

## Supplementary Information

**Supplementary Movie 1:** FRET response of actinin-sstFRET expressing MDCK cells to an increase of shear stress from 0.06 to 10 dyn/cm<sup>2</sup>, showing an increase in FRET at higher flow rate. The arrow (top left) indicates high flow rate. The images were collected every 5 s for total 180 s.

**Supplementary Movie 2:** FRET ratio of actinin-sstFRET expressing MDCK cells in response to repeated increase in flow rate. The flow rate was increased from 0.3 to 50 μL/min (0.06 to 10 dyn/cm<sup>2</sup>) three times (indicated by the white arrow). The images were collected every 5 s for total 675 s.

**Supplementary Movie 3:** FRET ratio of cytochalasin-D treated MDCK cells in response to repeated increase of flow rate. Two cells were recorded in the same field, showing a continuous increase in FRET in top cell and a diminished response in bottom cell. The flow rate was increased from 0.3 to 50 μL/min (0.06 to 10 dyn/cm<sup>2</sup>) three times. The images were collected every 5 s for total 675 s.

**Supplementary Movie 4:** Typical FRET changes in cytochalasin-D treated MDCK cells showing no change in FRET signal upon flow stimulation. The flow rate was changed from 0.3 to 50 μL/min (0.06 to 10 dyn/cm<sup>2</sup>) three times. The arrows indicate the high flow rate. The images were collected every 5 s for total 675 s.