Supporting information for Chen *et al*. (2002) *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas.202614899.

## Supporting Materials and Methods

### Constant Ratio PCR (CR-PCR) and Chip Hybridization.

Absolutely RNA microprep kit (Stratagene) was used to isolate total RNA from small samples of sorted cells. Small amounts of RNA were quantitated by using the RiboGreen RNA quantitative kit (Molecular Probes). A Smart cDNA synthesis kit (CLONTECH), which was used for first-strand cDNA synthesis and amplification, was modified to adapt to CR-PCR protocol. The basic design of CR-PCR protocol is described in the Fig. 2 legend. Each modified oligo(dT) primer [poly(T-1), poly(T-2), and poly(T-3); below] contains a universal forward sequence (primer sequence underlined; black box in Fig. 2), a unique sequence tag (T-1, T-2, or T-3 primer sequence shown in italic; dark-blue and dark-orange boxes in Fig. 2), and oligo(dT) sequences. Each RNA sample (1 ng) was reverse-transcribed by using a designated poly(T) primer and the SMART oligo template-switching primer provided in the SMART cDNA synthesis kit (dark-green box linked to GGG in Fig. 2). This allows labeling mRNA from different cell populations with specific T-1, T-2, or T-3 tags by RT. Equal amounts of RT products from two or three samples were mixed and amplified with Universal

forward (black box in Fig. 2) and reverse PCR primer (dark-green box; sequences given below) by using advantage DNA polymerase. These Universal forward and reverse PCR primers are matched to the poly(T) primer (underline) and SMART oligo, respectively. Twenty amplification cycles were carried out to amplify cDNAs from 1 ng of total RNA. Amplified samples were then split into two or three equal fractions. T7 promoters (white box, Fig. 2) containing T1, T2, or T3 sequence tags that are matched to the corresponding polyT primers (dark-blue and dark-orange boxes; Fig. 2), the poly(T-1), poly(T–2), and poly(T-3) sequences specified above, were added selectively to the corresponding fraction of cDNAs in the mixture by linear extension using Advantage DNA polymerase. Biotinylated cRNAs were then synthesized and hybridized to Affymetrix U74Av2 mouse chips at 45°C for 16 h. Arrays were washed, stained, and scanned according to standard protocols. Raw gene-expression value was normalized by total signal intensity between the samples within the same amplification group. We identified genes that are differentially expressed among sample groups and clustered them according to the ratio and difference in fluorescent intensity.

RT Primers. poly(T-1), 5'-CTCACTATAGGGAGGCGG*ATCGC*$(T)_{24}$-3'; poly(T-2), 5'-CTCACTATAGGGAGGCGG*CAGCT*$(T)_{24}$-3'; and poly(T-3), 5'-CTCACTATAGGGAGGCGG*TCACG*$(T)_{24}$-3'.

PCR Amplification Primers. SMART du Universal – reverse, 5’-AAGCAGTGGTAACAACGCACACT-3’, and SMART du Universal – forward, 5’-CGACTCACTATAGGGAGGCGG-3’.

T7 Promoter Primers. T7-1, 5’-GGCCAGTGAATTGTAATACGACTCACTATAGGGAGGCGG*ATCGCT* -3’; T7-2, 5’-GGCCAGTGAATTGTAATACGACTCACTATAGGGAGGCGG*CAGCTT*-3’; and T7-3, 5’-GGCCAGTGAATTGTAATACGACTCACTATAGGGAGGCGG*TCACGT*-3’.