## Supplementary Information

# Gene expression analysis reveals early dysregulation of disease pathways and links *Chmp7* to pathogenesis of spinal and bulbar muscular atrophy

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## Supplementary Methods

### Primary culture

Primary spinal cord astrocyte cultures were established as previously described [1]. Spinal cords were isolated from postnatal day zero (P0) to P2 transgenic mouse pups. Cells were plated on poly-D-lysine coated plates in DMEM media supplemented with 10% foetal bovine serum and 1% penicillin and streptomycin (all Invitrogen). Cells were maintained for up to 2 weeks to allow cells to become confluent at which point the O2A progenitor cells and microglia were dislodged and removed. Cultures were immunoreactive for the astrocytic marker glial fibrillary acidic protein (GFAP) and plates were maintained at 37°C in 5% CO2 and 95% air.

In order to ensure differentially expressed genes were specific to motor neurons, we established cells cultured from meninges (predominantly fibroblasts removed from spinal cords dissected from embryos used for primary motor neuron cultures). These cultures were grown on poly-ornithine and laminin coated plates for 7 days *in vitro* in motor neuron culture media: neurobasal medium with 50U/mL penicillin, 50 µg/mL streptomycin, 2% B27 supplement, 25 µM 2-mercaptoethanol, 2% horse serum and 0.5 mM L-glutamine (all

Invitrogen), 0.1 ng/ml glial-derived neurotrophic factor, 0.1 ng/ml brain-derived neurotrophic factor and 0.5 ng/ml ciliary neurotrophic factor (all Peprotech). Cultures were treated with 50 nM dihydrotestosterone for 3 days before use. Cultures were maintained 7 days *in vitro* (DIV) in total at 37°C in 5% CO2 and 95% air.

**MN-1 cells**

MN-1 cells were maintained in Dulbecco's Modified Eagle's Medium with 10% foetal bovine serum (Thermo Fisher Scientific), 2 mM-glutamine and 100 units/ml penicillin/streptomycin. All the experiments were carried out in complete medium containing 10% charcoal dextran stripped-foetal bovine serum (Sigma-Aldrich), medium supplemented with 350 mg/ml G418 (Thermo Fisher Scientific). Cells were treated with 10 nM DHT for 48h before use.

**iPSC-derived patterned motor neuron precursor cells**

Induced pluripotent stem cells (iPSCs) (SB1, SB3 and SB6) from SBMA patients were kindly provided by Dr Kurt Fischbeck's lab at the National Institute for Neurological Disorders and Stroke (USA) through a material transfer agreement (MTA). Control 1 was available from Dr Rickie Patani's lab, UCL, UK and was reprogrammed using transfection of episomal plasmids as previously reported. [2]. Control 2 is commercially available from Coriell (cat number ND41866*C). iPSCs were maintained on Matrigel (Corning) with Essential 8 Medium media (Life Technologies) for Ctrl1 and Ctrl2 and mTESR-1 (Stem Cell Technologies) for SB1, SB3 and SB6. They were passaged using EDTA (Life Technologies, 0.5mM). iPSCs were plated to 100% confluency and then differentiated into neuroepithelium in chemically defined medium consisting of DMEM/F12 Glutamax, Neurobasal, L-Glutamine, N2 supplement, non-essential amino acids, B27 supplement, β-mercaptoethanol (all from Life Technologies) and insulin (Sigma). Between day 0-7 cells were treated with small molecules as follows: 1µM Dorsomorphin (Millipore), 2µM SB431542 (Tocris Bioscience) and 3.3 µM CHIR99021 (Miltenyi Biotec). At day 4 and day 12, the neuroepithelial layer was dissociated using dispase (GIBCO, 1 mg/ml) and re-plated on matrigel coated plates. From day 8 cells were patterned for 7 days with 0.5 µM retinoic acid and 1 µM purmorphomine. At day 14 spinal cord patterned motor neuron precursors (pMNs) were treated with 0.1 µM purmorphamine for a further 4 days. The precursors were harvested at day 18 for the experiments described in this paper. All cell cultures were maintained at 5% carbon dioxide and 37 C.

Total RNA was extracted from neural precursor cells cultures using the RNeasy mini kit (Qiagen). cDNA was prepared using superscript IV reverse transcriptase, according to manufacturer instructions (Thermo Fisher Scientific). qPCR was performed using TaqMan® Fast Advanced Master Mix (Thermo Fisher Scientific) with specific primers (Supplementary Information, Tables S5). Reactions were performed in triplicate and values normalised using β-actin (*Actb*). Relative quantification of gene expression was calculated via the comparative threshold cycle (ddCt) method [3].

## TrkB receptor assay

For the TrkB degradation assay, primary motor neuron cultures were grown as described for 7 DIV and were starved for 1 h in serum-free neurobasal medium without the TrkB ligand, BDNF. Cells were then incubated for the indicated time points with BDNF (10 ng/ml) and with cycloheximide (1 µg/ml), which inhibits synthesis of new protein. Cells were harvested in RIPA buffer (20 mM Tris pH 7.5, 150 mM NaCl, 1% NP-40, 0.5% deoxycholate, 0.1% SDS, 1 mM EDTA, 1mM EGTA, Halt Protease and Phosphatase Inhibitor Cocktail from Thermofischer) and analysed using Western Blot with antibodies against TrkB and β-actin.

## Animals

The SOD1-G93A model of Amyotrophic lateral sclerosis (ALS) harbouring a mutation in the *SOD1* gene is a widely used mouse model to recapitulate many of the features seen in ALS, the commonest form of motor neuron disease (MND) [4,5]. The generation and characterisation of the ALS4 SETX-R2136H mice carrying the R2136H mutation in the human SETX gene has been described previously [6]. Spinal cord from presymptomatic mice was used for qPCR analysis.

## Gene Ontology (GO) analysis and GO Term Enrichment

Differentially regulated genes were annotated using Gene Ontology (GO) classification, using PANTHER (http://www.pantherdb.org/). The Gene Ontology (GO) project describes gene products in terms of their associated biological processes, cellular components and molecular functions [7]. We examined higher level GO terms using GO slim terms, which are cut-down versions of the GO ontologies containing a subset of the terms in the whole GO giving a broad overview of the ontology content without the detail of the specific fine grained terms. To further understand the biological relevant significantly differentially regulated genes, we performed functional enrichment analysis using Cytoscape plug-ins ClueGO and CluePedia [8,9]. ClueGO analyses functional interaction of gene using GO information and database including, KEGG (Kyoto Encyclopedia of Genes and Genomes) and Reactome gene functional databases (http://www.reactome.org/). KEGG is a database of biological systems that integrates genomic, chemical and systemic functional information [10-12]. BioCarta (http://www.biocarta.com/) provides useful pathway information. ClueGO creates the first binary gene-term matrix with the selected terms and their associated genes and term-term similarity matrix is calculated using chance corrected kappa statistics. The kappa score measures the association strength between overlapping GO terms. A threshold of 0.3 (i.e., GO term pairs with kappa score ≥ 0.3 are connected in the ClueGO network).

Enrichment/depletion was calculated based on p-value corrected using hypothesis testing correction using the false discovery rate (FDR) method of Benjamin and Hochberg [13] and Storey [14]. CluePedia provides further insights into pathways by integrating experimental and in silico information and extends ClueGO functionality down to genes and miRNAs [8]. CluePedia calculates statistical correlation between differentially expressed genes and integrated variables using Pearson correlation, Spearman's rank, Distance correlation and Minimal Information Criteria (MIC). This allows investigation of a pathway by combining known and new, experimental derived information, about genes/proteins involved such as miRNAs influencing the expression of the genes.

### Transcription factor binding sites

oPOSSUM software (http://opossum.cisreg.ca/oPOSSUM3/) was used to identify transcription factor binding sites in the differentially regulated genes [15]. This allows identification of enriched potential transcription factor binding sites in promoter regions of genes. The mouse single site analysis (SSA) was used, with a 0.4 conservation cut-off and a matrix score threshold of 80% and by using by using 2000/0 on the upstream/downstream score.

### Immunofluorescence

Serial 10 μm transverse spinal cord sections were used for immunofluorescence staining. Sections were blocked and permeabilised at room temperature for 1 h in PBS containing 0.1% (w/v) Triton-X100 (v/v) and 5% serum (v/v) and incubated overnight at 4°C with the following primary antibodies: β-III tubulin (1:500, Biolegend), Chmp7 (1:250, Sigma UK), γ-H2AX (1:500, Millipore) and LAMP2 (1:250, Novus Biologicals) . Primary antibodies were detected with Alexa Fluor 488

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**Table S1. Transcripts upregulated in AR100 primary motor neurons**

| Probe Set ID | Gene Symbol | Gene Title | p-value | FC | GO biological process term |
|---|---|---|---|---|---|
| **1429159_at** | Itih5 | inter-alpha (globulin) inhibitor H5 | 0.009 | 1.86 | negative regulation of peptidase activity |
| **1428909_at** | A130040M12Rik | RIKEN cDNA A130040M12 gene | 0.035 | 1.81 | --- |
| **1436755_at** | Itih5 | inter-alpha (globulin) inhibitor H5 | 0.004 | 1.75 | negative regulation of peptidase activity |
| **1448550_at** | Lbp | lipopolysaccharide binding protein | 0.002 | 1.67 | leukocyte chemotaxis involved in inflammatory response |
| **1441946_at** | Itih5 | inter-alpha (globulin) inhibitor H5 | 0.026 | 1.55 | negative regulation of peptidase activity |
| **1416625_at** | Serping1 | serine (or cysteine) peptidase inhibitor, clade G, member 1 | 0.014 | 1.40 | negative regulation of complement activation, lectin pathway |
| **1437760_at** | Galnt12 | UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 12 | 0.044 | 1.37 | carbohydrate metabolic process |
| **1442899_at** | | | 0.044 | 1.33 | --- |
| **1444693_at** | Cacnb2 | calcium channel, voltage-dependent, beta 2 subunit | 0.007 | 1.33 | --- |
| **1419905_s_at** | Hpgd | hydroxyprostaglandin dehydrogenase 15 (NAD) | 0.017 | 1.30 | lipid metabolic process |
| **1421721_a_at** | Arnt | aryl hydrocarbon receptor nuclear translocator | 0.015 | 1.28 | response to hypoxia |
| **1424671_at** | Plekhf1 | pleckstrin homology domain containing, family F (with FYVE domain) member 1 | 0.048 | 1.27 | apoptotic process |
| **1417359_at** | Mfap2 | microfibrillar-associated protein 2 | 0.034 | 1.27 | platelet formation |
| **1422853_at** | Shc1 | src homology 2 domain-containing transforming protein C1 | 0.025 | 1.26 | MAPK cascade |
| **1460366_at** | Eml3 | echinoderm microtubule associated protein like 3 | 0.026 | 1.24 | proteolysis |
| **1460411_s_at** | AW548124 | expressed sequence AW548124 | 0.044 | 1.23 | ossification |
| **1456641_at** | 1190007F08Rik | RIKEN cDNA 1190007F08 gene | 0.035 | 1.23 | --- |
| **1453904_at** | 4930528G09Rik | RIKEN cDNA 4930528G09 gene | 0.045 | 1.22 | --- |
| **1422738_at** | Ddr2 | discoidin domain receptor family, member 2 | 0.046 | 1.22 | ossification |
| **1452289_a_at** | Rnf135 | ring finger protein 135 | 0.011 | 1.22 | protein ubiquitination |
| **1418394_a_at** | Cd97 | CD97 antigen | 0.016 | 1.22 | cell adhesion |
| **1452527_a_at** | P2rx4 | purinergic receptor P2X, ligand-gated ion channel 4 | 0.017 | 1.20 | regulation of sodium ion transport |
| **AFFX-18SRNAMur/X00686_M_at** | | | 0.028 | 1.20 | --- |
| **1427547_a_at** | Slc26a3 | solute carrier family 26, member 3 | 0.046 | 1.19 | transport |
| **1431203_at** | Sdccag8 | serologically defined colon cancer antigen 8 | 0.010 | 1.19 | establishment of cell polarity |
| **1453826_at** | Pard3b | par-3 partitioning defective 3 homolog B (C. elegans) | 0.005 | 1.19 | cell cycle |

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|---|---|---|---|---|---|
| **1417281_a_at** | Mmp23 | matrix metallopeptidase 23 | 0.006 | 1.18 | reproduction |
| **1422155_at** | Hist2h3c2 | histone cluster 2, H3c2 | 0.039 | 1.17 | negative regulation of transcription from RNA polymerase II promoter |
| **1437913_at** | Bcl2a1b | B-cell leukemia/lymphoma 2 related protein A1b | 0.004 | 1.17 | B cell homeostasis |
| **1458512_at** | Tle3 | transducin-like enhancer of split 3, homolog of Drosophila E(spl) | 0.048 | 1.17 | transcription DNA-dependent |
| **1448756_at** | S100a9 | S100 calcium binding protein A9 (calgranulin B) | 0.040 | 1.17 | chronic inflammatory response |
| **1424692_at** | 2810055F11Rik | RIKEN cDNA 2810055F11 gene | 0.047 | 1.16 | --- |
| **1418824_at** | Arf6 | ADP-ribosylation factor 6 | 0.022 | 1.16 | liver development |
| **1446313_at** | Zfp30 | zinc finger protein 30 | 0.043 | 1.16 | transcription DNA-dependent |
| **1425012_at** | Gng5 | guanine nucleotide binding protein (G protein), gamma 5 subunit | 0.012 | 1.15 | signal transduction |
| **1430397_at** | Got2 | glutamate oxaloacetate transaminase 2, mitochondrial | 0.016 | 1.15 | 2-oxoglutarate metabolic process |
| **1447850_x_at** | Zfand3 | zinc finger, AN1-type domain 3 | 0.045 | 1.15 | --- |
| **1451099_at** | Mbc2 | membrane bound C2 domain containing protein | 0.026 | 1.15 | --- |
| **1440649_at** | Tes | testis derived transcript | 0.021 | 1.14 | --- |
| **1420801_at** | Npas1 | neuronal PAS domain protein 1 | 0.030 | 1.14 | negative regulation of transcription from RNA polymerase II promoter |
| **1441535_at** | Mllt3 | myeloid/lymphoid or mixed lineage-leukemia translocation to 3 homolog (Drosophila) | 0.018 | 1.14 | --- |
| **1441179_at** | 9530020O07Rik | RIKEN cDNA 9530020O07 gene | 0.029 | 1.14 | --- |
| **1448626_at** | Cdk5rap1 | CDK5 regulatory subunit associated protein 1 | 0.015 | 1.14 | regulation of cyclin-dependent protein kinase activity |
| **1422699_at** | Alox12 | arachidonate 12-lipoxygenase | 0.029 | 1.14 | leukotriene metabolic process |
| **1428394_at** | Phyhd1 | phytanoyl-CoA dioxygenase domain containing 1 | 0.021 | 1.14 | pre-B cell differentiation |
| **1443029_at** | Ppfibp1 | PTPRF interacting protein, binding protein 1 (liprin beta 1) | 0.001 | 1.14 | --- |
| **1446869_at** | | | 0.042 | 1.14 | --- |
| **1442724_at** | AW060763 | expressed sequence AW060763 | 0.029 | 1.14 | embryo development |
| **1432878_at** | 4930544N03Rik | RIKEN cDNA 4930544N03 gene | 0.012 | 1.14 | --- |
| **1453926_at** | Rad54l | RAD54 like (S. cerevisiae) | 0.035 | 1.14 | double-strand break repair via homologous recombination |
| **1435622_at** | Hs3st3a1 | heparan sulfate (glucosamine) 3-O-sulfotransferase 3A1 | 0.012 | 1.13 | --- |
| **1453924_a_at** | Ptgfr | prostaglandin F receptor | 0.047 | 1.13 | signal transduction |
| **1417267_s_at** | Fkbp11 | FK506 binding protein 11 | 0.004 | 1.13 | protein peptidyl-prolyl isomerization |
| **1417860_a_at** | Spon2 | spondin 2, extracellular matrix protein | 0.017 | 1.13 | cell adhesion |

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|---|---|---|---|---|---|
| **1420195_at** | | | 0.034 | 1.13 | --- |
| **1452264_at** | Tenc1 | tensin like C1 domain-containing phosphatase | 0.023 | 1.13 | kidney development |
| **1416847_s_at** | Oas1d | 2'-5' oligoadenylate synthetase 1D | 0.021 | 1.13 | ovarian follicle development |
| **1441885_s_at** | | | 0.005 | 1.13 | --- |
| **1427880_at** | Uqcrq | ubiquinol-cytochrome c reductase, complex III subunit VII | 0.029 | 1.13 | transport |
| **1419541_at** | 4930403L05Rik | RIKEN cDNA 4930403L05 gene | 0.020 | 1.13 | regulation of signal transduction |
| **1453295_at** | Asxl2 | additional sex combs like 2 (Drosophila) | 0.003 | 1.13 | transcription DNA-dependent |
| **1436903_at** | Ubqln3 | ubiquilin 3 | 0.020 | 1.13 | --- |
| **1443325_at** | Erh | enhancer of rudimentary homolog (Drosophila) | 0.002 | 1.13 | --- |
| **1432139_at** | Srpk3 | serine/arginine-rich protein specific kinase 3 | 0.032 | 1.13 | protein phosphorylation |
| **1444324_at** | E530001K10Rik | RIKEN cDNA E530001K10 gene | 0.044 | 1.13 | --- |
| **1420894_at** | Tgfbr1 | transforming growth factor, beta receptor I | 0.045 | 1.12 | activation of MAPKK activity |
| **1440477_at** | | | 0.012 | 1.12 | --- |
| **1441561_at** | Fbxl3 | F-box and leucine-rich repeat protein 3 | 0.008 | 1.12 | protein ubiquitination |
| **1421220_at** | Ankrd17 | ankyrin repeat domain 17 | 0.014 | 1.12 | endoderm development |
| **1458486_at** | | | 0.030 | 1.12 | --- |
| **1430766_at** | 5033403F01Rik | RIKEN cDNA 5033403F01 gene | 0.008 | 1.12 | --- |
| **1424793_a_at** | Pbp2 | phosphatidylethanolamine binding protein 2 | 0.002 | 1.12 | negative regulation of peptidase activity |
| **1426197_at** | EG629860 | predicted gene, EG629860 | 0.004 | 1.12 | --- |
| **1438097_at** | Rab20 | RAB20, member RAS oncogene family | 0.038 | 1.11 | transport |
| **1447350_at** | | | 0.033 | 1.11 | --- |
| **1454580_at** | 5430427N15Rik | RIKEN cDNA 5430427N15 gene | 0.040 | 1.11 | --- |
| **1450621_a_at** | Hbb-y | hemoglobin Y, beta-like embryonic chain | 0.008 | 1.11 | negative regulation of transcription from RNA polymerase II promoter |
| **1430747_at** | Pqlc1 | PQ loop repeat containing 1 | 0.026 | 1.11 | --- |
| **1430321_at** | Krba1 | KRAB-A domain containing 1 | 0.039 | 1.11 | --- |
| **1442673_at** | Stam2 | signal transducing adaptor molecule (SH3 domain and ITAM motif) 2 | 0.037 | 1.11 | --- |
| **1438775_at** | | | 0.035 | 1.11 | --- |
| **1424660_s_at** | Crtc2 | CREB regulated transcription coactivator 2 | 0.011 | 1.11 | gluconeogenesis |
| **1423541_at** | 4930511I11Rik | RIKEN cDNA 4930511I11 gene | 0.023 | 1.11 | --- |
| **1422231_a_at** | Tnfrsf25 | tumor necrosis factor receptor superfamily, member 25 | 0.036 | 1.11 | signal transduction |
| **1457509_at** | Sbno1 | sno, strawberry notch homolog 1 (Drosophila) | 0.026 | 1.10 | --- |
| **1430505_at** | 1110032O16Rik | RIKEN cDNA 1110032O16 gene | 0.017 | 1.10 | --- |
| **1459363_at** | Atxn2 | ataxin 2 | 0.014 | 1.10 | negative regulation of receptor internalization |
| **1443840_x_at** | 1700010H22Rik | RIKEN cDNA 1700010H22 gene | 0.025 | 1.10 | --- |
| **1448003_at** | 1810043H04Rik | RIKEN cDNA 1810043H04 gene | 0.007 | 1.10 | --- |
| **1429871_at** | Hmmr | hyaluronan mediated motility receptor | 0.028 | 1.10 | --- |

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|---|---|---|---|---|---|
| | | (RHAMM) | | | |
| **1449178_at** | Pdlim3 | PDZ and LIM domain 3 | 0.036 | 1.10 | actin filament organization |
| **1445620_at** | | | 0.015 | 1.10 | --- |
| **1441697_at** | Maml1 | mastermind like 1 (Drosophila) | 0.010 | 1.10 | transcription DNA-dependent |
| **1449642_at** | Ebi2 | Epstein-Barr virus induced gene 2 | 0.043 | 1.10 | --- |
| **1459322_at** | Syne2 | synaptic nuclear envelope 2 | 0.039 | 1.10 | --- |
| **1446501_at** | A830053O21Rik | RIKEN cDNA A830053O21 gene | 0.043 | 1.10 | transcription DNA-dependent |
| **1441220_at** | Magi2 | membrane associated guanylate kinase, WW and PDZ domain containing 2 | 0.049 | 1.10 | --- |
| **1432009_at** | 4933415D12Rik | RIKEN cDNA 4933415D12 gene | 0.047 | 1.10 | --- |
| **1421898_a_at** | Mr1 | major histocompatibility complex, class I-related | 0.028 | 1.10 | antigen processing and presentation of peptide antigen via MHC class I |
| **1443084_at** | Ctso | cathepsin O | 0.007 | 1.09 | proteolysis |
| **1454326_at** | 1700017H01Rik | RIKEN cDNA 1700017H01 gene | 0.011 | 1.09 | --- |
| **1440782_at** | Skap1 | src family associated phosphoprotein 1 | 0.044 | 1.09 | positive regulation of transcription, DNA-dependent |
| **1439922_at** | Prrc1 | proline-rich coiled-coil 1 | 0.023 | 1.09 | --- |
| **1445153_at** | Abl1 | v-abl Abelson murine leukemia oncogene 1 | 0.029 | 1.09 | --- |
| **1425585_at** | Med12 | mediator of RNA polymerase II transcription, subunit 12 homolog (yeast) | 0.040 | 1.09 | neural tube closure |
| **1458532_at** | Mtr | 5-methyltetrahydrofolate-homocysteine methyltransferase | 0.022 | 1.09 | protein methylation |
| **1453305_at** | Iqcd | IQ motif containing D | 0.023 | 1.09 | --- |
| **1439081_at** | Mgea5 | meningioma expressed antigen 5 (hyaluronidase) | 0.012 | 1.09 | N-acetylglucosamine metabolic process |
| **1418578_at** | Dgka | diacylglycerol kinase, alpha | 0.008 | 1.09 | protein kinase C-activating G-protein coupled receptor signaling pathway |
| **1451443_at** | Nfix | nuclear factor I/X | 0.036 | 1.09 | negative regulation of transcription from RNA polymerase II promoter |
| **1437872_at** | AB112350 | cDNA sequence AB112350 | 0.033 | 1.09 | lipid metabolic process |
| **1429879_at** | 0610037L13Rik | RIKEN cDNA 0610037L13 gene | 0.008 | 1.09 | --- |
| **1439140_at** | 1700019P01Rik | RIKEN cDNA 1700019P01 gene | 0.017 | 1.09 | --- |
| **1430486_at** | Rad51l1 | RAD51-like 1 (S. cerevisiae) | 0.009 | 1.09 | DNA metabolic process |
| **1448839_at** | Ankrd47 | ankyrin repeat domain 47 | 0.041 | 1.09 | negative regulation of stress fiber assembly |
| **1443468_at** | | | 0.033 | 1.09 | --- |
| **1442547_at** | | | 0.027 | 1.09 | --- |
| **1441366_at** | | | 0.016 | 1.08 | --- |
| **1441568_at** | Osgin2 | oxidative stress induced growth inhibitor family member 2 | 0.032 | 1.08 | --- |
| **1430916_at** | Fancd2 | Fanconi anemia, complementation group D2 | 0.038 | 1.08 | DNA repair |
| **1421443_at** | Gpr110 | G protein-coupled receptor 110 | 0.018 | 1.08 | signal transduction |

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|---|---|---|---|---|---|
| **1428058_at** | Ahnak | AHNAK nucleoprotein (desmoyokin) | 0.026 | 1.08 | --- |
| **1440677_at** | EG245305 | predicted gene, EG245305 | 0.048 | 1.08 | regulation of transcription, DNA-dependent |
| **1439376_x_at** | | | 0.018 | 1.08 | transcription DNA-dependent |
| **1440126_at** | BC037704 | cDNA sequence BC037704 | 0.026 | 1.08 | --- |
| **1422565_s_at** | Nfic | nuclear factor I/C | 0.047 | 1.08 | negative regulation of transcription from RNA polymerase II promoter |
| **1443112_at** | Api5 | apoptosis inhibitor 5 | 0.040 | 1.08 | apoptotic process |
| **1448423_at** | Tmed4 | transmembrane emp24 protein transport domain containing 4 | 0.029 | 1.08 | transport |
| **1424304_at** | Tpcn2 | two pore segment channel 2 | 0.012 | 1.08 | transport |
| **1459520_at** | Gnb5 | guanine nucleotide binding protein, beta 5 | 0.022 | 1.08 | --- |
| **1442496_at** | Cdkl3 | cyclin-dependent kinase-like 3 | 0.023 | 1.08 | --- |
| **1459958_at** | Rsrc1 | arginine/serine-rich coiled-coil 1 | 0.023 | 1.08 | alternative nuclear mRNA splicing, via spliceosome |
| **1439970_at** | Wnk1 | WNK lysine deficient protein kinase 1 | 0.033 | 1.07 | --- |
| **1431916_at** | Hsd3b3 | hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 3 | 0.024 | 1.07 | steroid biosynthetic process |
| **1439110_at** | A930012O16Rik | RIKEN cDNA A930012O16 gene | 0.034 | 1.07 | --- |
| **1429110_a_at** | Nsun4 | NOL1/NOP2/Sun domain family, member 4 | 0.042 | 1.07 | methylation |
| **1441089_at** | Eif2c1 | eukaryotic translation initiation factor 2C, 1 | 0.047 | 1.07 | mRNA catabolic process |
| **1441755_at** | Mapk15 | mitogen-activated protein kinase 15 | 0.008 | 1.07 | negative regulation of transcription from RNA polymerase II promoter |
| **1458258_at** | | | 0.019 | 1.07 | --- |
| **1436631_at** | Ctnna1 | catenin (cadherin associated protein), alpha 1 | 0.037 | 1.07 | ovarian follicle development |
| **1453686_x_at** | Nphp1 | nephronophthisis 1 (juvenile) homolog (human) | 0.041 | 1.07 | spermatogenesis |
| **1443917_at** | Tmbim4 | transmembrane BAX inhibitor motif containing 4 | 0.024 | 1.07 | apoptotic process |
| **1424787_a_at** | Nrf1 | nuclear respiratory factor 1 | 0.037 | 1.07 | response to hypoxia |
| **1456297_at** | Slc46a2 | solute carrier family 46, member 2 | 0.045 | 1.07 | transport |
| **1456808_at** | 4933426M11Rik | RIKEN cDNA 4933426M11 gene | 0.017 | 1.07 | --- |
| **1446450_at** | Ttn | titin | 0.032 | 1.07 | in utero embryonic development |
| **1438762_at** | Tcf12 | transcription factor 12 | 0.048 | 1.06 | --- |
| **1450468_at** | Myoc | myocilin | 0.014 | 1.06 | --- |
| **1425675_s_at** | Ceacam1 | CEA-related cell adhesion molecule 1 | 0.027 | 1.06 | signal transduction |
| **1444482_at** | A130078K24Rik | RIKEN cDNA A130078K24 gene | 0.047 | 1.06 | --- |
| **1442578_at** | | | 0.027 | 1.06 | transcription DNA-dependent |
| **1440909_at** | Irgc1 | immunity-related GTPase family, cinema 1 | 0.039 | 1.06 | --- |
| **1417605_s_at** | Camk1 | calcium/calmodulin-dependent protein kinase I | 0.048 | 1.06 | protein phosphorylation |
| **1437840_s_at** | | | 0.028 | 1.06 | response to manganese ion |
| **1453969_at** | Prl8a1 | prolactin family 8, subfamily a, member 1 | 0.007 | 1.06 | --- |
| **1459060_at** | Shcbp1 | Shc SH2-domain binding protein 1 | 0.038 | 1.06 | --- |

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|---|---|---|---|---|---|
| **1433001_at** | 1700019L13Rik | RIKEN cDNA 1700019L13 gene | 0.041 | 1.06 | --- |
| **1429199_s_at** | Saal1 | serum amyloid A-like 1 | 0.037 | 1.06 | acute-phase response |
| **1419936_at** | Csnk2a2 | casein kinase 2, alpha prime polypeptide | 0.046 | 1.06 | --- |
| **1438694_at** | Csn1s2a | casein alpha s2-like A | 0.040 | 1.05 | transport |
| **1441321_at** | LOC552911 | hypothetical LOC552911 | 0.048 | 1.05 | --- |
| **1444394_at** | Acad9 | acyl-Coenzyme A dehydrogenase family, member 9 | 0.045 | 1.05 | metabolic process |
| **1416148_at** | Laptm4b | lysosomal-associated protein transmembrane 4B | 0.049 | 1.05 | transport |
| **1446879_at** | Grid2 | glutamate receptor, ionotropic, delta 2 | 0.029 | 1.05 | --- |
| **1416456_a_at** | Chia | chitinase, acidic | 0.029 | 1.05 | polysaccharide catabolic process |
| **1443655_s_at** | Pik3c2a | phosphatidylinositol 3-kinase, C2 domain containing, alpha polypeptide | 0.025 | 1.05 | exocytosis |
| **1422228_at** | Wnt8a | wingless-related MMTV integration site 8A | 0.018 | 1.05 | negative regulation of transcription from RNA polymerase II promoter |
| **1438783_at** | AW742560 | expressed sequence AW742560 | 0.026 | 1.05 | --- |
| **1451544_at** | Tapbpl | TAP binding protein-like | 0.016 | 1.05 | --- |
| **1442846_at** | Pbx1 | pre B-cell leukemia transcription factor 1 | 0.035 | 1.05 | --- |
| **1443475_at** | Hist1h3i | histone cluster 1, H3i | 0.033 | 1.05 | nucleosome assembly |
| **1457577_at** | | | 0.038 | 1.05 | --- |
| **1422187_at** | Gabrg3 | gamma-aminobutyric acid (GABA-A) receptor, subunit gamma 3 | 0.038 | 1.05 | transport |
| **1441677_at** | Smc4 | structural maintenance of chromosomes 4 | 0.040 | 1.04 | --- |
| **1441400_at** | 1810029B16Rik | RIKEN cDNA 1810029B16 gene | 0.041 | 1.04 | --- |
| **1421555_at** | Adrb3 | adrenergic receptor, beta 3 | 0.043 | 1.04 | diet induced thermogenesis |
| **1445446_at** | | | 0.041 | 1.04 | --- |
| **1434584_a_at** | Pramel5 | preferentially expressed antigen in melanoma like 5 | 0.031 | 1.03 | --- |
| **1441718_at** | Ubr5 | ubiquitin protein ligase E3 component n-recognin 5 | 0.022 | 1.03 | --- |
| **1431627_at** | Kif27 | kinesin family member 27 | 0.038 | 1.02 | microtubule-based movement |
| **1432568_at** | Cby3 | chibby homolog 3 (Drosophila) | 0.042 | 1.02 | --- |
| **1443117_at** | Eya1 | eyes absent 1 homolog (Drosophila) | 0.033 | 1.01 | establishment of mitotic spindle orientation |
| **1457413_at** | Ror1 | receptor tyrosine kinase-like orphan receptor 1 | 0.039 | 1.00 | --- |

**Table S2. Transcripts downregulated in AR100 primary motor neurons**

| Probe Set ID | Gene Symbol | Gene Title | p-value | FC | GO biological process term |
|---|---|---|---|---|---|
| **1430328_at** | Polr3f | polymerase (RNA) III (DNA directed) polypeptide F | 0.015 | -1.28 | transcription DNA-dependent |
| **1425446_at** | | | 0.008 | -1.26 | --- |
| **1454068_at** | 4930470H14Rik | RIKEN cDNA 4930470H14 gene | 0.024 | -1.25 | --- |
| **1433363_at** | A930036I15Rik | RIKEN cDNA A930036I15 gene | 0.023 | -1.24 | --- |
| **1451513_x_at** | Serpina1b | serine (or cysteine) preptidase inhibitor, clade A, member 1b | 0.025 | -1.24 | in utero embryonic development |
| **1444435_at** | Vps13b | vacuolar protein sorting 13B (yeast) | 0.039 | -1.23 | transport |
| **1442147_at** | Phka2 | phosphorylase kinase alpha 2 | 0.012 | -1.22 | --- |
| **1443933_at** | Mtac2d1 | membrane targeting (tandem) C2 domain containing 1 | 0.002 | -1.22 | --- |
| **1451892_at** | Kl | klotho | 0.01 | -1.22 | acute inflammatory response |
| **1430936_at** | Chmp7 | CHMP family, member 7 | 0.012 | -1.21 | transport |
| **1442639_at** | | | 0.028 | -1.21 | --- |
| **1432662_at** | 0610042E11Rik | RIKEN cDNA 0610042E11 gene | 0.032 | -1.21 | --- |
| **1421506_at** | Olfr78 | olfactory receptor 78 | 0.037 | -1.21 | signal transduction |
| **1434007_at** | Gyltl1b | glycosyltransferase-like 1B | 0.027 | -1.21 | --- |
| **1444665_at** | 4930402E16Rik | RIKEN cDNA 4930402E16 gene | 0.046 | -1.21 | glycine catabolic process |
| **1432449_at** | 4930564B18Rik | RIKEN cDNA 4930564B18 gene | 0.026 | -1.2 | --- |
| **1453509_at** | Lypd2 | Ly6/Plaur domain containing 2 | 0.003 | -1.2 | --- |
| **1439544_at** | Tbc1d5 | TBC1 domain family, member 5 | 0.009 | -1.2 | regulation of Rab GTPase activity |
| **1427572_at** | 2400001E08Rik | RIKEN cDNA 2400001E08 gene | 0.008 | -1.2 | --- |
| **1431021_at** | Cyb561d1 | cytochrome b-561 domain containing 1 | 0.012 | -1.19 | transport |
| **1427115_at** | Myh3 | myosin, heavy polypeptide 3, skeletal muscle, embryonic | 0.032 | -1.19 | --- |
| **1460067_at** | Ccr2 | chemokine (C-C motif) receptor 2 | 0.038 | -1.19 | angiogenesis |
| **1455445_at** | Cbln3 | cerebellin 3 precursor protein | 0.021 | -1.19 | --- |
| **1446218_at** | C230021G24Rik | RIKEN cDNA C230021G24 gene | 0.04 | -1.19 | --- |
| **1444749_at** | | | 0.006 | -1.19 | --- |
| **1457554_at** | Apob | apolipoprotein B | 0.032 | -1.18 | in utero embryonic development |
| **1416977_at** | Stam2 | signal transducing adaptor molecule (SH3 domain and ITAM motif) 2 | 0.015 | -1.18 | transport |
| **1420470_at** | Sult1c1 | sulfotransferase family, cytosolic, 1C, member 1 | 0.024 | -1.18 | sulfur compound metabolic process |
| **1432252_a_at** | 4933406K04Rik | RIKEN cDNA 4933406K04 gene | 0.028 | -1.18 | --- |
| **1442814_at** | Glcci1 | glucocorticoid induced transcript 1 | 0.037 | -1.18 | --- |
| **1432340_at** | 1700121N20Rik | RIKEN cDNA 1700121N20 gene | 0.025 | -1.18 | --- |
| **1447102_at** | Ninj2 | ninjurin 2 | 0.008 | -1.18 | --- |
| **1458895_at** | Ard1b | ARD1 homolog B (S. cerevisiae) | 0.025 | -1.18 | --- |
| **1424445_at** | Tm4sf5 | transmembrane 4 superfamily member 5 | 0.005 | -1.18 | --- |
| **1421222_at** | Fip1l1 | FIP1 like 1 (S. cerevisiae) | 0.024 | -1.18 | mRNA polyadenylation |
| **1442140_at** | Tnn | tenascin N | 0.048 | -1.18 | cell-matrix adhesion |

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|---|---|---|---|---|---|
| **1419175_a_at** | Btn1a1 | butyrophilin, subfamily 1, member A1 | 0.023 | -1.18 | negative regulation of cellular metabolic process |
| **1425145_at** | Il1rl1 | interleukin 1 receptor-like 1 | 0.022 | -1.17 | negative regulation of T-helper 1 type immune response |
| **1456949_at** | Fchsd2 | FCH and double SH3 domains 2 | 0.044 | -1.17 | --- |
| **1425215_at** | Ffar2 | free fatty acid receptor 2 | 0.041 | -1.17 | signal transduction |
| **1427214_at** | Agmat | agmatine ureohydrolase (agmatinase) | 0.023 | -1.17 | spermidine biosynthetic process |
| **1454210_at** | 4933428M09Rik | RIKEN cDNA 4933428M09 gene | 0.037 | -1.17 | --- |
| **1447065_at** | EG330070 | predicted gene, EG330070 | 0.033 | -1.17 | --- |
| **1446212_at** | | | 0.034 | -1.17 | --- |
| **1445905_at** | C79206 | expressed sequence C79206 | 0.036 | -1.17 | --- |
| **1432465_at** | 4933414I06Rik | RIKEN cDNA 4933414I06 gene | 0.049 | -1.16 | --- |
| **1445889_at** | Sorl1 | sortilin-related receptor, LDLR class A repeats-containing | 0.018 | -1.16 | --- |
| **1447455_at** | | | 0.042 | -1.16 | --- |
| **1429823_at** | Sgcg | sarcoglycan, gamma (dystrophin-associated glycoprotein) | 0.041 | -1.16 | cytoskeleton organization |
| **1419982_s_at** | | | 0.013 | -1.16 | --- |
| **1443043_at** | Otop2 | otopetrin 2 | 0.022 | -1.16 | --- |
| **1450274_at** | Gprc2a-rs5 | G protein-coupled receptor, family C, group 2, member A, related sequence 5 | 0.034 | -1.16 | --- |
| **1439628_x_at** | Rab38 | Rab38, member of RAS oncogene family | 0.04 | -1.16 | transport |
| **1444759_at** | Prickle1 | prickle like 1 (Drosophila) | 0.04 | -1.16 | neural tube closure |
| **1444516_at** | Ppfibp2 | protein tyrosine phosphatase, receptor-type, F interacting protein, binding protein 2 | 0.019 | -1.16 | --- |
| **1432118_at** | 2410012E07Rik | RIKEN cDNA 2410012E07 gene | 0.026 | -1.16 | --- |
| **1419621_at** | Ankrd2 | ankyrin repeat domain 2 (stretch responsive muscle) | 0.006 | -1.16 | --- |
| **1446233_at** | Adarb2 | adenosine deaminase, RNA-specific, B2 | 0.015 | -1.16 | --- |
| **1450604_at** | Olfr140 | olfactory receptor 140 | 0.01 | -1.16 | signal transduction |
| **1432239_at** | 1700102J08Rik | RIKEN cDNA 1700102J08 gene | 0.004 | -1.16 | --- |
| **1430932_at** | Slc9a8 | solute carrier family 9 (sodium/hydrogen exchanger), member 8 | 0.023 | -1.16 | transport |
| **1444424_at** | Sae1 | SUMO1 activating enzyme subunit 1 | 0.047 | -1.15 | --- |
| **1424401_at** | Aldh1l1 | aldehyde dehydrogenase 1 family, member L1 | 0.048 | -1.15 | one-carbon metabolic process |
| **1439714_at** | | | 0.005 | -1.15 | --- |
| **1432983_at** | 1700026H06Rik | RIKEN cDNA 1700026H06 gene | 0.043 | -1.15 | --- |
| **1458751_at** | | | 0.011 | -1.15 | --- |
| **1457224_at** | 2610528E23Rik | RIKEN cDNA 2610528E23 gene | 0.049 | -1.15 | --- |
| **1448002_x_at** | 2610001J05Rik | RIKEN cDNA 2610001J05 gene | 0.025 | -1.15 | --- |
| **1443092_at** | Mtmr3 | myotubularin related protein 3 | 0.022 | -1.15 | protein dephosphorylation |
| **1440606_at** | | | 0.006 | -1.15 | --- |
| **1428685_at** | Syce1 | synaptonemal complex central element protein 1 | 0.023 | -1.15 | cell cycle |
| **1432755_at** | 2210409O19Rik | RIKEN cDNA 2210409O19 gene | 0.021 | -1.15 | --- |

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|---|---|---|---|---|---|
| **1424592_a_at** | Dnase1 | deoxyribonuclease I | 0.026 | -1.15 | DNA catabolic process |
| **1446595_at** | Itsn2 | intersectin 2 | 0.029 | -1.15 | endocytosis |
| **1420253_at** | D2Ertd63e | DNA segment, Chr 2, ERATO Doi 63, expressed | 0.026 | -1.15 | --- |
| **1416725_at** | Tcf4 | transcription factor 4 | 0.029 | -1.15 | negative regulation of transcription from RNA polymerase II promoter |
| **1426139_a_at** | Ccrl1 | chemokine (C-C motif) receptor-like 1 | 0.023 | -1.15 | signal transduction |
| **1448872_at** | Reg3g | regenerating islet-derived 3 gamma | 0.045 | -1.15 | acute-phase response |
| **1431817_at** | Adh6-ps1 | alcohol dehydrogenase 6 (class V), pseudogene 1 | 0.027 | -1.15 | --- |
| **1441351_at** | Ankib1 | ankyrin repeat and IBR domain containing 1 | 0.039 | -1.14 | --- |
| **1442973_at** | C80865 | expressed sequence C80865 | 0.018 | -1.14 | --- |
| **1442719_at** | Prmt3 | protein arginine N-methyltransferase 3 | 0.016 | -1.14 | protein methylation |
| **1458962_at** | Tmed5 | transmembrane emp24 protein transport domain containing 5 | 0.019 | -1.14 | transport |
| **1459234_at** | Alox5ap | arachidonate 5-lipoxygenase activating protein | 0.04 | -1.14 | --- |
| **1420152_at** | | | 0.031 | -1.14 | --- |
| **1421684_at** | 2310051M13Rik | RIKEN cDNA 2310051M13 gene | 0.047 | -1.14 | proteolysis |
| **1424689_at** | Prss32 | protease, serine, 32 | 0.025 | -1.14 | proteolysis |
| **1442391_at** | 4930519H02Rik | RIKEN cDNA 4930519H02 gene | 0.043 | -1.14 | --- |
| **1446292_at** | Spata5 | spermatogenesis associated 5 | 0.031 | -1.14 | --- |
| **1459726_at** | Ptprn2 | protein tyrosine phosphatase, receptor type, N polypeptide 2 | 0.042 | -1.14 | --- |
| **1445052_at** | Pcdh9 | protocadherin 9 | 0.044 | -1.14 | --- |
| **1424627_at** | Cst12 | cystatin 12 | 0.01 | -1.14 | negative regulation of peptidase activity |
| **1419627_s_at** | Clec4n | C-type lectin domain family 4, member n | 0.038 | -1.14 | positive regulation of I-kappaB kinase |
| **1442765_at** | | | 0.022 | -1.13 | --- |
| **1423344_at** | Epor | erythropoietin receptor | 0.013 | -1.13 | heart morphogenesis |
| **1419969_at** | C77370 | expressed sequence C77370 | 0.033 | -1.13 | S phase of mitotic cell cycle |
| **1429010_at** | Snrp70 | U1 small nuclear ribonucleoprotein polypeptide A | 0.009 | -1.13 | nuclear mRNA splicing, via spliceosome |
| **1427891_at** | Gimap6 | GTPase, IMAP family member 6 | 0.01 | -1.13 | --- |
| **1446413_at** | 4930555G07Rik | RIKEN cDNA 4930555G07 gene | 0.028 | -1.13 | --- |
| **1440393_at** | Odz2 | odd Oz/ten-m homolog 2 (Drosophila) | 0.016 | -1.13 | --- |
| **1440617_at** | Cpa6 | carboxypeptidase A6 | 0.024 | -1.13 | proteolysis |
| **1420360_at** | Dkk1 | dickkopf homolog 1 (Xenopus laevis) | 0.043 | -1.13 | negative regulation of transcription from RNA polymerase II promoter |
| **1437352_at** | Acox3 | acyl-Coenzyme A oxidase 3, pristanoyl | 0.012 | -1.13 | lipid metabolic process |
| **1457442_at** | AW125324 | expressed sequence AW125324 | 0.029 | -1.12 | --- |
| **1449409_at** | Sult1c2 | sulfotransferase family, cytosolic, 1C, member 2 | 0.028 | -1.12 | sulfation |
| **1423147_at** | Mat1a | methionine adenosyltransferase I, alpha | 0.036 | -1.12 | S-adenosylmethionine biosynthetic process |
| **1454083_at** | 4930440C22Rik | RIKEN cDNA 4930440C22 gene | 0.012 | -1.12 | --- |
| **1439597_at** | 4932417H02Rik | RIKEN cDNA 4932417H02 gene | 0.001 | -1.12 | --- |
| **1456498_at** | Itga4 | integrin alpha 4 | 0.011 | -1.12 | blood vessel remodeling |

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|---|---|---|---|---|---|
| **1443273_at** | Epha3 | Eph receptor A3 | 0.007 | -1.12 | --- |
| **1437551_at** | 4930504E06Rik | RIKEN cDNA 4930504E06 gene | 0.046 | -1.12 | --- |
| **1460682_s_at** | Ceacam2 | CEA-related cell adhesion molecule 2 | 0.039 | -1.12 | signal transduction |
| **1420234_at** | | | 0.01 | -1.12 | --- |
| **1430682_at** | 4930555I21Rik | RIKEN cDNA 4930555I21 gene | 0.014 | -1.12 | --- |
| **1441189_at** | | | 0.005 | -1.12 | --- |
| **1451925_at** | Eda | ectodysplasin-A | 0.006 | -1.12 | hair follicle development |
| **1455466_at** | Gpr133 | G protein-coupled receptor 133 | 0.035 | -1.12 | cell surface receptor signaling pathway |
| **1456893_at** | | | 0.038 | -1.12 | --- |
| **1445054_at** | Foxn2 | forkhead box N2 | 0.016 | -1.12 | --- |
| **1426551_at** | Sidt1 | SID1 transmembrane family, member 1 | 0.036 | -1.12 | RNA interference |
| **1460322_at** | Chst3 | carbohydrate (chondroitin 6/keratan) sulfotransferase 3 | 0.029 | -1.12 | carbohydrate metabolic process |
| **1450996_at** | Fshb | follicle stimulating hormone beta | 0.013 | -1.12 | transforming growth factor beta receptor signaling pathway |
| **1444335_at** | Edem2 | ER degradation enhancer, mannosidase alpha-like 2 | 0.012 | -1.12 | --- |
| **1453254_at** | 1700034O15Rik | RIKEN cDNA 1700034O15 gene | 0.025 | -1.12 | --- |
| **1445486_at** | Lrrc49 | leucine rich repeat containing 49 | 0.033 | -1.12 | --- |
| **1444608_at** | Ankrd17 | ankyrin repeat domain 17 | 0.048 | -1.12 | --- |
| **1454081_at** | | | 0.034 | -1.12 | --- |
| **1456744_x_at** | Flcn | folliculin | 0.013 | -1.12 | regulation of protein phosphorylation |
| **1441184_at** | Trpc4ap | transient receptor potential cation channel, subfamily C, member 4 associated protein | 0.004 | -1.12 | ubiquitin-dependent protein catabolic process |
| **1431139_at** | Cdyl2 | chromodomain protein, Y chromosome-like 2 | 0.016 | -1.11 | metabolic process |
| **1453990_at** | 4930554G24Rik | RIKEN cDNA 4930554G24 gene | 0.024 | -1.11 | --- |
| **1452554_at** | EG667141 | predicted gene, EG667141 | 0.025 | -1.11 | --- |
| **1449860_at** | Higd1b | HIG1 domain family, member 1B | 0.019 | -1.11 | --- |
| **1441476_at** | Socs2 | suppressor of cytokine signaling 2 | 0.048 | -1.11 | lactation |
| **1449559_at** | Msx2 | homeo box, msh-like 2 | 0.042 | -1.11 | negative regulation of transcription from RNA polymerase II promoter |
| **1441126_at** | Farsb | phenylalanyl-tRNA synthetase, beta subunit | 0.033 | -1.11 | --- |
| **1454370_at** | 4930557B21Rik | RIKEN cDNA 4930557B21 gene | 0.03 | -1.11 | --- |
| **1419220_at** | Xirp1 | xin actin-binding repeat containing 1 | 0.047 | -1.11 | heart morphogenesis |
| **1450861_at** | Fancc | Fanconi anemia, complementation group C | 0.043 | -1.11 | myeloid cell homeostasis |
| **1448827_s_at** | Myh6 | myosin, heavy polypeptide 6, cardiac muscle, alpha | 0.045 | -1.11 | response to reactive oxygen species |
| **1453349_at** | Casz1 | castor homolog 1, zinc finger (Drosophila) | 0.014 | -1.11 | transcription DNA-dependent |
| **1446732_at** | | | 0.005 | -1.11 | --- |
| **1450364_a_at** | Havcr1 | hepatitis A virus cellular receptor 1 | 0.02 | -1.11 | phagocytosis, engulfment |
| **1432214_at** | 1700017D01Rik | RIKEN cDNA 1700017D01 gene | 0.016 | -1.11 | --- |
| **1458228_at** | | | 0.031 | -1.11 | --- |
| **1448731_at** | Il10ra | interleukin 10 receptor, alpha | 0.045 | -1.1 | cytokine-mediated signaling pathway |

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|---|---|---|---|---|---|
| **1457657_at** | | | 0.025 | -1.1 | --- |
| **1429361_at** | Pmch | pro-melanin-concentrating hormone | 0.033 | -1.1 | regulation of heart rate |
| **1425651_at** | Nhej1 | nonhomologous end-joining factor 1 | 0.024 | -1.1 | DNA repair |
| **1430657_at** | 1810034E14Rik | RIKEN cDNA 1810034E14 gene | 0.049 | -1.1 | --- |
| **1420571_at** | Prl6a1 | prolactin family 6, subfamily a, member 1 | 0.026 | -1.1 | --- |
| **1446083_at** | Hnrpd | heterogeneous nuclear ribonucleoprotein D | 0.028 | -1.1 | --- |
| **1432559_at** | 2210409D07Rik | RIKEN cDNA 2210409D07 gene | 0.028 | -1.1 | --- |
| **1446691_at** | Pdgfd | platelet-derived growth factor, D polypeptide | 0.027 | -1.1 | multicellular organismal development |
| **1457093_at** | Gapvd1 | GTPase activating protein and VPS9 domains 1 | 0.044 | -1.1 | endocytosis |
| **1445663_at** | | | 0.04 | -1.1 | --- |
| **1445333_at** | | | 0.038 | -1.1 | --- |
| **1423397_at** | Ugt2b5 | UDP glucuronosyltransferase 2 family, polypeptide B5 | 0.019 | -1.1 | metabolic process |
| **1436419_a_at** | 1700097N02Rik | RIKEN cDNA 1700097N02 gene | 0.028 | -1.1 | --- |
| **1423178_at** | Abi1 | abl-interactor 1 | 0.025 | -1.1 | somitogenesis |
| **1454219_at** | Dnajc2 | DnaJ (Hsp40) homolog, subfamily C, member 2 | 0.013 | -1.1 | G2 phase of mitotic cell cycle |
| **1450548_at** | Htr1f | 5-hydroxytryptamine (serotonin) receptor 1F | 0.012 | -1.1 | signal transduction |
| **1417447_at** | Tcf21 | transcription factor 21 | 0.021 | -1.1 | negative regulation of transcription from RNA polymerase II promoter |
| **1425450_at** | Chi3l4 | chitinase 3-like 4 | 0.01 | -1.1 | carbohydrate metabolic process |
| **1458766_at** | Pak7 | p21 (CDKN1A)-activated kinase 7 | 0.018 | -1.1 | --- |
| **1433036_at** | 4930564B12Rik | RIKEN cDNA 4930564B12 gene | 0.039 | -1.1 | --- |
| **1445951_at** | C86345 | expressed sequence C86345 | 0.003 | -1.1 | --- |
| **1457920_at** | C130026L21Rik | RIKEN cDNA C130026L21 gene | 0.038 | -1.1 | --- |
| **1427570_at** | Srpr | signal recognition particle receptor ('docking protein') | 0.016 | -1.1 | GTP catabolic process |
| **1440072_at** | E430022K19Rik | RIKEN cDNA E430022K19 gene | 0.023 | -1.1 | --- |
| **1459760_at** | Ndufs4 | NADH dehydrogenase (ubiquinone) Fe-S protein 4 | 0.019 | -1.1 | --- |
| **1446277_at** | | | 0.009 | -1.1 | --- |
| **1432732_at** | 5830437K03Rik | RIKEN cDNA 5830437K03 gene | 0.049 | -1.09 | --- |
| **1451661_at** | Akap4 | A kinase (PRKA) anchor protein 4 | 0.031 | -1.09 | transmembrane receptor protein serine |
| **1458055_at** | Dsc3 | desmocollin 3 | 0.014 | -1.09 | --- |
| **1432226_at** | 4930548G14Rik | RIKEN cDNA 4930548G14 gene | 0.043 | -1.09 | --- |
| **1452564_at** | Ofa | oncofetal antigen | 0.043 | -1.09 | --- |
| **1453268_at** | Thg1l | tRNA-histidine guanylyltransferase 1-like (S. cerevisiae) | 0.018 | -1.09 | tRNA modification |
| **1445603_at** | Sri | sorcin | 0.041 | -1.09 | --- |
| **1459187_at** | St3gal4 | ST3 beta-galactoside alpha-2,3-sialyltransferase 4 | 0.029 | -1.09 | --- |
| **1427478_at** | Usp12 | ubiquitin specific peptidase 12 | 0.021 | -1.09 | proteolysis |
| **1431470_a_at** | 4933414I15Rik | RIKEN cDNA 4933414I15 gene | 0.038 | -1.09 | --- |
| **1420962_at** | Hapln2 | hyaluronan and proteoglycan link protein 2 | 0.01 | -1.09 | cell adhesion |
| **1450229_at** | Med14 | mediator complex subunit 14 | 0.033 | -1.09 | transcription DNA-dependent |
| **1441862_at** | Ppox | protoporphyrinogen oxidase | 0.021 | -1.09 | porphyrin-containing compound |

| | | | | | |
|---|---|---|---|---|---|
| | | | | | biosynthetic process |
| **1445875_at** | A230057D06Rik | RIKEN cDNA A230057D06 gene | 0.028 | -1.09 | --- |
| **1433483_s_at** | C86187 | expressed sequence C86187 | 0.047 | -1.09 | --- |
| **1446051_at** | Gm1140 | gene model 1140, (NCBI) | 0.028 | -1.09 | --- |
| **1458573_at** | | | 0.023 | -1.09 | --- |
| **1457846_at** | Cox11 | COX11 homolog, cytochrome c oxidase assembly protein (yeast) | 0.011 | -1.09 | --- |
| **1458981_at** | Wdr70 | WD repeat domain 70 | 0.033 | -1.09 | --- |
| **1433039_at** | 4930555B11Rik | RIKEN cDNA 4930555B11 gene | 0.042 | -1.09 | --- |
| **1446878_at** | Lonrf2 | LON peptidase N-terminal domain and ring finger 2 | 0.005 | -1.09 | --- |
| **1445294_at** | | | 0.046 | -1.09 | --- |
| **1459567_at** | C77905 | expressed sequence C77905 | 0.025 | -1.09 | --- |
| **1445780_at** | | | 0.005 | -1.09 | --- |
| **1418725_at** | Dnajc5b | DnaJ (Hsp40) homolog, subfamily C, member 5 beta | 0.03 | -1.09 | protein folding |
| **1460472_at** | Cdk3 | cyclin-dependent kinase 3 | 0.026 | -1.09 | protein phosphorylation |
| **1445007_at** | Lrrc50 | leucine rich repeat containing 50 | 0.037 | -1.09 | --- |
| **1458257_at** | | | 0.036 | -1.09 | --- |
| **1433006_at** | 5430430B14Rik | RIKEN cDNA 5430430B14 gene | 0.046 | -1.09 | --- |
| **1453553_at** | | | 0.033 | -1.09 | --- |
| **1450609_at** | Lcn8 | lipocalin 8 | 0.021 | -1.09 | transport |
| **1429807_at** | 1700011H22Rik | RIKEN cDNA 1700011H22 gene | 0.048 | -1.09 | --- |
| **1445170_at** | 2700012I20Rik | RIKEN cDNA 2700012I20 gene | 0.025 | -1.09 | --- |
| **1431186_at** | Dlg5 | discs, large homolog 5 (Drosophila) | 0.02 | -1.08 | regulation of apoptotic process |
| **1459089_at** | RP23-292J1.1 | hypothetical LOC545637 | 0.027 | -1.08 | --- |
| **1426182_a_at** | Klrc1 | killer cell lectin-like receptor subfamily C, member 1 | 0.048 | -1.08 | --- |
| **1453477_at** | Zc3h6 | zinc finger CCCH type containing 6 | 0.049 | -1.08 | --- |
| **1440528_at** | | | 0.04 | -1.08 | --- |
| **1419764_at** | Chi3l3 | chitinase 3-like 3 | 0.044 | -1.08 | carbohydrate metabolic process |
| **1430096_at** | 2900017F05Rik | RIKEN cDNA 2900017F05 gene | 0.046 | -1.08 | --- |
| **1419346_a_at** | Svs5 | seminal vesicle secretory protein 5 | 0.022 | -1.08 | --- |
| **1445877_at** | Rfesd | Rieske (Fe-S) domain containing | 0.021 | -1.08 | oxidation-reduction process |
| **1441758_at** | Rhot1 | ras homolog gene family, member T1 | 0.027 | -1.08 | GTP catabolic process |
| **1442629_at** | Nt5c2 | 5'-nucleotidase, cytosolic II | 0.032 | -1.08 | --- |
| **1431633_x_at** | 4930526L06Rik | RIKEN cDNA 4930526L06 gene | 0.025 | -1.08 | --- |
| **1457222_at** | Creb5 | cAMP responsive element binding protein 5 | 0.011 | -1.08 | transcription DNA-dependent |
| **1446438_at** | Ubr1 | ubiquitin protein ligase E3 component n-recognin 1 | 0.027 | -1.08 | ubiquitin-dependent protein catabolic process |
| **1458611_at** | | | 0.031 | -1.08 | --- |
| **1450774_at** | Ly6g6d | lymphocyte antigen 6 complex, locus G6D | 0.041 | -1.08 | --- |
| **1432623_at** | Supv3l1 | suppressor of var1, 3-like 1 (S. cerevisiae) | 0.034 | -1.08 | mitochondrial mRNA catabolic process |
| **1453489_at** | 1700020O03Rik | RIKEN cDNA 1700020O03 gene | 0.047 | -1.08 | --- |

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|---|---|---|---|---|---|
| **1445439_at** | Epb4.9 | erythrocyte protein band 4.9 | 0.047 | -1.08 | cytoskeleton organization |
| **1459732_at** | | | 0.038 | -1.08 | --- |
| **1444332_at** | 9130019P16Rik | RIKEN cDNA 9130019P16 gene | 0.022 | -1.08 | --- |
| **1440778_x_at** | Zfp712 | zinc finger protein 712 | 0.041 | -1.08 | regulation of transcription, DNA-dependent |
| **1427818_at** | Bcl2 | B-cell leukemia/lymphoma 2 | 0.028 | -1.08 | --- |
| **1421637_at** | Slc5a4a | solute carrier family 5, member 4a | 0.046 | -1.08 | transport |
| **1432647_at** | Egfr | epidermal growth factor receptor | 0.044 | -1.08 | activation of MAPKK activity |
| **1437721_at** | Coro1c | coronin, actin binding protein 1C | 0.031 | -1.08 | actin cortical patch assembly |
| **1443994_at** | 6030407O03Rik | RIKEN cDNA 6030407O03 gene | 0.047 | -1.07 | --- |
| **1454970_at** | Ptcd1 | pentatricopeptide repeat domain 1 | 0.025 | -1.07 | --- |
| **1446541_at** | 4930434E21Rik | RIKEN cDNA 4930434E21 gene | 0.022 | -1.07 | --- |
| **1454417_at** | 4933408M05Rik | RIKEN cDNA 4933408M05 gene | 0.032 | -1.07 | --- |
| **1453827_at** | 1110035H17Rik | RIKEN cDNA 1110035H17 gene | 0.04 | -1.07 | --- |
| **1441524_at** | Tec | cytoplasmic tyrosine kinase, Dscr28C related (Drosophila) | 0.048 | -1.07 | --- |
| **1422707_at** | Pik3cg | phosphoinositide-3-kinase, catalytic, gamma polypeptide | 0.042 | -1.07 | positive regulation of acute inflammatory response |
| **1447104_at** | Arid1a | AT rich interactive domain 1A (Swi1 like) | 0.031 | -1.07 | --- |
| **1433370_at** | 4930474A20Rik | RIKEN cDNA 4930474A20 gene | 0.04 | -1.07 | --- |
| **1451781_at** | Nfatc2ip | nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2 interacting protein | 0.018 | -1.07 | cytokine production |
| **1442485_at** | 9030205A07Rik | RIKEN cDNA 9030205A07 Gene | 0.029 | -1.07 | --- |
| **1449782_at** | AA517650 | expressed sequence AA517650 | 0.014 | -1.07 | --- |
| **1432560_at** | 1700127D06Rik | RIKEN cDNA 1700127D06 gene | 0.025 | -1.07 | proteolysis |
| **1455661_at** | ENSMUSG00000054945 | predicted gene, ENSMUSG00000054945 | 0.043 | -1.07 | --- |
| **1459597_at** | Mtpn | myotrophin | 0.033 | -1.07 | catecholamine metabolic process |
| **1453585_at** | 1600010M07Rik | RIKEN cDNA 1600010M07 gene | 0.008 | -1.07 | --- |
| **1449801_at** | | | 0.043 | -1.07 | --- |
| **1452405_x_at** | A430107P09Rik | RIKEN cDNA A430107P09 gene | 0.015 | -1.06 | --- |
| **1449963_at** | 2310040M23Rik | RIKEN cDNA 2310040M23 gene | 0.016 | -1.06 | --- |
| **1458288_at** | 4933426I21Rik | RIKEN cDNA 4933426I21 gene | 0.031 | -1.06 | --- |
| **1422933_at** | Xlr5c | X-linked lymphocyte-regulated 5C | 0.049 | -1.06 | --- |
| **1420293_at** | Aytl2 | acyltransferase like 2 | 0.03 | -1.06 | phospholipid metabolic process |
| **1457739_at** | Ilf3 | interleukin enhancer binding factor 3 | 0.022 | -1.06 | transcription DNA-dependent |
| **1438644_x_at** | Commd9 | COMM domain containing 9 | 0.035 | -1.06 | --- |
| **1427779_a_at** | Cd4 | CD4 antigen | 0.034 | -1.06 | cytokine production |
| **1437089_at** | 4833409A17Rik | RIKEN cDNA 4833409A17 gene | 0.012 | -1.06 | oxidation-reduction process |
| **1457391_at** | Vamp3 | vesicle-associated membrane protein 3 | 0.016 | -1.06 | positive regulation of receptor recycling |
| **1460073_at** | Prkar1b | protein kinase, cAMP dependent regulatory, type I beta | 0.033 | -1.06 | --- |
| **1425370_a_at** | Erg | avian erythroblastosis virus E-26 (v-ets) oncogene related | 0.044 | -1.06 | transcription DNA-dependent |

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|---|---|---|---|---|---|
| **1421564_at** | Serpina3c | serine (or cysteine) peptidase inhibitor, clade A, member 3C | 0.026 | -1.06 | negative regulation of peptidase activity |
| **1421185_at** | Mettl7a | methyltransferase like 7A | 0.02 | -1.06 | metabolic process |
| **1441176_at** | | | 0.042 | -1.06 | --- |
| **1420108_at** | | | 0.043 | -1.06 | --- |
| **1444173_at** | | | 0.038 | -1.05 | --- |
| **1420379_at** | Slco1a1 | solute carrier organic anion transporter family, member 1a1 | 0.039 | -1.05 | transport |
| **1445957_at** | Evl | Ena-vasodilator stimulated phosphoprotein | 0.025 | -1.05 | --- |
| **1441106_at** | Zfand5 | zinc finger, AN1-type domain 5 | 0.009 | -1.05 | in utero embryonic development |
| **1428915_at** | Sirt5 | sirtuin 5 (silent mating type information regulation 2 homolog) 5 (S. cerevisiae) | 0.04 | -1.05 | protein deacetylation |
| **1436615_a_at** | Otc | ornithine transcarbamylase | 0.036 | -1.05 | urea cycle |
| **1444888_at** | AU022852 | expressed sequence AU022852 | 0.026 | -1.05 | --- |
| **1449608_a_at** | | | 0.032 | -1.05 | --- |
| **1432773_at** | 2610105M22Rik | RIKEN cDNA 2610105M22 gene | 0.025 | -1.05 | --- |
| **1443351_at** | Glra2 | glycine receptor, alpha 2 subunit | 0.032 | -1.05 | --- |
| **1450578_at** | Sry | sex determining region of Chr Y | 0.037 | -1.04 | transcription, DNA-dependent |
| **1458380_at** | Asxl1 | additional sex combs like 1 (Drosophila) | 0.043 | -1.04 | negative regulation of transcription from RNA polymerase II promoter |
| **1434068_s_at** | AI662270 | expressed sequence AI662270 | 0.032 | -1.04 | --- |
| **1449292_at** | Rb1cc1 | RB1-inducible coiled-coil 1 | 0.049 | -1.04 | autophagic vacuole assembly |
| **1449475_at** | Atp12a | ATPase, H+/K+ transporting, nongastric, alpha polypeptide | 0.042 | -1.04 | ATP biosynthetic process |
| **1435962_at** | Rps6 | ribosomal protein S6 | 0.036 | -1.03 | ribosomal small subunit assembly |
| **1427614_at** | Pip | prolactin induced protein | 0.048 | -1.03 | --- |
| **1444115_at** | | | 0.04 | -1.03 | --- |
| **1453275_at** | 2310002L13Rik | RIKEN cDNA 2310002L13 gene | 0.038 | -1.03 | --- |
| **1432690_at** | 9030407C09Rik | RIKEN cDNA 9030407C09 gene | 0.035 | -1.03 | --- |
| **1444382_at** | 4930579G22Rik | RIKEN cDNA 4930579G22 gene | 0.045 | -1.03 | --- |
| **1454433_at** | 6330526H18Rik | RIKEN cDNA 6330526H18 gene | 0.039 | -1.03 | --- |
| **1417812_a_at** | Lamb3 | laminin, beta 3 | 0.047 | -1.03 | cell adhesion |
| **1454216_at** | 4930408F14Rik | RIKEN cDNA 4930408F14 gene | 0.032 | -1.02 | gamete generation |
| **1444356_at** | Erc1 | ELKS/RAB6-interacting/CAST family member 1 | 0.036 | -1.02 | --- |

**Table S3. Identification of over-represented transcription factor binding sites (TFBS) a using oPOSSUM v3 software**

| TF | Class | Family | Target gene hits | Target TFBS hits | Z-score | Fisher score |
|---|---|---|---|---|---|---|
| **TBP** | Beta-sheet | TATA-binding | 182 | 624 | 12.828 | 9.602 |
| **PLAG1** | Zinc-coordinating | BetaBetaAlpha-zinc finger | 38 | 49 | 11.289 | 9.949 |
| **Foxa2** | Winged Helix-Turn-Helix | Forkhead | 196 | 775 | 8.741 | 7.903 |
| **Sox5** | Other Alpha-Helix | High Mobility Group | 219 | 1231 | 8.534 | 8.633 |
| **Evi1** | Zinc-coordinating | BetaBetaAlpha-zinc finger | 43 | 52 | 8.448 | 9.692 |
| **SOX9** | Other Alpha-Helix | High Mobility Group | 206 | 781 | 8.043 | 12.714 |
| **SRY** | Other Alpha-Helix | High Mobility Group | 248 | 1722 | 7.799 | 11.36 |
| **SRF** | Other Alpha-Helix | MADS | 26 | 26 | 7.631 | 9.825 |
| **HOXA5** | Helix-Turn-Helix | Homeo | 277 | 3370 | 7.52 | 11.997 |
| **Nkx2-5** | Helix-Turn-Helix | Homeo | 267 | 3208 | 7.432 | 8.658 |
| **Pdx1** | Helix-Turn-Helix | Homeo | 244 | 2227 | 6.709 | 6.404 |
| **ARID3A** | Helix-Turn-Helix | Arid | 249 | 2607 | 6.643 | 4.899 |
| **FOXD1** | Winged Helix-Turn-Helix | Forkhead | 206 | 1098 | 6.494 | 4.186 |
| **NR2F1** | Zinc-coordinating | Hormone-nuclear Receptor | 75 | 109 | 6.039 | 8.762 |
| **NFIL3** | Zipper-Type | Leucine Zipper | 112 | 260 | 5.915 | 6.119 |
| **IRF1** | Winged Helix-Turn-Helix | IRF | 93 | 211 | 5.864 | 2.941 |
| **NFATC2** | Ig-fold | Rel | 233 | 1646 | 5.136 | 5.502 |
| **INSM1** | Zinc-coordinating | BetaBetaAlpha-zinc finger | 120 | 285 | 5.1 | 8.237 |

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|---|---|---|---|---|---|---|
| **Sox17** | Other Alpha-Helix | High Mobility Group | 228 | 1185 | 4.869 | 8.993 |
| **FOXO3** | Winged Helix-Turn-Helix | Forkhead | 217 | 1212 | 4.804 | 5.095 |
| **Foxd3** | Winged Helix-Turn-Helix | Forkhead | 196 | 759 | 4.383 | 9.695 |
| **FOXI1** | Winged Helix-Turn-Helix | Forkhead | 207 | 812 | 4.296 | 11.43 |
| **Foxq1** | Winged Helix-Turn-Helix | Forkhead | 130 | 349 | 4.167 | 5.304 |
| **NR3C1** | Zinc-coordinating | Hormone-nuclear Receptor | 43 | 59 | 4.068 | 4.439 |
| **HNF1A** | Helix-Turn-Helix | Homeo | 55 | 71 | 4.011 | 8.206 |
| **Stat3** | Ig-fold | Stat | 167 | 431 | 3.928 | 10.858 |
| **Prrx2** | Helix-Turn-Helix | Homeo | 241 | 1863 | 3.889 | 8.937 |
| **HNF1B** | Helix-Turn-Helix | Homeo | 76 | 116 | 3.765 | 8.685 |
| **SPI1** | Winged Helix-Turn-Helix | Ets | 249 | 1746 | 3.602 | 9.943 |
| **Nobox** | Helix-Turn-Helix | Homeo | 210 | 1225 | 3.52 | 4.672 |
| **NKX3-1** | Helix-Turn-Helix | Homeo | 218 | 1155 | 3.49 | 8.181 |
| **CREB1** | Zipper-Type | Leucine Zipper | 161 | 373 | 3.076 | 11.689 |
| **SP1** | Zinc-coordinating | BetaBetaAlpha-zinc finger | 192 | 1169 | 3.015 | 9.049 |
| **MEF2A** | Other Alpha-Helix | MADS | 126 | 242 | 2.856 | 12.871 |
| **Arnt::Ahr** | Zipper-Type | Helix-Loop-Helix | 227 | 1317 | 2.581 | 10.868 |
| **T** | Beta-Hairpin-Ribbon | T | 35 | 44 | 2.393 | 4.303 |
| **Pou5f1** | Helix-Turn-Helix | Homeo | 57 | 79 | 2.377 | 6.394 |
| **CEBPA** | Zipper-Type | Leucine Zipper | 220 | 870 | 2.265 | 15.286 |

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|---|---|---|---|---|---|---|
| **PBX1** | Helix-Turn-Helix | Homeo | 74 | 110 | 2.232 | 7.219 |
| **FEV** | Winged Helix-Turn-Helix | Ets | 234 | 1416 | 2.041 | 6.592 |
| **Gata1** | Zinc-coordinating | GATA | 229 | 1327 | 2.007 | 6.35 |
| **CTCF** | Zinc-coordinating | BetaBetaAlpha-zinc finger | 53 | 66 | 1.955 | 5.538 |
| **YY1** | Zinc-coordinating | BetaBetaAlpha-zinc finger | 267 | 3003 | 1.834 | 8.352 |
| **MZF1_5-13** | Zinc-coordinating | BetaBetaAlpha-zinc finger | 217 | 1272 | 1.807 | 10.471 |
| **ELF5** | Winged Helix-Turn-Helix | Ets | 268 | 2043 | 1.482 | 16.25 |
| **PPARG::RXRA** | Zinc-coordinating | Hormone-nuclear Receptor | 102 | 195 | 1.345 | 6.214 |
| **Sox2** | Other Alpha-Helix | High Mobility Group | 47 | 62 | 1.313 | 4.769 |
| **FOXA1** | Winged Helix-Turn-Helix | Forkhead | 213 | 1065 | 1.073 | 4.919 |
| **NFKB1** | Ig-fold | Rel | 54 | 92 | 0.954 | 3.123 |
| **MIZF** | Zinc-coordinating | BetaBetaAlpha-zinc finger | 41 | 45 | 0.939 | 6.196 |
| **RREB1** | Zinc-coordinating | BetaBetaAlpha-zinc finger | 28 | 33 | 0.858 | 3.727 |
| **FOXF2** | Winged Helix-Turn-Helix | Forkhead | 69 | 102 | 0.822 | 5.467 |
| **Myb** | Helix-Turn-Helix | Myb | 204 | 1034 | 0.549 | 4.958 |
| **Tal1::Gata1** | Zipper-Type | Helix-Loop-Helix | 102 | 192 | 0.487 | 4.4 |
| **Pax6** | Helix-Turn-Helix | Homeo | 23 | 24 | 0.448 | 3.606 |
| **SPIB** | Winged Helix-Turn-Helix | Ets | 277 | 3020 | 0.331 | 14.922 |
| **Gfi** | Zinc-coordinating | BetaBetaAlpha-zinc finger | 217 | 1049 | 0.165 | 7.114 |
| **HNF4A** | Zinc-coordinating | Hormone-nuclear Receptor | 113 | 205 | 0.056 | 7.215 |
| **Ar** | Zinc-coordinating | Hormone-nuclear Receptor | 9 | 10 | -0.1 | 1.516 |

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|---|---|---|---|---|---|---|
| **Nkx3-2** | Helix-Turn-Helix | Homeo | 246 | 1653 | -0.184 | 9.21 |
| **ELK4** | Winged Helix-Turn-Helix | Ets | 100 | 170 | -0.187 | 5.87 |
| **NFYA** | Other Alpha-Helix | NFY CCAAT-binding | 98 | 163 | -0.213 | 6.992 |
| **ESR1** | Zinc-coordinating | Hormone-nuclear Receptor | 6 | 6 | -0.292 | 1.427 |
| **Nr2e3** | Zinc-coordinating | Hormone-nuclear Receptor | 113 | 217 | -0.508 | 6.129 |
| **STAT1** | Ig-fold | Stat | 106 | 177 | -0.517 | 6.67 |
| **GABPA** | Winged Helix-Turn-Helix | Ets | 151 | 351 | -0.714 | 8.983 |
| **ZNF354C** | Zinc-coordinating | BetaBetaAlpha-zinc finger | 267 | 3682 | -0.758 | 9.107 |
| **USF1** | Zipper-Type | Helix-Loop-Helix | 149 | 391 | -0.94 | 5.534 |
| **MYC::MAX** | Zipper-Type | Helix-Loop-Helix | 58 | 79 | -1.112 | 4.118 |
| **Ddit3::Cebpa** | Zipper-Type | Leucine Zipper | 94 | 155 | -1.271 | 5.781 |
| **PPARG** | Zinc-coordinating | Hormone-nuclear Receptor | 1 | 1 | -1.337 | 0.512 |
| **NHLH1** | Zipper-Type | Helix-Loop-Helix | 88 | 149 | -1.374 | 5.078 |
| **RXRA::VDR** | Zinc-coordinating | Hormone-nuclear Receptor | 8 | 8 | -1.383 | 1.327 |
| **EWSR1-FLI1** | Winged Helix-Turn-Helix | Ets | 3 | 3 | -1.487 | 0.788 |
| **Lhx3** | Helix-Turn-Helix | Homeo | 91 | 169 | -1.5 | 5.325 |
| **AP1** | Zipper-Type | Leucine Zipper | 255 | 1992 | -1.517 | 8.154 |
| **IRF2** | Winged Helix-Turn-Helix | IRF | 11 | 13 | -1.521 | 1.018 |
| **Spz1** | Other | Other | 98 | 221 | -1.639 | 1.563 |
| **HLF** | Zipper-Type | Leucine Zipper | 87 | 146 | -1.679 | 4.32 |
| **MZF1_1-4** | Zinc-coordinating | BetaBetaAlpha-zinc finger | 260 | 3158 | -1.864 | 10.733 |

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|---|---|---|---|---|---|---|
| **RUNX1** | Ig-fold | Runt | 217 | 1095 | -1.977 | 5.351 |
| **E2F1** | Winged Helix-Turn-Helix | E2F | 115 | 273 | -2.009 | 3.591 |
| **TEAD1** | Helix-Turn-Helix | Homeo | 84 | 136 | -2.041 | 4.236 |
| **Pax5** | Helix-Turn-Helix | Homeo | 18 | 22 | -2.12 | 1.306 |
| **Myc** | Zipper-Type | Helix-Loop-Helix | 131 | 304 | -2.146 | 6.194 |
| **TLX1::NFIC** | Helix-Turn-Helix::Other | Homeo::Nuclear Factor I-CCAAT-binding | 16 | 17 | -2.181 | 1.652 |
| **TP53** | Zinc-coordinating | Loop-Sheet-Helix | 0 | 0 | -2.268 | 0 |
| **Myf** | Zipper-Type | Helix-Loop-Helix | 148 | 455 | -2.332 | 2.935 |
| **NR4A2** | Zinc-coordinating | Hormone-nuclear Receptor | 232 | 1112 | -2.423 | 11.57 |
| **TAL1::TCF3** | Zipper-Type | Helix-Loop-Helix | 109 | 222 | -2.624 | 2.972 |
| **Tcfcp2l1** | Other | CP2 | 164 | 540 | -2.636 | 5.136 |
| **RELA** | Ig-fold | Rel | 105 | 209 | -2.639 | 3.448 |
| **znf143** | Zinc-coordinating | BetaBetaAlpha-zinc finger | 18 | 24 | -2.677 | 0.796 |
| **ELK1** | Winged Helix-Turn-Helix | Ets | 198 | 927 | -2.707 | 3.994 |
| **Hand1::Tcfe2a** | Zipper-Type | Helix-Loop-Helix | 206 | 964 | -2.722 | 4.279 |
| **Klf4** | Zinc-coordinating | BetaBetaAlpha-zinc finger | 219 | 1359 | -2.746 | 12.936 |
| **Arnt** | Zipper-Type | Helix-Loop-Helix | 105 | 222 | -2.827 | 3.718 |
| **MAX** | Zipper-Type | Helix-Loop-Helix | 128 | 266 | -3.016 | 5.247 |
| **NR1H2::RXRA** | Zinc-coordinating | Hormone-nuclear Receptor | 1 | 1 | -3.021 | 0.294 |
| **ESR2** | Zinc-coordinating | Hormone-nuclear Receptor | 37 | 45 | -3.658 | 2.18 |
| **RORA_2** | Zinc-coordinating | Hormone-nuclear Receptor | 56 | 74 | -4.052 | 2.998 |
| **NF-kappaB** | Ig-fold | Rel | 121 | 284 | -4.152 | 3.347 |

|  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|
| **EBF1** | Zipper-Type | Helix-Loop-Helix | 166 | 633 | -4.506 | 2.836 |
| **Mycn** | Zipper-Type | Helix-Loop-Helix | 126 | 293 | -4.645 | 4.532 |
| **REST** | Zinc-coordinating | BetaBetaAlpha-zinc finger | 2 | 2 | -4.774 | 0.289 |
| **Esrrb** | Zinc-coordinating | Hormone-nuclear Receptor | 170 | 458 | -4.829 | 6.914 |
| **HIF1A::ARNT** | Zipper-Type | Helix-Loop-Helix | 161 | 604 | -4.948 | 4.492 |
| **REL** | Ig-fold | Rel | 159 | 456 | -5.188 | 4.661 |
| **ZEB1** | Zinc-coordinating | BetaBetaAlpha-zinc finger | 264 | 2890 | -5.389 | 9.948 |
| **RORA_1** | Zinc-coordinating | Hormone-nuclear Receptor | 135 | 310 | -5.722 | 3.039 |
| **RXR::RAR_DR5** | Zinc-coordinating | Hormone-nuclear Receptor | 19 | 20 | -5.749 | 1.078 |
| **NFE2L2** | Zipper-Type | Leucine Zipper | 87 | 141 | -5.855 | 2.757 |
| **Zfp423** | Zinc-coordinating | BetaBetaAlpha-zinc finger | 81 | 150 | -5.89 | 2.23 |
| **Egr1** | Zinc-coordinating | BetaBetaAlpha-zinc finger | 82 | 158 | -6.43 | 1.969 |
| **Pax4** | Helix-Turn-Helix | Homeo | 0 | 0 | -6.728 | 0 |
| **Zfx** | Zinc-coordinating | BetaBetaAlpha-zinc finger | 149 | 427 | -7.532 | 8.335 |

## Table S4. qPCR primers

| Primer | Primer Sequence |
|---|---|
| Actb F | ACAACGGCTCCGGCATGTGCAAAG |
| Actb R | CATTCCCACCATCACACCCTGGTGC |
| Ampkb1 F | TCCGAAGAGATCAAGGCTCC |
| Ampkb1 R | GACTTCCTTTCCACCCCCTG |
| Atg5 F | TTTGGGCCATCAACCGGAAA |
| Atg5 R | CGCTCCGTCGTGGTCTGATA |
| Atg7 F | AGCGGCGACAGCATTAGGAT |
| Atg7 R | CCTCATGGCAGGAAAGCAGT |
| Atg12 F | CCTTAAACTGGTGGCCTCGG |
| Atg12 R | ACCATCACTGCCAAAACACTCA |
| Cox1 F | GGTCAACCAGGTGCACTTTT |
| Cox1 R | TGGGGCTCCGATTATTAGTG |
| Cox2 F | TGAAGACGTCCTCCACTCATGA |
| Cox2 R | GCCTGGGATGGCATCAGTT |
| Cox3 F | GCAGGATTCTTCTGAGCGTTCT |
| Cox3 R | GTCAGCAGCCTCCTAGATCATGT |
| Dram1 F | CTGTCGTCCCCATGATTGCC |
| Dram1 R | CAAAAGCCACGGTCCACTCA |
| Gadd45 F | TGGTGACGAACCCACATTCA |
| Gadd45 R | CGGGAGATTAATCACGGGCA |
| Ho-1 F | GGTCAGGTGTCCAGAGAAGG |
| Ho-1 R | CTTCCAGGGCCGTGTAGATA |
| Hprt1 F | GCGTTGGGCTTACCTCACTGCT |
| Hprt1 R | ATCGCTAATCACGACGCTGGG |
| Igfbp3 F | TAAGAAGAAGCAGTGCCGCC |
| Igfbp3 R | TTTCCCCTTGGTGTCGTAGC |
| Lamp 1 F | CAGTTTGGGATGAATGCCAGC |
| Lamp 1 R | TGGAGATGCTGAATGTGGGC |
| Lamp 2 F | CAAAAGGACAGTATTCTACAGCTCA |
| Lamp 2 R | TGATGGCGCTTGAGACCAAT |
| Map1lc3b F | TAATCAGACGGCGCTTGCAG |
| Map1lc3b R | CTCACCATGCTGTGCCCATT |
| Nd1 F | CCTTCGACCTGACCTGACAGAAGGA |
| Nd1 R | GATGCTCGGATCCATAGGAA |
| Nd5 F | GCTCTACCTCACCATCTCTTGC |
| Nd5 R | TCCAGTATGCTTACCTTGTTACG |
| Nqo1 F | TTCTCTGGCCGATTCAGAGT |
| Nqo1 R | GGCTGCTTGGAGCAAAATG |
| Nrf2 F | TTCCTCTGCTGCCATTAGTCAGTC |
| Nrf2 R | GCTCTTCCATTTCCGAGTCACTG |
| Pgc1a F | TTGCTAGCGGTCCTCACAGA |
| Pgc1a R | GGCTCTTCTGCCTCCTGA |
| Pgc1b F | CGCTCCAGGAGACTGAATCCAG |
| Pgc1b R | CTTGACTACTGTCTGTGAGGC |
| Pgk1 F | TCTTGGGAGGCGCTAAAGTTG |
| Pgk1 R | AAGGCCATTCCACCACCAAT |

| | |
|---|---|
| Pparg F | GGAAGACCACTCGCATTCCTT |
| Pparg R | TCGCACTTTGGTATTCTTGGAG |
| Pten F | GCGGAACTTGCAATCCTCAG |
| Pten R | ACATGAACTTGTCCTCCCGC |
| Sesn1 F | GACGGACTTTCAAACGCGG |
| Sesn1 R | TTCCAAGTTCCTCGACGCTC |
| Sesn2 F | CGCCACTCAGAGAAGGTTCA |
| Sesn2 R | ACGGGGTAGTCAGGTCATGT |
| Sqstm1/p62 F | GCCAGAGGAACAGATGGAGTC |
| Sqstm1/p62 R | AGCTTGGCCCTTCCGATTC |
| Tfam F | CCAAAAAGACCTCGTTCAGC |
| Tfam R | ATGTCTCCGGATCGTTTCAC |
| Tsc2 F | ACCATCAGGTCCGAAAGCTG |
| Tsc2 R | CGCGCCATCACCTTTTCAAT |

**Table S5. TaqMan qPCR primers**

| TaqMan primer | Primer Code |
|---|---|
| Actin beta | Mm01205647_g1 |
| Arnt | Mm00507836_m1 |
| Chmp1a | Mm00848170_s1 |
| Chmp1b | Mm00481091_s1 |
| Chmp2a | Mm01187331_g1 |
| Chmp2b | Mm00660653_m1 |
| Chmp3 | Mm01190252_m1 |
| Chmp4b | Mm00551493_m1 |
| Chmp4c | Mm00481500_m1 |
| Chmp5 | Mm00472587_m1 |
| Chmp6 | Mm01241413_g1 |
| Chmp7 | Mm00513649_m1 |
| Dkk1 | Mm00438422_m1 |
| Dkk2 | Mm01322146_m1 |
| Dkk3 | Mm00443800_m1 |
| Hprt1 | Mm01324427_m1 |
| Itih5 | Mm00552678_m1 |
| Pgk1 | Mm00435617_m1 |
| Serping1 | Mm00437834_m1 |
| | |
| Actin beta | Hs99999903_m1 |
| Chmp7 | Hs01061798_m1 |
| Pgk1 | Hs99999906_m1 |

## Supplementary Figure Legends

### Fig. S1. Differential gene expression from spinal and bulbar muscular atrophy and wild-type mice is specific to embryonic motor neurons.

(A) TaqMan qPCR was performed to determine mRNA expression of indicated genes using the "ddCT" method of relative comparison on WT, AR20 and AR100 motor neurons. *Itih5, Serping 1*, *Arnt* and *Chmp7* were dysregulated only in AR100 and not AR20 motor neurons treated with DHT, relative to WT (B) The values obtained using qPCR correlated with microarray data. (C) The differential expression of these genes in AR100 motor neurons was further affected by DHT. (D) qPCR was performed to determine mRNA expression of genes in WT and AR100 cells cultured from meninges removed from embryonic spinal cords (predominantly fibroblasts), with and without DHT treatment. The expression of genes is not altered in these cultures (F) Astroglial cultures did not demonstrate appreciable changes in mRNA expression and any changes in gene expression of *Serping 1* and *Arnt* were in the opposite direction to motor neurons. Data are displayed as mean ± SEM and are representative of independent experiments from three replicate cultures. Statistical analysis was performed using a one-way ANOVA followed by the Student–Newman–Keuls and Tukey's Honestly Significantly Different post hoc tests ($n \geq 3$, *$P<0.05$).

### Fig. S2. Gene ontology analysis of differentially expressed genes.

GO Slim analysis was performed on differentially expressed genes found to be altered in primary embryonic motor neurons from SBMA AR100 mice. In the Go slim biological process categories, significant genes were annotated to the signal transduction, anatomical structure development, transport, cell differentiation, cellular protein modification process, response to stress and cell death terms. In the molecular function categories, genes were mapped to the following terms: ion binding, signal transducer activity, DNA binding, enzyme binding, RNA binding and cytoskeletal protein binding

**Fig. S3. ClueGo protein-protein interaction network and CluePedia enrichment**

ClueGO analyses functional interaction of gene using GO information and KEGG, BioCarta and Reactome gene functional databases. A two-sided hypergeometric statistic was performed with kappa score threshold setting of 0.3. The kappa score measures the association strength between overlapping GO terms. Enrichment/depletion was calculated based on p-value corrected using the FDR method of Benjamini and Hochberg. CluePedia extends ClueGO functionality down to genes and miRNAs.

**Fig. S4. Gene set enrichment analysis and String protein-protein network generation.**

(A-B) GSEA analysis of gene expression data reveals upregulation of KEGG p53 pathway and enrichment of genes present in this pathway in AR100 motor neurons (shown in red) which were mapped using KEGG Mapper (https://www.genome.jp/kegg/mapper.html) and (B) enrichment of genes (in red) in KEGG Lysosome pathway.

**Fig. S5.** ***Chmp7*** **expression in models of motor neuron disease**

(A) qPCR was performed to determine the expression of *Chmp7* in the cortex of presymptomatic 3 month old AR100 and WT mice. (B) Spinal cord from presymptomatic 30 day old SOD1-G93A ALS mice was used to examine *Chmp7* expression**.** (C) qPCR was also used to examine expression of *Chmp7* in the spinal cord of presymptomatic 3 month old ALS4 SETX-R2136H mice. qPCR data are displayed as mean ± SEM and are representative of three independent experiments. Statistical analysis was performed using a two sample t-test (n=3, **P*<0.05).

**Fig. S6.** ***Lc3b*** **is upregulated in the spinal cord of SBMA mice**

(A) Genes associated with autophagy and the lysosome were examined using spinal cord from AR20 and AR100 presymptomatic mice of 3 months of age. Autophagy genes (*Atg5,* *Atg7, Atg12*, *Lc3b and p62*) were not altered in AR100 mice. (B) In spinal cord of 12 month old symptomatic AR100 mice, there was an increase in *Lc3b* expression. (C) LAMP2 and β-III tubulin staining of AR100 and AR20 spinal cord motor neurons. qPCR data are displayed as mean ± SEM and are representative of three independent experiments. Statistical analysis was performed using a two sample t-test ($n \geq 3$, $*P<0.05$)

**Fig. S7. Uncropped Western blot images.**

Uncropped Western blot images are displayed from Fig. 2F, G and I.

**Fig. S8. Aberrant CHMP7 localisation in AR100 SBMA mice**

Ventral horn spinal cord was immunostained with β-III tubulin and CHMP7. In presymptomatic 3 month old AR100 mice CHMP7 expression appeared reduced within motor neurons and was localised around the perinuclear region, while in WT mice CHMP7 displayed a more uniform pattern of staining within the cytoplasm and nucleus of motor neurons. Scale bars represent 20 μm.

**Fig. S1**

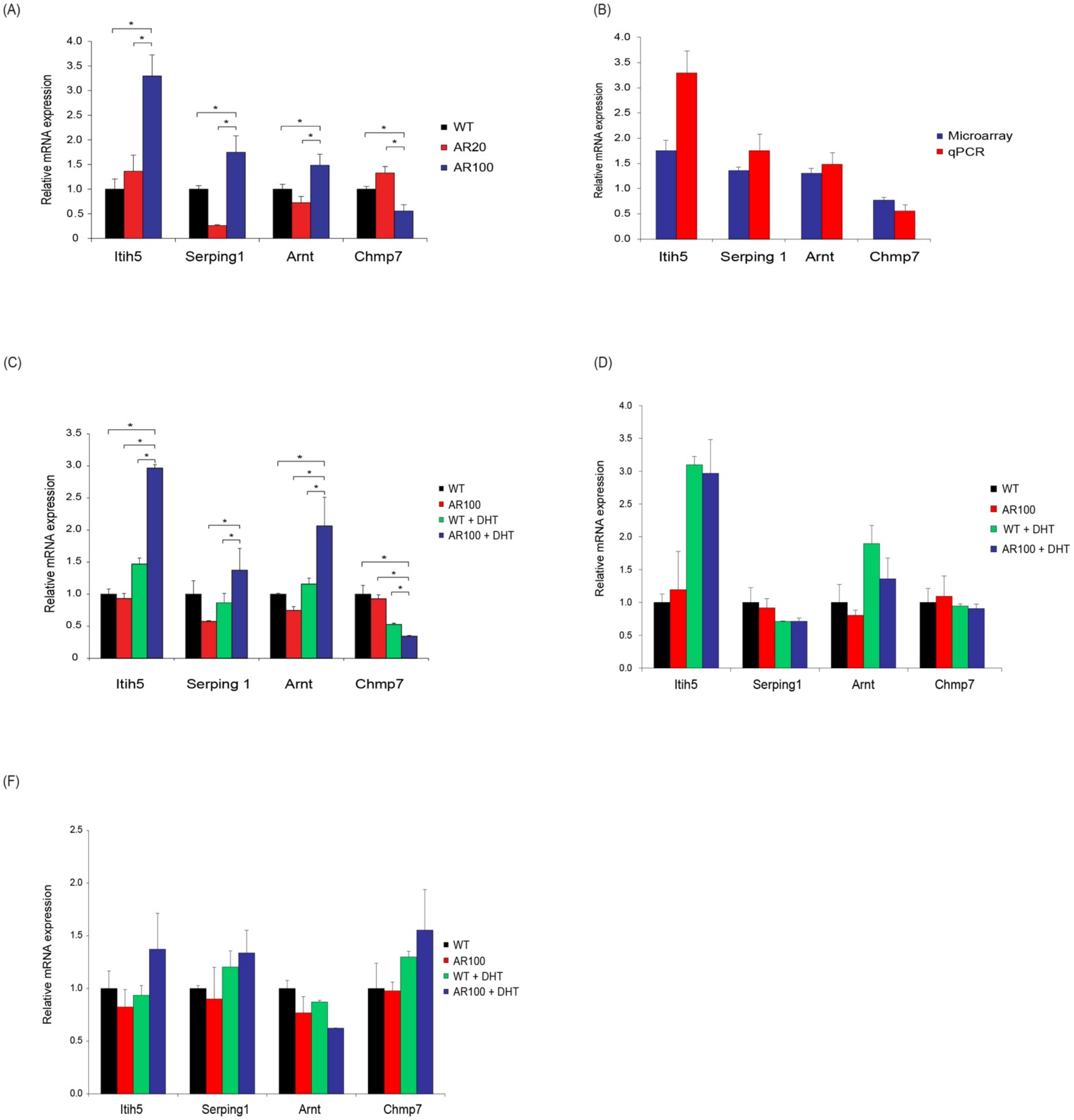
(A)
Relative mRNA expression
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
*
Itih5
Serping1
Arnt
Chmp7
WT
AR20
AR100
(B)
Relative mRNA expression
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Itih5
Serping 1
Arnt
Chmp7
Microarray
qPCR
(C)
Relative mRNA expression
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
*
Itih5
Serping 1
Arnt
Chmp7
WT
AR100
WT + DHT
AR100 + DHT
(D)
Relative mRNA expression
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Itih5
Serping1
Arnt
Chmp7
WT
AR100
WT + DHT
AR100 + DHT
(F)
Relative mRNA expression
2.5
2.0
1.5
1.0
0.5
0.0
Itih5
Serping1
Arnt
Chmp7
WT
AR100
WT + DHT
AR100 + DHT

**Fig. S2**

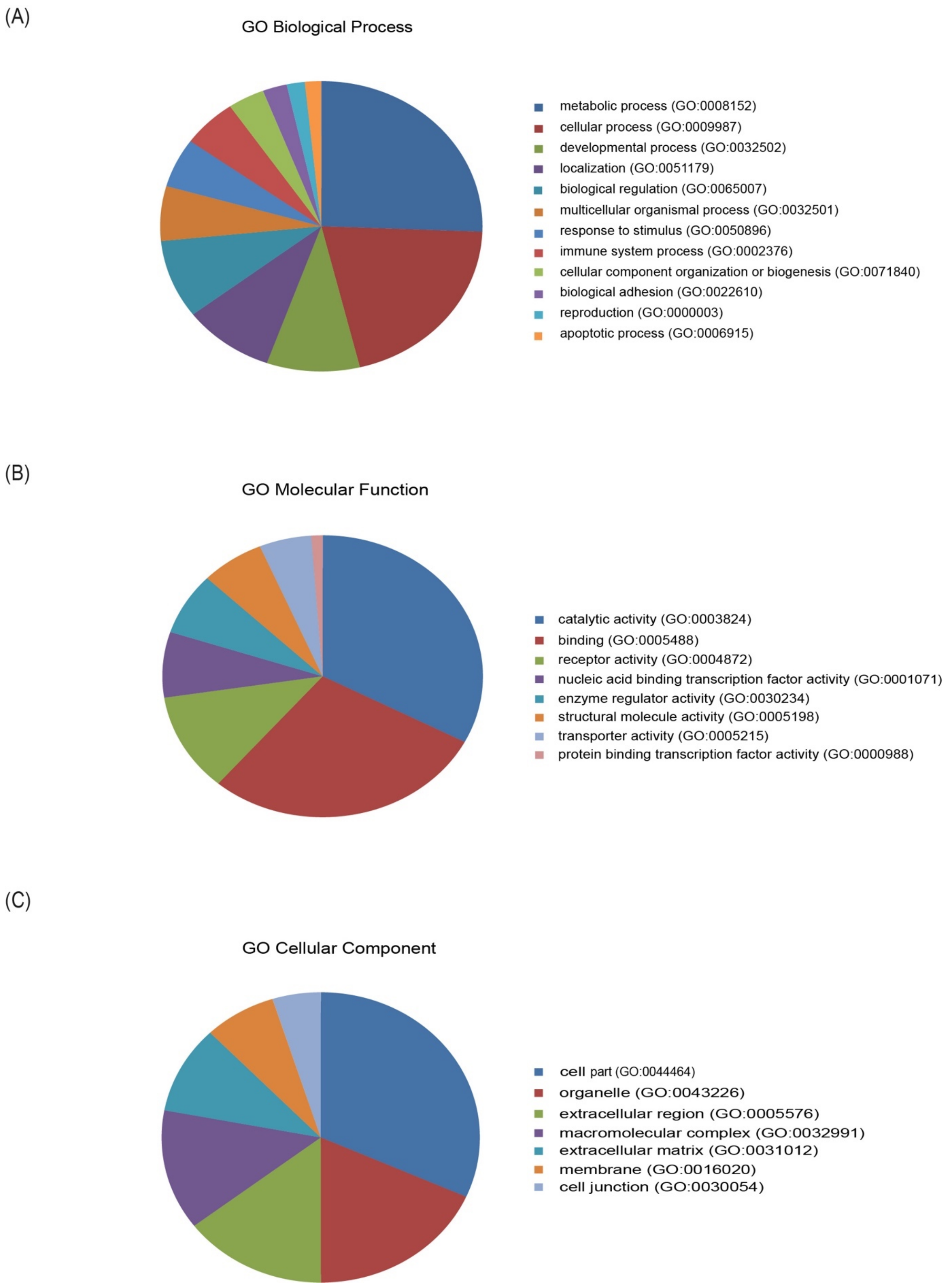
(A)
GO Biological Process
metabolic process (GO:0008152)
cellular process (GO:0009987)
developmental process (GO:0032502)
localization (GO:0051179)
biological regulation (GO:0065007)
multicellular organismal process (GO:0032501)
response to stimulus (GO:0050896)
immune system process (GO:0002376)
cellular component organization or biogenesis (GO:0071840)
biological adhesion (GO:0022610)
reproduction (GO:0000003)
apoptotic process (GO:0006915)
(B)
GO Molecular Function
catalytic activity (GO:0003824)
binding (GO:0005488)
receptor activity (GO:0004872)
nucleic acid binding transcription factor activity (GO:0001071)
enzyme regulator activity (GO:0030234)
structural molecule activity (GO:0005198)
transporter activity (GO:0005215)
protein binding transcription factor activity (GO:0000988)
(C)
GO Cellular Component
cell part (GO:0044464)
organelle (GO:0043226)
extracellular region (GO:0005576)
macromolecular complex (GO:0032991)
extracellular matrix (GO:0031012)
membrane (GO:0016020)
cell junction (GO:0030054)

**Fig. S3. (A) ClueGO protein-protein interaction network**

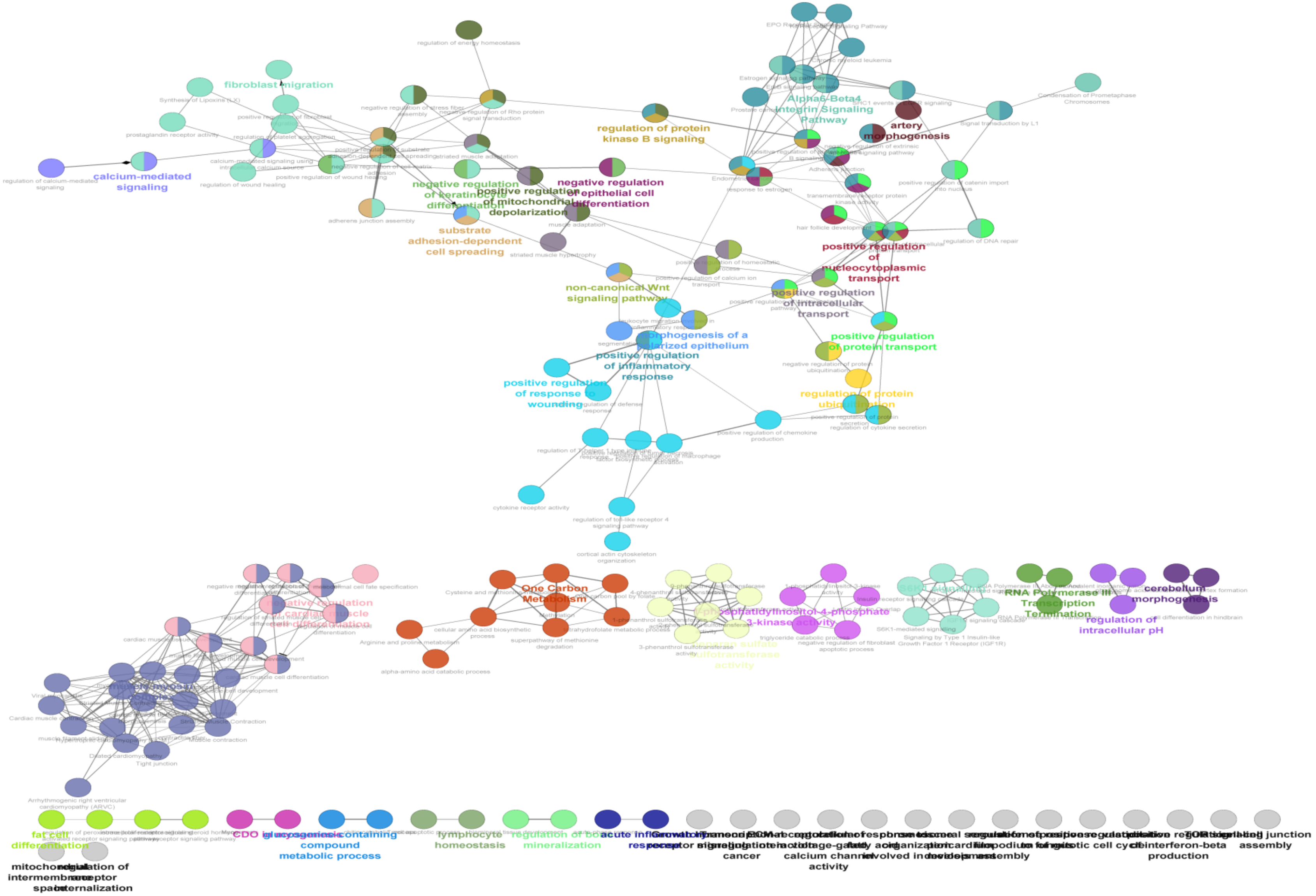

**Fig. S3. (B) CluePedia enrichment of ClueGO protein-protein interaction network**

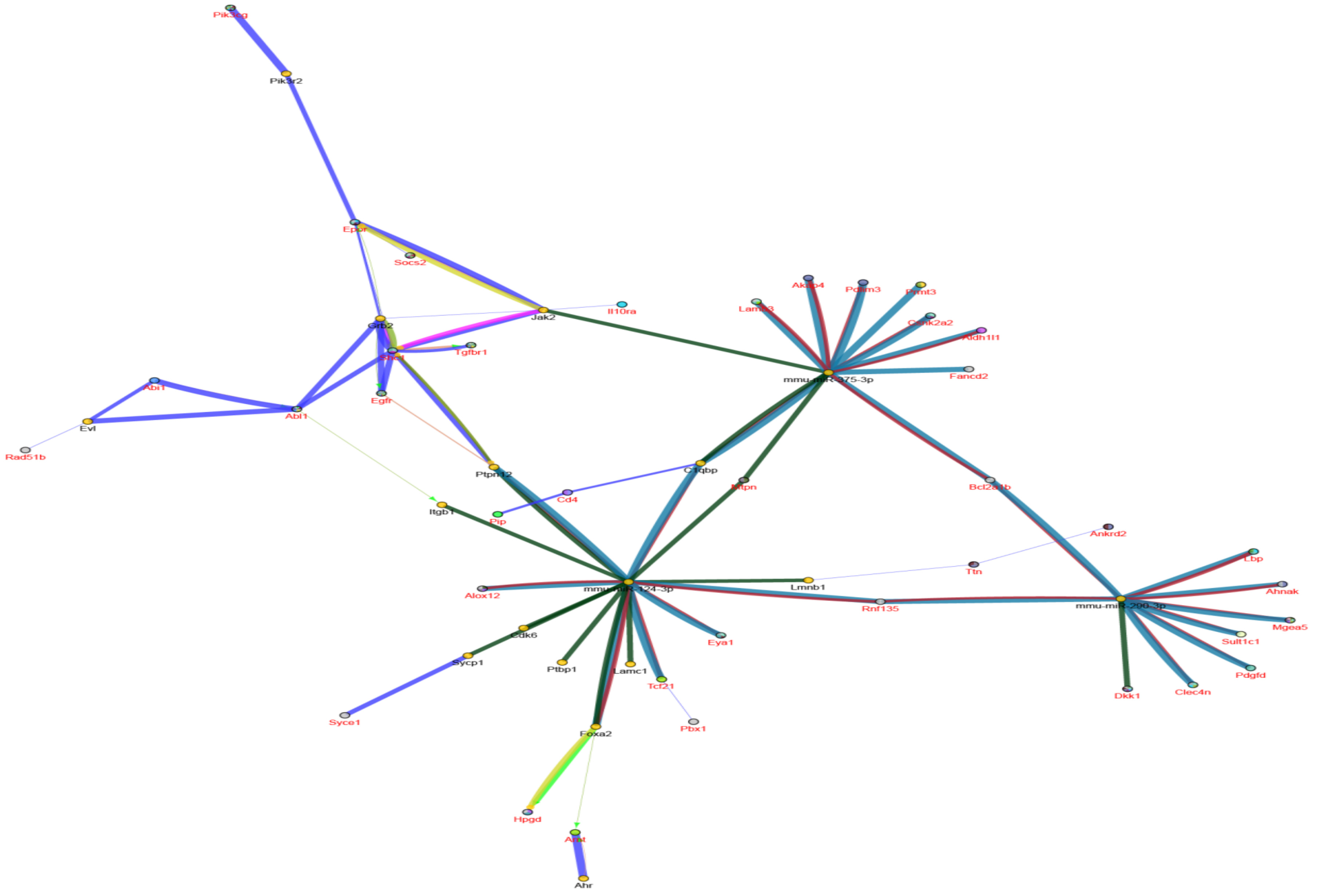

**Fig. S4. (A) GSEA p53 pathway analysis**

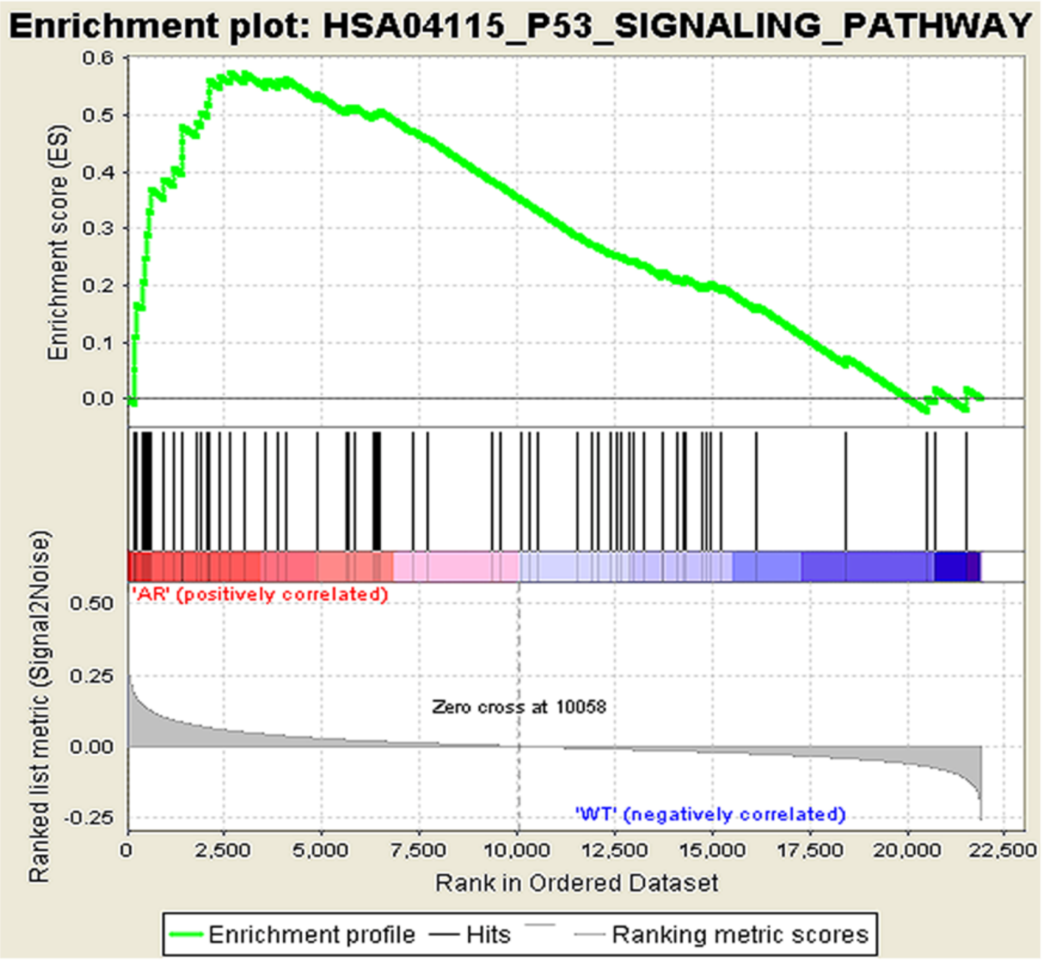

Fig. S4. (B) KEGG p53 pathway

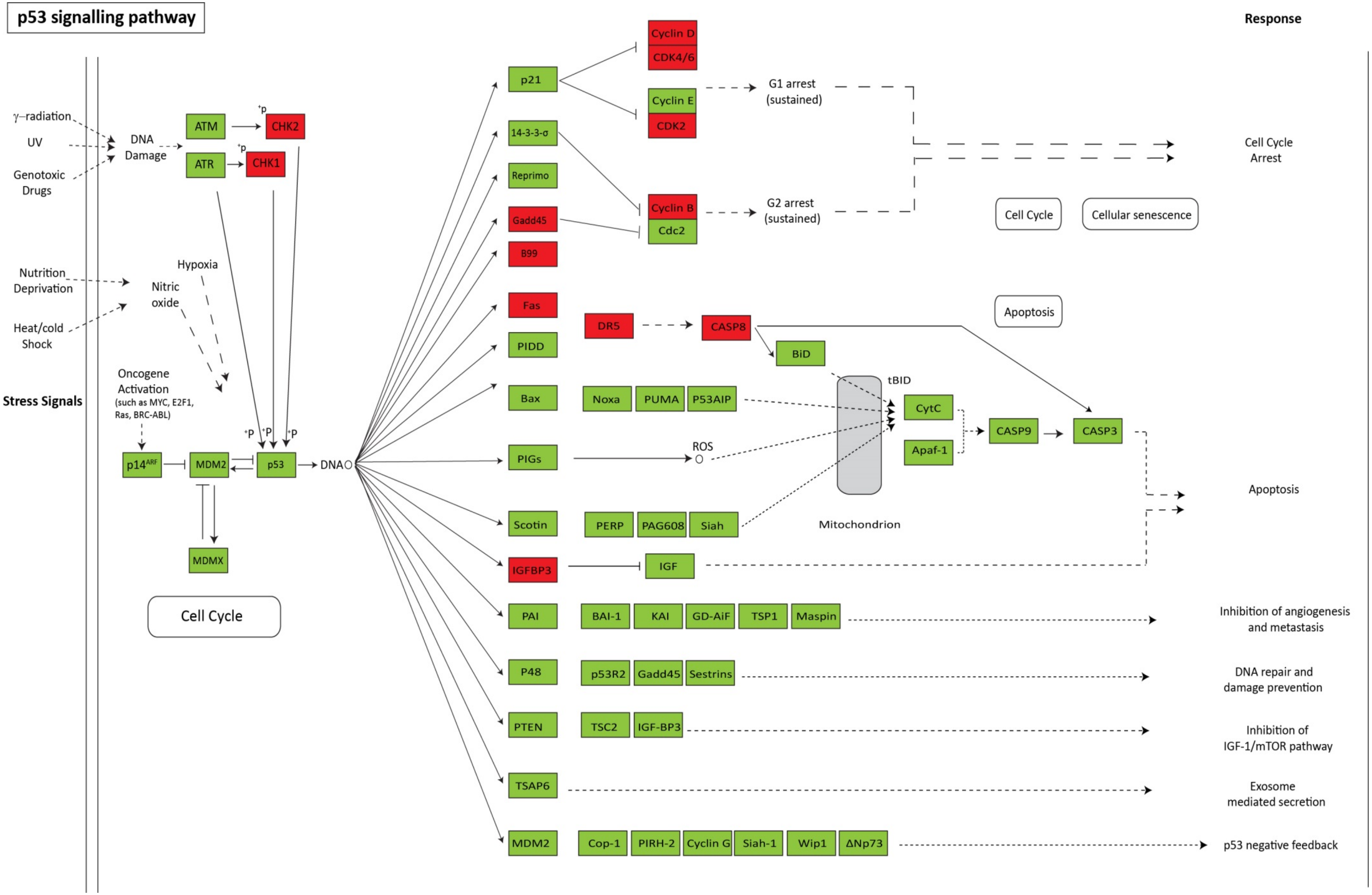

Fig. S4. (C) KEGG Lysosome

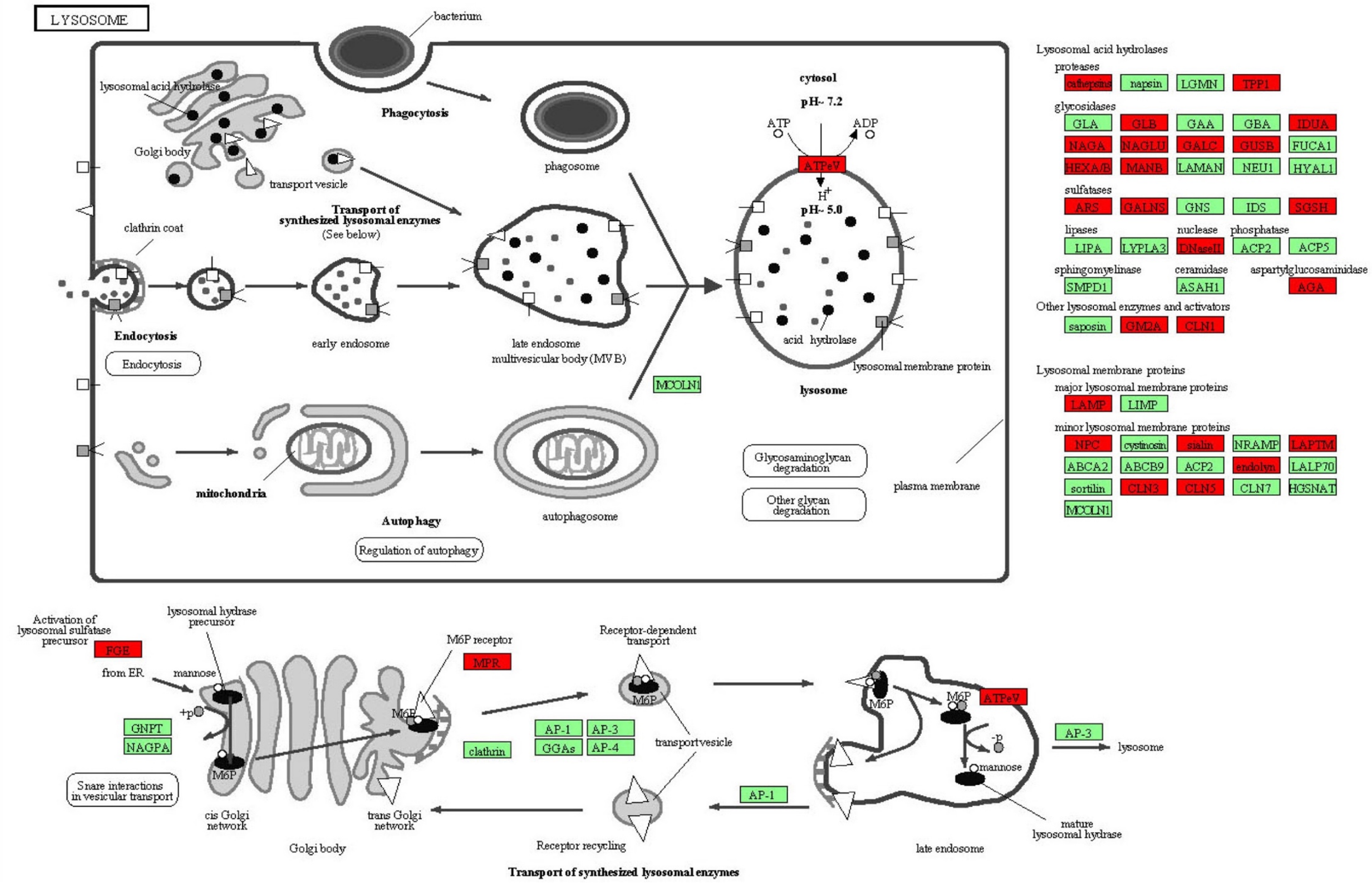

**Fig. S5**

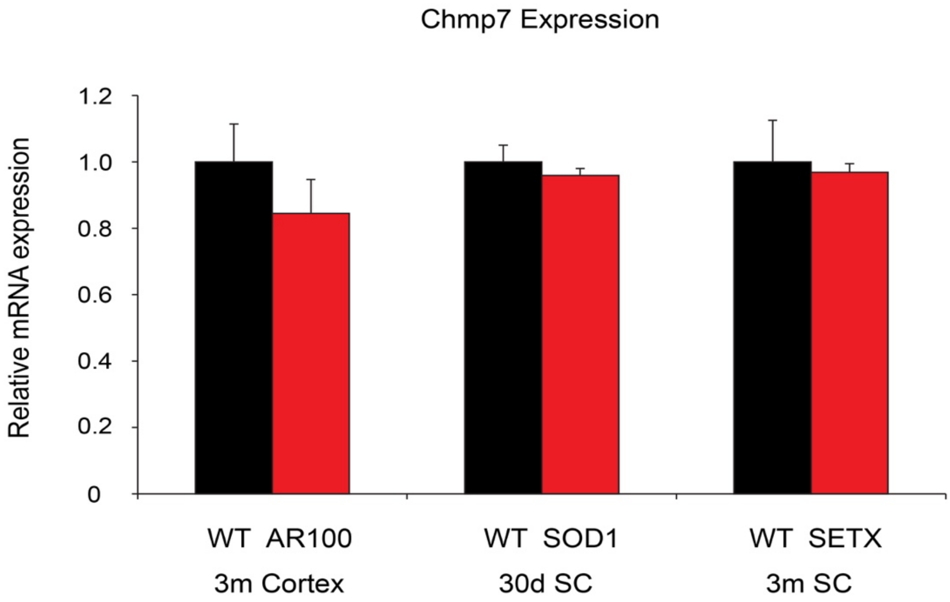
Chmp7 Expression
Relative mRNA expression
1.2
1.0
0.8
0.6
0.4
0.2
0
WT AR100
3m Cortex
WT SOD1
30d SC
WT SETX
3m SC

**Fig. S6**

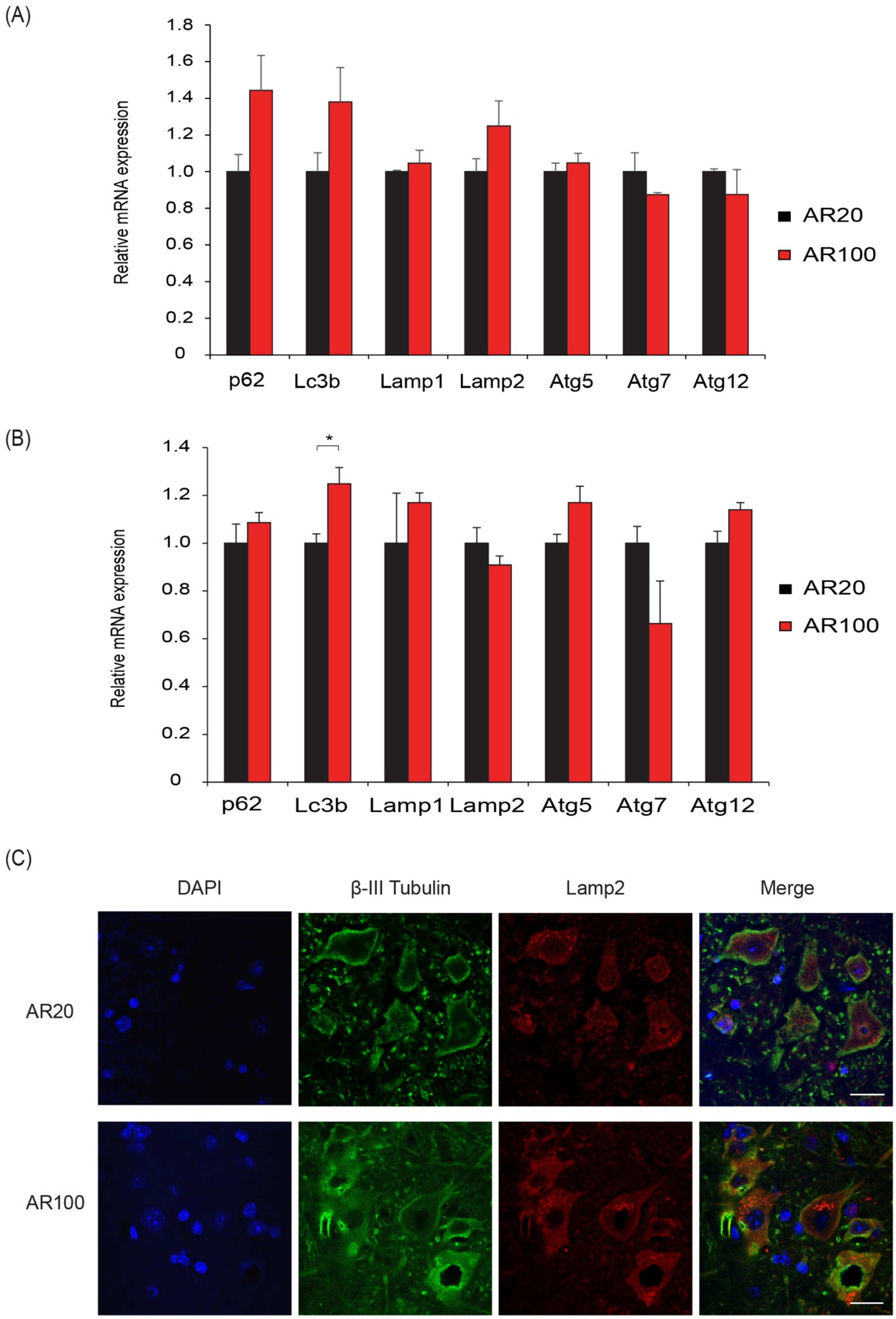
(A)
Relative mRNA expression
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
p62
Lc3b
Lamp1
Lamp2
Atg5
Atg7
Atg12
■ AR20
🟥 AR100
(B)
Relative mRNA expression
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
*
p62
Lc3b
Lamp1
Lamp2
Atg5
Atg7
Atg12
■ AR20
🟥 AR100
(C)
DAPI
β-III Tubulin
Lamp2
Merge
AR20
AR100

**Fig. S7.**

Fig. 2F

Fig. 2G

Fig. 2I

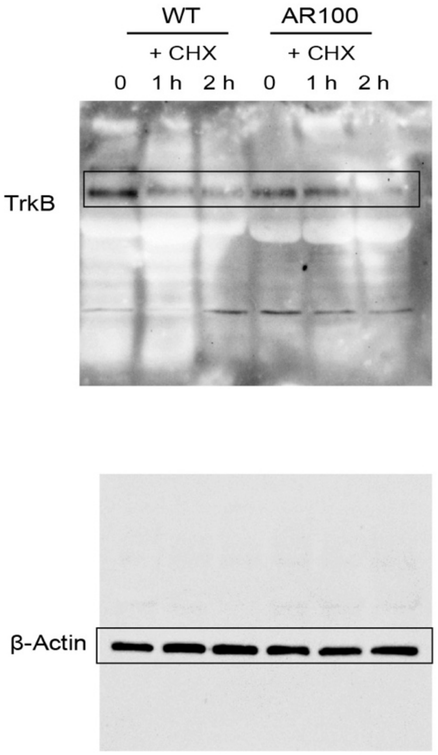

**Fig. S8.**