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Supplemental Information

Receptor-like Tyrosine Phosphatases CD45 and CD148 Have Distinct Functions in Chemoattractant-Mediated Neutrophil Migration and Response to *S. aureus*

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Inventory of supplemental information:

- Figure S1: Additional supporting data for main figure 3.
- Figure S2: Additional data mentioned in discussion and compared to Figure 4.
- Figure S3: Additional supporting data for main figure 6.
- Figure S4: Additional supporting data for main figure 7.

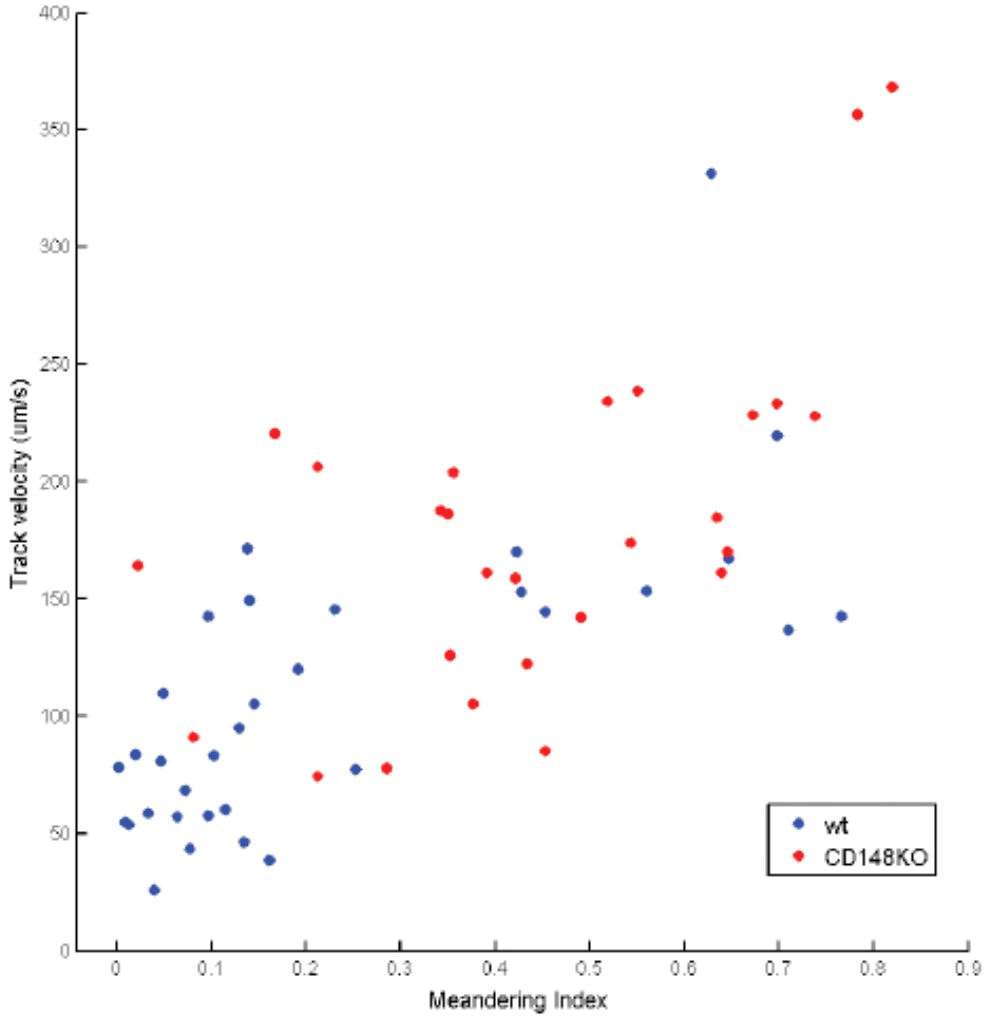

Figure S1. Two-dimensional plotting for in vitro chemotaxis assay (EZTAXIScan) shown in Fig 3. Each dot represents a cell track of the indicated genotype. Track velocity and meandering index were calculated as described in Fig. 3. The plot was created in Matlab.

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- Figure S1: Additional supporting data for main figure 3.
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- Movie M1, M2, M3, M4: Additional supporting data for main figure 3. The movies capture the raw data for the chemotaxis assay using EZ-TAXIScan.

- Movie M5, M6: Additional supporting data for main figure 6. The movies capture the raw data for the chemotaxis assay using EZ-TAXIScan.

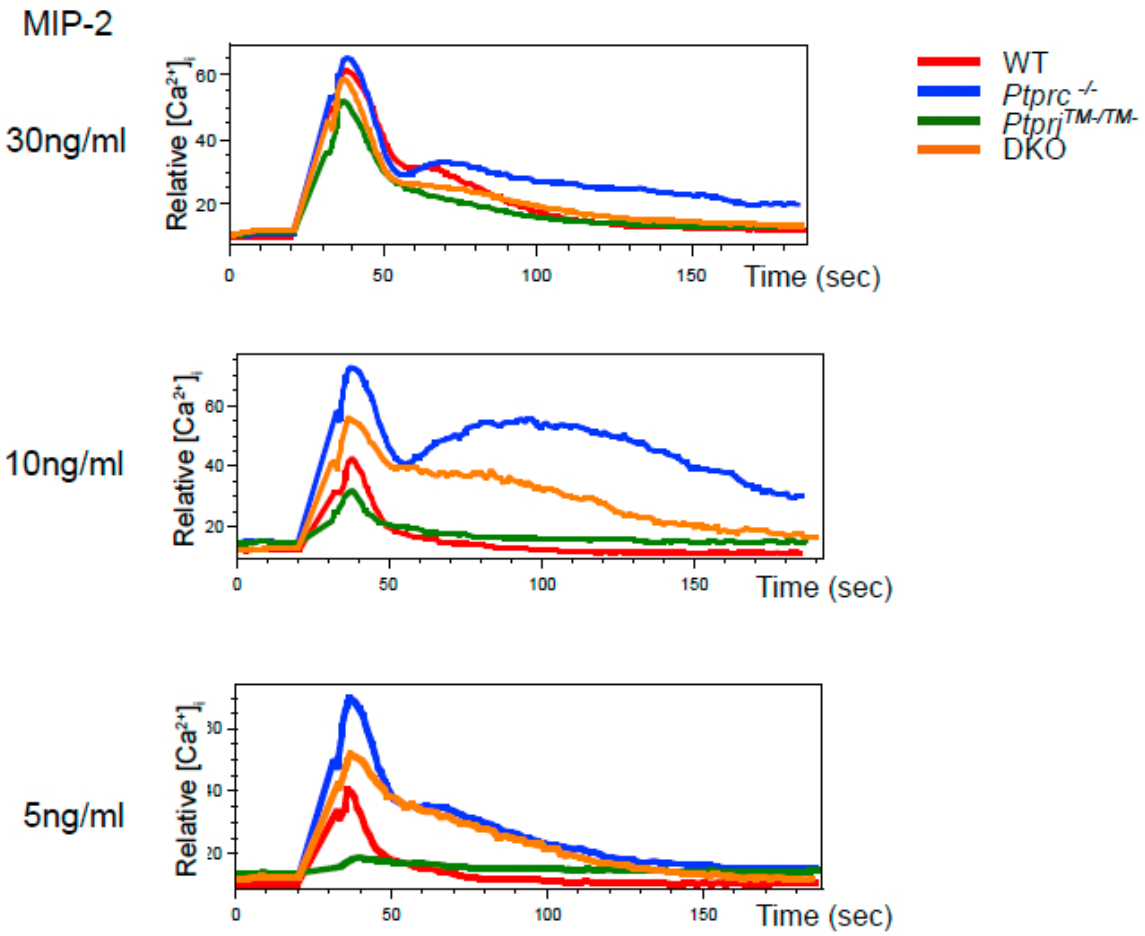

Figure S2.CD45 and CD148 differentially regulate MIP-2 mediated increase of intracellular Ca^{2+} in a dose dependent manner. Purified neutrophils from mice of the indicated genotypes were loaded with Fluo3-AM. Then intracellular free- Ca^{2+} concentrations were monitored before and after addition of different dose of MIP-2. Data are representative of three independent experiments.

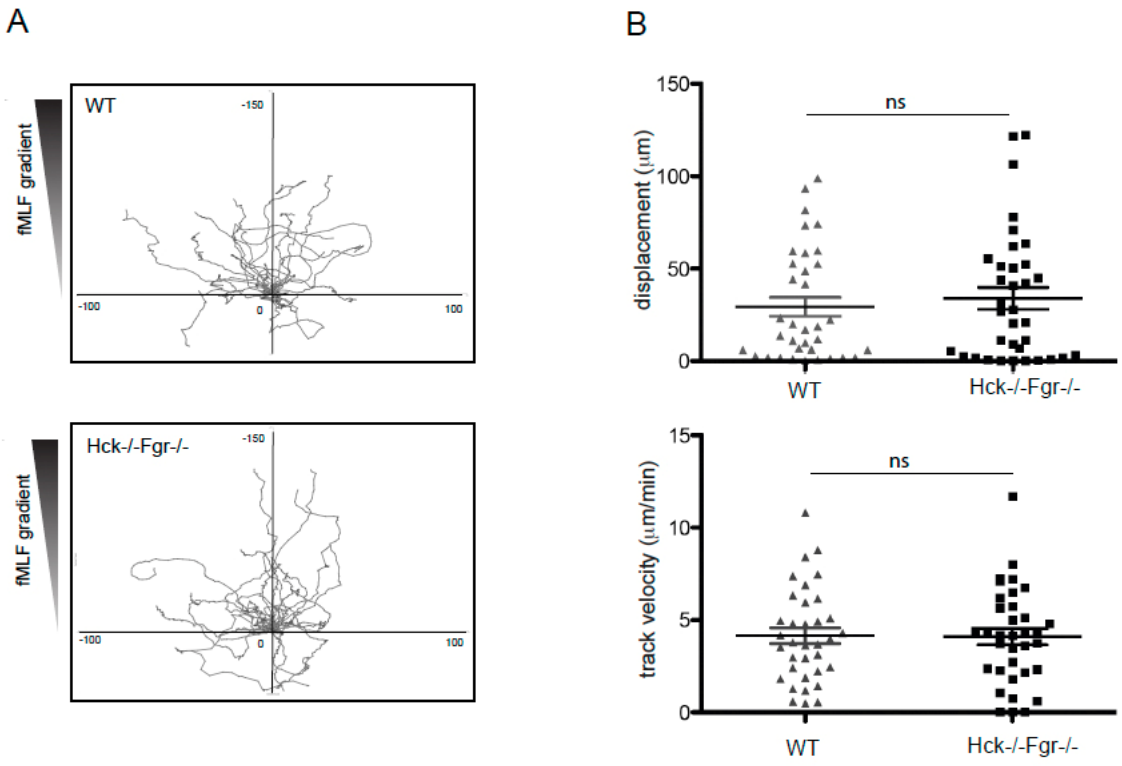

Figure S3. fMLF mediated chemotaxis was measured in an EZ-Taxiscan chamber as shown in Fig. 3. **A)** Center-zeroed tracks of individual neutrophils migrating towards reservoirs located at the top of the diagrams containing 0.5μM fMLF. The scales of each of the plots on the lefts are identical and equal in μm. **B)** Statistical analysis for the EZ-Taxiscan migration assay. p values were calculated by student t test.

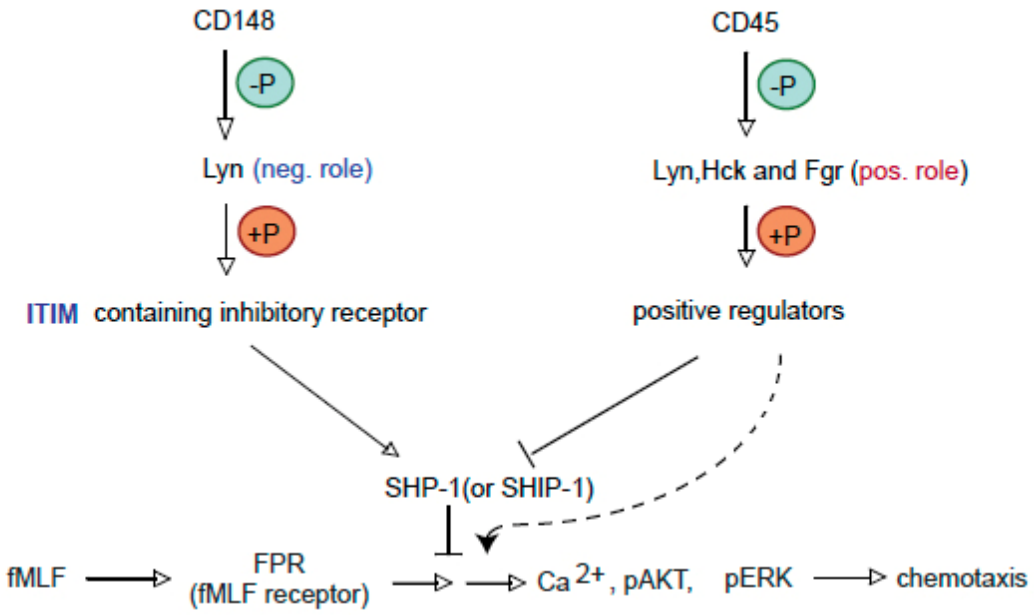

Figure S4. Working model of participation of CD45 and CD148 in chemoattractant mediated pathway. CD148 preferably targets Lyn by dephosphorylating its inhibitory tyrosine residue. Active Lyn is then able to phosphorylate an ITIM containing inhibitory receptor to recruit and activate the inhibitory phosphatase SHP-1. Loss of CD148 blocks these inhibitory events. In contrast, CD45 would differentially regulate all three SFKs in neutrophils, Hck,Fgr and Lyn, which positively regulates the pathway through an ITAM containing receptor or another, as yet unidentified positive regulator, thereby inhibiting the recruitment and activation of SHP-1. Loss of CD45 dampens the positive regulatory influence of the SFKs. Loss of both RPTPs eliminates any redundancy that CD148 provides in regulating SFKs.