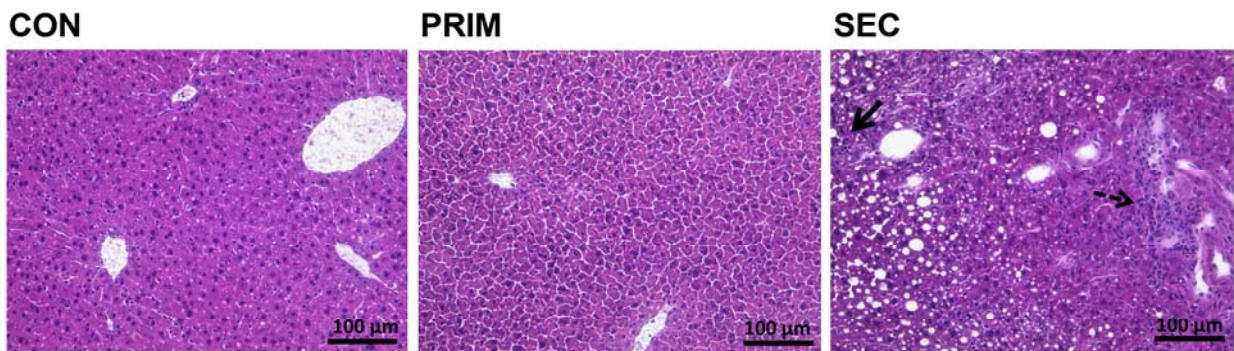# SUPPLEMENTARY DATA

Supplementary Figure S1.
(A) Representative images of the livers after hematoxylin-eosin staining. Full arrow: lobular inflammation; dashed arrow: portal inflammation.
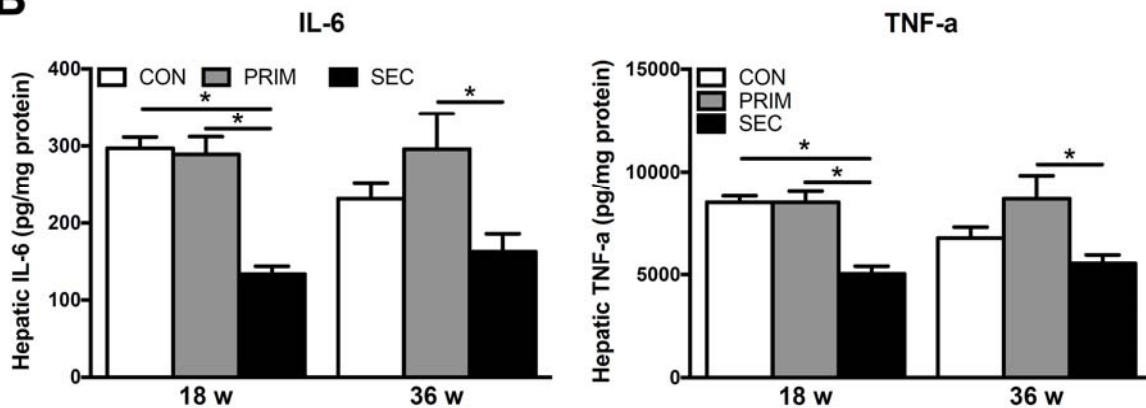(B) Hepatic levels of IL-6 and TNF-α cytokines.
(C) Composition of hepatic fatty acids was detected by 1H MRS *in vivo*. SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.
All data are presented as mean ± SEM; n=5-6 per group (B) and n=7-9 per group (C). *p<0.05, **p < 0.01; 2-way ANOVA with Bonferroni correction.
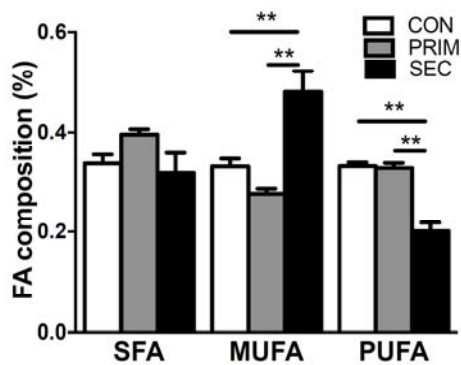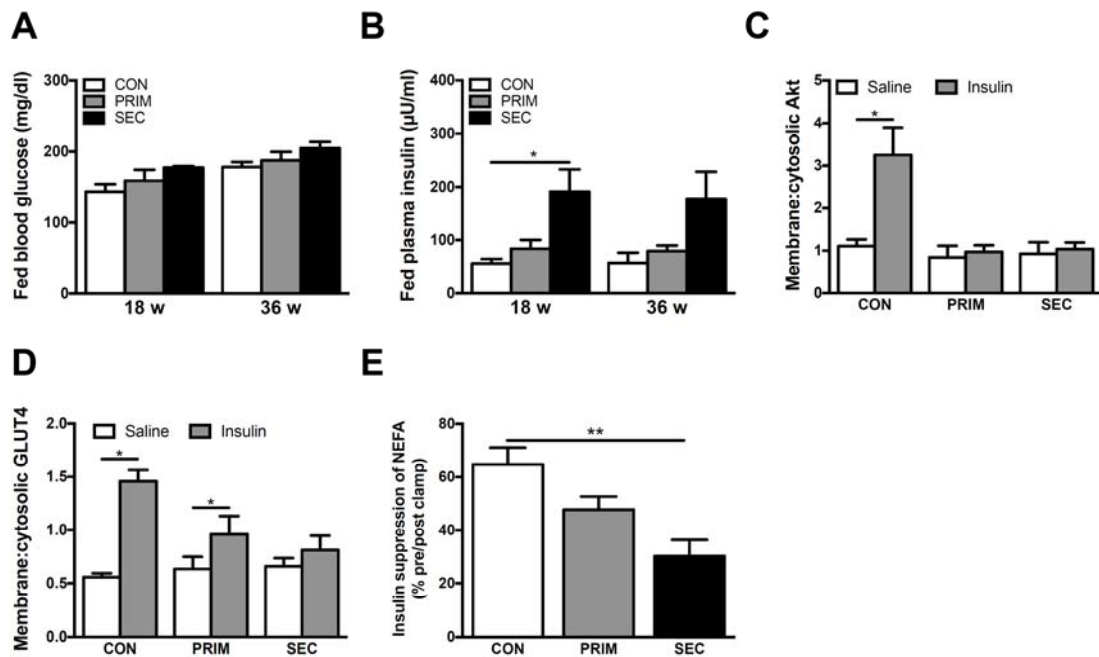
SUPPLEMENTARY DATA

Supplementary Figure S2. *Ex vivo* insulin sensitivity in 18 w old mice.
(A,B) Blood glucose (A) and plasma insulin (B) levels in fed state.
(C,D) Cytosolic-to-membrane translocation of Akt in the liver (A) and GLUT4 in the gastrocnemius muscle (B) of 18 w old mice. Tissues were collected 10 min after saline or insulin (1 U/kg) intraperitoneal injection under 6 h fasted conditions (n=6-8 per group).
(C) Insulin suppression of non-esterified fatty acids (NEFA) in the plasma of 36 w old mice from blood collected before the start of the clamp (6 h fasting) and at the end of the clamp (hyperinsulinemia-euglycemia) (n=5-8 per group).
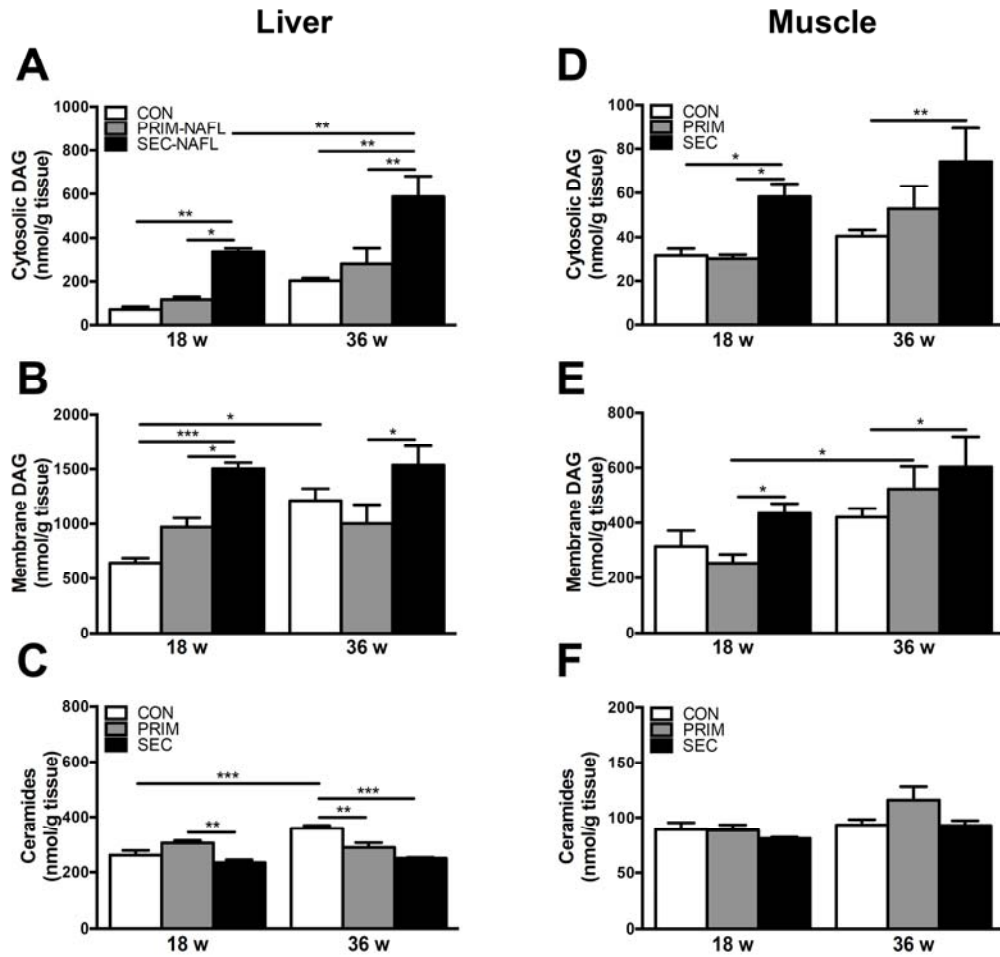All data are presented as mean ± SEM. *p < 0.05, **p < 0.01; 2-way ANOVA with Bonferroni correction.

SUPPLEMENTARY DATA

Supplementary Figure S3. Related to Figure 3.
Lipidomic analysis in the liver (A-C) and muscle (D-F) of 6 h fasted mice.
Total cytosolic (A,D) and membrane (B,E) diacylglycerols and total ceramides (C,F).
All data are presented as mean ± SEM (n=6-7 per group). *p < 0.05, **p < 0.01, ***p < 0.001; 2-way
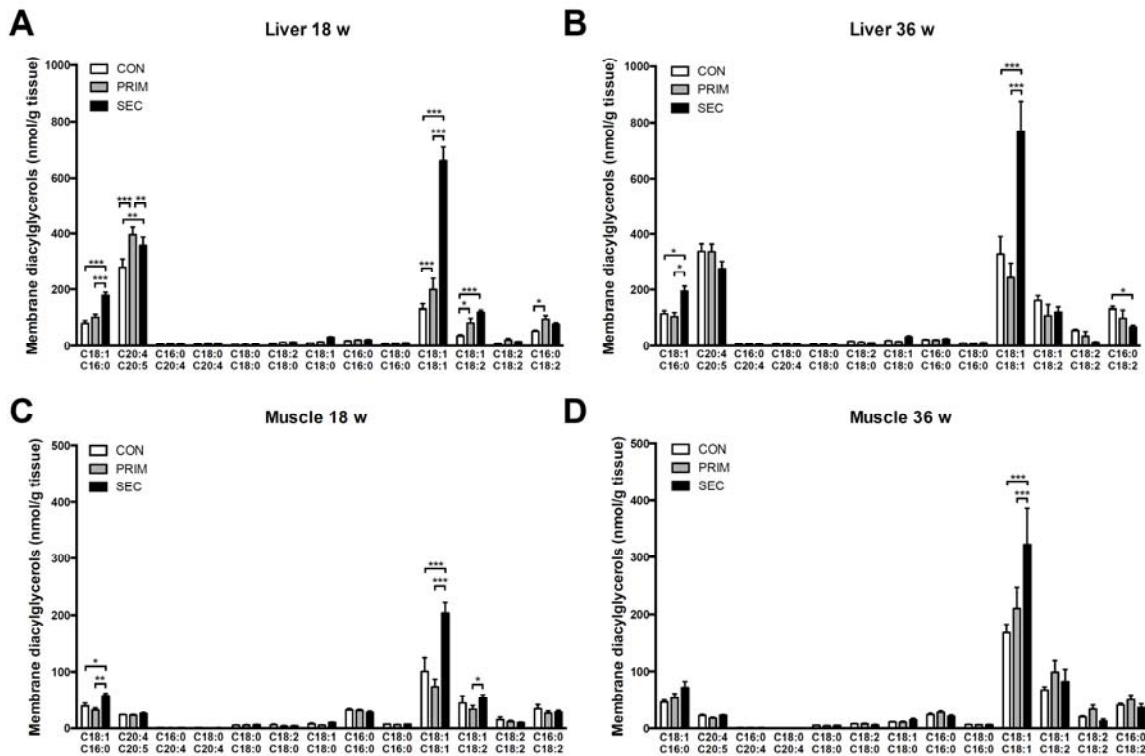ANOVA with Bonferroni correction.

SUPPLEMENTARY DATA

Supplementary Figure S4. Related to Figure 3.
Fatty acid composition of membrane diacylglycerols in the liver (A,B) and muscle (C,D) of 6 h fasted mice.
Membrane diacylglycerols of 18 w (A,C) and 36 w (B,D) old mice.
All data are presented as mean ± SEM (n=6-7 per group). *p < 0.05, **p < 0.01, ***p < 0.001; 2-way ANOVA with Bonferroni correction.
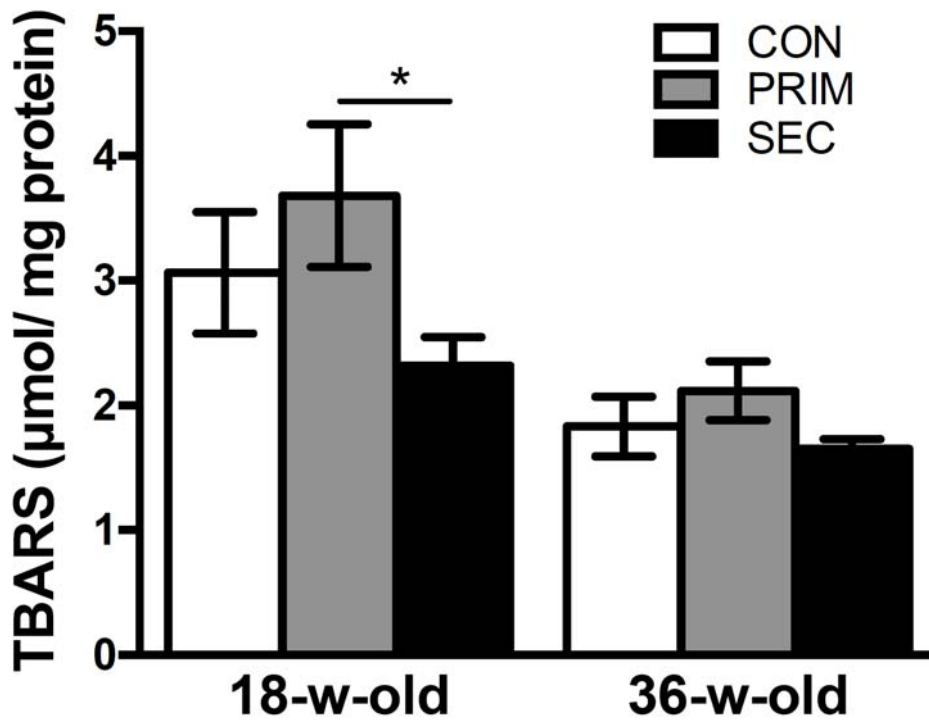
SUPPLEMENTARY DATA

Supplementary Figure S5. Related to Figure 5.
Hepatic lipid peroxidation.
Hepatic thiobarbituric acid reactive substances (TBARS) were used as marker of lipid peroxidation and normalized per protein content.
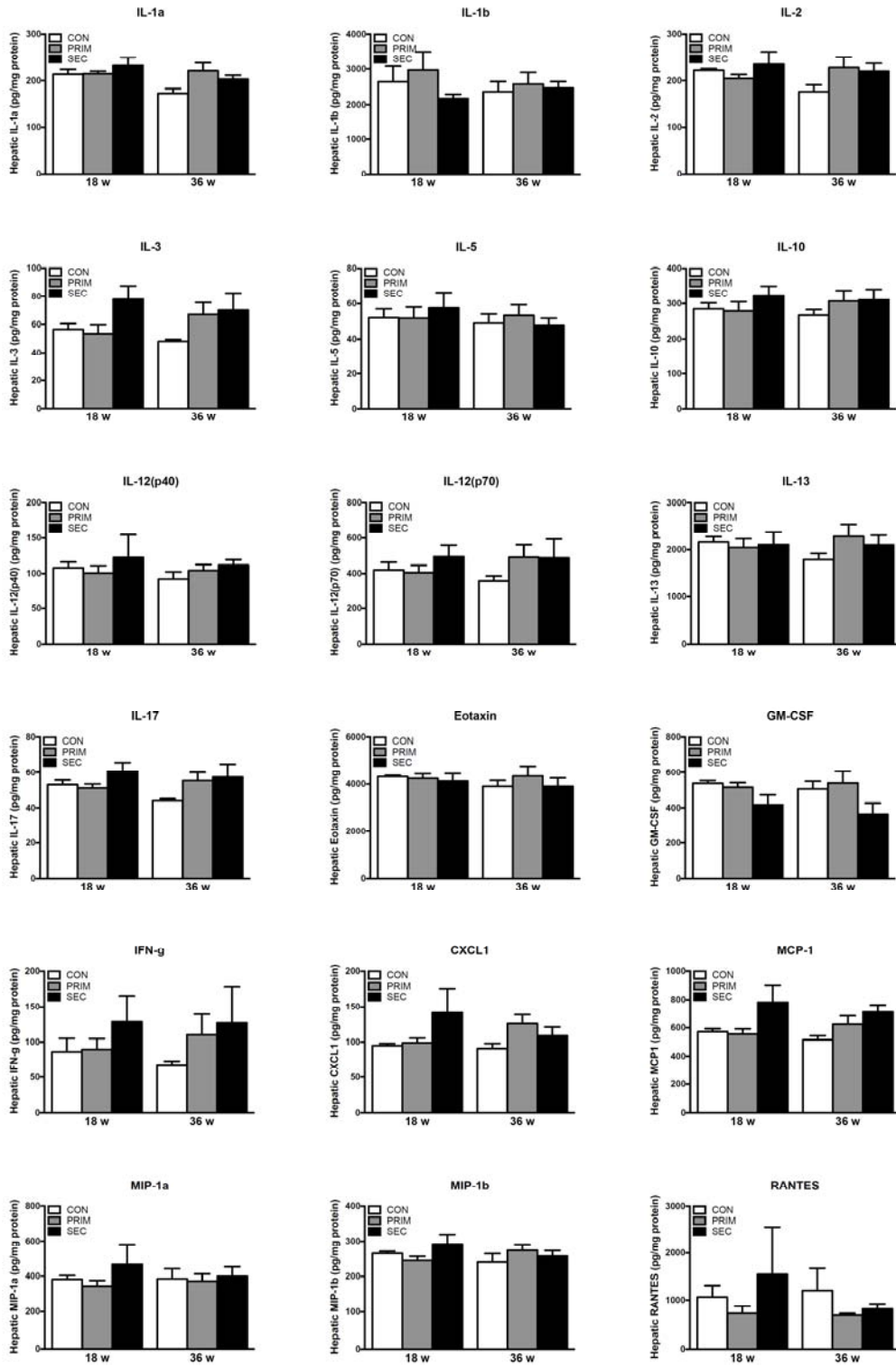All data are presented as mean ± SEM (n=6-7 per group). *p < 0.05; 2-way ANOVA with Bonferroni correction.

SUPPLEMENTARY DATA

Supplementary Figure S6.
The levels of hepatic cytokines.
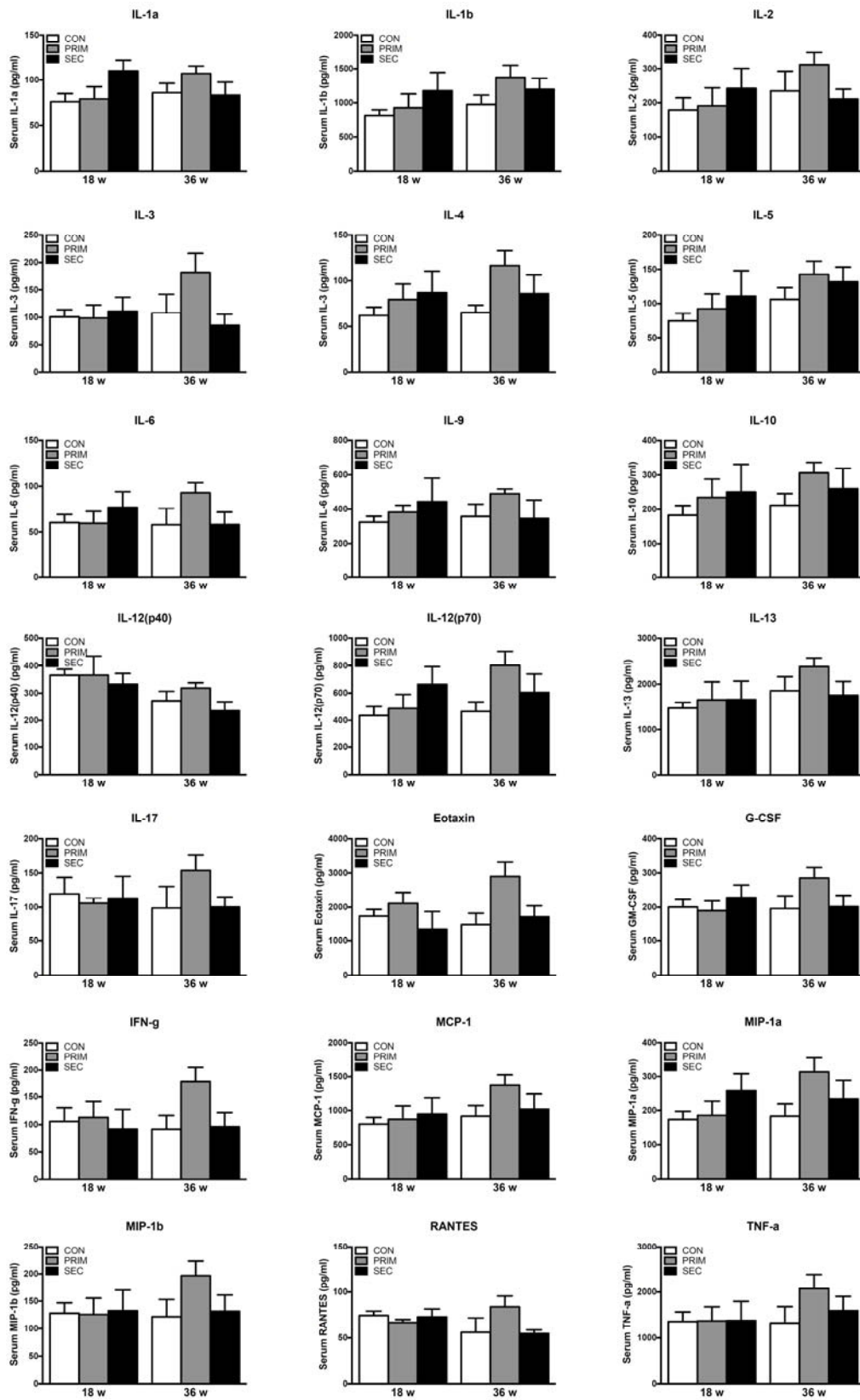All data are presented as mean ± SEM (n=5-6 per group). 2-way ANOVA with Bonferroni correction.

SUPPLEMENTARY DATA

Supplementary Figure S7.
The levels of circulating cytokines.
All data are presented as mean ± SEM (n=5-6 per group). 2-way ANOVA with Bonferroni correction.

SUPPLEMENTARY DATA

SUPPLEMENTARY DATA

Supplementary Table S1, related to Figure
Energy balance of CON, PRIM and SEC mice at the age of 18 w and 36 w

		18 w			36 w		
		CON	PRIM	SEC	CON	PRIM	SEC
Females (n)		8	9	6	10	7	7
Energy expenditure	Light	15.9 ± 0.5	14.3 ± 0.5	14.7 ± 0.5	15.5 ± 0.6	14.3 ± 0.5	14.1 ± 0.5
(kcal/h/kg)	Dark	19.8 ± 0.6	18 ± 0.9	18.6 ± 0.9	19.2 ± 0.7	17.8 ± 0.7	18.1 ± 0.8
Food intake	Light	36 ± 4	42 ± 4	58 ± 8	47 ± 6	46 ± 5	42 ± 5
(g/kg BW)	Dark	90 ± 8	91 ± 5	98 ± 11	105 ± 12	94 ± 6	95 ± 6
Water intake	Light	36 ± 5	55 ± 4	76 ± 14*	49 ± 5	51 ± 3	38 ± 8
(ml/kg BW)	Dark	97 ± 7	108 ± 10	126 ± 16	105 ± 4	110 ± 11	108 ± 7
Activity	Light	322 ± 33	450 ± 44	364 ± 36	337 ± 22	351 ± 27	235 ± 23
(Counts)	Dark	915 ± 55	1197 ± 93	895 ± 36‡	1320 ± 96	1007 ± 91#	656 ± 89#‡

Data are presented as means ± SEM. P values were calculated by repeated measures two-way ANOVA with Bonferroni post hoc analysis. *$P<0.05$ and #$P<0.01$ vs. CON at respective age and condition. ‡$P<0.01$ vs. PRIM at respective age and condition.