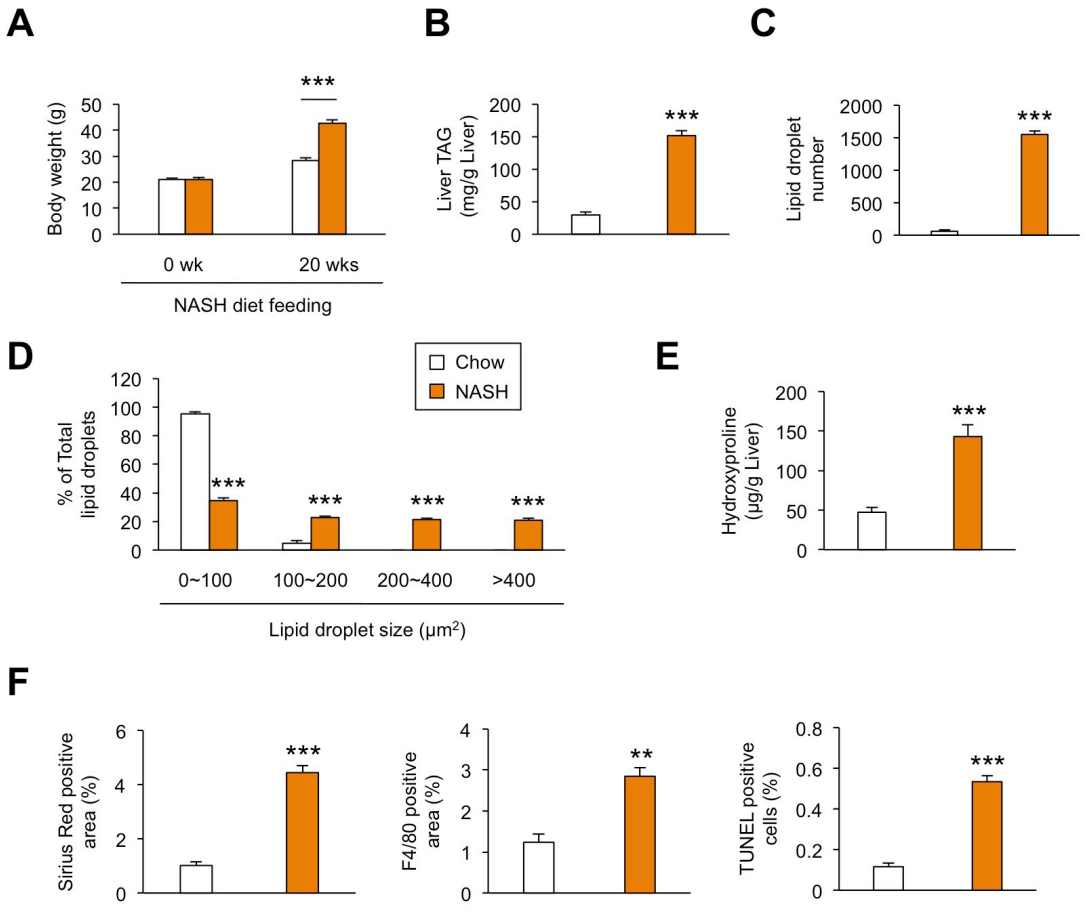


Supplemental Figure 1. Diet-induced NASH pathologies in mice.

The following parameters were measured in male C57BL/6J WT male mice fed chow (open, n=4) or NASH diet (orange, n=4) for 20 weeks, starting at 3 months of age. Data represent mean ± SEM. **p < 0.01, ***p < 0.001; two-tailed unpaired Student's t test.

(A) Body weight before and after 20 weeks of chow or NASH diet feeding.

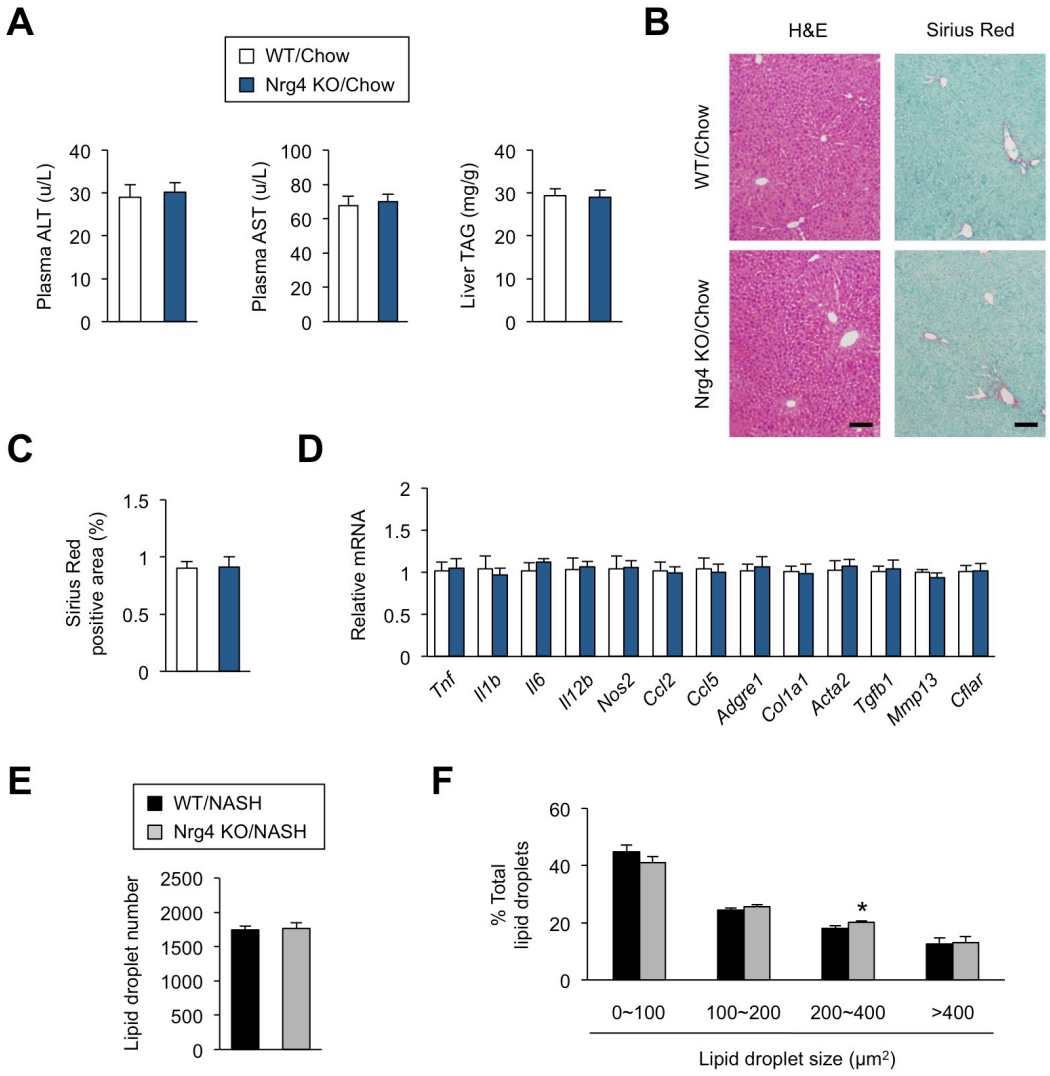
(B) Liver TAG content.

(C) Quantification of lipid droplet numbers based on the liver H&E staining images.

(D) Lipid droplet size analysis based on the liver H&E staining images.

(E) Liver hydroxyproline content.

(F) Quantification of Sirius Red, F4/80, and TUNEL staining images in Figure 1C.



Supplemental Figure 2. Hepatic and plasma parameters in WT and Nrg4 KO mice fed chow or NASH diet.

The parameters from (A) to (D) were measured in WT (open, n=5) and Nrg4 KO (blue, n=6) male mice fed standard chow. Mice were sacrificed for analysis at 8 months of age. Data represent mean ± SEM. Two-tailed unpaired Student's t test.

(A) Plasma ALT and AST levels and liver TAG content.

(B) H&E and Sirius Red staining of liver sections (bar. 100 μm).

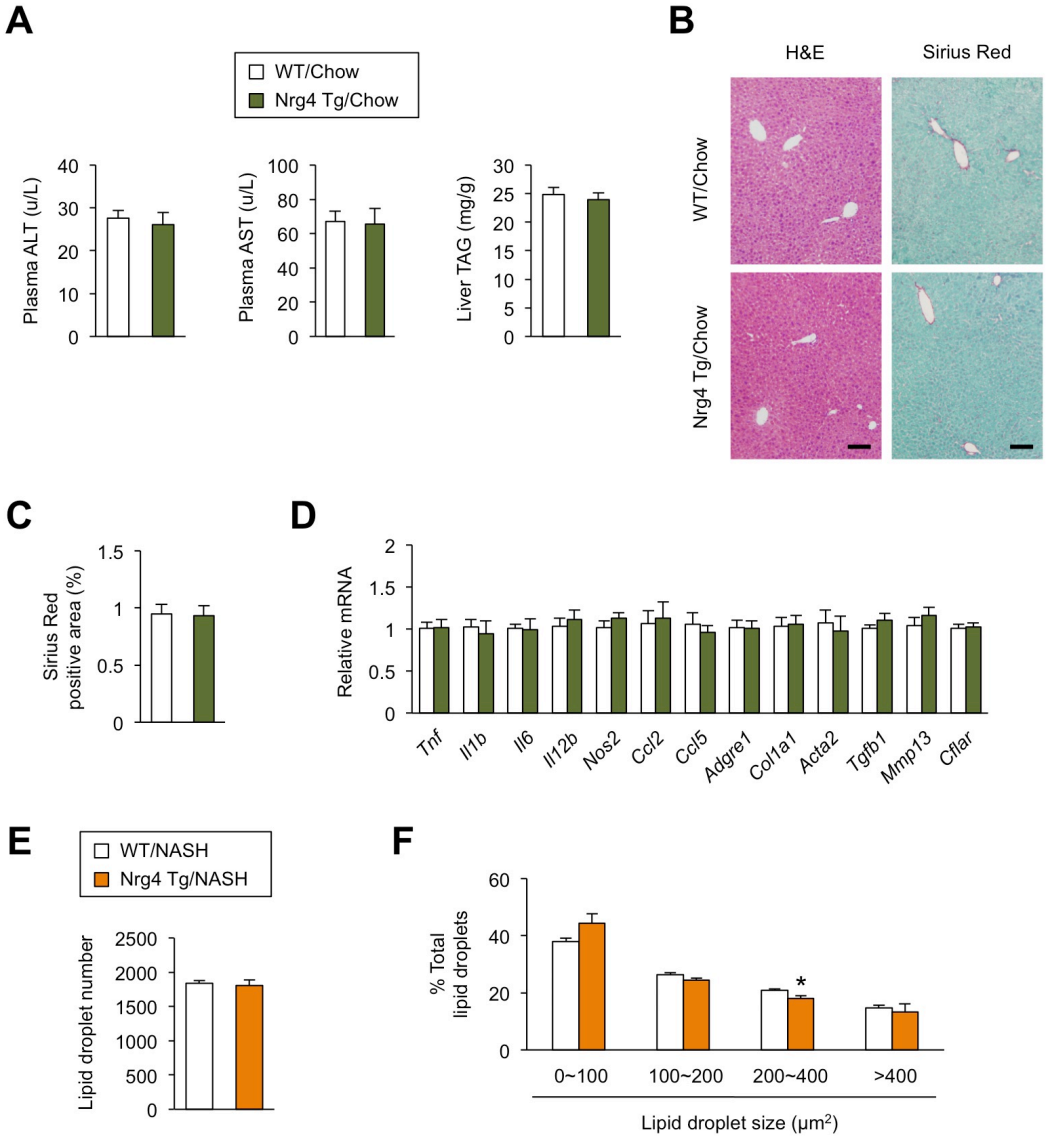
(C) Quantification of the Sirius Red staining images.

(D) qPCR analysis of hepatic gene expression.

The parameters from (E) to (F) were measured in mice fed NASH diet as described in Figure 2. Data represent mean ± SEM. *p < 0.05; two-tailed unpaired Student's t test.

(E) Quantification of lipid droplet number based on the liver H&E staining images.

(F) Lipid droplet size analysis based on the liver H&E staining images.



Supplemental Figure 3. Hepatic and plasma parameters in WT and Nrg4 Tg mice fed chow or NASH diet.

The parameters from (A) to (D) were measured in WT (open, n=7) and Nrg4 Tg (green, n=5) male mice fed standard chow. Mice were sacrificed for analysis at 8 months of age. Data represent mean ± SEM. Two-tailed unpaired Student's t test.

(A) Plasma ALT, AST levels and liver TAG content.

(B) H&E and Sirius Red staining of liver sections (bar. 100 μm).

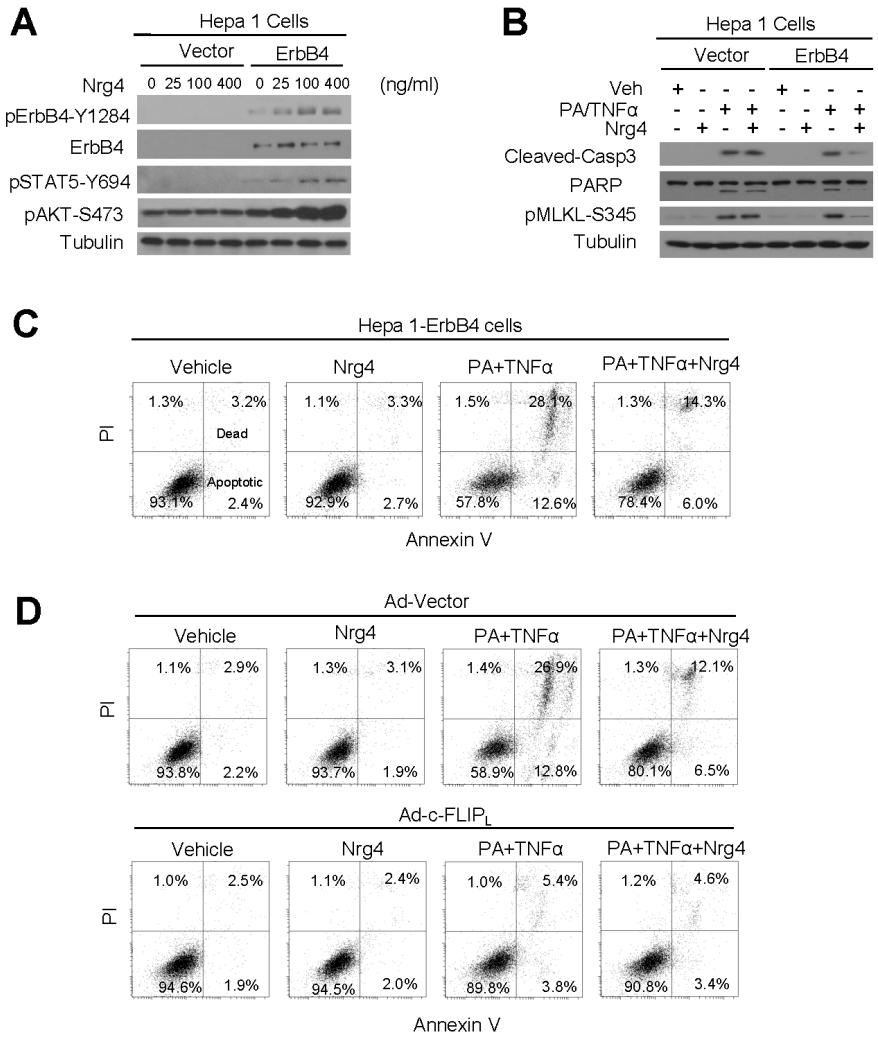
(C) Quantification of the Sirius Red staining images.

(D) qPCR analysis of hepatic gene expression.

The parameters from (E) to (F) were measured in mice fed NASH diet as described in Figure 3. Data represent mean ± SEM. *$p < 0.05$; two-tailed unpaired Student's t test.

(E) Quantification of lipid droplet number based on the liver H&E staining images.

(F) Lipid droplet size analysis based on the liver H&E staining images.



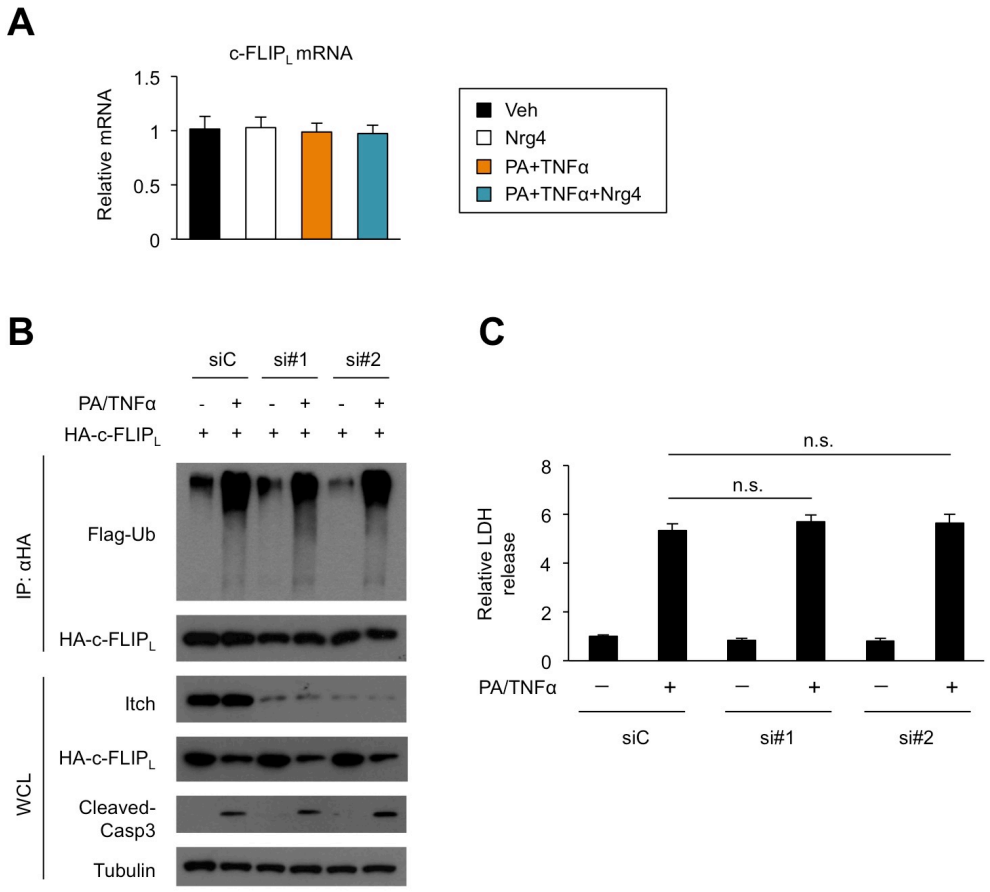
Supplemental Figure 4. Nrg4 suppresses hepatocyte cell death through c-FLIP$_L$ in Hepa 1 cells.

(A) Immunoblots of Hepa 1 cells stably expressing vector or ErbB4 and treated for 20 min with different concentrations of recombinant Nrg4.

(B) Immunoblots of total lysates from Hepa 1 cells stably expressing vector or ErbB4 and treated with 100 μM palmitic acid (PA) for 2 hrs followed by addition of 20 ng/ml TNFα and 100 ng/ml Nrg4 for 20 hrs.

(C) Representative flow cytometry plots for Figure 4G.

(D) Representative flow cytometry plots for Figure 5F.



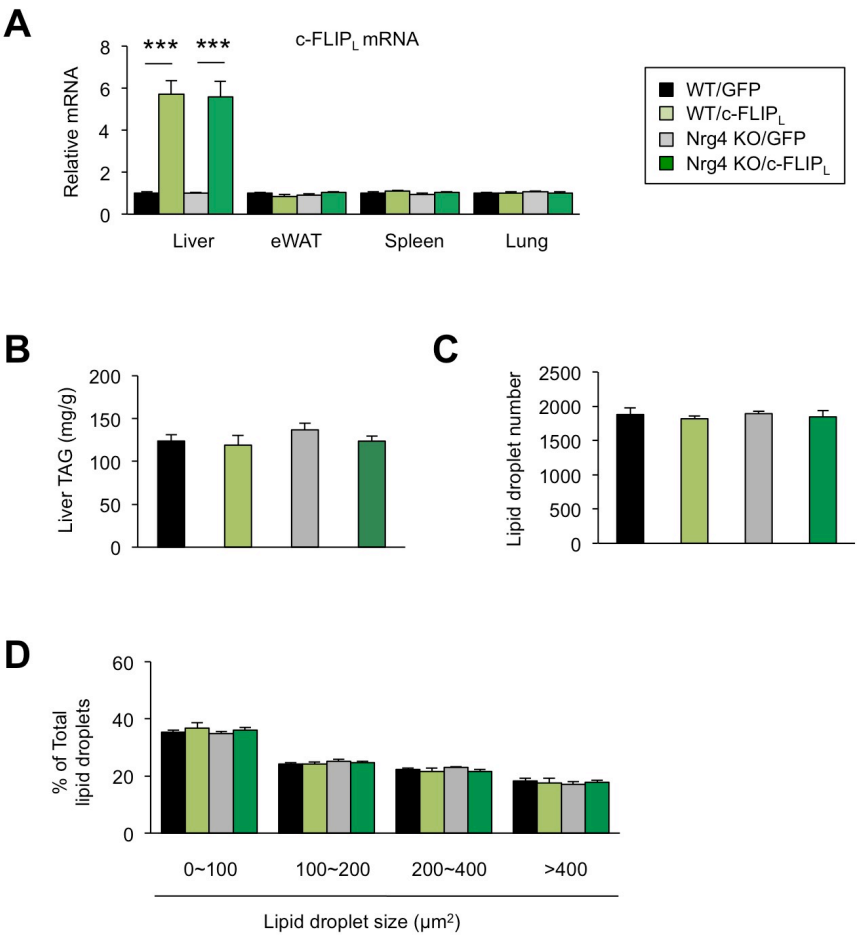
Supplemental Figure 5. c-FLIP$_L$ mRNA expression and siRNA knockdown of Itch.

(A) qPCR analysis of c-FLIP$_L$ mRNA expression. Hepa 1 cells stably expressing ErbB4 were treated with PA+TNFα in the absence or presence of 100 ng/ml Nrg4 for 6 hrs. Data represent mean ± SEM. one-way ANOVA.

The following experiments were performed in Hepa 1 cells. Cells were cultured in serum-free medium during treatment. WCL: whole cell lysates.

(B) Immunoblots of IP and WCL from Hepa 1 cells transfected with control siRNA (siC) or Itch siRNAs (si#1 and si#2) along with plasmids encoding HA-tagged c-FLIP$_L$ and Flag-tagged ubiquitin (Flag-Ub). Transfected cells were treated with or without PA/TNFα for 4 hrs.

(C) LDH release by Hepa 1 cells treated with or without PA/TNFα for 20 hrs. Data represent mean ± SEM. n.s.= no significant difference; one-way ANOVA.



Supplemental Figure 6. qPCR analysis of c-FLIP$_L$ mRNA expression and liver lipid droplet analysis.

The following experiments correspond to AAV-transduced mice described in Figure 7 and 8. Data represent mean ± SEM. ***$p < 0.001$, n.s.= no significant difference; one-way ANOVA.

(A) qPCR analysis of c-FLIP$_L$ mRNA expression.

(B) Liver TAG content.

(C) Quantification of lipid droplet number based on the liver H&E staining images.

(D) Lipid droplet size analysis based on the liver H&E staining images.

Supplemental Table 1. List of qPCR primers.

Human genes		
Gene name	**Forward primer**	**Reverse primer**
Il1b	TTCGACACATGGGATAACGAGG	TTTTTGCTGTGAGTCCCGGAG
Tnf	GAGGCCAAGCCCTGGTATG	CGGGCCGATTGATCTCAGC
Nos2	AGGGACAAGCCTACCCCTC	CTCATCTCCCGTCAGTTGGT
Col1a1	GTGCGATGACGTGATCTGTGA	CGGTGGTTTCTTGGTCGGT
Acta2	TGCCAACAACGTCATGTCG	CAGCGCGGTGATCTCTTTCT
Tgfb1	CAATTCCTGGCGATACCTCAG	GCACAACTCCGGTGACATCAA
36B4	AGGCGTCCTCGTGGAAGTGA	GCGGATCTGCTGCATCTGCT
Mouse genes		
Gene name	**Forward primer**	**Reverse primer**
Tnf	AGCCCCCAGTCTGTATCCTT	CTCCCTTTGCAGAACTCAGG
Il1b	TGGCAACTGTTCCTGAACTCAA	AGCAGCCCTTCATCTTTTGG
Il6	AGTTGCCTTCTTGGGACTGA	TCCACGATTTCCCAGAGAAC
Il12b	CCAGAGACATGGAGTCATAG	AGATGTGAGTGGCTCAGAGT
Nos2	GAGGCCCAGGAGGAGAGAGATCCG	TCCATGCAGACAACCTTGGTGTTG
Ccl2	AGGTCCCTGTCATGCTTCTG	TCTGGACCCATTCCTTCTTG
Ccl5	TGCCCACGTCAAGGAGTATTT	TTCTCTGGGTTGGCACACACT
Adgre1	ATCCTTGGCCATCCGGCAGA	GCAAAGCCAGGGTGGCAAGT
Col1a1	AAGAGGCGAGAGAGGTTTCC	AGAACCATCAGCACCTTTGG
Acta2	CTGACAGAGGCACCACTGAA	CATCTCCAGAGTCCAGCACA
Tgfb1	ACCATGCCAACTTCTGTCTGGGAC	ACAACTGCTCCACCTTGGGCTTG
Mmp13	TGCTTCCTGATGATGACGTTCAAGG	TGGGATGCTTAGGGTTGGGGTC
CFLAR	GCTCCAGAATGGGCGAAGTAA	ACGGATGTGCGGAGGTAAAAA
Nrg4	CCCAGCCCATTCTGTAGGTG	ACCACGAAAGCTGCCGATAG
36B4	GAAACTGCTGCCTCACATCCG	GCTGGCACAGTGACCTCACACG