Real-time quantitative RT-PCR. Total RNA was harvested from cells using TRIzol (Invitrogen) according to the manufacturer's directions. One microgram of total RNA was transcribed into cDNA using a First Strand cDNA Synthesis Kit (Fermentas) for reverse transcription-PCR (RT-PCR) according to the manufacturer's instructions, using random hexamers as primers. Five ul of cDNAs (diluted 1:50) were then amplified in PCR reactions containing 1.5 mM $MgCl_2$, 160 nM dNTPs, 1 unit of *Taq* polymerase (Fermentas), 12.5 nM fluorescein (Bio-Rad), SYBR Green (Invitrogen), and 500 nM of specific primer pairs:

NRIF3 sense: TTCTCAACTTTTGATAGCAACATCA,

NRIF3 antisense: AAAAATTGAATCACCCCAGTTT;

DIF-1 sense: CCGACTTCACCTTCTGGTTC,

DIF-1 antisense: AGGTTGTTGGGTTTCGAGG;

18S sense: CCATCCAATCGGTAGTAGCG,

18S antisense: GTAACCCGTTGAACCCCATT;

The amplified products were detected with a Bio-Rad iCycler (Bio-Rad). PCR program started with one cycle at 94^{o}C for 4 min, followed by 40 cycles of 94^{o}C for 20 s, 60^{o}C for 20 s, 72^{o}C for 20 s. Samples were normalized based on the amount of 18S ribosomal cDNA present, as determined by real-time PCR. A validation experiment was run to show that the efficiencies of all the targets amplifications are approximately equal. Using the comparative threshold cycle method (ref), relative levels were determined using samples from duplicate wells, each assayed in triplicate PCRs.

Ref: Applied Biosystems. 2008. Guide to performing relative quantitation of gene expression using real-time quantitative PCR. Applied Biosystems, Foster City, CA.