Combining dietary intervention with metformin treatment enhances NAFLD remission in mice fed a high-fat high-sucrose diet.

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	CD	HFHSD
Crude protein (%)	14.3	17.3
Crude fat (%)	4	23.2
Crude fibre (%)	4.1	5.0
Carbohydrate (available) (%)	48	47.6
Crude ash (%)	4.7	ND
Nitrogen free extracts (%)	43.5	ND
Energy density (Kcal/g (Kj/g))	2.9 (12.1)	4.7 (19.66)
Calories from protein (%)	20	14.7
Calories from fat (%)	13	44.6
Calories from carbohydrate (%)	67	40.7
Calcium (%)	0.7	0.62
Phosphorus (%)	0.6	0.33
Sodium (%)	0.1	0.13
Magnesium (%)	0.2	0.06
Potassium (%)	0.6	0.44
Chloride (%)	0.3	0.19
Caprid acid (C10:0) (%)	ND	0.42
Lauric acid (C12:0) (%)	ND	0.65
Myristic acid (C14:0) (%)	ND	2.46
Palmitic acid (C16:0) (%)	0.5	5.72
Palmitoleic acid (C16:1) (%)	ND	0.4
Stearic acid (C18:0) (%)	0.1	2.71
Oleic acid (C18:1) (%)	0.7	6.39
Linoleic acid (C18:2) (%)	2	1.67
Linolenic acid (C18:3) (%)	0.1	0.27
Cholesterol (mg/kg)	ND	576.6
Lysine (%)	0.7	1.37
Methionine (%)	0.4	0.45
Cystine (%)	0.3	0.35
Threonine (%)	0.5	0.74
Tryptophan (%)	0.2	0.2
Arginine (%)	0.8	0.64
Histidine (%)	0.4	0.49
Valine (%)	0.7	1.17
Isoleucine (%)	0.6	0.98
Leucine (%)	1.4	1.56
Phenylalanine (%)	0.7	0.86
Glycine (%)	0.7	0.31
Glutamic acid (%)	2.9	3.55
Aspartic acid (%)	0.9	1.17
Proline (%)	1.2	1.76
Alanine (%)	0.9	0.51
Serine (%)	0.7	0.98
Vitamin A (IU/kg)	17000	14068
Vitamin D_3 (IU/kg)	1200	1900
Vitamin E (IU/kg)	150	143
Vitamin K (as menadione) (mg/Kg)	58	1.4
Thiamin (B_1) (mg/Kg)	64	9.2
Riboflavin (B_2) (mg/Kg)	16	11.4
Pyridoxime (B_6) (mg/Kg)	17	10.9
Cyanocobalamin (B_{12}) (mg/Kg)	0.09	0.05
Nicotinic acid (mg/Kg)	84	57
Pantothenic acid (mg/Kg)	76	27.8
Folic acid (mg/Kg)	5	3.8
Biotin (mg/Kg)	0.57	0.38
Choline-Chloride (mg/Kg)	1030	1337
Iron (mg/Kg)	175	45.4
Manganese (mg/Kg)	100	12.9
Zinc (mg/Kg)	70	49.6
Copper (mg/Kg)	15	7.6
Iodine (mg/Kg)	6	0.23
Selenium (mg/Kg)	0.23	0.18

Table S1. Nutritional composition of chow diet (CD; TD.2014, Harlan Laboratories Inc., Madison, WI, USA) and high fat-high sucrose diet (HFHSD; TD.08811, Harlan Laboratories Inc., Madison, WI, USA)
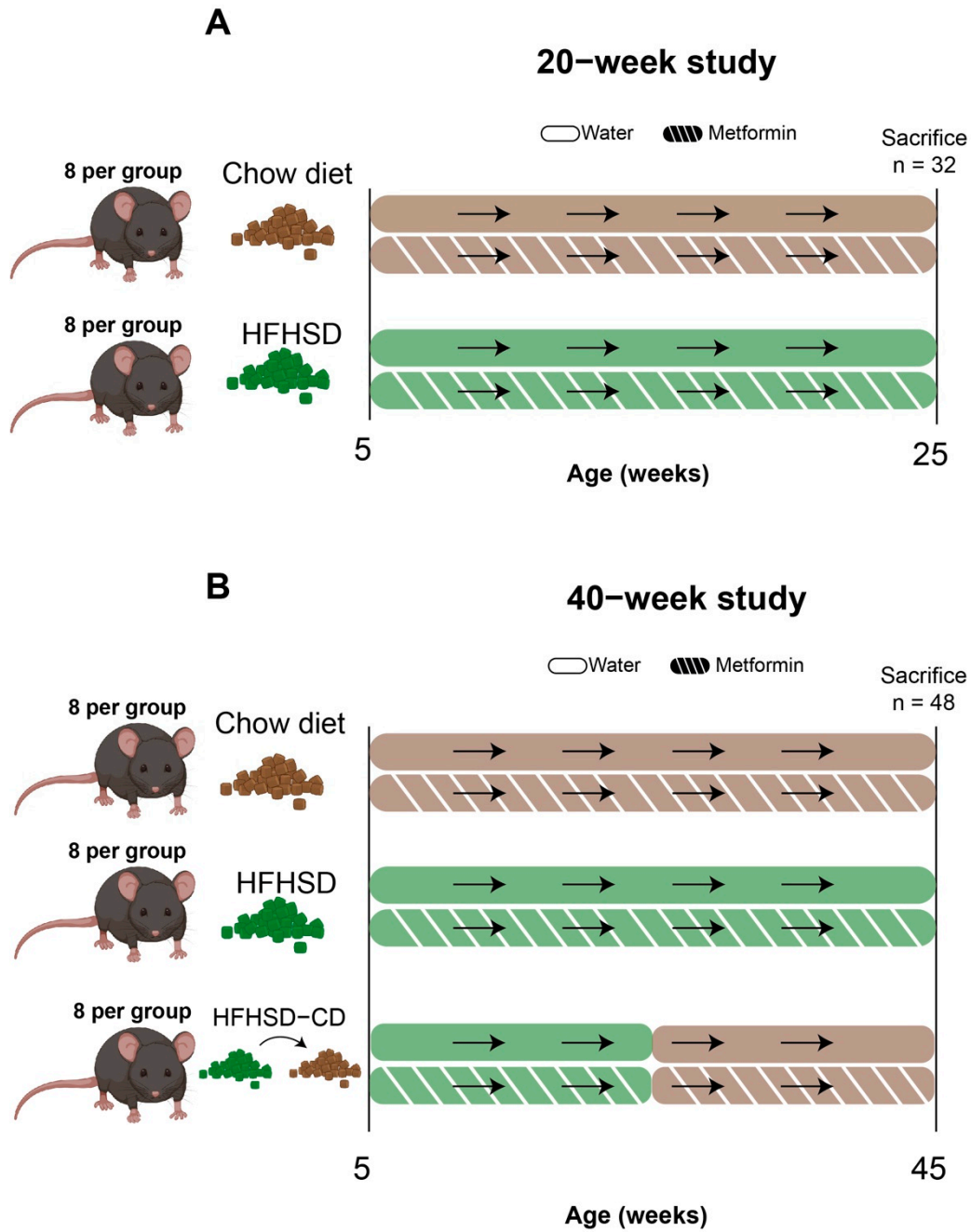
Fig. S1. Study design scheme
(**A**) Scheme of 20-week and (**B**) 40-week study design. HFHSD, high-fat high-sucrose diet.
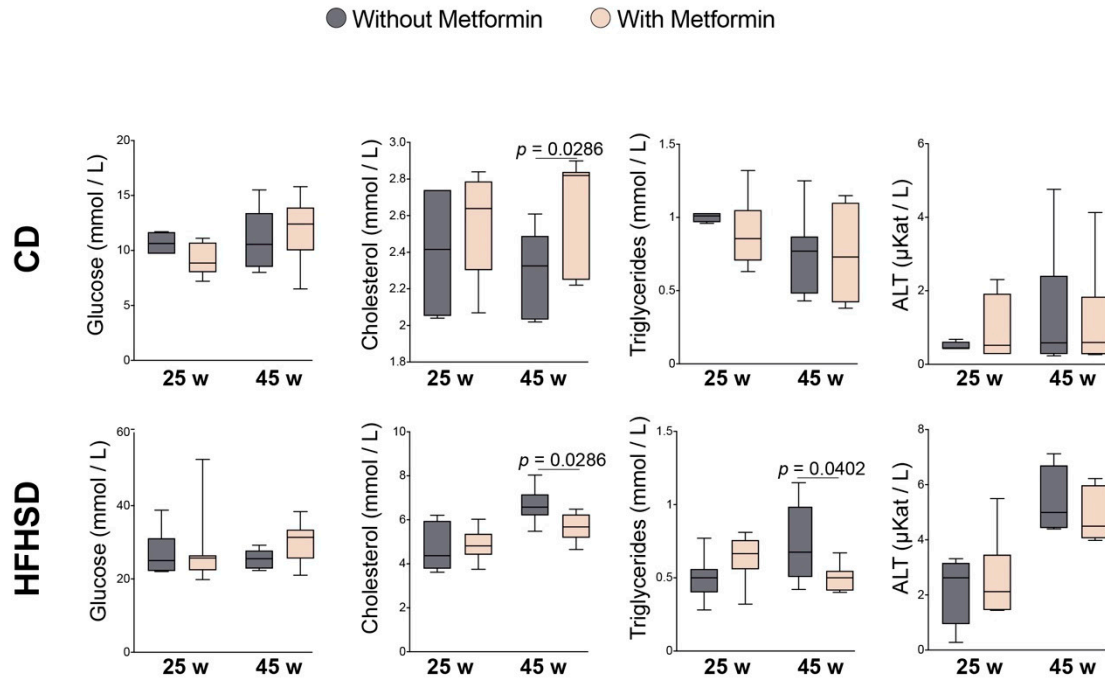
Fig. S2. Effects of metformin on biochemical variables
Glucose, cholesterol, and triglyceride concentrations, and alanine aminotransferase (ALT) activities in mice fed with chow or high-fat high-sucrose diets (CD and HFHSD, respectively) (n = 8/group). Data are presented as means, maximum and minimum. p values < 0.05 are considered significant. (Wilcoxon-rank sum test).
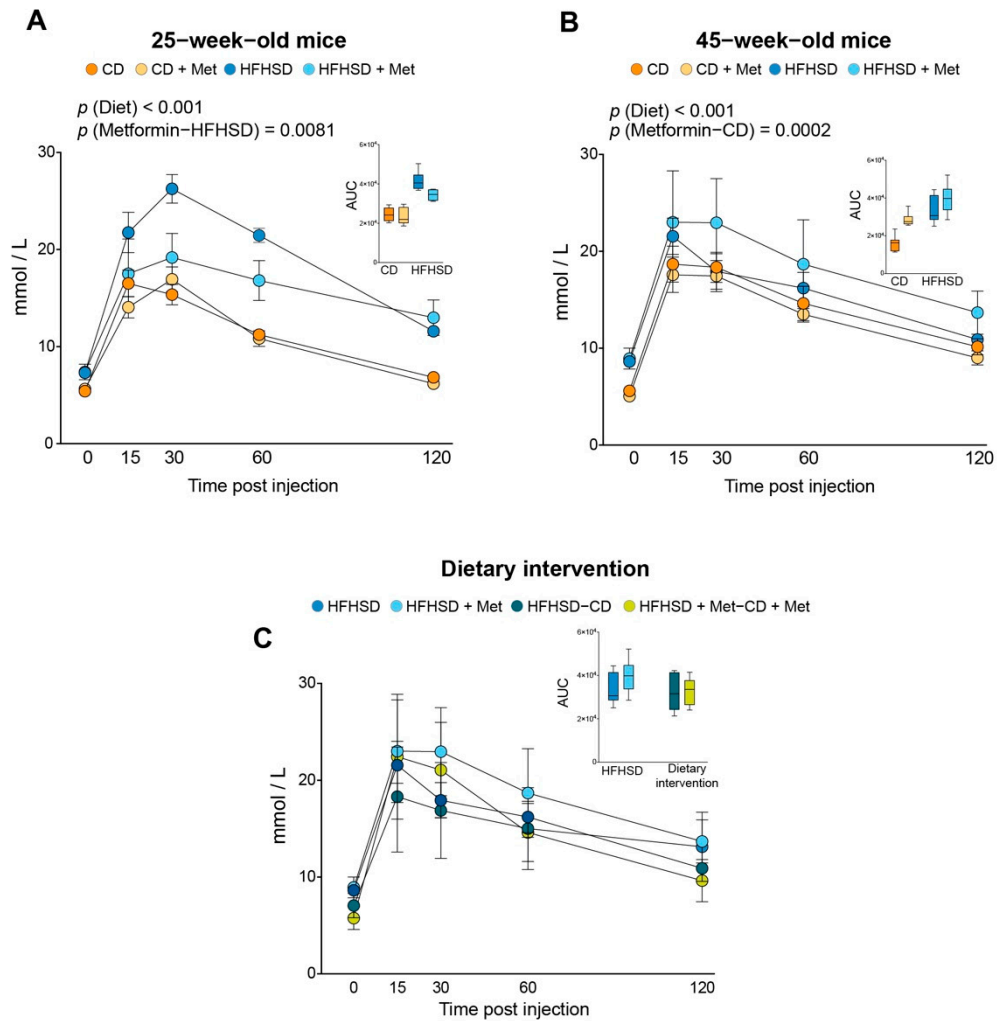
Fig. S3. Metformin does not improve glucose tolerance test in obese mice
(**A**) Glucose tolerance test curves and areas under the curve (AUC) of 25-week-old mice, and (**B**) 45-week-old mice. (**C**) Effect of dietary intervention, with or without metformin treatment, on glucose tolerance test (n = 8/group). Data are shown as means and SD. p values < 0.05 are considered significant. (Wilcoxon-rank sum test). CD: chow diet; HFHSD: high-fat high-sucrose diet; Met: metformin.
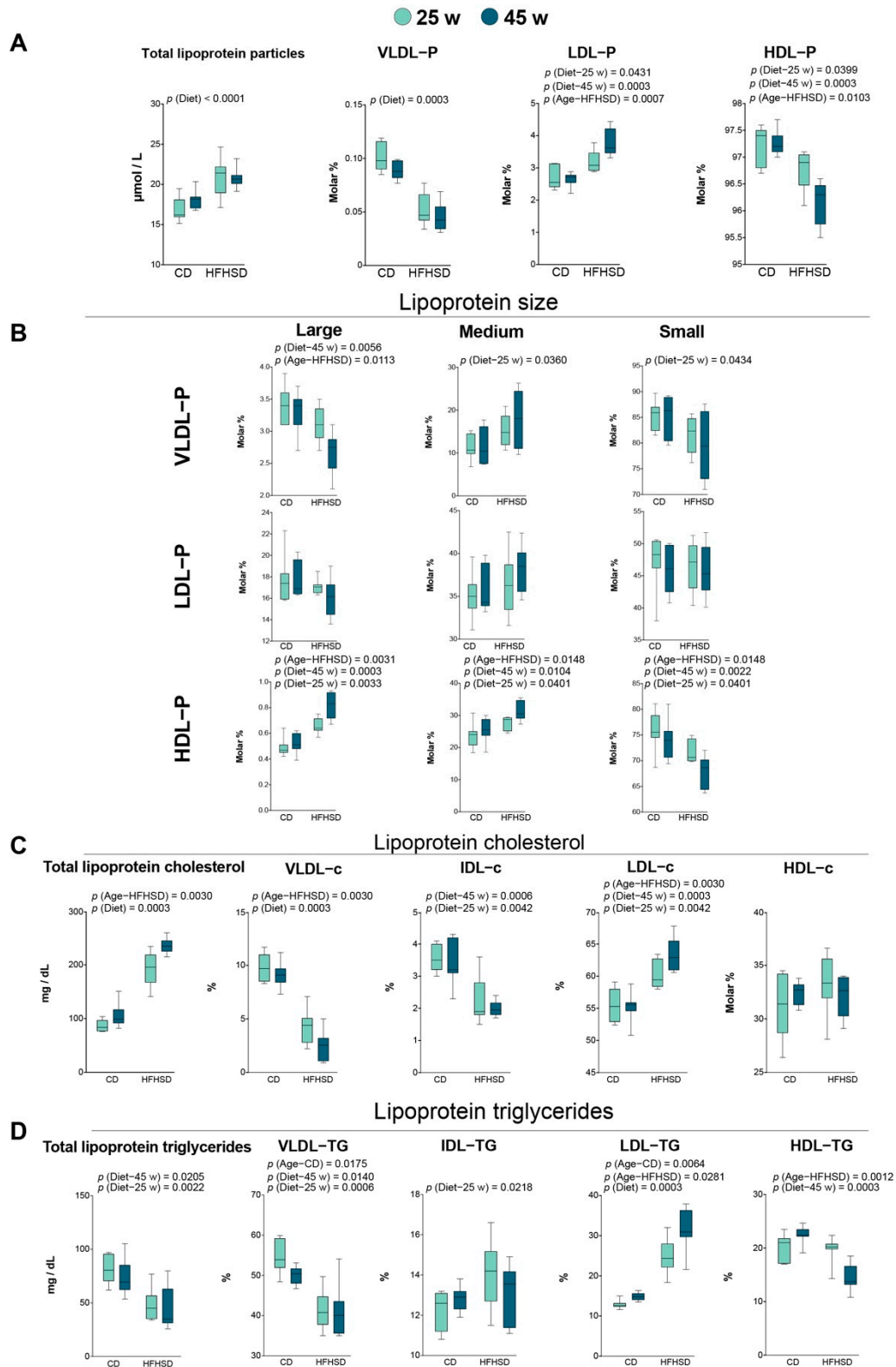
Fig. S4. High-fat high-sucrose diet and age adversely affect the lipoprotein profile
(**A**)Total concentration of lipoprotein particles and VLDL-p, LDL-p, and HDL-p molar abundance
(expressed as a percentage of total measured lipoprotein particles) (*n* = 4/group). (**B**) The molar
abundance of lipoprotein size (expressed as a percentage of total measured VLDL-p, LDL-p or HDL-
p) (*n* = 4/group). (**C**) Total lipoprotein cholesterol concentration and abundance of VLDL-c, IDL-c,

LDL-c and HDL-c (expressed as a percentage of total measured lipoprotein cholesterol) (n = 4/group). (**D**)Total lipoprotein triglyceride concentrations and abundance of VLDL-TG, IDL-TG, LDL-TG and HDL-TG (expressed as a percentage of total measured lipoprotein triglycerides) (*n* = 4/group). Data are shown as means, maximum and minimum. *p* values < 0.05 are considered significant. (Wilcoxon-rank sum test). CD: chow diet; HDL: High-density lipoproteins; HFHSD: high-fat high-sucrose diet; IDL: intermediate-density lipoproteins; LDL: low-density lipoproteins; p: particles; TG: triglycerides; VLDL: very low-density lipoproteins; w: weeks.
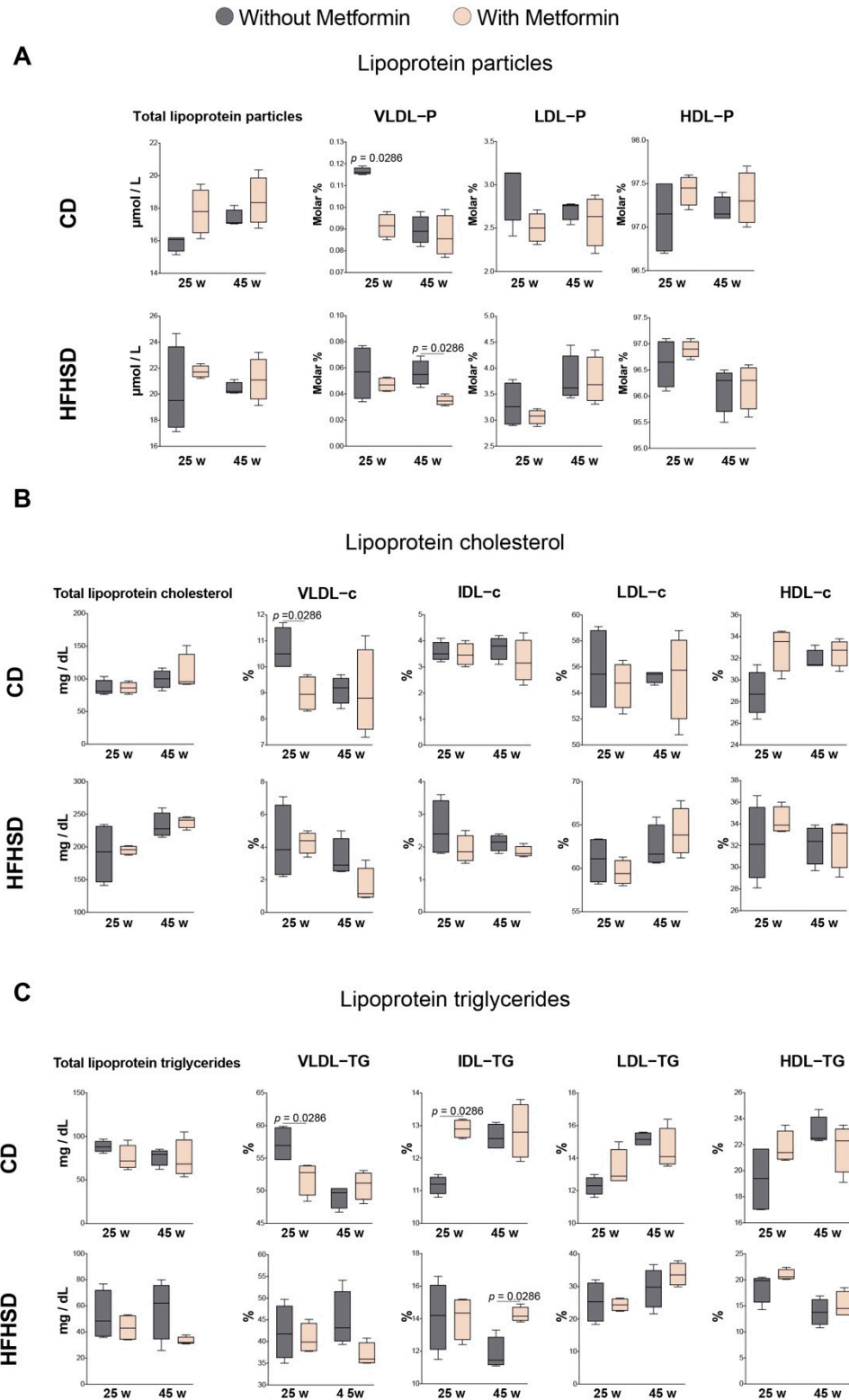
Fig. S5. Effects of metformin on lipoproteins profiles

(**A**) Concentration of total lipoprotein particles and VLDL-p, LDL-p and HDL-p molar abundance (expressed as a percentage of total measured lipoprotein particles) in CD or HFHSD-fed mice (*n* = 4/group). (**B**) Total lipoprotein cholesterol concentration and abundance of VLDL-c, IDL-c, LDL-c and HDL-c (expressed as a percentage of total measured lipoprotein cholesterol) in CD or HFHSD-fed

mice (*n* = 4/group). (**C**)Total lipoprotein triglyceride concentrations and abundance of VLDL-TG, IDL-TG, LDL-TG and HDL-TG (expressed as a percentage of total measured lipoprotein triglycerides) in CD or HFHSD-fed mice (*n* = 4/group). Data are shown as means, maximum and minimum. *p* values < 0.05 are considered significant. (Wilcoxon-rank sum test). CD: chow diet; HDL: high-density lipoproteins; HFHSD: high-fat high-sucrose diet; IDL: intermediate-density lipoproteins; LDL: low-density lipoproteins; p: particles; TG: triglycerides; VLDL: very low-density lipoproteins; w: weeks.

Fig. S6. Effects of metformin on lipoprotein particle size

(**A-C**) The molar abundance of lipoprotein size (expressed as a percentage of total measured VLDL-p, LDL-p or HDL-p) in CD or HFHSD-fed mice (*n* = 4/group). Data are shown as means, maximum and minimum. *p* values < 0.05 are considered significant. (Wilcoxon-rank sum test). CD: chow diet; HDL: high-density lipoproteins; HFHSD: high-fat high-sucrose diet; IDL: intermediate-density lipoproteins; LDL: low-density lipoproteins; p: particles; TG: triglycerides; VLDL: very low-density lipoproteins; w: weeks.
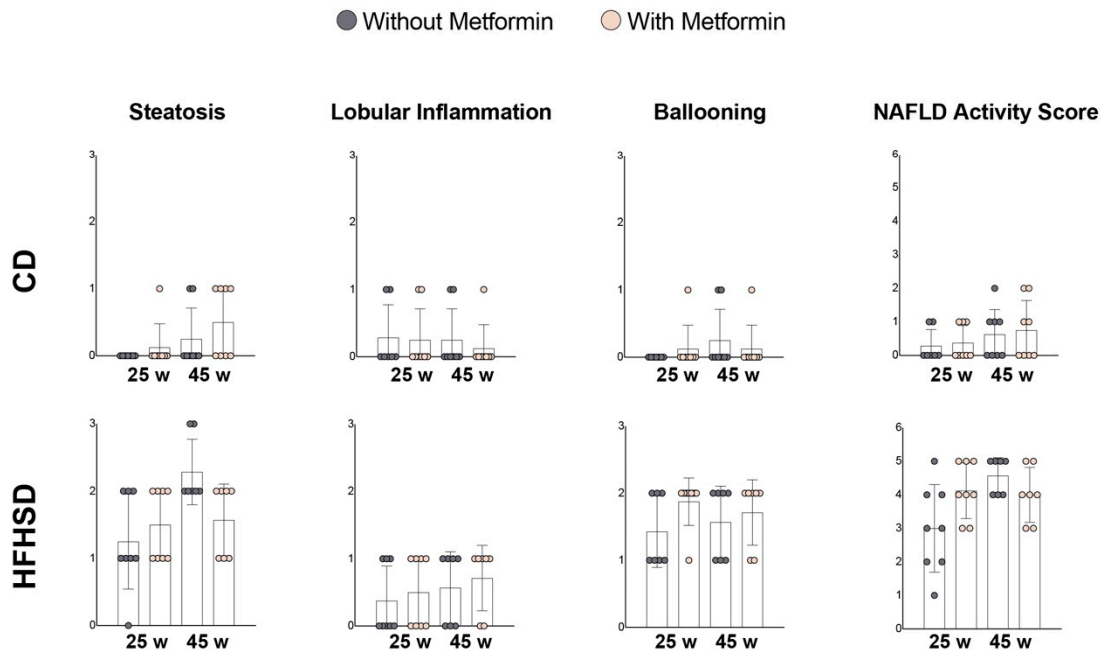
Fig. S7. Effects of metformin on hepatic histology
Hepatic steatosis, lobular inflammation, ballooning, and NAFLD activity scores in CD or HFHSD-fed mice with or without metformin treatment (n = 8/group). Data shown as means and SD. p values < 0.05 are considered significant. (Wilcoxon-rank sum test). CD: chow diet; HFHSD: high-fat high sucrose diet; w: weeks.
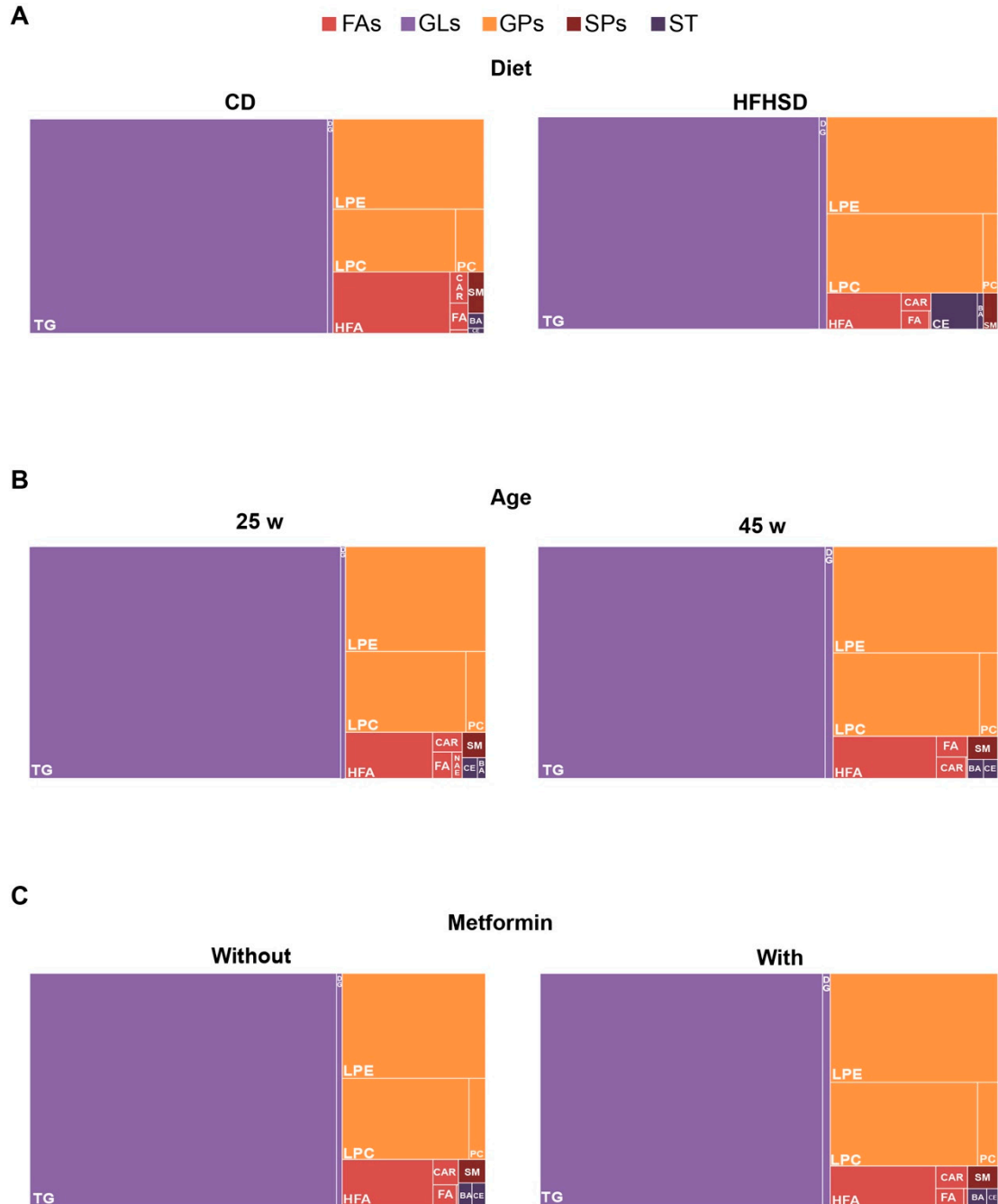
Fig. S8. Hepatic lipid signature according to diet, age, and metformin treatment
Treemaps show the relative distribution of main lipid categories and classes (*n* = 4/group). These relative distributions serve as a comparative metric of hepatic lipidome between (**A**) dietary treatments, (**B**) age, (**C**) metformin treatment. FAs, fatty acyls; GLs, glycerolipids; GPs, glycerophospholipids; BA: bile acids; CAR: carnitines; CE: cholesterol esters; DG, diglycerides; FA: fatty acids; HFA: hydroxy fatty acids; LPC: lysophosphatidylcholine; LPE: lysophosphatidylethanolamine; PC: phosphatidylcholines; SM: sphingomyelins; SPs: sphingophospholipids, ST: sterol lipids; w: weeks.
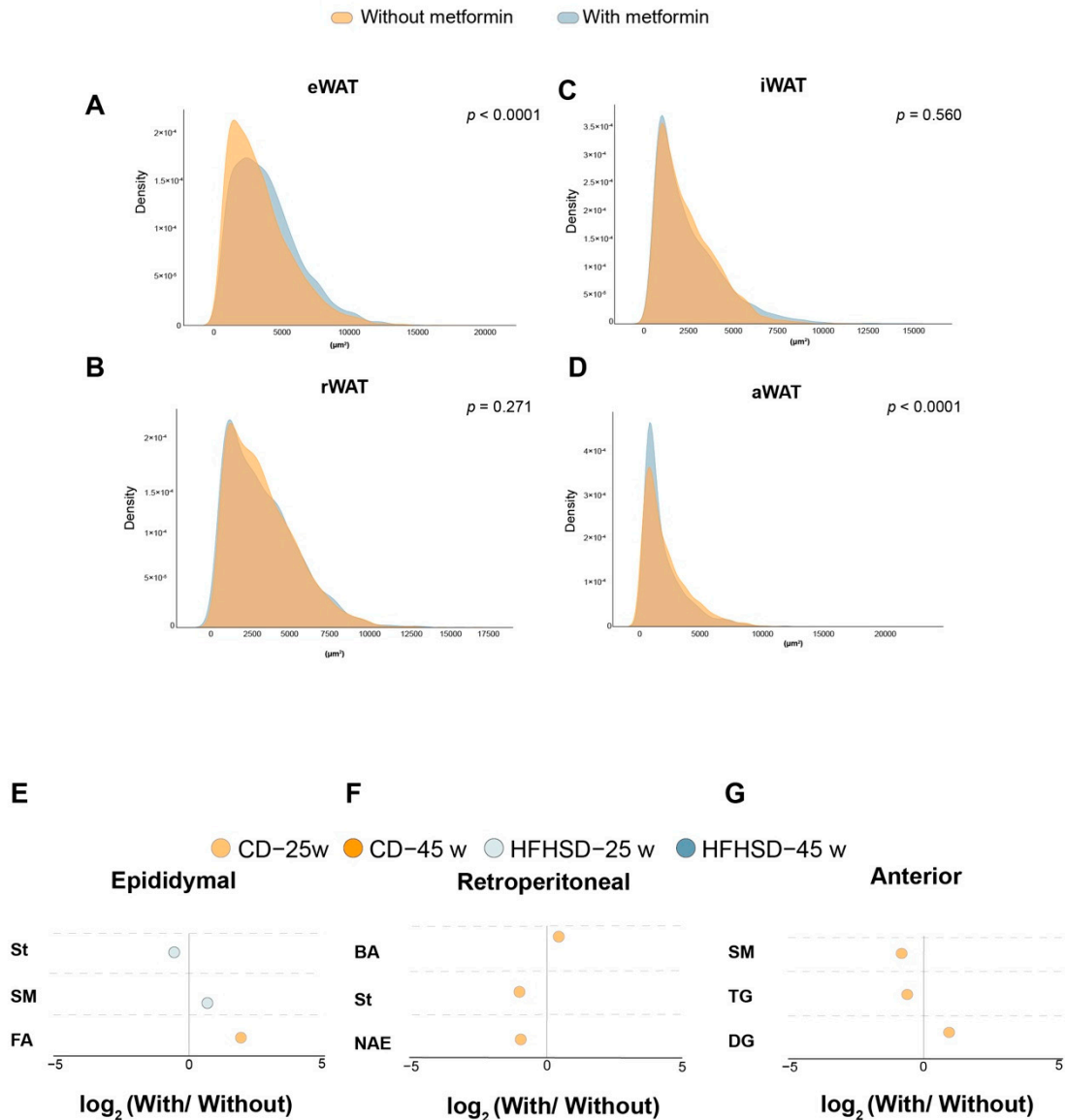
Fig. S9. Adipocyte lipid signature according to metformin treatment

Density plots show the relative frequency of histological adipocyte size from (**A**) epididymal (eWAT),
(**B**) retroperitoneal (rWAT), (**C**) inguinal (iWAT) and (**D**) anterior (aWAT) white adipose tissues. The
metformin treatment highlighted in sky-orange (mice without metformin) and sky-blue (mice with
metformin) (n = 8/group). Representation of significant lipid classes, showing the mean $\log_2$ (with
metformin / without metformin) in (E) epididymal, (F) retroperitoneal, and (**G**) anterior adipocytes (n =
4/group). Groups of mice represented in sky-orange (25-week-old mice with CD) or orange (45-week-
old mice with CD) (n = 4/group) and sky-blue (25-week-old mice with HFHSD) or blue (45-week-old
mice with HFHSD) dots (n = 4/group). p values < 0.05 are considered significant. (Wilcoxon-rank sum
test). BA: bile acids; CD: chow diet; DG: diglycerides; FA: fatty acids; HFHSD: high-fat high-sucrose
diet; NAE: N-acyl-ethanolamine's; SM: sphingomyelins; St: steroid hormones; TG: triglycerides; w:
weeks.

CD   HFHSD

A eWAT   *p* < 0.0001

C iWAT   *p* < 0.0001

B rWAT   *p* = 0.0002

D aWAT   *p* < 0.0001

E

Without Met−25 w   With Met−25 w   Without Met−45 w   With Met−45 w

Epididymal

SFA   MUFA   PUFA

CD   HFHSD

BA
St
LPE
LPC
HFA
NAE

log$_2$ (HFHSD / CD)

F

Retroperitoneal

SFA   MUFA   PUFA

CD   HFHSD

BA
St
LPE
LPC
PC
NAE

log$_2$ (HFHSD / CD)

G

Inguinal

SFA   MUFA   PUFA

CD   HFHSD

BA
St
SM
CAR

log$_2$ (HFHSD / CD)

H

Anterior

SFA   MUFA   PUFA

CD   HFHSD

BA
St
SM
PC
HFA
FA

log$_2$ (HFHSD / CD)

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Fig. S10. Adipocyte lipid signature according to dietary treatment
Density plots show the relative frequency of histological adipocyte size from (**A**) epididymal (eWAT), (**B**) retroperitoneal (rWAT), (**C**) inguinal (iWAT) and (**D**) anterior (aWAT) white adipose tissues. Diets of mice colored in sky-orange (CD-fed mice) and sky-blue (HFHSD-fed mice) (n = 8/group). Representation of significant lipid classes showing the mean $\log_2$ (HFHSD-fed mice / CD-fed mice) and the unsaturation changes in (**E**) epididymal (top left), (**F**) retroperitoneal (top right), (**G**) inguinal (bottom left) and (**H**) anterior (bottom right) adipocytes. Groups of mice represented in sky-orange (without metformin) or orange (with metformin) dots in 25-week-old mice (n = 4/group) and sky-blue (without metformin) or blue (with metformin) dots in 45-week-old mice (n = 4/group). p values < 0.05 are considered significant. (Wilcoxon-rank sum test). BA: bile acids; CAR: carnitines; CD: chow diet; DG: diglyceride; FA: fatty acid; HFA: hydroxy fatty acids; HFHSD: high-fat high-sucrose diet; LPC: lysophosphatidylcholines; LPE: lysophosphatidylethanolamines; Met: metformin; MUFA: monounsaturated fatty acids; NAE: N-acyl-ethanolamine's; PC: phosphatidylcholines; PUFA: polyunsaturated fatty acids; SM: sphingomyelins; SFA: saturated fatty acids; ST: steroid hormones; TG: triglycerides; w: weeks.
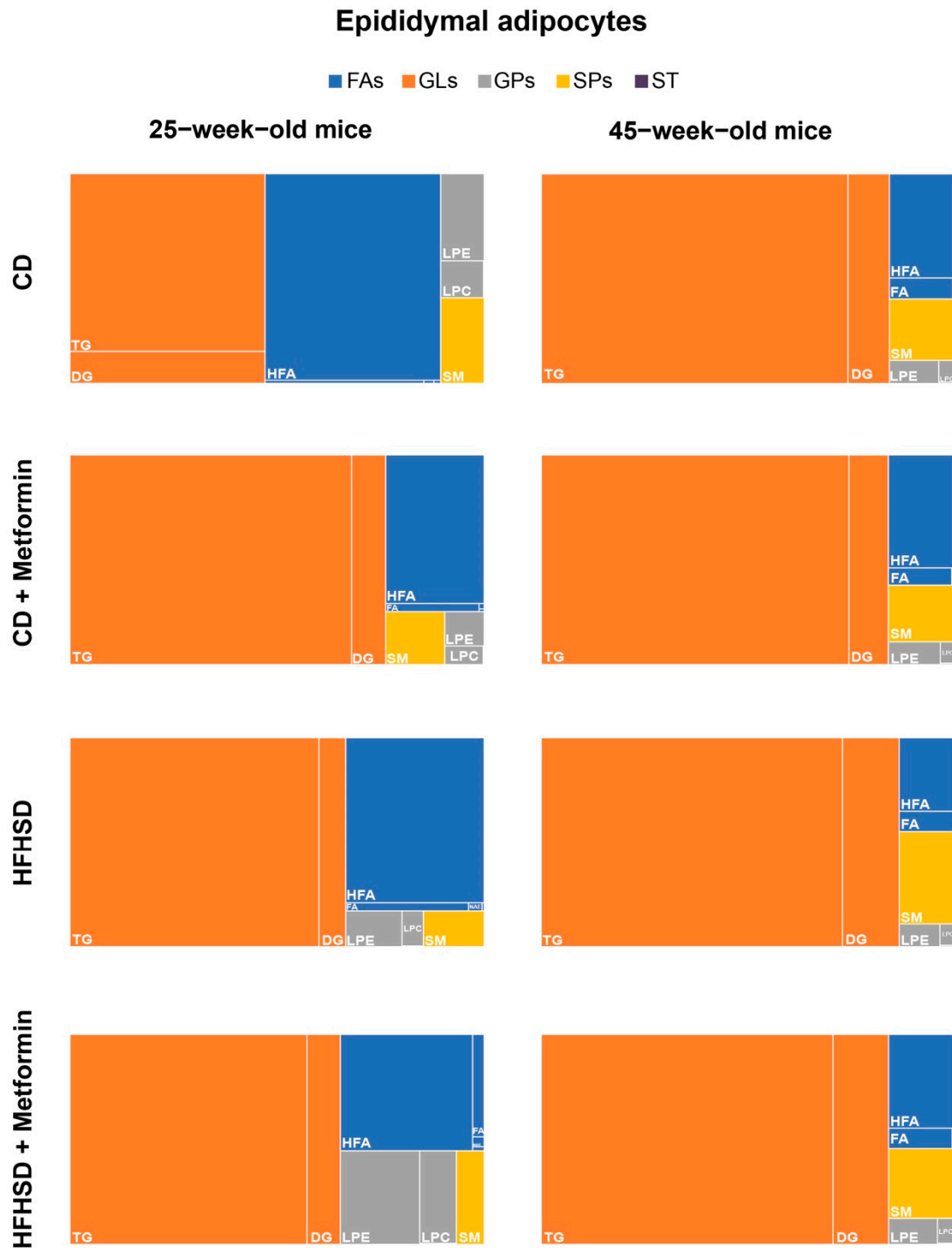
Epididymal adipocytes

Fig. S11. Lipid class distribution in epididymal adipocytes
Treemaps show the relative distribution of main lipid categories and classes in epididymal adipocytes
(*n* = 4/group). These relative distributions serve as a comparative metric of epididymal lipidome
between dietary treatments, age, and metformin treatment. BA: bile acids; CAR: carnitine; CD: chow
diet; DG: diglycerides; FA: fatty acids; FAs: fatty acyls; GLs: glycerolipids; GPs: glycerophospholipids,
HFA: hydroxy fatty acids; HFHSD: high-fat high sucrose diet; LPC: lysophosphatidylcholines; LPE:
lysophosphatidylethanolamines; NAE: N-acyl-ethanolamine's; PC: phosphatidylcholines; SM:
sphingomyelins; SPs: sphingophospholipids; St: steroid hormones; ST: sterol lipids, TG: triglycerides.

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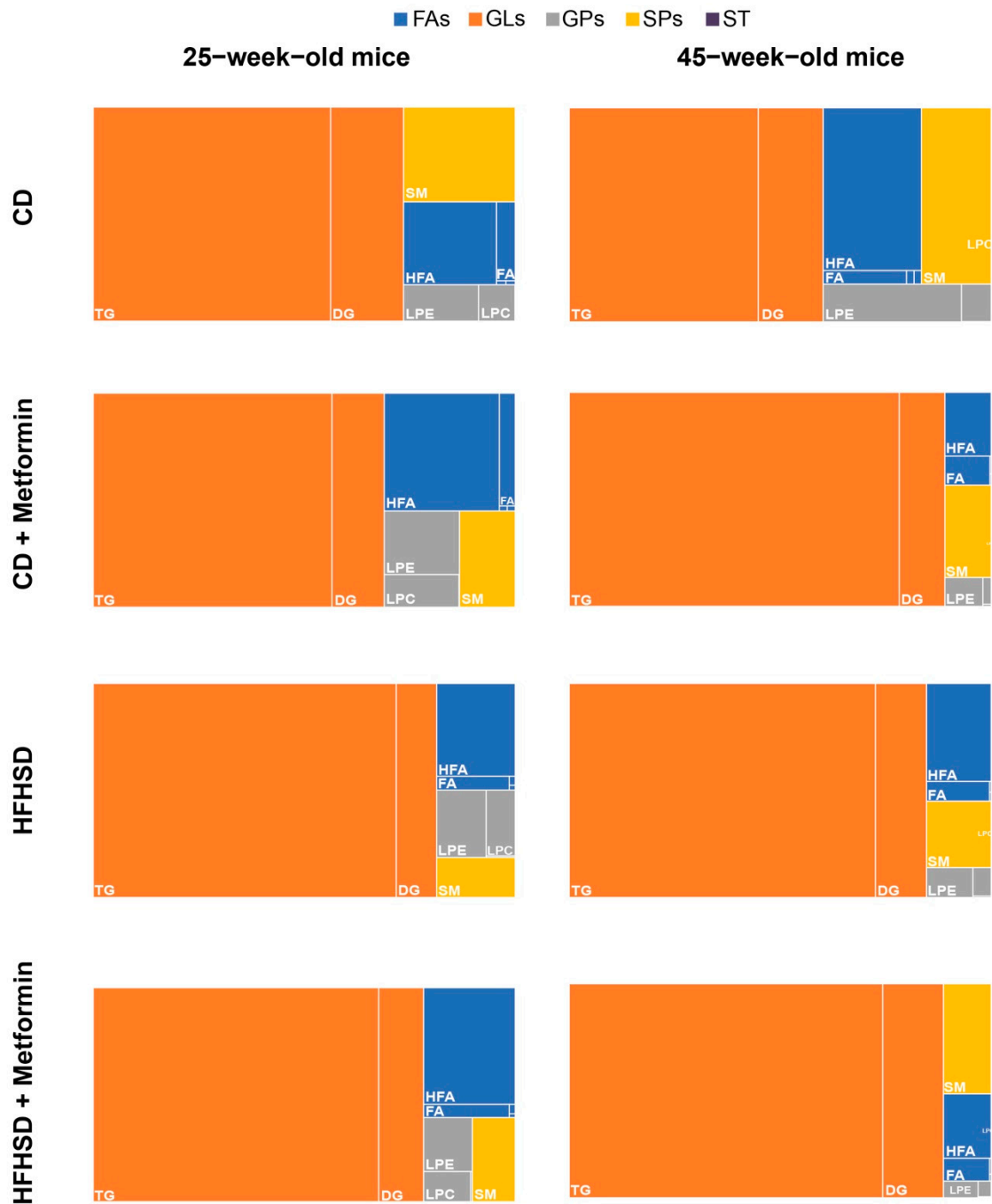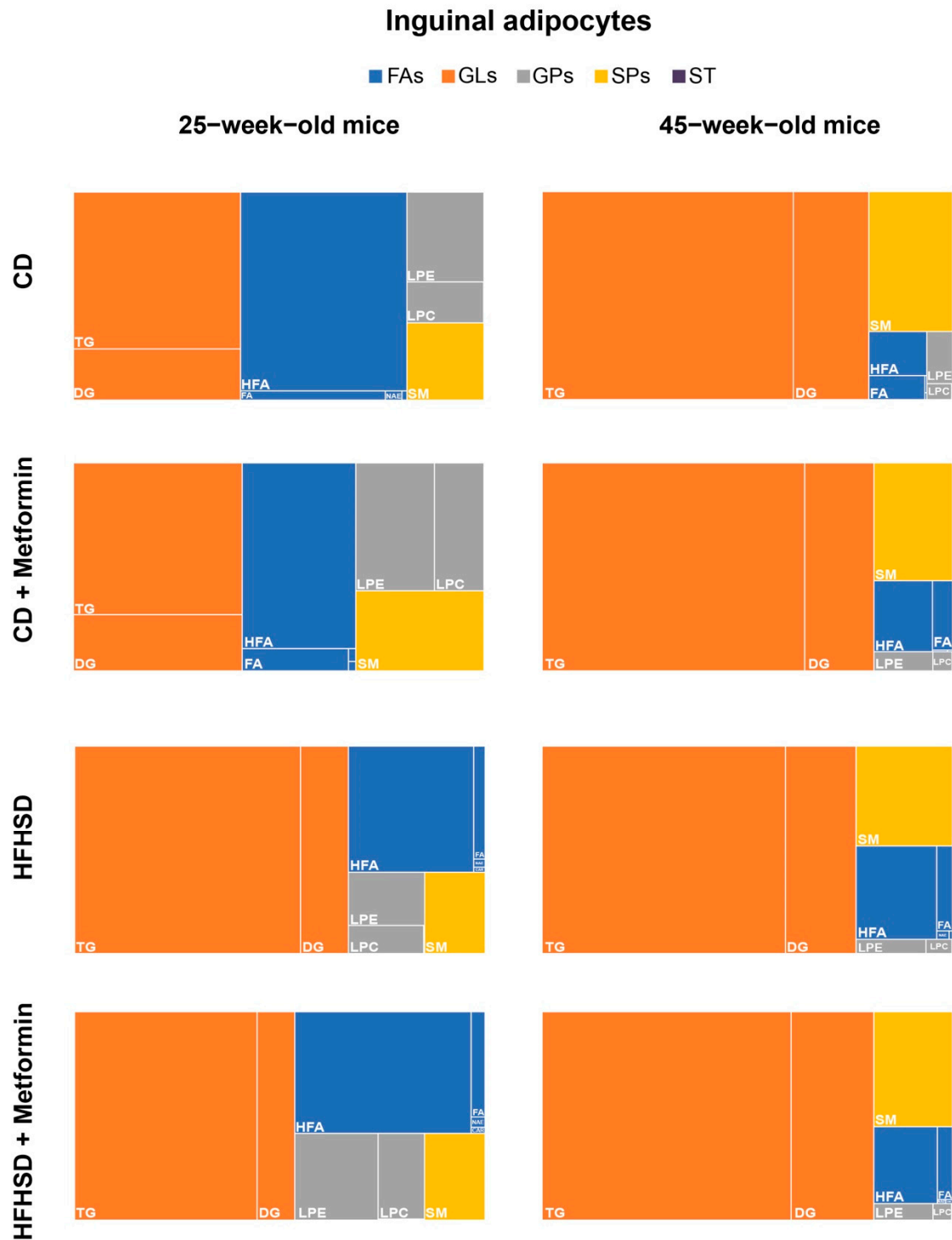# Retroperitoneal adipocytes

■ FAs ■ GLs ■ GPs ■ SPs ■ ST

Fig. S12. Lipid class distribution in retroperitoneal adipocytes

Treemaps show the relative distribution of main lipid categories and classes in retroperitoneal adipocytes (*n* = 4/group). These relative distributions serve as a comparative metric of retroperitoneal lipidome between dietary treatments, age, and metformin treatment. BA: bile acids; CAR: carnitine; CD: chow diet; DG: diglycerides; FA: fatty acids; FAs: fatty acyls; GLs: glycerolipids; GPs: glycerophospholipids, HFA: hydroxy fatty acids; HFHSD: high-fat high sucrose diet; LPC: lysophosphatidylcholines; LPE: lysophosphatidylethanolamines; NAE: N-acyl-ethanolamine's; PC: phosphatidylcholines; SM: sphingomyelins; SPs: sphingophospholipids; St: steroid hormones; ST: sterol lipids, TG: triglycerides.
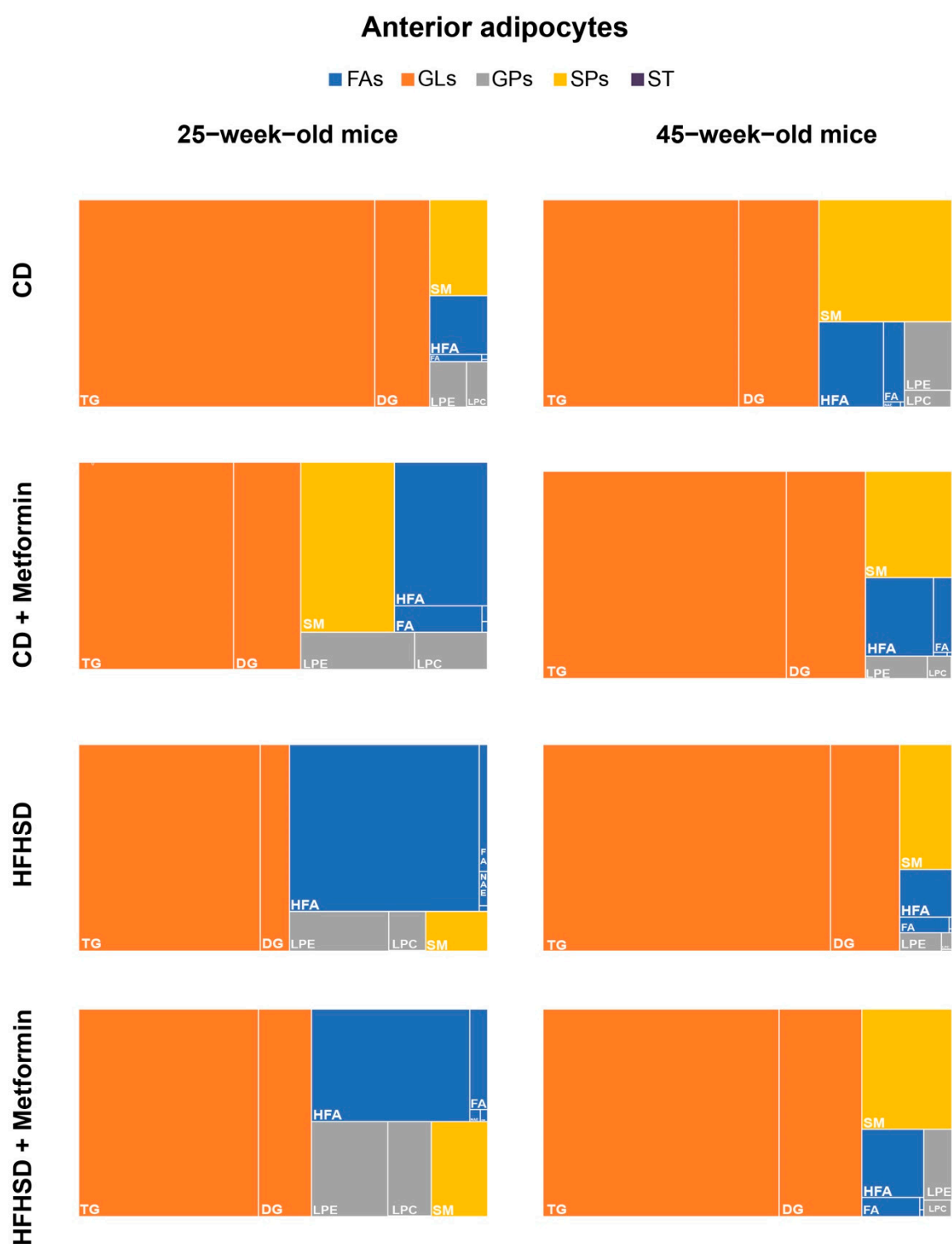
Fig. S13. Lipid class distribution in inguinal adipocytes
Treemaps show the relative distribution of main lipid categories and classes in inguinal adipocytes (*n* = 4/group). These relative distributions serve as a comparative metric of inguinal lipidome between dietary treatments, age, and metformin treatment. BA: bile acids; CAR: carnitine; CD: chow diet; DG: diglycerides; FA: fatty acids; FAs: fatty acyls; GLs: glycerolipids; GPs: glycerophospholipids, HFA: hydroxy fatty acids; HFHSD: high-fat high sucrose diet; LPC: lysophosphatidylcholines; LPE: lysophosphatidylethanolamines; NAE: N-acyl-ethanolamine's; PC: phosphatidylcholines; SM: sphingomyelins; SPs: sphingophospholipids; St: steroid hormones; ST: sterol lipids, TG: triglycerides.
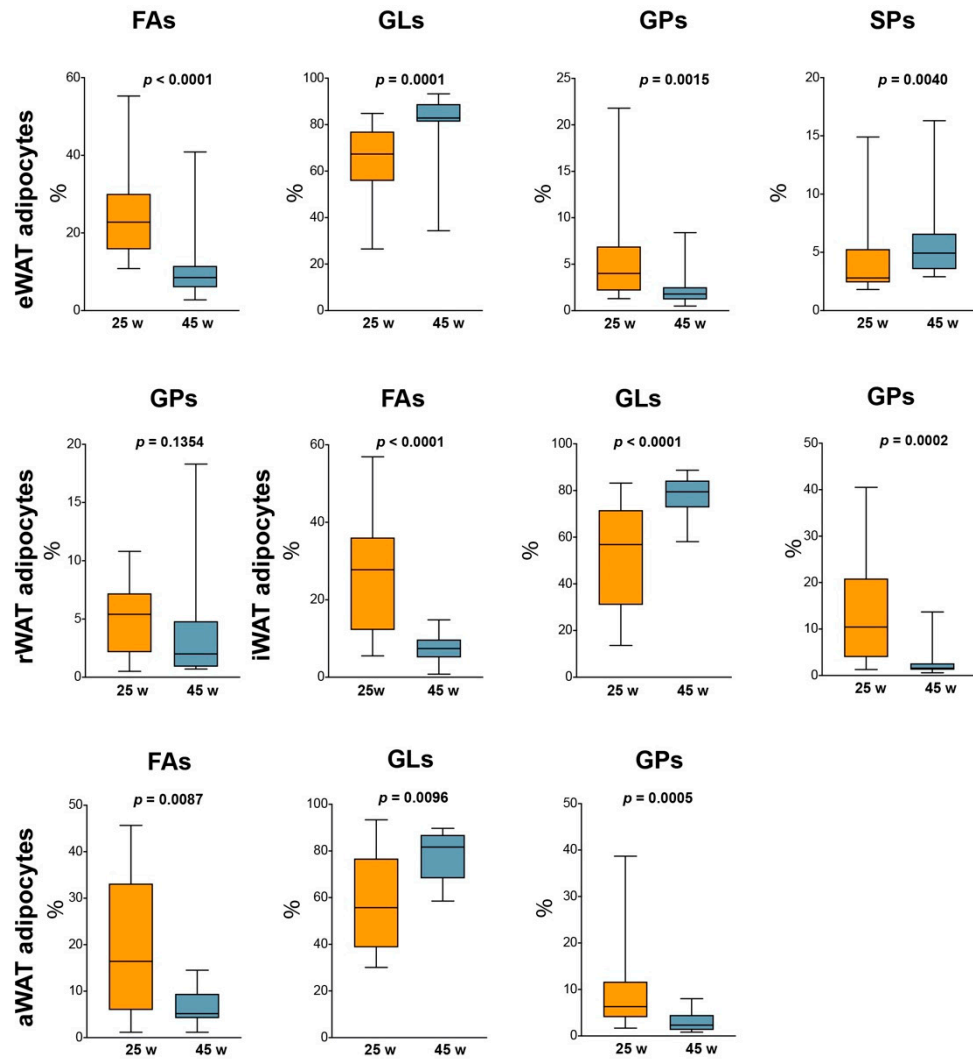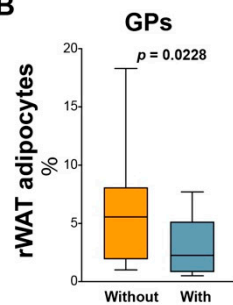
Fig. S14. Lipid class distribution in anterior adipocytes
Treemaps show the relative distribution of main lipid categories and classes in anterior adipocytes (*n* = 4/group). These relative distributions serve as a comparative metric of anterior lipidome between dietary treatments, age, and metformin treatment. BA: bile acids; CAR: carnitine; CD: chow diet; DG: diglycerides; FA: fatty acids; FAs: fatty acyls; GLs: glycerolipids; GPs: glycerophospholipids, HFA: hydroxy fatty acids; HFHSD: high-fat high sucrose diet; LPC: lysophosphatidylcholines; LPE: lysophosphatidylethanolamines; NAE: N-acyl-ethanolamine's; PC: phosphatidylcholines; SM: sphingomyelins; SPs: sphingophospholipids; St: steroid hormones; ST: sterol lipids, TG: triglycerides.
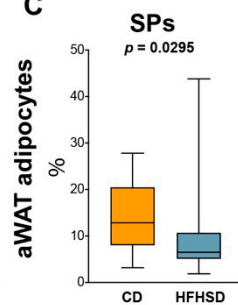
A

Fig. S15. Age, diet and metformin effects in adipocyte lipid categories

(**A**) Box plots show the lipid category alterations in visceral and subcutaneous adipocytes according to age, (**B**) metformin and (**C**) diet (*n* = 4/group). Data are presented as means, maximum and minimum. *p* values < 0.05 are considered significant. (Wilcoxon-rank sum test). aWAT: anterior white adipose tissue; CD: show diet; eWAT: epididymal white adipose tissue; FAs: fatty acyls; GLs: glycerolipids; GPs: glycerophospholipids; HFHSD: high-fat high sucrose diet; iWAT: inguinal white adipose tissue; rWAT: retroperitoneal white adipose tissue; SPs: sphingophospholipids, ST: sterol lipids.
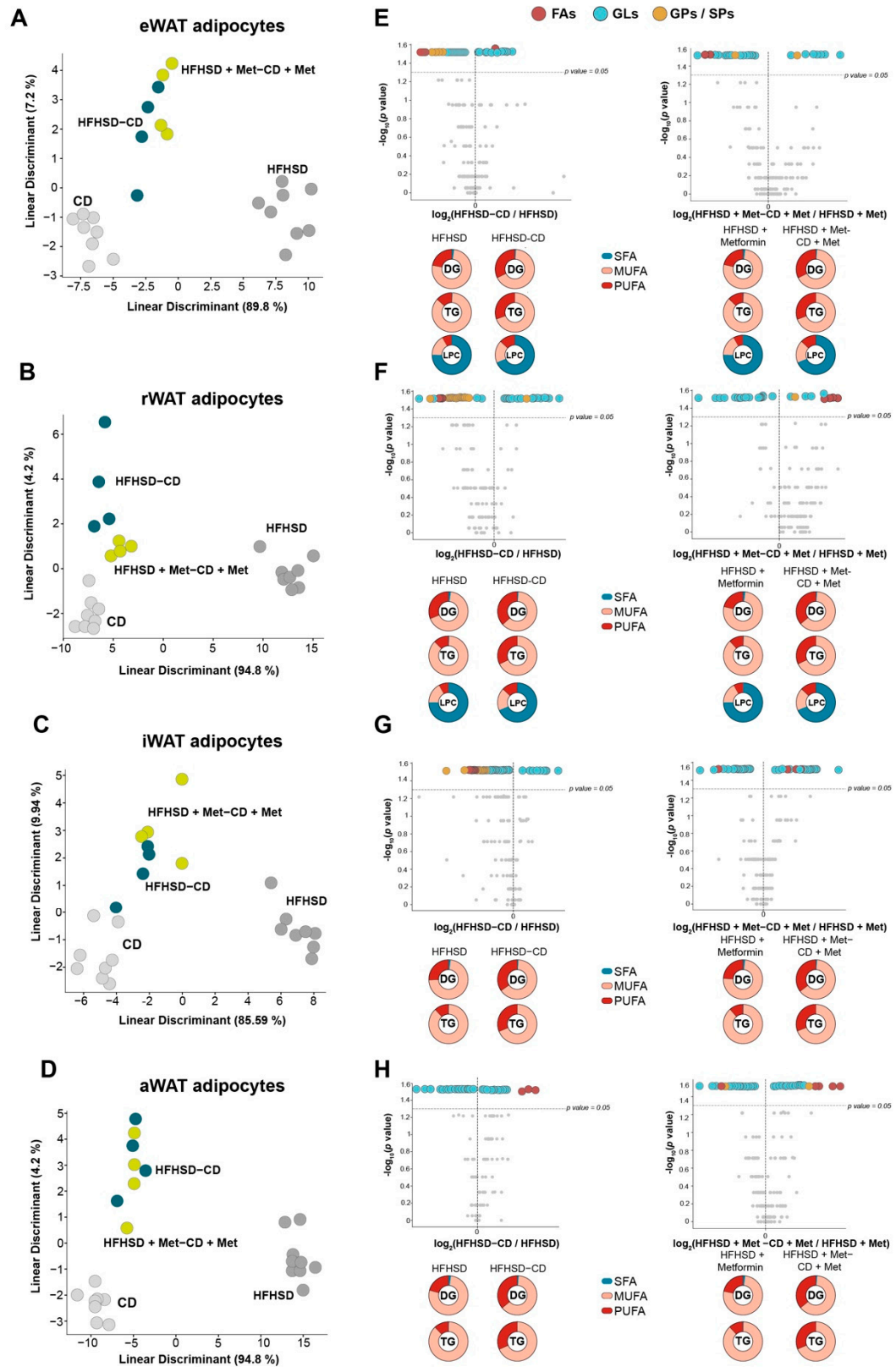
Fig. S16. Dietary intervention increased the lipid unsaturation in adipocytes
Lipid signature analysis between dietary intervention with or without metformin in obese mice in (**A**) epididymal (eWAT), (**C**) retroperitoneal (rWAT), (**E**) inguinal (iWAT), (**G**) anterior (aWAT) adipocytes (*n* = 4/group). (**B**) Volcano plots of the effect of dietary intervention with or without metformin

administration in (**B-D**) visceral and (**F-H**) subcutaneous adipocytes (n = 4/group). Significant lipid species are colored according to the lipid category. Levels of significance below 0.05 were considered significant (Wilcoxon-rank sum test).CD: chow diet; DG: diglycerides; FAs: fatty acyls; GLs: glycerolipids; GPs: glycerophospholipids; HFHSD: high-fat high-sucrose diet LPC: lysophosphatidylcholines; Met: metformin; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids, SFA: saturated fatty acids; SPs: sphingophospholipids; TG: triglycerides.