

**Supplementary Table S1.** Calorimetric temperature shifts of SPHINX on 64 different kinases.

| Target ID | T <sub>m</sub> Shift (°C) | Protein Family |
|-----------|---------------------------|----------------|
| SRPK1     | 9.82                      | CMGC           |
| SRPK3     | 7.93                      | CMGC           |
| SRPK2     | 3.5                       | CMGC           |
| PIM3      | 1.31                      | CAMK           |
| CLK1      | 1.05                      | CMGC           |
| STK33     | 0.94                      | CAMK           |
| CLK2      | 0.84                      | CMGC           |
| STK16     | 0.84                      | Other          |
| PKMYT1    | 0.79                      | Other          |
| SNF1LK2   | 0.75                      | CAMK           |
| CDC42BPA  | 0.71                      | AGC            |
| MYLK2     | 0.55                      | CAMK           |
| NEK1      | 0.55                      | Other          |
| GUCY2D    | 0.54                      | Other          |
| AKT3      | 0.43                      | AGC            |
| VRK2      | 0.43                      | CK1            |
| DCAMKL1   | 0.38                      | CAMK           |
| SLK       | 0.32                      | STE            |
| CSNK2A2   | 0.3                       | Other          |
| MAPK8     | 0.29                      | CMGC           |
| CDC42BPB  | 0.27                      | AGC            |
| Aurora C  | 0.21                      | Other          |
| ZAK       | 0.2                       | TKL            |
| CHEK2     | 0.17                      | CAMK           |
| LOC340156 | 0.17                      | CAMK           |
| PLK4      | 0.14                      | Other          |
| PAK4      | 0.13                      | STE            |
| RPS6KA1   | 0.12                      | AGC            |
| STK38L    | 0.12                      | AGC            |
| CSNK1G1   | 0.11                      | CK1            |
| TNIK      | 0.05                      | STE            |
| CSNK1G3   | 0.04                      | CK1            |
| PIM1      | 0.03                      | CAMK           |
| PCTK1     | 0.02                      | CMGC           |
| STK17B    | -0.06                     | CAMK           |
| MAPK13    | -0.11                     | CMGC           |
| DYRK2     | -0.13                     | CMGC           |
| MAPK9     | -0.13                     | CMGC           |
| CAMKK2    | -0.15                     | Other          |
| RPS6KA6   | -0.2                      | AGC            |
| CSNK2A1   | -0.24                     | Other          |
| GRK1      | -0.26                     | AGC            |
| OSR1      | -0.29                     | STE            |
| STK10     | -0.29                     | STE            |
| ACVR1     | -0.3                      | TKL            |
| CDK8      | -0.35                     | CMGC           |
| FES       | -0.36                     | TK             |
| STK17A    | -0.36                     | CAMK           |
| ADRBK2    | -0.37                     | AGC            |
| CDKL1     | -0.37                     | CMGC           |
| GSG2      | -0.37                     | Other          |
| CDK2      | -0.38                     | CMGC           |
| BMX       | -0.43                     | Kinase-TK      |
| STK24     | -0.45                     | STE            |
| GPRK5     | -0.48                     | AGC            |
| PRKD2     | -0.48                     | CAMK           |
| MAP2K2    | -0.49                     | STE            |
| PRKCL1    | -0.5                      | AGC            |
| YANK1     | -0.54                     | AGC            |
| MAPK3     | -0.55                     | CMGC           |
| MST4      | -0.57                     | STE            |
| CLK3      | -0.61                     | CMGC           |

|        |       |      |
|--------|-------|------|
| ACVR2  | -0.71 | TKL  |
| DYRK1A | -0.74 | CMGC |