**Supporting Information:** 

## Substrate stiffness coupling TGF-\u00df1 modulates migration and traction force of

## MDA-MB-231 human breast cancer cells in vitro

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Figure S1:



Figure S1: Wound healing rate between the first and second 12 hours

(A) Bar plot shows that there was no difference in wound healing rate of MDA-MB-231 cultured on increasing stiffness substrates without TGF- $\beta$ 1 treatment between the first and second 12 hours.

(B) Bar plot shows that the wound healing rate of MDA-MB-231 cultured on increasing stiffness substrates with TGF- $\beta$ 1 treatment of the second 12 hours was significantly lower than that of the first 12 hours. (\*\*p<0.01, \*\*\*p<0.001)

Figure S2:



Figure S2: Migration behavior of MCF7 on PA gel with different stiffness and either with or not TGF-β1 treatment, and traction force generated by MCF7 in response to TGF-β1

(A-C) Bar plots show that migration speed (A), MSD (B), and migration persistence ( $\beta$  index; C) of MCF7 cultured on stiffer substrate (60 kPa) were significantly larger than that of MCF7 cultured on softer substrate (5 kPa), and the migration behaviors were further enhanced with the addition of TGF- $\beta$ 1.

**(D)** Strain energy displays the traction force generation of MCF7 in response to TGF-β1. (\*p<0.05, \*\*p<0.01, \*\*\*p<0.001)

Figure S3:



Figure S3: Traction force generation of MDA-MB-231 cultured on 5 kPa gel either with or not TGF-β1 treatment

Bar plot showing the strain energy of MDA-MB-231 cultured on 5 kPa PA gel either without (referred as control) and with TGF- $\beta$ 1 (referred as +TGF- $\beta$ 1) treatment. (\*p<0.05)

Figure S4:



Figure S4: The effects of substrate stiffness and TGF-β1 on focal adhesion (FA) structure

(A-C) Quantitative analysis of focal adhesion (FA) area (A), shape index (B), and length (C) of MDA-MB-231 cultured on increasing stiffness substrate and either with or not TGF- $\beta$ 1 treatment corresponding to the images shown in figure 6. (\*\*p<0.01, \*\*\*p<0.001)