

1 **Title:** Construction of a multi-tissue cell atlas reveals cell-type-specific regulation of
2 molecular and complex phenotypes in pigs

3

4 **Running Title:** Cell-type-specific gene regulation in pigs

5

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99 **Abstract**

100 The systematic characterization of cellular heterogeneity among tissues and cell-type-specific
101 regulation underlying complex phenotypes remains elusive in pigs. Within the Pig Genotype-
102 Tissue Expression (PigGTEx) project, we present a single-cell transcriptome atlas of adult pigs
103 encompassing 229,268 high-quality nuclei from 19 tissues, annotated to 67 major cell types.
104 Besides cellular heterogeneity within and across tissues, we further characterize prominent
105 tissue-specific features and functions of muscle, epithelial, and immune cells. Through
106 deconvoluting 3,921 bulk RNA-seq samples from 17 matching tissues, we dissect thousands
107 of genetic variants with cell-type interaction effects on gene expression (ieQTL). By
108 colocalizing these ieQTL with variants associated with 268 complex traits, we provide new
109 insights into the cellular mechanisms behind these traits. Moreover, we highlight that
110 orthologous genes with cell-type-specific regulation in pigs exhibit significant heritability
111 enrichment for some human complex phenotypes. Altogether, our work provides a valuable
112 resource and highlights novel insights in cellular regulation of complex traits for accelerating
113 pig precision breeding and human biomedical research.

114

115 **Keywords:** PigGTEx; Single-nucleus RNA-seq; Cellular deconvolution; Gene regulation;
116 Cell-type-trait association

117 **Introduction**

118 The cell is a fundamental structural, biological, and evolutionary unit of life and plays a key
119 role in orchestrating the development and homeostasis of all living beings through global
120 intercellular interactions. Multicellular organisms, including mammals, are generally
121 composed of over 400 distinct cell types that are distinct in morphology and function (1-5).
122 Genome-wide association studies (GWASs) have revealed that over 90% of phenotype-
123 associated genetic variants lie within non-coding regions, suggesting that these variants might
124 influence complex phenotypes through gene expression modulation (6-8). The limited overlaps
125 between bulk expression quantitative trait loci (eQTL) and GWAS signals suggest that many
126 candidate variants might regulate biological processes and then complex phenotypes through
127 cell-type-specific mechanisms (9-12). Single-cell omics studies have shown that the substantial
128 disorders in cellular activity, identity, and composition play a crucial role in the development
129 of complex traits and diseases, both within and across individuals (5, 13-16), highlighting the
130 importance of constructing a multi-tissue single-cell atlas for functionally understanding
131 genotype-phenotype associations. In addition, a better understanding of molecular and cellular
132 mechanisms underpinning complex phenotypes will be an important initial step in generating
133 new avenues for precision breeding in agriculture and therapeutic solutions for similar human
134 diseases (13, 14, 17).

135

136 As an important farm animal species, the domestic pig (*Sus scrofa*) is not only an abundant
137 source of animal protein worldwide but also serves as a valuable human biomedical model and
138 an optimal organ donor for xenotransplantation (18). Numerous studies in pigs have delineated
139 significant QTL underlying complex traits of economic importance (19, 20), leading to vast
140 improvements in pig breeding programs and production efficiency. However, the systematic
141 interpretation of molecular mechanisms underlying complex phenotypes in pigs lags behind
142 human and mouse research due to limitations in functional data availability. The ongoing
143 Functional Annotation of Animal Genomes (FAANG) and Farm animal Genotype-Tissue
144 Expression projects (FarmGTE) are global efforts to provide catalogues of functional
145 elements and variants in pigs at tissue level (21-23). The next step is to explore the cell-type-
146 dependent biological consequences of trait-associated variants as tissues contain numerous cell
147 types (24). Although some studies have conducted single-cell/nucleus RNA-seq (scRNA-seq
148 and snRNA-seq) analyses in pigs, they primarily focused on elucidating the cellular
149 heterogeneity and trajectories of lineage specification in a limited range of tissue types (25-32).

150 The cell-type-specific biological impacts of genetic variants on complex traits by integrating
151 single-cell RNA-sequencing with large-scale pig genetics data still need to be explored.
152
153 To further fine-map the causative genetic variants and decipher their cellular impacts on both
154 molecular and complex phenotypes in pigs, we first constructed a single-nucleus transcriptome
155 atlas by profiling a total of 319,433 nuclei from 19 major tissue types, representing 261 major
156 cell clusters. Dissection of muscle, epithelial and immune cells depicted the cellular
157 heterogeneity across these tissues and revealed a number of critical master regulators (*i.e.*,
158 GATA4 and ZBTB11) driving cell identity. Through cellular deconvolution of PigGTEX
159 tissues, cell-type interaction expression QTL (ieQTL) mapping, and the integrative analysis
160 with GWAS results of 268 complex traits, we pinpoint the cell-type-specific contexts in which
161 trait-associated genetic variants regulate the transcriptional activity and result in phenotypic
162 variation. Moreover, we demonstrate that orthologous genes with cell-type-specific regulation
163 in pigs exhibit significant heritability enrichment for many human complex phenotypes.
164 Overall, this study enriches and enhances rich and open resources (<http://piggtex.farmgtex.org/>
165 and <https://dreamapp.biomed.au.dk/pigatlas/>) for charting the cell-cell transcriptome
166 variability within and across tissues and expands our understanding of the connections between
167 genetic variants and phenotypes at single-cell resolution in pigs. Our results provide relevant
168 information for the development of future precision breeding strategies in pigs and human
169 biomedical research.

170

171 **Results**

172 **Global landscape of single-nucleus transcriptomic reference atlas from 19 pig tissues**

173 To generate a comprehensive multi-tissue single-cell transcriptomic reference atlas of pigs, we
174 performed snRNA-seq in 19 tissues/organs from two adult Meishan pigs (one male and one
175 female) using 10 × Genomics technology, including subcutaneous adipose, cerebellum,
176 cerebrum, colon, duodenum, heart, hypothalamus, ileum, jejunum, kidney, liver, lymph node,
177 skeletal muscle, ovary, pancreas, pituitary gland, spleen, testis, and uterus (Fig. 1a). Initially,
178 we profiled 16,812 nuclei and sequenced over 660 million raw reads per tissue on average (Fig.
179 1a). After quality control (see Methods for details), we obtained transcriptomic data for a total
180 of 229,268 high-quality nuclei across all the 19 tissues (Supplementary Fig. 1). We first
181 assessed the transcriptional similarity by comparing our snRNA-seq data with that from a
182 previous study across seven common tissues (27). The Spearman correlation values between

183 the two pseudo-bulk single-cell transcriptomic profiles were high for all tissues, ranging from
184 0.653 to 0.825 (Supplementary Fig. 2), suggesting globally consistent transcriptional profiles
185 of samples between these two single-cell RNA-seq studies at the bulk tissue level.

186
187 The complete snRNA-seq dataset was grouped into 77 cell clusters and manually annotated as
188 67 major cell types based on the expression of canonical marker genes from the literature (Fig.
189 1b-c, Supplementary Fig. 3, and Supplementary Table 1). All tissues and cell types showed
190 sufficient transcriptional abundance, with a median of 4,008 unique molecular identifiers (UMI)
191 and 2,064 transcribed genes per nucleus, therefore displaying higher expression than the
192 previously reported single-cell data in pigs (27). The global cell atlas revealed that a majority
193 of cell types, like cardiomyocytes, enterocytes, and hepatocytes, exhibited a high tissue
194 specificity regarding gene expression (Fig. 1d), reflecting their specialized functions. Notably,
195 several prevalent cell types, such as immune cells, endothelial cells, and fibroblasts, were
196 commonly shared among tissues. To gain a deeper understanding of cellular heterogeneity
197 within each tissue, we generated individual visualizations in the hierarchy with Uniform
198 Manifold Approximation and Projection (UMAP), resulting in an average of 14 main cell types
199 per tissue (Supplementary Figs. 4-5). Of note, the ileum showed 24 putative cell subpopulations,
200 consistent with its highest cell-type diversity evaluated by the Shannon entropy index (Fig. 1c-
201 d and Supplementary Figs. 4-5). Additionally, we compared cellular signatures of tissues
202 shared by our work and the previous study (27), and in general, found a high consistency in
203 both cell annotation, distribution, and expression (Supplementary Fig. 6). However, some cell
204 types or marker genes, such as *ADIPOQ* in adipose tissue, *DOCK4* in heart, and *CD163* in
205 liver, displayed distinct expression levels and patterns between the two studies (Supplementary
206 Fig. 6). This discrepancy might be attributed to differences in tissue sampling regions and
207 experimental protocols. To further probe the intercellular relationships, we conducted an
208 unsupervised hierarchical clustering analysis for all these 67 cell types based on their
209 transcriptomic profiles (Fig. 1e). These cell types could be largely classified into nine different
210 functional groups of cells, including endocrine, endothelial, epithelial, germline, immune, islet,
211 muscle, neural and stromal cells. Remarkably, we observed a higher similarity among cell types
212 within the nine major lineages rather than among tissues, suggesting that cell clustering was
213 primarily driven by cell type and that these neighboring cell types possibly had similar
214 functions (Fig. 1e). To evaluate the dynamics of cell state in each cell type, we computed the
215 cell cycling index as described previously (3). Germline cells exhibited a greater cell division
216 capacity than other cells, while the endothelial, stromal, and muscle compartments, which are

217 known to be largely quiescent, had low cycling indices (Fig. 1f and Supplementary Fig. 7).
218 Notably, the epithelial cells presented the highest variations in cell states, suggesting great
219 functional diversity of epithelial cell subpopulations.
220

221 **Distinct transcriptional patterns among three types of muscle cells**

222 The muscular system is a complex collection of organs that allow movement through the
223 contraction of muscle fibers and is also the main production target of the pig industry, with the
224 aim to provide high-quality protein in the form of meat. There are three distinct muscle types
225 in the body, namely skeletal, cardiac, and smooth muscle, each with unique cellular
226 morphologies and functions (33). We found that skeletal muscle cells and cardiomyocytes
227 accounted for 66.43% and 25.09% of total cells in muscle and heart, respectively, while smooth
228 muscle cells could be found in eight tissues with an average proportion of 2.52%
229 (Supplementary Figs. 4-5). To provide a more detailed view of the three muscle cell types, we
230 extracted a total of 10,117 muscle cells from corresponding tissues according to cell type
231 annotations and performed the dimension reduction analysis. As expected, t-SNE inspection
232 and dendrogram showed a clear separation among the three major muscle cell types, and each
233 specifically expressed its classical marker genes (Fig. 2a-b), like *MYH7*, *MYBPC2*, and *TNNI1*
234 for skeletal muscle cells, *MYBPC3* and *TNNI2* for cardiac muscle cells, and *ACTA2*, *MYH11*,
235 and *RYR2* for smooth muscle cells. We observed a preferential grouping of skeletal muscle
236 cells with cardiac muscle cells since both belong to striated muscle tissue and share similar
237 structural and functional characteristics (34). In addition, skeletal and smooth muscle cells
238 could be further partitioned into multiple subclusters in the hierarchy (Fig. 2b), suggesting their
239 subtle context-dependent functions. To examine global transcriptional differences among the
240 three muscle cell types, we performed a pair-wise differential gene expression analysis. In total,
241 we identified 1,250 differentially expressed genes (DEGs) across the three myocyte subtypes
242 (Fig. 2c). The 343 DEGs in skeletal muscle cells were significantly enriched in striated muscle
243 contraction, while DEGs in cardiac muscle cells were mainly involved in cardiac muscle tissue
244 development. Smooth muscle cell-specific genes were enriched in the extracellular matrix
245 organization (Fig. 2c). Further analysis of transcription factor (TF) activity revealed many
246 remarkable regulons in the control of muscle cell type specification (Fig. 2d). For example,
247 *MYOD1*, *MYOG*, and *FOXO4* served as master TFs responsible for skeletal muscle cell
248 development, while certain members of the GATA and TBX families showed unique
249 regulatory roles in the cardiac and smooth muscle cell types, respectively.
250

251 In addition to characterizing differences across the three main muscle cell types, we further
252 probed cellular heterogeneity within skeletal and smooth muscle cells separately. Our analysis
253 of pig myonuclei in skeletal muscle confirmed the presence of *MYH7* type I (slow-twitch) and
254 *TNNC2* type II (fast-twitch, IIa/b, and IIx) myofibers (Fig. 2b), consistent with a previous study
255 in monkeys (5). A pairwise comparison between type I and type II myofibers uncovered 209
256 DEGs (Fig. 2e). Notably, type I myofiber-specific genes were enriched in several fundamental
257 pathways related to molecular structure and function like muscle contraction and sarcomere
258 organization (Supplementary Fig. 8a), while the upregulated genes in type II myofibers were
259 essential for metabolic pathways such as phosphorylation and glycolysis (Supplementary Fig.
260 8b). By examining DEGs of these two types of myofibers previously reported in humans (35,
261 36), we observed a strong positive Pearson correlation of 0.945 regarding fold changes of the
262 shared genes between pigs and humans (Supplementary Fig. 8c), implying that the process of
263 muscle fiber specialization might be highly conserved between these two species. Furthermore,
264 we identified several critical master regulators, including METTL3, MYF6, and SIX4, which
265 displayed distinct regulatory activities in type I, IIa/b, and IIx myonuclei (Fig. 2f). By
266 conducting RNA velocity analysis in myofibers together with satellite cells (known as skeletal
267 muscle stem cells), we further explored the differentiation trajectory of muscle fibers. Our
268 results revealed clear myogenesis from satellite cells to mature muscle fibers (Fig. 2g), which
269 were driven by several fundamental genes with dynamic expressions across distinct cell states
270 such as *MYH7* and *PRKG1*. Interestingly, the type IIa/b fibers displayed intermediate cell states
271 and characteristics during the fast-to-slow fiber-type switch. In the smooth muscle cell
272 compartment, we found distinct gene signatures and tissue enrichment among these six cell
273 subtypes (Fig. 2h-i). For instance, SMC_1, which was preferably located in the intestine,
274 showed much higher activity of *MYH11* and *DMD*, while SMC_6, mainly from testis, exhibited
275 exclusively high expression of *MYO1B* and *RGS5*. These results suggested that the same cell
276 types undergo subtle processes of functional differentiation depending on the original tissue
277 contexts in which they reside.

278

279 **About the similarity and heterogeneity of intestinal epithelial cells**

280 Epithelia are sheets of cells that cover most body surfaces, line internal cavities, and compose
281 certain glands. They perform a wide range of biological functions, including protection,
282 absorption, and secretion (37). First, we pursued to investigate the primary characteristics and
283 functions of epithelial cells, given their high abundance and diversity in the different organs.
284 We obtained a total of 57,049 epithelial cells from eight tissues and identified their tissue-

285 specific expression patterns and functions through the global t-SNE and hierarchical clustering
286 (Fig. 3a and Supplementary Fig. 9). Epithelial cells from the duodenum, jejunum, ileum, and
287 colon, representing the digestive system in the present study, exhibited closer relationships
288 with other cells from the same digestive system than with cells from other systems. As expected,
289 epithelial cells from the intestines showed a strong digestive and metabolic capacity, such as
290 microvillus organization and intestinal absorption, compared to other subtypes (Fig. 3b-c). We
291 then extracted intestinal stem cells, enterocytes, and enteroendocrine cells for further
292 exploration, as these cell types might play pivotal roles in feed efficiency traits in pigs (38-40).
293 Intestinal stem cells expressed high levels of *OLFM4* and *LGR5* and could be further
294 subdivided into two subtle subtypes according to the differential expression levels of these two
295 markers (Fig. 3d). We defined four enterocyte subgroups by the transcriptional patterns of
296 canonical enterocyte markers (for example, *MUC13*, *SI*, *FUT8*, *APOB*, and *BEST4*), including
297 enterocyte progenitors, immature enterocytes, mature enterocytes, and $BEST4^+$ enterocytes.
298 Enteroendocrine cells, which are specialized gut epithelial cells that produce and release
299 hormones in the intestine (40), displayed a higher expression of *RAB3C*, *CHGA*, and *STXBP5L*
300 when compared to other intestinal epithelial cells. Enrichment analyses of cell types across
301 tissues revealed that intestinal stem cells were mainly located in the ileum and, to some extent,
302 in the jejunum and colon, while enterocytes were more abundant in the duodenum and colon
303 (Fig. 3e).

304
305 To further characterize the lineage relationships and cell states among intestinal stem cells,
306 enterocytes, and enteroendocrine cells, we conducted the pseudotime analysis and cell cycling
307 index prediction (3, 41). Both analyses revealed that intestinal stem cells and enterocyte
308 progenitors exhibited a great capacity for differentiation into enterocytes and enteroendocrine
309 cells, as evidenced by their high proliferative states (Fig. 3f and Supplementary Fig. 10a-b).
310 The differentiation trajectory of these intestinal epithelial cells was highly similar among the
311 four individual intestine segments (Supplementary Fig. 10c-f). Functional annotation analyses
312 based on the Gene Ontology (GO) database demonstrated that gene signatures of each cell
313 subgroup in intestinal stem cells were mainly enriched in cell cycle-related biological processes
314 as expected (Fig. 3g). The highly expressed genes in $BEST4^+$ enterocytes were over-
315 represented in cell development and morphogenesis, which was highly different from the
316 functions of immature and mature enterocytes. The gene sets restricted in enteroendocrine cells
317 were significantly enriched in signal release and protein secretion (Fig. 3g). The distinct
318 transcriptional profiles and functions of these cell types can be attributed to their diverse gene

319 regulatory programs ([Supplementary Fig. 11](#)). By inferring the TF activity across the trajectory,
320 we found that three master regulators, NFIB, STAT1, and ZBTB11, play essential roles in
321 enterocyte lineage specification by a coordinated sequential activation ([Fig. 3h and](#)
322 [Supplementary Fig. 12](#)). To compare the structures and intensities of cell-cell communication
323 across the four gut segments, we employed CellChat (42) to identify potential ligand-receptor
324 pairs among the major cell types. Our results revealed that EGF, PDGF, and BMP signaling
325 pathways were major communicating pathways in the porcine intestine segments ([Fig. 3i and](#)
326 [Supplementary Fig. 13](#)). Although the global interaction patterns were similar, the strength of
327 intercellular interactions was different across intestine segments. For instance, compared with
328 the colon, we observed stronger intercellular interactions among enterocytes, epithelial cells,
329 and intestinal stem cells in small intestine tissues. We further mapped ligand-receptor pairs in
330 specified cell subpopulations across different organs to understand the rewiring of molecular
331 interactions regulating cell-cell interactions. Notably, the “NAMPT-INSR” and “GHRL-
332 GHSR” ligand-receptor pairs were specific in interactions between enterocytes. Overall, our
333 findings highlight the importance of dynamic information exchange between different cells in
334 contributing to the diverse digestive functions of different intestine sections.

335

336 **A cross-tissue reference of immune cell types and states**

337 The immune system is a complex network of cell types distributed throughout the whole body
338 and provides protection against bacteria, viruses, and other pathogens. Understanding the
339 specific and shared features of tissue-resident immune cells is crucial for deciphering the
340 molecular mechanisms underlying immune responses and ultimately for accelerating precision
341 breeding of disease resistance in pigs. We identified a total of 45,491 immune cells prevailing
342 in 17 tissues, including T cells, B cells, natural killer cells (NK), macrophages, and other tissue-
343 resident immune cells ([Fig. 4a and Supplementary Fig. 14](#)). Hierarchical clustering analysis
344 revealed three main branches of immune cells: myeloid and lymphoid lineages, as well as
345 microglia, which are brain-resident macrophages ([Fig. 4b](#)). As expected, each tissue has its
346 own immune compartments, with specific immune cell compositions. For example, the four
347 major parts of the brain exclusively contain microglia cells. A large population of B cells was
348 evident in the spleen, whereas lymph nodes were enriched for multiple T cell types. We next
349 subdivided and reanalyzed the immune dataset to explore further heterogeneity within
350 macrophages and T cells, which were abundantly present across tissues. All tissue-resident
351 macrophages, together with monocytes, were divided into 13 more granular subsets, which
352 were supported by the expression of well-established marker genes ([Fig. 4c](#)). These

353 macrophage subgroups exhibited clear tissue-type separation and preference, although certain
354 subsets were shared by multiple tissues (Fig. 4b). For instance, the M1 macrophage subgroups
355 were enriched in muscle and liver, while M2 macrophages were mainly located in ileum and
356 ovary. To further dissect cell-type-specific transcriptional profiling, we performed pair-wise
357 differential expression analyses and identified 2,903 genes with restricted expression in one or
358 a few cell types (Supplementary Fig. 15a). Functional enrichment analysis evidenced the
359 presence of overrepresented biological processes for each macrophage subtype, which
360 recapitulated cell-type-specific functions regarding resident tissues and niches as well as
361 putative cellular states (Fig. 4d-f and Supplementary Fig. 15b). Furthermore, cell-type-specific
362 transcriptional programs were combinatorially controlled by several TFs with overlapping
363 expression patterns. The regulons KLF3 and CEBPB were exclusively expressed in monocyte
364 subsets and showed a gradual decrease in expression levels across the monocyte-to-
365 macrophage differentiation trajectory (Fig. 4g).

366

367 T cells play a crucial role in eliciting and controlling the adaptive immune response (43). We
368 identified seven T cell clusters based on known gene signatures, with CD4⁺ and CD8⁺ T cells
369 showing a distinct separation, while the remaining clusters were designated as general T cells
370 due to the absence of significant CD4 or CD8 surface molecules (Fig. 4h). CD4⁺ and CD8⁺ T
371 cells in our data were further divided into two subtle clusters, respectively, based on the
372 transcriptional differences of several classical markers like *CD3E* and *NCALD*. While these
373 annotated T cell clusters were observed in 14 organs, their relative proportion and enrichment
374 varied greatly across different organs (Fig. 4b). CD4⁺ T cells were primarily located in lymph
375 nodes and jejunum, whereas CD8⁺ T and NK cells were more abundant in heart and ovary. To
376 understand their potential diverse biological functions, we identified DEGs among these T cell
377 subtypes and then carried out a functional annotation. The majority of T cells shared several
378 enriched GO terms, like T cell activation and T cell receptor signaling pathway, suggesting
379 their shared immune functions regardless of tissue origins. Specifically, signatures of CD4⁺ T
380 cells were enriched for cell-cell adhesion, whereas CD8⁺ T cells had enhanced biological
381 functions in nuclear division and regulation of antigen receptor-mediated signaling pathway
382 (Fig. 4i and Supplementary Fig. 16). The distinct transcriptional profiles and molecular
383 functions were attributed mainly to the specific TF network (Fig. 4j). Overall, our study
384 provides valuable insights into the diversity and complexity of T cell populations across
385 different organs and sheds light on their roles in regulating the immune response.

386

387 **Genetic mapping and functional implications of cell-type-specific eQTL**

388 Bulk tissue samples often contain a high degree of cellular heterogeneity, which can mask
389 genetic effects that are active only in specific cell types within the sampled tissue. To address
390 this, we explored ieQTL by performing the cell-type deconvolution analysis of 3,921 bulk
391 RNA-seq samples in the PigGTEX project via this newly built cross-tissue cell atlas. First, we
392 tested the cell estimation performance of the CIBERSORT algorithm (44) in pigs by
393 deconvoluting pseudo-bulk samples generated from simulation studies using the SCDC
394 software (45). By employing the gene signature matrix built from our liver snRNA-seq data,
395 we observed that the estimated cell proportions from pseudo-bulk samples were highly
396 correlated with the putative cell populations identified in the liver snRNA-seq, with the highest
397 correlation in Hepatocyte_1 subtype (Pearson's $r = 0.841$, p -value $< 2.2 \times 10^{-16}$, **Supplementary**
398 **Fig. 17a-d**). This result indicated the feasibility and accuracy of our cellular deconvolution
399 pipeline in pigs. To identify cell-type-specific eQTL in an unbiased manner, we performed
400 eQTL deconvolution analysis by integrating our cross-tissue snRNA-seq data with the large-
401 scale bulk RNA-seq collections from the PigGTEX project.

402

403 The pseudo-bulk gene expressions of our snRNA-seq data were significantly correlated with
404 those of PigGTEX bulk samples across all the 17 matching tissues, with correlation coefficients
405 ranging from 0.498 (colon) to 0.745 (spleen), implying sufficient concordance for the
406 subsequent integration (**Supplementary Fig. 17e**). We thus estimated the relative cell fractions
407 of these 17 PigGTEX tissues using the snRNA-seq signature matrix of the respective tissues,
408 where sample sizes of PigGTEX tissues varied from 44 (kidney) to 1,321 (muscle). Overall,
409 most samples were well-deconvoluted (p -value < 0.05 , 1,000-times permutations) and revealed
410 a striking variability in cellular composition across the PigGTEX samples (**Fig. 5a** and
411 **Supplementary Fig. 18**). The number of putative cell types detected in deconvoluted samples
412 ranged from six (uterus) to 23 (ileum) (**Supplementary Fig. 17f**). In particular, the predicted
413 abundance of cell types in muscle and heart displayed considerable inter-individual variations,
414 with certain cell types in some samples even being totally missing, while colon and
415 hypothalamus showed less heterogeneous cell fractions across samples (**Supplementary Fig.**
416 **18**). To map ieQTL, we performed a linear regression analysis that models an interaction term
417 between estimated cell fractions and genotypes (46). We detected a total of 5,168 protein-
418 coding genes with at least one significant ieQTL (ieGenes) across cell types and tissues (**Fig.**
419 **5b**), with around a third of these ieQTL validated using the allele-specific expression approach.
420 Of note, muscle exhibited the highest number of significant ieGenes, followed by cerebrum,

421 testis, liver, and adipose tissues (Fig. 5c). The discovery power of ieGenes in tissue was
422 significantly correlated with its sample size (Fig. 5b). We detected an average of 114 ieGenes
423 across 79 cell types from 14 tissues. Among them, type IIx myonuclei had the largest number
424 of ieGenes ($n = 797$), whereas ileum Paneth cells only had one ieGene. For instance, the effects
425 of *rs3472489394* and *rs330736093* on *MANBA* and *SKOR2* significantly interacted with the
426 enrichment of type IIx myonuclei in muscle and Leydig cells in the testis, respectively (Fig.
427 5d-e and Supplementary Fig. 19a).

428

429 Furthermore, to explore the cellular effects of trait-associated variants, we performed a
430 colocalization analysis between ieQTL and GWAS hits of 268 complex traits in pigs
431 (Supplementary Table 2). Of the putative ieQTL, 305 loci colocalized with at least one pig
432 GWAS hit (Fig. 5f), indicating a potential involvement in the genetic control of complex traits.
433 By comparing GWAS colocalization results between standard PigGTEX eQTL and the newly
434 detected ieQTL, we found that a substantial proportion of GWAS signals ($> 81.96\%$) could be
435 colocalized by both ieQTL and eQTL (Fig. 5f-h, Supplementary Fig. 19b, and Supplementary
436 Table 3). For example, we found a promising colocalization between the *MANBA* gene in
437 muscle and loin muscle depth (Fig. 5g), which was supported by both ieQTL (posterior
438 probability of colocalization (PP4) = 0.88) and standard eQTL (PP4 = 0.82). Of note, there
439 were 37 ieQTL-specific GWAS colocalizations (Supplementary Table 3), representing 19
440 complex traits, which indicated the cell-specific regulation of these traits and their potential
441 cellular origin. We also discovered that some GWAS hits missed by bulk eQTL could be
442 retrieved by ieQTL. A noteworthy example was the Leydig cell ieQTL of *SKOR2* in testis (Fig.
443 5h), which colocalized with the GWAS signal for the number of born alive at birth (PP4 =
444 0.78), whereas the standard eQTL from bulk testis tissues did not (PP4 = 0.34). These results
445 together showcased the substantial potential of our cell atlas in dissecting the genetic control
446 of the transcriptome and complex phenotypes at single-cell resolution in pigs.

447

448 **Association of cell types with complex traits and diseases in pigs and humans**

449 Although ieQTL have provided new potential target genes and variants potentially underlying
450 GWAS loci, the causal cell types of complex phenotypes are yet to be fully understood. To
451 systematically infer the relevance of cell types with complex traits and diseases, we conducted
452 the GWAS signal enrichment analyses using the signature genes of each cell type. The complex
453 traits collected in the PigGTEX project (Supplementary Table 2) were grouped into five main
454 categories, including reproduction traits ($n = 71$), health traits ($n = 61$), meat and carcass traits

455 (n = 50), production traits (n = 19), and exterior traits (n = 6). Of the 263 high-resolution cell
456 clusters in all 19 tissues, 222 (84.41%) showed significant enrichments for at least one
457 phenotype category after multiple testing correction ([Supplementary Fig. 20](#)). For instance, the
458 litter size relevant traits were maximally enriched in the immune cell cluster, implying the
459 existence of critical relationships between immune function and piglet survival
460 ([Supplementary Fig. 21](#)). Notably, many reproduction traits, such as total number born alive
461 (NBA), total number of piglets born (TNB), and the number of stillborn pigs (NBS), showed a
462 significant enrichments in neuronal cell types such as oligodendrocyte in cerebrum and
463 cerebellum, in addition to Leydig cells in testis, endothelial cells in ovary and lumen cells in
464 uterus ([Fig. 6a](#) and [Supplementary Fig. 22](#)). Moreover, several production and growth traits,
465 including average daily gain (ADG), backfat thickness (BFT), and loin muscle area (LMA),
466 were enriched not only in three skeletal myocytes but also in pituitary somatotropes, intestine
467 enterocytes, and pancreatic acinar cells ([Fig. 6a](#)). However, we did not find any significant
468 enrichment for health and exterior traits, possibly due to their relatively low GWAS power. To
469 validate the results, we partitioned the heritability of two production traits, backfat thickness,
470 and loin muscle depth, by cell types in a large population of over 26,000 genotyped individuals
471 ([Fig. 6b-c](#)). As expected, we observed the enriched heritability of muscle depth trait in type IIx
472 myonuclei. Likewise, backfat thickness showed a remarkable enrichment for enterocytes in the
473 duodenum and enteroendocrine cells in the jejunum and colon. Although both results were
474 obtained from two datasets with different sample sizes and distinct enrichment approaches,
475 they showed to some extent consistency ([Fig. 6a-c](#)). Furthermore, through examining the gene-
476 traits/disorders from Online Mendelian Inheritance in Animals database (OMIA,
477 <https://omia.org/>), we identified notable cell-type-specific expression programs of many
478 essential genes. For example, *APOE*, a major risk factor gene for Alzheimer's disease (47),
479 showed higher transcription levels in the pig astrocyte and microglia subtypes compared to
480 other cell types. High levels of *CD163* expression (an essential receptor for the porcine
481 reproductive and respiratory syndrome (48)) were mainly observed in the Kupffer cells and
482 other macrophages.

483
484 To explore whether our pig cell atlas could help to understand the cellular mechanisms of
485 complex traits and diseases in humans, we quantified the heritability enrichment of 137 human
486 complex phenotypes ([Supplementary Table 4](#)) across the 261 annotated cell types (two cell
487 clusters defined as unknown types were discarded) via the stratified linkage disequilibrium
488 score regression analysis (LDSC). We retrieved 15,354 one-to-one pig-human orthologous

489 protein-coding genes from the Ensembl dataset (version 104) for the following analyses. Our
490 results revealed a total of 1,547 significant associations (the corrected enrichment p -value <
491 0.05) between pig cell types and human complex phenotypes (Fig. 6g, **Supplementary Fig. 23**,
492 and **Supplementary Table 5**). As expected, we observed significant enrichments of several
493 neurological and psychiatric phenotypes, such as multiple sclerosis, schizophrenia, and bipolar
494 disorder, in neural cell types, including excitatory neurons and neural progenitor cells from the
495 cerebrum, as well as in certain immune cell clusters such as microglia from cerebrum and
496 macrophages from pituitary. Additionally, metabolic traits, including type 2 diabetes and
497 cholesterol-related phenotypes, showed expected associations with hepatocytes, pancreatic
498 duct cells, and ileum goblet cells, as well as interesting associations with several skeletal
499 muscle and intestine cell populations. Moreover, our analysis revealed some novel
500 relationships between GWAS traits and cell types. For instance, we found enriched heritability
501 of several intestine diseases, such as Crohn's disease and diverticular disease, in cell clusters
502 corresponding to brain-resident immune cells (5, 15), in addition to enterocytes and immune
503 cells from the four intestine segments. For fasting insulin and glucose traits, we found
504 significant enrichments in adipocytes from adipose and skeletal muscle cells and enterocytes
505 from the intestines. Similarly, we observed striking enrichments of anthropometric traits,
506 including height, waist-hip ratio, and body fat percentage, not only in intestinal stem cells,
507 fibro-adipogenic progenitor cells from skeletal muscle, and adipocyte from adipose but also in
508 multiple cell populations from testis and ovary. Overall, our pig snRNA-seq data provided new
509 comprehensive insights into trait-relevant cell types in both pigs and humans, which will boost
510 the unraveling of molecular and cellular mechanisms underlying complex phenotypes and the
511 potential utilization of pigs as human biomedical models for certain diseases.

512

513 **Discussion**

514 The domestic pig (*Sus scrofa*) is a valuable livestock species that contributes significantly to
515 both agricultural and biomedical research. Recent studies, including our PigGTEx project, have
516 revealed that many traits-associated variants are located in non-coding regions and affect the
517 spatiotemporal expression of candidate genes in a context-specific (tissue- or cell-type-specific)
518 fashion. However, the impacts of genetic variations on these regulatory pathways and how they
519 vary across trait-relevant cell types have not been explored in pigs. To bridge the gaps between
520 genetic variants and phenotypes at single-cell resolution, we performed a comprehensive
521 analysis by integrating a cross-tissue snRNA-seq atlas with the large-scale PigGTEx datasets.
522 This work not only establishes a comprehensive single-cell reference map as a baseline for

523 dissecting cellular heterogeneity within and across tissues but also highlights a more powerful
524 strategy for identifying trait-critical cellular signatures and cell-type-specific eQTL in pigs.

525
526 The present study employed single-nucleus RNA-seq to profile gene expression in 229,268
527 high-quality cells from 19 tissues in pigs, similar to a recent study (27) which constructed the
528 first single-cell transcriptomic atlas of 222,526 cells across 20 swine tissues. Compared with
529 that work, our dataset represents a broader range of pig organ sources covering nine major
530 body systems and especially comprises several highly important tissues in pig production
531 performance, such as skeletal muscle, four intestine segments, and three reproductive organs.
532 Given the large diversity in the chosen tissues, the two studies demonstrate a good complement
533 and represent very significant contributions to the efforts of the pig single-cell consortium. In
534 line with single-cell landscapes in other species (1, 2, 4, 5, 49-51), we identified primary cell
535 classes based on known canonical marker genes and captured a few rare cell types like Purkinje
536 cells from the brain and enteroendocrine cells from the intestine, which may facilitate our
537 understanding of cell lineage trajectory and tissue homeostasis. Our pig cross-tissue cell atlases
538 clarify the heterogeneous characteristics in cellular compositions and molecular properties
539 within and across tissues. For example, we delineated the global transcriptional divergence and
540 transition pattern among three dominant myofiber types (type I, IIa/b, and IIx) and revealed
541 evolutionarily conserved similarity in pivotal genes specializing myofiber, such as *MYH7* and
542 *MYBPC2* across mammals (35, 36, 52). This finding may have important implications for
543 improving meat quality and quantity, which are largely affected by myofiber characteristics
544 and proportions in pigs (53, 54). Type II myonuclei exhibited a notable enrichment in metabolic
545 processes, indicating their crucial involvement in metabolic traits and syndromes, *i.e.*, meat
546 production and fat deposition in pigs and type 2 diabetes and obesity in humans (35, 55-57).
547 Our data also demonstrate the prevalence of epithelial and immune cells across different tissue
548 contexts and offer a more detailed understanding of cell compartments. Although some cells
549 of a common type are shared across tissues, subpopulations are specifically enriched in
550 particular tissues. These tissue-resident epithelial and immune subsets are specialized to fulfill
551 the specific functional demands of different tissues, probably owing to unique local
552 environments or niches (50, 58).

553

554 Although our PigGTEX project has provided a compendium of genetic regulatory effects across
555 pig tissues and functional variants underlying complex traits (24), a comprehensive
556 understanding of gene regulation at the single-cell resolution for most non-coding loci is still

557 lacking. To address this issue, emerging approaches such as single-cell eQTL and heritability
558 enrichment analyses have been extensively used in deciphering complex human traits and
559 diseases (14-17, 46) but have yet to be systematically applied in pig studies. As a critical
560 component of the PigGTEX project, our work offers an in-depth dissection of the genetic effects
561 of trait-critical cellular signatures and cell-type-specific eQTL, in addition to the
562 comprehensive pig cell reference map, setting it apart from other recent single-cell studies (27).
563 We revealed that around 15% of the loci that co-localized with GWAS traits showed significant
564 cell-type specificity, underscoring the advantages of single-cell eQTL analysis over the
565 standard bulk eQTL approach. The proportion missed by bulk studies is slightly lower than
566 what has been described in humans (46), which might be attributed to the limited sample size
567 in our work. By linking individual cell types to complex traits, we identified substantial cell-
568 type-trait associations that are consistent with previous studies (5, 15, 16, 35), suggesting high
569 functional conservation of major cell types among mammal species (52). Furthermore, we
570 mapped several unique associations between cell types and important phenotypes in pigs, such
571 as the driving roles of myofiber cell types for meat production traits and Leydig cells from the
572 testis for reproduction traits. Overall, our results provide meaningful insights into previously
573 cryptic molecular and cellular mechanisms behind traits of economic importance and offer new
574 opportunities for precision breeding in pigs.

575
576 Despite the significant findings of our study, several limitations must be noted. Firstly, the
577 current dataset comprises only one male and one female pig and is not an exhaustive
578 characterization of all pig organs. As such, we cannot fully capture the complete single-cell
579 picture and inter-individual variation in cellular composition, potentially limiting our ability to
580 explore rare cell types and map entire trait-associated cellular signatures. Secondly, compared
581 with single-cell RNA-seq, our single-nucleus RNA-seq can only profile nuclear transcripts and
582 not cytoplasmic transcripts. Different library preparation protocols may result in a reasonable
583 proportion difference in specific cell types, such as muscle, neural, and immune cells, despite
584 globally consistent detection performance in gene number and cell type diversity between them
585 (59, 60). Lastly, the sample size of certain tissues used in cellular deconvolution and heritability
586 partitioning analyses is relatively small, limiting the statistical power to detect causative trait-
587 associated cell types and single-cell eQTL. Therefore, future studies that incorporate larger
588 sample sizes, a broader range of tissues, and multiple complementary single-cell approaches
589 will be required to provide robust evidence and facilitate a more comprehensive interpretation
590 of our findings.

591
592 In summary, this study presents a compendium of high-resolution body-wide single-cell
593 transcriptional landscape, provides a deeper understanding of the expression patterns and
594 functions of tissue-specific and shared cell types, and illuminates the intricate cell-cell
595 interactions governing tissue homeostasis. Through pioneering single-cell eQTL and
596 colocalization analyses in pigs, we pinpointed the likely causative cell-type-associated variants
597 and genes underlying traits of economic importance. Additionally, thousands of cell-type-trait
598 associations were discovered, and previously unexplored biological mechanisms were
599 explicated using heritability enrichment analysis. Collectively, these findings will significantly
600 enhance our understanding of cross-tissue and cross-individual variations of cellular
601 phenotypes and highlight promising trait-associated determinants (variants and cell types) for
602 advancing the fields of future pig breeding and human biomedical research.

603
604 **Methods**

605 **Ethics statement**
606 All animal protocols and procedures were implemented in compliance with the Guide for the
607 Care and Use of Experimental Animals established by the Ministry of Agriculture and Rural
608 Affairs (Beijing, China) and were approved by the Institutional Animal Care and Use
609 Committee of the Chinese Academy of Agricultural Sciences. Prior to tissue sampling, the pigs
610 were humanely euthanized as necessary to minimize their suffering.

611
612 **Tissue collection and single-nucleus suspension**

613 One male and one female Meishan pig, aged 180 days, were obtained from a commercial pig
614 farming company managed under the same conditions (Nantong, Jiangsu). Nineteen tissues,
615 including adipose, cerebellum, cerebrum, colon, duodenum, heart, hypothalamus, ileum,
616 jejunum, kidney, liver, lymph, muscle, ovary, pancreas, pituitary, spleen, testis, and uterus,
617 were freshly harvested from postmortem samples. Each tissue was kept on ice and minced into
618 5-10 pieces weighing approximately 50-100 mg each on ice with sterilized scissors. Tissue
619 samples were then snap-frozen in liquid nitrogen and stored at -80°C until nuclear extraction
620 was performed. Single-nucleus isolation was conducted as previously described (28, 59).
621 Briefly, tissue samples were homogenized using the Dounce homogenizer with 25 strokes of
622 the loose pestle A and then 25 strokes of the tight pestle B in 1 ml of ice-cold homogenization
623 buffer. After this, the mixture was filtered through a 40-µm cell strainer into a 1.5-ml tube. To
624 collect dissociated single nuclei, the sample was centrifuged at 500g for 5 min at 4°C, and the

625 supernatant was discarded. After centrifugation, the nuclear pellet was resuspended using an
626 appropriate amount of 1× PBS/0.5% BSA with RNase inhibitor, filtered through a 40-μm cell
627 strainer, and counted. A final concentration of 1,000 nuclei per μl was used for library
628 preparation.

629

630 **Single-nucleus RNA-seq library preparation and sequencing**

631 The single-nucleus RNA-seq libraries were prepared following the standard protocol supplied
632 by 10× Genomics (Berry Genomics, Beijing, China). In brief, isolated nuclei were captured in
633 droplets with gel beads in the Chromium Controller. Following the RNA reverse transcription
634 step, emulsions were broken, and barcoded cDNA was purified with Dynabeads, after which
635 PCR amplification was performed. The amplified cDNA was then used for 3' gene expression
636 library construction. Then, indexed libraries were constructed according to the manufacturer's
637 recommendations. After quality control, eligible libraries were sequenced on the Novaseq 6000
638 platform (Illumina) in a 150 bp paired-end manner. The first 28 bp in read 1 captured both the
639 16 bp 10× barcode and the 12 bp UMI.

640

641 **Preprocessing of snRNA-seq data**

642 The Sscrofa11.1 reference assembly (61) in FASTA format and annotated gene model in GTF
643 format were downloaded from the Ensembl database (<ftp://ftp.ensembl.org/pub/release-101/>).
644 Raw snRNA-seq data were aligned to the pig reference genome and subjected to barcode
645 assignment and unique molecular identifier (UMI) counting using the commands
646 recommended by the CellRanger pipeline (10× Genomics, CA, USA). Given that snRNA-seq
647 captures both unspliced pre-mRNA and mature mRNA, we used the *include-introns* option for
648 counting exonic and intronic reads together. The filtered gene expression matrix was used for
649 further analysis with the Seurat package (62). To ensure the accuracy and robustness of our
650 results, we removed ambient RNA and potential doublets using DecontX (63) and
651 DoubletFinder (64) with default settings. We also filtered out low-quality nuclei expressing
652 less than 200 genes or more than 5,000 genes, and less than 500 UMIs or more than 15,000
653 UMIs, as well as those exceeding 5% of mitochondrial content. During the gene filter step, all
654 genes not expressed in at least three nuclei were removed. In addition, to balance our dataset
655 in subsequent analyses, we randomly selected 20,000 nuclei from the spleen, as it had a much
656 higher number (n = 53,444) of captured nuclei compared to other tissues.

657

658 **Cell clustering and cell type annotation**

659 After filtering, the remaining high-quality data were log-normalized and scaled to account for
660 cell-to-cell variation with regression on the number of UMIs and percentage of mitochondrial
661 genes. Subsequently, PCA linear dimensionality reduction analysis was performed, followed
662 by t-Distributed Stochastic Neighbor Embedding (t-SNE) and Uniform Manifold
663 Approximation and Projection (UMAP) visualization approaches using the Scanorama tool
664 (65), to capture the global cell type landscapes across tissues. For individual clustering, each
665 tissue dataset was visualized using the Seurat package (62). Parameters used in each function
666 were manually curated to obtain the optimal clustering of cells by adjusting the number of
667 principal components and resolutions on a per-dataset basis. We employed the *FindAllMarkers*
668 or *FindMarkers* function with default parameters to identify marker genes of each cluster and
669 annotated each cell type based on known classical markers from extensive published literature.
670 The Pearson or Spearman correlation coefficients among cell types were calculated using the
671 average expression of the top 1,000 highly variable features, and we used the pheatmap
672 package (<https://github.com/raivokolde/pheatmap>) to visualize the results. Besides, the
673 expression of marker genes in different cell types was visualized with the ggplot2 R package.
674

675 **Cell type diversity estimation**

676 Shannon entropy was calculated to evaluate cell type diversity in each tissue with a previously
677 published method (14) according to the formula $-\sum_x(p_x \times \log_2(p_x))$, where p_x is the
678 proportion of each cell type x in a tissue. The entropy value per tissue was plotted using the
679 ggplot2 R package.
680

681 **Pseudotime trajectory inference and RNA velocity analysis**

682 The cell lineage trajectory was inferred using Monocle 3 (41) according to the standard tutorial.
683 We used the built-in DDRTree algorithm for dimensional reduction and visualization after
684 constructing the cell trajectory. Notably, the root state of the inferred trajectory was specified
685 based on existing biological knowledge. Furthermore, we predicted the velocity streams and
686 latent time assignments from sorted bam files using the dynamical model implemented in
687 scVelo (66).
688

689 **Cell cycle index estimation**

690 To further infer dynamic information about cell state, we calculated a cell cycle index for each
691 cell type with a previously published method (3). Typically, progenitor cells with rapidly

692 dividing capacity display higher cycling indices, whereas cell types that are known to be largely
693 quiescent exhibit lower values.

694

695 **Cell-cell interaction analysis**

696 To investigate cellular communication patterns between different cell types, we used the
697 CellChat package (42) with default parameters, which is a manually curated database of
698 literature-supported ligand-receptor interactions in humans and mice. To run CellChat analysis
699 in pig datasets, we mapped pig gene symbols to human orthologs. Ligand-receptor pairs with
700 p -value < 0.05 were considered to be significant.

701

702 **Tissue enrichment of clusters**

703 We estimated the enrichment of each cell cluster across tissues, as previously described (67).
704 In brief, we calculated the observed and expected cell numbers in each cell cluster to compute
705 the ratio ($R_{o/e}$) between the two values using the epitools R package. We considered a cluster
706 to be enriched in a specific tissue if $R_{o/e} > 1$.

707

708 **Gene ontology (GO) enrichment analysis**

709 Gene Ontology (GO) analysis was performed using the clusterProfiler 4.0 (68) and
710 org.Hs.eg.db annotation package, considering that genome-wide annotation is incomplete in
711 pigs. The Benjamini-Hochberg (BH) procedure was used for the multiple testing corrections,
712 and only GO terms with an adjusted p -value smaller than 0.05 were retained.

713

714 **Single-cell regulatory network analysis**

715 To uncover cell-type-specific transcription regulons and construct gene regulation networks
716 (GRNs), we conducted single-cell regulatory network inference and clustering analysis using
717 the SCENIC suite (69) with the default parameters. The original expression matrix was
718 normalized with Seurat and fed into SCENIC to build a coexpression network using the built-
719 in GRNBoost2 algorithm. The activity of regulons in each cell was calculated by the AUCell
720 algorithm.

721

722 **Cellular deconvolution analysis using CIBERSORT**

723 For each tissue, we first identified differentially expressed genes specific to each cell type using
724 the *Findmarkers* function in the Seurat package. We then selected the top 50 genes with the
725 most significant overexpression, based on adjusted p -value (< 0.05) and average \log_2 fold

726 change (> 0.5), to build the gene expression signature matrix for the cell-type reference set. To
727 predict the abundances of cell types in a mixed cell population for each tissue, we collected the
728 RNA-seq expression matrix of 17 matching bulk tissues with our snRNA-seq data from the
729 PigGTEX database (<http://piggtex.farmgtx.org/>). Subsequently, the CIBERSORT tool (44)
730 was selected for cellular deconvolution analysis, given its great resolving power (70). To test
731 the robustness of cellular deconvolution, we first used the *generateBulk_norep* function in the
732 SCDC package (45) to obtain the transcript per million (TPM) matrix of 1,000 pseudo bulk
733 samples (default parameters) with known cell type distribution based on our liver snRNA-seq
734 data. Then we used CIBERSORT to perform deconvolution on these samples using the TPM
735 matrix of signature genes from each cell type in pig liver. The number of permutation tests was
736 set to 1,000 times to determine the significance level, and $p < 0.05$ was regarded as statistical
737 significance. Finally, we calculated the Spearman correlation coefficient between the known
738 and predicted cell type distribution of hepatocyte cells to assess the accuracy of CIBERSORT
739 deconvolution in our pig dataset.

740

741 **Cell type interaction *cis*-eQTL mapping**

742 To detect whether a *cis*-eQTL explicitly affects gene expression in a given cell type, we
743 performed cell type interaction *cis*-eQTL (ieQTL) mapping for 17 bulk tissues of PigGTEX.
744 We used the cell type composition (i.e., enrichment score) estimated from CIBERSORTx as
745 above and only considered cell types with an enrichment score > 0 in at least 20 samples and/or
746 20% of samples within a tissue. For each tissue-cell type pair, we performed ieQTL mapping
747 via a linear regression model implemented in TensorQTL (v1.0.3) (71), which included an
748 interaction term between genotype and cell type enrichment score:

749
$$\mathbf{y} \sim \mathbf{g} + \mathbf{b} + \mathbf{g} \times \mathbf{b} + A$$

750 where \mathbf{y} is the vector of gene expression values (i.e., the inverse normal transformed TMM),
751 \mathbf{g} is the genotype dosage (i.e., 0/1/2) vector of the tested SNP from PigGTEX samples, \mathbf{b} is the
752 enrichment score of a given cell type predicted from snRNA-seq data, $\mathbf{g} \times \mathbf{b}$ is the interaction
753 term between genotype and enrichment score, and A represents the covariates (i.e., genotype
754 PCs and PEER factors, detailed in PigGTEX pilot phase). For the ieQTL mapping, we only
755 considered SNPs within ± 1 Mb of transcription start sites (TSS) of each gene. We eliminated
756 those SNPs with minor allele frequency (MAF) < 0.05 in the top and/or bottom 50% of samples
757 sorted by the enrichment score of each cell type, using TensorQTL (v1.0.3) with parameter: --
758 maf_threshold_interaction 0.05. To correct for the multiple testing at the gene level, we used

759 eigenMT (72) in TensorQTL for calculating the top nominal p-value of each gene. We then
760 computed the genome-wide significance of genes using the Benjamini-Hochberg FDR
761 correction on the eigenMT-corrected *p*-values and defined as ieGene that with at least one
762 significant ieQTL (*i.e.*, FDR-corrected *p*-value < 0.05).

763

764 **Allele-specific expression validation of ieQTL**

765 We used allele-specific expression (ASE) data at the individual level to validate the discovered
766 ieQTL. We first estimated the effect size (*i.e.*, allelic fold change, aFC) of the top ieQTL for
767 each ieGene from ASE data using the script phaser_cis_var.py in phASER (v1.1.1) (73) and
768 considered only ieQTL with nominally significant ASE (*p*-value < 0.05) data in more than ten
769 heterozygous individuals with more than eight reads for a gene. To filter out outlier samples in
770 ASE data, we applied the median absolute deviation (MAD) based on Hampel's test to the
771 allelic imbalance (AI) ratio values ($|\frac{\text{Reference reads}}{\text{Total reads}} - 0.5|$) across samples (46, 74). When a
772 sample had $|\text{AI}_i - \text{median}(\text{AI})| \geq 4.5 \times \text{MAD}$, where $\text{MAD} = \text{median}(|\text{AI}_i - \text{median}(\text{AI})|)$
773 and AI_i is the allelic imbalance ratio value for the *i*th individual, we defined it as an outlier and
774 eliminated it in the validation process. Within a given tissue, we determined that an ieQTL was
775 validated by ASE data if it presented a nominally significant (*p*-value < 0.05) Pearson's
776 correlation between allelic fold change (aFC) of an ASE locus and cell type enrichment score
777 across samples.

778

779 **Colocalization between ieQTL and GWAS loci**

780 To identify shared association variants between the ieQTL and GWAS loci retrieved from the
781 PigGTEX project, we performed colocalization analysis using the Bayesian statistical
782 procedure implemented in Coloc (v5.1) (75). Briefly, we used the summary statistics of ieQTL
783 for each ieGene and its matched GWAS loci as input for Coloc. We only considered the GWAS
784 loci with at least one SNP with a *p*-value < 1×10^{-5} . We obtained posterior probabilities PP4
785 from the *coloc.abf* function with default parameters, where PP4 represents the probabilities of
786 a shared variant affecting both the gene expression of a given cell type and the complex trait.
787 We defined ieGene-trait pairs with $\text{PP4} > 0.5$ as significant colocalization. In addition, to
788 compare whether eQTL differ from ieQTL in terms of colocalization with GWAS loci, we used
789 the same pipeline employed for ieQTL scanning to perform the colocalization analysis for
790 eQTL for each ieGene and its matched GWAS loci as well.

791

792 **Genetic mapping of cell type specificity for complex traits in pigs and humans**

793 To uncover associations of traits with cell types, we performed an enrichment analysis of
794 significant GWAS loci and cell-type-specific genes using the LOLA (v1.22.0) R package (76).
795 Specifically, we extracted the top 200 cell-type-specific genes sorted in ascending order by the
796 *p*-value for each of the 19 available tissues and created an annotation based on the genomic
797 regions of these candidate genes for each cell type-tissue pair. We then examined the GWAS
798 summary statistics of 268 pig complex traits and selected significant genetic variants with *p* <
799 5×10^{-8} for each trait (24), using all tested SNPs of the 268 GWAS summaries as the
800 background set. Finally, we calculated the significance level (*p*-value) of the enrichment fold
801 using Fisher's exact test with FDR correction and defined trait-tissue-cell type trios with *p*-
802 value < 0.05 as significant enrichment. Furthermore, we expanded our enrichment analysis to
803 a larger Duroc population (> 26,000 individuals) from a commercial company, given that the
804 current GWAS dataset is relatively small. We performed heritability enrichment analysis for
805 backfat thickness and loin muscle depth traits with genomic partitioning of quantitative genetic
806 variance similar to (77). A total of 11.7 M imputed variants that had been quality-controlled
807 were grouped into two sets: one containing variants within ± 10 Kb of the top genes specific to
808 each cell type, and the other containing the remaining variants. Per-variant heritability
809 enrichment was calculated for each cell type-specific variant set.

810

811 To test the enrichment of genes associated with human traits and diseases for each specific pig
812 cell type, we collected the GWAS summary statistics of 137 human complex traits from the
813 UK Biobank and public literature (Supplementary Table 4). We converted cell-type-specific
814 genes in pigs to the corresponding human orthologous genes with one-to-one mapping with the
815 Ensembl database. Finally, we employed linkage disequilibrium (LD) score regression analysis
816 (<https://github.com/bulik/ldsc>) (78, 79) to partition the heritability based on 262 annotations,
817 including 261 cell-type-specific gene lists and one base annotation including all SNPs.
818 Heritability enrichment was calculated as the proportion of trait heritability contributed by
819 SNPs in the annotation over the total proportion of SNPs in that annotation. We reported the
820 coefficient *p*-value as a measure of the association of each cell type with the traits. All plots
821 showed the predicted z-score of partitioned LD score regression.

822

823 **Statistics and reproducibility**

824 If not specified, all statistical analyses and data visualization were performed in the R
825 environment. No statistical method was used to predetermine sample size, no data were

826 excluded from the analyses, and all analyses were not randomized, ensuring maximum
827 reproducibility.

828

829 **Data availability**

830 Raw sequencing reads generated by this work were deposited in the National Center for
831 Biotechnology Information database under the accession number GSE233285. Analysis codes
832 in this work are available at <https://github.com/chenlijuan009/PigCellAtlas>.

833

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847

848 **Author contributions**

849 G.Y. and L.F. conceived and designed the study. X.Q., Z.C., and L.Y. were responsible for
850 sample collection. L.C., H.L., J.T., Z.W., X.C., Jinghui L. H.Z., Z.B., and J.J. conducted
851 bioinformatic analysis. L.C. and Z.W. performed snRNA-seq analyses. H.L., X.P., and J.T.
852 contributed to eQTL mapping and cellular deconvolution. J.T., X.C., Jinghui L., J.J., Z.Z., and
853 Jiaqi L. were responsible for GWAS data collection and analysis in pigs and humans. G.Y.,
854 H.L., J.T., and Jinghui L. wrote the initial draft of the manuscript. G.Y., L.F., J.J., G.E.L., F.W.,
855 L.L., Y.L., G.S., M.S.L., M.B., D.C.P., P.K.M., M.F., A.C., M.A., C.L., C.K.T., and O.M.
856 revised the manuscript. All authors read and approved the final manuscript.

857

858 **Competing interests**

859 The authors declare no competing interests.

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861 References

- 862 1. X. Han, R. Wang, Y. Zhou, L. Fei, H. Sun, S. Lai, A. Saadatpour, Z. Zhou, H. Chen, F. Ye, D. Huang, Y. Xu, W. Huang, M. Jiang, X. Jiang, J. Mao, Y. Chen, C. Lu, J. Xie, Q. Fang, Y. Wang, R. Yue, T. Li, H. Huang, S. H. Orkin, G. C. Yuan, M. Chen, G. Guo, Mapping the Mouse Cell Atlas by Microwell-Seq. *Cell* **173**, 1307 (2018).
- 863 2. X. Han, Z. Zhou, L. Fei, H. Sun, R. Wang, Y. Chen, H. Chen, J. Wang, H. Tang, W. Ge, Y. Zhou, F. Ye, M. Jiang, J. Wu, Y. Xiao, X. Jia, T. Zhang, X. Ma, Q. Zhang, X. Bai, S. Lai, C. Yu, L. Zhu, R. Lin, Y. Gao, M. Wang, Y. Wu, J. Zhang, R. Zhan, S. Zhu, H. Hu, C. Wang, M. Chen, H. Huang, T. Liang, J. Chen, W. Wang, D. Zhang, G. Guo, Construction of a human cell landscape at single-cell level. *Nature* **581**, 303-309 (2020).
- 864 3. C. Tabula Sapiens, R. C. Jones, J. Karkanias, M. A. Krasnow, A. O. Pisco, S. R. Quake, J. Salzman, N. Yosef, B. Bulthaup, P. Brown, W. Harper, M. Hemenez, R. Ponnusamy, A. Salehi, B. A. Sanagavarapu, E. Spallino, K. A. Aaron, W. Concepcion, J. M. Gardner, B. Kelly, N. Neidlinger, Z. Wang, S. Crasta, S. Kolluru, M. Morri, A. O. Pisco, S. Y. Tan, K. J. Travaglini, C. Xu, M. Alcantara-Hernandez, N. Almanzar, J. Antony, B. Beyersdorf, D. Burhan, K. Calcuttawala, M. M. Carter, C. K. F. Chan, C. A. Chang, S. Chang, A. Colville, S. Crasta, R. N. Culver, I. Cvijovic, G. D'Amato, C. Ezran, F. X. Galdos, A. Gillich, W. R. Goodyer, Y. Hang, A. Hayashi, S. Houshdaran, X. Huang, J. C. Irwin, S. Jang, J. V. Juanico, A. M. Kershner, S. Kim, B. Kiss, S. Kolluru, W. Kong, M. E. Kumar, A. H. Kuo, R. Leylek, B. Li, G. B. Loeb, W. J. Lu, S. Mantri, M. Markovic, P. L. McAlpine, A. de Morree, M. Morri, K. Mrouj, S. Mukherjee, T. Muser, P. Neuhofer, T. D. Nguyen, K. Perez, R. Phansalkar, A. O. Pisco, N. Puluca, Z. Qi, P. Rao, H. Raquer-McKay, N. Schaum, B. Scott, B. Seddighzadeh, J. Segal, S. Sen, S. Sikandar, S. P. Spencer, L. C. Steffes, V. R. Subramaniam, A. Swarup, M. Swift, K. J. Travaglini, W. Van Treuren, E. Trimm, S. Veizades, S. Vijayakumar, K. C. Vo, S. K. Vorperian, W. Wang, H. N. W. Weinstein, J. Winkler, T. T. H. Wu, J. Xie, A. R. Yung, Y. Zhang, A. M. Detweiler, H. Mekonen, N. F. Neff, R. V. Sit, M. Tan, J. Yan, G. R. Bean, V. Charu, E. Forgo, B. A. Martin, M. G. Ozawa, O. Silva, S. Y. Tan, A. Toland, V. N. P. Vemuri, S. Afik, K. Awayan, O. B. Botvinnik, A. Byrne, M. Chen, R. Dehghannasiri, A. M. Detweiler, A. Gayoso, A. A. Granados, Q. Li, G. Mahmoudabadi, A. McGeever, A. de Morree, J. E. Olivieri, M. Park, A. O. Pisco, N. Ravikumar, J. Salzman, G. Stanley, M. Swift, M. Tan, W. Tan, A. J. Tarashansky, R. Vanheusden, S. K. Vorperian, P. Wang, S. Wang, G. Xing, C. Xu, N. Yosef, M. Alcantara-Hernandez, J. Antony, C. K. F. Chan, C. A. Chang, A. Colville, S. Crasta, R. Culver, L. Dethlefsen, C. Ezran, A. Gillich, Y. Hang, P. Y. Ho, J. C. Irwin, S. Jang, A. M. Kershner, W. Kong, M. E. Kumar, A. H. Kuo, R. Leylek, S. Liu, G. B. Loeb, W. J. Lu, J. S. Maltzman, R. J. Metzger, A. de Morree, P. Neuhofer, K. Perez, R. Phansalkar, Z. Qi, P. Rao, H. Raquer-McKay, K. Sasagawa, B. Scott, R. Sinha, H. Song, S. P. Spencer, A. Swarup, M. Swift, K. J. Travaglini, E. Trimm, S. Veizades, S. Vijayakumar, B. Wang, W. Wang, J. Winkler, J. Xie, A. R. Yung, S. E. Artandi, P. A. Beachy, M. F. Clarke, L. C. Giudice, F. W. Huang, K. C. Huang, J. Idoyaga, S. K. Kim, M. Krasnow, C. S. Kuo, P. Nguyen, S. R. Quake, T. A. Rando, K. Red-Horse, J. Reiter, D. A. Relman, J. L. Sonnenburg, B. Wang, A. Wu, S. M. Wu, T. Wyss-Coray, The Tabula Sapiens: A multiple-organ, single-cell transcriptomic atlas of humans. *Science* **376**, eabl4896 (2022).
- 865 4. Y. Liao, L. Ma, Q. Guo, W. E. X. Fang, L. Yang, F. Ruan, J. Wang, P. Zhang, Z. Sun, H. Chen, Z. Lin, X. Wang, X. Wang, H. Sun, X. Fang, Y. Zhou, M. Chen, W. Shen, G.

907 Guo, X. Han, Cell landscape of larval and adult *Xenopus laevis* at single-cell resolution.
908 *Nature Communications* **13**, 4306 (2022).

909 5. L. Han, X. Wei, C. Liu, G. Volpe, Z. Zhuang, X. Zou, Z. Wang, T. Pan, Y. Yuan, X.
910 Zhang, P. Fan, P. Guo, Y. Lai, Y. Lei, X. Liu, F. Yu, S. Shangguan, G. Lai, Q. Deng,
911 Y. Liu, L. Wu, Q. Shi, H. Yu, Y. Huang, M. Cheng, J. Xu, Y. Liu, M. Wang, C. Wang,
912 Y. Zhang, D. Xie, Y. Yang, Y. Yu, H. Zheng, Y. Wei, F. Huang, J. Lei, W. Huang, Z.
913 Zhu, H. Lu, B. Wang, X. Wei, F. Chen, T. Yang, W. Du, J. Chen, S. Xu, J. An, C. Ward,
914 Z. Wang, Z. Pei, C. W. Wong, X. Liu, H. Zhang, M. Liu, B. Qin, A. Schambach, J.
915 Isern, L. Feng, Y. Liu, X. Guo, Z. Liu, Q. Sun, P. H. Maxwell, N. Barker, P. Munoz-
916 Canoves, Y. Gu, J. Mulder, M. Uhlen, T. Tan, S. Liu, H. Yang, J. Wang, Y. Hou, X.
917 Xu, M. A. Esteban, L. Liu, Cell transcriptomic atlas of the non-human primate *Macaca*
918 *fascicularis*. *Nature* **604**, 723-731 (2022).

919 6. J. Lonsdale, J. Thomas, M. Salvatore, R. Phillips, E. Lo, S. Shad, R. Hasz, G. Walters,
920 F. Garcia, N. Young, B. Foster, M. Moser, E. Karasik, B. Gillard, K. Ramsey, S.
921 Sullivan, J. Bridge, H. Magazine, J. Syron, J. Fleming, L. Siminoff, H. Traino, M.
922 Mosavel, L. Barker, S. Jewell, D. Rohrer, D. Maxim, D. Filkins, P. Harbach, E.
923 Cortadillo, B. Berghuis, L. Turner, E. Hudson, K. Feenstra, L. Sabin, J. Robb, P.
924 Branton, G. Korzeniewski, C. Shive, D. Tabor, L. Qi, K. Groch, S. Nampally, S. Buia,
925 A. Zimmerman, A. Smith, R. Burges, K. Robinson, K. Valentino, D. Bradbury, M.
926 Cosentino, N. Diaz-Mayoral, M. Kennedy, T. Engel, P. Williams, K. Erickson, K.
927 Ardlie, W. Winckler, G. Getz, D. DeLuca, D. MacArthur, M. Kellis, A. Thomson, T.
928 Young, E. Gelfand, M. Donovan, Y. Meng, G. Grant, D. Mash, Y. Marcus, M. Basile,
929 J. Liu, J. Zhu, Z. Tu, N. J. Cox, D. L. Nicolae, E. R. Gamazon, H. K. Im, A.
930 Konkashbaev, J. Pritchard, M. Stevens, T. Flutre, X. Wen, E. T. Dermitzakis, T.
931 Lappalainen, R. Guigo, J. Monlong, M. Sammeth, D. Koller, A. Battle, S. Mostafavi,
932 M. McCarthy, M. Rivas, J. Maller, I. Rusyn, A. Nobel, F. Wright, A. Shabalina, M.
933 Feolo, N. Sharopova, A. Sturcke, J. Paschal, J. M. Anderson, E. L. Wilder, L. K. Derr,
934 E. D. Green, J. P. Struewing, G. Temple, S. Volpi, J. T. Boyer, E. J. Thomson, M. S.
935 Guyer, C. Ng, A. Abdallah, D. Colantuoni, T. R. Insel, S. E. Koester, A. R. Little, P. K.
936 Bender, T. Lehner, Y. Yao, C. C. Compton, J. B. Vaught, S. Sawyer, N. C. Lockhart,
937 J. Demchok, H. F. Moore, The Genotype-Tissue Expression (GTEx) project. *Nat. Genet.*
938 **45**, 580-585 (2013).

939 7. F. Aguet, S. Anand, K. G. Ardlie, S. Gabriel, G. A. Getz, A. Graubert, K. Hadley, R. E.
940 Handsaker, K. H. Huang, S. Kashin, X. Li, D. G. MacArthur, S. R. Meier, J. L. Nedzel,
941 D. T. Nguyen, A. V. Segrè, E. Todres, B. Balliu, A. N. Barbeira, A. Battle, R.
942 Bonazzola, A. Brown, C. D. Brown, S. E. Castel, D. F. Conrad, D. J. Cotter, N. Cox, S.
943 Das, O. M. de Goede, E. T. Dermitzakis, J. Einson, B. E. Engelhardt, E. Eskin, T. Y.
944 Eulalio, N. M. Ferraro, E. D. Flynn, L. Fresard, E. R. Gamazon, D. Garrido-Martín, N.
945 R. Gay, M. J. Gloudemans, R. Guigó, A. R. Hame, Y. He, P. J. Hoffman, F.
946 Hormozdiari, L. Hou, H. K. Im, B. Jo, S. Kasela, M. Kellis, S. Kim-Hellmuth, A.
947 Kwong, T. Lappalainen, X. Li, Y. Liang, S. Mangul, P. Mohammadi, S. B.
948 Montgomery, M. Muñoz-Aguirre, D. C. Nachun, A. B. Nobel, M. Oliva, Y. Park, Y.
949 Park, P. Parsana, A. S. Rao, F. Reverter, J. M. Rouhana, C. Sabatti, A. Saha, M.
950 Stephens, B. E. Stranger, B. J. Strober, N. A. Teran, A. Viñuela, G. Wang, X. Wen, F.
951 Wright, V. Wucher, Y. Zou, P. G. Ferreira, G. Li, M. Melé, E. Yeger-Lotem, M. E.
952 Barcus, D. Bradbury, T. Krubit, J. A. McLean, L. Qi, K. Robinson, N. V. Roche, A. M.
953 Smith, L. Sabin, D. E. Tabor, A. Undale, J. Bridge, L. E. Brigham, B. A. Foster, B. M.
954 Gillard, R. Hasz, M. Hunter, C. Johns, M. Johnson, E. Karasik, G. Kopen, W. F.
955 Leinweber, A. McDonald, M. T. Moser, K. Myer, K. D. Ramsey, B. Roe, S. Shad, J.
956 A. Thomas, G. Walters, M. Washington, J. Wheeler, S. D. Jewell, D. C. Rohrer, D. R.

957 Valley, D. A. Davis, D. C. Mash, P. A. Branton, L. K. Barker, H. M. Gardiner, M.
958 Mosavel, L. A. Siminoff, P. Fliceck, M. Haeussler, T. Juettemann, W. J. Kent, C. M.
959 Lee, C. C. Powell, K. R. Rosenbloom, M. Ruffier, D. Sheppard, K. Taylor, S. J.
960 Trevanion, D. R. Zerbino, N. S. Abell, J. Akey, L. Chen, K. Demanelis, J. A. Doherty,
961 A. P. Feinberg, K. D. Hansen, P. F. Hickey, F. Jasmine, L. Jiang, R. Kaul, M. G. Kibriya,
962 J. B. Li, Q. Li, S. Lin, S. E. Linder, B. L. Pierce, L. F. Rizzardi, A. D. Skol, K. S. Smith,
963 M. Snyder, J. Stamatoyannopoulos, H. Tang, M. Wang, L. J. Carithers, P. Guan, S. E.
964 Koester, A. R. Little, H. M. Moore, C. R. Nierras, A. K. Rao, J. B. Vaught, S. Volpi,
965 The GTEx Consortium atlas of genetic regulatory effects across human tissues. *Science*
966 **369**, 1318-1330 (2020).

967 8. F. Aguet, A. A. Brown, S. E. Castel, J. R. Davis, Y. He, B. Jo, P. Mohammadi, Y. Park,
968 P. Parsana, A. V. Segrè, B. J. Strober, Z. Zappala, B. B. Cummings, E. T. Gelfand, K.
969 Hadley, K. H. Huang, M. Lek, X. Li, J. L. Nedzel, D. Y. Nguyen, M. S. Noble, T. J.
970 Sullivan, T. Tukiainen, D. G. MacArthur, G. Getz, A. Addington, P. Guan, S. Koester,
971 A. R. Little, N. C. Lockhart, H. M. Moore, A. Rao, J. P. Struewing, S. Volpi, L. E.
972 Brigham, R. Hasz, M. Hunter, C. Johns, M. Johnson, G. Kopen, W. F. Leinweber, J. T.
973 Lonsdale, A. McDonald, B. Mestichelli, K. Myer, B. Roe, M. Salvatore, S. Shad, J. A.
974 Thomas, G. Walters, M. Washington, J. Wheeler, J. Bridge, B. A. Foster, B. M. Gillard,
975 E. Karasik, R. Kumar, M. Miklos, M. T. Moser, S. D. Jewell, R. G. Montroy, D. C.
976 Rohrer, D. Valley, D. C. Mash, D. A. Davis, L. Sabin, M. E. Barcus, P. A. Branton, N.
977 S. Abell, B. Balliu, O. Delaneau, L. Frésard, E. R. Gamazon, D. Garrido-Martín, A. D.
978 H. Gewirtz, G. Glinner, M. J. Gloudemans, B. Han, A. Z. He, F. Hormozdiari, X. Li, B.
979 Liu, E. Y. Kang, I. C. McDowell, H. Ongen, J. J. Palowitch, C. B. Peterson, G. Quon,
980 S. Ripke, A. Saha, A. A. Shabalin, T. C. Shimko, J. H. Sul, N. A. Teran, E. K. Tsang,
981 H. Zhang, Y.-H. Zhou, C. D. Bustamante, N. J. Cox, R. Guigó, M. Kellis, M. I.
982 McCarthy, D. F. Conrad, E. Eskin, G. Li, A. B. Nobel, C. Sabatti, B. E. Stranger, X.
983 Wen, F. A. Wright, K. G. Ardlie, E. T. Dermitzakis, T. Lappalainen, F. Aguet, K. G.
984 Ardlie, B. B. Cummings, E. T. Gelfand, G. Getz, K. Hadley, R. E. Handsaker, K. H.
985 Huang, S. Kashin, K. J. Karczewski, M. Lek, X. Li, D. G. MacArthur, J. L. Nedzel, D.
986 T. Nguyen, M. S. Noble, A. V. Segrè, C. A. Trowbridge, T. Tukiainen, N. S. Abell, B.
987 Balliu, R. Barshir, O. Basha, A. Battle, G. K. Bogu, A. Brown, C. D. Brown, S. E.
988 Castel, L. S. Chen, C. Chiang, D. F. Conrad, N. J. Cox, F. N. Damani, J. R. Davis, O.
989 Delaneau, E. T. Dermitzakis, B. E. Engelhardt, E. Eskin, P. G. Ferreira, L. Frésard, E.
990 R. Gamazon, D. Garrido-Martín, A. D. H. Gewirtz, G. Glinner, M. J. Gloudemans, R.
991 Guigo, I. M. Hall, B. Han, Y. He, F. Hormozdiari, C. Howald, H. Kyung Im, B. Jo, E.
992 Yong Kang, Y. Kim, S. Kim-Hellmuth, T. Lappalainen, G. Li, X. Li, B. Liu, S. Mangul,
993 M. I. McCarthy, I. C. McDowell, P. Mohammadi, J. Monlong, S. B. Montgomery, M.
994 Muñoz-Aguirre, A. W. Ndungu, D. L. Nicolae, A. B. Nobel, M. Oliva, H. Ongen, J. J.
995 Palowitch, N. Panousis, P. Papasaikas, Y. Park, P. Parsana, A. J. Payne, C. B. Peterson,
996 J. Quan, F. Reverter, C. Sabatti, A. Saha, M. Sammeth, A. J. Scott, A. A. Shabalin, R.
997 Sodaei, M. Stephens, B. E. Stranger, B. J. Strober, J. H. Sul, E. K. Tsang, S. Urbut, M.
998 van de Bunt, G. Wang, X. Wen, F. A. Wright, H. S. Xi, E. Yeger-Lotem, Z. Zappala, J.
999 B. Zaugg, Y.-H. Zhou, J. M. Akey, D. Bates, J. Chan, L. S. Chen, M. Claussnitzer, K.
1000 Demanelis, M. Diegel, J. A. Doherty, A. P. Feinberg, M. S. Fernando, J. Halow, K. D.
1001 Hansen, E. Haugen, P. F. Hickey, L. Hou, F. Jasmine, R. Jian, L. Jiang, A. Johnson, R.
1002 Kaul, M. Kellis, M. G. Kibriya, K. Lee, J. Billy Li, Q. Li, X. Li, J. Lin, S. Lin, S. Linder,
1003 C. Linke, Y. Liu, M. T. Maurano, B. Molinie, S. B. Montgomery, J. Nelson, F. J. Neri,
1004 M. Oliva, Y. Park, B. L. Pierce, N. J. Rinaldi, L. F. Rizzardi, R. Sandstrom, A. Skol, K.
1005 S. Smith, M. P. Snyder, J. Stamatoyannopoulos, B. E. Stranger, H. Tang, E. K. Tsang,
1006 L. Wang, M. Wang, N. Van Wittenberghe, F. Wu, R. Zhang, C. R. Nierras, P. A.

1007 Branton, L. J. Carithers, P. Guan, H. M. Moore, A. Rao, J. B. Vaught, S. E. Gould, N.
1008 C. Lockart, C. Martin, J. P. Struewing, S. Volpi, A. M. Addington, S. E. Koester, A. R.
1009 Little, G. T. Consortium, a. Lead, D. A. Laboratory, C. Coordinating, N. I. H. p.
1010 management, c. Biospecimen, Pathology, Q. T. L. m. w. g. e, D. A. Laboratory, G.
1011 Coordinating Center —Analysis Working, G. Statistical Methods groups—Analysis
1012 Working, G. g. Enhancing, N. I. H. C. Fund, Nih/Nci, Nih/Nhgri, Nih/Nimh, Nih/Nida,
1013 N. Biospecimen Collection Source Site—, Genetic effects on gene expression across
1014 human tissues. *Nature* **550**, 204-213 (2017).

1015 9. A. S. E. Cuomo, A. Nathan, S. Raychaudhuri, D. G. MacArthur, J. E. Powell, Single-
1016 cell genomics meets human genetics. *Nat Rev Genet*, (2023).

1017 10. H. Mostafavi, J. P. Spence, S. Naqvi, J. K. Pritchard, Limited overlap of eQTLs and
1018 GWAS hits due to systematic differences in discovery. *bioRxiv*,
1019 2022.2005.2007.491045 (2022).

1020 11. N. J. Connally, S. Nazeen, D. Lee, H. Shi, J. Stamatoyannopoulos, S. Chun, C. Cotsapas,
1021 C. A. Cassa, S. R. Sunyaev, The missing link between genetic association and
1022 regulatory function. *eLife* **11**, e74970 (2022).

1023 12. B. D. Umans, A. Battle, Y. Gilad, Where Are the Disease-Associated eQTLs? *Trends
1024 Genet* **37**, 109-124 (2021).

1025 13. K. A. Jagadeesh, K. K. Dey, D. T. Montoro, R. Mohan, S. Gazal, J. M. Engreitz, R. J.
1026 Xavier, A. L. Price, A. Regev, Identifying disease-critical cell types and cellular
1027 processes by integrating single-cell RNA-sequencing and human genetics. *Nat. Genet.*
1028 **54**, 1479-1492 (2022).

1029 14. G. Eraslan, E. Drokhlyansky, S. Anand, E. Fiskin, A. Subramanian, M. Slyper, J. Wang,
1030 N. Van Wittenberghe, J. M. Rouhana, J. Waldman, O. Ashenberg, M. Lek, D. Dionne,
1031 T. S. Win, M. S. Cuoco, O. Kuksenko, A. M. Tsankov, P. A. Branton, J. L. Marshall,
1032 A. Greka, G. Getz, A. V. Segre, F. Aguet, O. Rozenblatt-Rosen, K. G. Ardlie, A. Regev,
1033 Single-nucleus cross-tissue molecular reference maps toward understanding disease
1034 gene function. *Science* **376**, eabl4290 (2022).

1035 15. K. Zhang, J. D. Hocker, M. Miller, X. Hou, J. Chiou, O. B. Poirion, Y. Qiu, Y. E. Li,
1036 K. J. Gaulton, A. Wang, S. Preissl, B. Ren, A single-cell atlas of chromatin accessibility
1037 in the human genome. *Cell* **184**, 5985-6001 e5919 (2021).

1038 16. H. K. Finucane, Y. A. Reshef, V. Anttila, K. Slowikowski, A. Gusev, A. Byrnes, S.
1039 Gazal, P. R. Loh, C. Lareau, N. Shores, G. Genovese, A. Saunders, E. Macosko, S.
1040 Pollack, C. Brainstorm, J. R. B. Perry, J. D. Buenrostro, B. E. Bernstein, S.
1041 Raychaudhuri, S. McCarroll, B. M. Neale, A. L. Price, Heritability enrichment of
1042 specifically expressed genes identifies disease-relevant tissues and cell types. *Nat.
1043 Genet.* **50**, 621-629 (2018).

1044 17. X. Sheng, Y. Guan, Z. Ma, J. Wu, H. Liu, C. Qiu, S. Vitale, Z. Miao, M. J. Seasock, M.
1045 Palmer, M. K. Shin, K. L. Duffin, S. S. Pullen, T. L. Edwards, J. N. Hellwege, A. M.
1046 Hung, M. Li, B. F. Voight, T. M. Coffman, C. D. Brown, K. Susztak, Mapping the
1047 genetic architecture of human traits to cell types in the kidney identifies mechanisms of
1048 disease and potential treatments. *Nat. Genet.* **53**, 1322-1333 (2021).

1049 18. J. K. Lunney, A. Van Goor, K. E. Walker, T. Hailstock, J. Franklin, C. Dai, Importance
1050 of the pig as a human biomedical model. *Sci. Transl. Med.* **13**, eabd5758 (2021).

1051 19. Z.-L. Hu, C. A. Park, J. M. Reecy, Bringing the Animal QTLdb and CorrDB into the
1052 future: meeting new challenges and providing updated services. *Nucleic Acids
1053 Research* **50**, D956-D961 (2021).

1054 20. M. A. Groenen, A decade of pig genome sequencing: a window on pig domestication
1055 and evolution. *Genet. Sel. Evol.* **48**, 23 (2016).

1056 21. S. Liu, Y. Gao, O. Canela-Xandri, S. Wang, Y. Yu, W. Cai, B. Li, R. Xiang, A. J.
1057 Chamberlain, E. Pairo-Castineira, K. D'Mellow, K. Rawlik, C. Xia, Y. Yao, P. Navarro,
1058 D. Rocha, X. Li, Z. Yan, C. Li, B. D. Rosen, C. P. Van Tassell, P. M. Vanraden, S.
1059 Zhang, L. Ma, J. B. Cole, G. E. Liu, A. Tenesa, L. Fang, A multi-tissue atlas of
1060 regulatory variants in cattle. *Nat. Genet.* **54**, 1438-1447 (2022).

1061 22. Z. Pan, Y. Yao, H. Yin, Z. Cai, Y. Wang, L. Bai, C. Kern, M. Halstead, G.
1062 Chanthavixay, N. Trakooljul, K. Wimmers, G. Sahana, G. Su, M. S. Lund, M. Fredholm,
1063 P. Karlskov-Mortensen, C. W. Ernst, P. Ross, C. K. Tuggle, L. Fang, H. Zhou, Pig
1064 genome functional annotation enhances the biological interpretation of complex traits
1065 and human disease. *Nat Commun* **12**, 5848 (2021).

1066 23. L. Andersson, A. L. Archibald, C. D. Bottema, R. Brauning, S. C. Burgess, D. W. Burt,
1067 E. Casas, H. H. Cheng, L. Clarke, C. Couldrey, B. P. Dalrymple, C. G. Elsik, S. Foissac,
1068 E. Giuffra, M. A. Groenen, B. J. Hayes, L. S. Huang, H. Khatib, J. W. Kijas, H. Kim,
1069 J. K. Lunney, F. M. McCarthy, J. C. McEwan, S. Moore, B. Nanduri, C. Notredame,
1070 Y. Palti, G. S. Plastow, J. M. Reecy, G. A. Rohrer, E. Sarropoulou, C. J. Schmidt, J.
1071 Silverstein, R. L. Tellam, M. Tixier-Boichard, G. Tosser-Klopp, C. K. Tuggle, J. Vilkki,
1072 S. N. White, S. Zhao, H. Zhou, F. Consortium, Coordinated international action to
1073 accelerate genome-to-phenome with FAANG, the Functional Annotation of Animal
1074 Genomes project. *Genome Biol* **16**, 57 (2015).

1075 24. J. Teng, Y. Gao, H. Yin, Z. Bai, S. Liu, H. Zeng, L. Bai, Z. Cai, B. Zhao, X. Li, Z. Xu,
1076 Q. Lin, Z. Pan, W. Yang, X. Yu, D. Guan, Y. Hou, B. N. Keel, G. A. Rohrer, A. K.
1077 Lindholm-Perry, W. T. Oliver, M. Ballester, D. Crespo-Piazuelo, R. Quintanilla, O.
1078 Canela-Xandri, K. Rawlik, C. Xia, Y. Yao, Q. Zhao, W. Yao, L. Yang, H. Li, H. Zhang,
1079 W. Liao, T. Chen, P. Karlskov-Mortensen, M. Fredholm, M. Amills, A. Clop, E.
1080 Giuffra, J. Wu, X. Cai, S. Diao, X. Pan, C. Wei, J. Li, H. Cheng, S. Wang, G. Su, G.
1081 Sahana, M. S. Lund, J. C. M. Dekkers, L. Kramer, C. K. Tuggle, R. Corbett, M. A. M.
1082 Groenen, O. Madsen, M. Gòdia, D. Rocha, M. Charles, C.-j. Li, H. Pausch, X. Hu, L.
1083 Frantz, Y. Luo, L. Lin, Z. Zhou, Z. Zhang, Z. Chen, L. Cui, R. Xiang, X. Shen, P. Li,
1084 R. Huang, G. Tang, M. Li, Y. Zhao, G. Yi, Z. Tang, J. Jiang, F. Zhao, X. Yuan, X. Liu,
1085 Y. Chen, X. Xu, S. Zhao, P. Zhao, C. Haley, H. Zhou, Q. Wang, Y. Pan, X. Ding, L.
1086 Ma, J. Li, P. Navarro, Q. Zhang, B. Li, A. Tenesa, K. Li, G. E. Liu, Z. Zhang, L. Fang,
1087 A compendium of genetic regulatory effects across pig tissues. *bioRxiv*,
1088 2022.2011.2011.516073 (2022).

1089 25. L. Zhang, M. Guo, Z. Liu, R. Liu, Y. Zheng, T. Yu, Y. Lv, H. Lu, W. Zeng, T. Zhang,
1090 C. Pan, Single-cell RNA-seq analysis of testicular somatic cell development in pigs. *J
1091 Genet Genomics* **49**, 1016-1028 (2022).

1092 26. J. E. Wiarda, J. M. Trachsel, S. K. Sivasankaran, C. K. Tuggle, C. L. Loving, Intestinal
1093 single-cell atlas reveals novel lymphocytes in pigs with similarities to human cells. *Life
1094 Science Alliance* **5**, e202201442 (2022).

1095 27. F. Wang, P. Ding, X. Liang, X. Ding, C. B. Brandt, E. Sjöstedt, J. Zhu, S. Bolund, L.
1096 Zhang, L. P. M. H. de Rooij, L. Luo, Y. Wei, W. Zhao, Z. Lv, J. Haskó, R. Li, Q. Qin,
1097 Y. Jia, W. Wu, Y. Yuan, M. Pu, H. Wang, A. Wu, L. Xie, P. Liu, F. Chen, J. Herold, J.
1098 Kalucka, M. Karlsson, X. Zhang, R. B. Helmig, L. Fagerberg, C. Lindskog, F. Pontén,
1099 M. Uhlen, L. Bolund, N. Jessen, H. Jiang, X. Xu, H. Yang, P. Carmeliet, J. Mulder, D.
1100 Chen, L. Lin, Y. Luo, Endothelial cell heterogeneity and microglia regulons revealed
1101 by a pig cell landscape at single-cell level. *Nature Communications* **13**, 3620 (2022).

1102 28. L. Han, C. P. Jara, O. Wang, Y. Shi, X. Wu, S. Thibivilliers, R. K. Wóycicki, M. A.
1103 Carlson, W. H. Velander, E. P. Araújo, M. Libault, C. Zhang, Y. Lei, Isolating and
1104 cryopreserving pig skin cells for single-cell RNA sequencing study. *PLoS One* **17**,
1105 e0263869 (2022).

1106 29. J. Zhu, F. Chen, L. Luo, W. Wu, J. Dai, J. Zhong, X. Lin, C. Chai, P. Ding, L. Liang,
1107 S. Wang, X. Ding, Y. Chen, H. Wang, J. Qiu, F. Wang, C. Sun, Y. Zeng, J. Fang, X.
1108 Jiang, P. Liu, G. Tang, X. Qiu, X. Zhang, Y. Ruan, S. Jiang, J. Li, S. Zhu, X. Xu, F. Li,
1109 Z. Liu, G. Cao, D. Chen, Single-cell atlas of domestic pig cerebral cortex and
1110 hypothalamus. *Science Bulletin* **66**, 1448-1461 (2021).
1111 30. L. Zhang, J. Zhu, H. Wang, J. Xia, P. Liu, F. Chen, H. Jiang, Q. Miao, W. Wu, L. Zhang,
1112 L. Luo, X. Jiang, Y. Bai, C. Sun, D. Chen, X. Zhang, A high-resolution cell atlas of the
1113 domestic pig lung and an online platform for exploring lung single-cell data. *J Genet
1114 Genomics* **48**, 411-425 (2021).
1115 31. J. Herrera-Uribe, J. E. Wiarda, S. K. Sivasankaran, L. Daharsh, H. Liu, K. A. Byrne, T.
1116 P. L. Smith, J. K. Lunney, C. L. Loving, C. K. Tuggle, Reference Transcriptomes of
1117 Porcine Peripheral Immune Cells Created Through Bulk and Single-Cell RNA
1118 Sequencing. *Front. Genet.* **12**, (2021).
1119 32. W. Tang, Y. Zhong, Y. Wei, Z. Deng, J. Mao, J. Liu, T. G. Valencak, J. Liu, H. Xu, H.
1120 Wang, Ileum tissue single-cell mRNA sequencing elucidates the cellular architecture
1121 of pathophysiological changes associated with weaning in piglets. *BMC Biology* **20**,
1122 123 (2022).
1123 33. H. L. Sweeney, D. W. Hammers, Muscle Contraction. *Cold Spring Harb. Perspect.
1124 Biol.* **10**, (2018).
1125 34. K. Mukund, S. Subramaniam, Skeletal muscle: A review of molecular structure and
1126 function, in health and disease. *Wiley Interdiscip. Rev. Syst. Biol. Med.* **12**, e1462
1127 (2020).
1128 35. P. Orchard, N. Manickam, C. Ventresca, S. Vadlamudi, A. Varshney, V. Rai, J. Kaplan,
1129 C. Lalancette, K. L. Mohlke, K. Gallagher, C. F. Burant, S. C. J. Parker, Human and
1130 rat skeletal muscle single-nuclei multi-omic integrative analyses nominate causal cell
1131 types, regulatory elements, and SNPs for complex traits. *Genome Res.* **31**, 2258-2275
1132 (2021).
1133 36. A. B. Rubenstein, G. R. Smith, U. Raue, G. Begue, K. Minchev, F. Ruf-Zamojski, V.
1134 D. Nair, X. Wang, L. Zhou, E. Zaslavsky, T. A. Trappe, S. Trappe, S. C. Sealfon,
1135 Single-cell transcriptional profiles in human skeletal muscle. *Sci. Rep.* **10**, 229 (2020).
1136 37. J. Günther, H.-M. Seyfert, The first line of defence: insights into mechanisms and
1137 relevance of phagocytosis in epithelial cells. *Semin. Immunopathol.* **40**, 555-565 (2018).
1138 38. R. Elmentait, N. Kumasaka, K. Roberts, A. Fleming, E. Dann, H. W. King, V.
1139 Kleshchevnikov, M. Dabrowska, S. Pritchard, L. Bolt, S. F. Vieira, L. Mamanova, N.
1140 Huang, F. Perrone, I. Goh Kai'En, S. N. Lisgo, M. Katan, S. Leonard, T. R. W. Oliver,
1141 C. E. Hook, K. Nayak, L. S. Campos, C. Dominguez Conde, E. Stephenson, J.
1142 Engelbert, R. A. Botting, K. Polanski, S. van Dongen, M. Patel, M. D. Morgan, J. C.
1143 Marioni, O. A. Bayraktar, K. B. Meyer, X. He, R. A. Barker, H. H. Uhlig, K. T.
1144 Mahbubani, K. Saeb-Parsy, M. Zilbauer, M. R. Clatworthy, M. Haniffa, K. R. James,
1145 S. A. Teichmann, Cells of the human intestinal tract mapped across space and time.
1146 *Nature* **597**, 250-255 (2021).
1147 39. A. Ali, H. Tan, G. E. Kaiko, Role of the Intestinal Epithelium and Its Interaction With
1148 the Microbiota in Food Allergy. *Front. Immunol.* **11**, 604054 (2020).
1149 40. A. L. Haber, M. Biton, N. Rogel, R. H. Herbst, K. Shekhar, C. Smillie, G. Burgin, T.
1150 M. Delorey, M. R. Howitt, Y. Katz, I. Tirosh, S. Beyaz, D. Dionne, M. Zhang, R.
1151 Raychowdhury, W. S. Garrett, O. Rozenblatt-Rosen, H. N. Shi, O. Yilmaz, R. J. Xavier,
1152 A. Regev, A single-cell survey of the small intestinal epithelium. *Nature* **551**, 333-339
1153 (2017).

1154 41. J. Cao, M. Spielmann, X. Qiu, X. Huang, D. M. Ibrahim, A. J. Hill, F. Zhang, S.
1155 Mundlos, L. Christiansen, F. J. Steemers, C. Trapnell, J. Shendure, The single-cell
1156 transcriptional landscape of mammalian organogenesis. *Nature* **566**, 496-502 (2019).
1157 42. S. Jin, C. F. Guerrero-Juarez, L. Zhang, I. Chang, R. Ramos, C. H. Kuan, P. Myung, M.
1158 V. Plikus, Q. Nie, Inference and analysis of cell-cell communication using CellChat.
1159 *Nat Commun* **12**, 1088 (2021).
1160 43. D. D. Chaplin, Overview of the immune response. *J Allergy Clin Immunol* **125**, S3-23
1161 (2010).
1162 44. A. M. Newman, C. L. Liu, M. R. Green, A. J. Gentles, W. Feng, Y. Xu, C. D. Hoang,
1163 M. Diehn, A. A. Alizadeh, Robust enumeration of cell subsets from tissue expression
1164 profiles. *Nat. Methods* **12**, 453-457 (2015).
1165 45. M. Dong, A. Thennavan, E. Urrutia, Y. Li, C. M. Perou, F. Zou, Y. Jiang, SCDC: bulk
1166 gene expression deconvolution by multiple single-cell RNA sequencing references.
1167 *Brief Bioinform* **22**, 416-427 (2021).
1168 46. S. Kim-Hellmuth, F. Aguet, M. Oliva, M. Muñoz-Aguirre, S. Kasela, V. Wucher, S. E.
1169 Castel, A. R. Hamel, A. Viñuela, A. L. Roberts, S. Mangul, X. Wen, G. Wang, A. N.
1170 Barbeira, D. Garrido-Martín, B. B. Nadel, Y. Zou, R. Bonazzola, J. Quan, A. Brown,
1171 A. Martinez-Perez, J. M. Soria, G. Getz, E. T. Dermitzakis, K. S. Small, M. Stephens,
1172 H. S. Xi, H. K. Im, R. Guigó, A. V. Segrè, B. E. Stranger, K. G. Ardlie, T. Lappalainen,
1173 Cell type-specific genetic regulation of gene expression across human tissues. *Science*
1174 **369**, (2020).
1175 47. Y. Yamazaki, N. Zhao, T. R. Caulfield, C. C. Liu, G. Bu, Apolipoprotein E and
1176 Alzheimer disease: pathobiology and targeting strategies. *Nat. Rev. Neurol.* **15**, 501-
1177 518 (2019).
1178 48. H. Van Gorp, W. Van Breedam, J. Van Doorsselaere, P. L. Delputte, H. J. Nauwynck,
1179 Identification of the CD163 protein domains involved in infection of the porcine
1180 reproductive and respiratory syndrome virus. *J. Virol.* **84**, 3101-3105 (2010).
1181 49. J. J. Wu, S. Zhu, F. Gu, T. G. Valencak, J. X. Liu, H. Z. Sun, Cross-tissue single-cell
1182 transcriptomic landscape reveals the key cell subtypes and their potential roles in the
1183 nutrient absorption and metabolism in dairy cattle. *J Adv Res* **37**, 1-18 (2022).
1184 50. J. Qu, F. Yang, T. Zhu, Y. Wang, W. Fang, Y. Ding, X. Zhao, X. Qi, Q. Xie, M. Chen,
1185 Q. Xu, Y. Xie, Y. Sun, D. Chen, A reference single-cell regulomic and transcriptomic
1186 map of cynomolgus monkeys. *Nat Commun* **13**, 4069 (2022).
1187 51. H. Li, J. Janssens, M. De Waegeneer, S. S. Kolluru, K. Davie, V. Gardeux, W. Saelens,
1188 F. P. A. David, M. Brbić, K. Spanier, J. Leskovec, C. N. McLaughlin, Q. Xie, R. C.
1189 Jones, K. Brueckner, J. Shim, S. G. Tattikota, F. Schnorrer, K. Rust, T. G. Nystul, Z.
1190 Carvalho-Santos, C. Ribeiro, S. Pal, S. Mahadevaraju, T. M. Przytycka, A. M. Allen,
1191 S. F. Goodwin, C. W. Berry, M. T. Fuller, H. White-Cooper, E. L. Matunis, S. DiNardo,
1192 A. Galenza, L. E. O'Brien, J. A. T. Dow, H. Jasper, B. Oliver, N. Perrimon, B.
1193 Deplancke, S. R. Quake, L. Luo, S. Aerts, D. Agarwal, Y. Ahmed-Braimah, M.
1194 Arbeitman, M. M. Ariss, J. Augsburger, K. Ayush, C. C. Baker, T. Banisch, K. Birker,
1195 R. Bodmer, B. Bolival, S. E. Brantley, J. A. Brill, N. C. Brown, N. A. Buehner, X. T.
1196 Cai, R. Cardoso-Figueiredo, F. Casares, A. Chang, T. R. Clandinin, S. Crasta, C.
1197 Desplan, A. M. Detweiler, D. B. Dhakan, E. Donà, S. Engert, S. Floc'hlay, N. George,
1198 A. J. González-Segarra, A. K. Groves, S. Gumbin, Y. Guo, D. E. Harris, Y. Heifetz, S.
1199 L. Holtz, F. Horns, B. Hudry, R. J. Hung, Y. N. Jan, J. S. Jaszcak, G. Jefferis, J.
1200 Karkanias, T. L. Karr, N. S. Katheder, J. Kezos, A. A. Kim, S. K. Kim, L. Kockel, N.
1201 Konstantinides, T. B. Kornberg, H. M. Krause, A. T. Labott, M. Laturney, R. Lehmann,
1202 S. Leinwand, J. Li, J. S. S. Li, K. Li, K. Li, L. Li, T. Li, M. Litovchenko, H. H. Liu, Y.
1203 Liu, T. C. Lu, J. Manning, A. Mase, M. Matera-Vatnick, N. R. Matias, C. E.

1204 McDonough-Goldstein, A. McGeever, A. D. McLachlan, P. Moreno-Roman, N. Neff,
1205 M. Neville, S. Ngo, T. Nielsen, C. E. O'Brien, D. Osumi-Sutherland, M. N. Özel, I.
1206 Papatheodorou, M. Petkovic, C. Pilgrim, A. O. Pisco, C. Reisenman, E. N. Sanders, G.
1207 Dos Santos, K. Scott, A. Sherlekar, P. Shiu, D. Sims, R. V. Sit, M. Slaidina, H. E. Smith,
1208 G. Sterne, Y. H. Su, D. Sutton, M. Tamayo, M. Tan, I. Tastekin, C. Treiber, D. Vacek,
1209 G. Vogler, S. Waddell, W. Wang, R. I. Wilson, M. F. Wolfner, Y. E. Wong, A. Xie, J.
1210 Xu, S. Yamamoto, J. Yan, Z. Yao, K. Yoda, R. Zhu, R. P. Zinzen, Fly Cell Atlas: A
1211 single-nucleus transcriptomic atlas of the adult fruit fly. *Science* **375**, eabk2432 (2022).
1212 52. J. Li, J. Wang, P. Zhang, R. Wang, Y. Mei, Z. Sun, L. Fei, M. Jiang, L. Ma, W. E. H.
1213 Chen, X. Wang, Y. Fu, H. Wu, D. Liu, X. Wang, J. Li, Q. Guo, Y. Liao, C. Yu, D. Jia,
1214 J. Wu, S. He, H. Liu, J. Ma, K. Lei, J. Chen, X. Han, G. Guo, Deep learning of cross-
1215 species single-cell landscapes identifies conserved regulatory programs underlying cell
1216 types. *Nat. Genet.* **54**, 1711-1720 (2022).
1217 53. Y. M. Choi, B. C. Kim, Muscle fiber characteristics, myofibrillar protein isoforms, and
1218 meat quality. *Livestock Science* **122**, 105-118 (2009).
1219 54. R. E. Klont, L. Brocks, G. Eikelenboom, Muscle fibre type and meat quality. *Meat Sci*
1220 **49s1**, S219-229 (1998).
1221 55. L. Jin, Q. Tang, S. Hu, Z. Chen, X. Zhou, B. Zeng, Y. Wang, M. He, Y. Li, L. Gui, L.
1222 Shen, K. Long, J. Ma, X. Wang, Z. Chen, Y. Jiang, G. Tang, L. Zhu, F. Liu, B. Zhang,
1223 Z. Huang, G. Li, D. Li, V. N. Gladyshev, J. Yin, Y. Gu, X. Li, M. Li, A pig BodyMap
1224 transcriptome reveals diverse tissue physiologies and evolutionary dynamics of
1225 transcription. *Nat Commun* **12**, 3715 (2021).
1226 56. J. Talbot, L. Maves, Skeletal muscle fiber type: using insights from muscle
1227 developmental biology to dissect targets for susceptibility and resistance to muscle
1228 disease. *Wiley Interdiscip Rev Dev Biol* **5**, 518-534 (2016).
1229 57. S. T. Joo, G. D. Kim, Y. H. Hwang, Y. C. Ryu, Control of fresh meat quality through
1230 manipulation of muscle fiber characteristics. *Meat Sci* **95**, 828-836 (2013).
1231 58. C. Domínguez Conde, C. Xu, L. B. Jarvis, D. B. Rainbow, S. B. Wells, T. Gomes, S.
1232 K. Howlett, O. Suchanek, K. Polanski, H. W. King, L. Mamanova, N. Huang, P. A.
1233 Szabo, L. Richardson, L. Bolt, E. S. Fasouli, K. T. Mahbubani, M. Prete, L. Tuck, N.
1234 Richoz, Z. K. Tuong, L. Campos, H. S. Mousa, E. J. Needham, S. Pritchard, T. Li, R.
1235 Elmentaite, J. Park, E. Rahmani, D. Chen, D. K. Menon, O. A. Bayraktar, L. K. James,
1236 K. B. Meyer, N. Yosef, M. R. Clatworthy, P. A. Sims, D. L. Farber, K. Saeb-Parsy, J.
1237 L. Jones, S. A. Teichmann, Cross-tissue immune cell analysis reveals tissue-specific
1238 features in humans. *Science* **376**, eabl5197 (2022).
1239 59. M. Slyper, C. B. M. Porter, O. Ashenberg, J. Waldman, E. Drokhlyansky, I. Wakiro, C.
1240 Smillie, G. Smith-Rosario, J. Wu, D. Dionne, S. Vigneau, J. Jane-Valbuena, T. L.
1241 Tickle, S. Napolitano, M. J. Su, A. G. Patel, A. Karlstrom, S. Gritsch, M. Nomura, A.
1242 Waghray, S. H. Gohil, A. M. Tsankov, L. Jerby-Arnon, O. Cohen, J. Klughammer, Y.
1243 Rosen, J. Gould, L. Nguyen, M. Hofree, P. J. Tramontozzi, B. Li, C. J. Wu, B. Izar, R.
1244 Haq, F. S. Hodi, C. H. Yoon, A. N. Hata, S. J. Baker, M. L. Suva, R. Bueno, E. H.
1245 Stover, M. R. Clay, M. A. Dyer, N. B. Collins, U. A. Matulonis, N. Wagle, B. E.
1246 Johnson, A. Rotem, O. Rozenblatt-Rosen, A. Regev, A single-cell and single-nucleus
1247 RNA-Seq toolbox for fresh and frozen human tumors. *Nat. Med.* **26**, 792-802 (2020).
1248 60. J. Ding, X. Adiconis, S. K. Simmons, M. S. Kowalczyk, C. C. Hession, N. D.
1249 Marjanovic, T. K. Hughes, M. H. Wadsworth, T. Burks, L. T. Nguyen, J. Y. H. Kwon,
1250 B. Barak, W. Ge, A. J. Kedaigle, S. Carroll, S. Li, N. Hacohen, O. Rozenblatt-Rosen,
1251 A. K. Shalek, A. C. Villani, A. Regev, J. Z. Levin, Systematic comparison of single-
1252 cell and single-nucleus RNA-sequencing methods. *Nat. Biotechnol.* **38**, 737-746 (2020).

1253 61. A. Warr, N. Affara, B. Aken, H. Beiki, D. M. Bickhart, K. Billis, W. Chow, L. Eory,
1254 H. A. Finlayson, P. Fliceck, C. G. Giron, D. K. Griffin, R. Hall, G. Hannum, T. Hourlier,
1255 K. Howe, D. A. Hume, O. Izuogu, K. Kim, S. Koren, H. Liu, N. Manchanda, F. J.
1256 Martin, D. J. Nonneman, R. E. O'Connor, A. M. Phillippe, G. A. Rohrer, B. D. Rosen,
1257 L. A. Rund, C. A. Sargent, L. B. Schook, S. G. Schroeder, A. S. Schwartz, B. M.
1258 Skinner, R. Talbot, E. Tseng, C. K. Tuggle, M. Watson, T. P. L. Smith, A. L. Archibald,
1259 An improved pig reference genome sequence to enable pig genetics and genomics
1260 research. *Gigascience* **9**, (2020).

1261 62. R. Satija, J. A. Farrell, D. Gennert, A. F. Schier, A. Regev, Spatial reconstruction of
1262 single-cell gene expression data. *Nat. Biotechnol.* **33**, 495-502 (2015).

1263 63. S. Yang, S. E. Corbett, Y. Koga, Z. Wang, W. E. Johnson, M. Yajima, J. D. Campbell,
1264 Decontamination of ambient RNA in single-cell RNA-seq with DecontX. *Genome Biol*
1265 **21**, 57 (2020).

1266 64. C. S. McGinnis, L. M. Murrow, Z. J. Gartner, DoubletFinder: Doublet Detection in
1267 Single-Cell RNA Sequencing Data Using Artificial Nearest Neighbors. *Cell Syst* **8**,
1268 329-337.e324 (2019).

1269 65. B. Hie, B. Bryson, B. Berger, Efficient integration of heterogeneous single-cell
1270 transcriptomes using Scanorama. *Nat. Biotechnol.* **37**, 685-691 (2019).

1271 66. V. Bergen, M. Lange, S. Peidli, F. A. Wolf, F. J. Theis, Generalizing RNA velocity to
1272 transient cell states through dynamical modeling. *Nat. Biotechnol.* **38**, 1408-1414
1273 (2020).

1274 67. R. Xue, Q. Zhang, Q. Cao, R. Kong, X. Xiang, H. Liu, M. Feng, F. Wang, J. Cheng, Z.
1275 Li, Q. Zhan, M. Deng, J. Zhu, Z. Zhang, N. Zhang, Liver tumour immune
1276 microenvironment subtypes and neutrophil heterogeneity. *Nature* **612**, 141-147 (2022).

1277 68. T. Wu, E. Hu, S. Xu, M. Chen, P. Guo, Z. Dai, T. Feng, L. Zhou, W. Tang, L. Zhan, X.
1278 Fu, S. Liu, X. Bo, G. Yu, clusterProfiler 4.0: A universal enrichment tool for
1279 interpreting omics data. *Innovation (Camb)* **2**, 100141 (2021).

1280 69. S. Aibar, C. B. González-Blas, T. Moerman, V. A. Huynh-Thu, H. Imrichova, G.
1281 Hulselmans, F. Rambow, J. C. Marine, P. Geurts, J. Aerts, J. van den Oord, Z. K. Atak,
1282 J. Wouters, S. Aerts, SCENIC: single-cell regulatory network inference and clustering.
1283 *Nat. Methods* **14**, 1083-1086 (2017).

1284 70. H. Jin, Z. Liu, A benchmark for RNA-seq deconvolution analysis under dynamic testing
1285 environments. *Genome Biol* **22**, 102 (2021).

1286 71. A. Taylor-Weiner, F. Aguet, N. J. Haradhvala, S. Gosai, S. Anand, J. Kim, K. Ardlie,
1287 E. M. Van Allen, G. Getz, Scaling computational genomics to millions of individuals
1288 with GPUs. *Genome Biol* **20**, 228 (2019).

1289 72. J. R. Davis, L. Fresard, D. A. Knowles, M. Pala, C. D. Bustamante, A. Battle, S. B.
1290 Montgomery, An Efficient Multiple-Testing Adjustment for eQTL Studies that
1291 Accounts for Linkage Disequilibrium between Variants. *Am. J. Hum. Genet.* **98**, 216-
1292 224 (2016).

1293 73. S. E. Castel, P. Mohammadi, W. K. Chung, Y. Shen, T. Lappalainen, Rare variant
1294 phasing and haplotypic expression from RNA sequencing with phASER. *Nat Commun*
1295 **7**, 12817 (2016).

1296 74. S. E. Castel, A. Levy-Moonshine, P. Mohammadi, E. Banks, T. Lappalainen, Tools and
1297 best practices for data processing in allelic expression analysis. *Genome Biol* **16**, 195
1298 (2015).

1299 75. C. Giambartolomei, D. Vukcevic, E. E. Schadt, L. Franke, A. D. Hingorani, C. Wallace,
1300 V. Plagnol, Bayesian test for colocalisation between pairs of genetic association studies
1301 using summary statistics. *PLoS Genet* **10**, e1004383 (2014).

1302 76. N. C. Sheffield, C. Bock, LOLA: enrichment analysis for genomic region sets and
1303 regulatory elements in R and Bioconductor. *Bioinformatics* **32**, 587-589 (2016).

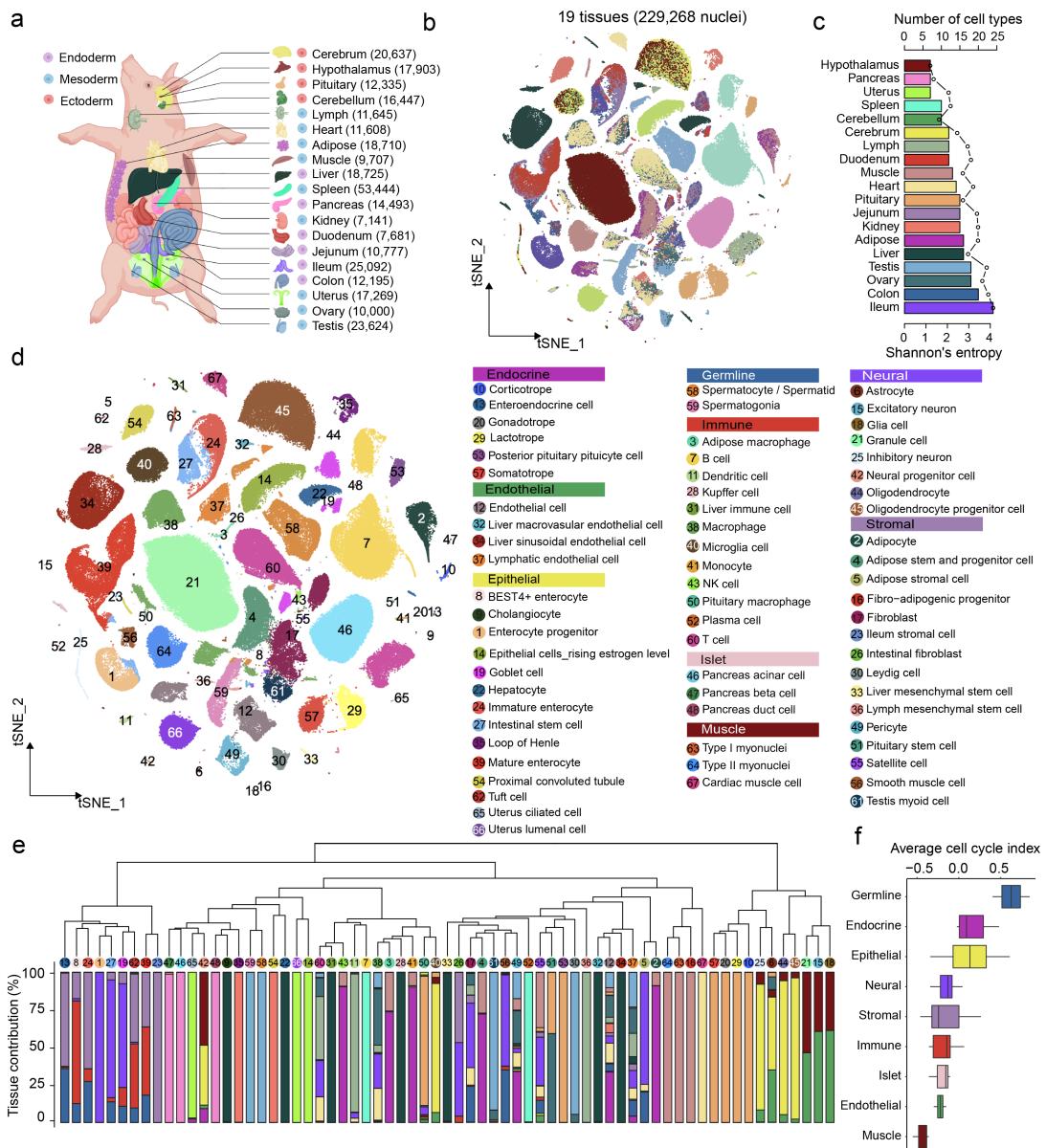
1304 77. A. Marceau, Y. Gao, R. L. t. Baldwin, C. J. Li, J. Jiang, G. E. Liu, L. Ma, Investigation
1305 of rumen long noncoding RNA before and after weaning in cattle. *BMC Genomics* **23**,
1306 531 (2022).

1307 78. H. K. Finucane, B. Bulik-Sullivan, A. Gusev, G. Trynka, Y. Reshef, P. R. Loh, V.
1308 Anttila, H. Xu, C. Zang, K. Farh, S. Ripke, F. R. Day, S. Purcell, E. Stahl, S. Lindstrom,
1309 J. R. Perry, Y. Okada, S. Raychaudhuri, M. J. Daly, N. Patterson, B. M. Neale, A. L.
1310 Price, Partitioning heritability by functional annotation using genome-wide association
1311 summary statistics. *Nat. Genet.* **47**, 1228-1235 (2015).

1312 79. B. K. Bulik-Sullivan, P. R. Loh, H. K. Finucane, S. Ripke, J. Yang, N. Patterson, M. J.
1313 Daly, A. L. Price, B. M. Neale, LD Score regression distinguishes confounding from
1314 polygenicity in genome-wide association studies. *Nat. Genet.* **47**, 291-295 (2015).

1315

1316 **Figures**



1317

1318 **Fig. 1 | Single-nucleus transcriptomic landscape across 19 frozen tissues in adult pigs.**

1319 **a**, Schematic diagram showing 19 primary pig tissues collected for snRNA-seq in this study. The cartoons used to generate this illustration were purchased from BioRender.com. The number of nuclei profiled per tissue is denoted in parentheses.

1320 The number of nuclei profiled per tissue is denoted in parentheses.

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1322 **b**, t-SNE visualization of single-nucleus profiles (dots) colored by tissues.

1323 **c**, Bar plot displaying the number and diversity of cell types identified in each of the 19 tissues.

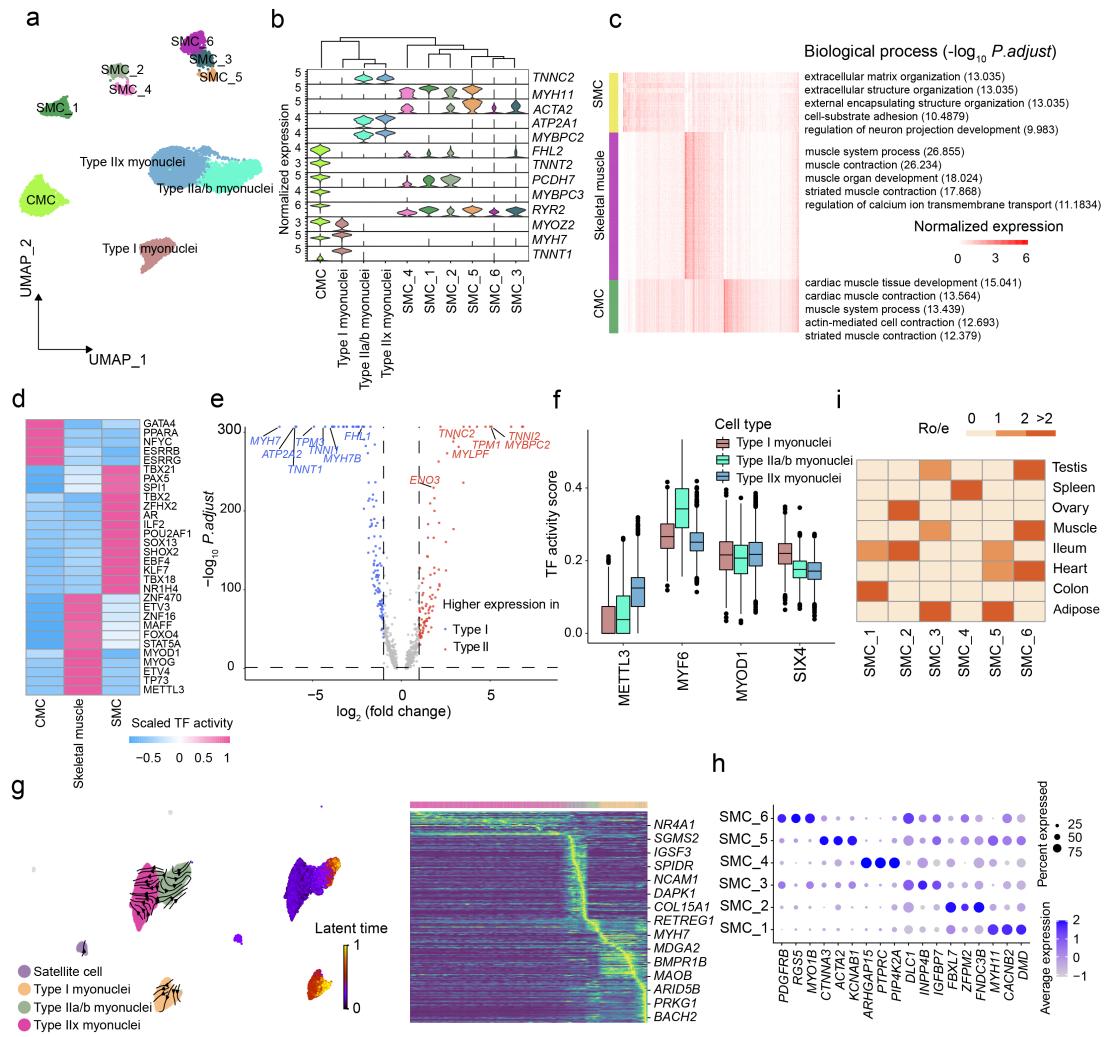
1324 Entropy shown by dotted line was calculated as described in Methods.

1325 **d**, t-SNE visualization of single-nucleus profiles (dots) colored by major cell types. All cell
1326 types are categorized into nine top-level cell lineages, and cell type annotation is provided in
1327 the legend to the right.

1328 **e**, Cellular relationship and composition across tissues. The dendrogram was created by
1329 hierarchical clustering based on the transcriptional levels of each cell type. The bar chart
1330 represents the relative contributions of tissues to each cell type.

1331 **f**, Cell state prediction of nine top-level cell lineages. Cells with higher cell cycling index are
1332 more proliferative. The horizontal line in the boxplots corresponds to the median, the box
1333 bounds indicate the 25th and 75th percentiles and the whiskers represent 1.5 times the
1334 interquartile range. Values outside the whiskers are displayed as points.

1335



1336

1337 Fig. 2 | Identification and characterization of three muscle cell types.

1338 **a**, UMAP visualization of all muscle cells from eight tissues. Each dot represents one nucleus,
1339 with colors coded according to manually annotated cell types. CMC, cardiac muscle cell; SMC,
1340 smooth muscle cell.

1341 **b**, Violin plots showing the normalized expression levels of marker genes for three major
1342 muscle cell types.

1343 **c**, Significantly enriched biological process terms of specific gene signatures in three major
1344 muscle cell types. Numbers between parentheses represent significance expressed as -log₁₀
1345 (adjusted p-value).

1346 **d**, Transcription factors with different activity scores among three major muscle cell types.

1347 **e**, Volcano plot displaying differentially expressed genes between type I and type II myonuclei.

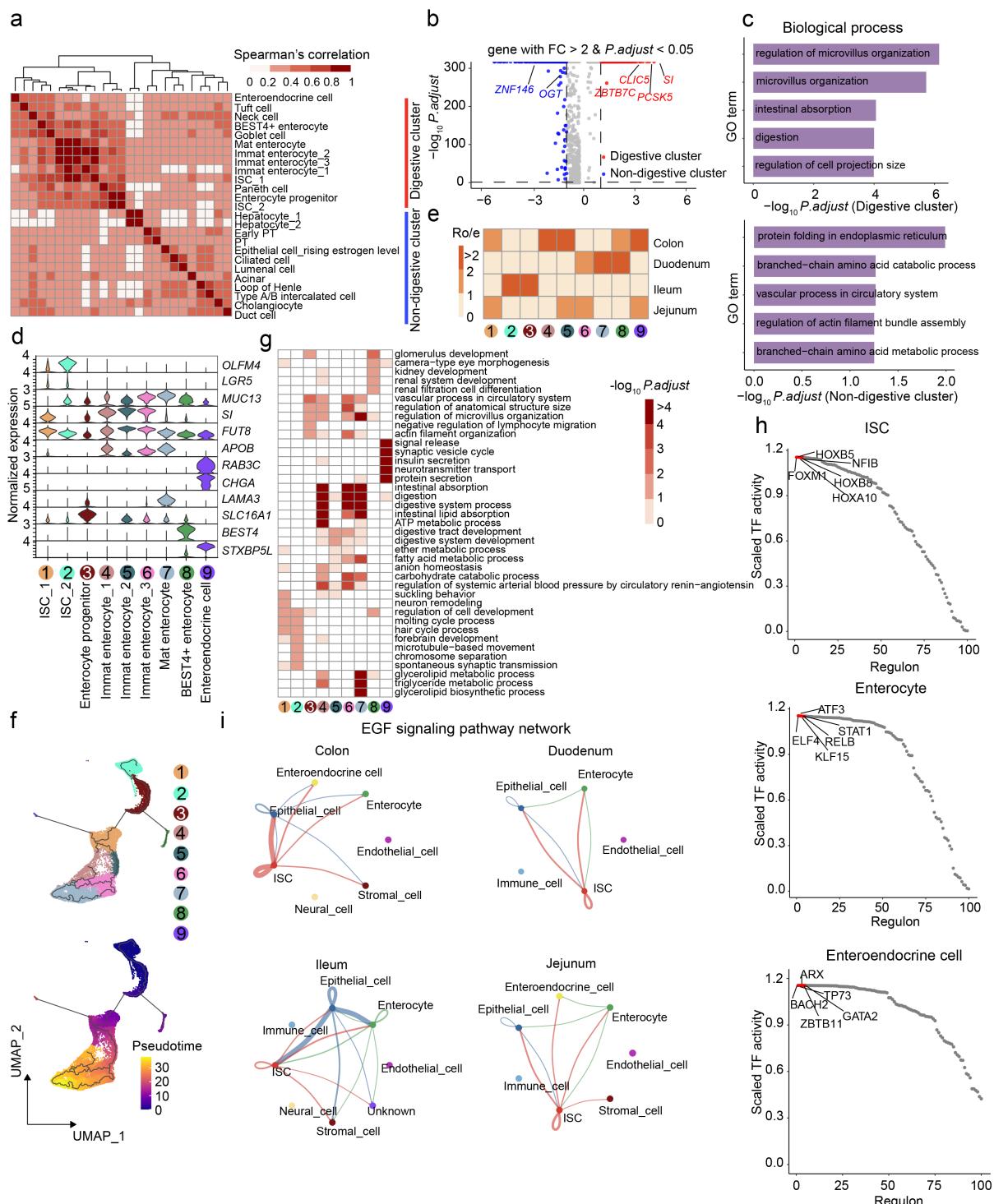
1348 **f**, Four candidate transcription factors with distinct activity scores in type I, IIa/b, and IIx
1349 myonuclei.

1350 **g**, RNA velocity analysis demonstrating state transition from satellite cells to myofiber in the
1351 skeletal muscle tissue. The arrows represent a flow derived from the ratio of unspliced to
1352 spliced transcripts, which in turn predicts dynamic changes in cell identity. Heatmap on the
1353 right demonstrates stereotyped changes in gene expression trajectory.

1354 **h**, Dot plot showing the expression levels of selected marker genes for each smooth cell cluster.

1355 **i**, Heatmap indicating the tissue preference of each cell population across different tissues
1356 revealed by $R_{o/e}$ (ratio of observed cell number to expected cell number).

1357



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1359 **Fig. 3 | Shared and tissue-specific molecular features for epithelial cell compartments.**

1360 **a**, Heatmap showing Spearman correlation coefficient between 25 epithelial cell subtypes
1361 which could be broadly classified into digestive and non-digestive groups.

1362 **b**, Volcano plot displaying differentially expressed genes between the digestive and non-
1363 digestive clusters. Dots in the volcano plot highlight up-regulated genes in each group.

1364 **c**, Functional annotation of up-regulated genes in each group. Top enriched biological
1365 processes terms are listed.

1366 **d**, Violin plots showing the normalized expression levels of marker genes for each cell subtype.

1367 **e**, Heatmap indicating the tissue preference of each cell population across four intestinal
1368 segments revealed by $R_{o/e}$ (ratio of observed cell number to expected cell number).

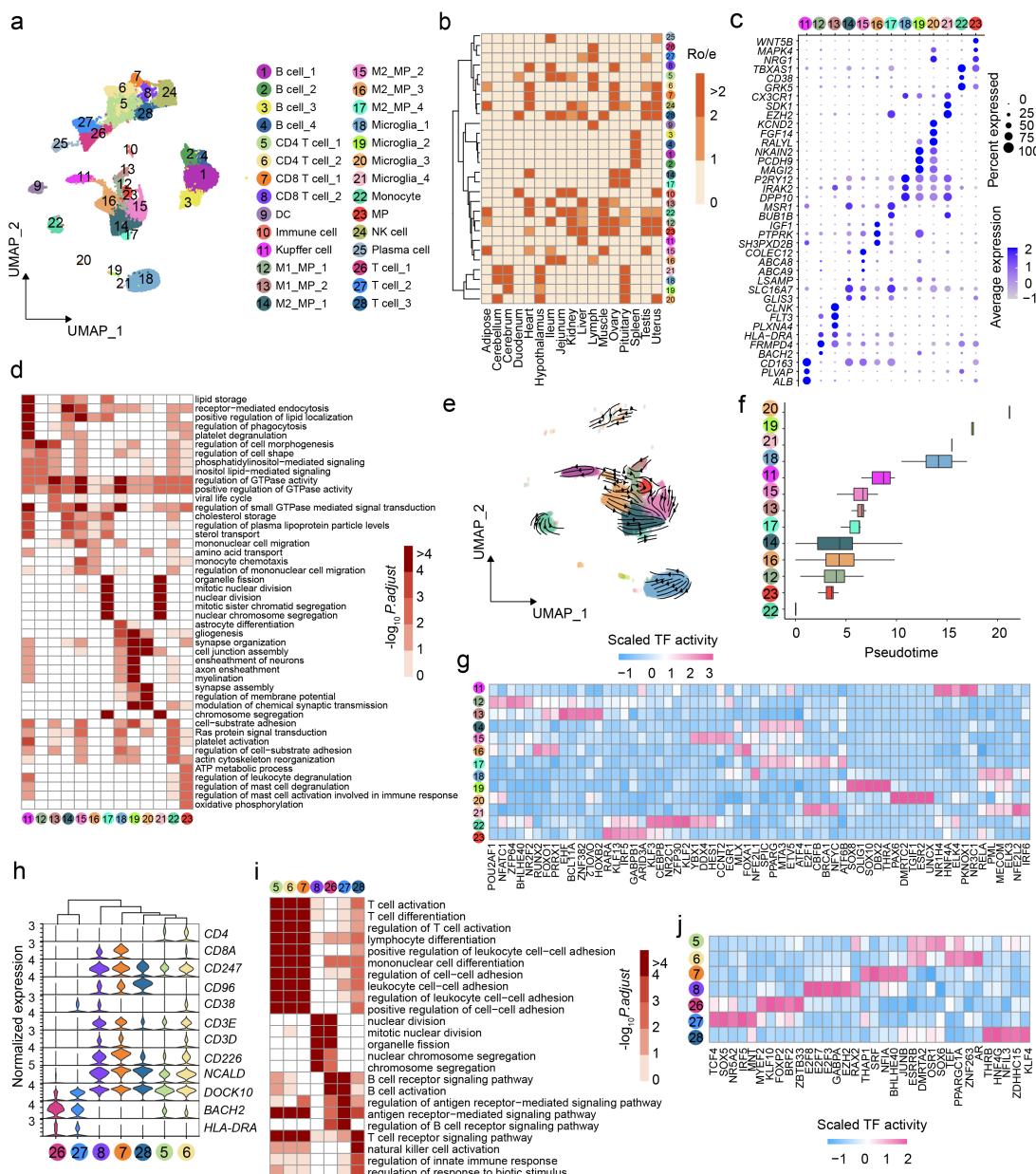
1369 **f**, UMAPs showing the pseudotime differentiation trajectories of intestinal stem cells,
1370 enterocytes, and enteroendocrine cells, respectively.

1371 **g**, Heatmap representing the enrichment of biological process terms in epithelial cell subtypes.

1372 **h**, Scatter plots showing the top 100 regulons of the three major epithelial cell subtypes. Each
1373 regulon is ordered by activity score, and the top five regulons with high activity are highlighted
1374 in red.

1375 **i**, The inferred EGF signalling pathway network among the major cell types in four intestinal
1376 segments. The edge width represents the communication probability, and a thicker edge line
1377 indicates a stronger signal.

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1379

1380 **Fig. 4 | Immune cell heterogeneity across tissues in pigs.**

1381 **a**, UMAP visualization of immune cell types across different tissues. Each dot represents one

1382 cell, with colors coded according to manually annotated cell types.

1383 **b**, Heatmap indicating the tissue preference of annotated immune cell types across different

1384 tissues revealed by $R_{\text{o/e}}$ (ratio of observed cell number to expected cell number).

1385 **c**, Dot plot showing the expression levels of selected marker genes for each cell cluster.

1386 **d**, Heatmap representing the enrichment of biological process terms for monocyte and

1387 macrophage lineages residing in different tissues.

1388 **e**, UMAP showing the pseudotime differentiation trajectories of monocyte and macrophage

1389 lineages.

1390 **f**, Box plots denoting the distribution of estimated pesudotime value for each cell type by
1391 Monocle3.

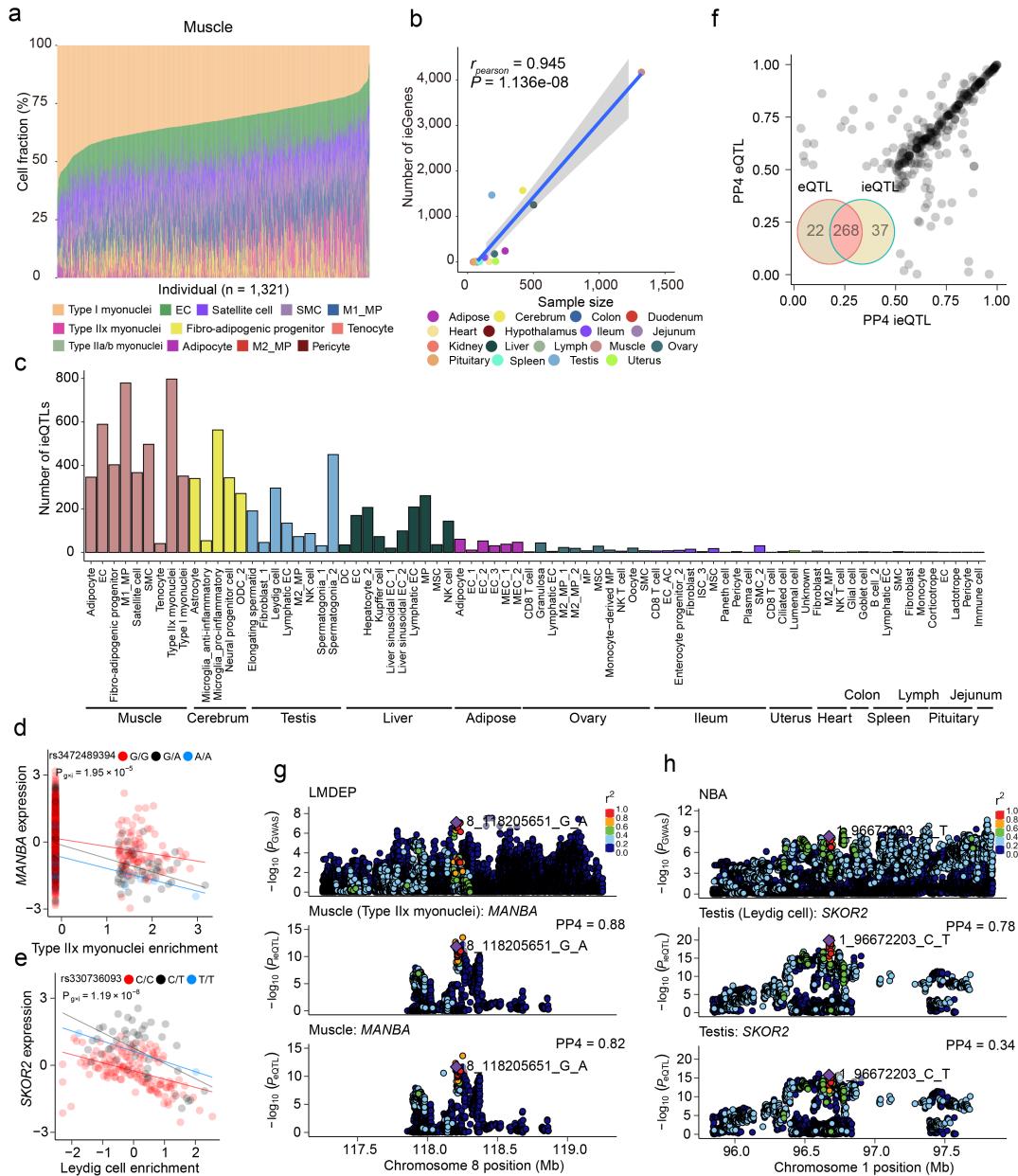
1392 **g**, Heatmap showing transcription factors with distinct activity scores in six major myeloid cell
1393 compartments.

1394 **h**, Violin plots showing the normalized expression levels of marker genes for T cell populations.

1395 **i**, Heatmap representing the enrichment of biological process terms for T cell subtypes in
1396 different tissues.

1397 **j**, Heatmap showing transcription factors with different activity scores among different T cell
1398 subtypes.

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1401 **Fig. 5 | Cell-type-dependent activities of genetic variants on gene expression and pig traits.**

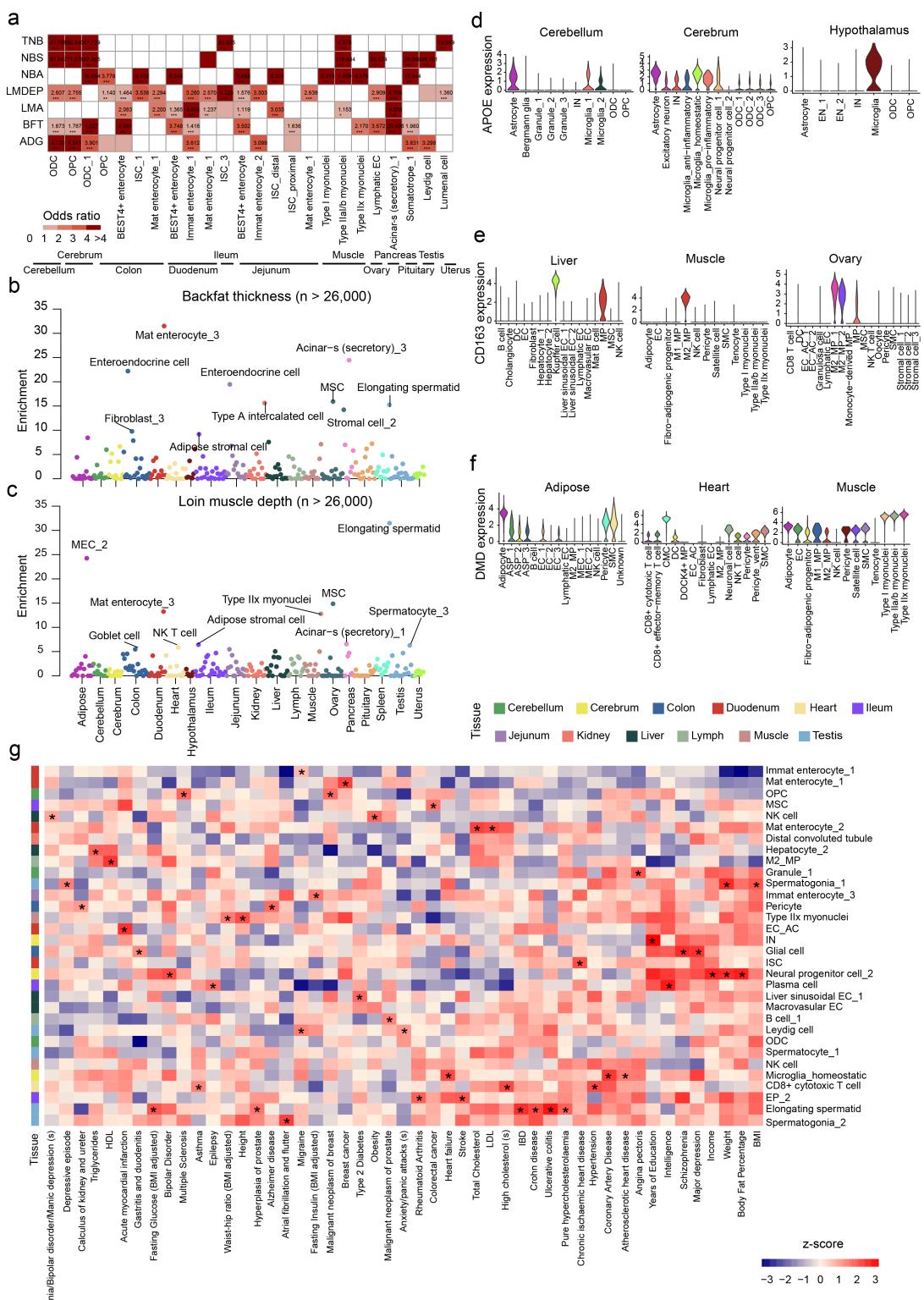
1402 **a**, Stacked bar plots showing the fraction of cell types estimated in PigGTEx RNA-seq samples
1403 based on our snRNA-seq reference matrix in muscle tissue.

1404 **b**, Scatter plot showing the estimated number of ieGenes versus sample sizes for 17 tissues
1405 estimated using public bulk RNA-seq datasets.

1406 **c**, Number of cell type interaction QTL (ieQTL) discovered in each cell type-tissue
1407 combination at FDR < 5%.

1408 **d**, An ieQTL of *MANBA* showing cell-type-specific effects in type Ix myonuclei from muscle.
1409 Each point represents an individual and is colored by three genotypes. Both gene expression

1410 levels and cell type enrichment values are inverse normal transformed across samples. The
1411 lines are fitted by a linear regression model using the geom_smooth function from ggplot2
1412 (v3.3.2) in R (v4.0.2).
1413 **e**, An ieQTL of *SKOR2* showing cell-type-specific effects in type IIx myonuclei from muscle.
1414 Each point represents an individual and is colored by three genotypes. Both gene expression
1415 levels and cell type enrichment values are inverse normal transformed across samples. The
1416 lines are fitted by a linear regression model using the geom_smooth function from ggplot2
1417 (v3.3.2) in R (v4.0.2).
1418 **f**, Overlaps between ieQTL and eQTL detected by traditional bulk RNA-seq.
1419 **g**, Aligned Manhattan plots of pig GWAS, ieQTL, and eQTL at the *MANBA* locus for loin
1420 muscle depth trait (LMDEP). SNPs are colored according to the magnitude of linkage
1421 disequilibrium (r^2) between adjacent SNPs pairs.
1422 **h**, Aligned Manhattan plots of pig GWAS, ieQTL, and eQTL at the *SKOR2* locus for number
1423 born alive trait (NBA). SNPs are colored according to the magnitude of linkage disequilibrium
1424 (r^2) between adjacent SNPs pairs.



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1426 Fig. 6 | Association of single-cell transcriptomic profiles with complex traits in pigs and

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1428 **a**, Heatmap showing representative significant associations between cell types and traits in pigs.

1429 Definitions for abbreviations and complete results are provided in Supplementary Table 2.

1430 **b**, Evaluation of the enrichment of backfat thickness trait in putative cell types by scRNA-seq
1431 data. Each circle represents a cell-type-trait association from a large-scale population dataset.
1432 **c**, Evaluation of the enrichment of loin muscle depth trait in putative cell types by scRNA-seq
1433 data. Each circle represents a cell-type-trait association from a large-scale population dataset.
1434 **d**, Cell-type-specific expression patterns of *APOE* in the cerebellum, cerebrum, and
1435 hypothalamus. The *APOE* gene is a key candidate associated with
1436 hyperlipidemia/atherosclerosis from the OMIA database.
1437 **e**, Cell-type-specific expression patterns of *CD163* in the liver, muscle, and ovary. The *CD163*
1438 gene is an essential receptor linked to resistance/susceptibility to the porcine reproductive and
1439 respiratory syndrome (PRRS) virus from the OMIA database.
1440 **f**, Cell-type-specific expression patterns of *DMD* in the adipose, heart, and muscle. The *DMD*
1441 gene plays a vital role in muscular dystrophy from the OMIA database.
1442 **g**, Heatmap showing enrichment of pig cell types (indicated on the right) associated with
1443 selected human traits and diseases (indicated at the bottom). The colored boxes indicate
1444 selected enriched patterns. Definitions for abbreviations and complete results are provided in
1445 Supplementary Tables 4 and 5.