

***animal journal***

**High-throughput untargeted metabolomics reveals metabolites and metabolic pathways that differentiate two divergent pig breeds**

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**Supplementary Table S1.** Super-pathways, sub-pathways and metabolites measured in the whole pig population and included in the study.

Super-pathway	Sub-pathway	Metabolites (n.)	
		Raw dataset	Final dataset
Unknown (unnamed metabolites)	Unknown (unnamed metabolites)	68	57
Lipid	Sphingomyelins	25	25
Lipid	Lysophospholipid	23	22
Lipid	Phosphatidylcholine (PC)	19	16
Lipid	Fatty Acid, Dicarboxylate	19	19
Lipid	Fatty Acid, Monohydroxy	15	9
Lipid	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	14	12
Lipid	Diacylglycerol	14	10
Lipid	Secondary Bile Acid Metabolism	13	11
Lipid	Plasmalogen	11	11
Lipid	Phosphatidylethanolamine (PE)	9	9
Lipid	Phospholipid Metabolism	7	7
Lipid	Medium Chain Fatty Acid	7	2
Lipid	Long Chain Saturated Fatty Acid	7	6
Lipid	Long Chain Monounsaturated Fatty Acid	6	6
Lipid	Fatty Acid Metabolism (Acyl Glycine)	6	4
Lipid	Sterol	5	5
Lipid	Phosphatidylinositol (PI)	5	5
Lipid	Fatty Acid Metabolism (Acyl Choline)	5	0
Lipid	Dihydrosphingomyelins	5	5
Lipid	Ceramides	5	3
Lipid	Lysoplasmalogen	4	4
Lipid	Hexosylceramides (HCER)	4	4
Lipid	Fatty Acid, Dihydroxy	4	4
Lipid	Fatty Acid Metabolism (Acyl Carnitine, Long Chain Saturated)	4	3
Lipid	Endocannabinoid	4	4
Lipid	Primary Bile Acid Metabolism	3	3
Lipid	Glycerolipid Metabolism	3	3
Lipid	Fatty Acid, Amino	3	3
Lipid	Fatty Acid Metabolism (also BCAA Metabolism)	3	2
Lipid	Fatty Acid Metabolism (Acyl Carnitine, Monounsaturated)	3	3
Lipid	Fatty Acid Metabolism (Acyl Carnitine, Dicarboxylate)	3	2
Lipid	Corticosteroids	3	3
Lipid	Sphingosines	2	2
Lipid	Sphingolipid Synthesis	2	2
Lipid	Mevalonate Metabolism	2	2
Lipid	Inositol Metabolism	2	1
Lipid	Fatty Acid, Branched	2	0
Lipid	Fatty Acid Metabolism (Acyl Carnitine, Medium Chain)	2	2
Lipid	Dihydroceramides	2	1
Lipid	Carnitine Metabolism	2	2
Lipid	Short Chain Fatty Acid	1	1
Lipid	Progestin Steroids	1	0
Lipid	Phosphatidylserine (PS)	1	1
Lipid	Monoacylglycerol	1	0
Lipid	Lactosylceramides (LCER)	1	1
Lipid	Ketone Bodies	1	1
Lipid	Glycosphingolipid Sulfates	1	1
Lipid	Fatty Acid Metabolism (Acyl Carnitine, Short Chain)	1	1
Lipid	Fatty Acid Metabolism (Acyl Carnitine, Polyunsaturated)	1	1
Lipid	Fatty Acid Metabolism (Acyl Carnitine, Hydroxy)	1	0
Lipid	Ceramide PEs	1	1
Amino Acid	Leucine, Isoleucine and Valine Metabolism	28	25
Amino Acid	Tryptophan Metabolism	18	17
Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism	18	16
Amino Acid	Urea cycle; Arginine and Proline Metabolism	17	16
Amino Acid	Histidine Metabolism	16	14
Amino Acid	Lysine Metabolism	15	14
Amino Acid	Tyrosine Metabolism	11	11
Amino Acid	Glycine, Serine and Threonine Metabolism	10	10
Amino Acid	Glutamate Metabolism	8	7
Amino Acid	Alanine and Aspartate Metabolism	7	7
Amino Acid	Polyamine Metabolism	6	5
Amino Acid	Glutathione Metabolism	6	6
Amino Acid	Phenylalanine Metabolism	5	5

Amino Acid	Lactoyl Amino Acid	5	4
Amino Acid	Creatine Metabolism	4	4
Amino Acid	Guanidino and Acetamido Metabolism	2	2
Xenobiotics	Food Component/Plant	28	0
Xenobiotics	Benzoate Metabolism	19	0
Xenobiotics	Chemical	10	0
Xenobiotics	Drug - Topical Agents	3	0
Nucleotide	Pyrimidine Metabolism, Uracil containing	11	11
Nucleotide	Pyrimidine Metabolism, Cytidine containing	8	7
Nucleotide	Purine Metabolism, (Hypo)Xanthine/Inosine containing	8	8
Nucleotide	Purine Metabolism, Adenine containing	6	6
Nucleotide	Purine Metabolism, Guanine containing	5	5
Nucleotide	Pyrimidine Metabolism, Thymine containing	2	2
Nucleotide	Pyrimidine Metabolism, Orotate containing	2	1
Nucleotide	Purine and Pyrimidine Metabolism	1	1
Peptide	Gamma-glutamyl Amino Acid	13	13
Peptide	Dipeptide	6	4
Peptide	Acetylated Peptides	4	4
Peptide	Dipeptide Derivative	2	2
Peptide	Polypeptide	1	0
Cofactors and Vitamins	Nicotinate and Nicotinamide Metabolism	6	4
Cofactors and Vitamins	Ascorbate and Aldarate Metabolism	6	5
Cofactors and Vitamins	Tocopherol Metabolism	3	2
Cofactors and Vitamins	Hemoglobin and Porphyrin Metabolism	3	1
Cofactors and Vitamins	Pantothenate and CoA Metabolism	2	2
Cofactors and Vitamins	Vitamin B6 Metabolism	1	1
Cofactors and Vitamins	Vitamin A Metabolism	1	1
Cofactors and Vitamins	Riboflavin Metabolism	1	1
Cofactors and Vitamins	Pterin Metabolism	1	1
Carbohydrate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	6	6
Carbohydrate	Pentose Metabolism	5	5
Carbohydrate	Aminosugar Metabolism	5	5
Carbohydrate	Fructose, Mannose and Galactose Metabolism	4	4
Carbohydrate	Disaccharides and Oligosaccharides	1	0
Carbohydrate	Advanced Glycation End-product	1	1
Energy	TCA Cycle	7	7
Energy	Oxidative Phosphorylation	1	1
Partially Characterized Molecules	Partially Characterized Molecules	7	4

**Supplementary Table S2.** Full information of metabolites (n. 100) that showed differences in concentration between breeds in the 962 analysed pigs. Metabolites are sorted based on the Mean Decrease Gini (MDG) value (from the highest to the lowest).

General information about metabolites					Detection of metabolites					Network analysis			
Order	HMDB	Metabolite name	Super-pathway	Sub-pathway	Detection method	Breed having a higher concentration	$\Delta\%$	MDG	AUC	Cluster	Average Shortest Path	Betweenness Centrality	Node Degree
1	NA	glycosyl ceramide (d18:2/24:1, d18:1/24:2)	Lipid	Hexosylceramides (HCER)	BORUTA_AND_SPLSD A	IDU	-193.60	0.0488	0.928	Cluster of lipids	3.0000	0.0000	3
2	NA	X-15503	NA	NA	BORUTA	ILW	59.40	0.0406	0.896	Cluster of amino acids (small) 2	1.0000	0.0556	4
3	HMDB0000715	kynurenate	Amino Acid	Tryptophan Metabolism	BORUTA	ILW	57.70	0.0381	0.893	Cluster of amino acids (small) 2	1.0000	0.0556	4
4	HMDB0001434	3-methoxytyrosine	Amino Acid	Tyrosine Metabolism	BORUTA_AND_SPLSD A	IDU	-47.82	0.0324	0.878	Singleton	0.0000	0.0000	0
5	NA	hydroxypalmitoyl sphingomyelin (d18:1/16:0(OH))	Lipid	Sphingomyelins	BORUTA_AND_SPLSD A	IDU	-45.80	0.0321	0.881	Cluster of lipids	1.6957	0.1692	10
6	HMDB0000684	kynurenine	Amino Acid	Tryptophan Metabolism	BORUTA_AND_SPLSD A	ILW	43.35	0.0317	0.876	Cluster of amino acids (small) 2	1.2500	0.0000	3
7	HMDB0011172	gamma-glutamylvaline	Peptide	Gamma-glutamyl Amino Acid	BORUTA_AND_SPLSD A	IDU	-102.66	0.0316	0.827	Cluster of lipids	3.5217	0.0000	1
8	NA	glycosyl-N-palmitoyl-sphingosine (d18:1/16:0)	Lipid	Hexosylceramides (HCER)	BORUTA	IDU	-115.74	0.0310	0.894	Cluster of lipids	2.1304	0.2372	4
9	NA	glycosyl ceramide	Lipid	Hexosylceramides	BORUTA_AND_SPLSD	IDU	-	0.030	0.877	Cluster of	3.0000	0.0000	3

		(d18:1/20:0, d16:1/22:0)		(HCER)	A		161.17	5		lipids			
10	HMDB0003357	N - d e l t a - acetylmithine	Amino Acid	Urea cycle; Arginine and Proline Metabolism	BORUTA	ILW	56.20	0.0276	0.868	Cluster of amino acids (big) 1	2.0714	0.0833	6
11	NA	X-24736	NA	NA	BORUTA_AND_SPLSD A	ILW	62.35	0.0240	0.864	Cluster of amino acids (big) 1	2.2143	0.0247	4
12	NA	glycosyl-N-stearoyl-sphingosine (d18:1/18:0)	Lipid	Hexosylceramides (HCER)	BORUTA	IDU	-140.08	0.0230	0.866	Cluster of lipids	3.0000	0.0000	3
13	NA	1 - m e t h y l - 5 - imidazolelactate	Amino Acid	Histidine Metabolism	BORUTA	ILW	43.73	0.0228	0.862	Cluster of amino acids (big) 1	1.8571	0.1511	5
14	HMDB0011745	N-acetylmethionine	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism	BORUTA_AND_SPLSD A	ILW	51.92	0.0169	0.780	Singleton	0.0000	0.0000	0
15	HMDB0240342	N-acetylkynurenine (2)	Amino Acid	Tryptophan Metabolism	BORUTA	ILW	54.78	0.0147	0.816	Cluster of amino acids (small) 2	1.0000	0.0556	4
16	HMDB0000479	3-methylhistidine	Amino Acid	Histidine Metabolism	BORUTA_AND_SPLSD A	ILW	32.14	0.0142	0.828	Cluster of amino acids (big) 1	2.5000	0.0027	2
17	HMDB0000355	3 - h y d r o x y - 3 - methylglutarate	Lipid	Mevalonate Metabolism	BORUTA	ILW	57.43	0.0136	0.808	Other connected component	1.0000	0.0000	1
18	HMDB0000522	3- methylglutaconate	Amino Acid	Leucine, Isoleucine and Valine Metabolism	BORUTA	ILW	54.32	0.0135	0.819	Other connected component	1.0000	0.0000	1
19	NA	glycine conjugate of	Partially Characterized	Partially Characterized Molecules	BORUTA_AND_SPLSD A	IDU	-70.76	0.0127	0.784	Other connected	1.0000	0.0000	1

		C6H10O2 (2)	Molecules							compone nt			
20	HMDB024064 4	sphingomyelin (d18:2/24:2)	Lipid	Sphingomyelins	BORUTA	IDU	-39.56	0.010 3	0.768	Singleton	0.0000	0.0000	0
21	HMDB001016 8	palmitoyl dihydrospingo myelin (d18:0/16:0)	Lipid	Dihydrospingomyelins	BORUTA	IDU	-31.39	0.010 0	0.807	Cluster of lipids	1.6957	0.0117	11
22	HMDB04988	1 - m e t h y l - 5 - imidazoleacetate	Amino Acid	Histidine Metabolism	BORUTA	ILW	64.25	0.009 2	0.752	Singleton	0.0000	0.0000	0
23	HMDB009469 2	N-methylalanine	Amino Acid	Alanine and Aspartate Metabolism	BORUTA	ILW	40.75	0.008 9	0.711	Singleton	0.0000	0.0000	0
24	HMDB000210 8	S- methylcysteine	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism	BORUTA	IDU	-41.43	0.007 6	0.790	Singleton	0.0000	0.0000	0
25	HMDB000286 9	campesterol	Lipid	Sterol	BORUTA_AND_SPLSD A	IDU	-56.72	0.007 0	0.774	Cluster of lipids	1.9565	0.0079	7
26	HMDB000098 2	5-methylcytidine	Nucleotide	Pyrimidine Metabolism, Cytidine containing	BORUTA	ILW	26.74	0.006 7	0.772	Other connecte d compone nt	1.0000	0.0000	1
27	HMDB000003 3	carosine	Amino Acid	Histidine Metabolism	BORUTA	ILW	35.33	0.006 6	0.768	Cluster of amino acids 1 (big)	2.6429	0.1429	2
28	HMDB000056 2	creatinine	Amino Acid	Creatine Metabolism	BORUTA	ILW	12.69	0.006 5	0.748	Cluster of amino acids 1 (big)	1.8571	0.4835	4
29	HMDB024062 0, HMDB02406 09, HMDB0240 622	sphingomyelin (d18:1/17:0, d17:1/18:0, d19:1/16:0)	Lipid	Sphingomyelins	BORUTA	IDU	-28.33	0.006 3	0.785	Cluster of lipids	1.6957	0.0155	11
30	HMDB000070 9	cysteinylglycine disulfide	Amino Acid	Glutathione Metabolism	BORUTA_AND_SPLSD A	ILW	24.94	0.006 3	0.754	Singleton	0.0000	0.0000	0
31	NA	2 ' - O - methylcytidine	Nucleotide	Pyrimidine Metabolism, Cytidine containing	BORUTA	IDU	-15.88	0.006 0	0.741	Other connecte d compone	1.0000	0.0000	1

										nt			
32	HMDB0240610, HMDB0240632	sphingomyelin (d18:1/20:1, d18:2/20:0)	Lipid	Sphingomyelins	BORUTA_AND_SPLSD A	IDU	-22.99	0.0059	0.775	Cluster of lipids	2.1739	0.0006	4
33	HMDB0011211	1-(1-enyl-palmitoyl)-2-linoleoyl-GPC (P-16:0/18:2)	Lipid	Plasmalogen	BORUTA	IDU	-28.16	0.0057	0.749	Cluster of lipids	2.5652	0.0870	2
34	HMDB0240668	sphingomyelin (d18:2/23:1)	Lipid	Sphingomyelins	BORUTA_AND_SPLSD A	IDU	-30.78	0.0054	0.784	Cluster of lipids	2.1304	0.0049	7
35	HMDB0000701	hexanoylglycine	Lipid	Fatty Acid Metabolism (Acyl Glycine)	BORUTA_AND_SPLSD A	IDU	-175.59	0.0053	0.760	Singleton	0.0000	0.0000	0
36	HMDB0240328	2'-O-methyluridine	Nucleotide	Pyrimidine Metabolism, Uracil containing	BORUTA	IDU	-23.31	0.0053	0.749	Other connected component	1.0000	0.0000	1
37	NA	X-17354	NA	NA	BORUTA	ILW	47.80	0.0053	0.744	Singleton	0.0000	0.0000	0
38	HMDB0000679	homocitrulline	Amino Acid	Urea cycle; Arginine and Proline Metabolism	BORUTA	ILW	44.80	0.0051	0.753	Cluster of amino acids (big)	2.0714	0.0668	6
39	HMDB0000875	trigonelline (N'-methylnicotinate)	Cofactors and Vitamins	Nicotinate and Nicotinamide Metabolism	BORUTA	ILW	22.32	0.0051	0.767	Cluster of amino acids (big)	2.4286	0.1429	2
40	HMDB0002264	2-hydroxyoctanoate	Lipid	Fatty Acid, Monohydroxy	BORUTA	ILW	42.63	0.0051	0.752	Other connected component	1.0000	0.0000	1
41	HMDB0000273	thymidine	Nucleotide	Pyrimidine Metabolism, Thymine containing	BORUTA	ILW	26.38	0.0050	0.745	Singleton	0.0000	0.0000	0
42	HMDB0000092	dimethylglycine	Amino Acid	Glycine, Serine and Threonine Metabolism	BORUTA	IDU	-21.38	0.0048	0.720	Cluster of amino acids (big)	3.3571	0.0000	1
43	HMDB006217	methionine	Amino Acid	Methionine, Cysteine, SAM and Taurine	BORUTA_AND_SPLSD	ILW	26.17	0.004	0.767	Cluster of amino	2.7857	0.0000	3

	4	sulfone		Metabolism	A			8		acids 1 (big)			
44	HMDB0000089	cytidine	Nucleotide	Pyrimidine Metabolism, Cytidine containing	BORUTA	ILW	36.09	0.0047	0.756	Other connecte d compone nt	1.0000	0.0000	1
45	HMDB0240636, HMDB0240615	sphingomyelin (d18:2/24:1, d18:1/24:2)	Lipid	Sphingomyelins	BORUTA	IDU	-21.48	0.0044	0.751	Cluster of lipids	1.8696	0.0168	9
46	NA	X-23593	NA	NA	BORUTA	ILW	23.80	0.0041	0.745	Cluster of amino acids 1 (big)	1.6429	0.4817	7
47	HMDB0012104	sphingomyelin (d18:1/22:1, d18:2/22:0, d16:1/24:1)	Lipid	Sphingomyelins	BORUTA	IDU	-18.23	0.0041	0.753	Cluster of lipids	1.6087	0.0601	13
48	HMDB0028930	leucylhydroxypro line	Peptide	Dipeptide Derivative	BORUTA	ILW	46.34	0.0038	0.732	Cluster of amino acids 1 (big)	3.5714	0.0000	1
49	HMDB0000929	tryptophan	Amino Acid	Tryptophan Metabolism	BORUTA	IDU	-19.25	0.0037	0.752	Singleton	0.0000	0.0000	0
50	HMDB0240634, HMDB0011696, HMDB0240614	sphingomyelin (d18:2/23:0, d18:1/23:1, d17:1/24:1)	Lipid	Sphingomyelins	BORUTA	IDU	-21.06	0.0036	0.747	Cluster of lipids	1.6957	0.0143	11
51	NA	phenylacetylalan ine	Peptide	Acetylated Peptides	BORUTA	IDU	-76.87	0.0036	0.723	Singleton	0.0000	0.0000	0
52	HMDB0240253	N-acetyltaurine	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism	BORUTA	IDU	-40.11	0.0035	0.728	Singleton	0.0000	0.0000	0
53	HMDB0028929	leucylglycine	Peptide	Dipeptide	BORUTA	ILW	29.14	0.0034	0.711	Singleton	0.0000	0.0000	0
54	NA	gamma- glutamylcitrulline	Peptide	Gamma-glutamyl Amino Acid	BORUTA	ILW	41.13	0.0034	0.722	Cluster of peptides	1.4000	0.0000	3
55	NA	gamma- glutamyl-alpha-	Peptide	Gamma-glutamyl Amino Acid	BORUTA	ILW	42.03	0.0033	0.709	Cluster of peptides	1.2000	0.0333	4



		lysine											
56	HMDB0010169	palmitoyl sphingomyelin (d18:1/16:0)	Lipid	Sphingomyelins	BORUTA	IDU	-14.40	0.0033	0.761	Cluster of lipids	1.4348	0.3530	14
57	NA	sphingomyelin (d18:1/19:0, d19:1/18:0)	Lipid	Sphingomyelins	BORUTA	IDU	-24.40	0.0033	0.748	Cluster of lipids	1.7391	0.0144	10
58	HMDB0000695	4 - m e t h y l - 2 - oxopentanoate	Amino Acid	Leucine, Isoleucine and Valine Metabolism	BORUTA	IDU	-11.24	0.0030	0.692	Cluster of amino acids 3 (small)	1.0000	0.0000	2
59	HMDB0006469	linoleoylcarnitine (C18:2)	Lipid	Fatty Acid Metabolism (Acyl Carnitine, Polyunsaturated)	BORUTA_AND_SPLSD A	IDU	-48.67	0.0030	0.742	Other connected component	1.0000	0.0000	1
60	NA	X-25172	NA	NA	BORUTA	IDU	-22.38	0.0030	0.716	Singleton	0.0000	0.0000	0
61	HMDB0000192	cystine	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism	BORUTA	ILW	29.69	0.0030	0.648	Singleton	0.0000	0.0000	0
62	HMDB0011738	gamma-glutamylglutamine	Peptide	Gamma-glutamyl Amino Acid	BORUTA	ILW	37.50	0.0030	0.729	Cluster of peptides	1.2000	0.0333	4
63	NA	X-23423	NA	NA	BORUTA	ILW	33.80	0.0029	0.715	Cluster of amino acids 1 (big)	2.2143	0.0027	4
64	HMDB0000019	3 - m e t h y l - 2 - oxobutyrate	Amino Acid	Leucine, Isoleucine and Valine Metabolism	BORUTA	IDU	-14.52	0.0028	0.695	Cluster of amino acids 3 (small)	1.0000	0.0000	2
65	HMDB0001123	anthranilate	Amino Acid	Tryptophan Metabolism	BORUTA	ILW	26.28	0.0027	0.713	Singleton	0.0000	0.0000	0
66	HMDB0012107	sphingomyelin (d18:1/24:1, d18:2/24:0)	Lipid	Sphingomyelins	BORUTA	IDU	-20.01	0.0027	0.740	Cluster of lipids	1.5652	0.0781	14
67	HMDB0240617, HMDB02406	sphingomyelin (d17:1/16:0, d18:1/15:0,	Lipid	Sphingomyelins	BORUTA	IDU	-31.01	0.0027	0.760	Cluster of lipids	1.6087	0.1803	13

	08	d16:1/17:0)											
68	HMDB0240670, HMDB0240672, HMDB0240669	sphingomyelin (d18:1/22:2, d18:2/22:1, d16:1/24:2)	Lipid	Sphingomyelins	BORUTA	IDU	-17.95	0.0025	0.728	Cluster of lipids	2.3478	0.0000	4
69	HMDB0000099	cystathionine	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism	BORUTA	IDU	-6.30	0.0025	0.658	Singleton	0.0000	0.0000	0
70	HMDB0002712	1,5-anhydroglucitol (1,5-AG)	Carbohydrate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	BORUTA	IDU	-35.83	0.0025	0.729	Singleton	0.0000	0.0000	0
71	NA	6-bromotryptophan	Amino Acid	Tryptophan Metabolism	BORUTA	IDU	-25.55	0.0023	0.711	Singleton	0.0000	0.0000	0
72	HMDB0006335, HMDB0001492	gamma-tocopherol/beta-tocopherol	Cofactors and Vitamins	Tocopherol Metabolism	BORUTA	IDU	-53.23	0.0023	0.719	Singleton	0.0000	0.0000	0
73	HMDB0011741	gamma-glutamyltyrosine	Peptide	Gamma-glutamyl Amino Acid	BORUTA	ILW	33.89	0.0023	0.697	Cluster of peptides	1.0000	0.1167	5
74	HMDB0000148	glutamate	Amino Acid	Glutamate Metabolism	BORUTA	IDU	-19.85	0.0023	0.710	Other connected component	1.0000	0.0000	1
75	HMDB0000498	2R,3R-dihydroxybutyrate	Lipid	Fatty Acid, Dihydroxy	BORUTA	ILW	17.73	0.0022	0.709	Singleton	0.0000	0.0000	0
76	HMDB0029159	gamma-glutamylthreonine	Peptide	Gamma-glutamyl Amino Acid	BORUTA	ILW	23.10	0.0020	0.717	Cluster of peptides	1.4000	0.0000	3
77	NA	X-18913	NA	NA	BORUTA	ILW	32.63	0.0019	0.690	Other connected component	1.0000	0.0000	1
78	HMDB0012102	sphingomyelin (d18:1/20:0, d16:1/22:0)	Lipid	Sphingomyelins	BORUTA	IDU	-13.05	0.0018	0.706	Cluster of lipids	1.9565	0.0059	7
79	HMDB0000730	isobutyrylglycine	Amino Acid	Leucine, Isoleucine and Valine Metabolism	BORUTA	IDU	-41.86	0.0017	0.686	Other connected	1.0000	0.0000	1

										compone nt			
80	NA	ascorbic acid 3-sulfate	Cofactors and Vitamins	Ascorbate and Aldarate Metabolism	BORUTA	IDU	-26.91	0.0017	0.685	Singleton	0.0000	0.0000	0
81	HMDB000044 2, HMDB0000357, HMDB0000011	3-hydroxybutyrate (BHBA)	Lipid	Ketone Bodies	BORUTA	IDU	-43.09	0.0017	0.699	Singleton	0.0000	0.0000	0
82	NA	2-hydroxyheptanoate	Lipid	Fatty Acid, Monohydroxy	BORUTA	ILW	26.70	0.0016	0.671	Singleton	0.0000	0.0000	0
83	HMDB0011667	gamma-glutamylglycine	Peptide	Gamma-glutamyl Amino Acid	BORUTA	ILW	38.17	0.0016	0.672	Cluster of peptides	1.0000	0.1167	5
84	HMDB0059766	picolinoylglycine	Lipid	Fatty Acid Metabolism (Acyl Glycine)	BORUTA	ILW	28.67	0.0016	0.697	Cluster of amino acids 2 (small)	1.2500	0.0000	3
85	NA	X-11850	NA	NA	BORUTA	IDU	-45.02	0.0016	0.677	Other connected component	1.0000	0.0000	1
86	HMDB0000191	aspartate	Amino Acid	Alanine and Aspartate Metabolism	BORUTA	IDU	-22.63	0.0016	0.665	Other connected component	1.0000	0.0000	1
87	HMDB0002038	N6-methyllysine	Amino Acid	Lysine Metabolism	BORUTA	IDU	-13.11	0.0015	0.533	Singleton	0.0000	0.0000	0
88	HMDB0001893	alpha-tocopherol	Cofactors and Vitamins	Tocopherol Metabolism	BORUTA	ILW	11.15	0.0015	0.577	Cluster of lipids	2.2174	0.0000	3
89	HMDB0000271	sarcosine	Amino Acid	Glycine, Serine and Threonine Metabolism	BORUTA	ILW	19.50	0.0015	0.658	Singleton	0.0000	0.0000	0
90	NA	X-22162	NA	NA	BORUTA	IDU	-17.65	0.0015	0.656	Singleton	0.0000	0.0000	0
91	HMDB0000682	3-indoxyl sulfate	Amino Acid	Tryptophan Metabolism	BORUTA	IDU	-29.02	0.0015	0.703	Singleton	0.0000	0.0000	0
92	HMDB000669	pro-hydroxy-pro	Amino Acid	Urea cycle; Arginine and	BORUTA	ILW	15.00	0.001	0.684	Cluster of amino	2.6429	0.1429	2

	5			Proline Metabolism				4		acids 1 (big)			
93	HMDB0011517	1-arachidonoyl-GPE (20:4n6)	Lipid	Lysophospholipid	BORUTA	ILW	8.18	0.0012	0.594	Other connected component	1.0000	0.0000	1
94	HMDB0000194	anserine	Amino Acid	Histidine Metabolism	BORUTA	ILW	41.37	0.0012	0.700	Cluster of amino acids 1 (big)	3.5714	0.0000	1
95	HMDB0001383	sphinganine-1-phosphate	Lipid	Sphingolipid Synthesis	BORUTA	ILW	28.66	0.0012	0.560	Singleton	0.0000	0.0000	0
96	HMDB0000172	isoleucine	Amino Acid	Leucine, Isoleucine and Valine Metabolism	BORUTA	IDU	-7.76	0.0012	0.690	Cluster of amino acids 3 (small)	1.0000	0.0000	2
97	NA	X-11843	NA	NA	BORUTA	IDU	-34.32	0.0011	0.658	Other connected component	1.0000	0.0000	1
98	HMDB0007883	1-myristoyl-2-arachidonoyl-GPC (14:0/20:4)	Lipid	Phosphatidylcholine (PC)	BORUTA	ILW	11.71	0.0011	0.626	Cluster of lipids	2.5217	0.0000	2
99	HMDB0240294	2-O-methylascorbic acid	Cofactors and Vitamins	Ascorbate and Aldarate Metabolism	BORUTA	IDU	-9.78	0.0008	0.628	Singleton	0.0000	0.0000	0
100	HMDB0007969	1-palmitoyl-2-palmitoleoyl-GPC (16:0/16:1)	Lipid	Phosphatidylcholine (PC)	BORUTA	ILW	8.41	0.0005	0.630	Cluster of lipids	2.5217	0.0000	2

Abbreviations: AUC = Area Under the Curve, HMDB = Human metabolome database, IDU = Italian Duroc, ILW = Italian Large White, SPLSDA = Sparse partial least squares discriminant analysis

**Supplementary Table S3.** Over-represented pathways ( $P_{\text{FDR}} < 0.05$ ) on the RaMP-DB database, based on the metabolites discriminating between the two pig breeds.

Metabolite Set	Source database	Total <sup>1</sup>	Hits <sup>2</sup>	Expect	Enrichment ratio <sup>3</sup>	<i>P</i>	FDR corrected <i>P</i>	Overlapping metabolites
Metabolism of amino acids and derivatives	Reactome	283	14	2.05	6.83	2.12E-09	7.04E-06	alpha-Ketoisovaleric acid; Carnosine; L-Cystathionine; Sarcosine; Creatinine; Kynurenine; Kynurenic acid; L-Tryptophan; L-Aspartic acid; Ketoleucine; Glutamic acid; Isoleucine; Dimethylglycine; Anserine
SLC transporter disorders	Reactome	80	8	0.579	13.82	5.81E-08	9.64E-05	Cytidine; L-Cystine; Thymidine; L-Tryptophan; L-Aspartic acid; Glutamic acid, 3-Hydroxybutyric acid; Isoleucine
Disorders of transmembrane transporters	Reactome	98	8	0.71	11.27	2.89E-07	2.71E-04	Cytidine; L-Cystine; Thymidine; L-Tryptophan; L-Aspartic acid; Glutamic acid, 3-Hydroxybutyric acid; Isoleucine
Leucine, isoleucine and valine metabolism	WikiPathways	67	7	0.485	14.43	3.27E-07	2.71E-04	Isoleucine; Isobutyrylglycine; Ketoleucine; 3-Hydroxybutyric acid; alpha-Ketoisovaleric acid; 3-Hydroxymethylglutaric acid; 3-Methylglutaconic acid;
Amino acid metabolism	WikiPathways	109	8	0.789	10.14	6.62E-07	3.90E-04	3-Hydroxybutyric acid; L-Cystathionine; Glutamic acid; Isoleucine; L-Aspartic acid; L-Tryptophan; Ketoleucine; alpha-Ketoisovaleric acid
Biochemical pathways: part I	WikiPathways	445	14	3.22	4.35	7.07E-07	3.90E-04	alpha-Ketoisovaleric acid; Cytidine; Dimethylglycine; Glutamic acid; Isoleucine; L-Aspartic acid; L-Cystine; Sarcosine; Thymidine; Kynurenine; Ketoleucine; Kynurenic acid; L-Tryptophan; 2-Aminobenzoic acid
SLC-mediated transmembrane transport	Reactome	155	9	1.12	8.04	8.64E-07	4.09E-04	Cytidine; L-Cystine; Thymidine; Creatinine; L-Tryptophan; L-Aspartic acid; Glutamic acid; 3-Hydroxybutyric acid; Isoleucine;
Amino acid transport defects (IEMs)	WikiPathways	27	5	0.196	25.51	1.03E-06	4.25E-04	Glutamic acid; L-Aspartic acid; L-Tryptophan; L-Cystine; Isoleucine;
Tryptophan catabolism	Reactome	33	5	0.239	20.92	2.93E-06	1.08E-03	Kynurenine; Kynurenic acid; L-Tryptophan; Isoleucine
Urea cycle and metabolism of amino groups	WikiPathways	34	5	0.246	20.33	3.41E-06	1.13E-03	Glutamic acid; L-Aspartic acid; Sarcosine; Creatinine; N2-Acetylornithine
Transport of small molecules	Reactome	208	9	1.51	5.96	1.01E-05	3.03E-03	Cytidine; L-Cystine; Thymidine; Creatinine; L-Tryptophan; L-Aspartic acid; Glutamic acid; 3-Hydroxybutyric acid; Isoleucine
Tryptophan catabolism leading to NAD <sup>+</sup> production	WikiPathways	23	4	0.167	23.95	1.81E-05	5.00E-03	Kynurenine; Kynurenic acid; L-Tryptophan; 2-Aminobenzoic acid;

Transcription/Translation	SMPDB	25	4	0.181	22.10	2.56E-05	6.53E-03	Glutamic acid; Isoleucine; L-Aspartic acid; L-Tryptophan
Transport of inorganic cations/anions and amino acids/oligopeptides	Reactome	52	5	0.377	13.26	2.91E-05	6.77E-03	L-Cystine; L-Tryptophan; L-Aspartic acid; Glutamic acid; Isoleucine;
Tryptophan metabolism	KEGG	53	5	0.384	13.02	3.20E-05	6.77E-03	Glutamic acid;Kynurenine; Kynurenic acid; L-Tryptophan; 2-Aminobenzoic acid;
beta-Alanine metabolism	KEGG	28	4	0.203	19.70	4.08E-05	6.77E-03	Carnosine; Glutamic acid; L-Aspartic acid; Anserine
Carnosinuria, carnosinemia	SMPDB	28	4	0.203	19.70	4.08E-05	6.77E-03	Carnosine; Glutamic acid; L-Aspartic acid; Anserine
GABA-Transaminase Deficiency	SMPDB	28	4	0.203	19.70	4.08E-05	6.77E-03	Carnosine; Glutamic acid; L-Aspartic acid; Anserine
Ureidopropionase Deficiency	SMPDB	28	4	0.203	19.70	4.08E-05	6.77E-03	Carnosine; Glutamic acid; L-Aspartic acid; Anserine
Kynurenine pathway and links to cell senescence	WikiPathways	28	4	0.203	19.70	4.08E-05	6.77E-03	Kynurenic acid; 2-Aminobenzoic acid; L-Tryptophan; Kynurenine;
Amino acid transport across the plasma membrane	Reactome	32	4	0.232	17.24	7.03E-05	1.11E-02	L-Cystine; L-Tryptophan; Glutamic acid; Isoleucine
Branched-chain amino acid catabolism	Reactome	37	4	0.268	14.93	1.26E-04	1.90E-02	alpha-Ketoisovaleric acid; Ketoleucine; Glutamic acid; Isoleucine;
Cysteine and methionine catabolism	WikiPathways	39	4	0.282	14.18	1.55E-04	2.24E-02	L-Cystine; Glutamic acid;Dimethylglycine;L-Cystathionine
Pyrimidine metabolism and related diseases	WikiPathways	43	4	0.311	12.86	2.28E-04	3.06E-02	Cytidine; Thymidine; Glutamic acid; L-Aspartic acid
3-Phosphoglycerate dehydrogenase deficiency	SMPDB	49	4	0.355	11.27	3.80E-04	3.06E-02	Dimethylglycine; L-Cystathionine;Glutamic acid; Sarcosine
Dihydropyrimidine Dehydrogenase Deficiency (DHPD)	SMPDB	49	4	0.355	11.27	3.80E-04	3.06E-02	Dimethylglycine; L-Cystathionine;Glutamic acid; Sarcosine
Dimethylglycine Dehydrogenase Deficiency	SMPDB	49	4	0.355	11.27	3.80E-04	3.06E-02	Dimethylglycine; L-Cystathionine;Glutamic acid; Sarcosine
Hyperglycinemia, non-ketotic	SMPDB	49	4	0.355	11.27	3.80E-04	3.06E-02	Dimethylglycine; L-Cystathionine;Glutamic acid; Sarcosine
Non Ketotic Hyperglycinemia	SMPDB	49	4	0.355	11.27	3.80E-04	3.06E-02	Dimethylglycine; L-Cystathionine;Glutamic acid; Sarcosine
Sarcosinemia	SMPDB	49	4	0.355	11.27	3.80E-04	3.06E-02	Dimethylglycine; L-Cystathionine;Glutamic acid; Sarcosine
Methionine Metabolism	KEGG	51	4	0.369	10.84	4.44E-04	3.06E-02	Dimethylglycine; L-Cystathionine;Glutamic acid; Sarcosine

Valine, leucine and isoleucine degradation	KEGG	51	4	0.369	10.84	4.44E-04	3.06E-02	alpha-Ketoisovaleric acid; Glutamic acid; Isoleucine;Ketoleucine;
2-Methyl-3-Hydroxybutyryl CoA Dehydrogenase Deficiency	SMPDB	51	4	0.369	10.84	4.44E-04	3.06E-02	alpha-Ketoisovaleric acid; Glutamic acid; Isoleucine;Ketoleucine;
3-Hydroxy-3-Methylglutaryl-CoA Lyase Deficiency	SMPDB	51	4	0.369	10.84	4.44E-04	3.06E-02	alpha-Ketoisovaleric acid; Glutamic acid; Isoleucine;Ketoleucine;
3-hydroxyisobutyric acid dehydrogenase deficiency	SMPDB	51	4	0.369	10.84	4.44E-04	3.06E-02	alpha-Ketoisovaleric acid; Glutamic acid; Isoleucine;Ketoleucine;
3-hydroxyisobutyric aciduria	SMPDB	51	4	0.369	10.84	4.44E-04	3.06E-02	alpha-Ketoisovaleric acid; Glutamic acid; Isoleucine;Ketoleucine;
3-Methylcrotonyl Coa Carboxylase Deficiency Type I	SMPDB	51	4	0.369	10.84	4.44E-04	3.06E-02	alpha-Ketoisovaleric acid; Glutamic acid; Isoleucine;Ketoleucine;
3-Methylglutaconic Aciduria Type I	SMPDB	51	4	0.369	10.84	4.44E-04	3.06E-02	alpha-Ketoisovaleric acid; Glutamic acid; Isoleucine;Ketoleucine;
3-Methylglutaconic Aciduria Type III	SMPDB	51	4	0.369	10.84	4.44E-04	3.06E-02	alpha-Ketoisovaleric acid; Glutamic acid; Isoleucine;Ketoleucine;
3-Methylglutaconic Aciduria Type IV	SMPDB	51	4	0.369	10.84	4.44E-04	3.06E-02	alpha-Ketoisovaleric acid; Glutamic acid; Isoleucine;Ketoleucine;
Beta-Ketothiolase Deficiency	SMPDB	51	4	0.369	10.84	4.44E-04	3.06E-02	alpha-Ketoisovaleric acid; Glutamic acid; Isoleucine;Ketoleucine;
Isobutyryl-coa dehydrogenase deficiency	SMPDB	51	4	0.369	10.84	4.44E-04	3.06E-02	alpha-Ketoisovaleric acid; Glutamic acid; Isoleucine;Ketoleucine;
Isovaleric acidemia	SMPDB	51	4	0.369	10.84	4.44E-04	3.06E-02	alpha-Ketoisovaleric acid; Glutamic acid; Isoleucine;Ketoleucine;
Isovaleric Aciduria	SMPDB	51	4	0.369	10.84	4.44E-04	3.06E-02	alpha-Ketoisovaleric acid; Glutamic acid; Isoleucine;Ketoleucine;
Maple Syrup Urine Disease	SMPDB	51	4	0.369	10.84	4.44E-04	3.06E-02	alpha-Ketoisovaleric acid; Glutamic acid; Isoleucine;Ketoleucine;
Methylmalonate Semialdehyde Dehydrogenase Deficiency	SMPDB	51	4	0.369	10.84	4.44E-04	3.06E-02	alpha-Ketoisovaleric acid; Glutamic acid; Isoleucine;Ketoleucine;
Methylmalonic Aciduria	SMPDB	51	4	0.369	10.84	4.44E-04	3.06E-02	alpha-Ketoisovaleric acid; Glutamic acid; Isoleucine;Ketoleucine;
Propionic Acidemia	SMPDB	51	4	0.369	10.84	4.44E-04	3.06E-02	alpha-Ketoisovaleric acid; Glutamic acid; Isoleucine;Ketoleucine;
Histidine catabolism	Reactome	22	3	0.159	18.87	4.83E-04	3.13E-02	Carnosine; Glutamic acid;Anserine

Biomarkers for urea cycle disorders	WikiPathways	22	3	0.159	18.87	4.83E-04	3.13E-02	Glutamic acid; L-Aspartic acid;Homocitrulline
Defective SLC1A3 causes episodic ataxia 6 (EA6)	Reactome	5	2	0.0362	55.25	5.01E-04	3.13E-02	L-Aspartic Acid; Glutamic acid
Defective SLC1A1 is implicated in schizophrenia 18 (SCZD18) and dicarboxylic aminoaciduria (DCBXA)	Reactome	5	2	0.0362	55.25	5.01E-04	3.13E-02	L-Aspartic Acid; Glutamic acid
Gut-liver indole metabolism	WikiPathways	5	2	0.0362	55.25	5.01E-04	3.13E-02	Indoxyl sulfate; L-Tryptophan
NAD biosynthesis II (from tryptophan)	WikiPathways	24	3	0.174	17.24	6.28E-04	3.83E-02	Glutamic acid; Kynurenine, L-Tryptophan
Glycine, serine and threonine metabolism	KEGG	56	4	0.406	9.85	6.36E-04	3.83E-02	Dimethylglycine; L-Cystathionine; Sarcosine; Glutamic acid;Glutamic acid; Sarcosine
Metabolism	Reactome	1370	19	9.91	1.92	7.11E-04	4.21E-02	3-Hydroxybutyric acid; alpha-Ketoisovaleric acid; Carnosine; Cytidine; L-Cystathionine; Sarcosine; Thymidine; Creatinine; Kynurenine; Kynurenic acid; L-Tryptophan; Sphinganine 1-phosphate; alpha-Tocopherol; L-Aspartic acid; Ketoleucine; Glutamic acid; Isoleucine; Dimethylglycine; Anserine
Trans-sulfuration, one-carbon metabolism and related pathways	WikiPathways	58	4	0.42	9.52	7.27E-04	4.23E-02	Dimethylglycine; L-Cystathionine; Sarcosine; Glutamic acid

Abbreviations: FDR = False Discovery Rate, SLC = Solute carrier, GABA = Gamma-aminobutyric acid.

<sup>1</sup> No. of metabolites annotated in the set.

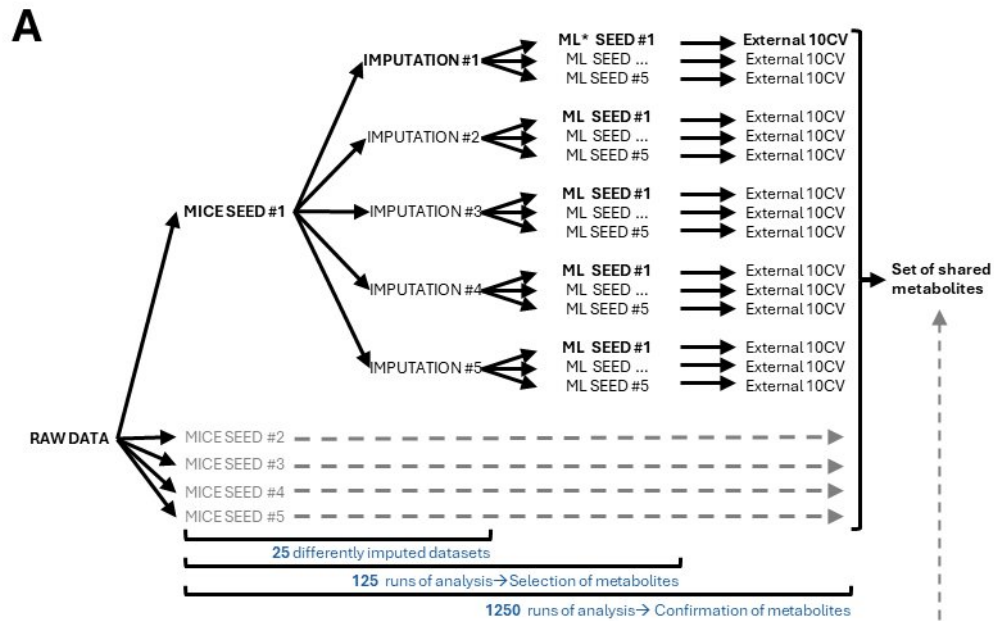
<sup>2</sup> Selected metabolites belonging to the set.

<sup>3</sup> Enrichment Ratio is computed by Hits / Expected, where hits = observed hits; expected = expected hits.

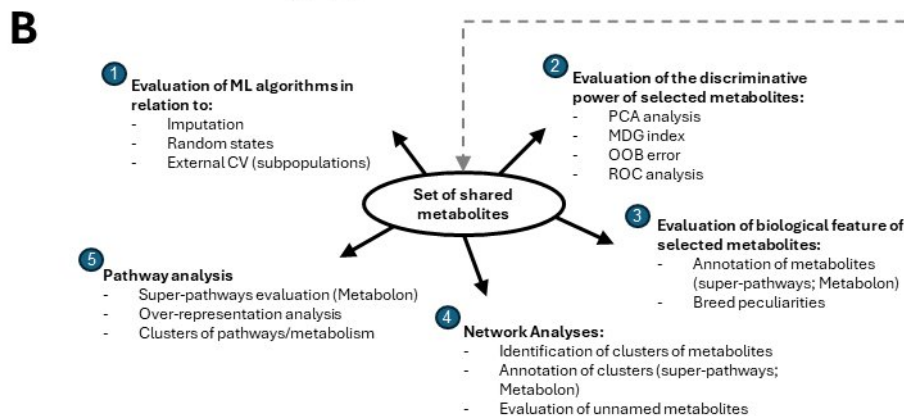


**Supplementary Figure S1.** Bioinformatics analyses adopted in the study. A) Schema of the pipeline used for the identification of differentially abundant metabolites between the two pig breeds. B) Methodological and biological evaluation of metabolite selections results.

Abbreviations: CV = Cross validation, MDG = Mean Decrease Gini, OOB = Out-of-bag, PCA = Principal Component Analysis, ROC = Receiver Operating Characteristic, sPLS-DA = sparse Partial Least Squares Discriminant Analysis

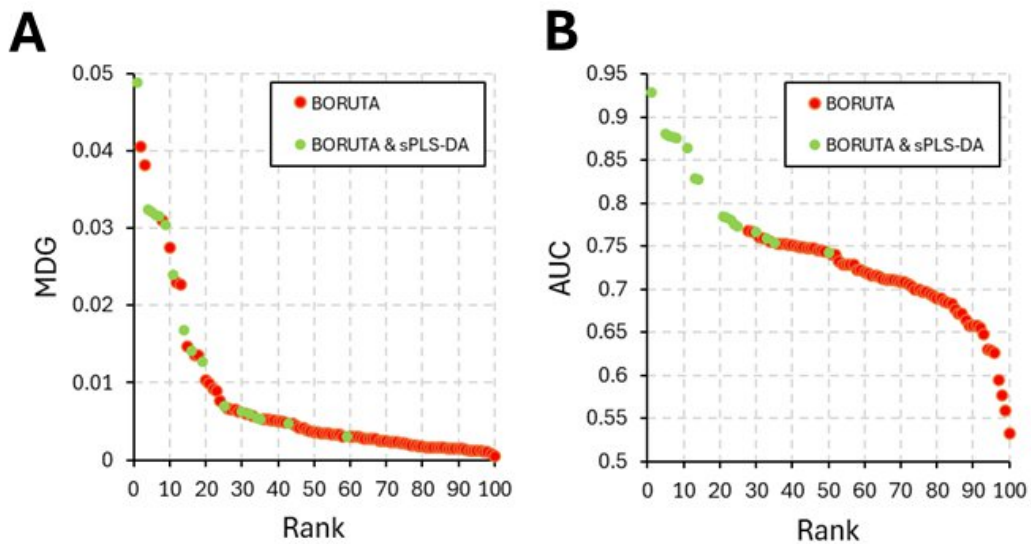


\*Machine Learning (ML) approaches: Boruta and sPLS-DA

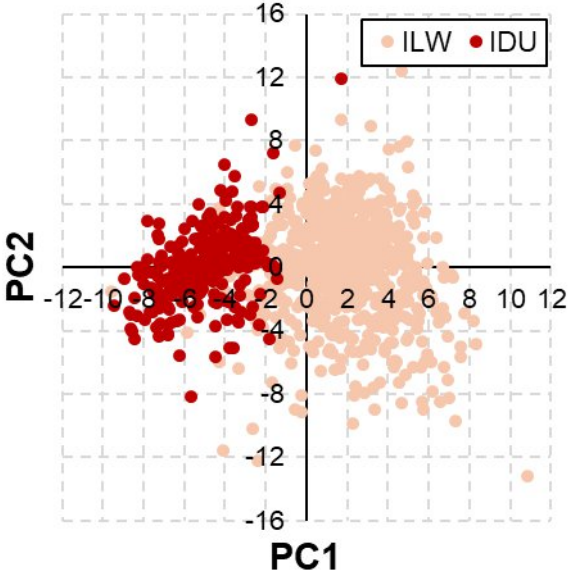


**Supplementary Figure S2.** Importance and discriminative power of selected metabolites discriminating between the two pig breeds. A) Metabolites are ranked based on the Mean Decrease Gini (MDG) index obtained in the Random Forest analysis; B) Metabolites are ranked based on the Area Under the Curve (AUC) obtained from the Receiver Operating Characteristic (ROC) analysis.

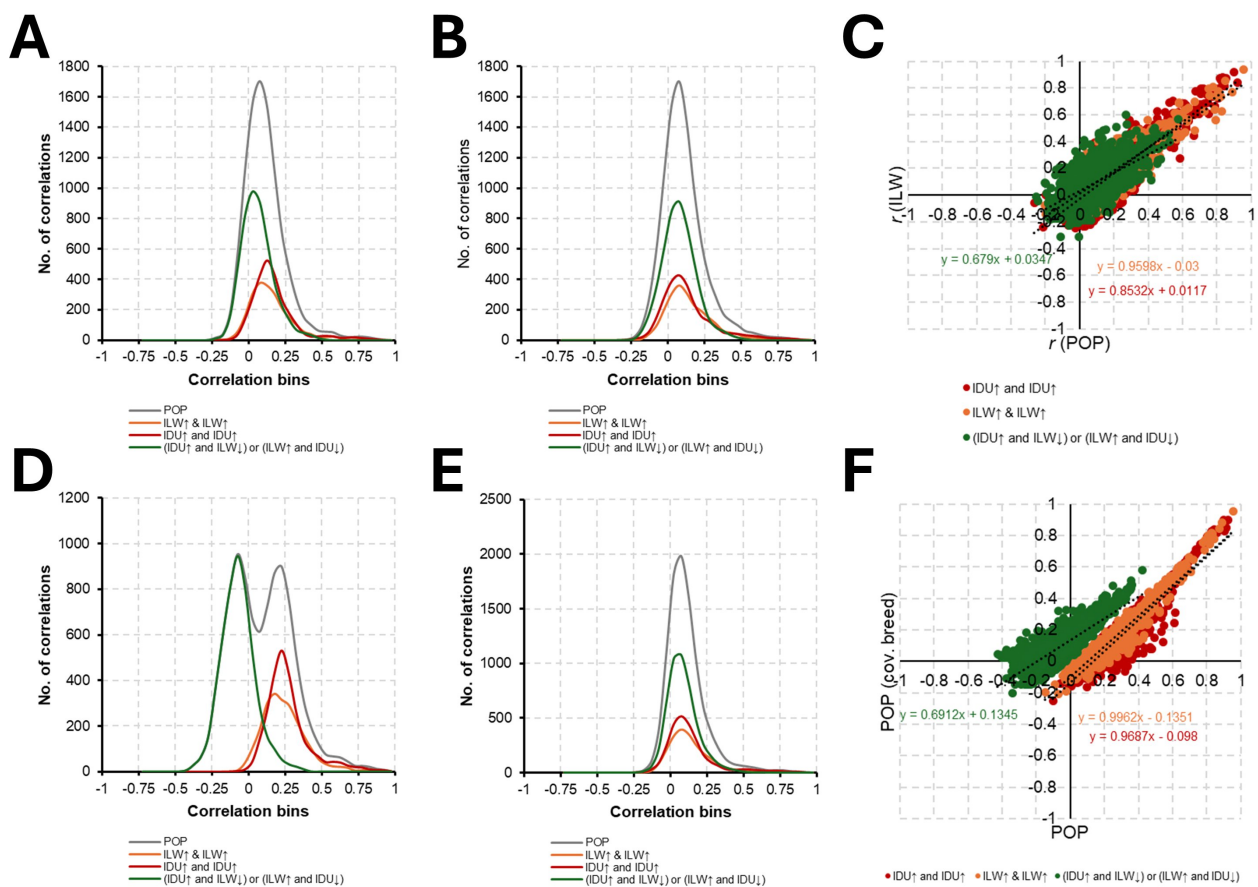
Abbreviations: sPLS-DA = sparse Partial Least Squares Discriminant Analysis



**Supplementary Figure S3.** Principal Component Analysis (PCA) of all pigs from Italian Duroc (IDU) and Italian Large White (ILW), based on metabolites selected only by BORUTA (n. 83). The first two Principal Components (PC) are shown.



**Supplementary Figure S4.** Correlation coefficients between selected metabolites in the Italian Large White (ILW), Italian Duroc (IDU) and the whole population (POP). A) Distribution of correlations in ILW pigs. Data are presented considering all correlations (gray line) and stratified based on classes of metabolite pairs [both metabolites have higher concentrations in ILW (orange line); one metabolite is higher in one population and the opposite (green line); both metabolites have higher concentrations in IDU (red line)]. B) Distribution of correlations in IDU pigs. Data are presented considering all correlations and stratified based on classes of metabolite pairs; C) Relationship between correlations obtained in ILW and IDU; D) Distribution of correlations in the POP; E) Distribution of correlations in the POP with metabolites corrected including the breed as covariate in the model; F) Relationship between uncorrected (no breed as a covariate in the model) and corrected correlations (breed as a covariate in the model; cov.breed) at the POP level.



**Supplementary Figure S5.** Relationship between corrected and uncorrected correlations in the Italian Large White (ILW), Italian Duroc (IDU) pigs, and the whole pig population (POP). A-B) ILW and IDU vs POP (uncorrected); C-D) ILW and IDU vs POP (corrected; cov.breed). Data are stratified based on classes of metabolite pairs [both metabolites have higher concentrations in ILW (orange dots); one metabolite is higher in one population and the opposite (green dots); both metabolites have higher concentrations in IDU (red dots)].

