Supporting Methods

Cloning and Purification of DSCR1 Fragments. Full-length (FL) DSCR1 was PCR amplified with the primers 5'-CGC**GGATCC**ATGGAGGAGGTGGACCTGCAG-3' and 5'-CCG**GAATT**CTCAGCTGAGGTGGATCGGCGT-3' (BamHI and EcoRI restriction sites added are in boldface). DSCR1 C-terminal fragments A30, K49, R68, T88, D102, K118, and ex7 were amplified with the forward primers 5'-CGC**GGATCC**GCCAAAT-TTGAGTCCCTCTTTAGGACG-3', 5'-CGC**GGATCC**AAGAGCTTCAAACGAGTC-AGAATAAAC-3', 5'-CGC**GGATCC**AGGCTCCAGCTGCATAAGACTGAGTTT-3', 5'-CGC**GGATCC**ACCTTACACATAGGAAGCTCACACCTG-3', 5'-CGC**GGATCC**-GACAAGCAGTTTCTGATCTCCCCTCCC-3', 5'-CGC**GGATCC**AAACAAGTGGA-AGATGCGACCCCAGTC-3', and 5'-CGC**GGATCC**GGAGAAAAGTATGAATTGC-ACGCAGCG-3', respectively. DSCR1 fragments ex5/6, ex6, ex6_D102, and ex6_K118 were amplified by using the above forward primers with the reverse primer 5'-CCG**GA-ATT**CTCATGGCCCCAGCTTGGAGATGGCATA-3'. The PCR products were digested and ligated into the pGEX-6P-1 vector (Amersham).

For biochemical experiments, FL DSCR1 and DSCR1-ex7 (exon 7 of DSCR1 that is encoded by residues 141–197) were purified to homogeneity. These constructs were expressed in *Escherichia coli* Rosetta strains (Novagen), and when the cell density reached OD 600 of 0.6, protein expression was induced with 1 mM IPTG overnight at 16ºC or 2 hrs at 37ºC for DSCR1 and DSCR1-ex7, respectively. Cell pellets were resuspended in lysis buffer [50 mM Tris (pH 8)/300 mM NaCl/10% glycerol/1 mM DTT/1 mM PMSF/1 mM benzamidine] and lysed with a cell disrupter. Lysates were clarified by centrifugation (15,000 × *g* for 1 h). Soluble extracts were incubated with glutathione-Sepharose beads overnight at 4ºC, and the resin was washed extensively with lysis buffer. The GST tag was cleaved on resin by an overnight incubation at 4ºC with 0.5 mg of recombinant rhinovirus 3C protease. DSCR1 or DSCR1-ex7 were eluted and applied onto a HiPrep 26/60 Sephacryl S-100 column (Amersham), which was equilibrated with buffer [20 mM Tris (pH 7.5)/50 mM NaCl/2 mM DTT/250 μM PMSF]. Fractions containing pure protein were pooled.

Cloning and Purification of Calcineurin Fragments. The A and B subunits of calcineurin (CNα) were expressed by using the bicistronic pETCNα construct (40). $CN_{\Delta 372}$ and $CN_{\Delta 347}$ were generated by inserting a stop codon after residues C372 and M347, respectively. In all these constructs, the B subunit was expressed from an internal ribosomal binding sequence immediately preceding the ORF for the *CnB* gene. For biochemical experiments, CNα and $CN_{\Delta 347}$ were purified to homogeneity and were expressed in *E. coli* BL21(DE3) cells, and, when cell density reached OD 600, protein expression was induced with 1 mM IPTG at 37ºC for 3 h. Cell pellets were resuspended in lysis buffer [50 mM Tris (pH 8)/300 mM NaCl/10% glycerol/1 mM CaCl2/0.5 mM EDTA/5 mM β-mercaptoethanol and 1 mM PMSF/1 mM benzamidine/1 mM pepstatin A/1 μg/ml aprotinin/1 μg/ml leupeptin] and lysed with a cell disrupter. Soluble extracts were loaded onto a DE52 (Whatman) resin, and the flow-through was loaded onto a HiTrap metal chelating column (Amersham) charged with 100 mM NiSO4. After washing extensively with lysis buffer, the column was further washed with 20 column

volumes of lysis buffer containing 15 mM imidazole. The proteins were eluted with a linear 0–400 mM imidazole gradient in lysis buffer. Fractions containing the proteins were diluted until salt concentrations were ≈75 mM NaCl before loading onto a HiTrap Q XL column (Amersham). The proteins were eluted with a linear 100–800 mM NaCl gradient in lysis buffer, concentrated, and applied to a HiLoad 26/60 Superdex 200 column (Amersham). Fractions containing pure protein were collected and pooled. Because the $CN_{\Delta 347}$ fragment lacks the CnB binding domain, the overexpressed CnB subunit separates from the catalytic domain of calcineurin during purification.

CD Spectroscopy. Purified DSCR1-ex7 was exchanged into an appropriate buffer [10 nM Na phosphate (pH 7.5), 50 mM NaSO4, and 2 mM DTT] for CD and concentrated to 0.2 mg/ml. CD spectra were recorded on a CD spectrometer (model 62DS, Aviv Associates, Lakewood, NJ) fitted with a thermostated cell holder. Sample spectra were measured at 10ºC by using a quartz sample cell (Uvonics, Plainview, NY) with a path length of 1 mm. The following acquisition parameters were used: scan range, 320–200 nm; data interval, 1 nm; averaging time, 10 s; spectral bandwidth, 1.5 nm. CD spectra obtained with buffer solutions were subtracted from the protein spectra.

Calcineurin Phosphatase Activity Towards pNPP. Phosphatase assays using *p*-nitrophenyl phosphate (pNPP, Pierce) were performed in a 50-μl reaction volume using 50 nM CNα or $CN_{\Delta 347}$ and FL DSCR1 or FLR1-ex7 at a concentration range of 0–100 nM. The concentration of pNPP was varied from 0 to 150 mM for each concentration of DSCR1 (FL) and (ex7). Blank tubes containing only buffer [100 nM Tris, pH 7.5, 100 mM NaCl, 1 mM DTT, 1 mM CaCl2, 1 mM MnCl2, 100 μg/ml BSA, and 200 nM calmodulin (Sigma)] were prepared to determine background levels of hydrolyzed product, *p*-nitrophenol (PNP). The enzyme was preincubated in buffer for 10 min at 30ºC, and the reactions were initiated by the addition of pNPP. The mixtures were incubated for a further 30 min at 30ºC, allowing for a ≈1% product formation. The reactions were terminated by the addition of 950 μl of 1 M NaOH, and the amount of PNP generated was measured by absorbance at 405 nm by using a DU640 spectrophotometer (Beckman Couter). The absorbance values were corrected by dilution factors, and the background PNP hydrolysis activity was subtracted from the results. The final absorbance values were converted into the amount of PNP released by using the extinction coefficient (13,700 $cm^{-1} \cdot M^{-1}$) and divided by the reaction time (30 min). Each reaction was carried out in triplicate, and the data were plotted as velocity (nmol of PNP/min) versus pNPP concentration. The data were fitted to nonlinear regression equations [**1**] and [**2**] for competitive and noncompetitive inhibition, respectively, by using SIGMAPLOT 8.0.

$$v = \{V\mathrm{max}[S]\} / \{K\mathrm{m}(1+[I] / Ki) + [S]\} \quad [1]$$

$$v = \{V\mathrm{max}[S] / K\mathrm{m}(1 + [I] / Kii)\} / (K\mathrm{m} + [S]), \quad [2]$$

where v is the velocity, Vmax is the maximum velocity, [S] is substrate concentration, Km is the Michaelis constant, [I] is inhibitor concentration, and Ki and Kii are the dissociation constants for the enzyme-inhibitor complex for competitive and noncompetitive inhibitors, respectively.