

Supplemental figure legends

Supplemental Figure 1. Impact of siRNA targeting of Dragon on IL-6, MCP-1, TNF α , IL-1 β and IFN- γ mRNA expression in RAW264.7 cells stimulated with LPS. RAW264.7 were transfected with control or Dragon siRNA, followed by incubation of LPS. The cells were then analyzed for mRNA levels of IL-6, MCP-1, TNF- α , IL-1 β , IFN- γ and Dragon. The expression levels of these factors are expressed as a fraction of values from the respective controls (LPS treated cells transfected with control siRNA). The efficacy of Dragon siRNA was shown in the inset. *, $p < 0.05$; **, $p < 0.01$.

Supplemental Figure 2. Impact of siRNA targeting of Dragon on IL-6 expression in mouse PASM, HUVEC, mouse IMCD3 and C2C12 cells. mRNA levels were measured 46 h after cells were transfected with control and Dragon siRNAs by quantitative real-time PCR, were normalized to RPL19 mRNA levels, and are expressed as a fraction of values from cells treated with control siRNA (left panels). The efficacy of Dragon siRNA in each cell type was shown in the respective right panels. **, $p < 0.01$; ***, $p < 0.001$.

Supplemental Figure 3. Impact of siRNA targeting of Smad4 on IL-6 and Id-1 expression in RAW264.7 macrophages. mRNA levels for Smad4 (A), IL-6 (B) and Id1 (C) were measured 46 h after cells were transfected with control and Smad4 siRNAs by quantitative real-time PCR, were normalized to RPL19 mRNA levels, and are expressed as a fraction of values from cells treated with control siRNA. **, $p < 0.01$.

Supplemental Figure 4. Generation of RGMb knockout mice. (A) Targeting strategy for homologous recombination in ES cells to eliminate RGMb function. The second coding exon was disrupted with an eGFP-IRES-NLS-LacZ-pA-TK-Neo cassette. Exons are indicated in yellow. (B) Genotyping of Dragon knockout mice. Genomic DNA samples are digested with EcoRI. Probe for Southern analysis is depicted in dark blue. (C) Northern blot analysis with RNA extracted from the brains of wild-type and RGMb knockout mice. Methylene Blue (MB) staining served as a loading control. (D) RT-PCR

analysis for RGMb mRNA expression in the lungs of wild-type and RGMb knockout mice. RT-PCR for actin was used as control for RNA quality.

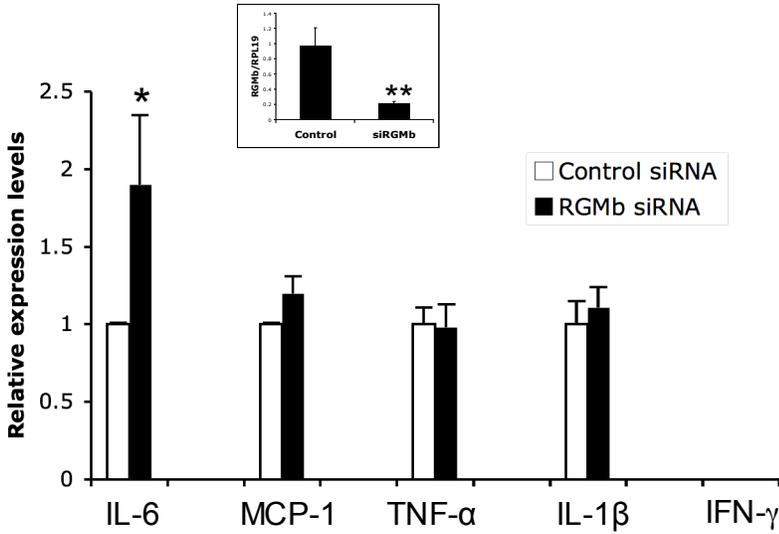
Supplemental Figure 5. IL-6 mRNA expression levels in the spleen, colon and muscle were not altered in Dragon knockout mice. Total RNA was extracted from spleens, colons and femoral muscles of wild type (WT) and Dragon knockout (KO) mice at ages of day 10-12. mRNA levels were measured by quantitative real-time PCR, were normalized to RPL19 mRNA levels, and are expressed as a fraction of values from WT samples.

Supplemental Figure 6. MCP-1, TNF α IL-1 β and IFN- γ mRNA expression is up regulated in the lung of Dragon knockout mice. Total RNA from lungs and livers of wild type (WT) and Dragon knockout (KO) mice at ages of day 10-12 was extracted. mRNA levels for MCP-1, TNF α IL-1 β and IFN- γ were measured by quantitative real-time PCR, were normalized to RPL19 mRNA levels, and are expressed as a fraction of values from the respective WT samples. **, $p < 0.01$; *** $p < 0.001$ versus wild type.

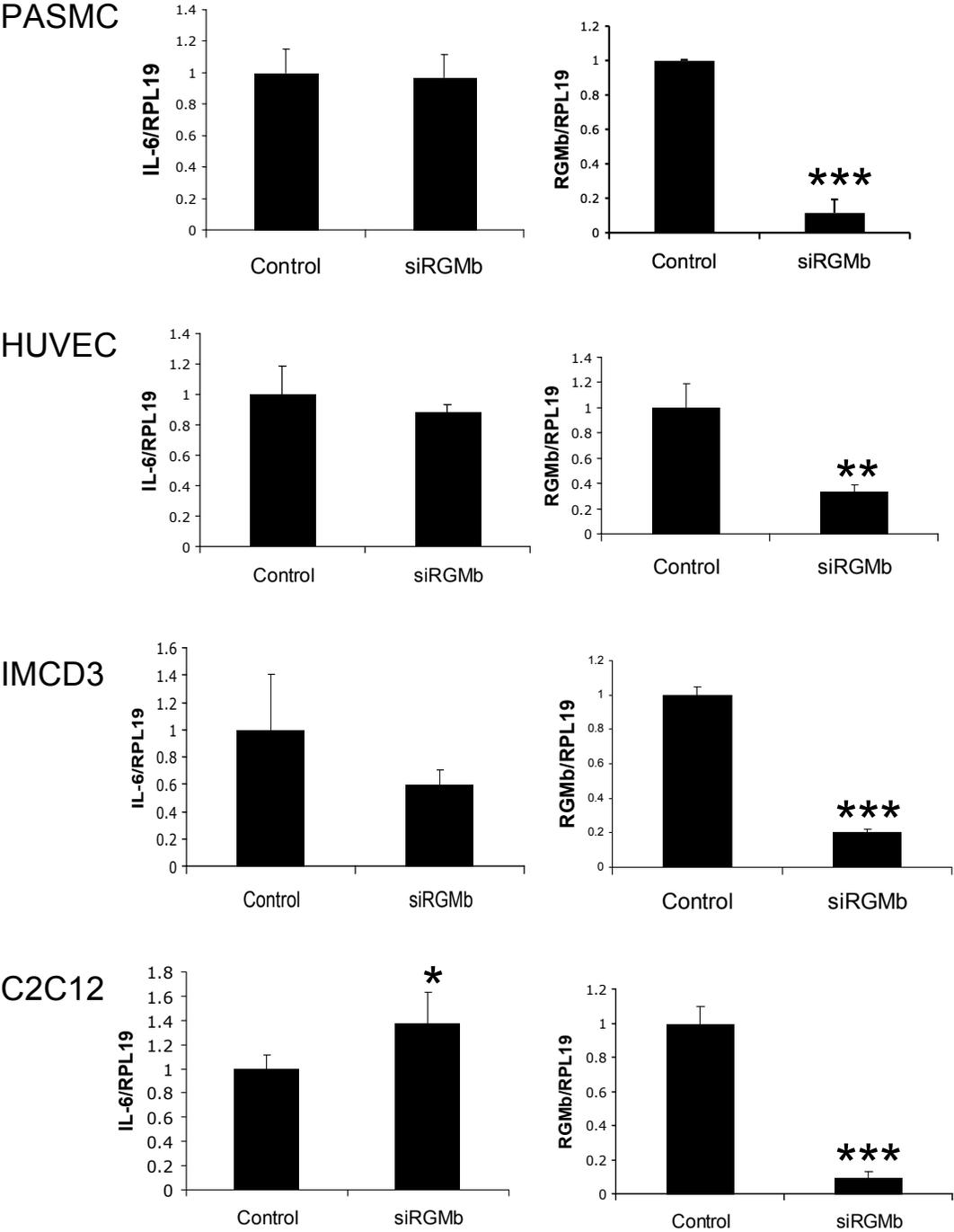
Supplemental Figure 7. Flow cytometry identification and enumeration of various (immune) cell populations in the lungs of 10-12 day old Dragon KO or WT littermates. (A) Flow profile for identification of macrophages, neutrophils, CD11b⁺ dendritic cells, Ly-6Chi and Ly-6Clo monocytes, and other cells. (B-C) Absolute (B) and relative (C) numbers of the above cell types in whole lungs.

Supplemental Figure 1

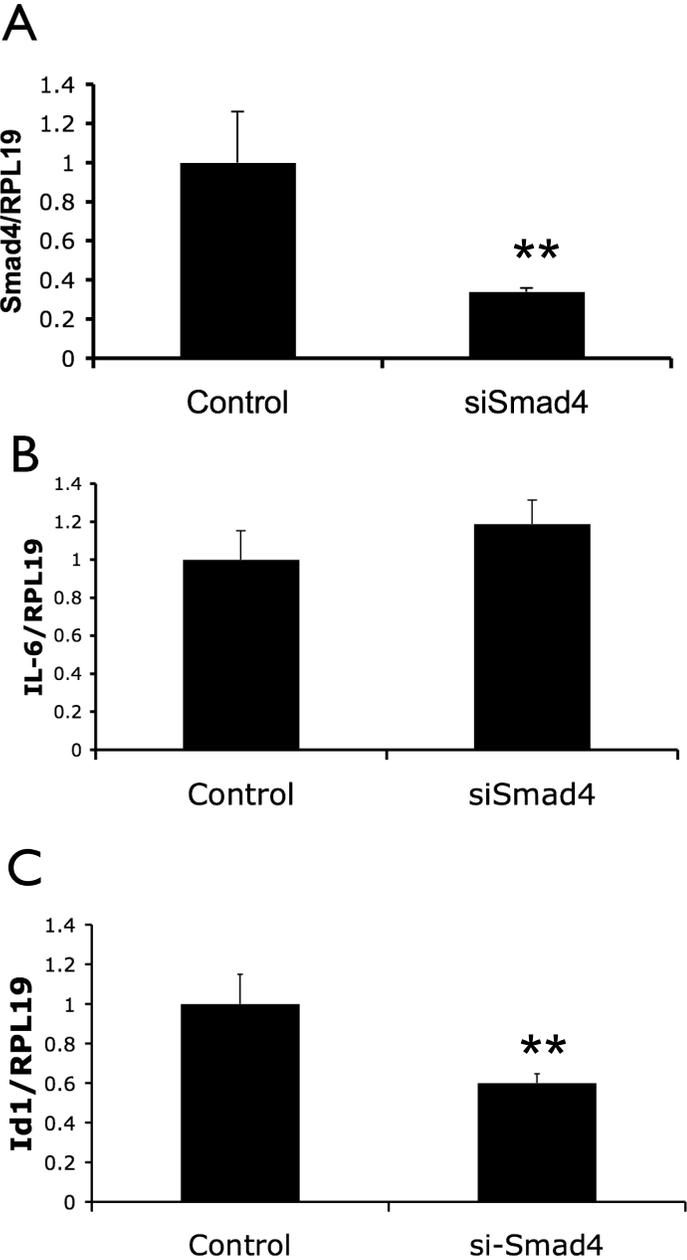
RAW cells
LPS stimulation



Supplemental Figure 2



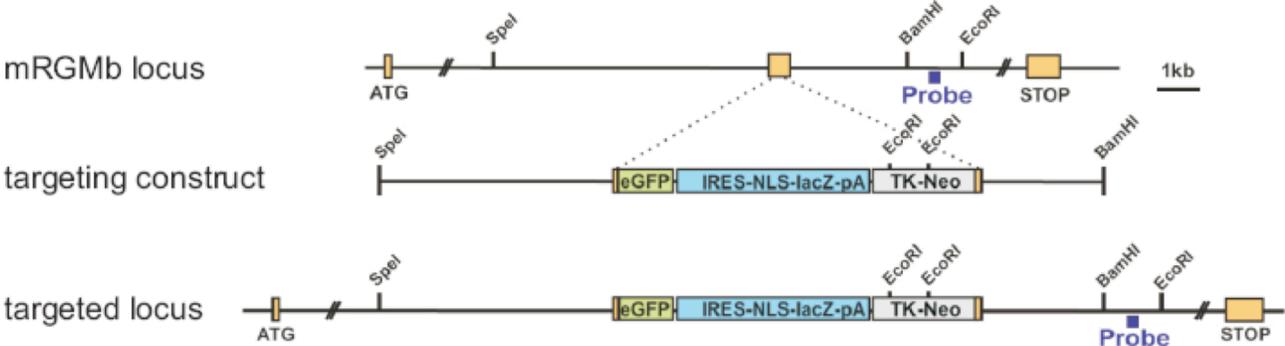
Supplemental Figure 3



Supplemental Figure 4

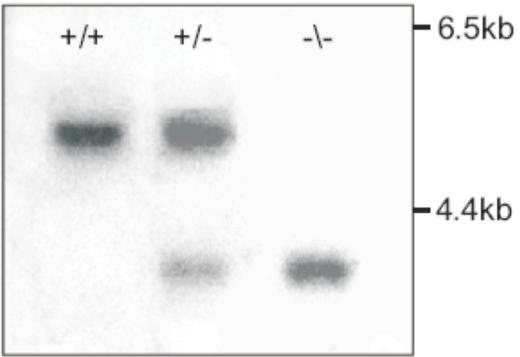
A

Generation of RGMb deficient mouse



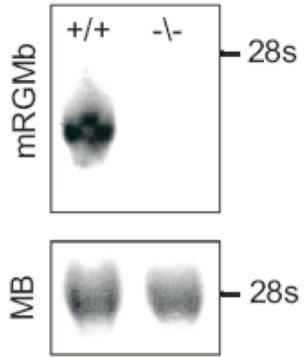
B

Southern

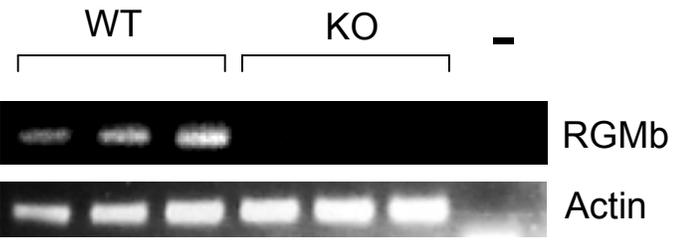


C

Northern

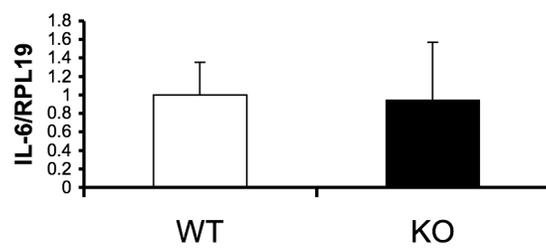


D

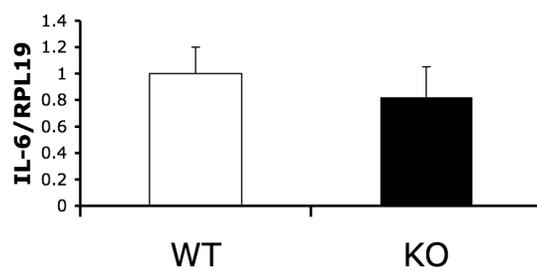


Supplemental Figure 5

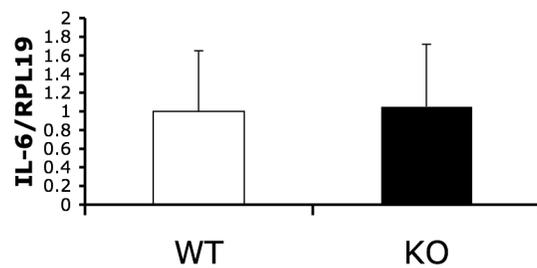
Spleens/D10-12



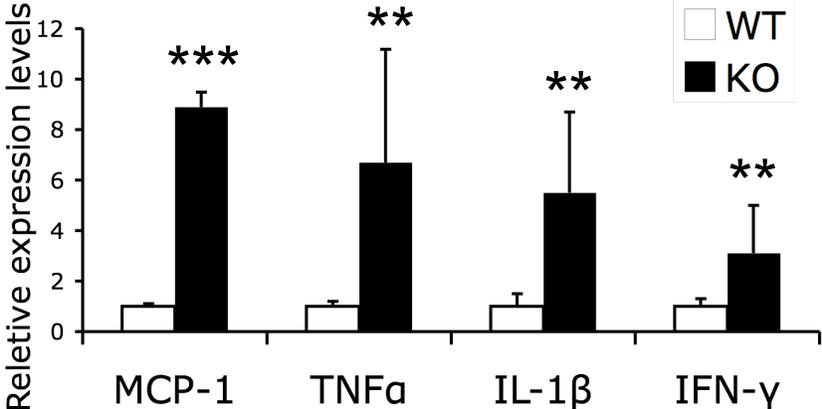
Colons/D10-12



Muscles/D10-12

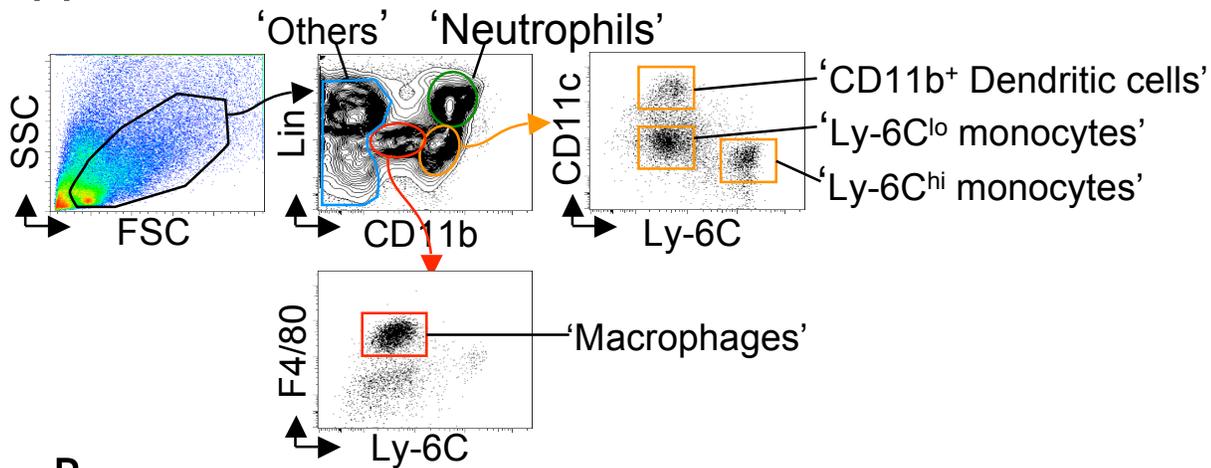


Supplemental Figure 6

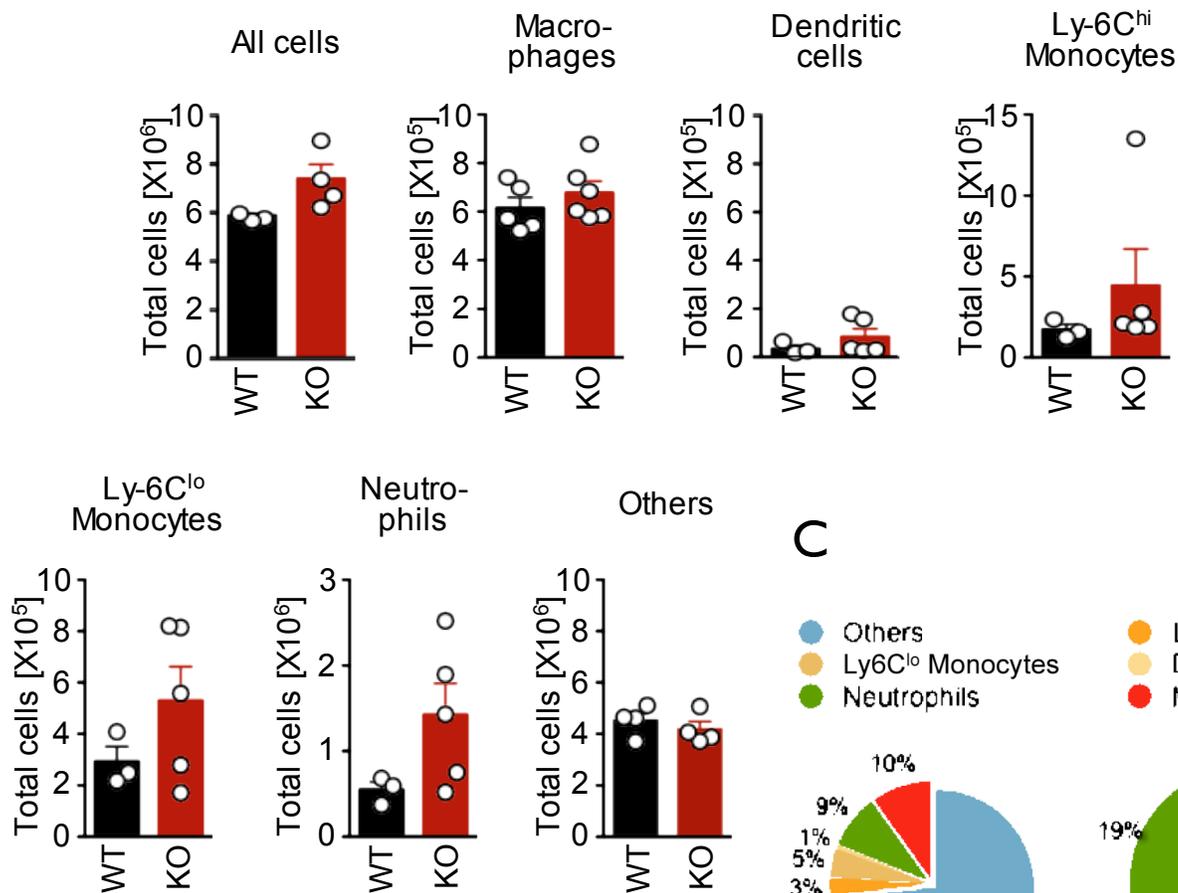


Supplemental Figure 7

A



B



C

