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Supplemental information

Preclinical characterization of the Toll-like receptor 7/8 antagonist MHV370 for lupus therapy

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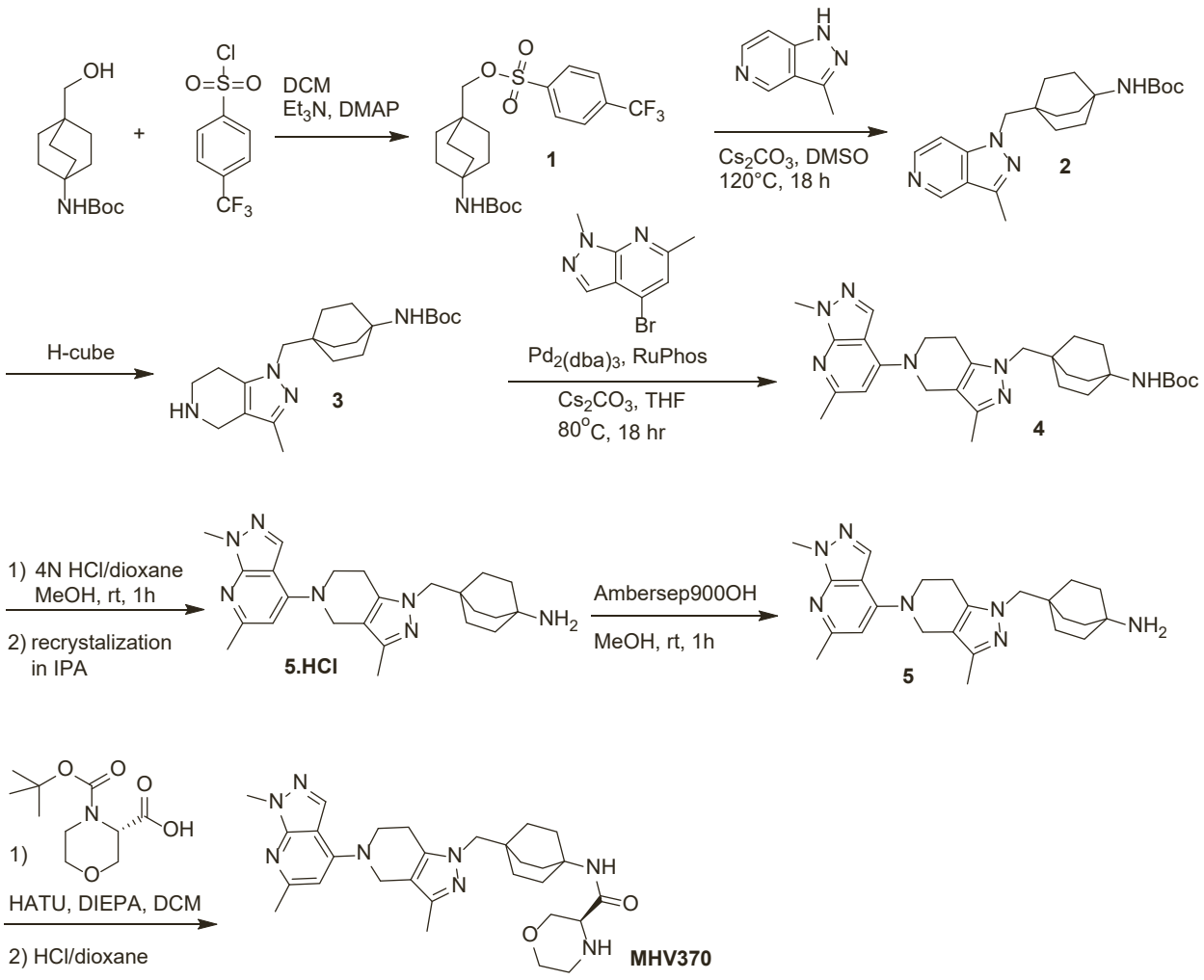
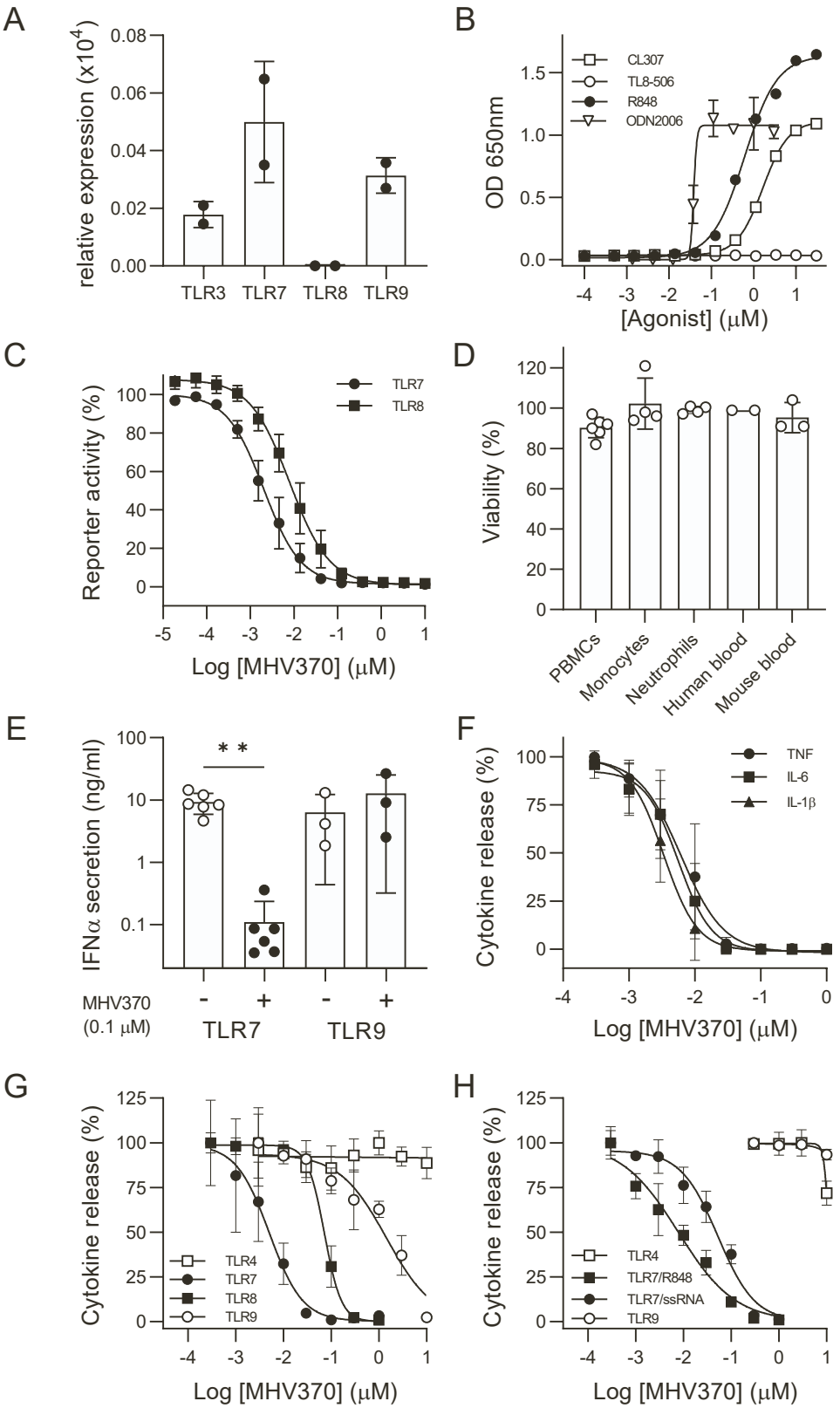
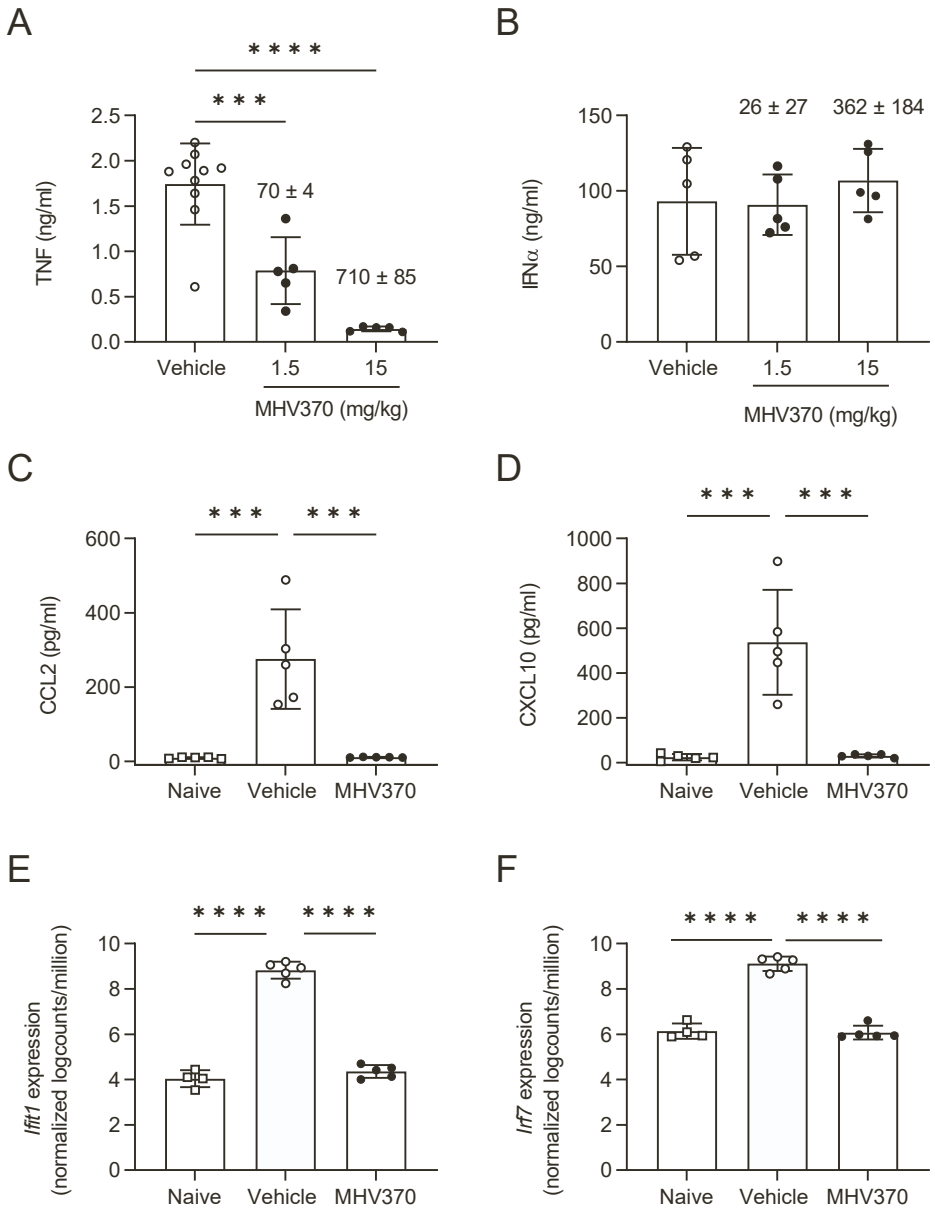


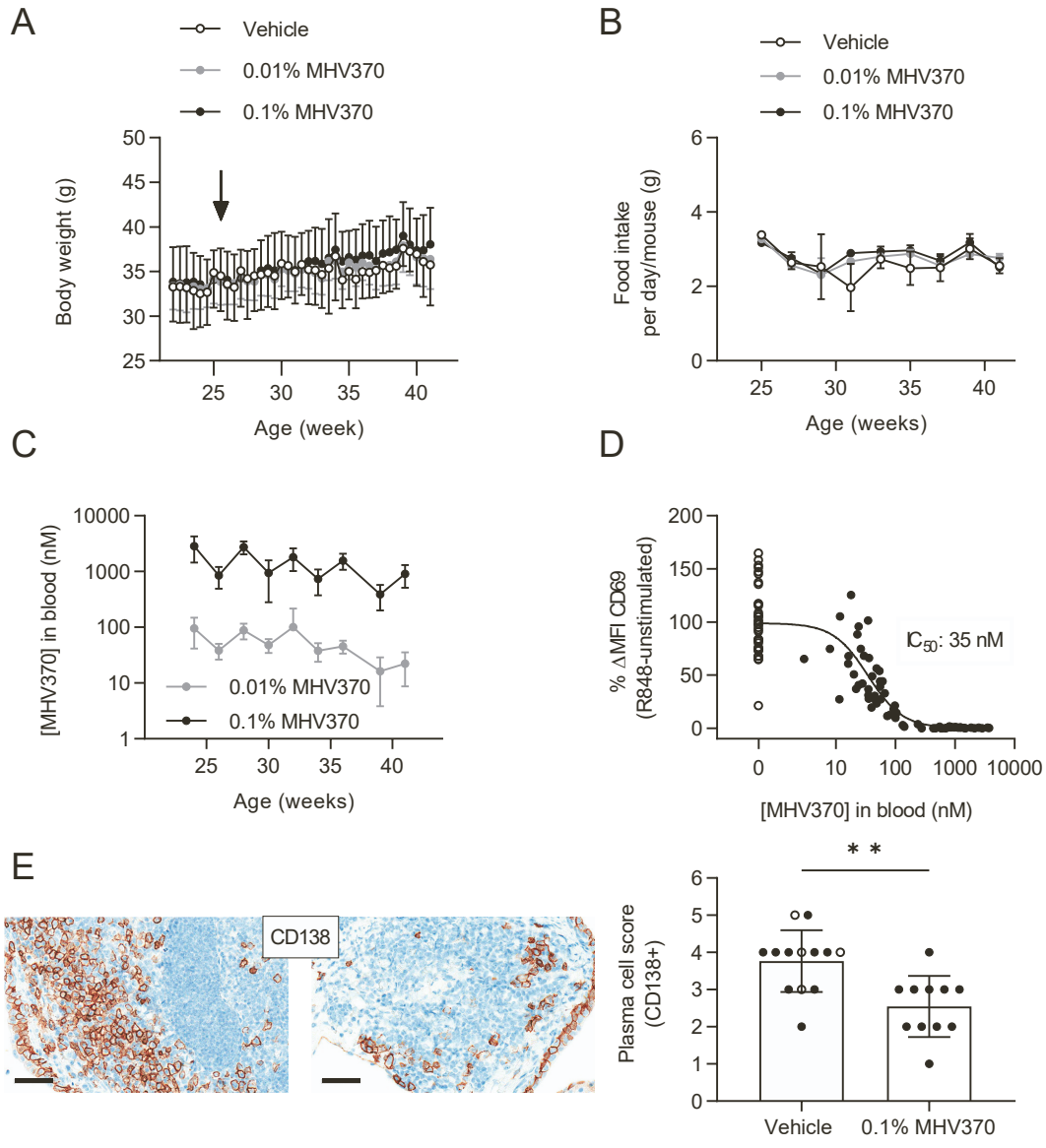
Supplementary Figure 1: Synthesis scheme of MHV370, related to STAR methods, related to Figure 1 and STAR methods. Description of synthesis steps in the STAR method section.


A
relative expression (x10^4)
0.08
0.06
0.04
0.02
0.00
TLR3
TLR7
TLR8
TLR9
B
OD 650nm
CL307
TL8-506
R848
ODN2006
[Agonist] (μM)
C
Reporter activity (%)
TLR7
TLR8
Log [MHV370] (μM)
D
Viability (%)
PBMCs
Monocytes
Neutrophils
Human blood
Mouse blood
E
IFNα secretion (ng/ml)
* *
MHV370
(0.1 μM)
TLR7
TLR9
F
Cytokine release (%)
TNF
IL-6
IL-1β
Log [MHV370] (μM)
G
Cytokine release (%)
TLR4
TLR7
TLR8
TLR9
Log [MHV370] (μM)
H
Cytokine release (%)
TLR4
TLR7/R848
TLR7/ssRNA
TLR9
Log [MHV370] (μM)

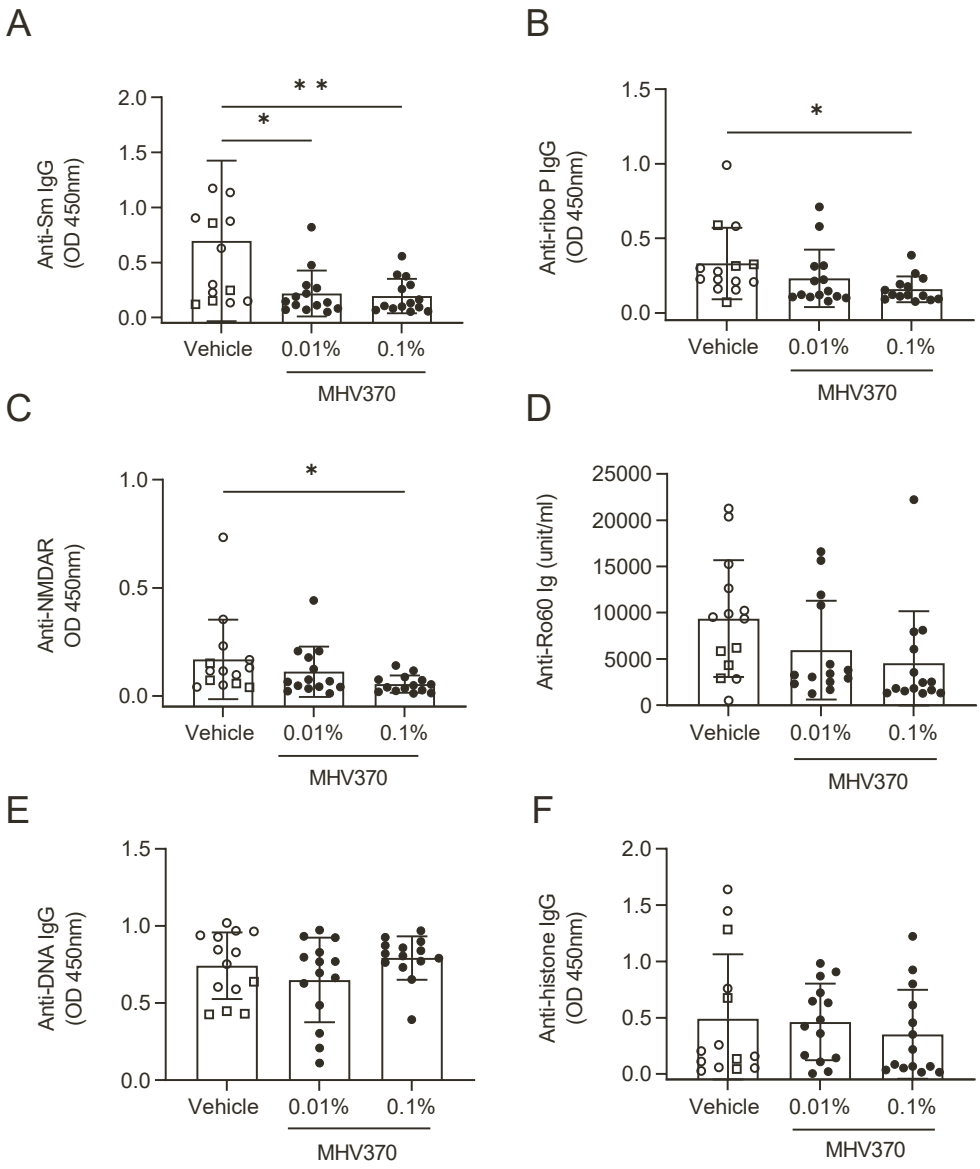
Supplementary Figure 2: MHV370 inhibits TLR responses in human and murine cells, related to Figure 1. **(A)** Expression of selected TLRs in Ramos B cells, two independent experiments. Means ± SD. **(B)** Reporter gene activity in Ramos B cells stimulated with agonists for TLR7 (CL307), TLR8 (TL8-506), TLR7/8 (R848) or TLR9 (ODN2006), means ± SD, representative of n=2-4 experiments. **(C)** Reporter gene activity from HEK cells transfected with TLR7 (circles) or TLR8 (squares), following R848-stimulation, means of six experiments ± SD. **(D)** Cell viability in the presence of 10 μM MHV370 from each human cell type or mouse blood. Data points are means of triplicate measurements and normalized to cell viability without MHV370, bars represent means 2-6 donors ± SD.**(E)** IFNα (TLR7/ssRNA, TLR9/ODN2216 driven) release from isolated human pDCs. Data points are means of duplicate measurements, bars represent means of six (TLR7) or three (TLR9) donors ± SD. **(F)** Cytokine release from isolated human monocytes following TLR8/TL8-506-stimulation. Data points are means of triplicate measurements ± SD of 6 donors. **(G)** TLR agonist driven IFNα (TLR7/ssRNA, TLR9/ODN2216) or TNF (TLR4/LPS, TLR8/ssRNA) release from human PBMCs. Data points are means of triplicate measurements ± SD. **(H)** TLR agonist driven IFαN (TLR7/ssRNA, TLR9/ODN1585) or TNF (TLR7/R848, TLR4/LPS) release from murine blood. Data points are means of duplicate measurements ± SD. Panels F-H, are normalized to release without MHV370 and for Panel G, H show one representative of n experiments from individual donors (for n, see Table 1). **, $p<0.01$, ***, $p<0.001$ one-way ANOVA with Šídák's comparison test, comparing MHV370 groups to vehicle group.



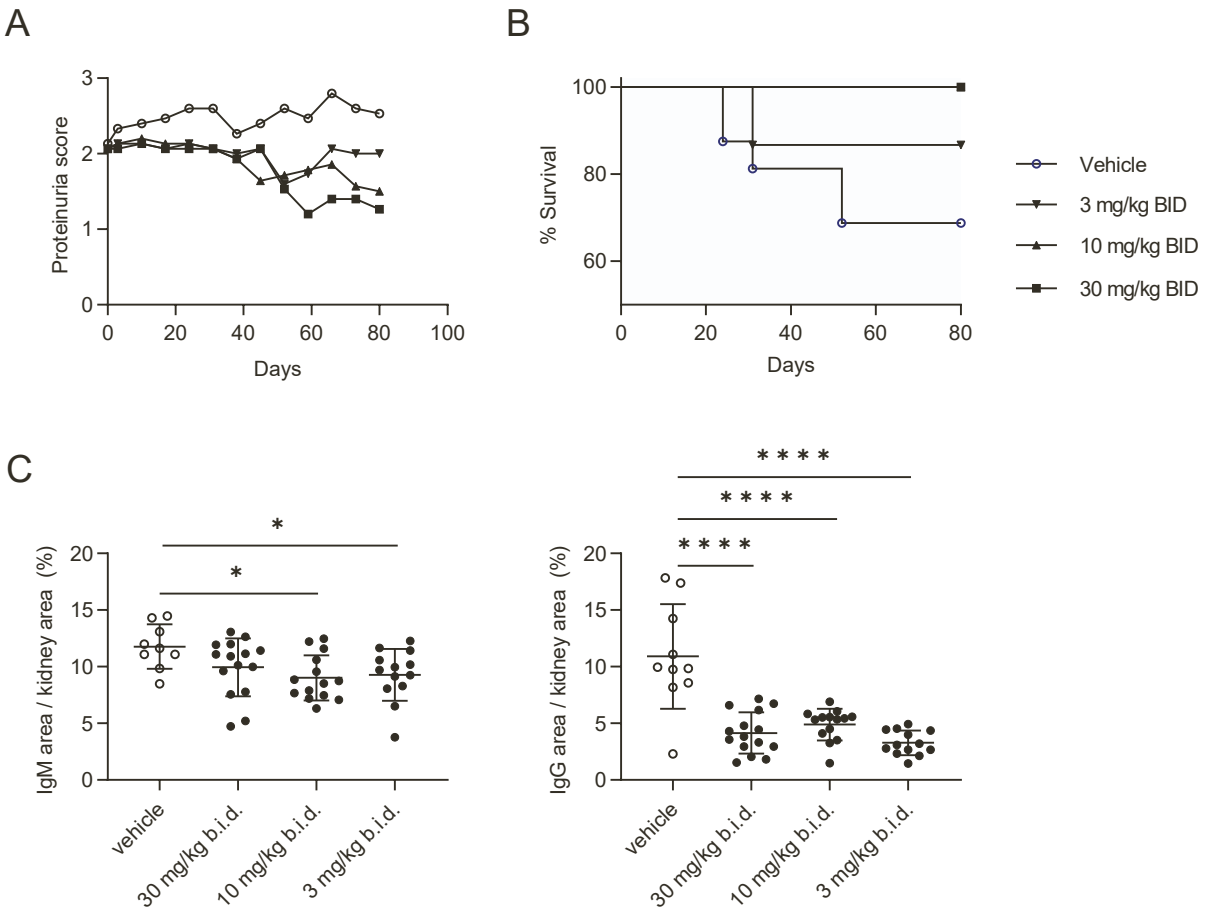
Supplementary Figure 3: MHV370 inhibits acute and chronic TLR7 responses in mice. Related to Figures 2, 3. (A) TLR7/ssRNA-induced TNF and **(B)** TLR9/ODN1585-induced IFNα in mouse plasma following MHV370 p.o. or vehicle treatment. Mean MHV370 blood exposures are indicated above the bars (as nM ± SD), symbols show individual mice, bars show means ± SD. **(C, D)** Cytokine expression on day 14 after R848 treatment q.d. with vehicle or MHV370 treatment (5 mg/kg b.i.d.) and (**E,F)** expression of specific ISGs in blood on day 14 after R848 treatment q.d. and following 7 d therapeutic treatment with 15 mg/kg MHV370 b.i.d or vehicle, triplicate measurements. Data points in A-F represent individual mice with means ± SD. **, $p<0.01$, ***, $p<0.001$; ****, $p<0.0001$, ANOVA with Tukey's post test.



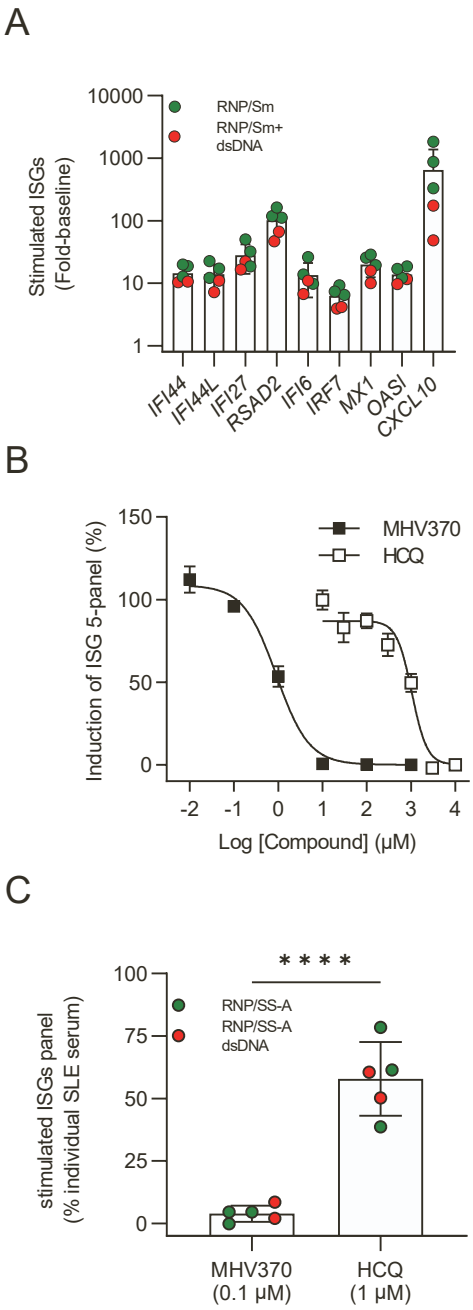
Supplementary Figure 4: Body weight and food intake of MHV370 dosed NZBW/F1 mice, related to Figure 5
(A) Body weight of NZB/W F1 mice treated with MHV370-laced or vehicle food. MHV370 treatment started at 23 weeks of age (arrow). **(B)** Calculated daily food intake per mouse. Calculation based on 3-4 cages per group. Panels A, B show means ± SD of 14 mice per group. All AUC differences were non-significant (one-way ANOVA, Dunnett's post-test). **(C)** MHV370 blood exposure during food dosing. Means ± SD of 12-14 mice. **(D)** *Ex vivo* CD69 expression on B cells (weeks 26, 28 and 41) vs. corresponding MHV370 blood exposures. Data points represent individual mice, open circles represent mice from vehicle group, **(E)** CD138+ plasma cell infiltration in kidneys (left) and plasma cell score (right), scale bars 50 µm, Open circles, vehicle-treated mice which were terminated prematurely as they reached the humane endpoint of the license. Data points represent individual mice, bars represent means ± SD. *p<0.05, **p<0.01, ***p=0.001, ****p<0.0001, Mann-Whitney test.



Supplementary Figure 5: Serum autoantibodies in NZBW F1 mice at termination, related to Figure 5. (A) anti-Smith IgG, **(B)** anti-riboP IgG, **(C)** anti-NMDAR-IgG, **(D)** anti-Ro60 IgG, **(E)** anti-DNA-IgG and **(F)** anti-histone IgG. Data points represent individual mice; open squares represent vehicle-treated mice which were terminated prematurely, as they reached the humane endpoint of the license. Bars represent means ± SD. *p<0.05, **p<0.01; ANOVA with Dunnett's post-test, comparing MHV370-dosed groups to vehicle group.



Supplementary Figure 6: Independent confirmation of *in vivo* efficacy of MHV370 in the NZB/W F1 model of lupus following twice daily dosing, related to Figure 5. (A) Proteinuria score by urine dipstick measurement, data points are means of n=15 mice per group, mice were included at an average proteinuria score of 2 (corresponding to 0.3-1 mg/ml). **(B)** Survival over time, starting at n=15 mice per group. **(C)** Ratio of the area of histological IgM staining (left) or IgG staining (right) on total kidney section area at termination of the experiment, expressed as percent. Data points are individual mice, horizontal bars are means ± SD. *<0.05, **<0.01, ***, p<0.001, ****, p<0.0001; one-way ANOVA with Dunnett's post-test.



Supplementary Figure 7: MHV370 inhibits ISGs, related to Figure 6. (A) Stimulation of individual ISGs from PBMCs using SLE sera and NE. Data points represent mean gene expression from 2-4 PBMC donors stimulated with individual SLE patient sera (n=5) complexed with NE, and two technical replicates per stimulation. **(B)** Expression of a five-gene ISG panel in human PBMCs stimulated with ssRNA **(Table S6)**, in presence of MHV370 (closed symbols) or HCQ (open symbols). Data are normalized to expression levels without compound. Means ± SEM of technical triplicates, one representative of two independent experiments. IC_{50} values for each condition are indicated in **Table S7**. **(C)** Inhibition of a five gene ISG panel (comprising *IFI44*, *IFI44L*, *IFI6*, *RSAD2*, *IFI27*) on immune-complex stimulated PBMCs, in presence of MHV370 or HCQ. Data points are mean ISG panel expression data from 2-4 PBMC donors stimulated with individual patient sera (*n=5*) complexed with NE, and two technical replicates per stimulation. ****, p<0.0001, unpaired t-test. Colours indicate autoantibody profiles of individual SLE patients, bars show means ± SD.

Supplementary Table 1: Human and murine probes for qPCR, related to STAR methods. TaqMan probes were obtained from Thermo Fisher Scientific Cat#4331182.

Assay ID	Gene Symbol	Gene Name
Hs01124252_g1 Mm99999072_m1	*CXCL10* *Cxcl10*	Chemokine (C-X-C motif) ligand 10
Hs00895608_m1 Mm01218004_m1	*MX1* *Mx1*	Myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse)
Hs00984387_m1	*OASL*	2'-5'-oligoadenylate synthetase-like
Hs01014809_g1 Mm00516791_g1	*IRF7* *Irf7*	Interferon regulatory factor 7
Hs00951349_m1 Mm00505670_m1	*IFI44* *Ifi44*	Interferon induced protein 44
Hs00915292_m1 Mm00518988_m1	*IFI44L* *Ifi44L*	Interferon induced protein 44 like
Hs00242571_m1	*IFI6*	Interferon induced protein 6
Hs01086370_m1	*IFI27*	Interferon induced protein 27
Hs00369813_m1 Mm00491265_m1	*RSAD2* *Rsad2*	Radical S-adenosyl methionine domain-containing protein 2
Hs99999901_s1	*18S rRNA*	Ribosomal RNA
Mm00446968_m1	*Hprt*	Hypoxanthine guanine phosphoribosyl transferase
Mm02619580_g1	*Actx*	Beta actin
Hs00152933_m1	*TLR3*	Toll like receptor 3
Hs00152971_m1	*TLR7*	Toll like receptor 7
Hs00152972_m1	*TLR8*	Toll like receptor 8
Hs00152973_m1	*TLR9*	Toll like receptor 9

Supplementary Table 2: MHV370 inhibition of TLR7/8 driven cytokines in human monocytes, related to Figure 1. IC_{50} values (nM) shown are the mean ± SEM of (*n*) separate experiments or donors with each dataset performed at least in triplicate.

Cytokine	TLR7/8 (ssRNA)	TLR7/8 (R848)	TLR8 (TL8-506)
TNF	70 ± 15 (4)	54 ± 11 (6)	6.7 ± 2.3 (6)
IL-6	99 ± 21 (4)	67 ± 13 (6)	6.1 ± 1.6 (6)
IL-1β	26 ± 5.0 (4)	32 ± 5.9 (6)	3.4 ± 1.1 (6)

Supplementary Table 3: MHV370 inhibition of TLR7/8 driven cytokines in human PBMCs, related to Figure 1. IC_{50} values (nM) shown are the mean ± SEM of (*n*) separate experiments or donors with each dataset performed at least in triplicate. n.s= no stimulated IFNα release detectable, n.d.=not determined

Cytokine	TLR7/8 (ssRNA)	TLR7/8 (R848)	TLR8 (TL8-506)
IFNα	4.1 ± 0.4 (39)	n.s	n.s
CXCL10	45 ± 13 (4)	76 ± 5.9 (2)	4.6 ± 1.9 (4)
TNF	70 ± 7.4 (37)	21 ± 3.4 (16)	18 ± 2.3 (6)
IL-6	80 ± 8.0 (16)	16 ± 2.6 (12)	11 ± 1.5 (4)
IL-1β	40 ± 7.8 (8)	13 ± 3.5 (7)	7.6 ± 1.1 (4)
IL-12	7.0 ± 1.1 (4)	n.d	n.d

Supplementary Table 4: MHV370 inhibition of TLR7 driven B cell markers, related to Figure 1. IC_{50} values (nM) shown are the mean ± SEM of (*n*) separate experiments or donors with each dataset performed in duplicate.

Inhibition of B cell markers	
IL-6	0.7 ± 0.2 (2)
pAkt	5.0 ± 0.5 (2)
CD69	4.7 ± 0.2 (4)
IgM	1.1 ± 0.2 (2)

Supplementary Table 5: Autoantibody titers of SLE patients, related to Figure 6. Titer (IgG) levels of SLE patient sera assessed against a panel of autoantigens as described in the Methods. Titer levels >100 IU/ml (dsDNA) and >1 index (RNP/Sm, RNP70, Sm, SS-A, SS-B, Scl70, Centromer B, Jo-1) are considered positive and indicated in bold.

	Serum autoantibody IgG (index or IU/ml*)								
Sera ID	**dsDNA***	**RNP/Sm**	**RNP70**	**Sm**	**SS-A**	**SS-B**	**Scl70**	**Centromer B**	**Jo-1**
SLE1	**505**	0.6	**2.7**	**3.0**	0.8	0.1	0.6	0.1	0.2
SLE2	**146**	0.3	0.1	0.2	0.2	0.1	0.6	0.2	0.2
SLE3	8	**3.7**	**3.5**	0.3	0.6	0.1	0.7	0.1	0.2
SLE4	**497**	0.2	0.4	0.2	**2.8**	0.3	0.6	0.1	0.2
SLE5	**156**	0.3	0.6	0.3	0.1	0.1	0.6	0.1	0.2
SLE6	**143**	**2.8**	**2.9**	0.3	0.2	0.2	0.9	0.1	0.2
SLE7	**172**	0.4	**3.3**	**3.4**	**6.3**	0.3	1.0	0.1	0.3
SLE8	26	**1.1**	0.1	0.9	**3.7**	0.8	0.6	0	0.1
SLE9	4	**2.4**	**3.2**	0.1	**1.7**	0.1	0.6	0	0.1
SLE10	**200**	**2.1**	0.7	0.5	**3.4**	3	0.7	0	0.1
SLE11	8	**2.9**	**1.3**	**2.3**	0.7	0.1	0.7	0	0.1
SLE12	**224**	**4.6**	**1.3**	**4**	**2.6**	0.1	0.5	0	0.1
SLE13	**1064**	**2.9**	**2.9**	**2.7**	**1.7**	0.1	0.5	0.2	0.2
SLE14	**128**	**3.4**	**6.0**	0.4	**2.9**	**5.7**	0.5	0.1	0.1
SLE15	80	**3.4**	**2.7**	**2.4**	**1.3**	0.1	0.5	0.0	0.1
SLE16	36	**3.7**	**8.7**	**4.8**	**3.1**	0.6	0.4	0.1	0.1
SLE17	**73**	**0.3**	**2.8**	**2.4**	**2.0**	0.3	0.4	0.1	0.1
SLE18	**1866**	0.4	0.9	0.8	0.3	0.1	0.4	0.1	0.1
SLE19	**400**	0.1	0.4	0.4	0.3	0.1	0.5	0.1	0.1
SLE20	**2017**	0.2	0.3	0.2	0.2	0.0	0.4	0.1	0.1
SLE21	**966**	0.4	0.8	0.1	0.1	0.0	0.4	0.0	0.1

Supplementary Table 6: Inhibition of ssRNA driven IFN transcripts in human PBMCs, related to Figure 6. MHV370 inhibition of specific IFN-related transcripts compared to HCQ. IC_{50} values of MHV370 compared HCQ and percentage inhibition at doses 100 nM and 1000 nM respectively. Data shown are the mean ± SEM. from two individual donors measured in biological triplicates. A 5-ISGs panel comprises of transcript genes of *IFI44*, *IFI44L*, *IFI6*, *IFI27* and *RSAD2*.

	MHV370		**HCQ**	
Transcript	**IC_{50} (nM)**	**% Inhibition**	**IC_{50} (nM)**	**% Inhibition**
IRF7	1.0 ± 0.3	88 ± 2	2800 ± 1200	6 ± 6
MX1	0.8 ± 0.1	96 ± 1	2400 ± 900	6 ± 14
OASL	1.1 ± 0.2	91 ± 0	2500 ± 1000	-7 ± 10
CXCL10	0.7 ± 0.3	99 ± 1	1900 ± 700	14 ± 24
IFI44	1.3 ± 0.1	93 ± 1	5000 ± 2700	4 ± 19
IFI44L	1.6 ± 0.2	95 ± 2	2200 ± 800	14 ± 18
IFI6	0.6 ± 0.3	96 ± 0	2300 ± 900	27 ± 23
IFI27	0.4 ± 0.0	96 ± 1	3700 ± 2200	31 ± 21
RSAD2	0.8 ± 0.1	98 ± 1	3100 ± 1700	17 ± 27
5-ISGs	**1.0 ± 0.2**	**96 ± 1**	**3300 ± 500**	**18 ± 4**

Supplementary Table 7. MHV370 inhibition of pathway responses in SLE patients, related to Figure 6. IC_{50} values (nM) shown are the mean ± SEM of (n) separate experiments or donors with each dataset performed in triplicate.

		MHV370 inhibition (IC_{50}, nM)	
Pathway	**Response**	**HV**	**SLE**
TLR7	pAKT	19 ± 3.0 (5)	17 ± 7.0 (4)
	CD69	7.0 ± 2.0 (5)	3.0 ± 0.3 (4)
	IFNα	3.0 ± 1.0 (4)	8.0 ± 6.0 (3)
TLR8	TNF	12 ± 3.0 (5)	17 ± 10 (5)