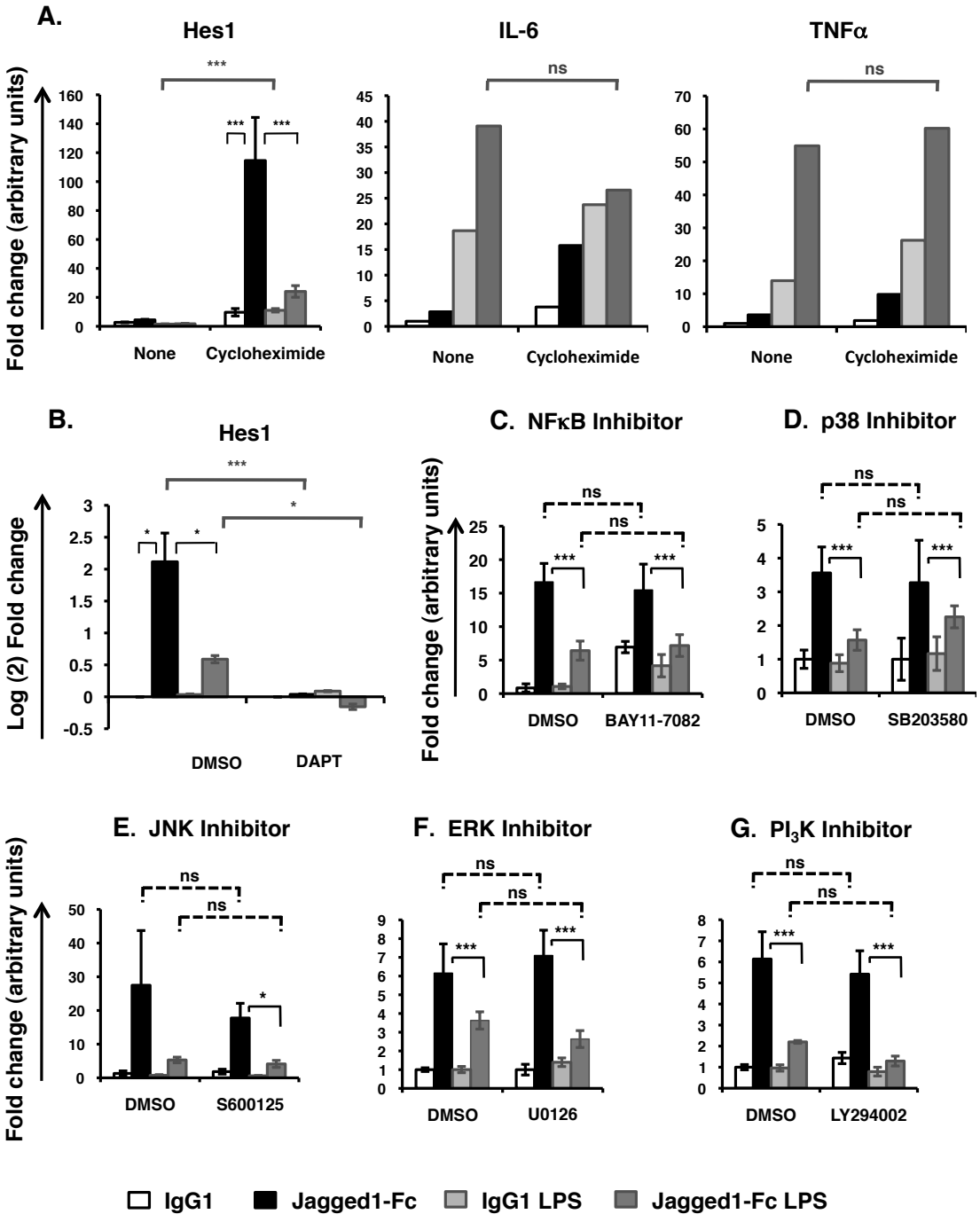
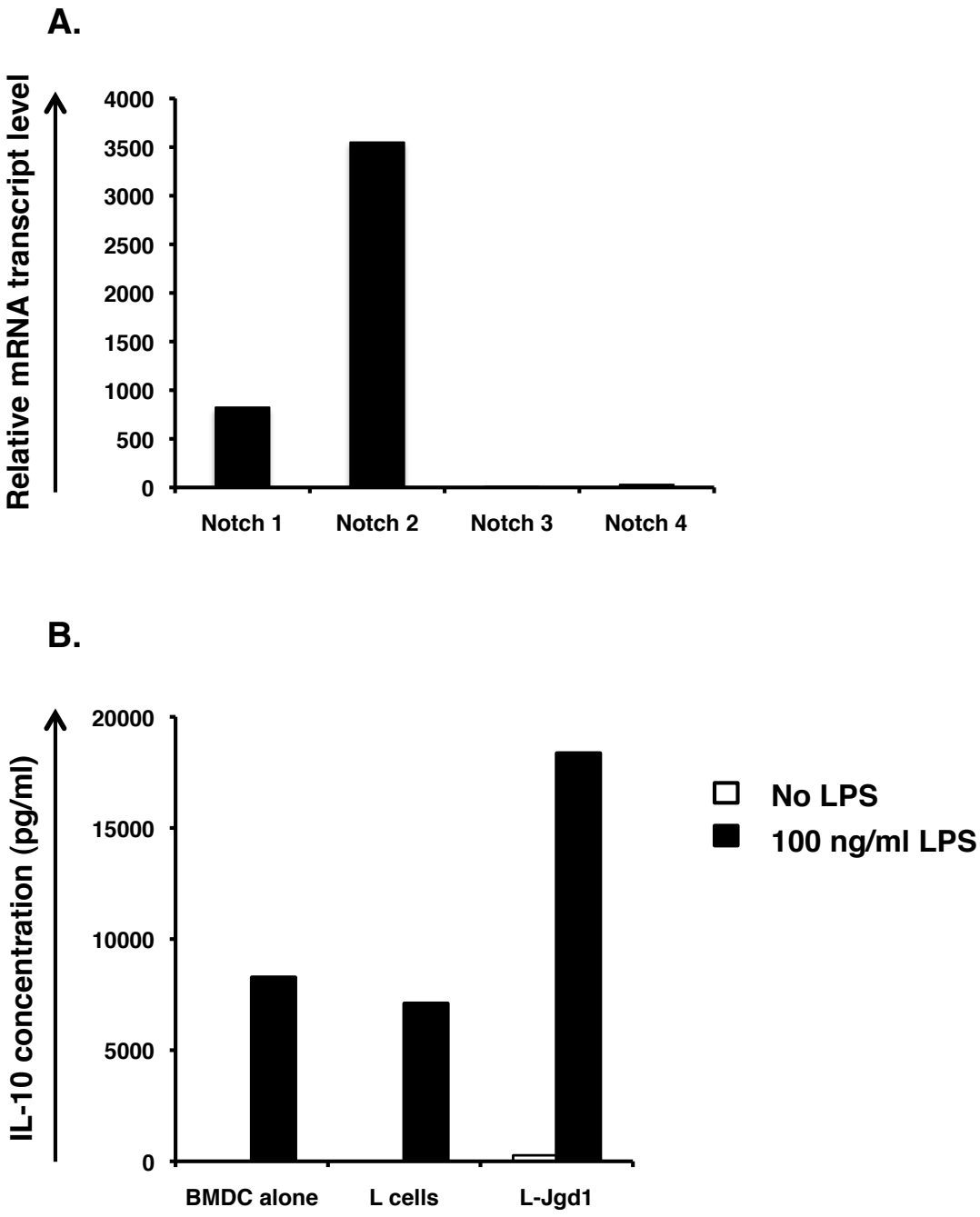
Supplementary Figures



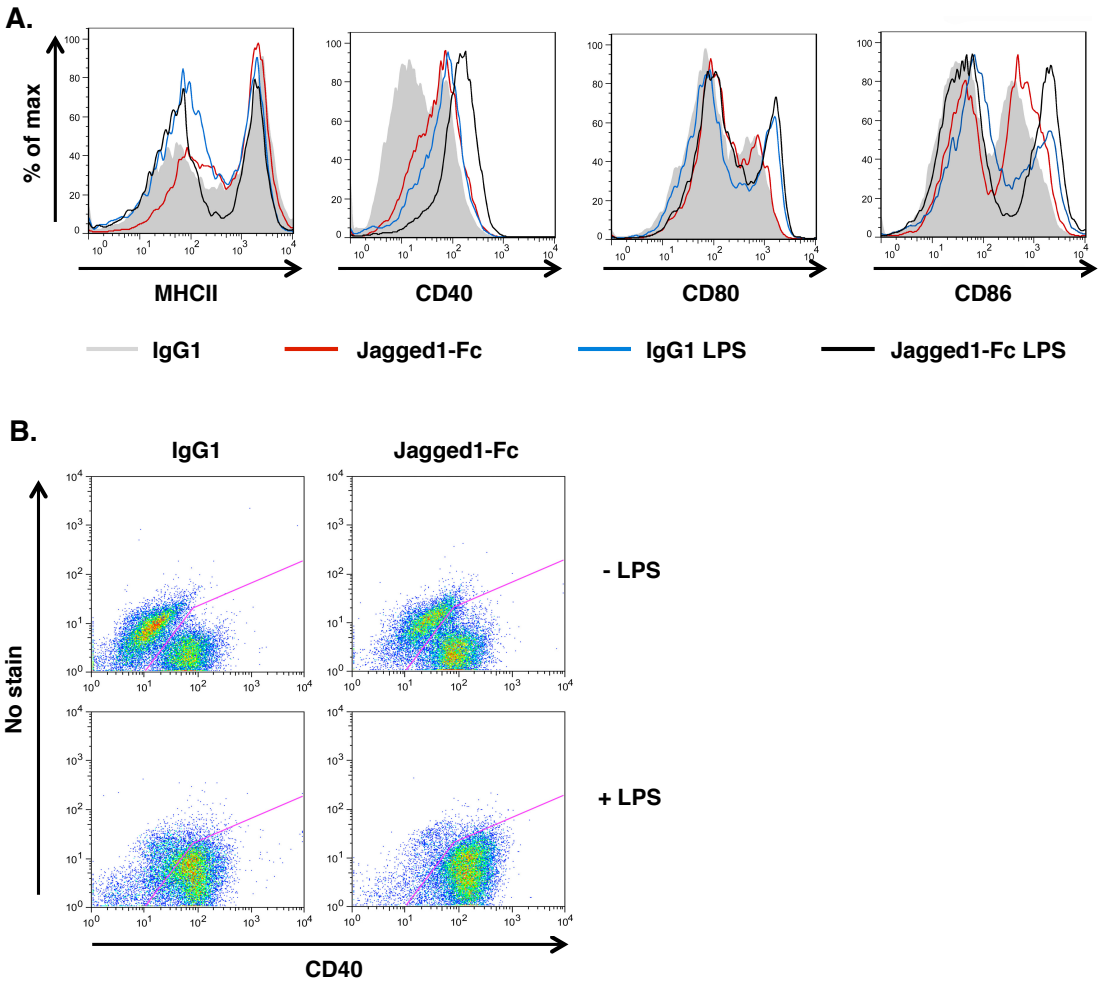
Supplementary Figure S1 Hes1 is directly induced by γ-secretase dependent Notch signaling and suppression of Jagged1-Fc induced Hes1 by LPS is not significantly altered by inhibition of NFκB, MAPK, or PI_3K activity. BMDC were pretreated with (**A**) 10 μg/ml cycloheximide, (**B**) 10 μM of DAPT, (**C**) BAY11-

7082, (**D**) SB203580, (**E**) SP600125, (**F**) U0126, (**G**) LY294002 or a corresponding quantity of vehicle control (DMSO) for 1 hour and then stimulated with Jagged1-Fc or IgG1 in the presence or absence of 100 ng/ml LPS for 4 h. Relative levels of Hes1, IL-6 or TNFα mRNA transcripts were measured after 4 h by qrt-PCR. Data in all parts are mean ± SD of triplicates and are representative of at least 3 independent experiments. Two-way ANOVA with Bonferroni post-test was used to statistically compare stimuli; $P<0.05$ was considered significant. * = $P<0.05$, ** = $P<0.01$, *** = $P<0.001$, ns = not significant.



Supplementary Figure S2 BMDC express Notch1 and Notch2 mRNA and Notch ligation of DC augments LPS induced IL-10. **(A)** Relative steady state expression levels of Notch receptor mRNA transcripts present in BMDC. (**B**) BMDC were co-cultured with the parental L cell line or L cells expressing Delta-like1 (L-ΔL1) or Jagged1 (L-Jgd1) at a ratio of 1:1 in the presence or absence of 100 ng/ml LPS. IL-10

present in the supernatant after culture for 24 h was measured by ELISA and data shown are representative of 3 independent experiments.



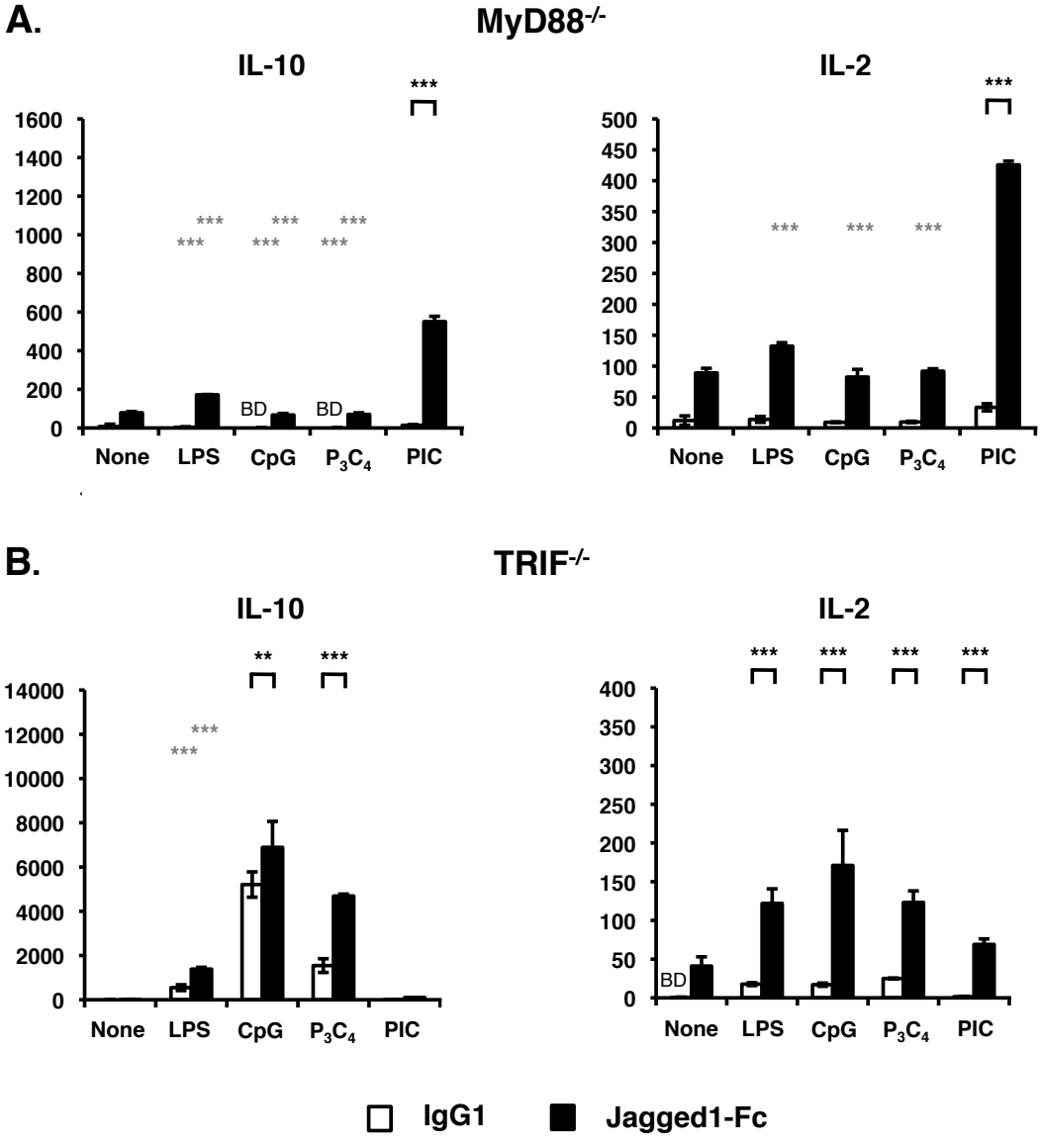
C.

	IgG1	Jagged1-Fc	IgG1 LPS	Jagged1-Fc LPS
% MHCII⁺ cells	46	50	35	35
MHCII MFI	1204	1238	723	772
% CD40⁺ cells	41	58	71	89
CD40 MFI	43	75	87	161
% CD80⁺ cells	35	41	36	40
CD80 MFI	248	303	436	526
% CD86⁺ cells	40	52	38	38
CD86 MFI	300	480	598	769

Supplementary Figure S3 Jagged1-Fc enhances surface CD40 expression.

BMDC were stimulated with plate bound IgG1 or Jagged1-Fc in the presence or absence of 100 ng/ml LPS for 24 h. Surface CD40, CD80, CD86 and MHC Class II

expression was measured by flow cytometry. Histograms are shown in **A**, representative dot-plots for CD40 expression are shown in **B**, while the percentage of $CD40^+$, $CD80^+$, $CD86^+$, and MHC class II^+ cells are given in **B**. Data are representative of two independent experiments.



Supplementary Figure S4 MyD88 is required for Notch modulation of CpG and Pam_3CSK_4 induced cytokines while TRIF is required for Notch modulation of Poly [I:C] induced cytokine expression. BMDC from $MyD88^{-/-}$ (**A**) or $TRIF^{-/-}$ (**B**)

mice were stimulated with plate bound IgG1 or Jagged1-Fc in the absence (none) or presence of 100 ng/ml LPS, 0.5 μg/ml CpG, 1 μg/ml Pam_3CSK_4 (P_3C_4) or 100 μg/ml Poly [I:C] (PIC)for 24 hours. Accumulation of IL-10, and IL-2 protein in the supernatant was measured by ELISA. Data shown are mean ± standard deviation of cell culture triplicates from a representative example of data obtained from two mice per genotype. Two-way ANOVA with Bonferroni post-test was used to judge whether observed differences were statistically significant; $P<0.05$ was considered significant. Black stars indicate a statistically significant difference between cells stimulated with IgG1 and Jagged1-Fc. Grey stars indicate a statistically significant difference between wild-type and $MyD88^{-/-}$ or wild-type and $TRIF^{-/-}$ respectively. Absence of stars indicates that the difference is not significant. * = $P<0.05$, ** = $P<0.01$, *** = $P<0.001$. BD = below the detection limit of the assay.