avGFP	super ecliptic pHluorin GFP	GEVI ArcLight	GEVI Marina
S65	T65	T307	T307
R96	R96	R338	R338
S147	D147	D389	A389
H148	H148	H390	A390
N149	Q149	Q391	Q391
Y151	Y151	Y393	Y393
R168	R168	R410	R410
Y200	Y200	Y442	A442
S202	F202	F444	F444
T203	T203	T445	T445
Q204	T204	T446	T446
S205	S205	S447	S447
A206	T206	T448	T448
L221	L221	L463	L463
E222	E222	E464	E464
F223	F223	F465	F465
A227	A227	D469	D469

Figure S1: The list of amino acid residues in fluorescent protein that were targeted for creating mutagenic libraries described in the paper. The shown numbering of amino acid residues is from starting Methionine in avGFP, super ecliptic pHluorin GFP, GEVI ArcLight and GEVI Marina.

	% △F/F	t_{On1} (ms)	t_{On2} (ms)	% fast t_{On}	t_{Off1} (ms)	t_{Off2} (ms)	% fast t_{Off}
ArcLight	-39.5±1.2	12.3±1.8	34.7±3.14	38±6	14.1±0.4	x	x
ArcLight A389 A390	17.8±1.2	3.6±0.6	30.3±3.0	37±2	12.1±0.6	46.8±5.6	68± 7
ArcLight A389 A390 V442 Marina	29.2±2.0	28.8±2.4	x	x	15.6±1.5	59.4±5.8	61± 4

Figure S2. Size and speed of voltage dependent response of GEVI Marina. Novel GEVI Marina is showing similar kinetic properties as parent ArcLight. Comparison of % ΔF/F, activation and deactivation kinetics of parent probe ArcLight (n=4 cells) and two mutants with reversed voltage-dependent response, ArcLight A389 A390 (n= 6 cells) and ArcLight A389 A390 V442 (n=12 cells). Data are plotted as mean ± s.e.m.

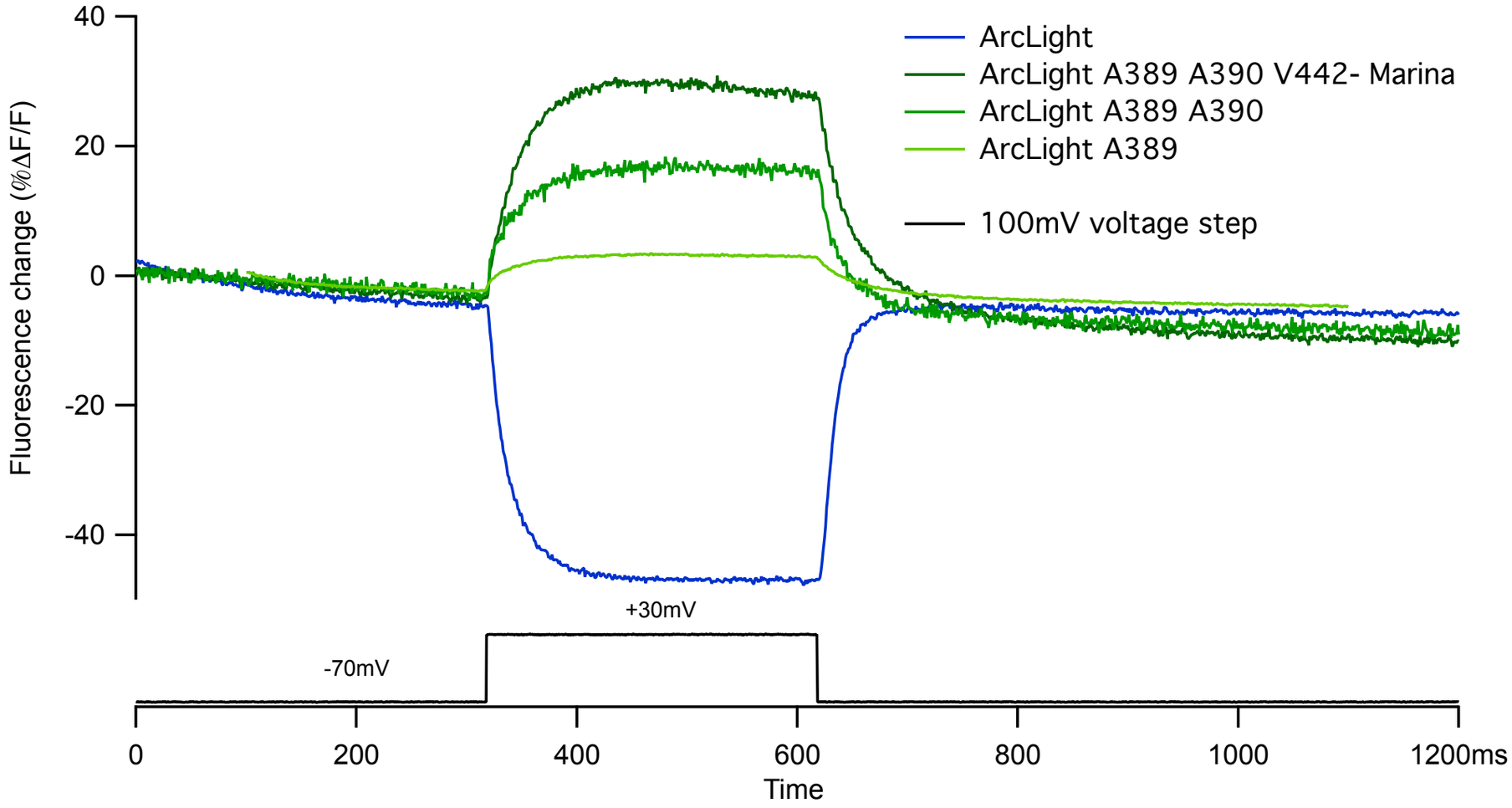

Figure S3. Same traces as in Figure 3. are shown here without bleach correction. HEK293 cells transiently expressing various GEVIs were simultaneously electrically recorded using whole-cell patch clamp (300ms/ 100mV depolarizing steps from -70 mV holding potential) and imaged with a high-speed (1000 Hz) CCD camera. Depolarization changes in membrane potential cause decrease in fluorescence intensity in ArcLight (in blue) and increase in fluorescence intensity in ArcLight D389A (light green), ArcLight A389 H390A (green) and ArcLight A389 A390 Y442V-Marina (dark green). All traces are unfiltered single trials. Excitation light intensity measured at the sample plane was 18 mW/mm^2.

GEVI name	FP	FP insertion site within *CiVSD*	AA residue at 65 in eGFP (in GEVI)	△F orientation (at 400nm)	△F orientation (at 488nm)	Addgene #
epArcLight	Ecliptic pHluorin	S249	S65 (S317)	+	**-**	85803
ArcLight (S249)	Super Ecliptic phluorin	S249	T65 (T317)	no signal	-	36855
ArcLightCo (Q239)	Super Ecliptic phluorin	Q239	T65 (T307)	no signal	-	85844
ArcLight A389 A390	Super Ecliptic phluorin	Q239	T65 (T307)	no signal	+	n/a
ArcLight S307 A389 A390	Super Ecliptic phluorin	Q239	S65 (S307)	no signal	+	n/a
ArcLight T307 A389 A390 V442 (Marina)	Super Ecliptic phluorin	Q239	T65 (T307)	no signal	+	74216

Figure S4. The list of GEVIs used in the experiments with results shown in Figure 7.