SUPPLEMENTARY INFORMATION

Identification of novel GSK-3β hits using competitive biophysical assays

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Supplementary figures
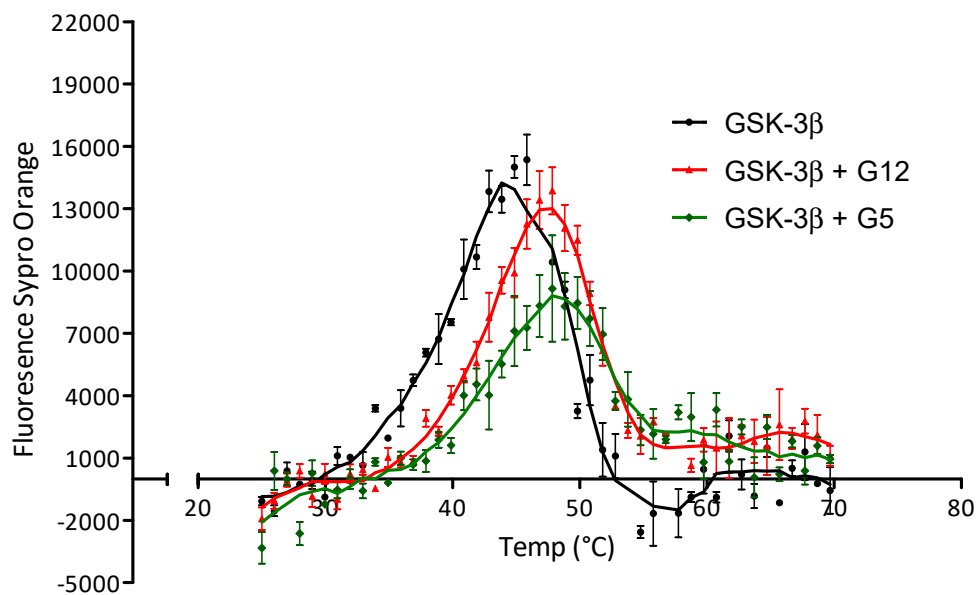
Supplementary Figure S1

Figure S1. Thermal Shift Assay of GSK-3β alone (black trace), in the presence of G5 (green trace) and G12 (red trace) compounds. G5 and G12 stabilize GSK-3β protein and lead to an increase in melting temperature (T_m) of 3.2 °C and 2.5 °C, respectively. Error bars denote Standard Deviation (SD).

Supplementary Figure S2

Bar chart of Activity (% control) for a panel of kinases:

Abl(h), AMPKa(h), ASK1(h), Aurora-A(h), CaMKIα(h), CDK1/Cyclin B(h), CDK2/Cyclin A(h), CDK6/Cyclin D3(h), CDK7/Cyclin H/MAT1(h), CDK9/Cyclin T1(h), CHK1(h), CK2α2(h), c-RAF(h), DRAK1(h), eEF-2K(h), EGFR(h), EphA5(h), EphB4(h), Fyn(h), GSK3β(h), IGF-1R(h), IKKα(h), IRAK4(h), JAK2(h), KDR(h), LOK(h), Lyn(h), MAPKAP-K2(h), MEK1(h), MLK1(h), MnK1(h), MSK2(h), MST1(h), mTOR(h), NEK2(h), P70S6K(h), PAK2(h), PDGFRβ(h), Pim-1(h), PKA(h), PKBα(h), PKCα(h), PKCθ(h), PKG1α(h), PRAK(h), ROCK-I(h), Rsk1(h), SAPK2a(h), SRPK1(h), TAK1(h), PI3Kβ(h), PI3Kδ(h), PI3Kα(h)
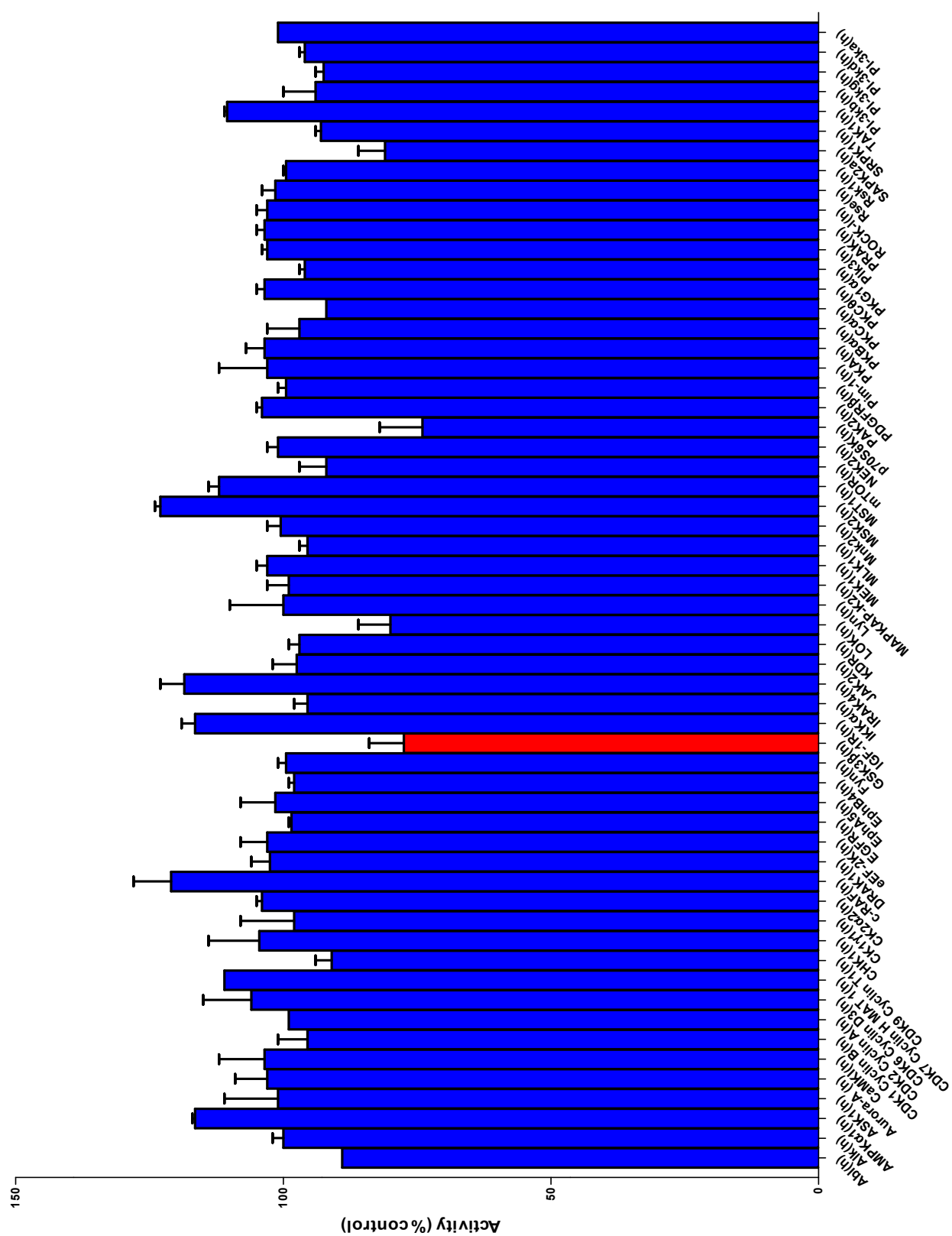
Figure S2. Bar Graphs showing G5 inhibitory effect on different kinases of the commercial panel (Eurofins service); all the experiments were performed using 20 μM of compound and ATP concentrations ± 15 μM of each K_m. Error bars denote SD.
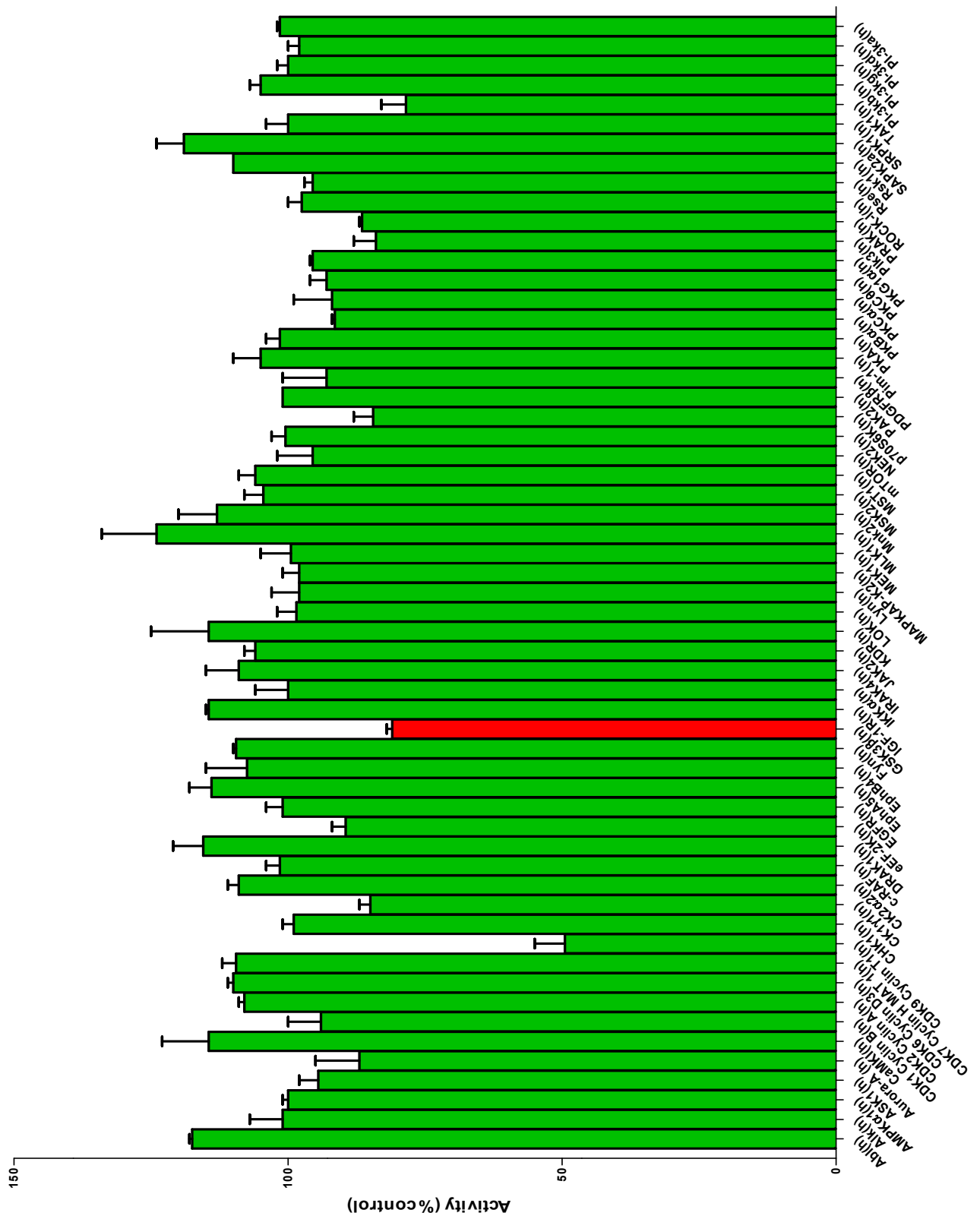
Supplementary Figure S3

Figure S3. Bar Graphs showing G12 inhibitory effect on different kinases of the commercial panel (Eurofins service); all the experiments were performed using 20 μM of compound and ATP concentrations ± 15 μM of each K_m. Error bars denote SD.

Supplementary Table

	G5	G12
Data reduction		
Resolution (Å)	2.5-48.8 (2.5-2.59)	2.2-89 (2.2-2.26)
Rmerge	0.388 (3.619)	0.319 (3.86)
Mean(I/sd(I))	7.9(0.8)	6.9 (0.6)
Completeness	100(100)	95.6 (87.8)
Unique Reflections	44494 (4582)	61582(4107)
Multiplicity	13.4 (12.9)	12.2(11.4)
$CC_{1/2}$	0.992 (0.39)	0.993(0.37)
Refinement		
Resolution (Å)	2.5-48.8	2.2-89
Rwork/Rfree	0.225/0.268	0.24/0.27
RMS bonds (Å)	0.0086	0.0075
RMS angles (º)	1.808	1.573

Table S1. Table reporting refinement statistics and values used for solving structure of GSK-3β in complex with G5 and G12 compounds