Supplementary information: data collection and processing methods used for data displayed in this publication.

**Data collection and labeling.** The FacsXpert protocol design tool ([http://scienceXperts.com](http://scienceXperts.com)) was used to collect information for composing stain sets, labeling axes, defining subjects and samples, adding compensation (single stain) controls and recording these and information relevant to other aspects of the protocol design process. When the protocol design was complete, a summary file automatically formatted by FacsXpert was used to pass the protocol information to the DIVA software ([http://BD.com](http://BD.com)) controlling the Stanford-modified 3-laser 14-parameter FACS instrument (Flasher II) with which data were collected. Spherotec 197 Rainbow beads (Spherotec) were used to standardize the instrument before initiation of data collection and whenever nozzle clogs or other problems required re-initiation of data collection.

For each analyzed sample, the DIVA software packages the uncompensated data, plus the protocol and instrument information, into a separate Flow Cytometry Standard (FCS) file and stores these files on its local computer. If a compensation matrix was defined with the DIVA compensation utility, this matrix is also packaged with the data in the FCS files.

**Data storage.** Once data collection is complete, the user triggers a specially-designed data storage utility running on the local DIVA computer to move the data files to the central Stanford Shared FACS Facility DataStore for long-term storage. This data storage system, available as ScienceDataStore from ScienceXperts.com ([http://scienceXperts.com](http://scienceXperts.com)), provides long-term secure storage for large data files and serves these files readily over the Internet to cooperating data analysis programs. In addition, it provides the user with a specific link that can be used to call for a copy of the data from the DataStore.

**Data import and transformation for logicle data display.** The FlowJo FACS data analysis package ([http://treestar.com](http://treestar.com)) was used for studies shown here. Data were called back from the DataStore by supplying FlowJo with the link that specifies the data location. FlowJo imports data for display on logarithmic or on logicle axes depending on the preferences that are set. Setting used for the data presented here are shown in the MAC screen view on the right. (Note: The location in FlowJo for setting the preferences is different on the MAC and PC. Therefore, please refer to the FlowJo website for instructions.) See figure.

**Fluorescence compensation.** As part of the data loading process, FlowJo automatically associates the axis labels and other protocol information with the appropriate components of the data. If there is a compensation matrix in the datafile, FlowJo will automatically associate it with the appropriate data set. Alternatively, FlowJo can be triggered to compute the necessary compensation matrices based on user specification of the appropriate compensation (single-stain) controls in the data set. Once the matrices have been computed, they can be applied to the appropriate samples and the compensated data for the samples used for further analyses.

**Gating.** Compensated data were displayed and gated sequentially as shown in the accompanying figure (see below).
Propidium iodide (PI) was included in this stain to reveal dead cells, which are revealed as cells having fluorescence values >10^5 on the PECy5 scale. The few dead cells present in this data set were gated out in a FSc by PECy5 display before the lymphocytes gates were set.