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Distinct interleukin-6 production in IPL and TAFRO subtypes of idiopathic multicentric Castleman disease

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Abstract

Idiopathic multicentric Castleman disease (iMCD) is a rare lymphoproliferative disorder characterized by systemic inflammation and lymphadenopathy. Two major clinical subtypes, idiopathic plasmacytic lymphadenopathy (iMCD-IPL) and iMCD with thrombocytopenia, anasarca, fever, renal dysfunction/reticulin fibrosis, and organomegaly (iMCD-TAFRO), exhibit distinct pathophysiologic mechanisms. While interleukin-6 (IL-6) is known to be elevated in iMCD, the differences in IL-6 production sources between subtypes remain unclear. We examined the source of IL-6 production and its transcriptional regulation across iMCD subtypes using immunohistochemistry (IHC), in situ hybridization (ISH), and gene expression profiling. IHC and ISH revealed that plasma cells were the predominant IL-6-expressing cells in iMCD-IPL, whereas vascular endothelial cells expressed IL-6 in iMCD-TAFRO. Plasma cells in iMCD-IPL exhibited stronger IL-6 protein expression than in iMCD-TAFRO. Gene expression analysis revealed upregulation of *XPB1*, *MZB1*, *DERL3*, *SSR4*, *FKBP11*, *FKBP2*, *PIM2*, *RABAC1*, and *SDF2L1* in iMCD-IPL, implying endoplasmic reticulum stress and plasma cell differentiation in IL-6 dysregulation. Our findings suggest that *XPB1*-mediated IL-6 production may contribute to iMCD-IPL pathogenesis, potentially explaining its favorable responses to IL-6 blockade therapy. In contrast, IL-6 production in iMCD-TAFRO may be predominantly from vascular endothelial cells, suggesting that elevated serum IL-6 is a secondary phenomenon of the cytokine storm in this subtype. Future studies should clarify how proteomics and gene expression profiling can inform subtype-specific therapeutic strategies in iMCD.

1. Introduction

Idiopathic multicentric Castleman disease (iMCD) is a rare, non-clonal lymphoproliferative disorder characterized by systemic lymphadenopathy and inflammation^{1,2}. Two clinical subtypes, iMCD with idiopathic plasmacytic lymphadenopathy (iMCD-IPL) and iMCD with thrombocytopenia, anasarca, fever, renal dysfunction/reticulin fibrosis, and organomegaly (iMCD-TAFRO), display distinct clinicopathological and treatment responses^{3,4}. iMCD-IPL patients present an indolent clinical course, and exhibit plasmacytic lymph node histology and hypergammaglobulinemia. In contrast, iMCD-TAFRO is characterized by aggressive clinical symptoms with TAFRO features. Histologically, iMCD-TAFRO showed marked vascularization in atrophic germinal centers and the interfollicular area. Patients with iMCD-TAFRO often require intensive care unit admissions or renal replacement therapy. In addition, the high levels of inflammatory cytokines and gene expression patterns observed in iMCD-TAFRO suggest a pathologic state of cytokine storm^{5,6}.

While IL-6 inhibitors have been the first-line treatment for iMCD over the past decade⁷, patients with different iMCD subtypes exhibit variable treatment responses^{3,8,9}. As previously reported, patients with iMCD-IPL usually have favorable responses to interleukin 6 (IL-6) blockade therapy with siltuximab or tocilizumab^{3,10}, with sustained remissions over time^{11,12}. However, patients with iMCD-TAFRO are more likely to show marginal responses to IL-6-directed therapy¹³ and often require combined high-dose glucocorticoids, rituximab, or either lymphoma- or myeloma-based systemic chemotherapy^{3,14,15}. Furthermore, there are reports that TNF inhibitors and BTK inhibitors are effective^{16,17}. The divergent treatment outcomes suggest underlying subtype-specific pathogenic differences, potentially related to the role of IL-6 and its producing cells in each subtype.

Despite the differences in clinical responses, patients with iMCD-IPL and iMCD-TAFRO often exhibit similarly elevated serum IL-6 levels^{3,18}. A recent study in Japanese cohorts has revealed that one-quarter of patients with TAFRO symptoms do not have lymphadenopathy¹⁹. The pathological differences between TAFRO without lymphadenopathy and TAFRO with lymphadenopathy (including iMCD-TAFRO) remain unclear, but they may be due to differences in the expression of inflammation-related genes in the lymph nodes. Furthermore, a recent gene expression study revealed significant enrichment of the PI3K-Akt signaling pathway in iMCD-TAFRO, suggesting that non-IL-6 cytokines may be the primary disease driver of its pathogenesis²⁰. Previous studies have identified vascular endothelial cells or B cells as the source of IL-6 in iMCD lymph nodes, but did not compare subtypes²¹⁻²⁴. Thus, the present study aimed to elucidate the cellular sources of IL-6 and associated transcriptional regulators in iMCD-IPL versus iMCD-TAFRO using immunohistochemistry (IHC), in situ hybridization (ISH), and gene expression analysis.

2. Methods

2.1 Patients and iMCD Subtype Definition

Sixty-four patients with iMCD-IPL (n = 50) or iMCD-TAFRO (n = 14) were included in the study. All cases were retrieved from pathology consultation files at Okayama University, Japan, and met the diagnostic criteria for iMCD¹. IgG4-related disease was ruled out in all cases based on established exclusion criteria^{25,26}. Currently, three sets of diagnostic criteria are used for iMCD-IPL^{4,27-29}, and these criteria differ slightly. In this study, iMCD-IPL was defined according to the criteria defined by Kojima et al., including (1) prominent polyclonal hypergammaglobulinemia (gamma globulin >4.0 g/dL or serum IgG level >3500 mg/dL), (2) generalized lymphadenopathy, (3) absence of definite autoimmune disease, and (4) sheet-like infiltration of mature plasma cells²⁸. There are several diagnostic criteria for iMCD-TAFRO^{30,31}, and this study

used the international definition³¹. iMCD-TAFRO was defined as those with iMCD-consistent lymph node pathology meeting at least four clinical criteria (thrombocytopenia, anasarca, fever/hyperinflammatory status, and organomegaly), in addition to supplementary criteria, including renal dysfunction, or pathological features in the bone marrow such as reticulin fibrosis or megakaryocyte hyperplasia³¹. Furthermore, histological subtypes of iMCD were classified based on international criteria¹³. All patients were serologically or immunohistochemically negative for KSHV/HHV8. This study was approved by the Institutional Review Board of Okayama University (protocol number 2007-033) and was performed in accordance with the tenets of the Declaration of Helsinki.

2.2 Histology, Immunohistochemistry, sample quantification, and In situ hybridization

All lymph node specimens were fixed in 10% formaldehyde and embedded in paraffin. Formalin-fixed paraffin-embedded (FFPE) tissue blocks were sliced into 3- μ m thickness stained with hematoxylin and eosin. IL-6 immunohistochemical staining was performed in all cases, and H scores were calculated. Additionally, IL-6 and IL-6 receptor (IL-6R) mRNA expression was visualized in two iMCD-IPL and two iMCD-TAFRO cases (Online Supplementary Appendix).

2.3 Whole Transcriptome Analysis

Whole transcriptome analysis was performed on 20 Japanese patients with iMCD-IPL (n=12), iMCD-not otherwise specified (NOS) (n=6), and iMCD-TAFRO (n=2) with frozen lymph node lesions. Cases that did not meet iMCD-IPL and iMCD-TAFRO criteria were defined as iMCD-NOS^{6,20}. However, all iMCD-NOS included in the study were iMCD-TAFRO-like cases that did not completely meet the criteria for iMCD-TAFRO, although they had pleural and/or ascites effusions and showed hypervascular histology. The details of the iMCD-NOS patients included in this study are

summarized in Supplemental Table 2. Total RNA extraction and sequencing are described in the Online Supplementary Appendix. Following this, deconvolution analysis was performed using the abs mode of CIBERSORTx³² (<https://cibersortx.stanford.edu>) with transcripts per million (TPM) values from whole transcriptome analysis.

2.4 Gene expression profiling

Gene expression profiling was performed using the nCounter platform (NanoString Technologies, Seattle, WA, USA) in 24 iMCD-IPL and seven iMCD-TAFRO cases with sufficient FFPE tissue that passed the RNA quality check. RNA extraction from FFPE tissue and analysis are described in the Online Supplementary Appendix.

2.5 Statistical Analysis

For categorical data, we used the chi-square test. The Wilcoxon rank sum test was used to compare two continuous variables. All p-values were calculated using two-sided tests, and a threshold of 0.05 was used to measure statistical significance. Statistical analyses were performed using R version 4.2.1 (<https://cran.r-project.org>).

3. Results

3.1 Clinical findings

Table 1 summarizes the clinical characteristics. There was no significant age difference between iMCD-IPL and iMCD-TAFRO ($p = 0.644$). Consistent with previous reports and subtype definitions, iMCD-IPL patients had higher platelet counts and immunoglobulin (IgG, IgA, IgM) levels than iMCD-TAFRO patients ($p < 0.001$). Serum IL-6 levels were comparable between subtypes ($p = 0.234$).

3.2 Histological, Immunohistochemical, and ISH Findings

Histological findings of iMCD-IPL and iMCD-TAFRO are shown in Figures 1 and 2. iMCD-IPL lymph nodes showed normal to hyperplastic germinal centers with an expanded interfollicular area featuring sheet-like plasma cell proliferation (**Figure 1A**); hemosiderin deposition was frequently observed (**Figure 1B**). In contrast, iMCD-TAFRO lymph nodes had atrophic germinal centers with prominent vascular proliferation (**Figure 2A**). “Whirlpool” vessels often appeared within the atrophic germinal centers (**Figure 2B**). iMCD-TAFRO cases also showed variable degrees of plasmacytosis; some cases had extensive plasma cell proliferation alongside marked vascularity. In iMCD-TAFRO, plasma cells are not arranged in sheet-like formations but are interspersed among proliferating blood vessels and lymphocytes (**Figure 2C, D**).

IL-6 IHC showed significantly stronger IL-6 protein staining in iMCD-IPL than in iMCD-TAFRO (**Figure 3**). In iMCD-IPL, the interfollicular zones contained sheet-like mature plasma cell proliferation (**Figure 3A**) with granular cytoplasmic IL-6 staining (**Figure 3C**). Vascular endothelial cells in iMCD-IPL were also IL-6-positive, though less intensely than the plasma cells (**Figure 3E**). In iMCD-TAFRO, some cases demonstrated interfollicular plasmacytosis (**Figure 3B**) with IL-6-positive plasma cells (**Figure 3D**), and IL-6 staining was likewise seen in vascular endothelial cells (**Figure 3F**). Quantitatively, the IL-6 H-score was significantly higher in iMCD-IPL than in iMCD-TAFRO ($p < 0.001$; **Figure 3G**).

ISH corroborated the IHC results: IL-6 mRNA signals appeared primarily in plasma cells in iMCD-IPL, whereas in iMCD-TAFRO, they were detected in vascular endothelial cells (**Figure 4A, B**). Additionally, in iMCD-IPL, we observed slight signals in vascular endothelial cells compared to those in plasma cells (**Supplementary Figure 1**). Similarly, IL-6

receptor mRNA was localized to plasma cells in iMCD-IPL and endothelial cells in iMCD-TAFRO (**Figure 4C, D**).

3.3 Whole Transcriptome Analysis

The results of the whole transcriptome analysis show the correlation of transcripts per million (TPM) between iMCD cases (**Figure 5**). Notably, several iMCD-IPL samples (IPL1, 2, 4, 5, 6, 8) clustered tightly together along with one iMCD-NOS and one iMCD-TAFRO case (NOS1 and TAFRO2), whereas the remaining iMCD-IPL cases (IPL3, 7, 9, 10, 11, 12) fell into separate clusters. All iMCD-IPL cases classified in the separate clusters showed a Distribution Value of fragments ≥ 200 nucleotides (DV200) of less than 95%. Non-hierarchical cluster analysis was performed to identify the upregulated genes in these case groups. Focusing on the dominant iMCD-IPL cluster, we identified 105 genes significantly upregulated in iMCD-IPL (**Supplemental Figure 2**). By contrast, clustering of the 105 genes showed a mixed gene expression pattern in iMCD-TAFRO and iMCD-NOS cases. The 105 genes included plasma cell-related genes such as *XBPI* and *MZB1*. Considering the possibility that the gene signature characteristic of iMCD-IPL depends on the amount of plasma cells in the constituent cells, we performed deconvolution analysis (**Supplemental Figure 3**). As a result, no significant difference in the amount of plasma cells was observed between iMCD-IPL and iMCD-TAFRO ($p = 0.536$).

3.4 Gene expression profiling

Targeted gene expression profiling (nCounter) was performed on all available cases for the 105 genes to validate the RNA-seq findings. The top 10 most highly expressed genes across all samples were *IGLL5*, *XBPI*, *MZB1*, *DERL3*, *SSR4*, *FKBP11*, *PIM2*, *FKBP2*, *RABAC1*, and *SDF2L1* (**Supplemental Figure 4**). Notably, nine of these genes (*XBPI*, *MZB1*, *DERL3*, *SSR4*, *FKBP11*, *FKBP2*, *PIM2*, *RABAC1*, *SDF2L1*) were expressed at significantly higher levels in iMCD-IPL than

in iMCD-TAFRO (**Figure 6**; $p < 0.05$ for each with varying levels of significance as shown). The rank order of gene expression by subtype is shown in **Supplemental Figure 5**.

4. Discussion

The present study provides the first direct evidence of differential IL-6 production pathways between iMCD-IPL and iMCD-TAFRO, as determined through IHC, ISH, and gene expression analysis. Our study provides the first direct evidence of distinct IL-6 production mechanisms in iMCD-IPL versus iMCD-TAFRO. Previous reports noted IL-6 signaling in iMCD endothelial cells but did not differentiate between subtypes^{21,23}. We found that IL-6 production by vascular endothelial cells is far more prominent in the iMCD-TAFRO subtype. In iMCD-TAFRO, we observed activation of the PI3K-Akt pathway and upregulation of cytokine storm-related genes such as TNF α , IL-1R, MTOR, and VEGFA^{6,19}; however, no significant difference in IL-6 gene expression was observed. Therefore, the elevation of serum IL-6 in iMCD-TAFRO is suggested to be a consequence of the subsequent cytokine storm rather than a primary disease driver^{5,34,35}.

In contrast, in iMCD-IPL, we detected IL-6 protein, as well as IL-6 and IL-6R mRNAs, within the proliferating plasma cells, supporting the hypothesis of autocrine IL-6 production. Notably, the gene expression data showing high *XBPI* in iMCD-IPL further reinforces an autocrine and possibly a paracrine mechanism, as *XBPI* is known to drive IL-6 secretion (**Figure 7**). *XBPI* is a transcription factor implicated in multiple myeloma, where it enhances IL-6 secretion, plasma cell survival, and angiogenesis³⁶⁻³⁸. Furthermore, *XBPI* is an ER stress-related gene, and its expression is enhanced during the differentiation of B cells into plasma cells in lymph nodes^{36,39}. Given that vascular proliferation is not considerably pronounced in iMCD-IPL²⁰, *XBPI* activation in iMCD-IPL may likely be driven by factors other than angiogenesis, consistent with an

alternative mechanism of IL-6 upregulation in this subtype.

The constitutive, *XBPI*-driven IL-6 cycle may explain clinical features in iMCD-IPL patients, including hypergammaglobulinemia and thrombocytosis. While the role of *XBPI* in promoting plasma cell survival may also contribute to the chronic nature of iMCD-IPL, further investigation is needed to identify the trigger for *XBPI* activation that leads to excessive plasma cell differentiation and IL-6 production. Furthermore, gene expression analysis in this study revealed that iMCD-IPL was associated with increased expression of ER stress-related genes, including *XBPI*. ER stress is closely related to plasma cells in lymph nodes, and it is crucial to determine whether the enhancement of this gene signature is dependent on the component cells of iMCD-IPL and iMCD-TAFRO. Deconvolution analysis revealed no significant difference in the estimated number of plasma cells between iMCD-IPL and iMCD-TAFRO, suggesting that the difference in IL-6 activity between the two groups may be attributed to their distinct characteristics rather than the quantity of plasma cells. Histologically, plasma cells in iMCD-TAFRO were observed to be mixed with vascular and lymphocytes, in contrast to the sheet-like proliferation observed in iMCD-IPL. The distribution areas and appearance patterns of plasma cells in iMCD-IPL and iMCD-TAFRO are considered to be different.

We noted that IL-6 protein staining in iMCD-IPL plasma cells was markedly stronger than in vascular endothelial cells. Additionally, IL-6 staining was more intense in plasma cells in iMCD-IPL than in iMCD-TAFRO. The discrepancy likely does not reflect the sheer overproduction of IL-6, but rather the intracellular, autocrinally produced IL-6 retained within iMCD-IPL plasma cells, rather than being secreted. Such a chronic autocrine IL-6 signaling mechanism could sustain ongoing plasma cell proliferation and related symptoms in iMCD-IPL, which needs to be verified in future studies.

Conversely, the predominance of endothelial-derived IL-6 in iMCD-TAFRO may reflect a different pathophysiology and explain why IL-6 inhibitors have limited efficacy in some patients with iMCD-TAFRO. It is worth noting that IL-6 IHC H-scores were significantly higher in iMCD-IPL; one may need to consider that this could be influenced by the greater number of plasma cells in iMCD-IPL tissues, which may not truly reflect the actual amount of IL-6. However, as most iMCD-TAFRO cases did have various degrees of plasmacytosis with weak IL-6-positive cells, and we quantified H-scores in hotspots for each case, differences in staining intensity may likely account for the higher H-scores in iMCD-IPL.

We found similarly high *IGLL5* expression in both iMCD-IPL and iMCD-TAFRO, but the significance of this observation may differ by subtype. In iMCD-IPL, *IGLL5* upregulation is likely attributable to the abundance of plasma cells, as *IGLL5* is located in the immunoglobulin lambda locus and is involved in B-cell development⁴⁰. In contrast, the unexpectedly high *IGLL5* in iMCD-TAFRO might represent an intriguing paradox, suggesting that different cell types or signals drive *IGLL5* in iMCD-TAFRO. Notably, *IGLL5* is also upregulated in multiple myeloma and in tumor-infiltrating immune cells, suggesting a broad role in immune regulation and inflammation^{41,42}. One potential hypothesis is that in iMCD-TAFRO, *IGLL5* expression might originate from non-plasma cell populations such as activated B cells, dendritic cells, or myeloid-derived suppressor cells, in the setting of widespread endothelial activation and a severe cytokine storm. Future studies should employ single-cell RNA sequencing and spatial transcriptomics to pinpoint the cellular source of *IGLL5* in iMCD and clarify its role in immune dysregulation. The approaches may also determine whether *IGLL5* could serve as a useful biomarker or therapeutic target in specific iMCD subtypes. Several other genes were highly expressed in iMCD-IPL relative to TAFRO, including *DERL3*, *SSR4*, *FKBP2*, and *FKBP11*. *DERL3* encodes a Derlin family protein in the ER membrane activated by ER stress⁴³. *SSR4* encodes a component of the translocation-associated protein (TRAP) complex,

which helps translocate proteins across the ER membrane⁴⁴. *FKBP2* and *FKBP11* encode FK506-binding proteins that are upregulated during ER stress; notably, *FKBP11* is induced during *XPB1*-driven differentiation of B cells into plasma cells⁴⁵. The expression patterns support the hypothesis that ER stress-driven IL-6 production by plasma cells is a key feature of iMCD-IPL.

From a clinical standpoint, the present findings underscore the need for subtype-tailored therapy in iMCD. IL-6 blockade remains the first-line treatment, and the results validate its particular importance in iMCD-IPL, where early anti-IL-6 therapy could interrupt the *XPB1*-driven autocrine IL-6 loop and plasma cell proliferation. However, for patients with relapsed/refractory diseases against IL-6 blockade, there is currently no standard second-line therapy^{8,46}. Rituximab, an anti-CD20 B-cell therapy, has a category 2 indication for iMCD in the United States; however, its level of evidence is limited by the retrospective nature of the data and small sample sizes⁴⁶. Given the potential key role of *XPB1* in iMCD-IPL, further studies should explore whether advanced molecular profiling, such as proteomics or gene expression profiling, can guide second-line or later treatment decisions or predict responses to novel targeted therapies.

This study has a few limitations. First, the results of ISH staining were qualitative rather than quantitative due to the limited availability of the lymph node specimen. Second, due to the nature of bulk gene expression analysis, we were unable to identify the responsible cells expressing each gene. Single-cell gene expression analyses may be beneficial in identifying cells with upregulated genes in iMCD-IPL, including *XPB1* and *MZB1*. In the future, more experiments are required to investigate whether *XPB1* inhibition suppresses IL-6 production. Third, the mechanism of action of IL-6 in vascular endothelial cells was not clear in this study. IL-6 has both trans-signaling and classical signaling pathways, and their effects

are different. Since it has been reported that trans-signaling in vascular endothelial cells can amplify inflammatory responses, IL-6 trans-signaling may also be involved in iMCD-TAFRO. To clarify this point, future single-cell analysis is needed.

In conclusion, our study elucidates distinct IL-6-producing cells and hypothesized regulatory mechanisms in iMCD subtypes, with *XBP1* potentially playing a pivotal role in IL-6 overproduction in iMCD-IPL. Future studies are warranted to clarify the roles of ER stress and IL-6 and IL-6R in each iMCD subtype. These insights may advance our understanding of iMCD pathogenesis and highlight potential molecular targets for subtype-specific precision therapies.

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Table 1. Comparison of clinical findings between iMCD-IPL and iMCD-TAFRO

	iMCD-IPL (n=50)	iMCD-TAFRO (n=14)	p-value
Age (median [IQR])	54.0 (62.0–44.5)	49.0 (65.5–41.8)	0.644
Sex (M/F)	27/23	11/3	
Laboratory findings (median [IQR])			
WBC, $\times 10^3/\mu\text{L}$ [n=43]	8.0 (9.6–5.7)	9.0 (12.4–5.7)	0.456
Plt, $\times 10^3/\mu\text{L}$ [n=56]	387 (439–286)	35 (54–22)	<0.001
Hb, g/dL [n=57]	10.3 (11.5–9.1)	9.0 (11.3–7.5)	0.269
CRP, mg/dL [n=55]	6.5 (8.8–3.4)	6.0 (16.1–1.9)	0.992
Serum IL-6, pg/mL [n=16]	37.5 (52.5–23.6)	15.6 (26.6–10.7)	0.234
IgG, mg/dL [n=52]	4727.0 (6014.5–4274.3)	1317.7 (1560.3–1193.1)	<0.001
IgM, mg/dL [n=41]	215.0 (271.2–154.0)	73.7 (96.3–63.0)	<0.001
IgA, mg/dL [n=41]	553.6 (686.0–450.0)	188.6 (227.3–169.0)	<0.001

Significance was calculated using the Mann–Whitney U test. Significant p-values are shown in bold. IQR, interquartile range; SD, standard deviation; WBC, white blood cell; Plt, platelet; Hb, hemoglobin; CRP, C-reactive protein; IL-6, interleukin 6; Ig, immunoglobulin. Normal ranges: WBC, $3.3\text{--}8.6 \times 10^3/\mu\text{L}$; Plt, $158\text{--}348 \times 10^3/\mu\text{L}$; Hb, $13.7\text{--}16.8$ g/dL (male), $11.6\text{--}14.8$ g/dL (female); CRP, $0.00\text{--}0.30$ mg/dL; IL-6, $0.0\text{--}4.0$ pg/mL; IgG, $870\text{--}1700$ mg/dL; IgM, $31\text{--}200$ mg/dL (male), $52\text{--}270$ mg/dL (female); IgA, $110\text{--}410$ mg/dL.

Figure legends

Figure 1. Histopathological features of iMCD-IPL

A: Interfollicular areas are expanded, and germinal centers show normal to hyperplastic. Scale bar = 200 μ m. B: The sheet-like proliferation of mature plasma cells in the interfollicular areas and hemosiderin deposition are observed. Scale bar = 50 μ m.

Figure 2: Histopathological features of iMCD-TAFRO

A, B: iMCD-TAFRO without plasmacytosis. Atrophic germinal centers and severe vascularization are observed. Scale bar = 200 μ m (A). The proliferation of whirlpool vessels within the germinal centers was observed. Scale bar = 100 μ m. (B). C, D: iMCD-TAFRO with plasmacytosis. An Atrophic germinal center with a whirlpool vessel was observed. Scale bar = 100 μ m (C). In the interfollicular area, prominent vascular proliferation and plasma cells admixed with lymphocytes were observed. Scale bar = 50 μ m (D).

Figure 3. IL-6 immunohistochemical staining in iMCD-IPL and iMCD-TAFRO

A: iMCD-IPL. Sheet-like proliferation of mature plasma cells was observed in the interfollicular area. Scale bar = 50 μ m. B: iMCD-TAFRO. Marked vascularization with plasmacytosis was observed in the interfollicular area. Scale bar = 100 μ m. C: IL-6 immunostaining of iMCD-IPL. The cytoplasm of the plasma cells in the interfollicular area showed a granular staining pattern. Scale bar = 50 μ m. D: iMCD-TAFRO. IL-6 protein expression was detected in plasma cells, weaker than in iMCD-IPL. Scale bar = 50 μ m. E: Vascular endothelial cells in iMCD-IPL showed weaker IL-6 protein expression than plasma cells. Scale bar = 50 μ m. F: iMCD-TAFRO. IL-6 protein expression was detected in the cytoplasm of vascular endothelial cells. Scale bar = 50 μ m. G: Comparison of IL-6 Hscore between iMCD-IPL and iMCD-TAFRO. The IL-6

staining pattern was calculated on a four-point scale (0–3) and the H-score. The H-score was calculated for cell staining using the following formula: $H\text{-score} = (\% \text{ at } 0) \times 0 + (\% \text{ at } 1+) \times 1 + (\% \text{ at } 2+) \times 2 + (\% \text{ at } 3+) \times 3$, as described previously³³. The IL-6 H-score was significantly higher in the iMCD-IPL group ($p < 0.001$). iMCD-IPL (median [IQR]): 162.1 (115.7–196.0), iMCD-TAFRO (median [IQR]): 36.4 (17.7–58.3)

Figure 4. IL-6 and IL-6 receptor in situ hybridization in iMCD-IPL and iMCD-TAFRO

A: iMCD-IPL. IL-6 mRNA signals were observed mainly in plasma cells (yellow arrowheads). Scale bar = 50 μm . B: iMCD-TAFRO. IL-6 mRNA signals were detected in vascular endothelial cells (yellow arrowheads). No IL-6 mRNA signals were detected in the plasma cells. Scale bar = 50 μm . C: iMCD-IPL. IL-6 receptor (IL-6R) mRNA signals were detected mainly in plasma cells (yellow arrowheads). Scale bar = 50 μm . D: iMCD-TAFRO. IL-6R mRNA signals were observed in vascular endothelial cells (yellow arrowheads). No IL-6R mRNA signals were detected in the plasma cells. Scale bar = 50 μm .

Figure 5: Whole transcriptome analysis in iMCD subtypes

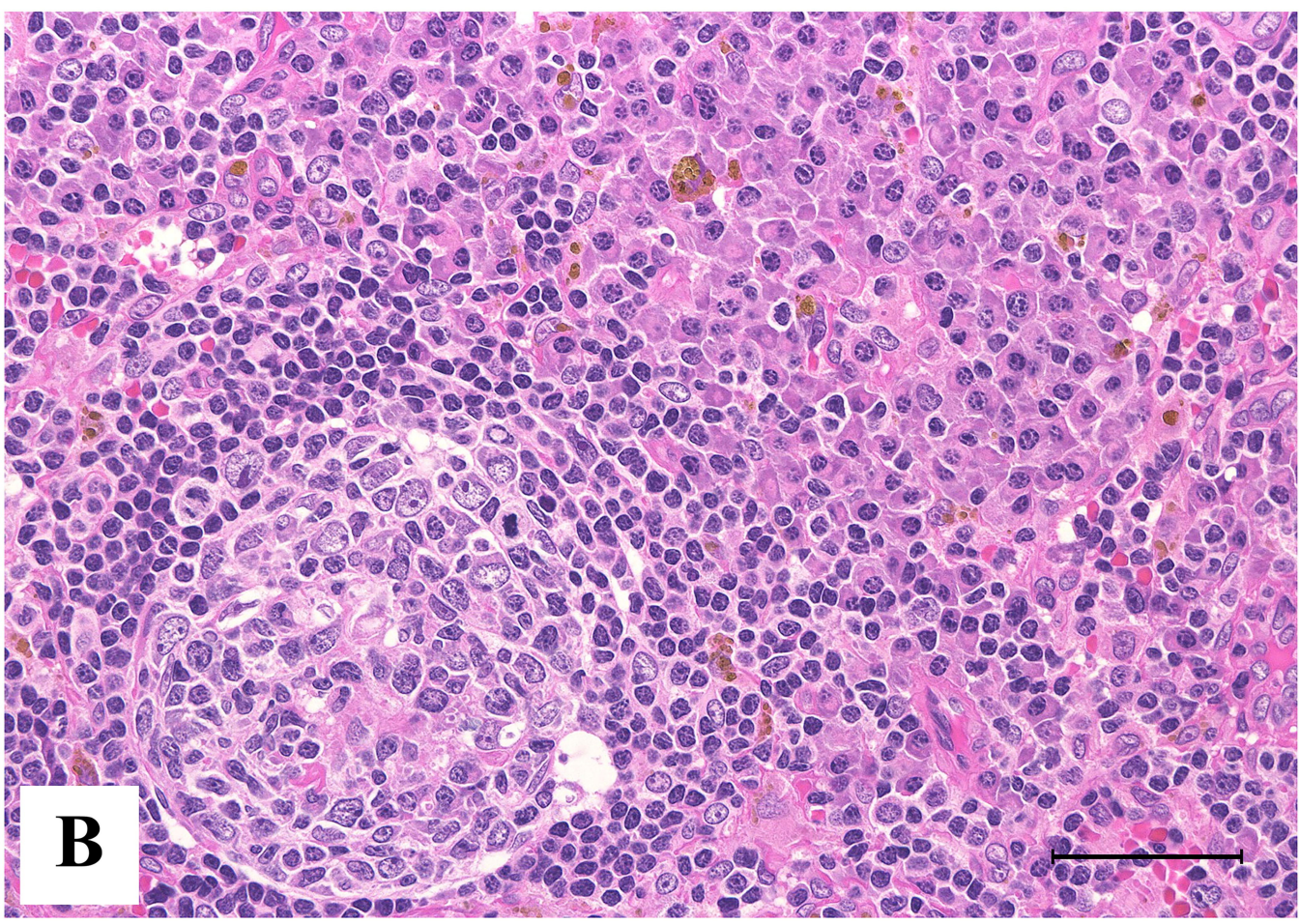
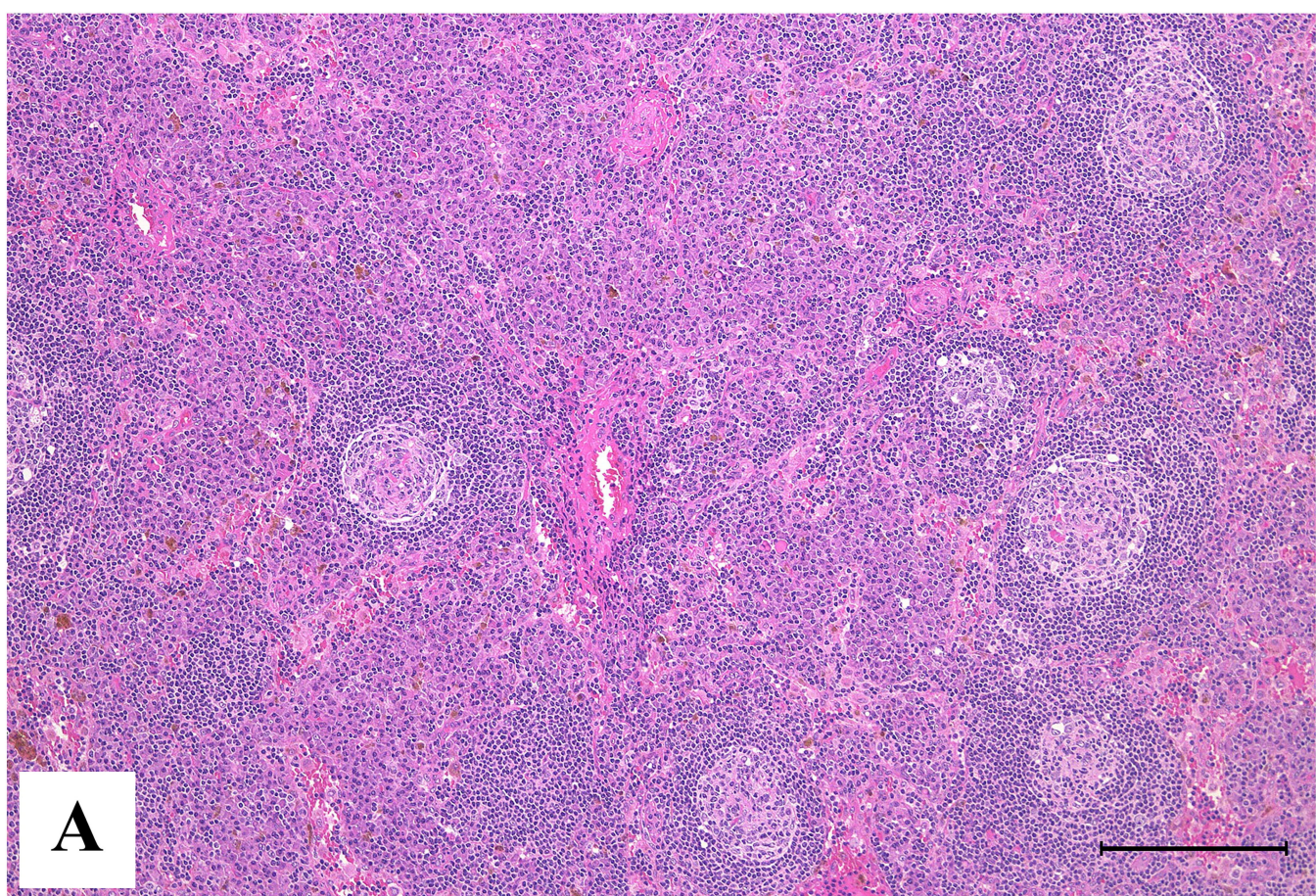
Correlation analysis between iMCD cases based on transcripts per million (TPM) using 750 highly variable genes. Strong positive correlations were observed in the cluster containing IPL1, 2, 4, 5, 6, 8, NOS1, and TAFRO2.

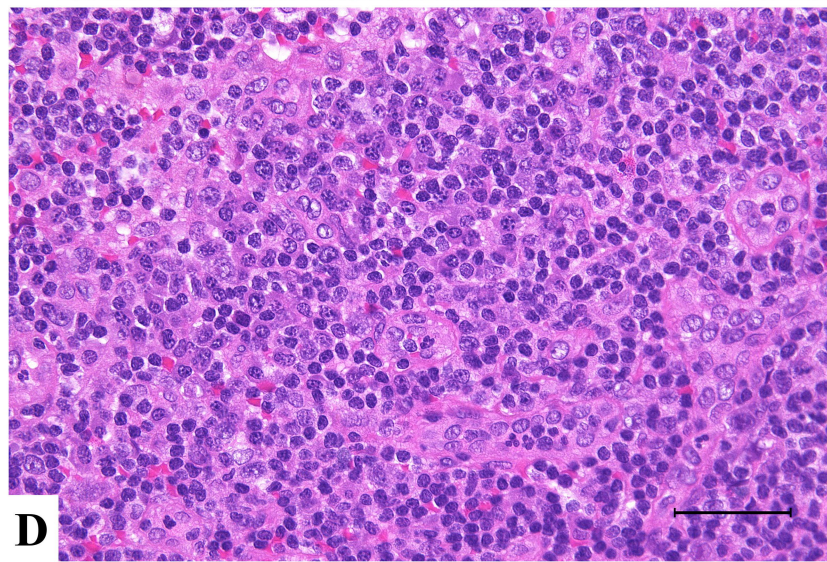
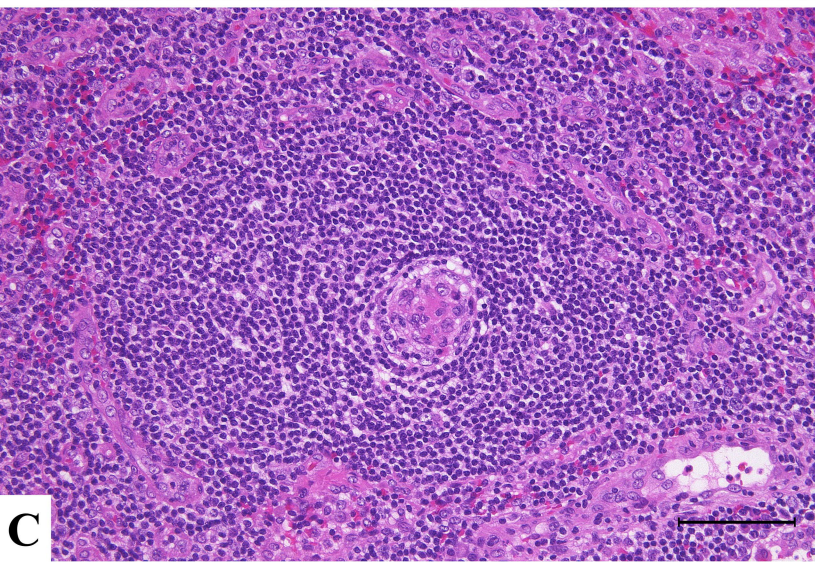
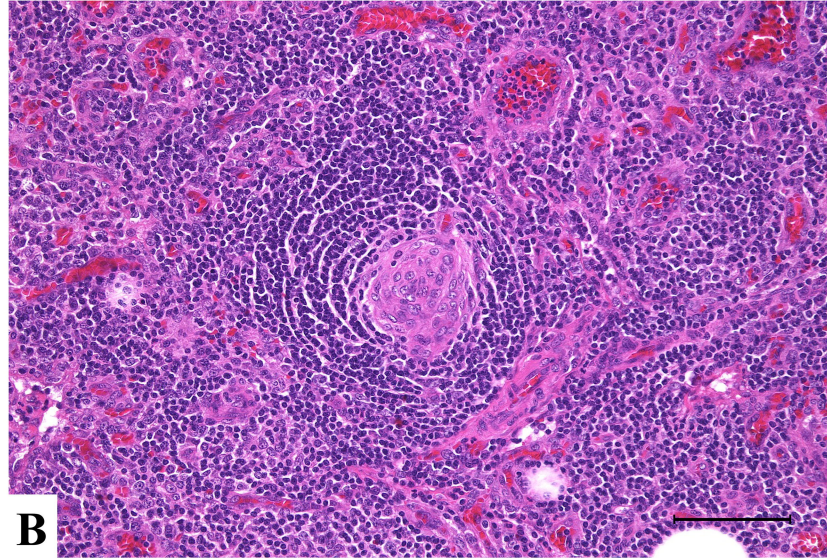
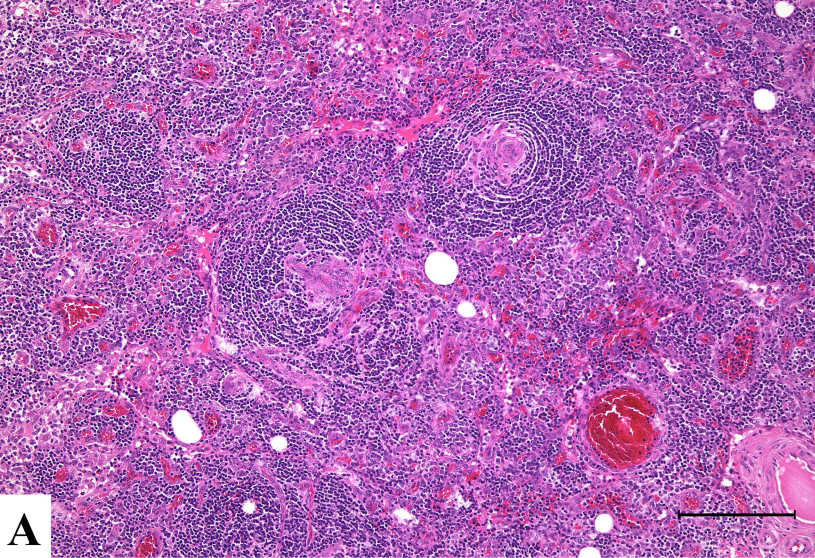
Figure 6: Gene expression analysis in iMCD-IPL and iMCD-TAFRO using a custom panel

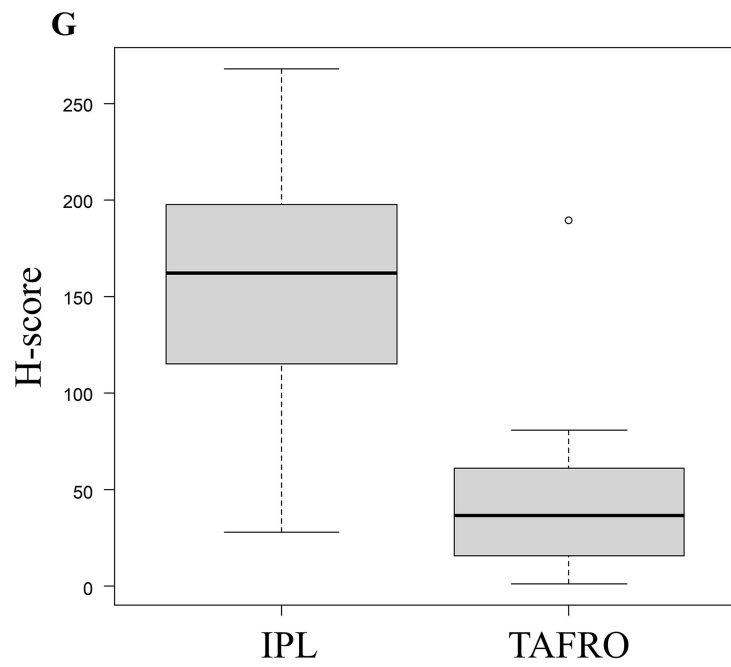
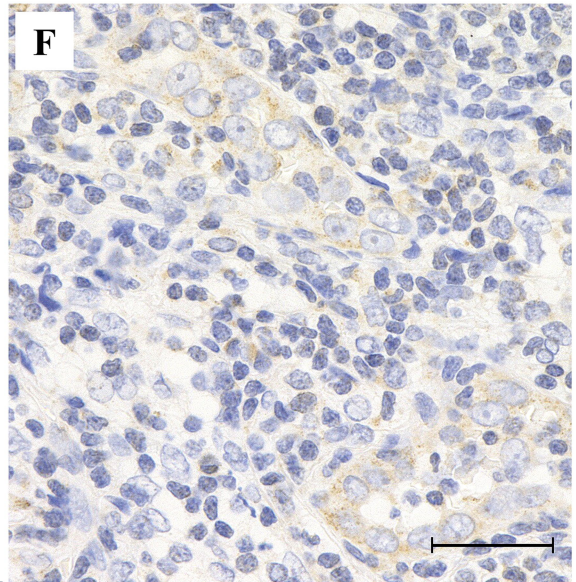
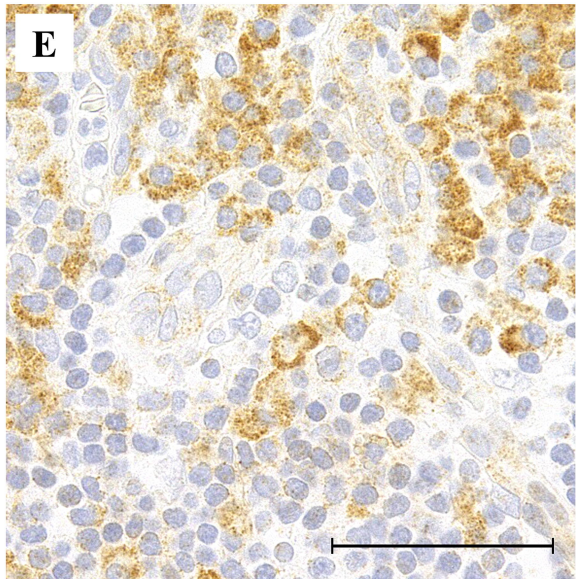
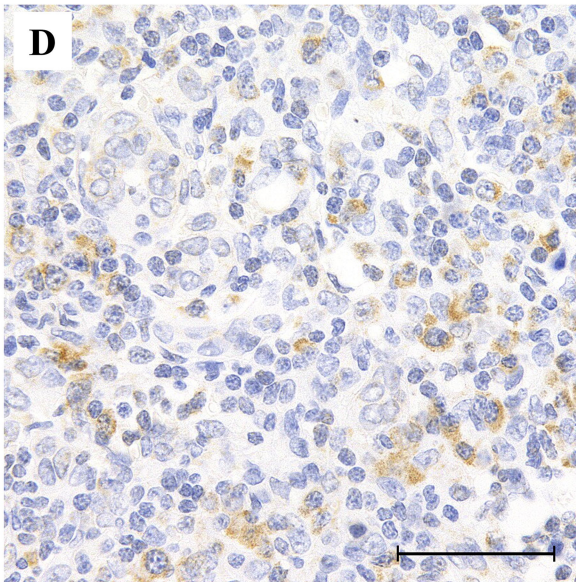
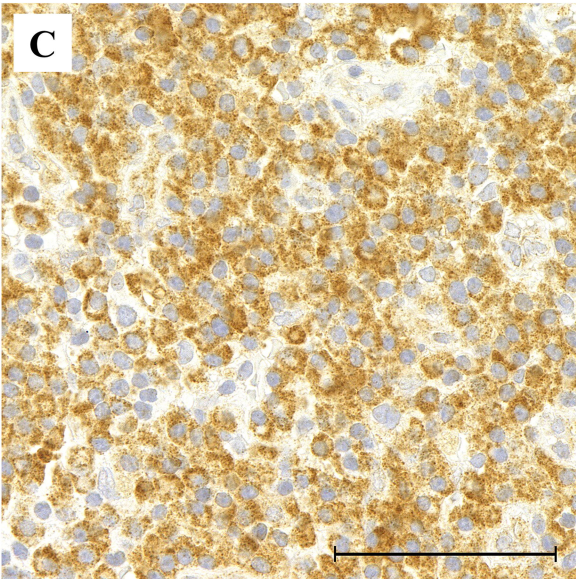
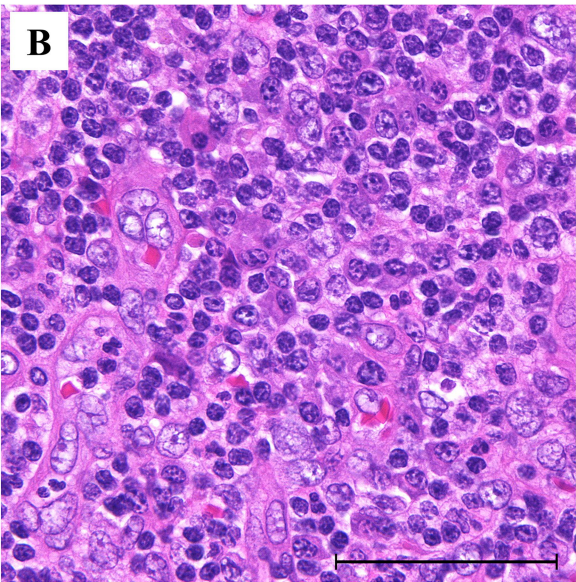
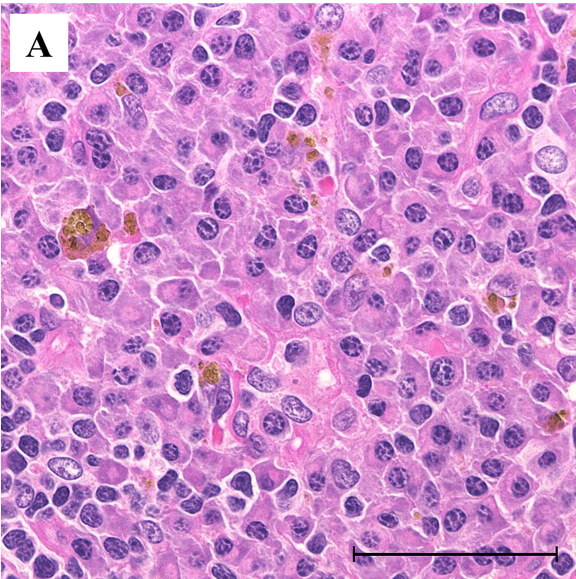
Comparison of expression levels between iMCD-IPL and iMCD-TAFRO for the top 10 expressed genes (*IGLL5*, *XPB1*, *MZB1*, *DERL3*, *SSR4*, *FKBP11*, *PIM2*, *FKBP2*, *RABAC1*, and *SDF2L1*). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Figure 7. Pathogenesis and gene expression of iMCD-IPL.

The pathological hypothesis of iMCD-IPL is based on the IHC, ISH, and gene expression analysis results. An unknown trigger enhances B cell differentiation into plasma cells and causes plasma cell proliferation. In this model, plasma cells produce immunoglobulins, resulting in the expansion of the endoplasmic reticulum (ER). Consequently, ER stress-related genes are upregulated, and *XBP1* may be constantly activated, thereby becoming involved in IL-6 autocrine production by plasma cells. Immunoglobulin production may upregulate *MZB1* and enhance ER stress.

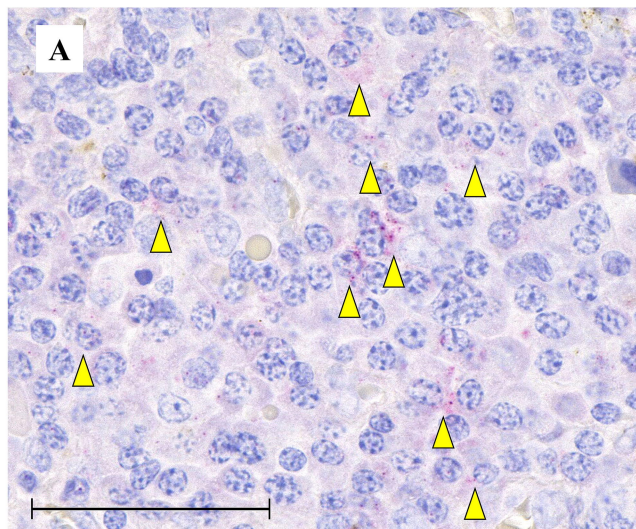




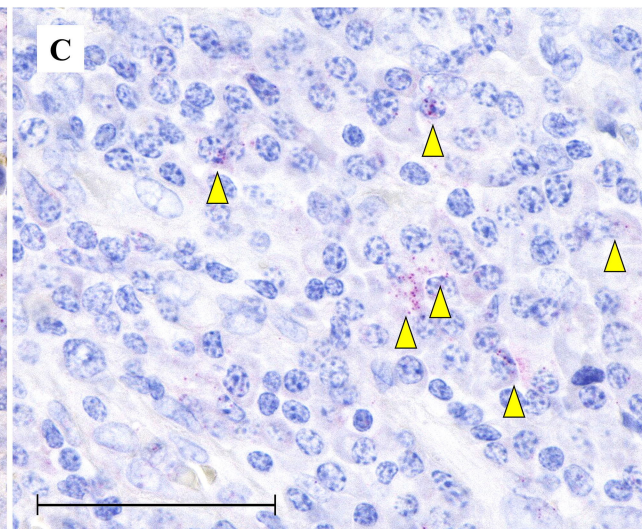


iMCD-IPL

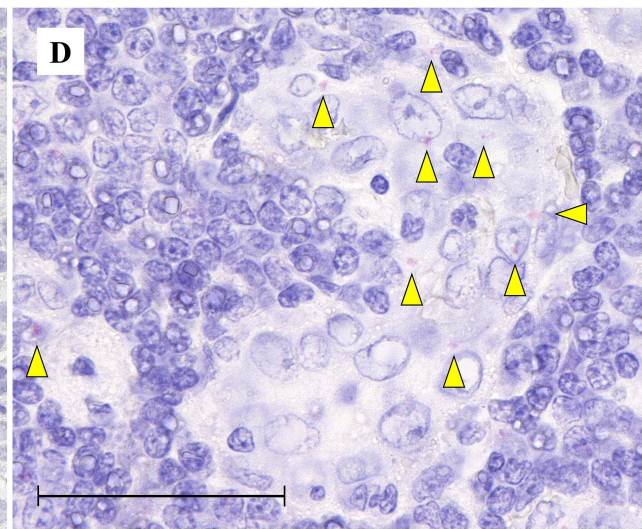
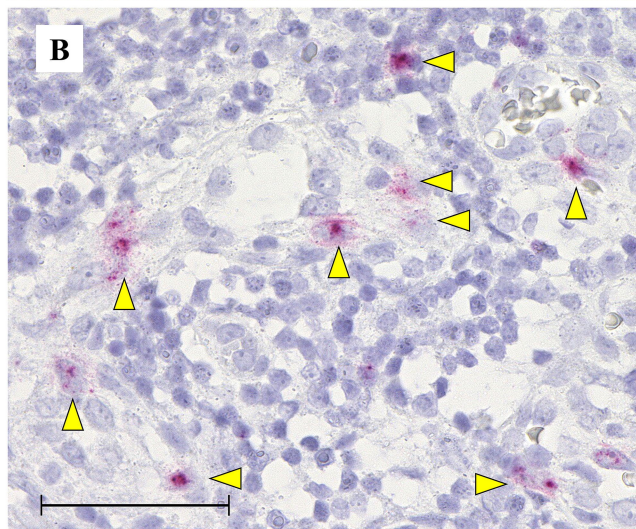
IL-6

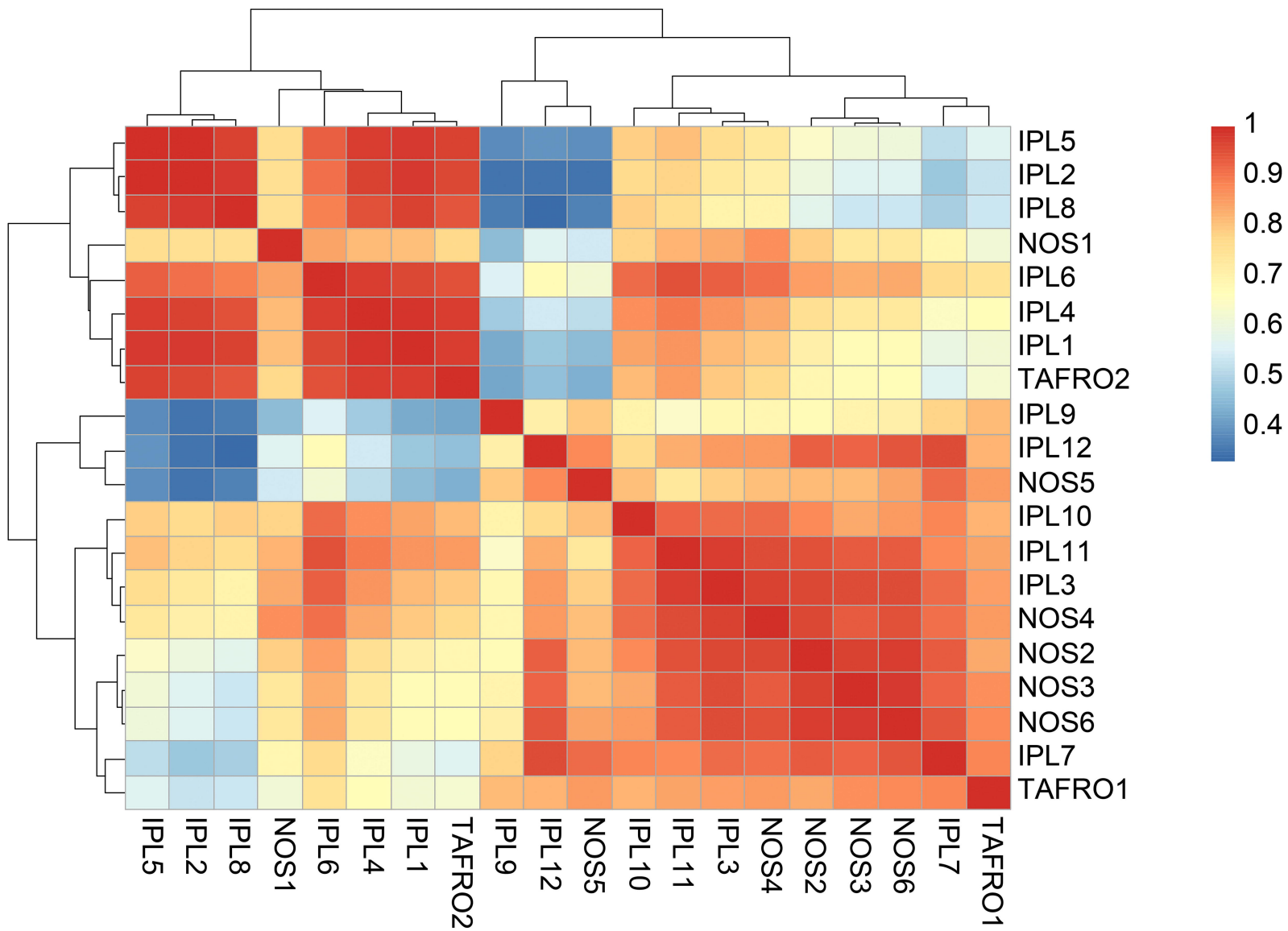


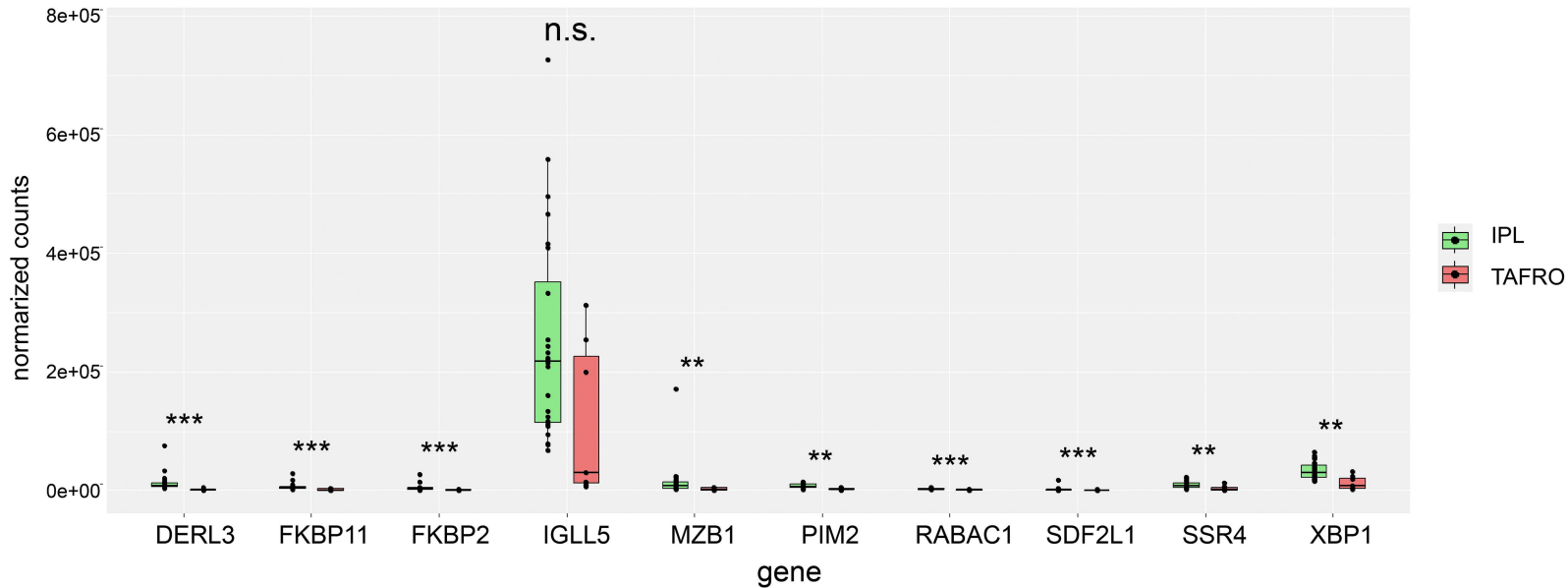
IL-6R



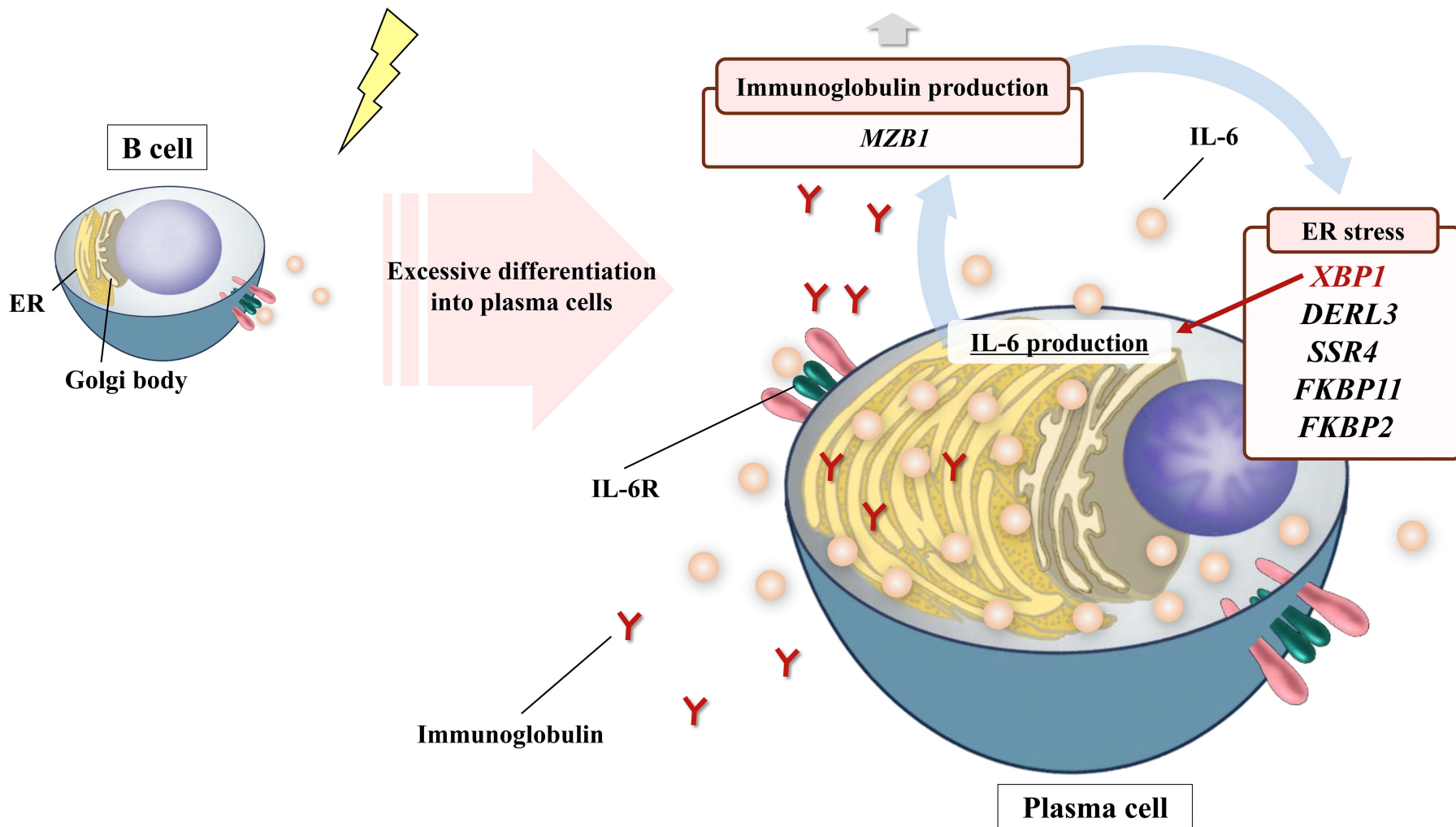
iMCD-TAFRO







Unknown trigger



Supplementary Methods

Immunohistochemistry, sample quantification, and In situ hybridization

Immunohistochemical staining was performed on an automated Bond-III instrument (Leica Biosystems, Wetzlar, Germany) using the primary antibody of IL-6 (10C12, 1:200, Leica Biosystems). Internal positive and negative control cells were confirmed. The slides were scanned using a Nanozoomer whole-slide scanner (Hamamatsu Photonics, Hamamatsu, Japan) at 40X magnification and analyzed using QuPath (version 0.4.3, University of Edinburgh, UK). Single-cell detection was performed using QuPath. Each cell was scored from 0 (negative) to 3 (strongly positive) according to diaminobenzidine intensity, and the H-score was calculated. The H-score was calculated for cell staining using the following formula: $H\text{-score} = (\% \text{ at } 0) \times 0 + (\% \text{ at } 1+) \times 1 + (\% \text{ at } 2+) \times 2 + (\% \text{ at } 3+) \times 3$, as described previously. Analysis was performed on more than 3000 cells in hotspots on the slides.

IL-6 and IL-6 receptor (IL-6R) mRNA expression was visualized in FFPE sections from two iMCD-IPL and two iMCD-TAFRO cases using the ViewRNA ISH Tissue Evaluation Kit (Thermo Fisher Scientific, Waltham, MA, US). Briefly, deparaffinized lymph node sections were boiled for 10 minutes and treated with a protease solution for 10 minutes. After formalin fixation, the ViewRNA type 1 Probe Set (Thermo Fisher Scientific) against IL-6 (VA1-13526-VT) and IL-6R (VA1-11611-VT) was used to hybridize the samples for two hours. Signals were amplified by hybridization with the PreAmplifier Mix and Amplifier Mix, allowing for the specific amplification of the target-specific signal using branched DNA technology. They were then developed using Label Probe 1-AP with FastRed substrate.

Whole Transcriptome Analysis

Total RNA was extracted from the frozen specimens using the RNeasy Tissue Mini Kit (Qiagen, Venlo, Netherlands) according to the manufacturer's instructions. RNA was quantified using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, United States). Following processing using the poly A method, the remaining RNAs were fragmented using a fragmentation buffer and reverse-

transcribed into cDNA with random primers. RNA sequencing was performed using the NovaSeq 6000 system (Illumina, San Diego, CA, United States)

Gene expression profiling

To extract RNA from FFPE tissue samples, we used a Maxwell RSC RNA FFPE kit (Promega Corporation, Madison, WI, USA) according to the manufacturer's instructions. Based on the whole transcriptome analysis results, a custom panel on the nCounter platform (NanoString Technologies, Seattle, WA, USA) was created that included 105 genes that were uniformly upregulated in iMCD (Supplemental Table 1) and seven housekeeping genes. RNA extracted from FFPE tissues was hybridized overnight at 65 °C with probes, processed on the nCounter prep station, and analyzed using a nCounter digital analyzer.

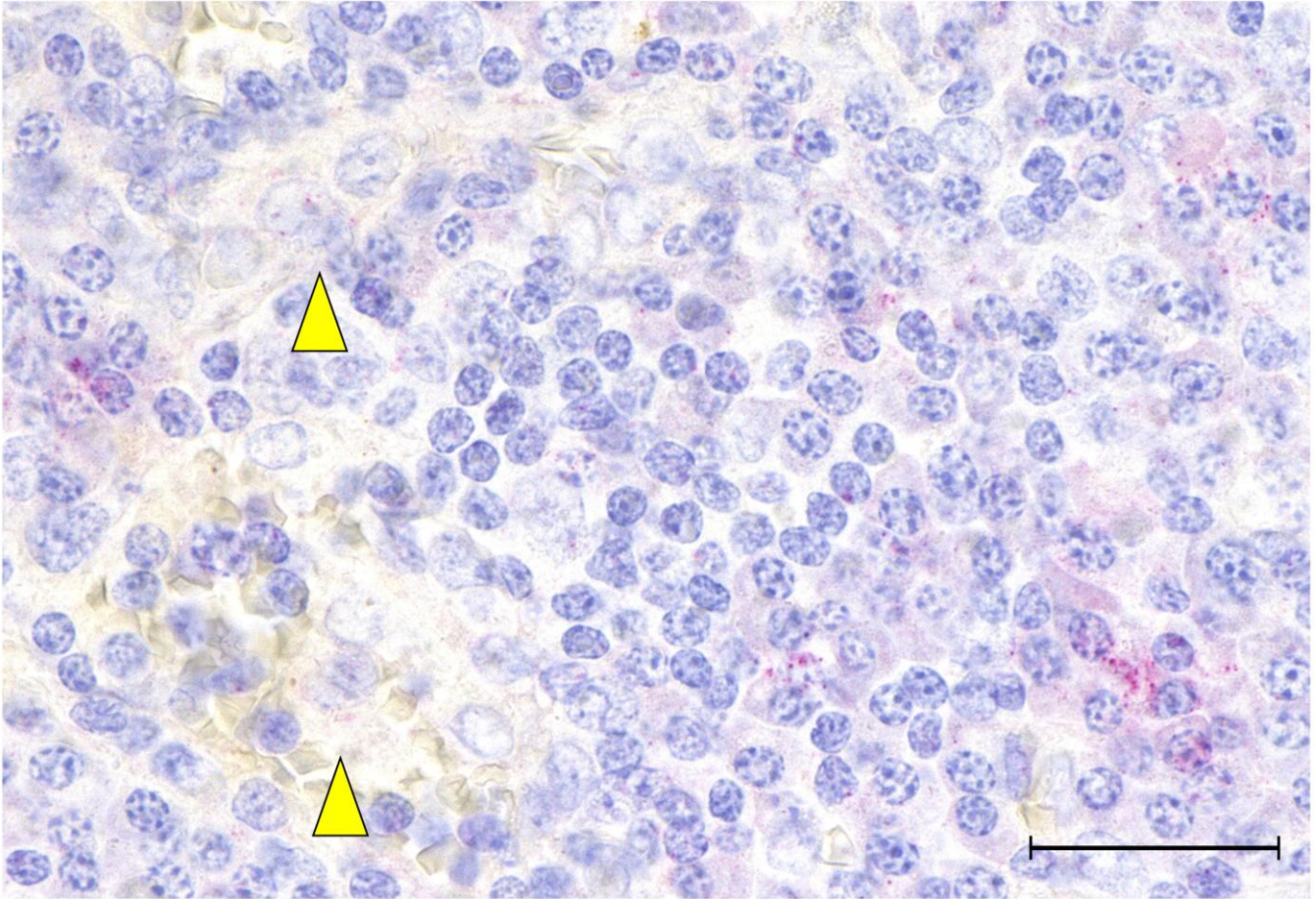
Supplemental table1. Gene sets included in custom panel

<i>ABHD14A</i>	<i>HM13</i>	<i>SDC1</i>
<i>ALDH1L2</i>	<i>HSP90B1</i>	<i>SDF2L1</i>
<i>AMPD1</i>	<i>ICAM4</i>	<i>SEC11C</i>
<i>APLP1</i>	<i>IGLL5</i>	<i>SEL1L</i>
<i>ARSA</i>	<i>INHBE</i>	<i>SERPINI1</i>
<i>ARX</i>	<i>JCHAIN</i>	<i>SIL1</i>
<i>ATAD3C</i>	<i>JSRP1</i>	<i>SLAMF7</i>
<i>ATF5</i>	<i>KCNA5</i>	<i>SLC1A5</i>
<i>B9D1</i>	<i>KRTCAP2</i>	<i>SLC35B1</i>
<i>BSCL2</i>	<i>LAX1</i>	<i>SLC38A5</i>
<i>C17orf107</i>	<i>LTC4S</i>	<i>SLC3A2</i>
<i>C3orf70</i>	<i>MANF</i>	<i>SLC6A9</i>
<i>CERCAM</i>	<i>MC4R</i>	<i>SMIM1</i>
<i>CFAP54</i>	<i>MEI1</i>	<i>SMOX</i>
<i>CHAC1</i>	<i>MSLN</i>	<i>SMPDL3B</i>
<i>CHPF</i>	<i>MZB1</i>	<i>SPAG4</i>
<i>CLDN14</i>	<i>NUCB2</i>	<i>SPCS2</i>
<i>CNKSRI</i>	<i>OVOL3</i>	<i>SRM</i>
<i>COMTD1</i>	<i>P2RX1</i>	<i>SSR4</i>
<i>CRELD2</i>	<i>PDK1</i>	<i>STARD5</i>
<i>CRYBA4</i>	<i>PIM2</i>	<i>TASIR3</i>
<i>CTH</i>	<i>PLPP5</i>	<i>TECR</i>
<i>CYBA</i>	<i>PODXL2</i>	<i>TMEM125</i>
<i>DCC</i>	<i>PPCDC</i>	<i>TMEM208</i>
<i>DERL3</i>	<i>PRDMI</i>	<i>TMEM238</i>
<i>DNAJB9</i>	<i>PRDX4</i>	<i>TP53INP1</i>
<i>DNAJC1</i>	<i>PRKCG</i>	<i>TPTE2</i>
<i>ELL2</i>	<i>PRSSI6</i>	<i>TRIM55</i>
<i>ERLEC1</i>	<i>PTP4A3</i>	<i>TSPAN1</i>
<i>FCRLB</i>	<i>QPCTL</i>	<i>TXNDC11</i>
<i>FKBP11</i>	<i>RAB26</i>	<i>TXNDC15</i>
<i>FKBP2</i>	<i>RABAC1</i>	<i>TXNDC5</i>
<i>GGT1</i>	<i>RPN2</i>	<i>WIP11</i>
<i>GNB3</i>	<i>RRBP1</i>	<i>XBP1</i>
<i>HID1</i>	<i>SCAMP5</i>	<i>ZBP1</i>

Supplemental Table2: Clinical findings of iMCD-NOS

No.	iMCD subtype	Age	Sex	(T) Thrombocytopenia ($<10 \times 10^4/\mu\text{l}$)	Plet ($\times 10^4/\mu\text{l}$)	(A) Anasarca (Pleural effusion, ascites, or subcutaneous edema)	(F) Fever or hyperinflammatory status (Fever $\geq 37.5^\circ\text{C}$ or CRP ≥ 2.0 mg/dL)	CRP (mg/dL)	(R) Renal insufficiency (eGFR ≤ 60 , Cre >1 mg/dL (F), >1.3 mg/dL (M), or renal failure necessitating hemodialysis)	eGFR (mL/min/1.73 m ²)	Cre (mg/dl)	Reticulin fibrosis	(O) Organomegaly (lymphadenopathy in two or more regions, hepatomegaly, or splenomegaly)
1	NOS	49	F	-	36.6	+	+	3.21	-	82	0.6		+
2	NOS	51	F	-	24.1	+	+	29.4	+	54.7	0.86	+	+
3	NOS	49	F	-	35.8	+	+	4.1	-	104.7	0.48	-	+
4	NOS	74	M	-	22	+	-	0.9	-	N/A	1.17	N/A	+
5	NOS	49	M	-	12.2	+	+	6	+	35.1	1.72	N/A	+
6	NOS	54	F	-	40.4	-	+	7.26	+	46.6	0.98	N/A	+

The reference considered was the laboratory data before the lymph node biopsy. The thresholds for each data point are determined based on the iMCD-TAFRO consensus criteria [31]. iMCD-TAFRO was defined as meeting at least four clinical criteria (thrombocytopenia, anasarca, fever/hyperinflammatory status, and organomegaly), renal dysfunction or reticulin fibrosis, and pathological criteria for lymph nodes. Patient 3 presented with renal failure and did not require hemodialysis.

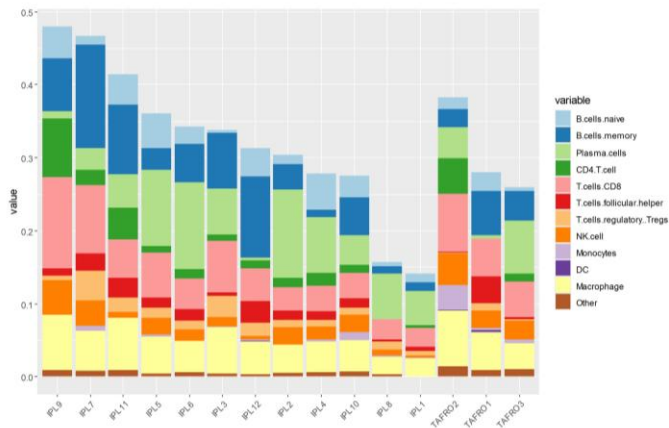


Supplemental Figure 1: In situ hybridization of IL-6 in iMCD-IPL

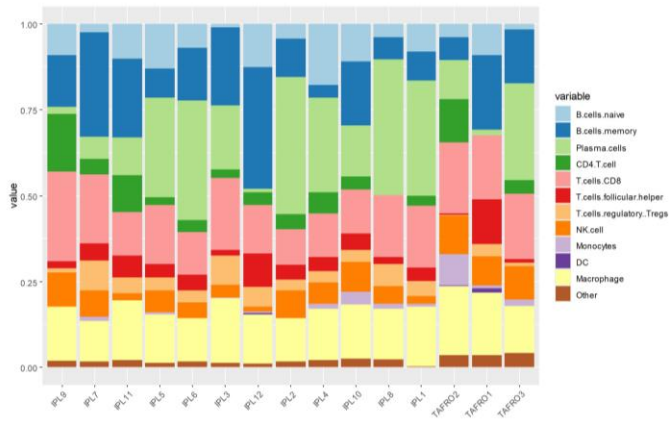
The signals of IL-6 mRNA were slightly detected in vascular endothelial cells (yellow arrowheads). These signals were weaker than those in plasma cells of iMCD-IPL (Figure 4A) and vascular endothelial cells of iMCD-TAFRO (Figure 4B).

Scale bar = 50 μ m.

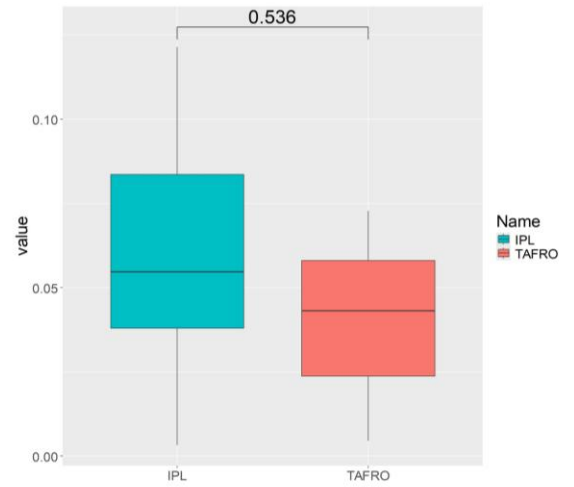
A



B



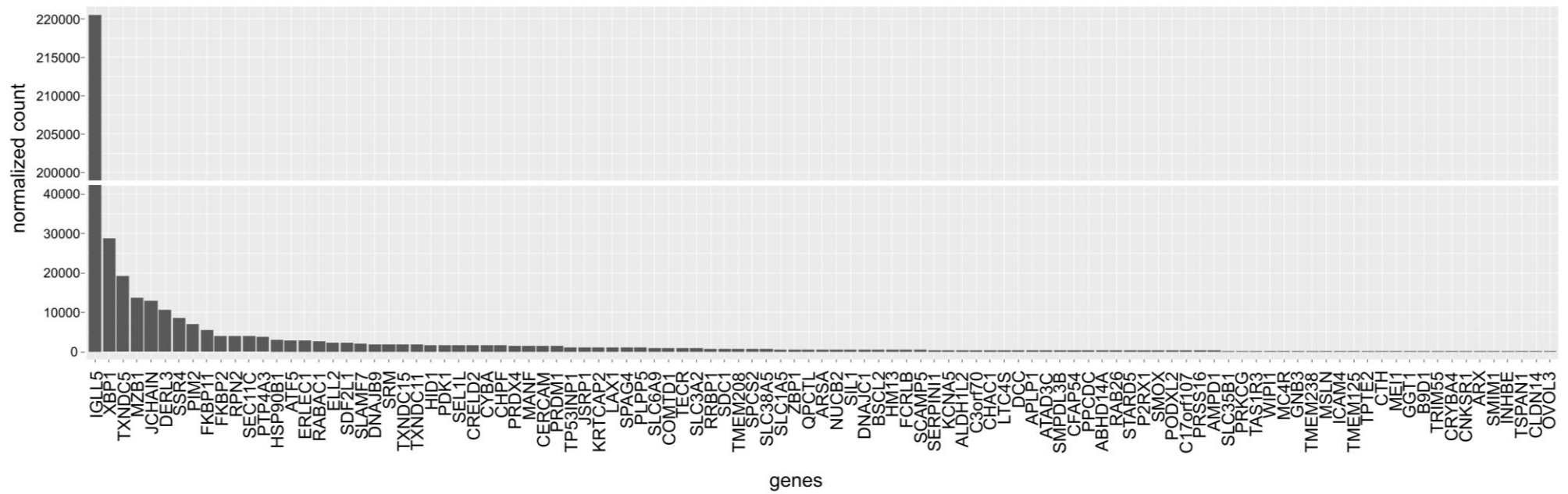
C



Supplemental Figure 3: Deconvolution analysis in iMCD-IPL and iMCD-TAFRO.

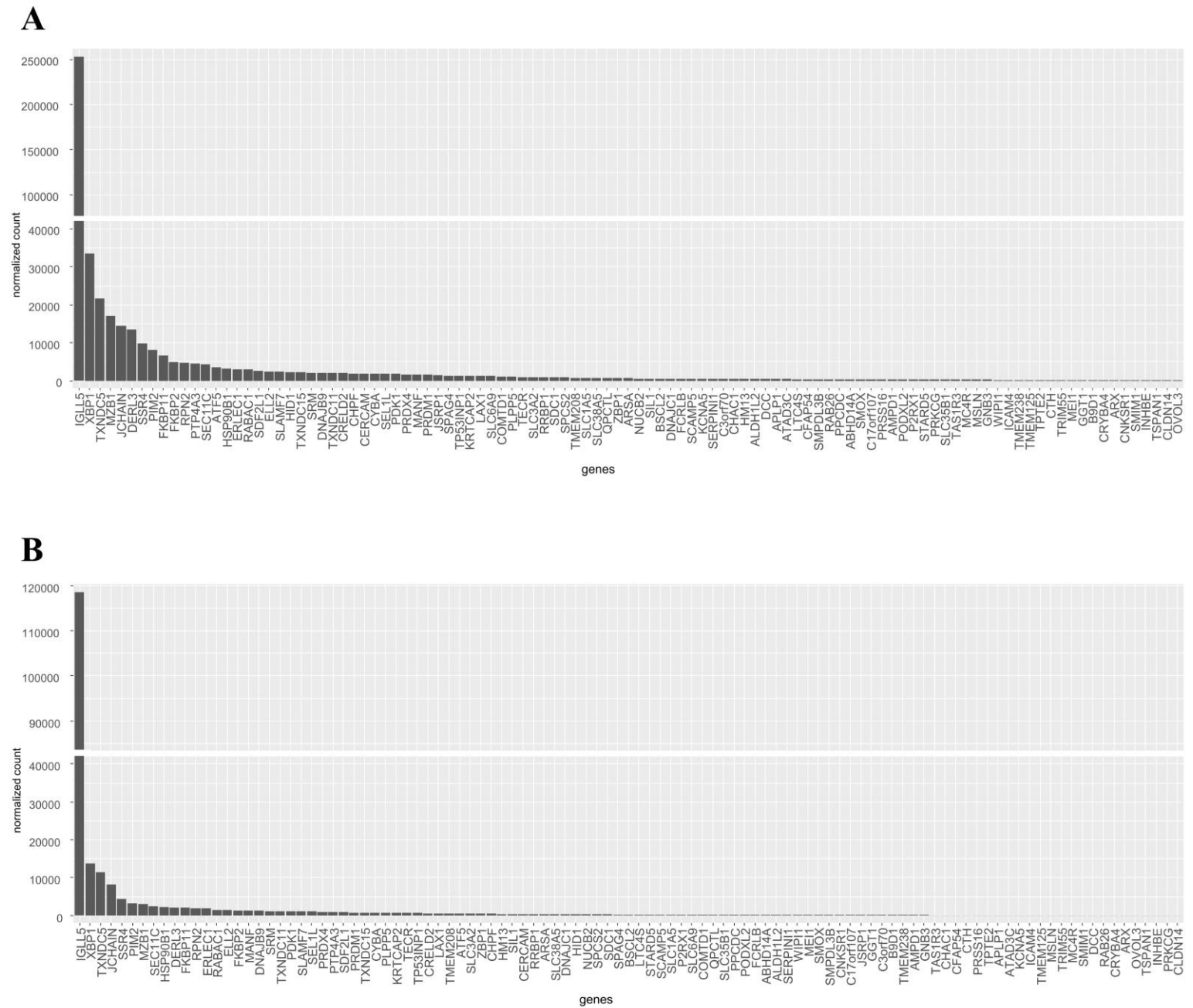
A, B: The cell amounts for each case were estimated using the abs mode of CIBERSORTx based on the transcripts per million (TPM) results of transcriptomic analysis. LM22 provided by CIBERSORTx was used as a reference. C: A comparison of the amount of plasma cells between iMCD-IPL and iMCD-TAFRO was performed based on the results of CIBERSORTx. No significant difference was observed in the estimated amount of plasma cells between iMCD-IPL and iMCD-TAFRO ($P=0.536$).

The Mann-Whitney U test was used for the test, and $P < 0.05$ was used to measure statistical significance.



Supplemental Figure 4: Gene expression analysis in iMCD-IPL and iMCD-TAFRO using a custom panel

The normalized count was calculated for all cases and is shown in order of expression level.



Supplemental Figure 5: The average gene expression level of iMCD subtypes in the nCounter analysis.

The normalized count of cases was calculated. A: iMCD-IPL. B: iMCD-TAFRO