Supplementary Methods S1. Analysis of multimer binding & Immunophenotyping

Dextramer and/or tetramer samples were gated as follows: 1) SSC-A/FSC-A dot plot to set lymphocyte gate; 2) FSC-H/FSC-A dot plot to exclude doublets; 3) FVS510-A/FSC-A dot plot to set viability gate**;** 4) CD8/FSC-A dot plot to set gate for $CD8^{high}$ lymphocytes, excluding NK cells ($CD8^{low}$) and 5) multimer/CD8 dot plot to select dextramer/$tetramer^{+}$ $CD8^{+}$ lymphocytes.

Immunophenotype panel 1 was gated as follows: FSC-H/FSC-A dot plot to exclude doublets, FVS510-A/FSC-A dot plot to set viability gate, SSC-A/FSC-A dot plot to gate lymphocytes, CD3/FSC-A dot plot to set gate for CD3+ T cells, CD8/CD4 dot plot to select CD3+CD8+ and CD3+CD4+ T cells. CD3+CD8+ and CD3+CD4+ T cells where individually checked for CCD7/CD45RA expression to dissect naïve, effector, central memory and effector memory subsets[7] as well as for their PD-1 levels. Regulatory T cells (Tregs) were defined within the CD3+CD4+ T cells gate according to CD25hi CD127lo status while the combination of CXC3 and CCR6 guided the distinction between T helper lineages (Supplementary Figure S2).

Similarly, for panel 2 doublets were gated out in a FSC-H/FSC-A dot plot before putting dead cells (FVS+) and CD3 T cells into one dump channel. Viable, non-T cells were divided into CD19+ and CD19- subsets with the CD19+ subset ultimately giving rise to B cells based on simultaneous CD20 expression. The CD3-CD19- non-T, non-B-cell subset was used to distinguish between HLA-DR+ and DR- myeloid lineages and NK cells: DR+ cells led to monocytes subsets according to CD14/CD16 expression for classical, intermediate and non-classical monocytes. DR- cells on the other hand gave DR-CD11b+CD14-/loCD15hi granulocytic MDSCs. Non-T, non-B-cell that were negative for CD20 and CD14 gave rise to CD11chi/DRhi myeloid DCs, CD123hi/DR+/hi plasmacytoid DCs and CD56+CD16+/- NK cells. Non-T, non-B cells also included CD14+CD11b+DR-/lo monocytic MDSCs (Supplementary Figure S3).

Supplementary Table S1. Antibodies and dextramers used for flow cytometric analysis

ANTIGEN	CLONE	IOSTYPE	FORMAT	COMPANY
CD4	RPA-T4	mouse IgG	FITC	BD Pharmingen
CD8	RPA-T8	mouse IgG	Alexa Fluor 700	BD Pharmingen
CD25	M-A251	mouse IgG	PE	BD Pharmingen
CD127	HIL-7R-M21	mouse IgG	PE-Cy7	BD Pharmingen
CCR6 (CD196)	11A9	mouse IgG	BV650	BD Horizon
CXCR3 (CD183)	1C6	mouse IgG	PE-CF594	BD Horizon
CD45RA	HI100	mouse IgG	APC	BioLegend
CD3e	UCHT1	mouse IgG	PerCP-Cy5.5	BD Pharmingen
CCR7 (CD197)	3D12	rat IgG	BV605	BD Horizon
PD-1	EH12.2H7	mouse IgG	BV421	BioLegend
CD3e	UCHT1	mouse IgG	BV510	BD Horizon
HLA-DR	G46-6	mouse IgG	FITC	BD Pharmingen
CD11c	B-ly6	mouse IgG	Alexa Fluor 700	BD Pharmingen
CD20	2H7	mouse IgG	APC-H7	BD Pharmingen
CD11b	ICRF44	mouse IgG	PerCP-Cy5.5	BioLegend
CD14	MqP9	mouse IgG	PE	BD Pharmingen
CD16	3G8	mouse IgG	BV650	BD Horizon
CD15	HI98	mouse IgG	APC	BD Pharmingen
CD19	HIB19	mouse IgG	PE-Cy7	BD Pharmingen
CD56	B159	mouse IgG	PE-CF594	BD Horizon
CD123	7G3	mouse IgG	BV421	BD Horizon
CD8	RPA-T8	mouse IgG	PerCP-Cy5.5	BD Pharmingen
HLA-A2	BB7.2	mouse IgG	BB515	BD Horizon
Fixable Viability Stain	N/A	N/A	510	BD Horizon
Survivin dextramer	HLA-A*0201/LMLGEFLKL	N/A	PE	Immudex
JARID1B dextramer	HLA-A*0201/QLYALPCVL	N/A	FITC	Immudex
Mucin dextramer	HLA-A*0201/STAPPVHNV	N/A	PE	Immudex
Her2 dextramer	HLA-A*0201/KIFGSLAFL	N/A	APC	Immudex

Supplementary Table S2. Patients with one or more treatment positively-related (maximum grade possible, probable, definite) AEs by treatment arm

AE grade	Arm 1 (1 mg/kg, n=11)	Arm 2 (10 mg/kg, n=12)	Total (n=23)
	No. (%)	No. (%)	No. (%)
<3	6 (54.5)	10 (83.3)	16 (69.6)
3	4 (36.4)	1 (8.3)	5 (21.7)
4	1 (9.1)	1 (8.3)	2 (8.7)
5	0 (0)	0 (0)	0 (0)

Supplementary Table S3. Levels of circulating tumor-specific CD8+ T cells with reactivity for JARID1B, Muc1 or Her2 in breast cancer patients with HLA-A*0201 status[a].

patient / week	JARID1B T cells				Muc1 T cells				Her2 T cells			
	0	2	5	15	0	2	5	15	0	2	5	15
N05		0.049				0.123				0.000		
N10	0.768	0.201	0.407		0.177	0.089	0.104		0.124	0.112	0.088	
N14	0.235	0.271	0.207		0.197	0.398	0.162		0.072	0.134	0.094	
N15	0.160			0.623	0.077			0.085	0.106			0.058
U03	0.255	0.681	0.068		0.119	0.085	0.072		0.119	0.085	0.070	
U07	0.085	0.096	0.137	0.057	0.060	0.108	0.089	0.039	0.196	0.184	0.168	0.083

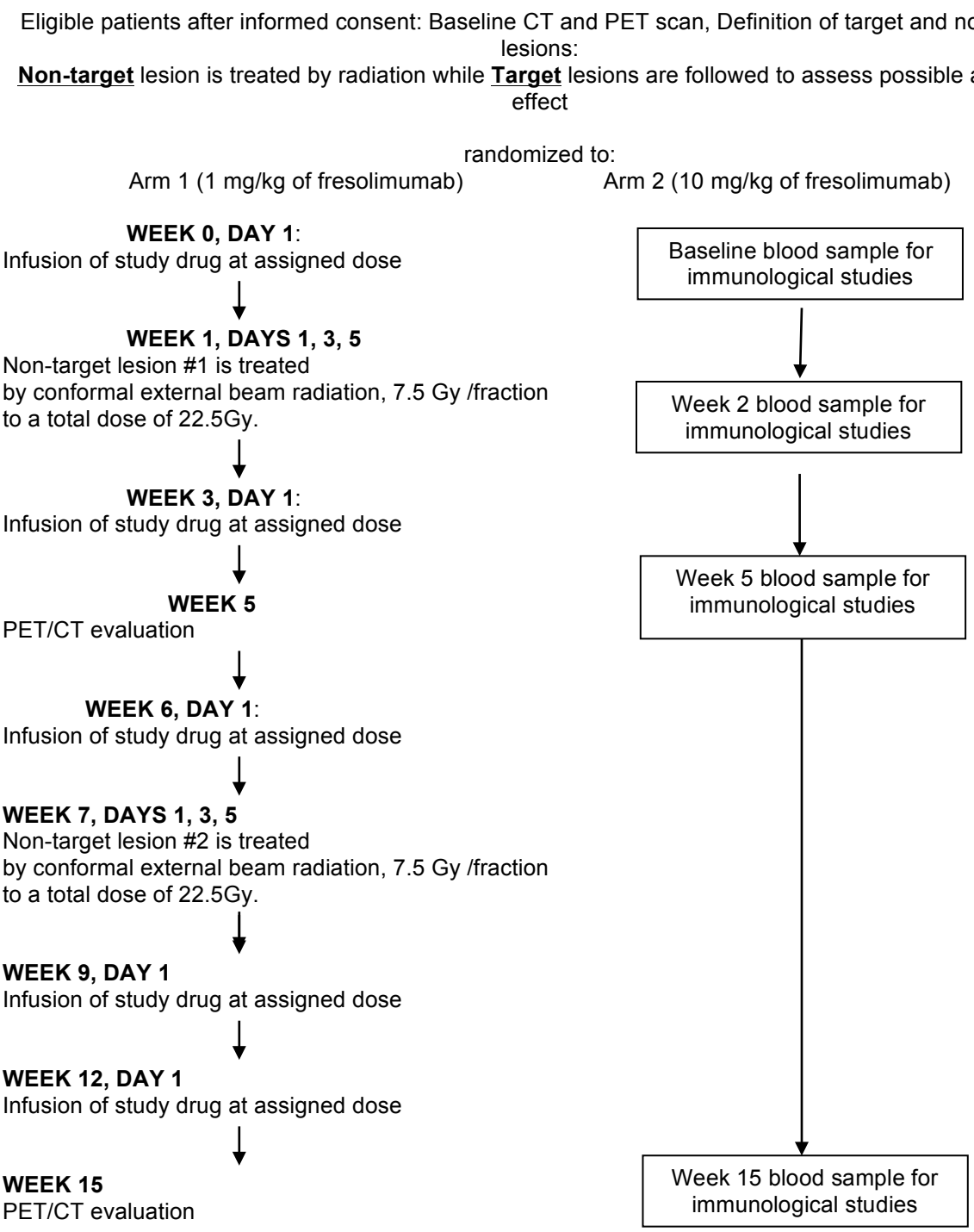
[a]Cells were stained with dextramers presenting the JARID1B (QLYALPCVL), Mucin-1 (STAPPVHNV) and Her2/neu (KIFGSLAFL) peptides and co-stained for $CD8^+$. Non-viable cells were excluded on grounds of high fixable viability stain up-take and the remaining sample was subjected to quality control requiring ≥10,000 viable events and ≥2,000 $CD8^+$ T cells. Data are $CD8^+$ T cells staining positive with the dextramer for each peptide [%]. Six healthy volunteer served as background control (LLD = median + IQR of % dextramer-reactive $CD8^+$ T cells in the healthy donors).

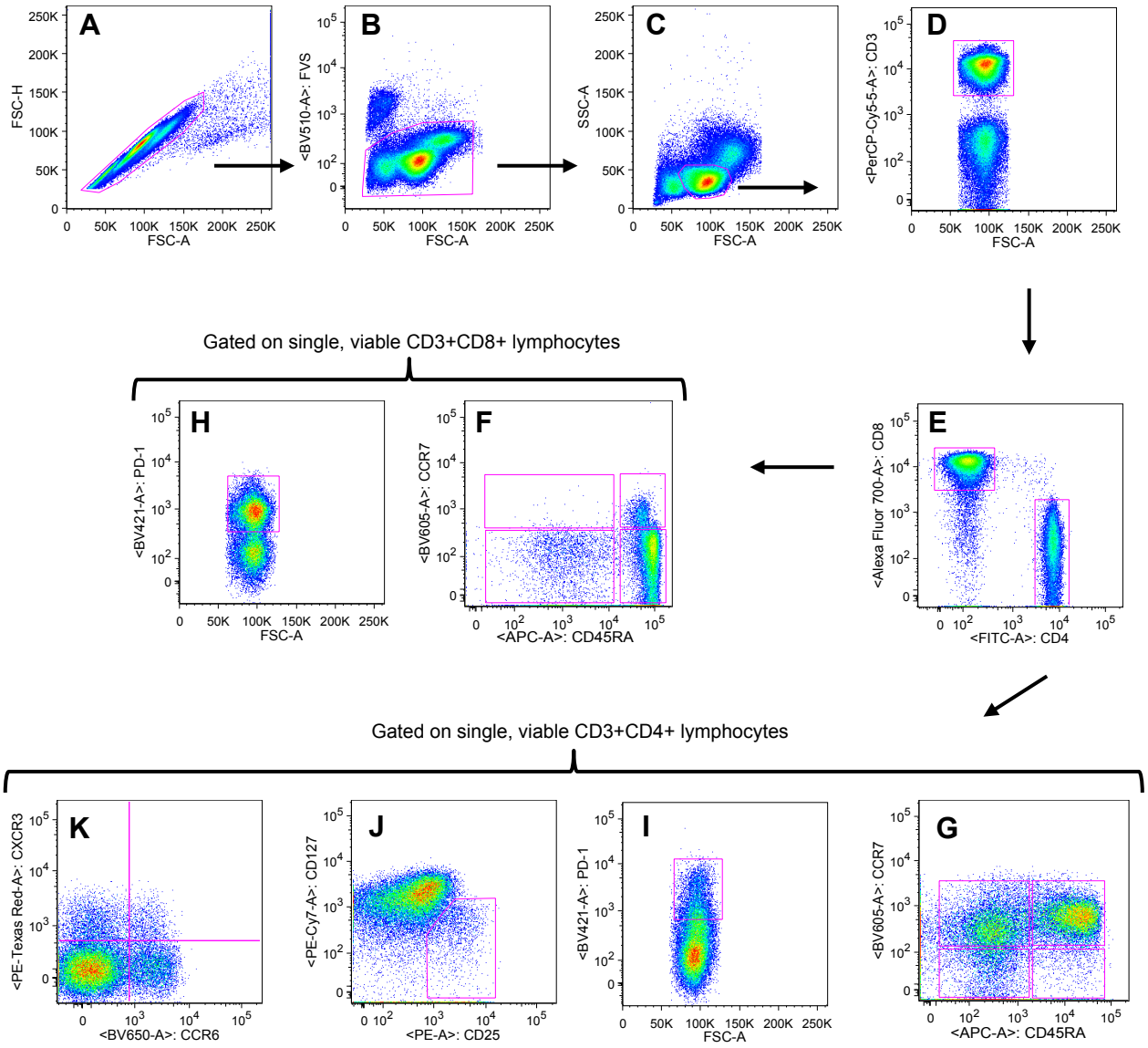
Supplementary Table S4. Humoral immune responses were not significantly affected following TGFβ blockade and radiation[a].

Patient ID	Fmab	week 0	week 5	week 15
N06	10mg	27	21	24
N09	1mg	25	5	
U02	10mg	17	18	
N03	1mg	7	3	1
U03	10mg	1	3	17
N11	10mg	0	0	6
N04	10mg	2	0	
U07	10mg	1	1	1
N05	1mg	1	0	
N13	1mg	1	2	
U04	1mg	0	0	1
U05	10mg	0	0	3
N01	1mg	0	0	
N02	10mg	0	0	1
N10	1mg	0	0	

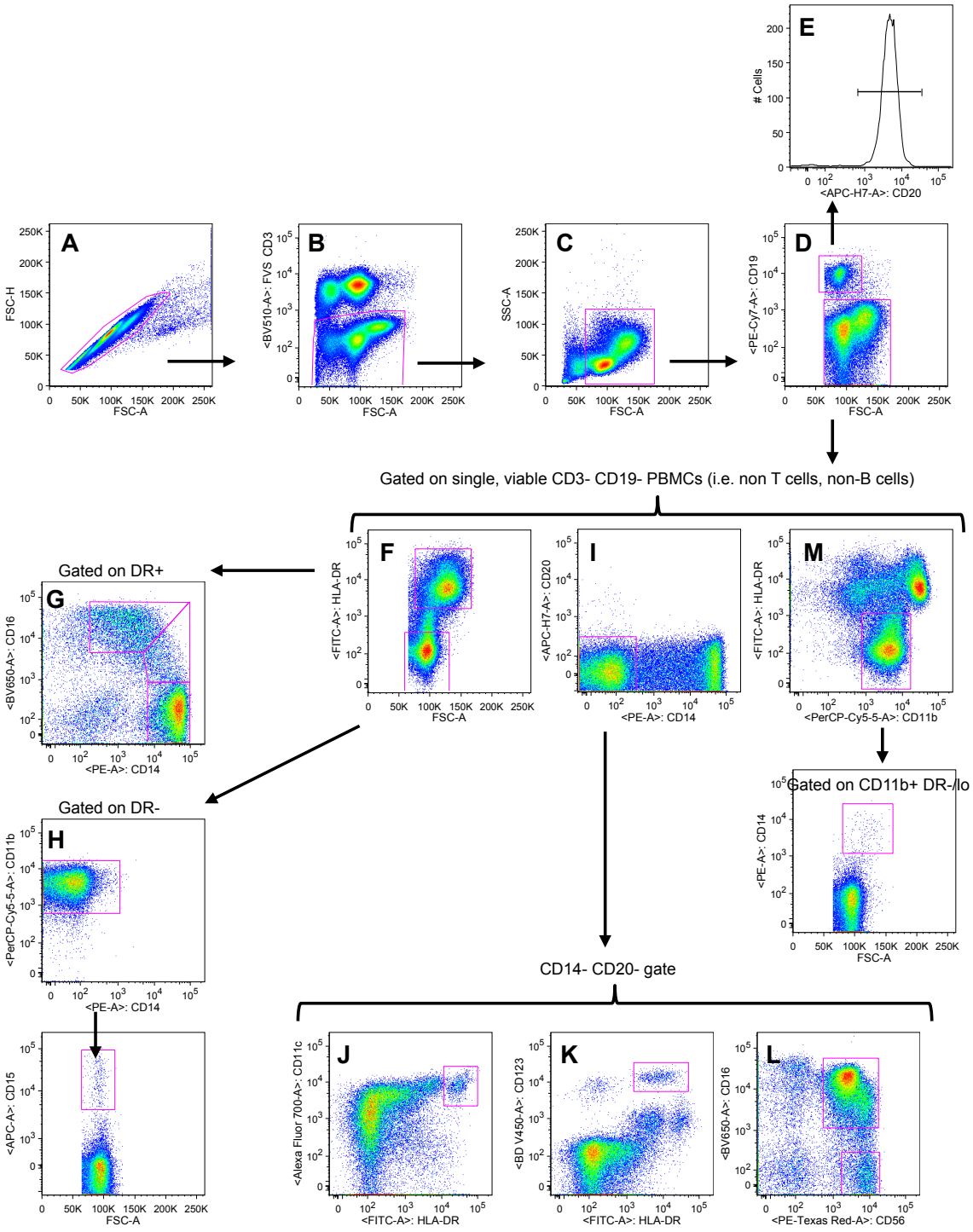
[a]Serum samples from 15 patients were tested for antibody reactivity against 34 different putative tumor-antigens by Seramatrix Corp. (Carlsbad, CA). A positive score was returned for any titer that was above 2x the 25th percentile of all antibodies, patients and time points. Data are cumulative positive titers in each patient.

Supplementary Figure S1: Study schema

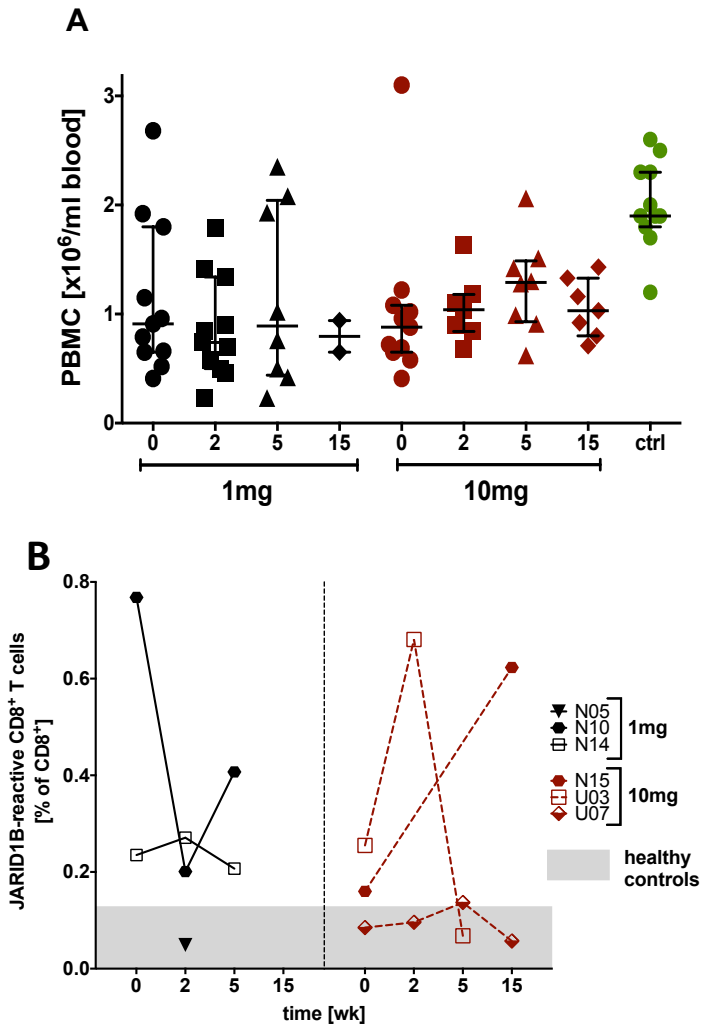




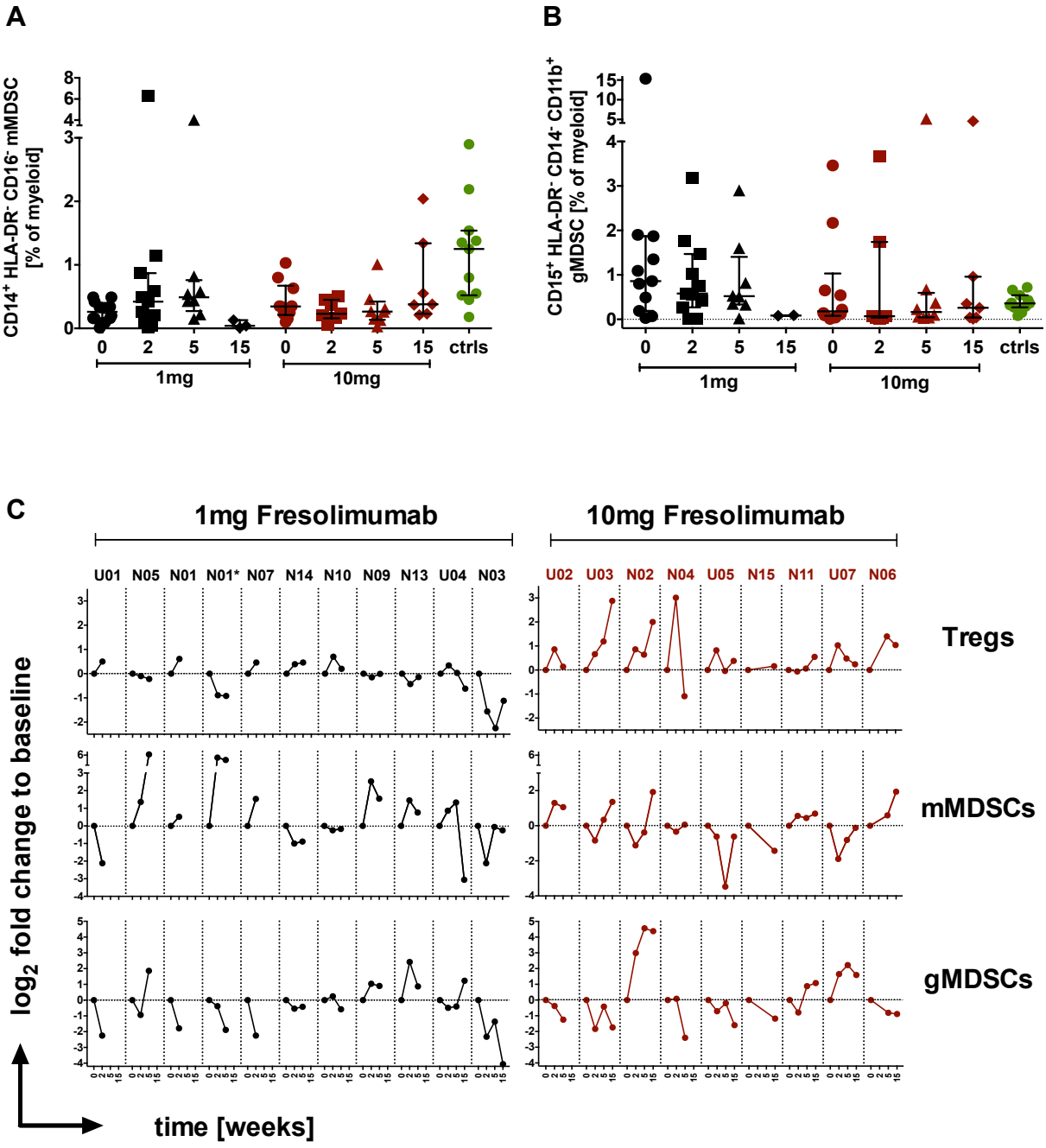
Supplementary Figure S2. Gating strategy in panel 1. A) FSC-H/FSC-A dot plot to exclude doublets; **B)** FVS510-A/FSC-A dot plot to set viability gate; **C)** SSC-A/FSC-A dot plot to gate in lymphocyte; **D)** CD3/FSC-A dot plot to set gate for CD3+ T cells; **E)** CD8/CD4 dot plot to select CD3+CD8+ and CD3+CD4+ T cells, which where individually checked for CCD7/CD45RA expression to dissect naïve, effector, central memory and effector memory subsets (**F** and **G**) as well as for their PD-1 levels (**H** and **I**); **J)** Regulatory T cells (Tregs) were defined within the CD3+CD4+ T cells gate according to CD25hi CD127lo status while the combination of CXC3 and CCR6 guided the distinction between T helper lineages **(K)**.



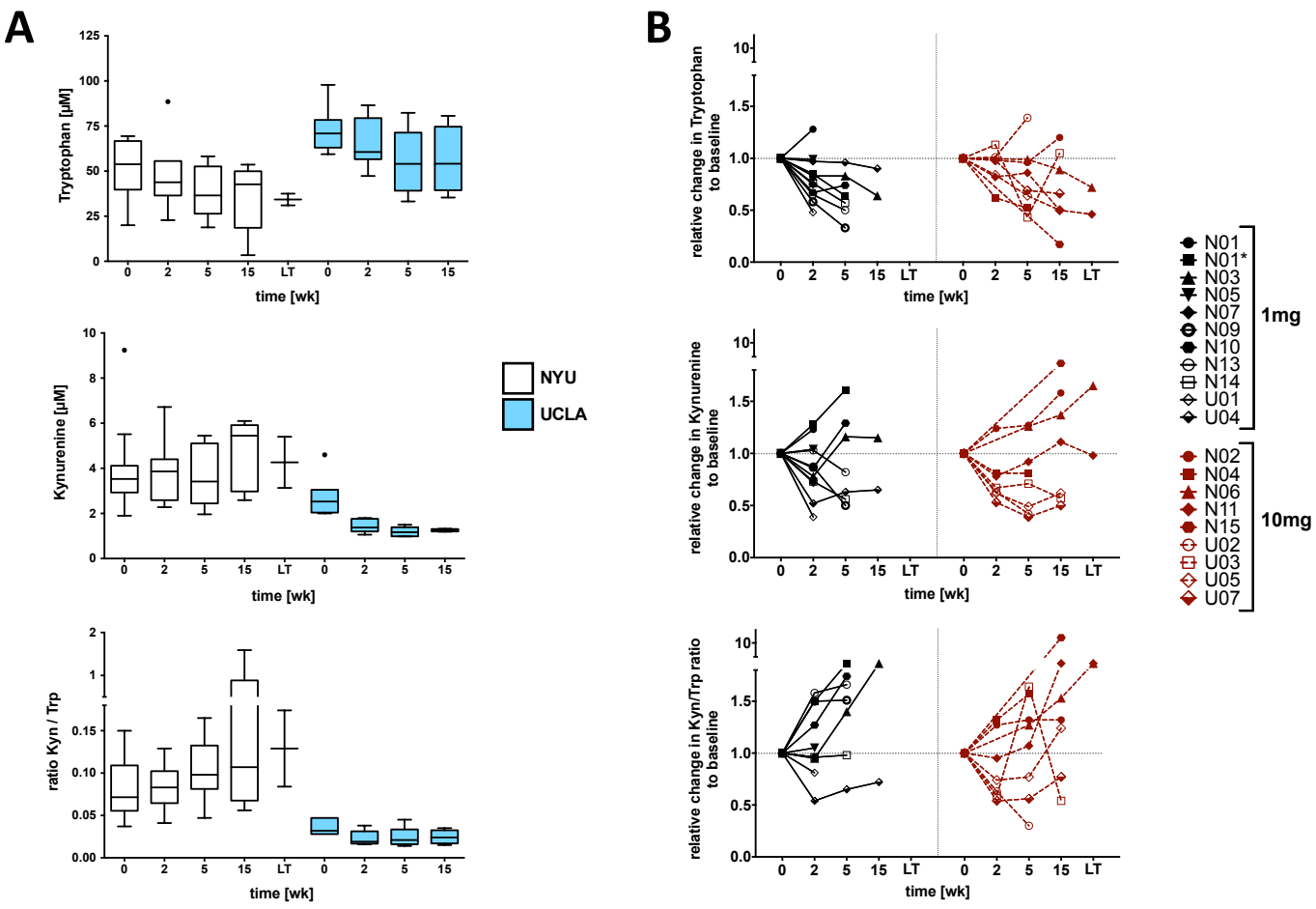
Supplementary Figure S3. Gating strategy in panel 2. A) FSC-H/FSC-A dot plot, gating out doublets; B) 510-A/FSC-A dot plot to exclude CD3+ lymphocytes and dead cells; C) viable non-T cells; D) CD19/FSC-A dot plot to select live CD3-CD19- and live CD3-CD19+ cells with the CD19+ subset ultimately enumerating B cells based on simultaneous CD20 expression (E); F) The CD19- subset was used to distinguish between HLA-DR+ and DR- myeloid lineages; G) DR+ cells led to monocytes subsets according to CD14/CD16 expression for classical, intermediate and non-classical monocytes; H) DR- cells on the other hand led to DR-CD11b+CD14-/loCD15hi granulocytic myeloid-derived suppressor cells (gMDSC); I) CD3-CD19-CD20-CD14- give rise to J) CD11chi/DRhi myeloid dendritic cells (DCs), K) CD123hi/DR+/hi plasmacytoid DCs and L) CD56+CD16+/- NK cells; M) non-T, non-B cells also include CD14+CD11b+DR-/lo monocytic myeloid-derived suppressor cells (mMDSC).



Supplementary Figure S4. **Changes in PBMC, survivin- and JARID1B-reactive CD8 T cells in response to TGFβ blockade and radiation. (A)** Absolute levels of PBMCs were maintained or rose in patients receiving fresolimumab, especially 10mg/kg. Green values are for healthy donors **(B)** Dextramer binding data are shown as % JARID-positive CD8+ T cells over the course of a 15 week treatment. The presumed threshold of median + IQR of n=11 healthy control levels is indicated in gray. (N=NYU patient; U=UCLA patient; black=1mg and red=10mg fresolimumab, green=11 healthy donors, N01 and N01* indicates a repeated draws at week 0 and week 2 due to significant treatment delay).



Supplementary Figure S5. Diametrically opposing dynamics in the myeloid suppressor compartment versus the suppressor T cell pool in response to TGFβ blockade and radiation. A) Myeloid cells with the monocytic (CD14+DR-CD16-) or **B)** the granulocytic (CD15+DR-CD14-CD11b+) myeloid-derived suppressor cell profile are shown as individual points. **C)** Changes in myeloid suppression is often diametrically opposed to those in Treg. Patients were ranked according to survival from shortest (left) to longest (right) within each treatment arm. (N=treated at NYU; U=treated at UCLA; black=1mg and red=10mg Fresolimumab, green=11 healthy volunteers)



Supplementary Figure S6. Changes in plasma levels of tryptophan and kynurenine in response to TGFβ blockade and radiation. Patient's plasma samples were analyzed in two batches according to treatment location (NYU vs UCLA) by liquid chromatographic/tandem mass spectrometry. **A)** Median and IQR (Tukey graph; whiskers 1.5xIQR) for each batch (NYU=white, UCLA=blue). **B)** log2 fold change to baseline for each patient over time (N=treated at NYU; U=treated at UCLA; black=1mg and red=10mg fresolimumab).