Carvalho et al, Effects of diet and development on the *Drosophila* lipidome

Supplementary Information

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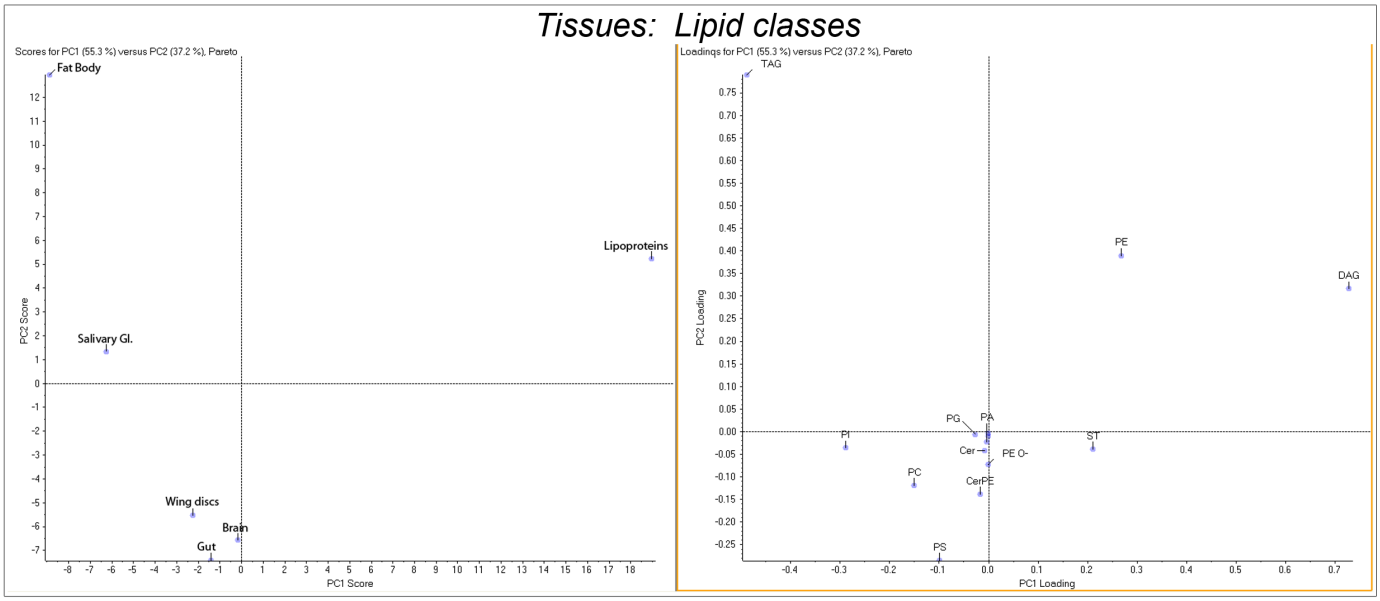
Table S1
Weighted average of fatty acid chain length and unsaturation in larval tissues and diets

Supplementary Datasets (available as a separate MS Excel file):

Dataset 1: Lipid species quantified in the different *Drosophila* diets
Dataset 2: Lipid species quantified in larval tissues of *Drosophila* fed on different diets
Dataset 3: Lipid species quantified over *Drosophila* development



Supplementary figure S1. Comparison of tissue lipid compositions, including neutral lipids. Depicted is the Principal Component Analysis (PCA) made by pareto analysis of the lipid class amounts quantified from different tissues and hemolymph lipoproteins of D. melanogaster early wandering 3rd instar larvae fed YF. Before performing the PCA, we calculated for each tissue the mol% represented by each lipid class with respect to total membrane lipids (not including TAG or DAG). Fat body and salivary gland lipid compositions are distinguished from the other tissues due to their content of TAGs. Lipoproteins are distinct from the other larval tissues due to their enrichment in DAGs and PE.

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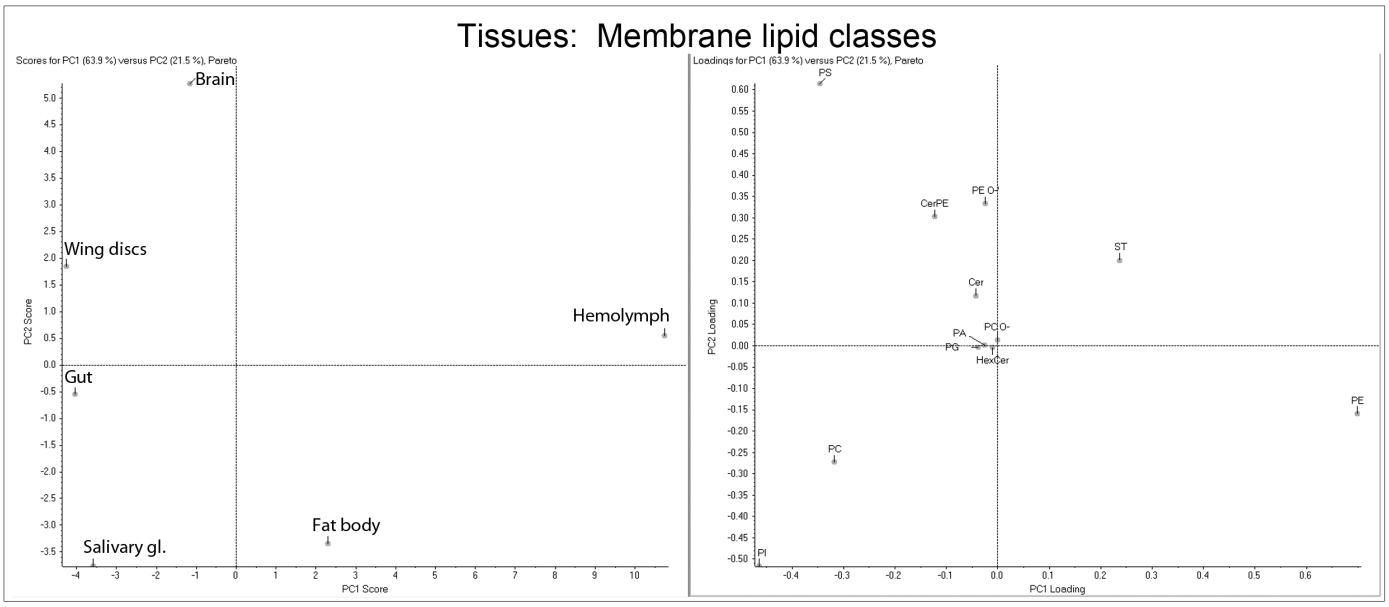

Supplementary figure S2. Comparison of tissue membrane lipid compositions. Depicted is the Principal Component Analysis (PCA) made by pareto analysis of the membrane lipid class amounts quantified in different tissues and hemolymph lipoproteins of D. melanogaster early wandering 3rd instar larvae fed YF. Before performing the PCA, we calculated for each tissue the mol% represented by each lipid class with respect to total membrane lipids (not including TAG or DAG).

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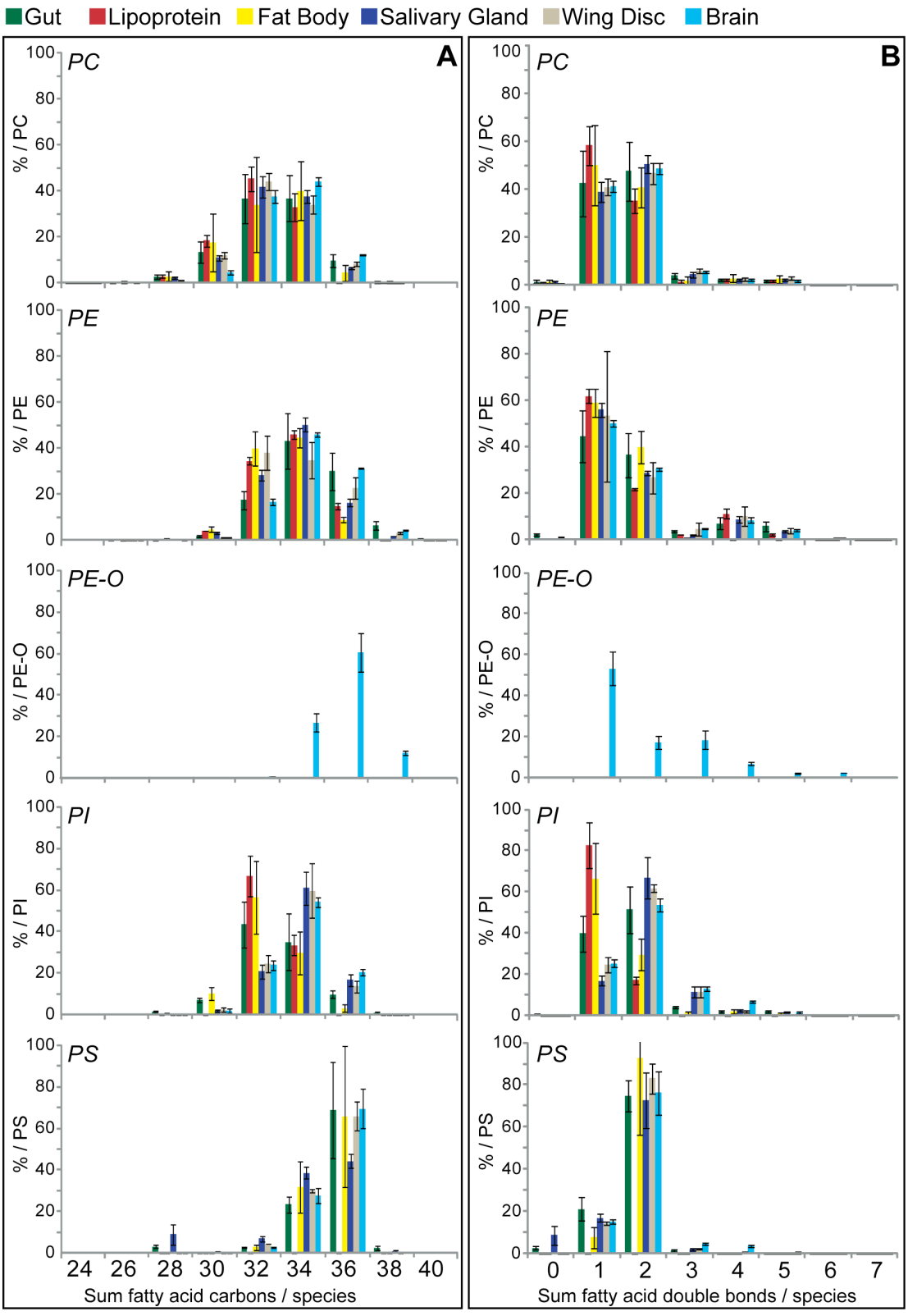
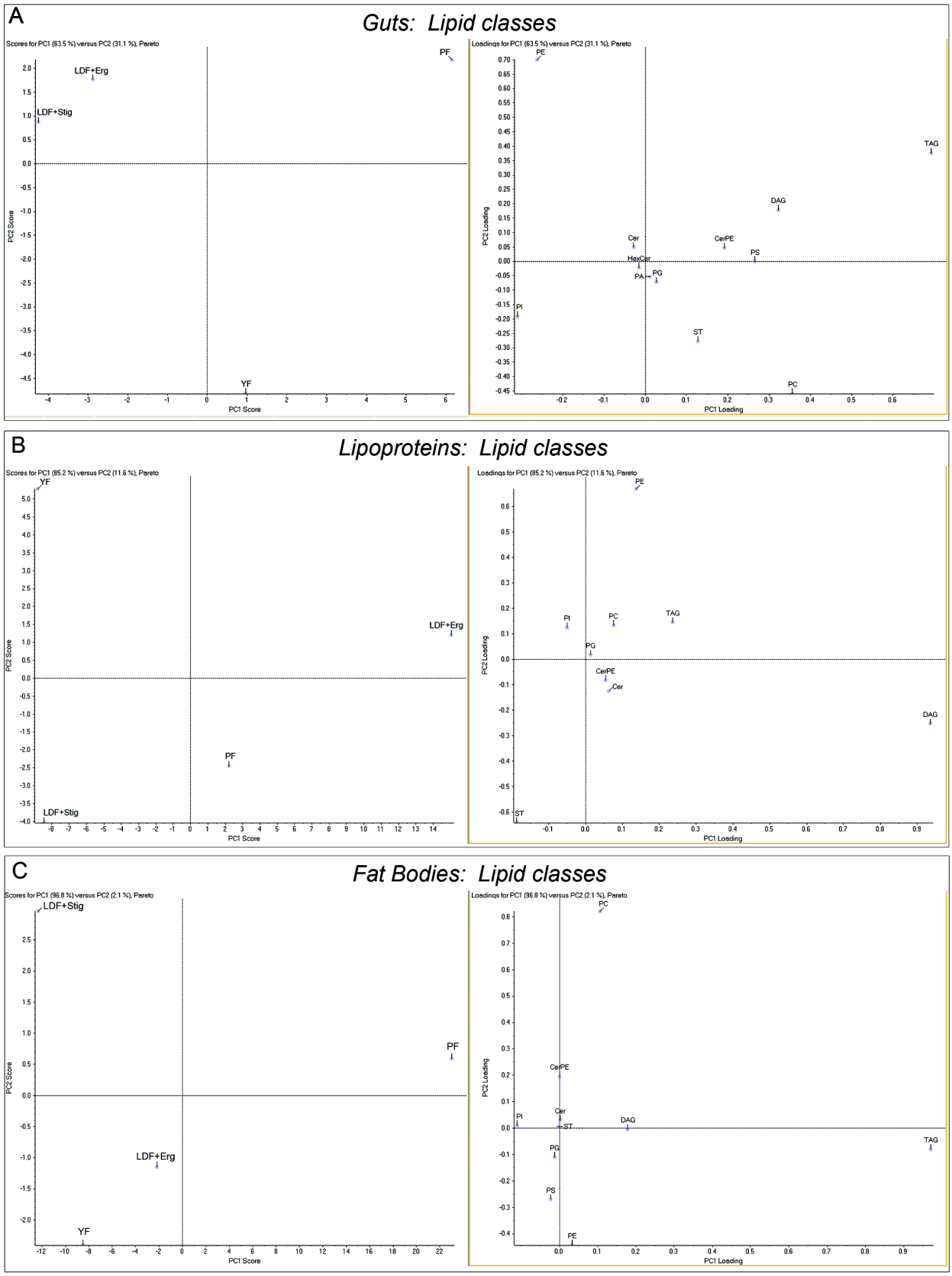
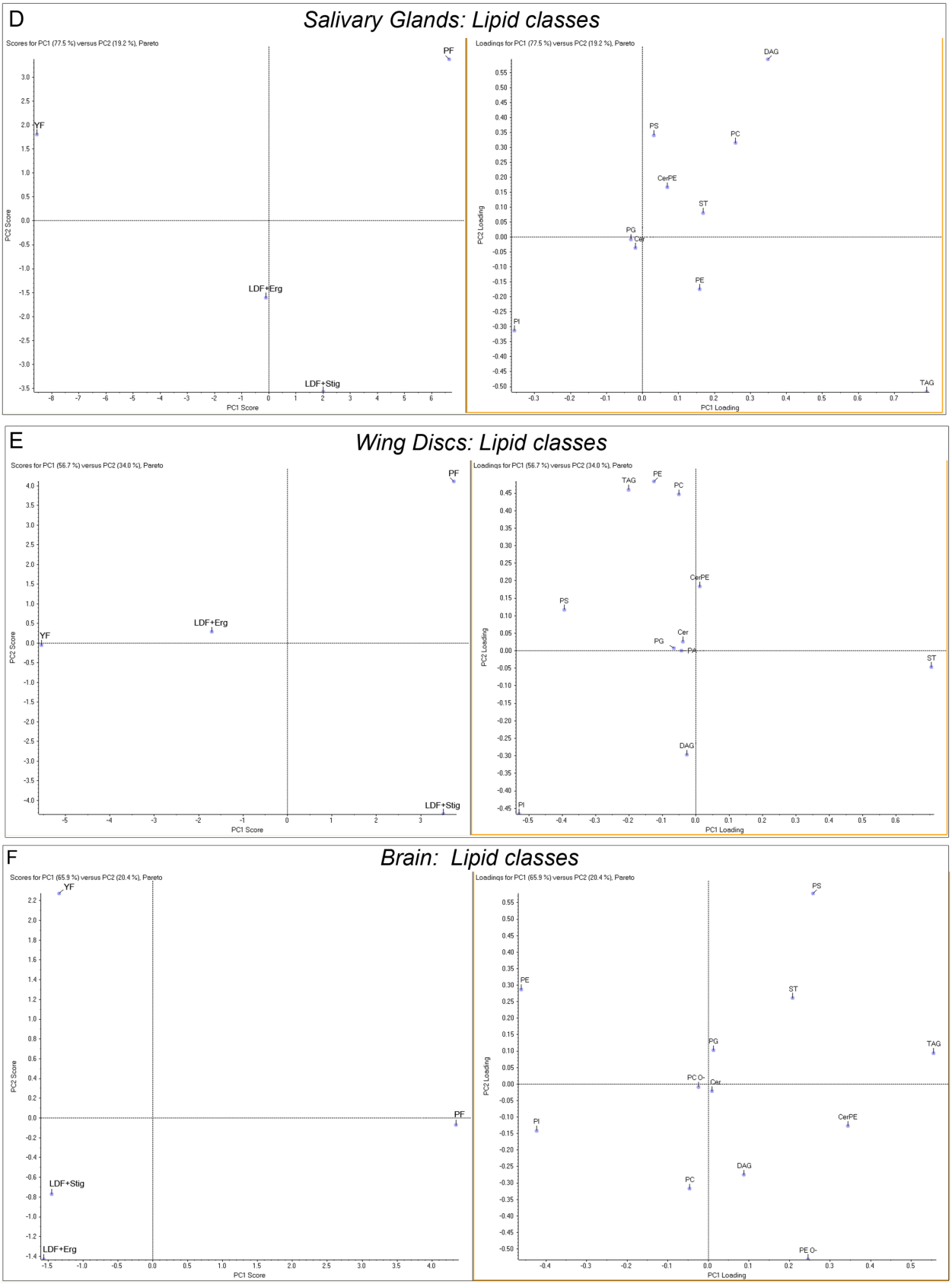


Supplementary figure S3. Combined fatty acid chain length and unsaturation in different phospholipid classes in different tissues. (A,B) show lipid species from different tissues (color coded as indicated) of early wandering 3rd instar larvae fed with YF. They depict the relative abundance within the indicated phospholipid classes of species containing the indicated combined fatty acid chain lengths (A) and number of double bonds (B). Error bars indicate standard deviation.



Supplementary figure S4. Effect of diet on tissue lipid class composition (including neutral lipids) (A-F) show different Principal Component Analyses (PCA) made by pareto analysis of the lipid class amounts (including TAG and DAG) present in tissues of larvae fed 4 different diets: YF, PF, LDF+ergosterol and LDF+stigmasterol. A separate analysis was performed for each tissue. (A) Guts. (B) Lipoproteins. (C) Fat bodies. (D) Salivary glands. (E) Wing discs. (F) Brain. ***(continues)***



Supplementary figure S4. Effect of diet on tissue lipid class composition (including neutral lipids) ***(continuation)*** Before performing the PCA, we calculated for each tissue the mol% represented by each lipid class with respect to total membrane lipids (not including TAG or DAG).

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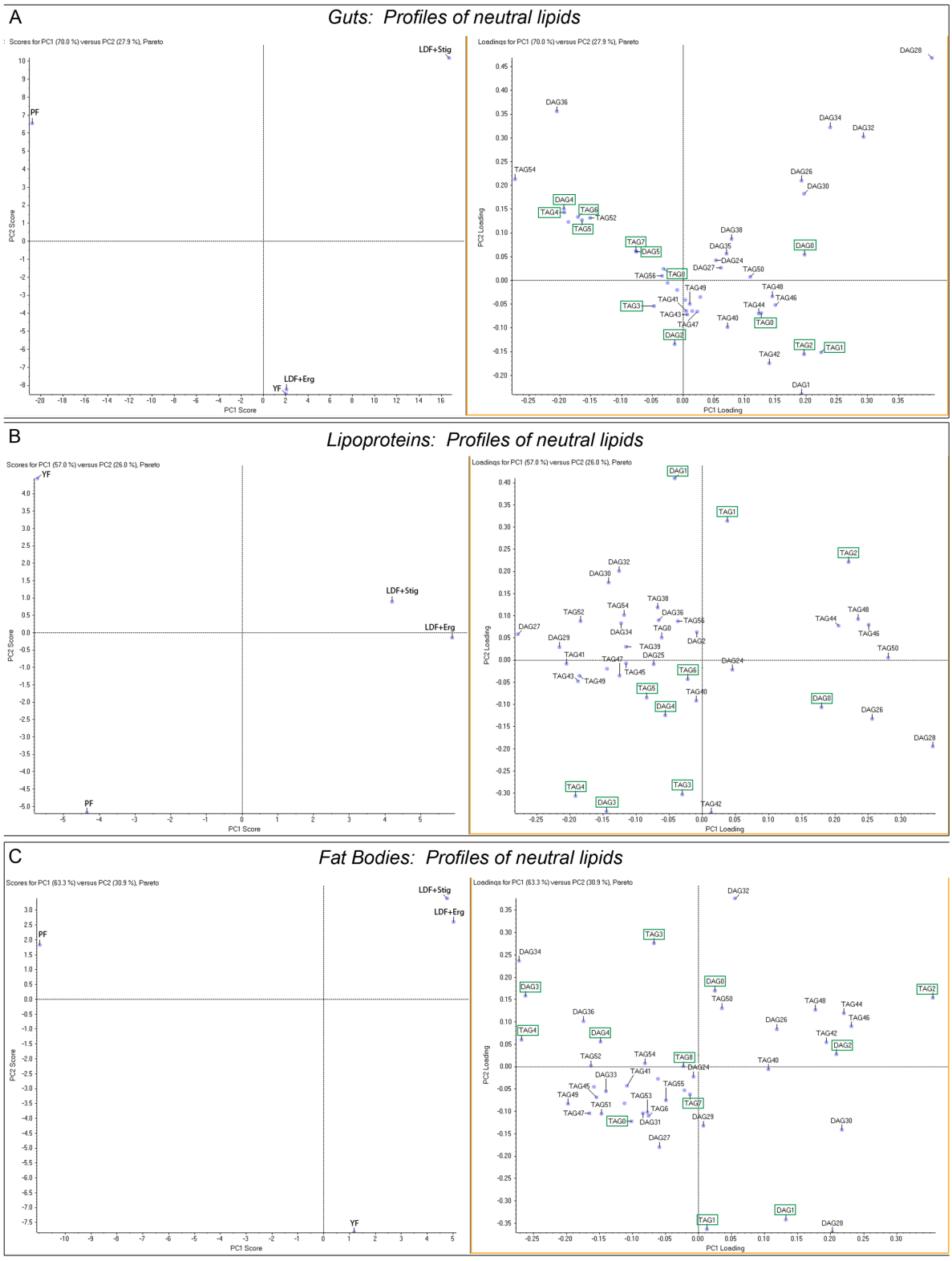
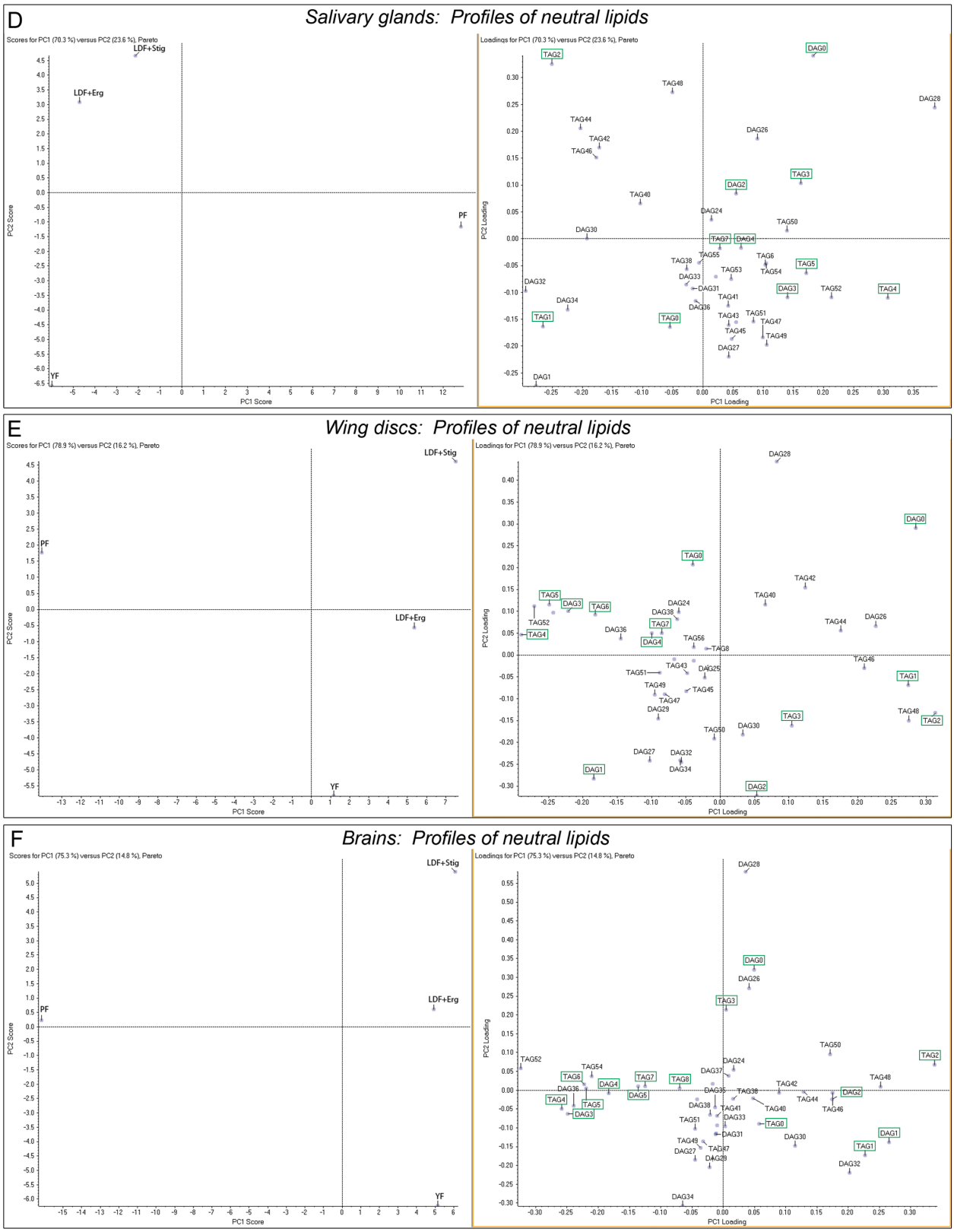
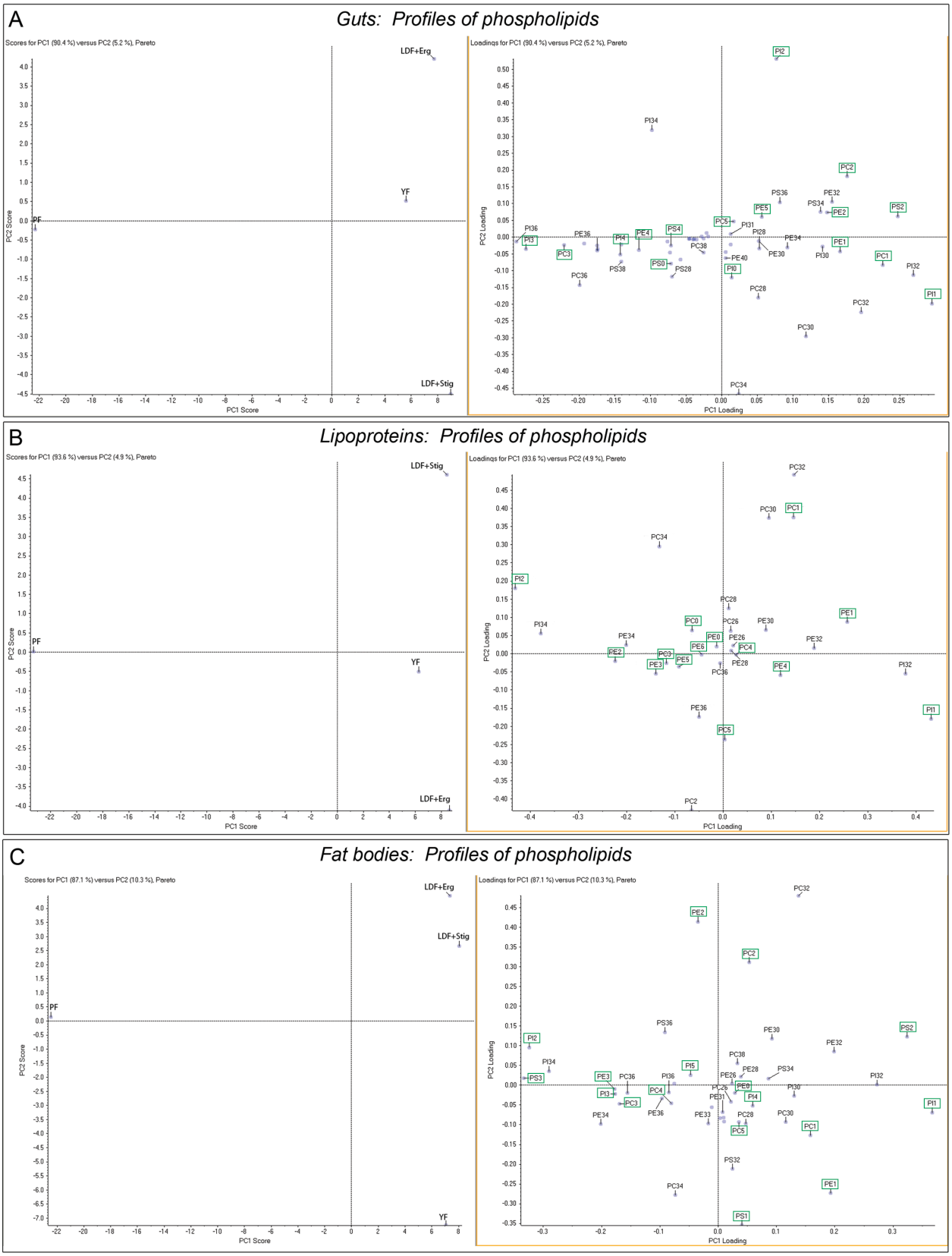


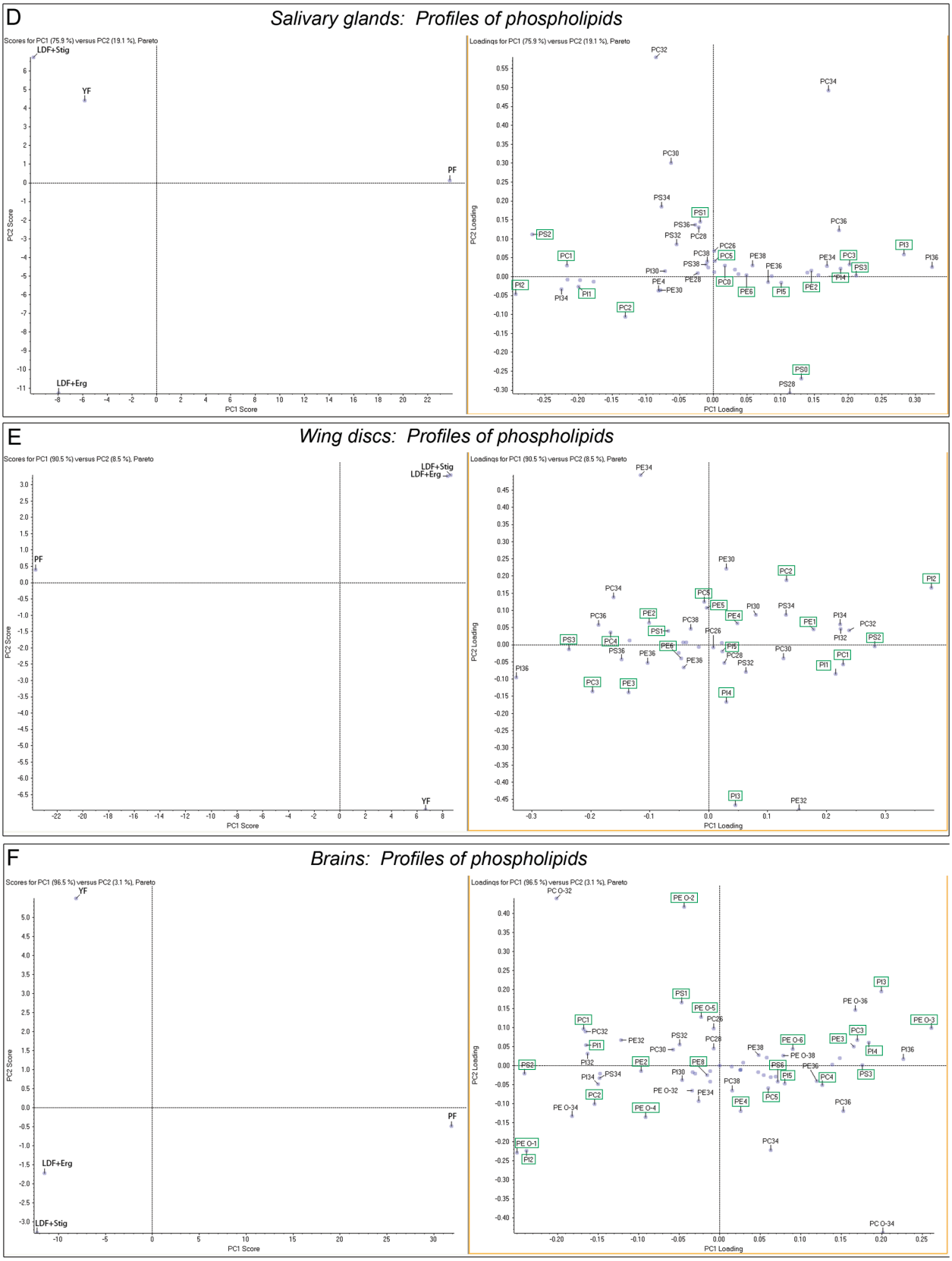
Supplementary figure S5. Effect of diet on neutral lipid species found in different tissues (A-F) show different Principal Component Analyses (PCA) made by pareto analysis of the neutral lipid species profiles from tissues of larvae fed with 4 different foods: YF, PF, LDF+ergosterol and LDF+stigmasterol. Before performing the PCA, the species profiles of TAGs and DAGs were determined by calculating the relative amounts of species with different combined fatty acid chain lengths and unsaturations. A separate analysis was performed for each tissue: (A) Guts. (B) Lipoproteins. (C) Fat bodies. (D) Salivary glands. (E) Wing discs. (F) Brain. ***(continues)***



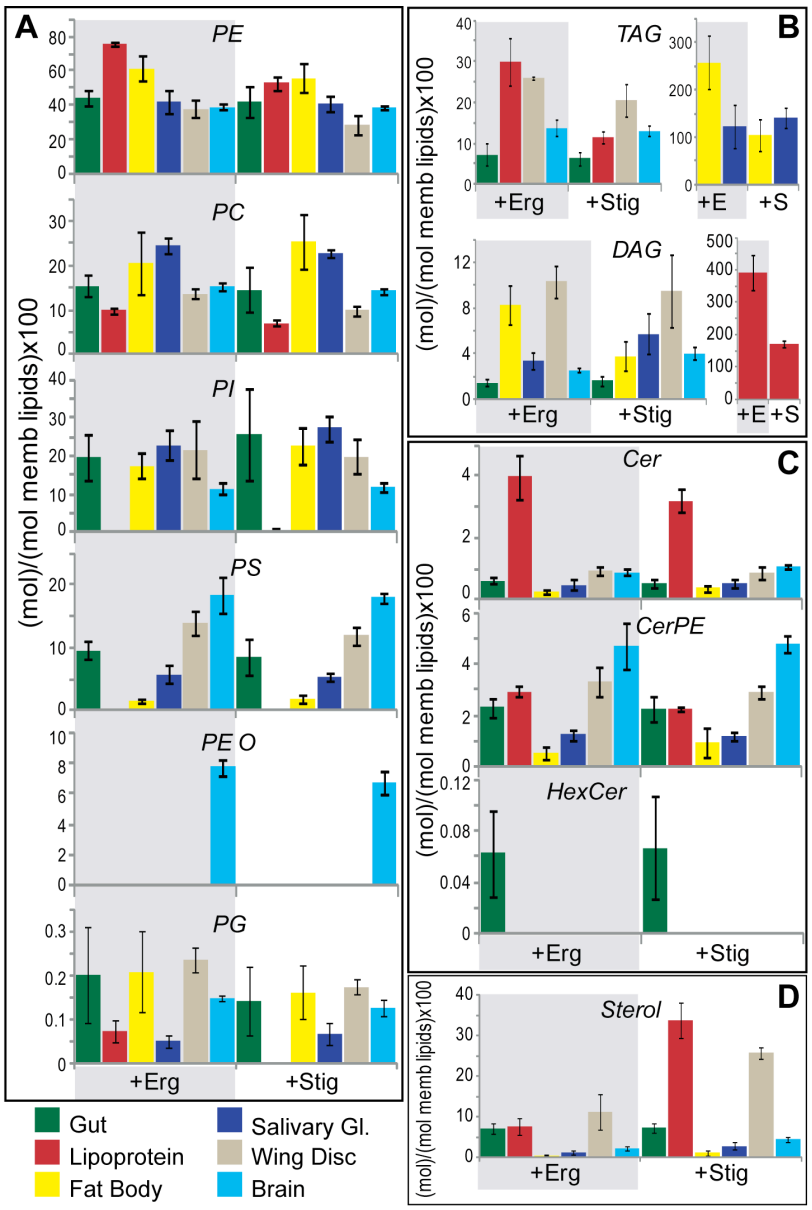
Supplementary figure S5. Effect of diet on neutral lipid species found in different tissues *(continuation)* TAG and DAG species in tissues of larvae fed PF are distinct from those of larvae fed with other foods in having longer and more unsaturated fatty acids. Labels with green boxes denote double bonds, unboxed labels denote fatty acid chain length.



Supplementary figure S6. Effect of diet on phospholipid species found in different tissues (A-F) show different Principal Component Analyses (PCA) made by pareto analysis of the phospholipid species profiles from tissues of larvae fed with 4 different foods: YF, PF, LDF+ergosterol and LDF+stigmasterol. Before performing the PCA, the phospholipid species profiles were determined by calculating the relative amounts of species with different combined fatty acid chain lengths and unsaturations. A separate analysis was performed for each tissue. ***(continues)***



Supplementary figure S6. Effect of diet on phospholipid species found in different tissues *(continuation)* Phospholipid species in tissues of larvae fed PF are distinct from those of larvae fed with other foods in having longer and more unsaturated fatty acids. Labels with green boxes denote double bonds, unboxed labels denote fatty acid chain length.



Supplementary figure S7. Effect of different dietary sterols on lipid class composition in different tissues (A-D) show the amounts of different lipid classes in tissues and hemolymph lipoproteins of early wandering 3rd instar larvae. Larvae were fed LDF supplemented either with ergosterol (+Erg or +E), shaded in grey, or with stigmasterol (+Stig or +S), not shaded. Each color represents one tissue, as indicated. The amounts of each lipid class were calculated as mole% with respect to all membrane lipids in the tissue. Membrane lipids include phospholipids, sphingolipids and sterols, but not DAG or TAG. (A) Phospholipids. (B) Neutral lipids. (C) Sphingolipids (D) Sterols. Error bars indicate standard deviation.

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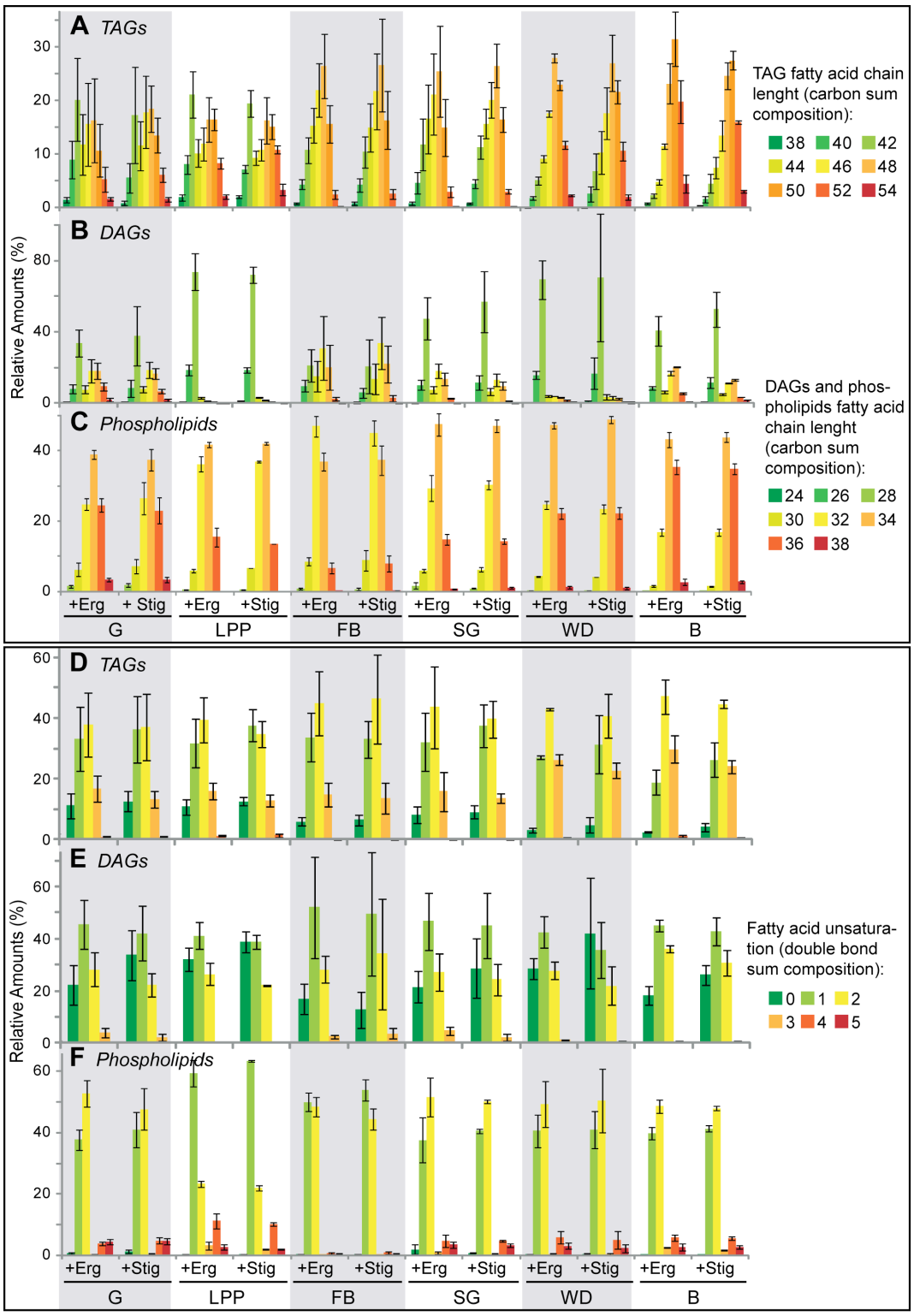

Supplementary figure S8. Different dietary sterols do not influence average fatty acid chain length or unsaturation in tissue lipids. Larvae were fed LDF either supplemented with ergosterol or stigmasterol, and their tissue lipid compositions were analyzed when they reached the beginning of the wandering stage. Depicted are the total combined fatty acid chain lengths (A) or total number of double bonds (B) per species, as a percentage of the total class or group of lipids. Error bars represent standard deviation. G=gut; LPP=lipoproteins; FB=fat body; SG=salivary glands; WD=wing discs; B=brain.

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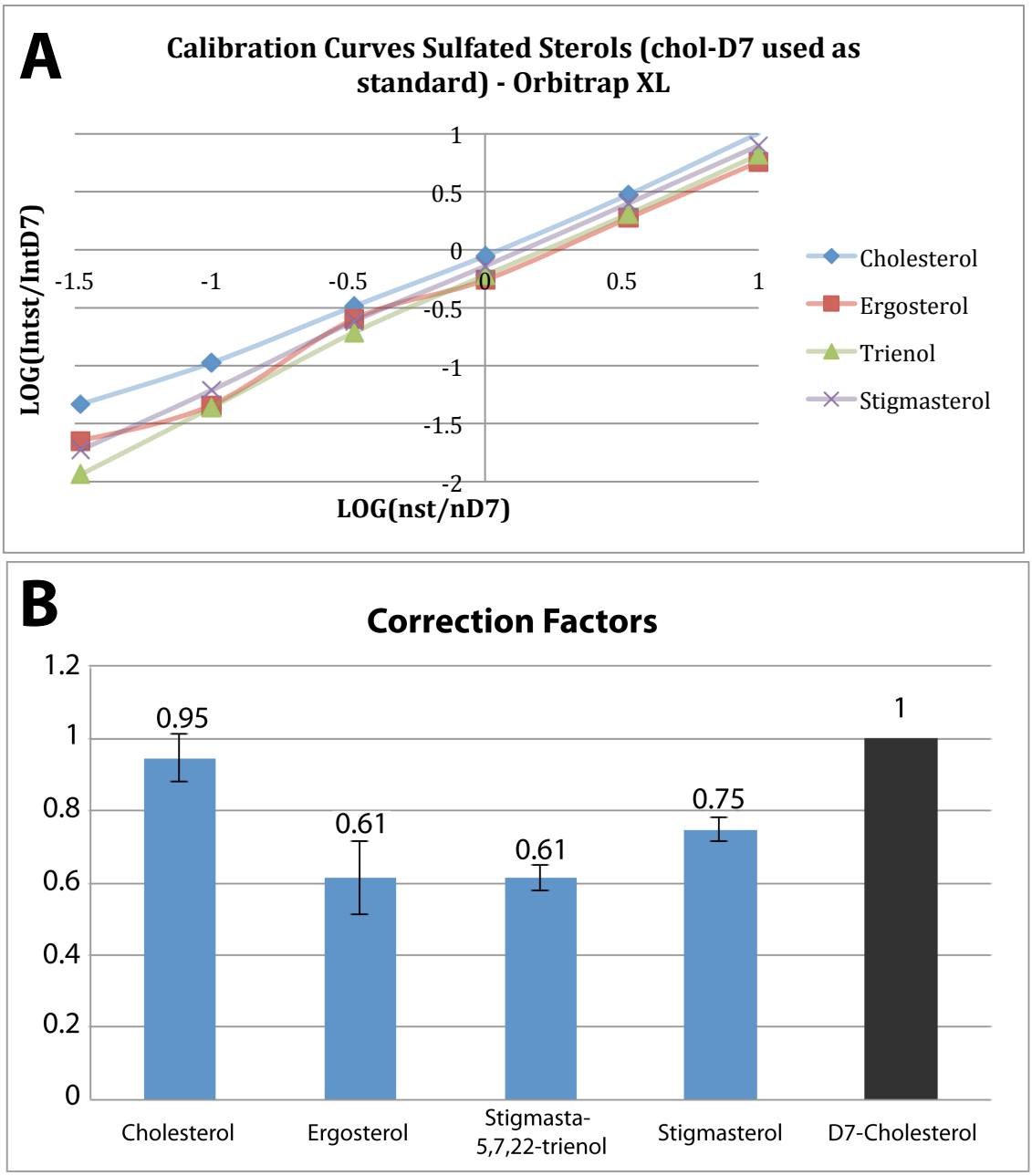

Supplementary figure S9. Calibration curves and correction factors for sterol standards.
(A) Calibration curves for sulfated sterols. X-axis: log of the concentration ratio of the indicated sterol and D7-cholesterol (internal standard); Y-axis: Log of the intensity ratio of the peaks of the indicated sulfated sterol and sulfated D7-cholesterol (internal standard). Concentrations of stock solutions of sterols (including D7-cholesterol) were calculated from the exact weight of samples taken from commercially available standards determined on the analytical balance BP 121S (Sartorius, Göttingen, Germany). Concentration of D7-cholesterol in the analyte was 2.5 µM.
(B) Correction factors controlling for differences in extraction, derivatisation yield and ionization efficiency of sterol standards and the internal standard D7-cholesterol that were computed from the calibration curves in panel A.

Supplementary table SI

Weighted average of fatty acid chain length and unsaturation in larval tissues and foods

		FA chain length and unsaturation		
Yeast Food		16.5:0.7		
Plant Food		17.8:1.5		
Larval Tissues	***Diets***	***TAG***	***DAG***	***PHOSPHOLIPIDS***
GUT	LDF+Erg	15.1:0.5	15.4:0.6	16.9:0.9
	LDF+Stig	15.4:0.5	15.2:0.5	16.8:0.9
	YF	15.3:0.5	15.4:0.6	16.9:0.9
	PF	17.1:1.3	16.9:1.2	17.3:1.2
LIPOPROTEIN	LDF+Erg	15.3:0.5	13.9:0.5	16.7:0.9
	LDF+Stig	15.4:0.5	13.9:0.4	16.6:0.8
	YF	15.4:0.5	14.1:0.5	16.6:0.8
	PF	15.3:0.6	13.9:0.5	16.9:1
FAT BODY	LDF+Erg	15.4:0.6	15.4:0.6	16.4:0.8
	LDF+Stig	15.4:0.6	15.5:0.6	16.4:0.7
	YF	15.5:0.6	15.2:0.6	16.5:0.7
	PF	15.7:0.7	15.9:0.7	16.8:0.9
SALIVARY GL	LDF+Erg	15.4:0.6	14.8:0.6	16.7:0.9
	LDF+Stig	15.4:0.5	14.5:0.5	16.7:0.9
	YF	15.5:0.5	15.0:0.6	16.8:0.9
	PF	15.9:0.8	14.2:0.6	17.1:1.1
WING DISC	LDF+Erg	16:0.6	14.1:0.5	16.9:0.9
	LDF+Stig	15.9:0.6	14.0:0.4	16.9:0.9
	YF	16.1:0.7	14.3:0.5	17:1
	PF	16.7:1	14.5:0.7	17.3:1.1
BRAIN	LDF+Erg	16.4:0.7	15.2:0.6	17.2:0.9
	LDF+Stig	16.1:0.6	14.7:0.5	17.2:0.9
	YF	16.1:0.6	15.4:0.6	17.2:0.9
	PF	16.9:1.2	15.5:0.9	17.6:1.3