Husso et al. 2023 Impacts of maternal microbiota and microbial metabolites on fetal intestine, brain and placenta



Supplementary Figure 1. Over-representation analysis of genes which were significantly differentially expressed (q < 0.05) in GF versus SPF fetal murine intestine, brain and placenta. Top 100 enriched ontology terms are shown. Metascape 3.5 using gene prioritization by evidence counting and selective GO clusters.



GO:0031400: negative regulation of protein modification process G0:0031400: negative regulation of protein modification process G0:0030522: intracellular receptor signaling pathway G0:0046626: regulation of insulin receptor signaling pathway G0:0019901: protein kinase binding G0:0030335: positive regulation of cell migration G0:1901214: regulation of neuron death G0:1902532: negative regulation of intracellular signal transduction G0:0048871: multicellular organismal-level homeostasis G0:00048343: carbohydrate transport GO:0008643: carbohydrate transport GO:0030099: myeloid cell differentiation GO:0045121: membrane raft GO:0014070: response to organic cyclic compound GO:0003725: double-stranded RNA binding GO:0002460: adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains GO:0051090: regulation of DNA-binding transcription factor activity GO:0001906: cell killing GO:0032647: regulation of interferon-alpha production GO:0016042: lipid catabolic process GO:0060759: regulation of response to cytokine stimulus GO:1901615: organic hydroxy compound metabolic process GO:0002252: immune effector process GO:0009725: response to hormone GO:0009725: response to normone GO:0031348: negative regulation of defense response GO:0008514: organic anion transmembrane transporter activity GO:0044242: cellular lipid catabolic process GO:0031329: regulation of cellular catabolic process GO:0045178: basal part of cell GO:0055088: lipid homeostasis GO:0035456: response to interferon-beta GO:0048002: antigen processing and presentation of peptide antigen GO:0051180: vitamin transport GO:0022600: digestive system process GO:0031528: microvillus membrane GO:0043122: regulation of I-kappaB kinase/NF-kappaB signaling GO:1901136: carbohydrate derivative catabolic process GO:0015850: organic hydroxy compound transport GO:0002683: negative regulation of immune system process GO:0010038: response to metal ion GO:0043230: extracellular organelle GO:0031581: hemidesmosome assembly GO:0010815: bradykinin catabolic process GO:0008270: zinc ion binding GO:0008270: zinc ion binding GO:0008255: negative regulation of cell population proliferation GO:0007584: response to nutrient GO:0022600: digestive system process GO:0007584: response to nutrient GO:0007584: response to nutrient GO:0002697: regulation of pattern recognition receptor signaling pathway GO:0002697: regulation of immune effector process GO:0002037: engulation infinite elector process GO:0140374: antiviral innate immune response GO:0009991: response to extracellular stimulus GO:0002012: regulation of small molecule metabolic process GO:0009743: response to carbohydrate GO:00042175: nuclear outer membrane-endoplasmic reticulum membrane network GO:001816: cytokine production GO:0019725: cellular homeostasis GO:0015291: secondary active transmembrane transporter activity GO:0048471: perinuclear region of cytoplasm GO:0005903: brush border GO:0009615: response to virus GO:0010876: lipid localization GO:0005902: microvillus GO:0000323: lytic vacuole GO:0008135: translation factor activity, RNA binding GO:000135: translation factor activity, RVA binding GO:0012729: mRNA binding GO:0010608: post-transcriptional regulation of gene expression GO:003044: regulation of chromosome GO:003044: regulation of chromosome organization GO:0006403: RNA localization GO:0005844: polysome GO:0005844: polysome GO:000520: amino acid metabolic process GO:0034399: nuclear periphery GO:0036464: cytoplasmic ribonucleoprotein granule GO:1904816: positive regulation of protein localization to chromosome, telomeric region GO:000028: ribosomal small subunit assembly GO:1901989: positive regulation of cell cycle phase transition GO:0016874: ligase activity GO:1901798: positive regulation of signal transduction by p53 class mediator GO:0022613: ribonucleoprotein complex biogenesis GO:0022613: ribonucleoprotein complex biogenesis GO:001944: vasculature development GO:008135: regulation of cellular response to stress GO:0005844: polysome GO:000135: regulation of cellular response to stress GO:0080135: regulation of cellular response to stress GO:0040008: regulation of growth GO:0050840: extracellular matrix binding GO:0008134: transcription factor binding GO:0031647: regulation of protein stability GO:0009123: nucleoside monophosphate metabolic process GO:0010638: positive regulation of organelle organization GO:0010638: positive regulation of organelle organization GO:0045598: regulation of fat cell differentiation GO:0043603: amide metabolic process GO:0051098: regulation of binding GO:0140657: ATP-dependent activity GO:0043467: regulation of generation of precursor metabolites and energy GO:0403829: positive regulation of protein localization GO:0006753: nucleoside phosphate metabolic process GO:0006790: sulfur compound metabolic process GO:0006730: sulfur compound metabolic process GO:0006730: multiple sheath Supplementary Figure 2. Over-representation GO:0043209: myelin sheath GO:0000278: mitotic cell cycle analysis of genes which were significantly GO:0062023: collagen-containing extracellular matrix GO:0019843: rRNA binding GO:0043021: ribonucleoprotein complex binding

downregulated or upregulated (q < 0.05) in GF versus SPF fetal murine intestine (DE) and genes with OPLS-DA variable importance in projection (VIP)>1 and negative or positive S-plot loadings. Top 100 enriched ontology terms are shown. Metascape 3.5 using gene prioritization by evidence counting.



Supplementary Figure 3. Over-representation analysis of genes which were significantly downregulated or upregulated (q < 0.05) in GF versus SPF fetal murine brain. Metascape 3.5 using gene prioritization by evidence counting.



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10 GO:0010632: regulation of epithelial cell migration GO:0051960: regulation of nervous system development GO:0051046: regulation of secretion GC:0051046: regulation of secretion GO:0046677: response to antibiotic GO:001235: positive regulation of apoptotic signaling pathway GC:0071634: regulation of transforming growth factor beta production GO:0050727: regulation of inflammatory response GO:0043408: regulation of MAPK cascade GO:0019216: regulation of Jipid metabolic process GO:0048471: perinuclear region of cytoplasm GO:0090909: negative regulation of canonical Wnt signaling pathway GO:0001775: cell activation GO:0070848: response to growth factor GO:0070848: response to growth factor GO:0045596: negative regulation of cell differentiation GO:0000323: lytic vacuole GO:0050839: cell adhesion molecule binding GO:0010942: positive regulation of cell death GO:0001632: positive regulation of proteolysis GO:000162: regulation of proteolysis GO:2001233: regulation of apoptotic signaling pathway GO:0001819: positive regulation of cytokine production GO:0048732: gland development GO:0010035: response to inorganic substance GO:0009611: response to wounding GO:0009725: response to hormone GO:0050900: leukocyte migration GO:0050865: regulation of cell activation GO:0001503: ossification GO:0031667: response to nutrient levels GO:0031667: response to nutrient levels GO:1903034: regulation of response to wounding GO:0019838: growth factor binding GO:0035239: tube morphogenesis GO:0030335: positive regulation of cell migration CO:0040008: regulation of cell migration GO:0040008: regulation of growth GO:0008285: negative regulation of cell population proliferation GO:0043588: skin development G0:0043588: skin development G0:0098609: cell-cell adhesion G0:0044057: regulation of system process G0:1904035: regulation of epithelial cell apoptotic process G0:0044703: multi-organism reproductive process G0:0045121: membrane raft G0:0010817: regulation of hormone levels G0:1901214: regulation of neuron death G0:00055080: monoatomic cation homeostasis G0:0042040: camera turo evid dovelament GO:0043010: camera-type eye development GO:0060191: regulation of lipase activity GO:0003158: endothelium development GO:0042063: gliogenesis GO:0043025: neuronal cell body GO:0005509: calcium ion binding GO:0051051: negative regulation of transport GO:0040113: negative regulation of intersport GO:0040013: negative regulation of locomotion GO:004589: developmental growth GO:1905953: negative regulation of lipid localization GO:0030510: regulation of BMP signaling pathway GO:0030510: regulation of BMP signaling pathway GO:0034103: regulation of tissue remodeling GO:0009896: positive regulation of catabolic process GO:0070161: anchoring junction GO:0006979: response to oxidative stress GO:0032103: positive regulation of response to external stimulus GO:0007167: enzyme-linked receptor protein signaling pathway GO:0000167: enzyme-linked receptor protein signaling pathway GO:0000041: transition metal ion transport GO:0032970: regulation of cardiocyte differentiation GO:0042176: regulation of cardiocyte differentiation GO:0042176: regulation of protein catabolic process GO:0051896: regulation of protein kinase B signaling GO:0001701: in utero embryonic development GO:002274: myeloid leukocyte activation GO:0002727: myeloid leukocyte activation GO:0002274: myeloid leukocyte activation GO:0015711: organic anion transport GO:0045744: negative regulation of G protein-coupled receptor signaling pathway GO:0045178: basal part of cell GO:0048662: negative regulation of smooth muscle cell proliferation GO:0048871: multicellular organismal-level homeostasis GO:0016209: antioxidant activity GO:00047204: ficeru membroarporic GO:001729: tissue morphogenesis GO:0048660: regulation of smooth muscle cell proliferation GO:0062023: collagen-containing extracellular matrix GO:0061024: membrane organization GO:0001894: tissue homeostasis GO:0048771: tissue remodeling GO:0060537: muscle tissue development GO:0031995: insulin-like growth factor II binding GO:0045807: positive regulation of endocytosis GO:0005583: fibrillar collagen trimer GO:001585: iorganic hydroxy compound transport GO:001585: iorganic hydroxy compound transport GO:0097435: supramolecular fiber organization GO:004194: cytolytic granule GO:0033886: response to methanol GO:0061448: connective tissue development CO:002120: costic ficerant hydrox GO:0032432: actin filament bundle GO:0003013: circulatory system process GO:0030500: regulation of bone mineralization GO:0043177: organic acid binding GO:0006869: lipid transport GO:0050873: brown fat cell differentiation GO:0030278: regulation of ossification GO:0046903: secretion

Supplementary Figure 4. Over-representation analysis of genes which were significantly downregulated or upregulated (q < 0.05) in GF versus SPF murine placenta (DE) and genes with OPLS-DA variable importance in projection (VIP)>1 and negative or positive S-plot loadings. Top 100 enriched ontology terms are shown. Metascape 3.5 using gene prioritization by evidence counting.



Supplementary Figure 5. Heatmaps for the interferon alpha and interferon gamma response gene sets.

a) Intestine Hallmark Interferon alpha Response, b) Intestine Hallmark Interferon gamma Response, c) Brain Hallmark Interferon alpha Response, d) Brain Hallmark Interferon gamma Response. Red = high expression, dark blue = lowest expression.



Supplementary Figure 6. Over-representation analysis of genes strongly associated with metabolites in fetal intestine.

Highest-scoring molecular features missing from GF fetuses (left) and highest-scoring annotated metabolites more abundant in SPF fetuses (right). Top 100 enriched ontology terms are shown. Here, non-selective picking of ontology clusters was used.



Supplementary Figure 7. Over-representation analysis of genes strongly associated with metabolites in fetal brain. Metabolites missing from GF fetuses are indicated by *. Top 100 enriched ontology terms are shown. Here, non-selective

picking of ontology clusters was used.



picking of ontology clusters was used.