Supplementary Figure 1 Detailed confocal images for experiments described in the main manuscript.

a

(a, b) Comparison of Lyn and Src N-terminal sequences in targeting protein constructs to the plasma membrane. In (a), NIH3T3 cells were transfected with Lyn11-CFP and Src13-YFP and imaged by confocal microscopy. In (b), RBL cells were transfected with Lyn11-FKBP2-CFP and Src13-FKBP2, stained using anti-HA antibody, labeled with Alexa594 (Covance) and imaged by confocal microscopy (Axiovert 100M, LSM 5 PASCAL, Zeiss; the HA tag was inserted after the second FKBP of the constructs). (c, d and e) Filopodia versus lamelipodia: Confocal fluorescence images of NIH3T3 cells expressing LDR and constitutively active Rac1 fused to CFP. The three figure panels show co-expression of these 2 constructs together with YF-Rac1DN (c), YF (d), or YF-Rac1DN combined with the dominant negative form of Cdc42 fused to CFP (e). Series of images were taken before and after addition of 5-mM iRap. Note that filopodia extend out from an edge of the lamellipodia after YF-Rac1DN translocates to the plasma membrane (inset images).

Lyn₁₁ b Lyn₁₁ Lyn₁₁ Src₁₃ Src₁₃ Src₁₃

C 5 min 10 min 15 min 20 min +iRap YF-Rac1_{DN} CFP-Rac1_{CA} d 15 min +iRap 5 min 10 min 20 min YF

CFP-Rac1_{CA}

е

YF-Rac1_{DN}

CFP-Rac1_{CA}

