**Fitting the topic model**

We used fastTopics [countClust2017, fastTopics2022] to fit a topic model to the (UMI?) counts, with *K* = 16 topics. fastTopics implements the following two-step approach to fit the topic model: (1) fit a non-negative matrix factorization based on a *Poisson model* (“Poisson NMF”) [hien2021]; (2) recover maximum-likelihood estimates (MLEs) of the topic model parameters by a simple reparameterization [fastTopics2022].

In detail, we took the following steps. First, we removed genes with very low expression (total UMI count ≤ 20). Therefore, the *K* = 16 topic model was fit to UMI counts for 364 samples and 28,209 genes. Second, we ran 20 expectation maximization (EM) updates, without extrapolation, to get close to an MLE solution (“prefitting phase”). This prefitting phase was implemented in R by calling fit\_poisson\_nmf from fastTopics with the following settings: numiter = 20, method = "em", init.method = "random", control = list(nc = 8). Third, we performed an additional 180 coordinate descent (CD) updates, with extrapolation, to improve the fit (“refinement phase”). This refinement phase was implemented by calling fit\_poisson\_nmf with the following settings: method = "scd", numiter = 180, control = list(numiter = 4, nc = 8, extrapolate = TRUE), in which the model fit was initialized using the fit obtained from the prefitting phase. Finally, the topic model was recovered from the Poisson NMF model by calling function poisson2multinom. The convergence diagnostics suggested that, after a total of 200 iterations of the Poisson NMF optimization, the parameter estimates were close to an MLE; the change in log-likelihood between successive iterations was less than 1 × 10-5, and the largest residual in the first-order (“Karush-Kuhn-Tucker”) conditions was less than 1.

Reassuringly, the estimated topics captured the predominant expression patterns, most of which identify the 13 tissues (the one exception was iLN and SP, which shared the same expression pattern). Two other topics (topics 1 and 6) captured variation specific to two tissues (LI and PBMC), and one topic (topic 9) captured changes in expression over time that were not specific to any one tissue.

*If necessary, we can add details here about fitting topic models separately to each tissue.*

**Visualizing the topic proportions**

The *n* × *K* matrix of topic proportions, *L*, where *n* denotes the number of RNA-seq samples and *K* is the number of topics, can be viewed as an embedding of the samples in a (*K* − 1)- dimensional space. A simple way to visualize this embedding in 2-d (or 3-d) is to apply a nonlinear dimensionality reduction technique such as *t*-SNE [tsne2008] to *L*. An alternative powerful approach, first suggested by [countClust2017], is to visualize all *K* − 1 dimensions simultaneously using a Structure plot, which has been used with great success in population genetics [rosenberg2002]. The Structure plot is essentially a stacked bar chart, in which bars correspond to samples (rows of *L*) and bar heights (in different colors, one for each topic) are determined by the topic proportions. To arrange the samples in the Structure plot, we first grouped the RNA-seq samples by tissue, then we ordered them within each tissue by time point.

**Differential expression analysis allowing for grades of membership**

To annotate the topics, we used the grade-of-membership differential expression (GoM DE) analysis methods developed in [GoM-DE2022] and implemented in the de\_analysis function in the fastTopics package. In brief, the GoM DE analysis is conceptually similar to a standard DE analysis (e.g., [soneson2018, wang2019]), but extends the idea of comparing expression between groups by allowing the cells to have *partial membership to multiple groups* (here, the groups are the topics in the topic model). We called the de\_analysis function with the following settings: shrink.method = "ash", pseudocount = 0.1 and control = list(ns = 1e5, nc = 8). We performed a second DE analysis, with the same settings, after merging topics 1 and 5 (capturing variation in PBMC expression), 2 and 13 (BM) and topics 6 and 14 (LI). The GoM DE analysis produces, for each gene *j* and topic *k*, estimates of differences in expression, and statistics quantifying support for these differences. In the de\_analysis interface, expression differences are defined by the “least extreme” log-fold change (“l.e. LFC”), which is defined for gene *j* and topic *k* as the log-fold change that is the smallest in magnitude among topic pairs (*k*, *l*). After computing initial estimates, the GoM DE analysis (with shrink.method = “ash”) performs an adaptive shrinkage step [ash2016], separately for each topic, to stabilize the l.e. LFC estimates. We used the posterior mean estimates, posterior standard errors, posterior z-scores (posterior mean/s.e.) and local false sign rates (*lfsr*) produced by the adaptive shrinkage step to report results of the GoM DE analysis. Note that the *lfsr* can be interpreted similarly to the *q*-value [qvalue2003, qvalue2003b], for example, although the *lfsr* tends to be more conservative than quantities such as the *q*-value that control for the false discovery rate [ash2016].

**Gene sets**

Mouse gene sets for the gene set enrichment analyses (GSEA) were compiled from the following gene set databases: NCBI BioSystems [biosystems2009]; Pathway Commons [pc2010, pc2019]; and MSigDB [gsea2005, msigdb2011, msigdb2015], which included Gene Ontology (GO) gene sets [go2000, go2020]. Specifically, we downloaded bsid2info.gz and biosystems\_gene.gz from the NCBI FTP site (https://ftp.ncbi.nih.gov/gene) on March 22, 2020; PathwayCommons12.All.hgnc.gmt.gz from the Pathway Commons website (https://www.pathwaycommons.org) on March 20, 2020; and msigdb\_v7.2.xml.gz from the MSigDB website (https://www.gsea-msigdb.org) on October 15, 2020. For the gene set enrichment analyses, we also downloaded the mouse gene information (“gene info”) file Mus\_musculus.gene\_info.gz from the NCBI FTP site on October 15, 2020. To facilitate integration of these gene sets into our analyses, we have compiled these gene sets into an R package [pathways2021].

We performed two gene set enrichment analyses. In the first GSEA, we included all gene sets other than the following MSigDB collections: C1, C3, C4 and C6, and gene sets labeled as “archived”. In the second GSEA, we focused on the curated pathways, specifically gene sets belonging to the GO and CP subcategories in the MSigDB C2 gene set collection. In both analyses, we removed gene sets with fewer than 10 genes and with more than 400 genes. After removing these gene sets, 21,442 candidate gene sets remained for the first analysis, and 8,939 gene sets remained for the second analysis.

**Computing environment for topic modeling and gene set enrichment analyses**

Most computations on real data sets were performed in R 3.5.1 [R2018], linked to the OpenBLAS 0.2.19 optimized numerical libraries, on Linux machines (Scientific Linux 7.4) with Intel Xeon E5-2680v4 (“Broadwell”) processors. For performing the model topic model optimization and DE analysis, which included multithreaded computations, as many as 8 CPUs and 16 GB of memory were used. More details about the computing environment, including the R packages used, were recorded in the workflowr [workflowr2019] pages in the companion code repository [TO DO: create Zenodo repository].

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