# Electronic Supplementary information

An automated screening platform for improving the performance of genetically encoded Ca²⁺ biosensors in mammalian cells

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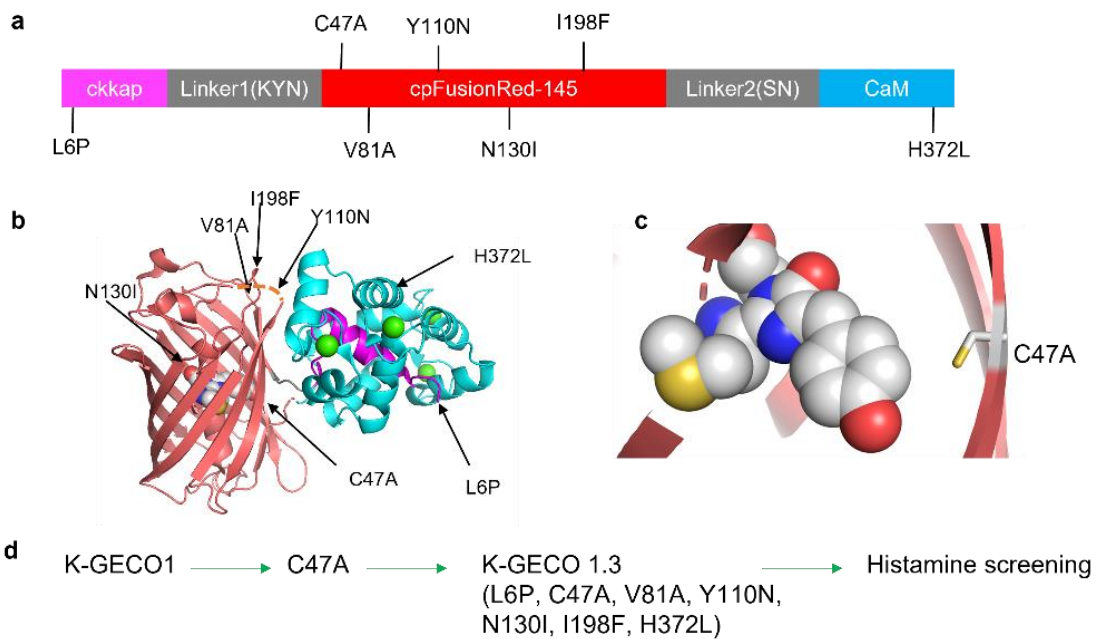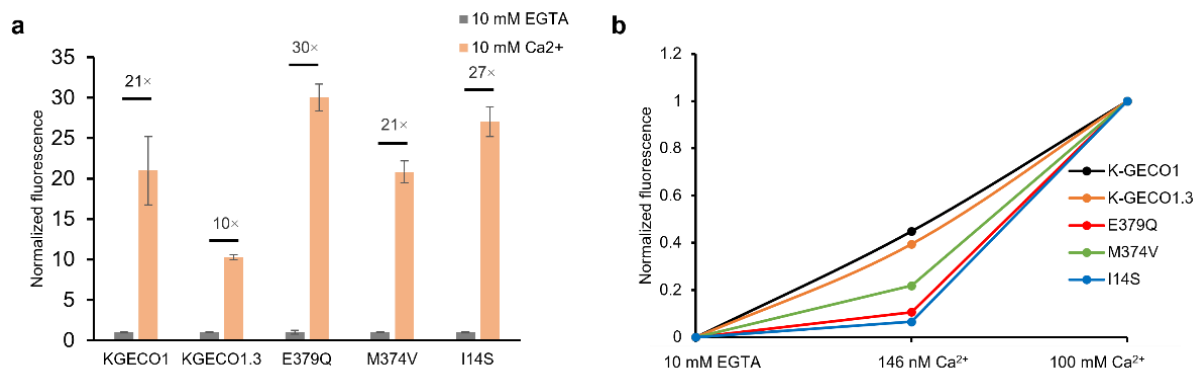
Supplementary figures
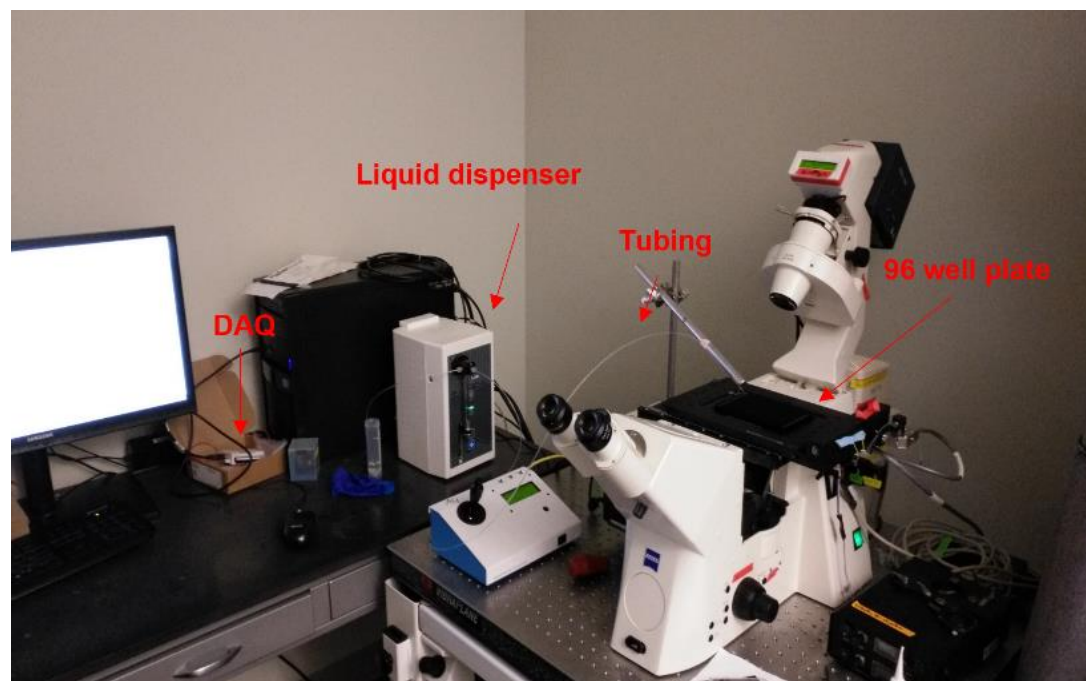
**Figure S1**. Experimental setup of the screening platform.
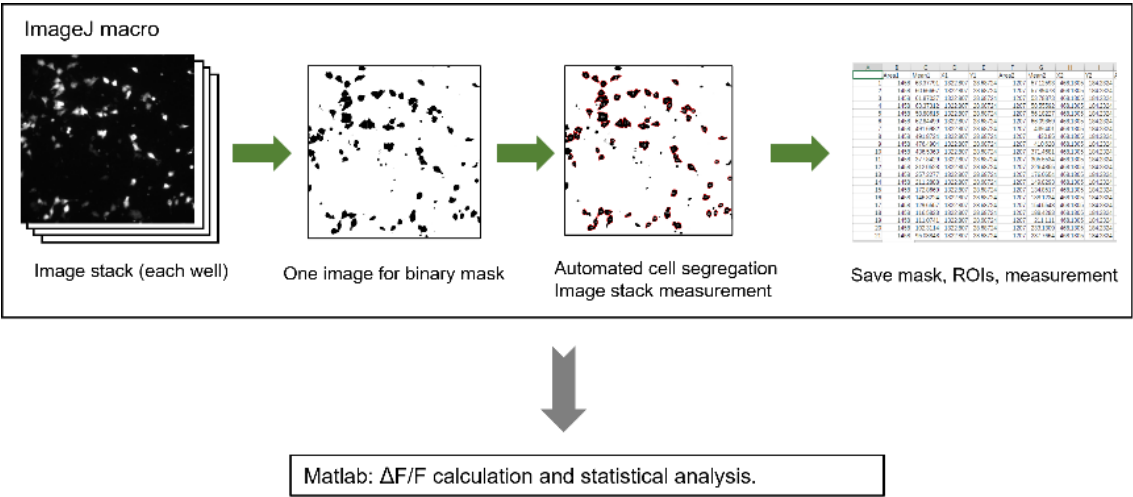
Figure S2. Schematic work flow of the image analysis.

Figure S3. Initial evolution of K-GECO1. (a) Schematic and (b) crystal structure (PDB ID:5UKG) of K-GECO1 labeled with mutations of K-GECO1.3. (c) Chromophore and C47 of K-GECO1. (d) Evolution trajectory from K-GECO1 to K-GECO1.3.

Figure S4. Dynamic range (a) and affinity (b) of K-GECO variants in bacterial lysates. Error bars represent standard deviation.
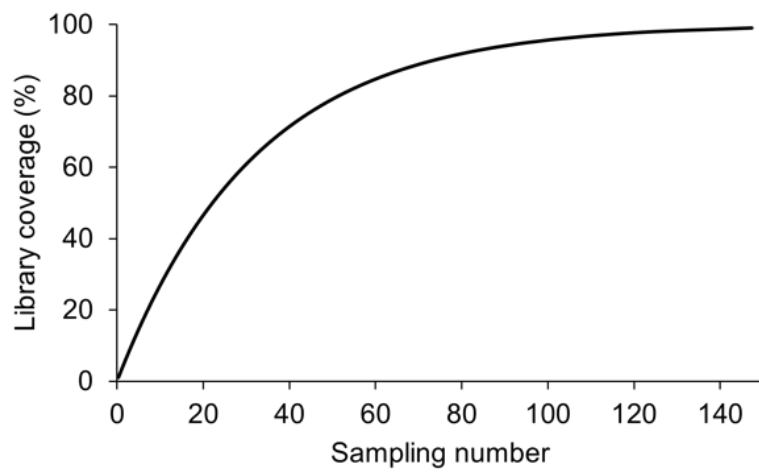
Figure S5. Library coverage at different sampling number in a single position NNK library.