SUPPLEMENTARY MATERIALS AND METHODS

Real-time RT-PCR analysis. Total RNA was isolated using the RNA STAT-60 reagent (Biogenesis, Poole, UK) according to the manufacturer's instructions. Reverse transcription was carried out with 8µg of RNA using an Moloney Murine Leukaemia Virus (M-MLV) based reverse transcriptase kit (Invitrogen) according to the manufacturer's instructions. Real-time RT-PCR amplification was carried out in a final volume of 10µl containing 5µl 2xSYBR green master mix (Invitrogen), 4µl primers (2µM), and 1µl cDNA using an Opticon DNA Engine Thermal Cycler (Bio-Rad Laboratories Inc., Waltham, MA). All amplifications were primed by pairs of chemically synthesised 18 to 22-mer oligonucleotides designed using published DNA sequences. Primer sequences were as follows: PDF (forward) 5'-CTGAATGGCTCTCAGATGCTC-3', PDF (reverse) 5'-GAGTCTTCGGAGTGCAACTCT-3', 18S (forward) 5'-CATTCGTATTGCGCCGCTA-3', 18S (reverse) 5'-CGACGGTATCTGATCGTCT-3'. Prior to each experiment, primer-specific annealing temperatures were optimised by performing temperature gradient analysis. Reaction conditions were: 50°C for 5 minutes, 95°C for 2 minutes, followed by 40 cycles of 95°C for 15 seconds, 57°C for 30 seconds, and 72°C for 1 minute. Melt curve analyses were used to examine the specificity of an amplified product and standard curves were generated to quantify the absolute expression levels of PDF and the 18S rRNA reference gene in each sample. The relative expression level of PDF in samples of interest was calculated by dividing the amount of normalized target by the value in an untreated calibrator sample.

Immunoblotting. Cells were harvested in ice-cold PBS, pelleted, and resuspended in PBS with protease inhibitors (Roche Diagnostics GmbH), 1 mmol/L sodium orthovanadate (Sigma), and 10 mmol/L sodium fluoride. Cells were then lysed by passing them through a 25-gauge needle 10 times and centrifuged at 13,200 rpm/4°C for 20 min to remove cell

debris. Protein concentration was determined using the bicinchoninic acid protein assay reagent (Pierce). Each protein sample (30 μg) was resolved on SDS-polyacrylamide gels (8%) and transferred to a PROTRAN BA 83 nitrocellulose membrane (Whatman, Schleicher & Schuell). Immunodetections were performed using anti-NAG-1 rabbit polyclonal antibody (Upstate Biotechnology, Charlottesville, VA) and poly(ADP-ribose) polymerase (PARP; eBioscience) mouse mAbs in conjunction with a horseradish peroxidase (HRP)–conjugated anti-rabbit or anti-mouse secondary antibodies (Amersham). Anti–phosphorylated Akt (Ser^{473}, Cell Signaling), anti-Akt (Cell Signaling), anti–phospho-extracellular signal-regulated kinase 1/2 (pErk1/2; Thr^{202}/Tyr^{204}, Cell Signalling, Beverly, MA) mouse mAb and anti-Erk1/2 (K-23, Santa Cruz) rabbit polyclonal antibodies were used in conjunction with an HRP-conjugated antimouse or antirabbit secondary antibody (Amersham). Equal loading was assessed using β-tubulin (Sigma) or glyceraldehyde-3-phosphate dehydrogenase (Biogenesis) mouse monoclonal primary antibodies. The Super Signal chemiluminescent system (Pierce) or ECL-plus (Amersham) were used for detection.

Flow cytometry. Cells were harvested in PBS/0.5mM EDTA and pelleted by centrifugation at 2000rpm/4°C for 5 minutes. Cell pellets were washed once with PBS/1% FCS, fixed in 70% ethanol, and stained with propidium iodide. Analysis was performed on a Beckman Coulter Epics XL flow cytometer (Miami, FL). Cells were gated on a dot plot display of forward scatter versus side scatter to extract aggregates and cell cycle populations were quantified using Wincycle histogram analysis software (Phoenix Flow Systems, San Diego, CA). Apoptosis was determined by evaluating the percentage of cells with DNA content <2N.