Figure S2
Confirmation that YC-nano-15 senses cytosolic Ca\(^{2+}\) levels

Ax2 cells expressing YC-nano-15 were treated with ionomycin (20 mM), a Ca\(^{2+}\) ionophore, in the presence of either 20 mM CaCl\(_2\) or EGTA for 15 min (19). The \([\text{Ca}^{2+}]_c\) is detected by FRET which is observed as the ratio of YFP/CFP emission. During incubation with ionomycin and EGTA, the ratio reduced and remained low for the duration of the experiment (15 min, data not shown). In the presence of high Ca\(^{2+}\), the ratio increased and peaked within 7.5 minutes, remaining high for the duration of the experiment (15 min, data not shown). These data demonstrate that the YFP/CFP emission ratio of YC-Nano-15 is responding to changes of intracellular Ca\(^{2+}\) as expected. Scale bar, 50 µm.