METHODS

In vivo challenge

In vivo challenge with T-dependent antigen was performed 4 months after transplantation as previously described.[E1] Briefly, serum samples were collected immediately before intravenous immunization with 100 μg of TNP-conjugated keyhole limpet hemocyanin (Biosearch Technologies, Novato, Calif). A boosting dose of 100 μg of TNP-conjugated keyhole limpet hemocyanin was injected intraperitoneally 3 weeks later, and serum samples were collected 7 days later.

Flow cytometry and TEC isolation

Single-cell suspensions were obtained from BM, spleen, thymus, and PB and stained with the mAbs listed in Table E1 in the Online Repository (at www.jacionline.org). Streptavidin phycoerythrin (PE)–cyanine 7 (Cy7) (BD PharMingen, San Jose, Calif) was used for the detection of biotinylated antibody, and a Live/Dead Fixable Dead Cell Stain Kit (Thermo Fisher Scientific, Waltham, Mass) was added to the antibody mix to exclude dead cells by flow cytometry. Samples were acquired on a FACSCanto II system (BD Biosciences, San Jose, Calif).

TECs were isolated as previously described.[E1] Cleaned thymus was digested, and TECs were enriched by depleting $CD45^+$ cells with anti-CD45 microbeads (Miltenyi Biotec, Cambridge, Mass) and immediately used for the staining with the following antibodies: anti-CD45 PE-Cy7 (clone 30-F11, BioLegend, San Diego, Calif), anti-EpCam peridinin chlorophyll protein–cyanine 5.5 (clone G8.8, BioLegend), anti-Ly51 PE (clone 6C3, Miltenyi Biotec), anti-MHC class II allophycocyanin-Cy7 (clone M5/114.15.2, BioLegend), and anti–*Ulex europeaus* agglutinin 1 (UEA1) (clone FL-1061, Vector Laboratories, Burlingame, Calif). mTECs were defined as $EpCam^+$ $Ly51^+$ $UEA1^{high}$ or $EpCam^+$ $Ly51^+$ $CD80^{high}$, and cortical TECs (cTECs) as $EpCam^+$ $Ly51^+$ $UEA1^{low}$ or $EpCam^+$ $Ly51^+$ $CD80^{low}$. A Live/Dead Fixable dead cell stain kit was used for assessing cell viability. Events were acquired on an LSR Fortessa flow cytometer (BD Biosciences, San Jose, Calif). All data were analyzed by using FlowJo software, version 10.5.2 (FlowJo, LLC, Ashland, Ore).

ELISA and autoantibody profile

Levels of IgG isotypes, IgM, and IgA were measured in plasma samples by multiplex assay (Beadlyte Mouse Immunoglobulin Isotyping kit, Millipore, Burlington, Mass) on a Luminex Magpix instrument (Luminex Corp, Austin, Tex). Anti-TNP specific antibody titers were measured by ELISA on serum samples after polystyrene plates were coated with 8 μg/mL of TNP(4)-BSA (Biosearch Technologies, United Kingdom). Serial dilutions of serum (from 1:100 to 1:72,900) were added, and the reaction was revealed using alkaline phosphatase–conjugated goat anti-mouse IgG (Sigma-Aldrich, Burlington, Mass), followed by addition of *p*-nitrophenylphosphate (Sigma-Aldrich). OD values were measured at 405 nm by using a Multiskan GO spectrophotometer (Thermo Fisher Scientific, Frederick, Md).

Protein arrays were used to screen for a broad panel of IgG autoantibodies. Protein arrays were used to screen for a broad panel of IgG autoantibodies. Autoantigen microarrays were manufactured, hybridized, and scanned by the Microarray Core Facility at the University of Texas Southwestern Medical Center (Dallas, Tex). Serum was collected from *Rag1-F971L* mice 16 weeks after transplantation and distinct conditioning regimens. Sera were also collected from WT and *MRL* mice, as negative and positive control groups, respectively.

Briefly, sera were incubated with an autoantigen array, and binding of autoantibodies was detected by using cyanine 3–labeled anti-IgG with a laser wavelength of 532 nm. As a quantitative measure of the ability to resolve true signal from background noise, we used the signal-to-noise ratio (SNR), which was calculated as follows: SNR = (Foreground median – Background median)/ SD (Background). SNR values equal or higher than 3 were considered representative of true signal. The net fluorescence intensity and net SNR for each antigen were calculated by subtracting all the negative control (PBS) effects that were included for each experiment as negative control. The antibody score was computed as follows: Antibody score = log2(NFI*SNR + 1), where NFI is the net fluorescence intensity. A heatmap was generated with R software based on log2 fold change in the antibody scores compared with the average for *Rag1-F971L* untreated mice.

Liver function

Alanine aminotransferase and aspartate aminotransferase levels were measured in plasma samples by using quantILab alanine aminotransferase/glutamate pyruvate transaminase and aspartate aminotransferase/oxaloacetate transaminase kits and an Ilab Aries instrument (Werfen-Italia, Instrumentation Laboratory, Milan, Italy), according to the manufacturer's instructions. All measures were preceded by the analysis of certified internal quality controls to verify the analytic performance of the test.

REFERENCE

E1. Capo V, Castiello MC, Fontana E, et al. Efficacy of lentivirus-mediated gene therapy in an Omenn syndrome recombination-activating gene 2 mouse model is not hindered by inflammation and immune dysregulation. J Allergy Clin Immunol 2018;142:928-41.e928.

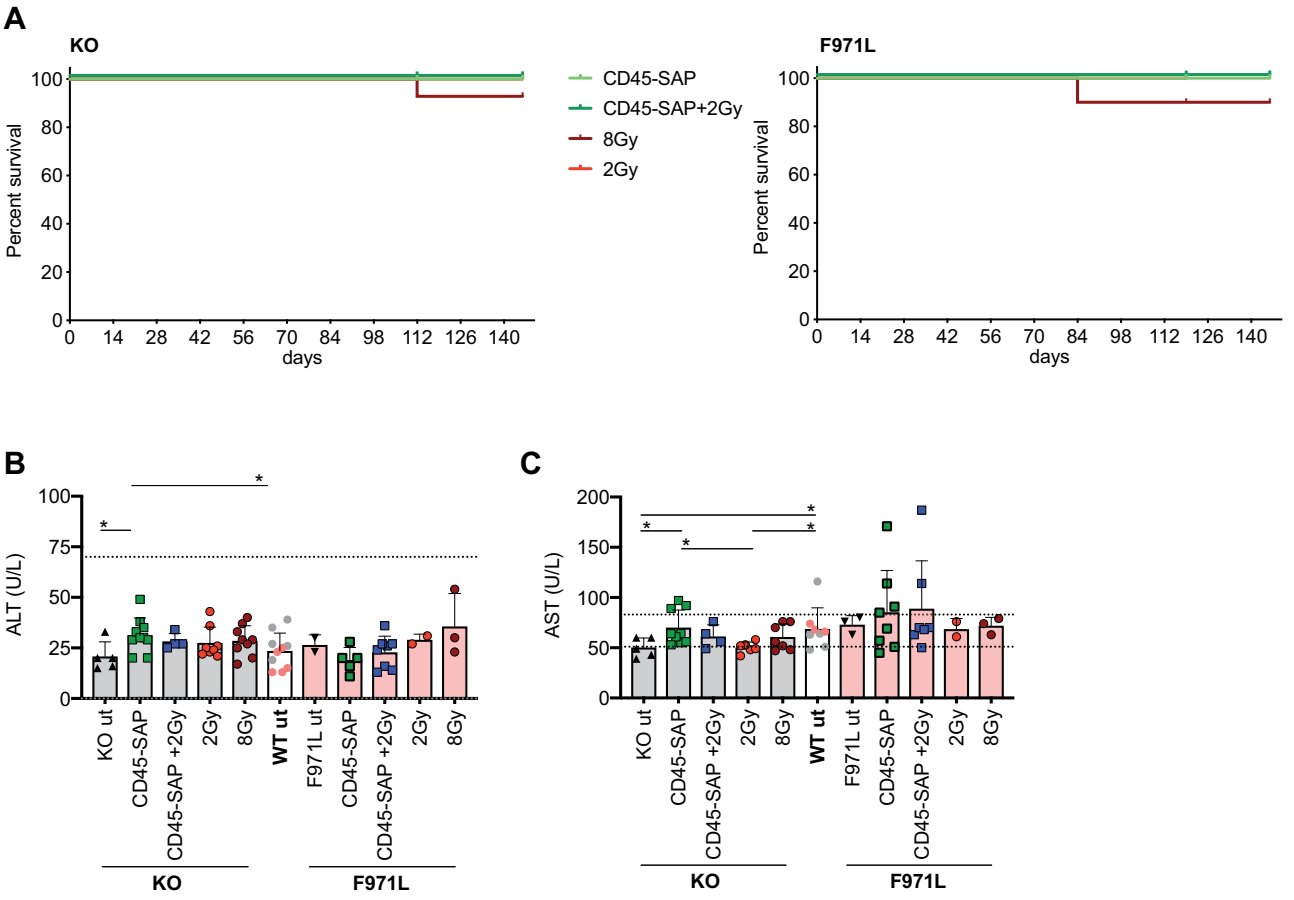

FIG E1. Survival rate of conditioned *Rag1*^{mut} mice. **A,** Kaplan-Meier survival curves for *Rag1-KO* (knockout [KO]) (*left panel*) and *Rag1-F971L* (F971L) (*right panel*) mice subjected to conditioning and transplantation. *Rag1-KO* mice were treated with the following: CD45-SAP (n = 16); CD45-SAP plus 2 Gy (n = 11); 8 Gy of irradiation (n = 13); and 2 Gy of irradiation (n = 11). *Rag1-F971L* mice were treated with the following: CD45-SAP (n = 11); CD45-SAP plus 2 Gy of irradiation (n = 11); 8 Gy of irradiation (n = 10); and 2 Gy of irradiation (n = 6). **B** and **C,** Liver function was analyzed by measuring alanine aminotransferase (ALT) (**B**) and aspartate aminotransferase (AST) (**C**) levels in plasma samples collected from *Rag1-KO* and *Rag1-F971L* mice undergoing various conditioning treatments when they were humanely killed (16 weeks after transplantation). Gray dots in the WT bar are values from samples collected at the SR-Tiget laboratory, and pink dots are values from samples collected at the National Institutes of Health laboratory. One-way ANOVA; Kruskal-Wallis test for multiple comparison; **P* < .05; ***P* < .005; ****P* < .0005; *****P* < .0001. Means ± SDs are shown. *ut,* Untreated.

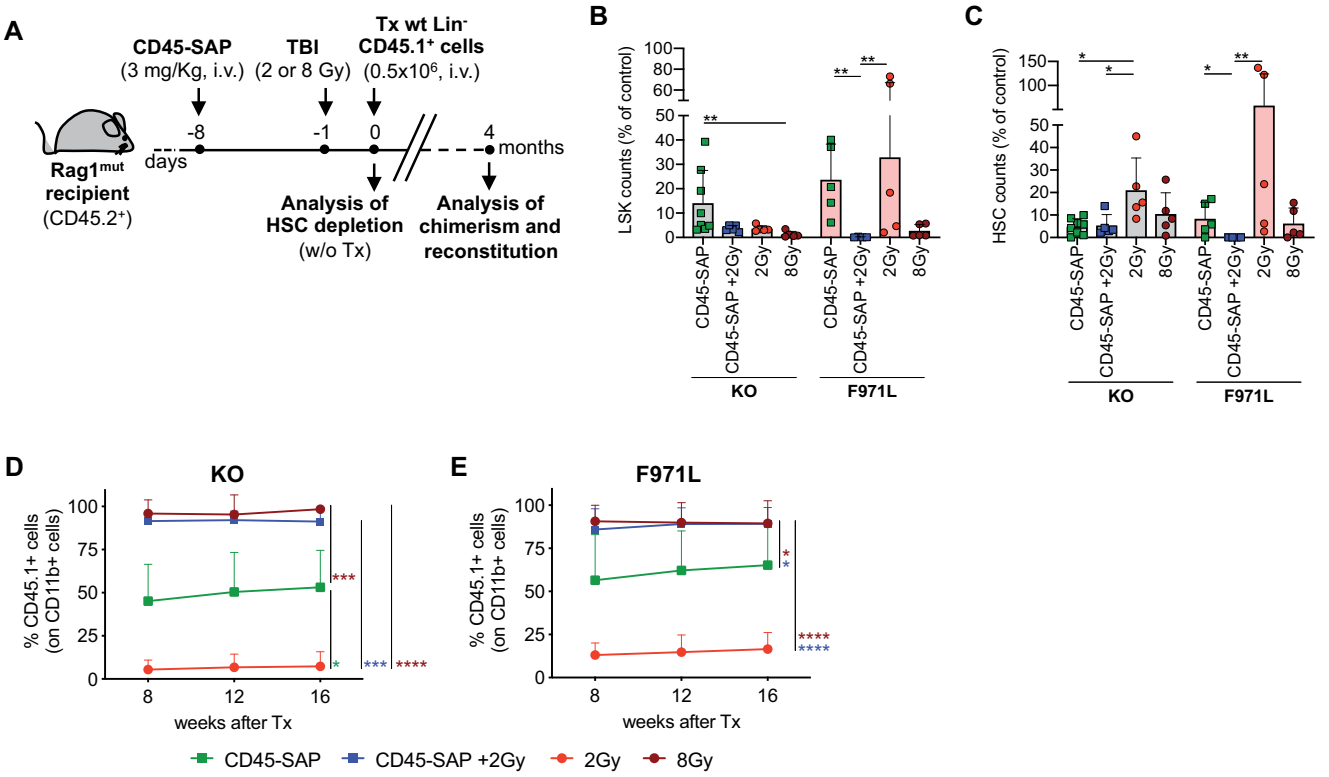

FIG E2. Depletion effect and engraftment level in conditioned *Rag1*^{mut} mice. **A,** Time line and scheme of the experiment. **B** and **C**, Absolute counts of LSK (**B**) and HSC (**C**) cells at day 0 are shown as percentages of the mean value of the respective untreated *Rag1*-mutant group. One-way ANOVA, Kruskal-Wallis test for multiple comparison. **D** and **E**, Analysis of myeloid chimerism frequency over time after transplant (Tx) in the PB of *Rag1-KO* (KO) mice (**D**) and *Rag1-F971L* (F971L) (**E**). One-way ANOVA, Kruskal-Wallis test for multiple comparison at 16 weeks after transplant. Asterisk colors indicate the groups of comparison. The group treated with CD45-SAP included 11 KO mice and 10 F971L mice; the group treated with CD45-SAP plus 2 Gy of TBI included 7 KO mice and 10 F971L mice; the group treated with 2 Gy of TBI included 11 KO mice and 6 F971L mice; and the group treated with 8 Gy of TBI included 12 KO mice and 10 F971L mice. **P* < .05; ***P* < .005; ****P* < .0005; *****P* < .0001. Means ± SDs are shown.

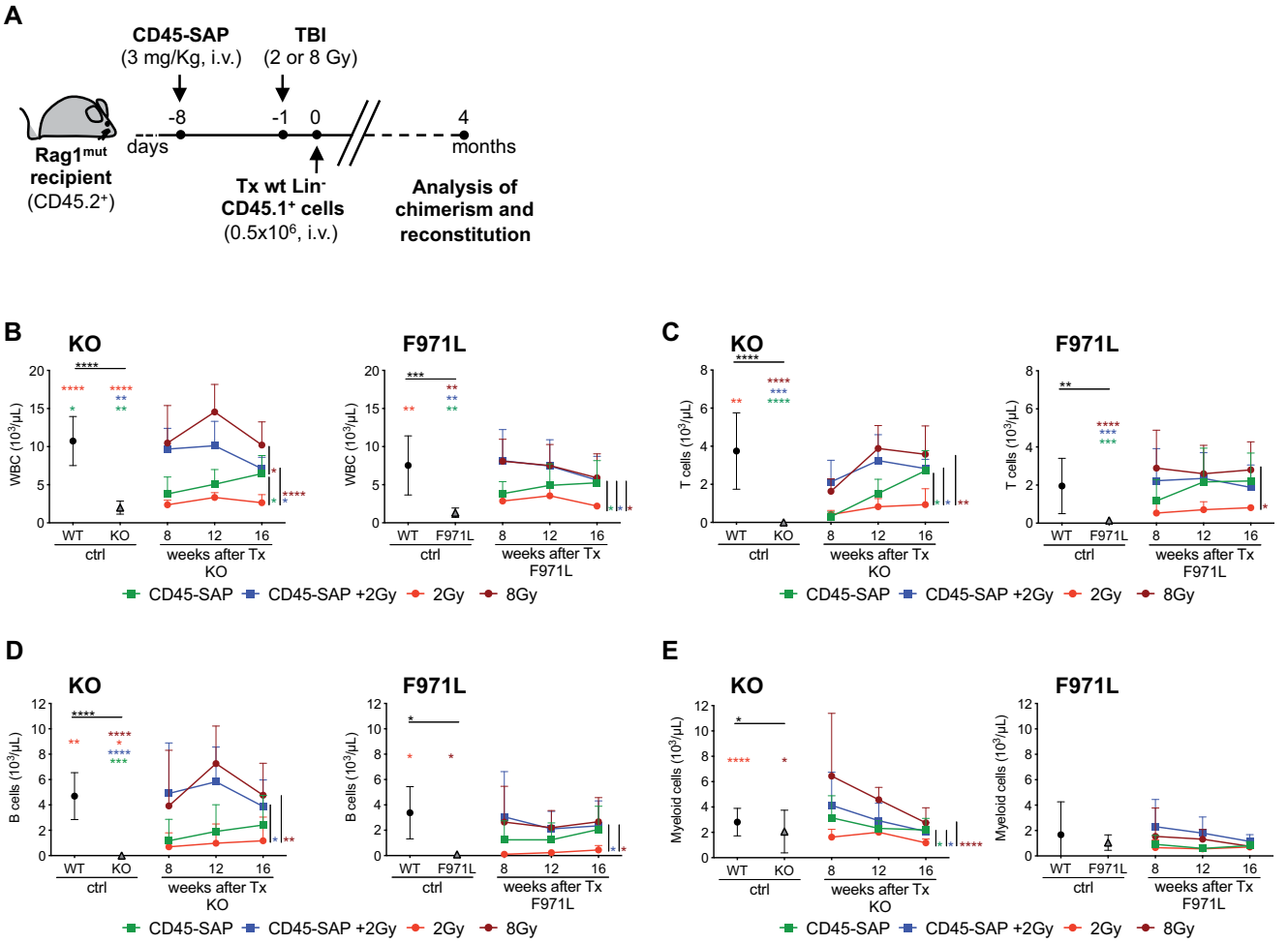

FIG E3. Kinetics of the immunologic reconstitution over time in the PB of conditioned *Rag1*^{mut} mice. **A,** Time line and scheme of the experiment. **B-F,** Analysis over time after transplant (Tx) WBC count (**B**), T-cell (CD3$^+$) count (**C**), B-cell (CD19$^+$ B220$^+$) count (**D**), and myeloid cells (CD11b$^+$) count (**E**) in the PB of *Rag1-KO* (knockout [KO]) (*left panel*) and *Rag1-F971L* (F971L) (*right panel*) mice. **B-F,** The group treated with CD45-SAP included 11 KO mice and 11 F971L mice; the group treated with CD45-SAP plus 2 Gy of TBI included 7 KO mice and 11 F971L mice; the group treated with 2 Gy of TBI included 11 KO mice and 7 F971L mice; and the group treated with 8 Gy of TBI included 11 KO mice and 10 F971L mice (WT control [ctrl] mice, n = 11-19 [*left panels*] and n = 8 [*right panels*]; KO ctrl mice, n = 11-16; and F971L ctrl mice, n = 7). One-way ANOVA, Kruskal-Wallis test for multiple comparison at 16 weeks after transplantation. **P* < .05; ***P* < .005; ****P* < .0005; *****P* < .0001. Means ± SDs are shown.

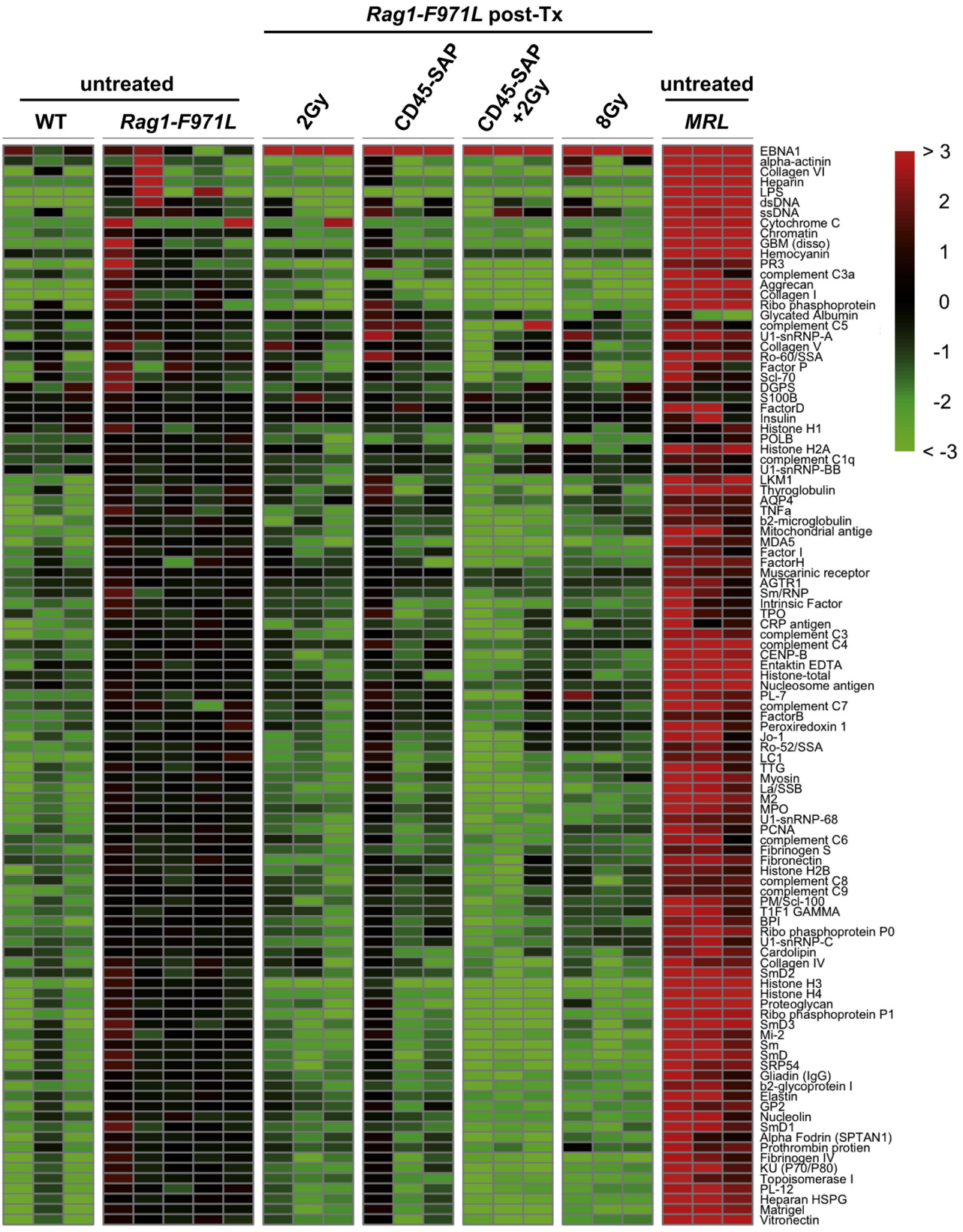

FIG E4. Autoantibody profile in *Rag1-F971L* mice subjected to conditioning and a transplant. Levels of IgG reactivity were tested against 123 antigens by autoantigen array in the sera of hypomorphic *Rag1-F971L* mice 16 weeks after transplant (post-Tx) and distinct conditioning regimens. The autoantibody profile of untreated WT, *Rag1-F971L,* and *MRL* mice are shown as control groups. Data are represented as a heatmap with colors corresponding to log2 fold change in antibody scores with the average for *Rag1-F971L* untreated mice.

TABLE E1. List of antibodies

Antibody	Clone	Source	Identifier	Dilution
B220 APC-Cy7	C363-16A	BioLegend	103310	1:200
B220 Pe-Cy7	RA36B2	BD Bioscience	552772	1:200
CD3 APC-Cy7	145-2C11	BD Bioscience	557596	1:100
CD4 Pacific Blue	GK1.5	BioLegend	100428	1:500
CD4 PerCP	RM/4-5	BD Bioscience	553052	1:500
CD8 APC	53-6.7	BioLegend	100712	1:400
CD8 Pe-Cy7	53-6.7	BD Bioscience	552877	1:400
CD8 Pacific Blue	53-6.7	BD Bioscience	558106	1:500
CD11b Pacific Blue	M1/70	BioLegend	101224	1:100
CD19 PE	1D3	BD Bioscience	553786	1:200
CD19 eFluor450	1D3	Thermo Fisher	48-0193-82	1:200
CD21 APC	7 E 9	BioLegend	123412	1:400
CD24 PE	M1/69	BD Bioscience	553262	1:400
CD25 PE	PC61	BioLegend	102008	1:200
CD25 APC	PC61	BD Bioscience	557192	1:100
CD43 biotinylated	S7	BD Bioscience	553269	1:100
CD44 FITC	IM7	BioLegend	103006	1:200
CD45.1 FITC	A20	BD Bioscience	553775	1:200
CD45.1 PE	A20	BD Bioscience	553776	1:200
CD45.1 APC-Cy7	A20	BD Bioscience	560579	1:200
CD45.1 Pacific Blue	A20	BioLegend	110722	1:200
CD45.2 PerCP5.5	104	BD Bioscience	552950	1:200
CD45.2 Pacific Blue	104	BioLegend	109820	1:200
CD48 PE	HM48-1	BioLegend	103405	1:100
CD62L APC	MEL-14	BD Bioscience	553152	1:200
CD150 APC	TC15-12F12.2	BioLegend	115910	1:100
cKit APC-Cy7	2BS	BioLegend	105826	1:100
IgM FITC	II/41	BD Bioscience	553437	1:200
Mouse lineage cocktail	17A2/RB6-8C5/6B2/ Ter119/M1/70	BioLegend	133310	1:20
Sca1 Pe-Cy7	D7	BioLegend	108114	1:100

APC, Allophycocyanin; *FITC*, fluorescein isothiocyanate; *PerCP*, peridinin chlorophyll protein.