

# Effects of Ethanol Feeding in Early-Stage NAFLD Mice Induced by Western Diet

## SUPPLEMENTARY MATERIAL

SUPPL. TABLE 1 – Ingredients of diets

<b>Suppl. Table 2 - animal diet composition</b>		
<b>Ingredients</b>	<b>Control-diet (V1534-300)</b>	<b>WD (S0279-S011)</b>
		19.5% casein and 1.25% cholesterol
<b>Crude protein</b>	19.0 %	28.0%
<b>Crude fat</b>	3.3 %	20.9%
<b>Crude fibers</b>	4.9 %	3.6%
<b>Crude ash</b>	6.4 %	4.4%
<b>Starch</b>	35.2 %	20.1%
<b>Sugar</b>	5.3 %	3.2%
<b>Vitamin A</b>	25,000 IE/kg chow	25,000 IE/kg chow
<b>Vitamin D<sub>3</sub></b>	1,500 IE/kg chow	1,500 IE/kg chow
<b>Vitamin E</b>	125 mg/kg chow	700 IE/kg chow
<b>Vitamin K<sub>3</sub></b>	20 mg/kg chow	20 mg/kg chow

<b>Copper</b>	5 mg/kg chow	5 mg/kg chow
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Manufacturer: ssniff Spezialdiäten GmbH, Soest, Germany

<b>Suppl. Table 2 – TaqMan® gene expression assays</b>		
<b>Gene</b>	<b>Assay-ID</b>	<b>Species</b>
<b><i>col1a1</i></b>	Mm00801666_g1	<i>Mus musculus</i>
<b><i>il1b</i></b>	Mm01336189_m1	<i>Mus musculus</i>
<b><i>ccl2</i></b>	Mm00441242_m1	<i>Mus musculus</i>
<b><i>ccr2</i></b>	Mm99999051_gH	<i>Mus musculus</i>
<b><i>fasn</i></b>	Mm01204974_m1	<i>Mus musculus</i>
<b><i>srebp-1c</i></b>	Mm00550338_m1	<i>Mus musculus</i>
<b><i>scd-1</i></b>	Mm00772290_m1	<i>Mus musculus</i>
<b><i>acta2</i></b>	Mm00725412_s1	<i>Mus musculus</i>

Manufacturer: Applied Biosystems, Waltham, MA

Supplementary Table 3 Gene identity, accession number and forward and reverse primers used for RT-PCR analysis of relative gene expression of human liver tissue.

<b>Gene</b>	<b>NCBI Accession number</b>	<b>Forward primer</b>	<b>Reverse primer</b>
<b>ACTA 2</b>	NM_001613	AATGGCTCTGGGCTCTGTAA	TTTGCTCTGTGCTTCGTAC
<b>CCR2</b>	NM_000647	CGGTGCTCCCTGTCATAAATC	TTGCCACAAAACCAAAGATC

<b>COL1A1</b>	NM_000088	TGTTTCAGCTTTGTGGACCTCC	CTGTACGCAGGTGATTGGTGC
<b>FASN</b>	NM_152871; NM_152872; NM_152873; NM_152874; NM_152875; NM_000043	CAAGGGATTGGAATTGAGGAC	ACCTGGAGGACAGGGCTTAT
<b>IL-1b</b>	NM_000576	TCCAGGGACAGGATATGGAGC	TCTTCAACACGCAGGACAGC

Suppl. Mat 1.

Computer Code for Quantification of Sirius Red Staining

// Sirius Red Quantification - Galaxy protocol - modified from  
<https://imagej.nih.gov/ij/docs/examples/stained-sections/index.html>

```

run("RGB Stack");

setSlice(2);

// change scale from inches to microns

run("Set Scale...", "distance=317 known=200 pixel=1 unit=um");

// erase scale bar

setBackground(255, 255, 255);

makeRectangle(1019, 906, 320, 44);

run("Clear", "slice");

run("Select None");

// set threshold

setAutoThreshold();

getThreshold(min, max)

setThreshold(0, 118);

// measure area and area fraction

run("Set Measurements...", "area area_fraction limit display redirect=None decimal=3");

run("Measure");

selectWindow("Results");

```

Suppl. Mat 2.

Computer Code for Quantification of Oil Red O Staining

```
// Galaxy Oil Red O quantification Macro
```

```
min=newArray(3);
```

```
max=newArray(3);
```

```
filter=newArray(3);
```

```
a=getTitle();
```

```
run("HSB Stack");
```

```
run("Convert Stack to Images");
```

```
selectWindow("Hue");
```

```
rename("0");
```

```
selectWindow("Saturation");
```

```
rename("1");
```

```
selectWindow("Brightness");
```

```
rename("2");
```

```
min[0]=227;
```

```
max[0]=255;
```

```
filter[0]="pass";
```

```
min[1]=58;
```

```
max[1]=255;
```

```
filter[1]="pass";
```

```
min[2]=89;
```

```
max[2]=255;
```

```
filter[2]="pass";
```

```
for (i=0;i<3;i++){
```

```
    selectWindow(""+i);
```

```

setThreshold(min[i], max[i]);

run("Convert to Mask");

if (filter[i]=="stop") run("Invert");
}

imageCalculator("AND create", "0", "1");

imageCalculator("AND create", "Result of 0", "2");

for (i=0;i<3;i++){

  selectWindow(""+i);

  close();

}

selectWindow("Result of 0");

close();

selectWindow("Result of Result of 0");

rename(a);

// Colour Thresholding-----

setAutoThreshold("Default");

//run("Threshold...");

setThreshold(21, 255);

run("Measure");

Suppl. Mat 3.

```

#### Computer Code for Quantification of F4/80 Staining

```

// F480 Quantification - Galaxy protocol - Max Thr is set at 100 - modified from
https://imagej.nih.gov/ij/docs/examples/stained-sections/index.html

// select the green channel, which has the best contrast

run("RGB Stack");

setSlice(2);

// change scale from inches to microns

run("Set Scale...", "distance=317 known=200 pixel=1 unit=um");

```

```
// erase scale bar
setBackgroundColor(255, 255, 255);
makeRectangle(1019, 906, 320, 44);
run("Clear", "slice");
run("Select None");
// set threshold
setAutoThreshold();
getThreshold(min, max)
setThreshold(0, 100);
// measure area and area fraction
run("Set Measurements...", "area area_fraction limit display redirect=None decimal=3");
run("Measure");
selectWindow("Results");
```