Supplemental Figure and Video Legends

Supplemental Figures

Figure S1. High resolution rolling characteristics of Th1 and Th2 Cells are similar. Instantaneous velocity plots were generated from VideoLab and RS-1 computer programs as described in Materials and Methods. The plots shown are representative of Th1 and Th2 cells interacting with MAEC stimulated with Th2 supernatant for 6 h. Within each plot, the left panel shows X-Y coordinates collected every 1/30th of a second as the cell passed from right to left across the field of view. The right panel is the conversion of X-Y coordinates into instantaneous velocities with respect to X-Y displacement over time (expressed as frame number). The rolling time (RT) is the total amount of time that a cell had an instantaneous velocity of ≤50 μm/sec and is indicated for each cell.

Figure S2. Antigen inexperinced naïve T cells do not support rolling. Naïve $CD4^+$ T cells purified from 9wk DO11.10 SCID mice and Th1 and Th2 polarized cells derived from DO11.10 mice were labeled with 2μM Cell Trace™calcein red-orange, AM (Th1 and Th2) or calcein, AM (naïve) and resuspended at a concentration of 1 x 10^6 cells/ml. T cells were combined at a 1:1 ratio and flowed over bEnd.3 cells with a shear stress of 0.5 dynes/cm^2. Endothelial cells were stimulated for 6 h with 10 ng/ml TNF-α. VideoLab software was used to digitize and track 3 minute sequences of videotape and an RS-1 program was used to determine the rolling time of each cell (see Materials and Methods). The top panels indicate the mean number of rolling cells from 10 min of footage taken from four randomly selected fields of view in one of four representative experiments. Error bars indicate SEM. The bottom panel shows pooled rolling times of interacting cells from four fields of view. # indicates statistical significance between T cell types. P <0.05.

Figure S3. Successive rounds of Th2 polarization does not affect rolling characteristics or P- and E-selectin ligand expression. Naïve $CD4^+$ T cells were cultured under Th2 polarizing conditions for one or two successive rounds of polarization to generate 7d and 14d Th2 cells respectively. **A.** Th2 cells were labeled with 2μM Cell

Trace™calcein red-orange, AM (14d Th2) or calcein, AM (7d Th2) and resuspended at a concentration of 1 x 10^6 cells/ml. T cells were combined at a 1:1 ratio and flowed over bEnd.3 cells with a shear stress of 0.5 dynes/cm^2. Endothelial cells were stimulated for 6 h with Th2 sup. VideoLab software was used to digitize and track 2 minute sequences of videotape and an RS-1 program was used to determine the rolling time of each cell (see Materials and Methods). The top panels indicate the mean number of rolling cells from 10 min of footage taken from four randomly selected fields of view in one of four representative experiments. Error bars indicate SEM. The bottom panel shows pooled rolling times of interacting cells from four fields of view. # indicates statistical significance between T cell types. $P<0.05$. **B.** Th2 cells cultured for 7 or 14 days as in **A** were re-stimulated with PMA and ionomycin for 6 h. Monensin was added for the final 4 h to stop cytokine secretion. T cells were surface stained with P- or E-selectin human IgM chimeras. Following surface staining, the cells were fixed, permeablized, and stained intracellularly for IL-4. The frequency of IL-4^+ cells that were bound P- and E-selectin hIgM was assessed by flow cytometry on the lymphoid gate. Quadrant percentiles of lymphocyte-gated cells are indicated.

Figure S4. Th1 and Th2 cell rolling is similar on a distinct endothelial line (bEnd.3), and is PSGL-1 dependent.

A. Th1 and Th2 cells were labeled with 2 μM Cell Trace™ calcein red-orange, AM (Th1) or calcein, AM (Th2), combined 1:1 and flowed with a shear stress of 0.5 dynes/cm^2 over bEnd.3 cells stimulated for 6 h with TNF-α (10 ng/ml, **left panels**), or Th2 supernatant (**right panels**). Data collection and analyses were performed as described in Materials and Methods. The upper panels indicate the mean number of rolling cells in 10 minutes of footage taken from four randomly selected fields of view and two representative experiments. Error bars indicate SEM. The middle panels show pooled rolling times of interacting cells from four fields of view. In the bottom panels, the average rolling velocity and SEM from four fields of view is shown. # indicates statistical significance between T cell subtypes. * indicates statistical significance between methods of endothelial cell stimulation ($P<0.05$).

B. Th1 and Th2 polarized cells derived from DO11.PSGL-1$^{-/-}$ mice were labeled with 2 μM calcein, AM (Th1) or Cell Trace™ calcein red-orange, AM (Th2) and combined 1:1 with wild-type (DO11.PSGL-1$^{+/+}$) polarized T cells of the same lineage. Cells were flowed at a shear stress of 0.5 dynes/cm^2 over bEnd.3 cells stimulated for 6 h with **(left panels)** TNF-α (10 ng/ml) or Th2 supernatant **(right panels)**. Data collection and analyses were performed as described in Materials and Methods. Error bars indicate SEM. Rolling time data are pooled from four fields of view for each condition and two representative experiments. † indicates statistical significance between wild-type and PSGL-1 deficient cells (P <0.05).

Figure S5. Th1 and Th2 cell rolling is comparable under elevated shear stress.
Th1 and Th2 cells were labeled with 2 μM Cell Trace™ calcein red-orange, AM (Th1) or calcein, AM (Th2), combined 1:1 and flowed with the indicated shear stress over bEnd.3 cells stimulated for 6 h with Th2 supernatant. Data collection and analyses were performed as described in Materials and Methods. The number of rolling Th1 cells (solid bars) and Th2 cells (open bars) were counted for 10 minutes of footage taken from four randomly selected fields of view in one representative experiment. Error bars indicate SEM.

Supplemental Videos

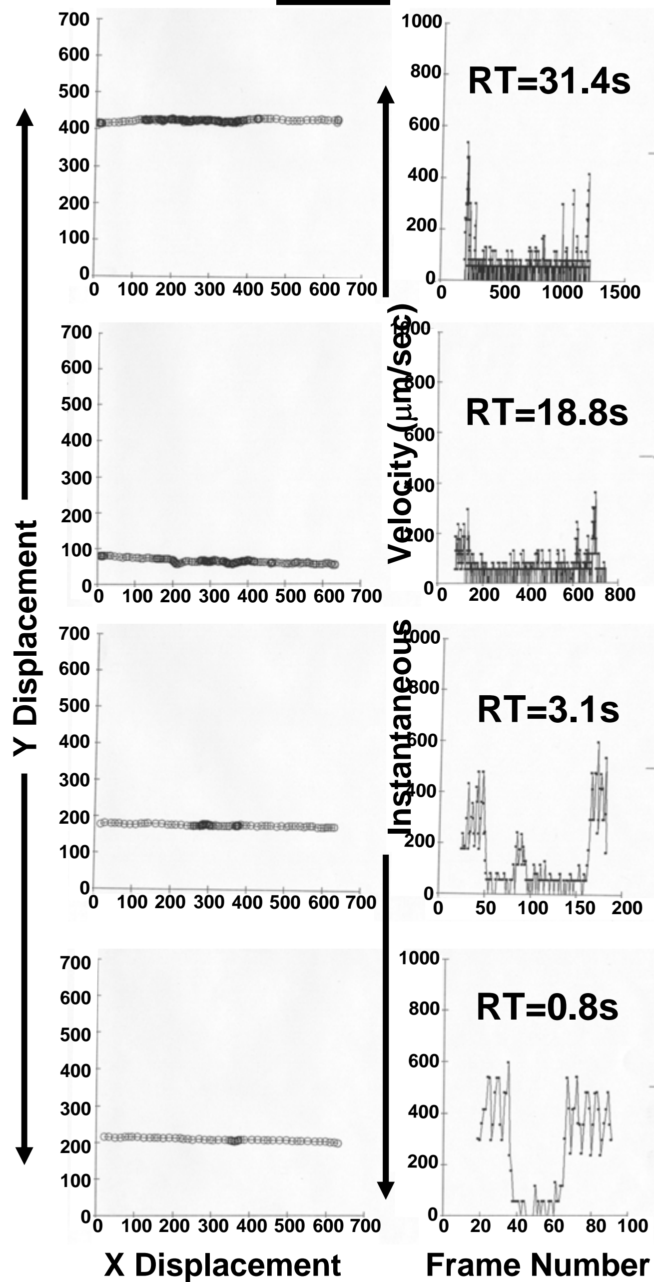
Video SV-1. Th1 and Th2 polarized populations do not interact with unstimulated MAEC under flow.
Th1 and Th2 cells were labeled with 2 μM Cell Trace calcein red-orange (**Th1-red**) or calcein, AM (**Th2-green**), combined 1:1, and flowed over confluent, unstimulated MAEC at a shear stress of 0.5 dynes/cm^2. Labeled T cells were illuminated using a halogen lamp and visualized at 10X magnification using an inverted microscope equipped to collect color images in real time (30 frames/s). The selected video represents 1000 frames of footage digitized using the Leitch Reality Digital Disk Recorder System. Note the absence of significant rolling interactions by either Th1 or Th2 populations.

Video SV-2. Wild-type DO11.10 but not DO11.PSGL-1$^{-/-}$ Th2 polarized populations roll on MAEC stimulated with TNF-α.

Th2 cells derived from wild-type or PSGL-1$^{-/-}$ mice were labeled with 2 μM Cell Trace calcein red-orange (**PSGL-1-deficient Th2-red**) or calcein, AM (**wild-type Th2-green**), combined 1:1, and flowed with a shear stress of 0.5 dynes/cm^2 over confluent MAEC stimulated for 6 h with TNF-α 10 ng/ml) Labeled T cells were illuminated using a halogen lamp and visualized at 10X magnification using an inverted microscope equipped with a color camera to collect images in real time (30 frames/s). Note the ablation of rolling interactions by PSGL-1-deficient Th2 cells relative to the wild-type control population.

Video SV-3. Both Th1 and Th2 polarized populations roll on MAEC stimulated with Th2-derived factors under flow.

Th1 and Th2 cells were labeled with 2 μM Cell Trace calcein red-orange (**Th1-red**) or calcein, AM (**Th2-green**) and analyzed as in **Video SV-1**. Note that both Th1 and Th2 cells demonstrate significant, comparable rolling interactions with the same activated endothelium under identical conditions of flow.



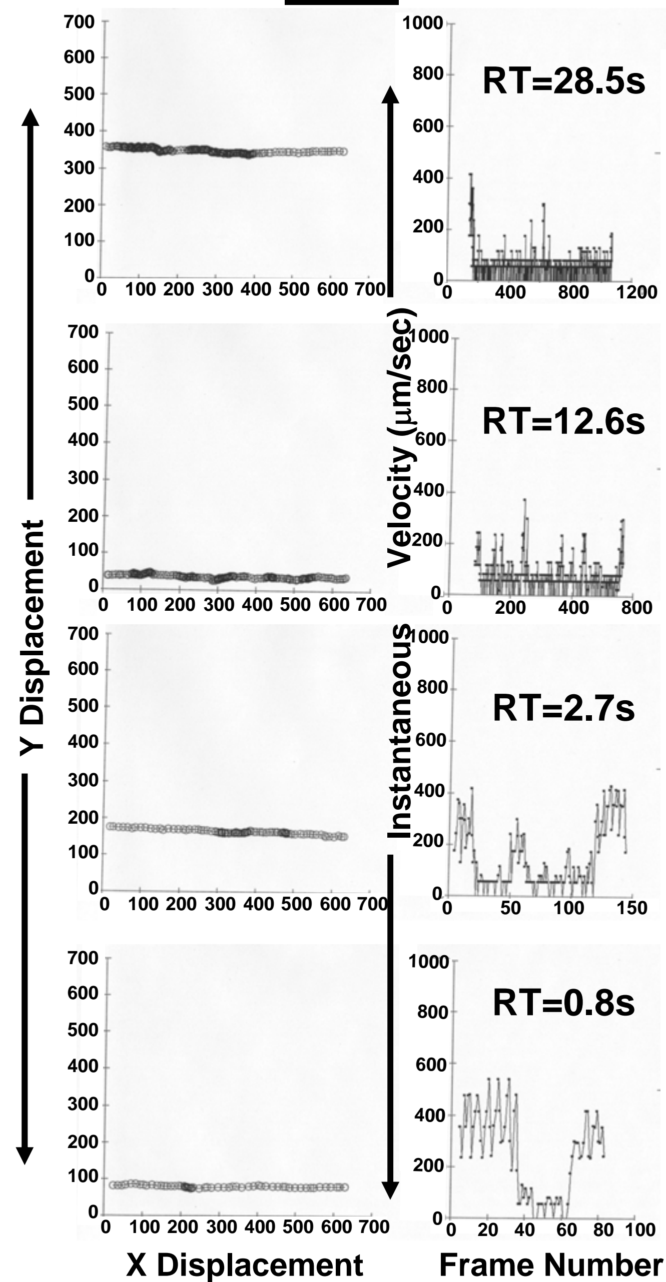
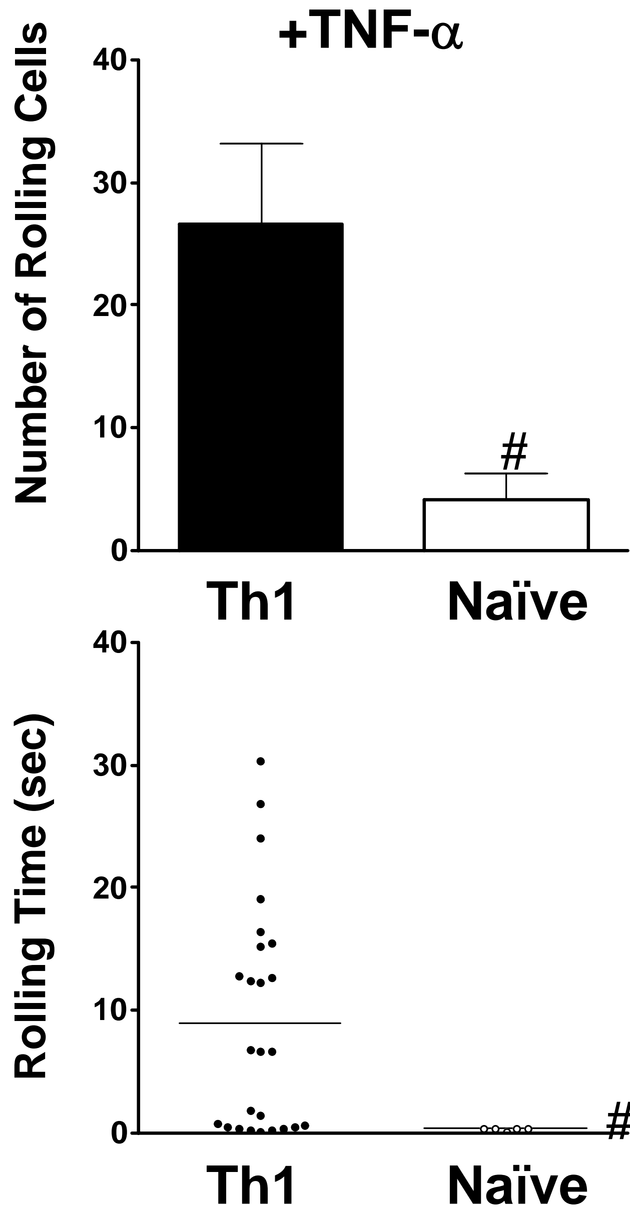


Fig. S1



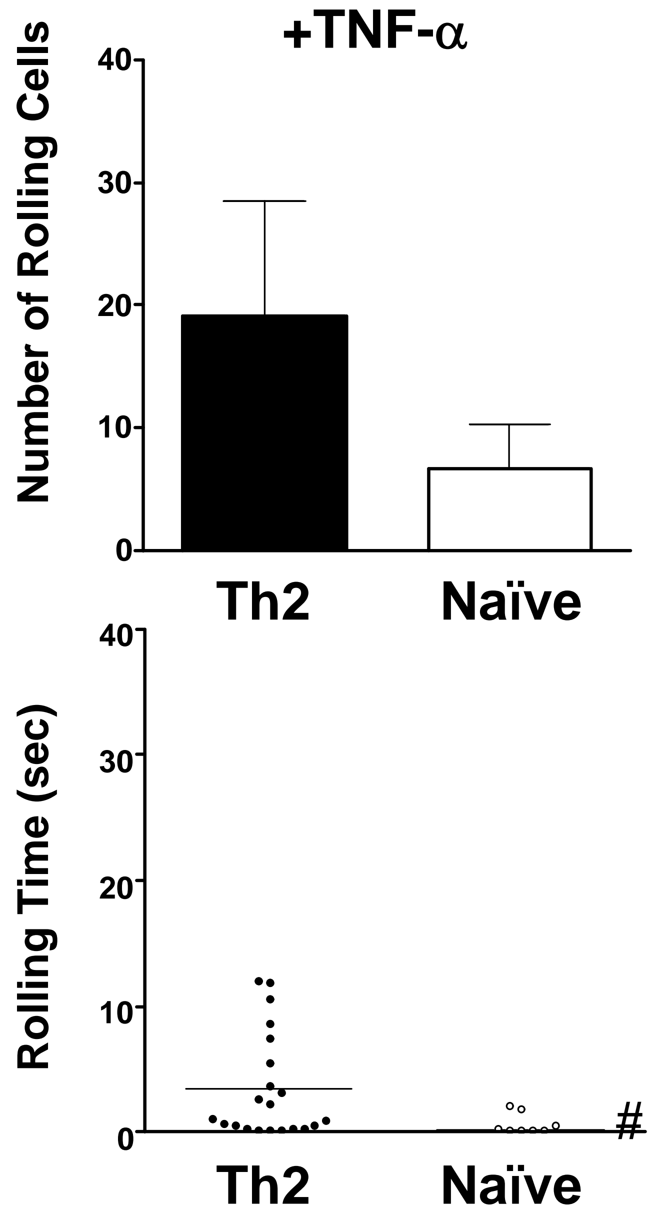
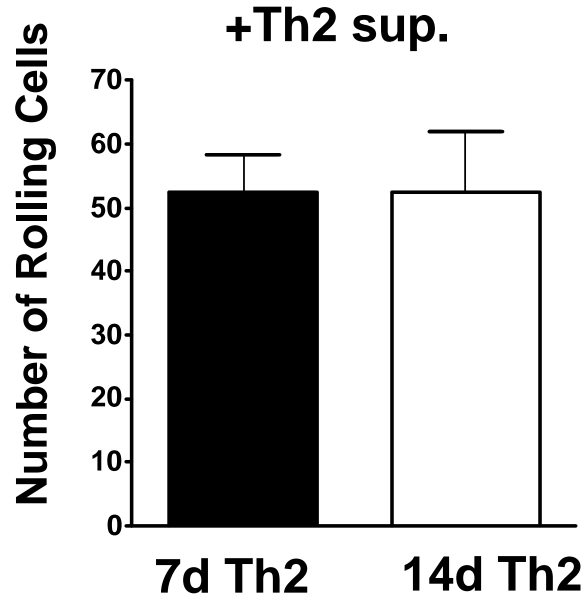
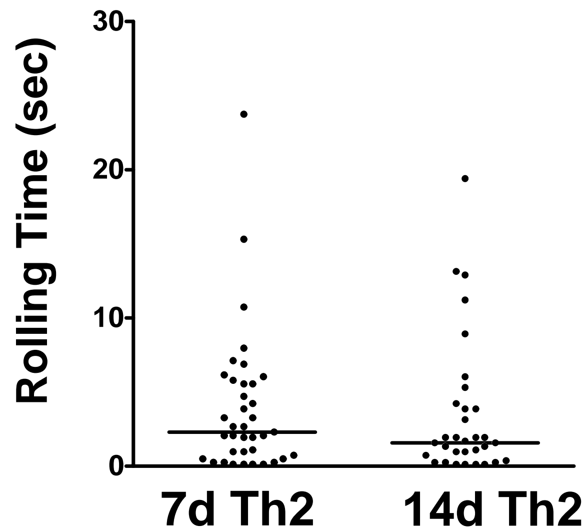


Fig. S2

A

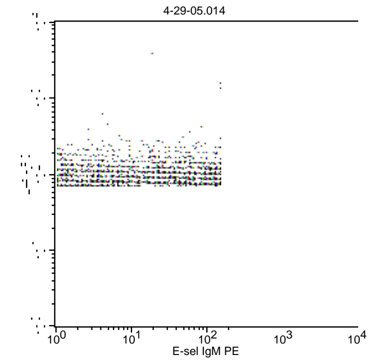




B

IL-4

7d Th2



IL-4

14d Th2

P-sel IgM

E-sel IgM

Fig. S3

A

+TNF-α

Number of Rolling Cells

0 10 20 30 40 50 60 70

#

Th1 Th2

+Th2 sup.

Number of Rolling Cells

0 10 20 30 40 50 60 70

*

Th1 Th2

Rolling Time (sec)

0 10 20 30 40 50 60 70

#

Th1 Th2

Rolling Time (sec)

0 10 20 30 40 50 60 70

*

Th1 Th2

Ave. Rolling Velocity (μm/sec)

0 25 50 75 100

#

Th1 Th2

Ave. Rolling Velocity (μm/sec)

0 25 50 75 100

*

Th1 Th2

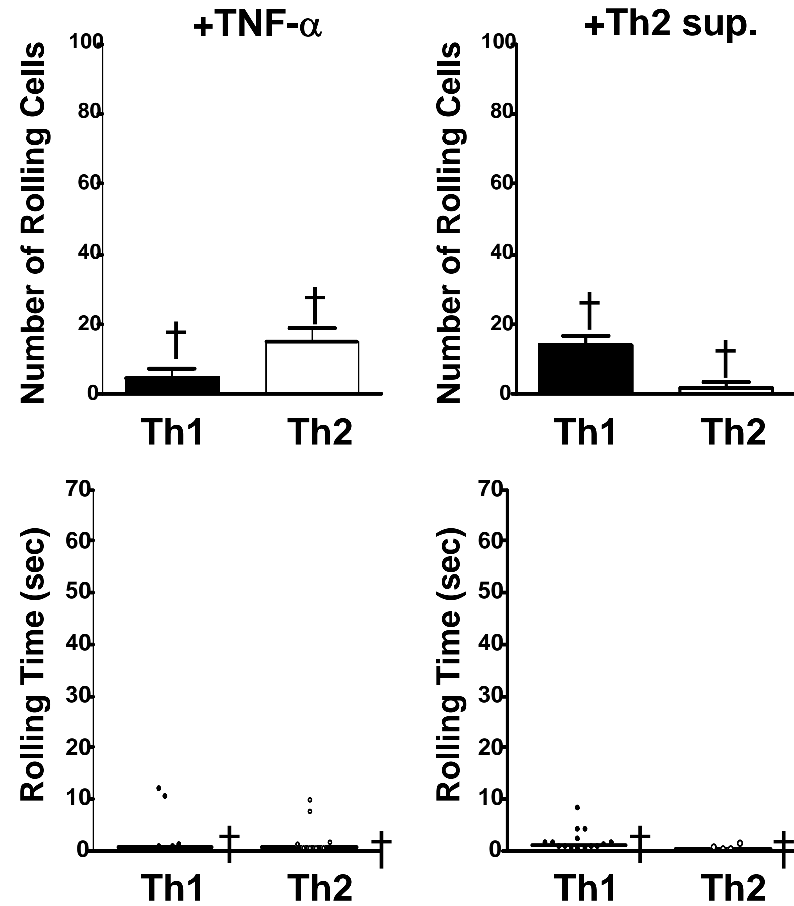


Fig. S4

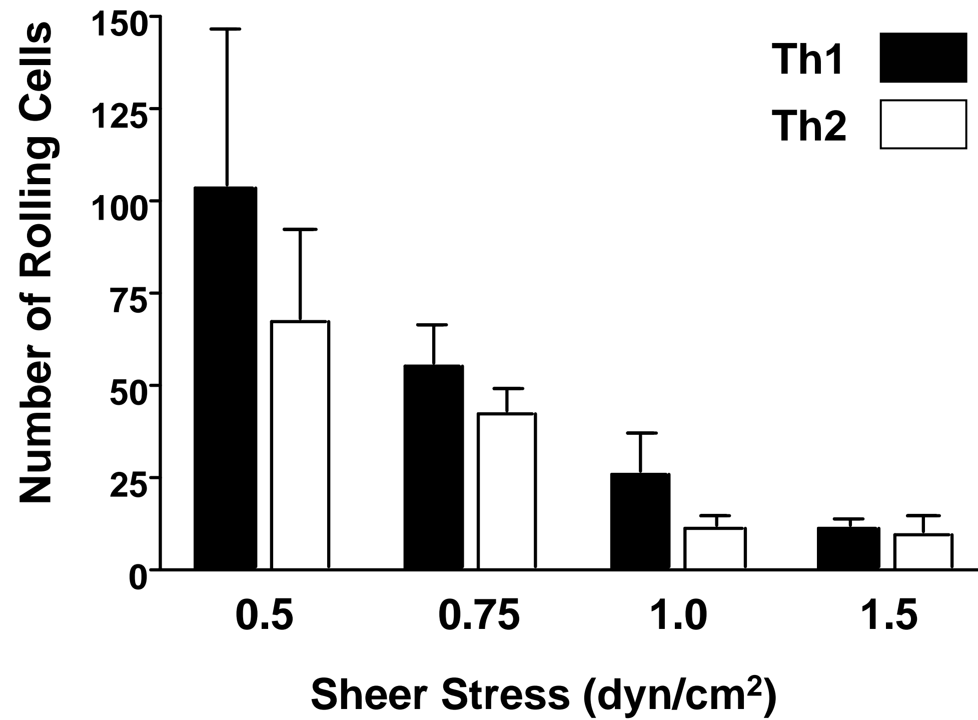


Fig. S5