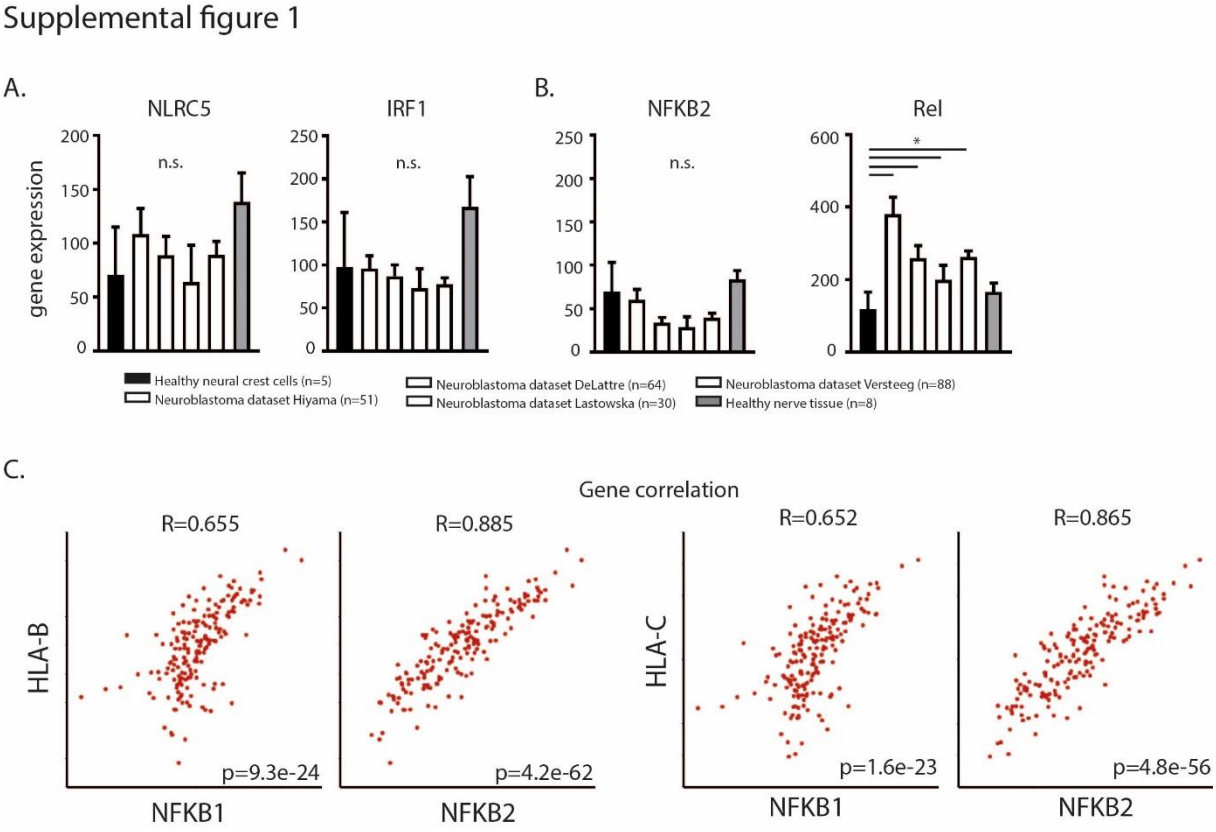
Supplemental Materials and Methods

Antibodies. anti-CD3 (Clone UCHT1, Sony Biotechnology); anti-CD8 (Clone RPA-T8, BD Biosciences); anti-LAMP1 (Clone H4A3, BD Biosciences); anti-TNF (Clone MAB11, Sony Biotechnology); anti-IFNγ (Clone 4S.B3, BD Biosciences); mouse-anti-human HLA-ABC (Clone W6/32, Biolegend); anti-actin (polyclonal, Santa Cruz); anti-RelA (Clone D14E12, Cell Signaling Techniques); anti-phospho-RelA (Clone 93H1, Cell Signaling Techniques); anti-RelB (Clone C1E4, Cell Signaling Techniques); anti-Rel (Clone D3B8S, Cell Signaling Techniques); anti-p100/p52 (Clone 18D10, Cell Signaling Techniques); anti-p105/p50 (Clone D7H5M, Cell Signaling Techniques); anti-IκBa (Clone 112B2, Cell Signaling Techniques); anti-TRAF3 (polyclonal, Cell Signaling Techniques); anti-NIK (polyclonal, Cell Signaling Techniques).

Plasmids and gRNA sequences. N4BP1- and TNIP1-knockout cells were generated using pXPR_001 (Addgene) and following gRNAs sequences: for N4BP1; 5'-(g)CTCCAAAGACCATCCGGGC-3' and 5'-(g)ATCTTTAACTCCTGCCCGGA-3' and for TNIP1; 5'–(g)ACTGGTGCTGGCTTGTCACT-3' and 5'-GGCTTGTCACTGGGGCATGC-3'. Where (g) represents a non-annealing nucleotide that was added to the actual gRNA to initiate gRNA transcription. Empty pxpr_001 vector was used as negative control. Mutations in ΔN4BP1 and ΔTNIP1 cell lines were validated by PCR using primers: Fw_N4BP1: 5'–GTTGTTTTTGCCTTAGTATGGGTCTTGC-3' ; Rv_N4BP1: 5'–ACCTACCAACCAGACTACAATATCTGC-3' ; Fw_TNIP1: 5'-AGGCCATTTTCTCAGACTACCTGG-3' ; Rv_TNIP1: 5'-CGATGGACTTGCCCAATATCACC-3'.
RelA-, RelB-, p100- and p105-knockout cells were generated using pL-CRISPR.SFFV.tRFP (Addgene) and gRNA sequences 5'–GCTTCCGCTACAAGTGCGAG-3' and 5'-(g)AGCGCCCCTCGCACTTGTAG-3' for RelA; 5'–GCCACGCCTGGTGTCTCGCG-3' and 5'–(g)TGGGGACACTAGTCGGCCCA-3' for RelB; 5'–(g)CCATCCCATGGTGGACTACC-3' and 5'-GGCACCAGGTAGTCCACCAT-3' for NFKB1; 5'–(g)AGAGGCTTCCGATTTCGATA-3' and 5'– (g)CTTCACAGCCATATCGAAAT-3' for NFKB2. N4BP1-His6 was purchased from Source Bioscience.

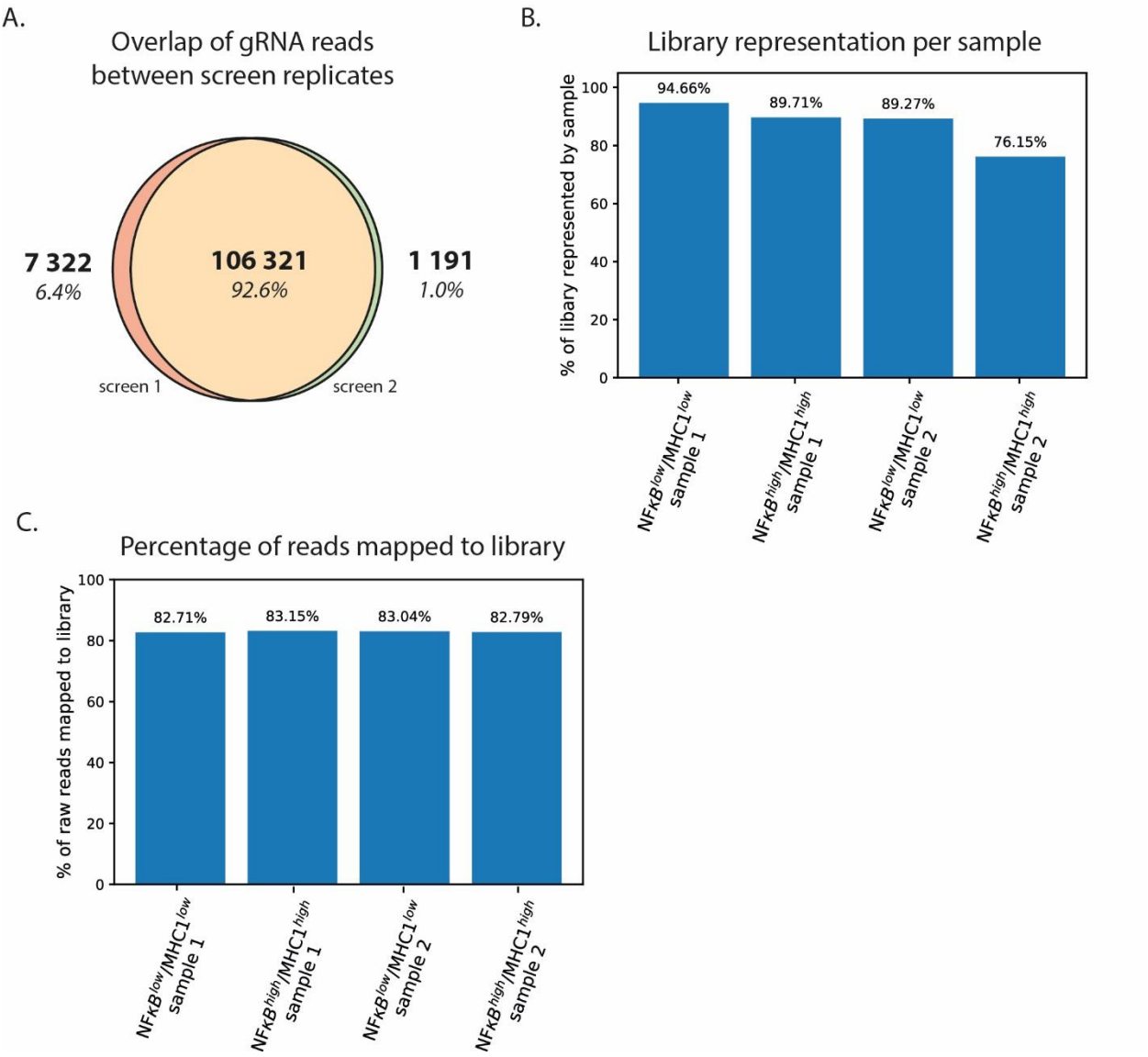
Gene expression analysis. Gene datasets were selected from the Gene Expression Omnibus: neural crest cells (GSE14340), neuroblastoma Hiyama cohort (GSE16237), neuroblastoma DeLattre cohort (GSE12460), neuroblastoma Lastowska cohort (GSE13136), neuroblastoma Versteeg cohort (GSE16476) and healthy nerve tissue (GSE7307). Gene expression data (U133 P2 microarray chip, MAS5.0 normalization) was accessed through the Genomics Analysis and Visualization Platform (r2.amc.nl).

Supplemental figure 1



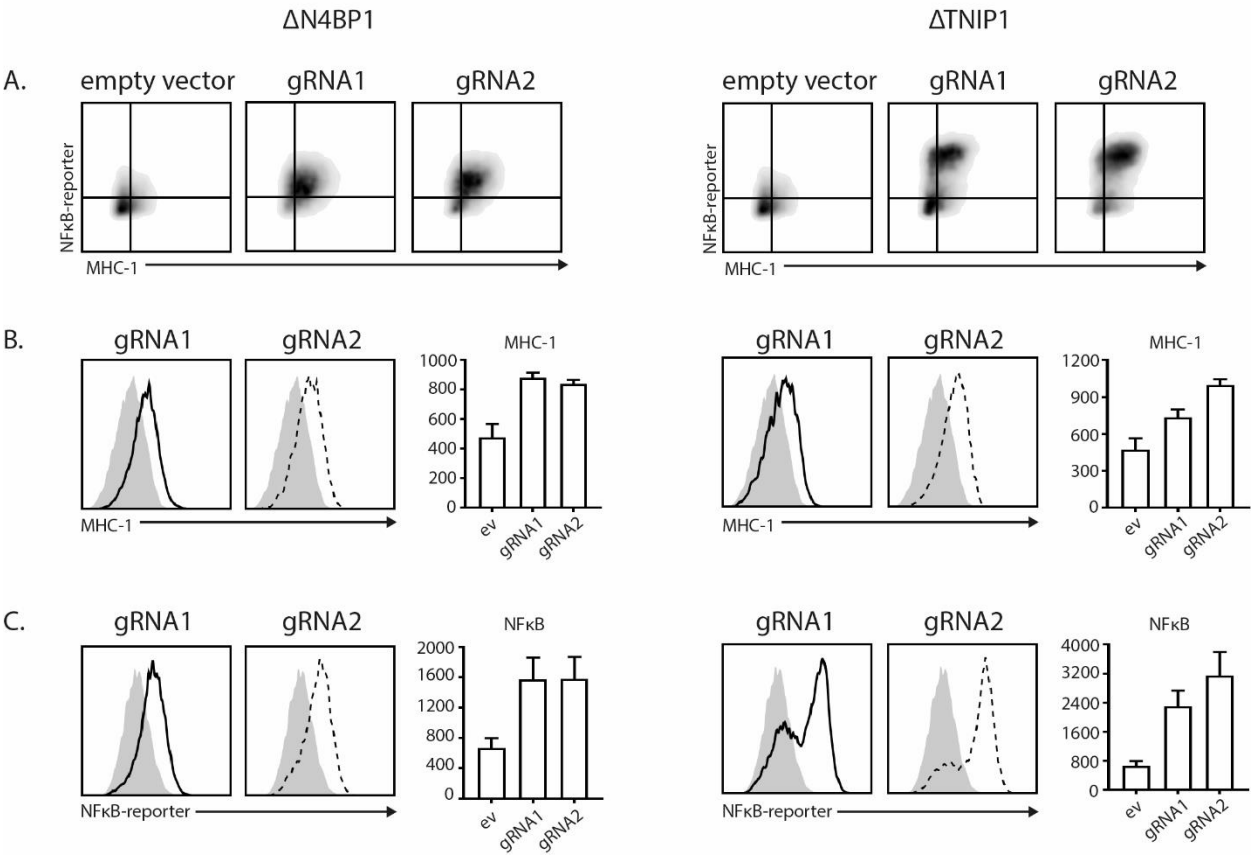
Supplemental figure 1. Gene expression of transcription factors regulating MHC-1 in neuroblastoma. Gene expression datasets of neural crest cells, healthy nerve tissue and neuroblastoma tumors were analyzed for the expression of NLRC5, IRF1 (A) and NF-κB alleles NFKB2 and REL (B). Two-way ANOVA test was used, $p<0.05$ was considered significant. C. Gene expression of MHC-1 alleles HLA-B and HLA-C in neuroblastoma tumor samples (cohort of 183 stage-4 neuroblastoma patients) was correlated with NFKB genes. Significance test for the Pearson correlation coefficient r was conducted, $p<0.5$ was considered significant.

Supplemental figure 2



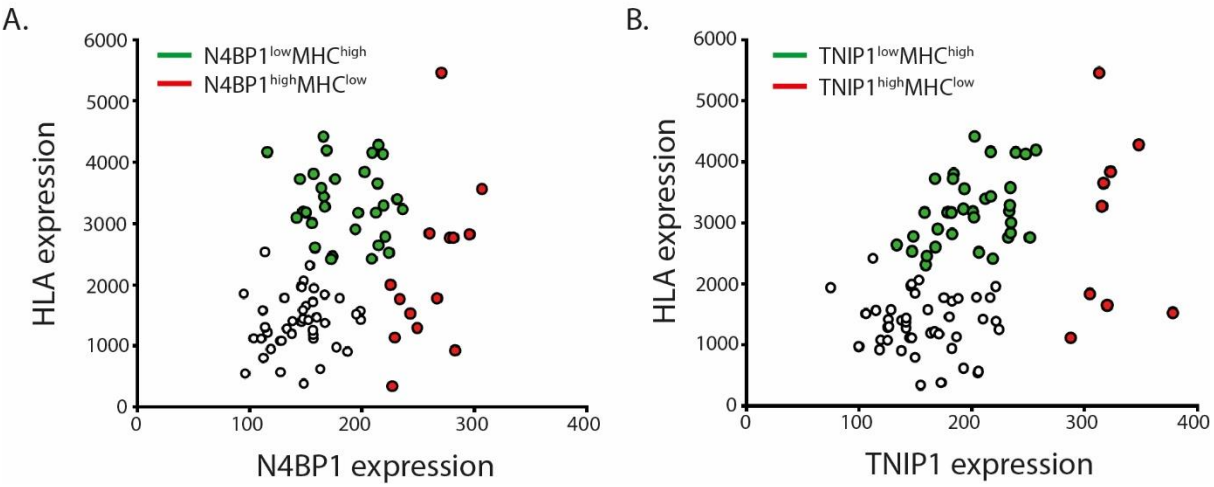
Supplemental figure 2. Reproducibility of CRISPR/Cas9 knock-out screen. A. Venn diagram showing the overlap of guide representation of the two screens. B. Percentage of total number of guides in the Gecko V2 library that is present in the sorted populations. C. Percentage of total number of raw reads mapped to Gecko V2 library for each population.

Supplemental figure 3



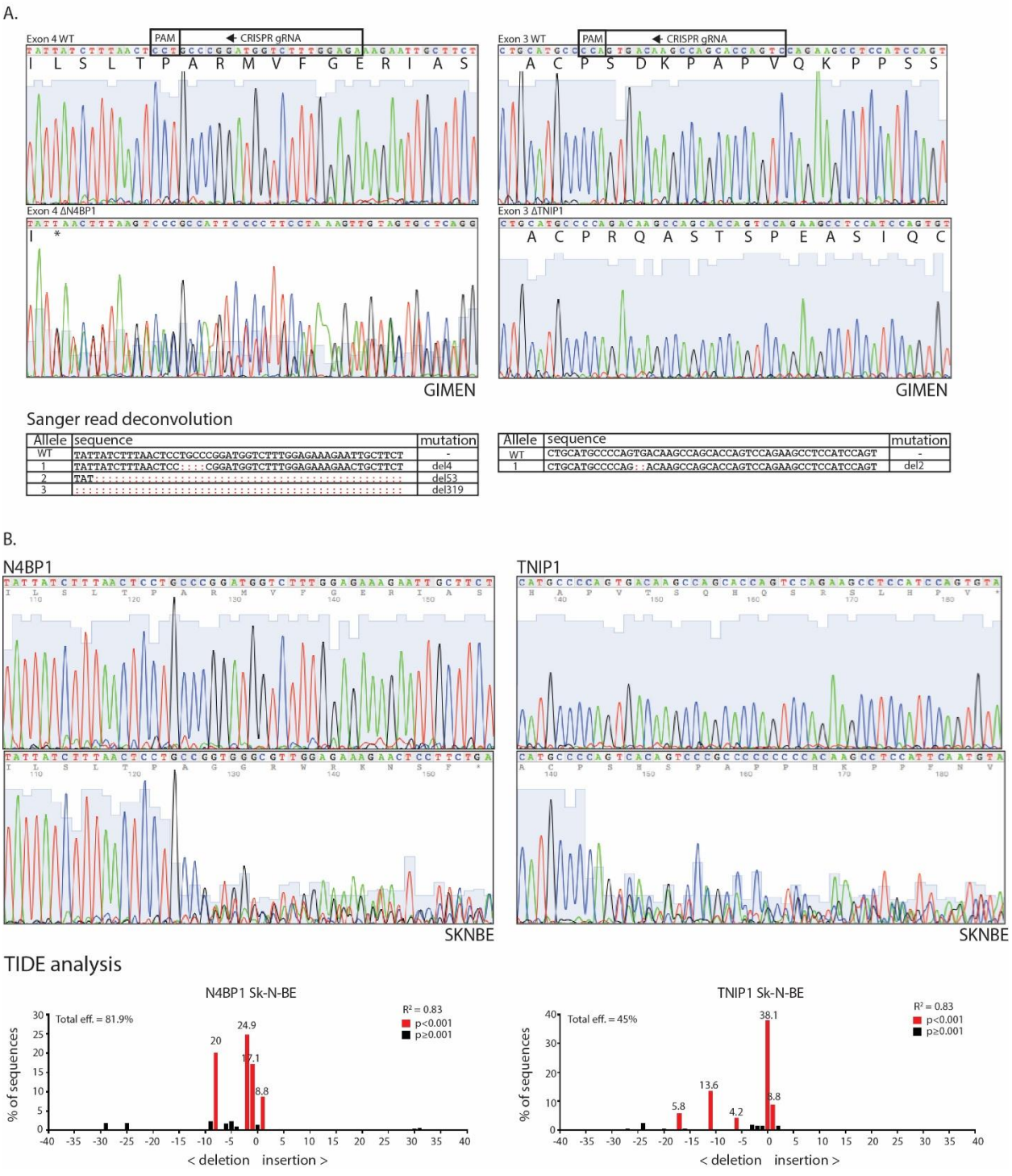
Supplemental figure 3. Validation of N4BP1 and TNIP1 as screen hits. Two different gRNAs are used to generate N4BP1- or TNIP1-depleted neuroblastoma cells. Activity of the NFκB-reporter (A and C) and surface MHC-1 expression (A and B) are measured by flow cytometry. From these N4BP1- or TNIP1-depleted cell populations, single cells were picked and grown out as single cell clones for use in following experiments.

Supplemental figure 4



Supplemental figure 4. Correlated expression of N4BP1 or TNIP1 with MHC-1 in neuroblastoma tumors. Gene expression dataset of 88 neuroblastoma tumors was analyzed for the combined expression of N4BP1 and HLA (A) or TNIP1 and MHC-1 (B). Three groups were defined as N4BP1/TNIP1^{low}MHC^{low} (white), N4BP1/TNIP1^{low}MHC^{high} (green) and N4BP1/TNIP1^{high}MHC^{low} (red).

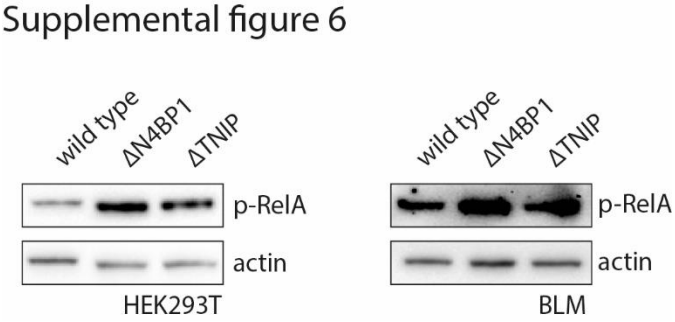
Supplemental figure 5



Supplemental figure 5. Generation of cells deficient for N4BP1 and TNIP1 using CRISPR/CAS9. To obtain ΔN4BP1 and ΔTNIP1 cell lines, CRISPR/CAS9 targeting the 4th exon of N4BP1 or the 3rd exon from TNIP1 were used to mutate the respective genes. gRNAs were cloned in pxpr_001 vector, encoding for both gRNA, CAS9 and puromycin resistance. Virus was obtained using standard protocols and cells were transduced. After 48 hours, cells were selected using 0.75 ug ml^{-1} puromycin for 48 hours or until non-

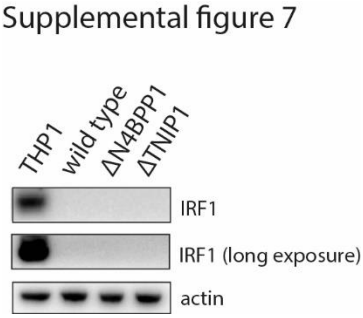
transduced control populations died. A. GIMEN clonal ΔN4BP1 and ΔTNIP1 cell lines were generated by limiting dilutions to ensure clonality. Allele annotations of the clonal cell lines are shown. N4BP1 is found mutated differently on 3 independent alleles while the same -2 mutation is found in all alleles in TNIP1 mutated cells. B. TIDE analysis for polyclonal ΔN4BP1 and ΔTNIP1 cell line, Sk-N-BE is shown as example.

Supplemental figure 6



Supplemental figure 6. Activation of NF-κB in non-neuroblastoma cell lines. Immunoblot analysis of phospho-RelA in WT, ΔN4BP1 and ΔTNIP1 mutants in HEK293T or BLM.

Supplemental figure 7



Supplemental figure 7. IRF1 expression in neuroblastoma WT and knock-out cells. Immunoblot analysis of IRF1 in WT, ΔN4BP1 and ΔTNIP1 GIMEN cells. THP1 lysates were used as positive control.