Supplementary material 1

HIP1 gene targeting. The pIFS promoter trap was used to target the HIP1 gene by replacing exon 8 and adjacent intronic sequences with an SA-IRES-βGeo-PA expression cassette. The targeting vector contained 7 Kb of 5'-homology and 3 Kb of 3'-homology which was amplified by polymerase chain reaction (PCR) from R1 ES cells and cloned into pIFS. The resulting vector was linearized and electroporated into R1 ES cells as described (Metzler et al., 1994). Homologous recombinant clones were identified by PCR which resulted in the amplification of a 3 Kb fragment using the sense primer p1 (5'-GAGTAGGTGTCATTCTATTCTGAGG) and anti-sense primer p2 (5'-CTCGTCGAACTGTCACATCAGTATCTCC). For characterization by Southern blot analysis genomic DNA from PCR positive clones was isolated and digested with EcoRI and NcoI, and hybridized with a radioactively labeled HIP1 fragment that was isolated from intron 10.

Southern blot analysis. Correct targeting at the HIP1 locus was confirmed by Southern blot analysis of DNA isolated from 5 targeted ES cell clones and the parental R1 ES cell line using a Southern probe isolated from intron 10 as shown in (Fig. 1A). Following digestion of DNA with EcoRI and NcoI, DNA fragments of the expected size were present, indicating normal (10.5 and 7.7 Kb) and targeted alleles (7.8 and 6.5 Kb).

PCR genotyping and RT-PCR. DNA was extracted from tails and 1 µg of DNA was used in 2 separate PCRs to determine the presence of the wild-type and targeted allele. For identification of the wild-type allele a HIP1 exon 8-specific sense primer p3 (5'-AACCTCCAGATGAGTGGACCC) and an intron 8-specific anti-sense primer p4 (5'-
CCTGTGCTCTGTCCTGCTTC) were used. For amplification of the targeted allele the neomycin-specific primer p1 was used in combination with the anti-sense primer p4. Total RNA was isolated from R1 ES cells and the RT-PCR reaction was performed as described previously (Chopra et al., 2000).