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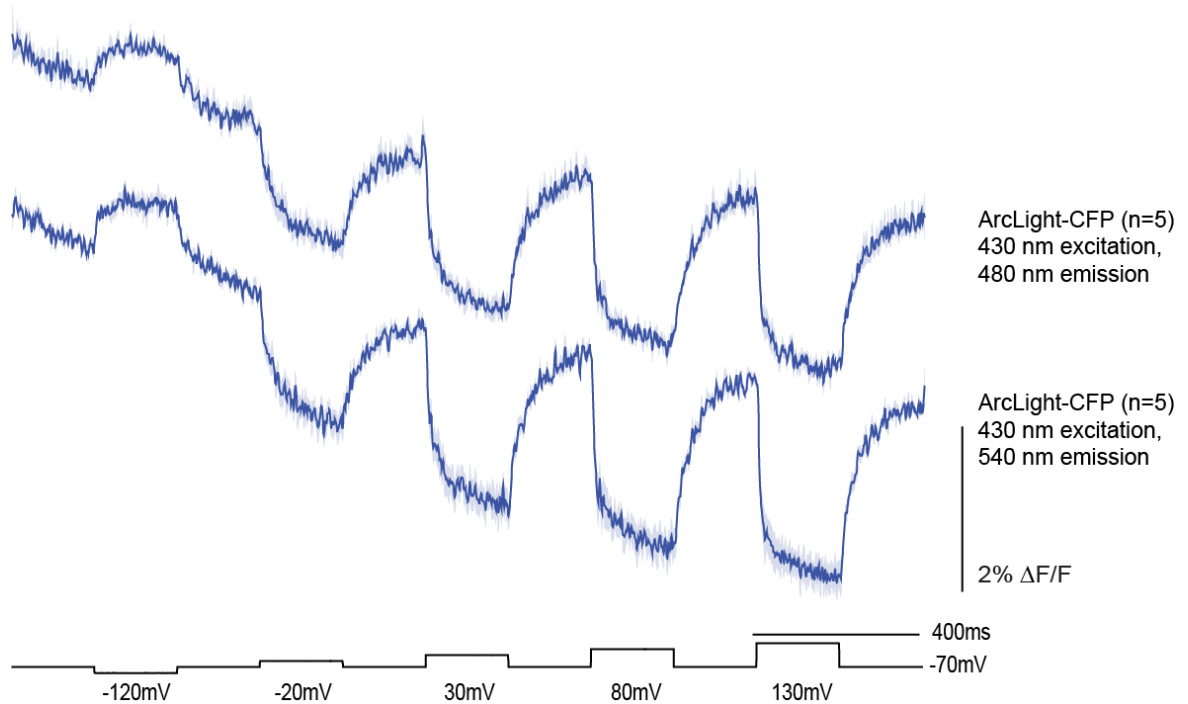
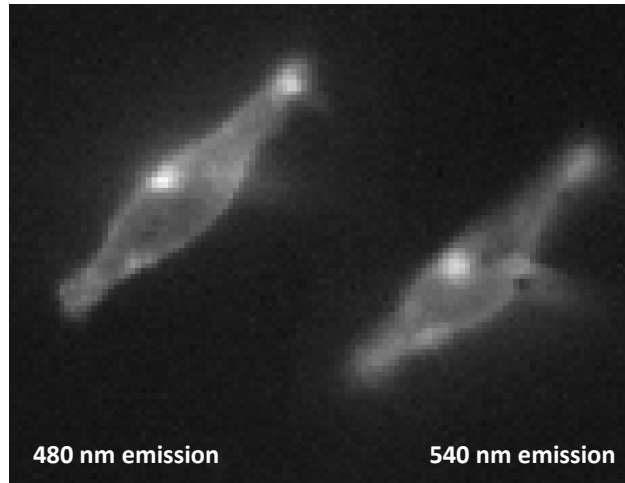
Supplemental information

**Improving the flexibility of genetically encoded voltage indicators via
intermolecular FRET**

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Supplemental Figure S1

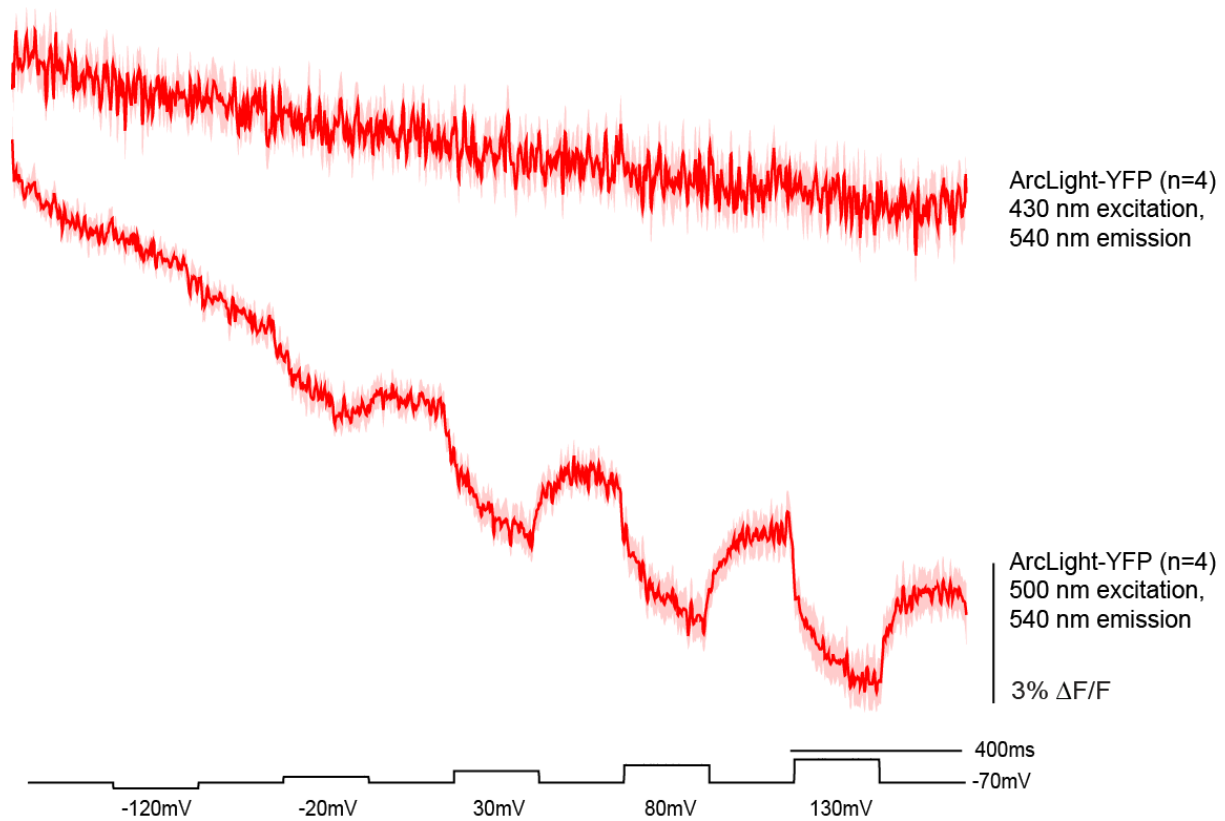
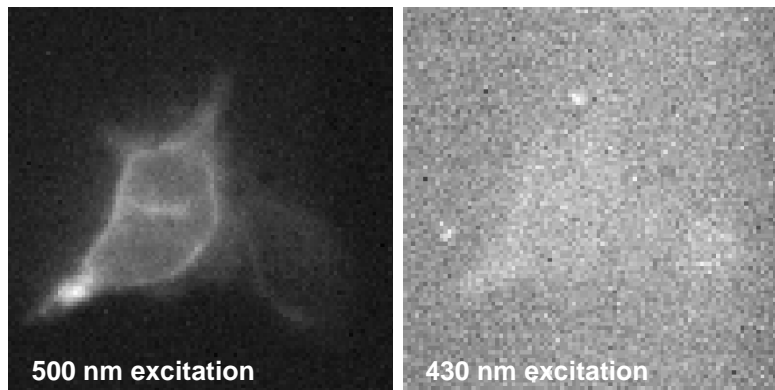
ArcLight-CFP expressing HEK cell



Supplemental figure S1. HEK cells expressing ArcLight-CFP only. When excited at 430 nm emission in both the 480 nm CFP emission channel and the 540 nm YFP emission channel can be detected. As a result, ArcLight-CFP also yields an optical signal in the YFP channel that dims upon depolarization of the plasma membrane.

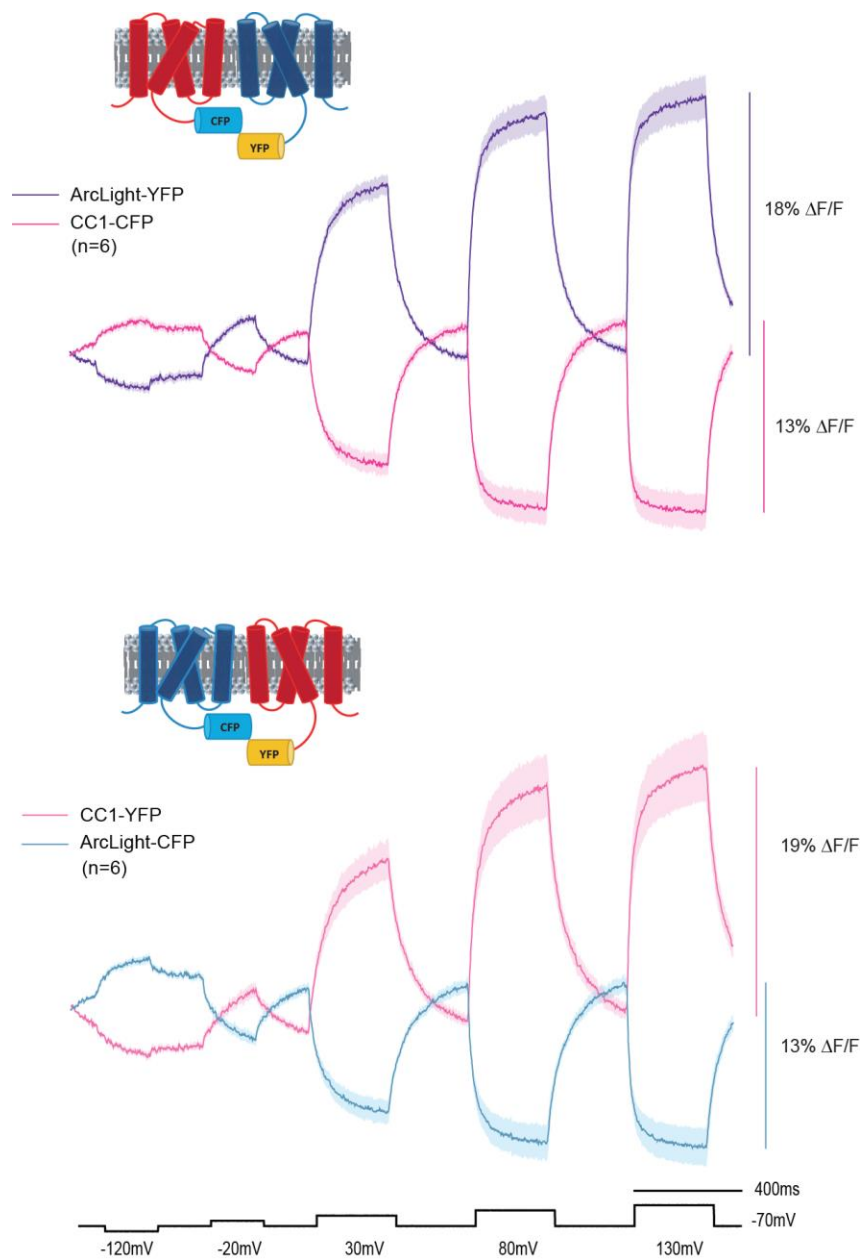
Supplemental Figure S2

ArcLight-YFP expressing HEK cell



Supplemental figure S2. HEK cells expressing ArcLight-YFP exhibit little fluorescence when excited at 430 nm resulting in no detectable voltage-dependent signal in the 540 nm YFP emission channel. When excited at 500 nm a voltage-dependent optical signal that dims upon depolarization of the plasma membrane can be seen.

Supplemental Figure S3



Supplemental figure S3. Voltage-dependent traces of inter-FRET pairs with heterogeneous voltage sensing domains (VSDs). Top traces - donor FP is attached to the CC1 VSD and the acceptor FP is attached to the ArcLight VSD. Bottom traces - donor FP is attached to the ArcLight VSD while the acceptor FP is attached to the CC1 VSD.

Supplemental Table S1

	$\Delta F/F$ at 200 mV depolarization	Signal polarity upon depolarization	Reference Figure
Single constructs			
ArcLight-CFP	3%	Downwards	Figure 2A
ArcLight-YFP	3%	Downwards	Figure 2B
Bongwoori-R3-CFP	3%	Downwards	Figure 3A
Bongwoori-R3-YFP	3%	Downwards	Figure 3B
ArcLight-mRuby2	2%	Downwards	Figure 5A
ArcLight-Clover	1.5%	Downwards	Figure 5B
CFP-YFP co-transfected constructs			
ArcLight-CFP ArcLight-YFP	11% 14%	Downwards Upwards	Figure 2C, 4, 6B
Bongwoori-R3-CFP Bongwoori-R3-YFP	8% 13%	Downwards Upwards	Figure 3C
ArcLight-CFP L221K ArcLight-YFP L221K	8% 13%	Downwards Upwards	Figure 4
ArcLight-CFP A206K ArcLight-YFP A206K	5% 10%	Downwards Upwards	Figure 4
ArcLight-CFP F223R ArcLight-YFP F223R	2% 4%	Downwards Upwards	Figure 4
CC1-CFP CC1-YFP	8% 15%	Downwards Upwards	Figure 6A
Bongwoori-R3-CFP Farnesylated-YFP	3% 2%	Downwards Upwards	Figure 7A
Farnesylated-CFP Bongwoori-R3-YFP	2% 1%	Downwards Upwards	Figure 7B
GFP-RFP co-transfected constructs			
ArcLight-Clover ArcLight-mRuby2	7% 11%	Downwards Upwards	Figure 5C
Farnesylated Clover ArcLight-mRuby2	3% 1%	Downwards Upwards	Figure 7C
CFP-RFP co-transfected constructs			
Bongwoori-R3-CFP Bongwoori-R3-dTomato	5% 15%	Downwards Upwards	Figure 5F
CFP-YFP-RFP tri-transfected constructs (*YFP emission was not recorded)			
Bongwoori-R3-CFP Bongwoori-R3-dTomato	10% 15%	Downwards Upwards	Figure 5D

ArcLight-CFP	7%	Downwards	Figure 5E
ArcLight-mRuby2	10%	Upwards	

Supplemental Table S1. Fluorescence changes of inter-FRET GEVIs with corresponding figure depiction.

Supplemental Table S2

Intermolecular FRET Constructs	N	State	Weighted τ (msec)	Fast τ (msec)	Slow τ (msec)	% fast
ArcLight-CFP	5	On	29 \pm 5	6 \pm 2	43 \pm 9	43 \pm 2
		Off	86 \pm 3	11 \pm 2	95 \pm 6	19 \pm 2
ArcLight-YFP		On	39 \pm 4	6 \pm 1	48 \pm 6	26 \pm 4
		Off	98 \pm 17	12 \pm 3	109 \pm 22	19 \pm 4
ArcLight-CFP A206K	5	On	11 \pm 2	4 \pm 1	22 \pm 2	51 \pm 5
		Off	85 \pm 31	10 \pm 3	111 \pm 40	22 \pm 1
ArcLight-YFP A206K		On	21 \pm 5	5 \pm 1	39 \pm 7	47 \pm 4
		Off	76 \pm 6	15 \pm 5	92 \pm 9	29 \pm 5
ArcLight-CFP L221K	4	On	17 \pm 5	5 \pm 2	43 \pm 15	55 \pm 8
		Off	100 \pm 16	12 \pm 3	140 \pm 25	18 \pm 4
ArcLight-YFP L221K		On	24 \pm 6	6 \pm 1	41 \pm 6	56 \pm 4
		Off	111 \pm 7	12 \pm 2	126 \pm 11	16 \pm 3
ArcLight-CFP F223R	5	On	20 \pm 4	6 \pm 2	73 \pm 34	65 \pm 9
		Off	118 \pm 20	12 \pm 2	176 \pm 29	20 \pm 5
ArcLight-YFP F223R		On	32 \pm 10	5 \pm 1	60 \pm 13	51 \pm 6
		Off	89 \pm 24	7 \pm 3	105 \pm 23	19 \pm 4
Bongwoori-R3 CFP	4	On	7 \pm 1	7 \pm 1	-	100
		Off	12 \pm 1	12 \pm 1	-	100
Bongwoori-R3 YFP		On	10 \pm 1	10 \pm 1	-	100
		Off	13 \pm 1	13 \pm 1	-	100
CC1-CFP	5	On	89 \pm 9	26 \pm 7	118 \pm 12	32 \pm 1
		Off	54 \pm 2	27 \pm 3	112 \pm 9	66 \pm 1
CC1-YFP		On	124 \pm 22	31 \pm 7	162 \pm 38	25 \pm 1
		Off	54 \pm 4	23 \pm 3	75 \pm 7	43 \pm 1
AL-CFP	6	On	53 \pm 4	18 \pm 2	77 \pm 5	48 \pm 7
		Off	75 \pm 4	15 \pm 1	87 \pm 5	24 \pm 1
CC1-YFP		On	84 \pm 8	38 \pm 3	136 \pm 28	44 \pm 8
		Off	76 \pm 4	19 \pm 2	91 \pm 8	24 \pm 3
CC1-CFP	6	On	66 \pm 5	21 \pm 1	78 \pm 6	20 \pm 1
		Off	64 \pm 5	20 \pm 2	82 \pm 5	31 \pm 1
AL-YFP		On	91 \pm 9	34 \pm 4	136 \pm 24	40 \pm 1
		Off	71 \pm 4	18 \pm 4	85 \pm 4	22 \pm 1

Table S2. Kinetics of CFP-YFP Intermolecular FRET GEVIs at 100mV depolarization tested in HEK 293 cells. Values are listed as mean \pm SEM (standard error of the mean). The Bongwoori-R3 construct only had a fast component while ArcLight and CC1 constructs had both fast and slow components. Excitation wavelength was 430 nm for the intermolecular FRET constructs. CFP and YFP emissions were recorded simultaneously using an optical splitter. Emission wavelength for CFP was 480 nm and emission wavelength for YFP was 540 nm.