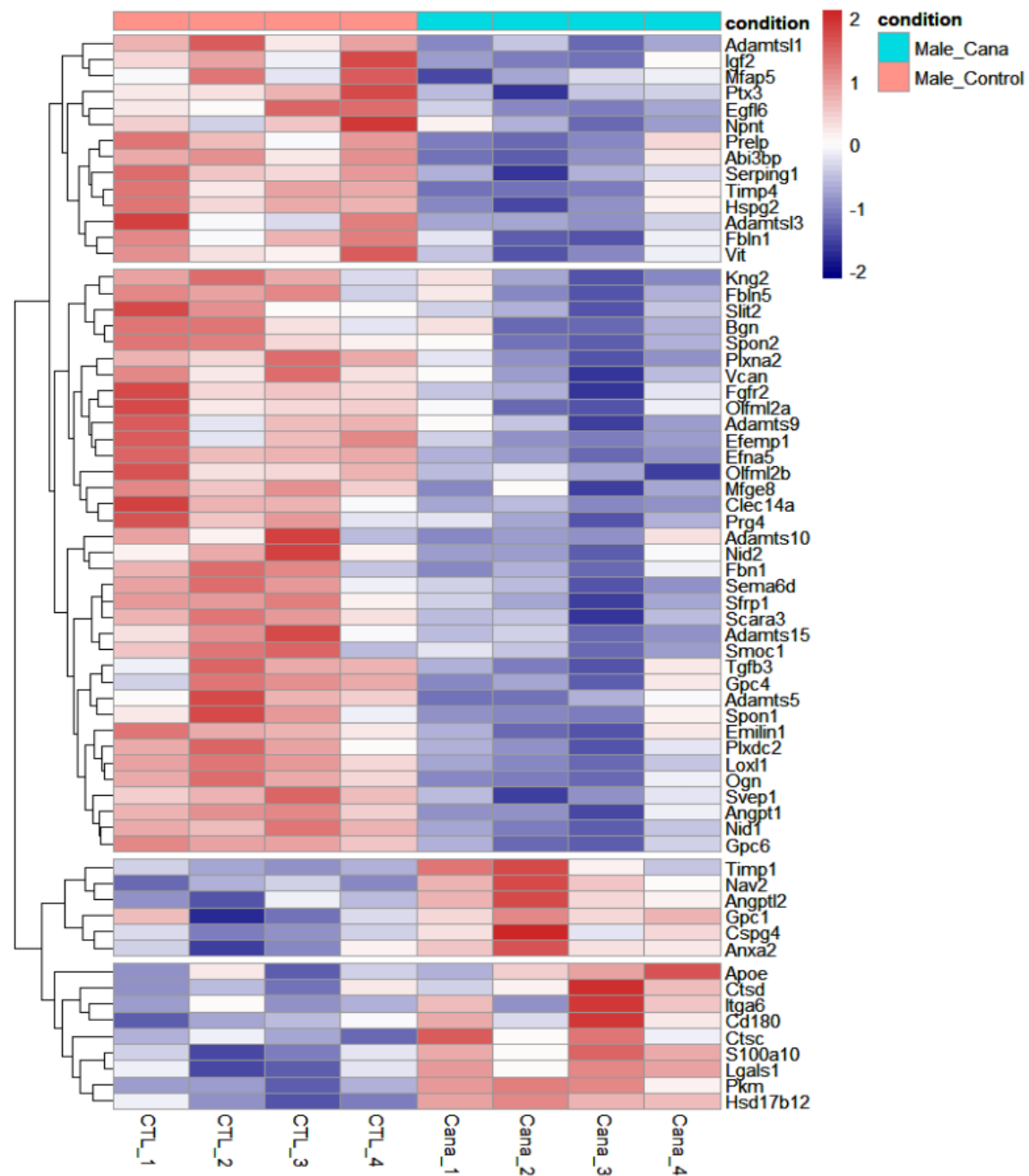


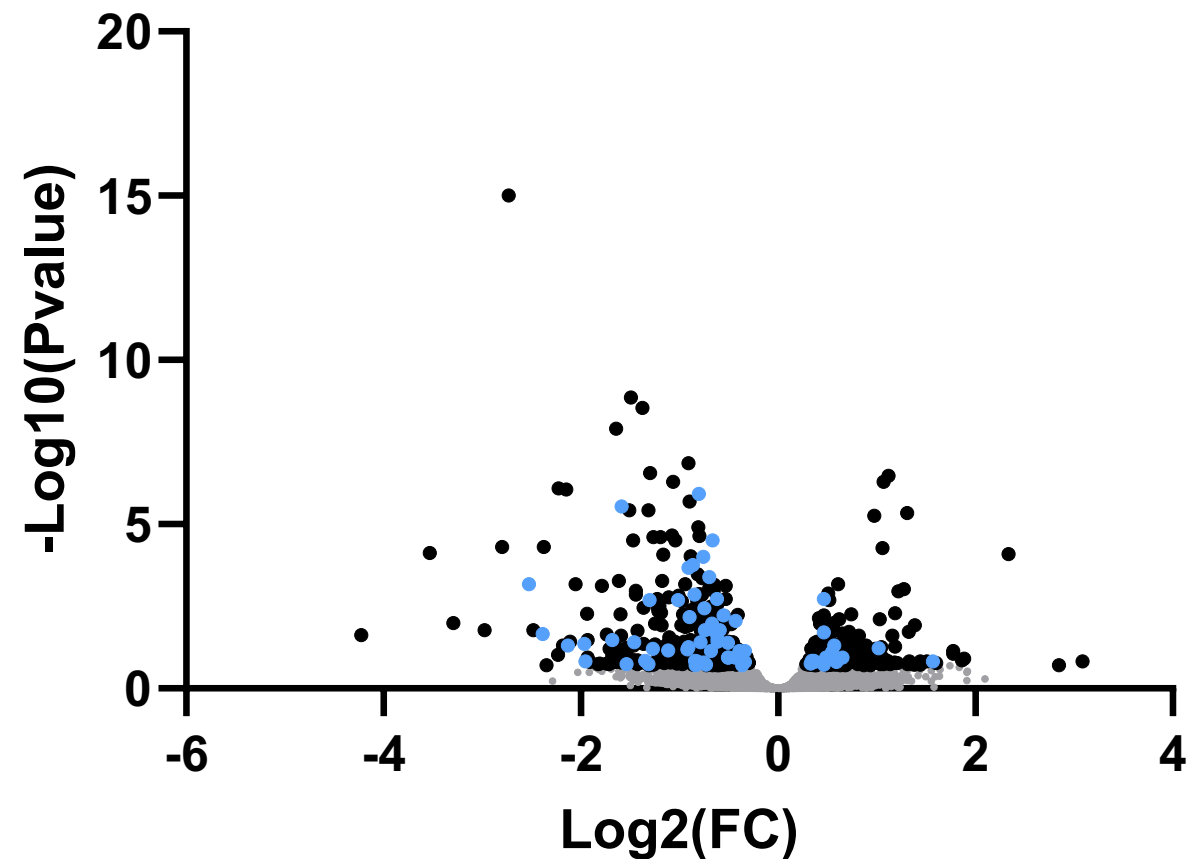
# Supplementary RNAseq data 1

## Ten-days cultures of primary osteoblasts from CANA-treated/untreated mice

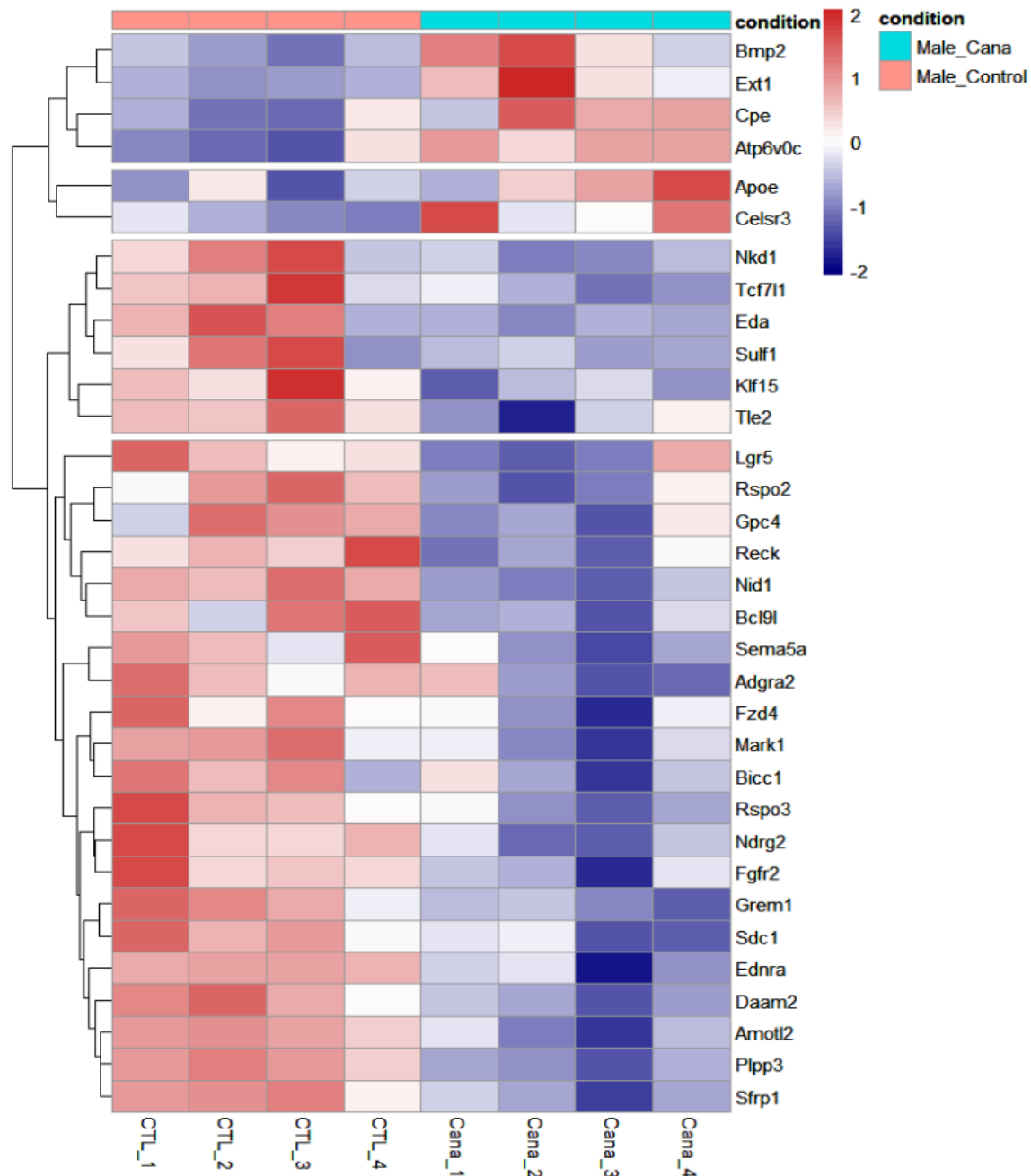
**RNAseq:** RNA was extracted from osteoblast cultures (10 days) using Qiagen RNeasy plus mini kit (Qiagen, cat# 74204). Subsequently, RNA-Seq analysis was conducted at the Wayne State University Genome Sciences Core. RNA quality was assessed using RNA ScreenTape on a 4200 TapeStation, and RNA libraries were prepared according to the QIAseq Stranded RNA Library Kits protocol. Following sequencing (NovaSeq 6000, 2 x 50 bp), the sequence reads were assembled and mapped to the mouse reference genome (Ensembl Mus\_musculus GRCm38.90) using HISAT2 v.2.1.0.13, with prior adapter trimming and quality checking. Counts quantification was determined using HTSeq-counts v0.6.0. Finally, Pairwise differential expression analysis was performed by the DESeq2 R/Bioconductor package (v. 1.34.0) (PMID: 25516281). Genes exhibiting expression changes greater than  $\pm 1.0$  log<sub>2</sub> fold change after treatments were further imputed in DAVID Bioinformatics Database NIAID/NIH for Gene Ontology (GO) analysis (PMID:19131956).



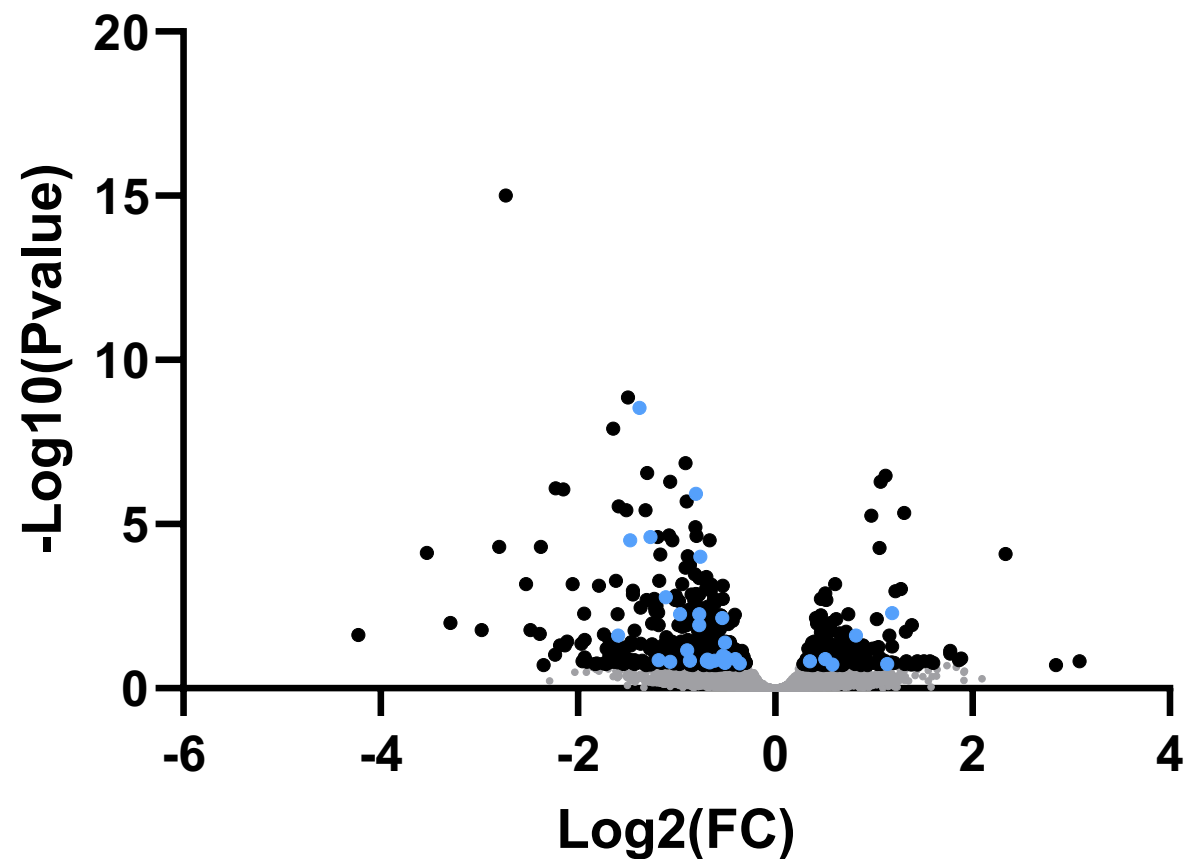
# **Males: Bone Marrow Culture Extracellular Matrix** **DEG: 50 downregulated, 15 upregulated**

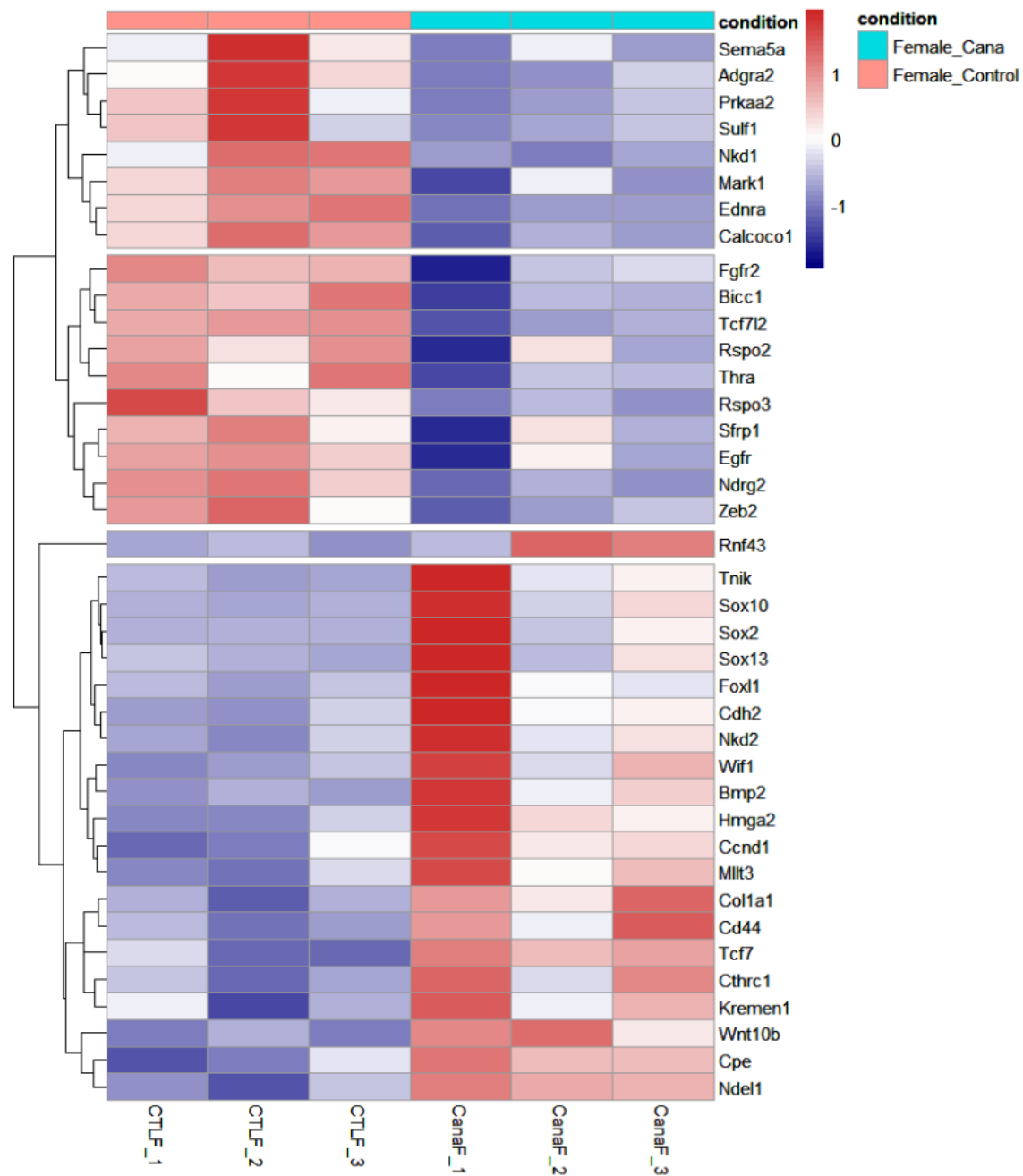






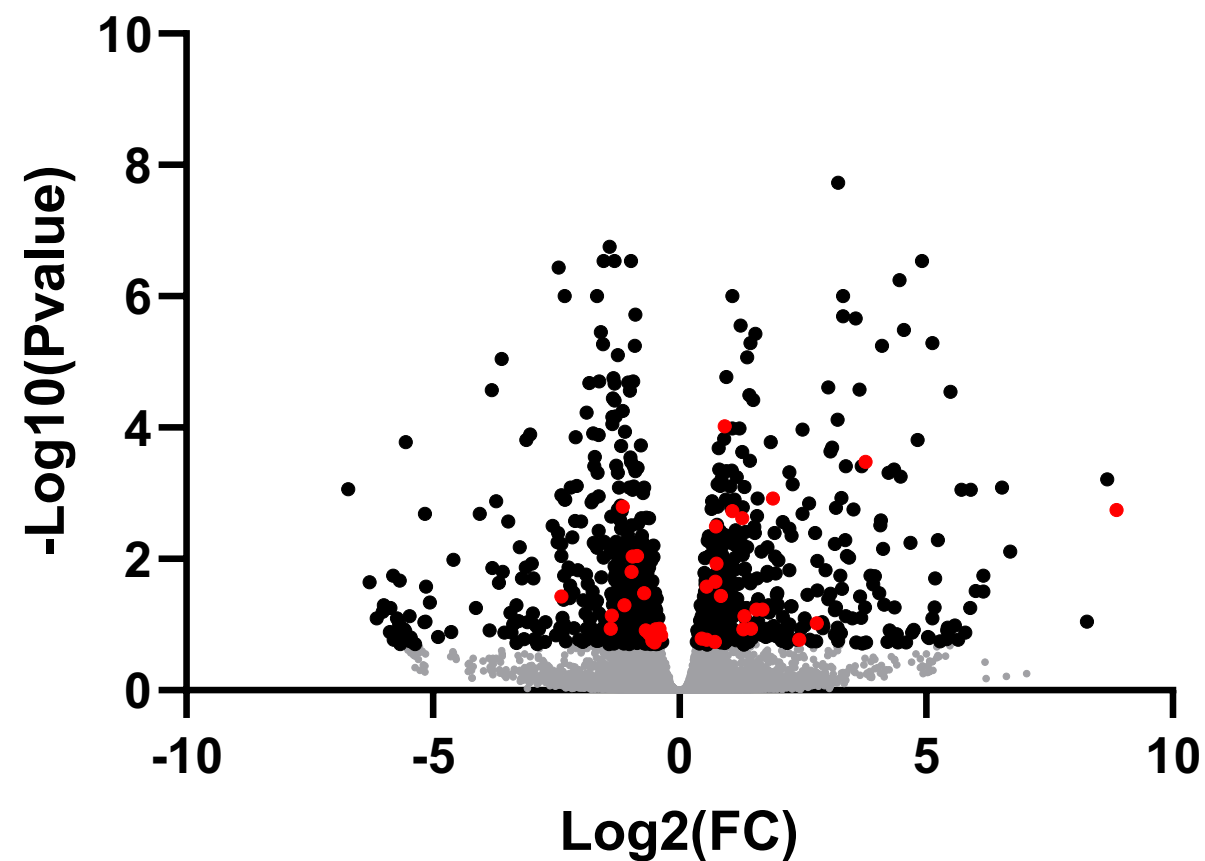
**Males: Bone Marrow Culture**  
**Wnt**  
**DEG: 27 downregulated, 6 upregulated**

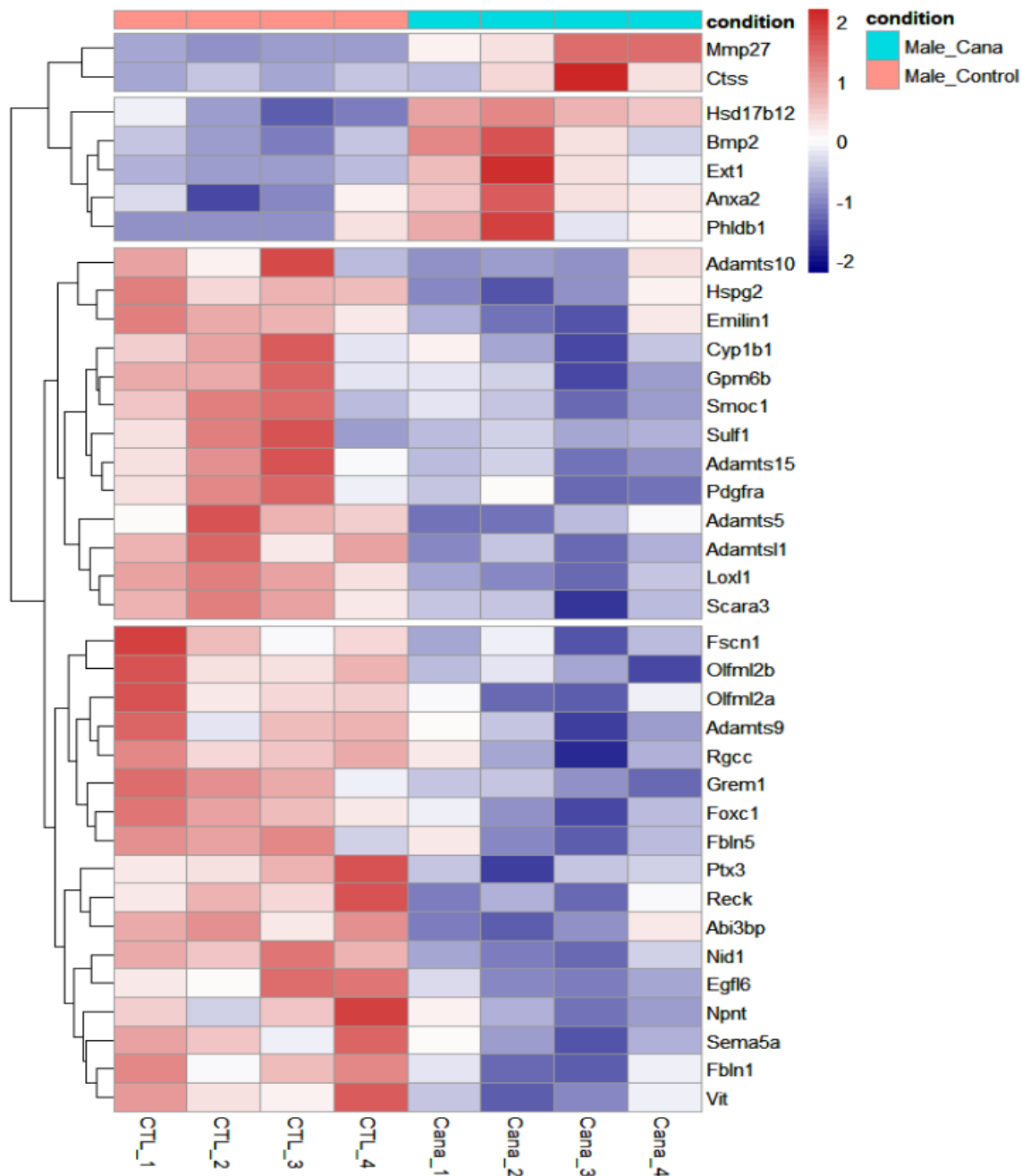




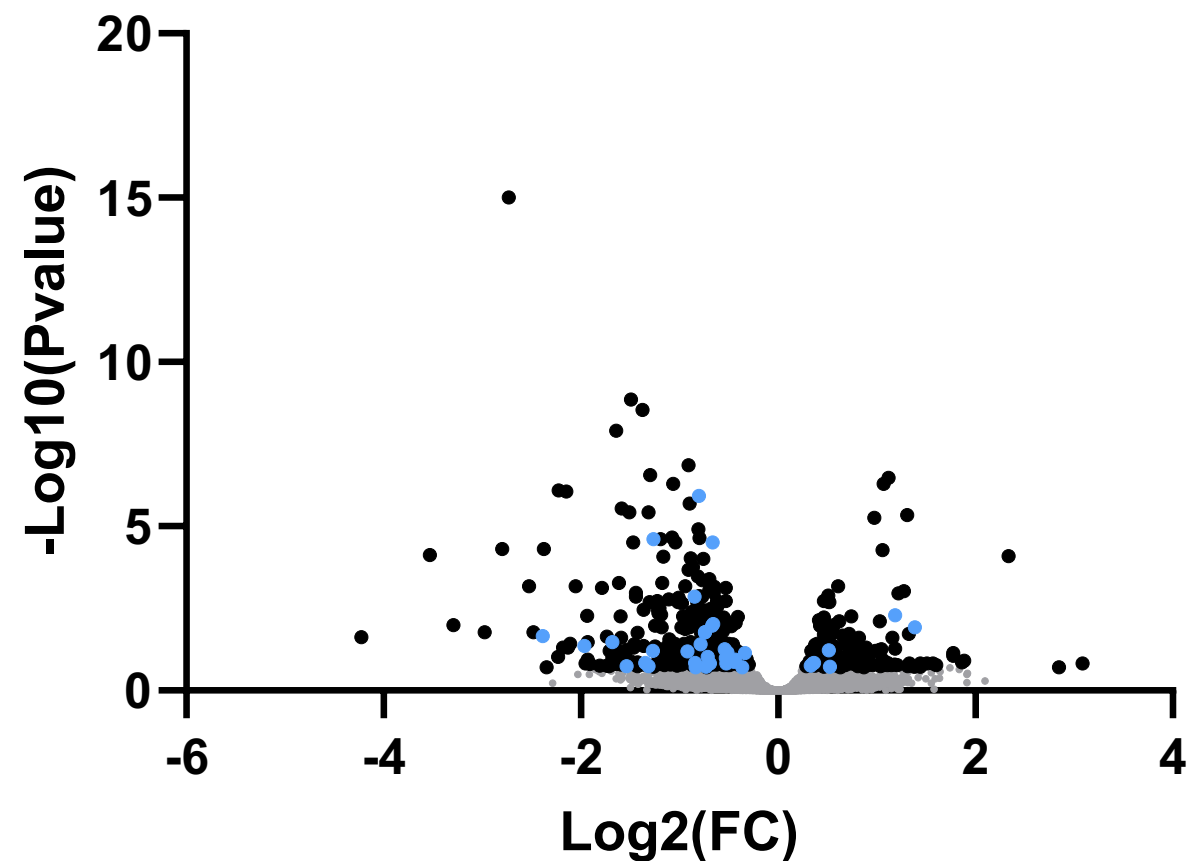
## Females: Bone Marrow Culture Wnt

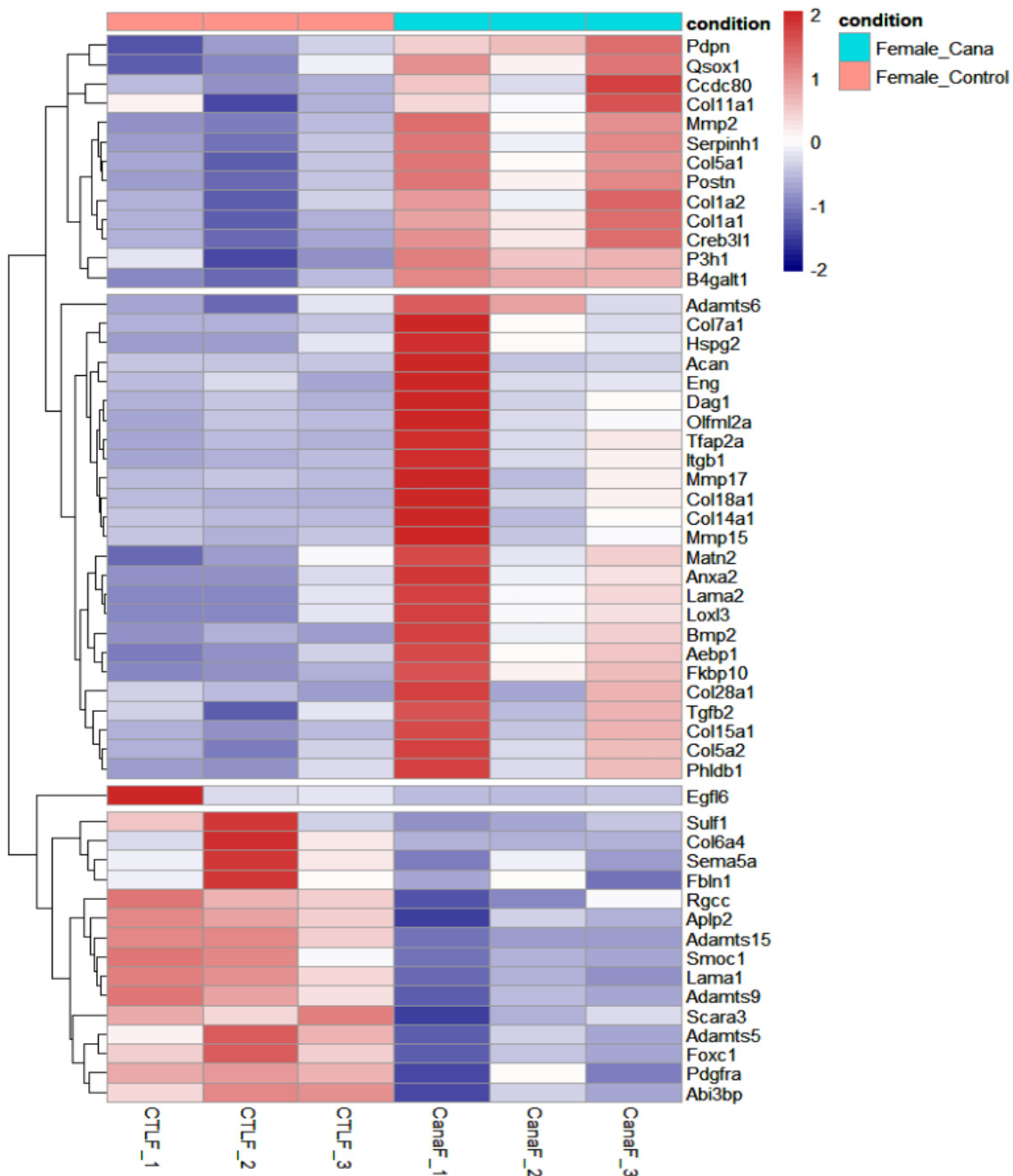
DEG: 18 downregulated, 21 upregulated



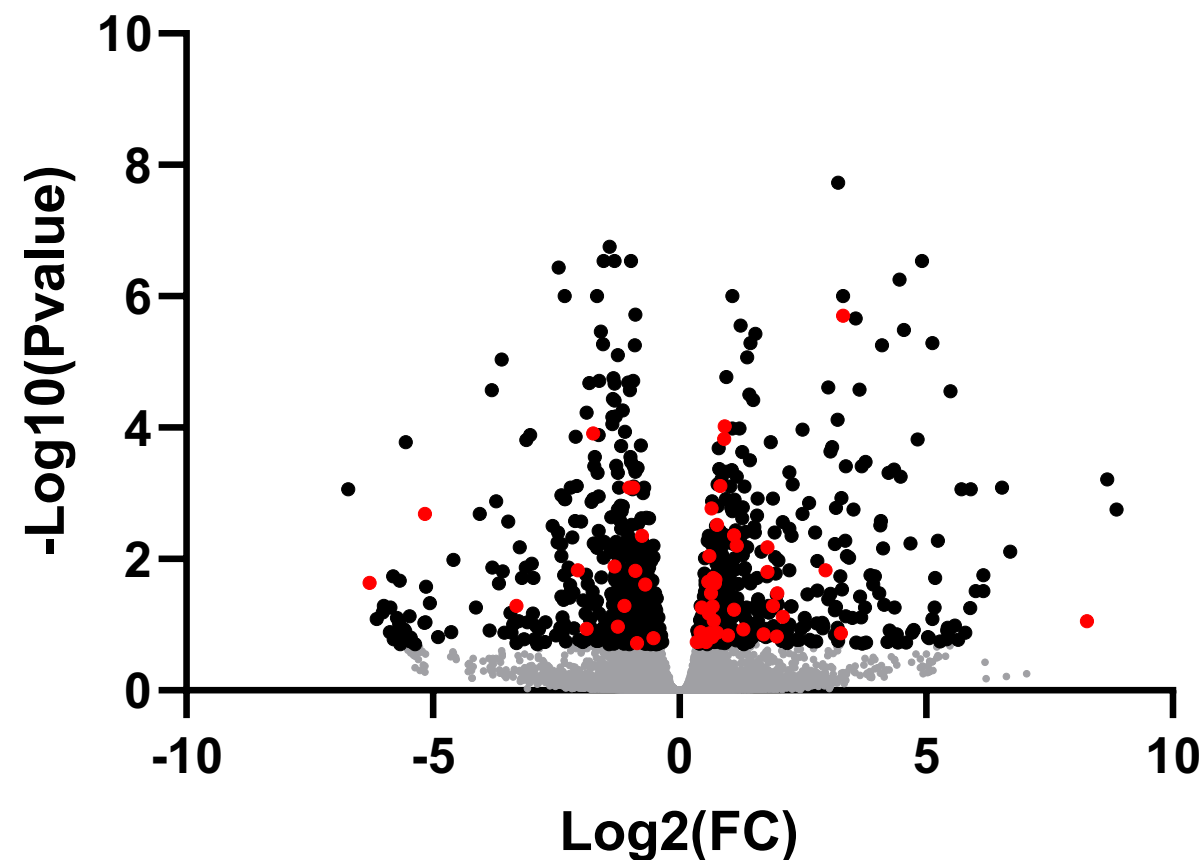


**Males: Bone Marrow Culture**  
**Extracellular Organization**  
 DEG: 30 downregulated, 7 upregulated

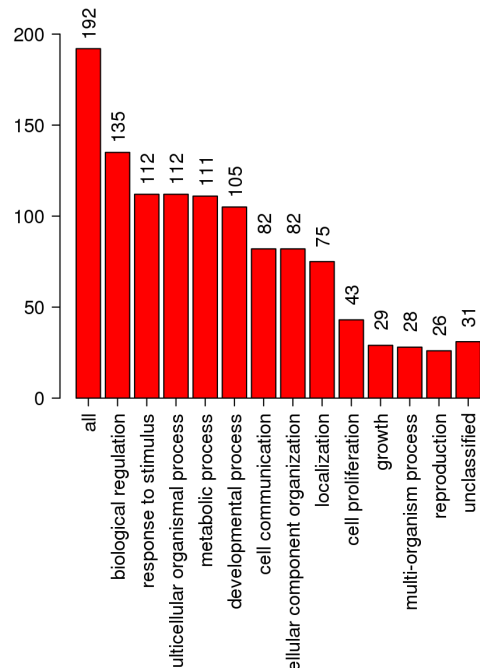




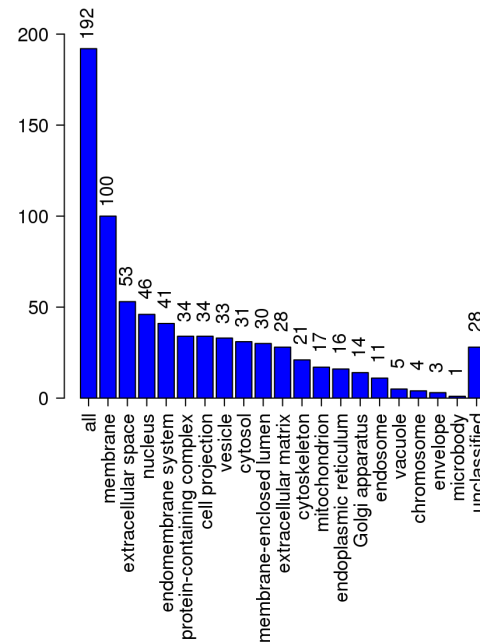
**Females: Bone Marrow Culture  
Extracellular Organization**  
DEG: 16 downregulated, 38 upregulated



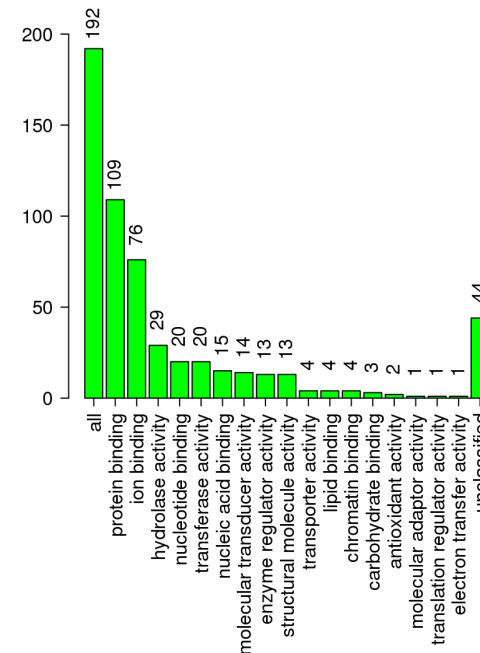
Bar chart of Biological Process categories



Bar chart of Cellular Component categories

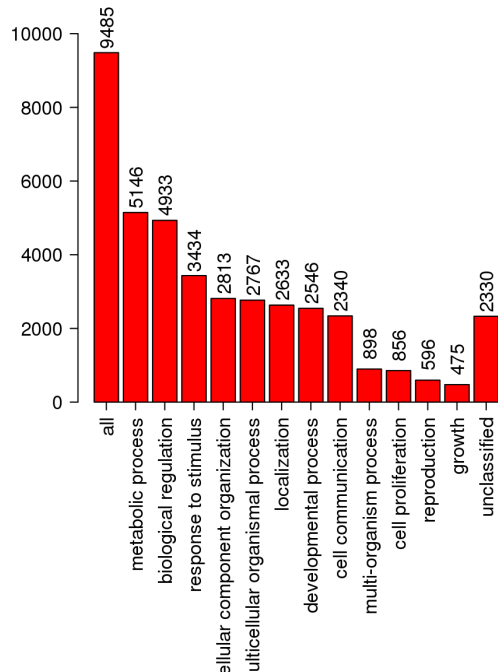


Bar chart of Molecular Function categories

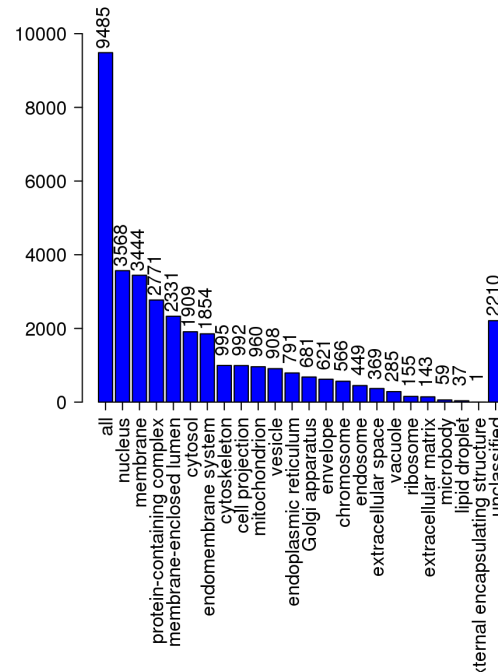


Male

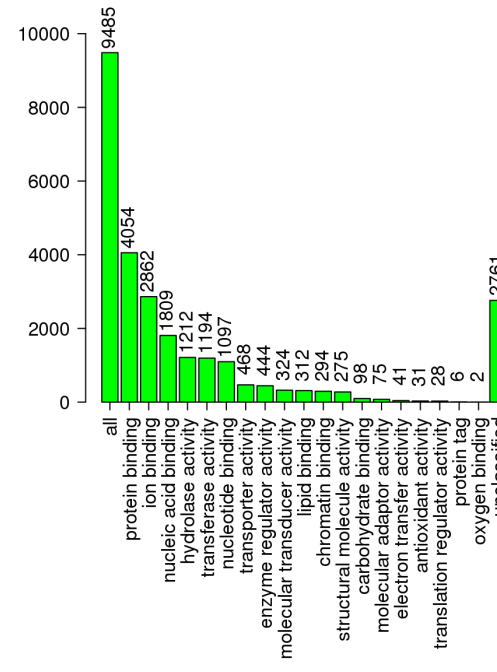
Bar chart of Biological Process categories



Bar chart of Cellular Component categories



Bar chart of Molecular Function categories



Female

GO BP OB cultures from Females CANA vs CTL			
Direction	Adj.Pval	Fold	Pathway
Up	1.86E-28	2.4	Anatomical structure morphogenesis
	1.41E-22	1.9	System development
	7.63E-21	1.8	Multicellular organism development
	8.34E-20	2.8	Cell adhesion
	8.34E-20	2.3	Cell development
	6.23E-18	2.4	Neurogenesis
	8.41E-18	1.8	Cellular developmental process
	1.51E-17	2.4	Tissue development
	5.62E-17	1.8	Cell differentiation
	6.43E-17	3.3	Cellular component morphogenesis
Down	1.44E-16	2.9	Lipid metabolic process
	7.81E-14	2.4	Small molecule metabolic process
	6.89E-11	4.5	Regulation of lipid metabolic process
	9.86E-10	2.8	Organic acid metabolic process
	9.86E-10	4	Fatty acid metabolic process
	9.86E-10	2.8	Carboxylic acid metabolic process
	1.03E-09	3.4	Monocarboxylic acid metabolic process
	1.03E-09	2.8	Oxoacid metabolic process
	2.88E-09	2.7	Cellular lipid metabolic process
	9.90E-09	1.8	Regulation of multicellular organismal process

GO BP OB cultures from Males CANA vs CTL			
Direction	Adj.Pval	Fold	Pathway
Up	6.50E-06	2.1	Immune system process
	6.50E-06	3.4	Inflammatory response
	2.27E-05	2.4	Cell migration
	2.85E-05	2.5	Immune response
	3.21E-05	2.4	Defense response
	8.58E-05	2.6	Regulation of cellular component movement
	1.19E-04	4.1	Adaptive immune response
	1.19E-04	2.2	Cell motility
	1.19E-04	2.2	Localization of cell
	1.28E-04	2.7	Regulation of cell migration
Down	4.19E-11	2.6	Cell adhesion
	4.35E-11	1.7	System development
	5.12E-11	2	Anatomical structure morphogenesis
	4.34E-09	1.6	Multicellular organism development
	4.34E-09	2.3	Cell migration
	6.47E-09	1.9	Cell surface receptor signaling pathway
	1.31E-08	1.7	Animal organ development
	2.39E-08	2.4	Circulatory system development
	2.81E-08	2.6	Regulation of cell migration
	2.81E-08	2.5	Tube development

**SGLT2 expression in primary osteoblasts:** We did not detect SLC5A2 gene expression in the RNAseq data of MSC cultures stimulated for ten days in osteogenic media.

Female (counts )	Slc5a2	Housekeeping gene (Rpl13a)	Male (counts )	Slc5a2	Housekeeping gene (Rpl13a)
CTLF1	0	719	CTTLM1	0	842
CTLF2	0	655	CTTLM2	2	596
CTLF3	8	1104	CTTLM3	0	491
CanaF1	1	1003	CTTLM4	2	557
CanaF2	0	734	CANAM1	1	923
CanaF3	6	1496	CANAM2	0	810
			CANAM3	8	586
			CANAM4	3	521
log2FC	-0.79	0.02	log2FC	1.66	0.00
padj	1.000	1.000	padj	1.000	1.000

A summary of available databases reporting SLC5A2 gene expression in BMSCs, osteoblasts, and osteoclasts.

Database (link)	Search by			Result
	Species	Cell type	Gene	
Single Cell Portal <a href="https://singlecell.broadinstitute.org/single_cell">https://singlecell.broadinstitute.org/single_cell</a>	Homo sapiens	mesenchymal stem cell OR osteoblast OR osteoclast	SLC5A2	No results found
Single Cell Portal <a href="https://singlecell.broadinstitute.org/single_cell">https://singlecell.broadinstitute.org/single_cell</a>	Mus musculus	mesenchymal stem cell OR osteoblast OR osteoclast	SLC5A2	No results found
Expression Atlas <a href="https://www.ebi.ac.uk/gxa/home">https://www.ebi.ac.uk/gxa/home</a>	Homo sapiens	mesenchymal stem cell	SLC5A2	hMSC-AT cell line: Expression level 0.6 TPM (very low)
		osteoblast OR osteoclast		No results found
Expression Atlas <a href="https://www.ebi.ac.uk/gxa/home">https://www.ebi.ac.uk/gxa/home</a>	Mus musculus	mesenchymal stem cell OR osteoblast OR osteoclast	SLC5A2	No results found
NCBI GEO Dataset <a href="https://www.ncbi.nlm.nih.gov/gds/advanced">https://www.ncbi.nlm.nih.gov/gds/advanced</a>	Homo sapiens	mesenchymal stem cell OR osteoblast OR osteoclast	SLC5A2	No items found
NCBI GEO Dataset <a href="https://www.ncbi.nlm.nih.gov/gds/advanced">https://www.ncbi.nlm.nih.gov/gds/advanced</a>	Mus musculus	mesenchymal stem cell OR osteoblast OR osteoclast	SLC5A2	No items found
MGI Gene eXpression Database --(RNA-Seq) <a href="https://www.informatics.jax.org/expression.shtml">https://www.informatics.jax.org/expression.shtml</a>	Mus musculus	bone marrow; Wild type only	SLC5A2	No results with expression data found
NCBI GEO Dataset <a href="https://www.ncbi.nlm.nih.gov/gds/advanced">https://www.ncbi.nlm.nih.gov/gds/advanced</a>	Mus musculus	all	SLC5A2	A comparative kidney transcriptome analysis of bicarbonate-loaded in srr-null mice

## RNAseq of **Bone marrow** from CANA-treated/untreated mice

**RNAseq:** RNA was extracted using Qiagen RNeasy plus mini kit (Qiagen, cat# 74204) from the bone marrow (BM) of the same samples that were used for the metabolome analysis. Subsequently, RNA-Seq analysis was conducted at the Wayne State University Genome Sciences Core. RNA quality was assessed using RNA ScreenTape on a 4200 TapeStation, and RNA libraries were prepared according to the QIAseq Stranded RNA Library Kits protocol. Following sequencing (NovaSeq 6000, 2 x 50 bp), the quality of raw data was checked by FastQC (v. 0.11.9), and the read counts were quantified using Salmon (v. 1.4.0) against the GRCm38.p6 mouse transcriptome reference (NCBI) database. Pairwise differential expression analysis was performed by the DESeq2 R/Bioconductor package (v. 1.34.0) (PMID: 25516281). Genes exhibiting expression changes greater than  $\pm 1.0$  log<sub>2</sub> fold change after treatments were further imputed in DAVID Bioinformatics Database NIAID/NIH for Gene Ontology (GO) analysis (PMID:19131956).



**SGLT2 expression in bone marrow:** We did not detect SLC5A2 gene expression in the RNAseq data of bone marrow extracted from mice treated with CANA for 1.5 months.

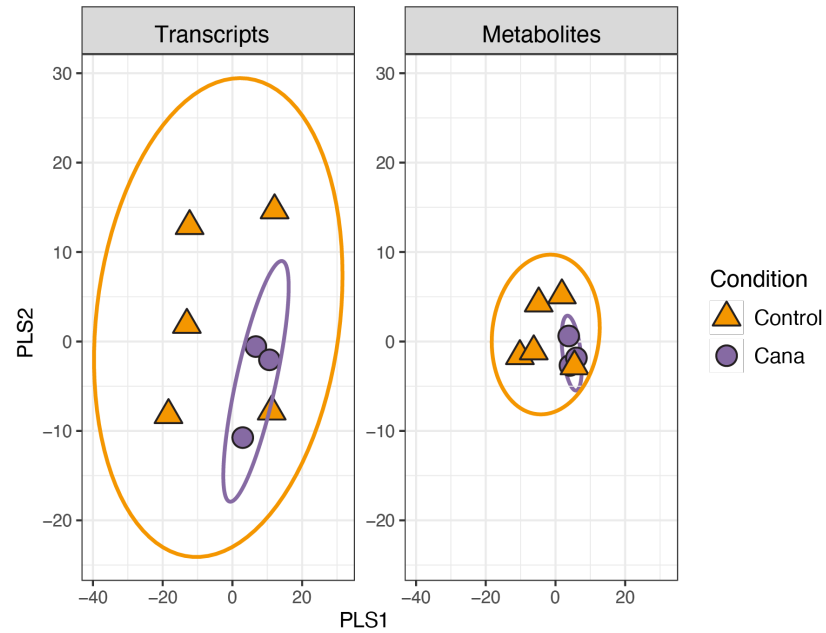
Male (counts )	Slc5a2	Housekeeping gene (Rpl13a)
MCTL_1	0	1973
MCTL_2	2	1660
MCTL_3	12	927
MCTL_4	0	1705
MCTL_5	0	1820
MCana_1	0	1334
MCana_2	0	2178
MCana_3	0	3033
log2FC	0.008	0.001
padj	1.000	1.000

# **IntegrOmics:**

## **Cortical bone tissue metabolome and bone marrow RNAseq**

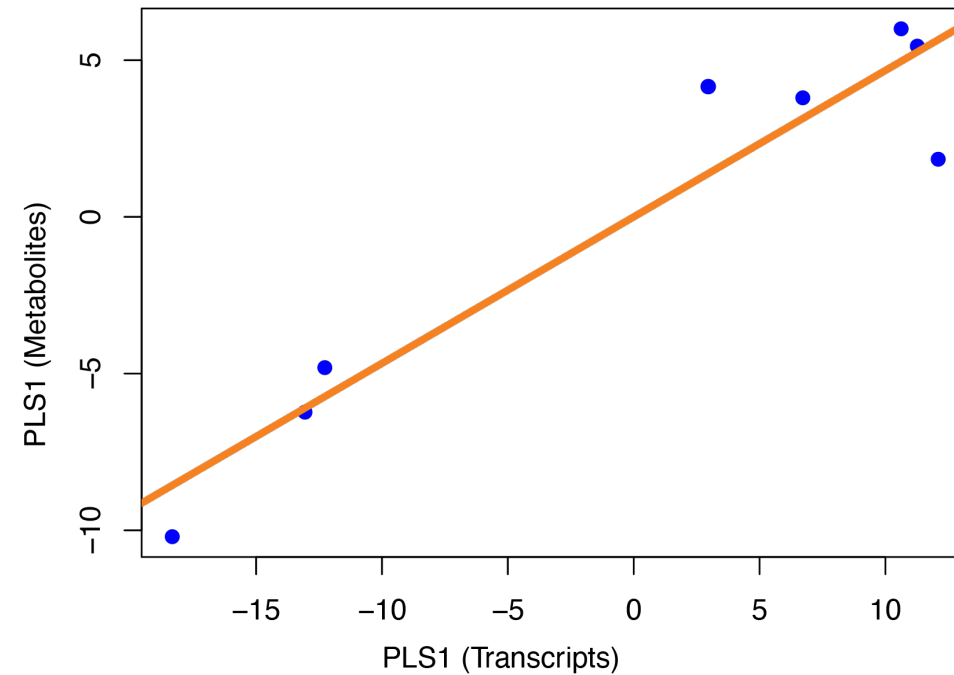
Partial least squares (PLS) regression analysis was done using the IntegrOmics R package v6.22.0 to investigate the correlations between the transcriptomics and metabolomics profiles (PMID:19706745).

STITCH ([www.stitch.embl.de](http://www.stitch.embl.de)) was used to further explore the biological relationships between metabolites-genes, metabolites-metabolites and genes-genes.



Sample plot from the PLS model performed on the cana data with two dimensions. Variates/Components associated to each data set (or block) are shown. Focusing only on the projection of the sample on the first component shows that the predictors in X (transcripts) tend to explain the group differences between control and cana. This agrees with the levels of the Y variable (metabolites).

The plot shows a clear separation between the Cana and Control groups along the first variate (Variate 1) - the ellipses do not overlap much (95% CI). This indicates that the first variate captures significant variation between these two groups due to the DEGs and metabolites.



Comp1 of X with Comp1 of Y:

The  $r$  value is 0.95, indicating a very strong positive correlation between the first component of X (DEG) and the first component of Y (significant metabolites). This suggests that these components are capturing similar or related underlying structures or patterns in the data from both blocks. This is confirmed by fitting a regression line using PLS scores.

# STITCH interactions

#node1	node2	Node1 string internal id	Node2 string internal id	Node1 external id	Node2 external id	Neighborhood on chromosome	Gene fusion	Phylogenetic cooccurrence	homology	coexpression	Experimentally determined interaction	Database annotated	Automated text mining	prediction	Combined score
Creb1	Rps6ka2	2103842	2096039	10090.ENSMUSP00000059973	10090.ENSMUSP00000024575	0	0	0	0	0	0.214	0.946	0.973	0	0.998
Creb1	Mapk3	2103842	2102406	10090.ENSMUSP00000059973	10090.ENSMUSP00000051619	0	0	0	0	0	0.152	0.954	0.956	0	0.998
Creb1	Mapk1	2103842	2095837	10090.ENSMUSP00000059973	10090.ENSMUSP00000023462	0	0	0	0	0	0.152	0.961	0.927	0	0.997
isoleucine	methionine	-100000791	-100000876	-1.CID100000791	-1.CID100000876	0	0	0	0.513	0	0	0.9	0.966	0	0.996
Mapk3	Rps6ka2	2102406	2096039	10090.ENSMUSP00000051619	10090.ENSMUSP00000024575	0	0	0	0.681	0	0.878	0.946	0.796	0	0.994
Mapk3	Mapk1	2102406	2095837	10090.ENSMUSP00000051619	10090.ENSMUSP00000023462	0	0	0.527	0.987	0	0.832	0.954	0.946	0	0.992
Abat	succinic semia.	2104402	-100001112	10090.ENSMUSP00000063548	-1.CID100001112	0	0	0	0	0	0.561	0.9	0.795	0	0.99
Rps6ka2	Mapk1	2096039	2095837	10090.ENSMUSP00000024575	10090.ENSMUSP00000023462	0	0	0	0.692	0	0.747	0.946	0.639	0	0.988
Rps6ka2	sunitinib	2096039	-103086686	10090.ENSMUSP00000024575	-1.CID103086686	0	0	0	0	0	0.866	0.795	0.534	0	0.986
Mat1a	methionine	2101069	-100000876	10090.ENSMUSP00000044288	-1.CID100000876	0	0	0	0	0	0.654	0.9	0.58	0	0.984
Tsc2	Rps6ka2	2109084	2096039	10090.ENSMUSP00000094986	10090.ENSMUSP00000024575	0	0	0	0	0	0.412	0.885	0.789	0	0.984
Lars	Lars2	2109130	2099660	10090.ENSMUSP00000095197	10090.ENSMUSP00000036710	0	0	0	0	0	0	0.8	0.906	0	0.98
Pdpk1	Rps6ka2	2110454	2096039	10090.ENSMUSP00000099991	10090.ENSMUSP00000024575	0	0	0	0.74	0	0.527	0.946	0.766	0	0.978
Adhfe1	succinic semia.	2112622	-100001112	10090.ENSMUSP00000116627	-1.CID100001112	0	0	0	0	0	0	0.9	0.776	0	0.976
Tsc2	Mapk3	2109084	2102406	10090.ENSMUSP00000094986	10090.ENSMUSP00000051619	0	0	0	0	0	0.27	0.908	0.667	0	0.975
Tsc2	Mapk1	2109084	2095837	10090.ENSMUSP00000094986	10090.ENSMUSP00000023462	0	0	0	0	0	0.374	0.908	0.586	0	0.974
Lars	methionine	2109130	-100000876	10090.ENSMUSP00000095197	-1.CID100000876	0	0	0	0	0	0.644	0.9	0.184	0	0.969
Lars	isoleucine	2109130	-100000791	10090.ENSMUSP00000095197	-1.CID100000791	0	0	0	0	0	0.644	0.9	0.17	0	0.968
Pdpk1	Tsc2	2110454	2109084	10090.ENSMUSP00000099991	10090.ENSMUSP00000094986	0	0	0	0	0	0	0.9	0.446	0	0.942
Pdpk1	Mapk1	2110454	2095837	10090.ENSMUSP00000099991	10090.ENSMUSP00000023462	0	0	0	0.671	0	0.119	0.9	0.28	0	0.915
Npepps	methionine	2093239	-100000876	10090.ENSMUSP00000001480	-1.CID100000876	0	0	0	0	0	0	0.9	0.143	0	0.911
Npepps	isoleucine	2093239	-100000791	10090.ENSMUSP00000001480	-1.CID100000791	0	0	0	0	0	0	0.9	0	0	0.9
Pdpk1	sunitinib	2110454	-103086686	10090.ENSMUSP00000099991	-1.CID103086686	0	0	0	0	0	0.14	0.795	0	0	0.818
Mapk3	sunitinib	2102406	-103086686	10090.ENSMUSP00000051619	-1.CID103086686	0	0	0	0	0	0	0.795	0.133	0	0.817
Mapk1	sunitinib	2095837	-103086686	10090.ENSMUSP00000023462	-1.CID103086686	0	0	0	0	0	0	0.795	0	0	0.795
Lars2	isoleucine	2099660	-100000791	10090.ENSMUSP00000036710	-1.CID100000791	0	0	0	0	0	0.644	0	0.166	0	0.694
Lars2	methionine	2099660	-100000876	10090.ENSMUSP00000036710	-1.CID100000876	0	0	0	0	0	0.644	0	0.142	0	0.686
Abat	Aldh11l	2104402	2098399	10090.ENSMUSP00000063548	10090.ENSMUSP00000032175	0.224	0	0	0	0.107	0.165	0	0.265	0	0.518
Aldh11l	succinic semia.	2098399	-100001112	10090.ENSMUSP00000032175	-1.CID100001112	0	0	0	0	0	0.512	0	0	0	0.512
Adhfe1	Aldh11l	2112622	2098399	10090.ENSMUSP00000116627	10090.ENSMUSP00000032175	0.363	0	0	0	0	0	0	0.245	0	0.499
Rps6ka2	isoleucine	2096039	-100000791	10090.ENSMUSP00000024575	-1.CID100000791	0	0	0	0	0	0	0	0.496	0	0.496
Adhfe1	isoleucine	2112622	-100000791	10090.ENSMUSP00000116627	-1.CID100000791	0	0	0	0	0	0	0	0.483	0	0.483
Hbq1a	methionine	2094901	-100000876	10090.ENSMUSP00000020535	-1.CID100000876	0	0	0	0	0	0.462	0	0	0	0.462
Tsc2	Creb1	2109084	2103842	10090.ENSMUSP00000094986	10090.ENSMUSP00000059973	0	0	0	0	0	0	0	0.407	0	0.407