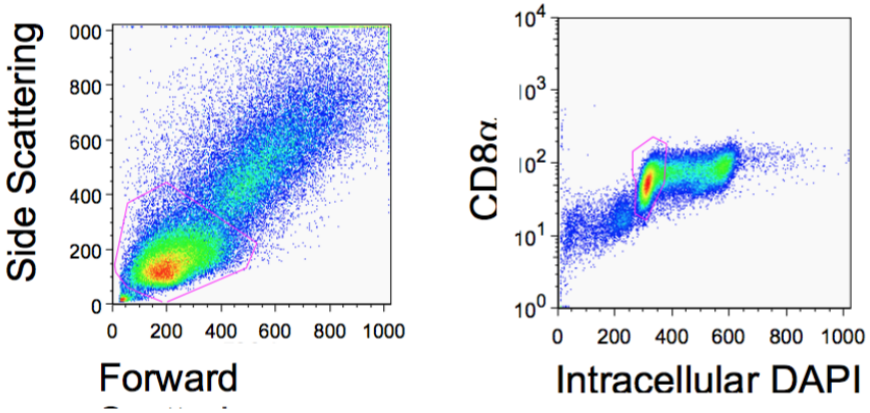
Dataset: FeinermanScience2008
Previously published in Feinerman et al. Science Vol. 321, Issue 5892, pp. 1081-1084 (2008).
MIFlowCyt-Compliant Items

Requirement	Please Include Requested Information
1.1. Purpose	To measure variability and robustness in T Cell activation
1.2. Keywords	ERK, MAP-Kinase, CD8, T-Cell, activation, SHP-1
1.3. Experiment variables	ppERK dose-response for different CD8 and SHP-1 levels in individual OT-1 T cell blasts
1.4. Organization name and address	Memorial Sloan Kettering Cancer Center, New York, NY, 10065, USA
1.5. Primary contact name and email address	Gregoire Altan-Bonnet gregoire.altan-bonnet@nih.gov
1.6. Date or time period of experiment	July 2007
1.7. Conclusions	Good agreement between theory and
1.8. Quality control measures	
2.1.1.1. (2.1.2.1., 2.1.3.1.) Sample description	OT-1 T cell cultures were prepared as follows. C57Bl/6N splenocytes were pulsed for 2hr with 100nM SIINFEKL peptide, then irradiated (3000RAD), washed once and used as stimulator/feeder cells. OT-1 cells were harvested from axillary, brachial and inguinal lymph nodes as well as spleen (splenocytes were treated with ACK lysis buffer to remove red blood cells), and mixed with SIINFEKL-pulsed B6 splenocytes in complete RPMI. After two days, cells were expanded by diluting 2 fold into medium containing 100 pM IL-2. After four days, the cells were again expanded by 2 fold dilution into medium with IL-2. After one more day of culture, cells were harvested and spun through a 1.09 density Ficoll- Paque Plus gradient (GE Healthcare) to remove dead cells. Live cells were recovered, washed twice in complete medium and resuspended at 1 million/ml in complete medium with 100pM IL-2. Cells were used for experiments between 6 and 8 days after primary stimulation.
2.1.1.2. Biological sample source description	
2.1.1.3. Biological sample source organism description	Splenocytes and lymphocytes were isolated from C57Bl/6N mice (Taconic Farms, Rockville, MD, USA) or H-2b OT-1 TCR transgenic mice (NIAID contract colony, Taconic Farms) on a Rag-2-/- background (S1) and used to prepare cultures of primary cells (see below). All mice were bred and maintained in accordance with the protocol (MSKCC#05-12-031) approved by the institutional animal care and use committee (IACUC) of Memorial Sloan-Kettering Cancer Center.
2.1.2.2. Environmental sample location	There were no environmental samples
2.3. Sample treatment description	The ppERK response to SRC and MEK inhibition was measured using primary OT-1 T-lymphocytes activated with RMA-S APCs. RMA-S cells were suspended in culture with 1 nM SIINFEKL peptide for 2 h at 37 °C, 5% CO2, and on a rotator to guarantee mixing. During this time we labelled OT-1 cells with an amine-reactive dye, CTV, according to the manufacture's protocol (Molecular Probes). This fluorescent tag was used to

	identify OT-1 cells in silico. We rested the OT-1 cells one hour after CTV staining, and then distributed them in a 96-well v-bottom plate. Each well was given various doses of SRC inhibitor and MEK inhibitor and kept at 37 °C for 5 min. Following the 5 min exposure to the inhibitors, we added the peptide pulsed RMA-S (10 RMA-S to 1 OT-1 T cell) and pelleted by centrifugation for 10 s at 460 rcf at room temperature. This step guaranteed that both cell types, OT-1 and RMA-S, came into contact. The cells were allowed to activate for 10 min, followed by fixing on ice in 2% PFA, and then permeabilized and stored in 90% MeOH at 20 °C.APCs were pulsed with serial dilutions of OVA or variant peptides for 2 hr at 37°C, then washed with T cell medium at the time of harvest, and resuspended with anti-CD8α (53-6.72)-Fab-coated T cells in their conditioned media in a V-bottom 96-well plate (Corning). Fab-coating was performed 10 min before cell use by incubating T cells with 10 μg/ml of Fab fragment. T:APC cell contacts were synchronized using a quick centrifugal spin (10s at 400g). Plates containing T:APC conjugates were placed on a water bath at 37°C and incubated for 5 min. Supernatants were then discarded, T:APC conjugates disrupted by vortexing, and cells resuspended in ice-cold 4% paraformaldehyde for 15 min. Cells were then permeabilized with ice-cold 90% methanol for 15 min on ice, and washed twice with FACS buffer.
2.4. Fluorescence reagent(s) description	Cells were then stained with rabbit-anti-endogenous protein (e.g., to SHP-1) and mouse- anti-ppERK (0.2μg/ml) for 30 min at room temperature. Secondary antibodies [anti-rat(FITC) + anti- rabbit(PE) + (anti-mouse(APC)] were then used at 2 μg/ml for 30min. Cells were loaded in FACS buffer containing 1 μg/ml DAPI and fluorescence acquired on a LSRII instrument (BDBioscience, San Diego, CA, USA), after compensation of fluorescence bleed-through between channels (see SOM#8 for an example of FACS data analysis).
3.1. Instrument manufacturer	BD Biosciences
3.2. Instrument model	LSRII (Marcel van den Brink Lab, MSKCC)
3.3. Instrument configuration and settings	Parameter voltages: FSC: SSC: FITC (pMEK): A-700 (CD8): PE (ppERK): Pacific Blue (CTV):
4.1. List-mode data files	All data for this experiment can be found in https://github.com/AmirErez/BimodalLogspaceCytA/tree/master/Data/FeinermanScience2008
4.2. Compensation description	Compensation samples were prepared the same way as the sample cells.

4.3. Data transformation details	Explained in main article
4.4.1. Gate description	See figures below for detailed visual description. Cells were gated for lymphocyte FSC-SSC, then CD8+DAPI-.
4.4.2. Gate statistics	See figures below
4.4.3. Gate boundaries	See figures below

Gating strategy:



Compensation (spillover matrix): single stain on vertical axis, spilled-into channels in horizontal axis. This plot was produced with FlowJo 9.9 (OSX, default settings).