Supplementary Information

FRCaMP, Red Fluorescent Genetically Encoded Calcium Indicator Based on Calmodulin from Schizosaccharomyces Pombe Fungus

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Supplementary Tables and Figures

Table S1. List of primers.

Primer	Primer sequence (5′-3′)
RSP-BglII	CTGAGATCTATGAGGAAACCGTTCCGTG
CaMSP-HindIII-r	CCAAGCTTCTACTTGGAAGAAATGACACGAGAGAATTC
MSP-3-5	ggtAGATCTatgAGAAAAnnsnnsnnsACTGCCTATAACGCTGTAC
MSP-6-8	ggtAGATCTatgAGAAAAACGTTCCGTnnsnnsnnsAACGCTGTACGTGCTTTC
MSP-9-11	ggtAGATCTatgAGAAAAACGTTCCGTACTGCCTATnnsnnsnnsCGTGCTTTC AACACTTGG
dRSP-BglII2	cgagatctGTGCCGCGGGTTTCCGAG
BamHI-bJun	TGGggatccgccaccatggTGAAGGCGGAGAGGAAGCGCATG
bJun-NheI-r	CAGgctagcGGTGGCGATGGATCTTCTAG
NheI-RSPC	ACCgctagcCTGGTAAGCAAGGGCGAGG
BamHI-bFos	TGGggatccgccaccatggTGGGTCGTGCGCAGTCCATCGG
bFos-NheI-r	CATgctagcGTGGTTCATGACTTTCTG
NheI-RSPN	CACgctagcATGAGGAAACCGTTCCGTG
RSPN-HindIII-r	GATaagcttCTACTTGTACAGCGCGTCCGTG

Table S2. In vitro ΔF/F response of truncated versions (with deleted M13-like peptide) of the purified FRCaM and GCaM6s indicators to the saturating calcium ion concentrations.

Indicator	ΔF/F		
	0-39 μM [a]	**0-820 μM** [a]	**0-2000 μM** [a]
FRCaM	0.17 ± 0.03	0.00 ± 0.04	0.04 ± 0.01
GCaM6s	0.16 ± 0.03	0.18 ± 0.02	0.05 ± 0.03

[a] 39, 820, and 2000 μM free calcium concentration corresponds to the 30 mM MOPS, 100 mM KCl, pH 7.20 buffer supplemented with either 10 mM CaEGTA, or 10 mM CaNTA or 2 mM $CaCl_2$, respectively. 0 free calcium concentration corresponds to the 30 mM MOPS, 100 mM KCl, pH 7.20 buffer supplemented with either 10 mM EGTA, or 10 mM NTA or 0 mM $CaCl_2$, respectively. Data were averaged across 8 repeats. SD is shown.

NES-FRCaMP-stop gene:

ATGCTTCAACTTCCTCCTCTTGAACGTCTTACTCTTTCGAGATCTATGAGGAAACCGTTCCGTGGC
GCGGGCAACGCTGTGCGTGCTTTCAGCACTTGGAAAAAGCTAGTGCCGCGGGTTTCCGAGTGGAT
GTACCCCGAGGACGGCGCCCTGAAGAGCGAGATCAGGAAGGGGCTGAGGCTGAAGGACGGCGG
CCACTACGCCGCCGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGC
CTACATCGTCGACATCGAGTTGGACATCTTGTCCCACAACGAGGACTACACCATCGTGGAACAGT
GCGAACGCGCCGTGGGCCGCCACCCCACCGGTGGCACGGACGCGCTGTACAAGGGAGGTACAG
GCTCCGGCGGGAGTCTGGTAAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGTTCAT
GCGCTTCAAGGTGCACATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGG
CGAGGGCCGCCCCTACGAGGCCTTCCAGACCGCTAGGCTGAAGGTGACCAAGGGTGGCCCCCTG
CCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCATGTACGGCTCCAAGGCCTACATTAAGCACCC
AGCCGACATCCCCGACTACTTCAAGCTGTCCTTCCCCGAGGGCTTCAGGTGGGAGCGCGTGATGA
GCTTCGAGGACGGCGGCATTATTCACGTTAATCAGGACTCCTCCCTGCAGGACGGCGTATTCATC
TACAAGGTGAAGCTGCTCGGCACCAACTTCCCCCCCGACGGCCCCGTAATGCAGAAAGAGACCA
TGGGCTGGGAGGCTTCCTACGAGATGACTACCCGTAACCTTACAGATGAGCAGATTGCGGAGTTC
CGTGAGGCCTTTTCGCTGCTTGATCGTGATCAGGATGGAAATATCACGTCCAATGAATTGGGTGT
GGTTATGAGGTCGTTAGGTCTATCGCCTACTGCCGCCGAATTACAAGATATGATTAATGAGGTCG
ATGCCGATGGTAATGGCACAATTGATTTTACCGAATTTTTGACAATGATGGCCCGGAAAATGAAG
GATACCGACGACGAAGAGGAAGTTCGCGAAGCCTTTAAAGTCTTCGATAAAGATGGAAGTGGAT
ACATTACAGTCGAGGAGCTGACTCATGTTCTTACAAGTCTCGGTGAACGTTTGTCTCGAGAAGAA
GTAGCCGATGTGATACGTGAAGCCGACTCCGATGGCGATGGTGTAATCAACTACGAAGAATTCT
CTCGTGTCATTTCTTCCAAGTAA

Figure S1. Nucleotide sequence of NES-FRCaMP protein.

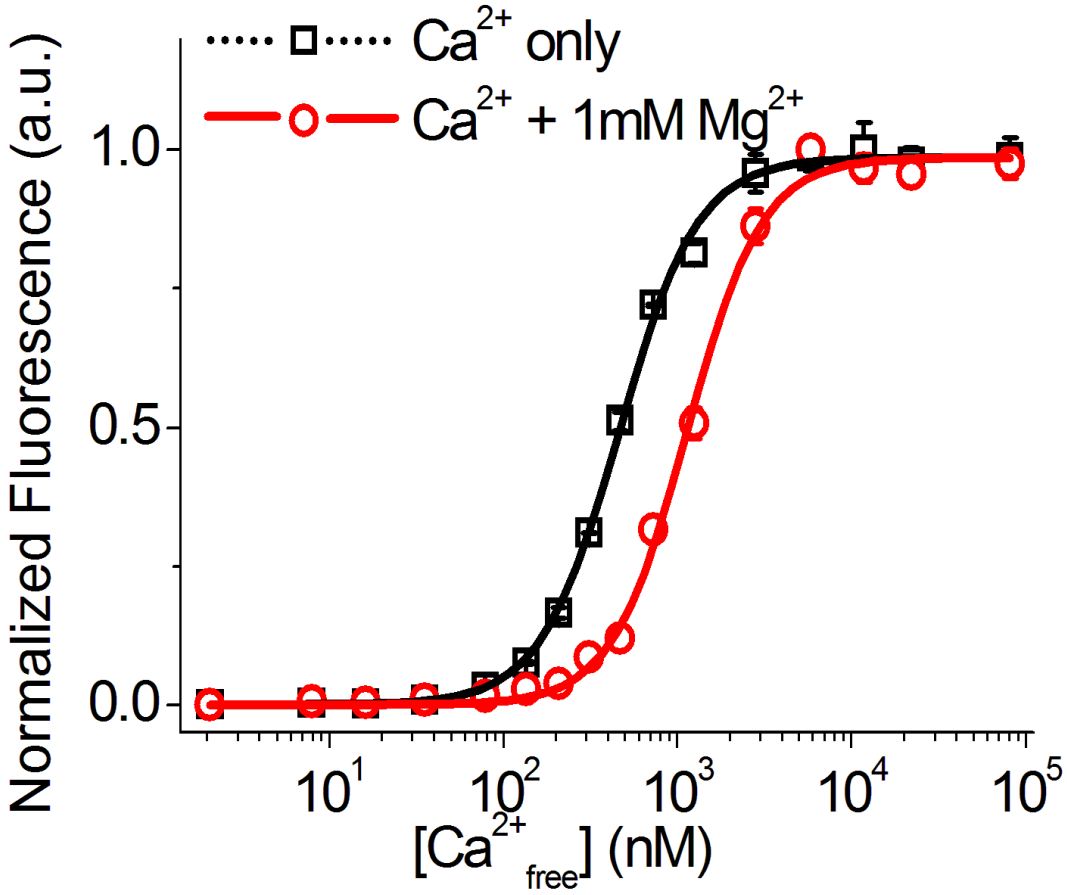

Figure S2. Calcium titration curves for R-GECO1.

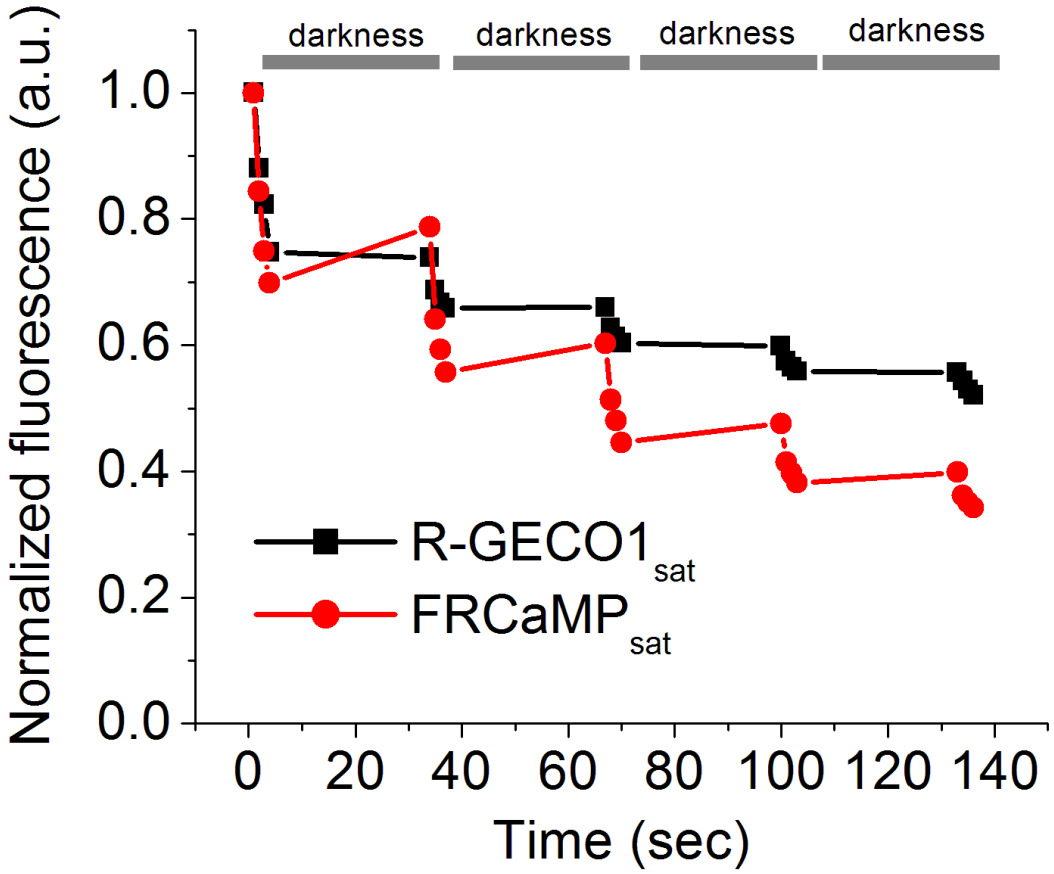

Figure S3. Photochromism in the FRCaMP$_{sat}$ indicator. 5 cycles of continuous metal halide lamp illumination were composed of photobleaching with yellow light (550/25BP) for 4 seconds followed by 30 seconds of darkness between cycles (marked as grey lines). Data were averaged across 6 drops in oil from 3 fields of view.

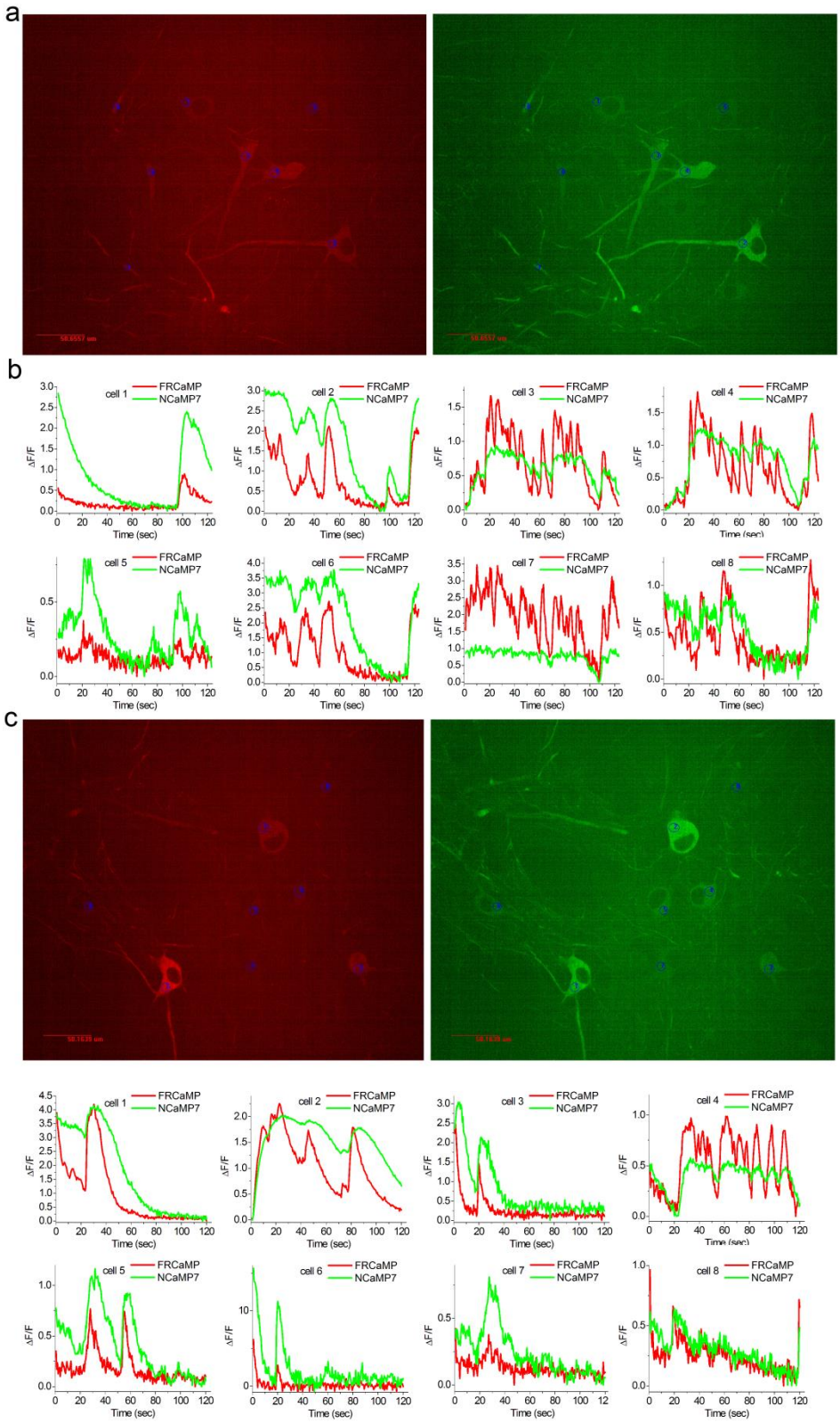

Figure S4. Calcium imaging of non-specific (spontaneous) activity of neuronal cultures co-expressing the FRCaMP and NCaMP7 calcium indicators. (**a, c**) Confocal images of two fields of view for neuronal cultures co-expressing the FRCaMP and NCaMP7 indicators. Scale bar, 50 μm. (**b, c**) Examples of ΔF/F traces for the 8 cells are shown for each of two fields of view. Neuronal cultures co-expressing the NES-FRCaMP and NES-NCaMP7 indicators were imaged on DIV 15th. Neuronal cultures were transduced on DIV 4th with the mixture of rAAVs carrying NES-FRCaMP and NES-NCaMP7.

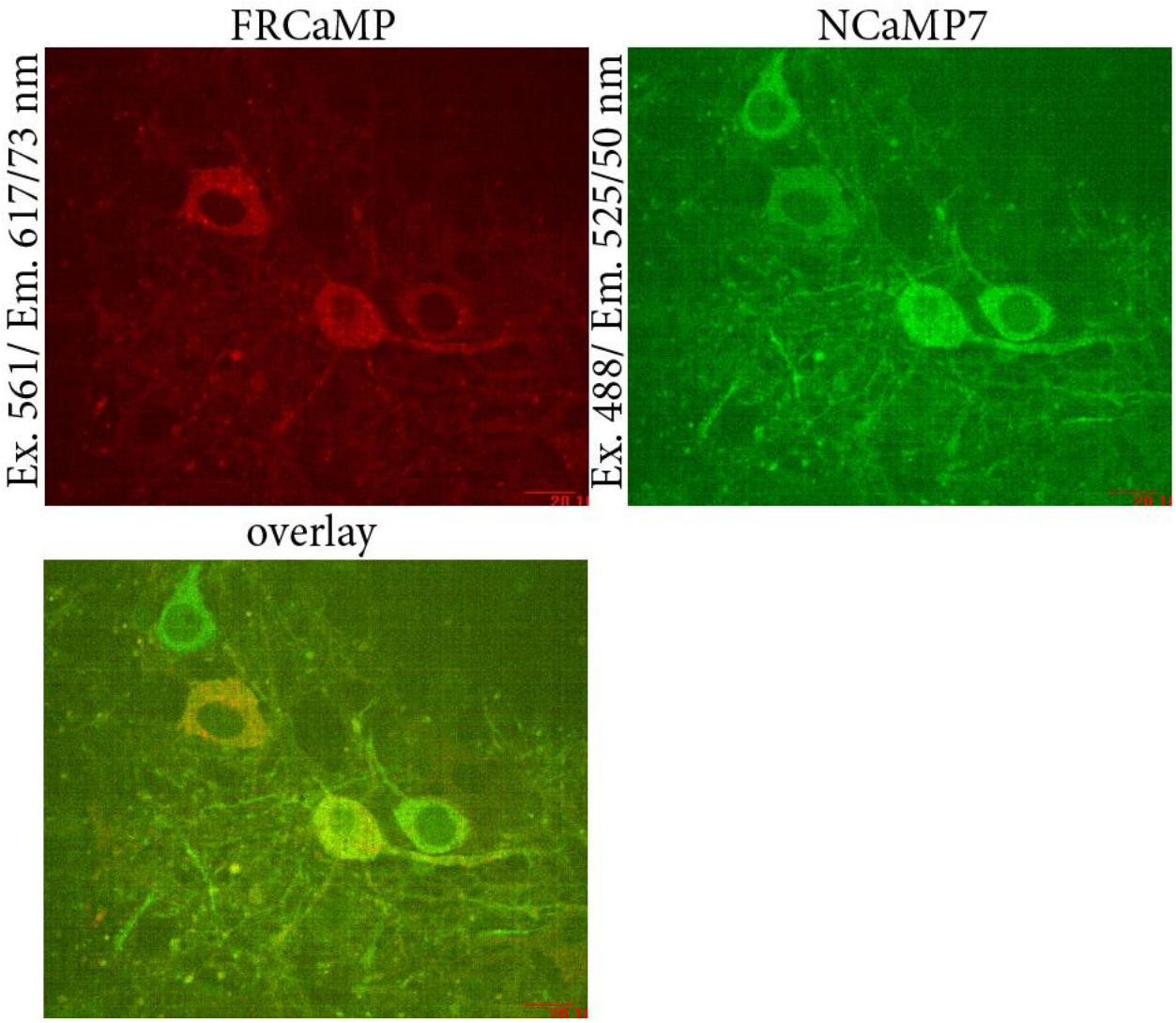

Figure S5. Puncta-like localization of the red FRCaMP indicator co-expressing with green NCaMP7 in cultured neurons. Confocal images of neurons co-expressing green NCaMP7 indicator with even distribution and red indicator FRCaMP with uneven puncta-like distribution. Neuronal cultures were imaged on DIV 22th. Scale bar, 20 μm.

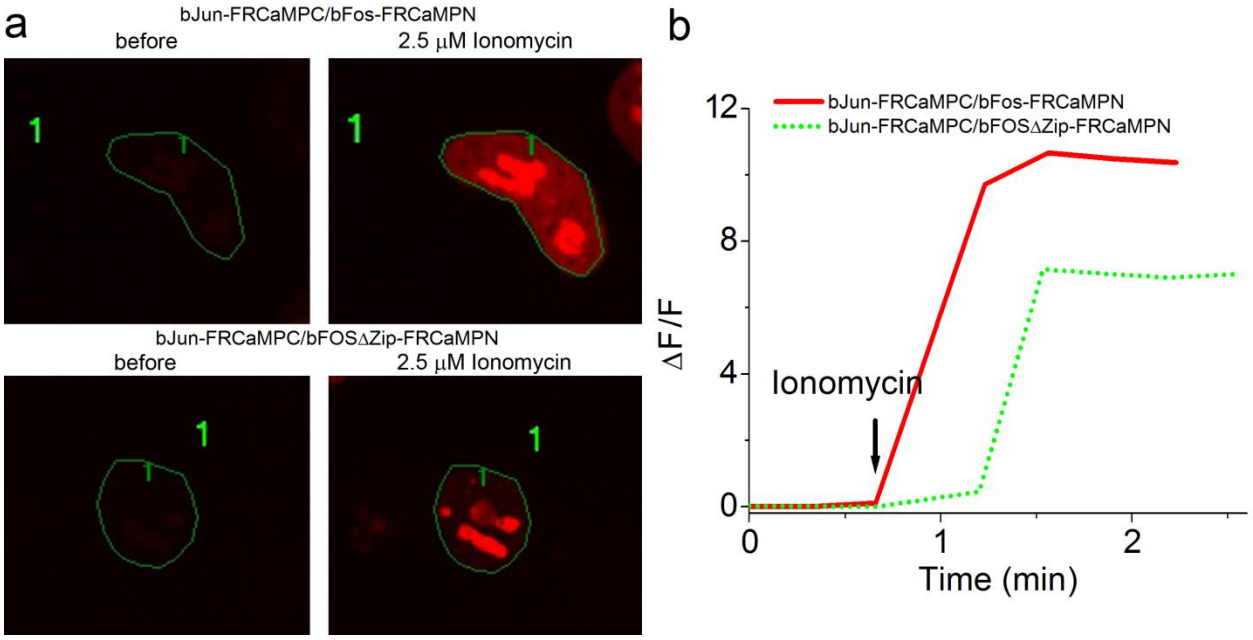

Figure S6. Localization and time-lapse response of the split-version of the FRCaMP indicator to Ca^{2+} variations in HeLa cells depending on the presence of the heterodimerizing (bJun-FRCaMPC/bFos-FRCaMPN) or non-heterodimerizing (bJun-FRCaMPC/bFOSΔZip-FRCaMPN) pair. **(a)** Zoomed area of the cell selected on Figure 7a. **(b)** The graph illustrates ΔF/F changes over time in red fluorescence of the split-version of the FRCaMP indicator in response to the addition of 2.5 μM of ionomycin depending on the presence of the heterodimerizing (bJun-FRCaMPC/bFos-FRCaMPN) or non-heterodimerizing (bJun-FRCaMPC/bFOSΔZip-FRCaMPN) pair. The changes on the graph correspond to the area indicated, as a numbered circle on the panel a, Figure 7 or panel a, Figure S5. Time of ionomycin addition is shown by arrow.

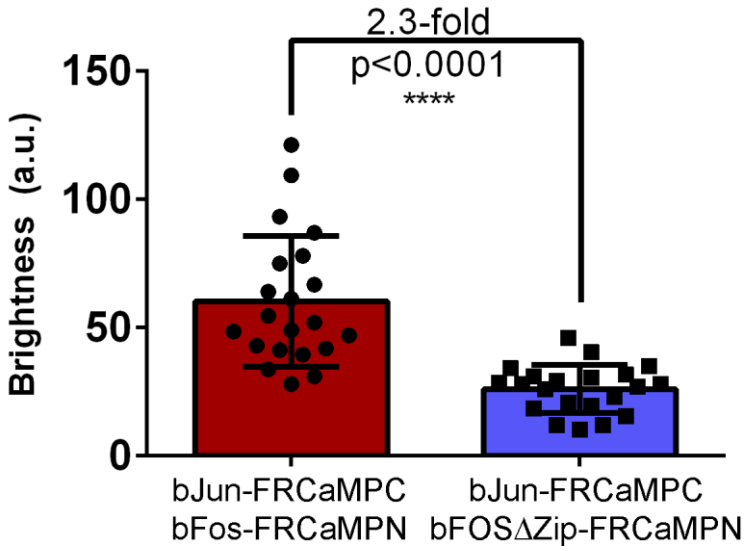

Figure S7. Brightness of the split-version of the FRCaMP indicator at low Ca^{2+} concentrations in HeLa cells depending on the the presence of the heterodimerizing (bJun-FRCaMPC / bFos-FRCaMPN) or non-heterodimerizing (bJun-FRCaMPC / bFOSΔZip-FRCaMPN) pair. Comparison of the averaged brightness **(**for the heterodimerizing bJun-FRCaMPC and bFos-FRCaMPN split calcium indicator and its control none-heterodimerizing bJun-FRCaMPC and bFos-FRCaMPN split calcium indicator in HeLa cells at physiological calcium concentration before ionomycin addition. Error bars are standard deviations across twenty one cells (three cultures). *p* values show statistical difference between the respective values. ****, *p* - value is lower than 0.0001.