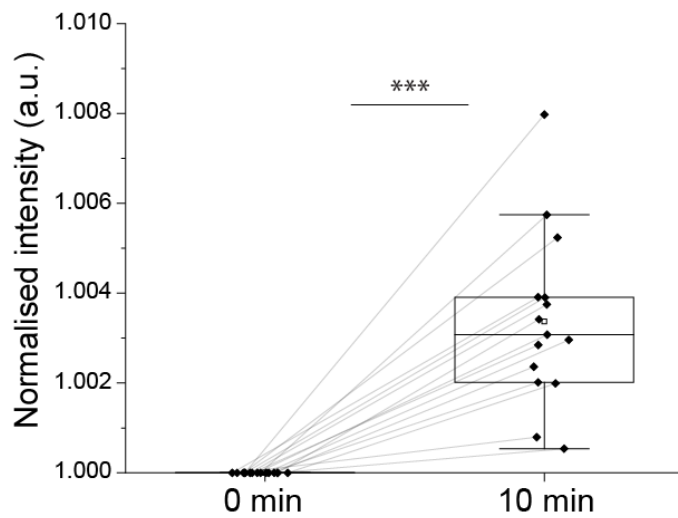
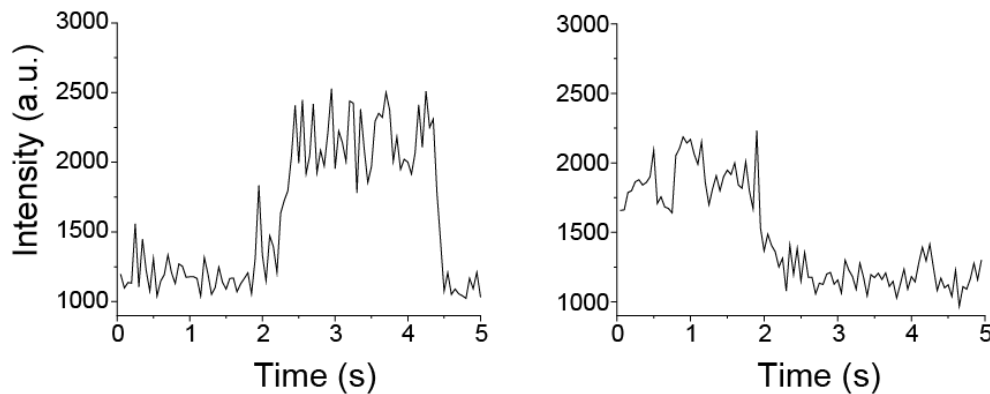

An optogenetic method for the controlled release of single molecules

In the format provided by the
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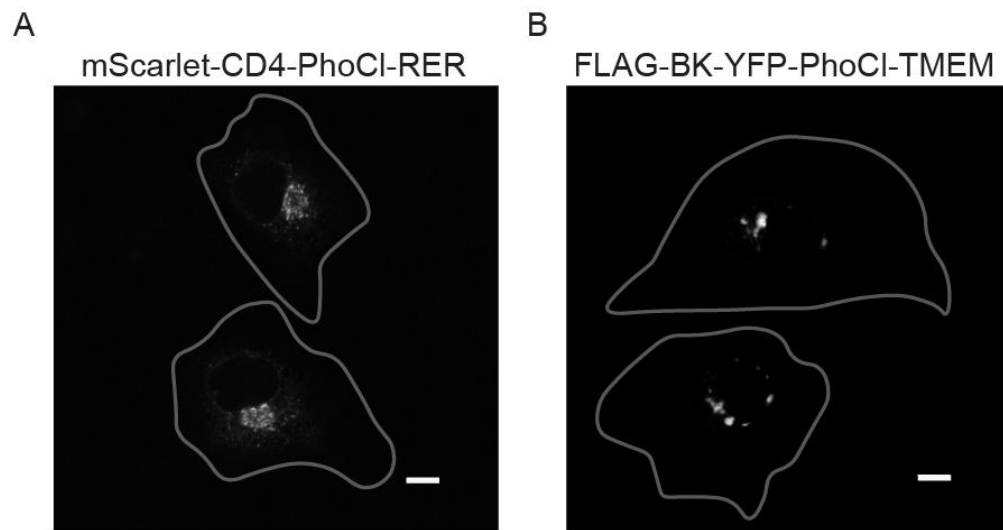
Supplementary Figures:



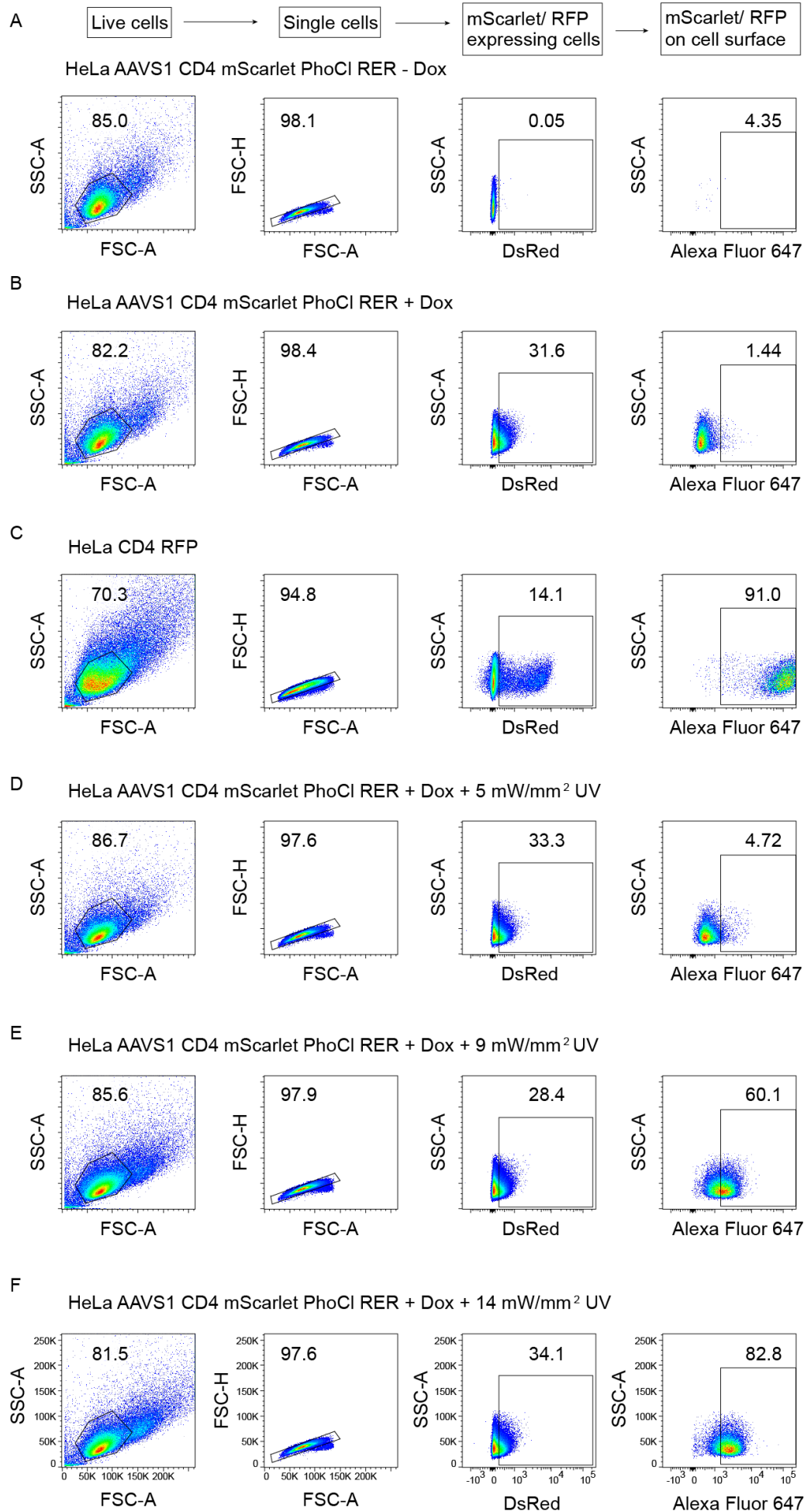
Supplementary Figure 1. Release of FGF2-GFP in the cytosol. Quantification of cytosolic FGF2-GFP before (1 ± 0) and 10 min after UV Illumination (1.05 ± 0.02) of CHO cells expressing TMEM-PhoCl-FGF2-GFP, $N = 1$, $n = 14$. Significance was tested using two-tailed paired sample sign test ($p = 10^{-4}$). Data in the legend are presented as mean $\pm$ SD. N = number of biological replicates, n = number of cells, SD = standard deviation. The center line in the box plot represents the median, the box represents the 25%-75% of the data, whiskers represent 1.5 interquartile range, the square box among the data points represents the mean.



Supplementary Figure 2. Single FGF2-GFP at the plasma membrane. Time traces of fluorescence emission from FGF2-GFP molecules on the plasma membrane.



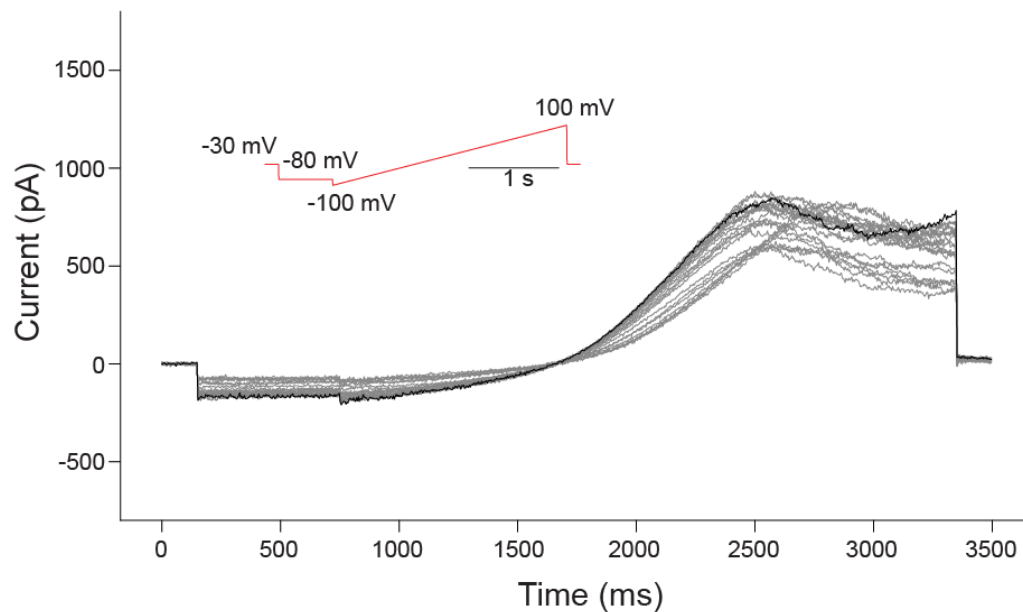
Supplementary Figure 3. Golgi localization of PhoCl caged proteins. (a) CV1 cells expressing mScarlet-CD4-PhoCl-RER (b) CV1 cells expressing FLAG-BK-YFP-PhoCl-TMEM. N = 3. Scale bars are 10 μ m.



Supplementary Figure 4. UV dose response of uncaging of mScarlet-CD4-PhoCl-RER. (a)

Representative negative control, HeLa cells without doxycycline (Dox)-based expression of mScarlet-CD4-PhoCl-RER, and the gating strategy used for the flow cytometry experiments. Briefly, live cells were selected from which single cells were gated for. Next, cells expressing mScarlet-CD4-PhoCl-RER or CD4-mRFP (positive control) were selected for. In the last step, cells with membrane localized mScarlet-CD4 or CD4-mRFP detected by AF647 labeled anti-RFP antibody were selected.

(b) Representative flow cytometry results from negative control, HeLa cells expressing mScarlet-CD4-PhoCl-RER under doxycycline but without UV treatment. **(c)** Representative flow cytometry results from positive control, HeLa cells overexpressing CD4-mRFP. **(d, e, f)** Representative flow cytometry results of HeLa cells expressing mScarlet-CD4-PhoCl-RER under doxycycline with increasing doses of UV treatment.



Supplementary Figure 5. Current kinetics of optogenetically released VRAC. Example ramp current traces from optogenetically released VRACs for 4 min upon switching bath solution to hypotonicity with maximal developed current trace shown in black. Ramp protocol shown red in inset. Note the fast inactivation at inside-positive voltages typical for LRRC8A/E-composed VRACs.

Primer list:

	Construct name	Fragment name		Primer sequence (5'-3')
1	TMEM-PhoCl-FGF2-GFP	TMEM115	For	aaaaaaa gctagc gccgccgcgatcg
			Rev	tttttt tgtaca cagcgtcgggggagc
2	TMEM-PhoCl-FGF2-GFP	PhoCl	For	aaaaaaa ctcgag gtgatccctgactactcaagcag
			Rev	tttttt aagctt ggtacctccacctcccgct
3	TMEM-PhoCl-FGF2-GFP	FGF2-GFP	For	aaaaaaa gaattc aa accatggcagccggg
			Rev	tttttt gtcgac ttactgtacagctcgtccatgc
4	mScarlet-CD4-PhoCl-RER (Fig2B,C)	mScarlet - CD4-PhoCl2c-RER	For	tagtcagctgacgcgtgctagcggatccatgtggcctcttgtt
			Rev	ttatcgatgcggccgcgctagctctagattagctggcgaaggc
5	mScarlet-CD4-PhoCl-RER_stable cells (Fig2D,E)	Tet promotor	For	ctccaaggccttaattcgaactagttaatgtgagttagctcactcattaggcac
			Rev	ggcgatctgacgggtcactaaac
6	mScarlet-CD4-PhoCl-RER_stable cells (Fig2D,E)	mScarlet-CD4-PhoCl2c-RER	For	gtttagtgaaccgtcagatcgccgccaccatgtggcctcttgttctgc
			Rev	tttgaataaccgcggaatacgcgtttagctggcgaaggctttcc
7	FLAG-BK-YFP-PhoCl-TMEM	FLAG-BK_fragment 1	For	atcctctagtcagctgacgcgtgaaccggtgccaccatggtgcataccatccat
			Rev	gacacgctgaactgtggccgttta
8	FLAG-BK-YFP-PhoCl-TMEM	BK_fragment 2	For	taaacggccacaagttcagcgtgc
			Rev	gtcgggtatgacgctgccgccgccaccgtaagccgctcttctgcac
9	CACNA1E-eGFP-PhoCl-TMEM115	PhoCl2c_PCR 1	For	gcatggacgagctgtacaagaccggtggcggcgccgagcgtcataccgactactcaagcagag
			Rev	tctagggtactttgtgaaca
10	CACNA1E-eGFP-PhoCl-TMEM115	PhoCl2c_PCR 2	For	gcatggacgagctgtacaag
			Rev	tgtgccgctccgctgtgccgctccgctctagggtactttgtgaaca
11	CACNA1E-eGFP-PhoCl-TMEM115	TMEM115	For	ggcggaggcggcacaggcggaggcggcacaatgcaacgtgccctgccca
			Rev	tcgacaagcttatcgatgcggccgcgctagcttacagcgtcggggagctgc
12	LRRC8A-GFP-PhoCl-TMEM	LRRC8A_fragment1	For	atcctctagtcagctgacgcgtgaaccggtgccaccatgattccggtgacagagctc
			Rev	ggatctccttgatgctggtgaactt
13	LRRC8A-GFP-PhoCl-TMEM	LRRC8A_fragment2	For	aagttcaccgacatcaaggagatcc
			Rev	gtcgggtatgacgctgccgccgccaccggtcttgtacagctcgtccatgc

14	LRRC8A-GFP-alfa-PhoCl-TMEM	Alfa_tag	For	aagttcaccgacatcaaggagatcc
			Rev	ggatctccttgatgtcggtgaactt
15	LRRC8A-PhoCl-TMEM	LRRC8A_fragment2	For	aagttcaccgacatcaaggagatcc
			Rev	atgacgctgccgccgccacccgcggcctgctccttgtagc
16	TMEM-PhoCl-IRAK4-mScarlet	TMEM-PhoCl	For	tttttggaggcctaggctacgcgtgccaccatgcaacgt
			Rev	actaccaccagaacctccacttcctcctctagggtactttgtgaaca
17	TMEM-PhoCl-IRAK4-mScarlet	IRAK4-mScarlet	For	ggaggaagtggaggtctggtggtagtatgaacaagccgttgacacat
			Rev	tgcaggctgactctagagtcgcggccgcttactgtacagct