Supplemental Information for

Nasal neuron PET imaging quantifies neuron generation and degeneration

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Tables and Figures

Supplemental Table 1 (appended to the end of the SI). GV1-57 does not show broad-spectrum kinase inhibition. A comprehensive kinase screen was completed by Reaction Biology Corp. using 5 μM non-radiolabeled GV1-57 in the presence of 1 μM ATP. Duplicate measurements were acquired against each kinase. The control compound, Staurosporine, was tested in the 10-dose IC_{50} mode with 4-fold serial dilutions starting at 20 or 100 μM. Alternate control compounds were tested in the 10-dose IC_{50} mode with 3-fold serial dilutions starting at 20 or 100 μM. For most kinases, there was no inhibition of kinase activity. The highest inhibition was of homeodomain interacting protein kinase 4 (HIPK4), with 51% inhibition at a concentration 15-fold higher than the measured ex vivo [^{11}C]GV1-57 IC_{50} (Supplemental Figure 5).

Supplemental Table 2. GV1-57 does not inhibit binding interactions with adenosine and purinergic receptors. Adenosine and purinergic receptor inhibition assays were completed by Eurofins Panlabs using 0.5 and 1.0 μM non-radiolabeled GV1-57. Eurofins Panlabs assays were binding displacement assays, and no significant inhibition of binding was detected. Labeled molecules used for determination of binding displacement were as follows: 1 nM [^{3}H]DPCPX (A1), 50 nM [^{3}H]CGS-21680 (A2A), 1.6 nM [^{3}H]MRS1754 (A2B), 0.5 nM [^{125}I]AB-MECA (A3), 8 nM [^{3}H]α, β-Methylene-ATP (P2X, general), 0.1 nM [^{35}S]ATP-αS (P2Y, general).

Receptor	Percent Inhibition*
A1	3 (0.5), -9 (1.0)
A2A	1 (0.5), 3 (1.0)
A2B	4 (0.5), 1 (1.0)
A3	-7 (0.5), -6 (1.0)
P2X	6 (0.5), -7 (1.0)
P2Y	-9 (0.5), -1 (1.0)

*Percent inhibition: ± percent inhibition (non-radiolabeled GV1-57, μM)

Supplemental Table 3. Individual olfactory sensory neuron influx rates during normative development. Rates (DVR/month) were calculated for individual rats. Between 5.5 and 12 months of age, rates of OSN population influx are significantly higher ($P < 0.005$) for animals starting with a DVR less than 5.5 compared to animals starting with a DVR greater than 6.0. The starting DVR is the DVR at 5.5 months of age.

Animal	Rate[a] (DVR/month)	DVR[b] (at 5.5 months)
1	0.084	6.76
2	0.30	5.38
3	0.23	5.04
4	0.26	4.40
5	0.12	6.24
6	0.11	6.92

[a] Calculated from 5.5–12 months of age
[b] t^* = 45 min

Supplemental Table 4. Olfactory sensory neuron regrowth rates following zinc sulfate tissue ablation. OSN regrowth rates (DVR/month) after $ZnSO_4$ treatment were calculated using the lowest post-$ZnSO_4$ DVR (lowest OSN population), the highest post-$ZnSO_4$ DVR (highest OSN population), and the intervening time interval between these DVR measurements. The OSN regrowth rates vary considerably across individual animals, with a 3.7-fold difference between the lowest and highest rate.

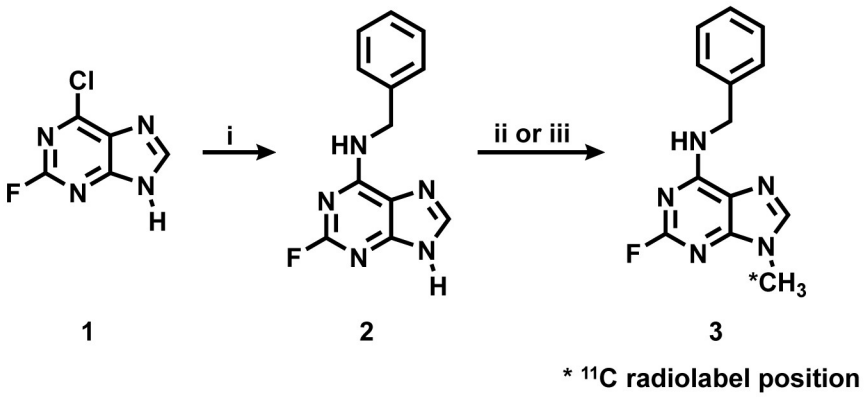
Animal	Rate (DVR/month)
1	0.55
2	1.5
3	0.96
4	2.0

Supplemental Table 5. Contingency table for classification of WT and rTg4510 animals at 3.7 months of age. Two trained radiologists used [^{11}C]GV1-57 images (SUV, 0–2.15) to classify individual 3.7 month old animals as having high or low radiotracer uptake in the olfactory epithelium (OE). The reference standard (*i.e.* gold standard) for this experiment was animal genotype (n = 3 per group).

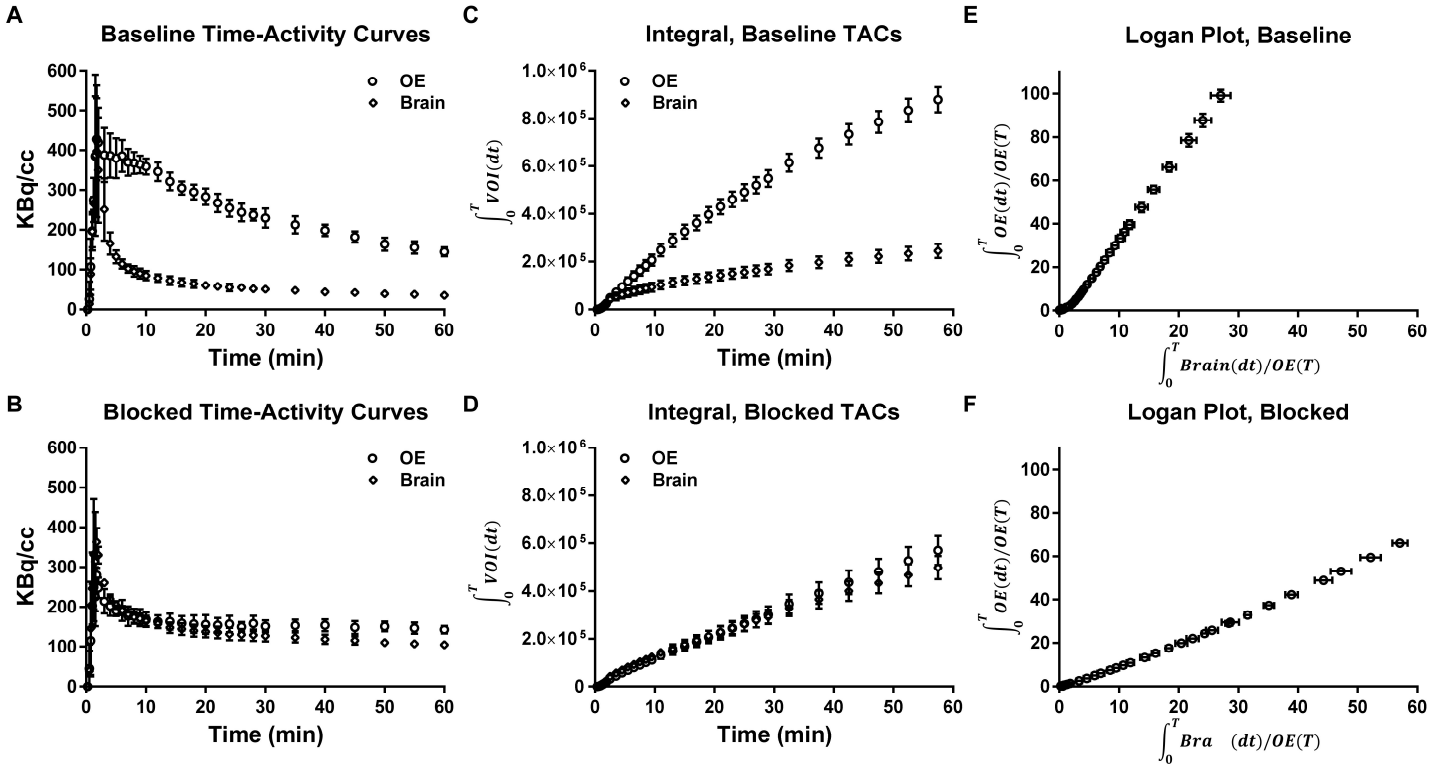
Genotype / [^{11}C]GV1-57 Image, 3.7 mo	Positive genotype, rTg4510	Negative genotype, WT
Positive scan, low uptake	3	1
Negative scan, high uptake	0	2

Supplemental Table 6. Contingency table for classification of WT and rTg4510 animals at 7 months of age. Two trained radiologists used [^{11}C]GV1-57 images (SUV, 0–2.15) to classify individual 7 month old animals as having high or low radiotracer uptake in the OE. The reference standard (*i.e.* gold standard) for this experiment was animal genotype (n = 3 per group).

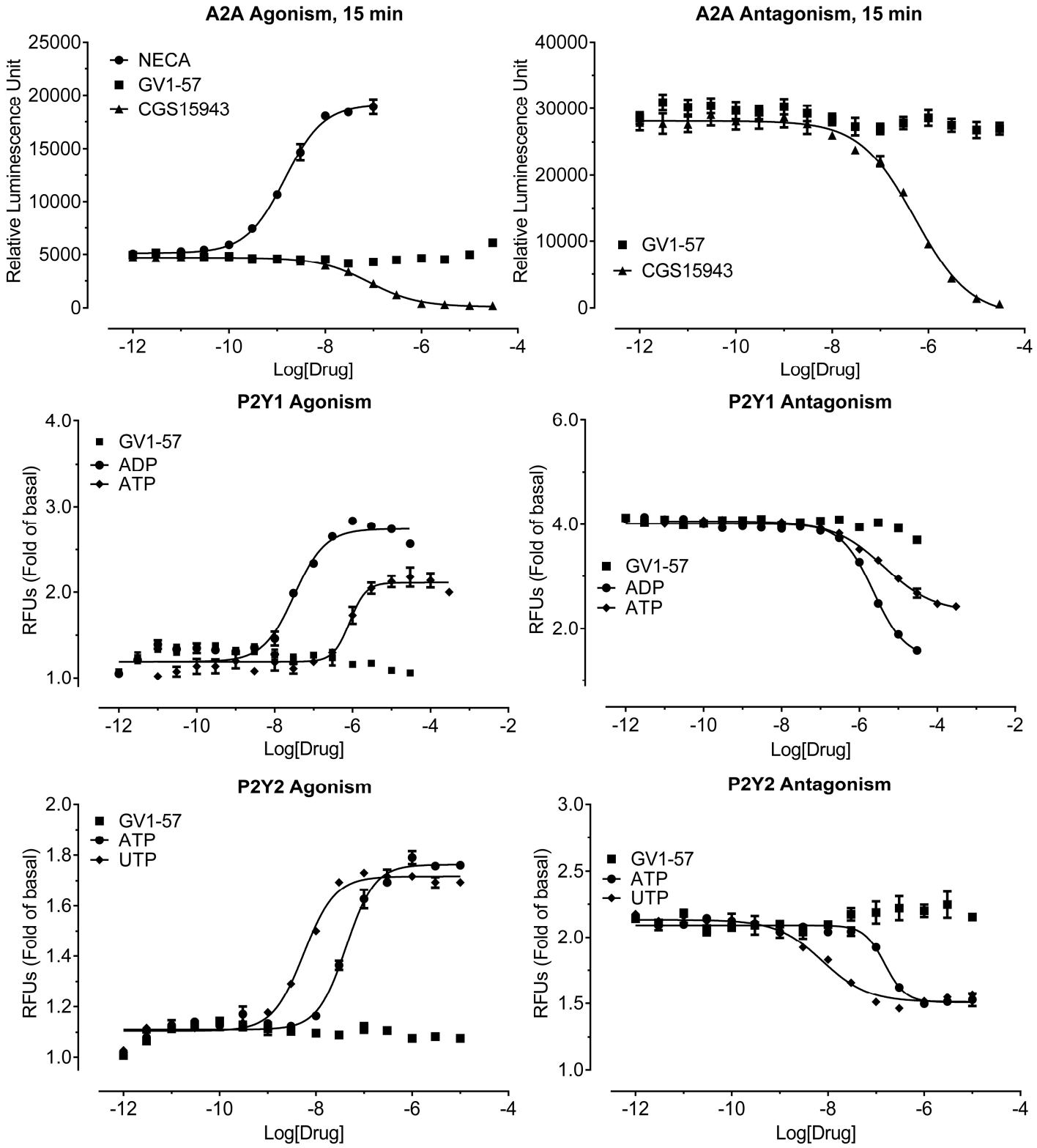
Genotype / [^{11}C]GV1-57 Image, 7 mo	Positive genotype, rTg4510	Negative genotype, WT
Positive scan, low uptake	3	0
Negative scan, high uptake	0	3



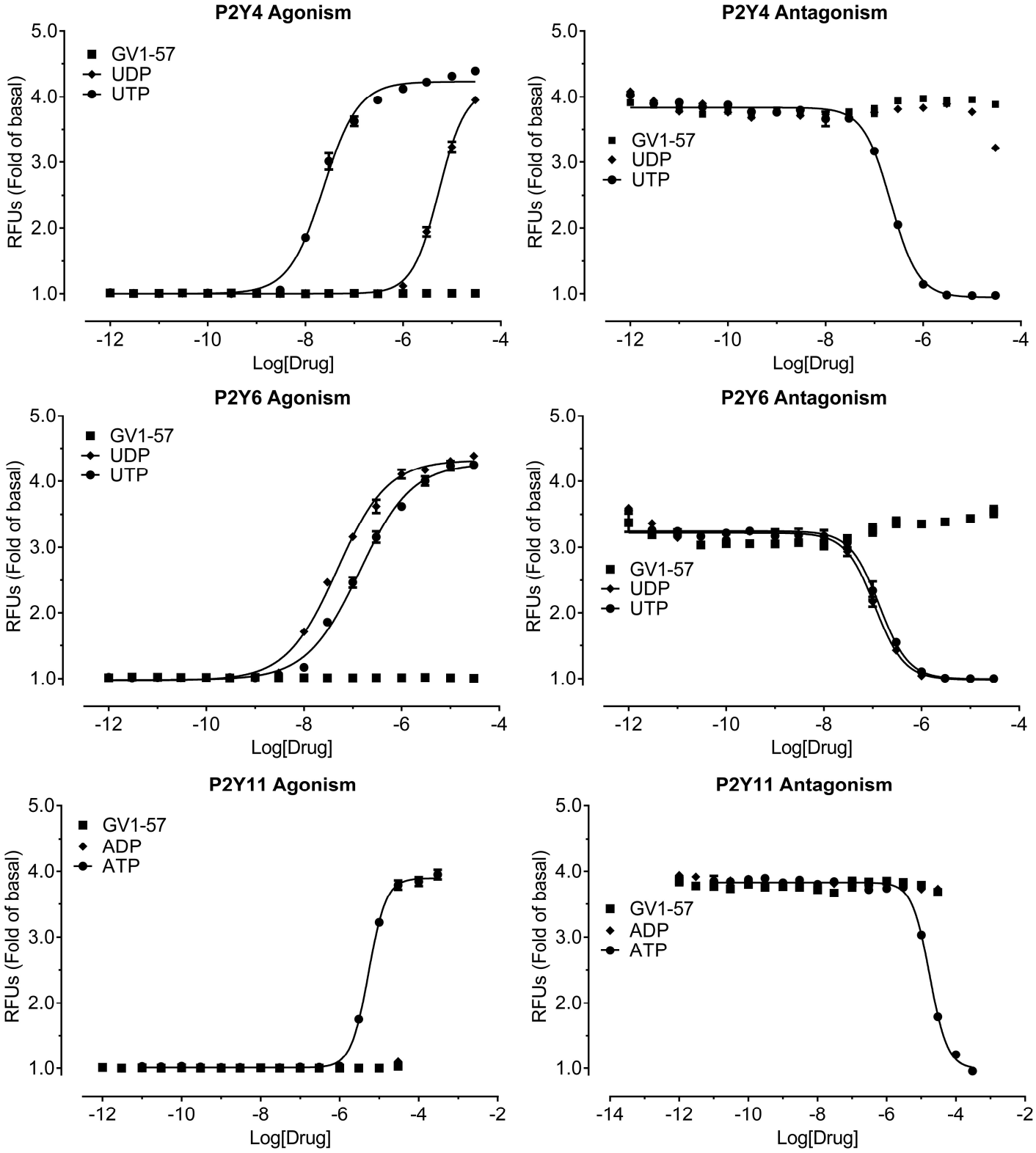
Supplemental Figure 1. Synthesis of non-radiolabeled GV1-57 and [^{11}C]GV1-57. Conditions: (i) Benzylamine, DIEA, *n*-BuOH, RT – 60 °C, 30 h, 92%. (ii) MeI, K_2CO_3, DMA, 7 h, 68%. (iii) [^{11}C]MeI, KOH, DMSO, 80 °C, 5 min, 14.2 ± 1.5% RCY, not decay corrected.



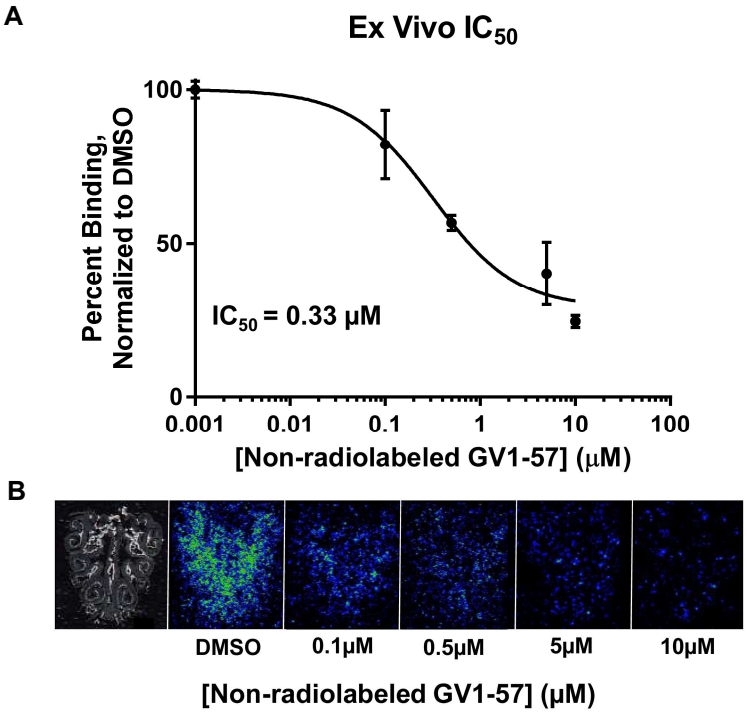
Supplemental Figure 2. Graphical representation of [^{11}C]GV1-57 Logan analysis. (**A, B**) Averaged time-activity curves (TACs) for the OE and the brain after injection with [^{11}C]GV1-57 (Baseline, **A**) or [^{11}C]GV1-57 and 16 mg/kg non-radiolabeled GV1-57 (Blocked, **B**). (**C, D**) Averaged integrals of the OE and brain TACs for animals injected with [^{11}C]GV1-57 (Baseline, **C**) or [^{11}C]GV1-57 and 16 mg/kg non-radiolabeled GV1-57 (Blocked, **D**). VOI y-axis label refers to either the OE or brain volume of interest. (**E, F**) Logan plots for animals injected with [^{11}C]GV1-57 (Baseline, **E**) or [^{11}C]GV1-57 and 16 mg/kg non-radiolabeled GV1-57 (Blocked, **F**). Treatment with 16 mg/kg non-radiolabeled GV1-57 occurred 5 min prior to [^{11}C]GV1-57 injection. Error bars are ± SEM, *n* = 3 for baseline and blocked groups.



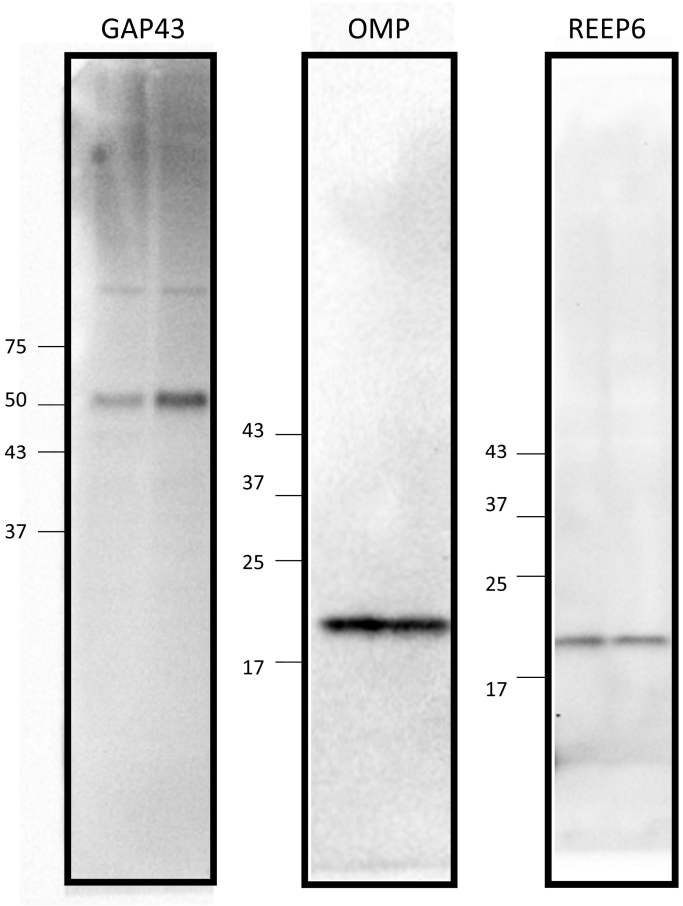
Supplemental Figure 3. GV1-57 does not inhibit activity of select adenosine and purinergic receptors. Adenosine (A2A) and purinergic (P2Y 1 and 2) receptor functional agonism and antagonism assays were completed by the PDSP using 3 pM – 30 μM non-radiolabeled GV1-57 (3, 10, 30, 100, 300 pM; 1, 3, 10, 30, 100, 300 nM; 1, 3, 10, 30 μM) (30). The data indicates no GV1-57 agonism or antagonism at these receptors. To desensitize the receptors, agonists were used in the antagonist functional assays, as follows: 30 nM NECA for A2A, 3 μM ADP for P2Y1, and 100 nM ATP for P2Y2.



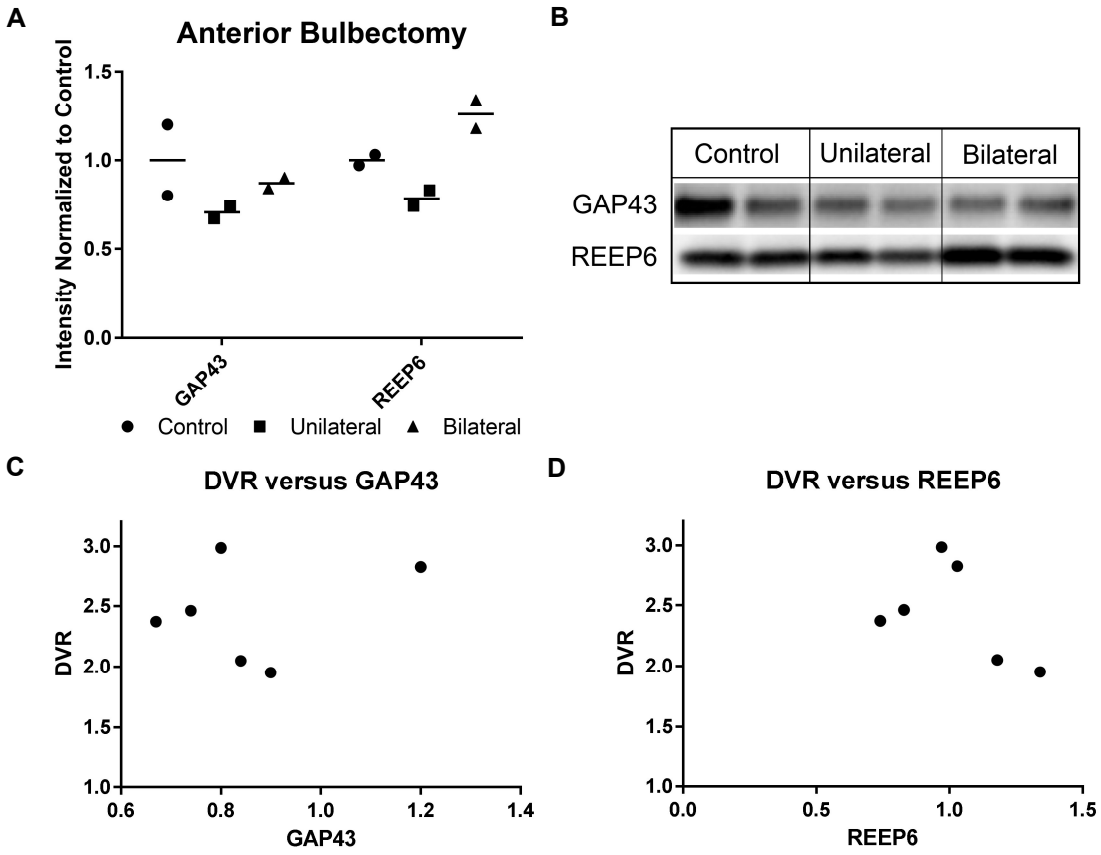
Supplemental Figure 4. GV1-57 does not inhibit activity of select adenosine and purinergic receptors. Purinergic (P2Y 4, 6, and 11) receptor functional agonism and antagonism assays were completed by the PDSP using 3 pM – 30 μM non-radiolabeled GV1-57 (3, 10, 30, 100, 300 pM; 1, 3, 10, 30, 100, 300 nM; 1, 3, 10, 30 μM) (30). The data indicates no GV1-57 agonism or antagonism at these receptors. To desensitize the receptors, agonists were used in the antagonist functional assays, as follows: 300 nM UTP for P2Y4, 100 nM UDP for P2Y6, and 100 μM ATP for P2Y11.



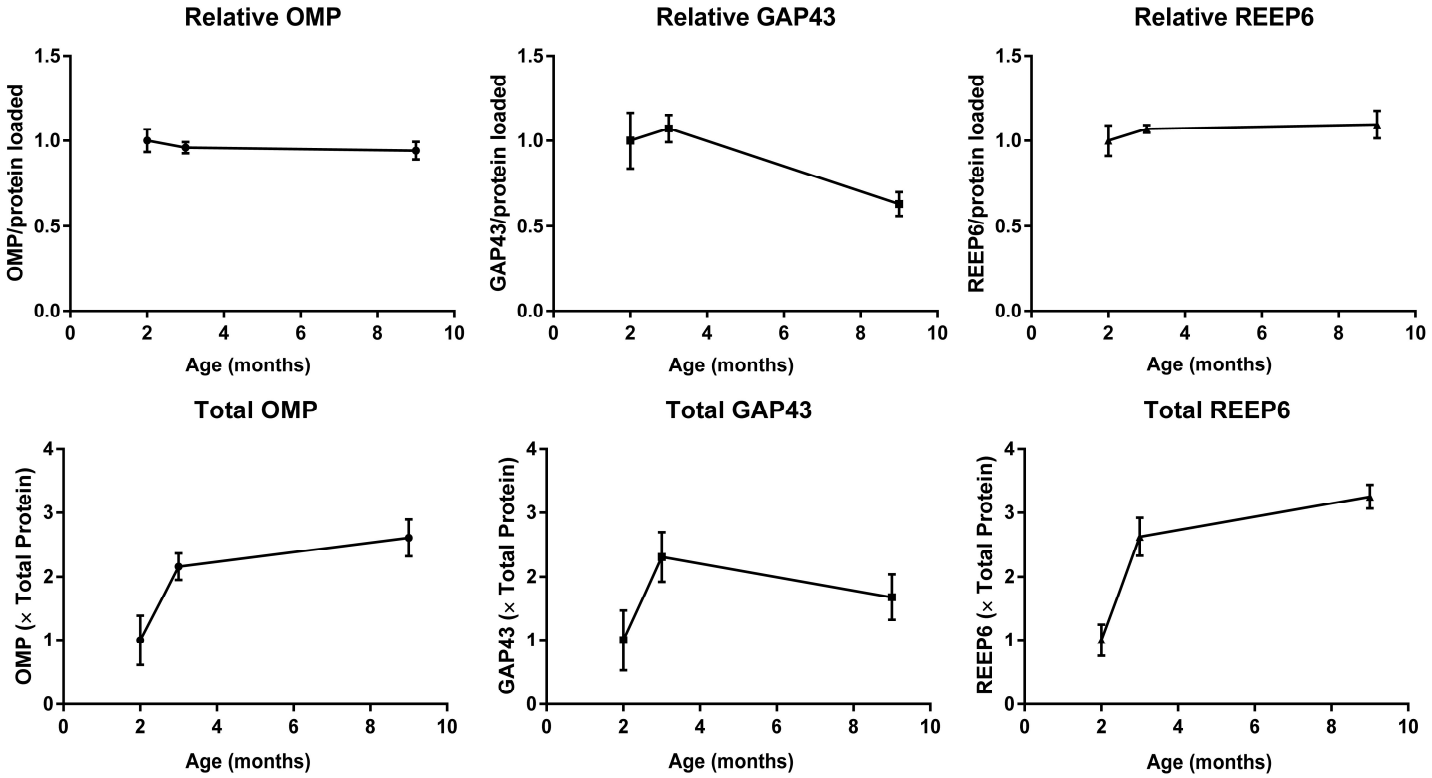
Supplemental Figure 5. [^{11}C]GV1-57 binding to olfactory epithelium sections is blocked by non-radiolabeled GV1-57. (**A**) Quantification of [^{11}C]GV1-57 autoradiography with coronal rat OE sections (10 µm) following a 20 min pre-incubation with non-radiolabeled GV1-57 (0–10 µM). Error bars are ± SEM; *n* = 8 sections/concentration. (**B**) Representative images of [^{11}C]GV1-57 binding to rat OE sections following pre-incubation with specified non-radiolabeled GV1-57 concentrations. Image on left is a digital scan of a representative coronal rat OE section that has been placed on a microscope slide.



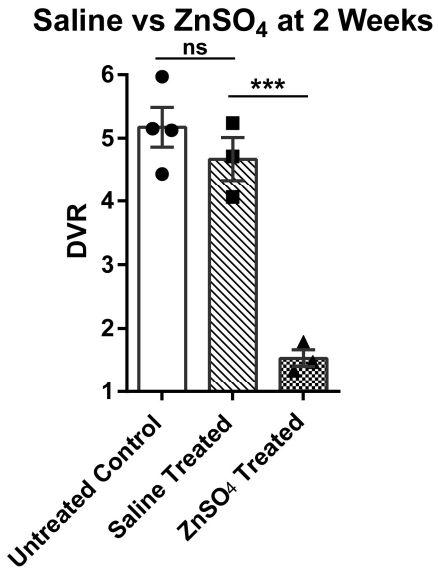
Supplemental Figure 6. Representative immunoblots for the cell-type-specific olfactory epithelium markers. Full immunoblots of rodent OE lysate indicate good selectivity of the chosen antibodies for anticipated protein molecular weights for GAP43 (25 kDa, apparent molecular weight ~43 kDa) (35), OMP (19 kDa), and REEP6 (22 kDa). The immunoblots shown in this figure are also shown in the left panel of Figure 4C.



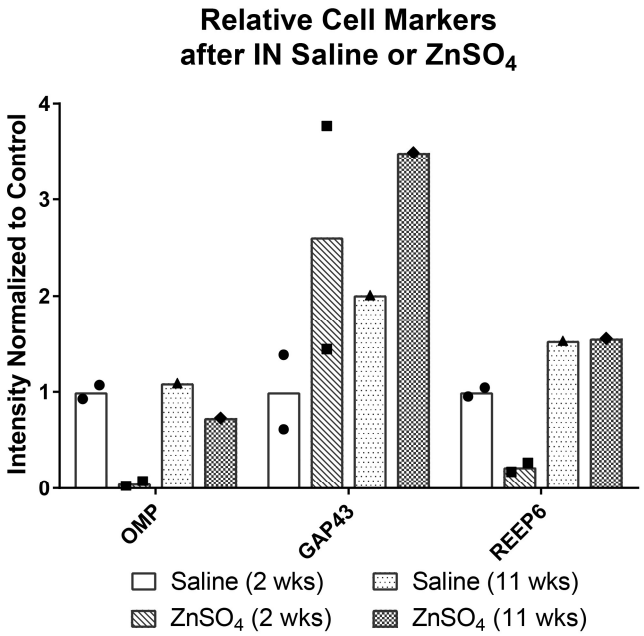
Supplemental Figure 7. Immature OSN and sustentacular cell markers do not correlate with [^{11}C]GV1-57 DVR following anterior bulbectomy. (**A**) Quantification of GAP43 (immature OSNs) and REEP6 (sustentacular cells) immunoreactivity to total OE lysate from control, unilateral bulbectomy and bilateral bulbectomy mice. For each antibody, band intensities were normalized to the average of the control bands. *n* = 2 per group (control, unilateral, or bilateral) (**B**) Representative images of developed GAP43 and REEP6 immunoblots. (**C**) Scatter plot of individual post-bulbectomy [^{11}C]GV1-57 DVRs (*t** = 45 min) and relative GAP43 immunoreactivities. There is no correlation between [^{11}C]GV1-57 DVR and GAP43 immunoreactivities (Spearman, r = -0.086, *P* = 0.92). (**D**) Scatter plot of individual post-bulbectomy [^{11}C]GV1-57 DVRs (*t** = 45 min) and relative REEP6 immunoreactivities. There is no correlation between REEP6 immunoreactivities (Spearman, r = -0.49, *P* = 0.36).



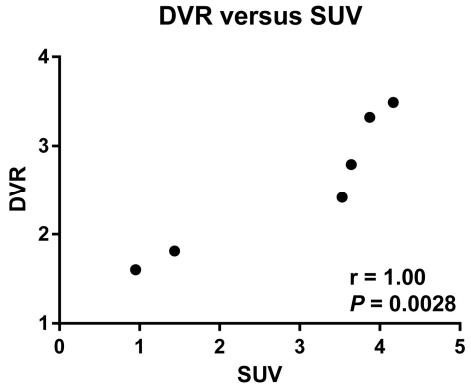
Supplemental Figure 8. Disparate effects of age on mature OSNs, immature OSNs, and sustentacular cells. Immunoblot analysis of mature OSNs (OMP), immature OSNs (GAP43), and sustentacular cells (REEP6) indicates that the mature OSNs and sustentacular cell markers remain at a constant level across development, relative to total protein. Alternatively, the immature OSN marker decreases during development, relative to total OE protein. To achieve a whole-tissue analysis of the cellular markers, the protein levels were multiplied by total protein extracted from septal OE tissue acquired from individual animals. This analysis approximates the whole-tissue, cellular-population analysis obtained from [^{11}C]GV1-57 imaging, and suggests that mature OSNs and sustentacular cells have an increasing cellular population (cellular influx) across the OE developmental period. Immature OSNs have an initially increasing population (cellular influx), which then trends downward between 3 and 9 months of age. This downward trend in the immature OSN population after 3 months of age might indicate an increased maturation rate of immature OSNs in conjunction with decreased rate of new immature OSN production. Error bars are ± SEM; *n* = 3 per age.



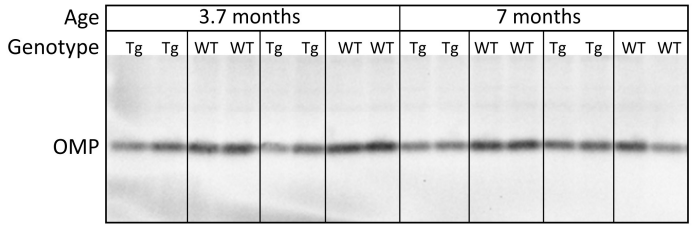
Supplemental Figure 9. Intranasal zinc sulfate significantly reduces [^{11}C]GV1-57 uptake. Quantification of [^{11}C]GV1-57 DVRs (t^* = 45 min) for untreated control rats and rats two-weeks-post intranasal treatment with saline or $ZnSO_4$. Error bars are ± SEM; n = 3–4 per group. ***P < 0.005 using a one-tailed Student's t test with a Bonferroni correction (α = 0.025) for multiple comparisons; ^{ns}P = 0.33 using a two-tailed Student's t test with a Bonferroni correction (α = 0.025) for multiple comparisons.



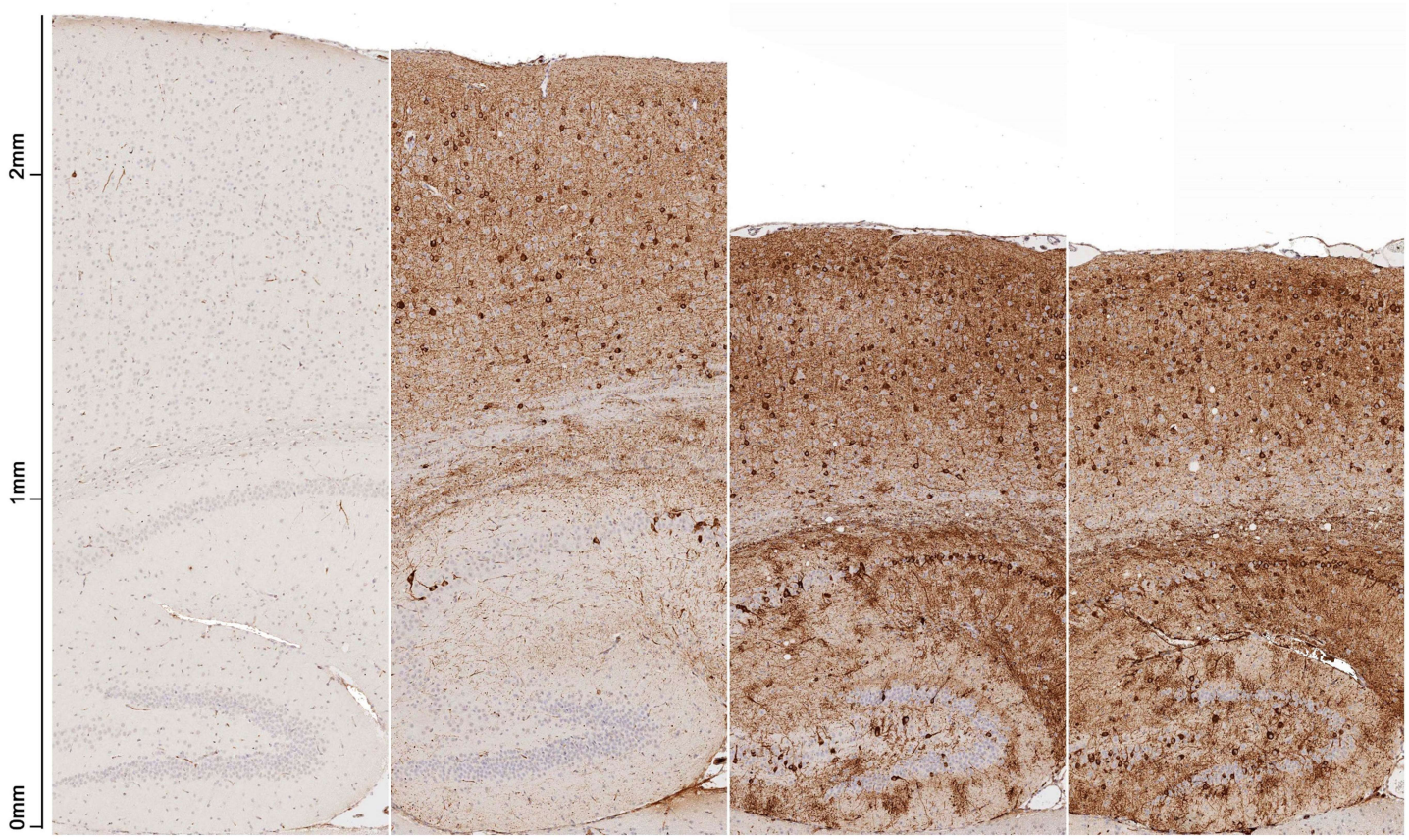
Supplemental Figure 10. Mature OSNs, but not immature OSNs or sustentacular cells, show alterations consistent with [^{11}C]GV1-57 imaging after saline and $ZnSO_4$ treatment. Quantitative analysis of OMP, GAP43, and REEP6 band intensity from OE tissue lysate from rats treated intranasally with saline (100 µl per nostril) or $ZnSO_4$ (10% in saline, 100 µl per nostril) two or eleven weeks prior to OE tissue harvesting. Band intensity normalized to average of saline bands for the respective marker protein. *n* = 1–2 per group.



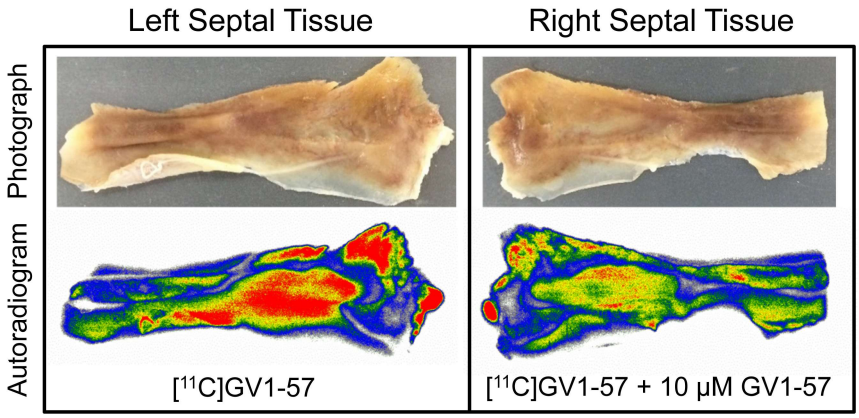
Supplemental Figure 11. Correlation of DVR and SUV analyses of [^{11}C]GV1-57 uptake in the olfactory epithelium. Plot of DVR (*t** = 45 min) versus SUV (3–45 min) shows significant correlation (Spearman, r = 1.00, *P* = 0.0028) between two quantitative measures of [^{11}C]GV1-57 uptake, indicating radiotracer versatility across quantitative analyses. Graph is comprised of data from a single, longitudinally-imaged, $ZnSO_4$-treated animal.



Supplemental Figure 12. Mature OSN population deficit in rTg4510 animals is confirmed by immunoblotting. Raw Western immunoblot data used for quantification of relative mature OSN populations in WT and rTg4510 animals (Figure 5C). For analysis, band intensities were background subtracted, multiplied by total extracted protein per sample, and normalized to the 3.7-month-old WT group. The mature OSN population measured by OMP is significantly ($P < 0.05$) lower in 7-month-old rTg4510 animals compared to age-matched WT controls. Within-age group comparisons were completed with a two-tailed Student's *t* test. $n = 4$ per group.



Supplemental Figure 13. Progressive tau-associated neurodegeneration in the rTg4510 mouse model. Cross-sectional analysis of brain tissue from rTg4510 mice shows a progressive increase in tau pathology between 2 and 8 months of age. Neuronal tau accumulation was minimal at 2 months of age and increased substantially by 4 months of age. At 6 months of age, severe cortical and hippocampal tau pathology was associated with significant tissue atrophy and neuronal loss. Tau-associated neurodegeneration was very severe by 8 months of age. Tau pathology was detected immunohistochemically using a phospho-specific tau antibody (PG-5; pSer409) in formalin-fixed paraffin-embedded tissue sections (sagittal plane). Images are representative of their respective time-points; *n* = 15–18 per age.



Supplemental Figure 14. [^{11}C]GV1-57 binding in *Papio anubis* nasal tissue. Tissue from either side of a male *Papio anubis* baboon's nasal septum was incubated with either [^{11}C]GV1-57 (left septal tissue) or [^{11}C]GV1-57 following a 20 min incubation with non-radiolabeled GV1-57 (10 μM, right septal tissue). The images on top are photographs of the left and right baboon septal tissue, and the images on the bottom are non-adjusted autoradiograms associated with the left and right septal tissue of the baboon.

Supplemental Table 1

Enzyme Activity Summary:

Kinase:	% Enzyme Activity (relative to DMSO controls) GV1-57 Data 1	% Enzyme Activity (relative to DMSO controls) GV1-57 Data 2	IC50 (M) Staurosporine*	IC50 (M) Alternate Control cpd*.	Alternate compound ID
ABL1	101.84	101.66	**2.76E-08**		
ABL2/ARG	95.98	93.43	**1.31E-08**		
ACK1	88.97	86.78	**7.05E-08**		
AKT1	97.63	94.49	**3.63E-09**		
AKT2	104.01	102.87	**1.41E-08**		
AKT3	103.23	102.94	**2.54E-09**		
ALK	99.44	97.39	**2.05E-09**		
ALK1/ACVRL1	110.62	107.35	**ND**	**7.85E-09**	**LDN193189**
ALK2/ACVR1	92.41	92.22	**ND**	**2.57E-08**	**LDN193189**
ALK3/BMPR1A	97.83	96.04	**ND**	**9.44E-09**	**LDN193189**
ALK4/ACVR1B	92.00	90.87	**ND**	**1.65E-07**	**LDN193189**
ALK5/TGFBR1	103.27	102.88	**ND**	**1.56E-07**	**LDN193189**
ALK6/BMPR1B	109.26	108.56	**ND**	**5.14E-09**	**LDN193189**
ARAF	89.95	87.57	**ND**	**1.89E-08**	**GW5074**
ARK5/NUAK1	98.17	94.67	**1.08E-09**		
ASK1/MAP3K5	90.26	88.46	**6.55E-09**		
Aurora A	88.75	88.64	**6.79E-10**		
Aurora B	96.98	95.87	**8.81E-09**		
Aurora C	95.67	95.00	**1.93E-09**		
AXL	102.97	102.61	**6.86E-09**		
BLK	92.00	90.17	**9.14E-10**		
BMPR2	95.21	95.02	**1.92E-06**		
BMX/ETK	100.85	99.88	**4.51E-09**		
BRAF	99.68	97.26	**ND**	**1.85E-08**	**GW5074**
BRK	84.59	84.34	**2.35E-07**		
BRSK1	97.24	96.87	**7.26E-10**		
BRSK2	109.54	107.52	**1.95E-09**		
BTK	97.17	97.03	**2.10E-08**		
c-Kit	110.98	110.25	**9.74E-08**		
c-MER	94.38	93.12	**5.92E-09**		
c-MET	94.49	93.59	**2.39E-08**		
c-Src	96.89	93.62	**1.86E-09**		
CAMK1a	100.84	99.14	**2.19E-09**		
CAMK1b	83.25	81.59	**3.61E-09**		
CAMK1d	92.72	92.70	**1.87E-10**		
CAMK1g	99.72	99.16	**4.22E-09**		
CAMK2a	95.79	94.94	**2.97E-11**		
CAMK2b	94.45	94.21	**3.20E-11**		
CAMK2d	101.79	101.59	**4.75E-11**		
CAMK2g	94.44	93.56	**3.27E-10**		
CAMK4	99.74	98.73	**9.07E-08**		
CAMKK1	106.80	103.56	**7.60E-08**		
CAMKK2	104.09	101.55	**1.82E-08**		
CDC7/DBF4	89.96	89.96	**5.50E-08**		
CDK1/cyclin A	93.73	92.56	**2.31E-09**		
CDK1/cyclin B	91.40	91.08	**1.34E-09**		
CDK1/cyclin E	102.97	99.88	**3.39E-09**		
CDK14/cyclin Y (PFTK1)	103.89	103.37	**1.21E-07**		
CDK16/cyclin Y (PCTAIRE)	94.06	93.62	**1.54E-08**		

CDK17/cyclin Y (PCTK2)	98.30	96.48	**1.32E-08**		
CDK18/cyclin Y (PCTK3)	113.73	113.51	**2.96E-08**		
CDK2/cyclin A	77.61	76.29	**6.71E-10**		
CDK2/Cyclin A1	71.81	71.54	**1.48E-09**		
CDK2/cyclin E	72.82	72.39	**1.43E-09**		
CDK2/cyclin O	69.00	68.07	**1.18E-09**		
CDK3/cyclin E	84.27	82.57	**2.43E-09**		
CDK4/cyclin D1	92.31	91.73	**1.83E-08**		
CDK4/cyclin D3	84.40	84.10	**4.54E-08**		
CDK5/p25	92.84	92.08	**2.25E-09**		
CDK5/p35	73.98	73.95	**1.44E-09**		
CDK6/cyclin D1	104.43	103.17	**5.89E-09**		
CDK6/cyclin D3	90.71	85.98	**9.38E-08**		
CDK7/cyclin H	98.88	97.91	**1.33E-07**		
CDK9/cyclin K	95.51	94.59	**3.79E-07**		
CDK9/cyclin T1	85.43	81.36	**4.39E-09**		
CHK1	96.33	94.69	**1.20E-10**		
CHK2	88.88	88.08	**5.72E-09**		
CK1a1	84.08	82.21	**2.47E-06**		
CK1a1L	103.37	101.71	**1.34E-06**		
CK1d	66.18	66.03	**ND**	**1.36E-07**	**D4476**
CK1epsilon	68.83	68.75	**ND**	**1.56E-07**	**D4476**
CK1g1	93.74	93.53	**3.78E-06**		
CK1g2	95.74	95.54	**1.52E-06**		
CK1g3	88.73	86.91	**1.83E-06**		
CK2a	87.38	86.30	**ND**	**7.41E-08**	**GW5074**
CK2a2	87.47	85.19	**1.33E-07**		
CLK1	102.12	99.35	**1.02E-08**		
CLK2	101.21	100.82	**3.57E-09**		
CLK3	98.15	95.78	**9.38E-07**		
CLK4	106.74	104.29	**9.29E-08**		
COT1/MAP3K8	105.42	105.33	**ND**	**1.05E-05**	**Ro-31-8220**
CSK	100.62	100.38	**1.32E-08**		
CTK/MATK	105.81	102.46	**1.20E-06**		
DAPK1	99.48	85.06	**9.56E-09**		
DAPK2	103.54	103.12	**2.89E-09**		
DCAMKL1	98.46	95.72	**2.09E-07**		
DCAMKL2	107.35	107.25	**1.41E-07**		
DDR1	102.29	100.97	**3.67E-09**		
DDR2	107.60	102.71	**4.44E-10**		
DLK/MAP3K12	85.73	85.68	**4.87E-08**		
DMPK	100.98	100.83	**1.56E-07**		
DMPK2	88.37	86.92	**2.89E-10**		
DRAK1/STK17A	93.78	92.66	**2.83E-08**		
DYRK1/DYRK1A	93.67	92.44	**2.93E-09**		
DYRK1B	85.63	85.11	**7.72E-10**		
DYRK2	68.27	68.27	**1.34E-07**		
DYRK3	93.40	92.39	**1.09E-08**		
DYRK4	99.08	98.80	**ND**	**GW5074**	
EGFR	92.82	92.22	**4.71E-08**		
EPHA1	91.35	91.01	**8.76E-08**		
EPHA2	106.81	105.28	**7.06E-08**		
EPHA3	94.83	94.29	**3.51E-08**		
EPHA4	95.96	95.84	**1.18E-08**		
EPHA5	100.52	97.71	**1.25E-08**		
EPHA6	96.99	96.08	**1.93E-08**		
EPHA7	91.63	89.47	**2.36E-08**		
EPHA8	95.04	91.61	**6.98E-08**		

EPHB1	91.60	89.92	**3.21E-08**		
EPHB2	96.71	96.05	**4.22E-08**		
EPHB3	106.84	105.31	**1.22E-06**		
EPHB4	97.09	97.05	**1.42E-07**		
ERBB2/HER2	97.23	96.50	**3.17E-08**		
ERBB4/HER4	89.20	88.67	**8.88E-08**		
ERK1	97.28	96.64	**5.12E-06**		
ERK2/MAPK1	82.87	82.22	**3.92E-06**		
ERK5/MAPK7	101.09	100.65	**5.12E-06**		
ERK7/MAPK15	83.75	82.43	**9.62E-09**		
ERN1/IRE1	94.42	92.64	**3.70E-08**		
ERN2/IRE2	98.93	98.85	**2.23E-08**		
FAK/PTK2	94.94	93.98	**1.44E-08**		
FER	111.21	110.50	**1.82E-10**		
FES/FPS	94.75	93.24	**1.74E-09**		
FGFR1	97.16	97.10	**2.66E-09**		
FGFR2	92.52	91.09	**8.57E-10**		
FGFR3	113.56	108.07	**6.83E-09**		
FGFR4	96.90	96.67	**1.02E-07**		
FGR	95.07	92.42	**6.58E-10**		
FLT1/VEGFR1	93.15	92.64	**9.69E-09**		
FLT3	90.15	90.10	**1.37E-09**		
FLT4/VEGFR3	108.79	107.14	**1.32E-09**		
FMS	99.93	97.96	**1.92E-09**		
FRK/PTK5	107.49	106.55	**5.68E-09**		
FYN	103.88	102.69	**1.88E-09**		
GCK/MAP4K2	96.28	94.70	**4.73E-10**		
GLK/MAP4K3	96.57	95.91	**7.30E-11**		
GRK1	98.88	97.83	**7.16E-08**		
GRK2	106.69	105.51	**1.21E-06**		
GRK3	101.29	100.52	**8.91E-07**		
GRK4	91.66	90.95	**6.21E-08**		
GRK5	107.91	107.04	**3.04E-08**		
GRK6	96.46	95.63	**5.37E-08**		
GRK7	98.83	97.54	**5.27E-09**		
GSK3a	85.16	83.49	**2.53E-09**		
GSK3b	90.05	89.64	**4.86E-09**		
Haspin	99.03	97.91	**2.80E-08**		
HCK	88.65	85.65	**2.68E-09**		
HGK/MAP4K4	89.58	88.57	**2.91E-10**		
HIPK1	100.44	98.40	**ND**	**2.31E-07**	**Ro-31-8220**
HIPK2	97.92	97.58	**1.39E-07**		
HIPK3	90.10	88.78	**2.08E-07**		
HIPK4	49.25	48.04	**1.58E-07**		
HPK1/MAP4K1	104.54	103.46	**ND**	**2.92E-08**	**Ro-31-8220**
IGF1R	96.69	95.73	**2.32E-08**		
IKKa/CHUK	98.81	97.06	**1.24E-07**		
IKKb/IKBKB	102.23	101.59	**2.15E-07**		
IKKe/IKBKE	104.58	102.33	**2.14E-10**		
IR	111.29	106.22	**8.36E-09**		
IRAK1	98.17	89.86	**1.29E-08**		
IRAK4	97.35	82.26	**3.04E-09**		
IRR/INSRR	79.80	79.28	**8.35E-09**		
ITK	100.76	100.29	**9.85E-09**		
JAK1	90.25	88.02	**4.74E-10**		
JAK2	94.77	93.95	**1.19E-10**		
JAK3	97.19	95.54	**7.89E-11**		
JNK1	88.77	87.83	**2.16E-07**		

JNK2	97.52	98.05	**8.54E-07**		
JNK3	92.26	90.95	**ND**	**5.37E-08**	**JNKi VIII**
KDR/VEGFR2	107.60	107.22	**4.12E-09**		
KHS/MAP4K5	91.47	90.03	**3.25E-10**		
KSR1	103.93	103.68	**6.12E-06**		
KSR2	101.32	98.70	**4.08E-06**		
LATS1	85.44	84.05	**8.59E-09**		
LATS2	92.85	91.88	**1.02E-08**		
LCK	99.25	95.84	**2.41E-09**		
LCK2/ICK	98.99	98.36	**4.25E-08**		
LIMK1	86.43	84.42	**2.70E-09**		
LIMK2	103.09	102.12	**2.61E-07**		
LKB1	106.13	104.38	**6.86E-08**		
LOK/STK10	108.89	108.18	**1.33E-08**		
LRRK2	99.09	98.18	**5.69E-09**		
LYN	98.08	97.63	**7.36E-10**		
LYN B	100.38	98.22	**1.32E-09**		
MAK	84.56	83.97	**2.02E-08**		
MAPKAPK2	112.41	110.09	**8.14E-08**		
MAPKAPK3	97.19	95.40	**2.47E-06**		
MAPKAPK5/PRAK	94.54	93.67	**8.52E-08**		
MARK1	101.77	101.37	**3.28E-10**		
MARK2/PAR-1Ba	95.14	93.54	**1.14E-10**		
MARK3	94.61	94.22	**2.60E-10**		
MARK4	98.38	98.00	**1.04E-10**		
MEK1	99.57	99.19	**1.15E-08**		
MEK2	89.56	89.04	**9.41E-09**		
MEK3	98.24	96.44	**5.19E-09**		
MEK5	100.51	100.10	**1.09E-08**		
MEKK1	100.82	99.35	**3.50E-07**		
MEKK2	92.54	91.07	**4.34E-08**		
MEKK3	86.93	85.87	**3.35E-08**		
MEKK6	97.50	96.66	**9.86E-08**		
MELK	90.70	89.41	**4.98E-10**		
MINK/MINK1	92.87	91.60	**4.22E-10**		
MKK4	98.36	98.16	**1.17E-06**		
MKK6	96.49	95.54	**2.44E-09**		
MKK7	101.78	101.59	**5.11E-07**		
MLCK/MYLK	90.48	90.29	**5.58E-08**		
MLCK2/MYLK2	93.28	92.21	**9.76E-09**		
MLK1/MAP3K9	96.96	96.42	**1.23E-09**		
MLK2/MAP3K10	92.21	87.38	**4.37E-09**		
MLK3/MAP3K11	95.64	91.45	**6.22E-09**		
MLK4	97.54	96.03	**1.15E-06**		
MNK1	100.69	100.10	**6.24E-08**		
MNK2	98.37	96.06	**1.46E-08**		
MRCKa/CDC42BPA	104.10	102.62	**1.60E-09**		
MRCKb/CDC42BPB	103.34	103.29	**9.28E-10**		
MSK1/RPS6KA5	99.90	97.95	**4.23E-10**		
MSK2/RPS6KA4	109.65	108.07	**2.62E-09**		
MSSK1/STK23	92.38	91.53	**1.70E-06**		
MST1/STK4	99.06	98.74	**1.51E-09**		
MST2/STK3	94.01	88.51	**3.78E-09**		
MST3/STK24	81.27	77.75	**1.46E-08**		
MST4	96.14	94.66	**2.15E-08**		
MUSK	79.24	76.20	**6.97E-08**		
MYLK3	103.76	102.68	**1.04E-07**		
MYLK4	98.94	98.54	**5.24E-08**		

MYO3A	95.94	93.10	**1.83E-08**		
MYO3b	106.03	101.51	**5.03E-09**		
NEK1	100.18	97.42	**8.14E-09**		
NEK11	85.18	81.22	**3.65E-07**		
NEK2	104.91	100.56	**1.07E-07**		
NEK3	110.27	106.95	**3.49E-05**		
NEK4	104.66	102.94	**4.93E-08**		
NEK5	91.01	88.72	**5.71E-08**		
NEK6	100.81	100.33	**ND**	**5.72E-05**	**PKR Inhibitor**
NEK7	89.43	86.28	**ND**	**6.79E-06**	**PKR Inhibitor**
NEK8	100.73	97.66	**3.65E-08**		
NEK9	91.38	89.03	**6.58E-08**		
NIM1	98.70	94.10	**7.90E-08**		
NLK	76.93	70.93	**5.38E-08**		
OSR1/OXSR1	95.43	94.95	**3.97E-08**		
P38a/MAPK14	93.56	91.39	**ND**	**1.60E-08**	**SB202190**
P38b/MAPK11	101.29	97.95	**ND**	**1.50E-08**	**SB202190**
P38d/MAPK13	104.65	102.82	**1.14E-07**		
P38g	104.91	100.86	**1.14E-07**		
p70S6K/RPS6KB1	98.52	98.46	**5.21E-10**		
p70S6Kb/RPS6KB2	98.85	98.63	**6.88E-10**		
PAK1	97.03	95.21	**3.91E-10**		
PAK2	100.63	98.95	**2.77E-09**		
PAK3	94.00	91.82	**3.50E-10**		
PAK4	96.35	95.08	**2.37E-08**		
PAK5	85.85	85.43	**1.76E-09**		
PAK6	98.67	98.17	**5.85E-08**		
PASK	98.89	97.74	**6.64E-09**		
PBK/TOPK	91.15	89.78	**4.85E-08**		
PDGFRa	95.79	95.66	**5.07E-10**		
PDGFRb	100.61	100.03	**2.28E-09**		
PDK1/PDPK1	81.75	81.33	**5.19E-10**		
PEAK1	94.64	94.46	**1.90E-09**		
PHKg1	98.44	96.86	**2.57E-09**		
PHKg2	98.80	98.41	**5.83E-10**		
PIM1	100.39	99.80	**4.27E-09**		
PIM2	102.87	102.57	**4.88E-09**		
PIM3	98.77	98.49	**9.90E-11**		
PKA	100.86	100.85	**5.79E-10**		
PKAcb	98.25	97.25	**4.86E-10**		
PKAcg	100.50	98.88	**1.84E-09**		
PKCa	95.38	94.59	**3.69E-10**		
PKCb1	88.33	87.72	**6.79E-09**		
PKCb2	94.55	94.19	**2.87E-09**		
PKCd	88.90	87.38	**4.28E-10**		
PKCepsilon	100.15	96.49	**1.98E-10**		
PKCeta	90.89	89.77	**2.42E-10**		
PKCg	94.83	93.95	**7.00E-10**		
PKCiota	88.86	88.06	**1.34E-08**		
PKCmu/PRKD1	90.30	89.17	**8.84E-10**		
PKCnu/PRKD3	98.49	98.43	**2.21E-09**		
PKCtheta	105.56	99.31	**4.16E-09**		
PKCzeta	98.98	95.21	**5.11E-08**		
PKD2/PRKD2	116.60	116.41	**9.58E-10**		
PKG1a	96.43	95.67	**1.04E-09**		
PKG1b	103.68	103.29	**2.57E-09**		
PKG2/PRKG2	101.05	98.30	**8.84E-09**		
PKN1/PRK1	114.33	105.42	**2.66E-09**		

PKN2/PRK2	107.59	104.77	**1.46E-08**		
PKN3/PRK3	102.66	98.53	**8.48E-09**		
PLK1	99.42	99.22	**1.23E-07**		
PLK2	97.93	97.90	**2.41E-07**		
PLK3	104.32	104.21	**1.43E-07**		
PLK4/SAK	97.33	96.74	**9.20E-09**		
PRKX	103.85	101.25	**1.81E-09**		
PYK2	101.09	100.97	**9.74E-09**		
RAF1	94.91	94.29	**ND**	**9.04E-09**	**GW5074**
RET	98.47	97.81	**2.36E-09**		
RIPK2	114.97	112.90	**3.89E-07**		
RIPK3	89.08	86.79	**ND**	**2.85E-06**	**GW5074**
RIPK5	97.76	94.99	**4.71E-08**		
ROCK1	99.76	99.74	**4.53E-10**		
ROCK2	103.11	102.97	**4.99E-10**		
RON/MST1R	99.10	96.20	**7.96E-08**		
ROS/ROS1	85.79	84.08	**1.23E-10**		
RSK1	96.54	95.19	**9.02E-11**		
RSK2	97.41	96.86	**9.48E-11**		
RSK3	104.60	103.72	**1.37E-10**		
RSK4	99.52	99.40	**1.06E-10**		
SBK1	96.76	96.62	**2.24E-07**		
SGK1	87.74	87.30	**5.73E-09**		
SGK2	90.62	88.14	**1.15E-08**		
SGK3/SGKL	101.94	100.65	**7.90E-08**		
SIK1	84.76	83.64	**4.40E-10**		
SIK2	95.18	93.76	**3.25E-10**		
SIK3	94.88	94.20	**6.46E-10**		
SLK/STK2	94.86	94.34	**1.59E-08**		
SNARK/NUAK2	95.73	94.73	**1.58E-09**		
SNRK	105.40	104.61	**5.43E-09**		
SRMS	90.83	90.20	**2.39E-06**		
SRPK1	93.09	91.89	**2.47E-08**		
SRPK2	95.79	94.25	**1.79E-07**		
SSTK/TSSK6	102.77	100.26	**1.65E-07**		
STK16	98.82	98.04	**2.66E-07**		
STK21/CIT	101.85	99.78	**4.33E-07**		
STK22D/TSSK1	68.59	67.59	**4.42E-11**		
STK25/YSK1	100.76	97.51	**4.98E-09**		
STK32B/YANK2	103.44	103.35	**3.66E-08**		
STK32C/YANK3	94.97	90.26	**1.46E-07**		
STK33	92.50	90.98	**3.68E-08**		
STK38/NDR1	98.16	97.74	**5.23E-09**		
STK38L/NDR2	92.48	92.13	**1.11E-09**		
STK39/STLK3	100.15	99.74	**2.63E-08**		
SYK	94.29	94.28	**2.56E-10**		
TAK1	100.99	98.96	**8.82E-08**		
TAOK1	107.69	105.48	**3.99E-10**		
TAOK2/TAO1	97.30	96.50	**4.21E-09**		
TAOK3/JIK	106.95	106.75	**2.13E-09**		
TBK1	103.78	101.91	**2.16E-09**		
TEC	102.71	101.32	**2.48E-08**		
TESK1	97.38	96.75	**1.63E-07**		
TGFBR2	107.82	104.59	**ND**	**1.36E-07**	**LDN193189**
TIE2/TEK	93.01	91.93	**2.52E-08**		
TLK1	98.48	97.86	**2.75E-08**		
TLK2	107.14	106.31	**3.08E-09**		
TNIK	98.73	98.26	**8.09E-10**		

TNK1	104.02	103.17	**2.03E-09**		
TRKA	96.62	96.40	**1.18E-09**		
TRKB	100.87	100.41	**8.02E-11**		
TRKC	85.55	85.25	**1.92E-10**		
TSSK2	95.50	94.66	**2.43E-09**		
TSSK3/STK22C	95.60	92.34	**3.06E-09**		
TTBK1	97.21	92.86	**ND**	**1.80E-05**	**SB202190**
TTBK2	92.08	89.86	**ND**	**5.07E-06**	**SB202190**
TXK	96.63	96.42	**2.49E-08**		
TYK1/LTK	100.28	98.89	**3.00E-08**		
TYK2	94.84	94.22	**1.54E-10**		
TYRO3/SKY	96.57	93.50	**1.85E-09**		
ULK1	118.66	118.46	**1.94E-08**		
ULK2	102.24	101.75	**2.31E-09**		
ULK3	97.28	96.12	**2.61E-09**		
VRK1	90.52	89.75	**ND**	**2.15E-07**	**Ro-31-8220**
VRK2	106.18	105.57	**ND**	**2.43E-05**	**Ro-31-8220**
WEE1	103.72	100.08	**ND**	**2.33E-06**	**Wee-1 Inhibitor**
WNK1	82.94	71.49	**1.07E-05**		
WNK2	89.93	86.71	**1.25E-06**		
WNK3	69.68	68.06	**ND**	**1.44E-06**	**Wee-1 Inhibitor**
YES/YES1	101.12	100.69	**1.96E-09**		
YSK4/MAP3K19	103.94	103.38	**5.37E-09**		
ZAK/MLTK	90.02	85.16	**ND**	**8.43E-07**	**GW5074**
ZAP70	110.24	108.05	**3.22E-09**		
ZIPK/DAPK3	97.23	95.79	**2.38E-09**		

*** Empty cells indicate no inhibition or compound activity that could not be fit to an IC50 curve**

ND **Indicates compound not tested against enzyme**