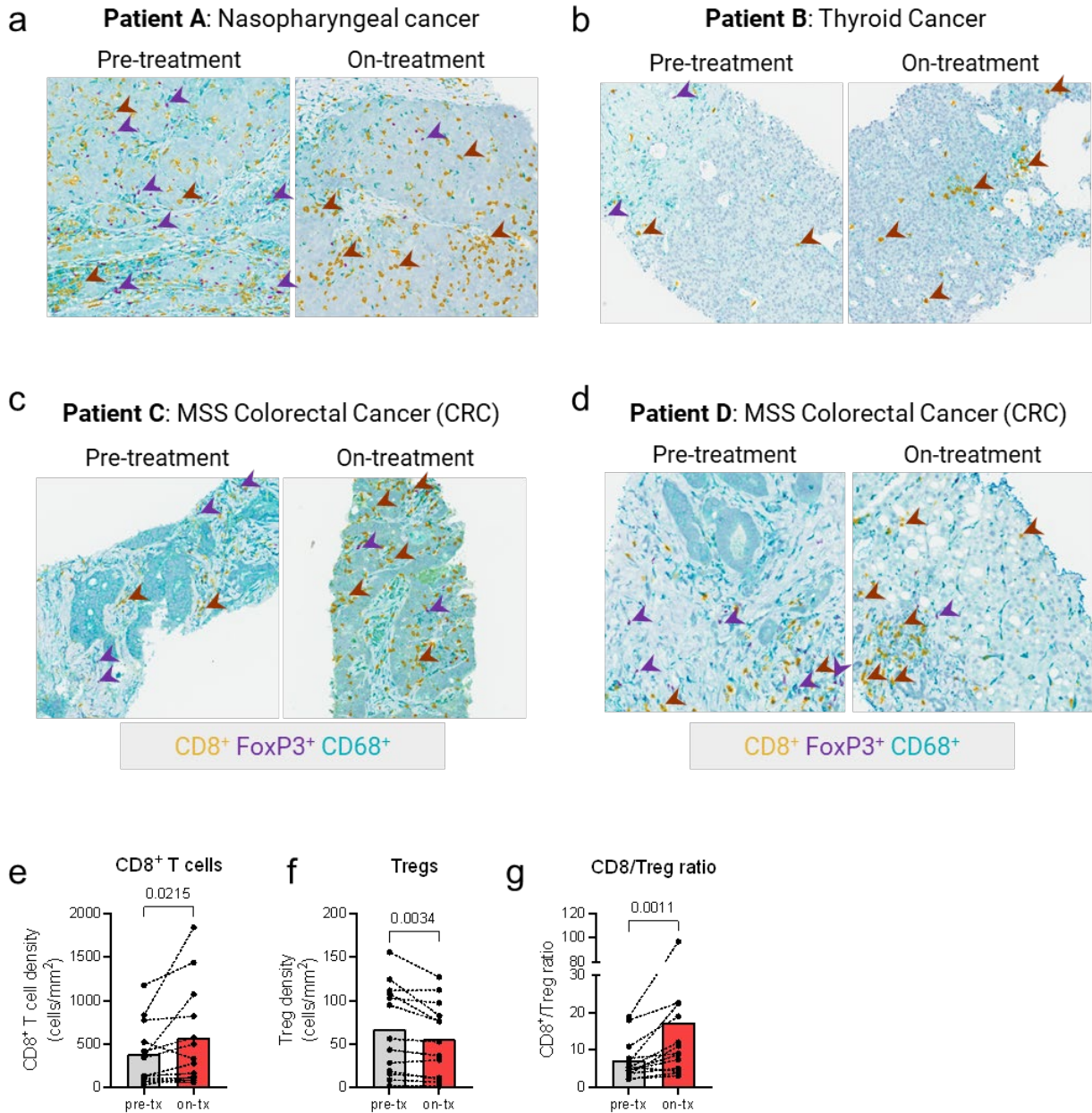


1 **Supplementary Figure S14**



2

3 **Supplementary Figure S14. Intratumoral Tregs and CD8⁺ T cell staining in patients with**
 4 **advanced solid cancers treated with botensilimab.** Triplex chromogenic
 5 immunohistochemistry on pre-treatment and on-treatment formalin fixed, paraffin-embedded
 6 tumor biopsies from patients treated with botensilimab monotherapy or in combination with

7 balstilimab (α PD-1). **(a)** Patient A, nasopharyngeal cancer (2 mg/kg botensilimab every three
8 weeks); **(b)** Patient B, thyroid cancer (2 mg/kg botensilimab every three weeks); **(c)** Patient C,
9 MSS-CRC (2 mg/kg botensilimab Q6W + 3 mg/kg balstilimab every two weeks); **(d)** Patient D,
10 MSS-CRC (1 mg/kg botensilimab every six weeks + 3 mg/kg balstilimab every 2 weeks). **(e)**
11 Quantitative analysis of **(e)** CD8⁺ T cells and **(f)** Treg cell densities, and **(g)** CD8/Treg ratio in
12 paired biopsies from 13 patients treated with botensilimab monotherapy or in combination with
13 balstilimab. Only samples with sufficient tissue and staining quality and containing ≥ 100 tumor
14 cells were evaluated. On-treatment biopsies were taken on cycle 2 day 1 for every six-week cohort
15 or cycle 3 day 1 for every three-week cohort. CD8 (yellow), FoxP3 (purple) CD68 (turquoise) are
16 shown. Tregs were defined as FoxP3⁺/CD8⁻ cells. Data are represented as paired data points
17 with group mean and analyzed with a two-tailed Wilcoxon matched-paired t test **(e, f)** ratio paired
18 *t*-test **(g)**.