Supplementary Material For:

An improved intracellular staining protocol for efficient detection of nuclear proteins in YFP-expressing cells

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Supplementary Materials and Methods

Mice
Rosa-YFP transgenic mice were purchased from Jackson Laboratory. Transgenic mice expressing the Vav-Cre transgene, in which the pan-hematopoietic promoter elements derived from the Vav1 oncogene were used to drive Cre recombinase expression, has been described3,4. EGFP-Foxp3 N-terminal knock-in mice were also described previously8. All animal experiments were carried out under protocols approved by the Institutional Animal Care and Usage Committee of the University of Pittsburgh (protocol approval number 0811074).

Flow cytometry
Flow cytometric analysis was performed on the BD FACSCalibur flow cytometer (BD Biosciences, San Jose, CA) and analyzed with CellQuest Pro software (BD Biosciences). Single cell suspensions were prepared from spleen, subjected to erythrocyte depletion in red blood cell lysis buffer (Sigma-Aldrich, St. Louis, MO), blocked with anti-CD16/32 antibody and stained with antibodies. The following antibodies were purchased from BD Biosciences: anti-CD16/32 (2.4G2), anti-CD4 PeCy5 (H129.9), anti-CD45-APC (30-F11), and anti-CD3-APC (145-2C11). Anti-CD25-APC (7D4) antibody was purchased from Miltenyi Biotec (Auburn, CA). Staining buffer: phosphate buffered saline (PBS, calcium and magnesium free, Invitrogen) supplemented with 1% bovine
Benchmarks

Table S1. Prefixation factors (PFs) under various prefixation conditions

<table>
<thead>
<tr>
<th>Prefixation Time (minutes)</th>
<th>2%</th>
<th>1%</th>
<th>0.5%</th>
<th>0.1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>60</td>
<td>30</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td>15</td>
<td>30</td>
<td>15</td>
<td>7.5</td>
<td>1.5</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>5</td>
<td>2.5</td>
<td>0.5</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>1</td>
<td>0.5</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Note: Prefixation factor (PF) = Prefixation time (minute) x Paraformaldehyde concentration (%). Cells treated with PFs in yellow could preserve sufficient YFP signals for FACS detection.

Intracellular staining of Foxp3 and Helios

Both the Foxp3 protein staining kit and the anti-helios antibody (Clone 22F6) were purchased from eBiosciences (San Diego, CA). Unless specified in the text, all procedures were performed following manufacturer’s suggested protocol. Permeabilization buffer contains 0.1% saponin and 0.09% sodium azide in PBS. Fixative/Permeabilization (Fix/Perm) buffer is prepared by supplementing permeabilization buffer with 4% paraformaldehyde.

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serum albumin (BSA, Sigma-Aldrich) and 0.1% sodium azide (Sigma-Aldrich).