Supplemental File E1. Quantitative PCR expression value normalization

High throughput quantitative PCR (qPCR) was performed using Fluidigm 96.96 Dynamic Arrays IFC (South San Francisco, CA, USA). Each array plate contains 96 assay inlets and 96 sample inlets, which allows for 9,216 reactions in one run. RNA was quantitated by a Nanodrop spectrophotometer (Wilmington, DE, USA). After multiplex pre-amplification of each sample with random hexamer primers (20 ng of total RNA, 15 cycles), 48 samples and 48 assays (primer-probe sets) were each run in duplicate on each Fluidigm plate using custom nested primers. Cycle threshold (Ct) values for duplicate assay inlets and duplicate sample inlets were analyzed for reproducibility of results. If one of the duplicate samples performed poorly across multiple assays, then that sample duplicate was dropped.

Healthy control and sarcoidosis samples were randomized to a total of ten Fluidigm 96.96 plates (representing 92,160 reactions). Each plate consisted of 75% sarcoidosis samples and 25% healthy control samples. For each unique sample-assay combination, the Ct values for the replicate reactions (up to 4) were averaged. Log2-transformed, normalized, relative expression values were generated by subtracting the inter-chip calibrated Ct value for a particular sample from the mean inter-chip calibrated Ct of the housekeeping genes for that sample. Next, within each individual plate, the Ct values were normalized to the Ct values of healthy control subjects by subtracting the mean Ct of the healthy controls from each sample on a per-gene basis, generating inter-chip calibrated Ct values to minimize batch effect.