Sample processing for mass cytometry

Cryopreserved PBMCs were thawed, washed twice with Cell Staining Buffer (CSB) (Fluidigm), and counted with an automated cell counter (Countess II - Thermo Fisher Scientific). Extracellular staining on live cells was done in CSB for 30 mins at room temperature, in 3-5^10^6 cells per sample. Cells were washed with 1X PBS (Fluidigm) and stained with 1 ml of 0.25 mM cisplatin (Fluidigm) for 1 min at room temperature for exclusion of dead cells. Samples were then washed with CSB and incubated with 1.6% PFA (Electron Microscopy Sciences) during 10 mins at room temperature. Samples were washed with CBS and barcoded using a Cell-ID™ 20-Plex Pd Barcoding Kit (Fluidigm) of lanthanide-tagged cell reactive metal chelators that will covalently label samples with a unique combination of palladium isotopes, then combined. Surface staining with antibodies that work on fixed epitopes was performed in CSB for 30 mins at room temperature (see **Antibody Table**). Cells were washed twice with CSB and fixed in Fix/Perm buffer (eBioscience) for 30 min, washed in permeabilization buffer (eBioscience) twice, then intracellular factors were stained in permeabilization buffer for 45 min at 4°C. Cells were washed twice with Fix/Perm Buffer and were labeled overnight at 4°C with Cell-ID Intercalator-Ir (Fluidigm) for DNA staining. Cells were then analyzed on a Helios instrument (Fluidigm).

To make all samples comparable, pre-processing of mass cytometry data included normalization within and between batches via polystyrene beads embedded with lanthanides as previously described (1). Files were debarcoded using the Matlab DebarcoderTool (2). Then normalization again between batches relative to a reference batch based on technical replicates (3). Gating was performed using Cell engine (Primitybio).

References

1. R. Finck *et al.*, Normalization of mass cytometry data with bead standards. *Cytometry A* **83**, 483-494 (2013).
2. E. R. Zunder *et al.*, Palladium-based mass tag cell barcoding with a doublet-filtering scheme and single-cell deconvolution algorithm. *Nat Protoc* **10**, 316-333 (2015).
3. R. P. Schuyler *et al.*, Minimizing Batch Effects in Mass Cytometry Data. *Front Immunol* **10**, 2367 (2019).