## **Supporting Information**

## Insight into the selectivity of Kir3.2 toward phosphatidylinositides

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Q197R



WT

**Figure S1. High-throughput expression screen of** *Pichia* **transformants.** Shown are photographs of pelleted cells in 96 deep well blocks after expression for two days. The mCherry fusion protein results in cell pellets red in color. The variation in protein expression observed here is commonly observed and previously reported (for example see [55, 59]).



**Figure S2. Identification and scale-up of well expressing Pichia transformants.** A) Photograph of screening block for Kir3.2 showing variations in protein expression. Red circles denote two candidates (C7 and G6) selected for scale up expression. B-C) Photograph of cell pellets from large-scale expression (4 L) of C7 and G6.



**Figure S3. Native mass spectra of purified Kir3.2 mutants after delipidation with DHPC.** The Kir3.2 mutant is labeled within each spectrum along with assignment of charge state. The mutant Kir3.2(K200N) has a resolved charge state distribution for an octameric assemble, which we suspect is an aggregate.

Lipid	Abbreviation	Tails (sn1-
		sn2)
1,2-dioctanoyl-phosphatidylinositol-4',5'-bisphosphate	PI(4,5)P <sub>2</sub> -d8	08:0-08:0
1,2-dioleoyl-phosphatidylinositol-4',5'-bisphosphate	PI(4,5)P <sub>2</sub> -do	18:1-18:1
1,2-dioleoyl-phosphatidylinositol-3',4'-bisphosphate	PI(3,4)P <sub>2</sub> -do	18:1-18:1
1,2-dioleoyl-phosphatidylinositol-4'-phosphate	PI(4)P <sub>2</sub> -do	18:1-18:1
1-stearoyl-2-arachidonoyl- phosphatidylinositol-4',5'-bisphosphate	PI(4,5)P <sub>2</sub> -sa	18:0-20:4
1-stearoyl-2-arachidonoyl- phosphatidylinositol-3',4',5'-bisphosphate	PI(3,4,5)P <sub>3</sub> -sa	18:0-20:4
1,2-dioleoyl-phosphatidylinositol	PI-do	18:1-18:1
1-palmitoyl-2-oleoyl-phosphatidylethanolamine	POPE	16:0-18:1
1-palmitoyl-2-oleoyl-phosphatidylcholine	POPC	16:0-18:1
1-palmitoyl-2-oleoyl-phosphatidic acid	РОРА	16:0-18:1
1-palmitoyl-2-oleoyl-phosphatidylserine	POPS	16:0-18:1
1-palmitoyl-2-oleoyl-phosphatidylglycerol	POPG	16:0-18:1
1-palmitoyl-2-(dipyrrometheneboron difluoride) undecanoyl-sn-glycero-3-	B-PE	16:0-
phosphoethanolamine		BODIPY
BODIPY FL Phosphatidylinositol 4,5-bisphosphate	B-PI(4,5)P <sub>2</sub>	BODIPY-6:0

Table S1. Full name and abbreviations for synthetic and BODIPY-modified lipids used in this study.

Protein	Detergent	Lipid	K <sub>D</sub>	Hill coefficient
Kir3.2	DDM	B-PE	6.7 ± 2.3	1.4 ± 0.1
Kir3.2 <sup>+</sup>	UDM	B-PE	5.6 ± 0.7	1.3 ± 0.2
Kir3.2 <sup>+</sup>	C <sub>12</sub> E <sub>8</sub>	B-PE	7.5 ± 2.7	1.1 ± 0.1
Kir3.2	DDM	B-PI(4,5)P <sub>2</sub>	*	*
Kir3.2 <sup>+</sup>	DDM	B-PI(4,5)P <sub>2</sub>	1.1 ± 0.1	1.2 ± 0.1
Kir3.2	C <sub>10</sub> E <sub>5</sub>	B-PI(4,5)P <sub>2</sub>	2.6 ± 0.9	0.8 ± 0.3
Kir3.2 <sup>+</sup>	C <sub>10</sub> E <sub>5</sub>	B-PI(4,5)P <sub>2</sub>	0.9 ± 0.1	2.7 ± 0.7
Kir3.2 <sup>K64Q†</sup>	C <sub>10</sub> E <sub>5</sub>	B-PI(4,5)P <sub>2</sub>	3.8 ± 0.3	1.3 ± 0.3
Kir3.2 <sup>R92P†</sup>	C <sub>10</sub> E <sub>5</sub>	B-PI(4,5)P <sub>2</sub>	8.7 ± 6.8	1.1 ± 0.2
Kir3.2 <sup>K194A†</sup>	C <sub>10</sub> E <sub>5</sub>	B-PI(4,5)P <sub>2</sub>	12.9 ± 2.7	0.9 ± 0.1

Table S2. Apparent binding parameters determined for WT and mutant Kir channels binding BODIPYmodified lipids. Reported is the average and standard deviation (n = 3).

<sup>†</sup>The fusion protein was treated with DHPC to remove contaminants. \*Regression of Hill model results in ambiguous values.

Group	Mutant	Expression	Hits	Scale-up	Successfully	Decipherable	Lipid
assigned	Name	Strain	In block	Expressed	purified	on MS	Binding
1	K64Q	KM71	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$
2	K90R	KM71	X	-	-	-	-
2	R92P	KM71	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$
3	K194A	KM71	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$
3	Q197A	KM71	$\checkmark$	Х	-	-	-
3	Q197K	KM71	$\checkmark$	$\checkmark$	$\checkmark$	Х	Х
3	Q197R	KM71	X	-	-	-	-
3	K199A	KM71	$\checkmark$	$\checkmark$	X	-	-
3	K199H	KM71	$\checkmark$	Х	-	-	-
3	K200A	KM71	X	-	-	-	-
3	K200N	KM71	$\checkmark$	$\checkmark$	$\checkmark$	X	-
3	K200R	KM71	Х	-	-	-	-

## Table S3. Summary of expression and purification of mutant Kir3.2 channels.

√ : Positive results
X : Negative results
- : Not performed

Lipid	Kir3.2	Kir <b>3.2</b> <sup>K64Q</sup>	Kir3.2 <sup>R92P</sup>	Kir3.2 <sup>K194A</sup>
PI(4,5)P2-d8	2.6	1.1	2.3	2.0
PI(4,5)P2-do	2.1	1.2	2.7	2.3
PI(3,4)P2-do	2.0	1.1	2.5	1.9
PI(4)P2-do	1.4	1.0	2.1	1.5
PI(4,5)P2-sa	2.2	1.5	2.6	2.3
PI(3,4,5)P3-sa	2.1	1.79	2.6	2.6
PI-do	1.0	0.9	1.4	1.2
POPE	0.4	0.4	0.9	0.4

Table S4. Representative average number of lipids bound to WT and mutant Kir3.2 channels.