Supplemental Materials and Methods

Oligonucleotides for shRNA hairpins

Fos#1 forward 5'-GAGAGGTGCTGCTGAGCGATAAAGCGGCTTCATTGATAAATAGTG
AAGCCACAGATGTA-3', Fos#1 reverse 5'-ATTCACCACCACTAGGCAGTAAAGCGGC
TTCATTGATAAATACATCTGTGGCTTCACT-3', Fos#2 forward 5'-GAGAGGTGCTGCT
GAGCGATCAGCGCGGACGTCAGAGATATAGTGAAGCCACAGATGTA-3', Fos#2 reve
rse 5'-ATTCACCACCACTAGGCAGTCAGCGCGGACGTCAGAGATATACATCTGTGGC
TTCACT-3', Jun#1 forward 5'-GAGAGGTGCTGCTGAGCGTAGAACAGGTTGCACAGC
TTAATAGTGAAGCCACAGATGTA-3', Jun#1 reverse 5'-ATTCACCACCACTAGGCAGA
GAACAGGTTGCACAGCTTAATACATCTGTGGCTTCACT-3', Jun#2 forward 5'-GAGAG
GTGCTGCTGAGCGCGGCGGAACTGCACAACCAGAATAGTGAAGCCACAGATGTA-3
', Jun#2 reverse 5'-ATTCACCACCACTAGGCATGGCGGAACTGCACAACCAGAATACA
TCTGTGGCTTCACT-3', ATF2#1 forward 5'-GAGAGGTGCTGCTGAGCGCGGCTATCA
TACTGCAGATAAATAGTGAAGCCACAGATGTA-3', ATF2#1 reverse 5'-ATTCACCACC
ACTAGGCATGGCTATCATACTGCAGATAAATACATCTGTGGCTTCACT-3', ATF2#2 fo
rward 5'-GAGAGGTGCTGCTGAGCGGCCATCCTCTAACAGACCAATATAGTGAAGCC
ACAGATGTA-3', ATF2#2 reverse 5'-ATTCACCACCACTAGGCATCCATCCTCTAACAG
ACCAATATACATCTGTGGCTTCACT-3', Luc forward 5'-GAGAGGTGCTGCTGAGCGA
GCTGCTGGTGCCAACCCTATTTAGTGAAGCCACAGATGTA-3', Luc reverse 5'-ATTC
ACCACCACTAGGCACGCTGCTGGTGCCAACCCTATTTACATCTGTGGCTTCACT-3'.

Cloning of HA-Ras expression constructs

The sequences of the mutagenic oligonucleotides were DN-Ras top 5'-GAGGTGTCGGGAAGAaCGCTTTGACGATACAG-3' and DN-Ras bottom 5'-CTGTATCGTCAAAGCGtTCTTCCCGACACCTC-3'. Lower case letter indicates position of the mutagenic nucleotide. An HA tag was also added to the N-terminus of Ha-Ras to differentiate the endogenous and ectopically expressed proteins. Oliognucleotides encoding the HA tag with flanking *Xho*I and *NotI* restriction sites were designed as follows: HA-tag top 5'-TCGAGATGTACCCATACGATGTTCCAG ATTACGCTGC-3' and HA-tag bottom 5'-GGCCGCAGCGTAATCTGGAACATCGTATG GGTACATC-3'. Underlined letters indicate the ORF of the HA insert. By annealing these complimentary oliognucleotides, the HA insert was created and cloned into the pDS vector. Subsequently, wild-type and dominant negative *Ha-ras* were cloned into pDS in-frame with the HA tag using *Not*I and *BssH*II restriction sites.

Preparation of virus stocks

Retroviral stocks were prepared from primary CEFs transfected with 10 μg of pBIS or pREV retroviral DNA, 0.3 μg of pCSV11S3 encoding an infectious genome of chicken syncytial virus (CSV), and 2.5M $CaCl_2$ mixed with an equal volume of BES-buffered saline solution (50mM BES, 280 mM NaCl, 1.5 mM Na2HPO4). For BIS-based viruses, cells were placed under G418 selection for 10-14 days before virus harvests were made and stored at -80°C. For REV-based viruses, viral supernatant fluids were harvested 5-7 days after transfection and stored at -80°C. RCASARNAi retroviral constructs were transfected as described above but without pCSV11S3. For the

production of DS viruses, the pTZDS-XB vector was completely digested with *Sal*I and ligated to the *Sal*I-digested pREP-A to create the replication-competent viruses. Ligated DNAs (4 μg) were transfected into CEF cultures as described above. Virus stocks were collected from the cultures 6-7 days after transfection and stored at -80°C.

For obtaining titers, virus stocks were bound to Hybond N+ nylon membrane (Amersham Biosciences, Piscataway, NJ) through a dot-blot transfer unit. DNA specific for BIS-based (a neomycin-resistance gene fragment), RCASARNAi (GFP), and DS-based viruses (875 bp *Sma*I-*Xho*I fragment from pTZDS-XB) randomly labeled with [^{32}P]dCTP (DECAprime II Kit, Ambion) was used as a probe. For titration of REV-based viruses, a [^{32}P]ATP labeled oligonucleotide probe specific to REV sequences was employed (Majid *et al.*, 2006). The hybridized probes were detected by phosphorimager analysis. Titers were normalized to titers of the control virus previously determined by immunohistochemical assays (Liss and Bose, 2002).

Northern blot analysis

Total RNA from cells grown in culture was prepared using the TriReagent (Ambion, Austin, TX), according to the manufacturer's instructions. Northern blots were performed as described previously (Majid *et al.*, 2006). Briefly, 10 μg of each RNA sample was separated on a 1% agarose-formaldehyde gel and transferred overnight onto a Hybond N+ nylon membrane (Amersham Biosciences). The membrane was dried and RNA crosslinked with UV. Equal loading and transfer of RNA was confirmed

by methylene blue staining. Membranes were hybridized at 55°C using UltraHyb solution (Ambion) with DNA probes labeled with [32P]dCTP by random priming (DECAprime II Kit, Ambion). The Probes used for the detection of genes are as follows: 1-kb *Xba*I fragment of chicken c-*jun*, 1.3-kb *Kpn*I fragment containing a full-length cDNA of c-*fos*, 0.26-kb *Pvu*II-*Hin*dIII fragment of chicken *fra-2*, and 1.5-kb *Hind*III-*Spe*I fragment of chicken ATF2.

Transfections and reporter assays

Reporter assays were performed as described previously (Majid *et al.*, 2006). Briefly, CEF cultures were transfected with 0.25 μg pRL-TK, 0.2 μg reporter vector, 0.1 μg empty pRc/RSV expression vector (Invitrogen Life Technologies, Carlsbad, CA) or one containing *c-rel* or *v-rel*. The pBluescript vector was used to maintain a total DNA concentration of 10 μg. The pGL2 reporter vectors containing five tandem Coll or Jun2 binding sites were a kind gift from Marc Castellazzi. The DNA was mixed with 2.5M $CaCl_2$ and an equal volume of 2X HEPES-buffered saline solution (50 mM HEPES, 280 mM NaCl, 10 mM KCl, 1.5 mM Na_2HPO_4, 12 μM dextrose). Eight hours later, cells were treated with glycerol shock solution (15% glycerol in 1xHBS). After an additional 24 hours, cells were harvested and luciferase activity measured, according to the manufacturer's instructions (Promega Corporation). Luciferase activity was normalized to values of Renilla luciferase activity to account for variations in transfection efficiency. The normalized luciferase activity obtained for cells transfected with the empty pRc/RSV

vector was considered baseline activity, and the fold induction by transfection of pRc/RSV containing c-rel or v-rel was determined by reference to this baseline.