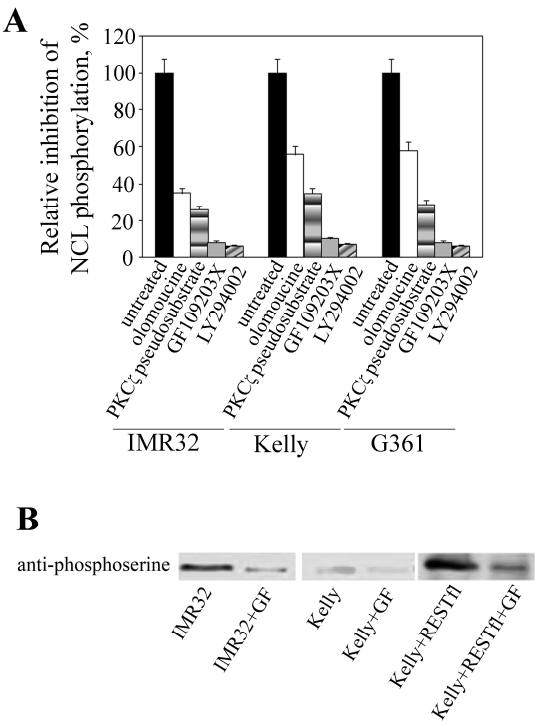
Supplemental Table 1. Oligonucleotides used for real-time quantitative PCR/reverse transcription-PCR, EMSA analysis and generation of plasmid constructs.

Name of detected gene/amplified sequence	Sequence	Used for
35bp RE	5'-GGCATCCTGAGGGGCGGGGCCGGGGGGCGGAGCCT-3' (S) 5'-AGGCTCCGCCCCCCGGCCCCGCCCCTCAGGATGCC-3' (A)	EMSA
mut35bp RE	5'-GGCATCCTGAGGTTCGGGTTCGGGGTTCGGAGCCT-3' (S) 5'-AGGCTCCGAACCCCGAACCCGAACCTCAGGATGCC-3' (A)	EMSA
irr35bp	5'-TTATCGCCACTGGCAGCAGCCACTGGTAACAGGAT-3' (S) 5'-ATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAA-3' (A)	EMSA
KpnI/F2145 R_mut35bp R_mut35bp_2nd AgeI/R	5'-CGCCGGTACCTGAATTCAGATTTGTGCACA-3' (S) 5'-AGGCTCCGAACCCCGAACCCGAACCTCAGGATGCC-3' (A) 5'-ATATACCGGTAAGATCCTCTTCCAGCCTCGAGCCGCTTCTGCGCTCAGCCCCCGCATTCTTTC GCTCCAGCCCGCAAGGCTCCGAACCCCGAACCCGAACCTCAGGATGCC-3' (A) 5'-ATATACCGGTAAGATCCTCTTCCAGCCTCGA-3' (A)	Cloning of the CD59 promoter with mutated 35bp RE
KpnI/F2145 R_irr1 R_irr2 R_irr3 R_irr4 R_irr_2nd AgeI/R	5'-CGCCGGTACCTGAATTCAGATTTGTGCACA-3' (S) 5'-TGGCGATAACTTGCCCTCCCAGGC-3' (A) 5'-TGCTGCCAGTGGCGATAACTTGCC-3' (A) 5'-ACCAGTGGCTGCTGCCAGTGGCGA-3' (A) 5'-ATCCTGTTACCAGTGGCTGCTGCC-3' (A) 5'-ATATACCGGTAAGATCCTCTTCCAGCCTCGAGCCGCTTCTGCGCTCAGCCCCCGCATTCTT TCGCTCCAGCCCGCAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAA-3' (A) 5'-ATATACCGGTAAGATCCTCTTCCAGCCTCGA-3' (A)	Cloning of the CD59 promoter with irrelevant 35bp
adaptor_F adaptor_R	5'-pGGCCGCGGATCCCCT-3' (S) 5'-AGGGGATCCGC-3' (A)	NotI-BamHI adaptor for subcloning of REST
CD59_REST/NCL	5'-CGAGGCAAGGGCATCCTGAGGGGCGGGGCCGGGGGGCGGAGCCTTGCGGGTCG-3' (S) 5'-CGACCCGCAAGGCTCCGCCCCCCGGCCCCGCCCCTCAGGATGCCCTTGCCTCG3' (A)	Cloning of the CD59 promoter fragment,

Mcl1_REST/NCL	5’-GATCCGGAGCCGCCGTTACGTAACCGGCACTCAGAGCGGCCCCGGCCCCGCCCCGGCCCGGCCGGGCAGCTGGTAGGTGCCGTGCGCAAG-3’ (S) 5'-GATCCTTGCGCACGGCACCTACCAGCTGCCCGGCCGGGCCGGGGCGGGGCCGGGGCCGCTCTGAGTGCCGGTTACGTAACGGCGGCTCCG-3' (A)	Cloning of the Mcl1 promoter fragment
ADCY5_REST	5'-CAGACACAGGACCTCCCATGGGGTGAGGGGTGGTGGTAC-3' (S) 5'-CACCACCCCTCACCCCATGGGAGGTCCTGTGTCTGGTAC-3' (A)	Cloning of the ADCY5 promoter fragment
CD59_REST/MCL_detect	5'-GAGAGAGATGGGTGCGAGAG-3' (S) 5'-CCTGTCTGAAGGGATGGTTG-3' (A)	QPCR detection of the CD59 promoter fragment
Mcl1_REST/NCL_detect	5'-TGTTCCGCATTCTGCAAG-3' (S) 5'-CTCCTCGCCCTTGCTCAC-3' (A)	QPCR detection of the Mcl1 promoter fragment
ADCY5_REST/NCL_detect	5'-GACGAGTCGGATCTCCCTTT-3' (S) 5'-CCCAGTACAAGCAAAAAGCA-3' (A)	QPCR detection of the ADCY5 promoter fragment
35bp RE_ChIP	5’-AGCCTGGGAGGGCAAG-3’ (S) 5’-TTTCGCTCCAGCCCGCA-3’ (A)	QPCR detection of the endogenous 35bp RE
CD59	5’-TAACCCAACTGCTGACTGCAA-3’ (S) 5’-TTTGGTAATGAGACACGCATCAA-3’(A)	QPCR analysis of expression
Mcl1	5’-ATGCTTCGGAAACTGGACAT-3’ (S) 5’-ATGGTTCGATGCAGCTTTCT-3’ (A)	QPCR analysis of expression
ADCY5	5’-GGAGGTGGGTCTGGAGGAG-3’ (S) 5’-AGCGGAATATCTGCAGCAAC-3’ (A)	QPCR analysis of expression
β-actin	5’-ACGGCCAGGTCATCACTATTG-3’ (S) 5’-AGTTTCATGGATGCCACAGGAT-3’ (A)	QPCR analysis of expression

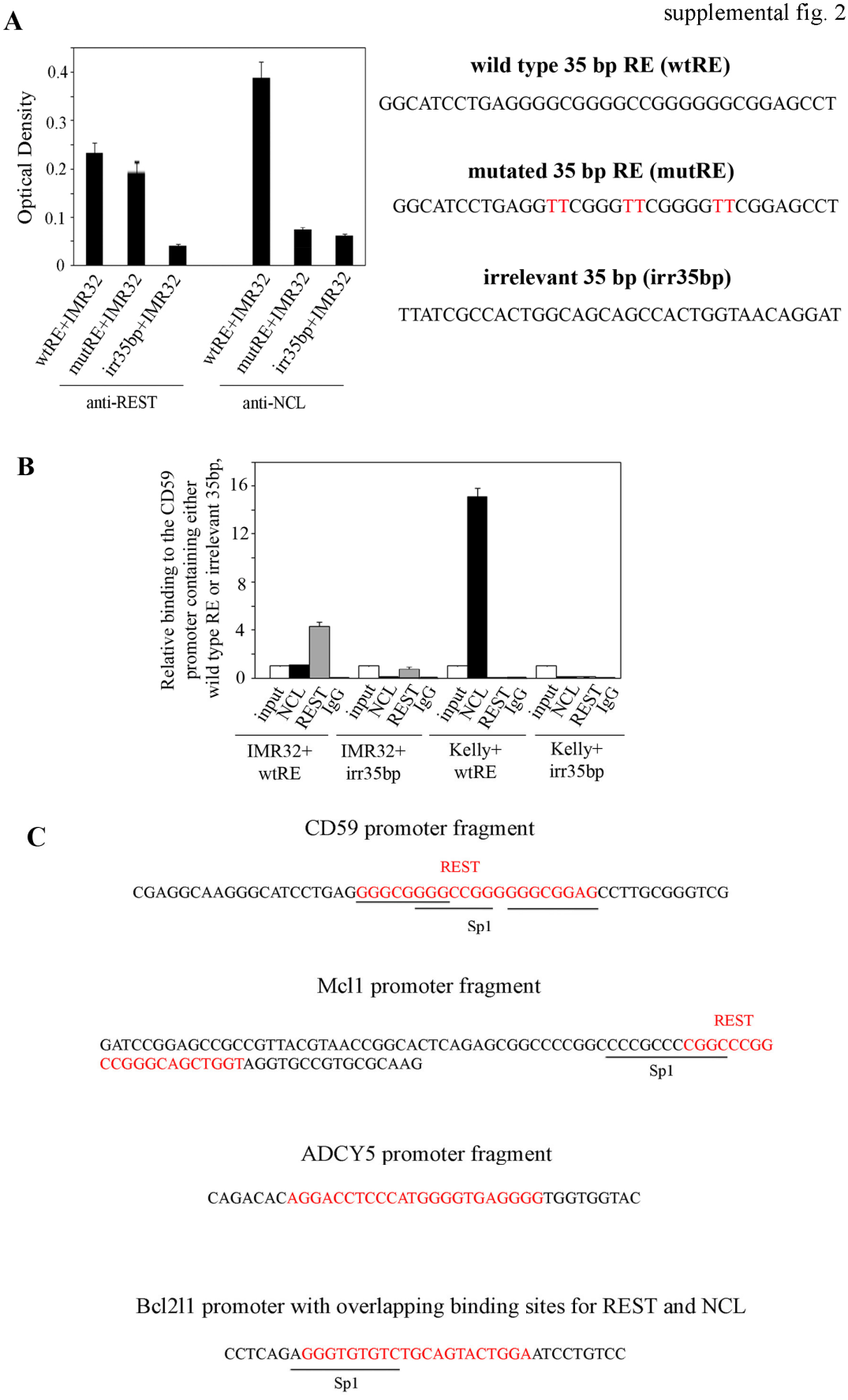
S, sense; A, antisense

Supplemental Figures



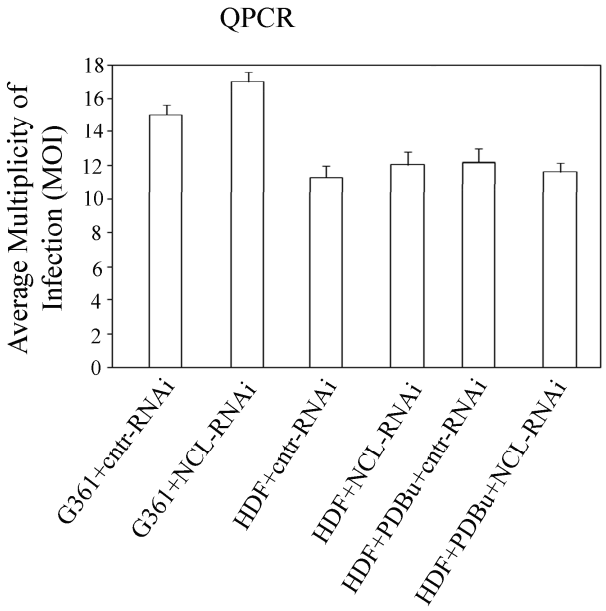
supplemental fig. 1

Suppl. Fig. 1. Effect of kinase inhibitors on phosphorylation of NCL and REST. (A) IMR32, Kelly and G361 cells were treated for 24 h with inhibitors of $p34^{cdc2}$ (olomoucin), PI3K (LY294002) and PKC (PKCξ pseudosubstrate, GF109203X) with dosages twice the IC50 recommended by the suppliers. Following the treatments, nuclear protein extracts were prepared and the effect of inhibitors on the phosphorylation status of NCL was determined by Western blotting with anti-NCL-phosphorylated (Thr76/Thr84) antibody. Results were analyzed densitometrically and presented as histograms. Phosphorylation in non-treated cells was set as 100%. Columns, results from three independent measurements; bars, SEM. (B) REST was immunoprecipitated from IMR32, Kelly and Kelly cells transfected with plasmid expressing full length REST treated with either 10 μM GF109203X (GF) inhibitor or DMSO as a control. Western blots were carried out with the immunoprecipitated RESTs (7 ng in each lane) and anti-phosphoserine to determine its phosphorylation status.



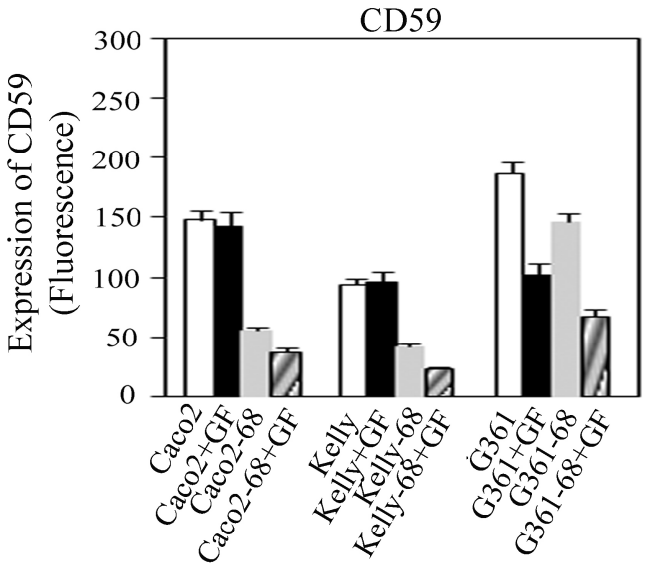
Suppl. Fig. 2. (A) DNA-binding ELISA for binding of REST and NCL to the 35bp RE. The assay was performed with a TransAm kit (Active Motif) using nuclear extracts from IMR32 cells following the supplier's protocol.

Oligonucleotides used in the assay are shown in the right panel: wtRE corresponds to the wild type 35 bp RE from the CD59 promoter; mutRE was designed to bind REST but not NCL (Sp1 binding sites were mutated) following the previously published consensus binding sequences for these two transcription factors (1-3); irr35bp is a 35 bp sequence that does not contain REST and Sp1/NCL binding sites. Columns, results from three independent experiments; bars, SEM. (B) Binding of REST and NCL to the wild type CD59 promoter (wtRE) or CD59 promoter in which the 35 bp RE has been replaced with the irr35bp shown in (A), determined by ChIP of chromatin fragments from IMR32 and Kelly cells transfected with either the wtRE or irr35bp promoter constructs. Binding was quantified by qPCR and was set as 1 for the input control of each transfection. Immunoprecipitation with non-immune rabbit IgG was carried out as a control for the assay background. Columns, results from three independent experiments; bars, SEM. (C) Schematic of *cd59*, *mcl1* and *adcy5* promoter fragments cloned into the pRRLSIN.cPPT.PGK-GFP.WPRE lentiviral vector for knocking down NCL expression. The binding site for REST is in red and the overlapping Sp1 binding sites (bind NCL) are underlined. A schematic of the *bcl2l1* gene, which regulates cell survival and apoptosis, with overlapping REST/NCL binding sites, is also given.



supplemental fig. 3

Suppl. Fig. 3. Assessment of multiplicity of infection (MOI). G361 and HDF cells (treated with PDBu or DMSO as a control) were infected with lentiviral particles containing the pRRLSIN.cPPT.PGK-GFP.WPRE-based constructs expressing either NCL-specific siRNA or a control siRNA. Cells were grown for 48 h and cells positive for GFP were separated by flow sorting. The MOI was assessed by qPCR comparison of the number of copies of the 35 bp RE from the CD59 promoter using genomic DNA purified from transformed and non-treated cells. For example, if the difference in copy number between transformed and non-treated cells was 7, we calculated MOI = 14 based on the fact that the genome is diploid while one viral particle brings only a single copy of the compared sequences.



supplemental figure 4

Suppl. Fig. 4. A cooperative effect of REST68 and GF treatment on suppression of CD59expression. Flow cytometry analysis of expression of CD59 on Caco2, Kelly and G361 cells as a result of REST68, GF or combined treatment. Columns: results from three independent measurements.

References

1. Jothi, R., Cuddapah, S., Barski, A., Cui, K. and Zhao, K. (2008) Genome-wide identification of in vivo protein-DNA binding sites from ChIP-Seq data. *Nucleic Acids Res*, **36**, 5221-5231.
2. Kadonaga, J., Courey, A., Ladika, J. and Tjian, R. (1988) Distinct regions of Sp1 modulate DNA binding and transcriptional activation. *Science*, **242**, 1566-1570.
3. Tsou, J., Chang, K., Wang, W., Tseng, J., Su, W., Hung, L., Chang, W. and Chen, B. (2008) Nucleolin regulates c-Jun/Sp1-dependent transcriptional activation of cPLA2alpha in phorbol ester-treated non-small cell lung cancer A549 cells. *Nucleic Acids Res*, **36**, 217-227.