

OMTN, Volume 13

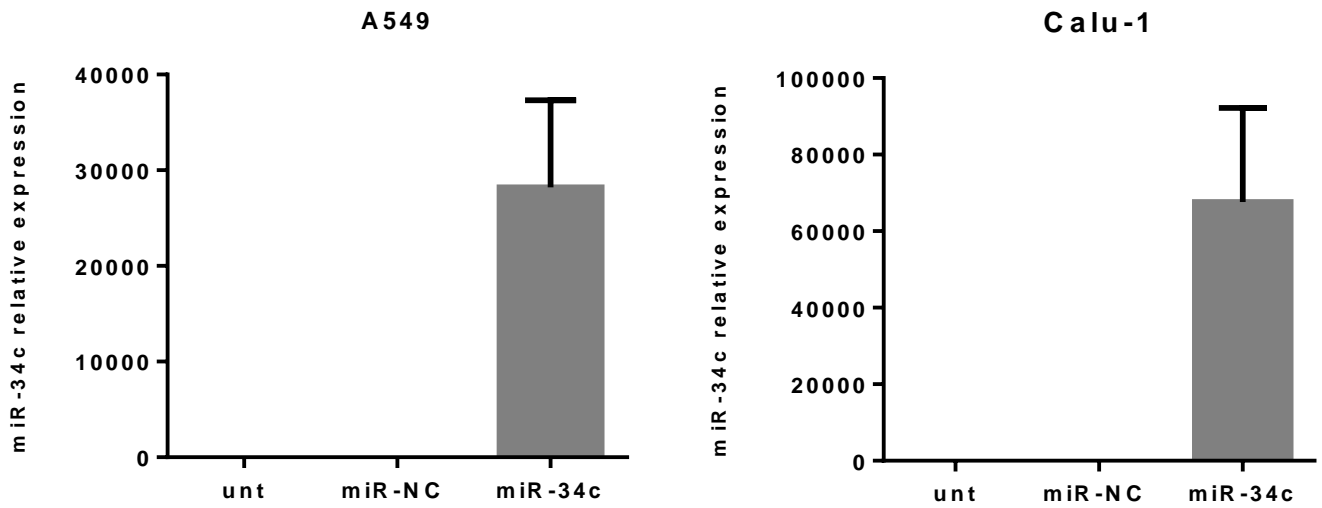
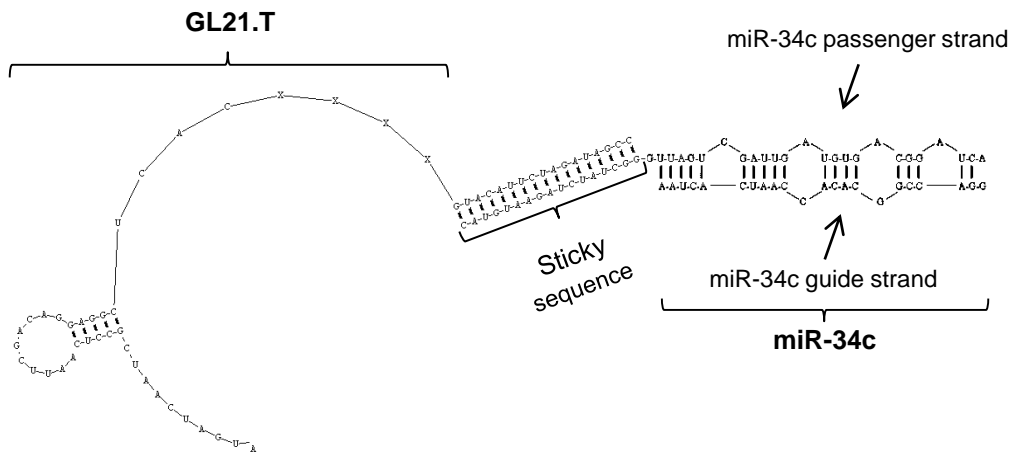
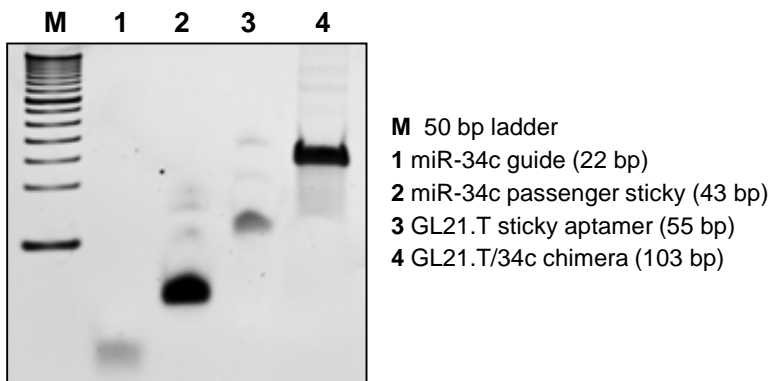
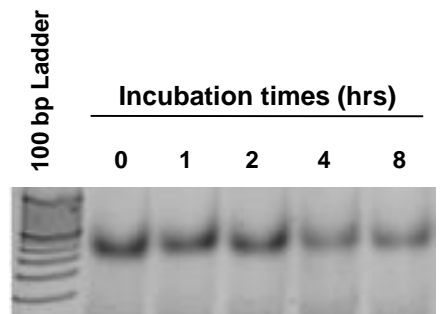
Supplemental Information

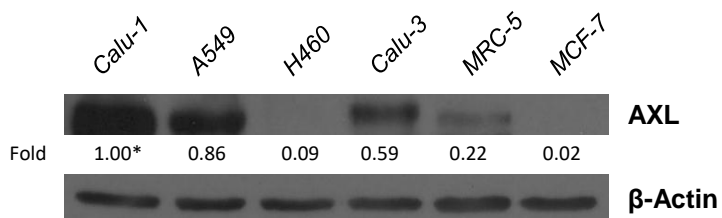
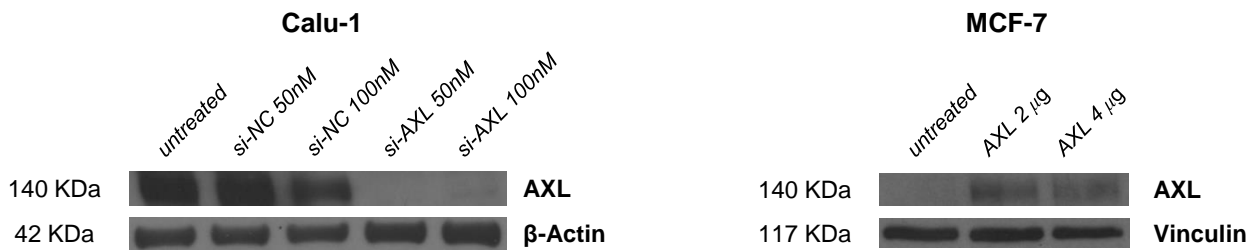
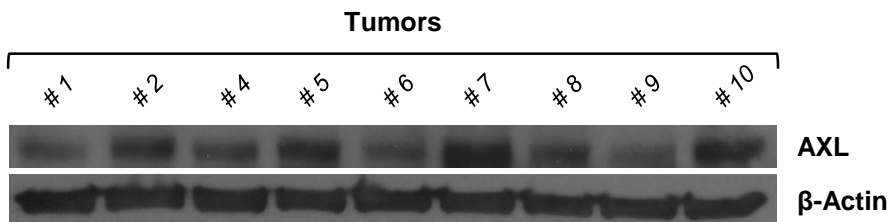
Aptamer-miR-34c Conjugate Affects Cell

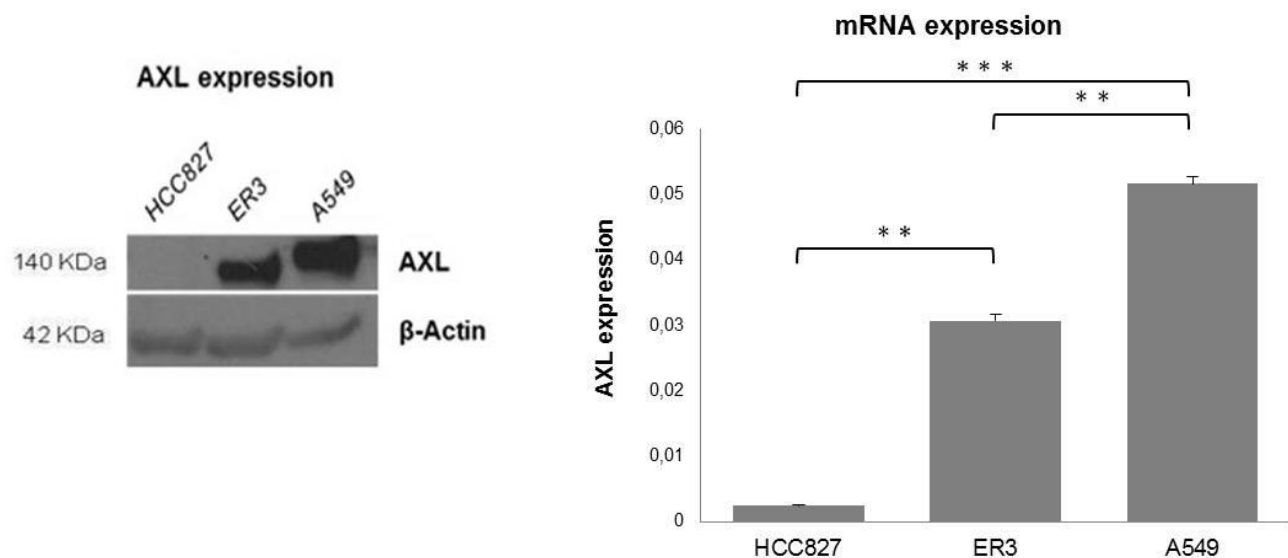
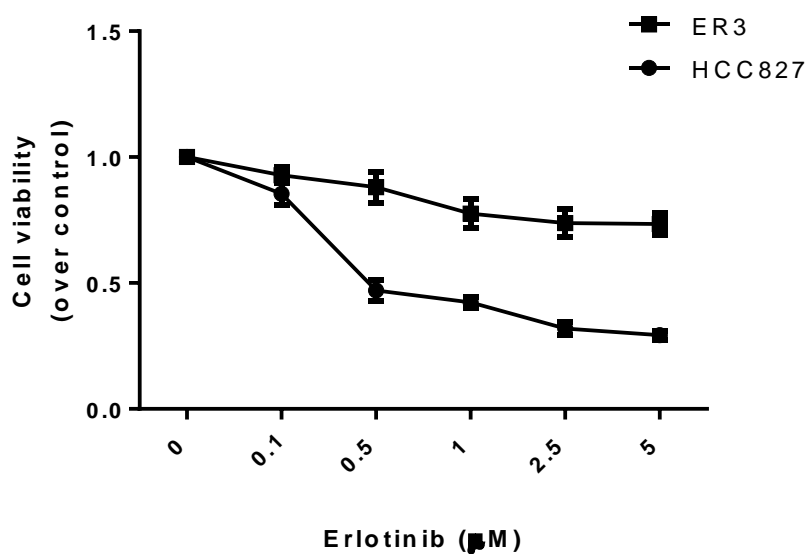
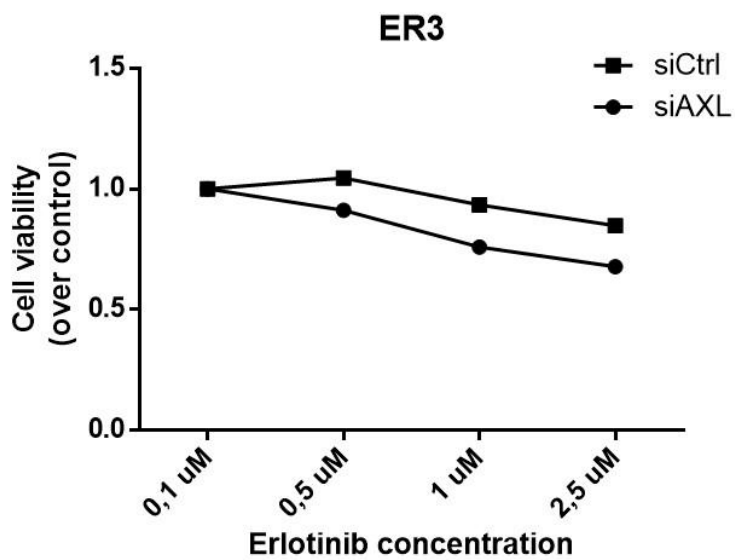
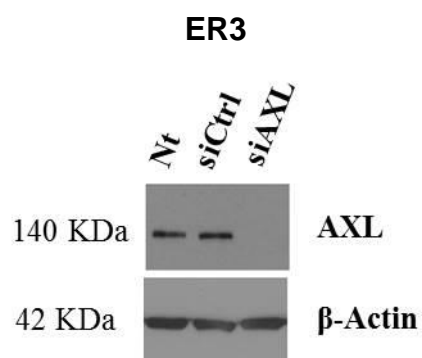
Proliferation of Non-Small-Cell Lung

Cancer Cells

Valentina Russo, Alessia Paciocco, Alessandra Affinito, Giuseppina Roscigno, Danilo Fiore, Francesco Palma, Marco Galasso, Stefano Volinia, Alfonso Fiorelli, Carla Lucia Esposito, Silvia Nuzzo, Giorgio Inghirami, Vittorio de Franciscis, and Gerolama Condorelli

A**B****C****D**

A**B****C**

A**B****C****D**

A

WT-ERL

WT+ERL

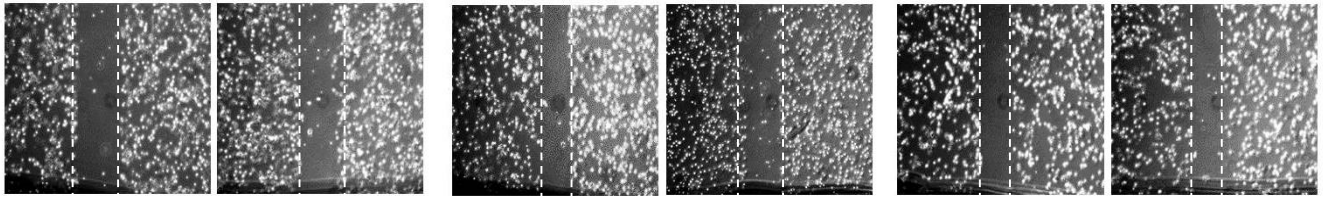
miR-NC -ERL

miR-NC +ERL

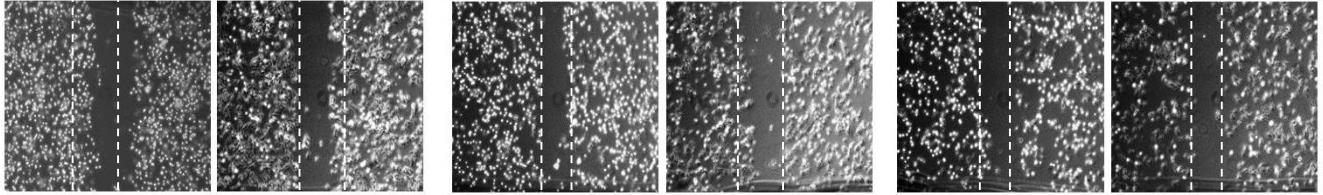
miR34c -ERL

miR34c +ERL

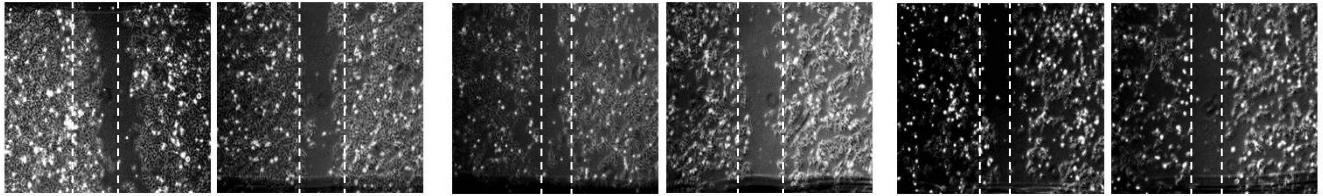
T0



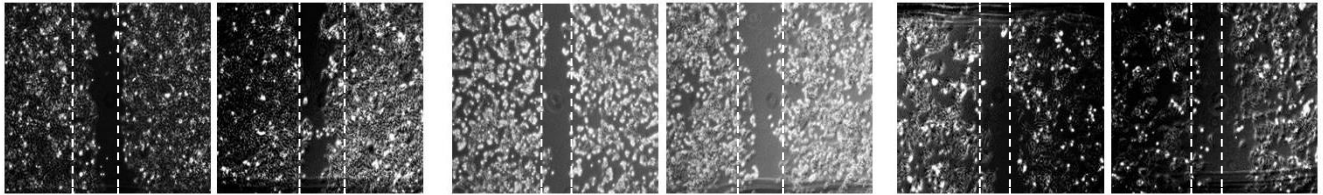
T24h



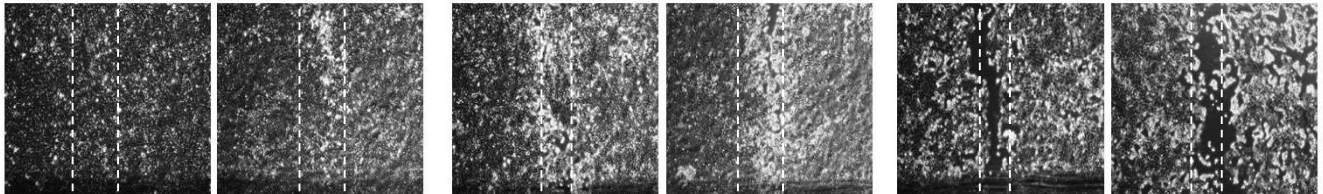
T48h



T72h



T144h



A

WT-ERL

WT+ERL

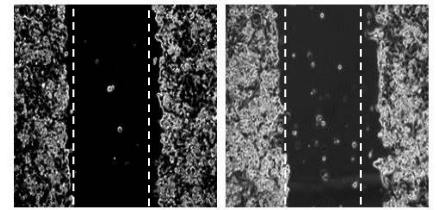
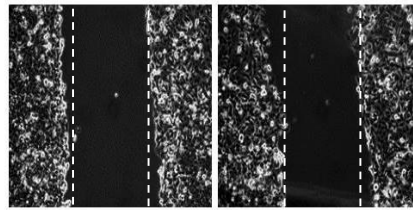
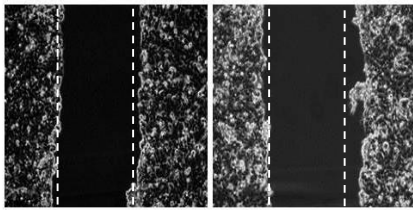
GL21.T-ERL

GL21.T+ERL

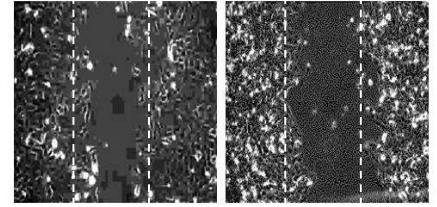
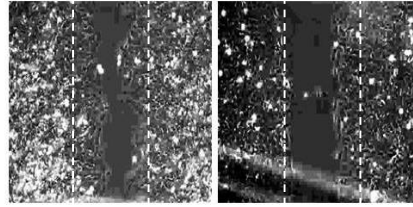
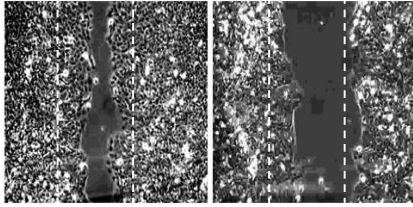
GL21.T-miR34c -ERL

GL21.T-miR34c +ERL

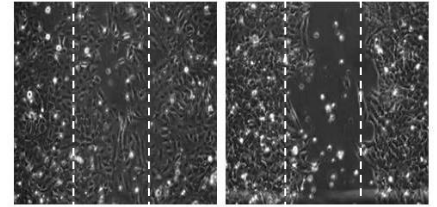
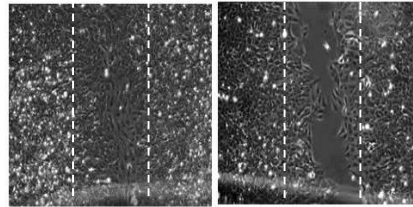
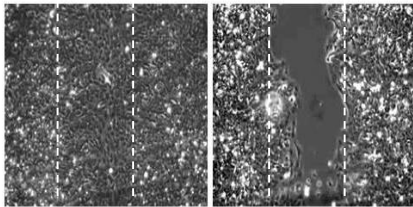
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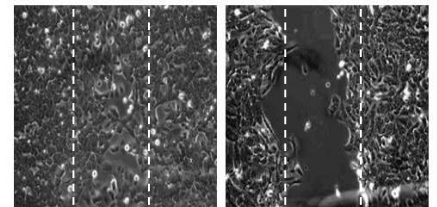
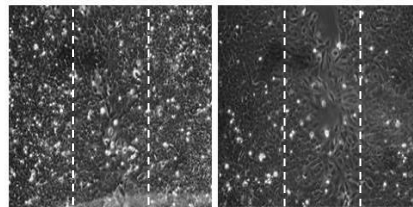
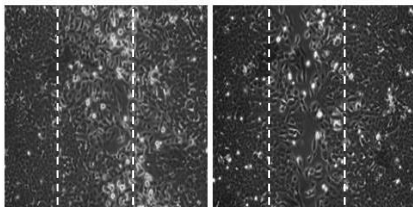
T24h



T48h



T72h



SUPPLEMENTAL Figure 1: *GL21.T-miR-34c design and folding.* **A)** A549 and Calu-1 cells were transfected with miR-NC or miR-34c-3p and, after 72 hrs, miR-34c-3p was quantified by RT-PCR. Bar graphs indicate mean value \pm SD. **B)** Secondary structure prediction of GL21.T-miR-34c conjugate, using RNA structure 5.3 program; **C)** To confirm annealing efficiency, all RNA sequences and annealed GL21.T-miR-34c conjugate were loaded on a 10% non-denaturing polyacrylamide gel visualized after staining with ethidium bromide; **D)** Binding of 200 nM GL21.T- miR-34c on A549 (AXL +). The Binding capability were measured by RT-qPCR and compared to GL21.T alone. **E)** The indicated RNA sequences, treated or untreated with recombinant Dicer, were loaded on a 12% non-denaturing polyacrilamide gel and visualized after staining with ethidium bromide. The arrows indicate bands corresponding to the Dicer cleavage products. **F)** GL21.T-miR34c chimera was incubated with 80% human serum up to 96 hrs. Loss of correct folding and RNA degradation was evaluated by electrophoresis on 10% non-denaturing polyacrylamide gel stained with ethidium bromide.

SUPPLEMENTAL Figure 2: *AXL expression in NSCLC cells.* **A)** AXL protein expression in different cell lines (Calu-1, A549, Calu-3, H460, MRC-5, and MCF-7) was evaluated by Western blot analysis. β -Actin was used as internal control. **B)** AXL protein expression in Calu-1 and MCF-7 cells, respectively transfected with si-AXL (or si-NC) and AXL cDNA. **C)** AXL protein expression in different primary lung cancer cells was evaluated by Western blot analysis. β -Actin was used as internal control.

SUPPLEMENTAL Figure 3: *AXL modulates response to Erlotinib in NSCLC cells.* **A)** AXL protein expression and AXL mRNA level in HCC827, ER3, A549 were analyzed by Western Blot and RT-PCR respectively. **B)** Dose-response curves (0-5 μ M) on HCC827 and ER3 cells viability were measured by MTT assay. Bar graphs indicate mean value \pm SD. **C)** Cell viability assay of ER3

cells transduced with a siRNA control or a siRNA targeting AXL in response to increasing doses of Erlotinib. **D)** AXL knockdown was validated by Western Blot.

SUPPLEMENTAL Figure 4: *miR-34c* affects cell migration in a wound-healing assay. **A)** ER3 cells were transfected with miR-34c-3p for 72 hrs, and then seeded onto 6-well plates at 80–90% confluency. A wound of approximately 1 mm in width was scratched with a 20 μ l pipette tip. Wound closure was monitored at the indicated time intervals and imaged with phase contrast microscopy on an inverted microscope (Olympus 1 \times 51 using a 5 \times phase contrast objective). The migration assay was performed in three independent experiments.

SUPPLEMENTAL Figure 5: *GL21.T/miR-34c* affects cell migration in a wound-healing assay. **A)** ER3 cells were seeded onto 6-well plates at 80–90% confluency and treated for 72 hrs with GL21.T/miR-34c. A wound of approximately 1 mm in width was scratched with a 20 μ l pipette tip. Wound closure was monitored at the indicated time intervals and imaged with phase contrast microscopy on an inverted microscope (Olympus 1 \times 51 using a 10 \times phase contrast objective). The migration assay was performed in three independent experiments.