**IFN-γ staining protocol**

Lineage deplete your cells to get cells of interest (for NK cells, use CD3, 4, 8, 19, Ter119)

Resuspend cells in Opti-Mem + IL-2 and IL-12 in 6 well plate  
IL-2: 1000 IU/mL (stock in freezer is at 1000 IU/uL)  
IL-12: 2 ng/mL (stock at 10 ug/mL)

(If should have 200,000-500,000 cells per well)

Leave for 8 hours @ 37°C

Add Golgi Plug (Brefeldin A) @ 1:1000 dilution (BD Biosciences)  
Brefeldin is in glycerol so will sink to bottom of well; make sure that you gently agitate the plate to spread it around a bit

Leave for 4 more hours @ 37°C

After 12 hrs total, cell surface stain as usual (step 1)

After staining for surface markers,  
Resuspend in 100 uL of Cytofix/Cytoperm for 10-20 minutes @ 4°C

Wash 2x with Perm/Wash Buffer

Resuspend in 100 uL Perm/Wash Buffer and add IFN-γ PE (1uL) or IgG-PE control (5 uL, depending on concentration) for 20 minutes at room temp. (preferred) or 30 minutes @ 4°C (step 2)

Wash 2x with Perm/Wash Buffer

Resuspend in FACS buffer for analysis
Staining examples:

<table>
<thead>
<tr>
<th>IFN-γ</th>
<th>control</th>
<th>“staining control”*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3</td>
<td>CD3</td>
<td>CD3</td>
</tr>
<tr>
<td>NK1.1</td>
<td>NK1.1</td>
<td>NK1.1</td>
</tr>
<tr>
<td>DX5</td>
<td>DX5</td>
<td>IgG</td>
</tr>
</tbody>
</table>

Cell surface staining *(step 1)*

* “Staining control” is done to demarcate your DX5+ population, which will be the most relevant one for analysis (CD3-DX5+). The staining control does not need to be processed beyond the first 2 washes with Perm/Wash buffer. Simply resuspend in FACS buffer and set aside until remainder of samples are ready.

A sample set-up plate may look like this:

<table>
<thead>
<tr>
<th>WT IFN-γ stain</th>
<th>WT control stain</th>
<th>“staining control”</th>
</tr>
</thead>
<tbody>
<tr>
<td>KO IFN-γ stain</td>
<td>KO control stain</td>
<td>Non-stimulated control: WT cells with OptiMem only (no IL-2 and IL-12), but stain to look for IFN-γ, which should not be produced</td>
</tr>
</tbody>
</table>