Supplementary Information:

Inflammatory cell death, PANoptosis, screen identifies host factors in coronavirus innate immune response as therapeutic targets

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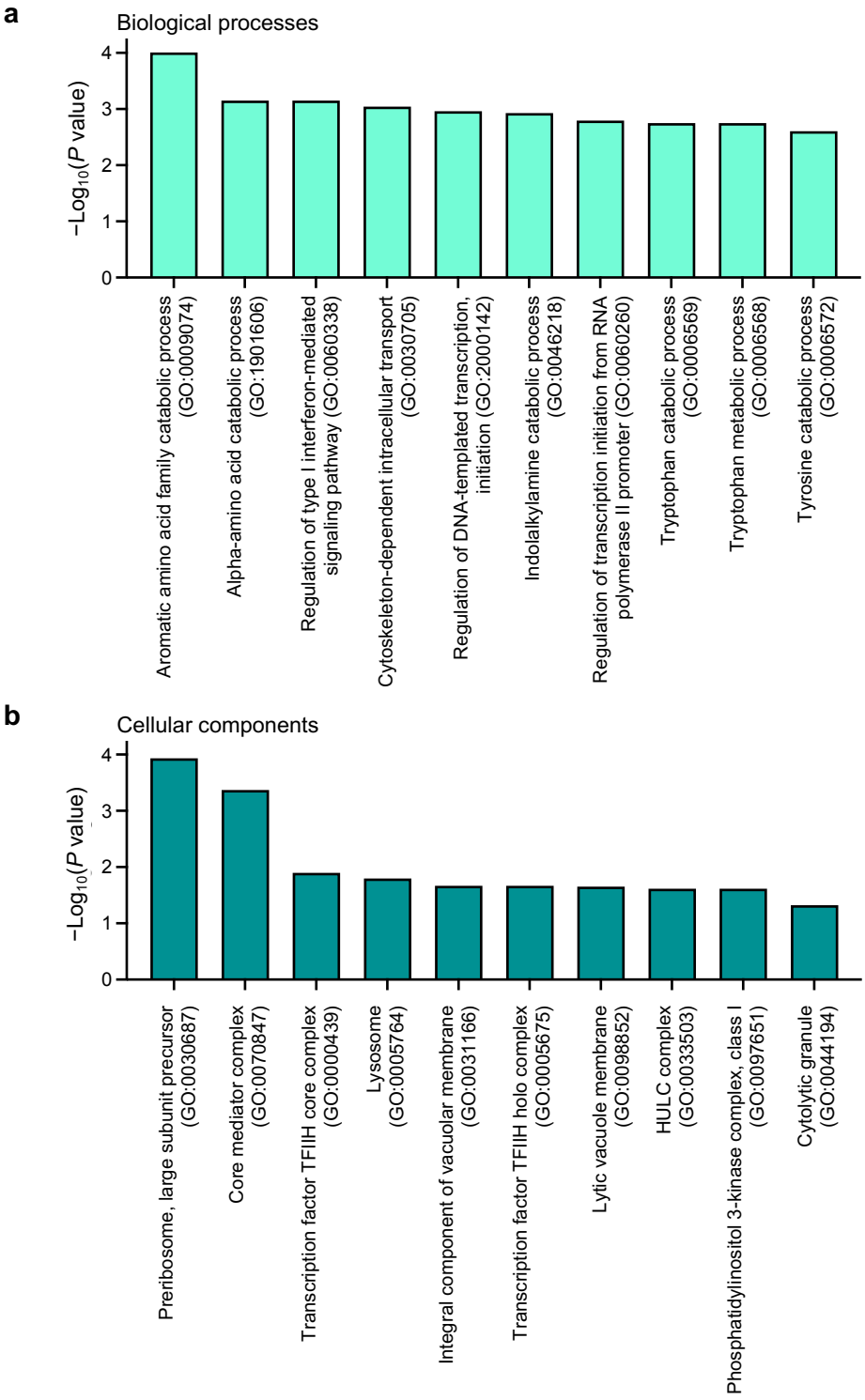
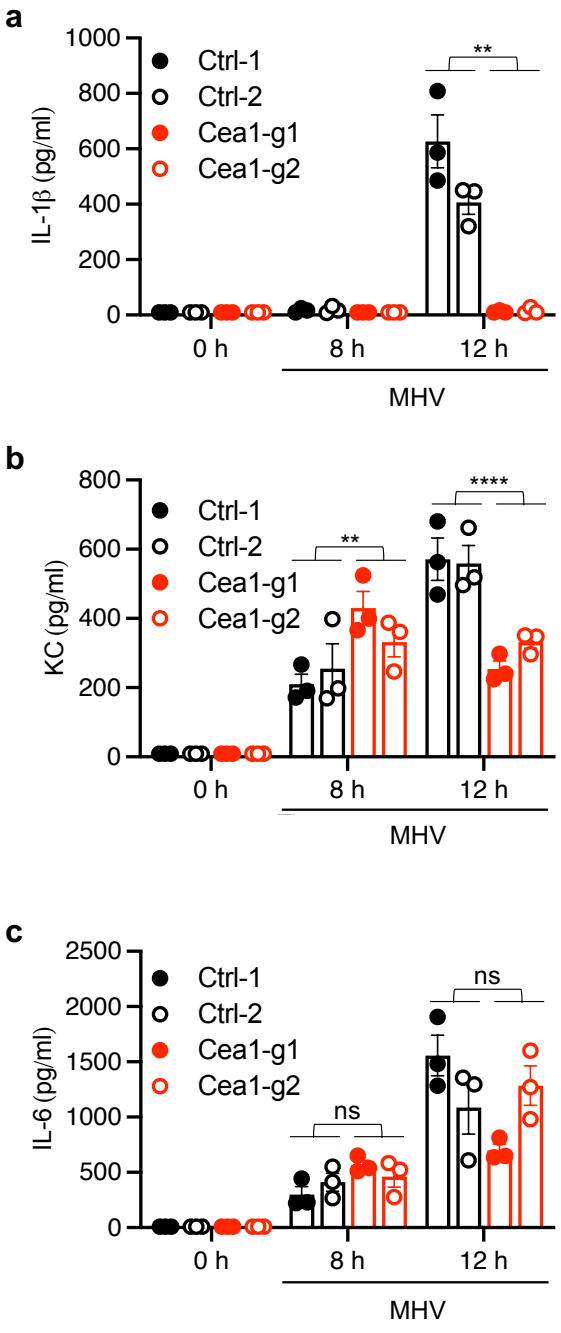
Contents:

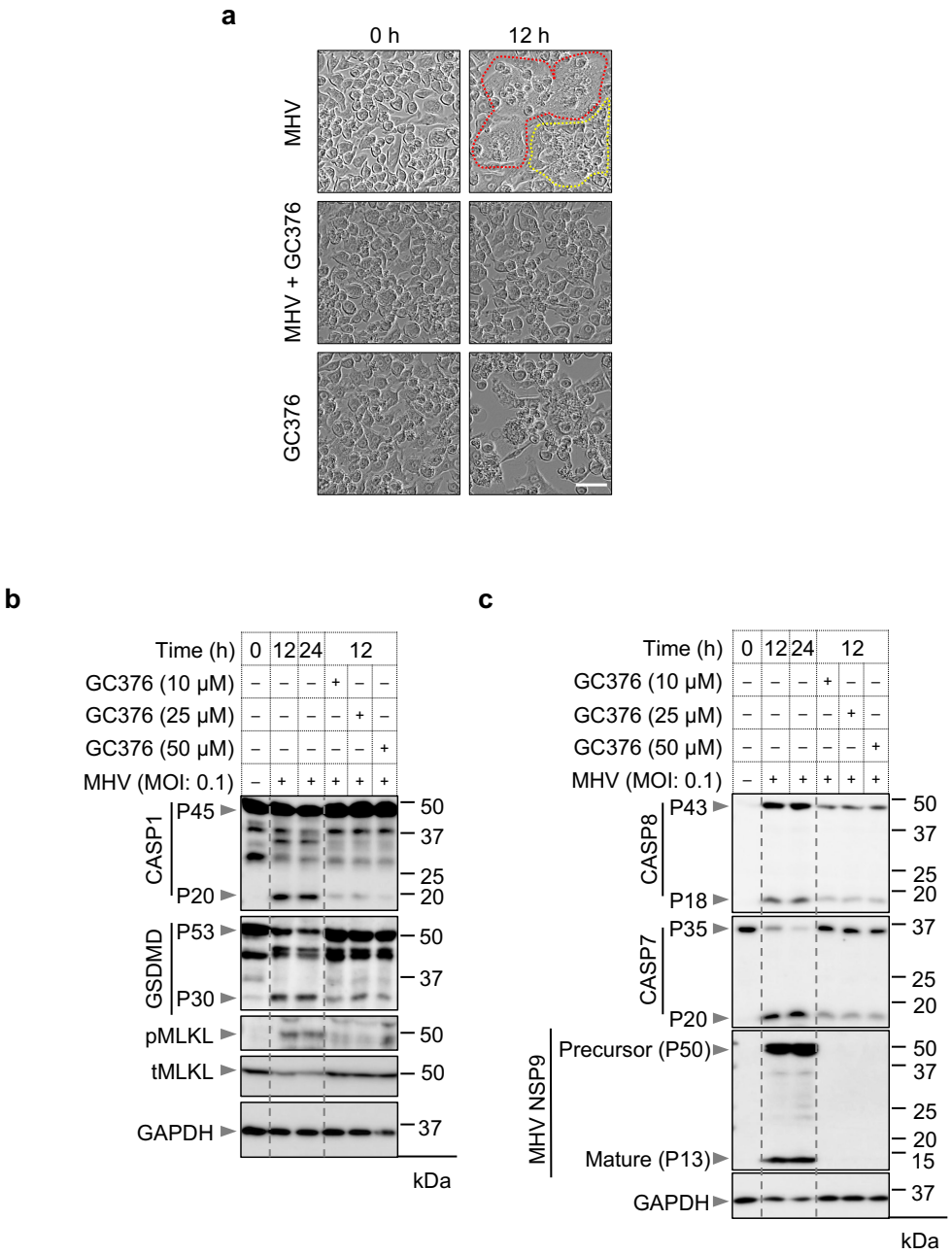


Supplementary Figure 1: GO term analysis identifies key biological processes and cellular components for MHV-mediated cell death.

a, b) The GO term analyses of biological processes (**a**) and cellular components (**b**) of the gene sets that were significantly enriched among the gRNAs in the mouse hepatitis virus (MHV) whole genome CRISPR screen using the Enrichr web-based tool (https://maayanlab.cloud/Enrichr/). The *P* values, **P* < 0.05, are considered statistically significant and included in the graphs.



Supplementary Figure 2: Loss of *Ceacam1* blocks MHV-mediated IL-1β and KC release. **a–c)** Cytokine release from mouse hepatitis virus (MHV; MOI 0.1)-infected immortalized bone marrow-derived macrophages (iBMDMs) with and without *Ceacam1* gRNA treatment with two different guides (Cea1-g1 and Cea1-g2) at the indicated timepoints. Data are shown as mean ± SEM and are presented from three biological replicates (a–c). Analysis was performed using the Student's t-test; ns, not significant, $^{**}P < 0.01$, $^{****}P < 0.0001$. Ctrl: Control with no gRNA.



Supplementary Figure 3: The viral replication inhibitor GC376 blocks MHV-induced PANoptosis.

a) Cell death analysis in mouse hepatitis virus (MHV; MOI 0.1)-infected immortalized bone marrow-derived macrophages (iBMDMs) with or without treatment with GC376, an inhibitor of viral replication that blocks the activity of the β-CoV main protease (M^{pro}). The yellow dotted line denotes syncytia, and the red dotted line denotes ballooning and dying syncytia. **b–c**) Immunoblot analysis of pro- (P45) and cleaved caspase-1 (P20; CASP1), pro- (P53) and activated (P30) gasdermin D (GSDMD), and phospho- (pMLKL) and total MLKL (tMLKL) (**b**); and cleaved caspase-8 (p43 and P18; CASP8), pro- (P35) and cleaved caspase-7 (P20; CASP7), and non-structural protein 9 (NSP9) precursor (P50 and P37) and mature forms (P13) in iBMDMs following the indicated treatments (**c**). GAPDH immunoblots were used as internal controls. The data presented are representative of two independent experiments (a–c). The scale bar is representative of 50 μm.

Fig. 3a: CASP1

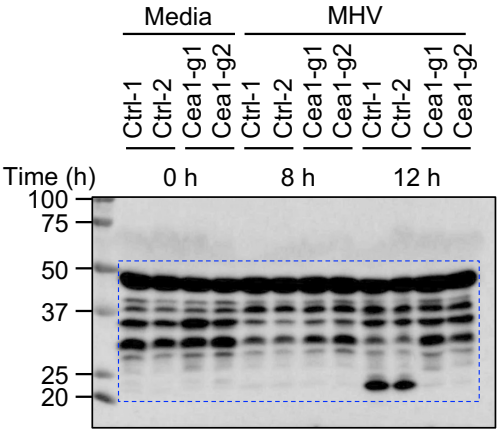


Fig. 3a: GSDMD

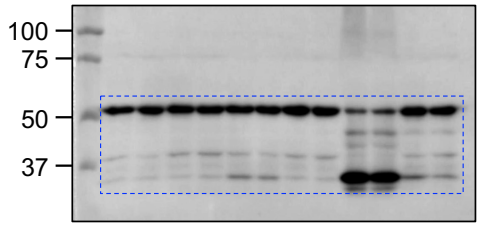


Fig. 3a: GSDME

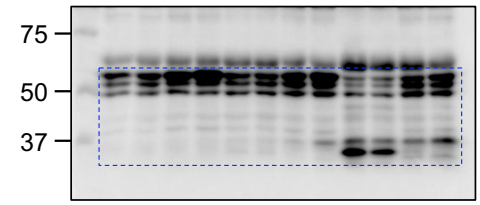


Fig. 3a: GAPDH

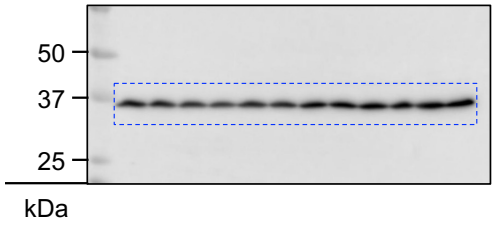


Fig. 3b: CASP8

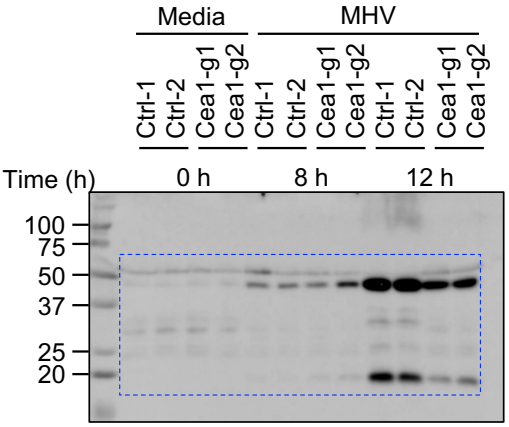


Fig. 3b: CASP7

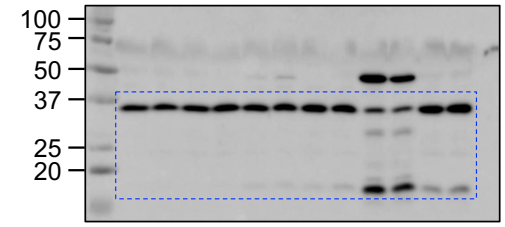


Fig. 3b: GAPDH

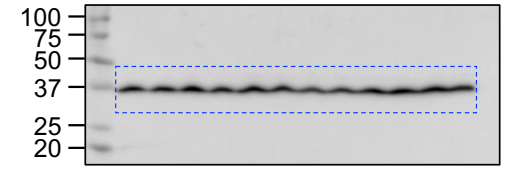


Fig. 3c: pMLKL

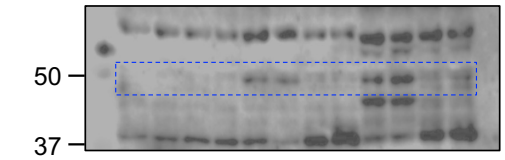


Fig. 3c: tMLKL

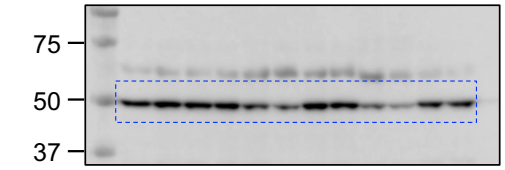
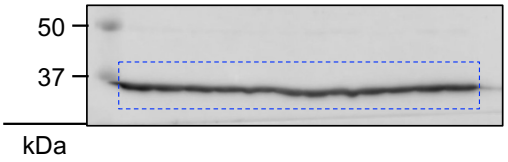


Fig. 3c: GAPDH



Supplementary Figure 4: Uncropped western blots for Figure 3.

Fig. 4c: MHV NSP9

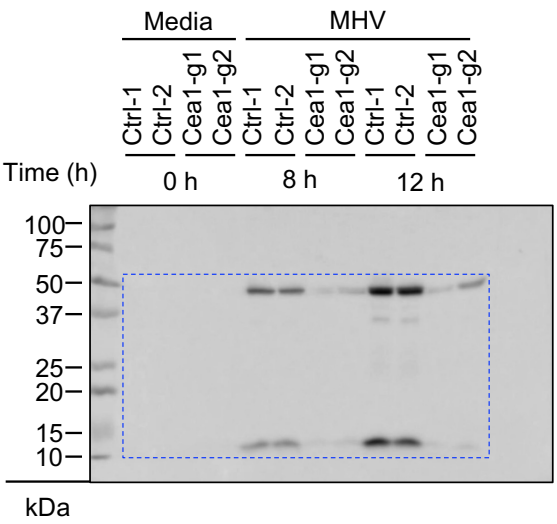
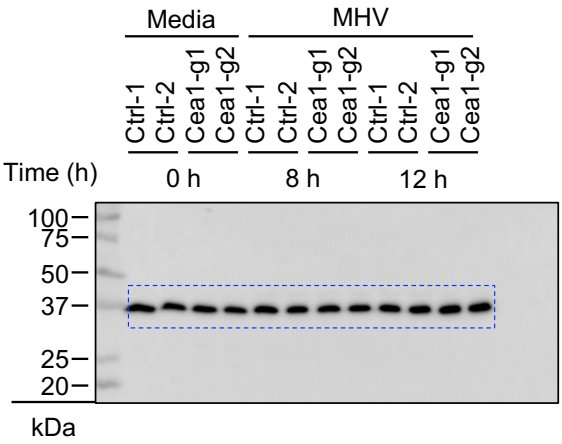


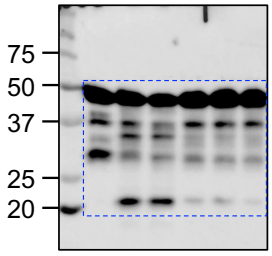
Fig. 4c: GAPDH



Supplementary Figure 5: Uncropped western blots for Figure 4.

Suppl. Fig. 3a: CASP1

Time (h)	0	12	24	12		
GC376 (10 µM)	–	–	–	+	–	–
GC376 (25 µM)	–	–	–	–	+	–
GC376 (50 µM)	–	–	–	–	–	+
MHV (MOI: 0.1)	–	+	+	+	+	+



Suppl. Fig. 3b: CASP8

Time (h)	0	12	24	12		
GC376 (10 µM)	–	–	–	+	–	–
GC376 (25 µM)	–	–	–	–	+	–
GC376 (50 µM)	–	–	–	–	–	+
MHV (MOI: 0.1)	–	+	+	+	+	+

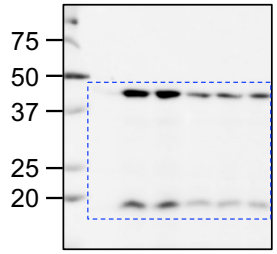


Fig. S3a: GSDMD

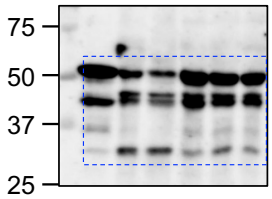


Fig. S3b: CASP7

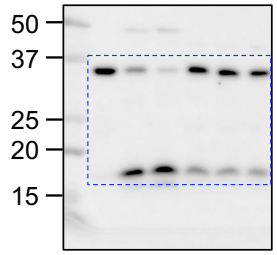


Fig. S3a: pMLKL

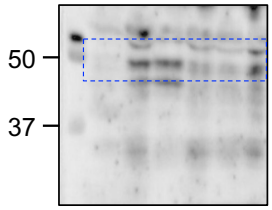


Fig. S3b: MHV NSP9

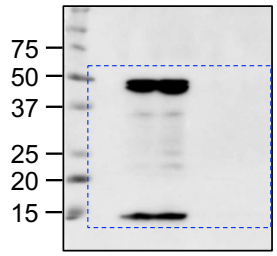
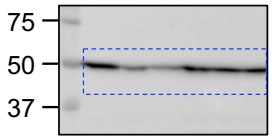


Fig. S3a: tMLKL



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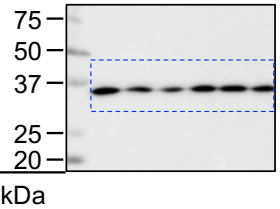
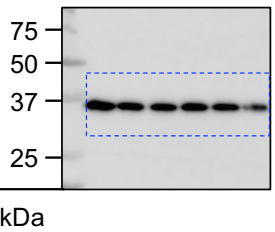


Fig. S3a: GAPDH



Supplementary Figure 6: Uncropped western blots for Supplementary Figure 3.