

## Supplementary Material

### **Blood Droplet-Based Cancer Diagnosis via Proteolytic Activity Measurement in Cancer Progression**

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### **S1. Atomic Force Microscopy (AFM) Imaging of the Surface of Nanomechanical Biosensor**

In order to verify the MMP-driven proteolysis that occurs on the surface of a nanomechanical biosensor, we consider the AFM imaging that is useful in visualization of the surface. Figure S1a shows the AFM images of the surface of a bare biosensor, peptide-functionalized biosensor, and the functionalized biosensor exposed to MMP2, respectively. It is shown that peptide functionalization of a bare biosensor (with its AFM height of ~1 nm) increases the AFM height into ~20 nm due to the size of peptide chain, while the exposure of

the peptide-functionalized biosensor to MMP2 decreases the AFM height into  $\sim 10$  nm owing to the length of cleaved peptide chain (Figure S1b). In addition, it is found that peptide functionalization of a bare biosensor increases its surface roughness into 3 nm, while the exposure of the peptide-functionalized biosensor to MMP2 reduces its surface roughness into  $\sim 1.7$  nm (Figure S1c). These AFM results confirm that MMP2-driven proteolysis occurs on the surface of the nanomechanical biosensor.

## **S2. Negative Control Experiments**

In order to validate the ability of our nanomechanical biosensor to interact with only MMP2, we consider negative control experiments by measuring the frequency dynamics of the biosensor when it was exposed to other MMP family such as MMP3, MMP9, and MMP14. As shown in Figure S2, the frequency dynamics of the biosensor was not affected by MMP3, MMP9, or MMP14, which confirms that the frequency dynamics of our biosensor is responsive only to MMP2.

## **S3. Nanomechanical Bioassay Using LLC-Transplanted Mouse Model**

As described in the paper, before we conducted the nanomechanical bioassay using H460-transplanted mouse model, we performed the nanomechanical bioassay with using LLC-transplanted mouse model. Figure S3a shows the mouse model, to which LLC (Lewis lung carcinoma) cells were transplanted. When compared with normal mouse, the tumor was clearly seen for LLC-transplanted mouse model (Figure S3a). The tumors at two different stages are shown in Figure S3b, and the weight of tumors at these two stages is provided in Figure S3c. Our results of nanomechanical bioassay are shown in Figure S3d, which suggests that the kinetics of MMP2-driven proteolysis is dependent on the tumor growth state. In particular, the total mass of cleaved peptide chains and the kinetic rate of proteolysis are shown to depend on the tumor growth state (Figure S3e).

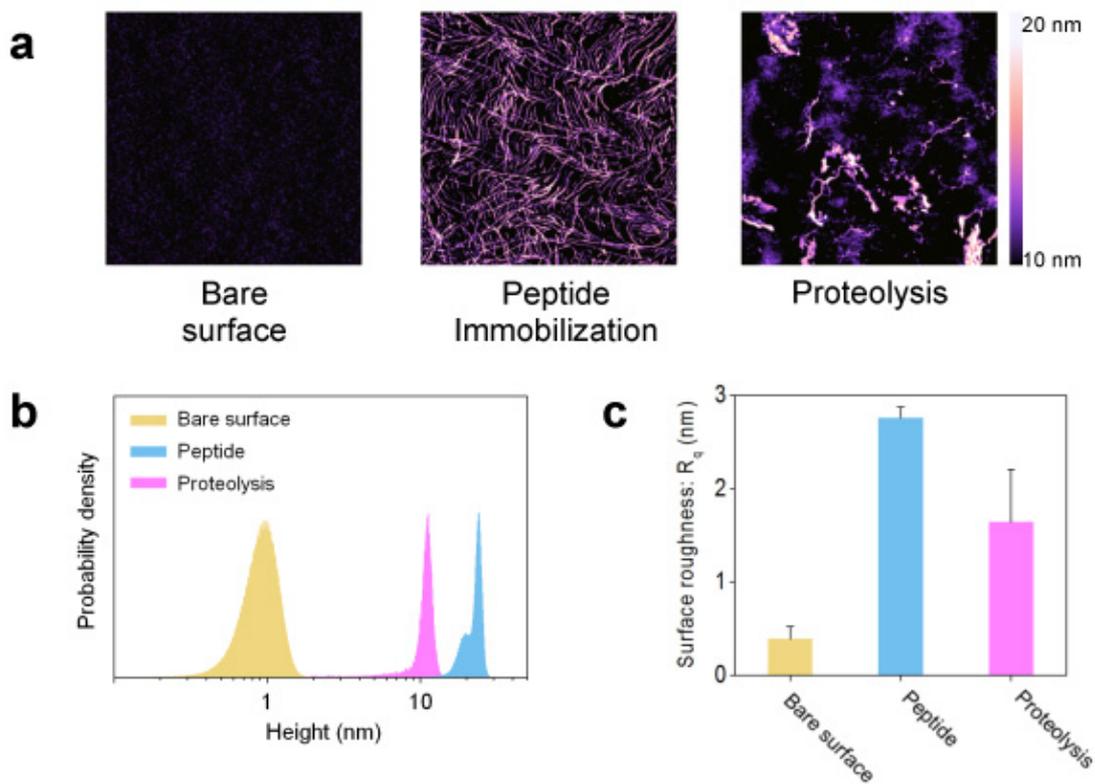
## **S4. Nanomechanical Quantitation of the Proteolytic Activity of MMP2 for Cancer Patients**

In this work, we consider 15 patients suffering from lung cancer at the same stage IV but different metastasis level. The lung of these cancer patients were shown in Figure S4.

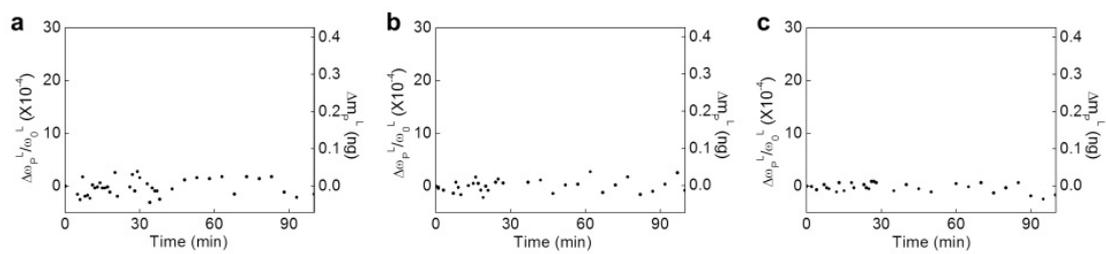
To validate the robustness of nanomechanical biosensor-based cancer diagnosis, we consider negative control experiments with using the blood droplet of 6 normal people. Figure S5 shows the frequency response of the biosensor when it was exposed to the blood droplet of normal people, who do not suffer from any cancer. It is shown that the resonant frequency of the biosensor was not affected when it was exposed to the blood droplet of normal people.

This suggests that our nanomechanical biosensor does not respond to the blood droplet of normal people.

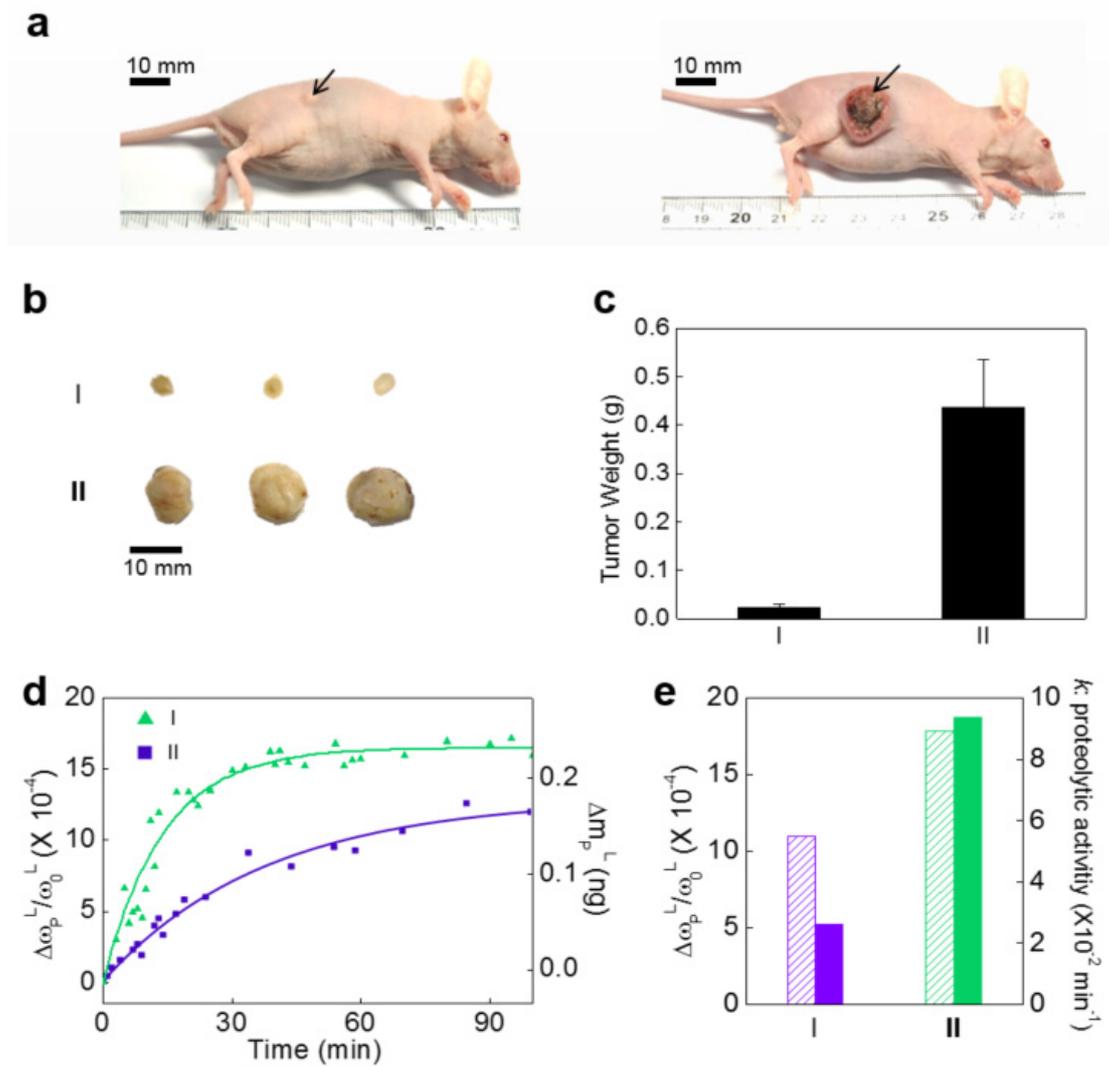
The frequency response of the nanomechanical biosensor to injection of the blood droplet of 15 cancer patients is shown in Figure S6. For all patients, the in situ frequency shift due to MMP2 is well dictated by Langmuir kinetic model. However, the total mass of cleaved peptide chains and the kinetic rate of proteolysis are varying between cancer patients, which is attributed to the different level of cancer metastasis (see the paper).



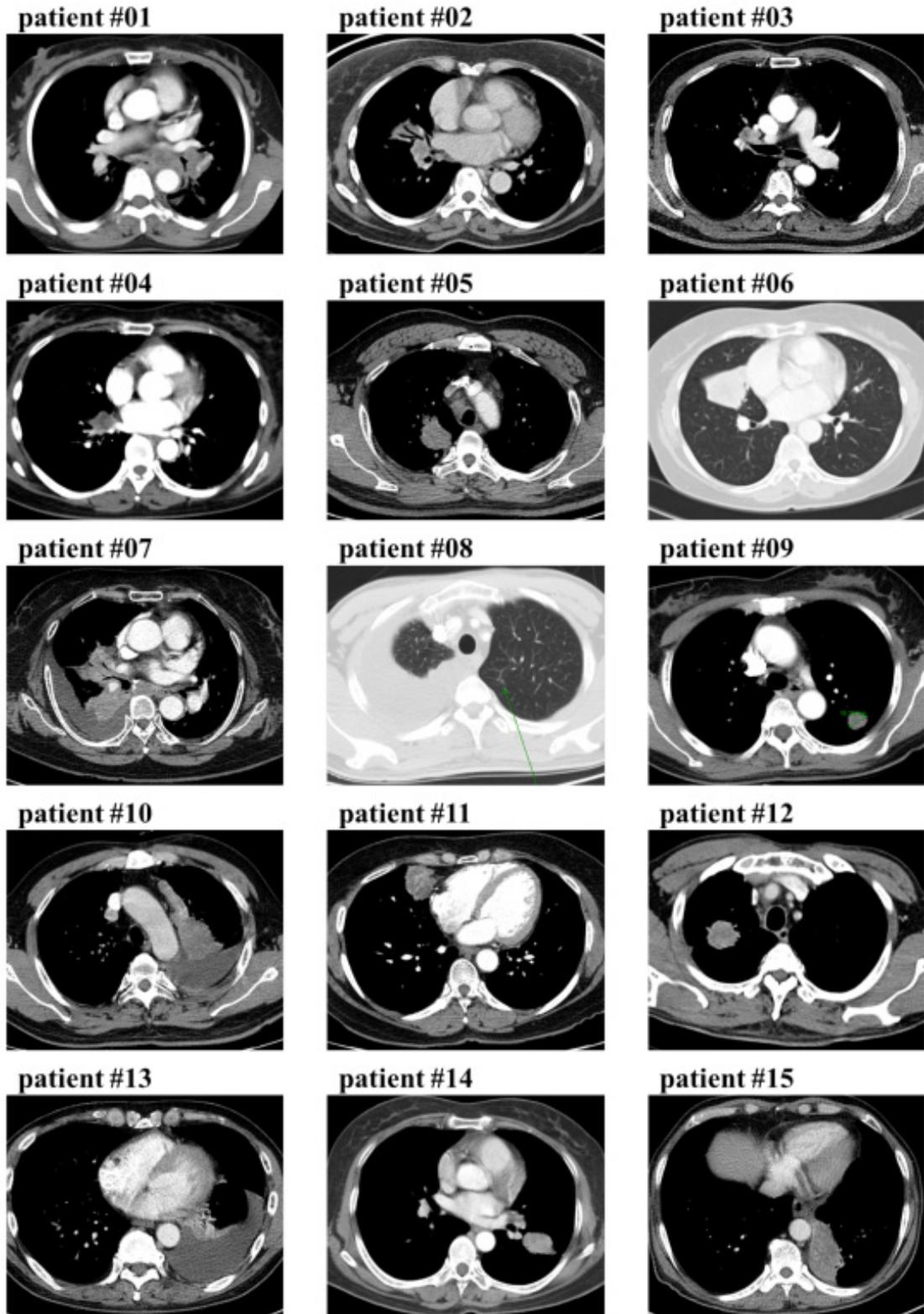
**Figure S1.** (a) AFM images of the surface of a bare biosensor (left), peptide-functionalized biosensor (middle), and the functionalized biosensor exposed to MMP2 (right), respectively. (b) Probability distribution of AFM heights for the bare biosensor (yellow), peptide-functionalized biosensor (blue), and the functionalized biosensor exposed to MMP2 (pink), respectively. (c) Surface roughness for the bare biosensor, peptide-functionalized biosensor, and the functionalized biosensor exposed to MMP2, respectively.



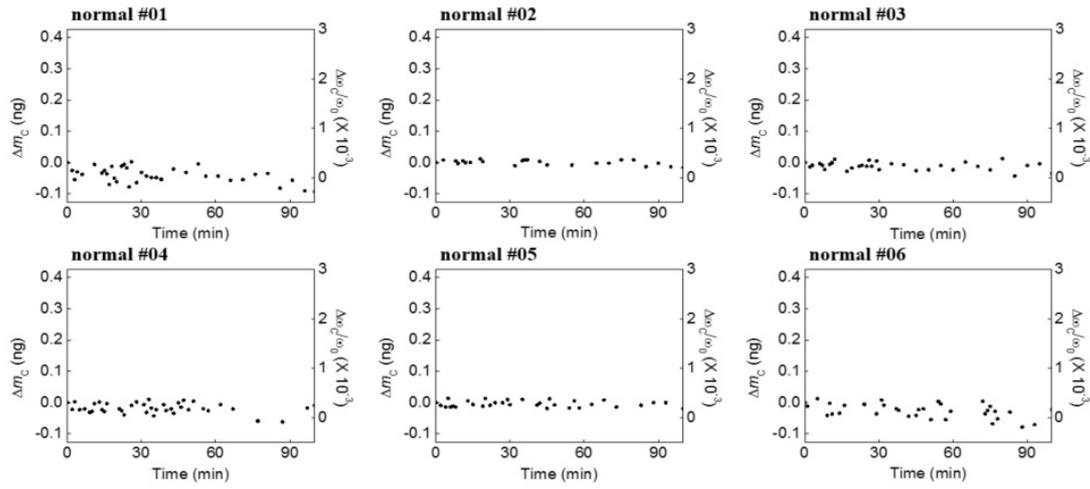
**Figure S2.** Negative control experiments: The frequency dynamics of a nanomechanical biosensor was monitored when it was exposed to (a) MMP3, (b) MMP9, or (c) MMP14.



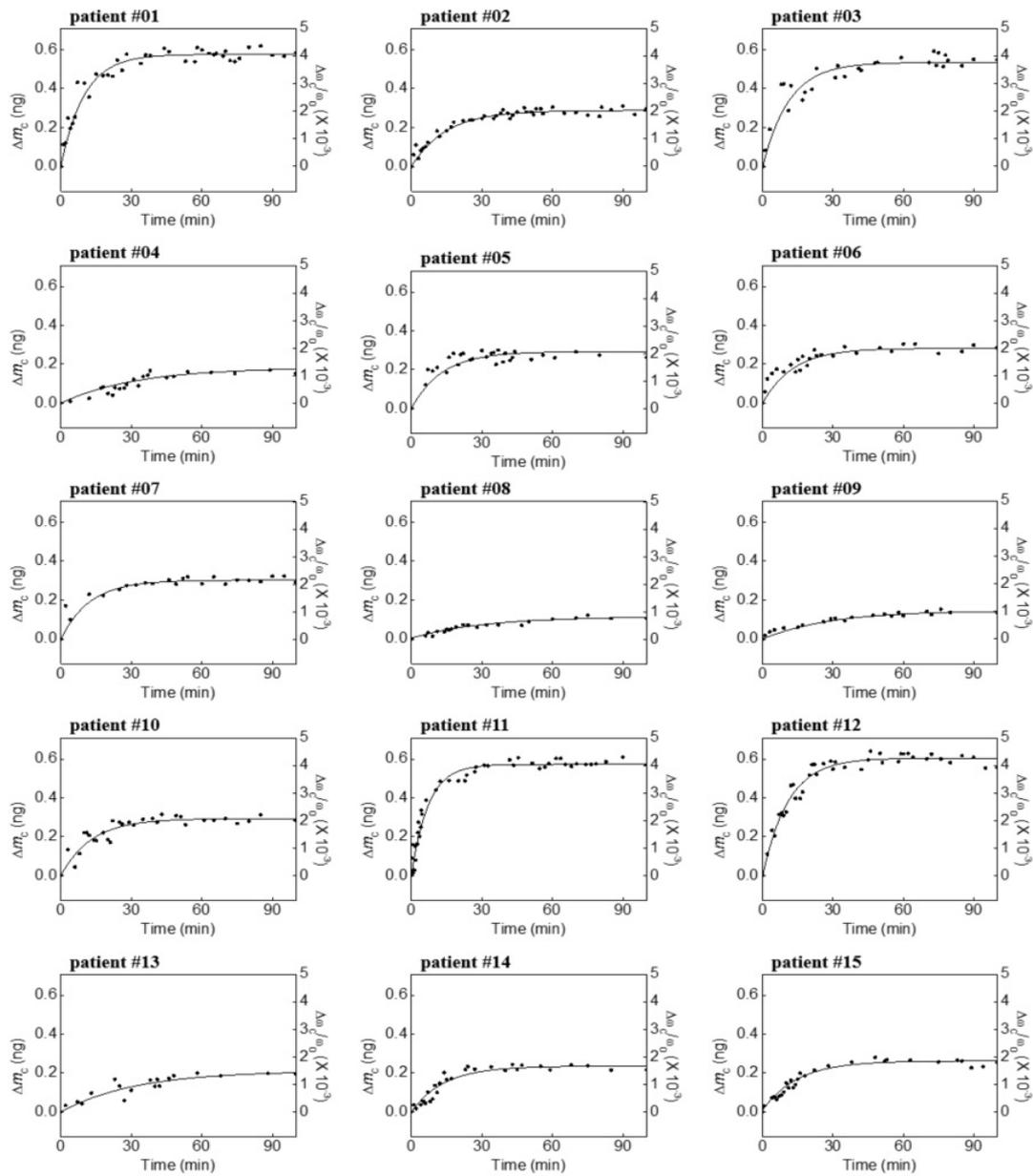
**Figure S3.** (a) Photographic images of normal mouse (left) and LLC-transplanted mouse (right), respectively. (b) Photographic images of tumors that appear on the mouse model at two different tumor growth states. (c) The weight of tumors at two different stages. (d) In situ real-time measurement of the frequency shift (or equivalently, the mass of cleaved peptide chains) due to MMP2 that is secreted from tumors appearing in the mouse model at two different tumor growth states. (e) Dependence of the total mass of cleaved peptide chains and the kinetic rate of proteolysis on the tumor growth states.



**Figure S4.** Computed tomography (CT) images of the lung of 15 cancer patients. The level of cancer metastasis is varying between patients.



**Figure S5.** Negative control experiments using the blood droplet of normal people. The frequency dynamics of a nanomechanical biosensor is not affected by injection of the blood droplet of normal people who do not suffer from any cancer.



**Figure S6.** In situ measurement of frequency shift (or equivalently, the mass of cleaved peptide chains) due to MMP2 that is likely to be secreted in the blood droplet of cancer patients. The measured frequency shifts (indicated as dots) for 15 cancer patients are well dictated by Langmuir kinetic model (represented as solid line). The value of frequency shift due to MMP2 varies between cancer patients, which is attributed to the different level of cancer metastasis (see the paper).