# Molecular spectrum of *BRAF, NRAS* and *KRAS* gene mutations in plasma cell dyscrasias: implication for MEK-ERK pathway activation

## Supplementary Material

## Supplementary Materials and Methods

### Mutation analyses

Genomic DNA was amplified using FastStart High Fidelity Polymerase (Roche) and fusion primers (Roche) containing M13 adapter sequences and the sequence-specific primers spanning *BRAF* exons 11 and 15 (RefSeq NM_004333.4) (exon 11 forward 5'-TCCCTCTCAGGCATAAGGTAA-3'; exon 11 reverse 5'-CGAACAGTGAATATTTCCTTTGAT-3'; exon 15 forward 5'-TCATAATGCTTGCTCTGATAGGA-3'; exon 15 reverse 5'-GGCCAAAAATTTAATCAGTGGA-3') [1], *NRAS* exons 2 and 3 (exon 2 forward 5'-ATGGAAGGTCACACTAGGGTT-3'; exon 2 reverse 5'-TCCTTTAATACAGAATATGGG-3'; exon 3 forward 5'-ACCCCCAGGATTCTTACAGA-3'; exon 3 reverse 5'-AGAGGTTAATATCCGCAAATG-3') [2], and *KRAS* exons 2, 3 and 4 (exon 2 forward 5'-TTAACCTTATGTGTGACATGTTCTAA-3'; exon 2 reverse 5'-GAAAGTAAAGTTCCCATATTAATGGT-3'; exon 3 forward 5'-GCACTGTAATAATCCAGACT-3'; exon 3 reverse 5'-CATGGCATTAGCAAAGACTC-3' [3]; exon 4 forward 5'-AGTTGTGGACAGGTTTTGAAAGA-3'; exon 4 reverse: 5'-ATTTCAGTGTTACTTACCTGTCTTG-3' (Primer-BLAST at http://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome)). Amplicon library A and B sequencing adapters and multiplex identifier (MID) tags were then added to both tails of amplicons by a second amplification step. PCR conditions were as follows: in the first amplification step, denaturating step at 94°C for 5 min followed by 25 cycles at 94°C (30 sec. per cycle), annealing step at 57°C (30 sec. per cycle), and extension at 72°C (45 sec. per cycle), followed by a final 7 min. extension at 72°C; in the second amplification step, denaturating step at 94°C for 5 min followed by 25 cycles at 94°C (20 sec. per cycle), annealing step at 55°C (20 sec. per cycle), and extension at 72°C (45 sec. per cycle), followed by a final 10 min. extension at 72°C. All PCR samples were purified using an AMPure XP (Agencourt) bead clean-up; samples were then quantitated using picogreen dye (Life Technologies) and the Victor X2 (Perkin Elmer) fluorometer. Samples were pooled together at equimolar ratios to prepare for Roche/454 pyrosequencing. The obtained amplicon library was added to the emulsion PCR at a ratio of 0.8 molecules per bead and subjected to deep sequencing on the Genome Sequencer Junior instrument (Roche-454 Life Sciences). The obtained sequencing reads were mapped to the reference sequence (RefSeq NG_007873.2 for *BRAF*, RefSeq NG_007572.1 for *NRAS*, RefSeq NG_007524.1 for *KRAS* ) and analyzed by the Amplicon Variant Analyzer software (Roche) to establish the mutant allele frequency.

To verify the occurrence of *BRAF* variants at transcriptional level, total RNA of mutated samples was converted to cDNA using M-MLV reverse transcriptase (Invitrogen) and random hexamers, and subjected to deep sequencing of the exon harboring the variant/s detected on genomic DNA. Sequence-specific exonic PCR primers were designed in the Primer 3 program (http://frodo.wi.mit.edu/primer3/) and are as follows: exon 11 forward 5'-CTCAGCGAGAAAGGAAGTCA-3'; exon 11 reverse 5'-GGAATAGCCCATGAAGAGTAGGA-3'; exon 15 forward 5'-AGATATTGCACGACAGACTGC-3'; exon 15 reverse 5'-TTCTGATGACTTCTGGTGCC-3'.

**Gene expression profiling and data analysis**

For gene expression analysis, samples were profiled on the GeneChip Human Gene 1.0 ST array (Affymetrix, Santa Clara, CA, USA) as previously described [4]. The raw intensity expression values were processed by Robust Multi-array Average procedure (RMA) [5], with the re-annotated Chip Definition Files from BrainArray libraries version 18.0.0 [6], available at http://brainarray.mbni.med.umich.edu. Sno/scaRNA expression profiles were excluded in the following supervised analyses by Significant Analysis of Microarrays (SAM). In the patients' dataset, supervised analyses on gene expression profiles were carried out using the SAM software version 4.00 (excel front-end publicly available at http://www-stat.stanford.edu/ B tibs/SAM/index.html) [7]. The cutoff point for statistical significance (at a *q*-value = 0) was determined by tuning the Δ parameter on the false discovery rate (FDR) and controlling the *q*-value of the selected gene lists. A higher stringency level ($90^{th}$ percentile FDR = 0) was also applied to the differentially expressed gene lists at *q*-value 0. The list of differentially expressed genes was submitted to ToppGene Suite portal (http://toppgene.cchmc.org) for functional enrichment analysis by using ToppFun application [8]. A *P*-value cut-off of 0.05 and FDR Benjamini and Hochberg correction method were applied to all the annotation terms in the default parameter set, and those with a *q*-value < 0.05 were defined as significantly enriched. For gene expression analysis of U266 cells treated with BRAF-inhibitor or untreated (two independent replicas for each condition), microarray data were globally analyzed by Gene Set Enrichment Analysis (GSEA) [9] by means of gene sets of the MSigDB database (http://www.broadinstitute.org/gsea/msigdb/index.jsp) consisting of 5-1 000 genes (C1 Positional and C4 Computational gene sets were not considered). Gene set permutations (1 000) were set and default parameters were applied for statistical analyses. Gene sets showing a false discovery rate lower than 5% were selected as significantly enriched in U266 treated and control phenotypes, respectively.

The principal component analysis of the samples was performed by singular value decomposition of the considered data expression matrix using *prcomp* function in *stats* package and visualized with *plot3d* function in *rgl* package for R software.

**Cell lines**

Details regarding the source of all cell lines have been previously reported by us [10, 11], except for delta-47, UTMC-2, MM.1S and MM1-144, that were kindly provided by Dr. G.Tonon - San Raffaele Scientific Institute, Milan. All cell lines have been recently authenticated by STR profiling and/or gene mutational analyses, and tested for mycoplasma contamination.

**Cell-based assays**

U266 cell line was maintained in RPMI 1640 Medium supplemented with 10% fetal calf serum. Vemurafenib (Sigma-Aldrich) was dissolved in dimethyl sulfoxide (DMSO) stock solutions and stored at -20°C. The drug was added to log phase cells and used at 30 μM, based on previuos experiments testing cell proliferation and survival in a dose-dependent manner (data not shown) that confirmed the very weak sensitivity of U266 to the drug [12].

For proliferation assays, U266 cells were plated in 6 or 96-well plates, at 0.5 x $10^6$ or 0.1 x $10^6$ cells/well respectively and were treated with Vemurafenib or vehicle (DMSO) as control for 6, 12, 24, 48, 72 hrs. Cell number and viability were assessed by Trypan Blue exclusion and cell proliferation was evaluated by Thiazolyl Blue Tetrazolium Bromide (MTT) assay (Sigma Aldrich, USA), following the manufacturer's instructions.

For cell cycle analysis, cells were then collected after 6, 12, 24, 48, 72 h, fixed in 70% ethanol, and stored at 4°C until analysis. Before cytofluorimetric analysis, $10^6$ cells were washed twice in PBS and incubated in staining solution (10 mg/ml propidium iodide, 100 mg/ml RNase) at room temperature in the dark for at least 2 h. Cell cycle profiles were assessed using a FACSCalibur device (Becton Dickinson, San Jose´, CA, USA) and analyzed using Multicycle AV™ software (version 4; De Novo Software). For apoptosis analysis, cells were harvested after 6, 12, 24, 48, 72 h and treated with Annexin V/7-aminoactinomycin D (7-AAD) solution (BD Pharmingen) according to the manufacturer's instructions. The fluorescent intensity of at least 20 000 cells from each sample was measured. Data were acquired using CellQuest software (BD, San Jose, CA) and analyzed using FCS Express software (version 4, De Novo Software).

**Western Blot Analysis**

Standard SDS-PAGE and Western blotting procedures were applied to total cell extracts. Anti-BRAF (F7) antibody (catalog no.sc-5284) was purchased from Santa Cruz

Biotechnology; anti-phospho-ERK1/2 (product no. M8159), anti-ERK1/2 (product no.M5670) and anti-B actin (product no. A2066) antibodies were purchased from Sigma-Aldrich.

**References**


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**Supplementary Table 1A: Clinical and molecular characteristics of 20 MM/PCL patients carrying non-synonymous *BRAF* mutations**

| *Sample name* | *Disease* | *Variant (GRCh38)* | *% of mutated sequencing reads* | *AA change* | *Sex* | *Age* | *PP*† | *del(13)** | *del(17p)** | *1q gain** | *1p loss** | *t(4;14)* | *t(11;14)* | *t(14;16)* | *t(14;20)* | *HD*° |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **MM-036** | MM | 140753355C>T | 5.61% | D594N | M | 65 | Gκ | + | - | + | nd | - | - | - | - | - |
| **MM-039** | MM | 140753333T>G | 28.53% | K601T | M | 50 | Gλ | - | - | - | nd | - | - | - | - | + |
| **MM-140** | MM | 140753355C>T | 1.68% | D594N | M | 62 | na | - | - | - | nd | - | + | - | - | - |
| **MM-177** | MM | 140753336A>T | 27.18% | V600E | M | 73 | Gκ | + | - | + | - | - | - | - | - | - |
| **MM-219** | MM | 140753354T>C | 7.78% | D594G | M | 73 | Aλ | + | - | - | - | - | - | - | - | + |
| **MM-224** | MM | 140753349C>G | 35.44% | G596R | F | 52 | Gκ | - | - | + | - | - | - | + | - | - |
| **MM-268** | MM | 140753336A>T | 29.57% | V600E | M | 77 | Gκ | - | - | - | - | - | - | - | - | - |
| **MM-295** | MM | 140753355C>T | 50.00% | D594N | F | 74 | λ | + | - | nd | nd | - | - | - | - | nd |
| **MM-313** | MM | 140753336A>T | 44.59% | V600E | M | 66 | Gλ | - | - | - | - | - | + | - | - | - |
| **MM-335** | MM | 140781602C>G | 50.52% | G469A | F | 68 | Aκ | + | - | - | - | - | - | + | - | - |
| **MM-411** | MM | 140753354T>C | 0.86% | D594G | M | 67 | Gλ | - | - | - | - | - | - | - | - | + |
| **MM-435** | MM | 140753354T>C | 22.22% | D594G | na | 42 | Gλ | - | - | - | - | - | - | - | - | + |
| **MM-441** | MM | 140753336A>T | 35.74% | V600E | na | na | Gλ | - | - | - | - | - | - | - | - | - |
| **MM-446** | MM | 140753336A>T | 26.48% | V600E | na | 52 | Gλ | - | - | + | - | - | - | - | - | + |
| **PCL-015** | pPCL | 140753336A>T | 41.18% | V600E | M | 78 | k | + | - | + | - | - | - | + | - | - |
| **PCL-023** | pPCL | 140781602C>G | 70.70% | G469A | M | 60 | Gκ | + | + | + | + | - | - | + | - | - |
| **PCL-026** | pPCL | 140753355C>T<br>140753379C>T | 43.05%<br>42.15% | D594N<br>E586K | F | 59 | Gκ | + | - | + | - | - | - | + | - | - |
| **PCL-028** | pPCL | 140753353A>C | 4.65% | D594E | F | 57 | κ | + | - | - | - | - | + | - | - | - |
| **PCL-042** | sPCL | 140781602C>G | 51.26% | G469A | F | 69 | Gλ | - | - | - | - | - | + | - | - | - |
| **PCL-043** | pPCL | 140753336A>T | 44.59% | V600E | F | 68 | Gλ | - | - | - | - | - | + | - | - | - |

†Paraprotein; *del(13), del(17), 1p loss and 1q gain were determined by FISH. °HD = presence of the hyperdiploid status on the basis of FISH evaluation criteria.

**Supplementary Table 1B: Clinical and molecular characteristics of the 167 MM/PCL patients analyzed for *BRAF* mutations**

| *Characteristic* | *All patients (n=167)* | | *BRAF wild type (n=147)* | | *BRAF mutated (n=20)* | | *P value*[a] |
|---|---|---|---|---|---|---|---|
| | *N* | *%* | *n* | *%* | *n* | *%* | |
| MM | 132 | *79* | 118 | *80.3* | 14 | *70* | n.s. |
| pPCL | 24 | *14.4* | 19 | *12.9* | 5 | *25* | |
| sPCL | 11 | *6.6* | 10 | *6.8* | 1 | *5* | |
| del(13q) | 79 | *47.6* | 70 | *47.9* | 9 | *45* | n.s. |
| chr 13 disomic patients | 87 | *52.4* | 76 | *52.1* | 11 | *55* | |
| del(17p) | 18 | *10.9* | 17 | *11.7* | 1 | *5* | n.s. |
| 17p disomic patients | 147 | *89.1* | 128 | *88.3* | 19 | *95* | |
| 1q gain | 68 | *43* | 61 | *43.9* | 7 | *36.8* | n.s. |
| 1q disomic patients | 90 | *57* | 78 | *56.1* | 12 | *63.2* | |
| 1p loss | 18 | *12.7* | 17 | *13.5* | 1 | *6.25* | n.s. |
| 1p disomic patients | 124 | *87.3* | 109 | *86.5* | 15 | *93.75* | |
| *IGH* trx | 82 | *49.7* | 72 | *49.6* | 10 | *50* | n.s. |
| no *IGH* trx | 83 | *50.3* | 73 | *50.4* | 10 | *50* | |
| hyperdiploid | 50 | *32.9* | 45 | *33.8* | 5 | *26.3* | n.s. |
| non-hyperdiploid | 102 | *67.1* | 88 | *66.2* | 14 | *73.7* | |

[a] Significance was assessed by Freeman-Halton extension of Fisher's exact test for disease type, and by Fisher's exact test for all other variables.

## Supplementary Table 2A: Non-synonymous *NRAS* mutations identified by NGS in MM/PCL patients

| *Variant (GRCh38)* | *AA change* | *dbSNP ID* | *COSMIC ID (v71)* | *MM literature** | *Mutated patients (percentage of mutated sequencing reads)* | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 114713908T>C | Q61R | rs11554290 | COSM584 | 1, 2, 3 | MM-424 (95.95%) | PCL-041 (90.50%) | MM-174 (80.77%) | PCL-039 (63.37%) | MM-212 (46.70%) | MM-034 (46.37%) | MM-049 (34.44%) | MM-435 (29.87%) | MM-241 (28.98%) | MM-381 (21.99%) | MM-261 (21.63%) | MM-229 (11.68%) | MM-387 (4.56%) |
| 114713909G>T | Q61K | rs121913254 | COSM580 | 1, 2, 3, 4 | MM-434 (88.27%) | MM-449 (84.36%) | PCL-005 (45.05%) | MM-246 (40.98%) | MM-330 (33.91%) | MM-414 (31.87%) | MM-087 (31.74%) | MM-448 (26.60%) | MM-037 (12.22%) | PCL-039 (8.26%) | MM-327 (4.28%) | MM-284 (5.86%) | |
| 114713907T>G | Q61H | / | COSM586 | 1, 3, 4 | MM-398 (47.45%) | MM-405 (43.42%) | MM-341 (31.48%) | MM-387 (18.46%) | PCL-027 (65.62%) | MM-302 (15.73%) | | | | | | | |
| 114716124C>G | G13R | rs121434595 | COSM569 | 1, 3, 4 | MM-372 (52.60%) | MM-066 (30.19%) | MM-302 (17.44%) | MM-284 (4.61%) | | | | | | | | | |
| 114716126C>T | G12D | rs121913237 | COSM564 | 3 | MM-334 (96.49%) | MM-422 (11.95%) | MM-295 (8.89%) | | | | | | | | | | |
| 114713907delT | E62Kfs*6 | / | / | / | MM-405 (2.67%) | MM-212 (1.28%) | MM-229 (1.27%) | | | | | | | | | | |
| 114713907T>A | Q61H | rs121913255 | COSM585 | 1, 3, 4 | MM-317 (48.82%) | MM-146 (6.11%) | | | | | | | | | | | |
| 114713908T>A | Q61L | rs11554290 | COSM583 | 3 | MM-276 (45.41%) | MM-448 (10.37%) | | | | | | | | | | | |
| 114716123C>T | G13D | rs121434596 | COSM573 | 3 | PCL-011 (93.06%) | | | | | | | | | | | | |
| 114713908_114713909TG>GT | Q61T | / | / | / | MM-314 (82%) | | | | | | | | | | | | |
| 114713878T>C | Y71C | / | / | / | MM-276 (45.95%) | | | | | | | | | | | | |
| 114713831T>A | S87C | / | / | / | MM-411 (45.45%) | | | | | | | | | | | | |
| 114713831delT | S87Afs*17 | / | / | / | MM-411 (2.24%) | | | | | | | | | | | | |

*1: Chapman *et al.*, Nature 2011; 2: Walker *et al.*, Blood 2012; 3: Lohr *et al.*, Cancer Cell 2014; 4: Bolli *et al.*, Nature Communications 2014.

**Supplementary Table 2B: Clinical and molecular characteristics of 40 MM/PCL patients carrying non-synonymous *NRAS* mutations**

| *Sample name* | *Disease* | *Variant (GRCh38)* | *% of mutated sequencing reads* | *AA change* | *Sex* | *Age* | *PP*[†] | *del(13)** | *del(17p)** | *1q gain** | *1p loss** | *t(4;14)* | *t(11;14)* | *t(14;16)* | *t(14;20)* | *HD°* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MM-405 | MM | 114713907T>G/g.114713907delT | 43.42%/2.67% | Q61H/E62Kfs*6 | M | 69 | Gκ | - | - | - | - | - | + | - | - | - |
| MM-034 | MM | 114713908T>C | 46.37% | Q61R | M | 71 | Gκ | - | - | - | - | - | - | - | - | + |
| MM-037 | MM | 114713909G>T | 12.22% | Q61K | F | 50 | Gκ,Aκ | + | - | - | - | - | + | - | - | - |
| MM-049 | MM | 114713908T>C | 34.44% | Q61R | M | 62 | κ | - | - | - | - | - | - | - | - | + |
| MM-066 | MM | 114716124C>G | 30.19% | G13R | F | 77 | Aκ | - | - | - | na | + | - | - | - | - |
| MM-087 | MM | 114713909G>T | 31.74% | Q61K | F | 84 | Gλ | + | - | + | na | + | - | - | - | - |
| MM-146 | MM | 114713907T>A | 6.11% | Q61H | M | 68 | Gλ | - | - | + | na | - | - | - | - | + |
| MM-174 | MM | 114713908T>C | 80.77% | Q61R | M | 85 | Aκ | - | - | - | - | - | - | - | - | + |
| MM-212 | MM | 114713908T>C/g.114713907delT | 46.70%/1.28% | Q61R/E62Kfs*6 | F | 55 | Gκ | - | - | - | - | - | + | - | - | - |
| MM-229 | MM | 114713908T>C/g.114713907delT | 11.68%/1.27% | Q61R/E62Kfs*6 | M | 75 | Gκ | - | - | - | - | - | - | - | - | - |
| MM-241 | MM | 114713908T>C | 28.98% | Q61R | M | 54 | Gκ | - | - | - | - | - | - | - | - | + |
| MM-246 | MM | 114713909G>T | 40.98% | Q61K | M | 71 | Aλ | - | - | - | - | - | + | - | - | - |
| MM-261 | MM | 114713908T>C | 21.63% | Q61R | F | 66 | Gκ | + | - | + | - | - | - | - | - | + |
| MM-276 | MM | 114713878T>C/g.114713908T>A | 45.95%/45.41% | Y71C/Q61L | F | 70 | Gκ | + | - | - | - | + | - | - | - | - |
| MM-284 | MM | 114716124C>G/g.114713909G>T | 4.61%/5.86% | G13R/Q61K | M | 49 | k | na | - | - | - | - | + | - | - | - |
| MM-295 | MM | 114716126C>T | 8.89% | G12D | F | 74 | λ | + | - | na | na | - | - | - | - | na |
| MM-302 | MM | 114716124C>G/g.114713907T>G | 17.44%/15.73% | G13R/Q61H | M | 65 | Aκ | - | - | + | - | - | - | - | - | + |
| MM-314 | MM | 114713908_114713909TG>GT | 82.04% | Q61T | F | 70 | Aλ | - | - | + | - | - | + | - | - | - |
| MM-317 | MM | 114713907T>A | 48.82% | Q61H | M | 56 | Gλ | - | - | + | - | - | + | - | - | - |
| MM-327 | MM | 114713909G>T | 4.28% | Q61K | F | 62 | absent | + | - | + | - | - | - | - | - | + |
| MM-330 | MM | 114713909G>T | 33.91% | Q61K | F | 61 | Aκ | + | - | + | - | + | - | - | - | - |
| MM-334 | MM | 114716126C>T | 96.49% | G12D | F | 45 | Gλ | + | + | + | + | - | - | - | - | + |
| MM-341 | MM | 114713907T>G | 31.48% | Q61H | M | 65 | Gκ | - | - | + | - | - | - | - | - | - |
| MM-372 | MM | 114716124C>G | 52.60% | G13R | M | 54 | Gk | - | - | + | - | - | + | - | - | - |
| MM-381 | MM | 114713908T>C | 21.99% | Q61R | F | 69 | Gκ | - | - | - | - | - | - | - | na | na |
| MM-387 | MM | 114713907T>G/g.114713908T>C | 18.46%/4.56% | Q61H/Q61R | F | 44 | Gκ | - | - | + | - | - | - | - | - | + |
| MM-398 | MM | 114713907T>G | 47.45% | Q61H | F | 65 | Gκ | + | - | + | - | - | + | - | - | - |
| MM-411 | MM | 114713831T>A/g.114713831delT | 45.45%/2.24% | S87C/ S87Afs*17 | M | 67 | Gλ | - | - | - | - | - | - | - | - | + |
| MM-414 | MM | 114713909G>T | 31.87% | Q61K | F | 67 | Gλ | - | - | - | - | - | + | - | - | - |
| MM-422 | MM | 114716126C>T | 11.95% | G12D | F | 74 | k | + | - | - | - | - | - | - | - | - |
| MM-424 | MM | 114713908T>C | 95.95% | Q61R | F | 84 | Gκ | + | - | - | + | - | - | - | - | + |
| MM-434 | MM | 114713909G>T | 88.27% | Q61K | F | 53 | κ | + | - | - | + | - | - | - | - | - |
| MM-435 | MM | 114713908T>C | 29.87% | Q61R | na | 42 | Gλ | - | - | - | - | - | - | - | - | + |
| MM-448 | MM | 114713909G>T/g.114713908T>A | 26.60%/10.37% | Q61K/Q61L | na | 59 | Aκ | + | - | + | - | - | - | - | - | - |
| MM-449 | MM | 114713909G>T | 84.36% | Q61K | na | 54 | Gκ | + | - | - | + | - | - | - | - | - |
| PCL-005 | sPCL | 114713909G>T | 45.05% | Q61K | M | 76 | Aκ | - | - | - | - | - | + | - | - | - |
| PCL-011 | sPCL | 114716123C>T | 93.06% | G13D | M | 76 | Gκ | + | + | + | - | - | - | + | - | - |
| PCL-027 | pPCL | 114713907T>G | 65.62% | Q61H | M | 65 | λ | - | + | - | - | - | + | - | - | - |
| PCL-039 | sPCL | 114713908T>C/g.114713909G>T | 63.37%/8.26% | Q61R/Q61K | M | 54 | Aκ | - | + | - | + | - | - | - | - | - |
| PCL-041 | sPCL | 114713908T>C | 90.50% | Q61R | M | na | Gκ | - | - | na | na | - | na | na | na | na |

[†]Paraprotein; *del(13), del(17), 1p loss and 1q gain were determined by FISH. °HD = presence of the hyperdiploid status on the basis of FISH evaluation criteria.

**Supplementary Table 2C: Clinical and molecular characteristics of the 167 MM/PCL patients analyzed for *NRAS* mutations**

| *Characteristic* | *All patients (n=167)* | | *NRAS wild type (n=127)* | | *NRAS mutated (n=40)* | | *P value*[a] |
|---|---|---|---|---|---|---|---|
| | *N* | *%* | *n* | *%* | *n* | *%* | |
| MM | 132 | *79* | 97 | *76.4* | 35 | *87.5* | |
| pPCL | 24 | *14.4* | 23 | *18.1* | 1 | *2.5* | 0.008 |
| sPCL | 11 | *6.6* | 7 | *5.5* | 4 | *10* | |
| del(13q) | 79 | *47.6* | 64 | *50.4* | 15 | *38.5* | n.s. |
| chr 13 disomic patients | 87 | *52.4* | 63 | *49.6* | 24 | *61.5* | |
| del(17p) | 18 | *10.9* | 14 | *11.2* | 4 | *10* | n.s. |
| 17p disomic patients | 147 | *89.1* | 111 | *88.8* | 36 | *90* | |
| 1q gain | 68 | *43* | 53 | *44.2* | 15 | *39.5* | n.s. |
| 1q disomic patients | 90 | *57* | *67* | *55.8* | 23 | *60.5* | |
| 1p loss | 18 | *12.7* | 13 | *12.1* | 5 | *14.3* | n.s. |
| 1p disomic patients | 124 | *87.3* | 94 | *87.9* | 30 | *85.7* | |
| *IGH* trx | 82 | *49.7* | 64 | *49.8* | 18 | *46.1* | n.s. |
| no *IGH* trx | 83 | *50.3* | 62 | *50.2* | 21 | *53.8* | |
| hyperdiploid | 50 | *32.9* | 37 | *32.2* | 13 | *35.1* | n.s. |
| non-hyperdiploid | 102 | *67.1* | 78 | *67.8* | 24 | *64.9* | |

[a] Significance was assessed by Freeman-Halton extension of Fisher's exact test for disease type, and by Fisher's exact test for all other variables.

**Supplementary Table 3A: Non-synonymous *KRAS* mutations identified by NGS in MM/PCL patients**

| *Variant (GRCh38)* | *AA change* | *dbSNP ID* | *COSMIC ID (v71)* | *MM literature** | *Mutated patients (percentage of mutated sequencing reads)* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 25227341T>G | Q61H | rs17851045 | COSM554 | 1, 2, 3, 4 | MM-055 (52.53%) | MM-442 (45.93%) | MM-430 (39.77%) | MM-440 (38%) | MM-327 (37.34%) | MM-202 (35.54%) | MM-151 (35.29%) | MM-402 (34.62%) | MM-200 (23.74%) |
| | | | | | MM-229 (12.07%) | MM-381 (9.51%) | MM-441 (8.45%) | PCL-046 (7.73%) | MM-300 (7.61%) | | | | |
| 25245350C>T | G12D | rs121913529 | COSM521 | 3 | MM-154 (45.28%) | MM-030 (45%) | MM-152 (31.69%) | MM-433 (31.78%) | MM-026 (30.59%) | MM-429 (21.47%) | PCL-046 (9.01%) | | |
| 25245351C>G | G12R | rs121913530 | COSM518 | 2, 3, 4 | MM-039 (46.94%) | PCL-038 (42.42%) | MM-445 (28.85%) | MM-159 (27.78%) | | | | | |
| 25245347C>T | G13D | rs112445441 | COSM532 | 1, 2, 3 | MM-385 (40.68%) | MM-308 (39.42%) | MM-151 (9.35%) | MM-219 (5.88%) | | | | | |
| 25227342T>C | Q61R | rs121913240 | COSM552 | 2, 3, 4 | MM-038 (40.82%) | MM-351 (28.66%) | MM-159 (3.75%) | | | | | | |
| 25245321G>T | Q22K | rs121913236 | COSM543 | 4 | MM-280 (50%) | MM-026 (32.94%) | | | | | | | |
| 25225628C>T | A146T | rs121913527 | COSM19404 | 1 | MM-413 (51.24%) | MM-131 (40.52%) | | | | | | | |
| 25245348C>A | G13C | rs121913535 | COSM527 | / | PCL-001 (48.64%) | MM-149 (29.13%) | | | | | | | |
| 25225713T>G | K117N | / | COSM19940 | 1, 3 | MM-310 (47.09%) | MM-269 (3.07%) | | | | | | | |
| 25245328C>G | L19F | / | COSM12703 | 1 | MM-386 (42.73%) | MM-335 (18.6%) | | | | | | | |
| 25245350C>A | G12V | rs121913529 | COSM520 | 3, 4 | MM-146 (24.58%) | MM-150 (8.93%) | | | | | | | |
| 25227334A>T | Y64N | / | / | 3 | MM-115 (54.2%) | | | | | | | | |
| 25227266A>T | N86K | / | / | / | MM-115 (44.27%) | | | | | | | | |
| 25245332G>T | A18D | / | COSM542 | 3 | PCL-021 (43.8%) | | | | | | | | |
| 25227334A>C | Y64D | / | / | 1 | MM-410 (41.96%) | | | | | | | | |
| 25245351C>A | G12C | rs121913530 | COSM516 | 4 | MM-243 (40.5%) | | | | | | | | |
| 25227348G>C | A59G | / | COSM28518 | 3 | MM-079 (23.13%) | | | | | | | | |
| 25227330C>A | S65I | / | / | / | PCL-009 (15.84%) | | | | | | | | |
| 25245350C>G | G12A | rs121913529 | COSM522 | 3, 4 | MM-140 (14.81%) | | | | | | | | |
| 25227342T>A | Q61L | rs121913240 | COSM553 | 3, 4 | MM-284 (9.54%) | | | | | | | | |
| 25245351C>T | G12S | rs121913530 | COSM517 | / | MM-004 (9.18%) | | | | | | | | |
| 25225657C>T | S136N | / | / | / | MM-202 (4.86%) | | | | | | | | |
| 25225627G>A | A146V | / | COSM19900 | 4 | MM-445 (2.53%) | | | | | | | | |

*1: Chapman *et al.*, Nature 2011; 2: Walker *et al.*, Blood 2012; 3: Lohr *et al.*, Cancer Cell 2014; 4: Bolli *et al.*, Nature Communications 2014.

## Supplementary Table 3B: Clinical and molecular characteristics of 49 MM/PCL patients carrying non-synonymous *KRAS* mutations

| *Sample name* | *Disease* | *Variant (GRCh38)* | *% of mutated sequencing reads* | *AA change* | *Sex* | *Age* | *PP†* | *del(13)** | *del(17p)** | *1q gain** | *1p loss** | *t(4;14)* | *t(11;14)* | *t(14;16)* | *t(14;20)* | *HD°* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MM-004 | MM | 25245351C>T | 9.18% | G12S | F | 58 | Gκ | - | na | na | na | - | - | + | - | na |
| MM-026 | MM | 25245321G>T/g.25245350C>T | 32.94%/30.59% | Q22K/G12D | F | 72 | κ | - | - | - | na | - | + | - | - | na |
| MM-030 | MM | 25245350C>T | 45.00% | G12D | M | 69 | Gλ | - | - | - | + | - | - | - | - | + |
| MM-038 | MM | 25227342T>C | 40.82% | Q61R | F | 67 | κ | + | - | - | - | - | - | - | - | + |
| MM-039 | MM | 25245351C>G | 46.94% | G12R | M | 50 | Gλ | - | - | - | na | - | - | - | - | + |
| MM-055 | MM | 25227341T>G | 52.53% | Q61H | F | 69 | Gκ | - | - | - | na | - | + | - | - | - |
| MM-079 | MM | 25227348G>C | 23.13% | A59G | F | 74 | Gκ+Gλ | - | - | - | - | - | - | - | - | + |
| MM-115 | MM | 25227334A>T/g.25227266A>T | 54.20%/44.27% | Y64N/N86K | F | 53 | Gλ | + | - | na | na | - | + | - | - | - |
| MM-131 | MM | 25225628C>T | 40.52% | A146T | M | 73 | Gκ | - | - | - | - | - | - | - | - | - |
| MM-140 | MM | 25245350C>G | 14.81% | G12A | M | 62 | Gκ | - | - | - | na | - | + | - | - | - |
| MM-146 | MM | 25245350C>A | 24.58% | G12V | M | 68 | Gλ | - | - | + | na | - | - | - | - | + |
| MM-149 | MM | 25245348C>A | 29.13% | G13C | F | 52 | Gλ | - | - | - | - | - | - | - | - | + |
| MM-150 | MM | 25245350C>A | 8.93% | G12V | F | 68 | Gλ | - | - | + | - | - | - | - | - | + |
| MM-151 | MM | 25227341T>G/g.25245347C>T | 35.29%/9.35% | Q61H/G13D | F | 71 | Gλ | - | - | - | - | - | - | - | - | + |
| MM-152 | MM | 25245350C>T | 31.69% | G12D | M | 66 | Gκ | - | - | - | na | - | - | - | - | + |
| MM-154 | MM | 25245350C>T | 45.28% | G12D | F | 71 | Gκ | + | - | + | - | - | - | + | - | - |
| MM-159 | MM | 25245351C>G/g.25227342T>C | 27.78%/3.75% | G12R/Q61R | M | 56 | κ | + | - | - | - | - | + | - | - | - |
| MM-200 | MM | 25227341T>G | 23.74% | Q61H | F | 63 | Aλ | - | - | - | - | - | - | - | - | + |
| MM-202 | MM | 25227341T>G/g.25225657C>T | 35.54%/4.86% | Q61H/S136N | M | 64 | Gκ | - | - | + | na | - | - | - | - | + |
| MM-219 | MM | 25245347C>T | 5.88% | G13D | M | 73 | Aλ | + | - | - | - | - | - | - | - | + |
| MM-229 | MM | 25227341T>G | 12.07% | Q61H | M | 75 | Gκ | - | - | - | - | - | - | - | - | - |
| MM-243 | MM | 25245351C>A | 40.50% | G12C | F | 68 | Gκ | - | - | + | - | - | - | - | - | + |
| MM-269 | MM | 25225713T>G | 3.07% | K117N | F | 67 | Gκ | + | - | - | - | - | - | - | - | + |
| MM-280 | MM | 25245321G>T | 50.00% | Q22K | M | 62 | Gλ | - | - | - | - | - | + | - | - | - |
| MM-284 | MM | 25227342T>A | 9.54% | Q61L | M | 49 | κ | na | - | - | - | - | + | - | - | - |
| MM-300 | MM | 25227341T>G | 7.61% | Q61H | M | 65 | Gκ | - | - | na | na | - | - | - | - | na |
| MM-308 | MM | 25245347C>T | 39.42% | G13D | M | 58 | Aκ | - | - | - | - | - | - | - | - | - |
| MM-310 | MM | 25225713T>G | 47.09% | K117N | M | 67 | Gl | - | - | - | - | - | + | - | - | - |
| MM-327 | MM | 25227341T>G | 37.34% | Q61H | F | 62 | absent | + | - | + | - | - | - | - | - | + |
| MM-335 | MM | 25245328C>G | 18.60% | L19F | F | 68 | Aκ | + | - | - | - | - | - | + | - | - |
| MM-351 | MM | 25227342T>C | 28.66% | Q61R | M | na | Aκ | - | - | - | - | - | - | - | - | - |
| MM-381 | MM | 25227341T>G | 9.51% | Q61H | F | 69 | Gκ | - | - | - | - | - | - | - | na | na |
| MM-385 | MM | 25245347C>T | 40.68% | G13D | F | 76 | Gλ | - | - | - | - | - | - | - | - | + |
| MM-386 | MM | 25245328C>G | 42.73% | L19F | F | 76 | Aλ | - | - | na | na | - | na | na | na | na |
| MM-402 | MM | 25227341T>G | 34.62% | Q61H | M | 67 | Gκ | + | - | - | - | - | + | - | - | - |
| MM-410 | MM | 25227334A>C | 41.96% | Y64D | F | 79 | λ | - | - | + | - | - | - | - | - | - |

| *Sample name* | *Disease* | *Variant (GRCh38)* | *% of mutated sequencing reads* | *AA change* | *Sex* | *Age* | *PP*† | *del(13)** | *del(17p)** | *1q gain** | *1p loss** | *t(4;14)* | *t(11;14)* | *t(14;16)* | *t(14;20)* | *HD*° |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MM-413 | MM | 25225628C>T | 51.24% | A146T | F | 51 | Gκ | + | - | + | - | - | - | - | - | + |
| MM-429 | MM | 25245350C>T | 21.47% | G12D | F | 63 | Gκ | - | - | + | - | - | - | - | - | + |
| MM-430 | MM | 25227341T>G | 39.77% | Q61H | na | 62 | Gλ | - | - | - | - | - | - | - | - | - |
| MM-433 | MM | 25245350C>T | 31.78% | G12D | M | 72 | κ | - | - | - | - | - | + | - | - | - |
| MM-440 | MM | 25227341T>G | 38.00% | Q61H | na | 61 | Aκ | - | - | - | + | - | - | - | - | + |
| MM-441 | MM | 25227341T>G | 8.45% | Q61H | na | na | Gλ | - | - | - | - | - | - | - | - | - |
| MM-442 | sPCL | 25227341T>G | 45.93% | Q61H | F | 65 | Gκ | + | - | + | + | - | - | - | - | - |
| MM-445 | MM | 25245351C>G/g.25225627G>A | 28.85%/2.53% | G12R/A146V | na | 65 | κ | + | - | + | - | - | + | - | - | - |
| PCL-001 | pPCL | 25245348C>A | 48.64% | G13C | F | 51 | κ | + | - | na | na | - | - | - | + | na |
| PCL-009 | sPCL | 25227330C>A | 15.84% | S65I | F | 77 | Gκ | + | - | + | - | + | - | - | - | - |
| PCL-021 | pPCL | 25245332G>T | 43.80% | A18D | M | 48 | Gλ | + | - | + | - | + | - | - | - | - |
| PCL-038 | pPCL | 25245351C>G | 42.42% | G12R | M | 57 | Gκ | + | - | + | + | - | - | - | - | + |
| PCL-046 | pPCL | 25227341T>G/g.25245350C>T | 7.73%/9.01% | Q61H/G12D | F | 50 | κ | + | - | + | - | - | - | - | - | + |

†Paraprotein; *del(13), del(17), 1p loss and 1q gain were determined by FISH. °HD = presence of the hyperdiploid status on the basis of FISH evaluation criteria.

**Supplementary Table 3C: Clinical and molecular characteristics of the 167 MM/PCL patients analyzed for *KRAS* mutations.**

| *Characteristic* | *All patients (n=167)* | | *KRAS wild type (n=118)* | | *KRAS mutated (n=49)* | | *P value*[a] |
|---|---|---|---|---|---|---|---|
| | *N* | *%* | *n* | *%* | *n* | *%* | |
| MM | 132 | *79* | 89 | *75.4* | 43 | *87.7* | n.s. |
| pPCL | 24 | *14.4* | 20 | *17* | 4 | *8.2* | |
| sPCL | 11 | *6.6* | 9 | *7.6* | 2 | *4.1* | |
| del(13q) | 79 | *47.6* | 62 | *52.5* | 17 | *35.4* | n.s. |
| chr 13 disomic patients | 87 | *52.4* | 56 | *47.5* | 31 | *64.6* | |
| del(17p) | 18 | *10.9* | 18 | *15.4* | 0 | *0* | 0.0019 |
| 17p disomic patients | 147 | *89.1* | 99 | *84.6* | 48 | *100* | |
| 1q gain | 68 | *43* | 53 | *46.5* | 15 | *34.1* | n.s. |
| 1q disomic patients | 90 | *57* | 61 | *53.5* | 29 | *55.9* | |
| 1p loss | 18 | *12.7* | 14 | *13.3* | 4 | *10.8* | n.s. |
| 1p disomic patients | 124 | *87.3* | 91 | *86.7* | 33 | *89.2* | |
| *IGH* trx | 82 | *49.7* | 64 | *54.7* | 18 | *37.5* | n.s. |
| no *IGH* trx | 83 | *50.3* | 53 | *45.3* | 30 | *62.5* | |
| hyperdiploid | 50 | *32.9* | 29 | *26.6* | 21 | *48.8* | 0.0124 |
| non-hyperdiploid | 102 | *67.1* | 80 | *73.4* | 22 | *51.2* | |

[a] Significance was assessed by Freeman-Halton extension of Fisher's exact test for disease type, and by Fisher's exact test for all other variables.

**Supplementary Table 4A: Modulated genes between *BRAF*/*NRAS*/*KRAS* mutated and wild type patients**

| *Gene* | *SAM score*[‡] | *Gene* | *SAM score* |
|---|---|---|---|
| *PLEKHA1** | -5.163765 | *ETV5** | 6.24113 |
| *ARL6IP5** | -4.342202 | *CD300A** | 5.77917 |
| *ZHX1** | -4.311072 | *SPRED2** | 5.37656 |
| *SMAD3** | -4.287826 | *DUSP6** | 4.36551 |
| *AHNAK** | -4.268764 | *NRXN1** | 4.23105 |
| *TBC1D1** | -4.237025 | *MORC1** | 4.20022 |
| *MTMR4** | -4.167526 | *F12** | 4.15845 |
| *COPZ2** | -4.103426 | *RRBP1** | 4.07138 |
| *RASGRP1** | -4.07288 | *TDO2** | 4.03133 |
| *FLNB* | -3.961337 | *LGALS3BP* | 3.97907 |
| *BMP2K* | -3.918772 | *PRKD2* | 3.94335 |
| *RIOK1* | -3.907891 | *ACVR1B* | 3.94093 |
| *TSHZ1* | -3.904952 | *ASPHD2* | 3.92401 |
| *NOTCH2* | -3.883492 | *GPR56* | 3.89699 |
| *ZNF681* | -3.859508 | *ACOXL* | 3.85393 |
| *DHCR24* | -3.82483 | *SPRED1* | 3.8092 |
| *LIFR* | -3.819499 | *LY9* | 3.76526 |
| *ATL3* | -3.818015 | *ABI3BP* | 3.74618 |
| *TGIF2* | -3.794277 | *DPEP1* | 3.70693 |
| *GLO1* | -3.776065 | *TMEM184B* | 3.70255 |
| *C6orf89* | -3.76636 | *PDE4D* | 3.69971 |
| *ZNF570* | -3.738105 | *P2RY6* | 3.68381 |
| *CYB5R1* | -3.727057 | *CASP4* | 3.66862 |
| *PHTF2* | -3.721193 | *MIR221* | 3.65834 |
| *DENND5A* | -3.687141 | *SEMA4D* | 3.65632 |
| *CD52* | -3.67123 | *MAPKAPK2* | 3.64609 |
| *ZADH2* | -3.659897 | *SIX4* | 3.64407 |
| *RRP8* | -3.635111 | | |
| *BCL2* | -3.627357 | | |
| *JAZF1* | -3.621626 | | |
| *CD86* | -3.612113 | | |
| *LAMTOR3* | -3.611917 | | |
| *SAMSN1* | -3.587501 | | |
| *NTAN1* | -3.586428 | | |
| *PLCL1* | -3.549426 | | |
| *LRIG3* | -3.547287 | | |
| *PPM1L* | -3.545335 | | |
| *GRB2* | -3.537068 | | |
| *NECAP2* | -3.522351 | | |
| *KDM1B* | -3.513606 | | |
| *KAT6A* | -3.51165 | | |
| *BCL11A* | -3.499956 | | |
| *NEU1* | -3.491002 | | |
| *KIAA1147* | -3.487048 | | |
| *NUDT7* | -3.455732 | | |
| *SLC23A2* | -3.446583 | | |
| *LSS* | -3.442877 | | |
| *LYPD6B* | -3.440096 | | |
| *ARHGEF3* | -3.436137 | | |
| *CLN3* | -3.423259 | | |
| *INSIG1* | -3.421565 | | |
| *LYN* | -3.382145 | | |
| *APBB1IP* | -3.378885 | | |
| *MYL2* | -3.372364 | | |
| *TOP3A* | -3.359096 | | |
| *AKAP5* | -3.358922 | | |
| *RPSAP58* | -3.357769 | | |
| *EDN1* | -3.355883 | | |
| *SQLE* | -3.353543 | | |

*Differentially expressed gene at the highest stringency level. [‡]Negative scores indicate downregulated genes in mutated cases.

**Supplementary Table 4B: Enriched biological processes in the MAPK mutation-associated 86 gene signature (*q* value<0.005)**

| *ID* | *Name* | *q value* | *Hit in Query List* |
|---|---|---|---|
| GO:0010563 | negative regulation of phosphorus metabolic process | 1.29E03 | *SEMA4D, AKAP5, PDE4D, SPRED2, CD300A, DUSP6, SPRED1, SAMSN1, LYN, EDN1, SMAD3* |
| GO:0045936 | negative regulation of phosphate metabolic process | 1.29E03 | *SEMA4D, AKAP5, PDE4D, SPRED2, CD300A, DUSP6, SPRED1, SAMSN1, LYN, EDN1, SMAD3* |
| GO:0042325 | regulation of phosphorylation | 1.29E03 | *PRKD2, SEMA4D, AKAP5, PDE4D, SPRED2, MAPKAPK2, CD300A, ARL6IP5, DUSP6, GRB2, LAMTOR3, SPRED1, BCL2, PLCL1, ACVR1B, SAMSN1, LYN, EDN1, SMAD3* |
| GO:0001932 | regulation of protein phosphorylation | 1.29E03 | *PRKD2, SEMA4D, AKAP5, PDE4D, SPRED2, MAPKAPK2, CD300A, DUSP6, LAMTOR3, SPRED1, BCL2, PLCL1, ACVR1B, SAMSN1, LYN, EDN1, SMAD3* |
| GO:0016126 | sterol biosynthetic process | 1.29E03 | *DHCR24, INSIG1, SQLE, LSS, CYB5R1* |
| GO:1902531 | regulation of intracellular signal transduction | 1.29E03 | *PRKD2, SEMA4D, RASGRP1, AKAP5, SPRED2, MAPKAPK2, TBC1D1, CD300A, ARL6IP5, DUSP6, GRB2, LAMTOR3, GPR56, PLEKHA1, SPRED1, BCL2, DENND5A, ARHGEF3, LYN, EDN1, NOTCH2* |
| GO:0023056 | positive regulation of signaling | 1.39E03 | *PRKD2, SEMA4D, AKAP5, SPRED2, NRXN1, MAPKAPK2, CD300A, ARL6IP5, GRB2, LAMTOR3, GPR56, SPRED1, BCL2, ACVR1B, LYN, EDN1, NOTCH2, SMAD3* |
| GO:0010647 | positive regulation of cell communication | 1.39E03 | *PRKD2, SEMA4D, AKAP5, SPRED2, NRXN1, MAPKAPK2, CD300A, ARL6IP5, GRB2, LAMTOR3, GPR56, SPRED1, BCL2, ACVR1B, LYN, EDN1, NOTCH2, SMAD3* |
| GO:1902533 | positive regulation of intracellular signal transduction | 1.39E03 | *PRKD2, SEMA4D, AKAP5, SPRED2, MAPKAPK2, CD300A, ARL6IP5, LAMTOR3, GPR56, SPRED1, BCL2, LYN, EDN1, NOTCH2* |
| GO:0048584 | positive regulation of response to stimulus | 1.39E03 | *PRKD2, SEMA4D, AKAP5, PDE4D, SPRED2, MAPKAPK2, CD86, CD300A, ARL6IP5, DUSP6, GRB2, LAMTOR3, GPR56, PLEKHA1, SPRED1, BCL2, ACVR1B, LYN, EDN1, NOTCH2, SMAD3* |
| GO:0001933 | negative regulation of protein phosphorylation | 1.39E03 | *SEMA4D, PDE4D, SPRED2, CD300A, DUSP6, SPRED1, SAMSN1, LYN, SMAD3* |
| GO:0019220 | regulation of phosphate metabolic process | 1.63E03 | *PRKD2, SEMA4D, RASGRP1, AKAP5, PDE4D, SPRED2, MAPKAPK2, TBC1D1, CD300A, ARL6IP5, DUSP6, GRB2, LAMTOR3, SPRED1, BCL2, PLCL1, DENND5A, ARHGEF3, ACVR1B, SAMSN1, LYN, EDN1, SMAD3* |
| GO:0002757 | immune response activating signal transduction | 1.63E03 | *PRKD2, PDE4D, MAPKAPK2, CD86, CD300A, DUSP6, GRB2, PLEKHA1, BCL2, LYN* |
| GO:0051174 | regulation of phosphorus metabolic process | 1.63E03 | *PRKD2, SEMA4D, RASGRP1, AKAP5, PDE4D, SPRED2, MAPKAPK2, TBC1D1, CD300A, ARL6IP5, DUSP6, GRB2, LAMTOR3, SPRED1, BCL2, PLCL1, DENND5A, ARHGEF3, ACVR1B, SAMSN1, LYN, EDN1, SMAD3* |
| GO:0009967 | positive regulation of signal transduction | 1.63E03 | *PRKD2, SEMA4D, AKAP5, SPRED2, MAPKAPK2, CD300A, ARL6IP5, GRB2, LAMTOR3, GPR56, SPRED1, BCL2, ACVR1B, LYN, EDN1, NOTCH2, SMAD3* |
| GO:0002764 | immune response regulating signaling pathway | 2.03E03 | *PRKD2, RASGRP1, PDE4D, MAPKAPK2, CD86, CD300A, DUSP6, GRB2, PLEKHA1, BCL2, LYN* |
| GO:0051094 | positive regulation of developmental process | 2.03E03 | *PRKD2, SEMA4D, TGIF2, AKAP5, NRXN1, CD86, ETV5, BCL2, BCL11A, ACVR1B, SIX4, LYN, EDN1, NOTCH2, SMAD3* |
| GO:0023014 | signal transduction by phosphorylation | 2.03E03 | *PRKD2, SPRED2, MAPKAPK2, CD300A, ARL6IP5, DUSP6, PPM1L, GRB2, LAMTOR3, SPRED1, ACVR1B, LYN, EDN1* |
| GO:0031324 | negative regulation of cellular metabolic process | 2.74E03 | *SEMA4D, TGIF2, AKAP5, PDE4D, SPRED2, CLN3, CD300A, INSIG1, DUSP6, KAT6A, JAZF1, SPRED1, RRP8, BCL2, BCL11A, SAMSN1, LYN, ZHX1, EDN1, NOTCH2, SMAD3* |
| GO:0043407 | negative regulation of MAP kinase activity | 2.74E03 | *SPRED2, CD300A, DUSP6, SPRED1, LYN* |
| GO:0042326 | negative regulation of phosphorylation | 2.74E03 | *SEMA4D, PDE4D, SPRED2, CD300A, DUSP6, SPRED1, SAMSN1, LYN, SMAD3* |
| GO:0009892 | negative regulation of metabolic process | 2.74E03 | *SEMA4D, TGIF2, AKAP5, PDE4D, SPRED2, CLN3, CD300A, INSIG1, DUSP6, KAT6A, JAZF1, SPRED1, RRP8, BCL2, BCL11A, ACVR1B, SAMSN1, LYN, ZHX1, EDN1, NOTCH2, SMAD3* |
| GO:0006468 | protein phosphorylation | 2.74E03 | *PRKD2, SEMA4D, RIOK1, AKAP5, PDE4D, SPRED2, BMP2K, MAPKAPK2, CD300A, DUSP6, LAMTOR3, SPRED1, BCL2, PLCL1, ACVR1B, SAMSN1, LYN, EDN1, SMAD3* |
| GO:2000026 | regulation of multicellular organismal development | 2.76E03 | *PRKD2, SEMA4D, TGIF2, NEU1, AKAP5, NRXN1, BMP2K, CD86, DUSP6, ETV5, GPR56, BCL2, BCL11A, ACVR1B, SIX4, LYN, EDN1, NOTCH2, SMAD3* |

| *ID* | *Name* | *q value* | *Hit in Query List* |
|---|---|---|---|
| GO:0002253 | activation of immune response | 2.77E03 | *PRKD2, PDE4D, MAPKAPK2, CD86, CD300A, DUSP6, GRB2, PLEKHA1, BCL2, LYN* |
| GO:0050853 | B cell receptor signaling pathway | 3.23E03 | *CD300A, PLEKHA1, BCL2, LYN* |
| GO:0002768 | immune response regulating cell surface receptor signaling pathway | 3.29E03 | *PRKD2, RASGRP1, PDE4D, CD86, CD300A, GRB2, PLEKHA1, BCL2, LYN* |
| GO:0030334 | regulation of cell migration | 3.29E03 | *DPEP1, PRKD2, SEMA4D, P2RY6, CD300A, GPR56, BCL2, ACVR1B, LYN, EDN1, SMAD3* |
| GO:0051270 | regulation of cellular component movement | 3.29E03 | *DPEP1, PRKD2, SEMA4D, PDE4D, P2RY6, CD300A, GPR56, BCL2, ACVR1B, LYN, EDN1, SMAD3* |
| GO:0000165 | MAPK cascade | 3.29E03 | *PRKD2, SPRED2, MAPKAPK2, CD300A, ARL6IP5, DUSP6, PPM1L, GRB2, LAMTOR3, SPRED1, LYN, EDN1* |
| GO:0032269 | negative regulation of cellular protein metabolic process | 3.62E03 | *SEMA4D, PDE4D, SPRED2, CLN3, CD300A, DUSP6, SPRED1, SAMSN1, LYN, EDN1, SMAD3* |
| GO:0016310 | phosphorylation | 3.73E03 | *PRKD2, SEMA4D, RIOK1, AKAP5, PDE4D, SPRED2, BMP2K, MAPKAPK2, CD300A, ARL6IP5, DUSP6, PPM1L, GRB2, LAMTOR3, SPRED1, BCL2, PLCL1, ACVR1B, SAMSN1, LYN, EDN1, SMAD3* |
| GO:0006695 | cholesterol biosynthetic process | 3.73E03 | *DHCR24, INSIG1, SQLE, LSS* |
| GO:0007167 | enzyme linked receptor protein signaling pathway | 4.06E03 | *LIFR, PRKD2, TGIF2, MTMR4, MAPKAPK2, CD86, DUSP6, PPM1L, GRB2, PLEKHA1, ARHGEF3, ACVR1B, LYN, NOTCH2, SMAD3* |
| GO:0043583 | ear development | 4.06E03 | *LRIG3, MAPKAPK2, INSIG1, TSHZ1, BCL2, SIX4, EDN1* |
| GO:0050851 | antigen receptor mediated signaling pathway | 4.06E03 | *PRKD2, PDE4D, CD300A, PLEKHA1, BCL2, LYN* |
| GO:0031399 | regulation of protein modification process | 4.06E03 | *PRKD2, SEMA4D, AKAP5, PDE4D, SPRED2, MAPKAPK2, CD300A, DUSP6, LAMTOR3, SPRED1, BCL2, PLCL1, ACVR1B, SAMSN1, LYN, EDN1, SMAD3* |
| GO:0043408 | regulation of MAPK cascade | 4.06E03 | *PRKD2, SPRED2, MAPKAPK2, CD300A, ARL6IP5, DUSP6, GRB2, LAMTOR3, SPRED1, LYN, EDN1* |
| GO:2000145 | regulation of cell motility | 4.08E03 | *DPEP1, PRKD2, SEMA4D, P2RY6, CD300A, GPR56, BCL2, ACVR1B, LYN, EDN1, SMAD3* |
| GO:0050776 | regulation of immune response | 4.11E03 | *PRKD2, RASGRP1, PDE4D, MAPKAPK2, CD86, CD300A, DUSP6, GRB2, PLEKHA1, BCL2, SAMSN1, LYN, SMAD3* |
| GO:0018193 | peptidyl amino acid modification | 4.90E03 | *PRKD2, SEMA4D, PDE4D, SPRED2, MAPKAPK2, CD300A, KAT6A, SPRED1, ASPHD2, BCL2, PLCL1, ACVR1B, SAMSN1, LYN* |

**Supplementary Table 5A: Description of the 150 genes at the top of the ranked list in GSEA analysis of U266 treated *versus* control cells**

| *GENE_SYMBOL* | *GENE_TITLE* | *SCORE* |
|---|---|---|
| *INHBE* | inhibin, beta E | 0.676317 |
| *CTH* | cystathionase (cystathionine gamma-lyase) | 0.634 |
| *STC2* | stanniocalcin 2 | 0.600332 |
| *FBXW10* | F-box and WD-40 domain protein 10 | 0.574939 |
| *TCP11L2* | t-complex 11 (mouse) like 2 | 0.532132 |
| *ESRP1* | epithelial splicing regulatory protein 1 | 0.509604 |
| *DDIT4* | DNA-damage-inducible transcript 4 | 0.498076 |
| *TRIB3* | tribbles homolog 3 (Drosophila) | 0.474272 |
| *GRB10* | growth factor receptor-bound protein 10 | 0.471464 |
| *ASS1* | argininosuccinate synthetase 1 | 0.458725 |
| *TMEM154* | transmembrane protein 154 | 0.457523 |
| *GDF15* | growth differentiation factor 15 | 0.452052 |
| *FAM106CP* | family with sequence similarity 106, member C, pseudogene | 0.448272 |
| *SESN2* | sestrin 2 | 0.437719 |
| *BEX2* | brain expressed X-linked 2 | 0.422433 |
| *PCK2* | phosphoenolpyruvate carboxykinase 2 (mitochondrial) | 0.41365 |
| *SLC35F1* | solute carrier family 35, member F1 | 0.405951 |
| *CREB5* | cAMP responsive element binding protein 5 | 0.403822 |
| *SLC10A5* | solute carrier family 10 (sodium/bile acid cotransporter family), member 5 | 0.401765 |
| *HMOX1* | heme oxygenase (decycling) 1 | 0.39905 |
| *SNORD116-18* | small nucleolar RNA, C/D box 116-18 | 0.397014 |
| *FDPSP2* | farnesyl diphosphate synthase pseudogene 2 | 0.385425 |
| *VSNL1* | visinin-like 1 | 0.381415 |
| *SNORD116-25* | small nucleolar RNA, C/D box 116-25 | 0.38046 |
| *SLC7A11* | solute carrier family 7, (cationic amino acid transporter, y+ system) member 11 | 0.379498 |
| *LOC101928082* | uncharacterized LOC101928082 | 0.371708 |
| *EIF4EBP1* | eukaryotic translation initiation factor 4E binding protein 1 | 0.36092 |
| *TUBE1* | tubulin, epsilon 1 | 0.358196 |
| *TRAV27* | T cell receptor alpha variable 27 | 0.356685 |
| *CBS* | cystathionine-beta-synthase | 0.354561 |
| *ISY1* | ISY1 splicing factor homolog (S. cerevisiae) | 0.354496 |
| *SNORD115-24* | small nucleolar RNA, C/D box 115-24 | 0.353983 |
| *ANK2* | ankyrin 2, neuronal | 0.351251 |
| *LOC101928574* | uncharacterized LOC101928574 | 0.351248 |
| *VLDLR* | very low density lipoprotein receptor | 0.34859 |
| *SLC43A1* | solute carrier family 43, member 1 | 0.342847 |
| *CXorf61* | chromosome X open reading frame 61 | 0.342714 |
| *STON1* | stonin 1 | 0.341989 |
| *CTAGE5* | CTAGE family, member 5 | 0.341841 |
| *PRSS8* | protease, serine, 8 (prostasin) | 0.340513 |
| *TRAV10* | T cell receptor alpha variable 10 | 0.340494 |
| *DDIT3* | DNA-damage-inducible transcript 3 | 0.336389 |
| *RPS10P7* | ribosomal protein S10 pseudogene 7 | 0.334938 |
| *CHAC1* | ChaC, cation transport regulator homolog 1 (E. coli) | 0.332567 |
| *FAM227B* | family with sequence similarity 227, member B | 0.332222 |
| *ALDH2* | aldehyde dehydrogenase 2 family (mitochondrial) | 0.331961 |
| *SLC6A9* | solute carrier family 6 (neurotransmitter transporter, glycine), member 9 | 0.330724 |
| *MIR186* | microRNA 186 | 0.328755 |
| *ZNF763* | zinc finger protein 763 | 0.328666 |
| *HIST1H2AJ* | histone cluster 1, H2aj | 0.327899 |
| *RNU5D-1* | RNA, U5D small nuclear 1 | 0.323913 |
| *ALOX5AP* | arachidonate 5-lipoxygenase-activating protein | 0.32156 |
| *FBXO32* | F-box protein 32 | 0.32125 |
| *ZC3H6* | zinc finger CCCH-type containing 6 | 0.316641 |
| *GPT2* | glutamic pyruvate transaminase (alanine aminotransferase) 2 | 0.314711 |

| GENE_SYMBOL | GENE_TITLE | SCORE |
|---|---|---|
| *COLGALT2* | collagen beta(1-O)galactosyltransferase 2 | 0.314565 |
| *WEE2-AS1* | WEE2 antisense RNA 1 | 0.314304 |
| *PPM1M* | protein phosphatase 1M (PP2C domain containing) | 0.313921 |
| *SNORA31* | small nucleolar RNA, H/ACA box 31 | 0.313363 |
| *C11orf74* | chromosome 11 open reading frame 74 | 0.313307 |
| *ZMAT1* | zinc finger, matrin type 1 | 0.313217 |
| *RND3* | Rho family GTPase 3 | 0.309211 |
| *CLGN* | calmegin | 0.305442 |
| *UGT1A10* | UDP glucuronosyltransferase 1 family, polypeptide A10 | 0.30265 |
| *CIR1* | corepressor interacting with RBPJ | 0.301674 |
| *CXorf22* | chromosome X open reading frame 22 | 0.299363 |
| *ALDH1L2* | aldehyde dehydrogenase 1 family, member L2 | 0.298943 |
| *RBM11* | RNA binding motif protein 11 | 0.298281 |
| *SNORD116-13* | small nucleolar RNA, C/D box 116-13 | 0.298082 |
| *TTLL1* | tubulin tyrosine ligase-like family, member 1 | 0.297329 |
| *CEBPB* | CCAAT/enhancer binding protein (C/EBP), beta | 0.296077 |
| *C2orf27A* | chromosome 2 open reading frame 27A | 0.295393 |
| *FAM129A* | family with sequence similarity 129, member A | 0.286598 |
| *KIAA1257* | KIAA1257 | 0.285531 |
| *LOC644717* | sarcoma antigen 2, pseudogene | 0.285277 |
| *AGMO* | alkylglycerol monooxygenase | 0.280998 |
| *PSAT1* | phosphoserine aminotransferase 1 | 0.278228 |
| *THAP9-AS1* | THAP9 antisense RNA 1 | 0.277759 |
| *SMPX* | small muscle protein, X-linked | 0.277239 |
| *COBLL1* | COBL-like 1 | 0.272505 |
| *WNT5A* | wingless-type MMTV integration site family, member 5A | 0.271617 |
| *MIR29A* | microRNA 29a | 0.271519 |
| *CBX4* | chromobox homolog 4 (Pc class homolog, Drosophila) | 0.270723 |
| *MT2A* | metallothionein 2A | 0.267114 |
| *ATF3* | activating transcription factor 3 | 0.26646 |
| *PDLIM3* | PDZ and LIM domain 3 | 0.265137 |
| *LYPLA1* | lysophospholipase I | 0.263954 |
| *PRH2* | proline-rich protein HaeIII subfamily 2 | 0.2622 |
| *SMIM14* | small integral membrane protein 14 | 0.261547 |
| *SCN4A* | sodium channel, voltage-gated, type IV, alpha | 0.260615 |
| *PEX5L* | peroxisomal biogenesis factor 5-like | 0.260427 |
| *RAB39B* | RAB39B, member RAS oncogene family | 0.259919 |
| *BEST1* | bestrophin 1 | 0.259136 |
| *MIR2682* | microRNA 2682 | 0.259071 |
| *LOC339803* | hypothetical protein LOC339803 | 0.25466 |
| *MAP1LC3B2* | microtubule-associated protein 1 light chain 3 beta 2 | 0.254636 |
| *FAAH2* | fatty acid amide hydrolase 2 | 0.254589 |
| *RNU5B-1* | RNA, U5B small nuclear 1 | 0.25144 |
| *ZNF117* | zinc finger protein 117 | 0.251039 |
| *SCARNA9* | small Cajal body-specific RNA 9 | 0.250541 |
| *NR5A2* | nuclear receptor subfamily 5, group A, member 2 | 0.249632 |
| *CHDC2* | calponin homology domain containing 2 | 0.249293 |
| *CCPG1* | cell cycle progression 1 | 0.248701 |
| *ZNF674* | zinc finger protein 674 | 0.248269 |
| *PIP5KL1* | phosphatidylinositol-4-phosphate 5-kinase-like 1 | 0.248112 |
| *INPP1* | inositol polyphosphate-1-phosphatase | 0.247267 |
| *IFRD1* | interferon-related developmental regulator 1 | 0.245181 |
| *LYPLAL1* | lysophospholipase-like 1 | 0.245028 |
| *KIF21B* | kinesin family member 21B | 0.244496 |
| *LRRC37A3* | leucine rich repeat containing 37, member A3 | 0.244306 |
| *TBC1D3P2* | TBC1 domain family, member 3 pseudogene 2 | 0.243629 |
| *OPN1LW* | opsin 1 (cone pigments), long-wave-sensitive (color blindness, protan) | 0.243267 |

| *GENE_SYMBOL* | *GENE_TITLE* | *SCORE* |
|---|---|---|
| *PCDHA1* | protocadherin alpha 1 | 0.243149 |
| *IGHV1-45* | immunoglobulin heavy variable 1-45 | 0.241021 |
| *ANKRD31* | ankyrin repeat domain 31 | 0.240202 |
| *KIF27* | kinesin family member 27 | 0.239263 |
| *OR2M3* | olfactory receptor, family 2, subfamily M, member 3 | 0.238097 |
| *MT1X* | metallothionein 1X | 0.237609 |
| *COX7B* | cytochrome c oxidase subunit VIIb | 0.23747 |
| *ZNF429* | zinc finger protein 429 | 0.237151 |
| *MBNL2* | muscleblind-like 2 (Drosophila) | 0.235401 |
| *ACSM3* | acyl-CoA synthetase medium-chain family member 3 | 0.2354 |
| *CYP19A1* | cytochrome P450, family 19, subfamily A, polypeptide 1 | 0.234959 |
| *SLC1A4* | solute carrier family 1 (glutamate/neutral amino acid transporter), member 4 | 0.233497 |
| *TP53* | tumor protein p53 (Li-Fraumeni syndrome) | 0.233082 |
| *RGMB* | RGM domain family, member B | 0.229311 |
| *SNORD116-6* | small nucleolar RNA, C/D box 116-6 | 0.226827 |
| *ARHGAP10* | Rho GTPase activating protein 10 | 0.226407 |
| *MST1* | macrophage stimulating 1 (hepatocyte growth factor-like) | 0.226056 |
| *RFPL4AL1* | ret finger protein-like 4A-like 1 | 0.225059 |
| *FAM45B* | family with sequence similarity 45, member B | 0.224498 |
| *ERO1LB* | ERO1-like beta (S. cerevisiae) | 0.223822 |
| *FBXO48* | F-box protein 48 | 0.223482 |
| *C5orf28* | chromosome 5 open reading frame 28 | 0.22115 |
| *DDR2* | discoidin domain receptor family, member 2 | 0.22074 |
| *TNFRSF10B* | tumor necrosis factor receptor superfamily, member 10b | 0.220335 |
| *AMY2B* | amylase, alpha 2B (pancreatic) | 0.219811 |
| *SNORD116-26* | small nucleolar RNA, C/D box 116-26 | 0.217503 |
| *TRNASUP1* | transfer RNA suppressor 1 (anticodon UUA) | 0.217355 |
| *PBLD* | phenazine biosynthesis-like protein domain containing | 0.217007 |
| *IGHV1-69* | immunoglobulin heavy variable 1-69 | 0.215382 |
| *IRAK1BP1* | interleukin-1 receptor-associated kinase 1 binding protein 1 | 0.214788 |
| *ARHGAP9* | Rho GTPase activating protein 9 | 0.214722 |
| *PHGDH* | phosphoglycerate dehydrogenase | 0.213742 |
| *PYCR1* | pyrroline-5-carboxylate reductase 1 | 0.213522 |
| *SCARNA9L* | small Cajal body-specific RNA 9-like | 0.213237 |
| *DEPTOR* | DEP domain containing MTOR-interacting protein | 0.212935 |
| *BCAT1* | branched chain aminotransferase 1, cytosolic | 0.212422 |
| *SHMT2* | serine hydroxymethyltransferase 2 (mitochondrial) | 0.211524 |
| *ALPK2* | alpha-kinase 2 | 0.211106 |

**Supplementary Table 5B: Description of the 150 genes at the bottom of the ranked list in GSEA analysis of U266 treated *versus* control cells**

| *GENE_SYMBOL* | *GENE_TITLE* | *SCORE* |
|---|---|---|
| *OR4M1* | olfactory receptor, family 4, subfamily M, member 1 | -0.18006 |
| *PRLR* | prolactin receptor | -0.18045 |
| *FAM19A1* | family with sequence similarity 19 (chemokine (C-C motif)-like), member A1 | -0.18057 |
| *TNFSF13* | tumor necrosis factor (ligand) superfamily, member 13 | -0.18059 |
| *TUBA3E* | tubulin, alpha 3e | -0.18091 |
| *FOS* | v-fos FBJ murine osteosarcoma viral oncogene homolog | -0.181 |
| *MIR146A* | microRNA 146a | -0.18117 |
| *EPGN* | epithelial mitogen homolog (mouse) | -0.18137 |
| *SNORD27* | small nucleolar RNA, C/D box 27 | -0.18153 |
| *LOC339524* | pothetical LOC339524 | -0.18153 |
| *OR7D4* | olfactory receptor, family 7, subfamily D, member 4 | -0.18161 |
| *IL5RA* | interleukin 5 receptor, alpha | -0.18194 |
| *SERPINB3* | serpin peptidase inhibitor, clade B (ovalbumin), member 3 | -0.18204 |
| *OR7E5P* | olfactory receptor, family 7, subfamily E, member 5 pseudogene | -0.1823 |
| *WBP4* | WW domain binding protein 4 (formin binding protein 21) | -0.18311 |
| *STARD4* | START domain containing 4, sterol regulated | -0.18332 |
| *VAV3* | vav 3 oncogene | -0.18398 |
| *S100A10* | S100 calcium binding protein A10 | -0.18437 |
| *SNORD115-32* | small nucleolar RNA, C/D box 115-32 | -0.18475 |
| *MIR101-2* | microRNA 101-2 | -0.1848 |
| *C4BPB* | complement component 4 binding protein, beta | -0.18487 |
| *CNN2* | calponin 2 | -0.18517 |
| *OR2J3* | olfactory receptor, family 2, subfamily J, member 3 | -0.18566 |
| *MPV17L* | MPV17 mitochondrial membrane protein-like | -0.18629 |
| *SMPDL3A* | sphingomyelin phosphodiesterase, acid-like 3A | -0.18643 |
| *ZNF492* | zinc finger protein 492 | -0.18661 |
| *IGHV3-35* | immunoglobulin heavy variable 3-35 | -0.18676 |
| *OR3A1* | olfactory receptor, family 3, subfamily A, member 1 | -0.18715 |
| *CYP2B7P1* | cytochrome P450, family 2, subfamily B, polypeptide 7 pseudogene 1 | -0.18722 |
| *SQLE* | squalene epoxidase | -0.1877 |
| *CYP4A22* | cytochrome P450, family 4, subfamily A, polypeptide 22 | -0.18797 |
| *PDIA3* | protein disulfide isomerase family A, member 3 | -0.18818 |
| *OR13J1* | olfactory receptor, family 13, subfamily J, member 1 | -0.18828 |
| *SAA2* | serum amyloid A2 | -0.18866 |
| *OR56A3* | olfactory receptor, family 56, subfamily A, member 3 | -0.18884 |
| *GAS6* | growth arrest-specific 6 | -0.18925 |
| *PLN* | phospholamban | -0.18931 |
| *C1QTNF9B-AS1* | C1QTNF9B antisense RNA 1 | -0.18973 |
| *LAX1* | lymphocyte transmembrane adaptor 1 | -0.19029 |
| *EGFL6* | EGF-like-domain, multiple 6 | -0.19069 |
| *RBM46* | RNA binding motif protein 46 | -0.19214 |
| *RFPL1* | ret finger protein-like 1 | -0.19246 |
| *SLCO4A1* | solute carrier organic anion transporter family, member 4A1 | -0.19335 |
| *BTN2A3P* | butyrophilin, subfamily 2, member A3, pseudogene | -0.19382 |
| *EFCAB2* | EF-hand calcium binding domain 2 | -0.19434 |
| *GYPB* | glycophorin B (MNS blood group) | -0.19494 |
| *KRTAP19-8* | keratin associated protein 19-8 | -0.19593 |
| *PBK* | PDZ binding kinase | -0.19827 |
| *IGHV3-33* | immunoglobulin heavy variable 3-33 | -0.19842 |
| *HSPH1* | heat shock 105kDa/110kDa protein 1 | -0.19858 |
| *MIR377* | microRNA 377 | -0.19874 |
| *PRICKLE1* | prickle homolog 1 (Drosophila) | -0.19891 |
| *ZNF300P1* | zinc finger protein 300 pseudogene 1 (functional) | -0.1992 |
| *CDC25A* | cell division cycle 25A | -0.20011 |
| *POU2F2* | POU domain, class 2, transcription factor 2 | -0.20045 |

| GENE_SYMBOL | GENE_TITLE | SCORE |
|---|---|---|
| *SHCBP1* | SHC SH2-domain binding protein 1 | -0.20052 |
| *TRAV8-3* | T cell receptor alpha variable 8-3 | -0.20052 |
| *PSG10P* | pregnancy specific beta-1-glycoprotein 10, pseudogene | -0.2013 |
| *BCL3* | B-cell CLL/lymphoma 3 | -0.20196 |
| *RPS27A* | ribosomal protein S27a | -0.20228 |
| *SNORA2A* | small nucleolar RNA, H/ACA box 2A | -0.20261 |
| *OR1N1* | olfactory receptor, family 1, subfamily N, member 1 | -0.20264 |
| *OR10G3* | olfactory receptor, family 10, subfamily G, member 3 | -0.20269 |
| *PKP2* | plakophilin 2 | -0.20317 |
| *RNU6-76P* | RNA, U6 small nuclear 76, pseudogene | -0.20332 |
| *PGM2L1* | phosphoglucomutase 2-like 1 | -0.20358 |
| *DTX3L* | deltex 3-like (Drosophila) | -0.20393 |
| *IFIT1* | interferon-induced protein with tetratricopeptide repeats 1 | -0.20488 |
| *GOLGA8DP* | golgin A8 family, member D, pseudogene | -0.20604 |
| *NETO1* | neuropilin (NRP) and tolloid (TLL)-like 1 | -0.20608 |
| *ADAM21* | ADAM metallopeptidase domain 21 | -0.2074 |
| *STON1-GTF2A1L* | STON1-GTF2A1L readthrough | -0.2077 |
| *PTP4A3* | protein tyrosine phosphatase type IVA, member 3 | -0.20809 |
| *MIR17HG* | microRNA host gene 1 (non-protein coding) | -0.20824 |
| *PCDH11Y* | protocadherin 11 Y-linked | -0.2088 |
| *FAM172BP* | family with sequence similarity 172, member B, pseudogene | -0.21178 |
| *HMGCR* | 3-hydroxy-3-methylglutaryl-Coenzyme A reductase | -0.212 |
| *MIR224* | microRNA 224 | -0.2121 |
| *LOC101929115* | uncharacterized LOC101929115 | -0.21248 |
| *TMEM75* | transmembrane protein 75 | -0.21334 |
| *PPT2-EGFL8* | PPT2-EGFL8 readthrough (NMD candidate) | -0.21366 |
| *SPRED2* | sprouty-related, EVH1 domain containing 2 | -0.21369 |
| *KLRC2* | killer cell lectin-like receptor subfamily C, member 2 | -0.21392 |
| *MIR103A2* | microRNA 103a2 | -0.21466 |
| *LOC730102* | hypothetical protein LOC730102 | -0.21508 |
| *TLR7* | toll-like receptor 7 | -0.21603 |
| *FASN* | fatty acid synthase | -0.21608 |
| *MSMO1* | methylsterol monooxygenase 1 | -0.21786 |
| *OR2T29* | olfactory receptor, family 2, subfamily T, member 29 | -0.2184 |
| *TMPRSS11B* | transmembrane protease, serine 11B | -0.22077 |
| *RPL13A* | ribosomal protein L13a | -0.22087 |
| *EGR1* | early growth response 1 | -0.22169 |
| *C3orf14* | chromosome 3 open reading frame 14 | -0.22297 |
| *SLA* | Src-like-adaptor | -0.2233 |
| *UGP2* | UDP-glucose pyrophosphorylase 2 | -0.22551 |
| *BCL6* | B-cell CLL/lymphoma 6 (zinc finger protein 51) | -0.22575 |
| *IGKV4-1* | immunoglobulin kappa variable 4-1 | -0.22598 |
| *E2F2* | E2F transcription factor 2 | -0.23065 |
| *LOC100288637* | OTU deubiquitinase 7A pseudogene | -0.23075 |
| *KRTAP21-1* | keratin associated protein 21-1 | -0.23221 |
| *SPANXN1* | SPANX family, member N1 | -0.23226 |
| *UGT2B28* | UDP glucuronosyltransferase 2 family, polypeptide B28 | -0.23258 |
| *KRTAP5-4* | keratin associated protein 5-4 | -0.23337 |
| *ACOXL* | acyl-Coenzyme A oxidase-like | -0.23394 |
| *PLA2G10* | phospholipase A2, group X | -0.23428 |
| *LDLR* | low density lipoprotein receptor (familial hypercholesterolemia) | -0.23852 |
| *C9orf153* | chromosome 9 open reading frame 153 | -0.24111 |
| *TPRX1* | tetra-peptide repeat homeobox 1 | -0.24176 |
| *NAIP* | NLR family, apoptosis inhibitory protein | -0.24236 |
| *AKAP5* | A kinase (PRKA) anchor protein 5 | -0.24382 |
| *HMGCS1* | 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (soluble) | -0.25189 |
| *MYH8* | myosin, heavy chain 8, skeletal muscle, perinatal | -0.2531 |

| *GENE_SYMBOL* | *GENE_TITLE* | *SCORE* |
|---|---|---|
| *PSG1* | pregnancy specific beta-1-glycoprotein 1 | -0.25377 |
| *ZNF718* | zinc finger protein 718 | -0.26188 |
| *MROH7* | maestro heat-like repeat family member 7 | -0.26352 |
| *LBH* | limb bud and heart development homolog (mouse) | -0.26527 |
| *ID3* | inhibitor of DNA binding 3, dominant negative helix-loop-helix protein | -0.26606 |
| *PRDM9* | PR domain containing 9 | -0.2697 |
| *DUSP6* | dual specificity phosphatase 6 | -0.27505 |
| *ZFP36* | zinc finger protein 36, C3H type, homolog (mouse) | -0.27713 |
| *IL6* | interleukin 6 (interferon, beta 2) | -0.28043 |
| *RNU6-23P* | RNA, U6 small nuclear 23, pseudogene | -0.28877 |
| *TBC1D3B* | TBC1 domain family, member 3B | -0.29116 |
| *ETV4* | ets variant gene 4 (E1A enhancer binding protein, E1AF) | -0.29659 |
| *MYOT* | myotilin | -0.29938 |
| *OCM2* | oncomodulin 2 | -0.31006 |
| *IDI1* | isopentenyl-diphosphate delta isomerase 1 | -0.31097 |
| *SPRED1* | sprouty-related, EVH1 domain containing 1 | -0.31603 |
| *IFNA7* | interferon, alpha 7 | -0.31626 |
| *MIR142* | microRNA 142 | -0.31715 |
| *DHCR24* | 24-dehydrocholesterol reductase | -0.31717 |
| *PRR23A* | proline rich 23A | -0.33145 |
| *TDGF1* | teratocarcinoma-derived growth factor 1 | -0.33705 |
| *INSIG1* | insulin induced gene 1 | -0.34427 |
| *GAGE1* | G antigen 1 | -0.34578 |
| *LOC286359* | hypothetical LOC286359 | -0.35466 |
| *ETV5* | ets variant gene 5 (ets-related molecule) | -0.38229 |
| *IFNA10* | interferon, alpha 10 | -0.38231 |
| *ARL2-SNX15* | ARL2-SNX15 readthrough (NMD candidate) | -0.38978 |
| *PARP9* | poly (ADP-ribose) polymerase family, member 9 | -0.39203 |
| *ZNF578* | zinc finger protein 578 | -0.40315 |
| *TRIM51* | tripartite motif-containing 51 | -0.40741 |
| *CCR1* | chemokine (C-C motif) receptor 1 | -0.40874 |
| *TRAV5* | T cell receptor alpha variable 5 | -0.41448 |
| *OR13C2* | olfactory receptor, family 13, subfamily C, member 2 | -0.42899 |
| *CD180* | CD180 molecule | -0.43024 |
| *MIR148A* | microRNA 148a | -0.43208 |
| *TRAV17* | T cell receptor alpha variable 17 | -0.46508 |
| *VTRNA1-1* | vault RNA 1-1 | -0.47056 |
| *GPRC5D* | G protein-coupled receptor, family C, group 5, member D | -0.5071 |

**Supplementary Table 6A: Gene sets enriched in U266 control cells**

| *NAME* | *NES* | *FDR q-value* |
|---|---|---|
| ROSTY_CERVICAL_CANCER_PROLIFERATION_CLUSTER | -2.50567 | 0 |
| KOBAYASHI_EGFR_SIGNALING_24HR_DN | -2.47026 | 0 |
| DUTERTRE_ESTRADIOL_RESPONSE_24HR_UP | -2.45821 | 0 |
| CROONQUIST_NRAS_SIGNALING_DN | -2.43549 | 0 |
| CROONQUIST_IL6_DEPRIVATION_DN | -2.42036 | 0 |
| GRAHAM_NORMAL_QUIESCENT_VS_NORMAL_DIVIDING_DN | -2.41937 | 0 |
| ZHANG_TLX_TARGETS_UP | -2.41358 | 0 |
| LEE_EARLY_T_LYMPHOCYTE_UP | -2.39146 | 0 |
| ZHAN_MULTIPLE_MYELOMA_PR_UP | -2.37026 | 0 |
| CHANG_CYCLING_GENES | -2.3691 | 0 |
| SCHMIDT_POR_TARGETS_IN_LIMB_BUD_UP | -2.36034 | 0 |
| SOTIRIOU_BREAST_CANCER_GRADE_1_VS_3_UP | -2.35916 | 0 |
| KANG_DOXORUBICIN_RESISTANCE_UP | -2.35159 | 0 |
| KONG_E2F3_TARGETS | -2.23723 | 4.46E-05 |
| GOBERT_OLIGODENDROCYTE_DIFFERENTIATION_UP | -2.24137 | 4.67E-05 |
| MANALO_HYPOXIA_DN | -2.24976 | 4.89E-05 |
| BENPORATH_ES_2 | -2.25888 | 5.13E-05 |
| REACTOME_CHOLESTEROL_BIOSYNTHESIS | -2.26001 | 5.40E-05 |
| GSE15750_DAY6_VS_DAY10_EFF_CD8_TCELL_UP | -2.26123 | 5.70E-05 |
| ISHIDA_E2F_TARGETS | -2.26645 | 6.04E-05 |
| FURUKAWA_DUSP6_TARGETS_PCI35_DN | -2.31819 | 6.41E-05 |
| VERNELL_RETINOBLASTOMA_PATHWAY_UP | -2.33317 | 6.84E-05 |
| ZHOU_CELL_CYCLE_GENES_IN_IR_RESPONSE_24HR | -2.33854 | 7.33E-05 |
| WINNEPENNINCKX_MELANOMA_METASTASIS_UP | -2.22891 | 8.12E-05 |
| BLUM_RESPONSE_TO_SALIRASIB_DN | -2.23351 | 8.46E-05 |
| ZHOU_CELL_CYCLE_GENES_IN_IR_RESPONSE_6HR | -2.20068 | 9.85E-05 |
| HORTON_SREBF_TARGETS | -2.2023 | 1.02E-04 |
| GOLDRATH_EFF_VS_MEMORY_CD8_TCELL_UP | -2.20359 | 1.05E-04 |
| ODONNELL_TFRC_TARGETS_DN | -2.21489 | 1.09E-04 |
| SARRIO_EPITHELIAL_MESENCHYMAL_TRANSITION_UP | -2.21638 | 1.13E-04 |
| ZHANG_TLX_TARGETS_60HR_DN | -2.22135 | 1.17E-04 |
| ODONNELL_TARGETS_OF_MYC_AND_TFRC_DN | -2.18752 | 1.20E-04 |
| WANG_RESPONSE_TO_GSK3_INHIBITOR_SB216763_DN | -2.18812 | 1.23E-04 |
| GRAHAM_CML_DIVIDING_VS_NORMAL_QUIESCENT_UP | -2.18997 | 1.27E-04 |
| MITSIADES_RESPONSE_TO_APLIDIN_DN | -2.17531 | 1.75E-04 |
| CHIANG_LIVER_CANCER_SUBCLASS_PROLIFERATION_UP | -2.16301 | 2.27E-04 |
| KEGG_STEROID_BIOSYNTHESIS | -2.12567 | 2.63E-04 |
| HOFFMANN_LARGE_TO_SMALL_PRE_BII_LYMPHOCYTE_UP | -2.12669 | 2.69E-04 |
| BURTON_ADIPOGENESIS_3 | -2.14326 | 2.77E-04 |
| AMUNDSON_GAMMA_RADIATION_RESPONSE | -2.11155 | 3.49E-04 |
| GSE15750_DAY6_VS_DAY10_TRAF6KO_EFF_CD8_TCELL_UP | -2.11248 | 3.58E-04 |
| PODAR_RESPONSE_TO_ADAPHOSTIN_DN | -2.10595 | 4.15E-04 |
| ZHANG_TLX_TARGETS_36HR_DN | -2.10456 | 4.29E-04 |
| CAFFAREL_RESPONSE_TO_THC_DN | -2.08356 | 9.54E-04 |
| BENPORATH_PROLIFERATION | -2.08173 | 9.56E-04 |
| FUJII_YBX1_TARGETS_DN | -2.07129 | 0.001047 |
| M_PHASE_OF_MITOTIC_CELL_CYCLE | -2.06083 | 0.001171 |
| GSE30962_PRIMARY_VS_SECONDARY_ACUTE_LCMV_INF_CD8_TCELL_UP | -2.06335 | 0.001177 |

| *NAME* | *NES* | *FDR q-value* |
|---|---|---|
| LY_AGING_OLD_DN | -2.05885 | 0.001186 |
| KEGG_TERPENOID_BACKBONE_BIOSYNTHESIS | -2.05962 | 0.001189 |
| YU_MYC_TARGETS_UP | -2.06141 | 0.001195 |
| TIEN_INTESTINE_PROBIOTICS_24HR_UP | -2.05638 | 0.001202 |
| MARKEY_RB1_CHRONIC_LOF_UP | -2.0486 | 0.001329 |
| MITOSIS | -2.04949 | 0.001335 |
| MARKEY_RB1_ACUTE_LOF_DN | -2.0437 | 0.001337 |
| EGUCHI_CELL_CYCLE_RB1_TARGETS | -2.0454 | 0.001342 |
| REACTOME_DNA_REPLICATION | -2.04318 | 0.001349 |
| PENG_LEUCINE_DEPRIVATION_DN | -2.04199 | 0.001396 |
| GARGALOVIC_RESPONSE_TO_OXIDIZED_PHOSPHOLIPIDS_TURQUOISE_DN | -2.03984 | 0.001425 |
| LY_AGING_PREMATURE_DN | -2.03722 | 0.001453 |
| M_PHASE | -2.0264 | 0.001719 |
| KAMMINGA_EZH2_TARGETS | -2.02676 | 0.001731 |
| PENG_GLUTAMINE_DEPRIVATION_DN | -2.0262 | 0.00174 |
| FINETTI_BREAST_CANCER_KINOME_RED | -2.01831 | 0.001986 |
| REACTOME_G1_S_SPECIFIC_TRANSCRIPTION | -2.01672 | 0.002018 |
| GARY_CD5_TARGETS_DN | -2.00731 | 0.002346 |
| CROONQUIST_NRAS_VS_STROMAL_STIMULATION_DN | -2.00023 | 0.002594 |
| PID_FOXM1PATHWAY | -2.00028 | 0.002632 |
| WILCOX_PRESPONSE_TO_ROGESTERONE_UP | -1.99054 | 0.00315 |
| LIU_IL13_PRIMING_MODEL | -1.98765 | 0.00334 |
| SMIRNOV_RESPONSE_TO_IR_6HR_DN | -1.98641 | 0.003409 |
| MISSIAGLIA_REGULATED_BY_METHYLATION_DN | -1.98407 | 0.003505 |
| MOLENAAR_TARGETS_OF_CCND1_AND_CDK4_DN | -1.98123 | 0.003668 |
| HORIUCHI_WTAP_TARGETS_DN | -1.9723 | 0.0042 |
| REACTOME_MITOTIC_M_M_G1_PHASES | -1.97297 | 0.004201 |
| WHITEFORD_PEDIATRIC_CANCER_MARKERS | -1.9688 | 0.004428 |
| REGULATION_OF_MITOSIS | -1.96696 | 0.004504 |
| LI_WILMS_TUMOR_ANAPLASTIC_UP | -1.96585 | 0.004538 |
| INACTIVATION_OF_MAPK_ACTIVITY | -1.96465 | 0.004572 |
| AFFAR_YY1_TARGETS_DN | -1.96296 | 0.004656 |
| GSE10239_NAIVE_VS_DAY4.5_EFF_CD8_TCELL_DN | -1.95866 | 0.004966 |
| IKEDA_MIR133_TARGETS_UP | -1.9544 | 0.005281 |
| MORI_LARGE_PRE_BII_LYMPHOCYTE_UP | -1.95038 | 0.005527 |
| REACTOME_CELL_CYCLE_MITOTIC | -1.94704 | 0.005724 |
| PUJANA_XPRSS_INT_NETWORK | -1.94754 | 0.005756 |
| SHEDDEN_LUNG_CANCER_POOR_SURVIVAL_A6 | -1.94109 | 0.006255 |
| REACTOME_MITOTIC_PROMETAPHASE | -1.93893 | 0.006489 |
| MYELOID_LEUKOCYTE_DIFFERENTIATION | -1.93734 | 0.006574 |
| PUJANA_BRCA_CENTERED_NETWORK | -1.93737 | 0.006637 |
| FOURNIER_ACINAR_DEVELOPMENT_LATE_2 | -1.9316 | 0.007106 |
| MUELLER_PLURINET | -1.92509 | 0.007672 |
| KAUFFMANN_MELANOMA_RELAPSE_UP | -1.92513 | 0.007756 |
| REICHERT_MITOSIS_LIN9_TARGETS | -1.92548 | 0.007796 |
| PID_PLK1_PATHWAY | -1.91885 | 0.008409 |
| TARTE_PLASMA_CELL_VS_PLASMABLAST_DN | -1.91183 | 0.009402 |
| GSE24634_TEFF_VS_TCONV_DAY7_IN_CULTURE_UP | -1.90901 | 0.009785 |
| VECCHI_GASTRIC_CANCER_EARLY_UP | -1.90755 | 0.009959 |

| *NAME* | *NES* | *FDR q-value* |
|---|---|---|
| AIYAR_COBRA1_TARGETS_DN | -1.90127 | 0.010795 |
| CHIARADONNA_NEOPLASTIC_TRANSFORMATION_KRAS_UP | -1.90134 | 0.010894 |
| REACTOME_GLUCURONIDATION | -1.89377 | 0.011913 |
| WHITFIELD_CELL_CYCLE_LITERATURE | -1.89411 | 0.011991 |
| FERREIRA_EWINGS_SARCOMA_UNSTABLE_VS_STABLE_UP | -1.88504 | 0.013656 |
| CHROMOSOME | -1.87943 | 0.01473 |
| REACTOME_E2F_MEDIATED_REGULATION_OF_DNA_REPLICATION | -1.87878 | 0.014736 |
| V$E2F_Q6_01 | -1.87675 | 0.014958 |
| WU_APOPTOSIS_BY_CDKN1A_VIA_TP53 | -1.86938 | 0.016366 |
| V$E2F_Q4 | -1.86613 | 0.017122 |
| GSE36476_CTRL_VS_TSST_ACT_72H_MEMORY_CD4_TCELL_YOUNG_DN | -1.86473 | 0.017343 |
| V$E2F_Q6 | -1.86231 | 0.017795 |
| JACKSON_DNMT1_TARGETS_UP | -1.86236 | 0.017958 |
| V$E2F1_Q3 | -1.86101 | 0.018107 |
| OXFORD_RALA_OR_RALB_TARGETS_UP | -1.85882 | 0.018468 |
| GAL_LEUKEMIC_STEM_CELL_DN | -1.85771 | 0.01855 |
| REACTOME_OLFACTORY_SIGNALING_PATHWAY | -1.85671 | 0.018677 |
| CELL_CYCLE_PHASE | -1.85472 | 0.019103 |
| GARGALOVIC_RESPONSE_TO_OXIDIZED_PHOSPHOLIPIDS_GREEN_UP | -1.85159 | 0.019629 |
| MARSON_BOUND_BY_E2F4_UNSTIMULATED | -1.84778 | 0.020681 |
| PUJANA_BRCA2_PCC_NETWORK | -1.84141 | 0.022741 |
| MIKKELSEN_PLURIPOTENT_STATE_UP | -1.83779 | 0.023408 |
| GSE3982_MAC_VS_TH1_DN | -1.83602 | 0.023412 |
| REN_BOUND_BY_E2F | -1.83629 | 0.023444 |
| WEST_ADRENOCORTICAL_TUMOR_MARKERS_UP | -1.83803 | 0.023501 |
| BERENJENO_TRANSFORMED_BY_RHOA_UP | -1.83856 | 0.023603 |
| ONDER_CDH1_TARGETS_1_DN | -1.83643 | 0.023619 |
| SGCGSSAAA_V$E2F1DP2_01 | -1.83459 | 0.023783 |
| SHEPARD_BMYB_TARGETS | -1.83406 | 0.023806 |
| TURASHVILI_BREAST_DUCTAL_CARCINOMA_VS_LOBULAR_NORMAL_UP | -1.83344 | 0.023828 |
| GSE24634_TREG_VS_TCONV_POST_DAY7_IL4_CONVERSION_UP | -1.8322 | 0.024116 |
| SENGUPTA_NASOPHARYNGEAL_CARCINOMA_UP | -1.83052 | 0.024494 |
| FRASOR_RESPONSE_TO_SERM_OR_FULVESTRANT_DN | -1.82939 | 0.024668 |
| DAUER_STAT3_TARGETS_DN | -1.82871 | 0.024769 |
| NADERI_BREAST_CANCER_PROGNOSIS_UP | -1.82703 | 0.025243 |
| V$E2F1_Q6 | -1.82417 | 0.025902 |
| CHROMOSOMAL_PART | -1.82366 | 0.025946 |
| PUJANA_BREAST_CANCER_WITH_BRCA1_MUTATED_UP | -1.82271 | 0.026028 |
| BURTON_ADIPOGENESIS_PEAK_AT_24HR | -1.82124 | 0.026395 |
| LU_TUMOR_VASCULATURE_UP | -1.81981 | 0.026758 |
| ZHAN_MULTIPLE_MYELOMA_CD2_DN | -1.81788 | 0.027279 |
| V$E2F1DP1RB_01 | -1.81724 | 0.027341 |
| GSE29614_CTRL_VS_DAY7_TIV_FLU_VACCINE_PBMC_DN | -1.8156 | 0.027703 |
| MORI_IMMATURE_B_LYMPHOCYTE_DN | -1.80745 | 0.030029 |
| NEGATIVE_REGULATION_OF_CYTOKINE_BIOSYNTHETIC_PROCESS | -1.80788 | 0.030096 |
| BIOCARTA_NKT_PATHWAY | -1.80845 | 0.030105 |
| MCDOWELL_ACUTE_LUNG_INJURY_UP | -1.80845 | 0.030319 |
| SISTER_CHROMATID_SEGREGATION | -1.806 | 0.030501 |
| LOPEZ_MESOTELIOMA_SURVIVAL_TIME_UP | -1.80246 | 0.031804 |

| *NAME* | *NES* | *FDR q-value* |
|---|---|---|
| GSE29614_DAY3_VS_DAY7_TIV_FLU_VACCINE_PBMC_DN | -1.80131 | 0.031832 |
| GSE22886_UNSTIM_VS_IL2_STIM_NKCELL_DN | -1.80164 | 0.031902 |
| TANG_SENESCENCE_TP53_TARGETS_DN | -1.79979 | 0.032238 |
| LY_AGING_MIDDLE_DN | -1.79848 | 0.03228 |
| V$E2F4DP1_01 | -1.79876 | 0.032331 |
| GAVIN_FOXP3_TARGETS_CLUSTER_P6 | -1.7971 | 0.032743 |
| RUIZ_TNC_TARGETS_DN | -1.79579 | 0.033173 |
| NEGATIVE_REGULATION_OF_MAP_KINASE_ACTIVITY | -1.79467 | 0.033194 |
| BARIS_THYROID_CANCER_DN | -1.79476 | 0.033356 |
| GSE13485_DAY3_VS_DAY7_YF17D_VACCINE_PBMC_DN | -1.79296 | 0.033658 |
| WEI_MYCN_TARGETS_WITH_E_BOX | -1.79224 | 0.033784 |
| CELLULAR_DEFENSE_RESPONSE | -1.79139 | 0.034031 |
| CASORELLI_ACUTE_PROMYELOCYTIC_LEUKEMIA_DN | -1.78855 | 0.035011 |
| CELL_CYCLE_CHECKPOINT_GO_0000075 | -1.78712 | 0.03553 |
| CHASSOT_SKIN_WOUND | -1.78383 | 0.036878 |
| XU_HGF_SIGNALING_NOT_VIA_AKT1_48HR_DN | -1.78179 | 0.037432 |
| SIMBULAN_UV_RESPONSE_NORMAL_DN | -1.7818 | 0.037657 |
| BASAKI_YBX1_TARGETS_UP | -1.7801 | 0.037975 |
| CERIBELLI_PROMOTERS_INACTIVE_AND_BOUND_BY_NFY | -1.77865 | 0.038379 |
| GSE36476_CTRL_VS_TSST_ACT_40H_MEMORY_CD4_TCELL_YOUNG_DN | -1.77775 | 0.038655 |
| GSE3982_EFF_MEMORY_CD4_TCELL_VS_TH1_DN | -1.77369 | 0.039578 |
| PID_AURORA_B_PATHWAY | -1.77544 | 0.039641 |
| BENPORATH_CYCLING_GENES | -1.77397 | 0.03966 |
| ROVERSI_GLIOMA_LOH_REGIONS | -1.77476 | 0.039747 |
| GSE10239_NAIVE_VS_KLRG1INT_EFF_CD8_TCELL_DN | -1.77415 | 0.039803 |
| MITOTIC_CELL_CYCLE | -1.77165 | 0.040045 |
| CHROMOSOMEPERICENTRIC_REGION | -1.77209 | 0.040051 |
| KORKOLA_TERATOMA | -1.77079 | 0.040263 |
| KEGG_OLFACTORY_TRANSDUCTION | -1.77005 | 0.04039 |
| SONG_TARGETS_OF_IE86_CMV_PROTEIN | -1.76934 | 0.040568 |
| GSE30962_ACUTE_VS_CHRONIC_LCMV_SECONDARY_INF_CD8_TCELL_DN | -1.76059 | 0.044475 |
| GSE1460_INTRATHYMIC_T_PROGENITOR_VS_NAIVE_CD4_TCELL_ADULT_BLOOD_UP | -1.7614 | 0.044513 |
| V$E2F_02 | -1.7609 | 0.044558 |
| WONG_ENDMETRIUM_CANCER_UP | -1.75917 | 0.044947 |
| MITOTIC_SISTER_CHROMATID_SEGREGATION | -1.75507 | 0.04706 |
| TURASHVILI_BREAST_DUCTAL_CARCINOMA_VS_DUCTAL_NORMAL_UP | -1.75334 | 0.047908 |
| LU_TUMOR_ENDOTHELIAL_MARKERS_UP | -1.75275 | 0.048044 |
| KUROZUMI_RESPONSE_TO_ONCOCYTIC_VIRUS_AND_CYCLIC_RGD | -1.75083 | 0.048362 |
| GCNP_SHH_UP_LATE.V1_UP | -1.75104 | 0.048485 |
| MALIK_REPRESSED_BY_ESTROGEN | -1.75139 | 0.048525 |
| REACTOME_PROCESSING_OF_CAPPED_INTRON_CONTAINING_PRE_MRNA | -1.74938 | 0.048894 |
| V$E2F1DP1_01 | -1.74752 | 0.049639 |

Gene sets are ordered according to FDR *q*-value. NES (normalized enrichment score) is also reported.

**Supplementary Table 6B: Gene sets enriched in U266 treated cells**

| *NAME* | *NES* | *FDR q-value* |
|---|---|---|
| GARGALOVIC_RESPONSE_TO_OXIDIZED_PHOSPHOLIPIDS_RED_UP | 2.351516 | 0 |
| REACTOME_AMINO_ACID_SYNTHESIS_AND_INTERCONVERSION_TRANSAMINATION | 2.37104 | 0 |
| KRIGE_RESPONSE_TO_TOSEDOSTAT_6HR_UP | 2.459397 | 0 |
| MTOR_UP.N4.V1_UP | 2.516844 | 0 |
| PENG_LEUCINE_DEPRIVATION_UP | 2.638843 | 0 |
| KRIGE_RESPONSE_TO_TOSEDOSTAT_24HR_UP | 2.659443 | 0 |
| BHATI_G2M_ARREST_BY_2METHOXYESTRADIOL_DN | 2.666824 | 0 |
| TIEN_INTESTINE_PROBIOTICS_24HR_DN | 2.67889 | 0 |
| PODAR_RESPONSE_TO_ADAPHOSTIN_UP | 2.694749 | 0 |
| PACHER_TARGETS_OF_IGF1_AND_IGF2_UP | 2.754331 | 0 |
| BLUM_RESPONSE_TO_SALIRASIB_UP | 2.762486 | 0 |
| ZHAN_MULTIPLE_MYELOMA_CD1_UP | 2.7971 | 0 |
| HELLER_SILENCED_BY_METHYLATION_DN | 2.81202 | 0 |
| ZHAN_MULTIPLE_MYELOMA_CD1_VS_CD2_UP | 2.872037 | 0 |
| KRIGE_AMINO_ACID_DEPRIVATION | 2.896582 | 0 |
| KEGG_GLYCINE_SERINE_AND_THREONINE_METABOLISM | 2.273052 | 3.01E-04 |
| AMUNDSON_RESPONSE_TO_ARSENITE | 2.241046 | 4.81E-04 |
| TERAMOTO_OPN_TARGETS_CLUSTER_7 | 2.241159 | 5.09E-04 |
| ONDER_CDH1_TARGETS_1_UP | 2.22951 | 5.58E-04 |
| WANG_HCP_PROSTATE_CANCER | 2.19506 | 0.0012 |
| FORTSCHEGGER_PHF8_TARGETS_UP | 2.175743 | 0.00169 |
| ALK_DN.V1_UP | 2.171733 | 0.001789 |
| KASLER_HDAC7_TARGETS_2_DN | 2.157696 | 0.002089 |
| PRAMOONJAGO_SOX4_TARGETS_UP | 2.144733 | 0.002684 |
| KAN_RESPONSE_TO_ARSENIC_TRIOXIDE | 2.126259 | 0.003574 |
| BMI1_DN_MEL18_DN.V1_UP | 2.108819 | 0.004551 |
| REACTOME_AMINO_ACID_TRANSPORT_ACROSS_THE_PLASMA_MEMBRANE | 2.089989 | 0.006055 |
| NEUTRAL_AMINO_ACID_TRANSMEMBRANE_TRANSPORTER_ACTIVITY | 2.079356 | 0.006766 |
| SCIBETTA_KDM5B_TARGETS_UP | 2.075699 | 0.006995 |
| XU_HGF_SIGNALING_NOT_VIA_AKT1_48HR_UP | 2.064639 | 0.007916 |
| IGARASHI_ATF4_TARGETS_DN | 2.061486 | 0.008095 |
| CONCANNON_APOPTOSIS_BY_EPOXOMICIN_UP | 2.047539 | 0.009946 |
| CHO_NR4A1_TARGETS | 2.041657 | 0.010372 |
| PARK_OSTEOBLAST_DIFFERENTIATION_BY_PHENYLAMIL_UP | 2.019906 | 0.013799 |
| NOJIMA_SFRP2_TARGETS_UP | 2.008785 | 0.015458 |
| HORIUCHI_WTAP_TARGETS_UP | 2.009873 | 0.015707 |
| SMITH_TERT_TARGETS_UP | 2.005849 | 0.015714 |
| FERRARI_RESPONSE_TO_FENRETINIDE_UP | 1.992631 | 0.018693 |
| MAHADEVAN_RESPONSE_TO_MP470_DN | 1.964147 | 0.027304 |
| HANN_RESISTANCE_TO_BCL2_INHIBITOR_DN | 1.954325 | 0.030298 |
| HUANG_FOXA2_TARGETS_UP | 1.947281 | 0.032153 |
| BOYAULT_LIVER_CANCER_SUBCLASS_G123_DN | 1.948409 | 0.032375 |
| KEGG_SELENOAMINO_ACID_METABOLISM | 1.941641 | 0.033749 |
| WANG_RESPONSE_TO_GSK3_INHIBITOR_SB216763_UP | 1.939409 | 0.033834 |
| GSE24634_IL4_VS_CTRL_TREATED_NAIVE_CD4_TCELL_DAY7_DN | 1.932102 | 0.036692 |
| HELLER_HDAC_TARGETS_SILENCED_BY_METHYLATION_DN | 1.92893 | 0.037313 |
| SASSON_FSH_RESPONSE | 1.904675 | 0.04931 |

Gene sets are ordered according to FDR *q*-value. NES (normalized enrichment score) is also reported.

**Supplementary Table 7: Clinical details of the 167 patients analyzed by NGS**

| *Sample* | *Sex* | *Age* | *Disease* | *Stage*[Δ] | *Phase*[‡] | *PP*[†] | *t(4;14)* | *t(11;14)* | *t(14;16)* | *t(14;20)* | *del(13)** | *del(17p)** | *1q gain** | *1p loss** | *HD°* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MM-004 | F | 58 | MM | IA | D | Gκ | - | - | + | - | - | na | na | na | na |
| MM-015 | M | 71 | MM | IIA | D | Gκ | - | + | - | - | - | - | - | - | - |
| MM-016 | M | 66 | MM | IIIB | D | Gκ | - | - | - | - | - | - | + | - | + |
| MM-026 | F | 72 | MM | IIIB | D | κ | - | + | - | - | - | - | - | na | na |
| MM-027 | M | 60 | MM | IA | D | Gκ | - | - | - | - | + | - | na | na | na |
| MM-030 | M | 69 | MM | IIIA | D | Gλ | - | - | - | - | - | - | - | + | + |
| MM-031 | M | 58 | MM | IIIA | D | Aκ | - | + | - | - | - | - | + | + | - |
| MM-034 | M | 71 | MM | IA | D | Gκ | - | - | - | - | - | - | - | - | + |
| MM-036 | M | 65 | MM | IIA | D | Gκ | - | - | - | - | + | - | + | na | - |
| MM-037 | F | 50 | MM | IIA | D | Gκ+Aκ | - | + | - | - | + | - | - | - | - |
| MM-038 | F | 67 | MM | IIA | D | κ | - | - | - | - | + | - | - | - | + |
| MM-039 | M | 50 | MM | IIA | D | Gλ | - | - | - | - | - | - | - | na | + |
| MM-042 | M | 54 | MM | IIIA | D | Aλ | + | - | - | - | + | - | + | - | + |
| MM-043 | F | 73 | MM | IA | D | Gκ+Gλ | - | - | - | - | - | - | + | - | - |
| MM-049 | M | 62 | MM | IIIB | D | κ | - | - | - | - | - | - | - | - | + |
| MM-055 | F | 69 | MM | IIIA | D | Gκ | - | + | - | - | - | - | - | na | - |
| MM-066 | F | 77 | MM | IIIA | D | Aκ | + | - | - | - | - | - | - | na | - |
| MM-069 | M | 63 | MM | IIA | D | Gκ | - | - | - | + | + | - | + | na | - |
| MM-078 | F | 59 | MM | IIIA | D | κ | - | - | - | - | + | - | - | na | + |
| MM-079 | F | 74 | MM | IIA | D | Gκ+Gλ | - | - | - | - | - | - | - | - | + |
| MM-087 | F | 84 | MM | IIIA | D | Gλ | + | - | - | - | + | - | + | na | - |
| MM-115 | F | 53 | MM | IIIA | D | Gλ | - | + | - | - | + | - | na | na | - |
| MM-123 | M | 55 | MM | IIIA | D | Gκ | + | - | - | - | + | - | + | - | - |
| MM-131 | M | 73 | MM | IA | D | Gκ | - | - | - | - | - | - | - | - | - |
| MM-140 | M | 62 | MM | IIIB | D | Gκ | - | + | - | - | - | - | - | na | - |
| MM-143 | M | 61 | MM | IIA | D | Gκ | - | - | - | - | - | - | + | - | + |
| MM-146 | M | 68 | MM | IIA | D | Gλ | - | - | - | - | - | - | + | na | + |
| MM-148 | F | 55 | MM | IA | D | Aκ | - | - | - | - | - | + | + | na | + |
| MM-149 | F | 52 | MM | IA | D | Gλ | - | - | - | - | - | - | - | - | + |
| MM-150 | F | 68 | MM | IIA | D | Gλ | - | - | - | - | - | - | + | - | + |
| MM-151 | F | 71 | MM | IA | D | Gλ | - | - | - | - | - | - | - | - | + |
| MM-152 | M | 66 | MM | IA | D | Gκ | - | - | - | - | - | - | - | na | + |

| *Sample* | *Sex* | *Age* | *Disease* | *Stage*[Δ] | *Phase*[‡] | *PP*[†] | *t(4;14)* | *t(11;14)* | *t(14;16)* | *t(14;20)* | *del(13)** | *del(17p)** | *1q gain** | *1p loss** | *HD°* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MM-154 | F | 71 | MM | IIA | D | Gκ | - | - | + | - | + | - | + | - | - |
| MM-159 | M | 56 | MM | IIA | D | κ | - | + | - | - | + | - | - | - | - |
| MM-174 | M | 85 | MM | IIA | D | Aκ | - | - | - | - | - | - | - | - | + |
| MM-177 | M | 73 | MM | IIIA | D | Gκ | - | - | - | - | + | - | + | - | - |
| MM-179 | M | 50 | MM | IIIA | D | Gλ | - | + | - | - | - | + | - | - | + |
| MM-195 | M | 62 | MM | IIA | D | Gκ | + | - | - | - | + | - | - | - | - |
| MM-200 | F | 63 | MM | IA | D | Aλ | - | - | - | - | - | - | - | - | + |
| MM-202 | M | 64 | MM | IIIA | D | Gκ | - | - | - | - | - | - | + | na | + |
| MM-206 | F | 73 | MM | IIA | D | Gκ | + | - | - | - | + | - | - | + | - |
| MM-207 | F | 68 | MM | IA | D | Aκ | - | - | - | - | + | - | + | - | - |
| MM-208 | M | 74 | MM | IIA | D | Aλ | + | - | - | - | - | - | - | - | - |
| MM-209 | F | 65 | MM | IIA | D | Gλ | - | - | - | - | - | - | - | - | + |
| MM-210 | M | 65 | MM | IIB | D | Aλ | - | - | - | - | - | - | - | - | - |
| MM-212 | F | 55 | MM | IIIA | D | Gκ | - | + | - | - | - | - | - | - | - |
| MM-213 | M | 66 | MM | IIIA | D | λ | - | + | - | - | + | - | - | - | - |
| MM-219 | M | 73 | MM | IIIA | D | Aλ | - | - | - | - | + | - | - | - | + |
| MM-224 | F | 52 | MM | IIIA | D | Gκ | - | - | + | - | - | - | + | - | - |
| MM-229 | M | 75 | MM | IIA | D | Gκ | - | - | - | - | - | - | - | - | - |
| MM-238 | M | 58 | MM | IIB | D | Gκ | - | - | - | - | + | - | + | - | - |
| MM-239 | F | 72 | MM | IIA | D | Aκ | - | - | - | - | + | - | - | - | + |
| MM-240 | M | 70 | MM | IA | D | Gλ | - | - | - | - | - | - | - | - | + |
| MM-241 | M | 54 | MM | IIA | D | Gκ | - | - | - | - | - | - | - | - | + |
| MM-242 | M | 69 | MM | IIIA | D | Aκ | - | - | - | - | + | - | - | - | + |
| MM-243 | F | 68 | MM | IIA | D | Gκ | - | - | - | - | - | - | + | - | + |
| MM-246 | M | 71 | MM | IIIA | D | Aλ | - | + | - | - | - | - | - | - | - |
| MM-252 | F | 77 | MM | IIA | D | Aλ | - | + | - | - | - | - | + | - | - |
| MM-253 | F | 45 | MM | IIA | D | λ | - | - | - | - | + | - | + | - | + |
| MM-256 | M | 58 | MM | IIIA | D | Aκ | - | - | + | - | + | - | - | - | - |
| MM-261 | F | 66 | MM | IIB | D | Gκ | - | - | - | - | + | - | + | - | + |
| MM-262 | M | 74 | MM | IA | D | λ | - | - | - | - | - | - | + | - | - |
| MM-263 | F | 65 | MM | IIB | D | κ | + | - | - | - | + | - | + | - | - |
| MM-267 | M | 74 | MM | IIIA | D | Gλ | - | - | - | - | + | - | - | - | + |
| MM-268 | M | 77 | MM | IIIB | D | Gκ | - | - | - | - | - | - | - | - | - |

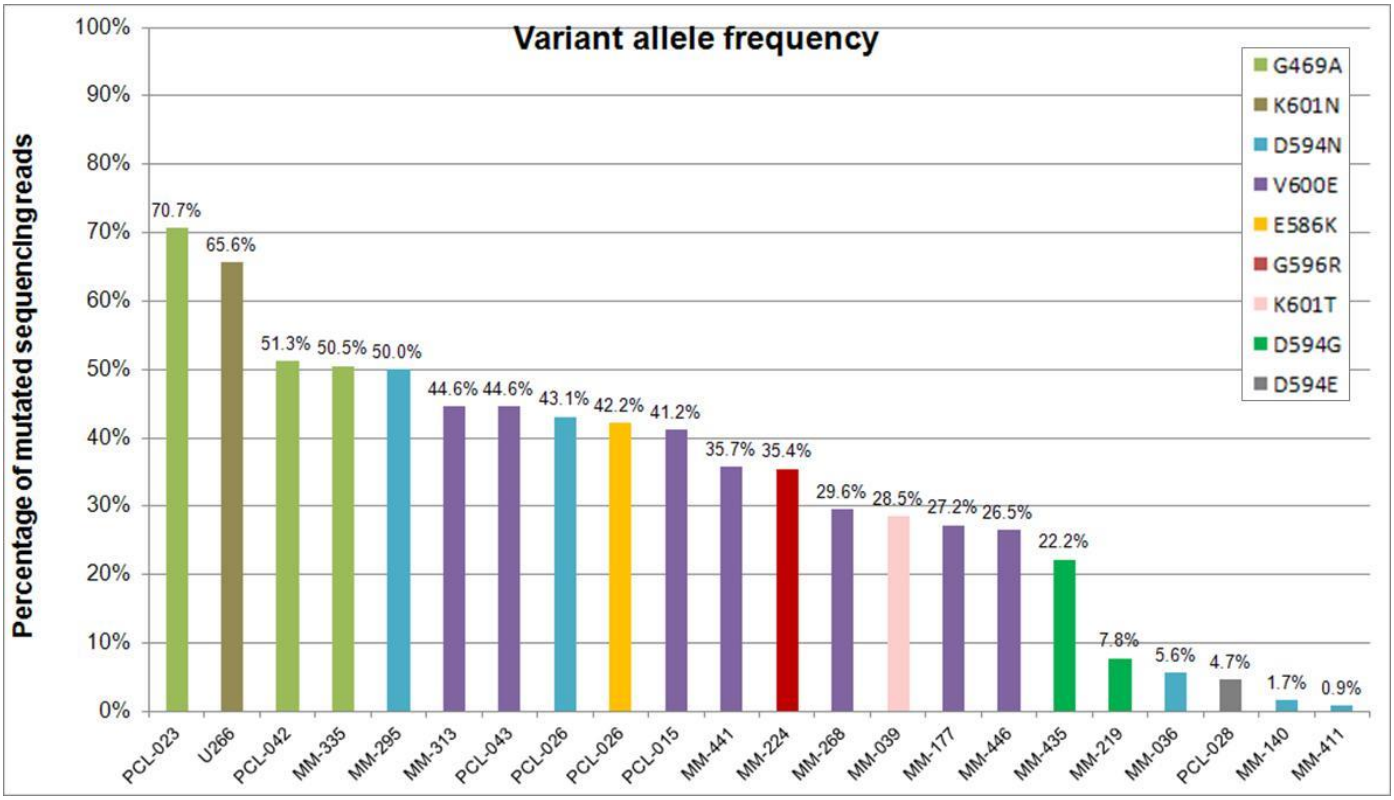
| *Sample* | *Sex* | *Age* | *Disease* | *Stage*[Δ] | *Phase*[‡] | *PP*[†] | *t(4;14)* | *t(11;14)* | *t(14;16)* | *t(14;20)* | *del(13)** | *del(17p)** | *1q gain** | *1p loss** | *HD°* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MM-269 | F | 67 | MM | IIA | D | Gκ | - | - | - | - | + | - | - | - | + |
| MM-271 | M | 76 | MM | IA | D | Gκ | - | - | - | - | + | - | - | - | - |
| MM-274 | M | 59 | MM | IIA | D | Gκ | + | - | - | - | - | - | - | - | - |
| MM-276 | F | 70 | MM | IIB | D | Gκ | + | - | - | - | + | - | - | - | - |
| MM-278 | M | 73 | MM | IIA | D | Gλ | - | - | - | - | - | - | - | + | + |
| MM-279 | F | 71 | MM | IIIA | D | Gλ | - | - | - | na | + | - | + | - | na |
| MM-280 | M | 62 | MM | IIIA | D | Gλ | - | + | - | - | - | - | - | - | - |
| MM-281 | F | 77 | MM | IIA | D | Gκ | - | - | - | - | - | - | - | - | - |
| MM-282 | M | 66 | MM | IIIA | D | Gκ | - | - | - | - | - | - | + | - | + |
| MM-284 | M | 49 | MM | IIIA | D | κ | - | + | - | - | na | - | - | - | - |
| MM-286 | F | 71 | MM | IIA | D | Gκ | - | - | - | - | + | - | - | - | - |
| MM-295 | F | 74 | MM | IIIA | D | λ | - | - | - | - | + | - | na | na | na |
| MM-300 | M | 65 | MM | IIB | D | Gκ | - | - | - | - | - | - | na | na | na |
| MM-301 | F | 72 | MM | IIA | D | Aλ | - | - | - | - | - | - | + | - | + |
| MM-302 | M | 65 | MM | IA | D | Aκ | - | - | - | - | - | - | + | - | + |
| MM-308 | M | 58 | MM | IA | D | Aκ | - | - | - | - | - | - | - | - | - |
| MM-313 | M | 66 | MM | IIIA | D | Gλ | - | + | - | - | - | - | - | - | - |
| MM-310 | M | 67 | MM | IIIA | D | Gλ | - | + | - | - | - | - | - | - | - |
| MM-314 | F | 70 | MM | IIIA | D | Aλ | - | + | - | - | - | - | + | - | - |
| MM-317 | M | 56 | MM | IIA | D | Gλ | - | + | - | - | - | - | + | - | - |
| MM-321 | M | 63 | MM | IIIA | D | Gκ | - | - | - | - | + | - | - | - | + |
| MM-327 | F | 62 | MM | IIA | D | absent | - | - | - | - | + | - | + | - | + |
| MM-330 | F | 61 | MM | IIIA | D | Aκ | + | - | - | - | + | - | + | - | - |
| MM-334 | F | 45 | MM | IA | D | Gλ | - | - | - | - | + | + | + | + | + |
| MM-335 | F | 68 | MM | IIA | D | Aκ | - | - | + | - | + | - | - | - | - |
| MM-340 | M | 46 | MM | IIIA | D | Gλ | - | + | - | - | + | - | - | - | - |
| MM-341 | M | 65 | MM | IIIA | D | Gκ | - | - | - | - | - | - | + | - | - |
| MM-343 | M | 74 | MM | IIIA | D | Aλ | - | + | - | - | - | - | - | - | - |
| MM-351 | M | na | MM | IIA | D | Aκ | - | - | - | - | - | - | - | - | - |
| MM-362 | F | 80 | MM | IIA | D | Gλ | - | - | - | - | + | + | + | - | - |
| MM-372 | M | 54 | MM | IA | D | Gκ | - | + | - | - | - | - | + | - | - |
| MM-375 | F | 78 | MM | IA | D | Gκ | + | - | - | - | - | + | - | - | - |
| MM-381 | F | 69 | MM | IA | D | Gκ | - | - | - | na | - | - | - | - | na |

| *Sample* | *Sex* | *Age* | *Disease* | *Stage*[Δ] | *Phase*[‡] | *PP*[†] | *t(4;14)* | *t(11;14)* | *t(14;16)* | *t(14;20)* | *del(13)** | *del(17p)** | *1q gain** | *1p loss** | *HD°* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MM-382 | M | 85 | MM | IIA | D | Gκ | - | - | - | - | + | + | na | na | na |
| MM-385 | F | 76 | MM | IIIA | D | Gλ | - | - | - | - | - | - | - | - | + |
| MM-386 | F | 76 | MM | IIA | D | Aλ | - | na | na | na | - | - | na | na | na |
| MM-387 | F | 44 | MM | IIIA | D | Gκ | - | - | - | - | - | - | + | - | + |
| MM-392 | F | 53 | MM | IA | D | Gλ | - | - | - | - | + | - | + | - | + |
| MM-398 | F | 65 | MM | IIA | D | Gκ | - | + | - | - | + | - | + | - | - |
| MM-402 | M | 67 | MM | IIA | D | Gκ | - | + | - | - | + | - | - | - | - |
| MM-405 | M | 69 | MM | IA | D | Gκ | - | + | - | - | - | - | - | - | - |
| MM-406 | M | 62 | MM | IIB | D | Aκ | - | + | - | - | - | - | + | - | na |
| MM-407 | F | 79 | MM | IIIA | D | λ | - | - | - | - | + | - | - | - | - |
| MM-410 | F | 79 | MM | IIIA | D | λ | - | - | - | - | - | - | + | - | - |
| MM-411 | M | 67 | MM | IIIA | D | Gλ | - | - | - | - | - | - | - | - | + |
| MM-413 | F | 51 | MM | IIA | D | Gκ | - | - | - | - | + | - | + | - | + |
| MM-414 | F | 67 | MM | IIIA | D | Gλ | - | + | - | - | - | - | - | - | - |
| MM-422 | F | 74 | MM | IIB | D | κ | - | - | - | - | + | - | - | - | - |
| MM-423 | F | 53 | MM | IIA | D | λ | + | - | - | - | + | - | - | - | - |
| MM-424 | F | 84 | MM | IA | D | Gκ | - | - | - | - | + | - | - | + | + |
| MM-425 | F | 65 | MM | IIA | D | Aλ | - | - | - | - | + | - | + | - | - |
| MM-428 | F | 65 | MM | IIA | D | Aλ | - | - | - | - | - | - | + | + | - |
| MM-429 | F | 63 | MM | IIIA | D | Gκ | - | - | - | - | - | - | + | - | + |
| MM-430 | na | 62 | MM | IIIA | D | Gλ | - | - | - | - | - | - | - | - | - |
| MM-431 | F | 70 | MM | IA | D | Gλ | + | - | - | - | + | - | + | - | - |
| MM-433 | M | 72 | MM | IIB | D | κ | - | + | - | - | - | - | - | - | - |
| MM-434 | F | 53 | MM | IIA | D | κ | - | - | - | - | + | - | - | + | - |
| MM-435 | na | 42 | MM | IIIA | D | Gλ | - | - | - | - | - | - | - | - | + |
| MM-437 | F | 72 | MM | IIB | D | λ | - | - | - | - | + | - | + | - | - |
| MM-440 | na | 61 | MM | IIA | D | Aκ | - | - | - | - | - | - | - | + | + |
| MM-441 | na | na | MM | IA | D | Gλ | - | - | - | - | - | - | - | - | - |
| MM-442 | F | 65 | sPCL | / | D | Gκ | - | - | - | - | + | - | + | + | - |
| MM-445 | na | 65 | MM | IIA | D | κ | - | + | - | - | + | - | + | - | - |
| MM-446 | na | 52 | MM | IIIA | D | Gλ | - | - | - | - | - | - | + | - | + |
| MM-447 | na | 57 | MM | IIA | D | Gκ | - | + | - | - | - | - | + | - | - |
| MM-448 | na | 59 | MM | IIA | D | Aκ | - | - | - | - | + | - | + | - | - |

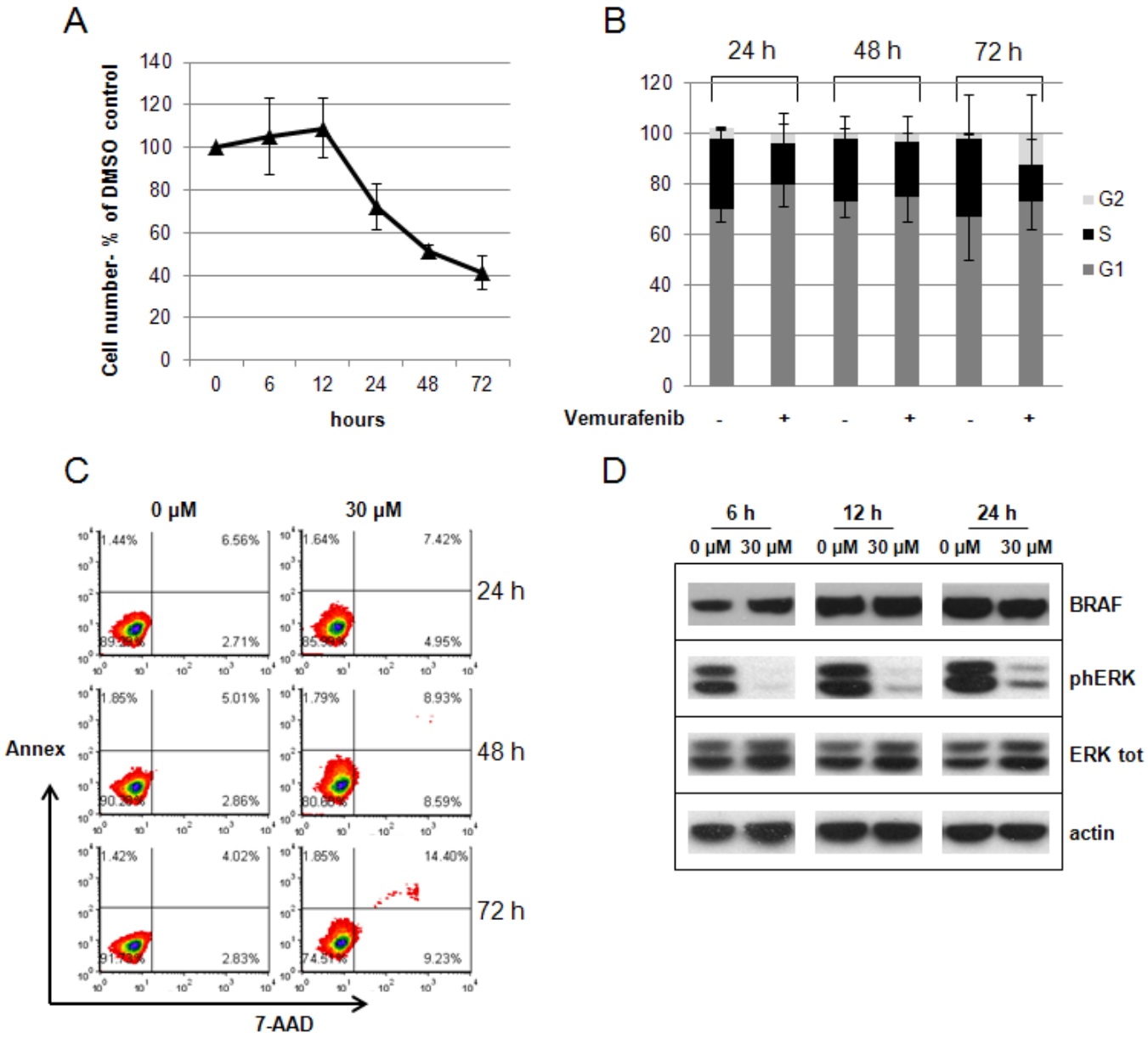
| *Sample* | *Sex* | *Age* | *Disease* | *Stage*[Δ] | *Phase*[‡] | *PP*[†] | *t(4;14)* | *t(11;14)* | *t(14;16)* | *t(14;20)* | *del(13)** | *del(17p)** | *1q gain** | *1p loss** | *HD°* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MM-449 | na | 54 | MM | IIA | D | Gκ | - | - | - | - | + | - | - | + | - |
| MM-464 | M | 75 | MM | IIIA | D | Gλ | - | - | - | - | + | - | + | - | - |
| PCL-001 | F | 51 | pPCL | / | D | κ | - | - | - | + | + | - | na | na | na |
| PCL-002 | F | 69 | sPCL | / | R | λ | - | + | - | - | + | na | - | na | na |
| PCL-004 | M | 72 | pPCL | / | D | Gκ | - | - | + | - | - | - | - | - | - |
| PCL-005 | M | 76 | sPCL | / | R | Aκ | - | + | - | - | - | - | - | - | - |
| PCL-007 | M | 65 | sPCL | / | R | κ | - | - | - | - | + | + | + | na | na |
| PCL-008 | M | 57 | pPCL | / | D | Gλ | - | - | + | - | + | - | - | na | na |
| PCL-009 | F | 77 | sPCL | / | R | Gκ | + | - | - | - | + | - | + | - | - |
| PCL-011 | M | 76 | sPCL | / | R | Gκ | - | - | + | - | + | + | + | - | - |
| PCL-012 | F | 62 | sPCL | / | R | Aκ | - | - | + | - | - | + | - | - | - |
| PCL-014 | M | 72 | pPCL | / | D | λ | - | + | - | - | + | + | - | + | - |
| PCL-015 | M | 78 | pPCL | / | D | κ | - | - | + | - | + | - | + | - | - |
| PCL-016 | F | 57 | pPCL | / | D | Gκ | - | - | + | - | + | - | + | + | - |
| PCL-017 | F | 68 | pPCL | / | D | Gκ | - | - | + | - | + | + | + | + | - |
| PCL-018 | F | 59 | pPCL | / | D | κ | - | + | - | - | + | + | - | - | - |
| PCL-019 | F | 67 | pPCL | / | D | Mκ | - | - | + | - | + | + | + | + | - |
| PCL-020 | F | 79 | pPCL | / | D | Gλ | - | - | - | - | - | - | + | - | - |
| PCL-021 | M | 48 | pPCL | / | D | Gλ | + | - | - | - | + | - | + | - | - |
| PCL-023 | M | 60 | pPCL | / | D | Gκ | - | - | + | - | + | + | + | + | - |
| PCL-026 | F | 59 | pPCL | / | D | Gκ | - | - | + | - | + | - | + | - | - |
| PCL-027 | M | 65 | pPCL | / | D | λ | - | + | - | - | - | + | - | - | - |
| PCL-028 | F | 57 | pPCL | / | D | κ | - | + | - | - | + | - | - | - | - |
| PCL-029 | M | 51 | pPCL | / | D | Aλ | - | - | + | - | - | - | - | - | - |
| PCL-030 | F | 52 | pPCL | / | D | κ | - | - | - | + | + | + | - | - | - |
| PCL-031 | F | 59 | sPCL | / | D | Gλ | - | - | + | - | - | + | + | - | - |
| PCL-032 | M | 51 | pPCL | / | D | Gκ | + | - | - | - | + | - | + | - | - |
| PCL-035 | F | 76 | pPCL | / | D | κ | - | + | - | - | - | - | - | - | - |
| PCL-036 | M | 72 | pPCL | / | D | Gκ | - | + | - | - | + | - | - | - | - |
| PCL-037 | M | 72 | pPCL | / | D | Aλ | - | + | - | - | + | - | - | - | - |
| PCL-038 | M | 57 | pPCL | / | D | Gκ | - | - | - | - | + | - | + | + | + |
| PCL-039 | M | 54 | sPCL | / | D | Aκ | - | - | - | - | - | + | - | + | - |
| PCL-041 | M | na | sPCL | / | D | Gκ | - | na | na | na | - | - | na | na | na |

| *Sample* | *Sex* | *Age* | *Disease* | *Stage*[Δ] | *Phase*[‡] | *PP*[†] | *t(4;14)* | *t(11;14)* | *t(14;16)* | *t(14;20)* | *del(13)** | *del(17p)** | *1q gain** | *1p loss** | *HD°* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PCL-042 | F | 69 | sPCL | / | D | Gλ | - | + | - | - | - | - | - | - | - |
| PCL-043 | F | 68 | pPCL | / | D | Gλ | - | + | - | - | - | - | - | - | - |
| PCL-046 | F | 50 | pPCL | / | D | κ | - | - | - | - | + | - | + | - | + |

[Δ]The Durie clinical staging system was adopted; [‡]D: Diagnosis; R: relapse; [†]Paraprotein; *del(13), del(17), 1p loss and 1q gain were determined by FISH.
°HD = presence of the hyperdiploid status on the basis of FISH evaluation criteria.




**Supplementary Figure 1: Percentage of variant *BRAF* sequencing reads on total sequencing reads for the nine non-synonymous somatic mutations identified by NGS analysis.** Horizontal axis: patients are ordered according to decreasing mutation load. Each color represents a different mutation (reported in the legend).

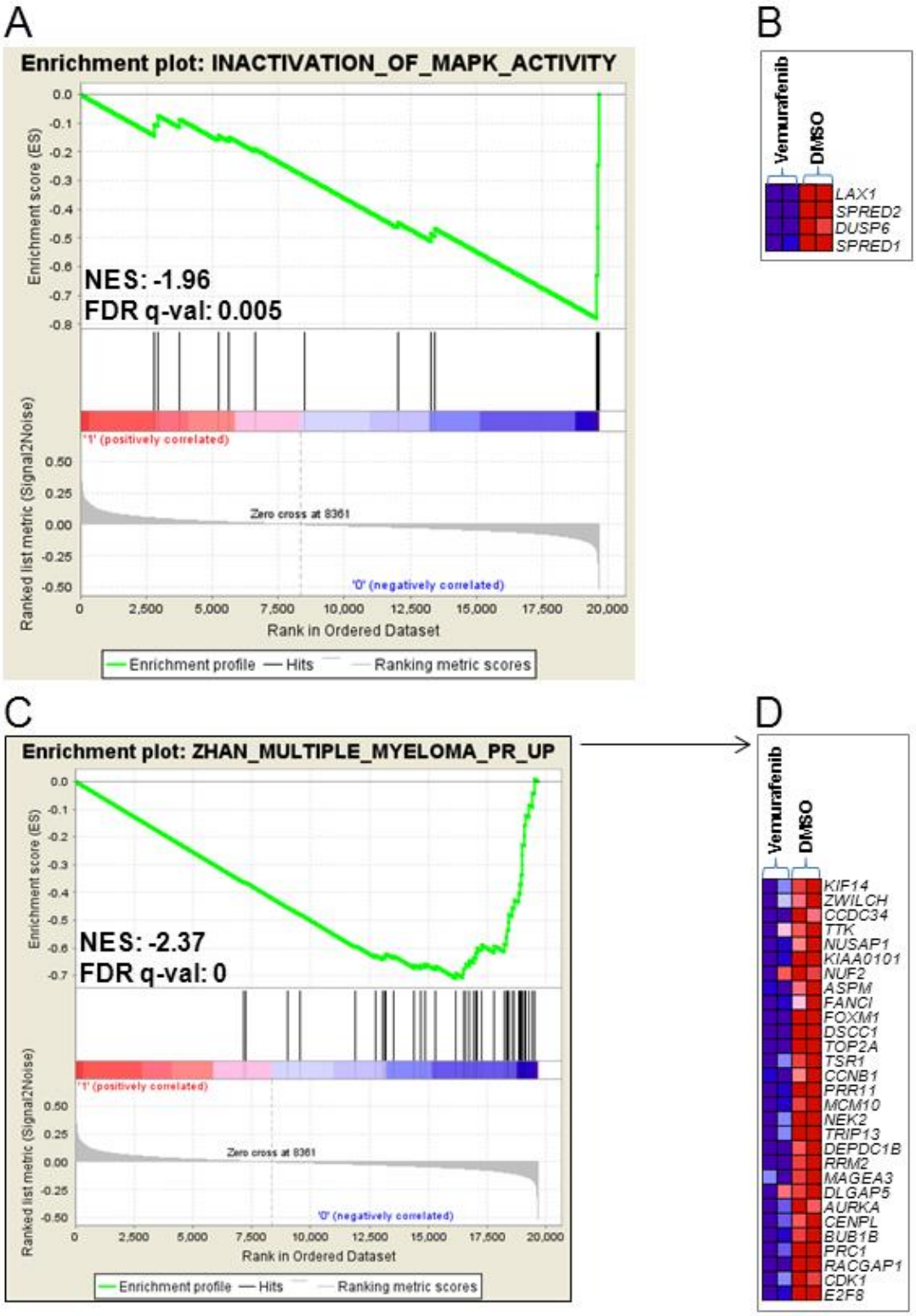



**Supplementary Figure 2: (A) Effect of Vemurafenib on the growth of U266 cells assayed by the trypan blue dye exclusion test.** Cells at a density of $0.5\times10^6$/ml were cultured for 72 h with Vemurafenib at the concentration of 30 µM. The data are presented as the mean±s.e. of three independent experiments.

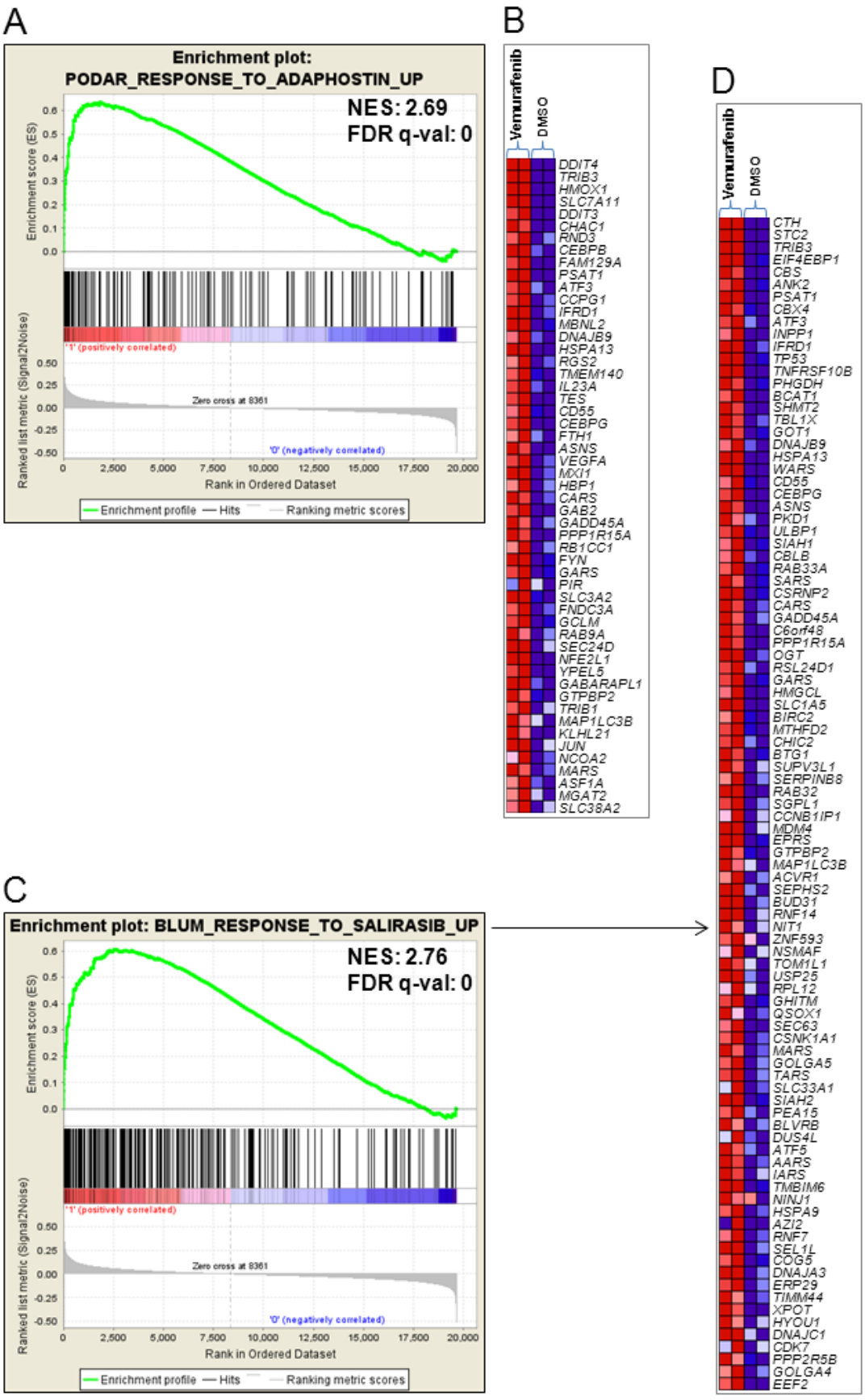
**(B) Bar graph representation of cell cycle of U266 cells treated with DMSO or with 30 µM Vemurafenib for 24, 48, and 72 hours, including standard deviations.** Dark grey are cells in G1 phase, black are cells in S phase, light grey cells in G2 phase. The data are presented as the mean±s.e. of three independent experiments.

**(C) Effect of Vemurafenib on the induction of apoptosis in U266 cells.** Cells were cultured with Vemurafenib at the concentration of 30µM. Cells were stained with FITC-annexin V (AN) and 7-amino-actinomycin D (7AAD) at each time point, and analyzed by flow cytometry. Represented data are from one experiment representative of two independent experiments.

**(D) Western blot analysis of U266 after 6-24 h of treatment with/without 30 µM Vemurafenib.** Cell lysates were analyzed for the expression of BRAF, phospho-ERK1/2 (Thr 202/Tyr 204), and total ERK1/2.




**Supplementary Figure 3:** (**A**, **C**) **GSEA enrichment plots of selected gene sets down-regulated in U266 cells treated with Vemurafenib with respect to DMSO controls.** The green curves show the enrichment score and reflect the degree to which each gene (black vertical lines) is represented at the bottom of the ranked gene list. Gene sets were considered enriched with an FDR <0.05. Normalized enrichment score (NES) and FDR are shown for both represented gene sets. **(B, D) Heat-maps in U266 treated and control cells of the genes constituting the leading edge subsets within the gene sets depicted in panels (A) and (C), respectively.**




**Supplementary Figure 4: (A, C) GSEA enrichment plots of selected gene sets up-regulated in U266 cells treated with Vemurafenib with respect to DMSO controls.** The green curves show the enrichment score and reflect the degree to which each gene (black vertical lines) is represented at the top of the ranked gene list. Gene sets were considered enriched with an FDR <0.05. Normalized enrichment score (NES) and FDR are shown for both

represented gene sets. **(B, D) Heat-maps in U266 treated and control cells of the genes constituting the leading edge subsets within the gene sets depicted in panels (a) and (c), respectively.**