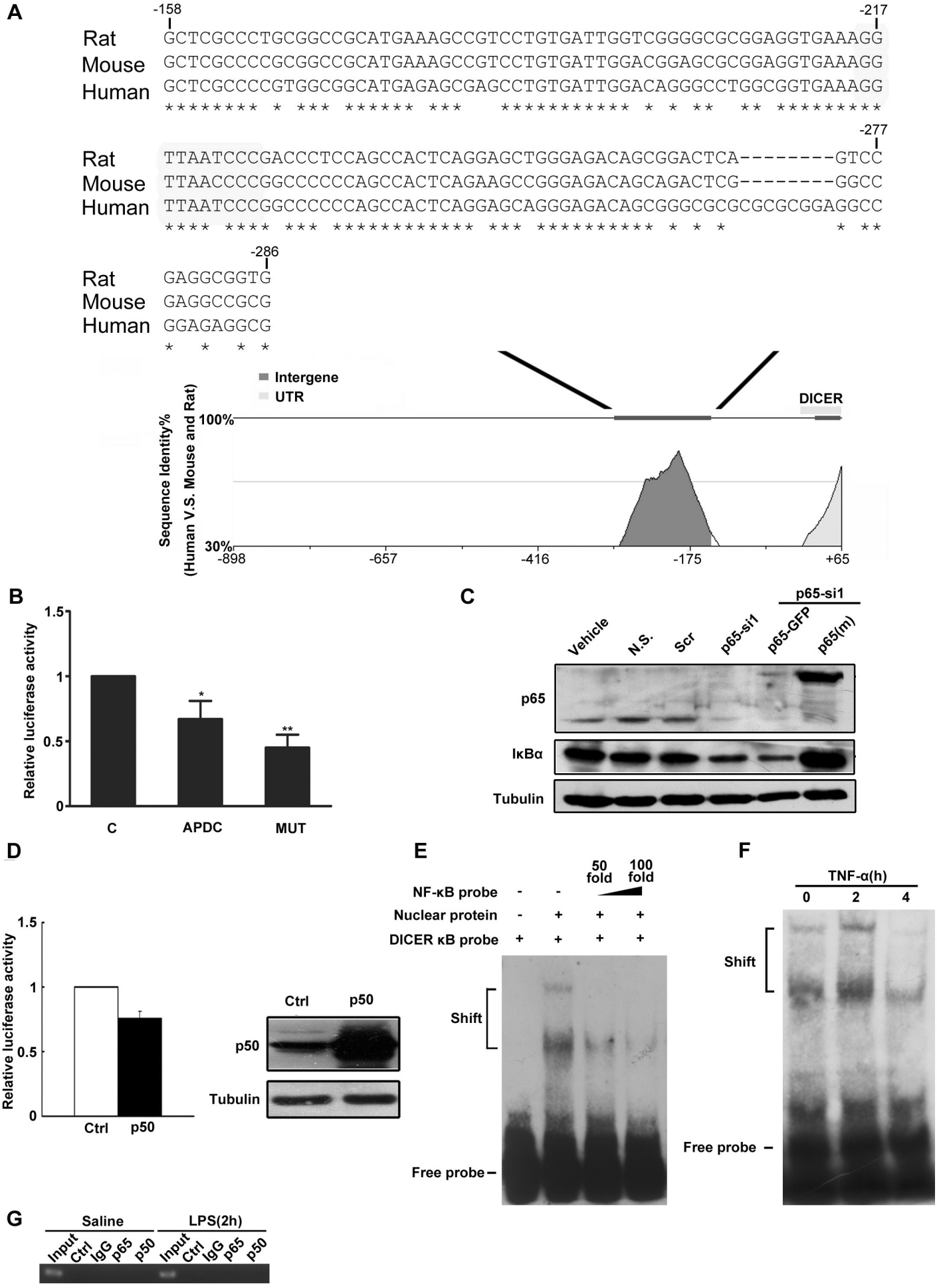
(A) Schematic diagram showed the putative binding sites of miR-16, miR-19, miR-125b, miR-130a and miR-181 in the 3′-UTR of TNF-α predicted by miRTarAS and TargetScan. (B) Huh7 cells were transfected with mimics and inhibitors of the indicated miRNAs for 72h, and the expression of TNF-α was determined. Data were means ± SEM of three independent experiments in triplicates.

Figure S5 Hepatocyte-specific deletion of Dicer.

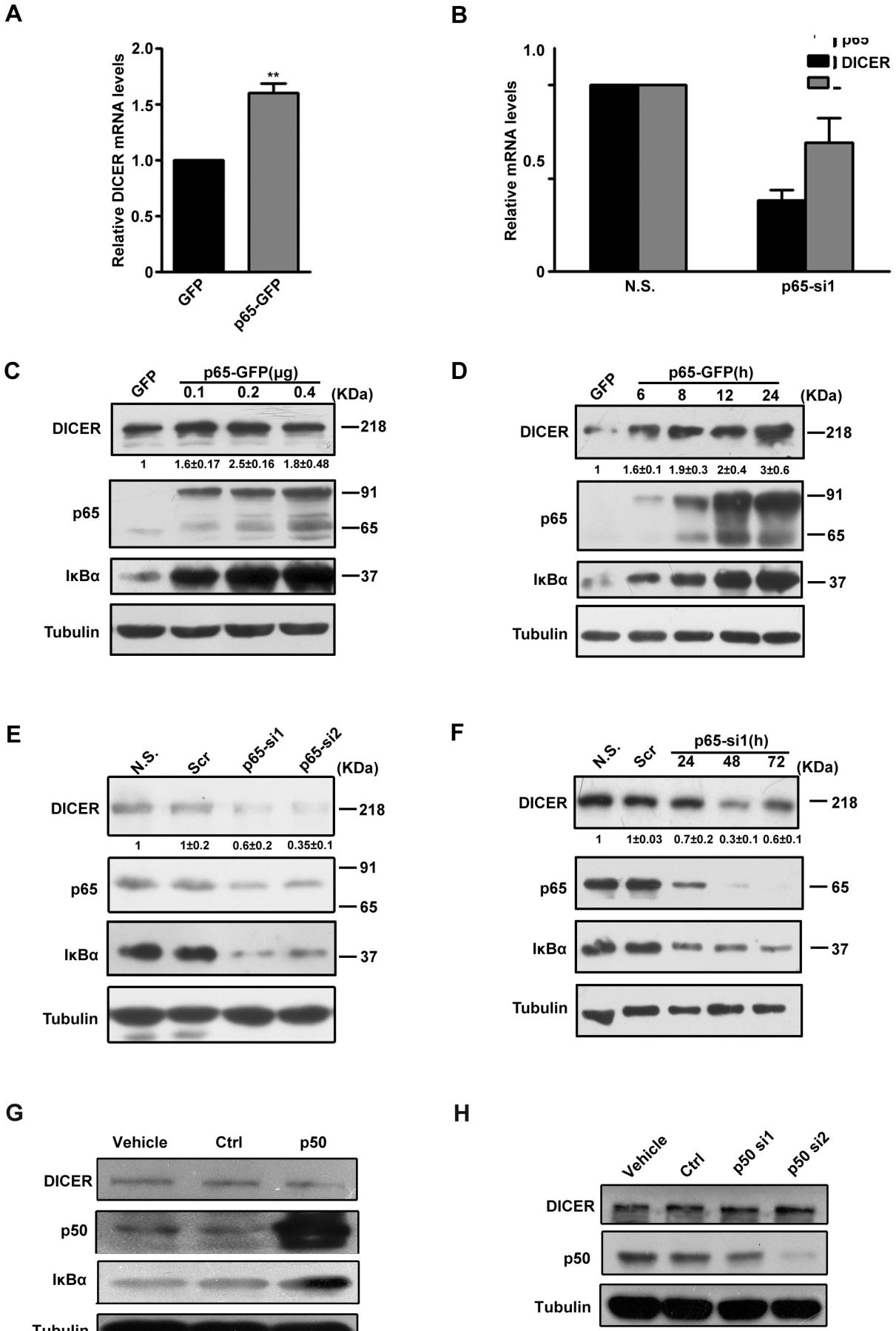
QRT-PCR analysis of Dicer expression in 12-week-old liver tissues from $Dicer^{F/F}$, $Dicer^{F/F}$AlbCre (A) and $Dicer^{F/F}$Mx1Cre (B) mice. *P<0.05, **P<0.01 vs $Dicer^{F/F}$. (C and D) qRT-PCR analysis of Dicer and miR-125b expression in $Dicer^{F/F}$, $Dicer^{F/+}$AlbCre and $Dicer^{F/F}$AlbCre mice. Each dot

represented measurement of an animal. Data were means ± SEM of 3 (for A and B) or 6 (for Cand D) animals in triplicates. *P<0.05 vs $DICER^{F/F}$. (E) $Dicer^{F/F}$, $Dicer^{F/+}$AlbCre and $Dicer^{F/F}$AlbCre mice were treated with LPS (10 μg/g) for 1h. Hepatic TNF-α mRNA levels were measured by qRT-PCR. Each dot represented measurement of an animal. Data were means ± SEM of 5-11 animals in triplicates. (F) The amount of serum TNF-α was measured by ELISA. Data were means ± SEM of five animals in triplicates. *P<0.05, **P<0.01 vs $Dicer^{F/F}$.

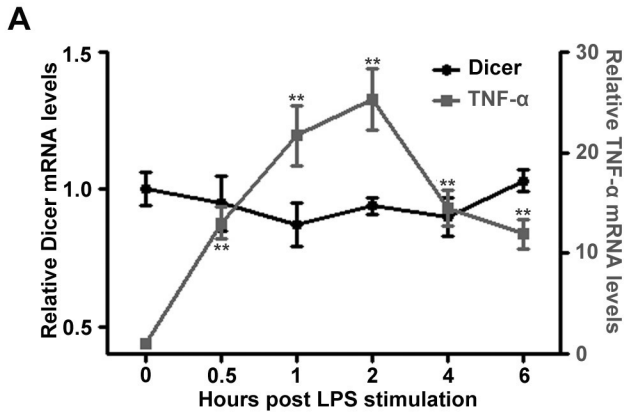
Figure S6 (A) F4/80 mRNA levels were determined using qRT-PCR in the livers of mice treated with PBS or liposome-encapsulated clodronate (Liposome) for 48h. Each dot represented the measurement of an animal. Horizontal bars indicated means ± SEM (n=8). **P<0.01 vs PBS. (B) TNF-α levels in primary-cultured hepatocytes isolated from $Dicer^{F/F}$AlbCre or $Dicer^{F/F}$ mice stimulated with LPS for 12h. Data were the means ± SEM (n=5). *P<0.05, **P<0.01 vs $Dicer^{F/F}$. (C) Peritoneal macrophages from $Dicer^{F/F}$ and $Dicer^{F/F}$AlbCre mice were treated with LPS (100ng/ml) for 6h. The TNF-α level was measured by ELISA. Data were means ± SEM of five animals in triplicates. Serum transaminase activity in $Dicer^{F/F}$ and $Dicer^{F/F}$AlbCre mice (D) and $Dicer^{F/F}$Mx1Cre mice (E) after treatment 6h with LPS (10 μg/g) was measured. Data were means ± SEM of five animals in triplicates (n=5).

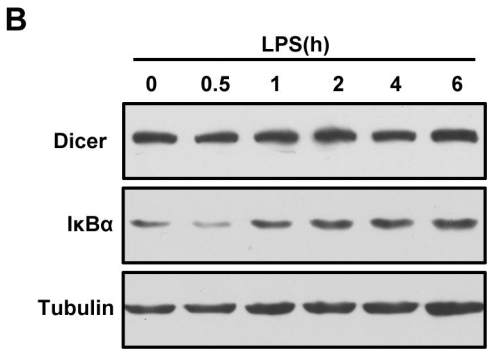


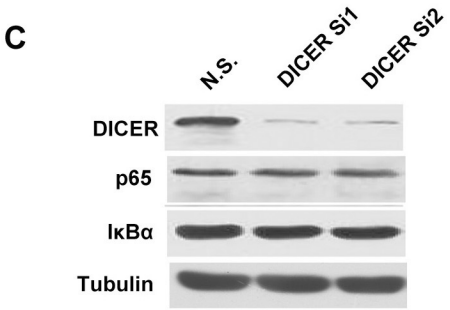
Supplementary Figure 1

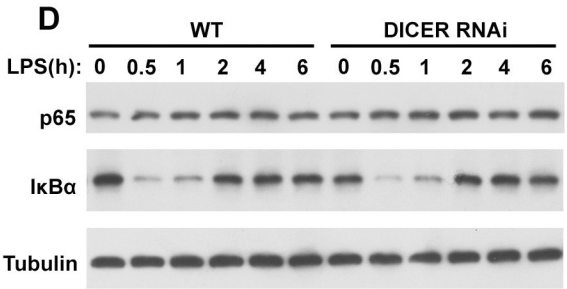


Supplementary Figure 2



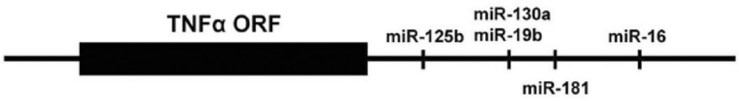






Supplementary Figure 3

A



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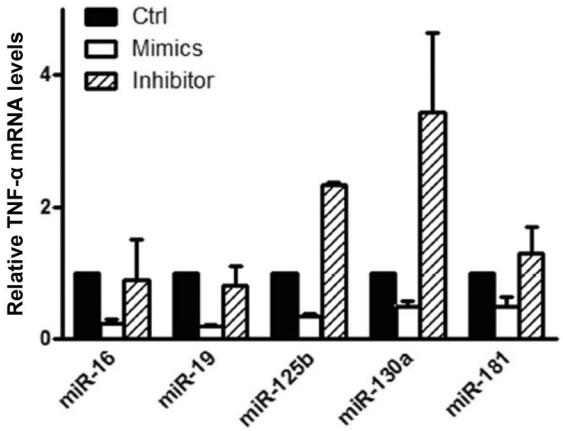
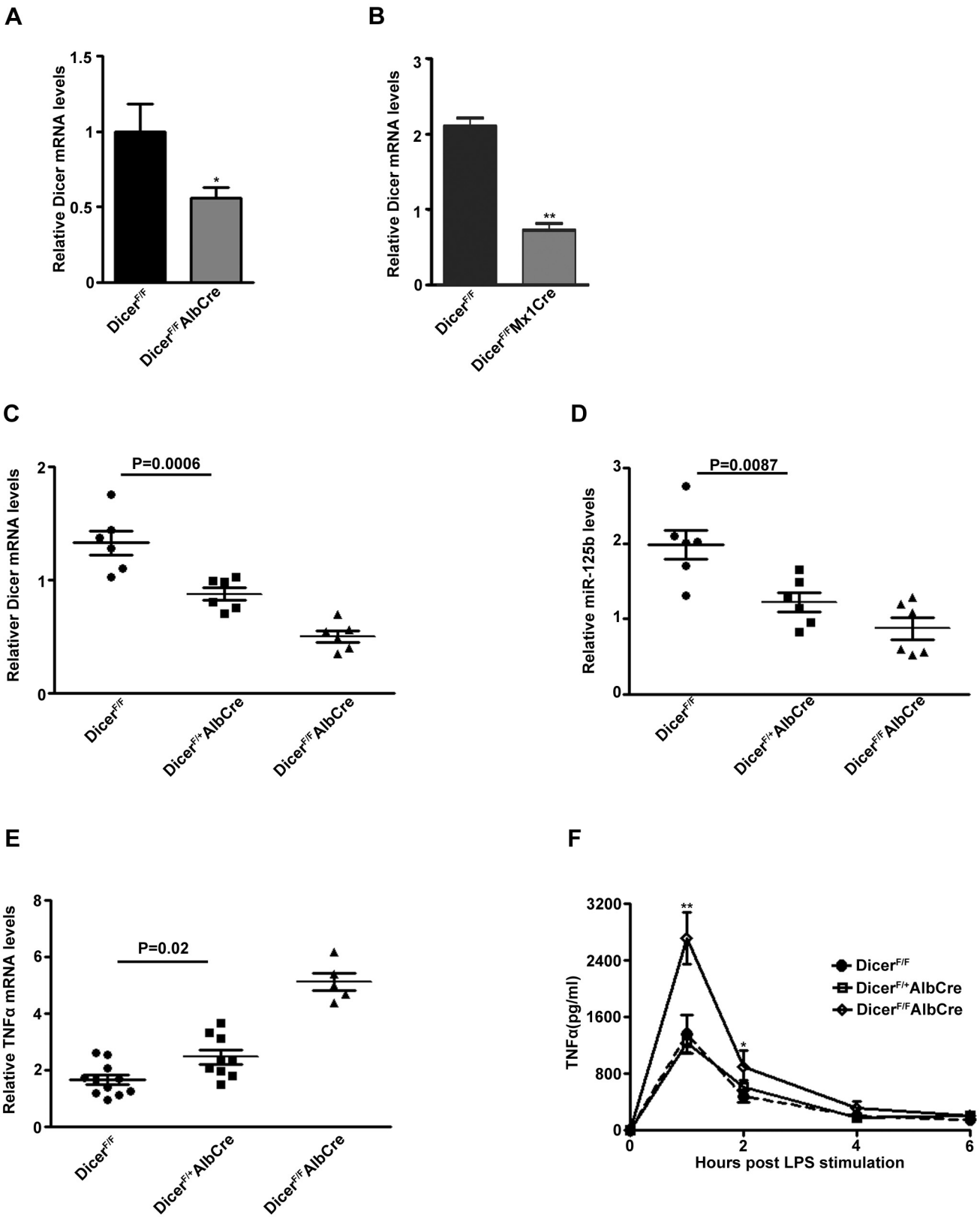
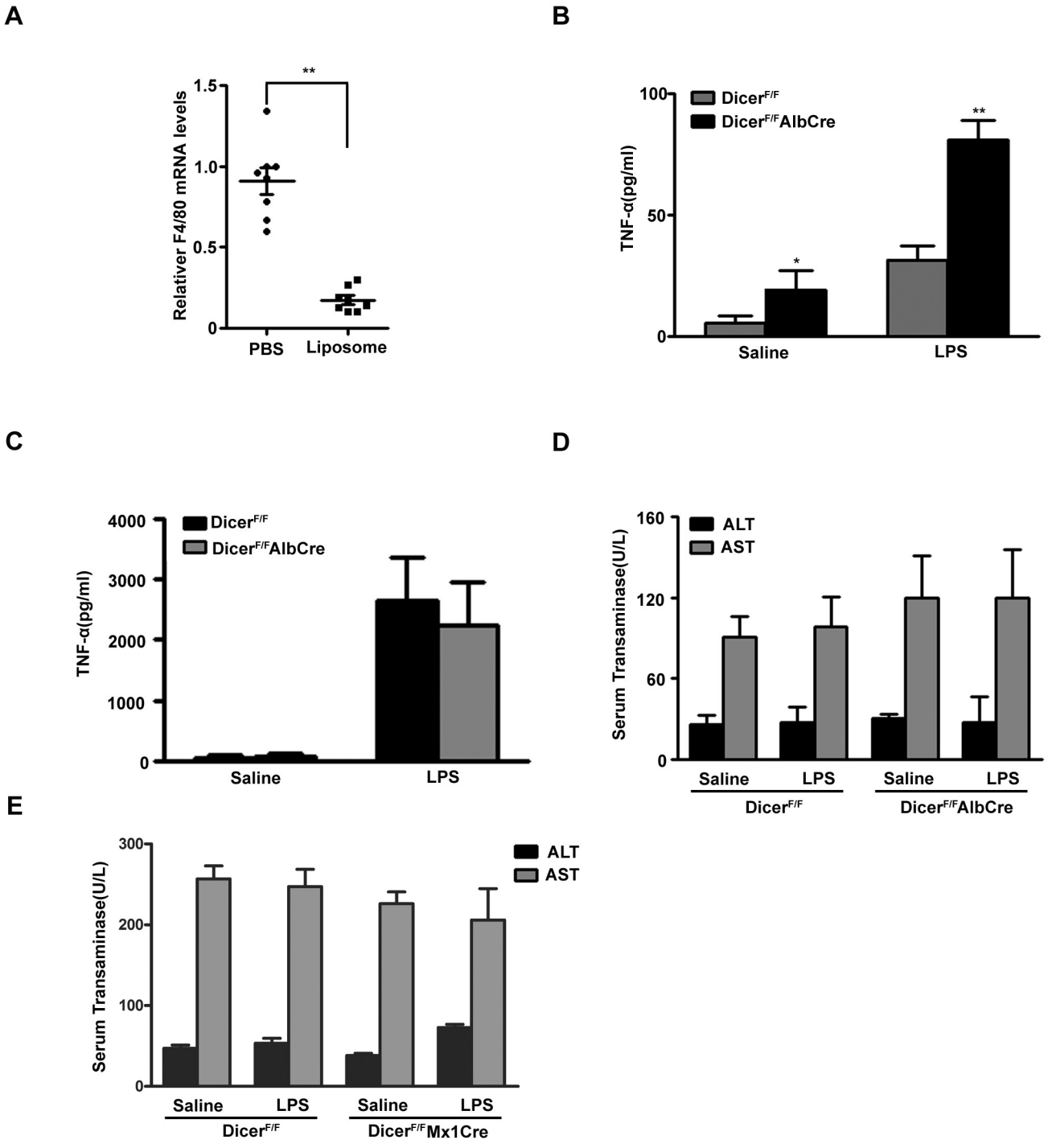


Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6