

Review

The biological pathways of Alzheimer disease: a review

Marco Calabrò, Carmela Rinaldi, Giuseppe Santoro and Concetta Crisafulli*

Department of Biomedical and Dental Sciences and Morphofunctional Imaging, University of Messina, Italy

* **Correspondence:** Email: ccrisafulli@unime.it; Tel: +39 0902213373; Fax: +39 090692449.

Supplementary material

Supplementary Box 1. Pharmacological Treatment

Two categories of drugs are presently used for AD treatment. The Cholinesterase Inhibitors (ChEI), act by increasing the level of acetylcholine, a neurotransmitter important for learning, memory and cognitive functions [1,2]. The latter is designed to block NMDA receptors and to slow down intracellular Ca^{2+} accumulation.

ChEIs

Tacrine: is the first ChEI to be approved specifically for the symptomatic treatment AD (mild to moderate). Tacrine present some adverse effects, in particular related to hepatotoxicity [3] and thus requires baseline and multiple follow-up liver enzyme determinations. This, along with its mutidose administration (10 mg 4 times daily), make it not the first choice for treatment, and limit its use to subjects not tolerating or not responding to other ChEIs. Regarding its pharmacokinetics properties, Tacrine is metabolized by the liver via the cytochrome P450 1A2 isoenzyme system. Therefore, potential interaction with other compounds metabolized by this isoenzymes should be considered. Tacrine should be avoided in patients with liver disease.

Donepezil: is the second ChEI approved for symptomatic treatment of mild to moderate AD [4]. Some adverse effects are present, in particular related to interaction with other compounds, like paroxetine, which increase the frequency of confusion and agitation states in treated AD subjects [5]. Donepezil is largely metabolized by the liver, although some of the dose is recovered in the urine as unchanged drug (11–17%) [6]. It is metabolized by the cytochrome P450 isoenzymes 2D6 and 3A4. Donepezil should be used with caution in subjects severe hepatic or renal disease.

Rivastigmine: This was the third ChEI approved by the FDA for symptomatic treatment of mild to moderate AD in the United States. Rivastigmine should be titrated every 4 weeks, as opposed to every 2 weeks, as recommended when the drug was first made available. Slower titration and taking rivastigmine with a full meal significantly improves tolerability, especially with regard to gastrointestinal side effects. One unique feature of rivastigmine that distinguishes it from other ChEIs is the very low risk of drug interactions in AD patients receiving multiple medications for “real-world” comorbidities [7]. This is because the metabolism of rivastigmine occurs primarily via enzymatic cleavage (hydrolysis) by cholinesterases at the site of action and does not require the cytochrome P450 enzyme system. The starting dose is 1.5 mg twice a day with meals (breakfast and supper), and this dose is increased by 3 mg/day, not faster than every 4 weeks (as tolerated), to a therapeutic dose of 6–12 mg/day. The highest tolerated dose is recommended, as there is some evidence that higher doses may provide greater benefits. A more rapid progression of AD while receiving placebo treatment was predictive of a significantly stronger patient response to rivastigmine therapy on various measures [8]. Laboratory monitoring is not required.

Continued on next page

Supplementary Box 1. Pharmacological Treatment

Galantamine: This was the fourth ChEI approved by the FDA for symptomatic treatment of mild to moderate AD in the United States. Metabolism is hepatic via glucuronidation and the cytochrome P450 isoenzymes 2D6 and 3A4; interactions with other drugs that are metabolized through this pathway are therefore possible. Caution should be used in patients with liver disease. The starting dose is 4 mg twice a day, and this dose is increased every 4 weeks. The therapeutic dose is 16–24 mg/day. A 6-month study showed no additional benefit and a higher rate of side effects with a dose of 32 mg/day [9]. Laboratory monitoring is not required.

NMDA Blockers

Memantine: a noncompetitive, highly voltage- dependent NMDA antagonist, has been approved for use in the treatment of dementia in Germany for over 10 years and recently was approved for use in the treatment of AD in the European Union. It has been found to be useful in more advanced (moderate to severe) cases of AD [10]. Patients with AD in the United States may import the drug or consider participating in double-blind, placebo-controlled studies currently underway at numerous sites all over the country. The FDA is currently reviewing the data for approval of its use in the United States. The recommended starting dose is 5 mg daily, which is increased to 10 mg daily after 4–6 weeks. Morning dosing of donepezil may be preferable in some patients who experience nightmares or insomnia. It may be taken without regard to meals unless gastrointestinal side effects occur, in which case it should be taken with meals. Although there is 1 case report of fulminant hepatitis with the concomitant use of donepezil and sertraline [11], laboratory monitoring of liver enzymes is not required.

Other compounds are also used (with less frequency) for AD treatment [2]. Unfortunately, none of them represent a definitive solution to the pathology.

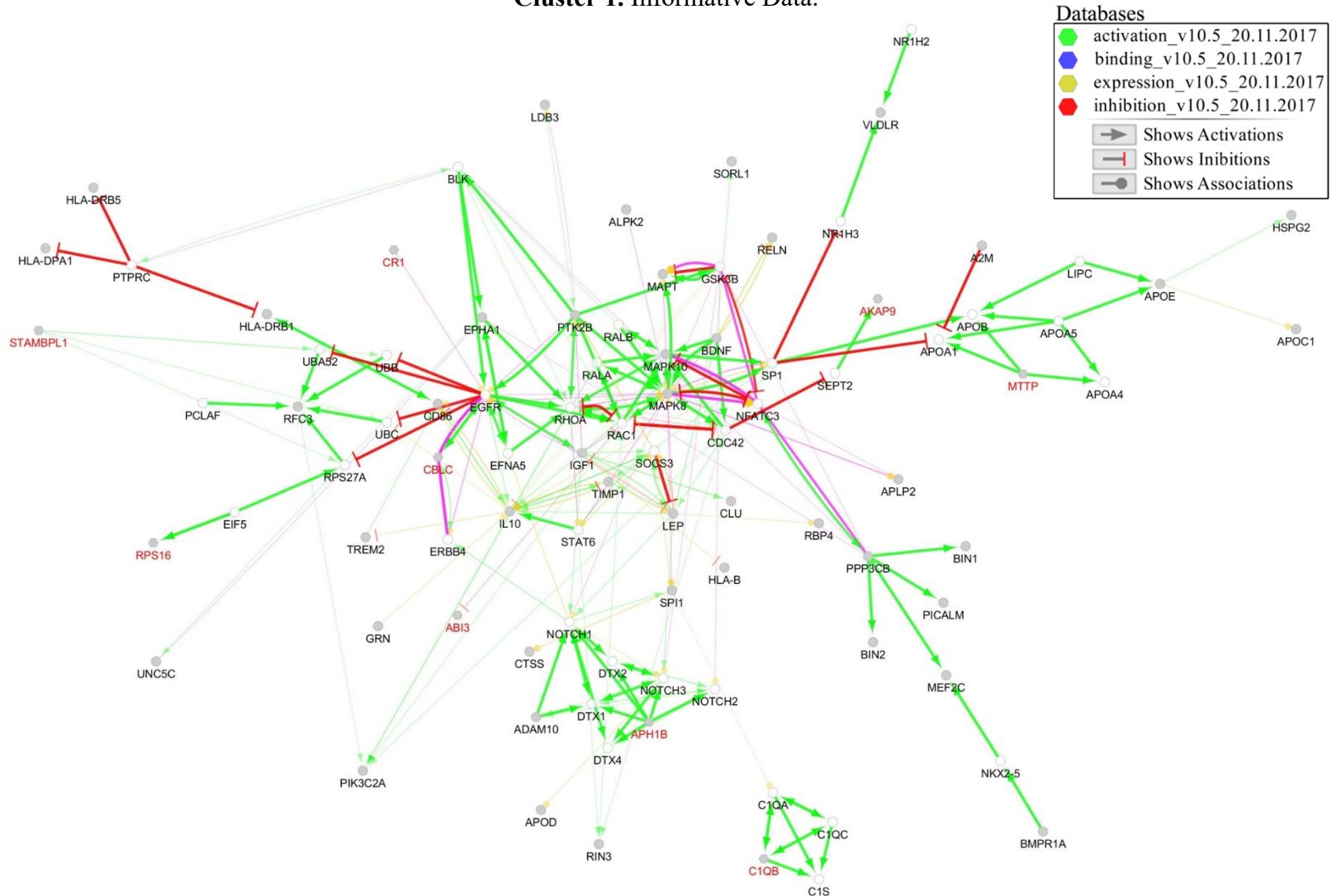
Supplementary Table 1. Genetic associations with Alzheimer's Disease in recent publications.

| Literature Data | Identified Associations |
|------------------------------|---|
| Known familiar genes [12–14] | |
| | Dominant |
| | APP, PSEN1, PSEN2 |
| | Risk |
| | APOE |
| [15] | |
| | Significant (after statistical correction) |
| | ABCA7, AC099552.4, BCAM, CBLC, GAS2L2, IGH3, MS4A6A, PILRA, STAG3, TREM2, ZNF655 |
| | Trend |
| | CBX3, DTNBP1, HLA-DPA1, LDB3, MUC4, NSF, OPRL1, RHBDD1, RPS16, SIRPB1 |
| [16] | |
| | Biomarkers |
| | APLP2, APOC1, ATAD1, BDNF, BMPR1A, CAPN2, CARD8, CTSS, GFRA2, GLRX3, GSN, HMGCS1, IGF1, ITPRIP, MAPJ1, MAPK10, MAPK8, PADI2, PCSK1, PFKFB3, PON2, PPP2R2B, PPP3CB, RBP4, RFC3, SEBPD, SERPINF1, SLC27A2, SMC3, STAMBPL1, TF, TIMP1, TMED10, TNFRSF1A, VLDLR |
| [17] | |
| | Potential AD Drug Targets |
| | A2M, ADAM12, APOD, C1QB, CD86, DNNP, GABRG2, HLA-B, HSPG2, IL-10, LEP, MTTP, NEK4, NOS1AP, PIK3C2A, RELN, TARBP1, TF |
| [18] | |
| | Novel associations |
| | AC074212.3, ADAM10, ADAMTS4, ALPK2, APH1B, CLNK, CNTNAP2, HESX1, HKAT8 |
| | Known AD candidate genes |
| | ABCA7, ABI3, BIN1, BZRAP2-AS1, CASS4, CD2AP, CD33, CLU, CR1, ECHDC3, EPHA1, HLA-DRB1, HS3ST1, INPPD5, MS4A6A, PICALM, PTK2B, SCIMP, SLC24A4, SORL1, SUZ12P1, TREM2, ZCWPW1 |

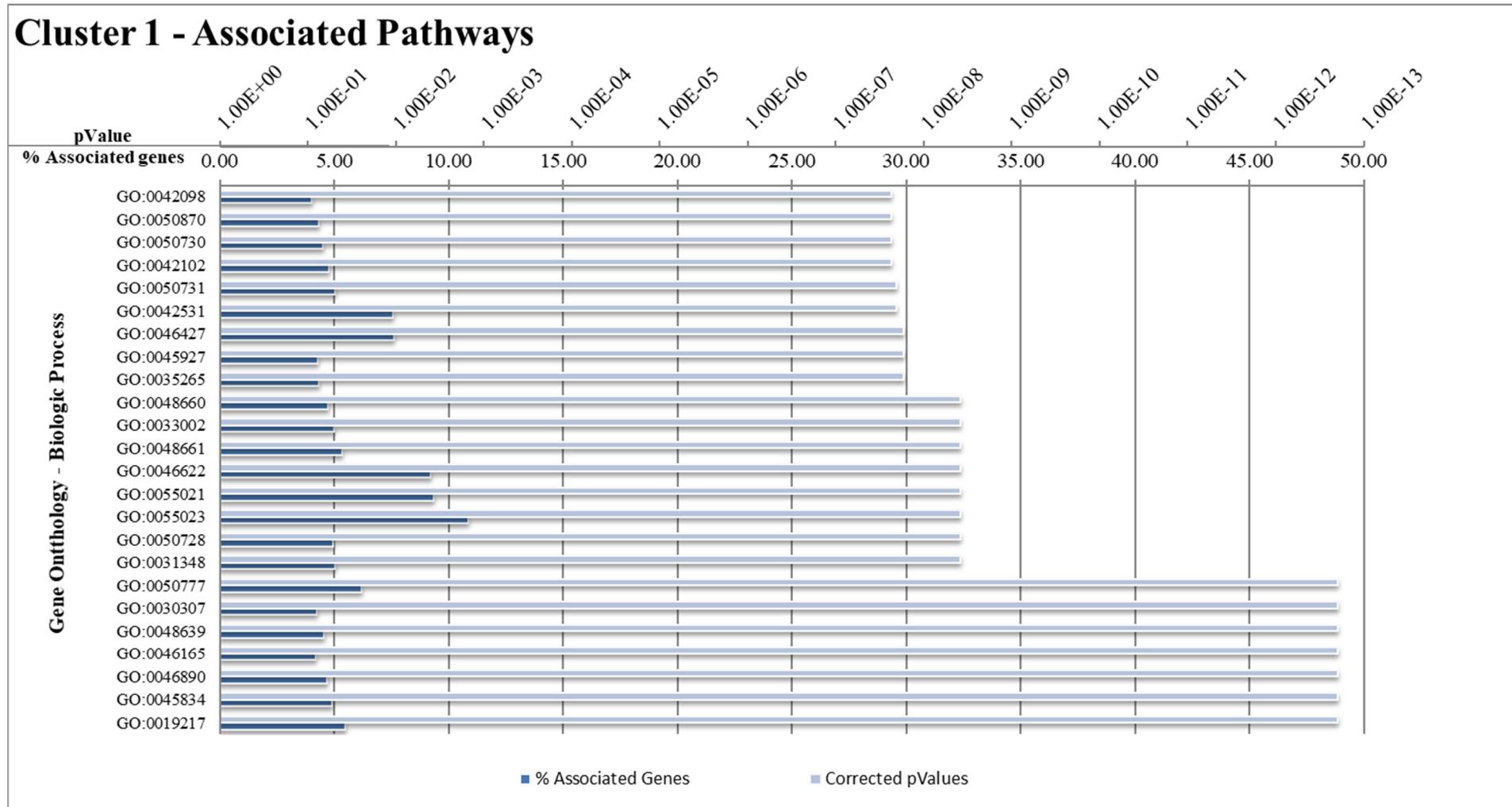
Continued on next page

| Literature Data | Identified Associations |
|-----------------|--|
| [19] | <p>Significant associations</p> <p>FERMT2, PICALM, SLC24A4</p> |
| | <p>Known AD candidate genes</p> <p>ABCA7, AKAP9, BIN1, CASS4, CD2AP, CD33, CELF1, CLU, CR1, EPHA1, GRN, HLA-DRB1, INPP5D, MAPT, MEF2C, MS4A6A, NME8, PLD3, PTK2B, RIN3, SORL1, TREM2, TREML2, ZCWPW1</p> |
| [20] | <p>Associated genes</p> <p>ABCA7, AKAP9, ADAM10, BIN1, CASS4, CD2AP, CD33, CELF1, CLU, CR1, DSG2, EPHA1, FERMT2, HLA-DRB5/DRB1, INPP5D, MEF2C, MS4A6A, NME8, PICALM, PLD3, PTK2B, RIN3, SLC24A4, SORL1, TREM2, UNC5C, ZCWPW1</p> |
| [21] | <p>Associated genes</p> <p>SPI1</p> |

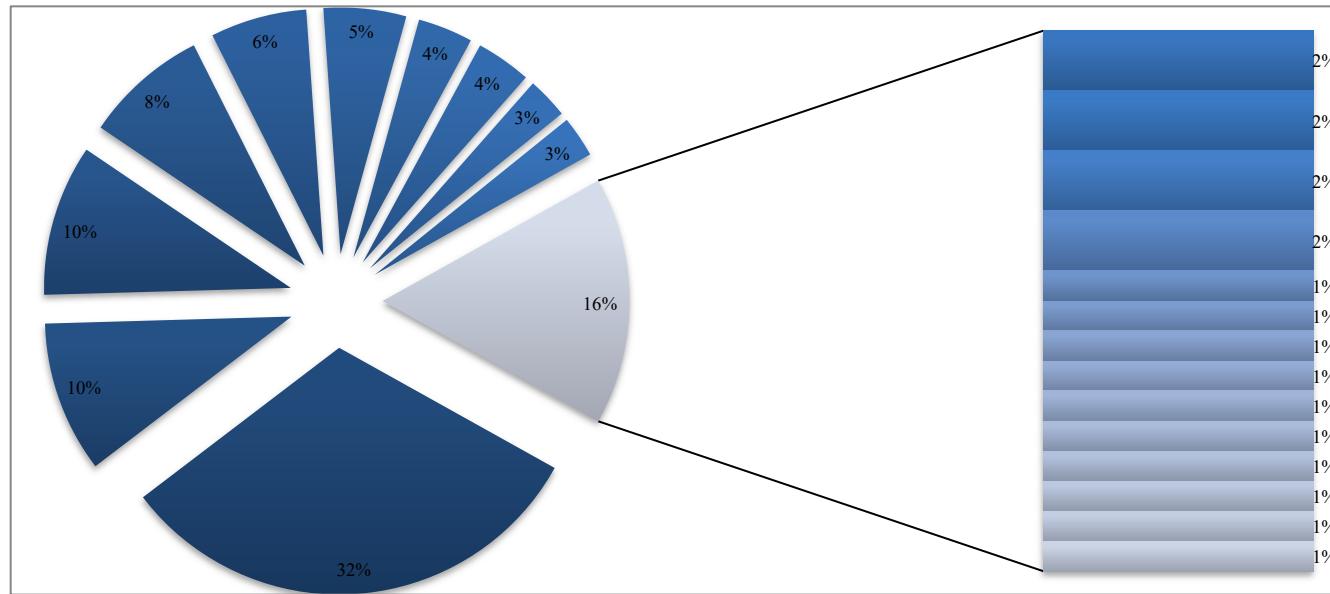
Cluster 1. Informative Data.



Cluster 1. Informative Data.



Cluster 1. Informative Data.



- 31.53% cholesterol transport
- 9.91% positive regulation of supramolecular fiber organization
- 6.31% regulation of peptidyl-tyrosine phosphorylation
- 3.60% regulation of cell size
- 2.70% positive regulation of growth
- 1.80% glial cell differentiation
- 1.80% regulation of nitric-oxide synthase activity
- 0.90% tissue regeneration
- 0.90% animal organ regeneration
- 0.90% positive regulation of small GTPase mediated signal transduction
- 0.90% positive regulation of hormone secretion
- 0.90% cardiac septum development

- 9.91% protein-lipid complex subunit organization
- 8.11% muscle cell proliferation
- 5.41% regulation of transcription from RNA polymerase II promoter in response to hypoxia
- 3.60% blood vessel endothelial cell migration
- 2.70% negative regulation of immune response
- 1.80% Notch signaling pathway
- 1.80% negative regulation of amyloid precursor protein catabolic process
- 0.90% regulation of humoral immune response
- 0.90% regeneration
- 0.90% artery morphogenesis
- 0.90% negative regulation of innate immune response
- 0.90% vasoconstriction

Cluster 1. Informative Data.

| Gene Ontology Identification Number | | Raw pValue | | Corrected pValue* | | % Associated Genes | Nr. Genes |
|-------------------------------------|---|------------|----------|-------------------|----------|--------------------|-----------|
| | | Term | Group | Term | Group | | |
| GO:0042098 | T cell proliferation | 5.31E-07 | 8.28E-15 | 2.55E-05 | 1.99E-13 | 4.02 | 10.00 |
| GO:0050870 | positive regulation of T cell activation | 6.43E-08 | 8.28E-15 | 4.05E-06 | 1.99E-13 | 4.33 | 11.00 |
| GO:0050730 | regulation of peptidyl-tyrosine phosphorylation | 1.15E-10 | 8.28E-15 | 1.00E-08 | 1.99E-13 | 4.52 | 15.00 |
| GO:0042102 | positive regulation of T cell proliferation | 4.36E-05 | 8.28E-15 | 3.49E-04 | 1.99E-13 | 4.76 | 6.00 |
| GO:0050731 | positive regulation of peptidyl-tyrosine phosphorylation | 2.96E-09 | 8.28E-15 | 2.25E-07 | 1.99E-13 | 5.02 | 12.00 |
| GO:0042531 | positive regulation of tyrosine phosphorylation of STAT protein | 2.98E-06 | 8.28E-15 | 1.01E-04 | 1.99E-13 | 7.59 | 6.00 |
| GO:0046427 | positive regulation of JAK-STAT cascade | 5.98E-08 | 8.28E-15 | 3.83E-06 | 1.99E-13 | 7.62 | 8.00 |
| GO:0045927 | positive regulation of growth | 9.82E-10 | 1.66E-10 | 8.06E-08 | 3.82E-09 | 4.29 | 14.00 |
| GO:0035265 | organ growth | 1.05E-06 | 1.66E-10 | 4.43E-05 | 3.82E-09 | 4.35 | 9.00 |
| GO:0048660 | regulation of smooth muscle cell proliferation | 2.30E-06 | 1.66E-10 | 8.50E-05 | 3.82E-09 | 4.73 | 8.00 |
| GO:0033002 | muscle cell proliferation | 3.25E-09 | 1.66E-10 | 2.40E-07 | 3.82E-09 | 4.98 | 12.00 |
| GO:0048661 | positive regulation of smooth muscle cell proliferation | 2.24E-05 | 1.66E-10 | 3.36E-04 | 3.82E-09 | 5.36 | 6.00 |
| GO:0046622 | positive regulation of organ growth | 9.36E-07 | 1.66E-10 | 4.12E-05 | 3.82E-09 | 9.23 | 6.00 |
| GO:0055021 | regulation of cardiac muscle tissue growth | 1.03E-07 | 1.66E-10 | 5.84E-06 | 3.82E-09 | 9.33 | 7.00 |
| GO:0055023 | positive regulation of cardiac muscle tissue growth | 3.50E-06 | 1.66E-10 | 1.05E-04 | 3.82E-09 | 10.87 | 5.00 |

Continued on next page

| Gene Ontology Identification Number | Raw pValue | | Corrected pValue* | | % Associated Genes | Nr. Genes | | |
|-------------------------------------|---|-------|-------------------|----------|--------------------|-----------|------|-------|
| | Term | Group | Term | Group | | | | |
| GO:0050728 | negative regulation of inflammatory response | | 1.67E-06 | 7.72E-10 | 6.53E-05 | 1.70E-08 | 4.94 | 8.00 |
| GO:0031348 | negative regulation of defense response | | 2.96E-09 | 7.72E-10 | 2.25E-07 | 1.70E-08 | 5.02 | 12.00 |
| GO:0050777 | negative regulation of immune response | | 1.60E-09 | 7.72E-10 | 1.25E-07 | 1.70E-08 | 6.18 | 11.00 |
| GO:0030307 | positive regulation of cell growth | | 1.29E-06 | 9.82E-10 | 5.14E-05 | 2.06E-08 | 4.25 | 9.00 |
| GO:0048639 | positive regulation of developmental growth | | 1.62E-07 | 9.82E-10 | 8.77E-06 | 2.06E-08 | 4.57 | 10.00 |
| GO:0046165 | alcohol biosynthetic process | | 5.48E-06 | 1.18E-09 | 1.43E-04 | 2.36E-08 | 4.21 | 8.00 |
| GO:0046890 | regulation of lipid biosynthetic process | | 2.91E-08 | 1.18E-09 | 2.00E-06 | 2.36E-08 | 4.68 | 11.00 |
| GO:0045834 | positive regulation of lipid metabolic process | | 3.93E-07 | 1.18E-09 | 1.96E-05 | 2.36E-08 | 4.89 | 9.00 |
| GO:0019217 | regulation of fatty acid metabolic process | | 3.93E-06 | 1.18E-09 | 1.10E-04 | 2.36E-08 | 5.47 | 7.00 |
| GO:0019218 | regulation of steroid metabolic process | | 6.51E-07 | 1.18E-09 | 3.00E-05 | 2.36E-08 | 5.59 | 8.00 |
| GO:0022600 | digestive system process | | 3.01E-06 | 1.18E-09 | 9.94E-05 | 2.36E-08 | 5.69 | 7.00 |
| GO:0008203 | cholesterol metabolic process | | 3.01E-09 | 1.18E-09 | 2.26E-07 | 2.36E-08 | 5.82 | 11.00 |
| GO:1905952 | regulation of lipid localization | | 9.92E-09 | 1.18E-09 | 7.05E-07 | 2.36E-08 | 6.13 | 10.00 |
| GO:0046889 | positive regulation of lipid biosynthetic process | | 9.74E-07 | 1.18E-09 | 4.19E-05 | 2.36E-08 | 6.73 | 7.00 |
| GO:0031102 | neuron projection regeneration | | 2.10E-05 | 1.18E-09 | 3.37E-04 | 2.36E-08 | 7.58 | 5.00 |
| GO:0034381 | plasma lipoprotein particle clearance | | 2.37E-06 | 1.18E-09 | 8.53E-05 | 2.36E-08 | 7.89 | 6.00 |
| GO:0060193 | positive regulation of lipase activity | | 3.47E-08 | 1.18E-09 | 2.36E-06 | 2.36E-08 | 8.16 | 8.00 |

Continued on next page

| Gene Ontology Identification Number | | Raw pValue | | Corrected pValue* | | % Associated Genes | Nr. Genes |
|-------------------------------------|--|------------|----------|-------------------|----------|--------------------|-----------|
| | | Term | Group | Term | Group | | |
| GO:0060191 | regulation of lipase activity | 5.45E-11 | 1.18E-09 | 4.85E-09 | 2.36E-08 | 8.46 | 11.00 |
| GO:1905953 | negative regulation of lipid localization | 9.36E-06 | 1.18E-09 | 2.15E-04 | 2.36E-08 | 8.93 | 5.00 |
| GO:0030301 | cholesterol transport | 1.56E-11 | 1.18E-09 | 1.41E-09 | 2.36E-08 | 9.48 | 11.00 |
| GO:0070328 | triglyceride homeostasis | 1.69E-07 | 1.18E-09 | 8.96E-06 | 2.36E-08 | 12.24 | 6.00 |
| GO:0033344 | cholesterol efflux | 8.66E-10 | 1.18E-09 | 7.18E-08 | 2.36E-08 | 12.90 | 8.00 |
| GO:0090207 | regulation of triglyceride metabolic process | 7.56E-08 | 1.18E-09 | 4.46E-06 | 2.36E-08 | 13.95 | 6.00 |
| GO:0032374 | regulation of cholesterol transport | 4.32E-10 | 1.18E-09 | 3.71E-08 | 2.36E-08 | 14.04 | 8.00 |
| GO:0034375 | high-density lipoprotein particle remodeling | 7.39E-08 | 1.18E-09 | 4.44E-06 | 2.36E-08 | 22.73 | 5.00 |
| GO:0051004 | regulation of lipoprotein lipase activity | 7.42E-11 | 1.18E-09 | 6.53E-09 | 2.36E-08 | 25.00 | 7.00 |
| GO:0043691 | reverse cholesterol transport | 1.80E-09 | 1.18E-09 | 1.39E-07 | 2.36E-08 | 25.00 | 6.00 |
| GO:0034380 | high-density lipoprotein particle assembly | 4.39E-08 | 1.18E-09 | 2.90E-06 | 2.36E-08 | 25.00 | 5.00 |
| GO:0051006 | positive regulation of lipoprotein lipase activity | 8.67E-09 | 1.18E-09 | 6.24E-07 | 2.36E-08 | 33.33 | 5.00 |
| GO:0010873 | positive regulation of cholesterol esterification | 1.36E-09 | 1.18E-09 | 1.07E-07 | 2.36E-08 | 45.45 | 5.00 |
| GO:0010810 | regulation of cell-substrate adhesion | 1.24E-07 | 3.89E-09 | 6.95E-06 | 7.40E-08 | 4.06 | 11.00 |
| GO:0031032 | actomyosin structure organization | 3.38E-07 | 3.89E-09 | 1.72E-05 | 7.40E-08 | 4.22 | 10.00 |
| GO:0010811 | positive regulation of cell-substrate adhesion | 1.27E-05 | 3.89E-09 | 2.54E-04 | 7.40E-08 | 4.58 | 7.00 |
| GO:1902905 | positive regulation of supramolecular fiber organization | 7.96E-09 | 3.89E-09 | 5.81E-07 | 7.40E-08 | 4.60 | 12.00 |

Continued on next page

| Gene Ontology Identification Number | | Raw pValue | | Corrected pValue* | | % Associated Genes | Nr. Genes |
|-------------------------------------|---|------------|----------|-------------------|----------|--------------------|-----------|
| | | Term | Group | Term | Group | | |
| GO:0034446 | substrate adhesion-dependent cell spreading | 2.02E-05 | 3.89E-09 | 3.44E-04 | 7.40E-08 | 5.45 | 6.00 |
| GO:0043149 | stress fiber assembly | 3.54E-06 | 3.89E-09 | 1.03E-04 | 7.40E-08 | 5.56 | 7.00 |
| GO:0110020 | regulation of actomyosin structure organization | 2.85E-06 | 3.89E-09 | 9.99E-05 | 7.40E-08 | 5.74 | 7.00 |
| GO:0001954 | positive regulation of cell-matrix adhesion | 3.92E-05 | 3.89E-09 | 3.53E-04 | 7.40E-08 | 6.67 | 5.00 |
| GO:0002040 | sprouting angiogenesis | 4.14E-06 | 5.75E-09 | 1.12E-04 | 1.04E-07 | 5.43 | 7.00 |
| GO:0043534 | blood vessel endothelial cell migration | 6.86E-08 | 5.75E-09 | 4.26E-06 | 1.04E-07 | 6.00 | 9.00 |
| GO:1903670 | regulation of sprouting angiogenesis | 5.68E-05 | 5.75E-09 | 2.84E-04 | 1.04E-07 | 6.17 | 5.00 |
| GO:0002042 | cell migration involved in sprouting angiogenesis | 3.01E-05 | 5.75E-09 | 3.91E-04 | 1.04E-07 | 7.04 | 5.00 |
| GO:0016101 | diterpenoid metabolic process | 2.85E-06 | 6.38E-09 | 9.99E-05 | 1.08E-07 | 5.74 | 7.00 |
| GO:0006695 | cholesterol biosynthetic process | 7.24E-06 | 6.38E-09 | 1.74E-04 | 1.08E-07 | 6.52 | 6.00 |
| GO:0015914 | phospholipid transport | 3.45E-06 | 6.38E-09 | 1.07E-04 | 1.08E-07 | 7.41 | 6.00 |
| GO:0006641 | triglyceride metabolic process | 1.02E-09 | 6.38E-09 | 8.26E-08 | 1.08E-07 | 7.75 | 10.00 |
| GO:0097006 | regulation of plasma lipoprotein particle levels | 5.88E-10 | 6.38E-09 | 5.00E-08 | 1.08E-07 | 8.20 | 10.00 |
| GO:0046503 | glycerolipid catabolic process | 9.33E-08 | 6.38E-09 | 5.41E-06 | 1.08E-07 | 9.46 | 7.00 |
| GO:0042632 | cholesterol homeostasis | 1.11E-09 | 6.38E-09 | 8.87E-08 | 1.08E-07 | 9.57 | 9.00 |
| GO:0071825 | protein-lipid complex subunit organization | 3.25E-12 | 6.38E-09 | 2.95E-10 | 1.08E-07 | 13.70 | 10.00 |
| GO:0019433 | triglyceride catabolic process | 7.56E-08 | 6.38E-09 | 4.46E-06 | 1.08E-07 | 13.95 | 6.00 |

Continued on next page

| Gene Ontology Identification Number | | Raw pValue | | Corrected pValue* | | % Associated Genes | Nr. Genes |
|-------------------------------------|---|------------|----------|-------------------|----------|--------------------|-----------|
| | | Term | Group | Term | Group | | |
| GO:0034369 | plasma lipoprotein particle remodeling | 6.22E-10 | 6.38E-09 | 5.22E-08 | 1.08E-07 | 18.92 | 7.00 |
| GO:0034378 | chylomicron assembly | 2.44E-08 | 6.38E-09 | 1.71E-06 | 1.08E-07 | 27.78 | 5.00 |
| GO:0007219 | Notch signaling pathway | 4.10E-08 | 4.10E-08 | 2.74E-06 | 6.55E-07 | 4.53 | 11.00 |
| GO:0008593 | regulation of Notch signaling pathway | 4.56E-05 | 4.10E-08 | 3.19E-04 | 6.55E-07 | 4.72 | 6.00 |
| GO:0031099 | regeneration | 4.84E-08 | 4.84E-08 | 3.15E-06 | 7.26E-07 | 4.45 | 11.00 |
| GO:0010001 | glial cell differentiation | 6.97E-08 | 6.97E-08 | 4.25E-06 | 9.76E-07 | 4.30 | 11.00 |
| GO:0048708 | astrocyte differentiation | 1.17E-05 | 6.97E-08 | 2.58E-04 | 9.76E-07 | 6.00 | 6.00 |
| GO:0051057 | positive regulation of small GTPase mediated signal transduction | 4.22E-07 | 4.22E-07 | 2.07E-05 | 5.49E-06 | 7.61 | 7.00 |
| GO:1902003 | regulation of amyloid-beta formation | 5.43E-07 | 7.45E-07 | 2.55E-05 | 8.93E-06 | 15.63 | 5.00 |
| GO:1902992 | negative regulation of amyloid precursor protein catabolic process | 7.39E-08 | 7.45E-07 | 4.44E-06 | 8.93E-06 | 22.73 | 5.00 |
| GO:0002920 | regulation of humoral immune response | 1.09E-06 | 1.09E-06 | 4.46E-05 | 1.20E-05 | 5.23 | 8.00 |
| GO:0090288 | negative regulation of cellular response to growth factor stimulus | 3.03E-05 | 2.62E-06 | 3.63E-04 | 2.09E-05 | 4.00 | 7.00 |
| GO:0090101 | negative regulation of transmembrane receptor protein serine/threonine kinase signaling pathway | 7.11E-06 | 2.62E-06 | 1.78E-04 | 2.09E-05 | 5.00 | 7.00 |
| GO:0030512 | negative regulation of transforming growth factor beta receptor signaling pathway | 6.39E-05 | 2.62E-06 | 2.56E-04 | 2.09E-05 | 6.02 | 5.00 |

Continued on next page

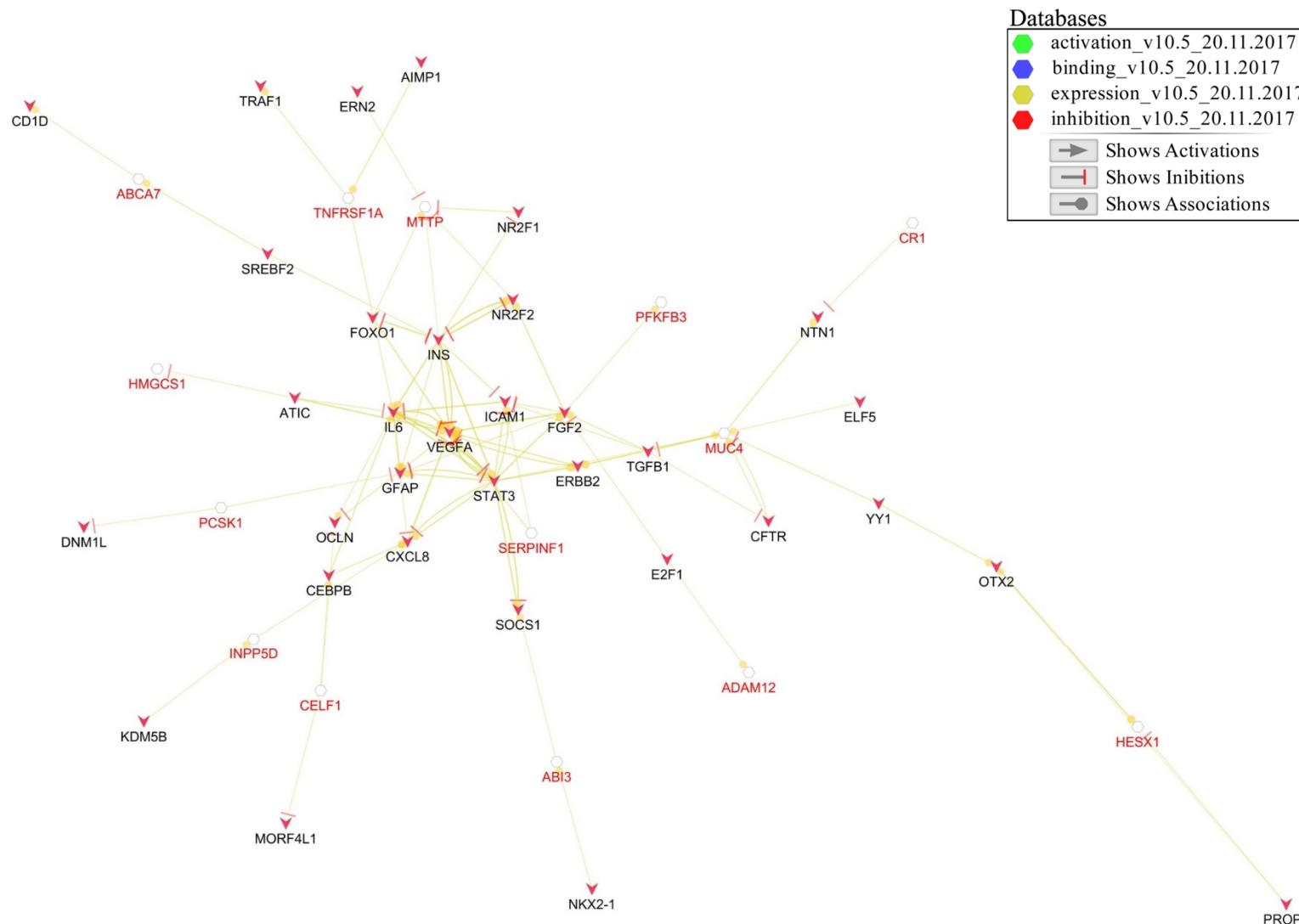
| Gene Ontology Identification Number | | Raw pValue | | Corrected pValue* | | % Associated Genes | Nr. Genes |
|-------------------------------------|--|------------|----------|-------------------|----------|--------------------|-----------|
| | | Term | Group | Term | Group | | |
| GO:0019985 | translesion synthesis | 3.06E-07 | 2.62E-06 | 1.59E-05 | 2.09E-05 | 11.11 | 6.00 |
| GO:0006296 | nucleotide-excision repair, DNA incision, 5'-to lesion | 3.13E-06 | 2.62E-06 | 1.00E-04 | 2.09E-05 | 11.11 | 5.00 |
| GO:0061418 | regulation of transcription from RNA polymerase II promoter in response to hypoxia | 1.49E-07 | 2.62E-06 | 8.20E-06 | 2.09E-05 | 12.50 | 6.00 |
| GO:0008361 | regulation of cell size | 7.27E-07 | 2.11E-06 | 3.27E-05 | 2.11E-05 | 4.55 | 9.00 |
| GO:0001952 | regulation of cell-matrix adhesion | 1.84E-06 | 2.11E-06 | 6.97E-05 | 2.11E-05 | 4.88 | 8.00 |
| GO:0061387 | regulation of extent of cell growth | 3.47E-05 | 2.11E-06 | 3.47E-04 | 2.11E-05 | 4.96 | 6.00 |
| GO:0048041 | focal adhesion assembly | 2.36E-05 | 2.11E-06 | 3.30E-04 | 2.11E-05 | 5.31 | 6.00 |
| GO:0048844 | artery morphogenesis | 2.37E-06 | 2.37E-06 | 8.53E-05 | 2.13E-05 | 7.89 | 6.00 |
| GO:0045824 | negative regulation of innate immune response | 1.32E-05 | 1.32E-05 | 2.50E-04 | 7.90E-05 | 8.33 | 5.00 |
| GO:0046887 | positive regulation of hormone secretion | 1.22E-05 | 1.22E-05 | 2.56E-04 | 8.53E-05 | 4.61 | 7.00 |
| GO:0051341 | regulation of oxidoreductase activity | 3.31E-05 | 3.31E-05 | 3.64E-04 | 1.66E-04 | 5.00 | 6.00 |
| GO:0050999 | regulation of nitric-oxide synthase activity | 1.95E-05 | 3.31E-05 | 3.52E-04 | 1.66E-04 | 7.69 | 5.00 |
| GO:0042246 | tissue regeneration | 4.74E-05 | 4.74E-05 | 2.84E-04 | 1.90E-04 | 6.41 | 5.00 |
| GO:0003279 | cardiac septum development | 6.68E-05 | 6.68E-05 | 2.00E-04 | 2.00E-04 | 4.41 | 6.00 |

Continued on next page

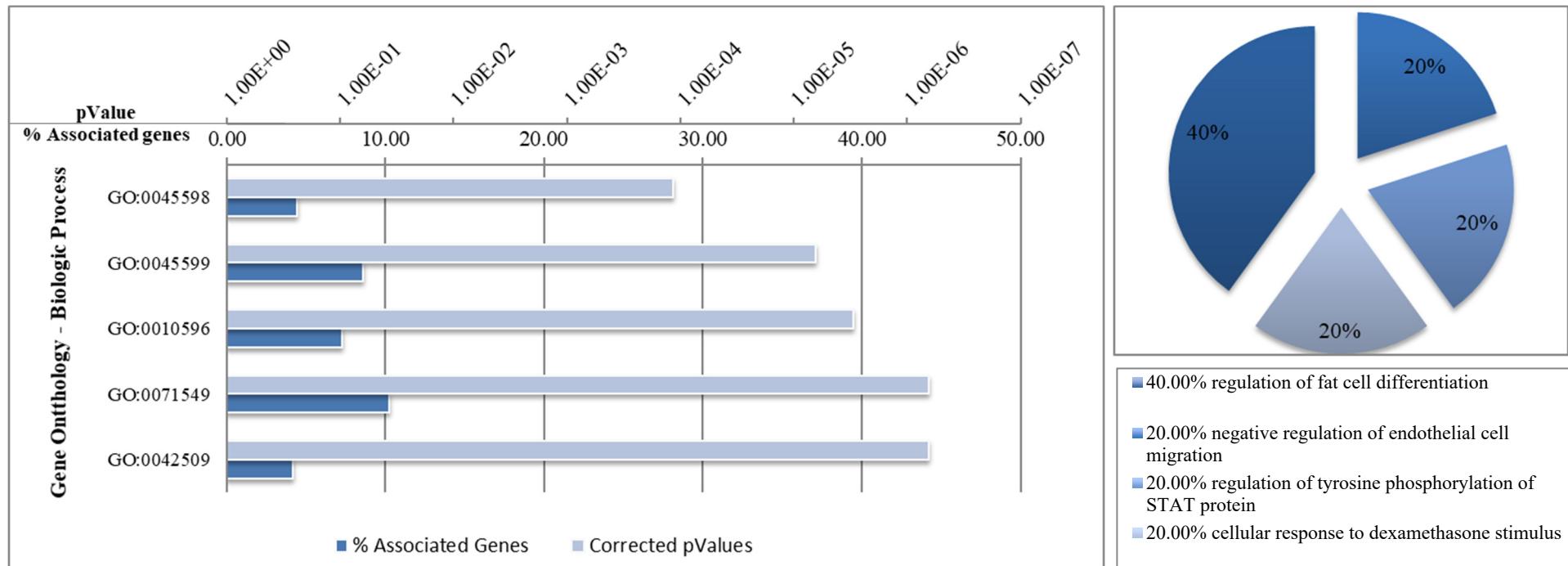
| Gene Ontology Identification Number | | Raw pValue | | Corrected pValue* | | % Associated Genes | Nr. Genes |
|-------------------------------------|---------------------------|------------|----------|-------------------|----------|--------------------|-----------|
| | | Term | Group | Term | Group | | |
| GO:0031100 | animal organ regeneration | 3.76E-04 | 3.76E-04 | 3.76E-04 | 3.76E-04 | 4.13 | 5.00 |
| GO:0042310 | vasoconstriction | 2.22E-04 | 2.22E-04 | 4.44E-04 | 4.44E-04 | 4.63 | 5.00 |

Note: The figure shows the enriched Cluster 1, derived from the initial selection of genes. Cluster 1 depicts the interacting genes and the type of interaction as shown in the figure legend. The Bar Chart associated with cluster 1 reports the percentage of genes belonging to a specific biological process (from Gene Ontology database) included in the pathway under analysis. It also shows the associations levels of biological processes with the cluster under analysis. The pie chart shows the overall degree of association of main biological processes involved with the cluster under investigation. To note, both the bar and pie chart report the top 25 biological processes. The table summarizes the details regarding the pathway analysis and shows all of the associated biological processes linked with the cluster under investigation.

Cluster 2. Informative Data.



Cluster 2. Informative Data.

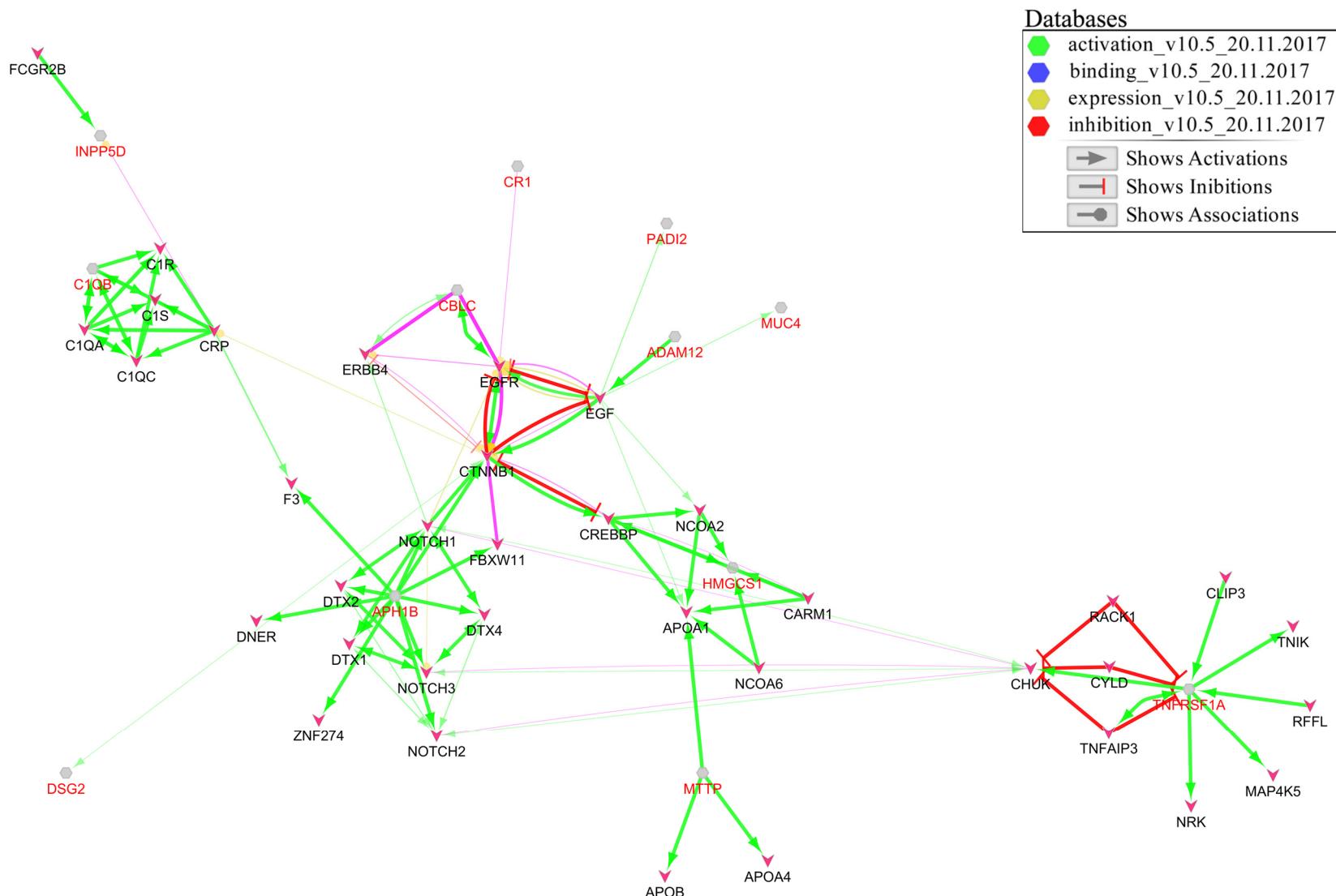


Cluster 2. Informative Data.

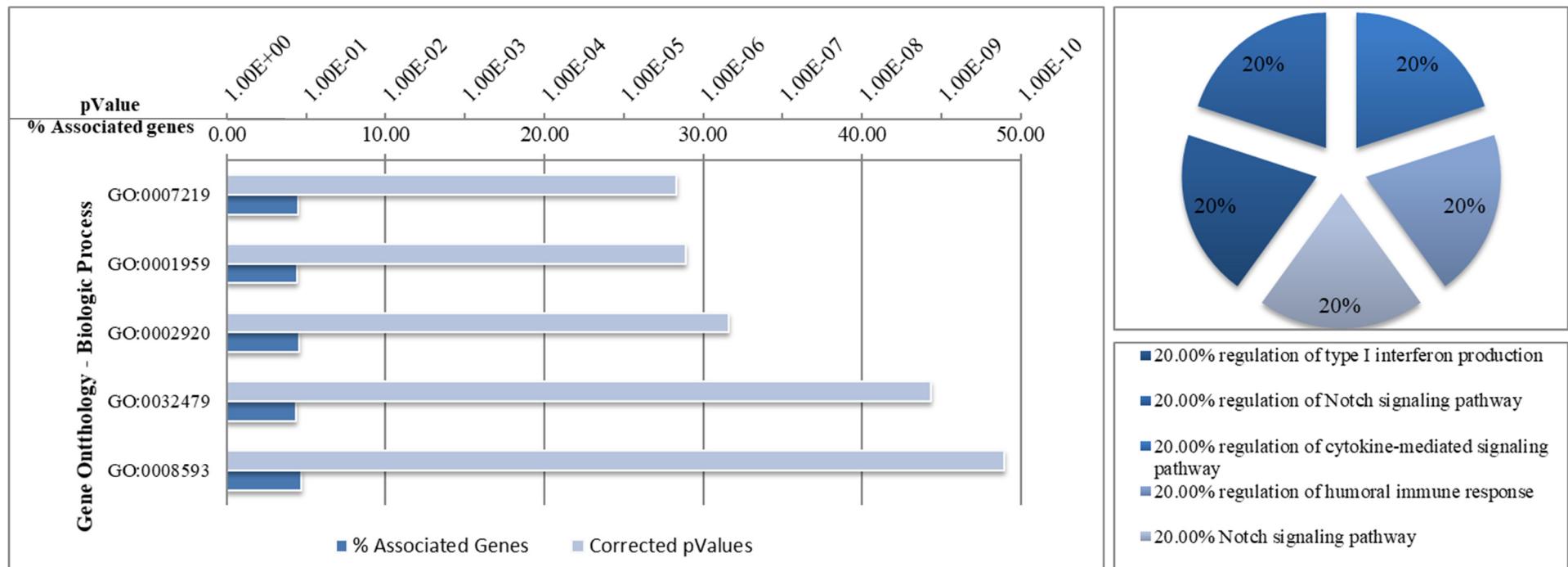
| Gene Ontology Identification Number | regulation of fat cell differentiation | Raw pValue | | Corrected pValue* | | % Associated Genes | Nr. Genes |
|-------------------------------------|--|------------|----------|-------------------|----------|--------------------|-----------|
| | | Term | Group | Term | Group | | |
| GO:0045598 | regulation of fat cell differentiation | 1.61E-07 | 8.06E-07 | 1.61E-07 | 6.45E-07 | 4.46 | 7.00 |
| GO:0045599 | negative regulation of fat cell differentiation | 4.09E-07 | 1.64E-06 | 1.61E-07 | 6.45E-07 | 8.62 | 5.00 |
| GO:0010596 | negative regulation of endothelial cell migration | 9.83E-07 | 2.95E-06 | 9.83E-07 | 2.95E-06 | 7.25 | 5.00 |
| GO:0071549 | cellular response to dexamethasone stimulus | 3.18E-06 | 6.36E-06 | 3.18E-06 | 6.36E-06 | 10.26 | 4.00 |
| GO:0042509 | regulation of tyrosine phosphorylation of STAT protein | 1.15E-04 | 1.15E-04 | 1.15E-04 | 1.15E-04 | 4.17 | 4.00 |

The figure shows the enriched Cluster 2, derived from the initial selection of genes, after exclusion of the genes involved in cluster 1. Cluster 2 depicts the interacting genes and the type of interaction as shown in the figure legend. The Bar Chart associated with cluster 2 reports the percentage of genes belonging to a specific biological process (from Gene Ontology database) included in the pathway under analysis. It also shows the associations levels of biological processes with the cluster under analysis. The pie chart shows the overall degree of association of main biological processes involved with the cluster under investigation. Cluster 2 genes seems to be mainly implicated with fat regulative processes. To note GO:0045599 - negative regulation of fat cell differentiation is a sub process of GO:0045598 - regulation of fat cell differentiation. Only main biological processes are represented in the pie chart. The table summarizes the details regarding the pathway analysis and shows all of the associated biological processes linked with the cluster under investigation.

Cluster 3. Informative Data.



Cluster 3. Informative Data.

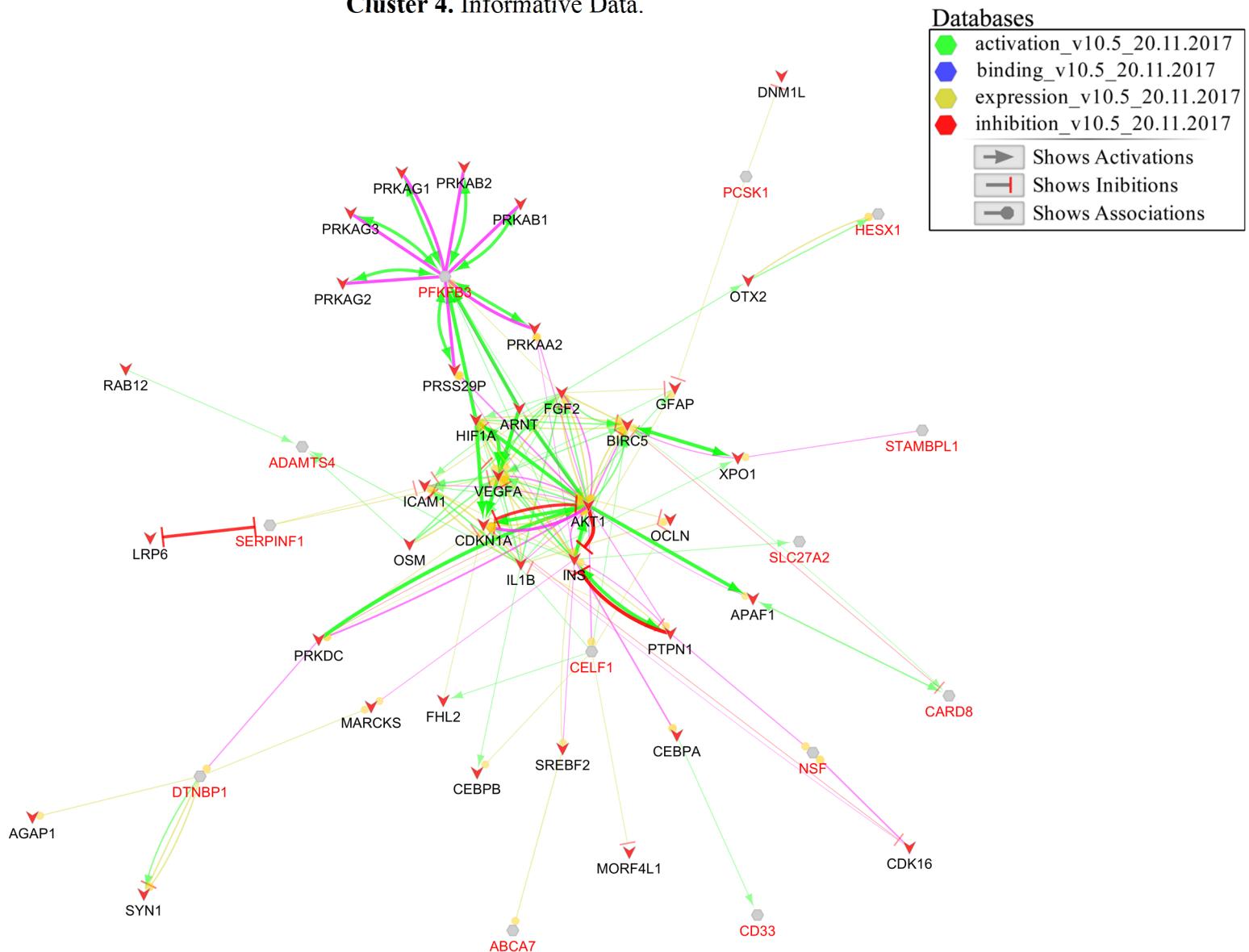


Cluster 3. Informative Data.

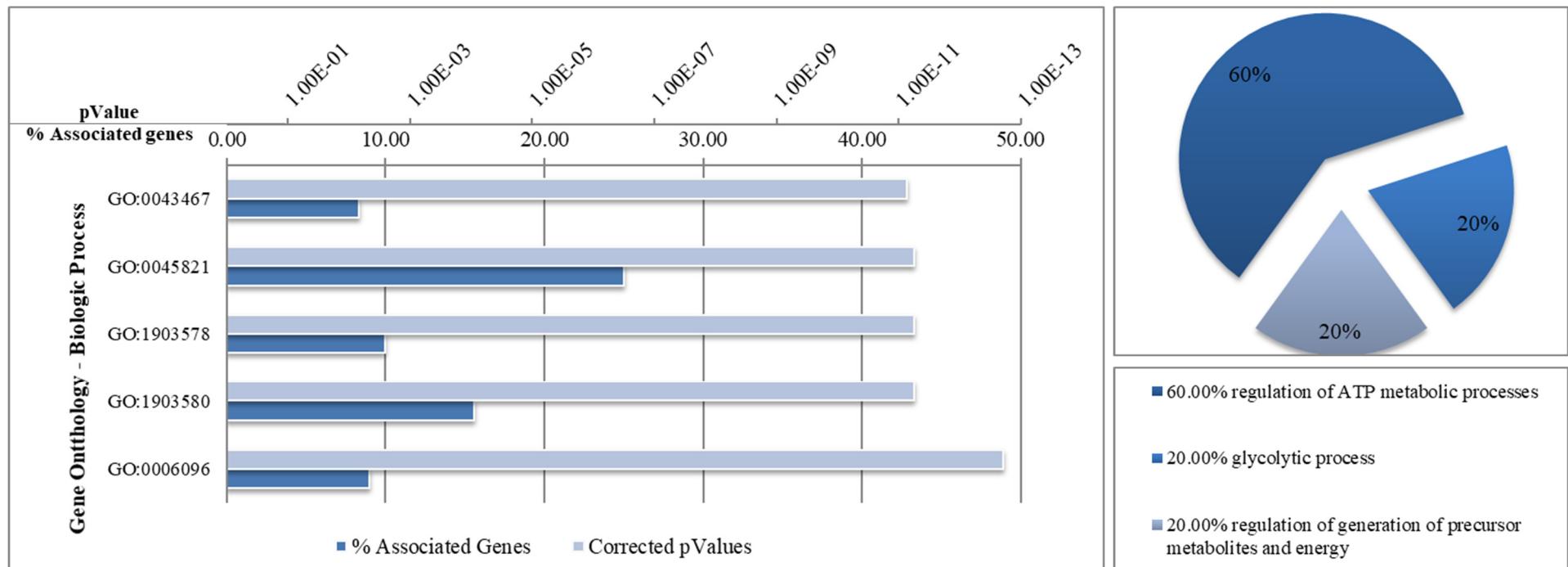
| Gene Ontology Identification Number | | Raw pValue | | Corrected pValue* | | % Associated Genes | Nr. Genes |
|-------------------------------------|---|------------|----------|-------------------|----------|--------------------|-----------|
| | | Term | Group | Term | Group | | |
| GO:0007219 | Notch signaling pathway | 3.26E-11 | 3.26E-11 | 1.63E-10 | 1.63E-10 | 4.53 | 11 |
| GO:0001959 | regulation of cytokine-mediated signaling pathway | 3.40E-10 | 3.40E-10 | 1.36E-09 | 1.36E-09 | 4.44 | 10 |
| GO:0002920 | regulation of humoral immune response | 1.57E-07 | 1.57E-07 | 4.71E-07 | 4.71E-07 | 4.58 | 7 |
| GO:0032479 | regulation of type I interferon production | 1.68E-06 | 1.68E-06 | 1.68E-06 | 1.68E-06 | 4.38 | 6 |
| GO:0008593 | regulation of Notch signaling pathway | 1.08E-06 | 1.08E-06 | 2.15E-06 | 2.15E-06 | 4.72 | 6 |

The figure shows the enriched Cluster 3, derived from the initial selection of genes, after exclusion of previous clusters. Cluster 3 depicts the interacting genes and the type of interaction as shown in the figure legend. The Bar Chart associated with cluster 3 reports the percentage of genes belonging to a specific biological process (from Gene Ontology database) included in the pathway under analysis. It also shows the associations levels of biological processes with the cluster under analysis. The pie chart shows the overall degree of association of main biological processes involved with the cluster under investigation. The table summarizes the details regarding the pathway analysis and shows all of the associated biological processes linked with the cluster under investigation.

Cluster 4. Informative Data.



Cluster 4. Informative Data.

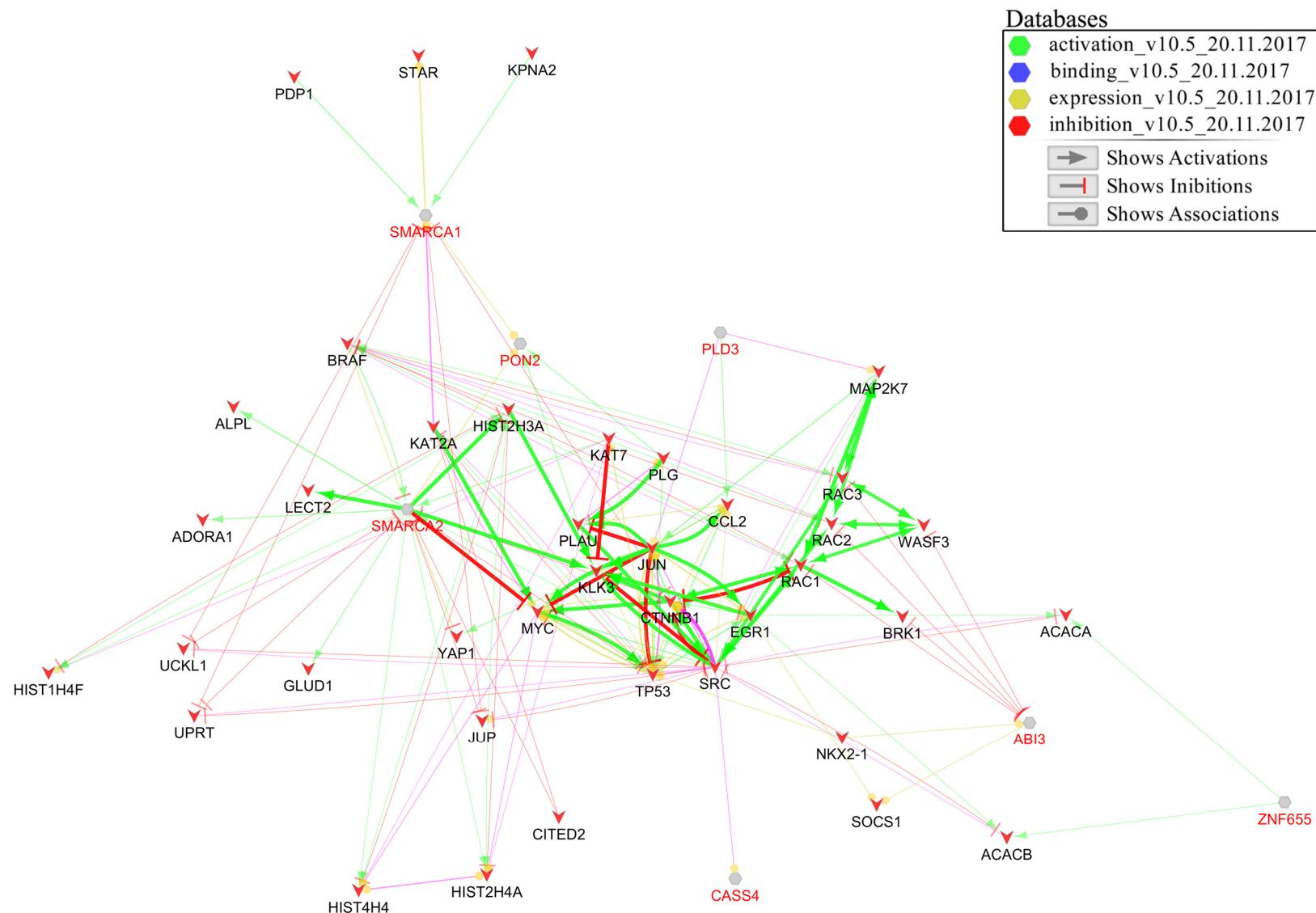


Cluster 4. Informative Data.

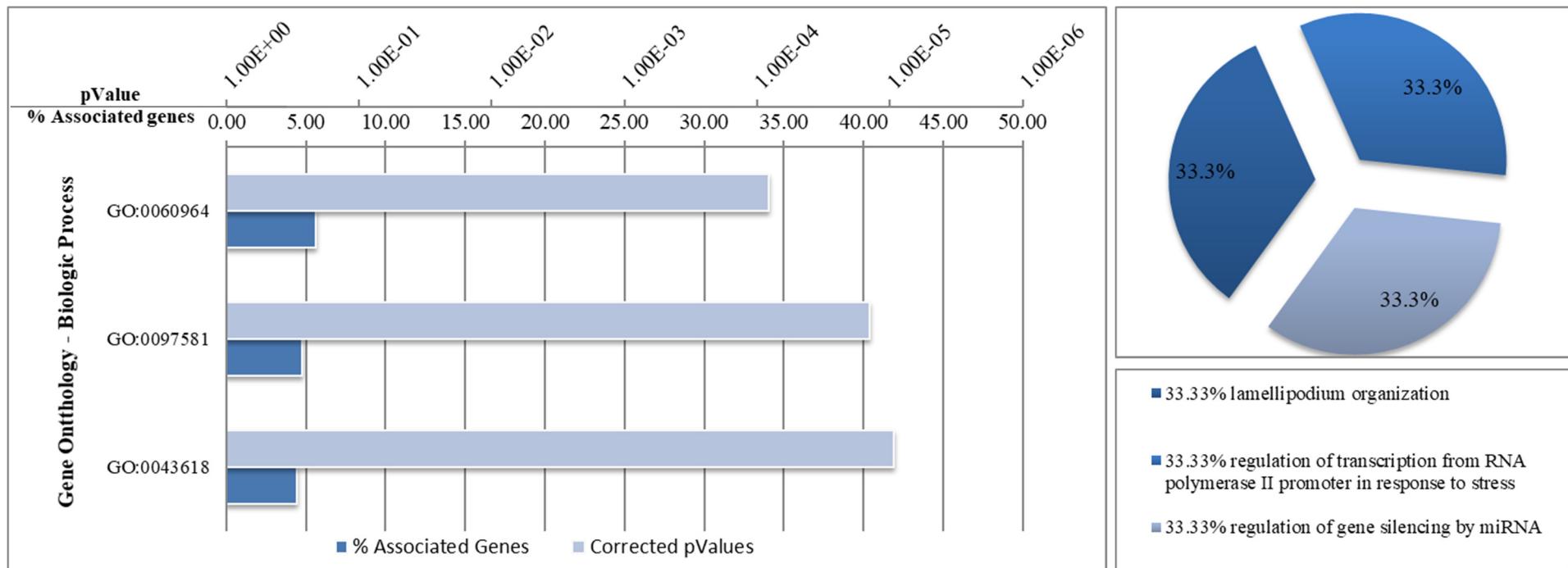
| Gene Ontology Identification Number | regulation of generation of precursor metabolites and energy | Raw pValue | | Corrected pValue* | | % Associated Genes | Nr. Genes |
|-------------------------------------|--|------------|----------|-------------------|----------|--------------------|-----------|
| | | Term | Group | Term | Group | | |
| GO:0043467 | regulation of generation of precursor metabolites and energy | 6.56E-14 | 6.56E-14 | 3.28E-13 | 1.97E-13 | 8.33 | 11 |
| GO:0045821 | positive regulation of glycolytic process | 4.45E-11 | 2.83E-12 | 4.45E-11 | 5.66E-12 | 25.00 | 6 |
| GO:1903578 | regulation of ATP metabolic process | 2.83E-12 | 2.83E-12 | 1.13E-11 | 5.66E-12 | 10.00 | 9 |
| GO:1903580 | positive regulation of ATP metabolic process | 3.53E-11 | 2.83E-12 | 7.06E-11 | 5.66E-12 | 15.56 | 7 |
| GO:0006096 | glycolytic process | 7.46E-12 | 7.46E-12 | 2.24E-11 | 7.46E-12 | 9.00 | 9 |

The figure shows the enriched Cluster 4, derived from the initial selection of genes, after exclusion of previous clusters. Cluster 4 depicts the interacting genes and the type of interaction as shown in the figure legend. The Bar Chart associated with cluster 4 reports the percentage of genes belonging to a specific biological process (from Gene Ontology database) included in the pathway under analysis. It also shows the associations levels of biological processes with the cluster under analysis. The pie chart shows the overall degree of association of main biological processes involved with the cluster under investigation. To note, only main biological processes are represented in the pie chart. The table summarizes the details regarding the pathway analysis and shows all of the associated biological processes linked with the cluster under investigation.

Cluster 5. Informative Data.



Cluster 5. Informative Data.

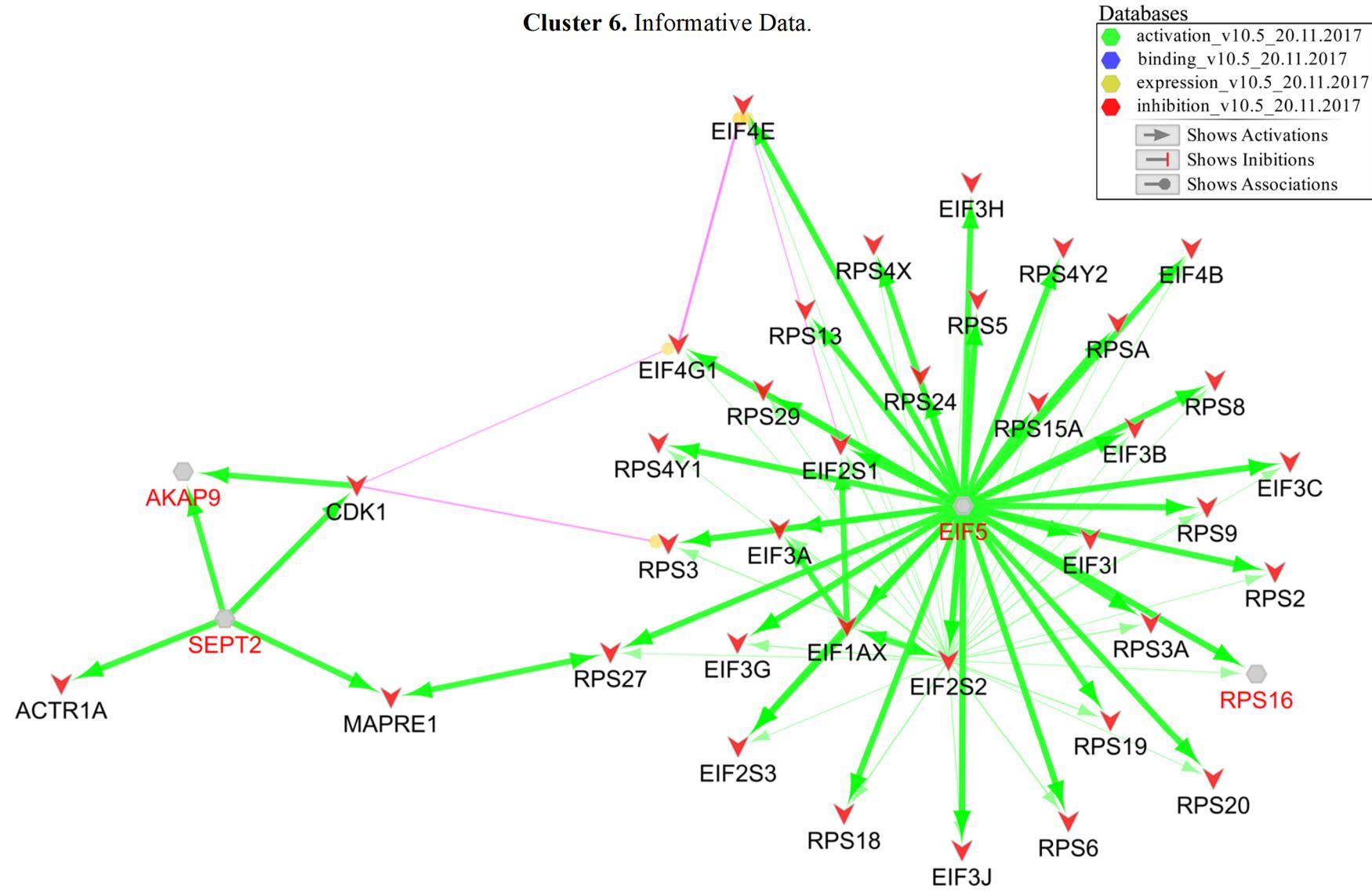


Cluster 5. Informative Data.

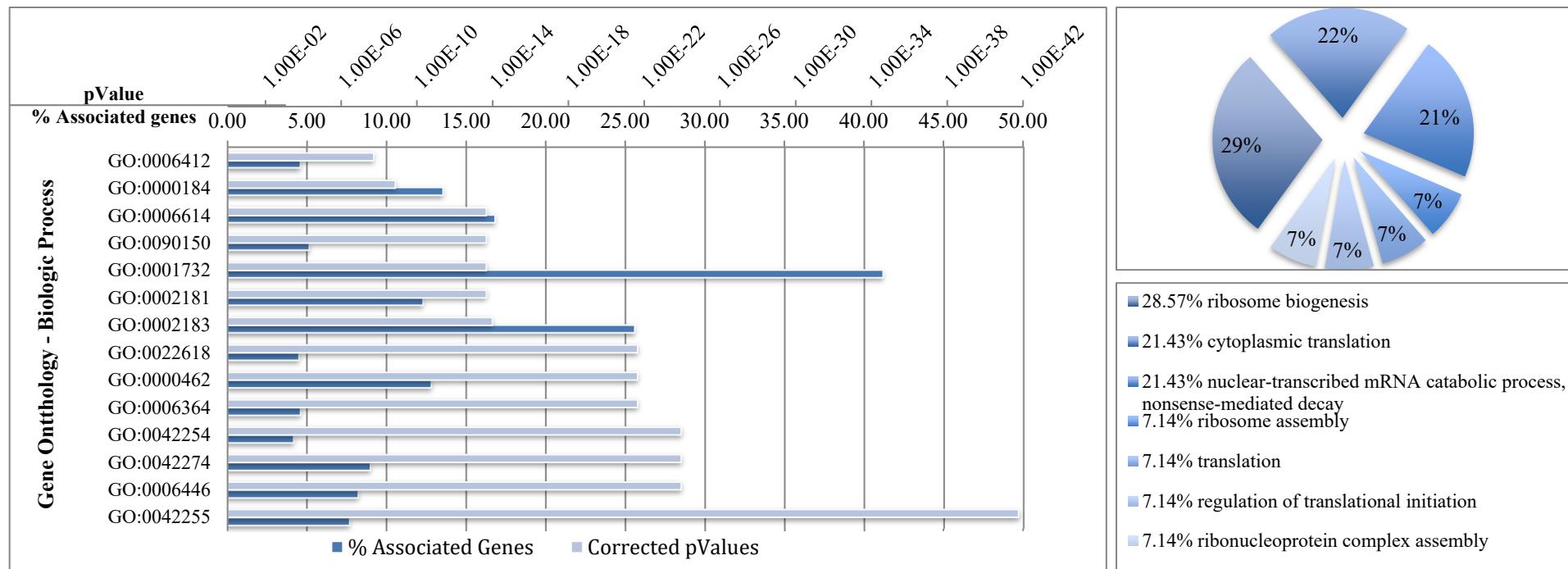
| Gene Ontology Identification Number | regulation of gene silencing by miRNA | Raw pValue | | Corrected pValue* | | % Associated Genes | Nr. Genes |
|-------------------------------------|---|------------|----------|-------------------|----------|--------------------|-----------|
| | | Term | Group | Term | Group | | |
| GO:0060964 | regulation of gene silencing by miRNA | 3.13E-06 | 3.13E-06 | 9.40E-06 | 9.40E-06 | 5.62 | 5 |
| GO:0097581 | lamellipodium organization | 7.07E-06 | 7.07E-06 | 1.41E-05 | 1.41E-05 | 4.76 | 5 |
| GO:0043618 | regulation of transcription from RNA polymerase II promoter in response to stress | 8.23E-05 | 8.23E-05 | 8.23E-05 | 8.23E-05 | 4.44 | 4 |

The figure shows the enriched Cluster 5, derived from the initial selection of genes, after exclusion of previous clusters. Cluster 5 depicts the interacting genes and the type of interaction as shown in the figure legend. The Bar Chart associated with cluster 5 reports the percentage of genes belonging to a specific biological process (from Gene Ontology database) included in the pathway under analysis. It also shows the associations levels of biological processes with the cluster under analysis. The pie chart shows the overall degree of association of main biological processes involved with the cluster under investigation. The table summarizes the details regarding the pathway analysis and shows all of the associated biological processes linked with the cluster under investigation.

Cluster 6. Informative Data.



Cluster 6. Informative Data.



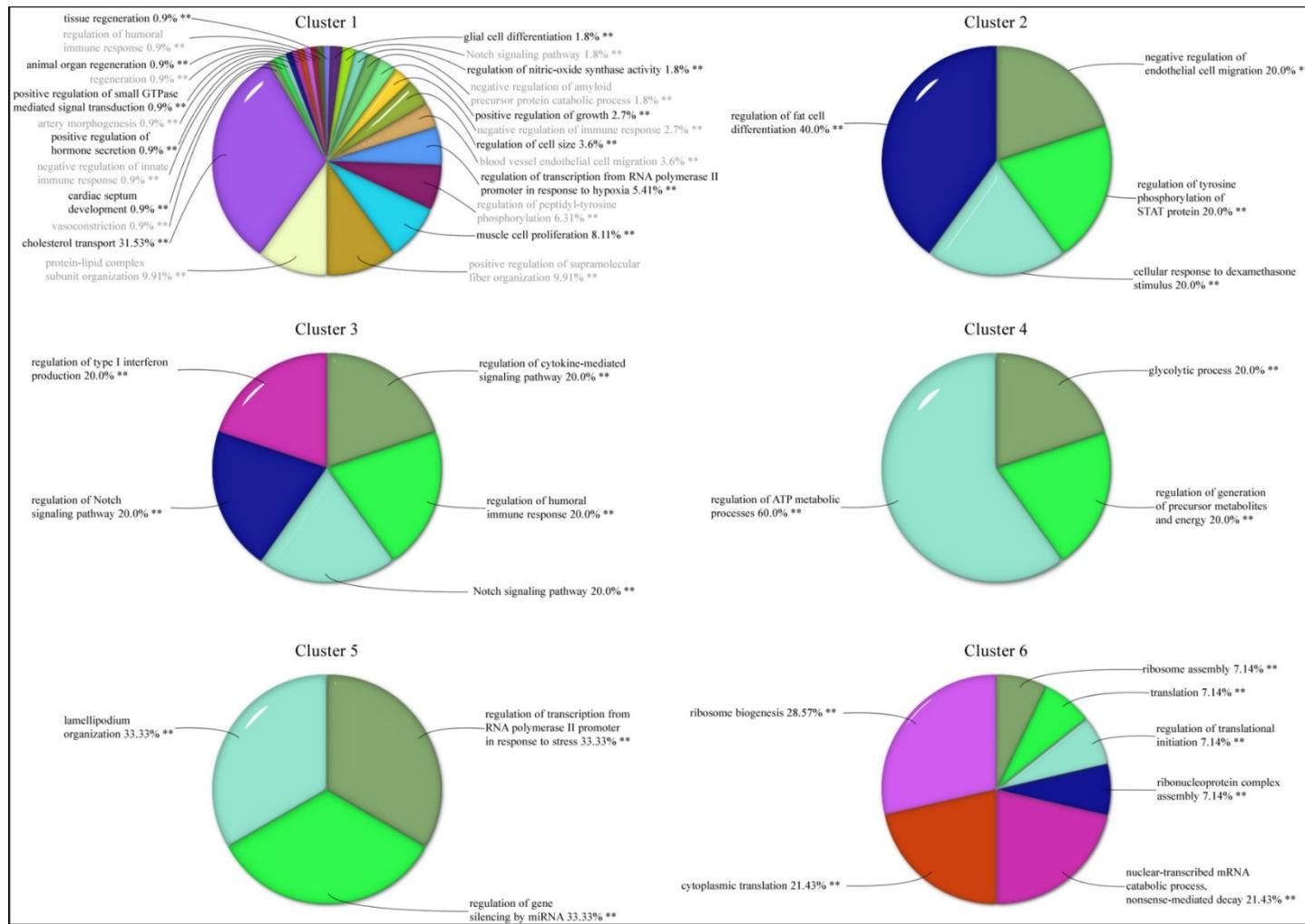
Cluster 6. Informative Data.

| Gene Ontology Identification Number | | Raw pValue | | Corrected pValue* | | % Associated Genes | Nr. Genes |
|-------------------------------------|---|------------|----------|-------------------|----------|--------------------|-----------|
| | | Term | Group | Term | Group | | |
| GO:0006412 | translation | 2.43E-43 | 2.43E-43 | 3.40E-42 | 1.70E-42 | 4.59 | 35 |
| GO:0000184 | nuclear-transcribed mRNA catabolic process, nonsense-mediated decay | 1.30E-33 | 1.85E-25 | 1.69E-32 | 1.11E-24 | 13.55 | 21 |
| GO:0006614 | SRP-dependent cotranslational protein targeting to membrane | 3.68E-32 | 1.85E-25 | 4.42E-31 | 1.11E-24 | 16.81 | 19 |
| GO:0090150 | establishment of protein localization to membrane | 2.86E-23 | 1.85E-25 | 3.15E-22 | 1.11E-24 | 5.15 | 20 |
| GO:0001732 | formation of cytoplasmic translation initiation complex | 3.01E-15 | 4.41E-23 | 2.11E-14 | 2.21E-22 | 41.18 | 7 |
| GO:0002181 | cytoplasmic translation | 4.41E-23 | 4.41E-23 | 4.41E-22 | 2.21E-22 | 12.30 | 15 |
| GO:0002183 | cytoplasmic translational initiation | 8.20E-21 | 4.41E-23 | 7.38E-20 | 2.21E-22 | 25.58 | 11 |
| GO:0022618 | ribonucleoprotein complex assembly | 2.56E-15 | 2.56E-15 | 2.05E-14 | 1.02E-14 | 4.50 | 14 |
| GO:0000462 | maturity of SSU-rRNA from tricistronic rRNA transcript | 2.32E-08 | 7.16E-15 | 2.32E-08 | 2.15E-14 | 12.82 | 5 |
| GO:0006364 | rRNA processing | 3.07E-12 | 7.16E-15 | 1.53E-11 | 2.15E-14 | 4.60 | 11 |
| GO:0042254 | ribosome biogenesis | 7.16E-15 | 7.16E-15 | 4.30E-14 | 2.15E-14 | 4.18 | 14 |
| GO:0042274 | ribosomal small subunit biogenesis | 1.80E-11 | 7.16E-15 | 7.19E-11 | 2.15E-14 | 8.99 | 8 |

Continued on next page

| Gene Ontology Identification Number | regulation of translational initiation | Raw pValue | | Corrected pValue* | | % Associated Genes | Nr. Genes |
|-------------------------------------|--|------------|----------|-------------------|----------|--------------------|-----------|
| | | Term | Group | Term | Group | | |
| GO:0006446 | regulation of translational initiation | 6.85E-10 | 6.85E-10 | 2.05E-09 | 1.37E-09 | 8.24 | 7 |
| GO:0042255 | ribosome assembly | 1.90E-08 | 1.90E-08 | 3.79E-08 | 1.90E-08 | 7.69 | 6 |

The figure shows the enriched Cluster 6, derived from the initial selection of genes, after exclusion of previous clusters. Cluster 6 depicts the interacting genes and the type of interaction as shown in the figure legend. The Bar Chart associated with cluster 6 reports the percentage of genes belonging to a specific biological process (from Gene Ontology database) included in the pathway under analysis. It also shows the associations levels of biological processes with the cluster under analysis. The pie chart shows the overall degree of association of main biological processes involved with the cluster under investigation. To note, only main biological processes are represented in the pie chart. The table summarizes the details regarding the pathway analysis and shows all of the associated biological processes linked with the cluster under investigation.



Supplementary Figure 1. Reports the overall summary of the biological processes associated ($p < 0.05$ with Bonferroni step down correction) with the clusters enriched from the initial list of genes (see Table1). Enrichment was based on STRING database (STRING-ACTIONS_v10.5) while pathway recognition used Gene Ontology Database (GO_BiologicalProcess-EBI-UniProt-GOA). Both analyses were performed on Cytoscape software [22], through ClueGO and CluePedia plugins [23].

References

1. Kumar A, Tsao JW (2018) *Alzheimer Disease*, in *StatPearls*, Treasure Island (FL).
2. Grossberg GT, Desai AK (2003) Management of Alzheimer's disease. *J Gerontol A Biol Sci Med Sci* 58: 331–353.
3. Watkins PB, Zimmerman HJ, Knapp MJ, et al. (1994) Hepatotoxic effects of tacrine administration in patients with Alzheimer's disease. *JAMA* 271: 992–998.
4. Winblad B, Wimo A, Engedal K, et al. (2006) 3-year study of donepezil therapy in Alzheimer's disease: effects of early and continuous therapy. *Dement Geriatr Cogn Disord* 21: 353–363.
5. Carrier L (1999) Donepezil and paroxetine: possible drug interaction. *J Am Geriatr Soc* 47: 1037.
6. Cacabelos R (2007) Donepezil in Alzheimer's disease: from conventional trials to pharmacogenetics. *Neuropsychiatr Dis Treat* 3: 303–333.
7. Grossberg GT, Stahelin HB, Messina JC, et al. (2000) Lack of adverse pharmacodynamic drug interactions with rivastigmine and twenty-two classes of medications. *Int J Geriatr Psychiatry* 15: 242–247.
8. Farlow MR, Hake A, Messina J, et al. (2001) Response of patients with Alzheimer disease to rivastigmine treatment is predicted by the rate of disease progression. *Arch Neurol* 58: 417–422.
9. Raskind MA, Peskind ER, Wessel T, et al. (2000) Galantamine in AD: a 6-month randomized, placebo-controlled trial with a 6-month extension. The Galantamine USA-1 Study Group. *Neurology* 54: 2261–2268.
10. Winblad B, Poritis N (1999) Memantine in severe dementia: results of the 9M-Best Study (Benefit and efficacy in severely demented patients during treatment with memantine). *Int J Geriatr Psychiatry* 14: 135–146.
11. Verrico MM, Nace DA, Towers AL (2000) Fulminant chemical hepatitis possibly associated with donepezil and sertraline therapy. *J Am Geriatr Soc* 48: 1659–1663.
12. Kruger J, Moilanen V, Majamaa K, et al. (2012) Molecular genetic analysis of the APP, PSEN1, and PSEN2 genes in Finnish patients with early-onset Alzheimer disease and frontotemporal lobar degeneration. *Alzheimer Dis Assoc Disord* 26: 272–276.
13. Montine KS, Olson SJ, Amarnath V, et al. (1997) Immunohistochemical detection of 4-hydroxy-2-nonenal adducts in Alzheimer's disease is associated with inheritance of APOE4. *Am J Pathol* 150: 437–443.
14. Farrer LA, Cupples LA, Haines JL, et al. (1997) Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. *JAMA* 278: 1349–1356.
15. Bis JC, Jian X, Kunkle BW, et al. (2020) Whole exome sequencing study identifies novel rare and common Alzheimer's-Associated variants involved in immune response and transcriptional regulation. *Mol Psychiatry* 25: 1859–1875.
16. Yao F, Zhang K, Zhang Y, et al. (2018) Identification of blood biomarkers for Alzheimer's disease through computational prediction and experimental validation. *Front Neurol* 9: 1158.
17. Han ZJ, Xue WW, Tao L, et al. (2018) Identification of novel immune-relevant drug target genes for Alzheimer's disease by combining ontology inference with network analysis. *CNS Neurosci Ther* 24: 1253–1263.

18. Jansen IE, Savage JE, Watanabe K, et al. (2019) Genome-wide meta-analysis identifies new loci and functional pathways influencing Alzheimer's disease risk. *Nat Genet* 51: 404–413.
19. Beecham GW, Vardarajan B, Blue E, et al. (2018) Rare genetic variation implicated in non-Hispanic white families with Alzheimer disease. *Neurol Genet* 4: e286.
20. Sancesario GM, Bernardini S (2018) Alzheimer's disease in the omics era. *Clin Biochem* 59: 9–16.
21. Rustenhoven J, Smith AM, Smyth LC, et al. (2018) PU.1 regulates Alzheimer's disease-associated genes in primary human microglia. *Mol Neurodegener* 13: 44.
22. Shannon P, Markiel A, Ozier O, et al. (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res* 13: 2498–2504.
23. Bindea G, Mlecnik B, Hackl H, et al. (2009) ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. *Bioinformatics* 25: 1091–1093.



AIMS Press

© 2021 the Author(s), licensee AIMS Press. This is an open access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>)