

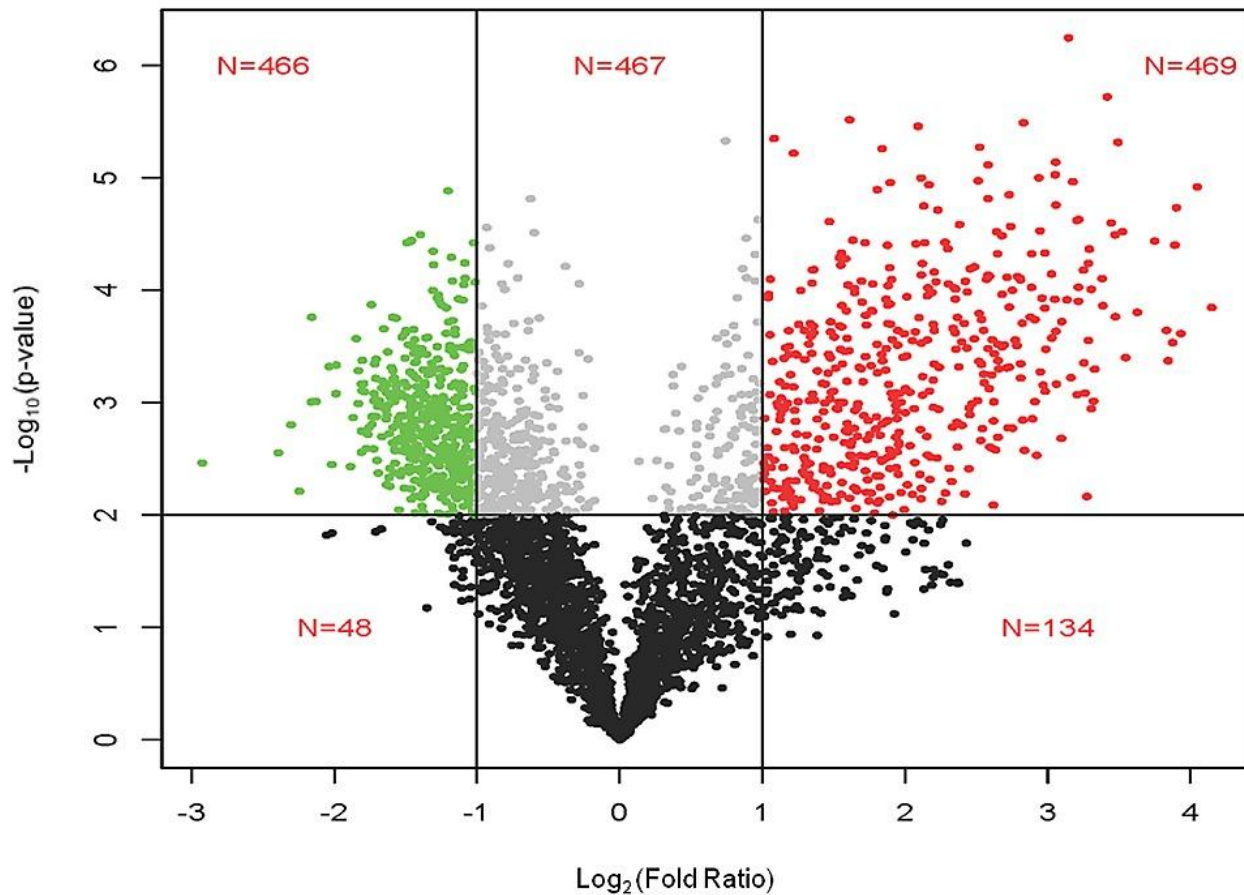
# Upcoming Workshops

- ◆ **10/26-27** - Introduction to RNA-Seq Analysis
- ◆ **11/9** - Single Cell Analysis
- ◆ **11/19** - Machine Learning

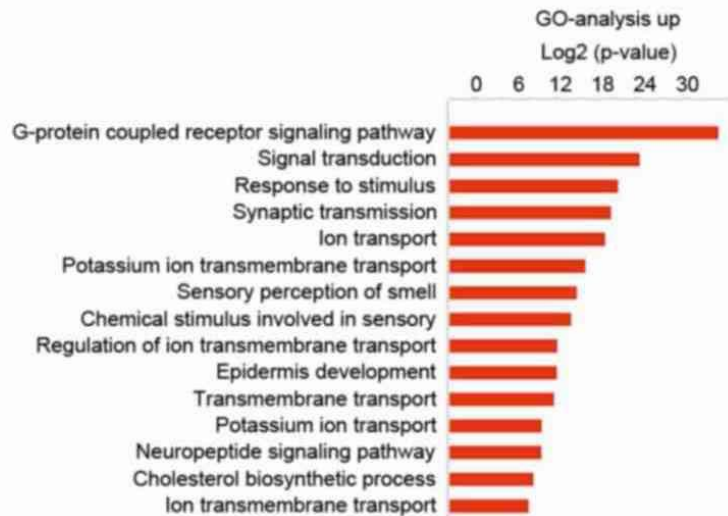
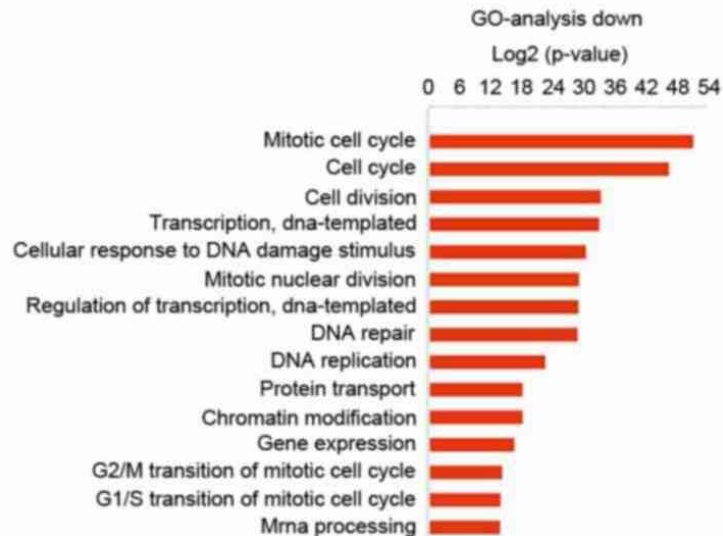
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6	RPS23P1	ENSG00000243504	2.87680167	0.0004883	0.00566507
7	RPS3AP44	ENSG00000234031	2.87189316	2.02E-05	0.00041197
8	RPS21	ENSG00000171858	1.78201017	5.40E-16	2.76E-13
9	ERGIC3	ENSG00000125991	1.04270392	4.08E-07	1.48E-05
10	ATP5F1E	ENSG00000124172	1.0419011	1.74E-07	6.97E-06
11	OST4	ENSG00000228474	1.04082316	8.61E-06	0.00020192
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13	BCR	ENSG00000186716	1.02217198	2.66E-08	1.37E-06
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36	ACTB	ENSG00000075624	-5.8670115	4.30E-06	0.00011161

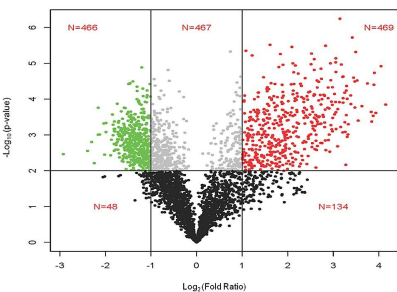
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27	OBSCN	ENSG00000154358	-3.93820271	0.0003276	0.00410635
28	TMEM14C	ENSG00000111843	-3.93798301	0.0022893	0.01811524
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30	COX4I1	ENSG00000131143	-4.91954634	2.37E-06	6.68E-05
31	CCT3	ENSG00000163468	-4.91242065	1.55E-05	0.00033124
32	SSR4	ENSG00000180879	-4.9086354	0.0002098	0.00286687
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34	NDUFA4	ENSG00000189043	-5.879366	1.02E-06	3.31E-05
35	NDUFS8	ENSG00000110717	-5.87563243	0.0022648	0.01795672
36	ACTB	ENSG00000075624	-5.8670115	4.30E-06	0.00011161



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# Functional Enrichment Analysis

DE Genes
ABL1
TP53
MDM2
...



Set 1	Set 2	Set 3	Set 4	Set 5	Set N
M	A	N	M	A	MDM2
R	D	C	R	C	RB1
C	T	L	C	L	CDK2
...	...	...	...	...	...



= GO Analysis



= Pathway Analysis



GO Term or Pathway	Ratio	P-Value	Z-Score	List of Genes
Cell Cycle	9/45	4.2E-12	2.5	MDM2, ABL1, RB1, ...
DNA Repair	9/52	2.6E-10	3.2	MDM2, ABL1, RB1, ...
TNF Signaling	8/40	1.1E-10	2.4	CDK2, TNFA1, ABL1, ...

# Functional Enrichment Analysis

## 1. Overrepresentation Analysis (ORA)

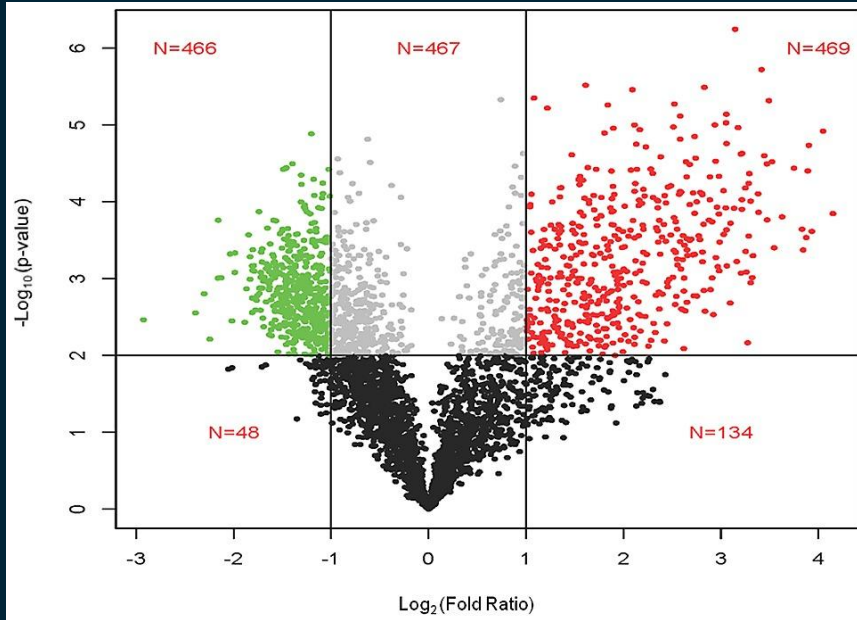
- Pick cutoffs
- Apply Fisher's Exact Test  
or Hypergeometric Test

## 2. Gene Set Enrichment Analysis (GSEA)

- Rank all genes
- Apply GSEA method



# 1. Overrepresentation Analysis (ORA)



## Pick cutoffs

- Log<sub>2</sub>FC
- Adjusted p-value
- Upregulated
- Downregulated
- Significantly changed

# 1. Overrepresentation Analysis (ORA)

## Apply statistical test

- Fisher's exact test or Hypergeometric test
- See detailed [descriptions](#) and [comparisons](#)

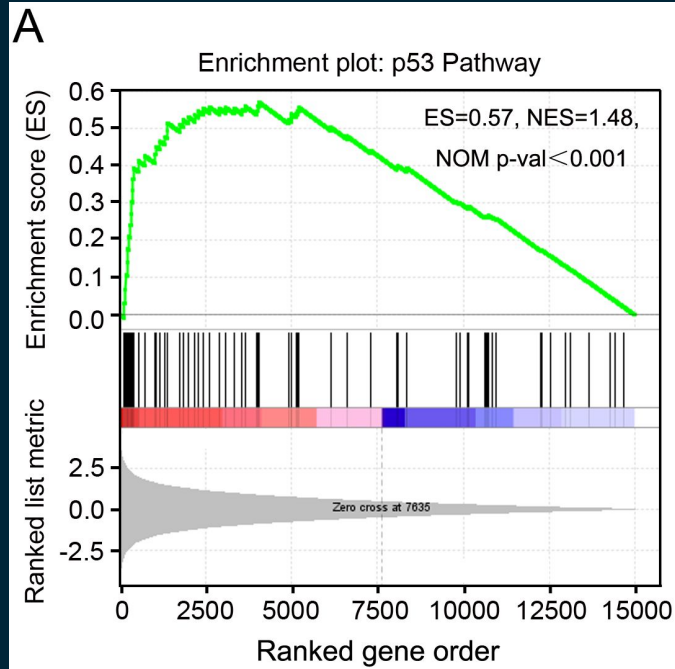
## Important factors

- Source of ontology and pathways sets
- Pre-filtering
- Post-filtering
- Use of background or reference set

## 2. Gene Set Enrichment Analysis (GSEA)

### Rank all genes

- $\text{sign}(\log_2\text{FC}) * -\log(\text{Pvalue})$



## 2. Gene Set Enrichment Analysis (GSEA)

### Apply statistical test

- [Subramanian, et al. PNAS 2005](#)

### Important factors

- Source of ontology and pathways sets
- Pre-filtering
- Post-filtering

### Advantages

- No arbitrary cutoffs
- Background is built into ranked gene set

*Which method should I use?*

**Both!**

# Survey of Tools

- [Enrichr](#) - simple test of 154 resources (ORA only)
  - Paste in human or mouse symbols
  - Browse and export results
  - Can't provide background set
  - See [documentation](#)
- [WebGestalt](#) - advanced test on select resources
  - Paste in symbols or identifiers ~15 species
  - Specify background set
  - Advanced options; advanced data visualizations
  - See [Wang, et al. NAR 2017](#)

# Survey of Tools

- *clusterProfiler* - R package
  - ORA and GSEA
  - Advanced input and configuration options
  - Built-in GO analysis; expansion options
  - Built-in data visualization; expansion options
  - See [documentation](#)

*Which tool should I use?*

**Multiple!**

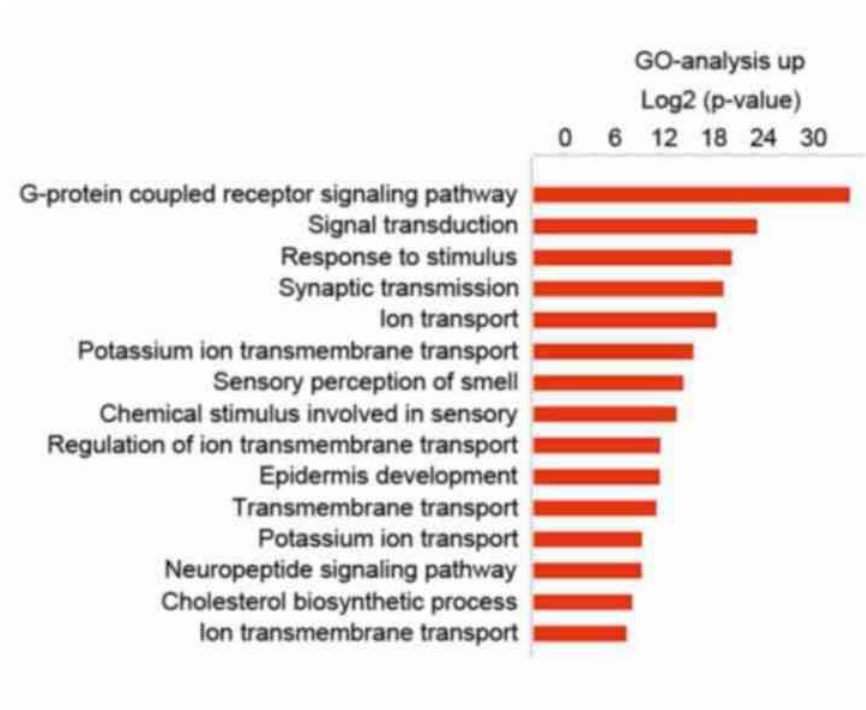


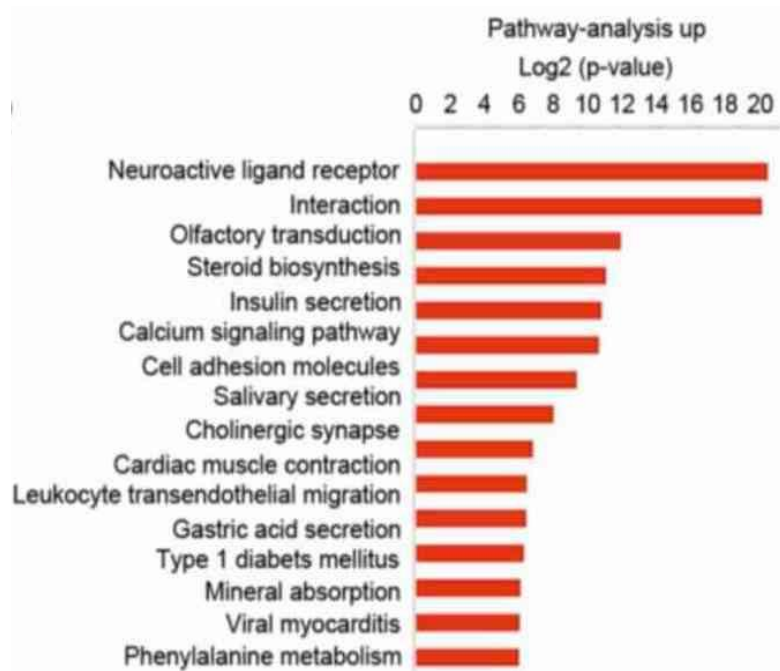
# Additional Reading

- [Huang 2009](#)
- [Tipney & Hunter 2010](#)
- [Kelder, Pico, et al. 2010](#)
- [Nguyen, et al. 2019](#)

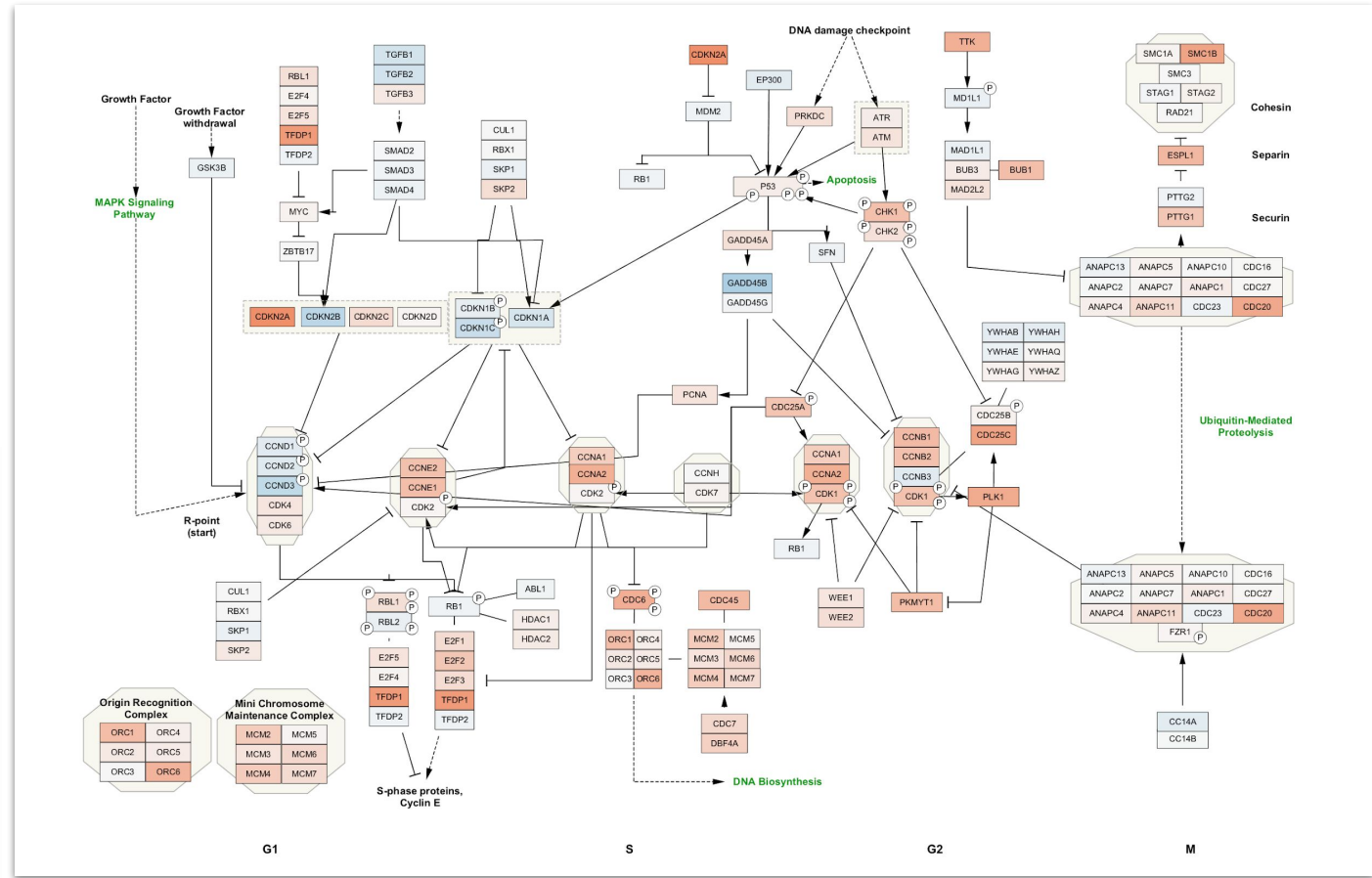
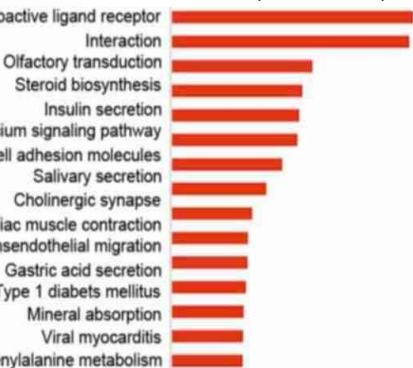


**GENEONTOLOGY**  
Unifying Biology





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1	OST4	ENSG00000228474	1.04082316	8.61E-06	0.000201
2	MIR663AHG	ENSG00000227195	1.03864396	1.16E-07	4.98E-4
3	ICK	ENSG00000186718	1.02217158	2.66E-08	1.37E-4
4	HST1H2AG	ENSG00000196787	1.02057118	9.58E-05	0.001488
5	SSR2	ENSG00000163479	1.01915311	4.30E-10	3.79E-4
6	EEF1A1	ENSG00000136508	-1.01124735	1.08E-07	4.62E-4
7	TMEM159	ENSG00000116538	-1.00983307	0.0017789	0.014985
8	CL2orf57	ENSG00000111678	-1.00909066	0.0009472	0.009422
9	ELOB	ENSG00000103363	-1.9700433	1.64E-05	0.000343
0	R2M1	ENSG00000116670	-1.92529859	3.78E-07	1.37E-4
1	EF3X	ENSG00000178982	-2.9604061	2.65E-06	7.30E-4
2	FBP	ENSG00000172500	-2.95401909	0.0005135	0.0059124
3	SCANAL7	ENSG00000251092	-2.95153973	1.74E-06	5.38E-4
4	HLA-B	ENSG00000234745	-2.94688319	6.68E-08	3.10E-4
5	C7orf26	ENSG00000146576	-3.94240445	0.0001522	0.002235
6	ITB	ENSG00000143543	-3.94165981	1.89E-06	5.60E-4
7	ORCN	ENSG00000154358	-3.93820271	0.0001276	0.004106
8	TMEM14C	ENSG00000111843	-3.93798301	0.0022893	0.018115
9	ATP5MG	ENSG00000167283	-3.93536081	0.0001484	0.002177
0	CDK4I	ENSG00000131143	-4.91393468	2.37E-06	6.88E-4
1	CCT3	ENSG00000163468	-4.91242005	1.55E-05	0.000331
2	SSR4	ENSG00000180879	-4.9086354	0.0002098	0.002666
3	SNHG22	ENSG00000267322	-4.90691154	9.03E-05	0.001446
4	NDUF44	ENSG00000189043	-5.8793366	1.02E-06	3.31E-4
5	NDUF58	ENSG00000110717	-5.87563241	0.0022648	0.017956
6	ACTB	ENSG00000075624	-9.8670111	4.30E-06	0.000111

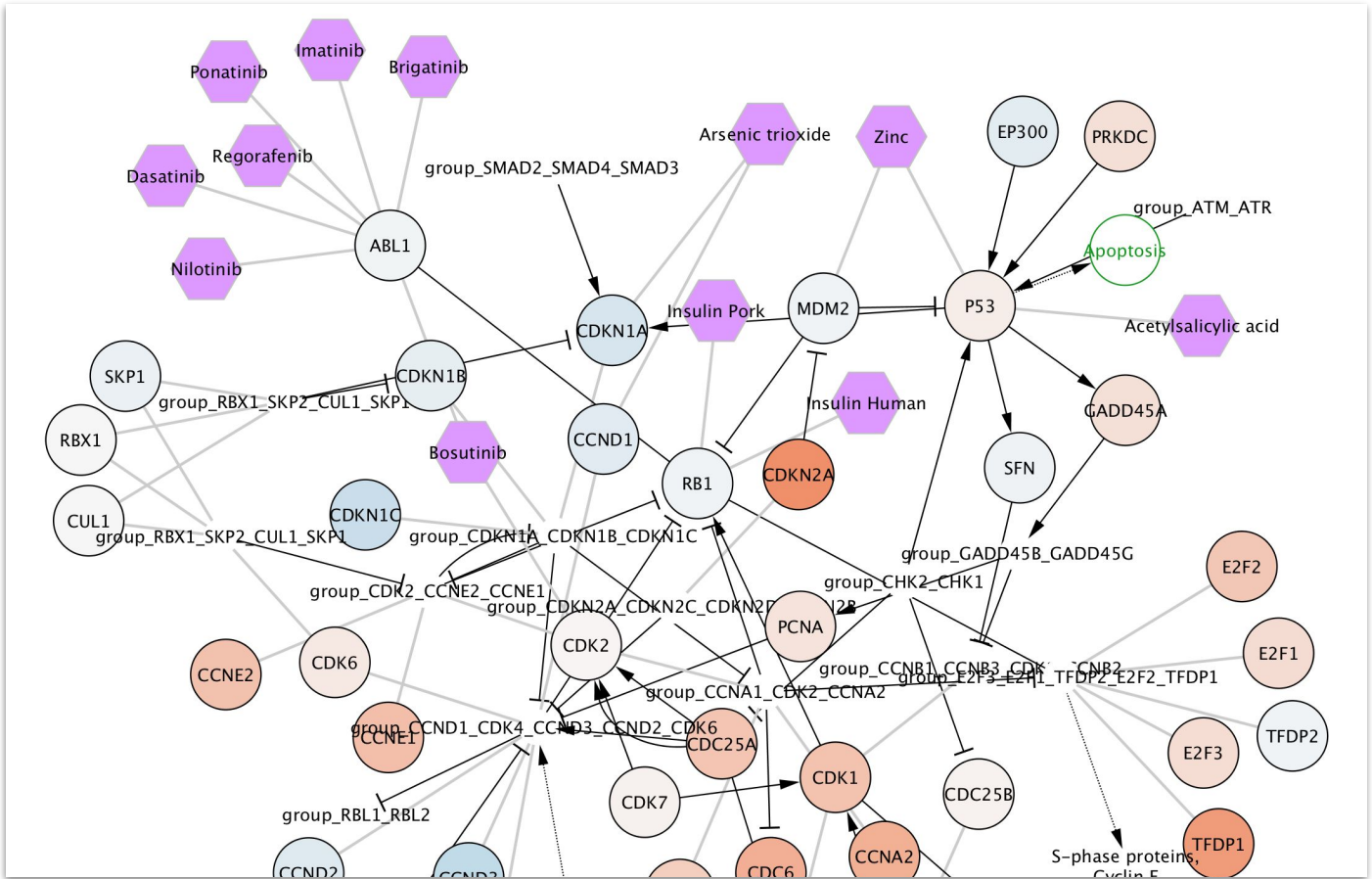
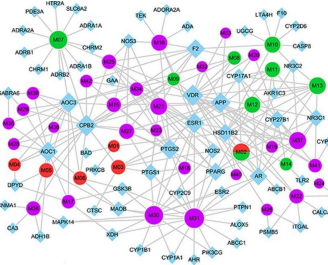


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1	IGK4	ENSG00000117594	4.97014526	5.80E-13	1.02E-1
1	CPFB3	ENSG00000181773	3.07384463	2.37E-06	6.48E-4
1	RPS20P10	ENSG00000233971	3.07366699	1.59E-05	0.0003374
1	RPS23P1	ENSG00000243504	2.87680167	0.0004883	0.0056655
1	RPS34P44	ENSG00000232021	2.87189316	2.02E-05	0.0004315
1	RPS31	ENSG00000171858	1.78201017	5.40E-16	2.76E-3
1	ERGIC3	ENSG00000125991	1.04270392	4.08E-07	1.48E-4
0	ATP9F1	ENSG00000124172	1.0419011	1.74E-07	6.97E-4
1	CDY4	ENSG00000228474	1.04063116	8.61E-06	0.0004071
2	MIR663AHG	ENSG00000227195	1.03864596	1.16E-07	4.98E-4
3	BCR	ENSG00000186716	1.02217198	2.66E-08	1.37E-4
4	HIST1H2AG	ENSG00000198787	1.02057118	9.38E-05	0.0014881
5	SSR2	ENSG00000163479	1.01915311	4.30E-10	3.79E-4
6	EFFA1	ENSG00000136508	-1.01124735	1.08E-07	4.62E-4
7	TMR31B9	ENSG00000116338	-1.00983307	0.017788	0.014905
8	CL12orf57	ENSG00000111678	-1.00950966	0.0009472	0.009422
9	ELOB	ENSG00000103363	-1.9700483	1.64E-05	0.000345
0	B2M	ENSG00000166710	-1.96259859	3.78E-07	1.37E-4
1	EFXK	ENSG00000178982	-2.9694961	2.65E-06	7.50E-4
2	FIBP	ENSG00000172500	-2.95401909	0.0005135	0.0059321
3	SCARNA17	ENSG00000251992	-2.95163973	1.74E-06	5.18E-4
4	HUA9	ENSG00000232745	-2.94688319	6.68E-08	3.10E-4
5	C7orf26	ENSG00000146576	-3.94240445	0.0001522	0.0022255
6	JTB	ENSG00000143543	-3.94165989	1.89E-06	5.00E-4
7	OBSCN	ENSG00000154358	-3.93820271	0.0002976	0.004198
8	TMR31AC	ENSG00000113841	-3.93798301	0.0023993	0.018115
9	ATP5MG	ENSG00000167283	-3.935336083	0.0001484	0.0021777
0	CDX41	ENSG00000131143	-4.919594834	2.23E-06	6.68E-4
1	CCT3	ENSG00000160488	-4.91412063	1.55E-05	0.000331
2	SSR4	ENSG00000186879	-4.9086354	0.0002098	0.002866
3	SNHG22	ENSG00000267822	-4.90693154	9.03E-05	0.001446
4	NDF4A	ENSG00000189043	-5.8793946	1.02E-06	3.31E-4
5	NDF5B	ENSG00000110717	-5.8756324	0.0023468	0.017969
6	ACTB	ENSG00000075624	-5.8670111	4.30E-06	0.000111



- Neuroactive ligand receptor
- Interaction
- Olfactory transduction
- Steroid biosynthesis
- Insulin secretion
- Calcium signaling pathway
- Cell adhesion molecules
- Salivary secretion
- Cholinergic synapse
- Cardiac muscle contraction
- Leukocyte transendothelial migration
- Gastric acid secretion
- Type 1 diabetes mellitus
- Mineral absorption
- Viral myocarditis
- Phenylalanine metabolism

### Drug-Target Interactions

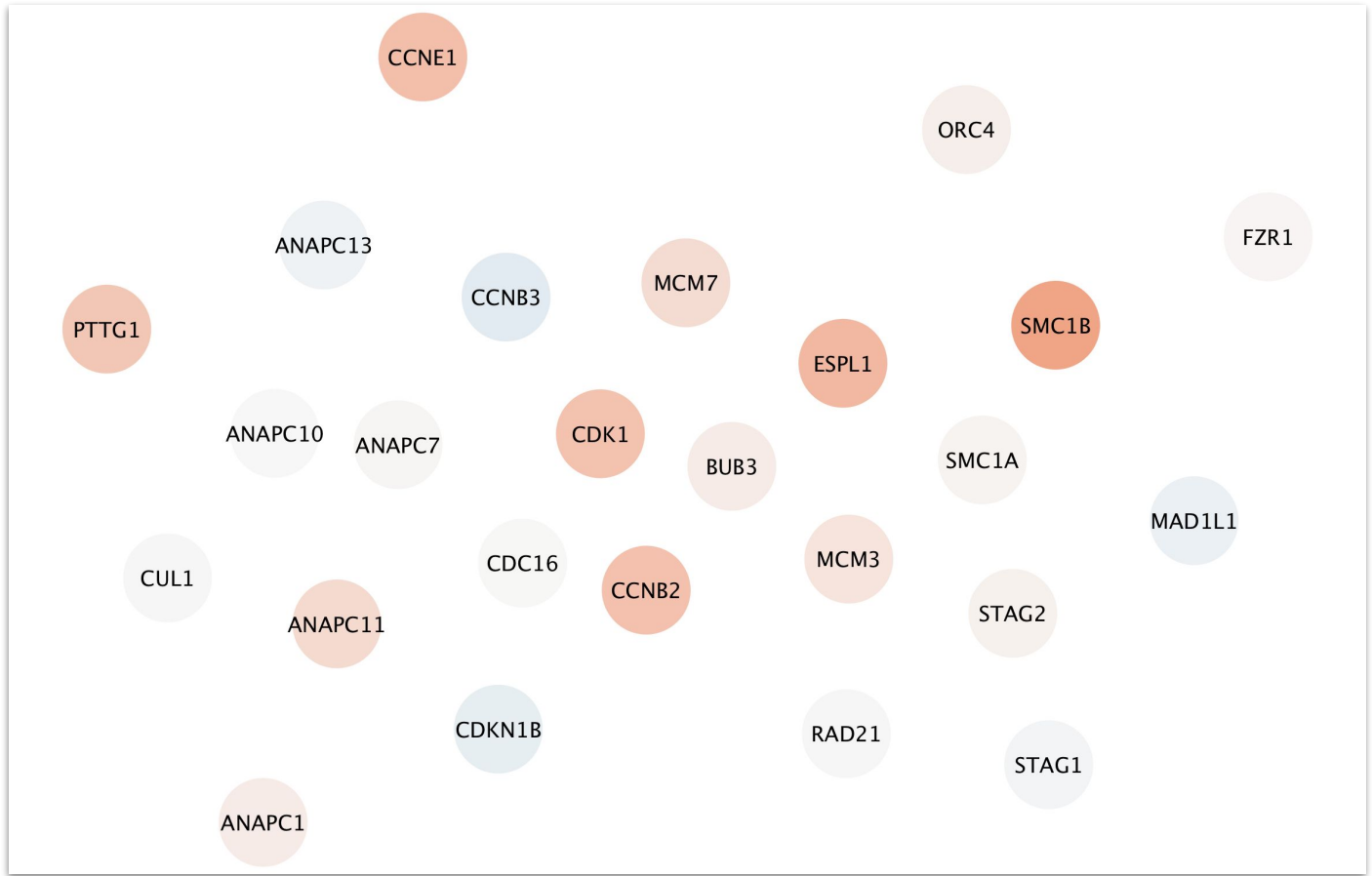
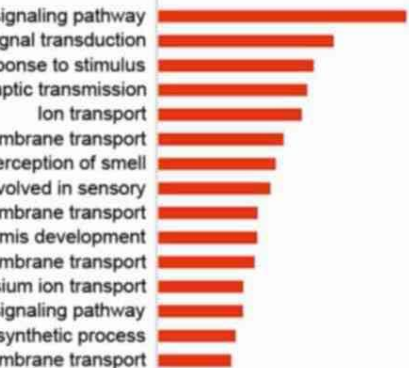


S-phase proteins, Cyclin E

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1	IGFB4	ENSG00000211594	4.57024526	5.80E-13	1.02E-1
1	GPR3	ENSG00000181773	3.07384469	2.37E-06	6.68E-4
1	RPS20P10	ENSG00000233971	3.0736699	1.59E-05	0.0003337
1	RPS23P1	ENSG00000233504	2.8760167	0.0004883	0.0056686
1	RPS1AP44	ENSG00000234031	2.87189316	2.02E-05	0.0004131
1	RPS21	ENSG00000171858	1.78201017	5.40E-16	2.76E-3
1	ENGIC3	ENSG00000123991	1.04270392	4.08E-07	1.48E-4
0	ATP5F4E	ENSG00000134172	1.04139011	1.74E-07	6.97E-4
1	OST4	ENSG00000228474	1.04082316	8.61E-06	0.0002031
2	MIR663AHG	ENSG00000227195	-1.03864596	1.16E-07	4.90E-4
3	BCR	ENSG00000138716	-1.02217198	2.66E-08	1.37E-4
4	HIST1H2AG	ENSG00000196787	-1.02057118	9.38E-05	0.0014883
5	SSR2	ENSG00000163479	-1.01915311	4.30E-10	3.79E-4
6	EFPA1	ENSG00000136508	-1.01124735	1.08E-07	4.62E-4
7	TMEM159	ENSG00000116358	-1.00983307	0.0017788	0.0149883
8	C12orf57	ENSG00000111678	-1.00900966	0.0009472	0.0094221
9	ELOB	ENSG00000103363	-1.9700433	1.64E-05	0.0003453
0	R2M	ENSG00000106710	-1.96259859	3.78E-07	1.37E-4
1	EF3K	ENSG00000178982	-2.9604061	2.65E-06	7.30E-4
2	FBP	ENSG00000172500	-2.95401909	0.0005135	0.0059121
3	SCARNAL7	ENSG00000231992	-2.95163973	1.74E-06	5.28E-4
4	HLA-B	ENSG00000234745	-2.94688319	6.68E-08	3.10E-4
5	C7orf26	ENSG00000146576	-3.94240445	0.0001522	0.0022239
6	JTB	ENSG00000143543	-3.94165989	1.89E-06	5.60E-4
7	OBSN	ENSG00000154835	-3.94020271	0.0003276	0.0041063
8	TMEM14C	ENSG00000111843	-3.93798301	0.0022893	0.0181153
9	ATP5MG	ENSG00000167283	-3.93536083	0.0001484	0.0021277
0	CCNA1	ENSG00000131143	-3.93534634	2.37E-06	6.98E-4
1	CCT3	ENSG00000163468	-4.91242055	1.55E-05	0.0003311
2	SSR4	ENSG00000180879	-4.9086354	0.0002098	0.0028661
3	SNHG22	ENSG00000267322	-4.90693154	9.03E-05	0.0014448
4	NDUF44	ENSG00000138043	-5.879386	1.02E-06	3.31E-4
5	NDUFS8	ENSG00000110717	-5.87363243	0.0022648	0.0179586
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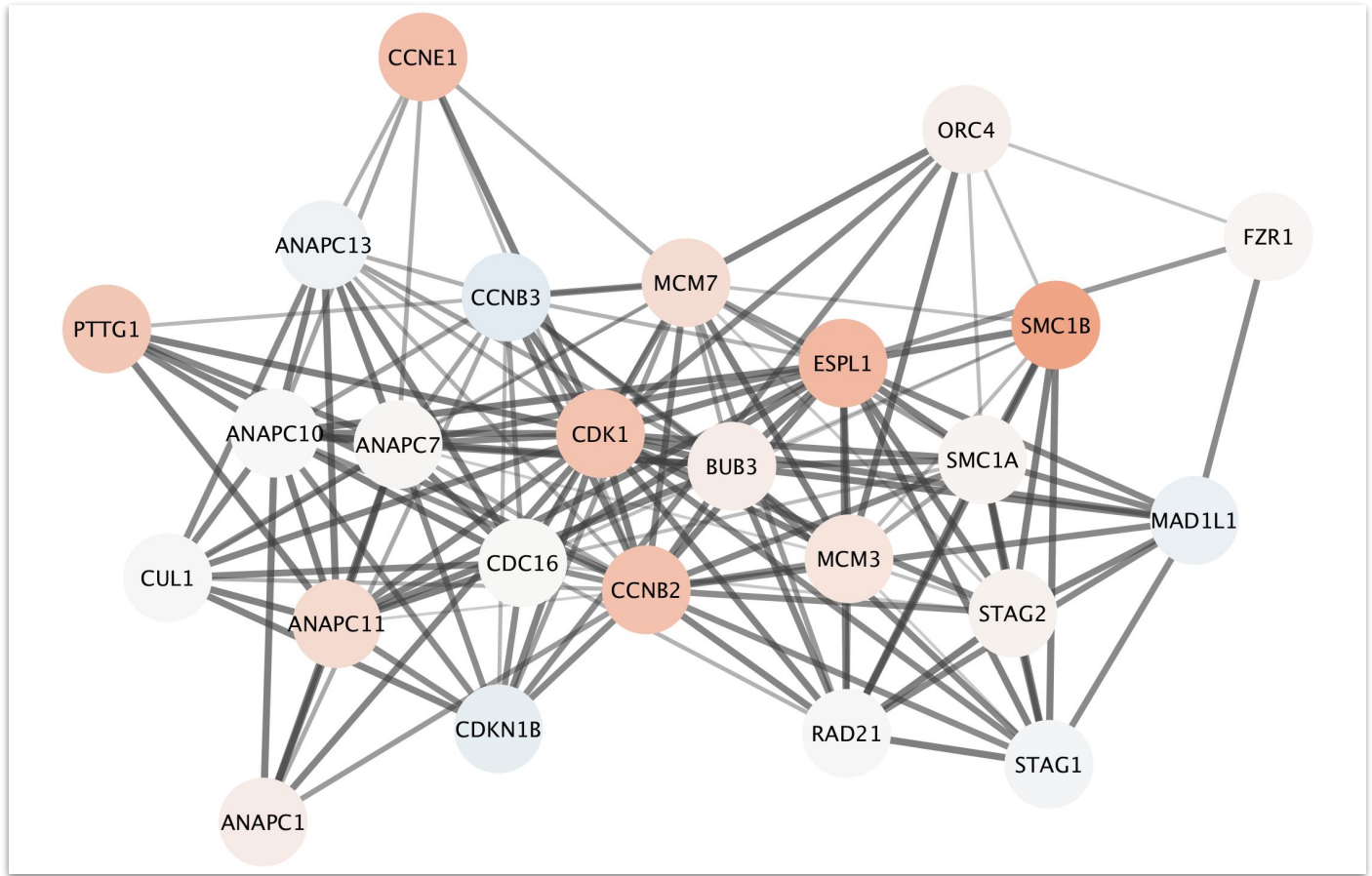
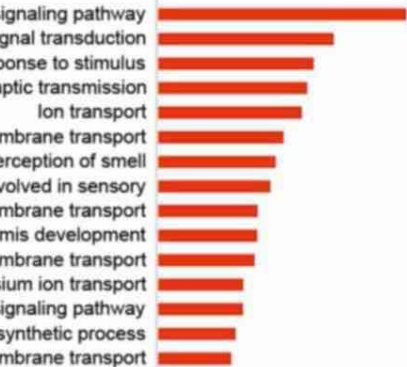
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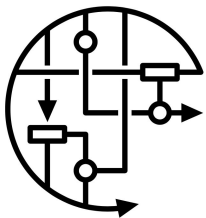


	A	B	C	D	E
1	hgnc_symbol	EnsemblID	logFC	Pvalue	FDR
1	FGFR2	ENSG00000137441	4.95372148	2.07E-11	2.52E-4
1	IGFBP4	ENSG00000211594	4.57024526	5.80E-13	1.02E-5
1	GPR3	ENSG00000181773	3.07384469	2.37E-06	6.68E-4
1	RPS20P10	ENSG00000233971	3.07366699	1.59E-05	0.0003337
1	RPS23P1	ENSG00000233504	2.87801607	0.0004883	0.0056686
1	RPS3AP44	ENSG00000234031	2.87189316	2.02E-05	0.0004131
1	RPS21	ENSG00000171858	1.78201017	5.40E-16	2.76E-5
1	ENGIC3	ENSG00000123991	1.04270392	4.08E-07	1.48E-4
0	ATP5F4E	ENSG00000134172	1.04139011	1.74E-07	6.97E-4
1	OST4	ENSG00000228474	1.04082316	8.61E-06	0.0002031
2	MIR663AHG	ENSG00000227195	-0.03864596	1.16E-07	4.90E-4
3	BCR	ENSG00000138716	-0.02121198	2.66E-08	1.37E-4
4	HIST1H2AG	ENSG00000196787	-0.02057118	9.38E-05	0.0014881
5	SSR2	ENSG00000163479	-1.01915311	4.30E-10	3.79E-4
6	EFPA1	ENSG00000136508	-1.01124735	1.08E-07	4.62E-4
7	TMEM159	ENSG0000011638	-1.00983307	0.0017788	0.0149851
8	C12orf57	ENSG00000111678	-1.00900966	0.0009472	0.0094221
9	ELOB	ENSG00000103363	-1.9700433	1.64E-05	0.0003451
0	R2M	ENSG00000106710	-1.96239859	3.78E-07	1.37E-4
1	EF3K	ENSG00000178982	-2.9604061	2.65E-06	7.30E-4
2	FBP	ENSG00000172500	-2.95401909	0.0005135	0.0059121
3	SCARNAL7	ENSG00000231992	-2.95163973	1.74E-06	5.18E-4
4	HLA-B	ENSG00000234745	-2.94688319	6.68E-08	3.10E-4
5	C7orf26	ENSG00000146576	-3.94240445	0.0001522	0.0022231
6	JTB	ENSG00000143343	-3.94165989	1.89E-06	5.60E-4
7	OBSCN	ENSG00000154345	-3.94020271	0.0003276	0.0041061
8	TMEM14C	ENSG00000111843	-3.93798301	0.0022893	0.0181151
9	ATP5MG	ENSG00000167283	-3.93536083	0.0001484	0.0021771
0	CCNA1	ENSG00000131143	-3.93294684	2.37E-06	6.98E-4
1	CCT3	ENSG00000168468	-4.91242055	1.55E-05	0.0003311
2	SSR4	ENSG00000180879	-4.9086354	0.0002098	0.0028681
3	SNHG22	ENSG00000267322	-4.90693154	9.03E-05	0.0014481
4	NDUF44	ENSG00000189043	-5.879396	1.03E-06	3.31E-4
5	NDUFS8	ENSG00000110717	-5.87563243	0.0022648	0.0179561
6	ACTB	ENSG00000075624	-5.8670115	4.30E-06	0.0001311



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