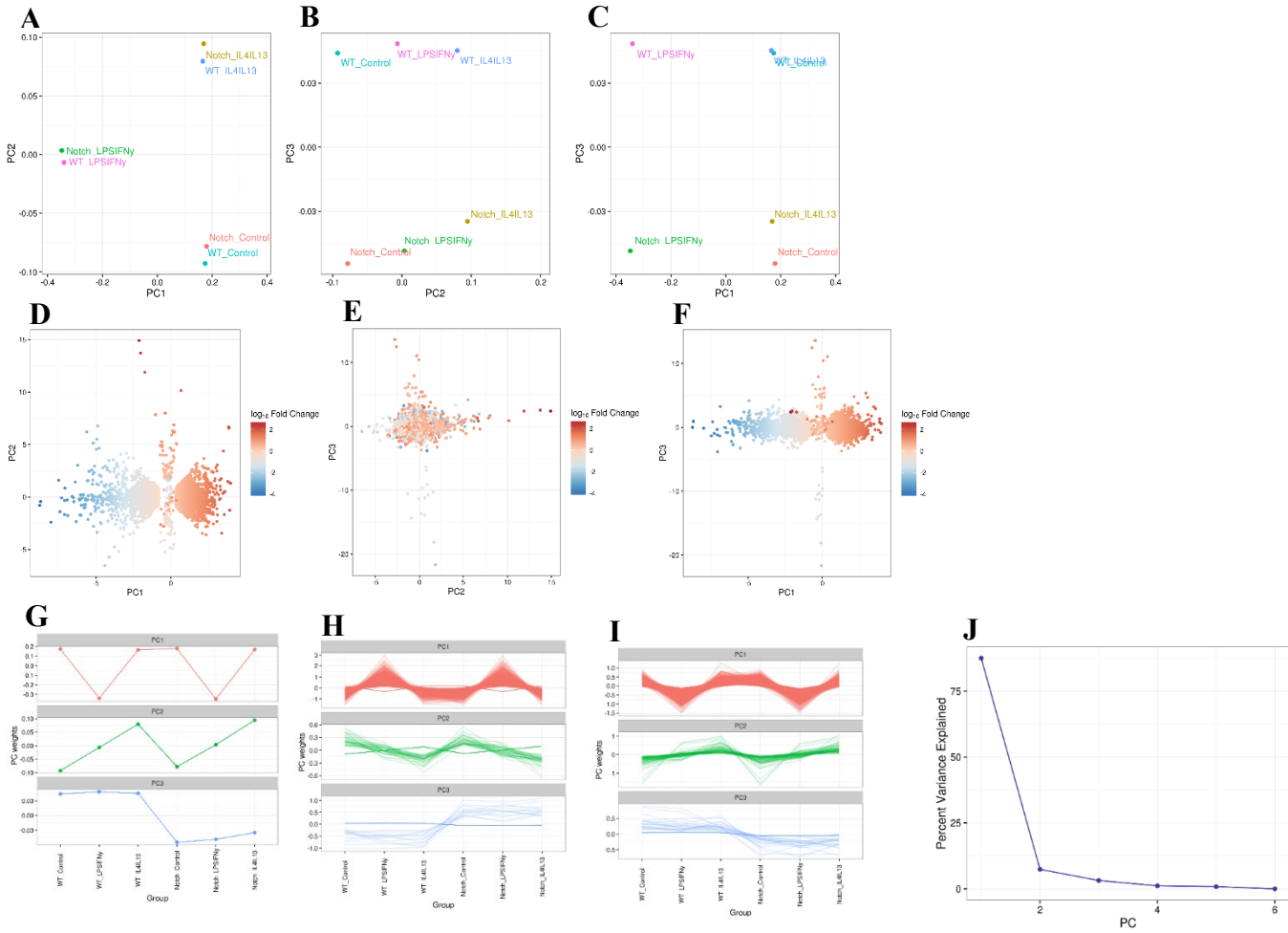


Supplementary Material

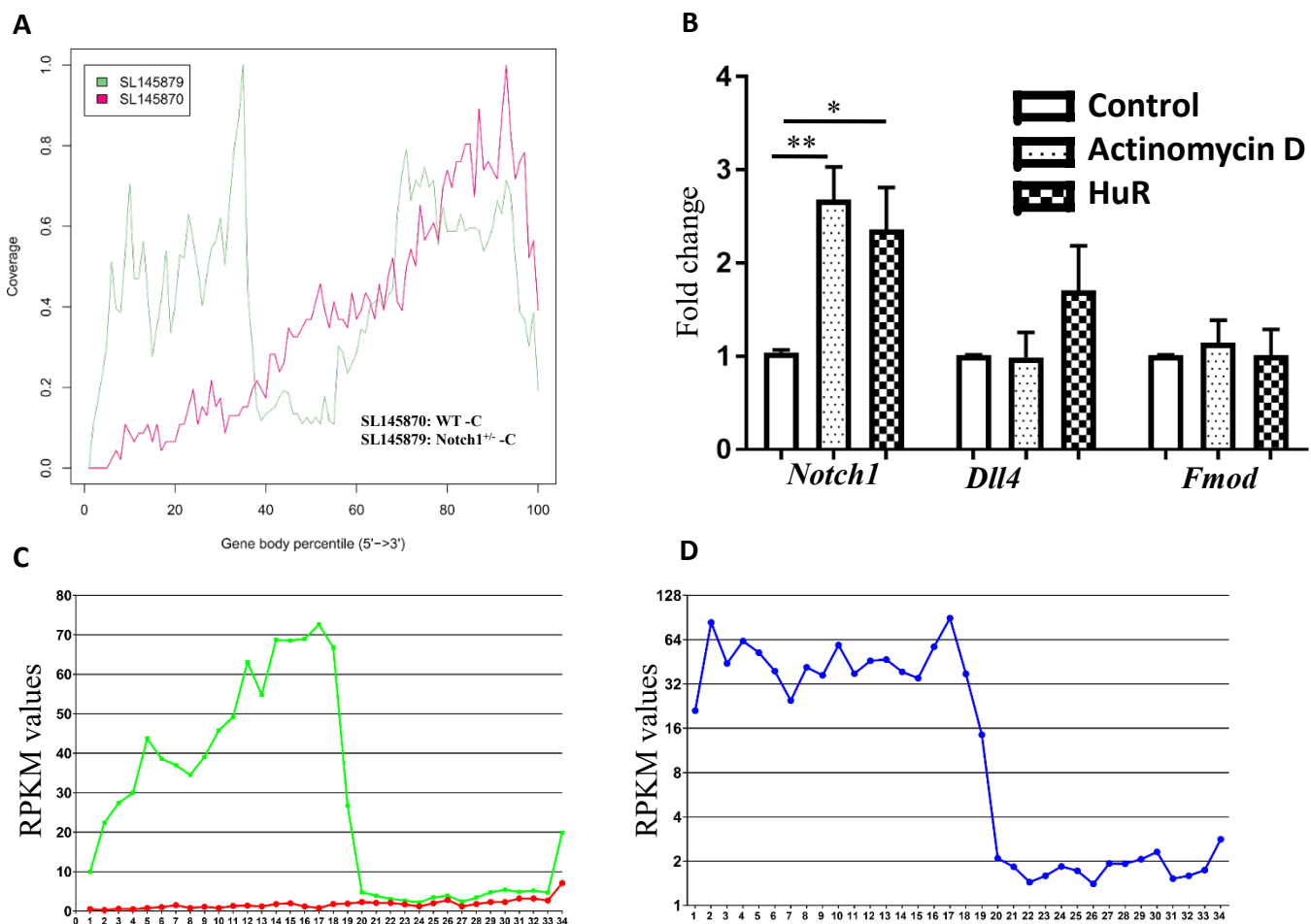
Transcriptomics Analysis Reveals New Insights into the Roles of Notch1 Signaling on Macrophage Polarization

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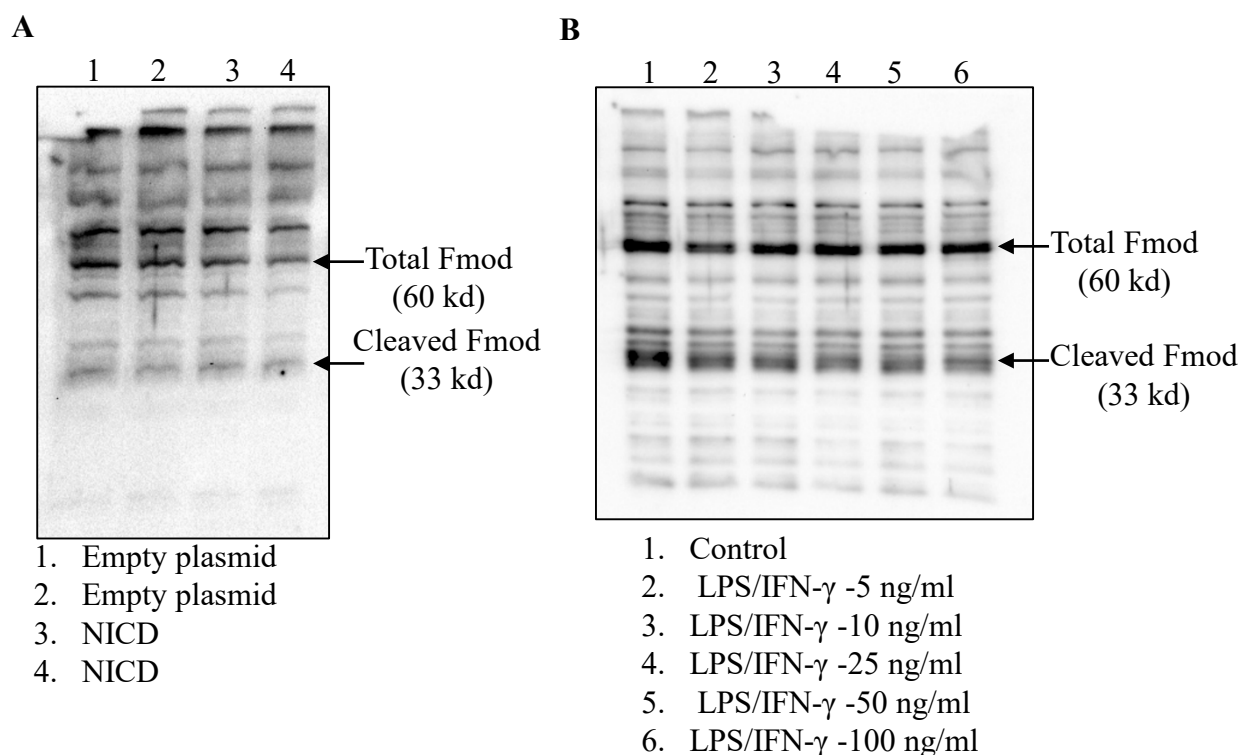
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Supplemental Figure 2: Principle component analysis of gene expression displaying the expression trends within the data set. **(A-C)** Separation of samples based on their PC composite values. Points are colored according to the group name, and the color scale is arbitrary. **(D-F)** Separation of genes based on their PC composite values. Only genes meeting the cluster criteria are shown. **(G)** Sample-specific PC Plots. RPKM for individual genes making up their respective principal components are shown in light color. Overall value for PC composite values are shown in bold color. **(H-I)** Genes correlated with positive or negative principal component. **(J)** Screeplot of the percent variance explained versus principal component number. To find genes that substantially contribute to each PC value, the correlation and fold-change in expression of each gene with the first three principal components was calculated. Genes with a positive or negative correlation of at least 0.9 and a fold change of at least 2.0 compared with the principal components are reported.



Supplemental Figure 4: (A) Read counts for *Notch1*^{+/-} and *WT* samples. (B) RNA Stabilizers (Actinomycin D (10 μ g/ml, or human recombinant HuR (100 ng/ml) increased the expression of *Notch1* in BMDMs without affecting the expression of *Dll4* or *Fmod*. (C) shows the mean *Notch1* RPKM values for the *WT* and *Notch1*^{+/-} BMDMs for each exon in the full-length transcript. (D) shows the ratio of the mean RPKM values for *Notch1*^{+/-} vs. *WT*. Note that the expression of *Notch1* mRNA is 30-60-fold higher in the *Notch1*^{+/-} vs. *WT* only for the undelated first 19 exons, consistent with dramatic up-regulation of the mutated *Notch1* allele. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.



Supplemental Figure 5: (A) Full WB showing the expression of total and cleaved Fmod in *WT* BMDMs 48h post transfection with empty or NICD plasmid. (B) Full WB showing the contents of total and cleaved Fmod in macrophages in response to increasing dose of LPS (0, 5, 10, 25, 50 or 100 ng/ml) for 24h.

Supplemental Table 1: List of top genes dysregulated by LPS/IFN- γ

■ Fold Change: up > 2 fold
■ Fold Change: down < 0.5 fold

S. No.	Gene ID	Gene Name	WT LPS/IFN- γ vs WT-M ϕ	Notch1 ^{+/-} LPS/IFN- γ vs Notch1 ^{+/-} M ϕ
1	17329	<i>Cxcl9</i>	26243.32	15172.68
2	18126	<i>Nos2</i>	14898.17	35964.89
3	16365	<i>Irg1</i>	8960.42	6616.27
4	19225	<i>Ptgs2</i>	7256.59	2268.96
5	15945	<i>Cxcl10</i>	7213.04	6245.32
6	16193	<i>Il6</i>	4387.86	4768.75
7	20304	<i>Ccl5</i>	3391.98	4754.51
8	13421	<i>Dnase1l3</i>	3288.47	3622.68
9	20210	<i>Saa3</i>	2817.02	2268.62
10	17472	<i>Gbp4</i>	2506.48	1659.34
11	20299	<i>Ccl22</i>	2199.95	2017.01
12	238393	<i>Serpina3f</i>	2021.20	2498.68
13	16160	<i>Il12b</i>	1959.08	4016.97
14	229898	<i>Gbp5</i>	1741.78	2242.01
15	12515	<i>Cd69</i>	1595.76	2054.14
16	60440	<i>Iigp1</i>	1494.22	1697.15
17	231655	<i>Oasl1</i>	1106.89	3758.70
18	14289	<i>Fpr2</i>	1026.45	898.70
19	109979	<i>Art3</i>	1023.14	1101.72
20	326623	<i>Tnfsf15</i>	990.52	642.62
21	24108	<i>Ubd</i>	906.18	717.80
22	21822	<i>Tgtp1</i>	904.47	1242.62
23	20293	<i>Ccl12</i>	875.29	673.83
24	16176	<i>Il1b</i>	873.11	1199.94
25	13011	<i>Cst7</i>	869.13	1111.09
26	14825	<i>Cxcl1</i>	845.78	284.09
27	56066	<i>Cxcl11</i>	779.22	550.88
28	626578	<i>Gbp10</i>	772.37	823.51
29	20310	<i>Cxcl2</i>	750.98	680.95
30	16161	<i>Il12rb1</i>	716.27	463.93
31	14962	<i>Cfb</i>	667.71	516.41
32	634650	<i>Gbp11</i>	667.59	513.69
33	100702	<i>Gbp6</i>	661.31	886.68
34	80885	<i>Hcar2</i>	657.11	711.28
35	64380	<i>Ms4a4c</i>	566.50	1319.13
36	17167	<i>Marco</i>	518.59	246.42
37	20555	<i>Slfn1</i>	482.35	953.07
38	12810	<i>Coch</i>	474.72	618.18
39	623121	<i>Pydc4</i>	469.02	867.32
40	12775	<i>Ccr7</i>	460.98	369.44
41	224796	<i>Clic5</i>	451.55	746.93
42	14990	<i>H2-M2</i>	431.79	264.50
43	20287	<i>Sct</i>	403.29	164.53
44	14468	<i>Gbp2b</i>	400.89	644.90
45	12349	<i>Car2</i>	384.47	816.98
46	99899	<i>Ifi44</i>	366.79	1092.65
47	12266	<i>C3</i>	337.74	260.37
48	20714	<i>Serpina3k</i>	335.78	221.05
49	22271	<i>Upp1</i>	334.34	411.95
50	17857	<i>Mx1</i>	328.68	1035.83

S. No.	Gene ID	Gene Name	WT LPS/IFN- γ vs WT-M ϕ	Notch1 ^{+/-} LPS/IFN- γ vs Notch1 ^{+/-} M ϕ
1	64214	<i>Rgs18</i>	0.01	0.01
2	71738	<i>Mamdc2</i>	0.01	0.01
3	17179	<i>Matk</i>	0.01	0.01
4	76408	<i>Abcc3</i>	0.01	0.02
5	71085	<i>Arhgap19</i>	0.02	0.01
6	22403	<i>Wisp2</i>	0.02	0.01
7	140483	<i>Hnmt</i>	0.02	0.03
8	107585	<i>Dio3</i>	0.02	0.02
9	18054	<i>Ngp</i>	0.02	0.05
10	381810	<i>Lpar5</i>	0.02	0.01
11	72324	<i>Plxdc1</i>	0.02	0.01
12	11565	<i>Adssl1</i>	0.02	0.02
13	19716	<i>Bex1</i>	0.02	0.04
14	19400	<i>Rapsn</i>	0.02	0.03
15	406217	<i>Bex4</i>	0.02	0.05
16	12097	<i>Bglap2</i>	0.02	0.04
17	12487	<i>Cd28</i>	0.02	0.01
18	140742	<i>Sesn1</i>	0.02	0.02
19	12096	<i>Bglap</i>	0.02	0.04
20	71706	<i>Slc46a3</i>	0.02	0.02
21	19016	<i>Pparg</i>	0.02	0.04
22	75744	<i>Svip</i>	0.02	0.04
23	194590	<i>Reps2</i>	0.02	0.03
24	21953	<i>Tnni2</i>	0.02	0.03
25	239405	<i>Rspo2</i>	0.02	0.02
26	11302	<i>Aatk</i>	0.02	0.02
27	26874	<i>Abcd2</i>	0.02	0.02
28	66447	<i>Mgst3</i>	0.03	0.03
29	12766	<i>Cxcr3</i>	0.03	0.02
30	16170	<i>Il16</i>	0.03	0.03
31	66355	<i>Gmpr</i>	0.03	0.01
32	97998	<i>Deptor</i>	0.03	0.02
33	14431	<i>Gamt</i>	0.03	0.02
34	12442	<i>Ccnb2</i>	0.03	0.01
35	269642	<i>Nat8l</i>	0.03	0.03
36	14456	<i>Gas6</i>	0.03	0.02
37	217262	<i>Abca9</i>	0.03	0.02
38	213573	<i>Cracr2b</i>	0.03	0.02
39	22402	<i>Wisp1</i>	0.03	0.03
40	233552	<i>Gdpd5</i>	0.03	0.02
41	213068	<i>Tmem71</i>	0.03	0.03
42	78771	<i>Mctp1</i>	0.03	0.03
43	16970	<i>Lrmp</i>	0.03	0.03
44	66425	<i>Pcp4l1</i>	0.03	0.03
45	240168	<i>Rasgrp3</i>	0.03	0.02
46	321019	<i>Gpr183</i>	0.03	0.03
47	381101	<i>Dnph1</i>	0.03	0.06
48	12925	<i>Crip1</i>	0.03	0.04
49	217946	<i>Cdca7l</i>	0.03	0.02
50	15985	<i>Cd79b</i>	0.03	0.04

Supplemental Table 2: List of top genes dysregulated by IL4/IL13.

■ Fold Change: up > 2 fold
■ Fold Change: down < 0.5 fold

S. No.	Gene ID	Gene Name	WT IL4/IL13 vs WT-Mφ	Notch1 ^{+/+} IL4/IL13 vs Notch1 ^{+/+} Mφ
1	104183	<i>Chil4</i>	661.44	1189.90
2	12655	<i>Chil3</i>	504.92	1049.84
3	58205	<i>Pdcd1lg2</i>	360.54	434.36
4	57262	<i>Retnla</i>	299.69	311.49
5	93726	<i>Rnase2a</i>	141.66	93.64
6	19662	<i>Rbp4</i>	129.07	295.54
7	12700	<i>Cish</i>	110.57	134.17
8	327766	<i>Tmem26</i>	106.27	101.68
9	229687	<i>Chil5</i>	87.30	130.85
10	229688	<i>Chil6</i>	62.31	98.82
11	16165	<i>Il13ra2</i>	52.81	61.25
12	20292	<i>Ccl11</i>	37.46	45.23
13	245126	<i>Tarm1</i>	31.21	25.26
14	17207	<i>Mcf2l</i>	22.45	27.39
15	16364	<i>Irf4</i>	20.35	17.29
16	215446	<i>Entpd3</i>	18.79	4.97
17	11846	<i>Arg1</i>	18.68	17.00
18	319930	<i>Ceacam19</i>	17.24	16.22
19	107221	<i>Ffar4</i>	16.54	17.14
20	11988	<i>Slc7a2</i>	15.83	14.05
21	13011	<i>Cst7</i>	15.76	17.93
22	245533	<i>Awat1</i>	15.74	17.64
23	327957	<i>Scimp</i>	14.35	7.89
24	70166	<i>Lipn</i>	13.46	7.88
25	16625	<i>Serpina3c</i>	13.45	11.72
26	14254	<i>Ftl1</i>	13.21	10.58
27	12703	<i>Socs1</i>	13.18	18.46
28	20299	<i>Ccl22</i>	13.08	13.81
29	104886	<i>Rab15</i>	12.54	12.63
30	12845	<i>Comp</i>	12.09	6.01
31	19224	<i>Ptgs1</i>	12.07	10.88
32	76477	<i>Pcolce2</i>	12.04	12.22
33	240327	<i>Gm4951</i>	11.76	9.62
34	406217	<i>Bex4</i>	11.50	14.89
35	56221	<i>Ccl24</i>	11.26	4.68
36	216864	<i>Mgl2</i>	10.91	14.95
37	19716	<i>Bex1</i>	10.77	15.12
38	110074	<i>Dut</i>	10.33	7.03
39	233079	<i>Ffar2</i>	9.96	15.32
40	108956	<i>Apol7c</i>	9.91	10.09
41	665033	<i>Col6a5</i>	9.46	6.96
42	380728	<i>Kcnh4</i>	9.26	7.80
43	101772	<i>Ano1</i>	9.24	2.17
44	71111	<i>Gpr39</i>	8.99	5.94
45	20293	<i>Ccl12</i>	8.77	4.79
46	15891	<i>Ibsp</i>	8.43	13.07
47	15220	<i>Foxq1</i>	8.42	13.01
48	15558	<i>Htr2a</i>	8.05	4.45
49	15937	<i>Ier3</i>	7.92	7.39
50	67573	<i>Lox14</i>	7.77	2.01

S. No.	Gene ID	Gene Name	WT IL4/IL13 vs WT-Mφ	Notch1 ^{+/+} IL4/IL13 vs Notch1 ^{+/+} Mφ
1	229499	<i>Fcrl1</i>	0.03	0.06
3	105349	<i>Akr1c18</i>	0.07	0.12
4	17392	<i>Mmp3</i>	0.07	0.09
5	12766	<i>Cxcr3</i>	0.08	0.06
6	56811	<i>Dkk2</i>	0.08	0.07
7	14985	<i>H2-M10.1</i>	0.09	1.00
8	66425	<i>Pcp4l1</i>	0.10	0.06
9	11670	<i>Aldh3a1</i>	0.10	0.16
10	14191	<i>Fgr</i>	0.11	0.30
11	23890	<i>Gpr34</i>	0.13	0.15
12	12273	<i>C5ar1</i>	0.13	0.13
13	57442	<i>Kcne3</i>	0.13	0.26
14	56620	<i>Clec4n</i>	0.14	0.10
15	14788	<i>Gpr162</i>	0.14	0.15
16	12161	<i>Bmp6</i>	0.14	0.11
17	232413	<i>Clec12a</i>	0.14	0.10
18	106952	<i>Arap3</i>	0.15	0.13
19	18591	<i>Pdgfb</i>	0.15	0.19
20	70261	<i>Chp2</i>	0.16	0.25
21	14451	<i>Gas1</i>	0.16	0.39
22	242425	<i>Gabbr2</i>	0.16	0.22
23	50723	<i>Icosl</i>	0.16	0.17
24	53945	<i>Slc40a1</i>	0.17	0.14
25	11522	<i>Adh1</i>	0.17	0.94
26	71738	<i>Mamdc2</i>	0.17	0.29
27	170757	<i>Adgrl4</i>	0.19	0.46
28	20513	<i>Slc1a6</i>	0.19	0.08
29	108723	<i>Card11</i>	0.20	0.44
30	14585	<i>Gfra1</i>	0.20	0.30
31	67888	<i>Tmem100</i>	0.20	0.31
32	74145	<i>F13a1</i>	0.20	0.14
33	723814	<i>Mir223</i>	0.21	0.54
34	75581	<i>Yipf7</i>	0.21	0.20
35	117590	<i>Asb10</i>	0.21	0.25
36	16010	<i>Igfbp4</i>	0.22	0.14
37	16633	<i>Klra2</i>	0.22	0.29
38	20303	<i>Ccl4</i>	0.22	0.25
39	75604	<i>Tm4sf5</i>	0.22	0.44
40	216799	<i>Nlrp3</i>	0.22	0.12
41	12504	<i>Cd4</i>	0.22	0.36
42	78303	<i>Hist3h2ba</i>	0.23	0.95
43	73708	<i>Dppa3</i>	0.23	0.41
44	12721	<i>Coro1a</i>	0.23	0.23
45	17384	<i>Mmp10</i>	0.24	0.58
46	320712	<i>Abi3bp</i>	0.24	0.27
47	212398	<i>Frat2</i>	0.24	0.31
48	56198	<i>Heyl</i>	0.24	0.77
49	11629	<i>Aif1</i>	0.24	0.20
50	11784	<i>Apba2</i>	0.24	0.26

Supplemental Table 3: List of primer sequences used in quantitative real-time reverse transcriptase PCR studies.

Gene Symbol	Forward Primer	Reverse Primer
<i>Rpl13a</i>	5'- TCC CTG CTG CTC TCA AGG-3'	5'- GCC CCA GGT AAG CAA ACT T-3'
<i>Notch1</i>	5'-CCG TTA CAT GCA GCA GTT TC-3'	5'-AGC CAG GAT CAG TGG AGT TG-3'
<i>Dll4</i>	5'-GAC CTG CGG CCA GAG ACT T-3'	5'-GAG CCT TGG ATG ATG ATT TGG-3'
<i>Jag1</i>	5'-GGC TTC TCA CTC AGG CAT GAT A-3'	5'-GTG GGC AAT CCC TGT GTT TT-3'
<i>RbpjK</i>	5'-AAG AGT CTC AAC CCT GTG CG-3'	5'-CAC TGT TGT GAA CTG GCG TG-3'
<i>Hey1</i>	5'-CGC GGA CGA GAA TGG AAA CT-3'	5'-TCT CGA TGA TGC CTC TCC GT-3'
<i>Hey2</i>	5'-TGG GGA GCG AGA ACA ATT AC-3'	5'-CCT CGC CAC TTC TGT TAA GC-3'
<i>HeyL</i>	5'-GGA ACA ACA GAG AAT GAA CCA ACC-3'	5'-TCT TGA CTT TGC CAG GGA TTA GC-3'
<i>Hes1</i>	5'-CCC CAG CCA GTG TCA ACA C-3'	5'-TGT GCT CAG AGG CCG TCT T-3'
<i>Casp4</i>	5'-CAA TGG CCG TAC ACG AAA GG-3'	5'-GCC CCA TAC CTC AGT GAG AGA T-3'
<i>Fmod</i>	5'-GTC CAC CTA CTA CGA CCC CT-3'	5'-GAC AGT CGC ATT CTT GGG GA-3'
<i>Alpl</i>	5'-TGG TAT GGG CGT CTC CAC AGT AAC C-3'	5'-CTT GGA GAG GGC CAC AAA GG-3'
<i>Colla1</i>	5'-GAG CGG AGA GTA CTG GAT CG-3'	5'-GTT CGG GCT GAT GTA CCA GT-3'
<i>Igfbp2</i>	5'-CTG CAC ATC CCC AAC TGT GA-3'	5'-CGC TGT CCG TTC AGA GAC AT-3'
<i>Lum</i>	5'-TCG AGC TTG ATC TCT CCT AT-3'	5'-TGG TCC CAG GAT CTT ACA GAA-3'

Figure-5 Hans

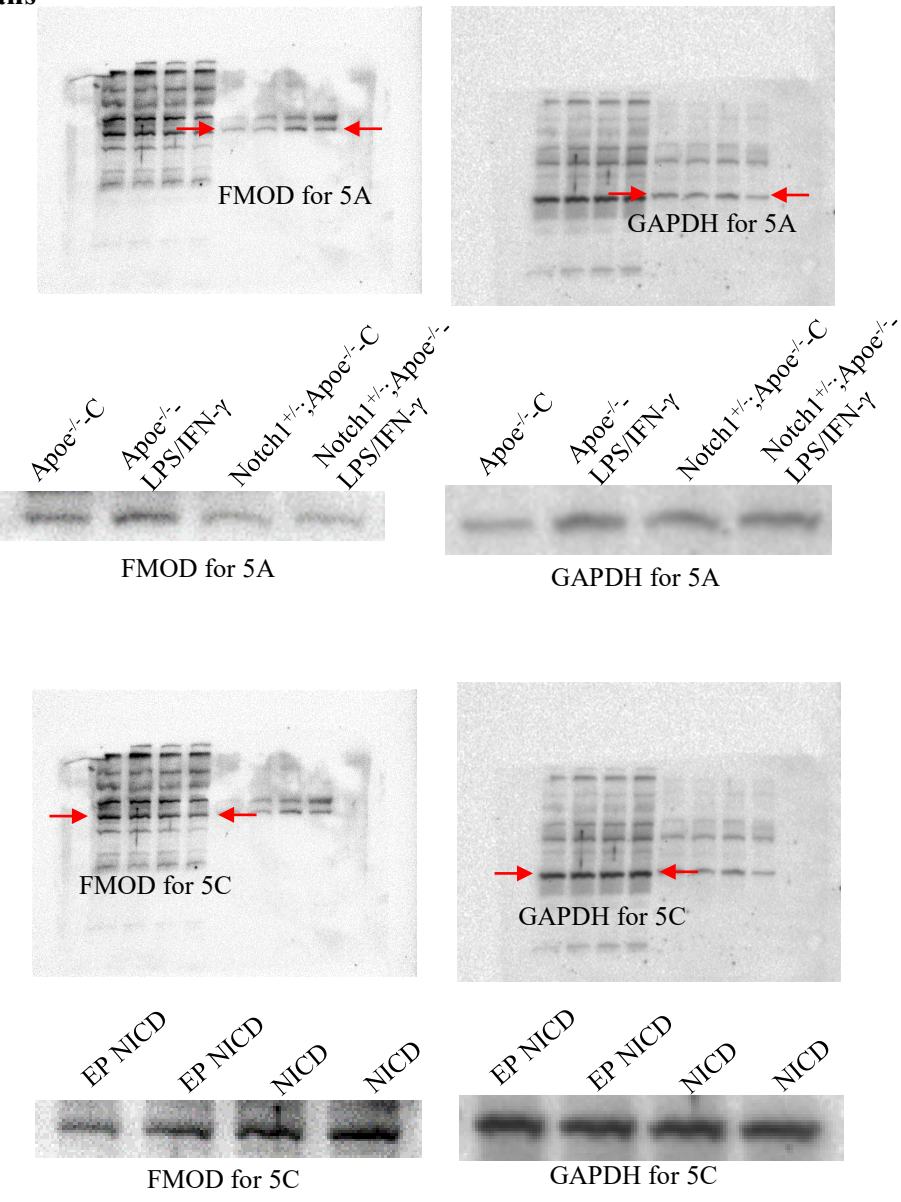


Figure-6 Hans

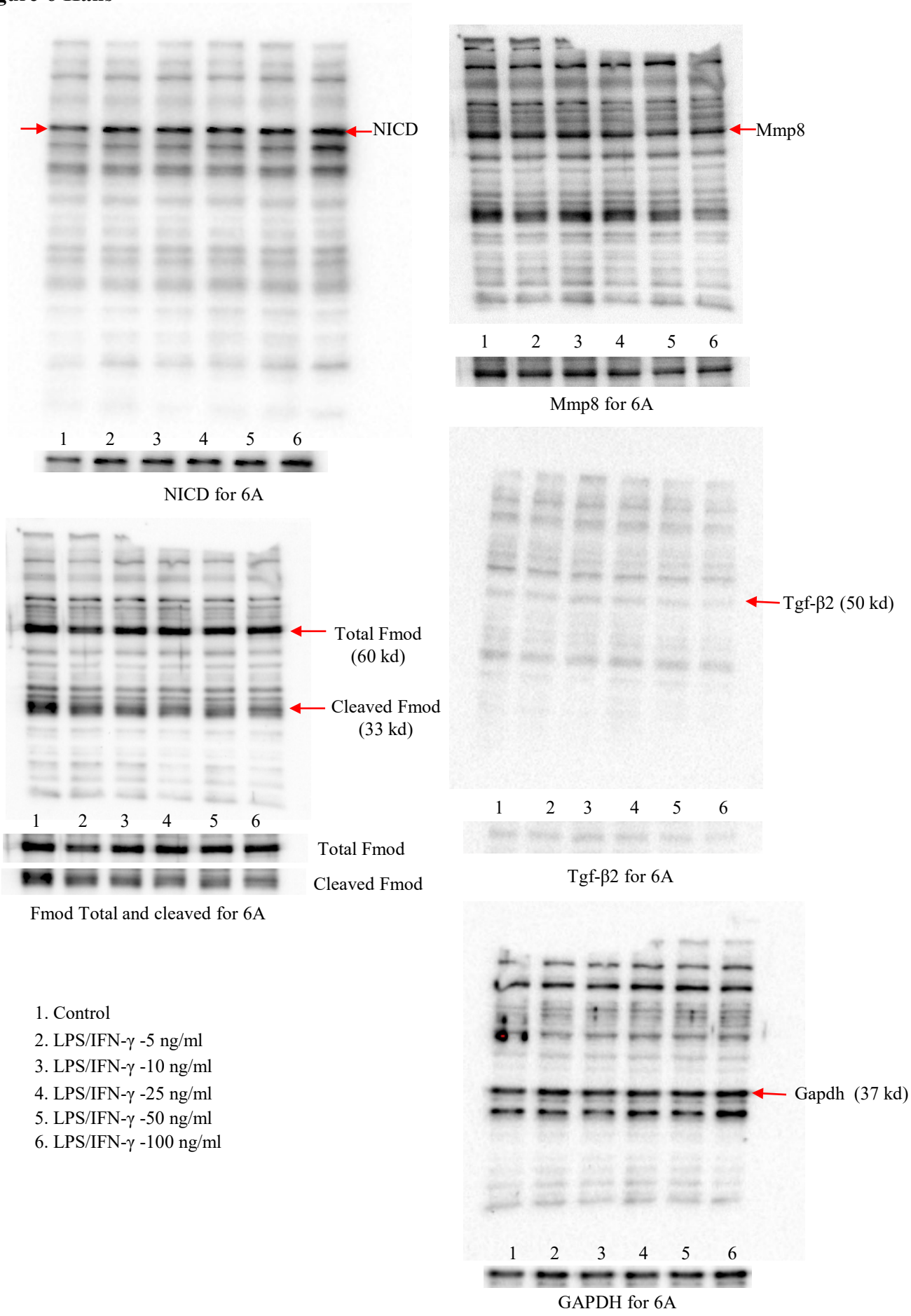
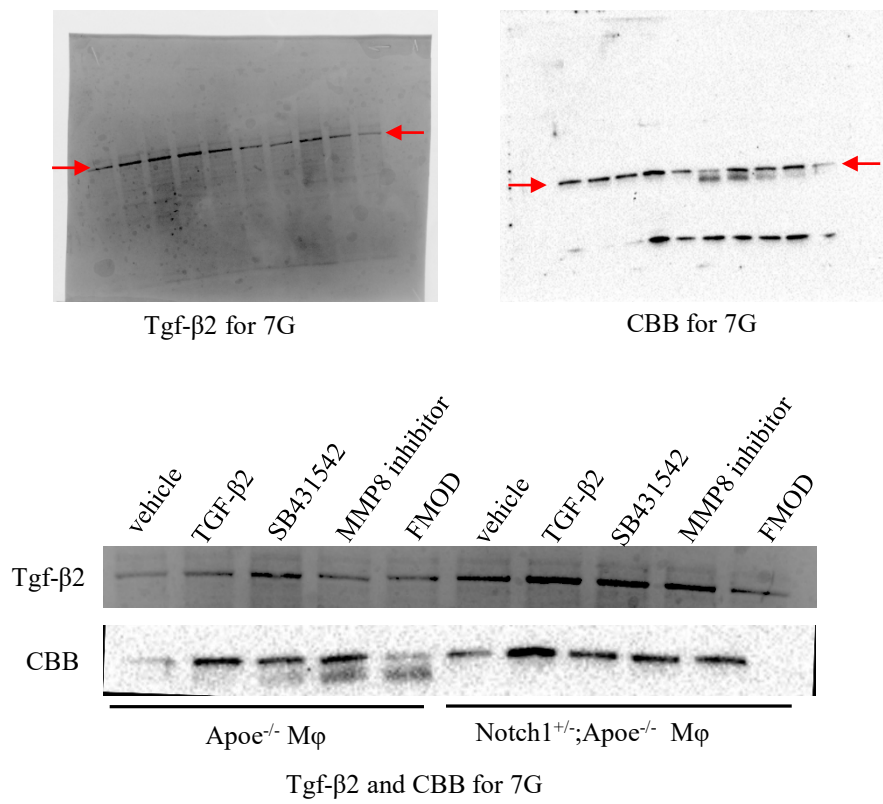
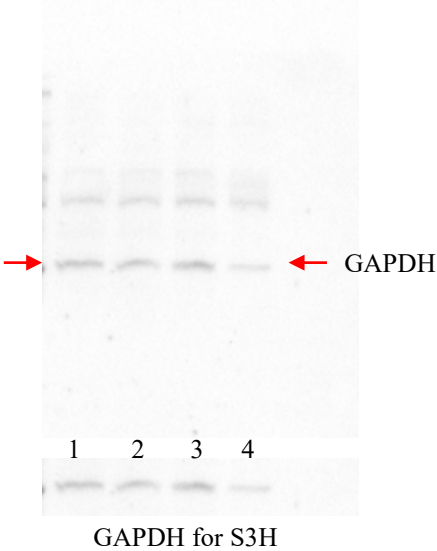
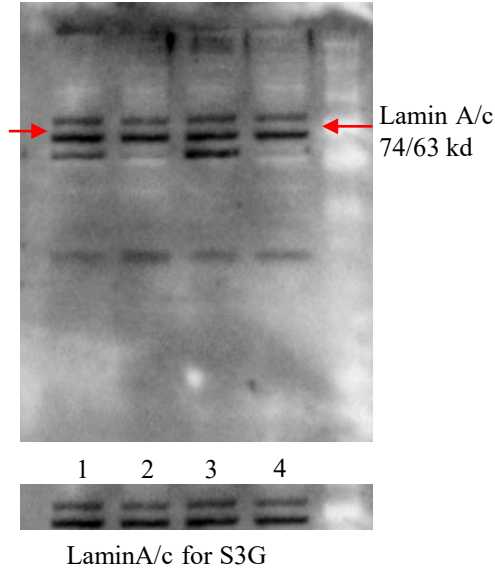
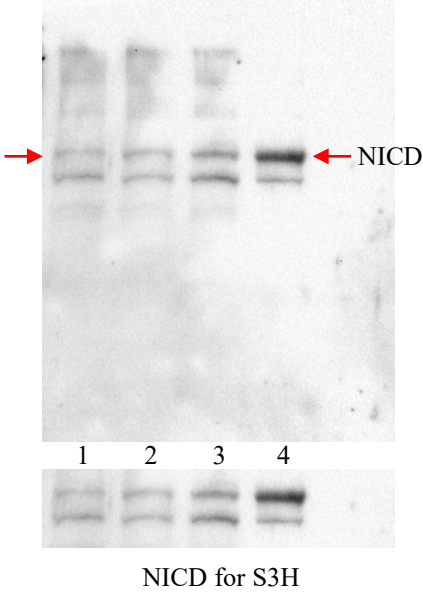
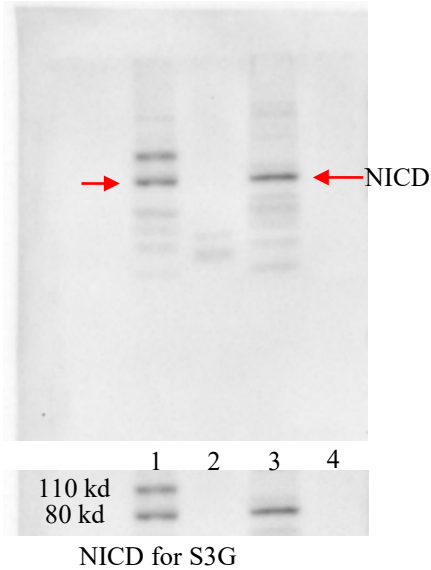


Figure-7 Hans



Supplemental Figure-3 Hans



- 1. Control
- 2. Notch1 siRNA
- 3. LPS
- 4. LPS+ Notch1 siRNA

- 1. Empty plasmid
- 2. Empty plasmid
- 3. NICD
- 4. NICD