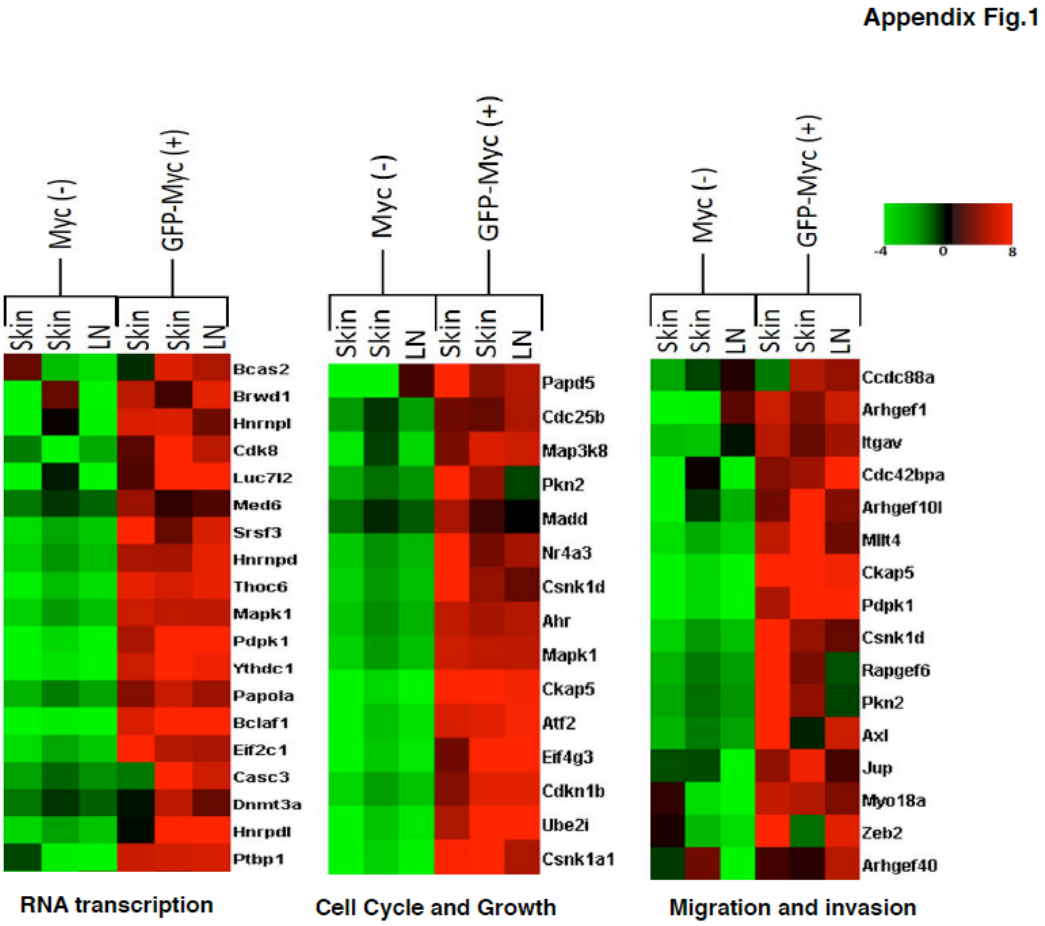
Appendix – table of contents:

Supplementary Material

Appendix Figure 1



Appendix Figure1: c-Myc expression in Tyr::Nras^{Q61K}INK4a$^{-/-}$ melanoma cells induces expression of RNA transcription, growth and migration classes of genes .

Heatmaps of RNA expression patterns comparing changes between GFP-cMyc(+) and GFP-c-Myc(-) cells indicating major alterations in RNA transcription, cell cycle and growth, migration and invasion pathways. Relative expression levels are color coded as indicated (green low expression – red high expression).

Appendix Table S1: Antibodies used

Antibody	Catalog number	Supplier	Application	Fluorochrome
c-Myc (Y69)	ab32072	Abcam	WB, IH	NA
c-Myc	ab11917	Abcam	WB	NA
PDK1	# 3062	Cell Signaling Technology	WB	NA
AMPKβ2	# 4148	Cell Signaling Technology	WB	NA
AMPK β1/2	# 4150	Cell Signaling Technology	WB	NA
Total Acetyl CoA carboxylase (ACC)	#3676	Cell Signaling Technology	WB	NA
phospho-ACC1 (Ser79)	#3661	Cell Signaling Technology	WB	NA
total Raptor	#2280	Cell Signaling Technology	WB	NA
phospho-Raptor (Ser792)	#2083	Cell Signaling Technology	WB	NA
total AMPKα	#2532	Cell Signaling Technology	WB	NA
phospho-AMPKα (Thr 172)	#2535	Cell Signaling Technology	WB	NA
phospho-ULK1 (Ser555)	#5869	Cell Signaling Technology	WB	NA
AMPKα1	07-350	Merck	WB	NA
AMPKα2	07-363	Merck	WB	NA
AMPKγ2	sc-19141	Santa Cruz	WB	NA
AMPKγ1	NA	Dr Kei sakamoto lab	WB	NA
Actin	#A5441	Sigma	WB	NA
α-tubulin	#T6074	Sigma	WB	NA
CD45	17-0451-83	ebioscience	Flow cytometry	APC
CD31	17-0311-82	ebioscience	Flow cytometry	APC
CD271	ab534	Abcam	Flow cytometry	NA
anti-rabbit secondary antibody	711-175-152	Jackson Immunoresearch	Flow cytometry	PE-Cy5

Abbreviations: WB – Western blot; IH – Immunohistochemistry; NA – non applicable

Materials & Methods – Supplemental Section:

Flow cytometry neural crest stem cell marker CD271. Single cell melanocyte suspensions ($5x10^5$ to $1x10^6$ cells) were resuspended in SM and stained with the following antibodies: α-CD45APC (eBioscience) and α-CD31APC (eBioscience). CD271 staining was performed as follows. Cells were in addition with α-CD271 antibody (Abcam) or normal rabbit serum (Invitrogen) followed by α-rabbit PE-Cy5 secondary antibody (Jackson Immunoresearch) for fluorescence detection. Dead cells were excluded using DAPI (Roche). $GFP^{+/-}$ and/or $GFP^{+/-}/CD271^{+/-}$ expression was analyzed on a Dako CyAn Flow Cytometer gated on $CD45^-$ $CD31^-$ $DAPI^-$ cells. Primary skin tumors from Tyr::$NRAS^{Q61K}$INK4a $^{-/-}$ mice were used as a negative control. Data was processed using FlowJo® software. The same staining protocol was used for sorting of $GFP^{+/-}$ and/or $GFP^{+/-}/CD271^{+/-}$ cells using a BC MoFlo $Astrios^{EQ}$ cell sorter. Purity of GFP^+ and GFP^- live cell populations ($CD31^-CD45^-DAPI^-$) were greater than 90%.

RNA-seq. RNA-seq data was mapped (Langmead and Salzberg 2012), mouse genome mm10) and analyzed using the mapping and the RNA-seq modules of HTSstation (http://htsstation.vital-it.ch/). Counts preprocessing and differential analysis was done with the R packages *limma* (Smyth 2004) and *voom* (Law et al. 2014). Genes with an adjusted p-value (Benjamini and Y. 1995) lower than 0.05 and an absolute log2 Fold-change greater than 1.5 were called differentially expressed.

Adenine nucleotide measurement

Sample preparation for adenine nucleotide analysis was based on (Gheldof et al. 2017). In brief, intracellular contents were extracted with hot 75% (v/v) methanol/ultrapure water and supernatants were evaporated to dryness. Samples were reconstituted in 100 µL of a solution of acetonitrile:water 60:40 (v/v). After 20 min centrifugation, 10 µL were transferred to glass vials for hydrophilic interaction ultra high performance liquid chromatography mass spectrometry (UHPLC-MS) analysis (Pluskal et al. 2010), and 5 µL were injected into the system. The UHPLC

consisted of a binary pump, a cooled autosampler, and a column oven (Vanquish UHPLC+ Focused, Thermo Scientific), connected to an orbitrap mass spectrometer (Orbitrap Fusion Lumos Tribrid, Thermo Scientific) equipped with a heated electrospray (H-ESI) source. Samples were injected into a HILIC analytical column (2.1 mm x 100 mm, 5 µm pore size, Merck SeQuant® ZIC®-pHILIC), guarded by a pre-column (2.1 mm x 20 mm, Merk SeQuant® ZIC®-pHILIC Guard Kit) operating at 35 °C. The mobile phase (10 mM ammonium acetate with 0.04% ammonium hydroxide, A, and acetonitrile, B) was pumped at 0.2 mL/min flow rate over a linear gradient of decreasing organic solvent (0.5-16 min, 90-25% B), followed by re-equilibration for a total running time of 30 min. On-the-fly alternating negative (3 kV) and positive (3.5 kV) ion modes was used for ionization and the following source parameters were applied: 20 sheath gas, 15 aux gas, ion transfer tube temperature 310 °C, vaporizer temperature 280 °C. MS acquisition was performed at 60000 orbitrap resolution, in centroid mode, by scanning between the mass range 85 to 850 Da. The software Xcalibur v4.1.31.9 (Thermo Scientific) was used for instrument control, data acquisition and processing. Positive ion mode extracted chromatograms using the corresponding accurate mass of AMP, ADP and ATP were integrated and used for relative comparison. Retention time and mass detection was confirmed by authentic standards.

References

Benjamini Y, Y. H. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society* **B 57**: 289-300.

Gheldof N, Moco S, Chabert C, Teav T, Barron D, Hager J. 2017. Role of sulfotransferases in resveratrol metabolism in human adipocytes. *Molecular nutrition & food research* **61**.

Langmead B, Salzberg SL. 2012. Fast gapped-read alignment with Bowtie 2. *Nature methods* **9**: 357-359.

Law CW, Chen Y, Shi W, Smyth GK. 2014. voom: Precision weights unlock linear model analysis tools for RNA-seq read counts. *Genome biology* **15**: R29.

Pluskal T, Nakamura T, Villar-Briones A, Yanagida M. 2010. Metabolic profiling of the fission yeast S. pombe: quantification of compounds under different temperatures and genetic perturbation. *Molecular bioSystems* **6**: 182-198.

Smyth GK. 2004. Linear models and empirical bayes methods for assessing differential expression in microarray experiments. *Statistical applications in genetics and molecular biology* **3**: Article3.