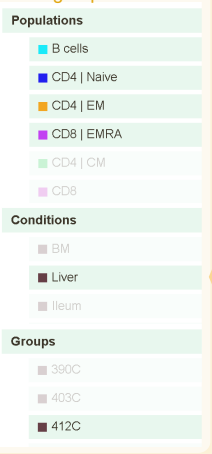
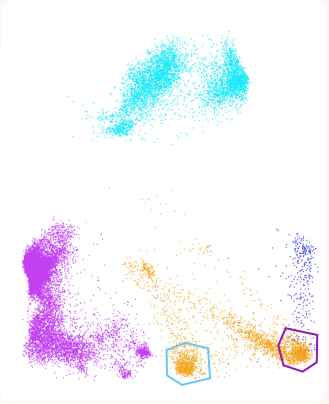
**QC and cleanup**
anomalous
signal **removal**


# FLOWAtlas

**filter** and
**colour** by conditions
and groups




**zoom**, **pan**, **draw**,
**discover** populations



## 1

FLOWJO

Open all files in FlowJo.
Ensure compensation matrix is correct
**Exclude** irrelevant events by gating out dead cells and aggregates
**Export** live single cells with only **compensated channels**
into new FCS files

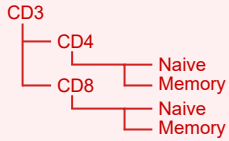
open exported files in a new FJ workspace

## 2

Annotate channels

| Panel A | | Panel B | | Panel C | |
|---|---|---|---|---|---|
| CD45 | BUV805 | CD45 | missing | CD45 | missing |
| CD19 | missing | CD19 | missing | CD19 | BV570 |
| IgM | missing | IgM | missing | CD3/IgD | BUV395 |
| IgD | missing | IgD | missing | CD4 | BUV805 |
| CD4 | BUV661 | CD4 | BUV805 | Foxp3/IgM | PE |
| Foxp3 | PE | Foxp3 | PE | | |

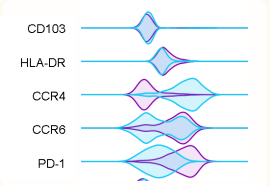
## 3



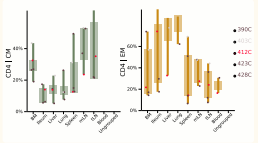

create panel-specific
**gating strategies** and
annotate
**equivalent populations**
using one common name

import into FA
**explore**

**differential** analysis of
**expression**




**differential** analysis of
population **abundances**




## 4

| | |
|---|---|
| Blood | Donor 390C |
| BM | Donor 403C |
| Ileum | Donor 412C |
| Liver | Donor 423C |
| Lung | Donor 428C |

assign all samples to
**groups** to ease data
filtering in FA

add **new populations** to FJ