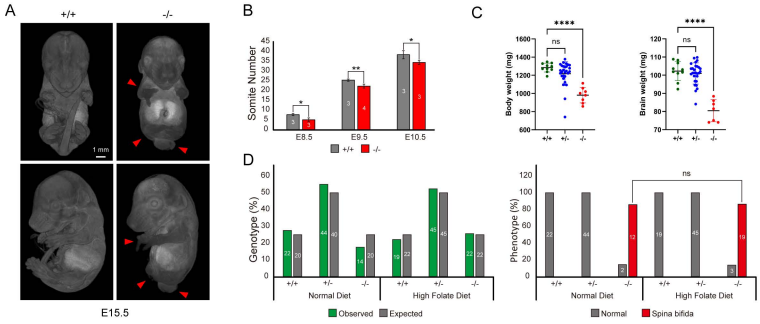


Fig. S1. *Tmem132a* deletion results in spina bifida, limb defects, and developmental delay. (A) Micro CT images of wild type and mutant embryos at E15.5 from https://www.mousephenotype.org/embryoviewer/?mgi=MGI:2147810. Red arrows indicate spina bifida, limb and digit defects in mutant embryos. There was no obvious preference of left or right side when the limb phenotype was unilateral. **(B, C)** Homozygous mutant embryos are developmentally delayed based on somite number in litter-matched embryos at E8.5, E9.5 and E10.5 (**B**, Student`s *t*-test. *$p \leq 0.05$, **$p \leq 0.01$), or body and brain weight at E18.5 **(C,** Student`s *t*-test. ****$p \leq 0.0001$). **(D)** Multi-generational folic acid supplementation in diet does not alter the Mendelian ratios of embryos of each genotype or the incidence of spina bifida due to loss of TMEM132A (assessed at E10.5, *p*-value as calculated by Fisher's exact test; ns=not significant). Numbers of embryos examined is indicated in each column.

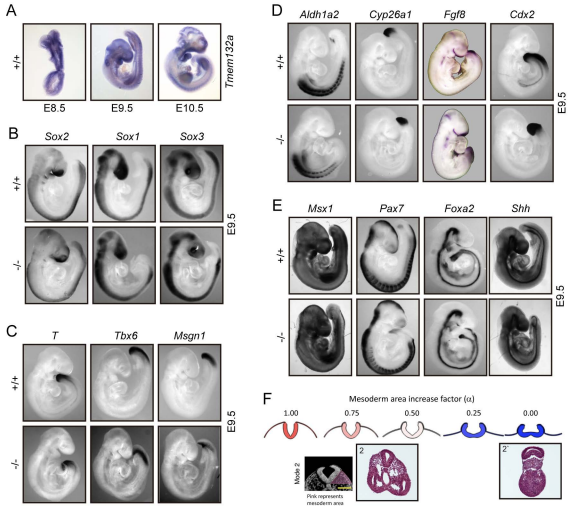


Fig. S2. Assessment of neuromesodermal progenitors (NMPs) and patterning markers. (A) Whole-mount in situ hybridization for *Tmem132a* expression in E8.5, E9.5 and E10.5 wild type embryos. **(B, C)** Whole-mount in situ hybridization showed relatively similar expression in wild type and homozygous mutant embryos at E9.5 for NMPs markers *Sox2* and *T* (*Brachyury*), neural lineage markers *Sox1* and *Sox3*, and mesodermal lineage markers *Tbx6* and *Msgn1,* suggesting no significant change in NMPs differentiation as a result of TMEM132A loss. **(D, E)** Whole-mount in situ hybridization showed relatively similar expression in wild type and mutant embryos at E9.5 of anterior-posterior patterning genes *Aldh1a2, Cyp26a1*, *Fgf8,* and *Cdx2* **(D)** and dorsal-ventral patterning genes *Msx1*, *Pax7*, *Foxa2* and *Shh* **(E)**. (**F**) Top line: modeling the effect of mesoderm expansion on DLHP formation by de Goederen, *et al*. *PNAS*, 2022. Bottom line: (Left) Mode 2 tissue section with pink illustrating determination of mesoderm area by de Goederen, *et al*. *PNAS*, 2022. (Middle & right) Images of wild type (Fig. 2C panel 2) and *Tmem132a* mutant (Fig. 2C panel 2'). Panel 2' shows a mesoderm area increase factor of α=0.

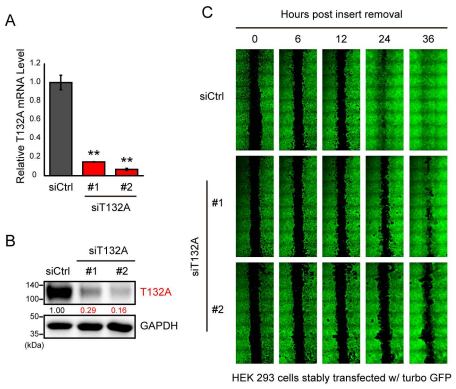


Fig. S3. TMEM132A regulates directional cell migration in wound healing assay in HEK 293 cells stably transfected with turbo GFP. (A, B) Real-time quantitative PCR **(A)** and immunoblotting **(B)** to assess the knockdown efficiency of siRNAs against TMEM132A or negative control in HEK 293 cells 48 hours post transfection. GAPDH serves as an internal control (for quantitative PCR, n≥3, Student`s *t*-test. ***p*≤0.01). Relative band intensity in immunoblotting assay normalized to GAPDH is given below the panel. **(C)** Wound healing assay performed in HEK 293 cells showed slower closing of the wound following knockdown of TMEM132A relative to negative control siRNA. Still images from videos at indicated time points are shown.

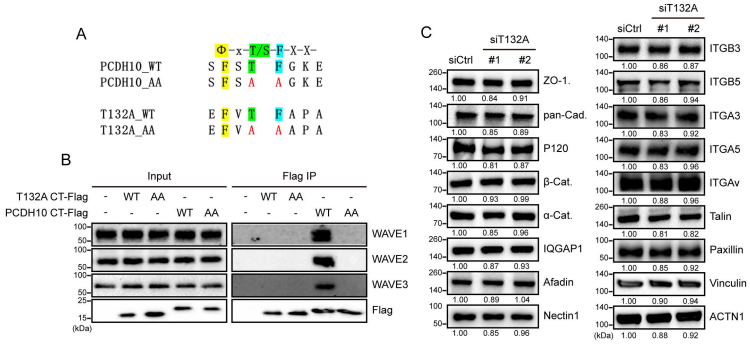
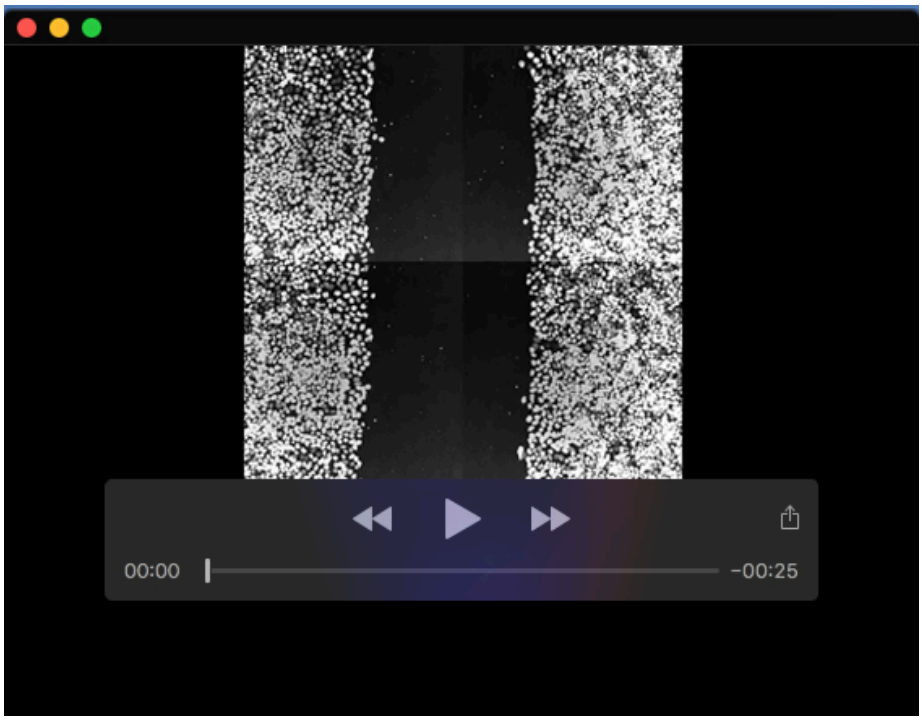
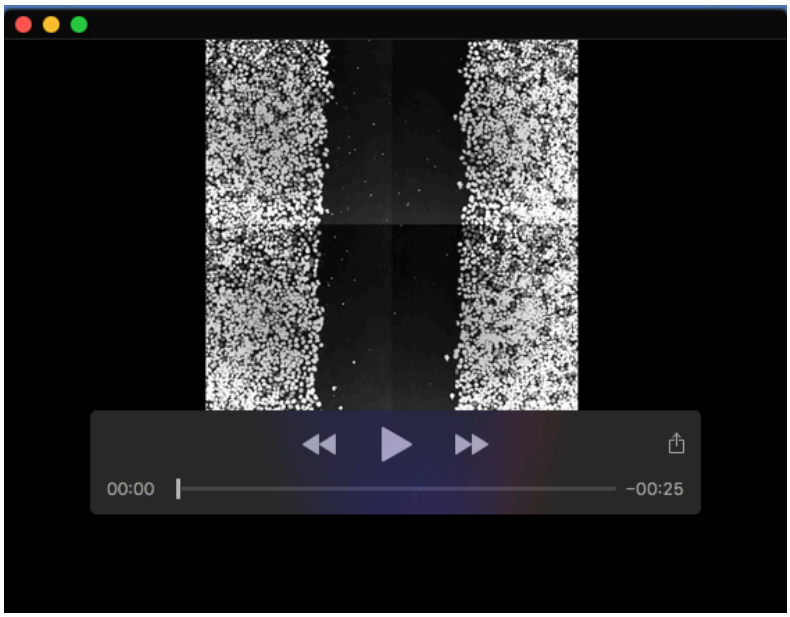


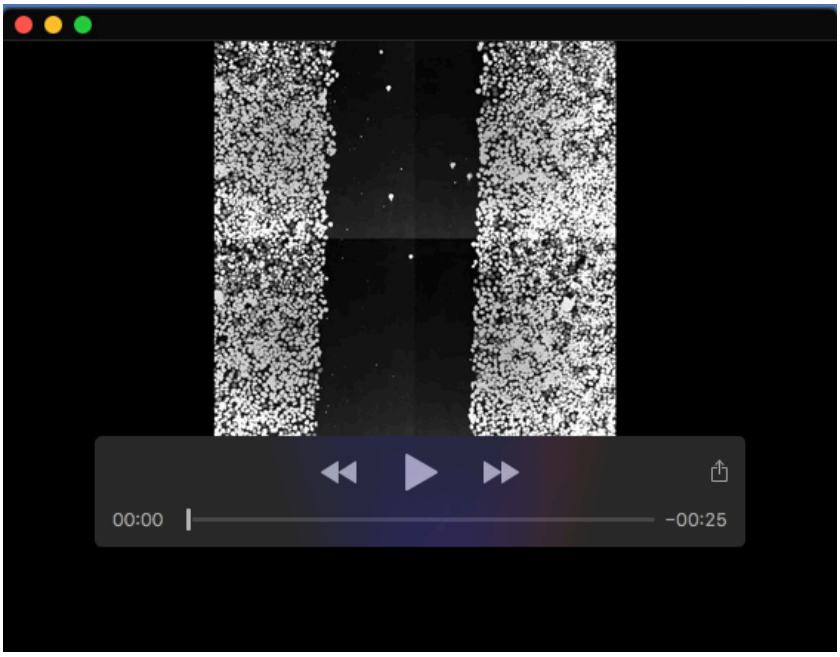
Fig. S4. TMEM132A does not interact with the WAVE regulatory complex (WRC). (A) WRC interacting receptor sequence (WIRS) and mutated form in C-terminus of human PCDH10, and the predicted WIRS and mutated form in mouse TMEM132A. **(B)** Overexpressed PCHD10, but not TMEM132A, interacts with WAVE1, 2 and 3 In HEK 293T cells, and disruption of the WIRS motif in PCDH10 abolishes this interaction. **(C)** Protein levels of apical tight junction protein ZO-1, the calcium-dependent adhesion junction proteins Cadherin(s), P120, β-Catenin, α-Catenin and IQGAP1, calcium-independent adhesion junction proteins Afadin and Nectin1, as well as α3, α5, αv, β3, β5, or other components of focal adhesion complex including Talin, Paxillin, Vinculin and ACTN1, were not affected by loss of TMEM132A in HeLa cells 48 hours post siRNA transfection. GAPDH in Figure 5A serves as an internal control.



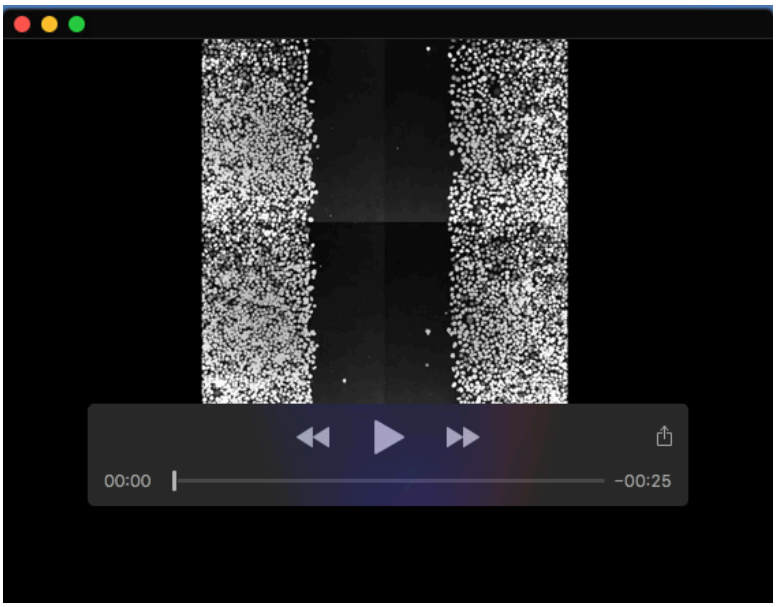
Movie 1. Live fluorescent imaging of HeLa cells transfected with negative control siRNA (area 1 of 3). Nuclei were visualized by SPY 505-DNA non-toxic staining (excitation: 512 nm, emission: 531 nm) and imaged using the green fluorescence channel (emission filter: 500-550 nm). Time-lapse microscopic images were taken every 10 minutes and 10 slides in Z-axis spanning approximately 15 μm was captured and maximum projected, and 10 frames per second (fps) videos were generated.



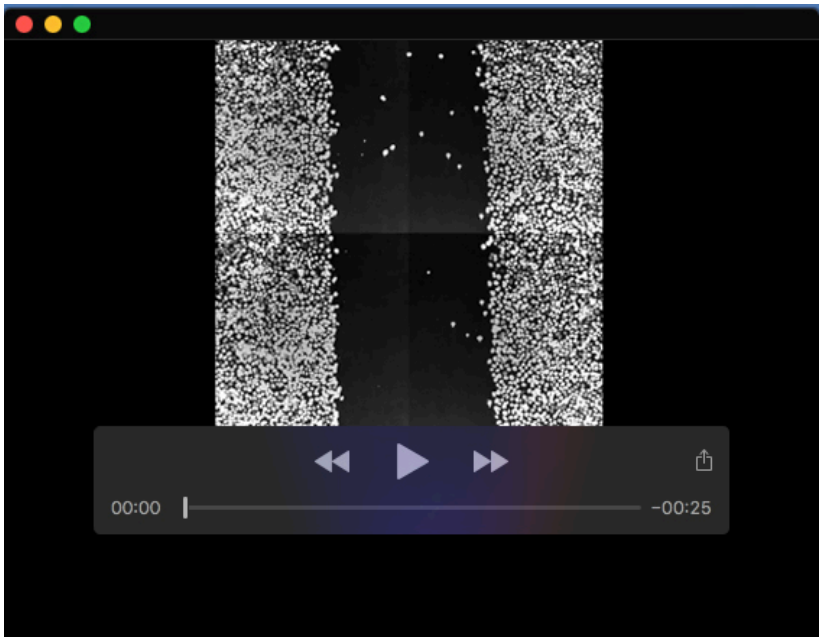
Movie 2. Live fluorescent imaging of HeLa cells transfected with negative control siRNA (area 2 of 3).



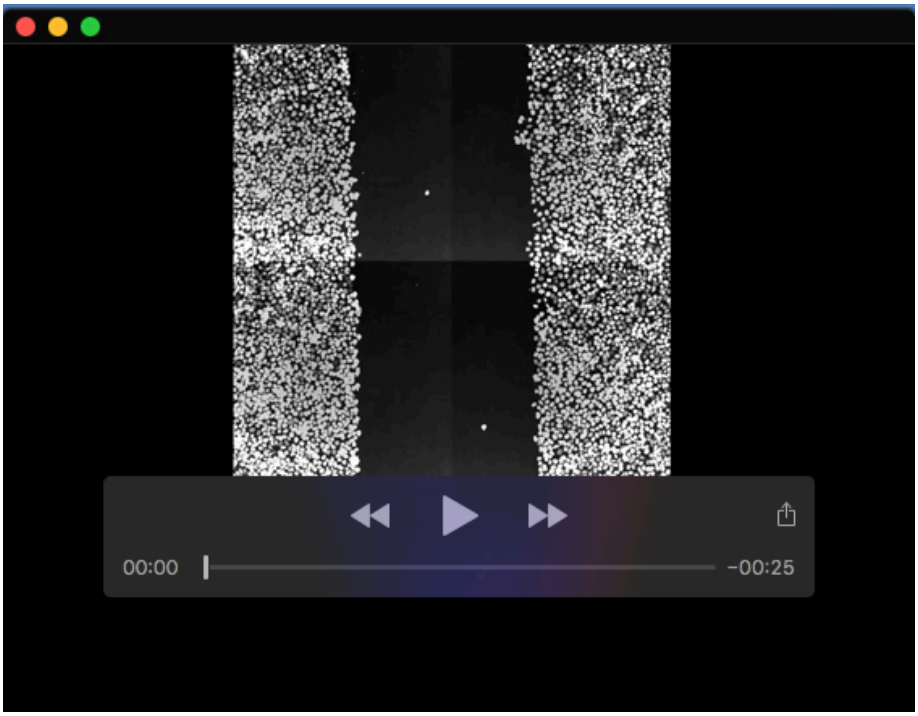
Movie 3. Live fluorescent imaging of HeLa cells transfected with negative control siRNA (area 3 of 3).



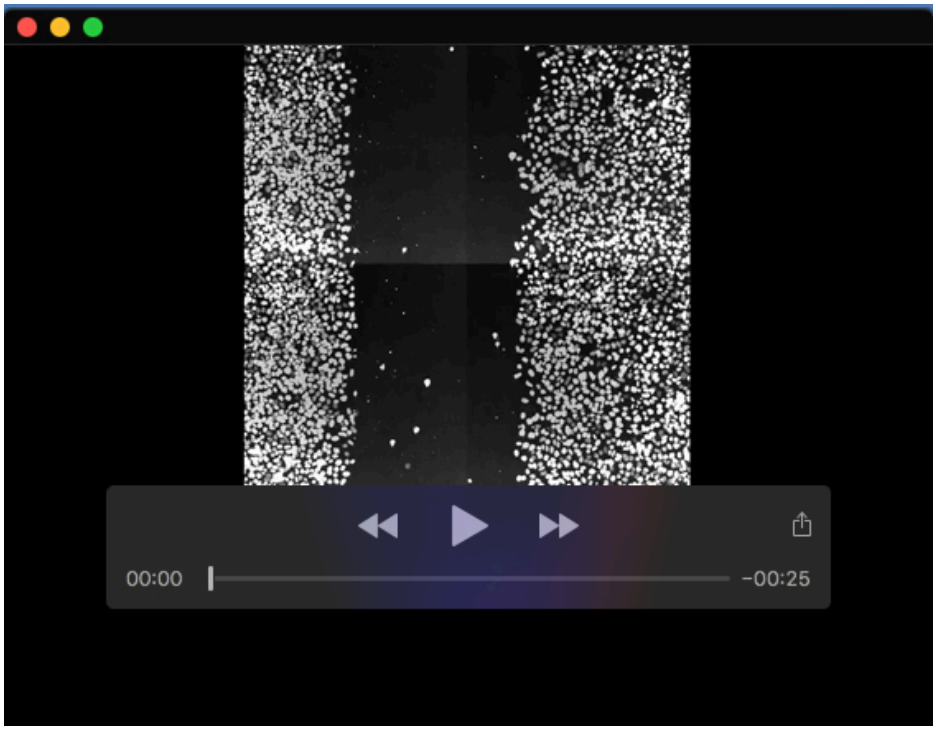
Movie 4. Live fluorescent imaging of HeLa cells transfected with TMEM132A-specific siRNA #1 (area 1 of 3). Nuclei were visualized by SPY 505-DNA non-toxic staining (excitation: 512 nm, emission: 531 nm) and imaged using the green fluorescence channel (emission filter: 500-550 nm). Time-lapse microscopic images were taken every 10 minutes and 10 slides in Z-axis spanning approximately 15 μm was captured and maximum projected, and 10 fps videos were generated.



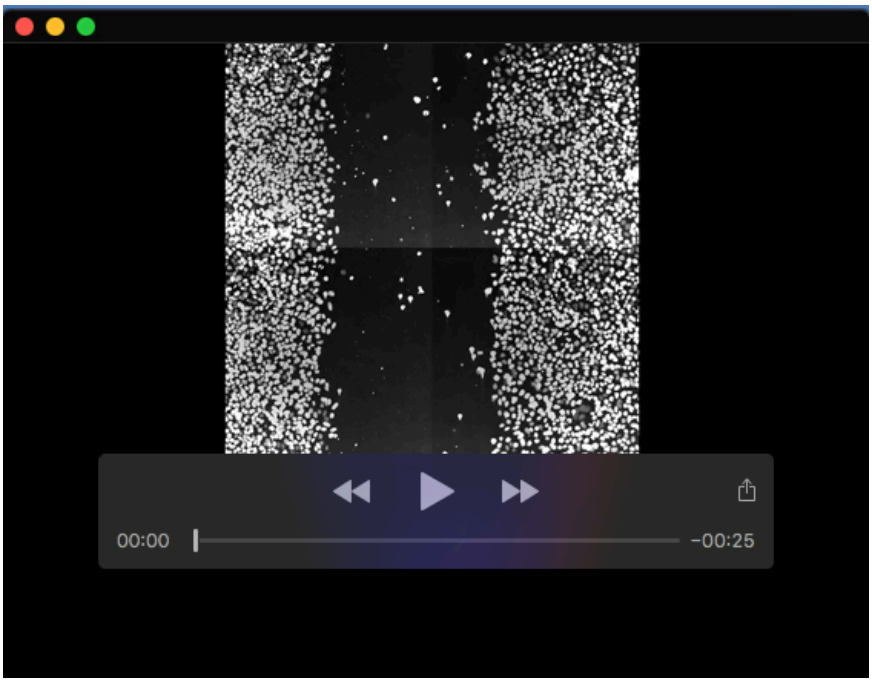
Movie 5. Live fluorescent imaging of HeLa cells transfected with TMEM132A-specific siRNA #1 (area 2 of 3).



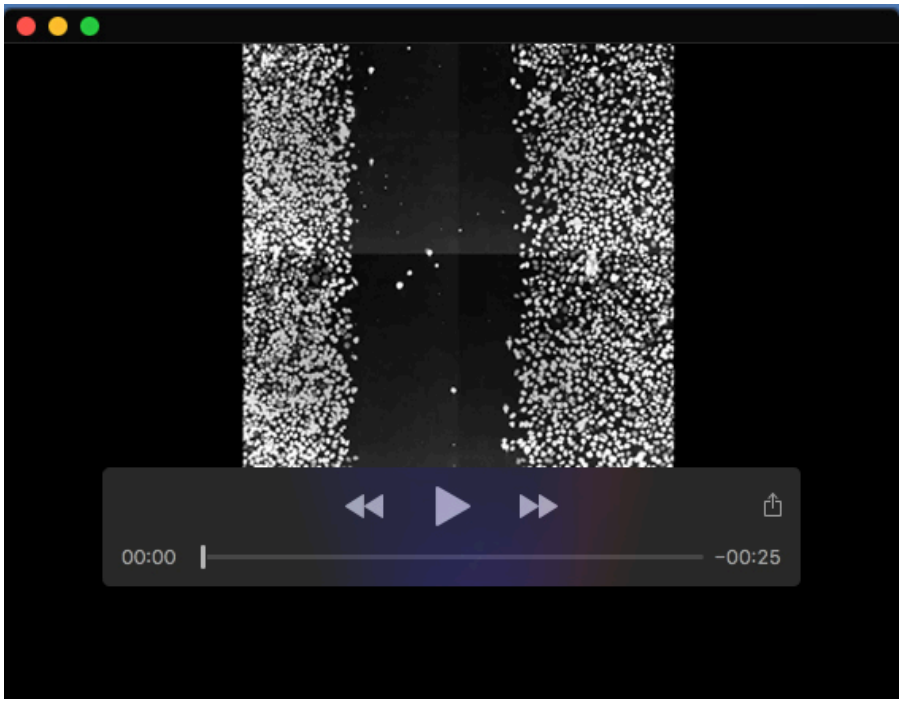
Movie 6. Live fluorescent imaging of HeLa cells transfected with TMEM132A-specific siRNA #1 (area 3 of 3).



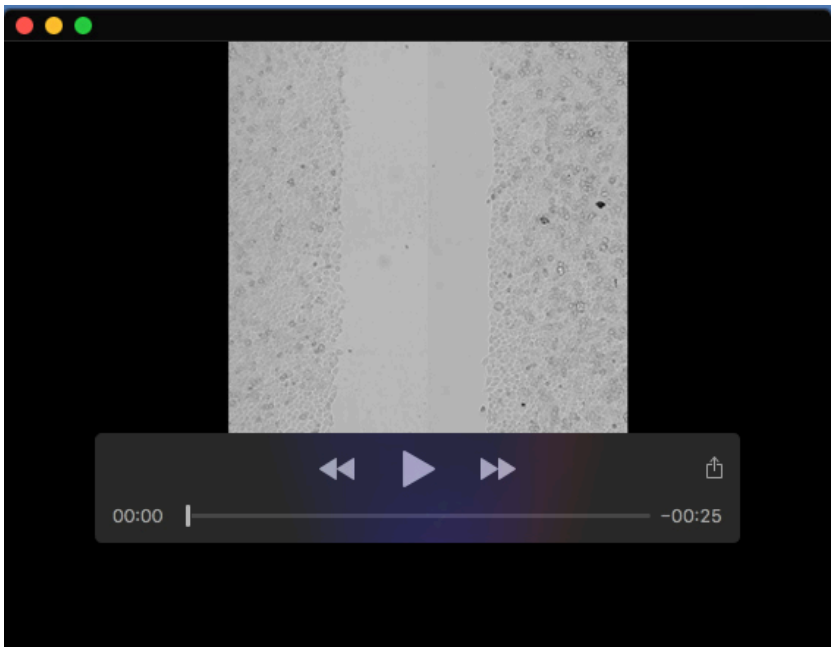
Movie 7. Live fluorescent imaging of HeLa cells transfected with TMEM132A-specific siRNA #2 (area 1 of 3). Nuclei were visualized by SPY 505-DNA non-toxic staining (excitation: 512 nm, emission: 531 nm) and imaged using the green fluorescence channel (emission filter: 500-550 nm). Time-lapse microscopic images were taken every 10 minutes and 10 slides in Z-axis spanning approximately 15 μm was captured and maximum projected, and 10 fps videos were generated.



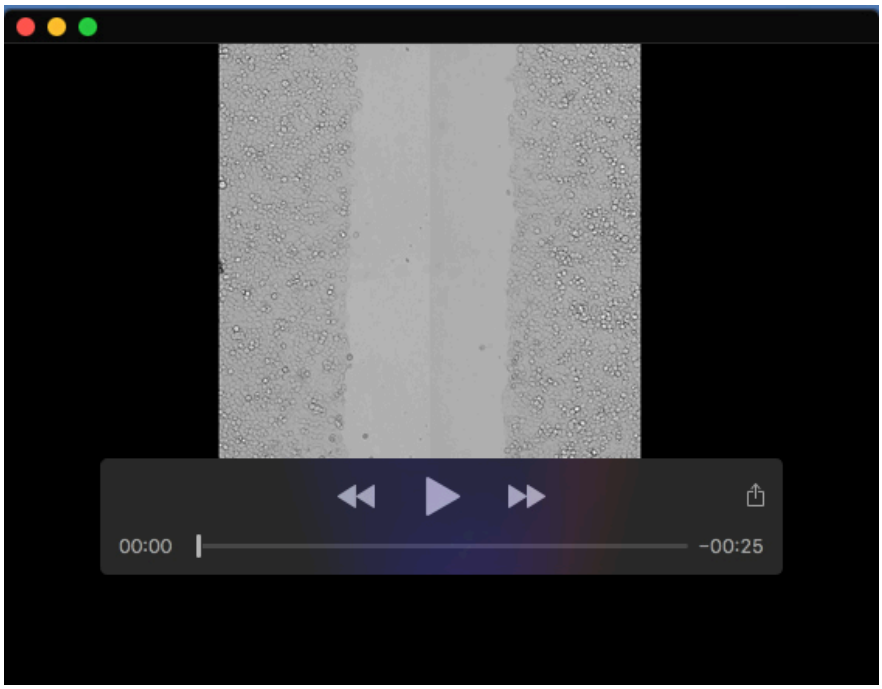
Movie 8. Live fluorescent imaging of HeLa cells transfected with TMEM132A-specific siRNA #2 (area 2 of 3).



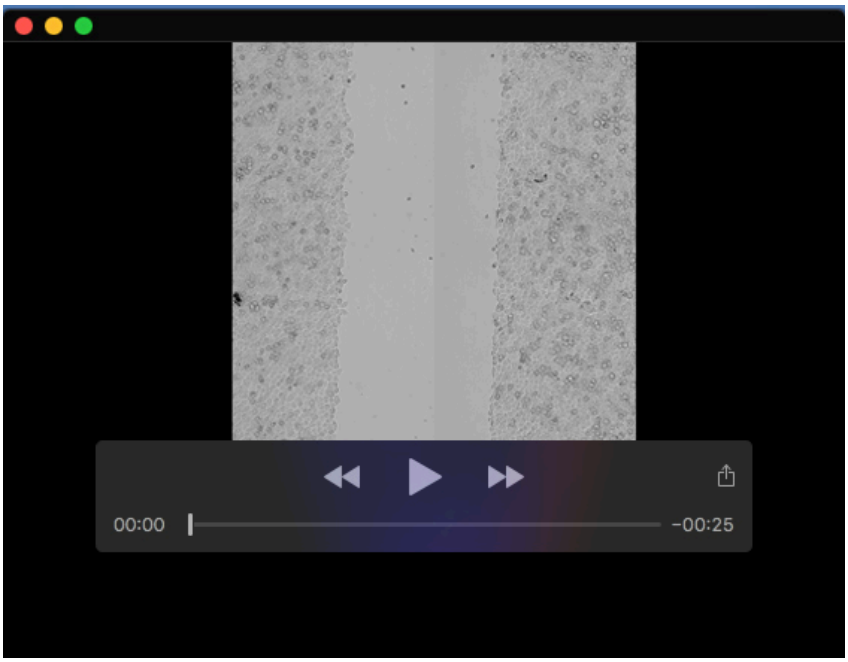
Movie 9. Live fluorescent imaging of HeLa cells transfected with TMEM132A-specific siRNA #2 (area 3 of 3).



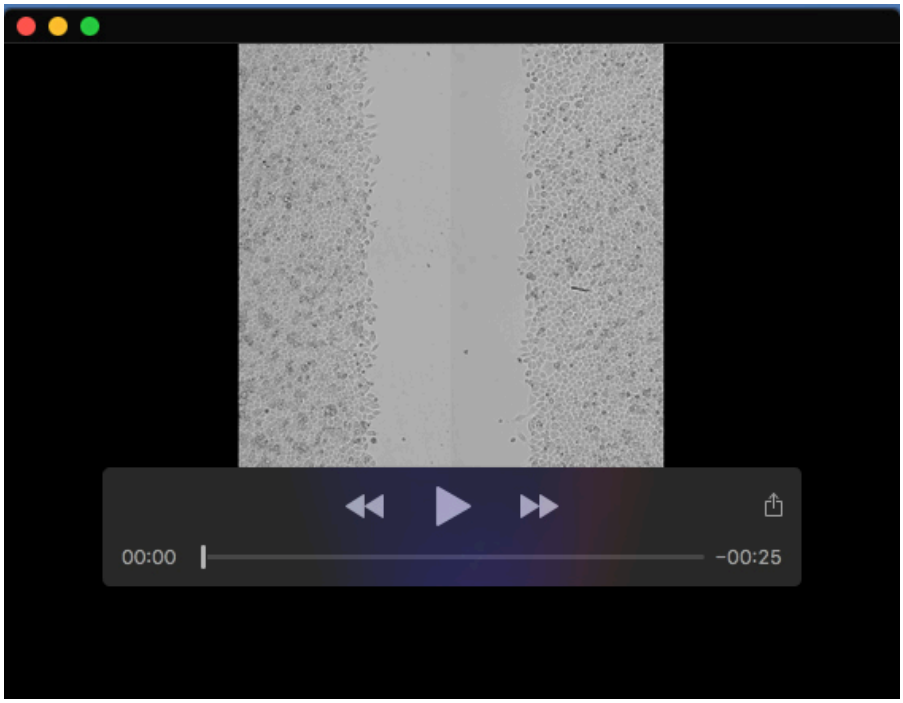
Movie 10. Live imaging of HeLa cells transfected with negative control siRNA in bright field. The same area as shown in supplementary movie 1 were also captured by bright field phase contrast microscopy. Time-lapse microscopic images were taken every 10 minutes and 10 slides in Z-axis spanning approximately 15 μm was captured and maximum projected, and 10 fps videos were generated.



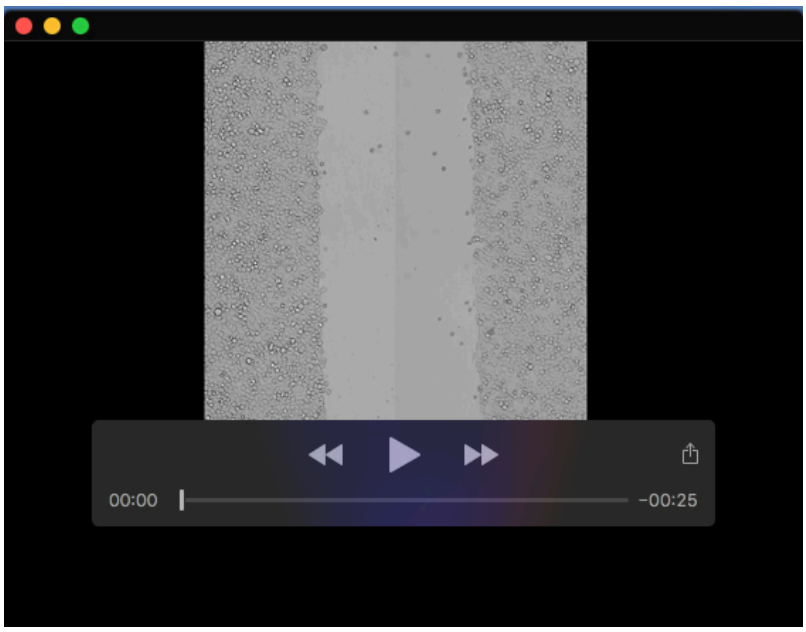
Movie 11. Live imaging of HeLa cells transfected with negative control siRNA in bright field. The same area as shown in supplementary movie 2.



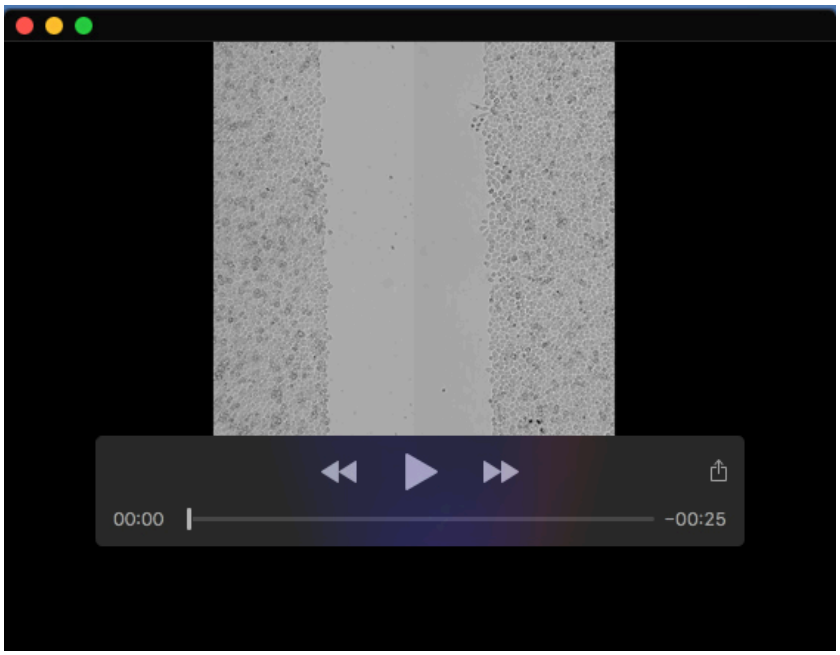
Movie 12. Live imaging of HeLa cells transfected with negative control siRNA in bright field. The same area as shown in supplementary movie 3.



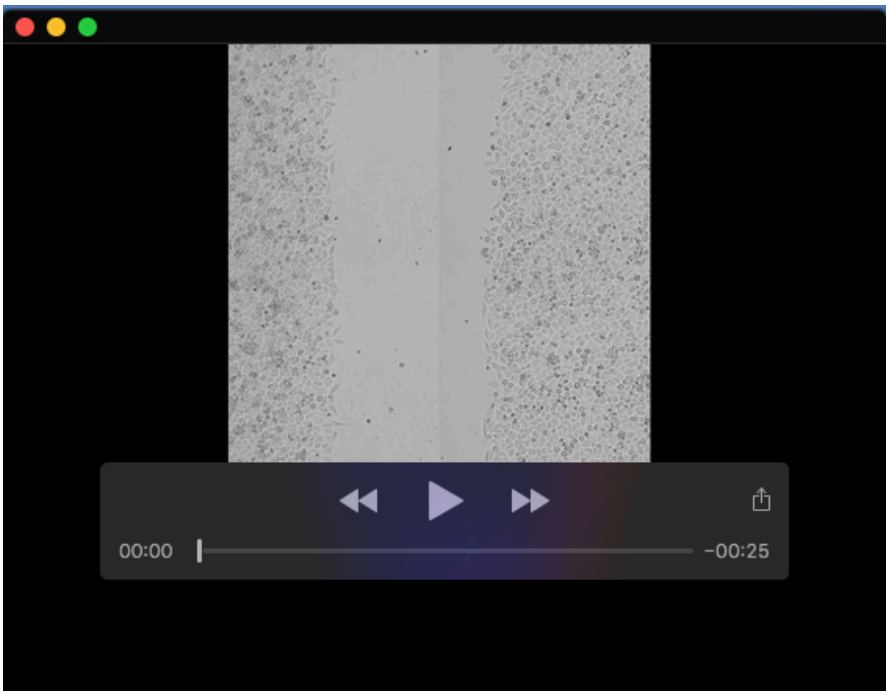
Movie 13. Live imaging of HeLa cells transfected with TMEM132A-specific siRNA #1 in bright field. The same area as shown in supplementary movie 4 were also captured by bright field phase contrast microscopy. Time-lapse microscopic images were taken every 10 minutes and 10 slides in Z-axis spanning approximately 15 μm was captured and maximum projected, and 10 fps videos were generated.



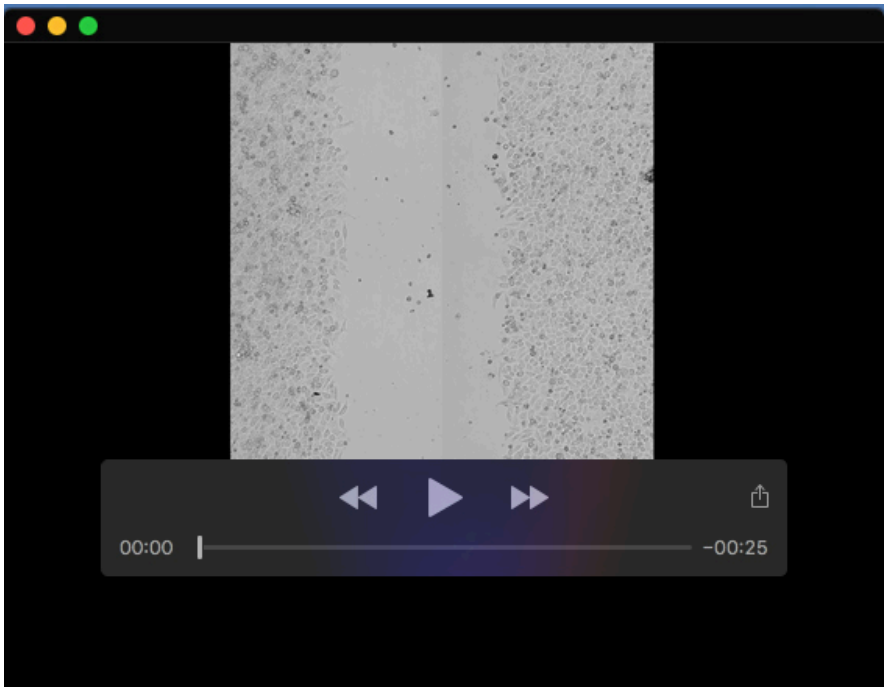
Movie 14. Live imaging of HeLa cells transfected with TMEM132A-specific siRNA #1 in bright field. The same area as shown in supplementary movie 5.



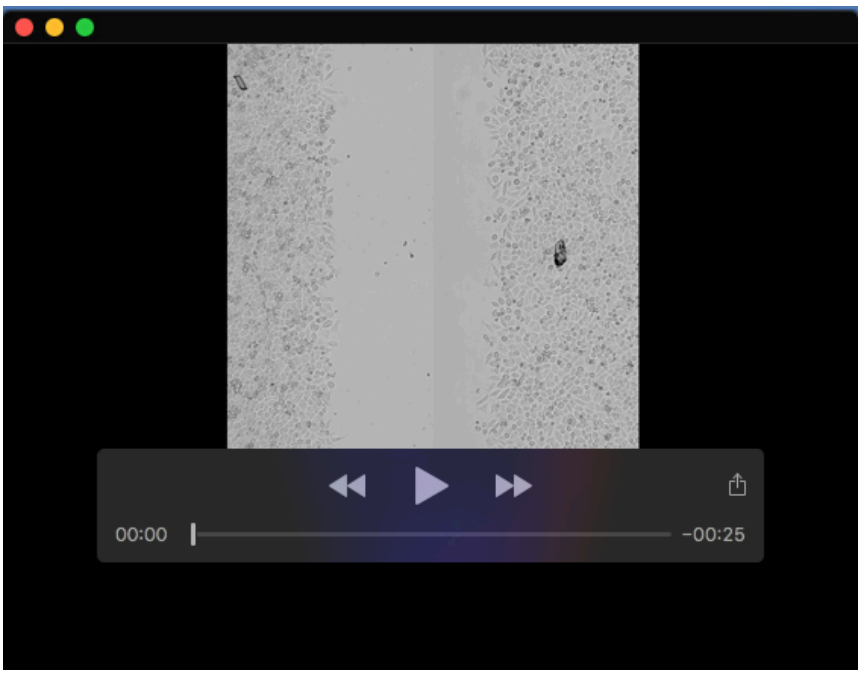
Movie 15. Live imaging of HeLa cells transfected with TMEM132A-specific siRNA #1 in bright field. The same area as shown in supplementary movie 6.



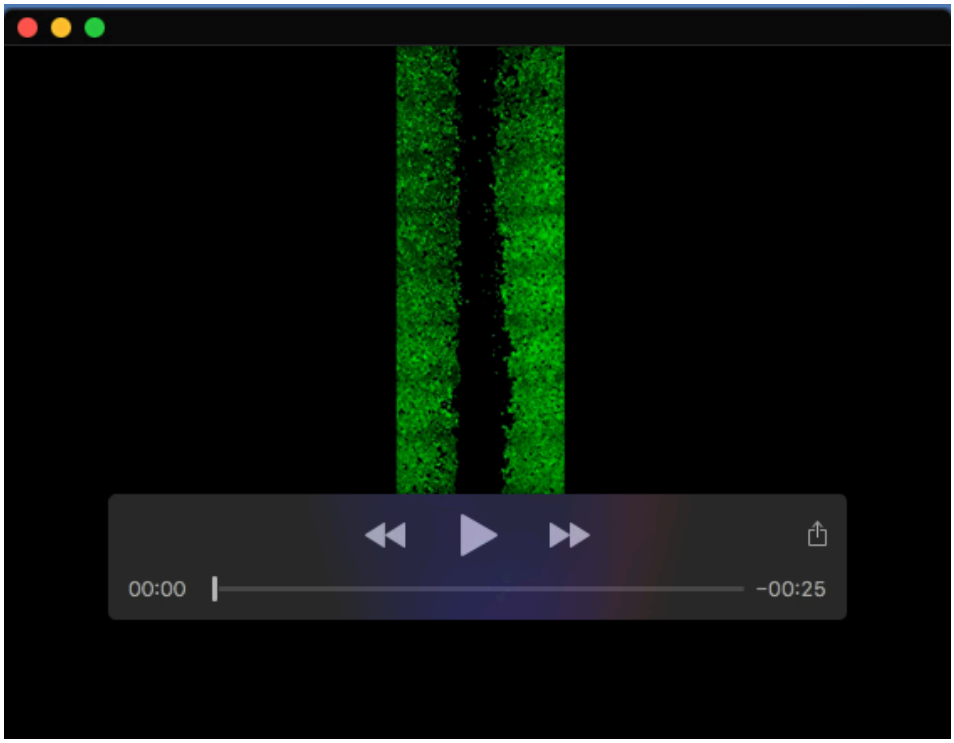
Movie 16. Live imaging of HeLa cells transfected with TMEM132A-specific siRNA #2 in bright field. The same area as shown in supplementary movie 7 were also captured by bright field phase contrast microscopy. Time-lapse microscopic images were taken every 10 minutes and 10 slides in Z-axis spanning approximately 15 μm was captured and maximum projected, and 10 fps videos were generated.



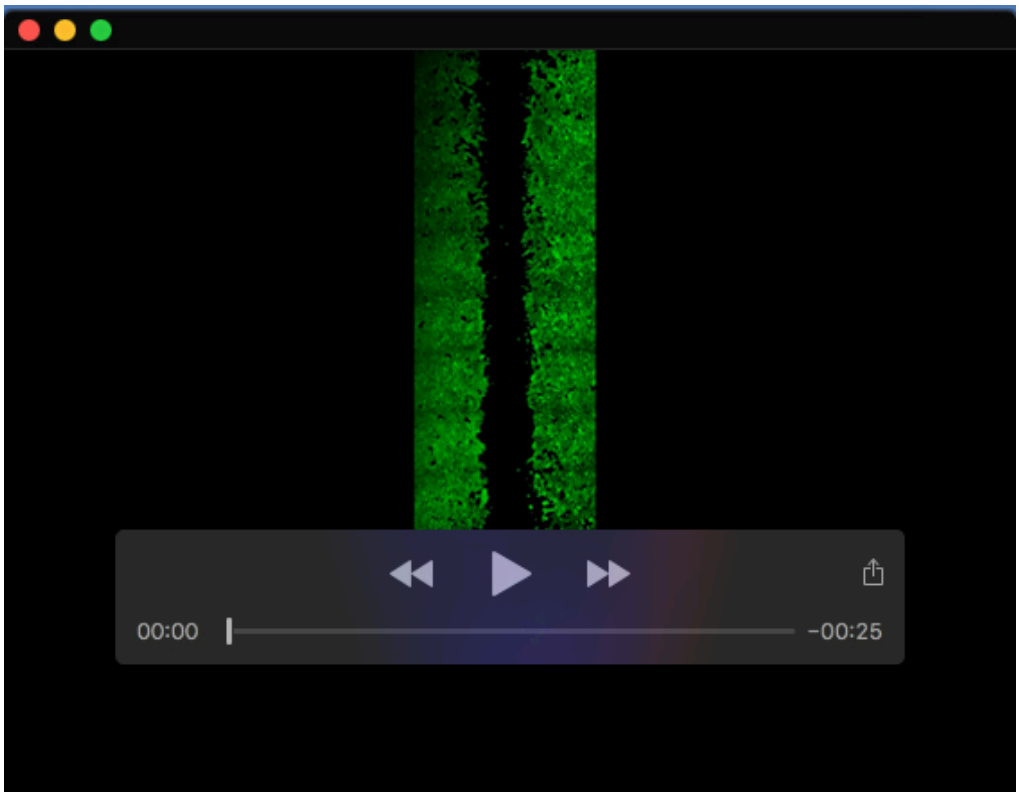
Movie 17. Live imaging of HeLa cells transfected with TMEM132A-specific siRNA #2 in bright field. The same area as shown in supplementary movie 8.



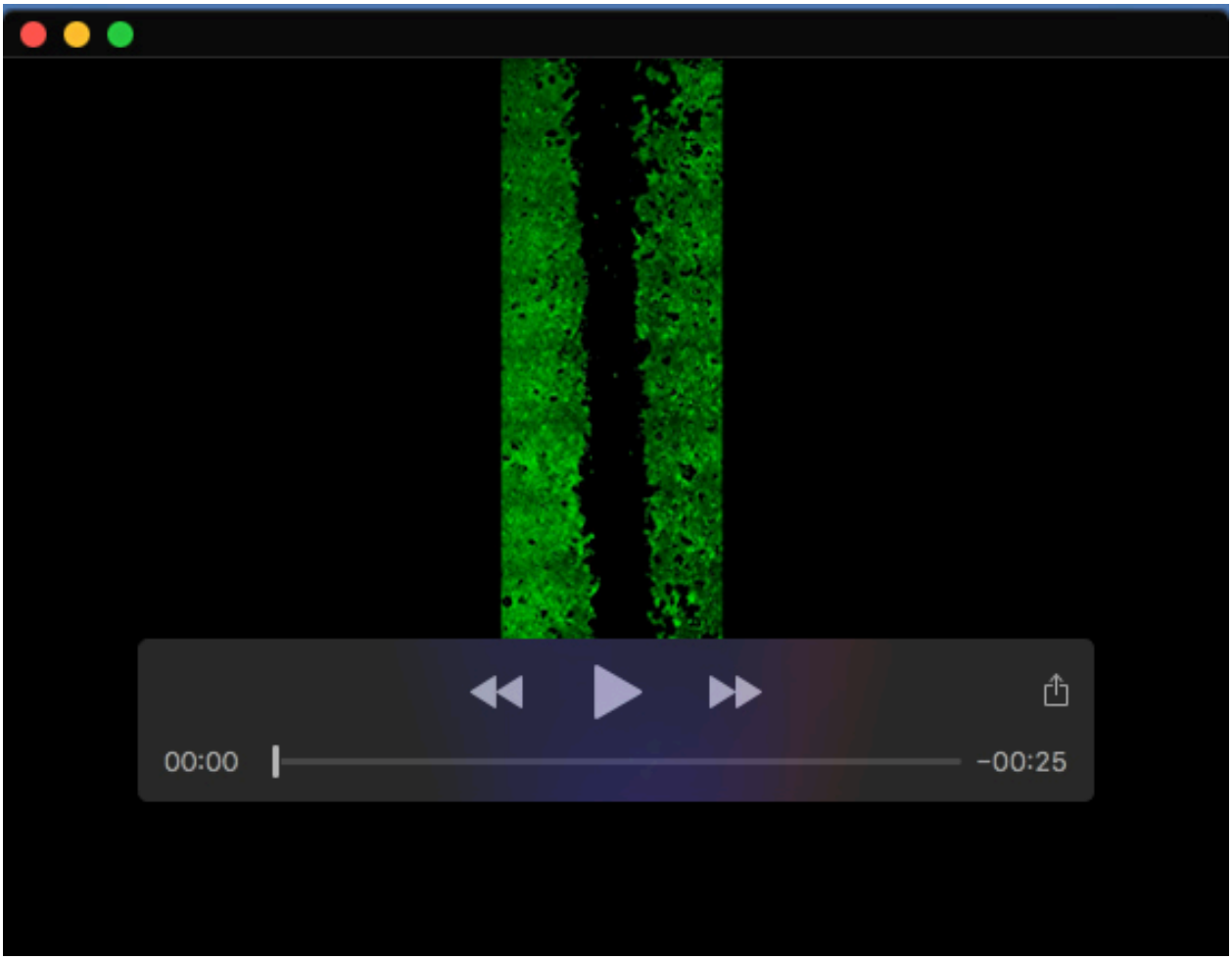
Movie 18. Live imaging of HeLa cells transfected with TMEM132A-specific siRNA #2 in bright field. The same area as shown in supplementary movie 9.



Movie 19. Live fluorescent imaging of HEK 293 cells transfected with turbo GFP (excitation: 482 nm, emission: 502 nm) **and negative control siRNA.** Microscopic images were captured through green fluorescence channel (emission filter: 500-550 nm). Time-lapse microscopic images were taken every 10 minutes and 10 slides in Z-axis spanning approximately 15 µm was captured and maximum projected, and 10 fps videos were generated.



Movie 20. Live fluorescent imaging of HEK 293 cells transfected with turbo GFP (excitation: 482 nm, emission: 502 nm) and TMEM132A-specific siRNA #1. Microscopic images were captured through green fluorescence channel (emission filter: 500- 550 nm). Time-lapse microscopic images were taken every 10 minutes and 10 slides in Z-axis spanning approximately 15 µm was captured and maximum projected, and 10 fps videos were generated.



Movie 21. Live fluorescent imaging of HEK 293 cells transfected with turbo GFP (excitation: 482 nm, emission: 502 nm) **and TMEM132A-specific siRNA #2.** Microscopic images were captured through green fluorescence channel (emission filter: 500- 550 nm). Time-lapse microscopic images were taken every 10 minutes and 10 slides in Z-axis spanning approximately 15 µm was captured and maximum projected, and 10 fps videos were generated.