

Supplementary Materials

Legends for Supplementary Figures and Movies

Figure S1

The scheme shows the general mechanism of calcium signaling stimulated by ATP. The lower right inset represents a typical time course of calcium response upon ATP stimulation.

Figure S2

A schematic cartoon of the activation mechanism of the Calcium FRET biosensor. This genetically encoded biosensor consists of an N-terminal ECFP, a Calmodulin (CaM), a M13, and a C-terminal YPet (a variant of EYFP). The ECFP and YPet are positioned separately with a weak FRET when calcium is absent. Calcium can cause the binding between CaM and M13. This action can result in the proximal position of ECFP and YPet to cause an increase of FRET. Hence, the emission ratio of I_{YPet}/I_{ECFP} with the excitation of ECFP should serve as a good indicator of calcium concentration.

Figure S3

(A) An image of micro-patterns on silicon wafer created by photolithography is shown on the left and an image of micro-patterns on cover glass surface generated by micro-contact printing is shown on the right. (B) A DIC image of a HUVEC cultured on a micro-patterned circle (left) and a representative YPet/ECFP emission ratio image of the cytosolic calcium biosensor expressed in this cell (right).

Figure S4

The decay curves of calcium signals upon ATP stimulation in HUVECs cultured on glass surface without patterns and under different treatments as indicated. The mean

decay curve (solid line) and the 95% confidence bands (dashed lines) under each condition were calculated using the LOESS method.

Figure S5

DIC images of two connected HUVECs on glass surface without patterns (left) or within a micro-patterned circle.

Movie 1: The YPet/ECFP emission ratio images of the cytosolic calcium biosensor in two connected HUVECs on glass surface without pattern.

Movie 2: The YPet/ECFP emission ratio images of the cytosolic calcium biosensor in two connected HUVECs within a micro-patterned circle.