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Supplemental Information

Suppression of Inflammasome Activation by IRF8 and IRF4 in cDCs Is Critical for T Cell Priming

Margaret M. McDaniel, Leah C. Kottyan, Harinder Singh, and Chandrashekhar Pasare

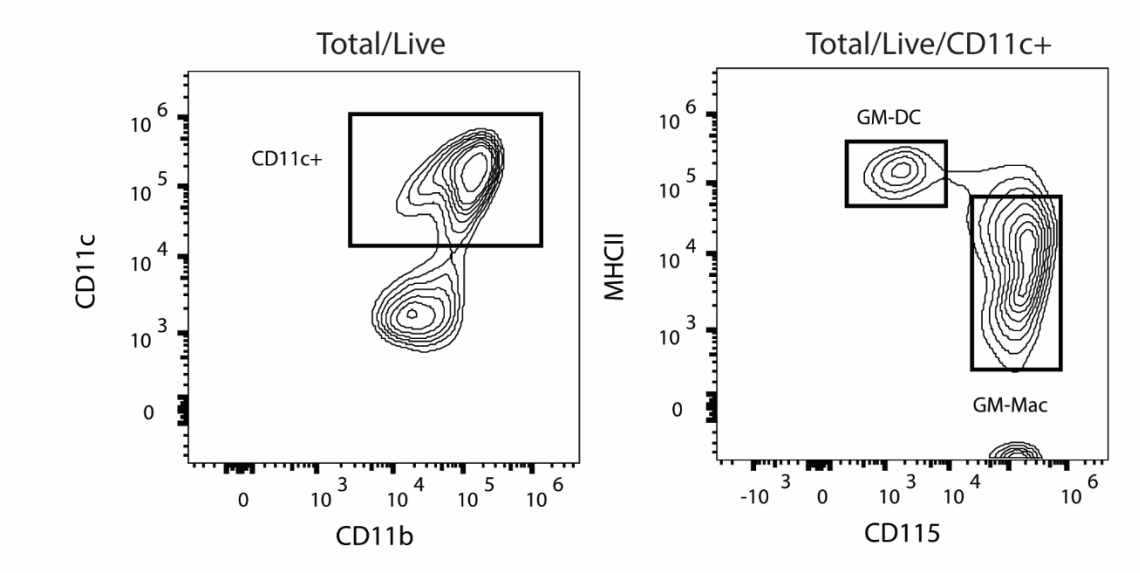


Figure S1 | Sorting strategy for GM-Macs and GM-DCs. Related to Figures 3 and 5**.** Representative image of the gating strategy for flow cytometric analysis and sorting of GM-Macs and GM-DCs within the GMCSF-derived BMDC culture.

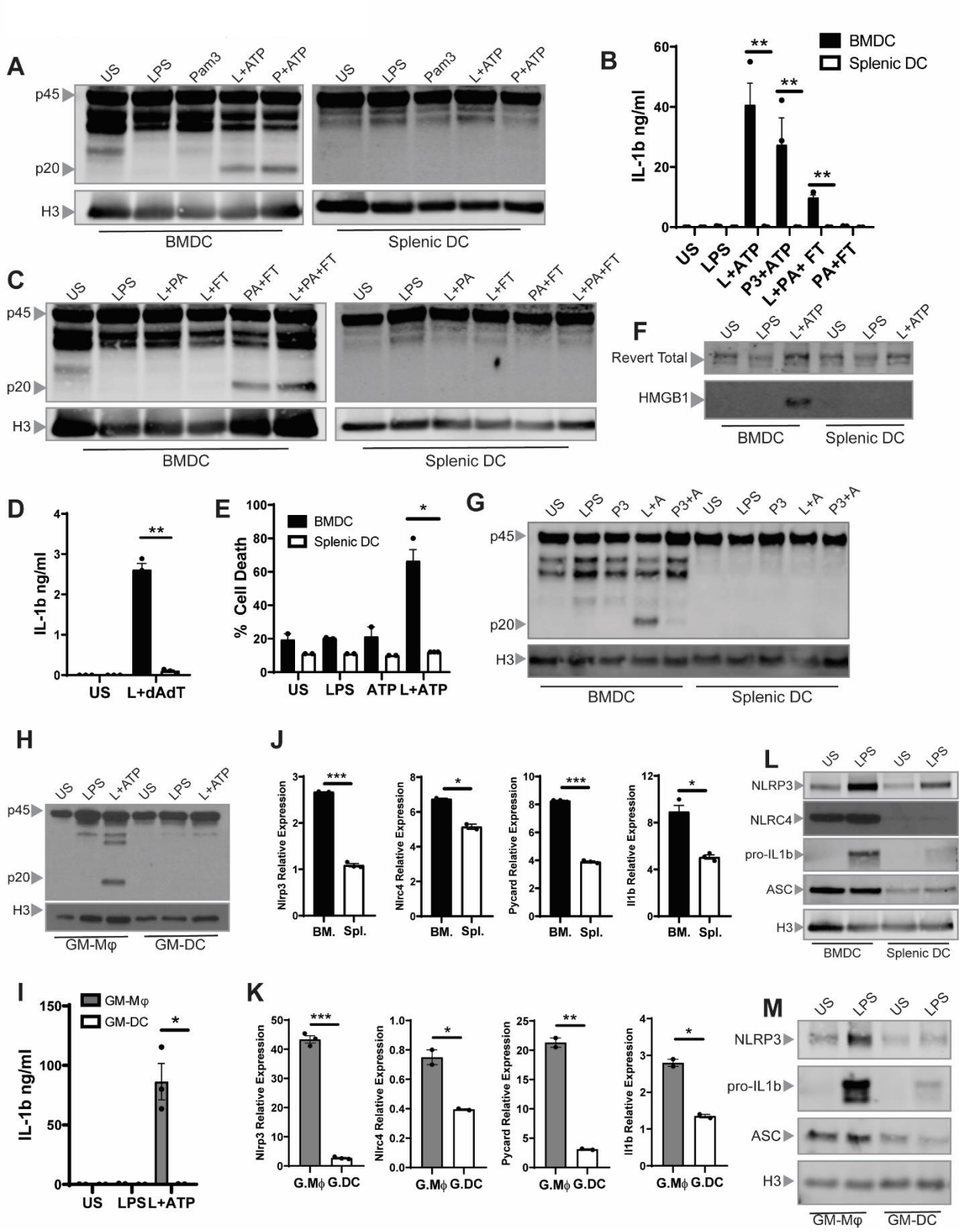
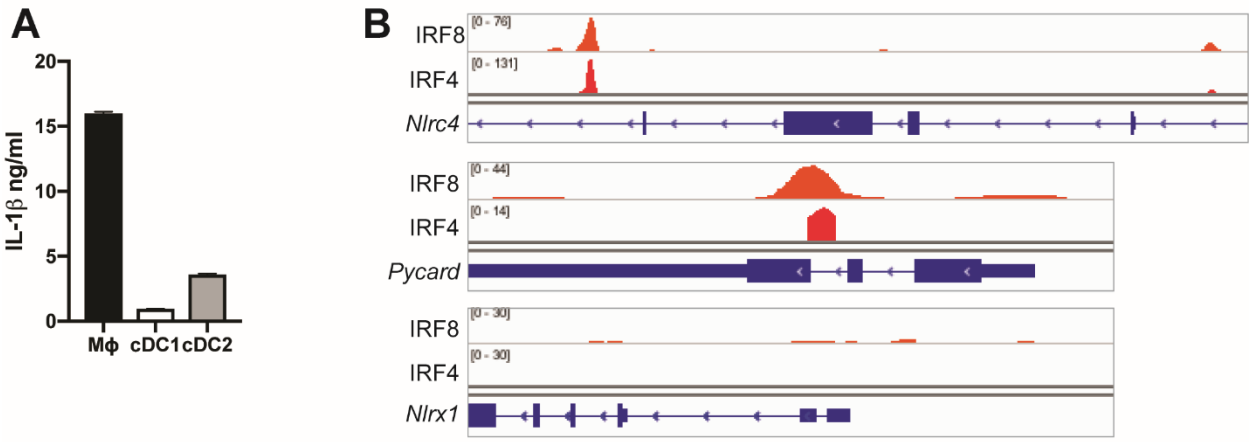
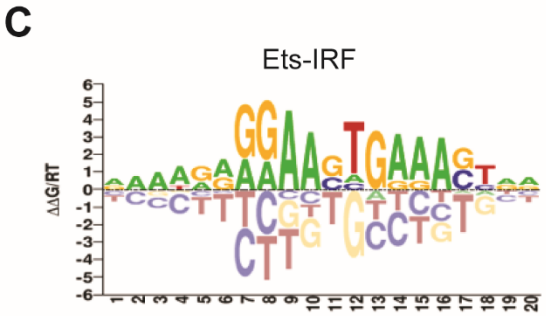

A
US
LPS
Pam3
L+ATP
P+ATP
p45
p20
H3
BMDC
Splenic DC
B
BMDC
Splenic DC
IL-1b ng/ml
US
LPS
L+ATP
P3+ATP
L+PA+ FT
PA+FT
C
L+PA
L+FT
PA+FT
L+PA+FT
F
Revert Total
HMGB1
D
US
L+dAdT
E
% Cell Death
ATP
G
P3
L+A
P3+A
H
GM-Mφ
GM-DC
J
Nlrp3 Relative Expression
Nlrc4 Relative Expression
Pycard Relative Expression
Il1b Relative Expression
BM.
Spl.
L
NLRP3
NLRC4
pro-IL1b
ASC
I
GM-Mφ
GM-DC
K
G.Mϕ
G.DC
M

Figure S2 | Splenic DCs and GM-DCs do not undergo inflammasome activation in response to canonical ligands and express lower levels of inflammasome machinery. Related to Figure 3. (A) Immunoblot analysis of pro-casp1 (p45) and cleaved casp1 (p20) in the lysates of WT BMDCs and $CD11c^+$ splenic DCs stimulated with LPS or PAM3 (100ng/ml) for 4hrs then ATP (5mM) for 30min. (B) IL-1β secretion was measured by ELISA in the supernatants from (A) and (C). (C) Immunoblot analysis of pro-casp1 (p45) and cleaved casp1 (p20) in the lysates of WT BMDCs and $CD11c^+$ splenic DCs stimulated with LPS for 4hrs then FlaTox (PA + LFn-FlaA, 1μg/ml) for 30min. (D) IL-1β secretion was measured by ELISA in the supernatants of BMDCs and $CD11c^+$ splenic DCs stimulated with LPS for 4hrs then transfected with poly(dA:dT) (2μg/ml) for 1hr. (E) Cell death was measured by LDH release in the supernatants of cultures from (A). Cell death was measured by HMGB1 release as detected by immunoblot analysis in the supernatant of (A). (G) Immunoblot analysis of pro-casp1 (p45) and cleaved casp1 (p20) in the lysates of WT BMDCs and $CD11c^+$ splenic DCs stimulated with LPS or Pam3 and ATP simultaneously for 30min. (H) Immunoblot analysis of pro-casp1 (p45) and cleaved casp1 (p20) in the lysates of FACS sorted GM-Macs and GM-DCs stimulated with LPS for 4hrs then ATP for 30min. (I) IL-1β secretion in the supernatants from (H) was measured by ELISA. (J) qPCR of designated mRNA in the lysates of BMDCs and $CD11c^+$ splenic DCs, or (K) FACS sorted GM-Macs and GM-DCs following 4hrs of LPS stimulation. (L) Immunoblot analysis of designated proteins in the lysates of BMDCs and $CD11c^+$ splenic DCs, or (M) FACS sorted GM-Macs and GM-DCs following 4hrs of LPS stimulation. Error bars indicate SEM; (B, D, E, and I-K) paired *t*-test, (A, C, F-H, L, and M) data representative of 3 independent experiments.





D

Gene Sequence	TF Motif	p-value
Pycard	IRF4	2.68E-06
Pycard	IRF8	6.08E-07
Il1b	IRF4	1.15E-05
Il1b	IRF4	8.16E-05
Il1b	IRF8	9.23E-05
Nlrc4	IRF4	6.75E-06
Nlrc4	IRF4	4.20E-05
Nlrc4	IRF8	4.47E-05
Nlrc4	IRF8	8.26E-05
Nlrp3	IRF4	1.6E-05
Nlrp3	IRF4	8.19E-05
Nlrp3	IRF8	2.17E-05
Nlrp3	IRF8	3.43E-05

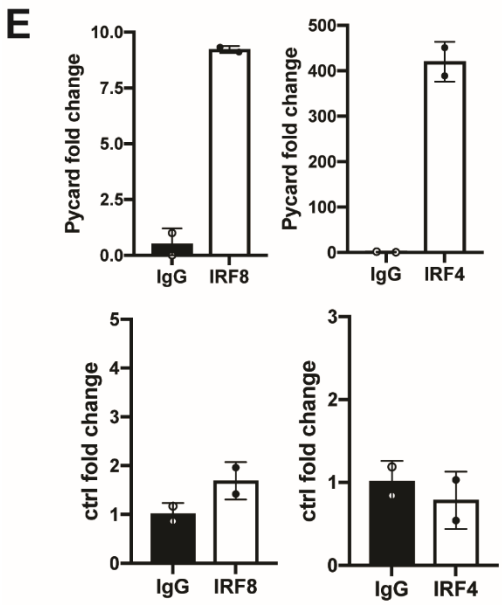


Figure S3 | IRF4 and IRF8 are predicted to bind similar regions of inflammasome-associated genes. Related to Figure 4. (A) IL-1β was measured by ELISA in the supernatant of FACS sorted splenic macrophages ($CD11b^+$), cDC1s ($CD11c^+CD8\alpha^+XCR1^+$), and cDC2s ($CD11c^+CD4^+SIRPa^+$) following stimulation with LPS for 4hrs then ATP for 30min. (B) ChIP-seq data from GSE53311 (Kc et al., 2014) and GSE40727 (Glasmacher et al., 2012) were analyzed with IGV. (C) Consensus motifs for IRF4 and IRF8 from the CIS-BP database (Lambert et al., 2018). (D) FIMO was used to confirm Ets-IRF binding motifs in the promoter or intronic regions of *Nlrp3*, *Nlrc4*, *Pycard*, and *Il1b* (Grant et al., 2011). P-value is defined as the probability that a random sequence of the same length as the motif matches that sequence with a better score. (E) qPCR of designated intronic and control regions were performed on chromatin immunoprecipitated with IRF8, IRF4, and IgG antibodies. Error bars indicate SEM; (A, E) data representative of 2 independent experiments.

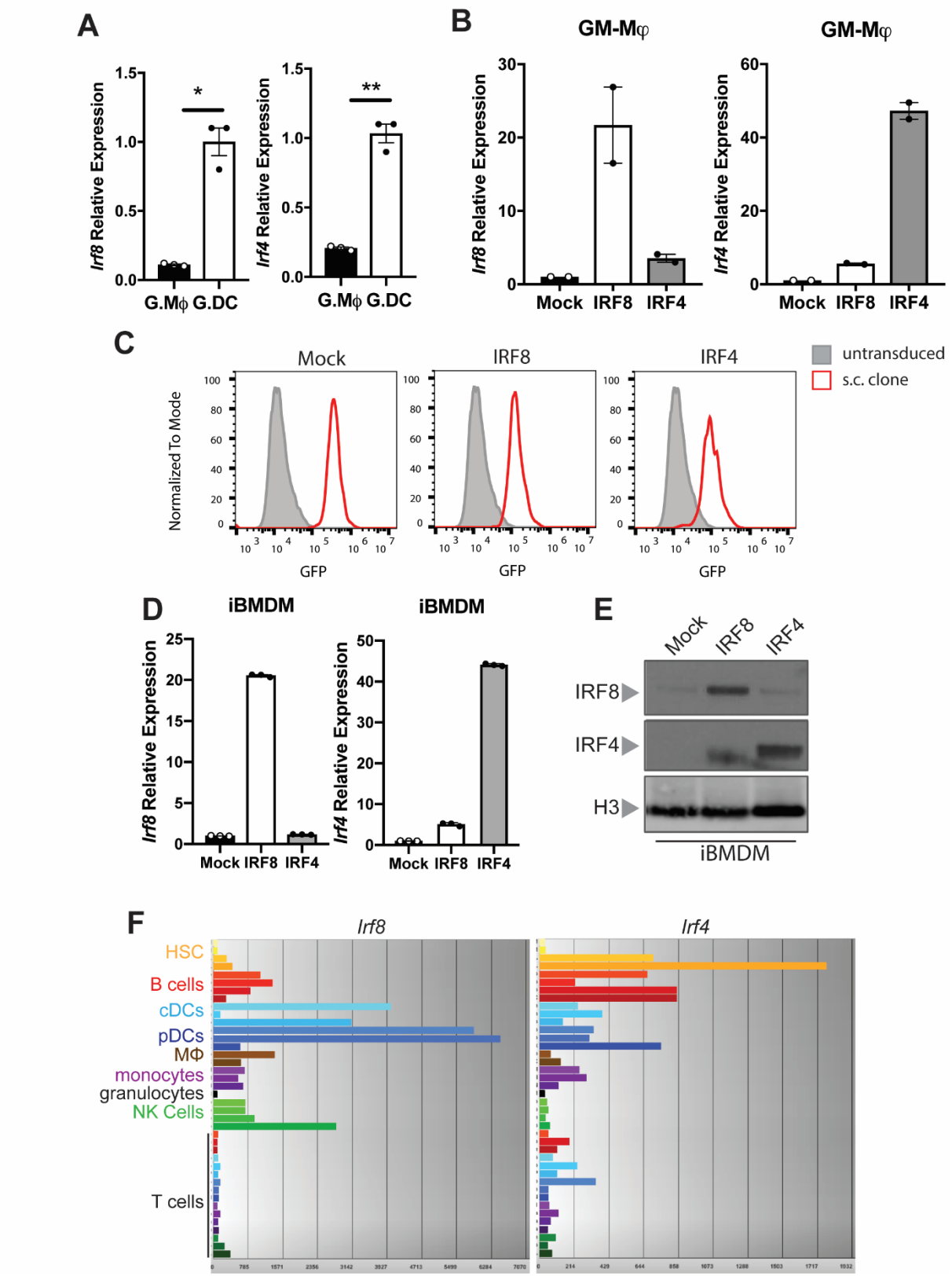

A
Irf8 Relative Expression
Irf4 Relative Expression
G.Mϕ G.DC
B
GM-Mφ
Irf8 Relative Expression
Irf4 Relative Expression
Mock IRF8 IRF4
C
Mock
IRF8
IRF4
untransduced
s.c. clone
Normalized To Mode
GFP
D
iBMDM
Irf8 Relative Expression
Irf4 Relative Expression
Mock IRF8 IRF4
E
Mock
IRF8
IRF4
IRF8
IRF4
H3
iBMDM
F
Irf8
Irf4
HSC
B cells
cDCs
pDCs
MΦ
monocytes
granulocytes
NK Cells
T cells

Figure S4 | Overexpression of IRF8 and IRF4 in BMDCs and iBMDMs. Related to Figure 5. (A) qPCR of *Irf8* and *Irf4* mRNA in the lysates of FACS sorted WT GM-Macs and GM-DCs. (B) qPCR of *Irf8* and *Irf4* mRNA in the lysates of FACS sorted GM-Macs following transduction with retrovirus expressing IRF8, IRF4, or control plasmid. (C) Representative flow plots of transduced iBMDMs populations following single cell GFP+ sorting and expansion. (D) qPCR of *Irf8* and *Irf4* mRNA in the lysates of iBMDMs from (C). Immunoblot analysis of IRF8 or IRF4 in the lysates of iBMDMs from (C). (F) The expression of *Irf8* and *Irf4* in murine immune cell types from the ImmGen microarray expression database of key immune populations. HSC, hematopoietic stem cell; Mφ, macrophage; NK natural killer. Error bars indicate SEM; data representative of 2 independent experiments.