

Supplementary materials

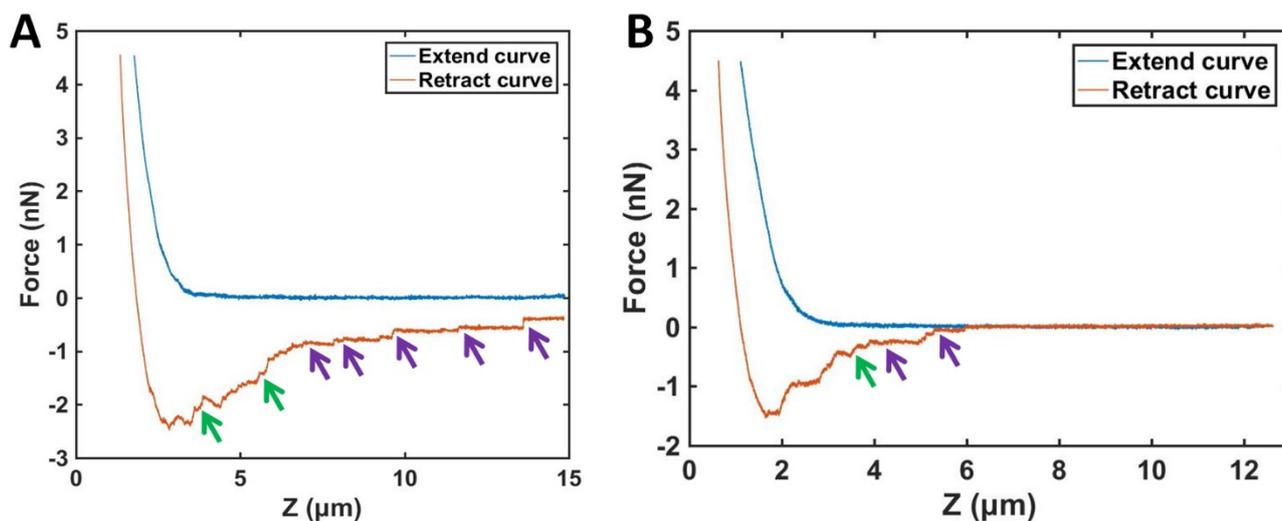


Fig. S1 (A, B) Two representative force curves obtained during AFM-based SCFS assay. The AFM tipless cantilever carrying a living MGC-803 cell was used to perform SCFS on a HMrSV5 cell grown on the coverslip. The purple arrows denote the events of membrane tether unbinding, and the green arrows denotes the events of force steps.

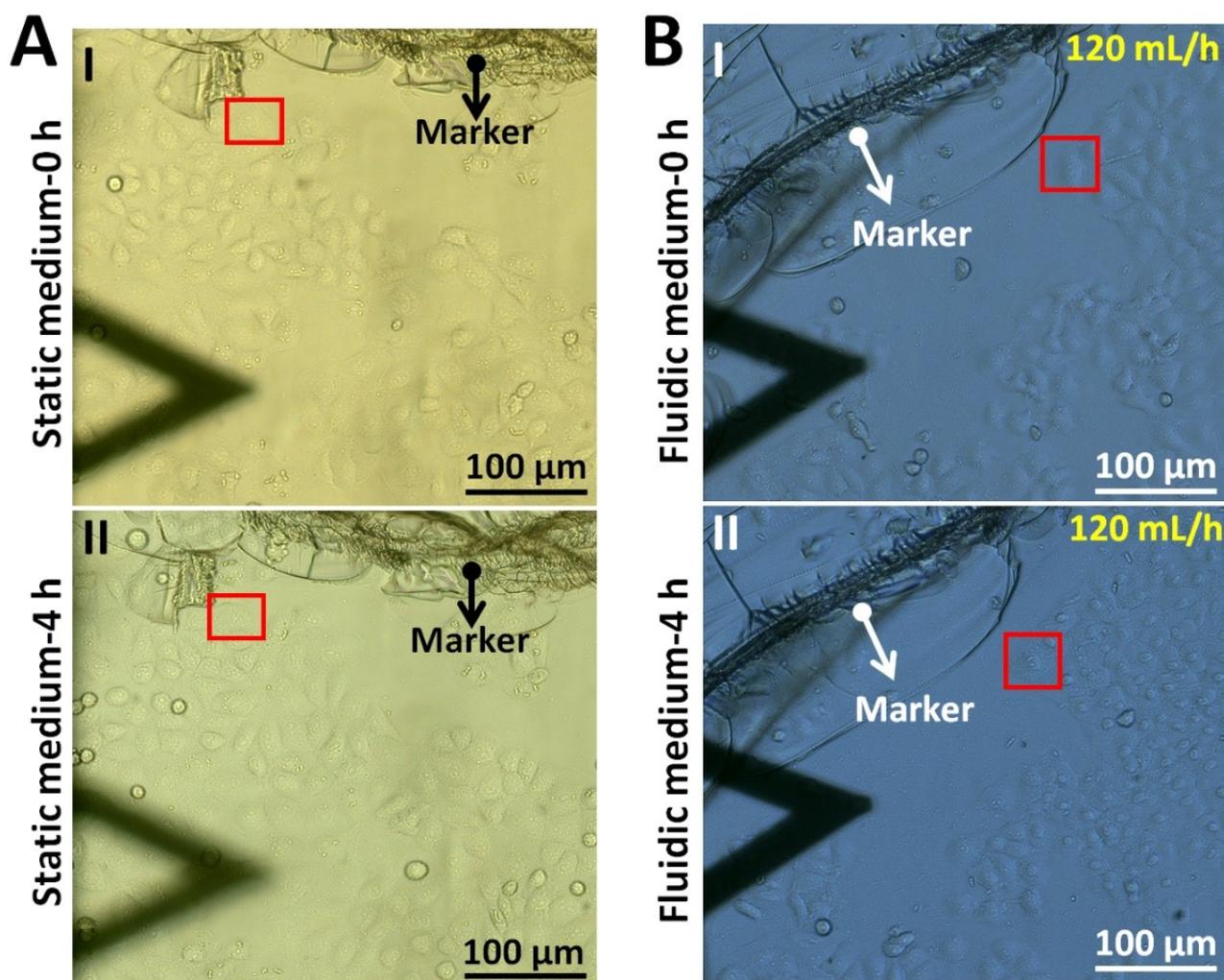


Fig. S2 Typical optical microscopy images of a same HMrSV5 cell (denoted by the red squares) before (I) and after (II) growth in static (A) or fluidic (the flow rate of the medium is 120 mL/h) cell culture medium (B), which was probed by the AFM single-cell (MGC-803 cell) probe. The physical markers were used to visually confirm the same cell.

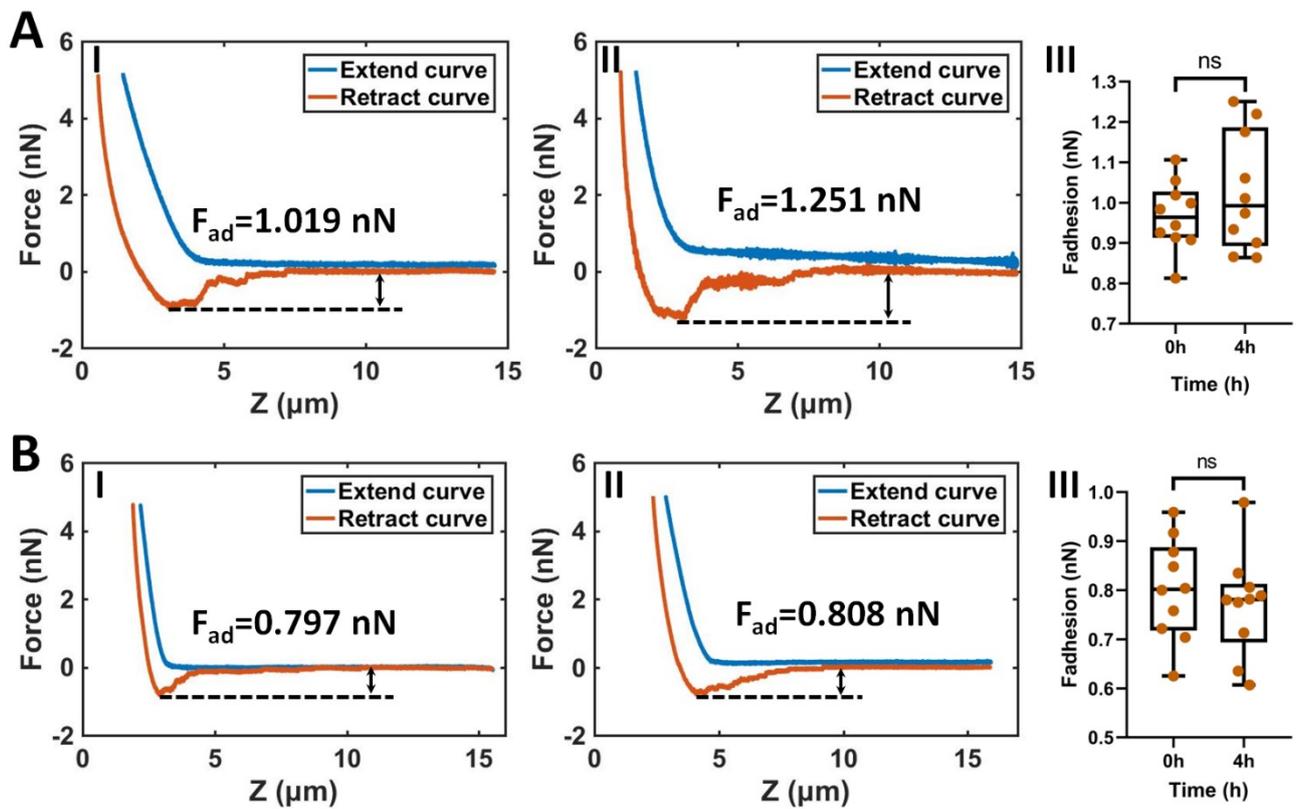


Fig. S3 Experimental results of utilizing the prepared single-cell probes to obtain force curves in the bare area of the coverslip before performing SCFS experiments on HMrSV5 cells. (A) Results of the MGC-803-modified AFM probes. (B) Results of the HGC-27-modified AFM probes. (I) A typical force curve obtained on the coverslip before cell growth by using a single-cell probe (0 h group). (II) A typical force curve obtained on the coverslip after cell growth by using another single-cell probe (4 h group). (III) Statistical results of the measured adhesion forces by the two single-cell probes before and after cell growth.

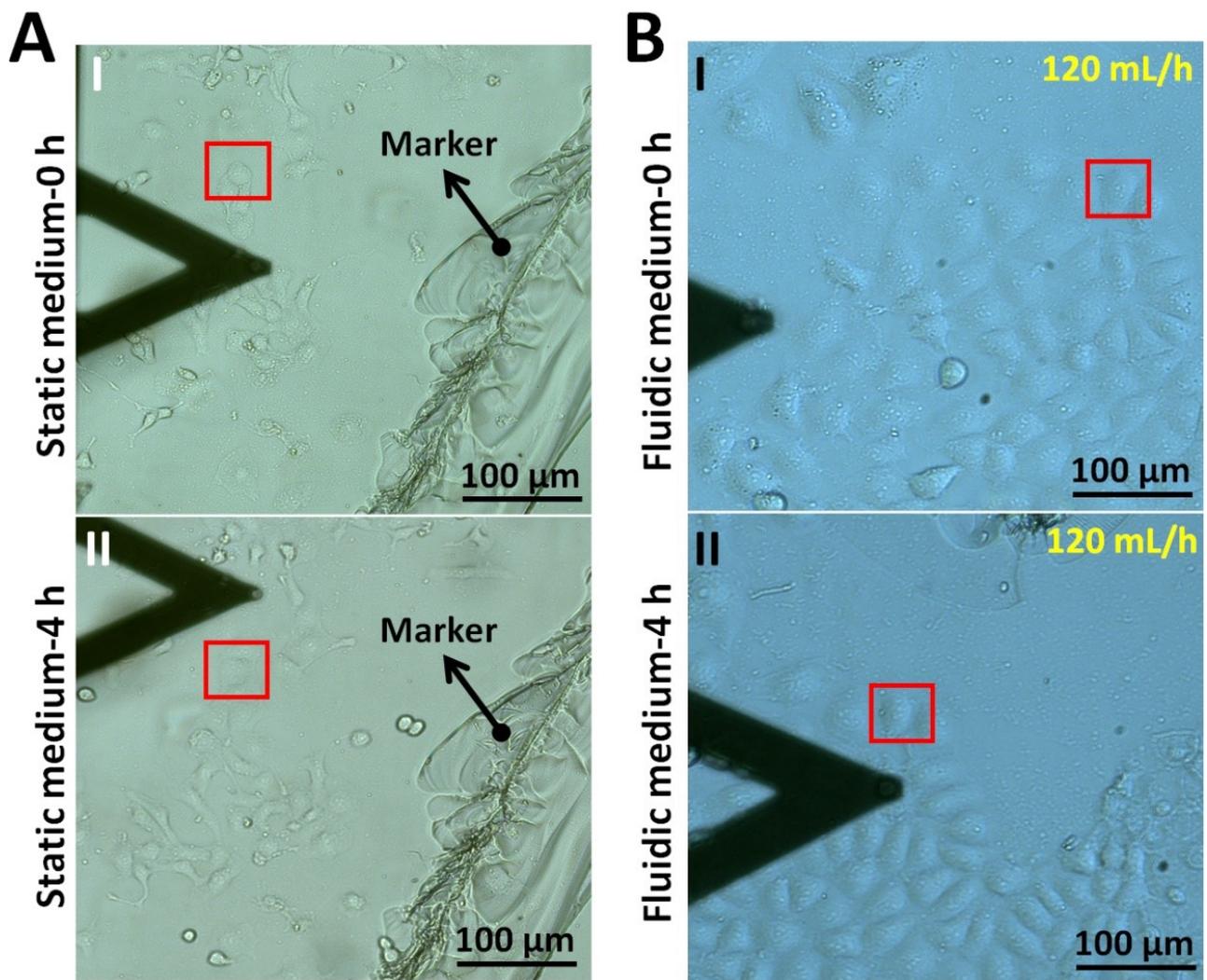


Fig. S4 Typical optical microscopy images of a same HMrSV5 cell (denoted by the red squares) before (I) and after (II) growth in static (A) or fluidic medium (B), which was probed by the AFM single-cell (HGC-27 cell) probe.

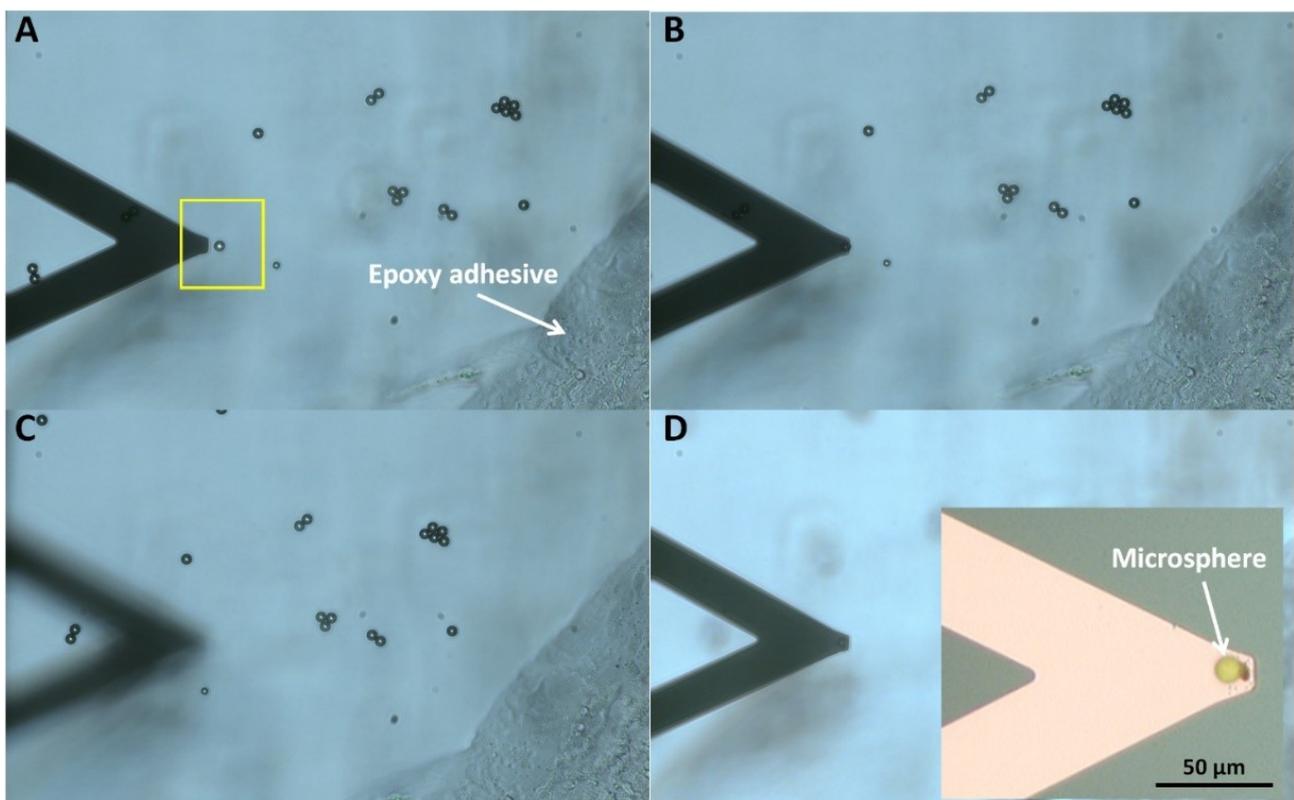


Fig. S5 Optical microscopy images showing the process of preparing a microsphere-modified AFM probe. (A) The

adhesive-coated AFM probe was controlled to gradually approach a single microsphere (denoted by the yellow square). When the AFM probe touched the microsphere (B), the AFM probe was controlled to retract (C). (D) The microsphere glued onto the AFM probe was discernible from the optical microscopy image. The inset in (D) is the enlarged optical image of the prepared spherical probe obtained by an upright optical microscope (HIROX Company, Tokyo, Japan).

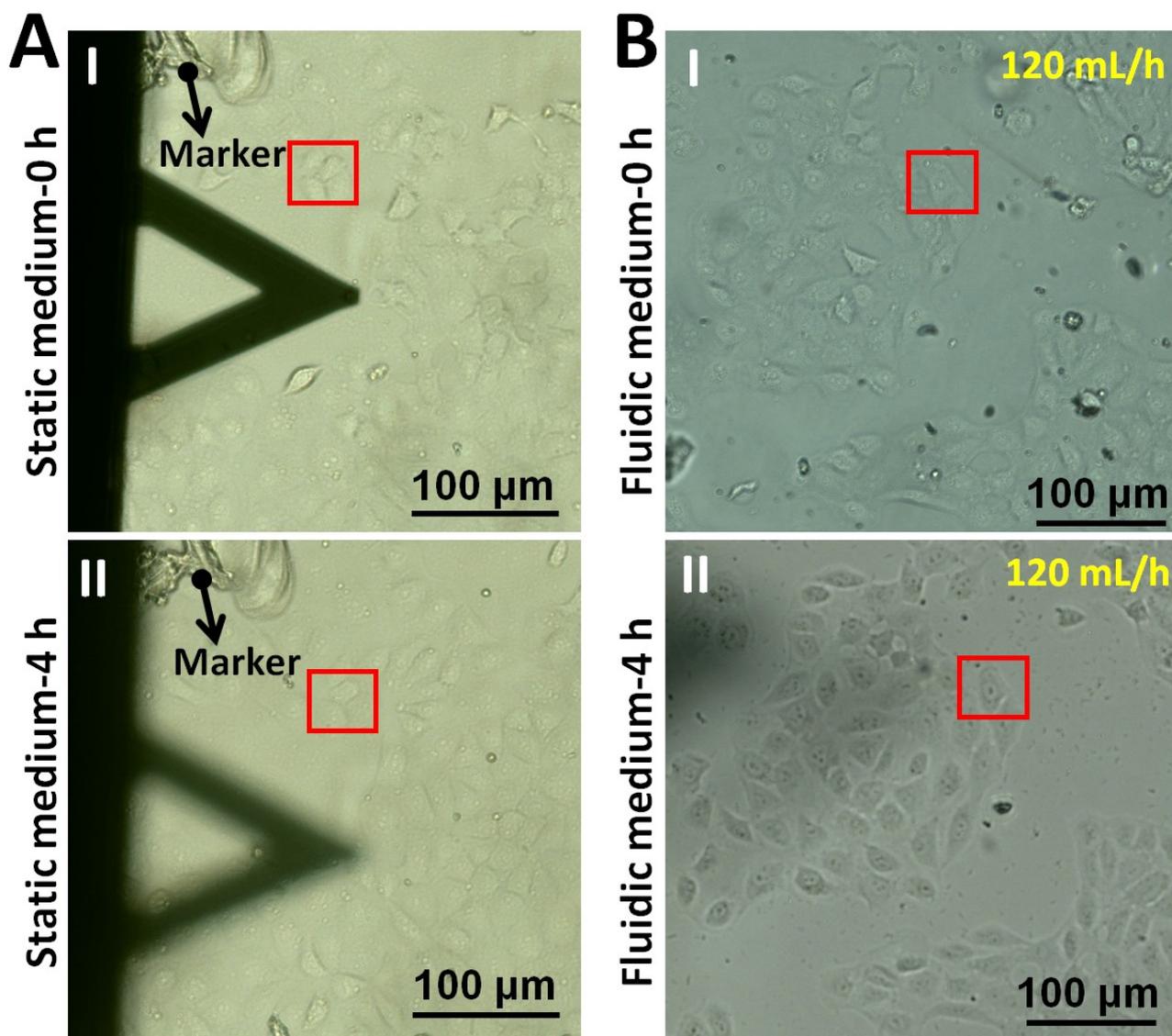


Fig. S6 Typical optical microscopy images of a same HMrSV5 cell (denoted by the red squares) before (I) and after (II) growth in static (A) or fluidic medium (B), which was probed by the AFM probe carrying a fibronectin-coated microsphere.

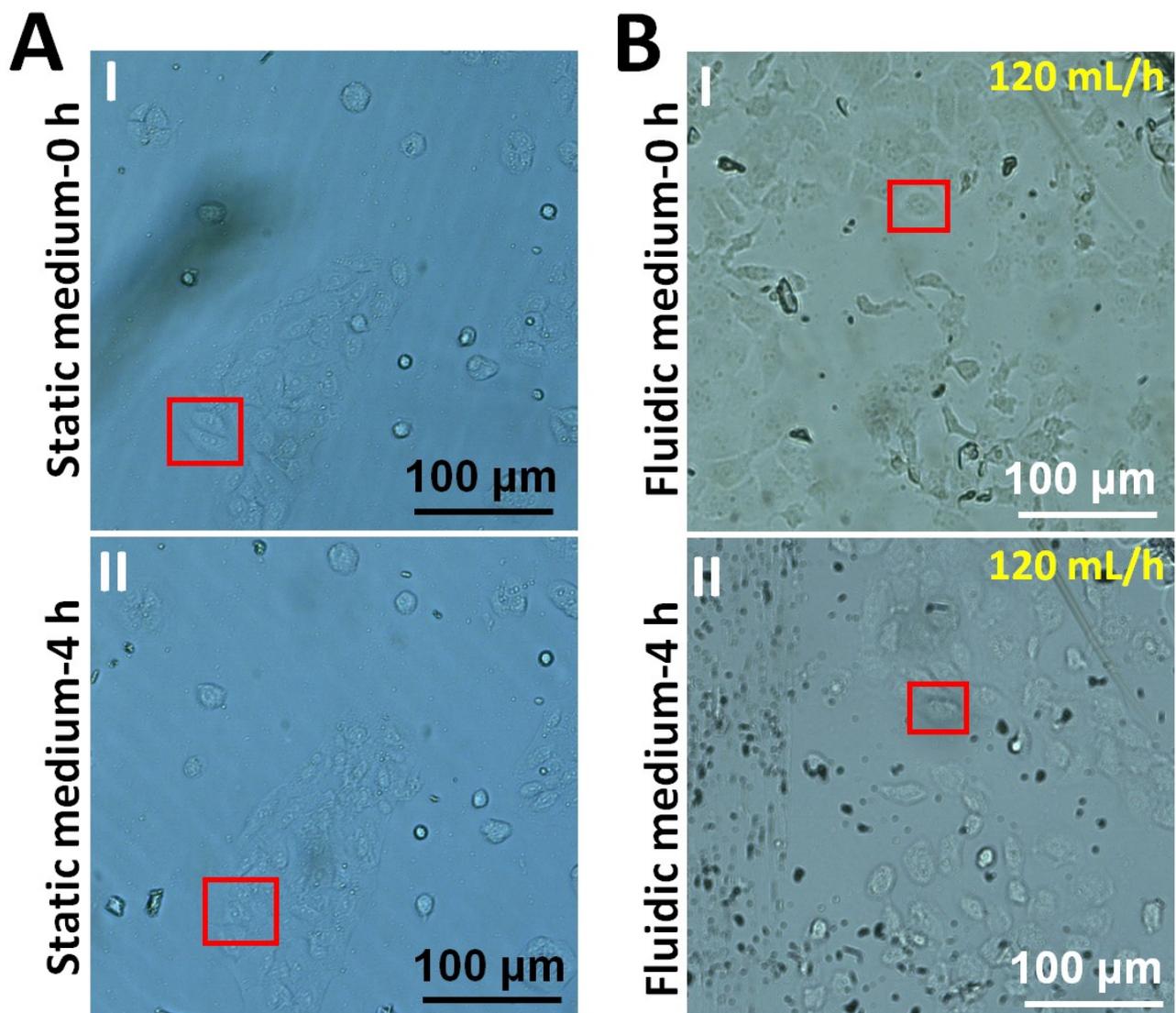


Fig. S7 Typical optical microscopy images of a same HMrSV5 cell (denoted by the red squares) before (I) and after (II) growth in static (A) or fluidic medium (B), which was probed by the AFM probe carrying a ConA-coated microsphere.

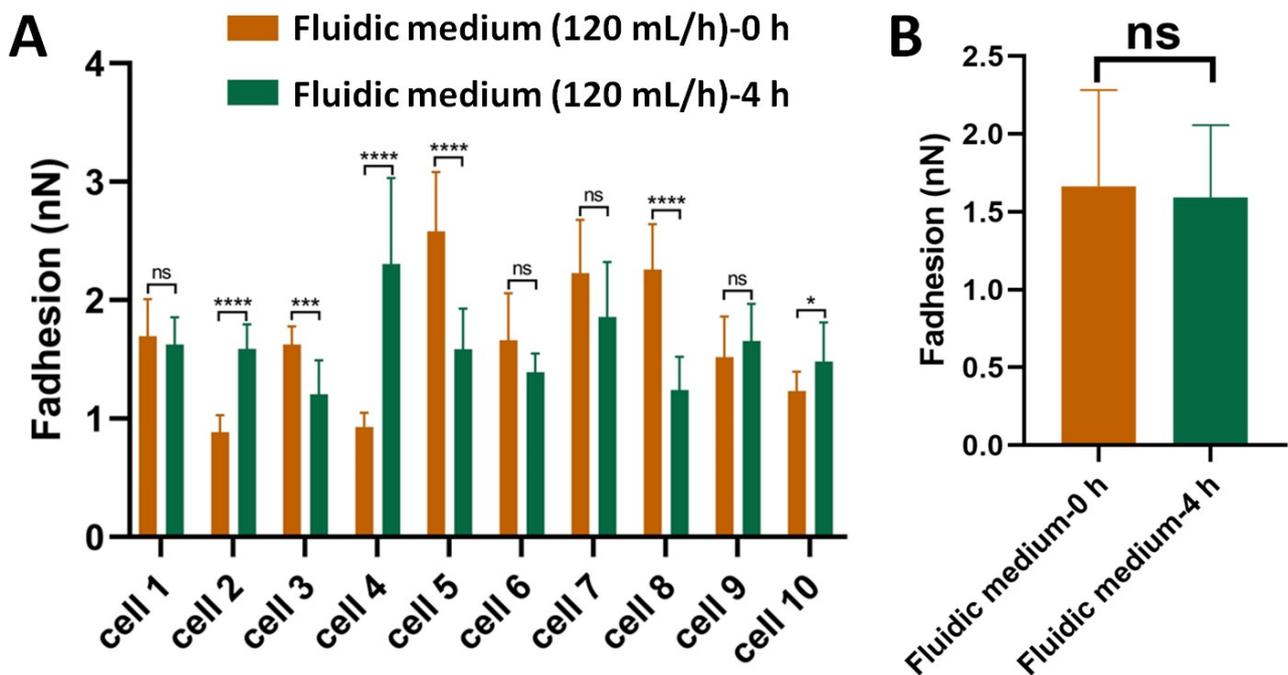


Fig. S8 Effects of fluid flow environment on the adhesion forces of HMrSV5 cells probed by an AFM probe carrying a ConA-coated microsphere. (A) Changes of the adhesion forces of ten same HMrSV5 cells before and after growth in fluidic flow medium. (B) Overall changes of the adhesion forces of the ten cells. Statistical significance was set at the following levels: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns not significant.

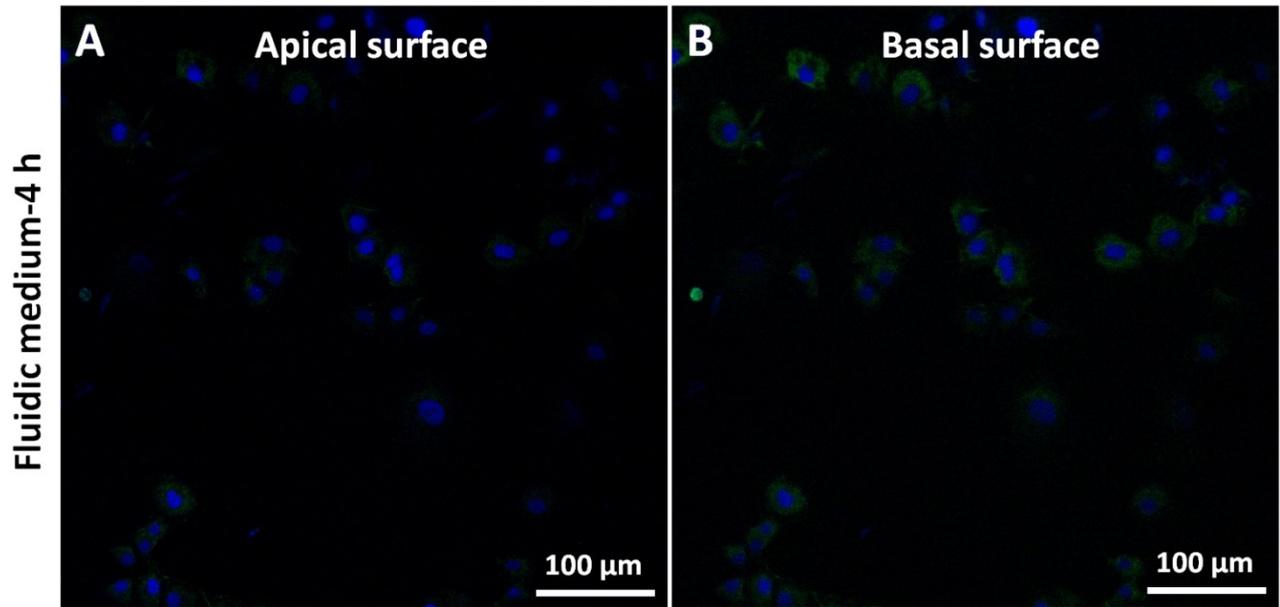


Fig. S9 More confocal fluorescence microscopy images of HMrSV5 cells after growth in fluidic cell culture medium for 4 h. (A) Results of the cell apical surface. (B) Results of the cell basal surface. E-cadherin molecules were stained with green fluorescence and nuclei were stained with blue fluorescence.