SI MATERIALS AND METHODS

**Determination of NOTA molecules per antibody**

The number of chelators conjugated to the mAbs was determined as described before, with minor modifications [1]. Briefly, 15 µg Ab (~0.1 nmol) was incubated in ammonium acetate solution (pH 8.2) with varying concentrations of natural Cu²⁺ (0.2–5 nmol) spiked with ⁶⁴Cu²⁺. The nat. Cu²⁺ solutions were prepared by dilution of a copper atomic absorption standard solution (TraceCERT, Sigma-Aldrich). After incubation for 1 h at room temperature, labeling samples were equally mixed with diethylenetriaminepentaacetic acid (DTPA; 0.2 g mL⁻¹) and the ratio of mAb-bound and unbound copper was subsequently determined by thin-layer chromatography (TLC) on iTLC SG microfiber paper impregnated with silica gel (Agilent Technologies) using DTPA as the eluent. Unbound Cu²⁺ eluted with the solvent front (R_f = 1.0) and Ab-bound activity remained at the origin (R_f = 0.0). Radioactivity distribution was measured by phosphorimaging on a Perkin Elmer Cyclone Plus. Determinations were performed in triplicate.
Flow cytometric antibody titration

Conservation of mAb binding affinity after NOTA conjugation was tested through side-by-side flow cytometric titration of conjugated and unconjugated α-PD-1 and α-PD-L1, respectively. 1×10^6 activated murine CD8 T cells (PD-1) and 1×10^6 IFN-γ-treated B16F10 melanoma cells (PD-L1) were incubated with serially diluted corresponding unconjugated and NOTA-conjugated antibodies (both rat anti-mouse). After washing, cells were stained with fluorochrome-conjugated goat anti-rat IgG mAb (eBioscience) and analyzed with a FACSVerse flow cytometer (BD Biosciences), with the mean fluorescence intensity plotted against the mAb concentration.

Detection of regulatory T cells

Tregs were detected in single-cell suspensions from spleens and lymph nodes of healthy C57BL/6 mice. Cells were first stained for α-CD3, α-CD4 (eBioscience), and α-CD25 (BD Horizon), and subsequently fixed, permeabilized and stained for intracellular FoxP3 with the anti-mouse FoxP3 staining set (eBioscience). Cells were analyzed using a BD FACSVerse flow cytometer with FACSuite software (Becton Dickinson).

**Fig. S1**

**A**

PD-1

![Graph](image)

**B**

PD-L1

![Graph](image)

**Fig. S1. Determination of NOTA molecules per antibody.**

Determination of the degree of NOTA modification per α-PD-1 (A) and α-PD-L1 (B) antibody molecules. Antibodies were incubated with varying concentrations of copper (0.2–5 nmol) spiked with $^{64}\text{Cu}$. $n = 3$ experiments each.
Fig. S2. Side-by-side flow cytometric titration.

Titration of NOTA-conjugated vs. unconjugated α-PD-1 (A) and α-PD-L1 (B). Serially diluted antibodies were incubated with activated murine T cells (PD-1+) or IFN-γ-treated B16 melanoma cells (PD-L1+) and analyzed as described above. n = 3.
Fig. S3. PD-1 expression on normal and regulatory CD4 T cells in untreated, healthy mice.

(A) The vast majority of PD-1+ cells in lymph nodes and spleen are CD3+ T cells. $n = 3$ mice. (B) Tregs and non-Tregs (CD4+ CD25+ FoxP3+) each account for approximately half of the PD-1+ CD4+ cells. $n = 3$ mice. (C) Representative histogram overlays of PD-1 expression on Treg and non-Treg CD4 T cells in lymph nodes and spleen.
Fig. S4. PD-L1 immunoPET/CT in naive mice

ImmunoPET/CT imaging of $^{64}$Cu-NOTA-PD-L1 24 h p.i. with coronal and sagittal whole-body 20-mm maximum intensity projection (MIP) scans shown together with 2-mm transverse MIPs indicated by white ticks.

Abbreviations: cLN – cervical lymph node, aLN – axillary lymph node, mLN – mesenteric lymph node, Li – liver, Sp – spleen, BAT – brown adipose tissue.
Fig. S5. PD-L1 immunoPET/CT detects interscapular BAT in mice.

Two coronal immunoPET sections of a representative, healthy and untreated WT mouse injected with $^{64}$Cu-NOTA-PD-L1 mAb (20 µg; 6.38 ± 0.35 MBq) 24 h p.i. Radiotracer uptake into interscapular butterfly-shaped BAT is indicated by white arrows.

Abbreviations: Sp – spleen, BAT – brown adipose tissue.
**Fig. S6. Strong induction of PD-1\textsuperscript{high} CD8\textsuperscript{+} TILs by combined immunoradiotherapy.**

Flow cytometric analysis of tumor-infiltrating cells 5 days post treatment with tumor radiotherapy (2×12 Gy) and injection of anti-PD-L1 plus anti-CTLA-4. Virtually all tumor-infiltrating cells were CD45\textsuperscript{+} leukocytes, the majority of which were PD-1\textsuperscript{high} CD3\textsuperscript{+} T cells. More than 80% of all T cells were CD8\textsuperscript{+} cytotoxic T cells. Most non-T cells did not show PD-1 expression.
**Fig. S7.** FACS PD-L1 expression analysis of B16 PD-L1 KO cells generated by CRISPR/Cas9 technology.

PD-L1 KO cells were generated as described in Materials & Methods. WT cells showed weak basal PD-L1 expression, which was strongly upregulated after 24 h of IFN-γ treatment. In contrast, PD-L1 KO cells neither showed basal nor IFN-γ-inducible PD-L1 expression.