- **1** Supporting Information for
- 2 ORIGINAL ARTICLE
- 3 Silybin A from Silybum marianum reprograms lipid metabolism to induce a
- 4 cell fate-dependent class switch from triglycerides to phospholipids

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## 52 Supplementary Figures

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Figure S1. Concentration- and time-dependent effects of silymarin and silybin on cellular PE levels. (A, B) HepG2 cells were treated with silymarin, silybin or vehicle (ethanol for silymarin, DMSO for silybin) at the indicated concentrations for 24 h (A) or with silymarin (10  $\mu$ g/ml), silybin (20  $\mu$ M) or vehicle (ethanol for silymarin, DMSO for silybin) for the indicated incubation times (B). Independent datasets connected by lines; n = 3 (A, B, silymarin) or n = 4 (B, silybin). \**P* < 0.05, \*\**P* < 0.01 vs.

61 vehicle control for the respective time point; two-tailed paired Student's *t*-test.

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64Figure S2. Effects of silymarin and silybin on cell number, membrane integrity and cell viability.65HepG2 cells were treated with silymarin and silybin (at the indicated concentrations), staurosporine66(STS, 1 μM) (C), or vehicle (ethanol for silymarin, DMSO for silybin and STS) for 24 h. (A) Cell67numbers. Individual values and mean + SEM; n = 3 (silymarin), or n = 4 (silybin). (B) Membrane68intactness measured by trypan blue staining. Individual values and mean + SEM; n = 3; effects of69silymarin (200 μg/ml) and silybin (300 μM) were assessed in independent experiments (A, B). (C)70Cell viability determined by MTT assay. Individual values and mean + SEM; n = 3 (silybin) or n = 471(silymarin and STS). \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001 vs. vehicle control; ordinary one-way</td>72ANOVA + Tukey HSD *post hoc* tests (C) and two-tailed paired Student's *t*-test (A and B, 200 μg/ml73silymarin and 300 μM silybin, C, STS).



- Figure S3. Influence of DGAT and ATGL inhibition on the phospholipid and TG content of hepatocytes. HepG2 cells were treated with the DGAT inhibitor PF 06424439 or the ATGL inhibitor atglistatin for 24-30 h. Total amounts of PE and TG were determined by UPLC-MS/MS. Individual values and mean + SEM; n = 3. \*P < 0.05 vs. vehicle control; two-tailed unpaired Student's *t*-test.







described in Figure 1. Heatmap showing the absolute abundance of phospholipid and TG species.
 Values are given as nmol / 1×10<sup>6</sup> cells for PC and units / 1×10<sup>6</sup> cells for PE, PS, PI, PG, SM and TG
 Data are presented as mean ± SEM. The color indicates percentage changes relative to control. Data

96 and the number of experiments are identical to Figure 1.

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A			
monocytes			
		P	C O
PC(16:0_16:0)	1.217 ± 0.397	1.485 ± 0.345	0.972 ± 0.06
PC(16:0_18:1)	0.681 ± 0.254	1.089 ± 0.276	0.456 ± 0.015
PC(18:0_18:1)	0.303 ± 0.099	0.45 ± 0.06	1.804 ± 0.069
PC(18:1_18:1)	0.329 ± 0.133	0.518 ± 0.152	0.374 ± 0.035
PC(18:1_20:1)	0.053 ± 0.023	0.08 ± 0.02	0.081 ± 0.016
PC(16:0_18:2)	0.096 ± 0.029	0.211 ± 0.034	0.178 ± 0.007
PC(18:0_18:2) PC(16:0_20:2)	0.045 ± 0.039	0.06 ± 0.028	0.038 ± 0.004
PC(16:0_20:3) PC(16:0_20:4)	0.094 + 0.032	0.164 + 0.039	0.232 + 0.008
PC(18:1_20:4)	0.037 ± 0.012	0.064 ± 0.013	0.093 ± 0.003
PC(18:0 20:3)	0.011 ± 0.004	0.017 ± 0.005	0.019 ± 0.002
PC(18:0 20:4)	0.012 ± 0.003	0.034 ± 0.004	0.028 ± 0.001
PC(16:0_22:5)	0.291 ± 0.078	0.347 ± 0.073	0.213 ± 0.025
PC(18:0_22:6)	0.058 ± 0.016	0.078 ± 0.008	0.021 ± 0.002
PC 32:0 E (16:0)	0.02 ± 0.008	0.023 ± 0.001	0.183 ± 0.02
PC 34:2 E (18:2)	0.128 ± 0.047	0.197 ± 0.046	0.255 ± 0.013
		F	Έ
PE(16:0 18:1)	0.057 ± 0.02	0.102 ± 0.031	0.148 ± 0.019
PE(18:0 18:1)	0.693 ± 0.289	0.978 ± 0.28	1.261 ± 0.067
PE(18:1_18:1)	0.156 ± 0.058	0.263 ± 0.087	0.204 ± 0.024
PE(18:1_20:1)	0.039 ± 0.017	0.076 ± 0.03	0.026 ± 0.002
PE(18:0_20:4)	0.483 ± 0.174	0.756 ± 0.206	1.781 ± 0.112
PE(18:0_22:5)	0.046 ± 0.02	0.069 ± 0.022	0.128 ± 0.007
PE 34:1 E (16:0)	0.158 ± 0.051	0.193 ± 0.053	0.247 ± 0.032
	control	silymarin	control
В			
HepG2		-	
		۲	
PC(16:0_16:0)	0.762 ± 0.058	0.943 ± 0.057	0.714 ± 0.083
PC(16:0_16:1)	1.369 ± 0.125	2.123 ± 0.056	$1.082 \pm 0.164$
DC/AC-0 40-4)			
PC(16:0_18:1)	3.377 ±0.246	4.94 ± 0.123	2.97 ±0.414
PC(16:0_18:1) PC(18:1_18:1) PC(16:0_18:3)	3.377±0.246 2.149±0.17	4.94 ± 0.123 3.275 ± 0.048	2.97 ±0.414 2.492 ±0.264
PC(16:0_18:1) PC(18:1_18:1) PC(16:0_18:2)	3.377 ±0.246 2.149 ±0.17 0.451 ±0.044	4.94±0.123 3.275±0.048 0.698±0.024	2.97 ±0.414 2.492 ±0.264 0.47 ±0.063
PC(16:0_18:1) PC(18:1_18:1) PC(16:0_18:2)	3.377±0.246 2.149±0.17 0.451±0.044	4.94 ±0.123 3.275±0.048 0.698±0.024	2.97 ±0.414 2.492 ±0.264 0.47 ±0.063
PC(18:0_18:1) PC(18:1_18:1) PC(16:0_18:2) PE(16:0_16:1) PE(16:0_16:1)	3.377±0.246 2.149±0.17 0.451±0.044	4.94 ± 0.123 3.275 ± 0.048 0.698 ± 0.024 F 0.255 ± 0.011	2.97 ±0.414 2.492 ±0.264 0.47 ±0.063 PE 0.196 ± 0.01
PC(16:0_18:1) PC(18:1_18:1) PC(16:0_18:2) PE(16:0_16:1) PE(16:0_18:1) PF(18:0_18:1)	3.377 ± 0.246 2.149 ± 0.17 0.451 ± 0.044 0.155 ± 0.013 0.67 ± 0.069 1.029 ± 0.128	4.94 ± 0.123 3.275 ± 0.048 0.698 ± 0.024 P 0.255 ± 0.011 1.129 ± 0.044 1.735 ± 0.11	2.97±0.414 2.492±0.264 0.47±0.063 PE 0.196±0.01 1.545±0.764 4.748±1.2
PC(16:0_18:1) PC(18:1_18:1) PC(16:0_18:1) PC(16:0_18:1) PC(16:0_18:1) PC(18:0_18:1) PC(18:0_18:1) PC(18:0_18:1)	3.377 ±0.246 2.149 ±0.17 0.451 ±0.044 0.155 ±0.013 0.67 ±0.069 1.029 ±0.128	4.94 ± 0.123 3.275 ± 0.048 0.698 ± 0.024 P 0.255 ± 0.011 1.129 ± 0.044 1.735 ± 0.11 1.588 ± 0.111	2.97±0.414 2.492±0.264 0.47±0.063 <b>*E</b> 0.136±0.01 1.545±0.764 4.748±1.2 1±0.219
PC(16:0_18:1) PC(18:1_18:1) PC(16:0_18:2) PE(16:0_18:1) PE(18:0_18:1) PE(18:0_18:1) PE(18:1_18:1) PE(18:1_18:1)	3.377±0.246 2.149±0.17 0.451±0.044 0.155±0.013 0.67±0.069 1.029±0.128 1.022±0.089 0.74±0.086	4.94 ±0.123 3.275±0.048 0.698±0.024 P 0.255±0.011 1.129±0.044 1.735±0.11 1.588±0.111 1.038±0.045	2.97 ±0.414 2.492 ±0.264 0.47 ±0.063 <b>*E</b> 0.1545 ±0.764 4.748 ±1.2 1±0.219 1.026 ±0.231
PC(16:0_18:1) PC(18:1_18:1) PC(16:0_18:2) PE(16:0_18:1) PE(18:0_18:1) PE(18:0_18:1) PE(18:0_18:1) PE(18:0_20:4)	3.377±0.246 2.149±0.17 0.451±0.044 0.155±0.013 0.67±0.069 1.029±0.128 1.022±0.089 0.74±0.086	4.94 ±0.123 3.275 ±0.048 0.698 ±0.024 P 0.255 ±0.011 1.129 ±0.044 1.735 ±0.11 1.588 ±0.111 1.038 ±0.045	2.97±0.414 2.492±0.264 0.47±0.063 PE 0.196±0.01 1.545±0.764 4.748±1.2 1±0.219 1.026±0.231 PS
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PC(16:0_18:1) PC(18:1_18:1) PC(16:0_18:2) PE(16:0_18:2) PE(16:0_18:1) PE(18:0_18:1) PE(18:0_20:4) PE(18:0_20:4) PS(16:0_18:1) PS(16:0_18:1)	3.377±0.246 2.149±0.17 0.451±0.044 0.155±0.013 0.67±0.069 1.029±0.128 1.022±0.089 0.74±0.086 0.115±0.015 0.132±0.014	4.94 ±0.123 3.275±0.048 0.698±0.024 P 0.255±0.051 1.129±0.044 1.735±0.11 1.588±0.111 1.038±0.045 P 0.147±0.01 0.187±0.008	2.97±0.414 2.492±0.264 0.47±0.063 <b>PE</b> 0.196±0.001 1.545±0.764 4.748±1.2 1±0.219 1.026±0.231 <b>PS</b> 0.109±0.009 0.672±0.072
PC(16:0_18:1) PC(18:1_18:1) PC(16:0_18:2) PE(16:0_18:1) PE(18:0_18:1) PE(18:0_18:1) PE(18:0_20:4) PS(16:0_18:1) PS(18:0_18:1) PS(18:0_18:1) PS(18:1_18:1)	3.377±0.246 2.149±0.17 0.451±0.044 0.155±0.043 0.67±0.069 1.029±0.128 1.022±0.089 0.74±0.086 0.116±0.015 0.132±0.014 0.866±0.092	4.94 ±0.123 3.275 ±0.048 0.698 ±0.024 P 0.255 ±0.044 1.129 ±0.044 1.735 ±0.11 1.588 ±0.111 1.038 ±0.045 P 0.147 ±0.01 0.187 ±0.008 1.056 ±0.033	2.97 ± 0.414 2.97 ± 0.414 2.492 ± 0.264 0.47 ± 0.063 PE 0.196 ± 0.01 1.545 ± 0.764 4.748 ± 1.2 1 ± 0.219 1.026 ± 0.231 PS 0.109 ± 0.009 0.672 ± 0.072 0.158 ± 0.012
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0.132 ± 0.014 0.806 ± 0.092 PS

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1.056 ± 0.053

0.109 ± 0.009

0.672 ± 0.072

 $0.158 \pm 0.01$ 

В

PS(16:0\_18:1) PS(18:0\_18:1) PS(18:1\_18:1)



hat gelöscht: normalized to the cell number (in relative units)

111 species. Values are given as nmol for PC and units for PE, PS, PI, PG, SM and TG. Data are presented as mean  $\pm$  SEM. The color indicates percentage changes relative to control. Data and the number of

experiments are identical to Figure 1.





117 118 119 120 121 122 Figure S7. (Lyso)phospholipid content of silymarin in comparison to hepatocytes. Absolute abundance of (lyso)phospholipids in 10  $\mu$ g silymarin or  $3 \times 10^5$  HepG2 cells, which corresponds to the treatment of hepatocytes with 10  $\mu$ g/ml under our experimental conditions. Lipid analysis of HepG2 cells

focused on those species from Figure S5 and S6 that are present in silymarin, i.e., PC(16:0/18:2). Single value or mean + SEM; n = 1 (silymarin) or n = 3 (HepG2 cells).





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hat gelöscht: Impact on intracellular membrane compartments and lipid droplets. HepG2 cells were incubated with silymarin (10 µg/ml), silybin (20 µM) or vehicle control (ethanol for silymarin, DMSO for silybin) for 24 h. (A) Cell diameter (µm) of HepG2 cells determined with a Vi-Cell<sup>TM</sup>XR cell counting system. Individual values and mean + SEM; n = 3. (B and C) Immunofluorescence staining of Golgi (B) and ER (C): anti-GM130 (red) was used as Golgi marker and anti-calnexin (red) as Em marker; nuclei were stained with DAPI (blue). (D) Left panel: Staining of lipid droplets with Oil Red O. Scale bar 50 µM. Right panel: Relative lipid droplets with Oil Red O. Scale bar 50 µM. Right panel: Relative lipid droplets with BODIPY493/503 (green); nuclei were visualized with DAPI (blue). Lipid droplets were counted in three independent experiments for 100 cells (each). Images are representative out of 3 independent experiments; magnified BODIPY493/503-stained section: 40×20 µm. Individual values and mean + SEM; n = 3. \*P < 0.05 vs. vehicle control. Two-tailed paired Student's *t*-test (A, D, E).



(B) membrane intactness measured by trypan blue staining. Individual values and mean + SEM; n = 3 (B) or n = 4 (A). *P* values vs. vehicle control; repeated measures one-way ANOVA + Tukey HSD *post* hoc tests.



193 Figure S10. Volcano plots showing the data from Fig. 6A-D adjusted for multiple comparisons.

Statistical calculations were performed by pairwise comparison of treatment and control groups using
 the GEO2R interactive webtool (https://www.ncbi.nlm.nih.gov/geo/geo2r/)<sup>1</sup>.

Adjusted *P* values were calculated by multiple *t*-tests, with correction for multiple comparisons

according to Benjamini and Hochberg (false discovery rate 5%) and autodetection for log-

198 transformation. The dashed line indicates an adjusted *P* value of 0.05.



201 Figure S11. Lipid metabolic enzymes differentially regulated by silymarin/silybin. Comparative 202 analysis of transcriptome data from silymarin-treated HepG2 and Huh7.5.1 hepatocarcinoma cells, 203 silybin-and silymarin-treated CaCo-2 colon carcinoma cells, and hepatocytes derived from HCV-204 infected mice receiving silybin. Radar plots indicating the fold change in PLA2G6, MBOAT2, PLD1, 205 DGAT2, LPIN2, PNPLA3, HSH17B1, MVK, CYP4F2, HADH, ACOX2, and CYP2C19 expression by 206 silymarin (HepG2, Huh7.5.1, CaCo-2) or silybin (hepatocytes, CaCo-2) relative to vehicle control. 207 Non-adjusted P values given vs. vehicle control; multiple two-tailed unpaired Student's t-tests. Data 208 are identical to Figure 4.

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212 213 Figure S12. Remodeling of *de novo* phospholipid biosynthesis and TG metabolism. (A-C) HepG2 214 cells were incubated with silymarin (10  $\mu$ g/ml), silybin (20  $\mu$ M) or vehicle (ethanol for silymarin, 215 DMSO for silybin) for 24 h; (A) mRNA levels of LPLAT/LPAAT1-3 normalized to β-actin 216 (LPLATs/LPAATs silybin) or GAPDH (LPLATs/LPAATs silymarin). Individual values and mean + 217 SEM as fold-change of control; n = 3 (LPLATs/LPAATs silymarin, LPLAT1/LPAAT1 silybin) and n 218 = 4 (LPLATs/LPAATs silybin except LPLAT1/LPAAT1). (B) Protein expression of DGAT1, DGAT2, 219 ATGL/PNPLA2, ACC1/2 and FASN, phosphorylation of ACC1/2. Individual values and mean + 220 SEM; n = 3 (DGAT1, DGAT2, ATGL, FASN silybin), n = 4 (pACC silybin, FASN silymarin), and n 221 = 5 (ACC, pACC silymarin). Individual values and mean + SEM; n = 3. Representative Western blots 222 223 224 are shown. (C) Effects of silymarin and silybin on the cellular content of malonyl-CoA, long-chain acyl-CoAs (normalized to the internal standard [13C3]-malonyl-CoA) and FFAs. Individual values and mean + SEM; n = 5 (silybin, malonyl-CoA and long-chain acyl-CoAs) and n = 6 (silymarin, malonyl-225 226 CoA and long-chain acyl-CoAs), n = 7 (silymarin, FFA) or n = 8 (silybin, FFA). \*P < 0.05 vs. vehicle controls; two-tailed paired Student's t-tests. 227

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235 236 237 238 239 labeled PE species, corrected for naturally occurring isotopes and normalized to internal standard and 240 241 cell number in nmol / 1×106 cells, as mean ± SEM, with color indicating percentage changes to the vehicle control. Data and the number of experiments are identical to Figure 4I. (C, D) The heatmap 242 243 244 245 246 247 248 and bar charts display the absolute amounts of isotopically labeled TG species, corrected for naturally occurring isotopes and normalized to the internal standard and cell count in nmol / 1×106 cells. Data are presented as mean ± SEM. The colors represent percentage changes relative to the vehicle control. Data and the number of experiments are identical to Figure 4J. Fatty acids that incorporated isotopically labeled sodium acetate (M+3) are indicated with asterisks (\*) and fatty acids that remained

in the molecule after fragmentation are indicated with primes ('). (B, D) Individual values and means + SEM; n = 3. \*P < 0.05, \*P < 0.001 vs. vehicle controls; two-tailed paired Student's *t*-tests.





250 251 Figure S14, Comparison of the silybin A effects in non-challenged hepatocytes and cell-based disease models of MAFLD and acute lipotoxicity. (A) HepaRG cells were treated with 0.1 mM palmitate (PA) 252 or a mixture of PA/oleate (OA) in a 1:2 ratio (in total 1 mM) together with vehicle (DMSO, 0.5%) or 253 compounds for 48 h. Relative lipid droplet content was determined by Oil Red O staining. (B-D) 254 HepaRG cells were either pre-treated with 0.1 mM palmitate (PA) or a mixture of PA/oleate (OA) in a 255 1:2 ratio (in total 1 mM) for 24 h followed by vehicle (DMSO, 0.5%) or silybin A (20  $\mu$ M) treatment 256 or cells were directly co-treated with vehicle (DMSO, 0.5%) or silybin A (20 µM), and the incubation 257 was prolonged for a further 24 h. Total levels of TG (B), PE (C), and PC (D) determined by UPLC-258 MS/MS. (E) Cell viability measured by MTT assay. (F) Viable cell numbers. (G) Cell membrane 259 integrity determined by trypan blue staining. Individual values and mean + SEM or  $\pm$  SEM, n = 2 (E, 0.1 mM PA) or n = 3 (A-G, except D, 0.1 mM PA). \*P < 0.05, \*\*\*P < 0.001 vs. control; two-tailed 260 261 unpaired Student's t-test.



266 Figure S15, Silymarin/silybin kinetically controls the mRNA levels of genes involved in drug 267 metabolism. Comparative analysis of transcriptome data from silymarin-treated HepG2 and Huh7.5.1 268 hepatocarcinoma cells, silybin- and silymarin-treated CaCo-2 colon carcinoma cells, and hepatocytes 269 derived from HCV-infected mice receiving silybin. (A-C) Volcano plots compare the expression of 270 proteins involved in drug metabolism upon silymarin (A-C) or silybin (C) treatment vs. vehicle 271 control. Statistical calculations were performed by pairwise comparison of treatment and control 272 groups using the GEO2R interactive webtool (https://www.ncbi.nlm.nih.gov/geo/geo2r/)<sup>1</sup>. P-values 273 were calculated by multiple t-tests without correction for multiple comparisons. The dashed line 274 indicates a P-value of 0.05. (D) Radar plots indicating the fold change in CYP2C9 by silymarin

275 (HepG2, Huh7.5.1, CaCo-2) or silybin (hepatocytes, CaCo-2) relative to vehicle control. Non-adjusted

276 *P* values are given vs. vehicle control; multiple two-tailed unpaired Student's *t*-tests.

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Figure S16, Impact of silybin on CYP expression in mouse liver. (A) Western blots for the densitometric data shown in Figure 8C are representative of n = 5 mice/group; (B) Protein

- concentration of the 9,000×g supernatant of the liver homogenate. Individual values and mean + SEM; n = 8 mice/group. \*\*P < 0.01 vs. vehicle control; two-tailed unpaired Student's *t*-test.
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### 287 Supplementary Notes

## 288 Supplementary Note 1: Cholesterol and CE metabolism

289 The effects of silybin/silymarin on CEs, the second major neutral lipid in lipid droplets after

- 290 TGs, are less consistent. In some settings, silybin/silymarin stimulates cholesterol
- biosynthesis by upregulating the transcription factor *SREBP1* (Figure 4A) and repressing its
  endoplasmic reticulum anchor *INSIG1* (Figure 4D and E), thereby favoring the transfer of *SREBP1* to the Golgi for proteolytic procession to the mature form<sup>2-4</sup>. On the contrary, several
- 294 SREBP-regulated target genes were downregulated (*ACACA, ELOVL6, MVK*, Figure 4A, E,
- Figure S11), except for FASN, which was upregulated as expected (Figure 4C). In other
- 296 settings, silybin/silymarin reduces the expression of cholesterol biosynthetic enzymes (MVK,
- 297 TM7SF2, Figure 4C, D, and E, and Figure 11). Such counter-regulation could be explained by
- initially increased levels of sterols, which then bind to the cholesterol sensor INSIG1 (Figure
- $\label{eq:second} 299 \qquad S6 \ D \ and \ E) \ and \ suppress \ SREBP1 \ signaling \ (along \ with \ target \ protein \ expression) \ without$
- 300 necessarily interfering with SREBP1 expression<sup>2</sup>. In addition to canonical cholesterol
- biosynthesis, increased lipoprotein and sterol uptake (*LRP2*) (Figure 4B and C) and possibly
- endosomal cholesterol transport (*STARD3NL*) (Figure 4C) may further add to the
- 303 accumulation of intracellular CE. In strong support of this hypothesis, silymarin
- administration to mice substantially elevated the hepatic CE content (Figure 1D). However,
- 305 the increase in esterified cholesterol levels did not seem to be translated into enhanced
- 306 cholesterol metabolism, as the expression of various sterol-metabolizing enzymes (*CYP1A1*,
- 307 *CYP1B1, CYP2C8, CYP3A4, CYP7A1, CYP11B2, CYP17A1, CYP19A1, CYP27A1, ABCB11,*
- SLC10A1, SLC27A5, HSD3B2, HSD17B1, HSD17B3, AKR1D1, AKR1C3, EBP, BAAT, STS)
  is largely repressed (Figure 4A, B, C, D and E, and Figure S11), with a few exceptions
- 310 (*CYP1A1, CYP1B1, CYP1B1, AKR1C3*, Figure 4B-D). Together, silymarin/silybin
- 311 consistently downregulate anabolic and catabolic sterol metabolism, while exerting complex,
- 312 partially opposite effects on cholesterol biosynthesis.

## 313 Supplementary Note 2: Vitamin A metabolism

- 314 In addition, silymarin/silybin differentially regulates a significant number of genes related to
- 315 vitamin A metabolism, including retinoic acid biosynthesis (CYP1B1, CYP3A4, ADH1B,
- ADH6, Figure 4A, Figure S14A-C), degradation (CYP2C18, CYP3A5, CYP26A1, CYP2C8,
- 317 Figure 8A and B, Figure S14B), and vitamin A storage (DGAT1, PNPLA3 and ATGL/
- B18 PNPLA2, Figure 4A, B, E, and F, Figure S11).

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330	Vitam	in A is stored as retinyl esters in lipid droplets in the liver and shares common	
331	metab	olic pathways with TG. This involves genetic risk factors for MAELD, such as DGAT1,	hat gelöscht: S
332	PNPL	A3, and ATGL/PNPLA2, which participate in retinol ester synthesis and degradation <sup>5,6</sup> .	
333	Vitam	in A plays a central role in the regulation of hepatic lipid metabolism, including	
334	lipoge	enesis, lipid transport, and lipid catabolism <sup>6</sup> , and disturbed vitamin A metabolism has	
335	been a	associated with MAFLD <sup>5,6</sup> . It is therefore remarkable that our comparative transcriptome	hat gelöscht: S
336	analys	sis revealed that numerous genes involved in vitamin A metabolism (including CYP1B1,	
337	CYP3.	A4, ADH1B, ADH6, CYP2C18, CYP3A5, CYP26A1, CYP2C8, DGAT1, PNPLA2/ATGL	
338	and po	ossibly PNPLA3) are subject to regulation by silybin/silymarin. Further studies are	
339	neede	d to explore whether the interference with vitamin A metabolism by silymarin/silybin	
340	affects	s hepatic lipid metabolism and influences the development and/or progression of	
341	MA <mark>F</mark> J	LD.	hat gelöscht: S
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