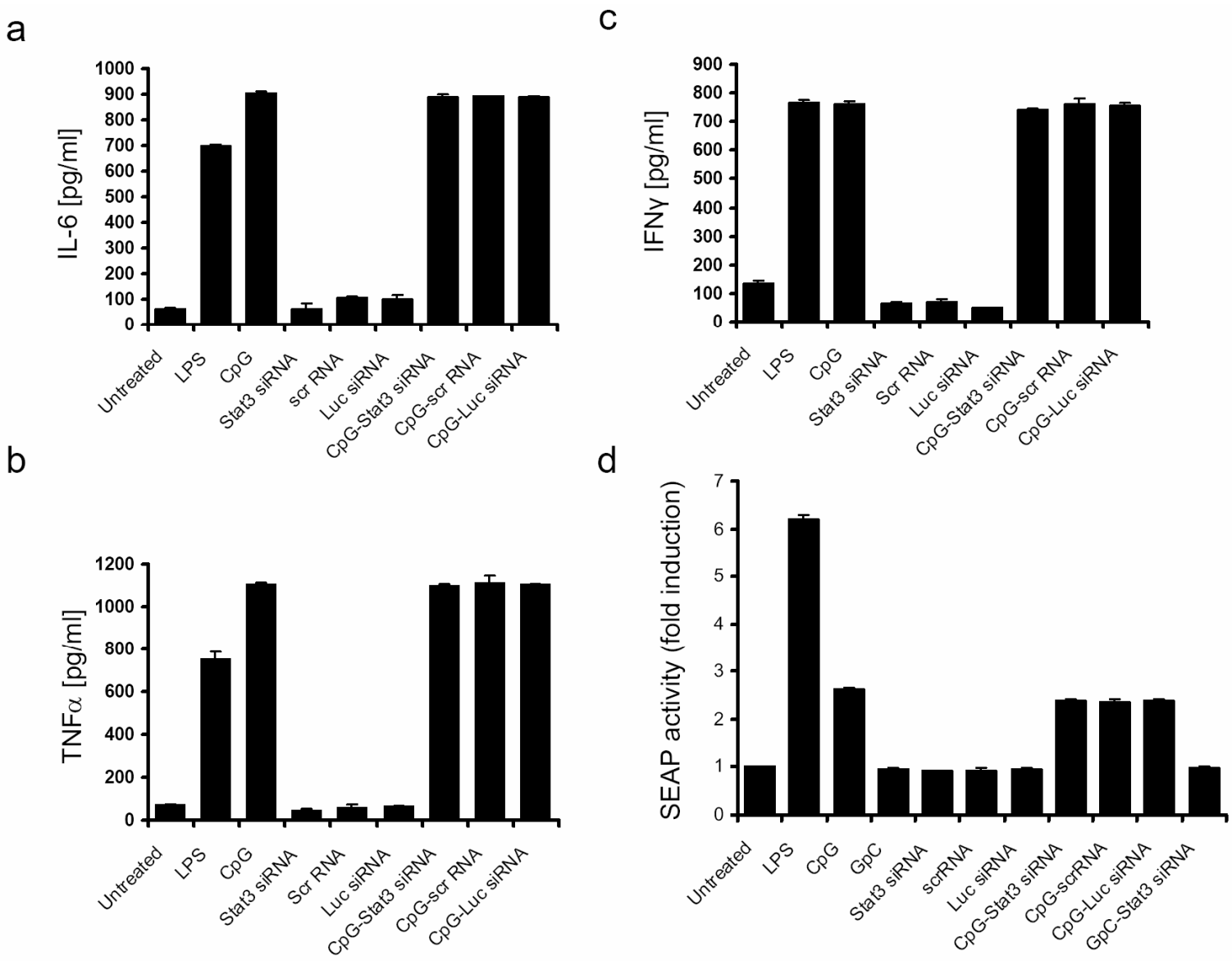
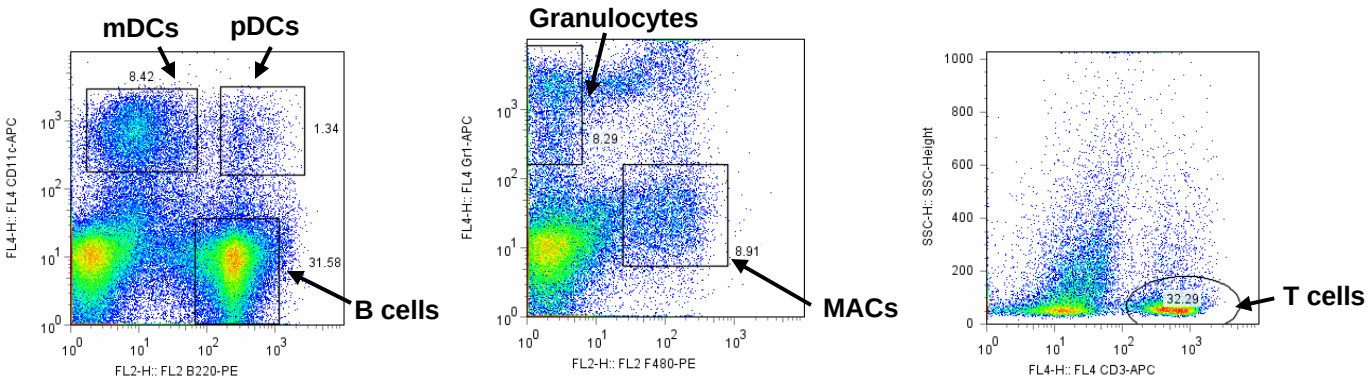



**Supplementary Fig. 1. Relative immunostimulatory properties of various oligonucleotide sequences used in the study.** (**a-c**) Freshly isolated splenocytes (pooled from three C57BL/6 mice) were cultured for 24 h in the presence of LPS (0.5 μg/ml) or various oligonucleotides (500 nM of each) as indicated. The secretion of proinflammatory mediators including IL-6 (**a**), TNFα (**b**) and IFNγ (**c**) into culture media was assessed using ELISA. (**d**) Immunostimulatory effects of various TLR agonists as measured using SEAP reporter gene assay. RAW-Blue™ cells (InvivoGen) were incubated for 24 h with LPS (5 μg/ml) or 500 nM of various oligonucleotides as indicated. The level of immune activation was assessed based on NF-κB/AP1-dependent induction of SEAP expression measured calorimetrically. Shown are representative results of two independent experiments analyzed in triplicates ± SEM.

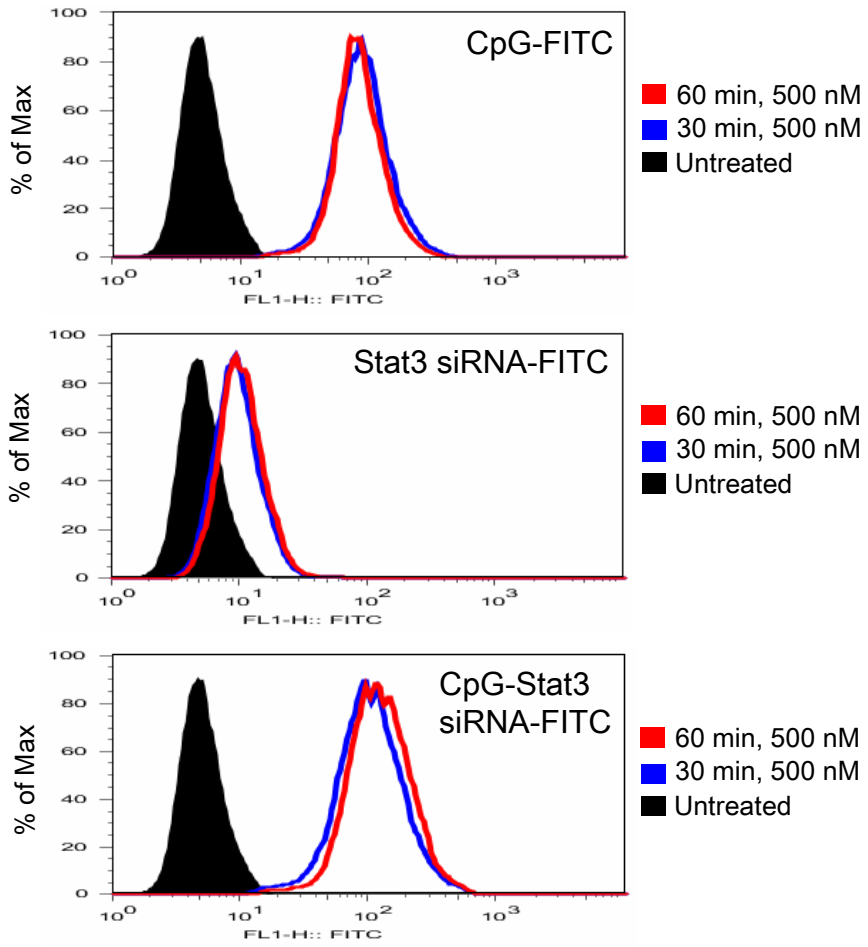
Supplementary Fig. 1a-d




**Supplementary Fig. 2. Gating of various immune cell subsets for the analysis of in vitro CpG-*Stat3* siRNA uptake.** FACS analysis was performed on single-cell suspensions of splenocytes prepared as described in the legend to Fig. 2b. The percentages of FITC-positive cells shown in Fig. 2b and Supplementary Table 1 were assessed in immune cell subtypes gated as indicated.

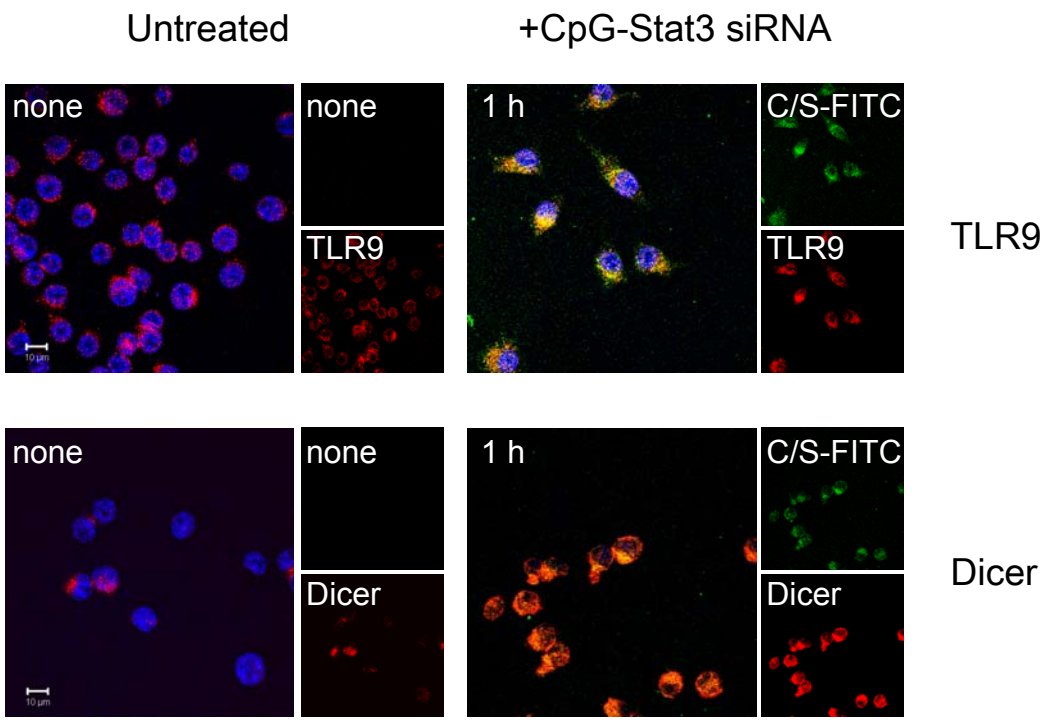
| | Untreated | 100 nM | 250 nM | 500 nM |
|---|---|---|---|---|
| **mDCs** | 0% | 75% | 94% | 97% |
| **pDCs** | 0% | 83% | 96% | 98% |
| **MACs** | 0% | 75% | 91% | 96% |
| **B cells** | 0% | 22% | 64% | 94% |
| **Granulocytes** | 0% | 16% | 28% | 29% |
| **Tcells** | 0% | 9% | 16% | 16% |

**Supplementary Table 1 (accompanying Fig. 2a). *In vitro* uptake of FITC-labeled CpG-Stat3 siRNA by various splenic immune cell populations.** Splenocytes were incubated for 3 h with various concentrations of CpG-*Stat3* siRNA labeled with fluorescein in the absence of any transfection reagents. Percentages of fluorescein-positive $CD11c^{+}B220^{-}$ non-plasmacytoid (mDCs) and $CD11c^{+}B220^{+}$ plasmacytoid (pDCs) DCs, $F4/80^{+}Gr1^{-}$ macrophages (MACs), $B220^{+}CD11c^{-}$ B cells, $Gr1^{+}F4/80^{-}$ granulocytes and $CD3^{+}$ T cells was assessed by FACS analysis. Shown are representative results from one of two independent experiments.

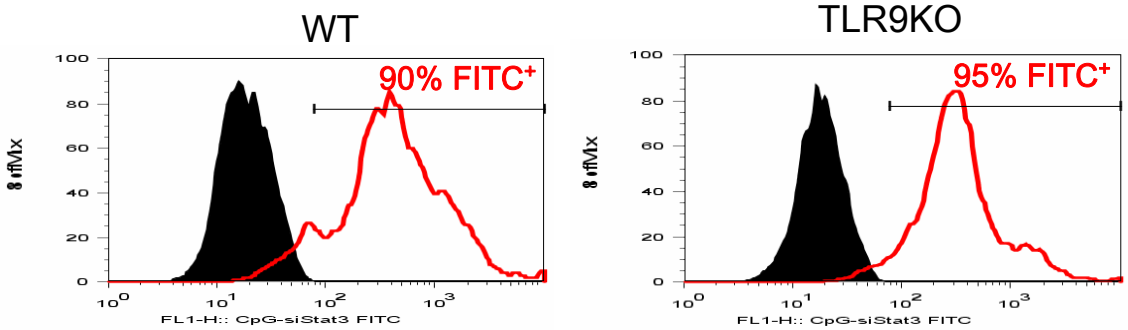



**Supplementary Fig. 3. Comparison of internalization kinetics of CpG ODN, siRNA and CpG-siRNA conjugate.** The uptake of FITC-labeled molecules by DC2.4 cells was analyzed by flow cytometry after incubation at 500 nM for indicated times.

Supplementary Fig. 3

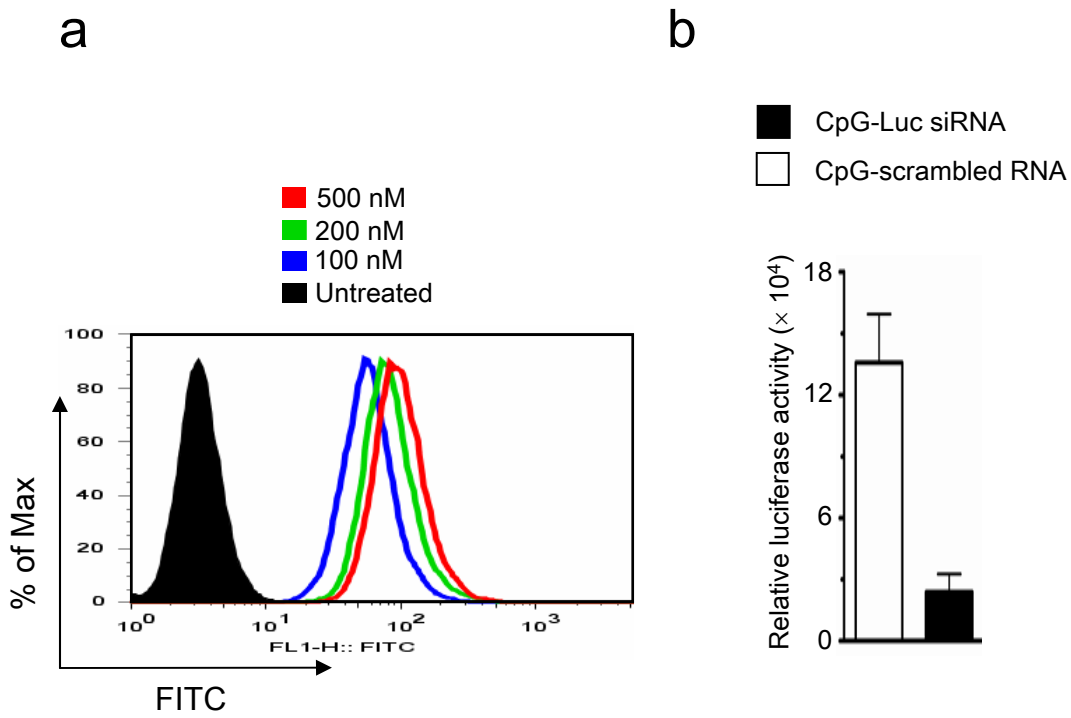



**Supplementary Figure 4. Colocalization of FITC-labeled CpG-Stat3 siRNA with TLR9 (top panels) and with Dicer (bottom panels) as shown by confocal microscopy.** DC2.4 cells were incubated with 500 nM of CpG-Stat3 siRNA for 1 h. Shown are confocal microscopy images at lower magnification to visualize similar colocalization pattern in the majority of analyzed cells; green – CpG-Stat3 siRNA-FITC (C/S-FITC), red – immunofluorescent detection of endogenous TLR9 or Dicer, blue – nuclear staining with Hoechst. All confocal imaging studies were performed at least twice with similar results. Scale bar = 10 μm.



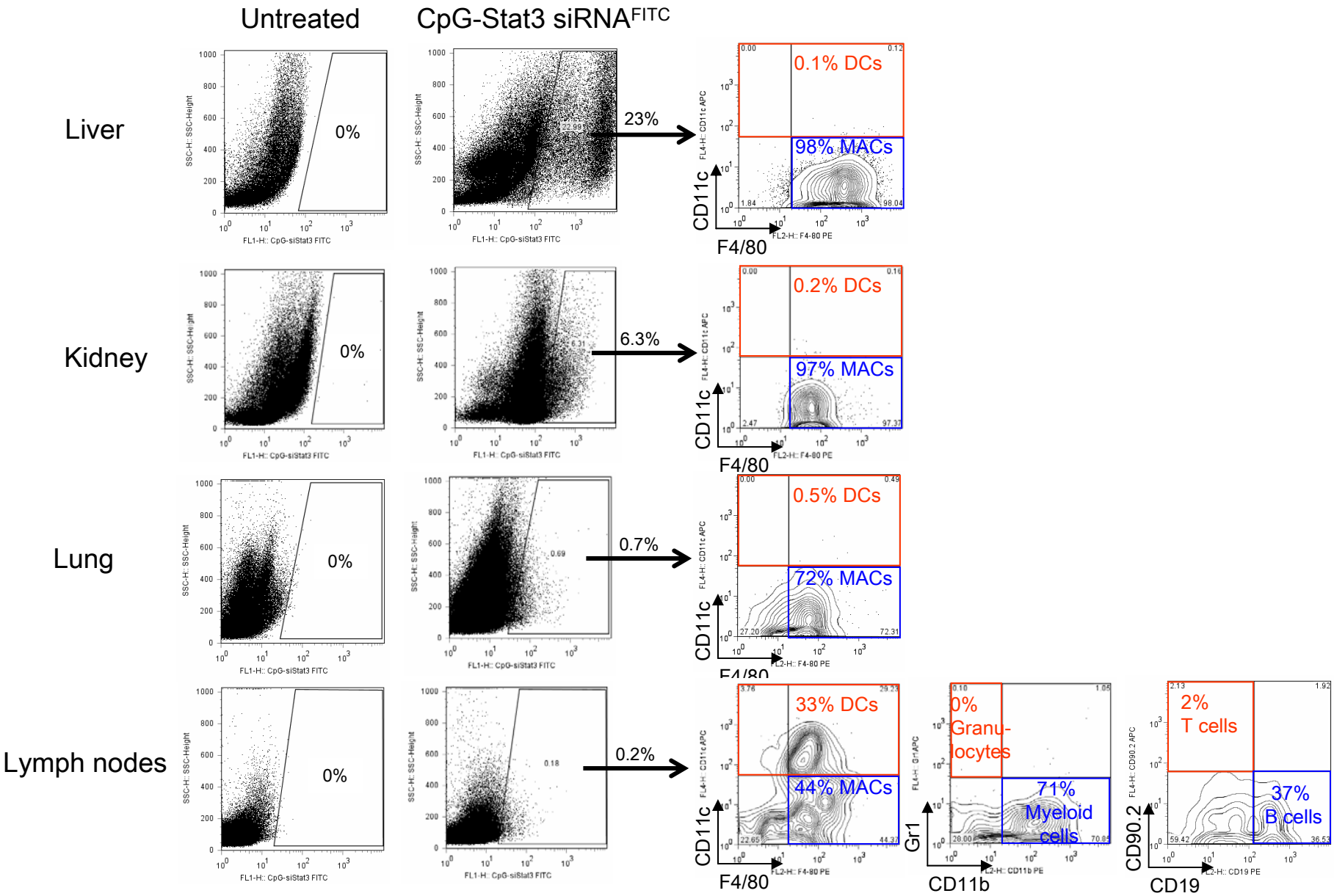

**Supplementary Fig. 5 . TLR9 is not required for uptake of FITC-labeled CpG-Stat3 siRNA.** Cultured bone marrow-derived DCs (day 9) were incubated for 1 h with 500 nM CpG-*Stat3* siRNA labeled with FITC. Shown are percentages of fluorescein-positive CD11c$^+$ cells as assessed by FACS.

Supplementary Fig. 5



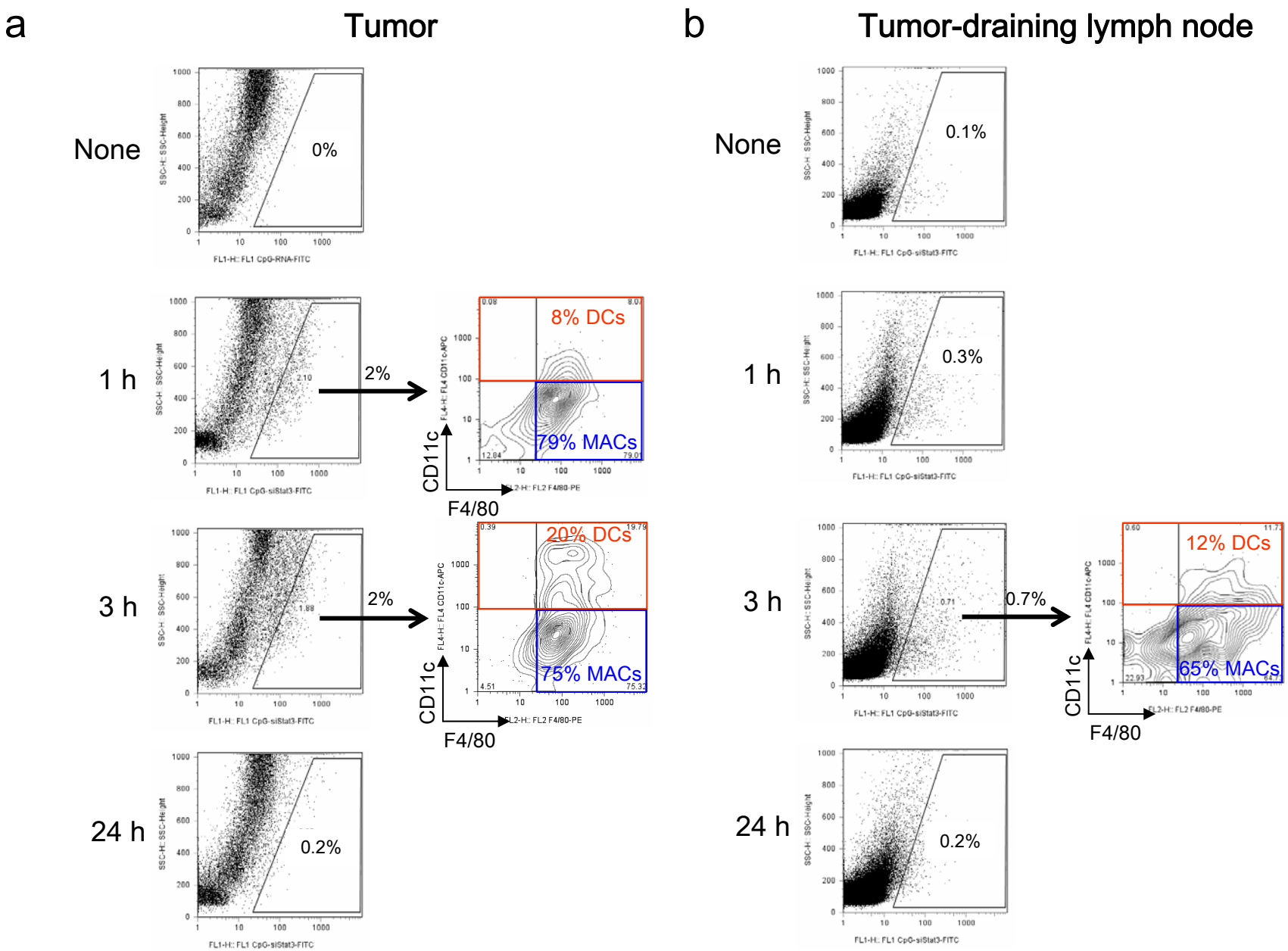

**Supplementary Fig. 6. CpG-siRNA uptake and gene silencing effects in A20 B cell lymphoma cells.** (**a**) A20 cells were cultured in the presence of the chimeric constructs for 24 h at indicated concentrations. (**b**) CpG-Luc siRNA silences gene expression as shown by reduction in luciferase activity. CpG-Luc siRNA and CpG-scrambled RNA were added to Luc-A20 cells (100 nM). Shown are averages ± SEM, $n = 5$.

Supplementary Fig 6ab



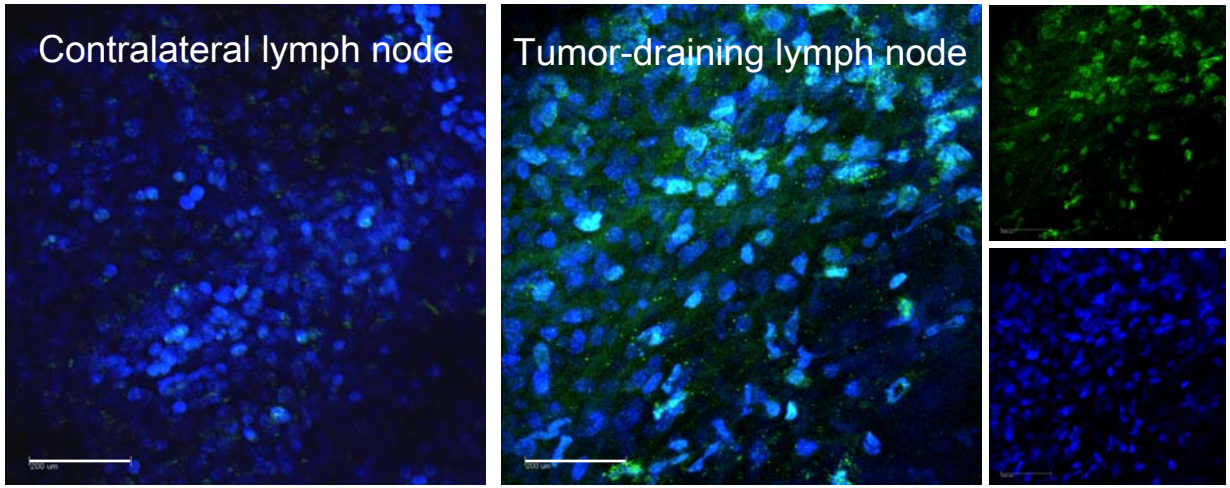

**Supplementary Fig. 7. Biodistribution of systemically injected CpG-Stat3 siRNA.** Mice were sacrificed 3 h after *i.v.* (retroorbital) injection of 100 μg FITC-labeled CpG-Stat3 siRNA. Harvested tissues were enzymatically dispersed into single cell suspension, enriched for mononuclear cells and analyzed by FACS for the presence of various immune cell subsets as indicated. Shown are representative results of two independent experiments using 2-3 mice analyzed individually.

**Supplementary Fig. 7**

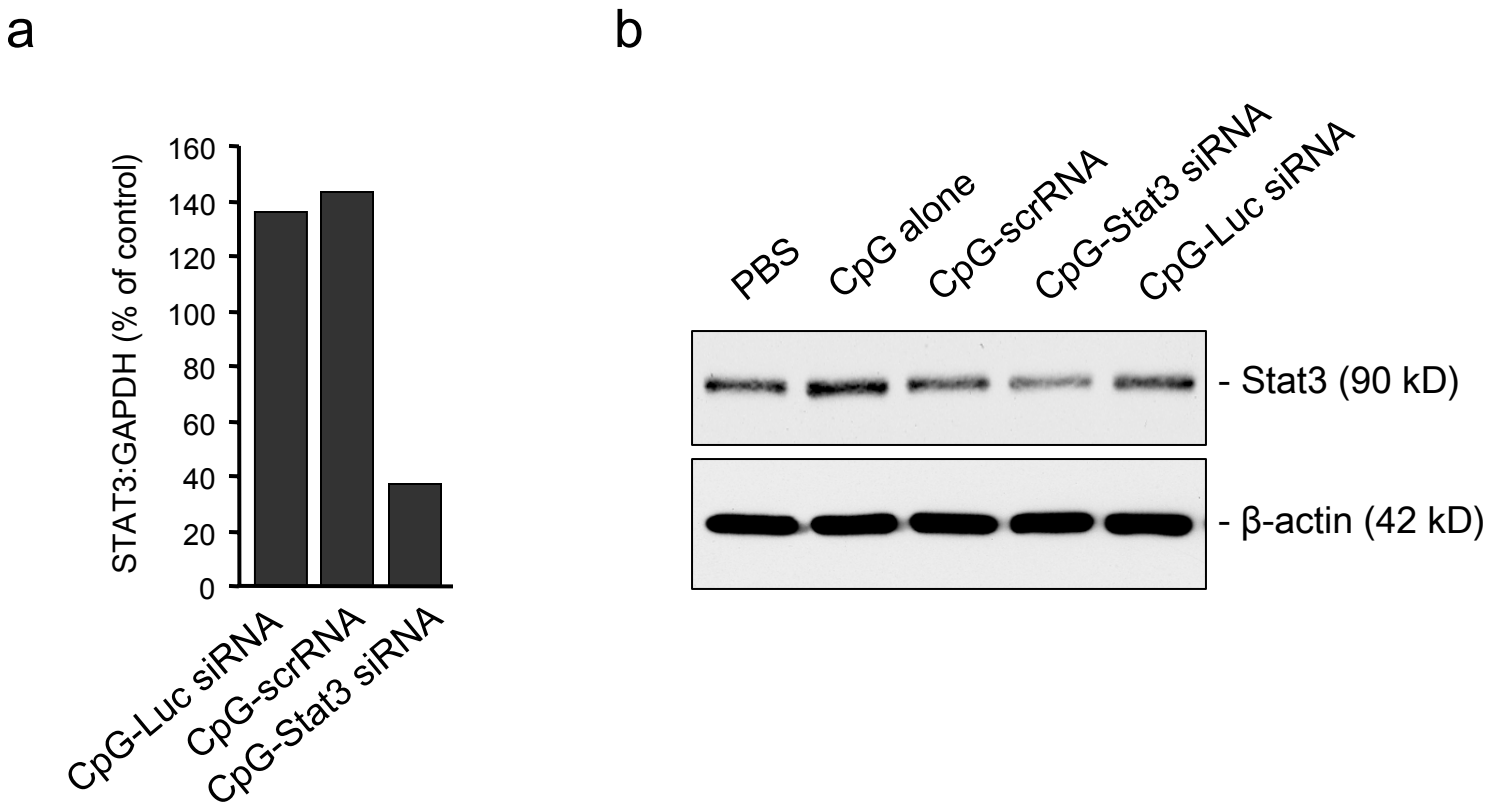



**Supplementary Fig. 8. Kinetics of CpG-Stat3 siRNA uptake *in vivo* following intratumoral injection.** Mice were treated by intratumoral injection of 20 μg FITC-labeled CpG-Stat3 siRNA at indicated times. Single-cell suspensions of tumors and tumor-draining lymph nodes were enriched for viable mononuclear cells and analyzed by FACS for the presence of F4/80$^{+}$CD11c$^{-}$ macrophages and CD11c$^{+}$ DCs. Shown are representative results of two independent experiments using 2-3 mice analyzed individually.

Supplementary Fig. 8ab

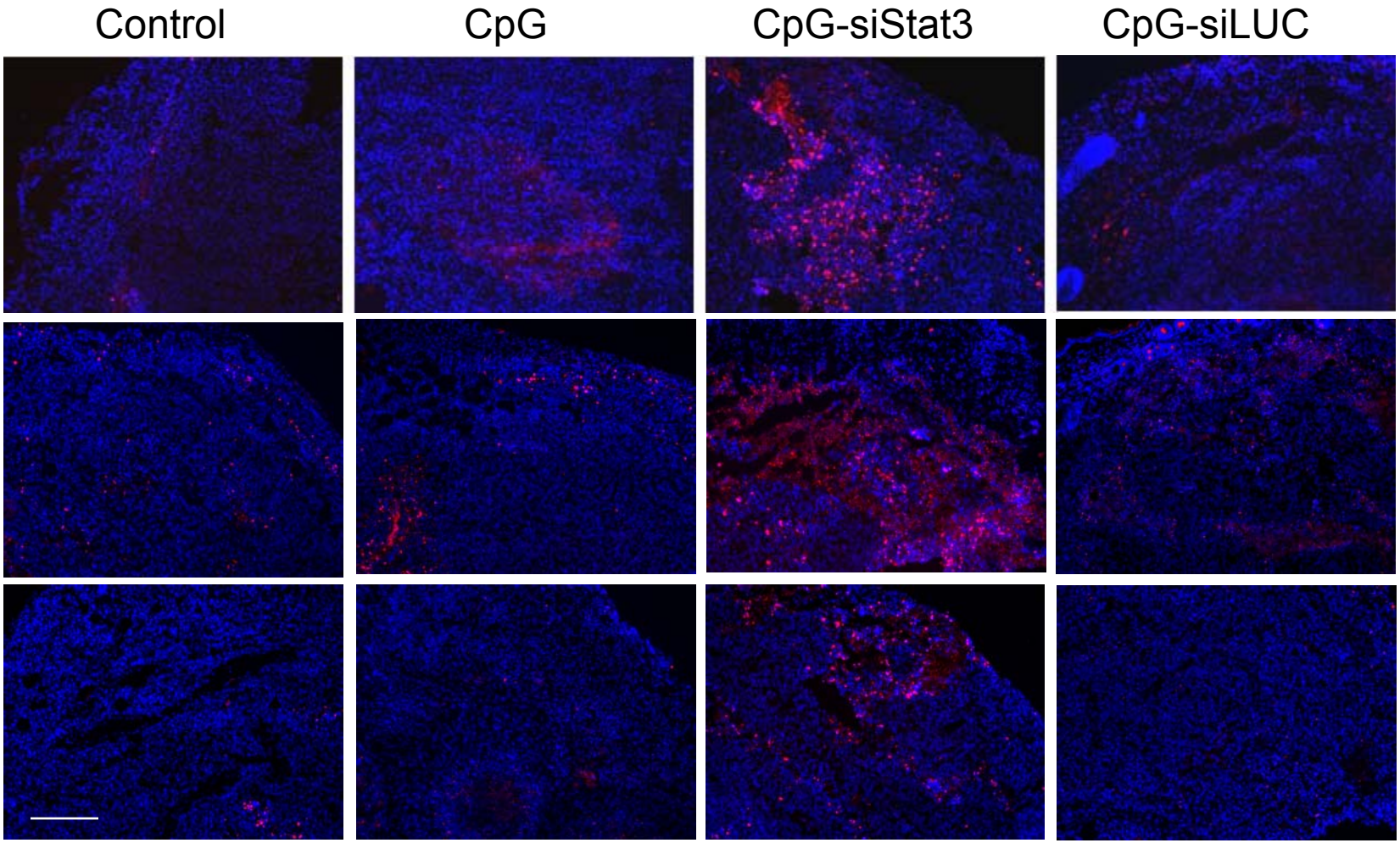



**Supplementary Fig. 9. *In vivo* uptake of intratumorally injected CpG-*Stat3* siRNA by myeloid cells.** Intravital two-photon microscopy on tumor-draining and contra-lateral lymph nodes at 1 h after single intratumoral injection of 20 μg FITC-labeled CpG-*Stat3* siRNA (green) together with intravenously injected Hoechst 33342 for nuclear staining (blue). For tumor-draining lymph node, the overlay image was split into green (upper right panel) and blue (lower right panel) channels to visualize better cellular localization of the injected CpG-*Stat3* siRNA. Scale bar = 200 μm.



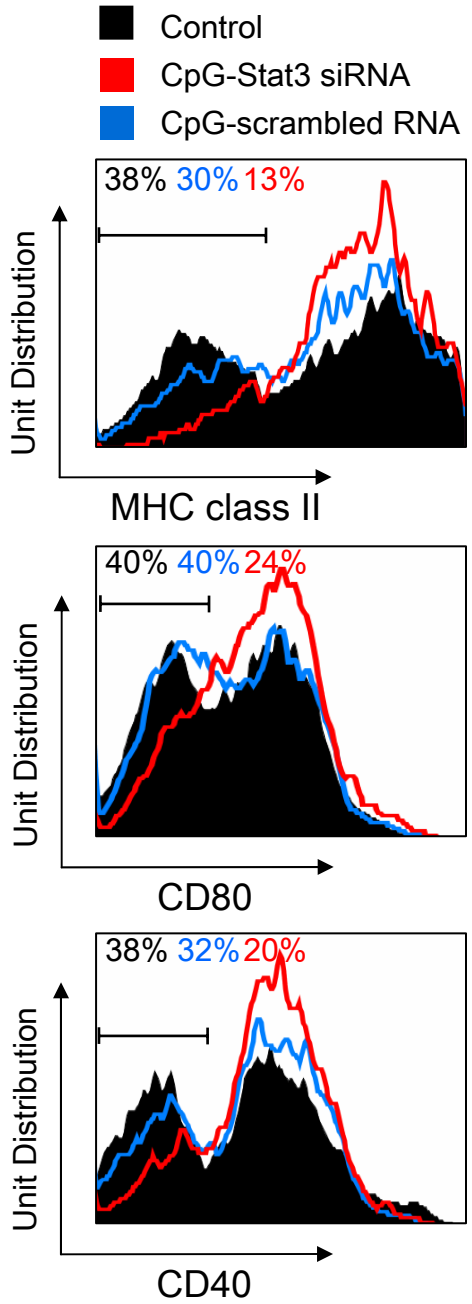

**Supplementary Fig. 10. Local treatment with CpG-Stat3 siRNA reduces Stat3 expression within total tumor-draining lymph nodes.** Mice with subcutaneous B16 tumors were treated by repeated peritumoral injections using various CpG-RNAs or PBS alone as indicated, every second day, starting six days after challenge with $1x10^5$ B16 cells using 7 mice/group. Single cell suspensions prepared from pooled tumor-draining lymph nodes were used for further analyses of Stat3 expression. (a) Local treatment using CpG-Stat3 siRNA leads to Stat3 silencing in tumor-draining lymph nodes. Shown are the results of real-time PCR for Stat3, normalized to GAPDH levels. The level of Stat3 expression in control PBS-treated sample is set as 100%. (b) Levels of Stat3 protein in total tumor-draining lymph node cells are reduced by CpG-Stat3 siRNA but not control conjugates, CpG-scrambled RNA and CpG-Luc siRNA. Representative results of Western blot analysis from one of two independent experiments are shown.

**Supplementary Fig. 10ab**



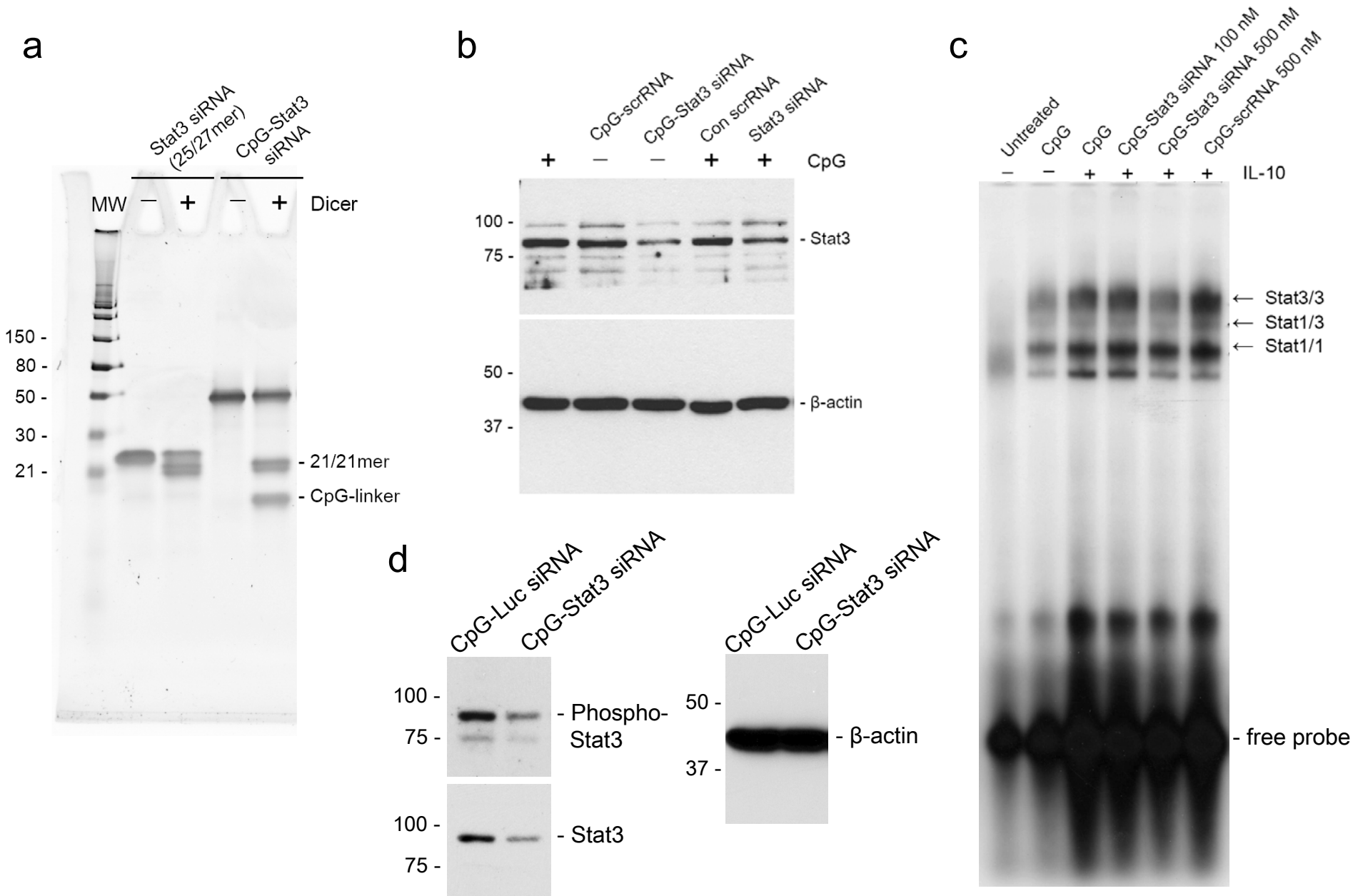

**Supplementary Figure 11. Augmented cell apoptosis within B16 tumors following peritumoral treatment with CpG-*Stat3* siRNA.** Frozen sections prepared from B16 tumors injected 3 times using PBS, CpG, or CpG-siRNA conjugates, were analyzed by immunofluorescence using antibodies specific to active caspase-3 to detect apoptosis (red) and counterstained with Hoechst (blue) for visualization of nuclei. Shown are representative results from two independent experiments using samples isolated from 4 individual mice; scale bar = 200 μm.

Supplementary Fig. 11




**Supplementary Fig. 12. Reduction of immature DCs in the tumor draining lymph nodes.** B16 tumor bearing mice were treated peritumorally with CpG-*Stat3* siRNA or CpG-scrambled RNA as described in Fig. 3d. Shown are results of flow cytometric analyses performed on single cell suspensions prepared from tumor-draining lymph nodes pooled from 5-6 mice; the percentages of DCs with low expression of MHC class II or co-stimulatory molecules are indicated.

Supplementary Fig. 12




Supplementary Fig. 13. Full-length representation of gels and blots presented in the main text in Figure 1c (a), Figure 1d (b), Figure 2f (c) and Figure 3d (d).

Supplementary Fig. 13a-d