Electric Supplementary Information for

Development of a FRET-based recombinant tension sensor to visualize cell–material interactions

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Table S1. Primer sequences for the construction of the plasmids pTrc[mKO-GPGGA8-mUKG-RGDS2], pTrc[mKO-RGDS2], and pTrc[mUKG-RGDS2].

Primer name	Sequence (5′–3′)
MCS-5	CGGATCCAAAGGGCCCTTTCATATGCCATGGAAAAAGCTTGGTAC
MCS-3	CAAGCTTTTTCCATGGCATATGTTTGGGCCCTTTGGATCCGAGCT
mKO/mUKG-5	GGGATCCAGATCT*ATGGTGAGCGTG*
mKO-3	**GGGGCCC***GGAGTGGGCCACGGCGTC*
mUKG-5	**G**CAT*ATGGTGAGCGTGATCAAGG*
mUKG-RGDS2-3	GAAGCTTA**ACTATCACCACGGCTATCACCACGC***TTGCTGGCCTGG CTG*
GPGGA8-1-5	**CGGAGGTGCAGGACCAGGAGGAGCAGGACCAGGTGGAGCAG GACCAGGTGGTGCTGGACCT**
GPGGA8-1-3	**ACCTGGTCCTGCTCCACCTGGTCCTGCTCCTCCTGGTCCTGCA CCTCCGGGCC**
GPGGA8-2-5	**GGAGGAGCAGGACCTGGAGGAGCAGGTCCAGGAGGAGCAGG ACCAGGAGGTGCACA**
GPGGA8-2-3	*TATG***TGCACCTCCTGGTCCTGCTCCTCCTGGACCTGCTCCTCCA GGTCCTGCTCCTCCAGGTCCAGCACC**
mKO-RGDS2-3	GAAGCTTA**ACTATCACCACGGCTATCACCACG***GGAGTGGGCCAC GG*

*Apa*I (GGGCCC), *Bam*HI (GGATCC), *Hin*dIII (AAGCTT), and *Nde*I (CATATG) are underlined; overhangs of *Apa*I, *Kpn*I (GGTACC), *Nde*I, and *Sac*I (GAGCTC) are underlined and gray; sequences matching the cDNA are italicized; sequences encoding $(RGDS)_2$ or $(GPGGA)_8$ are in bold.

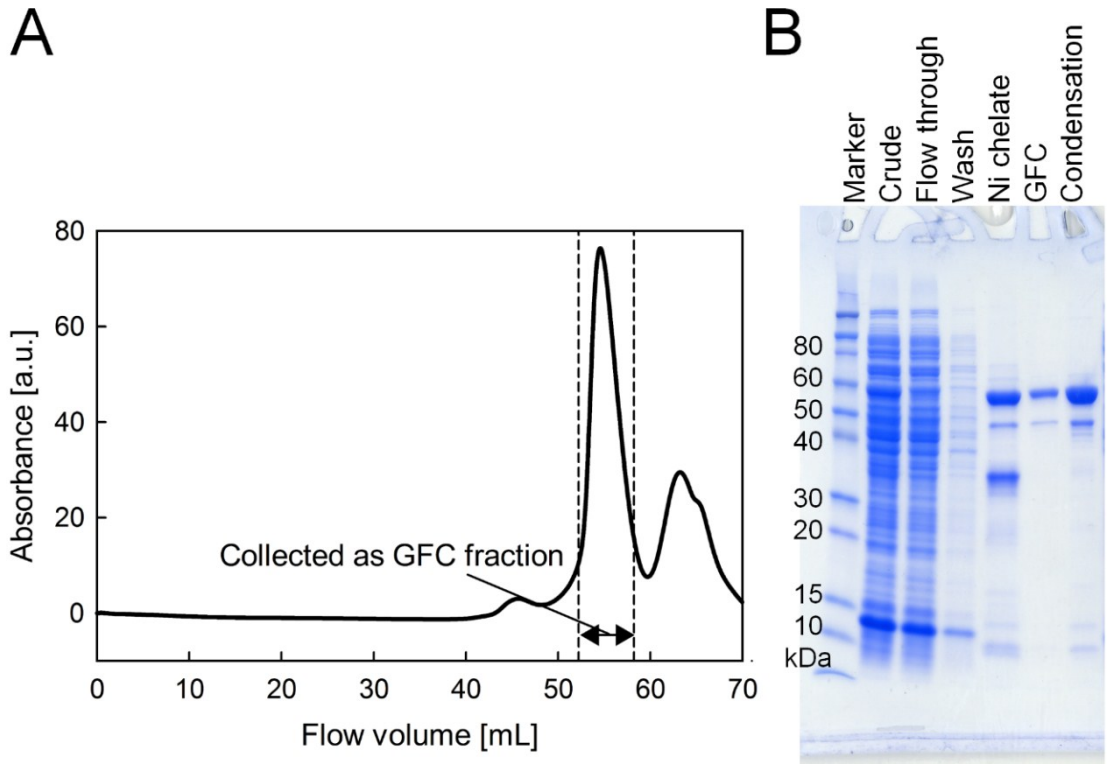

Figure S1. FPLC trace of rFRET-TS (A) and SDS-PAGE analysis (B) of solutions obtained in the rFRET-TS purification process.

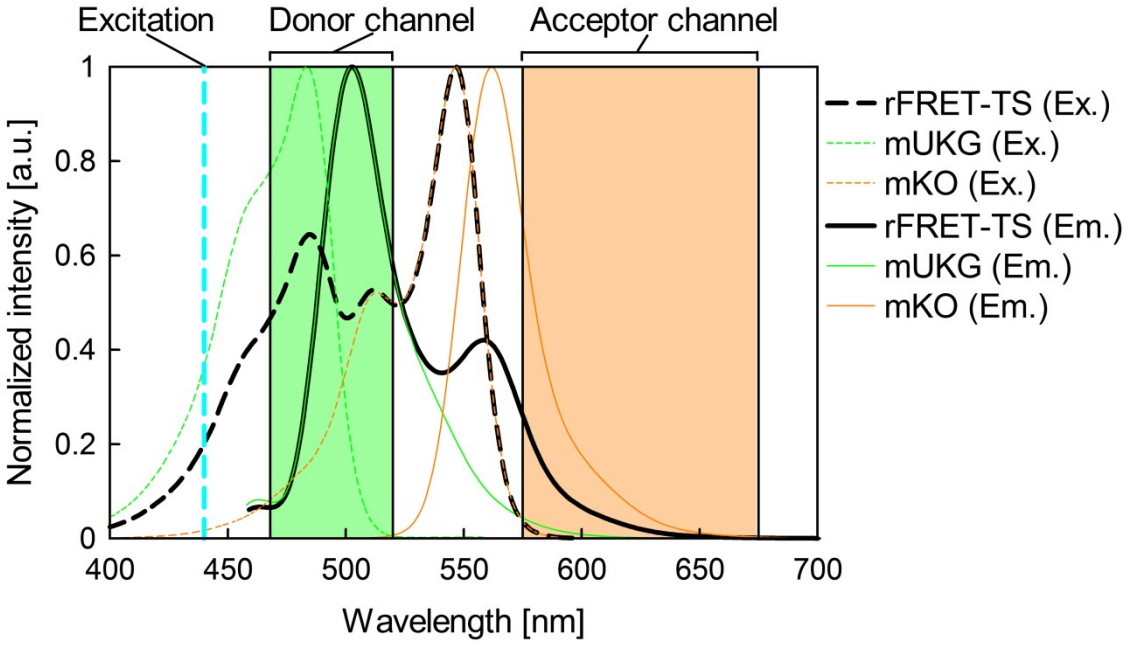

Figure S2. Excitation (Ex.) and emission (Em.) spectra of rFRET-TS, mUKG (donor), and mKO (acceptor), with CLSM settings for the fluorescent imaging of cells. The donor in rFRET-TS was excited selectively by a 440-nm laser. In addition, mUKG- and mKO-specific emissions were detected as donor and acceptor channels, respectively, by using appropriate dichroic mirrors and photomultiplier tubes with slits.

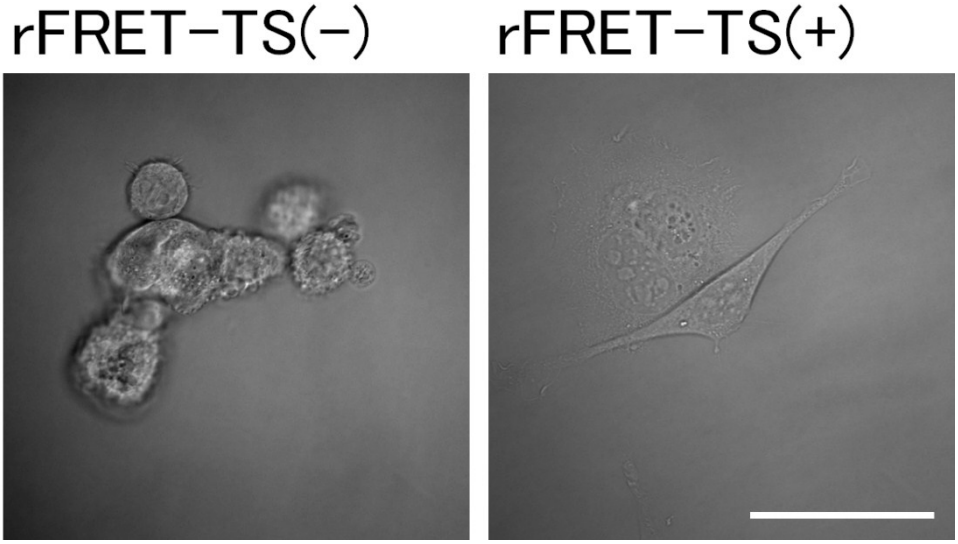

Figure S3. NIH3T3 fibroblasts seeded onto PEG-coated glass surfaces with (right) or without (left) the immobilization of rFRET-TS. Pictures were taken at 90 min post-seeding. Scale bar = 50 μm.

Fibroblasts did not adhere to the surface in the absence of rFRET-TS and showed a round shape. In contrast, cells adhered to the sensor-immobilized surface likely due to the cell-adhesion site in rFRET-TS ($[RGDS]_2$).

Movie S1. Movie of brightfield and FRET index images of fibroblasts on the rFRET-TS-immobilized glass surface. Donor and acceptor emissions were separated and detected simultaneously, with 440-nm excitation, at 5-min intervals. The donor and acceptor images were analyzed to obtain FRET index images by using FV10-ASW software (Olympus, Japan); the intensity of each pixel of the acceptor images was divided by that of the corresponding pixel of the donor images, with a chromatic modulation of 16. The bright and dark colors in the FRET index images indicate a low and high FRET index, respectively. Low FRET is induced by strong tension. The observations were finished within 24 h post cell-seeding.

Movie S2. Movie of brightfield and FRET index images of fibroblasts on the rFRET-TS-immobilized glass surface, with the actin depolymerizer cytochalasin D in the medium. Donor and acceptor emissions were separated and detected simultaneously, with 440-nm excitation, at 5-min intervals. Cytochalasin D was added to the medium at a final concentration of 4 μM immediately after one time point (i.e., at sixth frame), while imaging was continued. The observations were finished within 24 h post cell-seeding.