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## Supporting Information

## for

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**SLIC-1/sorting nexin 20: A novel sorting nexin that directs subcellular distribution of PSGL-1**

Ulrich Y. Schaff, Heather H. Shih, Meike Lorenz, Dianne Sako, Ron Kriz, Kim Milarski, Brian Bates, Boris Tchernychev, Gray D. Shaw and Scott I. Simon

**Supplemental Material:**

## Materials and methods

### Generation of SLIC-1/SNX20 knockout mice.

Lex-1 ES cells derived from the 129SvEvBrd mouse strain were targeted using standard conditions (Nagy, A., Gertsenstein, M., Vintersten, K., Behringer, R., Manipulating The Mouse Embryo a Laboratory Manual, Cold Spring Harbor Labboratory Press, Third Edition, 2003, Cold Spring Harbor, New York) ES cell genotypes were determined by Southern blot using a probe external to the targeting vector. This probe was generated using the following PCR primers: Forward, 5'-CTGTAGCCGAGTAAGAATCC; Reverse, 5'-CCAACATCTGCAGTGCTTTG. Mouse genotypes were determined by PCR using the following primers: 1: 5'-CTAGCCAGTACGCTTCACC; 2: 5'-CAATACTTCCTGTCTGGTCC'; 3: 5'-CCCACA AACCAGGACTAGC (see fig 1). Targeted ES cells were injected into recipient blastocysts and resulting chimeras were bred to establish a colony harboring the conditional allele. The deletion allele was generated by crossing mice to a transgenic strain expressing cre recombinase under the direction of the protamine promoter. Males harboring the conditional allele and the protamine-cre transgene transmitted the deletion allele to all subsequent offspring.

### Recruitment of neutrophils under shear and analysis

By assembling a vascular mimetic flow chamber over transfected L-cells expressing E-selectin and ICAM-1, we produced an environment favorable to PSGL-1 dependent neutrophil arrest. Neutrophils were diluted to a concentration of $1x10^6$ in HEPES buffer prior to shear experiment. The volumetric flow rate through the vascular mimetic microfluidic chamber was adjusted such that neutrophils were exposed to 1 dyne/$cm^2$ shear stress at the monolayer surface. Image sequences of neutrophils interacting with the E-selectin/ICAM-1 substrate were digitally captured at 4 frames per second for 6 minutes from 6 random microscope fields using image pro plus 4.1 (Mediacybernetics). The density of neutrophil recruitment was quantified by counting and averaging the number of rolling and arrested neutrophils in all of the microscope fields recorded for a given experiment. A neutrophil which moved less than 2 microns in 5 minutes was considered arrested, while any non-arrested neutrophil visibly interacting with the monolayer was considered rolling. The percentage of arrested neutrophils was quantified by the fraction of total interacting (rolling + arrested) neutrophils that arrested.

### Detection of PSGL-1 distribution in migrating neutrophils

In order to induce migration in neutrophils, the vascular mimetic flow chamber was annealed to a monolayer of murine bEND.3 endothelial cells (see Sikorski, EE J. Immunol. 1993). Endothelial cells were treated with 5 ng/mL recombinant murine IL-1β (R&D Systems) for 4 hours at 37C to increase expression of the chemoattractants and adhesion molecules required for trans-endothelial migration. After 5 minutes of interaction, neutrophils and monolayers were stained with 5 μg/mL anti-PSGL-1 conjugated to PE (2PH-1, BD Biosciences) for 30 minutes at 4C. Monolayers were then fixed with 2% paraformaldehyde for 30 minutes at 4C, washed with PBS, and mounted

on coverslides. Fluorescent images of PSGL-1 distribution were captured using a Nikon 1200 microscope equipped with a 100x oil objective (Nikon plan APO, NA=1.3). Migrating neutrophils exhibiting clustered PSGL-1 at the uropod were identified visually, and were recorded as a percentage of total observed neutrophils for each experiment.

## Fig S1

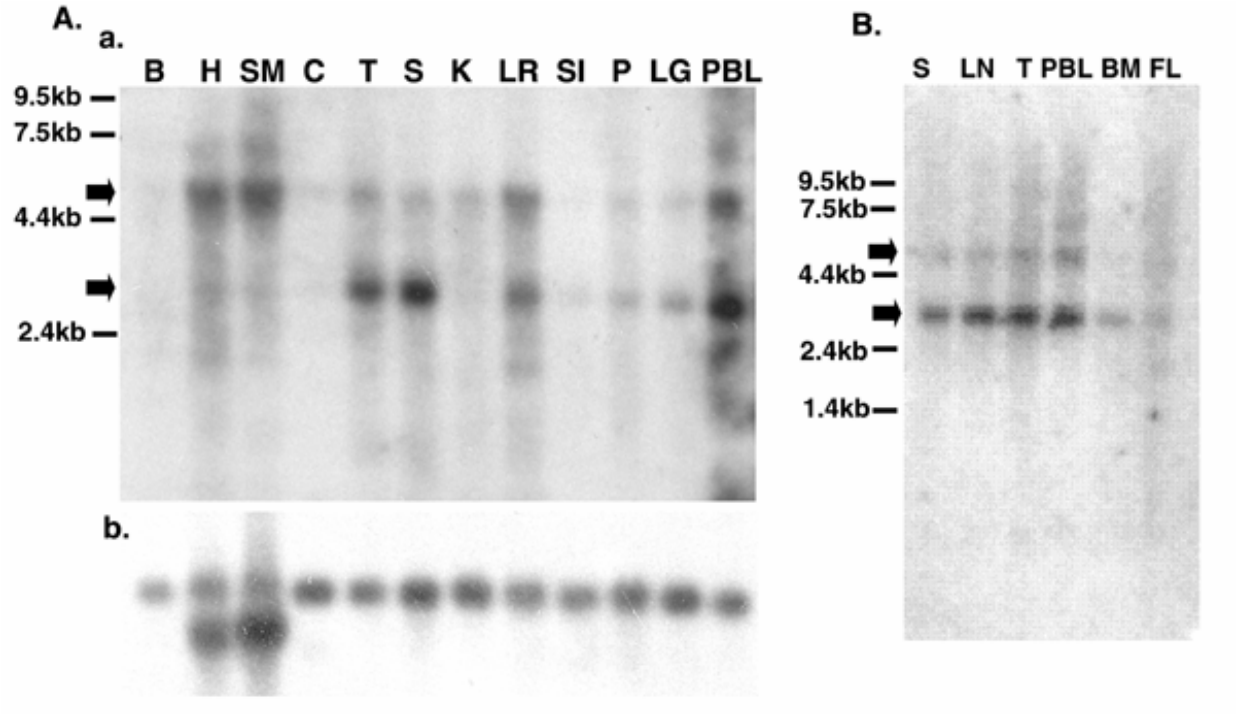
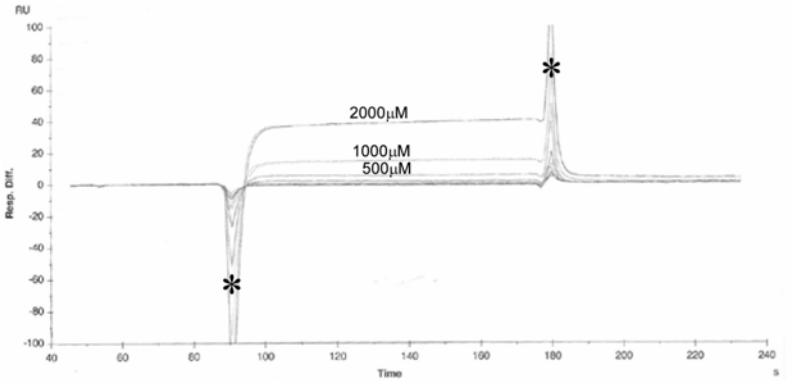



Fig. S1. **Northern blot analysis of SLIC-1 mRNA levels in human tissues.** A) A commercial multiple tissue blot was hybridized with SLIC-1 cDNA probe. B) The same blot was stripped and re-hybridized with control actin probe. B, brain; H, heart; SM, smooth muscle; C, colon; T, thymus; S, spleen; K, kidney; Lr, liver; SI, small intestine; P, placenta; Lg, lung; PBL, peripheral blood. C) A commercial immune system blot was hybridized with SLIC-1 cDNA probe. S, spleen; LN, lymph node; T, thymus; PBL, peripheral blood; BM, bone marrow; FL, fetal liver. Arrows indicate the 3-KB and the 5-KB SLIC-1 mRNA bands.

**Fig S2**

**Peptide 1.**
**HMYPVRNYSPTE**




**Peptide 2.**
**REDREGDDLTLH**

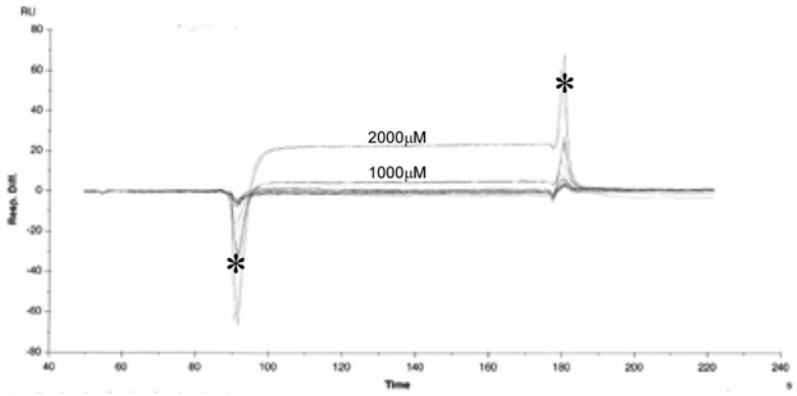



Fig. S2. **GST-SLIC-1 binds to PSGL-1 peptides.** Two peptides corresponding to regions in the cytoplamsic domain of PSGL-1 that interacted with GST-SLIC-1 in peptide array analysis were evaluated for GST-SLIC-1 binding in surface plasma resonance assays on a BIAcore instrument. An anti-GST antibody was first immobilized on the sensor chip, to which GST-SLIC-1 was subsequently captured. Increasing amounts of peptides showed binding to captured GST-SLIC-1. The spikes at the beginning and end of the injections are result of high concentrations of peptide.

**Fig S3**

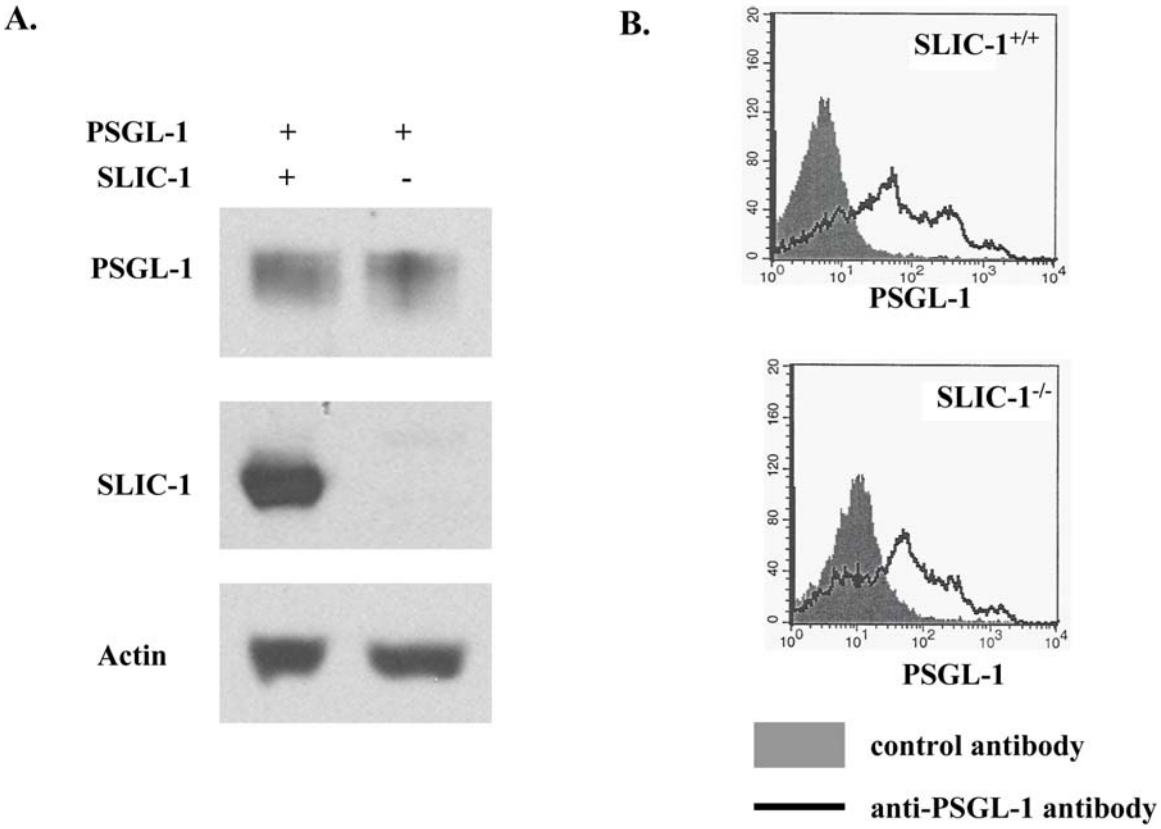



Fig S3. **PSGL-1 Expression levels are not affected by SLIC-1.** (A) Co-expressing SLIC-1 does not alter the expression levels of PSGL-1. 293 cells were transiently transfected with plasmids expressing PSGL-1 with or without SLIC-1. Total lysates were analyzed on Western blots. PSGL-1 and SLIC-1 were detected using an anti-PSGL-1 monoclonal antibody and a rabbit anti-SLIC-1 polyclonal antibody. Actin was detected with an anti-actin antibody and was used to indicate equal loading. (B) Cell-surface levels of PSGL-1 remain unchanged in myeloid cells in the absence of SNX20. Bone marrow myeloid progenitor cells were isolated from wildtype or SNX20-/- mice and subjected to flow cytometry analysis using an anti-PSGL-1 monoclonal antibody or control antibody.

**Fig S4**

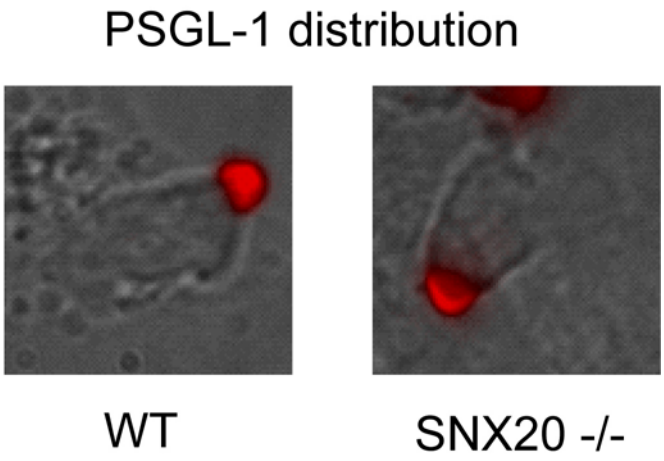



Fig S4. **SNX20 is not required for PSGL-1 redistribution during neutrophil migration.** Neutrophils were extracted from SNX20-/- and WT, stained with anti-PSGL-1 PE, and perfused over a monolayer of bEND.3 cells inflammed with 5 ng/mL IL-1β. Both types of neutrophils exhibited redistribution of PSGL-1 to the uropod of transmigrating cells indicating that SNX20 is not required for trafficking of surface expressed PSGL-1. There was no statistical difference in the fraction of SNX-/- and WT neutrophils that exhibited redistribution (45% vs. 48% respectively). Images are representative of 3 independent experiments between pairs of WT and SNX20-/- mice.