

Long Non-Coding RNAs Involved in Progression of non-Alcoholic Fatty Liver  
Disease to Steatohepatitis

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**Table S1. Plasma and clinical parameters of the MORE study population**

|                            | Number of individuals scored | Grade (number of individuals) |     |    |     |    |     |   |     |    |     |   |   |   |   |
|----------------------------|------------------------------|-------------------------------|-----|----|-----|----|-----|---|-----|----|-----|---|---|---|---|
|                            |                              | 0                             | 0,5 | 1  | 1,5 | 2  | 2,5 | 3 | 3,5 | 4  | 4,5 | 5 | 6 | 7 | 8 |
| Steatosis (0-3)            | 60                           | 16                            | 0   | 15 | 3   | 18 | /   | 8 | /   | /  | /   | / | / | / | / |
| Fibrosis (0-4)             | 58                           | 40                            | 0   | 9  | 0   | 5  | 1   | 2 | 1   | /  | /   | / | / | / | / |
| Ballooning (0-2)           | 60                           | 32                            | 1   | 24 | 0   | 3  | /   | 0 | /   | /  | /   | / | / | / | / |
| Lobular inflammation (0-3) | 60                           | 23                            | 2   | 23 | 0   | 7  | /   | 5 | /   | /  | /   | / | / | / | / |
| NASH grade (0-3)           | 60                           | 24                            | 0   | 24 | 0   | 8  | /   | 4 | /   | /  | /   | / | / | / | / |
| NASH stage (0-3)           | 59                           | 39                            | 0   | 12 | 0   | 4  | /   | 4 | /   | /  | /   | / | / | / | / |
| NAS score (0-8)            | 60                           | 16                            | 0   | 2  | 1   | 7  | 0   | 9 | 2   | 14 | 1   | 3 | 1 | 2 | 2 |

|                       | Number of individuals scored | No | Yes |
|-----------------------|------------------------------|----|-----|
| Microgranulomas       | 59                           | 53 | 6   |
| Large lipogranulomas  | 59                           | 42 | 17  |
| Portal inflammation   | 60                           | 46 | 14  |
| Acidofilic bodies     | 60                           | 54 | 6   |
| Pigmented macrophages | 60                           | 52 | 8   |
| Glucogenated nuclei   | 57                           | 48 | 9   |

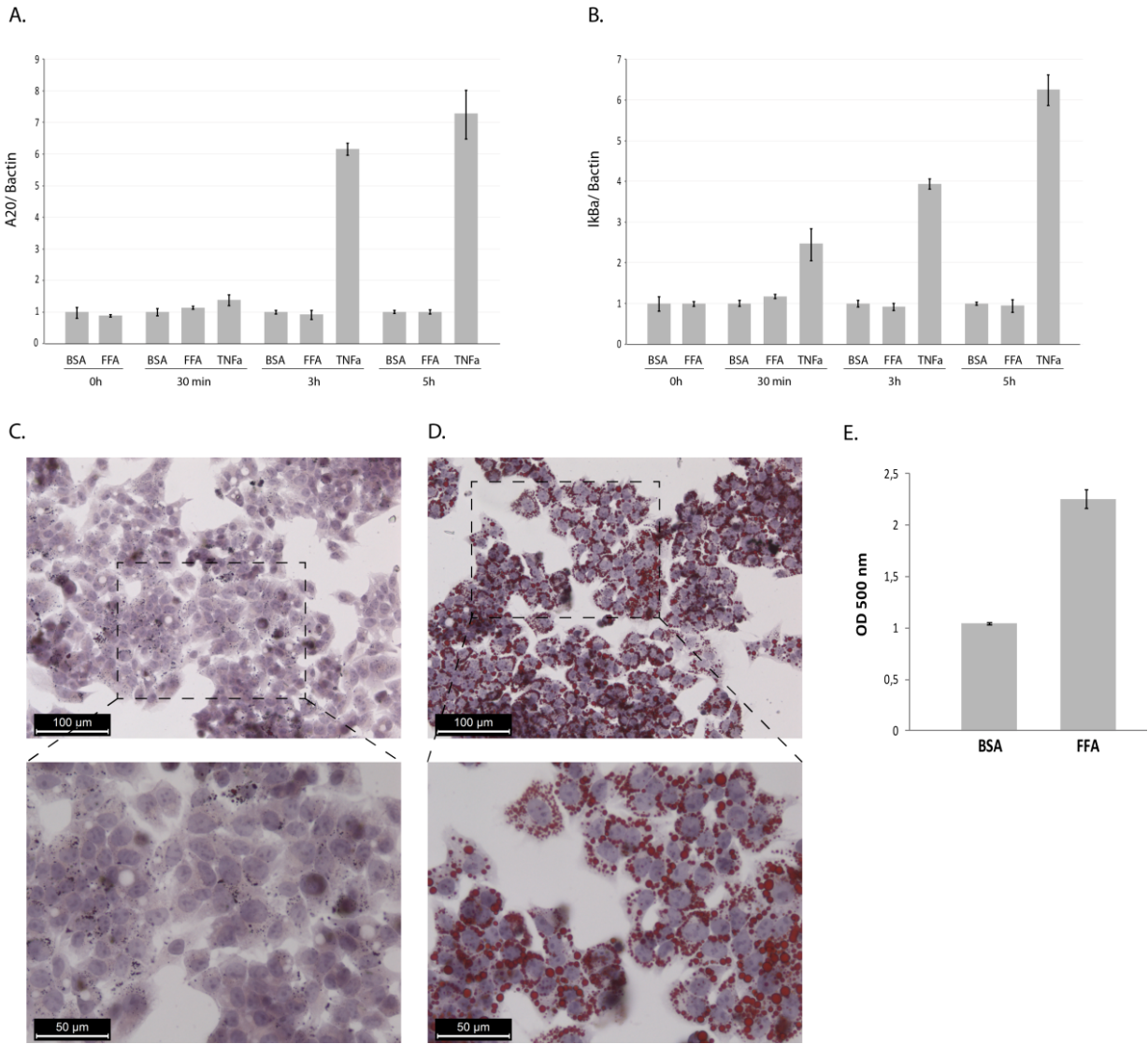
|                            | All individuals |           | Normal      |           | NAFLD       |           | NASH        |           |
|----------------------------|-----------------|-----------|-------------|-----------|-------------|-----------|-------------|-----------|
| Number of individuals      | 60              |           | 16          |           | 8           |           | 36          |           |
| Males/ Females             | 14/46           |           | 3/13        |           | 1/7         |           | 10/26       |           |
|                            | <u>Mean</u>     | <u>SD</u> | <u>Mean</u> | <u>SD</u> | <u>Mean</u> | <u>SD</u> | <u>Mean</u> | <u>SD</u> |
| Age (years)                | 44,05           | 9,21      | 41,31       | 8,68      | 43,00       | 7,23      | 45,50       | 9,72      |
| BMI (kg/m <sup>2</sup> )   | 46,42           | 10,09     | 42,50       | 6,27      | 45,06       | 7,02      | 48,46       | 11,54     |
| Waist/hip_ratio            | 1,00            | 0,12      | 0,96        | 0,11      | 1,02        | 0,12      | 1,02        | 0,12      |
| Glucose (mmol/L)           | 6,49            | 2,23      | 5,70        | 1,27      | 5,46        | 0,63      | 7,10        | 2,62      |
| Insulin (mU/L)             | 17,64           | 9,81      | 12,94       | 6,44      | 21,13       | 6,58      | 19,06       | 11,10     |
| HOMA-IR (mmol/L)           | 5,00            | 3,11      | 3,22        | 1,59      | 5,12        | 1,56      | 5,84        | 3,60      |
| HbA1c (mmol/L)             | 6,44            | 1,33      | 6,03        | 0,85      | 6,14        | 0,57      | 6,72        | 1,58      |
| Total Cholesterol (mmol/L) | 5,10            | 1,12      | 4,91        | 0,82      | 5,35        | 0,80      | 5,13        | 1,32      |
| HDL (mmol/L)               | 1,00            | 0,35      | 1,13        | 0,35      | 0,99        | 0,22      | 0,95        | 0,36      |
| LDL (mmol/L)               | 3,21            | 0,99      | 3,04        | 0,80      | 3,39        | 0,96      | 3,25        | 1,10      |
| TG (mmol/L)                | 2,29            | 2,23      | 1,65        | 0,77      | 2,12        | 0,90      | 2,67        | 2,84      |
| FFA (mmol/L)               | 0,77            | 0,86      | 0,47        | 0,24      | 0,68        | 0,22      | 0,96        | 1,09      |
| ALAT (U/L)                 | 24,78           | 9,72      | 19,88       | 4,87      | 28,00       | 8,82      | 26,32       | 10,93     |
| ASAT (U/L)                 | 23,40           | 12,13     | 13,88       | 5,16      | 26,00       | 11,49     | 27,26       | 12,38     |
| CRP (mg/L)                 | 9,82            | 8,24      | 7,62        | 6,62      | 11,95       | 12,11     | 10,29       | 7,87      |

**Table S2. Primer sequences**

| <b>Primer Name</b>       | <b>5'-3' primer sequence</b> |
|--------------------------|------------------------------|
| Bactin For               | AGCCTCGCCTTTGCCGA            |
| Bactin Rev               | GCGCGGCGATATCATCATC          |
| RP11-91K9.1 (lncTNF) For | CCCTGTGTGCTGGGATTAAA         |
| RP11-91K9.1 (lncTNF) Rev | TCCATATCAAGTGCATCAAGAA       |
| A20 For                  | CACTGTGTTTCATCGAGTACAG       |
| A20 Rev                  | GCAGTATCCTTCAAACATGGTG       |
| IKBA For                 | CTGGGCCAGCTGACACTAG          |
| IKBA Rev                 | AGTCATCATAGGGCAGCTCG         |
| U3 snoRNA For            | AACCCCGAGGAAGAGAGGTA         |
| U3 snoRNA Rev            | CACTCCCCAATACGGAGAGA         |
| DANCR For                | CGTCTCTTACGTCTGCGGAA         |
| DANCR Rev                | TGGCTTGTGCCTGTAGTTGT         |
| MALAT For                | GTGCTACACAGAAGTGGATTC        |
| MALAT Rev                | CCTCAGTCCTAGCTTCATCA         |
| NEAT For                 | CCTCCCTTTAACTTATCCATTAC      |
| NEAT Rev                 | TCTCTTCCTCCACCATTACCA        |
| DANCR For                | AGGAGTTCGTCTCTTACGTCT        |
| DANCR Rev                | TGAAATACCAGCAACAGGACA        |
| OIP5-AS1 For             | TGCGAAGAGACCACCAAA           |
| OIP5-AS1 Rev             | AGACAACATAGAGACACTTGCC       |

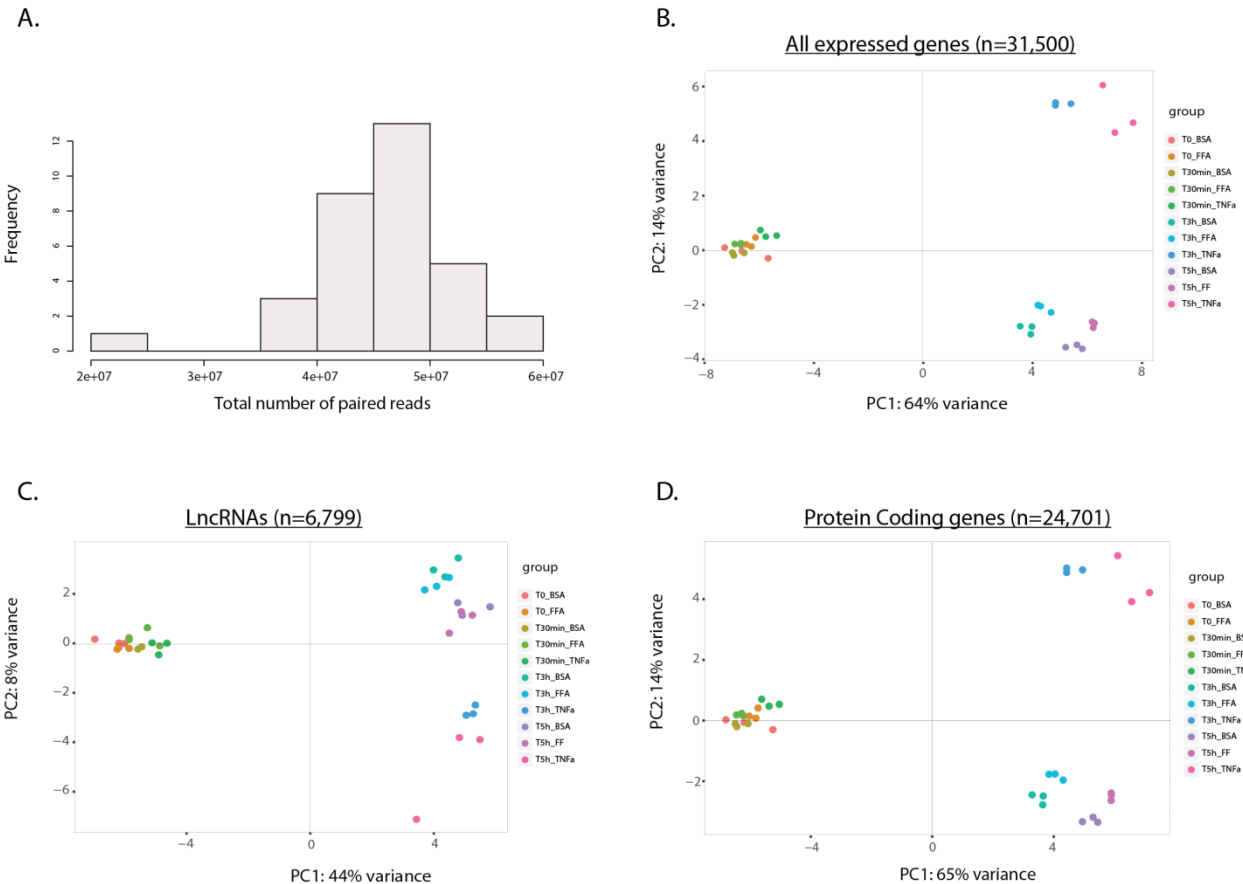
**Figure S1. Induction of inflammation and steatosis in HepG2 cells.**

**A.** qRT-PCR analysis assessing gene expression of A20 and **B.**  $\text{I}\kappa\text{B}\alpha$  genes to confirm successful  $\text{TNF}\alpha$  treatment. **C.** **D.** Microscopy images to confirm induction of cellular steatosis by performing Oil Red O staining on HepG2 cells (BSA treated cells in **C** and FFA treated cells in **D**). Upper figures represent 20x magnification and lower 40x magnification. **E.** Oil Red O extraction from lipid droplets of BSA (control) and FFA treated cells, measured by OD assessment on ELISA plate reader at 500 nm.

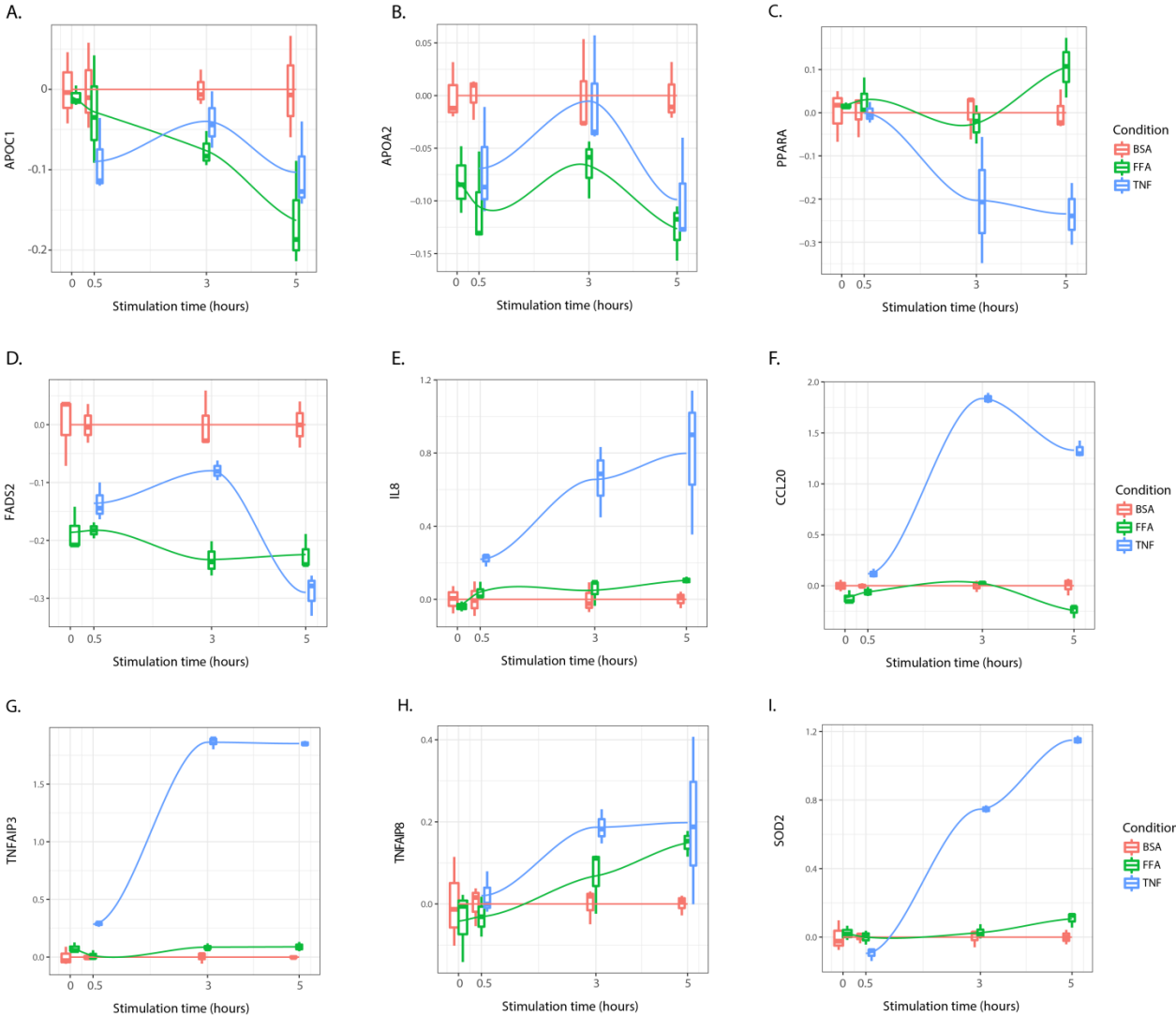


**Figure S2. RNA sequencing reads distribution and PC analysis.**

**A.** Distribution of total number of mapped sequencing reads from all 33 samples. **B.** Principal component analysis (PC1 on X-axis and PC2 on Y-axis) on all expressed genes; **C.** on lncRNAs alone and **D.** on protein coding gene alone.

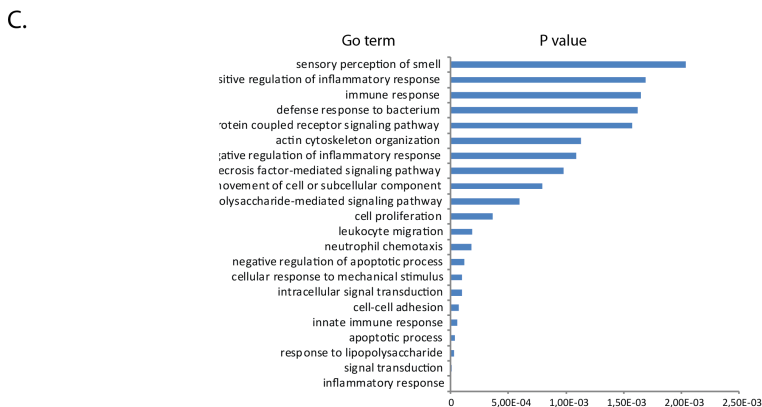
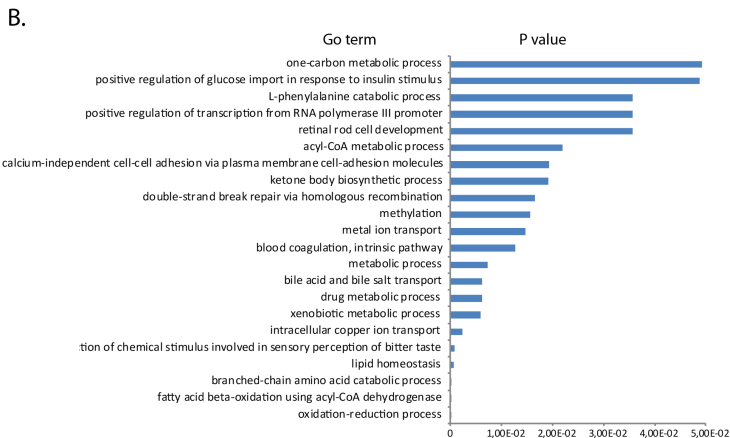
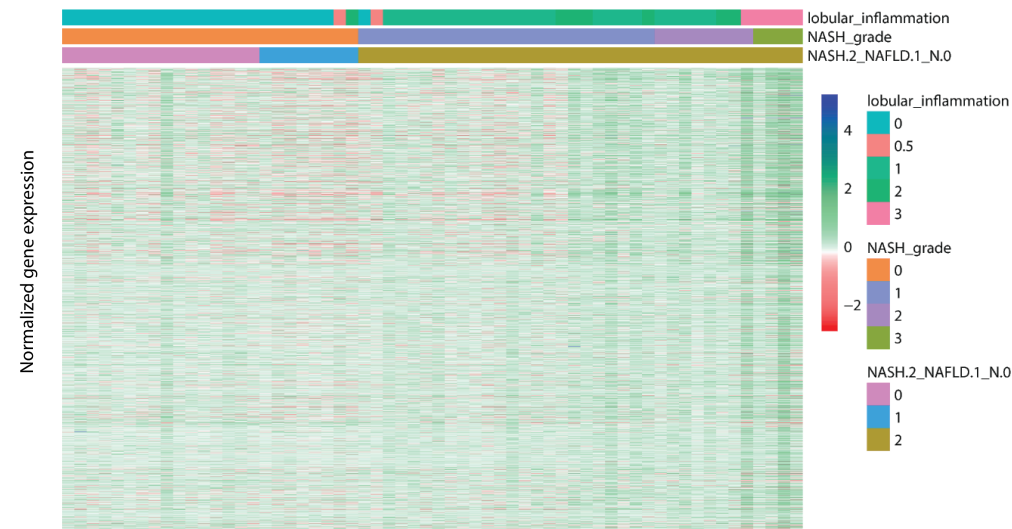


**Figure S3. Normalized expression levels of known NASH dis-regulated genes in the HepG2 dataset.**  
X-axis represents normalized gene expression level for the indicated gene and Y-axis represents duration (in hours) for the stimulation/ treatment condition of HepG2 cells: BSA in red line, FFA in green line and FFA+TNF $\alpha$  in blue line.



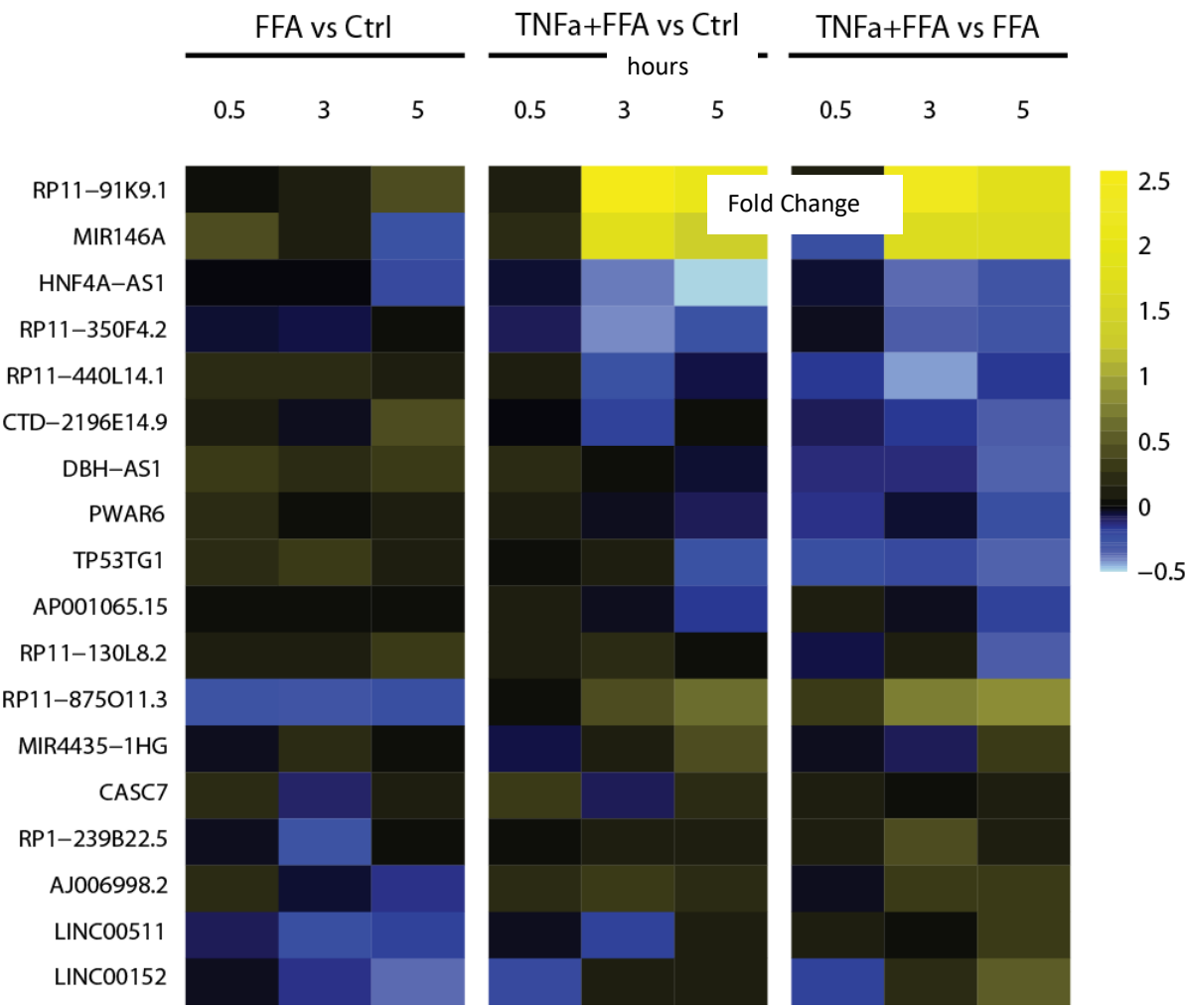
**Figure S4. Genes correlated with NAFLD phenotypes in human liver samples.**

**A.** Gene expression heatmap of NAFLD-associated genes (n=3,960) at FDR<0.1. **B.** Bar plot representing pathway-enrichment analysis on genes negatively associated with NAFLD/NASH. **C.** Bar plot representing pathway-enrichment analysis on genes positively associated with NAFLD/NASH. Enrichment P-values are presented on the X-axis, pathways on the Y-axis. GO term, Gene Ontology term.



**Figure S5. 18 lncRNAs which showed consistent expression patterns in challenged HepG2 cells and in human liver datasets.**

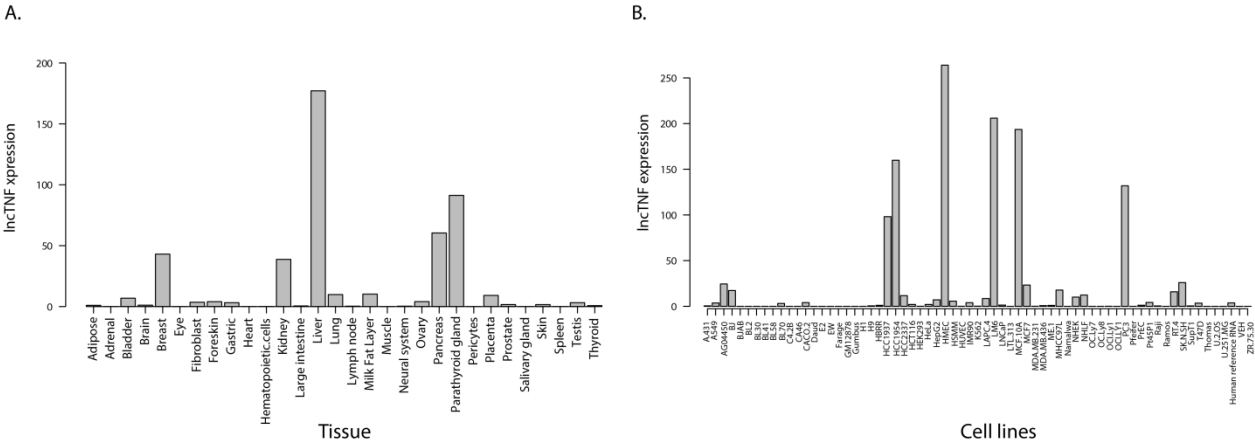
A heatmap representing the fold change of each lncRNA gene (Y-axis) relative to the BSA (ctrl) or FFA treatment conditions (X-axis).





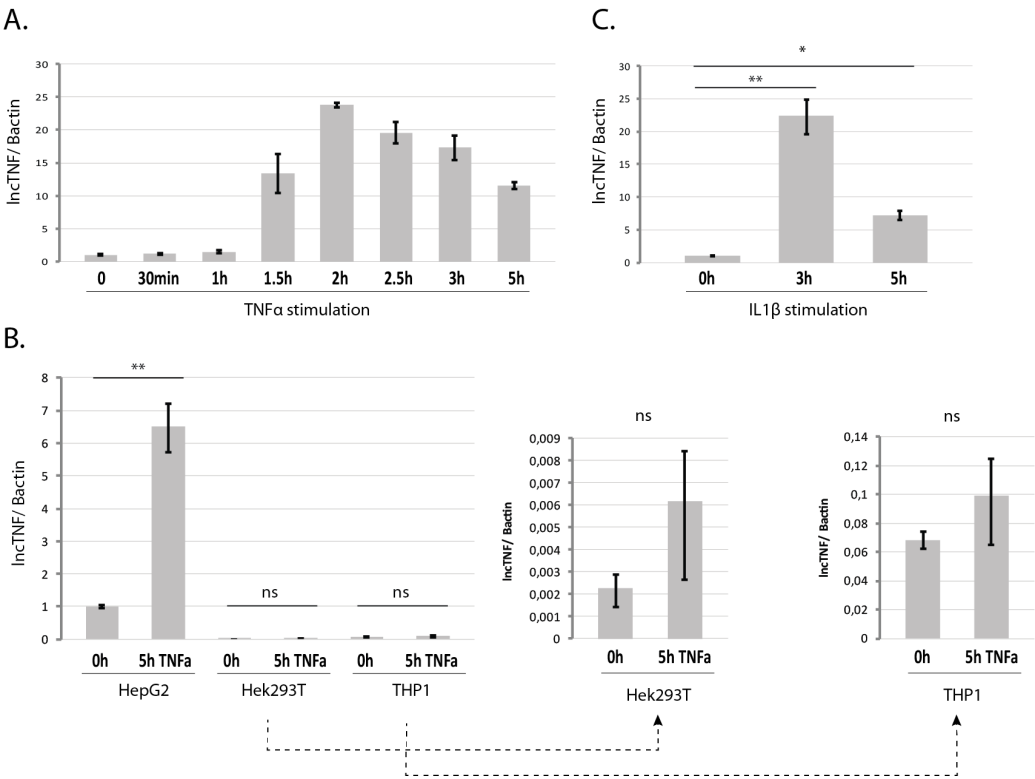
**Figure S6. Exprssion levels of lncTNF in publicly available RNAseq datasets (European Nucleotide Archive).**

**A.** Normalized expression levels of lncTNF (Y-axis) across 30 different tissues (X-axis). **B.** Normalized expression levels of lncTNF (Y-axis) across 66 different cell lines (X-axis).

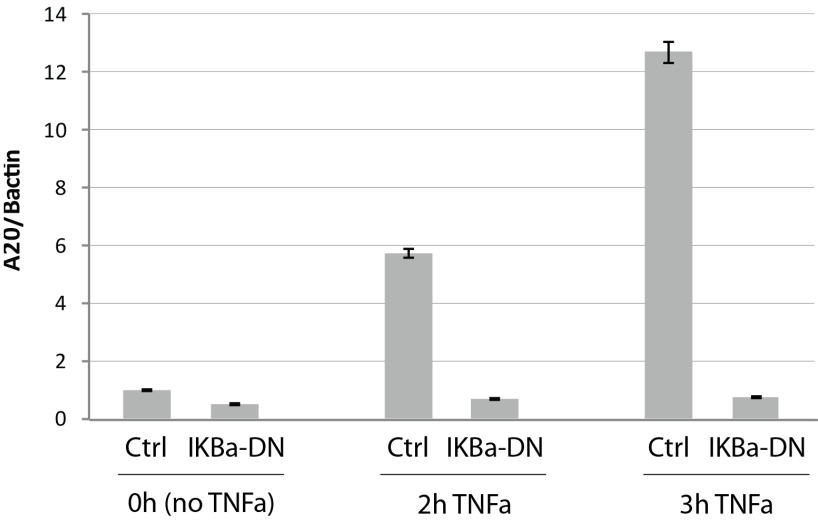


**Figure S7. qRT-PCR expression of lncTNF upon different cytokine treatments and among different cell lines.**

**A.** Expression of lncTNF relative to B-actin (Y-axis) in HepG2 cells untreated (0h) or treated with TNF $\alpha$  for 30min-5h time intervals (X-axis). **B.** Expression of lncTNF relative to B-actin in HepG2, Hek293T and THP1 cells in untreated (0h) or TNF $\alpha$  treated (5h) cells. **C.** Expression of lncTNF relative to B-actin in HepG2 cells untreated (0h) or treated with IL1 $\beta$  for 3 and 5h.



**Figure S8.** qRT-PCR measurement of A20 gene expression relative to B-actin gene (Y-axis) in HepG2 cells treated with Cre adenovirus (Ctrl) and Ad5I $\kappa$ B (I $\kappa$ B $\alpha$ -DN) adenovirus media (X-axis).



**Figure S9. Cellular localization of lncTNF in HepG2 cells.**

The localization was assessed by measuring nuclear/ cellular expression ratio of lncTNF. DANCER and OIPS-AS1 were used as controls for lncRNAs expressed in the cytoplasm, whereas MALAT1 and NEAT1 as controls for lncRNAs expressed in the nucleus.

