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Supplementary Materials for

Impact of mRNA chemistry and manufacturing process on innate immune activation

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This PDF file includes:

Supplemental Figures and Tables

Table S1-S4. Detailed gene lists, as shown from top to bottom for heat maps Figure 4E (Table S1), Figure 6 and Supplemental Figure S3A (Table S2), Supplemental Figure S3B (Table S3), and Supplemental Figure S3C (Table S4). The genes of the heat maps were grouped by hierarchical clustering using Pearson's correlation between genes as similarity/distance measure and average.

Table S5. Two-way ANOVA results for Figure 5A. The table represents the results from a 2-way ANOVA with Sidak's multiple comparisons post-test to compare WT to MAVS$^{-/-}$ mice, and Tukey's multiple comparisons post-test to compare treatment groups within a mouse strain from the serum hEPO quantitation data presented in Figure 5A.

Table S6. Two-way ANOVA results for Figure 5B. The table represents the results from a 2-way ANOVA with Sidak's multiple comparisons post-test to compare WT to MAVS$^{-/-}$ mice, and Tukey's multiple comparisons post-test to compare treatment groups within a mouse strain from the serum IFNα quantitation data presented in Figure 5B.

Table S7. Two-way ANOVA results for Figure 5C. The table represents the results from a 2-way ANOVA with Sidak's multiple comparisons post-test to compare WT to MAVS$^{-/-}$ mice, and Tukey's multiple comparisons post-test to compare treatment groups within a mouse strain from the activated B cells in the spleen data presented in Figure 5C.

Figure S1. NF-κB promoter activation in THP1-Dual, and A549-Dual cells. NF-κB promoter activation associated with mRNA transfected in THP1-Dual and A549-Dual cells, or TLR 7/8 (R848 and TL8506), RIG-I (5'ppp-dsRNA and 5'ppp-hpRNA) and STING (2'3'-cGAMP) agonists into **(A)** THP1-Dual cells, **(B)** MAVS$^{-/-}$ THP1-Dual cells, **(C)** MyD88$^{-/-}$ THP1-Dual cells, **(D)** A549-Dual cells, and **(E)** RIG-I$^{-/-}$ A549-Dual cells was

examined. NF-κB promoter activity **(A-E)** was measured through secreted alkaline phosphatase (SEAP) activity by a color-change substrate and reported as OD reading at 365nm. Data were from a single experiment, with n = 2-3 technical replicates.

Figure S2. Immune activation from hEPO mRNA incorporating uridine or 1mΨ, made by Process A or B; or poly(U) 19mer, poly(1mΨ) 19mer, 5'ppp-hpRNA, R848 and poly(I:C) in B6129SF2/J control or MAVS$^{-/-}$ mice (5 mice/group) at 6 hours post-injection. The level of **(A)** CCL4 and **(B)** CCL5 in serum was measured by Luminex. **(C)** The percentage of total (CD19^{+}) B cells in spleen was determined by flow cytometric analysis.

Figure S3. Immune activation from PBS, 5'ppp-hpRNA, R848, and poly(I:C) in B6129SF2/J control and MAVS$^{-/-}$ mice (5 mice/group) at 6 hours post-injection. Expression of splenic genes included in the Mouse Myeloid Innate Immunity Panel **(A)**, represented as Z-score, was evaluated by Nanostring analysis. Gene sets were grouped based on a cluster analysis of treatment groups with, **(B)** B6129SF2/J control and **(C)** MAVS$^{-/-}$ mice.

Table S1. Heatmap gene order for Figure 4E (C57BL/6). Shown top to bottom on heatmap.							
Cebpa	Nr2f6	Adamts17	Fcgr1	Irf7	Birc2	Il3ra	Fpr-rs5
Cd163	Itgam	Ccl27a	Ccr5	Isg15	Cd40	Tnfaip6	Ccl17
Csf1r	Igf1	Ephb6	Cd276	Stat1	Serpinb9	Rhoc	Cxcl3
Hpgd	Daglb	Rgs6	Casp1	Ccl12	Lif	Tgm2	Olr1
Mrc1	Ceacam1	Cd209e	C3	H2-T23	Il33	Myc	Scin
Acot11	Sema6d	Havcr1	Casp7	Trex1	Socs2	Cd69	Fzd9
Xcr1	Pglyrp1	Rasgrf2	Klrk1	Dpp4	Jun	Peli1	Aif1
Tlr11	Ccl6	Chil3	Plau	Sell	Ifnb1	Il15	Ros1
Itgax	Ccr3	Chil4	Kitl	Mmp13	Ccr7	Txn1	Pdzk1ip1
Cav1	Ccr2	Mmp8	Rin2	Tlr3	Fas	Anxa4	Mmp10
Pparg	Lrp5	Ceacam2	Cd38	H2-Q1	Traf1	Cd86	Hnf1b
Cd244	Tgfbr3	Cd1d2	Lamb3	Psmb9	Marcksl1	Map2k1	Cyr61
Havcr2	Cbr1	Ccr6	Tnf	Tlr9	Fscn1	Acod1	
Clec9a	Vsir	Mmp12	Sphk1	Tnfsf10	Icam1	Flnb	
Clec1b	Lipa	Kit	Tnfaip3	Tapbp	Gadd45b	Ccl7	
S100a9	Alox5ap	Ptger2	Cd80	Daxx	Il1rn	Il15ra	
S100a8	Cx3cr1	Thbd	Crem	Ifit1bl1	Ccl4	Ccl2	
Ly6g	Mapk14	S1pr1	Ifna1	Mx1	Ccl3	Ccrl2	
Cxcr2	Itgb7	Fzd6	Rgs16	Trafd1	Cdkn1a	Clic4	
Cdh4	Ptprb	Ms4a2	Cxcl5	Ly6c1	Nfkbiz	Atf3	
Col15a1	Il6ra	Trem2	Cxcl1	Psmb8	Emp1	Vwa5a	
Ccl9	Sema5a	Il9	Tnc	Cybb	Serpine1	Cxcl9	
Pdgfrb	Ifngr1	Mrc2	Il22	Tap1	Ccl5	Cxcl11	
Id1	Cxcr3	Fabp4	Ido1	Amica1	Tnfrsf12a	Irf1	
Csf3r	Flt3	Cd99	Tnfsf4	Cd274	Maff	Tap2	
H2-Q10	Fcer2a	Cxcl13	Edn1	Socs1	Ccl11	Stat3	
Nectin1	Il27ra	Cpa3	Cd70	Nampt	Adamts4	Hbegf	
Hdac6	Rapgef4	Siglec1	Cx3cl1	Cxcl10	Il6	Ldlr	
Dhrs3	H2-Ob	Ccl24	Csf3	Psme2	Ifng	Cxcl2	
Cd40lg	Smarcd3	Fgf10	Sele	Aoah	Il27	Rgs1	
Hdac5	Tlr5	Mok	Cldn1	Fcgr4	Adora2a	Rab20	
Ski	Cysltr1	Dusp2	Fpr-rs3	Lgals3	Ptafr	Hif1a	
Insr	Tlr12	Dusp1	Gch1	Ifit1bl2	Clec5a	Gem	
Mmp9	Id3	Ctla4	Cnksr1	Hdc	Ripk2	Nfil3	
Krba1	Klf10	Ptprk	Klk1	Kcnab1	Osm	Ptgs2	
H2-Eb1	Hes1	Hoxd4	Gzma	Il10	F11r	Cd83	
Itgb2	Ctsa	C3ar1	Nox1	Fosl1	Cxcl16	Areg	
Ptgs1	Ltb4r1	Nox4	Serinc2	Nod2	Ccl8	H2-Ea-ps	
Alox5	Mapk12	Tnfrsf8	Tlr7	Il12b	Tgfa	Cxcl14	
Tm7sf3	Cma1	Tnfrsf11a	Usp18	Socs3	Il1a	Cttnbp2	
Hspg2	Ptpn14	Fpr2	Mx2	Adamts9	Lag3	Fpr-rs4	

Table S2. Heatmap gene order for Figure 6 (Shown top to bottom on heatmap) **and Figure S3A** (Shown left to right on heatmap)					
Fcgr3	Tlr7	Ccl2	Nfkbia	Pdcd1	Tek
Cebpd	DDX58	Clic4	Cd80	Klk1	Cd1d1
Cyr61	Trex1	Il15ra	Emp1	Batf3	Cd180
Hdc	Tapbp	Atf3	Maff	Cx3cl1	Xcr1
Il1r2	Trafd1	Cd274	Serpine1	Ngf	Cxcl12
Il1b	Daxx	Vwa5a	Tnfrsf12a	Ly6g	Ptprb
Cd14	Mx1	Cxcl11	Adamts9	S100a9	Sema5a
Clec5a	Tnfsf10	Tlr9	Il1a	S100a8	Csf1r
C5ar1	DHX58	Kcnab1	Hbegf	Mmp8	Tlr11
Il1rap	Usp18	Klrk1	Cdkn1a	Ccr1	Cd163
Edn1	Ifit1bl1	Ifit1bl2	Cebpb	Cxcr2	Mrc1
Ptprk	IFIH1 a	Osm	Rab20	H2-Q10	Hpgd
Itga5	IFIH1 b	Cxcl1	Tgm2	Csf3r	Col14a1
Ier3	Mx2	Csf1	Il33	Prok2	Cbr1
Ifng	Irf7	Myd88	Il1rn	Sphk1	Mafb
Cxcl2	Stat1	Tgfa	Rhoc	Nos2	Ptgs1
Ccl11	Isg15	Rin2	Enc1	Fosl1	Ccr3
Plaur	H2-Q1	Sell	Fas	Trem1	Cav1
Ccr7	H2-T23	Cd86	Ccl4	Ear6	Sema6d
Cldn1	Fcgr1	Map2k1	Tnf	Ccl24	Lipa
Il12b	Cxcl9	Peli1	Ccl3	Cd244	Tgfbr3
Tnc	Fcgr4	Casp1	Acod1	Nectin1	Col1a2
Cxcl5	Txn1	Cd40	Crem	Ctsd	Itgax
Fpr1	Anxa4	Gem	Il6	Lpl	Fut4
Batf	PSMB10	Birc2	Adamts4	Pdgfrb	Cebpa
Tnfrsf4	Ccl12	Irf8	Hif1a	Ccl9	Crip1
Ccr5	Mmp13	Mob3c	Socs3	Ear3	H2-Ob
Ifna1	Il15	Nfkbiz	Jun	Des	Il6ra
Ifnb1	Ccrl2	Skil	Nfil3	Cxcr3	Acot11
Il3ra	Tap1	Nod1	Ptgs2	Cd40lg	Itgb7
Ccl8	Socs1	C3	Lif	Il27ra	Rapgef4
Nod2	Nampt	Aoah	Ccl7	Lat	Dusp6
Ikbke	Cxcl10	Fpr2	Stat3	Sesn1	Cx3cr1
Cd276	Psme2	Mif	Fscn1	Fcer2a	Vsir
Tnfaip6	Irf1	Ccl5	Socs2	Mmp12	Hdac5
Cd47	Tap2	Marcksl1	Serpinb9	Lat2	Mapk14
Tlr3	Psmb9	Flnb	Gadd45b	Ccr6	Nfatc2
Myc	Psmb8	Rgs16	Traf1	Tlr1	
Cybb	Ly6c1	Nlrp3	Cxcl16	S100a4	
Cd69	Icam1	Tlr2	Gzma	Ccr2	
Amica1	Rgs1	Tnfaip3	Il10	Kalrn	

Table S3. Heatmap gene order for Figure S3B (B6129SF2/J). Shown top to bottom on heatmap.					
Cxcl11	Birc2	Psmb8	Osm	Il1r2	Cd1d1
Ccr5	Rgs1	Tap2	Il1rap	Hdc	Xcr1
Fpr1	Cd40	Stat1	Ifit1bl2	Cd14	Tlr11
Fcgr1	Gem	Ly6c1	Il12b	Trem1	Sema5a
Fpr2	Cdkn1a	Trex1	Tnfrsf12a	C5ar1	Cxcl12
Aoah	Csf1	Isg15	Emp1	Prok2	Mrc1
Tlr2	Adamts9	Rin2	Itga5	H2-Q10	Csf1r
Nlrp3	Rab20	Sell	Crem	Csf3r	Ptgs1
Ikbke	Cebpb	Casp1	Ccl7	Il10	Hpgd
Nod2	Socs3	Mmp13	Nfil3	Ccl8	Col14a1
Il1a	Jun	Nod1	Ptgs2	Pdcd1	Cd163
Txn1	Klrk1	C3	Lif	Fcgr4	Lat
Cxcl9	Kcnab1	Tgm2	Ccr7	Batf	Il27ra
Traf1	Tlr7	Cd276	Hif1a	Tnfrsf4	Fut4
Serpinb9	Mx2	Tlr9	Enc1	Gzma	Cebpa
Gadd45b	Usp18	Ccl12	Il6	Ifnb1	Sesn1
Cxcl16	Daxx	PSMB10	Il33	Ifna1	Cxcr3
Socs2	Ifit1bl1	DDX58	Serpine1	Klk1	Cd40lg
Fscn1	Trafd1	Nfkbia	Cxcl2	Ear6	Nectin1
Nampt	Irf7	Maff	Ier3	Tgfbr3	Lipa
Cd274	Tapbp	Tnfaip3	Il1rn	Col1a2	Ccr3
Il15ra	Cd47	Cd80	Ccl11	Cd180	Cav1
Cxcl10	H2-T23	Adamts4	Plaur	Il6ra	Ctsd
Irf1	H2-Q1	Ccl4	Nos2	Dusp6	Crip1
Vwa5a	Mx1	Tnf	Fosl1	Tlr1	Itgax
Clic4	Amica1	Ccl3	Ifng	Hdac5	Fcer2a
Myd88	Peli1	Clec5a	Cxcl1	Acot11	Lpl
Il15	Tnfsf10	Cx3cl1	Cxcl5	Vsir	Ccl9
Rhoc	Map2k1	Marcksl1	Ngf	Cx3cr1	Pdgfrb
Anxa4	DHX58	Flnb	Ptprk	Nfatc2	Kalrn
Stat3	Cybb	Ccl5	Edn1	Mapk14	Des
Socs1	Tlr3	Mob3c	Tnc	Rapgef4	Tek
Nfkbiz	IFIH1 b	Rgs16	Fcgr3	Itgb7	S100a4
Hbegf	IFIH1 a	Tnfaip6	Batf3	H2-Ob	Ccr2
Tgfa	Myc	Mif	Sphk1	Ptprb	Cd244
Fas	Cd69	Cyr61	S100a9	Sema6d	Ear3
Ccl2	Cd86	Cebpd	S100a8	Mafb	Ccl24
Atf3	Skil	Il3ra	Mmp8	Cbr1	
Icam1	Tap1	Il1b	Cxcr2	Lat2	
Ccrl2	Psmb9	Acod1	Ccr1	Ccr6	
Irf8	Psme2	Cldn1	Ly6g	Mmp12	

Table S4. Heatmap gene order for Figure S3C (MAVS$^{-/-}$). Shown top to bottom on heatmap.					
Batf3	Cd80	Gadd45b	Icam1	Ifit1bl1	Crip1
Cx3cl1	Tnfaip3	Serpinb9	Irf1	Map2k1	Mafb
Ptprk	Tnfrsf12a	Hif1a	Ccl12	Cd69	Acot11
Fosl1	Adamts9	Rgs1	Mmp13	Myc	Sema6d
Nos2	Emp1	Fas	Kcnab1	Mx2	Fcer2a
Ngf	Ccr7	Tnfaip6	Nod1	Amica1	Ccr6
Trem1	Plaur	Nfil3	Trex1	Mx1	Mmp12
Fpr1	Cxcl16	Cebpb	Fcgr1	Ly6g	Cx3cr1
Ccl5	Maff	Rab20	Klrk1	Mmp8	Cxcr3
Gzma	Il10	Il33	Stat1	S100a8	Sesn1
Cyr61	Sphk1	Hbegf	H2-Q1	S100a9	Cd1d1
Cebpd	Edn1	Il1a	H2-T23	C5ar1	Cbr1
Fcgr3	Itga5	Il3ra	Ly6c1	H2-Q10	Mapk14
Ikbke	Cxcl2	Tlr9	Tapbp	Ccr1	Vsir
Cd14	Ier3	Tgfa	Irf7	Csf3r	Hdac5
Tnc	Cxcl1	Gem	PSMB10	Cxcr2	Nfatc2
Il1rap	Serpine1	Socs1	Psme2	Klk1	Il27ra
Clec5a	Il12b	Stat3	Tap1	Prok2	Itgb7
Il1b	Nod2	Clic4	Tap2	Ccl24	Rapgef4
Nlrp3	Ifna1	Vwa5a	Psmb8	Ear6	H2-Ob
Tlr2	Ifnb1	Cd274	Psmb9	Ccl9	Il6ra
Cldn1	Cd276	Cxcl10	Isg15	Cd244	Lipa
Cxcl5	Pdcd1	Il15	Ccr5	Ctsd	Ptgs1
Acod1	Tnfrsf4	Nampt	Tlr3	Lpl	Ccr2
Ifng	Batf	Osm	Cd47	Ccr3	Tek
Il6	Mif	Jun	Tlr7	Ear3	Cd163
Lif	Fcgr4	Socs3	Myd88	Des	Mrc1
Ptgs2	Fscn1	Cdkn1a	Casp1	Cav1	Csf1r
Adamts4	Rhoc	Nfkbiz	Rin2	Pdgfrb	Ptprb
Ccl3	Tgm2	Cd40	Sell	Lat	Hpgd
Ccl4	Ifit1bl2	Skil	Cd86	Kalrn	Sema5a
Crem	Aoah	Irf8	Peli1	S100a4	Col14a1
Il1r2	Fpr2	Mob3c	Cybb	Nectin1	Cxcl12
Hdc	Tnf	Ccl8	DDX58	Cd180	Tlr11
Ccl11	Socs2	Anxa4	DHX58	Lat2	Xcr1
Il1rn	Traf1	Txn1	Tnfsf10	Tlr1	Col1a2
Rgs16	Nfkbia	C3	Trafd1	Cd40lg	Tgfbr3
Csf1	Atf3	Cxcl9	IFIH1 a	Dusp6	
Enc1	Il15ra	Marcksl1	IFIH1 b	Fut4	
Ccl2	Ccrl2	Flnb	Daxx	Itgax	
Ccl7	Cxcl11	Birc2	Usp18	Cebpa	

Table S5. 2way ANOVA results for Figure 5A.				
Sidak's multiple comparisons post-test for Figure 5A				
Comparison	**Mean Diff.**	**95.00% CI of diff.**	**Significant?**	**Adjusted P Value**
WT - MAVS$^{-/-}$				
PBS	0	-640.0 to 640.0	No	>0.9999
5'ppp-hpRNA	0	-640.0 to 640.0	No	>0.9999
R848	0	-640.0 to 640.0	No	>0.9999
Poly I:C	0	-678.8 to 678.8	No	>0.9999
hEPO Process A (U)	83.27	-556.7 to 723.3	No	>0.9999
hEPO Process A (1mΨ)	1121	481.5 to 1762	Yes	<0.0001
hEPO Process B (U)	1072	432.4 to 1712	Yes	<0.0001
hEPO Process B (1mΨ)	823.8	145.0 to 1503	Yes	0.0078
Poly (U) 19mer	0	-678.8 to 678.8	No	>0.9999
Poly (1mΨ) 19mer	0	-640.0 to 640.0	No	>0.9999
Tukey's multiple comparisons post-test for Figure 5A				
Comparisons	**Mean Diff.**	**95.00% CI of diff.**	**Significant?**	**Adjusted P Value**
WT				
hEPO Process A (U) vs. hEPO Process A (1mΨ)	-1186	-1909 to -462.1	Yes	<0.0001
hEPO Process A (U) vs. hEPO Process B (U)	-975.7	-1699 to -252.2	Yes	0.0014
hEPO Process A (1mΨ) vs. hEPO Process B (1mΨ)	-65.39	-788.9 to 658.1	No	>0.9999
hEPO Process B (U) vs. hEPO Process B (1mΨ)	-275.3	-998.9 to 448.2	No	0.9633
Poly (U) 19mer vs. Poly (1mΨ) 19mer	0	-723.5 to 723.5	No	>0.9999
MAVS$^{-/-}$				
hEPO Process A (U) vs. hEPO Process A (1mΨ)	-147.4	-871.0 to 576.1	No	0.9996
hEPO Process A (U) vs. hEPO Process B (U)	13.43	-710.1 to 737.0	No	>0.9999
hEPO Process A (1mΨ) vs. hEPO Process B (1mΨ)	-363.1	-1130 to 404.4	No	0.8709
hEPO Process B (U) vs. hEPO Process B (1mΨ)	-523.9	-1291 to 243.5	No	0.4496
Poly (U) 19mer vs. Poly (1mΨ) 19mer	0	-767.4 to 767.4	No	>0.9999

Table S6. 2way ANOVA of data from Figure 5B.				
Sidak's multiple comparisons post-test for Figure 5B				
Comparison	**Mean Diff.**	**95.00% CI of diff.**	**Significant?**	**Adjusted P Value**
WT - MAVS$^{-/-}$				
PBS	120.1	-4012 to 4252	No	>0.9999
5'ppp-hpRNA	40072	35940 to 44204	Yes	<0.0001
R848	140	-3992 to 4272	No	>0.9999
Poly I:C	1592	-2790 to 5975	No	0.9711
hEPO Process A (U)	8298	4166 to 12430	Yes	<0.0001
hEPO Process A (1mΨ)	2547	-1585 to 6679	No	0.5638
hEPO Process B (U)	2815	-1317 to 6947	No	0.4212
hEPO Process B (1mΨ)	129.8	-4253 to 4512	No	>0.9999
Poly (U) 19mer	2797	-1586 to 7179	No	0.5146
Poly (1mΨ) 19mer	240.1	-3892 to 4372	No	>0.9999
Tukey's multiple comparisons post-test for Figure 5B				
Comparisons	**Mean Diff.**	**95.00% CI of diff.**	**Significant?**	**Adjusted P Value**
WT				
hEPO Process A (U) vs. hEPO Process A (1mΨ)	10761	6089 to 15432	Yes	<0.0001
hEPO Process A (U) vs. hEPO Process B (U)	5285	613.3 to 9956	Yes	0.0145
hEPO Process A (1mΨ) vs. hEPO Process B (1mΨ)	2449	-2222 to 7121	No	0.7873
hEPO Process B (U) vs. hEPO Process B (1mΨ)	7925	3254 to 12597	Yes	<0.0001
Poly (U) 19mer vs. Poly (1mΨ) 19mer	6684	2013 to 11356	Yes	0.0005
MAVS$^{-/-}$				
hEPO Process A (U) vs. hEPO Process A (1mΨ)	5009	338.0 to 9680	Yes	0.0258
hEPO Process A (U) vs. hEPO Process B (U)	-198.8	-4870 to 4472	No	>0.9999
hEPO Process A (1mΨ) vs. hEPO Process B (1mΨ)	32.27	-4922 to 4987	No	>0.9999
hEPO Process B (U) vs. hEPO Process B (1mΨ)	5240	285.7 to 10195	Yes	0.0296
Poly (U) 19mer vs. Poly (1mΨ) 19mer	4128	-826.8 to 9082	No	0.1864

Table S7. 2way ANOVA of data from Figure 5C.				
Sidak's multiple comparisons post-test for Figure 5C				
Comparison	**Mean Diff.**	**95.00% CI of diff.**	**Significant?**	**Adjusted P Value**
WT - MAVS$^{-/-}$				
PBS	0.514	-14.48 to 15.51	No	>0.9999
5'ppp-hpRNA	76.12	61.12 to 91.12	Yes	<0.0001
R848	8.88	-6.118 to 23.88	No	0.6188
Poly I:C	3.42	-12.49 to 19.33	No	0.9996
hEPO Process A (U)	18.38	3.382 to 33.38	Yes	0.007
hEPO Process A (1mΨ)	39.22	24.22 to 54.22	Yes	<0.0001
hEPO Process B (U)	14.64	-0.3575 to 29.64	No	0.0605
hEPO Process B (1mΨ)	3.665	-12.24 to 19.57	No	0.9992
Poly (U) 19mer	4.54	-11.37 to 20.45	No	0.9952
Poly (1mΨ) 19mer	0.108	-14.89 to 15.11	No	>0.9999
Tukey's multiple comparisons post-test for Figure 5C				
Comparisons	**Mean Diff.**	**95.00% CI of diff.**	**Significant?**	**Adjusted P Value**
WT				
hEPO Process A (U) vs. hEPO Process A (1mΨ)	27.16	10.21 to 44.11	Yes	<0.0001
hEPO Process A (U) vs. hEPO Process B (U)	18.88	1.925 to 35.83	Yes	0.0173
hEPO Process A (1mΨ) vs. hEPO Process B (1mΨ)	47.24	30.28 to 64.19	Yes	<0.0001
hEPO Process B (U) vs. hEPO Process B (1mΨ)	55.52	38.56 to 72.47	Yes	<0.0001
Poly (U) 19mer vs. Poly (1mΨ) 19mer	82.13	65.18 to 99.08	Yes	<0.0001
MAVS$^{-/-}$				
hEPO Process A (U) vs. hEPO Process A (1mΨ)	48	31.05 to 64.95	Yes	<0.0001
hEPO Process A (U) vs. hEPO Process B (U)	15.14	-1.815 to 32.09	No	0.1207
hEPO Process A (1mΨ) vs. hEPO Process B (1mΨ)	11.68	-6.301 to 29.67	No	0.5219
hEPO Process B (U) vs. hEPO Process B (1mΨ)	44.54	26.56 to 62.53	Yes	<0.0001
Poly (U) 19mer vs. Poly (1mΨ) 19mer	77.7	59.71 to 95.68	Yes	<0.0001

Figure S1.

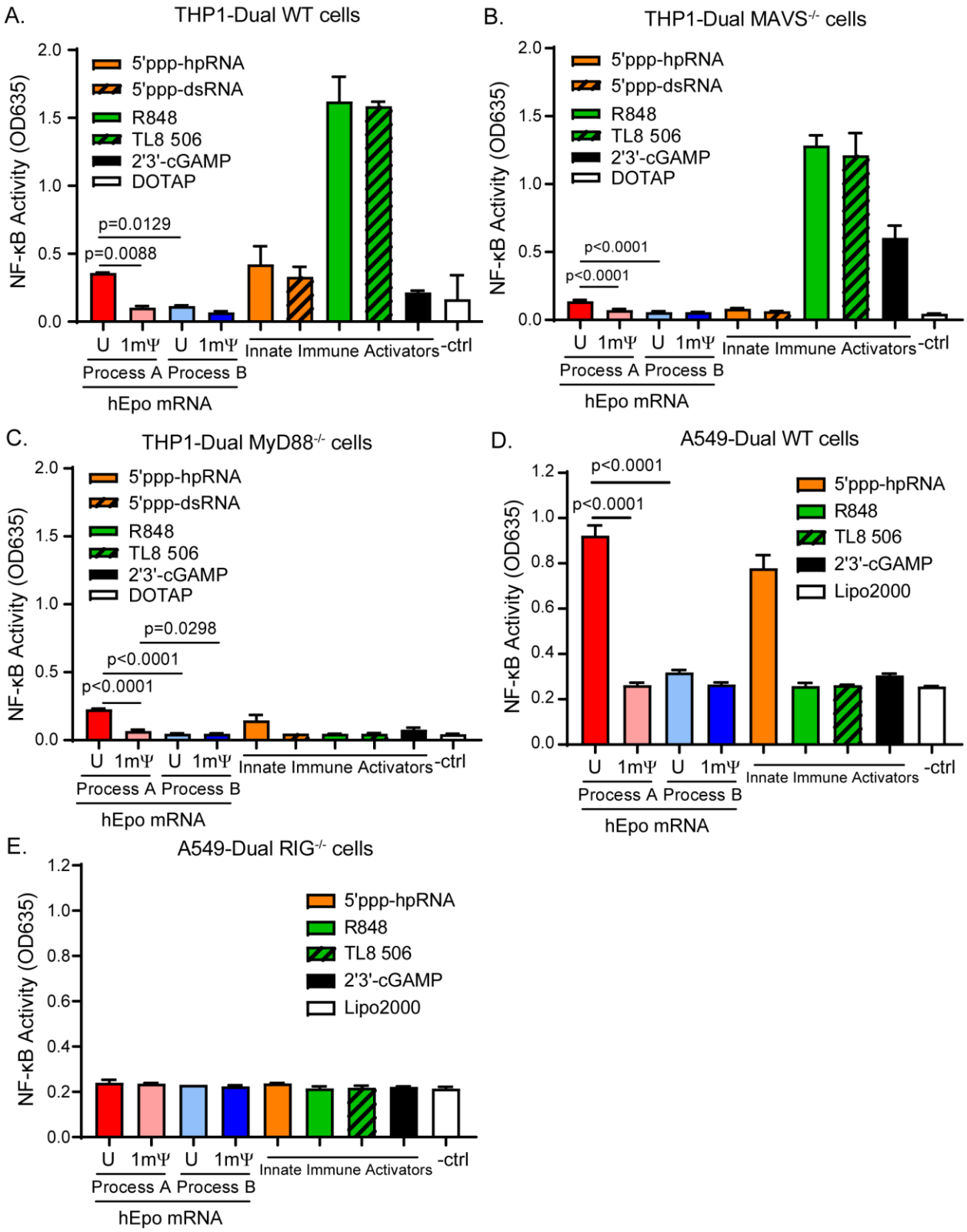

A.
THP1-Dual WT cells
NF-κB Activity (OD635)
5'ppp-hpRNA
5'ppp-dsRNA
R848
TL8 506
2'3'-cGAMP
DOTAP
p=0.0129
p=0.0088
U 1mΨ U 1mΨ
Innate Immune Activators
-ctrl
Process A Process B
hEpo mRNA
B.
THP1-Dual MAVS-/- cells
p<0.0001
p<0.0001
C.
THP1-Dual MyD88-/- cells
p=0.0298
p<0.0001
p<0.0001
D.
A549-Dual WT cells
Lipo2000
E.
A549-Dual RIG-/- cells

Figure S2.

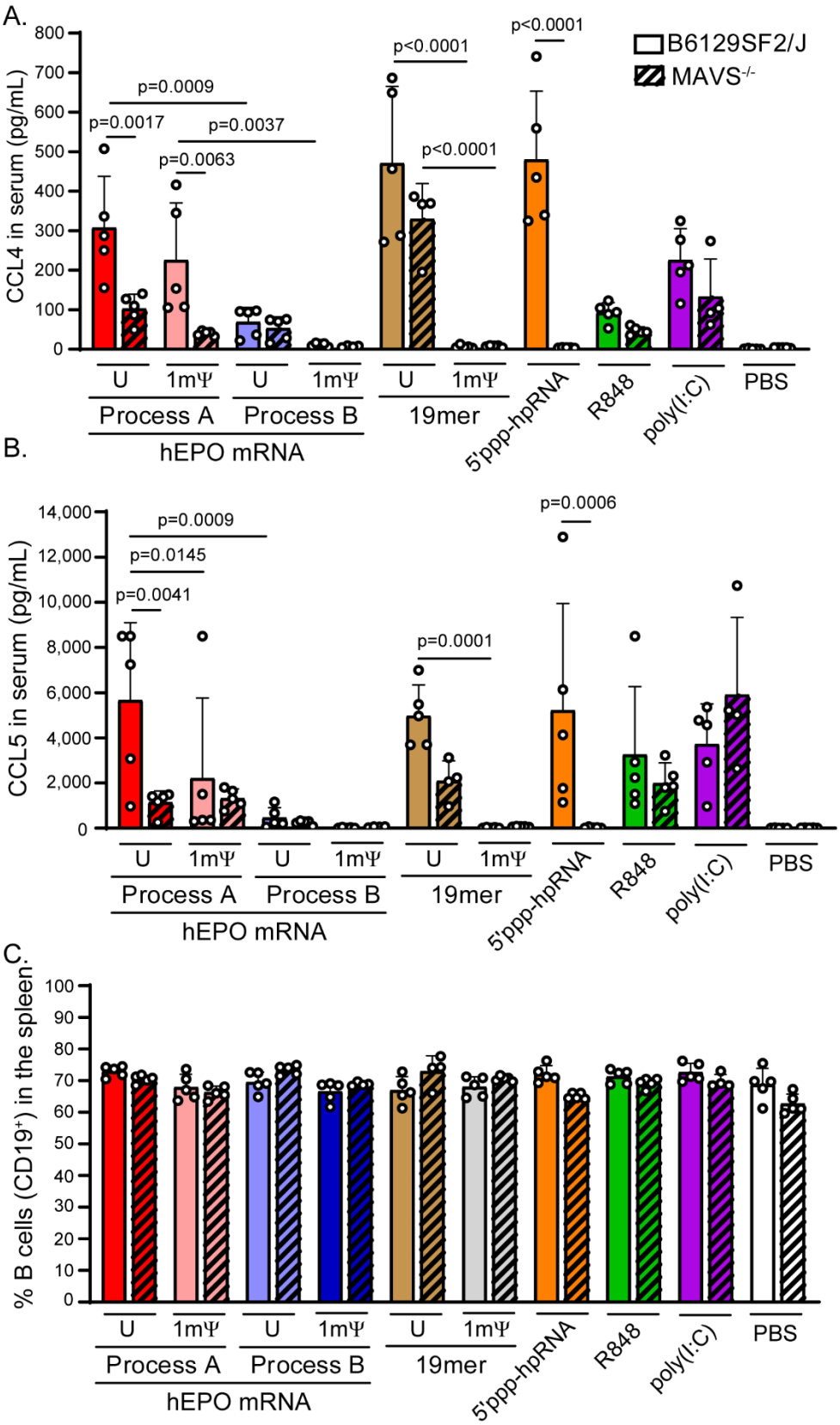
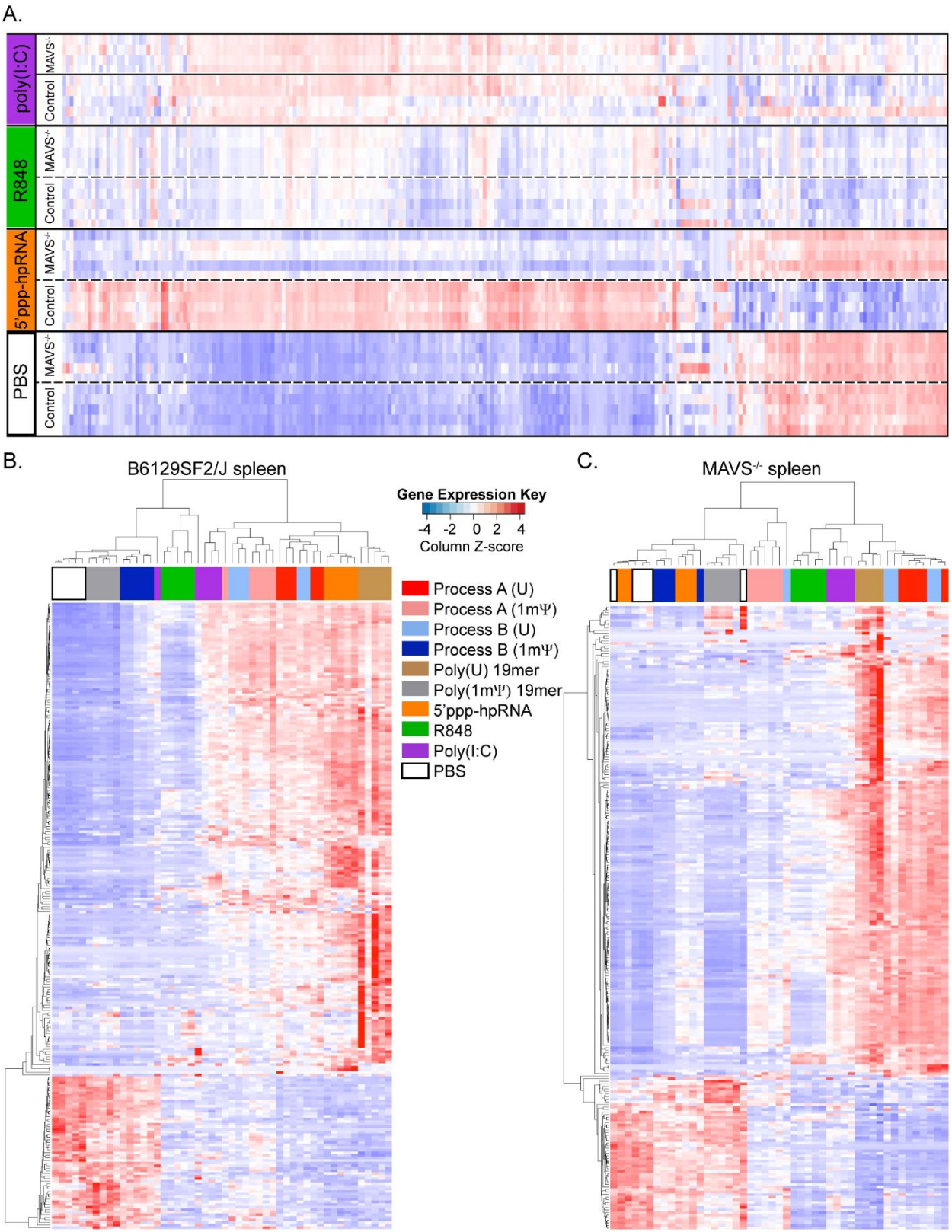

A.
CCL4 in serum (pg/mL)
B6129SF2/J
MAVS-/-
p=0.0009
p=0.0017
p=0.0037
p=0.0063
p<0.0001
p<0.0001
p<0.0001
U
1mΨ
Process A
Process B
hEPO mRNA
19mer
5'ppp-hpRNA
R848
poly(I:C)
PBS
B.
CCL5 in serum (pg/mL)
p=0.0009
p=0.0145
p=0.0041
p=0.0001
p=0.0006
C.
% B cells (CD19+) in the spleen

Figure S3.


A.
poly(I:C)
R848
5'ppp-hpRNA
PBS
MAVS-/-
Control
B.
B6129SF2/J spleen
Gene Expression Key
-4 -2 0 2 4
Column Z-score
Process A (U)
Process A (1mΨ)
Process B (U)
Process B (1mΨ)
Poly(U) 19mer
Poly(1mΨ) 19mer
5'ppp-hpRNA
R848
Poly(I:C)
PBS
C.
MAVS-/- spleen