Supplemental Figure S2: RT-PCR analyses

First strand cDNA was synthesized from total RNA templates using random primers and the superscript II reverse transcriptase (Invitrogen, Carlsbad, CA). One to two micrograms of total RNA was brought to 11 µl in diethyl pyrocarbonate (DEPC)-treated water and combined with 1 µl random hexamers (50 ng/µl). The mixture was heated at 65^{o}C for 5 min and then incubated on ice. The remaining components for reverse transcription were then added and incubated for 10 min as follows: 4 µl of 5x synthesis buffer, 2 µl 0.1 M DTT, 1 µl 10 mM dNTP mix and 1 µl (200 units) superscript reverse transcriptase. The reaction mix was left at room temperature (RT) for 10 min, incubated at 42^{o}C for 50 min and the reaction terminated by incubating at 70^{o}C for 15 min. Oligonucleotides corresponding to EGFP sequences were: 1) sense 5'-CCGACCACATGAAGCAGCACGA -3'; 2) antisense 5'-CGTTCTTCTGCTTGTCGGCCATG-3'. Oligonucleotides corresponding to b-actin sequences were: 1)sense 5'-ATGGATGACGATATCGCTGCGC-3'; 2)antisense 5'-TTCACGGTTGGCCTTAGGGTTCAG-3'.

PCR reactions contained (in a final volume of 50 µl): 5 µl of RT reaction (0.5-1 µg of first strand cDNA), 1 µM of sense and antisense primers, 25 µl of 2x PCR buffer HotStar Taq(Qiagen, Valencia, CA). Thirty cycles were performed on the samples using a MJ Mini Thermocycler (Biorad, Hercules, CA) as follows: 1) denaturation at 94^{o}C for 30 s; 2) annealing at 57^{o}C for 30 s; 3) extension at 72^{o}C for 30 s. This was followed by a final extension cycle at 72^{o}C for 10 min and a soak cycle at 4^{o}C. Reaction products were analyzed by electrophoresis on 2% agarose gels. Amplicon sizes were determined in agarose DNA gels following electrophoresis with 1Kb DNA ladder markers (Invitrogen, Carlsbad, CA).