Materials and Methods

Experimental protocol

Ten 10-week old male C57BL/6NRj were obtained from Janvier Labs and shipped to the Central laboratory animal husbandry of the University Medicine Rostock where they were euthanized by cervical dislocation the next day. Fzt:DU mice were bred inhouse in the Laboratory for Innovative Farm Animal Models[1]. Four 12-week old male mice were euthanized for the extraction of the hearts. All mice were handled in accordance to Directive 2010/63/EU on the protection of animals.

Hearts were pooled for each strain before using the Nuclei PURE Prep nuclei isolation kit (Sigma-Aldrich) according to the manufacturer´s instructions. In brief, hearts were rinsed with ice cold PBS, minced thoroughly, and further homogenized in chilled lysis solution using a gentleMACS dissociator. Cell debris and clumps were removed by using 40 µm strainers. Subsequently, lysate samples were mixed with chilled sucrose cushion solution, layered on 1.8 M sucrose cushion solution, and centrifuged for 45 min at 30,000 xg and 4 °C. Nuclei pellets were resuspended in chilled PBS containing 1 % BSA and 0.2 U/µl RNase inhibitor and cell debris was removed by a final filtration step. After centrifugation for 8 min at 600 xg and 4 °C, nuclei were resuspended in 1 ml Nuclei PURE storage buffer, snap-frozen in liquid nitrogen, and stored at -80 °C until processing.

Sequencing was conducted on the 10xGenomics system. Single cells were captured in droplet emulsions and scRNA-seq libraries were constructed as per the 10x Genomics protocol using GemCode Single-Cell 3' Gel Bead and Library V3 Kit. Libraries were sequenced on the NovaSeq 6000 Sequencing System (Illumina).

Computational data analysis

The snRNA-seq fastq data files were aligned with kallisto (v.0.46) to the generated mm10 genome (NCBI) index build reflecting the spliced and unspliced transcripts and subsequently quantified with bustools (v.0.39.3) as previously described[2]. The obtained transcript files have been incorporated into R by using the BUSpaRse R-package (v.0.99.25) to be able to use the downstream processing tool Seurat (v.3.1.1). For clustering, dimensionality was initially reduced by principal-component analysis and numbers of the most variable principal components were selected using heuristic methods implemented in Seurat. For an improved UMAP clustering, i.e. identification of small subgroups as well as batch corrections, we included the upstream processing algorithm harmony[3] (v.1.0). The RNA velocity was conducted with the velocyto R-package[4] (v.0.6). Sets of well-known marker genes for the respective cell types were used to annotate the identified cell types of the generated clusters. Novel cell cluster markers recently identified by other groups working with single nuclei data were applied for cluster annotation and found to be transferable to our datasets[5]. These and additional markers from the literature as well as the full UNIX and R-script can be freely obtained from our associated FairdomHub/iRhythmics instance. The raw .fastq data can be retrieved from Arrayexpress.

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