of WRC versus free Rac1-GMPPNP measured by equilibrium dialysis. Error bars indicate standard deviation in at least two independent measurements. $K_D$ estimated from Rac concentration at 50% saturation; curves are binding isotherms to guide the eye. $\Delta$WRC (containing WAVE1(1-186)) pink triangle, L697D/Y704D$_\text{Sra1}$ miniWRC black square, miniWRC gold diamond, E434K/F626A$_\text{Sra1}$-WRC green circle, R190D$_\text{Sra1}$-$\Delta$WRC blue cross, $\Delta$154$_\text{WAVE1}$-WRC (containing WAVE1(1-154)) cyan inverted triangle. c, WAVE1 meander region. Sra1 residues involved in binding Rac1 and WAVE1 Y151 are gold and blue sticks, respectively. Dashed oval indicates proposed Rac1-binding surface. Phosphorylated WAVE1 residues Tyr 125, Thr 138 and Tyr 151 are red sticks. d, Arp2/3 mediated pyrene-actin assembly assays with miniWRC (green) or miniWRC containing Y151E$_\text{WAVE1}$ (blue) or F686E$_\text{Sra1}$ (red). Control assay (orange) lacked WRC.

**Figure 4** | Model for cooperative membrane recruitment and activation of the WRC. a, b Electrostatic surface of miniWRC (red to blue = -5 kT e$^{-1}$ to +5 kT e$^{-1}$), oriented as in Figs. 1a and b, respectively. c, Schematic illustrating proposed WRC orientation at the plasma membrane and cooperative recruitment and activation by Rac and phospholipids. Phosphorylation sites in WAVE1 meander are indicated. It remains unclear what portions of the meander are disrupted by different stimuli.

**Additional Methods**

**Protein expression and purification.**
The proteins were handled with a modified version of the earlier protocol\textsuperscript{13}. Mutants were generated using QuikChange (Stratagene). To achieve maximal expression, His-tagged Sra1 (1-1253) and Nap1 (1-1128) were separately overexpressed in sf9 cells and Hi5 cells, respectively. Cells were lysed together and the Sra1:Nap1 complex was partially purified by a Ni-affinity column. For miniWRC, WAVE1-186VCA (WAVE1(1-186)-(GlyGlySer)\textsubscript{6}-VCA(485-559)), Abi2 (1-158) and full-length HSPC300 (1-75) were expressed separately in \textit{E. coli} as MBP-fusion proteins, purified with Amylose-affinity chromatography and assembled into the trimer subcomplex by incubation in the presence of 1\% NP-40 (Sigma) for 48 hours. The trimer was isolated using Source 15Q chromatography, and then mixed with the Sra1:Nap1 dimer at a 1:1.5 molar ratio overnight on ice to form the pentameric complex. Excess Sra1:Nap1 dimer was removed by an Amylose-affinity column. The eluted complex was further purified using Mono Q chromatography. The fusion tags were removed by treatment with TEV protease at room temperature for 4-6 hours. The sample was brought to homogeneity by a Superdex200 column equilibrated with protein buffer (200 mM NaCl, 12\% glycerol (w/v), 10 mM Tris-HCl, 5 mM DTT, pH 8.0), concentrated to \(\sim\)10 mg ml\(^{-1}\) and stored at -80 \(^\circ\)C. Selenomethionine labeled Sra1 and Nap1 were obtained by culturing insect cells in methionine free medium according to standard protocols (Expression Systems). \(\Delta WRC\) and \(\Delta 154_{WAVE1}\)WRC were produced the same as miniWRC, but contained WAVE1(1-186) and WAVE1(1-154), respectively.

**Crystallization**

Crystals were grown at 4 \(^\circ\)C by hanging-drop vapour diffusion methods. The native miniWRC crystals grew from 10 \% (w/v) glycerol, 4 \% PEG 10,000, 12-20 \% PEG 300, 100 mM Tris-HCl, 2 mM TCEP, 2 mM EDTA, pH 8.5 with protein to reservoir volume
ratio 1:1.8. Crystals form in space group $P2_12_12_1$, with $a = 103.5$ Å, $b = 113.8$ Å, $c = 323.0$ Å, and diffracted only to 4.2 Å. Extensive optimization failed to improve crystal quality. The crystals with selenomethionine labeled Sra1 and Nap1 grew from similar conditions in the same space group, with $a = 97.0$ Å, $b = 114.0$ Å, $c = 327.2$ Å, and diffracted to 2.3 Å.

**Data Processing and structure solution**

The structure of miniWRC was solved by experimental phasing using data processed with the HKL3000 suite\(^\text{37,39}\) (Supplementary Table 1). The initial phases were obtained by combining data from three crystals, two of which contained Se-labeled Sra1 and the third contained Se-labeled Nap1. There was a significant level of non-isomorphism between crystals. In case of the structure with Se-labeled Sra1, there was an additional component of non-isomorphism due to the breaking of crystal symmetry ($P2_1$ vs. $P2_12_12_1$). The symmetry violation was larger than experimental uncertainty, but still small enough to use the higher symmetry space group ($P2_12_12_1$) in structure solution. So the two data sets for the Sra1-labeled crystals scaled together were used for structure refinement in the $P2_12_12_1$ space group. Radiation induced non-isomorphism present in the collected data sets was corrected with the use of novel procedures\(^\text{40}\). The data were anisotropic, with the best direction diffracting to 2.29 Å. To fully use the data, structure factors were anisotropically sharpened to make the resolution fall-off isotropic. In consequence, the shell-based $R_{\text{merge}}$ values did not have statistical meaning in this case. To properly define the resolution we used an $I/\sigma(I)$ criterion, where $I$ represents averaged intensity after correcting for anisotropicity, and $\sigma(I)$ is defined for each reflection separately. The very small change of $R_{\text{free}}$ at higher resolutions is an indicator that the procedure worked correctly.
After all these corrections, the heavy atom substructure for the averaged Sra1-P2$_1$2$_1$2$_1$ was determined by ShelxD$^{41}$ called within the HKL3000 suite$^{37,39}$. Using initial phases, the positions of heavy atoms for the other data set (Se-labeled Nap1) were determined by searching for peaks in the anomalous difference map. The complete set of heavy atom positions was refined using MLPHARE$^{39}$ with anomalous differences only. Density modification was performed with Parrot$^{42}$, and initial model building with Buccaneer$^{43}$ (Supplementary Table 1). The rest of the model was built manually, using Coot$^{44}$ and Refmac$^{45}$, where experimental phases were used as an additional restraint.

Refinement was performed with Refmac, using the TLSMD server to generate TLS bodies for refinement$^{46}$, and the Molprobity server$^{47}$ to check the validity of the structure. The final refinement is summarized in Supplementary Table 1, which shows that 98.26%, 99.96% and 0.04% of the amino acid residues are in the favored, additional allowed and disallowed region of the Ramachandran plots, respectively.

**Equilibrium dialysis**

Equilibrium dialysis was performed at room temperature using a fast micro-equilibrium dialyzer (Harvard Apparatus). Rac1 (Q61L) was charged with GMP-PNP in pH 7.5 buffer containing 20 mM Tris-HCl, 150 mM NaCl, 14% (w/v) glycerol, 2 mM MgCl$_2$. WRC constructs were used at 4 μM. After reaching equilibrium (~ 40 hours), the concentrations of free Rac1 in one chamber and total Rac1 in the other chamber of the dialyzer were analyzed by SDS-PAGE gels, stained with Deep Purple (GE Healthcare) and quantified with ImageGauge (Fujifilm) by comparison to a standard curve generated.
from gels containing known concentrations of Rac1. Non-linear curve fitting to extract $K_D$ was performed using Prism 5. At Rac1 concentrations above ~10 µM, technical limitations including bubble formation, protein instability over an extended dialysis time (required by high solution viscosity) and loss, prevented us from obtaining reproducible data in this regime. Thus, $K_D$ was estimated from the approximate 50% saturation point in the assay.

**Actin polymerization and GST pulldown assays**

Arp2/3 complex mediated actin polymerization assays were performed as described\textsuperscript{13} using 4 µM actin (5% pyrene labeled) and 10 nM Arp2/3 complex in KMEI-20G buffer (20% (w/v) glycerol, 50 mM KCl, 1 mM MgCl$_2$, 1 mM EGTA and 10 mM imidazole pH 7.0). The data in Supplementary Fig. 12 used 1 µM actin (5% pyrene labeled) and 30 nM Arp2/3 complex in buffer lacking glycerol. 5 nM WAVE1-Abi2-HSPC300 trimer was used as the aggregated VCA. Different concentrations of WAVE1 VCA (residues 485-559) were also used.

GST pulldown experiments were performed using 40 µM GST-Rac1 (20 µM for $\Delta$WRC and $\Delta$t54-WRC comparison), 1.5 µM WRC constructs and 60 µL glutathione sepharose 4B resin in 0.2 ml pulldown buffer (10 mM Na-Hepes pH 7.0, 100 mM NaCl, 2 mM MgCl$_2$, 10% glycerol (w/v) and 2 mM DTT). After gentle mixing at room temperature for 30 min, the resin was spun down, washed three times with 0.4 ml pulldown buffer, and eluted with 30 mM reduced glutathione. The eluted proteins were resolved by SDS-PAGE and visualized with Coomassie blue.
Cell biology studies

HeLa cells were transfected with shVector-YFP control and various shWAVE2/HA-YFP-WAVE2 reconstitution vectors based on established protocols\textsuperscript{25,48}. We used shWAVE2 (GAGAAGAGAAAGCACAGGA), and made shRNA-resistant WAVE2 cDNA (GAaAAaAGgAAaCACAGGA) in order to generate HA-YFP suppression/reconstitution vectors as described\textsuperscript{25}. Transfectants were analyzed 72 hr post transfection by immunoprecipitation or immunofluorescence. Anti-HA affinity matrix and anti-HA-HRP were from Roche. Rabbit anti-Nap1 was generated using a synthetic peptide corresponding to amino acids 1117-1128 of human Nap1. Anti-Sra1/PIR121 was previously described\textsuperscript{47}. Anti-WAVE1 was obtained from Upstate Biotechnology Inc. Alexa Fluor-647 phalloidin was used (Invitrogen) to stain the actin filament. Images were obtained with an LSM-710 laser scanning confocal microscope (Carl Zeiss) and analyzed for the presence of lamellipodia formation. For quantification, greater than 200 cells for each transfected cell population, in at least three independent experiments, were blindly scored.

Electron microscopy

For negative staining electron microscopy, 4 µl of protein solution (10 µg ml\textsuperscript{-1}) was applied to glow discharged carbon coated 300-mesh Cu/Rh grids (Emsdiasum) and incubated for 30-60 s. Excess solution was blotted off with filter paper (Whatman #1), the grid was washed with 4 µl of protein buffer and stained with 2% uranyl acetate. Grids were imaged under low dose conditions (10-25 electrons/Å\textsuperscript{2}) on a FEI Tecnai G2 Spirit BioTwin electron microscope (FEI Co.) with a LaB6 filament operated at 120 kV at a nominal magnification of 30,000X. Images were recorded with a Gatan 2048x2048-pixel CCD camera (Gatan, Inc.) using 0.8–2.5 µm underfocus, with a final resolution of 3.63
Å/pixel on the object. Particles were picked manually using the boxer application in EMAN\textsuperscript{49}, normalized, and filtered to 22 Å. 10 class averages were generated using 9 iterations of reference-free classification (\textit{refine2d.py}), using a common reference to orient the classes to show the "upright" view. Classes with fewer than 8 particles were discarded automatically after each iteration.

**Calculation of surface conservation**

The conservation score were calculated using the Consurf Sever\textsuperscript{50}. Increasing conservation (scored from 1 to 9) was color coded in the figures by the spectra of white-to-green and white-to-cyan for Sra1 and Nap1, respectively. Residues scored 7-9, green in Sra1 and cyan in Nap1, are considered to be conserved, which typically have a single amino acid in ~80% of the sequences we examined. The sequences of Sra1 orthologs used in the calculation are: NP\textunderscore 055423.1(Hs\_Sra1), AAH72814.1, AAU05773.1, NP\textunderscore 974801.2, XP\textunderscore 001790637.1, EEN67132.1, NP\textunderscore 499949.2, Q6UK63.1, NP\textunderscore 650447.1, NP\textunderscore 997924.1, CAQ17050.1, NP\textunderscore 035500.2, XP\textunderscore 001379666.1, XP\textunderscore 001745727.1, EDO41734.1, NP\textunderscore 001048941.1, XP\textunderscore 001753041.1, XP\textunderscore 002468523.1, XP\textunderscore 002198076.1, EDV20545.1 and XP\textunderscore 002268225.1. The sequences of Nap1 orthologs used are: NP\textunderscore 038464.1(Hs\_Nap1), NP\textunderscore 181056.2, ABN04850.1, NP\textunderscore 001062406.1, EDQ74364.1, XP\textunderscore 001369085.1, XP\textunderscore 001232275.1, NP\textunderscore 058661.1, NP\textunderscore 001087969.1, XP\textunderscore 690388.2, CAA56333.1, NP\textunderscore 001137348.1, XP\textunderscore 002221623.1, NP\textunderscore 524214.1, XP\textunderscore 971119.1, EEC12517.1, XP\textunderscore 001184421.1, EDO45022.1, NP\textunderscore 502368.1, CAZ27842.1, XP\textunderscore 644083.1 and EAY21283.1.

**Total internal reflection fluorescence (TIRF) microscopy**
Actin (1 µM) was polymerized in the presence of Arp2/3 complex (10 nM), N-WASP VCA (0.1 µM) or active L841A/F844A/W845A_Sra1-miniWRC (0.1 µM) in KMEI buffer for 20 min, before adding Alexa-488-phalloidin (1:50 dilution). As a negative control, actin (1 µM) alone was polymerized for 1 hr. Alexa 488-phalloidin-bound filaments were diluted 1000-fold before adsorbing onto poly-D-lysine-coated glass-bottom dishes (Ted Pella) for 10 min. Filaments were imaged using a laser-based TIRF microscope (Olympus IX-71 base microscope), Micro-Manager 1.3 (Vale lab), and a Photometrics Cascade II 512 EMCCD camera.

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