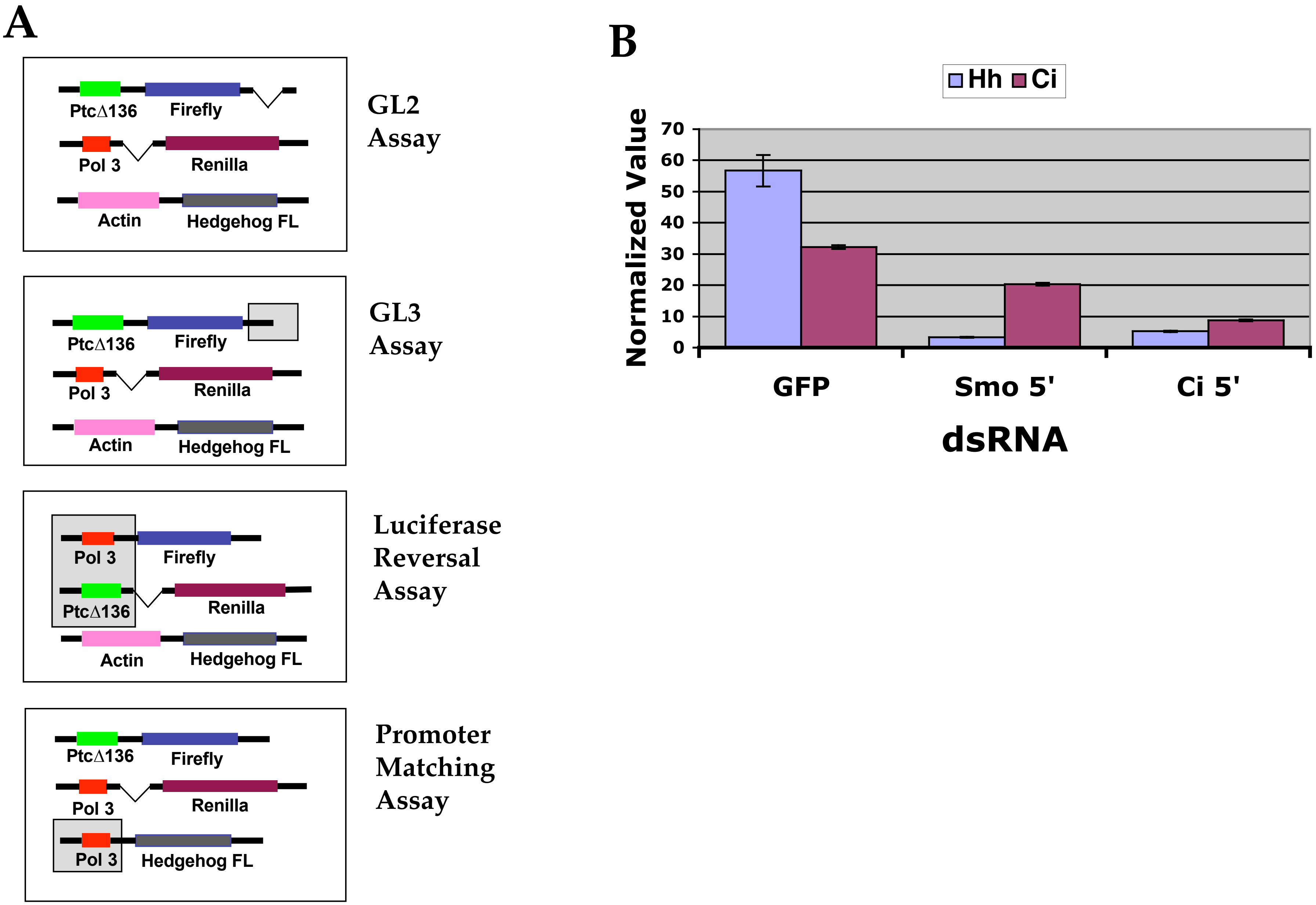
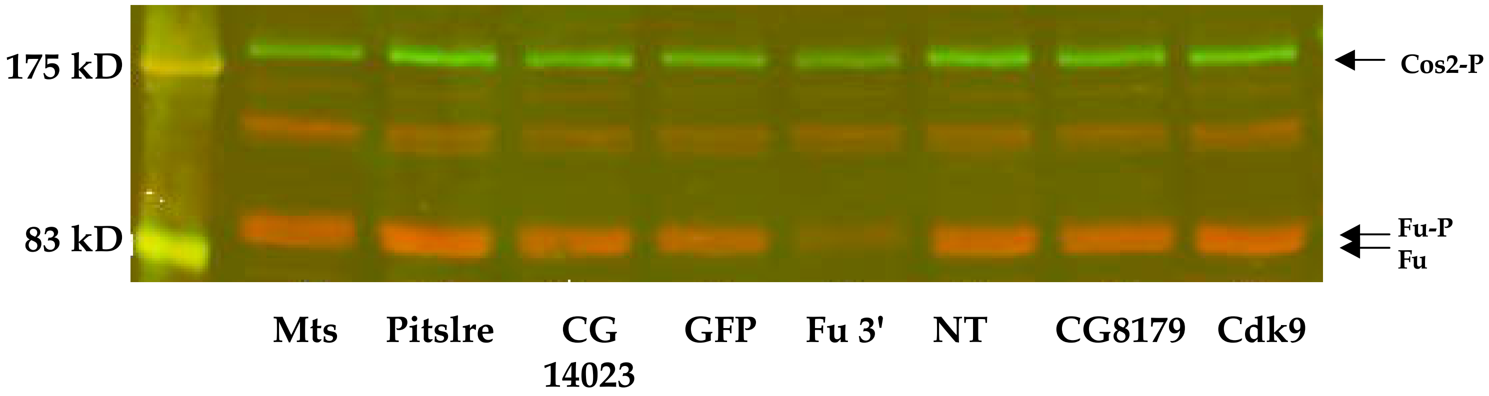
Supplementary Figure 1. Secondary Assays (A) Schematic representation of the secondary assays done to control for promoter or luciferase specific effects. The Hh-GL2 Assay is the original assay format. The Hh-GL3 assay is similar to the original reporter construct, but lacks a 3' intron that contributed to nonsense mediated decay of the reporter (see Materials and Methods). In the luciferase reversal assay, the promoters and luciferase constructs were swapped. In the promoter matching assay, the Hh expressing construct was driven by a Pol III promoter. (B) Ci overexpression partially activates the Hh signaling pathway and can partially rescue loss of reporter activity due to RNAi elimination of *smo*. Graphic representation of normalized luciferase values for the indicated stimulus (either Hh or Ci, in equal amounts) and dsRNA treatment. Ci expression activates the ptcΔ136 reporter to about half the level induced by Hh treatment. *smo* dsRNA almost completely eliminates reporter activity when Hh is used as stimulus, while *smo* dsRNA only reduces reporter by about 30% when Ci is used as stimulus.

Supplementary Figure 1



Supplementary Figure 2. Phosphorylation of Fu and Cos2 are not affected by reduction in *Cdk9*, *Pitslre*, or *mts*. Immunoblot of S2-Hh cell lysates treated using bathing RNAi to *Cdk9*, *Pitslre*, or *mts*. *GFP*, *CG14023*, and *CG8179* dsRNAs were added as negative controls, while *fu* dsRNA is included as a positive control for RNAi. NT indicates not treated. Hyperphosphorylated forms of Fu and Cos2 are indicated by arrows.

Supplementary Figure 2



Supplementary Table 1.
Potential Positive Regulators of Hh Signaling.

List of dsRNA's which decreased ptcΔ136 reporter activity by two or more standard deviations (an average Z-score of ≤ -2.0) in the primary screen. DRSC Amplicon is the unique number assigned to each dsRNA in the *Drosophila* RNAi Screening Center (DRSC) dsRNA library. FBgn is the Fly Base gene number. The "Z-score" column indicates the Z-score averaged over two replicates, and the Protein Domains describe the protein domains as annotated in Flybase release 3.1. Genes are sorted by CG number, and then by DRSC number.

Supplementary Table 1--Hits decreasing Hh reporter activity

CG#	Gene	Gene Name	DRSC Amplicon	FBgn	Z-Score	Protein Domains
CG10149	Rpn6	Proteasome p44.5 subunit	DRSC07541	FBgn0028689	-3.19	Tetratricopeptide repeat (TPR); Rpn6
CG1017	CG1017		DRSC08154	FBgn0035294	-2.19	
CG10198	Nup98		DRSC14209	FBgn0039120	-2.94	
CG10198	Nup98		DRSC14210	FBgn0039120	-3.46	
CG10305	RpS26	Ribosomal protein S26	DRSC03420	FBgn0004413	-2.61	FAD-linked reductases, C-terminal domain; RpS26, Ribosomal protein S26E
CG10325	abd-A	abdominal A	DRSC16897	FBgn0000014	-2.59	Homeobox domain, 'Homeobox' antennapedia-type protein, Homeodomain-like; abd-A
CG10423	RpS27	Ribosomal protein S27	DRSC14244	FBgn0039300	-3.5	Ribosomal protein S27E, Zinc beta-ribbon; CG10423
CG10484	Dox-A2	Diphenol oxidase A2	DRSC03318	FBgn0000486	-2.92	Tetratricopeptide repeat (TPR); Dox-A2
CG10541	Tektin-C	Tektin C	DRSC09741	FBgn0035638	-2.68	Tektin
CG10652	RpL30	Ribosomal protein L30	DRSC02087	FBgn0015745	-3.68	L30e-like; RpL30, Ribosomal protein L7AE, Ribosomal protein L30e
CG10869	CG10869		DRSC00290	FBgn0031347	-2.09	
CG10938	ProsMA5	Proteasome alpha subunit	DRSC07514	FBgn0016697	-3.07	Proteasome A-type subunit, Multispecific proteases of the proteasome, N-terminal nucleophile aminohydrolases (Ntn hydrol
CG10944	RpS6	Ribosomal protein S6	DRSC18712	FBgn0004922	-2.87	Ribosomal protein S6e
CG1101	Aly		DRSC12497	FBgn0010774	-2.68	RNA-binding region RNP-1 (RNA recognition motif), RNA-binding domain, RBD; aly
CG11121	so	sine oculis	DRSC07693	FBgn0003460	-2.21	Homeobox domain, Homeodomain-like; so
CG11194	Hey	Hairy/E(spl)-related with YRPW motif	DRSC07440	FBgn0027788	-3	Helix-loop-helix dimerization domain, Orange domain, Helix-loop-helix DNA-binding domain; Hey
CG11198	CG11198		DRSC06059	FBgn0033246	-2.08	Carbamoyl-phosphate synthase, Carboxyl transferase family, Biotin-requiring enzymes attachment site.
CG11276	RpS4	Ribosomal protein S4	DRSC11272	FBgn0011284	-2.9	Alpha-L RNA-binding motif; RpS4, S4 domain, Ribosomal protein S4E
CG11380	CG11380		DRSC18632	FBgn0040359	-2.01	
CG11405	A3-3		DRSC17225	FBgn0028550	-2.01	bZIP (Basic-leucine zipper) transcription factor fa, Fos transforming protein, Binding domain of Skn-1; A3-3
CG11416	CG11416		DRSC04111	FBgn0035025	-2.94	Prefoldin; CG11416
CG11502	svp	seven up	DRSC15311	FBgn0003651	-2.28	Ligand-binding domain of nuclear hormone receptor, C4-type steroid receptor zinc finger, Steroid hormone receptor, COUP transcription factor (2F nuclear receptor), Nuclear receptor ligand-binding domain; svp
CG11522	RpL6	Ribosomal protein L6	DRSC14323	FBgn0039857	-3	Ribosomal protein L6E
CG11561	smo	smoothened	DRSC00837	FBgn0003444	-5.16	Frizzled protein, G-protein coupled receptors family 2 (secretin-like
CG11624	CG11624		DRSC08703		-3.8	
CG11624			DRSC08703		-4.44	
CG11700	CG11700		DRSC17794	FBgn0029856	-3.31	Ubiquitin-like; CG11700
CG11711	Mob1		DRSC09117	FBgn0036172	-2.06	
CG11888	Rpn2		DRSC16839	FBgn0028692	-2.4	ARM repeat; Rpn2
CG11895	stan	starry night	DRSC05234	FBgn0024836	-2.24	GPS domain, G-protein coupled receptors family 2 (secretin-like, Conserved domain in several hormone receptors, Calcium-binding EGF-like domain, Cadherin domain, Cadherin; stan, Concanavalin A-like lectins/glucanases; stan, Membrane all-alpha; stan, EGF/Laminin; stan
CG11981	Prosbeta3		DRSC16801	FBgn0026380	-3.85	Proteasome B-type subunit, Multispecific proteases of the proteasome
CG11985	CG11985		DRSC14460	FBgn0040534	-2.05	
CG12000	CG12000		DRSC12186	FBgn0037314	-3.26	Proteasome B-type subunit, Multispecific proteases of the proteasome, N-terminal nucleophile aminohydrolases (Ntn hydrol
CG12050	CG12050		DRSC02152	FBgn0032915	-2.46	Trp-Asp repeat (WD-repeat); CG12050
CG12054	CG12054		DRSC14467	FBgn0039831	-2.36	C2H2 and C2HC zinc fingers; CG12054, Zinc finger, C2H2 type
CG12104	CG12104		DRSC08254	FBgn0035238	-2.49	HMG1/2 (high mobility group) box, HMG-box; CG12104
CG12238	l(1)G0084		DRSC19450	FBgn0026680	-3.75	PHD-finger
CG12254	Arc92		DRSC14483	FBgn0038760	-2.56	
CG12284	th	thread	DRSC11404	FBgn0003691	-2.29	Inhibitor of apoptosis (IAP) repeat; th
CG12323	Prosbeta5		DRSC07517	FBgn0029134	-2.04	Multispecific proteases of the proteasome, Proteasome B-type subunit
CG12324	RpS15Ab, RpS15Aa	Ribosomal protein S15Aa, Ribosomal protein S15Ab	DRSC06129	RpS15Aa: FBgn0010198, RpS15Ab: FBgn0033555	-2.86	RpS15Aa: Ribosomal protein S8, RpS15Aa: Ribosomal protein S8; RpS15A, RpS15Ab: Ribosomal protein S8, RpS15Ab: Ribosomal protein S8; CG12324
CG12348	Sh	Shaker	DRSC19214	FBgn0003380	-2.22	POZ domain; Sh, BTB/POZ domain

CG#	Gene	Gene Name	Amplicon	FBgn	Z-Score	Protein Domains
CG1263	RpL8	Ribosomal protein L8	DRSC08532	FBgn0024939	-2.51	Ribosomal protein L2, Translation proteins SH3-like domain; RpL8, Nucleic acid-binding proteins; RpL8
CG1263	RpL8	Ribosomal protein L8	DRSC08695	FBgn0024939	-2.68	Ribosomal protein L2, Translation proteins SH3-like domain; RpL8, Nucleic acid-binding proteins; RpL8
CG12740	RpL28	Ribosomal protein L28	DRSC08293	FBgn0035422	-2.71	Ribosomal L28e protein family
CG12750	CG12750		DRSC02179	FBgn0032678	-2.08	ARM repeat; CG12750, Middle domain of eIF4G
CG12775	RpL21	Ribosomal protein L21	DRSC03704	FBgn0032987	-2.96	Ribosomal protein L21E, Translation proteins SH3-like domain; CG12775
CG12822	CG12822, CG18853		DRSC20757	CG18853: FBgn0042173, CG12822: FBgn0033229	-2.29	
CG12852	CG12852		DRSC14558	FBgn0039573	-2.45	
CG12912	CG12912		DRSC06227	FBgn0033497	-3.62	
CG12977	CG12977		DRSC11687	FBgn0037052	-2.06	
CG13130	CG13130		DRSC02226	FBgn0040965	-3.07	
CG13165	CG13165		DRSC06292	FBgn0033707	-2.94	
CG13222	CG13222		DRSC06343	FBgn0033602	-2.06	
CG13333	CG13333		DRSC06368	FBgn0033855	-2.17	
CG13389	RpS13	Ribosomal protein S13	DRSC03419	FBgn0010265	-3.24	S15/NS1 RNA-binding domain; RpS13, Ribosomal protein S15
CG13445	CG13445		DRSC09992	FBgn0036532	-3.4	
CG13452	AGO2	Argonaute 2	DRSC09999	FBgn0046812	-2.79	
CG13847	CG13847		DRSC14697	FBgn0038967	-2.15	
CG13936	CG13936		DRSC08405	FBgn0035282	-2.53	
CG14023	CG14023		DRSC02353	FBgn0031698	-2.08	
CG14180	CG14180		DRSC10179	FBgn0036003	-2.06	
CG14206	RpS10b	Ribosomal protein S10b	DRSC19561	FBgn0031035	-3.52	
CG14210	CG33066, CG14210		DRSC19566	CG14210: FBgn0031040, CG33066: FBgn0053066	-2.75	
CG14342	CG14342		DRSC00372	FBgn0031324	-2.06	
CG14459	CG14459		DRSC11719	FBgn0037171	-2.39	
CG14494	CG14494		DRSC05695	FBgn0040740	-2.45	
CG14519	CG14519		DRSC14871	FBgn0039619	-2.1	
CG14641	CG14641		DRSC12227	FBgn0037220	-2.42	Zinc finger C-x8-C-x5-C-x3-H type, RNA-binding region RNP-1 (RNA recognition motif), RNA-binding domain, RBD; CG14641
CG14656	CG14656		DRSC12242	FBgn0037278	-2.52	
CG1475	RpL13A	Ribosomal protein L13A	DRSC12265	FBgn0037351	-4.02	Ribosomal protein L13, Ribosomal protein L13; CG1475
CG14792	sta	stubarista	DRSC18838	FBgn0003517	-2.5	Ribosomal protein S2, Ribosomal protein S2; sta
CG14813	deltaCOP	delta-coatomer protein	DRSC18760	FBgn0028969	-2.54	
CG1489	Pros45		DRSC20571	FBgn0020369	-2.5	AAA ATPase superfamily, P-loop containing nucleotide triphosphate hydrolas
CG14952	CG14952		DRSC08412	FBgn0035377	-2.14	
CG15157	CG15157		DRSC02458	FBgn0032675	-3.66	
CG1524	RpS14a	Ribosomal protein S14a	DRSC18710	FBgn0004403	-2.17	Translational machinery components; RpS14a, Ribosomal protein S11
CG15255	CG15255		DRSC01869	FBgn0028950	-2.08	Astacin (Peptidase family M12A) family, Neutral zinc metallopeptidases, zinc-binding region
CG1528	gammaCop	gamma-coatomer protein	DRSC16955	FBgn0028968	-2.07	
CG15442	RpL27A	Ribosomal protein L27A	DRSC00781	FBgn0010410	-3.18	Ribosomal proteins L15p and L18e; RpL27A, Ribosomal protein L27, Ribosomal protein L15
CG15476	CG15476		DRSC02487	FBgn0041000	-2.08	
CG15494	CG15494		DRSC02502	FBgn0040971	-2.32	
CG15552	Sox100B		DRSC15090	FBgn0024288	-2.36	HMG-box; Sox100B, HMG1/2 (high mobility group) box
CG15693	RpS20	Ribosomal protein S20	DRSC16836	FBgn0019936	-3.89 single	Ribosomal protein S10, Ribosomal protein S10; RpS20
CG15697	RpS30	Ribosomal protein S30	DRSC15119	FBgn0038834	-2.11	Ubiquitin-like; CG15697
CG15753	CG15753		DRSC19718	FBgn0030491	-2	
CG15783	CG15783		DRSC18089	FBgn0029772	-2.45	OAR domain
CG15814	CG15814		DRSC19730	FBgn0030873	-2.04	RING finger domain, C3HC4; CG15814
CG16901	sqd	squid	DRSC17066	FBgn0003498	-2.27	RNA-binding domain, RBD; sqd, RNA-binding region RNP-1 (RNA recognition motif)
CG16902	Hr4		DRSC18495	FBgn0023546	-2.18	C4-type steroid receptor zinc finger, Ligand-binding domain of nuclear hormone receptor, Vitamin D receptor, Steroid hormone receptor, Nuclear receptor ligand-binding domain; Hr4, FYVE/PHD zinc finger; Hr4
CG16918	CG16918		DRSC15165	FBgn0039574	-2.78	Trypsin-like serine proteases; CG16918, Serine proteases, trypsin family
CG16918	CG16918		DRSC15165	FBgn0039574	-3.51	Trypsin-like serine proteases; CG16918, Serine proteases, trypsin family
CG16941	CG16941		DRSC15166	FBgn0038464	-3.35	Ubiquitin-like; CG16941, SWAP / SURP

CG#	Gene	Gene Name	Amplicon	FBgn	Z-Score	Protein Domains
CG1708	cos	costa	DRSC07598	FBgn0000352	-2.8	Kinesin motor domain, P-loop containing nucleotide triphosphate hydrolas
CG17272	CG17272		DRSC15211	FBgn0038830	-1.8 single	EF-hand family, EF-hand; CG17272
CG17331	CG17331		DRSC02603	FBgn0032596	-2.67	Proteasome B-type subunit, Multispecific proteases of the proteasome, N-terminal nucleophile aminohydrolases (Ntn hydrol
CG17489	RpL5	Ribosomal protein L5	DRSC03801	FBgn0064225	-4.1	Ribosomal protein L18P/L5E, Translational machinery components; yip6
CG17521	Qm		DRSC11947	FBgn0024733	-3.17	Ribosomal protein L10E, Ribosomal protein L10e; qm
CG17888	Pdp1	PAR-domain protein 1	DRSC08897	FBgn0016694	-2.65	bZIP (Basic-leucine zipper) transcription factor fa
CG18174	Rpn11		DRSC03422	FBgn0028694	-2.82	
CG1821	RpL31	Ribosomal protein L31	DRSC06716	FBgn0025286	-3.92	Ribosomal protein L31e, Ribosomal protein L31e; RpL31
CG18468	CG18468		DRSC06759	FBgn0034217	-2.66	
CG18591	CG18591		DRSC02680	FBgn0031962	-2.03	Sm motif of small nuclear ribonucleoproteins, SNRN, Small nuclear ribonucleoprotein (Sm protein)
CG1874	CG1874		DRSC06787	FBgn0033425	-2.3	
CG2013	UbcD6	Ubiquitin conjugating enzyme	DRSC12370	FBgn0004436	-3.75	Ubiquitin-conjugating enzymes, Ubiquitin conjugating enzyme; UbcD6
CG2033	RpS15Ab, RpS15Aa	RpS15Aa: Ribosomal protein S15Aa, RpS15Ab: Ribosomal protein S15Ab	DRSC19831	RpS15Aa: FBgn0010198, RpS15Ab: FBgn0033555	-2.21	RpS15Aa: Ribosomal protein S8, RpS15Aa: Ribosomal protein S8; RpS15A, RpS15Ab: Ribosomal protein S8, RpS15Ab: Ribosomal protein S8; CG12324
CG2097	CG2097		DRSC12301	FBgn0037371	-2.89	ARM repeat; CG2097
CG2099	RpL35A	Ribosomal protein L35A	DRSC12302	FBgn0037328	-2.87	Globin, Ribosomal protein L35Ae
CG2125	ci	cubitus interruptus	DRSC17172	FBgn0004859	-3.38	Zinc finger, C2H2 type, C2H2 and C2HC zinc fingers; ci
CG2128	Hdac3		DRSC12352	FBgn0025825	-4.21	Arginase/deacetylase; HDAC3
CG2161	Rga	Regena	DRSC12364	FBgn0017550	-3.04	
CG2168	RpS3A	Ribosomal protein S3A	DRSC17168	FBgn0017545	-2.32	Ribosomal protein S3Ae
CG2184	Mlc2	Myosin light chain 2	DRSC16741	FBgn0002773	-2.59	EF-hand family, EF-hand; Mlc2
CG2685	CG2685		DRSC18463	FBgn0024998	-2.12	
CG2746	RpL19	Ribosomal protein L19	DRSC04649	FBgn0002607	-2.14	Ribosomal protein L19e, Ribosomal protein L19 (L19e); RpL19
CG2807	CG2807		DRSC00535	FBgn0031266	-3.56	ARM repeat; CG2807
CG2835	G-salpha60A	G protein salpha 60A	DRSC04616	FBgn0001123	-3.59	G-protein alpha subunit group S, Guanine nucleotide binding protein (G-protein), alp
CG2925	noi	noisette	DRSC12383	FBgn0014366	-2.55	RNA-binding protein C2H2 Zn-finger domain, Zinc finger, C2H2 type
CG2960	RpL40	Ribosomal protein L40	DRSC00782	FBgn0003941	-2.76	Ribosomal protein L40e, Ubiquitin-like; RpL40
CG2986	oho23B	overgrown hematopoietic organs at 23B	DRSC00833	FBgn0015521	-2.24	Ribosomal protein S21e
CG2998	RpS28b	Ribosomal protein S28b	DRSC18258	FBgn0030136	-2.03	Ribosomal protein S28e
CG30044	CG30044		DRSC06277	FBgn0050044	-2.79	
CG30126	CG30126		DRSC05769	FBgn0050126	-2.93	
CG30132	par-1		DRSC05792	FBgn0026193	-3.4	Eukaryotic protein kinase, Tyrosine kinase catalytic domain, Kinase associated domain 1, Serine/Threonine protein kinase family active site, Protein kinase-like (PK-like); par-1
CG30158	CG30158		DRSC04956	FBgn0050158	-3.01	P-loop containing nucleotide triphosphate hydrolas, Small GTPase, Ras subfamily
CG30361	mXr		DRSC05106	FBgn0050361	-2.57	G-protein coupled receptors family 3 (Metabotropic, Metabotropic glutamate receptor, Periplasmic binding protein-like I; CG18447, Periplasmic binding protein-like I; CG8692
CG30424	CG30424		DRSC04017	FBgn0050424	-2.07	
CG31111	CG31111		DRSC14649	FBgn0051111	-3.5	
CG31125	CG31125		DRSC13684	FBgn0051125	-3.44	
CG31145	CG31145		DRSC14670	FBgn0051145	-2.1	P-loop containing nucleotide triphosphate hydrolas
CG31209	CG31209		DRSC15701	FBgn0051209	-3.2	
CG31657	PNUTS		DRSC00638	FBgn0031291	-2.56	Zinc finger C-x8-C-x5-C-x3-H type, Conserved domain common to transcription factors T
CG3193	crn	crooked neck	DRSC18184	FBgn0000377	-2.95	N-terminal domain of phosphatidylinositol transfer, Protein prenylyltransferase; crn, Tetratricopeptide repeat (TPR); CG18842, C-terminal domain of phosphatidylinositol transfer, Profilin (actin-binding protein); CG18842
CG3193	crn	crooked neck	DRSC18185	FBgn0000377	-3.04	N-terminal domain of phosphatidylinositol transfer, Protein prenylyltransferase; crn, Tetratricopeptide repeat (TPR); CG18842, C-terminal domain of phosphatidylinositol transfer, Profilin (actin-binding protein); CG18842

CG#	Gene	Gene Name	Amplicon	FBgn	Z-Score	Protein Domains
CG3193	crn	crooked neck	DRSC18755	FBgn0000377	-2.77	N-terminal domain of phosphatidylinositol transfer, Protein prenylyltransferase; crn, Tetratricopeptide repeat (TPR); CG18842, C-terminal domain of phosphatidylinositol transfer, Profilin (actin-binding protein); CG18842
CG3195	RpL12	Ribosomal protein L12	DRSC04344	FBgn0034968	-3.01	Ribosomal protein L11
CG32029	CG32029		DRSC10646	FBgn0052029	-2.63	
CG3203	RpL17	Ribosomal protein L17	DRSC18293	FBgn0029897	-2.53	Ribosomal protein L22p / L17e, Ribosomal protein L22; CG3203
CG32046	CG32046		DRSC10171	FBgn0052046	-3.52	
CG32048	CG32048		DRSC08991	FBgn0052048	-2.09	PH domain-like; CG17357, Phosphotyrosine interaction (PID or PI)
CG32080	CG32080		DRSC09074	FBgn0052080	-2.41	
CG32133	CG32133		DRSC11048	FBgn0052133	-2.99	BRCT domain; CG8797, BRCT domain; CG6532
CG32139	Sox21b		DRSC10634	FBgn0042630	-2.02	HMG1/2 (high mobility group) box
CG32149	RhoGAP71E		DRSC10837	FBgn0036518	-2.21	GTPase activation domain, GAP; CG7396, GTPase activation domain, GAP; CG16980
CG32150	CG32150		DRSC09899	FBgn0052150	-2.3	
CG32253	CG11583		DRSC08202	FBgn0035524	-2.07	
CG32264	CG32264		DRSC08061	FBgn0052264	-2.05	
CG32296	CG32296		DRSC08263	FBgn0052296	-2.48	SAP domain
CG32308	CG32308		DRSC07967	FBgn0052308	-2.08	
CG32334	CG32334		DRSC07938	FBgn0052334	-2.46	
CG32381	unc-13-4A		DRSC10203	FBgn0035756	-4.11	C2 domain (Calcium/lipid-binding domain, CaLB); un
CG32432	CG32432		DRSC11703	FBgn0052432	-2.19	Ligand-binding domain of low-density lipoprotein r, Ligand-binding domain of low-density lipoprotein r, Spermadhesin, CUB domain; CG3116
CG32432	CG32432		DRSC11704	FBgn0052432	-2.7	Ligand-binding domain of low-density lipoprotein r, Ligand-binding domain of low-density lipoprotein r, Spermadhesin, CUB domain; CG3116
CG32474	dys	dysfusion	DRSC14897	FBgn0039411	-2.23	PAC motif, Helix-loop-helix DNA-binding domain; CG12561, Helix-loop-helix dimerization domain, PAS domain, PYP-like sensor domain; CG14552
CG32541	CG32541		DRSC19543	FBgn0052541	-2.53	Insulin-like; CG14188
CG32592	hiw	highwire	DRSC20338	FBgn0030600	-2.73	Regulator of chromosome condensation RCC1; hiw, B-box zinc finger superfamily, Immunoglobulin; hiw
CG32606	CG32606		DRSC19001	FBgn0052606	-2.22	
CG32676	CG32676		DRSC18442	FBgn0052676	-2.32	Ubiquitin-like; CG9725
CG32767	CG32767		DRSC18386	FBgn0052767	-2.22	
CG32776	CG32776		DRSC18119	FBgn0052776	-2.21	P-loop containing nucleotide triphosphate hydrolas, Small GTPase, Ras subfamily
CG3278	Tif-IA		DRSC03717	FBgn0032988	-2.29	
CG3278	Tif-IA		DRSC03726	FBgn0032988	-2.42	
CG32830	CG32830		DRSC01162	FBgn0052830	-2.76	
CG32955	CG32955		DRSC01163	FBgn0052955	-2.71	
CG32970	CG32970		DRSC01375	FBgn0052970	-2.51	
CG33106	mask	multiple ankyrin repeats single KH domain	DRSC16018	FBgn0043884	-3.52	KH-domain; CG6313, Ankyrin repeat; CG6313, Ankyrin repeat; CG6268, KH domain
CG33110	CG33110		DRSC14694	FBgn0053110	-2.31	
CG3314	RpL7A	Ribosomal protein L7A	DRSC18709	FBgn0014026	-3.11	L30e-like; RpL7A, Ribosomal protein L7A, Ribosomal protein L7AE, Ribosomal protein HS6
CG33141	sns	sticks and stones	DRSC06423	FBgn0024189	-2.89	Fibronectin type III; sns, Immunoglobulin; sns, Immunoglobulin-like, Immunoglobulin C-2 type
CG33141	sns	sticks and stones	DRSC07692	FBgn0024189	-2.83	Fibronectin type III; sns, Immunoglobulin; sns, Immunoglobulin-like, Immunoglobulin C-2 type
CG33183	Hr46	Hormone receptor-like in 46	DRSC06118	FBgn0000448	-2.14	C4-type steroid receptor zinc finger, Ligand-binding domain of nuclear hormone receptor, Steroid hormone receptor, Nuclear receptor ligand-binding domain; Hr46
CG33231	CG33231		DRSC08296	FBgn0053231	-2.49	
CG33277	CG33277		DRSC10335	FBgn0053277	-2.35	
CG3329	Prosbeta2	Proteasome beta2 subunit	DRSC11257	FBgn0023174	-2.52	Multispecific proteases of the proteasome, Proteasome B-type subunit
CG33352	CG33352		DRSC05013	FBgn0053352	-2.11	
CG33500	CG33500		DRSC09115	FBgn0053500	-2.02	
CG3395	RpS9	Ribosomal protein S9	DRSC11273	FBgn0010408	-3.71	Alpha-L RNA-binding motif; RpS9, S4 domain, Ribosomal protein S4
CG3411	bs	blistered	DRSC04676	FBgn0004101	-2.53	MADS-box domain, SRF-like; bs
CG3416	Mov34	Mov34	DRSC04624	FBgn0002787	-2.59	
CG3436	CG3436		DRSC00605	FBgn0031229	-2.23	
CG3455	Rpt4		DRSC18713	FBgn0028685	-2.06	P-loop containing nucleotide triphosphate hydrolas, AAA ATPase superfamily
CG3605	CG3605		DRSC00619	FBgn0031493	-3.06	
CG3661	RpL23	Ribosomal protein L23	DRSC04648	FBgn0010078	-3.38	Ribosomal protein L14b/L23e family, Ribosomal protein L14; RpL17A
CG3732	CG3732		DRSC04410	FBgn0034750	-2.92	
CG3751	RpS24	Ribosomal protein S24	DRSC04414	FBgn0034751	-3.35	Ribosomal protein S24e

CG#	Gene	Gene Name	Amplicon	FBgn	Z-Score	Protein Domains
CG3895	ph-d	polyhomeotic distal	DRSC18819	FBgn0004860	-2.24	
CG3922	RpS17	Ribosomal protein S17	DRSC11271	FBgn0005533	-3.17	Ribosomal protein S17e
CG3948	zetaCOP		DRSC11412	FBgn0040512	-2.73	Clathrin adaptor complex, small chain
CG3949	hoip	hoi-polloi	DRSC03546	FBgn0015393	-2.92	Ribosomal protein L7AE, Ribosomal protein HS6, L30e-like; hoip
CG3997	RpL39	Ribosomal protein L39	DRSC04651	FBgn0023170	-2.69	Ribosomal protein L39e, Ribosomal protein L39e; RpL46
CG40125	CG40125		DRSC00928	FBgn0058125	-2.24	
CG4013	Smr	Smrter	DRSC20288	FBgn0024308	-3.01	Aldehyde dehydrogenase family, Myb DNA binding domain, Homeodomain-like; Smr
CG40199	RpL15	Ribosomal protein L15	DRSC20963	FBgn0028697	-3.54	Ribosomal protein L15e, Ribosomal proteins L23 and L15e; RpL15
CG40278	RpL38	Ribosomal protein L38	DRSC07818	FBgn0040007	-3.63	Ribosomal L38e protein family
CG40341	CG40341		DRSC03750		-2.24	
CG4046	RpS16	Ribosomal protein S16	DRSC04442	FBgn0034743	-2.35	Ribosomal protein S9, Ribosomal protein S5 domain 2-like; CG4046
CG4087	RpLP1	Ribosomal protein LP1	DRSC00783	FBgn0002593	-3.38	60S Acidic ribosomal protein
CG4097	Pros26	Proteasome 26kD subunit	DRSC11256	FBgn0002284	-2.71	Multispecific proteases of the proteasome, Proteasome B-type subunit, N-terminal nucleophile aminohydrolases (Ntn hydrol
CG4111	RpL35	Ribosomal protein L35	DRSC18347	FBgn0029785	-3.04	Ribosomal protein L29, Ribosomal protein L29 (L29p); CG4111
CG4136	CG4136		DRSC18349	FBgn0029775	-2.74	Homeodomain-like; CG4136, Homeobox domain
CG4152	l(2)35Df	lethal (2) 35Df	DRSC03560	FBgn0001986	-2.17	DEAD/DEAH box helicase, Helicase C-terminal domain, P-loop containing nucleotide triphosphate hydrolas
CG4157	Rpn12		DRSC11275	FBgn0028693	-3.71	
CG4268	Pitslre		DRSC11874	FBgn0016696	-2.8	Eukaryotic protein kinase, Serine/Threonine protein kinase family active site, Protein kinase-like (PK-like); Pitslre
CG4379	Pka-C1	cAMP-dependent protein kinase 1	DRSC03399	FBgn0000273	-2.07	Eukaryotic protein kinase, Protein kinase C-terminal domain, Serine/Threonine protein kinase family active site, Protein kinase-like (PK-like); Pka-C1
CG4453	Nup153		DRSC19904	FBgn0061200	-2.16	
CG4453	Nup153		DRSC19904	FBgn0061200	-3.18	
CG4464	RpS19a	Ribosomal protein S19a	DRSC20281	FBgn0010412	-3.49	Ribosomal protein S19e
CG4482	mol	moladietz	DRSC01431	FBgn0028528	-2.6	
CG4501	bgm	bubblegum	DRSC03495	FBgn0027348	-2.72	Aminoacyl-transfer RNA synthetases class-II, AMP-dependent synthetase and ligase, Firefly luciferase-like; bgm
CG4585	CG4585		DRSC04475	FBgn0025335	-3.18	CDP-alcohol phosphatidyltransferase
CG4602	Srp54		DRSC03442	FBgn0024285	-2.02	RNA-binding region RNP-1 (RNA recognition motif), RNA-binding domain, RBD; Srp54
CG4609	fax	failed axon connections	DRSC11345	FBgn0014163	-3.08	Thioredoxin-like; fax, Glutathione S-transferases, C-terminal domain; fax
CG4651	RpL13	Ribosomal protein L13	DRSC03416	FBgn0011272	-2.15	Ribosomal protein L13e
CG4759	RpL27	Ribosomal protein L27	DRSC15638	FBgn0039359	-3.61	Ribosomal protein L27e
CG4863:CR31144	RpL3	Ribosomal protein L3	DRSC16834	FBgn0020910	-2.67	Ribosomal protein L3, Translation proteins; RpL3
CG4886	cyp33	cyclophilin-33	DRSC05666	FBgn0028382	-2.46	RNA-binding region RNP-1 (RNA recognition motif), Cyclophilin (peptidylprolyl isomerase); cyp33, RNA-binding domain, RBD; cyp33
CG4893	CG4893		DRSC10455	FBgn0036616	-2.02	
CG4897	RpL7	Ribosomal protein L7	DRSC03417	FBgn0005593	-3.86	Ribosomal protein L30, Ribosomal protein L30p/L7e; RpL7
CG4904	Pros35	Proteasome 35kD subunit	DRSC03401	FBgn0003151	-2.2	N-terminal nucleophile aminohydrolases (Ntn hydrol, Multispecific proteases of the proteasome, Proteasome A-type subunit
CG4912	eEF1delta		DRSC02790	FBgn0032198	-2.13	Elongation factor 1 beta/beta'/delta chain
CG4918	RpLP2	Ribosomal protein LP2	DRSC07539	FBgn0003274	-2.99	60S Acidic ribosomal protein
CG5166	Atx2	Ataxin-2	DRSC15727	FBgn0041188	-2.8	Sm motif of small nuclear ribonucleoproteins, SNRN
CG5179	Cdk9	Cyclin-dependent kinase 9	DRSC04601	FBgn0019949	-2.06	Eukaryotic protein kinase, Serine/Threonine protein kinase family active site, Protein kinase-like (PK-like); Cdk9
CG5271	RpS27A	Ribosomal protein S27A	DRSC03421	FBgn0003942	-2.14	Ubiquitin-like; RpS27A, Ribosomal protein S27a
CG5289	Pros26.4	Proteasome 26S subunit subunit 4 ATPase	DRSC16799	FBgn0015282	-3	AAA ATPase superfamily, P-loop containing nucleotide triphosphate hydrolas
CG5338	RpS19b	Ribosomal protein S19b	DRSC15773	FBgn0039129	-2.07	Ribosomal protein S19e
CG5360	CG5360		DRSC04498	FBgn0034873	-2.53	
CG5378	Rpn7		DRSC16841	FBgn0028688	-4.82	
CG5427	Oatp33Ea	Organic anion transporting polypeptide 33Ea	DRSC02859	FBgn0032433	-2.74	
CG5502	RpL4	Ribosomal protein L4	DRSC16833	FBgn0003279	-2.14	Ribosomal protein L4/L1e, Ribosomal protein L4; RpL1
CG5575	ken	ken and barbie	DRSC04696	FBgn0011236	-2.86	BTB/POZ domain, Zinc finger, C2H2 type, POZ domain; ken, C2H2 and C2HC zinc fingers; ken

CG#	Gene	Gene Name	Amplicon	FBgn	Z-Score	Protein Domains
CG5602	CG5602		DRSC04518	FBgn0034922	-2.64	DNA ligase/mRNA capping enzyme, catalytic domain;, ATP-dependent DNA ligase
CG5605	eRF1	eukaryotic release factor 1	DRSC11779	FBgn0036974	-2.4	eRF1-like proteins, Translational machinery components; eRF1, L30e-like; eRF1, N-terminal domain of eukaryotic peptide chain rele
CG5610	nAcRalpha-96Aa	nicotinic Acetylcholine Receptor alpha 96Aa	DRSC13670	FBgn0000036	-3.88	Neurotransmitter-gated ion-channel, Nicotinic acetylcholine receptor
CG5674	CG5674		DRSC02874	FBgn0032656	-2.7	
CG5684	CG5684		DRSC10537	FBgn0036239	-3.07	
CG5733	CG5733		DRSC06947	FBgn0034310	-2.14	
CG5758	CG5758		DRSC02889	FBgn0032666	-2.41	Beta-Ig-H3/Fasciclin domain
CG5786	ppan	peter pan	DRSC17030	FBgn0010770	-3.19 edge	
CG5827	RpL37A	Ribosomal protein L37A	DRSC02899	FBgn0028696	-2.68	Ribosomal L37ae protein family, Zn-binding ribosomal proteins; RpL37a
CG5920	sop	string of pearls	DRSC03614	FBgn0004867	-2.79	Ribosomal protein S5 domain 2-like; sop, Ribosomal protein S5, dsRNA-binding domain-like; sop
CG5931	CG5931		DRSC10559	FBgn0036548	-3.23	DEAD/DEAH box helicase, Helicase C-terminal domain, Protein prenylyltransferase; CG5931, P-loop containing nucleotide triphosphate hydrolas
CG5969	CG5969		DRSC11789	FBgn0036998	-2.22	
CG6015	CG6015		DRSC15948	FBgn0038927	-3.77	Trp-Asp repeat (WD-repeat); CG6015
CG6043	CG6043		DRSC02922	FBgn0032497	-3.85	
CG6064	TORC		DRSC10578	FBgn0036746	-2.96	
CG6141	RpL9	Ribosomal protein L9	DRSC03418	FBgn0015756	-3.57	Ribosomal protein L6, Ribosomal protein L6, signature 2, Ribosomal protein L6; RpL9
CG6223	betaCop	beta-coatomer protein	DRSC20312	FBgn0008635	-2.32	
CG6253	RpL14	Ribosomal protein L14	DRSC11269	FBgn0017579	-3.27	Ribosomal protein L14
CG6292	CycT	Cyclin T	DRSC11124	FBgn0025455	-3.21	Cyclin-like; CycT
CG6437	CG6437		DRSC04525	FBgn0034690	-2.27	Nucleotide-diphospho-sugar transferases; CG6437, Glycosyl transferase, family 2
CG6510	RpL18A	Ribosomal protein L18A	DRSC07538	FBgn0010409	-3.16	Ribosomal L18ae protein family
CG6551	fu	fused	DRSC20333	FBgn0001079	-3.03	Eukaryotic protein kinase, Serine/Threonine protein kinase family active site, Protein kinase-like (PK-like); fu
CG6625	Snap	Soluble NSF attachment protein	DRSC11285	FBgn0011712	-2.33	NSF attachment protein, Tetratricopeptide repeat (TPR); Snap
CG6694	CG6694		DRSC10696	FBgn0035900	-2.15	Zinc finger C-x8-C-x5-C-x3-H type
CG6699	beta'Cop	beta'-coatomer protein	DRSC03492	FBgn0025724	-2.34	
CG6779	RpS3	Ribosomal protein S3	DRSC16838	FBgn0002622	-3.14	KH domain, Ribosomal protein S3, Ribosomal protein S3 N-terminal domain-like; RpS3, Ribosomal protein S3 C-terminal domain; RpS3
CG6846	RpL26	Ribosomal protein L26	DRSC10726	FBgn0036825	-3.64	Ribosomal protein L24/bacterial NUSG, Ribosomal protein L24, Translation proteins SH3-like domain; CG6846
CG6905	CG6905		DRSC08577	FBgn0035136	-2.12	Homeodomain-like; CG6905, Myb DNA binding domain
CG6937	CG6937		DRSC16140	FBgn0038989	-2.25	RNA-binding domain, RBD; CG6937, RNA-binding region RNP-1 (RNA recognition motif)
CG6962	CG6962		DRSC16153	FBgn0037958	-2.06	
CG6987	SF2		DRSC16845	FBgn0040284	-2.35	RNA-binding region RNP-1 (RNA recognition motif), RNA-binding domain, RBD; SF2
CG7031	CG7031		DRSC16178	FBgn0039027	-3.89	
CG7109	mts	microtubule star	DRSC03574	FBgn0004177	-2.84	Serine/threonine specific protein phosphatase, Metallo-dependent phosphatases; mts
CG7219	CG7219		DRSC03029	FBgn0031973	-2.33	Serpins; CG7219, Serpins
CG7269	Hel25E	Helicase at 25E	DRSC03342	FBgn0014189	-3.34	DEAD/DEAH box helicase, Helicase C-terminal domain, P-loop containing nucleotide triphosphate hydrolas
CG7283	RpL10Ab	Ribosomal protein L10Ab	DRSC10798	FBgn0036213	-2.86	Ribosomal protein L1; CG7283, Ribosomal protein L1
CG7292	Rrp6		DRSC16223	FBgn0038269	-2.22	3'-5' exonuclease, HRDC domain, Ribonuclease H-like; CG7292
CG7305	CG7305		DRSC16225	FBgn0038557	-2.57	
CG7424	RpL36A	Ribosomal protein L36A	DRSC03055	FBgn0031980	-2.49	Ribosomal protein L44E, Zn-binding ribosomal proteins; CG7424
CG7425	eff	effete	DRSC16940	FBgn0011217	-2.79	Ubiquitin-conjugating enzymes, Ubiquitin conjugating enzyme; eff
CG7434	RpL22	Ribosomal protein L22	DRSC18707	FBgn0015288	-4.32	Histone H1 and H5 family, Ribosomal L22e protein family
CG7481	RhoGAP18B		DRSC20049	FBgn0030986	-2.57	GTPase activation domain, GAP; CG7481
CG7490	RpLP0	Ribosomal protein LP0	DRSC11878	FBgn0000100	-2.22	Ribosomal protein L10, 60S Acidic ribosomal protein
CG7525	Tie	Tie-like receptor tyrosine kinase	DRSC08104	FBgn0014073	-3.17	Eukaryotic protein kinase, Tyrosine kinase catalytic domain, Protein kinase-like (PK-like); Tie
CG7622	RpL36	Ribosomal protein L36	DRSC18708	FBgn0002579	-2.99	Ribosomal protein L36E

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CG7664	crp	cropped	DRSC03515	FBgn0001994	-2.54	Helix-loop-helix dimerization domain, Helix-loop-helix DNA-binding domain; crp
CG7726	RpL11	Ribosomal protein L11	DRSC07537	FBgn0013325	-3.64	Ribosomal protein L5, Ribosomal protein L5; RpL11
CG7757	CG7757		DRSC10912	FBgn0036915	-2.1	
CG7762	Rpn1		DRSC11274	FBgn0028695	-2.6	ARM repeat; Rpn1
CG7776	E(Pc)	Enhancer of Polycomb	DRSC07408	FBgn0000581	-2.38	
CG7835	CG7835		DRSC20058	FBgn0030879	-2.18	
CG7913	PP2A-B'		DRSC16337	FBgn0042693	-2.49	Protein phosphatase 2A regulatory B subunit (B56 fa
CG7923	Fad2		DRSC11165	FBgn0029172	-2.27	Fatty acid desaturase, type 1
CG7926	Axn	Axin	DRSC14120	FBgn0026597	-2.23	Regulator of G Protein Signaling (RGS) domain, DIX domain, Regulator of G-protein signaling, RGS; Axn
CG7939	RpL32	Ribosomal protein L32	DRSC16835	FBgn0002626	-2.69	Ribosomal protein L32e; RpL32, Ribosomal protein L32e
CG7961	alphaCop	alpha-coatomer protein	DRSC08706	FBgn0025725	-3.01	
CG7977	RpL23A	Ribosomal protein L23A	DRSC08694	FBgn0026372	-3.37	Ribosomal L23 protein, Histone H1 and H5 family, Ribosomal proteins L23 and L15e; RpL23a
CG8108	CG8108		DRSC09675	FBgn0027567	-3.31	Zinc finger, C2H2 type
CG8118	mam	mastermind	DRSC05468	FBgn0002643	-2.26	
CG8179	CG8179		DRSC07091	FBgn0034020	-2.48	SH3-domain; CG8179
CG8264	Bx42	Bx42	DRSC17743	FBgn0004856	-2.95	
CG8332	RpS15	Ribosomal protein S15	DRSC07151	FBgn0034138	-2.84	Ribosomal protein S19; CG8332, Ribosomal protein S19
CG8367	cg	combgap	DRSC07650	FBgn0000289	-4.51	C2H2 and C2HC zinc fingers; cg, Zinc finger, C2H2 type
CG8415	RpS23	Ribosomal protein S23	DRSC07169	FBgn0033912	-2.84	Ribosomal protein S12, Nucleic acid-binding proteins; CG8415
CG8426	l(2)NC136		DRSC04908	FBgn0033029	-3.23	
CG8435	CG8435		DRSC07179	FBgn0034084	-2.56	
CG8580	CG8580		DRSC11000	FBgn0035773	-3.52	
CG8615	RpL18	Ribosomal protein L18	DRSC11016	FBgn0035753	-3.79	Ribosomal protein L18e, Ribosomal proteins L15p and L18e; CG8615
CG8787	Asx	Additional sex combs	DRSC05924	FBgn0000142	-2.51	
CG8857	RpS11	Ribosomal protein S11	DRSC07289	FBgn0033699	-3.89	Ribosomal protein S17, Nucleic acid-binding proteins; CG8857
CG8877	CG8877		DRSC07293	FBgn0033688	-2.48	
CG8900	RpS18	Ribosomal protein S18	DRSC07540	FBgn0010411	-2.69	Ribosomal protein S13, Ribosomal protein S13; RpS18
CG8922	RpS5a	Ribosomal protein S5a	DRSC20282	FBgn0002590	-4.49	Ribosomal protein S7, Ribosomal protein S7; RpS5
CG8936	Arpc3B		DRSC20126	FBgn0065032	-2.23	
CG9091	RpL37a	Ribosomal protein L37a	DRSC20149	FBgn0030616	-3.91	Ribosomal protein L37e, Zn-binding ribosomal proteins; CG9091
CG9094	CG9094		DRSC07936	FBgn0035184	-2.79	
CG9176	cngl	CNG channel-like	DRSC20318	FBgn0029090	-2.08	cAMP-binding domain-like; cngl, Cyclic nucleotide gated / K+ ion channel TM region, Potassium channel, Membrane all-alpha; cngl
CG9282	RpL24	Ribosomal protein L24	DRSC03185	FBgn0032518	-3.99	Ribosomal protein L24E, Glucocorticoid receptor-like (DNA-binding domain);
CG9306	CG9306		DRSC03193	FBgn0032511	-2.58	
CG9324	CG9324		DRSC03201	FBgn0032884	-3.25	
CG9327	Pros29	Proteasome 29kD subunit	DRSC04644	FBgn0003150	-2.24	N-terminal nucleophile aminohydrolases (Ntn hydrol, Multispecific proteases of the proteasome, Proteasome A-type subunit
CG9354	RpL34b		DRSC16475	FBgn0037686	-2.05	Ribosomal protein L34e
CG9429	Crc	Calreticulin	DRSC16614	FBgn0005585	-2.08	Calreticulin family
CG9533	rut	rutabaga	DRSC20367	FBgn0003301	-2.55	Guanylate cyclase, Adenylyl and guanylyl cyclase catalytic domain; ru
CG9667	CG9667		DRSC16535	FBgn0037550	-2.38	
CG9817	CG9817		DRSC18446	FBgn0030219	-2.23	Zinc finger, C2H2 type
CG9842	Pp2B-14D	Protein phosphatase 2B at 14D	DRSC20270	FBgn0011826	-2.15	Serine/threonine specific protein phosphatase, Metallo-dependent phosphatases; Pp2B-14D
CG9885	dpp	decapentaplegic	DRSC00118	FBgn0000490	-4.48	Inhibin alpha chain, Cystine-knot cytokines; dpp
CG9930	E5	E5	DRSC16585	FBgn0008646	-2.39	Homeodomain-like; E5, Helix-turn-helix / lambda; and other repressors, Homeobox domain
CR31615	His-Psi:CR31616, His-Psi:CR31615		DRSC03760	His-Psi:CR31615: FBgn0051615, His-Psi:CR31616: FBgn0051616	-3.1	
			DRSC00133		-2.06	
			DRSC00181		-2.78	
			DRSC00209		-3.02	
			DRSC00244		-2.18	
			DRSC00245		-5.67	
			DRSC00405		-2.63	
			DRSC00938		-2.16	
			DRSC00977		-4.19	

CG#	Gene	Gene Name	Amplicon	FBgn	Z-Score	Protein Domains
			DRSC01077		-4.75	
			DRSC01082		-3.87	
			DRSC01088		-2.56	
			DRSC01091		-3.9	
			DRSC01151		-3.56	
			DRSC01243		-2.24	
			DRSC01284		-2.29	
			DRSC01382		-2.25	
			DRSC01392		-2.8	
			DRSC01492		-2.15	
			DRSC01498		-3.01	
			DRSC01624		-2.19	
			DRSC01729		-2.48	
			DRSC01800		-2.53	
			DRSC01970		-2.27	
			DRSC02439		-2.31	
			DRSC03669		-3.4	
			DRSC03679		-2.69	
			DRSC03777		-4.07	
			DRSC04825		-2.63	
			DRSC04827		-4.06	
			DRSC04925		-3.73	
			DRSC04952		-3.47	
			DRSC04953		-2.02	
			DRSC05037		-5.04	
			DRSC05108		-5.49	
			DRSC05225		-3.12	
			DRSC05231		-3.15	
			DRSC05259		-2.87	
			DRSC05297		-3.08	
			DRSC05322		-3.23	
			DRSC05339		-2.56	
			DRSC05360		-2.61	
			DRSC05475		-2.17	
			DRSC05564		-3.23	
			DRSC05623		-3.82	
			DRSC05644		-3.24	
			DRSC05653		-2.59	
			DRSC05691		-3.91	
			DRSC05715		-4.17	
			DRSC05797		-3.03	
			DRSC05818		-2.49	
			DRSC05871		-2.02	
			DRSC05899		-3.64	
			DRSC06303		-2.03	
			DRSC06353		-3.17	
			DRSC07851		-2.2	
			DRSC07855		-2.13	
			DRSC07876		-2.17	
			DRSC07932		-2.3	
			DRSC07982		-2.5	
			DRSC07995		-2.27	
			DRSC08039		-2.23	
			DRSC08080		-2.08	
			DRSC08092		-4.75	
			DRSC08743		-2.33	
			DRSC08878		-2.02	
			DRSC08903		-2.54	
			DRSC08908		-2.75	
			DRSC08917		-2.03	
			DRSC08919		-3.62	
			DRSC08923		-2.05	
			DRSC08929		-2.08	
			DRSC08973		-4.51	
			DRSC09001		-2.25	
			DRSC09014		-2.76	
			DRSC09065		-2.74	
			DRSC09077		-2.94	
			DRSC09089		-2.39	
			DRSC09409		-2.87	
			DRSC09601		-2.31	
			DRSC09612		-2.25	
			DRSC10097		-2.11	
			DRSC10152		-4.34	
			DRSC11453		-2.28	
			DRSC11456		-2.35	
			DRSC12666		-2.13	
			DRSC12796		-2.32	

CG#	Gene	Gene Name	Amplicon	FBgn	Z-Score	Protein Domains
			DRSC12841		-2.2	
			DRSC13109		-2.01	
			DRSC13294		-2.56	
			DRSC13978		-3.46	
			DRSC14042		-3.15	
			DRSC14046		-2.3	
			DRSC14061		-4.36	
			DRSC14674		-3.78	
			DRSC14730		-2.5	
			DRSC14786		-2.52	
			DRSC15105		-2.01	
			DRSC17234		-3.37	
			DRSC17234		-2.25	
			DRSC17236		-2.05	
			DRSC17248		-2.61	
			DRSC17270		-2.06	
			DRSC17343		-2.96	
			DRSC17344		-2.05	
			DRSC17378		-2.42	
			DRSC17379		-3.6	
			DRSC17473		-2.5	
			DRSC17488		-2.01	
			DRSC17612		-2.84	
			DRSC17617		-3.99	
			DRSC17710		-2.26	
			DRSC18003		-2.33	
			DRSC18889		-2.42	
			DRSC18905		-2.72	
			DRSC18937		-2.37	
			DRSC18999		-3.52	
			DRSC19008		-2.12	
			DRSC19029		-3.21	
			DRSC19104		-2.08	
			DRSC19193		-3.81	
			DRSC19200		-4.26	
			DRSC19255		-3.56	
			DRSC19272		-3.83	
			DRSC19301		-2.47	
			DRSC20442		-2.01	
			DRSC20534		-2.23	
			DRSC20751		-2.54	
			DRSC21055		-3.14	

Supplementary Table 2.
Potential Negative Regulators of Hh Signaling.

List of dsRNA's which increased ptcΔ136 reporter activity by 3 or more standard deviations (an average Z-score of ≥ +3.0). Columns are the same as in Supplemental Table 1. As described in the Material and Methods, a stricter standard was adopted in accepting increased scores due both to edge effects and the higher lability of the Renilla signal, which caused artificially high normalized luciferase values.

Supplementary Table 2--Hits increasing Hh reporter activity

CG#	Gene	Gene Name	DRSC Amplicon	FBgn	Z-Score	Protein Domains
CG10042	MBD-R2		DRSC14180	FBgn0038016	5.37	Methyl-CpG binding domain, Zinc finger, C2H2 type, PHD-finger, Tudor domain, DNA-binding domain; CG10042
CG10095	dpr15		DRSC14188	FBgn0037993	4.64	Immunoglobulin C-2 type, Immunoglobulin and major histocompatibility complex, Immunoglobulin-like, Immunoglobulin; CG10095
CG10279	Rm62	Rm62	DRSC12365	FBgn0003261	5.99	ATP-dependent helicase, DEAD-box, DEAD/DEAH box helicase, Helicase C-terminal domain, P-loop containing nucleotide triphosphate hydrolas
CG10306	CG10306		DRSC04056	FBgn0034654	3.93	
CG10706	SK	small conductance calcium-activated potassium channel	DRSC17888	FBgn0029761	4.06	Membrane all-alpha; SK, Potassium channel
CG10712	Chro	Chromator	DRSC11630	FBgn0044324	3.66	Chromo domain-like; CG10712, Chromo domain
CG11184	Upf3		DRSC04092	FBgn0034923	5.12	
CG1135	CG1135		DRSC08180	FBgn0035489	4.98	SMAD/FHA domain; CG1135
CG12079	CG12079		DRSC08243	FBgn0035404	3.06	Respiratory-chain NADH dehydrogenase 30 Kd subunit
CG12607	CG12607		DRSC08287	FBgn0035545	9.41	
CG1322	zfh1	Zn finger homeodomain 1	DRSC17098	FBgn0004606	3.27	Zinc finger, C2H2 type, Homeobox domain, Homeodomain-like; zfh1, C2H2 and C2HC zinc fingers; zfh1
CG1492	CG1492		DRSC19605	FBgn0030361	4.52	Gamma-glutamyltranspeptidase
CG1559	Upf1		DRSC19667	FBgn0030354	7.41	P-loop containing nucleotide triphosphate hydrolas
CG17077	pnt	pointed	DRSC17028	FBgn0003118	3.2	Ets-domain, HSF/ETS DNA-binding domain, Sterile alpha motif (SAM)/Pointed domain, SAM/Pointed domain; pnt
CG1716	CG1716		DRSC19765	FBgn0030486	3.25	WW domain; CG1716, HMG-I and HMG-Y DNA-binding domain (A+T-hook)
CG18041	CG18041		DRSC15301	FBgn0039710	4.96	
CG18214	trio		DRSC08527	FBgn0024277	4.89	DBL homology domain; trio, Spectrin repeat; trio, Cytochrome c; trio, Dbl domain (dbl/cdc24 rhoGEF family), PH domain-like; trio
CG1915	sls	sallimus	DRSC08670	FBgn0003432	3.03	Immunoglobulin; sls, Fibronectin type III; CG18242, Immunoglobulin; CG18857, Immunoglobulin; CG18242, Immunoglobulin-like, Immunoglobulin subtype, Immunoglobulin C-2 type, Immunoglobulin and major histocompatibility complex
CG2065	CG2065		DRSC06813	FBgn0033204	3.97	Insect alcohol dehydrogenase family, Glucose/ribitol dehydrogenase, Short-chain dehydrogenase/reductase (SDR) superfami, 2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase (En, NAD(P)-binding Rossmann-fold domains; CG2065
CG2253	Upf2		DRSC18218	FBgn0029992	4.78	Middle domain of eIF4G, ARM repeat; CG2253
CG2411	ptc	patched	DRSC05117	FBgn0003892	4.84	Patched family
CG2411	ptc	patched	DRSC07669	FBgn0003892	3.31	Patched family
CG30053	CG30053		DRSC07260	FBgn0050053	4.95	
CG3025	mof	males absent on the first	DRSC18795	FBgn0014340	3.12	Chromo domain, Acyl-CoA N-acyltransferases (Nat);
CG32050	CG32050		DRSC10173	FBgn0052050	3.06	
CG32202	CG32202		DRSC09989	FBgn0052202	3.59	
CG32566	CG9132		DRSC20159	FBgn0030791	3.09	
CG32688	Hk	Hyperkinetic	DRSC18674	FBgn0001203	3.06	NAD(P)-linked oxidoreductase; hk
CG32743	Smg1		DRSC18366	FBgn0052743	7.34	Phosphatidylinositol 3- and 4-kinase, Protein kinase-like (PK-like); CG4549
CG32743	Smg1		DRSC18367	FBgn0052743	6.49	Phosphatidylinositol 3- and 4-kinase, Protein kinase-like (PK-like); CG4549
CG3412	slmb	supernumerary limbs	DRSC17056	FBgn0023423	3.38	Skp1-Skp2 dimerization domains; slmb, Trp-Asp repeat (WD-repeat); slmb
CG4003	pont	pontin	DRSC17029	FBgn0040078	3.22	P-loop containing nucleotide triphosphate hydrolas, AAA ATPase superfamily
CG40218	CG40218		DRSC21138		8.31	
CG4035	eIF-4E	Eukaryotic initiation factor 4E	DRSC11342	FBgn0015218	4.62	Eukaryotic translation initiation factor 4E (eIF-4E, Translation initiation factor eIF4e; eIF-4E
CG4699	CG4699		DRSC15625	FBgn0038364	19.79	
CG4903	MESR4	Misexpression suppressor of ras 4	DRSC06901	FBgn0034240	3.17	Elongation factor Ts (EF-Ts), dimerization domain;, Zinc finger, C2H2 type
CG5499	His2Av	Histone H2A variant	DRSC16702	FBgn0001197	4.62	Histone-fold/TFIID-TAF/NF-Y domain, Histone H2A, Histone-fold; His2Av
CG5838	Dref	DNA replication-related element factor	DRSC03319	FBgn0015664	6.48	BED finger
CG6054	Su(fu)	Suppressor of fused	DRSC16871	FBgn0005355	3.71	
CG6105	l(2)06225		DRSC02928	FBgn0010612	3.59	
CG7752	CG7752		DRSC11848	FBgn0037066	7.74	C2H2 and C2HC zinc fingers; CG7752, Zinc finger, C2H2 type, FYVE/PHD zinc finger; CG7752
CG7938	Sry-beta	Serendipity beta	DRSC16868	FBgn0003511	3.15	Zinc finger, C2H2 type

CG#	Gene	Gene Name	Amplicon	FBgn	Z-Score	Protein Domains
CG8048	Vha44	Vacuolar H+ ATPase 44kD C subunit	DRSC07572	FBgn0020611	3.71	
CG8233	CG8233		DRSC07116	FBgn0033897	8.05	alpha/beta-Hydrolases; CG8233
CG8277	CG8277		DRSC10975	FBgn0035823	3.25	Translation initiation factor eIF4e; CG8277, Eukaryotic translation initiation factor 4E (eIF-4E
CG8954	Smg5		DRSC03124	FBgn0019890	5.25	
CG9233	fu2		DRSC03324	FBgn0029173	4.01	C2H2 and C2HC zinc fingers; fu2, MHC antigen-recognition domain; fu2, Zinc finger, C2H2 type
CG9504	CG9504		DRSC20187	FBgn0030597	3.1	FAD/NAD(P)-binding domain; CG9504, FAD-linked reductases, C-terminal domain; CG9504, Glucose-methanol-choline (GMC) oxidoreductase
CG9748	bel	belle	DRSC16555	FBgn0000171	6.5	DEAD/DEAH box helicase, ATP-dependent helicase, DEAD-box, Helicase C-terminal domain
CG9750	rept	reptin	DRSC11388	FBgn0040075	3.96	P-loop containing nucleotide triphosphate hydrolas
CG9924	CG9924		DRSC16580	FBgn0038192	3.08	BTB/POZ domain, TRAF domain; CG9924, POZ domain; CG9924
			DRSC05810		3.41	
			DRSC12025		3.73	
			DRSC12677		3.27	
			DRSC12923		5.32	
			DRSC13172		5.25	
			DRSC13783		3.44	
			DRSC13841		14.24	
			DRSC14928		4.79	
			DRSC17256		3.68	

Supplementary Table 3.
Orthologs of Potential Hh Regulators.

Table of hits identified from the primary screen that have human, mouse, yeast, and/or *C. elegans* orthologs. The average Z-score from the primary screen is indicated, as are the Entrez, Unigene, and Homologene identifiers.

Supplementary Table 3-- Hits with Known Orthologs

3.1 Target	Gene	DRSC Amplicon	Entrez Gene	Unigene	Homologene	Human	Mouse	C. elegans	Yeast
Decreases									
CG7109	mts	DRSC03574	45959	Dm.4245	3063	PPP2CB	Ppp2cb	4M623	PPH22, PPH21
CG11561	smo	DRSC00837	33196	Dm.21517	7325	FZD4	Fzd4		
CG10869	CG10869	DRSC00290	33337	Dm.7506	32639	FLJ90575	4921513E08Rik		
CG7269	Hel25E	DRSC03342	33781	Dm.3332	5567	BAT1	Bat1a		SUB2
CG3436	CG3436	DRSC00605	33180	Dm.1591	3538	HPRP8BP	0610009C03Rik	1E771, XL916	
CG3605	CG3605	DRSC00619	33514	Dm.285	6678	SF3B2	Sf3b2	5A609	CUS1
CG13389	RpS13	DRSC03419	34149	Dm.3535	791	RPS13	Rps13	rps-13	RPS13
CG6699	beta'Cop	DRSC03492	45757	Dm.2980	3499	COPB2	Copb2	4K227, 1N14	SEC27
CG4501	bgm	DRSC03495	44117	Dm.20327	9044	BG1	MGI:2385656	3O630, 4L76	
CG4152	l(2)35Df	DRSC03560	48782	Dm.1748	6257	KIAA0052	2610528A15Rik	4K593	MTR4
CG5920	sop	DRSC03614	34309	Dm.3451	20642	RPS2, LOC91561, MGC27348, LOC286444, LOC342808, LOC346643, LOC401974	Rps2, LOC194481, LOC207695, LOC215495, LOC230301, LOC235979, LOC238756, LOC244229, LOC245606, LOC277589	rps-2	RPS2
CG4912	eEF1delta	DRSC02790	34363	Dm.11017	23404	EEF1D, LOC126037	Eef1d		
CG12750	CG12750	DRSC02179	35099	Dm.11546	24205	KIAA1604			
CG4379	Pka-C1	DRSC03399	34284	Dm.2136	21942	PRKACB	Prkacb	kin-1	TPK1, TPK2
CG5602	CG5602	DRSC04518	37791	Dm.19278	197	LIG1	Lig1		CDC9
CG8426	l(2)NC136	DRSC04908	35501	Dm.2133	7149	CNOT3	Cnot3	ntl-3	NOT3
CG3278	Tif-IA	DRSC03717	35454	Dm.485	6342	RRN3, LOC94431, LOC388238	E130302O19Rik	3F90	RRN3
CG6437	CG6437	DRSC04525	37516	Dm.9285	2519	UGCG	Ugcg	2G205, XS315, 5Q366	
CG9324	CG9324	DRSC03201	35342	Dm.19856	9308	C13orf12	2510048O06Rik		
CG2746	RpL19	DRSC04649	37995	Dm.3682	758	RPL19, LOC285658	Rpl19	rpl-19	RPL19B, RPL19A
CG2835	G-salpha60A	DRSC04616	37805	Dm.4160	1565	GNAL		gsa-1	
CG3732	CG3732	DRSC04410	37588	Dm.3693	3984	ZNF265		2D362	
CG10149	Rpn6	DRSC07541	36638	Dm.3085	2108	PSMD11	Psmd11	rpn-6	RPN6
CG8877	CG8877	DRSC07293	36304	Dm.574	4706	PRPF8	Prpf8	prp-8	PRP8
CG8415	RpS23	DRSC07169	36576	Dm.4964	799	RPS23	Rps23, LOC269121, LOC269435	rps-23	RPS23A, RPS23B
CG5733	CG5733	DRSC06947	37078	Dm.682	11755	PCNT1		npp-2	
CG1017	CG1017	DRSC08154	38256	Dm.9436	4332	MFAP1	Mfap1	1J216	
CG7961	alphaCop	DRSC08706	38199	Dm.2217	3218	COPA	Copa	1D464	COP1
CG6905	CG6905	DRSC08577	38062	Dm.797	957	CDC5L	Cdc5l		
CG7977	RpL23A	DRSC08694	38208	Dm.7339	15121	RPL23A, LOC122585, MGC70863, LOC285214, LOC341511, LOC388574, LOC389101, LOC400725, LOC400814	BC029892	rpl-25.1	
CG7726	RpL11	DRSC07537	37235	Dm.700	36046	RPL11, LOC401727	Rpl11	rpl-11.1	
CG8435	CG8435	DRSC07179	36789	Dm.9146	6350	FLJ10374	2900016D05Rik		
CG10938	ProsMA5	DRSC07514	36951	Dm.3609	2084	PSMA5	Psma5	pas-5	PUP2
CG3395	RpS9	DRSC11273	39108	Dm.6943	787	RPS9	3010033P07Rik, LOC272691	rps-9	RPS9B, RPS9A
CG8580	CG8580	DRSC11000	38821	Dm.11452	9988	C6orf166	LOC380615	1E706	
CG32253	CG11583	DRSC08202	326206	Dm.21145	10133	BRIX	1110064N10Rik	3J135	BRX1
CG5684	CG5684	DRSC10537	39366	Dm.6800	22262			ccf-1	POP2
CG7762	Rpn1	DRSC11274	40174	Dm.3192	2101	PSMD2	Psmd2, LOC215879	rpn-1	
CG3329	Prosbeta2	DRSC11257	39628	Dm.4370	2093	PSMB7	Psmb7	pbs-2	PUP1
CG5969	CG5969	DRSC11789	40270		8238	QPCT	Qpct	5L85	
CG6292	CycT	DRSC11124	39961	Dm.20508	947	CCNT1	Ccnt1		
CG6625	Snap	DRSC11285	40233	Dm.2107	2839	NAPA	Napa	5I613	SEC17
CG5931	CG5931	DRSC10559	39737	Dm.973	5859	ASCC3L1, LOC400613	A330064G03Rik	2N18	BRR2
CG4097	Pros26	DRSC11256	39855	Dm.2536	2087	PSMB1	Psmb1	pbs-6	PRE7
CG5605	eRF1	DRSC11779	40240	Dm.2095	3475	ETF1	Etf1	5G915	SUP45
CG14641	CG14641	DRSC12227	40529	Dm.3575	9980	RBM22	Rbm22		
CG7292	Rrp6	DRSC16223	41798	Dm.7279	31105	EXOSC10	Exosc10	2K881C	
CG2097	CG2097	DRSC12301	40709	Dm.11484	3543	SYMPK	LOC225468	5J61	
CG1475	RpL13A	DRSC12265	40687	Dm.4531	6152	RPL13A, LOC283340, LOC387841, LOC387850, LOC387930. LOC399810	Rpl13a		RPL16A, RPL16B
CG11981	Prosbeta3	DRSC16801	41079	Dm.6972	2089	PSMB3	Psmb3, LOC240289, LOC384862	pbs-3	PUP3
CG12000	CG12000	DRSC12186	40639	Dm.11642	2090	PSMB4	Psmb4	pbs-7	PRE4
CG2013	UbcD6	DRSC12370	40610	Dm.2229	2503	UBE2A	Ube2a		RAD6
CG6962	CG6962	DRSC16153	41436	Dm.1159	9813	FLJ20297	4122402O22Rik		
CG2925	noi	DRSC12383	40678	Dm.4519	4949	SF3A3	Sf3a3	2I814	PRP9
CG2128	Hdac3	DRSC12352	44446	Dm.3642	2884	HDAC3	Hdac3		RPD3
CG6015	CG6015	DRSC15948	42593	Dm.20226	5716	CDC40		1L531	CDC40
CG16941	CG16941	DRSC15166	42048	Dm.4173	4294	SF3A1	Sf3a1	prp-21	
CG10198	Nup98	DRSC14209	42816	Dm.14811	3930	NUP98	Nup98		
CG33106	mask	DRSC16018	50070	Dm.7426	35296	ANKRD17			
CG5378	Rpn7	DRSC16841	42641	Dm.3424	7157	p44S10	2400006A19Rik	rpn-7	RPN7
CG5289	Pros26.4	DRSC16799	42828	Dm.2368	2095	PSMC1	Psmc1, LOC240657, LOC328691	rpt-2	RPT2
CG2184	Mlc2	DRSC16741	43587	Dm.1488	373	MYL5, MYLC2PL		mlc-2	
CG5502	RpL4	DRSC16833	43349	Dm.2104	748	RPL4	Rpl4	rpl-4	RPL4B, RPL4A
CG3193	crn	DRSC18755	31208	Dm.3140	6462	CRNKL1	Crnkl1	5G423	CLF1
CG12054	CG12054	DRSC14467	43694	Dm.4416	18291	JAZF1	AI591476		MIG1
CG11888	Rpn2	DRSC16839	43449	Dm.6875	2100	PSMD1		rpn-2	RPN2

3.1 Target	Gene	DRSC Amplicon	Entrez Gene	Unigene	Homologene	Human	Mouse	C. elegans	Yeast
CG14813	deltaCOP	DRSC18760	45250	Dm.6542	1250	ARCN1	Arcn1, LOC224732	3H712	RET2
CG2998	RpS28b	DRSC18258	31897	Dm.3643	804	RPS28	Rps28	4C425	RPS28B, RPS28A
CG10944	RpS6	DRSC18712	31700	Dm.2623	20271	RPS6	Rps6		
CG3203	RpL17	DRSC18293	31613	Dm.7261	13361		LOC226640, LOC383883	1B631	
CG8264	Bx42	DRSC17743	31840	Dm.2932	5294	SKIIP	Skiip	skp-1	
CG2807	CG2807	DRSC00535	33235	Dm.18458	6696	SF3B1	Sf3b1	3F354	HSH155
CG8922	RpS5a	DRSC20282	32700	Dm.2690	783	RPS5	Rps5	rps-5	RPS5
CG1489	Pros45	DRSC20571	33105	Dm.2162	2098	PSMC5	Psmc5	3D953, rpt-6	RPT6
CG15814	CG15814	DRSC19730	32765	Dm.198	10132	RNF121	Rnf121	3F260	
CG6223	betaCop	DRSC20312	32820	Dm.2883	5664	COPB	Copb1	2D377	SEC26
CG4087	RpLP1	DRSC00783	33214	Dm.2270	777	RPLP1	Rplp1	rpa-1	RPP1B, RPP1A
CG2960	RpL40	DRSC00782	33629	Dm.19778	21893		Gm1863, LOC384950		RPL40B, RPL40A
CG18174	Rpn11	DRSC03422	33738	Dm.3343	4240	PSMD14	Psmd14	rpn-11	RPN11
CG15442	RpL27A	DRSC00781	33654	Dm.295	21779		LOC237361, LOC239245, LOC268695, LOC270015, LOC270037, LOC383802	1C638	RPL28
CG5827	RpL37A	DRSC02899	44783	Dm.1819	773	RPL37A	Rpl37a	rpl-43	RPL43B, RPL43A
CG18591	CG18591	DRSC02680	34080	Dm.23392	22314	SNRPE, LOC148064, LOC158352	Snrpe		SME1
CG4904	Pros35	DRSC03401	34359	Dm.2599	2080	PSMA1	Psma1	pas-6	PRE5
CG7424	RpL36A	DRSC03055	34098	Dm.7214	776	RPL36AL, LOC255701, LOC284230, LOC390096, LOC392248. LOC392381	Rpl36al	2L388	RPL42A, RPL42B
CG5271	RpS27A	DRSC03421	34420	Dm.2888, Dm.4835	2217	RPS27A	Rps27a, LOC213533, LOC213898, LOC383115		RPS31
CG6141	RpL9	DRSC03418	34526	Dm.403	541	LOC388147		rpl-9	RPL9A, RPL9B
CG3949	hoip	DRSC03546	44173	Dm.20299	3672	NHP2L1	Gm1857, LOC381807	2K948	SNU13
CG4897	RpL7	DRSC03417	34352	Dm.3483	751	RPL7, LOC389305	Rpl7, LOC194742, LOC208522, LOC268809, Gm1874, LOC381646, LOC381756, 1700073E17Rik	rpl-7	RPL7A, RPL7B
CG9306	CG9306	DRSC03193	34747	Dm.6297	3669	NDUFB9	Ndufb9	3H471	
CG9282	RpL24	DRSC03185	34754	Dm.424	763	RPL24	Rpl24, LOC270006	rpl-24.1	RPL24A, RPL24B
CG17331	CG17331	DRSC02603	34999	Dm.11371	2088	PSMB2	Psmb2	pbs-4	PRE1
CG7664	crp	DRSC03515	34956	Dm.6530	2424	TFAP4	Tfap4		
CG10305	RpS26	DRSC03420	35098	Dm.6957	21736	LOC158200, LOC219542, LOC283479, LOC286513, LOC286539, LOC338611, RPS26, LOC390009, LOC400156, LOC401470		rps-26	RPS26A, RPS26B
CG3278	Tif-IA	DRSC03726	35454	Dm.485	6342	RRN3, LOC94431, LOC388238	E130302O19Rik	3F90	RRN3
CG12050	CG12050	DRSC02152	35374	Dm.1478	32771	FLJ12519	2410118I19Rik		
CG10484	Dox-A2	DRSC03318	35176	Dm.4848	2102	PSMD3	Psmd3	rpn-3	RPN3
CG9327	Pros29	DRSC04644	37378	Dm.712	2083	PSMA4	Psma4	pas-3	PRE9
CG10652	RpL30	DRSC02087	44059	Dm.1865	766	RPL30	Rpl30	rpl-30	RPL30
CG12775	RpL21	DRSC03704	35453	Dm.11743	759	RPL21, LOC387753, LOC388143, LOC388471, LOC388532, LOC388621	Rpl21, LOC210534, LOC218274, LOC227992, LOC229920, LOC232399, LOC238198, LOC268676, LOC380736, LOC380995, LOC381084, LOC381162, LOC381374, LOC381440, LOC381920, LOC382211, LOC382248, LOC383964, LOC384940		RPL21A, RPL21B
CG3751	RpS24	DRSC04414	37589	Dm.19983	800	RPS24	Rps24, LOC210668, LOC225058, LOC380888, LOC383815	rps-24	RPS24B, RPS24A
CG3416	Mov34	DRSC04624	37894	Dm.770	2104	PSMD7	Psmd7	rpn-8	RPN8
CG3661	RpL23	DRSC04648	37628	Dm.6664	755	RPL23, LOC402120	Rpl23	rpl-23	RPL23A, RPL23B
CG4046	RpS16	DRSC04442	37580	Dm.7604	794	RPS16	Rps16	rps-16	RPS16B, RPS16A
CG3195	RpL12	DRSC04344	45329	Dm.7505	31003	RPL12, LOC389387, LOC389974	Rpl12	rpl-12	
CG5179	Cdk9	DRSC04601	37586	Dm.4856	965	CDK9	Cdk9	cdk-9	
CG3997	RpL39	DRSC04651	37849	Dm.14062	775	RPL39, RPL39L	Rpl39, 4930517K11Rik	rpl-39	RPL39
CG33183	Hr46	DRSC06118	36073	Dm.5036	5041	RORB	Rorb	nhr-23	
CG12323	Prosbeta5	DRSC07517	45269	Dm.1454	2091	PSMB5	Psmb5	pbs-5	PRE2
CG33352	CG33352	DRSC05013	2768716		14435	LOC92154	BC060632		
CG12324	RpS15Ab, RpS15Aa	DRSC06129	36142, 44150	Dm.1427	793	RPS15A, LOC125910, LOC389382, LOC391035, LOC391656	LOC237512, Rps15a	rps-22	RPS22B, RPS22A
CG11198	CG11198	DRSC06059	35761	Dm.11366	32537	ACACA	Acac		
CG8367	cg	DRSC07650	36571	Dm.2240	5069	ZNF32	Zfp637		
CG13222	CG13222	DRSC06343	36193	Dm.14040	36721			pqn-32	
CG4918	RpLP2	DRSC07539	36855	Dm.2691	778	RPLP2	Rplp2	rpa-2	

3.1 Target	Gene	DRSC Amplicon	Entrez Gene	Unigene	Homologene	Human	Mouse	C. elegans	Yeast
CG8900	RpS18	DRSC07540	37292	Dm.20558	5747	RPS18	Rps18, LOC382762, LOC382827, LOC383131, LOC384017, LOC384525		
CG6510	RpL18A	DRSC07538	36985	Dm.669	757	RPL18A, LOC400068	2510019J09Rik	rpl-20Co	RPL20B, RPL20A
CG8332	RpS15	DRSC07151	36851	Dm.6178	792	RPS15, LOC148640, LOC401019	Rps15, LOC381772	rps-15	RPS15
CG1263	RpL8	DRSC08695	44251	Dm.6647	32141		Rpl8	rpl-2	
CG1263	RpL8	DRSC08532	44251	Dm.6647	32141		Rpl8	rpl-2	
CG12740	RpL28	DRSC08293	38397	Dm.14669	768	RPL28	Rpl28	rpl-28	
CG32296	CG32296	DRSC08263	38338	Dm.13305	10826	MKL2	Mrtf-B		
CG32381	unc-13-4A	DRSC10203	38801	Dm.21296	20844	BAIAP3	Gm937	XD240	
CG10541	Tektin-C	DRSC09741	38653	Dm.5022	7973	TEKT1	Tekt1	XE623	
CG7283	RpL10Ab	DRSC10798	39338	Dm.20863	5152	RPL10A, LOC137107, LOC389308	Rpl10a	rpl-1	RPL1B, RPL1A
CG11276	RpS4	DRSC11272	39484	Dm.951	781	RPS4X, RPS4Y1, LOC126235, RPS4Y2	Rps4x	4H848	RPS4A, RPS4B
CG3922	RpS17	DRSC11271	39088	Dm.5973	795	RPS17, LOC402057	Rps17, LOC382492	rps-17	RPS17B, RPS17A
CG7923	Fad2	DRSC11165	44006	Dm.3344	23447			fat-6, fat-5	
CG4157	Rpn12	DRSC11275	39845	Dm.981	2105	PSMD8		rpn-12	RPN12
CG3948	zetaCOP	DRSC11412	39862	Dm.20098	9366	COPZ1	Copz1	2L163	RET3
CG32133	CG32133	DRSC11048	39552	Dm.24000	33004		A630073D07Rik		
CG6064	TORC	DRSC10578	39970	Dm.11355	32620	MECT1			
CG4893	CG4893	DRSC10455	39813	Dm.5901	21389	TPPP	2900041A09Rik		
CG7490	RpLP0	DRSC11878	40451	Dm.7933	6517	RPLP0, LOC220717	Arbp	rpa-0	RPP0
CG6846	RpL26	DRSC10726	40060	Dm.6097	764	RPL26	Rpl26		
CG7757	CG7757	DRSC10912	40172	Dm.13639	3447	PRPF3	Prpf3	3L514	PRP3
CG17521	Qm	DRSC11947	43864	Dm.1411	4386	RPL10, LOC284393, LOC389342	Rpl10	rpl-10	RPL10
CG9667	CG9667	DRSC16535	40960		6283	KIAA1160	5830446M03Rik	5L480	ISY1
CG2099	RpL35A	DRSC12302	40654	Dm.1069	10946	RPL35A	Rpl35a, LOC383767, LOC384380		
CG11985	CG11985	DRSC14460	50007		12841	SF3B5	1110005L13Rik		
CG9429	Crc	DRSC16614	41166	Dm.2457	3205	CALR	Calr	crt-1	
CG9354	RpL34b	DRSC16475	41120	Dm.4884	5819			4N33	RPL34B, RPL34A
CG4863:CR31144	RpL3	DRSC16834	41347	Dm.6703	13398		LOC230616, LOC242809, LOC380868. Gm1899		
CG11502	svp	DRSC15311	41491	Dm.20676	21158	NR2F1	Nr2f1		
CG10325	abd-A	DRSC16897	42037	Dm.3286	20947	HOXB7	Hoxb7	mab-5	
CG6987	SF2	DRSC16845	53443	Dm.4443	31411	SFRS1	Sfrs1	rsp-3	HRB1
CG9930	E5	DRSC16585	45396		17092	GSH1	Gsh1	XP650	
CG7425	eff	DRSC16940	41785	Dm.4048	2506	UBE2D2, LOC388784	1700013N18Rik	let-70	UBC5, UBC4
CG7913	PP2A-B'	DRSC16337	42169	Dm.3799	2037	PPP2R5C	Ppp2r5c	5L508	
CG5786	ppan	DRSC17030	42502	Dm.3008	5690	PPAN	Ppan	lpd-6	SSF2, SSF1
CG15697	RpS30	DRSC15119	42470	Dm.7408	1513	FAU	Fau	rps-30	RPS30A
CG7305	CG7305	DRSC16225	42151	Dm.25477	8988	RIMS1	Rims1		
CG15693	RpS20	DRSC16836	42464	Dm.2391	6063			rps-20	
CG10198	Nup98	DRSC14210	42816	Dm.14811	3930	NUP98	Nup98		
CG10423	RpS27	DRSC14244	43039	Dm.20253	803	RPS27, LOC389112	Rps27	rps-27	RPS27A, RPS27B
CG6937	CG6937	DRSC16140	42662	Dm.6006	12216	MKI67IP	Mki67ip		
CG4759	RpL27	DRSC15638	43103	Dm.6265	765	RPL27, LOC401947	Rpl27	rpl-27	RPL27B, RPL27A
CG31145	CG31145	DRSC14670	42784	Dm.10845	8909	FAM20B	C530043G21Rik		
CG32474	dys	DRSC14897	43174	Dm.18571	3716	SIM2	Sim2		
CG31111	CG31111	DRSC14649	318595	Dm.23785	18102	TBRG1	Tbrg1		
CG6779	RpS3	DRSC16838	42761	Dm.20234	31005	RPS3	Rps3	rps-3	
CG5338	RpS19b	DRSC15773	42830	Dm.20517	796			rps-19	RPS19A, RPS19B
CG12852	CG12852	DRSC14558	43365		21066	TNRC11	Tnrc11	5N91	
CG15552	Sox100B	DRSC15090	45039	Dm.3273	7950	SOX8	Sox8		
CG11522	RpL6	DRSC14323	43723	Dm.6176	31001	RPL6, LOC285900	Rpl6	rpl-6	
CG2685	CG2685	DRSC18463	31259	Dm.6933	9466	WBP11	Wbp11	2K463	
CG2168	RpS3A	DRSC17168	43768	Dm.6768	15353		LOC268700, LOC270030, LOC270099, LOC380925, LOC381850		
CG14792	sta	DRSC18838	31106	Dm.4723	27634		LOC380682, LOC380692, LOC381114, LOC381515		
CG3193	crn	DRSC18185	31208	Dm.3140	6462	CRNKL1	Crnkl1	5G423	CLF1
CG7622	RpL36	DRSC18708	31009	Dm.14146	6660	RPL36, LOC391209	LOC239658, LOC242643	rpl-36	RPL36A, RPL36B
CG1528	gammaCop	DRSC16955	43717	Dm.1895	22214		Copg	4K919	SEC21
CG3193	crn	DRSC18184	31208	Dm.3140	6462	CRNKL1	Crnkl1	5G423	CLF1
CG3895	ph-d	DRSC18819	44889		23119	PHC2	Phc2		
CG11700	CG11700	DRSC17794	31564		7987	UBB	Ubb, Gm1821	ubq-1	UBI4
CG3455	Rpt4	DRSC18713	31567	Dm.4	2099	PSMC6	Psmc6	rpt-4	
CG3314	RpL7A	DRSC18709	31588	Dm.7159	31002	RPL7A	Rpl7a		
CG4111	RpL35	DRSC18347	31483	Dm.19648	31432	RPL35, LOC154880, LOC341604, LOC388754, LOC390876	Rpl35		
CG1524	RpS14a	DRSC18710	47218	Dm.6665	4103	RPS14	Rps14	rps-14	RPS14A, RPS14B
CG2033	RpS15Ab, RpS15Aa	DRSC19831	36142, 44150	Dm.1427	793	RPS15A, LOC125910, LOC389382, LOC391035, LOC391656	LOC237512, Rps15a	rps-22	RPS22B, RPS22A
CG9842	Pp2B-14D	DRSC20270	32624	Dm.21227	728	PPP3CA	Ppp3ca	tax-6	CNA1, CMP2
CG32592	hiw	DRSC20338	32429	Dm.3259	9005	MYCBP2	Phr1	rpm-1	
CG9533	rut	DRSC20367	32406	Dm.2613	10896	ADCY1	Adcy1	acy-3	
CG8936	Arpc3B	DRSC20126	32696	Dm.19161	4178	ARPC3, LOC387805	Arpc3	arx-5	ARC18

3.1 Target	Gene	DRSC Amplicon	Entrez Gene	Unigene	Homologene	Human	Mouse	C. elegans	Yeast
CG14210	CG33066, CG14210	DRSC19566	318844, 32958	Dm.6071	5941	MGC2574		1O20	CGR1
CG7481	RhoGAP18B	DRSC20049	32898	Dm.3638	33731			gei-1	
CG9885	dpp	DRSC00118	33432	Dm.4767	926	BMP2	Bmp2		
CG32955	CG32955	DRSC01163	318268	Dm.1555	33819			klp-18, klp-10C	
CG4482	mol	DRSC01431	34872	Dm.3630	16043	NIP	9030623N16Rik, BC019755		
CG11895	stan	DRSC05234	36125	Dm.4360	1078	CELSR2	Celsr2		
CG14494	CG14494	DRSC05695	50213		36965	KIAA1817			
CG30132	par-1	DRSC05792	2768852		36096	MARK3	Mark3		
CG30126	CG30126	DRSC05769	246472		33482	FOXP2	Foxp2		
CG4886	cyp33	DRSC05666	36984	Dm.2854	36550		LOC381106, LOC383442		
CG32264	CG32264	DRSC08061	38438	Dm.15742	11392	PHACTR4	Phactr4		
CG11711	Mob1	DRSC09117	39293	Dm.4055	12477	HCCA2	1110017M21Rik		
CG17888	Pdp1	DRSC08897	45588	Dm.1594	33568			pqn-24	
CG5610	nAcRalpha-96Aa	DRSC13670	42918	Dm.2364	36034	CHRNA5	Chrna5	unc-63	
CG12348	Sh	DRSC19214	32780	Dm.7088	21034	KCNA2	Kcna2	2K57	
CG11121	so	DRSC07693	35662	Dm.4727	4360	SIX1	Six1	ceh-34	
CG8615	RpL18	DRSC11016	38794	Dm.7265	31004	RPL18	Rpl18		
CG8857	RpS11	DRSC07289	36321	Dm.576	789	RPS11	Rps11	rps-11	RPS11A, RPS11B
CG1101	Aly	DRSC12497	44029	Dm.3104	5722	THOC4	Thoc4, Refbp2, LOC226957, LOC235966, LOC327811, LOC385297	aly-2, aly-3	
CG9091	RpL37a	DRSC20149	32446	Dm.14847	772	RPL37, LOC147655, LOC346950	Rpl37	2P101, rpl-37	RPL37A, RPL37B
CG14206	RpS10b	DRSC19561	32953	Dm.6225	788	RPS10, LOC389127	Rps10	rps-10	RPS10A, RPS10B
CG7434	RpL22	DRSC18707	31022	Dm.6921	14006	LOC256441, LOC389175, LOC402098. LOC402100			
CG7926	Axn	DRSC14120	43565	Dm.7154	3420	AXIN2	Axin2		
CG7939	RpL32	DRSC16835	43573	Dm.7621	771	RPL32, LOC392202, LOC392447	Rpl32, LOC242084	rpl-32	RPL32
CG2986	oho23B	DRSC00833	33487	Dm.2727	798	RPS21	Rps21	rps-21	RPS21B, RPS21A
CG4651	RpL13	DRSC03416	34329	Dm.21541	5568	RPL13	Rpl13	rpl-13	RPL13A, RPL13B
CG16901	sqd	DRSC17066	41666	Dm.7189	22580	HNRPAB	Hnrpab	4H89	
Increases									
CG8954	Smg5	DRSC03124	34804	Dm.2100	9095	EST1B	BC024683		
CG11184	Upf3	DRSC04092	37792	Dm.756	11307	UPF3B	Upf3b	smg-4	
CG10306	CG10306	DRSC04056	37475	Dm.3730	8292	eIF3k	1200009C21Rik	eif-3.K	
CG8233	CG8233	DRSC07116	36560	Dm.615	9949	FLJ10081	4632411B12Rik		
CG8048	Vha44	DRSC07572	36826	Dm.7688	1281	ATP6V1C1	Atp6v1c1	vha-11	VMA5
CG18214	trio	DRSC08527	43974	Dm.803	20847	HAPIP	Hapip		
CG12079	CG12079	DRSC08243	38378	Dm.11274	3346	NDUFS3	Ndufs3	1H106	
CG1135	CG1135	DRSC08180	38478	Dm.3951	4622	MCRS1	Mcrs1		
CG4035	eIF-4E	DRSC11342	45525	Dm.7012	1488	EIF4E	Eif4e, Gm206	ife-3	CDC33
CG6054	Su(fu)	DRSC16871	41565	Dm.2196	9262	SUFU	Sufu		
CG3412	slmb	DRSC17056	42504	Dm.2387	7646	BTRC	Btrc	lin-23	
CG5499	His2Av	DRSC16702	43229	Dm.2346	36329	H2AFV			
CG1322	zfh1	DRSC17098	43650	Dm.4708	9421	ZNF580	Zfp580		
CG1559	Upf1	DRSC19667	32153	Dm.3617	2185			smg-2	NAM7
CG32566	CG9132	DRSC20159	2768882		5869	DOM3Z	Dom3z	dom-3	RAI1
CG2065	CG2065	DRSC06813	35707	Dm.5789	6770	RDH13	Rdh13	dhs-24	
CG2411	ptc	DRSC07669	35851	Dm.2630	223	PTCH	Ptch1		
CG1915	sls	DRSC08670	44013	Dm.2974	25517			pqn-43	
CG9750	rept	DRSC11388	40092	Dm.7319	4856	RUVBL2	Ruvbl2	4H682	RVB2
CG9748	bel	DRSC16555	45826	Dm.7823	8069		Ddx3y, D1Pas1		DBP1
CG4003	pont	DRSC17029	53439	Dm.1557	2749	RUVBL1	Ruvbl1	5K460	RVB1
CG9924	CG9924	DRSC16580	41704	Dm.10784	20788	SPOP	Spop	mel-26	
CG10706	SK	DRSC17888	31456	Dm.11909	1694	KCNN1	Kcnn1	XG9	
CG2253	Upf2	DRSC18218	31724	Dm.17613	6101	UPF2	Upf2		NMD2
CG32688	Hk	DRSC18674	31955	Dm.4020	22588	KCNAB2	Kcnab2		
CG2411	ptc	DRSC05117	35851	Dm.2630	223	PTCH	Ptch1		
CG6105	l(2)06225	DRSC02928	46069	Dm.7708	25670		LOC231046		

Supplementary Table 4.

Summary Information and Scores for Genes tested in Secondary Assays.

Hh average and Hh+GL2 average are the average scores of the assays in which Act5C-Hh and the control vector were cotransfected with the ptcΔ136-GL3 and ptcΔ136-GL2 reporter, respectively. Reversed Average indicates the scores of the secondary assay in which a Pol III-GL3 control reporter was cotransfected with a ptcΔ136-Renilla experimental reporter. The Pol III-Hh average is the score of the assay in which Pol III-Hh was substituted for Act5C-Hh and cotransfected with the ptcΔ136-GL3 reporter. "Secondary Average" is the average of these first four secondary assay scores. For the No Hh Induction experiment, empty Act5C vector was substituted for Act5C-Hh and cotransfected with the ptcΔ136-GL3 reporter. For the Ci overexpression assay, an equal amount of Act5C-Ci (full-length) was substituted for the Act5C-Hh and cotransfected with the ptcΔ136-GL3 reporter. Values are percentage change relative to the GFP controls with differences greater than -25% or +50% indicated with orange and blue highlighting, respectively. Scores are arranged with hits which pass all of the first four secondary assays listed at top, followed by hits which pass progressively fewer of these four secondary assays. Hits which reduce Hh signaling are listed before those that increase Hh signaling and then, within these categories, hits are listed from higher to lower No Hh assay scores. The "Percent change from Renilla average" column indicates the percentage change from the mean plate Renilla score and gives an approximate measure of the change in cell number of transfected cells. Renilla scores less than 50% of the plate average are highlighted in pink, while Renilla scores 50% or greater than the plate average are highlighted in gray. Both the single and averaged raw Z-scores from primary screen replicates 1 and 2 are listed, as is the value that one plate standard deviation corresponds to in terms of percentage of the original primary plate average value. Also listed are the number of potential off-target genes as described in the Supplementary Note. Entrez Gene, Unigene, and Homologene identifiers are also listed. Occasionally, a particular CG# appears more than once in the secondary assay CG list because more than one dsRNA targeting that CG# was included in the secondary screening plates. Scores for the Smo, Ci, Firefly Luciferase ("Luc"), th, and GFP dsRNA control wells are also listed at the bottom of the table. At the bottom of each secondary assay column, the average, normalized luciferase score for the GFP dsRNA control wells in each plate is listed to give a sense of the actual normalized values for each secondary assay. If known, human, mouse, yeast, and *C. elegans* orthologs are listed. Protein domains, as annotated in Flybase, are indicated. Groups of genes with similar function have been color coded according to the legend at the bottom of the table.

Supplementary Table 4--Scores and Summary Information for genes tested in Secondary Assays.

CG/DRSC#	Gene	Number Potential Secondary Targets	Hh+GL2 Average	Hh Average	Reversed Average	Pol III-Hh Average	Secondary Average	Ci Average	No Hh Average	% Change from Plate Renilla Average	Primary Averaged Z-Score	Z-Score #1	Replicate #1 Standard Deviation as % of Plate Mean	Z-Score #2	Replicate #2 Standard Deviation as % of Plate Mean	DRSC Amplicon	Entrez Gene
CG1708	cos	0	-54.6	-72.7	-63.5	-59.1	-62.5	61.3	123.1	28.0	-2.80	-3.31	12.71	-2.29	13.43	DRSC07598	35653
CG3949	hoip	0	-42.3	-75.7	-59.6	-71.4	-62.2	19.5	113.4	-93.5	-2.92	-1.71	17.75	-4.13	13.14	DRSC03546	44173
CG11700	CG11700	3	-68.7	-76.2	-70.2	-62.3	-69.4	27.4	52.9	-88.5	-3.31	-3.47	20.09	-3.15	19.21	DRSC17794	31564
CG1874	CG1874	0	-40.5	-25.0	-45.4	-31.8	-35.7	38.4	45.0	-9.5	-3.75	-3.60	13.37	-3.90	12.85	DRSC06787	35983
CG5684	CG5684	0	-35.0	-34.0	-36.0	-32.3	-34.3	-0.1	37.3	71.6	-3.07	-3.48	13.72	-2.65	14.47	DRSC10537	39366
CG10325	abd-A	381	-55.6	-63.4	-66.4	-29.5	-53.7	-33.0	34.3	-80.5	-2.59	-3.56	14.50	-1.62	26.15	DRSC16897	42037
CG7109	mts	0	-48.6	-62.4	-62.6	-62.9	-59.1	42.4	18.6	-53.6	-2.84	-3.65	15.06	-2.03	17.64	DRSC03574	45959
CG10198	Nup98	0	-29.9	-59.1	-31.5	-36.7	-39.3	-16.3	13.8	-59.7	-2.94	-3.78	12.75	-2.10	12.23	DRSC14209	42816
CG2161	Rga	0	-35.4	-28.7	-34.9	-25.3	-31.1	3.3	12.7	28.9	-2.32	-2.94	12.97	-1.69	18.60	DRSC12364	40683
CG10198	Nup98	0	-48.8	-67.1	-50.4	-42.7	-52.3	-28.0	2.3	-19.0	-3.46	-3.51	14.46	-3.41	9.37	DRSC14210	42816
CG8108	CG8108	4	-57.2	-50.2	-67.7	-52.4	-56.9	-36.4	2.1	-30.6	-3.31	-3.34	14.68	-3.28	21.62	DRSC09675	39161
CG8367	cg	174	-79.9	-80.2	-77.7	-61.7	-74.9	-43.7	1.9	-87.5	-4.51	-4.25	14.15	-4.76	13.58	DRSC07650	36571
CG4453	Nup153	0	-36.7	-47.0	-39.8	-36.0	-39.9	-42.1	-0.2	-34.3	-3.18	-3.12	13.88	-3.24	9.66	DRSC19904	32630
CG11502	svp	310	-51.2	-56.2	-40.7	-27.1	-43.8	-35.7	-3.3	-70.5	-2.28	-2.81	16.35	-1.75	21.63	DRSC15311	41491
CG14519	CG14519	25	-33.1	-54.7	-52.9	-46.7	-46.9	-35.8	-4.8	-87.9	-2.10	-2.69	16.01	-1.51	21.24	DRSC14871	43419
CG4453	Nup153	0	-41.4	-62.1	-40.9	-43.8	-47.1	-44.0	-10.2	-16.8	-2.16	-3.52	12.61	-0.80	11.16	DRSC19904	32630
CG6043	CG6043	340	-66.5	-55.8	-64.0	-41.1	-56.8	-54.4	-11.4	-62.7	-3.85	-3.54	11.53	-4.16	10.91	DRSC02922	34732
DRSC00977		2	-34.9	-48.9	-34.4	-31.8	-37.5	-34.8	-12.9	12.5	-4.19	-4.15	11.59	-4.22	8.73	DRSC00977	
CG9324	CG9324	0	-34.1	-57.8	-63.2	-55.9	-52.7	-33.0	-15.0	18.0	-3.25	-2.99	12.71	-3.51	13.43	DRSC03201	35342
CG32046	CG32046	6	-46.0	-63.5	-53.0	-61.6	-56.0	-62.0	-15.5	12.0	-2.09	-3.07	14.68	-1.11	21.62	DRSC10171	39145
CG7913	PP2A-B'	87	-62.1	-63.2	-50.2	-42.9	-54.6	-55.2	-16.1	-52.7	-2.51	-2.40	22.65	-2.62	11.27	DRSC16337	42169
CG13936	CG13936	0	-41.7	-49.7	-47.1	-45.2	-46.0	-23.5	-16.1	48.2	-2.53	-3.60	13.85	-1.46	17.56	DRSC08405	38242
CG6551	fu	0	-58.3	-72.5	-72.9	-73.5	-69.3	-8.0	-16.2	43.3	-3.03	-5.34	12.85	-0.72	23.15	DRSC20333	32855
DRSC03777		0	-40.6	-54.1	-38.8	-43.6	-44.3	-33.2	-17.2	171.2	-4.07	-3.95	9.69	-4.20	10.35	DRSC03777	
CG6699	beta'Cop	0	-31.4	-73.4	-34.7	-64.6	-51.0	-29.4	-17.6	3.9	-2.34	-2.20	15.71	-2.48	16.93	DRSC03492	45757
CG14813	deltaCOP	0	-33.1	-66.6	-36.3	-66.0	-50.5	-16.9	-18.7	-50.0	-2.54	-2.45	20.61	-2.62	18.83	DRSC18760	45250
DRSC08092		12	-47.8	-65.6	-58.1	-53.4	-56.2	-52.9	-18.9	-17.2	-4.75	-4.41	11.66	-5.09	11.52	DRSC08092	
CG16901	sqd	0	-35.2	-46.0	-28.8	-50.7	-40.2	-15.4	-20.5	-2.1	-2.27	-2.68	20.67	-1.86	22.91	DRSC17066	41666
CG8922	RpS5	0	-30.9	-62.6	-36.3	-39.1	-42.3	-30.2	-22.5	49.1	-4.49	-4.24	12.61	-4.74	11.16	DRSC20282	32700
CG4912	eEF1delta	0	-27.5	-42.6	-34.0	-29.9	-33.5	-52.4	-23.5	-23.5	-2.13	-1.71	15.71	-2.56	16.93	DRSC02790	34363
CG4157	Rpn12	0	-48.0	-66.2	-56.7	-56.9	-56.9	-40.3	-23.5	-26.5	-3.71	-3.33	16.76	-4.10	13.32	DRSC11275	39845
DRSC05108		2	-65.7	-73.5	-73.8	-75.9	-72.2	-51.1	-23.8	-52.9	-5.49	-4.46	11.67	-6.52	10.71	DRSC05108	
DRSC20534		17	-33.7	-46.9	-53.9	-53.2	-46.9	29.1	-24.8	-68.3	-2.23	-3.88	12.85	-0.57	30.81	DRSC20534	
CG4013	Smr	513	-54.9	-60.8	-52.9	-27.3	-49.0	-50.8	-25.3	-39.5	-3.01	-3.37	14.94	-2.65	21.22	DRSC20288	32225
CG40341	CG40341	1	-29.4	-46.4	-32.3	-47.0	-38.8	-40.7	-26.4	1.1	-2.24	-2.09	9.69	-2.39	10.35	DRSC03750	
CG15552	Sox100B	0	-34.7	-44.8	-37.7	-53.3	-42.6	-36.5	-27.0	39.2	-2.36	-2.86	16.01	-1.85	21.24	DRSC15090	45039
CG2184	Mlc2	0	-49.6	-55.2	-62.1	-56.0	-55.7	-3.6	-28.4	0.1	-2.12	-2.18	20.61	-2.06	18.83	DRSC16741	43587
DRSC05037		1	-55.9	-62.3	-70.6	-65.5	-63.6	-29.7	-28.9	36.7	-5.04	-4.57	11.67	-5.51	10.71	DRSC05037	
CG7757	CG7757	0	-27.7	-47.7	-32.1	-36.8	-36.1	-7.9	-30.0	-2.5	-2.10	-2.38	12.89	-1.82	16.59	DRSC10912	40172
CG4602	Srp54	0	-35.4	-55.0	-25.5	-57.3	-43.3	-26.7	-30.2	86.2	-2.02	-2.45	15.71	-1.58	16.93	DRSC03442	34312
CG4501	bgm	0	-55.0	-75.6	-29.7	-74.5	-58.7	-40.6	-30.3	-10.7	-2.72	-2.85	15.71	-2.59	16.93	DRSC03495	44117
CG6292	CycT	4	-48.8	-77.2	-32.1	-53.7	-52.9	-7.0	-30.9	-28.4	-3.21	-3.93	13.88	-2.49	13.21	DRSC11124	39961
CG8435	CG8435	0	-36.7	-56.9	-38.8	-42.3	-43.7	-34.9	-31.7	-22.1	-2.56	-3.11	12.91	-2.02	14.61	DRSC07179	36789
DRSC05797		19	-30.2	-46.6	-37.6	-36.0	-37.6	-36.0	-31.9	-47.7	-3.03	-2.75	12.24	-3.32	11.47	DRSC05797	
CG7425	eff	0	-54.1	-59.8	-63.0	-65.7	-60.6	-48.8	-33.0	-14.3	-2.79	-3.08	14.50	-2.49	26.15	DRSC16940	41785
CG2807	CG2807	0	-33.8	-67.9	-32.6	-51.1	-46.4	-30.1	-33.1	-23.0	-3.59	-4.89	12.61	-2.28	11.16	DRSC00535	33235

CG/DRSC#	Gene	Number Potential Secondary Targets	Hh+GL2 Average	Hh Average	Reversed Average	Pol III-Hh Average	Secondary Average	Ci Average	No Hh Average	% Change from Plate Renilla Average	Primary Averaged Z-Score	Z-Score #1	Replicate #1 Standard Deviation as % of Plate Mean	Z-Score #2	Replicate #2 Standard Deviation as % of Plate Mean	DRSC Amplicon	Entrez Gene
DRSC00405		1	-39.0	-51.6	-38.5	-53.4	-45.6	-37.8	-33.3	-38.7	-2.62	-3.48	12.61	-1.77	11.16	DRSC00405	
CG8936	Arpc3B	0	-43.0	-64.4	-40.4	-66.0	-53.5	-53.6	-33.6	-16.9	-2.23	-3.34	13.38	-1.11	23.15	DRSC20126	32696
CG11985	CG11985	0	-27.4	-29.9	-34.1	-32.8	-31.0	-23.1	-33.6	-28.6	-2.05	-2.84	12.97	-1.25	18.60	DRSC14460	50007
CG8877	CG8877	0	-30.4	-71.0	-26.0	-35.4	-40.7	-37.8	-34.0	-17.4	-2.48	-2.32	20.40	-2.63	18.24	DRSC07293	36304
CG33231	CG33231	1	-40.2	-67.4	-37.1	-60.4	-51.2	-30.7	-34.3	-23.2	-2.35	-2.45	12.91	-2.26	14.61	DRSC08296	2769003
CG4893	CG4893	0	-27.7	-37.2	-39.0	-61.9	-41.5	-21.8	-34.6	-45.2	-2.02	-1.49	16.76	-2.54	13.32	DRSC10455	39813
DRSC14042		0	-31.5	-43.5	-51.8	-34.5	-40.3	-39.9	-35.2	-8.2	-3.15	-2.48	15.32	-3.82	7.96	DRSC14042	
CG3436	CG3436	0	-33.8	-65.6	-34.7	-70.4	-51.1	-52.3	-35.8	12.8	-2.23	-2.74	15.06	-1.72	17.64	DRSC00605	33180
CG5378	Rpn7	0	-58.7	-75.5	-61.1	-67.1	-65.6	-49.3	-36.2	-32.7	-4.82	-4.76	12.75	-4.88	12.23	DRSC16841	42641
CG5969	CG5969	0	-38.8	-60.8	-38.9	-63.8	-50.6	-42.1	-37.0	5.3	-2.22	-2.25	13.88	-2.19	13.21	DRSC11789	40270
CG31111	CG31111	0	-54.1	-67.6	-41.3	-72.7	-58.9	-40.6	-37.3	1.0	-3.44	-3.55	14.46	-3.33	9.37	DRSC14649	318595
CG7434	RpL22	1	-51.7	-60.5	-28.4	-74.0	-53.6	-38.4	-37.4	70.2	-4.32	-4.56	13.88	-4.08	9.66	DRSC18707	31022
CG11416	CG11416	6	-29.2	-56.7	-41.6	-40.9	-42.1	-49.0	-37.8	-10.8	-2.94	-3.02	12.71	-2.86	13.43	DRSC04111	37924
CG11981	Prosbeta3	0	-50.0	-70.8	-47.3	-58.3	-56.6	-48.8	-37.9	-10.6	-3.85	-3.59	14.22	-4.12	14.16	DRSC16801	41079
CG13130	CG13130	7	-47.1	-61.7	-57.4	-65.3	-57.9	-42.2	-38.3	13.8	-3.07	-2.89	17.75	-3.26	13.14	DRSC02226	50439
CG8179	CG8179	371	-41.2	-60.6	-50.4	-60.0	-53.1	-37.9	-38.4	16.9	-2.14	-2.22	15.06	-2.07	8.53	DRSC07091	36712
CG4609	fax	0	-53.3	-73.5	-42.1	-66.9	-59.0	-42.2	-38.6	18.3	-3.08	-2.80	13.88	-3.37	13.21	DRSC11345	39826
CG5758	CG5758	5	-29.3	-45.1	-25.7	-43.0	-35.8	-33.8	-38.8	-1.6	-2.41	-2.18	11.53	-2.63	10.91	DRSC02889	35083
CG5179	Cdk9	0	-30.3	-62.7	-29.2	-55.3	-44.4	-20.0	-39.0	7.0	-2.06	-2.08	13.11	-2.04	18.68	DRSC04601	37586
CG1821	RpL31	0	-58.4	-78.5	-44.7	-77.4	-64.8	-34.7	-39.1	-10.2	-3.92	-3.32	13.37	-4.52	12.85	DRSC06716	35988
CG12750	CG12750	0	-28.1	-60.4	-52.3	-50.0	-47.7	-33.8	-39.7	-84.5	-2.08	-1.75	15.71	-2.42	16.93	DRSC02179	35099
DRSC14061		36	-55.3	-71.2	-53.7	-50.6	-57.7	-45.6	-39.8	18.1	-4.36	-3.72	15.32	-5.00	7.96	DRSC14061	
CG6846	CG6846	0	-52.2	-74.0	-48.8	-77.7	-63.2	-36.0	-40.4	-46.6	-3.64	-4.57	12.89	-2.72	16.59	DRSC10726	40060
CG1475	CG1475	0	-52.2	-83.5	-39.4	-76.5	-62.9	-36.5	-41.3	22.5	-4.02	-3.65	14.22	-4.39	14.16	DRSC12265	40687
CG7835	CG7835	14	-45.9	-39.0	-32.0	-50.7	-41.9	-39.0	-42.1	-61.3	-2.18	-2.73	13.38	-1.62	23.15	DRSC20058	32771
CG7219	CG7219	0	-44.7	-45.7	-46.3	-61.7	-49.6	-10.2	-42.1	-41.4	-2.33	-2.35	17.75	-2.32	13.14	DRSC03029	34091
CG4585	CG4585	0	-46.3	-69.0	-27.6	-63.8	-51.7	-39.8	-42.4	14.8	-3.18	-3.54	12.71	-2.83	13.43	DRSC04475	37782
CG1483	Map205	0	-42.0	-62.3	-34.2	-61.6	-50.0	-35.2	-42.7	-31.0	-1.96	-1.78	20.61	-2.14	18.83	DRSC16732	43765
CG3193	crn	0	-41.5	-80.3	-50.9	-77.9	-62.6	-46.7	-42.7	-2.2	-3.01	-3.30	20.61	-2.72	18.83	DRSC18755	31208
CG3278	Tif-IA	0	-45.1	-60.2	-29.0	-53.6	-47.0	-47.5	-42.8	76.6	-2.76	-2.80	13.66	-2.73	12.94	DRSC03726	35454
CG3278	Tif-IA	0	-44.7	-61.7	-32.6	-56.4	-48.9	-48.2	-43.0	1.2	-2.41	-2.82	12.71	-2.01	13.43	DRSC03717	35454
CG32767	CG32767	6	-37.1	-37.6	-40.6	-53.3	-42.2	-42.7	-43.1	-41.5	-2.21	-2.24	20.09	-2.18	19.21	DRSC18386	31430
DRSC00245		8	-54.5	-52.4	-77.9	-72.0	-64.2	-54.4	-43.3	-43.9	-5.66	-5.57	9.83	-5.76	6.73	DRSC00245	
CG8580	CG8580	0	-52.1	-72.8	-58.0	-69.8	-63.2	-61.0	-43.4	46.6	-3.52	-4.37	13.72	-2.66	14.47	DRSC11000	38821
CG9885	dpp	0	-36.0	-62.8	-34.7	-70.6	-51.0	-56.9	-43.4	-32.3	-4.48	-5.01	9.83	-3.95	6.73	DRSC00118	33432
CG3193	crn	1	-44.4	-71.8	-56.1	-73.7	-61.5	-21.9	-45.3	-9.3	-3.04	-4.12	15.76	-1.95	25.47	DRSC18184	31208
CG14210	.4210, CG33	0	-51.3	-60.0	-37.4	-59.3	-52.0	-38.9	-45.4	14.5	-2.75	-3.88	12.85	-1.63	30.81	DRSC19566	318844, 3295
CG13445	CG13445	6	-32.0	-57.7	-40.1	-48.8	-44.7	-47.4	-46.7	-10.7	-3.40	-3.00	16.76	-3.80	13.32	DRSC09992	39719
CG2097	CG2097	0	-51.3	-63.3	-25.9	-54.0	-48.6	-48.6	-48.2	49.8	-2.87	-2.77	14.22	-2.97	14.16	DRSC12301	40709
CG17489	yip6	1	-60.3	-65.5	-47.0	-76.1	-62.2	-15.6	-50.3	-32.1	-4.10	-3.56	13.66	-4.65	12.94	DRSC03801	
CG6015	CG6015	0	-43.4	-76.4	-32.2	-71.5	-55.9	-57.0	-54.1	42.7	-3.76	-3.59	12.75	-3.94	12.23	DRSC15948	42593
CG10541	Tektin-C	0	-50.5	-57.7	-29.6	-61.3	-49.8	-37.5	-54.2	24.5	-2.93	-3.13	13.08	-2.72	21.97	DRSC09741	38653
CG6987	SF2	0	-44.5	-69.2	178.1	-43.0	5.4	-82.9	-56.0	95.7	-2.35	-3.49	14.50	-1.21	26.15	DRSC16845	53443
CG12238	CG12238	2	-63.6	-69.4	-66.2	-64.8	-66.0	-61.7	-65.4	38.8	-3.75	-3.74	12.61	-3.76	11.16	DRSC19450	32965
CG40218	CG40218	1	287.9	127.0	146.2	161.4	180.6	154.7	181.7	-47.1	8.31	9.50	15.06	7.12	8.53	DRSC21138	
CG1135	CG1135	0	174.1	76.1	130.8	199.9	145.2	152.8	155.5	-48.2	4.98	5.03	13.72	4.93	14.47	DRSC08180	38478

CG/DRSC#	Gene	Number Potential Secondary Targets	Hh+GL2 Average	Hh Average	Reversed Average	Pol III-Hh Average	Secondary Average	Ci Average	No Hh Average	% Change from Plate Renilla Average	Primary Averaged Z-Score	Z-Score #1	Replicate #1 Standard Deviation as % of Plate Mean	Z-Score #2	Replicate #2 Standard Deviation as % of Plate Mean	DRSC Amplicon	Entrez Gene
CG10139	CG10139	0	545.0	239.2	129.0	189.0	275.5	294.3	330.5	-61.4	7.20	8.40	20.40	6.01	18.24	DRSC05972	36626
CG5499	His2Av	0	164.1	101.2	109.5	113.1	122.0	124.6	165.1	-64.7	4.62	4.85	20.61	4.39	18.83	DRSC16702	43229
CG8233	CG8233	1	520.6	316.1	137.8	428.8	350.8	663.8	394.9	-82.9	8.05	8.50	20.40	7.60	18.24	DRSC07116	36560
CG4699	CG4699	0	1077.1	375.5	361.6	306.0	530.0	656.3	741.4	-91.7	19.79	21.86	12.75	17.72	12.23	DRSC15625	41911
CG14023	CG14023	302	-26.2	-46.0	-34.4	-13.7	-30.1	17.4	99.7	-77.6	-2.08	-1.88	15.06	-2.29	17.64	DRSC02353	33761
CG12977	CG12977	250	-37.4	-33.9	-35.3	7.1	-24.9	-39.5	13.7	-64.2	-2.06	-2.85	12.89	-1.27	16.59	DRSC11687	40334
CG14952	CG14952	6	-27.7	-45.3	-42.6	-11.4	-31.8	-33.9	4.8	-63.2	-2.14	-1.61	13.85	-2.66	17.56	DRSC08412	38348
CG32381	unc-13-4A	233	-28.2	-34.7	-27.6	1.9	-22.2	-41.1	2.7	-48.4	-2.45	-2.68	13.08	-2.22	21.97	DRSC10203	38801
CG32296	CG32296	62	-35.9	-46.6	-41.8	-0.8	-31.3	-65.5	-1.3	-38.2	-2.08	-2.54	13.85	-1.63	17.56	DRSC08263	38338
CG32776	CG32776	123	-43.8	-35.4	-27.7	12.5	-23.6	-45.6	-10.2	-79.2	-2.29	-2.16	20.09	-2.41	19.21	DRSC18119	31344
CG12054	CG12054	187	-42.5	-44.8	-39.3	-6.3	-33.2	-44.1	-14.9	-60.7	-2.36	-2.21	20.61	-2.51	18.83	DRSC14467	43694
CG32474	dys	387	-55.3	-56.9	-52.4	-16.1	-45.2	-52.3	-17.9	-79.7	-2.53	-2.00	14.46	-3.06	9.37	DRSC14897	43174
CG14459	CG14459	341	-60.6	-36.9	-30.7	-22.1	-37.6	-43.2	-19.4	23.3	-2.39	-1.07	12.89	-3.71	16.59	DRSC11719	40476
CG12852	CG12852	385	-56.9	-47.5	-60.8	-18.0	-45.8	-37.7	-21.2	-72.3	-2.44	-3.19	16.01	-1.70	21.24	DRSC14558	43365
CG31145	CG31145	161	-40.5	-30.7	-27.4	-18.3	-29.2	-44.5	-22.3	4.6	-3.20	-3.27	14.46	-3.13	9.37	DRSC14670	42784
CG33110	CG33110	210	-28.6	-46.7	-47.5	-12.9	-33.9	-59.0	-24.2	-66.4	-3.11	-2.63	14.46	-3.60	9.37	DRSC14694	42659
CG13165	CG13165	235	-35.8	-34.6	-33.5	9.3	-23.6	-50.1	-30.7	-40.1	-2.94	-2.75	14.15	-3.14	13.58	DRSC06292	36330
CG8787	Asx	12	-42.9	-29.0	-43.3	-18.1	-33.3	-54.4	-33.7	-52.1	-2.51	-2.73	14.15	-2.28	13.58	DRSC05924	36612
CG5575	ken	80	-41.1	-43.2	-35.4	11.9	-27.0	-28.9	-34.0	-67.7	-2.86	-2.45	12.71	-3.28	13.43	DRSC04696	37785
CG7481	RhoGAP18B	1	-59.5	-60.6	-29.8	-18.3	-42.1	-65.3	-47.8	59.0	-2.57	-4.45	12.85	-0.70	30.81	DRSC20049	32898
DRSC14786		390	-40.2	-38.6	-30.7	-2.9	-28.1	-52.3	-19.1	-58.0	-2.52	-2.52	22.65	-2.52	11.27	DRSC14786	
DRSC14674		559	-53.6	-57.2	-69.8	-6.3	-46.7	-39.8	21.4	-90.4	-3.78	-3.44	14.46	-4.11	9.37	DRSC14674	
CG7776	E(Pc)	0	-46.0	-52.3	-12.8	-37.0	-37.0	-14.2	6.0	48.4	-2.38	-2.39	14.15	-2.38	13.58	DRSC07408	36238
CG7269	Hel25E	0	-45.1	-57.5	-6.4	-42.5	-37.9	-27.9	-4.8	-60.6	-3.34	-4.03	15.06	-2.65	17.64	DRSC03342	33781
CG7664	crp	0	-48.1	-43.2	-21.5	-28.1	-35.2	-41.1	-6.3	100.5	-2.54	-2.07	11.53	-3.01	10.91	DRSC03515	34956
CG9094	CG9094	0	-31.4	-47.3	7.8	-29.4	-25.1	-37.0	-11.1	182.3	-2.79	-2.62	11.66	-2.97	11.52	DRSC07936	38117
CG13847	CG13847	107	-30.3	-46.8	-23.5	-27.9	-32.1	-29.4	-12.4	-60.0	-2.15	-2.19	12.75	-2.11	12.23	DRSC14697	42636
CG32149	RhoGAP71E	209	-35.0	-44.9	-23.6	-38.1	-35.4	-58.3	-17.3	-6.5	-2.30	-1.87	16.76	-2.74	13.32	DRSC10837	39693
CG30044	CG30044	1	-38.0	-53.8	-3.0	-48.0	-35.7	-35.4	-18.7	81.2	-2.93	-3.32	14.15	-2.54	13.58	DRSC06277	36371
CG5602	CG5602	0	-39.5	-50.4	-16.1	-51.1	-39.3	-34.0	-20.0	35.0	-2.64	-2.41	12.71	-2.87	13.43	DRSC04518	37791
CG2013	UbcD6	0	-31.6	-47.0	-6.6	-35.1	-30.1	-45.0	-20.1	34.2	-2.20	-2.32	14.22	-2.09	14.16	DRSC12370	40610
CG15255	CG15255	0	-32.7	-46.5	-13.9	-46.8	-35.0	-41.7	-20.2	73.3	-2.08	-1.57	11.53	-2.58	10.91	DRSC01869	34913
CG32592	hiw	5	-32.2	-44.2	-20.5	-27.4	-31.1	-33.3	-24.4	42.2	-2.22	-3.01	13.38	-1.42	23.15	DRSC20338	32429
CG9429	Crc	0	-25.8	-41.3	-7.5	-36.1	-27.7	-49.4	-25.6	37.4	-2.08	-2.82	16.35	-1.34	21.63	DRSC16614	41166
CG2128	HDAC3	0	-49.2	-68.1	-24.6	-39.1	-45.3	-41.2	-25.9	170.2	-3.04	-3.02	14.22	-3.07	14.16	DRSC12352	44446
CG6962	CG6962	0	-47.4	-66.2	-23.8	-54.0	-47.8	-32.2	-28.1	-3.6	-2.06	-2.08	14.22	-2.03	14.16	DRSC16153	41436
CG32955	CG32955	2	-45.8	-58.5	-22.1	-54.9	-45.3	-19.0	-29.7	48.5	-2.51	-2.46	11.59	-2.56	8.73	DRSC01163	318268
CG4268	Pitslre	1	-32.6	-59.0	-24.4	-40.3	-39.1	-29.5	-29.7	-3.9	-2.80	-2.66	13.88	-2.93	13.21	DRSC11874	40292
CG32253	CG32253	0	-41.2	-63.2	-19.0	-52.4	-43.9	-46.6	-30.1	48.1	-2.05	-2.27	13.72	-1.84	14.47	DRSC08202	326206
CG14656	CG14656	5	-45.7	-56.8	-23.7	-47.2	-43.3	-41.4	-31.6	137.3	-2.52	-3.19	12.89	-1.84	16.59	DRSC12242	40597
CG12254	Arc92	537	-34.0	-49.5	-21.8	-26.9	-33.0	-47.3	-32.3	-15.3	-2.56	-2.18	12.75	-2.95	12.23	DRSC14483	42385
CG18468	CG18468	0	-35.4	-51.0	-4.0	-33.2	-30.9	-38.1	-33.9	81.9	-2.66	-2.59	12.91	-2.72	14.61	DRSC06759	36957
CG9282	CG9282	0	-45.4	-69.8	-21.0	-69.2	-51.4	-7.6	-34.7	16.0	-3.99	-3.58	11.53	-4.40	10.91	DRSC03185	34754
CG2685	CG2685	0	-55.4	-55.2	-2.8	-41.4	-38.7	-64.1	-35.0	97.4	-2.14	-2.97	15.76	-1.30	25.47	DRSC18463	31259
CG9176	cngl	3	-35.0	-46.4	-23.6	-47.7	-38.2	-21.1	-35.1	44.3	-2.08	-2.67	13.38	-1.48	23.15	DRSC20318	32468
CG5786	ppan	0	-42.4	-57.6	-21.0	-48.4	-42.3	-49.9	-35.2	91.2	-3.19	edge	22.65	-3.19	11.27	DRSC17030	42502

CG/DRSC#	Gene	Number Potential Secondary Targets	Hh+GL2 Average	Hh Average	Reversed Average	Pol III-Hh Average	Secondary Average	Ci Average	No Hh Average	% Change from Plate Renilla Average	Primary Averaged Z-Score	Z-Score #1	Replicate #1 Standard Deviation as % of Plate Mean	Z-Score #2	Replicate #2 Standard Deviation as % of Plate Mean	DRSC Amplicon	Entrez Gene
CG3732	CG3732	8	-32.1	-56.6	0.2	-28.9	-29.4	-45.8	-36.7	99.5	-2.92	-2.81	12.71	-3.03	13.43	DRSC04410	37588
CG32150	CG32150	16	-29.2	-43.5	-22.5	-28.9	-31.0	-37.8	-37.0	29.8	-2.07	-1.19	16.76	-2.95	13.32	DRSC09899	39727
CG7292	Rrp6	0	-34.7	-51.7	-3.5	-35.5	-31.3	-60.7	-37.1	6.7	-2.22	-2.02	14.22	-2.42	14.16	DRSC16223	41798
CG31657	CG4124	0	-51.4	-62.2	-1.4	-54.6	-42.4	-49.9	-37.9	56.2	-2.94	-3.11	15.06	-2.78	17.64	DRSC00638	318873
CG6937	CG6937	1	-39.9	-59.5	-19.6	-36.9	-39.0	-50.0	-37.9	141.4	-2.25	-1.45	14.46	-3.05	9.37	DRSC16140	42662
CG5605	eRF1	0	-44.7	-57.8	-8.3	-50.8	-40.4	-64.4	-39.0	48.2	-2.40	-2.23	13.88	-2.58	13.21	DRSC11779	40240
CG15814	CG15814	0	-29.1	-33.1	-18.6	-45.3	-31.5	-23.4	-41.8	-4.6	-2.04	-2.38	12.61	-1.70	11.16	DRSC19730	32765
CG33106	mask	1	-33.3	-50.1	-24.4	-43.6	-37.8	-49.4	-42.4	18.0	-2.31	-2.92	12.75	-1.70	12.23	DRSC16018	50070
CG5427	CG5427	0	-36.6	-58.5	-2.8	-54.7	-38.1	-44.9	-43.7	16.5	-2.74	-3.30	15.71	-2.18	16.93	DRSC02859	34660
CG9842	Pp2B-14D	1	-27.0	-40.6	-12.1	-30.6	-27.6	-45.0	-43.8	43.3	-2.15	-2.64	13.38	-1.66	23.15	DRSC20270	32624
CG4152	l(2)35Df	0	-40.9	-57.1	-9.4	-46.4	-38.5	-63.5	-47.4	18.1	-2.17	-2.40	15.71	-1.93	16.93	DRSC03560	48782
CG12104	CG12104	0	-53.0	-61.8	12.6	-50.7	-38.2	-49.6	-56.8	195.3	-2.49	-2.64	12.91	-2.33	14.61	DRSC08254	38187
CG16918	CG16918	121	-62.9	-4.5	-65.1	-28.9	-40.4	-32.6	-3.5	-90.0	-3.51	-3.98	13.88	-3.05	9.66	DRSC15165	43366
CG7031	CG7031	357	-51.9	-7.5	-32.7	-28.7	-30.2	-30.0	-26.0	-35.0	-3.89	-3.69	14.46	-4.09	9.37	DRSC16178	42704
CG8264	Bx42	0	-23.9	-68.9	-32.0	-45.2	-42.5	-19.9	-0.7	-71.8	-2.94	-3.23	17.50	-2.66	16.83	DRSC17743	31840
CG15476	CG15476	2	-17.6	-44.6	-49.1	-37.4	-37.1	-58.0	-3.3	12.7	-2.08	-1.93	13.66	-2.23	12.94	DRSC02487	50474
CG7961	alphaCop	0	-16.7	-64.6	-41.2	-62.2	-46.2	-23.1	-8.8	-15.1	-3.01	-3.49	12.91	-2.53	14.61	DRSC08706	38199
CG6223	betaCop	0	-16.1	-57.1	-29.4	-63.7	-41.6	-23.3	-14.0	-22.7	-2.32	-2.64	12.61	-2.00	11.16	DRSC20312	32820
CG1528	gammaCop	0	-17.1	-56.9	-25.7	-56.1	-38.9	-16.0	-19.0	-18.0	-2.07	-2.38	15.76	-1.75	25.47	DRSC16955	43717
CG5931	CG5931	0	-22.6	-64.1	-39.3	-42.3	-42.1	-23.4	-19.5	-40.2	-3.23	-3.25	13.88	-3.20	13.21	DRSC10559	39737
CG5360	CG5360	0	-21.6	-46.2	-33.3	-36.0	-34.3	-51.9	-24.4	48.5	-2.53	-2.87	12.71	-2.19	13.43	DRSC04498	37723
DRSC00209		6	-4.4	-31.8	-30.7	-28.2	-23.8	-32.5	-20.6	-10.2	-3.02	-3.35	9.83	-2.69	6.73	DRSC00209	
CG7752	CG7752	0	165.2	73.4	48.0	92.4	94.8	37.3	131.2	-38.4	7.74	8.37	13.88	7.11	13.21	DRSC11848	40351
CG10279	Rm62	1	171.9	57.6	16.3	80.0	81.4	48.9	71.1	-23.1	5.99	6.58	14.22	5.39	14.16	DRSC12365	40739
CG1492	CG1492	0	75.8	107.9	48.4	76.6	77.2	116.1	55.4	-56.1	4.52	4.93	14.94	4.11	21.22	DRSC19605	32164
CG18041	CG18041	0	204.5	44.7	144.9	127.8	130.4	121.1	128.5	-27.8	4.96	4.84	20.61	5.08	18.83	DRSC15301	43529
CG30053	CG30053	0	55.3	43.3	-29.8	72.4	35.3	23.6	47.1	-20.3	4.95	6.09	14.15	3.81	13.58	DRSC07260	246419
CG1559	Upf1	0	82.3	-40.6	35.6	-27.5	12.4	-38.5	-26.7	23.0	7.41	7.25	17.50	7.58	16.83	DRSC19667	32153
CG31209	CG31209	141	-40.0	-34.2	-15.5	-4.8	-23.7	-47.5	-15.0	-58.4	-2.56	-2.19	22.65	-2.94	11.27	DRSC15701	42403
CG33141	sns	265	-37.1	-36.1	-16.5	-7.9	-24.4	-50.0	-16.3	24.6	-2.83	-3.42	13.37	-2.25	12.85	DRSC06423	44097
CG6064	CG6064	212	-32.9	-36.0	17.1	-9.1	-15.2	-42.7	-21.7	96.8	-2.96	-2.19	16.76	-3.72	13.32	DRSC10578	39970
CG5674	CG5674	106	-31.3	-40.0	-22.0	-19.0	-28.1	-50.8	-22.8	81.7	-2.70	-2.35	11.53	-3.06	10.91	DRSC02874	35072
CG32676	CG32676	129	-31.1	-42.7	-22.3	-7.7	-25.9	-49.4	-29.4	-73.3	-2.22	-2.76	14.94	-1.69	21.22	DRSC18442	32005
CG33183	Hr46	289	-29.7	-38.9	-12.7	-20.4	-25.4	-35.4	-13.5	-64.3	-2.49	-2.90	13.37	-2.09	12.85	DRSC06118	36073
CG10869	CG10869	0	-28.2	-33.2	-13.6	-20.3	-23.8	3.3	-29.8	-21.0	-2.09	-2.19	15.06	-2.00	17.64	DRSC00290	33337
CG5733	CG5733	0	-26.6	-54.4	-5.3	-22.6	-27.2	-38.2	10.9	37.1	-2.14	-2.15	12.91	-2.13	14.61	DRSC06947	37078
CG3895	ph-d	84	-26.3	-42.3	-22.8	-23.1	-28.6	-45.0	-30.5	-45.1	-2.24	-3.02	15.76	-1.46	25.47	DRSC18819	44889
CG12912	CG12912	538	-37.8	-22.8	11.8	-34.0	-20.7	-22.9	-28.9	120.8	-3.62	-3.98	13.37	-3.26	12.85	DRSC06227	36075
CG8426	l(2)NC136	0	-35.4	-16.3	-46.2	13.5	-21.1	77.6	45.4	-26.9	-3.23	-3.13	12.71	-3.33	13.43	DRSC04908	35501
DRSC01970		138	-27.5	-9.1	-25.7	-19.9	-20.5	-15.0	-15.3	-37.8	-2.27	-2.51	11.53	-2.02	10.91	DRSC01970	
DRSC14928		13	139.8	31.6	70.6	36.1	69.5	-20.1	50.0	-46.7	4.79	6.59	16.35	2.98	21.63	DRSC14928	
CG16941	CG16941	0	-24.6	-60.1	-14.6	-42.6	-35.5	-41.2	-34.9	-16.0	-3.35	-3.73	12.75	-2.98	12.23	DRSC15166	42048
CG3605	CG3605	0	-24.2	-67.3	-20.3	-36.1	-37.0	-39.1	-36.9	26.4	-3.06	-3.27	15.06	-2.86	17.64	DRSC00619	33514
CG6437	CG6437	0	-24.0	-57.0	-8.0	-31.4	-30.1	-27.7	-11.5	128.3	-2.27	-1.87	12.71	-2.67	13.43	DRSC04525	37516
CG6905	CG6905	0	-23.0	-57.6	-19.2	-39.8	-34.9	-34.4	-21.3	-22.1	-2.12	-1.86	12.91	-2.38	14.61	DRSC08577	38062
CG9667	CG9667	1	-22.7	-58.9	-13.2	-36.1	-32.7	-41.7	-30.1	124.9	-2.38	-2.66	12.97	-2.10	18.60	DRSC16535	40960

CG/DRSC#	Gene	Number Potential Secondary Targets	Hh+GL2 Average	Hh Average	Reversed Average	Pol III-Hh Average	Secondary Average	Ci Average	No Hh Average	% Change from Plate Renilla Average	Primary Averaged Z-Score	Z-Score #1	Replicate #1 Standard Deviation as % of Plate Mean	Z-Score #2	Replicate #2 Standard Deviation as % of Plate Mean	DRSC Amplicon	Entrez Gene
CG2925	noi	0	-19.7	-67.9	-22.9	-44.1	-38.6	-41.8	-38.1	34.2	-2.76	-2.91	14.22	-2.60	14.16	DRSC12383	40678
CG17272	CG17272	0	-18.9	-34.5	2.0	-36.4	-21.9	-33.5	-19.0	80.4	-2.58	-3.36	22.65	-1.80	11.27	DRSC15211	42465
CG6694	CG6694	0	-9.0	-48.3	-22.3	-34.0	-28.4	-45.8	5.6	-49.1	-1.99	-1.78	13.08	-2.20	21.97	DRSC10696	38968
CG6625	Snap	0	-7.9	-52.2	-10.1	-33.8	-26.0	-8.5	2.6	-31.8	-2.33	-2.67	13.88	-1.99	13.21	DRSC11285	40233
CG7923	Fad2	2	-5.8	-47.5	-24.1	-44.5	-30.5	-7.9	-0.2	-32.3	-2.27	-1.92	14.68	-2.62	21.62	DRSC11165	44006
CG3948	zetaCOP	0	-5.1	-47.5	-11.0	-35.3	-24.7	-13.2	-0.3	-10.7	-2.73	-4.93	16.76	-0.54	13.32	DRSC11412	39862
CG14180	CG14180	1	-2.2	-36.4	-14.8	-31.3	-21.2	-41.8	-12.1	-19.3	-2.06	-1.74	14.68	-2.39	21.62	DRSC10179	39101
CG7525	Tie	4	-11.5	-31.5	-22.3	-27.1	-23.1	-26.1	-29.3	15.6	-3.17	-3.09	11.66	-3.25	11.52	DRSC08104	38559
CG14342	CG14342	2	-10.3	-35.7	-33.6	-18.9	-24.6	-38.6	-14.1	-45.4	-2.06	-2.55	12.61	-1.57	11.16	DRSC00372	33310
CG7926	Axn	0	-9.2	-29.9	-28.1	-7.2	-18.6	-43.7	-22.9	-13.8	-2.23	-2.62	13.88	-1.85	9.66	DRSC14120	43565
CG18591	CG18591	0	-3.7	-47.2	-39.2	-21.8	-28.0	-22.7	-14.0	18.7	-2.03	-1.06	17.75	-3.00	13.14	DRSC02680	34080
CG33141	sns	454	-20.3	-37.3	-26.0	-6.3	-22.5	-48.1	-29.1	32.4	-2.14	-2.25	13.37	-2.03	12.85	DRSC07692	44097
DRSC02439		4	-6.4	-27.9	-43.3	-13.6	-22.8	-30.1	-23.8	-48.3	-2.31	-1.92	11.53	-2.70	10.91	DRSC02439	
DRSC08743		0	-7.2	-29.7	-44.6	-24.8	-26.6	-6.3	2.4	10.2	-2.33	-1.86	11.66	-2.81	11.52	DRSC08743	
CG4035	eIF-4E	1	86.1	-12.6	83.0	11.0	41.9	30.5	-17.1	-14.5	4.62	4.48	13.72	4.75	14.47	DRSC11342	45525
CG32688	Hk	11	89.7	-19.5	59.8	33.3	40.8	-46.7	-9.7	68.1	3.06	3.65	14.94	2.48	21.22	DRSC18674	31955
CG17077	pnt	75	97.2	39.1	103.3	40.0	69.9	31.2	-6.3	-80.2	3.20	2.22	14.46	4.19	9.37	DRSC17028	42757
CG18214	trio	25	50.0	-24.7	90.4	-2.4	28.4	2.2	16.7	-57.4	4.89	4.66	12.91	5.12	14.61	DRSC08527	43974
CG5825	His3.3A	1	141.3	27.6	60.4	21.2	62.6	-8.8	233.3	-79.2	2.96	3.95	15.64	1.98	16.26	DRSC03343	33736
CG1322	zfh1	0	72.7	7.2	2.2	76.5	39.7	26.9	39.3	-2.2	3.27	3.67	20.61	2.87	18.83	DRSC17098	43650
CG4903	MESR4	0	85.1	31.6	16.8	61.3	48.7	36.9	52.7	-65.7	3.17	4.90	12.26	1.45	20.02	DRSC06901	36986
CG3025	mof	1	126.7	47.9	6.0	92.9	68.4	56.4	65.5	-27.4	3.12	4.65	20.09	1.58	19.21	DRSC18795	31518
CG9750	rept	0	63.6	-43.6	26.7	-8.7	9.5	-14.2	-3.5	129.7	3.96	5.31	12.89	2.62	16.59	DRSC11388	40092
CG8954	Smg5	0	183.5	-29.5	11.1	-16.1	37.3	-28.3	-29.8	-8.4	5.25	5.92	15.71	4.58	16.93	DRSC03124	34804
CG7305	CG7305	421	-46.2	-1.8	-10.7	-5.7	-16.1	-20.4	-23.8	-66.5	-2.57	-1.79	22.65	-3.36	11.27	DRSC16225	42151
CG15494	CG15494	21	-34.1	-24.3	-9.5	-7.7	-18.9	-61.9	-29.9	-45.4	-2.32	-2.41	11.53	-2.23	10.91	DRSC02502	50445
CG9533	rut	556	-28.9	-20.0	-14.4	15.4	-11.9	-51.6	-28.7	-52.7	-2.55	-3.63	13.38	-1.46	23.15	DRSC20367	32406
CG13452	AGO2	95	-22.6	-30.7	9.1	-15.5	-14.9	7.7	1.9	26.4	-2.79	-2.13	16.76	-3.46	13.32	DRSC09999	39683
CG14641	CG14641	0	-19.8	-34.0	22.7	-3.9	-8.7	-46.8	-29.1	31.6	-2.42	-2.25	13.88	-2.59	13.21	DRSC12227	40529
CG2835	G-salpha60A	0	-18.6	-44.3	-16.5	-23.9	-25.8	-16.0	-17.0	39.9	-2.55	-2.57	12.71	-2.54	13.43	DRSC04616	37805
CG32029	CG32029	20	-18.0	-30.2	-11.5	-14.1	-18.4	-23.5	-19.3	21.0	-2.55	-2.53	13.08	-2.58	21.97	DRSC10646	38990
CG9930	E5	143	-17.9	-27.0	5.3	-10.9	-12.6	-52.5	-19.2	-51.2	-2.39	-3.19	14.50	-1.59	26.15	DRSC16585	45396
CG11194	Hey	358	-17.5	-26.3	10.7	4.9	-7.1	-45.7	-22.5	32.1	-3.00	-2.19	13.37	-3.81	12.85	DRSC07440	35764
CG11405	A3-3	458	-12.5	-27.0	-11.6	-4.2	-13.8	-49.1	-20.8	-13.2	-2.01	-3.00	15.76	-1.02	25.47	DRSC17225	43867
CG1915	sls	0	-12.1	-27.8	14.3	-6.1	-7.9	-25.8	-2.7	81.2	3.03	0.80	13.85	5.25	17.56	DRSC08670	44013
CG1017	CG1017	0	-10.0	-47.4	-15.1	-15.4	-22.0	-27.3	-22.3	-27.0	-2.19	-2.28	12.91	-2.11	14.61	DRSC08154	38256
CG9504	CG9504	0	-9.6	-28.3	24.9	-15.8	-7.2	-29.1	-18.1	83.5	3.10	-0.15	13.38	6.35	23.15	DRSC20187	32423
CG13333	CG13333	5	-3.5	-31.8	-22.9	-14.0	-18.1	-39.5	-5.6	-5.0	-2.17	-1.81	14.15	-2.53	13.58	DRSC06368	36509

Supplementary Table 4

CG/DRSC#	Gene	Number Potential Secondary Targets	Hh+GL2 Average	Hh Average	Reversed Average	Pol III-Hh Average	Secondary Average	Ci Average	No Hh Average	% Change from Plate Renilla Average	Primary Averaged Z-Score	Z-Score #1	Replicate #1 Standard Deviation as % of Plate Mean	Z-Score #2	Replicate #2 Standard Deviation as % of Plate Mean	DRSC Amplicon	Entrez Gene
CG32133	CG32133	9	2.6	-25.6	3.4	0.1	-4.9	-24.6	-19.4	-33.3	-2.02	-1.12	16.76	-2.91	13.32	DRSC11048	39552
CG4886	cyp33	0	15.7	-17.9	-46.6	-18.3	-16.8	-17.0	2.8	-40.5	-2.46	-2.32	12.24	-2.59	11.47	DRSC05666	36984
DRSC05475		6	5.7	-23.8	-25.1	-0.7	-11.0	-44.6	-27.9	-23.0	-2.17	-2.09	12.51	-2.26	12.19	DRSC05475	
CG32202	CG32202	0	62.7	8.8	16.5	16.8	26.2	35.4	50.1	30.9	3.59	3.51	16.76	3.67	13.32	DRSC09989	326199
CG10095	CG10095	0	67.5	-23.2	44.6	-11.3	19.4	-9.9	-15.2	56.7	4.64	4.69	16.35	4.59	21.63	DRSC14188	41473
CG8048	Vha44	0	72.2	-4.6	27.7	32.7	32.0	-0.1	0.8	-48.1	3.71	4.37	12.91	3.06	14.61	DRSC07572	36826
CG4003	pont	0	76.8	-19.3	38.0	-0.8	23.7	-2.2	1.5	15.4	3.22	3.40	16.35	3.04	21.63	DRSC17029	53439
CG10712	Chro	0	99.4	29.6	31.9	14.4	43.8	23.8	80.7	-28.7	3.66	3.91	13.88	3.40	13.21	DRSC11630	40508
CG11184	Upf3	0	101.2	-18.6	25.6	-2.5	26.4	-39.1	-22.9	-20.4	5.12	5.51	12.71	4.73	13.43	DRSC04092	37792
CG32743	Smg1	0	102.2	-15.6	-11.7	-23.6	12.8	-11.1	-26.3	-15.0	7.34	8.78	17.50	5.89	16.83	DRSC18366	31625
CG2253	Upf2	0	113.1	-8.3	17.8	-7.6	28.7	5.4	-23.9	-1.5	4.77	5.70	20.09	3.85	19.21	DRSC18218	31724
CG9748	bel	3	139.8	11.0	44.5	41.3	59.2	-3.5	17.1	-8.7	6.50	7.95	12.97	5.05	18.60	DRSC16555	45826
CG32743	Smg1	0	159.2	-9.2	13.5	10.0	43.4	-25.0	-21.7	-4.5	6.49	8.32	20.09	4.66	19.21	DRSC18367	31625
CG10306	CG10306	0	26.3	-11.8	76.4	30.9	30.5	-29.5	-10.2	-11.0	3.93	3.85	12.71	4.01	13.43	DRSC04056	37475
CG3412	slmb	0	36.2	35.8	70.3	9.5	37.9	126.6	1.8	-25.9	3.38	2.86	12.75	3.91	12.23	DRSC17056	42504
CG32541	CG32541	49	-17.4	-23.5	-8.7	-17.5	-16.8	-26.1	-16.9	6.2	-2.73	-4.34	12.85	-1.12	30.81	DRSC19543	32889
CG15783	CG15783	269	-16.0	-22.4	1.4	-1.5	-9.6	-10.6	-23.1	18.7	-2.45	-2.07	13.88	-2.84	9.66	DRSC18089	31467
CG1101	Aly	0	-14.8	-20.7	14.8	5.4	-3.8	-48.0	-15.5	58.2	-2.68	-1.39	15.06	-3.96	8.53	DRSC12497	44029
CG12607	CG12607	0	-11.8	-24.5	-9.7	-0.6	-11.6	-31.1	-11.8	6.4	4.86	-0.36	13.08	10.08	21.97	DRSC08287	38547
CG32139	Sox21b	103	-10.3	-15.5	5.6	4.2	-4.0	-43.9	-14.7	-19.3	-2.21	-1.82	16.76	-2.61	13.32	DRSC10634	39569
CG32432	CG32432	51	-7.8	-21.7	-17.6	1.5	-11.4	-42.4	-9.6	-66.5	-2.23	-2.84	12.89	-1.61	16.59	DRSC11704	40310
CG13222	CG13222	130	-4.5	-18.0	6.3	-9.4	-6.4	-31.8	-20.8	-9.6	-2.06	-1.36	14.15	-2.75	13.58	DRSC06343	36193
DRSC17378		0	4.8	-3.5	3.4	-18.6	-3.5	-41.5	-20.7	-26.8	-2.42	-1.00	15.32	-3.84	7.96	DRSC17378	
CG1546	PH4alphaSG	0	2.3	-23.3	7.6	-6.7	-5.0	-19.3	-21.6	36.4	2.93	0.52	16.01	5.33	21.24	DRSC15037	43623
CG8397	CG8397	0	2.3	-16.0	2.6	2.2	-2.2	-24.2	-9.5	19.9	2.87	0.17	12.26	5.56	20.02	DRSC07162	36767
CG9233	fu2	0	7.7	-11.6	14.7	-7.5	0.8	-23.3	-8.2	8.5	4.01	1.11	17.75	6.92	13.14	DRSC03324	34177
CG12822	8853, CG12	0	12.3	-6.9	-4.9	5.0	1.4	12.6	8.1	-20.3	-2.29	-2.11	9.92	-2.46	6.08	DRSC20757	35737, 24651
CG10706	SK	0	22.2	-6.4	45.5	19.1	20.1	0.0	-6.4	19.6	4.05	0.76	20.09	7.35	19.21	DRSC17888	31456
CG8277	CG8277	0	23.0	-16.4	32.0	-0.6	9.5	-23.4	-17.7	3.3	1.70	1.20	13.08	2.20	21.97	DRSC10975	38878
CG32050	CG32050	4	28.5	7.3	37.7	38.2	27.9	22.6	-4.4	-11.3	3.06	3.63	14.68	2.50	21.62	DRSC10173	39154
CG12079	CG12079	0	30.1	-16.4	29.1	14.8	14.4	1.3	13.2	4.4	3.06	2.61	13.72	3.51	14.47	DRSC08243	38378
CG14084	CG14084	0	39.4	-6.8	45.1	43.2	30.2	-12.6	-8.1	12.2	2.91	2.46	12.89	3.37	16.59	DRSC10088	40094
DRSC17256		0	33.7	5.2	3.3	13.5	13.9	-10.2	-7.1	-18.9	3.68	7.02	15.32	0.35	7.96	DRSC17256	
Ci 5'			-89.0	-92.6	-91.5	-93.7	-91.7	-72.4	37.7	25.8							
Ci 5'			-89.0	-91.0	-88.7	-94.2	-90.7	-73.4	30.5	40.8							
Ci 5'			-89.5	-90.9	-88.4	-88.8	-89.4	-72.8	24.7	39.6							
GFP			2.4	1.2	-1.3	2.5	1.2	0.2	1.2	-44.2							
GFP			-2.4	-1.2	1.3	-2.5	-1.2	-0.2	-1.2	-51.6							
GFP			1.5	0.1	-1.3	4.8	1.3	0.2	-1.2	-18.2							
GFP			-1.5	-0.1	1.3	-4.8	-1.3	-0.2	1.2	-25.5							
GFP			0.6	0.7	-2.1	2.0	0.3	-1.8	4.8	-27.8							
GFP			-0.6	-0.7	2.1	-2.0	-0.3	1.8	-4.8	-29.4							
Luc			-96.8	-96.5	979.5	-96.1	172.6	-88.9	-90.2	96.7							
Luc			-97.2	-95.9	992.7	-95.0	176.1	-88.0	-91.2	138.5							
Luc			-97.2	-96.3	1027.6	-96.7	184.4	-90.0	-92.1	84.1							
Smo 5'			-93.2	-95.2	-95.4	-96.9	-95.2	-37.8	-15.7	1.1							
Smo 5'			-93.3	-94.2	-92.7	-97.2	-94.3	-38.7	-16.5	46.3							
Smo 5'			-93.5	-94.1	-93.1	-96.9	-94.4	-34.1	-13.8	39.3							
th			-21.0	-51.2	-48.8	-35.1	-39.0	52.9	377.1	-98.0							
th			-8.7	-25.6	-33.6	-46.4	-28.6	87.1	393.6	-99.1							

CG/DRSC#	Gene	Number Potential Secondary Targets	Hh+GL2 Average	Hh Average	Reversed Average	Pol III-Hh Average	Secondary Average	Ci Average	No Hh Average	% Change from Plate Renilla Average	Primary Averaged Z-Score	Z-Score #1	Replicate #1 Standard Deviation as % of Plate Mean	Z-Score #2	Replicate #2 Standard Deviation as % of Plate Mean	DRSC Amplicon	Entrez Gene
th			-18.9	-23.5	-29.0	-44.4	-29.0	107.4	396.2	-99.0							

	Hh+GL2 Average	Hh Average	Reversed Average	Pol III-Hh Average	Secondary Average	Ci Average	No Hh Average
Plate #1 Normalized GFP Average	37.558	72.893	0.323	116.194		32.680	4.640
Plate #2 Normalized GFP Average	40.854	58.423	0.389	134.677		32.454	4.467
Plate #3 Normalized GFP Average	37.598	55.583	0.381	126.394		31.510	4.458

Legend

🟨 = Splicing Component

🟥 = Transcription Factor

🟩 = Ribosomal Component

🟧 = RNA regulatory Factor

⬜ = COPI Component

🟪 = Kinase

🟥 = Lipid Synthesis Factor

🟦 = Proteasomal Component

🟪 = Ubiquitin/Ubiquitin Ligase

🟩 = PP2A Component

🟧 = Nucleoporin

Supplementary Table 4--

CG/DRSC#	Gene	Unigene	Homologene	Human	Mouse	C. elegans	Yeast	Protein Domains
CG1708	cos	Dm.5775	14086					Kinesin motor domain, P-loop containing nucleotide triphosphate hydrolas
CG3949	hoip	Dm.20299	3672	NHP2L1	Gm1857, LOC	2K948	SNU13	Ribosomal protein L7AE, Ribosomal protein HS6, L30e-like; hoip
CG11700	CG11700		7987	UBB	Ubb, Gm1821	ubq-1	UBI4	Ubiquitin-like; CG11700
CG1874	CG1874	Dm.16231						
CG5684	CG5684	Dm.6800	22262			ccf-1	POP2	
CG10325	abd-A	Dm.3286	20947	HOXB7	Hoxb7	mab-5		Homeobox domain, 'Homeobox' antennapedia-type protein, Homeodomain-like; abd-A
CG7109	mts	Dm.4245	3063	PPP2CB	Ppp2cb	4M623	PPH22, PPH21	Serine/threonine specific protein phosphatase, Metallo-dependent phosphatases; mts
CG10198	Nup98	Dm.14811	3930	NUP98	Nup98			
CG2161	Rga	Dm.2486	34327					
CG10198	Nup98	Dm.14811	3930	NUP98	Nup98			
CG8108	CG8108	Dm.7293	34162					Zinc finger, C2H2 type
CG8367	cg	Dm.2240	5069	ZNF32	Zfp637			C2H2 and C2HC zinc fingers; cg, Zinc finger, C2H2 type
CG4453	Nup153		34304					
CG11502	svp	Dm.20676	21158	NR2F1	Nr2f1			Ligand-binding domain of nuclear hormone receptor, C4-type steroid receptor zinc finger, Steroid hormone receptor, COUP transcription factor (2F nuclear receptor), Nuclear receptor ligand-binding domain; svp
CG14519	CG14519							
CG4453	Nup153		34304					
CG6043	CG6043	Dm.418	36900					
DRSC00977								
CG9324	CG9324	Dm.19856	9308	C13orf12	2510048O06Rik			
CG32046	CG32046	Dm.918						
CG7913	PP2A-B'	Dm.3799	2037	PPP2R5C	Ppp2r5c	5L508		Protein phosphatase 2A regulatory B subunit (B56 fa
CG13936	CG13936		16421					
CG6551	fu	Dm.17302						Eukaryotic protein kinase, Serine/Threonine protein kinase family active site, Protein kinase-like (PK-like); fu
DRSC03777								
CG6699	beta'Cop	Dm.2980	3499	COPB2	Copb2	4K227, 1N14	SEC27	
CG14813	deltaCOP	Dm.6542	1250	ARCN1	Arcn1, LOC22	3H712	RET2	
DRSC08092								
CG16901	sqd	Dm.7189	22580	HNRPAB	Hnrpab	4H89		RNA-binding domain, RBD; sqd, RNA-binding region RNP-1 (RNA recognition motif)
CG8922	RpS5	Dm.2690	783	RPS5	Rps5	rps-5	RPS5	Ribosomal protein S7, Ribosomal protein S7; RpS5
CG4912	eEF1delta	Dm.11017	23404	EEF1D, LOC12	Eef1d			Elongation factor 1 beta/beta'/delta chain
CG4157	Rpn12	Dm.981	2105	PSMD8		rpn-12	RPN12	
DRSC05108								
DRSC20534								
CG4013	Smr	Dm.7194	25869					Aldehyde dehydrogenase family, Myb DNA binding domain, Homeodomain-like; Smr
CG40341	CG40341							
CG15552	Sox100B	Dm.3273	7950	SOX8	Sox8			HMG-box; Sox100B, HMG1/2 (high mobility group) box
CG2184	Mlc2	Dm.1488	373	MYL5, MYLC2PL		mlc-2		EF-hand family, EF-hand; Mlc2
DRSC05037								
CG7757	CG7757	Dm.13639	3447	PRPF3	Prpf3	3L514	PRP3	
CG4602	Srp54	Dm.2072	14360			rsp-7		RNA-binding region RNP-1 (RNA recognition motif), RNA-binding domain, RBD; Srp54
CG4501	bgm	Dm.20327	9044	BG1	MGI:2385656	3O630, 4L76		Aminoacyl-transfer RNA synthetases class-II, AMP-dependent synthetase and ligase, Firefly luciferase-like; bgm
CG6292	CycT	Dm.20508	947	CCNT1	Ccnt1			Cyclin-like; CycT
CG8435	CG8435	Dm.9146	6350	FLJ10374	2900016D05Rik			
DRSC05797								
CG7425	eff	Dm.4048	2506	UBE2D2, LOC	1700013N18R	let-70	UBC5, UBC4	Ubiquitin-conjugating enzymes, Ubiquitin conjugating enzyme; eff
CG2807	CG2807	Dm.18458	6696	SF3B1	Sf3b1	3F354	HSH155	ARM repeat; CG2807

CG/DRSC#	Gene	Unigene	Homologene	Human	Mouse	C. elegans	Yeast	Protein Domains
DRSC00405								
CG8936	Arpc3B	Dm.19161	4178	ARPC3, LOC38	Arpc3	arx-5	ARC18	
CG11985	CG11985		12841	SF3B5	1110005L13Rik			
CG8877	CG8877	Dm.574	4706	PRPF8	Prpf8	prp-8	PRP8	
CG33231	CG33231							
CG4893	CG4893	Dm.5901	21389	TPPP	2900041A09Rik			
DRSC14042								
CG3436	CG3436	Dm.1591	3538	HPRP8BP	0610009C03R	1E771, XL916		
CG5378	Rpn7	Dm.3424	7157	p44S10	2400006A19R	rpn-7	RPN7	
CG5969	CG5969		8238	QPCT	Qpct	5L85		
CG31111	CG31111	Dm.23785	18102	TBRG1	Tbrg1			
CG7434	RpL22	Dm.6921	14006	LOC256441, LOC389175, LOC402098, LOC402100				Histone H1 and H5 family, Ribosomal L22e protein family
CG11416	CG11416	Dm.774	23851					Prefoldin; CG11416
CG11981	Prosbeta3	Dm.6972	2089	PSMB3	Psmb3, LOC2	pbs-3	PUP3	Proteasome B-type subunit, Multispecific proteases of the proteasome
CG13130	CG13130	Dm.1554						
CG8179	CG8179	Dm.11920						SH3-domain; CG8179
CG4609	fax	Dm.6723	14641			2M555, 2N599		Thioredoxin-like; fax, Glutathione S-transferases, C-terminal domain; fax
CG5758	CG5758	Dm.19847	17872					Beta-Ig-H3/Fasciclin domain
CG5179	Cdk9	Dm.4856	965	CDK9	Cdk9	cdk-9		Eukaryotic protein kinase, Serine/Threonine protein kinase family active site, Protein kinase-like (PK-like); Cdk9
CG1821	RpL31	Dm.20585	24659					Ribosomal protein L31e, Ribosomal protein L31e; RpL31
CG12750	CG12750	Dm.11546	24205	KIAA1604				ARM repeat; CG12750, Middle domain of eIF4G
DRSC14061								
CG6846	CG6846	Dm.6097	764	RPL26	Rpl26			Ribosomal protein L24/bacterial NUSG, Ribosomal protein L24, Translation proteins SH3-like domain; CG6846
CG1475	CG1475	Dm.4531	6152	RPL13A, LOC2	Rpl13a		RPL16A, RPL1	Ribosomal protein L13, Ribosomal protein L13; CG1475
CG7835	CG7835	Dm.3606						
CG7219	CG7219	Dm.18230	15985					Serpins; CG7219, Serpins
CG4585	CG4585	Dm.3875						CDP-alcohol phosphatidyltransferase
CG1483	Map205	Dm.1892						
CG3193	crn	Dm.3140	6462	CRNKL1	Crnkl1	5G423	CLF1	N-terminal domain of phosphatidylinositol transfer, Protein prenylyltransferase; crn, Tetratricopeptide repeat (TPR); CG18842, C-terminal domain of phosphatidylinositol transfer, Profilin (actin-binding protein); CG18842
CG3278	Tif-IA	Dm.485	6342	RRN3, LOC94	E130302O19R	3F90	RRN3	
CG3278	Tif-IA	Dm.485	6342	RRN3, LOC94	E130302O19R	3F90	RRN3	
CG32767	CG32767	Dm.6248						
DRSC00245								
CG8580	CG8580	Dm.11452	9988	C6orf166	LOC380615	1E706		
CG9885	dpp	Dm.4767	926	BMP2	Bmp2			Inhibin alpha chain, Cystine-knot cytokines; dpp
CG3193	crn	Dm.3140	6462	CRNKL1	Crnkl1	5G423	CLF1	N-terminal domain of phosphatidylinositol transfer, Protein prenylyltransferase; crn, Tetratricopeptide repeat (TPR); CG18842, C-terminal domain of phosphatidylinositol transfer, Profilin (actin-binding protein); CG18842
CG14210	4210, CG33	Dm.6071	5941	MGC2574		1O20	CGR1	
CG13445	CG13445	Dm.15267						
CG2097	CG2097	Dm.11484	3543	SYMPK	LOC225468	5J61		ARM repeat; CG2097
CG17489	yip6							Ribosomal protein L18P/L5E, Translational machinery components; yip6
CG6015	CG6015	Dm.20226	5716	CDC40		1L531	CDC40	Trp-Asp repeat (WD-repeat); CG6015
CG10541	Tektin-C	Dm.5022	7973	TEKT1	Tekt1	XE623		Tektin
CG6987	SF2	Dm.4443	31411	SFRS1	Sfrs1	rsp-3	HRB1	RNA-binding region RNP-1 (RNA recognition motif), RNA-binding domain, RBD; SF2
CG12238	CG12238	Dm.11278						PHD-finger
CG40218	CG40218							
CG1135	CG1135	Dm.3951	4622	MCRS1	Mcrs1			SMAD/FHA domain; CG1135

CG/DRSC#	Gene	Unigene	Homologene	Human	Mouse	C. elegans	Yeast	Protein Domains
CG10139	CG10139	Dm.4657						
CG5499	His2Av	Dm.2346	36329	H2AFV				Histone-fold/TFIID-TAF/NF-Y domain, Histone H2A, Histone-fold; His2Av
CG8233	CG8233	Dm.615	9949	FLJ10081	4632411B12Rik			alpha/beta-Hydrolases; CG8233
CG4699	CG4699	Dm.10603	34852					
CG14023	CG14023	Dm.13484	32943					
CG12977	CG12977	Dm.24997						
CG14952	CG14952							
CG32381	unc-13-4A	Dm.21296	20844	BAIAP3	Gm937	XD240		C2 domain (Calcium/lipid-binding domain, CaLB); un
CG32296	CG32296	Dm.13305	10826	MKL2	Mrtf-B			SAP domain
CG32776	CG32776	Dm.20557						P-loop containing nucleotide triphosphate hydrolas, Small GTPase, Ras subfamily
CG12054	CG12054	Dm.4416	18291	JAZF1	AI591476		MIG1	C2H2 and C2HC zinc fingers; CG12054, Zinc finger, C2H2 type
CG32474	dys	Dm.18571	3716	SIM2	Sim2			PAC motif, Helix-loop-helix DNA-binding domain; CG12561, Helix-loop-helix dimerization domain, PAS domain, PYP-like sensor domain; CG14552
CG14459	CG14459							
CG12852	CG12852		21066	TNRC11	Tnrc11	5N91		
CG31145	CG31145	Dm.10845	8909	FAM20B	C530043G21Rik			P-loop containing nucleotide triphosphate hydrolas
CG33110	CG33110	Dm.13232	27926					
CG13165	CG13165		16139					
CG8787	Asx	Dm.2970						
CG5575	ken	Dm.2792	34064					BTB/POZ domain, Zinc finger, C2H2 type, POZ domain; ken, C2H2 and C2HC zinc fingers; ken
CG7481	RhoGAP18B	Dm.3638	33731			gei-1		GTPase activation domain, GAP; CG7481
DRSC14786								
DRSC14674								
CG7776	E(Pc)	Dm.564	37048					
CG7269	Hel25E	Dm.3332	5567	BAT1	Bat1a		SUB2	DEAD/DEAH box helicase, Helicase C-terminal domain, P-loop containing nucleotide triphosphate hydrolas
CG7664	crp	Dm.6530	2424	TFAP4	Tfap4			Helix-loop-helix dimerization domain, Helix-loop-helix DNA-binding domain; crp
CG9094	CG9094	Dm.15919						
CG13847	CG13847	Dm.6236						
CG32149	RhoGAP71E	Dm.14535	27685					GTPase activation domain, GAP; CG7396, GTPase activation domain, GAP; CG16980
CG30044	CG30044	Dm.12830						
CG5602	CG5602	Dm.19278	197	LIG1	Lig1		CDC9	DNA ligase/mRNA capping enzyme, catalytic domain;, ATP-dependent DNA ligase
CG2013	UbcD6	Dm.2229	2503	UBE2A	Ube2a		RAD6	Ubiquitin-conjugating enzymes, Ubiquitin conjugating enzyme; UbcD6
CG15255	CG15255	Dm.12043	15035					Astacin (Peptidase family M12A) family, Neutral zinc metallopeptidases, zinc-binding region
CG32592	hiw	Dm.3259	9005	MYCBP2	Phr1	rpm-1		Regulator of chromosome condensation RCC1; hiw, B-box zinc finger superfamily, Immunoglobulin; hiw
CG9429	Crc	Dm.2457	3205	CALR	Calr	crt-1		Calreticulin family
CG2128	HDAC3	Dm.3642	2884	HDAC3	Hdac3		RPD3	Arginase/deacetylase; HDAC3
CG6962	CG6962	Dm.1159	9813	FLJ20297	4122402O22Rik			
CG32955	CG32955	Dm.1555	33819			klp-18, klp-10C		
CG4268	Pitslre	Dm.1019	26837					Eukaryotic protein kinase, Serine/Threonine protein kinase family active site, Protein kinase-like (PK-like); Pitslre
CG32253	CG32253	Dm.21145	10133	BRIX	1110064N10R	3J135	BRX1	
CG14656	CG14656	Dm.17188						
CG12254	Arc92	Dm.1258	32918					
CG18468	CG18468	Dm.3715						
CG9282	CG9282	Dm.424	763	RPL24	Rpl24, LOC27	rpl-24.1	RPL24A, RPL2	Ribosomal protein L24E, Glucocorticoid receptor-like (DNA-binding domain);
CG2685	CG2685	Dm.6933	9466	WBP11	Wbp11	2K463		
CG9176	cngl	Dm.2910	35132					cAMP-binding domain-like; cngl, Cyclic nucleotide gated / K+ ion channel TM region, Potassium channel, Membrane all-alpha; cngl
CG5786	ppan	Dm.3008	5690	PPAN	Ppan	lpd-6	SSF2, SSF1	

CG/DRSC#	Gene	Unigene	Homologene	Human	Mouse	C. elegans	Yeast	Protein Domains
CG3732	CG3732	Dm.3693	3984	ZNF265		2D362		
CG32150	CG32150	Dm.15266						
CG7292	Rrp6	Dm.7279	31105	EXOSC10	Exosc10	2K881C		3'-5' exonuclease, HRDC domain, Ribonuclease H-like; CG7292
CG31657	CG4124		27555					Zinc finger C-x8-C-x5-C-x3-H type, Conserved domain common to transcription factors T
CG6937	CG6937	Dm.6006	12216	MKI67IP	Mki67ip			RNA-binding domain, RBD; CG6937, RNA-binding region RNP-1 (RNA recognition motif)
CG5605	eRF1	Dm.2095	3475	ETF1	Etf1	5G915	SUP45	eRF1-like proteins, Translational machinery components; eRF1, L30e-like; eRF1, N-terminal domain of eukaryotic peptide chain rele
CG15814	CG15814	Dm.198	10132	RNF121	Rnf121	3F260		RING finger domain, C3HC4; CG15814
CG33106	mask	Dm.7426	35296	ANKRD17				KH-domain; CG6313, Ankyrin repeat; CG6313, Ankyrin repeat; CG6268, KH domain
CG5427	CG5427	Dm.7250	36899					
CG9842	Pp2B-14D	Dm.21227	728	PPP3CA	Ppp3ca	tax-6	CNA1, CMP2	Serine/threonine specific protein phosphatase, Metallo-dependent phosphatases; Pp2B-14D
CG4152	l(2)35Df	Dm.1748	6257	KIAA0052	2610528A15R	4K593	MTR4	DEAD/DEAH box helicase, Helicase C-terminal domain, P-loop containing nucleotide triphosphate hydrolas
CG12104	CG12104	Dm.7697						HMG1/2 (high mobility group) box, HMG-box; CG12104
CG16918	CG16918	Dm.24110						Trypsin-like serine proteases; CG16918, Serine proteases, trypsin family
CG7031	CG7031	Dm.13734						
CG8264	Bx42	Dm.2932	5294	SKIIP	Skiip	skp-1		
CG15476	CG15476							
CG7961	alphaCop	Dm.2217	3218	COPA	Copa	1D464	COP1	
CG6223	betaCop	Dm.2883	5664	COPB	Copb1	2D377	SEC26	
CG1528	gammaCop	Dm.1895	22214		Copg	4K919	SEC21	
CG5931	CG5931	Dm.973	5859	ASCC3L1, LO(	A330064G03F	2N18	BRR2	DEAD/DEAH box helicase, Helicase C-terminal domain, Protein prenylyltransferase; CG5931, P-loop containing nucleotide triphosphate hydrolas
CG5360	CG5360	Dm.13577	16247					
DRSC00209								
CG7752	CG7752	Dm.18621	26839					C2H2 and C2HC zinc fingers; CG7752, Zinc finger, C2H2 type, FYVE/PHD zinc finger; CG7752
CG10279	Rm62	Dm.1520	34088					ATP-dependent helicase, DEAD-box, DEAD/DEAH box helicase, Helicase C-terminal domain, P-loop containing nucleotide triphosphate hydrolas
CG1492	CG1492	Dm.19697						Gamma-glutamyltranspeptidase
CG18041	CG18041	Dm.1370	34902					
CG30053	CG30053	Dm.4052	37052					
CG1559	Upf1	Dm.3617	2185			smg-2	NAM7	P-loop containing nucleotide triphosphate hydrolas
CG31209	CG31209	Dm.5097						
CG33141	sns	Dm.14097	27917					Fibronectin type III; sns, Immunoglobulin; sns, Immunoglobulin-like, Immunoglobulin C-2 type
CG6064	CG6064	Dm.11355	32620	MECT1				
CG5674	CG5674	Dm.12153						
CG32676	CG32676	Dm.11844	34283					Ubiquitin-like; CG9725
CG33183	Hr46	Dm.5036	5041	RORB	Rorb	nhr-23		C4-type steroid receptor zinc finger, Ligand-binding domain of nuclear hormone receptor, Steroid hormone receptor, Nuclear receptor ligand-binding domain; Hr46
CG10869	CG10869	Dm.7506	32639	FLJ90575	4921513E08Rik			
CG5733	CG5733	Dm.682	11755	PCNT1		npp-2		
CG3895	ph-d		23119	PHC2	Phc2			
CG12912	CG12912		34628					
CG8426	l(2)NC136	Dm.2133	7149	CNOT3	Cnot3	ntl-3	NOT3	
DRSC01970								
DRSC14928								
CG16941	CG16941	Dm.4173	4294	SF3A1	Sf3a1	prp-21		Ubiquitin-like; CG16941, SWAP / SURP
CG3605	CG3605	Dm.285	6678	SF3B2	Sf3b2	5A609	CUS1	
CG6437	CG6437	Dm.9285	2519	UGCG	Ugcg	2G205, XS315, 5Q366		Nucleotide-diphospho-sugar transferases; CG6437, Glycosyl transferase, family 2
CG6905	CG6905	Dm.797	957	CDC5L	Cdc5l			Homeodomain-like; CG6905, Myb DNA binding domain
CG9667	CG9667		6283	KIAA1160	5830446M03F	5L480	ISY1	

CG/DRSC#	Gene	Unigene	Homologene	Human	Mouse	C. elegans	Yeast	Protein Domains
CG2925	noi	Dm.4519	4949	SF3A3	Sf3a3	2I814	PRP9	RNA-binding protein C2H2 Zn-finger domain, Zinc finger, C2H2 type
CG17272	CG17272	Dm.6142	26929					EF-hand family, EF-hand; CG17272
CG6694	CG6694	Dm.15477	34752					Zinc finger C-x8-C-x5-C-x3-H type
CG6625	Snap	Dm.2107	2839	NAPA	Napa	5I613	SEC17	NSF attachment protein, Tetratricopeptide repeat (TPR); Snap
CG7923	Fad2	Dm.3344	23447			fat-6, fat-5		Fatty acid desaturase, type 1
CG3948	zetaCOP	Dm.20098	9366	COPZ1	Copz1	2L163	RET3	Clathrin adaptor complex, small chain
CG14180	CG14180	Dm.15450						
CG7525	Tie	Dm.13034	15284					Eukaryotic protein kinase, Tyrosine kinase catalytic domain, Protein kinase-like (PK-like); Tie
CG14342	CG14342							
CG7926	Axn	Dm.7154	3420	AXIN2	Axin2			Regulator of G Protein Signaling (RGS) domain, DIX domain, Regulator of G-protein signaling, RGS; Axn
CG18591	CG18591	Dm.23392	22314	SNRPE, LOC1	Snrpe		SME1	Sm motif of small nuclear ribonucleoproteins, SNRN, Small nuclear ribonucleoprotein (Sm protein)
CG33141	sns	Dm.14097	27917					Fibronectin type III; sns, Immunoglobulin; sns, Immunoglobulin-like, Immunoglobulin C-2 type
DRSC02439								
DRSC08743								
CG4035	eIF-4E	Dm.7012	1488	EIF4E	Eif4e, Gm206	ife-3	CDC33	Eukaryotic translation initiation factor 4E (eIF-4E, Translation initiation factor eIF4e; eIF-4E
CG32688	Hk	Dm.4020	22588	KCNAB2	Kcnab2			NAD(P)-linked oxidoreductase; hk
CG17077	pnt	Dm.2370						Ets-domain, HSF/ETS DNA-binding domain, Sterile alpha motif (SAM)/Pointed domain, SAM/Pointed domain; pnt
CG18214	trio	Dm.803	20847	HAPIP	Hapip			DBL homology domain; trio, Spectrin repeat; trio, Cytochrome c; trio, Dbl domain (dbl/cdc24 rhoGEF family), PH domain-like; trio
CG5825	His3.3A	Dm.2931, Dm	21103	H3F3B, LOC3	H3f3b, LOC21	his-72, his-71	HHT2	Histone-fold/TFIID-TAF/NF-Y domain, Histone H3, Histone-fold; His3.3A
CG1322	zfh1	Dm.4708	9421	ZNF580	Zfp580			Zinc finger, C2H2 type, Homeobox domain, Homeodomain-like; zfh1, C2H2 and C2HC zinc fingers; zfh1
CG4903	MESR4	Dm.2764	34061					Elongation factor Ts (EF-Ts), dimerization domain;, Zinc finger, C2H2 type
CG3025	mof	Dm.2952						Chromo domain, Acyl-CoA N-acyltransferases (Nat); mof
CG9750	rept	Dm.7319	4856	RUVBL2	Ruvbl2	4H682	RVB2	P-loop containing nucleotide triphosphate hydrolas
CG8954	Smg5	Dm.2100	9095	EST1B	BC024683			
CG7305	CG7305	Dm.25477	8988	RIMS1	Rims1			
CG15494	CG15494	Dm.20355						
CG9533	rut	Dm.2613	10896	ADCY1	Adcy1	acy-3		Guanylate cyclase, Adenylyl and guanylyl cyclase catalytic domain; ru
CG13452	AGO2	Dm.9677	27684					
CG14641	CG14641	Dm.3575	9980	RBM22	Rbm22			Zinc finger C-x8-C-x5-C-x3-H type, RNA-binding region RNP-1 (RNA recognition motif), RNA-binding domain, RBD; CG14641
CG2835	G-salpha60A	Dm.4160	1565	GNAL		gsa-1		G-protein alpha subunit group S, Guanine nucleotide binding protein (G-protein), alp
CG32029	CG32029	Dm.5882						
CG9930	E5		17092	GSH1	Gsh1	XP650		Homeodomain-like; E5, Helix-turn-helix / lambda; and other repressors, Homeobox domain
CG11194	Hey	Dm.516						Helix-loop-helix dimerization domain, Orange domain, Helix-loop-helix DNA-binding domain; Hey
CG11405	A3-3	Dm.10455	34711					bZIP (Basic-leucine zipper) transcription factor fa, Fos transforming protein, Binding domain of Skn-1; A3-3
CG1915	sls	Dm.2974	25517			pqn-43		Immunoglobulin; sls, Fibronectin type III; CG18242, Immunoglobulin; CG18857, Immunoglobulin; CG18242, Immunoglobulin-like, Immunoglobulin subtype, Immunoglobulin C-2 type, Immunoglobulin and major histocompatibility complex
CG1017	CG1017	Dm.9436	4332	MFAP1	Mfap1	1J216		
CG9504	CG9504							FAD/NAD(P)-binding domain; CG9504, FAD-linked reductases, C-terminal domain; CG9504, Glucose-methanol-choline (GMC) oxidoreductase
CG13333	CG13333	Dm.604						

CG/DRSC#	Gene	Unigene	Homologene	Human	Mouse	C. elegans	Yeast	Protein Domains
CG32133	CG32133	Dm.24000	33004		A630073D07Rik			BRCT domain; CG8797, BRCT domain; CG6532
CG4886	cyp33	Dm.2854	36550		LOC381106, LOC383442			RNA-binding region RNP-1 (RNA recognition motif), Cyclophilin (peptidylprolyl isomerase); cyp33, RNA-binding domain, RBD; cyp33
DRSC05475								
CG32202	CG32202	Dm.23823						
CG10095	CG10095	Dm.13690						Immunoglobulin C-2 type, Immunoglobulin and major histocompatibility complex, Immunoglobulin-like, Immunoglobulin; CG10095
CG8048	Vha44	Dm.7688	1281	ATP6V1C1	Atp6v1c1	vha-11	VMA5	
CG4003	pont	Dm.1557	2749	RUVBL1	Ruvbl1	5K460	RVB1	P-loop containing nucleotide triphosphate hydrolas, AAA ATPase superfamily
CG10712	Chro		26848					Chromo domain-like; CG10712, Chromo domain
CG11184	Upf3	Dm.756	11307	UPF3B	Upf3b	smg-4		
CG32743	Smg1	Dm.18753	35121					Phosphatidylinositol 3- and 4-kinase, Protein kinase-like (PK-like); CG4549
CG2253	Upf2	Dm.17613	6101	UPF2	Upf2		NMD2	Middle domain of eIF4G, ARM repeat; CG2253
CG9748	bel	Dm.7823	8069		Ddx3y, D1Pas1		DBP1	DEAD/DEAH box helicase, ATP-dependent helicase, DEAD-box, Helicase C-terminal domain
CG32743	Smg1	Dm.18753	35121					Phosphatidylinositol 3- and 4-kinase, Protein kinase-like (PK-like); CG4549
CG10306	CG10306	Dm.3730	8292	eIF3k	1200009C21R	eif-3.K		
CG3412	slmb	Dm.2387	7646	BTRC	Btrc	lin-23		Skp1-Skp2 dimerization domains; slmb, Trp-Asp repeat (WD-repeat); slmb
CG32541	CG32541	Dm.4349						Insulin-like; CG14188
CG15783	CG15783	Dm.23769						OAR domain
CG1101	Aly	Dm.3104	5722	THOC4	Thoc4, Refbp2	aly-2, aly-3		RNA-binding region RNP-1 (RNA recognition motif), RNA-binding domain, RBD; aly
CG12607	CG12607	Dm.5459						
CG32139	Sox21b	Dm.955	26804					HMG1/2 (high mobility group) box
CG32432	CG32432	Dm.4337	16618					Ligand-binding domain of low-density lipoprotein r, Ligand-binding domain of low-density lipoprotein r, Spermadhesin, CUB domain; CG3116
CG13222	CG13222	Dm.14040	36721			pqn-32		
DRSC17378								
CG1546	PH4alphaSG	Dm.1907	3094	P4HA2	P4ha2			Tetratricopeptide repeat (TPR); CG1546
CG8397	CG8397	Dm.9139	34656			3F971		
CG9233	fu2	Dm.3460	15994					C2H2 and C2HC zinc fingers; fu2, MHC antigen-recognition domain; fu2, Zinc finger, C2H2 type
CG12822	8853, CG12	Dm.6978						
CG10706	SK	Dm.11909	1694	KCNN1	Kcnn1	XG9		Membrane all-alpha; SK, Potassium channel
CG8277	CG8277	Dm.7849	1488					Translation initiation factor eIF4e; CG8277, Eukaryotic translation initiation factor 4E (eIF-4E
CG32050	CG32050	Dm.15438						
CG12079	CG12079	Dm.11274	3346	NDUFS3	Ndufs3	1H106		Respiratory-chain NADH dehydrogenase 30 Kd subunit
CG14084	CG14084	Dm.998	4287	BET1	Bet1		BET1	
DRSC17256								
Ci 5'								
Ci 5'								
Ci 5'								
GFP								
GFP								
GFP								
GFP								
GFP								
GFP								
Luc								
Luc								
Luc								
Smo 5'								
Smo 5'								
Smo 5'								
th								
th								

CG/DRSC#	Gene	Unigene	Homologene	Human	Mouse	C. elegans	Yeast	Protein Domains
th								

Plate #1 Normalized GFP
Plate #2 Normalized GFP
Plate #3 Normalized GFP

Supplemental Methods

Primary Screening

The primary assay was a transfection-based, dual-luciferase assay, using a previously described Hh reporter in which a portion of the *ptc* promoter, termed ptcΔ136, drives firefly luciferase[1](outlined in Figure 1A). The experimental signal of the ptcΔ136 reporter was normalized by the signal from a Pol III-Renilla luciferase reporter to control for cell number and transfection efficiency. These two reporters were cotransfected, along with a construct expressing full-length Hh and the dsRNA present in each well, into *Drosophila* clone 8 cells and the firefly and the Renilla luciferase activities assayed after five days of incubation. Primary screening for the Hh assay was conducted in white, opaque, polystyrene 384 well tissue culture plates (Corning-Costar #3704) into which an average of 75 ng of dsRNA, in 5 μL of 1 mM Tris, pH=7.0, was preloaded into each well. The development of the genome-wide dsRNA library has been previously described[2]. Transfection screening was conducted using Effectene (Qiagen) according to the following modifications of the manufacturer's protocol. A master mix was made such that each 9 μL contained 50 ng of the ptcΔ136-GL2 experimental reporter, 15 ng of the Pol III-RL control reporter, and 40 ng of the pAc5C-Hh expressing vector in Buffer EC (Qiagen). The master mix was mixed by inversion, and then enhancer (Qiagen) was added such that each 10 μL of the EC-DNA mix contained 1 μL of enhancer. Effectene was then added to give 0.3 μL of Effectene per 10.3 μL of the EC-DNA-enhancer mix, and this final master mix was mixed by inversion. 10 μL per well of the master mix was dispensed into the screening plates using a MultiDrop 384 (Thermo Labsystems) liquid dispenser and the plate mixed by brief vortexing. After incubation for 5 minutes at room temperature, 35 μL of complete M3 medium containing 25,000 clone 8 cells was aliquoted per well using the MultiDrop 384 and dispersed by gentle shaking. The plates were incubated in a humidified chamber in a humidity controlled incubator for 5 days at 25 °C.

To determine Luciferase values for Firefly and Renilla luciferases, all but 20 μL of cell culture medium was aspirated off the cells. Dual-Glo Luciferase (Promega) was then used essentially according to the manufacturer's instructions with 20μL of each reagent aliquoted per well. Reagents were dispensed using a MicroFill (BioTek) and then luciferase levels read using a Analyst HT plate reader (Molecular Dynamics).

We should note that at the end of our 5 day assay period, clone 8 cells are generally confluent within the wells of a 384 well plate. In our hands, clone 8 cells exhibit roughly a 5-10% transfection rate as judged by GFP transfection (K.N., data not shown). Consequently, even cells whose viability may be reduced by a transfected dsRNA are still found in a confluent cell environment.

To identify genes whose reduction by RNAi affected Hh signaling, the Firefly luciferase values were normalized by the Renilla luciferase values to give the Normalized Luciferase score. Using the Normalized Luciferase Value, a Z-score for each well was determined using the interior-well plate average and interior-well plate standard deviation. Z-scores are a measure of how different a particular value (a normalized luciferase value in the case of our data) is from the mean in terms of the number of standard deviations from that mean. The interior wells are all wells except those on the outer edges of the plates, which we found empirically it was best not to include in these calculations due to occasional edge effects found in some plates. Z-scores from duplicate screens were averaged and the average Z-scores used to identify hits. Hits were defined as wells with a Z-score <-2.0 or Z-score >+3.0. We used the higher Z-score criteria for hits greater than zero because of our observation that false increases in Z-score were far more likely to occur due to false decreases in the primary Renilla value than vice versa. Since edge effects could cause these artificial increases in normalized luciferase scores, we also manually curated increased scores, eliminating those that only occurred in edge wells or only occurred once in the two replicates. For plates 25-31 and 39-45 of replicate #1, a occlusion in a dispensing pin resulted in misaliquoting of luciferase reagents to the J rows, and so the J rows were left out of the calculations of plate average and plate standard deviations for these plates.

Secondary Screening
Several criteria were used to determine which genes to examine in secondary screens. First, we did not test all the components of the proteasome and ribosome, whose function seems likely to rely on the presence of most or all of the components. Rather, we simply picked 3 representatives of the proteasome and 6 of the ribosome to test in the secondary assays while leaving out the remaining dsRNAs targeting these complexes. We also picked only 24 of the total of 127 Heidelberg annotation hits to retest. This is because the Heidelberg annotations are based on a gene prediction program with much more relaxed gene identification criteria. Many of the putative novel genes which it identifies do not appear to target legitimate genes (although they may target non-coding RNAs)[2-4]. The great majority of them also have a large number of potential off-targets (see Supplemental Note). Thus, 194 dsRNAs targeting ribosomal, proteasomal, and Heidelberg genes were not tested in secondary assays. We also chose not do secondaries on a further 60 hits for a variety of reasons, usually having to do with large numbers of off-targets, low initial assay scores, and/or lack of a cDNA for the purported gene. This left us with 255 hits on which to conduct secondary assays (Supplementary Table 4).

To set up the secondary screening plates, the original T7 tailed amplicons were reamplified as described above. Master dsRNA plates containing experimental and control dsRNAs at 20 ng per μL were then prepared in deep well, 96 well plates. From these master dsRNA plates, 96 well secondary test plates were prepared by aliquoting 10 μL (=200 ng) of dsRNA per well, sealing, and storing at –80°C until use.

As almost all of the wells in the secondary screening plates contained dsRNAs identified as substantially changing Hh reporter activity, we could not use plate averages or standard deviations to judge which dsRNA was a hit in this assay format. In place of the plate average/standard deviation, we instead scored the hits by their percentage change versus GFP dsRNA controls included in the secondary screening plates. Hits were considered to be anything that reduced reporter activity by 25% or more or increased reporter activity by 50% or more. When the original screening protocol was conducted on the secondary screening plates, 78% of the genes rescored as hits, giving only a 22% false positive rate for hits determined from the primary screen.

The rationale and design of each of the secondary assays is discussed below:

mRNA stability: During the primary round of screening, we noticed that five components involved in Nonsense Mediated Decay (NMD) appeared as hits that strongly increased reporter activity (see table below). The NMD pathway is an RNA surveillance mechanism that identifies transcripts containing premature termination codons and subjects them to increased rates of degradation[5]. The parent vector of the ptcΔ136 reporter, pGL2 (Promega), contained an intron after the luciferase stop codon (Figure 1A), which could trigger NMD of the ptcΔ136 luciferase reporter mRNA. We therefore created a new reporter vector in pGL3 (Promega), called ptcΔ136-GL3, which contained the same promoter sequence driving luciferase, but lacked the intron after the luciferase stop codon (see DNA Constructs and Cloning in the Materials and Methods). In secondary screening with this new reporter, we found that the dsRNAs targeting NMD pathway members and several genes that increased reporter activity no longer scored as increased hits, but 80% of the dsRNAs that affected Hh signaling in the primary screen repeated as hits (Supplementary Table 4).

CG#	Gene	DRSC Amplicon	Average Z-Score	Hh GL3	Hh GL2
CG11184	Upf3	DRSC04092	5.12	-0.04	1.01
CG8954	Smg5	DRSC03124	5.25	-0.07	1.84
CG1559	Upf1	DRSC19667	7.41	-0.23	0.82
CG32743	Smg1	DRSC18367	6.49	0.05	1.59
CG2253	Upf2	DRSC18218	4.78	-0.02	1.13
CG32743	Smg1	DRSC18366	7.34	-0.05	1.02

Luciferase Reversal: To test whether some of the hits could be genes that caused a preferential accumulation of Renilla or Firefly Luciferase independent of Hh signaling (through regulation of mRNA stability, translation, and/or protein stability), we reversed the two reporter vectors, creating an assay in which the ptcΔ136 promoter drives Renilla luciferase while the Pol III promoter drives Firefly luciferase. If a particular RNAi target changes normalized luciferase values simply by differentially affecting one of the two luciferases, then trading the two luciferases should substantially shift the percentage change in the opposite direction of that previously determined. After testing the reversed constructs in secondary assays, we found that most dsRNAs repeated as hits using this assay and found only two genes whose reporter signature was actually reversed by interchange of the promoter-luciferase constructs (Supplementary Table 4).

Promoter Matching: To control for the possibility that some hits could be due to differential regulation of the Actin promoter driving Hh and the Pol III promoter driving Renilla in our original assay, we conducted secondary assays in which Hh was driven by the Pol III promoter. Thus, we created a construct in which Hh was driven by the Pol III promoter and conducted the secondary assays again with Pol III-Renilla and the ptcΔ136-GL3 reporter. As can be seen in Supplementary Table 4, 65% of hits repeat in this assay and, for those hits that do repeat, the Z-scores are actually stronger than those we observe in the assay using Act5C-Hh, suggesting that the use of the two different promoters was actually reducing the resolution of the assay in some cases.

Screening of the secondary plates was conducting using the same protocol used for screening primary plates except that the assays were done in 96 well plates with 125 ng of ptcΔ136-pGL3 experimental reporter, 37.5 ng of the Pol III-RL control reporter, and 100 ng of the Act5C-Hh transfected per well. In the 96 well GL2 assay, 125 ng of ptcΔ136-pGL2 was used instead of ptcΔ136-pGL3. For the "Luciferase Reversal" assay, 37.5 ng of ptcΔ136-RL and 125 ng of Pol III-GL3 were used as experimental and control reporter, respectively. For the "Promoter matching" control, 100 ng of Pol III-Hh was used instead of 100 ng of Act5C-Hh. For the "No Induction" screen, the empty pAct5C parent vector was substituted for the Act5C-Hh.

Primers used to make non-DRSC dsRNAs against *Drosophila* genes.

Amplicon Name	Sense Primer	Antisense Primer
5' Ptc	TAATACGACTCACTATAGGGAATGGACCGCGACAGCCTCCCACGCGTTC	TAATACGACTCACTATAGGGATTGGTTGAGGCCTGGTATAACGACCGC
mid Ptc	TAATACGACTCACTATAGGGACTTCTACAACTATCTGTCGGCATGGGC	TAATACGACTCACTATAGGGACGCCAACGCATAAGACCACCAGCAGAAGC
3' Ptc	TAATACGACTCACTATAGGGAATGACATCCGTTGGCAACCGACAGCGCCGC	TAATACGACTCACTATAGGGATAAAGTTATAGCTGCGCACCGCCCTGC
5' Ci	TAATACGACTCACTATAGGGCATCATGGACGCCTACGCGTTACCTACATATTTTCC	TAATACGACTCACTATAGGGCGCGGCCGCGTTTACCACGTTGTTGTAACAACGGTC
5' Smo	TAATACGACTCACTATAGGGCACAATTAAACCAGGATATCCCGAGTCC	TAATACGACTCACTATAGGGCGCGGCCGCATCCACCTCACTGAAAGCCATCGTGG
3' Smo	TAATACGACTCACTATAGGGCAGATCTATCATGCTTAATGAATTCCTGCAGAAAAATGGC	TAATACGACTCACTATAGGGCCTTTGAAGGCAGCAATAACATTTTGAGTTTGTC
Su(fu) 5'	TAATACGACTCACTATAGGGCATGGCCGAGGCGAATTTGGACAAAAAACCTGAGG	TAATACGACTCACTATAGGGCGCGATCCATGTTAGTCACCAGCCAATCACC
Cos 3'	TAATACGACTCACTATAGGGCCAGCCGCCTCCGCCCATCGATCCTGAGTCG	TAATACGACTCACTATAGGGCGTTTCGACGACTTGCGTCCTGGATAATTATCTTGTTCTTCTG
Fu 3'UTR	TAATACGACTCACTATAGGGCTGAATGTTGCCCTGGAAGTAGTCGAGC	TAATACGACTCACTATAGGGCTAGCTGAATGGTTGACTACACCTTAGCAG
Fu 3'	TAATACGACTCACTATAGGGCGGGTGCATGTTGGCCCTGATGAGCT	TAATACGACTCACTATAGGGCGGTGACGAAAAAAGTGAAGTGACTGATCTC

Amplicon Name	Sense Primer	Antisense Primer
	GTGTGC	
Hh 5'	TAATACGACTCACTATAGGGCGGATCCATAATACCATAAATCTGAATAACAAACGTG	TAATACGACTCACTATAGGGCGCGGCCGCCTTGCTCATCAAGCGATCCGCTCCGGTGCC
Sqd KN Raspec 3' UTR	TAATACGACTCACTATAGGGCGACGGACAGGGATCATACGGCGGCTATGGCGGCGGCTAC	TAATACGACTCACTATAGGGCGTTTGAATGGGAAACACAACAACGTGGAAATTTTGG
CG4268 Pitslre KN exon 2	TAATACGACTCACTATAGGGCGCACTAGAACGGGATCATCGATATGACTATCGG	TAATACGACTCACTATAGGGCCCCTCCCGGCGATCACTTCTCTCGACCCGATGC
CG1017 KN exon 3	TAATACGACTCACTATAGGGCAAGAGGAGCGACGCCAGGAGCTGCGCCAGAACC	TAATACGACTCACTATAGGGCCGCCGGCGTGTTCGTTGTGGAACTTGATGTTGC
CG7109 mts KN exon 3	TAATACGACTCACTATAGGGCTTTCGGAAACCTTTAACAACACAAACGGCCTGAC	TAATACGACTCACTATAGGGCCATCCAGTTCCATAAGAGCCGCTTGGTTGC
CG33297 Areg KN exon 3	TAATACGACTCACTATAGGGCGAGTTTGGCCACCGTAGAGGAAACCGTGGTGCG	TAATACGACTCACTATAGGGCAGATACTCCGTCTCAACGACCTTGGCAAACTCG
CG33297 Areg KN exon 6	TAATACGACTCACTATAGGGCGCTTGTTCTGCCTGAATGTTTTGGCAGAGG	TAATACGACTCACTATAGGGCTCTGTGTCTGTGTTCAGTTTGTCGAGTGTGGGC
CG6235 tws KN exon 4	TAATACGACTCACTATAGGGCGAGCGTGACAAGTCGTTCGGCGGCTACAACACG	TAATACGACTCACTATAGGGCTATCACATAGTATTGTGCCCTTAGAGCTCG
CG6235 tws KN 5' UTR	TAATACGACTCACTATAGGGCTTCTTTCTTCCCCTCTACATGCTTAAGTCGTGC	TAATACGACTCACTATAGGGCCCGATTTTAAAGTTTCAAAAAGTTGAGCGCG
CG5643 wbd KN exon 3	TAATACGACTCACTATAGGGCCGTTGCAGTTGCAGATTGGTCAGGACGCGAACG	TAATACGACTCACTATAGGGCGCTGCAGCTCAACATCCTGAGAATTTCTGTAGC
CG5643 wdb KN exon 7	TAATACGACTCACTATAGGGCTAATCGAGGAGAACAATGCGGTCATCATGCCG	TAATACGACTCACTATAGGGCCTTGTAGCTGGAGGTAAGCTCGTCGAACAGTTTCG
CG5643 wdb KN exon 5	TAATACGACTCACTATAGGGCCTTGCAACCTATTTCGCACGTTGCCGCCCAG	TAATACGACTCACTATAGGGCCACGCTTGCCGATAGTTGCTTGGAAATCCTGCG
CG7913 B’ KN RAspec exon 3	TAATACGACTCACTATAGGGCTCCTCTGCGGCGGCTGTGCCGTCGTCCGCCGCC	TAATACGACTCACTATAGGGCGTAGAATAGATTCCGGTCGCGAGCCCTTCCCGG
CG7913 B’ KN RE exon 2sp	TAATACGACTCACTATAGGGCGACAACAACAGCAGCATCTTCAGTTGTGGAAACC	TAATACGACTCACTATAGGGCACTCTCTTTGATACCGTTAGTGGCCGATGCCGG
CG7913 B’ KN exon 5	TAATACGACTCACTATAGGGCCCCGAATGGTGCCGAATTCGATCCGGAGGAGG	TAATACGACTCACTATAGGGCTTTCCATAGATGCGATGTAAAACAGTCTTCAGG
CG7913 B’ KN exon 7	TAATACGACTCACTATAGGGCCGCACAGTCCCAAGGAGGTTATGTTTTTGAACGAGC	TAATACGACTCACTATAGGGCTTGCTTGTAGTTCTTGCTGCACTCGTCAAAAAGCCG
CG4733 B" KN exon 5	TAATACGACTCACTATAGGGCAGCACTTCTACGTGATTTACTGCAAGTTTTGGG	TAATACGACTCACTATAGGGCCTGCTGCTCCTCGTAGAAGTATTCCAGTTCG

Primers used to generate siRNAs against Mouse genes

Fly CG	Fly Locus Link	Gene	Mouse Locus Link	Left Primer*	Right Primer*	Size
CG5179	37586	Cdk9	107951	taatacgactcactataggTGGACCTCATTGACAAGCTG	taatacgactcactataggCGTTCAAATTCCGTCTGGTT	244
CG4268	40292	Pitslre	12537	taatacgactcactataggTCTGCACATCACCGTACCAT	taatacgactcactataggTTCACTGGTGCTCCCTTCTT	294
CG6292	39961	Cyclin T	12455	taatacgactcactataggGTGCGTTTGGTTCCCAGAAG	taatacgactcactataggTGGCCACAGGGAGTCTCATT	291
CG6699	45757	beta'Cop	50797	taatacgactcactataggAGTCGGAGCAGAAGTGGAAA	taatacgactcactataggTCTCCCTCCACAGTTTCACC	333
CG7961	38199	alpha Cop	12847	taatacgactcactataggTGGCAGCCTACTTCACACAC	taatacgactcactataggCCTCAGCCCAATCACATCTT	392
CG6625	40233	Snap	108124	taatacgactcactataggGCTGGAGCAGTACCAGAAGG	taatacgactcactataggCTCGTCACCCTGAATGGTCT	355
CG14813	45250	delta COP	213827	taatacgactcactataggCAGCTACAGACGCATCCAAA	taatacgactcactataggACTCCAGGCTGCCACTCTTA	391
CG3948	39862	zeta COP	56447	taatacgactcactataggAAATCGCTCTGTTGGAAGGA	taatacgactcactataggCTGACTGCAGCACCTGAGAC	327
CG1528	43717	gamma Cop	54161	taatacgactcactataggTGCTGTGCTCATGAACTTCC	taatacgactcactataggTCCAAGACCACACGGTTGTA	267
CG1017	38256	CG1017	67532	taatacgactcactataggCTTGGCTTCCCACATTGACT	taatacgactcactataggGTAGTTATGGCTGGCCTGGA	396
CG3193	31208	crooked neck	66877	taatacgactcactataggGGTGGAAAGTGATGCGGAAG	taatacgactcactataggCCCAAAGCTCTTCTGGCAAA	291

*--Lower case letters indicate T7 Polymerase Binding Site.

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Additional Genes and Protein Complexes Affecting Hh Signaling

Our screen identified several interesting hits and categories of hits that we did not have space to discuss in detail in the main body of the text. In this section we will discuss several of these hits and surmise how they may interact with both known Hh regulators and the candidate hits we identify in the screen. In addition, a brief section expanding upon the possible role of splicing factors in Hh signaling is included.

SNAP and the COPI complex play a role in Hh signaling

One of the large protein complexes we identified was the COPI complex. The COPI complex is involved in vesicular trafficking and consists of seven core components: the alpha, beta, beta', gamma, delta, zeta, and epsilon subunits, of which we identified all but the epsilon subunit(Figure 3A and Supplementary Table 4)[1-3]. The alpha and delta subunits were also identified as factors which increased basal reporter activity in another RNAi screen for Hh components[4]. Originally identified as regulating retrograde Golgi to endoplasmic reticulum (ER) transport, the COPI complex has since been implicated in retrograde and anterograde transport between the plasma membrane and the Golgi apparatus and in endosomal transport[1]. All six COPI subunits scored as moderate to strong hits, reducing Hh signaling by 45-70% (Figure 3A). When we examined how the COPI subunits scored in the Ci assay, we found that the scores of all the COPI subunits were reduced to less than half of their original Hh values. These findings suggested that the COPI complex exerts most of its effects upstream of or in parallel to Ci (Figure 3B and Table 1).

One other hit from the screen was annotated as a vesicular trafficking protein; the *Drosophila* homolog of the Soluble NSF Attachment Protein or *SNAP* (*CG6625*)(Figure 3A). SNAP is involved in vesicular transport and fusion, and its function has been especially well characterized in synaptic vesicle regulation[5]. *SNAP* RNAi reduced Hh reporter activity by ~50% when Hh was expressed, while *SNAP* RNAi only reduced reporter activity by ~10% when Ci was expressed, suggesting that *SNAP* also works primarily upstream of Ci (Figure 3B).

To determine if the COPI complex and SNAP are involved in Shh signaling, we used siRNAs to vertebrate SNAP and five vertebrate COPI components to assay Hh reporter activity in a mouse Shh signaling assay. As shown in Figure 3C, mouse alpha, beta, and delta COP, all reduce Shh signaling in NIH3T3 cells by greater than 80% while siRNAs to Zeta COP, gamma COP, and SNAP all reduce reporter activity to 50% that of normal. In this assay, alpha COP siRNAs actually reduce reporter activity more than siRNA's to mSmo, possibly indicative of a more profound requirement for COPI complex members in Shh signaling in this cell type. Thus, the COPI complexes and SNAP play conserved roles in the insect and vertebrate Hh signaling pathways.

The CCR4-NOT Deadenylation Complex Regulates Hh Signaling.

Degradation of mRNAs is an important process in regulation of gene expression. Deadenylation is the rate-limiting step in mRNA decay. In metazoans, the CCR4-NOT complex is the major mRNA deadenylation enzyme and has been well conserved in evolution. The CCR4-NOT complex consists of several subunits, of which the founding yeast components, CCR4 and CAF1/POP2, appear to contain the enzymatic activity involved in cleavage of the poly-A tail[6]. Up to five members of the NOT family of proteins are accessory members of this complex, binding the core deadenylating enzymes and presumably regulating them in an as yet undefined fashion. Following cleavage of the poly-A tail by this complex, the 5' cap is removed and the rest of the mRNA is degraded by exonucleases[6,7].

In our screen, we isolated three components of the CCR4-NOT complex: *Rga*, which encodes a *NOT2* ortholog, *CG5684*, which encodes a *CAF1* ortholog, and *l(2)NC136*, which encodes a *NOT3/5* ortholog[8] (Supplementary Table 1). Targeting of *CG5684* and *Rga* by RNAi reduced Hh signaling in all four secondaries, while *l(2)NC136* only reduced signaling in the GL2 and Pol III-Hh secondaries. None of these three genes significantly reduced Renilla control reporter activity, suggesting that they did not substantially reduce cell number in dsRNA transfected cells (Supplementary Table 4). When reduced by RNAi, *Rga* and *CG5684* had no effect on Ci induced ptcΔ136 reporter activity, indicating that they act upstream of Ci. *l(2)NC136*, however, increased Ci induced reporter activity, indicating a more complex interaction with Ci (Supplementary Table 4). We should also note that *CG1884*, which encodes the *Drosophila* ortholog of the *NOT1* gene, had an average Z-score of -1.95, and, thus, just barely missed being scored as a hit.

How might this deadenylation complex be regulating Hh signaling? The CCR4/NOT complex has recently been shown to be involved in localization and degradation of *Hsp83* transcripts in *Drosophila*[9]. Smaug, an RNA binding protein involved in translational repression, binds *Hsp83* mRNAs and recruits the CCR4/NOT complex to these mRNAs, leading to their degradation. It is therefore possible that one of the RNA binding proteins we isolated in our screen binds to a mRNA encoding a inhibitor of the Hh pathway and recruits the CCR4/NOT complex to that mRNA. Degradation of the inhibitory mRNA then contributes to the activation of Hh signaling. Interestingly, (as described in the main text) we isolated several RNA binding proteins that regulate Hh signaling, including *sqd*, *CG2097*, and *CG8435*.

Lipid Synthesis Genes

Five *Drosophila* genes involved in lipid synthesis were also identified in the primary screen. All five of these were retested in secondary assays. dsRNAs directed against two genes, *bgm* (*CG4501*) and *CG4585*, passed all four secondary assays and thus seem to target legitimate Hh regulators. One of these hits, the protein encoded by the *bgm* (*CG4501*) gene, is involved in the metabolism of very long-chain fatty acids. The *bgm* gene was originally isolated as a mutant that caused degeneration of the adult central nervous system and increased the amount of very long-chain fatty acids (VLCFAs) in *Drosophila*[10]. VLCFAs accumulate in these mutants because *bgm* is required to activate these fatty acids for further anabolic and catabolic events, such as oxidation, chain elongation, and/or phospholipid synthesis[11-15]. Interestingly, mammalian orthologs of *bgm*, refered to as either hsBG1 or lipidosin, also cause VLCFA accumulation and appear to be localized to mitochondrial membranes and microsomes found near mitochondria[14].

CG4585 is a gene without a clear mammalian ortholog. However, *CG4585* does contain a phosphatidylglycerophosphate synthase domain covering roughly one quarter of its length. Phosphatidylglycerophosphate synthesis is the rate-limiting step of cardiolipin synthesis[16]. Cardiolipin is a unique, dimeric phospholipid found in all eukaryotes which contains four acyl chains. It is found exclusively in mitochondrial membranes, as is phosphatidylglycerophosphate synthase itself[17,18]. Cardiolipin appears to have several biological roles, including modulation of respiratory chain proteins and mitochondrial protein import[19,20].

Whether these genes are truly involved in Hh signaling and exactly what role these genes may be playing in this pathway are issues that should be further investigated. It is, however, interesting to note that both of these genes are involved in lipid metabolism and both are localized to the mitochondrion. Perhaps a lipid or lipid derivative produced by these enzymes on the mitochondrion moves to a cellular compartment containing a Hh signaling factor and modulates its activity.

TIF-IA and rRNAs

In this paper, we have shown that the ribosome plays an important positive regulatory role in Hh signaling. As ribosomal RNAs play critical roles in the structure and catalytic activity of the ribosome, one would expect that disruption of these RNAs would similarily affect Hh signaling. Unfortunately, the dsRNA library we used does not contain amplicons directed against rRNAs, so we could not examine this idea directly. One of our hits, however, indicated that rRNAs likely do play a positive role in Hh signaling.

TIF-IA is one of the principal transcription factors that regulates transcription of ribosomal RNAs (rRNAs) through its interaction with the RNA Polymerase I transcription factor complex[21]. Elimination of TIF-IA causes growth inhibition, nucleolar disruption, cell-cycle arrest, and induction of apoptosis[22]. Two dsRNAs targeting TIF-IA are present in the DRSC library and both were identified as strong positive regulators of Hh signaling in our assay. Both amplicons also reduce basal activity of the reporter, but, interestingly, do not reduce cell viability as judged by Renilla signal intensity (Supplementary Table 4). While identification of Tif-IA suggests that rRNAs play a role in Hh signaling, we should note that we did not identify any of the components of the RNA Polymerase I holoenzyme in the primary screen, which would also be expected to be positive regulators of Hh signaling.

Alternative Splicing Control of Hh Signaling

Based on our data identifying large numbers of splicing factors, and, in particular, many splicing factors known to be principally involved in splice site selection, we are compelled to ask the following question: If alternative splicing is important in Hh signaling, then what is the identity of the alternatively spliced mRNA? At present, there is no information on alternative splicing events in the *Drosophila* Hh signaling pathway. However, in vertebrates, both *Ptc* orthologs has been proven to have alternative transcriptional start sites and alternatively spliced isoforms. *Ptch*, the predominant *ptc* gene in vertebrates, has a least five different mRNA transcripts, with alternative transcriptional start sites in these transcripts encoding three different protein isoforms[23]. The second vertebrate *ptc* gene, *Ptch2*, has at least 8 different transcripts, resulting from two alternative splicing events and one intron read-through event[24,25]. Indeed, it has been found that one of the three Ptch protein isoforms is upregulated during Hh signaling and has reduced inhibitory capabilities with respect to Hh signaling[26]. Thus, it is possible that *Drosophila ptc* could be subject to alternative splicing regulated by the factors we have identified in this paper, producing Ptc proteins with differing inhibitory capabilities.

Other Issues Relevant to dsRNAi Screening for Hh Components

Comparison to a previous Hh RNAi Screen.

Previously, Lum et al. conducted a large scale RNAi screen in which dsRNAs targeted against ~6,000 genes were tested using a conditioned media assay to identify new components of the *Drosophila* Hh pathway. Our assay differs from that of Lum et al[4] in several important respects. First, our screen is a genome-wide screen while the Lum et al screen only covered roughly 40% of the genome. Second, the previous study used as the source of Hh a conditioned medium containing a truncated and artificially soluble form of Hh, known as Hh-N. We chose to use cotransfected, full length Hh as the stimulus in our assay. While this meant that we risked missing some genes that may be involved in Hh transport or surface reception, we obtained a higher signal to noise ratio. Transfection of full length Hh also avoided the potential complications attendant with using a conditioned media containing undefined growth factors, signaling molecules, and/or hormones. Third, our assay is of higher sensitivity, given that we identify a greater proportion of the known Hh signaling genes, a far greater proportion of the ribosomal proteins, and more of the COPI complex proteins than did the Hh-N conditioned media study.

With respect to the genes identified in each screen, the Hh-N conditioned media screen identified 5 novel genes (out of 21 total novel genes) that we did not identify. Discounting uncertain genes, the ribosomal genes, and the proteasomal complex genes, we identified over 150 genes that were not reported in the Hh-N screen. And, in spite of our more stringent criteria for hits--Z scores of less than -2.0 or greater than +3.0-- we identified more of the known components of the Hh signaling pathway than the previous Hh-N screen. While the Hh-N screen identified five out of the 16 known components of Hh signaling, we identified 9 and would have identified a tenth component if we lowered our scoring threshold to match those in the Hh-N study (Casein Kinase 1 alpha had a average Z-score of -1.83 in our primary screen and therefore would have been identified with a Z-score threshold of -1.75 as in Lum et al.[4]). Why we did not identify the four other genes found in the Hh-N conditioned media screen is not yet clear. In the case of *dlp*, a GPI linked protein for which there is in vivo evidence for its role in coreception and/or transport of morphogens such as Hh and Wg[4,27-31], it is likely that expression of Hh in the assayed cells partially alleviates the requirement for Dlp in Hh signaling. *CG9211* may have been missed for a similar reason, as it is also a membrane associated protein. However, *caupolican*, a homeodomain containing gene, and *CG6745*, a pseudouridine synthase, should have been identified in our screen if they are involved in Hh signaling. These genes might not have been identified in our screen because they are specifically required for Hh-N stimulated Hh signaling versus full length Hh signaling, or because loss of the gene products has a more acute effect in the Hh-N assay. In the case of *caupolican*, however, we tested three new, distinct dsRNA's to try and find a effect on Hh signaling, but only found an effect with a *caup* dsRNA that had multiple off target effects (data not shown).

Off-Target Effects

Not surprisingly, some sequences in known *Drosophila* transcripts contain continuous 21 bp matches to other known transcripts. dsRNAs which have 21 bp overlaps with other known transcripts could potentially target those transcripts for RNAi as well as the intended target. While most of the dsRNAs in our collection are ~500 bp and the majority of potential 21 bp fragments generated by a particular dsRNA would be specific, it is nonetheless conceivable that a single overlapping fragment could efficiently target an unintended mRNA as well as the intended target. Indeed, we have some evidence that some hits are a result of these off-target effects. However, this phenomena seems to be largely limited to dsRNAs which have more than ten potential off-target genes. In the limited testing that we have conducted so far, dsRNAs that have less than 10-20 potential off target genes are usually having an effect because of RNAi against the intended target and not the unintended target. This was ascertained by making and testing new dsRNAs without potential off-target effects but to different regions of the genes than the original library dsRNA targeted (data not shown). The first Table shown at the end of this Supplementary Note lists those amplicons which do not have potential off target effects, while the next Table lists those amplicons which do have potential off targets and the number of off target mRNA's that could be targeted by that dsRNA. Potential off target effects were determined using the DRSC algorithm found at the DRSC's website at http://flyrnai.org and are defined as any transcript having at least one continuous 21 bp segment exactly matching a sequence found in the amplicon. Some potential off-target genes may have more than one 21 bp overlap and/or may have overlaps longer than 21 bp. To confirm that genes identified as hits but represented by a single amplicon in the DRSC collection were legitimate, we also made new, no off-target dsRNAs targeting some of these genes and tested them in our assay. As shown for three of our hits, the majority of genes tested in this way are still identified as hits in the Hh assay (Supplementary Figure 3).

Edge Effects

Edge effects, in the context of our assay, are defined as artificially high Z-scores found principally along the outer edge rows (that is, the A and P rows) of 384 well assay plates. These artificially high Z-scores are due to low Renilla luciferase scores in these edge wells: the Firefly luciferase scores for these wells do not seem, on average, to be different from the plate average. We do not know why the Renilla luciferase values for these edge wells are artificially low, but we speculate that it is related to the particular chemistry of the Renilla luciferase reaction. These edge effects seem to occur at random in our screen. The end result of the appearance of these edge effects is that some true Hh regulators whose dsRNA was in an edge row, such as *wdb*, were not scored as hits in the primary assay.

However, we believe that relatively few genes were missed in our assay because of edge effects. To test if we had actually missed a significant number of hits in edge rows, we made two 384 well plates that contained the edge rows (that is, the A and P rows from the original 384 well assay plates) from screening assay plates 1-14. These rows were arrayed such that none of the rows were now in edge rows in these edge row test plates. We then tested these edge row plates using the original screening conditions. In the original primary screen we had found 13 hits, both up and down, from the A and P rows of assay plates 1-14. When we rescreened using the new edge well test plates, we found 16 hits, both up and down. Of these hits from the edge well plates, 10 of the 13 found in the primary screen were re-identified. This left six that were possibly new hits not identified in the primary screen (however, we should note that two of the new hits found in the edge plates had Z scores of -1.8 or lower and so just barely missed being scored in the primary screen). While we only did this edge row assay once, we believe that the results indicate that very few hits (at most, approximately 6 out of over 672 wells retested, or roughly 0.9%) were missed because of the edge row effects. This seems to be due to the fact that decreases in normalized luciferase score can still appear in edge wells, and it is mainly, although not exclusively, increases in scores that are masked by the edge well effect. Since we had stricter criteria for increases--and hence did not identify many increases in the primary screen--this is probably why the number of hits did not change substantially when the edge row plates were tested.

Cell Density and Cell Number Effects

As has previously been noted, cell density can modulate the efficiency of several signaling pathways. However, this is not a major concern in our assay system. Clone 8 cells have a low transfection efficiency, with only 5-10% of the cells being transfected and all the remaining cells being untransfected, as judged by GFP transfections. The untransfected cells appear to grow normally and so, by the end of our 5 day assay period, these untransfected cells have generally grown to confluence, thus providing even those cells that may be retarded in their growth due to a particular dsRNA a confluent, or nearly confluent, cell environment. Cell number effects--that is the number of cells actually affected by the dsRNA--only seem to be a concern at very low transfected cell numbers caused by RNAi against genes such as *thread*. For all dsRNAs except those that most severely reduce viable cell number, the average firefly and renilla luciferase numbers for GFP control assays in this 384 well format were generally at least 20 times that of background in spite of the low transfection efficiency, and so the scores are not substantially affected by background fluctuations.

PP2A Analysis

In our primary Hh screen, the A regulatory subunit was not identified as a hit, and the only B subunit that was identified was one of the two *PP2A-B′* dsRNAs. This particular *PP2A-B′* dsRNA had multiple potential off-target effects (see above) and so we were uncertain as to the validity of this hit. We did not identify *wdb* in the primary screen because the only *wdb* dsRNA in the collection is in an edge well whose Z-score was likely artificially raised due to the above noted edge effects. The A regulatory subunit dsRNAs were not in edge rows. One of the two dsRNAs in the collection targeting *CG33297* does not seem to work in our assay (Figure 6C) and the other did not show up as a hit in the a primary assay, while it was identified as positive regulator of Hh signaling in our testing of specific PP2A components.

dsRNAs without Potential Off-Target Effects

CG#	Gene	DRSC Amplicon	Number of Potential Secondary Targets
CG10042	MBD-R2	DRSC14180	0
CG10095	dpr15	DRSC14188	0
CG10149	Rpn6	DRSC07541	0
CG1017	CG1017	DRSC08154	0
CG10198	Nup98	DRSC14209	0
CG10198	Nup98	DRSC14210	0
CG10305	RpS26	DRSC03420	0
CG10306	CG10306	DRSC04056	0
CG10423	RpS27	DRSC14244	0
CG10484	Dox-A2	DRSC03318	0
CG10541	Tektin-C	DRSC09741	0
CG10652	RpL30	DRSC02087	0
CG10706	SK	DRSC17888	0
CG10712	Chro	DRSC11630	0
CG10869	CG10869	DRSC00290	0
CG10938	ProsMA5	DRSC07514	0
CG1101	Aly	DRSC12497	0
CG11184	Upf3	DRSC04092	0
CG11276	RpS4	DRSC11272	0
CG1135	CG1135	DRSC08180	0
CG11522	RpL6	DRSC14323	0
CG11561	smo	DRSC00837	0
CG11888	Rpn2	DRSC16839	0
CG11981	Prosbeta3	DRSC16801	0
CG11985	CG11985	DRSC14460	0
CG12000	CG12000	DRSC12186	0
CG12050	CG12050	DRSC02152	0
CG12079	CG12079	DRSC08243	0
CG12104	CG12104	DRSC08254	0
CG12284	th	DRSC11404	0
CG12323	Prosbeta5	DRSC07517	0
CG12324	RpS15Ab, RpS15Aa	DRSC06129	0
CG12607	CG12607	DRSC08287	0
CG1263	RpL8	DRSC08532	0
CG1263	RpL8	DRSC08695	0
CG12740	RpL28	DRSC08293	0
CG12750	CG12750	DRSC02179	0
CG12775	RpL21	DRSC03704	0
CG12822	CG12822, CG18853	DRSC20757	0
CG1322	zfh1	DRSC17098	0
CG13389	RpS13	DRSC03419	0
CG13936	CG13936	DRSC08405	0
CG14206	RpS10b	DRSC19561	0
CG14210	CG33066, CG14210	DRSC19566	0
CG14641	CG14641	DRSC12227	0
CG1475	RpL13A	DRSC12265	0
CG14792	sta	DRSC18838	0
CG14813	deltaCOP	DRSC18760	0
CG1489	Pros45	DRSC20571	0
CG1492	CG1492	DRSC19605	0

CG#	Gene	DRSC Amplicon	Number of Potential Secondary Targets
CG15255	CG15255	DRSC01869	0
CG1528	gammaCop	DRSC16955	0
CG15442	RpL27A	DRSC00781	0
CG15552	Sox100B	DRSC15090	0
CG1559	Upf1	DRSC19667	0
CG15693	RpS20	DRSC16836	0
CG15697	RpS30	DRSC15119	0
CG15814	CG15814	DRSC19730	0
CG16901	sqd	DRSC17066	0
CG16941	CG16941	DRSC15166	0
CG1708	cos	DRSC07598	0
CG1716	CG1716	DRSC19765	0
CG17272	CG17272	DRSC15211	0
CG17331	CG17331	DRSC02603	0
CG17521	Qm	DRSC11947	0
CG18041	CG18041	DRSC15301	0
CG18174	Rpn11	DRSC03422	0
CG1821	RpL31	DRSC06716	0
CG18468	CG18468	DRSC06759	0
CG18591	CG18591	DRSC02680	0
CG1874	CG1874	DRSC06787	0
CG1915	sls	DRSC08670	0
CG2013	UbcD6	DRSC12370	0
CG2033	RpS15Ab, RpS15Aa	DRSC19831	0
CG2097	CG2097	DRSC12301	0
CG2099	RpL35A	DRSC12302	0
CG2125	ci	DRSC17172	0
CG2128	Hdac3	DRSC12352	0
CG2161	Rga	DRSC12364	0
CG2168	RpS3A	DRSC17168	0
CG2184	Mlc2	DRSC16741	0
CG2253	Upf2	DRSC18218	0
CG2411	ptc	DRSC07669	0
CG2411	ptc	DRSC05117	0
CG2685	CG2685	DRSC18463	0
CG2746	RpL19	DRSC04649	0
CG2807	CG2807	DRSC00535	0
CG2835	G-salpha60A	DRSC04616	0
CG2925	noi	DRSC12383	0
CG2960	RpL40	DRSC00782	0
CG2986	oho23B	DRSC00833	0
CG2998	RpS28b	DRSC18258	0
CG30053	CG30053	DRSC07260	0
CG30158	CG30158	DRSC04956	0
CG30424	CG30424	DRSC04017	0
CG31111	CG31111	DRSC14649	0
CG31657	PNUTS	DRSC00638	0
CG3193	crn	DRSC18185	0
CG3193	crn	DRSC18755	0
CG3195	RpL12	DRSC04344	0
CG3203	RpL17	DRSC18293	0
CG32202	CG32202	DRSC09989	0
CG32253	CG11583	DRSC08202	0
CG32308	CG32308	DRSC07967	0
CG32743	Smg1	DRSC18366	0
CG32743	Smg1	DRSC18367	0
CG3278	Tif-IA	DRSC03717	0
CG3278	Tif-IA	DRSC03726	0
CG3314	RpL7A	DRSC18709	0
CG3329	Prosbeta2	DRSC11257	0
CG3395	RpS9	DRSC11273	0

CG#	Gene	DRSC Amplicon	Number of Potential Secondary Targets
CG3412	slmb	DRSC17056	0
CG3416	Mov34	DRSC04624	0
CG3436	CG3436	DRSC00605	0
CG3455	Rpt4	DRSC18713	0
CG3605	CG3605	DRSC00619	0
CG3661	RpL23	DRSC04648	0
CG3751	RpS24	DRSC04414	0
CG3922	RpS17	DRSC11271	0
CG3948	zetaCOP	DRSC11412	0
CG3949	hoip	DRSC03546	0
CG4003	pont	DRSC17029	0
CG40125	CG40125	DRSC00928	0
CG40199	RpL15	DRSC20963	0
CG40278	RpL38	DRSC07818	0
CG4046	RpS16	DRSC04442	0
CG4097	Pros26	DRSC11256	0
CG4111	RpL35	DRSC18347	0
CG4152	l(2)35Df	DRSC03560	0
CG4157	Rpn12	DRSC11275	0
CG4453	Nup153	DRSC19904	0
CG4453	Nup153	DRSC19904	0
CG4464	RpS19a	DRSC20281	0
CG4501	bgm	DRSC03495	0
CG4585	CG4585	DRSC04475	0
CG4602	Srp54	DRSC03442	0
CG4609	fax	DRSC11345	0
CG4651	RpL13	DRSC03416	0
CG4699	CG4699	DRSC15625	0
CG4759	RpL27	DRSC15638	0
CG4863	RpL3	DRSC16834	0
CG4893	CG4893	DRSC10455	0
CG4897	RpL7	DRSC03417	0
CG4903	MESR4	DRSC06901	0
CG4904	Pros35	DRSC03401	0
CG4912	eEF1delta	DRSC02790	0
CG4918	RpLP2	DRSC07539	0
CG5179	Cdk9	DRSC04601	0
CG5271	RpS27A	DRSC03421	0
CG5338	RpS19b	DRSC15773	0
CG5360	CG5360	DRSC04498	0
CG5378	Rpn7	DRSC16841	0
CG5427	Oatp33Ea	DRSC02859	0
CG5499	His2Av	DRSC16702	0
CG5602	CG5602	DRSC04518	0
CG5605	eRF1	DRSC11779	0
CG5684	CG5684	DRSC10537	0
CG5733	CG5733	DRSC06947	0
CG5786	ppan	DRSC17030	0
CG5838	Dref	DRSC03319	0
CG5920	sop	DRSC03614	0
CG5931	CG5931	DRSC10559	0
CG5969	CG5969	DRSC11789	0
CG6015	CG6015	DRSC15948	0
CG6054	Su(fu)	DRSC16871	0
CG6105	l(2)06225	DRSC02928	0
CG6141	RpL9	DRSC03418	0
CG6223	betaCop	DRSC20312	0
CG6253	RpL14	DRSC11269	0
CG6437	CG6437	DRSC04525	0
CG6510	RpL18A	DRSC07538	0
CG6551	fu	DRSC20333	0
CG6625	Snap	DRSC11285	0

CG#	Gene	DRSC Amplicon	Number of Potential Secondary Targets
CG6694	CG6694	DRSC10696	0
CG6699	beta'Cop	DRSC03492	0
CG6779	RpS3	DRSC16838	0
CG6846	RpL26	DRSC10726	0
CG6905	CG6905	DRSC08577	0
CG6962	CG6962	DRSC16153	0
CG6987	SF2	DRSC16845	0
CG7109	mts	DRSC03574	0
CG7219	CG7219	DRSC03029	0
CG7269	Hel25E	DRSC03342	0
CG7283	RpL10Ab	DRSC10798	0
CG7292	Rrp6	DRSC16223	0
CG7424	RpL36A	DRSC03055	0
CG7425	eff	DRSC16940	0
CG7490	RpLP0	DRSC11878	0
CG7622	RpL36	DRSC18708	0
CG7664	crp	DRSC03515	0
CG7726	RpL11	DRSC07537	0
CG7752	CG7752	DRSC11848	0
CG7757	CG7757	DRSC10912	0
CG7762	Rpn1	DRSC11274	0
CG7776	E(Pc)	DRSC07408	0
CG7926	Axn	DRSC14120	0
CG7939	RpL32	DRSC16835	0
CG7961	alphaCop	DRSC08706	0
CG7977	RpL23A	DRSC08694	0
CG8048	Vha44	DRSC07572	0
CG8264	Bx42	DRSC17743	0
CG8277	CG8277	DRSC10975	0
CG8415	RpS23	DRSC07169	0
CG8426	l(2)NC136	DRSC04908	0
CG8435	CG8435	DRSC07179	0
CG8580	CG8580	DRSC11000	0
CG8615	RpL18	DRSC11016	0
CG8857	RpS11	DRSC07289	0
CG8877	CG8877	DRSC07293	0
CG8900	RpS18	DRSC07540	0
CG8922	RpS5a	DRSC20282	0
CG8936	Arpc3B	DRSC20126	0
CG8954	Smg5	DRSC03124	0
CG9091	RpL37a	DRSC20149	0
CG9094	CG9094	DRSC07936	0
CG9233	fu2	DRSC03324	0
CG9282	RpL24	DRSC03185	0
CG9306	CG9306	DRSC03193	0
CG9324	CG9324	DRSC03201	0
CG9327	Pros29	DRSC04644	0
CG9354	RpL34b	DRSC16475	0
CG9429	Crc	DRSC16614	0
CG9504	CG9504	DRSC20187	0
CG9750	rept	DRSC11388	0
CG9885	dpp	DRSC00118	0
CG9924	CG9924	DRSC16580	0
		DRSC00133	0
		DRSC00181	0
		DRSC00244	0
		DRSC00938	0
		DRSC01382	0
		DRSC01392	0
	mol	DRSC01431	0
		DRSC01492	0
		DRSC01498	0

CG#	Gene	DRSC Amplicon	Number of Potential Secondary Targets
		DRSC01800	0
		DRSC03669	0
		DRSC03679	0
		DRSC03777	0
	RpL39	DRSC04651	0
		DRSC04953	0
		DRSC05339	0
		DRSC05564	0
		DRSC05653	0
	cyp33	DRSC05666	0
		DRSC06303	0
		DRSC07851	0
		DRSC07876	0
		DRSC07982	0
		DRSC07995	0
		DRSC08039	0
		DRSC08743	0
		DRSC08908	0
		DRSC08917	0
		DRSC08929	0
		DRSC09001	0
		DRSC09014	0
		DRSC09089	0
		DRSC09601	0
		DRSC14042	0
		DRSC14046	0
		DRSC17236	0
		DRSC17344	0
		DRSC17378	0
		DRSC18937	0
		DRSC19104	0
		DRSC20751	0
		DRSC21055	0
		DRSC05810	0
		DRSC12025	0
		DRSC12677	0
		DRSC13172	0
		DRSC12923	0
		DRSC13841	0
		DRSC13783	0
		DRSC17256	0

dsRNAs with Potential Off-target Effects

CG#	Gene	DRSC Amplicon	Number of Potential Secondary Targets
CG10279	Rm62	DRSC12365	1
CG10325	abd-A	DRSC16897	381
CG10944	RpS6	DRSC18712	2
CG11121	so	DRSC07693	252
CG11194	Hey	DRSC07440	358
CG11198	CG11198	DRSC06059	3
CG11380	CG11380	DRSC18632	62
CG11405	A3-3	DRSC17225	458
CG11416	CG11416	DRSC04111	6
CG11502	svp	DRSC15311	310
CG11624		DRSC08703	5

CG#	Gene	DRSC Amplicon	Number of Potential Secondary Targets
CG11700	CG11700	DRSC17794	3
CG11711	Mob1	DRSC09117	7
CG12054	CG12054	DRSC14467	187
CG12238	l(1)G0084	DRSC19450	2
CG12254	Arc92	DRSC14483	537
CG12348	Sh	DRSC19214	409
CG12852	CG12852	DRSC14558	385
CG12912	CG12912	DRSC06227	538
CG12977	CG12977	DRSC11687	250
CG13130	CG13130	DRSC02226	7
CG13165	CG13165	DRSC06292	235
CG13222	CG13222	DRSC06343	130
CG13333	CG13333	DRSC06368	5
CG13445	CG13445	DRSC09992	6
CG13847	CG13847	DRSC14697	107
CG14023	CG14023	DRSC02353	302
CG14180	CG14180	DRSC10179	1
CG14342	CG14342	DRSC00372	2
CG14459	CG14459	DRSC11719	341
CG14494	CG14494	DRSC05695	358
CG14519	CG14519	DRSC14871	25
CG14656	CG14656	DRSC12242	5
CG14952	CG14952	DRSC08412	6
CG15157	CG15157	DRSC02458	69
CG1524	RpS14a	DRSC18710	1
CG15476	CG15476	DRSC02487	2
CG15494	CG15494	DRSC02502	21
CG15753	CG15753	DRSC19718	519
CG15783	CG15783	DRSC18089	269
CG16902	Hr4	DRSC18495	283
CG17077	pnt	DRSC17028	75
CG17489	RpL5	DRSC03801	1
CG17888	Pdp1	DRSC08897	587
CG18214	trio	DRSC08527	25
CG2065	CG2065	DRSC06813	1
CG30044	CG30044	DRSC06277	1
CG30126	CG30126	DRSC05769	280
CG30132	par-1	DRSC05792	282
CG3025	mof	DRSC18795	1
CG30361	mXr	DRSC05106	4
CG31125	CG31125	DRSC13684	3
CG31145	CG31145	DRSC14670	161
CG31209	CG31209	DRSC15701	141
CG3193	crn	DRSC18184	1
CG32029	CG32029	DRSC10646	20
CG32046	CG32046	DRSC10171	6
CG32048	CG32048	DRSC08991	468
CG32050	CG32050	DRSC10173	4
CG32080	CG32080	DRSC09074	16
CG32133	CG32133	DRSC11048	9
CG32139	Sox21b	DRSC10634	103
CG32149	RhoGAP71E	DRSC10837	209
CG32150	CG32150	DRSC09899	16
CG32264	CG32264	DRSC08061	79
CG32296	CG32296	DRSC08263	62
CG32334	CG32334	DRSC07938	37
CG32381	unc-13-4A	DRSC10203	233
CG32432	CG32432	DRSC11703	267
CG32432	CG32432	DRSC11704	51
CG32474	dys	DRSC14897	387
CG32541	CG32541	DRSC19543	49
CG32566	CG9132	DRSC20159	1

CG#	Gene	DRSC Amplicon	Number of Potential Secondary Targets
CG32592	hiw	DRSC20338	5
CG32606	CG32606	DRSC19001	345
CG32676	CG32676	DRSC18442	129
CG32688	Hk	DRSC18674	11
CG32767	CG32767	DRSC18386	6
CG32830	CG32830	DRSC01162	153
CG32955	CG32955	DRSC01163	2
CG32970	CG32970	DRSC01375	408
CG33106	mask	DRSC16018	1
CG33110	CG33110	DRSC14694	210
CG33141	sns	DRSC06423	265
CG33141	sns	DRSC07692	454
CG33183	Hr46	DRSC06118	289
CG3411	bs	DRSC04676	295
CG3732	CG3732	DRSC04410	8
CG3895	ph-d	DRSC18819	84
CG4013	Smr	DRSC20288	513
CG40218		DRSC21138	1
CG40341		DRSC03750	1
CG4035	eIF-4E	DRSC11342	1
CG4087	RpLP1	DRSC00783	4
CG4136	CG4136	DRSC18349	381
CG4268	Pitslre	DRSC11874	1
CG4379	Pka-C1	DRSC03399	1
CG5166	Atx2	DRSC15727	395
CG5289	Pros26.4	DRSC16799	1
CG5575	ken	DRSC04696	80
CG5610	nAcRalpha-96Aa	DRSC13670	39
CG5674	CG5674	DRSC02874	106
CG5758	CG5758	DRSC02889	5
CG5827	RpL37A	DRSC02899	3
CG6043	CG6043	DRSC02922	340
CG6064	TORC	DRSC10578	212
CG6292	CycT	DRSC11124	4
CG6937	CG6937	DRSC16140	1
CG7031	CG7031	DRSC16178	357
CG7305	CG7305	DRSC16225	421
CG7434	RpL22	DRSC18707	1
CG7481	RhoGAP18B	DRSC20049	1
CG7525	Tie	DRSC08104	4
CG7835	CG7835	DRSC20058	14
CG7913	PP2A-B'	DRSC16337	87
CG7923	Fad2	DRSC11165	2
CG7938	Sry-beta	DRSC16868	1
CG8108	CG8108	DRSC09675	4
CG8179	CG8179	DRSC07091	371
CG8233	CG8233	DRSC07116	1
CG8332	RpS15	DRSC07151	1
CG8367	cg	DRSC07650	174
CG8787	Asx	DRSC05924	12
CG9176	cngl	DRSC20318	3
CG9533	rut	DRSC20367	556
CG9667	CG9667	DRSC16535	1
CG9748	bel	DRSC16555	3
CG9817	CG9817	DRSC18446	394
CG9842	Pp2B-14D	DRSC20270	1
CG9930	E5	DRSC16585	143
CR31615	His-Psi:CR31616, His-Psi:CR31615	DRSC03760	3
		DRSC00209	6

CG#	Gene	DRSC Amplicon	Number of Potential Secondary Targets
		DRSC00245	8
		DRSC00405	1
		DRSC00977	2
		DRSC01077	28
		DRSC01082	34
		DRSC01088	199
		DRSC01091	32
		DRSC01151	21
		DRSC01243	15
		DRSC01284	20
		DRSC01624	1
		DRSC01729	29
		DRSC01970	138
		DRSC02439	4
		DRSC04825	5
		DRSC04827	30
		DRSC04925	2
		DRSC04952	3
	CG33352	DRSC05013	323
		DRSC05037	1
		DRSC05108	2
		DRSC05225	55
		DRSC05231	31
	stan	DRSC05234	149
		DRSC05259	405
		DRSC05297	29
		DRSC05322	3
		DRSC05360	29
	mam	DRSC05468	112
		DRSC05475	6
		DRSC05623	234
		DRSC05644	10
		DRSC05691	5
		DRSC05715	45
		DRSC05797	19
		DRSC05818	1
		DRSC05871	385
		DRSC05899	20
		DRSC06353	408
		DRSC07855	3
		DRSC07932	16
		DRSC08080	101
		DRSC08092	12
	CG33231	DRSC08296	1
		DRSC08878	4
		DRSC08903	32
		DRSC08919	492
		DRSC08923	11
		DRSC08973	10
		DRSC09065	7
		DRSC09077	28
	CG33500	DRSC09115	8
		DRSC09409	28
		DRSC09612	28
	AGO2	DRSC09999	95
		DRSC10097	28
		DRSC10152	38
	CG33277	DRSC10335	210
		DRSC11453	3
		DRSC11456	30
		DRSC12666	437
		DRSC12796	204

CG#	Gene	DRSC Amplicon	Number of Potential Secondary Targets
		DRSC12841	6
		DRSC13109	29
		DRSC13294	31
		DRSC13978	30
		DRSC14061	36
		DRSC14674	559
		DRSC14730	68
		DRSC14786	390
		DRSC14928	13
		DRSC15105	469
	CG16918	DRSC15165	121
		DRSC17234	163
		DRSC17248	368
		DRSC17270	258
		DRSC17343	3
		DRSC17379	198
		DRSC17473	503
		DRSC17488	35
		DRSC17612	231
		DRSC17617	28
		DRSC17710	24
		DRSC18003	142
	CG32776	DRSC18119	123
		DRSC18889	6
		DRSC18905	4
		DRSC18999	31
		DRSC19008	211
		DRSC19029	154
		DRSC19193	22
		DRSC19200	30
		DRSC19255	12
		DRSC19272	40
		DRSC19301	1
		DRSC20442	1
		DRSC20534	17

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Supplementary Figure 3. New dsRNAs targeted to regions of gene transcripts still demonstrate the same effect on Hh signaling. An example of three new dsRNAs designed to regions of the mRNA which were not targeted by the DRSC library dsRNA but which still have the same effect on ptcΔ136 reporter activity. The genes targeted are *Pitslre* (*CG4268*), *CG1017*, and *sqd* (*CG16901*) and were tested in the . In the case of *sqd*, the new dsRNA was designed to target only the RA isoform. These dsRNA’s were designed to lack any potential off-target effects. The reduction in Hh reporter activity for these new dsRNA's is very similar to that seen with the library dsRNAs.

Supplementary Figure 3