1 Supplemental Material for

2	Insiahts	gained from	sinale-cell	analvsis	of immune	cells in	Tofacitinib
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3 treatment of Vogt-Koyanagi-Harada disease

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17 Figures S1 to S7





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Figure S1. The inhibitory effects of TOFA on PBMC in vitro and clustering strategy in CyTOF.

- 21 The flow cytometry histograms (left) and box plots (right) showing the phosphorylation of STAT1
- 22 (A) and STAT3 (B) in CD3+ T cells (n = 6/group).
- 23 **C.** The cytotoxicity of tofacitinib on PBMCs (n = 4/group).
- 24 The clustering strategy of major immune cell populations (D) identifying 4 PBMC types based on
- 25 the scaled expression heatmap of discriminative gene for each cluster (E). Then NK&T, BC and
- 26 MYE (D) were re-clustered and identified classical subsets based on the scaled expression
- 27 heatmap of discriminative gene for each cluster (F).
- 28 Significance in A-B was calculated using two-tailed unpaired Student's t-test; **P < 0.01.



0 Figure S2. Clustering strategy in scRNA-seq.

The clustering strategy of major immune cell populations (A) identifying 5 PBMC types (left) based on the scaled expression heatmap of discriminative gene for each cluster (right). Then NK&T (B), BC (C) and MYE (D) were re-clustered and identified classical subsets (left) based on the scaled expression heatmap of discriminative gene for each cluster (right). Color scheme is based on z-score distribution from -2 (blue) to 2 (red).

- 36 The full names of the cell types in CyTOF and scRNA-seq are as follows: NK&T, NK and T cells;
- 37 BC, B cells; MYE, myeloid cells; CD4Na, CD4+ naive T cell; CD4Tcm, central memory CD4+ T
- 38 cell; CD4Tem, effector memory CD4+ T cell; CD4Treg, regulatory CD4+ T cell; CD4CTL, cytotoxic
- 39 CD4+ T cell; CD8Na, CD8+ naive T cell; CD8Tem, effector memory CD8+ T cell; CD8CTL,
- 40 cytotoxic CD8+ T cell; CD4-CD8-, CD4-CD8- double-negative T cell; CD4+CD8+, CD4+CD8+
- 41 double-positive T cell; Proliferating TC, proliferating T cell; NK1, CD16 (FCGR3A)-
- 42 CD56(NCAM1)^{bright} NK; NK2, CD16(FCGR3A)+ CD56(NCAM1)^{dim} CD57(B3GAT1)- NK; NK3,
- 43 CD16(FCGR3A)+ CD56(NCAM1)^{dim} CD57(B3GAT1)+ late NK; NBC, naive B cell; MBC, memory
- 44 B cell; ABC, autoimmune-associated B cell; PC, plasma cell; CMC, classical monocyte; NMC,
- 45 nonclassical monocyte; IMC, intermediate monocyte; CDC, conventional DC; PDC, plasmacytoid
- 46 DC; CD45-, CD45- cells; MEGA, megakaryocyte; CD34+, CD34+ cells.



Figure S3. Reconstitution of the circulating cellular ecosystem by VKH.

- A. t-SNE plot of immune cells in HC and VKH groups.
- **B.** The percentage of MC in PBMCs between HC and VKH groups (n = 5/group).
- 51 C. The flow cytometry histogram showing the expression of CD3 and CD14, and the percentage
- 52 53 of CD14+ MC in PBMCs between HC and VKH groups (n = 5/group).
- D. Violin plot showing the expression of CD45RA, LEF1, CD45RO, CCR4 and T-bet in CD4+ or
- 54 CD8+ T cells between HC and VKH groups in CvTOF.
- 55 E. t-SNE plot of immune cells, and the color indicating the number of VKH-DEGs.
- 56 Significance in B, C was calculated using two-tailed unpaired Student's t-test; *P < 0.05, ****P <
- 57 0.0001.



60 Figure S4. TOFA treatment downregulated cell activation of NKs and TCs.

- 61 Violin plot showing the expression of CD45RA, CD45RO, LEF1, CCR4 and T-bet in CD4+(**A**) or 62 CD8+(**B**) T cells between VKH and TOFA groups in CyTOF.
- 63 **C.** The percentage of CD4+ TCs subsets in CD4+ T cells in VKH and TOFA groups (n = 5/group).
- 64 **D.** The percentage of CD8+ subsets in CD8+ TCs in VKH and TOFA groups (n = 5/group).
- 65 **E.** Violin plot showing the expression of CCL5 and GZMK in NK cells between VKH and TOFA groups in CyTOF.
- F. Violin plot showing the expression of CCL5 and GZMB in CD8+ T cells between VKH and
 TOFA groups in CyTOF.
- 69 **G.** Representative GO biological process and pathways enriched in downregulated TOFA-DEGs
- 70 based on functional enrichment analysis in NK and CD8+ TCs subsets.
- 71 Significance in C-D was calculated using two-tailed paired t-test; ****P < 0.0001.



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Figure S5. TOFA treatment downregulated cell activation of BCs.

A. The pie plots showing the percentage of BCs subsets in HC, VKH and TOFA groups.

77 **B.** The percentage of NBC and MBC in BCs between HC and VKH groups (n = 5/group).

- 78 79 C. Volcano plot showing TOFA-DEGs in BC.
- D. The gene network showing PPI analysis of downregulated TOFA-DEGs in NBC subset.
- 80 Significance in **B** was calculated using two-tailed unpaired Student's t-test; $*^{P} < 0.01$.
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Figure S6. TOFA treatment downregulated cell activation of myeloid cells.

A. The expression levels of genes related to JAK-STAT signaling in myeloid cells subsets.

- **B.** The pie plots showing the percentage of CMCs-SCs in HC, VKH and TOFA groups.
- **C.** The gene network showing PPI analysis of downregulated TOFA-DEGs in CMC subset.
- **D.** The heatmap showing the levels of genes associated with specific pathways in CMC among
- 90 HC, VKH and TOFA groups.



- 95 Figure S7. TOFA treatment reduced the cell-cell interaction influenced by VKH.
- 96 **A.** The mRNA expression levels of *TNFAIP3*, *NOD1*, and *NOD2* were measured with real-time 97 quantitative PCR (n = 5/group).
- 98 **B.** Circle plot showing the downregulated L-R pairs in in TOFA/VKH comparison.
- 99 C. Relative contribution of each L-R pair in TNF signaling.
- 100 **D.** Relative contribution of each L-R pair in CCL signaling.
- 101 E. Circle plot showing IL6 signaling pathway network in HC, VKH and TOFA groups.
- 102 **F.** Relative contribution of each L-R pair in IL-6 signaling.
- 103 G. The heatmap showing the levels of MC-related *IL6*, DC-related *IL6R* and TC-related *IL6ST*
- among HC, VKH and TOFA groups.
- 105 **H.** Circle plot showing IFNG signaling pathway network in HC, VKH and TOFA groups.
- 106 I. Relative contribution of each L-R pair in IFNG signaling.
- 107 J. The heatmap showing the levels of NK-related *IFNGR1* and MC-related *IFNGR2* among HC,
- 108 VKH and TOFA groups.
- 109 **K.** Schematic of the drug discovery bioinformatics analysis.
- 110 Significance in **A** was calculated using two-tailed unpaired Student's t-test; *P < 0.05, **P < 0.01.