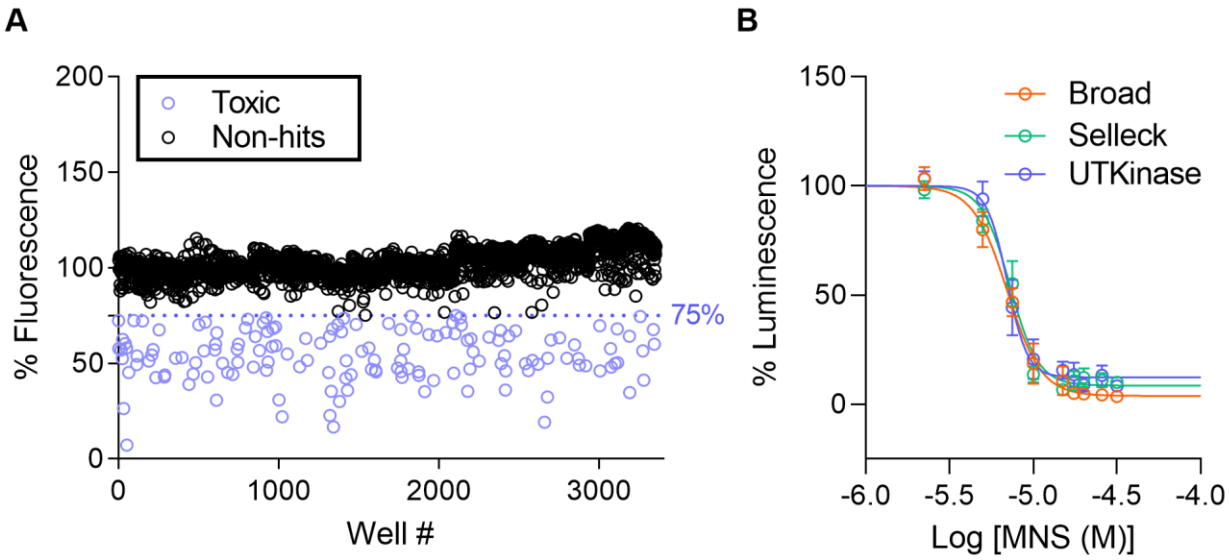
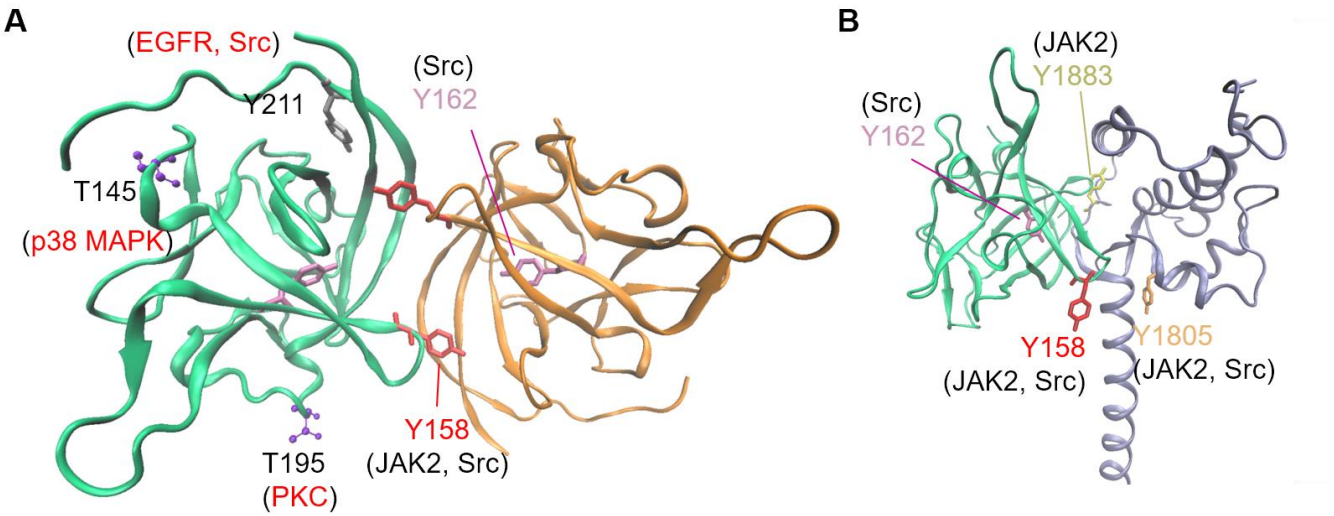
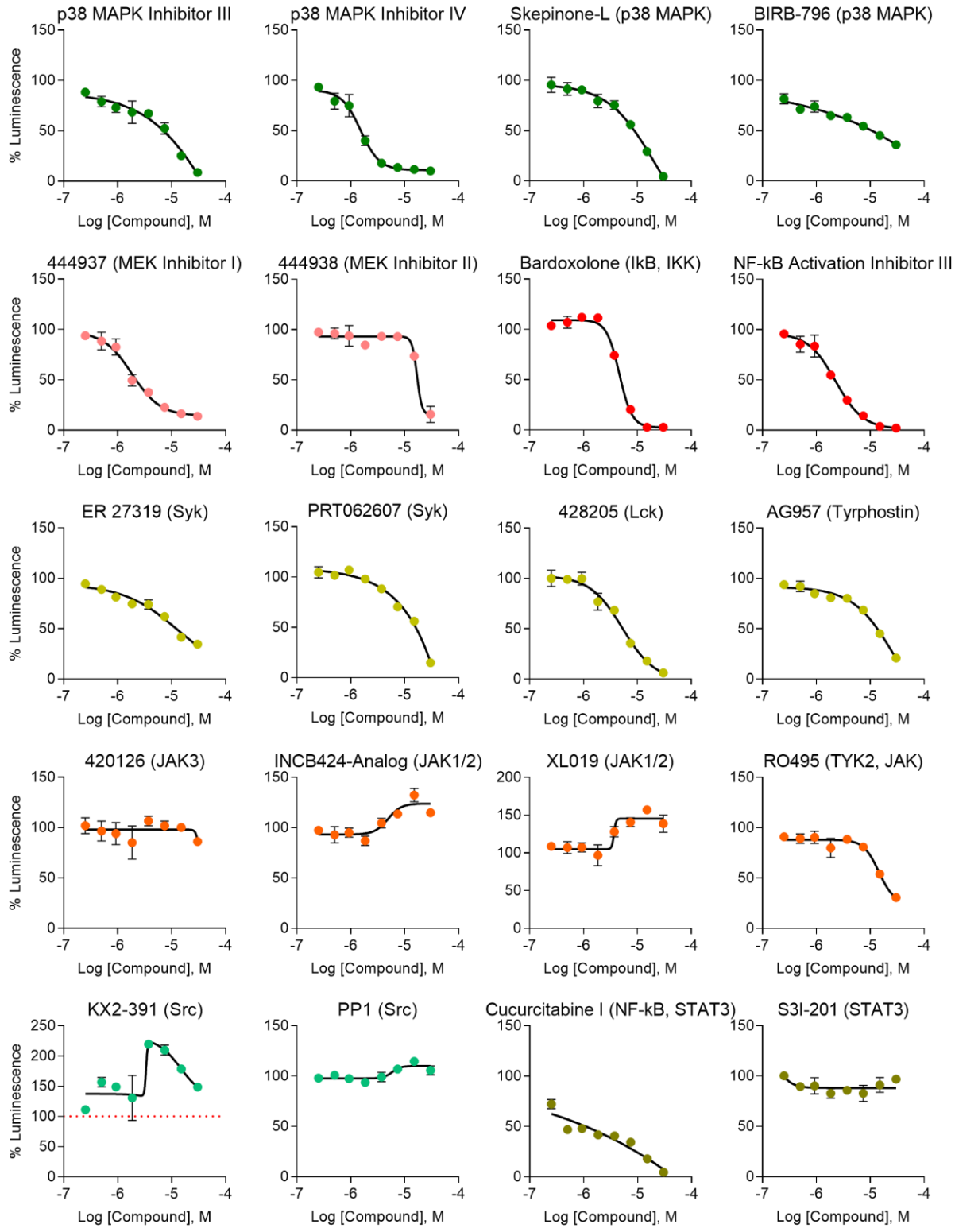
SUPPLEMENTARY MATERIAL (for online publication only)



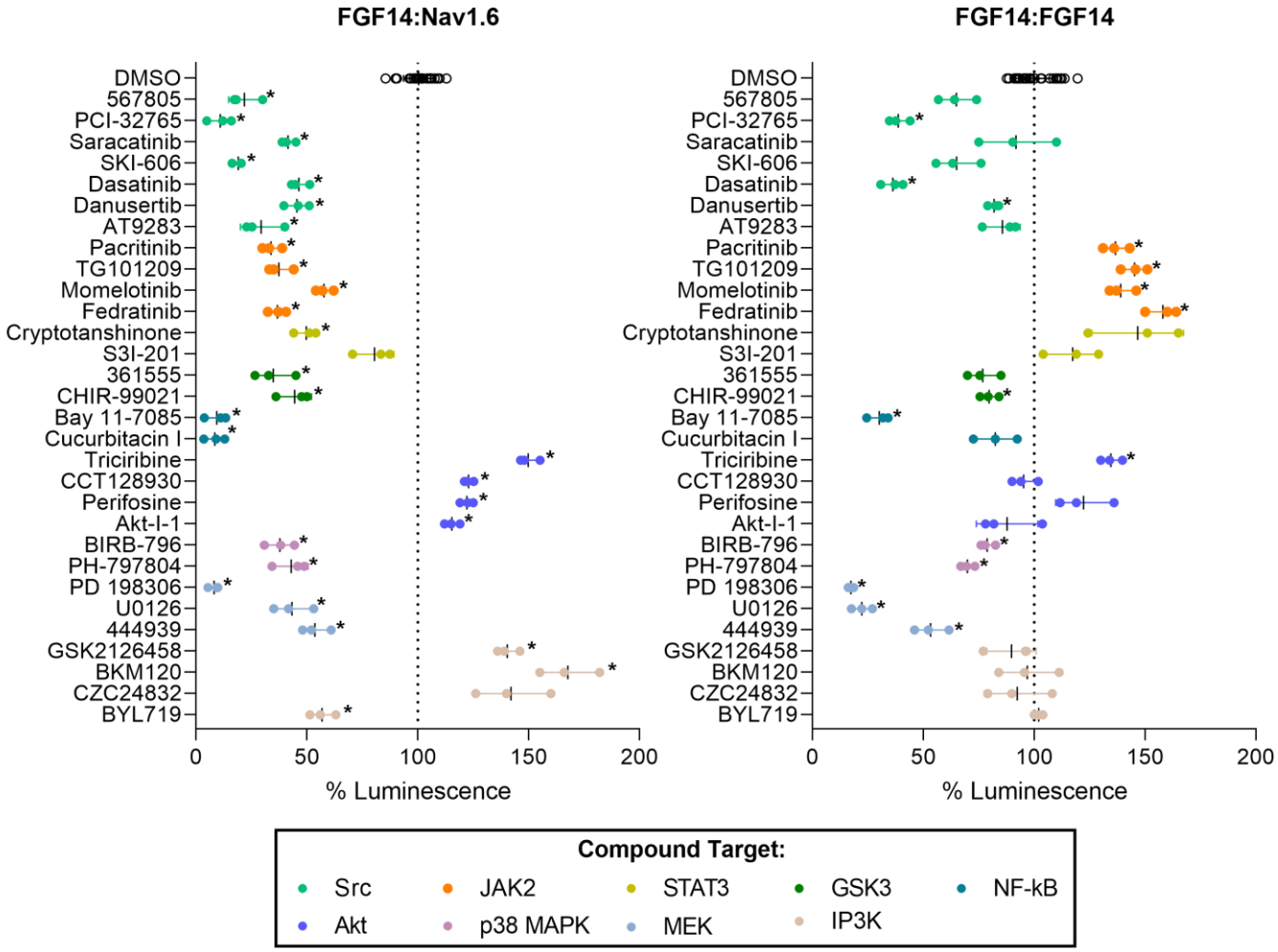
Supplementary Figure 1. HTS assay controls and toxicity counter-screen. (**A**) Cell viability assay results for the Broad, Selleck and UTKinase libraries. Cut-offs for compound toxicity were set at 75% fluorescence (relative to the mean for 0.3% DMSO controls). (**B**) Dose responses of the inhibitory control 3,4-Methylenedioxy-β-nitrostyrene (MNS) on screened plates (9 concentrations; *n* = 2 per concentration) to monitor and verify reproducibility between screened plates, ensuring that there were not changes in cell density or other cell behaviors, which often can be detected through changes in the concentration dependency[25]. For all plates of each library screened (Broad, Selleck, and UTKinase), the mean normalized luminescence ± SD is shown for each concentration with fitted non-linear regression curves.



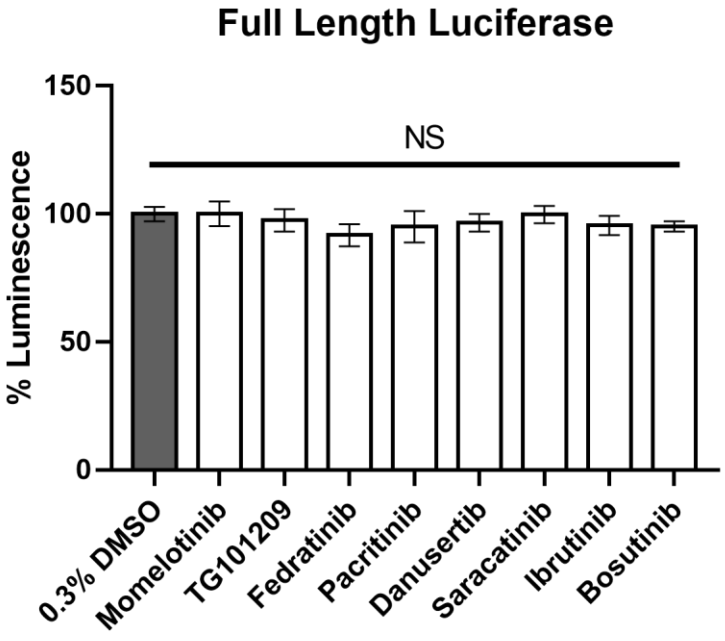
Supplementary Figure 2. Structural relationship of potential phosphorylation sites to the PPI interfaces of the FGF14:FGF14 homodimer and FGF14:Nav1.6 complex. (A) Model of the FGF14:FGF14 homodimer homology model, based on the FGF13 homodimer crystal structure (PDD: 3HBW) with monomer #1 as teal and monomer #2 as orange. Y158 on each is shown as red, while Y162 is shown as pink. Y162 is > 10Å from the PPI surface, and is unlikely to contribute to dimerization. On monomer #1 (teal), also showing other predicted phosphorylation sites that are not at the dimer interface, including T145, T195, and Y211. (**B**) Model of the FGF14:Nav1.6 C-tail complex, based on the FGF13:Nav1.5:CaM ternary complex crystal structure (PDB: 4DCK). Predicted Src and JAK2 phosphorylation sites on both FGF14 and Nav1.6 C-tail are shown. Also note that two SCN8A mutations leading to epileptic encephalopathies are found in the proximal portion of the C-terminus, including the missense mutations causing R1872W and R1871Q [56], which are 11 residues upstream of the Y1883 identified phosphomotif.



Supplementary Figure 3. Initial dose-dependency studies of selected hits for validation of HTS findings for other highly represented targets. Dose responses (8-point, *n* = 4 per concentration over two 384-well plates) were conducted against the FGF14:Nav1.6 complex using the LCA for HTS hits selected for further studies based on target clustering. Luminescence was normalized to per plate 0.3% DMSO controls, and data shown are mean normalized luminescence ± SD.



Supplementary Figure 4. Screening results for kinase inhibitors against the FGF14:Nav1.6 and FGF14:FGF14 homodimer. LCA results for screening inhibitors of top kinase targets, as selected based on proportion of screened compounds to hits (represented in Table 1), in combination with hypothesis-driven target selection using information including phospho-motif scans and homology modeling (represented in Table 2 and Suppl. Fig. 2). Transfected HEK293 cells were seeded in 384-well plates and treated with 0.3% DMSO (n = 32) or kinase inhibitors (30 μM; n = 3 per compound). Luminescence was normalized to per plate 0.3% DMSO controls. **Right**, LCA results for the FGF14:Nav1.6 complex. **Left**, LCA results for the FGF14:FGF14 homodimer. These data are represented in the form of a heat map in Figure 3B. Note that only JAK2 inhibitors demonstrated a consistent and opposing response between the FGF14:Nav1.6 complex and FGF14:FGF14 dimer; STAT3 inhibitors demonstrated a similar trend but were non-significant. Data are individual replicate values with mean normalized luminescence ± SD. Statistical significance was assessed using Brown-Forsythe and Welch ANOVA with post hoc Dunnett's T3 multiple comparisons test; *p<0.05.



Supplementary Figure 5. Validation of JAK2 inhibitors using the full-length luciferase assay. HEK293 cells were transiently transfected with the full length *photinus pyralis* luciferase and seeded in 96-well plates as previously described[20,25], and treated with 0.3% DMSO (*n* = 12) or JAK2 inhibitors (25 μM; *n* = 5 per compound) under conditions identical to those of the LCA. Lack of response against luciferase signifies that these inhibitor's effects on protein complexes using the LCA are not mediated by modulation of luciferase. Luminescence was normalized to 0.3% DMSO controls. Data are mean normalized luminescence ± SD. Significance was assessed using one-way ANOVA with post hoc Dunnett's multiple comparisons test.

Supplementary Table 1. Z'-factor and coefficient of variation for all screened plates from the Broad, Selleck, and UTKinase compound libraries. Z'-factor and coefficient of variation (CV) were calculated as described previously[25,34].

	Z'-Factor		
Library/Plate	Inhibitor	Enhancer	CV
Broad-1A	0.621	0.698	0.102
Broad-2A	0.674	0.746	0.088
Broad-1B	0.638	0.797	0.086
Broad-2B	0.671	0.808	0.096
Broad-1C	0.694	0.866	0.089
Broad-2C	0.658	0.765	0.093
SEL-1A	0.616	0.839	0.100
SEL-2A	0.738	0.803	0.065
SEL-3A	0.664	0.763	0.069
SEL-4A	0.749	0.875	0.060
SEL-1B	0.735	0.759	0.060
SEL-2B	0.716	0.858	0.072
SEL-3B	0.739	0.831	0.062
SEL-4B	0.520	0.700	0.070
SEL-1C	0.586	0.718	0.092
SEL-2C	0.557	0.675	0.106
SEL-3C	0.500	0.735	0.126
SEL-4C	0.548	0.711	0.120
UTK1A	0.633	0.731	0.070
UTK2A	0.560	0.746	0.093
UTK3A	0.681	0.759	0.064
UTK4A	0.500	0.778	0.057
UTK5A	0.622	0.740	0.087
UTK1B	0.174	0.652	0.099
UTK2B	0.625	0.736	0.063
UTK3B	0.666	0.759	0.073
UTK4B	0.614	0.792	0.092
UTK5B	0.639	0.764	0.068
UTK1C	0.836	0.863	0.050
UTK2C	0.880	0.876	0.033
UTK3C	0.910	0.823	0.023
UTK4C	0.738	0.830	0.071
UTK5C	0.702	0.924	0.085

Supplementary Table 2. Screened compounds targeting JAK2 and Src. The percent fluorescence intensity (% FI) from the CellTiter Blue (CTB) cell viability assay, as well as mean percent luminescence (% Lum), mean Z-score, and their respective standard deviations (SD) from the primary screening (LCA) is shown for each compound from *n* = 3 independent screenings in 384-well plates. Compounds with screening results near to the hit cut-offs are also shown. Statistical significance was assessed using Brown-Forsythe and Welch ANOVA with post hoc Dunnett's T3 multiple comparisons test; *p<0.05.

Library	Compound	Target (in order of selectivity)	% FI (CTB)	% Lum (LCA)	% Lum SD (LCA)	Z-Score (LCA)	Z-Score SD (LCA)	Signif?
Broad	BIO	GSK3, Pan-JAK	99.38	3.86	0.41	-10.33	0.22	Yes
Broad	Fedratinib	JAK2	101.76	37.94	2.00	-6.61	0.62	Yes
Broad	NVP-BSK805	JAK2	100.40	43.03	44.41	-5.91	4.59	Yes
Broad	momelotinib	JAK1/2	100.95	59.89	5.98	-4.30	0.60	Yes
Broad	ruxolitinib	JAK1/2	99.25	190.89	70.91	9.57	7.64	Yes
Selleck	WP1066	JAK2, STAT3/5, ERK1/2	85.91	9.34	3.37	-10.67	4.12	Yes
Selleck	Gandotinib (LY2784544)	JAK2, JAK1/3, FLT3, FGFR2	80.72	16.82	10.01	-9.45	3.18	Yes
Selleck	Pacritinib (SB1518)	JAK2, FLT3	100.37	38.31	11.31	-6.76	1.97	Yes
Selleck	NVP-BSK805 2HCl	JAK2	80.00	38.89	15.18	-6.57	1.71	Yes
Selleck	TG101209	JAK2, FLT3	85.58	36.58	12.97	-6.49	1.18	Yes
Selleck	TG101348 (SAR302503)	JAK2	93.54	41.73	13.30	-5.84	0.86	Yes
Selleck	AZ 960	JAK2	88.27	46.37	9.32	-5.64	1.38	Yes
Selleck	Tofacitinib (CP-690550) Citrate	JAK3	99.11	74.80	1.82	-2.18	0.71	No
Selleck	XL019	JAK1/2 > JAK3	106.34	139.69	18.71	5.87	2.63	Yes
UTK	420121	JAK3, JAK1, EGFR, TGM2	92.34	31.63	0.39	-9.24	2.51	Yes
UTK	WP1066	JAK2, STAT3/5, ERK1/2	92.85	11.70	6.19	-9.11	3.61	Yes
UTK	420126	JAK3 > JAK2	102.85	29.43	4.50	-8.84	1.11	Yes
UTK	ZM 39923 hydrochloride	JAK3, JAK1, EGFR, TGM2	99.52	26.02	15.32	-7.59	2.12	Yes
UTK	ZM 449829	JAK3, EGFR, JAK1 and CDK4	110.28	32.86	20.54	-7.07	2.68	Yes
UTK	ZM 449829	JAK3, EGFR, JAK1 and CDK4	106.51	32.67	20.68	-7.01	2.68	Yes
UTK	TG101209	JAK2, FLT3	94.65	41.16	12.43	-5.79	1.67	Yes
UTK	Pacritinib (SB1518)	JAK2, FLT3	95.32	43.54	3.68	-5.58	1.63	Yes
UTK	RO495	JAK (TYK2 family kinases)	102.28	48.67	4.50	-5.35	2.46	Yes
UTK	420104	JAK3, EGFR, Src, Abl, VEGFR	99.97	71.58	3.00	-3.34	1.11	No
UTK	INCB018424 (Ruxolitinib)	JAK1, JAK2	91.94	45.83	15.67	-3.18	1.03	No
UTK	CP690550 (Tofacitinib)	JAK3	101.79	73.55	3.80	-2.46	1.05	No
UTK	AG-490	JAK-2	107.10	75.98	6.42	-2.22	0.60	No
UTK	WHI-P131 (JAK3 Inhibitor 1)	JAK3	111.65	129.30	17.72	2.98	1.92	No
UTK	XL019	JAK1/2 > JAK3	97.70	154.82	26.57	5.34	1.75	Yes
UTK	Baricitinib (LY3009104)	JAK	97.63	141.36	16.32	5.46	3.75	Yes
UTK	INCB424-Analogue	JAK1,JAK2	98.90	150.25	16.53	5.95	1.91	Yes
Broad	ibrutinib	Src, BTK	97.43	35.52	5.57	-6.82	2.48	Yes
Broad	saracatinib	Src, Bcr-Abl	100.21	49.14	5.44	-5.41	1.95	Yes
Broad	dasatinib	Src, Bcr-Abl, many RTK	104.49	50.67	7.32	-5.22	0.63	Yes

Broad	KX2-391	Src, non-ATP competitive	99.74	203.46	35.83	10.90	6.59	Yes
Selleck	Rebastinib (DCC-2036)	Src, Bcr-Abl	105.65	35.74	7.18	-7.34	2.56	Yes
Selleck	Danusertib (PHA-739358)	Src, aurora	115.28	51.09	7.36	-4.41	1.61	Yes
Selleck	Ibrutinib (PCI-32765)	Src, BTK	76.72	52.96	17.02	-4.19	0.28	Yes
Selleck	Dasatinib	Src, Bcr-Abl	94.48	65.25	13.05	-3.11	0.24	Yes
Selleck	Src I1	Src family	97.19	74.13	10.11	-2.27	1.07	No
Selleck	PP1	Src family	107.74	112.33	38.43	2.32	2.96	No
Selleck	PP2	Src family	101.66	128.01	38.76	5.49	4.77	No
Selleck	KX2-391	Src, non-ATP competitive	93.04	173.26	27.55	10.85	7.81	Yes
UTK	Src Kinase Inhibitor I (567805)	Src > Lck	110.37	42.58	26.84	-8.22	3.09	Yes
UTK	Bosutinib(SKI-606)	Src-bcr-Abl	92.90	25.29	8.47	-7.89	1.96	Yes
UTK	Quercetin(Sophoretin)	Src, PI 3-K, PKC	99.45	31.79	7.32	-5.83	2.25	Yes
UTK	PCI-32765 (Ibrutinib)	Src	96.28	41.59	8.13	-5.43	1.58	Yes
UTK	NVP-BHG712	Src-Bcr-Abl, VEGFR, Raf	115.48	49.99	5.26	-5.37	2.63	Yes
UTK	Dasatinib	Src-bcr-Abl	94.44	46.37	2.72	-5.35	1.93	Yes
UTK	AT9283	Src-bcr-Abl, Aurora, FLT-3, JAK	97.65	32.24	6.70	-5.04	1.91	Yes
UTK	AZD0530(Saracatinib)	Src-bcr-Abl	93.47	52.51	10.43	-3.46	0.00	Yes
UTK	1-Naphthyl PP1	Src family	118.05	71.92	21.79	-2.46	1.59	No
UTK	PHA-739358(Danusertib)	Src-bcr-Abl, Aurora, FGFR	100.20	65.60	9.33	-2.18	0.64	Yes
UTK	KX2-391	Src, non-ATP competitive	103.11	221.92	25.01	15.20	5.79	Yes

Condition	Peak density (pA/pF)	Activation (mV)	K_{act} (mV)	Steady-state Inactivation (mV)	K_{inact} (mV)	Tau (τ) (ms)
GFP (DMSO)	-59.4 ± 6.0 (15)	-26.03 ± 1.1 (14)	3.5 ± 0.2 (12)	-62.6 ± 0.9 (12)	7.1 ± 0.4 (12)	1.2 ± 0.05 (10)
GFP (Fedratinib)	-51.5 ± 3.7 (13)	-25.7 ± 1.7 (13)	3.9 ± 0.2 (12)	-61.7 ± 1.0 (15)	6.0 ± 0.4 (11)	1.3 ± 0.07 (13)
FGF14-GFP (DMSO)	-24.9 ± 3.0 (16)[a]	-22.4 ± 1.1 (12)[c]	4.7 ± 0.2 (10)[f]	-59.8 ± 0.5 (12)[h]	6.3 ± 0.4 (12)	1.6 ± 0.1 (14)[i]
FGF14-GFP (Fedratinib)	-81.3 ± 11.3 (13)[b,j]	-30.5 ± 1.9 (13)[d,e]	2.8 ± 0.3 (11)[g]	-60.8 ± 1.3 (16)[n]	5.9 ± 0.6 (14)	1.4 ± 0.06 (12)
$FGF14^{Y158A}$-GFP (DMSO)	-23.97 ± 4.8 (12)	-22.06 ± 1.4 (12)	4.2 ± 0.3 (12)	-59.3 ± 2.0 (12)	8.5 ± 1.1 (12)	1.5 ± 0.12 (12)[k]
$FGF14^{Y158A}$-GFP (Fedratinib)	-26.65 ± 3.5 (13)	-22.69 ± 1.2 (13)	4.3 ± 0.4 (13)	-61.7 ± 2.2 (12)	8.4 ± 0.6 (12)	1.8 ± 0.11 (12)[l,m]

Supplementary Table 3. Effect of Fedratinib on Nav1.6-mediated currents in the presence of FGF14 or the $FGF14^{Y158A}$ mutant. Data are mean ± SEM (*n*).

[a] $P < 0.0001$, unpaired *t* tests compared to Nav1.6-GFP (DMSO)

[b] $P < 0.0001$, unpaired *t* tests compared to FGF14-GFP (DMSO).

[c] $P < 0.0358$, unpaired *t* tests compared to Nav1.6-GFP (DMSO).

[d] $P < 0.0019$, unpaired *t* tests compared to Nav1.6-GFP (DMSO).

[e] $P < 0.0495$, unpaired *t* tests compared to FGF14-GFP (DMSO).

[f] $P < 0.0017$, unpaired *t* tests compared to Nav1.6-GFP (DMSO).

[g] $P < 0.0001$, unpaired *t* tests compared to Nav1.6-GFP (DMSO).

[h] $P < 0.0144$, unpaired *t* tests compared to Nav1.6-GFP (DMSO).

[i] $P < 0.0052$, unpaired *t* tests compared to Nav1.6-GFP (DMSO).

[j] $P = 0.0883$, unpaired *t* tests compared to Nav1.6-GFP (DMSO).

[k] $P < 0.0268$, unpaired *t* tests compared to Nav1.6-GFP (DMSO).

[l] $P < 0.0003$, unpaired *t* tests compared to Nav1.6-GFP (DMSO).

[m] $P < 0.0147$, unpaired *t* tests compared to FGF14-GFP (Fedratinib).

[n] $P = 0.3072$, unpaired *t* tests compared to Nav1.6-GFP (DMSO).

Supplementary Table 3. Effect of Fedratinib on Passive and Active Electrical Properties of Hippocampal CA1 pyramidal neurons. Data are mean ± SEM (*n*).

Condition	Max Num. of AP	RMP (mV)	I_{thr} (pA)	V_{thr} (mV)	Max rise (mV/ms)	Max decay (mV/ms)	Cm (pF)	Rin (mΩ)	Tau (ms)
Fgf14$^{+/+}$ (DMSO)	16.3 ± 2.5 (4)	-69.8 ± 2.8 (4)	75.0 ± 16.6 (4)	-43.4 ± 2.1 (4)	315.6 ± 19.7 (4)	-85.1 ± 13.9 (4)	117.6 ± 16.8 (4)	169.4 ± 9.5 (4)	20.1 ± 3.3 (4)
Fgf14$^{+/+}$ (Fedratinib)	11.3 ± 1.4 (6)[a]	-68.1 ± 2.0 (6)	96.7 ± 12.8 (6)	-42.7 ± 3.0 (6)	291.0 ± 19.3 (6)	-64.7 ± 5.1 (6)	160.8 ± 31.2 (6)	121.5 ± 19.0 (6)	19.1 ± 4.0 (6)
Fgf14$^{-/-}$ (DMSO)	13.0 ± 3.9 (4)	-66.5 ± 2.1 (4)	72.5 ± 8.5 (4)	-40.3 ± 7.2 (4)	272.2 ± 56.1 (4)	-69.3 ±7.2 (4)	-79.5 ± 12.2 (4)	161.0 ± 19.1 (4)	12.5 ± 2.2 (4)
Fgf14$^{-/-}$ (Fedratinib)	11.8 ± 2.7 (5)	-63.2 ± 2.8 (5)	50.0 ± 11.4 (5)	-42.8 ± 1.3 (5)	264.4 ± 14.2 (5)	-105.5 ± 14.7 (5)	182.7 ± 47.1 (5)	181.0 ± 12.3 (5)	32.3 ± 7.5 (5)

[a] $p < 0.05$, unpaired t-test compared to FGF14$^{+/+}$ (DMSO)