Supplementary Information for

RPA1 binding to NRF2 switches ARE-dependent transcriptional activation to ARE-NRE-dependent repression

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This PDF file includes:

Supplementary text

Figs. S1 to S7

Tables S1 to S2

References for SI reference citations

Supplementary Information Text

Materials and Methods

Reagents, chemicals, and cell culture

Sulforaphane (SF), tert-butylhydroquinone (tBHQ), sodium arsenite (As(III)), brusatol, sphingosine-1-phosphate (S1P), and human thrombin (cell culture grade) were purchased from Sigma. Mouse embryonic fibroblasts (MEF) were isolated from wild type, *Nrf2* knockout (*Nrf2*^{-/-}), and *Keap1* knockout (*Keap1*^{-/-}) mice and cultured with DMEM (Corning) supplemented with 10% FBS (Atlanta Biological), 1% L-glutamine (Invitrogen), 1% Non-Essential Amino Acids (Invitrogen), 0.1% β-mercaptoethanol (Thermo Fisher Scientific), and 1% penicillin/streptomycin (Invitrogen). A549 lung epithelial cancer cells, SKOV3 ovarian adenocarcinoma cells, H1299 non-small cell lung cancer cells, and BEAS-2B lung epithelial cells were purchased from ATCC. Human primary pulmonary artery endothelial cells (HPAEC) were purchased from Lonza. A549, H1299 and SKOV3 cells were grown in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin. BEAS-2B cells were cultured in Ham's F-12 medium supplemented with 1% bovine hypothalamus extract (PromoCell), insulin (2 mg/mL, Sigma), epidermal growth factor (10 μg/mL, Millipore), transferrin (2.5 mg/ml, Sigma-Aldrich), cholera toxin (10 μg/mL, List Biological Laboratories, Inc.) and dexamethasone (0.05 mM, Sigma). HPAEC cells were cultured in complete medium (EBM-2, Lonza). All cells were maintained at 37℃ in a humidified incubator containing 5% CO_2.

Generation of *NRF2*^{-/-}, *RPA1*^{-/-} and *KEAP1*^{-/-} cells

NRF2 knockout (*NRF2*^{-/-}), RPA1 knockout (*RPA1*^{-/-}) and KEAP1 knockout (*KEAP1*^{-/-}) cells were generated using CRISPR-Cas9-mediated gene editing. A pair of single guide RNA (sgRNA) sequences was used to target coding sequences near the promoter region of each gene of interest. The sgRNA sequences used were as follows:

NRF2: sgRNA-A 5'-TATTTGACTTCAGTCAGCGA-3'

sgRNA-B 5'-TAGTTGTAACTGAGCGAAAA-3'

RPA1: sgRNA-A 5'- TGTATCCCCCTTCTGCATGA -3'

sgRNA-B 5'- TCATGCAGAAGGGGGATACA -3'

KEAP1: sgRNA-A 5'-AGCGTGCCCCGTAACCGCAT-3'

sgRNA-B 5'- GATCTACACCGCGGGCGGCT-3'

Each sgRNA pair was annealed and then ligated into the pSpCas9(BB)-2A-GFP plasmid. Cells were then cotransfected with 1μg of the pSpCas9(BB)-2A-GFP plasmid carrying sgRNA-A and 1μg of the pSpCas9(BB)-2A-GFP plasmid carrying sgRNA-B. GFP-positive cells were isolated using fluorescence-activated cell sorting (FACS) and subsequently plated at a low confluence for colony formation and isolation. Once colonies were obtained, individual clones were expanded, and their genomic DNA was isolated and successful homozygous knockout of the target genes of interest was confirmed by sequencing. Finally,

generation of *NRF2*^{-/-} and *RPA1*^{-/-} cell lines was confirmed by detecting loss of protein expression via immunoblot analysis.

Recombinant DNA molecules plasmid construction

The *MYLK* promoter region was synthesized by GenScript (Piscataway, NJ) and cloned into the pGL3-Basic vector (Promega). This region of DNA included 2,535 bp of sequence upstream of the MYLK promoter (NM_053025.3) located on chromosome 3q21, containing 2431 bp of 3' untranslated region, 97 bp of exon 1, and 7 bp of intron 1. To generate promoter deletion constructs, a series of primers was designed to amplify each fragment (2.5 kb: −2428∼ +100; 2.2 kb: −2111∼ +100; 1.9 kb: −1751∼ +100; 1.3 kb: −1211∼ +100; 0.9 kb: −831∼ +100; 0.4 kb: −271∼ +100). In addition, 41 nt, 25 nt, and 11 nt probes containing the ARE region were synthesized by Integrated DNA Technologies and cloned into the pGL4.22-Basic vector (Addgene) using MluI/BglII. The sequences can be found in SI Appendix, Fig. S2 and S3. Flag-tagged RPA1, HA-tagged RPA1 and His-tagged RPA1-full length (FL), domain 1 (D1), domain 2 (D2), and domain 3 (D3) were PCR-amplified using cDNAs reverse-transcribed from the mRNAs of HEK293T cells, then subcloned into the pCMV-Flag-5a vector (EcoRI/BamHI) and pET5b vector (NdeI/BamHI) respectively. The primer sequences are as follow:

FL-Forward   5'-ATGGTCGGCCAACTGAGCGAG-3'
FL-Reverse   5'-TCACATCAATGCACTTCTCCTG-3'
D1-Forward   5'-ATGGTCGGCCAACTGAGCGAG-3'
D1-Reverse   5'-TCACACTTTGGACTGTGTTCC-3'
D2-Forward   5'-GTGCCCATTGCCAGCCTCACTC-3'
D2-Reverse   5'-TCACGGCTTGTCGCCTTGGCCCA-3'
D3-Forward   5'-GACTACTTTAGTTCTGTGGCC-3'
D3-Reverse   5'-TCACATCAATGCACTTCTCCTG-3'

GST-tagged NRF2-wild type (WT), GST-tagged NRF2 Neh1 domain deletion (△Neh1), HA-tagged NRF2-WT, HA-tagged NRF2△Neh1, Flag-tagged NRF1/2/3 and His- tagged sMAFG vectors were generated as previously described (1, 2).

Transfection of cDNA and luciferase reporter assays

Transfection of cDNA was performed using Lipofectamine 3000 (Invitrogen) according to the manufacturer's instructions. Luciferase activity was measured using the Dual-luciferase reporter assay system (Promega). For relative luciferase activity analysis, the value of Firefly-luciferase was normalized to the value of *Renilla*-luciferase. In brief, 1×10^5 A549 cells/well were seeded into a 24-well plate and cultured for 16 h. 0.45 μg of plasmid DNA and 0.05 μg of hRluc/TK plasmid were cotransfected. After transfection for 24 h, the cells were treated with different compounds for another 16 h. Cells were then lysed in passive lysis buffer (Promega). Both Firefly and Renilla luciferase values were detected using the dual luciferase assay kit

(Promega) and luminometer (Model TD-20/20, Turner BioSystems, CA). Finally, the value of Firefly luciferase was normalized to the value of *Renilla* luciferase to obtain the relative luciferase activity. Values were further normalized to control groups where indicated.

mRNA extraction and real-time qRT-PCR analysis

Total mRNA was extracted using TRIzol (Invitrogen) according to the manufacturer's instructions. cDNA was then synthesized using 2 μg of mRNA and the Transcriptor first-strand cDNA synthesis kit (Promega). Real-time quantitative PCR (qRT-PCR) was then performed. The β-actin gene (*ACTB*) was used for qRT-PCR normalization and all experiments were performed in triplicate. Primer sequences are as follows:

Mouse-*MYLK*-Forward   5'-CCAAGGACCGGATGAAGAAATA-3'

Mouse -*MYLK*-Reverse   5'-CCCTGAGATCATTGCCATAGAG-3'

Mouse-*GCLM*-Forward   5'-TGGAGCAGCTGTATCAGTGG-3'

Mouse-*GCLM*-Reverse   5'-CAAAGGCAGTCAAATCTGGTG-3'

Mouse-*ACTB*-Forward   5'-AAGGCCAACCGTGAAAAGAT-3'

Mouse-*ACTB*-Reverse   5'-GTGGTACGACCAGAGGCATAC-3'

Human-*MYLK*-Forward   5'-CCAAGGACCGGATGAAGAAGTA-3'

Human-*MYLK*- Reverse   5'-CCCTGAGATCATTGCCATAGAG-3'

Human-*NRF2*-Forward   5'-ACACGGTCCACAGCTCATC-3'

Human-*NRF2*- Reverse   5'-TGTCAATCAAATCCATGTCCTG-3'

Human-*NQO1*-Forward   5'-ATGTATGACAAAGGACCCTTCC-3'

Human-*NQO1*-Reverse   5'-TCCCTTGCAGAGAGTACATGG-3'

Human-*GCLM*-Forward   5'-GACAAAACACAGTTGGAACAGC-3'

Human-*GCLM*-Reverse   5'-CAGTCAAATCTGGTGGCATC-3'

Human-*RPA1*-Forward   5'- ATACAAACATAAAGCCCATCC-3'

Human-*RPA1*-Reverse   5'- TTGCCAATCTTCACTCCAAC-3'

Human-*RASSF10*-Forward   5'-GCAGCAATGGGACAGCAAGA-3'

Human-*RASSF10*-Reverse   5'-TTCGCACATGGGCAAGGAGT-3'

Human-*TPD52L1*-Forward   5'-TTACTCCATTCGCCATTCCA-3'

Human-*TPD52L1*-Reverse   5'-CTGCCTCCATTAGGGTTCGT-3'

Human-*FAM110B*-Forward   5'-CCCACGCTCAAAGTGTTCGG-3'

Human-*FAM110B*-Reverse   5'-AAGGACTCGGCTGACTGCTCC-3'

Human-*NAV2*-Forward   5'-AGTTGGGAAGCAAGGTGGAG-3'

Human-*NAV2*-Reverse   5'-GAAATTCAAGCAGGCATCTATGTT-3'

Human-*PCNX1*-Forward   5'- GCAGCAACTATTAAAGGAGATA-3'

Human-*PCNX1*-Reverse   5'- TCATTGGAGACAAGACGAAA-3'

Human-*FOCAD*-Forward   5'-CAGTGCCCTGAAAGGTTAGA-3'

Human-*FOCAD*-Reverse   5'-CATCATCGCCTCTGTTGTCT-3'

Human-*ITGA1*-Forward   5'-TTACCCTGTGCTGTACCCAA-3'

Human-*ITGA1*-Reverse   5'-TTTCACTCCGAAGTTCTCCC-3'

Human-*TANC2*-Forward   5'-GATGCTGCTTACTGGTGGGAAAT-3'

Human-*TANC2*-Reverse   5'-TCACTGGAAGTGGCGGGACA-3'

Human-*ADGRG5*-Forward   5'-CTGCGGCTCATCTGTATCTACTTC-3'

Human-*ADGRG5*-Reverse   5'-GTTTCCTGGCTCCCTCCTTC-3'

Human-*SYT16*-Forward   5'-TGCCTGCGAAGATTTGGATG-3'

Human-*SYT16*-Reverse   5'-AGATGTGCCGTGGTGTTTGG-3'

Human-*CNIH3*-Forward   5'-GTGCTGCCAGAATACTCCAT-3'

Human-*CNIH3*-Reverse   5'-GAAATACCTCCAGAAGTGATAGAA-3'

Human-*EEFSEC*-Forward   5'-CCCAGATTTCCATCCCAACG-3'

Human-*EEFSEC*-Reverse   5'-GGACTTCACCTTCTTCACCACCT-3'

Human-*ACTB*-Forward   5'-CCCAGAGCAAGAGAGG-3'

Human-*ACTB*-Reverse   5'-GTCCAGACGCAGGATG-3'

Immunoblot (IB), immunofluorescence (IF), and immunohistochemical (IHC) analyses

IB, IF, and IHC were performed as previously described (3, 4). For IB, cells were harvested in sample buffer (62.5 mM Tris-HCl pH 6.9, 3% SDS, 10% glycerol, 5% β-mercaptoethanol, and 0.1% bromophenol blue). Lysates were boiled, sonicated, and resolved by SDS-PAGE, and then subjected to immunoblot analysis. For IF, the cells were fixed in 4% paraformaldehyde in PBS for 20 min, incubated with 0.2% Triton X-100 for 20 min, and blocked with 5% BSA for 1 h. Then the cells were incubated with Alexa Fluor® 568 phallotoxins antibody (1:40, Invitrogen) for 30 min. Images were acquired with a Zeiss Observer.Z1 fluorescent microscope using the Slidebook 4.2.0.11 software (Intelligent Imaging Innovations, Inc.). For IHC, the lung tissues were fixed in 10% buffered neutral formalin in PBS and embedded with paraffin. The embedded tissues were sectioned at a 5 µm thickness and then baked and deparaffinized. Sodium citrate buffer (0.01 M, pH=6.0) was used in antigen retrieval, and endogenous peroxidase was blocked with 0.3% H_2O_2. The slices were blocked with 5% BSA for 30 min and incubated with primary antibodies overnight at 4 °C. Staining was performed with the EnVision + System-HRP kit (Dako) according to the manufacturer's instructions. Primary antibodies against NRF2 (1:1000 for IB, 1:200 for IHC), MYLK (1:1000 for IB, 1:200 for IHC), KEAP1 (1:1000 for IB), NQO1 (1:1000 for IB), GCLM (1:1000 for IB), sMAF (1:1000 for IB), RPA1 (1:1000 for IB), HA (1:1000 for IB) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:3000 for IB) were purchased from Santa Cruz Biotechnology. The antibody against Flag (1:2000 for WB) as well as horseradish peroxidase (HRP)-conjugated secondary antibodies (1:3000 for IB) was purchased from Sigma. The Alexa Fluor® 568 phalloidin (1:100 for IF) was purchased from Invitrogen.

Transendothelial electrical resistance (TEER)

TEER measurements were performed using an electric cell-substrate impedance sensing (ECIS) system (Applied Biophysics, Troy, NY). HPAEC were plated in 96 well plates coated with collagen. After adhering to the dish, cells were treated with tBHQ, brusatol, or DMSO for 16 h and ECIS measurements were carried out. Thrombin (1 U/mL) was added to the wells and TEER measurements were obtained. Measurements were pooled and plotted as resistance versus time. Values are indicated as means ± SD.

Biotinylated-DNA pull-down assay

Biotinylated-DNA pull-down was performed with streptavidin beads. In brief, cells were lysed in RIPA buffer containing 1 mM DTT, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 1% protease inhibitor cocktail (Sigma). The cell lysates were pre-cleared with streptavidin beads (Invitrogen) and incubated with 2 µg biotinylated DNA probes which spanned the ARE containing sequences in the promoter regions of both human *MYLK* and mouse *Mylk* (41 bp). The DNA-protein complexes were further pulled down by streptavidin beads and complexes were washed three times, resolved by SDS-PAGE gel electrophoresis, and subjected to immunoblot analysis. The sequences of the 41 bp biotinylated DNA probes are shown in SI Appendix, Fig. S4A.

Small interfering RNA transfection

Human RPA1 siRNA (#5: SI02663696; #6: SI02663703; #9: SI05461946 and #10: SI05461953) and non-targeted control siRNA (SI03650318) were purchased from Qiagen. Hiperfect reagent (Qiagen) was used for siRNA transfection according to the manufacturer's protocol. In brief, 1×10^5 A549 cells/well were seeded into a 12-well plate and transfected with siRNA (5 nM) for 72 h. Cells were then used for luciferase assay or qRT-PCR analysis.

Chromatin immunoprecipitation assay (ChIP)

CHIP assay was performed according to the manufacturer's instructions (EZ-CHIPTM, Merck, Germany). In brief, A549 cells were seeded in a 150mm dish, and when they reached 95% confluence DNA-protein complexes were cross-linked by adding 1% formaldehyde in the medium for 10 min. Then, the cells were washed with cold PBS and suspended in 1 mL SDS lysis buffer containing 1 mM PMSF and 1% protease inhibitor cocktail (Sigma). All samples were sonicated for 9×20 s on ice, then centrifuged at 15,000 rpm for 30 min at 4°C. The solubilized chromatin (0.1 mL) was diluted with ChIP dilution buffer (0.9 mL) for ChIP assays, and 0.1 mL diluted solubilized chromatin was saved for total chromatin input. The chromatin was pre-cleared with protein G-agarose beads for 1 h at 4 °C and then incubated with 4 µg anti-NRF2 antibody (Santa Cruz Biotechnology), or rabbit normal IgG (Santa Cruz Biotechnology) for 16 h at 4 °C with rotation. The cross-linked immunoprecipitates and total chromatin input were reverse cross-linked respectively and the DNA was extracted via ethanol precipitation. Then, 1 µL of purified DNA was used for PCR detection

with primers specific for the *MYLK* promoter. For PCR amplification the following primers were used: forward 5'-GTAGATGAGAGGAAGCATCTC-3' and reverse 5'-GAGGTTAACAGCCGTCGATG -3'.

Immunoprecipitation analysis

For endogenous immunoprecipitation, A549 cell lysates were collected in radio-immunoprecipitation assay (RIPA) buffer containing: 10 mM sodium phosphate (pH 8.0), 1% Triton X-100, 150 mM NaCl, 1% sodium deoxycholate, and 0.1% SDS. 1 mM DTT, 1 mM PMSF, and 1% protease inhibitor cocktail (Sigma) were also added to the RIPA buffer. The cell lysates were pre-cleared with 10 μL of protein A-agarose beads (Invitrogen) for 1 h, and then incubated with 1 μg of antibody and 15 μL of protein A-agarose beads on a rotator at 4 °C for 16 h. The immunoprecipitated complexes were washed three times with RIPA buffer and eluted in sample buffer (50 mM Tris-HCl [pH 6.9], 2% SDS, 10% glycerol, 100 mM DTT, 0.1% bromophenol blue) by boiling for 5 min. Samples were then resolved by SDS-PAGE and subjected to immunoblot analysis. For immunoprecipitation of overexpressed proteins, HEK293T cells were transfected with empty vector or vectors expressing HA-NRF2-WT, HA-NRF2ΔNeh1, Flag-NRF1/2/3, HA-RPA1 and Flag-RPA1. After 24 h transfection, the cell lysates were collected with RIPA buffer and immunoprecipitated as before.

In vitro binding assays

GST-tagged NRF2, His-tagged RPA1-FL/D1/D2/D3 and His-tagged sMAFG proteins were expressed in *Escherichia coli* Rosetta (DE3) LysS cells and purified with glutathione sepharose 4B matrix (Amersham Biosciences) and Ni-IDA Agarose Beads (Qiagen). For the *in vitro* binding assay, the different purified proteins were mixed together and incubated in binding buffer (20 mM Tris-HCl pH 8.0, 1 mM β-mercaptoethanol, 3 mM EDTA, 150 mM Na_2Cl, 1% NP40, 0.02% Triton X-100, 1 mM DTT, 1 mM PMSF, and 1% protease inhibitor cocktail) in the presence of glutathione beads for 12 h at 4 °C. The beads were then washed six times with washing buffer (20 mM Tris-HCl pH 8.0, 1 mM β-mercaptoethanol, 3 mM EDTA, 150 mM Na_2Cl, 0.1% NP40, 0.02% Triton X-100, 1 mM DTT, 1 mM PMSF, and 0.1% protease inhibitor cocktail). The proteins were eluted by boiling in sample buffer, resolved by SDS-PAGE, and detected with Coomassie or silver staining, or immunoblot analysis.

ARE-NRE site identification and annotation

All nucleotide sequence iterations that match the ARE-NRE consensus sequence TGABNNNGCAAACTTCA were generated. These ARE-NRE were mapped to unique genomic loci on GRCh38 reference genome (SI Appendix, Table S1). The ChIPseeker package was used to annotate the identified sites. Unique "candidate genes" harboring an ARE-NRE within the promoter region (defined as ≤5 kb upstream of the transcription start site) or within the gene's first intron were used for later analysis.

Identification of differentially-expressed genes following RPA1 silencing in A549 cells

Total RNA from duplicate samples of A549-*RPA*$^{+/+}$, and two clones of RPA1-/- knockout A549 cells (A549-*RPA*$^{-/-}$-1 and A549-*RPA*$^{-/-}$-2) were isolated using Trizol followed by a column-based RNeasy kit (Qiagen). Ribosomal RNA was depleted using Ribo-Zero Gold rRNA Removal kit (Illumina), and prepared for sequencing using the NEB Ultra Directional RNA library prep kit for Illumina (NEB). Samples were run on HiSeq3000 Illumina Sequencing Platform as 50 bp single-end read runs. Output FASTQ files were mapped to hg38 reference genome using Tophat2. The resulting BAM files were sorted and indexed for downstream analyses, and can be accessed at the National Center for Biotechnology Information BioProject Database number 487650. We utilized an in-house shell script to generate a count matrix of the number of reads assigned to each gene within each sample. Subsequent analyses were conducted in R statistical programming environment. The DESeq2 package was used to identify differentially expressed genes between A549-*RPA*$^{+/+}$ and the pooled samples of A549-*RPA*$^{-/-}$-1 and A549-*RPA*$^{-/-}$-2 cells. Briefly, the count matrix was pre-filtered to remove genes that had zero read depth in any cell line. Size factors for normalization and dispersion values for statistical testings were calculated based on the entire transcriptome. Subsequently, the gene list was filtered to only include genes with the ARE-NRE sequence at the promoter or the first intron region (total 55 genes). *p*-value adjustment to correct for multiple testings was performed using the method described by Benjamini-Hochberg.

Data transformation for visualization purposes

Base mean value (μ_b) for a particular gene, as determined by the DESeq2 package, is an average of normalized counts for all samples. It represents the overall sequencing depth for a gene and therefore, gives an idea of the general expression level of that gene. For visualization purposes, these base mean values were logarithmically transformed using the following function $f(\mu_b) = 10\, ln\,(10\mu_b)$. Variance Stabilization Transformation (VST) of read counts is implemented in the DESeq2 package. This transformation derives homoscedastic data from discrete count data for easy interpretation. VST distances presented in the heat map (Fig. 6B), were calculated as control samples mean subtracted VST expression level.

Pre-clinical murine model of ventilation-induced lung injury (VILI)

All mice were handled according to the Guide for the Care and Use of Laboratory Animals, and the protocols were approved by the University of Arizona Institutional Animal Care and Use Committee. Mice received food and water *ad libitum*. Eight-week-old male C57BL/6J *Nrf2*$^{+/+}$;*Mylk*$^{+/+}$ (wild type, WT), *Nrf2*$^{-/-}$;*Mylk*$^{+/+}$ (*Nrf2*$^{-/-}$), *Nrf2*$^{+/+}$;*Mylk*$^{-/-}$ (*Mylk*$^{-/-}$), and *Nrf2*$^{-/-}$;*Mylk*$^{-/-}$ mice (25-27g) were randomly allocated to either the control group (N = 3) or VILI group (N = 6). For VILI experiments, mice were subjected to mechanical ventilation. In brief, the mice were anesthetized using ketamine/xylazine (i.p., 100/5 mg/kg, respectively), intubated with a 20-gauge IV catheter, and connected to a ventilator (Inspira, Harvard Apparatus). The ventilation parameters using room air were: respiratory rate = 75 breaths/min, tidal volume

= 40 mL/kg, and a positive and expiratory pressure of 0 cm H_2O for 4 h. Mice were constantly monitored and deep anesthesia was maintained throughout the whole experiment with ketamine/xylazine. The mice in the control groups were allowed to breathe spontaneously. After the treatments, the mice were euthanized and bronchoalveolar lavage (BAL) fluid was obtained via lung lavage with 1 mL HBSS (Invitrogen) through the tracheal cannula. To collect the cells, the BAL fluid was centrifuged at 500 $\times g$ for 20 min at 4°C, then cell pellets were resuspended in PBS and total cell counts were measured using the TC20 automated cell counter (BioRad). For differential BAL cell counts, cells were concentrated using a Cytospin 4 (Thermo Fisher Scientific) and the slides were stained using the Shandon Kwik-Diff kit (Thermo Fisher Scientific). Macrophages, neutrophils, and lymphocytes were identified using standard morphological criteria; at least 200 cells were examined per sample. The supernatant collected from the BAL fluid was centrifuged at 15,000 $\times g$ for 10 min at 4°C and the levels of IL-6 and TNF-α in each sample were detected with an ELISA kit (Invitrogen) following the manufacturer's instructions. Lungs were collected and divided as follows: two thirds were snap frozen in liquid nitrogen for total RNA extraction and protein assays, the other third was fixed with 10% buffered formalin, then embedded in paraffin for histological and immunohistochemical analyses.

Statistical analysis

Results are presented as mean ± SD for at least three independent experiments. Statistical analysis was performed using SPSS 17.0. Unpaired Student's t-tests were applied to compare the means of two groups. One-way ANOVA with Bonferroni's correction was used to compare the means of three or more groups. $p < 0.05$ was considered statistically significant.
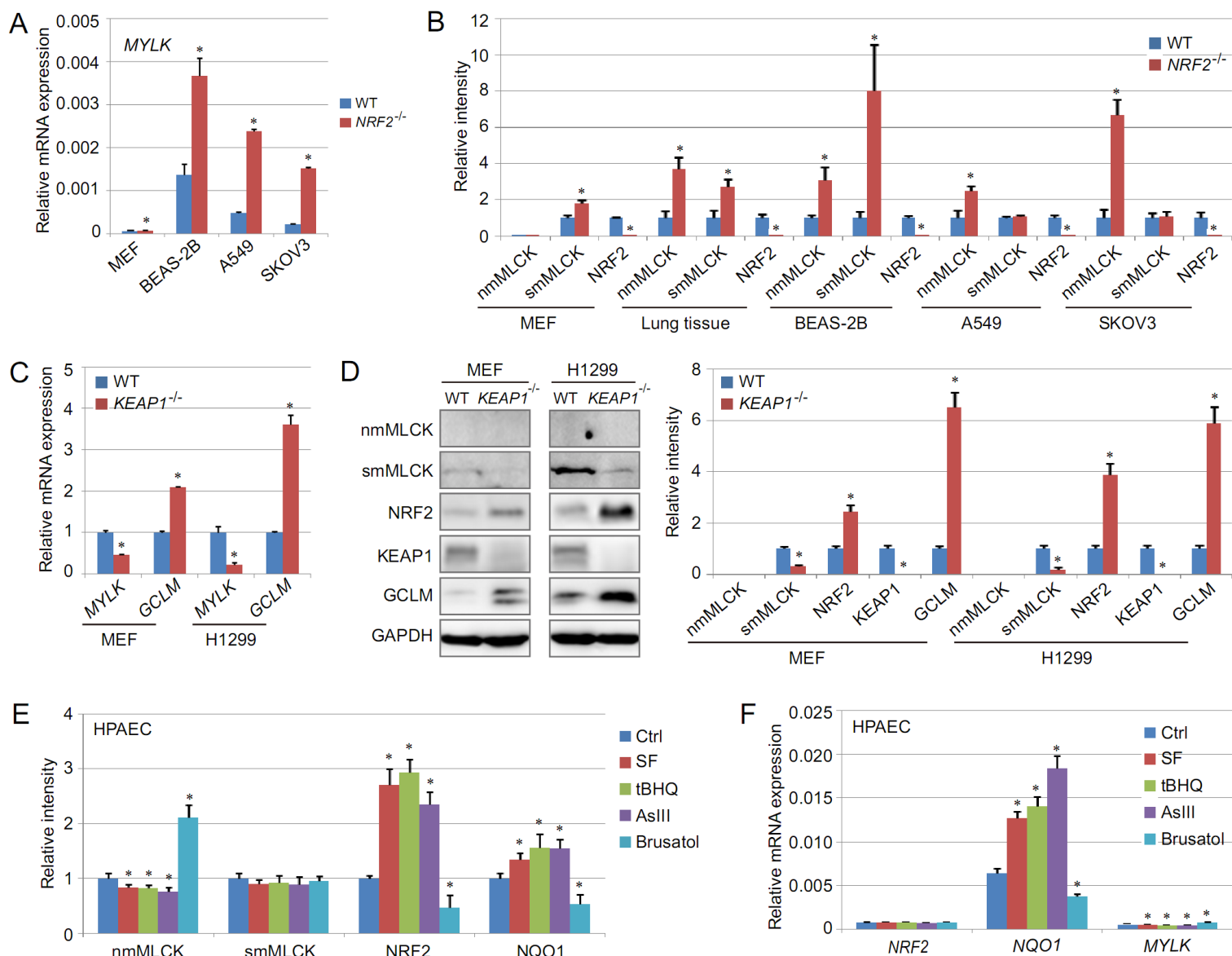
Fig. S1. NRF2 negatively regulates MYLK expression (Related to Fig. 1).

(A) Unnormalized results of Fig. 1A. (B) Quantification of immunoblot results in Fig. 1B. (C) qRT-PCR analysis of *MYLK* and *GCLM* expression in WT and *KEAP1*$^{-/-}$ cell lines. N = 3. Each gene was normalized to its control (Ctrl). Data are presented as mean ± SD. * $p < 0.05$. (D) Immunoblot analysis of WT and *KEAP1*$^{-/-}$ cell lines. Quantification is shown in the right panel, and data are presented as mean ± SD. * $p < 0.05$. (E) Quantification of immunoblot results in Fig. 1C. (F) Unnormalized results of Fig. 1D.

A
MYLK-ARE(41bp): 5'-ATTATTAGAATACCATGATTTTGCAAACTTCAATTAATTAA-3'
MYLK-mARE(41bp): 5'-ATTATTAGAATACCAACTTTTTCGAAACTTCAATTAATTAA-3'
MYLK-ARE(11bp):   5'-ATGATTTTGCA-3'
NQO1-ARE(11bp):   5'-GTGACTCAGCA-3'

B
MYLK-ARE(25bp): 5'-GAATACCATGATTTTGCAAACTTCA-3'
MYLK-mARE(25bp): 5'-GAATACCAACTTTTTCGAAACTTCA-3'
MYLK-ARE-mL(25bp): 5'-GTAAAGCATGATTTTGCAAACTTCA-3'
MYLK-ARE-mR(25bp): 5'-GAATACCATGATTTTGCAATCATGA-3'

C

D
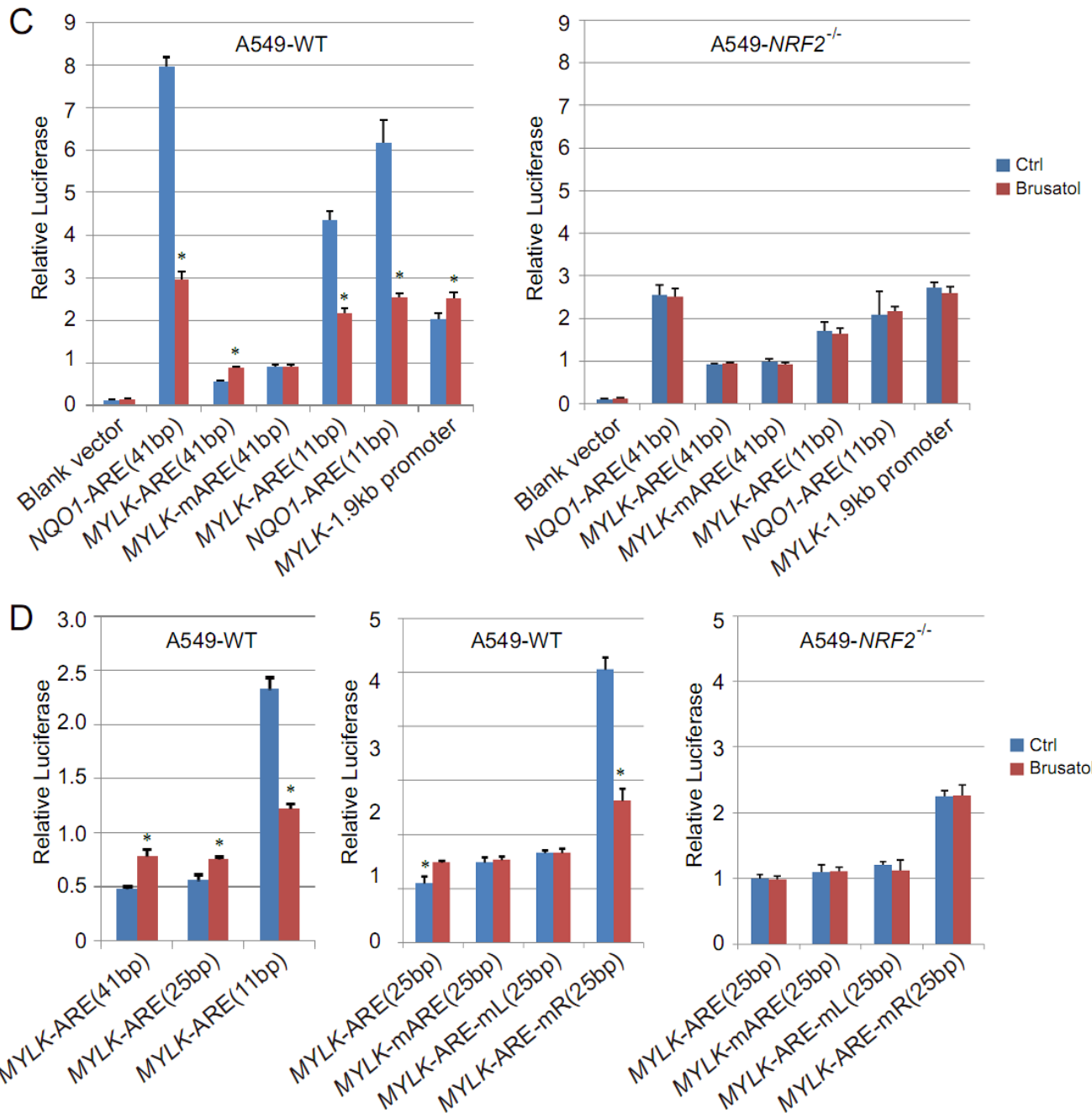
Fig. S2. An <u>N</u>RF2-<u>R</u>PA <u>E</u>lement (NRE) exists adjacent to the *MYLK* ARE. (Related to Fig. 2).

(A) Sequences of 11 bp core *nmMYLK*-ARE and an extended 41 bp *nmMYLK*-ARE. (B) Sequences of 25 bp *MYLK*-ARE (with 7 bp flanking the 11 bp core ARE), *MYLK*-mARE (mutations in the core ARE), *MYLK*-ARE-mL (5' or "left" flanking mutations*)*, and *MYLK*-ARE-mR (3' or "right" flanking mutations). (C-D) Unnormalized results of Fig. 2D and Fig. 2E.

A

MYLK-ARE(41bp): 5'-ATTATTAGAATACCATGATTTTGCAAACTTCAATTAATTAA-3'

NQO1-ARE(41bp): 5'-AATCGCAGTCACAGTGACTCAGCAGAATCTGAGCCTAGGG-3'
NQO1-ARE-NRE(41bp): 5'-AATCGCAGTCACAGTGACTCAGCAAACTTCAAGCCTAGGG-3'

GCLM-ARE(41bp): 5'-TTTCCTGGAAGACAATGACTAAGCAGAAATCGTAGCCGAGA-3'
GCLM-ARE-NRE(41bp): 5'-TTTCCTGGAAGACAATGACTAAGCAAACTTCATAGCCGAGA-3'

VEGFA-HRE(41bp): 5'-TGCATACGTGGGCTCCAACAGGTCCTCTTCCCTCCCAGTCA-3'
VEGFA-HRE-NRE(41bp): 5'-TGCATACGTGGGCTCCAACAGGTCCAACTTCATCCCAGTCA-3'

TNFα-KB(41bp): 5'-CATGGGTTTCTCCACCAAGGAAGTTTTCCGCTGGTTGAATG-3'
TNFα-KB-NRE(41bp): 5'-CATGGGTTTCTCCACCAAGGAAGTTTAACTTCAGTTGAATG-3'

CYP1A1-XRE(41bp): 5'-AGGCGCGGTGCCCAGGCGTTGCGTGAGAAGGACCGGAGGCC-3'
CYP1A1-XRE-NRE(41bp): 5'-AGGCGCGGTGCCCAGGCGTTGCGTGAACTTCACCGGAGGCC-3

B
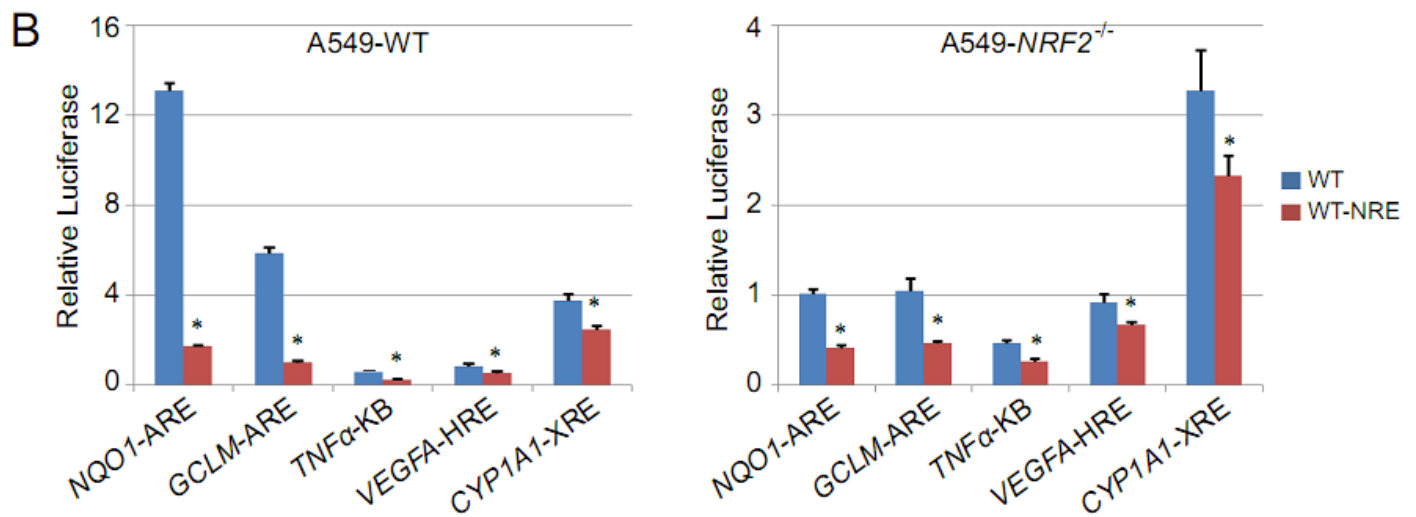
Fig. S3. NRE-mediated attenuation of *MYLK* transcription is ARE-specific (Related to Fig. 3).

(A) Sequences of *MYLK*-ARE, *NQO1*-ARE/ARE-NNRS, *GCLM*-ARE/ARE-NNRS, *VEGFA*-HRE/HRE-NNRS, *TNFα*-κB/κB-NNRS and *CYP1A1*-XRE/XRE-NNRS. (B) Unnormalized results of Fig. 3A.
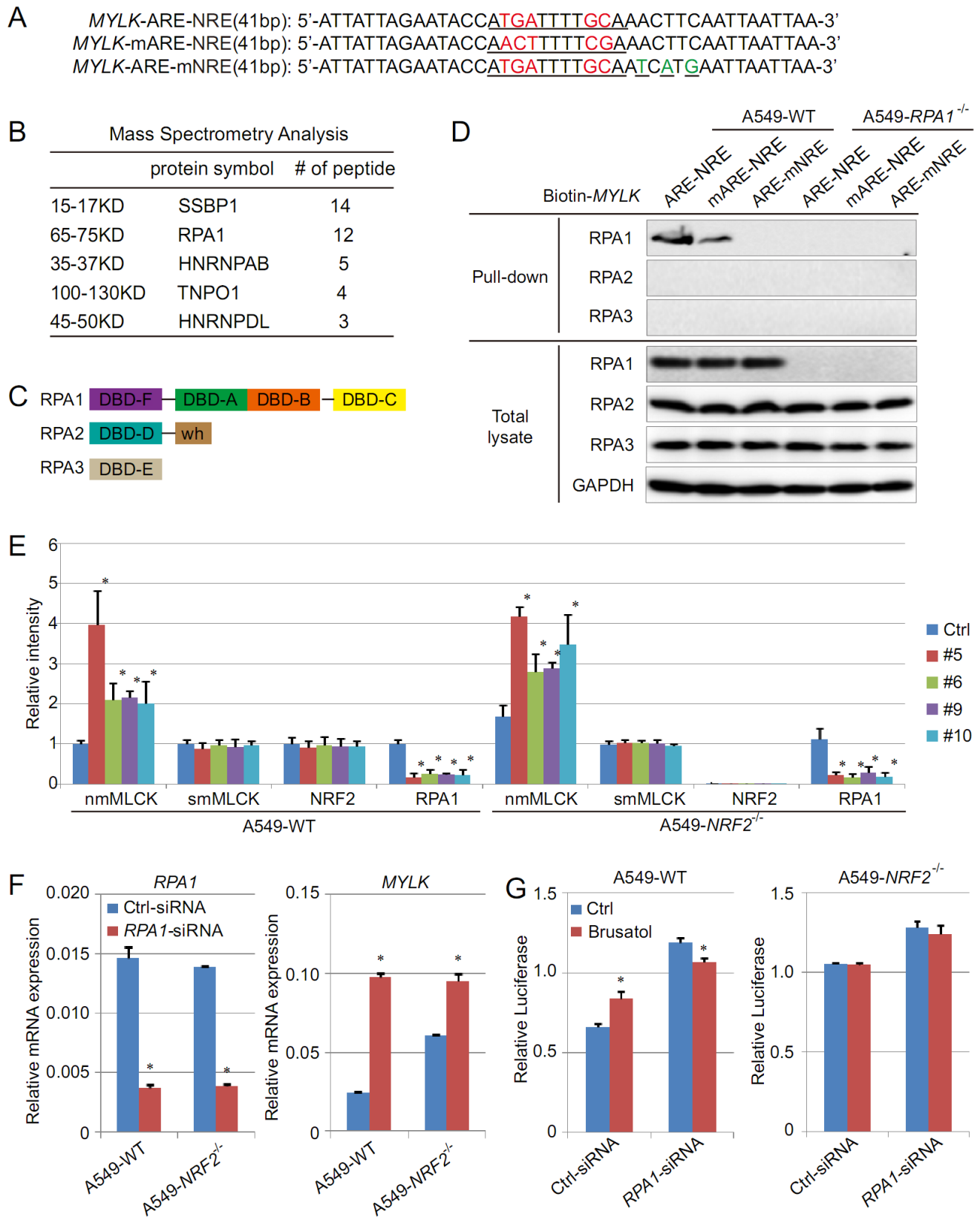
Fig. S4. Involvement of RPA1-NRE binding in repression of *MYLK* transcription (Related to Fig. 4).
(A) Sequence of biotinylated 41 bp double-stranded probes of *MYLK*-ARE-NNRS (wild type), *MYLK*-mARE-NNRS (mutation in ARE), and *MYLK*-ARE-mNNRS (mutation in NNRS). (B) The potential candidates identified by mass spectrometry. (C) Domain map of RPA1, RPA2 and RPA3. (D) Immunoblotting of biotinylated dsDNA probe pull-down of A549 WT and *RPA1*$^{-/-}$ cell lysates. (E) Quantification of immunoblot results in Fig. 4C. (F-G) Unnormalized results of Fig. 4D and Fig. 4E.
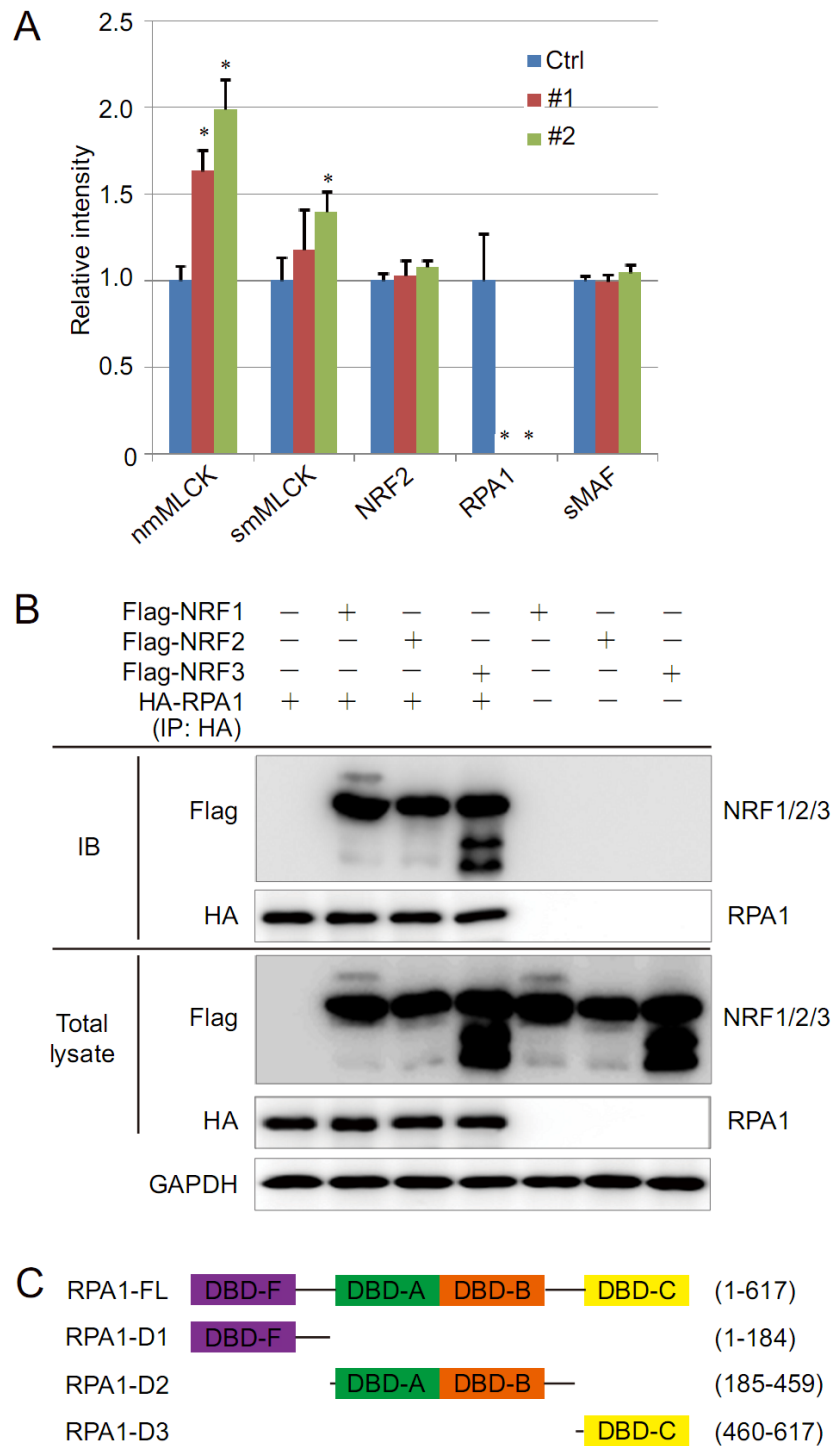
Fig. S5. RPA1 competes with sMAF to directly bind NRF2 (Related to Fig. 5).

(A) Quantification of immunoblot results in Fig. 5A. (B) Immunoprecipitation assay of HEK293 cells transfected with HA-tagged RPA1 and Flag-NRF1/2/3. (C) Domain map of RPA1-Full Length (FL), Domain 1 (D1), Domain 2 (D2), and Domain 3 (D3). The length of each respective peptide chain is shown on the right.

A

- Promoter (<=1kb) (0.72%)
- Promoter (1−2kb) (0.72%)
- Promoter (2−3kb) (1.44%)
- Promoter (3−4kb) (0.72%)
- Promoter (4−5kb) (1.2%)
- 3' UTR (0.48%)
- Other Exon (0.72%)
- 1st Intron (10.29%)
- Other Intron (43.3%)
- Downstream (<=3kb) (0.48%)
- Distal Intergenic (39.95%)

B

Relative mRNA expression

- A549-WT
- A549-$NRF2^{-/-}$

RPA1, RASSF10, MYLK, TPD52L1, FAM110B, NAV2, PCNX1, FOCAD, ITGA1, TANC2, ADGRG5, SYT16, CNIH3, EEFSEC
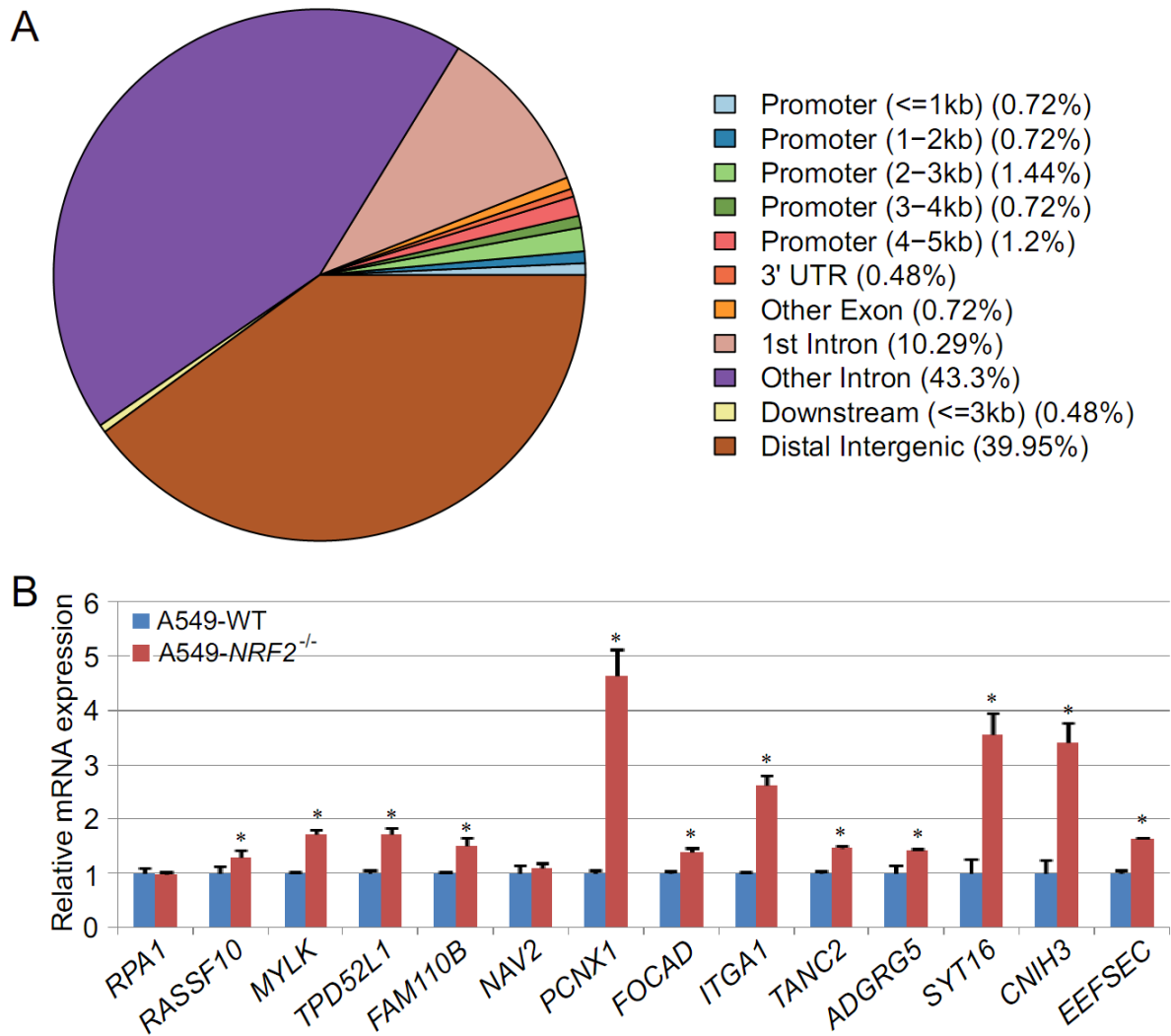
Fig. S6. NRF2-mediated negative transcriptional regulation is a fundamental mechanism controlling the expression of other genes (Related to Fig. 6).

(A) The location of the identified loci in SI Appendix Table S1. (B) qRT-PCR analysis of candidate gene mRNA expression in A549-WT and A549-$NRF2^{-/-}$ cells. N = 3. Data are presented as mean $\pm$ SD. * $p < 0.05$.
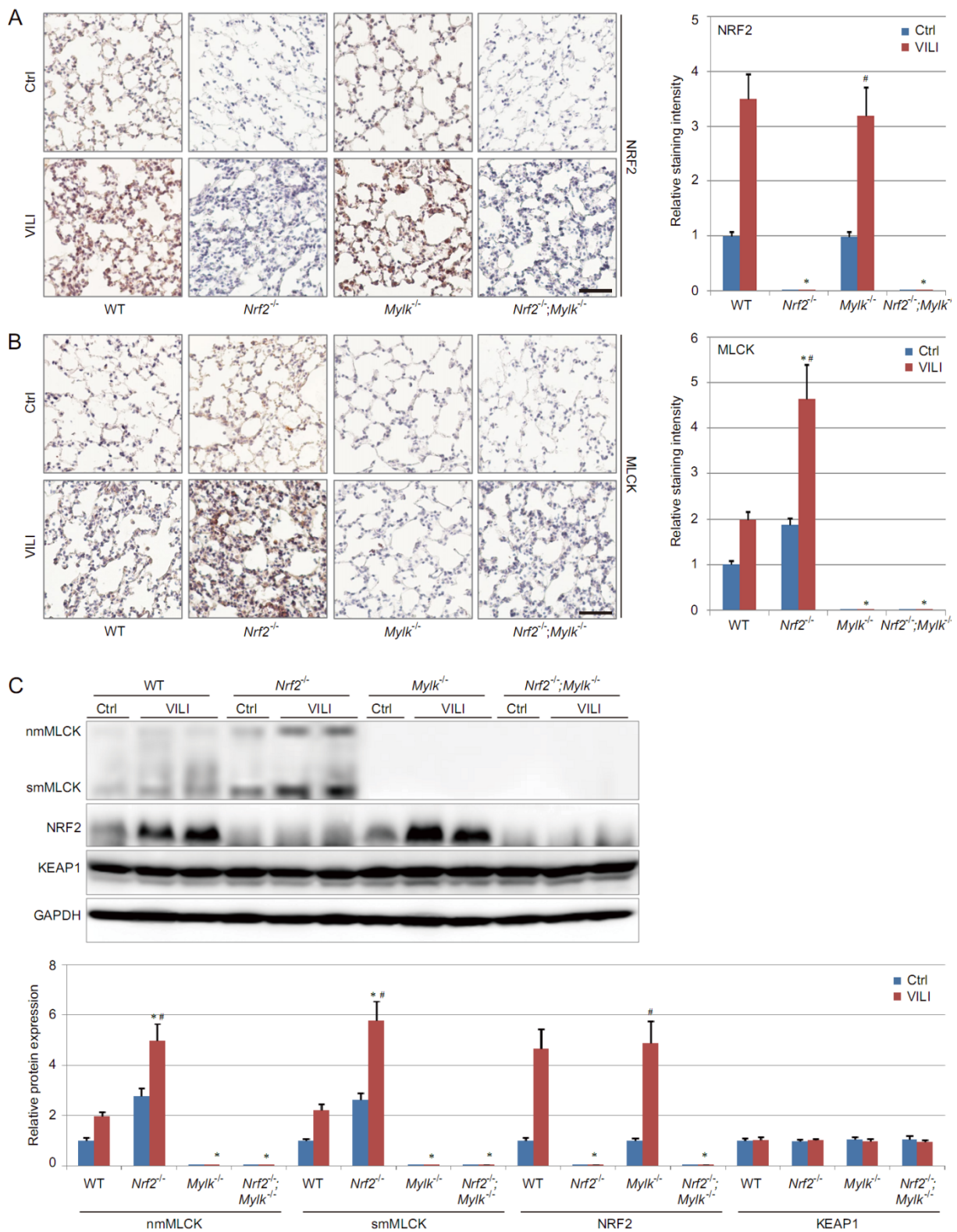
Fig. S7. NRF2-driven repression of MYLK expression attenuates inflammatory lung injury (Related to Fig. 7).

(A-B) Immunohistochemistry (IHC) of NRF2 and nmMLCK in lung tissue sections from control (Ctrl, N = 3) and VILI mice (N = 6). Scale bar = 50 μm. Quantification is shown on the right panel.

(C) Immunoblot detection of nmMLCK and smMLCK in lung tissues. A representative image (Ctrl group: n=1; VILI group: n =2) of the lung tissue from each group is shown first, followed by the relative quantification of total immunoblot results (Ctrl group: n=3; VILI group: n = 6).

Table S1. The 428 unique genomic loci containing the exact ARE-NNRS consensus sequence derived from the nmMYLK promoter (TGABNNNGCAAACTTCA) (Related to Fig. 6).

	Start	End	predicted site sequence	
chr4	155339372	155339389	GTGACAAAGCAAACTTCA	+
chr10	110549957	110549974	GTGACAAAGCAAACTTCA	-
chr10	17902864	17902881	CTGACAAAGCAAACTTCA	-
chr7	120146259	120146276	TTGATAAAGCAAACTTCA	-
chr14	78894141	78894158	TTGATAAAGCAAACTTCA	-
chr21	16900814	16900831	TTGATAAAGCAAACTTCA	-
chr5	155360389	155360406	TTGATTAAGCAAACTTCA	-
chrX	99028605	99028622	ATGATGAAGCAAACTTCA	+
chr9	8999267	8999284	TTGATGAAGCAAACTTCA	+
chr12	62412374	62412391	TTGATGAAGCAAACTTCA	-
chr1	198639790	198639807	TTGAGGAAGCAAACTTCA	-
chr3	115357115	115357132	TTGAGGAAGCAAACTTCA	-
chr6	138232839	138232856	TTGAGCAAGCAAACTTCA	-
chr5	27711727	27711744	TTGACATAGCAAACTTCA	+
chr2	44251528	44251545	ATGATATAGCAAACTTCA	+
chr5	117478945	117478962	TTGATATAGCAAACTTCA	+
chr10	120623397	120623414	TTGATATAGCAAACTTCA	+
chr2	133058872	133058889	CTGATATAGCAAACTTCA	+
chr6	75947425	75947442	CTGACTTAGCAAACTTCA	+
chr12	53611503	53611520	ATGAGTTAGCAAACTTCA	+
chr2	96363018	96363035	TTGATGTAGCAAACTTCA	+
chr6	70620583	70620600	TTGATGTAGCAAACTTCA	+
chr7	93714658	93714675	TTGATGTAGCAAACTTCA	+
chr8	66913019	66913036	TTGATGTAGCAAACTTCA	+
chr13	105358156	105358173	TTGATGTAGCAAACTTCA	+
chr15	43142491	43142508	TTGATGTAGCAAACTTCA	+
chr9	14038334	14038351	TTGATGTAGCAAACTTCA	-
chr13	79530451	79530468	TTGATGTAGCAAACTTCA	-
chr15	80227863	80227880	TTGATGTAGCAAACTTCA	-
chr20	19173616	19173633	TTGATGTAGCAAACTTCA	-
chr2	224376172	224376189	CTGATGTAGCAAACTTCA	+
chr9	39353073	39353090	CTGATGTAGCAAACTTCA	+
chr9	42181007	42181024	CTGATGTAGCAAACTTCA	+
chr9	60911756	60911773	CTGATGTAGCAAACTTCA	+
chr9	61187382	61187399	CTGATGTAGCAAACTTCA	+
chr9	66995187	66995204	CTGATGTAGCAAACTTCA	-
chr4	168800733	168800750	ATGACCTAGCAAACTTCA	+

chr4	157108839	157108856	TTGATCTAGCAAACTTCA	+
chr17	61740920	61740937	ATGATAGAGCAAACTTCA	-
chr4	167354944	167354961	GTGATAGAGCAAACTTCA	-
chr16	63113132	63113149	ATGAGAGAGCAAACTTCA	+
chr3	27157775	27157792	TTGATTGAGCAAACTTCA	+
chr13	40764746	40764763	ATGAGTGAGCAAACTTCA	+
chr13	52187621	52187638	ATGAGTGAGCAAACTTCA	+
chr1	84778707	84778724	GTGAGTGAGCAAACTTCA	+
chr9	15701482	15701499	CTGACGGAGCAAACTTCA	-
chr7	70175548	70175565	TTGATGGAGCAAACTTCA	+
chr8	9036293	9036310	TTGATGGAGCAAACTTCA	+
chrX	73204693	73204710	TTGATGGAGCAAACTTCA	+
chr14	50654128	50654145	TTGATGGAGCAAACTTCA	-
chr3	64425429	64425446	GTGAGGGAGCAAACTTCA	+
chr9	19793254	19793271	ATGACACAGCAAACTTCA	-
chr3	75758613	75758630	TTGACACAGCAAACTTCA	+
chr4	49604595	49604612	TTGACACAGCAAACTTCA	+
chr14	53722746	53722763	TTGACACAGCAAACTTCA	+
chr21	9105546	9105563	TTGACACAGCAAACTTCA	+
chr22	11943455	11943472	TTGACACAGCAAACTTCA	+
chr22	12785145	12785162	TTGACACAGCAAACTTCA	+
chr1_KI270707v1_random	10247	10264	TTGACACAGCAAACTTCA	+
chr3_GL000221v1_random	49494	49511	TTGACACAGCAAACTTCA	+
chr14_GL000009v2_random	100798	100815	TTGACACAGCAAACTTCA	+
chr14_GL000194v1_random	90869	90886	TTGACACAGCAAACTTCA	+
chrUn_KI270742v1	79447	79464	TTGACACAGCAAACTTCA	+
chrUn_GL000218v1	73233	73250	TTGACACAGCAAACTTCA	+
chr4	49192153	49192170	TTGACACAGCAAACTTCA	-
chr21	8789405	8789422	TTGACACAGCAAACTTCA	-
chr21	9355550	9355567	TTGACACAGCAAACTTCA	-
chr22	11449053	11449070	TTGACACAGCAAACTTCA	-
chr1_KI270706v1_random	29777	29794	TTGACACAGCAAACTTCA	-
chr1_KI270708v1_random	65464	65481	TTGACACAGCAAACTTCA	-
chr4_GL000008v2_random	113248	113265	TTGACACAGCAAACTTCA	-
chrUn_GL000224v1	31126	31143	TTGACACAGCAAACTTCA	-
chrUn_KI270743v1	98130	98147	TTGACACAGCAAACTTCA	-
chr3	151983716	151983733	GTGACACAGCAAACTTCA	-
chr10	74237064	74237081	CTGACACAGCAAACTTCA	-
chr14_KI270722v1_random	98433	98450	CTGACACAGCAAACTTCA	-
chr11	43238321	43238338	TTGATACAGCAAACTTCA	+
chr13	37402403	37402420	TTGATACAGCAAACTTCA	+
chrX	146005958	146005975	TTGATACAGCAAACTTCA	+

chr6	81415595	81415612	TTGATACAGCAAACTTCA	-
chr13	57849447	57849464	TTGATACAGCAAACTTCA	-
chrX	111475648	111475665	TTGATACAGCAAACTTCA	-
chr1	96127222	96127239	CTGATACAGCAAACTTCA	+
chr14	30326425	30326442	CTGATACAGCAAACTTCA	+
chr15	48164639	48164656	CTGATACAGCAAACTTCA	+
chr5	161540060	161540077	CTGATACAGCAAACTTCA	-
chr8	98530644	98530661	CTGATACAGCAAACTTCA	-
chr1	185165170	185165187	ATGAGACAGCAAACTTCA	-
chr15	86917022	86917039	TTGAGTCAGCAAACTTCA	+
chr3	43309442	43309459	ATGATGCAGCAAACTTCA	+
chr6	79316974	79316991	ATGATGCAGCAAACTTCA	-
chr1	57785945	57785962	TTGATGCAGCAAACTTCA	+
chr1	177260551	177260568	TTGATGCAGCAAACTTCA	+
chr2	5210473	5210490	TTGATGCAGCAAACTTCA	+
chr2	12992906	12992923	TTGATGCAGCAAACTTCA	+
chr2	161024631	161024648	TTGATGCAGCAAACTTCA	+
chr2	180813950	180813967	TTGATGCAGCAAACTTCA	+
chr2	194875966	194875983	TTGATGCAGCAAACTTCA	+
chr2	202702067	202702084	TTGATGCAGCAAACTTCA	+
chr2	237838054	237838071	TTGATGCAGCAAACTTCA	+
chr3	37037456	37037473	TTGATGCAGCAAACTTCA	+
chr3	154242734	154242751	TTGATGCAGCAAACTTCA	+
chr3	191045411	191045428	TTGATGCAGCAAACTTCA	+
chr4	4453286	4453303	TTGATGCAGCAAACTTCA	+
chr4	17456080	17456097	TTGATGCAGCAAACTTCA	+
chr4	117847457	117847474	TTGATGCAGCAAACTTCA	+
chr4	120846317	120846334	TTGATGCAGCAAACTTCA	+
chr4	156881099	156881116	TTGATGCAGCAAACTTCA	+
chr4	158440483	158440500	TTGATGCAGCAAACTTCA	+
chr4	161627179	161627196	TTGATGCAGCAAACTTCA	+
chr5	33209237	33209254	TTGATGCAGCAAACTTCA	+
chr5	92161723	92161740	TTGATGCAGCAAACTTCA	+
chr5	166190974	166190991	TTGATGCAGCAAACTTCA	+
chr6	70519531	70519548	TTGATGCAGCAAACTTCA	+
chr6	125153282	125153299	TTGATGCAGCAAACTTCA	+
chr6	131222883	131222900	TTGATGCAGCAAACTTCA	+
chr8	92448438	92448455	TTGATGCAGCAAACTTCA	+
chr8	116860424	116860441	TTGATGCAGCAAACTTCA	+
chr9	36920339	36920356	TTGATGCAGCAAACTTCA	+
chr10	75309390	75309407	TTGATGCAGCAAACTTCA	+
chr10	105456402	105456419	TTGATGCAGCAAACTTCA	+

chr11	13071304	13071321	TTGATGCAGCAAACTTCA	+
chr12	79255152	79255169	TTGATGCAGCAAACTTCA	+
chr12	125732049	125732066	TTGATGCAGCAAACTTCA	+
chr14	22763552	22763569	TTGATGCAGCAAACTTCA	+
chr14	46396328	46396345	TTGATGCAGCAAACTTCA	+
chr16	25671659	25671676	TTGATGCAGCAAACTTCA	+
chr17	55292277	55292294	TTGATGCAGCAAACTTCA	+
chr20	60710758	60710775	TTGATGCAGCAAACTTCA	+
chr1	28826775	28826792	TTGATGCAGCAAACTTCA	-
chr1	56278385	56278402	TTGATGCAGCAAACTTCA	-
chr1	161990703	161990720	TTGATGCAGCAAACTTCA	-
chr2	182468153	182468170	TTGATGCAGCAAACTTCA	-
chr3	24466916	24466933	TTGATGCAGCAAACTTCA	-
chr3	136414172	136414189	TTGATGCAGCAAACTTCA	-
chr4	99872526	99872543	TTGATGCAGCAAACTTCA	-
chr4	106146774	106146791	TTGATGCAGCAAACTTCA	-
chr4	141033044	141033061	TTGATGCAGCAAACTTCA	-
chr4	165791828	165791845	TTGATGCAGCAAACTTCA	-
chr4	187309987	187310004	TTGATGCAGCAAACTTCA	-
chr5	35593583	35593600	TTGATGCAGCAAACTTCA	-
chr5	62559190	62559207	TTGATGCAGCAAACTTCA	-
chr5	78313911	78313928	TTGATGCAGCAAACTTCA	-
chr5	87063440	87063457	TTGATGCAGCAAACTTCA	-
chr5	118870746	118870763	TTGATGCAGCAAACTTCA	-
chr6	23528735	23528752	TTGATGCAGCAAACTTCA	-
chr6	37913170	37913187	TTGATGCAGCAAACTTCA	-
chr6	47506886	47506903	TTGATGCAGCAAACTTCA	-
chr6	103811986	103812003	TTGATGCAGCAAACTTCA	-
chr6	112737464	112737481	TTGATGCAGCAAACTTCA	-
chr6	167536013	167536030	TTGATGCAGCAAACTTCA	-
chr7	17118658	17118675	TTGATGCAGCAAACTTCA	-
chr7	106621191	106621208	TTGATGCAGCAAACTTCA	-
chr7	125106037	125106054	TTGATGCAGCAAACTTCA	-
chr7	130117330	130117347	TTGATGCAGCAAACTTCA	-
chr7	138592231	138592248	TTGATGCAGCAAACTTCA	-
chr8	18858465	18858482	TTGATGCAGCAAACTTCA	-
chr8	58002927	58002944	TTGATGCAGCAAACTTCA	-
chr9	20653597	20653614	TTGATGCAGCAAACTTCA	-
chr9	74960803	74960820	TTGATGCAGCAAACTTCA	-
chr10	126653214	126653231	TTGATGCAGCAAACTTCA	-
chr12	1175242	1175259	TTGATGCAGCAAACTTCA	-
chr12	42494259	42494276	TTGATGCAGCAAACTTCA	-

chr12	44066828	44066845	TTGATGCAGCAAACTTCA	-
chr12	132627849	132627866	TTGATGCAGCAAACTTCA	-
chr13	84325016	84325033	TTGATGCAGCAAACTTCA	-
chr14	47013096	47013113	TTGATGCAGCAAACTTCA	-
chr15	95693482	95693499	TTGATGCAGCAAACTTCA	-
chr18	12443429	12443446	TTGATGCAGCAAACTTCA	-
chr18	61104347	61104364	TTGATGCAGCAAACTTCA	-
chr20	9396983	9397000	TTGATGCAGCAAACTTCA	-
chrX	138679821	138679838	TTGATGCAGCAAACTTCA	-
chr7	139686912	139686929	GTGATGCAGCAAACTTCA	+
chr19	43711241	43711258	GTGATGCAGCAAACTTCA	-
chr1	58331537	58331554	CTGATGCAGCAAACTTCA	+
chr2	9600758	9600775	CTGATGCAGCAAACTTCA	+
chr3	162261957	162261974	CTGATGCAGCAAACTTCA	+
chr4	2474704	2474721	CTGATGCAGCAAACTTCA	+
chr9	12399986	12400003	CTGATGCAGCAAACTTCA	+
chr11	30335481	30335498	CTGATGCAGCAAACTTCA	+
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chr12	60635359	60635376	CTGATGCAGCAAACTTCA	+
chr2	148686037	148686054	CTGATGCAGCAAACTTCA	-
chr3	44264604	44264621	CTGATGCAGCAAACTTCA	-
chr3	173439909	173439926	CTGATGCAGCAAACTTCA	-
chr6	28424430	28424447	CTGATGCAGCAAACTTCA	-
chr9	84088167	84088184	CTGATGCAGCAAACTTCA	-
chr11	49352905	49352922	CTGATGCAGCAAACTTCA	-
chr11	101043044	101043061	CTGATGCAGCAAACTTCA	-
chr11	107994657	107994674	CTGATGCAGCAAACTTCA	-
chr18	9390624	9390641	CTGATGCAGCAAACTTCA	-
chrX	38944286	38944303	TTGATCCAGCAAACTTCA	+
chr10	4432554	4432571	TTGAGCCAGCAAACTTCA	-
chr13	67737498	67737515	TTGACAATGCAAACTTCA	+
chr14	25089497	25089514	GTGACAATGCAAACTTCA	-
chrX	9936793	9936810	ATGATAATGCAAACTTCA	-
chr5	109677318	109677335	CTGATAATGCAAACTTCA	+
chr12	87038540	87038557	ATGACTATGCAAACTTCA	+
chr1	87237617	87237634	TTGACTATGCAAACTTCA	-
chr6	151616131	151616148	ATGATGATGCAAACTTCA	-
chr6	56883584	56883601	TTGATGATGCAAACTTCA	+
chr2	40005140	40005157	GTGATGATGCAAACTTCA	+
chr4	12110722	12110739	ATGAGGATGCAAACTTCA	+
chr4	12896112	12896129	ATGAGGATGCAAACTTCA	+
chr5	81793145	81793162	ATGATCATGCAAACTTCA	+

chr11	45340059	45340076	GTGATCATGCAAACTTCA	+
chr8	127188327	127188344	CTGATCATGCAAACTTCA	+
chr6	19008171	19008188	CTGAGCATGCAAACTTCA	-
chr11	58951187	58951204	TTGACATTGCAAACTTCA	+
chr6	144948772	144948789	ATGATATTGCAAACTTCA	+
chr17	27143774	27143791	CTGATATTGCAAACTTCA	+
chr1	190829922	190829939	TTGACTTTGCAAACTTCA	-
chr3	123885804	123885821	ATGATTTTGCAAACTTCA	-
chr15	93412339	93412356	TTGAGTTTGCAAACTTCA	-
chr10	80677388	80677405	TTGATGTTGCAAACTTCA	+
chrX	65113628	65113645	TTGATGTTGCAAACTTCA	+
chrX	89224849	89224866	TTGATGTTGCAAACTTCA	-
chrY	6553828	6553845	TTGATGTTGCAAACTTCA	-
chr9	70304733	70304750	CTGATGTTGCAAACTTCA	+
chr20	8378658	8378675	CTGATGTTGCAAACTTCA	-
chr22	46715379	46715396	ATGACCTTGCAAACTTCA	-
chr4	184726042	184726059	ATGACAGTGCAAACTTCA	+
chr2	145469832	145469849	CTGACAGTGCAAACTTCA	-
chr3	117213469	117213486	TTGATGGTGCAAACTTCA	+
chrX	145765513	145765530	TTGATACTGCAAACTTCA	+
chr20	42543131	42543148	TTGATACTGCAAACTTCA	-
chr8	100997012	100997029	ATGACTCTGCAAACTTCA	-
chr2	143086848	143086865	CTGATTCTGCAAACTTCA	+
chr4	104898403	104898420	ATGATGCTGCAAACTTCA	-
chr7	110240424	110240441	TTGATGCTGCAAACTTCA	+
chr7	66103946	66103963	TTGATGCTGCAAACTTCA	-
chr14	70916788	70916805	TTGATGCTGCAAACTTCA	-
chrX	138512535	138512552	TTGATGCTGCAAACTTCA	-
chr2	117187617	117187634	GTGATGCTGCAAACTTCA	+
chr7	138291175	138291192	CTGATGCTGCAAACTTCA	-
chr22	42599718	42599735	CTGAGGCTGCAAACTTCA	+
chr14	88008879	88008896	TTGATCCTGCAAACTTCA	-
chr12	44145855	44145872	ATGAGCCTGCAAACTTCA	-
chr3	50588755	50588772	ATGACAAGGCAAACTTCA	-
chr12	42789510	42789527	TTGACAAGGCAAACTTCA	+
chr1	204817990	204818007	CTGATAAGGCAAACTTCA	+
chr1	63174960	63174977	ATGATGAGGCAAACTTCA	-
chr2	168941740	168941757	TTGATGAGGCAAACTTCA	+
chr4	180278363	180278380	TTGATGAGGCAAACTTCA	+
chr11	133761729	133761746	TTGATGAGGCAAACTTCA	+
chr12	39268248	39268265	TTGATGAGGCAAACTTCA	+
chr14	61964745	61964762	TTGATGAGGCAAACTTCA	+

chr7	138929093	138929110	TTGATGAGGCAAACTTCA	-
chr12	82326025	82326042	TTGATGAGGCAAACTTCA	-
chrX	152348967	152348984	TTGATGAGGCAAACTTCA	-
chr3	197487111	197487128	ATGACCAGGCAAACTTCA	+
chr4	14092179	14092196	ATGATCAGGCAAACTTCA	+
chr8	138404466	138404483	ATGACATGGCAAACTTCA	-
chr3	58446008	58446025	TTGACATGGCAAACTTCA	-
chr2	111463890	111463907	GTGACATGGCAAACTTCA	-
chr22	46987810	46987827	CTGACATGGCAAACTTCA	-
chr5	58201511	58201528	ATGATATGGCAAACTTCA	+
chr2	189091383	189091400	TTGATATGGCAAACTTCA	+
chr6	56799413	56799430	TTGATATGGCAAACTTCA	+
chrX	37084810	37084827	TTGATATGGCAAACTTCA	+
chr3	21154904	21154921	TTGATATGGCAAACTTCA	-
chr3	114284342	114284359	TTGATATGGCAAACTTCA	-
chr3	146202615	146202632	TTGATATGGCAAACTTCA	-
chr4	36562556	36562573	TTGATATGGCAAACTTCA	-
chr12	51553013	51553030	TTGATATGGCAAACTTCA	-
chr6	116756656	116756673	CTGATATGGCAAACTTCA	+
chr3	80194479	80194496	ATGAGATGGCAAACTTCA	-
chr1	172924751	172924768	TTGATTTGGCAAACTTCA	-
chr18	23180293	23180310	GTGAGTTGGCAAACTTCA	-
chr1	84561632	84561649	TTGACGTGGCAAACTTCA	+
chr4	66697764	66697781	CTGACGTGGCAAACTTCA	-
chr8	77417871	77417888	ATGATGTGGCAAACTTCA	+
chr11	124988555	124988572	ATGATGTGGCAAACTTCA	-
chr1	105272225	105272242	TTGATGTGGCAAACTTCA	+
chr1	171599748	171599765	TTGATGTGGCAAACTTCA	+
chr2	6175252	6175269	TTGATGTGGCAAACTTCA	+
chr2	58064310	58064327	TTGATGTGGCAAACTTCA	+
chr2	95239468	95239485	TTGATGTGGCAAACTTCA	+
chr2	157354514	157354531	TTGATGTGGCAAACTTCA	+
chr3	15946074	15946091	TTGATGTGGCAAACTTCA	+
chr3	43975277	43975294	TTGATGTGGCAAACTTCA	+
chr3	104481765	104481782	TTGATGTGGCAAACTTCA	+
chr3	175254176	175254193	TTGATGTGGCAAACTTCA	+
chr4	73034369	73034386	TTGATGTGGCAAACTTCA	+
chr4	92372342	92372359	TTGATGTGGCAAACTTCA	+
chr5	25045123	25045140	TTGATGTGGCAAACTTCA	+
chr5	38082699	38082716	TTGATGTGGCAAACTTCA	+
chr5	52827617	52827634	TTGATGTGGCAAACTTCA	+
chr5	114457137	114457154	TTGATGTGGCAAACTTCA	+

chr5	125097120	125097137	TTGATGTGGCAAACTTCA	+
chr5	131630108	131630125	TTGATGTGGCAAACTTCA	+
chr6	83664848	83664865	TTGATGTGGCAAACTTCA	+
chr6	117242528	117242545	TTGATGTGGCAAACTTCA	+
chr6	139866669	139866686	TTGATGTGGCAAACTTCA	+
chr7	22485237	22485254	TTGATGTGGCAAACTTCA	+
chr7	138656016	138656033	TTGATGTGGCAAACTTCA	+
chr7	149362107	149362124	TTGATGTGGCAAACTTCA	+
chr8	38479897	38479914	TTGATGTGGCAAACTTCA	+
chr8	94660862	94660879	TTGATGTGGCAAACTTCA	+
chr8	136472387	136472404	TTGATGTGGCAAACTTCA	+
chr9	8323936	8323953	TTGATGTGGCAAACTTCA	+
chr9	77224865	77224882	TTGATGTGGCAAACTTCA	+
chr9	126822416	126822433	TTGATGTGGCAAACTTCA	+
chr10	25738289	25738306	TTGATGTGGCAAACTTCA	+
chr11	85621973	85621990	TTGATGTGGCAAACTTCA	+
chr11	88845159	88845176	TTGATGTGGCAAACTTCA	+
chr12	78797366	78797383	TTGATGTGGCAAACTTCA	+
chr12	82112704	82112721	TTGATGTGGCAAACTTCA	+
chr12	107013201	107013218	TTGATGTGGCAAACTTCA	+
chr14	31955119	31955136	TTGATGTGGCAAACTTCA	+
chr16	69546666	69546683	TTGATGTGGCAAACTTCA	+
chr17	63035074	63035091	TTGATGTGGCAAACTTCA	+
chr18	256845	256862	TTGATGTGGCAAACTTCA	+
chr18	22622938	22622955	TTGATGTGGCAAACTTCA	+
chr18	29292384	29292401	TTGATGTGGCAAACTTCA	+
chr18	35639206	35639223	TTGATGTGGCAAACTTCA	+
chr21	39654652	39654669	TTGATGTGGCAAACTTCA	+
chr21	40953131	40953148	TTGATGTGGCAAACTTCA	+
chr22	43558584	43558601	TTGATGTGGCAAACTTCA	+
chrX	68028147	68028164	TTGATGTGGCAAACTTCA	+
chrX	132790150	132790167	TTGATGTGGCAAACTTCA	+
chr1	32801595	32801612	TTGATGTGGCAAACTTCA	-
chr1	103057033	103057050	TTGATGTGGCAAACTTCA	-
chr1	213805243	213805260	TTGATGTGGCAAACTTCA	-
chr1	227932047	227932064	TTGATGTGGCAAACTTCA	-
chr2	33105842	33105859	TTGATGTGGCAAACTTCA	-
chr2	75248939	75248956	TTGATGTGGCAAACTTCA	-
chr2	210409362	210409379	TTGATGTGGCAAACTTCA	-
chr2	215467765	215467782	TTGATGTGGCAAACTTCA	-
chr3	119910632	119910649	TTGATGTGGCAAACTTCA	-
chr4	100171153	100171170	TTGATGTGGCAAACTTCA	-

chr4	106750564	106750581	TTGATGTGGCAAACTTCA	-
chr4	135496574	135496591	TTGATGTGGCAAACTTCA	-
chr4	144446938	144446955	TTGATGTGGCAAACTTCA	-
chr4	179941840	179941857	TTGATGTGGCAAACTTCA	-
chr5	63020026	63020043	TTGATGTGGCAAACTTCA	-
chr6	25072344	25072361	TTGATGTGGCAAACTTCA	-
chr7	13475516	13475533	TTGATGTGGCAAACTTCA	-
chr7	87235489	87235506	TTGATGTGGCAAACTTCA	-
chr7	124536867	124536884	TTGATGTGGCAAACTTCA	-
chr7	132862605	132862622	TTGATGTGGCAAACTTCA	-
chr8	98783609	98783626	TTGATGTGGCAAACTTCA	-
chr8	100321124	100321141	TTGATGTGGCAAACTTCA	-
chr9	93766935	93766952	TTGATGTGGCAAACTTCA	-
chr10	28297715	28297732	TTGATGTGGCAAACTTCA	-
chr10	32433752	32433769	TTGATGTGGCAAACTTCA	-
chr10	74411710	74411727	TTGATGTGGCAAACTTCA	-
chr10	92819722	92819739	TTGATGTGGCAAACTTCA	-
chr10	95739258	95739275	TTGATGTGGCAAACTTCA	-
chr11	19490271	19490288	TTGATGTGGCAAACTTCA	-
chr11	49788773	49788790	TTGATGTGGCAAACTTCA	-
chr11	83308791	83308808	TTGATGTGGCAAACTTCA	-
chr13	42937363	42937380	TTGATGTGGCAAACTTCA	-
chr13	112481923	112481940	TTGATGTGGCAAACTTCA	-
chr14	22081570	22081587	TTGATGTGGCAAACTTCA	-
chr18	3515073	3515090	TTGATGTGGCAAACTTCA	-
chr18	14014344	14014361	TTGATGTGGCAAACTTCA	-
chr18	78148178	78148195	TTGATGTGGCAAACTTCA	-
chr19	43888540	43888557	TTGATGTGGCAAACTTCA	-
chr21	41305871	41305888	TTGATGTGGCAAACTTCA	-
chrX	47852332	47852349	TTGATGTGGCAAACTTCA	-
chrX	86747781	86747798	TTGATGTGGCAAACTTCA	-
chrX	108603684	108603701	TTGATGTGGCAAACTTCA	-
chr1	214698595	214698612	GTGATGTGGCAAACTTCA	+
chr3	58798701	58798718	GTGATGTGGCAAACTTCA	+
chrX	7142984	7143001	GTGATGTGGCAAACTTCA	+
chr5	115361128	115361145	GTGATGTGGCAAACTTCA	-
chr12	45873927	45873944	GTGATGTGGCAAACTTCA	-
chr18	64172103	64172120	GTGATGTGGCAAACTTCA	-
chr2	34111947	34111964	CTGATGTGGCAAACTTCA	+
chr3	64428741	64428758	CTGATGTGGCAAACTTCA	+
chr3	128158446	128158463	CTGATGTGGCAAACTTCA	+
chr4	32209643	32209660	CTGATGTGGCAAACTTCA	+

chr4	113527085	113527102	CTGATGTGGCAAACTTCA	+
chr5	14800453	14800470	CTGATGTGGCAAACTTCA	+
chr7	135888536	135888553	CTGATGTGGCAAACTTCA	+
chr8	81943846	81943863	CTGATGTGGCAAACTTCA	+
chr8	129890270	129890287	CTGATGTGGCAAACTTCA	+
chr12	94414353	94414370	CTGATGTGGCAAACTTCA	+
chr12	106934210	106934227	CTGATGTGGCAAACTTCA	+
chr15	72766366	72766383	CTGATGTGGCAAACTTCA	+
chr20	24164182	24164199	CTGATGTGGCAAACTTCA	+
chrX	53915913	53915930	CTGATGTGGCAAACTTCA	+
chr1	51050421	51050438	CTGATGTGGCAAACTTCA	-
chr1	214508164	214508181	CTGATGTGGCAAACTTCA	-
chr2	213032885	213032902	CTGATGTGGCAAACTTCA	-
chr4	78829081	78829098	CTGATGTGGCAAACTTCA	-
chr4	92401837	92401854	CTGATGTGGCAAACTTCA	-
chr5	162556962	162556979	CTGATGTGGCAAACTTCA	-
chr7	25611670	25611687	CTGATGTGGCAAACTTCA	-
chr9	83670666	83670683	CTGATGTGGCAAACTTCA	-
chr12	45382955	45382972	CTGATGTGGCAAACTTCA	-
chr12	63786717	63786734	CTGATGTGGCAAACTTCA	-
chr14	23962265	23962282	CTGATGTGGCAAACTTCA	-
chr14	23997387	23997404	CTGATGTGGCAAACTTCA	-
chr14	24045343	24045360	CTGATGTGGCAAACTTCA	-
chr16	51293233	51293250	CTGATGTGGCAAACTTCA	-
chr16	57551269	57551286	CTGATGTGGCAAACTTCA	-
chr18	42077295	42077312	CTGATGTGGCAAACTTCA	-
chr20	8930130	8930147	CTGATGTGGCAAACTTCA	-
chr22	30842473	30842490	CTGATGTGGCAAACTTCA	-
chrX	127295412	127295429	CTGATGTGGCAAACTTCA	-
chr1	224598579	224598596	CTGAGGTGGCAAACTTCA	+
chr22	39788716	39788733	ATGACCTGGCAAACTTCA	+
chr14	53815170	53815187	TTGACCTGGCAAACTTCA	-
chr9	69299148	69299165	TTGATCTGGCAAACTTCA	-
chr18	1879222	1879239	TTGATCTGGCAAACTTCA	-
chr10	125806611	125806628	ATGAGCTGGCAAACTTCA	-
chr3	54950200	54950217	CTGAGCTGGCAAACTTCA	+
chr6	92772320	92772337	TTGATAGGGCAAACTTCA	-
chr12	52774278	52774295	GTGAGAGGGCAAACTTCA	+
chr7	116573723	116573740	TTGACTGGGCAAACTTCA	-
chr19	11014082	11014099	CTGATTGGGCAAACTTCA	+
chr13	52623929	52623946	ATGAGTGGGCAAACTTCA	-
chr7	3746409	3746426	TTGATGGGGCAAACTTCA	-

chr10	58728129	58728146	TTGATGGGGCAAACTTCA	-
chr18	78959489	78959506	TTGATGGGGCAAACTTCA	-
chr8	100833427	100833444	ATGACACGGCAAACTTCA	-
chr6	150556019	150556036	TTGATGCGGCAAACTTCA	+
chr2	222528935	222528952	TTGATGCGGCAAACTTCA	-
chr6	106878619	106878636	TTGATGCGGCAAACTTCA	-
chr14	23872511	23872528	CTGATGCGGCAAACTTCA	-
chr14	104917308	104917325	CTGATGCGGCAAACTTCA	-
chr17	41471151	41471168	CTGATGCGGCAAACTTCA	-
chr8	17710200	17710217	TTGATGTCGCAAACTTCA	-
chr3	14295273	14295290	TTGATTGCGCAAACTTCA	+
chr9	32326311	32326328	TTGATGCCGCAAACTTCA	-
chr6	82363319	82363336	TTGAGGCCGCAAACTTCA	-

Table S2. The 55 genes potentially regulated by the NRF2-RPA1 complex (Related to Figure 6)

	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
PTPRC	13.875275	0.145117189	0.392874	0.369373	0.71185	0.859227
GNGT1	0.8116207	-0.051592083	0.274281	-0.1881	0.850799	0.859227
SPATA31A1	0.1886614	0.02728467	0.153839	0.177359	0.859227	0.859227
SPATA31A6	0.1886614	0.02728467	0.153839	0.177359	0.859227	0.859227
SPATA31A7	0.3773227	0.052924561	0.153839	0.344026	0.730827	0.859227
SPATA31A3	0.1886614	0.02728467	0.153839	0.177359	0.859227	0.859227
MAFIP	458.86293	-0.597617441	0.120124	-4.97502	6.52E-07	5.13E-06
NDST3	253.16956	0.046553435	0.14786	0.314848	0.752877	0.859227
TARS	17921.897	-0.213485484	0.075457	-2.82925	0.004666	0.016265
ARRDC3	3079.6377	-0.113118508	0.202666	-0.55815	0.576741	0.859227
TPD52L1	3610.7657	1.336986881	0.133364	10.02507	1.18E-23	1.63E-22
ZNF503-AS1	28.35029	0.249225007	0.328437	0.758821	0.44796	0.724641
RASSF10	776.04531	1.625751032	0.124227	13.08696	3.91E-39	2.15E-37
OXA1L	4225.7138	-0.19015399	0.125616	-1.51377	0.130083	0.286183
OPRD1	1.0784277	-0.268791087	0.256244	-1.04897	0.294193	0.525428
LOC339975	30.731719	0.068560734	0.359813	0.190545	0.848882	0.859227
LOC10192938(	77.656974	0.060939806	0.238116	0.255925	0.798009	0.859227
ZFAND3	673.52295	0.03719575	0.107884	0.344774	0.730264	0.859227
FAM110B	52.911416	2.093879695	0.326201	6.418991	1.37E-10	1.51E-09
FOCAD	7448.2743	0.3017846	0.080531	3.74744	0.000179	0.000959
C9orf41-AS1	304.06667	-0.172040679	0.156358	-1.1003	0.271202	0.514349
LINC00924	281.68025	0.06313254	0.144842	0.435872	0.66293	0.859227
IRGC	0.1548732	-0.04963484	0.153839	-0.32264	0.746967	0.859227
RNF4	3163.4074	0.105191902	0.08541	1.231618	0.218092	0.444261
SLC8A1-AS1	7.0184953	-1.059265822	0.375732	-2.8192	0.004814	0.016265
MYLK	5066.7285	0.991947935	0.096019	10.33073	5.12E-25	9.38E-24
ARHGAP15	2.9103514	0.162388445	0.33514	0.484539	0.628004	0.859227
PCNX1	4494.9918	0.378884943	0.090813	4.172156	3.02E-05	0.000207
GPR65	741.80251	0.039546624	0.114876	0.344255	0.730654	0.859227
SYT16	14.729794	1.267117611	0.403964	3.136708	0.001709	0.007229
ZNF385D	0.1707071	-0.04963484	0.153839	-0.32264	0.746967	0.859227
TIGIT	3.629013	0.426622707	0.378022	1.128565	0.259081	0.50891
FAM162B	7.0415294	0.682923778	0.398667	1.713019	0.086709	0.207348
CABLES1	1011.5506	-1.565180784	0.125759	-12.4459	1.47E-35	4.05E-34
CTBS	473.09512	0.215801733	0.128298	1.682035	0.092562	0.212121
LOC10272487∠	122.33317	0.162292385	0.199514	0.81344	0.415966	0.693277
GRID2	0.9433068	0.108154049	0.177043	0.610891	0.541272	0.85057
ITGA1	197.34904	0.92746299	0.246479	3.762843	0.000168	0.000959
STEAP1B	239.2399	0.159791471	0.16397	0.974517	0.3298	0.566844
ZBTB43	1167.5567	-0.127994405	0.091224	-1.40308	0.160592	0.339714
TANC2	1597.3854	0.567735063	0.152222	3.729651	0.000192	0.000959
HS6ST2	1.7217019	-0.058852153	0.32279	-0.18232	0.855329	0.859227
LANCL1-AS1	101.77781	-0.646698341	0.23053	-2.80527	0.005027	0.016265
ENTPD1	2197.2548	-0.115727455	0.110773	-1.04472	0.29615	0.525428

Gene	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
NAV2	496.7791	0.726590534	0.137352	5.289995	1.22E-07	1.12E-06
EEFSEC	124.56365	0.477310169	0.201211	2.372189	0.017683	0.048628
ADGRG5	18.332703	1.497117155	0.415169	3.606046	0.000311	0.001425
CNIH3	27.244367	0.921189794	0.36408	2.530185	0.0114	0.033001
BANCR	0.1886614	0.02728467	0.153839	0.177359	0.859227	0.859227
KRT76	852.85909	-0.525222611	0.189633	-2.76967	0.005611	0.017146
LINC01510	11.797206	0.736540416	0.415219	1.773862	0.076086	0.190215
HNRNPA1L2	10647.351	-0.22541642	0.073316	-3.07459	0.002108	0.008281
KRT32	2.4513564	-0.687294884	0.350988	-1.95817	0.05021	0.131502
LINC01267	1.1348389	-0.104316523	0.21959	-0.47505	0.63475	0.859227
TPBG	2414.2215	-0.035804899	0.073317	-0.48836	0.625294	0.859227

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