Supplementary Information

ProxyPhos sensors for the detection of negatively charged membranes.

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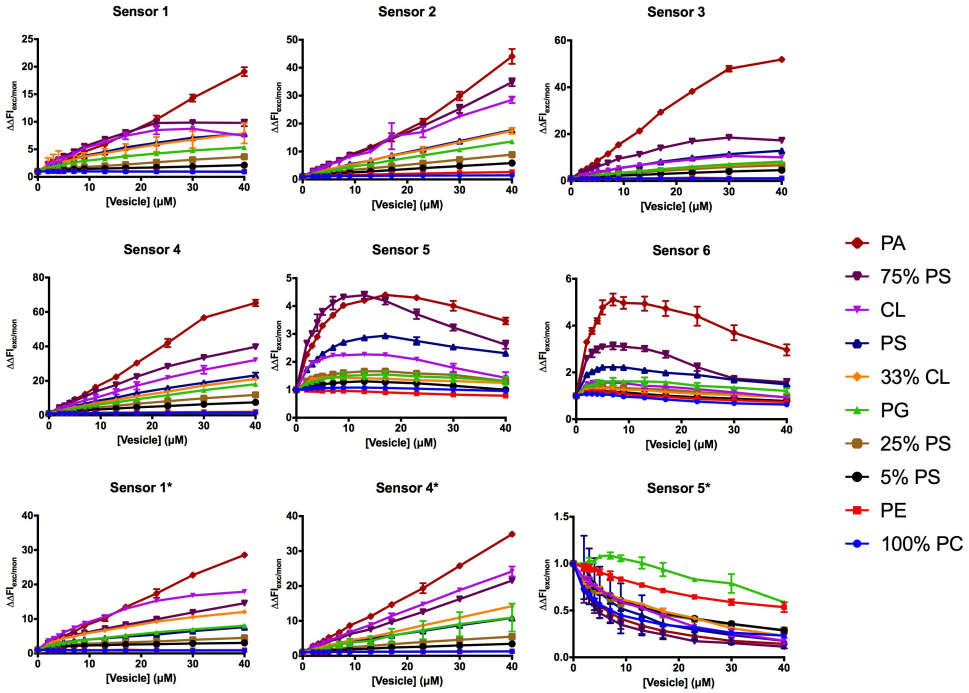
Supplementary Figures:

Figure S1. $\Delta\Delta FI_{exc/mon}$. $\Delta\Delta FI_{exc/mon}$ of all library sensors with all vesicles (50 mM HEPES, pH 7.5, 5% DMSO. (A) $\Delta\Delta FI_{exc/mon}$ for vesicle concentrations from 0-40 µM and (B) 0-200 µM. In all experiments, [ProxyPhos library sensors] = 25 µM; $\lambda_{exc/emi}$ = 350/476 nm and 350/376 nm.

A



B

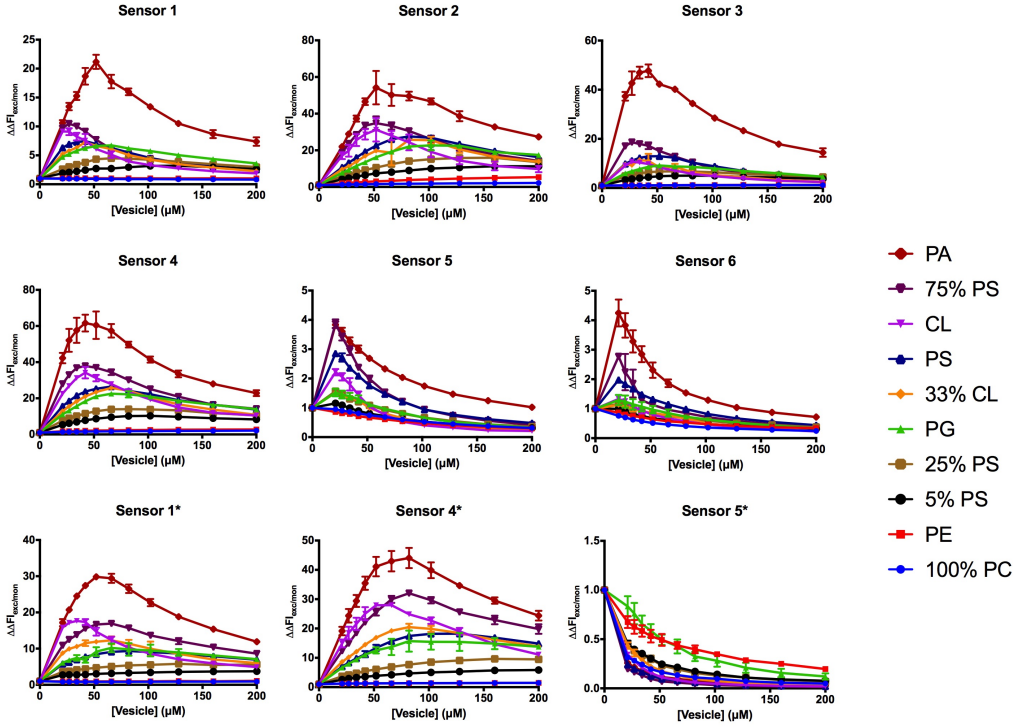
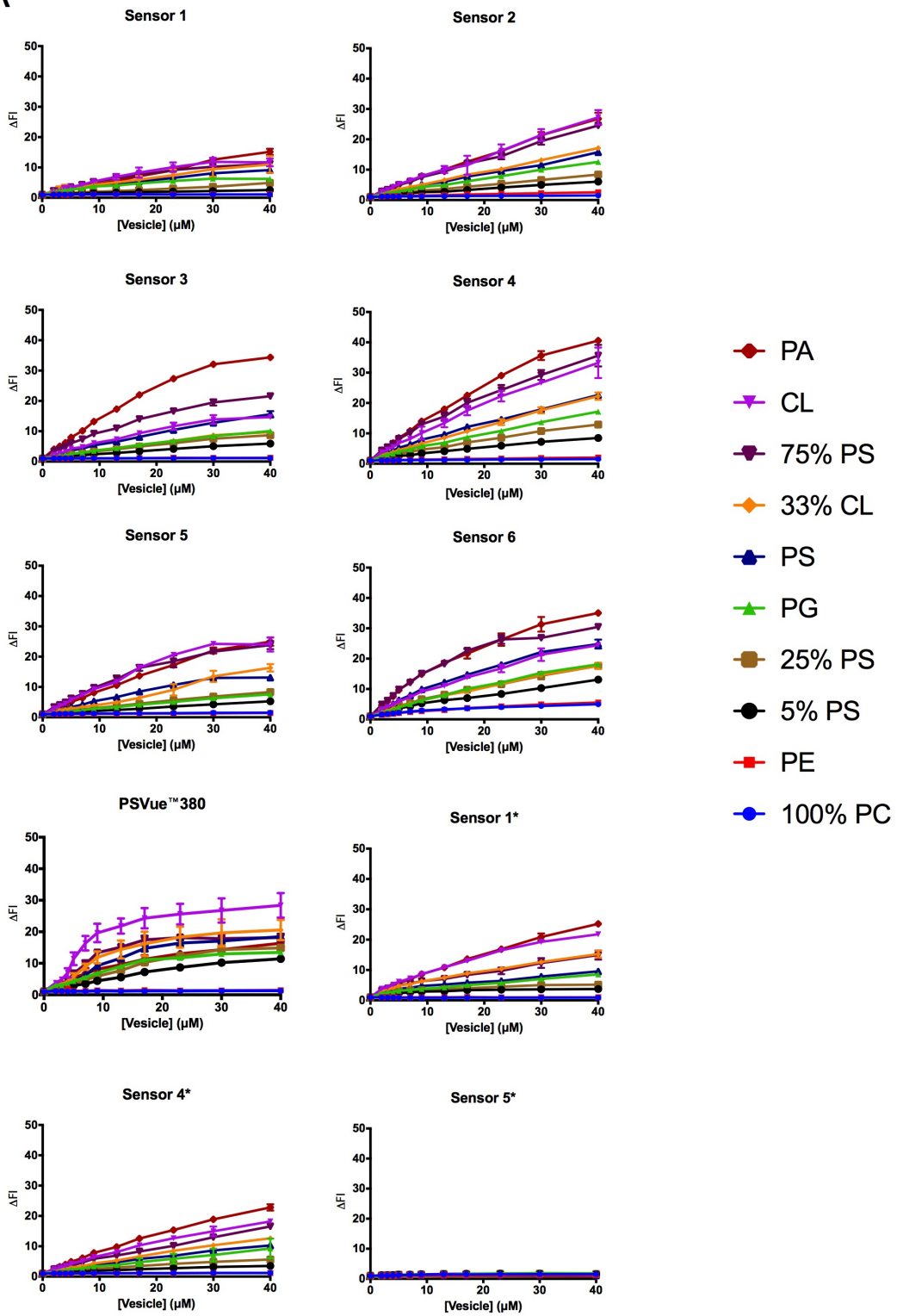

Sensor 1
Sensor 2
Sensor 3
Sensor 4
Sensor 5
Sensor 6
Sensor 1*
Sensor 4*
Sensor 5*
[Vesicle] (μM)
PA
75% PS
CL
PS
33% CL
PG
25% PS
5% PS
PE
100% PC

Figure S2. ΔFI_{exc}. ΔFI_{exc} of all library sensors and PSVue™380 with all vesicles (50 mM HEPES, pH 7.5, 5% DMSO). (A) ΔFI_{exc} for vesicle concentrations from 0-40 µM and (B) 0-200 µM. In all experiments, [ProxyPhos library sensors] = 25 µM; $\lambda_{exc/emi}$ = 350/476 nm and [PSVue™380] = 1 µM; $\lambda_{exc/emi}$ = 380/440 nm.

A



B

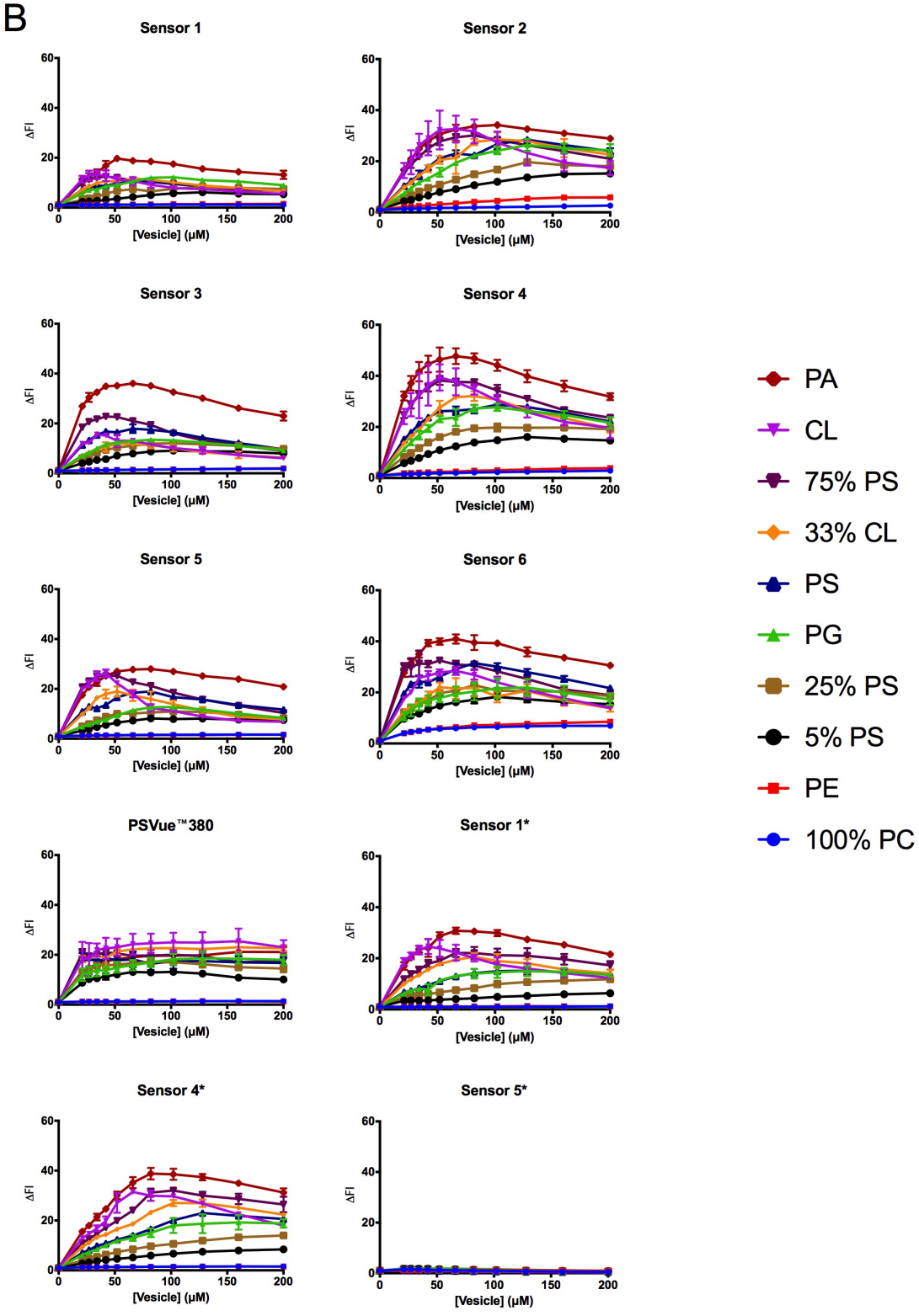
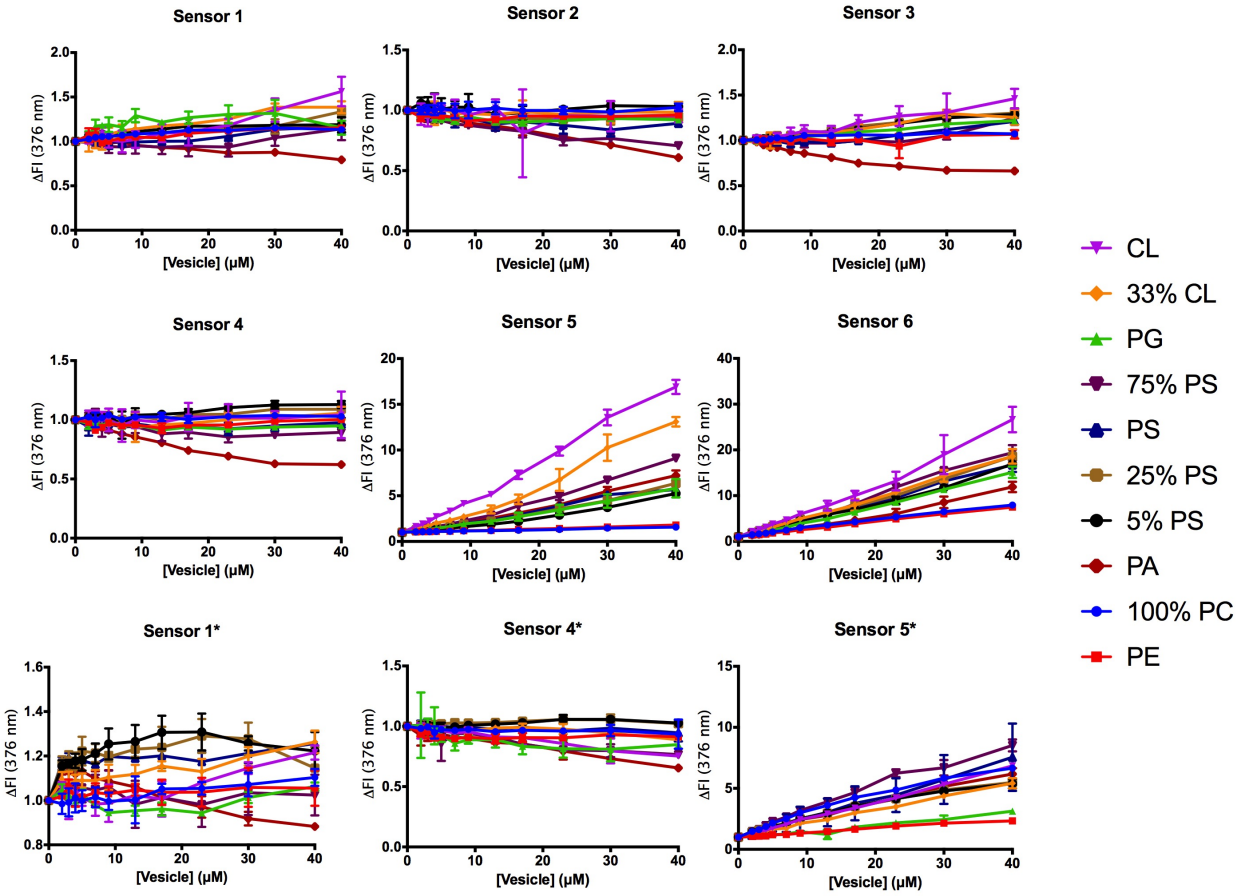

Sensor 1
Sensor 2
Sensor 3
Sensor 4
Sensor 5
Sensor 6
PSVue™380
Sensor 1*
Sensor 4*
Sensor 5*
ΔFI
[Vesicle] (μM)
0
20
40
60
0
50
100
150
200
PA
CL
75% PS
33% CL
PS
PG
25% PS
5% PS
PE
100% PC

Figure S3. ΔFI_{mon}. ΔFI_{mon} of all library sensors with all vesicles (50 mM HEPES, pH 7.5, 5% DMSO). (A) ΔFI_{mon} for vesicle concentrations from 0-40 µM and (B) 0-200 µM. In all experiments, [ProxyPhos library sensors] = 25 µM; $\lambda_{exc/emi}$ = 350/376 nm.

A



B

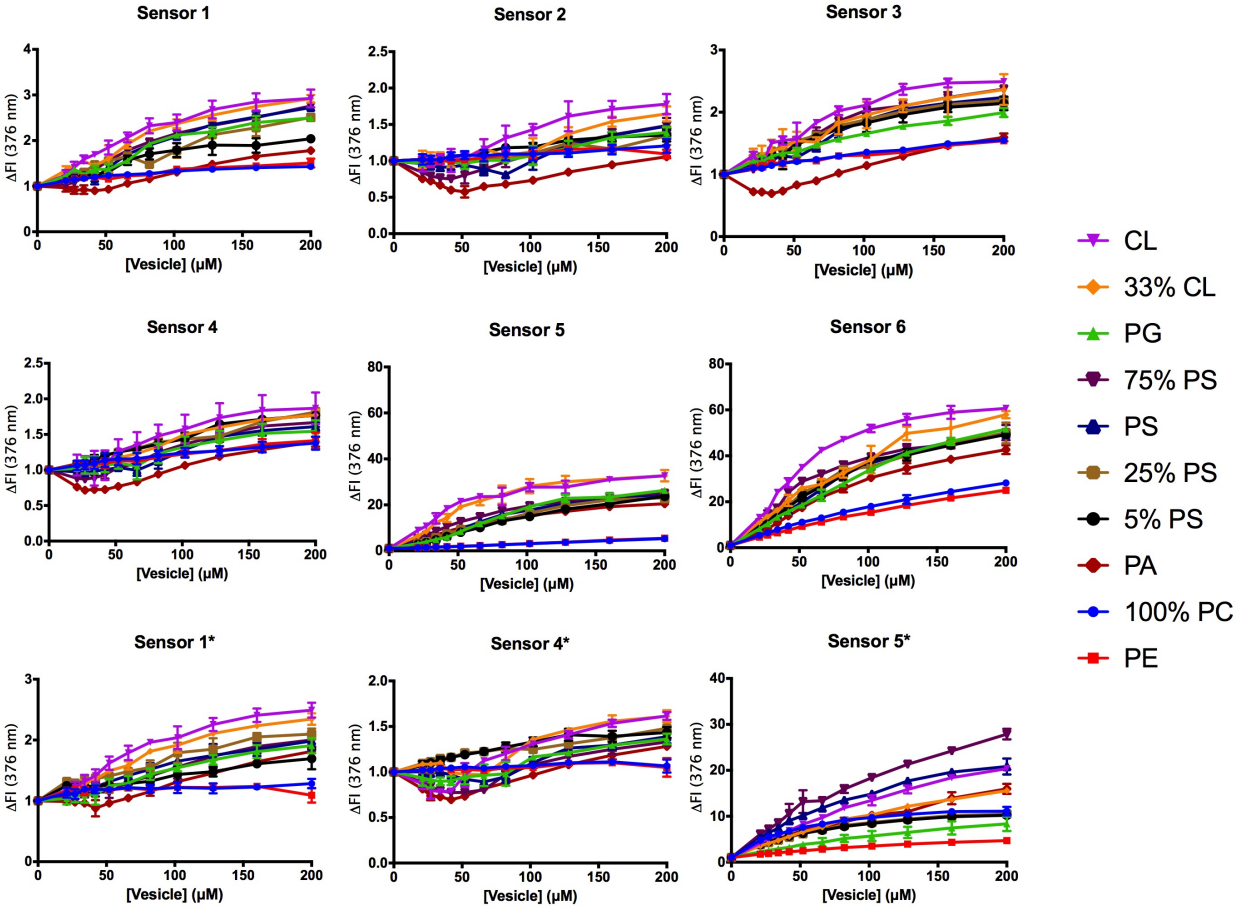
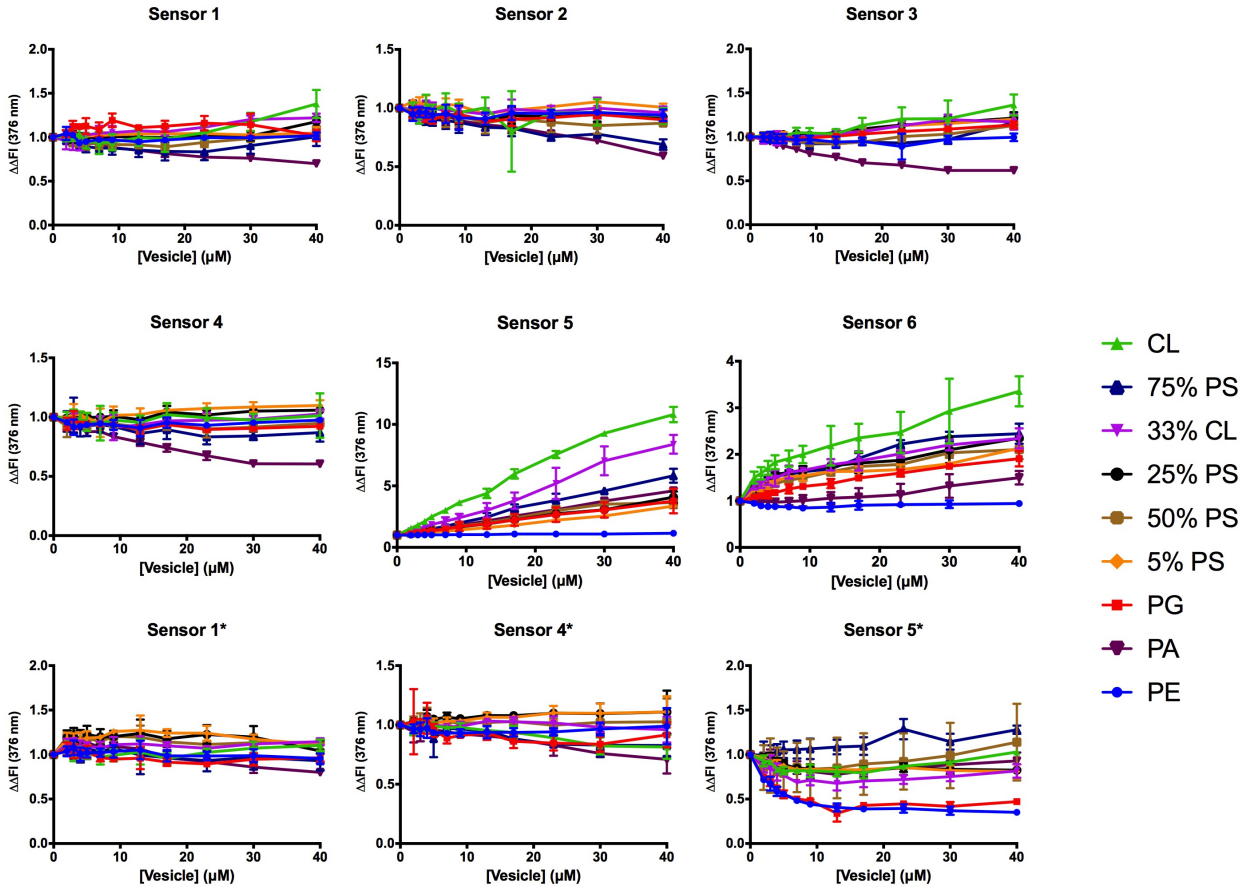

Sensor 1
Sensor 2
Sensor 3
Sensor 4
Sensor 5
Sensor 6
Sensor 1*
Sensor 4*
Sensor 5*
ΔFl (376 nm)
[Vesicle] (μM)
CL
33% CL
PG
75% PS
PS
25% PS
5% PS
PA
100% PC
PE

Figure S4. $\Delta\Delta FI_{mon.}$ $\Delta\Delta FI_{mon}$ of all library sensors with all vesicles (50 mM HEPES, pH 7.5, 5% DMSO). (A) $\Delta\Delta FI_{mon}$ for vesicle concentrations from 0-40 µM and (B) 0-200 µM. In all experiments, [ProxyPhos library sensors] = 25 µM; $\lambda_{exc/emi}$ = 350/376 nm.

A



B

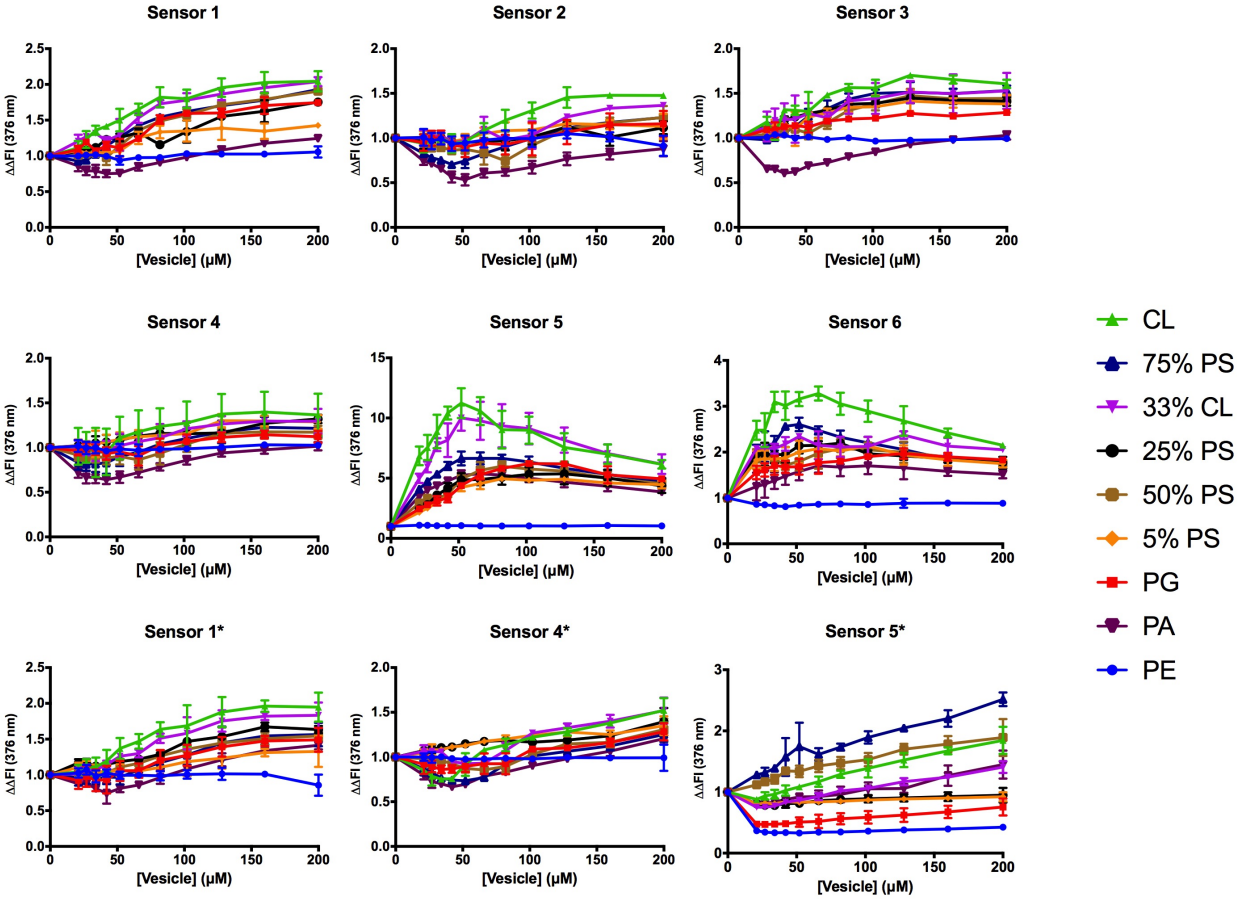
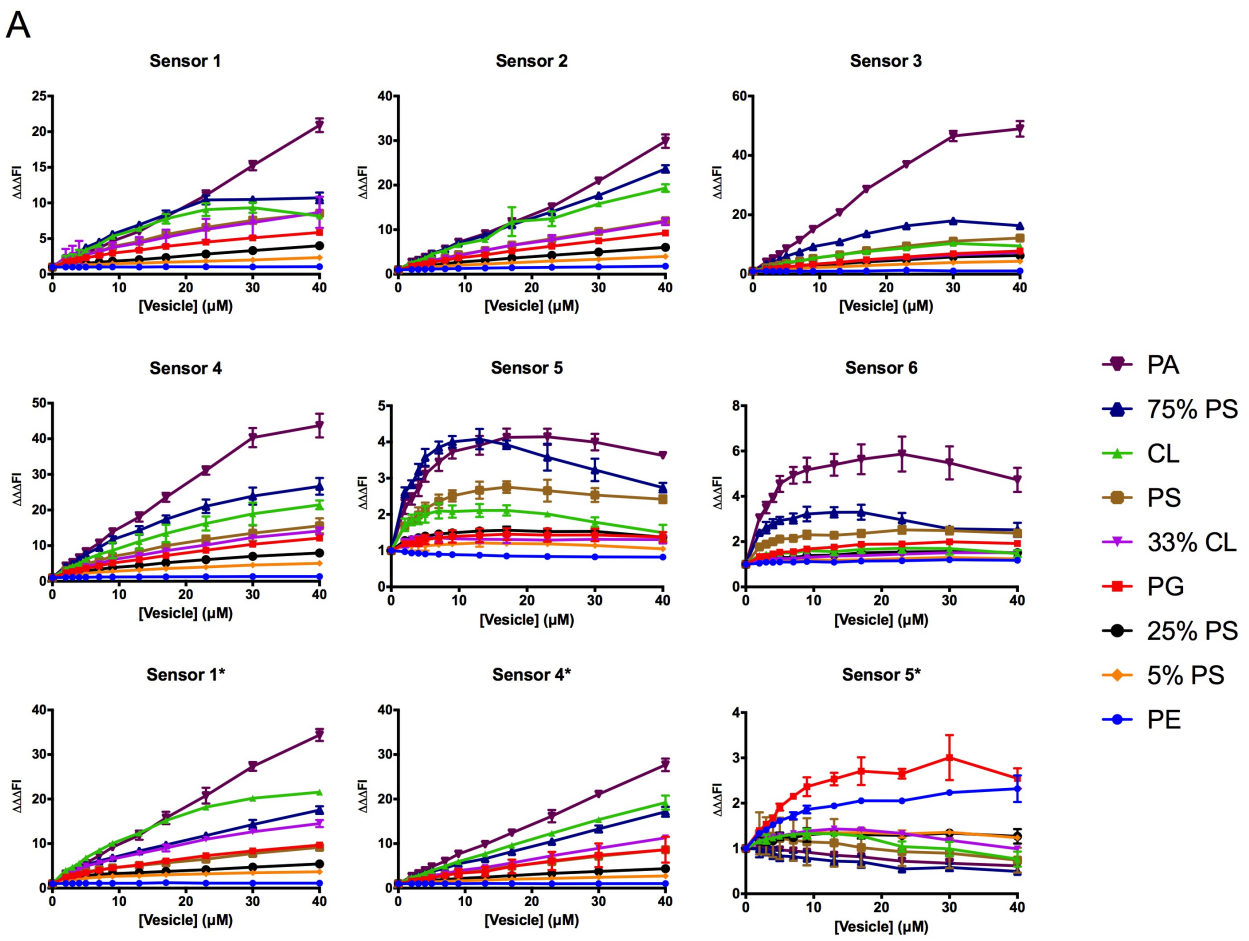

Sensor 1
Sensor 2
Sensor 3
Sensor 4
Sensor 5
Sensor 6
Sensor 1*
Sensor 4*
Sensor 5*
ΔΔFI (376 nm)
ΔΔFI (376 nm)
[Vesicle] (μM)
CL
75% PS
33% CL
25% PS
50% PS
5% PS
PG
PA
PE

Figure S5. ΔΔΔFI. ΔΔΔFI of all library sensors with all vesicles (50 mM HEPES, pH 7.5, 5% DMSO). (A) ΔΔΔFI for vesicle concentrations from 0-40 μM and (B) 0-200 μM. In all experiments, [ProxyPhos library sensors] = 25 μM; $\lambda_{exc/emi}$ = 350/476 nm and 350/376 nm.



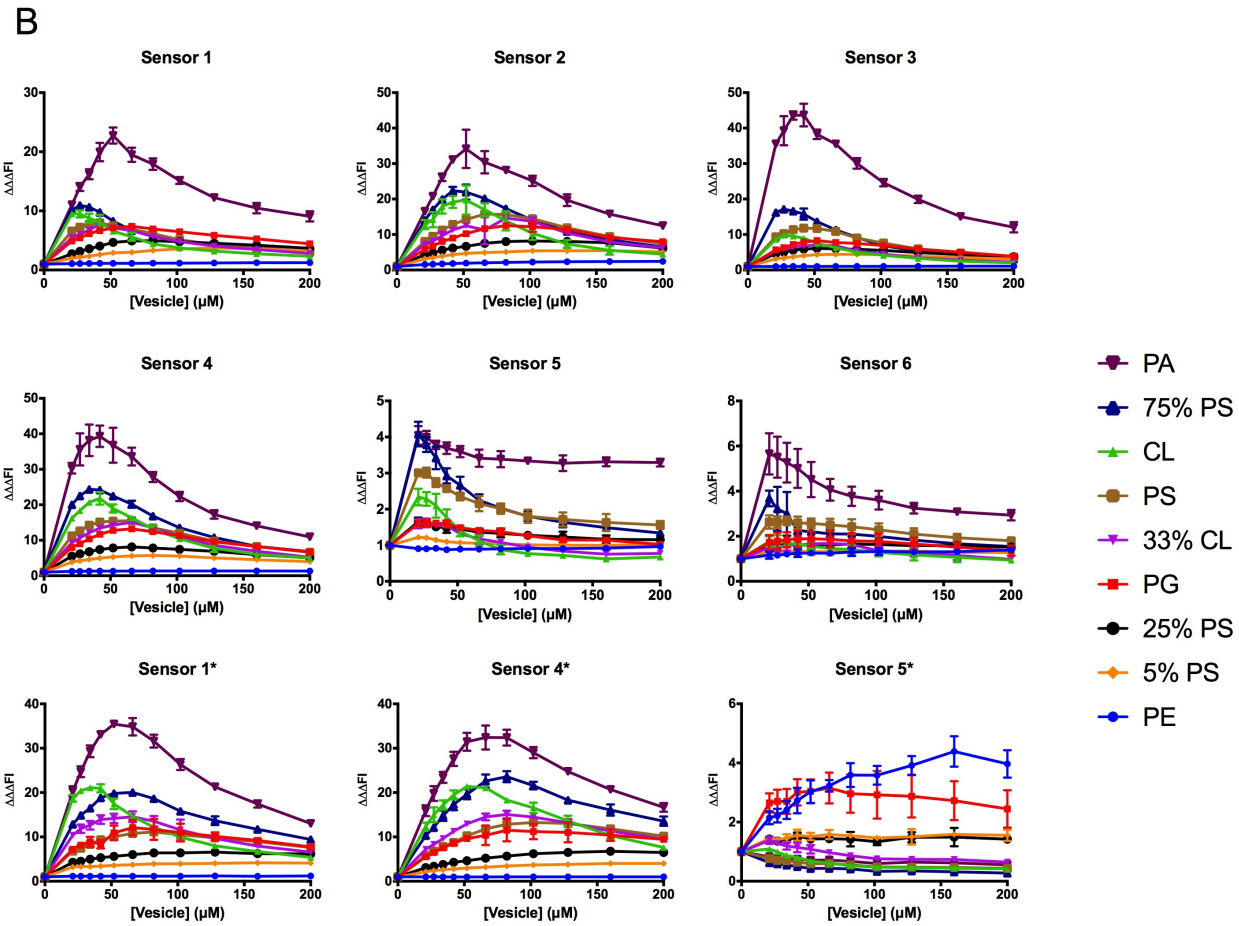


Figure S6. Optimal Sensor Concentration and Incubation Time Determination. (A) ΔFI_{exc} of 12.5 μM, 25 μM or 50 μM sensor 1 upon addition of PS vesicles (50 mM HEPES, pH 7.5, 10% DMSO). (B) Fluorescence intensity (FI_{exc}) of 25 μM sensor 1 in the presence of 150 μM PS vesicles, normalized to the intensity at time = 2 minutes (50 mM HEPES, pH 7.5, 5% DMSO). In both experiments, $\lambda_{ex/em}$ = 350/476 nm.

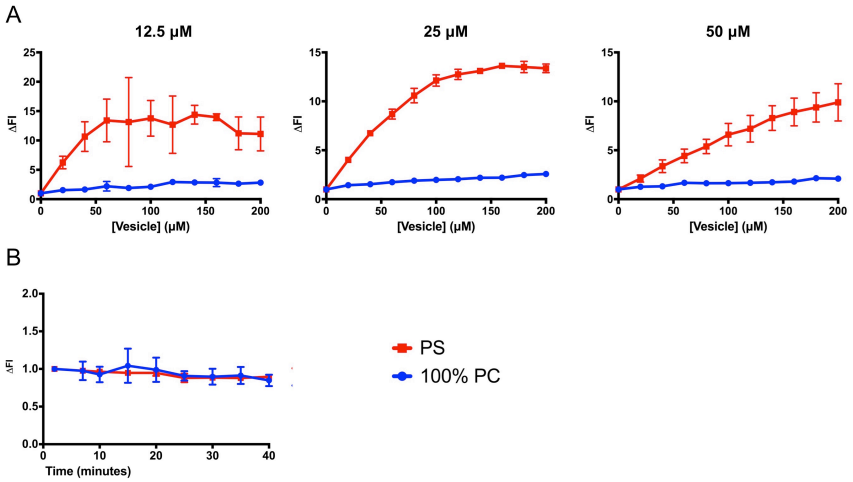


Figure S7. $\Delta\Delta FI_{exc}$ for PS-containing vesicles. $\Delta\Delta FI_{exc}$ of all ProxyPhos library sensors and PSVue™380 upon addtion of 5, 25, 50 and 75% PS vesicles (50 mM HEPES, pH 7.5, 5% DMSO). In all experiments, [ProxyPhos library sensors] = 25 μM; $\lambda_{ex/em}$ = 350/476 nm and [PSVue™380] = 1 μM; $\lambda_{ex/em}$ = 380/440 nm.

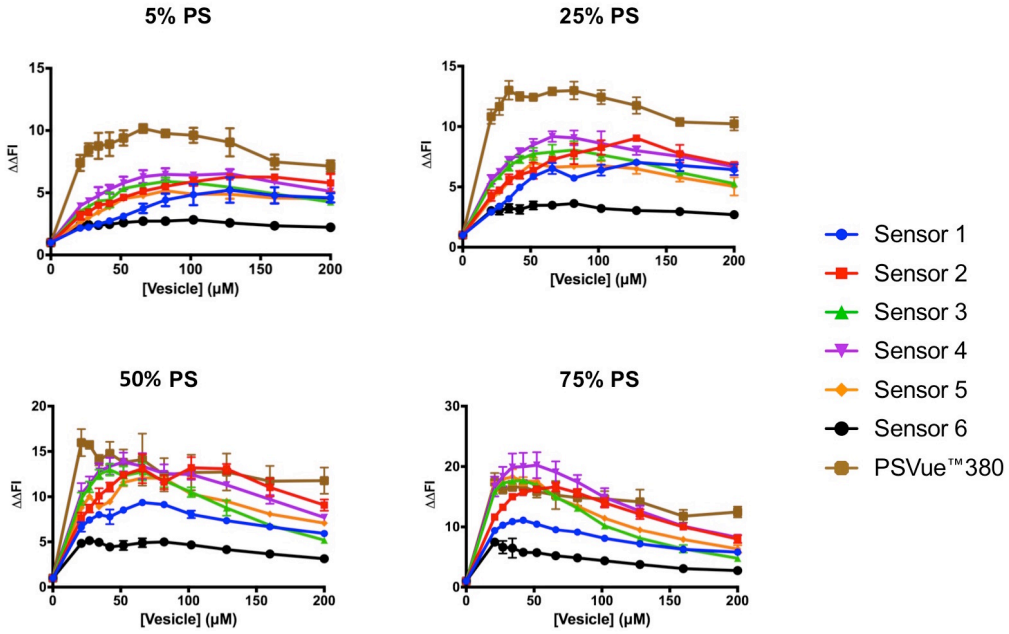


Figure S8. Short-chain lipid controls and phosphoserine. ΔFI_{exc} of all library sensors and PSVue™380 with DHPC, DHPS and O-Phospho-L-serine (50 mM HEPES, pH 7.5, 5% DMSO). (A) ΔFI_{exc} for analyte concentrations from 0-40 µM and (B) 0-200 µM. In all experiments, [ProxyPhos library sensors] = 25 µM; $\lambda_{exc/emi}$ = 350/476 nm and [PSVue™380] = 1 µM; $\lambda_{exc/emi}$ = 380/440 nm.

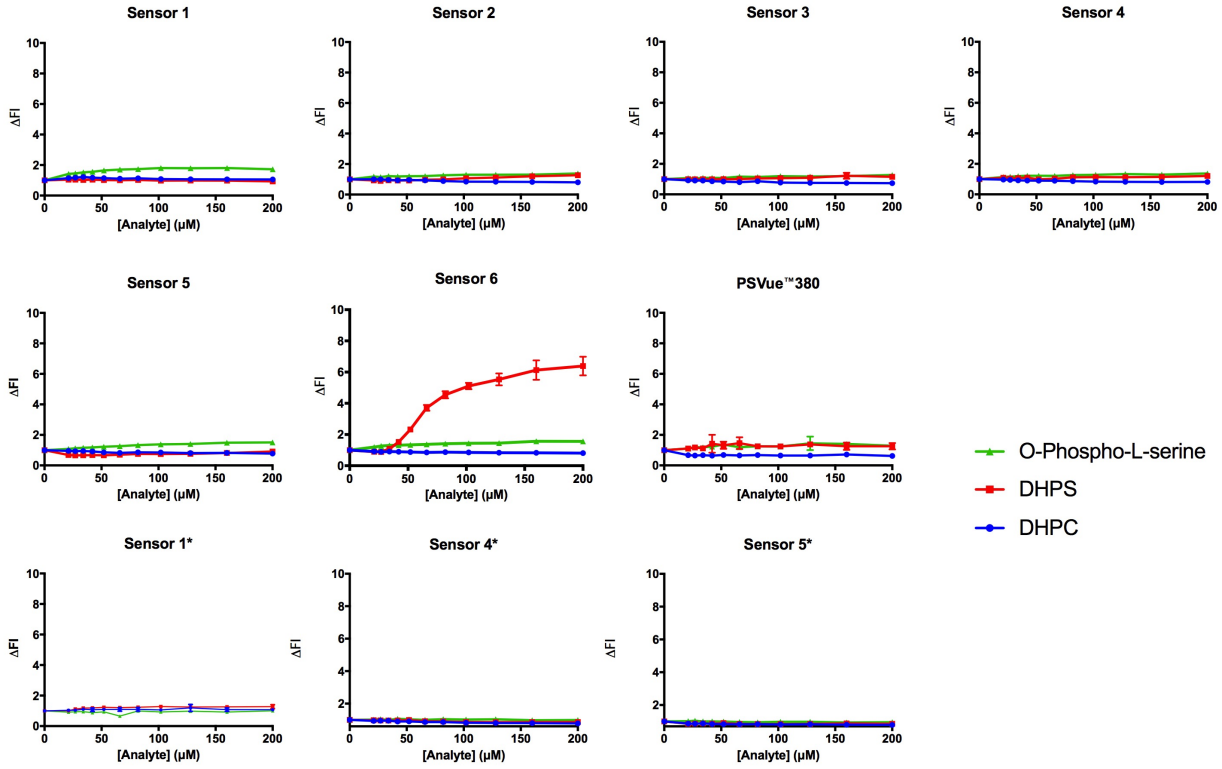


Figure S9. $\Delta\Delta FI_{exc}$ of Unmetallated ProxyPhos library sensors. $\Delta\Delta FI_{exc}$ of unmetallated sensors: 1*, 4* and 5* with all vesicles (50 mM HEPES, pH 7.5, 5% DMSO). (A) $\Delta\Delta FI_{exc}$ for vesicle concentrations from 0-40 µM and (B) 0-200 µM. In all experiments, [ProxyPhos library sensors] = 25 µM; $\lambda_{exc/emi}$ = 350/476 nm.

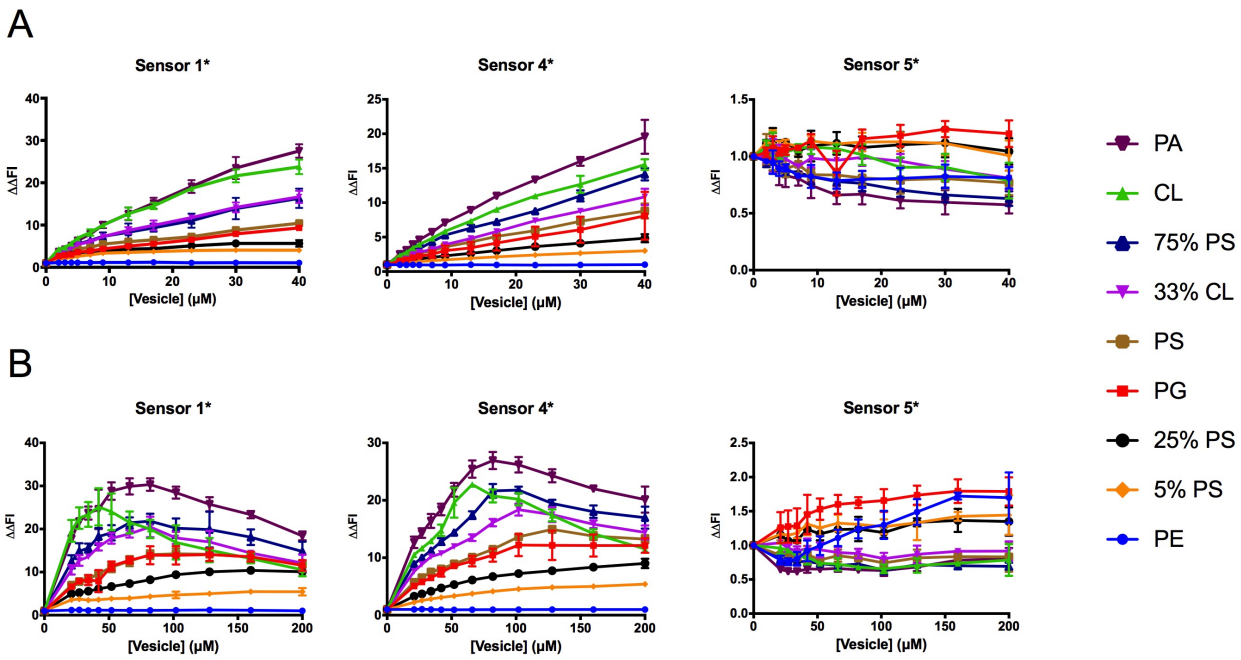


Figure S10. Excitation and emission spectra for all ProxyPhos library sensors. [ProxyPhos library sensors] = 50 µM in 50 mM HEPES, 10% DMSO, pH 7.5. All spectra recorded on a Tecan Infinite M1000.

Figure S11. Apoptosis Flow Cytometry Buffer Controls. A) Flow cytometry results of untreated (left panel) and camptothecin (10 μM) treated (right panel) MOLM-13 cells with HEPES buffer (50 mM HEPES, 75 mM NaCl, 1% BSA, 0.4% DMSO, pH 7.5) in PI channel. B) Flow cytometry results of untreated (left panel) and camptothecin (10 μM) treated (right panel) MOLM-13 cells with HEPES buffer in DAPI channel. All samples analyzed on CytoFLEX S (BeckmanCoulter) using near UV (exc: 375 nm, emi: 450/45 nm) and blue (exc: 488 nm, emi: 585/42 nm) lasers.

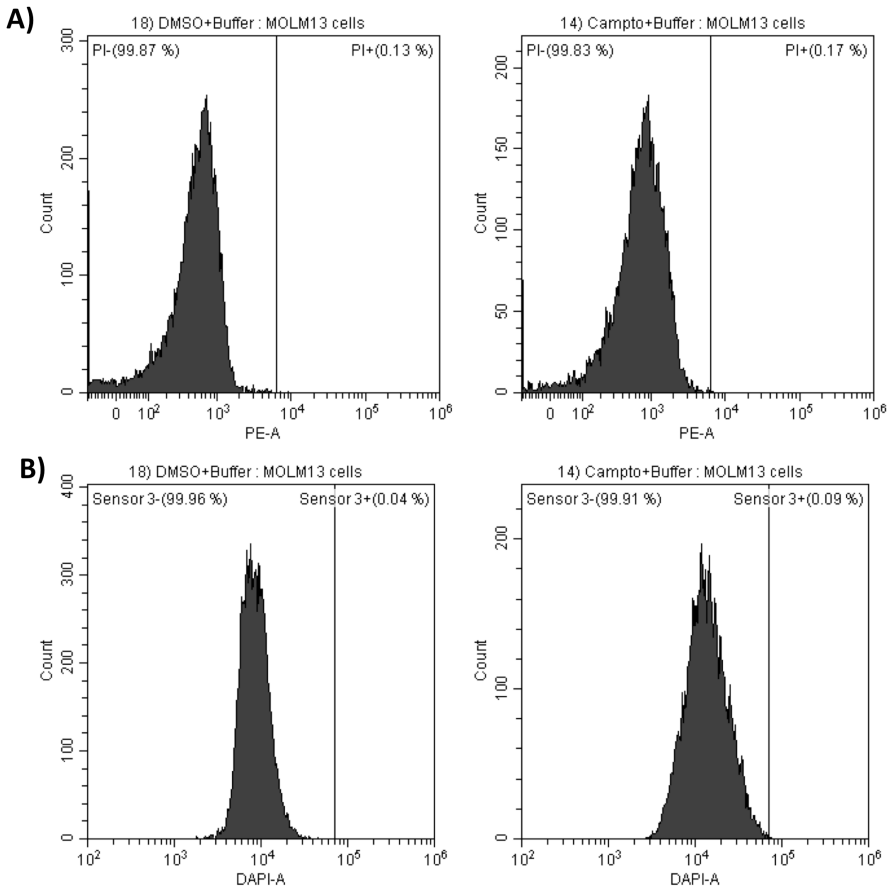


Figure S12. Apoptosis Flow Cytometry Single Stains. A) Flow cytometry results of untreated (left panel) and camptothecin (10 μM) treated (right panel) MOLM-13 cells with propidium iodide (0.02 μg/μL) in PI channel. B) Flow cytometry results of untreated (left panel) and camptothecin (10 μM) treated (right panel) MOLM-13 cells with sensor 3 (50 μM final) (top) or PSVue™380 (90.9 μM) (bottom) in DAPI channel. For all samples. buffer: 50 mM HEPES, 75 mM NaCl, 1% BSA, 0.4% DMSO, pH 7.5. All samples analyzed on CytoFLEX S (BeckmanCoulter) using near UV (exc: 375 nm, emi: 450/45 nm) and blue (exc: 488 nm, emi: 585/42 nm) lasers.

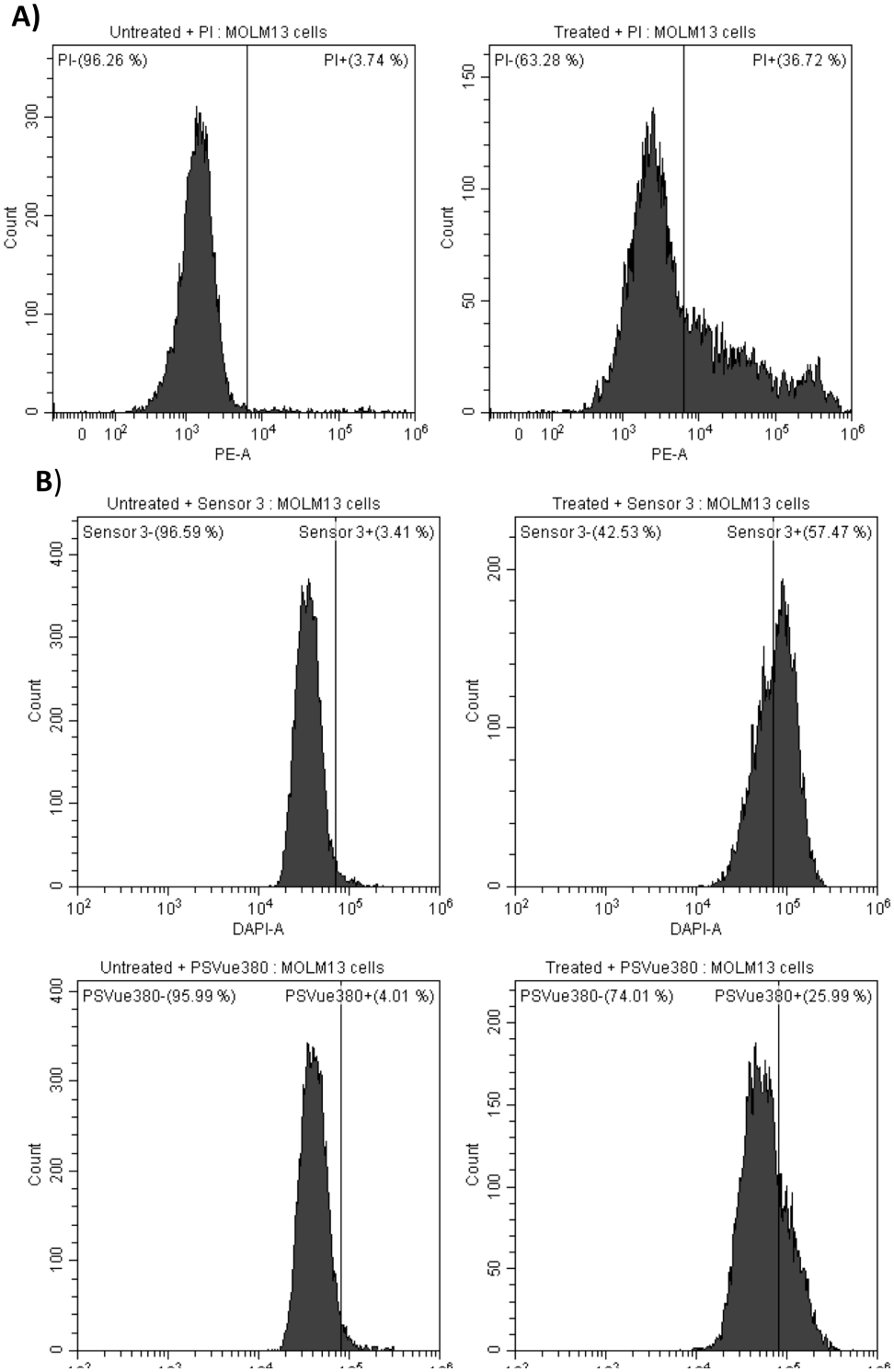
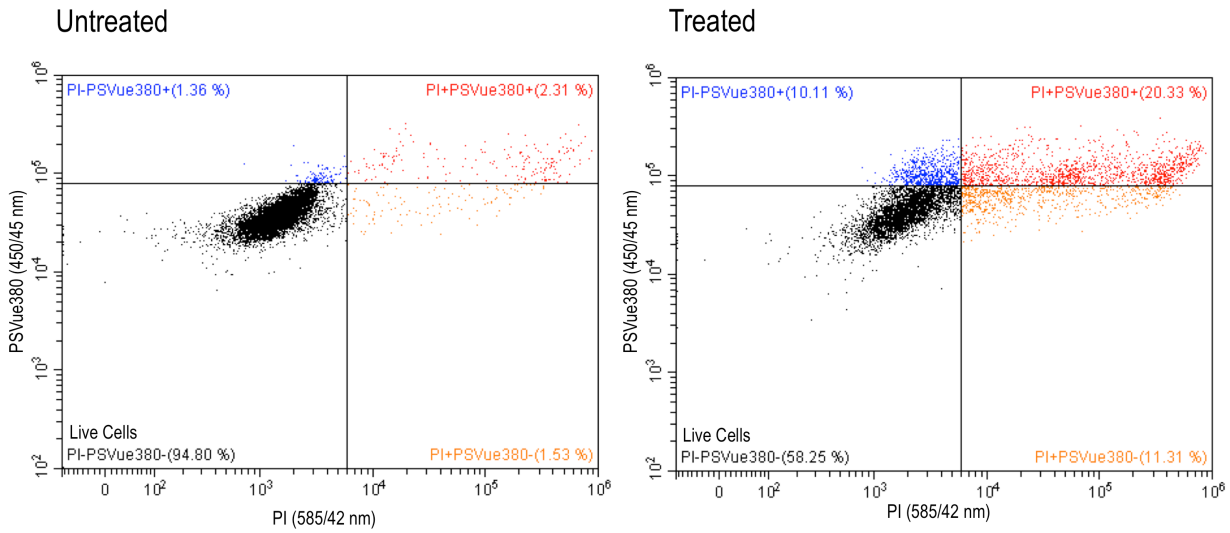


Figure S13. Proof of Concept Apoptosis Experiement with PSVue™380. Flow cytometry results of untreated (left panel) and camptothecin (10 µM) treated (right panel) MOLM-13 cells stained with PSVue™380 and prodium iodide (PI). Buffer: 50 mM HEPES, 75 mM NaCl, 1% BSA, 0.4% DMSO, pH 7.5. [PSVue™380] = 90.9 µM final. [PI] = 0.02 µg/µL final. Samples analyzed using Near UV (exc: 375 nm, emi: 450/45 nm) and blue (exc: 488 nm, emi: 585/42 nm) lasers.



Supplementary Notes

Supplementary Note 1 *List of synthetic phospholipid SUVs used*

100% PC = 100% POPC
PE = POPC:POPC 1:1
PG = POPC:POPG 1:1
5% PS = POPC:DOPS 95:5
25% PS = POPC:DOPS 3:1
PS = POPC:DOPS 1:1
75% PS = POPC:DOPS 1:3
PA = POPC:POPA 1:1
33% CL = POPC:TOCL 2:1
CL = POPC:TOCL 1:1

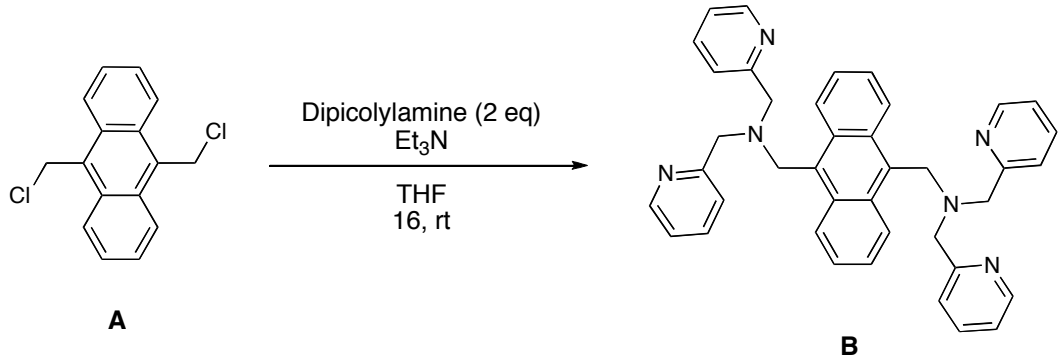
Supplementary Note 2 *List of Equations*

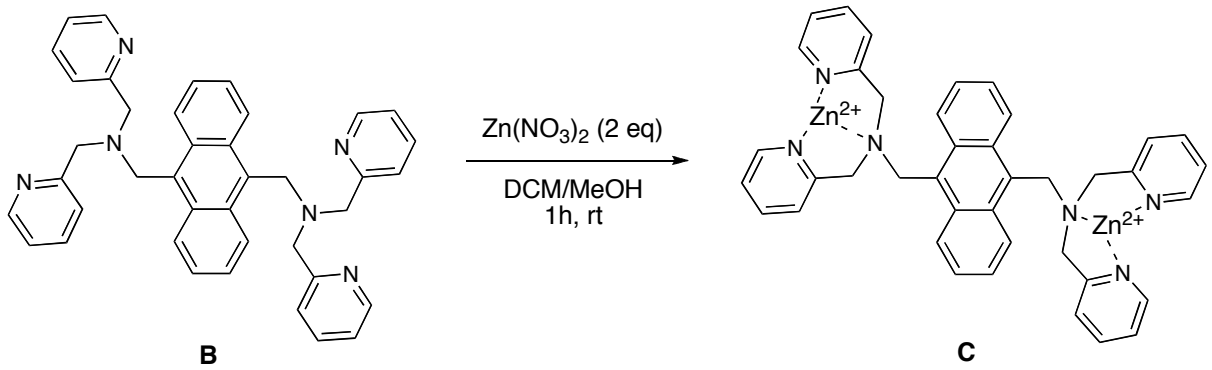
Equation S 1 $\Delta FI_{mon} = \frac{FI_{mon\ sensor+vesicle}}{FI_{mon\ sensor}}$

Equation S 2 $\Delta\Delta FI_{mon} = \frac{\Delta FI_{mon\ vesicle}}{\Delta FI_{mon\ 100\%\ PC}}$

Equation S 3 $\Delta\Delta\Delta FI = \frac{\Delta\Delta FI_{exc}}{\Delta\Delta FI_{mon}}$

Supplementary Note *3 Synthesis and Characterization of PSVue™380*





Synthesis of 1,8-bis[(2,2-dipicolylamino)methyl]anthracene (B) (Unmetallated PSVue™380):

9,10-bis(chloromethyl)anthracene **A** (100 mg, 0.363mmol, 1 eq) was dissolved in THF (1 mL) and cooled to 0 °C. 2,2'-dipicolylamine (0.131 ml, 0.726 mmol, 2 eq) and triethylamine (0.101 ml, 0.726 mmol, 2 eq) were dissolved in THF (1 mL) and added dropwise. The solution was stirred at room temperature for 16 h then filtered. The solvents were removed in vacuo. The residue was dissolved in DCM, washed with brine, dried over Na_2SO_4. After removal of the solvent in vacuo, the residue was purified by flash column chromatography (Hexanes/EtOAc) followed by recrystallization from EtOAc to give the desired product (98 mg, 45%) as a pale yellow powder.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (d, *J* = 5 Hz, 2H), 8.39 (dd, *J* = 7 Hz, 4 Hz, 2H), 7.50 (td, *J* = 8 Hz, 2 Hz, 2H), 7.42 (d, *J* = 8 Hz, 2H), 7.28 (d, *J* = 8 Hz, 2H), 7.02 (t, *J* = 5 Hz, 2H), 4.63 (s, 2H), 3.86 (s, 4H). Data in accordance with the literature.[1]

PSVue™380 (C):

To a solution of 1,8-bis[(2,2-dipicolylamino)methyl]anthracene **B** (35 mg, 0.0583 mmol) in DCM (2.1 mL) was added dropwise 116 mM $Zn(NO_3)_2$ in MeOH (1 mL, 0.1166 mmol)). After stirring for 1h at room temperature, the precipitate was filtered and washed with DCM to give 1 (42 mg, quantitative) as a pale yellow powder.

^{1}H NMR (400 MHz, DMSO-d_6+D_2O) δ 8.66 (bs, 2H), 8.09 (bs, 2H), 8.00 (bs, 2H), 7.68 (bs, 2H), 7.57 (bs, 2H), 7.28 (bs, 2H), 4.94 (s, 4H), 4.10 (d, 8 Hz, 4H), 3.67 (d, 8 Hz, 4H). ^{13}C NMR (125 MHz, DMSO-d_6+D_2O) δ 154.7, 148.7, 147.3, 141.8, 140.5, 131.9, 127.5, 126.9, 126.2, 125.9, 124.6, 58.0, 56.9, 55.8.

[1] J. AM. CHEM. SOC. 2002, 124, 6256-6258