SUPPLEMENTARY MATERIAL

Nuclear Envelope Lamin-A Couples Actin Dynamics with Immunological Synapse Architecture and T Cell Activation

José M. González-Granado et al.

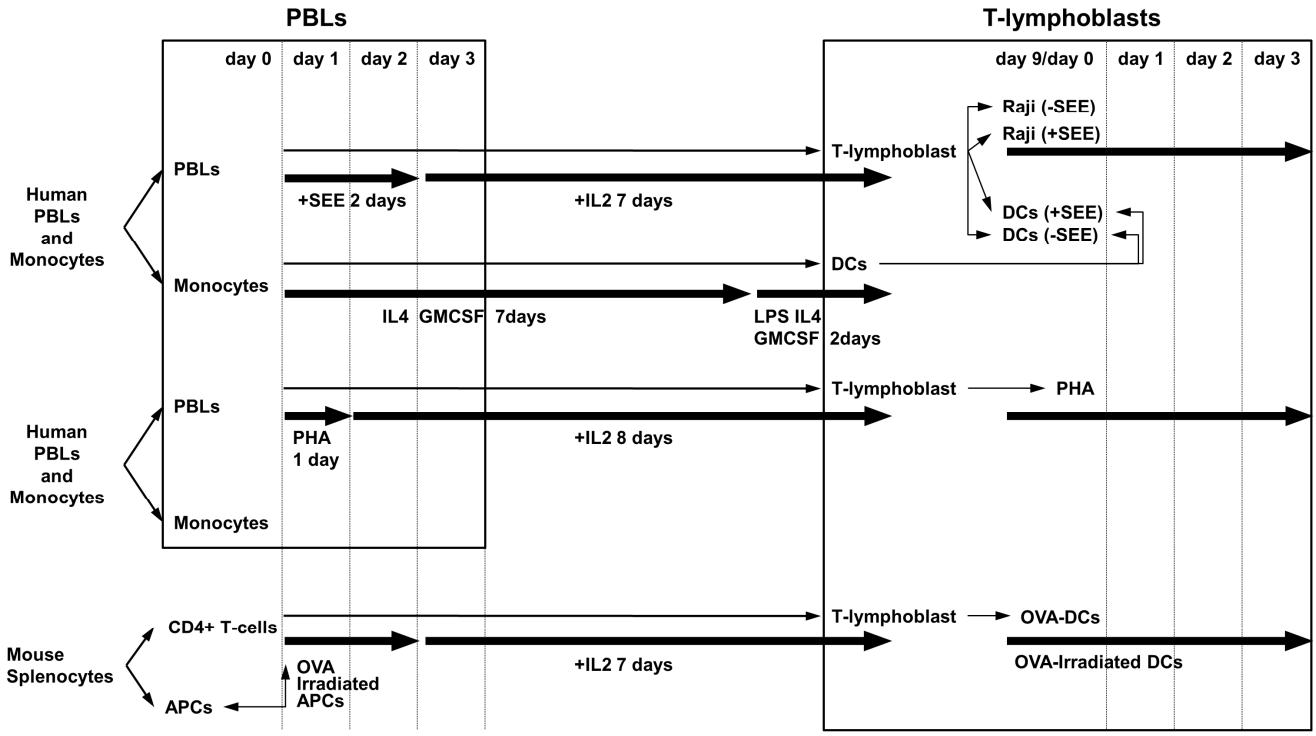

Fig. S1. Schemes of the protocols used to culture human PBLs and mouse splenocytes to generate human T lymphoblasts, human DCs, and mouse T lymphoblasts. Details on the isolation of the starting human and mouse populations of cells can be found in the Materials and Methods.

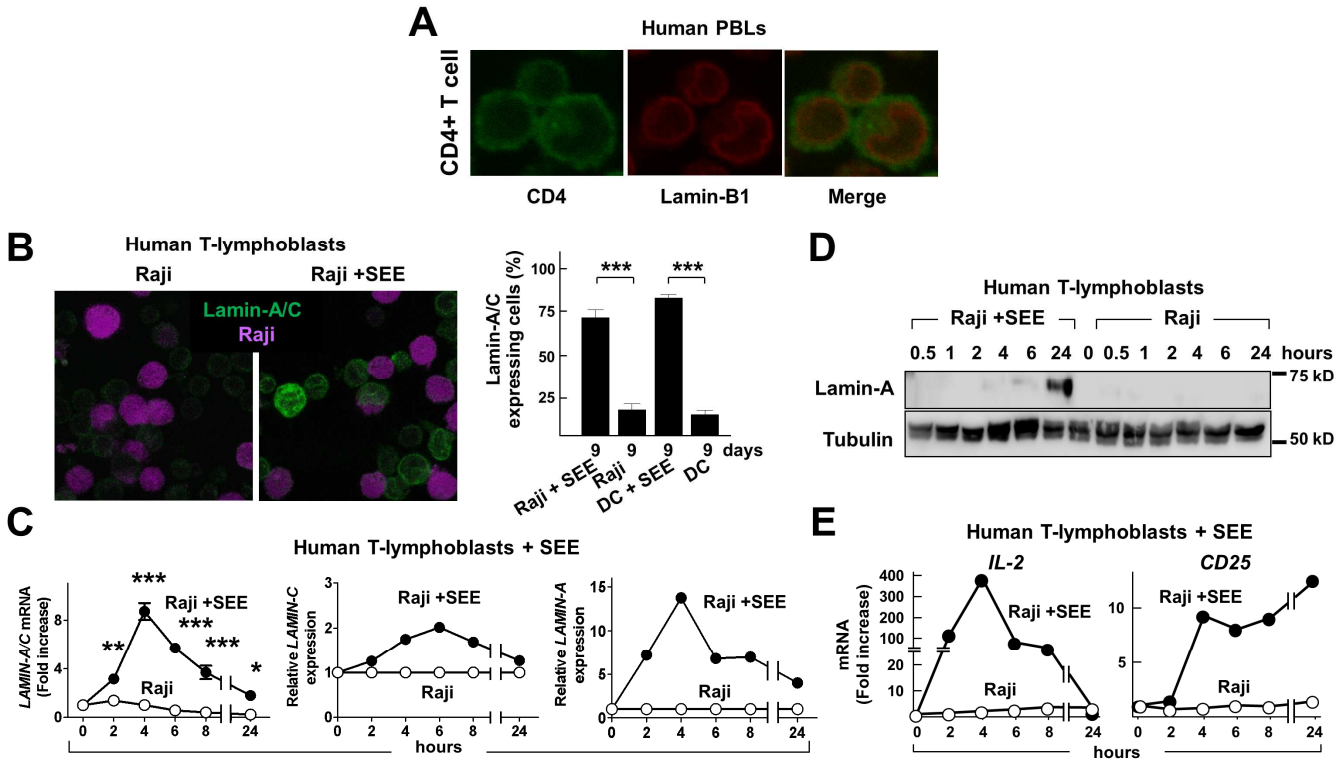

Fig. S2. Analysis of lamin-A/C and lamin-B1 in immune cells. (**A**) Human $CD4^+$ PBLs were incubated with antibodies against lamin-B (red) and CD4 (green) and were analyzed by confocal microscopy. A single confocal plane is shown. (**B**) Human $CD4^+$ T lymphoblasts were incubated with CMAC-labeled unloaded or SEE-loaded Raji cells or DCs, incubated with antibody against lamin-A/C (green), and then analyzed by confocal microscopy. Left: Representative images of $CD4^+$ T lymphoblasts that formed conjugates after incubation for 6 hours with Raji cells (purple). A single confocal plane is shown. Right: Graph shows quantification of the percentages of $CD4^+$ T lymphoblasts that have lamin-A/C upon stimulation with non-loaded or loaded Raji cells or DCs. (**C** to **E**) Human T lymphoblasts were incubated with either non-loaded or SEE-loaded Raji cells for the indicated times. (C) The relative abundances of *LAMIN-A/C*, *LAMIN-A*, and *LAMIN-C* mRNAs were quantified by RT-PCR analysis (Left: n=3; Middle and Right: n=1). (D) The presence of lamin-A protein was detected by Western blotting analysis. (E) The relative abundances of *IL-2* and *CD25* mRNAs were determined by qRT-PCR analysis to verify the activation of T-lymphoblasts.

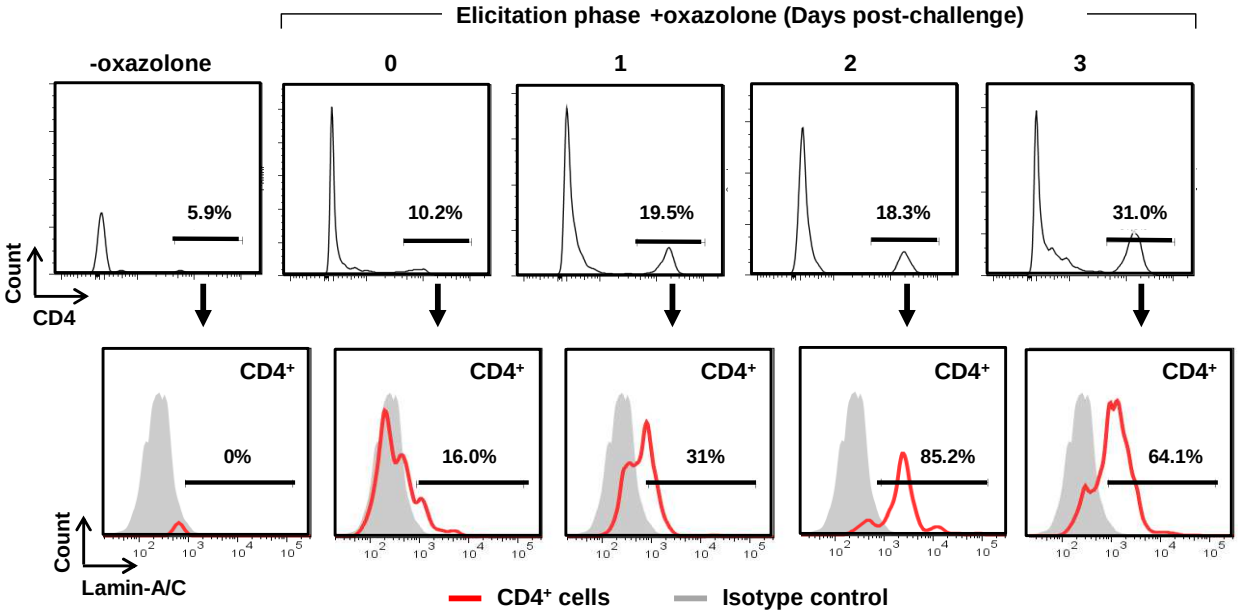

Fig. S3. CD4$^+$ T cells migrate to lymph nodes and have lamin-A/C in the hapten-induced contact hypersensitivity model. Representative flow cytometry histograms showing the percentages of CD4$^+$ lymphocytes (top) and lamin-A/C-containing CD4$^+$ cells (bottom) in the cervical lymph nodes from untreated (-oxazolone) and oxazolone-treated (+oxazolone) wild-type mice (tissue pooled from three mice). FITC-conjugated isotype control antibody is shown in filled gray.

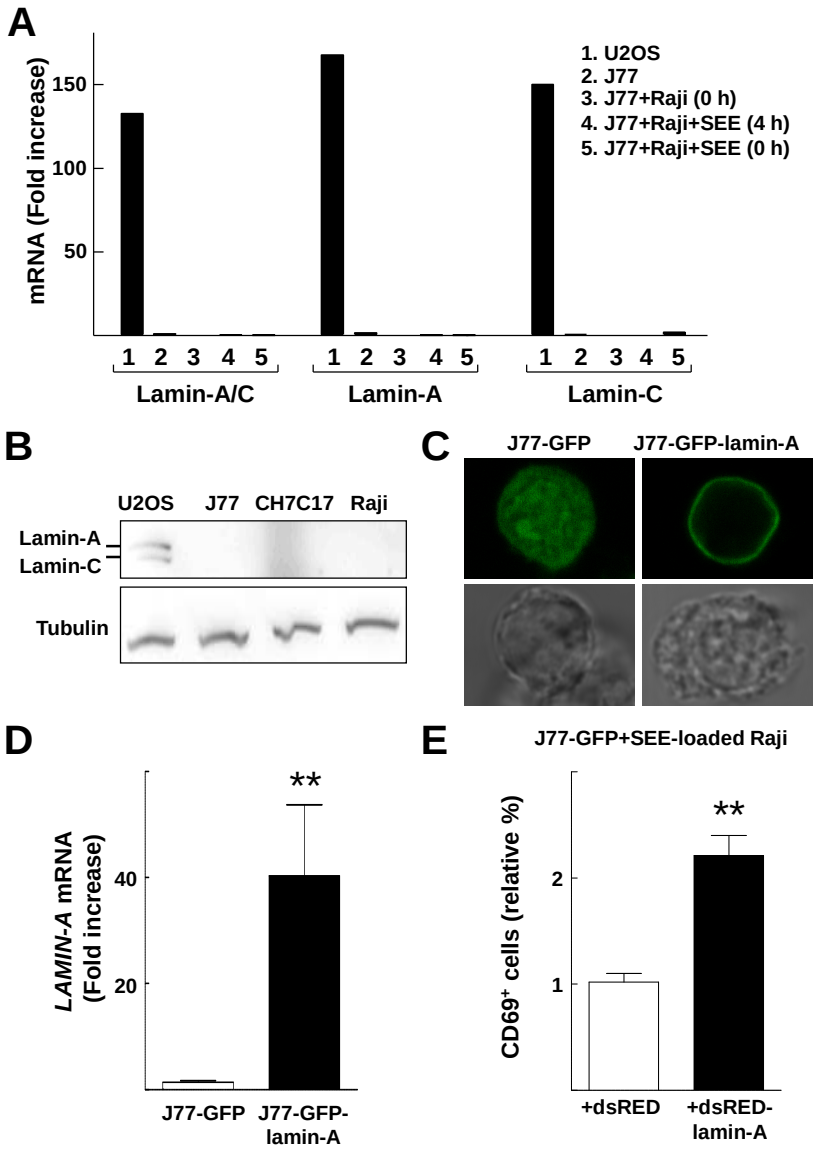

Fig. S4. J77 cells stably expressing GFP–lamin-A display enhanced T cell activation. (**A**) J77 T cells were incubated with non-loaded or SEE-loaded Raji cells for the indicated times. The relative amounts of lamin-A/C mRNA were determined by qRT-PCR analysis. Human osteosarcoma cells (U2OS) were used as a positive control for the detection of lamin-A/C mRNA. (**B**) U2OS, J77, CH7C17, and Raji cells were subjected to Western blotting analysis to detect lamin-A/C. (**C**) Confocal microscopic images of J77-GFP and J77-GFP-lamin-A cells showing GFP and bright field channels. A single confocal plane is shown. (**D**) Untransfected J77 cells and stably transfected J77-GFP and J77-GFP-lamin-A cells were subjected to qRT-PCR analysis to determine the relative amounts of lamin-A mRNA. Data are the fold-increase in *LAMIN-A* mRNA compared to that in untransfected J77 cells and are means ± SEM from three independent experiments. (**E**) J77-GFP transiently transfected with plasmids encoding dsRED or dsRED-lamin-A were incubated with SEE-loaded Raji cells for 16 hours, labeled with anti-CD69 antibody and analyzed by flow cytometry. The graph shows the percentages of $CD69^+$ cells relative to those among dsRED-expressing cells and are means ± SEM from three independent experiments.

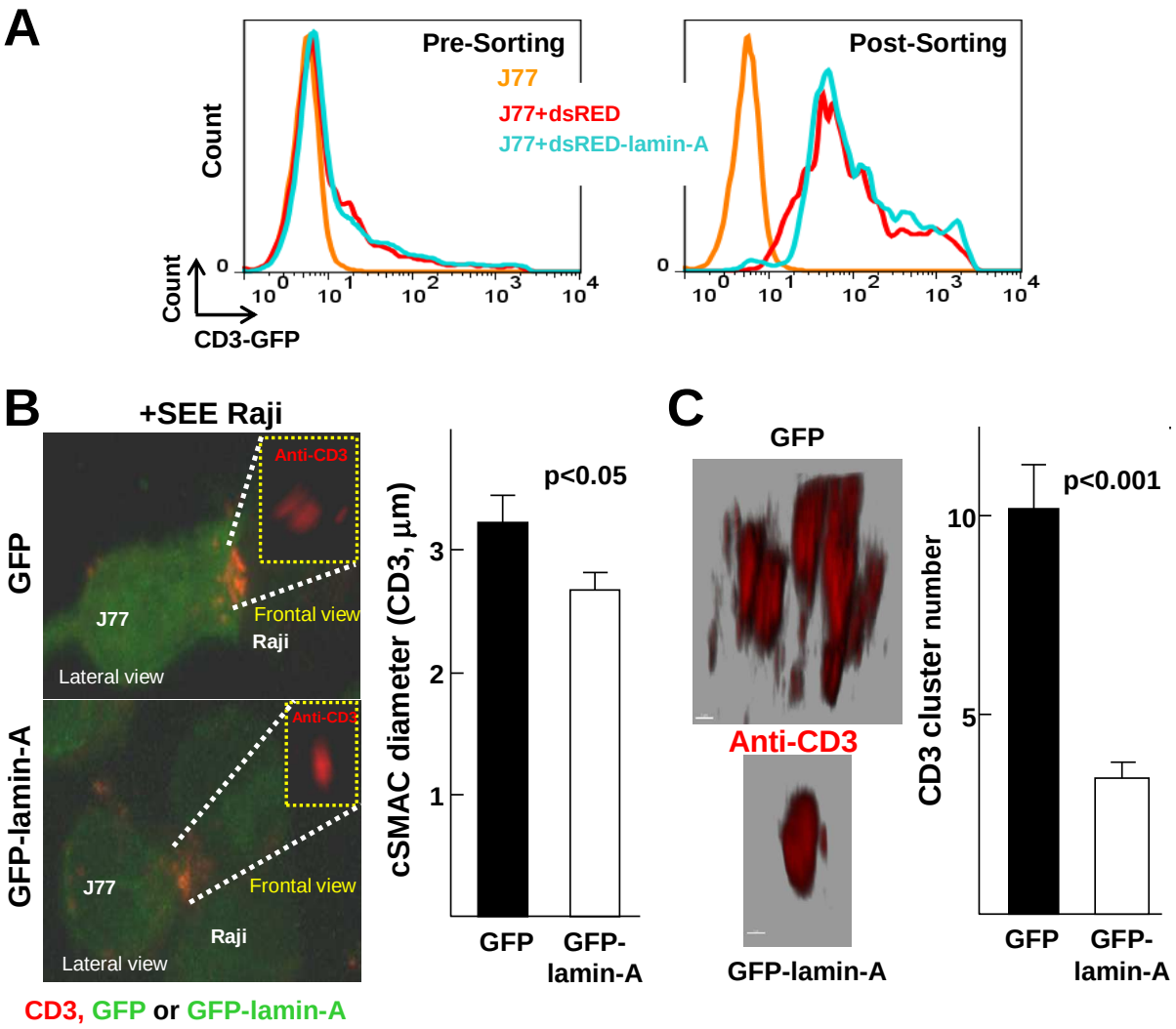

Fig. S5. Lamin-A facilitates the interactions between J77 cells and APCs and modulates CD3 movement and clustering at the immunological synapse. (**A**) J77 cells were cotransfected with plasmids encoding CD3ζ-EGFP plus either dsRED (red), or dsRED-lamin-A (blue), cultured for 24 hours and sorted by FACs to isolate $GFP^{+}dsRED^{+}$ cells. Flow cytometry histograms show the analysis of CD3ζ-EGFP expression before and after cell sorting. Untransfected J77 cells (orange) were used as a negative control for cell sorting. (**B** and **C**) J77-GFP and J77-GFP-lamin-A cells were allowed to form conjugates 30 min with SEE-loaded Raji cells, and then were incubated with antibody for CD3 and analyzed by confocal microscopy. (B) Representative confocal microscopic images and quantification of the the diameter of the cSMAC (µm). Data are means ± SEM of 100 conjugates from two independent experiments. (C) Representative 3D reconstruction of CD3 clustering at the contact area between the T cell and B cell, as well as quantification of the number of CD3 clusters at the immunological synapse. Data are means ± SEM of 100 conjugates from two independent experiments.

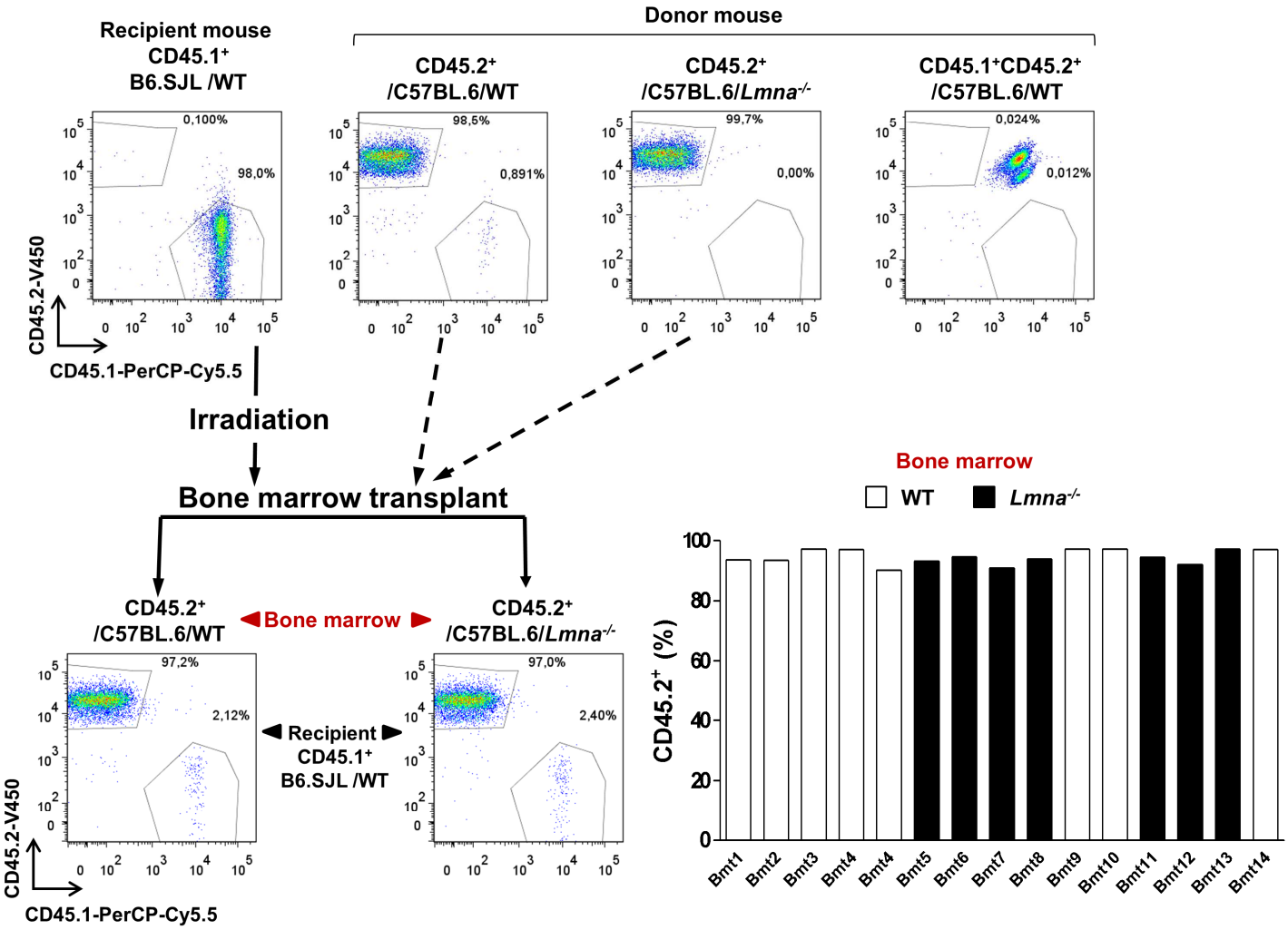

Fig. S6. Quantification of the numbers of CD45.1$^+$ and CD45.2$^+$ CD4$^+$ T cells in the blood of donor, recipient, and chimeric mice. For bone marrow transplant experiments (Fig. 2D), lethally-irradiated recipient CD45.1$^+$ B6.SJL wild-type (WT) mice were transplanted with bone marrow cells from C57BL. 6 CD45.2$^+$ WT or C57BL.6 CD45.2$^+$ *Lmna*$^{-/-}$ mice. Eight weeks after transplantation, chimerism in the recipient mice was assessed by flow cytometric analysis of blood cells stained with a combination of fluorescently labelled antibodies to detect CD45.1$^+$ cells from recipient mice and CD45.2$^+$ cells from the donors. The bar graph shows the percentage of CD45.2$^+$ cells in the blood of 14 recipient CD45.1$^+$ mice (Bmt1 to Bmt14) eight weeks after transplantation, which showed that more than 90% of cells were derived from the transplanted bone marrow cells. For adoptive transfer experiments (Fig. 2, E and F), B6.SJL CD45.1$^+$ WT recipient mice received CD4$^+$ T cells from C57BL.6 CD45.2$^+$ WT, C57BL.6 CD45.2$^+$ *Lmna*$^{-/-}$, or C57BL.6 CD45.1$^+$CD45.2$^+$ WT mice.

Movie 1. A-type lamins regulate several steps in the interaction between T cells and APCs. Representative movie of confocal video microscopy images of the interaction between J77-GFP cells (cyan and green) or J77-GFP-lamin-A (red) cells with SEE-loaded Raji cells (dark blue) at the indicated times.

Movie 2. Lamin-A regulates the movement of TCR-CD3 complexes in the plasma membrane. J77 cells were cotransfected with plasmid encoding CD3ζ-EGFP plus either plasmid encoding dsRED or dsRED-lamin-A. After 24 hours, $GFP^{+}dsRED^{+}$ cells were isolated by cell sorting, plated onto anti-CD3 antibody-coated coverslips and analyzed by TIRF microscopy at a penetration depth of ~90 nm. Representative movies are shown of CD3ζ-EGFP microclusters at the indicated times.