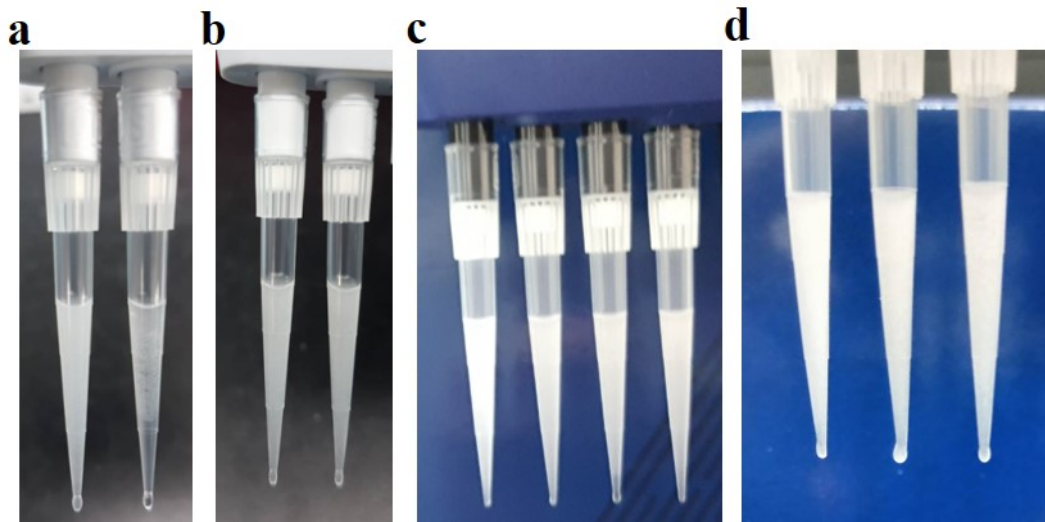
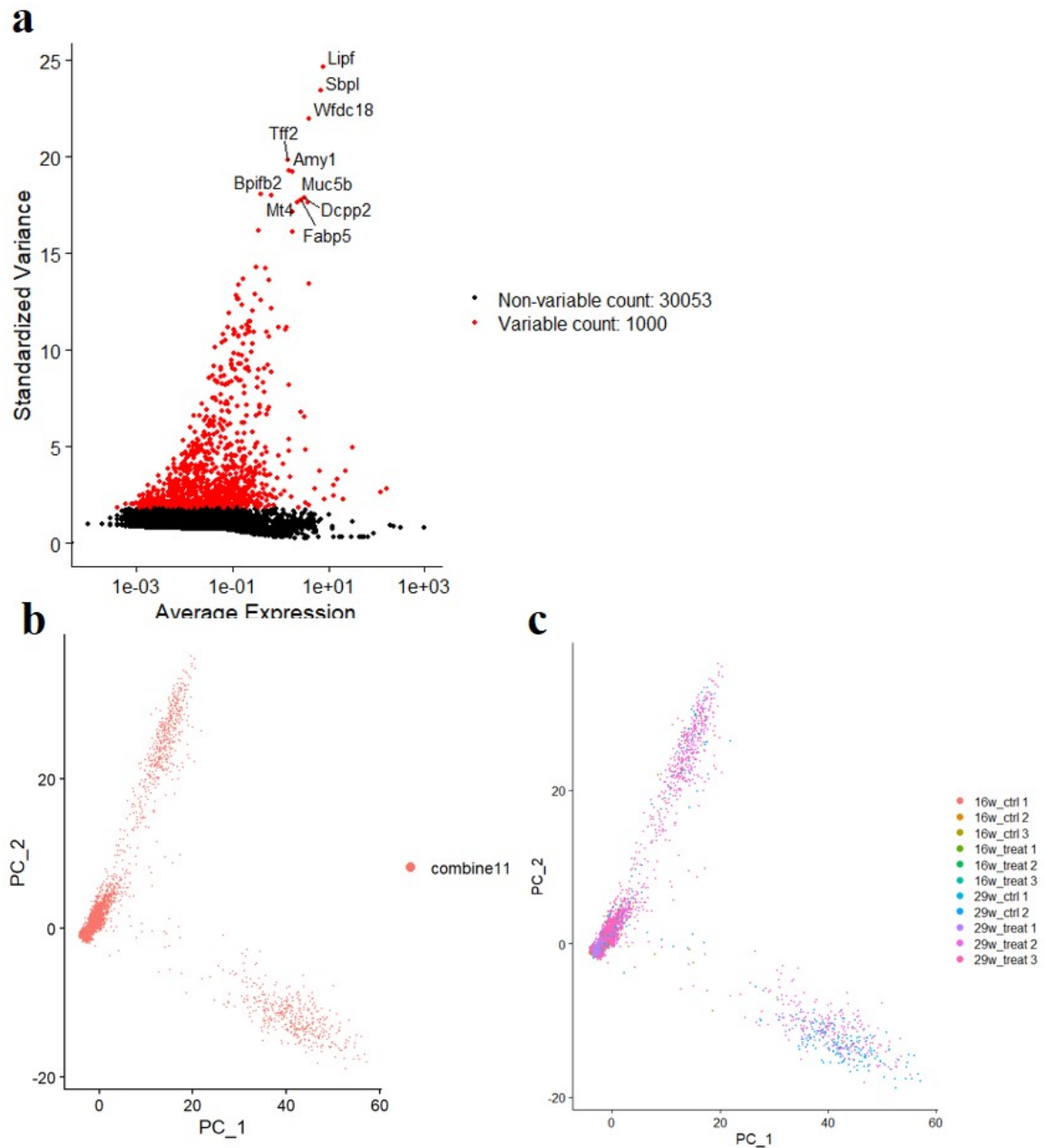


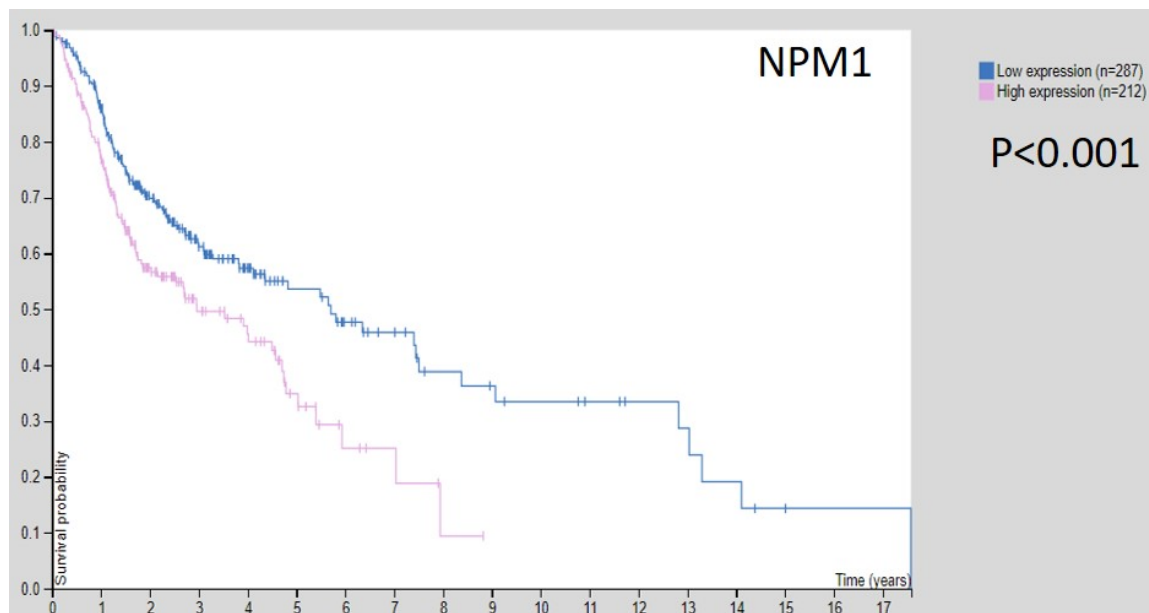
Supplement



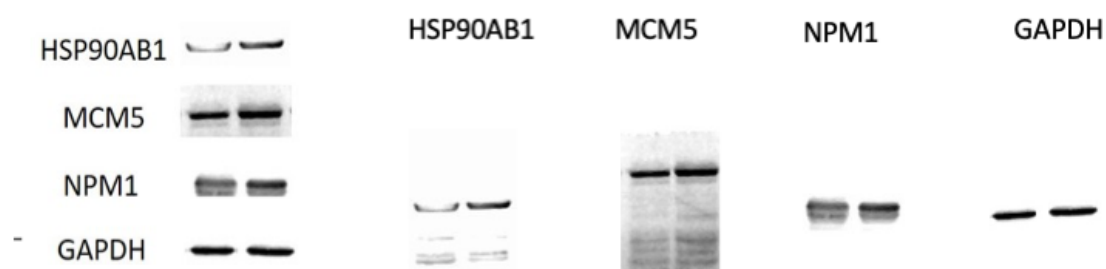
Supplemental Figure 1. Gel beads in emulsions (GEMs) generated by a single cell encapsulated in oil droplets. **(a)** After 29 weeks, cells in the experimental group (left) and control group (right) were encapsulated to produce GEMs; the control group exhibited a turbid, uneven distribution. **(b)** GEMs generated by repackaging cells obtained from the experimental and control groups at 29 weeks exhibited a uniform distribution. **(c)** The second batch of cells obtained from the experimental and control groups at 16 weeks generated GEMs after wrapping. **(d)** The second batch of cells obtained from the experimental and control groups at 29 weeks produced GEMs after wrapping.



Supplemental Figure 2. Calibration and linear dimensionality reduction data. **(a)** Variable feature plot displaying the expression levels of the 31 053 genes in all cells; the 1000 genes with the highest expression levels were selected for subsequent analysis, and the top 10 genes are labeled. **(c)** Principal component analysis was performed to determine the positions of 11 samples, which are indicated by a different color for each of the 11 samples.



Supplemental Figure 3. Effect of *NPM1* expression on the survival of patients with head and neck cancer. Data from The Cancer Genome Atlas database was analyzed to determine the relationship between *NPM1* mRNA expression and the survival rate of patients with head and neck cancer. The number of patient specimens with a high expression of *NPM1* mRNA was 212. The number of patient specimens with low expression of *NPM1* mRNA was 287. A high expression of *NPM1* was correlated with a lower likelihood of survival.



Supplemental Figure 4. The original PVDF membrane images of the Western Blotting of HSP90AB1, MCM5, NPM1, and GAPDH.

Protein extraction: the mouse tongues were cut with scissors and placed in a 1.5-ml microcentrifuge tube containing 2 μ l of protease inhibitor (Geneaid) and 198 μ l of Cytobuster Protein Extraction Reagent (Novagen, 71009-4). The tissues were ground using a micropestle, maintained at room temperature for 5 min and then centrifuged at 15 000 g for 5 min at 4°C. After centrifugation, the supernatant was transferred to a new microcentrifuge tube. With the protein extracted, the protein concentration was calculated so that protein samples could be prepared. To prepare these samples, 50 μ g of protein was combined with 1 μ l of reducing buffer (Lonza, 00193861B), 5 μ l of loading dye (Protein Aaasy Dye Reagent Concentrate, Bio-Rad, 500-0006) and a sufficient amount of water to yield a volume of 20 μ l; this mixture was then placed in a dry bath at a temperature of 100°C for 5 min. The protein samples were separated through sodium dodecyl sulfate polyacrylamide gel electrophoresis and then transferred to a polyvinylidene difluoride (PVDF; Millipore) membrane. The membrane was then placed in Tris-buffered saline combined with Tween 20 (TBST) and shaken on a flat rotary shaker for 1 h. The TBST solution was used to wash the PVDF membrane three times, with shaking at 50 rpm for 5 min each time. After the addition of the primary antibody, the solution was shaken at 50 rpm overnight on a flat rotary shaker at a temperature of 4°C (Supplemental Table 4). The next day, the solution was removed, and the PVDF membrane was washed three times with TBST, with shaking at 50 rpm for 5 min each time. Next, the secondary antibody was added, and the solution was shaken at 50 rpm on a flat rotary shaker for 1 h at room temperature. The PVDF membrane was washed with TBST three times, with shaking at 50 rpm for 5 min each time. Finally, Immobilon TM Western Chemiluminescent HRP Substrate (Millipore, P90720) was prepared at a ratio of 1:1. The appropriate amount was dropped onto the PVDF membrane to achieve coloration. Images were captured using a luminescence fluorescence image capture analyzer, and protein expression was quantified using Image J software.

Cell Line DNA Typing Report

Case Number: CID20130174
Report Date: 12/19/2013

Mission Biotech

10F-3, No.3, Yuanchi Street
Nangang, Taipei
Taiwan 115
Tel: 886 2 26557128
Email: service@missionbio.com.tw

Sample Information:

- i. Applicant Name: 謝達斌 Dar-BinShieh
- ii. Institution: 成功大學醫學院口腔醫學研究所 Institute of Oral Medicine and
Department of Stomatology, National Cheng Kung University, Tainan, Taiwan
- iii. Sample Description: HONE1
- iv. Sample type: Cell Pellet
- v. Sample Received Date: 12/10/2013

Allele table for the tested cell DNA

STR Locus	Repeat Numbers
D5S818	11,12
D13S317	10,12,13.3
D7S820	10
D16S539	9,10,11
vWA	14,16
TH01	7,9
Amelogenin	X
TPOX	8,12
CSF1PO	10,11
D21S11	27,30

Test Description:

Case Number: CID20130174
Test Date: 12/12/2013

Sample was extracted by Roche
MagNA Pure Compact System.
DNA conc. = 109.9 ng/μl
OD260/280 = 2.08
OD260/230 = 2.04

This test was performed by using the
Promega GenePrint® 10 System and
analyzed by ABI PRISM 3730 GENETIC
ANALYZER and GeneMapper® Software
V3.7.

Verified by:

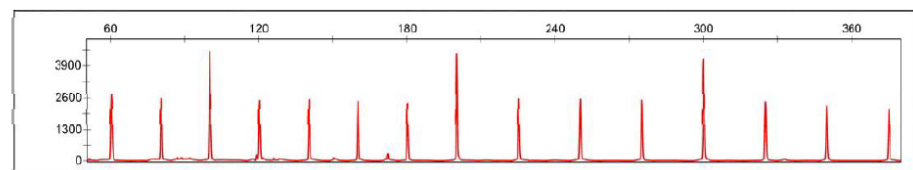
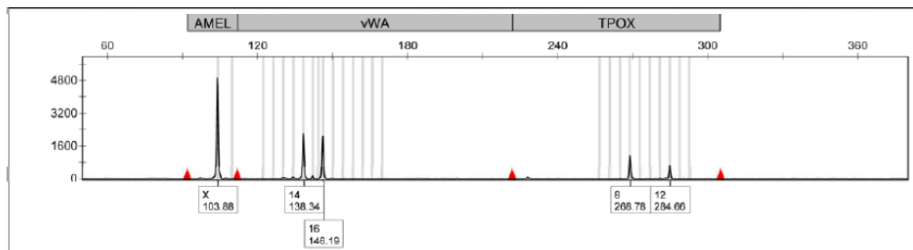
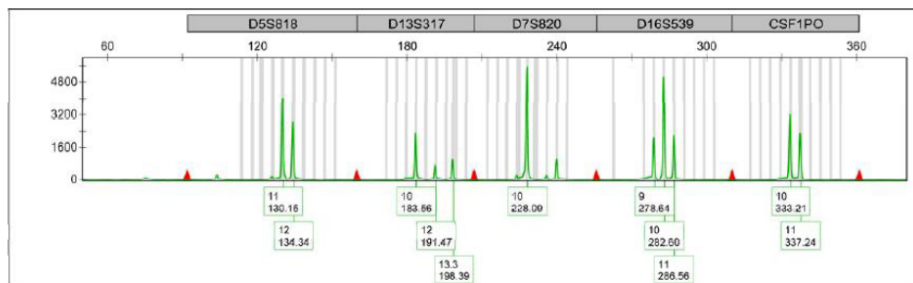
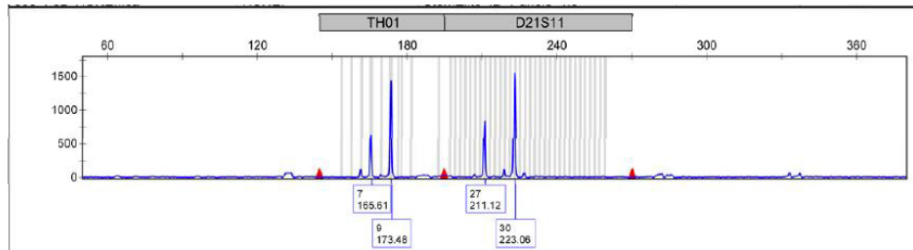
Laboratory Director (Title)

Liang Kuei Chang

12/16/2013

Allele Report

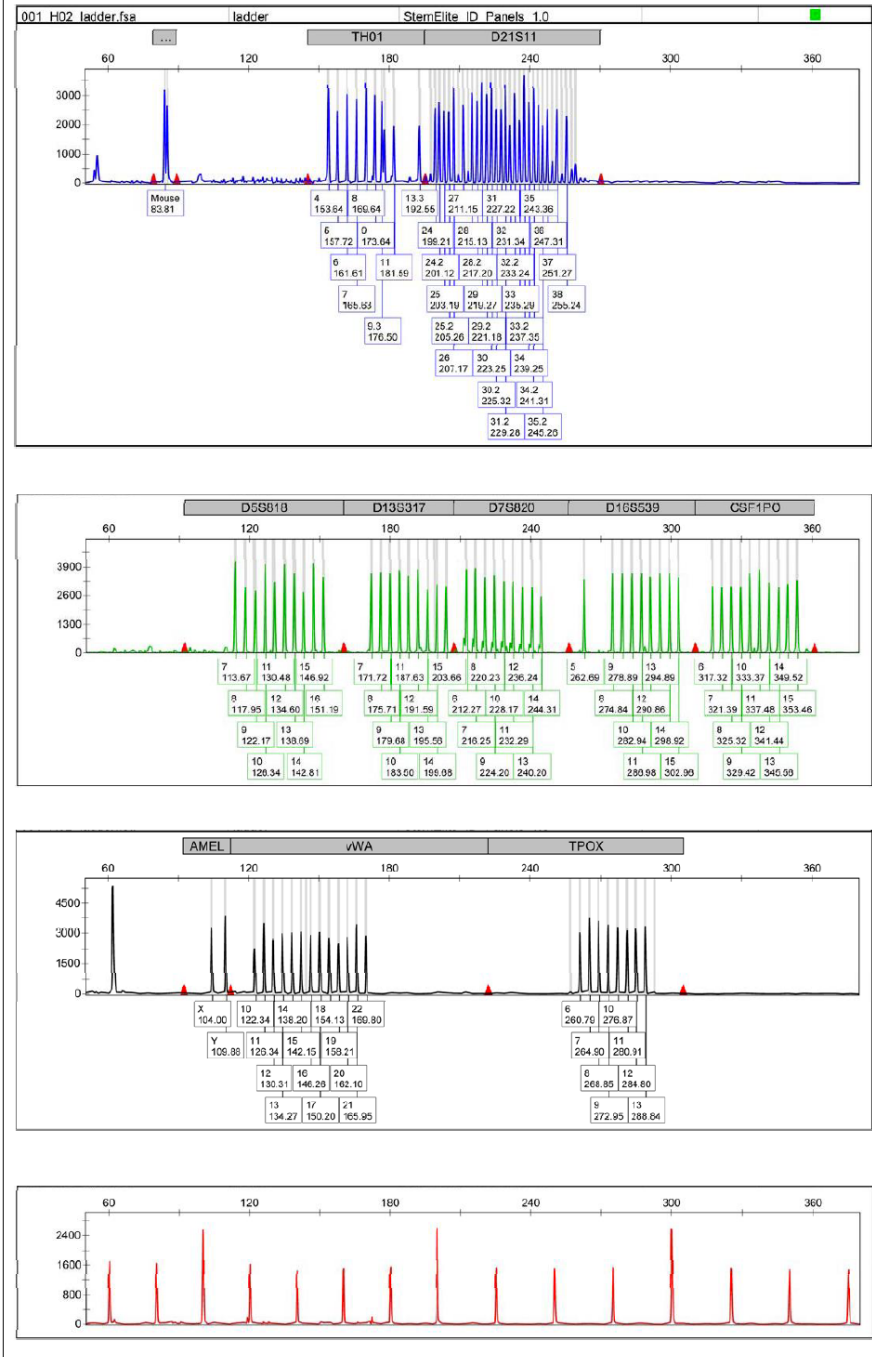
Case Number: CID20130174



Human Cell Line DNA Typing Report

	Dye	Sample File Name	Marker	Allele	Size	Height	Area
1	B,1	003_F02_HONE1.fsa	TH01	7	165.61	643	3065
2	B,2	003_F02_HONE1.fsa	TH01	9	173.48	1436	6638
3	B,3	003_F02_HONE1.fsa	D21S11	27	211.12	839	3967
4	B,4	003_F02_HONE1.fsa	D21S11	30	223.06	1561	7405
5	G,1	003_F02_HONE1.fsa	D5S818	11	130.15	4036	18612
6	G,2	003_F02_HONE1.fsa	D5S818	12	134.34	2883	13131
7	G,3	003_F02_HONE1.fsa	D13S317	10	183.56	2345	11125
8	G,4	003_F02_HONE1.fsa	D13S317	12	191.47	724	3490
9	G,5	003_F02_HONE1.fsa	D13S317	13.3	198.39	1055	4936
10	G,6	003_F02_HONE1.fsa	D7S820	10	228.09	5574	28551
11	G,7	003_F02_HONE1.fsa	D16S539	9	278.64	2075	10531
12	G,8	003_F02_HONE1.fsa	D16S539	10	282.6	5044	25460
13	G,9	003_F02_HONE1.fsa	D16S539	11	286.56	2183	11131
14	G,10	003_F02_HONE1.fsa	CSF1PO	10	333.21	3221	17027
15	G,11	003_F02_HONE1.fsa	CSF1PO	11	337.24	2337	12476
16	Y,1	003_F02_HONE1.fsa	AMEL	X	103.88	4944	23088
17	Y,2	003_F02_HONE1.fsa	vWA	14	138.34	2205	10390
18	Y,3	003_F02_HONE1.fsa	vWA	16	146.19	2085	9818
19	Y,4	003_F02_HONE1.fsa	TPOX	8	268.78	1132	5573
20	Y,5	003_F02_HONE1.fsa	TPOX	12	284.66	622	3233

Allelic Ladder



Human Cell Line DNA Typing Report

- *This STR analysis testing service is for research purposes only, and is not to be used for clinical diagnosis or applications involving humans.*
- *This test is just for typing the specific loci of the applied sample. The applicants may have to compare the results with the database of some bioresource institutes such as ATCC, JCRB or DSMZ by themselves.*



Genelabs Life science www.genelabs.com.tw

Phone: (02)26557678, (04)22633813, (06)2094380

Freecall: 0800-231914, 0800-094380



Cell Line DNA Typing Report

Case Number: CID20130175

Report Date: 12/19/2013

Mission Biotech

10F-3, No.3, Yuanchi Street

Nangang, Taipei

Taiwan 115

Tel: 886 2 26557128

Email: service@missionbio.com.tw

Sample Information:

- i. Applicant Name: 謝達斌 Dar-BinShieh
- ii. Institution: 成功大學醫學院口腔醫學研究所 Institute of Oral Medicine and
Department of Stomatology, National Cheng Kung University, Tainan, Taiwan
- iii. Sample Description: HONE1-CIS6
- iv. Sample type: Cell Pellet
- v. Sample Received Date: 12/10/2013

Allele table for the tested cell DNA

STR Locus	Repeat Numbers
D5S818	12
D13S317	10
D7S820	10,12
D16S539	9,10
vWA	14,16
TH01	6,7,9
Amelogenin	X
TPOX	8,12
CSF1PO	10,11
D21S11	27,30

Test Description:

Case Number: CID20130175

Test Date: 12/12/2013

Sample was extracted by Roche

MagNA Pure Compact System.

DNA conc.= 125.0 ng/ μ l

OD260/280 = 2.08

OD260/230 = 2.12

This test was performed by using the
Promega GenePrint® 10 System and
analyzed by ABI PRISM 3730 GENETIC
ANALYZER and GeneMapper® Software
V3.7.

Verified by:

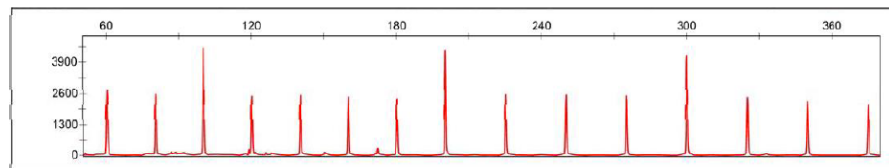
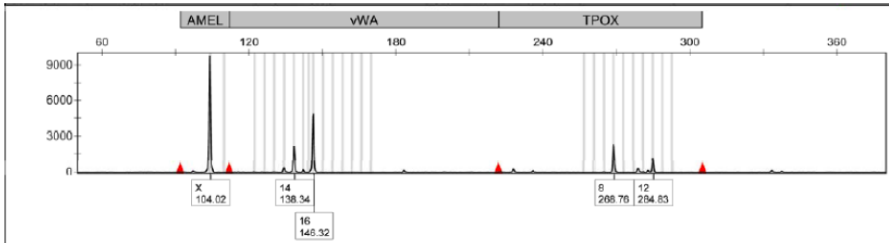
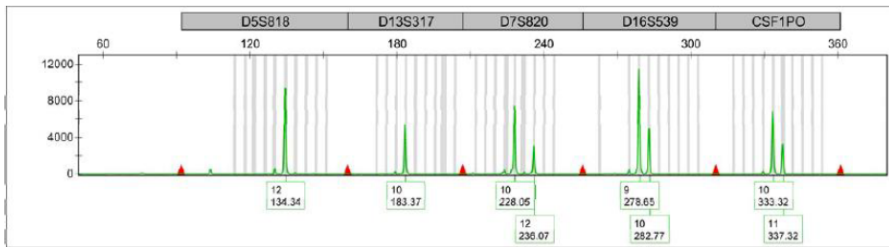
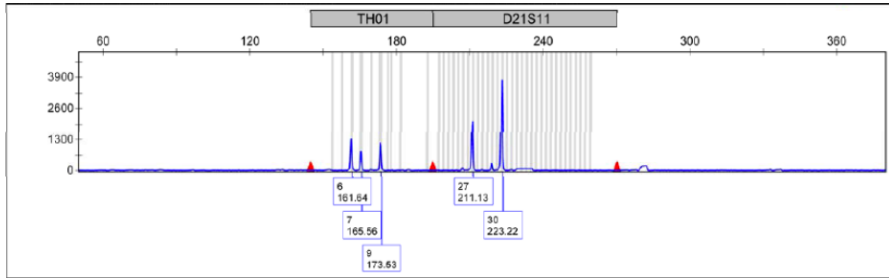
Laboratory Director (Title)

Liang Kuei Chang

12/16/2013

Allele Report

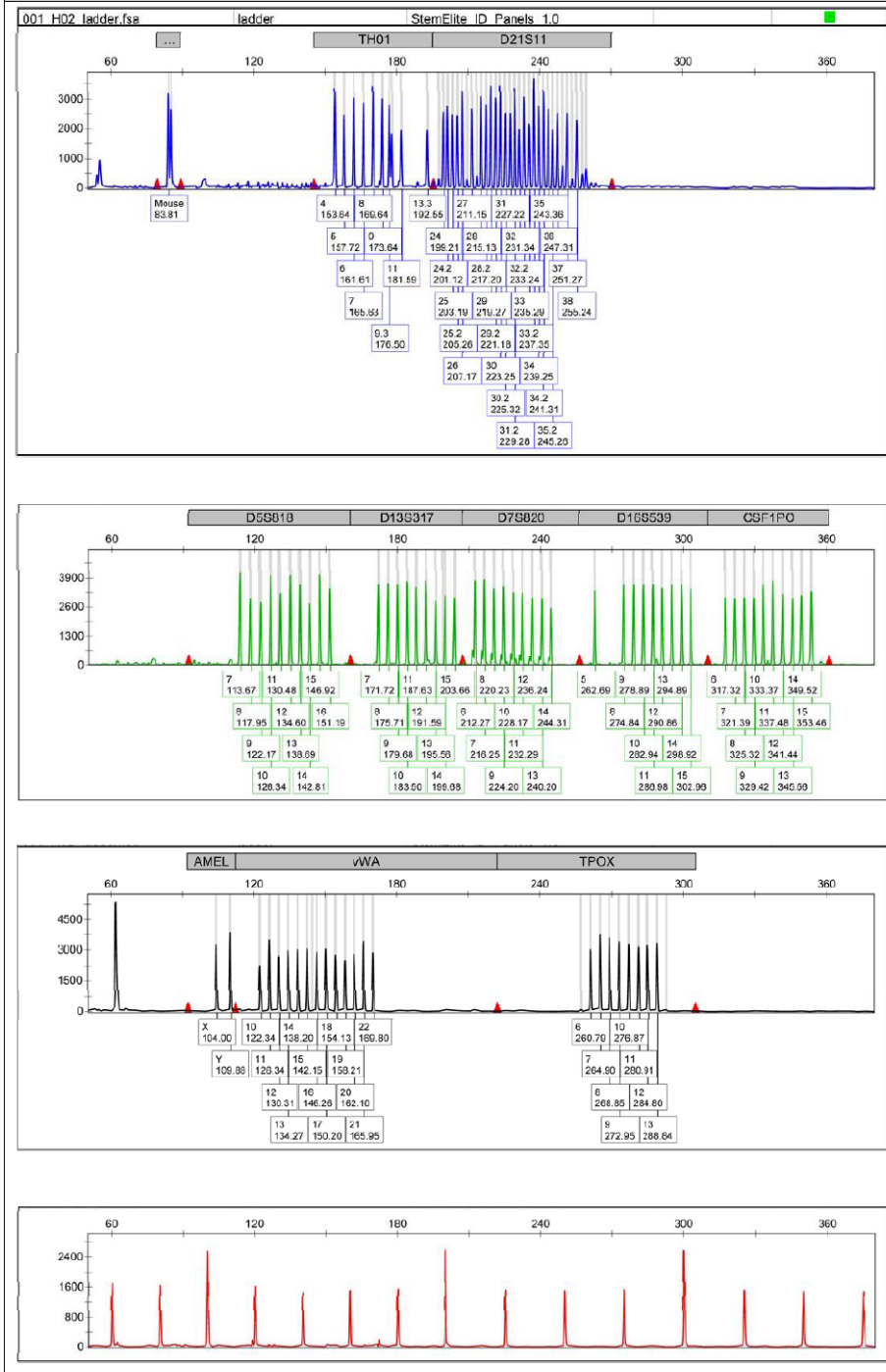
Case Number: CID20130175



Human Cell Line DNA Typing Report

	Dye	Sample File Name	Marker	Allele	Size	Height	Area
1	B,1	002_G02_HONE1-CIS6.fsa	TH01	6	161.64	1329	6112
2	B,2	002_G02_HONE1-CIS6.fsa	TH01	7	165.56	838	3681
3	B,3	002_G02_HONE1-CIS6.fsa	TH01	9	173.53	1171	5308
4	B,4	002_G02_HONE1-CIS6.fsa	D21S11	27	211.13	2075	9523
5	B,5	002_G02_HONE1-CIS6.fsa	D21S11	30	223.22	3787	17879
6	G,1	002_G02_HONE1-CIS6.fsa	D5S818	12	134.34	9331	43156
7	G,2	002_G02_HONE1-CIS6.fsa	D13S317	10	183.37	5408	25163
8	G,3	002_G02_HONE1-CIS6.fsa	D7S820	10	228.05	7498	36972
9	G,4	002_G02_HONE1-CIS6.fsa	D7S820	12	236.07	3087	15200
10	G,5	002_G02_HONE1-CIS6.fsa	D16S539	9	278.65	11493	57548
11	G,6	002_G02_HONE1-CIS6.fsa	D16S539	10	282.77	5021	25546
12	G,7	002_G02_HONE1-CIS6.fsa	CSF1PO	10	333.32	6806	34986
13	G,8	002_G02_HONE1-CIS6.fsa	CSF1PO	11	337.32	3270	16528
14	Y,1	002_G02_HONE1-CIS6.fsa	AMEL	X	104.02	9763	44820
15	Y,2	002_G02_HONE1-CIS6.fsa	vWA	14	138.34	2155	10390
16	Y,3	002_G02_HONE1-CIS6.fsa	vWA	16	146.32	4909	23029
17	Y,4	002_G02_HONE1-CIS6.fsa	TPOX	8	268.76	2347	11189
18	Y,5	002_G02_HONE1-CIS6.fsa	TPOX	12	284.83	1133	5511

Allelic Ladder



Human Cell Line DNA Typing Report

- *This STR analysis testing service is for research purposes only, and is not to be used for clinical diagnosis or applications involving humans.*
- *This test is just for typing the specific loci of the applied sample. The applicants may have to compare the results with the database of some bioresource institutes such as ATCC, JCRB or DSMZ by themselves.*



Genelabs Life science www.genelabs.com.tw

Phone: (02)26557678, (04)22633813, (06)2094380

Freecall: 0800-231914, 0800-094380



Supplemental Table 1.

Protocol for Generating Single-Cell 3' Gene Expression Libraries from Single Cells.

Steps	Procedures	Time	Store
Cell Preparation			
Cell Dissociation		1.5 h	
Step 1 – GEM Generation & Barcoding			
1-1	Prepare Reaction Mix	20 min	
1-2	Load Chromium Next GEM Chip G	10 min	
1-3	Run the Chromium Controller	18 min	
1-4	Transfer GEMs	3 min	
1-5	GEM-RT Incubation	55 min	4°C ≤72 h or -20°C ≤1 week
Step 2 – Post GEM-RT Cleanup & cDNA Amplification			
2-1	Post GEM RT-Cleanup – Dynabead	45 min	
2-2	cDNA Amplification	40 min	4°C ≤72 h or -20°C ≤1 week
2-3	cDNA Cleanup – SPRIselect	20 min	4°C ≤72 h -20°C ≤4 weeks
2-4	cDNA QC & Quantification	50 min	
Step 3 – 3' Gene Expression Library Construction			
3-1	Fragmentation, End Repair & A-tailing	50 min	
3-2	Post Fragmentation, End Repair & A-tailing Double Sided Size Selection – SPRIselect	30 min	
3-3	Adaptor Ligation	25 min	
3-4	Post Ligation Cleanup- SPRIselect	20 min	
3-5	Sample Index PCR	40 min	4°C ≤72 h
3-6	Post Sample Index PCR Double Sided Size Selection – SPRIselect	30 min	4°C ≤72 h or - 20°C long term
3-7	Post Library Construction QC	50 min	

First, 50% glycerol (JT Baker, 2136-01) was added to the Chromium Next GEM Chip G (Chip G; PN-1000120). Next, an appropriate amount of nuclease-free water (Thermo Fisher Scientific, R0581) and the corresponding amount of the cell suspension were added to the master mix [18.8 µl of RT Reagent B (PN2000165), 2.4 µl of Template Switch Oligo (PN3000228), 2.0 µl of Reducing Agent B (PN2000087) and 8.7 µl of RT Enzyme C (PN2000085)] to achieve a total volume of 75 µl. Subsequently, 70 µl of the combined master mix and cell suspension was added to the first row of Chip G (PN2000177). After single-cell 3' v3.1 gel beads (PN2000164) had been shaken for 30 s, 50 µl was extracted and added to the second row of Chip G, and 45 µl of partitioning oil (PN2000190) was added to the third row of Chip G. The 10X Gasket (PN370017) was then connected to the Chromium Controller and activated to coat the oil droplets. After processing, the gasket was removed and the chip holder was opened to form a 45° well. The volume of the first and second rows of Chip G was assessed, and then, 100 µl of gel beads in emulsion (GEMs) were transferred from the third row of Chip G to 8-row polymerase chain reaction (PCR) tubes. The GEMs then underwent incubation at a temperature of 53°C for 45 min and 85°C for 5 min before being maintained at 4°C.

To form Dynabeads, 125 µl of Recovery Agent (PN220016) was added to each sample, and the samples were kept at room temperature for 2 min. Next, 125 µl of the combined Recovery Agent and partitioning oil was extracted from the bottom of the tube, and a Dynabeads cleanup mix was prepared [182 µl of Cleanup Buffer (PN2000088), 8 µl of Dynabeads MyOne SILANE (PN2000048), 5 µl of Reducing Agent B (PN2000087), and 5 µl of nuclease-free water (Thermo Fisher Scientific, R0581)]. Each sample was supplemented with 200 µl of the cleanup mix and maintained at room temperature for 10 min. The samples were then placed in the 10X Magnetic Separator (PN120250) in the high position (10X Magnetic Separator-High) until the liquid was clear and then removed. Next, 300 µl of 80% alcohol was added to each sample. After 30 s, the alcohol was removed and 200 µl of

80% alcohol was added to each sample. After 30 s, the alcohol was removed again, and then, the sample was removed from the magnetic holder and centrifuged and placed in the 10X Magnetic Separator in the low position (10X Magnetic Separator-Low) to remove alcohol and air dry for 1 min. The samples were removed from the magnetic base, immediately supplemented with 35.5 µl of Elution Solution I [98 µl of elastic buffer (EB) (Qiagen, 19086), 1 µl of 10% Tween 20 (Sigma, P9416-50ML) and 1 µl of Reducing Agent B (PN2000087)], and then maintained at room temperature for 2 min. They were then returned to the magnetic base in the 10X Magnetic Separator-Low until the liquid was clear, and 35 µl of the samples were transferred to a new 8-row PCR tube.

After preparation of a cDNA amplification mix [50 µl of Amp Mix (PN2000047) and 15 µl of cDNA primers (PN2000089)], 35 µl of it was added to each sample (35 µl). A reaction was induced as follows: the temperature was set at 98°C for 3 min, 98°C for 15 sec, 63°C for 20 sec and 72°C for 1 min for 1 cycle and then 63°C for 20 sec and 72°C for 1 min for 11 cycles before being set at 72°C for 1 min and then maintained at 4°C. cDNA Cleanup-SPRIselect with shaking the SPRIselect reagent (Beckman Coulter, B23318); 60 µl (0.6X) was added to each sample, and then, the samples were maintained at room temperature for 5 min before being placed in the 10X Magnetic Separator-High until the liquid was clear. The supernatant was removed, and 200 µl of 80% alcohol was added to each sample. After 30 s, the alcohol was removed, and 200 µl of 80% alcohol was added to each sample. After 30 s, the alcohol was again removed. The sample was then removed from the magnetic stand, centrifuged and placed in the 10X Magnetic Separator-Low to remove the remaining alcohol and air dry for 2 min. The sample was then removed from the magnetic base, supplemented with 40.5 µl of EB (Qiagen, 19086) and maintained at room temperature for 2 min. Finally, 40 µl of the sample was transferred to a new 8-row PCR tube for measurement of the cDNA concentration. Fragmentation, end repair, and A-tailing were performed with a fragmentation mix [5 µl of Fragmentation Buffer (PN2000091) and 10 µl of Fragmentation Enzyme (PN2000090)] on ice. After 10 µl of cDNA was placed in a new 8-row PCR tube, 25 µl of EB (Qiagen, 19086) was added to each sample, and then, 15 µl of the fragmentation mix was added to each sample. The sample was placed in a thermal cycler under the following conditions: precool block at 4°C, fragmentation at 32°C for 5 min, end repair and A-tailing at 65°C for 30 min and finally maintenance at 4°C. Each sample was supplemented with 30 µl (0.6X) of shaken SPRIselect reagent and maintained at room temperature for 5 min before being placed in the 10X Magnetic Separator-High until the liquid was clear. After 75 µl of the supernatant had been placed in a new 8-row PCR tube, each sample was supplemented with 10 µl (0.8X) of shaken SPRIselect reagent (Beckman Coulter, B23318), maintained at room temperature for 5 min and then placed in the 10X Magnetic Separator-High until the liquid was clear. Next, 80 µl of the supernatant was removed, and 125 µl of 80% alcohol was added to each sample. After 30 s, the alcohol was removed and replaced with another 125 µl of 80% alcohol. After 30 s, the alcohol was again removed; samples were removed from the magnetic stand and then centrifuged and placed in the 10X Magnetic Separator-Low until the liquid was clear. The remaining alcohol was then removed, and samples were removed from the magnetic stand and centrifuged, supplemented with 50.5 µl of EB (Qiagen, 19086) and maintained at room temperature for 2 min. Next, samples were placed in the 10X Magnetic Separator-Low until the liquid was clear, and 50 µl of the samples were placed in a new 8-row PCR tube.

Adaptor ligation was performed using an adaptor ligation mix [20 µl of Ligation Buffer (PN2000092), 10 µl of DNA Ligase (PN220110) and 20 µl of Adaptor Oligos (PN2000094)]. After the addition of 50 µl of the adaptor ligation mix to each 50-µl sample, a temperature reaction was induced as follows: the temperature was set at 20°C 15 min and then maintained at 4°C. Each sample was supplemented with 80 µl (0.8X) of shaken SPRIselect reagent and maintained at room temperature for 5 min before being placed in the 10X Magnetic Separator-High until the liquid was clear. The supernatant was removed, and 200 µl of 80% alcohol was added to each sample. After 30 s, the alcohol was removed and replaced with 200 µl of 80% alcohol. After 30 s, the alcohol was again removed. Samples were taken out of the magnetic holder, centrifuged and placed in the 10X Magnetic Separator-Low. The remaining alcohol was removed, and the samples were air dried. After 2 min, the samples were removed from the magnetic holder and centrifuged. After being supplemented with 30.5 µl of EB, the samples were maintained at room temperature for 2 min and then placed in the 10X Magnetic Separator-Low until the liquid was clear. Next, 30 µl of the samples were placed in new 8-row PCR tubes.

A sample index PCR mix [50 µl of Amp Mix (PN2000047) and 10 µl of SI Primer (PN2000095)] (Supplemental Table 2) was prepared and used to perform sample index PCR. Each 30-µl sample was supplemented with 60 µl of the sample index PCR mix and 10 µl of the individual Chromium i7 Sample Index and then subjected to a temperature reaction under the following

conditions: temperature set at 98°C for 45 sec, 98°C for 20 sec, 54°C for 30 sec, 72°C for 20 sec and 98°C for 20 sec for 16 cycles and then set at 72°C for 1 min before being maintained at 4°C.

Post Sample Index PCR Double Sided Size Selection-SPRIselect Each sample was supplemented with 60 µl (0.6X) of shaken SPRIselect reagent and maintained at room temperature for 5 min. The samples were then placed in the 10X Magnetic Separator-High until the liquid was clear, and 150 µl of the supernatant was transferred to a new 8-row PCR tube. Each sample was supplemented with 20 µl of (0.8X) shaken SPRIselect reagent and maintained at room temperature for 5 min before being placed in the 10X Magnetic Separator-High until the liquid was clear; 165 µl of the supernatant was then removed, and 200 µl of 80% alcohol was added to each sample. After 30 s, the alcohol was removed, replaced with 200 µl of 80% alcohol and then removed again after 30 s. The samples were removed from the magnetic base and centrifuged, supplemented with 35.5 µl of EB and maintained at room temperature for 2 min before being placed in the 10X Magnetic Separator-Low until the liquid was clear. Finally, 35 µl of the samples was transferred to a new 8-row PCR tube for assessment of their concentration after establishment of a gene expression library.

Supplemental Table 2.

Sample indices of the NextSeq 500/550 High-Output v2.5 Kit (150 cycles) (20024907)

sample name	Index_Plate_Well	I7_Index_ID	index 1 sequence
TN-42_1	SI-GA-D11_1	SC0084	CTTTGCGG
TN-42_2	SI-GA-D11_2	SC0180	TGCACAAA
TN-42_3	SI-GA-D11_3	SC0276	AAGCAGTC
TN-42_4	SI-GA-D11_4	SC0372	GCAGTTCT
TN-43_1	SI-GA-C11_1	SC0083	GAGGATCT
TN-43_2	SI-GA-C11_2	SC0179	AGACCATA
TN-43_3	SI-GA-C11_3	SC0275	TCCTGCGC
TN-43_4	SI-GA-C11_4	SC0371	CTTATGAG
TN-44_1	SI-GA-B11_1	SC0082	G TTCCTCA
TN-44_2	SI-GA-B11_2	SC0178	AGGTACGC
TN-44_3	SI-GA-B11_3	SC0274	TAAGTATG
TN-44_4	SI-GA-B11_4	SC0370	CCCAGGAT

Supplemental Table 3.

Primers of the Real-Time Quantitative Polymerase Chain Reaction

<i>PSMA6</i> -RTF	GGTTTTGACCGCCACATTACC
<i>PSMA6</i> -RTR	GCCACCCTGGTTAATAGCCT
<i>EIF3B</i> -RTF	GGACCCGACCGACTTGAGA
<i>EIF3B</i> -RTR	TTGACCCGGAATGTGTGCTG
<i>MCM5</i> -RTF	AGCATTCGTAGCCTGAAGTCG
<i>MCM5</i> -RTR	CGGCACTGGATAGAGATGCG
<i>RANBP1</i> -RTF	CAAACTGTTCCGATTTGCCTC
<i>RANBP1</i> -RTR	GGCGTGATGTAGTGGTTGG
<i>HSP90AB1</i> -RTF	CGAAGTTGGACAGTGGTAAAGAG
<i>HSP90AB1</i> -RTR	TGCCCAATCATGGAGATGTCT
<i>NPM1</i> -RTF	GCACTTAGTAGCTGTGGAGGA
<i>NPM1</i> -RTR	TTCACTGGCGCTTTTCTTCA

RNA extraction: Mouse tongues were placed in a 1.5-ml microcentrifuge tube containing 1 ml of TRI reagent (Zymo, R2050-1-200). The tissues were cut with scissors, ground using a micropestle and then placed on ice for 5 min. After the addition of 200 µl of chloroform (PanReac AppliChem, A3691, 1000), it was shaken upside down to achieve an even distribution, placed on ice for 5 min and then centrifuged at 12 000 rpm at a temperature of 4°C for 15 min. The supernatant was transferred to a new 1.5-ml microcentrifuge tube and supplemented with an equal volume of isopropanol (PanReac AppliChem, A3928, 0500PE). The mixture was shaken upside down and mixed well. After resting on

ice for 10 min, it was then centrifuged at 12 000 rpm at a temperature of 4°C for 10 min to precipitate RNA. The supernatant was removed, leaving behind a white RNA pellet. After the addition of 1 ml of 70% alcohol (ECHO, 484000011107A-69EC) and a mixture of 5 diethyl pyrocarbonate (VWR, 71003-388) and water, the RNA pellet was washed and centrifuged at 7500 rpm at a temperature of 4°C for 5 min to remove the supernatant. The microcentrifuge tube was left open on a sterile operating table to air dry. After the alcohol had completely evaporated, 30 µl of diethyl pyrocarbonate water was added; RNA was purified, and its quality and concentration were assessed using a NanoDrop spectrophotometer before it was finally stored in a refrigerator at –80°C.

Reverse-transcription PCR (RT-PCR): in a PCR tube, 4 µl of 5X cDNA Synthesis Mix (PCR Biosystems, PB30.31-10) was combined with 1 µl of MlraScript 2.0 for cDNA synthesis (PCR Biosystems, PB30.31-10) and 2 µg of RNA with enough nuclease-free water (Thermo Fisher Scientific, R0581) to yield a total volume of 20 µl. The samples were placed in a PCR machine for a cDNA synthesis reaction with the temperature set at 55°C for 30 min and 95°C for 10 min before being maintained at 12°C. Subsequently, the cDNA underwent RT-PCR analysis in 8-row PCR tubes containing 10 µl of 2X qPCRBIO SyGreen Blue Mix (PCR Biosystems, PB20.16-01), 0.8 µl (10 µM) of forward primer, 0.8 µl (10 µM) of reverse primer, 1 µl of cDNA and 7.4 µl of nuclease-free water (Thermo Fisher Scientific, R0581) (Supplemental Table 3). The aforementioned q-PCR reaction mixture was placed in a real-time PCR apparatus, and real-time quantification was performed under the following conditions: temperature set at 95°C for 2 min, 95°C for 5 sec, 65°C for 30 sec and 95°C for 5 sec for 40 cycles and then 95°C for 15 sec, 60°C for 1 min and 95°C for 15 sec to melt the curve.

Supplemental Table 4.

Antibodies in the Western blot

Antibody	Brand	Dilution
Anti-BRK	Invitrogen	1:5000
Anti-GAPDH	proteintech	1:5000
Anti-MCM5	abcam	1:5000
Anti-NPM1	proteintech	1:3000
Anti-HSP90AB1	proteintech	1:2000
Goat anti-mouse IgG	Millipore	1:5000
Goat anti-rabbit IgG	Millipore	1:10000

Supplemental Table 5.

Significant Genes Expressed in Each of the 17 Cell Subtypes

cluster	order	gene	p_val	avg_logFC	pct.1	pct.2	p_val_adj
0	1	<i>Bpgm</i>	0.00E+00	1.3378	0.837	0.3	0.00E+00
0	2	<i>Fam46c</i>	0.00E+00	1.2019	0.786	0.244	0.00E+00
0	3	<i>Snca</i>	0.00E+00	1.1781	0.856	0.288	0.00E+00
0	4	<i>Mkrn1</i>	0.00E+00	1.1177	0.895	0.491	0.00E+00
0	5	<i>Epb41</i>	0.00E+00	1.0214	0.719	0.251	0.00E+00
0	6	<i>Hbb-bt</i>	0.00E+00	0.9622	0.995	0.489	0.00E+00
0	7	<i>Fam213a</i>	1.69E-279	0.9483	0.412	0.103	5.24E-275
0	8	<i>Hbb-bs</i>	0.00E+00	0.9466	0.998	0.709	0.00E+00
0	9	<i>Alas2</i>	0.00E+00	0.8486	0.934	0.351	0.00E+00
0	10	<i>Ube2o</i>	0.00E+00	0.7664	0.45	0.109	0.00E+00
0	11	<i>Fam220a</i>	0.00E+00	0.7386	0.716	0.282	0.00E+00
0	12	<i>Ube2l6</i>	0.00E+00	0.712	0.721	0.288	0.00E+00
0	14	<i>Isg20</i>	9.17E-252	0.656	0.625	0.252	2.85E-247
0	21	<i>Hba-a1</i>	9.07E-171	0.5559	0.996	0.53	2.82E-166
0	23	<i>Hba-a2</i>	1.45E-174	0.504	0.996	0.494	4.50E-170
0	30	<i>Car2</i>	1.84E-130	0.4005	0.357	0.13	5.72E-126
0	34	<i>Prdx2</i>	1.18E-166	0.3913	0.637	0.347	3.66E-162
0	39	<i>Gypa</i>	1.16E-80	0.3123	0.257	0.099	3.60E-76

cluster	order	gene	p_val	avg_logFC	pct.1	pct.2	p_val_adj
1	1	<i>Retnlg</i>	0.00E+00	4.4788	0.92	0.054	0.00E+00
1	2	<i>S100a9</i>	0.00E+00	3.3422	0.995	0.324	0.00E+00
1	3	<i>S100a8</i>	0.00E+00	3.341	0.996	0.312	0.00E+00
1	4	<i>Cxcl2</i>	0.00E+00	3.1766	0.656	0.057	0.00E+00
1	5	<i>Ifitm6</i>	0.00E+00	2.9945	0.876	0.078	0.00E+00
1	6	<i>Wfdc21</i>	0.00E+00	2.8492	0.94	0.067	0.00E+00
1	7	<i>Lcn2</i>	0.00E+00	2.795	0.95	0.12	0.00E+00
1	8	<i>Ngp</i>	0.00E+00	2.6969	0.811	0.081	0.00E+00
1	9	<i>Mmp9</i>	0.00E+00	2.6541	0.855	0.02	0.00E+00
1	10	<i>Ltf</i>	0.00E+00	2.6436	0.594	0.048	0.00E+00
1	13	<i>Camp</i>	0.00E+00	2.4163	0.705	0.117	0.00E+00
1	76	<i>Lyz2</i>	0.00E+00	1.4481	0.934	0.133	0.00E+00
1	84	<i>Chil3</i>	0.00E+00	1.3664	0.705	0.094	0.00E+00
1	141	<i>Hmgb2</i>	0.00E+00	1.1702	0.916	0.224	0.00E+00
1	145	<i>Serpina1a</i>	0.00E+00	1.1458	0.519	0.081	0.00E+00
1	191	<i>Hmgn2</i>	0.00E+00	0.9585	0.568	0.102	0.00E+00
1	528	<i>H2afz</i>	1.83E-233	0.4555	0.691	0.276	5.67E-229

cluster	order	gene	p_val	avg_logFC	pct.1	pct.2	p_val_adj
2	1	<i>Hbb-bt</i>	0.00E+00	1.0692	0.996	0.555	0.00E+00

2	2	<i>Hba-a2</i>	0.00E+00	0.9751	0.997	0.559	0.00E+00
2	3	<i>Hba-a1</i>	0.00E+00	0.9109	0.998	0.59	0.00E+00
2	4	<i>Bpgm</i>	5.35E-240	0.8089	0.895	0.363	1.66E-235
2	5	<i>Hbb-bs</i>	1.50E-160	0.7467	0.998	0.746	4.66E-156
2	6	<i>Mkrl1</i>	4.61E-187	0.7158	0.918	0.541	1.43E-182
2	7	<i>Alas2</i>	2.77E-187	0.7046	0.937	0.426	8.60E-183
2	8	<i>Snca</i>	4.73E-199	0.7026	0.869	0.36	1.47E-194
2	9	<i>Ube2l6</i>	5.24E-177	0.6432	0.792	0.336	1.63E-172
2	10	<i>Isg20</i>	1.08E-164	0.6122	0.716	0.29	3.34E-160
2	12	<i>Fam220a</i>	9.87E-153	0.5884	0.758	0.334	3.07E-148
2	19	<i>Gypa</i>	1.59E-101	0.3229	0.359	0.108	4.95E-97
2	24	<i>Fam213a</i>	9.35E-49	0.2984	0.34	0.152	2.90E-44
2	25	<i>Isca1</i>	5.16E-55	0.2684	0.344	0.147	1.60E-50

cluster	order	gene	p_val	avg_logFC	pct.1	pct.2	p_val_adj
3	1	<i>Hba-a2</i>	0.00E+00	1.1189	1	0.569	0.00E+00
3	2	<i>Hba-a1</i>	0.00E+00	1.0922	1	0.6	0.00E+00
3	3	<i>Hbb-bs</i>	8.33E-195	0.773	1	0.752	2.59E-190
3	4	<i>Alas2</i>	3.84E-36	0.4687	0.702	0.459	1.19E-31

3	5	<i>Ube2l6</i>	4.80E-13	0.2763	0.481	0.375	1.49E-08
3	6	<i>Fam220a</i>	8.58E-12	0.2576	0.479	0.368	2.66E-07
3	7	<i>Hbb-bt</i>	1.32E-45	0.2535	0.996	0.565	4.10E-41

cluster	order	gene	p_val	avg_logFC	pct.1	pct.2	p_val_adj
4	1	<i>ApoE</i>	0.00E+00	3.0049	0.525	0.063	0.00E+00
4	2	<i>Cd74</i>	0.00E+00	2.4612	0.344	0.029	0.00E+00
4	3	<i>H2-Ab1</i>	8.43E-252	2.4507	0.286	0.025	2.62E-247
4	4	<i>H2-Eb1</i>	6.63E-238	2.4167	0.256	0.021	2.06E-233
4	5	<i>H2-Aa</i>	9.64E-278	2.3344	0.28	0.02	2.99E-273
4	6	<i>Ctss</i>	0.00E+00	2.2366	0.678	0.021	0.00E+00
4	7	<i>Lyz2</i>	5.98E-209	2.0643	0.71	0.257	1.86E-204
4	8	<i>S100a4</i>	0.00E+00	1.9809	0.618	0.056	0.00E+00
4	9	<i>Plac8</i>	2.61E-246	1.9717	0.549	0.132	8.11E-242
4	10	<i>Psap</i>	2.95E-303	1.94	0.706	0.205	9.15E-299
4	29	<i>Chil3</i>	2.30E-55	1.3452	0.425	0.197	7.15E-51

4	113	<i>H2afz</i>	4.75E-155	0.9992	0.754	0.326	1.48E-150
4	143	<i>Lgals1</i>	9.89E-263	0.9463	0.522	0.088	3.07E-258
4	233	<i>H2-K1</i>	7.74E-158	0.7733	0.646	0.215	2.40E-153

cluster	order	gene	p_val	avg_logFC	pct.1	pct.2	p_val_adj
5	1	<i>Hba-a2</i>	1.55E-271	1.0144	0.997	0.574	4.82E-267
5	2	<i>Hba-a1</i>	1.00E-262	0.9697	0.997	0.605	3.11E-258
5	3	<i>Hbb-bs</i>	3.68E-170	0.7575	0.997	0.755	1.14E-165
5	4	<i>Hbb-bt</i>	5.24E-94	0.5807	0.996	0.57	1.63E-89
5	5	<i>Alas2</i>	4.73E-65	0.5144	0.825	0.452	1.47E-60
5	6	<i>Fam220a</i>	1.20E-29	0.344	0.583	0.362	3.72E-25
5	7	<i>Ube2l6</i>	8.72E-26	0.3002	0.575	0.368	2.71E-21
5	8	<i>Isca1</i>	3.99E-16	0.2816	0.274	0.16	1.24E-11

cluster	order	gene	p_val	avg_logFC	pct.1	pct.2	p_val_adj
6	1	<i>Dcn</i>	0.00E+00	4.8504	1	0.027	0.00E+00
6	2	<i>Gsn</i>	0.00E+00	4.5078	1	0.19	0.00E+00
6	3	<i>Plpp3</i>	0.00E+00	3.1037	0.93	0.032	0.00E+00
6	4	<i>Igfbp4</i>	0.00E+00	3.0014	0.983	0.046	0.00E+00
6	5	<i>Col3a1</i>	0.00E+00	2.9549	0.875	0.007	0.00E+00
6	6	<i>Serping1</i>	0.00E+00	2.8792	0.978	0.024	0.00E+00
6	7	<i>Mfap5</i>	0.00E+00	2.8517	0.945	0.004	0.00E+00
6	8	<i>Colla2</i>	0.00E+00	2.6834	0.95	0.004	0.00E+00
6	9	<i>Dpt</i>	0.00E+00	2.6559	0.92	0.003	0.00E+00
6	10	<i>Cdkn1c</i>	0.00E+00	2.6023	0.623	0.014	0.00E+00
6	12	<i>Igfbp5</i>	0.00E+00	2.5602	0.723	0.039	0.00E+00
6	31	<i>Sparc</i>	0.00E+00	2.2513	0.92	0.029	0.00E+00
6	51	<i>Igfbp7</i>	0.00E+00	1.9009	0.893	0.027	0.00E+00
6	68	<i>Mgp</i>	0.00E+00	1.7399	0.459	0.023	0.00E+00
6	69	<i>Ebfl</i>	0.00E+00	1.7373	0.845	0.043	0.00E+00
6	78	<i>Lgals1</i>	0.00E+00	1.6532	0.913	0.087	0.00E+00
6	90	<i>Sptbn1</i>	0.00E+00	1.5671	0.943	0.14	0.00E+00
6	281	<i>Timp3</i>	0.00E+00	0.9492	0.603	0.047	0.00E+00
6	370	<i>Psap</i>	4.50E-203	0.829	0.87	0.216	1.40E-198
6	466	<i>S100a4</i>	1.99E-114	0.7451	0.439	0.083	6.17E-110

6	541	<i>Hspb1</i>	1.40E-174	0.6929	0.449	0.061	4.36E-170
6	1043	<i>Nupr1</i>	3.27E-306	0.4709	0.701	0.097	1.02E-301
6	1180	<i>H2-K1</i>	2.78E-83	0.4197	0.686	0.228	8.64E-79
6	1237	<i>ApoE</i>	9.16E-61	0.4005	0.337	0.087	2.85E-56
6	1566	<i>Gng11</i>	5.64E-122	0.2874	0.367	0.055	1.75E-117
6	1582	<i>Cavin2</i>	2.70E-127	0.2771	0.334	0.044	8.38E-123

cluster	order	gene	p_val	avg_logFC	pct.1	pct.2	p_val_adj
7	1	<i>Car2</i>	0.00E+00	2.8052	0.975	0.148	0.00E+00
7	2	<i>Hmbs</i>	0.00E+00	2.6686	0.945	0.041	0.00E+00
7	3	<i>Hist1h1b</i>	0.00E+00	2.5751	0.549	0.04	0.00E+00
7	4	<i>Slc4a1</i>	0.00E+00	2.5201	0.897	0.081	0.00E+00
7	5	<i>Prdx2</i>	1.99E-278	2.469	0.972	0.388	6.19E-274

7	6	<i>Tmcc2</i>	0.00E+00	2.4167	0.589	0.021	0.00E+00
7	7	<i>Mki67</i>	0.00E+00	2.302	0.667	0.057	0.00E+00
7	8	<i>Gypa</i>	0.00E+00	2.1962	0.885	0.103	0.00E+00
7	9	<i>Blvrbl</i>	0.00E+00	2.1916	0.927	0.236	0.00E+00
7	10	<i>Rhd</i>	0.00E+00	2.1702	0.772	0.008	0.00E+00
7	84	<i>Hmgb2</i>	1.19E-71	1.1337	0.694	0.346	3.69E-67
7	106	<i>Stmn1</i>	1.25E-102	0.9878	0.373	0.072	3.88E-98
7	117	<i>Epb41</i>	1.05E-83	0.9477	0.762	0.337	3.25E-79
7	119	<i>Isg20</i>	3.63E-115	0.947	0.807	0.315	1.13E-110
7	133	<i>H2afz</i>	8.58E-32	0.9064	0.571	0.349	2.66E-27
7	175	<i>Fam46c</i>	7.08E-51	0.7843	0.707	0.35	2.20E-46
7	181	<i>Fam213a</i>	1.58E-43	0.7616	0.424	0.161	4.90E-39
7	200	<i>Ube2o</i>	6.06E-48	0.7235	0.456	0.173	1.88E-43
7	248	<i>Snca</i>	1.30E-65	0.6561	0.842	0.395	4.03E-61
7	265	<i>Alas2</i>	9.37E-53	0.6263	0.865	0.463	2.91E-48
7	323	<i>Hbb-bt</i>	7.12E-42	0.5302	0.992	0.584	2.21E-37
7	332	<i>Ube2l6</i>	1.16E-33	0.5184	0.689	0.371	3.60E-29
7	392	<i>Mkrnl</i>	2.18E-25	0.4394	0.835	0.569	6.77E-21
7	475	<i>Isca1</i>	1.77E-23	0.3105	0.356	0.16	5.49E-19
7	509	<i>Fam220a</i>	9.39E-14	0.2733	0.584	0.369	2.92E-09

cluster	order	gene	p_val	avg_logFC	pct.1	pct.2	p_val_adj
8	1	<i>Ccl5</i>	0.00E+00	2.8793	0.308	0.004	0.00E+00
8	2	<i>Cd3g</i>	0.00E+00	2.7169	0.838	0.002	0.00E+00
8	3	<i>Cd3d</i>	0.00E+00	2.3614	0.826	0.009	0.00E+00
8	4	<i>Trbc2</i>	0.00E+00	2.2912	0.843	0.008	0.00E+00
8	5	<i>Lgals1</i>	1.10E-218	2.1323	0.624	0.101	3.41E-214
8	6	<i>AW112010</i>	0.00E+00	2.1146	0.738	0.041	0.00E+00
8	7	<i>Trdc</i>	0.00E+00	2.1102	0.259	0.001	0.00E+00
8	8	<i>S100a4</i>	2.35E-200	2.025	0.547	0.081	7.30E-196
8	9	<i>Ms4a4b</i>	0.00E+00	2.0039	0.536	0.003	0.00E+00
8	10	<i>H2-K1</i>	9.12E-297	1.8895	0.96	0.221	2.83E-292
8	139	<i>Ctla2a</i>	1.28E-166	1.0647	0.425	0.052	3.98E-162
8	564	<i>H2afz</i>	1.21E-48	0.5118	0.744	0.344	3.76E-44
8	725	<i>Fgl2</i>	5.70E-69	0.4347	0.316	0.065	1.77E-64

cluster	order	gene	p_val	avg_logFC	pct.1	pct.2	p_val_adj
9	1	<i>Fabp5</i>	0.00E+00	4.6917	0.901	0.124	0.00E+00
9	2	<i>Mt4</i>	0.00E+00	3.9902	0.492	0.018	0.00E+00
9	3	<i>Lgals7</i>	0.00E+00	3.828	0.924	0.029	0.00E+00
9	4	<i>Gsto1</i>	0.00E+00	2.9759	0.772	0.047	0.00E+00
9	5	<i>Krt14</i>	0.00E+00	2.8359	0.736	0.012	0.00E+00
9	6	<i>Krt6a</i>	0.00E+00	2.8126	0.673	0.01	0.00E+00
9	7	<i>Hspb1</i>	0.00E+00	2.7549	0.858	0.052	0.00E+00
9	8	<i>Fam162a</i>	0.00E+00	2.6598	0.809	0.07	0.00E+00
9	9	<i>Krt17</i>	0.00E+00	2.6484	0.571	0.009	0.00E+00
9	10	<i>Sfn</i>	0.00E+00	2.6339	0.779	0.017	0.00E+00
9	67	<i>Tpm2</i>	0.00E+00	1.6655	0.667	0.03	0.00E+00
9	147	<i>Ly6d</i>	1.49E-222	1.1676	0.393	0.029	4.61E-218
9	280	<i>Fxyd3</i>	5.30E-270	0.8281	0.383	0.021	1.64E-265
9	390	<i>Tpm1</i>	9.57E-102	0.6723	0.452	0.088	2.97E-97
9	466	<i>Stmn1</i>	1.60E-48	0.5994	0.317	0.077	4.97E-44

cluster	order	gene	p_val	avg_logFC	pct.1	pct.2	p_val_adj
10	1	<i>Ccl21a</i>	0.00E+00	4.2395	0.746	0.004	0.00E+00
10	2	<i>Mmrn1</i>	0.00E+00	3.6658	0.993	0.003	0.00E+00
10	3	<i>Lyve1</i>	0.00E+00	3.4541	0.804	0.005	0.00E+00
10	4	<i>Igfbp5</i>	0.00E+00	3.2626	0.851	0.044	0.00E+00
10	5	<i>Timp3</i>	0.00E+00	3.2036	0.975	0.044	0.00E+00
10	6	<i>Cldn5</i>	0.00E+00	3.1977	0.978	0.007	0.00E+00
10	7	<i>Fgl2</i>	0.00E+00	2.9448	0.931	0.05	0.00E+00
10	8	<i>Cavin2</i>	0.00E+00	2.5556	0.917	0.032	0.00E+00
10	9	<i>Gng11</i>	0.00E+00	2.4836	0.942	0.043	0.00E+00
10	10	<i>Sptbn1</i>	0.00E+00	2.4217	0.971	0.149	0.00E+00
10	19	<i>Ctla2a</i>	0.00E+00	2.1758	0.851	0.043	0.00E+00
10	24	<i>Tm4sf1</i>	0.00E+00	2.0438	0.678	0.035	0.00E+00
10	40	<i>Egfl7</i>	0.00E+00	1.8363	0.826	0.028	0.00E+00
10	55	<i>Pecam1</i>	0.00E+00	1.6244	0.859	0.034	0.00E+00
10	229	<i>Serping1</i>	0.00E+00	0.9738	0.612	0.045	0.00E+00
10	296	<i>H2-K1</i>	1.92E-79	0.8962	0.717	0.233	5.97E-75
10	519	<i>Ptprb</i>	3.72E-242	0.7103	0.366	0.02	1.15E-237
10	755	<i>Psap</i>	8.61E-89	0.5901	0.754	0.227	2.68E-84
10	1196	<i>Apoe</i>	2.15E-30	0.4367	0.297	0.091	6.69E-26

cluster	order	gene	p_val	avg_logFC	pct.1	pct.2	p_val_adj
---------	-------	------	-------	-----------	-------	-------	-----------

11	1	<i>Igkc</i>	0.00E+00	5.496	0.802	0.03	0.00E+00
11	2	<i>Ighm</i>	0.00E+00	3.9795	0.885	0.043	0.00E+00
11	3	<i>Iglc1</i>	0.00E+00	3.6015	0.328	0	0.00E+00
11	4	<i>Cd79a</i>	0.00E+00	3.5534	0.927	0	0.00E+00
11	5	<i>Iglc2</i>	0.00E+00	3.2872	0.546	0.001	0.00E+00
11	6	<i>Ly6d</i>	0.00E+00	3.1772	0.752	0.021	0.00E+00
11	7	<i>Cd74</i>	0.00E+00	3.0573	0.71	0.035	0.00E+00
11	8	<i>Ebfl</i>	0.00E+00	3.0238	0.79	0.056	0.00E+00
11	9	<i>Vpreb3</i>	0.00E+00	2.9633	0.523	0.001	0.00E+00
11	10	<i>Cd79b</i>	0.00E+00	2.8537	0.748	0.004	0.00E+00
11	12	<i>H2-Aa</i>	0.00E+00	2.4219	0.557	0.026	0.00E+00
11	14	<i>H2-Ab1</i>	0.00E+00	2.2321	0.611	0.029	0.00E+00
11	16	<i>H2-Eb1</i>	0.00E+00	2.1231	0.55	0.024	0.00E+00
11	73	<i>H2-K1</i>	1.55E-67	1.4335	0.622	0.236	4.82E-63
11	290	<i>Ctss</i>	2.05E-38	0.6839	0.267	0.063	6.36E-34
11	327	<i>Plac8</i>	5.74E-08	0.5986	0.267	0.16	1.78E-03
11	342	<i>Hmgb2</i>	6.95E-01	0.5574	0.347	0.36	1.00E+00
11	347	<i>H2afz</i>	3.25E-02	0.5491	0.374	0.357	1.00E+00

cluster	order	gene	p_val	avg_logFC	pct.1	pct.2	p_val_adj
12	1	<i>Fabp4</i>	0.00E+00	3.2212	0.539	0.019	0.00E+00

12	2	<i>Mgp</i>	0.00E+00	2.936	0.65	0.024	0.00E+00
12	3	<i>Ctla2a</i>	0.00E+00	2.8107	0.78	0.046	0.00E+00
12	4	<i>Igfbp7</i>	1.94E-230	2.595	0.528	0.049	6.03E-226
12	5	<i>Tm4sf1</i>	0.00E+00	2.4388	0.791	0.034	0.00E+00
12	6	<i>Pecam1</i>	0.00E+00	2.4111	0.795	0.038	0.00E+00
12	7	<i>Vwf</i>	0.00E+00	2.4081	0.669	0.005	0.00E+00
12	8	<i>Sparc</i>	0.00E+00	2.3261	0.693	0.048	0.00E+00
12	9	<i>Egfl7</i>	0.00E+00	2.2837	0.772	0.031	0.00E+00
12	10	<i>Ptprb</i>	0.00E+00	2.2568	0.728	0.012	0.00E+00
12	18	<i>Gng11</i>	0.00E+00	1.9336	0.72	0.05	0.00E+00
12	22	<i>Sptbn1</i>	1.98E-209	1.86	0.827	0.154	6.15E-205
12	48	<i>Cavin2</i>	0.00E+00	1.5016	0.587	0.042	0.00E+00
12	62	<i>Hspb1</i>	1.01E-186	1.3927	0.555	0.064	3.12E-182
12	89	<i>Timp3</i>	5.22E-232	1.2266	0.587	0.056	1.62E-227
12	99	<i>Apoe</i>	2.21E-149	1.1712	0.567	0.085	6.86E-145
12	194	<i>Tpm1</i>	1.35E-109	0.9019	0.496	0.089	4.20E-105
12	231	<i>Plpp3</i>	1.14E-198	0.8541	0.539	0.055	3.53E-194
12	378	<i>H2-K1</i>	3.82E-50	0.7278	0.626	0.236	1.19E-45
12	575	<i>Igfbp4</i>	7.43E-87	0.5846	0.425	0.074	2.31E-82
12	984	<i>Ebfl</i>	4.02E-99	0.3779	0.433	0.066	1.25E-94
12	1138	<i>Psap</i>	4.46E-31	0.2845	0.559	0.233	1.39E-26

cluster	order	gene	p_val	avg_logFC	pct.1	pct.2	p_val_adj
13	1	<i>Camp</i>	2.72E-229	2.6261	1	0.216	8.43E-225
13	2	<i>Chil3</i>	3.24E-242	2.484	0.995	0.197	1.01E-237
13	3	<i>Ngp</i>	3.22E-221	2.2936	1	0.208	1.00E-216
13	4	<i>Hmgb2</i>	3.00E-176	2.2073	1	0.346	9.32E-172
13	5	<i>Hmgn2</i>	2.96E-271	2.1329	0.995	0.176	9.20E-267
13	6	<i>H2afz</i>	1.79E-172	2.0978	1	0.344	5.57E-168
13	7	<i>Serpinb1a</i>	2.44E-264	1.8599	0.972	0.149	7.58E-260
13	8	<i>Lcn2</i>	9.96E-170	1.8511	0.995	0.267	3.09E-165
13	9	<i>Ltf</i>	4.87E-247	1.7681	0.986	0.138	1.51E-242
13	10	<i>Stmn1</i>	0.00E+00	1.7229	0.944	0.066	0.00E+00
13	11	<i>Hist1h1b</i>	0.00E+00	1.7034	0.836	0.044	0.00E+00

13	14	<i>S100a9</i>	8.27E-103	1.5744	1	0.444	2.57E-98
13	18	<i>S100a8</i>	2.56E-100	1.5326	1	0.434	7.95E-96
13	22	<i>Mki67</i>	0.00E+00	1.424	0.935	0.062	0.00E+00
13	25	<i>Wfdc21</i>	4.30E-147	1.3742	0.991	0.222	1.33E-142
13	91	<i>Lyz2</i>	1.10E-109	0.9317	0.995	0.275	3.42E-105
13	163	<i>Ifitm6</i>	1.99E-117	0.7349	0.977	0.219	6.19E-113
13	363	<i>Plac8</i>	1.77E-96	0.507	0.729	0.151	5.48E-92

cluster	order	gene	p_val	avg_logFC	pct.1	pct.2	p_val_adj
14	1	<i>Lipf</i>	2.92E-63	6.8153	0.833	0.18	9.07E-59
14	2	<i>Sbpl</i>	4.10E-73	6.1474	0.972	0.264	1.27E-68
14	3	<i>Wfdc18</i>	3.52E-127	6.1158	0.986	0.132	1.09E-122
14	4	<i>2310057J18Rik</i>	3.25E-194	5.5834	0.972	0.076	1.01E-189
14	5	<i>Bpifb1</i>	2.78E-118	5.4949	0.944	0.128	8.63E-114
14	6	<i>Amy1</i>	1.14E-166	5.4836	0.972	0.09	3.54E-162
14	7	<i>Dcpp3</i>	8.86E-09	5.2994	0.361	0.152	2.75E-04
14	8	<i>Dcpp2</i>	5.43E-11	5.2414	0.375	0.14	1.69E-06
14	9	<i>Dcpp1</i>	6.22E-14	5.2379	0.347	0.101	1.93E-09
14	10	<i>Bpifa2</i>	1.34E-180	4.5083	0.792	0.05	4.15E-176
14	15	<i>Fxyd3</i>	7.77E-227	1.592	0.694	0.027	2.41E-222
14	21	<i>Wfdc2</i>	1.09E-95	1.1938	0.486	0.032	3.37E-91
14	29	<i>Nupr1</i>	6.30E-55	0.7189	0.708	0.116	1.96E-50

14	65	<i>H2-K1</i>	1.91E-14	0.4318	0.653	0.243	5.94E-10
----	----	--------------	----------	--------	-------	-------	----------

cluster	order	gene	p_val	avg_logFC	pct.1	pct.2	p_val_adj
15	1	<i>Tff2</i>	3.23E-99	6.0946	1	0.177	1.00E-94
15	2	<i>Muc5b</i>	7.10E-76	5.4813	0.956	0.216	2.20E-71
15	3	<i>Bpifb2</i>	9.30E-254	5.0452	0.926	0.046	2.89E-249
15	4	<i>Nupr1</i>	6.91E-140	4.8635	1	0.114	2.15E-135
15	5	<i>Agr2</i>	1.52E-292	4.7664	0.971	0.044	4.71E-288
15	6	<i>Gp2</i>	0.00E+00	3.8439	0.838	0.011	0.00E+00
15	7	<i>Wfdc2</i>	0.00E+00	3.7074	0.853	0.03	0.00E+00
15	8	<i>Fxyd3</i>	0.00E+00	3.1705	0.897	0.026	0.00E+00
15	9	<i>Tesc</i>	8.05E-290	2.772	0.809	0.029	2.50E-285
15	10	<i>Smgc</i>	0.00E+00	2.6877	0.75	0.007	0.00E+00
15	22	<i>Gsto1</i>	1.71E-115	2.0984	0.735	0.064	5.29E-111
15	34	<i>AW112010</i>	3.77E-112	1.8788	0.721	0.06	1.17E-107
15	121	<i>Sbpl</i>	3.64E-07	1.3402	0.485	0.268	1.13E-02
15	347	<i>Isg20</i>	5.84E-08	0.9249	0.588	0.332	1.81E-03
15	475	<i>H2-K1</i>	2.88E-08	0.7785	0.5	0.244	8.95E-04

cluster	order	gene	p_val	avg_logFC	pct.1	pct.2	p_val_adj
16	1	<i>Acta1</i>	5.64E-179	5.6089	1	0.047	1.75E-174
16	2	<i>Tnnc2</i>	5.87E-294	5.4455	1	0.026	1.82E-289
16	3	<i>Mylpf</i>	6.09E-180	5.2576	1	0.046	1.89E-175
16	4	<i>Tnni2</i>	3.51E-242	4.9991	0.949	0.029	1.09E-237
16	5	<i>Tnnt3</i>	8.79E-278	4.9315	0.949	0.025	2.73E-273
16	6	<i>Ckm</i>	9.00E-272	4.8803	0.949	0.025	2.80E-267
16	7	<i>Myl1</i>	0.00E+00	4.7277	0.923	0.014	0.00E+00
16	8	<i>Tpm2</i>	3.74E-173	4.5059	0.974	0.046	1.16E-168
16	9	<i>Tpm1</i>	1.94E-76	4.457	0.897	0.096	6.04E-72
16	10	<i>Mb</i>	0.00E+00	4.4245	0.846	0.014	0.00E+00
16	219	<i>Lgals1</i>	2.96E-05	1.1398	0.333	0.118	9.18E-01
16	224	<i>Fam162a</i>	1.20E-04	1.1099	0.256	0.091	1.00E+00
16	252	<i>Sbpl</i>	2.59E-02	0.8756	0.103	0.27	1.00E+00
16	268	<i>Isca1</i>	7.80E-02	0.7727	0.256	0.167	1.00E+00
16	325	<i>Hspb1</i>	2.30E-05	0.4166	0.256	0.075	7.14E-01

Supplemental Table 6.

Top Five Genes in the Seventh and Ninth Cell Subtypes With Significant Differences Between the 29- and 16-Week Experimental Groups

Cluster7:29w treat vs.16w treat

	p_val	avg_logFC	pct.1	pct.2	p_val_adj
<i>mt-Nd2</i>	1.41E-09	2.847555	0.933	0.312	4.38E-05
<i>Gm42418</i>	2.76E-08	1.519975	0.94	0.875	0.000857
<i>mt-Nd4l</i>	5.49E-08	1.861324	0.817	0.062	0.001706
<i>mt-Cytb</i>	1.01E-07	1.875175	0.944	0.625	0.00313
<i>mt-Nd3</i>	2.03E-07	0.868717	0.908	0.188	0.006301

Cluster9: 29w treat vs. 16w treat

	p_val	avg_logFC	pct.1	pct.2	p_val_adj
<i>Lnxl</i>	6.06E-15	-0.4813	0	0.222	1.88E-10
<i>Natd1</i>	6.06E-15	-0.4813	0	0.222	1.88E-10
<i>Gpr89</i>	6.06E-15	-0.53658	0	0.222	1.88E-10
<i>Krtap3-2</i>	6.06E-15	-2.93493	0	0.222	1.88E-10
<i>2300002M23Rik</i>	9.23E-15	-3.09765	0.015	0.444	2.87E-10

Supplemental Table 7.

Genes in the Seventh Subtype Involved in the MYC_targets_v1 Pathway in the Experimental Group According to Gene Function Enrichment Analysis

PROBE	GENE SYMBOL	GENE TITLE	RANK IN GENE LIST	RANK METRIC SCORE	RUNNING ES	CORE ENRICHMENT
RPS6	null	null	7	1.921929	0.023955	Yes
RPL14	null	null	16	1.717817	0.043869	Yes
RPS5	null	null	18	1.688999	0.069343	Yes
RPS2	null	null	20	1.622016	0.093772	Yes
RPS10	null	null	26	1.541237	0.113509	Yes
RPLP0	null	null	30	1.471963	0.133882	Yes
RPL18	null	null	39	1.404468	0.148911	Yes
PPIA	null	null	65	1.167021	0.145647	Yes
EEF1B2	null	null	67	1.160469	0.16288	Yes
RPL6	null	null	68	1.142827	0.180697	Yes
RPL34	null	null	81	1.045431	0.186696	Yes
NPM1	null	null	82	1.043903	0.20297	Yes
HSP90AB1	null	null	87	1.017407	0.215399	Yes
RACK1	null	null	100	0.947806	0.219875	Yes
RPL22	null	null	107	0.917201	0.229024	Yes
HNRNPD	null	null	115	0.863213	0.236473	Yes
NAP1L1	null	null	116	0.857354	0.24984	Yes
RPS3	null	null	119	0.829343	0.261053	Yes
PCNA	null	null	123	0.804367	0.271018	Yes
EIF4A1	null	null	127	0.787659	0.280723	Yes
SNRPD1	null	null	129	0.781525	0.292048	Yes
MCM7	null	null	132	0.777428	0.302452	Yes
DUT	null	null	137	0.761807	0.310895	Yes
HNRNPA3	null	null	142	0.738997	0.318983	Yes
NME1	null	null	149	0.709877	0.3249	Yes
CNBP	null	null	152	0.705802	0.334187	Yes
PSMA7	null	null	165	0.665208	0.334258	Yes
SET	null	null	166	0.662229	0.344582	Yes
NDUFAB1	null	null	168	0.656574	0.35396	Yes
RAN	null	null	169	0.655036	0.364172	Yes
U2AF1	null	null	172	0.650413	0.372595	Yes
RANBP1	null	null	177	0.648398	0.379271	Yes
EIF2S2	null	null	180	0.647028	0.387641	Yes

CCT7	null	null	189	0.630371	0.390602	Yes
GLO1	null	null	191	0.622106	0.399443	Yes
SNRPD2	null	null	213	0.579745	0.390455	Yes
PSMA2	null	null	216	0.578158	0.397752	Yes
HNRNPA1	null	null	218	0.573356	0.405833	Yes
CCT2	null	null	219	0.573042	0.414767	Yes
PCBP1	null	null	223	0.565737	0.421011	Yes
PSMD8	null	null	236	0.539554	0.419123	Yes
TCP1	null	null	245	0.524732	0.420437	Yes
HSPE1	null	null	247	0.523499	0.42774	Yes
SERBP1	null	null	253	0.517677	0.431519	Yes
CYC1	null	null	257	0.513842	0.436955	Yes
PSMD7	null	null	258	0.512522	0.444945	Yes
PRDX3	null	null	262	0.502667	0.450207	Yes
MCM2	null	null	269	0.494272	0.452762	Yes
SLC25A3	null	null	275	0.488874	0.456092	Yes
MCM6	null	null	281	0.481197	0.459302	Yes
EIF4H	null	null	290	0.472741	0.459806	Yes
MCM5	null	null	304	0.463321	0.45587	Yes
SRSF7	null	null	305	0.46248	0.46308	Yes
EIF3B	null	null	323	0.446794	0.455454	Yes
YWHAЕ	null	null	324	0.446039	0.462408	Yes
PABPC1	null	null	330	0.443984	0.465038	Yes
PHB2	null	null	333	0.442072	0.470213	Yes
RFC4	null	null	337	0.435477	0.474427	Yes
NHP2	null	null	346	0.428529	0.474241	Yes
SSB	null	null	350	0.426064	0.478308	Yes
PSMB2	null	null	371	0.412382	0.46757	No
HNRNPU	null	null	380	0.40774	0.46706	No
KPNB1	null	null	401	0.394307	0.45604	No
COX5A	null	null	408	0.388835	0.456952	No
YWHAQ	null	null	429	0.378294	0.445682	No
MCM4	null	null	439	0.373637	0.443782	No
CCT5	null	null	443	0.371106	0.446993	No
HNRNPC	null	null	447	0.370191	0.450189	No
CANX	null	null	456	0.36708	0.449045	No
EIF4G2	null	null	460	0.366306	0.452181	No
ETF1	null	null	463	0.365634	0.456164	No
USP1	null	null	504	0.346987	0.427239	No
PABPC4	null	null	554	0.330141	0.390326	No
C1QBP	null	null	560	0.327777	0.391144	No
HSPD1	null	null	564	0.326982	0.393667	No

EIF3D	null	null	581	0.32289	0.384967	No
ACP1	null	null	582	0.322836	0.39	No
EIF1AX	null	null	584	0.322747	0.394174	No
SNRPG	null	null	589	0.320656	0.395739	No
CCT3	null	null	624	0.310227	0.371391	No
NOP56	null	null	630	0.307446	0.371892	No
SNRPA1	null	null	643	0.300314	0.366274	No
UBA2	null	null	653	0.295413	0.363154	No
PRDX4	null	null	658	0.292359	0.364279	No
TARDBP	null	null	665	0.290024	0.36365	No
EIF2S1	null	null	667	0.289879	0.367311	No
SF3B3	null	null	684	0.284587	0.358014	No
LSM2	null	null	688	0.284135	0.359869	No
PSMC4	null	null	712	0.277822	0.344458	No
IMPDH2	null	null	724	0.275129	0.339305	No
CDK2	null	null	762	0.267001	0.311708	No
PTGES3	null	null	763	0.266373	0.315861	No
ERH	null	null	774	0.263113	0.311379	No
DEK	null	null	844	-0.28217	0.256551	No
PSMA4	null	null	910	-0.41634	0.207248	No
RRM1	null	null	936	-0.49423	0.193494	No
PSMD3	null	null	990	-0.60764	0.157473	No
TYMS	null	null	1008	-0.65224	0.15305	No
MAD2L1	null	null	1091	-0.79078	0.094992	No
HNRNPR	null	null	1133	-0.84623	0.072992	No
TRA2B	null	null	1225	-1.24548	0.014298	No
GNL3	null	null	1233	-1.28523	0.028326	No

Supplemental Table 8.

Genes in the Ninth Subtype Involved in the MYC_targets_v1 Pathway in the Experimental Group According to Gene Function Enrichment Analysis

PROBE	GENE SYMBOL	GENE_ TITLE	RANK IN GENE LIST	RANK METRIC SCORE	RUNNING ES	CORE ENRICHMENT
NPM1	null	null	11	1.81545	0.017986	Yes
SNRPD2	null	null	26	1.381676	0.029435	Yes
PPIA	null	null	27	1.357527	0.046157	Yes
SERBP1	null	null	31	1.31939	0.061216	Yes
NDUFAB1	null	null	35	1.294732	0.075971	Yes
RACK1	null	null	38	1.265074	0.090759	Yes
EIF4A1	null	null	64	1.14563	0.094923	Yes
PSMB2	null	null	65	1.141965	0.10899	Yes
PSMA7	null	null	73	1.114053	0.119927	Yes
HNRNPA3	null	null	83	1.057133	0.129368	Yes
CCT5	null	null	91	1.035708	0.139341	Yes
U2AF1	null	null	117	0.969301	0.141333	Yes
RPS2	null	null	119	0.958663	0.152744	Yes
SNRPD1	null	null	121	0.954391	0.164102	Yes
CCT2	null	null	125	0.94974	0.174608	Yes
EIF1AX	null	null	148	0.908348	0.177042	Yes
HNRNPA2B1	null	null	167	0.877369	0.180687	Yes
SRSF7	null	null	175	0.866202	0.188572	Yes
C1QBP	null	null	183	0.854403	0.196311	Yes
EIF4G2	null	null	187	0.845553	0.205533	Yes
HSPD1	null	null	197	0.827542	0.212146	Yes
LDHA	null	null	198	0.8259	0.222319	Yes
SNRPD3	null	null	206	0.805131	0.229452	Yes
ACP1	null	null	212	0.796114	0.237269	Yes
PSMA1	null	null	226	0.781197	0.241719	Yes
TCP1	null	null	234	0.778243	0.24852	Yes
HNRNPD	null	null	237	0.773912	0.257257	Yes
HSP90AB1	null	null	242	0.76801	0.265126	Yes
CCT4	null	null	257	0.747694	0.268765	Yes
RPS3	null	null	267	0.739954	0.274299	Yes
PSMA6	null	null	273	0.733269	0.281342	Yes
LSM2	null	null	274	0.732965	0.290371	Yes

MRPL23	null	null	280	0.725712	0.297321	Yes
RSL1D1	null	null	296	0.706402	0.300053	Yes
NME1	null	null	298	0.704029	0.308328	Yes
PSMD14	null	null	312	0.696356	0.311733	Yes
RANBP1	null	null	313	0.695745	0.320303	Yes
YWHAQ	null	null	314	0.69489	0.328863	Yes
PSMD7	null	null	323	0.681654	0.334076	Yes
RPLP0	null	null	329	0.6783	0.340442	Yes
EIF3B	null	null	337	0.673092	0.345948	Yes
PSMA4	null	null	338	0.671935	0.354225	Yes
RAD23B	null	null	348	0.664518	0.358829	Yes
RPS6	null	null	360	0.654325	0.362512	Yes
UBA2	null	null	373	0.645528	0.365689	Yes
GSPT1	null	null	377	0.642436	0.372409	Yes
FBL	null	null	388	0.633723	0.376236	Yes
SSBP1	null	null	393	0.629844	0.382403	Yes
SYNCRIP	null	null	427	0.603662	0.376707	Yes
PSMD1	null	null	434	0.596355	0.381665	Yes
RPS10	null	null	453	0.584197	0.381699	Yes
EIF2S1	null	null	455	0.582564	0.388477	Yes
DEK	null	null	456	0.58122	0.395637	Yes
CYC1	null	null	475	0.56754	0.395465	Yes
PSMC4	null	null	477	0.567122	0.402053	Yes
ABCE1	null	null	499	0.55219	0.400499	Yes
GNL3	null	null	505	0.549246	0.405275	Yes
COX5A	null	null	515	0.544633	0.408402	Yes
CBX3	null	null	520	0.53989	0.413461	Yes
CDK4	null	null	567	0.518266	0.401541	Yes
VDAC1	null	null	569	0.517363	0.407516	Yes
YWHAЕ	null	null	574	0.516021	0.41228	Yes
UBE2L3	null	null	601	0.498216	0.408071	Yes
PHB2	null	null	603	0.495808	0.413781	Yes
RNPS1	null	null	613	0.492135	0.416262	Yes
HNRNPU	null	null	642	0.477076	0.410996	No
NCBP2	null	null	645	0.475643	0.41606	No
HDAC2	null	null	681	0.459876	0.407797	No
PA2G4	null	null	689	0.455037	0.410617	No
RPL14	null	null	696	0.452547	0.413804	No
TRA2B	null	null	709	0.446332	0.414527	No
NAP1L1	null	null	791	0.418849	0.387454	No
TXNL4A	null	null	797	0.41702	0.390601	No
SRM	null	null	821	0.409868	0.386498	No

VBP1	null	null	823	0.409172	0.39114	No
ODC1	null	null	831	0.406248	0.393359	No
PSMA2	null	null	871	0.394101	0.382694	No
ERH	null	null	943	0.371203	0.359013	No
SNRPA1	null	null	970	0.36377	0.353148	No
SRPK1	null	null	979	0.361058	0.354412	No
CCT7	null	null	1004	0.352656	0.349206	No
RPL18	null	null	1017	0.349581	0.348737	No
CDC20	null	null	1032	0.345605	0.347424	No
KPNB1	null	null	1050	0.340422	0.344852	No
RPL34	null	null	1051	0.34029	0.349044	No
SNRPG	null	null	1065	0.337591	0.348029	No
UBE2E1	null	null	1085	0.33174	0.344555	No
HDGF	null	null	1093	0.329806	0.345832	No
BUB3	null	null	1129	0.322303	0.335875	No
RFC4	null	null	1144	0.318195	0.334223	No
XPOT	null	null	1147	0.31756	0.337339	No
IMPDH2	null	null	1212	0.305263	0.315632	No
HDDC2	null	null	1235	0.301811	0.310595	No
G3BP1	null	null	1251	0.299018	0.30831	No
APEX1	null	null	1260	0.298259	0.3088	No
GLO1	null	null	1276	0.294869	0.306464	No
COP55	null	null	1282	0.293796	0.308093	No
MRPL9	null	null	1285	0.292887	0.310905	No
EIF3D	null	null	1358	0.280467	0.285709	No
FAM120A	null	null	1368	0.279407	0.285569	No
ILF2	null	null	1375	0.277851	0.286605	No
SMARCC1	null	null	1379	0.276642	0.288818	No
HSPE1	null	null	1385	0.275385	0.290221	No
TRIM28	null	null	1405	0.272224	0.286014	No
PPM1G	null	null	1417	0.271045	0.284975	No
SNRPB2	null	null	1425	0.269277	0.285507	No
DUT	null	null	1433	0.26845	0.286028	No
PABPC4	null	null	1464	0.263897	0.277341	No
AIMP2	null	null	1513	0.255493	0.261388	No
PSMD8	null	null	1551	0.2511	0.249757	No
SRSF3	null	null	1672	-0.27793	0.205429	No
SLC25A3	null	null	1701	-0.28547	0.197804	No
PSMB3	null	null	1873	-0.35534	0.134135	No
CANX	null	null	1940	-0.39935	0.11279	No
EIF2S2	null	null	2032	-0.47492	0.082429	No
SET	null	null	2106	-0.54507	0.060094	No

PABPC1	null	null	2133	-0.59894	0.057126	No
SSB	null	null	2135	-0.60063	0.064127	No
PCBP1	null	null	2161	-0.6407	0.062071	No
NHP2	null	null	2190	-0.70586	0.059624	No
PGK1	null	null	2194	-0.7103	0.06718	No
HNRNPA1	null	null	2196	-0.71806	0.075627	No
CNBP	null	null	2230	-0.8301	0.072721	No
PTGES3	null	null	2257	-0.89594	0.073411	No
CCT3	null	null	2292	-0.99607	0.072151	No
IFRD1	null	null	2310	-1.02258	0.077983	No
TFDP1	null	null	2324	-1.06258	0.085899	No
EIF4H	null	null	2459	-1.3787	0.049559	No
POLD2	null	null	2574	-1.82379	0.026661	No