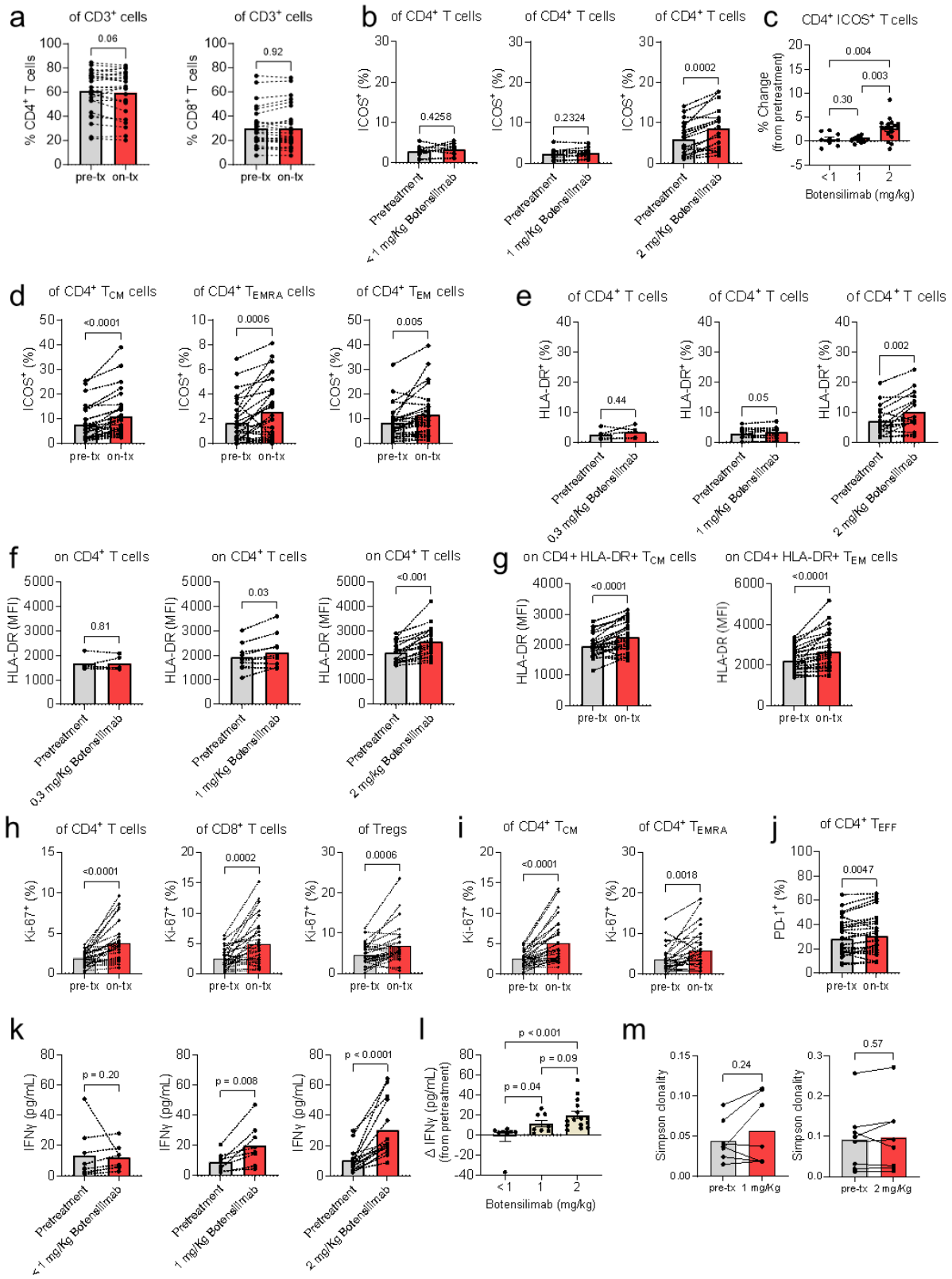


1 Supplementary Figure S13



3 **Supplementary Figure S13. Botensilimab induces a dose-dependent increase in peripheral**
4 **ICOS⁺ Ki-67⁺ and HLA-DR⁺ T cells and IFN γ in patients with advanced solid cancers.** Pre-
5 and on-treatment blood samples were obtained from patients (n = 37) treated with 0.1, 0.3, 1 or
6 2 mg/kg botensilimab monotherapy. On-treatment blood was drawn 7 days after the first dose.
7 Samples were analyzed by flow cytometry from cryopreserved peripheral blood mononuclear cells
8 to assess **(a)** the frequency of CD4⁺ and CD8⁺ T cells from patients treated with 1 and 2 mg/kg of
9 botensilimab (n =28), **(b)** the frequency of ICOS on CD4⁺ T cells from patients treated with 0.1
10 mg/kg (n=4), 0.3 mg/kg (n=5), 1 mg/kg (n=10) and 2 mg/kg (n=18) of botensilimab, **(c)** change in
11 frequency of ICOS on CD4⁺ T cells from baseline for each dose cohort, **(d)** the frequency of ICOS
12 on CD4⁺ central memory (T_{CM}, CD45RO⁺CCR7⁺), terminally differentiated effector memory cells
13 (T_{EMRA}, CD45RO⁻CCR7⁻) and effector memory (T_{EM}, CD45RO⁺CCR7⁻) T cell subsets from patients
14 treated with 1 and 2 mg/kg botensilimab (n =28), **(e)** the frequency of HLA-DR⁺ and **(f)** HLA-DR
15 mean fluorescence intensity (MFI) on CD4⁺ T cells from patients treated with 0.3 mg/kg (n=5), 1
16 mg/kg (n=10) and 2 mg/kg (n=18) botensilimab. **(g)** HLA-DR expression on CD4⁺ T_{CM} and T_{EM} T
17 cells. **(h)** Frequency of Ki-67⁺ CD4⁺, CD8⁺, regulatory T cells (Tregs, CD4⁺, CD127^{low/-}, CD25⁺),
18 **(i)** CD4⁺ T_{CM} and T_{EMRA} T cells, and **(j)** PD-1 on CD4⁺ effector (T_{EFF}; CXCR3⁺) T cells from patients
19 treated with 1 and 2 mg/kg of botensilimab (n=28). **(k)** Peripheral interferon gamma (IFN γ) levels
20 at pre- and on-treatment in plasma samples from patients treated with 0.1 or 0.3 (<1 mg/kg), 1 or
21 2 mg/kg botensilimab monotherapy (n=31). **(l)** Change in IFN γ secretion from baseline for each
22 dose cohort. On-treatment blood was drawn 24 hours after the first dose and samples analyzed
23 using a Meso Scale Discovery electrochemiluminescence assay. **(m)** Changes in blood T cell
24 receptor (TCR) Simpson clonality from patients treated with 1 mg/kg (n=7) or 2 mg /kg (n=8) of
25 botensilimab. Data are represented as paired data points with group mean **(a, b, d-k, m)** or mean
26 \pm s.e.m. **(c, l)**. Data were analyzed with a two-tailed Wilcoxon matched-paired t test **(a, b, d-k, m)**

- 27 or one-way ANOVA followed by a Kruskal-Wallis with a Benjamini, Krieger and Yekutieli's multiple
28 comparison test **(c, I)**.