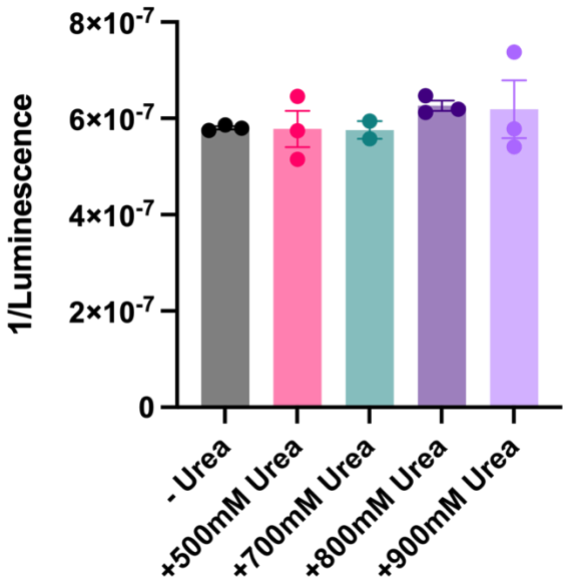
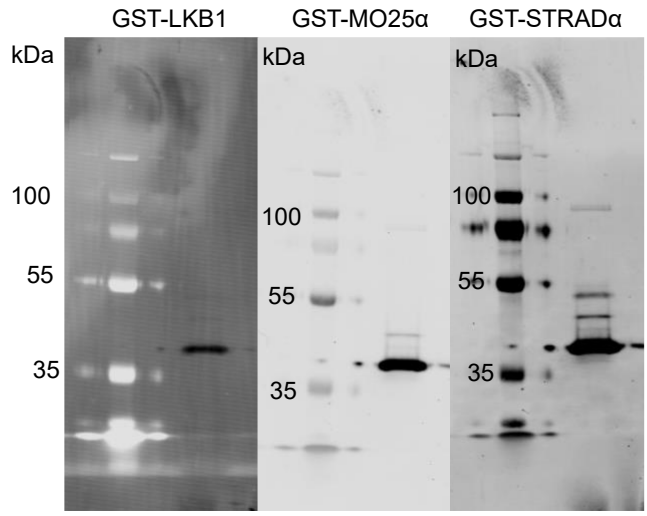
Supplementary Figures

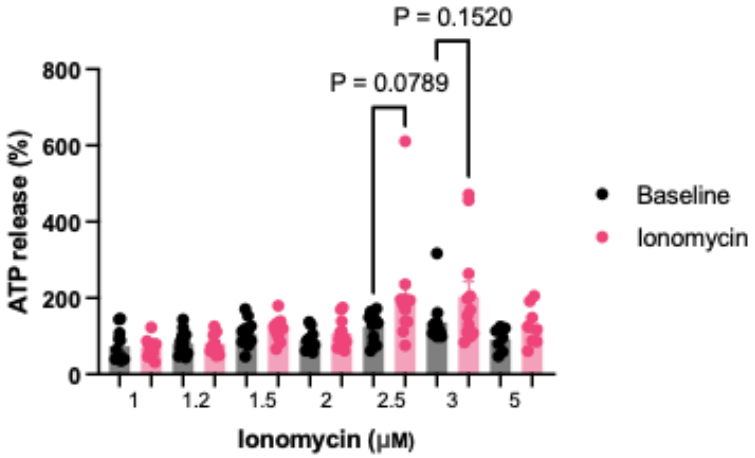
Chapter 3



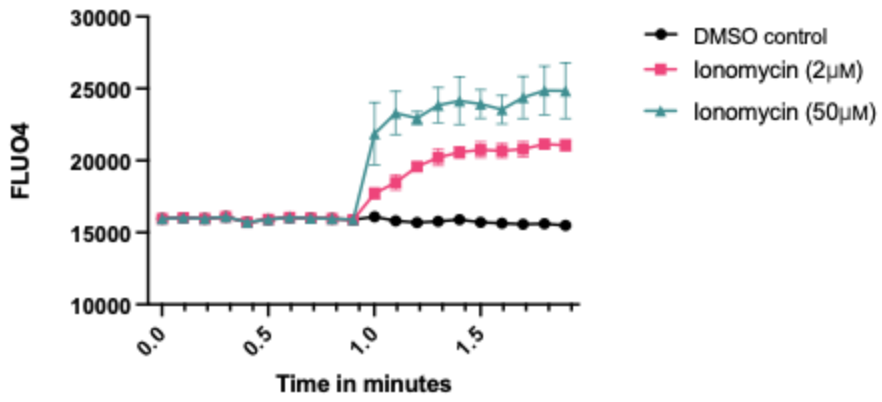
Supplementary Fig 3.1 **The effect of urea on SIK1 activity.** *In vitro* Kinase Glo Luminescence assay using dephosphorylated brain sample as substrate in 384-well plates. Varying concentrations of urea were incubated with 20nM of SIK1 kinase (500mM to 900mM of urea). Kinase buffer with no urea was used as a control. n=3.



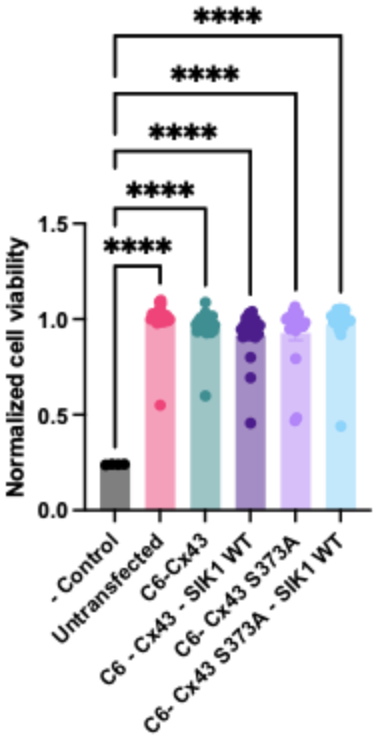
Supplementary Fig 3.2 **Full immunoblot of LKB1:STRADα:MO25α trimer.** Purified trimer was detected at the respective bands. PageRuler Prestained Protein Ladder was used. Corresponding bands are labeled.



Supplementary Fig 3.3 **Calcium ionophore (ionomycin) dose response curve in C6-Cx43 cells.** Extracellular ATP release (%) of Cx43-transfected C6 glioma cells in response to different doses of calcium ionophore (ionomycin) after 5 minutes. Each condition has a matched baseline condition without ionomycin addition to monitor Ca2+ induced extracellular ATP release. Mean ± SEM, n=7-12. Ordinary one-way ANOVA with Tukey's multiple comparisons test.



Supplementary Fig. 3.4 **Calcium Signaling assay of C6 glioma cells.** Calcium release, measured with FLUO4 dye, from WT C6 glioma cells with DMSO control, 2uM and 50uM of calcium ionophore (ionomycin) over 2 minutes of continuous fluorescence reading. One minute of baseline reading with native media (HBSS-Ca2+) was taken. DMSO or ionomycin were added after baseline and fluorescence continued to be recorded for another minute. Mean ± SEM, n=2.

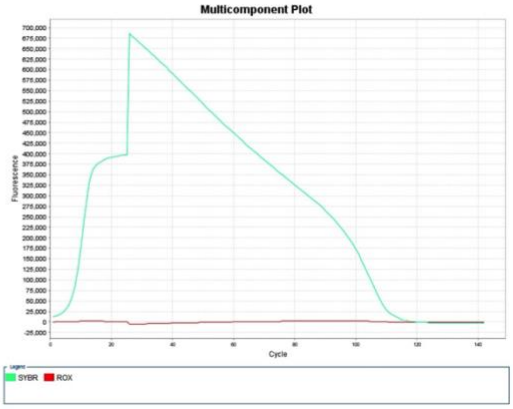


Supplementary Fig 3.5 **Presto Blue Cell Viability assay.** Cell viability of C6 glioma cells normalized to no cell control wells. Cells were either untransfected or transfected with Cx43-WT, Cx43 S373A or co-transfected with SIK1-WT. Raw fluorescence readings at 560/590nm (excitation/emission) were taken. Ordinary one-way ANOVA with Tukey's multiple comparisons test. **** $p<0.0001$. Mean ± SEM, n=4-19.

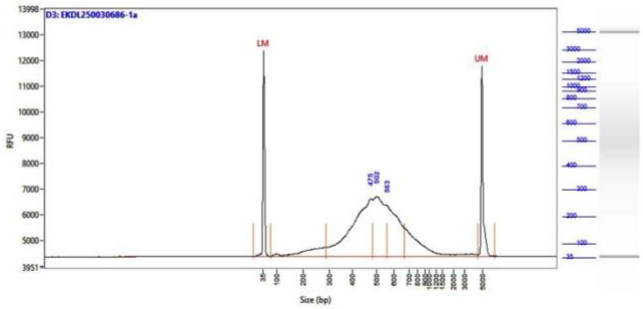
a

Label	RNA ratios A260/A280	RNA ratios A260/A230	Qubit Broad Range Concentration (ng/ul)	RIN number
SIKC23.1j WT	2.1	2.17	184	8.9
SIKC24.2e WT	2.11	1.93	69.2	9.1
SIKC24.1f WT	2.08	2.31	174	8.9
SIKC23.2i WT	2.11	1.8	55.6	9.1
SIKC23.2g KI	2.08	2.24	97	9.1
SIKC23.2h WT	2.1	1.9	91.2	9.2
SIKC23.1h KI	2.1	2.21	144	9.1
SIKC24.1e KI	2.1	1.75	208	8.9
SIKC23.3a KI	2.09	2.28	232	8.9
SIKC23.5a KI	2.08	2.11	134	9.1
SIKC23.2a KI	2.09	2.05	142	9
SIKC23.3h WT	2.08	2.07	68.8	9.3
SIKC24.4a KI	2.11	1.43	384	8.5
SIKC24.1a WT	2.09	2.31	300	8.8
SIKC24.2f WT	2.09	2.14	113	8.7
SIKC24.2a KI	2.08	1.94	98.2	9.1
SIKC26.1c WT	2.07	2.23	172	9.1

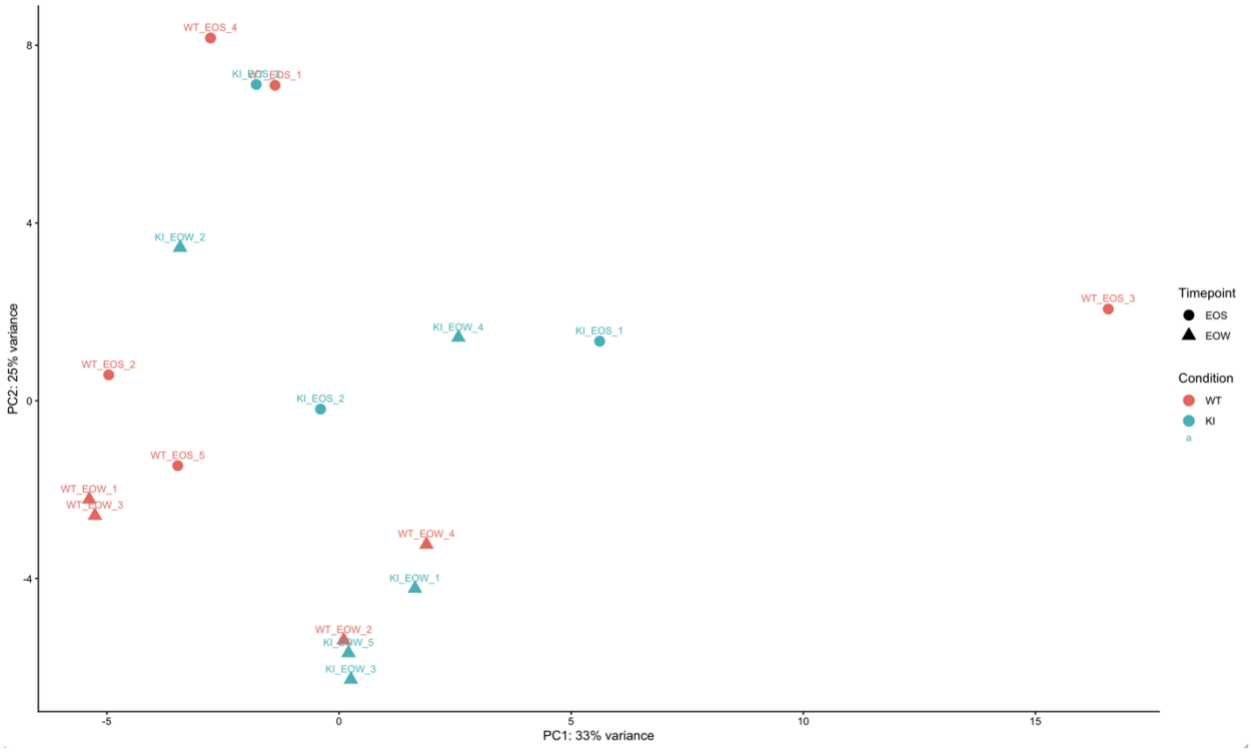
b



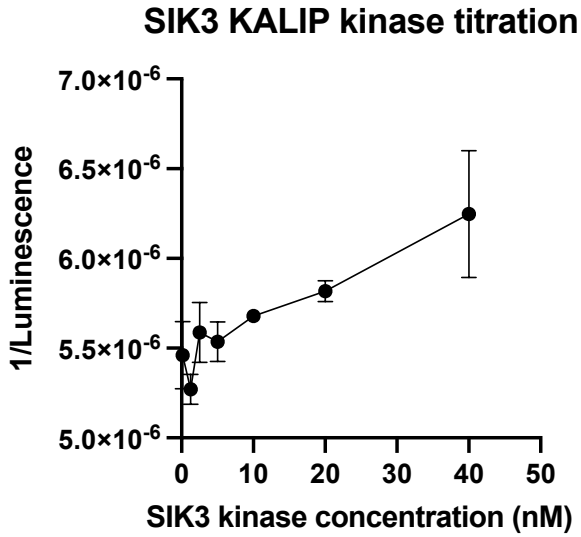
c



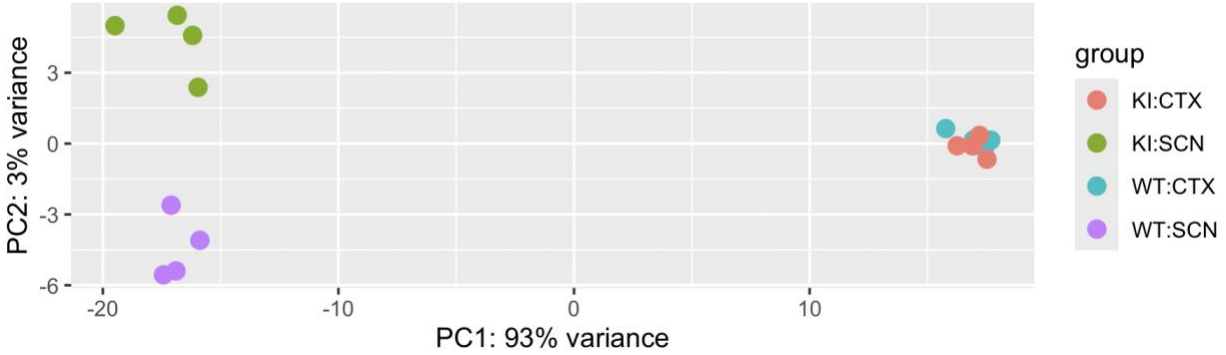
Supplementary Fig. 4.1 **SIK3 BRB-seq Quality Control (QC) plots.** (a) RNA extraction measurements corresponding to individual cortex samples including RNA ratios (A260/A280 and A260/A230) from NanoDrop Spectrophotometer, concentration (ng/ul) from Qubit Broad Range and RNA integrity number (RIN) from TapeStation. (b) Multicomponent plot from qPCR in BRB-seq protocol to determine optimal ampification cycles. (c) Fragmentation analysis of cDNA quality from Novogene.



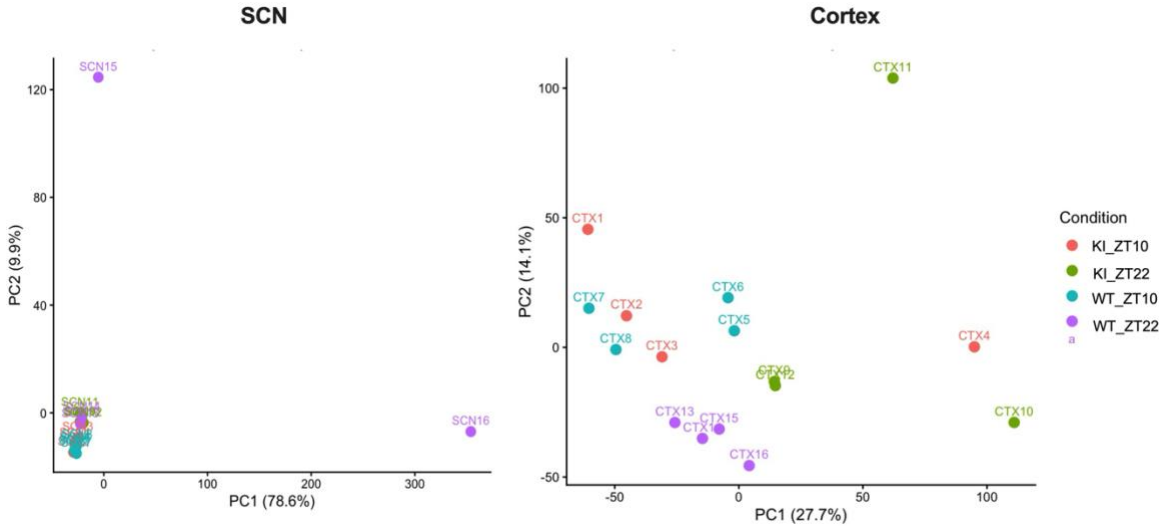
Supplementary Fig. 4.2 **PCA plot of SIK3 BRBseq.** PCA plot showing the distribution of samples for QC and determining outliers. PCA plot generated by RStudio.



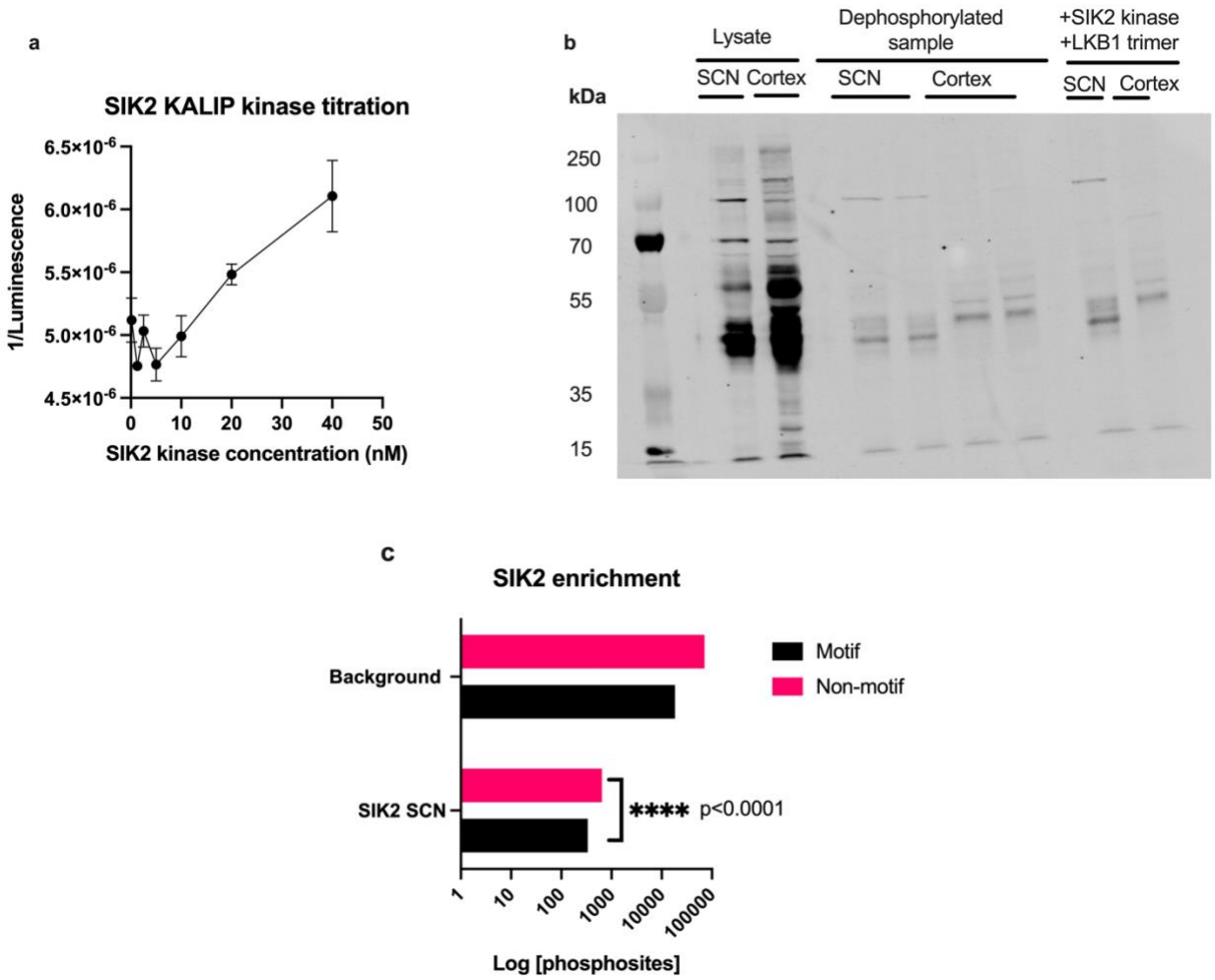
Supplementary Fig 4.3 **Determining optimal KALIP conditions for SIK3 kinase.** *In vitro* Kinase Glo Luminescence assay using dephosphorylated cortex tissue lysate as substrate in 384-well plates. SIK3 kinase titration curve with two-fold serial dilution of SIK2 kinase from 0.125nM to 40nM at 30 minutes of reaction time. Linear range from 5nM to 40nM fitted with simple linear regression. SIK3 kinase activity measured as 1/Luminescence (inverse). n=3.



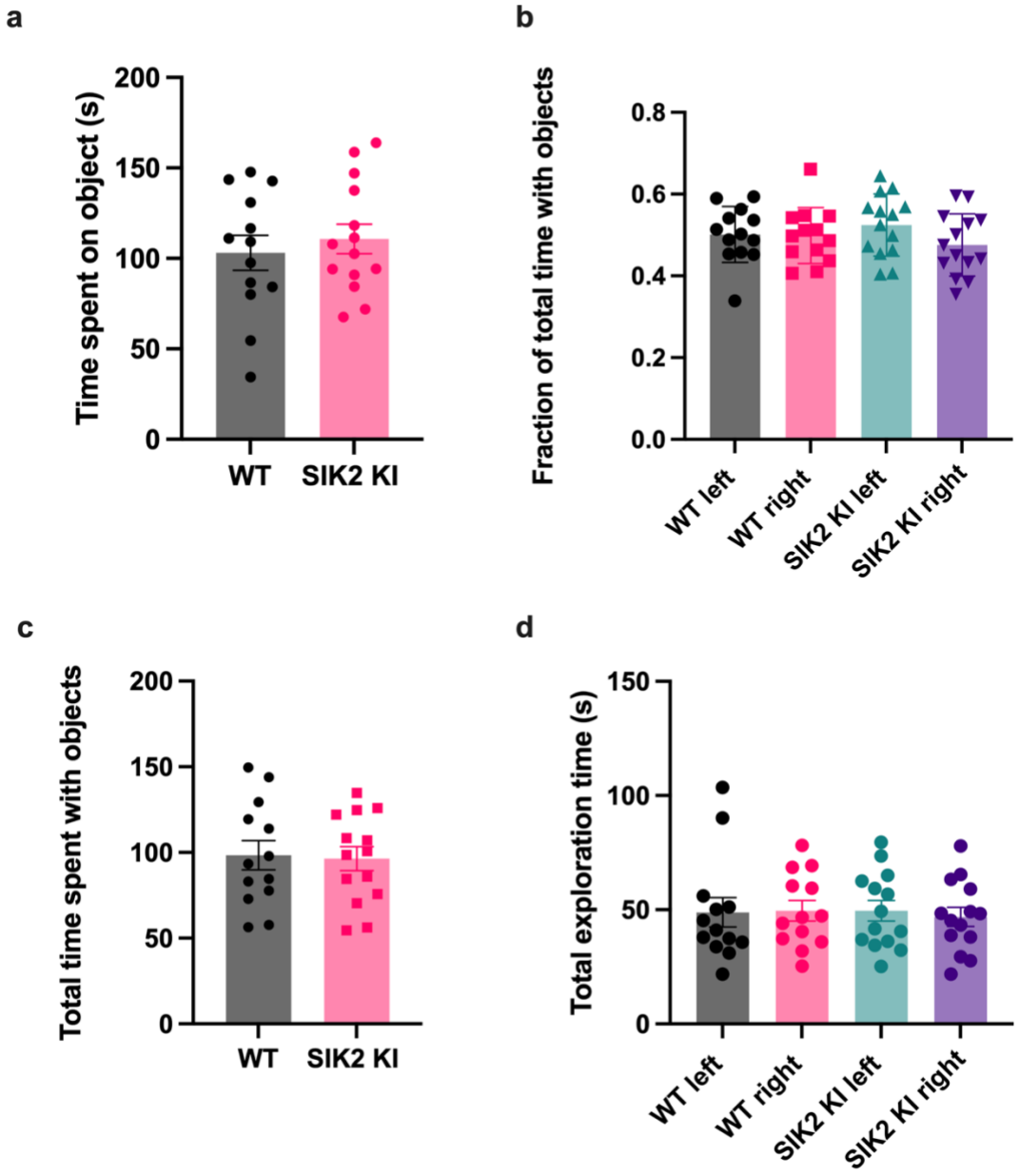
Supplementary Fig. 5.1 **PCA analysis of SCN and Cortex RNAseq**. PCA plot showing the distribution of samples for QC and determining outliers. PCA plot generated by RStudio. KI= knock-in animals; WT= wild-type animals; CTX = cortex.



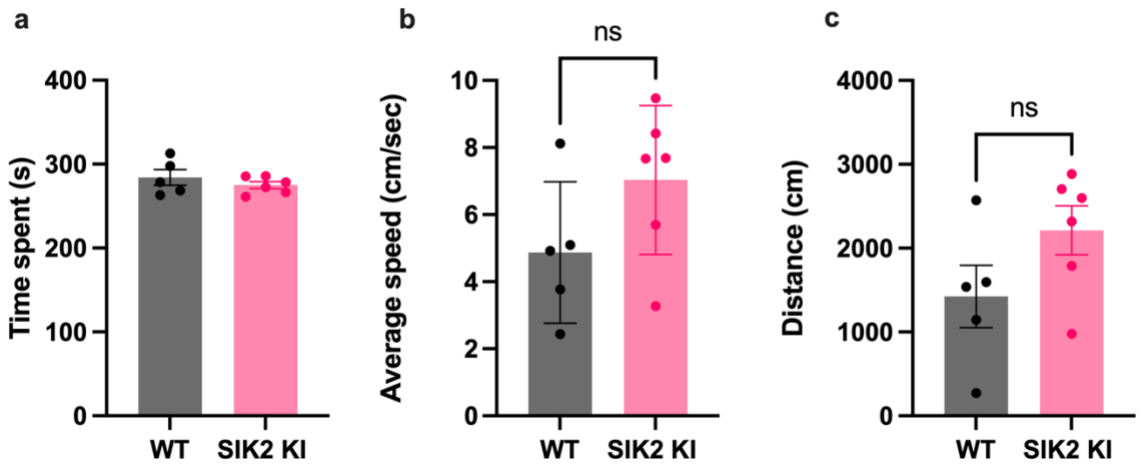
Supplementary Fig. 5.2 **PCA analysis of SCN and cortex phosphoproteomics**. PCA plot showing the distribution of samples for QC and determining outliers. PCA plot generated by RStudio. KI= knock-in animals; WT= wild-type animals.



Supplementary Fig 5.3 **Determining optimal KALIP conditions for SIK2 kinase.** (a) *In vitro* Kinase Glo Luminescence assay using dephosphorylated cortex tissue lysate as substrate in 384-well plates. SIK2 kinase titration curve with two-fold serial dilution of SIK2 kinase from 0.125nM to 40nM at 30 minutes of reaction time. Linear range from 5nM to 40nM fitted with simple linear regression. SIK2 kinase activity measured as 1/Luminescence (inverse). n=3. (b) Immunoblot of pAMPK substrate motif with lanes showing lysate, dephosphorylated sample, kinase reaction with SIK2 kinase and LKB1:STRADa: MO25a trimer complex of SCN and cortex tissue. PageRuler Plus Prestained Ladder was used. (c) Motif enrichment analysis using Johnson et al as background signal for SIK2. “Motif” was defined as a site that has a motif percentile of over 80%. Number of sites was plotted for both the background and SIK2 KALIP SCN dataset comparing number of “motif” and “non-motif” sites. Chi-squared test, Two-sided p-value. Odds ratio 2.208, p value <0.0001.



Supplementary Fig. 5.4 **Novel Object Recognition (NOR) training and testing.** Training sessions were conducted where WT and SIK2 KI animals were allowed to explore the same objects on opposite sides of the arena for 5 minutes to test for animal innate preferences. (a) The total time WT and SIK2 KI animals spent exploring objects during the 5-minute recorded interval during the training session. (b) The total time spent on objects was split into “left” and “right” objects for WT and SIK2 KI animals during the training session. (c) Total time spent on objects and (d) total exploration time on “left” and “right” objects were also recorded for the testing sessions. n=13-14 per group.



Supplementary Fig. 5.5 **Y-maze activity parameters.** Mobility and exploration parameters of individual WT and SIK2 KI animals were analyzed. (a) Total exploration time in seconds of the arms, (b) average moving speed in arms (cm/sec) and (c) total distance travelled (cm) were recorded. ns = $p>0.05$, Two-tailed unpaired t-test. Data points are mean ± SEM, n=6 per group.