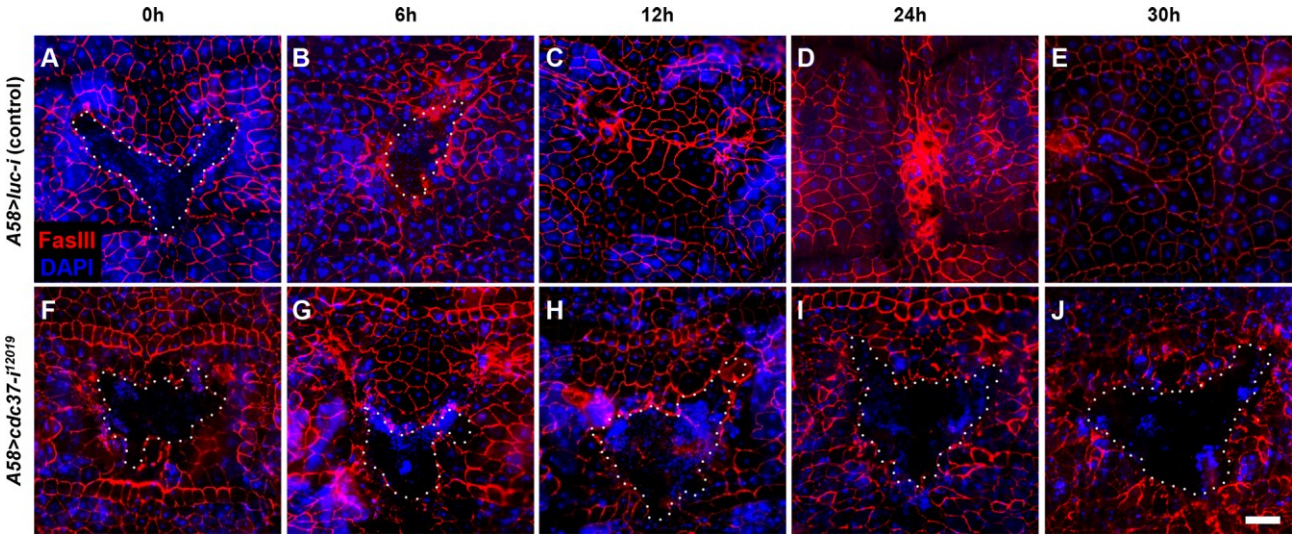
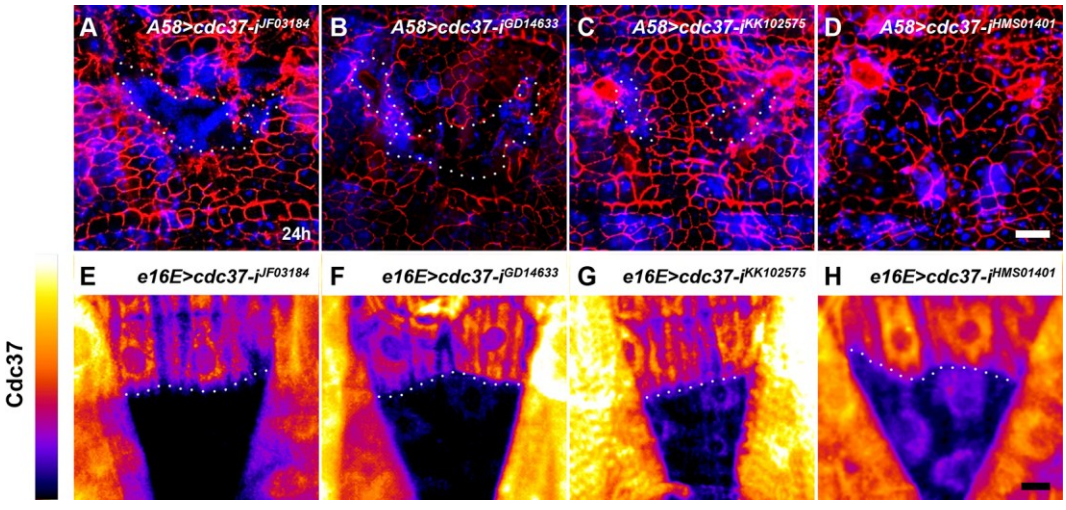
Supplemental Materials

Molecular Biology of the Cell

Lee et al.

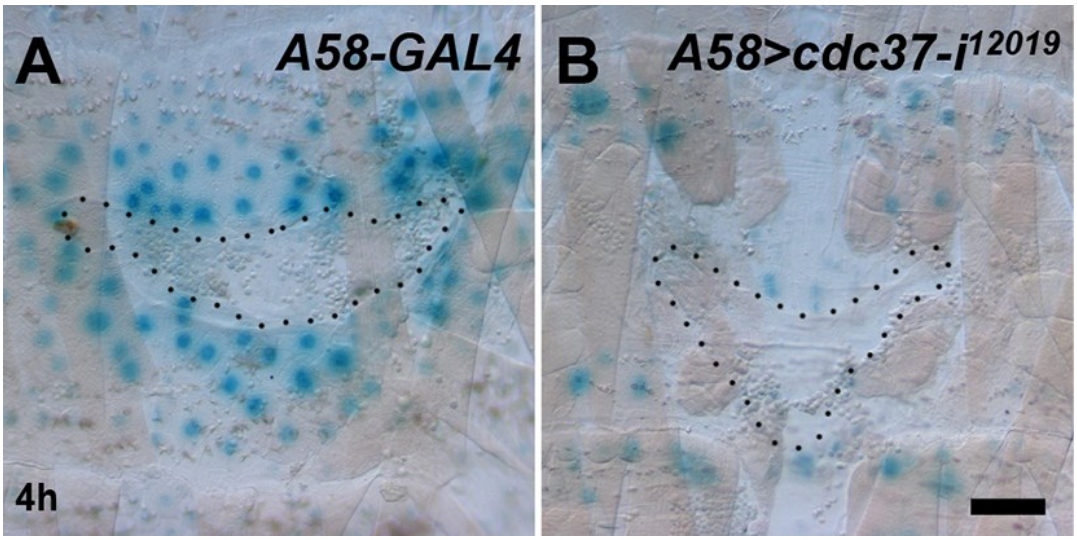


Supplemental Figure S1. Examination of wound closure at different time points. The epidermis of late third instar larvae was examined at the indicated times after injury in A58>luc-i (control; A-E) or A58>cdc37-i (F-J). (A, F) 0 h. (B, G) 6 h. (C, H) 12 h. (D, I) 24 h. (E, J) 30 h. Cell boundaries were stained red with anti-FasIII antibody, and the nuclei were stained blue with DAPI. The white-dotted line indicates the wound margin. The strong blue staining patterns in the wound hole show hemocytes attached to the wound site. Scale bar: 100 μm.

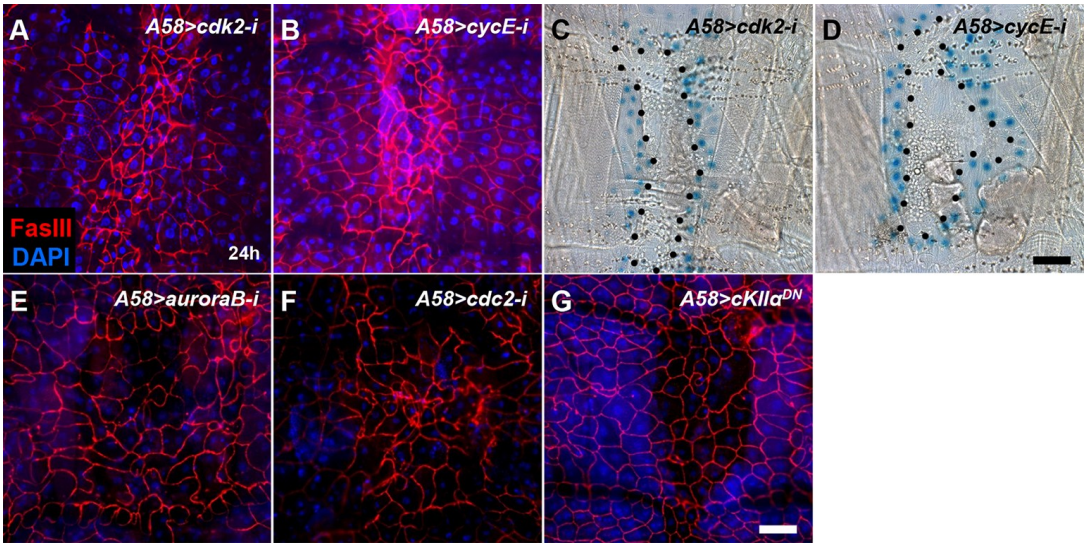


Supplemental Figure S2. Defects in wound healing observed in other cdc37 RNAi lines. (A-D) The open wound phenotype was confirmed in other cdc37 RNAi lines 24 h after injury. The cell boundary was stained red using anti-FasIII antibody, and the nuclei were stained blue with DAPI. The white-dotted line indicates the wound hole. (A) A58>cdc37-iJF03184. (B) A58>cdc37-iGD14633. (C) A58>cdc37-iKK102575. (D) A58>cdc37-iHMS01401. For each genotype, at least eight larvae were examined. (E-H) RNAi knockdown was confirmed by immunostaining with anti-Cdc37 antibody. e16E-GAL4 was active only in the posterior half of each segment, leaving the

anterior half as an internal control. The dotted line indicates the anterior-posterior compartment boundary. Anterior is up. (E) e16E>cdc37-iJF03184. (F) A58>cdc37-iGD14633. (G) A58>cdc37-iKK102575. (H) A58>cdc37-iHMS01401. Scale bar: 100 μm (A-D); 25 μm (E-H)

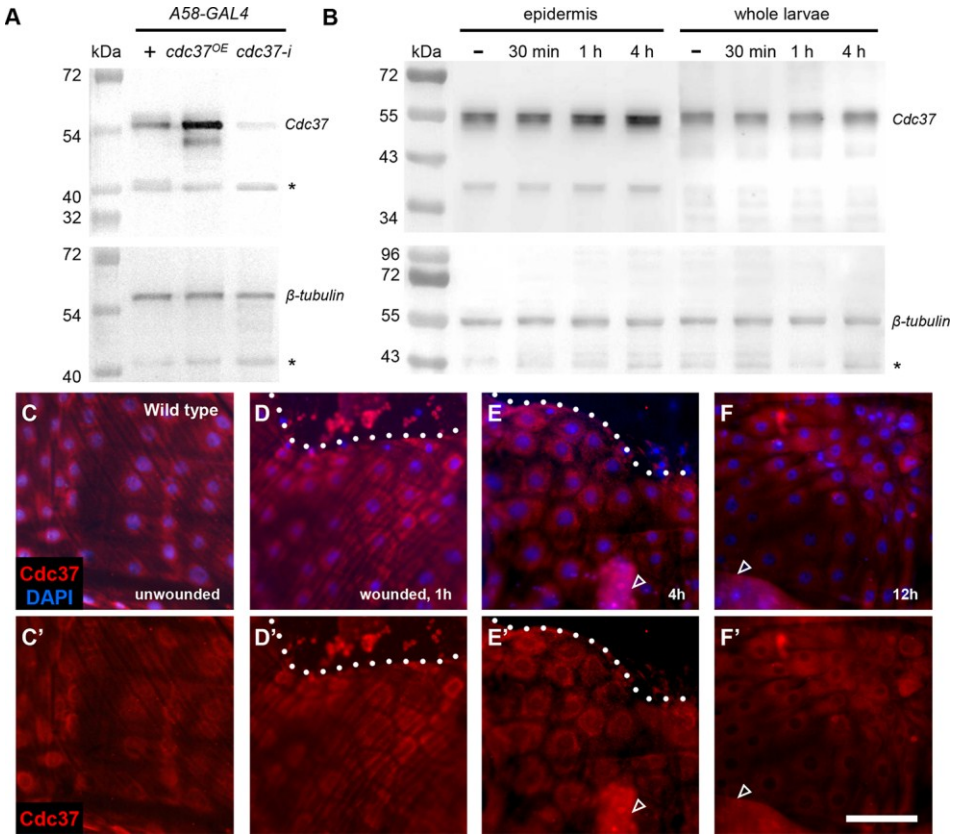


Supplemental Figure S3. Examination of JNK pathway activation via the puc-lacZ reporter at 4 h after injury. β-galactosidase expression was visualized with X-gal staining in blue. The black-dotted line indicates the wound margin. (A) A58-only control. (B) A58>cdc37-i12019. Scale bar: 100 μm.



Supplemental Figure S4. Wound healing phenotypes in various larval genotypes. (A, B, E-G) Wound closure was examined 24 h after injury. Cell boundaries were visualized by immunohistochemistry using anti-FasIII antibody in red, and cell nuclei were stained blue with DAPI. (C, D) Activation of the JNK pathway was examined 4 h after injury using the msn-lacZ

reporter. β-galactosidase expression was visualized by X-gal staining in blue. The black-dotted line indicates the wound margin. (A, C) A58>cdk2-i. (B, D) A58>cycE-i. (E) A58>auroraB-i. (F) A58>cdc2-i. (G) A58>ckIIαDN. Scale bar: 100 μm.



Supplemental Figure S5. Generation of anti-Cdc37 antibody and analysis of Cdc37 protein expression. (A, B) Western blot analysis showing the specificity of a newly generated anti-Cdc37 antibody (A; epidermis) and changes in Cdc37 protein levels after epidermal injury (B; epidermis and whole larvae). β-tubulin was used as a loading control. The asterisks indicate non-specific bands. (C-F') Examination of possible changes in protein levels and distribution of Cdc37 in unwounded (C, C'), or wounded epidermis 1 h (D, D'), 4 h (E, E'), and 12 h (F, F') after injury. The protein levels of Cdc37 appeared slightly increased 4 h after injury, but Cdc37 did not show polar distribution patterns, monitored up to 12 h after injury. Cdc37 was stained red with anti-Cdc37 antibody (Cutforth and Rubin, 1994), and the cell nuclei were stained blue with DAPI. The white-dotted line indicates the wound margin. The arrowheads indicate non-epidermal tissue debris. Scale bar: 100 μm.