Farnham Lab Protocol for Peripheral blood mononuclear cells (PBMC)

1. **Cell isolation.**
   Whole blood was mixed 1:1 with PBS and layered onto histopaque in 50mL tubes. The tubes were spun at 400xg for 30 minutes with the brake off. The plasma was carefully removed and discarded, and the buffy coat was transferred to a fresh 50mL tube and ~20mLs of PBS was added. Cells were spun at 2400rpm (r=180mm) for 10 minutes. PBS was removed and the cells were resuspended in 2 mls PBS, then topped up to 20mL and spun again. The resulting cell pellet was resuspended to 10mLs using PBS and a sample taken for a cell count. The cells were then re-spun and suspended in warm sterile medium to approx 5x10>5 cells / mL.

2. **Crosslinking reaction.**
   The cells were crosslinked in PBS containing 1% formaldehyde for 10 min at room temperature on oscillating platform shaker.The reaction was stopped by adding glycine to a final concentration of 0.125 M. After 5 min, cells were washed twice with ice-cold PBS (Ca+2/Mg+2 free) and centrifuged at 1,500-2,000 x g for 5 min at 4°C. The supernatant was discarded and the cell pellet was flash frozen in liquid nitrogen and stored at - 80°C.