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Supplemental Information

The Splicing Factor hnRNP M Is a Critical Regulator of Innate Immune Gene Expression in Macrophages

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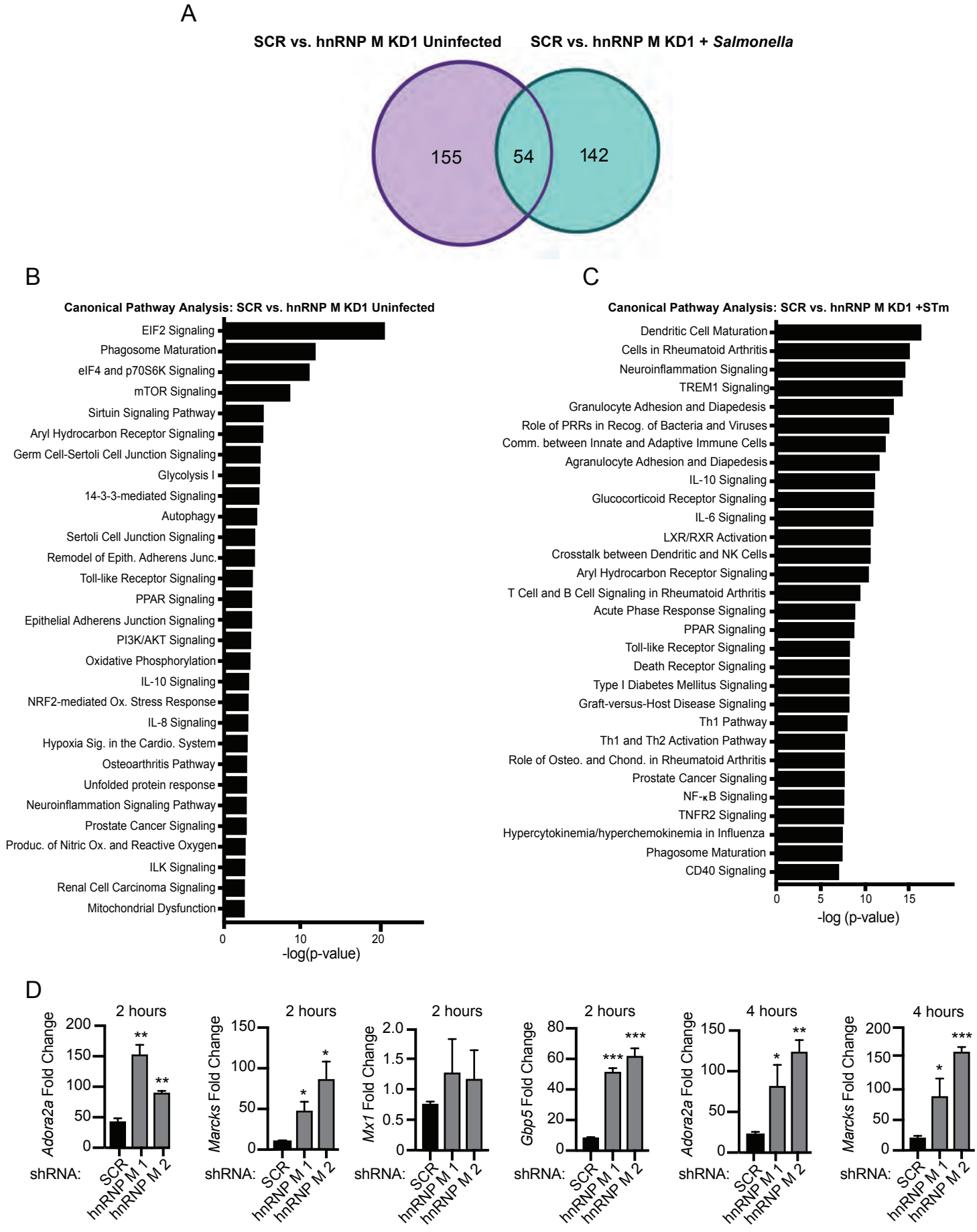
Supplemental Information
West et al. 2019



Supplemental Figure S1

Figure S1. Related to Figure 1. HnRNP M regulates expression of specific immune genes during *Salmonella* Typhimurium Infection.

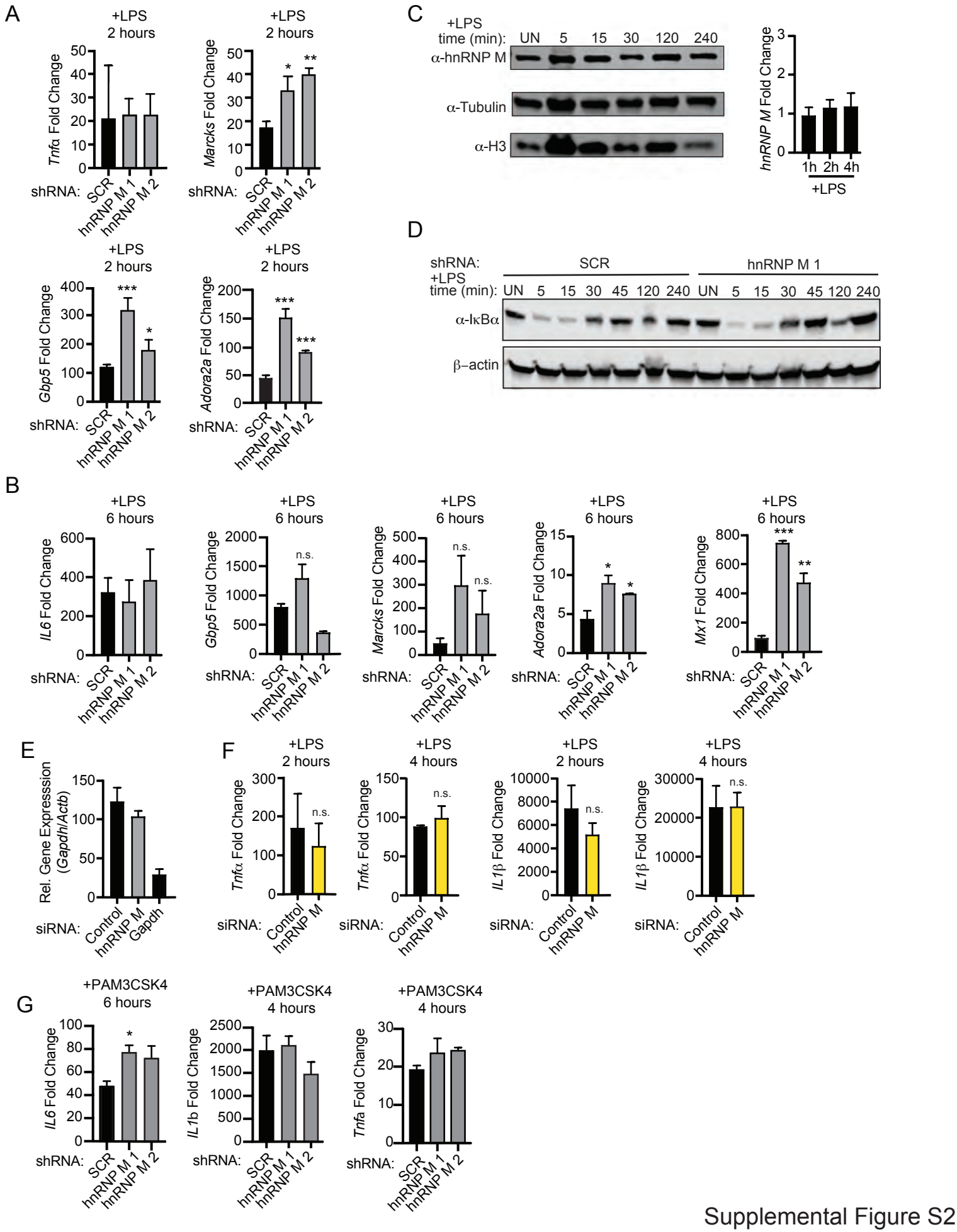
(a) Venn diagram to represent overlap between hnRNP M-regulated transcripts in uninfected and *Salmonella*-infected macrophages. (b) Complete ingenuity pathway analysis for SCR vs. hnRNP M KD uninfected macrophages. (c) Complete ingenuity pathway analysis for SCR vs. hnRNP M KD *Salmonella*-infected macrophages. (d) Gene expression by RT-qPCR in SCR, hnRNP M KD 1, and hnRNP M KD 2 2hr post-*Salmonella* infection for *Adora2a, Marcks, Mx1, Gbp5,* and 4hr for *Adora2a* and *Marcks*.

RNA-Seq Uninfected		RNA-Seq +*Salmonella*	
GENE NAME	FOLD INDUCTION	GENE NAME	FOLD INDUCTION
Hpgd	13.90711	Fabp7	6.765286
Cxcr4	9.354822	Ms4a6c	5.454133
Ms4a6c	7.745006	Ms4a4c	4.956656
Csf3r	7.714053	Ms4a6d	4.288573
Fabp7	5.976491	Il6	3.957798
S1pr1	5.387236	Marcks	3.499546
Cd33	4.585221	Gm16685	3.373171
Ms4a6d	4.420621	Adora2a	3.23969
Neurl3	3.949566	Ighm	3.19321
Pros1	3.932567	Slc28a2	3.177709
Prss46	3.472838	Cfb	3.166876
Prss50	3.46898	Slamf9	3.116945
St6gal1	3.419711	Rpl27-ps3	3.017322
Ighm	3.19321	Mx1	3.008578
Cd36	3.104169	Cd36	2.828894
Il10ra	3.085386	Ctsc	2.787805
Gm12166	2.956821	C3	2.569081
Fabp4	2.923715	Fcgr3	2.480588
Aph1c	2.916988	Fabp3	2.4082
Fosb	2.85088	Smpdl3a	2.352208
Atf3	2.832853	Pld4	2.271779
Tnfrsf1b	2.814419	Nectin2	2.247367
Tgfbr1	2.740644	Dusp1	2.241607
Fcgr3	2.73009	Cd300e	2.21221
Clec4a3	2.694922	Gm5431	2.182886
Il7r	2.691738	Havcr2	2.170636
Tmem86a	2.672264	Fcgr2b	2.150546
Cav2	2.651061	Spen	2.146736
Gm6377	2.615475	C3ar1	2.115022
Cd300c2	2.58364	Cd300c2	2.104473
Fabp3	2.555074	Siglece	2.089605
Smpdl3a	2.51753	Edn1	2.089051
Ptafr	2.485913	Cd276	2.056106
Pld4	2.390038	Tgm2	2.027966
Plk2	2.379248	Fcgr4	1.997097
Btg2	2.363222	Gm10260	1.99323
C3ar1	2.210747	Il10ra	1.978782
Wfdc17	2.139324	Sema4a	1.955406
Cd276	2.135795	Ccrl2	1.938053
Itgam_1	2.060784	Ass1	1.925226
Ctsc	2.051702	Adgre1	1.866584
Egr2	2.046845	Il4i1	1.857245
Tgm2	2.039586	Pik3r6	1.817064
Egr1	1.995767	Bcl3	1.802364
Dusp1	1.995471	Tnfsf9	1.799192
Mpeg1	1.96516	Fcgr1	1.797059
Bhlhe41	1.959191	Igsf6	1.796179
Xdh	1.939404	Lacc1	1.793885
Lst1	1.919394	Zc3h12c	1.793278
Lsp1	1.897235	Gngt2	1.786466

Gnpda1	1.891593	Gm4070	1.762879
Tlr13	1.876798	Plk2	1.735775
Myo1f	1.868713	Bcl2a1a	1.731698
Rap2b	1.824333	Rsad2	1.728779
Jun	1.818452	Lsp1	1.728275
Arl11	1.803489	Cmpk2	1.716878
Rnf26_1	1.800696	Fabp4	1.709066
Havcr2	1.800502	Stat5a	1.705524
Fblim1	1.779344	Napsa	1.704077
Mcoln2	1.774978	Ppp1r15a	1.686405
Rnf128	1.761964	Gk	1.68233
Rab32	1.75113	Myo1f	1.677152
Tnfaip2	1.726583	Gpnmb	1.675334
Dhrs3	1.725571	Arhgef3	1.672991
Litaf	1.714146	Tnfaip3	1.661885
Ctsf	1.705514	Nfkbiz	1.658248
Cxcl16	1.703797	Syk	1.651344
Gstm1	1.702554	Tagap	1.645342
Gngt2	1.701373	Itgal	1.638434
Zfp385a	1.688385	Tmem51	1.636726
Pbxip1	1.687734	Mpeg1	1.635068
Hist1h1c	1.68698	Cebpb	1.63348
Gpnmb	1.672091	Dusp2	1.627537
Napsa	1.658448	Ctsf	1.621343
Fos	1.652633	Fam129a	1.620868
Cotl1	1.646704	Zfp36	1.62075
Slc15a4	1.638645	Saa3	1.617704
Tmem51	1.636726	Slc44a1	1.610821
Id3	1.631679	Ldlr	1.600648
Slc44a1	1.628823	Ptafr	1.600574
Ier2	1.613946	Fam177a	1.598248
Msrb1	1.611736	Birc3	1.591189
Il10rb	1.603	Anpep	1.587615
Bcl2a1d	1.597259	Myo1g	1.58755
Ctnnb1	1.585479	Tnfrsf1b	1.587515
Slc6a8	1.582178	Il18	1.582494
Nceh1	1.57352	Tnfaip2	1.546983
Sptssa	1.571322	Cxcl16	1.538268
Gyg	1.565705	Traf1	1.533961
Rgs2	1.565309	Gbp5	1.531947
Plin2	1.563214	Blnk	1.531359
B4galt1	1.550249	Atf3	1.530953
Rnh1	1.536697	Gadd45b	1.526009
Tbc1d16	1.521571	Lrrc25	1.515623
Zfp36	1.515133	Jak2	1.509944
Fabp5	1.507761	Icam1	1.501014
Cybb	1.506247	Capg	-1.43733
Anpep	1.50344	Ubald2	-1.50115
Alox5ap	1.500911	Rab31	-1.50144
Pdia3	-1.50295	Pdia3	-1.51841
Tubb6	-1.50417	Atp1b3	-1.5477
Tmsb10	-1.50845	Snx9	-1.56831
Sumo2	-1.53189	Ets2	-1.60419
Ccl3	-1.54719	Plau	-1.60747

Pip5k1b	-1.56974	Naa25	-1.60934
Hmga2	-1.58317	Gm26619	-1.6641
Atp1b3	-1.58414	Mrps6	-1.67118
Ank	-1.5913	Spp1	-1.67374
Cpne8	-1.59717	Tfdp1	-1.67919
Fcrl1	-1.61248	Serinc2	-1.69061
Rbpj	-1.61302	Tmem98	-1.70829
Tfdp1	-1.61335	Dhrs9	-1.71239
Slc6a12	-1.61529	Rpl10-ps3	-1.73337
Snx9	-1.62045	Spink5	-1.77682
Prelid2	-1.64498	Epn2	-1.79498
Slc16a3	-1.65921	Ccz1	-1.81366
Fosl2	-1.65925	Ier3	-1.86244
Mrps6	-1.68031	Acy1	-1.8627
Acot7	-1.71671	Ccl17	-1.86471
Oas2	-1.71742	Ccnd1	-1.87697
Ehd1	-1.75186	Lif	-1.88307
Ccnd1	-1.78697	Rgs16	-1.91798
Marcksl1	-1.81918	Csf3	-1.99019
Bnip3	-1.84262	Npl	-2.01377
Ak4	-1.84773	Timp1	-2.02017
Ets2	-1.85673	Hmga2	-2.04688
Acy1	-1.86399	Ccl2	-2.14895
Isg15	-1.88973	Dmpk	-2.16457
Odc1	-1.95677	Tnfsf15	-2.17432
Gm28037	-1.96705	Odc1	-2.24711
Layn	-1.96945	Pdia3	-2.34015
Naa25	-2.00186	Scin	-2.42501
Plekha3	-2.06644	Car2	-2.49682
Ccz1	-2.12175	Kbtbd11	-2.58657
Gm9803	-2.13322	Ccl7	-2.59416
Rtp4	-2.18593	Hnrnpm	-2.72047
Npl	-2.19264	Gm18445	-2.80579
Spp1	-2.25665	Slc6a4	-2.88423
Dmpk	-2.4654	Dmwd	-3.67138
Emp2	-2.65804	Mmp9	-3.86347
Hnrnpm	-2.69036	Tnfsf8	-4.12014
Fosl1	-2.78138	Sema7a	-4.72083
Spink5	-2.80455		
Gm18445	-2.80579		
Tmem26	-3.02585		
Flt1	-3.09266		
Serinc2	-3.27645		
Rgs16	-3.53479		
Car2	-3.73217		
Gm21987	-4.1013		
Dmwd	-4.16092		
Slc6a4	-4.72442		
Mmp9	-6.9746		
1810011H11Rik	2.333564		
AB124611	1.783388		

Table S1



Supplemental Figure S2

Figure S2. Related to Figure 2. hnRNP M-dependent gene expression profiles are similar amongst diverse immune stimuli.

(a) *Tnfα, Marcks, Gbp5,* and *Adora2a* expression by RT-qPCR in LPS-treated cells of SCR, hnRNP M KD 1, and hnRNP M KD 2 2hr post-treatment. (b) *IL6, Gbp5, Marcks, Adora2a,* and *Mx1* expression by RT-qPCR in SCR, hnRNP M KD1, and hnRNP M KD 2 6hr post-LPS treatment. (c) Western blot analysis of endogenous hnRNP M in RAW 264.7 macrophages treated with LPS for 5, 15, 30, 120, and 240 mins. RT-qPCR of *hnRNP M* expression in RAW 264.7 cells treated with LPS for 1hr, 2hr, and 4hr. (d) Western blot analysis of αIKβ in SCR control and hnRNP M KD 1 macrophages treated with LPS for 5, 15, 30, 45, 120, and 240 mins. (e) RT-qPCR demonstrating effective depletion of GAPDH in siRNA transfected BMDMs. (f) RT-qPCR of *Tnfα* and *IL1ß* in negative control and hnRNP M siRNA BMDMs treated with 10 ng/ml of LPS for 2hrs and 4hrs. (g) RT-qPCR of *IL6, Tnfα,* and *IL1ß* in SCR control and hnRNP M KD macrophages treated with PAM3CSK4 for 6hrs and 4hrs.

+ *Salmonella*

Upregulated genes			Downregulated genes		
	GUGGUGG	GGUUGGUU		GUGGUGG	GGUUGGUU
Fabp7			Ubald2	+	
Ms4a6c	+		Rab31	+	+
Ms4a4c	+	+	Pdia3	+	+
Ms4a6d	+		Atp1b3	+	+
Il6	+		Snx9	+	+
Marcks	+		Ets2	+	
Gm16685	+		Plau	+	
Adora2a	+		Naa25	+	+
Slc28a2	+		Gm26619	+	
Cfb			Mrps6	+	
Slamf9			Spp1	+	
Mx1	+		Tfdp1	+	+
Cd36	+	+	Serinc2	+	
Ctsc	+		Tmem98	+	
C3	+	+	Dhrs9		
Fcgr3	+	+	Rpl10-ps3		
Fabp3			Spink5	+	
Smpd13a	+		Epn2	+	+
Pld4	+		Ccz1	+	
Nectin2	+	+	Ier3		
Dusp1			Acy1	+	
Cd300e	+		Ccl17		
Gm5431	+	+	Ccnd1	+	
Siglece	+		Lif	+	
Havcr2	+	+	Rgs16	+	
Fcgr2	+		Csf3		
Spen	+	+	Npl	+	+
C3ar1	+		Timp1	+	
Cd300c2	+		Hmga2	+	+
Edn1			Ccl2		
Cd276			Dmpk	+	
Tgm2	+	+	Tnfsf15	+	
Fcgr4	+	+	Odc1	+	
Gm10260			Gm9260		
IL10ra	+		Scin	+	+
Sema4a	+		Car2		
Ccr12			Kbtbd11	+	
Ass1	+		Ccl7		
Adgre	+	+	Hnrnpm	+	
Pik3r6	+		Slc6a4	+	+
Il4i1	+		Dmwd		
Bcl3	+		Mmp9	+	
Tnfsf9			Tnfsf8	+	
Fcgr1	+		Sema7a	+	
Igsf6	+	+			
Lacc1	+	+			
Zc3h12c	+				
Gngt2					
Plk2					
Gm4070	+				
Bcl2a1a					
Rsad2	+				
Lsp1	+	+			
Cmpk2	+				
Fabp4	+				
Stat5a	+	+			
Napsa	+				
Ppr1r15a					
Gk	+	+			
Myo1f	+				
Gpnmb	+	+			

Arhgef3	+	+			
Tnfaip3	+				
Nfkbiz	+	+			
Syk	+	+			
Tagap		+			
Itgal	+	+			
Mpeg1					
Cebpb					
Dusp2					
Ctsf					
Fam129a	+				
Zfp36					
Saa3	+				
Slc44a1	+	+			
Ldlr	+				
Ptafr	+	+			
Acp2	+				
Fam177a	+				
Birc3	+				
Anpep	+				
Myo1g	+				
Tnfrsf1b	+	+			
Il18	+				
Tnfaip2					
Cxcl16					
Traf1	+	+			
Gbp5	+				
Blnk	+				
Atf3					
Lrrc25	+				
Jak2	+	+			
Gadd45b					
Icam1	+	+			

Table S2

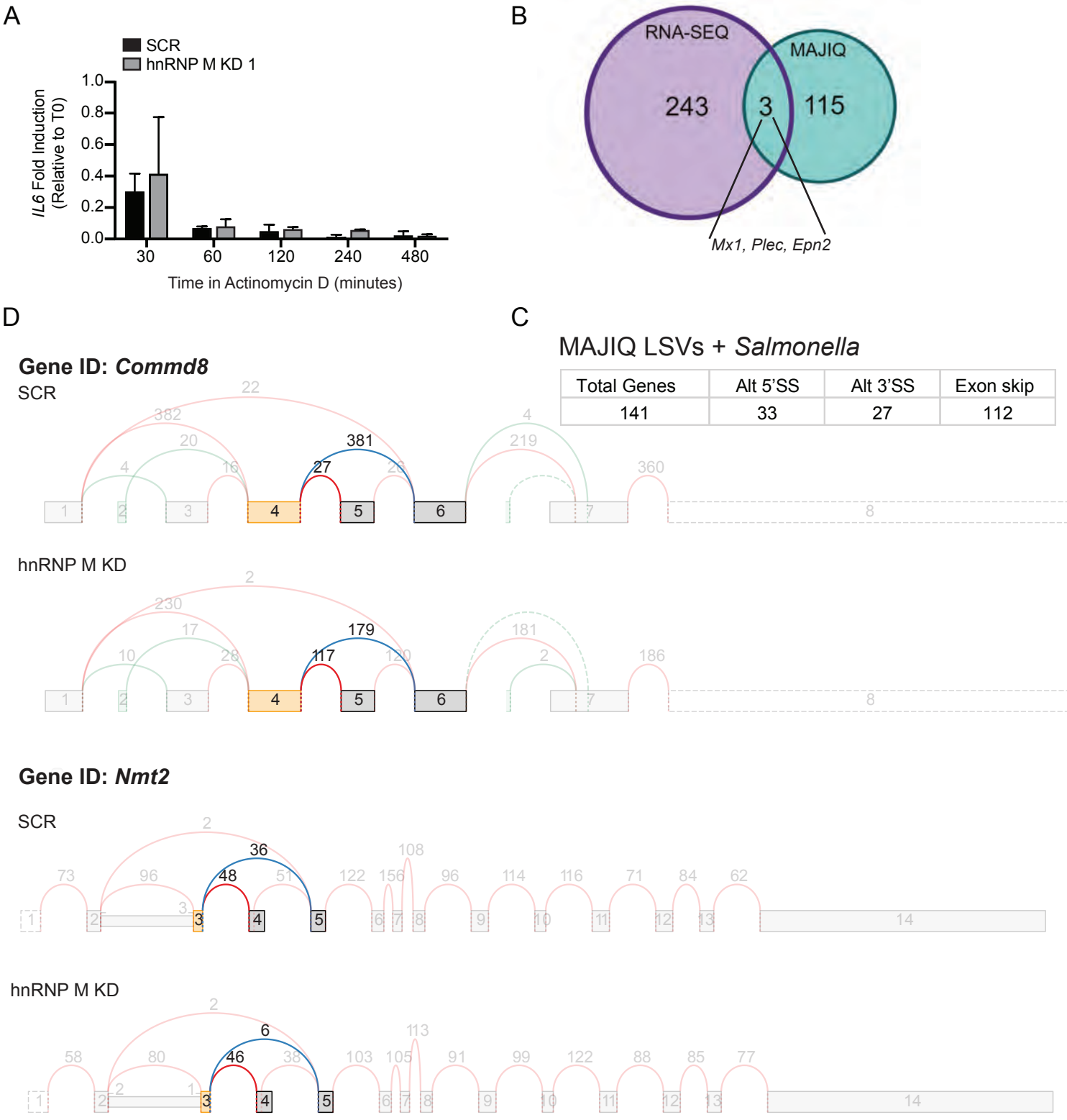


Figure S3. Related to Figure 3. hnRNP M influences gene expression outcomes at the level of pre-mRNA splicing.
(a) IL6 expression by RT-qPCR in SCR, hnRNP M KD1 cells treated with LPS for 1hr then treated with Actinomycin D for 0, 30, 60, 120, 240, and 480 mins. (b) Venn diagram to represent overlap between hnRNP M-regulated transcripts identified via RNA-Seq analysis (unique genes identified in Salmonella and uninfected analyses) and hnRNP M-dependent LSVs identified via MAJIQ analysis (total number of unique LSV events from both Salmonella and uninfected conditions). (c) Categorization of alternative splicing events identified via MAJIQ in uninfected vs. Salmonella-infected samples. (d) Full VOILA-generated tracks for Commd8 and Nmt2. Significant LSVs are shown in color.

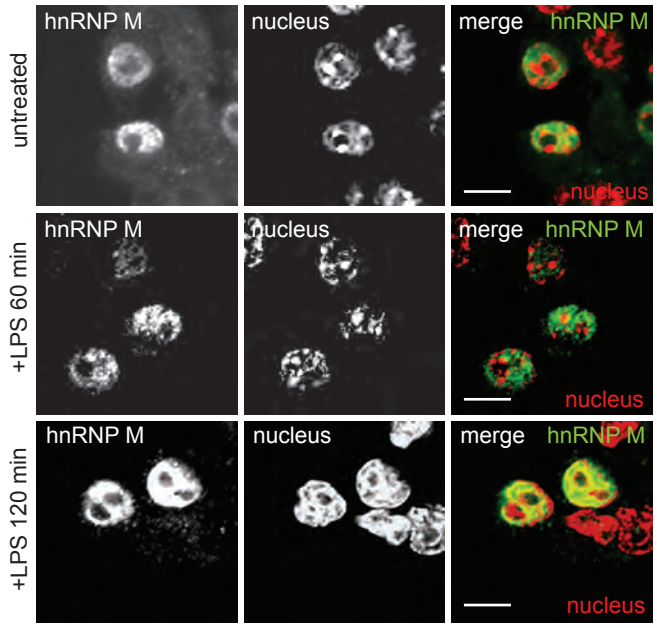
MAJIQ GENES (UN+SAL)	Found in eCLIP (ENCODE)	RNA-Seq Uninfected	Found in eCLIP (ENCODE)	RNA-Seq +*Salmonella*	Found in eCLIP (ENCODE)
1190007I07Rik				Fabp7	
Acvr1	*	Hpgd		Ms4a6	
Aldh3a2		Cxcr4		Ms4a4c	
Amotl1	*	Ms4a6c		Ms4a6d	
Asxl1	*	Csf3r		Il6	
Atxn2	*	Fabp7		Marcks	*
Auh	*	S1pr1		Gm16685	
Ccne2		Cd33		Adora2a	*
Chka	*	Ms4a6d		Ighm	*
Cnot4	*	Neurl3		Slc28a2	
Commd8		Pros1	*	Cfb	
Dcaf8	*	Prss46		Slamf9	
Desi2	*	Prss50		Rpl27-ps3	
Dock10		St6gal1	*	Mx1	
Dtx2	*	Ighm		Cd36	
Ercc8		Cd36		Ctsc	*
Frmd4a	*	Il10ra		C3	
Gfod1	*	Gm12166		Fcgr3	
Gm29247		Fabp4		Fabp3	
Hps5		Aph1c		Smpdl3a	
Ist1	*	Fosb		Pld4	
Kat6a	*	Atf3		Nectin2	
Kat6a		Tnfrsf1b		Dusp1	
Klf7	*	Tgfbr1	*	Cd300e	
Ktn1	*	Fcgr3		Gm5431	
Lrmp		Clec4a3		Havcr2	
Mdm4	*	Il7r		Fcgr2b	
Mx1		Tmem86a		Spen	*
Nmt2	*	Cav2		C3ar1	
Numb		Gm6377		Cd300c2	
Pak1	*	Cd300c2		Siglece	
Pds5b	*	Fabp3		Edn1	
Phactr4	*	Smpdl3a	*	Cd276	*
Plec	*	Ptafr		Tgm2	
Plxnc1		Pld4		Fcgr4	
Rpl22	*	Plk2		Gm10260	
Sec14l1	*	Btg2		Il10ra	
Senp1		C3ar1		Sema4a	
Senp6	*	Wfdc17		Ccrl2	
Smc6		Cd276	*	Ass1	*
Tbc1d7		Itgam_1		Adgre1	
Tex30		Ctsc	*	Il4i1	*
Tmem87a	*	Egr2		Pik3r6	
Trmu	*	Tgm2		Bcl3	
Ubqln1	*	Egr1	*	Tnfsf9	
Xpo6		Dusp1		Fcgr1	
Zfyve27	*	Mpeg1		Igsf6	
Zmynd8	*	Bhlhe41		Lacc1	
1110051M20Rik		Xdh		Zc3h12c	
4833420G17Rik		Lst1		Gngt2	
Ankra2		Lsp1		Gm4070	
Aplp2	*	Gnpda1		Plk2	
Atf2	*	Tlr13		Bcl2a1a	
Ccdc77		Myo1f		Rsad2	
Ccdc82	*	Rap2b		Lsp1	
Clk4	*	Jun		Cmpk2	
Csnk1d	*	Arl11		Fabp4	
Def8	*	Rnf26_1		Stat5a	*
Dmac2		Havcr2		Napsa	
Ehmt1	*	Fblim1		Ppp1r15a	

Emsy	*	Mcoln2	*	Gk	
Epn2	*	Rnf128		Myo1f	
Fbxo34	*	Rab32		Gpnmb	
Foxd2os		Tnfaip2	*	Arhgef3	*
Foxo3	*	Dhrs3	*	Tnfaip3	*
Gabpb2		Litaf	*	Nfkbiz	*
Ggta1		Ctsf		Syk	
Gm46430		Cxcl16		Tagap	
Golgb1	*	Gstm1		Itgal	
Herc2	*	Gngt2		Tmem51	*
Lair1		Zfp385a		Mpeg1	
Luzp1	*	Pbxip1		Cebpb	
Lyar		Hist1h1c		Dusp2	
Mettl2		Gpnmb		Ctsf	
Mycbp2	*	Napsa		Fam129a	
Myo18a	*	Fos		Zfp36	
Ncapg2	*	Cotl1		Saa3	
Nsmaf	*	Slc15a4	*	Slc44a1	*
Panx1		Tmem51		Ldlr	
Patz1	*	Id3		Ptafr	
Ppcdc		Slc44a1	*	Fam177a	
Ppp2r5c	*	Ier2	*	Birc3	
Pradc1		Msrb1		Anpep	
Prepl		Il10rb		Myo1g	
Prr14	*	Bcl2a1d		Tnfrsf1b	
Ptger4		Ctnnb1	*	Il18	
Ptpn4	*	Slc6a8		Tnfaip2	*
Rnf20		Nceh1		Cxcl16	
Snx12		Sptssa	*	Traf1	
Sptan1		Gyg		Gbp5	
Srrm2	*	Rgs2		Blnk	
St7	*	Plin2		Atf3	
Tmem161b	*	B4galt1	*	Gadd45b	
Tmem229b		Rnh1		Lrrc25	
Tmem80		Tbc1d16		Jak2	*
Ttc13	*	Zfp36		Icam1	
Vegfa		Fabp5		Capg	*
Zmym3		Cybb		Ubald2	
		Anpep		Rab31	*
		Alox5ap		Pdia3	
		Pdia3		Atp1b3	*
		Tubb6		Snx9	*
		Tmsb10		Ets2	*
		Sumo2		Plau	*
		Ccl3		Naa25	
		Pip5k1b	*	Gm26619	
		Hmga2	*	Mrps6	*
		Atp1b3	*	Spp1	
		Ank	*	Tfdp1	*
		Cpne8	*	Serinc2	
		Fcrl1		Tmem98	
		Rbpj	*	Dhrs9	
		Tfdp1	*	Rpl10-ps3	*
		Slc6a12		Spink5	
		Snx9	*	Epn2	*
		Prelid2	*	Ccz1	
		Slc16a3		Ier3	
		Fosl2		Acy1	
		Mrps6	*	Ccl17	
		Acot7		Ccnd1	
		Oas2		Lif	
		Ehd1		Rgs16	
		Ccnd1		Csf3	
		Marcksl1	*	Npl	
		Bnip3	*	Timp1	

		Ak4	*	Hmga2	*
		Ets2	*	Ccl2	
		Acy1		Dmpk	
		Isg15		Tnfsf15	
		Odc1	*	Odc1	*
		Gm28037		Pdia3	
		Layn		Scin	
		Naa25	*	Car2	
		Plekha3		Kbtbd11	*
		Ccz1		Ccl7	
		Gm9803		Hnrnpm	*
		Rtp4		Gm18445	
		Npl		Slc6a4	
		Spp1		Dmwd	
		Dmpk		Mmp9	
		Emp2		Tnfsf8	
		Hnrnpm		Sema7a	*
		Fosl1			
		Spink5			
		Gm18445			
		Tmem26			
		Flt1			
		Serinc2			
		Rgs16			
		Car2			
		Gm21987			
		Dmwd			
		Slc6a4			
		Mmp9			
		1810011H11Rik			
		AB124611			

Table S3

A



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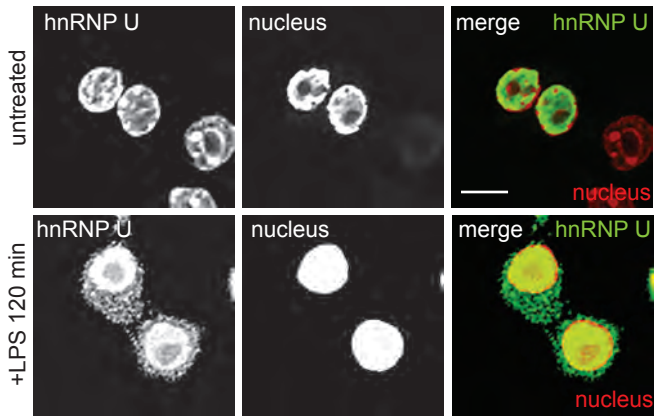

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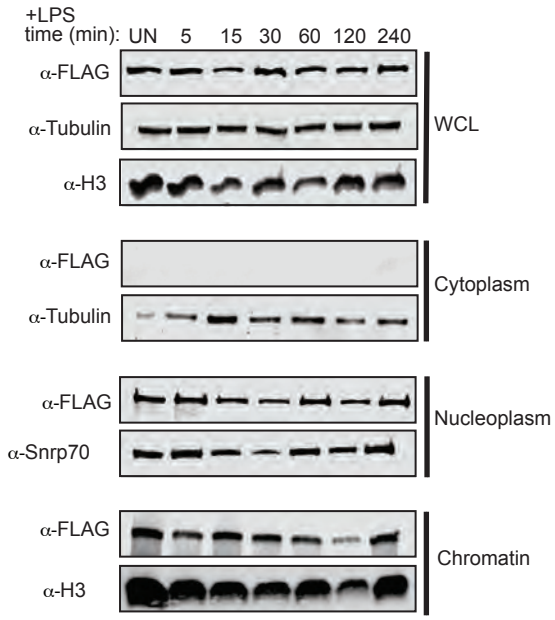

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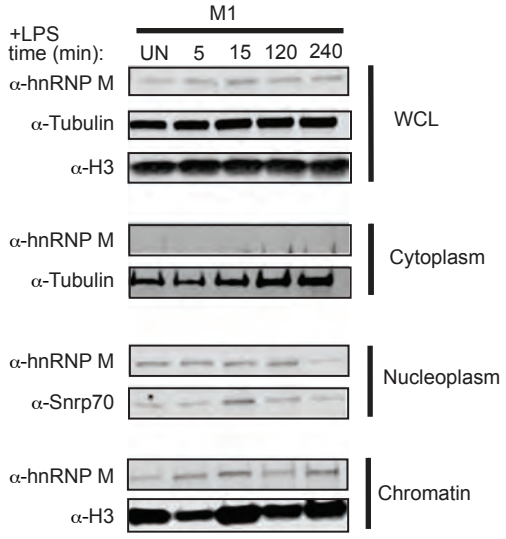


E

Supplemental Figure S4

Figure S4. Related to Figure 4. hnRNP M associates with chromatin at the *IL6* genomic locus. (a) Immunofluorescence microscopy of 3xFLAG-hnRNP M in untreated and LPS-treated macrophages (1hr and 2hr post-treatment). (b) Immunofluorescence microscopy of hnRNP U in untreated and LPS-treated macrophages (2hr post-treatment). (c) Western blot of whole cell lysate, cytoplasm, nucleoplasm, and chromatin of fractionated stable 3xFL-hnRNP M-expressing macrophages over a time-course of LPS treatment. (d) Western blot analysis of cellular fractions with anti-hnRNP M and loading controls of cytoplasm (tubulin), nucleoplasm (Snrp70) and chromatin (H3) fractions of uninfected and LPS stimulated hnRNP M KD 1 macrophages. (e) RNA sequence of *IL6* introns 2 and 3. Consensus or near-consensus hnRNP M binding sites are highlighted in yellow.

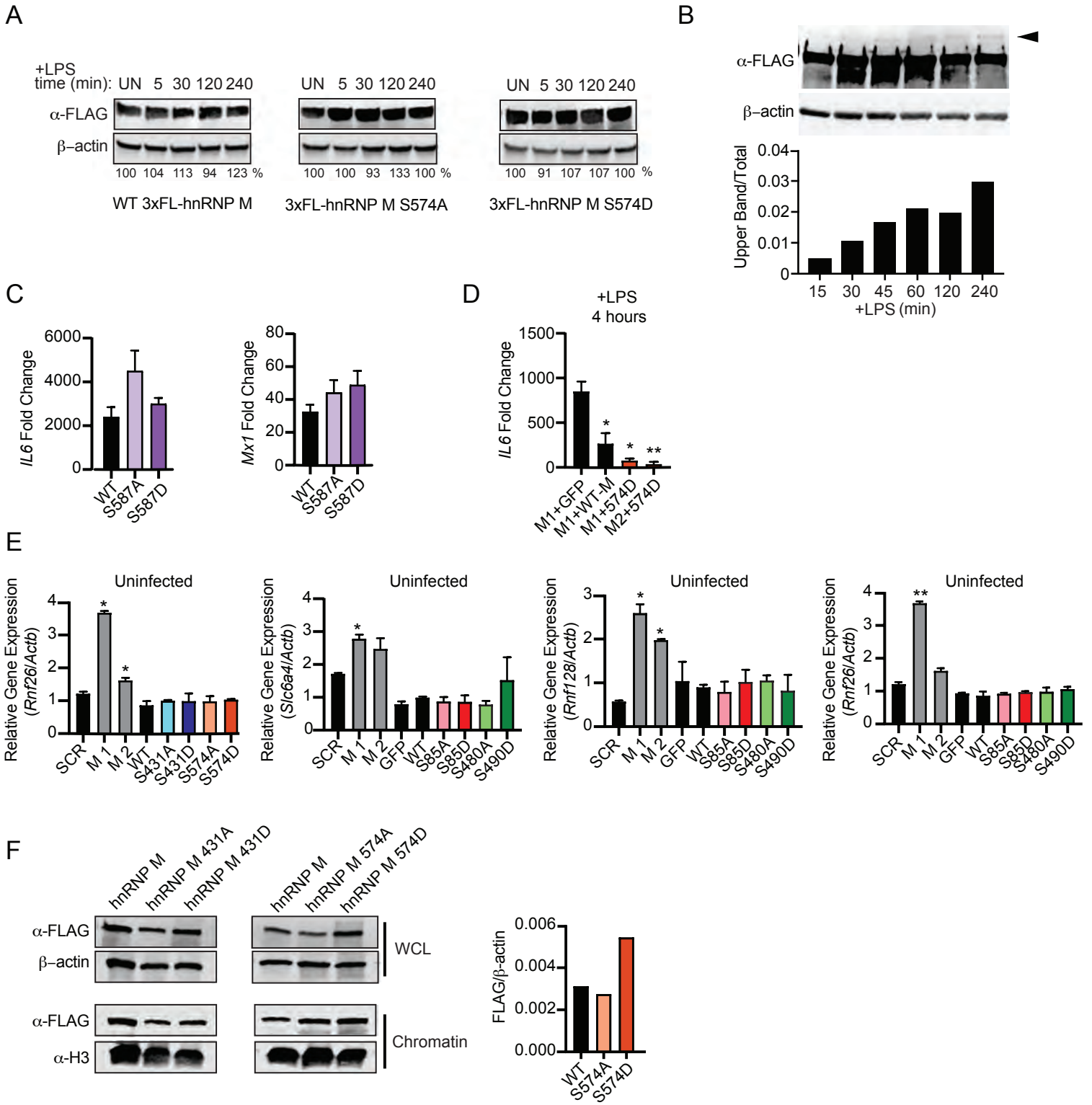


Figure S5. Related to Figure 5. Specific phosphorylation of hnRNP M controls its ability to repress expression of innate immune transcripts.

(a) Western blot analysis of 3xFL-hnRNP M WT, S574A, S574D phoshomutants stably expressed in RAW 264.7 macrophages treated with LPS for 5, 30, 120, and 240 mins. (b) Western blot analysis of phosphorylation events of 3xFL-hnRNP M WT stably expressed in RAW 264.7 macrophages treated with LPS for 15, 30, 45, 60, 120, and 240 mins. (c) IL6 and Mx1 expression by RT-qPCR, 2hr post-LPS treatment in 3xFL-hnRNP M WT and 3xFLhnRNP M S587A/D-expressing macrophages. (d) IL6 expression by RT-qPCR, 4hr post-LPS treatment in hnRNP M KD 1 macrophages complemented with 3xFL-GFP, 3xFL-hnRNP M WT, or S574D and hnRNP M KD 2 macrophages complemented with S574D. (e) Expression by RT-qPCR of Rnf26, Slc6a4, Rnf128 for 3xFL-hnRNP M WT, hnRNP M KD 1 and hnRNP M KD 2, and 3xFL-S431A/D, S574A/D, S85A/D, S480A/D-expressing macrophages. (f) Western blot of whole cell lysate and chromatin of fractionated stable 3xFL-hnRNP M WT, 3xFL-hnRNP M 431A/D and 3xFL-hnRNP M 574A/D-expressing macrophages. FLAG expression was quantified over βactin.

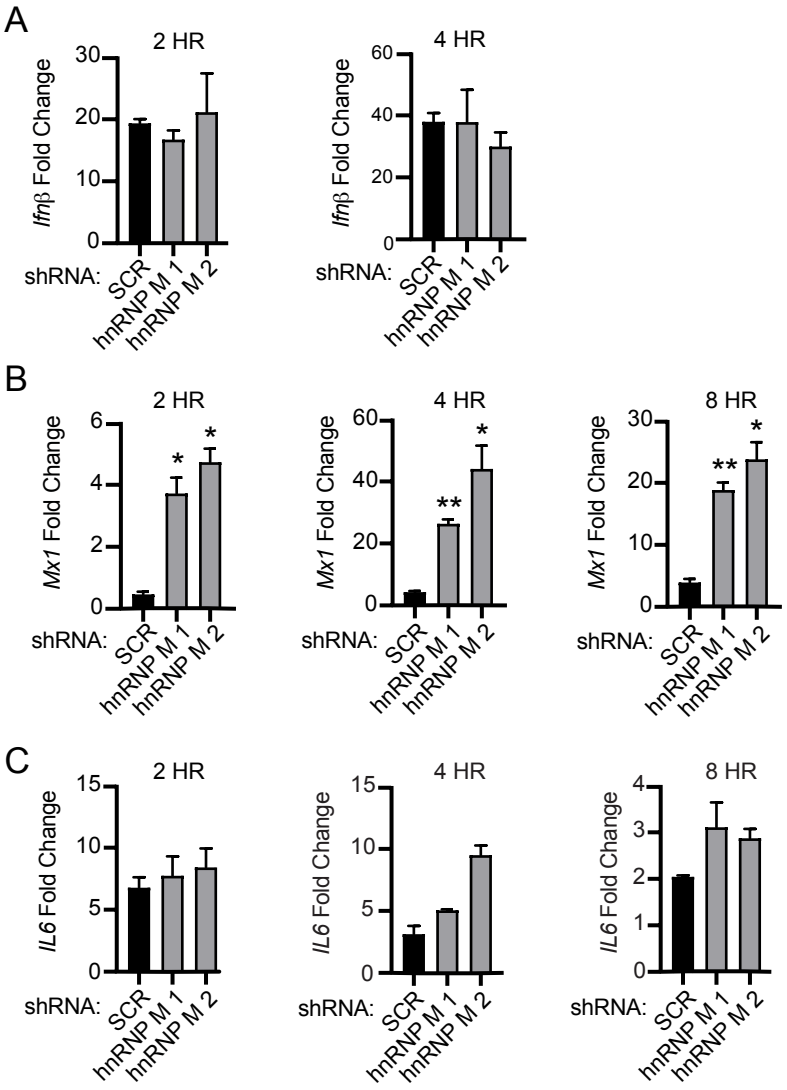
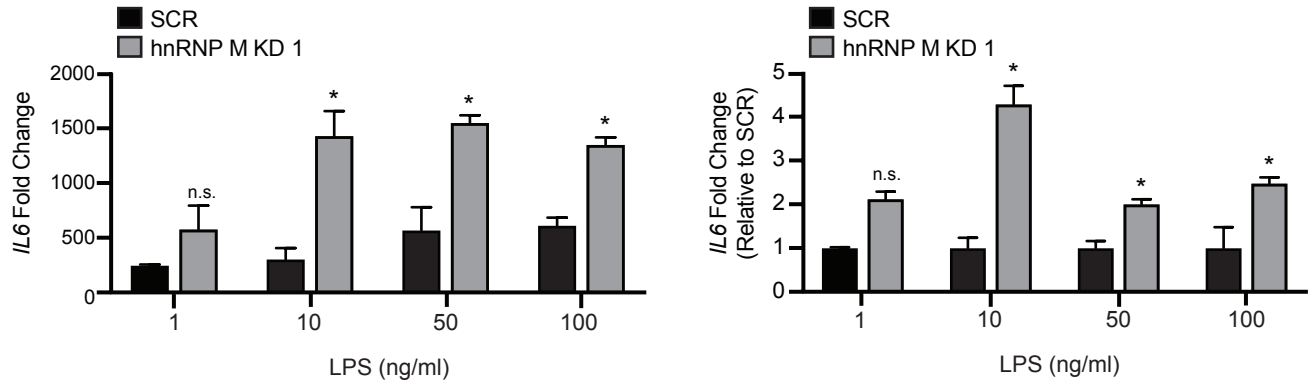


Figure S6. Related to Figure 6. hnRNP M influences innate immune gene expression during viral infection.
(a) RT-qPCR of Ifnβ mRNA levels in SCR control and hnRNP M KD cells at 2hr and 4hr post-infection, MOI=0.1. (b) RT-qPCR of Mx1 transcript in VSV infected SCR control and hnRNP M KD cells at 2hr, 4hr, and 8hr post-infection, MOI=0.1. (c) RT-qPCR of IL6 transcript in SCR control and hnRNP M KD cells at 2hr, 4hr, and 8hr post-infection, MOI=0.1. All figures are representative of 2 biological replicates.

A



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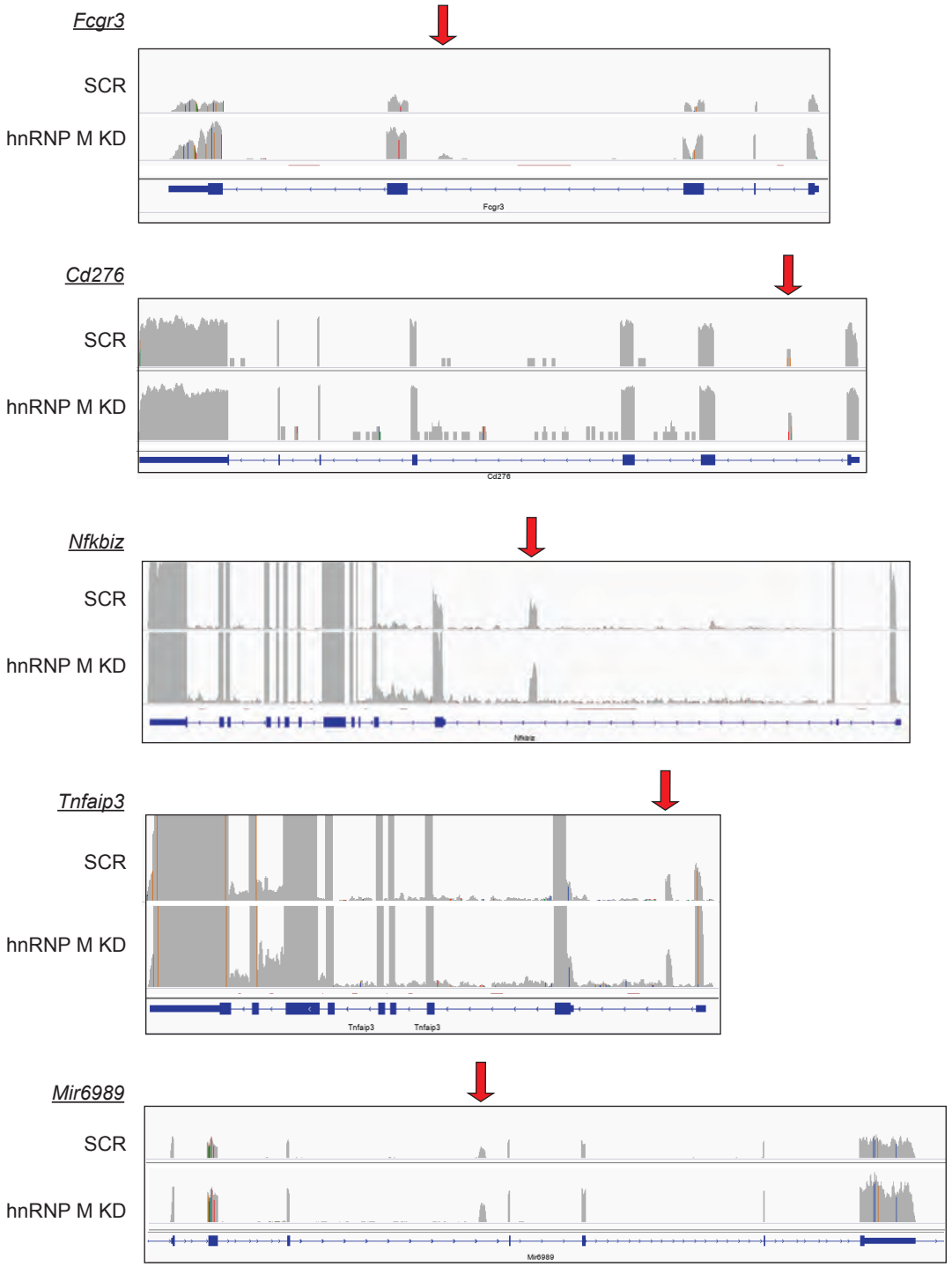

Supplemental Figure S7

Figure S7. Related to Figure 7. hnRNP M can respond to low levels of innate immune stimuli and may influence differential inclusion of cryptic exons.
(a) RT-qPCR of *IL6* mRNA levels in SCR control and hnRNP M KD cells at 2h treated with 100ng/ml, 50ng/ml, 10ng/ml and 1ng/ml. Data is expressed as fold-change over time = 0 as well as fold-change relative to SCR. (a) is representative of 2 biological replicates with values indicating means ± SD, n=2. Statistical significance was determined using two-tailed students' *t*-test. *P < 0.05, **P < 0.01, n.s.= not significant. (b) Screenshots of IGV viewer of *Salmonella*-infected SCR and hnRNP M KD RNA-Seq reads at *Fcgr3, Cd276, Nfkbiz, Tnfaip3,* and *Mir6989*. Red arrows indicate potential cryptic exons.

Primer: *Rnf26* F: AGAGCGGAAGAAGTGTGTTATC R: ATGAGGATCTCAGTGCAAGC
Primer: *Rnf128* F: CTTCAGTACAAGGAGCAGATGAG R: ATCCACGGCCACAGAATTT
Primer: *Slc6a* F: GTGTGTGAGGTTTAAGGTGAGTAG R: CAGAGGCCATTAGTGACATACC
Primer: *IL6* exon 1 F: GGGATGTCTGTAGCTCATTCTG R: AGAGGAACTTCATAGCGGTTTC
Primer: *IL6* intron 1 F: GCCCAACTGTGCTATCTGCT R: TCAGTCCCAAGAAGGCAACT
Primer: *IL6* exon 2 F: AGTTGCCTTCTTGGGACTGA R: GTCTCCTCTCCGGACTTGTG
Primer: *IL6* exon junction 1-2 F: GTCAATTCCAGAAACCGCTATG R: GGACTTGTGAAGTAGGGAAGG
Primer: *IL6* exon 2 F: TTGCCTTCTTGGGACTGATG R: TTAAGCCTCCGACTTGTGAAG
Primer: *IL6* intron 2 F: TGGTGCTTGTTGTAAGAGGTG R: TGCAAGTGCATCATCGTTGT
Primer: *IL6* exon junction 2-3 F: CTTCACAAGTCGGAGGCTTAAT R: GCAAGTGCATCATCGTTGTTC
Primer: *IL6* exon 3 F: GCAATGGCAATTCTGATTGTATGC R: ATCCAGTTTGGTAGCATCCATC
Primer: *IL6* intron 3 F: GAAAGAACTGACTTCCTTTTCCA R: GGTACTCCAGAAGACCAGAGGA
Primer: *IL6* exon 4 F: TTTCCTCTGGTCTTCTGGAGTA R: TGTATCTCTCTGAAGGACTCTGG
Primer: *IL6* intron 4 F: TGTGCAATATTTAACCAGTCTTTG R: GGAAATTGGGGTAGGAAGGA
Primer: *IL6* exon junction 3-4 F: GAAATGATGGATGCTACCAAACTG R: ACTCCAGAAGACCAGAGGAA
Primer: *IL6* exon 5 F: CCCAATTTCCAATGCTCTCCTA R: GGTTTGCCGAGTAGATCTCAAA
Primer: *IL6* exon junction 4-5 F: ACAAAGCCAGAGTCCTTCAG R: GTTAGGAGAGCATTGGAAATTGG

Primer: *Mx1* F: TCTGAGGAGAGCCAGACGAT R: ACTCTGGTCCCCAATGACAG
Primer: *TNF* F: ATGGCCTCCCTCTCATCAGT R: GTTTGCTACGACGTGGGCTA
Primer: *Gbp5* exon 4 F: AGGAGGCATCCAGGTCAAC R: TGTGTTCTCTATGGAAGGCAGA
Primer: *Ifnβ* set 1 F: GCAAGCTCAGGATCGCTATTA R: GGGACTCTTCCATCCTGAGT
Primer: *IRF7* F: CTTCAGCACTTTCTTCCGAGA R: TGTAGTGTGGTGACCCTTGC
Primer: *Commd8* F (exon 3): TGAGTGGAAGCATGTTCTCG R (exon 5): GCTGCGCACAAGAAATATCA
Primer: *Nmt2* F (exon 3): CATCTGGCAGCAGATTTCAG R (exon 5): GTGGCCTCATCAATGTTCCT
Primer: *IFIT* F: AGCTTTAGGGCAAGGAGAAC R: CGTAGCCTATCGCCAAGATTTA
Primer: *hnRNP M* KD check F: TTTGACCGAGCCATTGAGAT R: CTTCCTGGCTACTCCAGGTG
Primer: *Adora2a* set 3 F: GCTATTGCCATCGACAGATACA R: GTACCACGTCCTCAAACAGAC
Primer: *Marcks* set 2 F: GTGCCCAGTTCTCCAAGAC R: TTTACGTGGCCATTCTCCTG
CHIP Primer: IL6 1 F: CAGCAGTGGGATCAGCACTAA R: CCCAGTGGTCTCTTGGCTATC
CHIP Primer: IL6 2 F: TGAGGCTAGCCCTAAGAAGCA R: CCATCAAGACATGCTCAAGTGC
CHIP Primer: IL6 3 F: GAAAACCGGCAAGTGAGCAG R: CCTCTGGCGGAGCTATTGAG
CHIP Primer: IL6 4 F: TTTGAGTGGAGGTTGGGAAGG R: TGAGGGAAAAGGAATCCCCAC
CHIP Primer: IL6 5 F: TTTGACTCTGCTTGACAGAAAGG R: TGGTTCTACACACCCAGACCT
CHIP Primer: IL6 6 F: AACGATGATGCACTTGCAGA R: ACGTGCAAGAGAGAACCTTGA

CHIP Primer: IL6 7 F: ACAGGTAAAGGCCCACTATGC R: GTGTAGGCCTGGATCCTTCC
CHIP Primer: IL6 8 F: GGGAAGGAGTGAGGGTCAGAA R: GCATCAGCTGACCTCCTGAATTA
CHIP Primer: IL6 9 F: TCCCTAGGTGGGGTAGTCTC R: GGGGAACAAAATACCCATGGAAG
CHIP Primer: IL6 end F: AGTGGCAGACAGAACAGTAAGG R: TTTGACTCACCCAAGCCAGG
CHIP Primer: Intergenic Control F: ATTTTGTGCTGCATAACCTCCT R: TAGCAACATCCTAAGCTGGACA
Primer: VSV-G F: CAAGTCAAAATGCCCAAGAGTCACA R: TTTCCTTGCATTGTTCTACAGATGG
Primer: VSV-M F: ATGATCCGAATCAATTAAGATATG R: GGGACGTTTCCCTGCCATTCCGATG
Primer: shRNA pSICO SCR: F: TCCTAGGTTAAGTCGCCCTTTCAAGAGATAGA AGGGCGACTTAACCTAGGTTTTTTC R: TCGAGAAAAAACCTAGGTTAAGTCGCCCTTCTCTTGAA AGGGCGACTTAACCTAGGA
Primer: shRNA hnRNP M1: F: TGGAAGATGCTAAAAGGACAATTCAAGAGATTGTCCTT TAGCATCTTCCTTTTTTC R: TCGAGAAAAAAGGAAGATGCTAAAGGACAATCTCTTGA ATTGTCCTTTAGCATCTTCC
Primer: shRNA hnRNP M2: F: TGCACAGTATTTGTAGCAAATTCAAGAGATTTGCTAC AAATACTGTGCTTTTTTC R: TCGAGAAAAAAGCACAGTATTTGTAGCAAATCTCTTGAA TTTGCTACAAATACTGTGC

Table S4