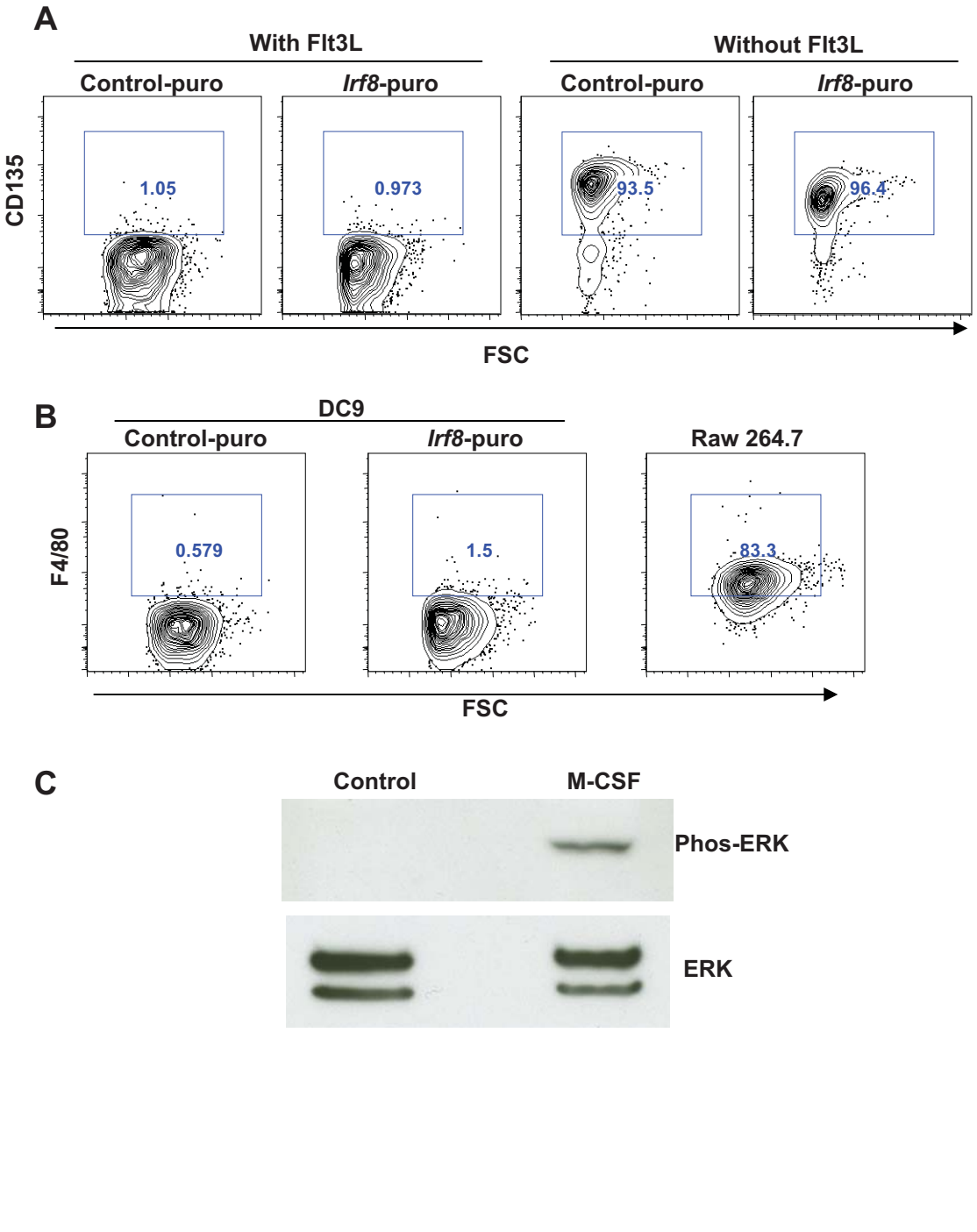
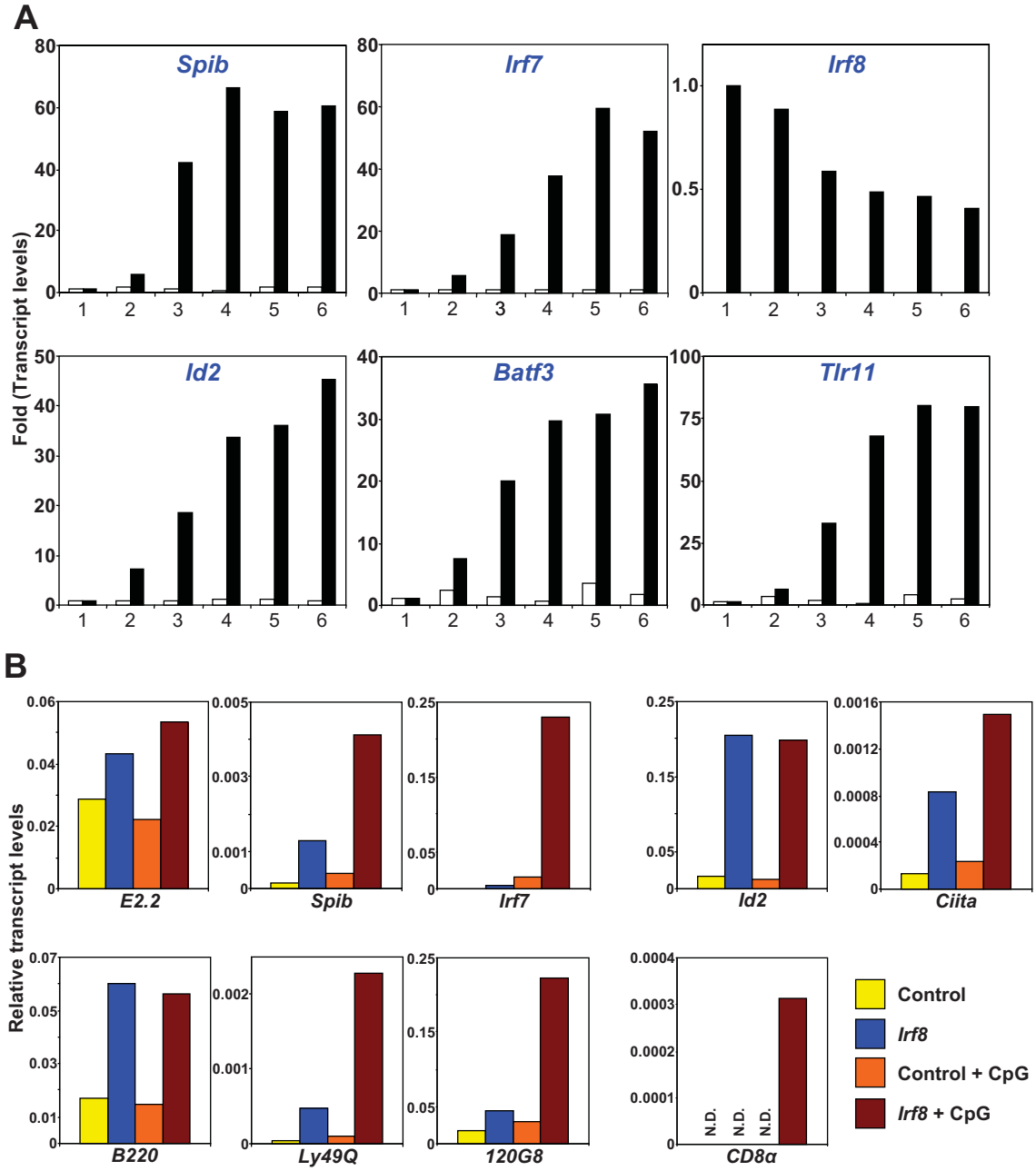
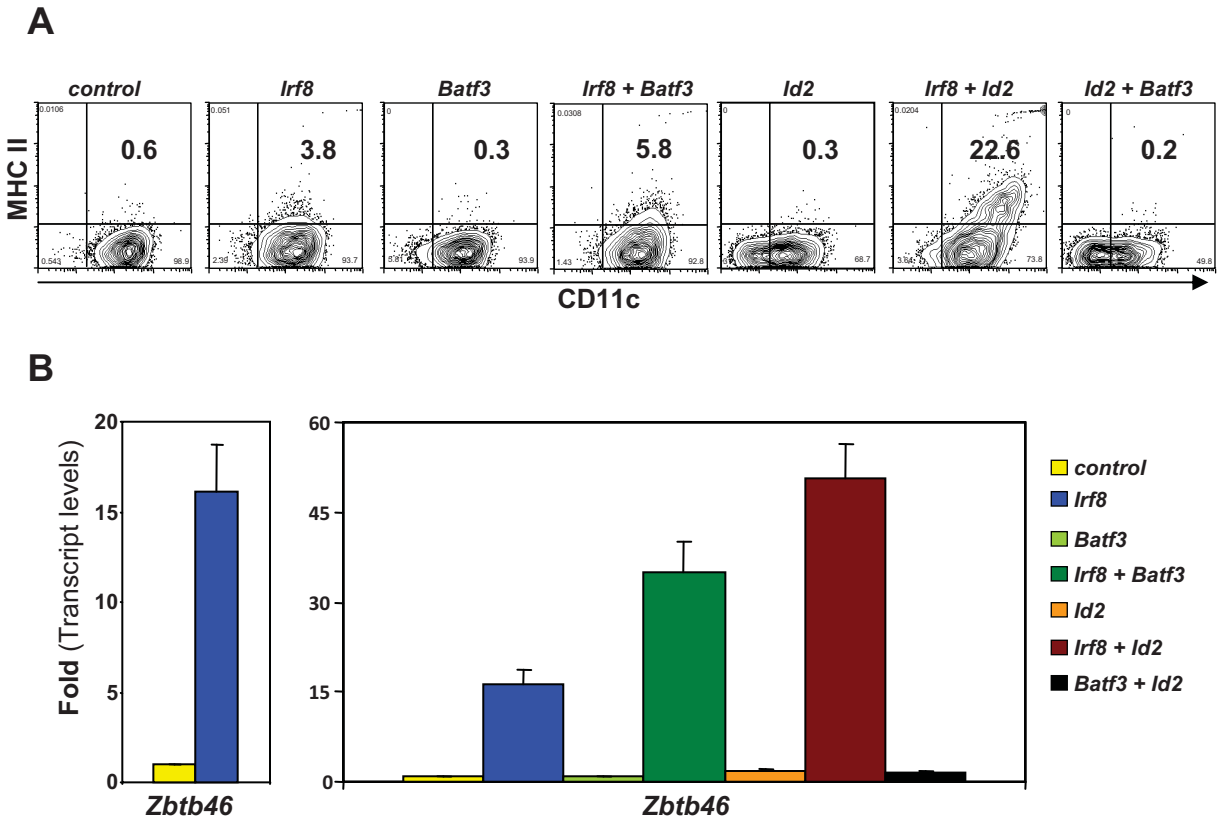
Supplemental Figure 1



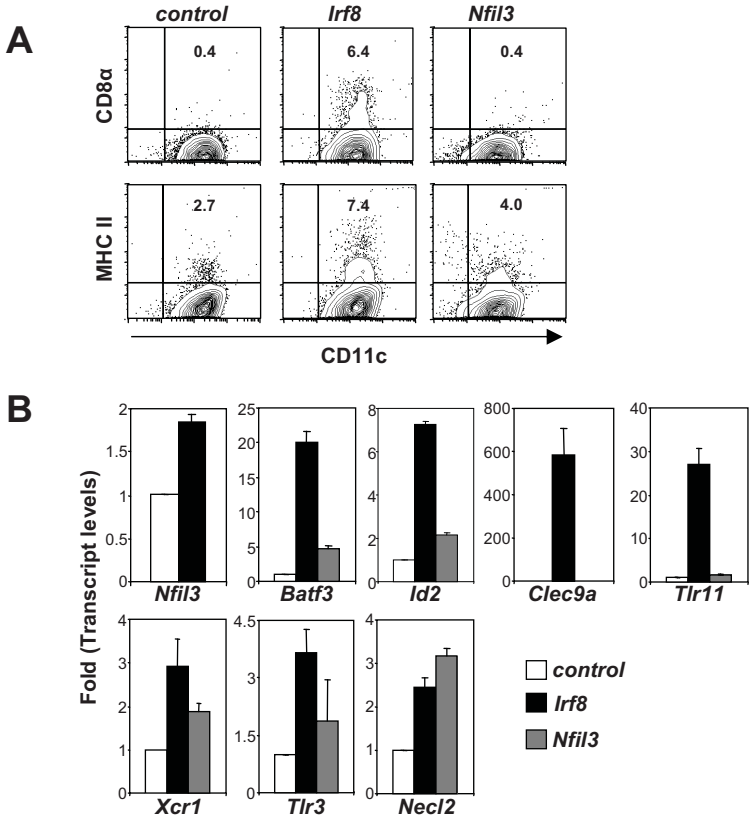
Supplemental Figure 2



Supplemental figure 3



Supplemental Figure 4



Legends for supplemental data

Supplemental figure 1: Characterization of DC9 cell line. (A) DC9 cells were transduced with control and *Irf8* expressing retroviruses and cells were selected for 48 hr with puromycin. Selected populations were cultured in absence of Flt3-L for 12 hrs and CD135 (Flt3 receptor) expression was examined by flow cytometry. In the presence of Flt3-L, due to continuous signaling CD135 expression could not be detected but by removing Flt3-L for a brief period, CD135 can be ubiquitously detected on DC9 cells. (B) Monocyte-macrophage specific marker F4/80 could not be detected on control or *Irf8* expressing cell population; suggesting that *Irf8* expression didn't lead to macrophage differentiation of DC9 cells. RAW264.7 cells were used as a staining control. (C) DC9 cells were cultured in serum starved condition and treated with M-CSF (50 ng/ml) for 1 hr along with serum containing medium. M-CSF stimulation led to efficient phosphorylation implying that M-CSFR expression on DC9 cells can transduce signals efficiently.

Supplemental Figure 2: *Irf8* expression led to increase in the pDC and CD8α^+ DC specific transcript levels in DC9 cells. (A) DC9 cells were transduced with Mig-control-IRES-hCD8t (white bars) and Mig-*Irf8*-IRES-hCD8t (black bars) retroviruses. Cells were purified with anti-human CD8 microbeads everyday post transduction and gene expression levels were measured by real time PCR. On day 6 expression levels of *Spib,*

Irf7, Batf3, Id2 and Tlr11 genes increased 40-80 fold from the expression levels of respective population on day1. X-axis shows the time point post transduction in days. (B) DC9 cells were transduced with MSCV-control-puro and MSCV-*Irf8* puro retroviruses and selected for 48 hrs with puromycin. Selected populations were stimulated with CpG for 6 hrs and transcript levels of pDC and CD8α$^+$ DC specific genes were studied by real time PCR (N.D. = not detected).

Supplemental figure 3: *Batf3* and *Id2* co-expression with *Irf8*, resulted in a synergistic effect. DC9 cells were transduced with MSCV-puro retroviruses expressing genes as indicated above the graph, cells were selected for 48 hrs with puromycin. (A) Unstimulated cells were examined for the MHCII expression by flow cytometry. Population of the cells co-expressing *Irf8* and *Id2* showed high levels of MHCII expression and its level increased further upon CpG stimulation as shown in figure 4C. (B) *Irf8* expression increased cDC specific *Zbtb46* gene transcript levels and the expression of *Zbtb46* was synergistically increased by co-expression of *Id2* or *Batf3* with *Irf8* (this data is in addition to Figure 5).

Supplemental Figure 4: *Nfil3* expression is not sufficient for the CD8α$^+$ DC development. Control, *Nfil3* and *Irf8* expressing MSCV-puro retroviruses were transduced in DC9 cells. Populations were selected with puromycin antibiotic for 48 hrs. (A) *Nfil3* expression in DC9 cells did not lead to appearance of CD8α marker upon CpG (1826, 1μg/ml, 24 hrs) treatment where as MHCII expression levels were induced at lower levels in comparison to *Irf8* expressing cells. (B) RT-PCR analysis on unstimulated

populations showed that expression of *Nfil3* led to increase in *Batf3* and *Id2* transcript levels; though respective expression levels were higher in *Irf8* expressing cells. Other $CD8\alpha^+$ DC specific gene transcripts were also lower in *Nfil3* expressing cells compared to *Irf8* expressing population. Data are representative of two independent experiments.