Supplementary Figure 1 | Rolling leukocytes form slings in mouse cremaster venules. DyLight-488-conjugated-anti-LFA-1 (green) mAb was injected (via a catheter connected to the right carotid artery) into WT mice to stain all leukocytes green and the rolling in cremaster venules was recorded using a 100X 1.45 NA oil immersion objective and intravital epifluorescence microscopy. Images were saturated to reveal slings, which makes the cells appear bigger than their normal size (~7.5 µm). (a-f) Six representative leukocytes before, during, and after sling formation. (a) A sling appears in the front of a rolling leukocyte at \( t = 0.1 \) s (sling marked with white arrow) and the cell wraps the sling around itself by \( t = 0.2 \) s (sling still visible marked with white arrow). (b) A rolling leukocyte forms a sling in the front at \( t = \)}
0.2 s (sling marked with white arrow). As the cell rolls over the sling, the sling comes closer to the vessel wall and becomes clearly visible at $t = 0.5$ s (sling marked with white arrow). (c) A rolling leukocyte forms a sling in the front at $t = 0.4$ s (sling marked with white arrow) and then rolls over the sling by $t = 0.6$ s. (d) A rolling leukocyte forms a sling in the front at $t = 0.1$ s (sling marked with white arrow) and rolls over the sling by $t = 0.4$ s. (e) A rolling leukocyte forms a sling in the front at $t = 0.1$ s (sling marked with white arrow) and starts to roll over the sling by $t = 0.2$ s (sling still visible marked with white arrow). (f) A rolling leukocyte forms a sling in the front at $t = 0.2$ s (sling marked with white arrow) and then rolls over the sling by $t = 0.6$ s. Scale bars 5 µm. Thick grey horizontal arrows denote the direction of rolling.
Supplementary Figure 2 | Aspect ratio of tether anchorage points increases linearly with maximum tether length. (a) Sequence of qDF images of a DiI-stained neutrophil rolling on P-selectin. The tether anchorage point (white arrowhead) elongates with the increasing length of tether. Wall shear stress 10 dyn/cm\(^2\). Images were processed to reveal tether anchorage points. P-selectin 20 molecules/µm\(^2\). TIRF excitation 561 nm laser and incidence angle \(\theta = 70^\circ\). Scale bars 5 µm. Grey thick arrow denotes the direction of rolling. (b-d) Aspect ratio of tether anchorage points plotted as a function of the maximum tether length (horizontal distance between the cell center and tether anchorage point before tether detachment from the substrate) at shear stresses of (b) 6, (c) 8, and (d) 10 dyn/cm\(^2\). P-selectin 20 molecules/µm\(^2\). Data representative of three
independent experiments. (e) Linear correlation analysis of the data in b-d. $a$, y intercept; $b$, slope; $\sigma_a$, uncertainty in $a$; $\sigma_b$, uncertainty in $b$; $r^2$, linear correlation coefficient.

### Supplementary Figure 3 | Fraction of the forward force and torque acting on a rolling neutrophil balanced by slings and tethers.

<table>
<thead>
<tr>
<th></th>
<th>6 dyn/cm²</th>
<th>8 dyn/cm²</th>
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<tr>
<td>$F_{\text{cell}}$</td>
<td>272.5 pN</td>
<td>363.5 pN</td>
</tr>
<tr>
<td>$F_{\text{SL/Te}(2 \text{ SL &amp; 2Te})}$</td>
<td>88% of $F_{\text{cell}}$</td>
<td>66% of $F_{\text{cell}}$</td>
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<tr>
<td>$\Gamma_{\text{cell}}$</td>
<td>374 pN µm</td>
<td>499 pN µm</td>
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<tr>
<td>$\Gamma_{\text{SL/Te}(2 \text{ SL &amp; 2Te})}$</td>
<td>100% of $\Gamma_{\text{cell}}$</td>
<td>100% of $\Gamma_{\text{cell}}$</td>
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</tbody>
</table>

Two slings and two tethers, each with inflection/anchorage point 10 µm from the cell center; $F_{\text{cell}}$, total forward force on the cell; $F_{\text{SL/Te}(2 \text{ SL & 2Te})}$, forward force balanced by 2 slings and 2 tethers; $\Gamma_{\text{cell}}$, total torque on the cell; $\Gamma_{\text{SL/Te}(2 \text{ SL & 2Te})}$, torque balanced by 2 slings and 2 tethers. Refer to Methods for details on force and torque estimation.
Supplementary Figure 4 | Footprints of naïve CD4T cells rolling on peripheral node addressin (PNAd). Naïve CD4T cells isolated from the spleen of WT mice were stained with membrane dye DiI and allowed to roll on human PNAd into a microfluidic device at a shear stress of 1.8 dyn/cm². Footprints of rolling cells were recorded at different time points using qDF. TIRF excitation 561 nm laser and incidence angle $\theta = 70^\circ$. Scale bar 5 µm. Thick-grey horizontal arrow denotes the direction of rolling.

41. Hemmerich, S., Butcher, E.C., & Rosen, S.D. Sulfation-dependent recognition of high
Supplementary Figure 5| Sling formation by Th1 cells rolling on P-selectin. (a) Naïve CD4T cells were in vitro differentiated into Th1 cells and the differentiation was verified by their ability to produce γ-interferon (IFN) following activation. (b) Th1 cells were tested for their ability to bind soluble recombinant murine P-selectin-Fc. Red curve (P-selectin-Fc + APC-anti-
human-IgG), blue curve (APC-anti-human-IgG), and green (no IgG-Fc). (c) In vitro differentiated Th1 cells stained with membrane dye DiI were allowed to roll on P-selectin in a microfluidic device and footprints of rolling cells visualized using qDF. TIRF excitation 561 nm laser, incidence angle $\theta = 70^\circ$. A membrane tether (anchorage point marked with a white arrowhead at $t = 0$ s) following detachment at the rear of the rolling cell is laid in the front of the cell as a sling (marked with white arrow at $t = 5$ s). The cell rolls forward relocating the sling to the rear ($t = 7$ and 18.5s). P-selectin 20 molecules/µm$^2$. Wall shear stress 8 dyn/cm$^2$. Thick-grey horizontal arrow denotes the direction of rolling. Images were processed to reveal slings and tether anchorage points. Scale bars 5 µm.
Supplementary Figure 6 | PSGL-1 expressed on the tips of microvilli. Isolated mouse bone marrow neutrophils stained with membrane dye DiI (red) and DyLight-488-conjugated-non-blocking-anti-PSGL-1 mAb (green) were allowed to roll on P-selectin in a microfluidic device and footprints visualized using DqDF. (a) DqDF image of a bone marrow neutrophil from a WT mouse rolling on P-selectin. First column-dual color image showing membrane (red) and PSGL-1 (green). Corresponding single color images of PSGL-1 (green) and membrane (red) shown in second and third column, respectively. Slings marked with white solid arrows. Thick grey horizontal arrow denotes the direction of rolling. TIRF excitation 488 and 561 nm lasers and incidence angle, $\theta = 70^\circ$. Scale bars 5 µm. P-selectin 20 molecules/µm$^2$. Wall shear stress 10 dyn/cm$^2$. (b) The DiI image (red image in the third column of a) was used to generate a 3D-reconstruction of the footprint$^{12}$ and the PSGL-1 image (green image in the second column of a)
overlaid to reveal the PSGL-1 distribution on slings, tether anchorage points, and microvilli (hills) vs. cell surface (valleys). Thick white horizontal arrow denotes the direction of rolling.
Supplementary Figure 7 | PSGL-1 expressed on the tips of microvilli (dye swap). Isolated-mouse-bone marrow neutrophils stained with membrane dye DiO (green) and Alexa-Fluor-568-conjugated-non-blocking-anti-PSGL-1 mAb (red) were allowed to roll on P-selectin in a microfluidic device and footprints visualized using DqDF. (a) DqDF image of a bone marrow neutrophil from a WT mouse rolling on P-selectin. First column-dual color image showing membrane (green) and PSGL-1 (red). Corresponding single color images of membrane (green) and PSGL-1 (red) are shown in second and third column, respectively. (b) The DiO image (green image in second column of a was used to generate a 3D-reconstruction of the footprint as described previously\textsuperscript{12} and the PSGL-1 image (red image in third column of a) was overlaid to reveal the PSGL-1 distribution on slings, tether anchorage points, and microvilli (hills) vs. cell
surface (valleys). TIRF excitation 488 and 561 nm lasers and incidence angle, $\theta = 70^\circ$. Scale bars 5 µm. Thick arrow denotes the direction of rolling.
**Supplementary Figure 8| PSGL-1 is distributed in patches on slings.** Mouse bone marrow neutrophils stained with Alexa-Fluor-568-conjugated-non-blocking-anti-PSGL-1 mAb (red) were allowed to roll on P-selectin and footprints visualized using qDF. TIRF excitation 561 nm laser and incidence angle $\theta = 70^\circ$. qDF images of a rolling neutrophil show PSGL-1 expression on the sling (white arrow at $t = 1$ s), tether anchorage points (white arrowhead at $t = 1$ s), and also cell surface. Wall shear stress 10 dyn/cm$^2$. P-selectin 20 molecules/µm$^2$. Thick-grey horizontal arrow denotes the direction of rolling. Scale bars 5 µm.
**Supplementary Figure 9** | PSGL-1 staining absent in the footprints of PSGL-1 deficient neutrophils. Isolated-bone marrow neutrophils from PSGL-1 deficient (Selplg^-/-) mice stained with membrane dye DiO (green) and Alexa-Fluor-568-conjugated-non-blocking-anti-PSGL-1 mAb (red) were allowed to roll on E-selectin and footprints visualized using DqDF. TIRF excitation 488 and 561 nm lasers and incidence angle, $\theta = 70^\circ$. First row-dual color image showing membrane (green) and PSGL-1 (red-staining absent). Corresponding single color images of membrane (green) and PSGL-1 (red-staining absent) shown in second and third row, respectively. The PSGL-1 (red) staining is absent in the dual color (row 1) and the single color (row 3) images. Wall shear stress 10 dyn/cm². E-selectin coating concentration 1 µg/ml. Scale
bars 5 µm. Tether anchorage points and slings marked with white arrowheads and white arrows, respectively. Thick-grey horizontal arrow denotes the direction of rolling.
**Supplementary Figure 10** | **Microvilli preserved as lobes on slings-Example 1.** Isolated-mouse bone marrow neutrophils rolling on P-selectin in a microfluidic device were fixed and visualized using scanning electron microscopy. View from top of the cell. Scanning electron micrograph showing lobe-like structures (marked with white dashed arrows) on the slings. Scale bar 5 µm. Thick white arrow denotes the direction of rolling. Wall shear stress 10 dyn/cm². P-selectin 20 molecules/µm². Image processed to reveal lobes on slings.
Supplementary Figure 11| Microvilli preserved as lobes on slings-Example 2. Isolated-mouse bone marrow neutrophils rolling on P-selectin in a microfluidic device were fixed and visualized using scanning electron microscopy. View from top of the cell. Scanning electron micrograph showing lobe-like structures (marked with white dashed arrows) on the slings. Scale bar 5 µm. Thick white arrow denotes the direction of rolling. Wall shear stress 10 dyn/cm². P-selectin 20 molecules/µm². Image processed to reveal lobes on slings.
### Supplementary Figure 12| Measurements from SEM images

<table>
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<th>Value</th>
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<td>Diameter of a mouse neutrophil measured from SEM images</td>
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<td>Mean diameter of sling based on SEMs after correction for shrinkage</td>
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<td>Mean diameter of microvilli based on SEMs after correction for shrinkage</td>
<td>271 ± 48 (s.d.) nm</td>
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<tr>
<td>Mean length of microvilli based on SEMs after correction for shrinkage</td>
<td>698 ± 152 (s.d.) nm</td>
</tr>
<tr>
<td>Total number of microvilli per 7.5 µm neutrophil based on SEMs</td>
<td>1043 ± 97 (s.d.)</td>
</tr>
<tr>
<td>Estimate for the number of microvilli that contribute to the formation of a 10 µm sling</td>
<td>9</td>
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<tr>
<td>Estimate for the spacing between the tips of microvilli preserved as lobes on a 10 µm sling</td>
<td>1.32 µm</td>
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**Supplementary Figure 12| Measurements from SEM images.** Table showing the estimated distance between adjacent microvilli tips preserved as lobes on a 10 µm long sling. Estimates are based on measurements from SEM images of 11 neutrophils. Microvilli and slings were considered cylindrical in shape.
**Supplementary Figure 13** | Step-wise peeling of slings. Isolated mouse bone marrow neutrophils stained with membrane dye DiO (green) and Alexa-Fluor-568-conjugated-non-blocking-anti-PSGL-1 mAb (red) were allowed to roll on P-selectin in a microfluidic device and footprints visualized using DqDF. TIRF excitation 488 and 561 nm lasers and incidence angle, $\theta = 70^\circ$. P-selectin 20 molecules/µm$^2$. Wall shear stress 10 dyn/cm$^2$. Scale bars 5 µm. The same cell is shown in **Figure 3c**. Images were saturated to reveal membrane (green) in the tether anchorage points and slings. PSGL-1 patches on the slings appear yellow (red + green). The rolling neutrophil has two slings (marked with red solid arrows as SL#1 and 2) and three long tethers (anchorage points marked with red dashed arrows as Te#1, 2, and 3). Rolling is from left
to right. As described in Figure 3d-e and Supplementary figure 14-15, when a PSGL-1 patch becomes load-bearing, the bond force acting on the patch pulls the patch out of the TIRF evanescent wave (~100 nm from the cover slip\(^4\)). As a result, the PSGL-1 staining (yellow spot) in the patch falls below detection limit and thus, seems to disappear. Based on this, a patch or a tether anchorage point was considered as failed only when both the PSGL-1 staining (yellow spot) and the green membrane underneath disappeared. The PSGL-1 patches at the inflection point of SL#1 and 2 are visible as yellow spots (marked with solid white arrow) at \(t = -0.8\) s when the slings are not yet loaded. As the cell rolls forward the patches marked with white arrows at the inflection point of both the slings at \(t = -0.8\) s become load bearing at \(t = 0\) s which is evident by the loss of PSGL-1 staining (yellow) in these patches. The loading of slings cause the cell to slow down. The two load-bearing patches on SL#1 fail somewhere between \(t = 0\) and \(0.2\) s allowing the cell to roll forward by a small distance (displacement relative to black vertical dashed line) and the next two downstream patches on SL#1 become load-bearing, which is again evident by the loss of PSGL-1 staining in these patches which was visible at \(t = 0\) s (yellow spots). The first load-bearing patch on SL#1 fails somewhere between \(t = 0.2\) and \(0.4\) s allowing the cell to roll to the right by a small distance. The unloaded patches on SL#1 are clearly visible as yellow spots. The remaining load-bearing patch on SL#1, the load-bearing patch on SL#2, and Te#2 fail somewhere between \(t = 1\) s and \(1.2\) s. The cell jumps forward and the load is transferred to the next downstream patches on SL#1 and SL#2. The new load-bearing patch on SL#1 shows decreased PSGL-1 staining following loading at \(t = 1.2\) s which was evident as a yellow spot at \(t = 1\) s when unloaded. The new load-bearing patch on SL#2 is hidden under the cell and becomes visible as the cell rolls forward, however, it was visible as a yellow spot (marked with white dashed arrow) when unloaded at \(t = -0.8\) s. The displacement generated by
this failure event is much larger than that generated by failure of individual patches at $t = 0.2$ and $0.4$ s. This event differs from previous ones by the simultaneous failure of Te#2. As the cell tries to roll forward, the PSGL-1 staining in the tether anchorage point of Te#1 and the unloaded patch on SL#1 starts to disappear, showing that these attachment points are being pulled by the rolling cell. A new tether Te#4 (anchorage point marked with red dashed arrow) forms by $t = 1.6$ s. Although the PSGL-1 staining is not visible, the green membrane in the anchorage points of Te#1, 3, and 4 is still visible, confirming that tethers have not failed. Te#4 fails somewhere between 1.8 and 2 s and the cell jumps forward. One patch on SL#1 and Te#1 and 3 all fail somewhere between 2 and 2.2 s allowing the cell to again jump forward and the last patch on the SL#1 becomes load-bearing (marked with red solid arrow at $t = 2.2$ s). The PSGL-1 staining on the last patch is not visible although the membrane (green; marked with red solid arrow at $t = 2.2$ s) is visible. The last patch on SL#1 fails somewhere between 2.2 and 2.4 s. The failure of the last patch is equivalent to failure of a tether because no new patches are available on the SL#1 to bear the load. Thus, the cell jumps forward as it would do in the event of a tether failure. The SL#2 (marked with red arrow at $t = 2.4$ s) is still present and sharing the load. This example demonstrates that failure of PSGL-1 patches on slings does not result in as large a forward displacement of the cell as a tether failure does. The only exception is the failure of last patch on the sling which is equivalent to a tether failure (refer the schematic in Fig. 3f). The effect of each failure event on the position of the cell is also shown in a graph in Figure 3g.
Supplementary Figure 14 | Estimation of strain in the PSGL-1 patches following load-bearing. Isolated mouse bone marrow neutrophils stained with membrane dye DiI were allowed to roll on P-selectin in a microfluidic device and footprints visualized using qDF. TIRF
excitation 561 nm laser and incidence angle, $\theta = 70^\circ$. Wall shear stress 10 dyn/cm$^2$. (a) Color maps showing the $z$-position (30-220 nm) of the membrane from the substrate in three PSGL-1 patches immediately before and after becoming load-bearing. Patch #1 is unloaded at 0 s (white circle) and becomes loaded at 1 s. Patch #2 is unloaded at 1 s (white circle) and becomes loaded at 1.3 s. Patch #3 is unloaded at 1.3 s (white circle) and becomes loaded at 2 s. The $z$-distance (nm) encoded by the color is defined in the color bar on top. White-dashed vertical lines connect a patch immediately before and after becoming load-bearing. Grey horizontal arrow denotes the direction of rolling. Refer to Methods for details. (b) Mean $z$-distance from the substrate for 10x10 pixel regions containing each of the three patches in the color maps before (U) and after (L) becoming load-bearing. ‘*’ $p < 0.05$. Error bars are s.e.m.
Supplementary Figure 15 | The intensity of PSGL-1 patches drops following loading.

Isolated mouse bone marrow neutrophils stained with membrane dye DiO (green) and Alexa-Fluor-568-conjugated-non-blocking-anti-PSGL-1 mAb (red) were allowed to roll on P-selectin in a microfluidic device and footprints visualized using DqDF. TIRF excitation 488 and 561 nm lasers and incidence angle, $\theta = 70^\circ$. P-selectin 20 molecules/µm². Wall shear stress 10 dyn/cm². (a, b) DqDF images of two cells showing a PSGL-1 patch on the sling immediately before and after becoming load-bearing. Cell #1-the white dashed line connects the PSGL-1 patch that is not load-bearing at $t = 0$ s (visible as red spot) but becomes load-bearing at $t = 1$ s (red spot not
visible). Cell #2-the white dashed line connects the PSGL-1 patch that is not load-bearing at $t = 0$ s (visible as red spot) but becomes load-bearing at $t = 0.2$ s (red spot becomes less bright). Scale bars 5 µm. Thick grey horizontal arrows denote the direction of rolling. Images processed to reveal slings. (c) Mean pixel intensity of the red fluorescence for the PSGL-1 patches shown in a and b. U-mean intensity when the patch is unloaded. L-mean intensity when the patch is loaded. ‘*’ $p < 0.05$. Error bars are s.e.m.
**Supplementary Figure 16** | Rolling is stabilized by step-wise peeling of slings. Same conditions as Figure 3g and Supplementary figure 13. Position of a rolling neutrophil plotted as a function of time over a 1.2 s period of rolling. The rolling neutrophil has one sling and one long tether referred as SL#1 and Te#1, respectively. Closed black circles denote the experimentally measured position of the cell at different time points 0.12 s apart. Red solid line denotes SL#1. Red closed triangle denotes failure of a PSGL-1 patch on SL#1 and also marks the midpoint of the 0.12 s interval during which the patch fails. Red dashed arrow denotes the lifetime of Te#1 starting from the time when the tether forms and ending when it fails. Wall shear stress 10 dyn/cm². P-selectin 20 molecules/µm². Te#1 did not fail during the 1.2 s period of rolling shown.
Supplementary Figure 17 | Rolling is stabilized by step-wise peeling of slings. Same conditions as Figure 3g and Supplementary figure 13. Position of a rolling neutrophil plotted as a function of time over a 1.6 s period of rolling. The rolling neutrophil has one sling and two long tethers referred as SL#1 and Te#1 and 2, respectively. Closed black circles denote the experimentally measured position of the cell at each time point 0.2 s apart. Red solid line denotes SL#1. Red closed triangle denotes the failure of a PSGL-1 patch on SL#1 (shown at the midpoint of the 0.2 s interval during which the patch fails). Red-five-point-star symbol at $t = 1.5$ s (midpoint of the 0.2 s interval) denotes the failure of the last PSGL-1 patch on SL#1 resulting in complete detachment of SL#1. Red dashed arrows denote Te#1 and 2, respectively, starting from
the time when the tether forms and ending when it fails (midpoint of the 0.2 s interval). Wall shear stress 10 dyn/cm². P-selectin 20 molecules/µm².
Supplementary Figure 18| Slings slow down rolling leukocytes in vivo. DyLight-488-conjugated-anti-LFA-1 mAb (green) was injected (via a catheter connected to the right carotid artery) into WT mice and the rolling of leukocytes in cremaster venules was recorded using a 100X 1.45 NA oil immersion objective and intravital epifluorescence microscopy. Images were processed to reveal slings and the instantaneous rolling velocity was measured for four cells over 1-1.5 s following sling detection. (a) Cell 1; cell detaches from the vessel wall at t = 1 s. (b) Cell 2. (c) Cell 3. (d) Cell 4. Red vertical arrow denotes the time when the sling becomes visible. In all four cells, the rolling velocity decreased for 0.5-1 s following sling appearance. The time of detection of a sling in a rolling leukocyte in intravital observations does not necessarily represent the time of formation of the sling; the sling could have already existed before.
Supplementary Figure 19| Scanning electron micrograph showing the peeling of a sling.

Isolated-mouse bone marrow neutrophils rolling on P-selectin in a microfluidic device were fixed and visualized using scanning electron microscopy. View from top of the cell. The inflection point of the sling is marked with a white thin arrow. Same situation as shown in step 3 of Figure 3f. Image was processed to reveal the inflection point of the peeling sling. Part of the sling is hidden under the cell. White arrowhead marks a tether which is being pulled. Wall shear stress 10 dyn/cm². P-selectin 20 molecules/µm². Thick-white vertical arrow denotes the direction of rolling. Scale bar 5 µm.
Supplementary Figure 20| LFA-1 is expressed uniformly on the sling. Isolated mouse bone marrow neutrophils stained with membrane dye DiI (red) and DyLight-488-conjugated-anti-mouse-LFA-1 mAb (green) were allowed to roll on P-selectin in a microfluidic device and footprints visualized using DqDF. Top row-dual color footprint of a bone marrow neutrophil from a WT mouse rolling on P-selectin showing membrane (red) and LFA-1 (green). Corresponding single color images of LFA-1 (green) and membrane (red) are shown in the second and third row, respectively. Refer to Supplementary movie 9 for dual color time series of the same cell. TIRF excitation 488 and 561 nm lasers and $\theta = 70^\circ$. Wall shear stress 10 dyn/cm$^2$. P-selectin 20 molecules/µm$^2$. Thick-grey arrow denotes the direction of rolling. Sling marked with white solid arrow. Tether anchorage point marked with white arrowhead. Scale bars 5 µm.
Supplementary Figure 21| LFA-1 is expressed over the entire length of slings. Mouse bone marrow neutrophils stained with only Dylight-488-conjugated-anti-LFA-1 mAb (green) were allowed to roll on P-selectin and footprints visualized using qDF. TIRF excitation 488 nm laser and incidence angle $\theta = 70^\circ$. qDF footprint of a rolling neutrophil at different time points reveals LFA-1 expression on sling (green; marked with white arrow at $t = 1$ and 2 s), tether anchorage points (marked with white arrowhead at $t = 1$ and 2 s), and cell surface. Wall shear stress 10 dyn/cm$^2$. P-selectin 20 molecules/µm$^2$. Thick-grey horizontal arrow denotes the direction of rolling. Scale bars 5 µm.
Supplementary Figure 22| LFA-1 staining absent in the footprints of LFA-1 deficient neutrophils. Isolated-bone marrow neutrophils from LFA-1 deficient (Itgal⁻/⁻) mice stained with membrane dye DiI (red) and Dylight-488-conjugated-anti-LFA-1 mAb (green) were allowed to roll on P-selectin and footprints visualized using DqDF. TIRF excitation 488 and 561 nm lasers and incidence angle \( \theta = 70° \). First row-dual color image showing membrane (red) and LFA-1 (green-staining absent). Corresponding single color images of LFA-1 (green-staining absent) and membrane (red) are shown in second and third row, respectively. LFA-1 staining (green) is absent in the dual color (row 1) and single color (row 2) images. Wall shear stress 10 dyn/cm², P-selectin 20 molecules/µm². Scale bars 5 µm. Tether anchorage points and slings marked with
white arrowheads and white arrows, respectively. Thick-grey horizontal arrow denotes the direction of rolling.
Supplementary Figure 23: PSGL-1 is distributed in patches while LFA-1 is uniformly expressed on slings. Mouse bone marrow neutrophils stained with both Dylight-488-conjugated-anti-LFA-1 mAb (green) and Alexa-Fluor-568-conjugated-non-blocking-anti-PSGL-1 mAb (red) were allowed to roll on P-selectin and footprints visualized using DqDF. TIRF excitation 488 and 561 nm lasers. TIRF incidence angle $\theta = 70^\circ$. Dual color footprints (column 1) of a rolling neutrophil at different time points (top to bottom) show distribution of PSGL-1 (red spots) and LFA-1 (green) in the slings (white arrows), tether anchorage points (white arrowheads), and cell surface. Corresponding LFA-1 (green) and PSGL-1 (red) single color qDF images at each time point are shown in column 2 and 3, respectively. Wall shear stress 10 dyn/cm$^2$. P-selectin 20 molecules/µm$^2$. Thick-grey horizontal arrows denote the direction of rolling. Scale bars 5 µm.
**Supplementary Figure 24** Nature of PSGL-1 and LFA-1 expression on slings is independent of the choice of fluorochrome. The experiment shown in **Supplementary figure 23** was repeated with the two fluorochromes swapped between PSGL-1 and LFA-1. Mouse bone marrow neutrophils stained with both Alexa-Fluor-568-conjugated-anti-LFA-1 mAb (red) and DyLight-488-conjugated-non-blocking-anti-PSGL-1 mAb (green) were allowed to roll on P-selectin and footprints visualized using DqDF. Three different cells are shown. Top row- Dual color image showing LFA-1 (red), PSGL-1 (green) and colocalization (yellow). Corresponding single color images of LFA-1 (red) and PSGL-1 (green) are shown in second and third row, respectively. Slings are marked with white solid arrows. TIRF excitation 488 and 561 nm lasers. TIRF incidence angle $\theta=70^\circ$. Wall shear stress 10 dyn/cm$^2$. P-selectin 20 molecules/µm$^2$. Thick-grey horizontal arrow denotes the direction of rolling. Scale bars 5 µm.
Supplementary Figure 25 | Expression of ICAM-1 and ICAM-2 on mouse blood and bone marrow neutrophils. ICAM-1 and ICAM-2 expression on mouse blood and bone marrow neutrophils by flow cytometry. (a) ICAM-1 expression on mouse whole blood neutrophils. (b) ICAM-1 expression on mouse bone marrow neutrophils. Red curve-staining with anti-mouse ICAM-1 Ab. Blue curve-isotype control Ab. Representative of three experiments. (c) Relative Fluorescence Intensity (RFI) of ICAM-1 in blood and bone marrow neutrophils. RFI was calculated by subtracting mean fluorescence intensity (MFI) of the cells stained with isotype control Ab from the MFI of cells stained with anti-ICAM-1 mAb. n = 3 experiments. N.D, not detected. (d) ICAM-2 expression on mouse whole blood neutrophils. (e) ICAM-2 expression on mouse bone marrow neutrophils. Red curve-staining with anti-mouse ICAM-2 mAb. Blue curve-
isotype control Ab. Representative of three experiments. (f) RFI of ICAM-2 in blood and bone marrow neutrophils. RFI was calculated by subtracting MFI of the cells stained with isotype control Ab from the MFI of cells stained with anti-ICAM-2 mAb. $n = 3$ experiments. BM, bone marrow. Error bars are s.d.
Supplementary Figure 26 | Slings enable LFA-1-ICAM-2 interactions in *trans* on rolling neutrophils. Bone marrow neutrophils from WT or LFA-1 deficient (Itgal<sup>−/−</sup>) mice either untreated or blocked with function blocking anti-LFA-1 (TIB217) mAb or anti-ICAM-2 (3C4) mAb were allowed to roll on cover glass coated with P-selectin or both P-selectin and ICAM-1 in a microfluidic device at a wall shear stress of 10 dyn/cm<sup>2</sup>. Mean rolling velocity of WT neutrophils (black; *n* = 159 cells) rolling on P-selectin, LFA-1 deficient neutrophils (grey; *n* = 173 cells) rolling on P-selectin, WT neutrophils blocked with anti-LFA-1 mAb (black; *n* = 142 cells) rolling on P-selectin, WT neutrophils blocked with anti-ICAM-2 mAb (black; *n* = 98 cells) rolling on P-selectin, WT neutrophils (black; *n* = 127 cells) rolling on P-selectin and ICAM-1,
and WT neutrophils blocked with anti-LFA-1 mAb (black; \( n = 146 \) cells) rolling on P-selectin and ICAM-1. P-selectin 20 molecules/µm\(^2\). ‘*’ \( p < 0.01 \) relative to WT neutrophils without any blocking on P-selectin. ‘†’ \( p < 0.05 \) relative to WT neutrophils without any blocking on P-selectin and ICAM-1. Error bars are s.e.m.
**Supplementary Figure 27** | ICAM-2 minimally expressed on slings but abundant on cell surface. Mouse bone marrow neutrophils stained with both Alexa-Fluor-568-conjugated-anti-LFA-1 mAb (red) and Alexa-Fluor-488-conjugated-anti-ICAM-2 mAb (green) were allowed to roll on P-selectin in a microfluidic device and footprints visualized using DqDF. First column-sequence (top-bottom) of dual color footprints of a rolling neutrophil showing LFA-1 (red) and ICAM-2 (green). The corresponding single color images of LFA-1 (red) and ICAM-2 (green) are shown in column two and three, respectively. TIRF excitation 488 and 561 nm laser. TIRF incidence angle, $\theta = 70^\circ$. Wall shear stress 10 dyn/cm$^2$. P-selectin 20 molecules/µm$^2$. Thick-grey horizontal arrows denote the direction of rolling. Slings are marked with white solid arrows. Scale bars 5 µm.
Supplementary Figure 28 | Specificity of ICAM-2 staining on rolling neutrophils. Isolated-bone marrow neutrophils from WT mice stained with both Alexa-Fluor-568-conjugated-anti-LFA-1 mAb (red) and Alexa-Fluor-488-Rat-IgG2α Ab (green; isotype matched control Ab for Alexa-Fluor-488-anti-ICAM-2 mAb) were allowed to roll on P-selectin in a microfluidic device and footprints visualized using DqDF. Top row—dual color footprint. Single color footprints are shown in second and third row, respectively. LFA-1 (red) visible in dual color (top row) and single color (middle row). Green staining absent in dual color image (top row) and single color image (bottom row). TIRF excitation 488 and 561 nm laser. TIRF incidence angle, $\theta=70^\circ$. Wall shear stress 10 dyn/cm$^2$. P-selectin 20 molecules/μm$^2$. Thick-grey horizontal arrow denotes the direction of rolling. Slings are marked with white solid arrows. Scale bars 5 μm.
Supplementary Note 1| Slings appear bright in qDF, suggesting that they are very close to the substrate. Analysis of 7 slings shows that the average minimum distance of a sling from the substrate is $89 \pm 37$ (s.d.) nm, which is similar to the resting length of the P-selectin-PSGL-1 bond ($\sim 70$ nm$^{42-44}$). To obtain a high resolution top view of rolling neutrophils, we fixed cells while rolling in the microfluidic channels, keeping track of the flow direction, and subjected them to scanning electron microscopy. Based on the SEMs, the slings were found to have an average diameter of $209 \pm 6$ (s.e.m.; $n = 9$) nm.


Supplementary Note 2 | Slings were rarely observed at 4 dyn/cm². In vitro cultured endothelial cells have been shown²⁵,²⁶ to express P-selectin at a molecular density of 10-50 molecules/µm², which formed the base for our choice of 20 molecules/µm² in this study. At 10 and 20 molecules/µm², we observed that 80% of rolling neutrophils formed slings at a wall shear stress of 10 dyn/cm² (no stable rolling was observed on P-selectin molecular density below 10 molecules/µm²).
Supplementary Note 3| In order to test whether formation of slings is associated with stabilized rolling at high shear stress, we tested the ability of mouse naïve CD4T cells to roll on immobilized PNAd (ligand for L-selectin^{15}) in a microfluidic device. Rolling naïve CD4T cells did not form long tethers or slings and could not roll at shear stress higher than 2 dyn/cm^{2} (Supplementary fig. 4). However, CD4T cells differentiated into Th1 cells^{27} formed tethers at the rear and slings in the front and were able to roll on P-selectin at a shear stress of 8 dyn/cm^{2} (Supplementary fig. 5).
Supplementary Note 4| Sling wrapping is less tight and allows for a longer (more favorable) lever arm when LFA-1 does not interact with ICAM-2. We think that this effect is normally masked by the (stronger) reduction in rolling velocity afforded by extended LFA-1 with ICAM-2 on endothelial cells. ICAM-2 is known to be constitutively expressed on endothelial cells\(^\text{21}\).
LEGENDS FOR SUPPLEMENTARY MOVIES

**Supplementary Movie 1**| Slings formed by DiI-stained mouse bone marrow neutrophil rolling on P-selectin. Image processed to reveal slings. TIRF excitation 561 nm, incidence angle $\theta = 70^\circ$. P-selectin 20 molecules/µm². Wall shear stress 10 dyn/cm². View from the bottom. Frame rate 5 s⁻¹.

**Supplementary Movie 2**| Sling formed by an *EGFP* neutrophil rolling on P-selectin in whole blood of *Lyz2-EGFP* mouse. Image processed to reveal sling. TIRF excitation 488 nm, incidence angle $\theta = 70^\circ$. P-selectin 20 molecules/µm². Wall shear stress 8 dyn/cm². View from the bottom. Frame rate 2 s⁻¹.

**Supplementary Movie 3**| Wrapping of slings around a DiI-stained mouse bone marrow neutrophil rolling on P-selectin. TIRF excitation 561 nm, incidence angle $\theta = 70^\circ$. P-selectin 20 molecules/µm². Wall shear stress 10 dyn/cm². View from the bottom. Frame rate 8 s⁻¹.

**Supplementary Movie 4**| Wrapping of sling by a leukocyte rolling in the cremaster venule of a WT mouse. Image processed to reveal sling. Cell stained with DyLight-488-anti-LFA-1 mAb (green). View from side of the vessel. Straight lines denote vessel walls. Frame rate 1 s⁻¹.

**Supplementary Movie 5**| Sling formation by a leukocyte rolling in the cremaster venule of a WT mouse. Image processed to reveal sling. Cell stained with DyLight-488-anti-LFA-1 mAb (green). View from top of the vessel. Straight lines denote vessel walls. Frame rate 1 s⁻¹.

**Supplementary Movie 6**| Tether (arrowhead) swings over to become a sling (arrow). DiI-stained mouse bone marrow neutrophil rolling on P-selectin. Image processed to reveal sling.
TIRF excitation 561 nm, \( \theta = 70^\circ \). P-selectin 20 molecules/\( \mu \text{m}^2 \). Wall shear stress 10 dyn/cm\(^2\). View from the bottom. Frame rate 2 s\(^{-1}\).

**Supplementary Movie 7**| Tether (arrowhead) swings over to become a sling (arrow). WT mouse bone marrow neutrophil rolling on P-selectin. DIC microscopy. P-selectin 20 molecules/\( \mu \text{m}^2 \). Wall shear stress 10 dyn/cm\(^2\). View from the bottom. Frame rate 4 s\(^{-1}\).

**Supplementary Movie 8**| Step-wise peeling of a sling. PSGL-1 patches (red spots) visible on sling (green). TIRF excitation 561 and 488 nm, incidence angle \( \theta = 70^\circ \). P-selectin 20 molecules/\( \mu \text{m}^2 \). Wall shear stress 10 dyn/cm\(^2\). View from the bottom. Frame rate 5 s\(^{-1}\).

**Supplementary Movie 9**| Staining of LFA-1 on a rolling neutrophil. Membrane (red) and LFA-1 (green). TIRF excitation 561 and 488 nm, incidence angle \( \theta = 70^\circ \). P-selectin 20 molecules/\( \mu \text{m}^2 \). Wall shear stress 10 dyn/cm\(^2\). View from the bottom. Frame rate 3 s\(^{-1}\).