

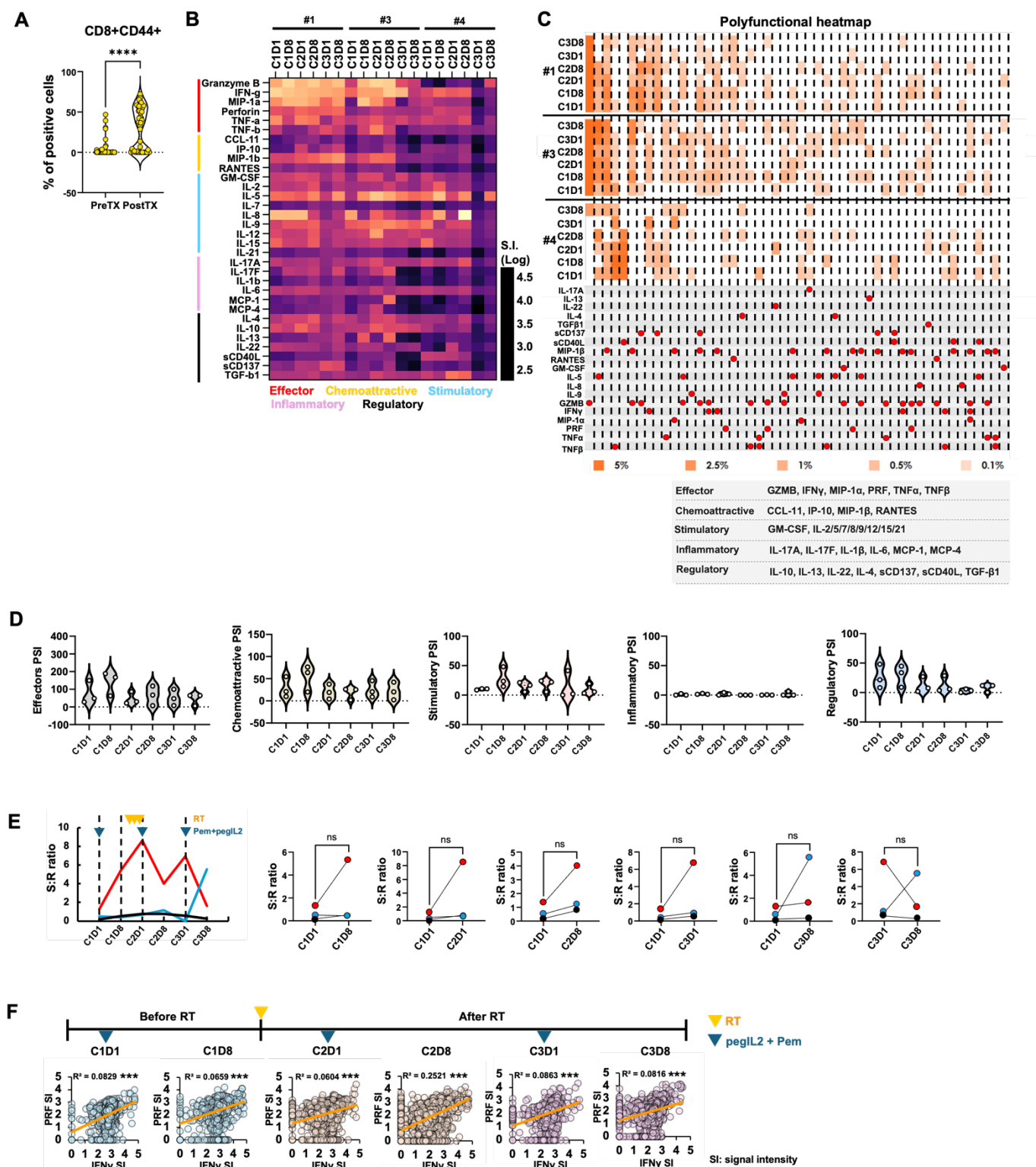


Figure S1. Single-cell proteomics to assess CD8⁺ T cell functionality in patients with recurrent or metastatic HNSCC patients. (A) Multispectral immunohistochemistry analysis of memory T cells (CD8⁺CD44⁺) infiltration. *P* values: **** ≤ 0.0001 by unpaired T test. (B-D) Live⁺CD8⁺ T cells sorted from patients' peripheral blood mononuclear cells (PBMCs) were subjected to single-cell multiplex secretome analysis to screen treatment-induced phenotype changes in CD8⁺ T cells. (B) Signal intensity (S.I.) heatmap from detected protein. (C) Polyfunctional heatmap showing single- and multi-protein expression of single cells in each patient at each treatment cycle. (D) Polyfunctionality Strength Index (PSI) from each

cycle of each CD8 T cell subset. **(E)** Stimulatory:regulatory (S:R) ratio from each cycle. *P* values: ns, not significant by paired T test **(F)** Correlation within single cells of expression level of indicated proteins. *P* values: *** ≤ 0.001 by two-tailed test.

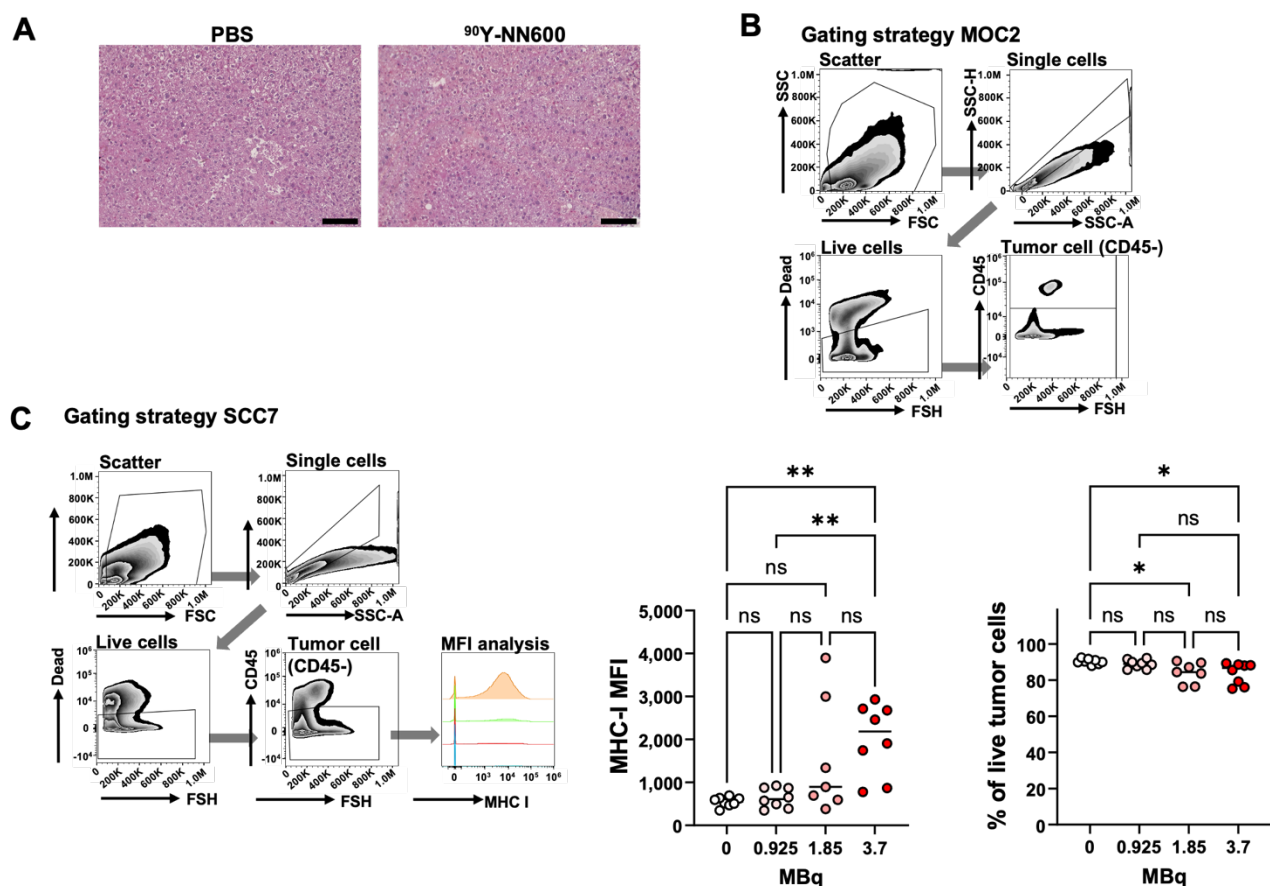
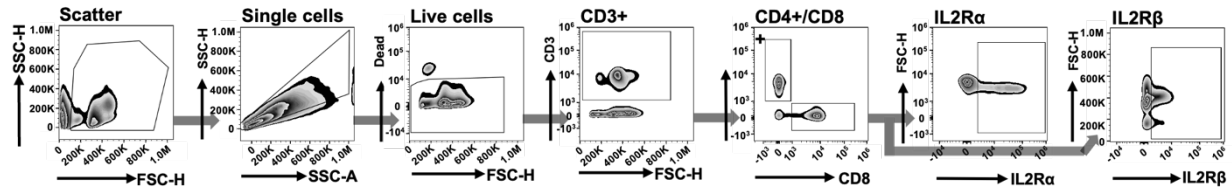


Figure S2. (A) C57Bl/6 mice with MOC2 tumors were treated with PBS or ^{90}Y -NN600 on day 1. Fixed livers were H&E stained on day 25 post ^{90}Y -NN600 administration. Scale bar 50 μm . (B) Flow cytometry gating strategy (left) and MHC I and live $^{+}$ tumor cell (CD45 $^{-}$) analysis in SCC7 tumor (right). (n=7-8/group) *P* values: * ≤ 0.05 ; ** ≤ 0.01 ; ns, not significant by one-way ANOVA with Tukey's correction for multiple comparisons.

A Gating strategy



B

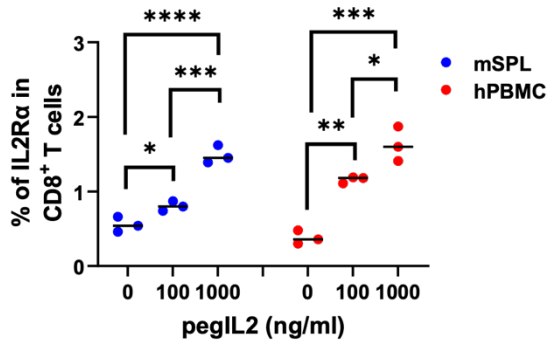


Figure S3. (A) Flow cytometry gating strategy. (B) Healthy human donor PBMCs (hPBMC) and naïve mouse splenocytes (mSPL) were cultured in the presence of pegIL2 for 72h, then IL2Rα-expressing CD8+ T cells were quantified. *P* values: * ≤ 0.05 ; ** ≤ 0.01 ; *** ≤ 0.001 , **** ≤ 0.0001 by one-way ANOVA with Tukey's correction for multiple comparisons.

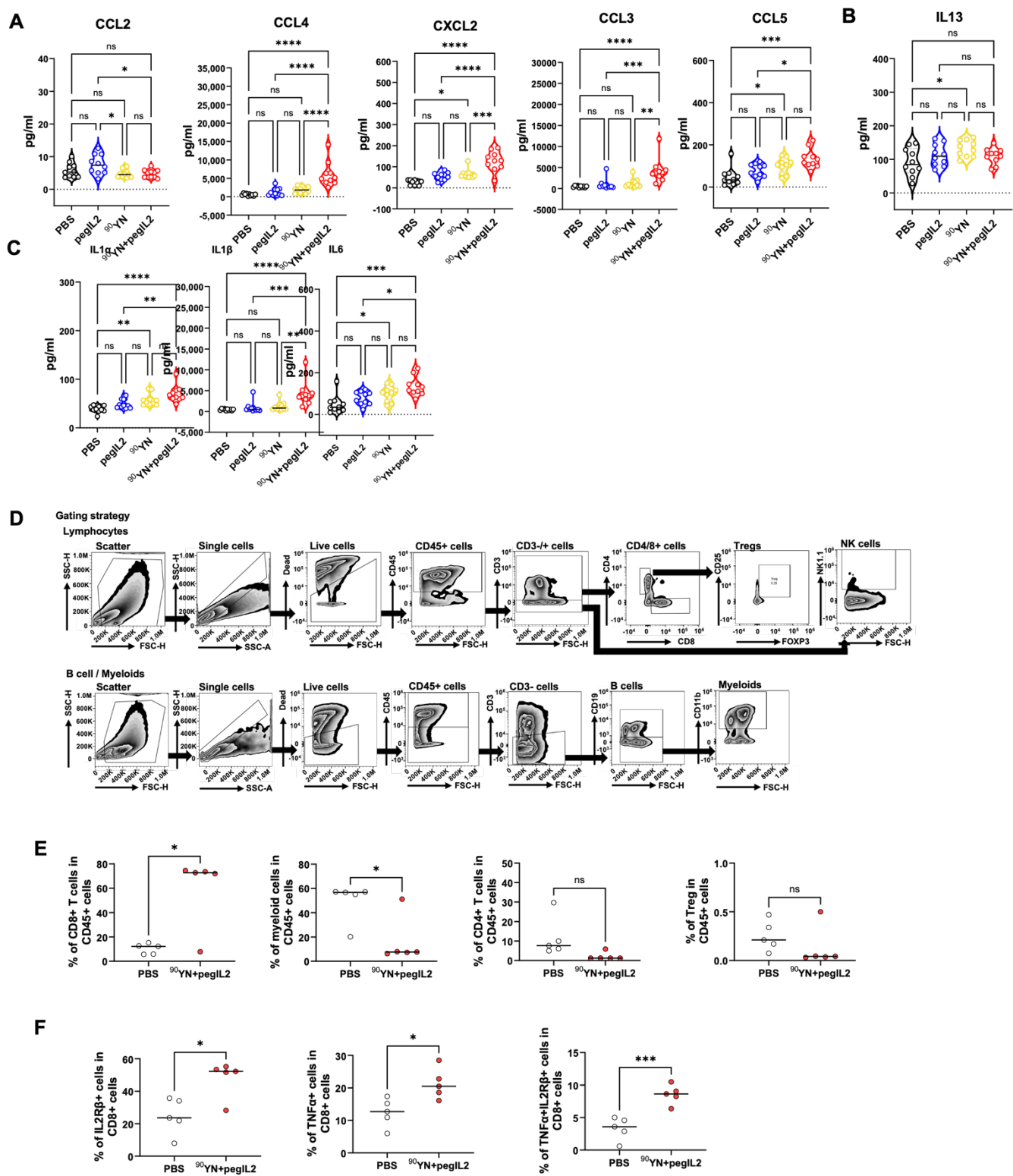


Figure S4. (A) Cytokine and (B-C) chemokine concentration from tumor multiplex analysis. (n=10/group) *P* values: * ≤ 0.05 ; ** ≤ 0.01 ; *** ≤ 0.001 ; ns: not significant by one-way ANOVA with Tukey's correction for multiple comparisons. (D) Flow cytometry gating strategy. (E) Tumor-infiltrated immune cells and (F) IL2R β and TNF α expression on CD8 $^{+}$ T cells at day 17 post-treatment in SCC7-bearing mice. (n=5/group), *P* values: * ≤ 0.05 ; ns: not significant by unpaired T-test.

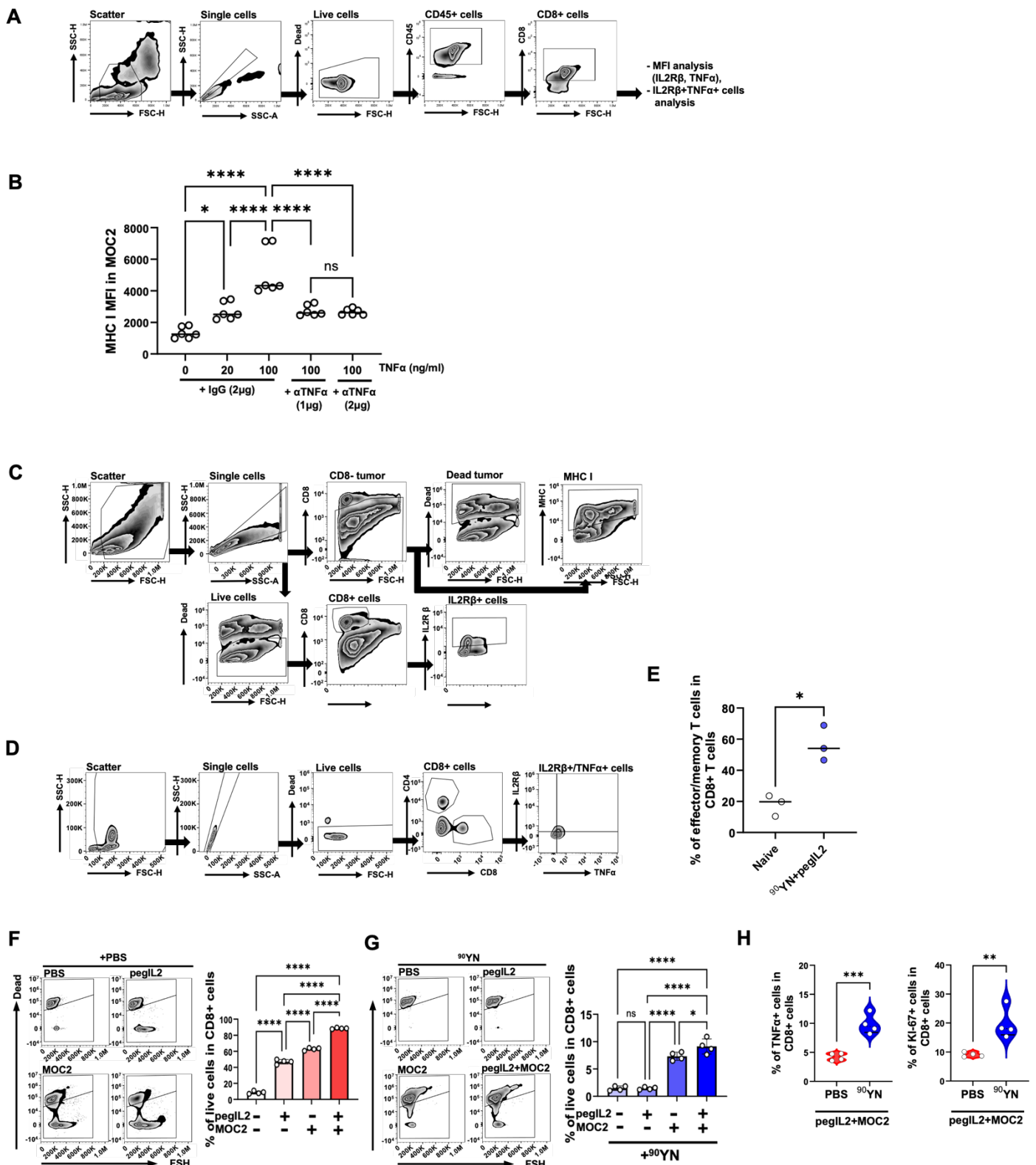


Figure S5. (A) Flow cytometry gating strategy. (B) Validating recombinant mouse TNF α and anti-TNF α antibody efficacy on MHC I expression in MOC cells. *P* values: * ≤ 0.05 ; **** ≤ 0.0001 by one-way ANOVA with Tukey's correction for multiple comparisons (C and D) Flow cytometry gating strategy. (E) Mice rendered tumor-free by ^{90}Y -NM600 + pegIL2 or control tumor-naïve mice were injected with MOC2 intravenously. Blood was collected 72 hours after injection and effector memory T cells (CD44 $^+$ CD62L $^+$)

in CD8⁺ T cells were quantified by flow cytometry. *P* values: * ≤ 0.05 by unpaired T-test. **F-H**, CD8⁺ T cells sorted from naïve mice were cultured alone or with pre-plated MOC2 cells in the presence of PBS, BEMPEG, ⁹⁰Y-NM600, or ⁹⁰Y-NM600+BEMPEG together. After 30 hours incubation, (**F** and **G**) live⁺CD8⁺ T cells and (**H**) TNF α ⁺ and Ki-67⁺ in CD8⁺ T cells were analyzed by flow cytometry. *P* values: * ≤ 0.05 ; ** ≤ 0.01 ; *** ≤ 0.001 ; **** ≤ 0.0001 ; ns: not significant by one-way ANOVA with Tukey's correction for multiple comparisons (**F** and **G**) and unpaired T-test (**H**).

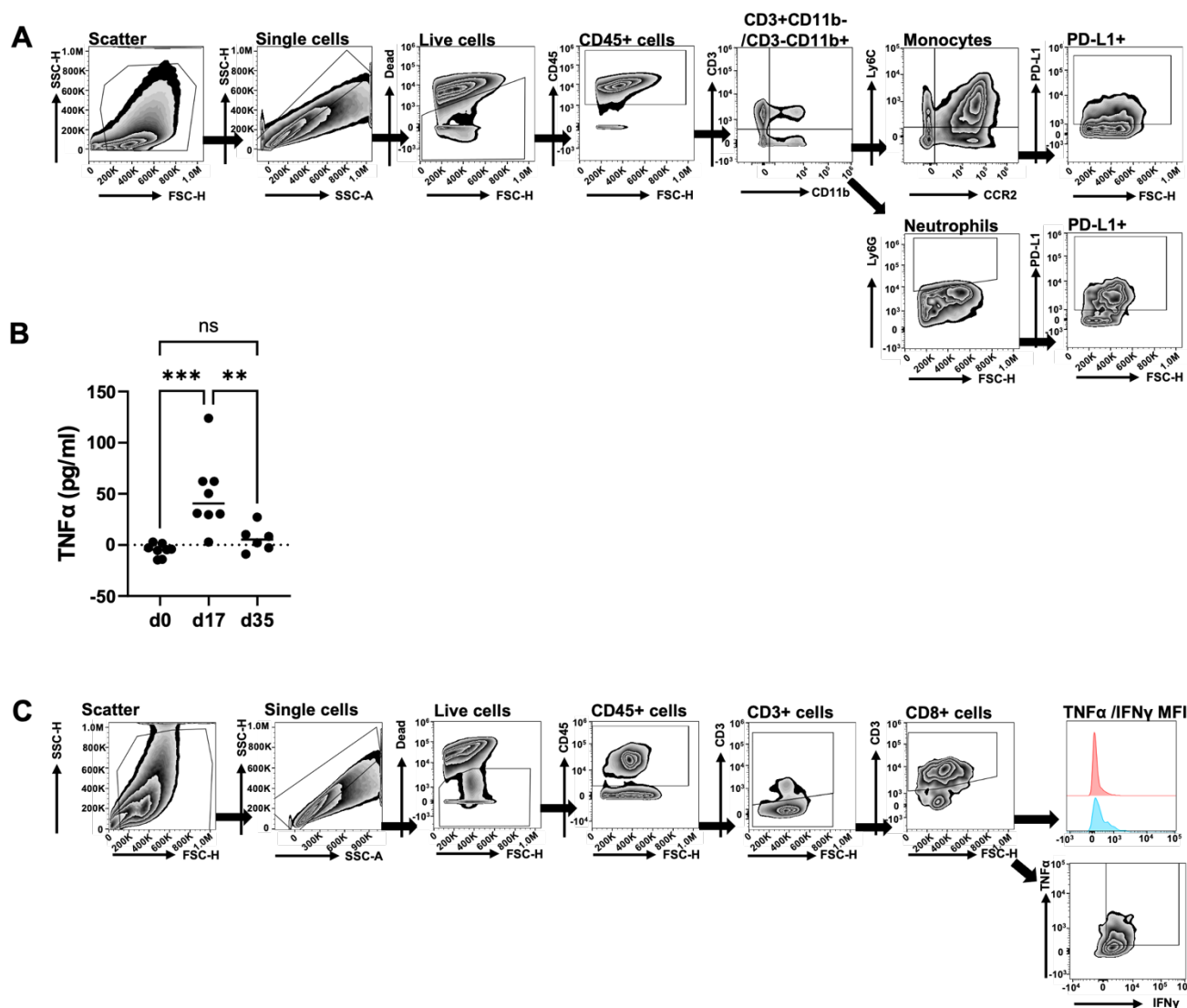


Figure S6. (A) Flow cytometry gating strategy. (B) TNF α serum concentrations from ^{90}Y -NM600 + pegIL2-treated MOC2-bearing mice at the indicated day. (n=6-8/group) *P* values: ** ≤ 0.01 ; *** ≤ 0.001 ; ns: not significant by one-way ANOVA with Tukey's correction for multiple comparisons (C) Flow cytometry gating strategy.

Table S1. Primer sequence list

Murine	Primer sequences 5' -3'	
Genes	Forward	Reverse
<i>CD274</i>	CCAGCCACTTCTGAGCATGA	CTTCTCTTCCCACTCACGGG
<i>Ifnb</i>	CCCTATGGAGATGACGGAGA	CTGTCTGCTGGTGGAGTTCA
<i>Oas2</i>	TAAGAGGCTGCTCCGATGGT	GACGTCAAGGTATGCATCTTGGT
<i>Oas3</i>	TTTCTCAGTCAAAGGCGTCCA	TCTATCCAGTGTTCTCCGTCTG
<i>Mx1</i>	AGCTCACCTCCCACATCTGTAA	GCTTGCACTCTGATGACTGCTAT
<i>Tnf</i>	AAGTTCCCAAATGGCCTCCC	CCTAACTGCCCTTCCTCCATC
<i>Tgfb1</i>	CTATTGCTTCAGCTCCACAG	GACAGAAGTTGGCATGGTAG
<i>Trex1</i>	CCATTTCTCAGGGACTTCCA	AGCTCAGCTTTGCTCAGACC
<i>Prf1</i>	TTCGGGAACCAAGCTACACC	AGTAATGTGTGCAGGGGCTC
<i>Gzmb</i>	AAAGGCAGGGGAGATCATCG	AGGCTGCTGATCCTTGATCG
<i>Ifng</i>	AACAAGGCGAAAAAGGATGC	TCATTGAATGCTTGGCGCTG
<i>Ccl2</i>	TTTGAATGTGAAGTTGACCCGT	GTGCTTGAGGTGGTTGTGGA
<i>Ccl3</i>	ACATCATGAAGGTCTCCACCAC	CCATATGGCGCTGAGAAGACT
<i>Ccl4</i>	CCTCCCCTTCCTGCTGTTT	GTCTCATAGTAATCCATCACAAAGC
<i>Ccl5</i>	GAAGGAACCGCCAAGTGTGT	CCTAGCTCATCTCCAAATAGTTGAT
<i>Cxcl10</i>	CGCTGCAACTGCATCCATA	TAGGCTCGCAGGGATGATTTC
<i>Cxcl11</i>	TGTGACATCCTGGGAACGTCT	TTCCAAGACAGCAGAGGGTCA
<i>H2-k1</i>	GAGCTGTGGTGGCTTTTGTG	GATCAGAGGTCTGGGAGCCT
<i>H2-d1</i>	TGGTGCTGCAGAGCATTACA	GCGTTCCCGTTCTTCAGGTA
<i>Hprt</i>	AGCCTAAGATGAGCGCAAGT	GGCCACAGGACTAGAACACC

Table S2: Patient Characteristics

Characteristic	n for category (% or Range)
Sex	
Male	5 (100)
Female	0 (0)
Median age	65 y (33-79)
Histology	
SCC	5 (100)
Ethnicity	
White	5 (100)
Other	0 (0)
Smoking status	
Current/former	2 (40)
Never smoker	3 (60)
Prior therapy	
Chemoradiation	3 (60)
Surgery	2 (40)
Number of Lesions	3 (2-4)
Cycles of NKTR-214	3 (1-6)
Site of RT	
Head & Neck	2 (40)
Thorax	2 (40)
Liver	1 (20)

Abbreviation: SCC, squamous cell carcinoma

Table S3: Summary of adverse events

Adverse Events Among 5 Patients Evaluable for Toxicity					
Adverse Event	No. (% of patients)				
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Dry Mouth	2 (40)	0	1 (20)	0	0
Anemia	2 (40)	0	0	0	0
Constipation	1 (20)	0	0	0	0
Dry Skin	1 (20)	0	0	0	0
Eczema	1 (20)	0	0	0	0
Edema limbs	1 (20)	0	0	0	0
Edema limbs	1 (20)	0	0	0	0
Fatigue	3 (60)	0	0	0	0
Fever	1 (20)	0	0	0	0
Flu-like symptoms	1 (20)	0	0	0	0
Hyperthyroidism	2 (40)	0	1 (20)	0	0
Hypoglycemia	1 (20)	0	0	0	0
Hyponatremia	0	1 (20)	0	0	0
Hypothyroidism	0	2 (40)	0	0	0
Lipase increased	1 (20)	0	0	0	0
Lymphocyte count decreased	2 (40)	1 (20)	1 (20)	0	0
Neutrophil count decreased	1 (20)	0	0	0	0
Oral Pain	1 (20)	0	1 (20)	0	0
Pruritus	2 (40)	0	0	0	0
Rash maculo-papular	0	1 (20)	0	0	0
Thrush	0	0	1 (20)	0	0
White blood cell decreased	1 (20)	0	0	0	0
Lipase increased	1 (20)	0	0	0	0
Eosinophilia	4 (80)	0	0	0	0
Lymphocyte count decreased	0	1 (20)	0	1 (20)	0
Neutrophil count decreased	1 (20)	0	0	0	0

Table S4. EORTC QLQ-C30 Score

EORTC QLQ-30 Score (Median, %)					
Dimension	Screening	C1D8	C2D8	C3D8	30-Day Post
QL2	66.7	50	70.8	75	62.5
PF2	100	93.3	90	93.3	93.3
RF2	100	100	66.7	91.7	83.3
EF	66.7	66.7	87.5	83.3	83.3
CF	83.3	83.3	100	91.7	83.3
SF	83.3	83.3	100	91.7	91.7
FA	13.3	20	16.7	13.3	23.3
NV	0	16.7	16.7	16.7	0
PA	33.3	33.3	25	25	16.7
DY	0	0	0	0	0
SL	33.3	33.3	16.7	16.7	0
AP	33.3	33.3	16.7	16.7	33.3
CO	0	0	0	0	0
DI	0	0	0	0	16.7
FI	0	0	0	0	0

*QL2 Global health status, PF2 Physical functioning, RF2 Role functioning, EF Emotional functioning, CF Cognitive functioning, SF Social functioning, FA Fatigue, NV Nausea and vomiting, PA Pain, DY Dyspnea, SL Insomnia, AP Appetite loss, CO Constipation, DI Diarrhea, FI Financial difficulties

Table S5. EORTC QLQ-H&N35

EORTC H&N35 Scoring					
Dimension	Screening	C1D8	C2D8	C3D8	30-Day Post
HNPA	25	33.3	20.8	0	29.1
HNSW	8.3	8.3	20.8	8.3	20.8
HNSE	16.6	0	0	8.3	16.6
HNSP	11.1	0	16.6	5.5	22.2
HNSO	25	16.6	16.6	12.5	45.8
HNSC	0	0	6.6	6.6	10
HNSX	0	16.6	0	16.6	16.6
HNTE	0	0	0	0	16.6
HNOM	0	0	16.6	16.6	0
HNDR	66.6	66.6	50	50	33.3
HNSS	66.6	33.3	33.3	16.6	33.3
HNCO	33.3	66.6	33.3	16.6	50
HNFI	0	33.3	16.6	16.6	0
HNPK	60	80	75	50	50
HNNU	60	40	75	50	0
HNFE	20	20	0	0	0
HNWL	40	20	0	0	75
HNWG	40	20	25	25	0

*HNPA Pain, HNSW Swallowing, HNSE Senses problem, HNSP Speech problems, HNSO Trouble with social eating, HNSC Trouble with social contact, HNSX Less sexuality, HNTE Teeth, HNOM Opening mouth, HNDR Dry mouth, HNSS Sticky saliva, HNCO Coughing, HNFI Felt ill, HNPk pain killers, HNNU Nutritional supplements, HNFE Feeding tube, HNWL Weight loss, HNWG Weight gain

Table S6. EQ-5D Scoring

EQ-5D Score					
Dimension	Baseline n or median (%; range)	C1D8 n or median (%; range)	C2D8 n or median (%; range)	C3D8 n or median (%; range)	30 Day n or median (%; range)
Mobility					
No problems	4 (80%)	4 (80%)	4 (100%)	3 (75%)	3 (75%)
Slight problems	0	1 (20%)	0	1 (25%)	1 (25%)
Moderate problems	0	0	0	0	0
Severe problems	1 (20%)	0	0	0	0
Unable to walk about	0	0	0	0	0
Self-care					
No problems	4 (80%)	4 (80%)	4 (100%)	4 (100%)	4 (100%)
Slight problems	1 (20%)	1 (20%)	0	0	0
Moderate problems	0	0	0	0	0
Severe problems	0	0	0	0	0
Unable to wash or dress	0	0	0	0	0
Usual activities					
No problems	4 (80%)	3 (60%)	3 (75%)	3 (75%)	3 (75%)
Slight problems	0	2 (40%)	1 (25%)	1 (25%)	0
Moderate problems	0	0	0	0	1 (25%)
Severe problems	1 (20%)	0	0	0	0
Unable to do usual activities	0	0	0	0	0
Pain/discomfort					
No pain/discomfort	1 (20%)	0	2 (50%)	2 (50%)	2 (50%)
Slight pain/discomfort	2 (40%)	2 (40%)	1 (25%)	0	0
Moderate pain/discomfort	2 (40%)	3 (60%)	1 (25%)	1 (25%)	1 (25%)
Severe pain/discomfort	0	0	0	0	1 (25%)
Extreme pain/discomfort	0	0	0	1 (25%)	0
Anxiety/Depression					
Not anxious/depressed	2 (40%)	4 (60%)	2 (50%)	2 (50%)	3 (75%)
Slight anxious/depressed	3 (60%)	2 (40%)	2 (50%)	1 (25%)	1 (25%)
Moderate anxious/depressed	0	0	0	1 (25%)	0
Severe anxious/depressed	0	0	0	0	0
Extremely anxious/depressed	0	0	0	0	0
Your Health					
	81 (50-90)	65 (55-87)	87.5 (60-100)	80 (40-100)	65 (50-95)

