Nuclear pore complex-mediated modulation of TCR signaling is required for naïve CD4⁺ T cell homeostasis

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Nuclear pore complexes (NPCs) are channels connecting the nucleus with the cytoplasm. We report that loss of the tissue-specific NPC component Nup210 causes a severe deficit of naïve CD4⁺ T cells. Nup210-deficient CD4⁺ T lymphocytes develop normally but fail to survive in the periphery. The decreased survival results from both an impaired ability to transmit tonic T cell receptor (TCR) signals and increased levels of Fas, which sensitize Nup210⁻/⁻ naïve CD4⁺ T cells to Fas-mediated cell death. Mechanistically, Nup210 regulates these processes by modulating the expression of Cav2 (encoding Caveolin-2) and Jun at the nuclear periphery. Whereas the TCR-dependent and CD4⁺ T cell-specific upregulation of Cav2 is critical for proximal TCR signaling, cJun expression is required for STAT3-dependent repression of Fas. Our results uncover an unexpected role for Nup210 as a cell-intrinsic regulator of TCR signaling and T cell homeostasis and expose NPCs as key players in the adaptive immune system.

Lymphocytes are integral players in the adaptive immune response. After development in the thymus, mature T cells recirculate among the blood, lymph, and secondary lymphoid organs, where they scan antigen-presenting cells for their cognate antigen. The maintenance of the circulating naïve T cell population is the result of a balance among thymic output, survival, and homeostatic proliferation. Naïve T cell homeostasis is essential for maintaining the functional TCR repertoire necessary for ensuring immunity against foreign antigens while avoiding self-reactivity. NPCs are aqueous channels that span the nuclear envelope. Although NPCs have traditionally been known as regulators of nucleocytoplasmic transport, it has become evident that they also have multiple transport-independent functions including the regulation of gene expression and chromatin organization. NPCs are built from 32 different proteins known as nucleoporins. Although the structure of the NPC is conserved in all cells, the expression of several nucleoporins varies among different cell types and tissues, and mutations in various nucleoporins result in tissue-specific diseases. Thus, NPCs can be specialized to perform cell-type-specific functions. In support of this idea, we have recently reported that the tissue-specific nucleoporin Nup210 (ref. ³) is a critical regulator of skeletal muscle physiology. Although Nup210 expression is absent in myoblasts, its incorporation into the NPCs of differentiating myotubes is both required and sufficient for myogenesis and myofiber maturation.

We also discovered that Nup210 deletion in mice specifically decreases the number of circulating naïve CD4⁺ T lymphocytes. We discovered that CD4⁺ T cells from Nup210-deficient mice, compared with wild-type mice, have reduced tonic TCR signaling, thus compromising their survival in the periphery, and fail to properly activate in response to TCR ligation. We found that Nup210 mediates proximal TCR signaling by modulating induction of the lipid-raft protein Cav2 after TCR activation. The findings that the Cav2 gene is present at NPCs and that its efficient activation requires Nup210 support the emerging idea that NPCs act as scaffolds for the regulation of inducible genes. We also found that Nup210 is critical for the proper expression of cJun, which, together with STAT3, prevents expression of the Fas death receptor. Our findings reveal a cell-intrinsic role of Nup210 in the regulation of CD4⁺ T cell homeostasis and establish tissue-specific NPCs as key modulators of TCR signaling.

Results

Nup210⁻/⁻ mice have fewer numbers of CD4⁺ T lymphocytes. While analyzing Nup210 mRNA levels in tissues from adult mice, we found that this nucleoporin showed high expression in immune organs, including the spleen, lymph nodes, and bone marrow. Analysis of immune-cell subsets revealed that T and B lymphocytes expressed higher levels of Nup210 than did eosinophils, macrophages, monocytes, and neutrophils. These results are consistent with publicly available ImmGen data. To investigate the function of Nup210 in the immune system, we generated a constitutive Nup210-knockout mouse line (Nup210⁻/⁻) through a deletion of exon 2 that completely abolished expression of the protein. These results were observed in naïve CD4⁺ T cells and were also found in aged mice. The Nup210⁻/⁻ cells showed correct localization of nucleoporins and normal nucleocytoplasmic transport. In agreement with our previous findings, Nup210⁻/⁻ cells showed no defects in NPC assembly or function. Analysis of the blood and bone marrow of Nup210⁻/⁻ mice, compared with wild type, showed significantly fewer white blood cells.
specific to lymphocytes but no detectable alterations in erythrocytes or myeloid cells (Fig. 2a.b and Supplementary Fig. 2a). Detailed characterization of the lymphocyte populations in the spleen revealed no changes in B cells or spleen cellularity (Supplementary Fig. 2b,c), but lower proportions and numbers of CD3+ T lymphocytes (Fig. 2c.d). Further analysis of the CD3+ population showed an abnormal CD4+/CD8+ T cell ratio (1.4±0.05 in Nup210+/- versus 0.6±0.02 in Nup210+/+; Fig. 2e) that resulted from substantially fewer (~60%) CD4+ T lymphocytes (Fig. 2f.g). Importantly, we found no alterations in the numbers of CD8+ T cells, although their proportion within the CD3+ population was elevated because of the lower number of CD4+ T cells (Fig. 2f.g). Within the CD4+ T cell population from Nup210-knockout compared with wild-type mice, we observed a lower percentage of naïve cells and a higher percentage of effector and central memory subsets (Fig. 2h). This abnormal distribution resulted from a strikingly lower number (>75%) of naïve CD4+ T cells (Fig. 2i). A similarly low number of naïve CD4+ T cells was observed in the blood (Supplementary Fig. 2d–f). The numbers of TCRγδ T cells, natural killer cells, natural killer T cells, and regulatory T cells were not altered in Nup210-/- mice compared with wild type (Supplementary Fig. 2g–j). These findings indicate that Nup210 is critical for the maintenance of the naïve CD4+ T cell population.

Nup210-/- mice have normal T cell development and circulation. To address whether the deficit in peripheral naïve CD4+ T lymphocytes was caused by abnormal T cell development, we analyzed T cell precursors in the thymus. We found no differences in the number or proportion of double-negative (DN1–DN4) or double-positive thymocyte populations between Nup210+/- and Nup210-/- mice (Fig. 3a–d and Supplementary Fig. 3a,b). Furthermore, we observed equal numbers of single-positive CD4+ and CD8+ T cells (Supplementary Fig. 3c) and confirmed that Nup210-/- and Nup210+/- mice produced equal numbers of naïve CD4+ T cells (Supplementary Fig. 3d). These findings indicate that T cells develop normally in Nup210-/- mice and suggest that the lower numbers of naïve CD4+ T cells in Nup210-/- mice are caused by abnormalities in the periphery.

After they exit the thymus, naïve T cells continuously recirculate among the blood, lymph, and secondary lymphoid organs. The low number of CD4+ T cells in the spleen and blood of Nup210-/- mice may have resulted from abnormal retention in lymph nodes, as
has been shown for mice lacking the kinase TBK1 (ref. 14), or from aberrant migration to nonlymphoid organs, as has been observed in mice lacking the transcription factor KLF2 (ref. 15). Analysis of peripheral and mesenteric lymph nodes showed the same low numbers of naïve CD4+ T cells and no changes in the CD8+ T cell population (Fig. 3e–h and Supplementary Fig. 3e–g), thus indicating that Nup210 deficiency does not cause T cell retention in secondary lymphoid organs. To determine whether naïve Nup210+/− CD4+ T cells abnormally localized to nonlymphoid organs, we isolated RNA from tissues of Nup210+/− and Nup210−/− mice and analyzed the distribution of CD4+ T cells by measuring Cd4 mRNA levels15. In agreement with the observed low numbers of CD4+ T cells in the

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**Fig. 2 | Nup210 is required for naïve CD4+ T cell homeostasis.** a, b, Analysis of white blood cell (WBC; a) and red blood cell (RBC; b) concentrations in the blood of Nup210+/+ and Nup210−/− mice. Lym, lymphocytes; Mon, monocytes; Gra, granulocytes; HGB, hemoglobin; HCT, hematocrit; MCV, mean cell volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDWc, red cell distribution width; PLT, platelet; PCT, platelet hematocrit; MPV, mean platelet volume; PDWc, platelet distribution width. c–i, Flow cytometric analysis of Nup210+/+ and Nup210−/− spleens. c, Gating strategy used to define splenic CD3+ T cells. d, Splenic CD3+ T cell numbers. e, Splenic CD4+/CD8− T cell ratios. f, Gating strategy used to define splenic CD4+ and CD8+ T cell populations. g, Splenic CD4+ and CD8+ T cell numbers. h, Gating strategy used to identify naïve (CD62L+/CD44−), effector memory (CD62L−/CD44+), and central memory (CD62L+/CD44+) splenic CD4+ T cells. i, Splenic naïve and memory CD4+ T cell numbers. Data are mean ± s.e.m. (a,b,d,e,g,i), each symbol represents an individual mouse; mice per group: n = 4 (a,b), n = 28 or 29 (d), n = 35 or 39 (e), n = 37 or 41 (g), and n = 33 or 36 (i). Data are representative of 2 (a,b), 8 (c), 11 (f), and 10 (h) independent experiments, or are pooled from 8 (d), 11 (e,g), and 10 (i) independent experiments. NS, not significant (P > 0.05), *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001 (two-tailed unpaired Student’s t test).
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organ analyzed (Fig. 3i). These results indicate that Nup210 deple-

tion does not cause naïve CD4+ T cells to be abnormally retained in

lymph nodes or mislocalized to nonlymphoid tissues.

Nup210 has a cell-intrinsic role in survival of naïve CD4+ T cells. The normal development and circulation of CD4+ T cells in

Nup210−/− mice suggested that the phenotype observed might have resulted from survival defects in the periphery. To investigate this

possibility, we isolated T cells from Nup210+/+ and Nup210−/− mice and labeled them with the fluorescent tracking dyes CFSE and

CTV, mixed at a 1:1 ratio, then transferred them into lymphoreplete recipient mice. In agreement with an intrinsic survival defect, the

number of naïve CD4+ T cells recovered from spleens and lymph

nodes 7 d after cotransfer was significantly lower for Nup210−/− than

wild type mice (Fig. 4a and Supplementary Fig. 4a). Moreover, the naïve CD4+ T cells isolated from the Nup210−/− mice exhibited

higher expression of the proapoptotic factor Fas, greater staining with a cell-death marker, and more rapid death, after being cultured

spleen and lymph nodes of Nup210−/− mice, CD4 expression was low

in these tissues, but there were no compensatory increases in any

organ analyzed (Fig. 3). These results indicate that Nup210 deple-
tion does not cause naïve CD4+ T cells to be abnormally retained in

lymph nodes or mislocalized to nonlymphoid tissues.

Fig. 3 | Nup210−/− mice have normal T cell development and circulation. a–d, Flow cytometric analysis of thymic populations of Nup210+/+ and Nup210−/− mice. a, Gating strategy used to subdivide double-negative (DN) populations into DN1 (CD44+CD25−), DN2 (CD44+CD25+), DN3 (CD44−CD25−), and DN4 (CD44 CD25+). b, Quantification of thymocyte populations from a. c, Gating strategy used to define single-positive CD4+ and CD8+, double-positive (DP), and DN thymocyte populations. d, Quantification of thymocyte populations from a. e–h, Flow cytometric analysis of Nup210+/+ and Nup210−/− mouse peripheral lymph nodes (pLN; axillary, brachial and inguinal). e, Total cell number. f, CD4+/CD8+ T cell ratios. g, CD4+ and CD8+ T cell numbers. h, Number of naïve/memory CD4+ T cells. i, Cd4 mRNA levels in different tissues from Nup210+/+ and Nup210−/− mice, analyzed by qPCR. Cd4 expression is normalized to that of Gapdh and Hprt. Ax. LN, axillary lymph node; Int., intestine. Data are mean ± s.e.m., with each symbol representing an individual mouse (b–h); mean ± s.d., with each symbol representing a technical replicate (i); mice per group: n = 8 (b), n = 14 or 15 (d), n = 19 or 24 (e), n = 26 or 28 (f), n = 19 or 22 (g), n = 15 or 17 (h); n = 3 technical replicates of one biological sample from each genotype, each prepared by pooling cells from n = 2 mice per group (i). Data are representative of 2 (a,i) or 4 (c) independent experiments, or are pooled from 2 (b), 4 (d,h), 5 (e,g), or 9 (f) independent experiments. NS, not significant (P > 0.05). **P ≤ 0.01, ****P < 0.0001 (two-tailed unpaired Student’s t test).
**Fig. 4** | Nup210 is required for peripheral naïve CD4+ T lymphocyte survival. 

- **a.** Flow cytometry showing the number of naïve CD4+ (CD4+CD44hi) T cells recovered from spleens (Spl), peripheral lymph nodes (pLN), and mesenteric lymph nodes (mLN) 7 d after cotransfer of a 1:1 mixture of CFSE-labeled Nup210+/+ and CTV-labeled Nup210−/− T cells into lymphopenic Nup210−/− hosts. Values are normalized to the ratio at time of transfer. 
- **b.** Flow cytometry showing Fas expression and mean fluorescence intensity (MFI) in resting naïve CD4+ (CD4+CD62LloCD44hi) splenic T cells, as determined with a viability dye. 
- **c.** Flow cytometry showing the percentage of live naïve CD4+ (CD4+CD44lo) T cells (%), Flow cytometry showing the percentage of slow-proliferating cells in each peak of division is indicated. 

**Nup210** is required for peripheral naïve CD4+ T cells. We observed that naïve CD4+ T cells to enter peripheral lymph nodes, known as homing, locally in secondary lymphoid organs. Therefore, the ability of naïve T cells to homeostatically maintain naïve T cells requires the expression of Nup210.

To further confirm that Nup210 has a cell-intrinsic function in CD4+ T cell survival, we crossed mice in which exon 2 of Nup210 was flanked by loxP sites (Nup210fl/f) with Cd4CreER T2 mice, to specifically delete Nup210 in CD4-expressing cells. We observed a decrease in the CD4+ T cell population within 3 weeks of tamoxifen-induced Nup210 ablation, in agreement with our previous data (Fig. 4i). These findings indicate that Nup210 expression is necessary for the survival and maintenance of peripheral naïve CD4+ T cells.

**Nup210 is required for efficient TCR signaling.** Naïve T cell homeostasis is largely maintained by two signals: the survival cytokine IL-7 and interaction of the TCR with self-peptides loaded on major histocompatibility complexes (MHCs), a process also known as tonic TCR signaling. These homeostatic signals are received locally in secondary lymphoid organs. Therefore, the ability of naïve T cells to enter peripheral lymph nodes, known as homing, is essential for their survival. We observed that naïve CD4+ T cells from Nup210−/− mice, compared with wild type, showed a slight...
Fig. 5 | Nup210+/− naive CD4+ T cells show features of altered TCR signaling. **a**, Flow cytometry showing the number of naïve CD4+ (CD46CD44lo) T cells recovered from peripheral lymph nodes (pLN) and mesenteric lymph nodes (mLN) 18 h after cotransfer of a 1:1 mixture of CFSE-labeled Nup210+/− and CTV-labeled Nup210−/− T cells into lymphoreplete Nup210−/− hosts. Values were normalized to the ratio at the time of transfer. **b**, Flow cytometric analysis of STAT5 (pY694) expression in unstimulated (dashed lines) or IL-7-treated (solid lines) CD4+CD44lo T cells. **c**, Mean fluorescence intensity (MFI) values are shown as a percentage of Nup210+/− T cells recovered from peripheral lymph nodes (pLN) and mesenteric lymph nodes (mLN) 18 h after cotransfer of a 1:1 mixture of CFSE-labeled and CTV-labeled Nup210+/− T cells into lymphoreplete Nup210−/− hosts. **d**, Scatter plot depicting the differentially expressed genes in Nup210+/− cells. Genes are organized by log2 fold change (fold change) and −log (P value). Genes below the cutoff (dashed line, q value of 0.05) are shown in gray. Downregulated genes are shown in blue, and upregulated genes are shown in red. **e**, Heat map illustrating the log2 (fold change) values of upregulated and downregulated genes, respectively. **f**, Hierarchical clustering of differentially expressed genes (q value <0.05). Each sample (#1-3) represents a pool of 4 mice. AP-1 pathway members are depicted. Red and blue colors show upregulated and downregulated genes, respectively. **g**, Heat map illustrating the log2 (fold change) values of selected genes in Nup210+/− relative to Nup210−/− mice. **h**, qPCR analysis of Junb and Fosb expression in naïve CD4+ (CD46CD46CD44CD44CD25+) T cells normalized to Hprt. **i**, Flow cytometric analysis of JunB expression (I) and MFI (J) in unstimulated CD46CD46CD44CD44CD25 Fopx3− T cells. **j**, Heat map illustrating the log2 (fold change) values of selected genes in Nup210+/− relative to Nup210−/− mice. Data are mean±s.e.m. (a-j); or mean±s.d. (c); each symbol represents an individual mouse (a-j), a technical replicate (c), or a biological sample (h); n = 3 or 4 host mice (a); n = 3 technical replicates of one biological sample from each genotype, each prepared by pooling cells from n = 2 or 3 mice per group (c); n = 3 biological samples from each genotype, each prepared by pooling cells from n = 4 mice per group (h); n = 4 or 5 mice per group (j). Data are representative of 2 (a-c) or 3 (d-g) independent experiments, or are pooled from 3 (h,i) independent experiments. In **d-g**, false discovery rate-adjusted P values were calculated with Benjamini–Hochberg correction for multiple testing with an allowed false discovery rate of 0.05. NS, not significant (P > 0.05), *P ≤ 0.05, **P ≤ 0.01 (two-tailed unpaired Student’s t test).
Fig. 6 Nup210 is required for TCR signaling. **a.** Immunoblot analysis of p-Lck (Y394) and total Lck protein levels in Nup210+/+ and Nup210−/− naïve CD4+ T cells. Hsp90 was used as a loading control. Activation with sodium peroxycyanate was used as a positive control. Secondary Ab, fluorescently labeled secondary-antibody-stained sample. MFI is shown relative to that of Nup210+/+ mice. **b, d.** Flow cytometric analysis of p-PLC-γ1 (Y783) expression (d) and MFI (e) in resting naïve CD4+ (CD4+CD62L+CD44+CD25−) T cells from Nup210+/+ and Nup210−/− mice. Activation with sodium peroxycyanate was used as a positive control. Secondary Ab, fluorescently labeled secondary-antibody-stained sample. MFI is shown relative to that of Nup210+/+ mice. **c, f.** Flow cytometric analysis of Nur77 expression in Nup210−/− or Nup210−/− CD4+CD44+ T cells stimulated for 16 h with soluble (sol.) anti-CD3 mAb. Values are displayed relative to sol. anti-CD3-stimulated Nup210+/+ cells. **g.** Flow cytometric analysis of Nur77 expression in unstimulated (left) or TCR-stimulated (right) Nup210+/+ and Nup210−/− CD4+CD44+ T cells. TCR stimulation was performed with plate-bound anti-CD3 and anti-CD28 mAbs, and soluble IL-2 for 14 h. FMO, fluorescence minus one. **h.** Flow cytometric analysis of Nur77 levels in CD4+ T cells stimulated as in g or without IL-2 supplementation. Values shown are relative to those of control-treated cells. **i, l.** Flow cytometric analysis of CFSE and CTV dilutions 48 h after stimulation of CFSE-labeled Nup210+/+ and CTV-labeled Nup210−/− CD4+CD44+ T cells with plate-bound anti-CD3/anti-CD28 mAbs and soluble IL-2. The percentage of cells that underwent proliferation is indicated. **j.** Quantification of the percentage of cells from each genotype, each prepared by pooling cells from 4 mice (f); n = 3 technical replicates of 1 biological sample from each genotype, each prepared by pooling cells from 5 or 10 mice per group (j). Data are representative of 2 (a, f, i) or 3 (b, d, g, h) independent experiments, or are pooled from 3 (c, e) independent experiments. NS, not significant (P > 0.05), *P ≤ 0.05, **P ≤ 0.001, ***P ≤ 0.0001 (two-tailed unpaired Student’s t test).

downregulation in the expression of CD62L (Supplementary Fig. 5a), a homing receptor required for this process17. To determine whether Nup210−/− naïve CD4+ T cells were able to migrate into peripheral lymph nodes, we adoptively transferred a mixture of CFSE-labeled Nup210+/+ and CTV-labeled Nup210−/− T cells into wild-type recipient mice. Analysis of peripheral and mesenteric lymph nodes 18 h after transfer showed equal recovery of Nup210+/+ and Nup210−/− cells (Fig. 5a and Supplementary Fig. 5b), thus indicating that naïve CD4+ T cells lacking Nup210 can efficiently home and further confirming their ability to circulate normally.

These findings suggest that the survival defects of Nup210−/− naïve CD4+ T cells might result from alterations in sensing survival
that interactions result in low levels of TCR signaling (tonic signaling), incompatibility complexes displayed on antigen-presenting cells. These between the survival cytokine IL-7, which binds the IL-7 receptor and subsequently triggers phosphorylation of the transcription factor STAT5 and promotes cell survival. To determine whether Nup210 depletion affects IL-7 signaling, we cultured Nup210–/– J14 SLP-76-EYFP cells expressing control (Ctr; empty vector) or Cav2 after stimulation with soluble anti-CD3. Hsp90 was used as a loading control. Data are representative of 3 independent experiments (\(n \geq 200\) cells per group (e.g.): representative of 2 independent experiments (h). NS, not significant (P > 0.05), **P ≤ 0.001, ****P ≤ 0.0001 (two-tailed unpaired Student’s t test). Scale bars, 5 μm.

The second survival signal received in lymph nodes results from TCR engagement with self-peptides loaded on major histocompatibility complexes displayed on antigen-presenting cells. These interactions result in low levels of TCR signaling (tonic signaling), which are essential for survival of naïve T cells. TCR stimulation initiates a signaling cascade that results in activation of the transcription factor NFAT and upregulation of the AP-1 family members Jun and Fos. Therefore, expression of AP-1 factors has long been used as a readout for TCR signaling. Our whole-transcriptome sequencing (RNA-seq) analysis of unstimulated naïve CD4+ T cells (>99% purity; Supplementary Fig. 5c) showed significantly lower levels of members of the AP-1 family in Nup210–/– than in wild type, and this pathway was the most significantly altered in the dataset (Fig. 5d–g and Supplementary Table 1). Our analysis also showed significant alterations in the NFAT pathway (data not shown) and elevated levels of the apoptotic markers Fas and PUMA in Nup210–/– naïve CD4+ T cells (Fig. 5g), results consistent with their low survival. The low levels of the AP-1 factors Junb and Fos in unstimulated Nup210–/– naïve CD4+ T lymphocytes were confirmed through real-time PCR and flow cytometric analysis (Fig. 5h–j). These findings suggest that Nup210–/– naïve CD4+ T cells have lower levels of basal TCR signaling. To confirm this possibility, we isolated unstimulated Nup210+/+ and Nup210–/– naïve CD4+ T cells and determined the phosphorylation levels of downstream signals in secondary lymphoid organs. Within lymph nodes, T cells encounter the survival cytokine IL-7, which binds the IL-7 receptor and subsequently triggers phosphorylation of the transcription factor STAT5 and promotes cell survival. To determine whether Nup210 depletion affects IL-7 signaling, we cultured Nup210+/+ and Nup210–/– naïve CD4+ T cells in varying concentrations of IL-7, then measured STAT5 phosphorylation. We observed no difference in the dose-dependent increase in phospho- (p-) STAT5 levels between Nup210+/+ and Nup210–/– cells (Fig. 5b,c), thus indicating that Nup210–/– naïve CD4+ T cells can sense IL-7 efficiently.
Articles confirm that Nup210-deficient cells have defective basal TCR signaling, we analyzed the levels of both Lck modifications. All Tyr394 residue alone or in combination with the inhibitory Tyr505 residue26, we studied Lck catalytic activity when it is phosphorylated at the activating Tyr394 residue, as reported, the addition of IL-2 did not alter Nur77 induction29,30. In Nup210−/− relative to wild type, we also detected a lower frequency of naïve CD4+ T cells that upregulated the early activation marker CD69 (Supplementary Fig. 6c,d) and less cell division in response to TCR engagement (Fig. 6i,j). Altogether, these findings indicate that the TCR-signaling defect caused by Nup210 deficiency cannot be rescued by optimal activation conditions and demonstrate that Nup210 is critical for proper TCR signaling during CD4+ T cell activation and proliferation.

Nup210 regulates early TCR signaling by promoting Cav2 expression. To dissect the molecular mechanism of Nup210 regulation of TCR signaling and survival, we used CRISPR–Cas9 to knock out Nup210 in human Jurkat J14 T cells expressing the TCR adaptor protein SLP-76 tagged with EYFP31. Single-guide-RNA oligonucleotides targeting exon 2 resulted in full depletion of Nup210 in these cells (Fig. 7a,b). Analogously to the primary Nup210−/− naïve CD4+ T cells, Nup210-depleted J14 cells, compared with wild type, showed lower levels of cFos in unstimulated conditions (Fig. 7b) and less activation in response to CD3-induced TCR stimulation (Fig. 7b,c and Supplementary Fig. 7a). Because TCR ligation leads to rapid formation of SLP-76-containing TCR microclusters at the plasma membrane21,22, clustering of SLP-76/EYFP in these cells has been used to control cells, Nup210−/− cells did not induce Nur77 after TCR ligation, regardless of IL-2 stimulation (Fig. 6g,h). As previously reported, the addition of IL-2 did not alter Nur77 induction29,30. In Nup210−/− relative to wild type, we also detected a lower frequency of naïve CD4+ T cells that upregulated the early activation marker CD69 (Supplementary Fig. 6c,d) and less cell division in response to TCR engagement (Fig. 6i,j). Altogether, these findings indicate that the TCR-signaling defect caused by Nup210 deficiency cannot be rescued by optimal activation conditions and demonstrate that Nup210 is critical for proper TCR signaling during CD4+ T cell activation and proliferation.

TNC TCR effectors, including Lck, Zap70, and PLC-γ1 (ref. 23). Because Lck is catalytically active when it is phosphorylated at the activating Tyr394 residue alone or in combination with the inhibitory Tyr505 residue26, we studied the levels of both Lck modifications. All these factors showed lower phosphorylation levels in naïve CD4+ T cells from Nup210−/− mice, compared with wild type, at steady state (Fig. 6a–e and Supplementary Fig. 6a,b), thus indicating that Nup210 depletion disrupts TCR-signaling events. To further confirm that Nup210-deficient cells have defective basal TCR signaling, we stimulated naïve CD4+ T cells with soluble anti-CD3 monoclonal antibody (mAb), which has previously been used to mimic in vivo tonic signaling27. We found that induction of Nur77, encoded by an early TCR-responsive gene28, was strongly impaired in Nup210−/− CD4+ T cells (Fig. 6f). Altogether, these observations indicate that Nup210 is required to sustain basal/tonic TCR signaling of naïve CD4+ T cells to promote survival.

Nup210 is required for CD4+ T lymphocyte activation. The discovery that Nup210 is required for proper tonic TCR signaling in naïve CD4+ T cells prompted us to ask whether this nucleoporin might also be important for TCR-dependent activation. To investigate this possibility, we cultured naïve populations of Nup210+/+ and Nup210−/− CD4+ T cells in the presence of immobilized anti-CD3 mAb plus costimulatory anti-CD28 mAb, in the presence or absence of exogenous IL-2, and measured Nur77 protein levels. In contrast to control cells, Nup210−/− cells did not induce Nur77 after TCR ligation, regardless of IL-2 stimulation (Fig. 6g,h). As previously reported, the addition of IL-2 did not alter Nur77 induction29,30. In Nup210−/− relative to wild type, we also detected a lower frequency of naïve CD4+ T cells that upregulated the early activation marker CD69 (Supplementary Fig. 6c,d) and less cell division in response to TCR engagement (Fig. 6i,j). Altogether, these findings indicate that the TCR-signaling defect caused by Nup210 deficiency cannot be rescued by optimal activation conditions and demonstrate that Nup210 is critical for proper TCR signaling during CD4+ T cell activation and proliferation.

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Fig. 8 | Cav2 and Jun genes localize to the nuclear periphery, and Nup210 is required for repression of the proapoptotic receptor Fas. DNA-FISH analysis of Cav2 and Nup62 in Nup210+/+ naïve CD4+ T cells. Gene loci are shown in red, and the nuclear-periphery marker Lamin B1 is in green. a, Quantification of the percentage of nuclei from a showing one or both loci associated with the nuclear periphery from 3D nuclear reconstructions (additional data in Supplementary Fig. 8a). b, qPCR analysis of Cav1, Cav2, and Irf4 mRNA levels in Nup210+/+ naïve CD4+ T cells unstimulated or activated with soluble anti-CD3. Expression is normalized to that of Hprt. c, DNA FISH of Jun in Nup210+/+ naïve CD4+ T cells. Red, gene loci; green, Lamin B1. d, ChIP analysis of STAT3 binding to the Fas promoter in unstimulated NUP210+/+ or NUP210−/− J14 SLP-76-EYFP cells. Binding of STAT3 was quantified by qPCR. e, Flow cytometric viability analysis of Nup210−/− or Nup210+/+ naïve CD4+ T cells cocultured with splenocytes from TCRγ−/− TCRδ−/− mice and soluble Fas ligand (FasL). Viability was determined through annexin V and propidium iodide staining. Data are representative of 3 independent experiments (a,d); mean ± s.d. pooled from three independent experiments, n ≥ 200 cells quantified from n = 4, 5 or 8 individual fields, with symbols showing individual field quantification (b); mean ± s.e.m., n = 5 mice per time point, representative of one experiment (c); mean ± s.d., with each symbol representing a technical replicate, n = 3, representative of two independent experiments (e); mean ± s.e.m., with each symbol representing an individual mouse, n = 9 mice per group, data pooled from two independent experiments (f). *P ≤ 0.05, ****P ≤ 0.0001 (two-tailed unpaired Student’s t test). Scale bars, 5 μm.
as a reporter for proximal TCR activation. Although it is unknown whether clustering occurs during tonic TCR signaling in vivo, we found that stimulation with soluble anti-CD3, which mimics tonic TCR signaling in vitro, led to SLP-76 clustering in J14 T cells (Fig. 7d). Nup210 depletion in these cells completely abolished SLP-76-cluster formation in response to TCR activation (Fig. 7d,e), thus confirming that Nup210 is critical for early TCR signaling and indicating a conserved role of Nup210 in TCR activation in humans.

The formation of clusters and signaling assemblies induced by TCR ligation is mediated by actin cytoskeletal rearrangements, a process requiring the lipid-raft protein Caveolin-1 (Cav1) and Cav3. Whereas Cav3 is selectively expressed in muscle tissues, Cav1 and Cav2 are more ubiquitous and can form heterooligomeric complexes. Immunofluorescence analysis of Cav1 and Cav2 expression in J14 SLP-76-EYFP cells showed that both proteins were expressed at low levels, but only Cav2 was upregulated in response to TCR stimulation with soluble anti-CD3 (Fig. 7f). Cav2 upregulation was not observed in Nup210-depleted cells, thus indicating a critical role of Nup210 in Cav2 induction (Fig. 7f). Because Cav1 was found to be required for TCR signaling in CD8 T-cell subsets but not CD4 T cells, and J14 cells express the CD4 but not the CD8 coreceptor, these findings suggest the possibility that Cav2 may be required for TCR signaling specifically in CD4 T cells. Such a requirement would explain the CD4 T-cell–restricted phenotype of Nup210+ mice. To confirm that Nup210 modulates proximal TCR signaling through Cav2, we performed rescue experiments. We determined the ability of wild-type and Nup210-depleted J14 T cells transduced with control or Cav2-expressing lentiviruses to initiate TCR signaling by analyzing SLP-76 clustering and Lck phosphorylation. Cav2 overexpression did not induce spontaneous activation or increase the activation of wild-type cells, but was sufficient to partially reestablish SLP-76 clustering after TCR activation in Nup210-depleted cells (Fig. 7g and Supplementary Fig. 7b). Ectopic Cav2 expression also restored Lck phosphorylation at the activating Tyr394 residue and promoted dephosphorylation of the inhibitory Tyr505 (Fig. 7b). These results confirm that Nup210 mediates proximal TCR signaling by promoting Cav2 expression. Interestingly, Cav2 was not able to rescue the TCR-induced increase in the expression of downstream genes (Supplementary Fig. 7c), thus indicating that Nup210 regulates TCR-induced gene expression through another mechanism.

The Cav2 gene localizes to the nuclear periphery. Our previous work has indicated that Nup210 regulates muscle physiology by modulating gene expression at the nuclear periphery. To determine the intranuclear localization of the Cav2 gene, we performed fluorescence in situ hybridization (FISH) in primary naïve CD4 T cells. We found that the Cav2 gene but not the Nup62 gene, whose expression is not regulated by Nup210, localized to the nuclear periphery (Fig. 8a,b and Supplementary Fig. 8a). Recent work has suggested that NPCs can act as scaffolds for regulation of inducible poised genes. Interestingly, analysis of histone modifications from published data showed that the Cav2 promoter had active (K4-trimethylated histone H3) and repressive (K27-trimethylated histone H3) marks (Supplementary Fig. 8b). The presence of bivalent histone marks in a promoter is believed to maintain genes in a silent or low-expression state while keeping them poised for rapid activation or stable silencing. Notably, the Cav1 gene, which is positioned next to the Cav2 gene, showed only the repressive K27-trimethylated histone H3 mark (Supplementary Fig. 8b), thus indicating that localization at the nuclear periphery is not sufficient to maintain a poised state. To determine whether the Cav2 gene at NPCs might be poised for rapid activation in response to TCR stimulation, we determined its kinetics of expression by analyzing the mRNA levels of Cav1 and Cav2 in primary naïve CD4 T cells activated ex vivo. Whereas no changes in Cav1 expression were observed under these conditions, Cav2 expression was rapidly upregulated, with kinetics similar to that of the early TCR-responsive gene Irf4 (Fig. 8c). Upregulation of Cav2 was not observed in CD8 T lymphocytes (Supplementary Fig. 8c), thus further supporting the idea that Cav2 function might be restricted to CD4 T cells. Our results are consistent with earlier observations that the Cav1 and Cav2 genes are independently regulated at the transcriptional level and, additionally indicate that in CD4 T cells, the Cav2 gene is set to be rapidly activated at the nuclear periphery in response to TCR stimulation in a Nup210-dependent manner.

Nup210 is required for repression of the proapoptotic receptor Fas. Our RNA-seq analysis indicated that AP-1 was the most significantly altered pathway in Nup210-deficient naïve CD4 T cells (Fig. 5e–j). FISH analyses in naïve CD4 T lymphocytes showed that the Jun gene also localizes to NPCs (Fig. 8b,d), thus suggesting that Nup210 might regulate its activity at the nuclear periphery. Previous studies have found that c-Jun works together with STAT3 in repressing the expression of Fas, encoding a cell-death receptor. Because the association of STAT3 with the Fas promoter depends on c-Jun, and this AP-1 factor is strongly downregulated in Nup210-deficient cells, our findings suggest that in the absence of Nup210, recruitment of STAT3 to the Fas promoter might be impaired, thus resulting in derepression of this gene. To test this possibility, we analyzed the levels of STAT3 at the Fas gene promoter in wild-type and Nup210-depleted J14 cells by using chromatin immunoprecipitation (ChIP). We found that NUP210−/− cells, compared with wild-type cells, exhibited significantly lower recruitment of STAT3 to the Fas promoter (Fig. 8e), even though the levels and localization of STAT3 were not affected (Supplementary Fig. 8d,e). These findings explain the higher levels of Fas in naïve CD4 T lymphocytes of Nup210−/− mice than in wild type (Fig. 4b,c and Fig. 5f,g). To test whether elevated Fas levels render these cells more susceptible to cell death, we incubated Nup210−/− and Nup210+/− naïve CD4 T cells with Fas ligand and measured cell survival. We found that Nup210−/− cells showed higher cell death than did wild-type cells (Fig. 8f). The same result was observed for wild-type and Nup210-depleted J14 cells (Supplementary Fig. 8f). Altogether, our findings indicate that the death of peripheral naïve CD4 T cells in Nup210−/− mice results from a combination of their inability to sense survival tonic TCR signals and their higher sensitivity to Fas-mediated cell death (Supplementary Fig. 8g).

Discussion

Accumulating evidence indicates the existence of specialized NPCs; however, the physiological functions of these structures remain largely unknown. Here, we discovered that deletion of the cell-type-specific nucleoporin Nup210 in mice results in a dramatic decrease in circulating naïve CD4 T cells. Although the development and migration of these cells occur normally, the survival of peripheral naïve CD4 T lymphocytes in Nup210−/− animals is compromised. The increased death of CD4 T cells results from an impairment in tonic TCR signaling, which prevents proper transmission of the survival signals provided by the TCR–self peptide/MHC interactions, and from elevated levels of Fas, which sensitizes naïve CD4 T lymphocytes to cell death. We found that Nup210 regulates these processes by modulating the expression of Cav2 and Jun genes present at NPCs. Whereas TCR-stimulation-dependent upregulation of Cav2 is critical for proximal TCR signaling, c-Jun expression is required for STAT3-dependent repression of the Fas receptor. Our results uncover an unexpected role of Nup210 as a cell-intrinsic regulator of TCR signaling and T cell homeostasis, and expose NPCs as key players in the adaptive immune system.

Connections between NPCs and the immune system have recently emerged. Mouse heterozygous for the nucleoporins...
Nup96 and Sec13 show low levels of MHC class I and class II proteins in antigen-presenting cells\(^1\). Low MHC expression in Nup96\(^{-}\)Tc-r mice indirectly affects T cell expansion and the response to vesicular stomatitis virus infections\(^1\). We observed that naïve CD4\(^{+}\) T cells from Nup210-deficient mice, compared with wild type, showed lower survival and activation as a result of their inability to properly transmit TCR signals, thus indicating that NPCs also regulate T lymphocyte activity in a cell-intrinsic manner. Interestingly, the SLP-76 adaptor protein has recently been found to interact with the NPC-associated protein RanGAP1 during T cell activation. This association is required for the nuclear accumulation of the transcription factors NFAT and NF-κB\(^2\). These findings suggest that TCR activation can modulate the transport function of NPCs. Reciprocally, our findings indicate that NPCs modulate proximal and distal TCR signaling. We thus propose the existence of a positive regulatory loop between NPCs and TCRs that ensures proper transmission of tonic and activation TCR signals.

Several reports have shown a critical role of Cav1 in the initiation and downstream TCR-signaling cascade\(^3\). Cav1 has previously been shown to be important for TCR signaling in CD8\(^{+}\) but not CD4\(^{+}\) T cells\(^4\). Here we report that TCR activation in naïve CD4\(^{+}\) T cells but not CD8\(^{+}\) cells results in specific upregulation of Cav2. This selective TCR-activation-induced increase in Cav2 expression is required for proximal TCR signaling and is dependent on the expression of Nup210. These findings raise the possibility that Cav1 and Cav2 may have specialized roles selectively regulating TCR signaling in different T lymphocyte subsets. In this context, the CD4\(^{+}\)-specific upregulation of Cav2 and its dependence on Nup210 may explain why depletion of this nucleoporin affects only CD4\(^{+}\) T cell survival.

In muscle, Nup210 regulates genes that localize to NPCs by recruiting the transcription factor MEF2C\(^5\). The finding that the Cav2 and jun genes localize to the nuclear periphery indicates that the role of Nup210 in regulating NPC-associated genes is conserved in T cells. The observation that the nuclear-periphery-associated Cav2 gene is rapidly induced in response to TCR activation supports the idea that nuclear pores act as scaffolds for the regulation of inducible/poised genes\(^6,7\) and suggests that NPCs also act as a hub for the regulation of TCR-signaling-related genes. Which transcription factors regulate the activity of TCR-signaling genes at the nuclear periphery are currently unknown, but NFAF1, a central transcription factor in TCR signaling, has recently been found to interact with Nup210 in T cells\(^8\). How Nup210, with its small NPC/nuclear-facing domain\(^9\), regulates gene expression is unclear. Most probably, and similarly to its yeast counterpart Pom152, Nup210 interacts with other nuclear-envelope components\(^10\) and other soluble factors, thereby forming a transcriptional-regulatory complex at the nuclear periphery. It is also important to consider that Nup210 might function outside NPCs. Lapetina et al. have recently described novel non-NPC structures at the nuclear envelope of Saccharomyces cerevisiae that contain several nucleoporins and are involved in chromatin regulation\(^11\).

Methods

Methods, including statements of data availability and any associated accession codes and references, are available at https://doi.org/10.1038/s41590-018-0103-5.

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Author contributions
J.B. designed the experimental approach, performed experiments, analyzed data, and cowrote the manuscript; S.S. performed experiments, analyzed data, and provided critical input; E.C. and R.T. assisted with adoptive transfer experiments and provided critical input; L.M.B. provided reagents and critical expertise; M.A.D. designed the experimental approach, analyzed data, provided oversight and critical expertise, and cowrote the manuscript.

Competing interests
The authors declare no competing interests.

Additional information
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Methods
Mice. Nup210floxed/"Nup210" mice were generated by nuclear injection of homologous DNA (Center for Mouse Genome Modification (CMGM); University of Connecticut Health Center). To generate Nup210+ mice, Nup210+ mice were crossed with Ifpr1floxed mice (stock no. 004302, Jackson Laboratory). A marker-assisted speed congenic breeding strategy was used to 99.9% backcross the mice to the C57BL/6J strain. To generate Nup210fl/CD4CreER2 mice, Nup210+ mice were crossed with CD4CreER2+ mice. CD4CreER2+ and TCRγ2 (B6.129P2-Tg(R2-stop(Ta1325A))/J) mice were from The Jackson Laboratory (stock no. 022356 and 002122, respectively). All animals were bred in specific-pathogen-free facilities at the Sanford Burnham Prebys Medical Discovery Institute. All experiments were approved by the Institutional Animal Care and Use Committee of the Sanford Burnham Prebys Medical Discovery Institute and were performed in accordance with institutional guidelines and regulations. Male and female mice were used at 6–10 weeks of age.

In vivo tamoxifen treatment. Nup210fl/CD4CreER2 mice were administered 3 mg of tamoxifen (Sigma), suspended in corn oil, intraperitoneally once daily for 4 d. Cre-induced Nup210 deletion was verified by PCR genotyping and immunofluorescence.

Cell culture. Mouse naive CD4+ T cells (1.0 × 10^6/ml) were cultured in RPMI-1640 medium containing 2.05 mM l-glutamine and supplemented with 10% heat-inactivated FBS, 10 mM HEPES, 1% nonessential amino acids, 1 mM sodium pyruvate, penicillin–streptomycin, and 55 μM 2-mercaptoethanol. The T14 SL-76 hyper-IgM cell line was a gift from L. E. Samelson (Laboratory of Cellular and Molecular Biology, NCI, NIH). T14 cells were maintained in RPMI-1640 medium containing 2.05 mM l-glutamine and supplemented with 10% FBS, 2 mM Glutamax, and penicillin–streptomycin.

Viral transduction. For CRISPR-Cas9 gene editing14, T14 SL-76-EVFF cells were transduced with tetracycline-inducible Cas9 lentivirus, then selected with 1.25 μg/ml puromycin (Gibco). Cas9-expressing cells were then transfected with lentiviruses expressing scramble or Nup210 CRISPR gRNAs5 and selected with 10 μg/ml of chloramphenicol (Gibco). Cas9 expression was induced with 2 μg/ml doxycycline (Clontech) for 3 d. Doxycycline was removed from the medium, and cells were single-cell cloned. Clones depleted of Nup210 were identified through immunofluorescence. Two or more clones for each cell line were used for functional studies. For the Cav2-rescue studies, scrambled or Nup210-knockout CRISPR3 cells were transduced with lentiviruses expressing human Cav2 (NM_001323.4) or empty vector and selected with 0.5 mg/ml hygromycin (Gibco). Lentiviral vectors were produced by VectorBuilder.

Ex vivo and in vitro T cell activation. Naive CD4+ T cells were prepared from spleens and peripheral lymph nodes through negative selection. Cells were incubated with anti-CD16/32 (93; BioLegend 101302) and with biotin-labeled antibodies to B220 (RA3-6B2; BioLegend 103204), CD11b (M1/70; BioLegend 101204), CD4 (14; N18; BioLegend 117307), CD91 (MB19; 1; BioLegend 101904), CD24 (M1/69; BioLegend 101804), CD8 (53-6.7; BioLegend 100704), CD25 (MAD1; BioLegend 100140), CD3 (53-6.7; BioLegend 100721), and P-Stat5 (A85-15; BioLegend 100929), then incubated with streptavidin-APC (BioLegend 100152). Cells were cultured on 96-well plates at 1.0 × 10^6/ml in T cell culture medium either with soluble or lentiviruses expressing scramble or Nup210 CRISPR gRNAs5 and selected with 10 μg/ml of chloramphenicol (Gibco). Cas9 expression was induced with 2 μg/ml doxycycline (Clontech) for 3 d. Doxycycline was removed from the medium; and cells were single-cell cloned. Clones depleted of Nup210 were identified through immunofluorescence. Two or more clones for each cell line were used for functional studies. For the Cav2-rescue studies, scrambled or Nup210-knockout CRISPR3 cells were transduced with lentiviruses expressing human Cav2 (NM_001323.4) or empty vector and selected with 0.5 mg/ml hygromycin (Gibco). Lentiviral vectors were produced by VectorBuilder.

Fluorescence-activated cell sorting (FACS). Single-cell suspensions were prepared from spleens and peripheral lymph nodes or bone marrow, then stained for flow cytometry as indicated in the ‘Flow cytometry’ section. Before sorting, cells were resuspended in FACS buffer (PBS containing 1% FBS). Live CD4+ and CD8+ T cells were gated by side scatter and forward scatter. Flow cytometric analysis of the flow cytometry data was performed using FlowJo software (Tree Star).

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Purity was verified after each sort and was found to be >99%. RiboLock RNase Inhibitor (10 U per sample; Thermo Fisher Scientific) was used for staining neutrophils (CD45^+Ly-6G^-CD11b^-F4/80^+), macrophages (CD45^+F4/80^+CD11b^+), monocytes (CD45^+CD11b^-Ly-6G^-Ly6C^-), eosinophils (CD45^-Siglec-F^-), CD4^+ T cells (CD45^+CD3^-CD4^+), CD8^+ T cells (CD45^+CD3^-CD8^-), and B cells (CD45^+B220^-CD3^-), which were sorted at 4°C on a BD FACS Aria II Cell Sorter (BD Biosciences) with an 85-μm needle into lysis buffer (PureLink RNA Micro Kit, Thermo Fisher Scientific). Bone marrow cells were flushed from the tibia and femur with PBS containing 1.2% FBS and 5 mM EDTA.

**RNA sequencing.** A total of three biological replicates of FACS-sorted cells were used for each genotype, each consisting of cells pooled from four mice. RNA extraction was performed with an RNeasy Plus Micro Kit (Qiagen), and RNA integrity was confirmed with an Agilent 2100 Bioanalyzer (Agilent Technologies). Poly(A)^+ RNA was purified with a NEBiNExt Poly(A) mRNA Magnetic Isolation Module, and barcoded libraries were made with a NEBiNExt Ultra Directional RNA Library Prep Kit for Illumina (NEB). Libraries were pooled and single-end sequenced (1 x 75) on the Illumina NextSeq 500 platform with a High output V2 kit (Illumina). Read data was processed in BaseSpace (http://www.basespace.illumina.com). Reads were aligned to the Mus musculus genome (mm10) in the BaseSpace RNA-Seq Alignment app v1.1 and TopHat2 aligner (http://tophat.cbcb.umd.edu/) with default settings. Differential transcript expression was determined with the Cufflinks Cuffdiff package (http://cufflinks.cbcb.umd.edu/). Pathway analysis was performed with MetaCore software (Thomson Reuters) and Ingenuity Pathway analysis. Hierarchical clustering was performed by differentially expressed genes (q value <0.05) in three Nup210^-/+ and three Nup210^-/- naive CD4^+ T cell samples was performed on standardized data (gene expression rescaled for each sample to mean value of 0 and s.d. of 1) in Partek Genomic Suite 6.6. Clustering was performed with an average linkage algorithm and Euclidian distance as a dissimilarity measure.

**qPCR.** Total RNA extraction was performed from primary cells or homogenized tissues (Qagen TissueLyser LT, five cycles of 1 min at 50 Hz) with a PureLink RNAqPCR. Was performed with an average linkage algorithm and Euclidian distance as a sample to mean value of 0 and s.d. of 1) in Partek Genomic Suite 6.6. Clustering was performed with an average linkage algorithm and Euclidian distance as a dissimilarity measure.

**DNA fluorescence in situ hybridization.** Immunomagnetically isolated cells were spotted onto poly-l-lysine–coated coverslips and left to settle for 15–30 min at RT. Cells were then processed for immune–DNA FISH as described previously, with the following modifications. Digoxygenin (DIG)-labeled probes were prepared by labeling 1 μg of BAC DNA with DIG-Nick Translation Mix (Sigma-Aldrich) and cells were subjected to IF analysis with an anti-DIG antibody (Sigma-Aldrich 1133089001). The following BAC probes from BACPAC resources, CHORI were used: Cav2 BAC (RP23-48418), cjun BAC (RP24-282818), and Nup62 BAC (RP23-40332D).

**Electron microscopy.** FACS-purified naive CD4^+ T cells were fixed in 2.5% glutaraldehyde with 2% paraformaldehyde in 0.15 M cacodylate buffer containing 2 mM calcium chloride, pH 7.4 for 1 h at 4°C. Cells were then embedded in 2% low-melting-point agarose (Sigma). The pellet was fixed in 1% osmium tetroxide/1.5% potassium ferrocyanide in buffer at 4°C in the dark, then stained en bloc with 2% uranyl acetate at 4°C in the dark, and then subjected to a graded dehydration series (50%, 70%, 90%, 100%, 100%). Samples were then rapidly infiltrated in Spur's resin with a Ted Pella PELCO BioWave microwave processing unit, embedded in flat-bottom tubes, and cured at 60°C overnight. 70-nm ultrathin sections were then cut on a Leica UC7 ultramicrotome, and cells were examined on a Zeiss Libra 120 KVP PLUS Energy Filtered Transmission Electron Microscope at nominal magnifications of 8,000× and 16,000×.

**Immunoblotting.** Immunoblotting was performed as described previously, with the following modifications. Protein extracts were obtained with RIPA lysis buffer (Sigma) or Tris-NP40 buffer (50 mM Tris-HCl, 150 mM NaCl, 0.5% NP-40, 1 mM EDTA, 1 mM MgCl2, 1 mM DTT) (J11 cells) containing protease and phosphatase inhibitors (Pierce Halt Protease and Phosphatase Inhibitor Cocktail, Thermo Fisher Scientific) and 1 mM PMSF (Sigma). Tissues were lysed with a Qagen TissueLyser LT (five cycles of 30 s at 50 Hz). Protein concentration was determined with a Pierce BCA reagent (Thermo Fisher Scientific). LDS Sample Buffer premixed with NuPAGE Sample Reducing Agent (Life Technologies) was added, and samples were incubated for 10 min at 70°C. For western blot analysis, 100 μg of protein was resolved by SDS–PAGE on NuPage Novex 3–8% Tris-aceate or Bolt 4–12% Bis-Tris Plus protein gels (Life Technologies), then blotted to nitrocellulose membranes with an iBlot2 system. The following antibodies were used: anti-Cd3e (CD3-12, Cell Signaling Technology 4443); anti-Nup210 (Bethyl Laboratories PA5-27623); anti-Nup107 (kind gift from M. Hetzer, Salk Institute); anti-Nup210 (Bethyl Laboratories A301-795A); anti-Nup98 (39A3; Cell Signaling Technology 2598P); anti-Nup98 (F2; Santa Cruz Biotechnology sc-374400); anti-Lamin A (Sigma Aldrich L1293); and anti-Lamin B1 (Abcam ab6684); anti-Cav1 (Cell Signaling Technology 3238), and anti-Cav2 (65; BD Transduction Laboratories). Nup62 was detected with a mouse anti-human Nup62 BAC (RP23-40332D).

**Fluorescence recovery after photobleaching.** Primary hepatocytes were isolated as described previously. Cells were transfected with a plasmid expressing NES-Tomato-NLS with Lipofectamine 3000 (Life Technologies). 48 h after transfection, cells were imaged with a Leica SP8 confocal microscope. Nuclear Tomato signal was photobleached with maximum laser power for 3 s. Recovery was recorded for 10 min, and transport rates were analyzed in LAS X software.

**DNA sequencing.** Immunomagnetically isolated cells were sorted on poly-l-lysine–coated coverslips and left to settle for 15–30 min at RT. Cells were then processed for immune–DNA FISH as described previously, with the following modifications. Digoxygenin (DIG)-labeled probes were prepared by labeling 1 μg of BAC DNA with DIG-Nick Translation Mix (Sigma-Aldrich) and cells were subjected to IF analysis with an anti-DIG antibody (Sigma-Aldrich 1133089001). The following BAC probes from BACPAC resources, CHORI were used: Cav2 BAC (RP23-48418), cjun BAC (RP24-282818), and Nup62 BAC (RP23-40332D).

**Electron microscopy.** FACS-purified naive CD4^+ T cells were fixed in 2.5% glutaraldehyde with 2% paraformaldehyde in 0.15 M cacodylate buffer containing 2 mM calcium chloride, pH 7.4 for 1 h at 4°C. Cells were then embedded in 2% low-melting-point agarose (Sigma). The pellet was fixed in 1% osmium tetroxide/1.5% potassium ferrocyanide in buffer at 4°C in the dark, then stained en bloc with 2% uranyl acetate at 4°C in the dark, and then subjected to a graded dehydration series (50%, 70%, 90%, 100%, 100%). Samples were then rapidly infiltrated in Spur's resin with a Ted Pella PELCO BioWave microwave processing unit, embedded in flat-bottom tubes, and cured at 60°C overnight. 70-nm ultrathin sections were then cut on a Leica UC7 ultramicrotome, and cells were examined on a Zeiss Libra 120 KVP PLUS Energy Filtered Transmission Electron Microscope at nominal magnifications of 8,000× and 16,000×.
5′-GGGAGGCTCCATTGATTCAG-3′ designed to amplify a 189-bp fragment of the Fas core promoter, as previously described41.

Data analysis. GraphPad Prism software v7.0a (GraphPad Software) was used to prepare graphs and to perform statistical analysis. The scatter plot depicting the differentially expressed genes in Nup210−/− naïve CD4+ T cells was prepared in R. ChIP-seq data for H3K4me3 and H3K27me3 in mouse naïve CD4+ T cells was accessed with the Cistrome Data Browser55 and visualized with the UCSC Genome Browser56.

Statistical analysis. Two-tailed unpaired Student's t test was used to compare outcomes (GraphPad Prism), and the resulting P values are indicated. For the RNA-seq samples, false discovery rate–adjusted P values were calculated with the Benjamini–Hochberg correction for multiple testing with an allowed false discovery rate of 0.05 (Cufflinks).

Reporting Summary. Further information on experimental design is available in the Nature Research Reporting Summary linked to this article.

Data availability. The RNA-seq datasets generated during the current study are available in the NCBI biorepository under accession number PRJNA438345. The remaining data that support the findings of this study are available from the corresponding author upon reasonable request.

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Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

- **n/a** Confirmed
- **X** The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- **X** An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- **X** The statistical test(s) used AND whether they are one- or two-sided
  - Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- **X** A description of all covariates tested
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- **X** A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- **X** For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
  - Give P values as exact values whenever suitable.
- **X** For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- **X** For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- **X** Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated
- **X** Clearly defined error bars
  - State explicitly what error bars represent (e.g. SD, SE, CI)

Software and code

Policy information about availability of computer code

**Data collection**

Flow cytometry data were acquired on a BD LSRFortessa (BD Biosciences), on a BD LSRFortessa X-20 (BD Biosciences), or on a ZE5 (Bio-Rad). qPCR data were collected on an ABI 7900HT Real-Time PCR System (ThermoFisher Scientific) or on a CFX384 Real-Time PCR Detection System (Bio-Rad). Confocal microscopy images were taken using a Leica SP8 confocal microscope (Leica Microsystems). Immunoelectron microscopy images were collected on a Zeiss Libra 120kV PLUS Energy Filtered Transmission Electron Microscope. RNA sequencing data were collected on a NextSeq 500 sequencer (Illumina).

**Data analysis**

Flow cytometry data were analyzed using FlowJo software v10.0.8r1 (Tree Star, Inc.). GraphPad Prism software v7.0a (GraphPad Software, Inc.) was used to prepare graphs and to perform statistical analyses. Two-tailed unpaired Student’s t test was used to compare outcomes and resulting P-values are indicated in the figures and respective legends. RNA-seq reads were aligned using the BaseSpace RNA-Seq Alignment app v1.1 app and TopHat2 aligner. Differential transcript expression was determined using the Cufflinks Cuffdiff package. False discovery rate (FDR) adjusted P-values were calculated using the Benjamini-Hochberg correction for multiple testing with an allowed false discovery rate of 0.05 (Cufflinks). The scatter plot depicting the differentially expressed genes (DEG) in Nup210-/- naïve CD4+ T cells was prepared using R. Pathway analysis was performed using MetaCore software (Thomson Reuters) and Ingenuity® Pathway Analysis (IPA®, QIAGEN). ChiP-seq data for H3K4me3 and H3K27me3 in mouse naïve CD4+ T cells were accessed using the Cistrome Data Browser and visualized using the UCSC Genome Browser. Microscopy data were analyzed using the Leica Application Suite X software v3.1.5.16308 (Leica Microsystems), ImageJ v2.0.0-rc-s4/1.51h (NIH), and Adobe Photoshop CS5.1 v12.1 x64 (Adobe).

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For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.
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All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

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- A list of figures that have associated raw data
- A description of any restrictions on data availability

The RNA-seq datasets generated during the current study are available in the NCBI biorepository at https://www.ncbi.nlm.nih.gov/bioproject/438343. The remaining data that support the findings of this study are available from the corresponding author upon request.

Field-specific reporting

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Life sciences

Study design

All studies must disclose on these points even when the disclosure is negative.

Sample size
Sample size was determined according to standard practices in the field. The sample size and associated statistics are indicated in the figures and respective legends.

Data exclusions
No data were excluded from the analyses.

Replication
All attempts at replication were successful.

Randomization
No randomization was performed in this study.

Blinding
Mouse genotypes were not known at time of data acquisition and analysis.

Materials & experimental systems

Policy information about availability of materials

n/a Involved in the study

Unique materials

Antibodies

Eukaryotic cell lines

Research animals

Human research participants

Unique materials

Obtaining unique materials

The J14 SLP-76-EYFP cell line was a kind gift of Dr. Lawrence E. Samelson (Laboratory of Cellular and Molecular Biology, National Cancer Institute, National Institutes of Health).

Antibodies

Antibodies used

Antibodies used in this work are listed below. All antibodies are from commercial sources unless otherwise stated.

- Anti-mouse CD3ε (clone 145-2C11, BioLegend catalog# 100341)
- Anti-mouse CD3ε (clone 145-2C11, BioLegend catalog# 100308)
- Anti-mouse CD3 δ (clone 17A2, BD Biosciences catalog# 555274)
- Anti-mouse CD4 (clone GK1.5, BioLegend catalog# 100424)
- Anti-mouse CD4 (clone GK1.5, BioLegend catalog# 100406)
- Anti-mouse CD4 (clone GK1.5, BioLegend catalog# 100451)
- Anti-mouse CD4 (clone GK1.5, BioLegend catalog# 100410)
- Anti-mouse CD4 (clone GK1.5, BioLegend catalog# 100408)
- Anti-mouse CD4 (clone GK1.5, BD Biosciences cat # 563790)
Validation

All antibodies used in this study were either previously validated by other researchers or by the companies where we purchased them from. When no prior validation had been performed, we validated the antibodies in-house by immunofluorescence, western blotting, or flow cytometry.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s) The J14 SLP-76-EYFP cell line was a kind gift of Dr. Lawrence E. Samelson (Laboratory of Cellular and Molecular Biology, National Cancer Institute, National Institutes of Health).

Authentication The J14 cell line was obtained from Dr. Samelson and we did not further authenticate it.

Mycoplasma contamination The J14 cell line tested negative for Mycoplasma contamination by enzyme detection.
Commonly misidentified lines
(See KLAC register)

No commonly misidentified cell lines were used.

Research animals

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Animals/animal-derived materials

Nup210flox/flox (Nup210f/f) mice were generated by nuclear injection of homologous DNA (The Center for Mouse Genome Modification (CMGM); University of Connecticut Health Center). To generate Nup210-/- mice, Nup210f/f mice were then crossed with HprtCre mice (Stock No. 004302, The Jackson Laboratory). A marker-assisted speed congenic breeding strategy was used to 99.9% backcross the mice to the C57BL/6J strain. To generate Nup210f/f-Cd4CreERT2 mice, Nup210f/f mice were crossed with Cd4CreERT2 mice (Stock No. 022356, The Jackson Laboratory). TCRbeta-TCRdelta- (B6.129P2-Tcrbtm1Mom Tcrdtm1Mom/J) mice were from The Jackson Laboratory (Stock No. 002122). Male and female mice were used at 6-10 weeks of age.

Method-specific reporting

n/a

Involved in the study

- ChIP-seq
- Flow cytometry
- Magnetic resonance imaging

Flow Cytometry

Plots

Confirm that:

- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Staining and washes were performed in 96-well plates. Unless otherwise stated, all incubations were performed in Staining Buffer (HBSS containing 1.2% FBS) for 30 minutes at 4°C. Negative and positive controls were generated for each experiment by pooling Nup210+/+ and Nup210-/- samples. Primary antibodies were diluted in Staining Buffer at a final dilution of 1:100 or 1:200; except anti-Nur77, which was used at 1 μg per sample, and anti-pSTAT5, which was used at 0.06 μg per sample. Anti-Rabbit secondary antibodies conjugated to Alexa Fluor 488 or Alexa Fluor 647 (Life Technologies) were used at 1:1,000.

- Cell surface staining: cells isolated from spleens and peripheral lymph nodes (2 x 10^6 cells per sample) were stained with LIVE/DEAD® Fixable Aqua Dead Cell Stain Kit (Life Technologies) or with Zombie NIR Fixable Viability Kit (BioLegend) diluted in PBS at room temperature (RT) for 10 minutes, followed by incubation with fluorochrome-conjugated primary antibodies. For CCR7 surface staining, incubation was performed for 30 minutes at 37°C. Cells were either immediately analyzed by flow cytometry or fixed in 1% formaldehyde in PBS for later analysis.

- Intracellular staining of pLck, pPLC-g1, pZap70, and pSTAT5: after viability and surface staining, cells (0.2 x 10^6 cells per sample) were fixed in 1.85% formaldehyde for 7 minutes at 37°C, washed in ice-cold PBS, and post-fixed in 90% methanol for 30 minutes at 4°C. After washing in ice-cold PBS, cells were stained with antibodies against pLck, pPLC-g1, pZap70, pSTAT5, or matching isotype control, diluted in Staining Buffer. A fluorescently conjugated secondary antibody diluted in Staining Buffer was subsequently used.

- Intracellular staining of Nur77, JunB, and Foxp3: after viability and surface staining, 0.2 x 10^6 cells per sample were fixed in BD Cytofix/Cytoperm (BD Biosciences) followed by post-fixation using the Foxp3/Transcription Factor Fixation/Permeabilization Concentrate and Diluent (eBioscience) as per the manufacturer’s instructions. Intracellular staining was performed with Nur77, JunB or Foxp3 antibodies diluted in Permeabilization Buffer (eBioscience). For JunB, a fluorescently conjugated secondary antibody diluted in Permeabilization Buffer was subsequently used.

Instrument

Flow cytometry data were acquired in a BD LSRFortessa (BD Biosciences), in a BD LSRFortessa X-20 (BD Biosciences), or in a ZE5 (Bio-Rad).

Software

Flow cytometry data were acquired using the BD FACSDIVA Software (BD Biosciences; in the case of acquisition in a BD LSRFortessa or in a BD LSRFortessa X-20); or using the Everest software (Bio-Rad; in the case of acquisition using a ZE5 Cell Analyzer). Flow cytometry data were analyzed using Flowjo software v10.0.8r1 (Tree Star, Inc.). GraphPad Prism software v7.0a (GraphPad Software, Inc.) was used to prepare graphs and to perform statistical analyses.

Cell population abundance

Within the splenic and lymph node combined CD4+CD25- T cell population, naïve CD4+ T cells represent ~85% in Nup210+/+ mice, and ~70% in Nup210-/- mice. Refer to Supplementary Figure 5c for the gating strategy used to isolate naïve CD4+ T cells by FACs, as well as representative examples of the purity before and after FACs. Refer to Figure 2 c, f, and h for representative plots of T cells in Nup210+/+ and Nup210-/- mice.

Gating strategy

For all experiments, lymphocytes were initially gated based on their size in a FSC-A vs. SSC-A scatter plot. Aggregates were then
Gating strategy

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.