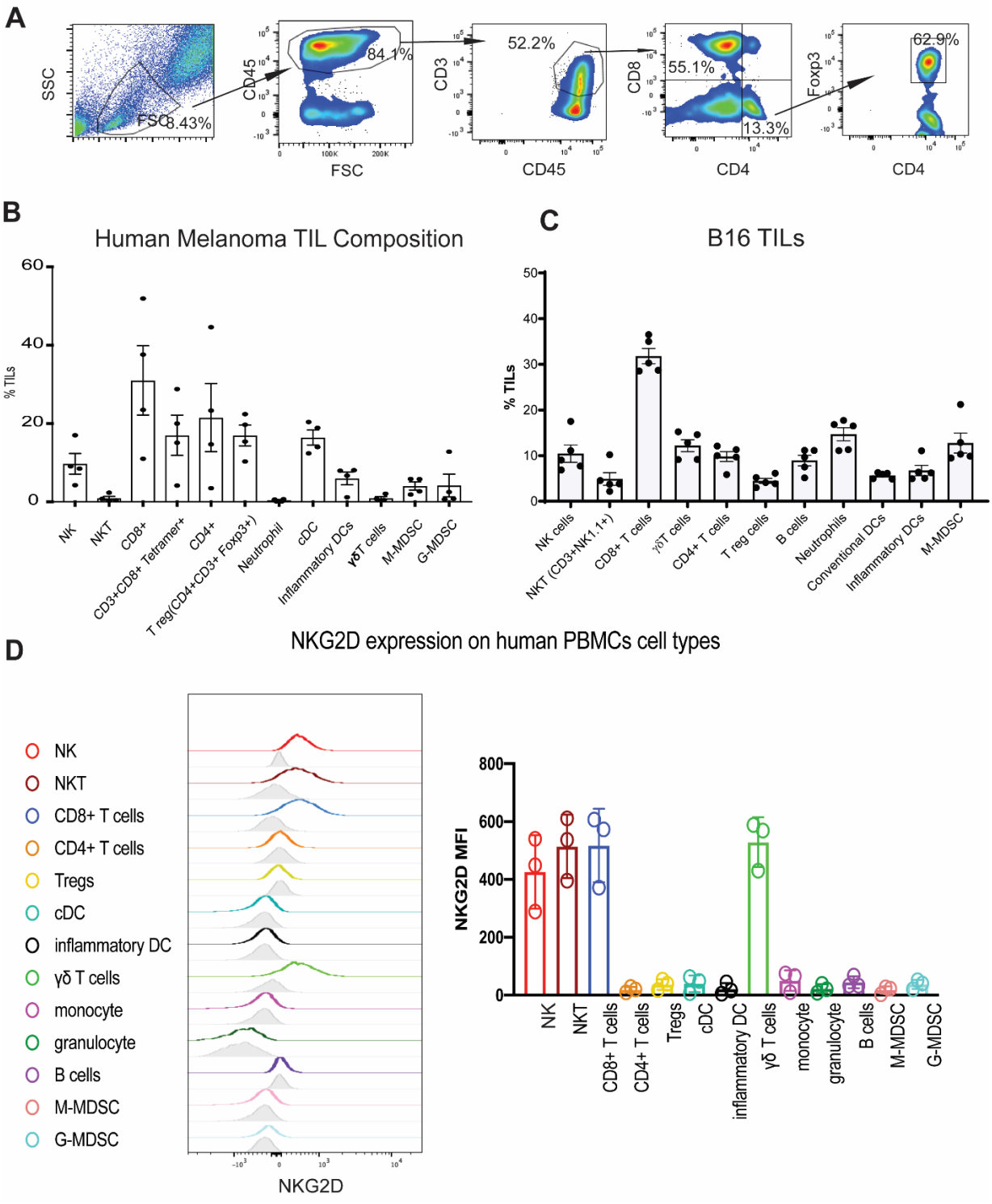
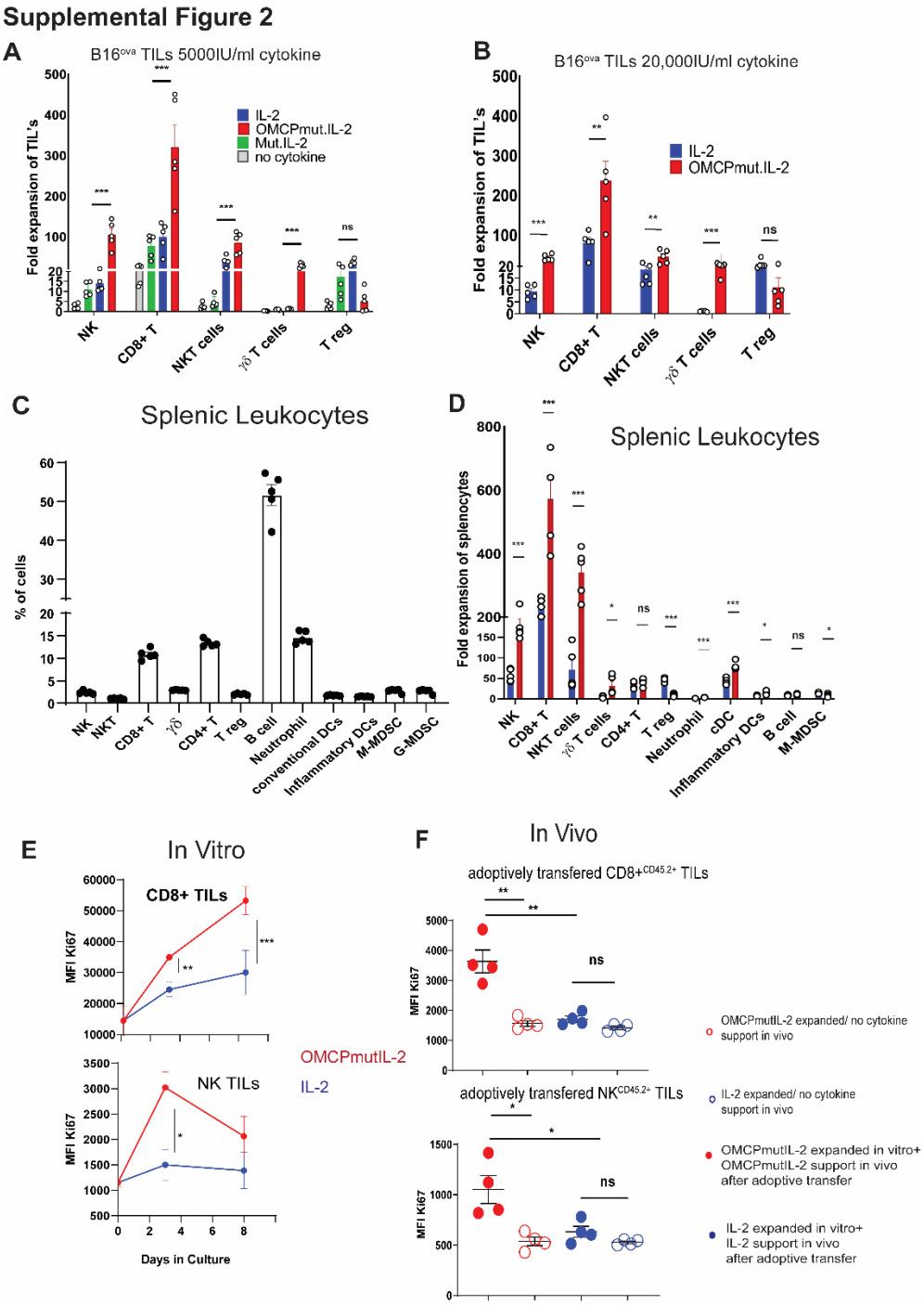
**Supplemental Figure 1**

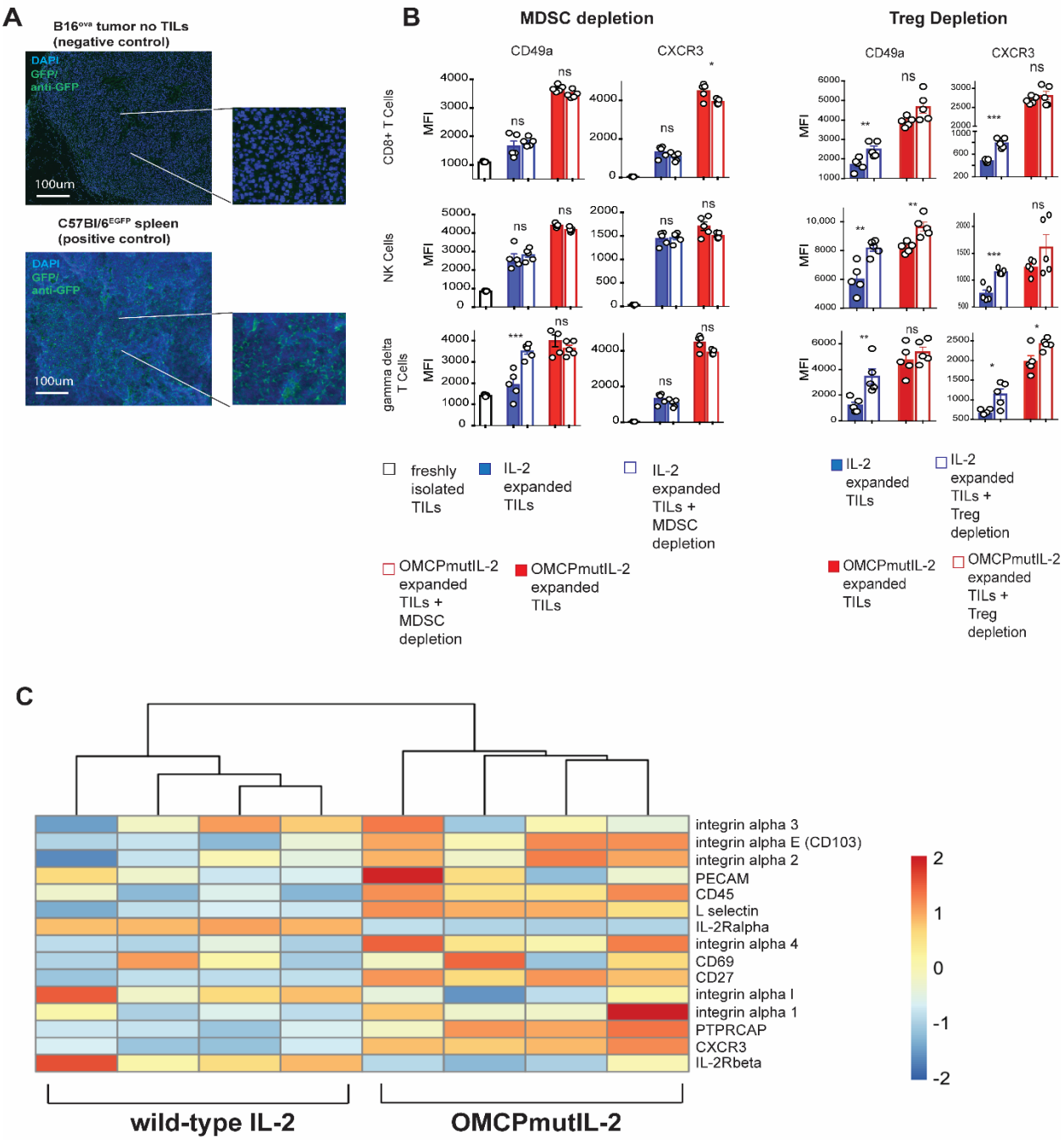



**Supplemental Figure 1:** (A) Representative example of gating strategy for TILs in B16$^{ova}$ melanoma. (B) Flow cytometric analysis of TILs from resected human melanoma specimens. (C) TIL composition of B16 melanoma. (D) Expression of NKG2D on various leukocyte subsets from human PBMCs.




**Supplemental Figure 2:** (A) Expansion of TILs with OMCPmutIL-2, wild-type IL-2, mutIL-2, or no cytokine. Line indicates t-test comparison mutIL-2 vs OMCPmutIL-2. (B) Expansion of TILs in 20,000IU cytokine/ml. (C) Relative distribution of various leucocytes from a resting B6 spleen. (D) Relative expansion of splenic leucocytes expanded in OMCPmutIL-2 (red) or wild-type IL-2 (blue). (E ) Mitosis of $CD8^+$ T cells and NK cells during *in vitro* TIL culture as measured by KI-67 MFI. (F) Mitosis of adoptively transferred $CD8^{+CD45.2+}$ and $NK^{CD45.2+}$ cells into $B16^{ova}$-bearing $B6^{CD45.1+}$ congenic mice as measured by KI-67 expression levels. Transferred TILs evaluated in the tumor bed. ***=p<.001; **=p<.01; *=p<.05

## Supplemental Figure 3




**Supplemental Figure 3:** (A) Immunohistochemical analysis of B16$^{ova}$ tumor that did not receive C57Bl/6$^{EGFP}$ TILs (negative imaging control) (top) and spleen of C57Bl/6$^{EGFP}$ mouse (positive imaging control)(bottom). Relative expression, by median fluorescence intensity (MFI), of CD49a or CXCR3 on TIL-resident CD8$^{+}$ T cells, NK cells or γδ T cells after either MDSC or Treg depletion prior to expansion in IL-2 (blue) or OMCPmutIL-2 (red). *p<.05; **p<.01, ***p<.001, NS p>.05

**Supplemental Table**

| **Supplemental Table: SPR Binding** | | | |
|---|---|---|---|
| | **$k_{on}$ ($10^6$ $m^{-1}s^{-1}$)** | **koff ($s^{-1}$)** | **$K_D$ (nM)** |
| mCD25:wild-type IL-2 | 0.97 ± 0.25 | 0.038 ± 0.01 | 42.7 ± 11.8 |
| mNKG2D:OMCPmutIL-2 | 2.6 ± 0.58 | 0.017 ± 0.005 | 7.3 ± 1.8 |