
























Supplementary data 2, Bone Tissue Metabolome

UM-HET3 mice placed on CANA-containing diet (180ppm) for 1.5 month.

Bone tissue metabolome: Six-month old male and female control and UM-HET3 mice were treated with control or CANA-containing diet for 1.5 months (n=5 per sex and treatment). Cortical bone metabolome was extracted in 4% formic acid from femur and tibia flushed of marrow and cleaned of soft tissue and the periosteum. Samples were analyzed by LC-MS/MS in the NYU Mass Spectrometry Core for Neuroscience. One μL samples were applied to a Thermo Scientific™ Hypersil Gold C18 100 x 1 mm column (ThermoScientific, Waltham, Massachusetts, USA) that was maintained at a constant temperature of 50 °C and was equilibrated with mobile phase “A” containing 0.1% (v/v) formic acid/liter HPLC grade water. Metabolites were eluted with a 0-60% gradient of methanol containing 0.1% (v/v) formic acid (mobile phase “B”) at a flow rate of 50 $\mu\text{L}/\text{min}$, over 22 min, followed by 60-100% B over 3 min, then back to 0% B at 27 min. This method was adapted from Deng et al. [70]. Each sample was analyzed twice, once in positive ion mode and once in negative mode, using data-dependent acquisition. Up to five most abundant precursors from the survey scans acquired at a resolution of 120,000 over a scan range of m/z 200-2,000 were selected with an isolation window of 1.7 Th and fragmented by higher-energy collisional dissociation with a normalized collision energy setting of 30. Maximum ion injection time for the survey and MS/MS scans was 75 ms and the ion target values for MS and MS/MS scans were set to 3×10^6 and 1×10^5 respectively. Raw mass spectrometry data from both positive and negative ion mode analyses were used to search Biocyc (version 28.0, released April 2, 2024, includes 20,050 databases), Human Metabolome Database ((Version 5.0, released Jan 2022, includes 220,945 compounds), KEGG (Version 110.0, released April 1, 2024, includes 19,337 compounds), NIST (released 2023, version NIST23, includes 1,934,658 MS/MS spectra of 210,195 precursor ions from 49,590 compounds), and mzCloud (released 2024, includes 38,244 compounds and 24,271,766 spectra) libraries using Compound Discoverer 3.1 (Thermo Scientific, Waltham, MA, USA) for metabolite identification. The Ingenuity Pathway Analysis (IPA; QIAGEN, Redwood City, CA) program was employed for analyses of mammalian endogenous and non-mammalian exogenous metabolites and their respective gene networks and identifications of top canonical pathways. Analyses include those metabolites found to differ in their concentrations for each comparison between groups, which included those of M CTL vs F CTL, F CTL vs F CANA, M CTL vs M CANA, and M CANA vs F CANA. Fold change of the peak height concentration for each metabolite was calculated in the direction of the second comparator for each comparison and Log2 transformed. These transformed values were uploaded into IPA datasets and then analyzed. Default analysis settings were employed. The searchable databases included mouse, rat, and human, which constitute all conserved mammalian metabolic processes currently published. Each dataset comparison was analyzed as a Core Metabolomics Analysis of the transformed values. For each comparison, the molecules with significant values generated a short list of molecules that was uploaded to the IPA database. IPA top canonical pathways that explain the differences between the compared groups were identified, and the gene network architecture that explains the metabolome was created. Each top canonical pathway that explains the major differences between groups was compared to known transcription regulator genes, which constitutes the “overlap” and its corresponding p-value [Supplement Data 2]. The raw mass spectrometry data of the metabolomics analysis are available at MassIVE (UCSD) and can be accessed at <https://massive.ucsd.edu/ProteoSAFe/static/massive.jsp> the dataset identifier MSV000094557. Reviewers can access by using the following user name and password: User name: MSV000094557 Password: a

Key

Network Shapes

	Canonical Pathway		Ion Channel
	Complex/Group		Kinase
	Chemical/Toxicant		Ligand-dependent Nuclear Receptor
	Cytokine		Mature microRNA
	Disease		microRNA
	Drug		Other
	Enzyme		Peptidase
	Function		Phosphatase
	Fusion gene/product		Transcriptional Regulator
	G-Protein Coupled Receptor		Translational Regulator
	Growth Factor		Transmembrane Receptor
			Transporter

Graphical Summary Legend

Predicted Activity



Predicted activation



Predicted inhibition

Relationships between Nodes



Leads to activation



Leads to inhibition



Inferred relationship



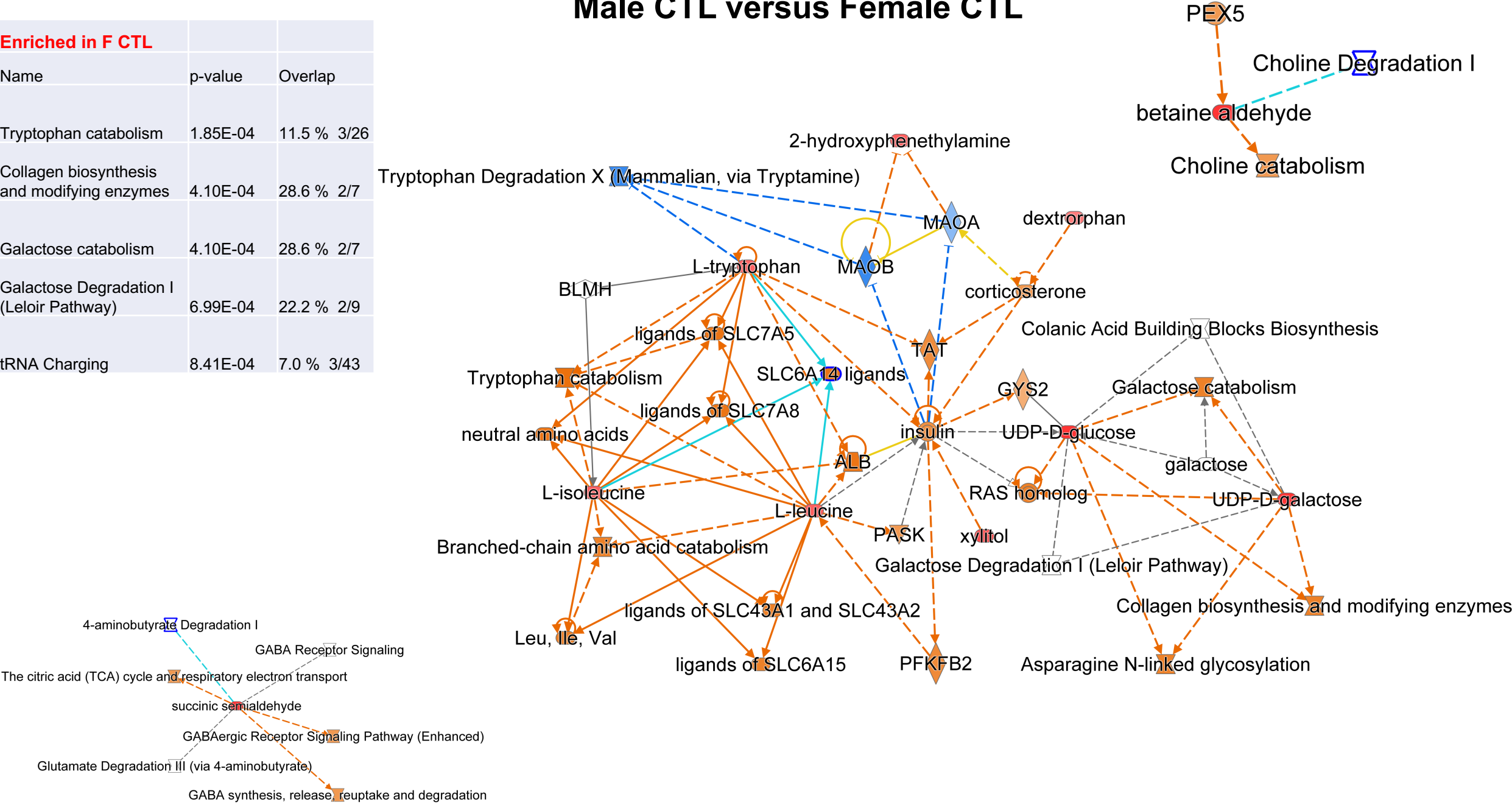
Indirect interaction



Direct interaction

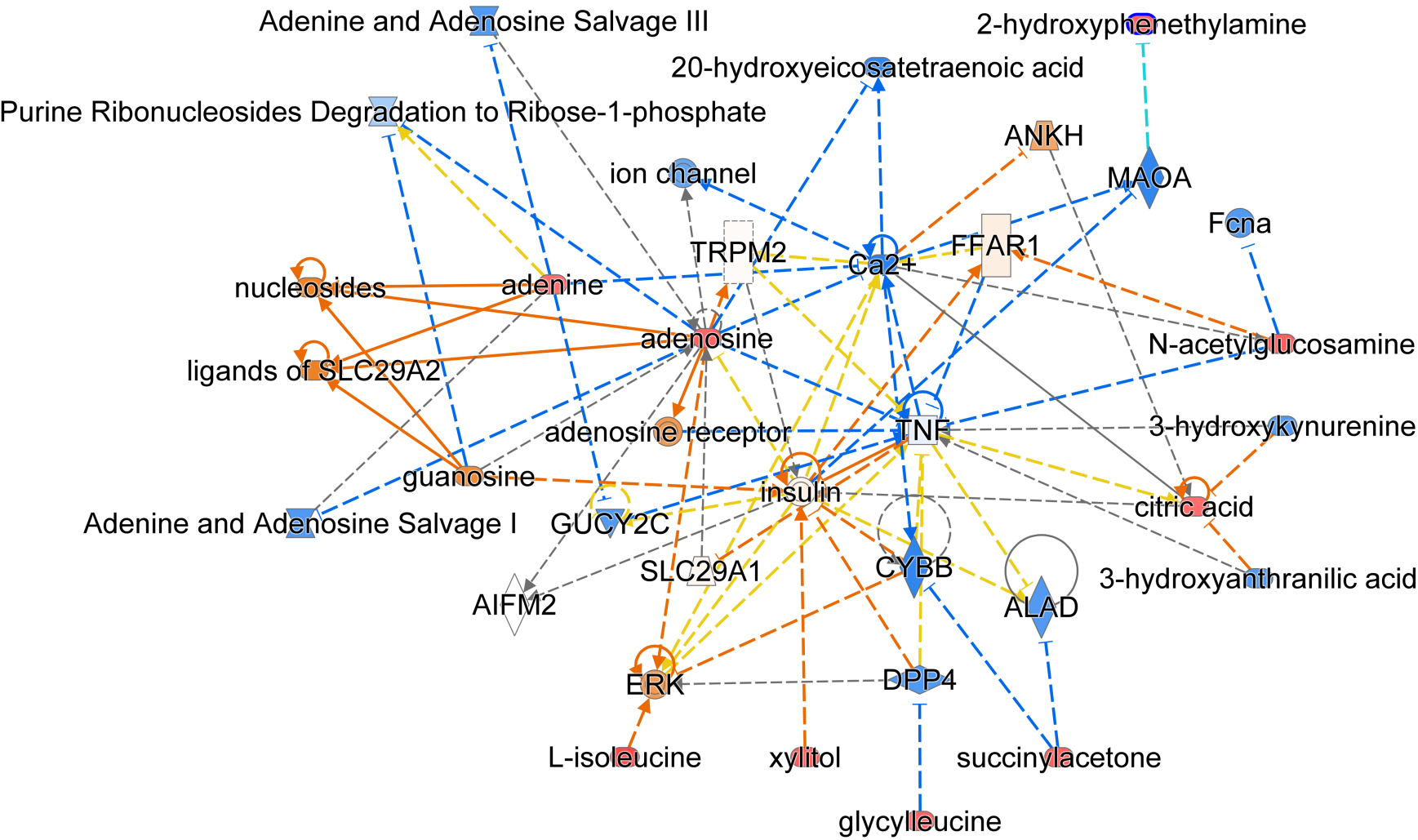
Male CTL versus Female CTL

Enriched in F CTL		
Name	p-value	Overlap
Tryptophan catabolism	1.85E-04	11.5 % 3/26
Collagen biosynthesis and modifying enzymes	4.10E-04	28.6 % 2/7
Galactose catabolism	4.10E-04	28.6 % 2/7
Galactose Degradation I (Leloir Pathway)	6.99E-04	22.2 % 2/9
tRNA Charging	8.41E-04	7.0 % 3/43

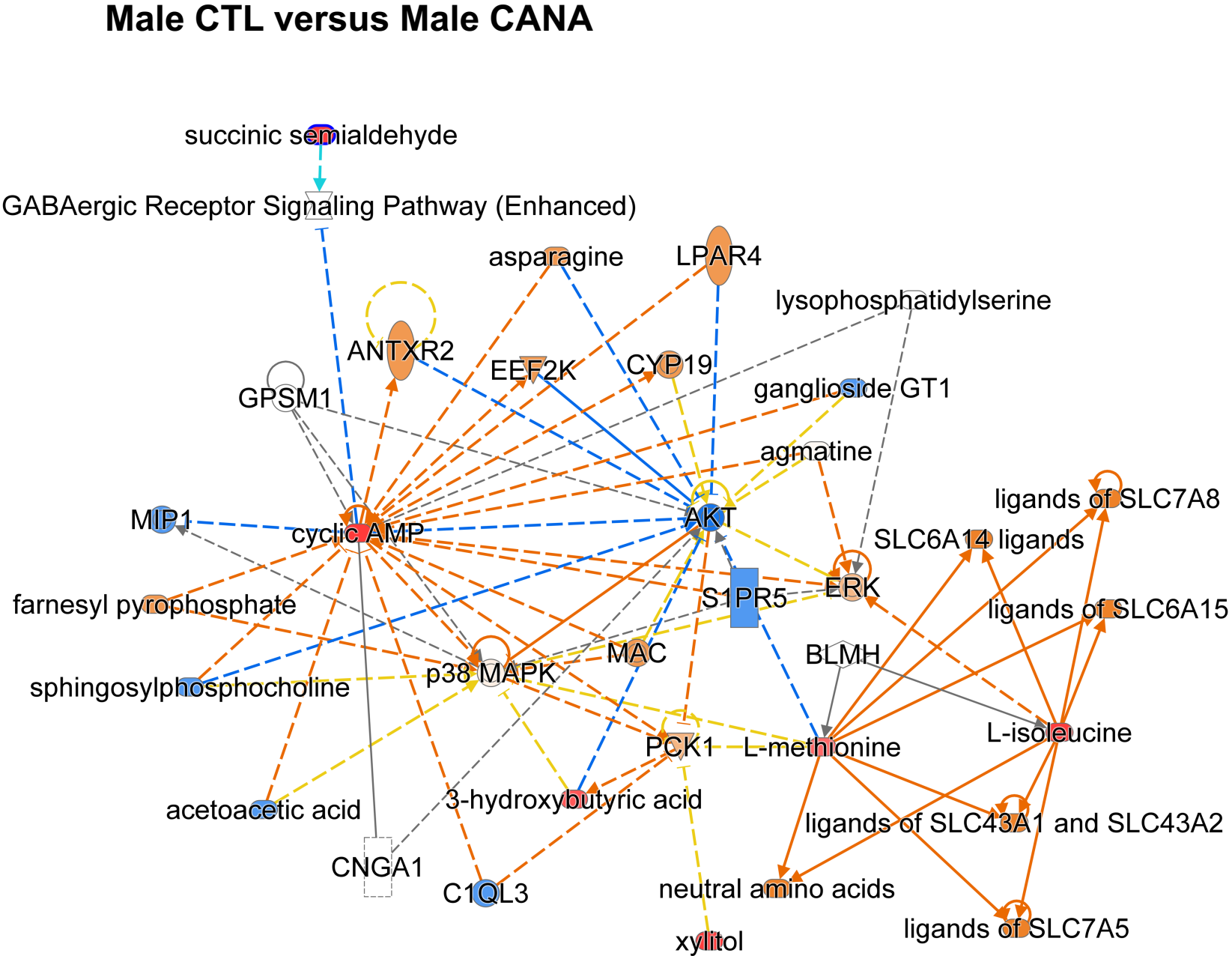


Female CTL versus Female CANA

Enriched in F CANA		
Name	p-value	Overlap
Adenine and Adenosine Salvage I	2.69E-04	28.6 % 2/7
Adenine and Adenosine Salvage III	5.73E-04	20.0 % 2/10
Purine Ribonucleosides Degradation to Ribose-1-phosphate	8.38E-04	16.7 % 2/12
S-methyl-5'-thioadenosine Degradation II	1.52E-02	25.0 % 1/4
Adenine and Adenosine Salvage VI	1.52E-02	25.0 % 1/4



Enriched in M CANA		
Name	p-value	Overlap
GABAergic Receptor Signaling Pathway (Enhanced)	2.41E-04	20.0 % 2/10
FXR/RXR Activation	2.41E-04	20.0 % 2/10
Transport of bile salts and organic acids, metal ions and amine compounds	4.50E-04	4.3 % 3/69
Tryptophan catabolism	1.71E-03	7.7 % 2/26
ROBO SLIT Signaling Pathway	2.54E-03	100.0 % 1/1



Male CANA versus Female CANA

Network 1 : M CANA vs F CANA for IPA - 2024-04-02 07:17 PM : M CANA vs F CANA for IPA : M CANA vs F CANA for IPA - 2024-04-02 07:17 PM

Enriched in F CANA		
Name	p-value	Overlap
Transport of bile salts and organic acids, metal ions and amine compounds	6.30E-05	7.2 % 5/69
Branched-chain amino acid catabolism	1.23E-03	9.1 % 3/33
Leucine Degradation I	5.53E-03	11.8 % 2/17
Tryptophan catabolism	1.28E-02	7.7 % 2/26
Synthesis of Prostaglandins (PG) and Thromboxanes (TX)	1.79E-02	6.5 % 2/31

