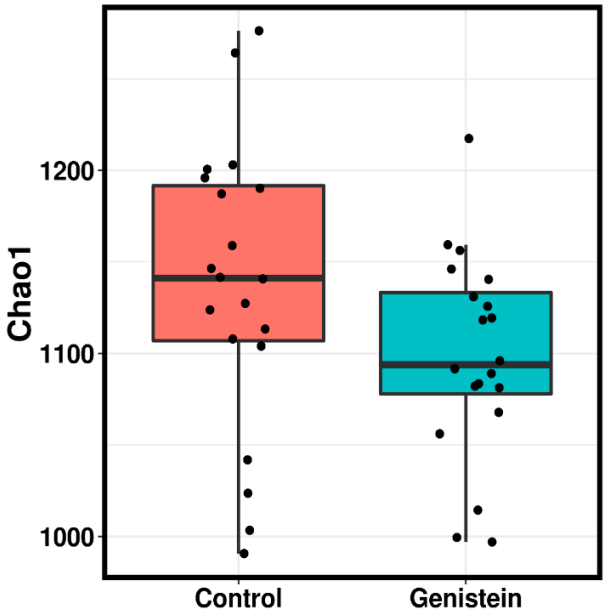
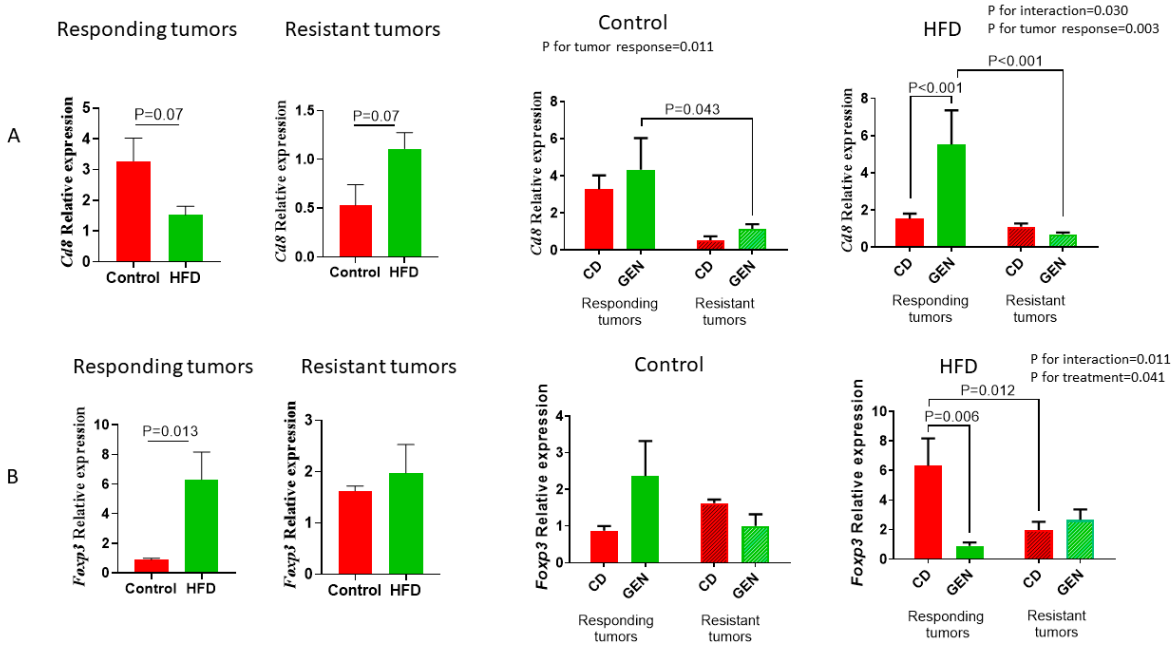
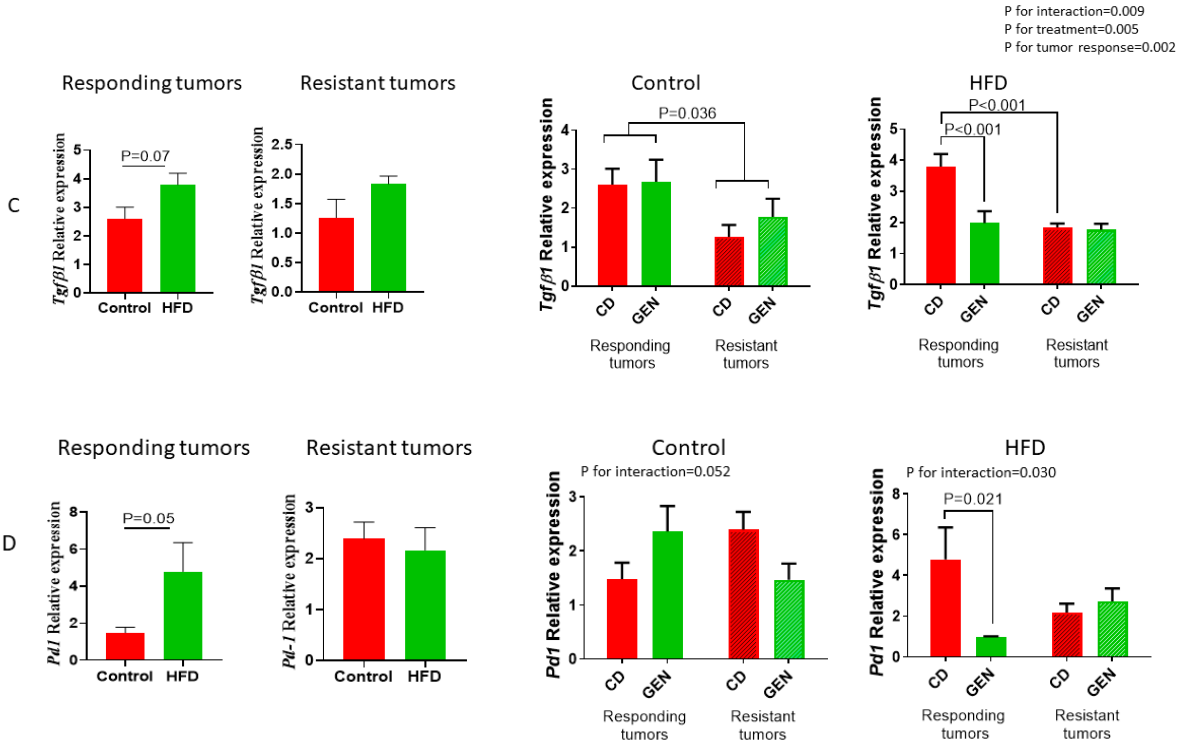
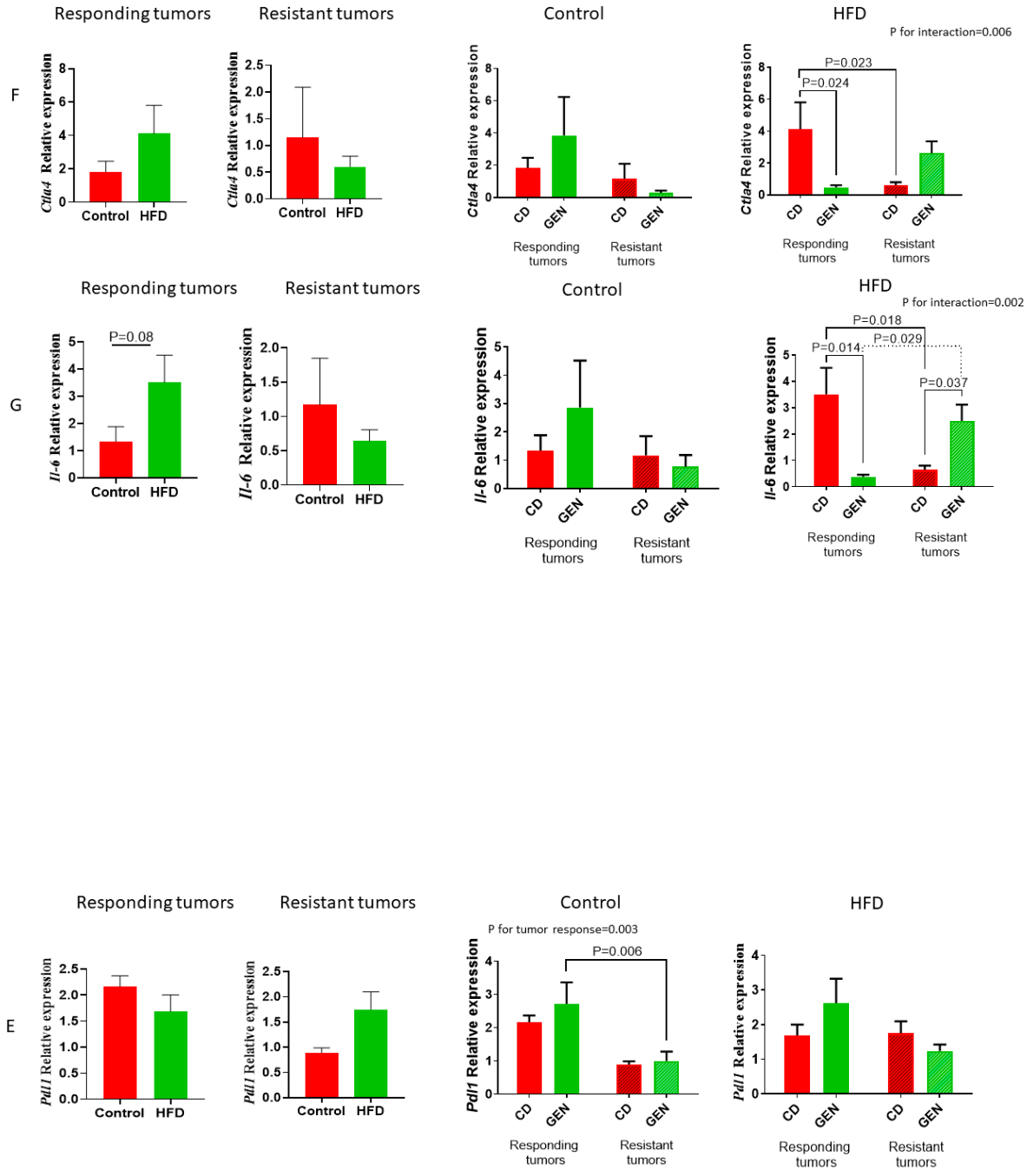



**Supplementary Fig. S1.** The genistein supplementation significantly reduced Chao 1 microbial richness. N = 20 per group. P < 0.05.

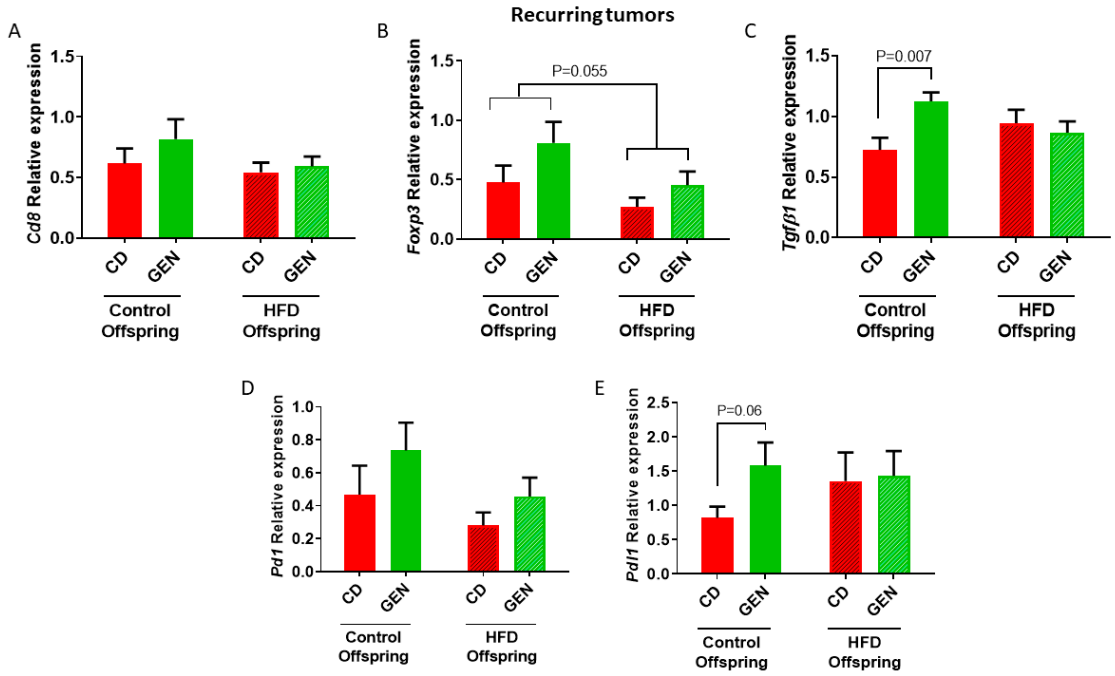



P for interaction=0.009
P for treatment=0.005
P for tumor response=0.002
C
Responding tumors
Resistant tumors
Control
HFD
Tgfβ1 Relative expression
P=0.07
Control HFD
P=0.036
P<0.001
P<0.001
CD
GEN
Responding tumors
Resistant tumors
D
Pd1 Relative expression
Pd-1 Relative expression
P=0.05
P for interaction=0.052
P for interaction=0.030
P=0.021




**Supplementary Fig. S2. Genistein reverses markers of immunosuppression in the tamoxifen responsive mammary tumors in the HFD offspring.** Gene expression of *Cd8a* (A), *Foxp3* (B), *Tgfβ1* (C), *Pd1* (D), *Pdl1* (E), *Ctla4* (F), *Il6* (G) in mammary tumors of control and HFD offspring treated with tamoxifen and supplemented with genistein. Maternal HFD significantly increased expression of *Foxp3* (P=0.013), *Pd1* (P=0.05) and non-significantly increased *Tgfb1* (P=0.07), *Il6* (P=0.08) and decreased *Cd8* (P=0.07) in responding tumors. Maternal HFD did not significantly change expression of any of the genes in resistant tumors. Genistein did not change expression of any gene in responding and resistant tumors of control offspring compared to untreated control. Among HFD offspring,

genistein supplementation increased expression of *Cd8* (P<0.001) and decreased expression of *Foxp3* (P=0.006), *Tgfβ1* (P<0.001), *Pd1* (P=0.021), *Ctla4* (P=0.024) and *Il-6* (P=0.014) in responding tumors. In resistant tumors, genistein increased expression of *Il-6* (P=0.037) among HFD offspring. Differences according to t test when comparing the effects of Maternal exposure only and Two-Way ANOVA followed by Holm-Sidak test when comparing the effects of Maternal exposure and offspring treatment. Means ± SEM, n=2-11 are shown.




**Supplementary Fig. S3. Effects of genistein supplementation on recurring tumors.** Gene expression of *Cd8a* (A), *Foxp3* (B), *Tgfβ1* (C), *Pd1* (D) and *Pdl1* (E) in recurring mammary tumors of control and HFD offspring treated with tamoxifen and supplemented with genistein after tamoxifen response. Genistein supplementation significantly and non-significantly increased expression of *Tgfβ1* (P=0.007) and *Pdl1* (P=0.06), respectively, in the control offspring compared to untreated control. Genistein did not change expression of *Cd8*, *Foxp3* and *Pd1* in both control and HFD offspring and *Tgfb1* and *Pdl1* in HFD offspring. Differences according to Two-Way ANOVA followed by Holm-Sidak test for *Foxp3* and *Tgfb1* and t test for *Pdl1*. Means ± SEM, n=7-9 are shown.

**Supplementary Table S1.** Primers used in quantitative real-time PCR

| Gene | Sequence |
|---|---|
| *Cd8*_ Forward | GCGATATTTACATCTGGGCACC |
| *Cd8*_ Reverse | AATTTCTCTGAAGGTCTGGGC |
| *Ctla4*_ Forward | GTACAAAACTCACCTGCAGC |
| *Ctla4*_ Reverse | AAGGTTGGGTCACTTGTATGG |
| *Foxp3*_ Forward | TGCCACCTGGGATCAATGTG |
| *Foxp3*_ Reverse | CGTGGGAAGGTGCAGAGTAGAGC |

| | |
|---|---|
| *Hprt1*_ Forward | 5′-GCC CTT GAC TAT AAT GAG CAC T-3′ |
| *Hprt1*_ Reverse | 5′-CCG CTG TCT TTT AGG CTT TG-3′ |
| *Il6*_ Forward | TTGCCTTCTTGGGACTGATG |
| *Il6*_ Reverse | GTGGTATCCTCTGTGAAGTCTC |
| *Pd1*_ Forward | ATATCCCAGACCCTCACCCAA |
| *Pd1*_ Reverse | TTCTCTGGCCTCTGACATACCT |
| *Pdl1*_ Forward | AACGTGACCAGCGTTCTGAG |
| *Pdl1*_ Reverse | GGTACAGGCAGTTCTGGGATG |
| *Tgfβ1*_forward | 5′- CCTGAGTGGCTGTCTTTTGA -3′ |
| *Tgfβ1*_reverse | 5′- CGTGGAGTACATTATCTTTGCTG -3′ |

**Supplementary Table S2.** Metabolites significantly dysregulated in gut contents of the offspring of dams fed a high-fat diet (HFD). *N* = 20 per group. CD: offspring of the dams fed a control or low-fat diet.

| HMDB ID | Compound_name | *P* value | Fold (HFD/CD) | ESI | RT [min] | Monoisotopic_ Mass | Delta (ppm) |
|---|---|---|---|---|---|---|---|
| HMDB0000491 | 3-Methyl-2-oxovaleric acid | 8.36E-04 | 0.40 | negative | 3.018 | 130.06 | 16 |
| HMDB0006528 | Docosapentaenoic acid (22n-3) | 1.16E-03 | 0.56 | positive | 11.502 | 330.26 | 0 |
| HMDB0000254 | Succinic acid | 1.29E-03 | 2.83 | negative | 1.184 | 118.03 | 13 |
| HMDB0028854 | Glycylvaline | 3.89E-03 | 0.70 | negative | 1.238 | 174.10 | 11 |
| HMDB0028735 | Asparaginyl-Leucine | 5.13E-03 | 1.81 | positive | 1.738 | 245.14 | 1 |
| HMDB0010378 | 5,8,11-Eicosatrienoic acid | 5.13E-03 | 0.67 | positive | 11.806 | 306.26 | 0 |
| HMDB0240261 | LysoPI(18:0/0:0) | 6.15E-03 | 2.48 | negative | 9.859 | 600.33 | 4 |
| HMDB0000756 | Hexanoylcarnitine | 6.15E-03 | 1.44 | positive | 4.146 | 259.18 | 2 |
| HMDB0000156 | L-Malic acid | 1.12E-02 | 1.81 | negative | 0.915 | 134.02 | 14 |
| HMDB0000210 | Pantothenic acid | 1.22E-02 | 0.72 | negative | 1.998 | 219.11 | 8 |
| HMDB0032549 | N-Undecylbenzenesulfonic acid | 1.32E-02 | 1.84 | negative | 8.245 | 312.18 | 1 |
| HMDB0059915 | 4-Dodecylbenzenesulfonic Acid | 1.32E-02 | 1.78 | negative | 8.862 | 326.19 | 0 |
| HMDB0094651 | Pyroglutamylvaline | 1.32E-02 | 1.48 | positive | 1.826 | 228.11 | 0 |
| HMDB0000687 | L-Leucine | 1.32E-02 | 0.74 | negative | 1.316 | 131.09 | 15 |
| HMDB0062121 | Dihydroferulic acid | 1.43E-02 | 1.65 | positive | 4.357 | 196.07 | 2 |
| HMDB0041607 | 2-Phenoxyethanol | 1.55E-02 | 1.55 | positive | 3.611 | 138.07 | 2 |
| HMDB0094646 | 2-Pentanamido-3-phenylpropanoic acid | 1.95E-02 | 0.42 | positive | 3.851 | 249.14 | 2 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| HMDB0002658 | 6-Hydroxynicotinic acid | 1.95E-02 | 1.65 | negative | 2.832 | 139.03 | 14 |
| HMDB0061660 | 2(R)-hydroxydocosanoic acid | 2.11E-02 | 1.94 | negative | 12.79 | 356.33 | 1 |
| HMDB0000867 | Ribonic acid | 2.11E-02 | 0.75 | negative | 1.425 | 166.05 | 4 |
| HMDB0000182 | L-Lysine | 2.11E-02 | 0.59 | positive | 1.323 | 146.11 | 2 |
| HMDB0000070 | Pipecolic acid | 2.27E-02 | 0.70 | positive | 1.328 | 129.08 | 2 |
| HMDB0031491 | 2,3-Hexanedione | 2.45E-02 | 0.52 | positive | 3.254 | 114.07 | 4 |
| HMDB0001448 | Sulfate | 2.63E-02 | 1.92 | negative | 1.063 | 97.97 | 21 |
| HMDB0000300 | Uracil | 2.63E-02 | 0.77 | negative | 0.971 | 112.03 | 19 |
| HMDB0028932 | Leucyl-Isoleucine | 2.63E-02 | 1.41 | positive | 3.636 | 244.18 | 1 |
| HMDB0002024 | Imidazoleacetic acid | 2.83E-02 | 0.76 | negative | 1.252 | 126.04 | 16 |
| HMDB0060390 | 4-Hydroxyphenyl acetate | 2.83E-02 | 0.78 | negative | 2.639 | 152.05 | 13 |
| HMDB0028991 | Phenylalanylaspartic acid | 2.83E-02 | 1.51 | positive | 3.416 | 280.11 | 1 |
| HMDB0037293 | 2-Acetylpyrrolidine | 2.83E-02 | 1.36 | positive | 1.444 | 113.08 | 3 |
| HMDB0000549 | gamma-Butyrolactone | 3.04E-02 | 1.83 | positive | 1.322 | 86.04 | 7 |
| HMDB0000176 | Maleic acid | 3.04E-02 | 1.48 | negative | 0.941 | 116.01 | 18 |
| HMDB0001999 | Eicosapentaenoic acid | 3.26E-02 | 1.17 | positive | 3.667 | 302.22 | 11 |
| HMDB0000824 | propionylcarnitine | 3.50E-02 | 1.21 | positive | 3.363 | 217.13 | 1 |
| HMDB0000138 | Glycocholic acid | 3.75E-02 | 1.66 | positive | 5.873 | 465.31 | 1 |
| HMDB0011757 | N-Acetylvaline | 3.75E-02 | 1.70 | positive | 1.818 | 159.09 | 1 |
| HMDB0011732 | 2-Keto-L-gluconate | 3.75E-02 | 1.67 | negative | 0.897 | 194.04 | 9 |
| HMDB0028757 | Aspartyl-Leucine | 3.75E-02 | 0.59 | negative | 2.708 | 246.12 | 5 |
| HMDB0028804 | Glutaminylphenylalanine | 4.02E-02 | 1.61 | positive | 3.338 | 293.14 | 1 |
| HMDB0001406 | Niacinamide | 4.02E-02 | 2.13 | positive | 1.693 | 122.05 | 3 |
| HMDB0031654 | 3-Aminobutanoic acid | 4.30E-02 | 1.79 | positive | 1.323 | 103.06 | 6 |
| HMDB0029638 | 4-Methylbenzaldehyde | 4.60E-02 | 0.07 | positive | 1.647 | 120.06 | 3 |
| HMDB0031598 | 2,3-Pentanedione | 4.60E-02 | 0.49 | positive | 1.351 | 100.05 | 4 |
| HMDB0094704 | N-Propionylmethionine | 4.60E-02 | 0.69 | positive | 3.887 | 205.08 | 2 |
| HMDB0032616 | Sinapic acid | 4.60E-02 | 1.92 | positive | 4.245 | 224.07 | 1 |
| HMDB0001186 | N1-Acetylspermine | 4.60E-02 | 0.40 | positive | 1.238 | 244.23 | 1 |
| HMDB0001227 | Thymidine 5'-monophosphate | 4.60E-02 | 2.02 | negative | 1.128 | 322.06 | 4 |
| HMDB0094701 | N-Acetylproline | 4.91E-02 | 0.71 | positive | 3.882 | 157.07 | 3 |
| HMDB0001432 | Agmatine | 4.91E-02 | 2.65 | positive | 1.232 | 130.12 | 2 |
| HMDB0000562 | Creatinine | 4.91E-02 | 1.52 | positive | 1.332 | 113.06 | 4 |
| HMDB0000301 | Urocanic acid | 4.91E-02 | 0.73 | negative | 0.901 | 138.04 | 14 |

**Supplementary Table S3.** The Lilikoi algorithm identified biological pathways significantly enriched in the fecal samples in the offspring of dams maternal high-fat diet or with or without genistein supplementation. HFD: The offspring of dams fed a maternal high-fat diet. CD: the offspring of dams fed a maternal control diet. CD/GEN: rats from control dams with the genistein supplementation. CD/CD: rats from control dams without the genistein supplementation. HFD/GEN: rats from HFD dams with the genistein supplementation. HFD/CD: rats from HFD dams without the genistein supplementation. GEN: with genistein supplementation. CD: without genistein supplementation.

| Pathway | Gain Ratio | Enriched |
|---|---|---|
| Warburg Effect | 0.42 | HFD vs CD |
| | | |
| Arginine and Proline Metabolism | 0.42 | GEN vs CD |
| Arginine: Glycine Amidinotransferase Deficiency (AGAT Deficiency) | 0.42 | GEN vs CD |
| Creatine deficiency, guanidinoacetate methyltransferase deficiency | 0.42 | GEN vs CD |
| Guanidinoacetate Methyltransferase Deficiency (GAMT Deficiency) | 0.42 | GEN vs CD |
| Hyperornithinemia with gyrate atrophy (HOGA) | 0.42 | GEN vs CD |
| Hyperornithinemia-hyperammonemia-homocitrullinuria [HHH-syndrome] | 0.42 | GEN vs CD |
| Hyperprolinemia Type I | 0.42 | GEN vs CD |
| Hyperprolinemia Type II | 0.42 | GEN vs CD |
| L-arginine:glycine amidinotransferase deficiency | 0.42 | GEN vs CD |
| Ornithine Aminotransferase Deficiency (OAT Deficiency) | 0.42 | GEN vs CD |
| Prolidase Deficiency (PD) | 0.42 | GEN vs CD |
| Prolinemia Type II | 0.42 | GEN vs CD |
| | | |
| Disulfiram Action Pathway | 0.57 | HFD/GEN vs HFD/CD |
| Nicotinate and Nicotinamide Metabolism | 0.57 | HFD/GEN vs HFD/CD |
| 3-Phosphoglycerate dehydrogenase deficiency | 0.47 | HFD/GEN vs HFD/CD |
| Ammonia Recycling | 0.47 | HFD/GEN vs HFD/CD |
| Carnitine Synthesis | 0.47 | HFD/GEN vs HFD/CD |
| Dihydropyrimidine Dehydrogenase Deficiency (DHPD) | 0.47 | HFD/GEN vs HFD/CD |
| Dimethylglycine Dehydrogenase Deficiency | 0.47 | HFD/GEN vs HFD/CD |
| Glycine and Serine Metabolism | 0.47 | HFD/GEN vs HFD/CD |
| Hyperglycinemia, non-ketotic | 0.47 | HFD/GEN vs HFD/CD |
| Ibuprofen Action Pathway | 0.47 | HFD/GEN vs HFD/CD |
| Non Ketotic Hyperglycinemia | 0.47 | HFD/GEN vs HFD/CD |
| Sarcosinemia | 0.47 | HFD/GEN vs HFD/CD |
| Amino Sugar Metabolism | 0.46 | HFD/GEN vs HFD/CD |
| G(M2)-Gangliosidosis: Variant B, Tay-sachs disease | 0.46 | HFD/GEN vs HFD/CD |
| Salla Disease/Infantile Sialic Acid Storage Disease | 0.46 | HFD/GEN vs HFD/CD |
| Sialuria or French Type Sialuria | 0.46 | HFD/GEN vs HFD/CD |
| Tay-Sachs Disease | 0.46 | HFD/GEN vs HFD/CD |
| | | |
| 2-ketoglutarate dehydrogenase complex deficiency | 0.57 | CD/GEN vs CD/CD |
| Citric Acid Cycle | 0.57 | CD/GEN vs CD/CD |
| Congenital lactic acidosis | 0.57 | CD/GEN vs CD/CD |
| Fumarase deficiency | 0.57 | CD/GEN vs CD/CD |
| Mitochondrial complex II deficiency | 0.57 | CD/GEN vs CD/CD |
| Pyruvate dehydrogenase deficiency (E2) | 0.57 | CD/GEN vs CD/CD |
| Pyruvate dehydrogenase deficiency (E3) | 0.57 | CD/GEN vs CD/CD |
| Methylenetetrahydrofolate Reductase Deficiency (MTHFRD) | 0.47 | CD/GEN vs CD/CD |
| Tryptophan Metabolism | 0.47 | CD/GEN vs CD/CD |
| Carnitine Synthesis | 0.46 | CD/GEN vs CD/CD |

**Supplementary Table S4**. Top 20 important metabolites based on mean decrease accuracy identified by the Random Forest algorithms contributing to the classification of the genistein supplementation status.

| HMDB ID | ESI | Compound | MeanDecreaseAccuracy | MeanDecreaseGini |
|---|---|---|---|---|
| HMDB0000884 | negative | Ribothymidine | 6.87 | 1.27 |
| HMDB0003306 | negative | Phloretin | 5.92 | 1.02 |
| HMDB0000875 | positive | Trigonelline | 4.83 | 0.43 |
| HMDB0011500 | negative | LysoPE(14:0/0:0) | 4.82 | 0.54 |
| HMDB0004157 | positive | Stercobilinogen | 4.36 | 0.48 |
| HMDB0004157 | negative | Stercobilinogen | 3.98 | 0.45 |
| HMDB0000054 | positive | Bilirubin | 3.69 | 0.40 |
| HMDB0000257 | negative | Thiosulfate | 3.63 | 0.38 |
| HMDB0000054 | negative | Bilirubin | 3.60 | 0.33 |
| HMDB0000151 | positive | Estradiol | 3.59 | 0.35 |
| HMDB0004284 | negative | Tyrosol | 3.26 | 0.29 |
| HMDB0002670 | negative | Naringenin | 2.70 | 0.35 |
| HMDB0000626 | negative | Deoxycholic acid | 2.33 | 0.14 |
| HMDB0002639 | positive | Sulfolithocholylglycine | 2.32 | 0.10 |
| HMDB0035248 | positive | 2,6-Dimethylpyrazine | 2.30 | 0.19 |
| HMDB0000017 | negative | 4-Pyridoxic acid | 2.28 | 0.18 |
| HMDB0005199 | positive | (R)-Salsolinol | 2.28 | 0.27 |
| HMDB0011177 | positive | Phenylalanylproline | 2.16 | 0.15 |
| HMDB0000254 | negative | Succinic acid | 2.07 | 0.10 |
| HMDB0034423 | negative | 2-Deoxycastasterone | 1.99 | 0.13 |