# Supporting Information

## Guo et al. 10.1073/pnas.1411683111

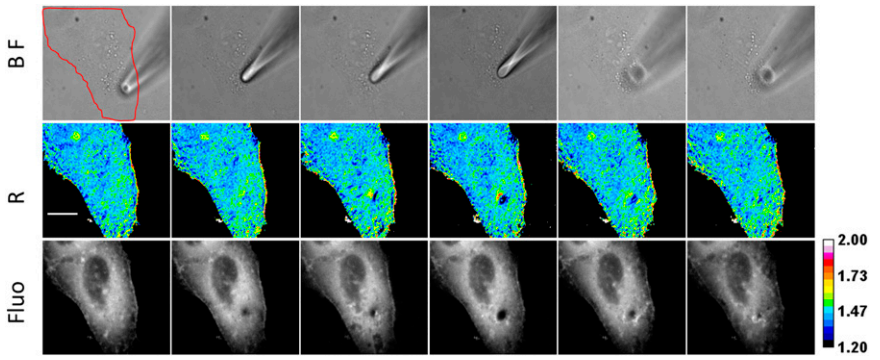



**Fig. S1.** MDCK cpA control cells pressed by micropipettes. Red line outlined the cell in bright-field image. BF, bright field; Fluo. fluorescence of FRET channel; R, anisotropy FRET ratio. *R* images are pseudocolored by an ImageJ 16-color map of 1.20–2.0. (Scale bar, 20 μm.)

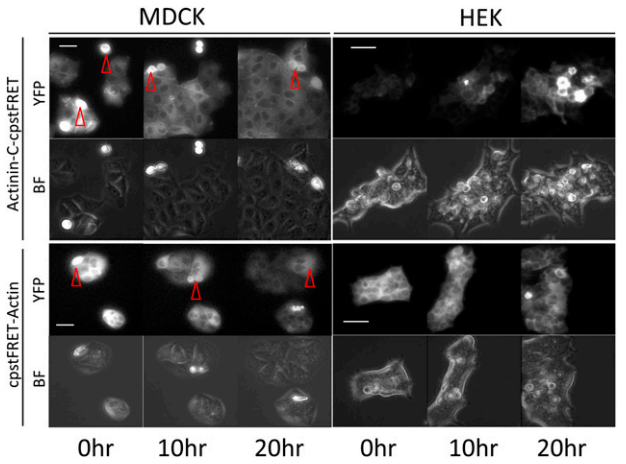



**Fig. S2.** Stable cell lines expressing cpstFRET–Actin and acitnin–C–cpstFRET chimeras show unaffected cell physiology. A total of 13 cell lines were created in this work (Table S1). MDCK and HEK stable cell lines expressing actin and actinin constructs were cultured in media in 5% $CO_2$ chamber on a heated stage. A 20-h time lapse of each cell line was recorded to monitor the cell proliferation. YFP, YFP channel signal from cpVenus. Using the Zeiss Definite Focus, we monitored at least five cell colonies simultaneously. All cells went through mitosis and proliferation. Arrowheads point at dividing cells. (Scale bar, 50 μm.)

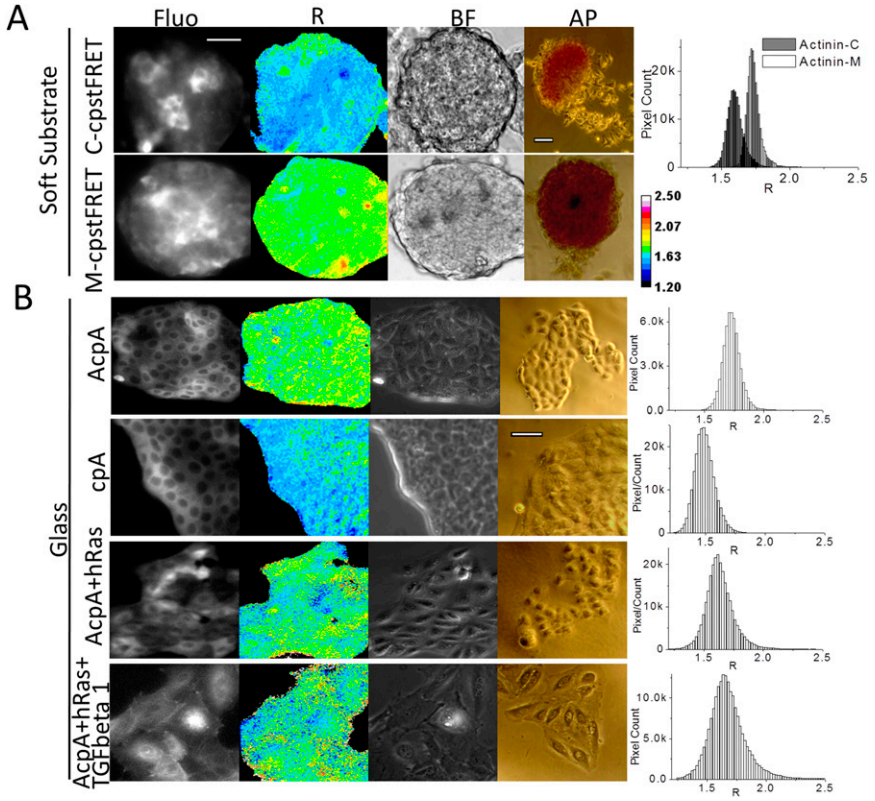


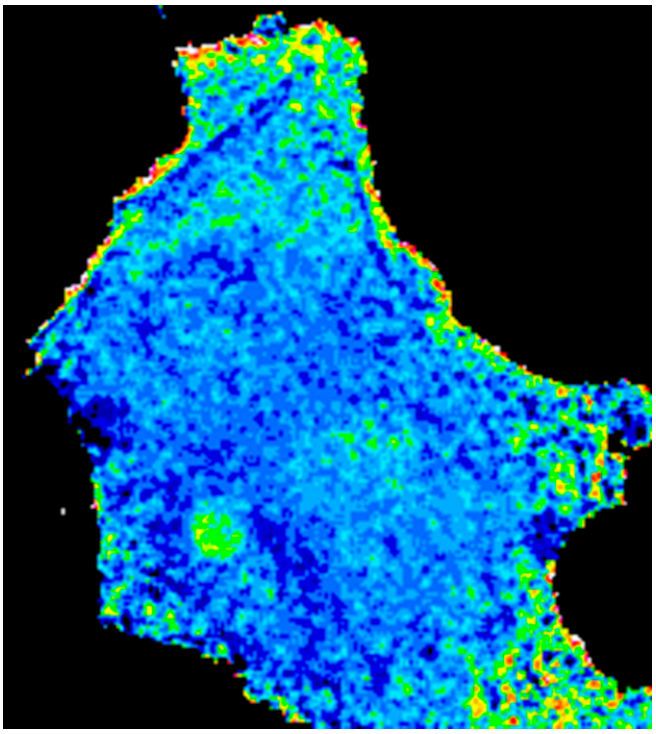

**Fig. S3.** Actinin cytoskeleton stress in the stem cells reprogrammed from HEK and MDCK stable cell lines. (*A*) HEK actinin stable cell lines cultured on soft substrate (PDMS). M–cpstFRET, actinin–M–cpstFRET; C–cpstFRET, Actinin–C–cpstFRET. The stress in actinin in EBs remained at resting stress level. AP, alkaline phosphatase staining of EBs and cells; BF, bright-field images of embryonic bodies and cells; Fluo, FRET signal; *R* anisotropy ratio represents stress in actinin. Pixel count distribution plots on the right were generated from all pixels from at least 10 *R* images. (Scale bar, 50 μm.) (AP scale bar, 100 μm.) (*B*) MDCK actin stable cell lines cultured on glass. AcpA, MDCK stable line expressing actin–cpstFRET–actin cassette; cpA, MDCK stable line expressing cpstFRET–actin; +hRas, MDCK stable cell lines expressing hRas gene. hRas MDCK stable cell lines were derived from MDCK AcpA or cpA stable cell lines. +TGFbeta 1, cell cultures were supplied with 5 μg/mL of TGF-β1. Pixel count distribution plots on the right were created using all pixel values from at least 10 *R* images. All *R* images are presented with an ImageJ 16-color map of 1.20–2.50. (Scale bar, 50 μm.) (AP scale bar, 100 μm.)

**Table S1. Stable cell lines**

| Parental cell line | Gene | Plasmid constructs | Antibiotic, concentration |
|---|---|---|---|
| HEK | Actinin–M–cpstFRET | pEG–Actinin–M–cpstFRET, created in F.S. laboratory | Geneticin, 600 μg/mL |
| HEK | Actinin–C–cpstFRET | pEG–Actinin–C–cpstFRET, created in F.S. laboratory | Geneticin, 600 μg/mL |
| HEK | Actin–cpstFRET–Actin | pEG–Actin–cpstFRET–Actin, created in F.S. laboratory | Geneticin, 600μg/mL |
| HEK | cpstFRET–Actin | pEG–cpstFRET–Actin, created in F.S. laboratory | Geneticin, 600 μg/mL |
| MDCK | Actin–cpstFRET–Actin | pEG–Actin–cpstFRET–Actin, created in F.S. laboratory | Geneticin, 800 μg/mL |
| MDCK | cpstFRET–Actin | pEG–cpstFRET–Actin, created in F.S. laboratory | Geneticin, 800 μg/mL |
| MDCK | Actinin–M–cpstFRET | pEG–Actinin–M–cpstFRET, created in F.S. laboratory | Geneticin, 800 μg/mL |
| MDCK | Actinin–C–cpstFRET | pEG–Actinin–C–cpstFRET, created in F.S. laboratory | Geneticin, 800 μg/mL |
| MDCK (Actin–cpstFRET–Actin) | hRas | pBabe–puro Ras V12, Addgene | Puromycin, 2 μg/mL |
| MDCK (cpstFRET–Actin) | hRas | pBabe–puro Ras V12, Addgene | Puromycin, 2 μg/mL |
| HEK | Snail | pBabe–puro Snail, Addgene | Puromycin, 2 μg/mL |
| HEK | E-cadherin shRNA | pLKO.1 hygro shRNA E-cadherin, Addgene | Hygromycin, 100 μg/mL |
| HEK | E-caderin | pWZL blast E-cadherin, Addgene | Blasticidin, 5 μg/mL |

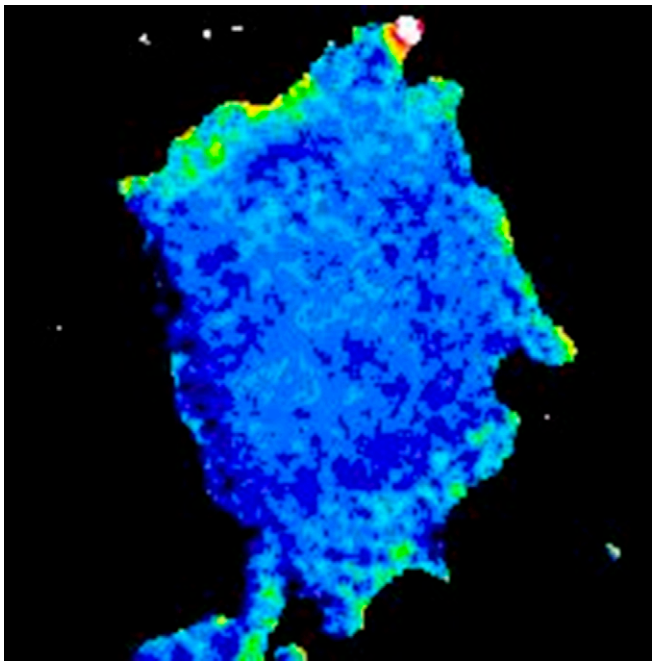
**Table S2. Primers for gene constructs**

| Plasmid constructs | Vector | Primers | Direction | Sequence, 5′–3′ |
|---|---|---|---|---|
| pEG–cpstFRET | pEGFP-C1 | BamH1 cpstFRET | Forward | GTACCAGGATCCATGGGCGGCGTGCAG |
| | pEGFP-C1 | Apa1 cpstFRET | Reverse | GGATCCCGGGCCCTTTAACTACCGCGTGGCAC |
| pET–cpVenus | PET-52b+ | BamH1 cpVenus | Forward | GGTACCAGGATCCATGGGCGGCGTGCAGCTCGC |
| | PET-52b+ | Sac1 cpVenus | Reverse | CCAGAGCGAGCTCGTCCTCGATGTTGTGGCGGATC |
| pET–cpCerulean | PET-52b+ | BamH1 cpCerulean | Forward | GTACCAGGATCCATGGGCAGCGTGCAGCTCGCCGACC |
| | PET-52b+ | Sac1 cpCerulean | Reverse | GGCACCAGAGCGAGCTCGTCCTCGATGTTGTGGCGGATC |
| pET–cpstFRET | pET-52b+ | BamH1 cpstFRET | Forward | GGTACCAGGATCCATGGGCGGCGTGCAGCTCGCCG |
| | pET-52b+ | Sac1 cpstFRET | Reverse | CCAGAGCGAGCTCGTCCTCGATGTTGTGGCG |
| pEG–Actinin | pEGFP-C1 | Nhe1 Actinin | Forward | AGATCCGCTAGCATGGACCATTATGATTCTCAGCAAACC |
| | pEGFP-C1 | Kpn1 Actinin | Reverse | GCCCGCGGTACCTTAGAGGTCACTCTCGCCGTACAGC |
| pEG–Actinin–M–cpstFRET | pEGFP-C1 | Age1 cpstFRET | Forward | GAGAACCGGACCGGTATGGGCGGCGTGCAGCTCGCCG |
| | pEGFP-C1 | Not1 cpstFRET | Reverse | CGGGCACGCGGCCGCTACTACCGCGTGGCACCAGAGCGAGCTCG |
| pEG–Actinin–C–cpstFRET | pEGFP-C1 | Age1 cpstFRET | Forward | GTGACCTCACCGGTATGGGCGGCGTGCAGCTCGCCGACCACTACC |
| | pEGFP-C1 | Not1 cpstFRET | Reverse | GTACCTTAGCGGCCGCTACTACCGCGTGGCACCAGAGCGAG |
| pEG–cpstFRET–β-Actin | pEGFP-C1 | Sac1 Actin | Forward | CGAGGACGAGCTCGGTGGGGGAGGAGGTATGGATGATGATATCGCC-GCGCTCG |
| | pEGFP-C1 | Apa1 Actin | Reverse | GTGGATCCCGGGCCCTTTAGAAGCATTTGCGGTGGACGATGGAGGGGC |
| pEG–β-Actin–cpstFRET–β-Actin | pEGFP-C1 | Nhe1 Actin | Forward | CAGATCCGCTAGCATGGATGATGATATCGCCGCGCTCGTCGTCGAC |
| | pEGFP-C1 | Kpn1 Actin | Reverse | GATCCTGGTACCCCGCCTCCTCCGAAGCATTTGCGGTGGACGATGGAG-GGGCCG |



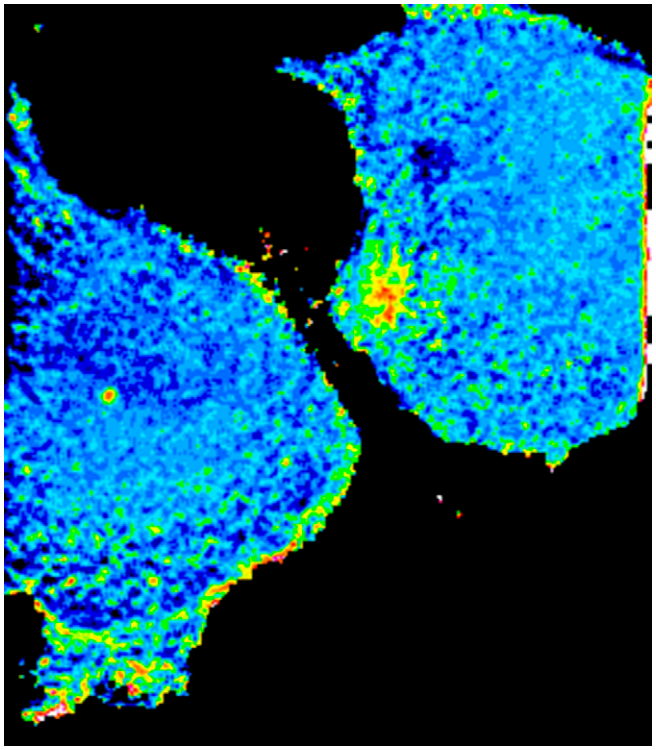
**Movie S1.** Anisotropy ratio *R* video of cpA-expressing MDCK cells challenged by anisotonic osmotic pressure. The video is presented with a 16-color map of ImageJ with the same range as in Fig. 3. Inset words show the time points of administration of distilled water and exchanging back to saline.

Movie S1



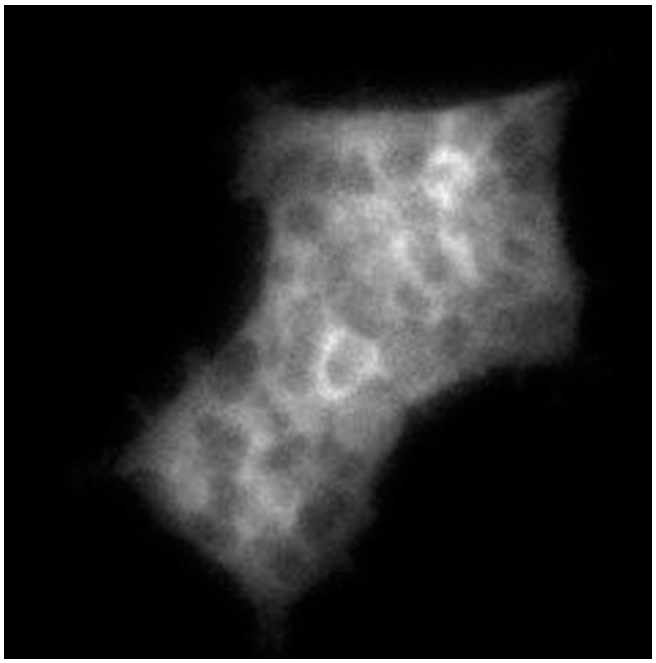
**Movie S2.** Anisotropy ratio *R* video of cpA-expressing HEK cells treated by 5 μM cytochalasin D. The video is presented with a 16-color map of ImageJ with the same range as in Fig. 3. Inset words show the time points of administration of the drug and washoff.

Movie S2



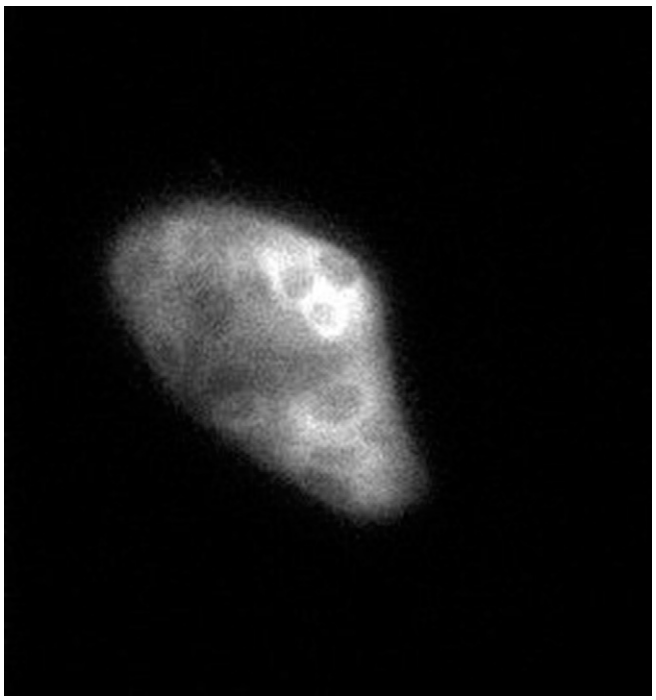
**Movie S3.** Anisotropy ratio *R* video of cpA-expressing HEK cells under the treatments of the 10 mM of caffeine. The video is presented with a 16-color map of ImageJ with the same range as in Fig. 3. Inset words show the time points of administration of the drug and washoff.

Movie S3



**Movie S4.** A 20-h time-lapse sequence video of AcpA-expressing HEK cells going through division and proliferation. The video is presented with the YFP channel from cpVenus.

Movie S4



**Movie S5.** A 20-h time-lapse sequence video of AcpA-expressing MDCK cells going through division and proliferation. The video is presented with the YFP channel from cpVenus.

Movie S5