Supplemental Table 1

Kinetic analysis of FRAP in transfected CHO cells expressing PSGL-1-YFP constructs

PSGL-1/cell treatment	τ[a] (s)	F_{max}[b]
WT/DMSO	88 ± 8	0.7 ± 0.0
WT/Latrunculin B	61 ± 4[c]	0.8 ± 0.0[c]
SRKS/DMSO	97 ± 5	0.8 ± 0.0[c]
SRKS/Latrunculin B	58 ± 3[c]	0.8 ± 0.0[c]
MYVR/DMSO	60 ± 8	0.7 ± 0.0
MYVR/Latrunculin B	62 ± 2	0.8 ± 0.0

[a] Time constant for fluorescence recovery

[b] Maximum fluorescence recovery

[c] $P < 0.01$ compared to WT

FRAP curves (see Supplemental Figure 1) from 5 independent trials with 3 to 5 measurements per trial were fitted to a function for monoexponential recovery of fluorescence (see Materials and methods) to derive τ and F_{max}. The data represent the mean ± SEM.

Supplemental figure legends

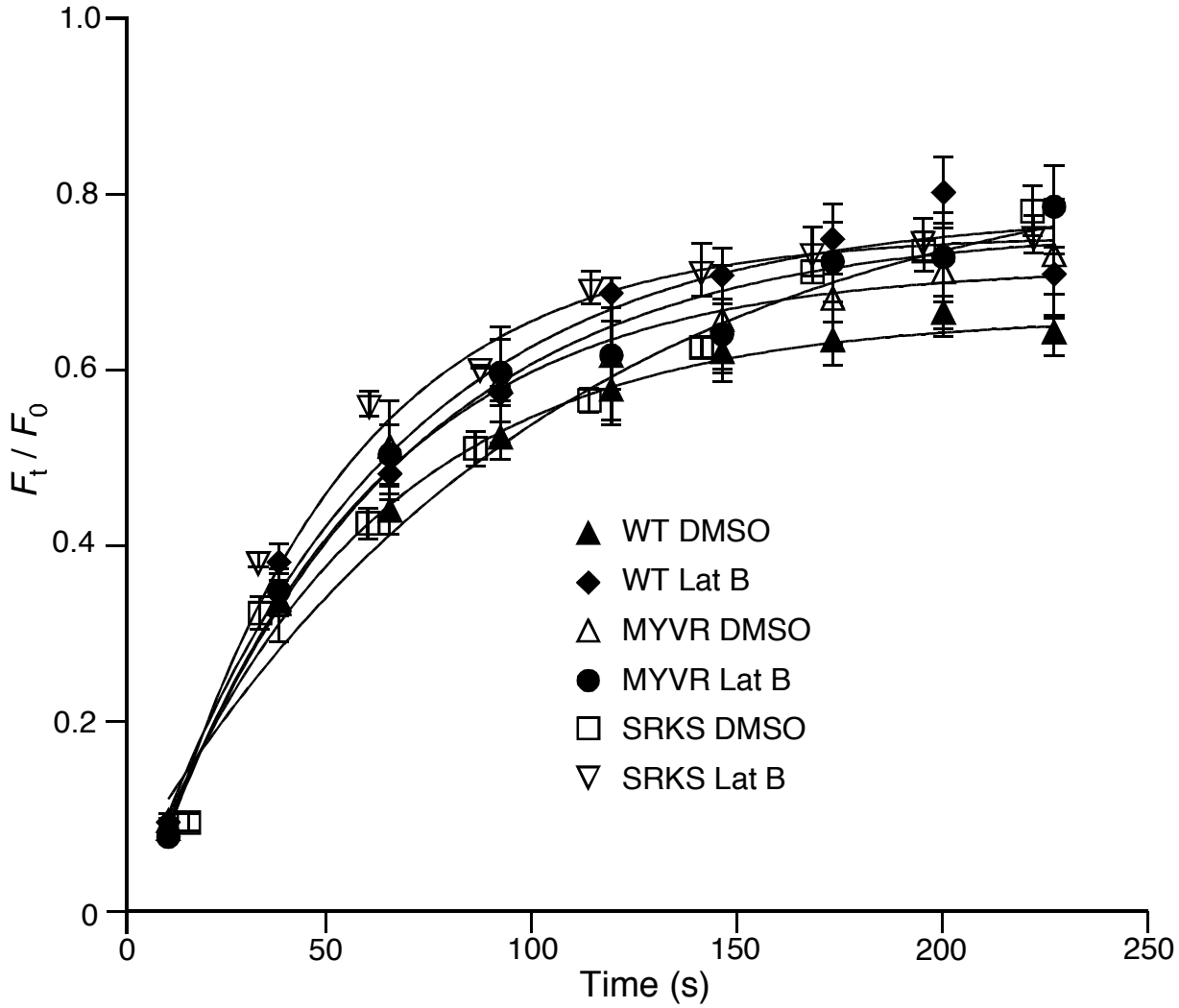
Supplemental Figure 1. **Juxtamembrane residues in the cytoplasmic domain of PSGL-1 restrain its membrane mobility.** Plot of normalized fluorescence intensity values vs. time after photobleaching a small area of peripheral membrane of transfected CHO cells expressing the indicated PSGL-1-YFP construct in the presence or absence of DMSO or latrunculin B (Lat B). The symbols for SRKS/DMSO and SRKS/Lat B are offset slightly to the left for clarity. The experimental conditions were similar to those in Figure 1D and Figure 2, except that a smaller area of plasma membrane (12 μm^2) was photobleached. FRAP curves from 5 independent trials with 3 to 5 measurements per trial were fitted to a function for monoexponential recovery of fluorescence (see Materials and methods) to derive τ and F_{max} as reported in Supplemental Table 1. The data represent the mean ± SEM.

Supplemental Figure 2. **BMDMs express myeloid and macrophage markers and integrins $\alpha_M\beta_2$ and $\alpha_L\beta_2$.** (**A** and **B**) BMDMs were incubated with the indicated FITC-labeled mAb and analyzed by flow cytometry. The data are representative of three experiments.

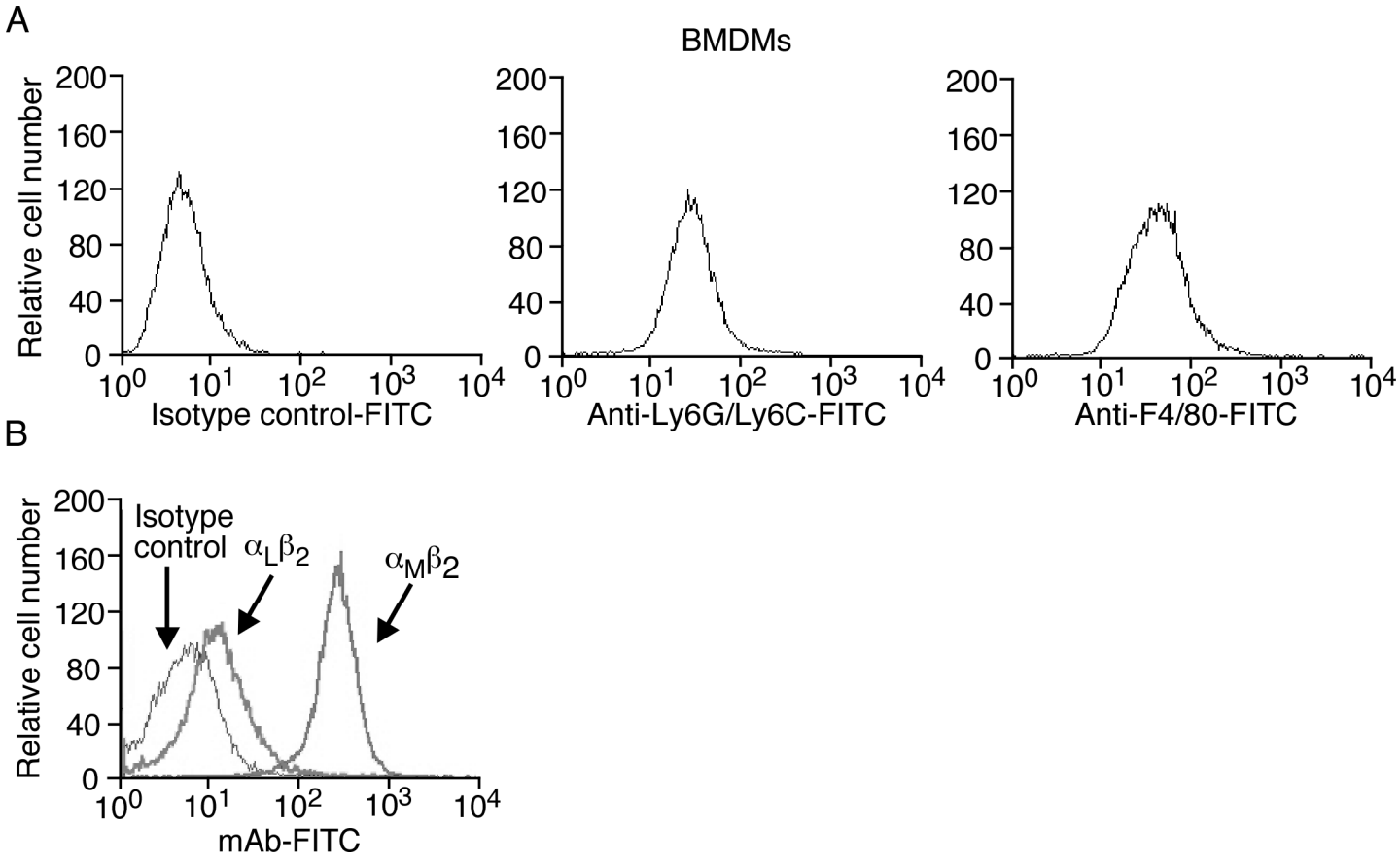
Supplemental Figure 3. **Treating cells with latrunculin B disrupts the organization of actin filaments.** Murine bone marrow neutrophils (**A**), murine BMDMs (**B**), or human neutrophils (**C**) were untreated or treated with DMSO or latrunculin B (Lat B). The cells were then permeabilized, fixed, stained with PE-conjugated phalloidin, and analyzed by confocal microscopy. The data are representative of three experiments. Scale bar, 2 μm.

Supplemental Figure 4. **Treating neutrophils or BMDMs with latrunculin B does not alter global surface expression of PSGL-1.** Murine neutrophils, BMDMs, or human neutrophils were incubated with DMSO or latrunculin B and then fixed. Murine neutrophils and BMDMs were incubated with PE-conjugated anti-murine PSGL-1 mAb 2PH1 or an isotype control mAb. Human neutrophils were incubated with anti-human PSGL-1 mAb PL1 or an isotype control mAb and then with FITC-conjugated goat-anti-murine IgG. The cells were analyzed by flow cytometry. The data are representative of three experiments.

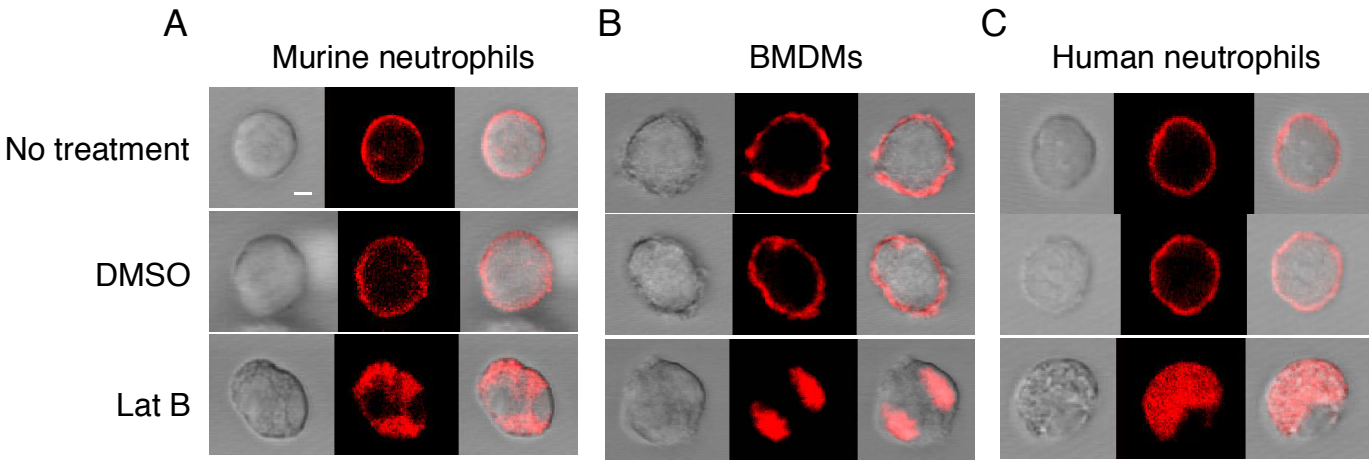
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4