

Supplemental Table 1: Commercial sources and sequences for antibodies and primers

Antibody sources

| Primary Antibody against: | Company/catalog number |
|---|---------------------------------|
| rabbit anti-ZO-1 | Life Technologies 61-7300 |
| guinea pig anti-occludin | Hycult HP9047 |
| rabbit anti-claudin-3 | Invitrogen 34-1700 |
| mouse NFKB p65 | Santa Cruz sc-8008 |
| rabbit anti-phospho-NF- κ B p65 (serine 536) | Abcam, ab86299 |
| mouse anti-HSC70 | Santa Cruz sc-7298 |
| rabbit anti-importin- α 5 | Proteintech, 18137-1-AP |
| rabbit anti- β actin | Cell Signaling 4967 |
| Secondary Antibody: | |
| donkey anti-rabbit 488 | Molecular Probes, A21206 |
| goat anti-guinea pig 568 | Molecular Probes , A11075 |
| donkey anti-rabbit | Santa Cruz, sc-2313 |
| goat anti-guinea pig | Santa Cruz, sc-2438 |
| goat anti-mouse | Santa Cruz sc-2005 |
| goat anti-mouse 488 | Life Technologies REFA11029 |

Primer sequences (mRNA)

| Gene of interest | Forward | Reverse |
|-------------------------|-------------------------------|--------------------------------|
| Rat | | |
| TNF α | CAA GGA GGA GAA GTT CCC AA | CTC TGC TTG GTG GTT TGC TA |
| IP10/CXCL10 | GGG CCA TAG GAA AAC TTG AA TC | CAT TGT GGC AAT GAT CTC AAC AT |
| IL-6 | TCA ACT CCA TCT GCC CTT CAG | AAG GCA ACT GGC TGG AAG TCT |
| Mouse | | |
| TNF α | CCCTCACACTAGATCATCTTCT | GCTACGACGTGGGCTACAG |
| IL-6 | TAGTCCTTCCCTACCCCAATTCC | TTGGTCCTTAGCCACTCCTC |
| MCP-1 | AGGTCCCTGTCATGCTTCTG | TCTGGACCCATTCTTCTTG |
| ZO-1 | TGGGAACAGCACACAGTGAC | GCTGGCCCTCCTTTTAACAC |
| Occludin | ACCCGAAGAAAGATGGATCG | CATAGTCAGATGGGGGTGGA |
| Claudin-3 | CCTCATCGTGGTGTCCATCC | CGTCTCGTCTTGTACGCAGT |

Primer sequences (miRNA)

| miRNA of interest | Sequence/catalog number | |
|--|--------------------------------|--|
| Syn_rno-mir-181b-1-3P mimic, QIAGEN | CUCACUGAACAAUGAAUGCAA | |
| AllStars Negative Control(control mimic) | Cat No. 1027280, QIAGEN | |
| Rn_miR-181b-1* miScript primer assay | Cat No. MS00026873, QIAGEN | |
| Hs_SNORD68-11 | Cat No. MS00033712, QIAGEN | |

Supplementary Table 2: MicroRNAs identified by Next Generation Sequencing that were down regulated in Kupffer cells from ethanol-fed rats compared to pair-fed controls.

| Gene ID | Fold-change | Regulation | Accession |
|----------------|--------------------|-------------------|------------------|
| MI0012590_1 | -2.1150098 | Down | rno-miR-362 |
| MI0015421_1 | -2.5144277 | Down | rno-miR-3559-3p |
| MI0015477_1 | -2.2176843 | Down | rno-miR-741-3p |
| MI0015460_1 | -2.4241643 | Down | rno-miR-3585-3p |
| MI0012608_2 | -2.0592384 | Down | rno-miR-201 |
| MI0000885_1 | -2.2398326 | Down | rno-miR-100 |
| MI0000933_2 | -2.0746253 | Down | rno-miR-190 |
| MI0006148_1 | -2.686254 | Down | rno-miR-455 |
| MI0006148_2 | -3.2648048 | Down | rno-miR-455* |
| MI0000872_2 | -2.096135 | Down | rno-miR-31 |
| MI0000877_ | -2.0589275 | Down | rno-miR-34a* |
| MI0003527_1 | -2.028425 | Down | rno-miR-541 |
| MI0003724_1 | -2.314985 | Down | rno-miR-497 |
| MI0000939_1 | -2.841826 | Down | rno-miR-195 |
| MI0000939_2 | -3.5002127 | Down | rno-miR-195* |
| MI0000593_1 | -2.4073894 | Down | rno-miR-301a* |
| MI0000841_1 | -2.4408545 | Down | rno-miR-10a-5p |
| MI0000921_1 | -4.920171 | Down | rno-miR-152* |
| MI0000921_2 | -2.4939632 | Down | rno-miR-152 |
| MI0000926_2 | -4.134409 | Down | rno-miR-181b-1* |
| MI0000941_1 | -3.0407896 | Down | rno-miR-199a-5p |
| MI0000941_2 | -3.4742124 | Down | rno-miR-199a-3p |
| MI0000954_2 | -2.0321853 | Down | rno-miR-214 |
| MI0000859_2 | -2.558785 | Down | rno-miR-27b* |
| MI0000853_1 | -2.2368307 | Down | rno-miR-23b |
| MI0000898_1 | -3.8153477 | Down | rno-miR-126* |
| MI0000898_2 | -3.5592475 | Down | rno-miR-126 |
| MI0000918_2 | -3.1053104 | Down | rno-miR-145 |
| MI0000916_1 | -2.598191 | Down | rno-miR-143 |
| MI0000916_2 | -2.0461056 | Down | rno-miR-143* |

Supplementary Table 3: MicroRNAs identified by Next Generation Sequencing that were down regulated in Kupffer cells from ethanol-fed rats compared to pair-fed controls and in turn up-regulated in response to HA35.

| Gene ID | Fold-change | Regulation | Accession |
|----------------|--------------------|-------------------|------------------|
| MI0012590_1 | 2.296203 | Up | rno-miR-362 |
| MI0000877_2 | 2.051095 | Up | rno-miR-34a* |
| MI0000926_2 | 2.453269 | Up | rno-miR-181b-1* |

Supplemental Figure legends

Supplemental Figure 1: Pipeline for Next generation sequencing data analysis.

Bioinformatics analysis involved first removing the custom 3' adapter sequence from the reads. Reads were then aligned to mature miRNAs sequences. Finally, the total number of matches to each mature miRNA was incorporated into an expression profile. For the current analysis, only miRNAs down-regulated by ethanol feeding were used for target prediction.

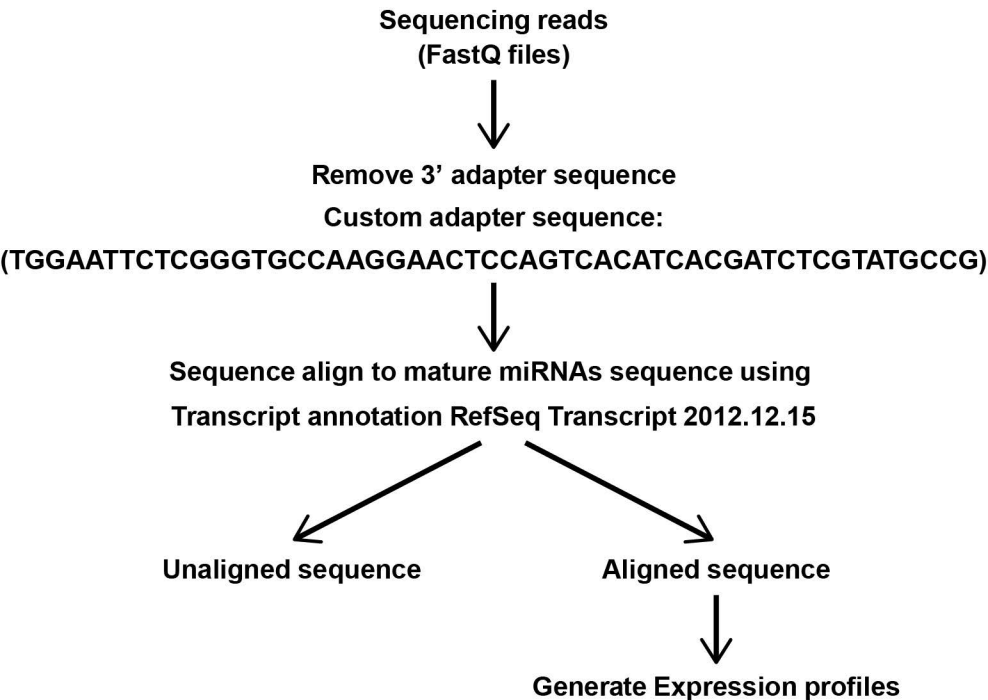
Supplemental Figure 2. microRNA regulatory pathways in Kupffer cells isolated from ethanol- and pair-fed rats. Targeted gene analysis identified the network of the interactions generated by thirty microRNAs whose expression was down-regulated more than 2.0-fold in Kupffer cells isolated from ethanol-fed rats compared to pair-fed rats. Targets were predicted using the TargetScan Bioinformatics Tool. Based on the highly predicted targets for these miRNAs the following pathways were predicted to be regulated: Nuclear-cytoplasmic transport (red), NF κ B (Green) and Inflammatory Pathway (Purple). Targets regulated by increasing numbers of miRNAs are positioned more towards the center of the network.

Supplemental Figure 3. Expression of ZO-1, occluding and claudin-3 protein and mRNA in ethanol- and pair-fed mice treated or not with HA35.

C57BL/6J mice were allowed free access to an ethanol containing diet for 4 days (2 days at 1% (v/v) ethanol, followed by 2 days 6% (v/v) ethanol) or pair-fed an isocaloric control diet. Mice were gavaged once daily for the last 3 days of ethanol feeding with HA35 at 1.5mg/kg body

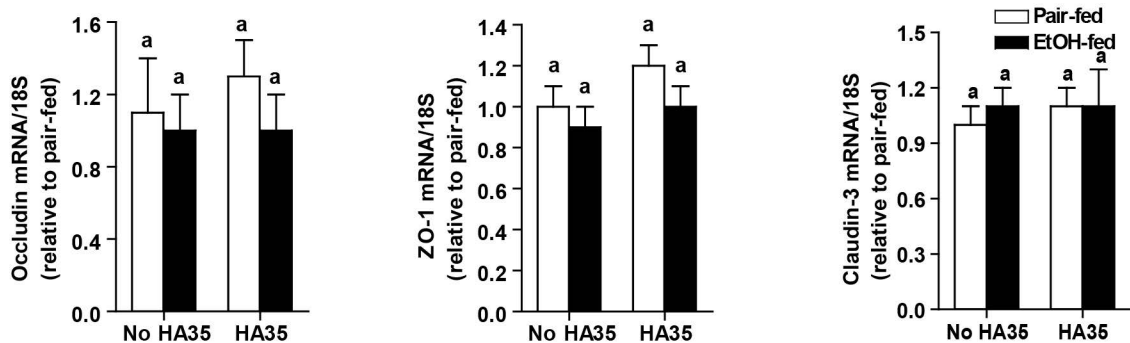
weight or saline control the last three days of the study. **(A)** Expression of ZO-1, occludin and claudin-3 mRNA in proximal colon was measured by qRT-PCR and normalized to 18S rRNA. **(B)** Proximal colon was lysed and expression of ZO-1, occludin and claudin-3 measured by Western blot. Expression was normalized to HSC70. Semi-quantification was performed using Carestream Imaging Software. Values are expressed as mean \pm SEM. Different alphabetical superscripts denote statistical differences between groups, $p < 0.05$; for pair-fed $n=4$ and for ethanol-fed $n=6$.

Supplemental Figure 1



Supplemental Figure 3

A



B

