

Molecular difference between WASP and N-WASP critical for chemotaxis of T-cells towards SDF-1 α

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Figure S1: Expression of N-WASP is lower than WASP in primary T cells. Quantitative real time PCR analysis of WASP and N-WASP expression at mRNA level in non-activated and activated splenocytes. *** $P < 0.001$

Figure S2: S1 WASP shRNA was specific to WASP. Expression of WASP-GFP, N-WASP-GFP and WASP_R-GFP in HEK293T cells transfected with or without S1 WASP shRNA using anti-GFP (α -GFP) antibody.

Figure S3: WASP Δ ^{I30} together with WIP can rescue the growth defect of *las17 Δ* strain.

A) Viability at 24 and 37°C of *las17 Δ* yeast strain IDY166 transformed with full length WT WASP or WASP Δ ^{I30} expressing plasmids with WIP or empty vector (Vect).

B) Western blot analysis of WASP and WASP Δ ^{I30} expressed with WIP or empty vector (Vect) in *las17 Δ* yeast strain.

Figure S4: I30 region is critical for Nck1 and Toca1 induced conformation change

A) Quantification of fluorescence signal from 100 *S. cerevisiae* cells transformed with NLS-WIP + WASP sensor or NLS-WIP + WASP Δ ^{I30} sensor together with (1) Vector or (2) Nck1 (3) Toca1 expressing plasmids. *** $P < 0.001$ compared to WASP sensor + NLS WIP + Vector. ** $P < 0.01$

B) Analysis of expression of the WASP sensors or WIP in *S. cerevisiae* cells. Anti-Hexokinase (α -Hex) was used for endogenous control.

Figure S5: I30 region interacts with Hck.

(A) Schematic diagram showing the WASP and WASP deletion mutants generated for His-tag pull down assay.

(B) HEK293T cells were transfected with Hck together with (1) Vector (2) WASP-His, (3) WASP^{ΔI30}-His, (4) WASP₁₃₈₋₃₂₀-His (5) WASP₁₃₈₋₃₂₀^{ΔI30}-His. The WASP or WASP deletion mutants were isolated by His-tag pull-down assay. * represent the degradation product of WASP or its deletion mutants.

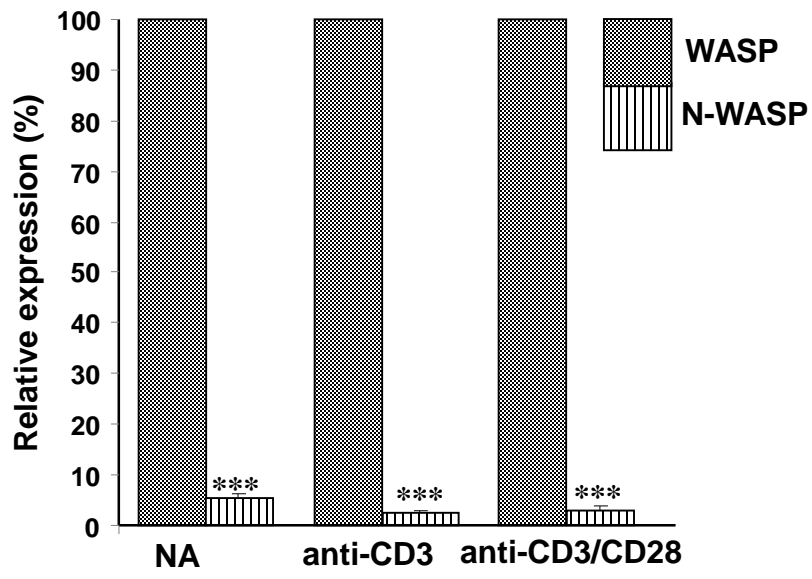
(C) **Deletion of I30 region enhanced Hck mediated WASP phosphorylation.** HEK293T cells were transfected with WASP-His or WASP^{ΔI30}-His together with (1) Vector, (2) Hck-GFP. WASP or WASP^{ΔI30} was isolated by His-tag pull-down assay. Hck expression was detected using anti-GFP antibody. Tyrosine-phosphorylation levels of WASP and WASP^{ΔI30} was detected using 4G10 antibody.

Figure S6: Directionality of migration is random in N-WASP expressing Jurkat^{W^KD} T-cells.

Time lapsed images of WASP_R or N-WASP expressing Jurkat^{W^KD} T-cells in Dunn chamber assay.

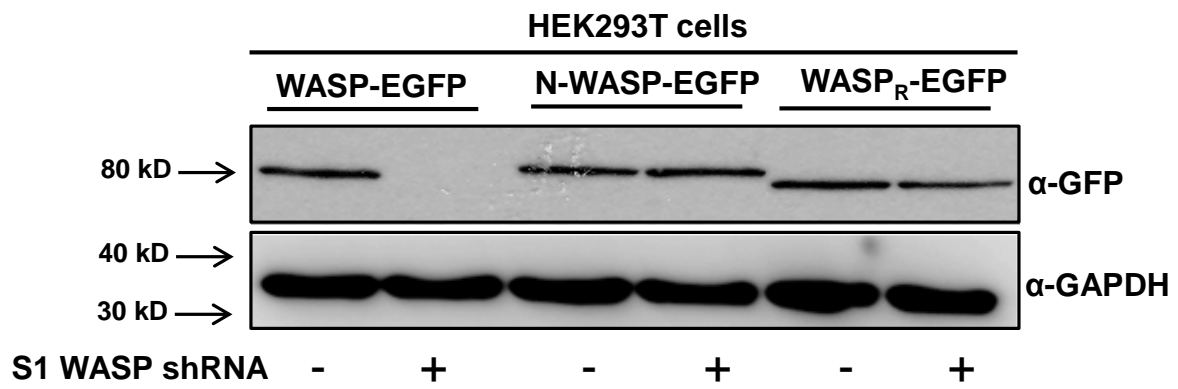
Supplementary

S1)



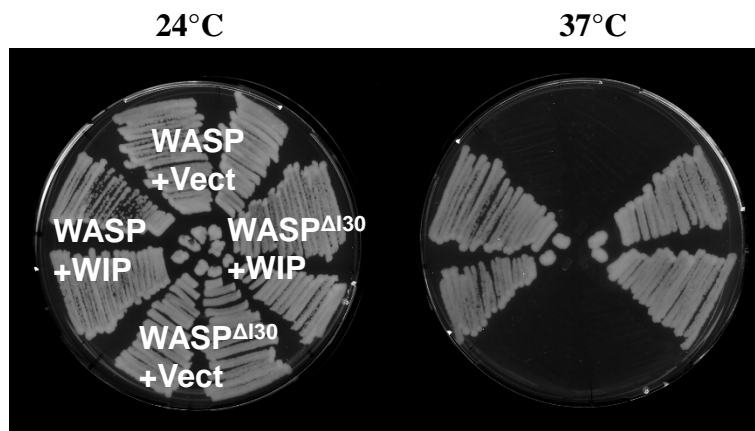
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S2)

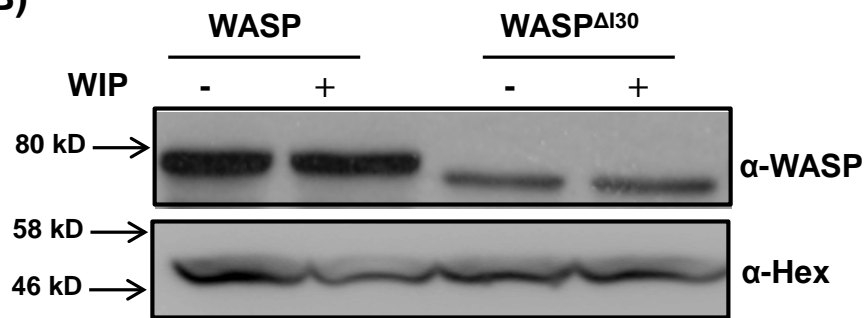


S3)

A)

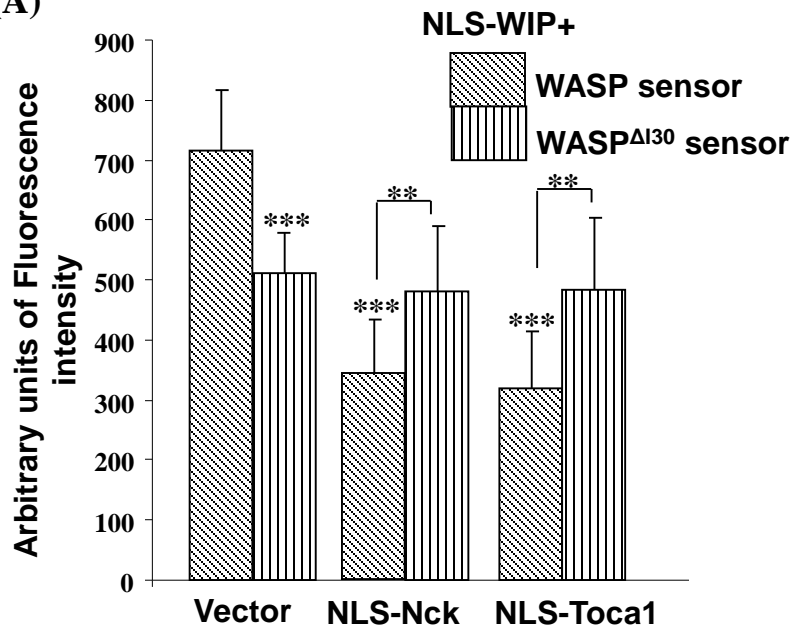


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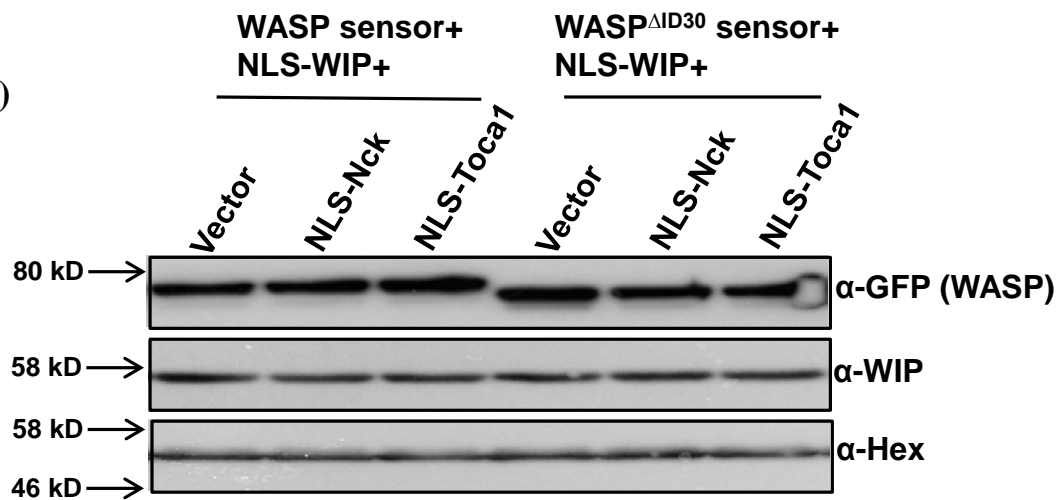


S4)

(A)

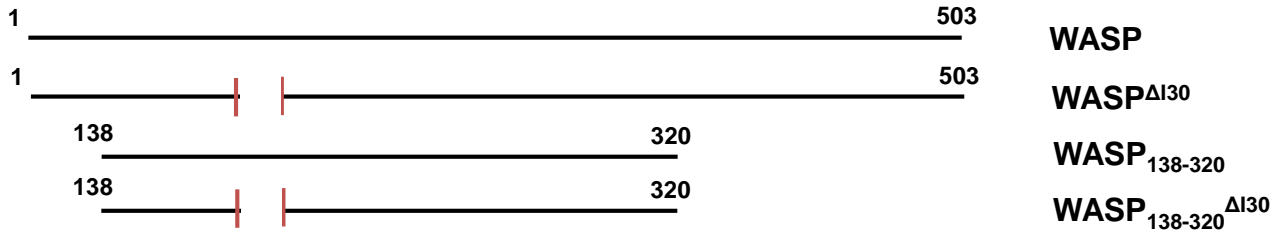


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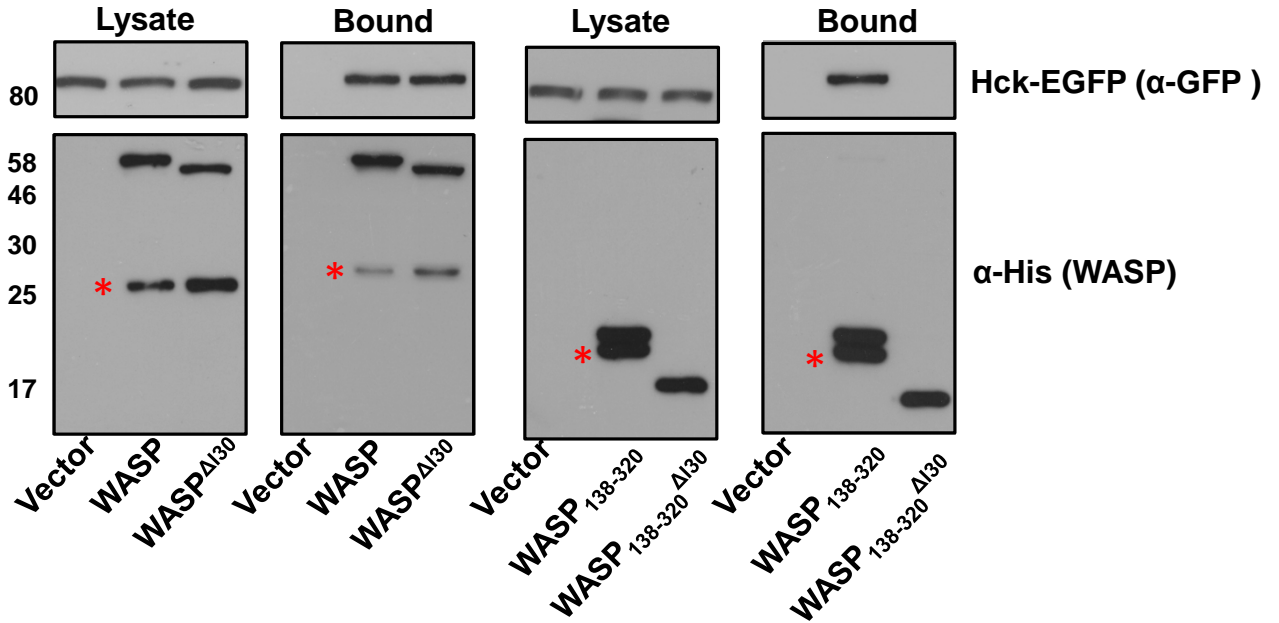


S5)

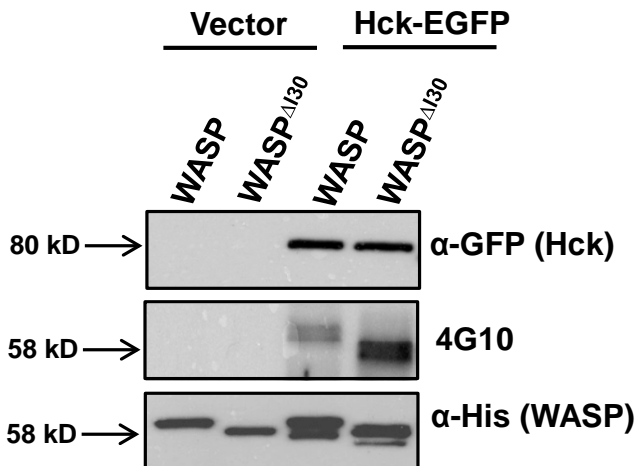
A)



B)



C)



S6)

Jurkat^{W_{KD}} T-cells+

