Supplemental Information

	Only WD			WD plus *C.pn* infection		
	Ldlr $^{-/-}$	***Nlrp3*** $^{-/-}$***Ldlr*** $^{-/-}$	***Casp1*** $^{-/-}$***Ldlr*** $^{-/-}$	***Ldlr*** $^{-/-}$	***Nlrp3*** $^{-/-}$***Ldlr*** $^{-/-}$	***Casp1*** $^{-/-}$***Ldlr*** $^{-/-}$
Total Cholesterol (mg/dL)	1638±207	1675.30±289	1545.83±292	1610±208	1597±240	1498±306
LDL (mg/dL)	377±56	384±52	386±44	390±65	349±62	386±44
HDL (mg/dL)	91±12	94±13	96±12	87±13	89±15	96±9
TG (mg/dL)	124±28	148±24	128±27	134±24	149±21	139±27

Table S1. Related to Figure 1. Plasma lipid profile

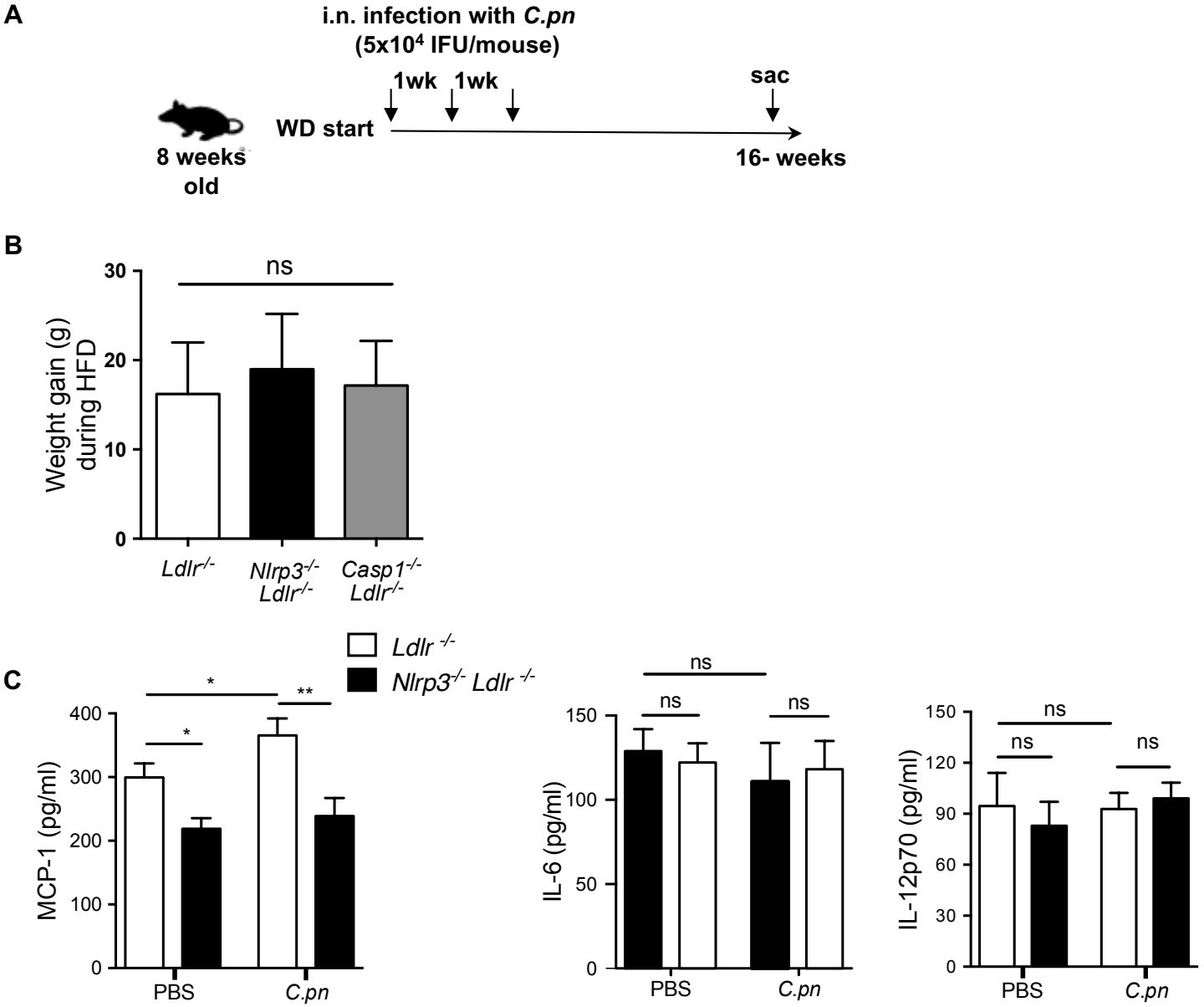

Figure S1. Related to Figure 1. The role of the Nlrp3 inflammasome in *C.pn* infection-accelerated atherosclerosis in *Ldlr*$^{-/-}$ mice.

(A) Experimental scheme. *Nlrp3*$^{-/-}$*Ldlr*$^{-/-}$, *Casp1*$^{-/-}$*Ldlr*$^{-/-}$ and control *Ldlr*$^{-/-}$ mice were fed WD for 16 weeks with or without intranasal *C.pn* (5x10^4 IFU/mouse) infection three times (one week apart for first three consecutive weeks of WD).

(B) Weight gain of *Nlrp3*$^{-/-}$*Ldlr*$^{-/-}$, *Casp1*$^{-/-}$*Ldlr*$^{-/-}$ and control *Ldlr*$^{-/-}$ mice infected with C.pn and fed a WD for 16 weeks. (n=13-15/group).

(C) Serum concentrations of MCP-1, IL-12p70 and IL-6 were measured by ELISA (n=8). *Nlrp3*$^{-/-}$*Ldlr*$^{-/-}$ and control *Ldlr*$^{-/-}$ mice were fed WD for 16 weeks and some group of mice were infected intranasally with *C.pn*.

All data are mean±SD. Significance was determined using One-Way ANOVA with Tukey's post-hoc test. *p<0.05, **p<0.01. Both male and female mice were used.

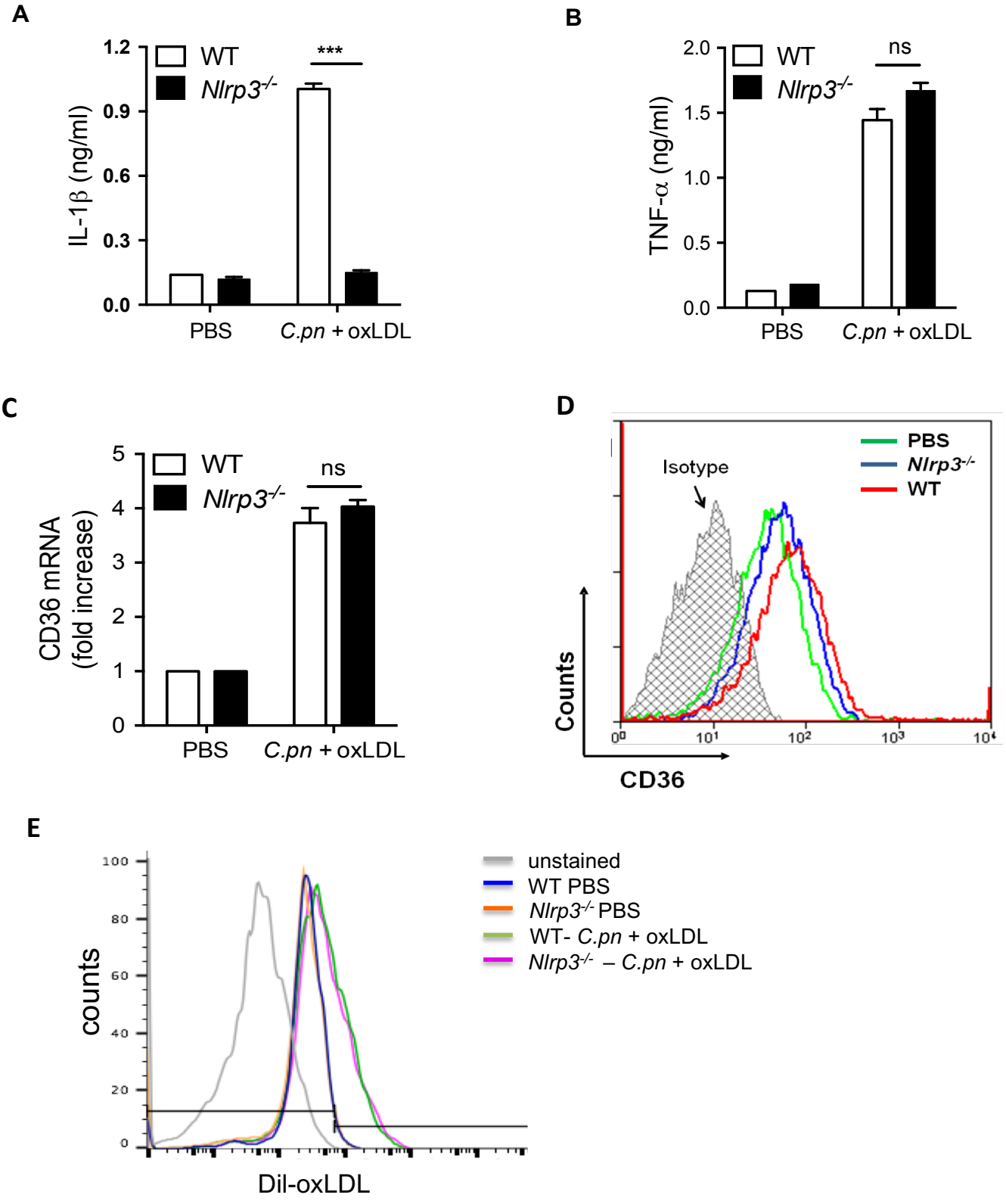

Figure S2. Related to Figure 3. NLRP3 does not affect TNFα production or CD36.
(A-B) The production of IL-1β (A) and TNF-α (B) in culture medium determined by ELISA in peritoneal macrophages from WT and *Nlrp3*$^{-/-}$ mice.
(C) CD36 mRNA by RT-PCR.
(D) CD36 expression by FACS analysis.
(E) The cells were treated with or without C.pn + oxLDL for 24h and incubated with with 20 ug/ml Dil-oxLDL for 3h. Intracellular Dil-oxLDL were measured by FACS.
All data are mean±SD. Significance was determined using One-Way ANOVA with Tukey's post-hoc test. *p<0.05, **p<0.01. Both male and female mice were used.

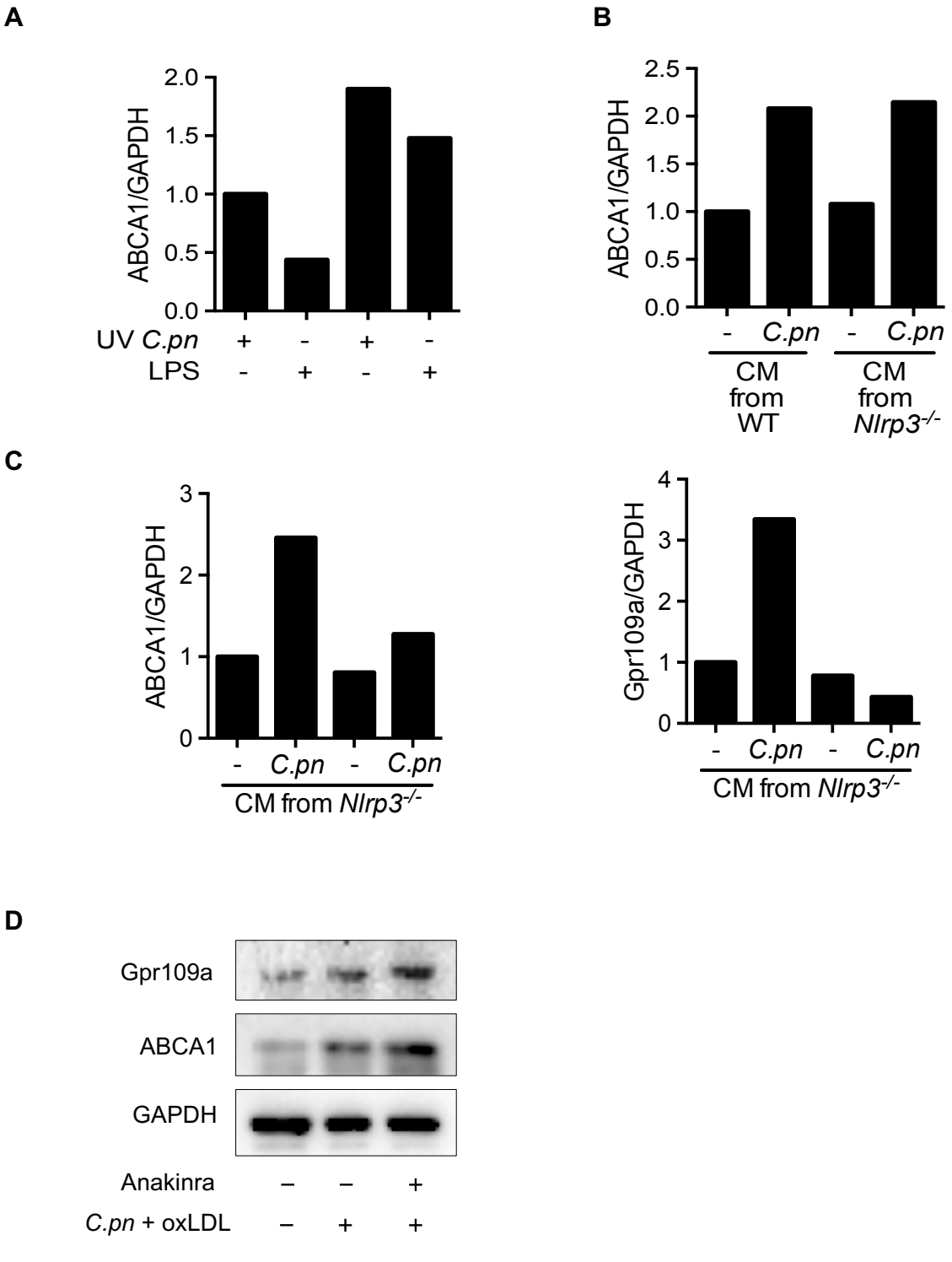

Figure S3. Related to Figure 4. Gpr109a-ABCA1 pathway is upregulated by β-hydroxybutyrate produced by *C.pn*-infected macrophages.

(A) Band densitometry for western blot analysis shown in Figure 4H.
(B) Band densitometry for western blot analysis shown in Figure 4I.
(C) Band densitometry for western blot analysis shown in Figure 4J.
(D) The expression of Gpr109a and ABCA1 is also regulated by IL-1R1 mediated signaling in human macrophages. Human monocyte-derived macrophages (MDM) were co stimulated with *C.pn* and oxLDL for 16h with or without 10 ug/ml anakinra. The protein level of Gpr109a and ABCA1 was determined by Western blotting. A typical picture of 4 separate experiments is shown. Both male and female mice were used.

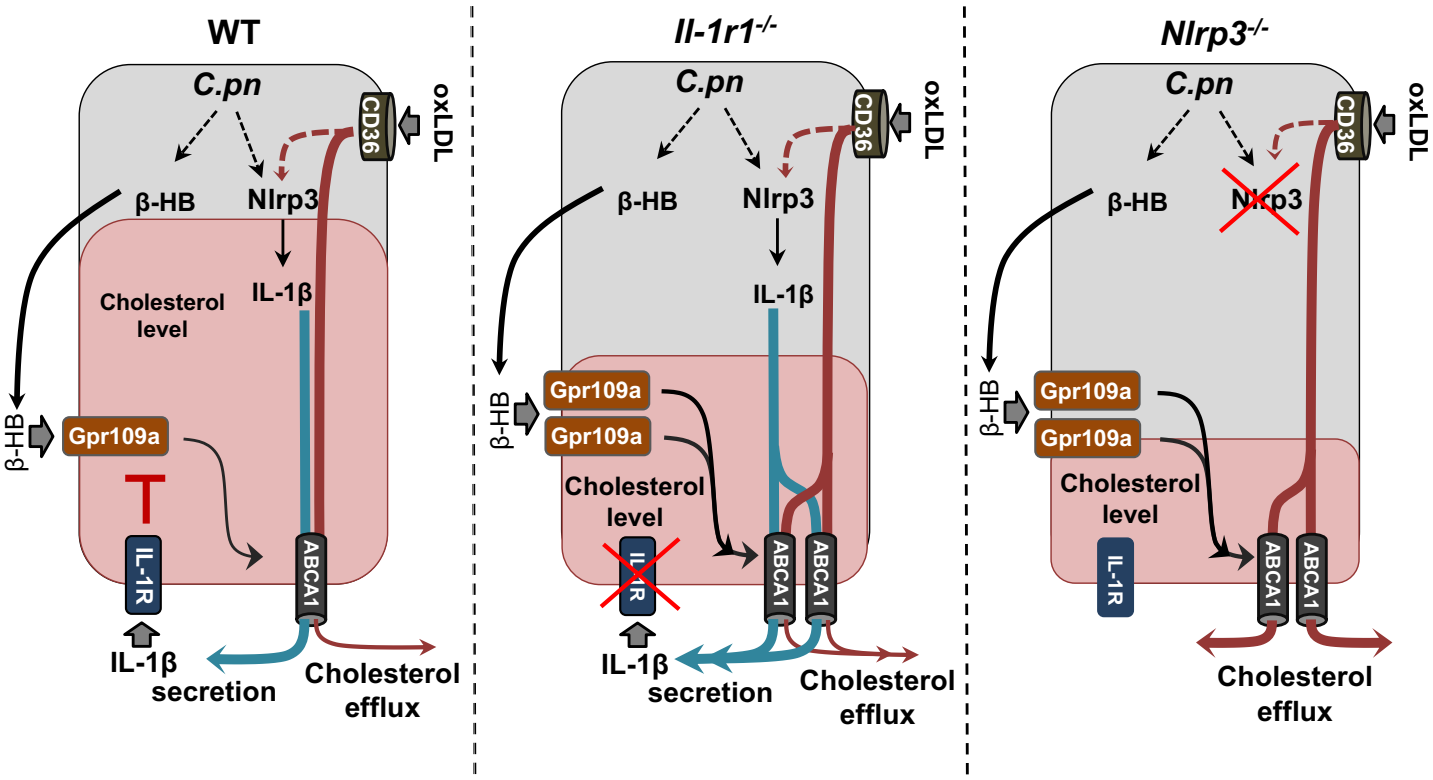

Figure S4. Related to Figures 4 and 5. <u>Schematic of auto regulatory IL-1β feedback loop</u>. *C.pn*-induced Nlrp3 inflammasome activation and IL-1β release play a critical role in cholesterol accumulation and foam cell formation.

In WT macrophages infected with C.pn, β-hydroxybutyrate (β-HB) engages its receptor (Gpr109a) and induces ABCA1 expression and cholesterol efflux. However, C.pn infection also induces NLPR3 activation and IL-1β release (sharing the ABCA1 transporter with cholesterol), and the extracellular IL-1β in turn induces a negative feedback loop that suppresses the expression of Gpr109a and ABCA1. This feedback mechanism leads to diminished cholesterol efflux, and accumulation of cholesterol inside the cell (increased pink).

In IL-1R1-deficient macrophages infected with C.pn, the released (exogenous) IL-1β- induced feedback loop is interrupted as IL-1β can not signal. This leads to increased β-HB-induced Gpr109a and ABCA1 expression and increased cholesterol efflux (less pink compared to WT macrophages). However, IL-1β is still being made and it shares the ABCA1 transporter with cholesterol to exit the cell.

In NLRP3-deficient macrophages infected with C.pn, there is no production of mature IL-1β, and therefore, there is no exogenous IL-1β -induced negative feedback loop for the expression of Gpr109a/ABCA1. Therefore, Gpr109 and ABCA1 expression is higher in NLRP3-deficient macrophage. Since there is no IL-1β made by the cell, there is also no competition for ABCA1 transporter for cholesterol efflux (the least amount of pink).

Figure S4

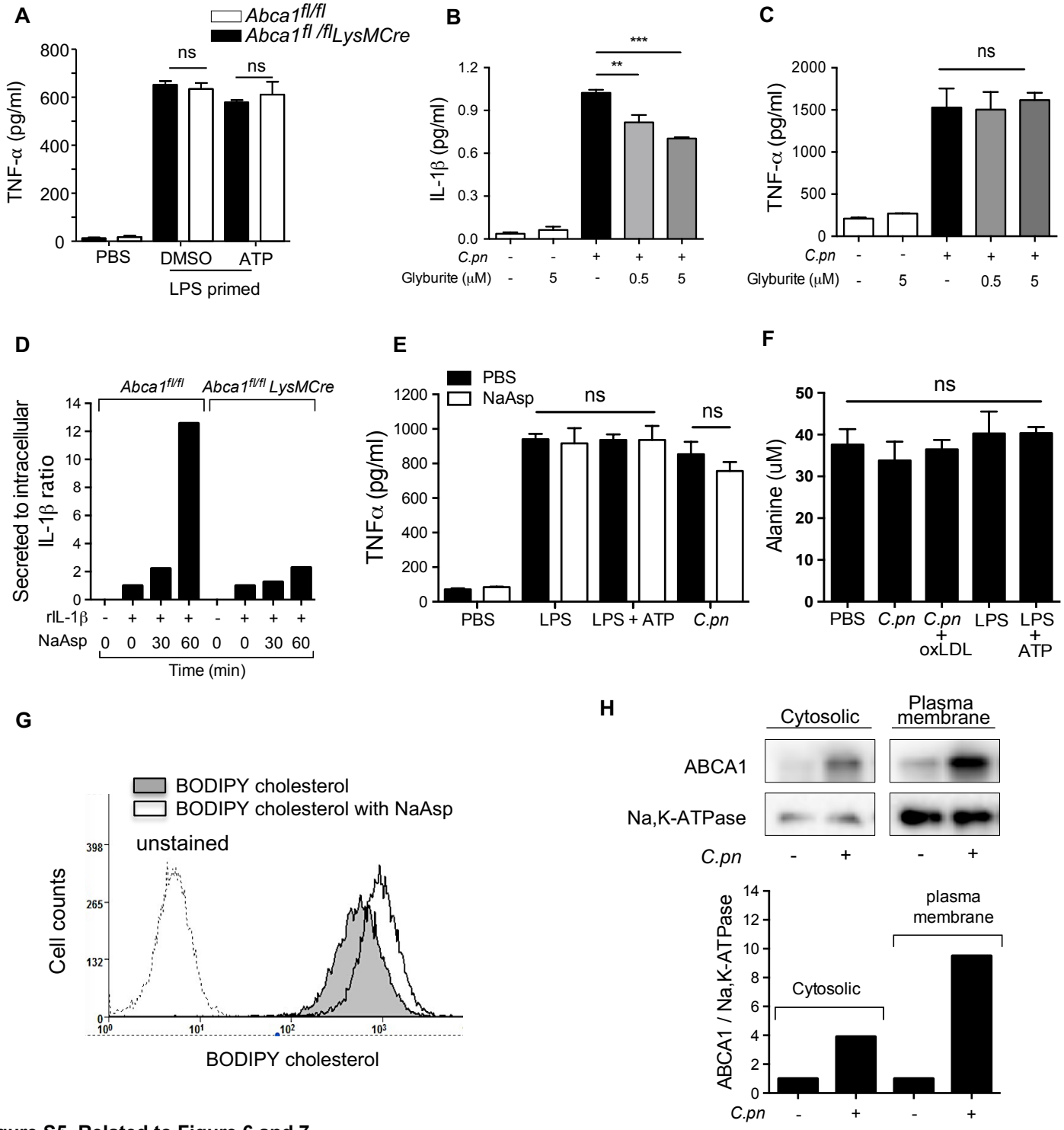

Figure S5. Related to Figure 6 and 7.

(A) The cells were stimulated as mentioned in Fig 6E. Secreted TNF-α was measured by ELISA.

(B and C) The cells were infected with *C.pn* for 24h and glyburide was added the last 8h. Secreted IL-1β (B) and (C) TNF-α were measured by ELISA in cell supernatants.

(D) Band densitometry for western blot analysis shown in Figure 6H.

(E) The cells were stimulated as mentioned in Fig 6I. Secreted TNF-α was measured by ELISA.

(F) The WT cells were treated as mentioned in Figure 7B. Intracellular alanine was determined by alanine assay.

(G) BODIPY-cholesterol was added to WT macrophages with or without NaAsp. Intracellular BODIPY cholesterol level by FACS.

(H) *IL1r1*$^{-/-}$ peritoneal macrophages were infected with *C.pn* for 24h. Plasma membrane protein fraction was isolated by a membrane protein isolation kit according to the manufacturer's protocol. ABCA1 and Na, K- ATPase protein (cell membrane protein marker as loading control) was determined by western blotting. The quantification of band densitometry is shown below.

A typical picture of three independent experiments are shown in F-G. Values are expressed as means ± SD; n=3 performed each in triplicate in A-E. Statistical significance was determined using One-Way ANOVA with Tukey's post-hoc test. *p<0.05, **p<0.01, ns- not significant. Both male and female mice were used.

Figure S5