Identification of TREM2 Macrophages in Human Infectious Disease

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INTRODUCTION

- Triggering receptor expressed on myeloid cells 2 (TREM2) is an innate immunomodulatory receptor found on myeloid-lineage immune cells, including dendritic cells, monocytes, and tissue-resident macrophages.

- Although a mechanistic understanding of TREM2 function and signaling remain elusive, the role of TREM2 macrophages have recently been highlighted in diseases characterized by altered lipid metabolism and chronic inflammation, including atherosclerosis, obesity, cancer, and Alzheimer's disease.[20,21,22]

- In a recent study by Ma *et al.,* 2021, TREM2 macrophages were identified for the first time in human infectious disease within lepromatous leprosy lesions at high expression levels.[22]

Methodology: Bioinformatics

Figure 1.
Our lab previously curated a list of consensus signature genes for "10 SPP1" and "18 TREM2," two clusters of macrophages that express TREM2 at different levels and as well as different biomarker genes. Using online multiple list comparators, the 16 common genes were used to overlap TREM2 scRNA-seq data in the papers reviewed in this study.

Venny 2.1.0 [24]

Overlapping TREM2 macrophage biomarker genes identified in 23 studies in the literature

Gene name	Number of clusters in which the gene is significant*	Studies (References)*
TREM2	26	Common1018, 1,2,3,4,5,6, Leprosy myeloid,7,8,9,10,11,12,13,14,15,16,18
APOE	23	1,2,3,4,5,6, Leprosy myeloid,7, 8,14,15,16,17,18,19
CTSB	22	1,20,2,3,4, Leprosy myeloid,7,8,11,12,13,14,15,17,18,19
C1QA	19	2,3,4,5, Leprosy myeloid,8,9,10,11,12,13,14,17,19
C1QB	19	2,3,4,5, Leprosy myeloid,8,9,10,11,12,13,14,17,19
C1QC	19	2,3,4,5, Leprosy myeloid,8,9,10,11,12,13,14,19
LGMN	19	2,4,5, Leprosy myeloid,8,9,10,11,12,14,15,16,18,19
CD68	18	1,2,3,4,5,7,8,9,12,13,14,15,17,19
CTSD	18	1,2,3,4, Leprosy myeloid,7,8,9,10,11,13,14,15,17,18,19
GPNMB	18	Common1018, 1,20,2,3,4, Leprosy myeloid,7,8,9,11,12,13,14,15,17,18
SPP1	18	1,20,2,3,6, Leprosy myeloid,7,8,9,11,12,13,14,15,16,18,19
CD63	17	20,2,3,4, Leprosy myeloid,7,8,9,11,12,13,14,17,18
CTSL	17	1,2,3,7,8,9,11,12,13,14,15,17,18,19
LGALS3	17	1, Casanova-Acebes M, Leprosy myeloid,7,8,9,11,12,13,14,17,19
PLD3	17	1,20,2,3, Leprosy myeloid,7,8,9,10,11,12,14,15,17,18
CAPG	16	1,2,3,4, Leprosy myeloid,7,8,9,11,13,14,17,17,18,19
CSTB	16	1,2,3,4, Leprosy myeloid,7,8,9,11,14,17,18,19
CTSS	16	2,3,4,5, Leprosy myeloid,8,9,10,11,12,14,17,19
GRN	16	2,3,4, Leprosy myeloid,8,9,11,12,13,14,17,19
LIPA	16	Common1018,1,2,4,6, Leprosy myeloid,8,9,11,13,14,15,16,17,18
C3AR1	15	2, Leprosy myeloid,Acne TREM2,8,11,12,14,16,17,18,19
CREG1	15	2,4,5, Leprosy myeloid,8,11,12,14,15,16,17,18,19
CTSZ	15	20,2,4, Leprosy myeloid,7,8,9,10,11,14,17,18,19
LAMP1	15	1,2,3,4,8,11,12,14,15,16,17,18,19
LGALS1	15	2,3, Leprosy myeloid,7,8,9,11,12,14,17,18,19
NPC2	15	20,2,3,4, Leprosy myeloid,7,8,9,11,12,13,14,16,18,19

Table 1. A list of TREM2 consensus signature genes curated in this study.

*Some studies include more than one cluster in which scRNA-seq data for TREM2 macrophages are significant.

Results: Building a TREM2 consensus sequence and assessing gene function

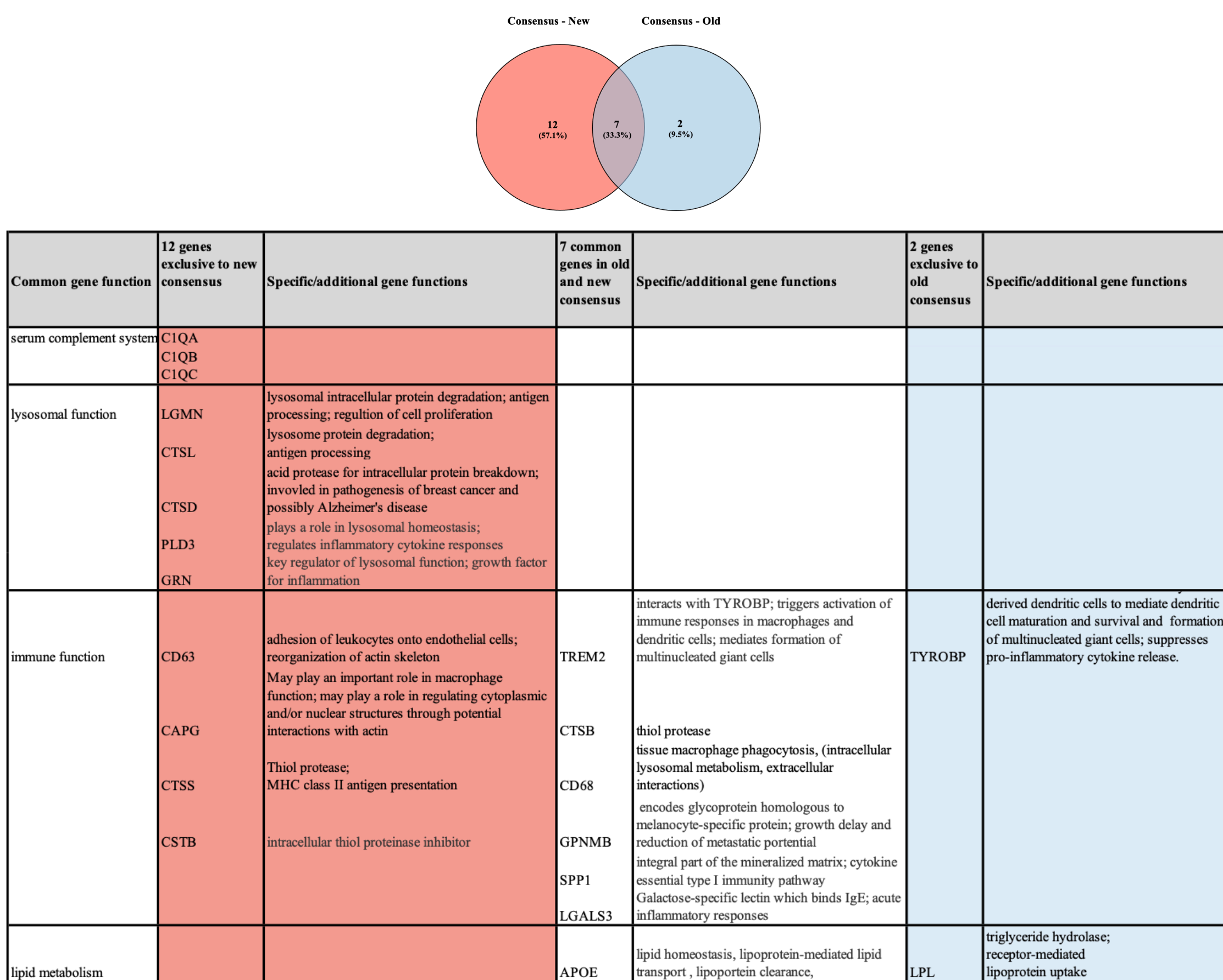| Common gene function	12 genes exclusive to new consensus	Specific/additional gene functions	7 common genes in old and new consensus	Specific/additional gene functions	2 genes exclusive to old consensus	Specific/additional gene functions
serum complement system	C1QA C1QB C1QC					
lysosomal function	LGMN	lysosomal intracellular protein degradation; antigen processing; regulation of cell proliferation				
	CTSL	lysosome protein degradation; antigen processing				
	CTSD	acid protease for intracellular protein breakdown; involved in pathogenesis of breast cancer and possibly Alzheimer's disease				
	PLD3	plays a role in lysosomal homeostasis; regulates inflammatory cytokine responses				
	GRN	key regulator of lysosomal function; growth factor for inflammation				
immune function	CD63	adhesion of leukocytes onto endothelial cells; reorganization of actin skeleton	TREM2	interacts with TYROBP; triggers activation of immune responses in macrophages and dendritic cells; mediates formation of multinucleated giant cells	TYROBP	derived dendritic cells to mediate dendritic cell maturation and survival and formation of multinucleated giant cells; suppresses pro-inflammatory cytokine release.
	CAPG	May play an important role in macrophage function; may play a role in regulating cytoplasmic and/or nuclear structures through potential interactions with actin	CTSB	thiol protease tissue macrophage phagocytosis, (intracellular lysosomal metabolism, extracellular interactions)		
	CTSS	Thiol protease; MHC class II antigen presentation	CD68	encodes glycoprotein homologous to melanocyte-specific protein; growth delay and reduction of metastatic potential		
	CSTB	intracellular thiol proteinase inhibitor	GPNMB	integral part of the mineralized matrix; cytokine essential type I immunity pathway		
			SPP1			
			LGALS3	Galactose-specific lectin which binds IgE; acute inflammatory responses		
lipid metabolism			APOE	lipid homeostasis, lipoprotein-mediated lipid transport , lipoprotein clearance,	LPL	triglyceride hydrolase; receptor-mediated lipoprotein uptake

Table 2. The "New" list of consensus signature genes generated by this study (19 genes) consisted of 10 more genes than the "Old" list curated previously by our lab (9 genes). The "New" list of consensus signature genes generated by this study (19 genes) were overlapped with the "Old" list curated previously by our lab (9 genes) using Venny 2.1.0[23]. Gene functions were searched in GeneCards, and the genes were grouped by similar function.

Methodology: Immunohistochemical staining

Immunoperoxidase Labeling

Figure 2. All biopsies were taken from patients before treatment.

Identification of TREM2 in leprosy biopsies

Figure 3. TREM2 protein expression was evaluated in human leprosy sections: L-lep (lepromatous leprosy), top; T-lep (tuberculoid leprosy), bottom. TREM2, IgG3 (isotype control), and CD68 (positive control, macrophage marker) were detected by the immunoperoxidase technique. Original magnification: x200. Scale bars: 40µm.

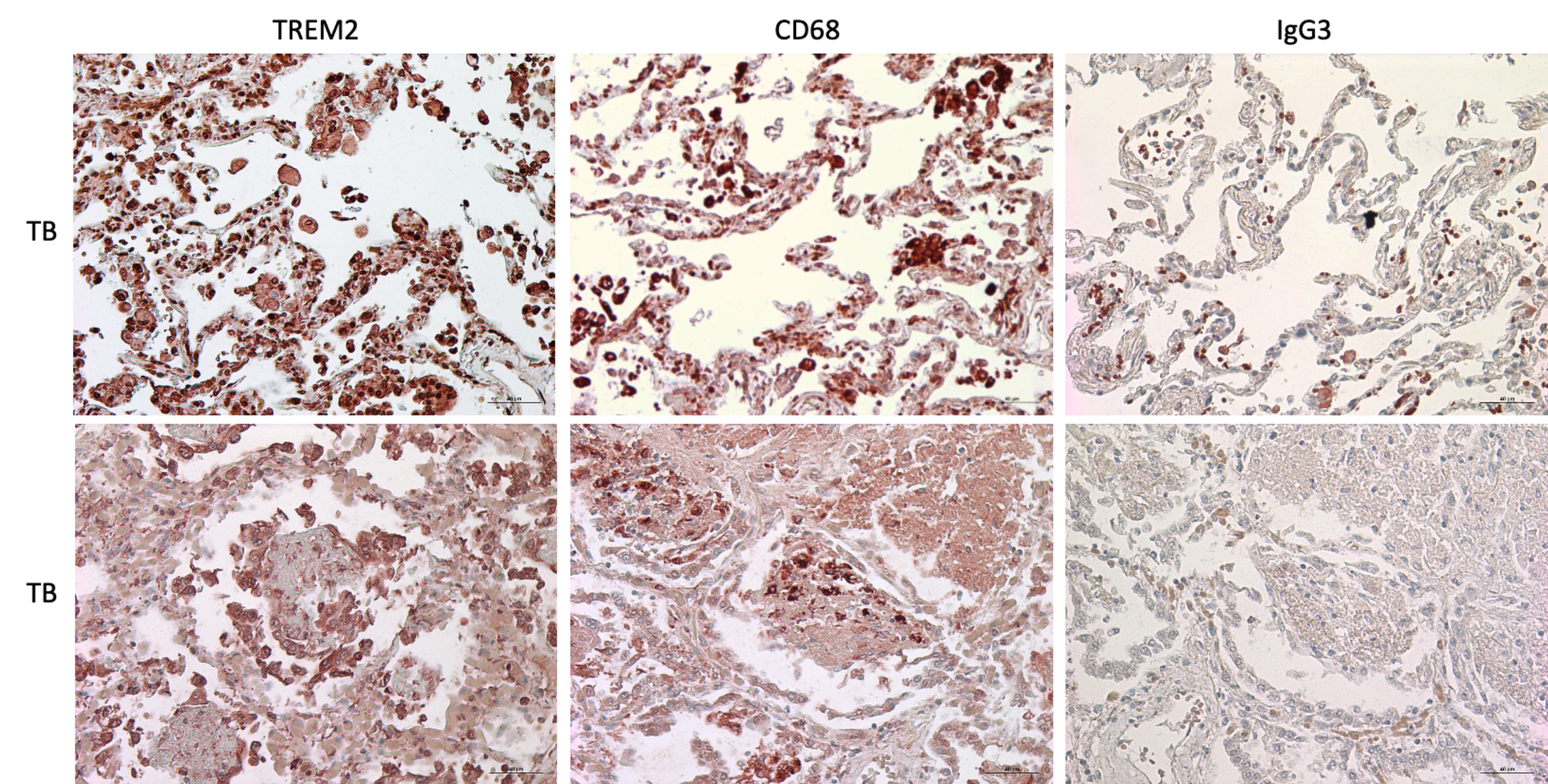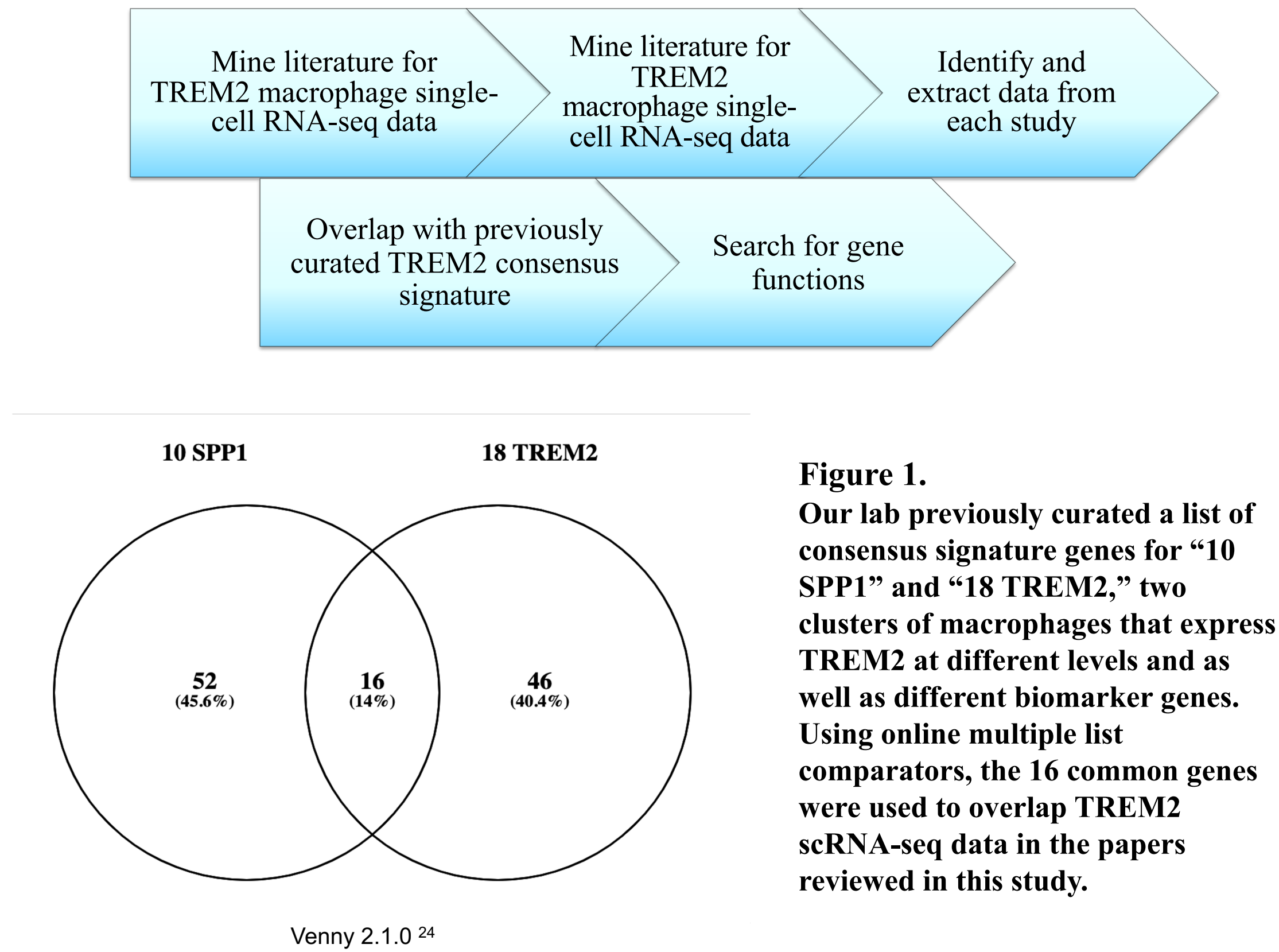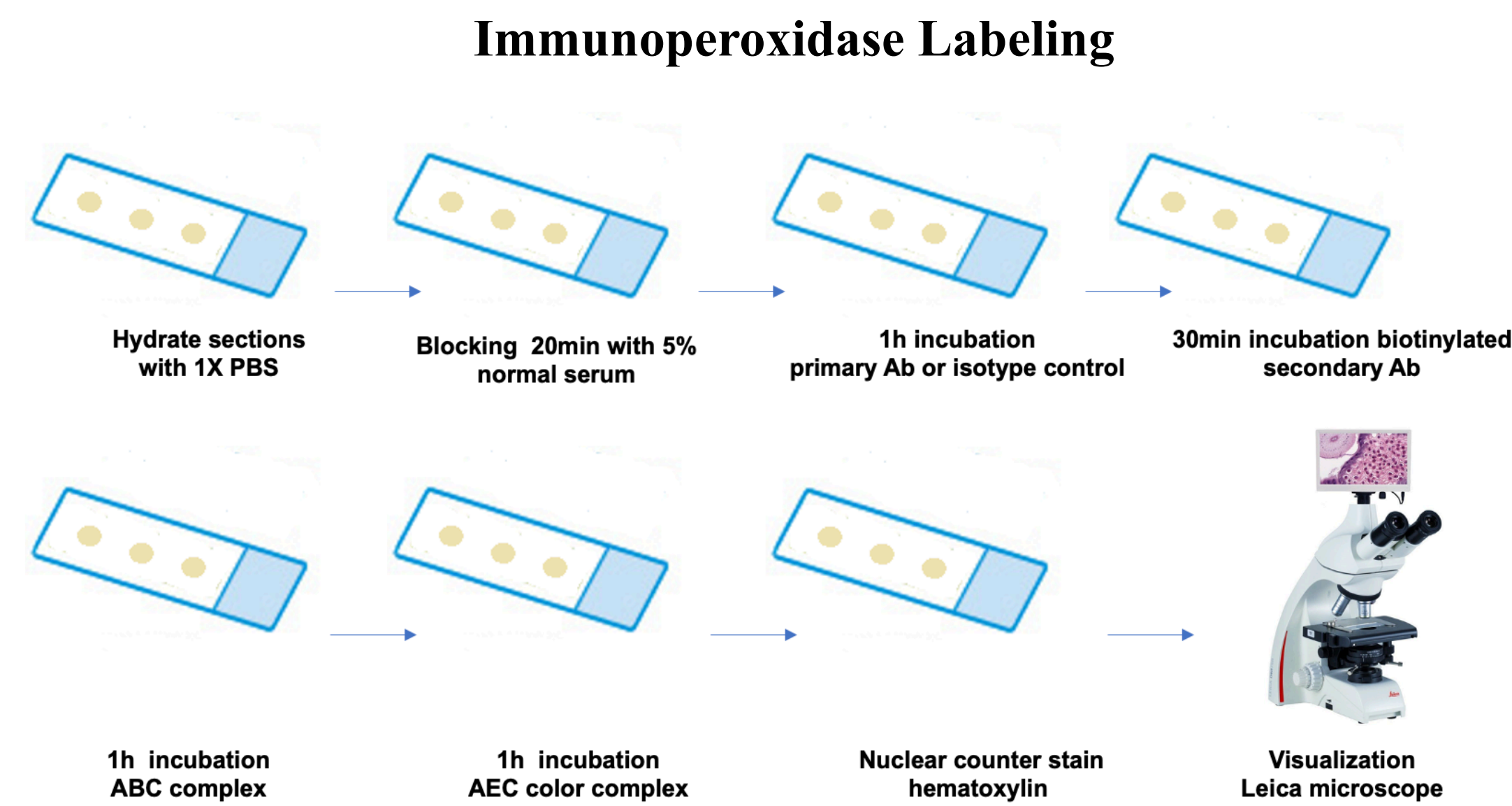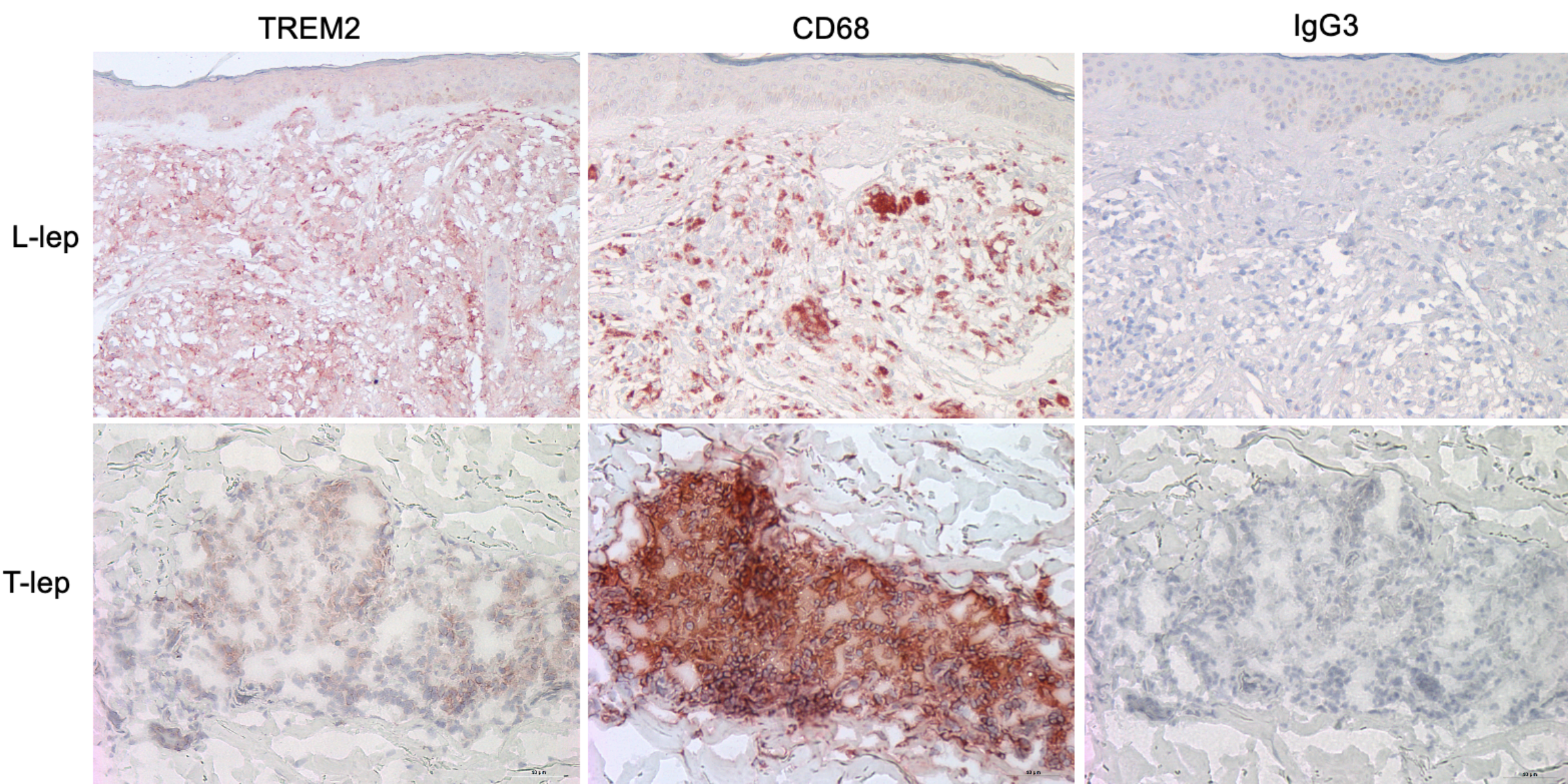## Identification of TREM2 in lung tuberculosis biopsies

Figure 4. TREM2 protein expression was evaluated in human pulmonary tuberculosis sections. TREM2, IgG3 (isotype control), and CD68 (positive control, macrophage marker) were detected by the immunoperoxidase technique. Original magnification: x200. Scale bars: 40µm.

DISCUSSION

- In this study, our list of TREM2 consensus signature genes was expanded and improved using scRNA-seq data analysis. The genes exclusively identified in the "New" consensus included genes involved in normal lysosomal function and homeostasis, as well as immunity and inflammation (Table 2).

- Immunohistochemical staining demonstrated that TREM2 expression in leprosy and tuberculosis samples was more positive in the core of granulomas where macrophage aggregates are localized, and was noted to be especially positive for foamy macrophage cells. Additionally, TREM2 expression is stronger in L-lep (disseminated leprosy) than T-lep (self-limiting leprosy) (Figure 3).

- The validation of TREM2 protein expression in leprosy and tuberculosis sections in this study supports the finding by Ma *et al.* that TREM2 macrophages may play a role in human infectious disease, including the pathology of mycobacterial infections.

- Although TREM2 macrophages have recently been increasingly identified across diverse pathologies, and has been newly verified to be present in sites of disease for human infectious disease samples, TREM2 signaling and function still remains unclear. Thus, performing single-cell transcriptomic analysis to generate TREM2 macrophage consensus signatures may help elucidate the role of TREM2 in pathogenesis.

REFERENCES

1. Jaitin DA, Adlung L, Thaiss CA, et al. Lipid-Associated Macrophages Control Metabolic Homeostasis in a Trem2-Dependent Manner. Cell. 2019;178(3):686-698.e614.
2. Cochain C, Vafadarnejad E, Arampatzi P, et al. Single-Cell RNA-Seq Reveals the Transcriptional Landscape and Heterogeneity of Aortic Macrophages in Murine Atherosclerosis. Circ Res. 2018;122(12):1661-1674.
3. Yao Y, Li H, Chen J, et al. TREM-2 serves as a negative immune regulator through Syk pathway in an IL-10 dependent manner in lung cancer. Oncotarget: Vol 7, No 20. 2016.
4. Wang ECE, Dai Z, Ferrante AW, Drake CG, Christiano AM. A Subset of TREM2(+) Dermal Macrophages Secretes Oncostatin M to Maintain Hair Follicle Stem Cell Quiescence and Inhibit Hair Growth. Cell Stem Cell. 2019;24(4):654-669.e656.
5. Xiong X, Kuang H, Ansari S, et al. Landscape of Intercellular Crosstalk in Healthy and NASH Liver Revealed by Single-Cell Secretome Gene Analysis. Mol Cell. 2019;75(3):644-660.e645.
6. Xue D, Tabib T, Morse C, Lafyatis R. Transcriptome landscape of myeloid cells in human skin reveals diversity, rare populations and putative DC progenitors. Journal of Dermatological Science. 2020;97(1):41-49.
7. Depuydt MAC, Prange KHM, Slenders L, et al. Microanatomy of the Human Atherosclerotic Plaque by Single-Cell Transcriptomics. Circ Res. 2020;127(11):1437-1455.
8. Harasymowicz NS, Rashidi N, Savadipour A, et al. Single-cell RNA sequencing reveals the induction of novel myeloid and myeloid-associated cell populations in visceral fat with long-term obesity. The FASEB Journal. 2021;35(3):e21417.
9. Obradovic A, Chowdhury N, Ager C, et al. Abstract PO009: Single-cell protein activity inference identifies tumor-enriched macrophages associated with early post-surgical disease recurrence. Cancer Research. 2021;81(5 Supplement):PO009-PO009.
10. Dong Y, D'Mello C, Pinsky W, et al. Oxidized phosphatidylcholines found in multiple sclerosis lesions mediate neurodegeneration and are neutralized by microglia. Nature Neuroscience. 2021;24(4):489-503.
11. Hornburg M, Desbois M, Lu S, et al. Single-cell dissection of cellular components and interactions shaping the tumor immune phenotypes in ovarian cancer. Cancer Cell. 2021.
12. Xiong D, Wang Y, You M. A gene expression signature of TREM2hi macrophages and γδ T cells predicts immunotherapy response. Nature Communications. 2020;11(1):5084.
13. Krishna C, DiNatale RG, Kuo F, et al. Single-cell sequencing links multiregional immune landscapes and tissue-resident T cells in ccRCC to tumor topology and therapy efficacy. Cancer Cell. 2021;39(5):662-677.e666.
14. Fidler TP, Xue C, Yalcinkaya M, et al. The AIM2 inflammasome exacerbates atherosclerosis in clonal haematopoiesis. Nature. 2021;592(7853):296-301.
15. Zou G, Wang J, Xu X, et al. Cell subtypes and immune dysfunction in peritoneal fluid of endometriosis revealed by single-cell RNA-sequencing. Cell & Bioscience. 2021;11(1):98.
16. Pozebo Antunes AR, Scheyltjens I, Lodi F, et al. Single-cell profiling of myeloid cells in glioblastoma across species and disease stage reveals macrophage competition and specialization. Nature Neuroscience. 2021;24(4):595-610.
17. Habermann AC, Gutierrez AJ, Bui LT, et al. Single-cell RNA sequencing reveals profibrotic roles of distinct epithelial and mesenchymal lineages in pulmonary fibrosis. Sci Adv. 2020;6(28):eaba1972.
18. Casanova-Acebes M, Dalla E, Leader AM, et al. Tissue-resident macrophages provide a pro-tumorigenic niche to early NSCLC cells. Nature. 2021.
19. Guerrero-Juarez CF, Dedhia PH, Jin S, et al. Single-cell analysis reveals fibroblast heterogeneity and myeloid-derived adipocyte progenitors in murine skin wounds. Nature Communications. 2019;10(1):650.
20. Deczkowska A, Weiner A, Amit I. The Physiology, Pathology, and Potential Therapeutic Applications of the TREM2 Signaling Pathway. Cell. 2020;181(6):1207-1217.
21. Kober DL, Brett TJ. TREM2-Ligand Interactions in Health and Disease. J Mol Biol. 2017;429(11):1607-1629.
22. Ma F, Hughes TK, Teles RMB, et al. The cellular architecture of the antimicrobial response network in human leprosy granulomas. Nature Immunology. 2021;22(7):839-850.
23. Oliveros JC. Venny 2.1.0, https://bioinfogp.cnb.csic.es/tools/venny/; 2007 [accessed].
24. www.genecards.org Stelzer G, Rosen R, Plaschkes I, Zimmerman S, Twik M, Fishilevich S, Iny Stein T, Nudel R, Lieder I, Mazor Y, Kaplan S, Dahary D, Warshawsky D, Guan - Golan Y, Kohn A, Rappaport N, Safran M, and Lancet D. The GeneCards Suite: From Gene Data Mining to Disease Genome Sequence Analysis , Current Protocols in Bioinformatics(2016), 54:1.30.1

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