Number abnormal/Total number of mice evaluated Organ/Tissue	Ctrl-1/Ctrl-2	*Ptpn1*/Ctrl-2	Ctrl-1/*Ptpn2*	*Ptpn1*/*Ptpn2*	Significant?
Heart	0/6	0/6	0/6	0/6	ns
Lung	0/6	0/6	0/6	0/6	ns
Thymus	0/6	0/6	0/6	6/6	Yes
Salivary glands	0/6	0/6	0/6	0/6	ns
Lymph nodes	0/6	2/6	1/6	0/6	ns
Trachea	0/6	0/6	0/6	0/6	ns
Thyroids	0/6	0/6	0/6	0/6	ns
Liver	0/6	0/6	0/6	6/6	Yes
Gallbladder	0/6	0/6	0/6	0/6	ns
Spleen	0/6	1/6	1/6	6/6	Yes
Kidney	0/6	0/6	0/6	0/6	ns
Adrenal glands	0/6	0/6	0/6	0/6	ns
Pancreas	0/6	0/6	0/6	0/6	ns
Stomach	0/6	0/6	0/6	0/6	ns
Small intestine	0/6	0/6	1/6	0/6	ns
Colon	0/6	0/6	0/6	0/6	ns
Brain	0/6	0/6	0/6	0/6	ns
Eye	0/6	0/6	0/6	0/6	ns
Mammary	0/6	0/6	0/6	0/6	ns
Skin	0/6	0/6	0/6	0/6	ns
Knee joint	0/6	0/6	0/6	0/6	ns
Bone	0/6	0/6	0/6	6/6	Yes
Muscle	0/6	0/6	0/6	0/6	ns
Spinal cord	0/6	0/6	0/6	0/6	ns
Seminal vesicles	0/6	0/6	0/6	0/6	ns
Coagulating glands	0/6	0/6	0/6	0/6	ns
Urinary bladder	0/6	0/6	0/6	0/6	ns
Prostate	0/6	0/6	0/6	0/6	ns
Testicle	4/4*	5/5*	6/6	6/6	ns
Epididymis	0/6	0/6	0/6	0/6	ns
Bulbourethral gland	0/6	0/6	0/6	0/6	ns
Urethra	0/6	0/6	0/6	0/6	ns

Supplementary Table 1: All organs evaluated by necropsy in Ctrl-1/Ctrl-2, PTPN1/Ctrl-2, Ctrl-1/PTPN2, and PTPN1/PTPN2 BMCs 4 weeks after bone marrow implantation. All abnormalities are highlighted in orange. Significant pathologic differences are indicated. *Testicles not recovered for 3 mice. Statistical significance was assessed by a two-sided Fisher's exact test.

Bone Marrow

Clustering Iteration 0

Cluster	Classification	Reason
0	non-specific	large cluster containing multiple lineages
1	neutrophils	S100a8, S100a9, Ncf1, Cd177, Lcn2
2	?	small cluster (one cell)
3	myeloid/granulocyte progenitors	Mpo, Calr; mt* overexpression, with Hspa5, indicates likely cell stress

Clustering Iteration 1

Cluster	Classification	Reason
0-0	Cxcr4-hi mac/mono	Cxcr4 high, Mpeg1
0-1	B progenitor/doublet?	Igll1, high mt* expression indicating QC issue
0-2	cycling	Top2a, Mki67
0-3	T cells	Cd3d, Ccl5, Nkg7
0-4	Prdx-hi mac/mono	Prdx-high
0-5	?	few cells
0-6	?	few cells
0-7	?	few cells
0-8	B cells and progenitors	Vpreb3, Cd37, Cd79
1-0	Cebpe-hi/pre-Neu	high expression of secondary granules, Cebpe
1-1	Cepd-hi/imm-Neu	high expression of tertiary granules, Cebpd
1-2	?	few cells
1-3	proliferating neutrophil progenitor	Mki67
1-4	?	few cells
1-5	B/granulocyte doublet	Cd79, Ighm, Vpreb3
1-6	Stfa-hi.1	Stfa2, Stfa2l1, Stfa3
1-7	Cxcr2-hi/Csf3r-hi	Cxcr2, Csf3r
1-8	Stfa-hi.2	Stfa2l1, Stfa2, Stfa1, Stfa3
2	no further clustering annotation performed	
3	no further clustering annotation performed	

Clustering Iteration 2

Cluster	Classification	Reason
0-0-0	C3-hi mac/mono	C3
0-0-1	pDC	Tcf4, Irf8
0-0-2	?	few cells
0-0-3	?	few cells
0-2-0	B cell (pre-pro)	Mki67, Cd79, Vpreb3, Cd43
0-2-1	Calr-hi	Calr
0-3	no further clustering annotation performed	
0-4	no further clustering annotation performed	
0-8-0	pre-B	Il7r+
0-8-1	immature B	IL7r-
0-8-2	?	
0-8-3	pro-B	Cd43+
1	no further clustering annotation performed	
2	no further clustering annotation performed	
3	no further clustering annotation performed	

Spleen

Clustering Iteration 0

Cluster	Classification	Reason
0	non-neutrophil	large cluster, many lineages present
1	neutrophils	S100a8, S100a9, Lcn2, Ngp

Clustering Iteration 1

Cluster	Classification	Reason
0-0	T cells	Cd3, Lck, Nkg7
0-1	mac/mono	Fcer1g, Mpeg1
0-2	?	mt* high indicating cell stress/QC concern, relatively small cluster
0-3	?	few cells
0-4	plasma cells	Jchain, immunoglobulin genes
0-5	B cells	Cd79, Cd37, immunoglobulins
1-0	neutrophils	Ltf, Ly6g
1-1	proliferating neutrophils	Mki67
1-2	Stfa*-hi	Stfa1, Stfa2, Stfa3

Clustering Iteration 2

Cluster	Classification	Reason
0-0-0	progenitor exhausted/naive T cell	Tcf7
0-0-1	activated CD8	Gzmb, Ccl5
0-0-2	activated CD4	Ikzf1, Ikzf2
0-1-0	Lyz2, Ccr2	Lyz2, Ccr2
0-1-1	Cd74, mhc-ii, slamf7	Cd74, Mhc-ii, Slamf7
0-1-2	mhc-ii	Mhc-ii
0-1-3	cycling	Mki67
0-1-4	Ccr1, Tgfbi, Cd3, Gzmb, Il1r2, Gzma, Mmp8	Ccr1, Tgfbi, Cd3, Gzmb, Il1r2, Gzma, Mmp8
0-1-5	pDC	Tcf4, Irf8
0-1-6	?	few cells
0-1-7	Cd11a, Fcer1g, Cd36, Cd300	Cd11a, Fcer1g, Cd36, Cd300
0-1-8	C1q	C1q
0-1-9	S100a8, S100a9, Sell	S100a8, S100a9, Sell

Supplementary Table 2: Cell cluster annotations for scRNAseq data from Ctrl-1/Ctrl-2, PTPN1/Ctrl-2, Ctrl-1/PTPN2, and PTPN1/PTPN2 BMCs.

Number abnormal/Total number of mice evaluated Organ/Tissue	Ctrl-2/FRT-Ctrl-1	*Ptpn1*/FRT-Ctrl-1	Ctrl-2/FRT-*Ptpn2*	*Ptpn1*/FRT-*Ptpn2*	Significant?
Heart	0/8	0/8	0/8	0/8	ns
Lung	0/8	0/8	0/8	0/8	ns
Thymus	0/8	0/8	0/8	8/8	Yes
Salivary glands	0/8	0/8	0/8	0/8	ns
Lymph nodes	0/8	0/8	0/8	0/8	ns
Trachea	0/8	0/8	0/8	0/8	ns
Thyroids	0/8	0/8	0/8	0/8	ns
Liver	0/8	0/8	0/8	0/8	ns
Gallbladder	0/8	0/8	0/8	0/8	ns
Spleen	0/8	0/8	0/8	0/8	ns
Kidney	0/8	0/8	0/8	0/8	ns
Adrenal glands	0/8	0/8	0/8	0/8	ns
Pancreas	0/8	0/8	0/8	0/8	ns
Stomach	0/8	0/8	0/8	0/8	ns
Small intestine	0/8	0/8	0/8	8/8	Yes
Colon	0/8	0/8	0/8	2/8	ns
Brain	0/8	0/8	0/8	0/8	ns
Eye	0/8	0/8	0/8	0/8	ns
Mammary	0/8	0/8	0/8	0/8	ns
Skin	0/8	0/8	0/8	0/8	ns
Knee joint	0/8	0/8	0/8	0/8	ns
Bone	0/8	0/8	0/8	0/8	ns
Muscle	0/8	0/8	0/8	0/8	ns
Spinal cord	0/8	0/8	0/8	0/8	ns
Seminal vesicles	0/8	0/8	0/8	0/8	ns
Coagulating glands	0/8	0/8	0/8	0/8	ns
Urinary bladder	0/8	0/8	0/8	0/8	ns
Prostate	0/8	0/8	0/8	0/8	ns
Testicle	8/8	8/8	8/8	8/8	ns
Epididymis	0/8	0/8	0/8	0/8	ns
Bulbourethral gland	0/8	0/8	0/8	0/8	ns
Urethra	0/8	0/8	0/8	0/8	ns

Supplementary Table 3: All organs evaluated by necropsy in Ctrl-2/FRT-Ctrl-1, PTPN1/FRT-Ctrl-1, Ctrl-2/FRT-PTPN2, and PTPN1/FRT-PTPN2 BMCs 10 weeks after bone marrow implantation. All abnormalities are highlighted in orange. Significant pathologic differences are indicated. Statistical significance was assessed by a two-sided Fisher's exact test.

Supplementary Note 1

Plasmids

Plasmids were synthesized by Genscript. The plasmids and full sequences for the six plasmids used in this study have been deposited on Addgene. For single gene knockout studies, we utilized pXPR_053 (Addgene ID: 113591). pXPR_053 contains the human U6 promoter (with Lac operator site) for expression of an sgRNA, as well as the human PGK promoter for expression of the fluorophore Vex (violet-excited GFP). pXPR_071 (Addgene ID: 164558) is a modified form of pXPR_053 that produces higher titer lentivirus and has the following changes: (1) removal of Lac operator site from U6 promoter, (2) use of tracrRNA V4 scaffold (pXPR_053 has tracrRNA V1), and (3) codon optimization of the Vex fluorophore for murine systems. For C-CHIME studies we utilized pXPR_219 (Addgene ID: 164559). pXPR_219 is a modified form of pXPR_071 with the following change: addition of a second sgRNA cassette containing the mouse U6 promoter driving transcription of an sgRNA with tracrRNA V1. This cassette contains cloning sites for BfuAI as well as a stuffer to enable efficient cloning via BfuAI. For I-CHIME studies we utilized pXPR_070 (Addgene ID: 164265) and pXPR_068 (Addgene ID: 164555). pXPR_070 is a modified form of pXPR_071 with the following change: introduction of a stuffer into the stem loop of the Broad GPP chRNA V2 tracrRNA. This stuffer contains transcriptional stops on the proximal 5' and 3' ends and is flanked by loxP sequences in the same directional orientation. pXPR_068 is a modified form of pXPR_071 with the following change: introduction of a stuffer into the stem loop of the Broad GPP chRNA V2 tracrRNA. This stuffer contains transcriptional stops on the proximal 5' and 3' ends and is flanked by FRT sequences in the same directional orientation. For L-CHIME studies we also utilized pXPR_070. For S-CHIME studies we utilized pXPR_220 (Addgene ID: 164560), a modified form of pXPR_068 with the following changes: addition of a second sgRNA cassette containing a mouse U6 promoter driving transcription of an sgRNA followed by tracrRNA V1. This cassette contains cloning sites for BfuAI as well as a stuffer to enable efficient cloning via BfuAI.

Plasmids available on Addgene:

1. pXPR_053
2. pXPR_071
3. pXPR_219
4. pXPR_220
5. pXPR_070
6. pXPR_068

Supplementary Note 2

Imaging

H&E stained sections were imaged with a Hamamatsu ORCA-Flash4.0 V3 Digital CMOS camera attached to a Nikon Ti inverted microscope used for widefield imaging. The microscope had a Prior ProScanIII motorized stage, a Nikon Z drive focus accessory, and a Lumencor SOLA LED light engine. All images were acquired with the Nikon Elements Acquisition Software Version 5.21.03 build 1489. A 12-bit depth and no binning were used for imaging. For all images, the auto-exposure setting was used to ensure the color saturation did not exceed 4095. In order to take 10x images of the spleen and bone marrow with the Plan Apo Lambda 10x/0.45 Air DIC N1 objective, the XY large image option was used (with different fields of view for different organs) with 15% overlap between images. For each sample, a focus map was created for the 10x scan. After the XY images were taken, they were stitched together (Image → ND processing → Stitch multipoint to large image → select Image Registration and Optimal Path) in the Nikon Elements Acquisition Software. Bone marrow images taken at 40x magnification used the Plan Fluor 40x/1.30 Oil DIC N2 objective. Small intestinal images taken at 20x magnification used the Plan Apo 20x/0.75 Air Ph2 DM objective. Data was saved as .nd2 files. To match the color contrast and brightness between images, the images were opened in Fiji (Image J, 64-bit) and duplicated. The channels (1, 2, and 3) were then merged for the duplicated image. The images were then adjusted to have a similar orientation. Image resolution was reduced in Fiji to decrease figure file sizes. The image elements were overlaid and flattened, and the image was saved. The brightness and contrast were adjusted to match a selected image for the 10x spleen images using Adobe Photoshop 2022.

Image analysis

<u>Bone marrow quantification</u>

Fiji was used to quantify the number of cells in the 40x bone marrow images. The files were opened in Fiji using the hyperstack option. The image was then duplicated and the colors were merged. The StarDist plugin was utilized to count the nuclei (plugins → StarDist → StarDist 2D → H&E nuclei → ok). After duplicating this image, the location and number of dots were saved in the ROI manager. The dots were then deleted and the image converted to an 8-bit image (image → type → 8-bit). The dots were then added back in as black dots from the ROI manager. The threshold was adjusted prior to inverting the colors. Finally, the number of nuclei was analyzed using the analyze particles function. The count values were recorded and the inverted filled image was saved as a reference image.

<u>Spleen quantification</u>

Fiji v1.53q and QuPath v0.3.0 were used to quantify white pulp regions in the spleen. For the images that were >2GB, the image was reopened with Nikon Elements Acquisition Software Version 5.21.03 build 148 and cropped (image → crop) until the file size was <2GB. The images were then opened in Fiji and an RGB composite image was created. In order to train a classifier in QuPath, cropped images were created. Next, a project was created in QuPath and the cropped images were dragged into the new project as Brightfield (H&E) images. Each cropped image was opened and a rectangle was drawn around the entire image. A training image was created by merging the cropped images and the classifications were set for the desired regions (background, red pulp, and white pulp for the spleens). Then, a cell detection was applied (detection image was set as Hematoxylin OD, the requested pixel size was 0.2, the background radius was 75, median filter radius was set at 0, sigma was 0.1, minimum area was 5, maximum area was 25, the threshold was 0.1, and the maximum background intensity was 2). Smoothed features were then added (analyze → calculate features → add smoothed features → 25 μm → run) and the classifier was trained (classify → object classification → train object classifier → live update). The default settings were used for the classification. To apply the classifier to the remaining images, the images were imported into QuPath as Brightfield (H&E) images. The cell detection and smoothed features were run on these images. Then, images were exported as RGB with overlays and the downsample factor changed to 5.0. Classified images were opened in Fiji and split into red, green, and blue channels (note only red/green channels were used as they correspond to red and white pulp regions, respectively). The images were thresholded and background regions removed. Then, particles were analyzed and the percentage of white pulp area was calculated by dividing the red channel area by the sum of the red and green channel areas.

Supplementary Note 3
Single-cell RNAseq analyses

mRNA Count Matrix Generation

Sequencing output (bcl files) were demultiplexed using the mkfastq command from cellranger v6.1.2 (10x Genomics, v6.1.2 used throughout), using a reference prepared from the M. musculus genome GRCm39 and gene annotations from Ensembl release 106 using the cellranger mkref command. Demultiplexed fastq files then underwent alignment and mRNA count quantification via the cellranger count command, using the STAR 2.7.2a aligner (packaged within cellranger), with feature-barcode quantification (cell-hashing) via the following feature reference:

id,name,read,pattern,sequence,feature_type
Hash1,B0301_TotalSeqB,R2,5PNNNNNNNNNN(BC),ACCCACCAGTAAGAC,Antibody Capture
Hash2,B0302_TotalSeqB,R2,5PNNNNNNNNNN(BC),GGTCGAGAGCATTCA,Antibody Capture
Hash3,B0303_TotalSeqB,R2,5PNNNNNNNNNN(BC),CTTGCCGCATGTCAT,Antibody Capture
Hash4,B0304_TotalSeqB,R2,5PNNNNNNNNNN(BC),AAAGCATTCTTCACG,Antibody Capture

Count Matrix Merging, pre-processing, and QC

Subsequent analysis was performed in python v3.9.5, using the following packages and versions:

- loompy v3.0.6 (https://github.com/linnarsson-lab/loompy)
- numpy v1.21.5 (https://github.com/numpy/numpy)
- scipy v1.7.3 (https://github.com/scipy/scipy)
- sklearn v1.1.1 (https://github.com/scikit-learn/scikit-learn)
- umap-learn v0.5.2 (https://github.com/lmcinnes/umap)
- matplotlib v3.5.1 (https://github.com/matplotlib/matplotlib)
- seaborn 0.11.2 (https://github.com/mwaskom/seaborn)
- pandas v1.3.5 (https://github.com/pandas-dev/pandas)
- panopticon v0.3 (https://github.com/scyrusm/panopticon/)

Output files (filtered_feature_bc_matrix.h5) from cellranger count were converted to loom files with the panopticon command panopticon.utilities.convert_10x_h5. Created looms were combined with the loompy.combine command. In each experimental group, we obtained the following unique barcodes that were identified as cells via cellranger:

Bone marrow: Ctrl-1/Ctrl-2 (13,239), Ctrl-1/*Ptpn2* (14,340), *Ptpn1*/Ctrl-2 (11,578), *Ptpn1/Ptpn2* (10,952)
Spleen: Ctrl-1/Ctrl-2 (12,879), Ctrl-1/*Ptpn2* (12,480), *Ptpn1*/Ctrl-2 (13,055), *Ptpn1/Ptpn2* (10,085)

Normalized count matrices were generated via the panopticon.preprocessing.generate_count_normalization command, which computes the "log2(TP10k+1)" transformation:

$$g_i' = log_2(\frac{10^5 g_i}{\sum_i g_i} + 1)$$

g_i represents the raw count expression of the i'th gene, and the sum is computed over all counted genes in a cell. Where relevant, log2(TP10k+1) counts were also standardized, either over all cells ("log2(TP10k+1)_cell_standardized") or genes ("log2(TP10k+1)_gene_standardized"), using the panopticon.preprocessing.generate_standardized_layer command.

Feature barcoding (cell-hashing) was converted into hashtag predictions, as inspired by step one of the DSB procedure[49], and as implemented in panopticon.preprocessing.generate_antibody_prediction. Cells with no or multiple predicted hashtags were removed from downstream analysis (the latter as suspected doublets/multiplets).

Cell complexity (the number of unique, detected genes associated with a cell barcode) was estimated using the panopticon.preprocessing.generate_cell_and_gene_quality_metrics command. Cells with complexity less than 1,000 were removed from downstream analysis. Following this quality-control, we retained the following numbers of cells in each experimental group:
Bone marrow: Ctrl-1/Ctrl-2 (9,115), Ctrl-1/*Ptpn2* (8,876), *Ptpn1*/Ctrl-2 (8,302), *Ptpn1/Ptpn2* (7,639)
Spleen: Ctrl-1/Ctrl-2 (10,364), Ctrl-1/*Ptpn2* (9,941), *Ptpn1*/Ctrl-2 (9,357), *Ptpn1/Ptpn2* (5,912)

Dimensionality reduction, clustering, cluster annotation
We then split this joint dataset into separate datasets for spleen- and bone marrow-derived data. Principal component analysis (PCA) was used as initial dimensionality reduction (as implemented using the panopticon.analysis.generate_incremental_pca). Each dataset was visualized using umap, as implemented in the panopticon.analysis.generate_embedding command, using the "log2(TP10k+1)" normalization. Cell clustering was performed using the panopticon.analysis.generate_clustering command using the

“log2(TP10k+1)_cell_standardized” which, in brief, clusters cells using hierarchical clustering with a cosine distance metric (equivalent to a correlation distance metric when using standardized counts), using hierarchical clustering as implemented in sklearn.cluster.AgglomerativeClustering, using an average linkage. The optimal number of clusters (between 2 the cube root of the number of cells being clustered) was then selected via silhouette score, as implemented in sklearn.metrics.silhouette_score (when the maximum silhouette score is less than 0.1, no clustering is performed). This procedure was then repeated within each cluster for a total of 4 such rounds of clustering/subclustering.

Cluster transcriptional markers were generated using the panopticon.analysis.get_differential_expression_dict, which, in brief, performs differential expression (via panopticon.analysis.get_cluster_differential_expression) between clusters and their complementary clusters within a clustering round. For example, subclusters that were split in round n+1 of this clustering procedure would only be compared against subclusters which stem from the same supercluster in round n. These markers were then used to annotate clusters, as described in Supplementary Table 2.

Visualization

Repertoire plotting (stacked bar plots indicating the relative abundance of clusters in each experimental group, and the relative makeup of each experiment group by cluster) were generated with the function panopticon.visualization.repertoire_plot, using the options normalize=True, stack_order='matched'. Stacked bars indicate the relative abundance of a cluster across experimental groups, the option pre_normalize_by_cohort=True was used. Heatmaps indicating key marker genes were generated with the function panopticon.visualization.cluster_differential_expression_heatmap, with doublets and other undetermined cells (as denoted by classification “?”) removed, with gene-standardized gene expression. Markers were determined with the function panopticon.analysis.get_differential_expression_dict, over ‘log2(TP10k+1)’ gene expression values.

References

49. Mulè, M. P., Martins, A. J. & Tsang, J. S. Normalizing and denoising protein expression data from droplet-based single cell profiling. *Nat Commun* **13**, 2099 (2022).