Study Proposal

Unravel the dom pathway associated with recurrent in Acute Myeloid Leukemia

Group Members

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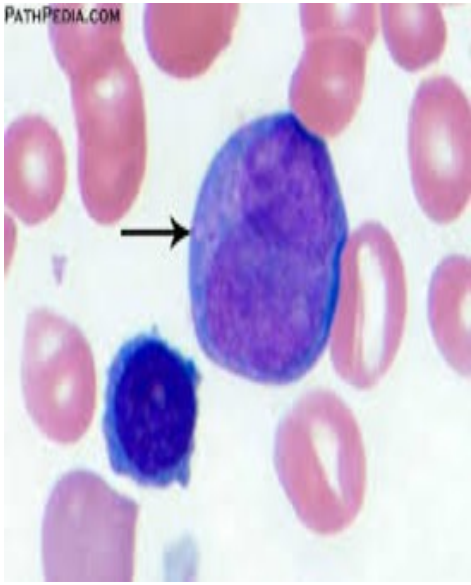
Main Points

- Introduction
- Aim of the work
- Methodology
- Initial Results

Introduction

AML



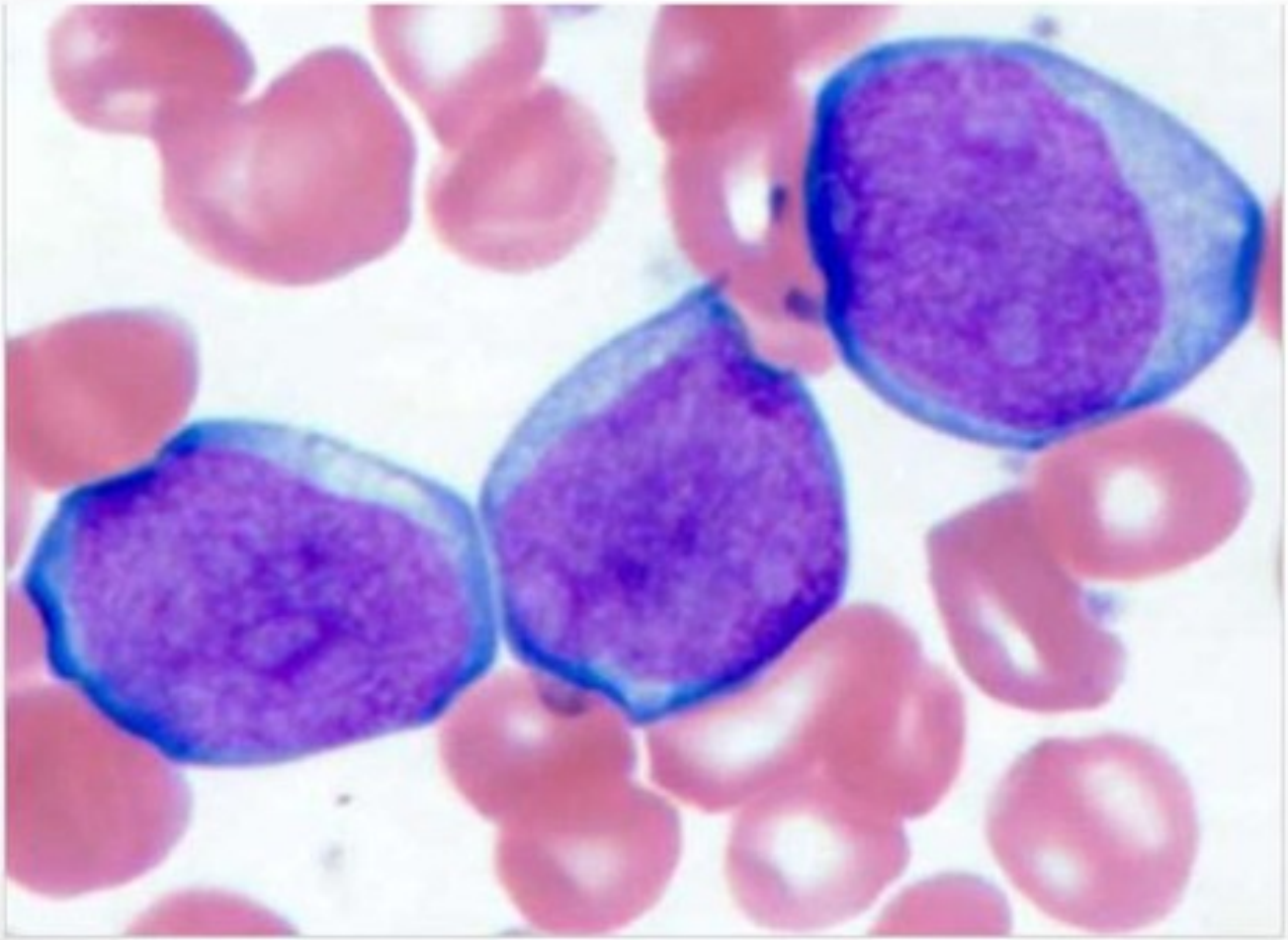
Acute myeloid leukemia (AML) is a malignant clonal hematopoietic stem cell disorder of the bone marrow characterized by the presence of more than 20% blasts in the bone marrow.

The underlying **pathophysiology** in AML consists of a maturational arrest of bone marrow cells in the earliest stages of development.

The **symptoms** of AML result from bone marrow failure such as anemia and thrombocytopenia and/or symptoms resulting from organ infiltration with leukemic cells and/or such as lymphadenitis .

Risk factors: antecedent hematologic disorders, familial syndromes, environmental exposures, and drug exposures. However, most patients who present with de novo AML have no identifiable risk factor.

[ACUTE MYELOGENOUS LEUKEMIA, M1, BLOOD]. The defining feature of a myeloblast is fine nuclear chromatin and scant to moderate amount of cytoplasm. Note that myeloblasts may not show any cytoplasmic granules or Auer rods as in this case. The correct identification rests upon immunophenotyping which shows expression of myelomonocytic antigens such as CD13, CD15, CD33, CD117, and myeloperoxidase. The blasts generally also express CD34 and HLA-DR.



Male

Site	Current Trend 5 Year AAPC	Delay-Adjusted Incidence Rates Cases per 100,000
Prostate	-7.6* (-10.5 - -4.7)	118.2
Lung and bronchus	-2.4* (-2.8 - -2.0)	73.2
Colon and rectum	-1.9* (-3.2 - -0.6)	46.5
Urinary bladder	-0.8* (-1.0 - -0.7)	36.8
Melanoma of the skin	+2.3* (2.0 - 2.6)	27.4
Non-Hodgkin lymphoma	-0.2 (-0.5 - 0.1)	23.7
Kidney and renal pelvis	+1.1* (0.5 - 1.8)	22.3
Leukemia	+1.6* (1.1 - 2.1)	19.0
Oral cavity and pharynx	+1.3* (1.0 - 1.6)	17.7
Pancreas	+1.0* (1.0 - 1.1)	14.5
Liver and intrahepatic bile duct	+2.8* (2.0 - 3.6)	12.5
Stomach	-0.3 (-0.7 - 0.1)	9.4
Myeloma	+2.5* (2.0 - 3.0)	8.7
Esophagus	-1.6* (-2.3 - -1.0)	8.1
Brain and other nervous system	-0.2* (-0.3 - -0.1)	7.9
Thyroid	+2.4* (1.3 - 3.5)	7.3
Larynx	-2.3* (-2.4 - -2.1)	6.1

Female

Site	Current Trend 5 Year AAPC	Delay-Adjusted Incidence Rates Cases per 100,000
Breast	+0.4* (0.1 - 0.7)	125.6
Lung and bronchus	-1.2* (-1.4 - -1)	53.3
Colon and rectum	-1.7* (-2.9 - -0.6)	35.2
Corpus and uterus, NOS	+1.2* (1.1 - 1.4)	26.3
Thyroid	+1.9* (1.1 - 2.7)	21.6
Melanoma of the skin	+1.2* (0.5 - 1.8)	16.8
Non-Hodgkin lymphoma	-0.4* (-0.7 - -0.2)	16.3
Ovary	-1.6* (-1.7 - -1.4)	11.8
Leukemia	+1.4* (1 - 1.8)	11.5
Kidney and renal pelvis	+0.4* (0.1 - 0.7)	11.5
Pancreas	+1.1* (0.9 - 1.2)	11.2
Urinary bladder	-0.8* (-1 - -0.6)	9.1
Cervix	-1.0* (-1.3 - -0.7)	7.7
Oral cavity and pharynx	+0.8* (0.4 - 1.1)	6.5
Myeloma	+1.6* (0.7 - 2.5)	5.7
Brain and other nervous system	-0.7* (-1.2 - -0.2)	5.7
Stomach	+0.1 (-0.6 - 0.8)	4.7
Liver and intrahepatic bile duct	+3.8* (3.5 - 4.2)	4.3

NATIONAL CANCER STATISTICS REPORTS(2018)

Diagnosis of AML

- Blood tests
- Bone marrow aspiration and biopsy
- Analysis of genetic abnormalities
- Diagnostic imaging.
- Immunophenotyping
- Molecular analysis

WHO classification of AML

1. Acute myeloid leukemia with recurrent genetic abnormalities
2. Acute myeloid leukemia with multilineage dysplasia
3. AMl and MDS therapy related (alkylating agents, topoisomerase II inhibitor…etc.
4. AMl not otherwise categorized

Risk stratification for AML patients on the basis of genetic abnormalities (ELN guidelines, 2017).

- Genetic abnormalities with favorable risk are as follows:
- t(8;21)(q22;q22.1); RUNX1-RUNX1T1
- inv(16)(p13.1q22) or t(16;16)(p13.1;q22) ; CBFB-MYH11
- Mutated NPM1 and Biallelic mutated CEBPA

Genetic abnormalities with intermediate risk are as follows:

- Mutated NPM1 and high allelic ratio (≥0.5) FLT3-ITD
- Wild-type NPM1 without FLT3-ITD or with low allelic ratio FLT3-ITD
- t(9;11)(p21.3;q23.3); MLLT3-KMT2A

Genetic abnormalities with adverse risk are as follows:

- t(6;9)(p23;q34.1); DEK- NUP214
- t(v;11q23.3); KMT2A rearranged
- t(9;22)(q34.1;q11.2); BCR- ABL1
- inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); GATA2, MECOM(EVI1)
- −5 or del(5q); −7; −17/abn(17p)
- Complex karyotype or monosomal karyotype
- Wild-type NPM1 and high allelic ratio FLT3-ITD
- Mutated RUNX1, Mutated ASXL1 and Mutated TP53

AML prognosis

- Seven genes were associated with prognosis: CD34, RHOC, SCRN1, F2RL1, FAM92A1, MIR155HG, and VWA8. The fewer the genes with high expression, the better the prognosis (Marcucci et al., 2014).

- The five-year survival rate for AML is 26.9 percent.

What is Relapse

- AML may have successfully completed treatment.

- However, as can happen sometimes, the leukaemia has returned.

- When the levels of leukaemic cells have risen beyond those considered remission (when all tests show absence of leukaemia), this is called a relapse or recurrence of leukaemia.

Observation (Research Question)

- AML relapse affects about 50% of all patients who achieved remission after initial treatment, and can occur several months to several years after treatment.

- AML may have relapsed due to a variety of factors.

- One of these factors is the resistant to treatment due to genetic variation or genetics dysfunction.

Hypothesis

There are significant pathways associated with recurrence of Acute Myeloid Leukemia.

Objective

- Understanding the downstream pathways associated with the recurrence of Acute Myeloid Leukemia

To achieve the goal of this study

- We will study **differential miRNA expression**, and **RNA-seq**, in Acute Myeloid Leukemia patients (Primary tumors and recurrent).

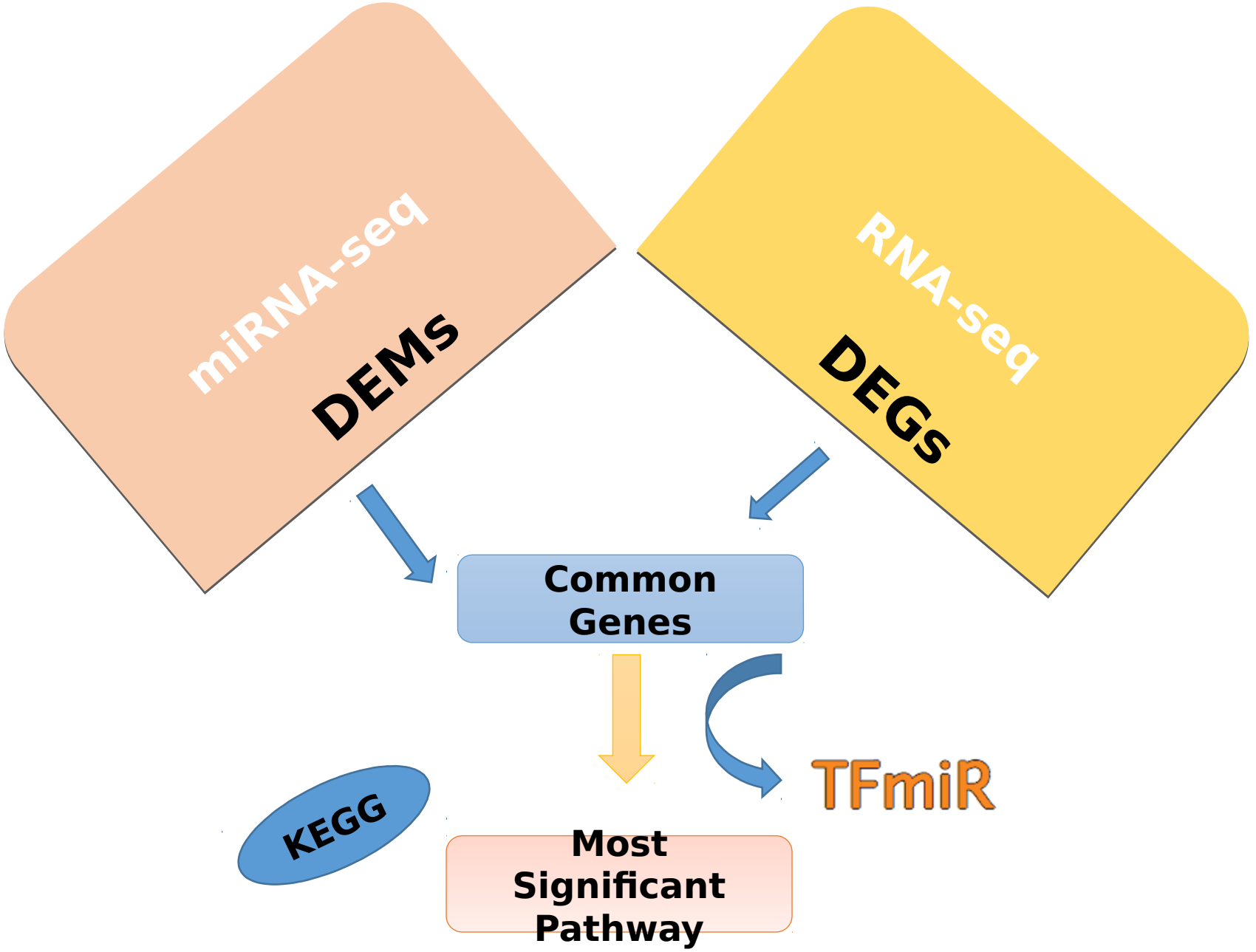
- Integrate the **data generated** from previous step by using appropriate tools to find the **significant pathway** associated with **recurrence of AML.**

Methodology

Sample selection

- Download all dataset of AML tumours

	Primary tumours from bone marrow	Recurrence from bone marrow
miRNA data set	255 cases	42 cases
RNA seq Data set	119	40

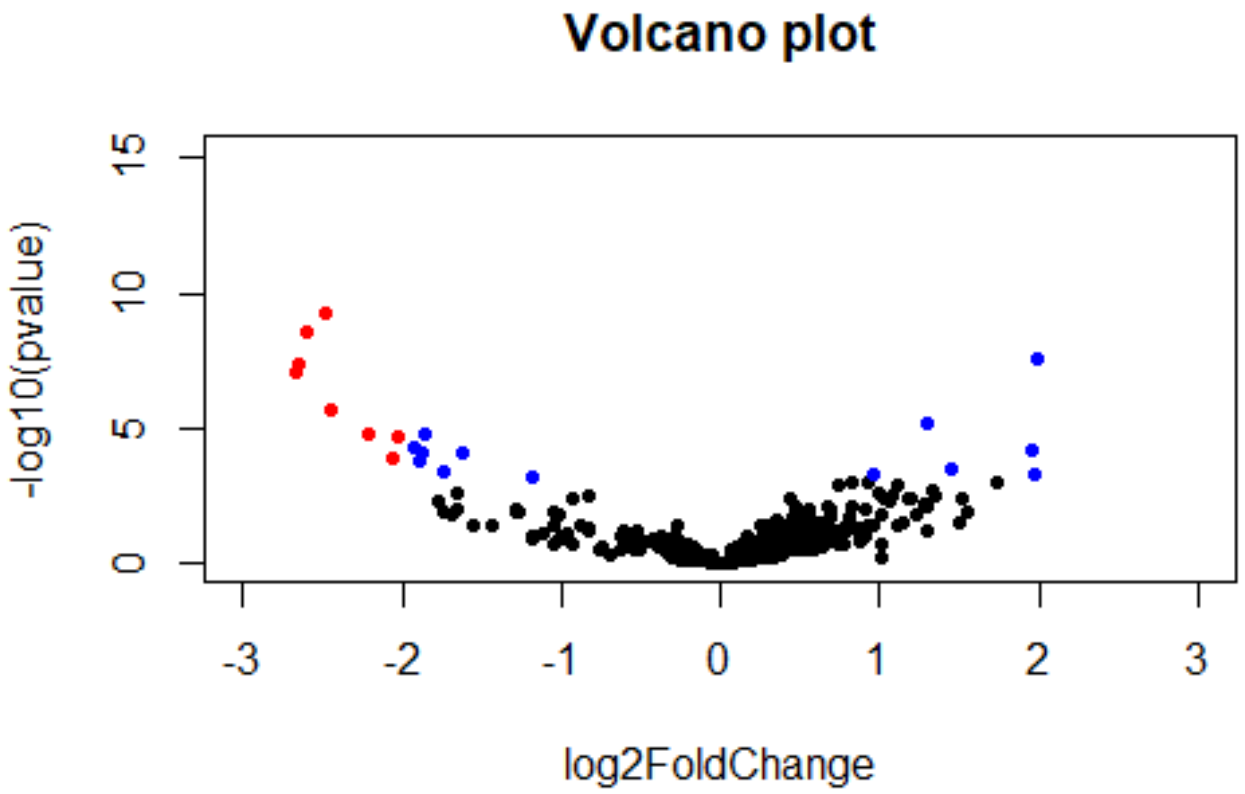

miRNA-seq
DEMs
RNA-seq
DEGs
Common Genes
TFmiR
KEGG
Most Significant Pathway

Initial Results

25 miRNA significant with adjusted pvalue < 0.05 and log2(2)

	baseMean	log2FoldC	lfcSE	stat	pvalue	padj
hsa-mir-7641-1	29.44508	6.056714	0.749604	8.079884	6.48E-16	4.21E-13
hsa-mir-7641-2	19.67615	6.046636	0.859949	7.031389	2.04E-12	6.64E-10
hsa-mir-379	724.5316	-2.49193	0.401101	-6.21274	5.21E-10	1.13E-07
hsa-mir-410	27.07937	-2.60019	0.436378	-5.95858	2.54E-09	4.13E-07
hsa-mir-4449	78.50451	1.982652	0.356968	5.554147	2.79E-08	3.62E-06
hsa-mir-381	60.85513	-2.65927	0.483549	-5.49949	3.81E-08	4.12E-06
hsa-mir-889	30.34589	-2.67544	0.49928	-5.35861	8.39E-08	7.78E-06
hsa-mir-411	7.039928	-2.4465	0.513097	-4.76811	1.86E-06	0.000150859
hsa-let-7b	95696.49	1.294539	0.285683	4.531388	5.86E-06	0.000422552
hsa-mir-127	455.8992	-1.86616	0.430907	-4.33078	1.49E-05	0.000964299
hsa-mir-136	13.80084	-2.21771	0.515128	-4.30516	1.67E-05	0.00098449
hsa-mir-758	31.71836	-2.03499	0.477033	-4.26592	1.99E-05	0.00107667
hsa-mir-337	15.60307	-1.93071	0.472792	-4.08364	4.43E-05	0.002213414
hsa-mir-509-3	4.784286	1.960953	0.486822	4.028068	5.62E-05	0.002606995
hsa-mir-452	83.11318	-1.87181	0.472097	-3.96487	7.34E-05	0.003177268
hsa-mir-153-1	22.30786	-1.62671	0.413735	-3.93178	8.43E-05	0.003420181
hsa-mir-496	3.283908	-2.06029	0.533034	-3.86522	0.000111	0.004237153
hsa-mir-369	12.18659	-1.89127	0.500092	-3.78186	0.000156	0.005612506
hsa-mir-6718	329.5474	1.452101	0.403388	3.599761	0.000319	0.010879611
hsa-mir-495	8.484046	-1.74767	0.488317	-3.57896	0.000345	0.011193891
hsa-mir-3115	5.312561	1.967197	0.567818	3.464487	0.000531	0.015671685
hsa-mir-455	46.60818	-1.18994	0.349556	-3.40414	0.000664	0.018728924
hsa-mir-509-1	5.279913	1.728134	0.522452	3.307739	0.000941	0.025115355
hsa-mir-182	14381.47	1.110066	0.339192	3.27268	0.001065	0.02560739
hsa-mir-612	3.39427	1.326661	0.424311	3.126621	0.001768	0.039572839

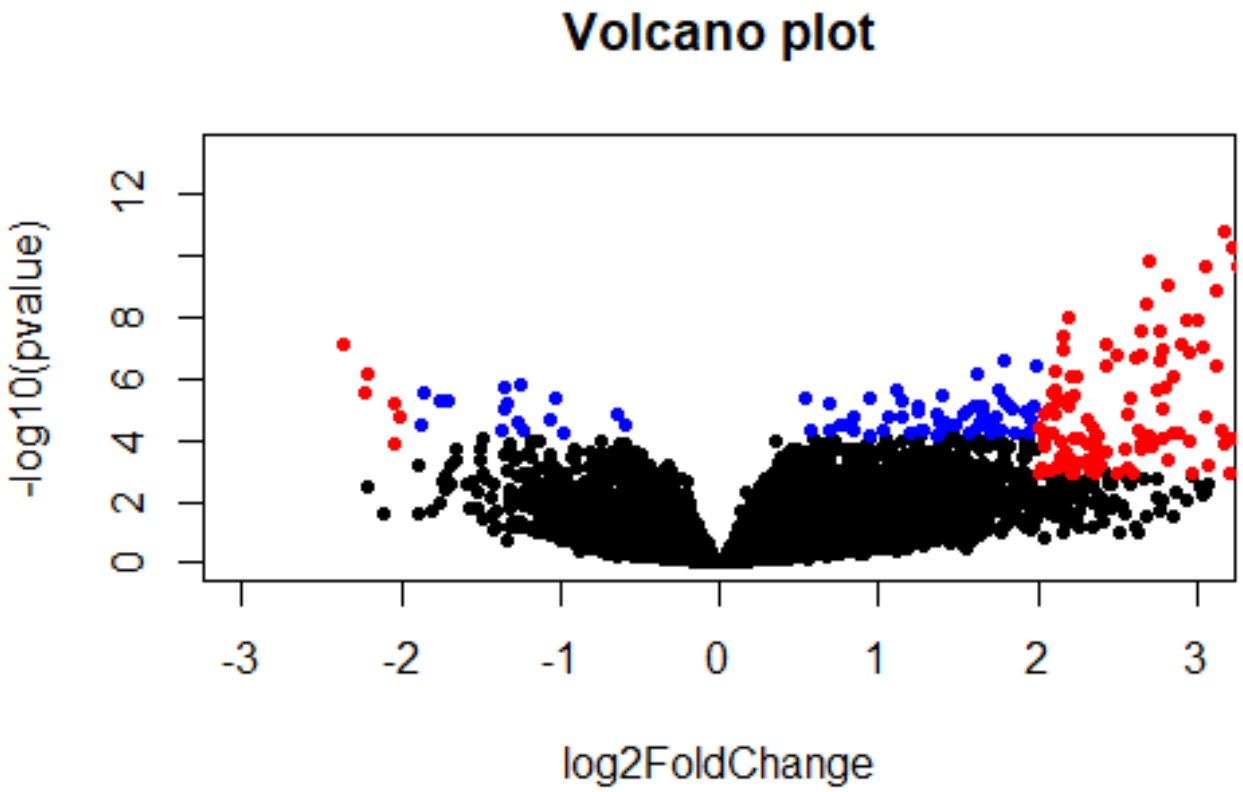
miRNA-seq Volcano plot



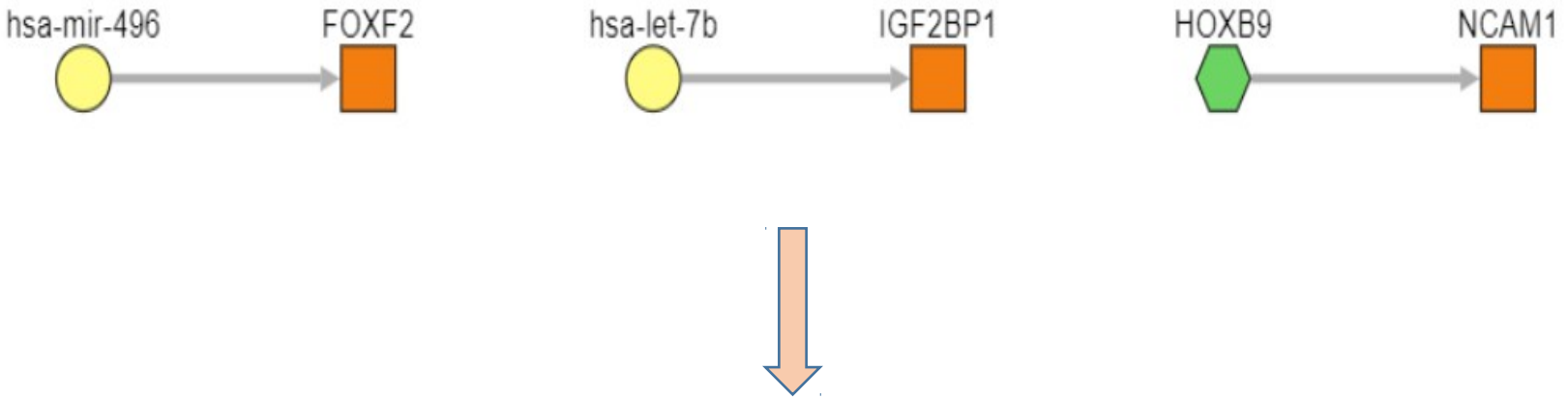
352 RNA gene significant with adjusted P value < 0.05 and log2(2)

		baseMean	log2FoldC	lfcSE	stat	pvalue	padj
1	COL6A6	115.6411	3.700412	0.489899	7.553414	4.24E-14	9.01E-10
2	DNAH8	113.0436	3.559166	0.483842	7.356047	1.89E-13	2.01E-09
3	SOX11	49.19598	3.171394	0.471591	6.724875	1.76E-11	1.25E-07
4	SLC13A3	64.07639	3.226491	0.493207	6.541858	6.08E-11	3.23E-07
5	DSC3	27.03315	4.90586	0.759013	6.463473	1.02E-10	4.35E-07
6	DPEP3	259.4182	2.696767	0.42169	6.395134	1.60E-10	5.68E-07
7	DOK6	66.56734	3.249592	0.5127	6.338187	2.32E-10	6.40E-07
8	PBX1	328.5789	3.04633	0.481053	6.332629	2.41E-10	6.40E-07
9	MYCT1	146.3447	3.747975	0.60289	6.216681	5.08E-10	1.20E-06
10	COL6A5	111.1015	3.60518	0.583422	6.179367	6.44E-10	1.37E-06
11	GREB1	63.18018	2.821455	0.460422	6.127982	8.90E-10	1.72E-06
12	C2orf66	54.78546	3.114745	0.513154	6.069805	1.28E-09	2.09E-06
13	GABRE	109.2002	3.995801	0.657806	6.074433	1.24E-09	2.09E-06
14	HIF3A	74.26774	2.676317	0.454649	5.88655	3.94E-09	5.99E-06
15	COL11A1	45.80198	4.185657	0.72102	5.805189	6.43E-09	9.11E-06
16	PRSS2	186.2395	3.673364	0.638536	5.752795	8.78E-09	1.17E-05
17	GPM6B	713.3656	2.190371	0.382835	5.721448	1.06E-08	1.32E-05
18	LOC33926	56.82729	2.933322	0.514872	5.697192	1.22E-08	1.39E-05
19	MYO3B	34.88362	2.992778	0.525862	5.691186	1.26E-08	1.39E-05
20	POU4F1	1422.572	3.584585	0.630526	5.685075	1.31E-08	1.39E-05
21	CGA	28.01071	4.759376	0.846405	5.623048	1.88E-08	1.90E-05
22	MEG3	377.998	2.763813	0.497693	5.553254	2.80E-08	2.71E-05
23	KIAA1210	10.0667	2.650384	0.478625	5.537496	3.07E-08	2.84E-05
24	TCF23	11.73652	2.149679	0.391969	5.484315	4.15E-08	3.68E-05
25	ST6GAL2	41.3135	2.89709	0.537394	5.391001	7.01E-08	5.96E-05
26	TNFRSF19	104.0592	-2.36743	0.440178	-5.37834	7.52E-08	6.15E-05
27	CDC42BPA	464.4693	2.428003	0.453123	5.358379	8.40E-08	6.61E-05
28	PADI3	20.09387	3.035836	0.56939	5.331738	9.73E-08	7.39E-05
29	CATSPERB	13.50521	2.1535	0.405383	5.312254	1.08E-07	7.67E-05
30	CLEC2L	32.47119	2.788574	0.524538	5.31625	1.06E-07	7.67E-05
31	RNF182	295.6891	2.957155	0.561674	5.26489	1.40E-07	9.51E-05
32	ROBO2	61.63967	4.368174	0.830271	5.261144	1.43E-07	9.51E-05
33	SLITRK5	124.4172	2.647307	0.504862	5.243627	1.57E-07	0.000101
34	SHROOM3	13.69313	2.496943	0.47836	5.219804	1.79E-07	0.000112
35	ROBO1	400.1422	2.607243	0.503427	5.17899	2.23E-07	0.000135
36	ANKRD18I	246.3614	2.76872	0.53833	5.143165	2.70E-07	0.000155
37	SYT5	27.04488	1.783071	0.346559	5.145066	2.67E-07	0.000155
38	TEX15	7.935702	3.114595	0.61168	5.091873	3.55E-07	0.000198
39	C3orf70	22.73454	1.983509	0.391759	5.063086	4.13E-07	0.000225
40	GAD1	414.9702	2.424851	0.479426	5.057819	4.24E-07	0.000225
41	NLGN1	25.20063	3.353505	0.66527	5.040817	4.64E-07	0.00024
42	HMCN1	16.43089	2.102792	0.419696	5.01028	5.44E-07	0.000275
43	CCL2	629.5979	-2.21857	0.445889	-4.9756	6.50E-07	0.000314
44	FREM1	91.15981	3.469913	0.697101	4.977636	6.44E-07	0.000314
45	SERPINI1	262.947	1.623072	0.326661	4.968674	6.74E-07	0.000318
46	STAC	84.38588	2.843648	0.575705	4.939421	7.84E-07	0.000362
47	HSPB7	27.89201	2.199233	0.445808	4.93314	8.09E-07	0.000366
48	KBTBD12	28.76664	2.242599	0.457198	4.905097	9.34E-07	0.000414
49	MT1H	28.31572	3.71258	0.759727	4.886731	1.03E-06	0.000445
50	HLX	4462.382	-1.24581	0.259775	-4.79572	1.62E-06	0.000689

mRNA-seq Volcano plot

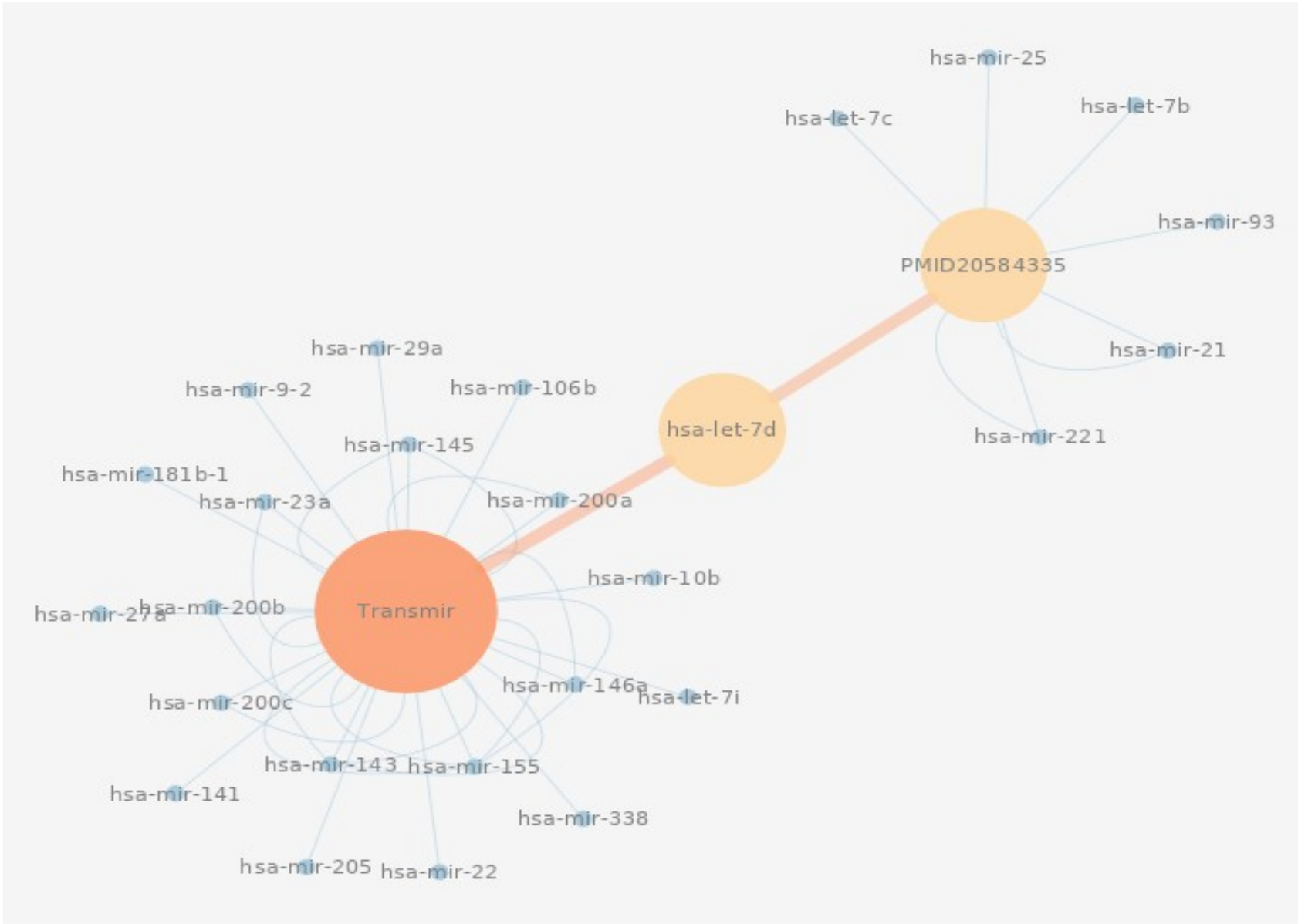


TFmiR



IGF2BP1: Insulin Like Growth Factor 2 MRNA Binding Protein 1
Its role in AML: **IGF2BP1** was confirmed to be a novel downstream target of **LIN28B** (Lin-28 Homolog B) via **let-7 miRNA** in **AML**. Notably, over expression of LIN28B increased **tumorigenicity,** while silencing LIN28B led to **slow tumor growth** in vivo.

Cytoscape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hsa-mir-10b
hsa-mir-27a
hsa-mir-200b
Transmir
hsa-mir-146a
hsa-let-7i
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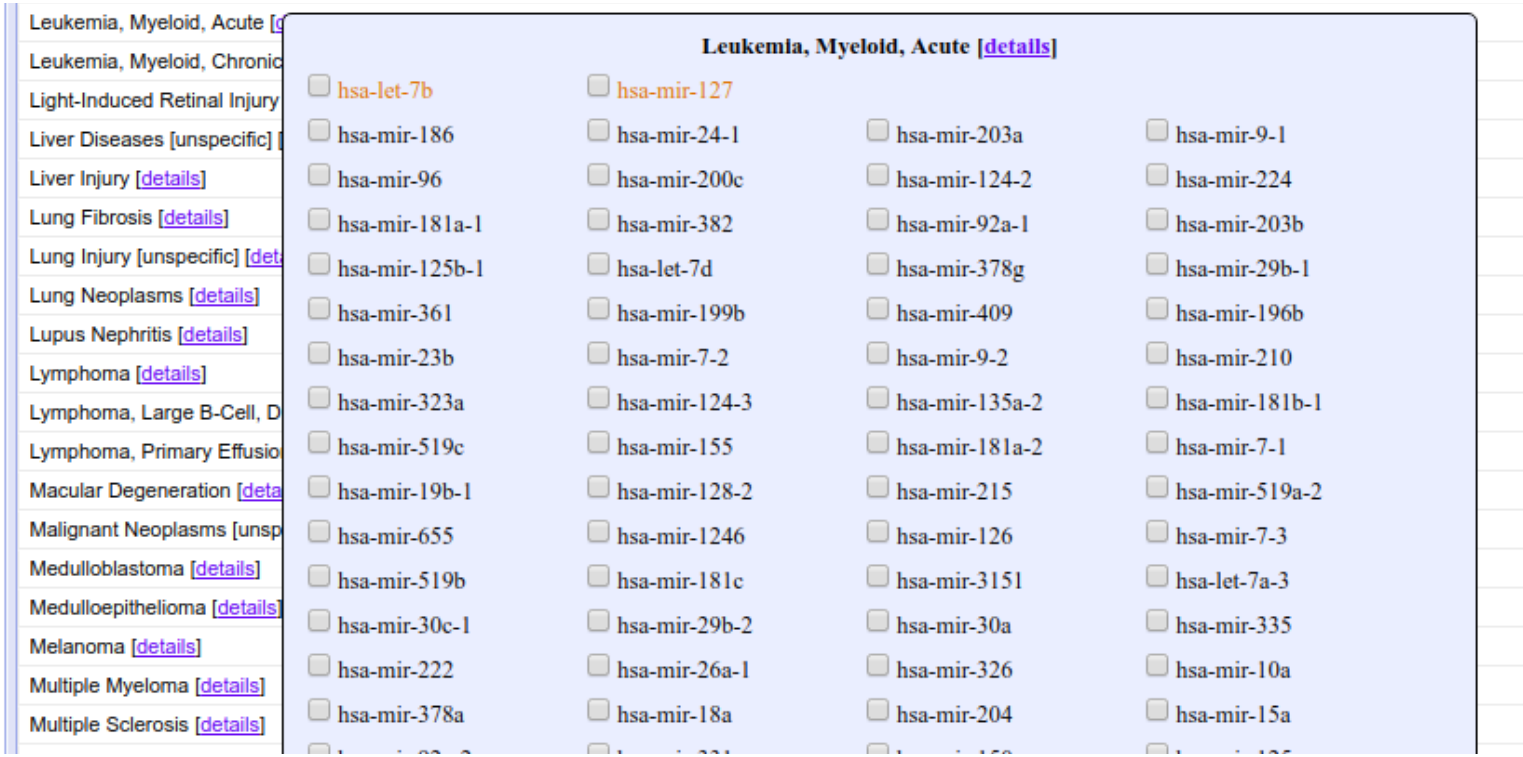
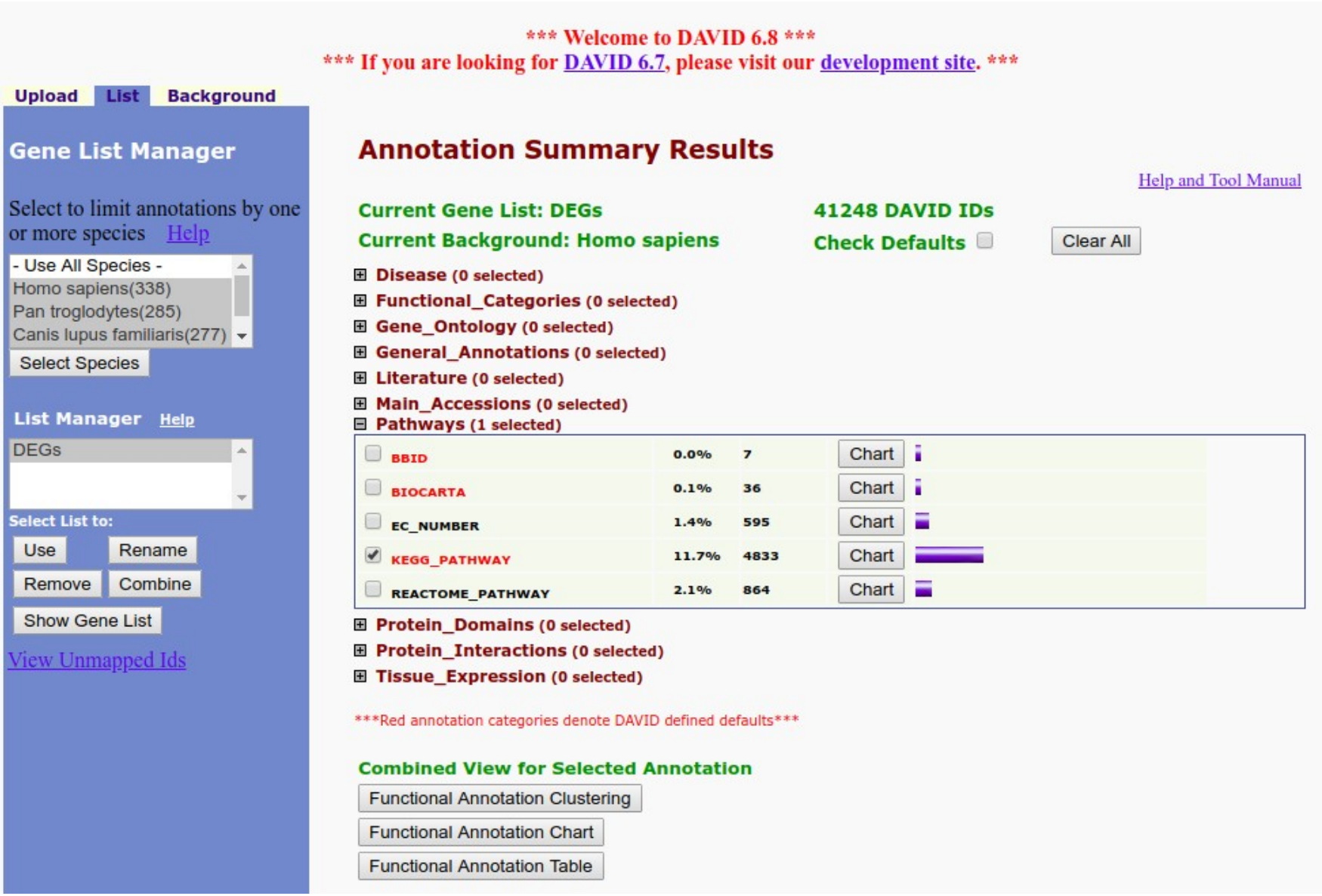
hsa-mir-22

TAM

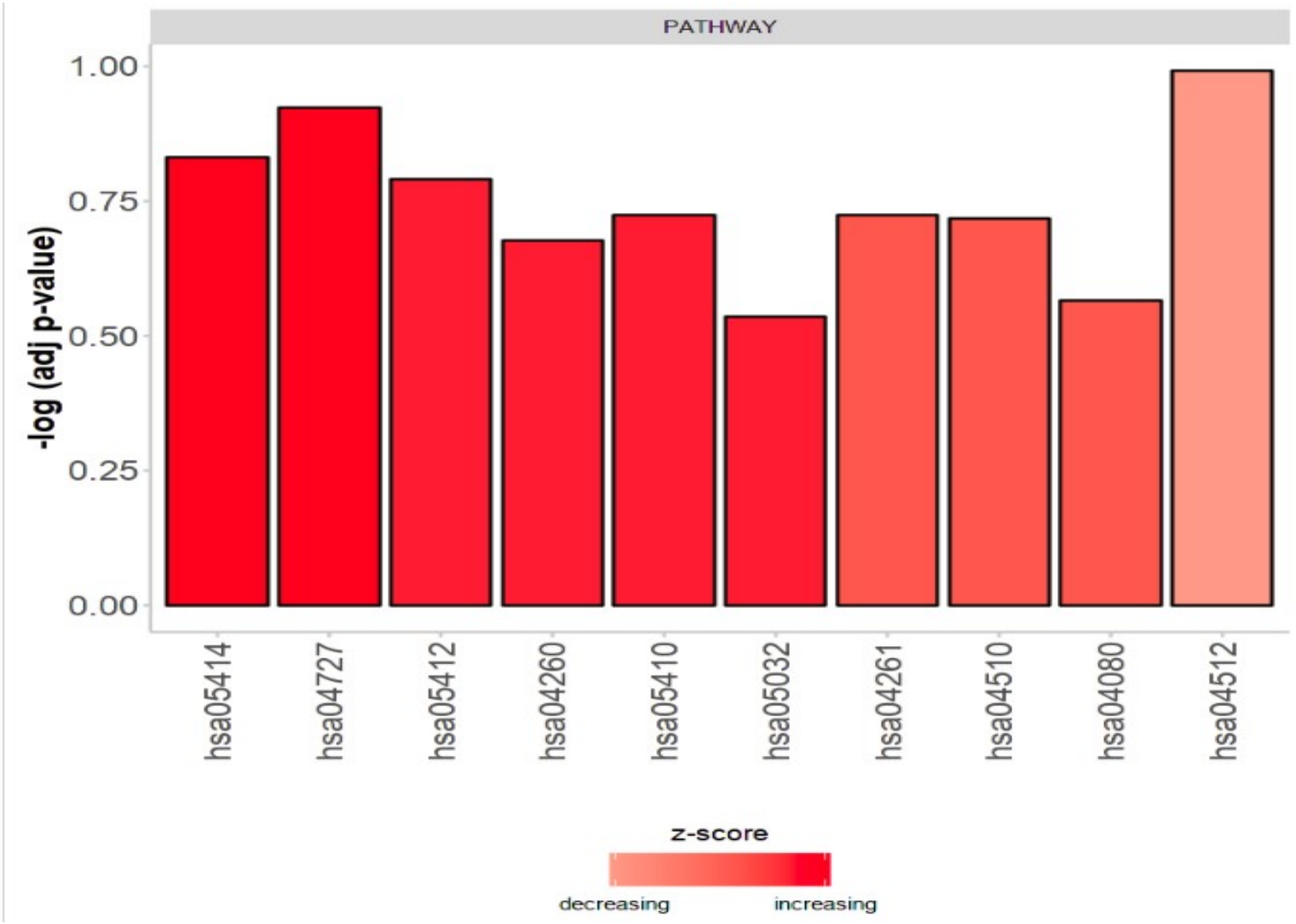


Term	miRNA	PMID
Leukemia, Myeloid, Acute	hsa-let-7b	18056805 23391324 22348345
Leukemia, Myeloid, Acute	hsa-mir-127	18478077

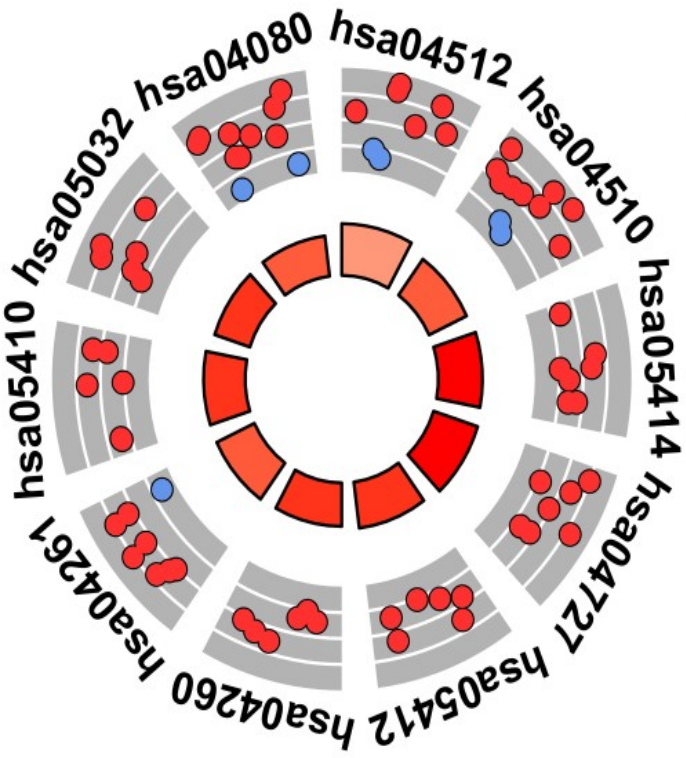
David


*** Welcome to DAVID 6.8 ***
*** If you are looking for DAVID 6.7, please visit our development site. ***
Upload
List
Background
Gene List Manager
Select to limit annotations by one or more species
Help
- Use All Species -
Homo sapiens(338)
Pan troglodytes(285)
Canis lupus familiaris(277)
Select Species
List Manager
Help
DEGs
Select List to:
Use
Rename
Remove
Combine
Show Gene List
View Unmapped Ids
Annotation Summary Results
Help and Tool Manual
Current Gene List: DEGs
41248 DAVID IDs
Current Background: Homo sapiens
Check Defaults
Clear All
Disease (0 selected)
Functional_Categories (0 selected)
Gene_Ontology (0 selected)
General_Annotations (0 selected)
Literature (0 selected)
Main_Accessions (0 selected)
Pathways (1 selected)
BBID 0.0% 7 Chart
BIOCARTA 0.1% 36 Chart
EC_NUMBER 1.4% 595 Chart
KEGG_PATHWAY 11.7% 4833 Chart
REACTOME_PATHWAY 2.1% 864 Chart
Protein_Domains (0 selected)
Protein_Interactions (0 selected)
Tissue_Expression (0 selected)
Red annotation categories denote DAVID defined defaults
Combined View for Selected Annotation
Functional Annotation Clustering
Functional Annotation Chart
Functional Annotation Table

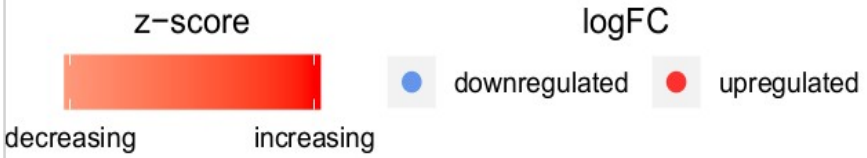
GoPlot (KEGG Pathways)



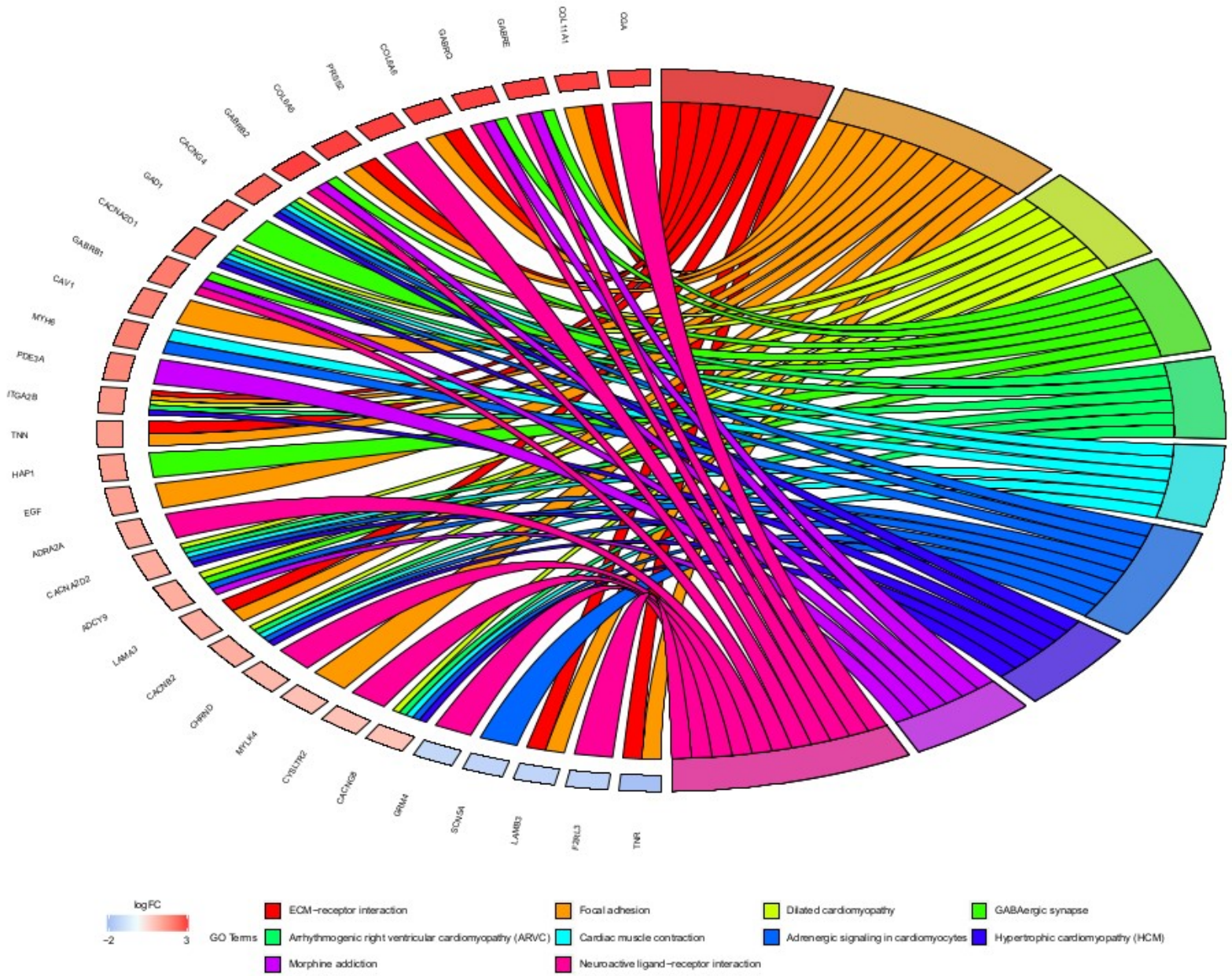
GoPlot (KEGG Pathways)



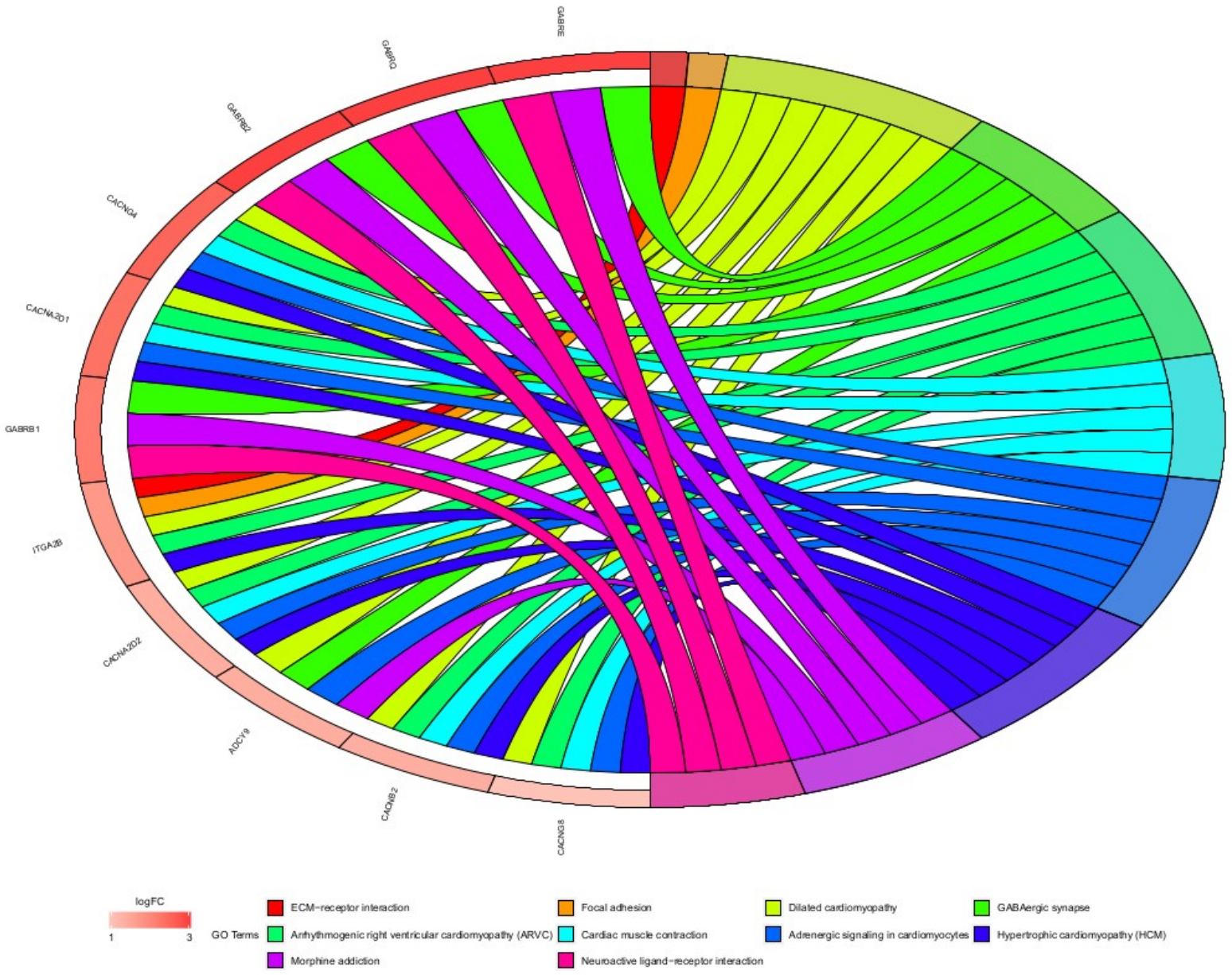
ID	Description
hsa04512	ECM−receptor interaction
hsa04510	Focal adhesion
hsa05414	Dilated cardiomyopathy
hsa04727	GABAergic synapse
hsa05412	Arrhythmogenic right ventricular cardiomyopathy (A
hsa04260	Cardiac muscle contraction
hsa04261	Adrenergic signaling in cardiomyocytes
hsa05410	Hypertrophic cardiomyopathy (HCM)
hsa05032	Morphine addiction
hsa04080	Neuroactive ligand−receptor interaction



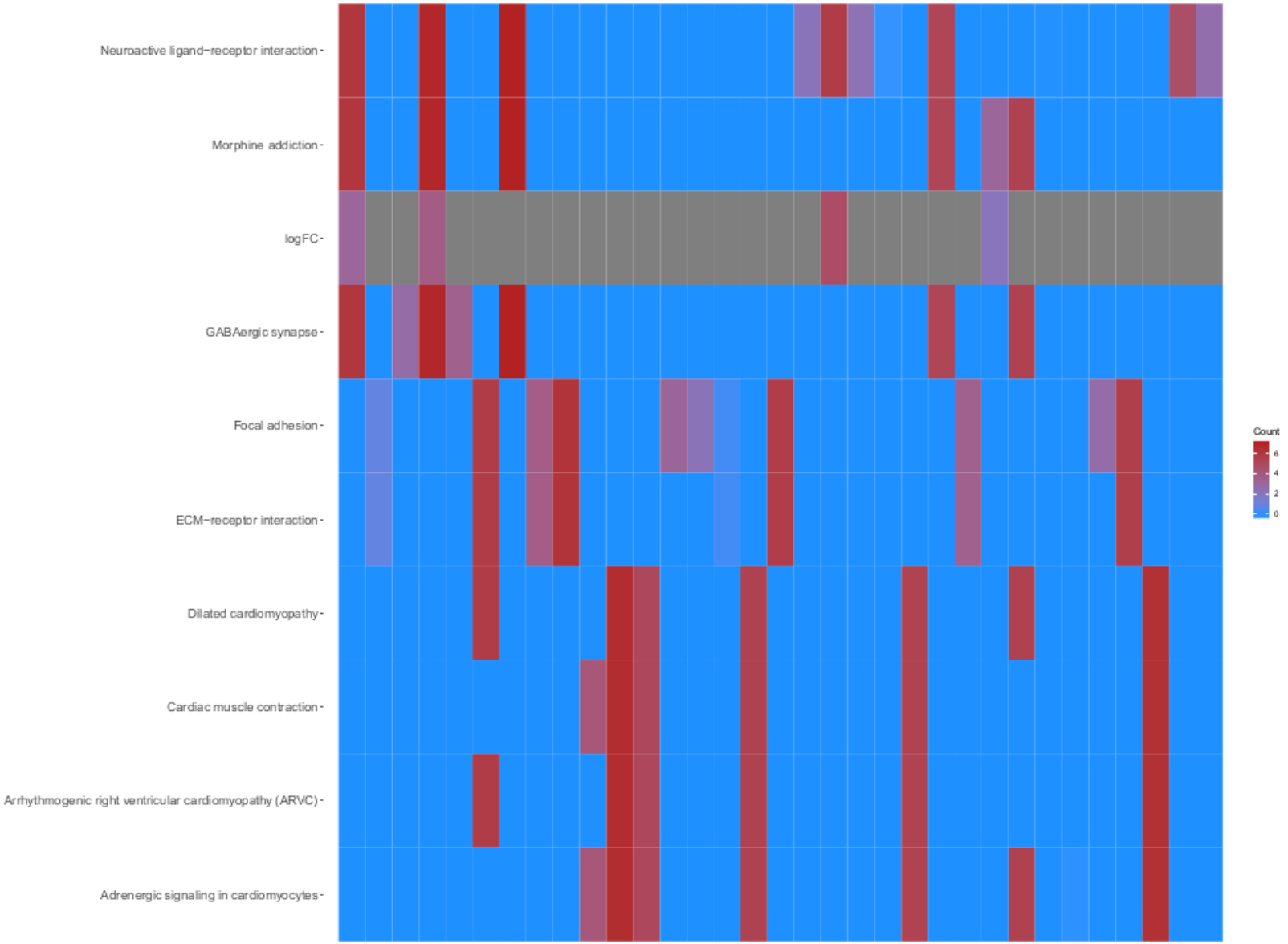
GoPlot (KEGG Pathways)



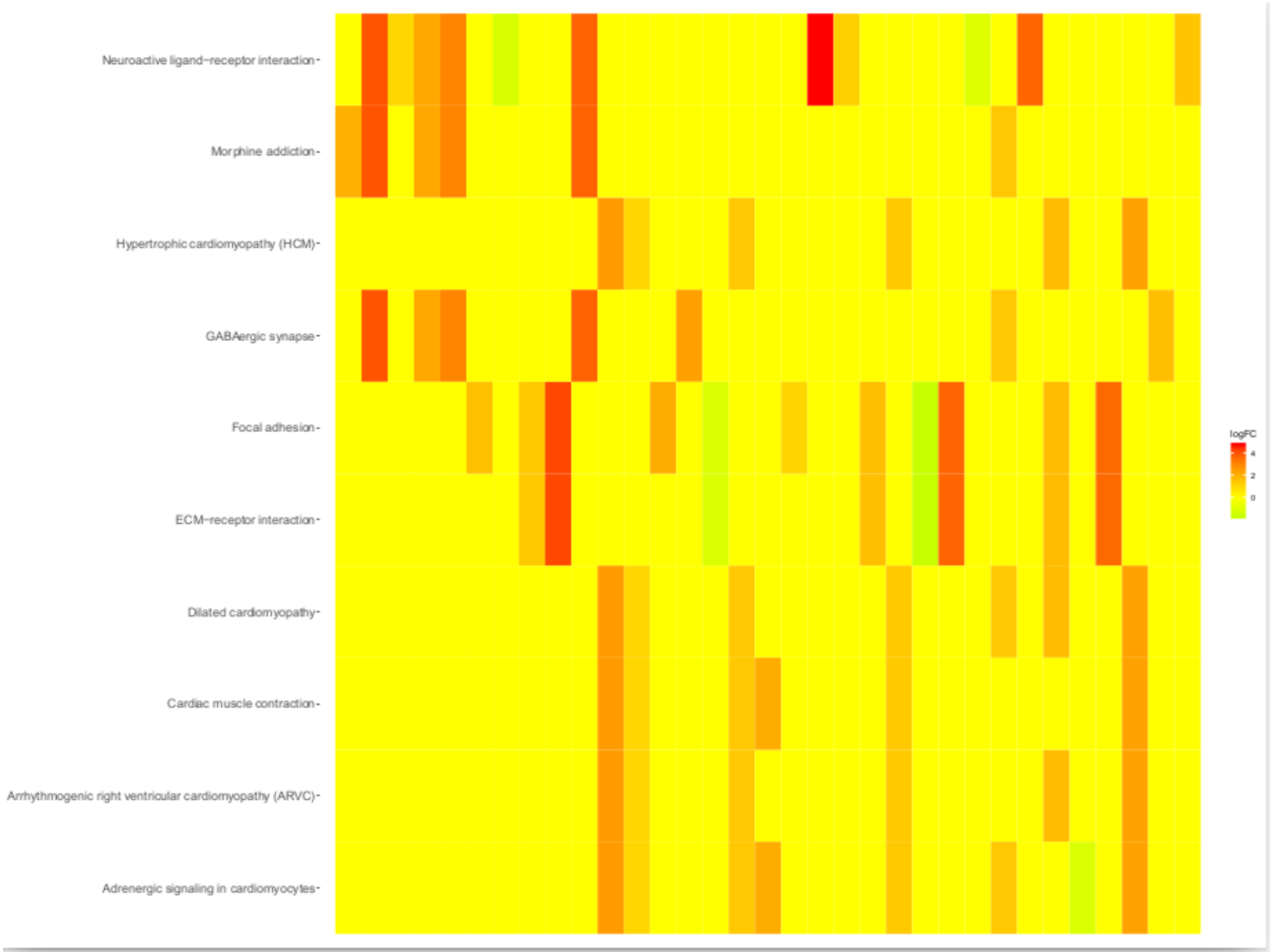
GoPlot (KEGG Pathways)



GoPlot (KEGG Pathways)

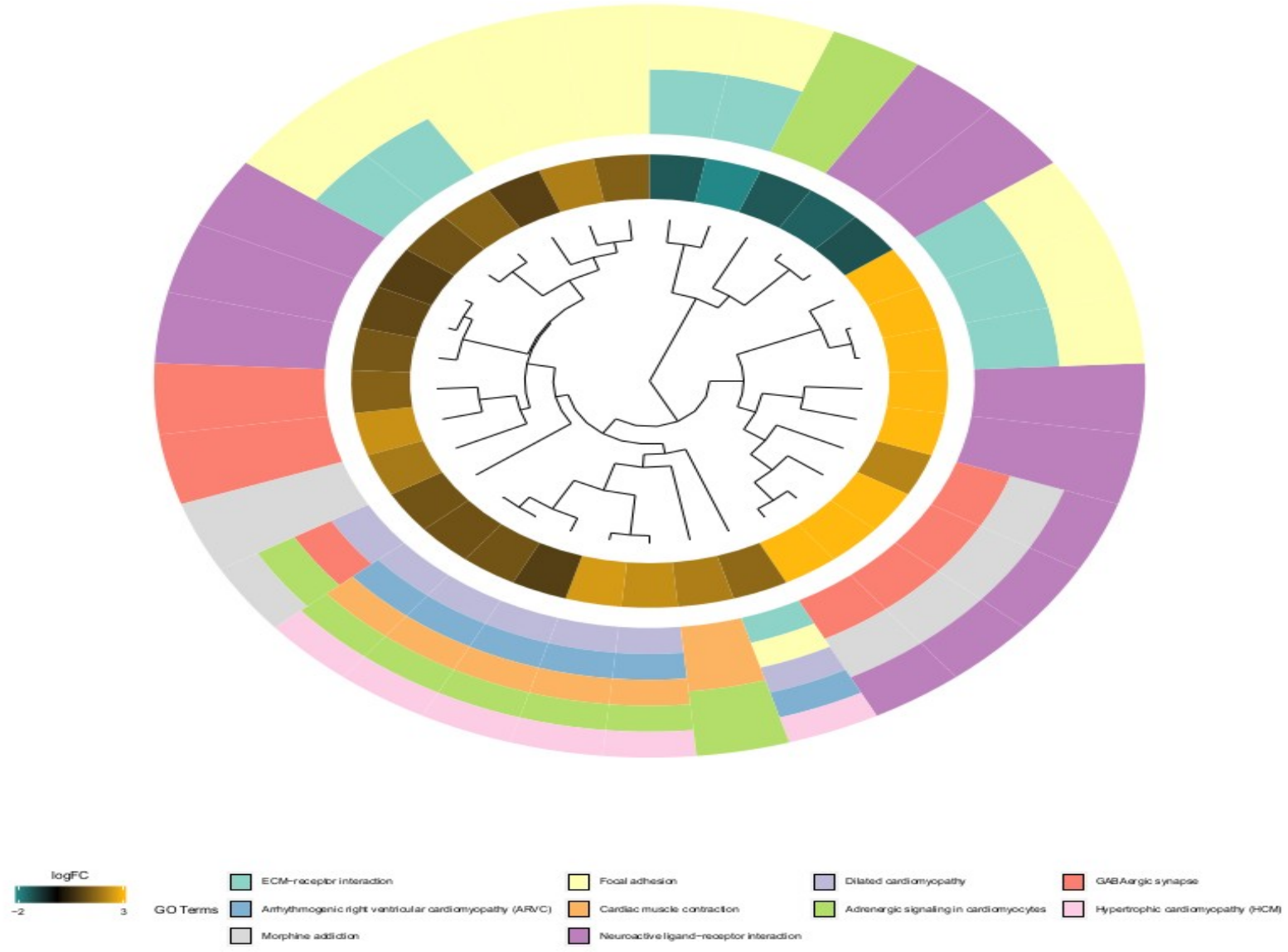


GoPlot (KEGG Pathways)

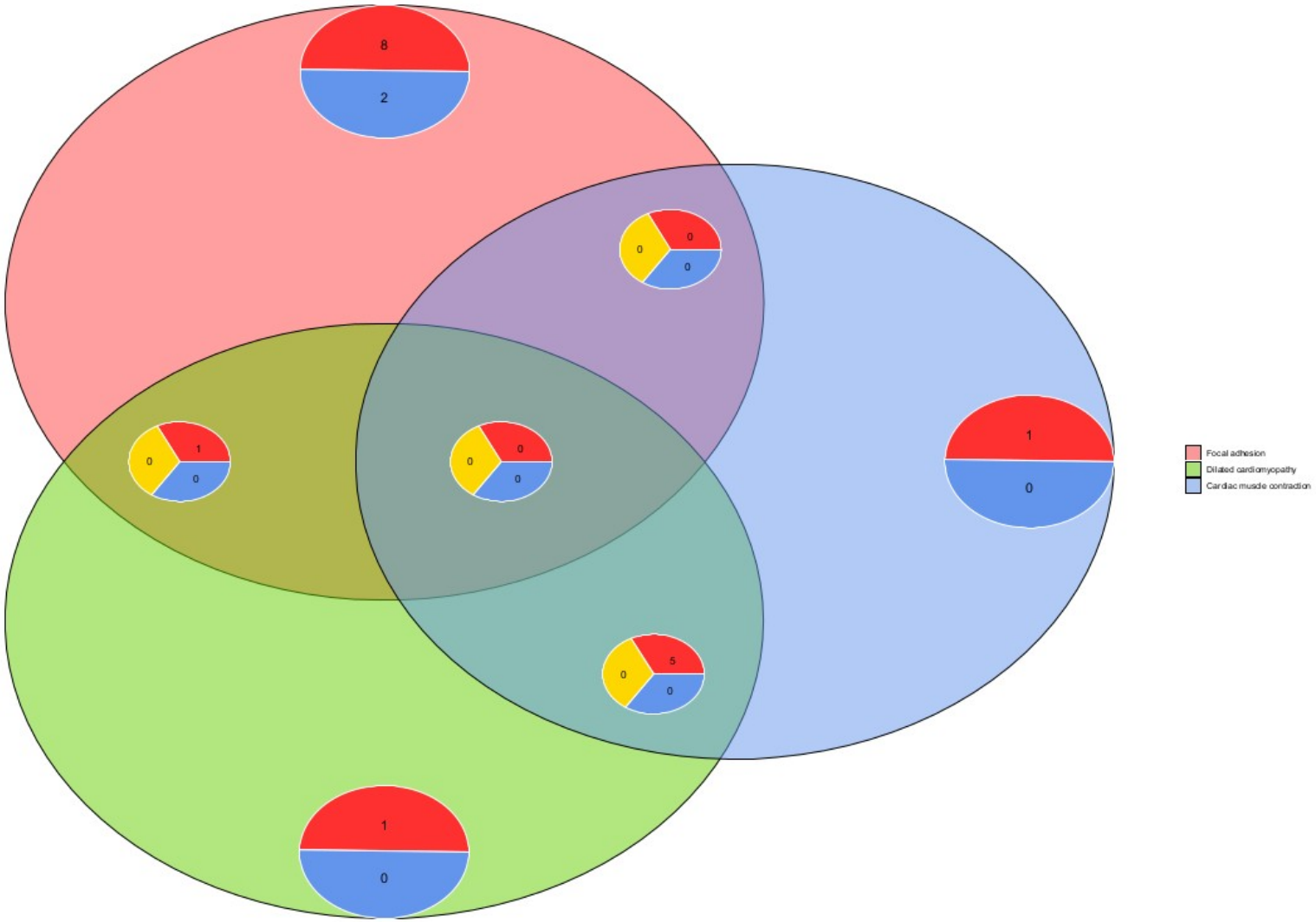


GoPlot (KEGG Pathways)

GoPlot (KEGG Pathways)



GoPlot (KEGG Pathways)



Thank you