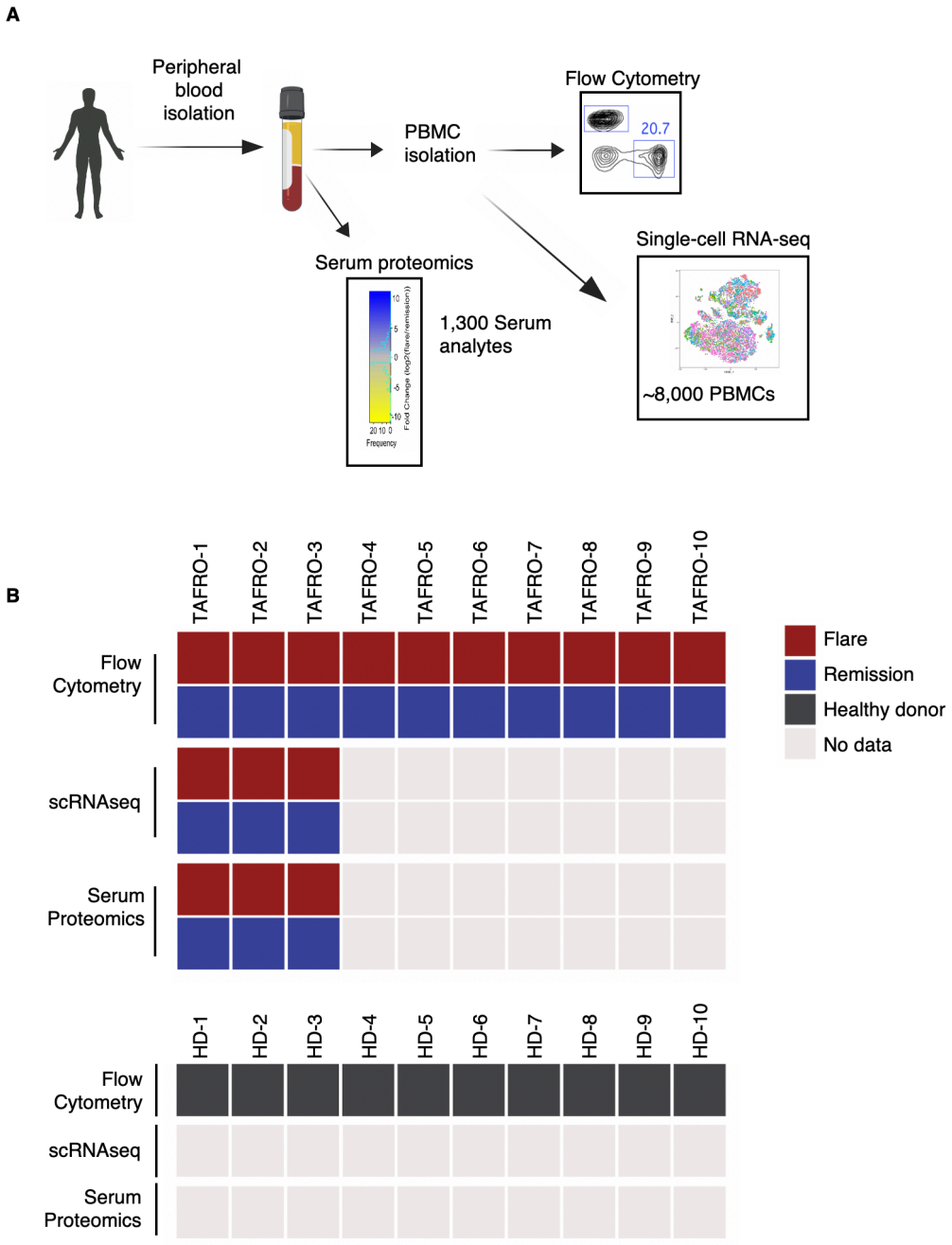
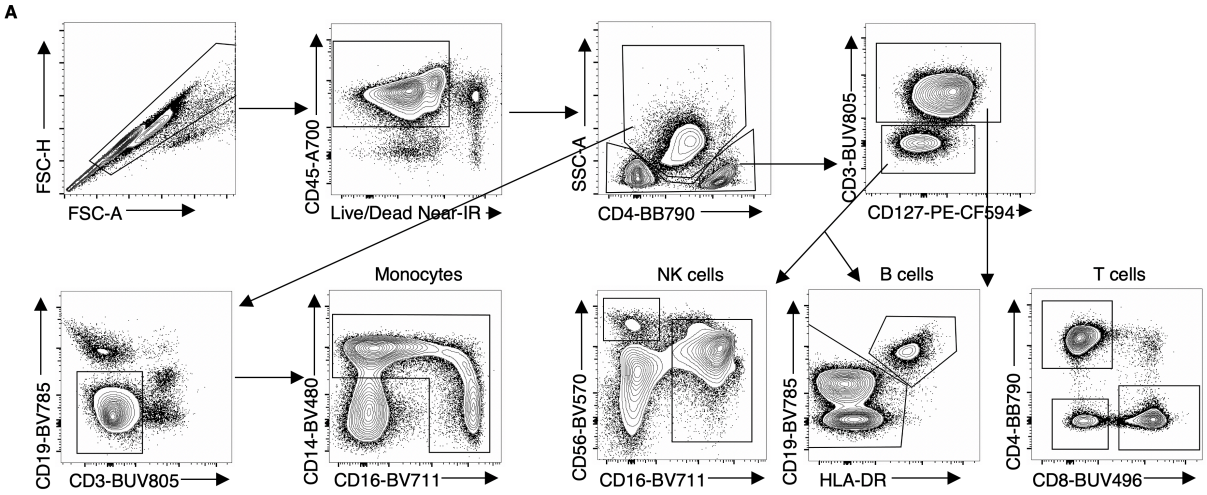
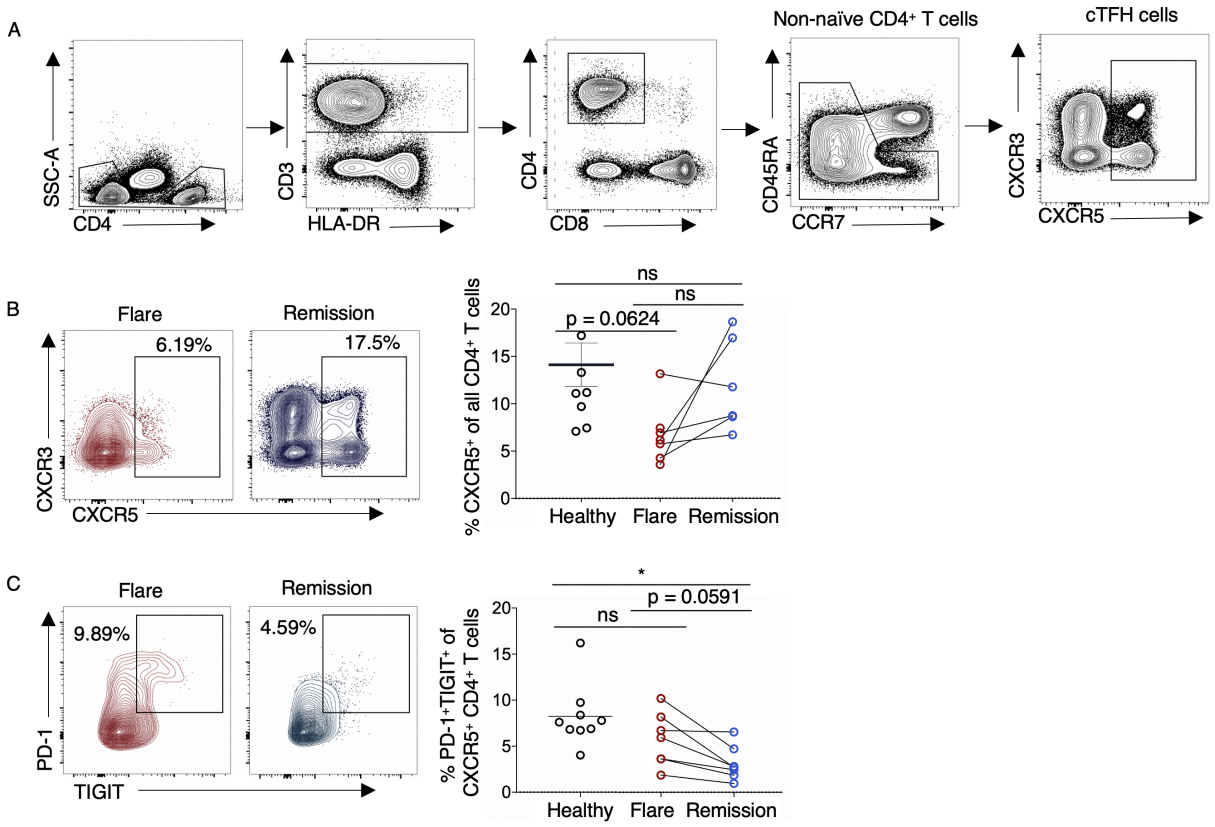


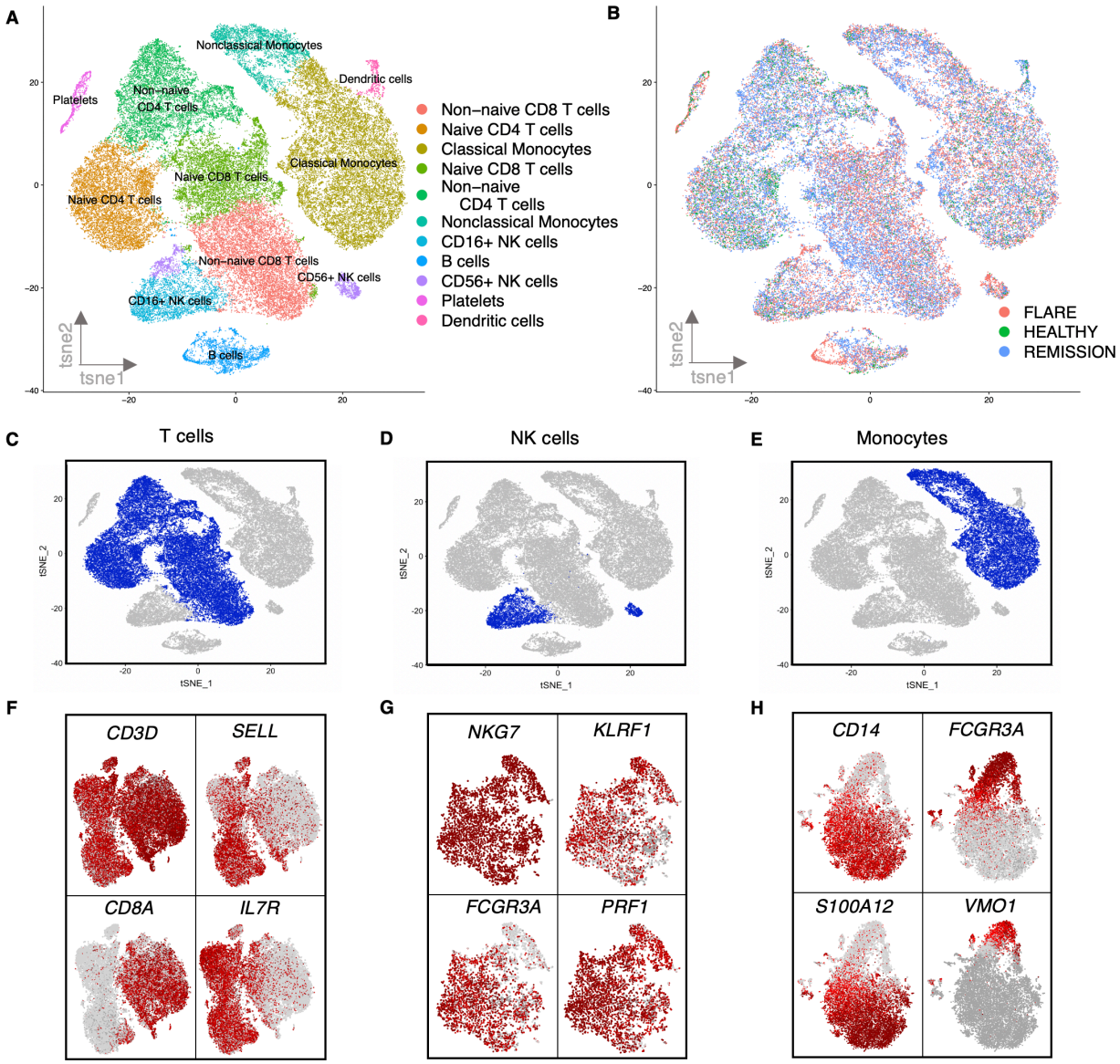
Supplemental Figure 1: Utilization of a multiomics approach to investigate circulating immune cell populations during iMCD-TAFRO flare and remission. (A) Schematic describing workflow for multiomics approach involving isolation of serum and PBMCs from the peripheral blood of iMCD-TAFRO patients during flare and remission and subsequent downstream analyses. **(B)** Cohort of 10 iMCD-TAFRO patients and 10 age and sex-matched healthy donors with analyzes of PBMCs and serum from flare and remission as indicated by flow cytometry, scRNAseq, and serum proteomics. Functional studies were performed in a separate cohort due to limited availability of additional samples from the original cohort. Created with BioRender.com.



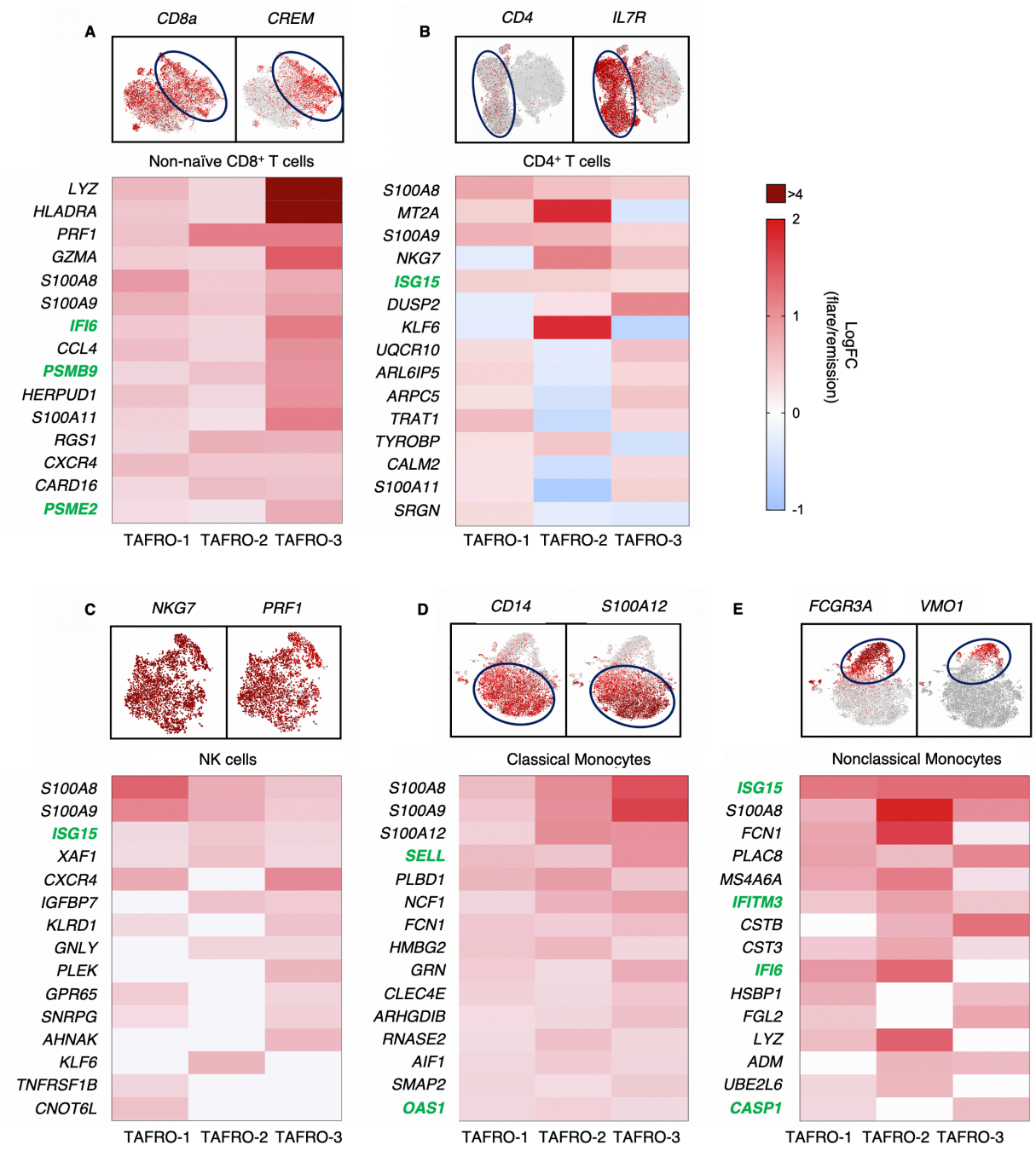
Supplemental Figure 2. Flow cytometric gating strategy. (A) Gating of live singlets followed by identification of monocytes, NK cells, B cells, and T cells.



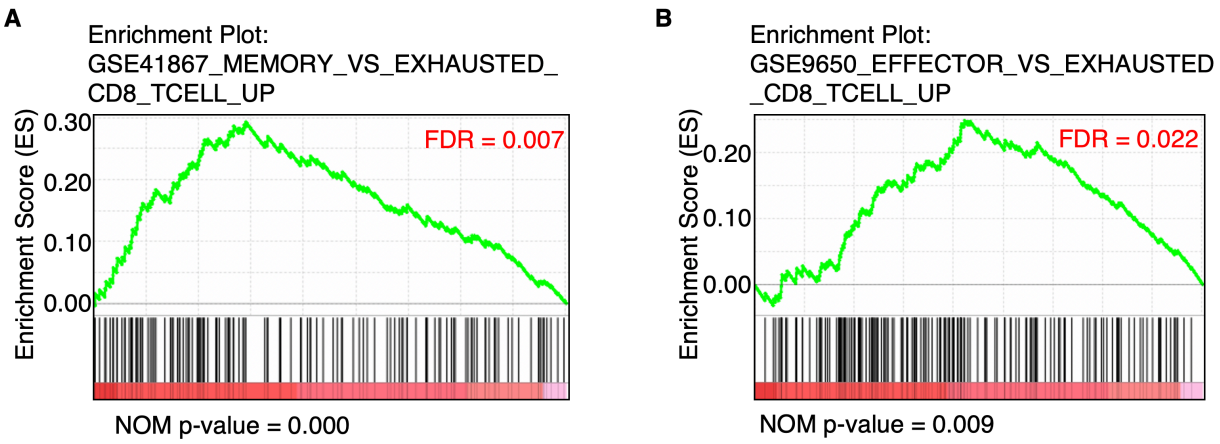
Supplemental Figure 3. Circulating T Follicular Helper (Tfh) CD4⁺ T cells appear more activated during iMCD-TAFRO flare when compared to remission. A. Identification of CD4⁺ T cells (previously gated as singlets, live, CD45⁺, CD3⁺) and subsequent gating of non-naïve CD4⁺ T cells lacking co-expression of CD45RA and CCR7 and circulating T follicular helper (cTFH) cells expressing CXCR5. **B.** Representative plots and comparison of CXCR5⁺ CD4⁺ T cell frequencies. **C.** Representative plots and comparison of cTFH co-expressing PD-1 and TIGIT. Error bars represent mean ± s.e.m. P values are based on paired two-tailed T tests between remission and flare samples and unpaired two-tailed T tests between healthy donor and flare with corrections for multiple comparisons. *P<0.05.



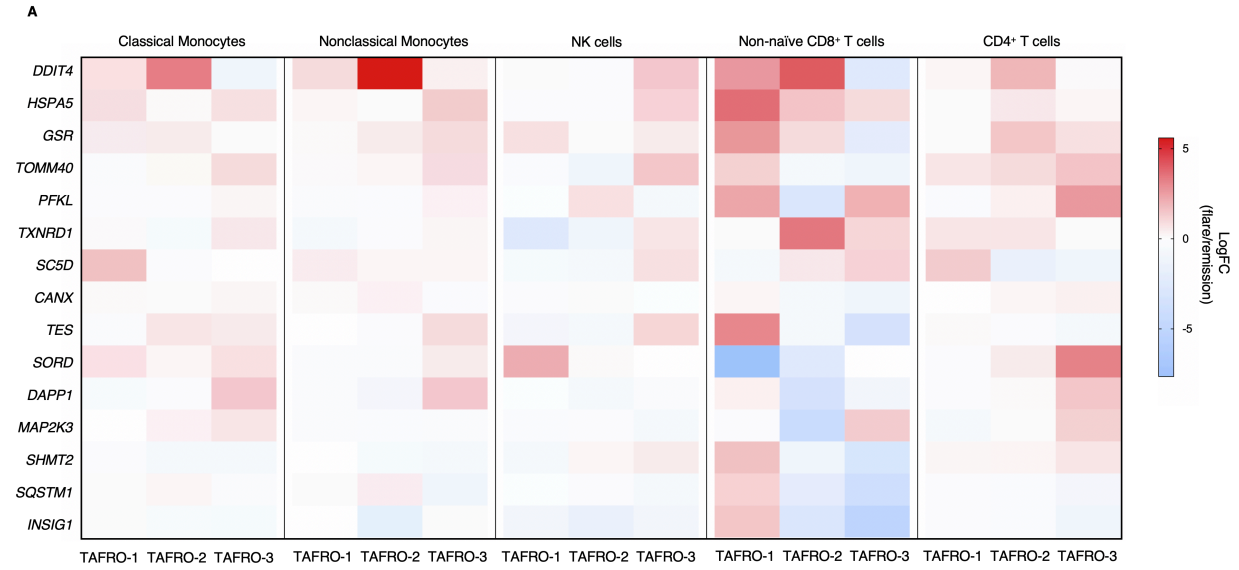
Supplemental Figure 4. Identification of immune cell populations in the scRNAseq dataset. A. TSNE plot clustering all cells within the scRNAseq dataset and labelling of immune cell populations within each cluster. **B.** TSNE plot representing significant overlap of cells from flare (red), remission (blue), and healthy donors (green) across the cell populations identified in A. C-F. Subdivision of immune cell populations within clusters (highlighted in blue) for T cells **(C)**, NK cells **(D)**, and Monocytes **(E)**.



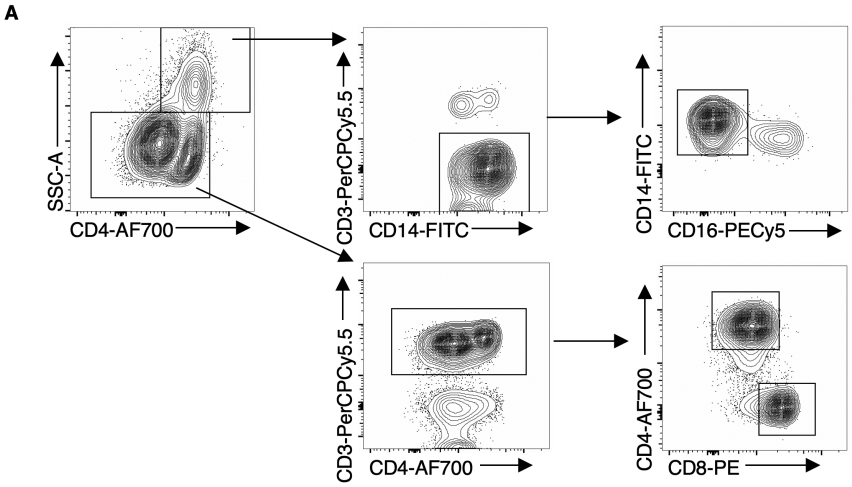
Supplemental Figure 5. Differential gene expression analysis across CD8⁺ T cells and monocytes between flare and remission. A-D. TSNE Clustering and Identification of non-naïve CD8⁺ T cells **(A)**, CD4⁺ T cells **(B)**, NK cells **(C),** classical monocytes **(D)**, and nonclassical monocytes **(E)** within the scRNAseq dataset and heatmap reporting the natural log fold-change (LogFC) of the Top 15 significantly differentially expressed genes between flare and remission. Type I Interferon genes highlighted in green.



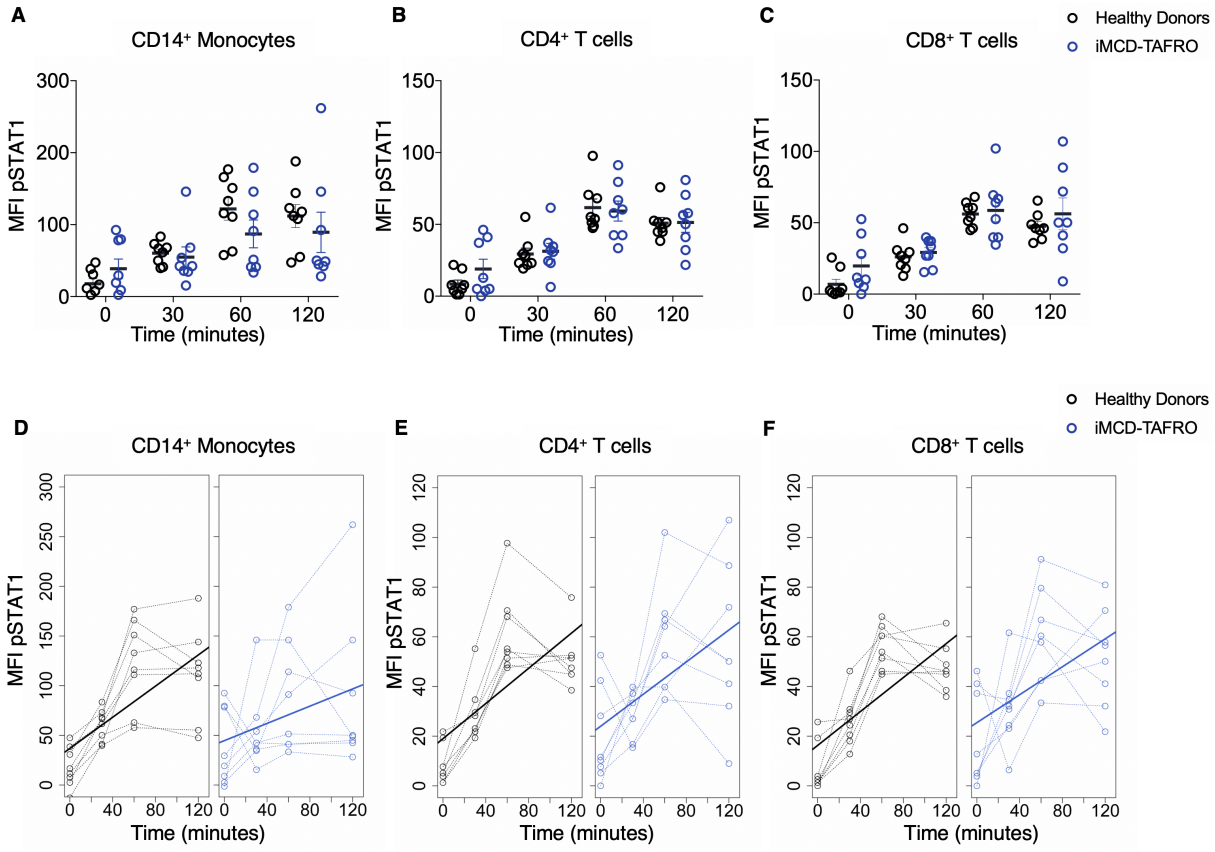
Supplemental Figure 6. CD8⁺ T cells are activated and do not display an exhausted CD8⁺ T cell gene signature during flare. Gene set enrichment analysis of non-naïve CD8 T cells from three iMCD-TAFRO patients comparing the average expression between flare and remission of genes within the GSE41867_MEMORY_VS_EXHAUSTED_CD8_TCELL_UP gene set **(A)** and GSE9650_EFFECTOR_VS_EXHAUSTED_CD8_TCELL_UP gene set **(B)**.



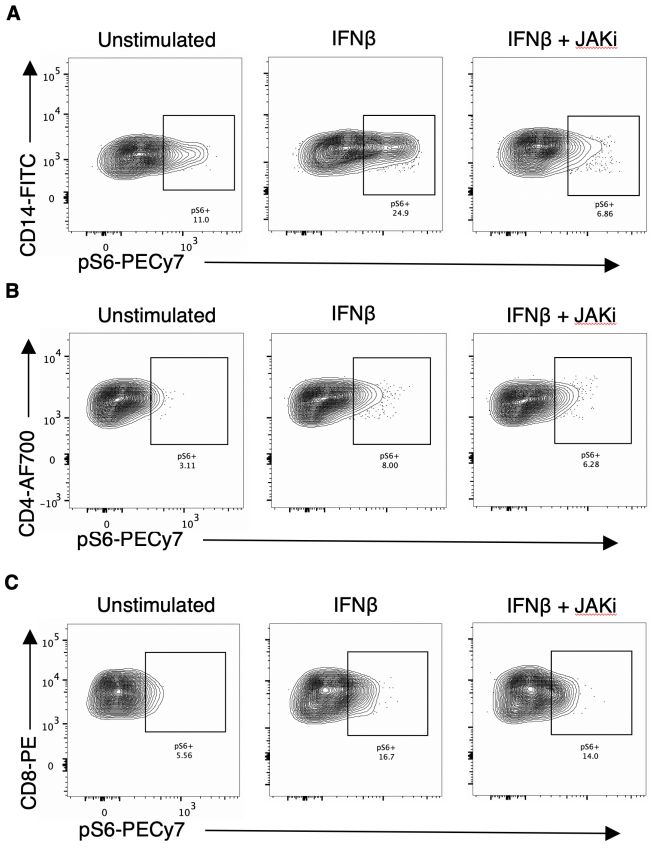
Supplemental Figure 7. Gene expression of mTORC1 signaling genes across monocytes, NK cells, and T cells between flare and remission. A. Heatmap reporting the log fold-change (LogFC) of genes from the HALLMARK_MTORC1_SIGNALING gene set between flare and remission for TAFRO-1, TAFRO-2, and TAFRO-3.



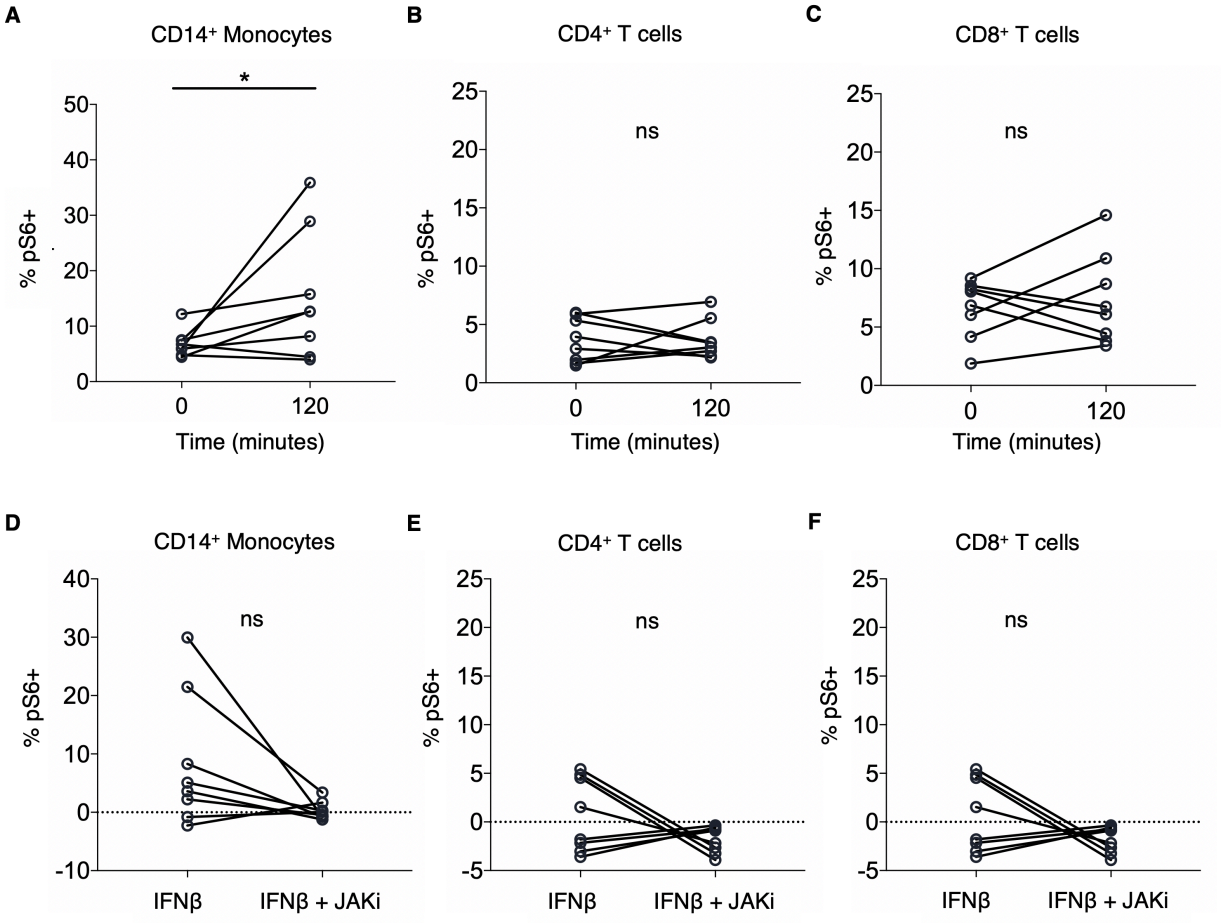
Supplemental Figure 8. Gating strategy for phospho-flow cytometry. Cells were previously gated for singlets and live cells. Monocyte and lymphocyte populations were gated using SSC-A and CD4. $CD14^+$ monocytes were identified within the monocyte gate as $CD3^-$ $CD14^+$. T cells were identified within the lymphocyte gate as $CD3^+$ and either $CD4^+$ Or $CD8^+$ T cells.



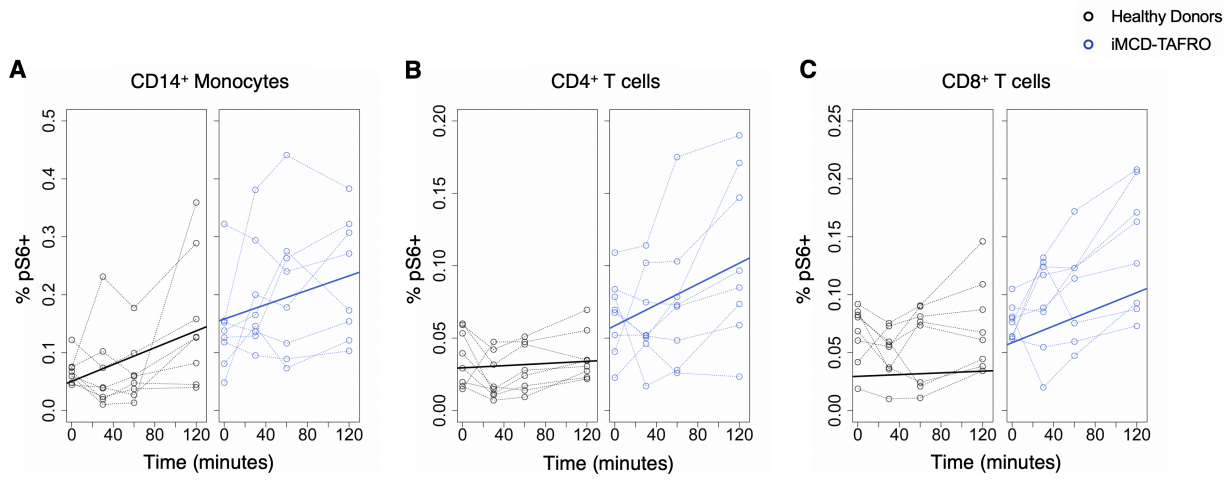
Supplemental Figure 9. Flow cytometric characterization of phosphorylated STAT1 following treatment with IFNβ. A-C. Monocytes and non-naïve CD4+ and CD8+ T cells were characterized from iMCD patients in remission (n = 8) and age and sex-matched healthy donors (n = 8) by flow cytometry to identify phosphorylation of STAT1 upon stimulation with 100ng/ml IFNB. Groups show the mean fluorescence intensity (MFI) of pSTAT1 (Y701) within **(A)** CD14+ classical monocytes, **(B)** CD4+ T cells, and **(C)** CD8+ T cells following 0, 30, 60, or 120 stimulation. Data are mean ± SEM. Statistics were based on two-way repeated ANOVA with Bonferroni-corrected post-hoc T tests between healthy donor (HD) and iMCD-TAFRO remission samples at each timepoint of stimulation **D-F.** Time evolution (dotted line) of the mean fluorescence intensity of pSTAT1 (Y701) within CD14+ cells, CD4+ cells, and CD8+ cells. A linear mixed effects model was constructed to evaluate the change in MFI overtime for iMCD patients and healthy donors. The model consisted of time point and group fixed effects and individual subject random effect..**(D)** The MFI of pSTAT1 increased significantly over time for CD14+ classical monocytes from both iMCD-TAFRO patients in remission (β1-iMCD-TAFRO = 0.43 [95%CI: 0.026, 0.84] 1/min) and healthy donors (β1-HD = 0.78 [95%CI: 0.49, 1.08] 1/min). **(E)** The MFI of pSTAT1 increased significantly over time for CD4+ T cells from both iMCD patients in remission (β1-iMCD-TAFRO = 0.28 [95%CI: 0.12, 0.44] 1/min) and healthy donor cells (β1-HD = 0.35 [95%CI: 0.22, 0.49] 1/min). **(F)** The MFI of pSTAT1 increased significantly over time for CD8+ T cells: The dependence on time was significant for iMCD-TAFRO cells in remission (β1-iMCD = 0.32 [95%CI: 0.14, 0.50] 1/min) and healthy donor cells (β1-HD = 0.34 [95%CI: 0.22, 0.46] 1/min). The β1 coefficients reported above are in units of percentage change per minute.



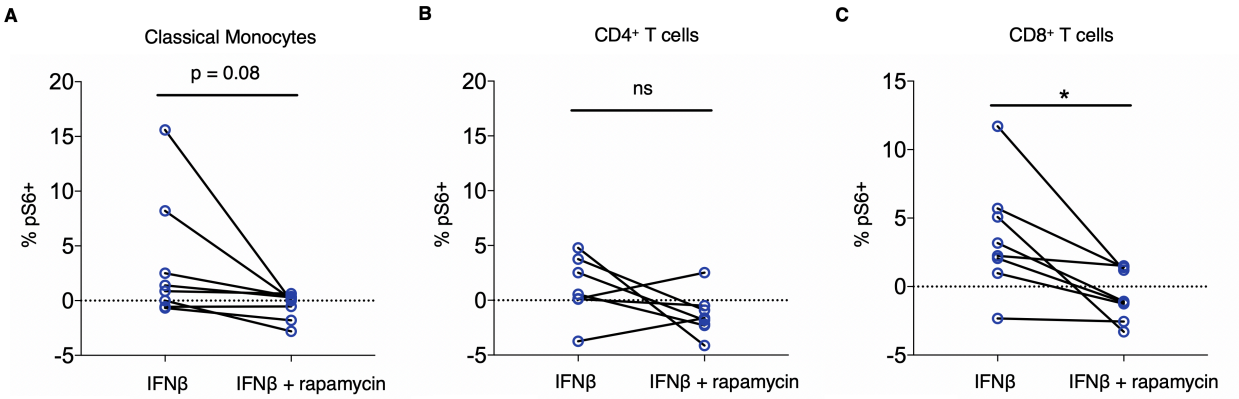
Supplemental Figure 10. Representative flow plots for gating of pS6⁺ cells within iMCD patient immune cell populations. A-C. Representative gating for pS6⁺ cells within unstimulated, IFNβ treated, or IFNβ and JAKi treated CD14⁺ monocytes **(A)**, CD4⁺ T cells **(B),** and CD8⁺ T cells **(C)**.



Supplemental Figure 11. Stimulation of healthy donor cells with IFNβ and treatment with JAK inhibition. A-C. Percent of healthy donor cells expressing phosphorylated ribosomal protein S6 (S235/S236) upon stimulation with IFNβ for 0 or 120 minutes within CD14+ classical monocytes **(A)**, CD4+ T cells **(B)**, and CD8+ T cells **(C)**. **D-F.** Comparison of the percent of cells expressing phosphorylated ribosomal protein S6 (S235/S236) from paired iMCD-TAFRO samples from remission treated with either 100ng/ml IFNβ alone or both IFNβ and 1uM JAKi withinclassical monocytes **(D)**, CD4+ T cells **(E)**, and CD8+ T cells **(F)**. P values are based on one-tailed, paired T tests between treatment groups. ns = not significant. *P<0.05.



Supplemental Figure 12. Flow cytometric characterization of phosphorylated S6 protein following treatment with IFNB. A-C. Time evolution (dotted line) of the frequency of pS6+ cells within CD14+ classical monocytes **(A)**, CD4+ T cells **(B)**, and CD8+ T cells **(C)** from iMCD-TAFRO patients in remission and age and sex-matched healthy donors (HD) following treatment with 100ng/ml IFNβ. The four-time points for each subject were fitted with a line (solid). The frequency of pS6+ cells increased significantly over time for CD14+ classical monocytes from both iMCD (β1-iMCD-TAFRO = 0.066 [95%CI: 0.013, 0.12] 1/min) and healthy donor (β1-HD = 0.072 [95%CI: 0.022, 0.12] 1/min). The frequency of pS6+ cells increased significantly over time for CD4+ T cells from iMCD-TAFRO patients in remission (β1-iMCD-TAFRO = 0.036 [95%CI: 0.015, 0.057] 1/min) but it was not significant for the healthy donor cells (β1-HD = 0.004 [95%CI: -0.006, 0.014] 1/min). The same case was found for CD8: The dependence on time was significant for iMCD cells in remission (β1-iMCD-TAFRO = 0.052 [95%CI: 0.030, 0.075] 1/min) but not for healthy donor cells (β1-HD = 0.009 [95%CI: -0.007, 0.027] 1/min). The β1 coefficients reported above are in units of percentage change per minute.



Supplemental Figure 13. Stimulation of iMCD patient cells from remission with IFNβ and treatment with rapamycin (Sirolimus). A-C. Comparison of the percent of cells expressing phosphorylated ribosomal protein S6 (S235/S236) from paired iMCD-TAFRO samples from remission (n = 8) treated with either 100ng/ml IFNβ alone or both IFNβ and 100nM rapamycin within CD14+ classical monocytes **(A)**, CD4+ T cells **(B),** and CD8+ T cells **(C)**. P values are based on one-tailed, paired T tests between treatment groups. ns = not significant. *P<0.05.

Supplemental Table 1. Antibodies used for Flow Cytometry

Marker	Fluorochrome	Clone	Company	Cat #
CD11c	BUV395	B-ly6	BD	563788
CD8	BUV496	RPA-T8	BD	564804
CD8	BV570	RPA-T8	Biolegend	301038
CD8	PE-Cy5.5	RPA-T8	Thermo Fisher	35-0088-42
CD45RA	BUV563	HI100	BD	565702
CD45RA	BV650	HI100	BD	563963
CD38	BUV661	HIT2	BD	565069
CD25	BUV737	2A3	BD	564385
CD25	BV785	BC96	Biolegend	302638
CD3	BUV805	UCHT1	BD	565515
CD3	BV711	UCHT1	Biolegend	300464
CD3	APC-R700	UCHT1	BD	565119
PD1	BV421	EH12.2H7	Biolegend	329920
PD1	APC	EH12.2H7	Biolegend	329908
PD1	PE-Cy7	EH12.2H7	Biolegend	329918
CD14	BV480	MΦP9	BD	566190
CD14	BV510	63D3	Biolegend	367124
CD56	BV570	HCD56	Biolegend	318330
HLA-DR	BV605	G46-6	BD	562845
CD27	BV650	O323	Biolegend	302828
CD27	BV785	O323	Biolegend	302832
CD16	BV711	3G8	Biolegend	302044
CD19	BV785	HIB19	Biolegend	302240
CD19	BV510	HIB19	Biolegend	302242
CD138	FITC	MI15	BD	552723
CD4	BB790	SK3	BD	624296
CD4	PE-Cy5.5	S3.5	Thermo Fisher	MHCD0418
CD4	BV570	RPA-T4	Biolegend	300534
CD123	PE	9F5	BD	561050
CD127	PE-CF594	HIL-7R-M21	BD	562397
CD127	BV421	A019D5	Biolegend	351310
CD69	PE-Cy5	FN50	Biolegend	310908
CD34	PE-Cy5.5	581	Thermo Fisher	CD34-581-18
CD21	PE-Cy7	Bu32	Biolegend	354912
CXCR5	A647	RF8B2	BD	558113
CD45	A700	HI30	BD	560566
CCR7	APCCy7	G043H7	Biolegend	353212
CD161	PE-Cy5	DX12	BD	551138
TIGIT	PE-Cy7	MBSA43	Thermo Fisher	25-9500-42
LAG3	PE-eFluor610	3DS223H	Thermo Fisher	61-2239-42
Granzyme B	AF700	GB11	BD	560213
Tbet	PE	4B10	Biolegend	644810
Ki67	FITC	B56	BD	556026
Perforin	BV421	B-D48	Biolegend	353307
CCR4	PE-CF594	1G1	BD	565391
CCR6	BV605	G034E3	Biolegend	353420
CXCR3	BV711	G025H7	Biolegend	353732
ICOS	BB515	C398.4A	BD	565880
Foxp3	PE	259D/C7	BD	560046
RPS6 Phospho (Ser235/Ser236)	PE/Cy7	259D/C7	Biolegend	608605
STAT1 Phospho (Tyr701)	AF647	A17012A	Biolegend	666410

Supplemental Document 1. Extended patient clinical data.

iMCD-TAFRO-1 was a 48-year old male with a past medical history of Raynaud's disease who presented with nausea, vomiting, diarrhea, abdominal pain, body aches, chills, and a mild cough in March 2016. He deteriorated rapidly, experiencing acute respiratory failure requiring ventilation, renal failure requiring dialysis, hypotension requiring pressors, cytopenias (thrombocytopenia) requiring transfusions, anasarca (50 lb weight gain), myelofibrosis, and hepatosplenomegaly. A lymph node biopsy was consistent with iMCD with the plasmacytic histopathological subtype. He received the following treatment regimens: hydrocortisone and methylprednisolone (no response); methylprednisolone, prednisone, and siltuximab (partial response < 12 months); ciclosporin, cyclophosphamide, dexamethasone, etoposide, prednisone, rituximab, tocilizumab (complete response > 12 months).

iMCD-TAFRO-2 was a 47-year-old healthy male who no significant medical history who presented with severe worsening abdominal pain and fluid accumulation (ascites and pleural effusion) in September 2011 and was admitted. He then developed lymphadenopathy, persistent fevers, acute renal failure, weight loss, loss of appetite, and severe fatigue. He had a cervical lymph node resection that was consistent with iMCD with the mixed histopathological subtype. He received the following treatment regimens: prednisone (no response); prednisone and tocilizumab (no response); bortezomib, cisplatin, cyclophosphamide, dexamethasone, doxorubicin, etoposide, prednisone, rituximab, thalidomide, tocilizumab (complete response > 12 months).

iMCD-TAFRO-3 was a previously healthy 25-year-old male, who presented with 4 weeks of night sweats, fatigue, weight loss, generalized lymphadenopathy, eruptive cherry hemangiomatosis, and abdominal pain. His hospital course was notable for mutli-organ system failure, left eye blindness secondary to acute retinal hemorrhage, generalized anasarca, pericardial and pleural effusions, and severe muscle weakness. Laboratory findings included hyperinflammation with elevated CRP and ESR, renal dysfunction, hepatitis, anemia, thrombocytopenia, profound hypoalbuminemia, elevated ferritin, and IL-6. Radiologically, there was diffuse FDG-avid lymphadenopathy, hepatosplenomegaly, ascites, and pleural effusions. Lymph node biopsy was consistent with iMCD with the hypervascular histopathological subtype. He received the following treatment regimens: dexamethasone and methylprednisolone (no response); methylprednisolone, prednisone, and rituximab (partial response < 12 months); bortezomib, cyclophosphamide, dexamethasone, doxorubicin, etoposide, IVIG, methylprednisolone, prednisone, rituximab, siltuximab, and thalidomide (complete response > 12 months); bortezomib, cyclophosphamide, dexamethasone, doxorubicin, etoposide, methylprednisolone, prednisone, rituximab, siltuximab, thalidomide, and tocilizumab (complete response > 12 months); bortezomib, ciprofloxacin, dexamethasone, pentoxifylline, siltuximab, thalidomide, and tocilizumab (N/A - Treatment Started in Complete Remission); bortezomib, celecoxib, dexamethasone, siltuximab, and thalidomide (partial response < 12 months); bortezomib, ciclosporin, dexamethasone, IVIG, siltuximab, and thalidomide (no response); bortezomib, ciclosporin, cyclophosphamide, dexamethasone, doxorubicin, etoposide, IVIG, rituximab, and thalidomide (partial response < 12 months); IVIG and sirolimus (complete response > 12 months). Though this patient never received siltuximab +/- corticosteroids without having another drug initiated within 2 weeks and thus his response to siltuximab +/- corticosteroids is listed as not assessable in Table 1, he did fail to respond to siltuximab in the

acute setting and relapsed while on siltuximab monotherapy. Thus, a subjective review of this case suggests this patient is a non-responder to siltuximab +/- corticosteroids,

iMCD-TAFRO-4 was a 39-year-old male with a past medical history of ulcerative colitis, hypothyroidism, gall stones, and kidney stones, who presented with an elevated white blood count, CRP, anemia, thrombocytopenia, renal dysfunction, fatigue, fevers, night sweats, fluid accumulation, splenomegaly, and lymphadenopathy. Lymph node biopsy was consistent with iMCD with histopathological subtype not reported. He received the following treatment regimens: prednisone (no response); cyclophosphamide, vincristine, prednisone (complete response > 12 months); rituximab (no response); cyclophosphamide, hydroxydaunorubicin, vincristine, prednisone, rituximab (complete response < 12 months); rituximab, ifosfamide, carboplatin, etoposide (partial response > 12 months); prednisone and tocilizumab (complete response > 12 months).

iMCD-TAFRO-5 was a 39-year-old male with a past medical history of cholecystitis, congestive heart failure, who presented with elevated CRP, thrombocytopenia, proteinuria, fatigue, night sweats, severe fluid accumulation, hepatosplenomegaly, renal failure, thrombotic microangiopathy requiring intubation, and lymphadenopathy. Lymph node biopsy was consistent with iMCD with the hypervascular histopathological subtype. He received the following treatment regimens: prednisone (partial response < 12 months); siltuximab (complete response > 12 months).

iMCD-TAFRO-6 was a 46-year-old male with a past medical history of anxiety, kidney stones, gastroesophageal reflux disease and a family history of lupus who presented with abdominal pain in May 2018. He was admitted for abdominal pain and worsening pneumonia/pleural effusions. He was found to have bilateral adrenal gland hemorrhages, left renal vein thrombosis, pleural effusions, hepatosplenomegaly, lymphadenopathy, anemia and thrombocytopenia. A lymph node excisional biopsy was consistent with iMCD with the plasmacytic histopathological subtype. He received the following treatment regimens: prednisone (no response); dexamethasone, Prednisone, Rituximab, and Siltuximab (Complete Response >12 months). Though this patient received siltuximab +/- corticosteroids within 2 weeks of initiating rituximab and thus his response to siltuximab +/- corticosteroids is listed as not assessable in Table 1, he was worsening rapidly after the rituximab was given and dramatically improved once siltuximab was started. Thus, a subjective review of this case suggests this patient is a responder to siltuximab +/- corticosteroids.

iMCD-TAFRO-7 was a 67-year-old woman with a past medical history of hypertension who exhibited low platelets, fluid retention including large pleural effusions, renal dysfunction, splenomegaly, fatigue, night sweats, and abdominal pain. She was diagnosed with HHV-8 negative, HIV negative iMCD with the mixed histopathological subtype after a lymph node excision. She received the following treatment regimens: prednisone (partial response < 12 months); prednisone and rituximab (complete response > 12 months); etoposide, prednisone, and rituximab (complete response > 12 months).

iMCD-TAFRO-8 was a 13-year-old male with no significant past medical history who presented with fever, proteinuria, organomegaly and elevated CRP. The patient went on to develop thrombocytopenia, anasarca, and renal dysfunction. Lymph node biopsy was consistent with iMCD with hypervascular histopathological subtype. He received the following treatment regimens: methylprednisolone, prednisone, rituximab, sirolimus, and tocilizumab (partial

response > 12 months). Though this patient received tocilizumab +/- corticosteroids within 2 weeks of initiating rituximab and sirolimus and thus his response to tocilizumab +/- corticosteroids is listed as not assessable in Table 1, he was worsening rapidly after the tocilizumab was given and dramatically improved once sirolimus was started. Thus, a subjective review of this case suggests this patient is a non-responder to tocilizumab +/- corticosteroids.

iMCD-TAFRO-9 was a 17-year-old female with no past medical history who initially presented with fever, cough, shortness of breath, myalgias, decreased appetite, fatigue, ascites, pleural effusion, hepatosplenomegaly, and lymphadenopathy as well as anemia, thrombocytopenia, hypoalbuminemia, proteinuria, elevated creatinine, CRP, ESR, and alkaline phosphatase. Lymph node biopsy was consistent with iMCD with the hypervascular histopathological subtype. She received the following treatment regimens: dexamethasone and prednisolone (partial response < 12 months); bleomycin, dacarbazine, dexamethasone, doxorubicin, vinblastine (complete response < 12 months); tocilizumab (complete response > 12 months); dexamethasone, prednisolone, and tocilizumab (no response); sirolimus (complete response > 12 months). Though this patient experienced a complete response on tocilizumab that lasted over one year, she relapsed while on tocilizumab, suggesting that this patient is likely a non-responder to tocilizumab +/- corticosteroids.

iMCD-TAFRO-10 was a 63-year-old female patient with no significant past medical history who presented with nausea, vomiting, fever, splenomegaly, palpable axillary lymph nodes, fluid retention, thrombocytopenia, anasarca, elevated CRP, renal dysfunction, and reticulin fibrosis. Lymph node biopsy was consistent with iMCD with the hypervascular histopathological subtype. She received the following treatment regimens: methylprednisolone, rituximab, and tocilizumab (complete response > 12 months); sirolimus (partial response > 12 months). Though this patient received tocilicumab +/- corticosteroids within 2 weeks of initiating rituximab and thus her response to tocilizumab +/- corticosteroids is listed as not assessable in Table 1, she was worsening rapidly before the tocilizumab was given and dramatically improved once it was started. Thus, a subjective review of this case suggests this patient is likely to be a responder to tocilizumab +/- corticosteroids. However, she went on to relapse while on tocilizumab.

iMCD-TAFRO-11 was a 32-year-old female with a significant past medical history of GI-related disorders (IBS, GERD) and migraines, who developed diffuse body aches, fever, dry cough, shortness of breath, flank area pain, chest pain, and subsequently developed thrombocytopenia, anemia, hypoalbuminemia, diffuse lymphadenopathy, ascites, large pleural effusions, and an acute inflammatory pattern. Lymph node biopsy was consistent with iMCD with the hypervascular histopathological subtype. She received the following treatment regimens: prednisone (no response); dexamethasone, methylprednisolone, prednisone, and rituximab (Partial Response < 12 months); methylprednisolone and tocilizumab (complete response > 12 months).

iMCD-TAFRO-12 was a 48-year-old male with a past medical history of hypertension who presented with about a 1.5-month history of fever, intermittent throbbing frontal headaches, blurry vision, dry non-productive cough, vertigo, fever, arthralgia in his knees, diarrhea, nausea, dyspnea, lower extremity edema, increased abdominal girth, right upper quadrant pain, decreased appetite, thrombocytopenia, renal dysfunction, and organomegaly. Lymph node biopsy was consistent with iMCD with the mixed histopathological subtype. He received the following treatment regimens: methylprednisolone, prednisone, and rituximab (complete

response < 12 months); dexamethasone, IVIG, methylprednisolone, prednisone, rituximab (no response); dexamethasone, prednisone, and siltuximab (complete response > 12 months).

iMCD-TAFRO-13 was a 31-year-old female patient with a history of obesity, migraines, pseudotumor cerebri, and asthma, who presented with GI symptoms (nausea, vomiting, and abdominal pain), dyspnea, fatigue, fever, unintentional weight loss, disseminated lymphadenopathy, elevated ESR and renal dysfunction. Lymph node biopsy was consistent with iMCD with the mixed subtype. She received the following treatment regimens: ciclosporin (no response); siltuximab (no response); dexamethasone, prednisone, and siltuximab (complete response > 12 months).

iMCD-TAFRO-14 was an 18-year-old male with no significant past medical history who presented with left upper quadrant pain, lymphadenopathy and hepatosplenomegaly, thrombocytopenia, and fluid retention. Lymph node biopsies were consistent with iMCD with the hypervascular subtype. He received the following treatment regimens: siltuximab (no response). Though the patient did not meet the threshold of improvement required to achieve a partial response (improvement in the proportion of abnormal minor criteria from before treatment to the time of best response), this patient did experience modest improvement that led to a state of symptoms that was less severe than flare.

iMCD-TAFRO-15 was a 29-year-old woman with no significant medical history who presented with upper right quadrant pain, kidney failure, flu-like symptoms, renal failure requiring dialysis, anemia, proteinuria, and anorexia. Lymph node biopsies were consistent with iMCD with the mixed subtype. She received the following treatment regimens: prednisone (no response); ciclosporin and prednisone (no response); prednisone (no response); prednisone and siltuximab (no response); cyclophosphamide, dexamethasone, etoposide, prednisone, rituximab (complete response > 12 months).