**Supporting Information**

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I. General.

Egg yolk phosphatidylcholine (EYPC) was obtained from Avanti Polar Lipids Inc. 5(6)-carboxyfluorescein (CF) and 1-pyrenebutyric acid (PB) was purchased from Fluka; poly-L-arginine hydrochloride (pR), 1-pyreneacetic acid (PA), and sodium dodecyl sulfate (SDS) were from Sigma-Aldrich. Sodium laurate (SL) and sodium monododecyl phosphate (SDP) were obtained from Tokyo Chemical Industry (TCI), America. All salts and buffers of the best grade were available from Sigma-Aldrich, and used as received. CPPM1 and CPPM2 were synthesized and characterized as reported previously. Stock solution of CPPM was prepared in dimethyl sulfoxide (DMSO, Sigma). Fluorescence measurements were performed on a Jobin Yvon Fluorolog-3 equipped with a stirrer and temperature controller (all measurements were performed at 25 °C).

II. Preparation of Vesicles

Preparation of EYPC-LUV\(\rightleftharpoons\)CF. A thin lipid film was prepared by evaporating a solution of 25 mg EYPC in 2 mL CHCl\(_3\) on a rotary evaporator (40 °C) and then in vacuum overnight. After hydration (~ 1h) with 1.0 ml buffer A (10 mM Tris, 10 mM NaCl, 50 mM CF, pH 7.5) accompanied by occasional vortex, the resulting suspension was subjected to 5 freeze-thaw cycles (liquid N\(_2\) to freeze and 40 °C water bath to thaw), and 11 times extruded through a polycarbonate membrane (pore size 100 nm). Extra-vesicular components were removed by size exclusion chromatography (Sephadex G-50, Sigma-Aldrich) with buffer B (10 mM Tris, 107 mM NaCl, pH 7.5). The resulting vesicle solution was diluted with buffer B to give CF loaded LUVs stock solution having final lipid concentration of ~ 5.0 mM.
III. Activation Study

*Activation Study with Pyrenebutyrate.* 20 µl of pyrenebutyrate (PB) stock solution (pyrenebutyric acid in H₂O with 1 equivalent of NaOH) and 20 µl EYPC-LUVs⇒CF were added to 1.96 ml gently stirred, thermostated buffer B (10 mM Tris, 107 mM NaCl, pH 7.5) in a disposable plastic cuvette. The time dependent change in fluorescence intensity \( I_t \) (\( \lambda_{\text{ex}} = 492 \text{ nm}, \lambda_{\text{em}} = 517 \text{ nm} \)) was monitored during the addition of 20 µl CPPM or pR stock solution (final concentrations 2.5 µM CPPM and 0.25 µM pR, respectively) at \( t = 100 \text{ s} \), and addition of 40 µl 1.2 % (aq.) triton X-100 at the end of every experiment. Fluorescence time courses were normalized and converted into transmembrane activity \( I_F \) (equation S1, Figure S1a) and further converted into fractional activity \( Y \) using equation S2 (Figure S1b).

\[
I_t = \frac{(I_t - I_0)}{(I_\infty - I_0)} \quad \text{(S1)},
\]

where \( I_0 = I_t \) before CPPM addition and \( I_\infty = I_t \) after lysis. \( I_F \) at \( t = 600 \text{ s} \) just before lysis was defined as transmembrane activity \( Y \).

\[
Y = \frac{(Y_t - Y_o)}{(Y_\infty - Y_o)} \quad \text{(S2)},
\]

where \( Y_o \) is \( Y \) without activator, and \( Y_\infty \) is \( Y \) with excess activator. For Hill analysis, \( Y \) was plotted against varied pyrenebutyrate (PB) concentration (\( c_{PB} \)) at constant transport and lipid concentration, and fitted to the Hill equation S3 to give effective concentration \( EC_{50} \), \( Y_{\text{max}} \) and the Hill coefficient \( n \) (see Figure S1).

\[
Y = Y_o + \frac{(Y_{\text{max}} - Y_o)}{\left[1 + \frac{c_{PB}}{EC_{50}}\right]^n} \quad \text{(S3)},
\]

where \( Y_o \) is \( Y \) without activator, and \( Y_{\text{max}} \) is \( Y \) with excess activator.

Activation studies with the other activators, pyrene acetate (PA), sodium laurate (SL), sodium dodecyl phosphate (SDP), and sodium dodecyl sulfate (SDS) performed similarly as activation study with pyrenebutyrate (PB) and effective activator concentration \( EC_{50} \), \( Y_{\text{max}} \) and the Hill coefficient \( n \) were determined (see Table 1 and Table S1).
Figure S1. (A) Changes in CF emission ($I_F$) ($\lambda_{ex}$ 492 nm, $\lambda_{em}$ 517 nm) as a function of time ($t$) during addition of activator [0 (red curve), 0.1, 1, 5, 10, 20, 25, 50, 100 µM PB] at $t = 0$ s, CPPM1 (2.5 µM, final concentration) at $t = 100$ s to EYPC-LUVs$\supset$CF (50 µM EYPC), calibrated by final analysis ($I_F = 1.0$, with 40 µL 1.2% aqueous triton X-100). (B) Dose response curves for PB, with CPPM1 (2.5 µM) against EYPC-LUVs/CF vesicles (50 µM EYPC), with curve fit to Hill equation.
IV. Transporter Efficiency (E) Calculation

The effective transporter concentration $EC_{50}$ was denoted as activator concentration needed to reach $Y_{\text{max}}/2$ (see Table 1 in the manuscript). Transporter efficiency $E$ was calculated $^S$ as defined in eqn. S4 (see Table 2 in the manuscript and Table S1).

$$E = Y_{\text{max}} \times pEC_{50} / f$$  \hspace{1cm} (S4)

where $Y_{\text{max}}$ is the maximal activity in percent, $pEC_{50}$ the negative logarithm of the effective concentration ($EC_{50}$) in mM, and $f = 20.6$ as used for classical CPP pR-activator complex.$^S$

Table S1. $EC_{50}$, $Y_{\text{max}}$, and activator efficiency $E$ for the guanidine rich transporters.

| Activators | CPPM1 | |  | CPPM2 | |  | poly-arginine (pR)$^a$ | |  |
|------------|-------|-------|-----|-------|-------|-----|-----------------------|-------|
|            | $EC_{50}$ (µM) | $Y_{\text{max}}$ (%) | $n$ | $E$ | $EC_{50}$ (µM) | $Y_{\text{max}}$ (%) | $n$ | $E$ | $EC_{50}$ (µM) | $Y_{\text{max}}$ (%) | $n$ | $E$ |
| PB         | $20 \pm 1.3$ | $100 \pm 4.0$ | $2.2 \pm 0.3$ | $8.2$ | | | | | | | | | |
| PA         | $70 \pm 2.4$ | $85 \pm 1.0$ | $2.2 \pm 0.2$ | $4.8$ | | | | | | | | | |
| SL         | $15 \pm 2.0$ | $17 \pm 2.0$ | $1.3 \pm 0.4$ | $1.5$ | | | | | | | | | |
| SDP        | $25 \pm 3.1$ | $15 \pm 0.8$ | $2.1 \pm 0.5$ | $1.2$ | | | | | | | | | |
| SDS        | $10 \pm 1.0$ | $10 \pm 0.3$ | $2.3 \pm 0.7$ | $1.0$ | | | | | | | | | |
| PB         | $24 \pm 0.8$ | $100 \pm 2.0$ | $3.6 \pm 0.5$ | $7.9$ | | | | | | | | | |
| PA         | $88 \pm 3.6$ | $96 \pm 3.0$ | $3.5 \pm 0.4$ | $4.9$ | | | | | | | | | |
| SL         | $76 \pm 4.3$ | $17 \pm 0.6$ | $5.3 \pm 1.2$ | $0.9$ | | | | | | | | | |
| SDP        | $16 \pm 0.4$ | $30 \pm 0.4$ | $2.4 \pm 0.1$ | $2.6$ | | | | | | | | | |
| SDS        | $103 \pm 0.02$ | $14 \pm 2.0$ | $4.0 \pm 0.3$ | $0.7$ | | | | | | | | | |

*pR activation data incorporated from $^S$.}
V. Statistical Analysis

The results were analyzed using GraphPad Prism version 5.01. Data are reported as mean ± standard deviation. Three independent experiments were performed and the resulting mean of $EC_{50}$ and $Y_{\text{max}}$ values were compared by two-way analysis of variance (ANOVA) followed by Bonferroni’s post-test and $P < 0.05$ was considered statistically significant (Figure S2). Although this statistical analyses were performed based on only three data points for each $EC_{50}$ or $Y_{\text{max}}$ value, all the inferences drawn in the manuscript turned out statistically significant with $P < 0.001$. Statistical analyses were not performed for the $E$ values as equitation S3 includes both $EC_{50}$ and $Y_{\text{max}}$, which makes the overall error (standard deviation) evaluation complicated using two different error levels in a single parameter. Given the statistical significance of both $EC_{50}$ and $Y_{\text{max}}$, the differences in $E$ values are significant.

Figure S2. Statistical analyses with (A) $EC_{50}$ and (B) $Y_{\text{max}}$ were performed for different activators and CPPMs. Data represents three independent experiments; error bars represent mean ± s.d. (standard deviation). Statistical analyses were performed by two-way analysis of variance (ANOVA) with Bonferroni's post-test; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, ns, non significant. In (B) the $P$ values were shown comparing aromatic activator PB with other aliphatic activators; similar $P$ values were obtained while PA was compared with other aliphatic activators. Statistical analysis of PA with other activators is not shown in the figure (B) to avoid clumsiness.
VI. References

