Sphingosine 1-phosphate receptor 1 regulates the directional migration of lymphatic endothelial cells in response to fluid shear stress

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Supplemental Information

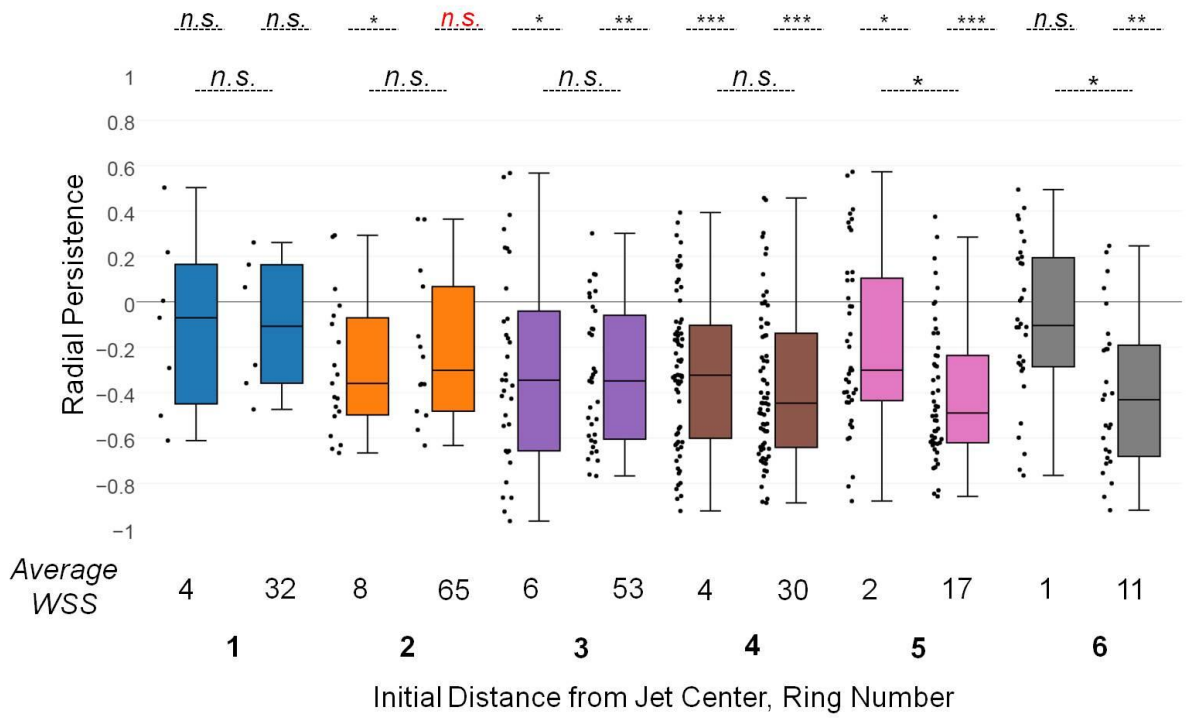

Figure S1. (A) Box-and-whisker plots showing the radial persistence of migration for tracked HLMVECs binned by initial starting position in response to 20 hours of impinging flow at a maximum WSS of 9 dynes/cm^2 (first plot per ring) and a maximum WSS of 72 dynes/cm^2 (second plot per ring). The average WSS at the given ring number is presented underneath each box-and-whisker plot. Positive values indicate persistent migration away from the jet center while negative values indicate migration toward the jet center. *N* = 200 cells per trial, taken from two independent experiments.

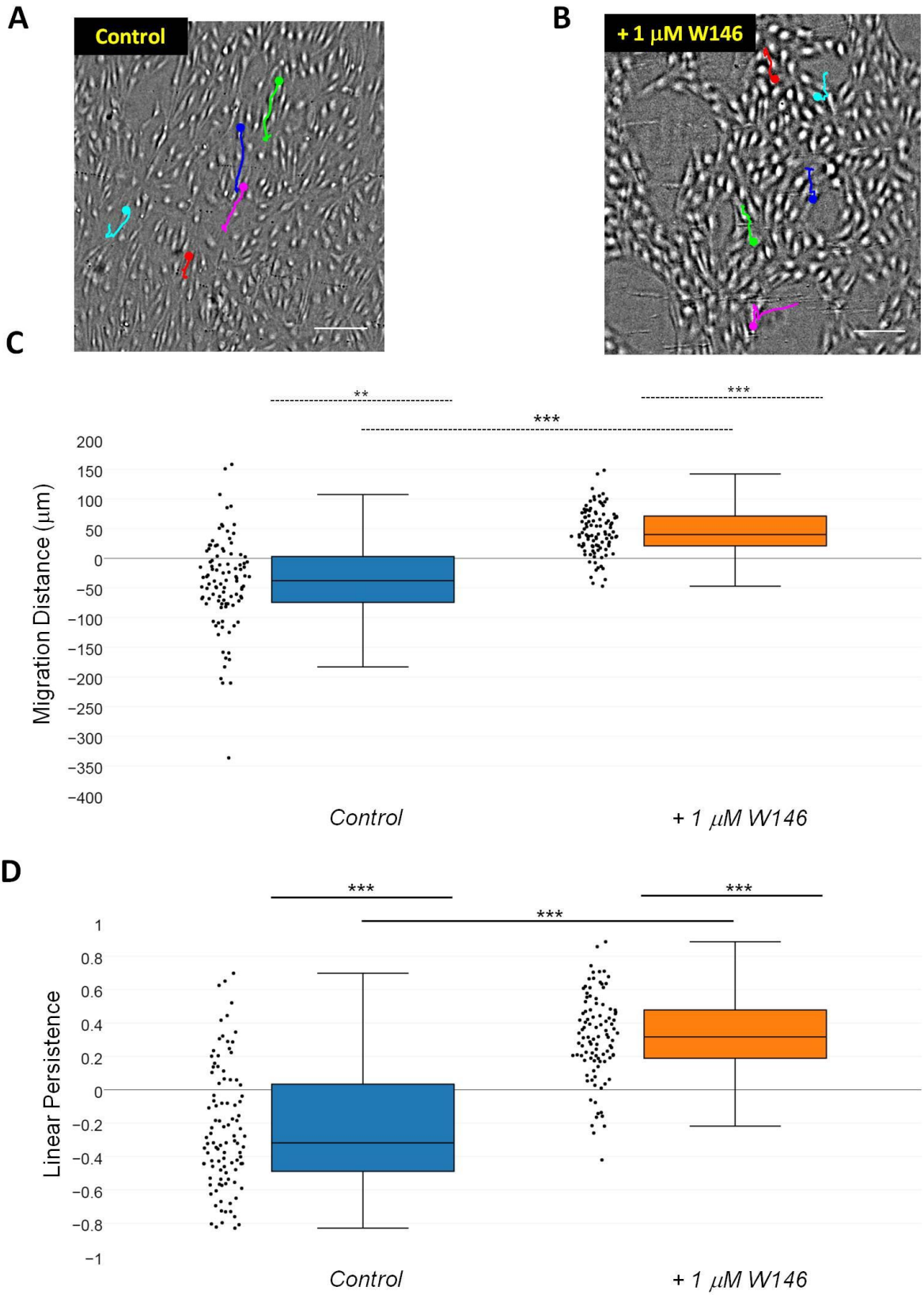
A
Control
B
+ 1 μM W146
C
**
***
***
200
150
100
50
0
−50
−100
−150
−200
−250
−300
−350
−400
Migration Distance (μm)
Control
+ 1 μM W146
D
***
***
***
1
0.8
0.6
0.4
0.2
0
−0.2
−0.4
−0.6
−0.8
−1
Linear Persistence
Control
+ 1 μM W146

Figure S2. HLMVECs exposed to a spatially uniform WSS of 12 dynes/cm^2 for 20 hours. (A) Untreated HLMVECs align with the flow direction and migrate against the flow (flow direction from top of image to bottom). Tracked cells are shown for reference with the migration trajectory (colored line) and dot indicating final position after 20 hours (B) HLMVECs treated with 1 μM W146 migrate with the flow direction (scale bar = 100 μm) (C, D) Box-and-whisker plots showing the linear displacement (C) and linear persistence of migration (D) for tracked cells binned by initial starting position. Positive values indicate migration with the direction of flow. *N* = 200 cells per trial, taken from two independent experiments.

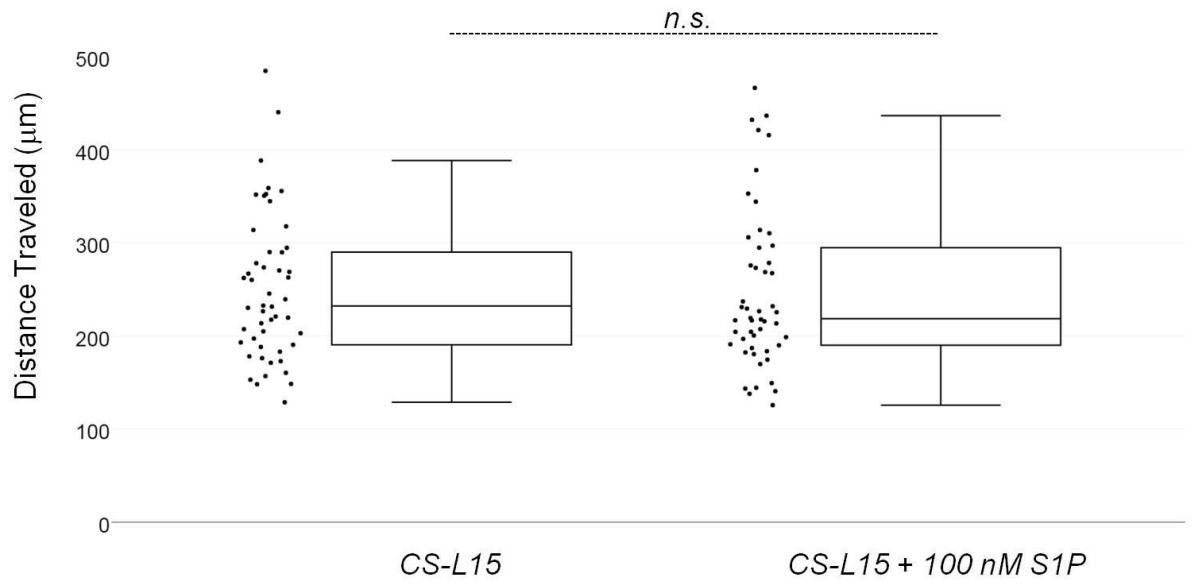

Figure S3. HLMVECs treated with charcoal-stripped (CS) media or CS media + 100 nM S1P were recorded in the absence of flow for 20 hours. The distribution of each cell's total distance traveled was calculated. N = 50 cells per trial.

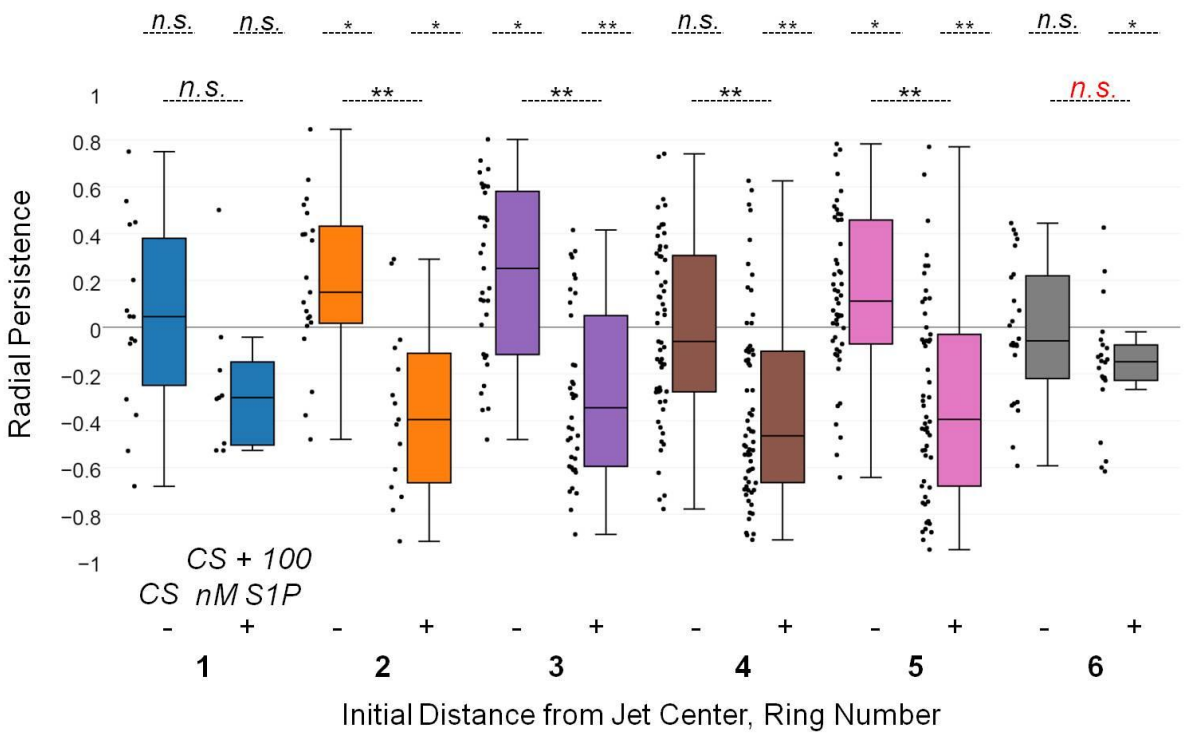

Figure S4. Box-and-whisker plots showing the radial persistence of migration for HLMVECs treated with CS–L15 (-) or CS–L15 with 100 nM S1P (+), in response to 20 hours of impinging flow at a maximum WSS of 9 dynes/cm^2. Positive values indicate persistent migration away from the jet center. *N* = 200 cells taken from two independent experiments.

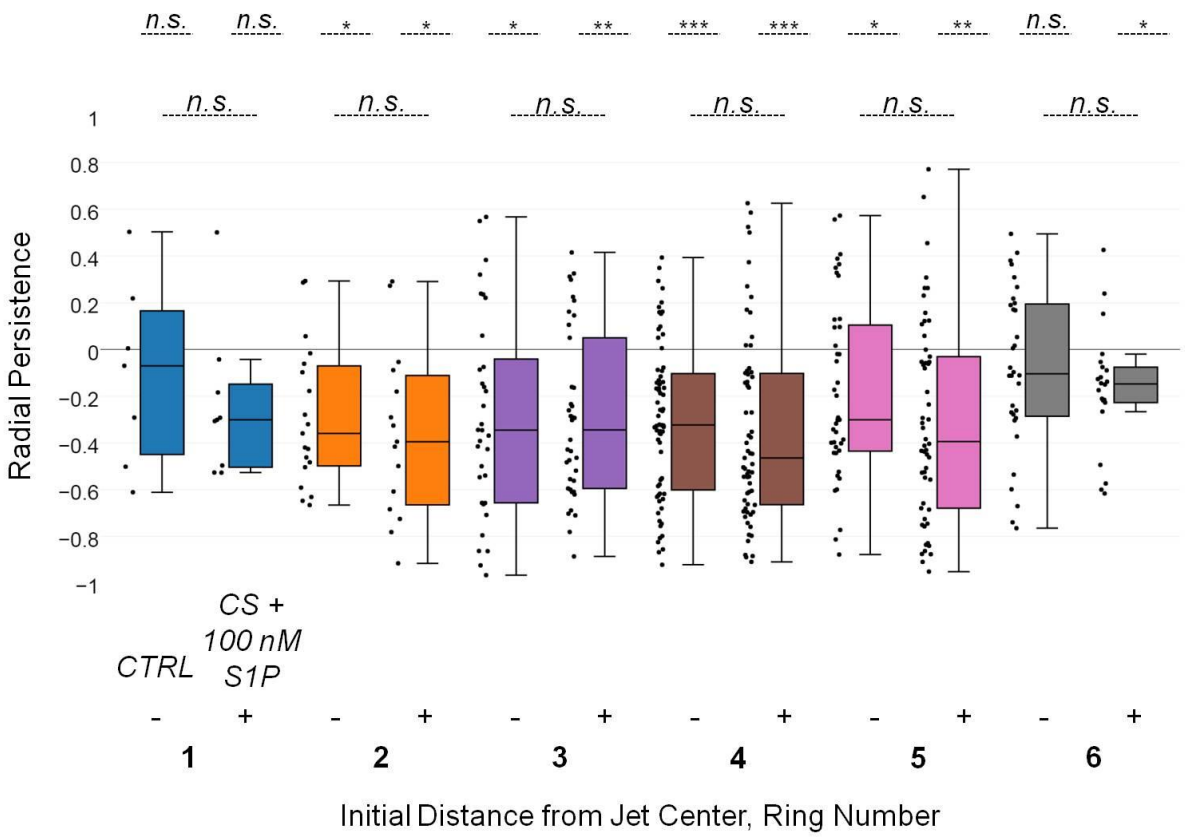

Figure S5. Box-and-whisker plots showing the radial persistence of migration for HLMVECs either untreated (-) or treated with CS–L15 with 100 nM S1P (+), in response to 20 hours of impinging flow at a maximum WSS of 9 dynes/cm^2. Positive values indicate persistent migration away from the jet center. *N* = 200 cells taken from two independent experiments.

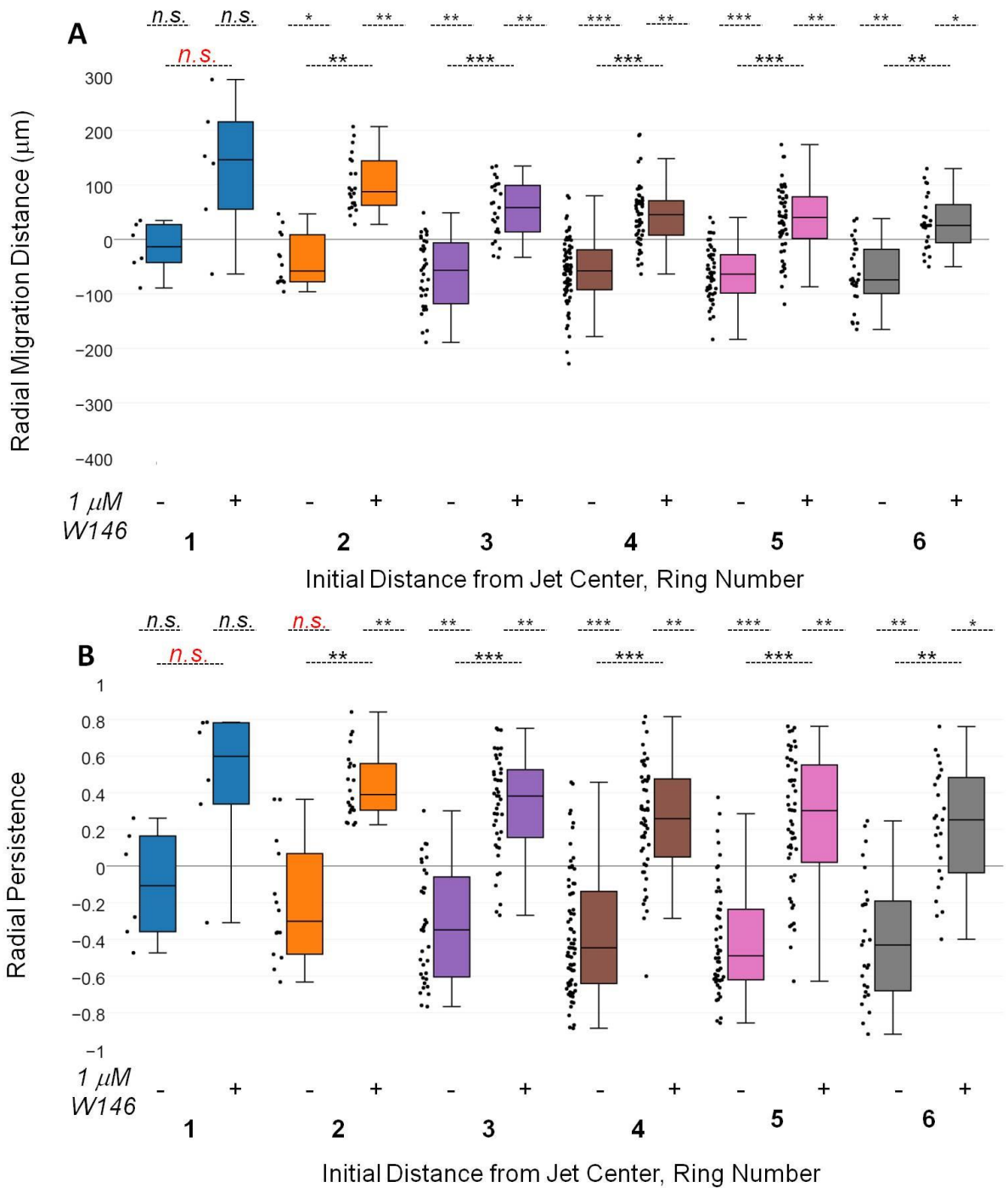

Figure S6. (A) Box-and-whisker plots showing the radial migration distance for both untreated (-) and HLMVECs treated with 1 μM W146 (+) in response to 20 hours of impinging flow at a maximum WSS of 72 dynes/cm^2. Positive values indicate migration away from the jet center. (B) Box-and-whisker plots showing the radial persistence of migration for both untreated (-) and HLMVECs treated with 1 μM W146

(+) in response to 20 hours of impinging flow at a maximum WSS of 72 dynes/cm^2. Positive values indicate persistent migration away from the jet center. *N* = 200 cells per trial taken from two independent experiments.

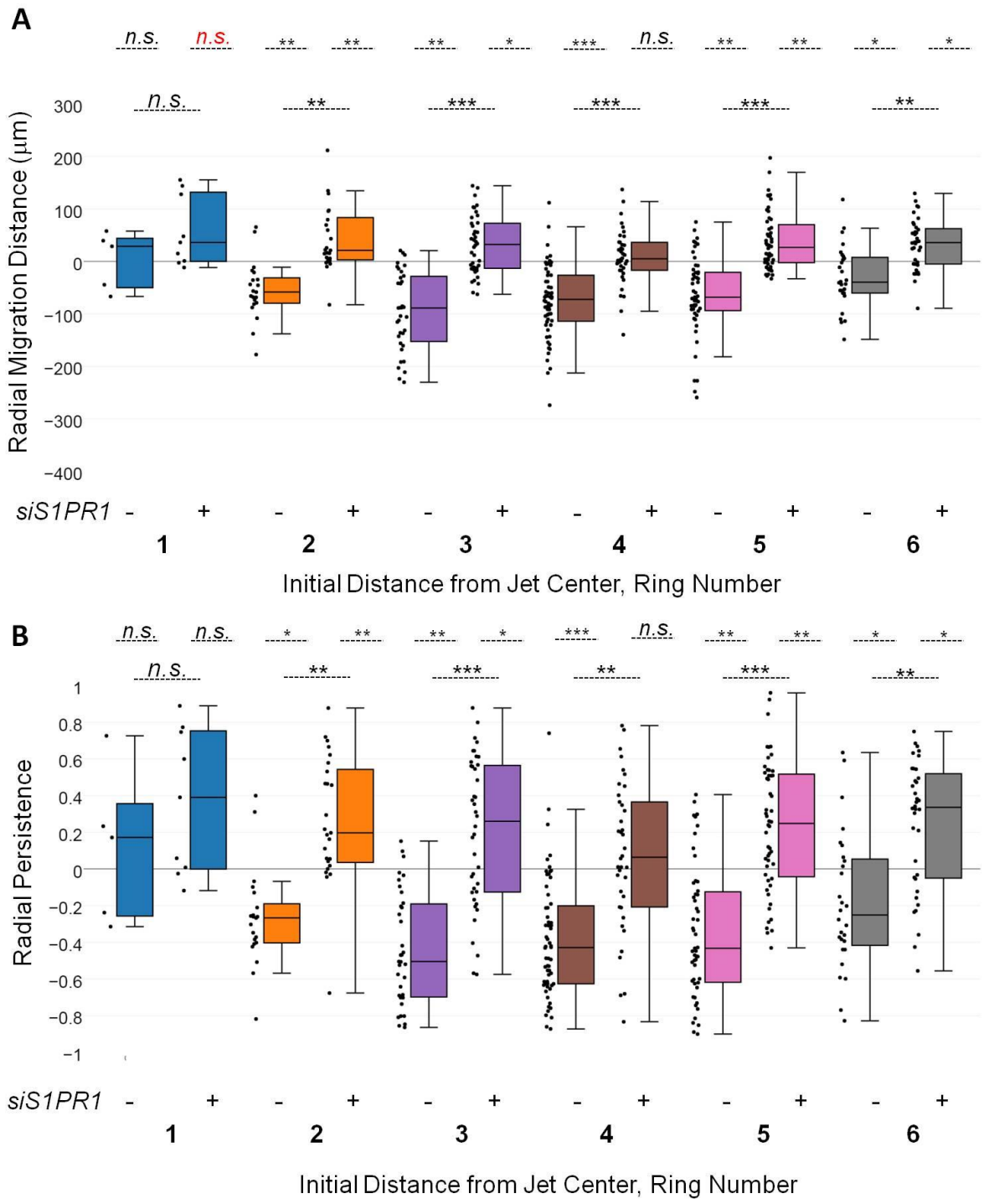

Figure S7. (A) Box-and-whisker plots showing the radial migration distance for both HLMVECs treated with scrambled siRNA (-) or siRNA targeting *S1PR1* (+) in response to 20 hours of impinging flow at a maximum WSS of 72 dynes/cm^2. Positive values indicate migration away from the jet center. (B) Box-

and-whisker plots showing the radial persistence of migration for both HLMVECs treated with scrambled siRNA (-) or siRNA targeting *S1PR1* (+) in response to 20 hours of impinging flow at a maximum WSS of 72 dynes/cm^2. Positive values indicate persistent migration away from the jet center. *N* = 200 cells per trial taken from two independent experiments.

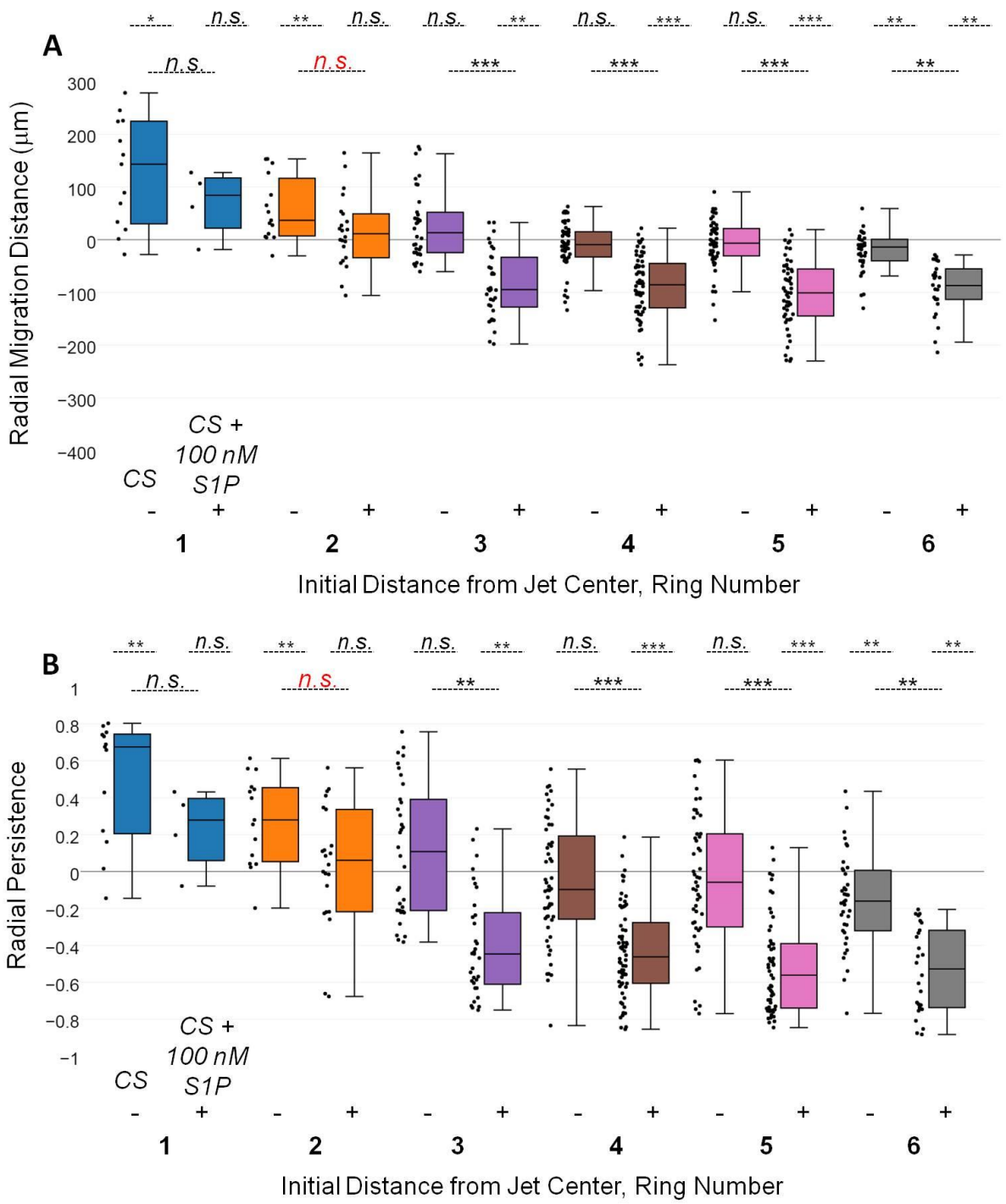

Figure S8. (A) Box-and-whisker plots showing the radial migration distance for both HLMVECs treated with CS–L15 (-) or CS–L15 with 100 nM S1P (+) in response to 20 hours of impinging flow at a maximum WSS of 72 dynes/cm^2. Positive values indicate migration away from the jet center. (B) Box-and-whisker plots showing the radial persistence of migration for both HLMVECs treated with CS–L15 (-) or CS–L15

with 100 nM S1P (+) in response to 20 hours of impinging flow at a maximum WSS of 72 dynes/cm^2. Positive values indicate persistent migration away from the jet center. *N* = 200 cells per trial taken from two independent experiments.

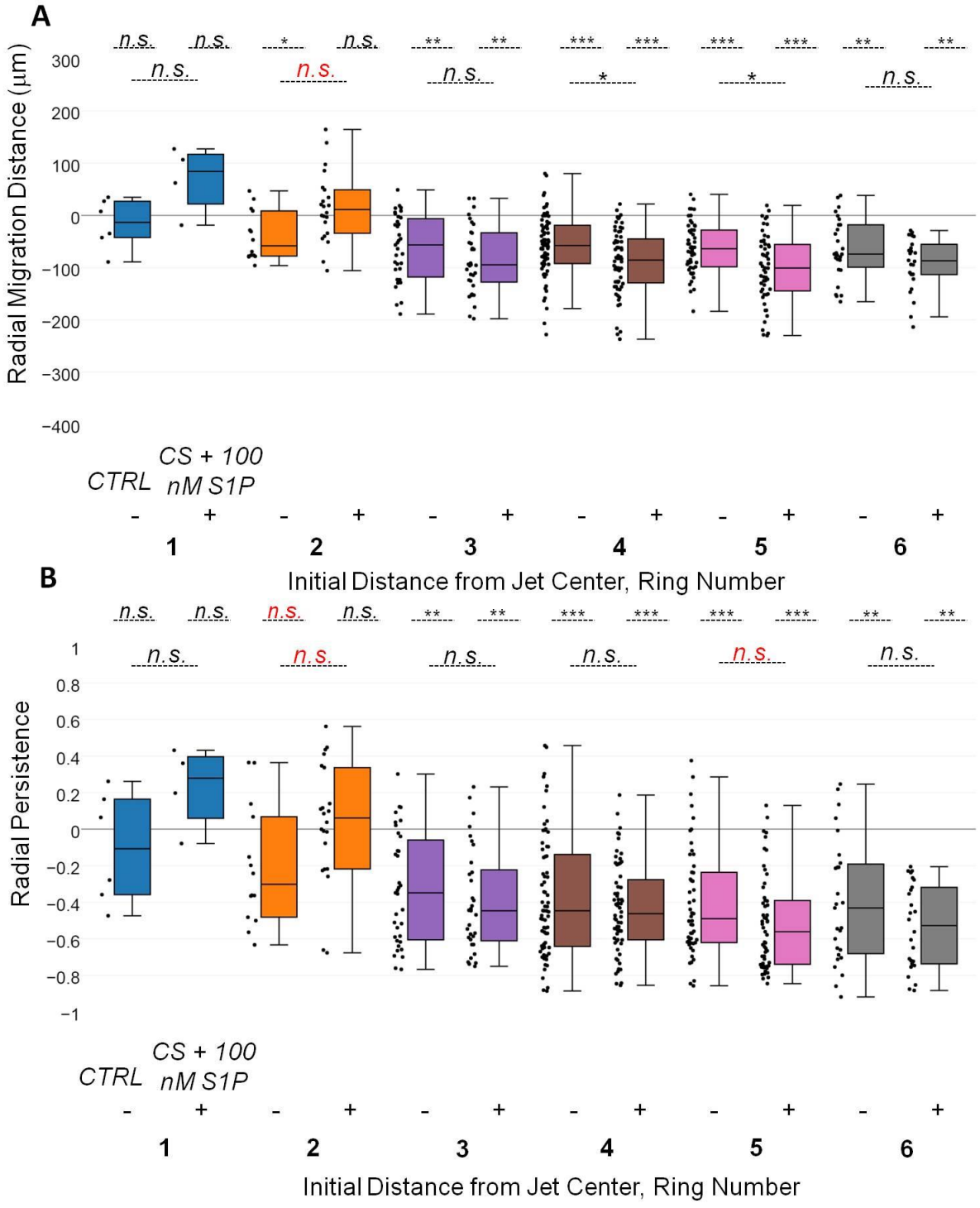

Figure S9. (A) Box-and-whisker plots showing the radial migration distance for both HLMVECs either untreated (-) or treated with CS–L15 with 100 nM S1P (+) in response to 20 hours of impinging flow at a maximum WSS of 72 dynes/cm^2. Positive values indicate migration away from the jet center. (B) Box-

and-whisker plots showing the radial persistence of migration for both HLMVECs either untreated (-) or treated with CS–L15 with 100 nM S1P (+) in response to 20 hours of impinging flow at a maximum WSS of 72 dynes/cm^2. Positive values indicate persistent migration away from the jet center. *N* = 200 cells per trial taken from two independent experiments.