

Collected SI Tables in numerical order. (Nyg et al.,)

Table S1. Ingenuity Pathway Analysis Summary for up-regulated genes in the intermediate growth phase

<i>Functional Analysis of gene subset¹</i>			
Category	Functions Annotation	p-value	# molecules
Nervous System Development and Function	neurotransmission	1,4E-09	36
Neurological Disease	Movement Disorders	1,8E-09	69
Cell Death and Survival	apoptosis	4,4E-09	127
Nervous System Development and Function	synaptic transmission	7,9E-09	31
Behavior	cognition	2,2E-07	31
Neurological Disease	neuromuscular disease	2,9E-07	55
Nervous System Development and Function	myelination	2,9E-07	18
Neurological Disease	Schizophrenia	6,1E-07	37
Cell-To-Cell Signaling and Interaction	synaptic depression	6,6E-07	14
Cellular Function and Maintenance	formation of plasma membrane projections	7,7E-07	36
Cell Death and Survival	cell death	7,9E-07	141
Neurological Disease	disorder of basal ganglia	8,7E-07	50
Nervous System Development and Function	quantity of neurites	1,0E-06	11
Cellular Growth and Proliferation	proliferation of cells	1,0E-06	148
Neurological Disease	progressive motor neuropathy	2,3E-06	30
Cellular Function and Maintenance	quantity of cellular protrusions	2,5E-06	15
Cellular Growth and Proliferation	arrest in growth of cells	4,0E-06	19
Cellular Assembly and Organization	formation of plasma membrane	5,0E-06	19
Nervous System Development and Function	morphology of nervous system	6,0E-06	48
Cellular Movement	cell movement of brain cancer cell lines	6,7E-06	12
<i>Canonical Pathway Analysis²</i>			
Top Canonical Pathway		p-value	
Wnt/ β -catenin Signaling		1,3E-04	
Induction of Apoptosis by HIV1		1,1E-04	
PI3K/AKT Signaling		1,2E-03	
Molecular Mechanisms of Cancer		1,3E-03	
Axonal Guidance Signaling		1,7E-03	
CDK5 Signaling		2,0E-03	
B Cell Receptor Signaling		2,6E-03	
Ephrin B Signaling		2,9E-03	
Clathrin-mediated Endocytosis Signaling		3,1E-03	
Synaptic Long Term Depression		3,1E-03	
Docosahexaenoic Acid (DHA) Signaling		3,3E-03	
Glutamate Receptor Signaling		3,3E-03	
Synaptic Long Term Potentiation		3,4E-03	
PTEN Signaling		3,4E-03	
Fatty Acid β -oxidation I		6,5E-03	
<i>Upstream Regulator Analysis³</i>			
Upstream Regulator	Activation z-score	p-value of overlap	Target molecules
TGFB1	4,11	1,6E-07	62
NFE2L2	3,48	3,1E-04	19
IGF1	3,40	1,3E-04	19
FGF2	3,20	4,1E-05	19
F2	3,09	5,0E-02	10
VEGFA	2,89	1,0E-03	13
BDNF	2,84	9,4E-06	15
SP1	2,82	3,7E-07	29
EGF	2,80	1,9E-06	26
SMAD3	2,80	1,6E-03	11
PDGF BB	2,76	7,3E-03	14
IFNG	2,70	2,3E-03	36
EPO	2,63	5,7E-03	13
AGER	2,62	1,4E-04	7
EGR1	2,60	3,6E-03	9
CR1L	-2,00	1,8E-03	4
FAS	-2,10	1,3E-02	15
TFAP2C	-2,22	2,5E-03	5
KRAS	-2,23	4,4E-03	14
SFTPA1	-2,24	2,4E-02	5

SPDEF	-2,45	3,3E-03	6
MYC	-2,73	2,4E-04	32

¹Top ranked biological functions that were most significant to the 477 genes eligible for analysis using a right tailed Fisher's exact test.

²Canonical pathway analysis identified from the Ingenuity Knowledge base that were most significant to the gene set.

³Upstream regulator analysis based on the 477 genes using Ingenuity Knowledge base. Top ranked regulators according to activation/inhibition z-score are displayed. The z-score is based on relationships between experimentally observed gene expression and function annotation data, as derived from the information compiled in the Ingenuity Knowledge Base. These relationships are associated with a direction of change that is either activating (z-score ≥ 2) or inhibiting (z-score ≤ -2). For activated upstream regulators predicted in this analysis, the top 15 regulators are displayed.

Table S2. Ingenuity Pathway Analysis Summary for up-regulated genes in the late growth phase

<i>Functional Analysis of gene subset¹</i>			
Category	Functions Annotation	p-Value	# molecules
Hair and Skin Development and Function	synthesis of melanin	1,3E-06	4
Amino Acid Metabolism	metabolism of melanin	6,5E-05	2
Developmental Disorder	albinism	1,9E-04	3
Cellular Development	differentiation of melanocytes	3,3E-04	3
Cellular Movement	migration of prostate cancer cells	4,5E-04	2
Hair and Skin Development and Function	pigmentation of skin	4,5E-04	2
Hair and Skin Development and Function	diluted color of fur	7,7E-04	2
Cardiovascular System Development and Function	size of cardiomyocytes	8,6E-04	3
Cancer	tumorigenesis of brain cancer cell lines	1,2E-03	2
Cellular Growth and Proliferation	proliferation of cells	1,4E-03	30
Cellular Movement	invasion of melanoma cells	1,6E-03	2
Cellular Movement	cell movement of tumor cell lines	1,8E-03	10
Organ Morphology	morphology of eye	2,2E-03	6
Cellular Movement	scattering of kidney cell lines	2,2E-03	2
Cancer	tumorigenesis of tumor cell lines	2,3E-03	4
<i>Canonical Pathway Analysis²</i>			
Top Canonical Pathway	p-value		
Formaldehyde Oxidation II (Glutathione-dependent)	9,37E-03		
Mechanisms of Viral Exit from Host Cells	1,51E-02		
Superoxide Radicals Degradation	2,79E-02		
Cell Cycle: G1/S Checkpoint Regulation	3,13E-02		
Glycogen Degradation II	4,15E-02		
<i>Upstream Regulator Analysis³</i>			
Upstream Regulator	Activation z-score	p-value of overlap	Target molecules in dataset
TNF	3,02	5,8E-04	16
MITF	2,4	2,4E-05	6
STAT4	2,22	2,6E-03	5

¹Top ranked biological functions that were most significant to the 97 genes eligible for analysis using a right tailed Fisher's exact test.

²Canonical pathway analysis identified from the Ingenuity Knowledge base that were significant to the gene set.

³Upstream regulator analysis based on the 97 genes using Ingenuity Knowledge base and ranked according to activation z-score. The z-score is based on relationships between experimentally observed gene expression and function annotation data, as derived from the information compiled in the Ingenuity Knowledge Base. Upstream regulators are categorized as activating when z-score \geq 2.

Table S3. Ingenuity Pathway Analysis Summary for up-regulated genes in the mixed-species sample (early metastatic growth in the brain)

<i>Functional Analysis of gene subset¹</i>			
Category	Functions Annotation	p-Value	# molecules
Cancer	uterine leiomyoma	2,9E-07	56
Protein Synthesis	translation of protein	1,4E-05	48
Cancer	leiomyomatosis	1,7E-05	59
Cardiovascular System Development and Function	abnormal morphology of dilated heart	2,8E-05	8
Protein Synthesis	expression of protein	4,4E-05	61
Cancer	cell transformation	5,6E-05	96
Nervous System Development and Function	growth of neurites	6,3E-05	87
Cancer	metastasis of tumor	1,1E-04	29
Nervous System Development and Function	outgrowth of neurites	1,4E-04	76
Protein Synthesis	synthesis of protein	1,4E-04	76
Cardiovascular Disease	bradycardia of heart	1,9E-04	4
Gene Expression	translation of RNA	3,2E-04	31
Embryonic Development	development of fetal membranes	3,5E-04	13
Small Molecule Biochemistry	metabolism of choline	3,9E-04	5
Cancer	tumorigenesis of tumor cell lines	4,2E-04	30
Cancer	metastasis	4,4E-04	98
Developmental Disorder	Growth Failure	5,0E-04	111
Cellular Growth and Proliferation	proliferation of cells	5,5E-04	535
Cell-mediated Immune Response	differentiation of effector T lymphocytes	7,6E-04	9
Cellular Function and Maintenance	function of endothelial cells	7,7E-04	12
<i>Canonical Pathway Analysis²</i>			
Top Canonical Pathway	p-value		
EIF2 Signaling	7,5E-04		
Ephrin Receptor Signaling	1,7E-03		
Colorectal Cancer Metastasis Signaling	1,7E-03		
DNA Double-Strand Break Repair by Homologous Recombination	3,5E-03		
Inhibition of Matrix Metalloproteases	3,5E-03		
HIF1 α Signaling	5,9E-03		
Acute Myeloid Leukemia Signaling	6,5E-03		
Ephrin A Signaling	8,5E-03		
Axonal Guidance Signaling	9,9E-03		
IL-15 Production	1,0E-02		
Role of Macrophages, Fibroblasts and Endothelial Cells in Rheumatoid Arthritis	1,1E-02		
Mouse Embryonic Stem Cell Pluripotency	1,4E-02		
Role of PKR in Interferon Induction and Antiviral Response	1,6E-02		
Myc Mediated Apoptosis Signaling	1,6E-02		
Integrin Signaling	1,8E-02		
FGF Signaling	2,0E-02		
Ephrin B Signaling	2,2E-02		
Glycine Betaine Degradation	2,3E-02		
GDP-mannose Biosynthesis	2,5E-02		
Regulation of eIF4 and p70S6K Signaling	2,6E-02		
<i>Upstream Regulator Analysis³</i>			
Upstream Regulator	Activation z-score	p-value of overlap	Target molecules in dataset
RAF1	4,69	9,2E-03	62
EGR2	4,55	1,9E-02	22
CD40LG	4,46	3,9E-02	51
MAP2K1	4,38	4,3E-04	30
ANGPT2	4,00	1,1E-02	20
GLI1	3,71	2,3E-03	30
MYCN	3,27	7,6E-05	45
FGFR1	3,26	4,4E-02	11
HNF4A	2,98	1,5E-02	246
MLL2	2,97	1,3E-04	10
RAC1	2,92	3,7E-02	14
FADD	2,85	1,4E-02	15
GNA12	2,69	8,1E-05	18
TP53	2,64	7,4E-03	153

CCNK	2,59	4,4E-02	7
EGFR	2,58	3,1E-02	35
MYOD1	2,58	2,6E-02	27
NFATC2	2,57	1,1E-02	24
IRF8	2,47	2,4E-03	18
SIX5	2,45	4,5E-02	6
ACSL5	-2,00	1,1E-01	4
ATP7B	-2,00	1,0E00	4
CBL	-2,12	1,4E-03	9
miR-92a-3p (and other miRNAs w/seed AUUGCAC)	-2,18	4,3E-02	5
mir-1	-2,20	8,6E-03	9
VIP	-2,40	4,0E-01	11
HIC1	-2,40	1,0E00	6
miR-122-5p (miRNAs w/seed GGAGUGU)	-3,41	4,5E-02	12
miR-145-5p (and other miRNAs w/seed UCCAGUU)	-3,41	6,6E-03	12
miR-483-3p (miRNAs w/seed CACUCCU)	-3,46	2,1E-03	12
COL18A1	-4,42	7,9E-04	20
miR-16-5p (and other miRNAs w/seed AGCAGCA)	-5,73	1,4E-03	34

¹Top ranked biological functions that were most significant to the 2412 genes eligible for analysis using a right tailed Fisher's exact test.

²Canonical pathway analysis identified from the Ingenuity Knowledge base that were most significant to the gene set.

³Upstream regulator analysis based on the 2412 genes using Ingenuity Knowledge base and ranked according to activation z-score. The z-score is based on relationships between experimentally observed gene expression and function annotation data, as derived from the information compiled in the Ingenuity Knowledge Base. Upstream regulators are categorized as activating when z-score \geq 2.

Table S4. Ingenuity Pathway Analysis Summary for genes present in K-means cluster 5

<i>Functional Analysis of gene subset¹</i>			
Category	Functions Annotation	p-Value	# molecules
Lipid Metabolism	translocation of lipid	7,8E-05	9
Skeletal and Muscular Disorders	limb girdle muscular dystrophy type 2a	1,4E-04	8
Skeletal and Muscular Disorders	muscular dystrophy	1,5E-04	17
Immunological Disease	immunodeficiency	2,8E-04	14
Skeletal and Muscular Disorders	facioscapulohumeral muscular dystrophy	3,1E-04	7
Embryonic Development	development of vertebral body	3,4E-04	4
Skeletal and Muscular Disorders	limb girdle muscular dystrophy	5,1E-04	10
Skeletal and Muscular Disorders	Miyoshi myopathy	5,4E-04	7
Reproductive System Development and Function	mass of testis	6,1E-04	19
Lipid Metabolism	translocation of cholesterol	6,5E-04	4
Cellular Growth and Proliferation	proliferation of follicular cells	7,5E-04	3
Amino Acid Metabolism	regulation of L-glutamic acid	7,5E-04	3
Cell Cycle	homologous recombination	9,6E-04	10
DNA Replication, Recombination, and Repair	homologous recombination	9,6E-04	10
Skeletal and Muscular Disorders	Duchenne muscular dystrophy	9,60E-04	9
Skeletal and Muscular Disorders	myopathy	1,1E-03	26
Immunological Disease	immunoglobulin deficiency	1,2E-03	5
Skeletal and Muscular Disorders	limb girdle muscular dystrophy type 2B	1,4E-03	7
Cell-To-Cell Signaling and Interaction	concentration of norepinephrine	1,5E-03	10
Hematopoiesis	differentiation of peripheral blood lymphocytes	1,8E-03	3
<i>Canonical Pathway Analysis²</i>			
Top Canonical Pathway	p-value		
Leptin Signaling in Obesity	3,1E-03		
Neuropathic Pain Signaling In Dorsal Horn Neurons	4,7E-03		
Docosahexaenoic Acid (DHA) Signaling	6,5E-03		
nNOS Signaling in Skeletal Muscle Cells	7,1E-03		
Gap Junction Signaling	7,6E-03		
Small Cell Lung Cancer Signaling	7,6E-03		
Axonal Guidance Signaling	9,0E-03		
Complement System	9,3E-03		
IGF-1 Signaling	9,5E-03		
Relaxin Signaling	1,0E-02		
Amyotrophic Lateral Sclerosis Signaling	1,0E-02		
iCOS-iCOSL Signaling in T Helper Cells	1,1E-02		
cAMP-mediated signaling	1,2E-02		
Thrombin Signaling	1,3E-02		
Melatonin Signaling	1,5E-02		
Growth Hormone Signaling	1,6E-02		
Myc Mediated Apoptosis Signaling	1,8E-02		
Ephrin A Signaling	2,0E-02		
Sphingosine-1-phosphate Signaling	2,3E-02		
Prolactin Signaling	2,5E-02		

¹Top ranked biological functions that were most significant to the 1201 genes eligible for analysis using a right tailed Fisher's exact test.

²Canonical pathway analysis identified from the Ingenuity Knowledge base that were most significant to the gene set.

Table S5. Ingenuity Pathway Analysis Summary for genes up-regulated in host brain stroma (mouse)

<i>Functional Analysis of gene subset¹</i>			
Category	Functions Annotation	p-Value	# molecules
Immune Cell Trafficking	cell movement of leukocytes	4,8E-14	45
Immune Cell Trafficking	leukocyte migration	3,1E-13	48
Immune Cell Trafficking	cell movement of myeloid cells	6,8E-12	33
Inflammatory Response	inflammatory response	1,1E-11	36
Hematological System Development and Function	quantity of leukocytes	1,8E-11	56
Hematological System Development and Function	quantity of blood cells	4,5E-11	60
Inflammatory Response	inflammation of organ	1,0E-10	40
Cellular Movement	migration of cells	1,2E-10	60
Cellular Function and Maintenance	function of leukocytes	2,1E-10	38
Immune Cell Trafficking	cell movement of phagocytes	4,9E-10	30
Inflammatory Disease	arthritis	8,1E-10	25
Inflammatory Disease	Rheumatic Disease	8,9E-10	26
Infectious Disease	infection of mammalia	2,9E-09	28
Cellular Movement	cell movement	5,1E-09	61
Cell-To-Cell Signaling and Interaction	activation of cells	5,1E-09	36
Cellular Movement	infiltration of cells	2,9E-08	26
Immune Cell Trafficking	homing of leukocytes	3,1E-08	20
Immune Cell Trafficking	chemotaxis of leukocytes	3,2E-08	19
Organismal Survival	organismal death	3,7E-08	70
Inflammatory Response	quantity of phagocytes	3,9E-08	28
<i>Canonical Pathway Analysis²</i>			
Top Canonical Pathway		p-value	
Cdc42 Signaling		6,7E-08	
Communication between Innate and Adaptive Immune Cells		8,7E-08	
Fcγ Receptor-mediated Phagocytosis in Macrophages and Monocytes		2,7E-06	
OX40 Signaling Pathway		1,2E-05	
Crosstalk between Dendritic Cells and Natural Killer Cells		1,6E-05	
Antigen Presentation Pathway		1,7E-05	
Allograft Rejection Signaling		2,5E-05	
Acute Phase Response Signaling		3,4E-05	
Remodeling of Epithelial Adherens Junctions		4,6E-05	
Cytotoxic T Lymphocyte-mediated Apoptosis of Target Cells		5,4E-05	
Dendritic Cell Maturation		6,4E-05	
Actin Cytoskeleton Signaling		1,1E-04	
Complement System		2,7E-04	
IL-10 Signaling		4,8E-04	
Toll-like Receptor Signaling		9,2E-04	
Systemic Lupus Erythematosus Signaling		1,4E-03	
Epithelial Adherens Junction Signaling		2,6E-03	
Production of Nitric Oxide and Reactive Oxygen Species in Macrophages		3,2E-03	
Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses		3,8E-03	
Autoimmune Thyroid Disease Signaling		4,4E-03	
<i>Upstream Regulator Analysis³</i>			
Upstream Regulator	Activation z-score	p-value of overlap	Target molecules in dataset
IKBKB	3,13	4,8E-11	19
TP53	2,10	4,9E-11	35
CHUK	3,35	1,5E-09	15
IFNG	3,13	1,8E-09	22
TLR4	3,26	5,9E-08	17
IKBKG	3,42	3,6E-07	12
BAX	2,14	6,0E-07	8
STAT3	2,81	9,0E-07	13
NOS2	2,38	4,7E-06	12
TNF	2,92	5,2E-06	15
TLR3	3,29	9,9E-06	15
MYD88	2,94	1,2E-05	11
TLR9	3,15	1,8E-05	12
IL6	2,40	2,1E-05	10
TICAM1	2,79	2,5E-05	10

XDH	-2,00	1,2E-04	4
ABCB4	-2,00	8,5E-04	4
SOCS3	-2,00	9,9E-04	4
SOCS1	-2,20	4,9E-03	5
APOE	-2,25	3,9E-08	16
INSIG1	-2,65	1,2E-03	7
ACOX1	-3,00	8,9E-04	9
TRIM24	-3,16	9,4E-06	10

¹Top ranked biological functions that were most significant to the 371 genes eligible for analysis using a right tailed Fisher's exact test.

²Canonical pathway analysis identified from the Ingenuity Knowledge base that were most significant to the gene set.

³Upstream regulator analysis based on the 371 genes using Ingenuity Knowledge base and ranked according to activation z-score. The z-score is based on relationships between experimentally observed gene expression and function annotation data, as derived from the information compiled in the Ingenuity Knowledge Base. These relationships are associated with a direction of change that is either activating (z-score ≥ 2) or inhibiting (z-score ≤ -2). For activated upstream regulators predicted in this analysis, the top 15 regulators are displayed.

Table S6. Ingenuity Pathway Analysis Summary for genes down-regulated in brain host stroma (mouse)

<i>Functional Analysis of gene subset¹</i>			
Category	Functions Annotation	p-Value	# molecules
Nervous System Development and Function	neurotransmission	4,6E-08	19
Nervous System Development and Function	synaptic transmission	7,3E-07	15
Behavior	learning	3,5E-06	18
Nervous System Development and Function	morphology of central nervous system	1,7E-05	23
Amino Acid Metabolism	metabolism of acidic amino acid	3,3E-05	3
Nervous System Development and Function	morphology of nervous system	3,9E-05	30
Nervous System Development and Function	morphology of hippocampus	4,0E-05	9
Cellular Movement	cell movement of neurons	4,3E-05	12
Nervous System Development and Function	NMDA-mediated synaptic current	8,0E-05	4
Nervous System Development and Function	morphology of brain	1,1E-04	20
Cell-To-Cell Signaling and Interaction	excitatory postsynaptic potential of neurons	1,1E-04	6
Behavior	behavior	1,4E-04	30
Nervous System Development and Function	migration of neurons	1,5E-04	11
Nervous System Development and Function	morphology of cerebral cortex	1,6E-04	11
Nervous System Development and Function	abnormal morphology of granule cell layer	1,9E-04	5
Nervous System Development and Function	formation of cerebral neocortex	2,3E-04	2
Embryonic Development	formation of heart tube	2,3E-04	2
Neurological Disease	Movement Disorders	3,5E-04	19
Nervous System Development and Function	action potential of neurons	3,7E-04	7
Nervous System Development and Function	paired-pulse facilitation of synapse	4,5E-04	5
<i>Canonical Pathway Analysis²</i>			
Top Canonical Pathway	p-value		
α -Adrenergic Signaling	2,5E-04		
Breast Cancer Regulation by Stathmin1	4,9E-04		
GABA Receptor Signaling	4,5E-04		
4-aminobutyrate Degradation I	6,8E-04		
γ -linolenate Biosynthesis II (Animals)	2,0E-03		
Glutamate Degradation III (via 4-aminobutyrate)	2,2E-03		
Ephrin B Signaling	4,1E-03		
nNOS Signaling in Neurons	4,7E-03		
Glutamate Receptor Signaling	7,8E-03		
Glycogen Degradation II	9,5E-03		
G Beta Gamma Signaling	9,7E-03		
G Protein Signaling Mediated by Tubby	1,1E-02		
Sertoli Cell-Sertoli Cell Junction Signaling	1,3E-02		
CCR5 Signaling in Macrophages	1,4E-02		
Glycogen Degradation III	1,4E-02		
Antiproliferative Role of Somatostatin Receptor 2	1,4E-02		
Glutamate Biosynthesis II	1,5E-02		
Glutamate Degradation X	1,5E-02		
Fatty Acid Activation	1,6E-02		
Gas Signaling	2,1E-02		
<i>Upstream Regulator Analysis³</i>			
Upstream Regulator	Activation z-score	p-value of overlap	Target molecules in dataset
ADORA2A	2,24	1,5E-02	5
NFE2L2	2,11	6,5E-03	11
miR-122-5p	-2,00	3,8E-03	4

¹Top ranked biological functions that were most significant to the 301 genes eligible for analysis using a right tailed Fisher's exact test.

²Canonical pathway analysis identified from the Ingenuity Knowledge base that were most significant to the gene set.

³Upstream regulator analysis based on the 301 genes using Ingenuity Knowledge base and ranked according to activation z-score. The z-score is based on relationships between experimentally observed gene expression and function annotation data, as derived from the information compiled in the Ingenuity Knowledge Base. Upstream regulators are categorized as activating when z-score ≥ 2 or inhibiting when z-score ≤ -2 .

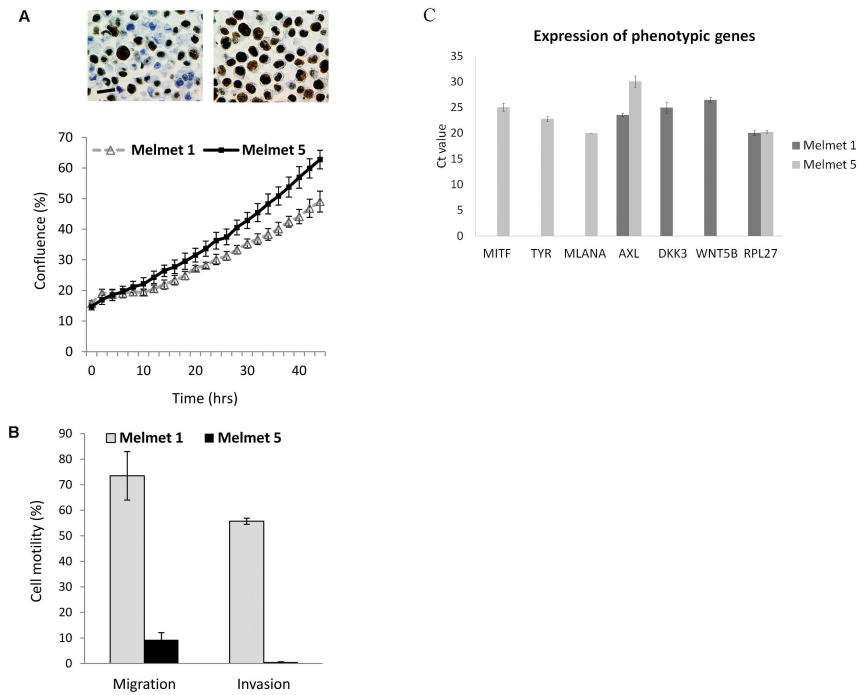
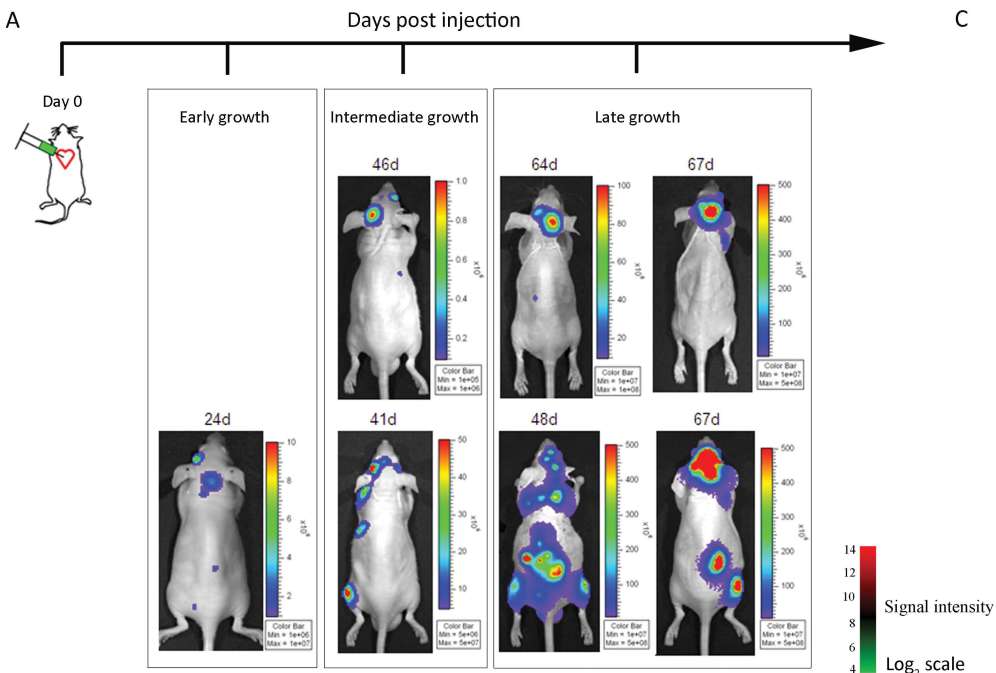
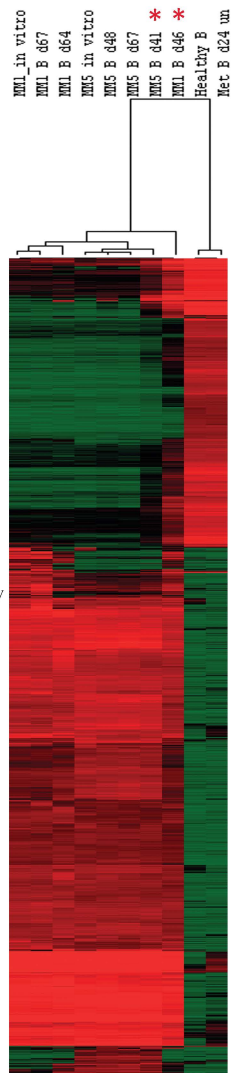


Figure S1. Phenotypic assignment of the Melmet 1 and Melmet 5 (A) Confluence was graphed over time to assess relative growth rates and relative proliferations was determined by Ki67 staining; (B) Migration through transwell membrane and invasion through Matrigel (24hrs); (C) Real time expression of phenotypic genes; *MITF*, *TYR* and *MLANA* (representative genes in proliferative signature), *AXL*, *DKK3* and *WNT5B* (representative genes in invasive signature). Each bar represents the Ct value measured for each gene (n=3).

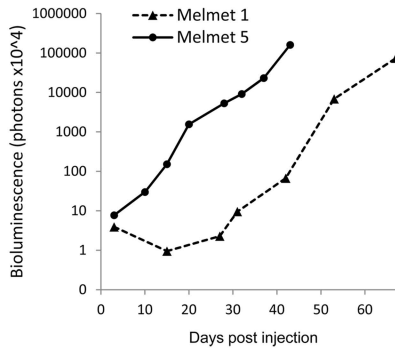
A



C



B



D

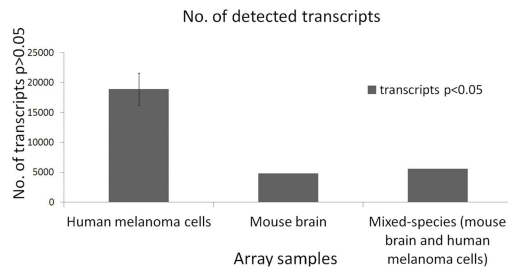


Figure S2. *In vivo* imaging and molecular characterization of samples harvested from an experimental mouse model. (A) *In vivo* imaging of metastatic growth at day of sacrifice showed distinct metastatic pattern for the two melanoma cell lines. The brain tissue samples were assigned into three groups representing different growth phases. (B) Brain colonization: tumor growth curves generated by measuring the increase in the bioluminescence signal (by IVIS) in the brain. Average values from 3 (MM1) and 4 (MM5) independent experiments including totally up to 19 and 26 animals, respectively. (C) Hierarchical clustering of genes with significant variance across all experimental sample conditions and cross-hybridization estimates. A total of 10 samples were analyzed; 6 brain metastasis, 2 *in vitro* cell cultures, one normal mouse brain and one mixed human/mouse sample. Gene vectors with SD ≥ 1.6 were selected for cluster analysis (1696/47324 transcripts). The two main gene cluster separate mouse derived samples from human tumor samples. The intermediate samples (*) showed a similar expression pattern as the mouse derived samples for a cluster of genes. (D) Cross-species hybridization rates on human-specific arrays. The bead-detection P-value statistic defined by the Genome Studio software was applied to quantify cross-hybridization of mouse transcripts on the Illumina Human HT-12v4 beadarray when standard stringent hybridization conditions were applied. The p-value statistic reports the confidence that a given transcript is detected above background and is typically influenced by low signal, high background or low stringency. The average number of probes statistically detected for the human melanoma samples was 39.9% (18888/47324). A pure mouse sample yielded positive detection for 10.2% (4824/47324) of the probes on the array while the mixed-species sample showed slightly increased detection of transcripts (11.8%, 5582/47324).

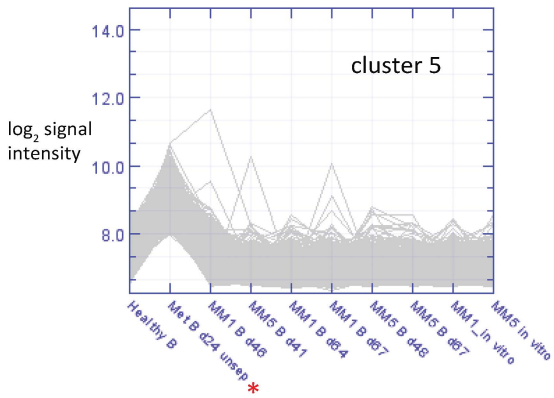
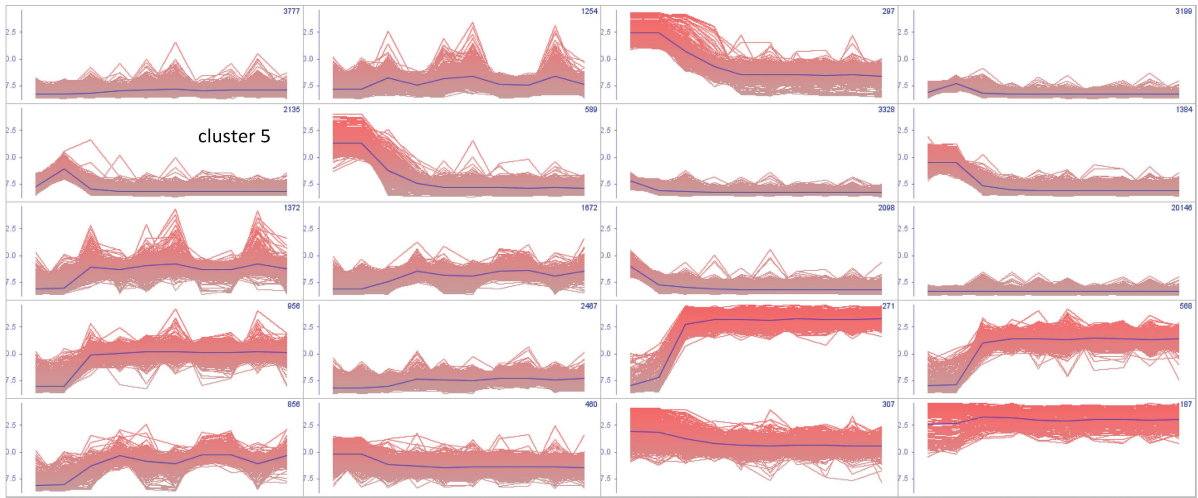


Figure S3. K-means clustering analysis. Unsupervised k-means clustering was performed whereby the dataset was distributed into 20 gene clusters. This partitioning of the data resulted in a satisfactory percentage of the variance explained by these 20 clusters. The peak in cluster 5 (enhanced box) depicts genes with elevated expression in the early growth phase sample (*) compared to the respective profiles of the normal mouse sample, *in vitro* samples and pure metastatic samples (all blue) harvested at later time points.

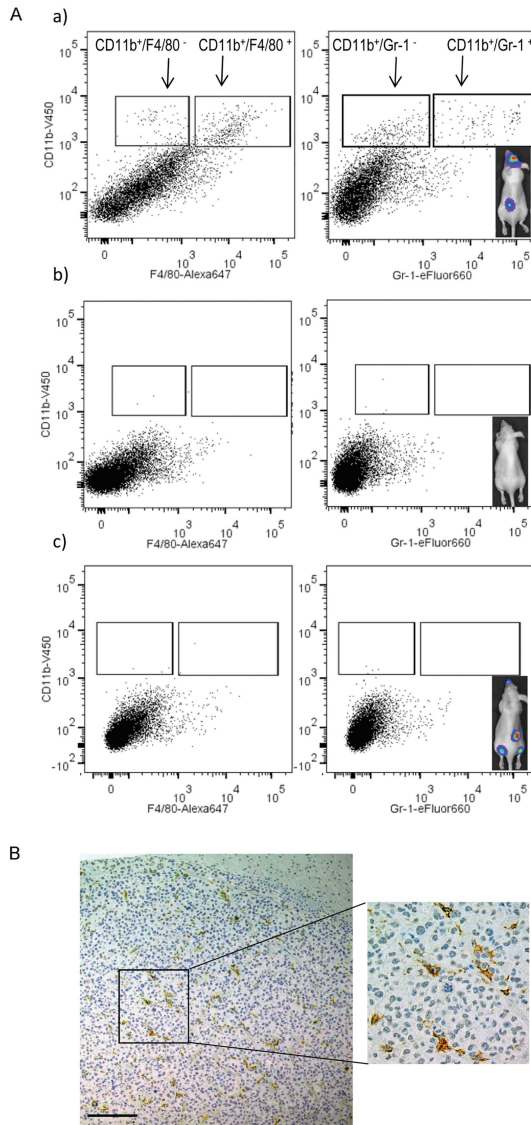


Figure S4. (A) Flow cytometric analysis of brain stroma cells stained with myeloid cell markers CD11b, F4/80 and Gr-1 in: a) metastatic brain; b) healthy brain; c) brain from the metastatic mouse with no metastases in the brain (illustrated by IVIS pictures in the inserts). Numbers indicate percentages of CD11b⁺ stroma cells negative (left quadrant) or positive (right quadrant) for F4/80 or Gr-1 (B) Immunohistochemical staining of brain metastases with anti-CD11b antibody.

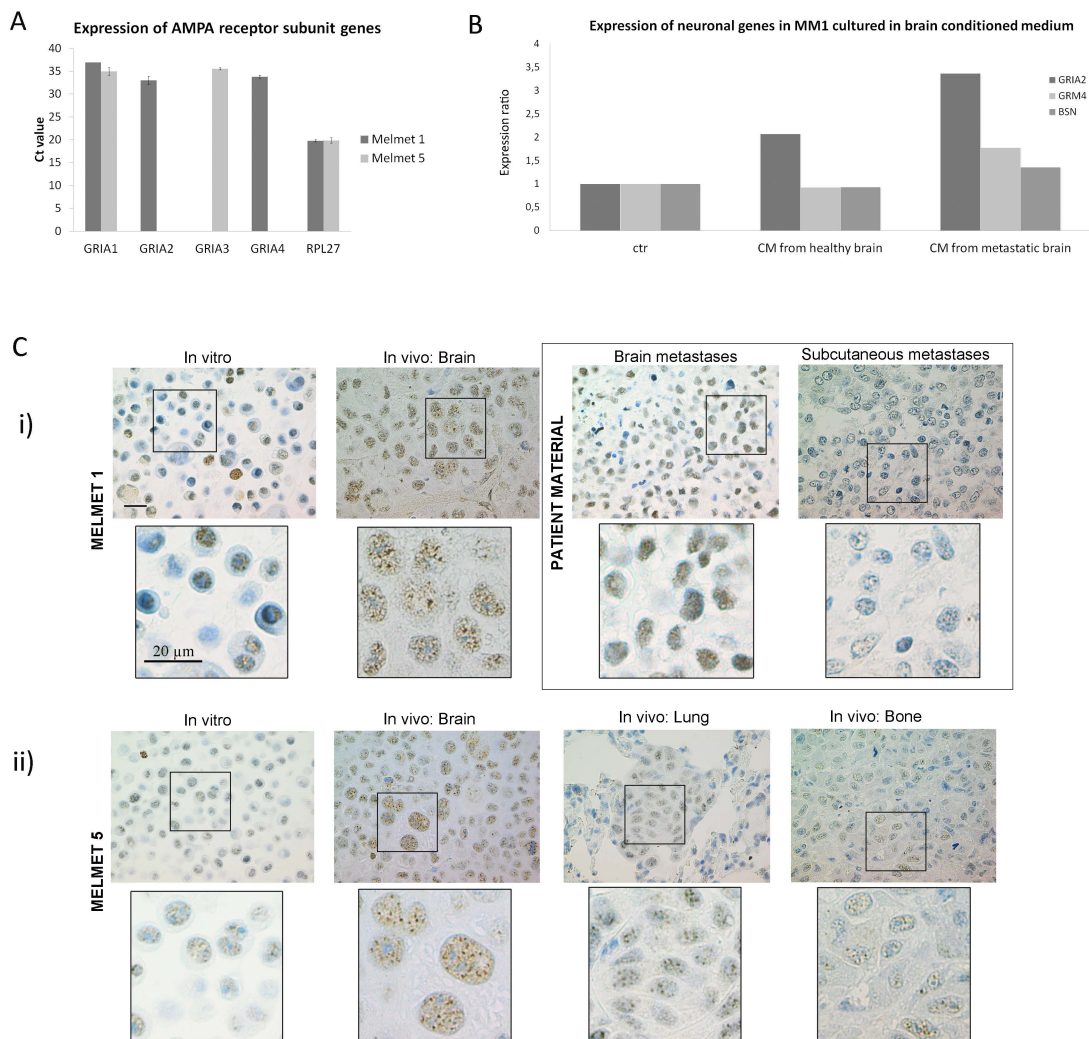


Figure S5. AMPA receptor expression *in vitro* and validation of induced neuron-like gene expression by the brain microenvironment. (A) Gene expression levels of AMPA receptor subunit genes in MM1 and MM5 cultured *in vitro*. Each bar represents the Ct value for each gene (n=3). (B) Analysis of neuronal gene expression in MM1 cultured in brain-conditioned medium. Real time RT-PCR analysis of neuron-like signature genes in MM1 grown in mouse brain-conditioned medium for 72hrs (n=2). The conditioned mediums were prepared from mouse brains in the following experimental conditions; normal healthy brain or metastatic brain. Brain-conditioned medium increased expression of *GRIA2*, consistent with *in vivo* gene expression data collected from MM1 brain metastases. (C) Antibody-based detection of *GRIA2* in *in vitro* and *in vivo* samples. Representative H&E sections stained with *GRIA2*-antibody. (i) Stained sections of fixed MM1 cells from *in vitro* cultures and MM1 brain metastasis obtained from the experimental metastasis animal model in nude mice. The brain section showed stronger staining of *GRIA2* with a nuclear localization. The brain metastasis section from the patient from which the MM1 cell line was derived, also showed stronger nuclear staining of *GRIA2* compared to the patient-matched subcutaneous lesion. (ii) Similar brain-specific staining pattern was observed in sections of fixed MM5 cells.