SUPPLEMENTARY FIGURES LEGENDS

Figure S1. Contribution of the membrane-interacting region in Rac1 to lamellipodia formation. (A) Cell morphologies and lamellipodial phenotypes of Rac1/2/3 KO cells (clone#1) transfected with myc-tagged Rac1L61 variants and stained for myc and the actin cytoskeleton by phalloidin. (B) Quantification of lamellipodial phenotypes performed as described for Figure 1C. n gives number of cells analysed, columns correspond to arithmetic means ± SEM from at least three independent experiments. Statistical significance was assessed for differences between the percentages of cells with "no lamellipodia" phenotype. *$p<0.05$ (two-sample, two-sided t-test). (C) Life cell imaging of Sra-1/PIR121 KO cells (clone #3) re-expressing indicated EGFP-tagged Sra-1 constructs. (D) Kymograph analysis showing membrane protrusion induced by respective construct. (E) Structural cartoon of the Sra-1 construct used in D and mediating assembly into a constitutively active WRC that lacks functional Rac binding sites (see also [7]). (F) Quantification of protrusion velocity of (C). n gives the number of cells analysed. Box and whisker plots represent data as follows: boxes correspond to 50% of all data points (25%–75%), and whiskers to 100% (0%–100%). Lines and red numbers in boxes correspond to medians. Statistical significance was assessed by nonparametric, Mann-Whitney-Rank-Sum test, *$p<0.05$.

Figure S2. Comparison of binding interfaces of small GTPases to the D site of Sra-1. (A) Quantification of lamellipodial phenotypes, done as described for Figure 1C. n gives number of cells analysed, data are arithmetic means ± SEM from at least three independent experiments. Statistical significance was assessed for differences between percentages of cells with "no lamellipodia" phenotype. ***$p<0.001$ (two-sample, two-sided t-test). (B) Sequence alignment of human GTPases. Rectangles mark amino acids that contact the D site of Sra-1. Sequence identity to Rac1 is indicated by green, difference by red colouring of respective amino acids. (C) Surface electrostatic potential of small GTPases capable of contacting the D site of Sra-1. Structures of GTPases were superimposed with Rac1 occupying the D site. Key residues in Sra-1 contacting Rac1 are shown as sticks. Differences in surface electrostatic potentials in the putative

binding interfaces or steric conflicts are encircled. Such differences seen in RhoA likely abolished potential responses comparable to RhoG or Cdc42 (see Figure 3). Note that the differences in Ras were so significant (7 circles in total) that we did not consider the latter to be of any relevance for WRC-driven lamellipodia formation in Rac-deficient cells.