

Supporting information

Targeted solvatochromic fluorescent probes for imaging lipid order in organelles under oxidative and mechanical stress

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Supporting figures

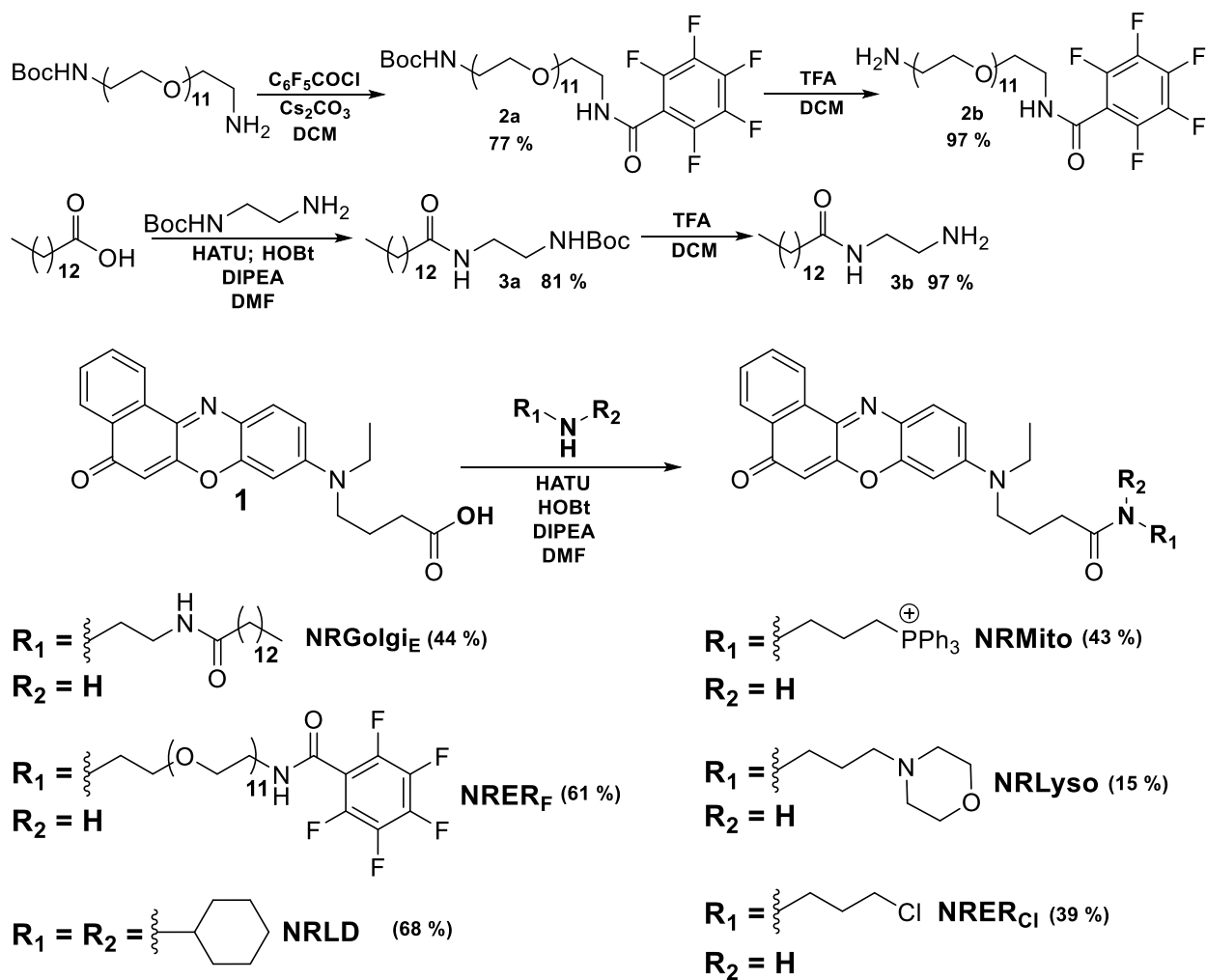
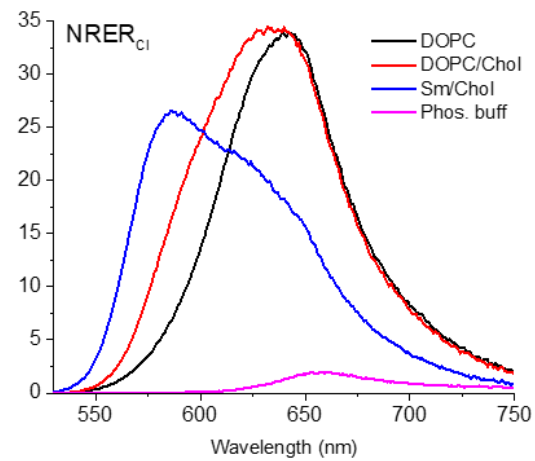
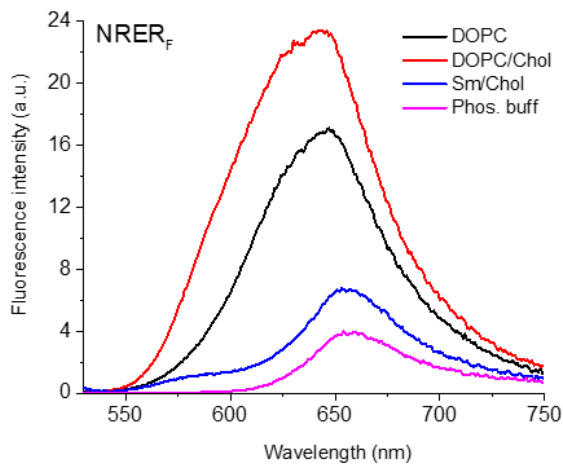
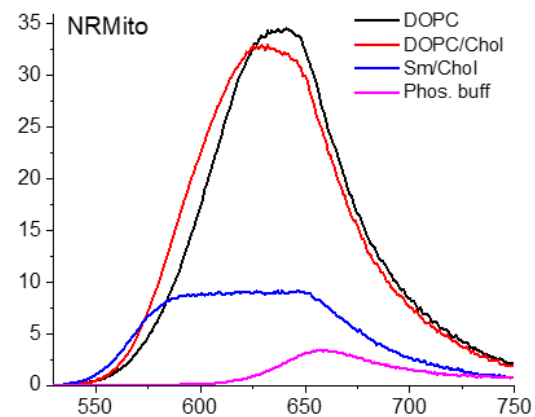
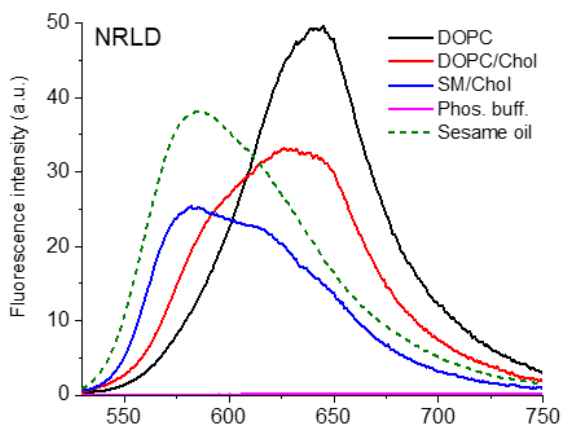
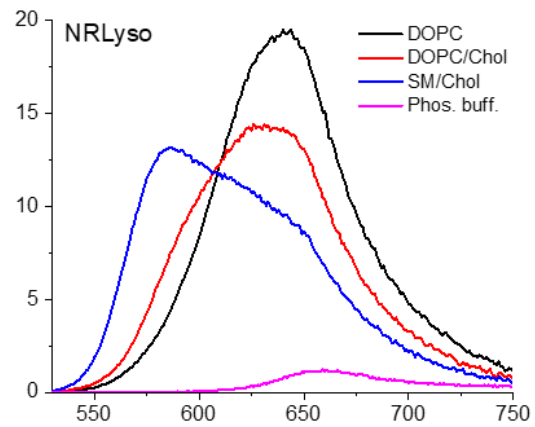
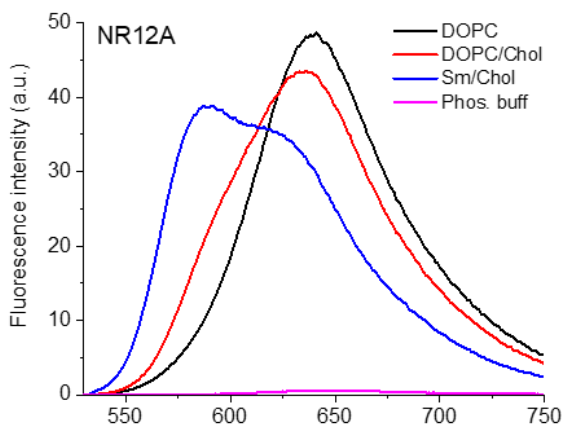


Figure S1. Organelle probe synthesis schemes.



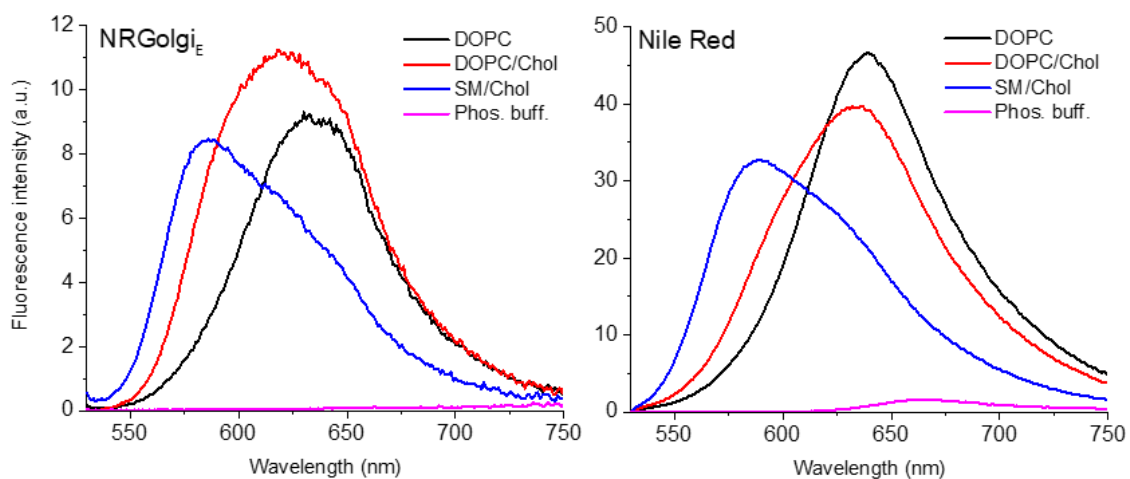


Figure S2. Fluorescence emission spectra of organelle probes NR12A, NRLyso, NRLD, NRMito, NRER_F, NRER_{Cl}, NRGolgi_E and Nile Red in lipid vesicles of different composition and phosphate buffer. NRLD was also measured in sesame oil. Probe concentration was 2 μ M. Total lipid concentration was 1 mM. Excitation wavelength was 520 nm.

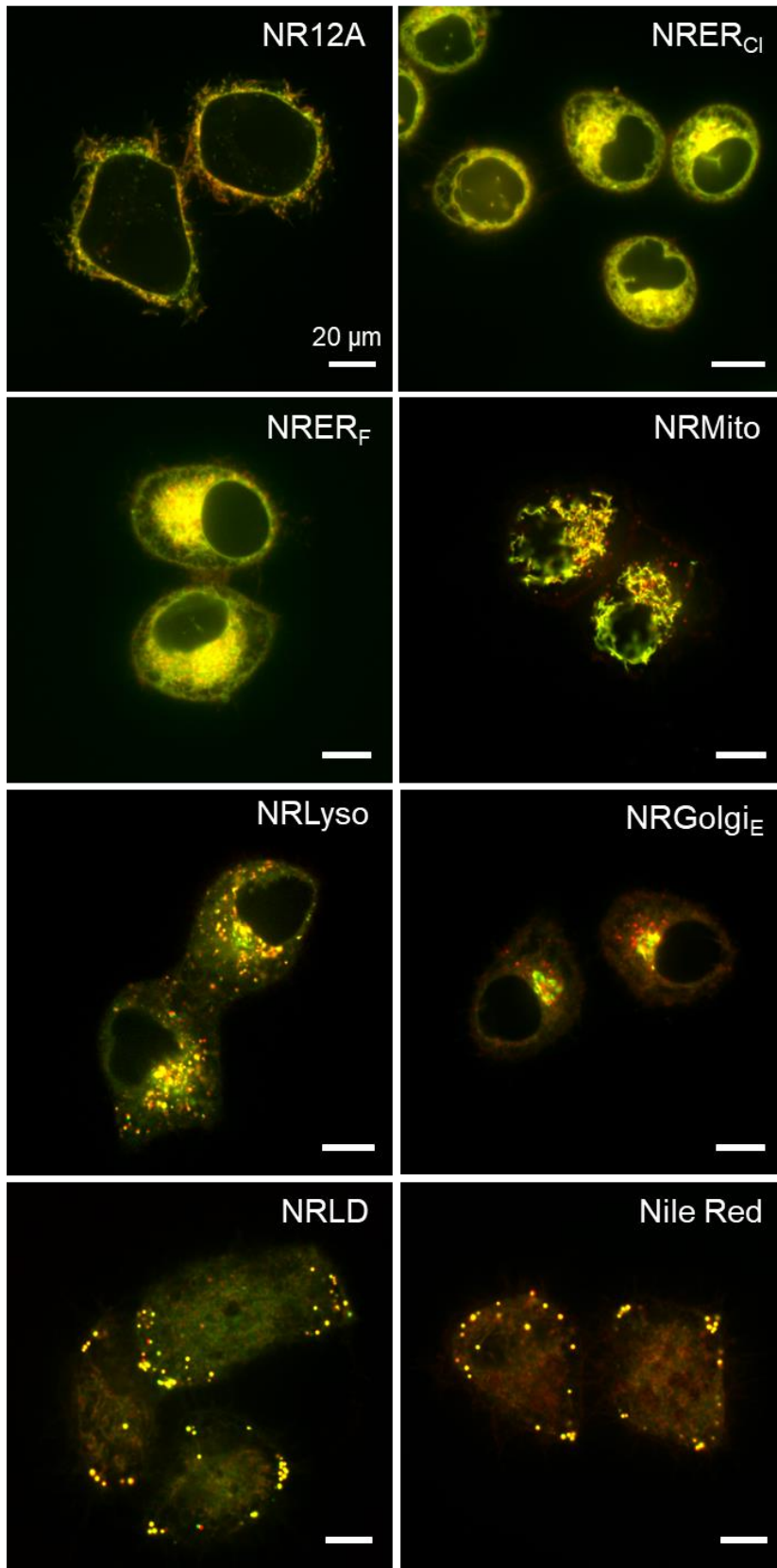


Figure S3. Zoom-in of composite colocalization microscopy images from Fig. 3.

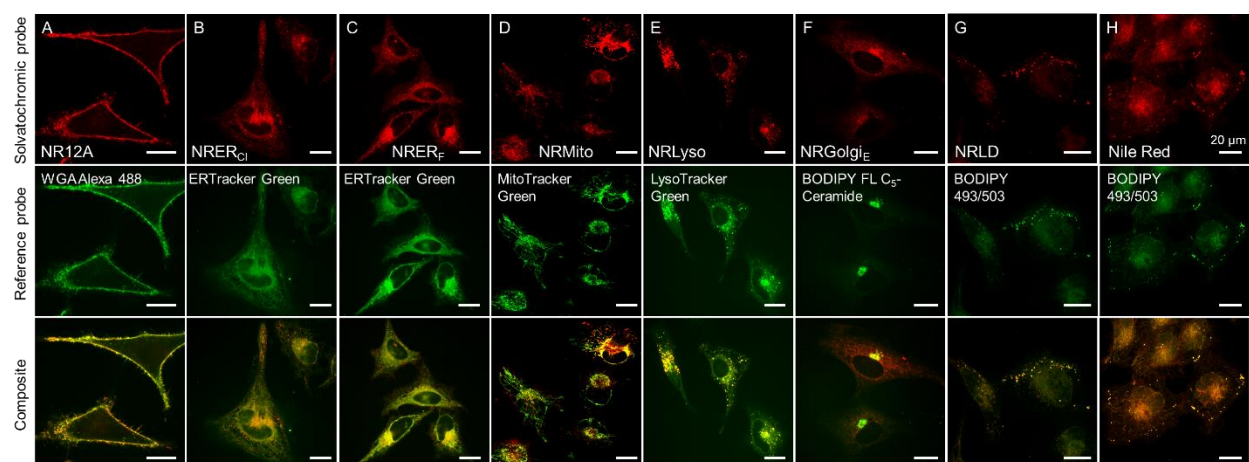
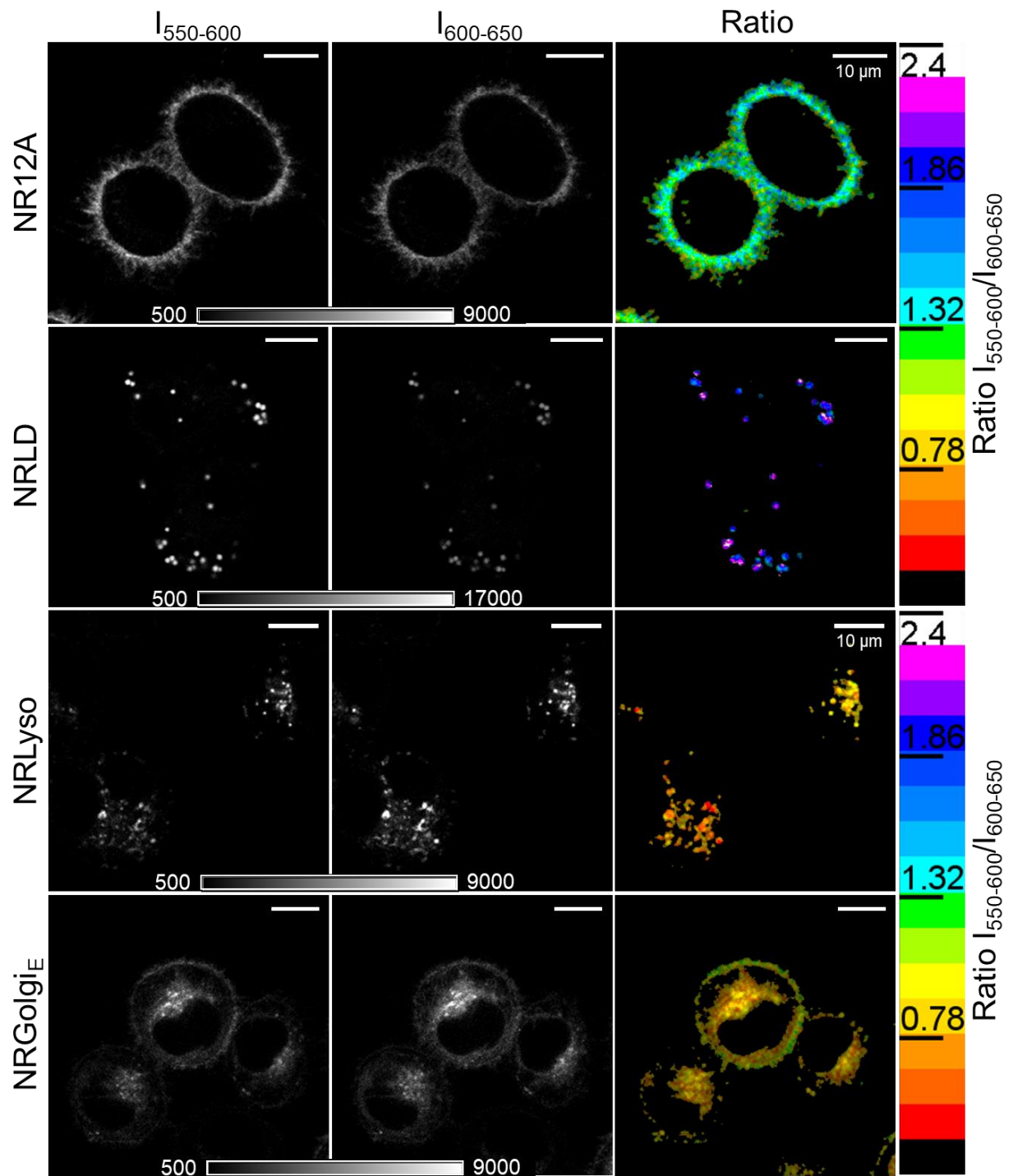


Figure S4. Imaging localization of solvatochromic organelle probes in live HeLa cells. Probe concentration 20 nM for NR12A; 50 nM for NRMito, NRLyso, NRLD, NRER_{C1}; 200 nM for NRER_F; 150 nM (in form of conjugate with β -cyclodextrine) for NRGolgi_E.



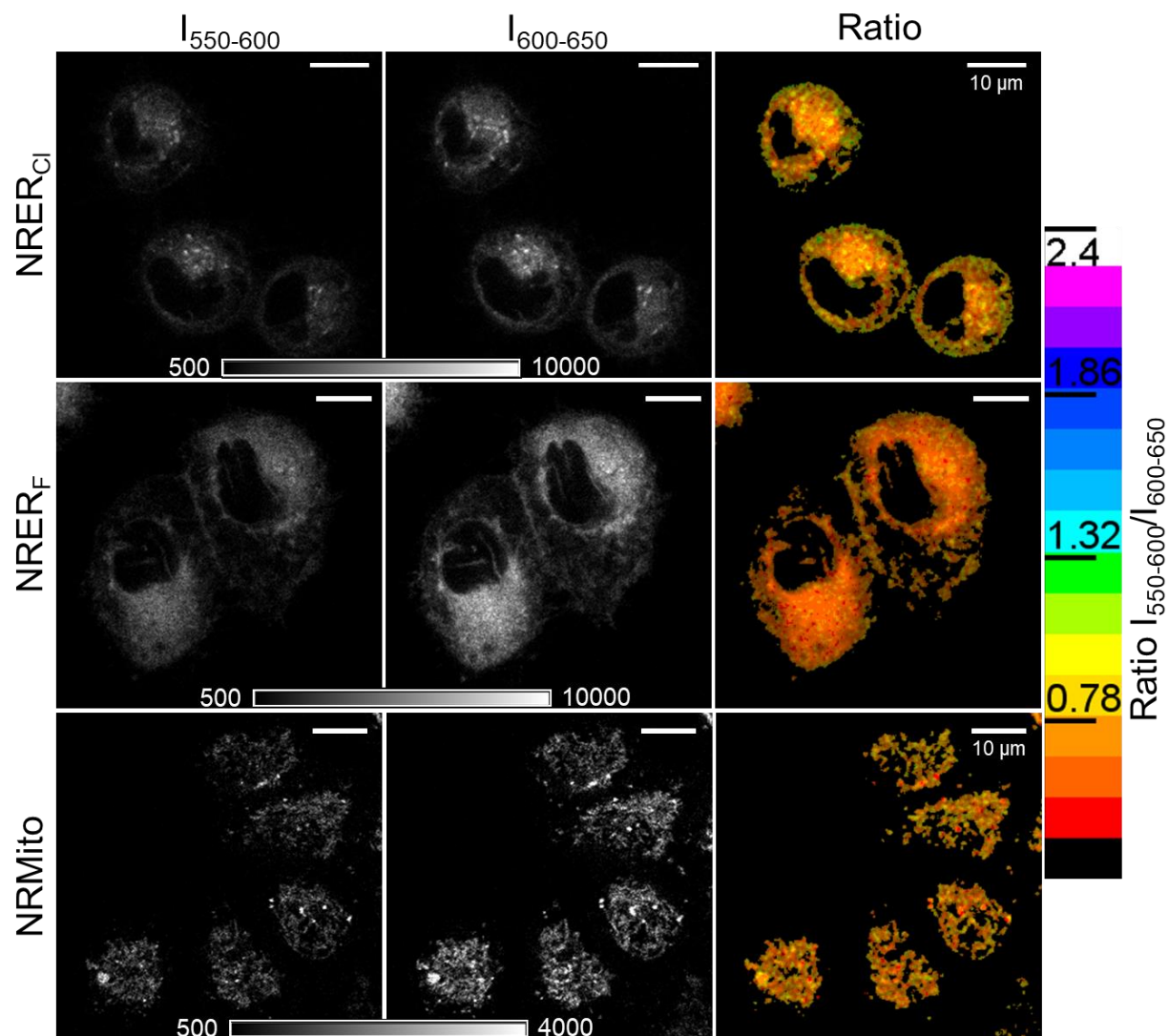
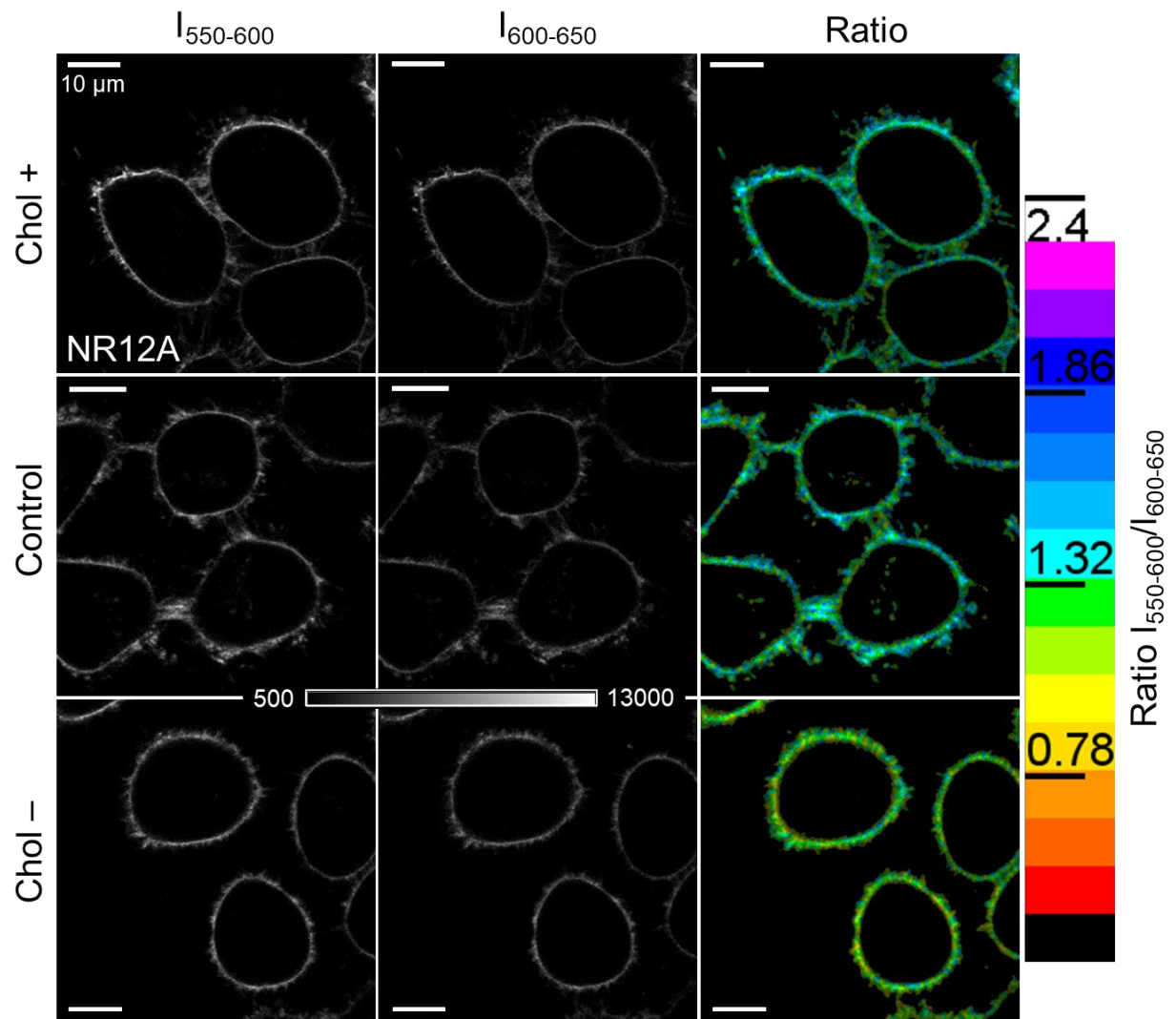
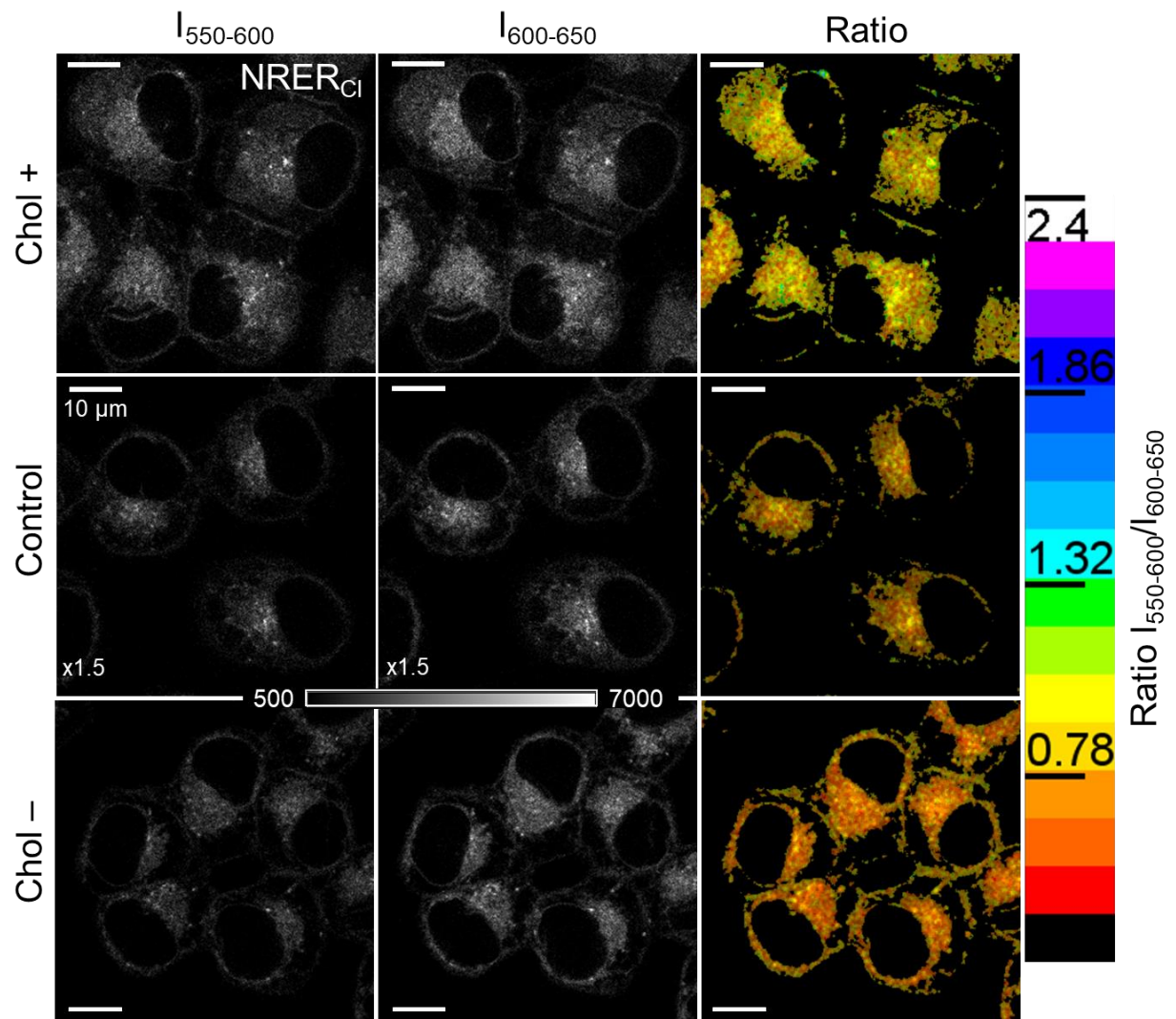
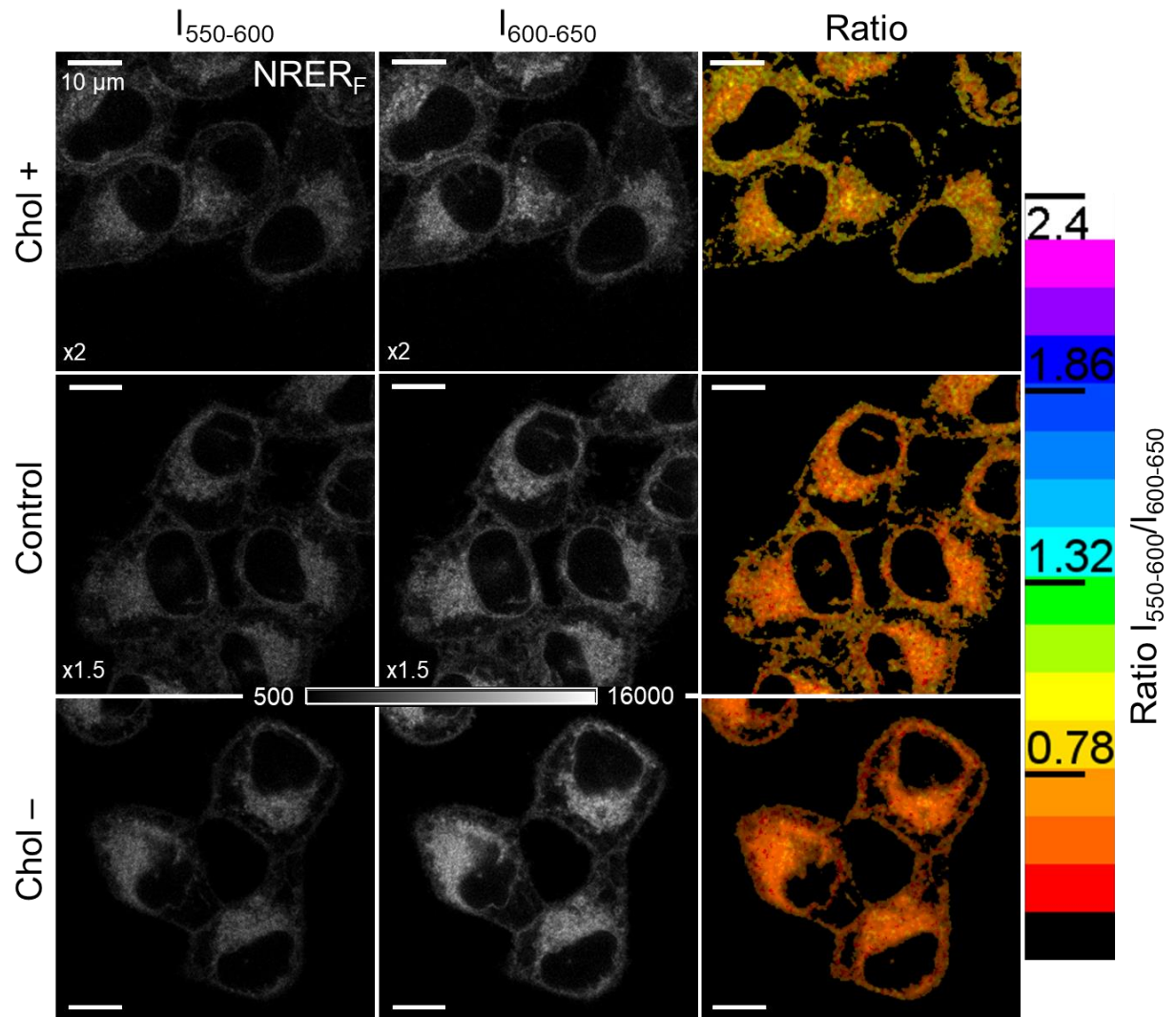
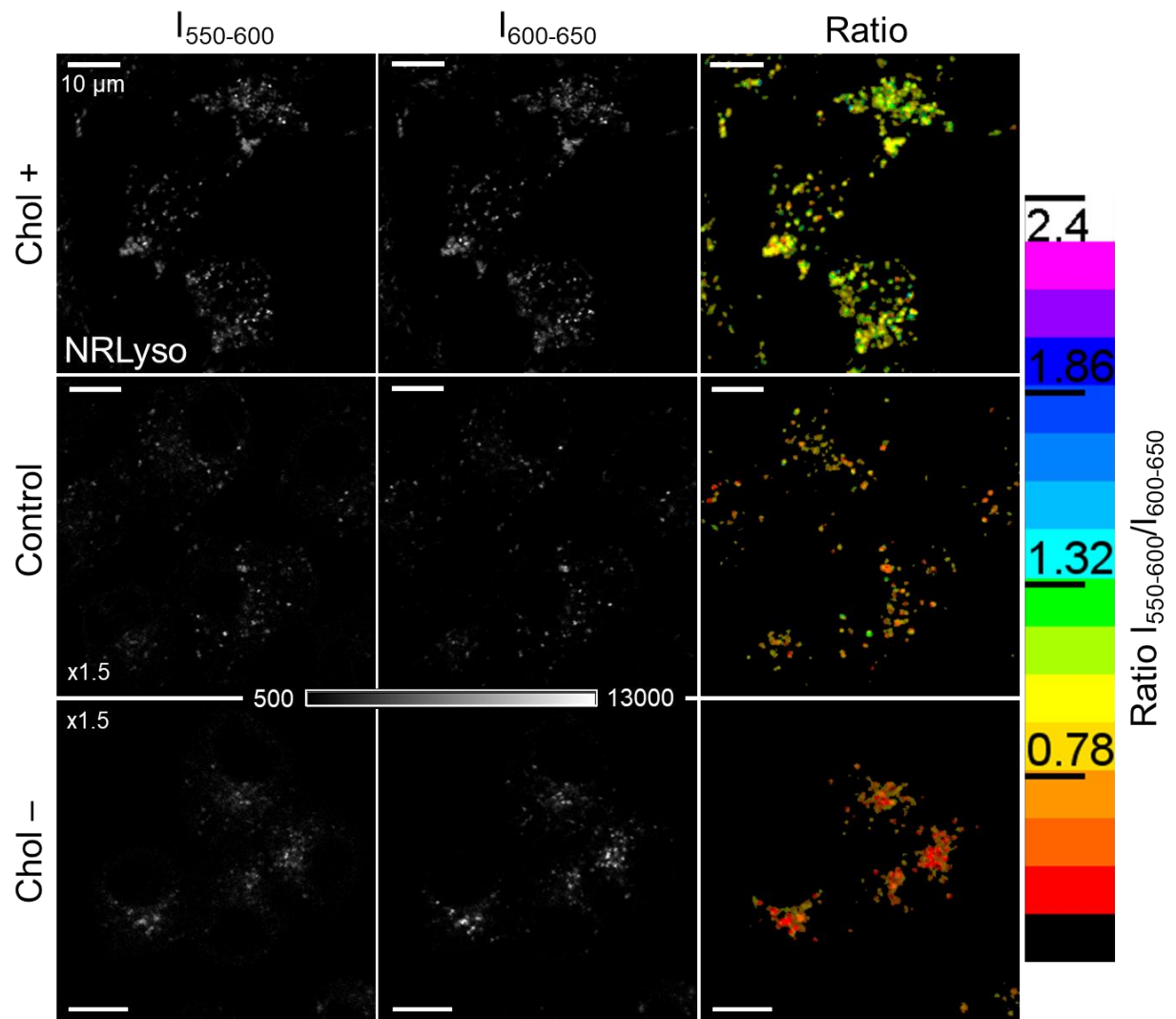


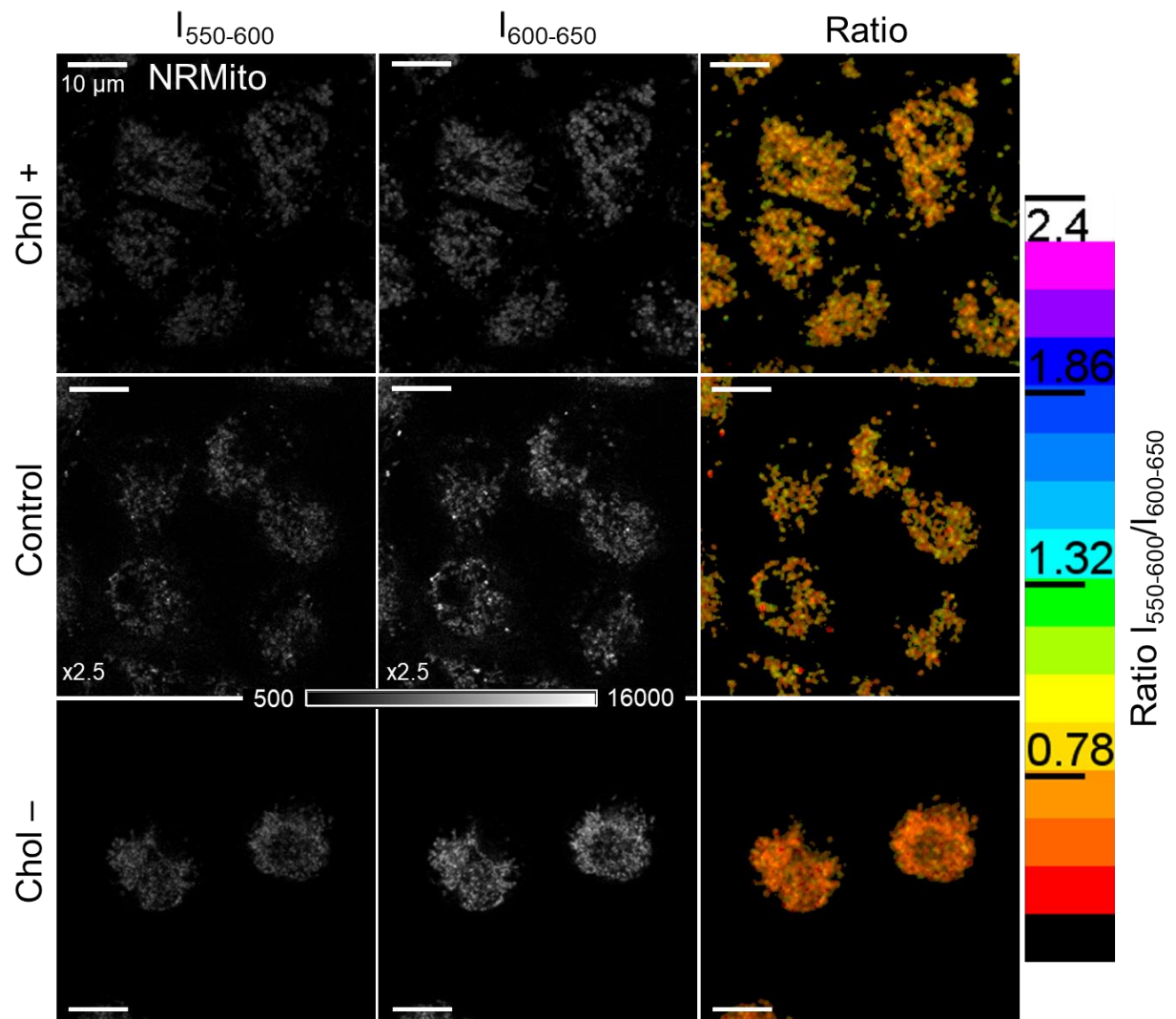
Figure S5. Ratiometric images of organelle probes NR12A, NRLyso, NRLD, NRMito, NRER_F, NRER_{Cl} and NRGolgi_E in KB cells. Probe concentration 20 nM for NR12A; 50 nM for NRMito, NRLyso, NRLD, NRER_{Cl}; 200 nM for NRER_F; 5 μ M (in form of BSA-conjugate) for NRGolgi_E.











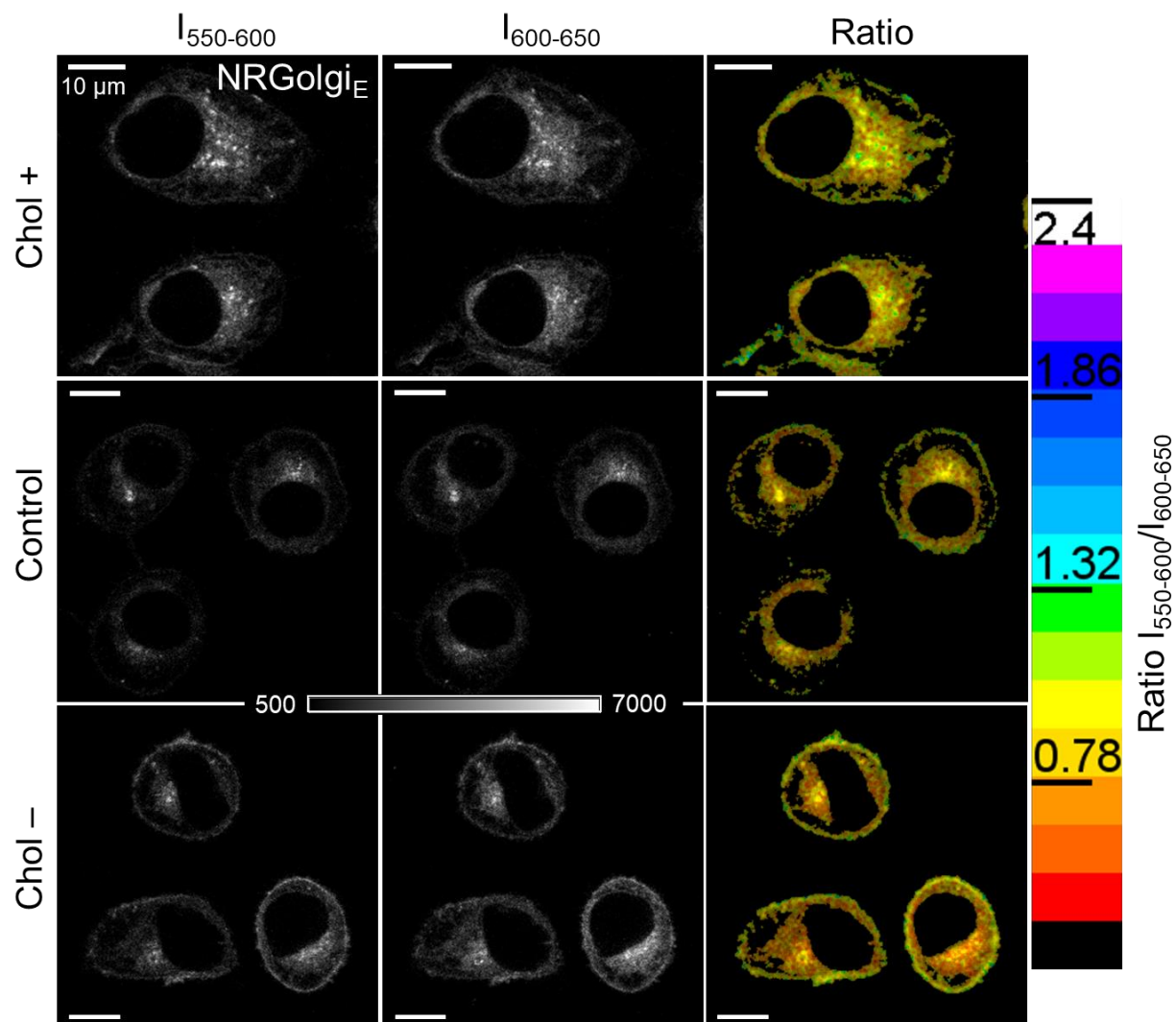


Figure S6. Ratiometric response of organelle probes NR12A, NRER_{Cl}, NRER_F, NRLyso, NRMito, and NRGolgi_E to cholesterol extraction or cholesterol enrichment induced in KB cells. Probe concentration 20 nM for NR12A; 50 nM for NRMito, NRLyso, NRLD, NRER_{Cl}; 200 nM for NRER_F; 2.5 μM (in form of BSA-conjugate) for NRGolgi_E.

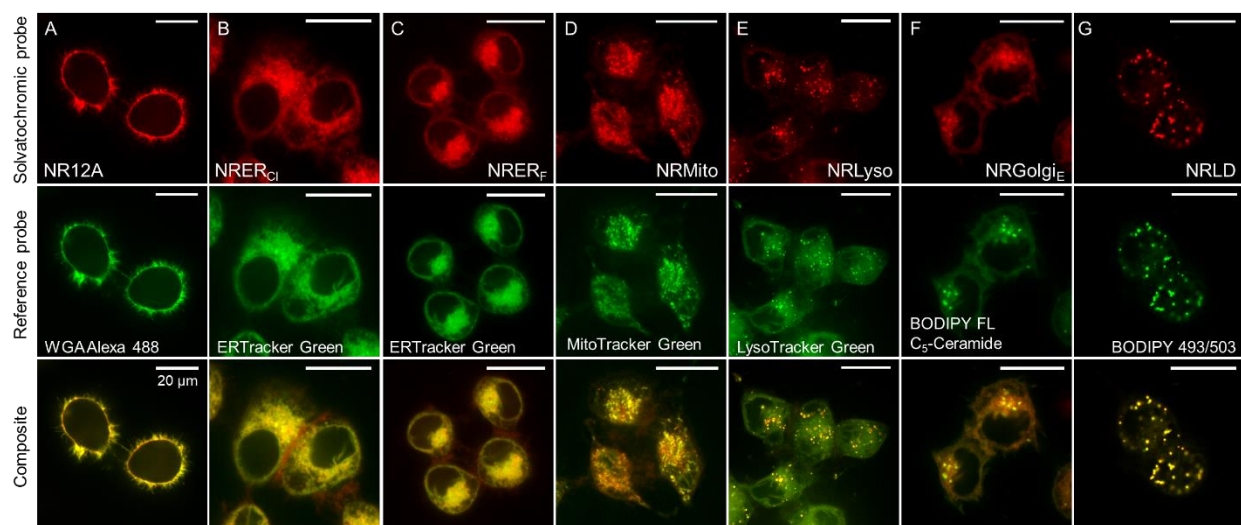
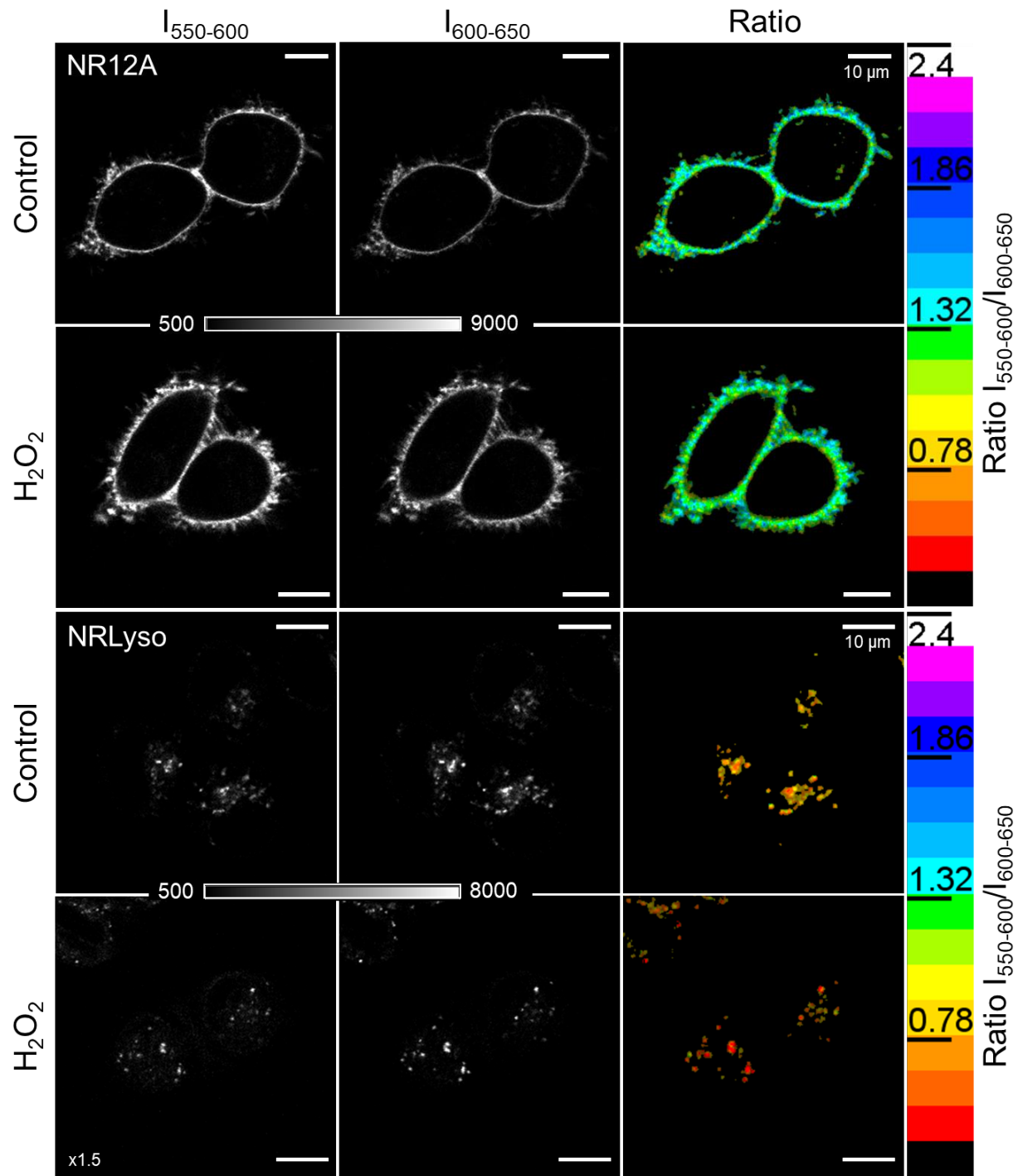
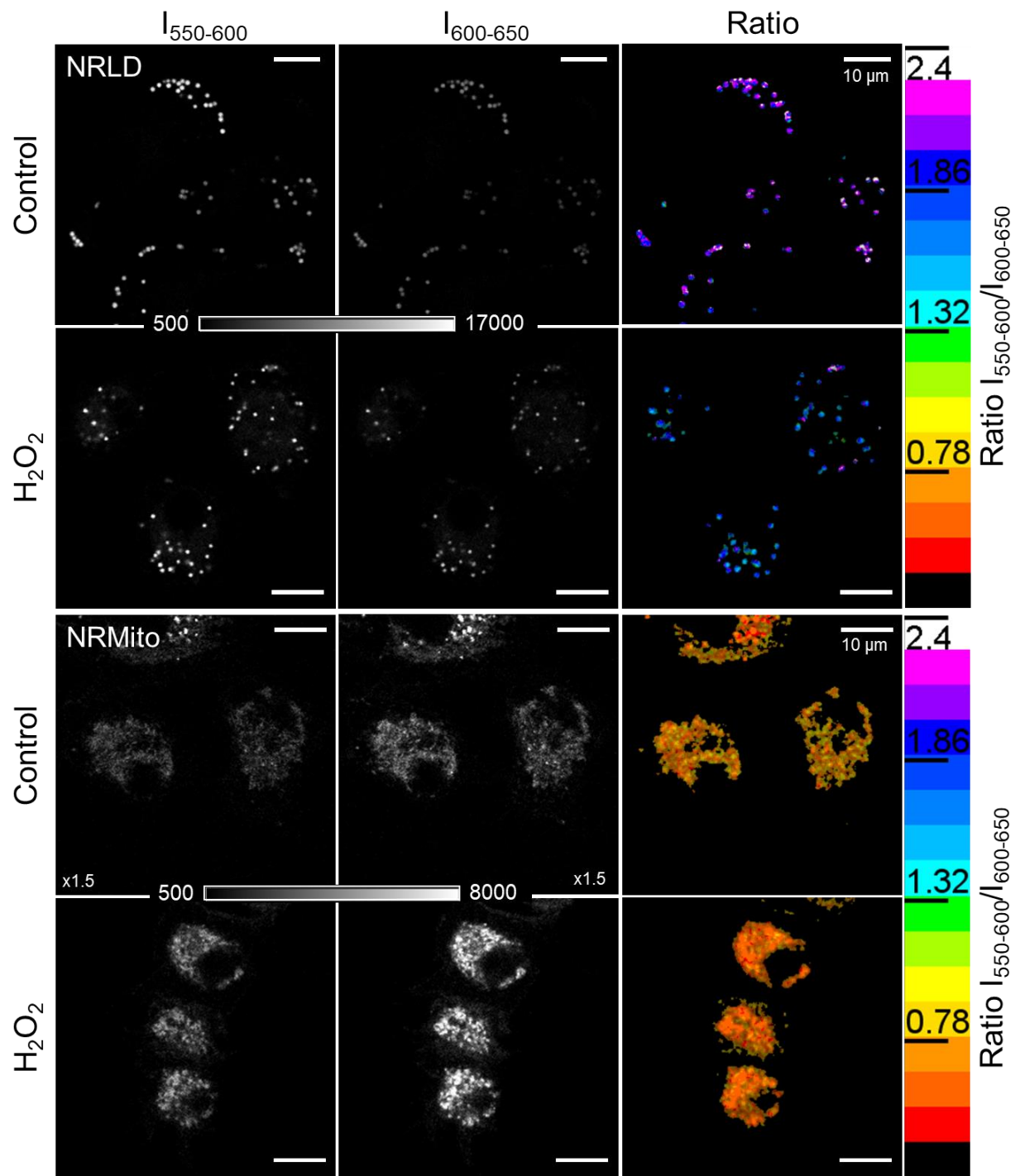
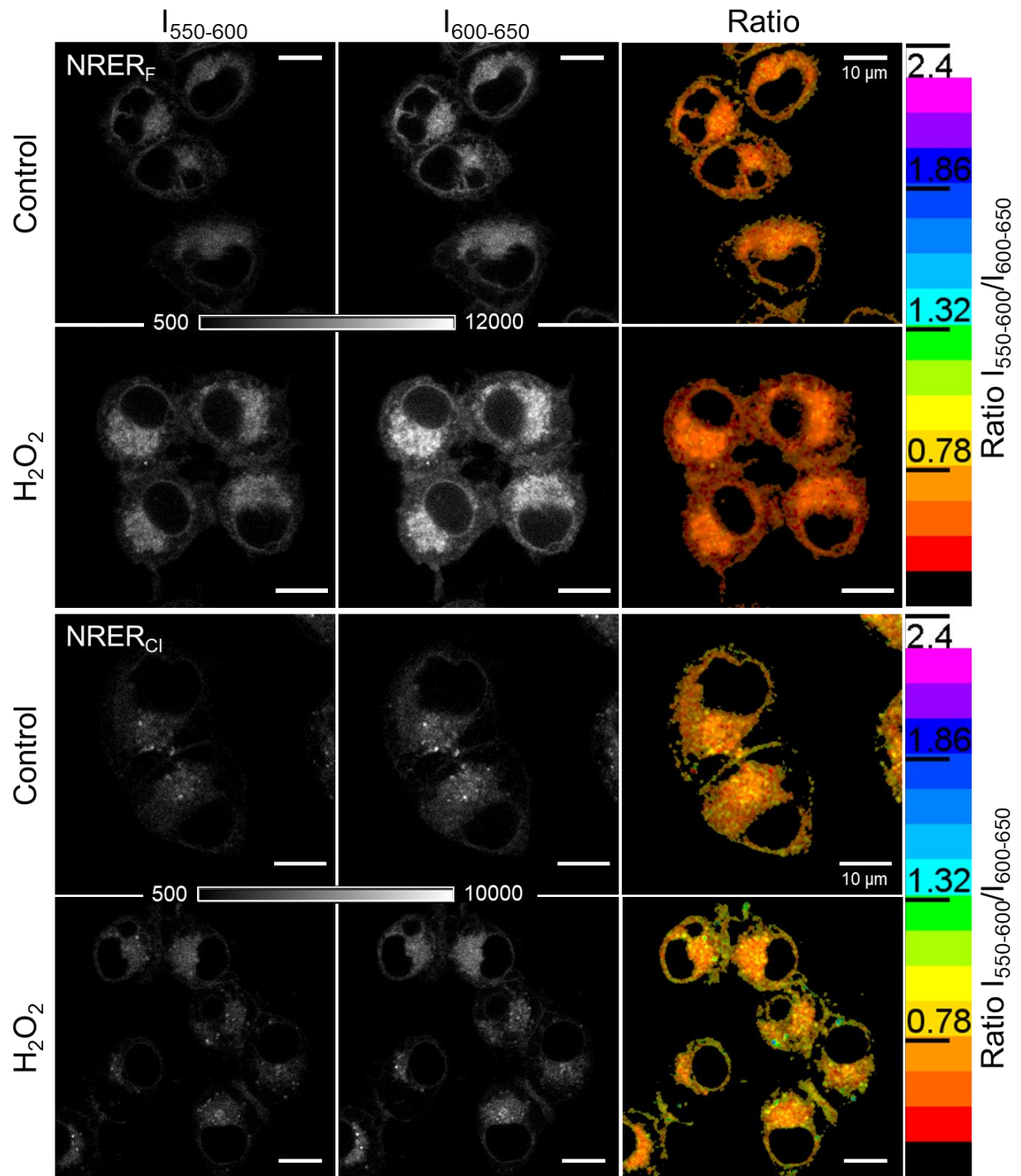


Figure S7. Imaging localization of solvatochromic organelle probes in live KB cells in oxidative stress conditions, using reference organelle-specific markers. Probe concentration 20 nM for NR12A; 50 nM for NRMito, NRLyso, NRLD, NRER_{C1}; 200 nM for NRER_F; 2.5 μM (in form of 1:1 conjugate with BSA) for NRGolgi_E.







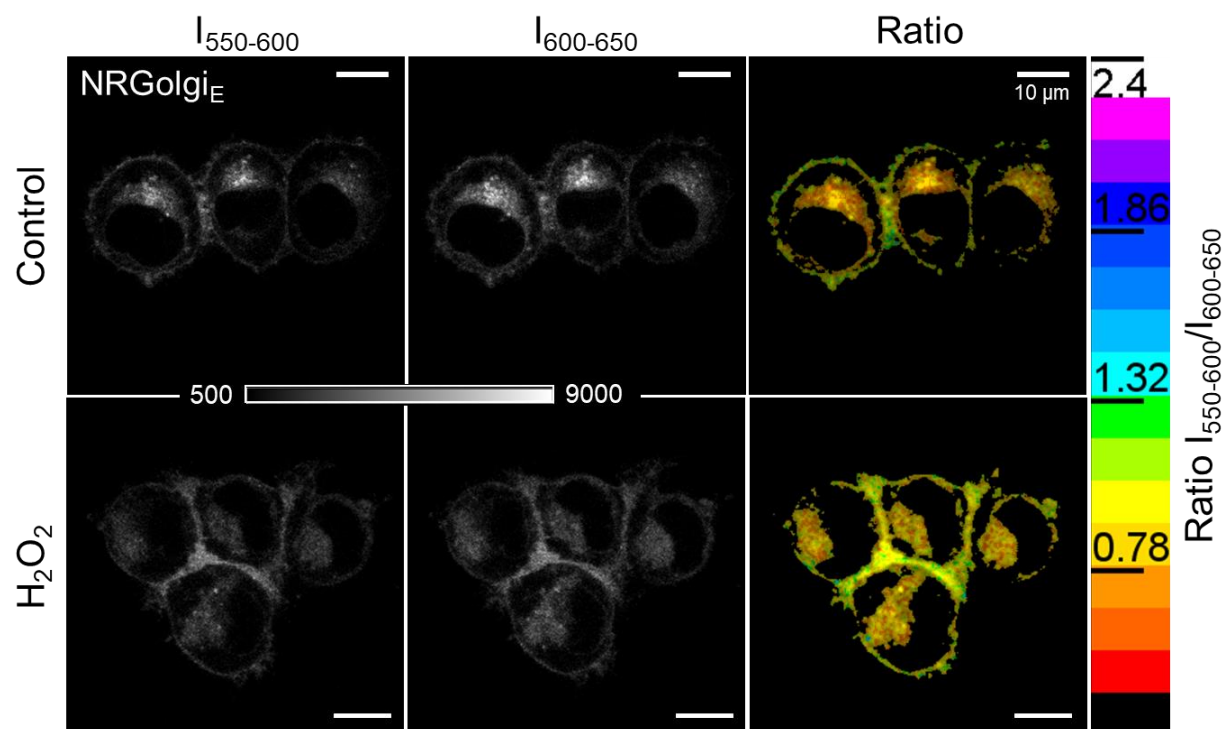


Figure S8. Ratiometric response of organelle probes NR12A, NRLyso, NRLD, NRMito, NRER_F, NRER_{Cl} and NRGolgi_E to oxidative stress induced in KB cells. Probe concentration 20 nM for NR12A; 50 nM for NRMito, NRLyso, NRLD, NRER_{Cl}; 200 nM for NRER_F; 5 μM (in form of BSA-conjugate) for NRGolgi_E.

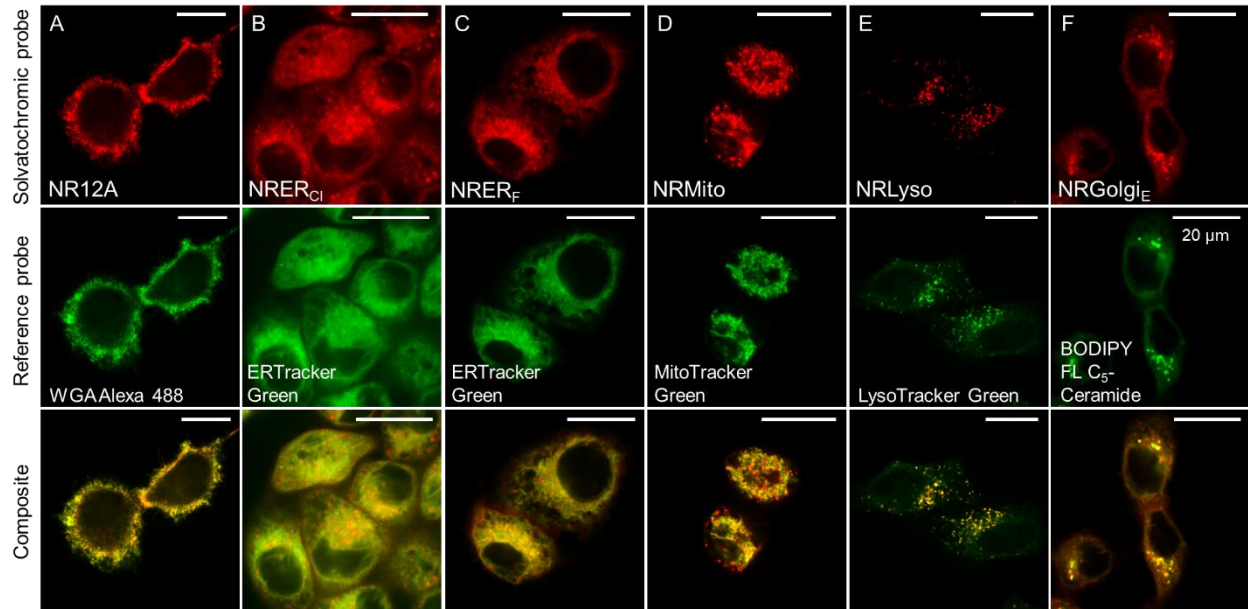
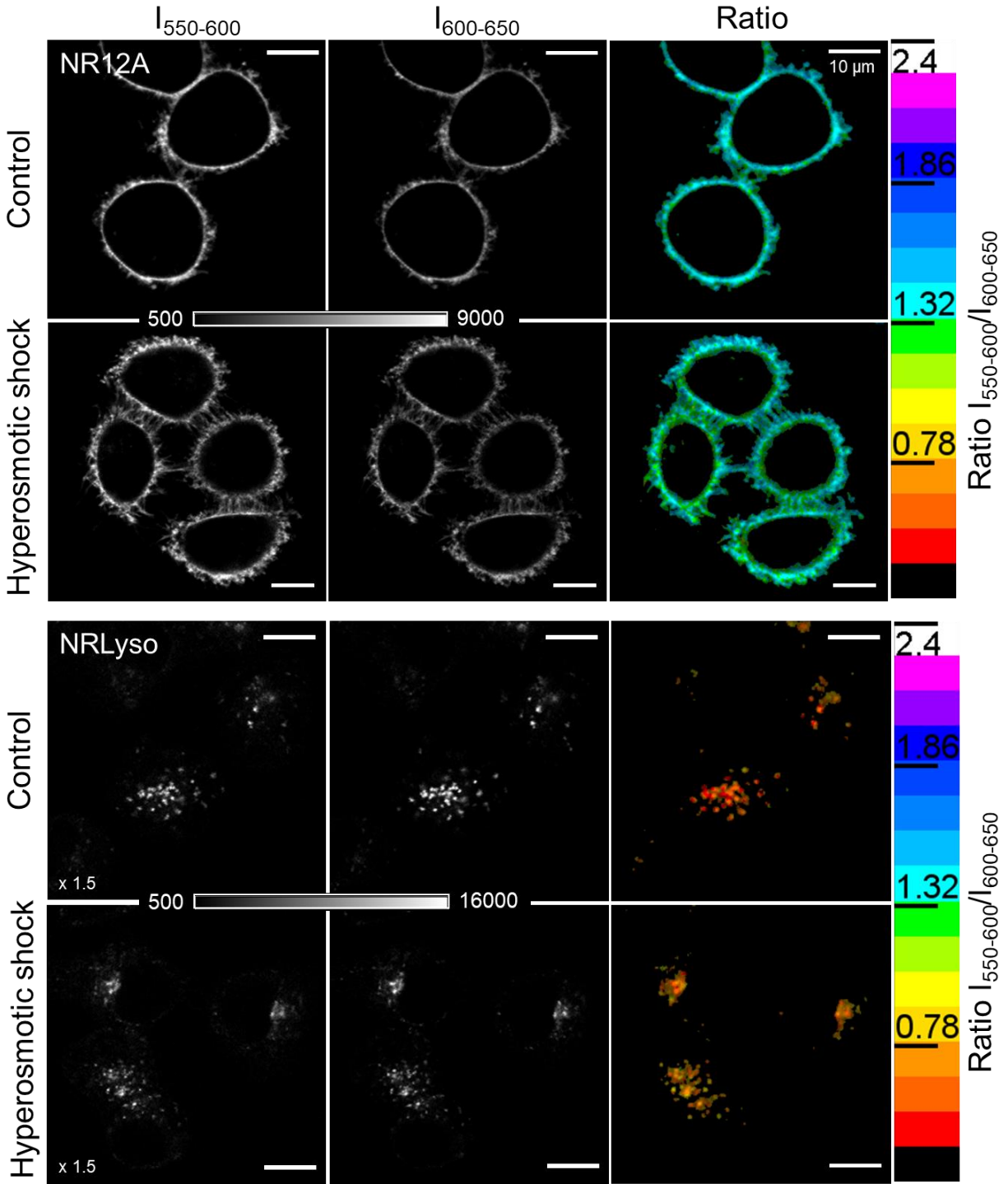
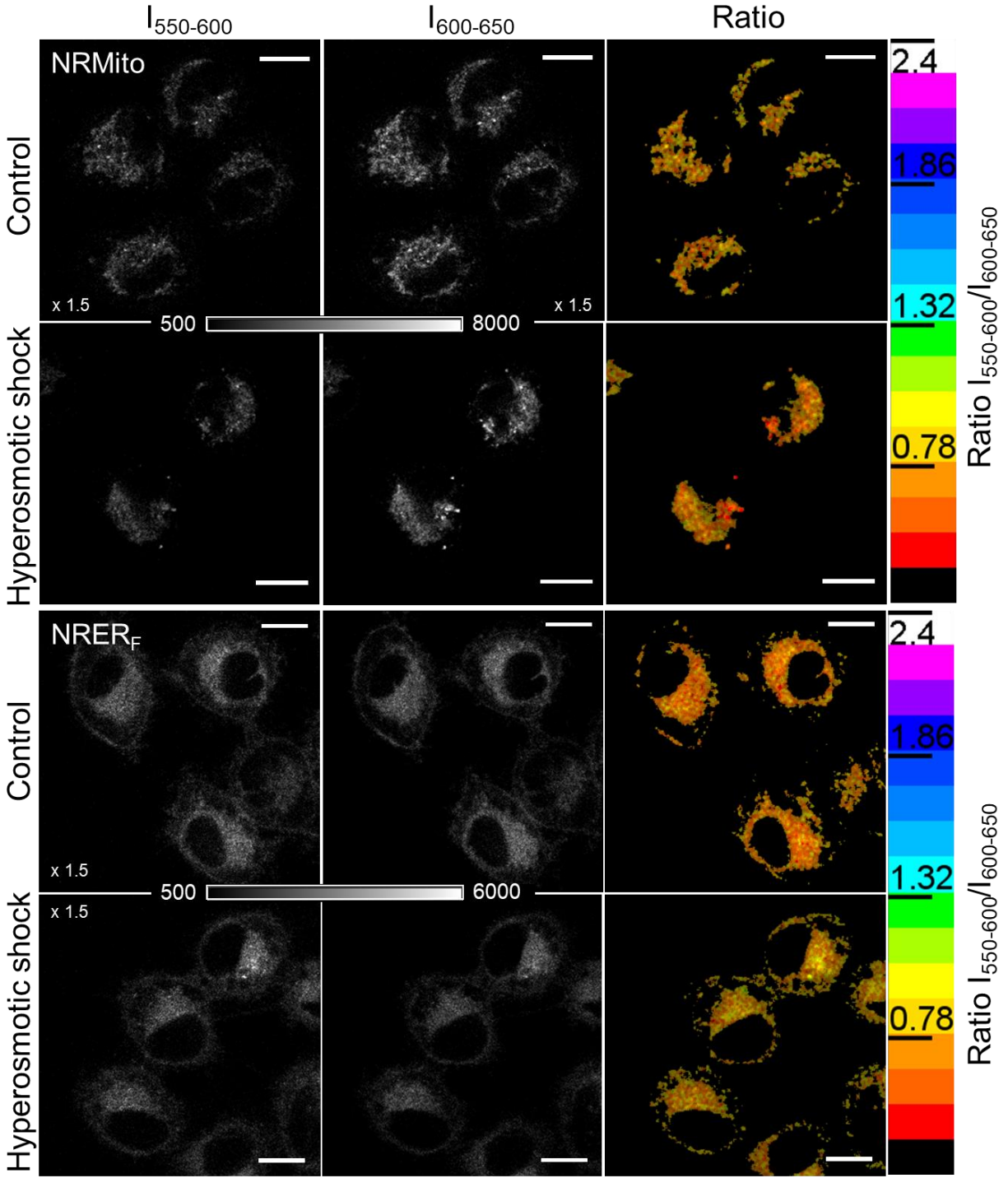


Figure S9. Imaging localization of solvatochromic organelle probes in live KB cells in hyperosmotic stress conditions, using reference organelle-specific markers. Probe concentration 20 nM for NR12A; 50 nM for NRMito, NRLyso, NRER_{C1}; 200 nM for NRER_F; 2.5 μM (in form of 1:1 conjugate with BSA) for NRGolgi_E.





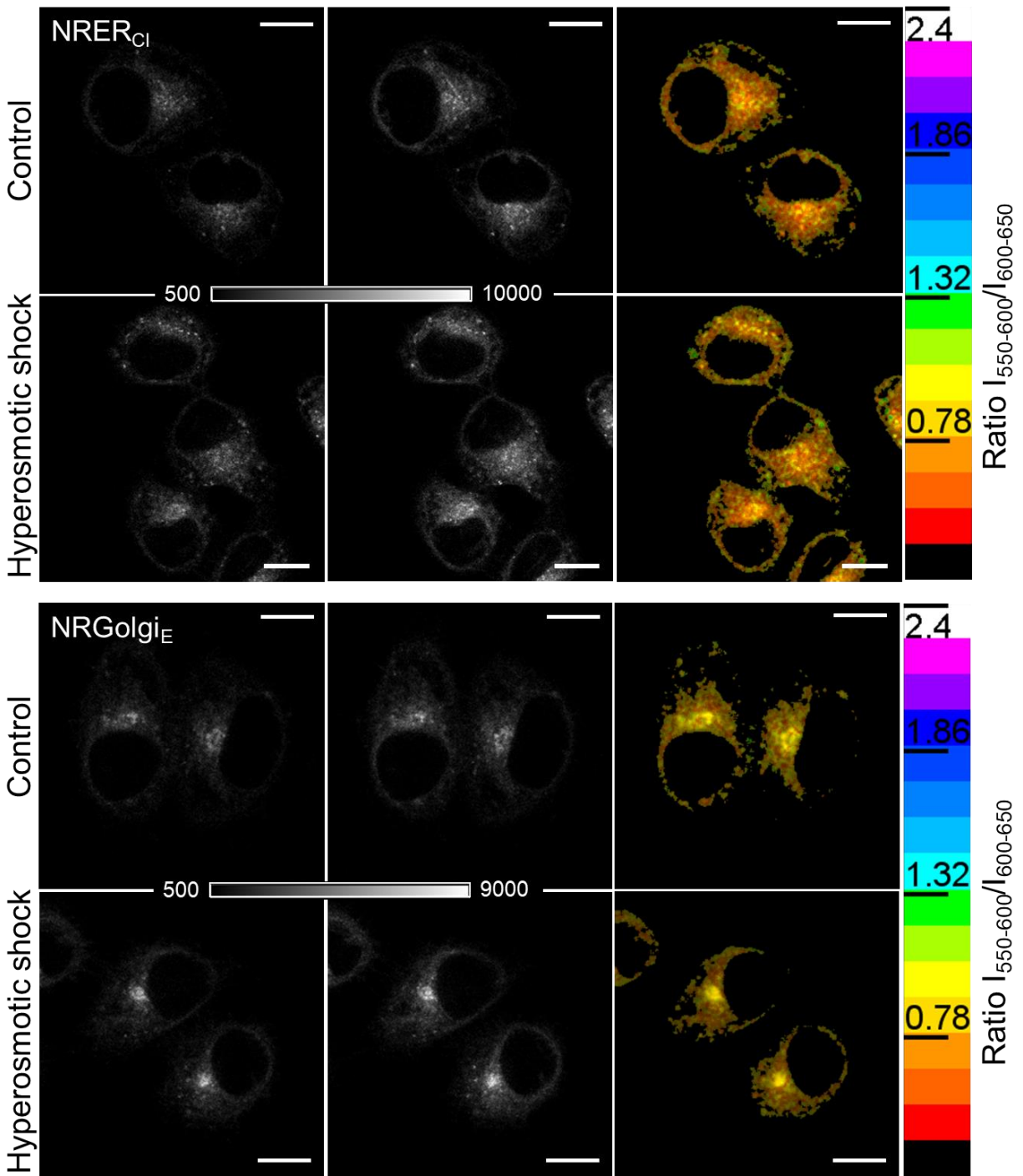


Figure S10. Ratiometric response of organelle probes NR12A, NRLyso, NRMito, NRER_F, NRER_{Cl} and NRGolgi_E to hyperosmotic shock induced in KB cells. Probe concentration 20 nM for NR12A; 50 nM for NRMito, NRLyso, NRER_{Cl}; 200 nM for NRER_F; 5 μ M (in form of BSA-conjugate) for NRGolgi_E.

Supporting tables

Table S1. Absorption and fluorescence properties of probes in solvents and lipid vesicles.^a

Probe name		Solvent or medium						
		DOPC	DOPC/ Chol	SM/ Chol	Phos. Buff.	MeOH	Acetone	Dioxane
NRMito	λ_{max} Abs	555	556	562	584	549	534	533
	λ_{max} Em	641	632	639	660	643	612	602
	QY, %	43	50	25	6.9	37	59	74
NRLyso	λ_{max} Abs	558	556	552	584	549	532	523
	λ_{max} Em	640	626	587	659	643	584	580
	QY, %	36	41	40	5.8	41	58	53
NRER _F	λ_{max} Abs	567	564	585	585	551	535	524
	λ_{max} Em	647	644	653	654	642	615	591
	QY, %	34	48	21	9.5	43	74	78
NRGolgi _E	λ_{max} Abs	520	528	514	512	550	533	522
	λ_{max} Em	630	618	586	736	642	615	586
	QY, %	21	31	28	0.44	41	77	78
NRER _{Cl}	λ_{max} Abs	550	554	549	583	549	534	521
	λ_{max} Em	639	632	586	653	645	613	584
	QY, %	38	55	56	6.5	42	86	75
NRLD	λ_{max} Abs	552	552	542	520	551	536	526
	λ_{max} Em	645	626	582	743	646	615	584
	QY, %	61	61	47	0.33	42	74	78
NR12A ^b	λ_{max} Abs	552	551	546	537	553	540	526
	λ_{max} Em	641	636	592	648	641	619	602
	QY, %	51	47	39	0.36	44	70	70
Nile Red ^b	λ_{max} Abs	549	554	541	586	551	532	520
	λ_{max} Em	639	636	589	666	641	615	585
	QY, %	55	71	52	4.2	38	70	74

^a Probe concentration was 2 μM . Total phospholipid concentration for all liposomes was 1 mM (phosphate buffer 20 mM, pH 7.4). Excitation wavelength was 520 nm. ^b Data from a previous work.¹

Table S2. Pearson's correlation coefficient values for colocalization of Nile Red organelle probes vs corresponding commercial organelle-specific markers in KB cells and HeLa cells.

Probe	Pearson's correlation coefficient			
	KB cells Control	HeLa cells Control	KB cells Oxidative stress	KB cells Hyperosmotic stress
NR12A	0.96	0.93	0.97	0.95
NRMito	0.92	0.55	0.91	0.83
NRLyso	0.89	0.82	0.86	0.89
NRGolgi _E	0.65	0.59	0.76	0.74
NRER _{C1}	0.95	0.82	0.95	0.93
NRER _F	0.98	0.89	0.96	0.95
NRLD	0.92	0.91	0.97	-
Nile Red	0.84	0.91	-	-

Table S3. Weighted arithmetic mean values for organelle probes in KB cells under various conditions.

Probe	Control (Chol)	Cholesterol extraction	Cholesterol enrichment	Control (H ₂ O ₂)	H ₂ O ₂	Control (HS)	Hyperosmotic shock
NR12A	1.319 ±0.005	1.21 ±0.02	1.346 ±0.006	1.311 ±0.008	1.26 ±0.03	1.39 ±0.01	1.38 ±0.01
NRMito	0.73 ±0.01	0.64 ±0.03	0.74 ±0.01	0.705 ±0.008	0.639 ±0.005	0.72 ±0.01	0.70 ±0.01
NRLyso	0.75 ±0.02	0.668 ±0.007	1.01 ±0.02	0.76 ±0.02	0.67 ±0.01	0.657 ±0.04	0.720 ±0.03
NRLD	-	-	-	1.93 ±0.02	1.81 ±0.07	-	-
NRER _{Cl}	0.792 ±0.004	0.742 ±0.008	0.888 ±0.007	0.749 ±0.005	0.80 ±0.01	0.762 ±0.008	0.767 ±0.004
NRER _F	0.657 ±0.003	0.599 ±0.006	0.755 ±0.008	0.638 ±0.004	0.62 ±0.01	0.726 ±0.009	0.786 ±0.005
NRGolgi _E	0.853 ±0.009	0.83298 ±0.007	0.920 ±0.004	0.848 ±0.008	0.91 ±0.02	0.84 ±0.01	0.824 ±0.008

References

(1) Danylchuk, D. I.; Moon, S.; Xu, K.; Klymchenko, A. S. Switchable Solvatochromic Probes for Live-Cell Super-Resolution Imaging of Plasma Membrane Organization. *Angew. Chem. Int. Ed.* 2019, 58, 14920-14924.