Supplemental Methods

T cell isolation

Human PBMCs were isolated using a Ficoll (GE Healthcare) density gradient in a SepMate PBMC Isolation Tube (STEMCELL Technologies). 5 x 10^7 cells were labeled with biotinylated antibodies (Supplemental Table 1). Negative selection was performed by depleting biotin-labeled cells with Biotin Binder Dynabeads (Life Technologies). The remaining cells were cultured in RPMI (Mediatech) supplemented with 10% fetal bovine serum (VWR), glutamine, antibiotics, and 20ng/mL recombinant human IL-2 (Peprotech). Cells were activated by the addition of Human T-Activator CD3/CD28 Dynabeads (Life Technologies).

Red blood cell lysis was performed, and CD8 T cells were isolated using negative selection with Biotin Binder Dynabeads following staining of 5 x 10^7 cells with biotinylated antibodies (Supplemental Table 1). The remaining cells were cultured in DMEM (Mediatech) supplemented with 20% fetal bovine serum, glutamine, non-essential amino acids, sodium pyruvate, HEPES, antibiotics, 2-mercaptoethanol, and 20ng/mL recombinant murine IL-2 (Peprotech). Cells were activated by the addition of Mouse T-Activator CD3/CD28 Dynabeads (Life Technologies).

Histology

Tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4 µm, and mounted on positively charged glass slides (Thermo Fisher Scientific). Slides were dried at 60°C for 20 min before dewaxing and staining with hematoxylin and eosin (H&E) using standard methods. H&E-stained sections were examined by a pathologist blinded to the experimental group assignments. Immunohistochemical staining was performed using the antibodies listed in Supplemental Table 1. For detection of Ly6B.2, the primary antibody concentration was 1:500, with heat-induced epitope retrieval using Epitope Retrieval solution 1 (ER1) for 20 minutes, incubation of the primary antibody for 60 minutes, and visualization with

rabbit anti-rat (BA-4001; Vector Laboratories) and the Bond Polymer Refine Detection kit (DS9800, Leica Biosystems). For detection of CD3, the primary antibody concentration was 1:1000, with heat-induced epitope retrieval using Cell conditioning media 2 (Ventana Medical Systems, Tucson, AZ) for 32 minutes followed antibody incubation for 60 minutes and visualization with DISCOVERY OmniMap anti-Rb HRP (760-4311; Ventana Medical Systems) and the DISCOVERY ChromoMap DAB kit (760-159; Ventana Medical Systems). After pathology analysis, IHC slides were digitized to 20x scalable whole slide images using the Aperio ScanScope XT (Leica Biosystems). ImageScope software and a modified version of the Aperio Color Deconvolution algorithm were used to assess the total percentage area of immunoreactivity for each IHC marker.

Supplemental Table 1

Antibody	Clone	Company	Target Species	Application
CD4	A161A1	BioLegend	Human	T Cell Isolation
CD19	HIB19	BioLegend	Human	T Cell Isolation
CD11b	ICRF44	BioLegend/ Invitrogen	Human	T Cell Isolation/ Flow Cytometry
CD24	ML5	BioLegend	Human	T Cell Isolation
B220	RA3-6B2	BioLegend	Human/ Mouse	T Cell Isolation
CD4	GK1.5	BioLegend	Mouse	T Cell Isolation/ Flow Cytometry
CD19	6D5	BioLegend	Mouse	T Cell Isolation
CD11b	M1/70	BioLegend	Mouse	T Cell Isolation
CD24	M1/69	BioLegend	Mouse	T Cell Isolation
TCRβ	H57-597	TONBO Biosciences	Mouse	Flow Cytometry
F4/80	BM8.1	TONBO Biosciences	Mouse	Flow Cytometry
NK1.1	PK136	TONBO Biosciences	Mouse	Flow Cytometry
Ly6C	HK1.4	Invitrogen	Mouse	Flow Cytometry
CD11c	N418	Invitrogen	Mouse	Flow Cytometry
CD8	53-6.7	Invitrogen	Mouse	Flow Cytometry
CD19	1D3	TONBO Biosciences	Mouse	Flow Cytometry
Ly6G	1A8	BioLegend	Mouse	Flow Cytometry
CD44	IM7	TONBO Biosciences	Mouse	Flow Cytometry
CD62L	MEL-14	TONBO Biosciences	Mouse	Flow Cytometry
CD25	PC61.5.3	BD Biosciences	Mouse	Flow Cytometry
STAT1 pY701		BD Biosciences	Human/ Mouse	Flow Cytometry
STAT3 pY705		BD Biosciences	Human/ Mouse	Flow Cytometry
STAT5 pY694		BD Biosciences/Cell Signaling Technology	Human/ Mouse	Flow Cytometry/ Western Blotting
STAT5	D2O6Y	Cell Signaling Technology	Mouse	Western Blotting

Cleaved Caspase 3	C92-605	BD Biosciences	Human/ Mouse	Flow Cytometry
BCL-2	BCL/10C4	BioLegend	Mouse	Flow Cytometry
BCL-xL	54H6	Cell Signaling Technology	Mouse	Flow Cytometry
MCL-1	D2W9E	Cell Signaling Technology	Mouse	Flow Cytometry
Ly6B.2	7/4	Novus Biologics	Mouse	Immunohistochemistry
CD3ε	M-20	Santa Cruz Biotechnology	Mouse	Immunohistochemistry
GR pS211		Cell Signaling Technology	Mouse	Western Blotting
GR	D6H2L	Cell Signaling Technology	Mouse	Western Blotting
β-actin	8H10D10	Cell Signaling Technology	Mouse	Western Blotting
P84		Genetex	Mouse	Western Blotting
Cytochrome c	6H2.B4	BioLegend	Human/ Mouse	Flow Cytometry

Supplemental Figures

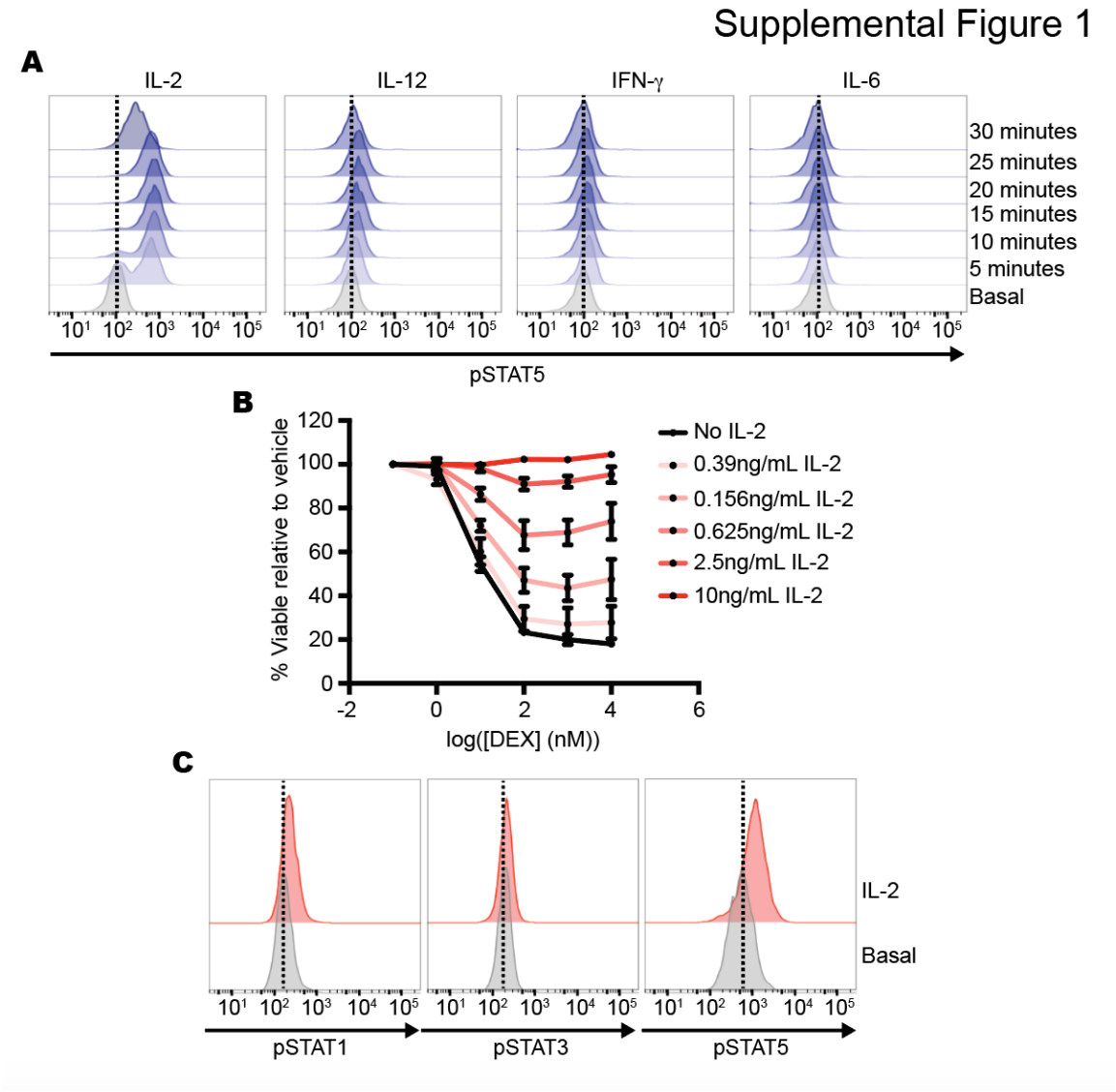

Fig. S1: STAT5-activating cytokines confer DEX resistance in murine CD8 T-cells. (A) Representative histograms of pSTAT5 in murine CD8 T cells following stimulation with 10ng/mL of the indicated cytokine for the indicated period of time. **(B)** Viability relative to vehicle control of murine CD8 T cells treated with increasing concentrations of DEX in the presence or absence of the indicated concentration of IL-2 for 24 hours in technical triplicate. **(C)** Representative histograms of pSTAT1, pSTAT3, and pSTAT5 in murine CD8 T cells in the basal state or

following stimulation with 10ng/mL IL-2 for 15 minutes. All data are representative of three independent experiments.

Supplemental Figure 2

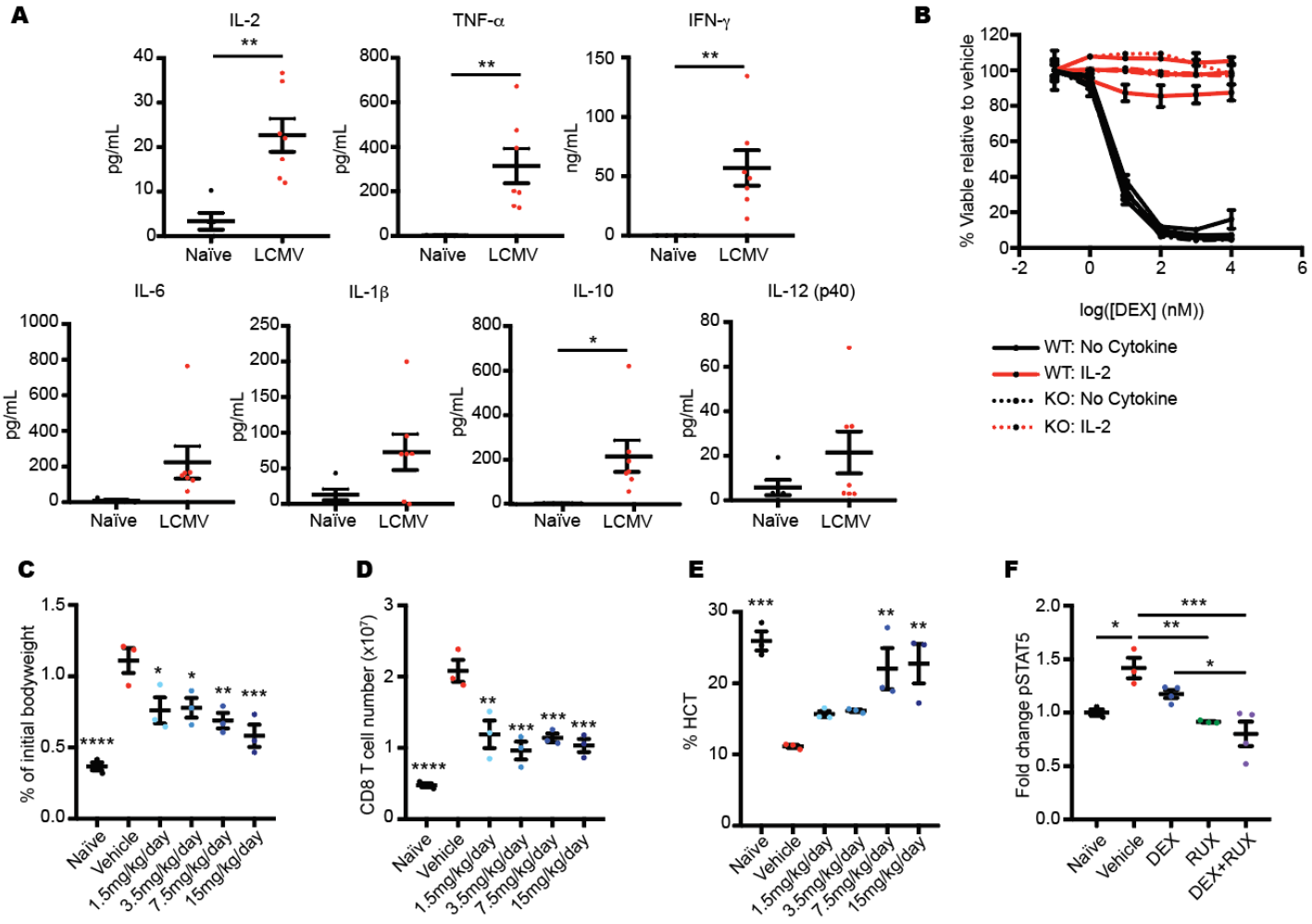

Fig. S2: HLH-like disease in *Prf1*$^{-/-}$ mice recapitulates key features of human HLH. (A) Levels of HLH-associated plasma proteins in plasma from naïve mice or LCMV-infected vehicle-treated mice on day 9 post-infection. **(B)** Viability relative to vehicle control of WT or *Prf1*$^{-/-}$ murine CD8 T cells treated with increasing concentrations of DEX in the absence or presence of 10ng/mL IL-2 for 24 hours in technical triplicate. **(C)** Bodyweight on day 9 post-infection relative to baseline bodyweight in naïve and vehicle- or DEX-treated mice. Statistical significance is relative to the vehicle-treated condition. **(D)** Number of CD8 T cells in spleens from naïve and vehicle- and drug-treated LCMV-infected mice on day 9 post-infection. Statistical significance is

relative to the vehicle-treated condition. **(E)** Hematocrit values in naïve mice and vehicle- and drug-treated LCMV-infected mice on day 9 post-infection. **(F)** Fold change in pSTAT5 in CD8 T cells from vehicle- and drug-treated LCMV-infected mice relative to CD8 T cells from naïve mice. Statistical significance is relative to the vehicle-treated condition. Statistical significance was assessed using two-sample t-tests (A) or one-way ANOVA with Tukey's method for multiple comparisons adjustment (C-F). ****p<0.0001, ***p<0.001, **p<0.01, *p<0.05.

Supplemental Figure 3

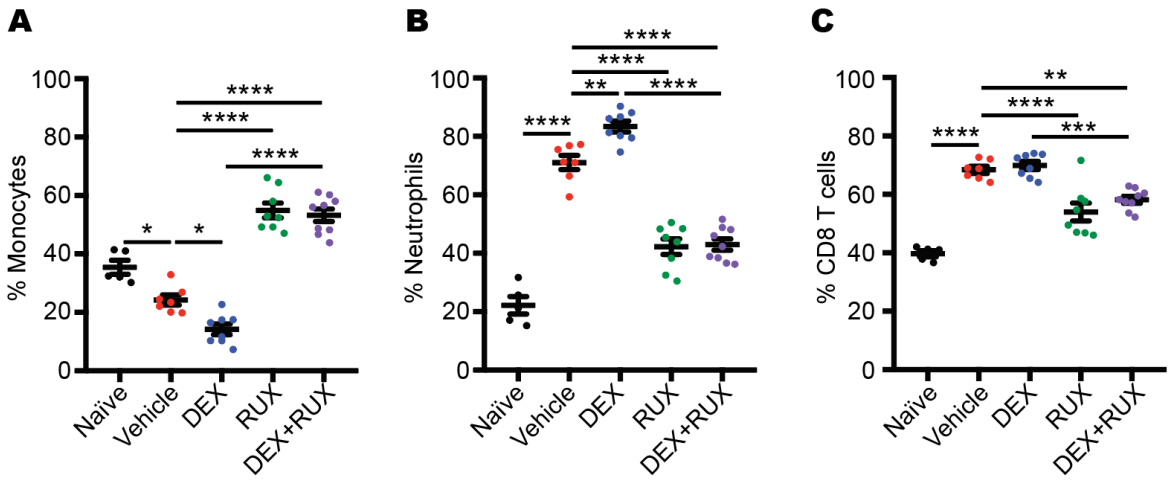

Fig. S3: Percentages of cell populations in vehicle- and drug-treated mice. (A-C) Percentages of monocytes **(A)**, neutrophils **(B)**, and CD8 T cells **(C)** in spleens from naïve and vehicle- and drug-treated LCMV-infected mice on day 9 post-infection. Statistical significance was assessed using one-way ANOVA with Tukey's method for multiple comparisons adjustment. ****p<0.0001, ***p<0.001, **p<0.01, *p<0.05.