## **Supplementary Information**

## Detection of nuclease activity using a simple fluorescence based biosensor

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**Figure S1** shows the emission intensity in absence and presence of the nuclease. At different substrate concentrations, initial signal obtained in the absence of enzyme is also different. This signal stays constant until the addition of enzyme, and then, depending on the enzyme amount, the signal increases as expected. This trend proves that the signal changing is solely dependent on the enzyme activities.



Figure S1: Emission intensity in the absence and presence of the nuclease enzyme.

We investigated the best time point to obtain the highest sensitivity in our measurements. In the analysis, we used data points between 0- 1250 U/mL to obtain the highest sensitivity as fast as possible. It was found that the lower enzyme concentrations have larger fluorescence response at earlier time points (**Figure S2**). Therefore, the average rate of intensity change was plotted as a function of enzyme activity and the highest sensitivity was obtained 6 minute after the addition of the enzyme. The highest sensitivity of this system was obtained between 25 U - 750 U/mL of S1 nuclease enzymes as shown by the inset.



Figure S2: Time dependent analysis of emission intensity.

The **Figure S3** shows a linear range with its R2 (0.9906) value within the period selected for the highest sensitivity. On other time points, this value decreases dramatically, as the lower concentrations of enzymes give larger fluorescence response at earlier time points. The signal difference between lower and higher concentration of enzymes increases depending on time (**Figure S3 (A-F) below**). The highest sensitivity is therefore obtained in 6 minutes following the enzyme addition.



A: Emission intensity with respect to enzyme activity in 6 minutes after enzyme addition.



**B:** Emission intensity with respect to enzyme activity in 7 minutes after enzyme addition.



C: Emission intensity with respect to enzyme activity in 8 minutes after enzyme addition.



**D:** Emission intensity with respect to enzyme activity in 10 minutes after enzyme addition.



E: Emission intensity with respect to enzyme activity in 30 minutes after enzyme addition.



**F:** Emission intensity with respect to enzyme activity in 60 minutes after enzyme addition.