Law et al., http://www.jcb.org/cgi/content/full/jcb.201304051/DC1

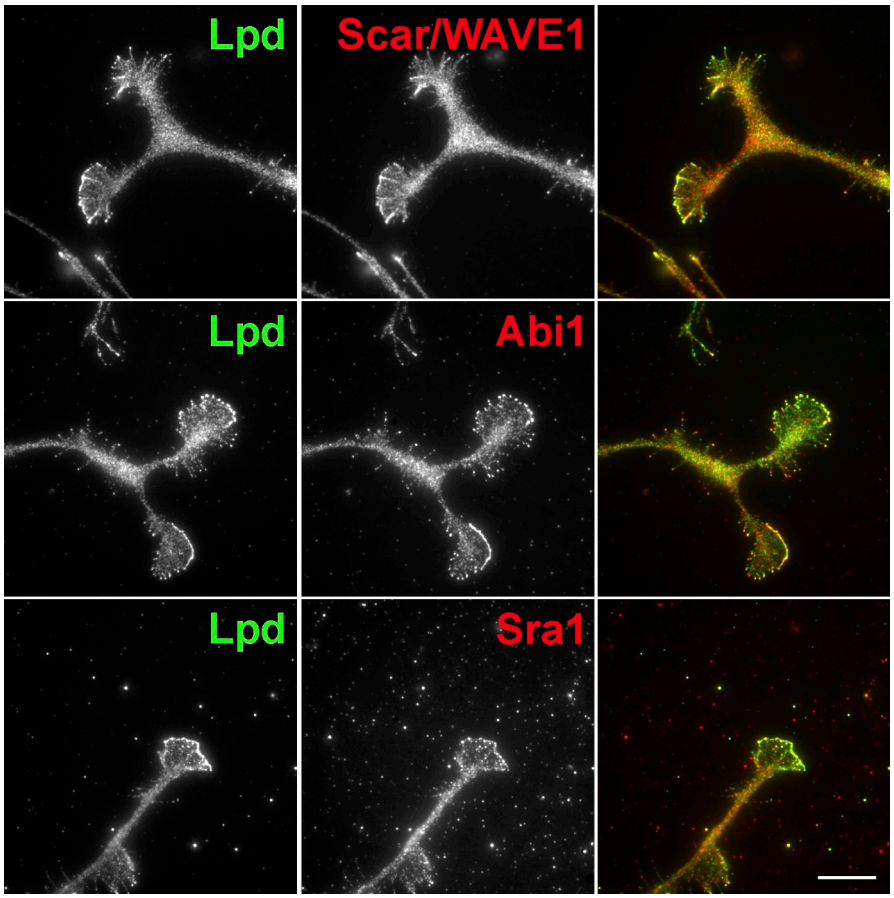


Figure S1. **Lamellipodin colocalizes with the Scar/WAVE complex at the leading edge of cells.** Endogenous Lpd (green) colocalizes with Scar/WAVE1, Abi1, and Sra1 (red) at the very edge of lamellipodia in CAD mouse neuronal cells. Bar, 25 µm.

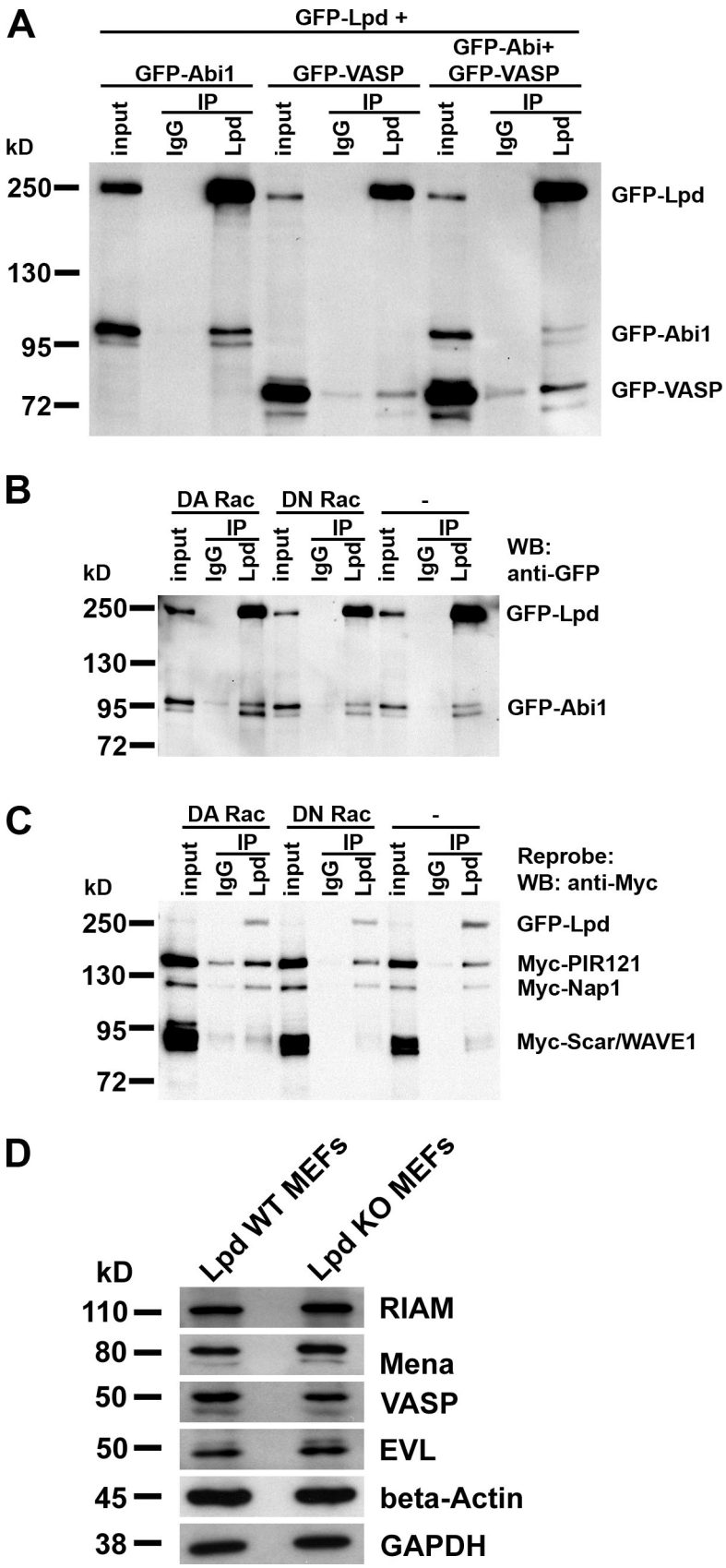


Figure S2. **Lpd is in complex with both Abi and VASP, the interaction between Lpd and the Scar/WAVE complex is positively regulated by active Rac, and expression of RIAM and Ena/VASP proteins is not changed in Lpd KO MEFs.** (A) Lpd forms a complex with both Abi and VASP. Immunoprecipitation using Lpd-specific antibodies or IgG control from HEK293 cell lysates expressing GFP-Lpd and GFP-Abi1 (left), GFP-Lpd and GFP-VASP (middle), or GFP-Lpd, GFP-Abi1, and GFP-VASP (right) show coimmunoprecipitation between Lpd and both GFP-Abi and GFP-VASP. Western blot: anti-GFP. (B) The interaction between Lpd and the Scar/WAVE complex is positively regulated by active Rac. Immunoprecipitation using Lpd-specific antibodies or IgG control from HEK293 cell lysates expressing GFP-Lpd, Myc-PIR121, Myc-Nap1, Myc-Scar/WAVE1, Myc-HSPC300, GFP-Abi1, and dominant-active Rac (DA Rac; left), dominant-negative Rac (DN Rac; middle), or empty vector control (right) show increased coimmunoprecipitation between Lpd, GFP-Abi, and Myc-PIR121, Myc-Nap1, and Myc-Scar/WAVE1 only when dominant-active Rac is coexpressed. Western blot: anti-GFP (B) and anti-Myc reprobe (C). Myc-HSPC300 is not shown. (D) Western blot analysis of whole cell lysates of Lpd WT and Lpd KO MEFs using specific anti-RIAM, -Mena, -VASP, and -EVL antibodies. β-Actin and GAPDH antibodies serve as loading controls.

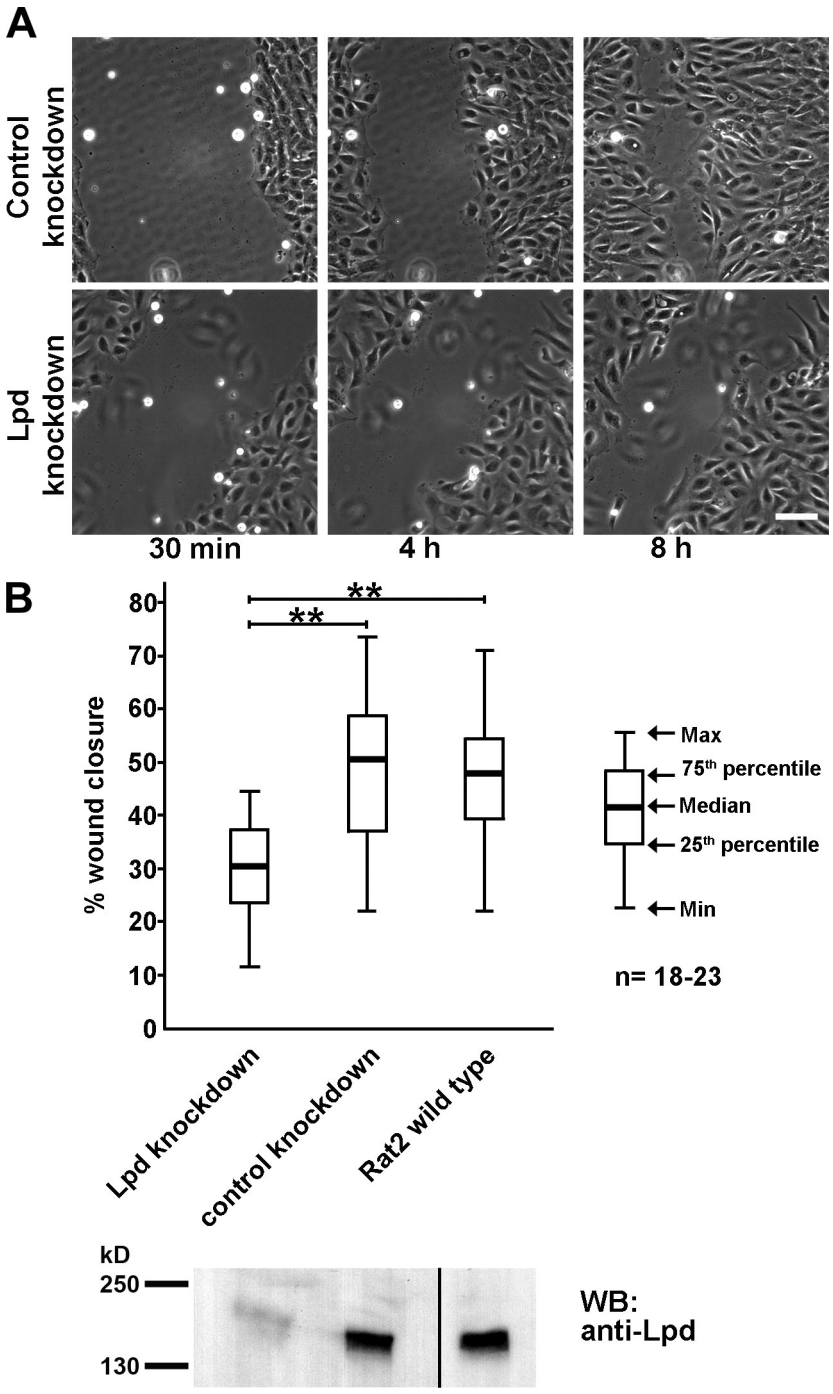


Figure S3. **Lpd regulates cell migration in Rat2 fibroblasts.** (A) A confluent layer of WT, scrambled control shRNA–, or Lpd-specific shRNA–expressing Rat2 fibroblasts was scratched, and the area of the scratch was measured at 0 and 8 h. (B) Area closure is shown as the percentage of wound closure. Values of three independent experiments are shown as box and whisker plots. **, $P \leq 0.01$; one-way ANOVA. Bar, 100 µm.

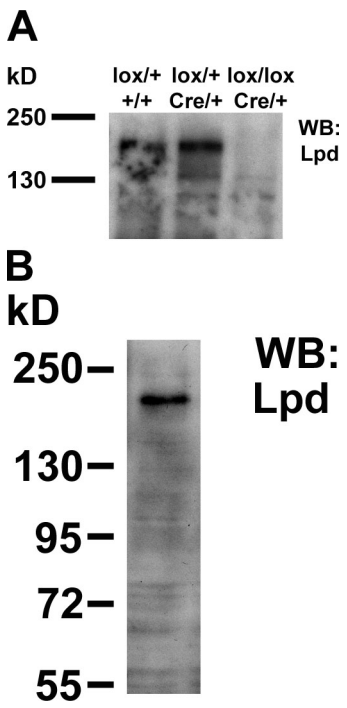


Figure S4. **Lpd is not expressed in Lpd KO mice but is expressed in melanoblasts.** (A) Lpd is not expressed in Lpd KO mice. Western blots of brain tissue lysates from WT β-actin-Cre$^{+/+}$;Lpd$^{flox/+}$, heterozygous β-actin-Cre$^{tg/+}$;Lpd$^{flox/+}$, and homozygous β-actin-Cre$^{tg/+}$;Lpd$^{flox/flox}$ mice showed loss of Lpd protein expression as expected only in the homozygous mice. Western blot: anti-Lpd. (B) Lpd is expressed in melanoblasts. Western blots of lysates from the melanoblast stem cell line melb-a (Sviderskaya et al., 1995) show expression of Lpd. Western blot: anti-Lpd.

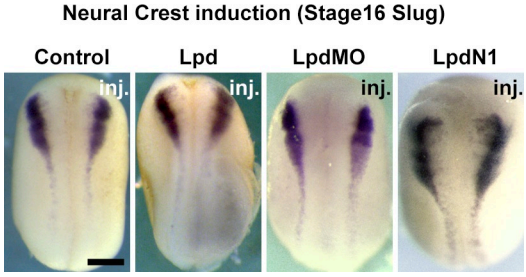
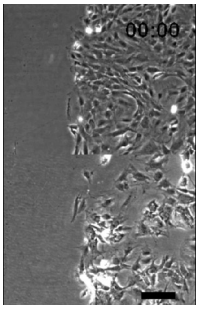
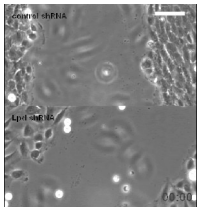


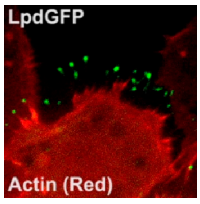
Figure S5. **Lpd does not affect NC induction.** (A) In situ hybridization for Slug (NC induction marker) in control embryos or those injected with Lpd mRNA, Lpd MO (LpdMO), or dominant-negative Lpd N1. No condition affects NC induction.



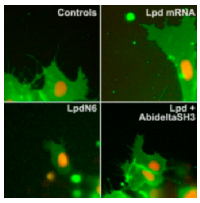
Video 1. **Lamellipodin KO MEFs are impaired in cell migration.** This movie shows WT control MEFs (Lpd WT MEFs; top) and Lpd KO MEFs (Lpd KO MEFs; bottom). Confluent monolayers were scratch-wounded and imaged every 5 min for 24 h by time-lapse phase contrast wide-field microscopy (IX81 [Olympus] with MetaMorph software [Molecular Devices]). Bar, 200 µm.



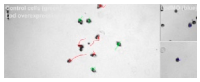
Video 2. **Lamellipodin regulates cell migration.** This movie shows scrambled control shRNA (top) and Lpd-specific shRNA expressing Rat2 fibroblasts. Confluent monolayers were scratch-wounded and imaged every 5 min for 8 h by time-lapse phase contrast wide-field microscopy (IX81 [Olympus] with MetaMorph software [Molecular Devices]). Note that the Lpd knockdown Rat2 cells show slow residual cell migration by extending filopodia. Bar, 100 µm.



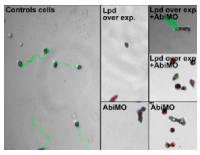
Video 3. **Localization of Lpd-GFP in *Xenopus* NC cells.** This movie shows the localization of Lpd-GFP in *Xenopus* NC cells coinjected with Life-Act-mCherry. Note that Lpd localizes at the tip of cell protrusions (lamellipodia and filopodia). Projection of a z stack acquired with a 100× objective lens on a Elmer spinning disk confocal [PerkinElmer] inverted IX81 microscope Olympus controlled by Volocity. Cells were imaged every 30 s for 5 min. The same sequence is looped three times.



Video 4. **Lpd regulates *Xenopus* NC cell lamellipodia via Scar/WAVE.** Examples of representative cell protrusions in control *Xenopus* NC cells (top left), NC cells overexpressing Lpd (top right), NC cells expressing Lpd dominant-negative N6 (bottom left), and NC cells coinjected with Lpd mRNA and Abi dominant-negative Abi-Δ-SH3 (bottom right). Note that Lpd overexpression promotes the formation of large protrusions. This effect is abolished by the coinjection of Abi dominant-negative. Cells were imaged every minute for 40 min using a 63× water-immersion objective lens on an upright microscope (Leica) equipped with a camera (Hamamatsu Photonics).



Video 5. **Lpd regulates cell migration in *Xenopus* NC cells.** This movie shows representative examples of control *Xenopus* NC cells (green tracks), NC cells overexpressing Lpd (red), and NC cells injected with Lpd MO (blue). Cells were imaged every 3 min for 3 h using a 10× objective on an inverted microscope (Axiovert; Carl Zeiss) equipped with a camera (Hamamatsu Photonics).



Video 6. **Lpd regulates *Xenopus* NC cell migration via Abi.** This movie shows representative examples of control *Xenopus* NC cells (left), NC overexpressing Lpd (top middle), NC cells injected with Abi MO (middle and right bottom), or cells coinjected with Lpd mRNA and Abi MO (right column, top and middle). Cells were imaged every 3.5 min for 3 h using a 10× objective lens on an inverted microscope (Axiovert; Zeiss) equipped with a camera (Hamamatsu Photonics).

Reference

Sviderskaya, E.V., W.F. Wakeling, and D.C. Bennett. 1995. A cloned, immortal line of murine melanoblasts inducible to differentiate to melanocytes. *Development*. 121:1547–1557.