

Supporting Online Material

Materials and Methods

Preparing dsRNA

Primer pairs with each primer encoding a T7 promoter sequence and gene-specific sequence were used to amplify PCR sequence product from a single exon using genomic DNA template or from a cDNA to generate template for *in vitro* dsRNA synthesis using T7 RNA polymerase (Ambion). See table S1 for list of primers used.

Overexpression of proteins

Proteins of interest were expressed using an Actin5C promoter to drive transcription of the corresponding cDNA inserted into the pACTIN-SV vector. Cells were transfected using Effectine (Qiagen) transfection reagent.

RNAi assay for Hh signaling cl-8 cells

The RNAi assay involves co-transfection of dsRNA (0.03 μ g for each gene targeted) and reporter plasmids (*ptc*-luciferase, to measure Hh response and *copia-Renilla*, to normalize for transfection efficiency) with Effectine followed by incubation for ~30 hrs to allow for protein turnover and degradation of targeted mRNA. Transfected cells in 24-well plates were then split into control or Hh-N-containing media and incubated for an additional 24 hrs. Cells were then lysed and luciferase activities in lysates measured using Dual-Luciferase Assay Kits (Promega). Fold induction upon stimulation with Hh following transfection of pooled dsRNAs was derived by dividing normalized reporter activity (firefly luciferase (L) to *Renilla* luciferase (R) ratio, L/R) in the presence of Hh by L/R in the absence of Hh (basal reporter activity). In most experiments, dsRNA targeting yellow fluorescent protein (YFP) was used as a control.

Generating and screening the dsRNA libraries

To prepare the library of predicted *Drosophila* kinases and phosphatases, primer pairs (Incyte) for each dsRNA to be synthesized were used to generate PCR products from genomic DNA with an average size of 419 bp. Oligonucleotides containing the T7 promoter were ligated to these products, followed by two rounds of amplification, and *in vitro* RNA synthesis. The dsRNAs in the phosphatase-kinase library represent all 351 kinases and phosphatases except for four (CT4320, CT4237, CT5085, and CT3650), whose annotations were initially made in error or were not represented in the Incyte primer library (see fig. S1). The DGCr1 collection contains 5,828 non-overlapping cDNAs, representing ~43% of predicted genes in the *Drosophila* genome (S1). The bulk of the DGCr1 library was generated using primers corresponding to sequences within the cloning vector, thus obviating the requirement for gene-

specific primers. However, dsRNA synthesis by this approach failed for 700 of the cDNAs within DGCr1, and an addendum to the DGCr1 library was synthesized with gene-specific primers and genomic DNA templates by the method used to produce the kinase-phosphatase library. In the DGCr1 screen, screening was performed in blocks that tested an average of 480 dsRNAs with 1,943 initial pools of dsRNAs representing 5,128 genes plus an additional 237 pools from an addendum library representing 682 recovered dsRNAs for a total of 5,810 out of 5,828 genes in DGCr1 (~99.7%).

Pools of dsRNA responsible for a decrease exceeding 1.75x standard deviations (STDEVs) below the average fold induction were tested again using the pooled dsRNAs as well as individual dsRNAs. Average basal reporter activities were also derived from each triplicate test of pooled dsRNAs, and those pools that resulted in an increase at least 4x STDEVs above the average basal reporter activity were further examined using individual dsRNAs from the pool. The average fold inductions and basal reporter activities from each of these screens were normalized to the lowest average values for each measurement from a single screen. dsRNA groups that are associated with a fold induction in excess of 1.5x STDEVs below the average or a basal reporter activity greater than 3x STDEVs above the average were further tested as described before.

Generating monoclonal antibodies against Dlp

cDNA corresponding to amino acids V⁵²³ to Q⁷⁰² of Dlp was ligated to DNA corresponding to an exogenous signal sequence on the 5' end and the human IgG Fc domain on the 3' end. The resulting cDNA was used to generate a secreted, heparan sulfate-modified fusion protein in Cos1 cells that was purified using Protein A sepharose from conditioned media lacking fetal calf serum. This protein was injected into mice and the spleen from a single mouse that demonstrated serum reactivity to the antigen was isolated and used to generate hybridomas at the Sloan-Kettering Hybridoma Core Facility. Hybridoma clones were first tested for ELISA reactivity and then for immunoprecipitation and Western blotting capabilities. The mAb from the clone 13G8 was used in all studies presented here.

RNAi assay for Wg signaling

The Wg assays were performed in Kc cells essentially as described for the Hh assay in cl-8 cells with the exception that the Super TopFlash reporter was used and conditioned media contains Wg protein secreted from a S2-Wg stable cell-line. The Super TopFlash plasmid incorporates a Wg-responsive promoter region with eight tandemly arrayed TCF consensus binding sequences that controls transcription of firefly luciferase, and was kindly provided by A. Kaykas and R. Moon.

Immunostaining in cl-8 cells

Cl-8 cells transfected with cDNAs were split the following day onto glass coverslips and then fixed in 3% paraformaldehyde the next day. Cells were then permeabilized with PBS/0.1% triton or simply incubated in PBS and then stained with either anti-Dlp mAb13G8 or anti-GFP polyclonal antibodies

followed by anti-mouse FITC or anti-rabbit Texas Red secondary antibodies, respectively.

Enzymatic treatments of Dlp

For enzymatic treatments of Dlp, immunoprecipitated material on Protein G sepharose were washed three times into PBS and then incubated at 32°C for 4 hours with 10U/ml of heparinase III or 5U/ml of chondroitinase ABC in the presence of protease inhibitors.

Injection of dsRNA into embryos

Injection of dsRNAs were performed essentially as described (S2). Anti-Wg mAb 4D4 was acquired from the Developmental Studies Hybridoma Bank (deposited by S.M.Cohen).

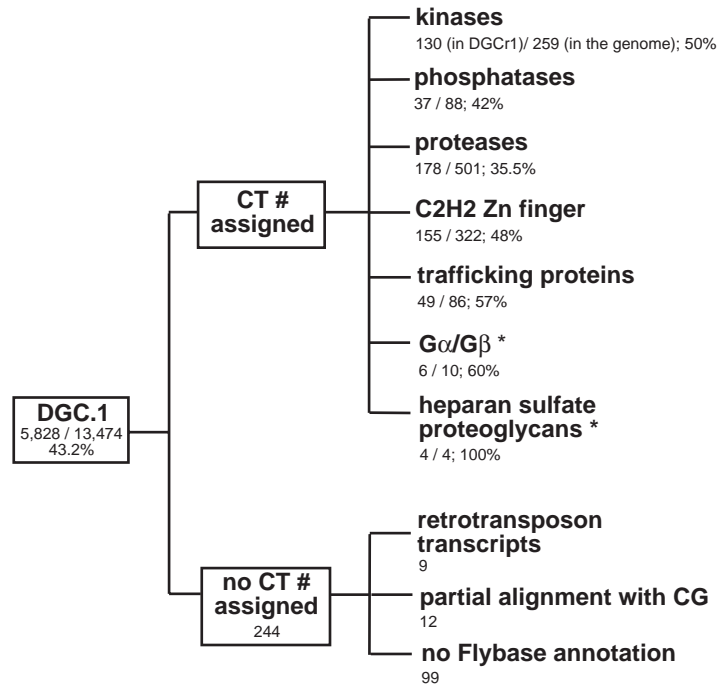
Fig. S1, Lum et al.

A.

| | Family | Members in family | Number of dsRNAs in library |
|---------------------|------------|-------------------|-----------------------------|
| Kinases | AGC | 30 | 29 |
| | CAMK | 25 | 25 |
| | CKI | 8 | 8 |
| | CMGC | 24 | 23 |
| | STE | 21 | 21 |
| | PTK | 32 | 31 |
| | OPK | 56 | 56 |
| | Atypical | 3 | 3 |
| | PIK | 13 | 13 |
| | DAG | 8 | 7 |
| | Choline K | 2 | 2 |
| | CGYC | 11 | 10 |
| | Unknown | 18 | 18 |
| | AC* | 12 | 12 |
| Total | 263 | 259 | |
| Phosphatases | STP | 28 | 28 |
| | RPTP/CPTP | 20 | 20 |
| | LMW-PTP | | |
| | DSP | 18 | 18 |
| | LMW-PTP | 2 | 2 |
| | IPP | 20 | 20 |
| Total | 88 | 88 | |

Annotation based on Morrison et. al, *J Cell Biol* 150, F57-62. (2000).

B.



5,828 = (published 5,849 - 21 duplicate clones) in DGCr1
13,474 = number of predicted genes in the genome

Table S1, Lum et al.

| Gene | Ref. Clone/ Sequence | Sense | Antisense |
|----------------------|---------------------------------|---------------|------------------|
| ptc | NM_078937 | 3921-3941 | 4628-4648 |
| smo | U87613 | 200-222 | 841-851 |
| cos2 | AF019250 | 3284-3304 | 3716-3736 |
| fu | X80468 | 904-924 | 1554-1576 |
| sufu | NM_080502 | 1064-1084 | 1679-1699 |
| ci | NM_079878 | 3416-3436 | 4115-4135 |
| pka-c1 | M18655 | 905-925 | 2261-2281 |
| slimb | AF032878 | 233-253 | 797-817 |
| dally | NM_079259 | 1776-1796 | 2299-2319 |
| dlp (UTR) | AY052017 | one-18 | 430-452 |
| dlp | AY052017 | 2035-2055 | 2539-2559 |
| sdc | U03282 | 348-368 | 870-890 |
| pcan | AJ271462 | 151-163 | 689-715 |
| frz 1 | X54648 | 1039-1059 | 1639-1659 |
| frz 2 | U65589 | 1-20 | 628-647 |
| arm | X54468 | 1810-1830 | 2392-2412 |
| pan | Y09125 | 2085-2105 | 2625-2645 |
| sgg | CT8875 | Incyte primer | Incyte primer |
| ck1e (#5) | AF055583 | 304-321 | 816-833 |
| ck1e (#6) | AF055583 | 834-851 | 1369-1386 |
| ck1 α (#3) | U55848 | 1244-1261 | 1667-1683 |
| ck1 α (3'UTR) | U55848 | 1459-1475 | 1667-1683 |
| ck1 α (5'UTR) | AI515490 | 1-18 | 403-420 |
| ck1 α (#4) | U55848 | 444-461 | 1172-1189 |
| yfp | pEYFP-Nuc | 613-630 | 1092-1109 |
| araucan | X95179 | 1382-1404 | 1890-1910 |
| caupolican | X95178 | 1673-1696 | 2297-2316 |
| combgap | AF276664 | 2180-2200 | 2702-2721 |
| ALPHA COP | AJ011114 | 3217-3241 | 3701-3724 |
| BETA COP | AF145656 | 1772-1791 | 2278-2298 |
| BETA' COP | AJ006523 | 1781-1805 | 2301-2324 |
| GAMMA COP | AF191563 | 210-232 | 746-769 |
| DELTA COP | NM_143755 | 1500-1519 | 1947-1968 |
| ZETA COP | NM_140653 | 72-90 | 630-649 |
| CG9211 | GH03927 | 1539-1560 | 2061-085 |

Table S2, Lum et al.

| dsRNA Pool | Family Name/ Transcript | CT# | dsRNA Pool | Family Name/ Transcript | CT# | dsRNA Pool | Family Name/ Transcript | CT# | dsRNA Pool | Family Name/ Transcript | CT# |
|------------|--|-------|------------|--------------------------------------|-------|------------|---------------------------------------|-------|------------|----------------------------------|-------|
| | AGC | | | | | | | | | | |
| 1 | 1 GEK | 13314 | 35 | 103 ERKA | 34260 | | 204 CKIIB | 35161 | | STP: PPP | |
| | 2 PK1 | 2222 | | 104 ERKA | 39192 | | 205 CKIIB | 41866 | | 299 PP1-like (e-113) | 25390 |
| | 3 PK1 | 42509 | | 105 DOA/CLK2 | 26257 | 69 | 206 CKIIB | 41870 | | 300 MTS/PP2A | 21977 |
| | 4 PK1 | 42507 | 36 | 106 DOA/CLK2 | 4592 | | 207 HIPK | 37856 | | 301 PP4-like (1e-97) | 36560 |
| 2 | 5 PK1 | 42511 | | 107 ERK7 (4e-96) | 7705 | | 208 CKIIX | 38735 | 101 | 302 RDGC/PP5 | 20426 |
| | 6 PK1 | 42513 | | 108 SGG/GSK3β | 8875 | 70 | 209 RAF | 9736 | | 303 PP5 (0.0) | 24679 |
| | 7 PK1 | 1275 | | 109 NEMO/NLK | 23293 | | 210 LIM-Domain Kinase (e-149) | 5560 | | 304 PP1 87B | 17842 |
| 3 | 8 AKT | 13304 | 37 | 110 CDK2/CDC2c | 29424 | | 211 EIF2 | 4285 | 102 | 305 PP1 96A | 20458 |
| | 9 AKT | 38917 | | STE | | 71 | 212 EIF2-like (4e-83) | 6726 | | 306 CAN A1/PP2B | 3539 |
| | 10 PKA CI | 14278 | | 111 MEK3/MKK3 | 14280 | | 213 MLK2 (e-129) | 7573 | | 307 PPV | 11529 |
| 4 | 11 PKG-like (PKG e-147) | 15549 | | 112 SLK (e-110) | 14372 | | 214 KSR | 7920 | 103 | 308 FLW/PP1B | 6778 |
| | 12 PKA C3 | 19108 | | 113 STE20 | 14490 | 72 | 215 BABO/TGFβ Type 1 receptor | 8241 | | 309 PP1 13C | 26196 |
| | 13 MAST 205 (e-140) | 20159 | | 114 MEKKS/ASK1 | 15203 | | 216 DIK2 | 8889 | | 310 PP2B 14D | 27780 |
| 5 | 14 PKC 53E | 20233 | | 115 STK3 (e-120) | 16551 | | 217 AURORA | 10220 | 104 | 311 PP4 | 3537 |
| | 15 PKC 53B | 20486 | | 116 MEK4 (1e-94) | 20838 | 73 | 218 SAX/Activin receptor | 5838 | | 312 PP4 | 3517 |
| | 16 PKC 53B | 42082 | | 117 CDC 7-related (4e-74) | 18162 | | 219 DLK/ZPK (e-138) | 25342 | | 313 PPY 55A | 30615 |
| 6 | 17 MSK | 19712 | | 118 CDC 7-related (e-40) | 25072 | | 220 SPRK2 (6e-51) | 24957 | 105 | 314 PPN 58A | 10905 |
| | 18 PKC _i (PKC _i 0.0) | 22025 | 40 | 119 CDC 7-related (e-40) | 25076 | 74 | 221 VRK (Vaccinia-related kin. 8e-22) | 25500 | | 315 PP1-like (e-113) | 10817 |
| | 19 ROCK1 | 27613 | | 120 GLK/KHS1 (GLK1 e-134) | 21217 | | 222 BUB1 | 5170 | | 316 PP2B | 27722 |
| 7 | 20 FOR/PKG | 42452 | | 121 STE20-like/SPAK | 23267 | | 223 PUNT/Activin receptor | 23870 | 106 | RPTP/CPTP | |
| | 21 PKC _i (PKC _i 0.0) | 28827 | 41 | 122 SEK1/MKK4 | 27508 | | 224 TKV/TGFβ receptor | 33585 | | 317 R-PTPX/IA2 (e-127) | 14210 |
| | 22 Fused | 20401 | | 123 SEK1/MKK4 | 38386 | 75 | 225 Sim. to PIM1 (KIAA0135 7e-81) | 10404 | | 318 | 14250 |
| 8 | 23 MNK/LK6 | 21460 | 42 | 124 PAK2 | 28905 | | 226 VRK (Vaccinia-related kin. e-60) | 19924 | | 319 R-PTP 4E | 21187 |
| | 24 MNK/LK6 | 21464 | | 125 PAK2 | 40904 | 76 | 227 PKU/TLK1 (e-159) | 9593 | 107 | 320 R-PTP _x (6e-94) | 22157 |
| | 25 BIN4 | 21577 | | 126 NEK1 (6e-59) | 30659 | | 228 PKU/TLK1 (e-159) | 9660 | | 321 LAR | 29268 |
| 9 | 26 NDR | 25011 | | 127 MST2 (e-140) | 31338 | | 229 MPSK/PSK | 2278 | | 322 R-PTP 69D | 30751 |
| | 27 CITRON (e-119) | 38848 | 43 | 128 TAO1 (e-146) | 33830 | 77 | 230 Similar to IRE (7e-44) | 23435 | 108 | 323 R-PTP 10D | 4920 |
| | 28 CITRON (e-119) | 29519 | | 129 PAK3 (e-101) | 34719 | | 231 PKN (e-44) | 15864 | | 324 R-PTP 99A | 6383 |
| 10 | 29 SKR | 34367 | | 130 NIK (e-122) | 37669 | | Atypical | | | 325 R-PTP 61F | 8979 |
| | 30 PKA C2 | 4596 | 44 | 131 NEK2 (1e-77) | 38234 | | 232 A6 (9e-99) | 10611 | 109 | 326 Novel (was CT18044) | 41322 |
| | 31 PKA-like (PKA C2 e-121) | 4640 | | 132 SOR | 9686 | 78 | 233 BCR (e-115) | 40020 | | 327 CSW | 13063 |
| 11 | 32 WTS/LATS | 4806 | | 133 HEP/MKK7 | 6871 | | 234 PDK | 3054 | | 328 MEG1 (e-144) | 2090 |
| | 33 PKC 98F | 4834 | 45 | 134 NINA C | 42491 | | PIK | | 110 | 329 PTPD1 (6e-54) | 26880 |
| | 34 SKK | 29454 | | 135 NINA C | 16120 | | 235 PI3K68D | 18836 | | 330 PTP-ER | 27816 |
| 12 | 35 PRK2 (e-161) | 6637 | | PTK | | 79 | 236 PI3K68D | 41611 | | 331 MEG2 (8e-85) | 7928 |
| | 36 PRK2 (e-161) | 6660 | | 136 RTK sim. to VEGFR/PDGFRα (1e-53) | 11013 | | 237 PI3K59F | 17070 | 111 | 332 Fragment (PTP TD14 e-101) | 26513 |
| | 37 GPRK2 (was GPRK1) | 40234 | 46 | 137 RET | 1245 | | 238 PI3K92E | 13632 | | 333 Fragment | 35613 |
| 13 | 38 PKG 21D | 10911 | | 138 SRC 64B | 1253 | 80 | 239 ATM (e-148) | 20331 | | 334 Fragment | 33841 |
| | 39 PKCδ (partial transcript) | 8675 | | 139 SRC 64B | 40878 | | 240 FRAP/TOR (FRAP 0.0) | 16317 | | 335 Fragment (PTPN6 5e-27) | 34505 |
| | CAMK | | 47 | 140 DNT-like (DNT 1e-57) | 13035 | | 241 FRAP-related (2e-51) | 14742 | 112 | DSP | |
| 14 | 40 SNF1A | 10258 | | 141 PR2/ACK | 13197 | | 242 MEI41/FRP1 | 24745 | | 336 CDC14 (e-124) | 22031 |
| | 41 MAPKAP | 10332 | 48 | 142 NRK | 13296 | | 243 PI4K (0.0) | 24817 | | 337 PTEN | 17882 |
| | 42 Sim. to C. elegans KIN1 & hC-TAK1 | 14856 | | 143 ABL | 13380 | | 244 PI4K (2e-80) | 13944 | 113 | 338 PRL | 16026 |
| | 43 SNF1-related (e-80) | 19136 | | 144 ROR | 15824 | | 245 PIP5K 59B | 28483 | | 339 TWINE | 15932 |
| 15 | 44 KP78a (C-TAK1 e-126) | 20848 | | 145 RTK? (TRKC 1e-37) | 1912 | 82 | 246 PIP5K 5786 | 21674 | | 340 STRING | 3224 |
| | 45 MLCK | 21482 | 49 | 146 SHARK (SVK/ZAP 70 related) | 41326 | | 247 PIP5K 102E (e-115) | 12323 | 114 | 341 PIR (e-159) | 5420 |
| | 46 MLCK | 37111 | | 147 Insulin receptor | 19952 | | 248 FYVE-containing PIP5K (3e-65) | 19856 | | 342 Homology to PIR (4e-16) | 32441 |
| 16 | 47 C-TAK1-like (C-TAK1 e-157) | 23618 | | 148 BTL/FGFR2 | 20816 | | 249 DGKβ | 3945 | | 343 MYOTUBULARIN | 26144 |
| | 48 C-TAK1-like (C-TAK1 e-157) | 37131 | 50 | 149 CSK (e-130CSK) | 21145 | 83 | 250 RDGA/Eye specific DGK | 30715 | 115 | 344 MYOTUBULARIN-like | 11849 |
| | 49 SNRK (e-136) | 24819 | | 150 SRC 29A | 41718 | | 251 DGKe | 25080 | | 345 | 31069 |
| | 50 CMG/CASK | 20820 | | 151 RTK similar to PDGFR (le-72) | 24332 | | 252 DGKδ (e-159) | 27810 | | 346 | 41365 |
| | 51 LOKI/CHK2 | 30511 | 51 | 152 ALK | 24459 | 84 | 253 DGKε (e-115) | 9029 | 116 | 347 MYOTUBULARIN-like (KIAA0371) | 12163 |
| | 52 CAMKIIs | 31200 | | 153 SRC 42A | 2448 | | 254 DGKε (e-126) | 18423 | | 348 | 13680 |
| | 53 CAMKIIs | 40503 | | 154 FPS | 25482 | | 255 Phosphoginase Kinase (6e-34) | 2650 | | 349 | 13770 |
| | 54 CAMKIIs | 40499 | 52 | 155 OFT/TRK (TRKA 3e-40) | 25769 | | 256 GYC (Guanylyl cyclase kin. 0.0) | 10689 | 117 | 350 | 13718 |
| | 55 EMK/KIAA0537 (2e-92) | 31947 | | 156 FAK | 28129 | 85 | 257 GYC (e-165) | 27662 | | 351 MKP-like (PUC 1e-18) | 28377 |
| | 56 CAKI | 32768 | | 157 EGFR | 28361 | | 258 GYC (0.0) | 30091 | | 352 MKP-like (DSP7 6e-43) | 33669 |
| 19 | 57 CAMK-like (1e-69) | 34154 | 53 | 158 FGFR1-like (FGFR 4e-62) | 28777 | | 259 GYC (0.0) | 30091 | 118 | 353 PUC/MKP | 23760 |
| | 58 EMK/KIAA0999 (e-97) | 34947 | | 159 DRL | 30099 | | 260 GYC 32E | 19618 | | 354 MKP-like (MKP1 7e-17) | 29124 |
| | 59 PROJECTIN | 3598 | | 160 DRL | 30136 | 86 | 261 GYC 76C | 25233 | | 355 MKP-like | 19498 |
| | 60 CAMK1 | 3727 | | 161 TOR | 3198 | | 262 GYC (0.0) | 10631 | | 356 MKP-like (MKP1 4e-27) | 35642 |
| | 61 CAMK1 | 3735 | 54 | 162 ACK2 (e-139) | 34843 | | 263 GYC (2e-71) | 17983 | 119 | 357 MKP-like (DSP3 3e-25) | 22699 |
| | 62 KP78b (C-TAK1 e-122) | 38153 | | 163 EPH | 3831 | | 264 GYC (0.0) | 10631 | | 358 MKP-like (VH1 3e-40) | 33824 |
| | 63 Sim. to myocardia SNF1-like kin. | 14053 | 55 | 164 SVH | 3980 | 87 | 265 GYC (0.0) | 13386 | | LMW-PTP | |
| | 64 CAMKIIB (e-105) | 39202 | | 165 HTL/FGFR1 | 39172 | | 266 GYCa 99B | 3044 | 120 | 359 LMW-PTP (3e-19) | 33927 |
| | 65 Hom. to DRAK-related kin. (e-60) | 5174 | | 166 JAK | 4219 | | 267 GYCb100B | 3618 | | 360 LMW-PTP (6e-26) | 27144 |
| | 66 MLCK-like (2e-80 MLCK) | 5322 | 56 | 167 DNT | 38779 | | 268 GYCd (1e-92) | 34707 | | IPP | |
| | 67 PHOSPHORYLASE KINASE | 5552 | | 168 DDR (DDR 2e-46) Part. transcript | 26866 | | 269 AC 13E (Adenylate cyclase kin.) | 26306 | 121 | 361 Inositol Triphosphate PPase | 11908 |
| | 68 PROJECTIN-like (9e-49) | 40324 | | 169 DDR (DDR 2e-46) Part. transcript | 33540 | | 270 AC 76E | 23940 | | 362 IPP (3e-50) | 21099 |
| | 69 PROJECTIN-like (9e-49) | 40326 | 57 | OPK | | | 271 AC 3 | 3791 | | 363 IPP (9e-35) | 29270 |
| | 70 PROJECTIN-like (9e-49) | 40322 | | 170 CACTUS IKK | 13812 | | 272 RUT | 26958 | | 364 IPP (2e-60) | 27664 |
| | 71 MLCK-like (e-32) | 28615 | | 171 WEE1 | 14568 | | 273 AC | 38106 | 124 | 365 IPP (Synaptojanin 0.0) | 20209 |
| 24 | CKI | | | 172 CHK1 | 14722 | | 274 AC | 18649 | | 366 IPP (2e-82) | 21268 |
| | 72 CKI-like (e-173) | 21414 | | 173 CHK1 | 14720 | | 275 AC (e-135) | 14186 | | 367 WUN | 4876 |
| | 73 CKI-like (e-173) | 39940 | 58 | 174 DYRK | 14734 | | 276 AC | 38110 | 125 | 374 | 8917 |
| | 74 CKI-like (8e-94) | 21551 | | 175 IRE-like (e-122) | 14850 | | 277 AC 78C | 29620 | | 375 WUN-like (WUN 1e-72) | 3034 |
| | 75 CKI-like (6e-78) | 28085 | | 176 TAK (TGFβ-activated kin. 2e-58) | 15429 | 91 | 278 AC | 6304 | | 376 IMP (1e-43) | 37805 |
| | 76 CKI | 30917 | | 177 GAK (Cyclin G-assoc. kin. e-146) | 1665 | | 279 AC (e-163) | 25794 | 126 | 377 IMP (3e-50) | 37791 |
| 26 | 77 CKI | 6528 | | 178 GAK (Cyclin G-assoc. kin. e-146) | 40880 | | 280 AC | 38108 | | 378 IMP (5e-32) | 37801 |
| | 78 DCO/CKIe | 6302 | 60 | 179 CDI/TSKI | 18533 | | Fragment/unknown | | | 379 IMP (1e-46) | 37797 |
| | 79 DCO/CKIe | 42623 | | 180 SRPK1 (9e-67) | 26050 | | 281 RAB/GAP domain and PK domain | 13402 | 127 | 380 IMP (1e-66) | 26653 |
| | 80 DCO/CKIe | 42625 | | 181 POLO/PLK1 | 20542 | | 282 | 1581 | | 381 IMP (5e-47) | 26645 |
| | 81 CKI-like (e-103) | 8263 | | 182 AURORA-like | 20576 | | 283 | 14670 | | STP: PPM | |
| | 82 CKI-like (e-109) | 8733 | | 183 SRPK2 (3e-77) | 20728 | | 284 | 22115 | 129 | 385 PP2C (7e-35) | 21997 |
| | 83 CKI-like | 36423 | | 184 PRP4 | 21738 | | 285 | 24364 | | 386 | 21999 |
| 28 | CMGC | | | 185 SAK/PLK | 22193 | | 286 | 27748 | | 387 PP2C (KIAA1072 2e-43) | 29142 |
| | 84 CDK7 | 11155 | | 186 TKK(3e-53) | 23319 | | 287 | 31887 | | 388 PP2C (KIAA0606 1e-49) | 29458 |
| | 85 PITSLRE | 13850 | | 187 MNB | 23674 | | 288 | 4060 | 130 | 389 PP2C (C. elegans PP2C e-101) | 8905 |
| | 86 MAK (e-123) | 14854 | | 188 MNB | 42249 | | 289 | 6100 | | 390 PP2C (C. elegans PP2C e-108) | 39335 |
| | 87 CDK4/6 | 15896 | | 189 Similar to KIAA1048 | 29762 | 95 | 290 | 9826 | | 391 | 5352 |
| | 88 CDK4/6 | 16072 | | 190 UNC 51-like (e-54) | 25478 | | 291 | 22173 | | 392 PP2Cβ (e-101) | 18937 |
| | 89 | | | | | | | | | | |

Figure S1. Overview of the kinase/phosphatase and DGCr1 dsRNA libraries. A. The kinase/phosphatase dsRNA library. Previously characterized and predicted transcripts derived from annotation of the *Drosophila* genome are categorized based on gene families. The number of predicted genes in each family and the number of corresponding dsRNA in the library are also indicated. See table S2 for complete list of transcripts and arrangement of dsRNAs into the pools used in the screen. B. The DGCr1 dsRNA library. The genes represented in DGCr1 are first divided into two groups on the basis of whether a Celera transcript (CT) number corresponding to the gene could be identified. Gene families that are of particular interest to the Hh pathway are indicated to the right and coverage of the library within each family is also indicated as (number of genes in the library / the predicted number of genes in the genome). Lists of gene families were assembled based on publicly available databases or a combination of literature and BLAST searches. Some examples of the types of genes within the group of 244 genes that do not have corresponding CT numbers are also noted. These genes apparently were not identified using computer-based gene-finding algorithms; 99 of these in addition do not have a Flybase entry and 9 correspond to retrotransposon transcripts. Twelve of these genes can be partially aligned to CT predictions, implying the existence of splicing variants or potential errors in computational splicing of the gene to generate a transcript.

Table S1. List of primers used to generate DNA templates for dsRNA synthesis. cDNA clones do not necessarily reflect the actual material used in PCR to generate subsequent templates for dsRNA synthesis but are noted here for reference purposes.

Table S2. List of individual kinases and phosphatases targeted in the kinase-phosphatase library. Names of the kinase and phosphatase families are in bold and abbreviated as in Morrison et al. Vertical lines indicate the pools of dsRNA used in the screen; pool number is on the left. Number to the right of each gene name designate CT number. Genes with multiple CT identifications were targeted multiple times.

References

- S1. G. M. Rubin *et al.*, *Science* **287**, 2222-4. (2000).
- S2. J. R. Kennerdell, R. W. Carthew, *Cell* **95**, 1017-26. (1998).