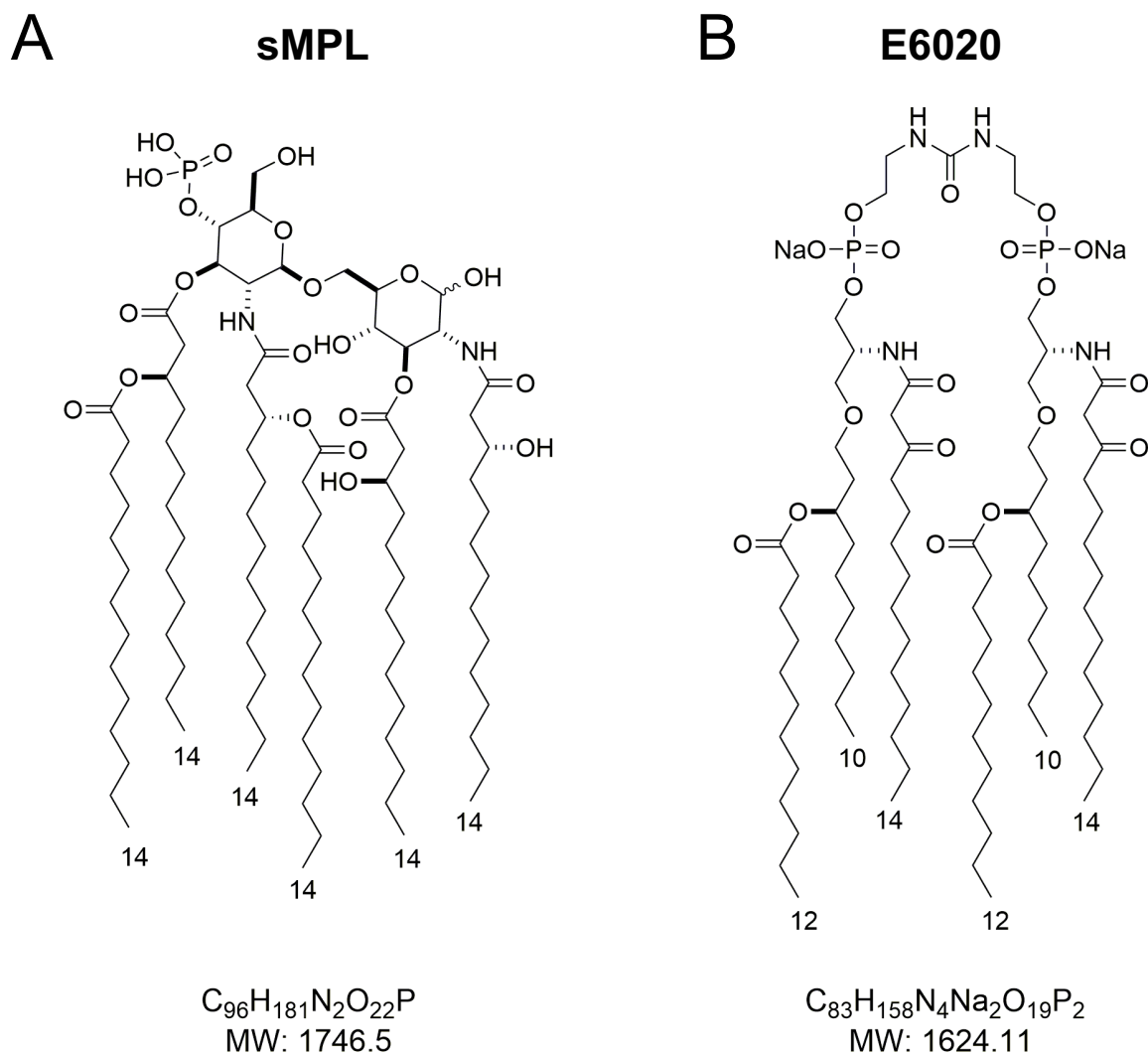


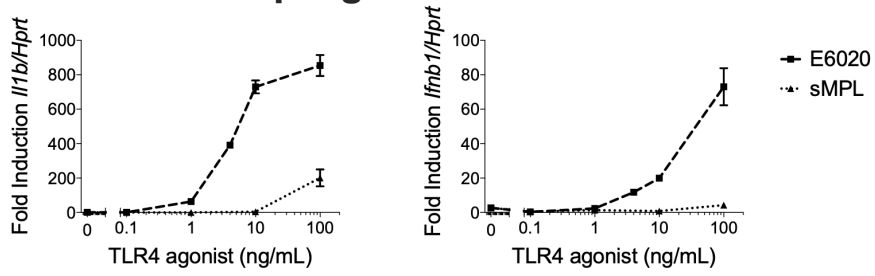
**Supplementary Materials to
Dissociation of TRIF bias and adjuvanticity**

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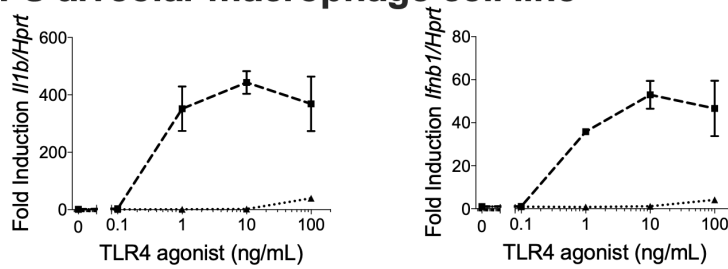


Supplementary Figure 1. Comparison of chemical structures of sMPL (PHAD®) and E6020.

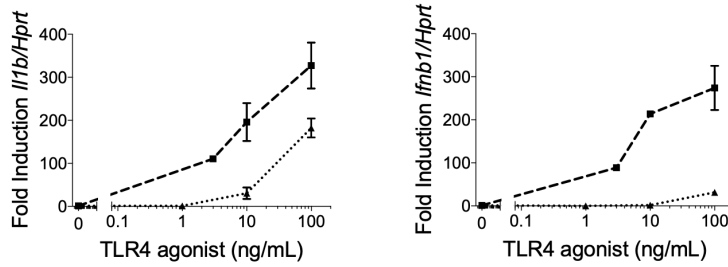
Murine macrophages RAW 264.7 macrophage cell line



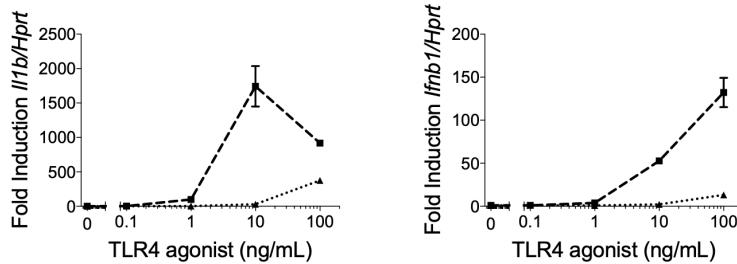
MH-S alveolar macrophage cell line



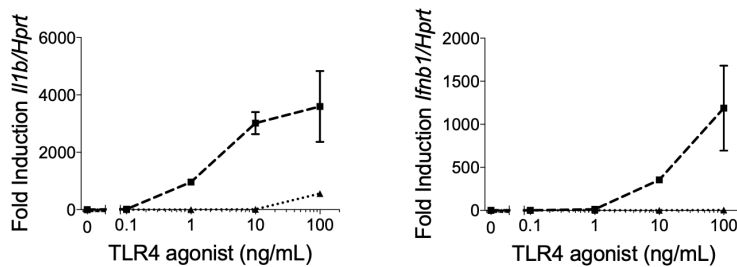
BMDM



Murine dendritic cells BMDC



Human macrophages THP-1 cell line



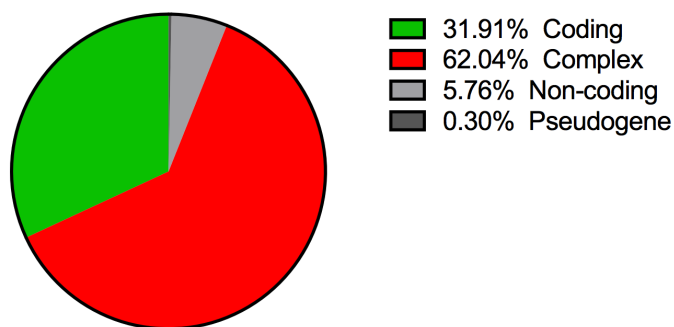
Supplementary Figure 2. Differential responses to sMPL and E6020 in diverse mouse and human antigen-presenting cells.

Murine RAW 264.7 and MH-S cell lines, C57BL/6J BMDM and BMDC, and human THP-1 cells, differentiated to macrophages with 100 nM PMA, were stimulated with 0.1 – 100 ng/mL of synthetic TLR4 agonists E6020 or sMPL (PHAD[®]) for 2 h (macrophages) or 4 h (dendritic cells). Levels of IL-1 β and IFN- β mRNA relative to HPRT mRNA, were measured by qRT-PCR. Enhanced signaling of both MyD88 and TRIF pathways and greater “TRIF-bias” by E6020 are preserved across these diverse cell types.

Supplementary Figure 3.

A.

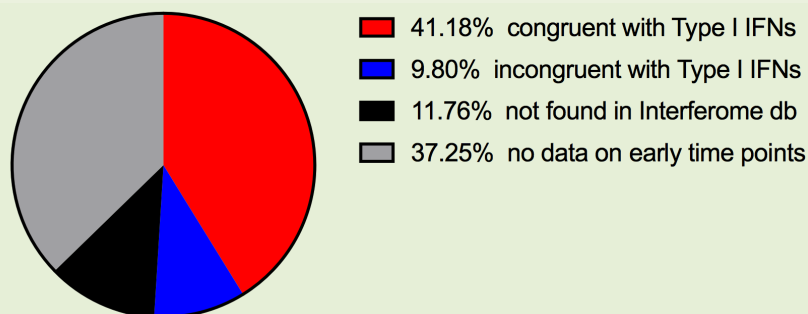
Breakdown of DE genes, FDR < 0.05



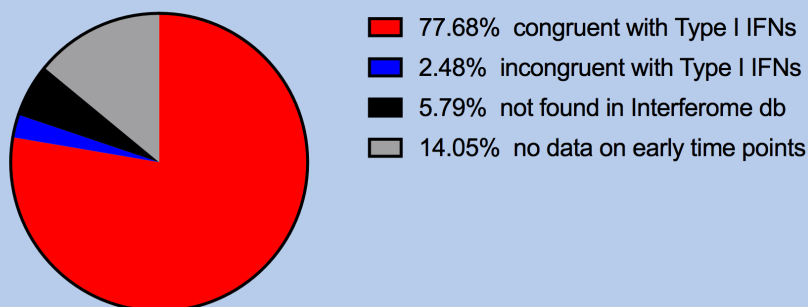
A. Grouping of differentially expressed (DE) genes (average of three biological replicates) showed > 2-fold difference in any comparison: sMPL vs. Control, E6020 vs. Control, or E6020 vs. sMPL) with false discovery rate (FDR) < 0.05. Protein-coding genes (Coding and Complex) made up the vast majority of DE genes that passed this QC threshold.

B.

Similarly regulated by sMPL and E6020



More induced/suppressed by E6020 than by sMPL



B. Highly differentially expressed protein coding genes (+/- 4-fold or more compared to untreated) were interrogated against Type I IFN expression data compiled in the Interferome.org database (db), and grouped as to whether or not their induction/suppression in our dataset was congruent with Type I IFNs. The majority of DE genes that were more induced by E6020 than by sMPL (blue background in Suppl. Table 2, above) are congruent with Type I IFN regulation.

Interferome.org reference:

Rusinova, I.; Forster, S.; Yu, S.; Kannan, A.; Masse, M.; Cumming, H.; Chapman, R.; Hertzog, P.J. INTERFEROME v2. 0: an updated database of annotated interferon-regulated genes. *Nucleic Acids Research*. 2013 January; 41 (database issue): D1040-D1046.

Supplementary Table I. Differentially expressed genes. Color coding: Green--Similar induction/suppression by both sMPL and E6020; Blue--Stronger induction/suppression by E6020 than sMPL (used as input for analysis in Supplementary Figure 1B). Colorless--Low induction by either sMPL or E6020.

Gene Symbol	Fold Change (linear) (sMPL vs. Control)	Fold Change (linear) (E6020 vs. Control)
<i>Il1b</i>	97.62	156.51
<i>Irg1</i>	67.01	83.30
<i>Ifit1</i>	2.93	78.10
<i>Mx1</i>	2.78	67.14
<i>Cxcl2</i>	72.96	63.01
<i>Tnfsf9</i>	21.24	59.48
<i>Mx2</i>	2.24	55.12
<i>Ccl5</i>	13.44	54.49
<i>Tgtp2</i>	< 2	41.30
<i>Tnf</i>	33.39	40.81
<i>Ccl4</i>	7.26	39.37
<i>Cxcl10</i>	4.95	37.27
<i>Cd69</i>	12.70	33.72
<i>Olr1</i>	19.08	32.12
<i>I830012O16Rik</i>	< 2	29.12
<i>Rnd1</i>	10.40	26.94
<i>Rsad2</i>	< 2	26.84
<i>Fam26f</i>	5.24	26.08
<i>Cmpk2</i>	< 2	24.45
<i>Nfkbiz</i>	12.91	23.75
<i>Il6</i>	< 2	23.27
<i>Tnfsf4</i>	< 2	22.54
<i>Il12b</i>	5.95	21.78
<i>Clec4e</i>	23.01	21.53
<i>Ifit2</i>	< 2	21.28
<i>Trim30c</i>	< 2	20.69
<i>Ptgs2</i>	< 2	20.25
<i>Tnfsf15</i>	2.23	20.08
<i>Oasl1</i>	2.03	19.48
<i>Cd40</i>	6.41	19.30
<i>Il23a</i>	< 2	16.80
<i>Cxcl9</i>	< 2	16.79
<i>Cxcl1</i>	18.69	16.60
<i>Slfn1</i>	< 2	16.15
<i>Ifih1</i>	2.18	15.80
<i>Serpinb2</i>	< 2	14.69
<i>Il27</i>	2.62	14.30
<i>F830016B08Rik</i>	< 2	14.22
<i>Nlrp3</i>	11.53	13.72
<i>Gbp2</i>	4.25	13.65
<i>Icam1</i>	9.22	13.40
<i>Il1a</i>	7.44	13.28
<i>Gbp2b</i>	3.93	13.03
<i>Gm4951</i>	< 2	12.38
<i>Ifit3</i>	< 2	12.37
<i>Gm12185</i>	< 2	12.33

<i>Gbp3</i>	2.47	12.3
<i>Edn1</i>	< 2	12.14
<i>Gbp10</i>	2.35	11.84
<i>Tarm1</i>	4.73	11.80
<i>Usp18</i>	< 2	11.35
<i>Gbp7</i>	2.53	11.28
<i>Gpr84</i>	14.29	11.00
<i>Gm6377</i>	5.14	10.98
<i>ligp1</i>	< 2	10.98
<i>Irf1</i>	4.66	10.57
<i>Nfkbie</i>	8.69	10.51
<i>Cxcl3</i>	8.41	10.38
<i>Ifi203</i>	3.56	10.28
<i>Ccl7</i>	< 2	9.63
<i>AW112010</i>	2.70	9.59
<i>Nfkbia</i>	6.94	9.33
<i>Mefv</i>	9.92	9.14
<i>Plet1</i>	< 2	8.99
<i>Gm14446</i>	< 2	8.98
<i>Serpine1</i>	< 2	8.81
<i>Ccl3</i>	6.04	8.80
<i>Sdc4</i>	3.64	8.73
<i>Slc7a2</i>	< 2	8.70
<i>Gbp4</i>	< 2	8.69
<i>Ccl2</i>	< 2	8.56
<i>Igtp</i>	< 2	8.51
<i>Rab11fip1</i>	4.84	8.51
<i>Tnfaip3</i>	5.12	8.46
<i>Parp14</i>	2.54	8.35
<i>Ifi44</i>	< 2	8.20
<i>Tlr2</i>	5.91	7.98
<i>Vcam1</i>	< 2	7.69
<i>Icosl</i>	10.36	7.63
<i>Adora2a</i>	4.87	7.62
<i>Ccl12</i>	< 2	7.58
<i>Isg15</i>	< 2	7.53
<i>Mmp13</i>	< 2	7.51
<i>H2-M2</i>	5.54	7.51
<i>Nupr1</i>	3.2	7.37
<i>Mndal</i>	2.39	7.31
<i>Cd83</i>	5.45	7.27
<i>Slfn8</i>	2.09	7.00
<i>Tnfp3</i>	5.53	6.72
<i>Rtp4</i>	< 2	6.70
<i>Gpr85</i>	4.77	6.64
<i>Irgm2</i>	< 2	6.42
<i>Ccrn4l</i>	< 2	6.41
<i>Ch25h</i>	< 2	6.41
<i>Pyhin1</i>	< 2	6.39
<i>Fnbp1l</i>	4.39	6.37
<i>Gbp11</i>	< 2	6.33

<i>Peli1</i>	2.41	6.26
<i>Gm4955</i>	< 2	6.17
<i>Il12a</i>	2.84	6.16
<i>Slc2a6</i>	3.85	6.15
<i>Slc25a37</i>	2.13	6.08
<i>Sele</i>	< 2	6.01
<i>Ehd1</i>	5.09	5.93
<i>Il15</i>	< 2	5.88
<i>Zc3h12c</i>	6.49	5.87
<i>Ripk2</i>	2.79	5.87
<i>Ccl2</i>	3.29	5.83
<i>Traf1</i>	4.27	5.79
<i>Cd80</i>	< 2	5.75
<i>AI504432</i>	< 2	5.70
<i>Xaf1</i>	< 2	5.65
<i>Slfn9</i>	< 2	5.53
<i>BC094916</i>	< 2	5.53
<i>Irf7</i>	< 2	5.48
<i>Zbp1</i>	< 2	5.48
<i>Cflar</i>	3.68	5.46
<i>AA467197</i>	< 2	5.41
<i>Oas1b</i>	< 2	5.34
<i>Mnda</i>	2.64	5.33
<i>Gbp9</i>	< 2	5.31
<i>Irgm1</i>	< 2	5.29
<i>Cish</i>	< 2	5.11
<i>Adm</i>	< 2	5.10
<i>Casp4</i>	3.06	5.01
<i>Zc3h12a</i>	3.33	4.85
<i>Ifi202b</i>	< 2	4.85
<i>Jak2</i>	2.77	4.83
<i>Gem</i>	3.01	4.83
<i>Stat2</i>	< 2	4.78
<i>Socs3</i>	< 2	4.77
<i>Rnd3</i>	< 2	4.74
<i>BC023105</i>	< 2	4.73
<i>Ccl22</i>	< 2	4.70
<i>Stx11</i>	3.05	4.55
<i>Samd9l</i>	< 2	4.54
<i>Pydc4</i>	< 2	4.53
<i>Rapgef2</i>	2.82	4.49
<i>Timp1</i>	< 2	4.47
<i>Helz2</i>	< 2	4.46
<i>Gpr132</i>	4.81	4.46
<i>Ms4a4c</i>	< 2	4.43
<i>Spred1</i>	3.16	4.42
<i>Malt1</i>	3.69	4.38
<i>Nfkb2</i>	2.99	4.38
<i>Slfn2</i>	3.40	4.33
<i>Pydc3</i>	< 2	4.30
<i>Ccnd2</i>	< 2	4.29
<i>Batf2</i>	< 2	4.28
<i>Mki67</i>	2.63	4.27
<i>Pstpip2</i>	3.16	4.27
<i>Phf11d</i>	< 2	4.27
<i>Tfec</i>	2.58	4.24

<i>Trim21</i>	< 2	4.18
<i>Nfkb1</i>	2.95	4.15
<i>E2f8</i>	2.86	4.13
<i>Ddx58</i>	< 2	4.10
<i>Il15ra</i>	< 2	4.10
<i>Csrnp1</i>	< 2	4.09
<i>Herc6</i>	< 2	4.04
<i>Zufsp</i>	< 2	4.03
<i>Cav1</i>	< 2	3.99
<i>Sp140</i>	< 2	3.95
<i>Lcp2</i>	2.76	3.88
<i>Phf11b</i>	< 2	3.88
<i>Oas2</i>	< 2	3.87
<i>Papd7</i>	< 2	3.86
<i>Nt5c3</i>	< 2	3.84
<i>Rassf4</i>	2.98	3.83
<i>Gvin1</i>	< 2	3.83
<i>Nr4a3</i>	< 2	3.81
<i>Tap1</i>	< 2	3.80
<i>Cdkn1a</i>	2.67	3.80
<i>Oas12</i>	< 2	3.79
<i>Daxx</i>	< 2	3.78
<i>Parp9</i>	< 2	3.75
<i>Ell2</i>	3.12	3.74
<i>Bcl2a1a</i>	2.63	3.71
<i>Gbp8</i>	< 2	3.70
<i>Ddx60</i>	< 2	3.69
<i>Klf7</i>	2.87	3.69
<i>Vcan</i>	< 2	3.68
<i>Ddhd1</i>	2.65	3.67
<i>Plat</i>	< 2	3.66
<i>Rasgrp1</i>	3.05	3.66
<i>Bcl3</i>	3.50	3.64
<i>Pvr</i>	< 2	3.64
<i>Cd274</i>	< 2	3.64
<i>Cd14</i>	< 2	3.63
<i>Cass4</i>	2.15	3.63
<i>Arhgef3</i>	2.65	3.61
<i>Hcar2</i>	2.35	3.61
<i>Rcan1</i>	< 2	3.59
<i>Tnfsf10</i>	< 2	3.59
<i>Rnf213</i>	< 2	3.58
<i>Nod2</i>	2.45	3.55
<i>Ptx3</i>	< 2	3.55
<i>Phf11a</i>	< 2	3.55
<i>Dtx3l</i>	< 2	3.54
<i>Inhba</i>	< 2	3.53
<i>Fpr2</i>	2.61	3.52
<i>Akna</i>	2.61	3.52
<i>Rel</i>	2.97	3.51
<i>Mfsd7a</i>	2.35	3.51
<i>Ifi204</i>	< 2	3.50
<i>Clec5a</i>	2.85	3.49
<i>Calcl</i>	< 2	3.46
<i>Trim30b</i>	< 2	3.43
<i>Met</i>	< 2	3.42

<i>Slfn3</i>	< 2	3.42
<i>Slfn4</i>	< 2	3.40
<i>C130026I21Rik</i>	< 2	3.38
<i>Tnip1</i>	2.83	3.37
<i>Itgal</i>	3.61	3.37
<i>Oas1g</i>	< 2	3.36
<i>Pnp</i>	< 2	3.35
<i>Slc7a11</i>	2.50	3.33
<i>Orai2</i>	2.52	3.31
<i>Bcl2a1c</i>	< 2	3.31
<i>Sap30</i>	< 2	3.30
<i>Tmem200b</i>	< 2	3.28
<i>Cfb</i>	< 2	3.27
<i>Tnfaip2</i>	2.61	3.26
<i>Rgcc</i>	< 2	3.25
<i>Kcna3</i>	< 2	3.25
<i>Kdm6b</i>	2.71	3.24
<i>Serpina3g</i>	< 2	3.21
<i>Sp110</i>	< 2	3.19
<i>Adamts4</i>	< 2	3.17
<i>LOC100041057</i>	< 2	3.14
<i>Cdc42ep2</i>	3.99	3.11
<i>Sod2</i>	2.67	3.11
<i>Arg2</i>	2.62	3.10
<i>Slfn5</i>	< 2	3.07
<i>Jag1</i>	< 2	3.06
<i>Trim30d</i>	< 2	3.05
<i>Rab32</i>	< 2	3.04
<i>Nos2</i>	< 2	3.04
<i>Fchsd2</i>	< 2	3.03
<i>Rgs16</i>	< 2	3.03
<i>Dyrk2</i>	2.20	3.00
<i>Pim1</i>	2.23	2.98
<i>Ralgds</i>	4.29	2.95
<i>Rnf19b</i>	2.81	2.95
<i>Setdb2</i>	< 2	2.95
<i>Pnp2</i>	< 2	2.94
<i>Eif2ak2</i>	< 2	2.94
<i>Gdap10</i>	< 2	2.92
<i>Fas</i>	2.99	2.92
<i>Oas1a</i>	< 2	2.91
<i>Spaca6</i>	2.31	2.90
<i>Abtb2</i>	< 2	2.89
<i>Marcks1</i>	< 2	2.89
<i>Mt2</i>	< 2	2.88
<i>Tank</i>	2.2	2.88
<i>Oaf</i>	< 2	2.88
<i>Tagap</i>	2.35	2.88
<i>1500012F01Rik</i>	< 2	2.87
<i>lfi35</i>	< 2	2.87
<i>Mtmr14</i>	2.22	2.86
<i>Ampd3</i>	2.47	2.86
<i>Stat1</i>	< 2	2.84
<i>Il17ra</i>	< 2	2.84
<i>Nfkbib</i>	2.06	2.83
<i>Adora2b</i>	< 2	2.83

<i>F10</i>	< 2	2.82
<i>N4bp1</i>	2.25	2.81
<i>Jarid2</i>	< 2	2.77
<i>Plek</i>	< 2	2.76
<i>Cdk5r1</i>	< 2	2.74
<i>Stat5a</i>	< 2	2.74
<i>Trim30a</i>	< 2	2.72
<i>Frmd6</i>	< 2	2.70
<i>Il33</i>	< 2	2.69
<i>Hivep2</i>	< 2	2.68
<i>Cd44</i>	< 2	2.64
<i>Sp110</i>	< 2	2.64
<i>Dcp2</i>	< 2	2.63
<i>Zhx2</i>	< 2	2.63
<i>Gm16181</i>	< 2	2.63
<i>Pilra</i>	2.66	2.63
<i>LOC664787</i>	< 2	2.62
<i>Mapkapk2</i>	< 2	2.61
<i>Serpina3n</i>	< 2	2.60
<i>Ube2l6</i>	< 2	2.59
<i>Kpna3</i>	< 2	2.59
<i>F3</i>	< 2	2.59
<i>Jdp2</i>	< 2	2.59
<i>Ets2</i>	2.64	2.58
<i>Serpina3f</i>	< 2	2.57
<i>Plscr1</i>	< 2	2.56
<i>Ms4a6c</i>	< 2	2.56
<i>Cst7</i>	< 2	2.56
<i>Zswim4</i>	2.33	2.54
<i>Znfx1</i>	< 2	2.53
<i>U90926</i>	< 2	2.53
<i>Itgav</i>	< 2	2.50
<i>Ier3</i>	< 2	2.50
<i>Errfi1</i>	< 2	2.50
<i>Pilrb2</i>	2.57	2.49
<i>Flrt3</i>	3.34	2.48
<i>Uba7</i>	< 2	2.48
<i>Cxcl11</i>	< 2	2.48
<i>Ahr</i>	< 2	2.47
<i>Sbds</i>	< 2	2.47
<i>Zfp131</i>	< 2	2.46
<i>Rhbdf2</i>	< 2	2.46
<i>Gch1</i>	< 2	2.46
<i>Parp8</i>	< 2	2.46
<i>Dhx58</i>	< 2	2.46
<i>Dusp2</i>	< 2	2.45
<i>Stk40</i>	< 2	2.45
<i>Tiparp</i>	< 2	2.42
<i>Bcl2l11</i>	< 2	2.42
<i>Ptges</i>	< 2	2.41
<i>Lacc1</i>	< 2	2.40
<i>LOC100041034</i>	< 2	2.40
<i>Src</i>	< 2	2.40
<i>1700047I17Rik2</i>	< 2	2.39
<i>Tlr7</i>	< 2	2.39
<i>Hdc</i>	< 2	2.39

<i>Cxcl16</i>	2.44	2.38
<i>Tmem171</i>	< 2	2.37
<i>Gadd45b</i>	< 2	2.37
<i>Hk2</i>	< 2	2.37
<i>Sav1</i>	< 2	2.36
<i>Parp12</i>	< 2	2.36
<i>AW011738</i>	< 2	2.36
<i>Sh3bp5</i>	< 2	2.35
<i>Fam46a</i>	< 2	2.34
<i>Prdm1</i>	3.11	2.34
<i>Htra4</i>	< 2	2.34
<i>Fam102b</i>	< 2	2.32
<i>Kctd12</i>	< 2	2.32
<i>Bcl2a1d</i>	< 2	2.32
<i>Ppm1k</i>	< 2	2.32
<i>Tnfrsf1b</i>	2.10	2.31
<i>1700047117Rik2</i>	< 2	2.31
<i>Rasa2</i>	< 2	2.31
<i>Csf2</i>	< 2	2.31
<i>Ube2f</i>	< 2	2.30
<i>Gm5431</i>	< 2	2.30
<i>Hspa1a</i>	< 2	2.29
<i>Zfp263</i>	2.55	2.29
<i>Gyk</i>	< 2	2.29
<i>P2ry13</i>	< 2	2.29
<i>Clic4</i>	< 2	2.28
<i>Ccdc86</i>	< 2	2.28
<i>Slc39a14</i>	2.13	2.28
<i>Naa25</i>	< 2	2.28
<i>Ccl9</i>	< 2	2.28
<i>Mkl1</i>	< 2	2.27
<i>Gas7</i>	< 2	2.27
<i>Itga4</i>	< 2	2.27
<i>Mapk14</i>	< 2	2.26
<i>Kcnn4</i>	< 2	2.25
<i>Klf2</i>	< 2	2.24
<i>Tshz1</i>	< 2	2.24
<i>Rasgef1b</i>	2.63	2.24
<i>LOC100041034</i>	< 2	2.24
<i>Zcchc2</i>	< 2	2.24
<i>Il10</i>	< 2	2.24
<i>Top1</i>	< 2	2.24
<i>Car13</i>	< 2	2.23
<i>Sgms2</i>	< 2	2.23
<i>Tlr1</i>	2.55	2.23
<i>Lcn2</i>	< 2	2.23
<i>Vezf1</i>	< 2	2.22
<i>Hivep1</i>	< 2	2.22
<i>Dusp1</i>	< 2	2.21
<i>Xkr8</i>	< 2	2.20
<i>Slc31a2</i>	2.40	2.20
<i>Tnfaip6</i>	< 2	2.19
<i>Mmp14</i>	2.15	2.19
<i>Slc4a7</i>	< 2	2.18
<i>Bcl2a1b</i>	< 2	2.18
<i>Fam129a</i>	< 2	2.17

<i>Zc3h7a</i>	< 2	2.17
<i>Pilrb1</i>	2.37	2.16
<i>Mtmr7</i>	< 2	2.15
<i>Cers6</i>	< 2	2.14
<i>Arl5c</i>	2.70	2.14
<i>Nod1</i>	< 2	2.14
<i>Apobec3</i>	< 2	2.14
<i>Agrn</i>	< 2	2.13
<i>Irf47</i>	< 2	2.13
<i>Ccnyl1</i>	< 2	2.12
<i>Ptprj</i>	< 2	2.12
<i>Irfb1</i>	< 2	2.12
<i>Myd88</i>	< 2	2.10
<i>Cdc14a</i>	< 2	2.09
<i>Foxp4</i>	< 2	2.09
<i>Psmb9</i>	< 2	2.09
<i>Snx20</i>	< 2	2.08
<i>Rbm7</i>	< 2	2.08
<i>Nfkbid</i>	< 2	2.08
<i>Nrip1</i>	2.17	2.08
<i>Clcf1</i>	< 2	2.07
<i>Sep11</i>	< 2	2.07
<i>Zfp800</i>	< 2	2.06
<i>Gsap</i>	2.75	2.06
<i>Nlrc5</i>	< 2	2.06
<i>Snx10</i>	< 2	2.05
<i>Enpp4</i>	< 2	2.05
<i>Fabp3</i>	< 2	2.05
<i>Relb</i>	2.08	2.04
<i>Baz1a</i>	< 2	2.04
<i>Ugcg</i>	< 2	2.04
<i>Marcks</i>	< 2	2.04
<i>Fam208b</i>	< 2	2.04
<i>Nipal1</i>	< 2	2.04
<i>Btg1</i>	2.29	2.03
<i>Abhd17c</i>	2.03	2.03
<i>Rap2c</i>	< 2	2.02
<i>Gtf2b</i>	< 2	2.01
<i>Rbpj</i>	< 2	2.01
<i>Klf3</i>	< 2	2.01
<i>Lancl2</i>	< 2	2.01
<i>Hck</i>	< 2	2.01
<i>Lztfl1</i>	< 2	2.01
<i>Rab20</i>	2.91	< 2
<i>Herpud1</i>	2.51	< 2
<i>Birc3</i>	2.42	< 2
<i>Samsn1</i>	2.41	< 2
<i>Vasp</i>	2.17	< 2
<i>Sowahc</i>	2.11	< 2
<i>Pdgfb</i>	2.08	> -2
<i>Skil</i>	2.01	< 2
<i>Irf8</i>	-2.17	> -2
<i>Sgk1</i>	> -2	-2.01
<i>N4bp2l1</i>	> -2	-2.02
<i>Zfp36l2</i>	> -2	-2.03
<i>Coq10a</i>	> -2	-2.03

<i>Prune</i>	> -2	-2.04
<i>Fam178a</i>	> -2	-2.05
<i>Wee1</i>	> -2	-2.07
<i>Tbc1d2</i>	> -2	-2.07
<i>Ercc6</i>	> -2	-2.07
<i>Clk4</i>	> -2	-2.07
<i>Tet3</i>	> -2	-2.07
<i>Ddx26b</i>	> -2	-2.08
<i>Dennd4c</i>	> -2	-2.09
<i>Ankrd12</i>	> -2	-2.09
<i>Vps18</i>	> -2	-2.10
<i>Flcn</i>	> -2	-2.11
<i>1810011H11Rik</i>	> -2	-2.12
<i>Lpxn</i>	> -2	-2.12
<i>Olfr111</i>	> -2	-2.16
<i>Gas2l3</i>	> -2	-2.19
<i>Bhlhe41</i>	> -2	-2.19
<i>Gm4787</i>	> -2	-2.19
<i>Fbxl20</i>	> -2	-2.20
<i>Tcp11l2</i>	> -2	-2.20
<i>Kcnj2</i>	> -2	-2.21
<i>Ldlrap1</i>	> -2	-2.23
<i>Kcnq1ot1</i>	> -2	-2.23
<i>Gadd45a</i>	> -2	-2.24
<i>Arl11</i>	> -2	-2.25
<i>Tmem126b</i>	> -2	-2.25
<i>Plekhn2</i>	> -2	-2.26
<i>Apobec1</i>	> -2	-2.27
<i>Ypel2</i>	> -2	-2.27
<i>B430306N03Rik</i>	> -2	-2.30
<i>Mnt</i>	> -2	-2.39
<i>Jun</i>	> -2	-2.39
<i>Rgs2</i>	-2.61	-2.41
<i>Lonrf3</i>	> -2	-2.41
<i>Rnf144b</i>	> -2	-2.46
<i>Tec</i>	> -2	-2.47
<i>Impact</i>	> -2	-2.47
<i>Stard9</i>	> -2	-2.47
<i>Klhl6</i>	> -2	-2.48
<i>Pwwp2b</i>	> -2	-2.51
<i>Cnr2</i>	> -2	-2.52
<i>Ddi2</i>	> -2	-2.55
<i>Rttn</i>	> -2	-2.55

<i>D130007C19Rik</i>	> -2	-2.55
<i>Cipc</i>	> -2	-2.60
<i>5430435G22Rik</i>	> -2	-2.61
<i>Gpr183</i>	> -2	-2.62
<i>Samd8</i>	> -2	-2.67
<i>Snx24</i>	> -2	-2.68
<i>Atf7ip</i>	> -2	-2.68
<i>Fnip2</i>	-2.02	-2.72
<i>Slc26a11</i>	> -2	-2.74
<i>Akap10</i>	> -2	-2.77
<i>Ddit3</i>	> -2	-2.78
<i>Ccdc125</i>	> -2	-2.79
<i>1700017B05Rik</i>	> -2	-2.84
<i>Slc30a1</i>	> -2	-2.84
<i>Pparg</i>	> -2	-2.85
<i>Lpin1</i>	> -2	-2.85
<i>Klhl24</i>	> -2	-2.86
<i>Trp53inp1</i>	> -2	-2.89
<i>Crebrf</i>	> -2	-2.90
<i>Sh2d3c</i>	> -2	-2.94
<i>Lpar6</i>	-2.42	-3.02
<i>Plekhn1</i>	> -2	-3.02
<i>Gpr157</i>	> -2	-3.04
<i>Atf7ip</i>	> -2	-3.18
<i>Tifab</i>	-2.16	-3.29
<i>Fgd4</i>	> -2	-3.31
<i>Foxo3</i>	-2.06	-3.59
<i>Fam83f</i>	-2.09	-3.70
<i>Irf2bp2</i>	> -2	-3.72
<i>Gadd45g</i>	-2.97	-3.75
<i>Fam214a</i>	-2.06	-3.89
<i>Lrrc14b</i>	-2.24	-3.89
<i>Arrdc3</i>	-2.4	-4.65
<i>Mafb</i>	> -2	-4.74
<i>Cebpa</i>	-2.82	-5.03
<i>Lyl1</i>	> -2	-5.16
<i>Cd300lb</i>	> -2	-5.37
<i>Sesn1</i>	> -2	-5.59
<i>Arhgap25</i>	-2.15	-8.15
<i>Cxcr4</i>	-2.18	-8.46
<i>Fbxo32</i>	> -2	-13.67

Supplementary Table II. Gene expression of A) MyD88-dependent genes, B) TRIF-dependent genes, and C) MyD88- and TRIF-co-dependent genes, 2 hours after stimulation with 100 ng/mL sMPL or E6020.

A Gene Symbol	Description	Fold Change sMPL vs Control	Fold Change E6020 vs Control	References for MyD88- dependence
<i>Ccl3</i>	chemokine (C-C motif) ligand 3	6.04	8.80	[56, 58]
<i>Cd38</i>	CD38 antigen	2.54	2.95	[56]
<i>Ch25h</i>	cholesterol 25-hydroxylase	3.73	6.41	[56]
<i>Clec4e</i>	C-type lectin domain family 4, member e	23.01	21.53	[59]
<i>Cxcl1</i>	chemokine (C-X-C motif) ligand 1	18.69	16.60	[56, 58, 60-62]
<i>Cxcl2</i>	chemokine (C-X-C motif) ligand 2	72.96	63.01	[56, 58-62]
<i>Cxcl3</i>	chemokine (C-X-C motif) ligand 3	8.41	10.38	[58]
<i>Ednrb</i>	endothelin receptor type B	< 2.00	2.54	[56]
<i>Fpr1</i>	formyl peptide receptor 1	4.70	4.30	[56]
<i>Fpr2</i>	formyl peptide receptor 2; formyl peptide receptor 3	2.61	3.52	[56]
<i>Il10</i>	interleukin 10	< 2.00	2.24	[58-61]
<i>Il1b</i>	interleukin 1 beta	97.62	156.51	[56, 58-64, 67]
<i>Malt1</i>	mucosa associated lymphoid tissue lymphoma translocation gene 1	3.69	4.38	[63]
<i>Nfkbie</i>	nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor, epsilon	8.69	10.51	[63]
<i>Nfkbiz</i>	nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor, zeta	12.91	23.75	[56, 58, 62]
<i>Pilra</i>	paired immunoglobulin-like type 2 receptor alpha	2.66	2.63	[56]
<i>Tank</i>	TRAF family member-associated Nf-kappa B activator	2.20	2.88	[63]
<i>Tlr2</i>	toll-like receptor 2	5.91	7.98	[59]
<i>Tnf</i>	tumor necrosis factor	33.39	40.81	[56, 59-64, 67-69]

B Gene Symbol	Description	Fold Change sMPL vs Control	Fold Change E6020 vs Control	References for TRIF-dependence
<i>Ccnd2</i>	cyclin D2	< 2.00	4.29	[57, 63]
<i>Cd40</i>	CD40 antigen	6.41	19.30	[57]
<i>Cdkn1a</i>	cyclin-dependent kinase inhibitor 1A (P21)	2.67	3.80	[64]
<i>Cfb</i>	complement factor B	< 2.00	3.27	[57]
<i>Cmpk2</i>	cytidine monophosphate (UMP-CMP) kinase 2, mitochondrial	< 2.00	24.45	[56, 60, 64]
<i>Cxcl10</i>	chemokine (C-X-C motif) ligand 10	4.95	37.27	[56-60, 63-65, 69, 70]
<i>Cxcl11</i>	chemokine (C-X-C motif) ligand 11	< 2.00	2.48	[56]
<i>Eif2ak2</i>	eukaryotic translation initiation factor 2-alpha kinase 2	< 2.00	2.94	[65]

<i>Gbp2</i>	guanylate binding protein 2	4.25	13.65	[64, 65]
<i>Gbp4</i>	guanylate binding protein 4	< 2.00	8.69	[64]
<i>Hspa1a</i>	heat shock protein 1A	< 2.00	2.29	[59]
<i>Ifi202b</i>	interferon activated gene 202B; interferon activated gene 205; interferon-activable protein 202-like	2.02	4.85	[57, 64]
<i>Ifi203</i>	interferon activated gene 203; flocculation protein FLO11-like; predicted gene 16340	3.56	10.28	[57, 64]
<i>Ifi204</i>	interferon activated gene 204	< 2.00	3.50	[57, 64]
<i>Ifi47</i>	interferon gamma inducible protein 47; olfactory receptor 56; interferon gamma inducible protein	< 2.00	2.13	[63, 64]
<i>Ifit1</i>	interferon-induced protein with tetratricopeptide repeats 1	2.93	78.10	[56-58, 60, 63, 65]
<i>Ifit2</i>	interferon-induced protein with tetratricopeptide repeats 2	< 2.00	21.28	[56, 63-65]
<i>Ifit3</i>	interferon-induced protein with tetratricopeptide repeats 3	< 2.00	12.37	[57, 58, 60, 63-65]
<i>Ifnb1</i>	interferon beta 1, fibroblast; interferon beta 1 fibroblast	< 2.00	2.12	[59, 64, 67-70]
<i>Igtp</i>	interferon gamma induced GTPase	< 2.00	8.51	[57, 63, 64]
<i>Iigp1</i>	interferon inducible GTPase 1	< 2.00	10.98	[63]
<i>Il15</i>	interleukin 15	< 2.00	5.88	[63]
<i>Il15ra</i>	interleukin 15 receptor, alpha chain	< 2.00	4.10	[57]
<i>Il23a</i>	interleukin 23, alpha subunit p19	3.18	16.80	[57]
<i>Il33</i>	interleukin 33	< 2.00	2.69	[66]
<i>Inhba</i>	inhibin beta-A	< 2.00	3.53	[65]
<i>Irf7</i>	interferon regulatory factor 7	< 2.00	5.48	[63-65]
<i>Irgm1</i>	immunity-related GTPase family M member 1	< 2.00	5.29	[57, 63, 64]
<i>Irgm2</i>	immunity-related GTPase family M member 2	< 2.00	6.42	[57]
<i>Isg15</i>	ISG15 ubiquitin-like modifier	< 2.00	7.53	[56, 64, 65]
<i>Mki67</i>	antigen identified by monoclonal antibody Ki 67	2.63	4.27	[56]
<i>Mmp13</i>	matrix metalloproteinase 13	2.37	7.51	[63]
<i>Mnda</i>	myeloid cell nuclear differentiation antigen	2.64	5.33	[64, 65]
<i>Ms4a4c</i>	membrane-spanning 4-domains, subfamily A, member 4C	< 2.00	4.43	[56]
<i>Mx1</i>	myxovirus (influenza virus) resistance 1	2.78	67.14	[63-65]
<i>Mx2</i>	myxovirus (influenza virus) resistance 2	2.24	55.12	[57, 63, 64]
<i>Nos2</i>	nitric oxide synthase 2, inducible	< 2.00	3.04	[63, 64, 69, 72]
<i>Nt5c3</i>	5-nucleotidase, cytosolic III	< 2.00	3.84	[64]
<i>Oas1b</i>	2-5 oligoadenylate synthetase 1B	< 2.00	5.34	[63]
<i>Oas2</i>	2-5 oligoadenylate synthetase 2	< 2.00	3.87	[63]
<i>Oasl1</i>	2-5 oligoadenylate synthetase-like 1	2.03	19.48	[56, 63, 65]
<i>Oasl2</i>	2-5 oligoadenylate synthetase-like 2	< 2.00	3.79	[65]
<i>Pyhin1</i>	pyrin and HIN domain family, member 1	< 2.00	6.39	[65]
<i>Ripk2</i>	receptor (TNFRSF)-interacting serine-threonine kinase 2	2.79	5.87	[57]
<i>Rsad2</i>	radical S-adenosyl methionine domain containing 2	< 2.00	26.84	[56-58, 60, 64]
<i>Slfn1</i>	schlafen 1	< 2.00	16.15	[63]
<i>Slfn8</i>	schlafen 8	2.09	7.00	[63, 64]

<i>Stat1</i>	signal transducer and activator of transcription 1	< 2.00	2.84	[64]
<i>Stat2</i>	signal transducer and activator of transcription 2	< 2.00	4.78	[64]
<i>Stat5a</i>	signal transducer and activator of transcription 5A	< 2.00	2.74	[65]
<i>Tap1</i>	transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)	< 2.00	3.8	[65]
<i>Tgtp2</i>	T cell specific GTPase 2; T cell specific GTPase 1	< 2.00	41.3	[64]
<i>Tlr3</i>	toll-like receptor 3	< 2.00	2.23	[57, 63]
<i>Tnfsf10</i>	tumor necrosis factor (ligand) superfamily, member 10	< 2.00	3.59	[57]
<i>Trim21</i>	tripartite motif-containing 21	< 2.00	4.18	[64]
<i>Trim30a</i>	tripartite motif-containing 30A	< 2.00	2.72	[64]
<i>Usp18</i>	ubiquitin specific peptidase 18	< 2.00	11.35	[64]

C Gene Symbol	Description	Fold Change sMPL vs Control	Fold Change E6020 vs Control	References for MyD88- and TRIF-dependence
<i>Ccl12</i>	chemokine (C-C motif) ligand 12	< 2.00	7.58	[57, 64]
<i>Ccl2</i>	chemokine (C-C motif) ligand 2	3.84	8.56	[58, 59, 64, 65]
<i>Ccl4</i>	chemokine (C-C motif) ligand 4	7.26	39.37	[56, 57, 60, 61]
<i>Ccl5</i>	chemokine (C-C motif) ligand 5	13.44	54.49	[56, 60, 64, 67, 70, 71]
<i>Cd14</i>	CD14 antigen	2.49	3.63	[59]
<i>Csf2</i>	colony stimulating factor 2 (granulocyte-macrophage)	< 2.00	2.31	[59, 61]
<i>Il12a</i>	interleukin 12a	2.84	6.16	[56, 59, 64]
<i>Il12b</i>	interleukin 12b	5.95	21.78	[57, 60, 61, 63, 64]
<i>Il1a</i>	interleukin 1 alpha	7.44	13.28	[56, 59-62]
<i>Il6</i>	interleukin 6	3.10	23.27	[56-61, 63, 64, 68, 69, 72]
<i>Irf1</i>	interferon regulatory factor 1	4.66	10.57	[56, 57, 60, 62, 64]
<i>Lztfl1</i>	leucine zipper transcription factor-like 1	< 2.00	2.01	[56]
<i>Met</i>	met proto-oncogene	< 2.00	3.42	[56]
<i>Myd88</i>	myeloid differentiation primary response gene 88	< 2.00	2.10	[59, 64]
<i>Nfkb1</i>	nuclear factor of kappa light polypeptide gene enhancer in B cells 1, p105	2.95	4.15	[59]
<i>Nfkbia</i>	nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor, alpha	6.94	9.33	[59, 62]
<i>Peli1</i>	pellino 1	2.41	6.26	[59]
<i>Pstpip2</i>	proline-serine-threonine phosphatase-interacting protein 2	3.16	4.27	[56]
<i>Ptgs2</i>	prostaglandin-endoperoxide synthase 2	8.76	20.25	[56, 59-61]
<i>Rel</i>	reticuloendotheliosis oncogene	2.97	3.51	[59, 64]
<i>Serpine1</i>	serine (or cysteine) peptidase inhibitor, clade E, member 1	2.94	8.81	[56, 60, 61]
<i>Slc7a2</i>	solute carrier family 7 (cationic amino acid transporter, y+ system), member 2	3.09	8.70	[56]

<i>Socs3</i>	suppressor of cytokine signaling 3	2.37	4.77	[56, 57, 62, 65]
<i>Tlr1</i>	toll-like receptor 1; toll-like receptor 1 (Tlr1), mRNA.	2.55	2.23	[59]
<i>Tnfaip3</i>	tumor necrosis factor, alpha-induced protein 3	5.12	8.46	[59, 62]
<i>Vcam1</i>	vascular cell adhesion molecule 1	4.71	7.69	[56]