

Supporting Information

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SI Text

Experimental Procedures

Microarray Hybridization and Data Analysis. A genome-wide set (>38K) of long mouse oligonucleotides obtained from Illumina and printed at the Advanced Technology Center (National Cancer Institute) was used in this study. This set is based on well-annotated sequence information derived largely from the Mouse Exonic Evidence-Based Oligonucleotide consortium (MEEBO) (<http://mmc.ucsf.edu/Meebo.html>). The detailed procedure for target preparation and hybridization on microarrays is available at <http://nciarray.nci.nih.gov/reference/NCIReference.shtml>. Briefly, 10 μg of total RNA, αMSH - or ASP-treated, or from vehicle-treated melanocytes were retro-transcribed and labeled with Cy3 or Cy5 (Amersham Pharmacia Biotech) in an indirect manner. Labeled cDNAs from treated and from control cells were cohybridized in a dye-swap arrangement to eliminate dye bias. Three biological replicates for each condition were hybridized in this manner, resulting in 6 replicated hybridizations for each time point. Hybridized arrays were scanned at 10- μm resolution on a GenePix 4000B microarray scanner running GenePix 5.0 software (Axon Instruments) at variable photomultiplier tube voltages (pmt) to obtain maximal signal intensities with 1% probe saturation. A second scan was performed at a lower pmt to avoid any saturated spots to obtain accurate signal values of saturated spots at the higher pmt setting. The intensity data from these two scans allowed us to detect higher numbers of genes than by single scan. Array data (images and sample intensity files) were deposited at the NCI microarray database (mAdb, <http://nciarray.nci.nih.gov/index.shtml>), filtered and normalized. Our quality standard required each spot to have a signal to background ratio of at least 2 for one of the two channels and at least 50 foreground pixels. Cy3: Cy5 intensity ratios were normalized by median centering of ratios for each array. After inverting the ratios of the reciprocal experiments, data were transformed to logarithmic scale to the base 2 for statistical calculations. The data were analyzed separately for each of the pmt scans and then were merged into a single set. Merging of two data sets was done considering that the normalized expression ratios are independent of pmt setting and signal saturation results in lower fold change. Any duplicated probes between the two pmt settings were therefore singled out by selecting either low or high pmt that gave highest differential expression among all arrays. Differentially expressed genes between αMSH - or ASP-treated versus control samples were identified using one-sample *t*-tests. Treated-to-control sample ratios were calculated using geometric averages of the replicates. Changes in gene expression were considered significant (at $P \leq 0.01$) when up- or down-regulated by at least 1.5-fold by ASP and/or by αMSH , after averaging the replicates, and when data were present for at least half of the six replicates. Global expression changes are considered to be statistically significant, since the number of genes differentially expressed at $P < 0.01$ were found to be about 5-fold larger than estimated false discoveries at this *P* value. Since multiple oligo probes may map to the same locus link, the numbers of genes indicated in the Tables refer to the features on the array. Functional classification was performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID, release 2.1) online tools <http://david.abcc.ncifcrf.gov/home.jsp> (1, 2). The gene-enrichment of functional pathways was measured by determining the number of genes belonging to the pathway in the list of

significantly altered genes weighed against the total murine genome using the Fisher Exact test.

Principal Component Analysis (PCA). PCA was performed using Partek Pro 5.1 (Partek) to allow the visual representation of variance in replicate averaged gene expression data. The correlation matrix was minimized to obtain principal components (PC). The percentage of total variance covered by each PC is shown as % on the respective axis in the 3D scatter plot. Examination of global expression patterns by PCA of $\approx 16,400$ features having no missing data revealed clustering of samples by drug (data not shown). From these, a subset of 1,715 features altered by 1.5-fold at $P < 0.01$ either by ASP or αMSH treatment at any time point were selected. PCA of these features was used to study the variance between the ASP and αMSH and with time.

Hierarchical Clustering. Hierarchical clustering of expression patterns was performed using Cluster and Tree View programs (3) for visualization of ASP and αMSH treatment response together. Replicate averages of logarithmic ratios were used for agglomerative clustering. Genes were clustered using $1-\rho$ as the distance measure where ρ is the correlation coefficient and the nodes were joined by an average linkage algorithm. Treated to untreated expression ratios are shown by heat map using red and green color codes for up- and down-regulation, respectively. The experiments were ordered by drug and time in columns of the heat map for visualization of temporal patterns following treatment with ASP or αMSH .

Immunoblotting. Melanocytes were harvested and solubilized in M-PER mammalian protein extraction buffer (Pierce Biotechnology, Rockford, IL, USA) containing Protease Inhibitor mixture (Roche). Protein concentrations of extracts were measured using the BCA protein assay kit (Pierce). Cell extracts were separated by SDS/PAGE under reducing conditions. After electrophoresis, proteins were transferred to Invitrolon polyvinylidene difluoride membranes (Invitrogen). Antigens were detected using the following antibodies: Dct (αPEP8), Tyr (αPEP7), Tyrp1 (αPEP1), Mart-1 (αPEP27h), Matp (αPEP30), Si (αPEP13), all from our own laboratory, Mif (monoclonal 6A5, was a gift from Dr. Heinz Arnheiter), *Gpnmb* (AF2330, R&D Systems), Rab38 (Novus Biological), Rab27a (20.1, BD Biosciences), Tcf4 (0.T.149, Gene Tex), Lef-1 (1C3, Gene Tex), CyclinD (AM29, Invitrogen), β -actin (AC-15, Abcam), GAPDH (Fl-335, Santa Cruz Biotechnology), TcfAp2a (3B5c, Developmental Studies Hybridoma Bank), CTGF (ab51704, Abcam), EphB2 (AF467, R&D), EphrinB2 (AF496, R&D), ErbB3 (AF4518, R&D), Fak2/Pyk2 (YE353, Epitomics), MelCam (H-62, Santa Cruz), and N-cadherin (EPR1792Y, Epitomics).

Transwell Migration, Invasion, and Wound Healing Assays. Transwell migration assays were performed using transwell Boyden chambers (8 μm PET membrane, BD Biosciences) coated for 2 h with fibronectin (10 $\mu\text{g}/\text{ml}$), then rinsed with PBS. Cells were then serum deprived for 24 h; 75,000 cells were seeded in the upper chamber and incubated at 37 $^{\circ}\text{C}$ for 8 h in media containing 5% FBS, in presence of ASP (10 nM), αMSH (100 nM) or were left untreated (Control). Cells remaining on the upper side of the filter were removed using cotton swabs; migrated cells were fixed and stained with Diff-Quick Stain Kit (Dade Behring); the average number of 4 chambers per condition was plotted as a

percentage of the maximal number of migrated cells. Each assay was repeated 3 times with similar results.

Invasion assays were performed using Growth Factor Reduced Matrigel Invasion Chambers (BD Biosciences); after Matrigel rehydration, 150,000 cells were seeded in the upper chamber and incubated at 37 °C for the indicated times. Treatment and staining of the cells were as described for the migration assay.

For the wound-healing assay, B16-F1 cells were seeded into 6-well culture plates. When the cells had grown to 80% confluence, a sterilized tip was used to scratch the cell monolayer. Cells were fixed and stained after 24 h and micrographs of representative fields were taken using a Leica light microscope.

Colony Growth Assay. Melan-a and B16F1 cells were seeded in 6-well plates (80,000 cells/plate) and cultured for 10 or 8 days, respectively, in the presence or absence of ASP, after which cells were fixed with 3% paraformaldehyde and nuclei were stained with

0.4% crystal violet solution in 20% ethanol. Pictures were taken and the crystal violet was then solubilized in 10% acetic acid; optical density was measured by spectrophotometry at 610 nm.

PCR Analysis. Total RNA was reverse-transcribed using SuperScript III (Invitrogen), as described above. PCR was performed using Takara's Ex Taq HS (Takara) DNA Polymerase and conditions: 5 min at 94 °C, (45 s at 94 °C, 60 s at 60 °C, and 2 min at 72 °C) × 30 or 35. Primers were as follows: Tcfap2a-sense 5'-CGGGTCGCCT-GTCGCTCCTCA-3'; Tcfap2a-antisense 5'-TTTCTTGCCACTT-GCTCATTG-3'; CTGF-sense 5'-TCGGTGTACCGCAGGTGA-GTC-3'; CTGF-antisense 5'-TCCACTCTGTGGTCTGGACC-AGG-3'; ErbB3-sense 5'-CTTACGGGACACAATGCTGA-3'; ErbB3-antisense; 5'-GGCAAACTTCCCATCGTAGA; Efnb2-sense 5'-ATATCTACATCAAATGGGTCTTTGG-3'; Efnb2-antisense 5'-TGCGGTGCTCCTGCGGTACTTGAGC-3'; N-cad-sense 5'-ACTGAGGAGCCGATGAAGGAACCAC-3'; N-cad-antisense 5'-GTTGATGATGAAGATGCCCGTTGGA-3'.

1. Dennis G, Jr, et al. (2003) DAVID: Database for annotation, visualization, and integrated discovery. *Genome Biol* 4:3.
2. Hosack DA, et al. (2003) Identifying biological themes within lists of genes with EASE. *Genome Biol* 4:R70.
3. Eisen MB, Spellman PT, Brown PO, Botstein D (1998) Cluster analysis and display of genome-wide expression patterns. *Proc Natl Acad Sci USA* 95:14863–14868.

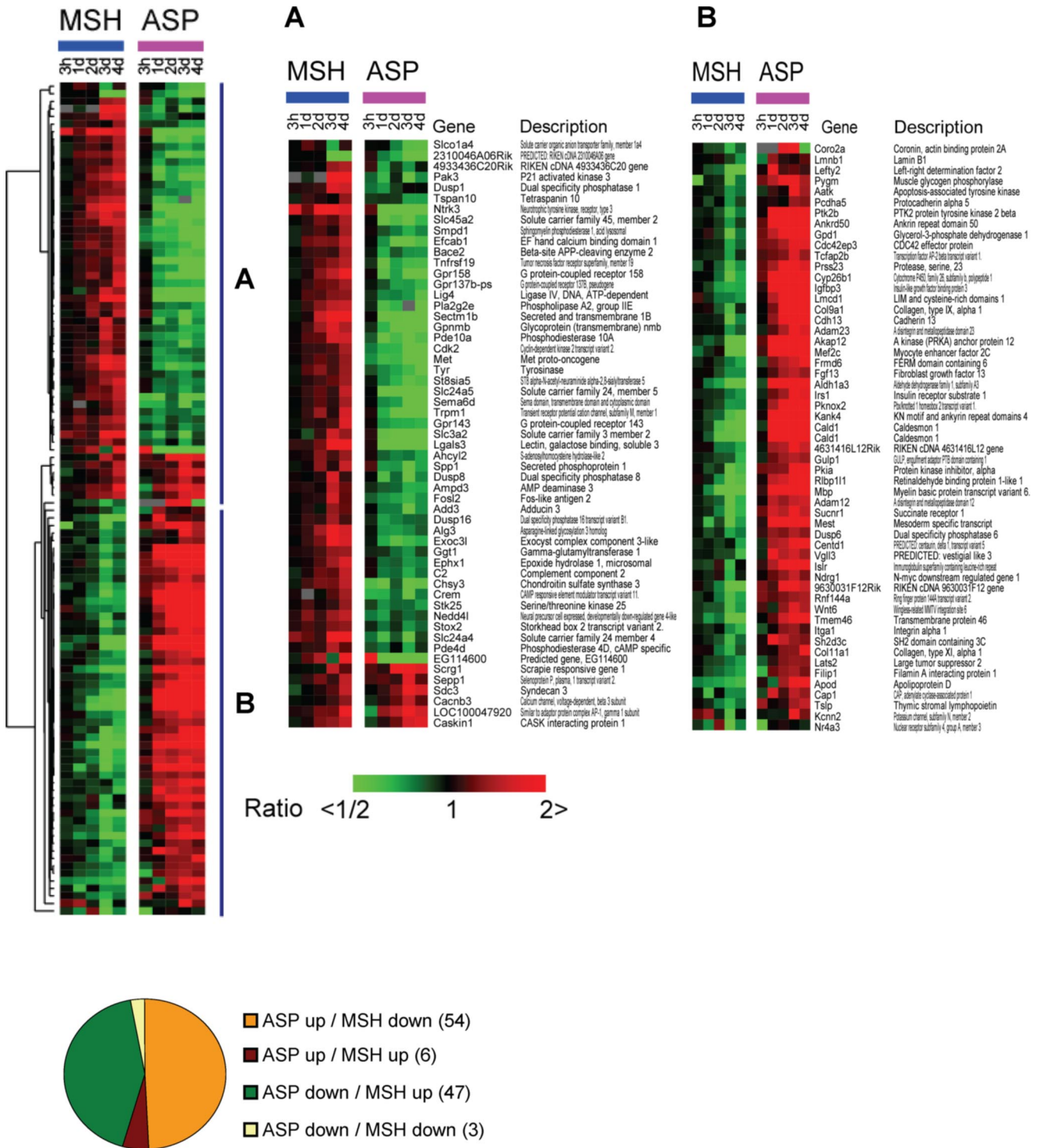


Fig. S2. Hierarchical clustering of genes significantly altered by both ASP and α MSH for at least one time point. The opposite regulatory effects of Mc1r agonist and inverse agonist, as expected, are emphasized by the heat map, as well as differences between the various times of treatment. Subclusters of genes up-regulated by α MSH for which 89% were down-regulated by ASP; and of genes down-regulated by α MSH for which 95% were up-regulated by ASP are shown. The log₂-fold changes of gene ratios are color coded as shown in the bar.

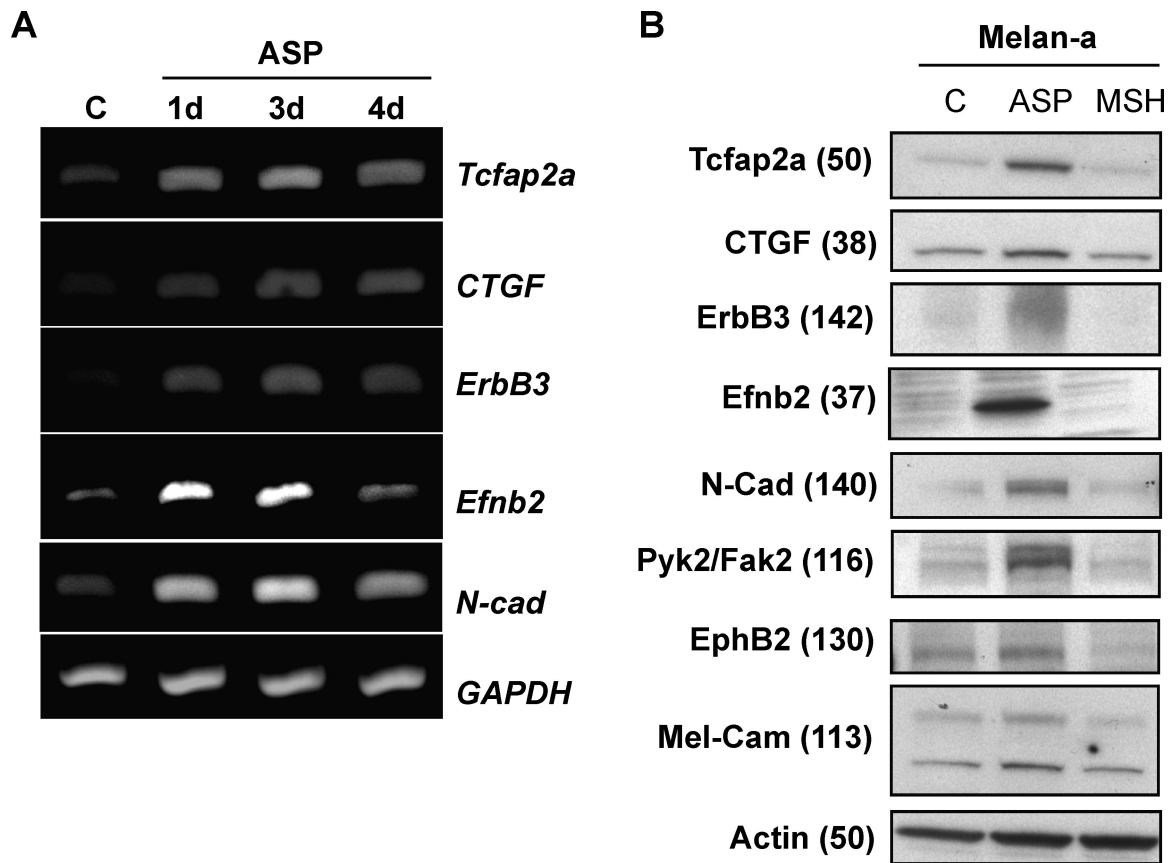


Fig. S3. Validation of the inducing effects of ASP on developmental genes and their products. (A) Semiquantitative PCR was performed on *Tcfap2a*, *CTGF*, *ErbB3*, *Efnb2* and *N-cadherin* in melan-a melanocytes treated with ASP for the different times indicated to confirm their mRNA expression levels. (B) Protein expression levels of those genes, as well as *Pyk2/Fak2*, *EphB2* and *Mel-Cam* (all involved in cell adhesion or migration during development), were investigated using immunoblotting after 3 days of treatment with ASP or α MSH. The concomitant induction of these molecules reveals novel targets of Mc1r signaling.

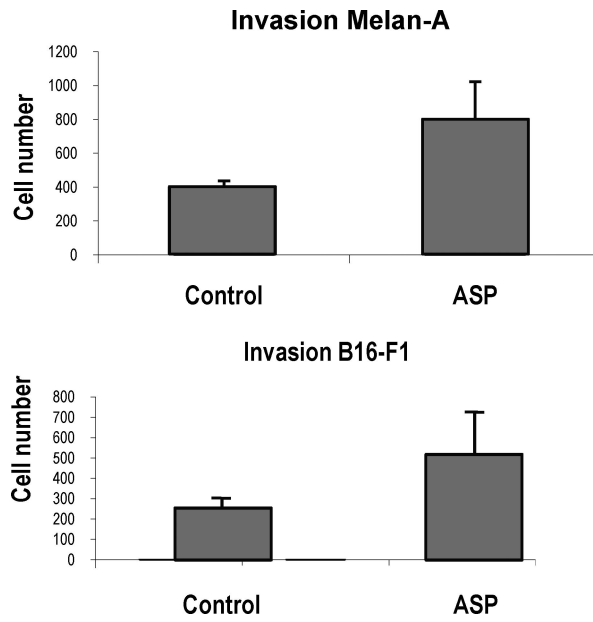


Fig. S4. ASP increases the invasiveness of melan-a melanocytes and of B16F1 melanoma cells. Cells were seeded in Matrigel Invasion Chambers in presence of ASP or were left untreated (Control). Melan-a and B16F1 cells were incubated at 37 °C for 24 or 8 h, respectively, and then were fixed and stained. Data are expressed as number of cells and are representative of at least 2 experiments.

Table S1. Detailed analysis using DAVID of over-represented GO Biological Process (BP), molecular function (MF), cellular component (CC), and Kegg pathways of the 110 genes significantly altered by both ASP and α MSH (treated/control ratio ≥ 1.5 ($P < 0.01$) for at least 1 time point over 5

Category	Term	Count	%	P value	Genes	List total	Pop hits	Pop total	Fold enrichment
GOTERM_BP_ALL	GO:0006796~phosphate metabolic process	12	11.11%	0.0117	Aatk, Met, Dusp6, Dusp8, Ptk2b, Ntrk3, Pak3, Cdk2, Dusp1, Igfbp3, Lats2, Dusp16	84	912	14977	2.3
GOTERM_BP_ALL	GO:0006793~phosphorus metabolic process	12	11.11%	0.0117	Aatk, Met, Dusp6, Dusp8, Ptk2b, Ntrk3, Pak3, Cdk2, Dusp1, Igfbp3, Lats2, Dusp16	84	912	14977	2.3
GOTERM_BP_ALL	GO:0048856~anatomical structure development	20	18.52%	0.0159	Cap1, Cdc42ep3, Farp2, Col11a1, Igfbp3, Lgals3, Sema6d, Mbp, Met, Wnt6, Lig4, Spp1, Cyp26b1, Nr4a3, Lefty2, Sepp1, Tcfap2b, Aldh1a3, Ntrk3, Mef2c	84	2058	14977	1.7
GOTERM_BP_ALL	GO:0043687~post-translational protein modification	15	13.89%	0.0255	Dusp6, Dusp8, Ptk2b, Pak3, Cdk2, Dusp16, Igfbp3, Rnf144, Aatk, Met, Nedd4l, Ntrk3, Bace2, Dusp1, Lats2	84	1435	14977	1.9
GOTERM_BP_ALL	GO:0033673~negative regulation of kinase activity	3	2.78%	0.0258	Pkia, Lats2, Dusp16	84	45	14977	11.9
GOTERM_BP_ALL	GO:0006469~negative regulation of protein kinase activity	3	2.78%	0.0258	Pkia, Lats2, Dusp16	84	45	14977	11.9
GOTERM_BP_ALL	GO:0051348~negative regulation of transferase activity	3	2.78%	0.0279	Pkia, Lats2, Dusp16	84	47	14977	11.4
GOTERM_BP_ALL	GO:0006583~melanin biosynthetic process from tyrosine	2	1.85%	0.0328	Slc45a2, Tyr	84	6	14977	59.4
GOTERM_BP_ALL	GO:0007399~nervous system development	9	8.33%	0.0329	Sema6d, Mbp, Met, Lig4, Sepp1, Farp2, Aldh1a3, Ntrk3, Mef2c	84	674	14977	2.4
GOTERM_BP_ALL	GO:0006470~protein amino acid dephosphorylation	4	3.70%	0.0342	Dusp6, Dusp8, Dusp1, Dusp16	84	128	14977	5.6
GOTERM_BP_ALL	GO:0022610~biological adhesion	9	8.33%	0.0342	Gpnmb, Adam12, Itga1, Spp1, Cdh13, Col9a1, Col11a1, Adam23, Pcdha5	84	679	14977	2.4
GOTERM_BP_ALL	GO:0007155~cell adhesion	9	8.33%	0.0342	Gpnmb, Adam12, Itga1, Spp1, Cdh13, Col9a1, Col11a1, Adam23, Pcdha5	84	679	14977	2.4
GOTERM_BP_ALL	GO:0006464~protein modification process	16	14.81%	0.0360	Dusp6, Dusp8, Ptk2b, St8 sia5, Pak3, Cdk2, Dusp16, Igfbp3, Aatk, Rnf144, Met, Nedd4l, Ntrk3, Bace2, Dusp1, Lats2	84	1648	14977	1.7
GOTERM_BP_ALL	GO:0032502~developmental process	25	23.15%	0.0368	Cdc42ep3, Col11a1, Pak3, Lgals3, Igfbp3, Aatk, Met, Wnt6, Lig4, Nr4a3, Sepp1, Tcfap2b, Mef2c, Cap1, Gulp1, Ndr1, Farp2, Slc45a2, Mbp, Sema6d, Cyp26b1, Spp1, Lefty2, Ntrk3, Aldh1a3	84	3028	14977	1.5
GOTERM_BP_ALL	GO:0043086~negative regulation of catalytic activity	3	2.78%	0.0424	Pkia, Lats2, Dusp16	84	59	14977	9.1
GOTERM_BP_ALL	GO:0016311~dephosphorylation	4	3.70%	0.0466	Dusp6, Dusp8, Dusp1, Dusp16	84	145	14977	4.9
GOTERM_BP_ALL	GO:0043412~biopolymer modification	16	14.81%	0.0489	Dusp6, Dusp8, Ptk2b, St8 sia5, Pak3, Cdk2, Dusp16, Igfbp3, Aatk, Rnf144, Met, Nedd4l, Ntrk3, Bace2, Dusp1, Lats2	84	1717	14977	1.7
GOTERM_BP_ALL	GO:0050790~regulation of catalytic activity	6	5.56%	0.0495	Cap1, Met, Centd1, Pkia, Lats2, Dusp16	84	360	14977	3.0
GOTERM_BP_ALL	GO:0006582~melanin metabolic process	2	1.85%	0.0541	Slc45a2, Tyr	84	10	14977	35.7
GOTERM_BP_ALL	GO:0042573~retinoic acid metabolic process	2	1.85%	0.0541	Cyp26b1, Aldh1a3	84	10	14977	35.7

Category	Term	Count	%	P value	Genes	List total	Pop hits	Pop total	Fold enrichment
GOTERM_BP_ALL	GO:0042438~melanin biosynthetic process	2	1.85%	0.0541	Slc45a2, Tyr	84	10	14977	35.7
GOTERM_BP_ALL	GO:0048731~system development	16	14.81%	0.0559	Farp2, Col11a1a, Igfbp3, Lgals3, Mbp, Sema6d, Met, Wnt6, Lig4, Spp1, Nr4a3, Sepp1, Tcfap2b, Aldh1a3, Ntrk3, Mef2c	84	1749	14977	1.6
GOTERM_BP_ALL	GO:0045859~regulation of protein kinase activity	4	3.70%	0.0647	Met, Pkia, Lats2, Dusp16	84	166	14977	4.3
GOTERM_BP_ALL	GO:0006811~ion transport	9	8.33%	0.0684	Slco1a4, Slc24a4, Trpm1, Col9a1, Slc24a5, Col11a1, Kcnn2, Cacnb3, Cdk2	84	784	14977	2.0
GOTERM_BP_ALL	GO:0006813~potassium ion transport	4	3.70%	0.0694	Slc24a4, Slc24a5, Kcnn2, Cdk2	84	171	14977	4.2
GOTERM_BP_ALL	GO:0006570~tyrosine metabolic process	2	1.85%	0.0697	Slc45a2, Tyr	84	13	14977	27.4
GOTERM_BP_ALL	GO:0007275~multicellular organismal development	19	17.59%	0.0702	Farp2, Col11a1a, Pak3, Igfbp3, Lgals3, Sema6d, Mbp, Met, Wnt6, Lig4, Spp1, Cyp26b1, Nr4a3, Lefty2, Sepp1, Tcfap2b, Aldh1a3, Ntrk3, Mef2c	84	2270	14977	1.5
GOTERM_BP_ALL	GO:0043549~regulation of kinase activity	4	3.70%	0.0713	Met, Pkia, Lats2, Dusp16	84	173	14977	4.1
GOTERM_BP_ALL	GO:0006468~protein amino acid phosphorylation	8	7.41%	0.0730	Aatk, Met, Ptk2b, Ntrk3, Pak3, Cdk2, Igfbp3, Lats2	84	660	14977	2.2
GOTERM_BP_ALL	GO:0051338~regulation of transferase activity	4	3.70%	0.0772	Met, Pkia, Lats2, Dusp16	84	179	14977	4.0
GOTERM_BP_ALL	GO:0019538~protein metabolic process	25	23.15%	0.0786	Ephx1, Dusp6, Dusp8, Col11a1a, Pak3, Cdk2, Dusp16, Igfbp3, Rnf144, Aatk, Adam12, Met, Ap1 g1, Nedd4l, Prss23, Bace2, Mest, Adam23, Lats2, Ptk2b, St8 sia5, Pygm, C2, Ntrk3, Dusp1	84	3268	14977	1.4
GOTERM_BP_ALL	GO:0007243~protein kinase cascade	5	4.63%	0.0797	Irs1, Met, Fgf13, Lats2, Dusp16	84	293	14977	3.0
GOTERM_BP_ALL	GO:0065009~regulation of a molecular function	6	5.56%	0.0804	Cap1, Met, Centd1, Pkia, Lats2, Dusp16	84	415	14977	2.6
GOTERM_BP_ALL	GO:0007169~transmembrane receptor protein tyrosine kinase signaling pathway	4	3.70%	0.0844	Irs1, Met, Ptk2b, Ntrk3	84	186	14977	3.8
GOTERM_BP_ALL	GO:0001501~skeletal development	4	3.70%	0.0854	Spp1, Col11a1a, Mef2c, Lgals3	84	187	14977	3.8
GOTERM_BP_ALL	GO:0009888~tissue development	5	4.63%	0.0868	Spp1, Nr4a3, Ntrk3, Col11a1a, Mef2c	84	302	14977	3.0
GOTERM_BP_ALL	GO:0048513~organ development	13	12.04%	0.0899	Col11a1a, Lgals3, Igfbp3, Met, Wnt6, Lig4, Spp1, Nr4a3, Sepp1, Tcfap2b, Ntrk3, Aldh1a3, Mef2c	84	1416	14977	1.6
GOTERM_BP_ALL	GO:0042445~hormone metabolic process	3	2.78%	0.0923	Cyp26b1, Aldh1a3, Bace2	84	92	14977	5.8
GOTERM_BP_ALL	GO:0044260~cellular macromolecule metabolic process	24	22.22%	0.0933	Dusp6, Ephx1, Dusp8, Ptk2b, St8 sia5, Col11a1a, Pak3, Cdk2, Igfbp3, Dusp16, Aatk, Rnf144, Met, Adam12, Pygm, Nedd4l, C2, Bace2, Prss23, Ntrk3, Mest, Adam23, Dusp1, Lats2	84	3165	14977	1.4
GOTERM_CC_ALL	GO:0044421~extracellular region part	23	21.30%	0.0037	Apod, Ephx1, Islr, Col11a1a, Igfbp3, Lgals3, Tnfrsf19, Met, Adam12, Wnt6, Spp1, Lefty2, Sepp1, Tyr, C2, Col9a1, Gpr143, Tslp, Bace2, Prss23, Ntrk3, Mest, Scrg1	89	2195	15845	1.9

Category	Term	Count	%	P value	Genes	List total	Pop hits	Pop total	Fold enrichment
GOTERM_CC_ALL	GO:0005576~extracellular region	23	21.30%	0.0094	Apod, Ephx1, Islr, Col11a1, Igfbp3, Lgals3, Tnfrsf19, Met, Adam12, Wnt6, Spp1, Lefty2, Sepp1, Tyr, C2, Col9a1, Gpr143, Tslp, Bace2, Prss23, Ntrk3, Mest, Scrg1	89	2375	15845	1.7
GOTERM_CC_ALL	GO:0005856~cytoskeleton	12	11.11%	0.0167	Cap1, Lmn1b1, Akap12, Cdc42ep3, Frmd6, Pknox2, Ptk2b, Cald1, Pde4d, Farp2, Add3, Lats2	89	957	15845	2.2
GOTERM_CC_ALL	GO:0005615~extracellular space	20	18.52%	0.0172	Apod, Ephx1, Islr, Igfbp3, Tnfrsf19, Met, Wnt6, Spp1, Lefty2, Sepp1, Tyr, C2, Col9a1, Gpr143, Tslp, Prss23, Ntrk3, Bace2, Mest, Scrg1	89	2064	15845	1.7
GOTERM_CC_ALL	GO:0005624~membrane fraction	8	7.41%	0.0175	Irs1, Ephx1, Met, Slco1a4, Cyp26b1, Cald1, Bace2, Pcdha5	89	481	15845	3.0
GOTERM_CC_ALL	GO:0005578~proteinaceous extracellular matrix	6	5.56%	0.0242	Adam12, Wnt6, Spp1, Col9a1, Col11a1, Lgals3	89	295	15845	3.6
GOTERM_CC_ALL	GO:0031012~extracellular matrix	6	5.56%	0.0268	Adam12, Wnt6, Spp1, Col9a1, Col11a1, Lgals3	89	303	15845	3.5
GOTERM_CC_ALL	GO:0000267~cell fraction	8	7.41%	0.0323	Irs1, Ephx1, Met, Slco1a4, Cyp26b1, Cald1, Bace2, Pcdha5	89	547	15845	2.6
GOTERM_CC_ALL	GO:0030864~cortical actin cytoskeleton	2	1.85%	0.0802	Cap1, Cald1	89	15	15845	23.7
GOTERM_CC_ALL	GO:0005737~cytoplasm	39	36.11%	0.0916	Dusp6, Ephx1, Cdc42ep3, Smpd1, Frmd6, Dusp8, Fgf13, Caskin1, Col11a1, Dusp16, Lgals3, Aatk, Ap1 g1, Nedd4l, Tyr, Cald1, Alg3, Lats2, Cap1, Gulp1, Akap12, Ndr1, Centd1, Ptk2b, Pde4d, Sh2d3c, Farp2, Add3, St8 sia5, Mbp, Irs1, Gpnmb, Pygm, Spp1, Pknox2, Col9a1, Aldh1a3, Ntrk3, Gpd1	89	5672	15845	1.2
GOTERM_MF_ALL	GO:0017017~MAP kinase tyrosine/serine/threonine phosphatase activity	4	3.70%	0.0000	Dusp6, Dusp8, Dusp1, Dusp16	92	10	16377	71.2
GOTERM_MF_ALL	GO:0033549~MAP kinase phosphatase activity	4	3.70%	0.0000	Dusp6, Dusp8, Dusp1, Dusp16	92	10	16377	71.2
GOTERM_MF_ALL	GO:0008138~protein tyrosine/serine/threonine phosphatase activity	4	3.70%	0.0014	Dusp6, Dusp8, Dusp1, Dusp16	92	40	16377	17.8
GOTERM_MF_ALL	GO:0005515~protein binding	47	43.52%	0.0021	Dusp6, Cdc42ep3, Fgf13, Crem, Cacnb3, Pak3, Lmcd1, Cdk2, Pcdha5, Igfbp3, Dusp16, Lgals3, Rnf144, Met, Adam12, Ap1 g1, Wnt6, Cdh13, Nedd4l, Tcfap2b, Tyr, Cald1, Tslp, Kcnn2, Adam23, Mef2c, Cap1, Sdc3, Apod, Gulp1, Itga1, Akap12, Islr, Ptk2b, Farp2, Add3, Irs1, Gpnmb, Tnfrsf19, Fosl2, Pygm, Spp1, Slc3a2, Lefty2, Pknox2, Coro2a, Ntrk3	92	5794	16377	1.4
GOTERM_MF_ALL	GO:0032403~protein complex binding	4	3.70%	0.0050	Irs1, Gpnmb, Spp1, Lgals3	92	62	16377	11.5
GOTERM_MF_ALL	GO:0042578~phosphoric ester hydrolase activity	7	6.48%	0.0084	Dusp6, Smpd1, Pde10a, Dusp8, Pde4d, Dusp1, Dusp16	92	317	16377	3.9

Category	Term	Count	%	<i>P</i> value	Genes	List total	Pop hits	Pop total	Fold enrichment
GOTERM_MF_ALL	GO:0016801~hydrolase activity, acting on ether bonds	2	1.85%	0.0489	Ephx1, 4631427C17Rik	92	9	16377	39.6
GOTERM_MF_ALL	GO:0008081~phosphoric diester hydrolase activity	3	2.78%	0.0578	Smpd1, Pde10a, Pde4d	92	70	16377	7.6
GOTERM_MF_ALL	GO:0004721~phosphoprotein phosphatase activity	4	3.70%	0.0588	Dusp6, Dusp8, Dusp1, Dusp16	92	159	16377	4.5
GOTERM_MF_ALL	GO:0008092~cytoskeletal protein binding	6	5.56%	0.0690	Cap1, Sdc3, Pknox2, Coro2a, Cald1, Farp2	92	395	16377	2.7
GOTERM_MF_ALL	GO:0046983~protein dimerization activity	5	4.63%	0.0743	Fosl2, Cdh13, Tcfap2b, Tyr, Crem	92	285	16377	3.1
GOTERM_MF_ALL	GO:0016788~hydrolase activity, acting on ester bonds	8	7.41%	0.0865	Dusp6, Pla2 g2e, Smpd1, Pde10a, Dusp8, Pde4d, Dusp1, Dusp16	92	685	16377	2.1
GOTERM_MF_ALL	GO:0004725~protein tyrosine phosphatase activity	3	2.78%	0.0979	Dusp6, Dusp8, Dusp1	92	95	16377	5.6
KEGG_PATHWAY	mmu04010:MAPK signaling pathway	8	7.41%	0.0116	Dusp6, Pla2 g2e, Dusp8, Fgf13, Cacnb3, Dusp1, Mef2c, Dusp16	42	252	4079	3.1
KEGG_PATHWAY	mmu04510:Focal adhesion	6	5.56%	0.0415	Met, Itga1, Spp1, Farp2, Col11a1, Pak3	42	192	4079	3.0
KEGG_PATHWAY	mmu04512:ECM-receptor interaction	4	3.70%	0.0513	Sdc3, Itga1, Spp1, Col11a1	42	84	4079	4.6

Table S2. ASP-altered genes coding for melanosomal proteins expressed in melanosomes of MNT-1 melanocytic cells

Gene	Description	ASP					MSH				
		3 h	1d	2d	3d	4d	3 h	1d	2d	3d	4d
aars	alanyl-tRNA synthetase	0.09	-0.38	-0.71	-0.76	-0.76	0.01	-0.06	-0.11	0.38	0.12
Amid	apoptosis-inducing factor (AIF)-like mitochondrion-associated inducer of death, transcript variant 2	0.02	-0.18	-0.04	-0.60	-0.39	0.14	-0.16	0.38	0.30	0.17
Ascc3	PREDICTED: activating signal cointegrator 1 complex subunit 3, transcript variant 1	0.12	-0.44	-0.50	-0.74	-0.36	0.13	0.35	-0.01	0.12	-0.13
Atp6v0a1	ATPase, H + transporting, lysosomal V0 subunit A1	-0.03	-0.32	-0.25	-0.60	-0.55	-0.04	0.18	0.22	0.29	0.54
Atp6v1 g1	ATPase, H + transporting, lysosomal V1 subunit G1	0.07	-0.28	-0.36	-0.59	-0.59	0.08	0.08	0.04	-0.08	0.25
Bace2	beta-site APP-cleaving enzyme 2	-0.08	-0.72	-0.34	-0.59	-0.62	-0.03	0.24	0.29	0.24	0.68
Cacybp	calcyclin binding protein	0.12	-0.12	-0.49	-0.64	-0.20	0.11	0.05	0.14	-0.11	-0.24
Cd44	CD44 antigen, transcript variant 2	-0.51	-0.17	-0.03	-0.97	-0.27	-0.71				
Cdk2	cyclin-dependent kinase 2, transcript variant 2	-0.50	-0.74	-0.76	-1.08	-1.13	0.06	0.23	0.24	0.33	0.69
Ctsb	cathepsin B	0.39	-0.18	-0.25	-0.53	-0.31	0.01	0.23	0.65	0.53	0.74
Dhcr7	7-dehydrocholesterol reductase	0.19	-0.17	-0.22	-0.51	-0.68	0.00	-0.06	0.15	0.08	0.51
Dusp3	dual specificity phosphatase 3 (vaccinia virus phosphatase VH1-related)	-0.29	-0.17	-0.97	-0.60	-0.05	-0.05	0.02	0.13	0.34	-0.17
Epb4.1l3	erythrocyte protein band 4.1-like 3	0.02	-0.37	-0.18	-0.59	-0.51	0.07	0.03	0.18	0.29	0.16
Ephx1	epoxide hydrolase 1, microsomal	0.09	-0.43	-0.37	-0.62	-0.53	0.01	0.08	0.34	0.66	0.91
Eprs	glutamyl-prolyl-tRNA synthetase	0.36	-0.46	-0.49	-0.88	-0.72	0.10	-0.09	0.05	0.20	0.07
Eps8	epidermal growth factor receptor pathway substrate 8	-0.31	-0.76	-0.81	-0.90	-0.96	0.05	0.20	-0.02	0.10	0.17
Fars1b	phenylalanine-tRNA synthetase-like, beta subunit	0.29	-0.32	-0.32	-0.72	-0.65	0.01	-0.17	0.09	-0.15	-0.03
Frrs1	ferric-chelate reductase 1	0.27	-0.55	-0.39	-0.62	-0.36	-0.07	0.05	0.26	0.09	0.24
Gpd2	glycerol phosphate dehydrogenase 2, mitochondrial	-0.04	-0.15	-0.41	-0.39	-0.63	-0.06	-0.03	0.00	0.34	0.17
Gpnmb	glycoprotein (transmembrane) nmb	-0.08	-0.75	-1.13	-1.37	-1.14	0.04	0.20	0.62	1.00	0.94
Gsr	glutathione reductase 1	0.26	-0.28	-0.69	-0.99	-0.58	0.02	0.04	0.08	0.22	0.13
Gstp1	glutathione S-transferase, pi 1	-0.02	-0.17	-0.61	-0.66	-0.39	-0.03	-0.07	0.29	0.10	0.42
Hbs1l	Hbs1-like (<i>S. cerevisiae</i>)	0.23	-0.45	-0.60	-0.17	-0.04	0.02	-0.04	0.01	0.00	0.29
Hspa4l	Heat shock protein 4 like	0.17	-0.20	-0.22	-0.81	-0.35	0.04	-0.23	0.04	-0.25	-0.02
Hspa9a	heat shock protein 9A	0.32	-0.71	-0.82	-1.05	-0.74	0.01	-0.25	0.09	0.59	0.07
Hyou1	hypoxia up-regulated 1	0.09	-0.29	0.10	-0.28	0.06	-0.05	-0.21	-0.09	0.61	-0.49
Iqgap1	IQ motif containing GTPase activating protein 1	0.03	0.06	-0.16	-0.93	-0.40	-0.06	0.12	0.21	0.59	0.10
Khgrp	KH-type splicing regulatory protein	-0.86	0.47	0.09	-0.16	0.13	-0.09	-0.21	0.12	-0.09	-0.33
Lars	PREDICTED: leucyl-tRNA synthetase, transcript variant 3	0.02	-0.33	-0.63	-0.80	-0.82	-0.03	-0.19	-0.07	0.12	-0.03
Lgals3	lectin, galactose binding, soluble 3	0.13	-1.93	-2.15	-2.52	-1.74	0.24	0.20	0.48	1.72	1.25
Lrrc59	leucine rich repeat containing 59	0.13	-0.41	-0.35	-0.67	-0.43	0.06	-0.04	0.09	0.17	0.08
Mif	macrophage migration inhibitory factor	-0.02	-0.54	-0.53	-0.90	-0.61	0.07	0.00	0.15	0.33	0.32
Mlana	PREDICTED: melan-A, transcript variant 1	0.16	-0.27	-0.72	-0.81	-0.61	-0.08	-0.03	-0.01	-0.23	0.05
Myh11	myosin, heavy polypeptide 11, smooth muscle	0.08	0.08	0.14	-0.11	-0.40	0.15	-0.08	0.18	0.08	0.65
Nars	asparaginyl-tRNA synthetase	0.16	-0.54	-0.86	-0.84	-0.79	0.01	-0.10	-0.20	0.29	-0.01
Nedd4l	neural precursor cell expressed, developmentally down-regulated gene 4-like	-0.49	-0.66	-0.23	-0.43	-0.31	0.16	0.55	0.26	0.64	0.80
Optn	optineurin	0.05	-0.61	-0.28	-0.35	-0.52	0.04	0.01	0.23	0.32	0.52
Pabpc1	Poly A binding protein, cytoplasmic 1	-0.06	-0.59	-0.16	-0.58	-0.07	0.05	-0.06	0.16	0.15	-0.16
Pabpc4	poly A binding protein, cytoplasmic 4, transcript variant 2	0.00	-0.35	-0.63	-0.47	-0.18	0.01	0.07	0.05	0.01	0.15
Pdcd8	programmed cell death 8	-0.18	-0.19	-0.50	-0.60	-0.48	0.02	0.08	-0.04	0.15	-0.05
Pfkfb	phosphofruktokinase, platelet	0.00	-0.30	-0.84	-0.66	-0.57	0.13	-0.23	-0.05	0.25	-0.06
Plscr1	phospholipid scramblase 1	-0.01	-0.28	-0.66	-0.52	-0.36	0.02	0.14	0.21	0.17	0.18
Pltp	phospholipid transfer protein	0.07	-0.28	-0.23	-0.81	-0.29	-0.11	-0.11	0.02	-0.02	0.22
Plxnc1	Plexin C1	0.12	0.08	-0.16	-0.34	-0.63	-0.09	0.29	0.17	-0.08	0.42
Prps1	phosphoribosyl pyrophosphate synthetase 1	0.03	-0.23	-0.52	-0.62	-0.22	0.07	-0.18	-0.09	-0.03	0.01
Psat1	phosphoserine aminotransferase 1	0.20	-0.34	-0.46	-0.71	-0.73	-0.07	-0.31	-0.18	0.00	-0.21
Rab27a	RAB27A, member RAS oncogene family	0.12	-0.40	-0.54	-0.92	-0.65	-0.07	0.31	0.14	0.11	0.31
Rab39b	RAB39B, member RAS oncogene family	0.33	-0.39	-0.33	-0.95	-0.61	0.58	0.24			
Rab5b	RAB5B, member RAS oncogene family	-0.42	-0.30	-0.08	0.09	0.00	0.08	0.10	0.35	0.46	0.74
Sars	seryl-aminoacyl-tRNA synthetase	-0.07	-0.59	-0.92	-0.84	-0.94	0.02	-0.16	0.01	0.44	0.25
Scpep1	serine carboxypeptidase 1	0.05	-0.17	-0.51	-0.59	-0.58	0.02	0.06	0.22	0.16	0.28

Gene	Description	ASP					MSH				
		3 h	1d	2d	3d	4d	3 h	1d	2d	3d	4d
Serpinf1	serine (or cysteine) peptidase inhibitor, clade F, member 1	0.21	-0.14	-0.11	-0.10	-0.23	0.06	-0.08	0.03	0.75	0.36
Sfxn1	sideroflexin 1	-0.01	-0.14	-0.47	-0.96	-0.68	0.16	0.08	-0.01	-0.05	0.19
Si	silver	0.05	-0.82	-0.01	-1.29	-1.13	-0.07	0.23	0.63	0.50	0.99
Slc16a6	solute carrier family 16 (monocarboxylic acid transporters), member 6, transcript variant 2	-0.42	-0.60	-0.47	-0.61	-0.53	0.15	-0.06	0.44	0.44	0.49
Slc1a4	CYS transporter - solute carrier family 1 (glutamate/neutral amino acid transporter), member 4	0.08	-0.74	-1.13	-1.56	-1.58	0.00	-0.26	-0.10	0.64	-0.06
Slc1a5	Solute carrier family 1 (neutral amino acid transporter), member 5	-0.21	-0.56	-0.88	-0.74	-0.48	-0.05	-0.52	0.21	0.47	-0.14
Slc24a5	solute carrier family 24, member 5	-0.01	-1.01	-1.10	-1.45	-1.32	0.04	0.25	0.40	0.21	0.81
Slc38a2	solute carrier family 38, member 2	0.29	-0.11	-0.36	-0.51	-0.62	0.11	-0.01	-0.13	0.05	-0.08
Slc39a14	Solute carrier family 39 (zinc transporter), member 14	-0.42	-0.37	-0.02	-0.80	-0.83	0.41	0.05	-0.38	0.19	0.36
Slc3a2	solute carrier family 3 (activators of dibasic and neutral amino acid transport), member 2	0.18	-0.97	-1.38	-1.37	-1.42	-0.03	-0.32	0.23	0.85	0.62
Slc45a2	solute carrier family 45, member 2	0.02	-1.15	-0.88	-1.33	-0.78	-0.03	0.18	0.48	0.35	0.72
Slc6a17	solute carrier family 6 (neurotransmitter transporter), member 17	-0.10	0.09	0.01	0.01	0.11	-0.03	0.06	0.21	0.36	0.63
Slc7a5	solute carrier family 7 (cationic amino acid transporter, y ⁺ system), member 5	0.06	-0.51	-1.16	-1.23	-1.11	-0.06	-0.28	-0.09	0.41	0.11
Tagln2	transgelin 2	-0.10	-0.64	0.00	-0.43	-0.19	0.14	-0.08	-0.08	0.28	0.27
Tars	threonyl-tRNA synthetase	-0.24	-0.39	-0.80	-0.82	-0.85	0.02	0.02	-0.10	0.00	0.05
Tfrc	transferrin receptor	-0.18	-0.57	-0.69	-0.74	-0.67	0.04	0.01	-0.19	0.13	-0.14
Tmem33	transmembrane protein 33, transcript variant 2	-0.67	-0.02	0.16	-0.89	-0.05	0.29	0.07	0.42	0.05	-0.24
Tnfaip3	tumor necrosis factor, alpha-induced protein 3	0.20	-0.60	-0.49	-0.62	-0.29	-0.41	-0.12	-0.02		-0.55
Tpi1	triosephosphate isomerase 1	0.15	-0.40	-0.67	-0.62	-0.44	0.03	0.04	-0.03	0.28	0.01
Trpv2	transient receptor potential cation channel, subfamily V, member 2	-0.07	-0.50	-0.47	-0.61	-0.40	0.08	0.04	0.20	0.24	0.44
Tspan10	tetraspanin 10	-0.23	-0.20	-0.21	-0.66	-0.65	-0.13	0.27	0.15	0.02	0.60
Tuba4	tubulin, alpha 4	-0.04	-0.34	-0.65	-0.59	-0.35	0.21	0.10	0.14	0.52	0.58
Tyr	tyrosinase	-0.32	-0.56	-0.68	-0.82	-1.18	-0.07	0.37	0.56	0.29	0.63
Tyrp1	tyrosinase-related protein 1	-0.25	-0.81	-0.46	-0.82	-0.65	-0.03	0.18	0.27	0.19	0.43
Yars	tyrosyl-tRNA synthetase	0.07	-0.16	-0.50	-0.59	-0.29	-0.01	-0.23	0.05	0.13	0.06
Abhd2	abhydrolase domain containing 2	-0.08	0.18	1.00	1.01	0.46	-0.09			-0.11	
Anxa11	annexin A11	0.00	0.26	0.80	0.61	0.60	0.04	-0.13	-0.19	0.03	-0.06
Arl6ip5	ADP-ribosylation factor-like 6 interacting protein 5	-0.24	0.09	0.47	0.60	0.48	0.03	0.00	0.25	0.09	-0.14
Atg9a	autophagy-related 9A (yeast)	0.13	0.12	0.12	0.59	0.62	-0.11	0.06	0.01	-0.14	0.08
Atp1b2	ATPase, Na ⁺ /K ⁺ transporting, beta 2 polypeptide	0.05	0.34	0.89	1.78	0.86	0.16	-0.35	0.11	-0.56	0.08
Atp1b2	ATPase, Na ⁺ /K ⁺ transporting, beta 2 polypeptide	0.05	0.34	0.89	1.78	0.86	0.08	-0.56	0.11	-0.35	0.16
Cald1	Caldesmon 1	0.06	1.20	1.32	1.60	1.85	-0.12	0.05	-0.44	-0.68	-0.84
Cap1	CAP, adenylate cyclase-associated protein 1 (yeast)	-0.29	0.89	0.29	0.78	0.62	-0.06	-0.86	0.03	-0.58	-0.56
Capg	capping protein (actin filament), gelsolin-like	0.08	-0.08	0.29	0.78	0.87	-0.07	-0.13	-0.01	0.33	0.27
Cd81	CD 81 antigen	0.25	0.54	0.29	0.62	0.64	-0.05	0.02	-0.03	-0.14	0.07
Cd82	CD82 antigen	0.08	-0.25	0.65	0.70	0.47		-0.11	0.31	0.10	0.28
Cnp1	cyclic nucleotide phosphodiesterase 1	0.03	0.53	0.86	1.26	0.93	-0.07	0.15		-0.09	0.02
Cst3	cystatin C (Cst3)	0.12	0.63	0.93	1.58	1.44	0.01	-0.17	-0.19	-0.35	-0.15
Dbn1	Drebrin 1	-0.30	0.07	-0.06	0.70	0.70	-0.11	-0.10	0.03	0.21	-0.08
Dhrs8	dehydrogenase/reductase (SDR family) member 8	-0.16	0.22	0.32	1.12	0.54	-0.17	-0.09		-0.20	0.45
Enah	enabled homolog (Drosophila)	0.18	0.10	0.83	0.62	0.25		-0.07	-0.01	-0.21	-0.01
Gla	galactosidase, alpha	-0.09	-0.55				-0.06	0.03	0.16	0.72	-0.18
Gnal	guanine nucleotide binding protein, alpha stimulating, olfactory type, transcript variant 1	0.47	-0.47	0.01			0.03	0.33	0.36	0.65	0.69
Gnb4	guanine nucleotide binding protein, beta 4	-0.07	0.43	0.14	0.60	0.35	-0.07	-0.10	-0.26	-0.23	-0.29
Gsn	gelsolin	0.01	0.42	0.15	0.60	0.32	-0.02	0.08	0.14	-0.34	0.06
Igf2r	insulin-like growth factor 2 receptor	-0.01	0.68	0.58	0.64	0.46	0.08	0.22	-0.37	-0.12	0.30
Leprot	leptin receptor overlapping transcript	0.19	0.27	-0.07	0.62	0.55	-0.03	-0.01	0.14	-0.14	0.03
Lrp1	low density lipoprotein receptor-related protein 1	-0.31	0.71	0.63	1.01	0.66	-0.09	-0.21	0.26	0.19	-0.47
Lrpap1	low density lipoprotein receptor-related protein associated protein 1	0.24	0.05	0.29	0.71	0.63	0.04	0.00	-0.03	0.28	0.18
Macf1	PREDICTED: microtubule-actin crosslinking factor 1, transcript variant 12	-0.91	0.69	0.19	0.13	0.11	-0.03	-0.19	0.00	0.24	-0.38
Man2b1	mannosidase 2, alpha B1	0.11	0.08	-0.05	0.20	0.04	-0.06	-0.03	0.19	0.40	0.59

Gene	Description	ASP				MSH					
		3 h	1d	2d	3d	4d	3 h	1d	2d	3d	4d
Mbc2	membrane bound C2 domain containing protein	0.15	0.16	0.49	0.32	-0.07	0.02	0.02	0.06	0.43	0.73
Myh14	myosin, heavy polypeptide 14	0.03	0.39	0.64	1.11	0.60	-0.17	0.06	0.38		
Ncstn	nicastrin	-0.79	0.28	0.24	0.72	0.40	-0.09	-0.16	0.09	0.25	-0.18
Ppfibp1	PTPRF interacting protein, binding protein 1 (liprin beta 1)	-0.26	0.22	0.26	0.60	0.32	-0.01	-0.05	-0.15	-0.09	-0.19
Prkar1a	Protein kinase, cAMP dependent regulatory, type I, alpha	-0.45	0.41	0.26	0.80	0.87	0.08	0.13	0.06	0.30	-0.15
Rab33b	RAB33B, member of RAS oncogene family	0.13	0.13	0.48	0.63	1.03	0.02	0.22	-0.02	-0.26	-0.35
S100a11	S100 calcium binding protein A11 (calizzarin)	0.04	0.33	0.84	1.10	1.02	0.05	-0.14	-0.13	-0.03	-0.08
Sema4c	PREDICTED: sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4C	-0.13	0.26	0.44	0.59	0.31	-0.11	-0.30	-0.20	0.05	-0.05
Serinc5	serine incorporator 5	-0.30	0.05	0.61	0.70	0.03	-0.30	-0.09	0.03	-0.13	-0.11
Serpine2	serine (or cysteine) peptidase inhibitor, clade E, member 2	0.15	0.50	0.95	1.21	0.47	0.05	-0.09	-0.10	-0.29	-0.36
Slc29a1	Solute carrier family 29 (nucleoside transporters), member 1	-0.10	0.22	0.34	0.72	0.80	0.05	-0.32	-0.02	-0.12	-0.14
Slc29a1	solute carrier family 29 (nucleoside transporters), member 1	-0.20	0.36	0.38	1.12	0.77	0.05	-0.10	0.14	-0.26	-0.27
Snx4	Sorting nexin 4	0.02	0.48	0.40	0.61	0.36	-0.05	-0.20	-0.21	-0.21	-0.31
Tln1	talin 1	-0.90	0.63	0.28	0.40	0.28	-0.08	-0.08	0.22	0.14	-0.68
Tmem106b	Transmembrane protein 106B	0.19	0.24	0.35	0.72	0.31	0.03	0.09	0.09	0.01	0.07
Tpm4	tropomyosin 4	0.08	0.05	0.03	0.39	0.60	0.12	0.08	0.03	-0.04	-0.12
Tspan14	tetraspanin 14	0.09	0.35	0.41	0.61	0.41	0.02	-0.08	-0.11	-0.18	-0.02
Tspan3	tetraspanin 3	0.06	0.25	0.32	0.98	0.78	0.05	-0.08	-0.21	-0.26	-0.12
Vcl	vinculin	0.02	0.08	0.74	0.75	0.48	-0.07	-0.04	0.13	0.19	0.22
Vim	vimentin	0.04	0.24	0.54	0.73	0.48	-0.10	0.22	-0.16	-0.26	-0.01
Vkorc1	vitamin K epoxide reductase complex, subunit 1	0.26	0.36	0.36	0.60	0.48	0.04	-0.08	0.09	-0.03	0.15

Table S3. ASP-altered genes coding for Wnt-signaling related proteins

Gene	Description	Log ASP/control
Tcf4	transcription factor 4	1.23
Ccnd1	cyclin D1	1.14
Lef1	lymphoid enhancer binding factor 1	1.13
Ppap2b	phosphatidic acid phosphatase type 2B	1.06
Plcb1	Phospholipase C, beta 1	1.04
Fzd7	frizzled homolog 7 (Drosophila)	0.95
Wnt6	wingless-related MMTV integration site 6	0.93
Lrp1	low density lipoprotein receptor-related protein 1	0.91
Tcf3	transcription factor 3	0.89
Tcf7l2	Transcription factor 7-like 2, T-cell specific, HMG-box	0.80
Fzd3	Frizzled homolog 3 (Drosophila)	0.79
Nfatc1	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent, transcript variant 1	0.78
Ctnnbip1	catenin beta interacting protein 1	0.76
Bcl9	B-cell CLL/lymphoma 9	0.74
Plcb4	phospholipase C, beta 4	0.73
Lrp5	low density lipoprotein receptor-related protein 5	0.73
Tle6	transducin-like enhancer of split 6, homolog of Drosophila E(spl)	0.69
Hbp1	High mobility group box transcription factor 1	0.68
Mapk8	Mitogen activated protein kinase 8	0.65
Fzd2	frizzled homolog 2 (Drosophila)	0.64
Tbl1xr1	PREDICTED: transducin (beta)-like 1X-linked receptor 1, transcript variant 2	0.61
Prickle1	prickle like 1 (Drosophila)	0.61
Prkacb	protein kinase, cAMP dependent, catalytic, beta	0.61
Daam2	dishevelled associated activator of morphogenesis 2	0.61
Dkk3	dickkopf homolog 3 (Xenopus laevis)	0.61
Cacybp	calyculin binding protein	-0.64

Table S4. Genes up-regulated by ASP, involved in development, cell-adhesion and/or extracellular-matrix (ECM) remodeling, analyzed from the microarray data

NSD including cell differentiation, neurogenesis, cell migration	Embryonic, vasculature, skeletal and muscle development	Others anatomical structure development	Cell-adhesion molecules	ECM	Metallo-endopeptidases	Cytoskeleton	Signaling molecules involved in migration
Ablim1	Adamts4	Crip2	Alcam	Agrn	Adam11	Ablim1	Arhgap6
Agt	Agt	Egr1	Amigo1	Bcan	Adam12	Acta2	Arhgef19
Alcam	Aldh1a1	Id3	Cdh2	Col10a1	Adam19	Actg2	Arhgef9
Aldh1a3	Aldh1a3	Itga6	Cdh13	Col11a1	Adam23	Actn4	Cdc42ep1
Amigo1	Aplp2	Kirrel3	Itga1	Col14a1	Adamts1	Actn4	Cdc42ep3
Cnp1	Col11a1	Lefty2	Itga3	Col1a1	Adamts4	Ctnnal1	Cdc42 se1
Dlx5	Col4a2	Mitf	Itga6	Col3a1	Adamts5	Ctnnbip1	Farp1
Efnb2	Col8a1	Npnt	Itga8	Col4a1	Adamts9	Ctnnd2	Mapk8
ErbB3	Ctgf	Nr4a3	Itgav	Col4a2	Adamtsl4	Myh14	Ptk2b
Foxd1	Cyp26b1	Peg10	Itgb3	Col5a1	Mme	Mylk	Rhob
Gbx2	Dlx5	Postn	Itgb5	Col5a3	Mmp15	Myo10	Rhobtb1
Itga3	Efnb2	Spry1	Jam2	Col8a1	Mmp17	Phactr1	Rhobtb3
Lamb1-1	Fgf1	Tns3	Mcam	Col9a1	Mmp2	Smarca2	Rhoj
Lef1	Gbx2		Pcdh1	Col9a2	Timp2	Smarcd1	Rock2
Mbp	Hoxa3		Pcdh11x	Ctgf		Smarcd3	Sh2d3c
Mef2c	Id1		Pcdh7	Dag1		Tpm1	Sh3 glb1
Ndrg2	Lect1		Pcdha5	Dmd		Tpm4	Shc1
Nrxn1	Lef1		Pcdhb14	Dmp1		Waspip	Shc2
Rapgef5	Mef2c		Pcdhb15	Dtna			
Rtn4r11	Mest		Pcdhb16	Ecm1			
Sema3d	Pdgfa		Pcdhb17	Efemp2			
Sema3f	Pdgfb		Pcdhb19	Emilin1			
Sema6a	Ppap2b		Pcdhb21	Fgf1			
Serpine2	Prelp		Pcdhb22	Hspg2			
Tcfap2a	Sox4		Pcdhgb4	Lama4			
Uchl1	Sox6		Pcdhgc5	Lama5			
	Vdr		Sdc2	Lamb1-1			
	Actg2		Sdc3	Lamb2			
	Dmd		Sdc4	Ltbp1			
	Igfbp3		Thbs1	Ltbp3			
				Matn2			
				Nid2			
				Npnt			
				Postn			
				Prelp			
				Tgfbr3			
				Tgm2			
				Wnt6			

All genes represented were increased by ASP by at least 1.5-fold ($P < 0.01$). The first 3 columns show genes up-regulated by 2-fold or more ($P < 0.01$).