**Fig. 1, Source Data 2**. Properties of lifetime standards and VoltageFluor dyes.

|  |  |  |  |
| --- | --- | --- | --- |
|  | %ΔF/F | %Δτ/τ | Lifetime (ns) |
| Fluorescein | N/A | N/A | 4.008 ± 0.009 |
| Erythrosin B | N/A | N/A | 0.083 ± 0.001 |
| VF2.1.Cl | 27 | 22.4 ± 0.4% | 1.77 ± 0.02 |
| VF2.0.Cl | 0 | 0.11 ± 0.05% | 3.482 ± 0.004 |

**Fig. 1, Source Data 2.** Properties of lifetime standards and VoltageFluor dyes.Fluorescein and erythrosin B standards were measured in drops of solution placed on a coverslip. For VF dyes, voltage sensitivities from intensity-based fluorescence imaging in HEK293T cells (%ΔF/F, percent change in fluorescence intensity for a voltage step from -60 mV to +40 mV) are from previously published work 30. Lifetime data were obtained from voltage-clamp electrophysiology of HEK293T cells loaded with 100 nM VF. Lifetime listed here is the average 0 mV lifetime from the electrophysiology calibration. % Δτ/τ is the percent change in lifetime corresponding to a 100 mV step from -60 mV to +40 mV. Lifetime sample sizes: fluorescein 25, erythrosin B 25, VF2.1.Cl 17, VF2.0.Cl 17. For lifetime standards, each measurement was taken on a separate day. VF2.1.Cl data in HEK293T is duplicated in Figure 2 – source data 1. Values are tabulated as mean ± SEM.