Supplementary Information

Heterogeneity of Intramuscular, Intermuscular, and Subcutaneous Fat in Laiwu Pigs: Insights from Targeted Lipidomics and Transcriptomics

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| Quantified lipid classes			
Abbrev.	Lipid classes		
PC	phosphatidylcholine	FFA	free fatty acid
PC O	Alkyl PC	AcCA	acylcarnitine
LPC	lyso-PC	DAG	diacylglycerol
PE	phosphatidylethanolamine	TAG	triacylglycerol
PE O	Alkyl PE	SL	sulfatide
LPE	lyso-PE	Cer	ceramide
PI	phosphatidylinositol	GluCer	glucosylceramide
LPI	lyso-PI	GalCer	galactosylceramide
PA	phosphatidic acid	LacCer	lactosylceramide
LPA	lyso-PA	S1P	sphingosine-1-phosphate
LPS	lyso-PS	Sph	sphingosine
PS	phosphatidylserine	Gb3	Ceramide trihexoside
PG	phosphatidylglycerol	GM3	monosialoganglioside
CL	cardiolipin	SM	sphingomyelins
BMP	bis(monoacylglycerol) phosphate		
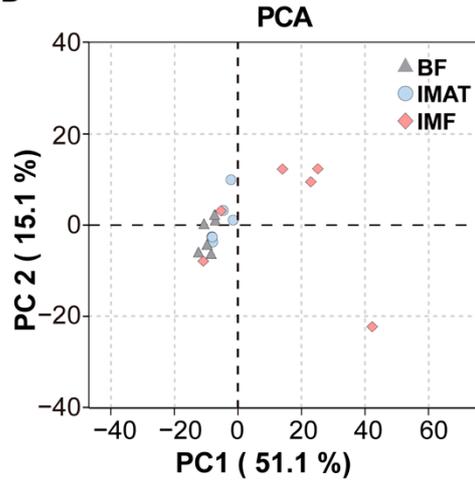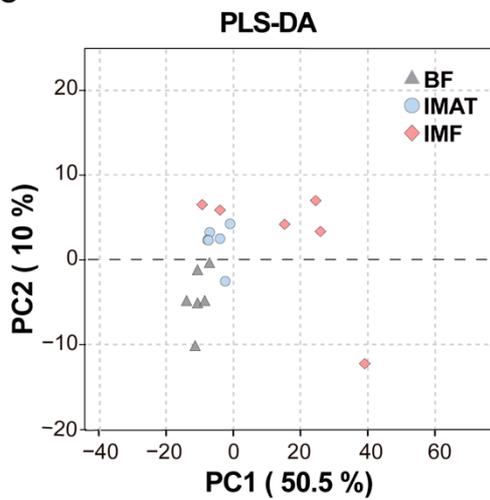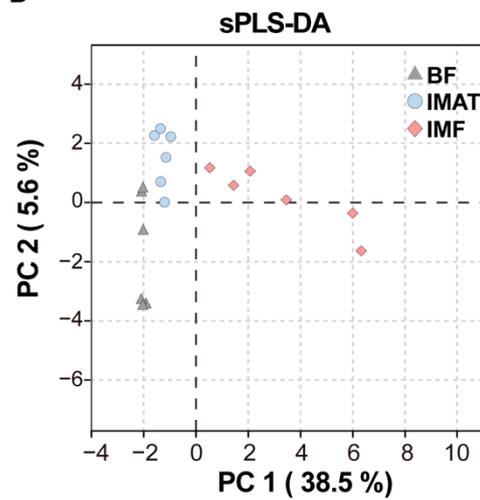
Figure S1. Multivariate data analysis of LC-MS lipidomics data from adipose tissue samples of 12-month-old Laiwu pigs. (A) Quantified lipid classes and their abbreviations used throughout the study. (B) Unsupervised PCA score plot. (C,D) Supervised PLS-DA and sPLS-DA models. Grey, light blue, and light red symbols represent inner BF, IMAT, and IMF samples from Laiwu pigs, respectively (*n* = 6).
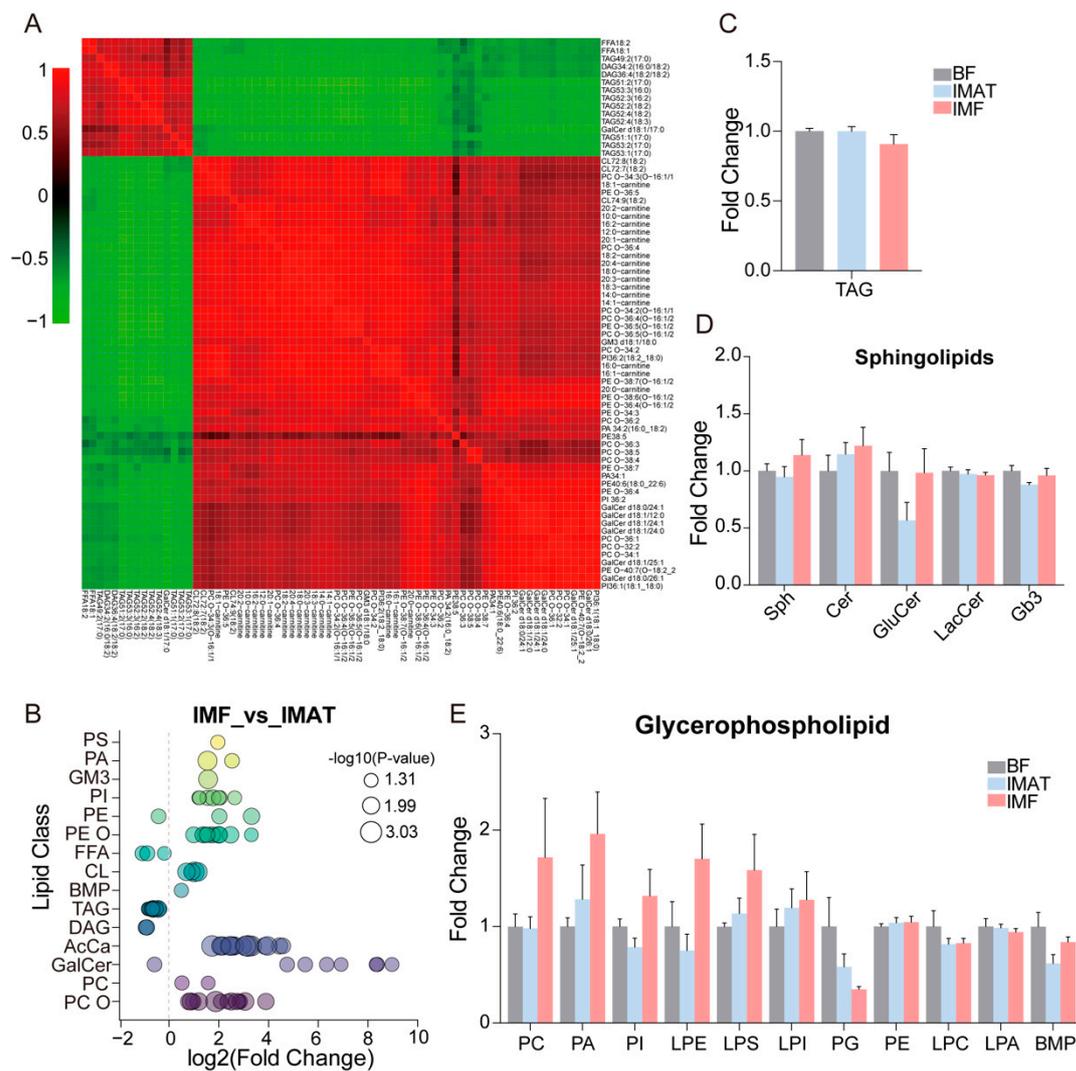
Figure S2. The overall composition of Lipid Classes altered in the IMF group. **(A)** The correlation matrix of these 70 significantly changed lipid species, with VIP > 1 and *p*-value < 0.05. The dotted color indicates the correlation coefficient: red means a positive correlation (1) and green means a negative correlation (−1). (B) Visualization of log2 fold changes in lipid species between the IMAT and IMF groups, with significance levels represented as -log10 *p*-value. Each dot represents a lipid species, and the dot size indicates significance. Only lipids with *p* < 0.05 are displayed. (C–E) The ion intensity fold change of lipid classes shows no significant difference (*n* = 6).
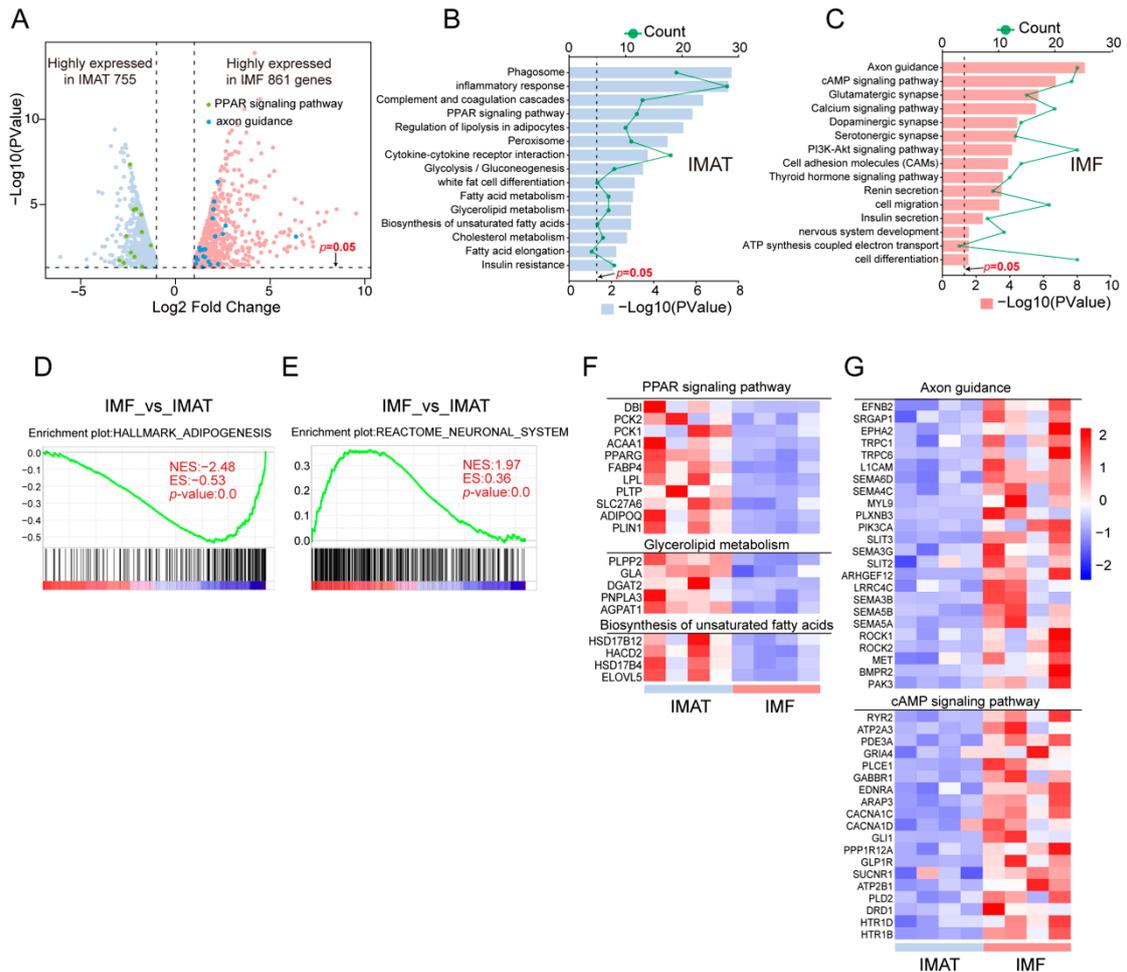
Figure S3. IMF exhibits a reduced capacity for lipid deposition and increased innervation compared to IMAT. (A) Representation of the differentially expressed genes between backfat and intramuscular adipose tissue. Genes overexpressed in IMAT and IMF are indicated in grey and pink, respectively. Genes enriched in the PPAR signaling pathway and axon guidance are highlighted. (B-C) GO analysis for highly expressed genes in IMF, and KEGG analysis for those in IMAT. (D-E) GSEA enrichment analyses between IMAT and IMF. (F) The heatmap of the genes involved in the PPAR signaling pathway, glycerolipid metabolism, and biosynthesis of unsaturated fatty acids. (G) The heatmap of the genes associated with axon guidance, and cAMP signaling pathway.

Table S1 The primers for RT-PCR

Gene	Name	Primer
18S	Forward	GTAACCCGTTGAACCCCATT
	Reverse	CCATCCAATCGGTAGTAGCG
CIDEA	Forward	GTCAAGGCCACCATGTACGA
	Reverse	AGCATTCGGAGCATGTACGT
GK	Forward	CGCTGAGGAAAGTGAAATCCG
	Reverse	TCGCGTCTTTGGAATCTACGA
UGT8	Forward	TGAACATGGCTTTGTCCTGG
	Reverse	TGCGGTAACCATTCTATGAGC
ADIPOQ	Forward	AGATTGGCTGGTTGATGCTCA
	Reverse	AAACCAGATGTCACACTTGCC
FABP4	Forward	GGCCAAACCCAACCTGATCA
	Reverse	CATCCCACTTCTGCACCTGT
PPARG	Forward	GTCATGGGTGAAACTCTGGGA
	Reverse	TGTCAACCATGGTCACCTCTTG
AGPAT1	Forward	CCCTATGTCGTCGTCTCCAA
	Reverse	AACCTCGGACATGACACTGA
PNPLA3	Forward	CTCCACCCATCCTTCAACCT
	Reverse	ACATATCGCTCGCCTCTGAA
ELOVL6	Forward	CCGGAAGTTTGCCATGTTCA
	Reverse	GCAGAAGAGCACAAGGTAGC
DGAT2	Forward	CCCTCATAGCTGCCTACTCC
	Reverse	GAGGAAAGACAGGACCCACT
FASN	Forward	CTGATCAAGGTGCTGCTGTC
	Reverse	CGAAGGAGTTTATGCCCACG
SCD	Forward	CTTCCTGATCATTGCCAACA
	Reverse	GCAAACCACCCTTCTCTTTG
ACACA	Forward	CGTGCAATCCGGTTTGTTGT
	Reverse	TGTTGTTGTTTGGGCCTCCT