

Supplemental Appendix 2 Analysis of CXCR4 expression using real-time quantitative RT-PCR

Commercial kits for RNA extraction (RNeasy, Qiagen, Valencia, CA, USA) and reverse transcription (SuperScript First-Strand Synthesis System, Invitrogen, Carlsbad, CA, USA) were used prior to amplification of specific cDNA using the primer pairs shown in Appendix Table 1. Human GAPDH was used as an internal control for loading. Real-time quantitative RT-PCR was performed using the ABI Prism 7500 sequence detector (Applied Biosystems; ABI, Foster. City, CA, USA) and the SYBR premix Ex Taq (Perfect Real Time, TaKaRa, Shiga, Japan). Reactions were performed at 95°C for 10 s, followed by 40 cycles of 95°C for 5 s, 60°C for 40 s, and a melt curve analysis to confirm the purity of the reaction products. A nontemplate control was included for both of the primer pairs. The amount of CXCR4 mRNA was determined from threshold cycle values normalized for GAPDH expression and then normalized to the value derived from cells at time 0 prior to the cytokine treatment. Each sample was assessed in triplicate. Experiments were performed at least three times.