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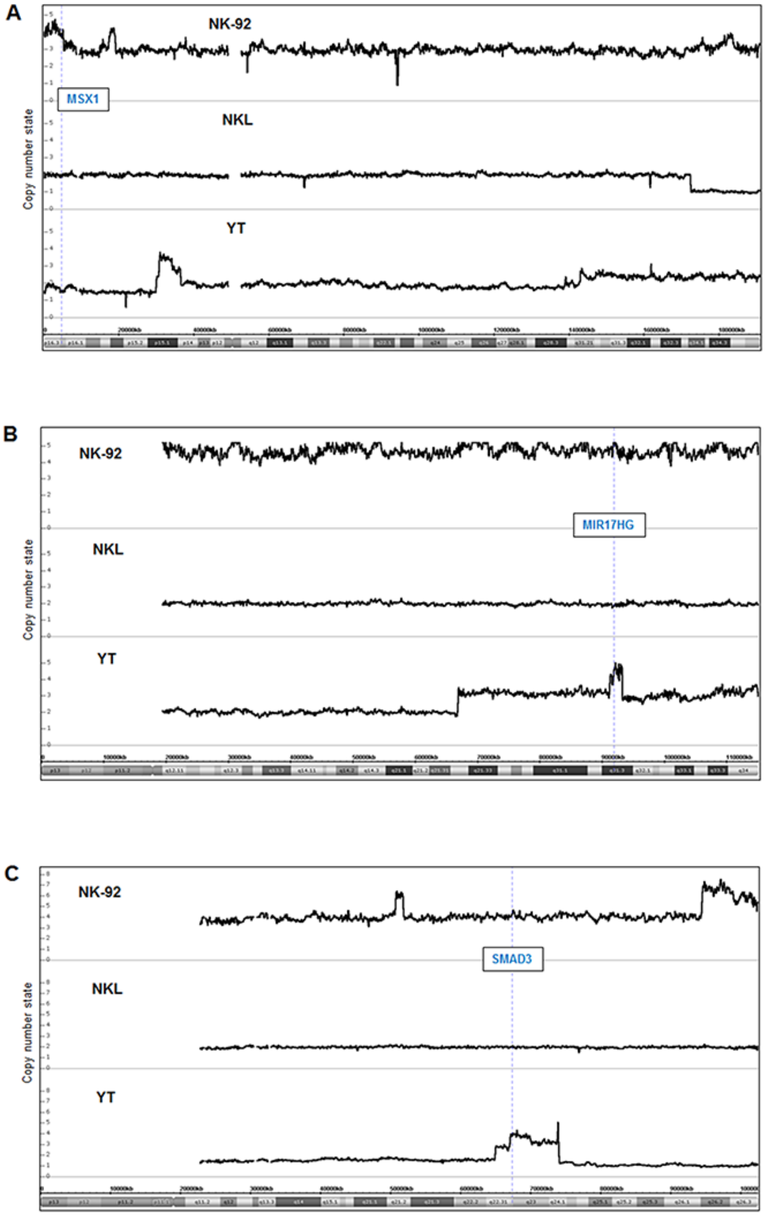
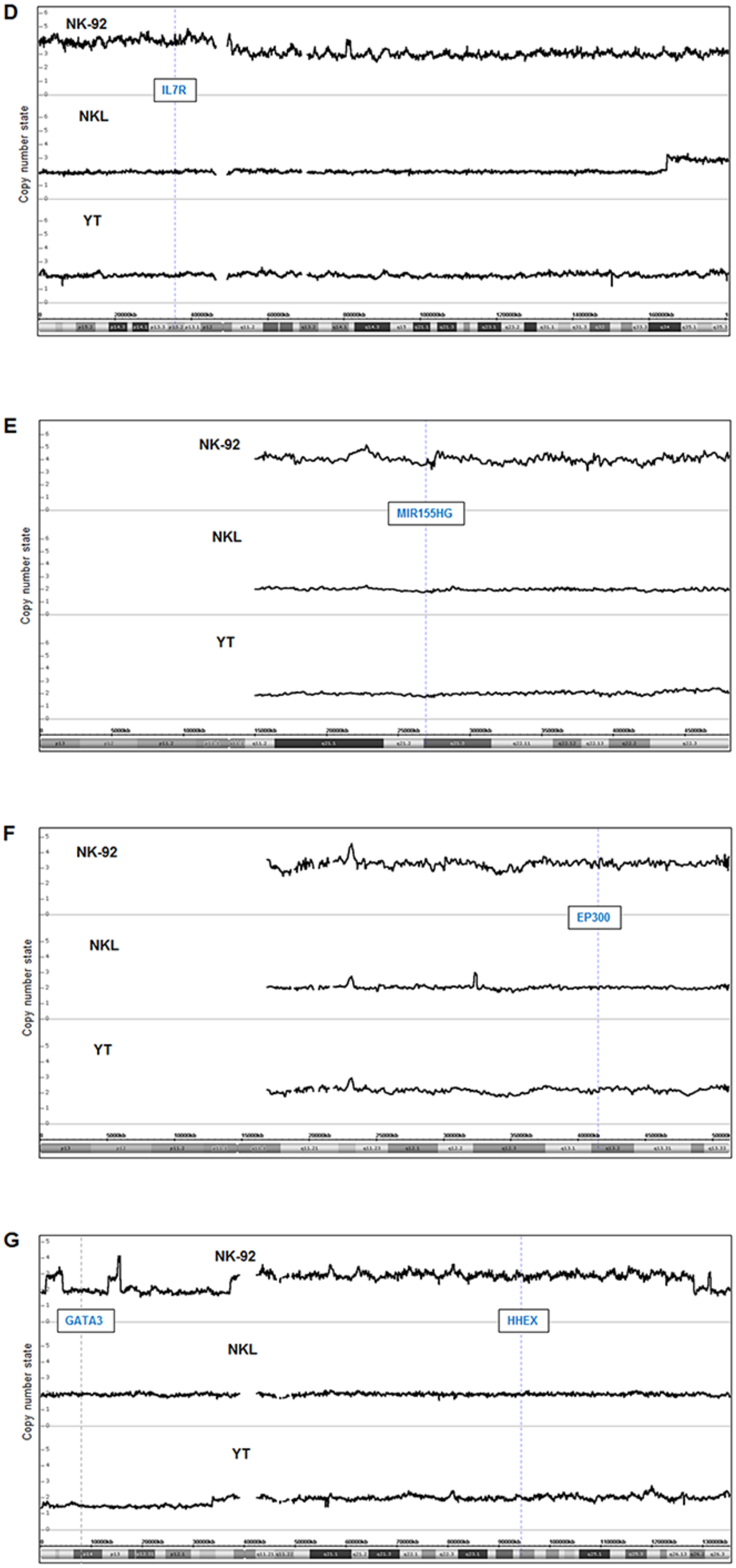
NKL homeobox gene MSX1 acts like a tumor suppressor in NK-cell leukemia

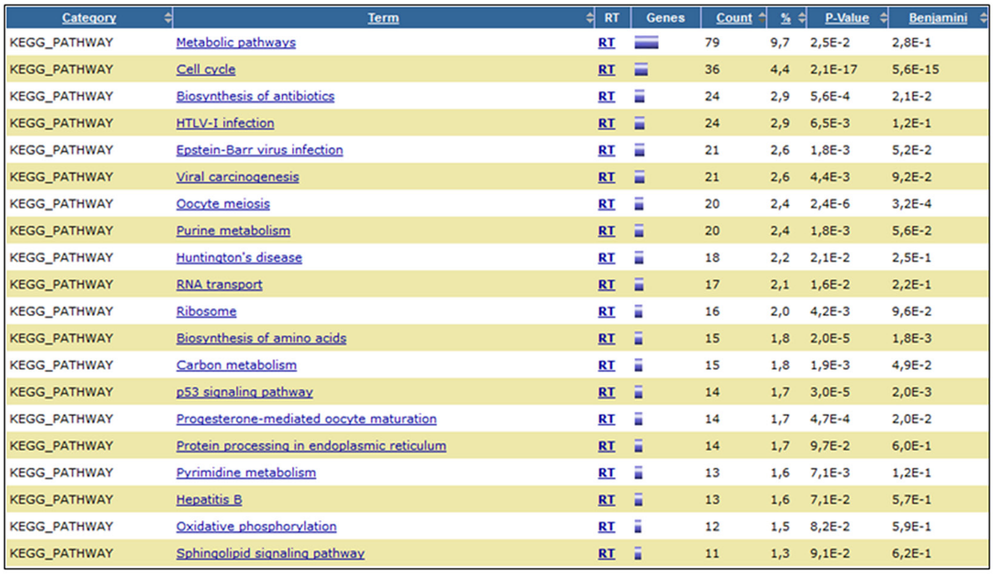
SUPPLEMENTARY MATERIALS



Supplementary Figure 1: Genomic profiling data. Data from selected chromosomes are shown to indicate copy number alterations and particular gene loci, including **(A)** MSX1, **(B)** MIR17HG, **(C)** SMAD3. (*Continued*)



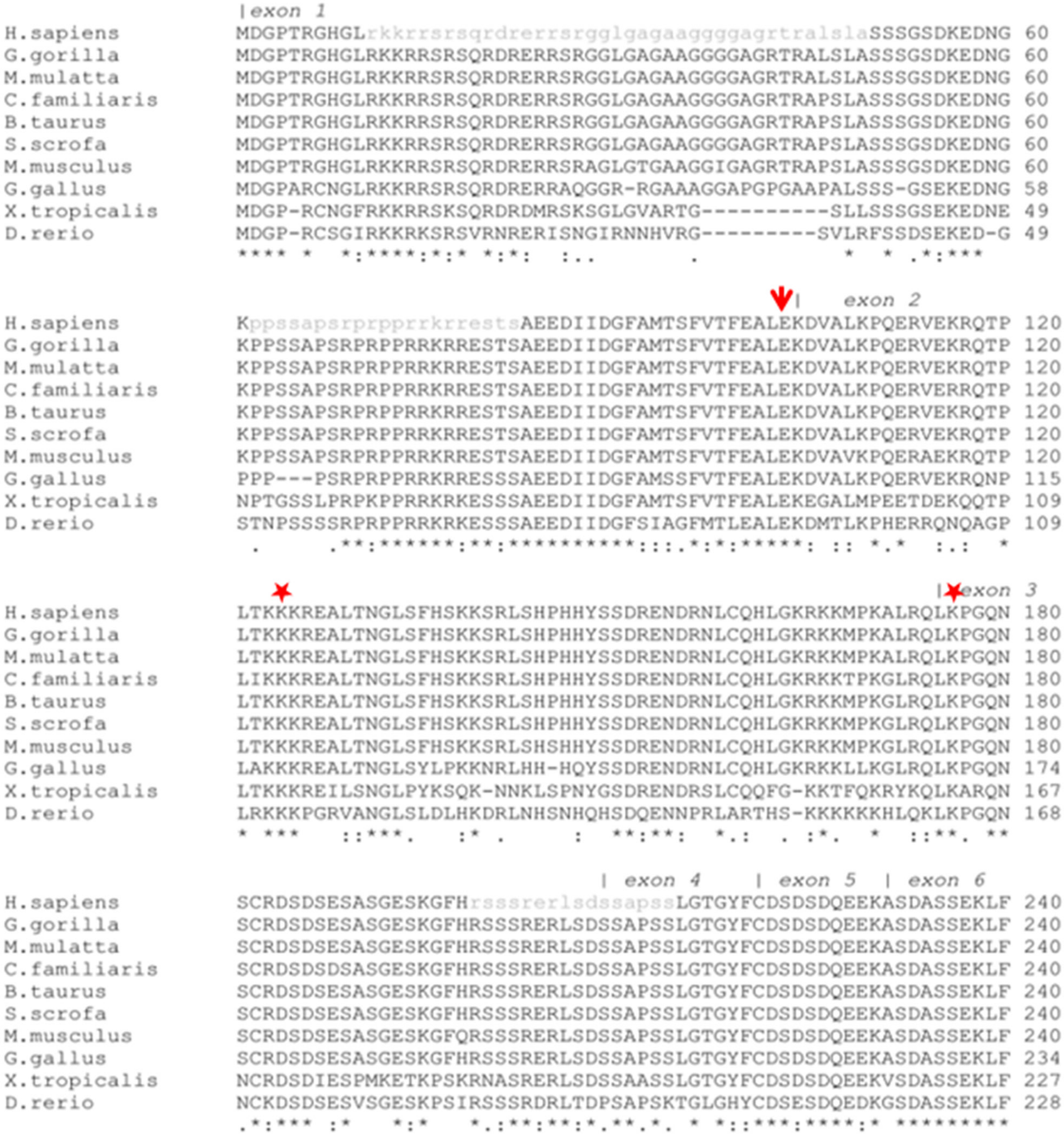
Supplementary Figure 1: Genomic profiling data. (***Continued***)Data from selected chromosomes are shown to indicate copy number alterations and particular gene loci, including **(D)** IL7R, **(E)** MIR155HG, **(F)** EP300, **(G)** GATA3 and HHEX.



Category	Term	RT	Genes	Count	%	P-Value	Benjamini
KEGG_PATHWAY	Metabolic pathways	RT	▬	79	9,7	2,5E-2	2,8E-1
KEGG_PATHWAY	Cell cycle	RT	▬	36	4,4	2,1E-17	5,6E-15
KEGG_PATHWAY	Biosynthesis of antibiotics	RT	▬	24	2,9	5,6E-4	2,1E-2
KEGG_PATHWAY	HTLV-I infection	RT	▬	24	2,9	6,5E-3	1,2E-1
KEGG_PATHWAY	Epstein-Barr virus infection	RT	▬	21	2,6	1,8E-3	5,2E-2
KEGG_PATHWAY	Viral carcinogenesis	RT	▬	21	2,6	4,4E-3	9,2E-2
KEGG_PATHWAY	Oocyte meiosis	RT	▬	20	2,4	2,4E-6	3,2E-4
KEGG_PATHWAY	Purine metabolism	RT	▬	20	2,4	1,8E-3	5,6E-2
KEGG_PATHWAY	Huntington's disease	RT	▬	18	2,2	2,1E-2	2,5E-1
KEGG_PATHWAY	RNA transport	RT	▬	17	2,1	1,6E-2	2,2E-1
KEGG_PATHWAY	Ribosome	RT	▬	16	2,0	4,2E-3	9,6E-2
KEGG_PATHWAY	Biosynthesis of amino acids	RT	▬	15	1,8	2,0E-5	1,8E-3
KEGG_PATHWAY	Carbon metabolism	RT	▬	15	1,8	1,9E-3	4,9E-2
KEGG_PATHWAY	p53 signaling pathway	RT	▬	14	1,7	3,0E-5	2,0E-3
KEGG_PATHWAY	Progesterone-mediated oocyte maturation	RT	▬	14	1,7	4,7E-4	2,0E-2
KEGG_PATHWAY	Protein processing in endoplasmic reticulum	RT	▬	14	1,7	9,7E-2	6,0E-1
KEGG_PATHWAY	Pyrimidine metabolism	RT	▬	13	1,6	7,1E-3	1,2E-1
KEGG_PATHWAY	Hepatitis B	RT	▬	13	1,6	7,1E-2	5,7E-1
KEGG_PATHWAY	Oxidative phosphorylation	RT	▬	12	1,5	8,2E-2	5,9E-1
KEGG_PATHWAY	Sphingolipid signaling pathway	RT	▬	11	1,3	9,1E-2	6,2E-1

Category	Term	RT	Genes	Count	%	P-Value	Benjamini
KEGG_PATHWAY	Pathways in cancer	RT	▬	23	3,5	1,5E-2	1,2E-1
KEGG_PATHWAY	Natural killer cell mediated cytotoxicity	RT	▬	20	3,0	2,5E-8	5,5E-6
KEGG_PATHWAY	Chemokine signaling pathway	RT	▬	18	2,7	2,0E-4	8,7E-3
KEGG_PATHWAY	Osteoclast differentiation	RT	▬	17	2,6	9,1E-6	9,7E-4
KEGG_PATHWAY	FoxO signaling pathway	RT	▬	16	2,4	4,9E-5	3,5E-3
KEGG_PATHWAY	MAPK signaling pathway	RT	▬	16	2,4	2,9E-2	1,7E-1
KEGG_PATHWAY	Tuberculosis	RT	▬	15	2,3	2,9E-3	4,4E-2
KEGG_PATHWAY	Endocytosis	RT	▬	15	2,3	5,9E-2	2,6E-1
KEGG_PATHWAY	Leukocyte transendothelial migration	RT	▬	14	2,1	1,9E-4	9,9E-3
KEGG_PATHWAY	Rap1 signaling pathway	RT	▬	14	2,1	2,8E-2	1,8E-1
KEGG_PATHWAY	Non-alcoholic fatty liver disease (NAFLD)	RT	▬	13	2,0	5,5E-3	6,8E-2
KEGG_PATHWAY	Focal adhesion	RT	▬	13	2,0	5,0E-2	2,5E-1
KEGG_PATHWAY	Regulation of actin cytoskeleton	RT	▬	13	2,0	5,8E-2	2,6E-1
KEGG_PATHWAY	Ras signaling pathway	RT	▬	13	2,0	8,6E-2	3,1E-1
KEGG_PATHWAY	Cytokine-cytokine receptor interaction	RT	▬	13	2,0	9,5E-2	3,2E-1
KEGG_PATHWAY	T cell receptor signaling pathway	RT	▬	12	1,8	7,5E-4	1,8E-2
KEGG_PATHWAY	Sphingolipid signaling pathway	RT	▬	12	1,8	2,6E-3	4,6E-2
KEGG_PATHWAY	Proteoglycans in cancer	RT	▬	12	1,8	8,1E-2	3,0E-1
KEGG_PATHWAY	NF-kappa B signaling pathway	RT	▬	11	1,7	7,3E-4	2,0E-2
KEGG_PATHWAY	Neurotrophin signaling pathway	RT	▬	11	1,7	7,9E-3	8,2E-2
KEGG_PATHWAY	Adrenergic signaling in cardiomyocytes	RT	▬	11	1,7	2,8E-2	1,8E-1
KEGG_PATHWAY	Transcriptional misregulation in cancer	RT	▬	11	1,7	6,3E-2	2,6E-1
KEGG_PATHWAY	Influenza A	RT	▬	11	1,7	7,6E-2	2,9E-1
KEGG_PATHWAY	Fc epsilon RI signaling pathway	RT	▬	10	1,5	4,8E-4	1,7E-2
KEGG_PATHWAY	B cell receptor signaling pathway	RT	▬	10	1,5	5,3E-4	1,6E-2
KEGG_PATHWAY	Fc gamma R-mediated phagocytosis	RT	▬	10	1,5	2,2E-3	4,2E-2
KEGG_PATHWAY	Choline metabolism in cancer	RT	▬	10	1,5	7,6E-3	8,7E-2
KEGG_PATHWAY	Chagas disease (American trypanosomiasis)	RT	▬	10	1,5	9,1E-3	9,0E-2
KEGG_PATHWAY	Platelet activation	RT	▬	10	1,5	3,4E-2	1,9E-1
KEGG_PATHWAY	Tight junction	RT	▬	10	1,5	4,5E-2	2,3E-1
KEGG_PATHWAY	Cell adhesion molecules (CAMs)	RT	▬	10	1,5	5,5E-2	2,6E-1
KEGG_PATHWAY	Hepatitis B	RT	▬	10	1,5	6,1E-2	2,5E-1
KEGG_PATHWAY	Oxytocin signaling pathway	RT	▬	10	1,5	9,3E-2	3,2E-1

Supplementary Figure 2: Gene-annotation enrichment analysis data (DAVID). The top 1000 differentially expressed genes between NK-cell lines and primary NK-cells were analyzed to identify deregulated cell functions according to KEGG pathways. Aberrantly activated functions are shown above, suppressed functions below.



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                  |exon 1
H.sapiens         MDGPTRGHGLrkkrrsrsqrdrerrsrgglgagaaggggagrtralslaSSSGSDKEDNG 60
G.gorilla         MDGPTRGHGLRKKRRSRSQRDRERRSRGGLGAGAAGGGGAGRTRALSLASSSGSDKEDNG 60
M.mulatta         MDGPTRGHGLRKKRRSRSQRDRERRSRGGLGAGAAGGGGAGRTRALSLASSSGSDKEDNG 60
C.familiaris      MDGPTRGHGLRKKRRSRSQRDRERRSRGGLGAGAAGGGGAGRTRAPSLASSSGSDKEDNG 60
B.taurus          MDGPTRGHGLRKKRRSRSQRDRERRSRGGLGAGAAGGGGAGRTRAPSLASSSGSDKEDNG 60
S.scrofa          MDGPTRGHGLRKKRRSRSQRDRERRSRGGLGAGAAGGGGAGRTRAPSLASSSGSDKEDNG 60
M.musculus        MDGPTRGHGLRKKRRSRSQRDRERRSRAGLGTGAAGGIGAGRTRAPSLASSSGSDKEDNG 60
G.gallus          MDGPARCNGLRKKRRSRSQRDRERRAQGGR-RGAAAGGAPGPGAAPALSSS-GSEKEDNG 58
X.tropicalis      MDGP-RCNGFRKKRRSKSQRDRDMRSKSGLGVARTG----------SLLSSSGSEKEDNE 49
D.rerio           MDGP-RCSGIRKKRKSRSVRNRERISNGIRNNHVRG---------SVLRFSSDSEKED-G 49
                  **** *  *:****:*:* *:*:  :..       .           *  * .*:***

                                                           |    exon 2
H.sapiens         KppssapsrprpprrkrrestsAEEDIIDGFAMTSFVTFEALEKDVALKPQERVEKRQTP 120
G.gorilla         KPPSSAPSRPRPPRRKRRESTSAEEDIIDGFAMTSFVTFEALEKDVALKPQERVEKRQTP 120
M.mulatta         KPPSSAPSRPRPPRRKRRESTSAEEDIIDGFAMTSFVTFEALEKDVALKPQERVEKRQTP 120
C.familiaris      KPPSSAPSRPRPPRRKRRESTSAEEDIIDGFAMTSFVTFEALEKDVALKPQERVERRQTP 120
B.taurus          KPPSSAPSRPRPPRRKRRESTSAEEDIIDGFAMTSFVTFEALEKDVALKPQERVEKRQTP 120
S.scrofa          KPPSSAPSRPRPPRRKRRESTSAEEDIIDGFAMTSFVTFEALEKDVALKPQERVEKRQTP 120
M.musculus        KPPSSAPSRPRPPRRKRRESTSAEEDIIDGFAMTSFVTFEALEKDVAVKPQERAEKRQTP 120
G.gallus          PPP---PSRPRPPRRKRRESSSAEEDIIDGFAMSSFVTFEALEKDVALKPQERVEKRQNP 115
X.tropicalis      NPTGSSLPRPKPPRRKRKESSSAEEDIIDGFAMTSFVTFEALEKEGALMPEETDEKQQTP 109
D.rerio           STNPSSSSRPRPPRRKRKESSSAEEDIIDGFSIAGFMTLEALEKDMTLKPHERRQNQAGP 109
                   .     .**:******:**:**********:::.*:*:*****: :: *.*  :.:  *

                                                                        |exon 3
H.sapiens         LTKKKREALTNGLSFHSKKSRLSHPHHYSSDRENDRNLCQHLGKRKKMPKALRQLKPGQN 180
G.gorilla         LTKKKREALTNGLSFHSKKSRLSHPHHYSSDRENDRNLCQHLGKRKKMPKALRQLKPGQN 180
M.mulatta         LTKKKREALTNGLSFHSKKSRLSHPHHYSSDRENDRNLCQHLGKRKKMPKALRQLKPGQN 180
C.familiaris      LIKKKREALTNGLSFHSKKSRLSHPHHYSSDRENDRNLCQHLGKRKKTPKGLRQLKPGQN 180
B.taurus          LTKKKREALTNGLSFHSKKSRLSHPHHYSSDRENDRNLCQHLGKRKKMPKGLRQLKPGQN 180
S.scrofa          LTKKKREALTNGLSFHSKKSRLSHPHHYSSDRENDRNLCQHLGKRKKMPKGLRQLKPGQN 180
M.musculus        LTKKKREALTNGLSFHSKKSRLSHSHHYSSDRENDRNLCQHLGKRKKMPKGLRQLKPGQN 180
G.gallus          LAKKKREALTNGLSYLPKKNRLHH-HQYSSDRENDRNLCQHLGKRKKLLKGLRQLKPGQN 174
X.tropicalis      LTKKKREILSNGLPYKSQK-NNKLSPNYGSDRENDRSLCQQFG-KKTFQKRYKQLKARQN 167
D.rerio           LRKKKPGRVANGLSLDLHKDRLNHSNHQHSDQENNPRLARTHS-KKKKKKHLQKLKPGQN 168
                  * ***   ::***.   :* .     :  **:**:  *.:  . :*.  *  ::**. **

                                            | exon 4     | exon 5   | exon 6
H.sapiens         SCRDSDSESASGESKGFHrsssrerlsdssapssLGTGYFCDSDSDQEEKASDASSEKLF 240
G.gorilla         SCRDSDSESASGESKGFHRSSSRERLSDSSAPSSLGTGYFCDSDSDQEEKASDASSEKLF 240
M.mulatta         SCRDSDSESASGESKGFHRSSSRERLSDSSAPSSLGTGYFCDSDSDQEEKASDASSEKLF 240
C.familiaris      SCRDSDSDSASGESKGFHRSSSRERLSDSSAPSSLGTGYFCDSDSDQEEKASDASSEKLF 240
B.taurus          SCRDSDSESASGESKGFHRSSSRERLSDSSAPSSLGTGYFCDSDSDQEEKASDASSEKLF 240
S.scrofa          SCRDSDSESASGESKGFHRSSSRERLSDSSAPSSLGTGYFCDSDSDQEEKASDASSEKLF 240
M.musculus        SCRDSDSESASGESKGFQRSSSRERLSDSSAPSSLGTGYFCDSDSDQEEKASDASSEKLF 240
G.gallus          SCRDSDSESASGESKGFHRSSSRERLSDSSAPSSLGTGYFCDSDSDQEEKASDASSEKLF 234
X.tropicalis      NCRDSDIESPMKETKPSKRNASRERLSDSSAASSLGTGYFCDSDSDQEEKVSDASSEKLF 227
D.rerio           NCKDSDSESVSGESKPSIRSSSRDRLTDPSAPSKTGLGHYCDSESDQEDKGSDASSEKLF 228
                  .*:*** :*   *:*   *.:**:**:*.**.*. * *::***:****:* *********
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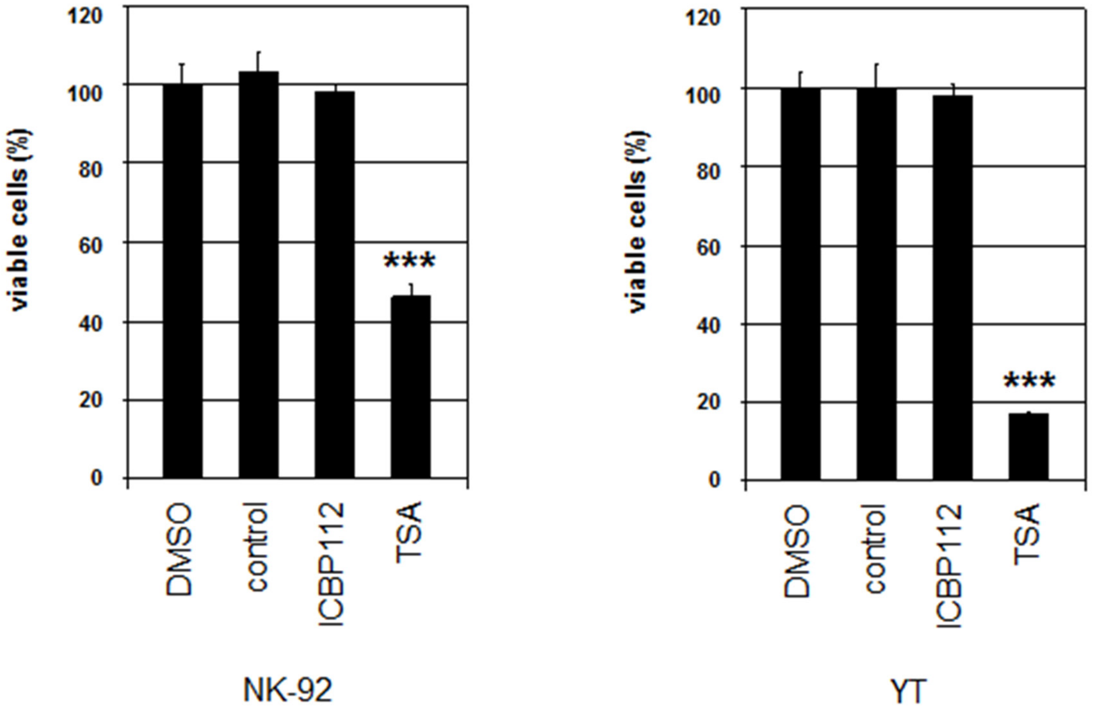
Supplementary Figure 3: AUTS2 alignment. The illustrated alignment was obtained from Beunders et al. (2013) [50], showing the N-terminal part of AUTS2 from the indicated vertebrates. Conserved amino acid residues are indicated by an asterisk, similar residues by dots. The red stars indicate the here identified mutated positions, the red arrow marks the position of deduced protein truncation.



Supplementary Figure 4: Cell viability analysis. MTT assay was performed in NK-92 (left) and YT (right) after treatment with histone acetyltransferase inhibitor ICBP112 and histone deacetylase inhibitor TSA. The statistical significance is indicated by asterisks.

Supplementary Table 1: Comparative expression profiling. The data demonstrate gene activities from primary NK-cells and NK-cell lines, showing (A) overexpressed and (B) downregulated genes between these two groups. Genes analyzed in this study are indicated in red and green, respectively.

See Supplementary Files 1A and 1B