**Legends for Supplemental Figures**

**Supplemental Figure 1. A.** Anti-CD40 antibody treatment is mediated by CD40. WT mice were either left untreated, injected with 100 μg Ratlg2a isotype control, or with 100 μg of anti-CD40 (clone IC-10), or anti-CD40 (clone FGK4.5). Additionally CD40−/− mice were injected with anti-CD40 (FGK4.5). Forty eight hours later, spleens were analyzed for populations of interest. Cells were gated as described in figure 1A. **B.** Appearance of Ly6Chi-CD11cHi cells in CD11b v. CD11c plots. Gating scheme, similar to figure 1A, except cells are only gated though the first 5 gates and displayed on a CD11c v. CD11b plot. **C.** Appearance of Ly6Chi-CD11cHi cells in CD11b v. CD11c plots at various time points after injection of mice with 100 μg anti-CD40 mAb. **D.** Comparison of surface CD11c levels of cDCs, Ly6Chi monocytes, Ly6CNeg monocytes, and Ly6Chi-CD11cHi cells. **E.** Similar populations of Ly6Chi-CD11cHi cells can be detected in the bone marrow (BM), mesenteric lymph nodes (MLN) and the blood of mice treated with anti-CD40 mAb.

**Supplemental Figure 2. Fate of Ly6CNeg monocytes using bead labeling. A.** Mice were injected with fluorescent beads and then at the indicated times monocytes (lineage negative, CD11b+, CD115+ cells) were analyzed for bead content. **B.** Similar to experiments in figure 3, mice were injected with fluorescent beads and then analyzed as indicated.
Supplemental Figure 3. Requirements for CD40 expression on monocyte activation in response to anti-CD40. (A). Wild type or CD40-/- mice were treated with anti-CD40 mAb for 24 hours to verify the specificity of the anti-CD40 and the requirement of CD40 for the response. Mixed bone-marrow chimeras were prepared by lethally irradiating wt or CD40-/- mice and reconstituting them with bone marrow cells from wt or CD40-/- as indicated. Mice were subsequently treated with anti-CD40 mAb and the spleens analyzed after 24 hr. (B). Ly6CHi monocytes were isolated from wt mice (CD45.1) and transferred into CD40-/- recipients (CD45.2). The recipient mice were either control-treated (PBS or rat IgG control, left column) or treated with anti-CD40 mAb (right column). 24 hours after treatment spleens were harvested for analysis as indicated.

Supplemental Figure 4. Production of TNF-α by populations in the spleens of mice 40 hrs after anti-CD40 treatment. Mice were treated with anti-CD40 for 40 hrs to induce the appearance of Ly6CHi-CD11cHi cells. Splenocytes were harvested and cultured in the presence of Brefeldin A (5 µg/ml) in either media alone, media with LPS (200 ng/ml), media with anti-CD40 mAb (5 µg/ml), or HK Listeria (5x10^7/ml) for 60 minutes. Cells were then analyzed for intracellular TNF-α by flow cytometry.
Drutman et al. Supplemental Figure 1

A

WT untreated
WT Rat IgG2a
WT anti-CD40 (IC10)
WT anti-CD40 (FGK4.5)
CD40-KO anti-CD40 (FGK4.5)

Ly6C
CD11c

B

gate scatter → gate live cells → gate singlets → gate non-B,T,NK,
Granulocyte → gate out PDCs → gate CD11b vs CD11c

SSC
FSC
7-AAD
FSC-Area
SSC
TCR/CD19/NK1.1/Ly6G
Siglec-H

C

control-treated
4 h
16 h
20 h
24 h
40 h

CD11c

D

CD11b(+) DCs
Ly6C(hi) Mo
Ly6c(neg) Mo
Ly6C(hi)-CD11c(hi)

E

control-treated
anti-CD40 - 40 h

BM
MLN
Blood

CD11c

# Cells
CD11c
Supplemental Figure 2

**A**

<table>
<thead>
<tr>
<th>Time after bead injection:</th>
<th>1h</th>
<th>24h</th>
<th>40h</th>
<th>48h</th>
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<tr>
<td>all monocytes</td>
<td>55%</td>
<td>44%</td>
<td>60%</td>
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<td>Bead + monocytes</td>
<td>76%</td>
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<td>96%</td>
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**B**

<table>
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<th>48h</th>
<th>64h</th>
<th>88h</th>
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<tr>
<td>all cells</td>
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<td></td>
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<tr>
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**CD11c**

Ly6C
### Supplemental Figure 3

#### A

<table>
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<tr>
<th>Treatment</th>
<th>Group</th>
<th>CD11c</th>
<th>Ly6C</th>
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<tbody>
<tr>
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<td>wt anti-CD40 24h</td>
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<td>CD40-/- anti-CD40 24h</td>
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<tr>
<td>CD40-/- BM into wt anti-CD40 24h</td>
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<tr>
<td>wt BM into CD40-/- anti-CD40 24h</td>
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<td>8.5</td>
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</table>

#### B

- Transferred wt Mo (CD45.1)
- Endogenous CD40-/- cells (CD45.2)
- Overlay

### Bone marrow chimeras

- control-treated
- anti-CD40 24h
Supplemental Figure 4

<table>
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<tr>
<th>Condition</th>
<th>CD11c&lt;sup&gt;+&lt;/sup&gt; Ly6C&lt;sup&gt;+&lt;/sup&gt;</th>
<th>Ly6C&lt;sup&gt;+&lt;/sup&gt; monocytes</th>
<th>CD11b(+) DCs</th>
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<tbody>
<tr>
<td>Isotype control stain</td>
<td>0.7%</td>
<td>1.0%</td>
<td>0.9%</td>
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<tr>
<td>Media</td>
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<td>0.6%</td>
<td>0.6%</td>
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<td>Media +BFA</td>
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<td>1.9%</td>
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<td>Media +BFA +LPS</td>
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<td>Media +BFA +anti-CD40</td>
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<td>5.1%</td>
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<tr>
<td>Media +BFA +HK Listeria</td>
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<td>5.5%</td>
<td>3.4%</td>
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</tbody>
</table>

Treatments include media, media +BFA, media +BFA +LPS, media +BFA +anti-CD40, media +BFA +HK Listeria.

Cell markers include CD11c<sup>+</sup>, Ly6C<sup>+</sup>, and CD11b(+) for different conditions.