

Supplemental Material

Supplemental Methods

Key resources

Reagent or Resource	Source	Resource Identifier (RRID) or Cat #
Antibodies		
6-phosphofructokinase/ fructose-2,6-biphosphatase 3	Cell Signalling Technology, Leiden, Netherlands	RRID:AB_2617178
Aldolase (C)	Abcam, Cambridge, U.K.	RRID:AB_2040466
Anti-mouse HRP	Thermo Fisher, Loughborough, U.K.	RRID:AB_933708
Anti-rabbit HRP	Thermo Fisher, Loughborough, U.K.	RRID:AB_933633
Beta actin	Abcam, Cambridge, U.K.	RRID:AB_722536
p-AMPK	Cell Signalling Technology, Leiden, Netherlands	RRID:AB_331250
AMPK	Cell Signalling Technology, Leiden, Netherlands	RRID:AB_490795
CD34-PE	BD Biosciences, U.K.	RRID:AB_400371
CD45-PerCP	Abcam, Cambridge, U.K.	RRID:AB_1139693
Enolase 1	Cell Signalling Technology, Leiden, Netherlands	RRID:AB_2246524
Fructose-1,6-bisphosphatase 1	Abcam, Cambridge, U.K.	RRID:AB_10864942
Glucose transporter 3	Abcam, Cambridge, U.K.	RRID:AB_2736916
Glucose transporter 5	Abcam, Cambridge, U.K.	RRID:AB_732614
Glyceraldehyde-3-phosphate dehydrogenase	Cell Signalling Technology, Leiden, Netherlands	RRID:AB_10622025
hCD33-APC	Biolegend, U.K.	RRID: AB_2565753
hCD45-FITC,	Biolegend, U.K.	RRID: AB_314393
Hexokinase 1	Cell Signalling Technology, Leiden, Netherlands	RRID:AB_2116996
Histone H1	Serotec, Oxford U.K.	RRID:AB_2114841
H-RAS	Santa-Cruz, Heidelberg, Germany	RRID:AB_631670
Hypoxia-inducible factor 1-alpha	BD Biosciences	RRID:AB_398271
Lactate dehydrogenase (A)	Cell Signalling Technology, Leiden, Netherlands	RRID:AB_2137173
mCD45-PerCP-Cy5.5	Biolegend, U.K.	RRID: AB_893340
Monocarboxylate transporter 4	Abcam, Cambridge, U.K.	RRID:AB_2050317
NOX2-PE	MBL Life science Nagoya, Japan,	RRID:AB_591389
N-RAS	Merck Milipore, Feltham, U.K.	RRID: AB_10683217
p38 ^{MAPK}	Cell Signalling Technology, Leiden, Netherlands	RRID:AB_330713

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Phosphofructokinase (P)	Cell Signalling Technology, Leiden, Netherlands	RRID:AB_2736917
Phosphoglucose isomerase	Abcam, Cambridge, U.K.	RRID: AB_2736921
Phosphoglycerate kinase	Abcam, Cambridge, U.K.	RRID: AB_2736923
Phosphoglycerate mutase 1	Cell Signalling Technology, Leiden, Netherlands	RRID: AB_2736922
Phospho-p38 ^{MAPK}	Cell Signalling Technology, Leiden, Netherlands	RRID:AB_331641
Pyruvate kinase (M) 1	Cell Signalling Technology, Leiden, Netherlands	RRID: AB_2715534
Pyruvate kinase (M) 2	Cell Signalling Technology, Leiden, Netherlands	RRID: AB_1904096
Phospho S6K1	Cell Signalling Technology, Leiden, Netherlands	RRID:AB_2285392
Total S6K1	Cell Signalling Technology, Leiden, Netherlands	RRID:AB_331676
Triphosphate isomerase	Abcam, Cambridge, U.K.	RRID: AB_2736924
α -Tubulin	Abcam, Cambridge, U.K.	RRID: AB_869989
UCP (D105V)	Cell Signalling Technology, Leiden, Netherlands	RRID: AB_2721818
Chemical, Peptides, Recombinant proteins		
2-NBDG	Life Technologies, Glasgow, U.K.	Cat # N13195
3-PO	Axon Medchem, Groningen, Netherlands	Cat # AXON2175
Diogenes TM	GeneFlow, Staffordshire U.K.	Cat # A2-0092
Diphenyleneiodonium	Sigma-Aldrich, Poole, U.K.	Cat # D2926
Glucose Oxidase	Sigma-Aldrich, Poole, U.K.	Cat # G6766
Granulocyte Colony-Stimulating Factor	Biolegend, U.K.	Cat # 578602
Human Fms-like tyrosine kinase-3 ligand	Biolegend, U.K.	Cat # 710802
Human Granulocyte-Macrophage Colony Stimulating Factor	Biolegend, U.K.	Cat # 572902
Human interleukin-3	Biolegend, U.K.	Cat # 578004
Human interleukin-6	Biolegend, U.K.	Cat # 570802
Human Stem Cell Factor	Biolegend, U.K.	Cat # 573902
Human Transferrin	Sigma-Aldrich, Poole, U.K.	Cat # 10652202001
Lipofectamine 2000	Invitrogen, Paisley, U.K.	Cat # 11668019
PEGylated Catalase	Sigma-Aldrich, Poole, U.K.	Cat # C4963
PFK158	Axon Medchem, Groningen, Netherlands	Cat # AXON2542
Recombinant HIF1 α	Abcam, Cambridge, UK	Cat # ab154478
Retronectin [®]	Takara Bio, Paris, France	Cat # T100A/B
Trizol	Life Technologies, Glasgow, U.K.	Cat # 15596026

Reagent or Resource	Source	Resource Identifier (RRID) or Cat #
UR13756	Gift of Dr Davis, Cardiff University, U.K.	N/A
VAS-2870	ENZO-life sciences, Exeter, U.K	Cat # 19205
Commercial Assays		
Ambion WT Expression Kit	Ambion and CBS services Cardiff U.K.	N/A
CD34+ Isolation Kit	Miltenyi Biotec, Bisley, U.K.	Cat # 130-046-701
CellTiter-Glo®	Promega, Southampton, U.K.	Cat # G7570
D-Glucose Assay Kit	Abcam, Cambridge, U.K.	Cat # ab136956
ECL Detection Kit	Life Technologies, Glasgow, U.K	Cat # 12994780
GeneChip® Human Exon 1.0 ST	Affymetrix; Thermo Fisher, Loughborough, U.K.	Cat # 900651
L-Lactate Assay Kit	Abcam, Cambridge, U.K.	Cat # ab65330
Metabolomics	Metabolon TM (http://www.metabolon.com/)	N/A
NOVEX western blotting	Life Technologies, Glasgow, U.K	N/A
Deposited Data		
Affymetrix Microarray	ArrayExpress	e-mexp-583
Experimental Models: Cell lines		
Human: 293T	ATCC, Middlesex, U.K.	RRID:CVCL_0063
Human: Mv4;11	ATCC, Middlesex, U.K.	RRID:CVCL_0064
Human: Phoenix	gift of Garry Nolan, Stanford University School of Medicine, Stanford	RRID:CVCL_H716
Human: THP-1	ECACC, Porton Down, Salisbury, UK	RRID:CVCL_0006
Experimental Models: Organisms/Strains		
NSG (NOD.Cg-Prkdc ^{scid} Il2rg ^{tm1Wjl/SzJ})	Jackson Laboratory	RRID:IMSR_JAX:005557
MRP8-NRAS ^{G12D}	Kind gift of Dr Omidvar, Cardiff University, U.K.	N/A
Experimental Models: Human primary cells		
Human Cord Blood	University Hospital Wales, U.K.	N/A
Primary AML Patient Blasts	UK MRC/NCRI AML 14-17 clinical trials, Cardiff University, U.K.	N/A
Recombinant DNA		
GFP-PINCO	Pier Pelicci, European Institute of Oncology, Milan, Italy (1)	N/A

Reagent or Resource	Source	Resource Identifier (RRID) or Cat #
H-RAS ^{G12V} – PINCO	Prof Darley (Cardiff University, UK)	N/A
N-RAS ^{G12D} – PINCO	Prof Darley (Cardiff University, UK)	N/A
PFKFB3	Eurofins MWG, Eberserg, Germany	N/A
shRNA control TRC2	Mission® Sigma, Dorset, U.K.	Cat # SHC202
shRNA HIF1 α (1)	Mission® Sigma, Dorset, U.K.	TRC2 N0000010819
shRNA HIF1 α (2)	Mission® Sigma, Dorset, U.K.	TRC2 N0000003810
shRNA HIF1 α (3)	Mission® Sigma, Dorset, U.K.	TRC2 N0000003808
shRNA PFKFB3 (1)	Mission® Sigma, Dorset, U.K.	TRC2 N0000314690
shRNA PFKFB3 (2)	Mission® Sigma, Dorset, U.K.	TRC2 N0000314746
shRNA PFKFB3 (3)	Mission® Sigma, Dorset, U.K.	TRC2 N0000314747
shRNA PFKFB3 (4)	Mission® Sigma, Dorset, U.K.	TRC2 N0000314748
shRNA PFKFB3 (5)	Mission® Sigma, Dorset, U.K.	TRC2 N0000381874
Software and Algorithms		
AIDA Image Analyser	Raytest, Straubenhardt, Germany	RRID:SCR_014440
FCS express v6	DeNovos Software, California, U.S.A	RRID:SCR_016431
Minitab v19	Minitab Ltd, Coventry, U.K.	RRID: SCR_014483
Partek Genomics Suite 6.0	Partek Inc U.S.A	RRID:SCR_011860

Affymetrix microarray analysis

RNA from transduced cultures were extracted and processed for hybridisation to Affymetrix GeneChip Human Exon 1.0 ST Array for whole-transcript expression analysis. All Exon array data were analysed using Partek Genomics Suite Software (Version 6.6; Partek Inc., MO, USA). For Partek analyses CEL files were imported using Affymetrix annotation files (NetAffx, Version na31. hg19). Exon-level data were filtered to include only those probe sets that are in the “core” meta-probe list which refers to probe sets that are supported by the most reliable evidence from RefSeq and full-length mRNA GenBank records containing complete CDS information. The core data include target-sequence, perfect-match, unique probe sets. For the supervised analysis, the “Full” probe data set was used and filtered to include only annotations that were characterised as involved in “glucose metabolism”. The RMA algorithm was used for Exon-level expression analysis. Adjustments were made for GC content and probe sequence on pre-background-

subtracted values. We used background correction, quantile normalization, \log_2 transformation, and median polishing for summarization. All image plots passed visual inspection. Hybridization controls had signal increases following concentration. Labelling control signal strengths followed the order Lys < Phe < Thr < Dap. Signal histograms and box plots were examined for raw and processed data.

The Affymetrix data for HSPC is available as supplemental material at <https://www.ebi.ac.uk/arrayexpress> (accession number e-mexp-583). A two-arm approach was applied in order to identify statistically differentially regulated genes. Firstly, statistically significant changing genes were identified using a paired t-test or 2-way ANOVA based on the following model; $Y_{ijk} = \mu + \text{Condition}_i + \text{Cord blood replicate}_j + \varepsilon_{ijk}$. Where Y_{ijk} represents the k^{th} observation on the i^{th} Condition j^{th} Cord blood replicate; μ is the common effect for the whole experiment; ε^{ijk} represents the random error present in the k^{th} observation on the i^{th} Condition j^{th} Cord blood replicate. The errors ε^{ijk} are assumed to be normally and independently distributed with mean 0 and standard deviation δ for all measurements. Cord blood replicate is a random effect and Condition represents experimental treatment e.g. mutant N-Ras^{G12D}. Fisher's Least Significant Difference (LSD) was performed for the following contrasts i) mutant N-RAS^{G12D} vs controls (GFP alone) or (ii) N-RAS^{G12D} vs N-RAS^{G12D}+DPI or (iii) GFP alone vs GFP alone +DPI (Figure 1A). Where possible the Benjamini-Hochberg multiple testing correction was applied to both these strategies, otherwise the level of significance was reduced to $P < 0.01$. Statistically significant genes were filtered to remove all genes that changed by less than 1.2-fold in each of the contrasts above. To identify mutant N-RAS^{G12D} gene changes that are mediated via ROS, the gene lists outlined above were combined in a Venn diagram to identify biological and statistically significant changing according to Figure 1A. Gene enrichment analysis was used to interpret the biological impact of differential gene expression of cells 'exposed' to oxidative stress. Gene Ontology (GO) enrichment analysis was undertaken using Metacore Pathway Analysis (MetacoreTM, Clarivate Analytics).

Supplemental Tables

Supplemental Table S1. Comparison of gene changes in human hematopoietic cord blood (CD34⁺) mixed progenitor cells transduced with N-RAS^{G12D} or control (expressing GFP alone). Microarray analyses were performed using Affymetrix Human Gene 1.0ST Genechips®.

Transcript ID	Gene Assignment (RefSeq ID)	Common Gene Name	p-value	Regulation (Fold-Change)	Regulation	Gene Ontology (GO) Accession number	GO Biological Process
2949299	ENST00000383413	LY6G6C	3.33E-08	-9.30178	Control down vs N-RAS		
3455890	NM_175834	KRT79	7.69E-06	-8.29868	Control down vs N-RAS		
3360456	NM_001005567	OR51B5	1.03E-09	-4.82082	Control down vs N-RAS	GO:0007608	sensory perception of smell
2735027	NM_001251830	SPP1	7.98E-05	-4.4816	Control down vs N-RAS	GO:0001503	ossification
3558375	ENST00000216341	GZMB	3.48E-06	-4.34065	Control down vs N-RAS	GO:0006508	proteolysis
3178147	NM_001257971	CTSL1	2.57E-05	-4.25934	Control down vs N-RAS	GO:0002250	adaptive immune response
4026010	NM_004961	GABRE	1.01E-05	-3.78265	Control down vs N-RAS	GO:0006810	transport
2953570	NM_018643	TREM1	0.000608837	-3.56154	Control down vs N-RAS	GO:0006959	humoral immune response
7385547	NM_002982	CCL2	0.000259351	-3.49656	Control down vs N-RAS	GO:0000165	MAPK cascade
3016148	NM_000602	SERPINE1	1.00E-04	-3.24133	Control down vs N-RAS	GO:0000302	response to reactive oxygen species
3456688	NM_020370	GPR84	1.29E-05	-3.22181	Control down vs N-RAS	GO:0008150	biological_process
3788976	NM_004163	RAB27B	4.15E-06	-3.2184	Control down vs N-RAS	GO:0006184	GTP catabolic process
3403015	NM_001975	ENO2	5.08E-05	-3.18563	Control down vs N-RAS	GO:0005975	carbohydrate metabolic process
4011008	NM_001184830	VSIG4	0.000225672	-3.07449	Control down vs N-RAS	GO:0006957	Complement activation
2879105	NM_030964	SPRY4	3.35E-07	-3.03003	Control down vs N-RAS	GO:0007275	multicellular organismal development
3464860	ENST00000279488	DUSP6	1.05E-05	-2.94658	Control down vs N-RAS	GO:0000188	inactivation of MAPK activity
2671728	NM_022842	CDCP1	2.91E-07	-2.80654	Control down vs N-RAS		
3654956	NM_014387	LAT	4.28E-06	-2.63323	Control down vs N-RAS	GO:0006955	immune response
2571510	ENST00000263341	IL1B	0.000163903	-2.63164	Control down vs N-RAS	GO:0000165	MAPK cascade
3870758	ENST00000570517	LILRA5	0.00048616	-2.46687	Control down vs N-RAS	GO:0045087	innate immune response
3091699	NM_006228	PNOC	1.56E-07	-2.3913	Control down vs N-RAS	GO:0007165	signal transduction
2358693	ENST00000368921	MLLT11	1.43E-07	-2.36277	Control down vs N-RAS	GO:0043065	positive regulation of apoptotic process
2692816	ENST00000296181	ITGB5	3.60E-07	-2.34162	Control down vs N-RAS	GO:0002474	antigen processing and presentation of peptide antigen
3662201	NM_005951	MT1H	0.000286967	-2.31976	Control down vs N-RAS	GO:0045926	negative regulation of growth

Transcript ID	Gene Assignment (RefSeq ID)	Common Gene Name	p-value	Regulation (Fold-Change)	Regulation	Gene Ontology (GO) Accession number	GO Biological Process
2699564	NM_182943	PLOD2	0.000118111	-2.31709	Control down vs N-RAS	GO:0001666	response to hypoxia
2773358	NM_002704	PPBP	8.38E-05	-2.3003	Control down vs N-RAS	GO:0002576	platelet degranulation
2453307	NM_001773	CD34	5.73E-07	-2.29289	Control down vs N-RAS	GO:0001894	tissue homeostasis
3507282	NM_002019	FLT1	7.77E-05	-2.28605	Control down vs N-RAS	GO:0000186	activation of MAPKK activity
3724545	NM_000212	ITGB3	8.50E-07	-2.24124	Control down vs N-RAS	GO:0001934	positive regulation of protein phosphorylation
4012178	ENST00000453707	CITED1	1.05E-06	-2.23623	Control down vs N-RAS	GO:0001656	metanephros development
3905875	ENST00000373313	MAFB	4.44E-06	-2.22667	Control down vs N-RAS	GO:0006351	transcription, DNA-dependent
2709132	NM_004454	ETV5	1.29E-06	-2.21491	Control down vs N-RAS	GO:0006355	regulation of transcription, DNA-dependent
2818517	NM_004385	VCAN	0.000306268	-2.20059	Control down vs N-RAS	GO:0007155	cell adhesion
3901055	NM_012072	CD93	1.20E-07	-2.16681	Control down vs N-RAS	GO:0006909	phagocytosis
2527747	NM_000578	SLC11A1	6.77E-05	-2.14937	Control down vs N-RAS	GO:0000060	protein import into nucleus, translocation
3869078	NM_033329	SIGLEC12	0.000104287	-2.14636	Control down vs N-RAS	GO:0007155	cell adhesion
2429261	NM_002524	NRAS	2.20E-07	-2.14477	Control down vs N-RAS	GO:0000165	MAPK cascade
3338192	NM_053056	CCND1	4.58E-06	-2.13858	Control down vs N-RAS	GO:0000080	G1 phase of mitotic cell cycle
2735759	ENST00000394980	MMRN1	3.70E-07	-2.12766	Control down vs N-RAS	GO:0002576	platelet degranulation
3299578	NM_003956	CH25H	0.000115365	-2.11985	Control down vs N-RAS	GO:0006629	lipid metabolic process
3345427	NM_015036	ENDOD1	2.17E-06	-2.09722	Control down vs N-RAS		
3622386	NM_001482	GATM	0.000541147	-2.09219	Control down vs N-RAS	GO:0006600	creatine metabolic process
3908358	ENST00000359930	SULF2	1.13E-05	-2.08273	Control down vs N-RAS	GO:0001822	kidney development
3887210	NM_004994	MMP9	5.80E-05	-2.08262	Control down vs N-RAS	GO:0001501	skeletal system development
3758510	NM_001986	ETV4	2.62E-06	-2.08204	Control down vs N-RAS	GO:0006351	transcription, DNA-dependent
3417842	NM_002332	LRP1	6.51E-05	-2.07352	Control down vs N-RAS	GO:0006898	receptor-mediated endocytosis
3076868	NM_013252	CLEC5A	4.28E-05	-2.06825	Control down vs N-RAS	GO:0002076	osteoblast development
3420316	ENST00000403681	HMGA2	0.000145477	-2.06393	Control down vs N-RAS	GO:0000122	negative regulation of transcription from RNA polymera
2878437	ENST00000302014	CD14	0.000387932	-2.06241	Control down vs N-RAS	GO:0006909	phagocytosis
3812385	ENST00000280200	CD226	0.000162212	-2.05972	Control down vs N-RAS	GO:0001816	cytokine production
3580769	ENST00000348956	CKB	2.20E-05	-2.05575	Control down vs N-RAS	GO:0006600	creatine metabolic process
3589141	NM_152594	SPRED1	0.00051876	-2.04069	Control down vs N-RAS	GO:0000188	inactivation of MAPK activity
3414296	NM_000486	AQP2	4.22E-05	-2.03535	Control down vs N-RAS	GO:0003097	renal water transport

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3750767	ENST00000395321	ALDOC	0.000106937	-2.03355	Control down vs N-RAS	GO:0001666	response to hypoxia
3577612	NM_001002236	SERPINA1	8.21E-07	-2.02758	Control down vs N-RAS	GO:0001666	response to hypoxia
3707335	NM_000173	GP1BA	3.09E-05	-2.01335	Control down vs N-RAS	GO:0007155	cell adhesion
3734379	NM_007261	CD300A	0.000116902	-2.00012	Control down vs N-RAS	GO:0007155	cell adhesion
3901041	ENST00000377103	THBD	0.000496945	-1.98116	Control down vs N-RAS	GO:0007565	female pregnancy
3753860	NM_002985	CCL5	3.78E-07	-1.96683	Control down vs N-RAS	GO:0000165	MAPK cascade
3388859	ENST00000532855	MMP12	0.000191212	-1.96301	Control down vs N-RAS		
3462816	ENST00000266671	PHLDA1	0.000406482	-1.95507	Control down vs N-RAS	GO:0006915	apoptotic process
3976639	NM_022825	PORCN	2.21E-06	-1.95168	Control down vs N-RAS	GO:0016055	Wnt receptor signaling pathway
3726691	ENST00000285238	ABCC3	2.32E-05	-1.90653	Control down vs N-RAS	GO:0006810	transport
3870611	NM_024318	LILRA6	0.000547687	-1.86805	Control down vs N-RAS		
3039177	NM_004956	ETV1	5.03E-06	-1.86698	Control down vs N-RAS	GO:0006355	regulation of transcription, DNA-dependent
2640579	NM_032242	PLXNA1	0.000111699	-1.85328	Control down vs N-RAS	GO:0007275	multicellular organismal development
2438575	ENST00000368199	SH2D2A	0.0001266	-1.85129	Control down vs N-RAS	GO:0001525	angiogenesis
2434139	NM_014849	SV2A	3.78E-05	-1.85083	Control down vs N-RAS	GO:0006836	neurotransmitter transport
3293280	NM_021129	PPA1	0.000227472	-1.84078	Control down vs N-RAS	GO:0006418	tRNA aminoacylation for protein translation
3151970	NM_014751	MTSS1	2.43E-06	-1.82939	Control down vs N-RAS	GO:0006928	cellular component movement
3529113	NM_138460	CMTM5	1.68E-05	-1.82812	Control down vs N-RAS	GO:0006935	chemotaxis
3681705	NR_003369	RRN3P2	5.12E-05	-1.82369	Control down vs N-RAS	GO:0001701	in utero embryonic development
3742627	ENST00000574081	SCIMP	3.13E-05	-1.81916	Control down vs N-RAS	GO:0070374	positive regulation of ERK1 and ERK2 cascade
3656990	NM_001145808	ITGAM	8.07E-07	-1.80306	Control down vs N-RAS	GO:0007155	cell adhesion
3557350	ENST00000397267	SLC22A17	0.000130184	-1.80097	Control down vs N-RAS	GO:0015891	siderophore transport
3569257	ENST00000216446	PLEK2	0.000419819	-1.79316	Control down vs N-RAS	GO:0030036	actin cytoskeleton organization
3215570	NM_000507	FBP1	7.66E-09	-1.79184	Control down vs N-RAS	GO:0005975	carbohydrate metabolic process
2599153	NM_022648	TNS1	3.72E-06	-1.78298	Control down vs N-RAS	GO:0007044	cell-substrate junction assembly
3046444	NM_003014	SFRP4	0.000257818	-1.78139	Control down vs N-RAS	GO:0001944	vasculature development
3960388	NM_003560	PLA2G6	0.000102186	-1.78108	Control down vs N-RAS	GO:0008219	cell death
3362159	NM_020645	NRIP3	0.000551347	-1.75779	Control down vs N-RAS	GO:0006508	proteolysis
2601414	ENST00000409840	SERPINE2	3.10E-05	-1.75699	Control down vs N-RAS	GO:0010757	negative regulation of plasminogen activation

Transcript ID	Gene Assignment (RefSeq ID)	Common Gene Name	p-value	Regulation (Fold-Change)	Regulation	Gene Ontology (GO) Accession number	GO Biological Process
3198346	NM_002839	PTPRD	0.000196156	-1.75448	Control down vs N-RAS	GO:0006470	protein dephosphorylation
3528864	ENST00000311852	MMP14	7.84E-05	-1.75208	Control down vs N-RAS	GO:0001503	ossification
3976341	NM_003254	TIMP1	1.59E-05	-1.74651	Control down vs N-RAS	GO:0001775	cell activation
3841357	NM_001130917	LILRA2	0.000298938	-1.74101	Control down vs N-RAS	GO:0006952	defense response
2945741	NM_014722	FAM65B	0.000170104	-1.73989	Control down vs N-RAS	GO:0007517	muscle organ development
3995254	ENST00000370306	GABRQ	6.19E-05	-1.72911	Control down vs N-RAS	GO:0006836	neurotransmitter transport
3839619	NM_014441	SIGLEC9	6.76E-05	-1.72874	Control down vs N-RAS	GO:0007155	cell adhesion
3892812	NM_016354	SLCO4A1	1.23E-05	-1.72266	Control down vs N-RAS	GO:0042403	thyroid hormone metabolic process
3281621	NM_020824	ARHGAP21	2.47E-05	-1.72214	Control down vs N-RAS	GO:0007030	Golgi organization
3980078	NM_001142503	STARD8	5.68E-07	-1.70835	Control down vs N-RAS	GO:0007264	small GTPase mediated signal transduction
3592401	NM_032413	C15orf48	0.000654438	-1.70231	Control down vs N-RAS		
3657041	NM_005353	ITGAD	1.49E-05	-1.7008	Control down vs N-RAS	GO:0006955	immune response
3635159	NR_028330	C15orf37	0.000134574	-1.69221	Control down vs N-RAS	GO:0009396	folic acid-containing compound biosynthetic process
3545130	NM_014909	VASH1	0.000673784	-1.68552	Control down vs N-RAS	GO:0001525	angiogenesis
3873160	NM_021158	TRIB3	1.54E-05	-1.66882	Control down vs N-RAS	GO:0006351	transcription, DNA-dependent
2902593	NM_001003693	LY6G6F	1.36E-05	-1.66224	Control down vs N-RAS		
3936515	NM_018943	TUBA8	8.17E-05	-1.65753	Control down vs N-RAS	GO:0007018	microtubule-based movement
3156307	NM_153831	PTK2	4.96E-06	-1.64554	Control down vs N-RAS	GO:0000226	microtubule cytoskeleton organization
3454821	NM_001033873	SMAGP	4.94E-05	-1.63504	Control down vs N-RAS		
3841862	NM_002000	FCAR	0.000643916	-1.6314	Control down vs N-RAS	GO:0006955	immune response
3340449	NM_007256	SLCO2B1	8.97E-05	-1.62824	Control down vs N-RAS	GO:0015711	organic anion transport
2374126	ENST00000367362	NR5A2	5.25E-05	-1.62488	Control down vs N-RAS	GO:0006355	regulation of transcription, DNA-dependent
3232349	NM_001242339	PFKP	2.86E-08	-1.62484	Control down vs N-RAS	GO:0005975	carbohydrate metabolic process
2600068	ENST00000248437	TUBA4A	1.47E-05	-1.62094	Control down vs N-RAS	GO:0000086	G2/M transition of mitotic cell cycle
3662808	NM_001145772	GPR56	5.11E-05	-1.61411	Control down vs N-RAS	GO:0007155	cell adhesion
3621080	NM_201631	TGM5	9.76E-05	-1.6038	Control down vs N-RAS	GO:0006464	cellular protein modification process
2908261	ENST00000336600	C6orf223	0.00012106	-1.60226	Control down vs N-RAS		
3890597	ENST00000356208	RBM38	1.80E-06	-1.60185	Control down vs N-RAS	GO:0006397	mRNA processing
3396107	ENST00000464067	ESAM	7.10E-06	-1.60092	Control down vs N-RAS	GO:0007156	homophilic cell adhesion

Transcript ID	Gene Assignment (RefSeq ID)	Common Gene Name	p-value	Regulation (Fold-Change)	Regulation	Gene Ontology (GO) Accession number	GO Biological Process
3635198	NM_001114735	BCL2A1	6.52E-05	-1.59391	Control down vs N-RAS	GO:0006915	apoptotic process
3979762	ENST00000519389	HEPH	1.53E-05	-1.58778	Control down vs N-RAS	GO:0006825	copper ion transport
3405748	NM_001423	EMP1	0.000269863	-1.58563	Control down vs N-RAS	GO:0007275	multicellular organismal development
2361154	NM_152280	SYT11	0.000474229	-1.58457	Control down vs N-RAS		
3218528	NM_005502	ABCA1	0.000568883	-1.58318	Control down vs N-RAS	GO:0002790	peptide secretion
3456732	ENST00000293379	ITGA5	9.59E-06	-1.58306	Control down vs N-RAS	GO:0001525	angiogenesis
2883440	NM_033274	ADAM19	2.53E-05	-1.57999	Control down vs N-RAS	GO:0006508	proteolysis
3434726	NR_033948	P2RX7	0.000504884	-1.57793	Control down vs N-RAS	GO:0002028	regulation of sodium ion transport
3662851	NM_170776	GPR97	0.000146461	-1.5776	Control down vs N-RAS	GO:0007218	neuropeptide signaling pathway
2427981	NM_020683	ADORA3	8.95E-05	-1.57111	Control down vs N-RAS	GO:0006954	inflammatory response
3188111	NM_000962	PTGS1	1.69E-06	-1.5701	Control down vs N-RAS	GO:0001516	prostaglandin biosynthetic process
3945651	NM_021822	APOBEC3G	0.000331342	-1.5685	Control down vs N-RAS	GO:0002230	positive regulation of defense response to virus by host
2320727	NM_001066	TNFRSF1B	1.64E-05	-1.56845	Control down vs N-RAS	GO:0006915	apoptotic process
3601955	ENST00000352410	MPI	7.70E-05	-1.56499	Control down vs N-RAS	GO:0005975	carbohydrate metabolic process
2489228	ENST00000348227	WDR54	1.14E-05	-1.56174	Control down vs N-RAS	GO:0006915	apoptotic process
3995804	NM_005629	SLC6A8	0.000204251	-1.55784	Control down vs N-RAS	GO:0006600	creatine metabolic process
3148463	NM_001146	ANGPT1	0.000414903	-1.55715	Control down vs N-RAS	GO:0001936	regulation of endothelial cell proliferation
3281703	NM_020200	PRTFDC1	6.87E-05	-1.55513	Control down vs N-RAS	GO:0006166	purine ribonucleoside salvage
7385611	NM_001258359	HPCAL1	5.62E-06	-1.55384	Control down vs N-RAS		
2775994	NM_006665	HPSE	0.000132173	-1.55195	Control down vs N-RAS	GO:0005975	carbohydrate metabolic process
2363808	NM_001002273	FCGR2B	2.29E-05	-1.54871	Control down vs N-RAS	GO:0006955	immune response
2933392	---	---	2.00E-06	-1.54777	Control down vs N-RAS		
3387537	ENST00000524717	MAML2	0.000448761	-1.54278	Control down vs N-RAS	GO:0006367	transcription initiation from RNA polymerase II promot
3204721	ENST00000378292	TPM2	1.59E-05	-1.542	Control down vs N-RAS	GO:0006936	muscle contraction
3191352	NM_014286	NCS1	1.94E-05	-1.53423	Control down vs N-RAS	GO:0010975	regulation of neuron projection development
3759137	NM_000419	ITGA2B	0.0001616	-1.53277	Control down vs N-RAS	GO:0002576	platelet degranulation
2772876	NM_014243	ADAMTS3	0.000315677	-1.5199	Control down vs N-RAS	GO:0006508	proteolysis
3726325	NM_022167	XYLT2	1.18E-05	-1.51257	Control down vs N-RAS	GO:0006024	glycosaminoglycan biosynthetic process
3883921	NM_006097	MYL9	0.000490309	-1.50525	Control down vs N-RAS	GO:0006936	muscle contraction

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3761164	NM_003726	SKAP1	5.25E-05	-1.50203	Control down vs N-RAS	GO:0045893	positive regulation of transcription, DNA-dependent
3225952	NM_022833	FAM129B	0.000377538	-1.49915	Control down vs N-RAS	GO:0043066	negative regulation of apoptotic process
3354443	NM_198277	SLC37A2	3.18E-06	-1.49772	Control down vs N-RAS	GO:0008643	carbohydrate transport
3902489	ENST00000376062	BCL2L1	0.000534995	-1.49323	Control down vs N-RAS	GO:0000910	cytokinesis
3721956	NM_016437	TUBG2	0.000540103	-1.4907	Control down vs N-RAS	GO:0000086	G2/M transition of mitotic cell cycle
3832992	NM_022835	PLEKHG2	2.93E-05	-1.48028	Control down vs N-RAS	GO:0006915	apoptotic process
3595846	NM_001040450	FAM63B	0.000368787	-1.4785	Control down vs N-RAS	GO:0008150	biological_process
3870361	NM_144687	NLRP12	6.20E-05	-1.47563	Control down vs N-RAS	GO:0006919	activation of cysteine-type endopeptidase activity involved
3743340	NM_080912	ASGR2	0.000479963	-1.47554	Control down vs N-RAS	GO:0007166	cell surface receptor signaling pathway
3599758	NM_001104554	PAQR5	2.07E-05	-1.47537	Control down vs N-RAS	GO:0007275	multicellular organismal development
2358360	NM_001202858	ECM1	0.000163204	-1.47532	Control down vs N-RAS	GO:0001503	ossification
3757917	NM_012232	PTRF	3.41E-05	-1.46971	Control down vs N-RAS	GO:0006355	regulation of transcription, DNA-dependent
3891342	NM_030773	TUBB1	0.000256593	-1.46806	Control down vs N-RAS	GO:0006457	protein folding
3179646	ENST00000375472	SUSD3	0.000129138	-1.46702	Control down vs N-RAS		
3649714	ENST00000300006	C16orf45	3.75E-05	-1.46645	Control down vs N-RAS		
3429857	NM_001145199	C12orf75	3.74E-05	-1.46579	Control down vs N-RAS		
3456049	ENST00000267082	ITGB7	8.77E-05	-1.45438	Control down vs N-RAS	GO:0007155	cell adhesion
3326635	NM_000610	CD44	6.41E-05	-1.45318	Control down vs N-RAS	GO:0007155	cell adhesion
2619480	NM_001296	CCBP2	4.93E-05	-1.45245	Control down vs N-RAS	GO:0006935	chemotaxis
3664785	NM_016951	CKLF	2.79E-05	-1.45052	Control down vs N-RAS	GO:0008283	cell proliferation
3967689	ENST00000217961	STS	0.000114689	-1.43717	Control down vs N-RAS	GO:0006665	sphingolipid metabolic process
3864551	NM_002659	PLAUR	0.000168976	-1.42612	Control down vs N-RAS	GO:0006501	C-terminal protein lipidation
3991889	NM_001078171	FAM127A	0.000393656	-1.42069	Control down vs N-RAS		
3755323	NM_007144	PCGF2	7.74E-05	-1.4202	Control down vs N-RAS	GO:0000122	negative regulation of transcription from RNA polymerase II
3670772	NM_198390	CMIP	2.05E-05	-1.41473	Control down vs N-RAS		
3731826	NM_002737	PRKCA	0.000310773	-1.40324	Control down vs N-RAS	GO:0001525	angiogenesis
3944690	ENST00000248901	CYTH4	3.55E-05	-1.40282	Control down vs N-RAS	GO:0016192	vesicle-mediated transport
3951719	NM_031890	CECR6	0.000400735	-1.40094	Control down vs N-RAS		

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3137530	NM_004318	ASPH	0.000340173	-1.40078	Control down vs N-RAS	GO:0006936	muscle contraction
3867965	NM_006270	RRAS	0.000217213	-1.39841	Control down vs N-RAS	GO:0006184	GTP catabolic process
3284302	NM_003873	NRP1	3.83E-05	-1.39561	Control down vs N-RAS	GO:0001569	patterning of blood vessels
3447694	NM_001178094	BCAT1	0.000211362	-1.39053	Control down vs N-RAS	GO:0000082	G1/S transition of mitotic cell cycle
3687494	NM_001040056	MAPK3	6.03E-05	-1.38628	Control down vs N-RAS	GO:0000165	MAPK cascade
3261820	NM_030912	TRIM8	0.000420297	-1.38096	Control down vs N-RAS	GO:0008150	no biological data available
3863021	NM_000660	TGFB1	5.64E-05	-1.37873	Control down vs N-RAS	GO:0000060	protein import into nucleus, translocation
3570373	NM_033262	SLC8A3	0.000453561	-1.37688	Control down vs N-RAS	GO:0006811	ion transport
3864646	NM_002250	KCNN4	0.000147527	-1.37561	Control down vs N-RAS	GO:0006813	potassium ion transport
3656829	NM_001122957	BCKDK	0.000291767	-1.3726	Control down vs N-RAS	GO:0009063	cellular amino acid catabolic process
3730322	NM_006039	MRC2	3.53E-06	-1.37164	Control down vs N-RAS	GO:0006897	endocytosis
3371003	NM_001258320	TP53I11	7.60E-05	-1.36998	Control down vs N-RAS	GO:0006950	response to stress
3850020	NM_031917	ANGPTL6	6.71E-05	-1.36179	Control down vs N-RAS	GO:0001525	angiogenesis
3888613	NM_005194	CEBPB	0.000266227	-1.36103	Control down vs N-RAS	GO:0001892	embryonic placenta development
2777714	NM_000345	SNCA	0.000139137	-1.35894	Control down vs N-RAS	GO:0001774	microglial cell activation
3768474	NM_017983	WIPI1	0.00050453	-1.35268	Control down vs N-RAS	GO:0000045	autophagic vacuole assembly
3869237	NM_001193306	FPR1	0.000330656	-1.35194	Control down vs N-RAS	GO:0000187	activation of MAPK activity
3846238	NM_174983	MFSD12	0.000257989	-1.35068	Control down vs N-RAS	GO:0006810	transport
2497161	NM_003853	IL18RAP	0.00064436	-1.3466	Control down vs N-RAS	GO:0006954	inflammatory response
3592511	ENST00000260324	SQRDL	0.000342847	-1.34335	Control down vs N-RAS	GO:0000096	sulfur amino acid metabolic process
3772090	ENST00000392467	TMC6	0.0004188	-1.33951	Control down vs N-RAS	GO:0006417	regulation of translation
3693314	NM_005550	KIFC3	3.23E-06	-1.33749	Control down vs N-RAS	GO:0007018	microtubule-based movement
3226041	NM_170600	SH2D3C	9.07E-05	-1.33744	Control down vs N-RAS	GO:0007254	JNK cascade
2888800	NM_004395	DBN1	0.00031225	-1.33668	Control down vs N-RAS	GO:0007015	actin filament organization
3381150	NM_001243784	PDE2A	4.86E-05	-1.33521	Control down vs N-RAS	GO:0000122	negative regulation of transcription from RNA polymerase
2452069	NM_002646	PIK3C2B	0.000351902	-1.33101	Control down vs N-RAS	GO:0008150	no biological data available
3120008	NM_019037	EXOSC4	0.000419394	-1.32989	Control down vs N-RAS	GO:0000288	nuclear-transcribed mRNA catabolic process, deadenylation
3770305	NM_006678	CD300C	0.000302612	-1.32821	Control down vs N-RAS	GO:0006968	cellular defense response
3526831	NM_007368	RASA3	6.29E-05	-1.32536	Control down vs N-RAS	GO:0007165	signal transduction

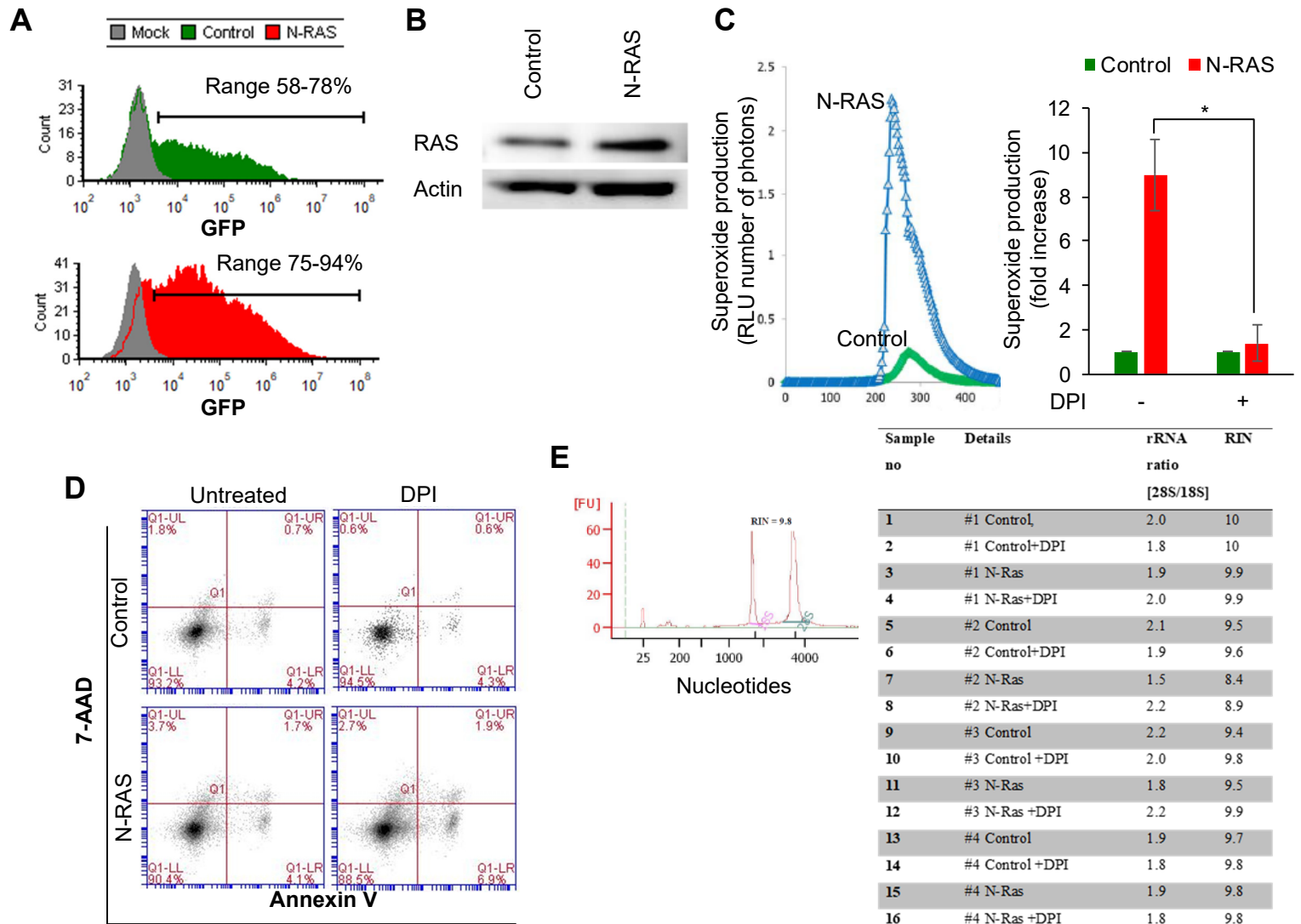
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3027204	ENST00000263552	TBXAS1	3.43E-05	-1.32472	Control down vs N-RAS	GO:0001516	prostaglandin biosynthetic process
3852880	NM_013447	EMR2	0.000444094	-1.32461	Control down vs N-RAS	GO:0006954	inflammatory response
3315024	NM_001109	ADAM8	4.94E-06	-1.32423	Control down vs N-RAS	GO:0000902	cell morphogenesis
3529725	ENST00000559919	REC8	1.96E-06	-1.31938	Control down vs N-RAS	GO:0001556	oocyte maturation
4000704	NM_003916	AP1S2	0.000377395	-1.31615	Control down vs N-RAS	GO:0006886	intracellular protein transport
3752709	ENST00000318217	MYO1D	0.000295803	-1.31504	Control down vs N-RAS	GO:0010923	negative regulation of phosphatase activity
3893910	NM_198723	TCEA2	6.89E-05	-1.31474	Control down vs N-RAS	GO:0006351	transcription, DNA-dependent
3654175	NM_000418	IL4R	0.00026485	-1.31321	Control down vs N-RAS	GO:0002532	production of molecular mediator involved in inflammatory re
3527514	NM_000270	PNP	0.000218987	-1.30803	Control down vs N-RAS	GO:0006139	nucleobase-containing compound metabolic process
3454006	NM_175736	FMNL3	0.000212358	-1.3072	Control down vs N-RAS	GO:0007010	cytoskeleton organization
2351854	ENST00000343534	C1orf162	1.04E-05	-1.29983	Control down vs N-RAS		
3836317	NM_003370	VASP	0.0004485	-1.29468	Control down vs N-RAS	GO:0001843	neural tube closure
2881239	ENST00000261799	PDGFRB	4.29E-05	-1.29352	Control down vs N-RAS	GO:0001701	in utero embryonic development
3305313	ENST00000278071	ITPRIP	0.000567556	-1.28765	Control down vs N-RAS		
4008170	NM_139289	AKAP4	0.000613622	-1.28587	Control down vs N-RAS	GO:0006928	cellular component movement
3832256	NM_021102	SPINT2	9.64E-05	-1.28373	Control down vs N-RAS	GO:0006928	cellular component movement
3020302	NM_001172895	CAV1	2.34E-05	-1.28182	Control down vs N-RAS	GO:0000122	negative regulation of transcription from RNA polymerase
2507896	NM_006895	HNMT	0.000239615	-1.27482	Control down vs N-RAS	GO:0002347	response to tumor cell
3060182	NM_000927	ABCB1	0.000494418	-1.27466	Control down vs N-RAS	GO:0000086	G2/M transition of mitotic cell cycle
3707095	NM_004313	ARRB2	0.000154339	-1.27142	Control down vs N-RAS	GO:0001932	regulation of protein phosphorylation
2616131	ENST00000330953	CCR4	0.00032708	-1.27003	Control down vs N-RAS	GO:0001764	neuron migration
2639734	NM_001024660	KALRN	0.000107846	-1.26932	Control down vs N-RAS	GO:0006468	protein phosphorylation
3317071	NM_001242932	LSP1	4.85E-08	-1.26699	Control down vs N-RAS	GO:0006928	cellular component movement
3829687	NM_000175	GPI	0.000224407	-1.26405	Control down vs N-RAS	GO:0001525	angiogenesis
3708462	NM_014716	ACAP1	1.51E-05	-1.26132	Control down vs N-RAS	GO:0006629	lipid metabolic process
2888648	NM_001031677	RAB24	0.000670137	-1.26029	Control down vs N-RAS	GO:0006914	autophagy
3820612	NM_001145056	SLC44A2	9.21E-06	-1.2538	Control down vs N-RAS	GO:0007165	signal transduction
3377669	NM_001130144	LTBP3	0.000466113	-1.24979	Control down vs N-RAS	GO:0007179	transforming growth factor beta receptor signaling pathwa

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3529508	ENST00000216780	PCK2	0.000148406	-1.24896	Control down vs N-RAS	GO:0005975	carbohydrate metabolic process
3752611	NM_022344	C17orf75	0.000293738	-1.2422	Control down vs N-RAS	GO:0007283	spermatogenesis
3607447	NM_007011	ABHD2	8.28E-05	-1.24146	Control down vs N-RAS	GO:0008150	no biological data available
2662331	NM_003656	CAMK1	8.71E-05	-1.24098	Control down vs N-RAS	GO:0006468	protein phosphorylation
3771602	NM_024599	RHBDF2	0.000173113	-1.24095	Control down vs N-RAS	GO:0006508	proteolysis
3960478	ENST00000400206	CSNK1E	0.000629253	-1.2408	Control down vs N-RAS	GO:0000086	G2/M transition of mitotic cell cycle
3540552	NM_178155	FUT8	0.000215516	-1.23423	Control down vs N-RAS	GO:0001701	in utero embryonic development
3410614	NM_139241	FGD4	0.000290373	-1.23327	Control down vs N-RAS	GO:0006915	apoptotic process
3816264	NM_032482	DOT1L	0.000250583	-1.23298	Control down vs N-RAS	GO:0034968	histone lysine methylation
2887048	NM_005990	STK10	4.40E-05	-1.22859	Control down vs N-RAS	GO:0006468	protein phosphorylation
2878809	NM_022481	ARAP3	0.000279899	-1.22746	Control down vs N-RAS	GO:0007010	cytoskeleton organization
2880361	NR_038902	JAKMIP2-AS1	0.000265617	-1.22137	Control down vs N-RAS		
2403215	NM_001042747	FGR	0.000291596	-1.22018	Control down vs N-RAS	GO:0002768	immune response-regulating cell surface receptor signalin
3151883	NM_194291	TMEM65	4.62E-06	-1.21775	Control down vs N-RAS		
3702547	ENST00000262428	COTL1	0.000217481	-1.21508	Control down vs N-RAS	GO:0008150	no biological data available
3255402	NM_018999	FAM190B	0.000412797	-1.21219	Control down vs N-RAS		
3598758	NR_027654	SMAD6	2.31E-05	-1.21099	Control down vs N-RAS	GO:0001657	ureteric bud development
3574121	NM_033104	STON2	0.000399932	-1.20629	Control down vs N-RAS	GO:0006886	intracellular protein transport
2405469	NM_198040	PHC2	5.40E-05	-1.20446	Control down vs N-RAS	GO:0007275	multicellular organismal development
3951732	NM_033070	CECR5	0.000660319	-1.2026	Control down vs N-RAS		
3403244	NM_014718	CLSTN3	0.000552207	-1.1965	Control down vs N-RAS	GO:0007156	homophilic cell adhesion
3996404	NM_001493	GDI1	0.000330545	-1.19636	Control down vs N-RAS	GO:0007165	signal transduction
3130113	ENST00000355904	GTF2E2	0.000101175	-1.19434	Control down vs N-RAS	GO:0006355	regulation of transcription, DNA-dependent
3822657	NM_078481	CD97	0.000129345	-1.19389	Control down vs N-RAS	GO:0006928	cellular component movement
3157901	NM_201384	PLEC	2.18E-05	-1.19347	Control down vs N-RAS	GO:0006915	apoptotic process
3743551	ENST00000356683	C17orf81	0.000432644	-1.19263	Control down vs N-RAS	GO:0006351	transcription, DNA-dependent
3204744	ENST00000314888	TLN1	8.67E-05	-1.19199	Control down vs N-RAS	GO:0002576	platelet degranulation
3631964	NM_001206796	PKM	0.000307982	-1.18478	Control down vs N-RAS	GO:0005975	carbohydrate metabolic process
3818897	NM_001166111	PNPLA6	0.000226017	-1.18005	Control down vs N-RAS	GO:0001525	angiogenesis

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2334052	ENST00000458657	C1orf228	0.000130811	-1.17864	Control down vs N-RAS		
3934729	ENST00000397850	ITGB2	3.94E-05	-1.17799	Control down vs N-RAS	GO:0006915	apoptotic process
3944882	NM_002305	LGALS1	0.000456275	-1.17503	Control down vs N-RAS	GO:0006915	apoptotic process
3735752	ENST00000436233	SEC14L1	0.000140686	-1.16914	Control down vs N-RAS	GO:0006810	transport
3835814	ENST00000252483	PVRL2	4.39E-05	-1.16738	Control down vs N-RAS	GO:0001675	acrosome assembly
3751590	NM_032854	CORO6	0.000180066	-1.16286	Control down vs N-RAS	GO:0030036	actin cytoskeleton organization
2376799	ENST00000367120	IKBKE	0.000109021	-1.15895	Control down vs N-RAS	GO:0002756	MyD88-independent toll-like receptor signaling pathway
4050590	NM_006647	NOXA1	0.000528186	-1.1553	Control down vs N-RAS	GO:0006801	superoxide metabolic process
3564210	NM_002863	PYGL	9.76E-05	-1.1544	Control down vs N-RAS	GO:0005975	carbohydrate metabolic process
3351564	ENST00000361417	PHLDB1	0.000176723	-1.15097	Control down vs N-RAS		
3837759	NM_000836	GRIN2D	0.000109386	-1.149	Control down vs N-RAS	GO:0001964	startle response
3771068	NM_033452	TRIM47	4.17E-05	-1.14562	Control down vs N-RAS		
3977067	ENST00000376327	PLP2	0.000549644	-1.14185	Control down vs N-RAS	GO:0006811	ion transport
3962839	NM_173050	SCUBE1	0.000294511	-1.13114	Control down vs N-RAS	GO:0006954	inflammatory response
3709244	NM_001005273	CHD3	0.000123369	-1.10941	Control down vs N-RAS	GO:0006351	transcription, DNA-dependent
3844021	---	---	0.000556388	1.11394	Control up vs N-RAS		
2747190	NM_001040261	DCLK2	0.000550545	1.13271	Control up vs N-RAS	GO:0035556	intracellular signal transduction
3831274	---	---	0.000655978	1.14386	Control up vs N-RAS		
2318656	NM_016831	PER3	0.00011279	1.15995	Control up vs N-RAS	GO:0000122	negative regulation of transcription from RNA polymerase II
3233322	NM_017782	FAM208B	0.00021648	1.17167	Control up vs N-RAS		
2420681	NM_018298	MCOLN3	5.54E-05	1.19411	Control up vs N-RAS	GO:0007626	locomotory behavior
3088213	NM_022071	SH2D4A	0.000216166	1.19634	Control up vs N-RAS	GO:0010923	negative regulation of phosphatase activity
2345239	NR_026985	LOC339524	0.000573727	1.22773	Control up vs N-RAS	GO:0015014	heparan sulfate proteoglycan biosynthetic process
2995076	NM_001080529	WIPF3	0.000551762	1.22818	Control up vs N-RAS	GO:0007275	multicellular organismal development
3975893	NM_014735	PHF16	0.000530995	1.2391	Control up vs N-RAS	GO:0043966	histone H3 acetylation
3173880	NM_004817	TJP2	8.36E-05	1.23927	Control up vs N-RAS	GO:0006915	apoptotic process
2481379	NM_006872	GTF2A1L	0.000323627	1.24136	Control up vs N-RAS	GO:0006355	regulation of transcription, DNA-dependent
3507003	NM_153371	LNX2	0.000598753	1.2472	Control up vs N-RAS	GO:0051260	protein homooligomerization
3132940	NM_020476	ANK1	2.91E-05	1.24926	Control up vs N-RAS	GO:0006887	exocytosis

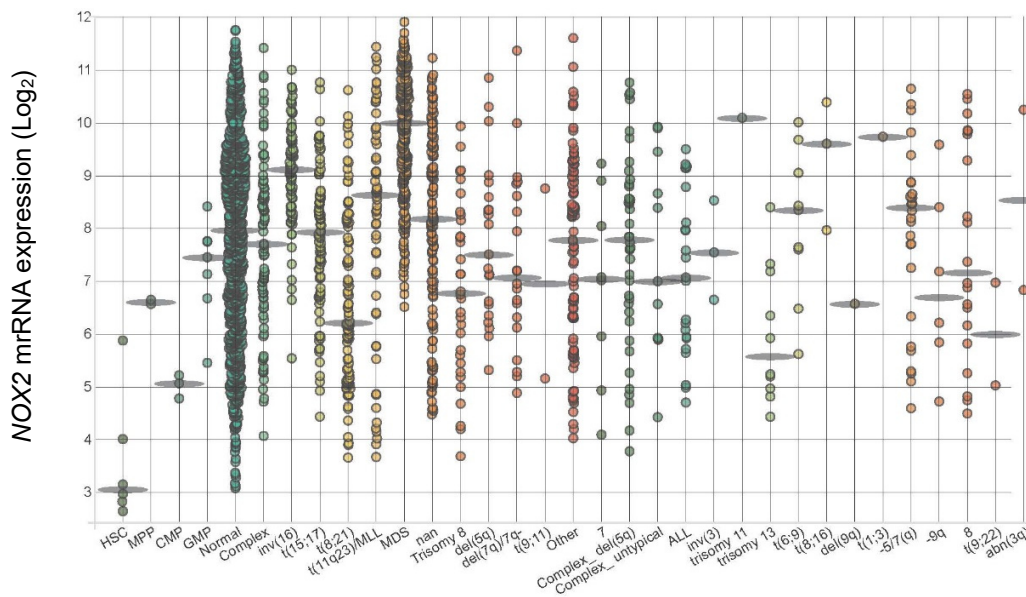
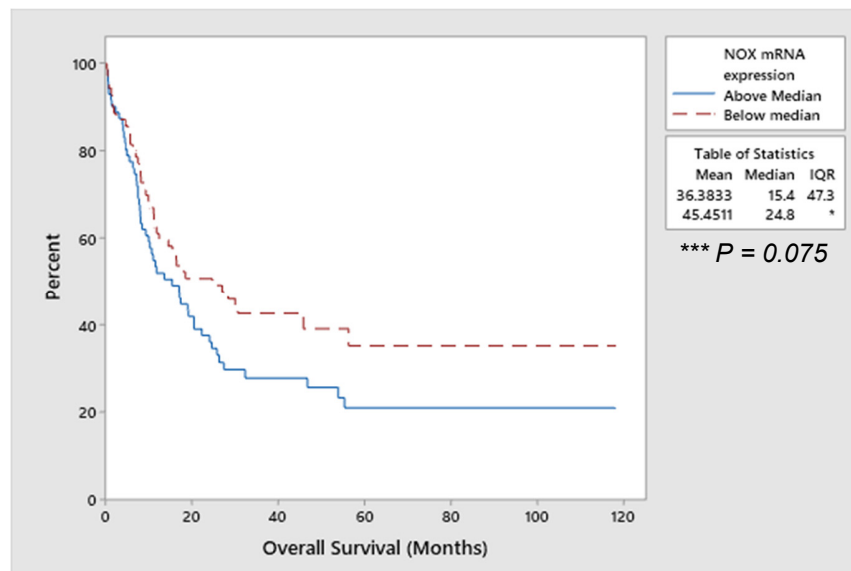
Transcript ID	Gene Assignment (RefSeq ID)	Common Gene Name	p-value	Regulation (Fold-Change)	Regulation	Gene Ontology (GO) Accession number	GO Biological Process
2933536	NM_020245	TULP4	0.00016489	1.25744	Control up vs N-RAS	GO:0006355	regulation of transcription, DNA-dependent
3901696	ENST00000432802	ACSS1	2.49E-05	1.27003	Control up vs N-RAS	GO:0006069	ethanol oxidation
2584787	NM_014900	COBLL1	0.000489745	1.28515	Control up vs N-RAS		
3858659	NM_032139	ANKRD27	0.000199712	1.31289	Control up vs N-RAS	GO:0045022	early endosome to late endosome transport
3403595	NM_016184	CLEC4A	0.000489718	1.33576	Control up vs N-RAS	GO:0007155	cell adhesion
3064158	ENST00000462107	TFR2	0.000245542	1.34614	Control up vs N-RAS	GO:0006508	proteolysis
3517251	ENST00000305425	DACH1	0.0003909	1.34645	Control up vs N-RAS	GO:0007275	multicellular organismal development
3419641	BC053903	SRGAP1	0.000334893	1.37042	Control up vs N-RAS	GO:0007264	small GTPase mediated signal transduction
3138204	NM_004820	CYP7B1	0.000444799	1.37709	Control up vs N-RAS	GO:0006699	bile acid biosynthetic process
3844744	NM_214710	PRSS57	0.000394019	1.38612	Control up vs N-RAS	GO:0006508	proteolysis
2880051	ENST00000394413	PPP2R2B	3.70E-05	1.38893	Control up vs N-RAS	GO:0006915	apoptotic process
2660617	NM_000564	IL5RA	0.000514733	1.40576	Control up vs N-RAS	GO:0002437	inflammatory response to antigenic stimulus
2349129	NM_001400	S1PR1	0.000285958	1.44731	Control up vs N-RAS	GO:0001525	angiogenesis
3301713	NM_013314	BLNK	0.000681544	1.45722	Control up vs N-RAS	GO:0006954	inflammatory response
2585400	ENST00000409672	SCN9A	2.93E-05	1.47085	Control up vs N-RAS	GO:0006814	sodium ion transport
2558736	NM_001617	ADD2	0.000596648	1.50901	Control up vs N-RAS	GO:0006811	ion transport
2557759	NM_015463	CNRIP1	5.30E-05	1.54189	Control up vs N-RAS		
2781138	NM_016269	LEF1	4.55E-05	1.66127	Control up vs N-RAS	GO:0000122	negative regulation of transcription from RNA polymerase II
4044363	ENST00000536471	CNR2	0.000676923	1.82158	Control up vs N-RAS	GO:0001975	response to amphetamine
3657253	NM_016633	AHSP	3.27E-05	2.28921	Control up vs N-RAS	GO:0006457	protein folding
2997789	ENST00000447769	GPR141	0.00068832	2.31209	Control up vs N-RAS	GO:0007186	G-protein coupled receptor signaling pathway
2727587	NM_000222	KIT	8.43E-06	2.37021	Control up vs N-RAS	GO:0000187	activation of MAPK activity

Supplemental Figures



Supplemental Fig. S1. Infection of human hematopoietic stem progenitor cells with mutant N-RAS^{G12D}.

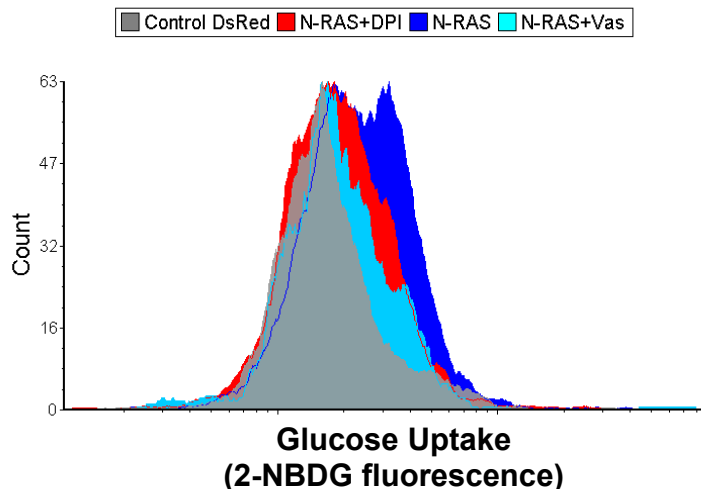
(A) Representative flow cytometric histograms showing percentage of CD34⁺ HSPC cells expressing GFP in cells infected with GFP alone (Control) or N-RAS^{G12D} (N-RAS)(n=7) analysed on day 3 of culture. GFP Histograms were gated to exclude cell debris based on forward and side scatter. Background auto fluorescence was established using HSPC subjected to the equivalent retroviral infection procedure but in the absence of retrovirus (mock). (B) Example western blot analysis of N-RAS protein expression in infected HSPCs. Actin was used as a loading control. (C) Superoxide production was measured using DiogenesTM (example chemiluminescent trace, left panel) in the presence or absence of NOX family inhibitor (diphenyleneiodonium, DPI) on day 7 (summary data (n=5), right panel). Cells were exposed to 100 nM DPI for 24 h prior to these measurements. (D) Bivariate flow cytometric data showing the survival of N-RAS infected HSPC compared to cells not treated with 100nm DPI (untreated) following 24 h incubation. Plots were gated to exclude small cell debris based on forward and side scatter. Quadrants delimit background auto fluorescence. (E) *Left panel*: Example Agilent Bioanalyzer 2100 electropherogram showing high RIN (RNA Integrity Number) indicating high quality RNA. *Right Panel*: Summary of RIN values and 28S/18S rRNA ratios. # indicates experimental replicate number. * denotes p<0.05, statistical significance was determined by Student's t-test.

A**B**

Supplemental Fig. S2. Over expression of *NOX2* mRNA in AML.

(A) *NOX2* (*CYBB*) mRNA expression data analysed using Bloodspot (<http://servers.binf.ku.dk/bloodspot/>) (2) and published online datasets. Human normal hematopoiesis data derived from GSE42519 (3). Data from Human AML blasts are derived from GSE13159 (4), GSE15434 (5), GSE61804 (6), GSE14468 (7) and The Cancer Genome Atlas (TCGA) (8). (B) Overall survival of AML patients stratified according to *NOX2* expression level using the AML TCGA dataset (excluding APL). ***Statistical significance analysed by Log-Rank. HSC, Hematopoietic stem cell Lin⁻ CD34⁺ CD38⁻ CD90⁺ CD45RA⁻; MPP, Multipotential progenitors Lin⁻ CD34⁺ CD38⁻ CD90⁻ 45RA⁻; CMP, Common myeloid progenitors cell Lin⁻ CD34⁺ CD38⁺ CD45RA⁻ CD123⁺; GMP, Granulocyte monocyte progenitors Lin⁻ CD34⁺ CD38⁺ CD45RA⁺ CD123⁺. Human AML subtype definitions are shown over-page.

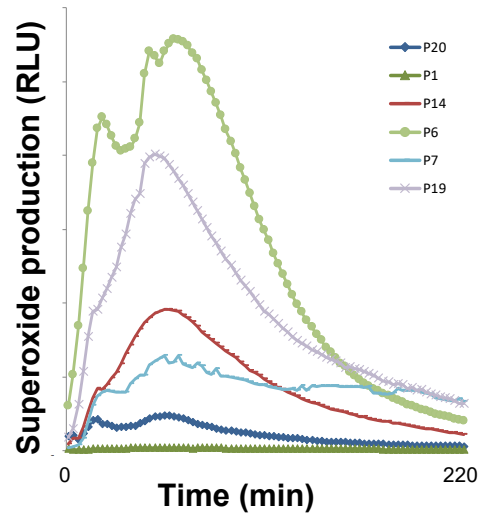
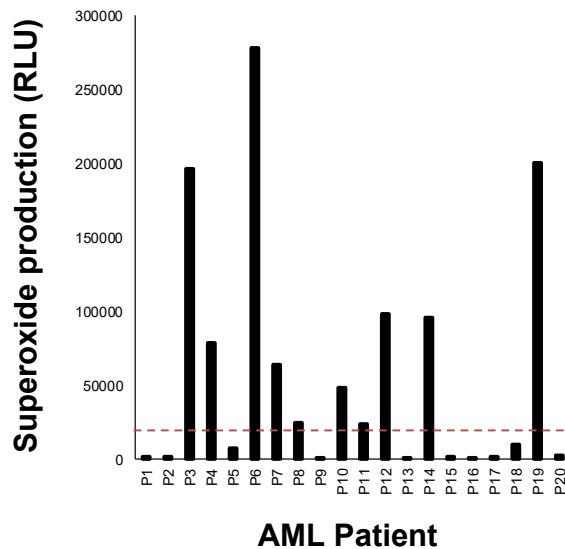
Normal	AML with Normal karyotype
Complex	AML with Complex karyotype
inv(16)	AML with inv(16)
t(15;17)	AML with t(15;17)
t(8;21)	AML with t(8;21)
t(11q23)/MLL	AML with t(11q23)/MLL
MDS	MDS
nan	AML with no karyotype information
Trisomy 8	AML with Trisomy 8
del(5q)	AML with del(5q)
del(7q)/7q-	AML with del(7q)/7q-
t(9;11)	AML with t(9;11)
Other	AML with Other abnormalities
7	AML with +7
Complex_ del(5q)	AML with Complex del(5q)
Complex_ untypical	AML with Complex untypical karyotype
ALL	ALL
inv(3)	AML with inv(3)
trisomy 11	AML with trisomy 11
trisomy 13	AML with trisomy 13
t(6;9)	AML with t(6;9)
t(8;16)	AML with t(8;16)
del(9q)	AML with del(9q)
t(1;3)	AML with t(1;3)
-5/7(q)	AML with -5/7(q)
-9q	AML with -9q
8	AML with +8
t(9;22)	AML with t(9;22)
abn(3q)	AML with abn(3q)



Supplemental Fig. S3. Glucose uptake in HSPC expressing mutant RAS.

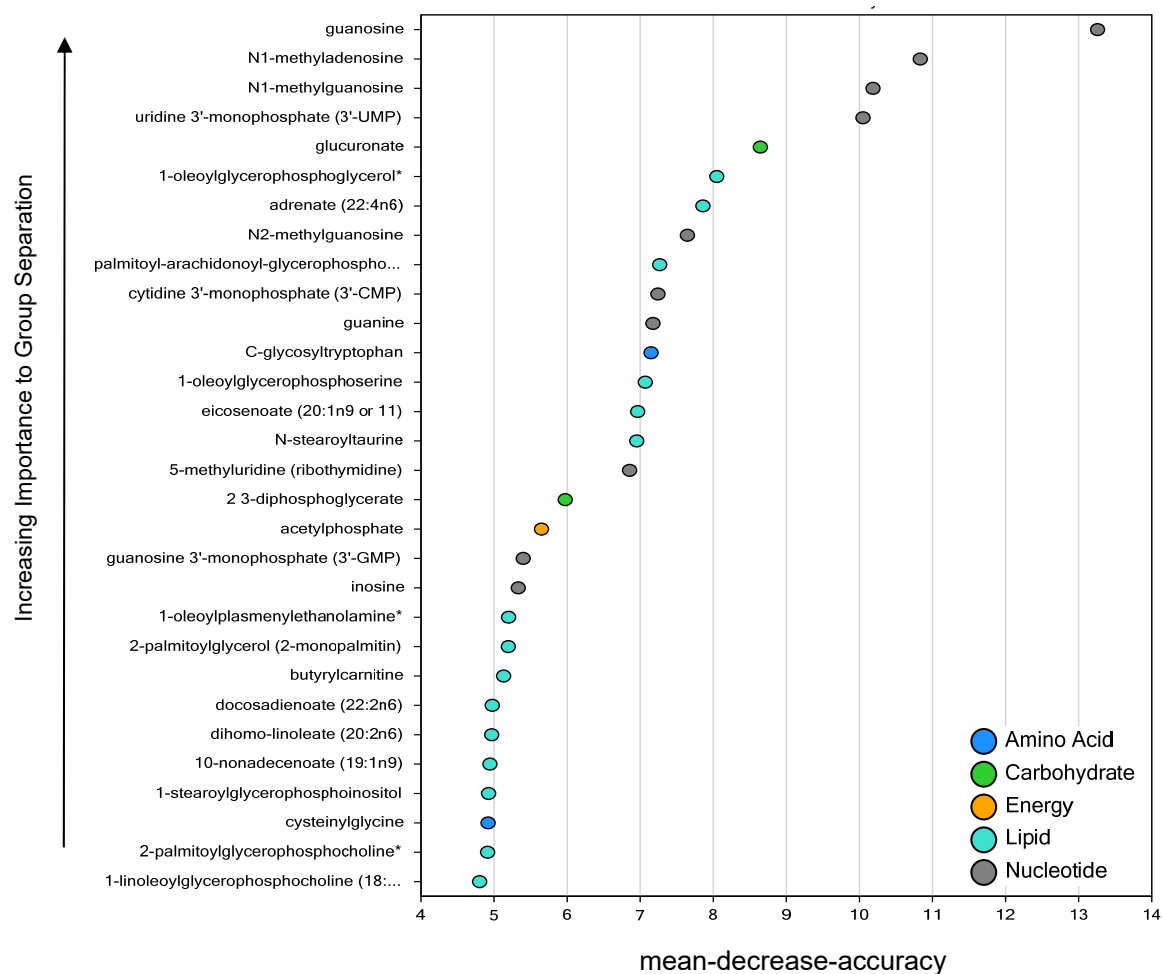
Representative flow cytometric histograms showing changes in glucose uptake in DsRed⁺ N-RAS^{G12D} cells compared to control or cells treated with NOX inhibitors (100nm DPI; 5 μ M VAS2870). Following infection, CD34⁺ cells were cultured for 3 further days (day 6 of development) at an initial seeding density of 1.5×10^5 cells/mL without growth factors. Glucose uptake was assayed at the single cell level using the glucose bioprobe 2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino)-2-D-deoxyglucose (2-NBDG) coupled with flow cytometry. Data were gated to exclude cell debris based on FSC/SSC.

2-NBDG is a fluorescent glucose derivative that is transported into the cell by glucose transporters and is phosphorylated to 2-NBDG-6-phosphate before being broken down into non-fluorescent derivatives. 2-NBDG emits fluorescence at a wavelength of 542nm which has a spectral overlap with GFP emission. Therefore, HSPC were alternatively infected with the PINCO retroviral expression vector that was modified to co-express DsRed instead of GFP.

A**B**

Supplemental Fig. S4. Validation of ROS production in AML patient blasts for the analysis of global biochemicals using ultra-high performance liquid chromatography-tandem MS (UPLC-MS/MS) and GC-MS.

Superoxide production was assayed from viable AML patients blasts *ex vivo* using Diogenes™ and chemiluminescence was detected using a Hidex® Chameleon luminometer every 5 min for up to 3 h. **(A)** Example chemiluminescent traces of a selection of AML blasts showing levels of superoxide production **from each patient (P)**. **(B)** Summary data of superoxide production in 20 primary AML blasts. AML patient blasts were stratified according to superoxide production above (AML-ROS^{High}) or below (AML-ROS^{Low}) the median (dotted line). RLU, Relative Light Units.



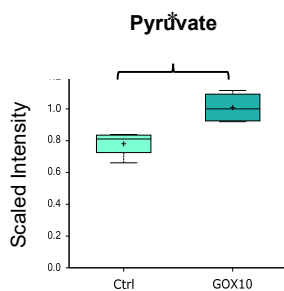
Supplemental Fig. S5. Random Forest analysis of AML ROS^{high} compared to AML ROS^{Low}.

Random Forest classification of AML-ROS^{High} compared to AML-ROS^{Low} gave a predictive accuracy of 85% based on their metabolic profiles which is better than random chance alone (50% accuracy for two groups), indicating that differences in biochemical profiles between groups may be sufficient for biomarker discovery.

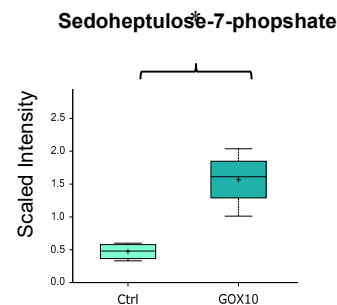
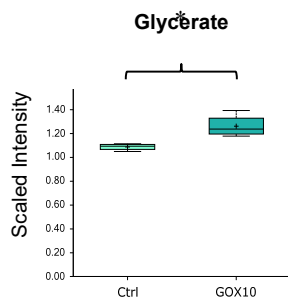
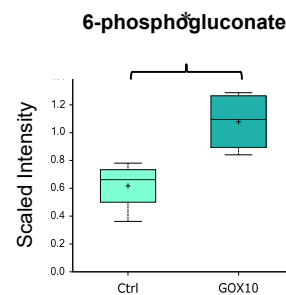
A

Biochemical Name	Platform	Comp ID	KEGG	HMDB	PubChem	GOX 10 Ctrl
fructose-6-phosphate	LC/MS polar	12021	C05345	HMDB00124	69507	0.71
Isobar: fructose 1,6-diphosphate, glucose 1,6-diphosphate,	LC/MS neg	46896				1.09
dihydroxyacetone phosphate (DHAP)	LC/MS pos early	15522	C00111	HMDB01473	668	0.85
3-phosphoglycerate	LC/MS neg	1414	C00597	HMDB00807	724	0.56
pyruvate	LC/MS polar	22250	C00022	HMDB00243	1060	1.29
lactate	LC/MS neg	527	C00186	HMDB00190	612	0.87
glycerate	LC/MS polar	1572	C00258	HMDB00139	752	1.16
6-phosphogluconate	LC/MS neg	15442	C00345	HMDB01316	91493	1.75
ribose 1-phosphate	LC/MS polar	1763	C00620	HMDB01489	439236	0.87
5-phosphoribosyl diphosphate (PRPP)	LC/MS neg	36840	C00119	HMDB00280	7339	0.90
sedoheptulose-7-phosphate	LC/MS neg	35649	C05382	HMDB01068	616	3.32

B (i)

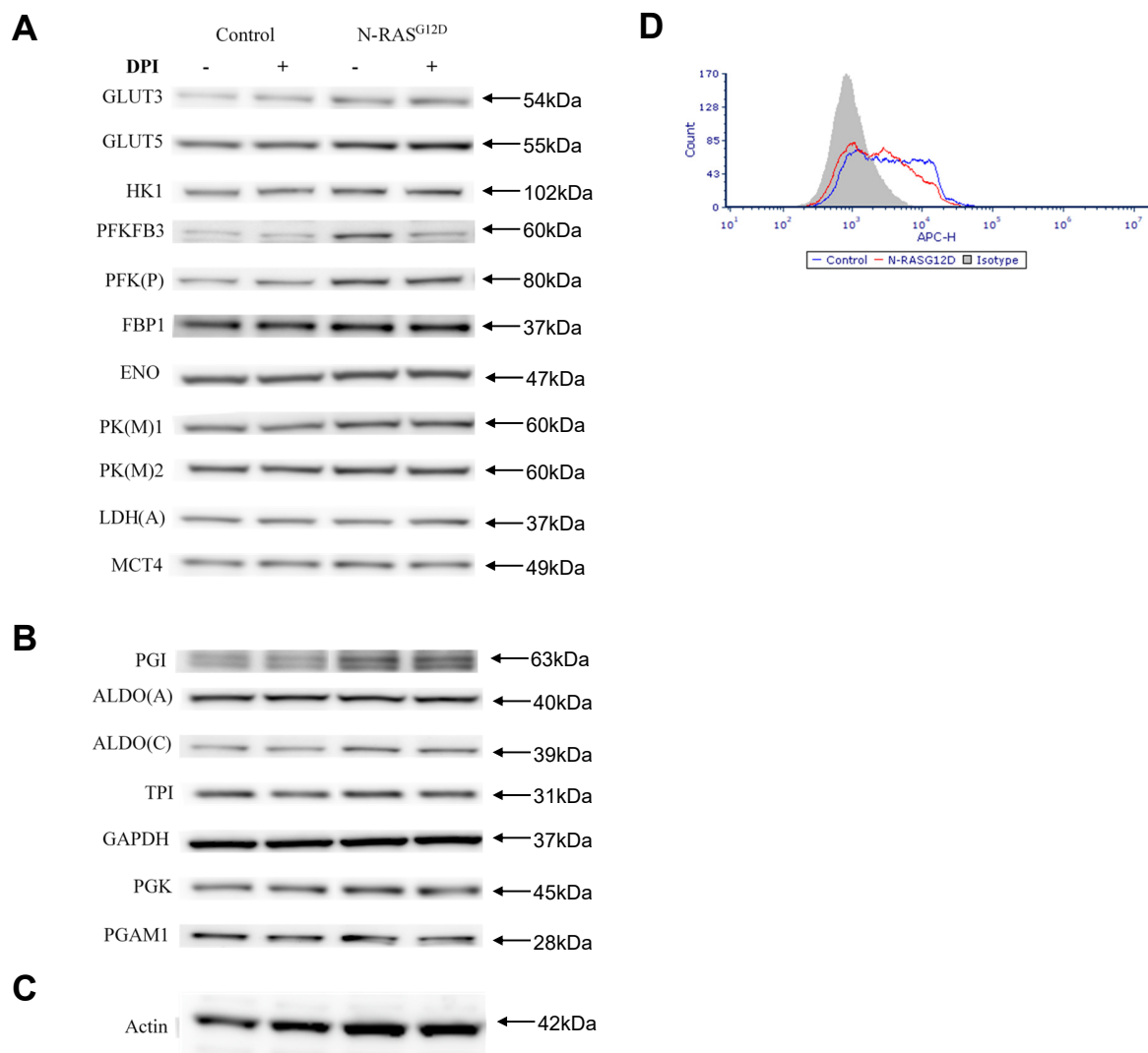


(ii)



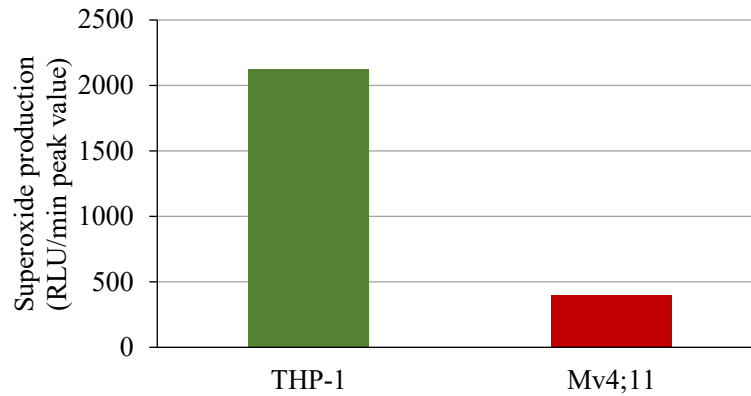
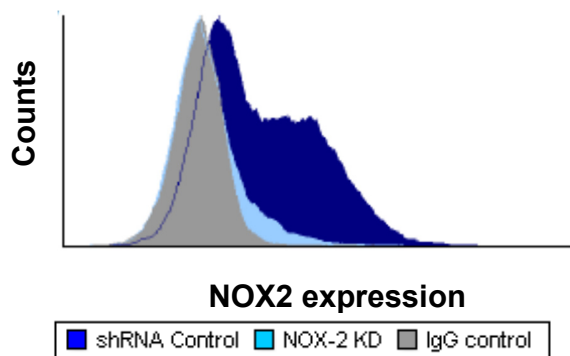
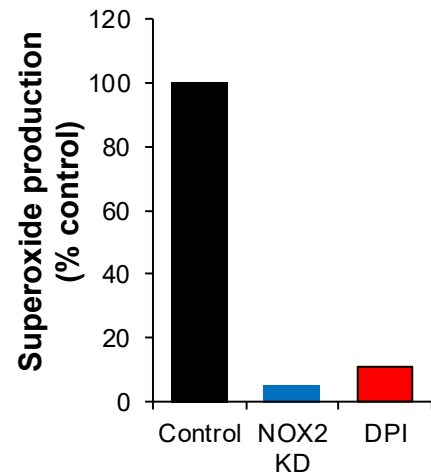
Supplemental Fig. S6. Treatment of the Mv4;11 AML cell line with H₂O₂ increases biochemical levels within the pentose phosphate pathway.

Data from biochemical profiling of Mv4;11 AML cells treated with 10 mU/mL glucose oxidase (GOX) or vehicle control (Ctrl) for 24hrs at 37°C followed by LC/MS analysis by Metabolon™ (A) Heat map of statistically significant biochemicals in Glycolysis, Gluconeogenesis, and Pyruvate Metabolism analysed by Welch's two sample t-Test. Red: indicates significant difference ($p \leq 0.05$) between the groups shown; metabolite ratio of ≥ 1.00 . Non-coloured text and cell: mean values are not significantly different for that comparison. (B) Levels of significant biochemicals normalised to total protein in (i) Glycolysis (ii) Pentose Phosphate Pathway (PPP).



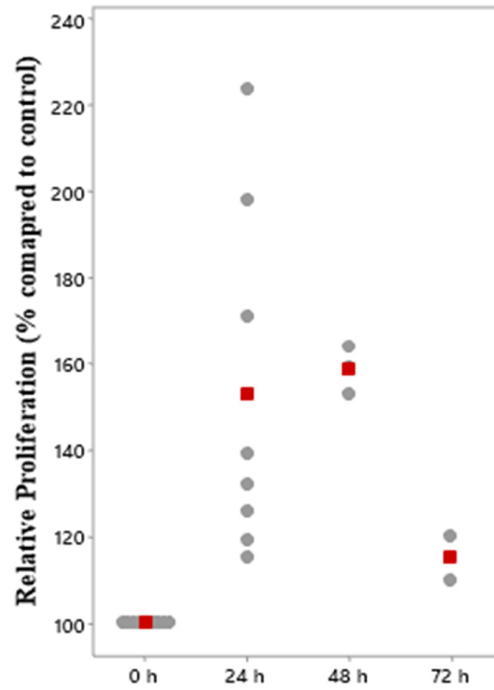
Supplemental Fig. S7. N-RAS^{G12D} and ROS dependent changes in protein expression of glycolytic enzymes.

Human HSPC control and N-RAS^{G12D} were incubated for 24h in the presence or absence of DPI (100nM) followed by whole cell protein extraction. Western blots showing expression (A) of ROS responsive genes identified in Figure 1C. (B) We also determined the protein expression of other glycolytic genes to determine if expression was affected by any non-transcriptional mechanisms. GLUT3 (SLC2A3), GLUT5 (SLC2A5), HK1, PGI (phosphoglucose isomerase), PFKFB3, PFK(P), FBP1, ALDO(A) (aldolase A), ALDO(C) (aldolase C), TPI (triphosphate isomerase), GAPDH (glyceraldehyde phosphate dehydrogenase), PGK (phosphoglycerate kinase), PGAM1 (phosphoglycerate mutase 1), ENO, PK(M)1 (pyruvate kinase muscle 1), PK(M)2 (pyruvate kinase muscle 2), LDH(A) and MCT4 (SLC16A3). (C) Actin was used as a loading control. (D) Glut5 cell surface protein expression measured by an indirect stain using anti-mouse IgG-APC. Note, no detectable expression was observed for Glut3. Gray filled histogram shows background isotype control.

A**B****C**

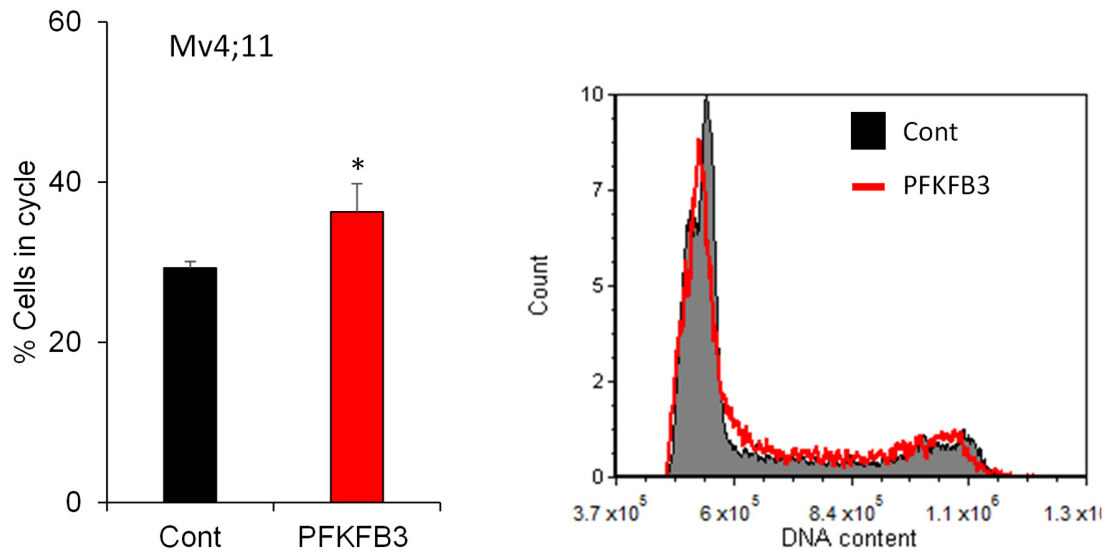
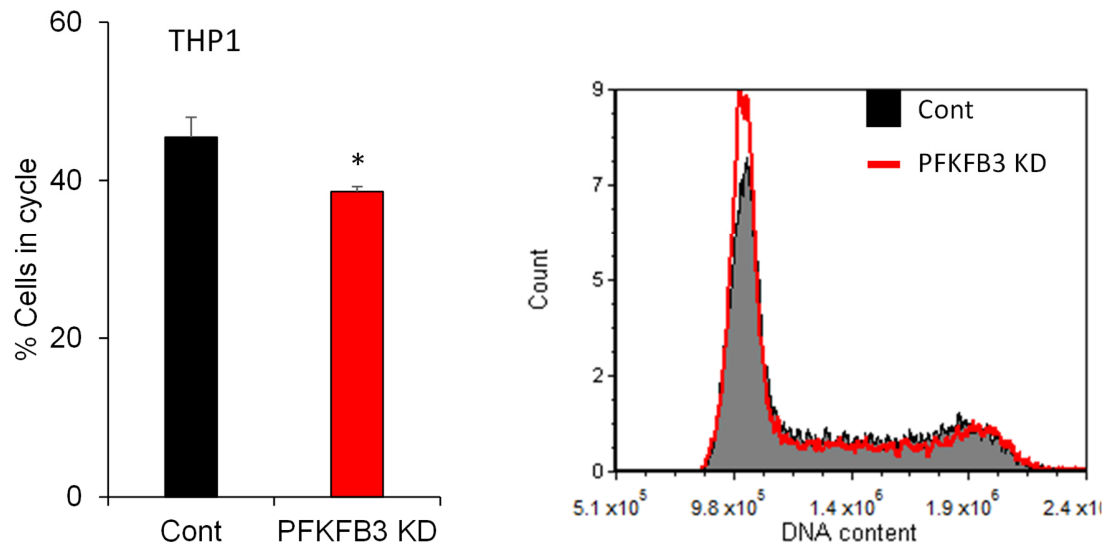
Supplemental Fig. S8. Validation of THP-1 AML cell line in which ROS production is ablated following NOX2 Knock down.

(A) Extracellular superoxide production in THP-1 and Mv4;11 AML cell lines. Peak superoxide production (relative luminescent units/min (RLU/min) measured using Diogenes™. (B) Cell surface NOX-2 expression in THP-1 cells transduced with Mission® Lentiviral shRNA vectors targeting non-mammalian gene (shRNA Control) or NOX2 (NOX2-KD) using anti-NOX2-PE conjugated antibody and compared to IgG1 isotype control. (C) Superoxide production (normalised to shRNA control) assayed using Diogenes™. Control THP-1 cells were also treated with DPI (100nM) overnight at 37°C.



Supplemental Fig. S9. Hydrogen peroxide (GOX) induces proliferation in Mv4;11 leukemia cells.

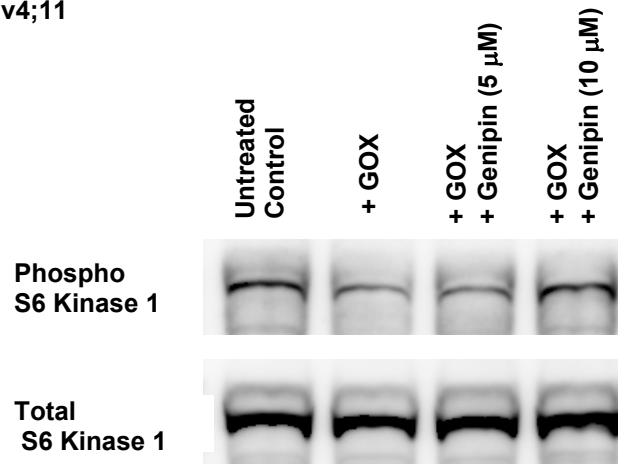
Percentage proliferation (normalised to control) of Mv4;11 cells treated with GOX for 24 h, 48 h or 72 h. Individual data points are shown as gray circles. Mean is indicated by red square. Note: proliferative response diminishes over time as GOX activity decreases.

A**B**

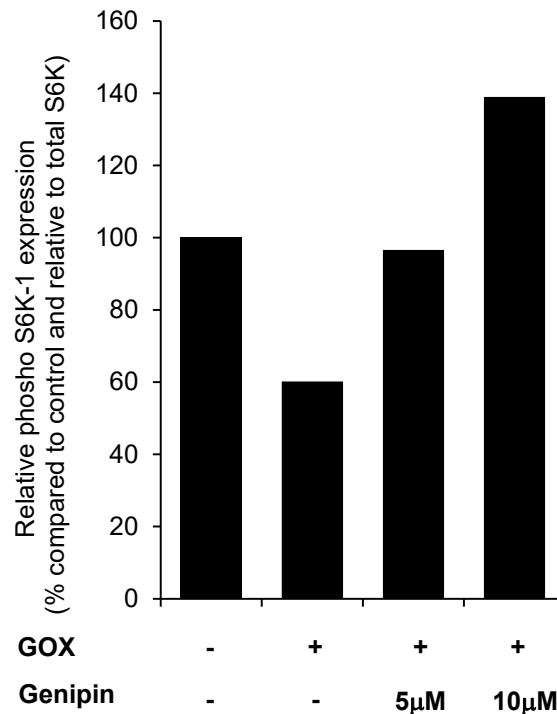
Supplemental Fig. S10. Effect of PFKFB3 overexpression and PFKFB3 knockdown on cell cycle distribution

(A) Effect of PFKFB3 overexpression on the cell cycle distribution of Mv4;11 cells with representative data in RH panel (n=3; p<0.05). (B) Effect of PFKFB3 knockdown on the cell cycle distribution of THP1 cells with representative data in RH panel (n=3; p<0.05).

A Mv4;11

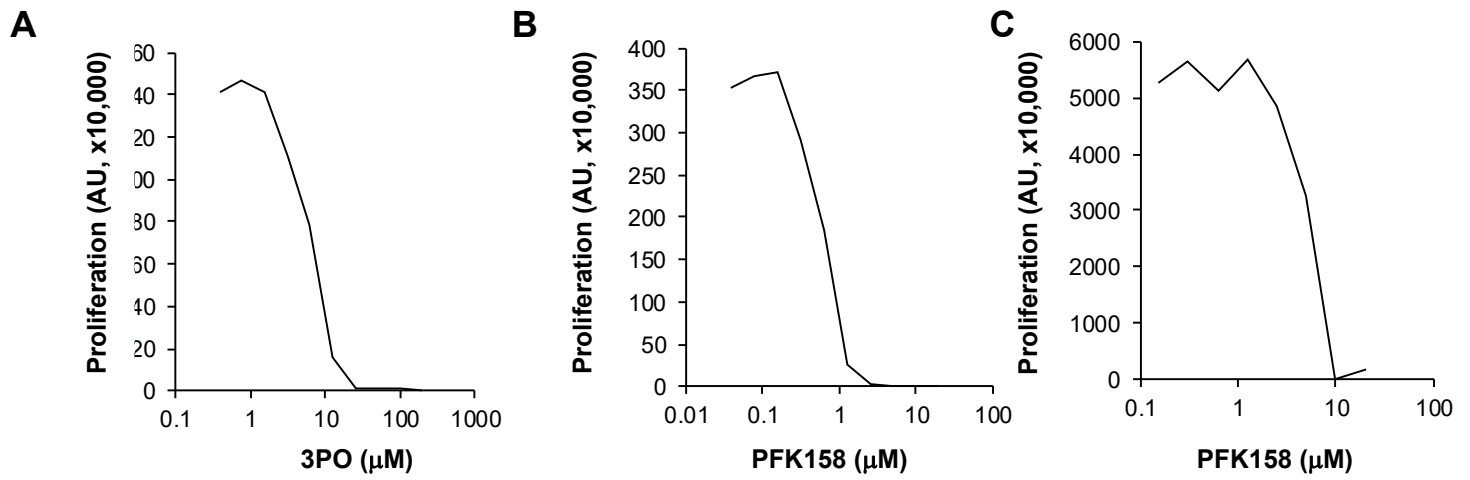


B



Supplemental Fig. S11. ROS induced changes in p-AMPK / mTORC1 pathway

(A) Western blot showing downstream activation of p-AMPK pathway upon 1 h pre-treatment of Mv4;11 cells with the UCP2 inhibitor Genipin (0-10 μM) followed by GOX treatment (20mU/mL) for 24 h. Membrane was re-probed with Total S6 Kinase 1. (B) Relative protein expression (as measured by pixel densitometry of equivalent regions of interest (ROI) between different samples on the same blot then normalised to total S6 Kinase and untreated control (n=1).



Supplemental Fig. S12. Determination of EC50 of 3PO and PFK158 on AML cell lines.

Mv4;11 cells were seeded at 1×10^5 and cultured for 24h in the presence of (A) 3PO or (B) PFK158 and subsequently assayed using CellTiter-Glo™ (n=1). (C) Similarly, THP-1 were seeded at 1×10^5 and cultured for 24h in the presence of PFK158 (n=1).

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