Electronic Supplementary Information

Identification of Type of Membrane Injury and Cell Death using Whole Cells-Based Proton-Sensitive Field-Effect Transistor Systems

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Hemolysis assay

Plasma components and buffy coat in defibrinated fresh sheep blood (Nippon Bio-test Laboratories, Tokyo, Japan) were removed by centrifugation at 1500 × *g* for 5 min. The isolated erythrocytes were washed three times with isotonic Tris-buffered saline (1 × TBS, pH 7.4). Four volumes of washed and packed erythrocytes was diluted with three volumes of TBS to produce a 57% hematocrit solution. Then, 75 μL of TBS containing a desired concentration of chemical compound was added to 175 μL of the erythrocyte solution (final hematocrit: 40%), followed by incubation on ice for 20 min, after which the supernatant was isolated by centrifugation at 1500 ×*g* for 5 min at 4°C. The amount of hemoglobin was determined from the absorbance at 543 nm (A_{543}) using a microplate reader (infiniteM200, Tecan, Mannedorf, Switzerland). The erythrocytes with water (100% hemolysis) and TBS (0% hemolysis) served as positive (p.c.) and negative (n.c.) controls. The degree of hemolysis was calculated as

$$[\text{Hemolysis}] = (A_{543} - A_{543,n.c.})/(A_{543,p.c.} - A_{543,n.c.})$$

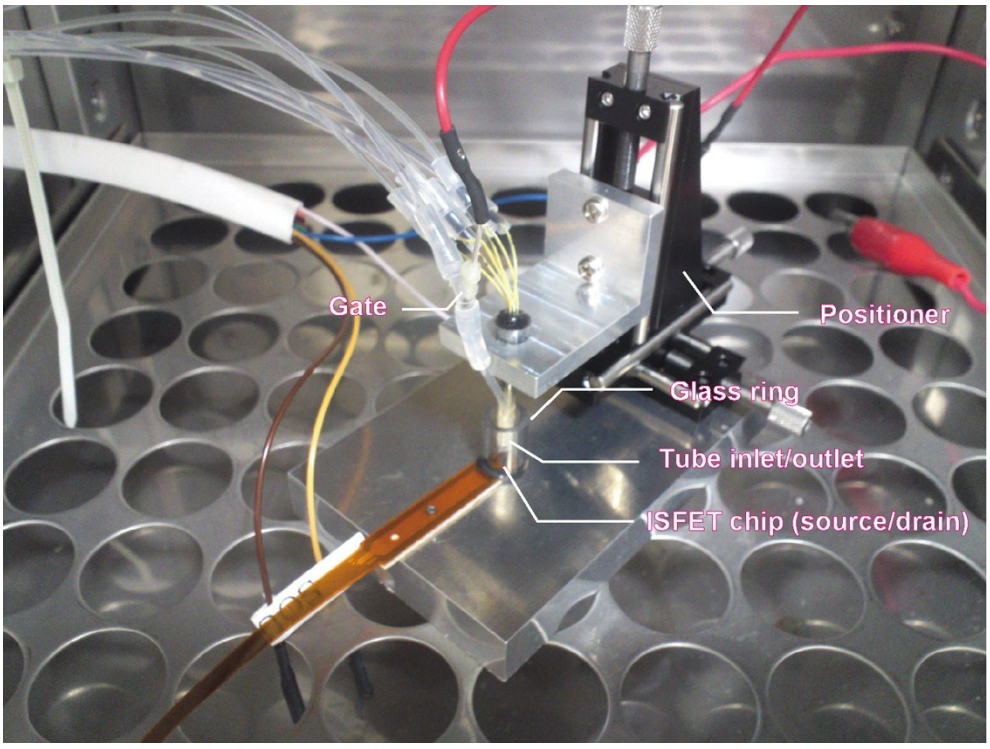

Fig. S1. A picture of the whole system including the sensor and well.

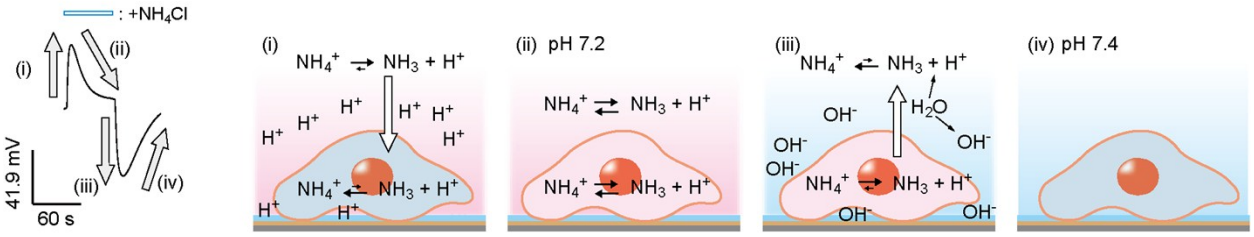

Fig. S2. Schematic illustrations explaining the mechanism for transient potential peaks during the exchange of NH_4Cl in the extracellular space.

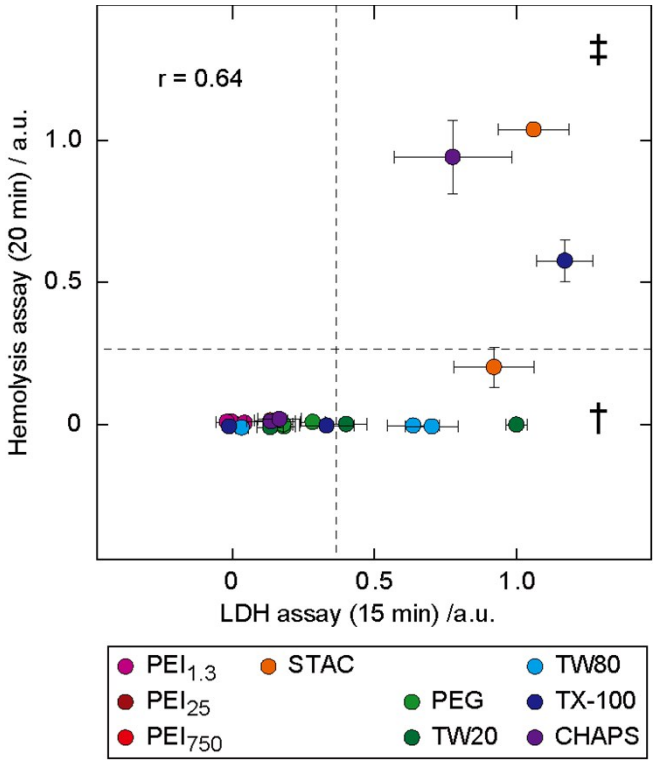

Fig. S3. Scattered plots between the hemolysis (20 min) and LDH (15 min) assays. † and ‡ represent the hemolysis^{-}/LDH^{+} and hemolysis^{+}/LDH^{+} regimes, respectively. Data points identify the two signals at set concentrations with the mean ± SD (n = 3). LDH signals were normalized by those obtained at 1.0 mg mL^{-1} TW20 for 15 min. Colours in symbol show chemical species. Dashed lines represent the thresholds. Correlation coefficient: r.

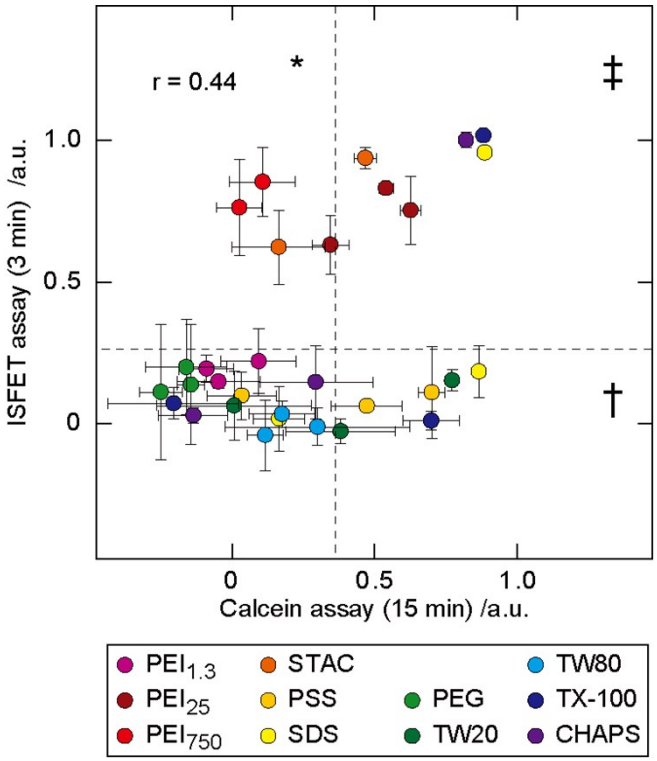

Fig. S4. Scattered plots between the ISFET assay and the calcein release assay. *, †, and ‡ represent the ISFET^{+}/calcein^{-}, ISFET^{-}/calcein^{+}, and ISFET^{+}/calcein^{+} regimes, respectively. The plots show the mean ± SD (n=3). r: correlation coefficient.

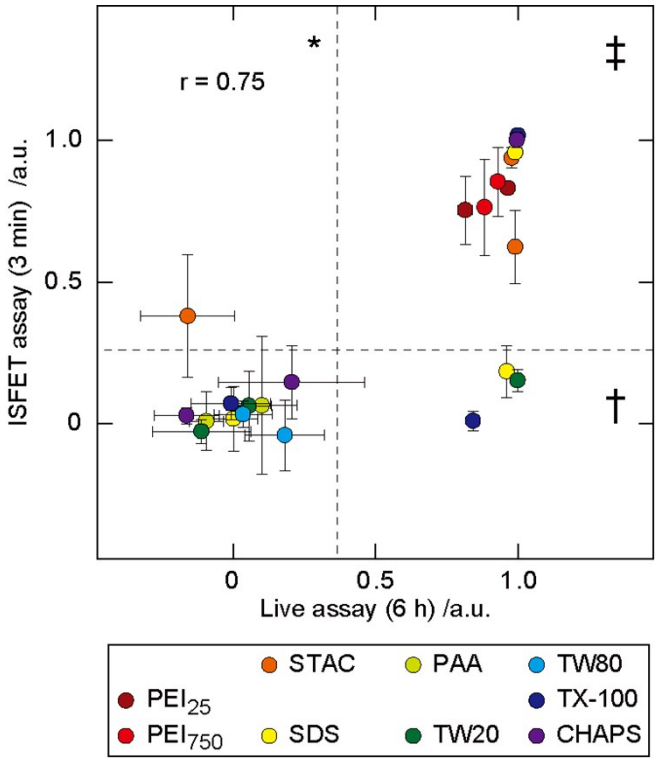

Fig. S5. Scattered plots between the ISFET and Live cell assays. *, †, and ‡ represent the ISFET$^+$/live$^-$, ISFET$^-$/live$^+$, and ISFET$^+$/live$^+$ regimes, respectively. The plots show the mean ± SD (n = 3). r: correlation coefficient.

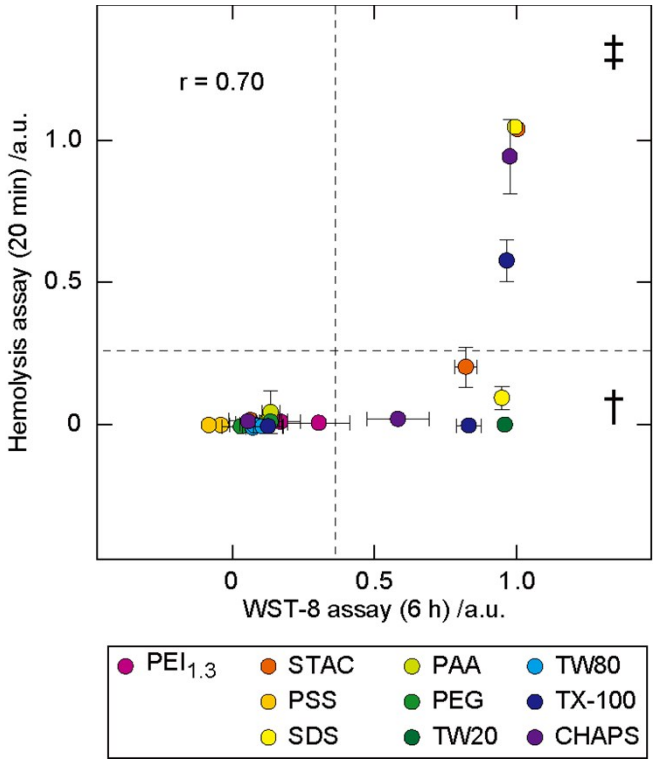

Fig. S6. A scattered plot between the hemolysis (20 min) and WST-8 (6 h) assays. † and ‡ represent the hemolysis^{-}/ WST-8^{+} and hemolysis^{+}/ WST-8^{+} regimes, respectively. The plots show the mean ± SD (n = 3). r: correlation coefficient

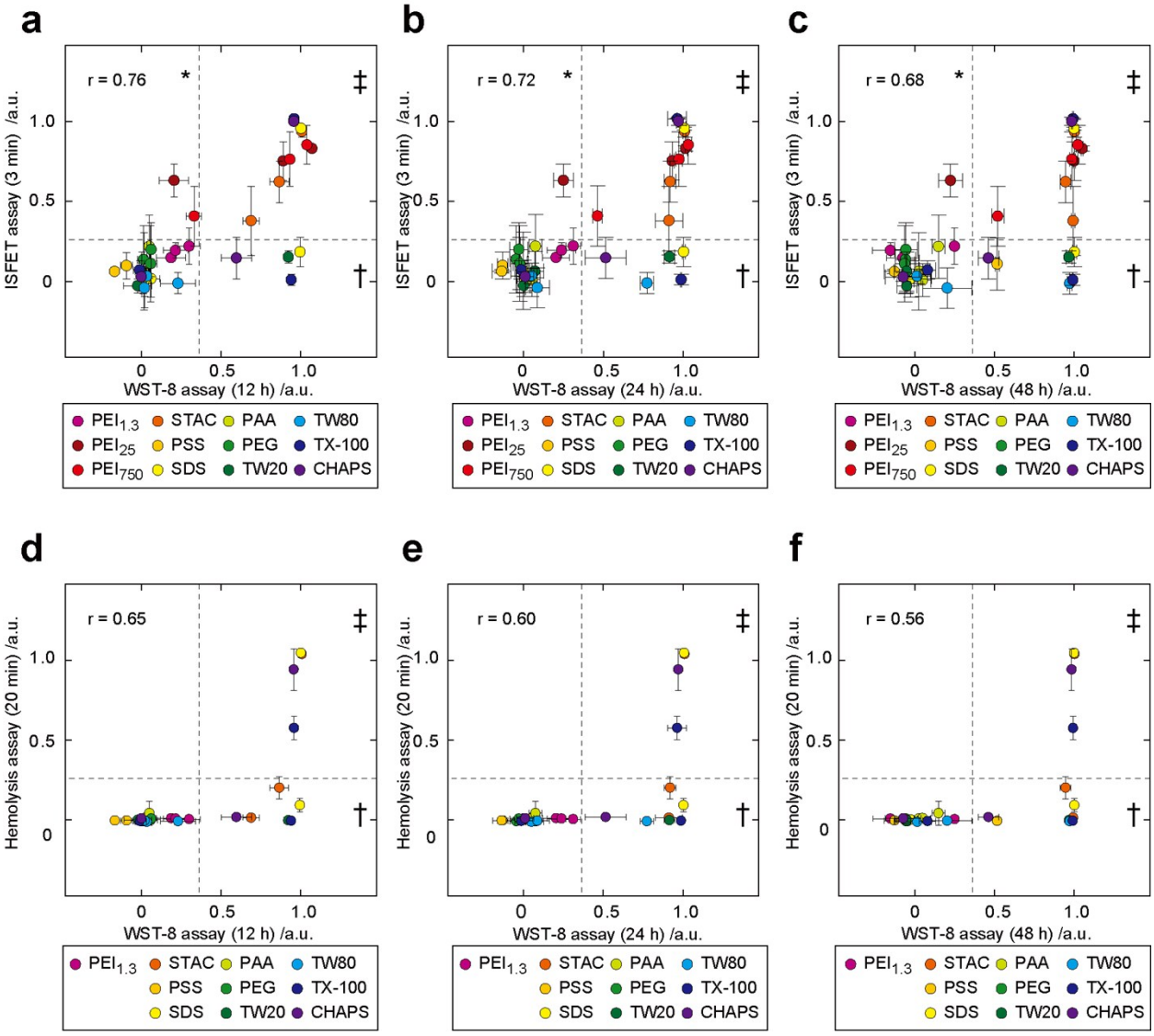

Fig. S7. Correlation diagrams. (**a–c**) Scattered plots that compare the ISFET assay with the WST-8 assay for 12 h (**a**), 24 h (**b**), and 48 h (**c**). *, †, and ‡ represent the ISFET^{+}/WST-8^{-}, ISFET^{-}/WST-8^{+}, and ISFET^{+}/WST-8^{+} regimes, respectively. (**d–f**) Scattered plots that compare the hemolysis assay with the WST-8 assay for 12 h (**d**), 24 h (**e**), and 48 h (**f**). † and ‡ represent the hemolysis^{-}/WST-8^{+}, and hemolysis^{+}/WST-8^{+} regimes, respectively. The plots show the mean ± SD (n = 3). r: correlation coefficient.

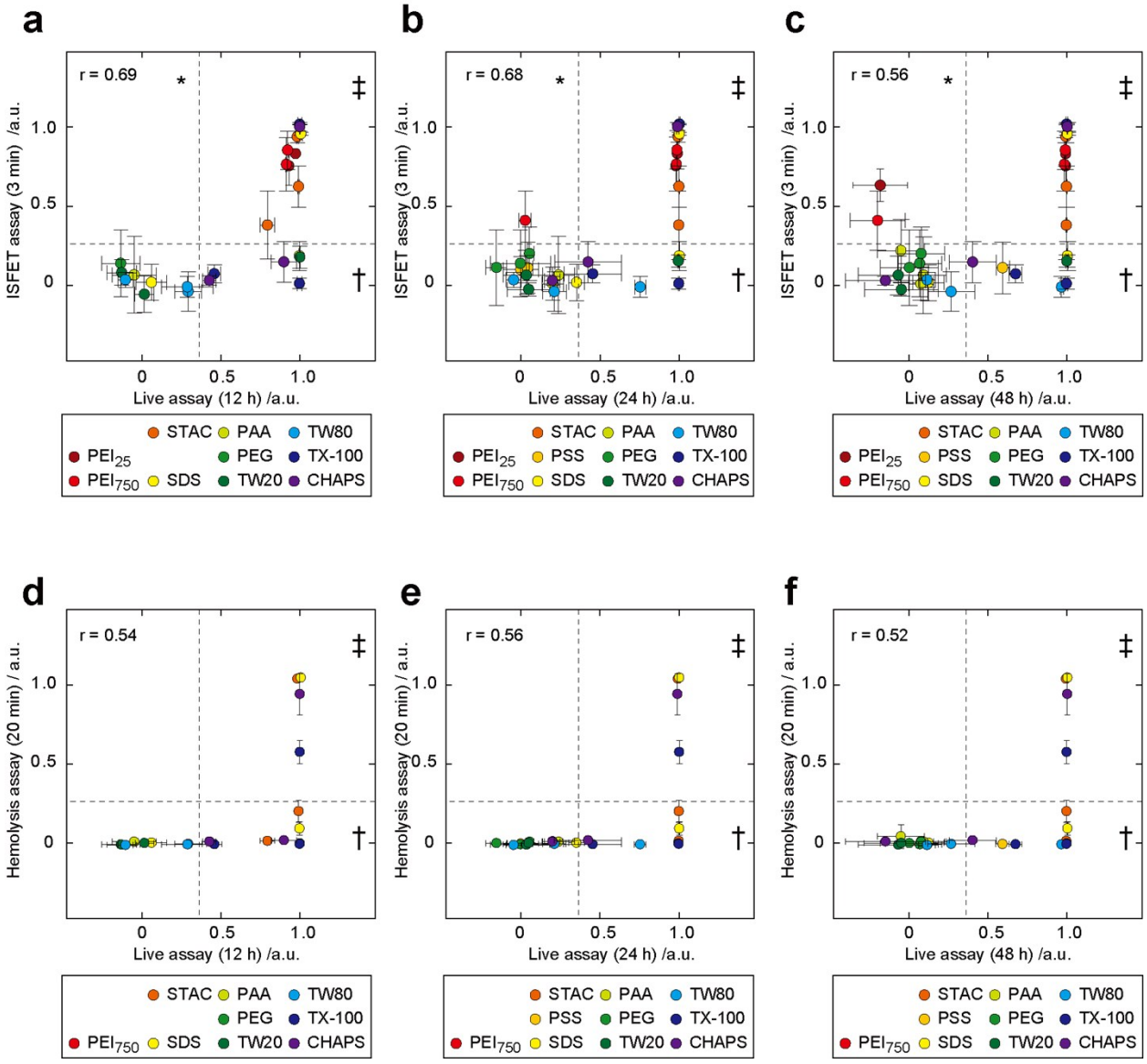

Fig. S8. Correlation diagrams. (**a–c**) Scattered plots that compare the ISFET assay with the live assay for 12 h (**a**), 24 h (**b**), and 48 h (**c**). *, †, and ‡ represent the ISFET^{+}/live^{-}, ISFET^{-}/live^{+}, and ISFET^{+}/live^{+} regimes, respectively. (**d–f**) Correlation diagrams that compare the hemolysis assay with the live assay for 12 h (**d**), 24 h (**e**), and 48 h (**f**). † and ‡ represent the hemolysis^{-}/live^{+}, and hemolysis^{+}/live^{+} regimes, respectively. The plots show the mean ± SD (n = 3). r: correlation coefficient.

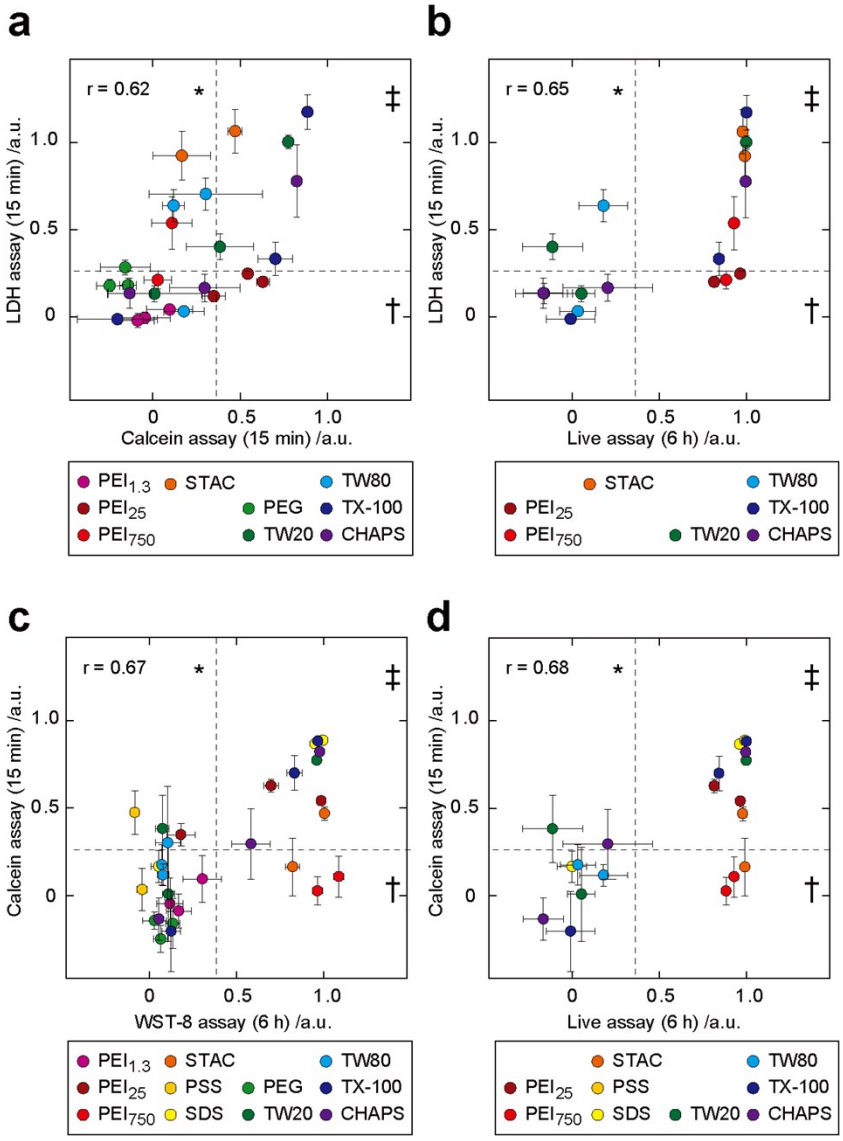

Fig. S9. Correlation diagrams. (**a–c**) Scattered plots that compare the following assays: the LDH assay and the calcein release assay (**a**), the LDH assay and the live assay (**b**), the calcein release assay and the WST-8 assay (**c**), and the calcein release assay and the live assay (**d**). The plots show the mean ± SD (n = 3). r: correlation coefficient.