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Tick Hemocytes at the Single Cell Level

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BACKGROUND

The tick *Ixodes scapularis* transmits several pathogens of public health relevance, including the spirochete *Borrelia burgdorferi* and the rickettsial agent *Anaplasma phagocytophilum*. To establish infection in ticks, these microbes must overcome the antimicrobial defenses generated by **hemocytes, or specialized immune cells**. However, the underpinnings of the tick cellular immune response are in its infancy, as the most significant contributions were made in the 1970s describing tick hemocytes based on morphological features. Particularly, three main subtypes are present in hard or soft ticks: prohemocytes, plasmatocytes and granulocytes. **Prohemocytes** are stem cells that give rise to other hemocytes, are the smallest cells and have a high nuclear/cytoplasmic ratio. **Granulocytes** are round/oval cells with variable sizes. Their cytoplasm is full of granular inclusions or vacuoles containing anti-microbial compounds. **Plasmatocytes** have few granular inclusions and vary in size and shape, as they contain several plasma membrane protrusions and/or cytoplasmic projections. Their main tasks are phagocytosis and wound healing. Although useful, this classification is not complete, as the full functional diversity, molecular characterization, and differentiation trajectories of tick hemocytes remains mostly elusive. In this work, we used scRNA-seq to define distinct subpopulations, lineages and molecular markers in *I. scapularis* hemocytes. **We hypothesize that the unknown genetic diversity of *I. scapularis* hemocytes can be revealed using scRNA-seq.**

METHODS

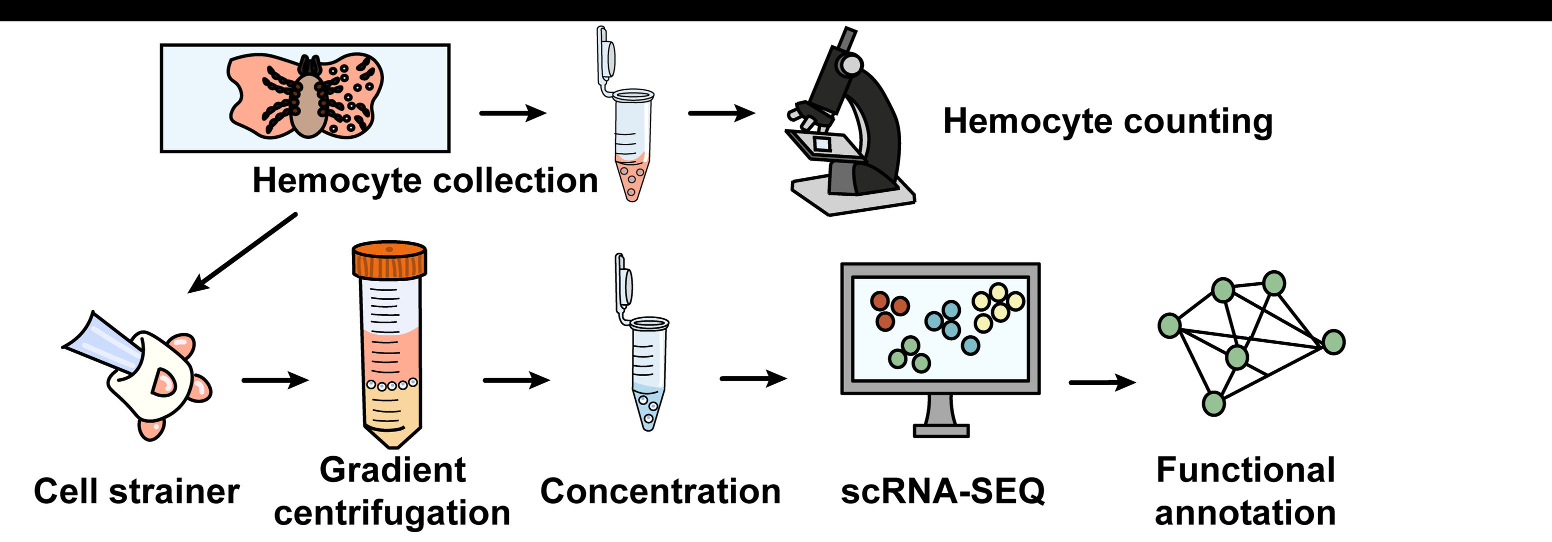


Figure 1: Tick hemocyte isolation and scRNA-seq. Hemocytes were isolated from *I. scapularis* nymphs, which were unfed, partially fed or fully fed on uninfected, *B. burgdorferi-* or *A. phagocytophilum*-infected mice. Ticks were covered in tick cell medium, their legs were incised and the hemolymph containing hemocytes was released into the medium. Then, hemocytes were collected, stained and counted using light microscopy, or were processed for scRNA-seq using 10X Genomics technologies (cell strainers, siliconized tips and vials, gradient centrifugations were employed to increase sample yield and quality).

RESULTS

1- Hemocyte composition changes during *I. scapularis* feeding and bacteria acquisition

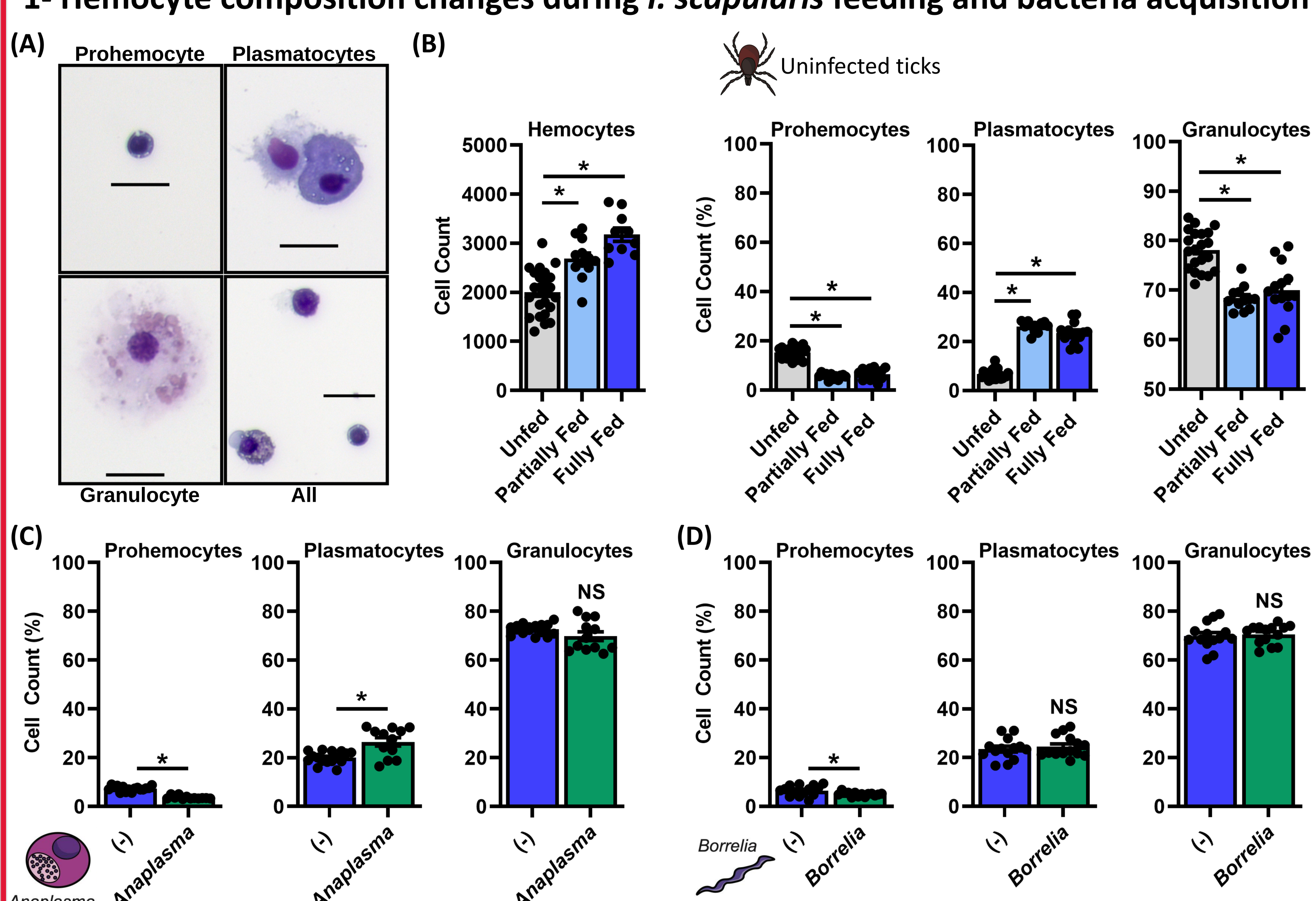
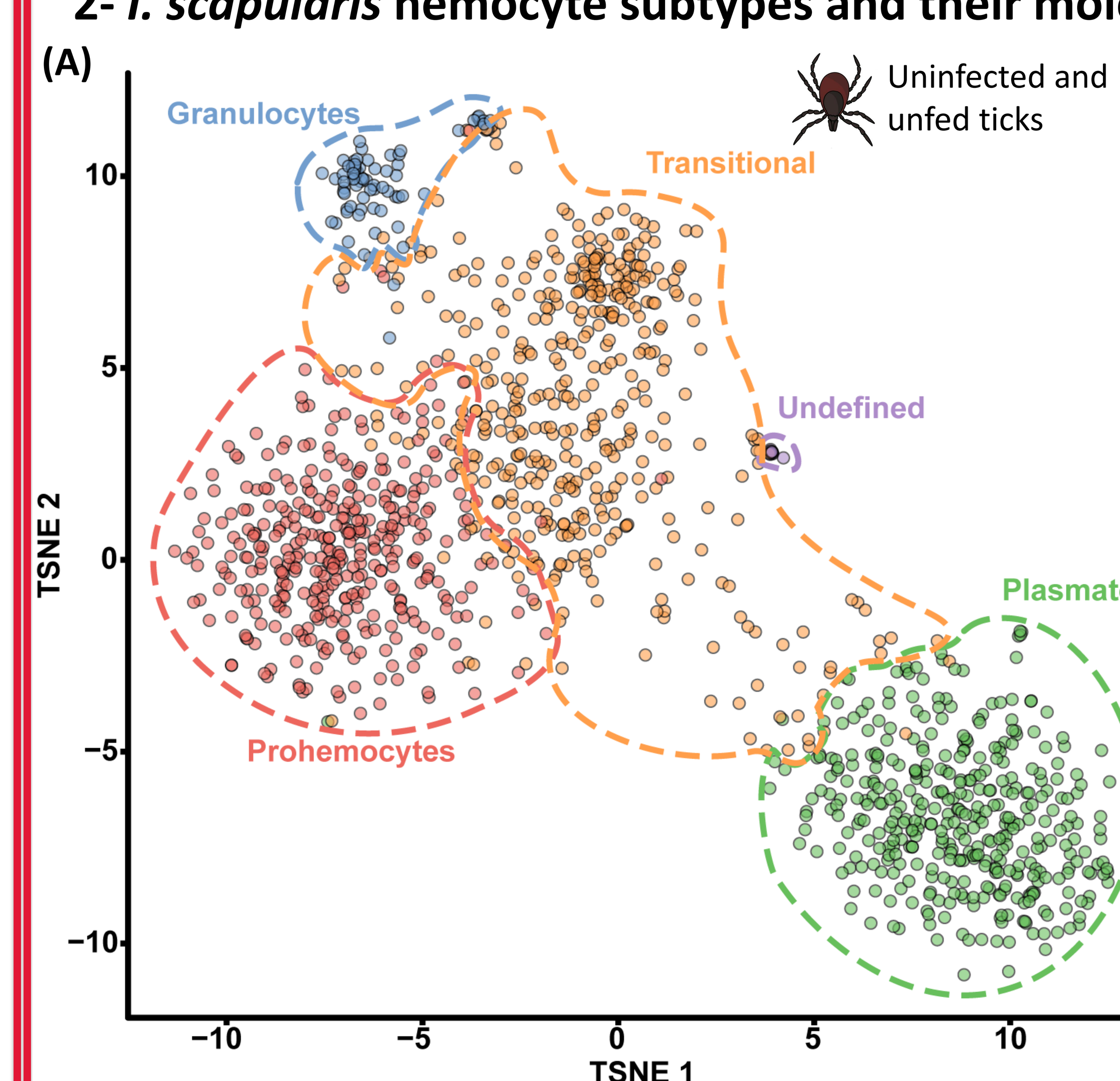


Figure 2: (A) The three main morphological subtypes of *I. scapularis* hemocytes were evaluated by light microscopy after Giemsa staining. Scale bars denote 20 µm. **(B-D)** Hemocyte numbers and types from unfed, partially fed (3 days) or fully fed (5 days) *I. scapularis* nymphs placed on **(B)** uninfected, **(C)** *A. phagocytophilum*-infected BL6 mice or **(D)** *B. burgdorferi*-infected C3H mice. Total number of hemocytes were counted using a hemocytometer. Each dot represents hemocytes from individual ticks, from three independent experiments. Error bars represent Mean+/-SD. Unpaired t-test. Asterisks indicate statistically significant difference.

2- *I. scapularis* hemocyte subtypes and their molecular markers reveled by scRNA-seq



(B)

Cluster	# of cells	# of DEGs (FDR<0.05)	Highest log_2FC value
Granulocytes	73	29	4.24
Transitional	437	69	0.43
Plasmatocytes	389	93	4.9
Prohemocytes	368	109	1.9
Undefined	10	9	6.84

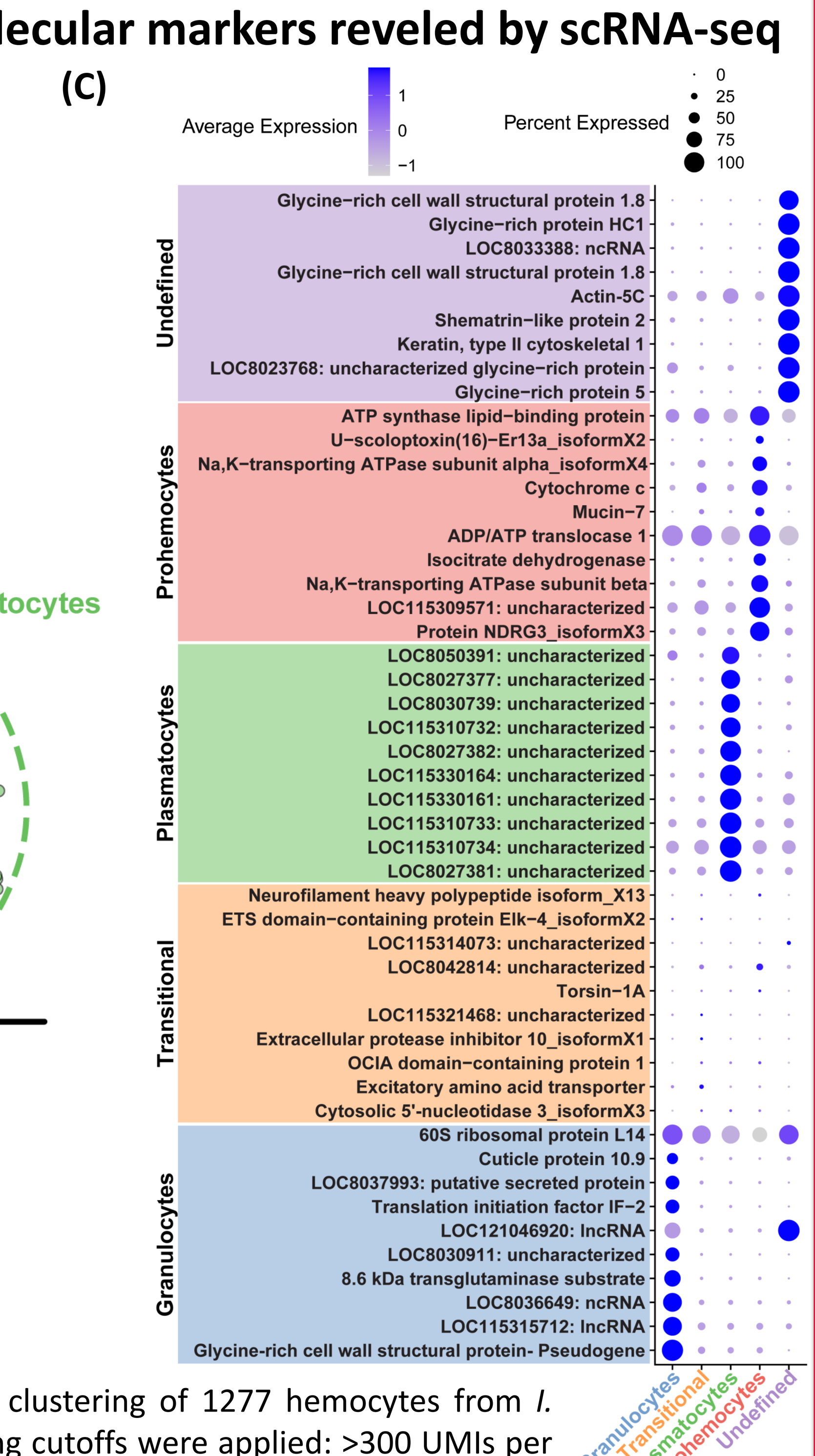


Figure 3: (A) t-Distributed Stochastic Neighbor Embedding (TSNE) plot clustering of 1277 hemocytes from *I. scapularis* unfed ticks, revealed five cell types (color coded). The following cutoffs were applied: >300 UMIs per cell; >150 genes per cell; <30% mito genes. **(B)** Number of cells, differentially expressed genes (DEGs) and highest gene Log_2FC value per hemocyte type. **(C)** Molecular markers of each hemocyte type (top DEGs).

3- Functional characterization of *I. scapularis* hemocyte subpopulations based on their gene expression

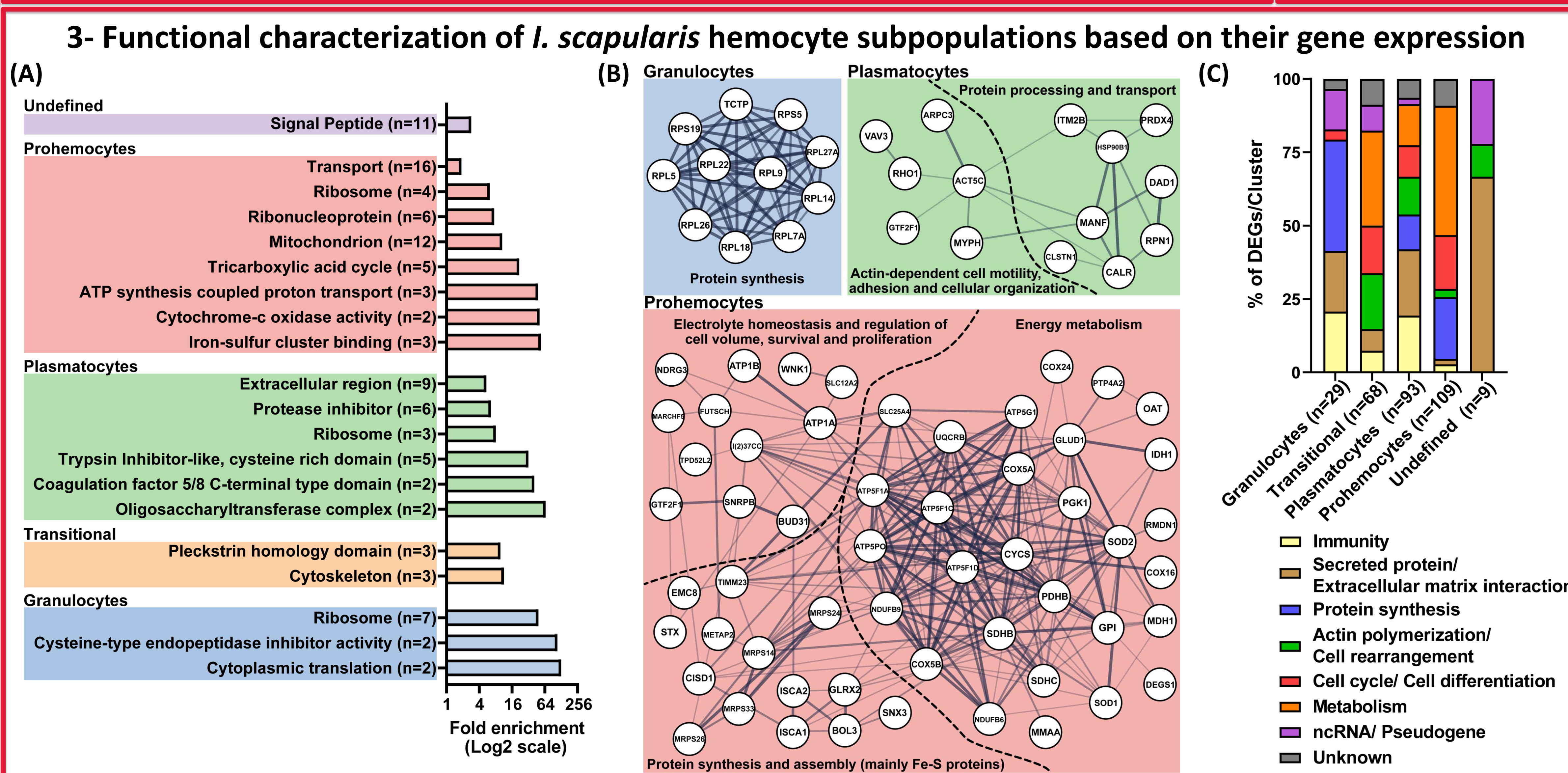


Figure 4: (A) GO and UNIPROT term enrichment analyses on the DEGs in each hemocyte clusters using DAVID (v2021). Only significant enrichments are depicted. Number of genes with a specific term associated are shown in parentheses. **(B)** Interactome enrichment analysis using STRING (v11.5). Thickness of the lines indicates the strength of data support. Only significant interactions are depicted, color coded by hemocyte clusters. **(C)** All DEGs per cluster were manually annotated using FlyBase, Uniprot and Interpro databases. Total number of DEGs per subpopulation are shown in parentheses.

4- Lineage differentiation pattern in *I. scapularis* hemocytes

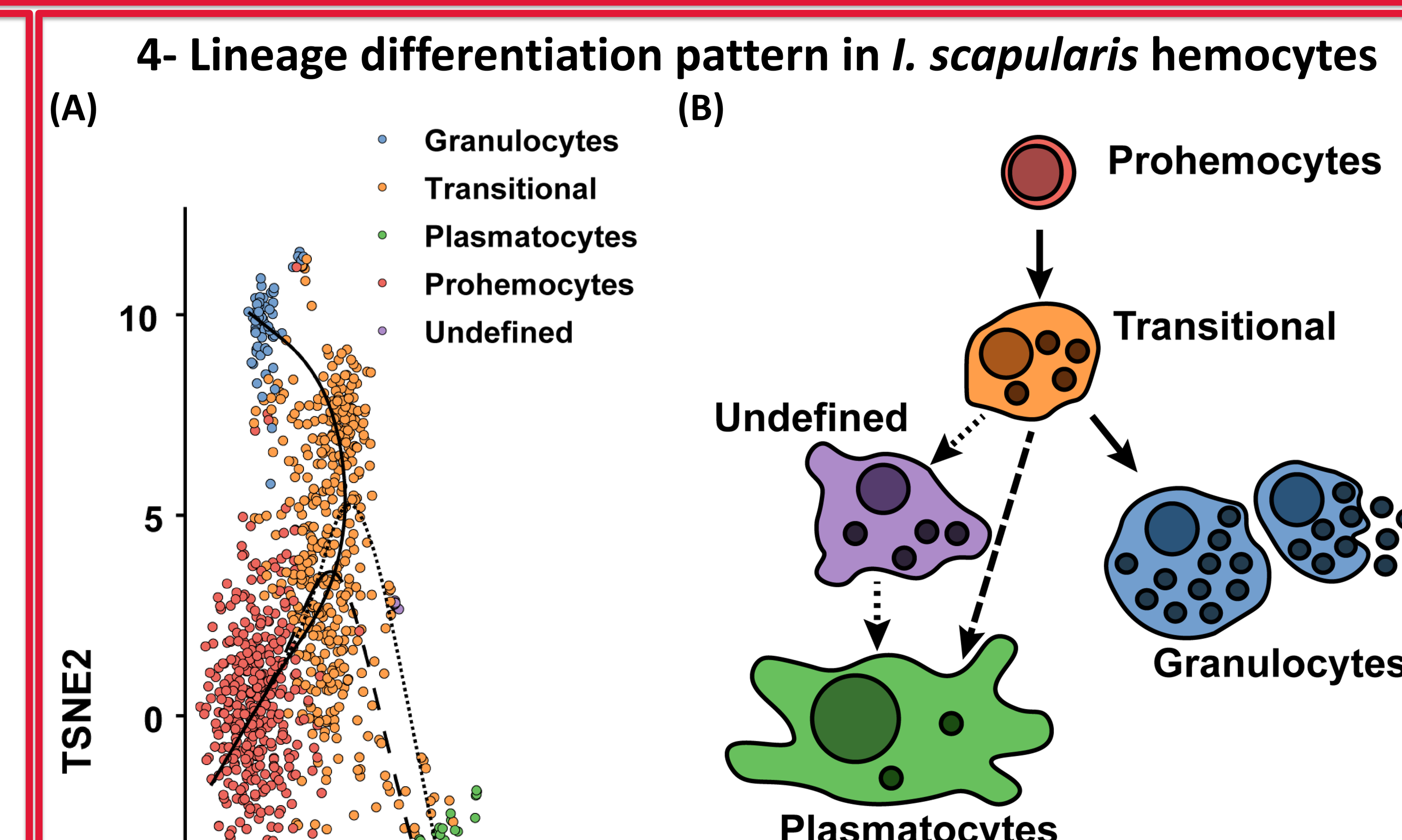


Figure 5: (A) Monocle3 revealed three distinct lineages of tick hemocytes in unsupervised pseudotime analysis, starting at the Prohemocyte cluster, indicated by three types of black lines. **(B)** Model of the lineage differentiation process in tick hemocytes, based on pseudotime analysis, molecular markers and functional annotation. Prohemocytes are the starting point, from where "Transitional" cells are differentiated and then give rise to more mature cell types, such as Plasmatocytes and Granulocytes.

CONCLUSIONS

- Blood feeding induces drastic changes in *I. scapularis* hemocyte physiology.
- scRNA-seq revealed molecular markers, lineage patterns and higher complexity in *I. scapularis* hemocytes subpopulations compared to previous morphological characterization.
- Our work provides a base for functionally characterizing tick hemocytes upon metabolic alterations or microbial infections and will serve as a valuable resource to the vector biology and rickettsial community.

ACKNOWLEDGEMENTS

- Dr. Uli Munderloh (Univ. of Minnesota) for providing ISE6 and IDE12 tick cell lines.
- NIH R01 AI116523 Funding.
- Fairbairn Family Lyme Research Initiative Funding.