Supplemental Materials

Molecular Biology of the Cell

Cheng and Mullins

Supplementary Figure 1. Generation of monoclonal VASP-eYFP B16F1 mouse melanoma cell line. (A) Dot plot from fluorescence activated cell sorting (FACS) of B16F1 cells transfected with Cas9/VASP-specific sgRNA and C-terminal eYFP tag repair template compared to WT B16F1 cells. (B) Endogenously tagged VASP-eYFP B16F1 cells display normal migratory behavior on laminin coated glass. Cell polarization leads to formation of a leading edge with correct VASP localization at lamellipodia, filopodia, and focal adhesions (Yellow arrowheads). Over time, the cell creates mature focal adhesions which is followed by new lamellipod formation and migration in a new direction. (C) The number of VASP clusters per cell in endogenously tagged VASP-eYFP cells or overexpressed GFP-VASP were calculated by counting the number of VASP clusters per 160 second time window using a Z-projection of the movie stack for easy visualization.

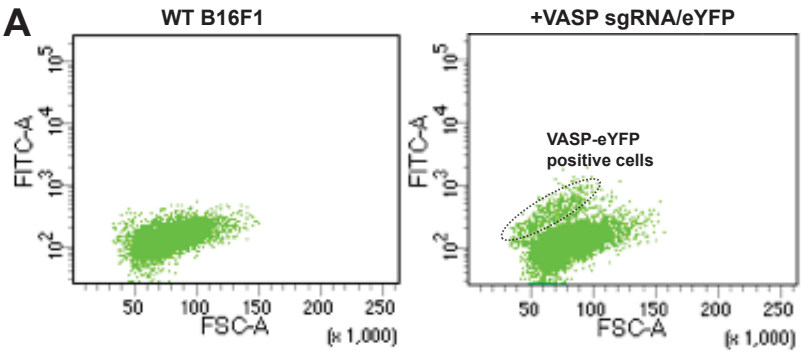
Supplementary Figure 2. Distinct dynamics within VASP/lamellipodin clusters compared to the entire leading-edge. (A) The ratio of VASP to lamellipodin intensities within clusters were compared to the ratio of VASP to lamellipodin intensities along every point on the leading edge of B16F1 cells. (B) Quantification of lateral skating movement of VASP/Lpd clusters (30 cluster tracks) vs. clusters that only contain Lpd (23 cluster tracks) along the leading edge. The starting position was normalized to begin at the same point for all tracks.

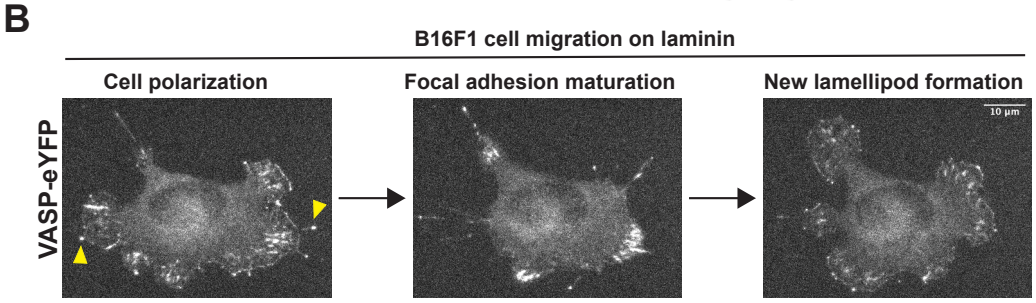
Supplementary Figure 3. Localization of endogenous and overexpressed IRSp53. (A) Immunofluorescence of endogenous IRSp53 (red) in VASP-eYFP (green) tagged B16F1 cells. Arrowheads: VASP-eYFP foci; yellow bar: filopodia shaft. (B) Verification of double CRISPR knock-in VASP-eYFP/IRSp53-tdTomato B16F1 cells by immunostaining with a polyclonal IRSp53 antibody and secondary Alexa 647 antibody. (C) Localization of overexpressed, N-terminally tagged mRuby2-IRSp53 in the context of the VASP-eYFP B16F1 CRISPR knock-in cell line. Inset: two zoomed examples of VASP-eYFP and mRuby2-IRSp53 localization in filopodia.

Supplementary Figure 4. Further characterization of size-dependent splitting of VASP/Lpd clusters at the leading edge. (A) Two additional examples of VASP/Lpd cluster splitting events.

Insets represent frames capturing the splitting event with white arrowhead tracking the chunk of VASP that is left behind in the cytoplasm upon splitting. (B) Alternative visualization strategy of the size-dependency of VASP/Lpd cluster instability. The X axis represents the time-averaged cluster size over a 160 second time window given by the integrated fluorescence intensity of either VASP-eYFP (left subplot) or Lpd-tdTomato (right subplot). The Y axis represents the relative deviations of the cluster size at every time point over the 160 second imaging window (Δ cluster size = time-averaged cluster size - cluster size(t).

Figure S1


A
WT B16F1
+VASP sgRNA/eYFP
FITC-A
FSC-A
(× 1,000)
VASP-eYFP
positive cells


B
B16F1 cell migration on laminin
Cell polarization
Focal adhesion maturation
New lamellipod formation
VASP-eYFP
10 µm

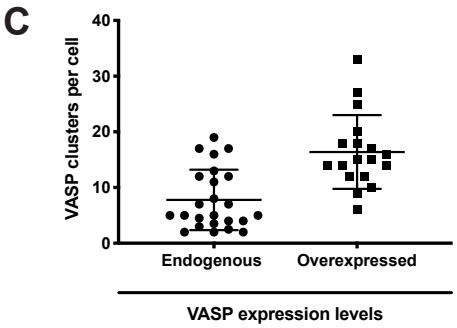

C
VASP clusters per cell
Endogenous
Overexpressed
VASP expression levels

Figure S2

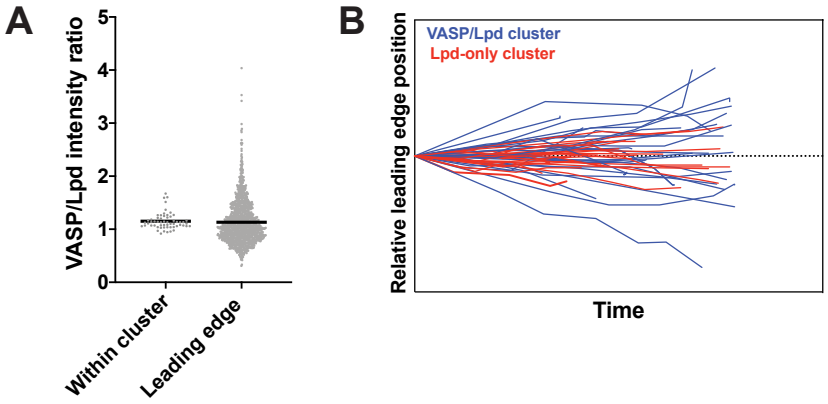

A
VASP/Lpd intensity ratio
0
1
2
3
4
5
Within cluster
Leading edge
B
VASP/Lpd cluster
Lpd-only cluster
Relative leading edge position
Time

Figure S3

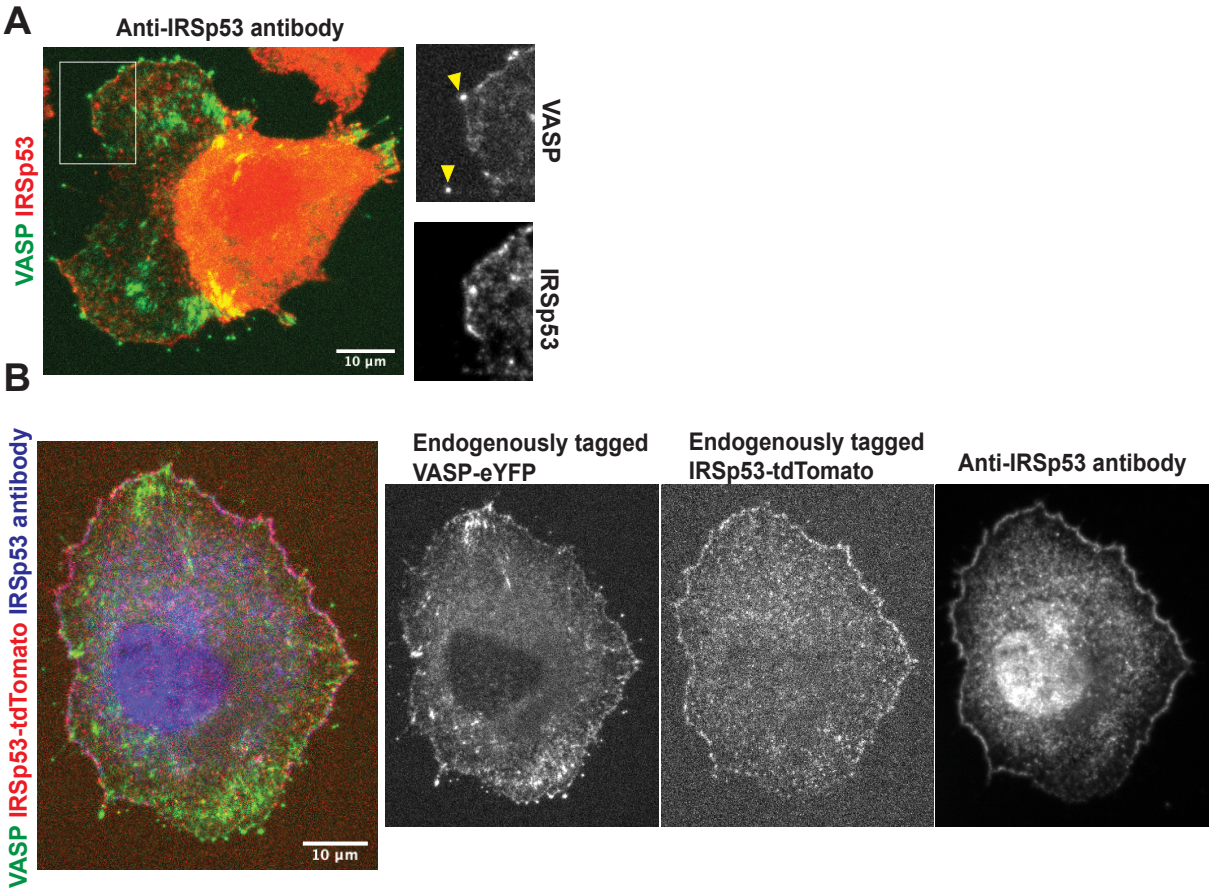
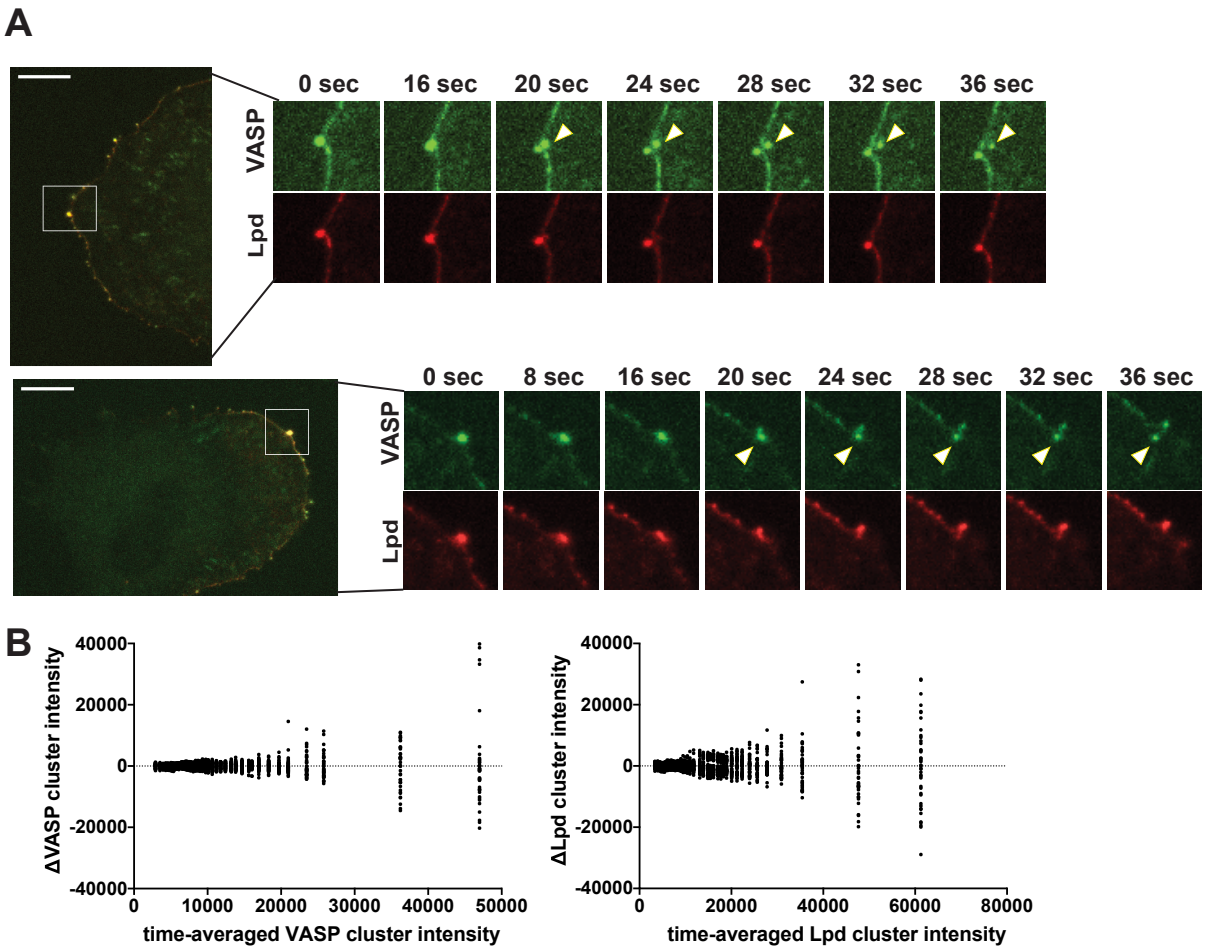

A
Anti-IRSp53 antibody
VASP IRSp53
10 µm
VASP
IRSp53
B
VASP IRSp53-tdTomato IRSp53 antibody
10 µm
Endogenously tagged
VASP-eYFP
Endogenously tagged
IRSp53-tdTomato
Anti-IRSp53 antibody
C
VASP mRuby2-IRSp53
1
2
10 µm
Endogenously tagged
VASP-eYFP
overexpressed
mRuby2-IRSp53
merged
1
2
5 µm

Figure S4


A
0 sec
16 sec
20 sec
24 sec
28 sec
32 sec
36 sec
VASP
Lpd
0 sec
8 sec
16 sec
20 sec
24 sec
28 sec
32 sec
36 sec
VASP
Lpd
B
ΔVASP cluster intensity
40000
20000
0
-20000
-40000
0
10000
20000
30000
40000
50000
time-averaged VASP cluster intensity
ΔLpd cluster intensity
40000
20000
0
-20000
-40000
0
20000
40000
60000
80000
time-averaged Lpd cluster intensity