SUPPLEMENTARY INFORMATION

The Loss of RGS Protein-$G\alpha_{i2}$ Interactions Results in Markedly Impaired Mouse Neutrophil Trafficking to Inflammatory Sites

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Running Title: RGS proteins modulate neutrophil trafficking

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Supplementary Figures.

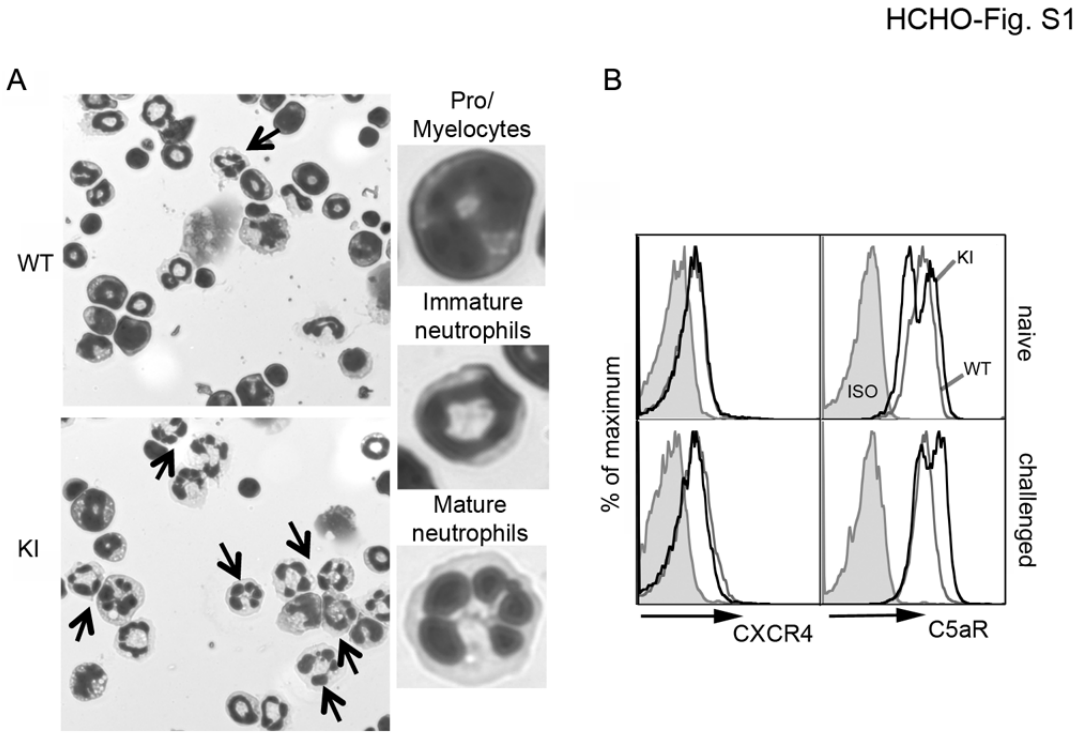

Figure S1 Photomicrographs and surface receptor expression of BM cells. (A) Representative photomicrographs of naïve BM cell cytospins. Cells were stained with Hema3. Arrows indicate mature neutrophils. Representative images of three BM neutrophil populations (promyelocytes/myelocytes, immature neutrophils, and mature neutrophils) are shown. (B) Expression of CXCR4 and C5aR on naïve or challenged BM neutrophils. 'ISO' represents neutrophils immunostained with isotype controls.

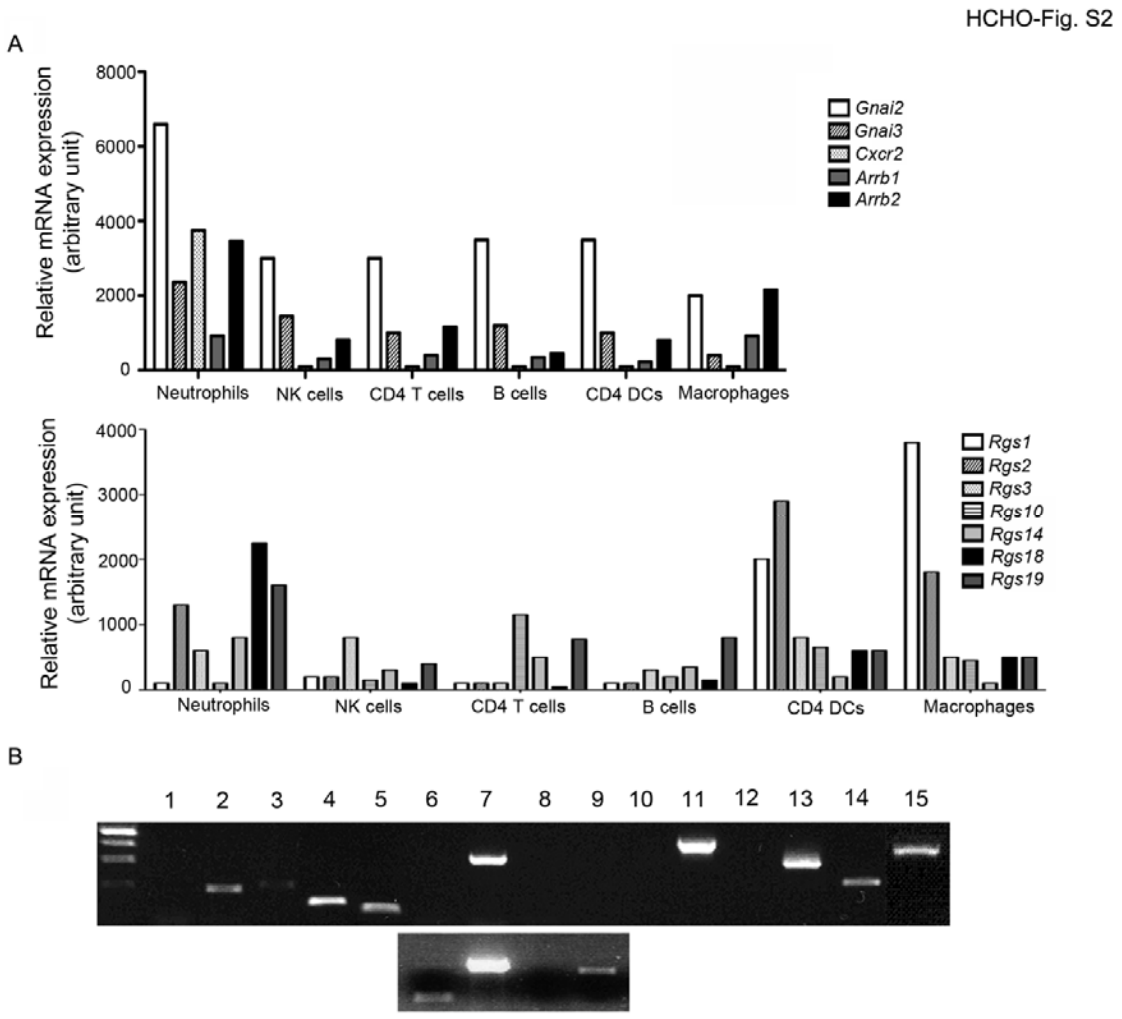

Figure S2. Expression of various Gα, β-arrestin, and RGS mRNAs in immune cells. (A) Relative mRNA expression data were obtained from Microarray experiments http://www.immgen.org/databrowsser/index.html. (B) Semi-quantitative RT PCR was performed with sorted BM Gr-1^{+}CD11b^{+} cells. A representative PCR result from two independent sorting experiments is shown. The PCR primers used were described previously (29, 30). 1-Gα_{i1}, 2-Gα_{i2}, 3-Gα_{i3}, 4-Gαq, 5-Gαs, 6-RGS1, 7-RGS2, 8-RGS3L, 9-RGS10, 10-RGS13, 11-RGS14, 12-RGS16, 13-RGS19, 14-actin, 15-RGS18. Lower panel shows a longer exposure, indicating a low expression of RGS1 and RGS10 mRNAs. Gα_{i1}, RGS3L, RGS13, and RGS16 mRNAs were not detected and therefore not shown.

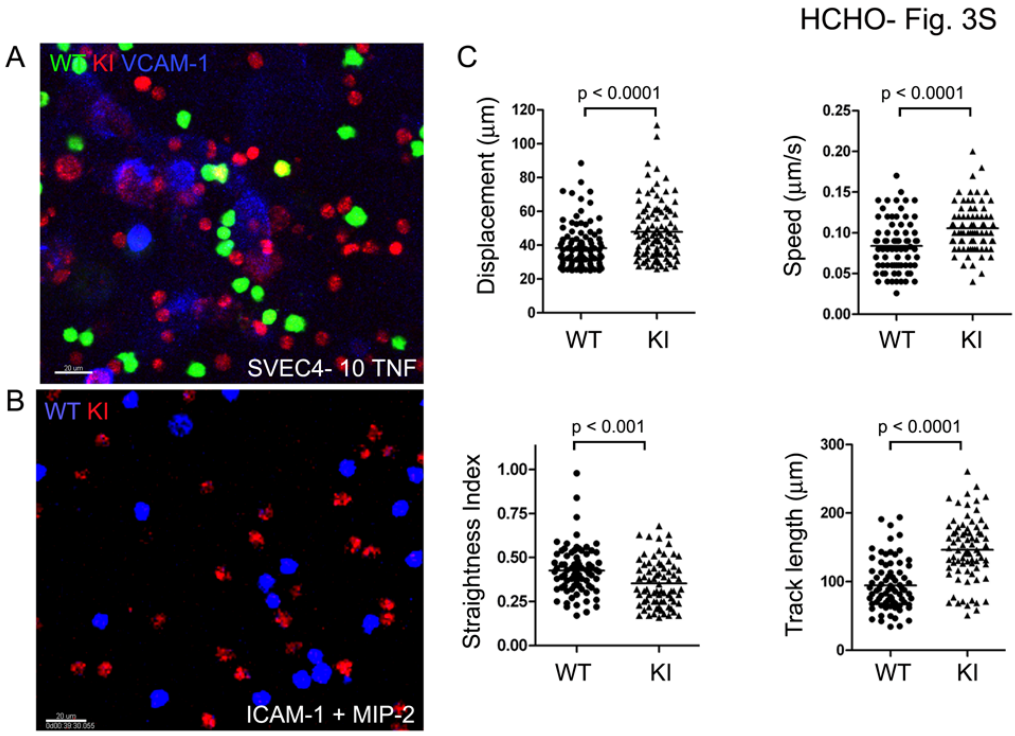

Figure S3. Adhesion of WT and KI neutrophils. (A) WT (green) and KI (red) bone marrow derived neutrophils adherent to a monolayer of SVEC4-10 cells previously treated with TNFα (20 ng/ml) for 24 hours. The monolayer was immunostained with a VCAM-1 antibody (blue). Scale bar 20 µm. (B) WT (blue) and KI (red) bone marrow derived neutrophils adherent to ICAM-1 and MIP-2 coated plate. Scale bar 20 µm. (C) Motility parameters of WT and KI neutrophils adherent to ICAM-1 + MIP-2 coated plates. Cells tracked every 10 seconds for 20 minutes using tracking function in Imaris.

Supplementary videos.

Video 1. Imaging WT and KI neutrophils part I. Adoptively transferred wild type (green) and KI (red) neutrophils in the ear of a wild type mouse. Images were taken for 42 minutes following laser damage. An image sequence of a 30 μm z-projection was acquired with 20x lens at a scanning speed of 17 second between frames. Collagen is blue. Frame rate is 20 frames/second.

Video 2. Imaging WT and KI neutrophils part II. Adoptively transferred wild type (green) and KI (red) neutrophils in the ear of a wild type mouse. Images were taken 45-104 minutes following laser damage. An image sequence of a 30 μm z-projection was acquired with 20x lens at a scanning speed of 17 second between frames. Collagen is blue. Frame rate is 20 frames/second.

Video 3. Imaging neutrophils in the ear of a LysM-EGFP WT mouse. Images were taken between 40-106 minutes following laser damage. An image sequence of a 30 μm z-projection was acquired with 20x lens at a scanning speed of 18 second between frames. Collagen is blue. Frame rate is 20 frames/second.

Video 4. Imaging neutrophils in the ear of a LysM-EGFP $G\alpha_{i2}^{G184S/WT}$ mouse. Images were taken for 40-106 minutes following laser damage. An image sequence of a 30 μm z-projection was acquired with 20x lens at a scanning speed of 18 second between frames. Collagen is blue. Frame rate is 20 frames/second.

Video 5. Imaging neutrophils in the ear of a LysM-EGFP $G\alpha_{i2}^{G184S/G184S}$ KI mouse. Images were taken for 50-100 minutes following laser damage. An image sequence of a 30 μm z-projection was acquired with 20x lens at a scanning speed of 18 second between frames. Blood vessels were outlined by infusing fluorescent Qdot 655 nanocrystals prior to imaging. Collagen is blue. Frame rate is 20 frames/second.