

## Materials and Methods

### (1) Constructs

16XSuperTopFlash (STF16): oligos containing 4 Tcf sites and an AscI overhang were multimerized and cloned into AscI/MluI sites of pTA(Clontech). 12XdTOP (dTF12): Tcf-binding sites were PCR cloned and multimerized to make the 12X “TOP” fragment. The TOP12-DNA fragment was cloned into the NheI site in the MCS of pGL3-luciferase vector (Promega) together with bp minimal promoter element from the *Drosophila Hsp70* gene, which was directionally cloned into the BglII/HinDIII sites. The primers used for PCR amplification of Hsp70 minimal promoter were: (Hsp.For) 5'-gaagatctgagcggcggagtataaatagaggc-3'; (Hsp.Rev) 5'-cccaagctccaattccctattcagagttctc -3', and TOP sites were: (TOP.For) 5'- gctctagagtgcacctgcagccc -3'; (TOP.Rev) 5'-ctagctagcgtcgactgaagctcctcc -3'. pMK33-Wg was a gift from R.Nusse (23) and PolIII-RL (polIII-Renilla Luciferase) was a gift from K. Nybakken. pAct-Rab5 expression construct was made by PCR amplification of Rab5 and blunt cloning of PCR product into the SpeI site of pact expression vector. The primers used for PCR were: (Rab5.For) 5'-gatcctccacattcgcatccgatcc-3'; (Rab5.Rev) 5'-ctagaatttcattttctacgaaggg-3'. All human cDNAs were amplified from a Human fetal brain or B-cell lymphoma cDNA libraries (Edge biosystems) or from cDNA derived from 293T cell mRNA. All full length ORF were inserted into pCS2+ (49). into the BamHI/XhoI sites. The accession number for all or the cDNAs used for the vertebrate experiments can be found in Supplemental Fig. S4. siRNA constructs were generated in pHippy (50). Sense and anti-sense oligos were annealed and cloned into the BsmBI site of pHippy. All siRNA sequences will be provided upon request.

### (2) RNAi Screen outline and cell culture

Wing imaginal disc-derived Clone 8 cells (20) were used in the RNAi experiment.

Transfections were performed in duplicate in 384-well plates using Effectene transfection reagent (Qiagen Inc.). The ratio of Luciferase reporter (STF16), normalization vector (PolIII-RL) and Inducer (pMK33-Wg23) DNA was 1:1:2 with 100ng of total DNA added per well. We used an inducible metallothionein promoter to drive *wg* expression (pMK33-Wg). dsRNAs were synthesized using *in vitro* transcription from PCR product templates which have T7 polymerase binding sites as linkers (as described in Boutros et al., 2004). All information related to PCR primers, length of PCR products and quality control for the amplicons, can be found through the DRSC link for a particular gene in Flybase (<http://flybase.bio.indiana.edu>). 80ng of dsRNA was added to each transfection reaction along with the total DNA. For the Wg screen we added the transfection mix together with the appropriate DNA constructs and the *Drosophila* cl8 cells into the 384-well screening plates containing individual dsRNAs (Supplementary Fig. S1). We waited 5 days to ensure RNAi-mediated degradation of the target mRNAs and complete depletion of target protein. The activity of the reporter-gene was assayed by measuring firefly (test reporter) and renilla luciferase (control reporter) with the “Dual-Glo” luciferase reagent (Promega) on the Analyst (Molecular Biosystems) plate reader. The protocol used for secondary screens remained unchanged from that of the primary screen except for the plate format, which was changed to 96-well plates to increase signal/noise ratio. Amount of dsRNA added to each well of 96-well plate was scaled up to 250ng/well.

### **(3) Data analysis**

Four distinct protocols were imposed on the raw data obtained from the primary screen to ascertain “hits”. 1. Normalized values (N), where  $N = \text{Firefly luciferase value} / \text{Renilla luciferase value}$ . Genes that were considered as hits either reduced Wg pathway activity by more than 1.5 SD (Standard Deviation) or increased reporter activity by more than 3

SDs with respect to the plate average (N). We chose a lower cut-off for genes that decreased reporter activity due to the nature of the inducibility of the reporter, which allows for greater dynamic range for activation, than for repression of the reporter. The same applies for the most of the following criteria. 2. Z-scores, that measure the deviation of N from the plate average in terms of the number of SDs. Genes with Z-scores less than  $-1.5$  (Fig. 2A, blue circle) or greater than  $2.5$  (Fig. 2A, red circle) were considered significant; 3.  $\log(\text{Normalized})$ . Log transformation of N values better fits the data points in a linear progression for both increases and decreases with respect to the plate average. Genes scoring  $\geq 2\text{SD}$  or  $\leq 2\text{SD}$  from plate average [ $\log(\text{N})$ ] were considered potential hits and finally; 4.  $\log(\text{Normalized}/\text{Plate Average of N})$ , which represents the fold change of a given normalized reporter activity value for a particular well from the plate average and a fold change of 2 or 0.5 from the plate average (N) were deemed to be significant. Genes that scored positive by at least two of the above-mentioned protocols were “cherry-picked” for further analyses in secondary screens. Note: The above-mentioned protocols for data analysis are not exhaustive. There could be other protocols imposed on the raw data to ascertain “hits”. Here we provide the examples of the protocols we used. The raw data for the entire screen would be made available for those who want to apply alternate statistical criteria to analyze the primary data at [www.flyrnai.org](http://www.flyrnai.org) post publication.

#### **(4) Fly stocks and immunohistochemistry**

The following fly strains were used in this study: C96-GAL4 on the third chromosome (drives expression of GAL4 at the wing margin, see Gustafson and Boulianne, 1996) and UAS-Rab5 (a kind gift from M. Gonzalez-Gaintan). C96-GAL4 males were crossed to UAS-Rab5 virgin females. Late 3rd instar larval wing imaginal discs were dissected and processed for antibody staining. Mouse monoclonal antibody against Wingless (4D4, partially purified) was commercially purchased from Developmental Studies Hybridoma

Bank (DHSB) and Senseless antibody (raised in Guinea Pig) was a gift from Dr. Hugo Bellen. Standard antibody staining techniques were used to detect Senseless and cytoplasmic Wingless proteins (described by Cadigan and Nusse, 1996) (51). Immunolabelling of extracellular Wingless was carried out using the protocol described in Baeg et al., 2001(52). The anti-Wingless antibody was used at 1:100 dilution and anti-Senseless at 1:1500.

### **(5) Mammalian Cell culture and reporter assays**

293T were obtained from (ATCC CRL-11268) and were grown in DMEM supplemented with 10% FBS and 1% Pen/Strep and were grown under standard conditions. All reporter assays were performed in 24 well dishes as described in Kaykas et al. (50, 53). Each well of a 24 well dish was transfected with lipofectamine plus (Invitrogen) using standard conditions with the following construct; 15 ng 16XSTF, 100 pg pRLCMV(Promega), 50 ng of pCS2+ or pools of 50ng of pHippy construct and brought up to 250 ng total with pCS2+. Cells were grown for 24 and treated an equal volume of L cell conditioned media or L cell conditioned media containing mouse Wnt3A. 24 hours after the addition of the conditioned media the cells were assayed for luciferase activity using the DLR luciferase assay kit (Promega) using a Berthold Mithras plate reader. All assays were normalized to Renilla levels and Cells transfected with GFP were set 1 fold activation which for most experiment represent 10,000-50,000 RLU's for 16XSTF (firefly luciferase) and 50,000-250,000 RLU's for Renilla luciferase.

### **(6) Injection of Zebrafish with mRNA and Morpholinos**

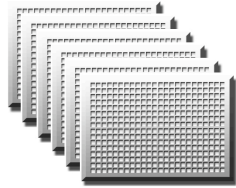
Sense mRNA was synthesized from pCS2+ using mMessage Machine (Ambion). All RNAs were resuspended in HEPES buffer or water prior to injection. Wnt8 ORF1 cDNA has been previously described (54). Morpholino antisense oligonucleotides were

obtained from Gene Tools (Philomath, OR). Morpholinos were dissolved in Danieau's buffer (Nasevicius and Ekker, 2000) prior to use. For all injections, 2-3 nl of the stock morpholino or mRNA at the concentration given in the experiment were injected at the one-cell stage of then wild-type strain of KWT zebrafish. All pictures were taken of live embryos submerged in methyl-cellulose with a ZiessAxiphot microscope with a digital camera attached.

# Figure S1

## Wg/Wnt Screen Outline

384 Well Plates with dsRNA's



Full Genome dsRNA Set=58 plates

Add Transfection Mix containing reporters



Add clone 8 Cells

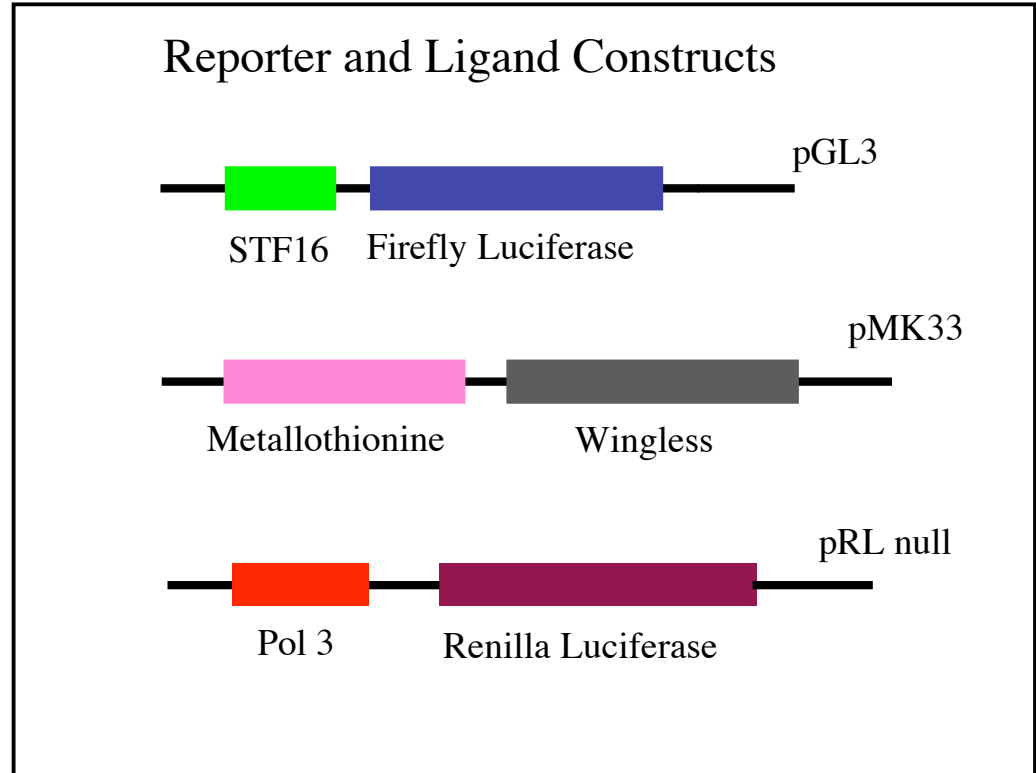


Incubate 5.5 Days



Dual-Glo Luciferase Assays

- 1) Firefly (Assay reporter)
- 2) Renilla (Normalization reporter)



### Controls in each plate

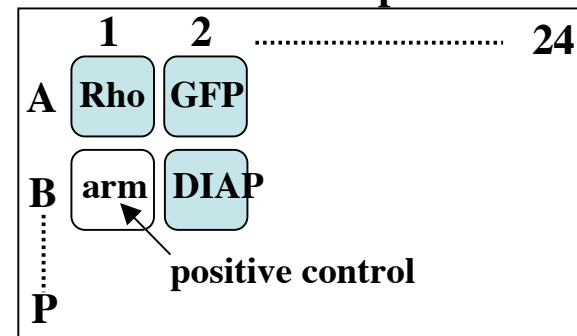
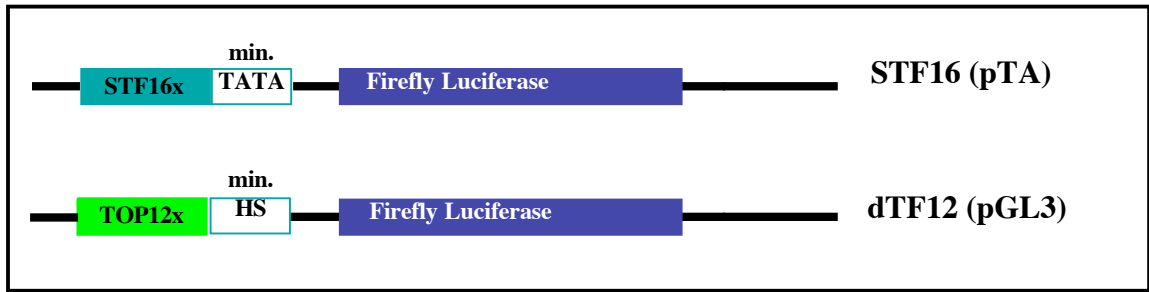
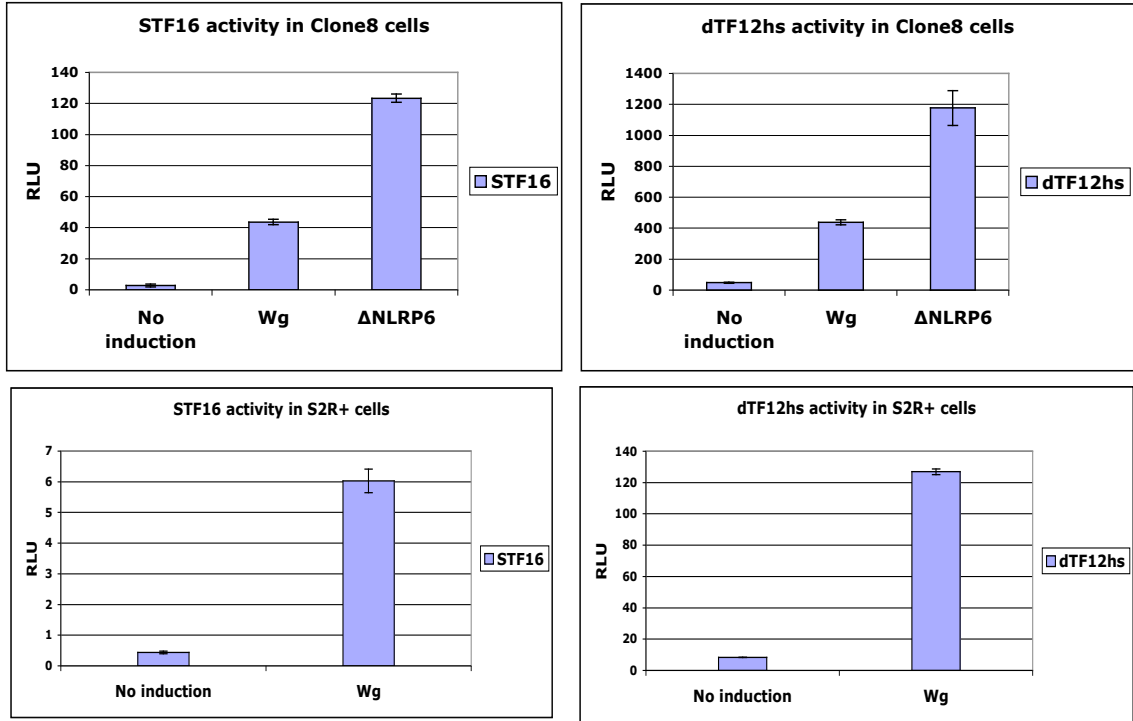


Figure S2

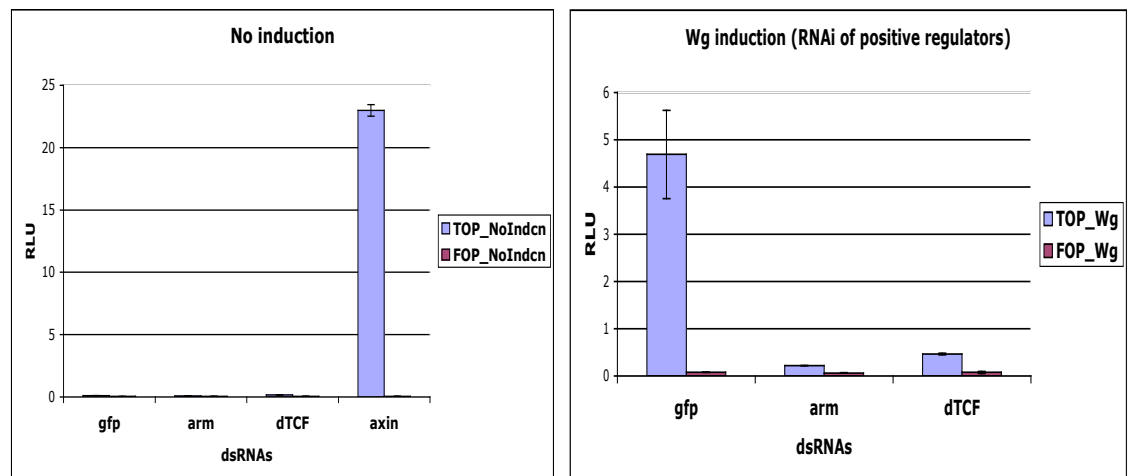
A



B

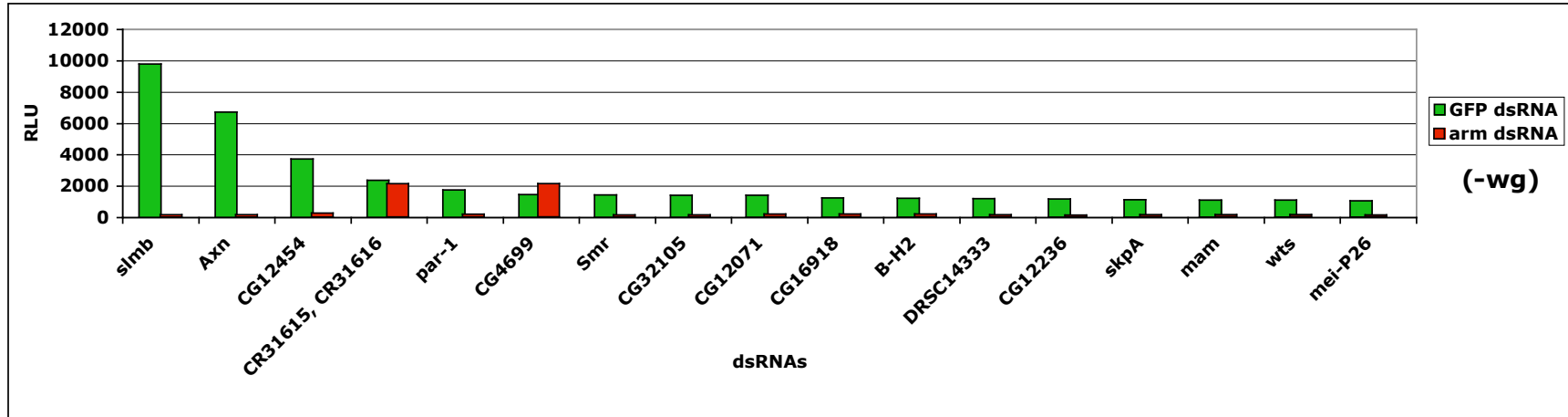


C

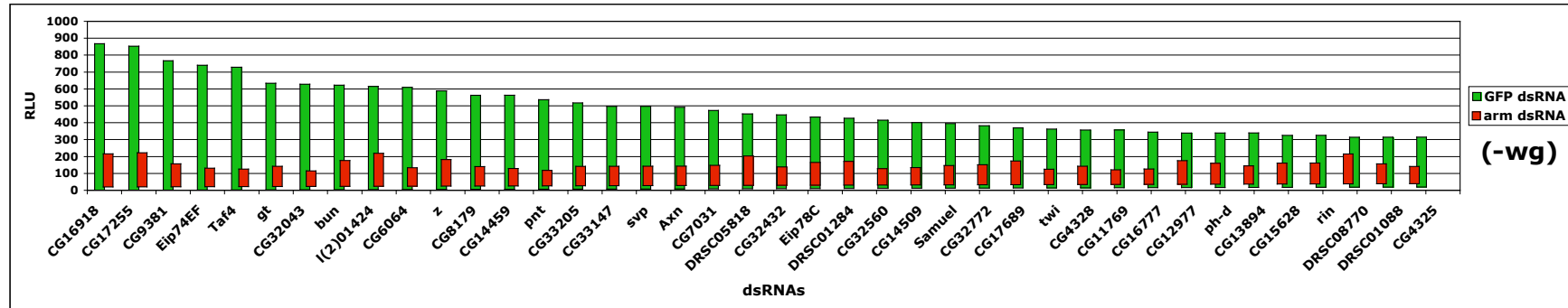


# Figure S3

Specific requirement of potential negative regulators for functional arm in the regulation of Wg-reporter (dTF12)



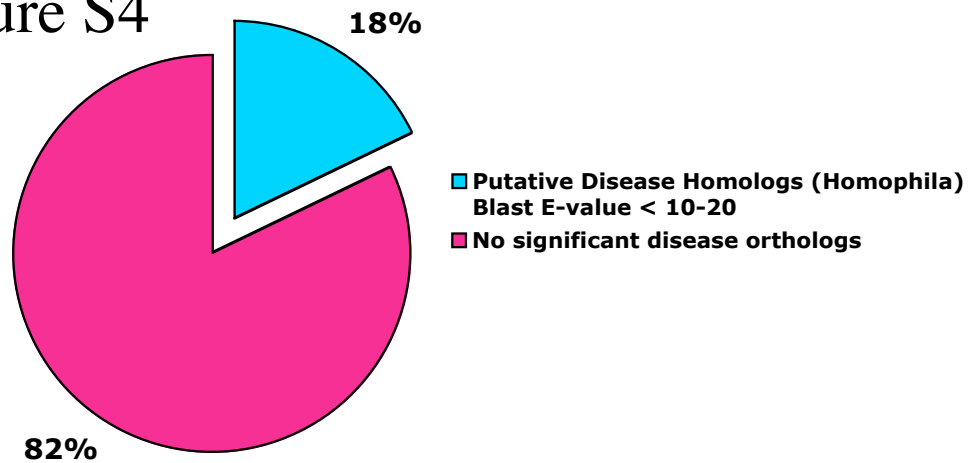
"strong" negative regulators



"moderate" negative regulators



# Figure S4



Rab5	FBgn0014010
ebi	FBgn0023444
CG14030	FBgn0031696
Pvr	FBgn0032006
CG12299	FBgn0032295
cdc2	FBgn0004106
dom	FBgn0020306
Bap55	FBgn0025716
Atx2	FBgn0041188
slmb	FBgn0023423
stg	FBgn0003525
arm	FBgn0000117
Axn	FBgn0026597
nej	FBgn0015624
CG6842	FBgn0027605
odd	FBgn0002985
Marcal1	FBgn0031655
Sos	FBgn0001965
Hr38	FBgn0014859
Lim3	FBgn0002023
Tbp	FBgn0003687
twi	FBgn0003900
CG8888	FBgn0033679
arr	FBgn0000119
SP2353	FBgn0034070
CG4328	FBgn0036274
CG32105	FBgn0052105
fz	FBgn0001085
Mi-2	FBgn0013591
CG7177	FBgn0037098
Chd3	FBgn0023395
CG32465	FBgn0052465
svp	FBgn0003651
wts	FBgn0011739
Trf2	FBgn0026758
fz4	FBgn0027342
CG32767	FBgn0052767
Rab39	FBgn0029959
B-H2	FBgn0004854
CG32560	FBgn0052560
CG32772	FBgn0052772
CG4136	FBgn0029775
Hr4	FBgn0023546

**Fly genes with potential disease-related human homologs**

## Figure legends for Supplementary Figures

### Supplementary Figure S1.

Screen outline: dsRNAs are aliquoted in 384-well plates. The entire genome is stored in 58 plates. Each plate has four control-wells, A1, A2, B1, B2 containing dsRNAs directed towards, Rho, GFP, arm, DIAP (*Drosophila* Inhibitor of Apoptosis). Clone8 cells and transfection mix containing reporter and expression vector driving Wg cDNA were added on Day1. At the end of ~5.5days both TOPFlash and control reporter activity was read using the Dual-Glo kit (Promega) in the Analyst GT (Molecular Devices) plate reader.

### Supplementary Figure S2.

(A) Schematic of two independent Wg-reporters, STF16 and dTF12 used in the high-density 384-well plate screen format. (B) Both STF16 and dTF12 display robust activity in clone8 and S2R+ cells upon induction with either Wg or  $\Delta$ Nlrp6 overexpression (in 96-well plates). (C) dsRNA knockdown of known negative regulator, Axin, activates the reporter in uninduced cells whereas knockdown of control positive regulators such as Arm and dTCF represses Wg-induced activation of the TOP-Flash reporter (in 96-well plates).

### Supplementary Figure S3.

$\beta$ -cat/arm is necessary for the activation of TOPflash reporter upon dsRNA-mediated knockdown of negative regulators: Y-axis represents relative luciferase activity (RLU) and the X-axis represents candidate dsRNAs co-transfected with either GFP or arm dsRNA. Green bars (control) represent the combined dsRNA-knockdown of individual negative regulators with that of GFP. Red bars (experimental) represent the combined knockdown of individual negative regulators with arm. Top panel comprise of the strongest negative regulators and bottom panel represents moderate to weak negative

regulators. Except of CR31615 and CG4699, the combined dsRNA-mediated knockdown of all negative regulators together with arm, suppresses reporter activity as compared to control (GFP).

#### **Supplementary Figure S4.**

Human disease relevance of candidate genes identified in the RNAi-screen for Wnt-Wg-pathway: Pie-chart representation of genes that have multiple potential disease-related human orthologs as curated from the “Homophila” database - <http://superfly.ucsd.edu/homophila/>. Blast E value of  $< 10^{-20}$  was used for this analysis. Homologs are based on sequence homologies and hence considered "highly-related" genes. For details, please refer to the primary paper (Reiter LT, Potocki L, Chien S, Gribskov M, Bier E., "A Systematic Analysis of Human Disease-Associated Gene Sequences In *Drosophila melanogaster*," Genome Research 11, 1114–1125, Cold Spring Harbor Laboratory Press), where sequence homologies of less than  $E < -10$  were considered highly-related.

Table S1A

HFA Amplicon ID	DRSC Amplicon ID	Gene	Phenotype (change in reporter activity)	Secondary Screen		Protein domain (InterPro, Flybase)	Molecular function (InterPro, Flybase)
				Primary Screen Z-score (Avg)	Fold change in reporter activity wrt GFP dsRNA		
HFA12490	DRSC12490	Antp	increased	6.68	>3	homeobox, homeodomain	Specific Transcription factor activity
HFA17148	DRSC17148	Arc70	increased	4.68	>2	TFII5, elonginA	Transcription factor activity
HFA00801	DRSC00801	aop	increased	2.44	>2	Ets-domain, HSF/ETS DNA-binding domain	Transcription factor activity, JNK, MAPKK cascade
HFA18738	DRSC18738	arm	decreased	-1.6	>3	Arm repeat	adhesion, transcription, signaling
HFA07451	DRSC07451	arr	decreased	-1.55	>3	Ligand binding dom. of LDL receptors	LDL-receptor activity
HFA15727	DRSC15727	Ahx2	increased	6.2	>3	Sm motif of snRNP	actin filament organization
HFA14120	DRSC14120	Axn	increased	7.4	>3	RGS(regulator of G-prot. Signaling)	beta-catenin binding
HFA14120	DRSC14120	Axn	increased	5.8	>3	RGS(regulator of G-prot. Signaling)	beta-catenin binding
HFA08379	DRSC08379	bab1	increased	1.68	>1.5	BTB/POZ, Homeo-domain	Transcription factor activity
HFA16555	DRSC16555	bel	increased	2.84	>3	ATP-dependent helicase, DEAD/DEAH box helicase	ATP dependent RNA helicase activity
HFA19335	DRSC19335	B-H2	increased	6.48	>3	homeobox, homeodomain	Specific Transcription factor activity
HFA16914	DRSC16914	bon	increased	3.3	>2	B-box, Zn finger, PHD	transcription co-activator activity
HFA02557	DRSC02557	bru-2	increased	6.95	>3	???	RNA-binding
HFA04676	DRSC04676	bs	increased	3.5	>3	MADS-Box domain, SRF-like	Transcription factor activity
HFA03500	DRSC03500	bun	increased	5.5	>3	TSC-22/dip/Bun family	Transcription factor activity
HFA12589	DRSC12589	Cop84Ad	increased	2.16	NC	???	structural component of larval cuticle
HFA03504	DRSC03504	cdc2	decreased	-0.88	>2	Protein Kinase	Ser/Thr kinase activity
HFA09698	DRSC09698	CG10107	increased	4.42	>3	Cysteine proteinases	cysteine-type peptidase activity
HFA06044	DRSC06044	CG11132	increased	9.8	>3	Myb DNA binding	ND
HFA18632	DRSC18632	CG11380	increased	3.81	>1.5	ND	ND
HFA14347	DRSC14347	CG11676	increased	3.3	>3	ND	nucleic acid metabolism
HFA14378	DRSC14378	CG11769	increased	4.93	>3	ND	ND
HFA14417	DRSC14417	CG11873	increased	4.2	>2.5	ND	ND
HFA19432	DRSC19432	CG11943	increased	3.3	>3	ND	ND
HFA14448	DRSC14448	CG11966	decreased	-1.2	NC	Zinc finger, C2H2 type	cell cycle regulation, transcription factor
HFA14458	DRSC14458	CG11983	decreased	-1.6	>2	ND	ND
HFA14467	DRSC14467	CG12054	increased	5.77	>2	Zn finger, C2H2&C2HC Zn fingers	ND
HFA14471	DRSC14471	CG12071	increased	3.66	>3	Zn finger, C2H2&C2HC Zn fingers	ND
HFA14477	DRSC14477	CG12207	increased	5.7	>3	LysM domain	ND
HFA17830	DRSC17830	CG12236	increased	5.05	>3	BTB/POZ, Zn finger	ND
HFA02158	DRSC02158	CG12299	increased	10.3	>1.5	ND	ND
HFA14515	DRSC14515	CG12425	increased	3.7	>3	ND	ND
HFA19458	DRSC19458	CG12454	increased	6.6	>3	ND	ND
HFA11680	DRSC11680	CG12768	increased	2.9	>2	ND	ND
HFA11687	DRSC11687	CG12977	increased	5.05	>3	ND	ND
HFA19513	DRSC19513	CG12993	decreased	-1.55	>2	ND	ND
HFA09981	DRSC09981	CG13310	decreased	-0.82	NC	ND	Spermadhesin_CUB domain
HFA10013	DRSC10013	CG13467	increased	2.8	>1.5	Doublecortin	ND
HFA10064	DRSC10064	CG13737	decreased	-1.02	NC	ND	ND
HFA02286	DRSC02286	CG13783	decreased	-1.1	>2	ND	ND
HFA14697	DRSC14697	CG13847	increased	4.48	>3	ND	ND
HFA0364	DRSC0364	CG13994	increased	4.1	>3	ND	ND
HFA02357	DRSC02357	CG14030	increased	5.3	>3	Protein Kinase	Ser/Thr kinase activity
HFA10110	DRSC10110	CG14107	decreased	-1.3	>2	ND	ND
HFA11719	DRSC11719	CG14459	increased	6.1	>3	ND	ND
HFA06467	DRSC06467	CG14966	increased	3.8	>3	ND	ND
HFA14861	DRSC14861	CG14509	increased	3.4	>2.5	ND	ND
HFA08452	DRSC08452	CG14996	increased	5.9	>3	Outer Arm of Dynein light chain	ND
HFA06562	DRSC06562	CG15610	increased	0.33	>1.5	PAH2 domain	ND
HFA02056	DRSC02056	CG15628	increased	0.33	>3	Acyl-CoA N-acetyltransferases,GCN5-related N-acetyltransferase	ND
HFA06580	DRSC06580	CG15709	decreased	-1.25	>2	ND	ND
HFA15152	DRSC15152	CG16777	increased	3.6	>3	ND	ND
HFA15165	DRSC15165	CG16918	increased	4.11	>3	serine protease, trypsin family	ND
HFA15165	DRSC15165	CG16918	increased	2.1	>3	serine protease, trypsin family	ND
HFA06649	DRSC06649	CG17048	decreased	-1.2	>1.5	RING finger domain, C3HC4	intracellular protein transport, development
HFA18123	DRSC18123	CG17255	increased	7.6	>2.5	ND	ND
HFA15222	DRSC15222	CG17304	increased	3.23	>3	ND	ND
HFA20865	DRSC20865	CG17514	increased	2.2	>2	Arm repeat	Translational activator
HFA21215	DRSC21215	CG17665	decreased	-0.54	>1.5	ND	ND
HFA06686	DRSC06686	CG17680	decreased	-1.4	>2	ND	ND
HFA10280	DRSC10280	CG17689	increased	4.4	>3	ND	ND
HFA02698	DRSC02698	CG18789	increased	4.4	>1.5	ND	intracellular protein transport
HFA12295	DRSC12295	CG20223	decreased	-1.35	>2	ND	ND
HFA19863	DRSC19863	CG22577	increased	4.4	>2.5	Protein kinase-like (PK-like)	casein kinase I activity
HFA18528	DRSC18528	CG2865	increased	4.2	>3	ND	signal transduction, cell communication
HFA16113	DRSC16113	CG31374	decreased	-1.9	>2	ND	ND
HFA14762	DRSC14762	CG31475	increased	4.2	>3	EF-hand	Calcium-ion binding
HFA10330	DRSC10330	CG32043	increased	4.8	>3	ND	ND
HFA10275	DRSC10275	CG32105	increased	6.4	>3	Homeobox, glucocorticoid receptor-like	Transcription factor activity
HFA08327	DRSC08327	CG32245	increased	4.6	>1.5	ND	ND
HFA11704	DRSC11704	CG32332	increased	3.5	>2	Ligand binding dom. of LDL receptors	ND
HFA12574	DRSC12574	CG32465	decreased	-1.75	>3	thioesterase, hydrolase	neurexin binding
HFA19967	DRSC19967	CG32560	increased	6.8	>3	ND	Ras GTPase activator activity
HFA19901	DRSC19901	CG32575	increased	2.5	>1.5	Zn finger C2H2-type	nucleic acid binding
HFA18044	DRSC18044	CG32705	increased	3.9	NC	Ferredoxin reductase-like, FAD-binding	ND
HFA18370	DRSC18370	CG32737	increased	7.5	>3	ND	ND
HFA18386	DRSC18386	CG32767	decreased	-0.9	>3	ND	nucleic acid binding
HFA18052	DRSC18052	CG32772	increased	6.3	>3	HMG box	nucleic acid binding
HFA05744	DRSC05744	CG33136	decreased	-1.94	>2	ND	ND
HFA06507	DRSC06507	CG33147	increased	5.2	>3	Nucleotide 3-Phosphate hydrolases	heparin-glucosamine 3-O-sulfotransferase activity
HFA10965	DRSC10965	CG33205	increased	4.3	>3	ND	ND
HFA02718	DRSC02718	CG3753	decreased	-0.85	NC	SNF2 related domain_DEAD/DEAH box helicase	helicase activity
HFA10385	DRSC10385	CG3891	increased	5.2	>3	CCAAT-binding transcription factor	Transcription factor activity
HFA06039	DRSC06039	CG4133	increased	2.63	>2	Zn finger C2H2 type	ND
HFA18349	DRSC18349	CG4136	increased	1.9	>3	homeobox, homeodomain	ND
HFA18516	DRSC18516	CG4325	increased	4.2	>3	RING finger, C3HC4	ND
HFA10410	DRSC10410	CG4328	increased	5.7	>1.5	Homeobox, glucocorticoid receptor-like	Transcription factor activity
HFA15625	DRSC15625	CG4699	increased	17.3	>3	ND	ND
HFA02785	DRSC02785	CG4841	increased	2.1	>1.5	ND	cell comm signal transduction
HFA15708	DRSC15708	CG5060	increased	2.1	>3	ND	ND
HFA10487	DRSC10487	CG5151	increased	5.3	>2.5	ND	ND
HFA15792	DRSC15792	CG5402	decreased	-1.39	>2	ND	ND
HFA02874	DRSC02874	CG5674	increased	3.29	>2	ND	ND
HFA15875	DRSC15875	CG5794	increased	6.8	>3	Arm repeat	ND
HFA10549	DRSC10549	CG5830	increased	2.7	>2	HAD-like	ND
HFA02914	DRSC02914	CG5953	increased	7.2	>3	ND	ND
HFA02922	DRSC02922	CG6043	increased	3.9	>3	ND	intracellular protein transport, exocytosis
HFA10578	DRSC10578	CG6064	increased	3.57	>3	ND	ND
HFA18661	DRSC18661	CG6121	increased	4.1	>2	Chromo domain, Acyl-CoA N-acetyltransferase	Histone acetyltransferase activity
HFA07000	DRSC07000	CG6546/Bap55	increased	4.1	>1.5	Actin-like ATPase domain	Cytoskeletal organization
HFA20000	DRSC20000	CG6606	decreased	-1.28	>2	Calcium-lipid binding, C2 domain	ND
HFA16125	DRSC16125	CG6834	decreased	-1.8	>2	ND	ND
HFA19338	DRSC19338	CG6842	increased	3.5	>1.5	AAA ATPase superfamily	ATPase activity, intracellular protein transport
HFA16178	DRSC16178	CG7031	increased	8.1	>3	ND	ND
HFA11813	DRSC11813	CG7177	increased	4.7	>3	protein kinase	ND
HFA10894	DRSC10894	CG7628	increased	4.1	>3	Phosphate transporter family	Phosphate transporter activity
HFA16325	DRSC16325	CG7837	decreased	-1.3	>2	BTB/POZ, ARM repeat	ND
HFA20069	DRSC20069	CG7990	increased	3.6	>2.5	ND	electron transport, oxidative phosphorylation
HFA07076	DRSC07076	CG8092	increased	3.5	>1.5	HMG	ND
HFA07091	DRSC07091	CG8179	increased	2.3	>3	SH3 domain	Protein kinase activity
HFA07101	DRSC07101	CG8192	increased	8	>3	Chitin binding, Tachyctin	ND
HFA18426	DRSC18426	CG8300	increased	3.7	>3	ND	ND
HFA11000	DRSC11000	CG8580	decreased	-1.35	>2	ND	ND
HFA11045	DRSC11045	CG8786	increased	2.8	>1.5	RING finger domain, C3HC4	intracellular protein transport, protein-peroxisome targeting
HFA07295	DRSC07295	CG8888	decreased	-1.3	NC	Short-chain dehydrogenase/reductase (SDR) superfamily ,NAD(P)-binding	oxidoreductase activity, acting on CH-OH group of donors
HFA20172	DRSC20172	CG9216	increased	1.9	>1.5	ND	cell cycle regulation
HFA16484	DRSC16484	mura	increased	9.3	>3	ND	ND
HFA11114	DRSC11114	Chd3	increased	4.5	NC	Chromo, DEAD, Helicase, SNF2	Chromatin-binding, helicase activity
HFA16918	DRSC16918	cic	decreased	-0.11	>2	ND	ND
HFA20231	DRSC20231	Klalpa	increased	11.4	>3	protein ser/thr kinase	Protein Ser/Thr kinase activity
HFA09881	DRSC09881	comm2	increased	4.7	>2	ND	ND

HFA14503	DRSC14503	cpo	increased	3.3	>3	RNA-binding, RNP	RNA binding
HFA03760	DRSC03760	His-Psi(CR31615,	increased	14.1	>3	pseudocoeane	pseudocoeane
HFA03514	DRSC03514	His-Psi(CR31616	increased	9.1	>3	Zn-finger	Transcription factor activity
HFA11859	DRSC11859	cro1	increased	4.1	>3	ND	ND
HFA10281	DRSC10281	CSN3	increased	3.5	>3	ND	ND
HFA10472	DRSC10472	dlp	increased	6.5	>3	ND	ND
HFA04558	DRSC04558	dom	increased	3.2	>3	SNF2, helicase	Transcription factor activity, helicase
HFA07402	DRSC07402	Dp	decreased	-1	>2	E2F dimerization domain	Transcription factor activity
HFA00814	DRSC00814	dronoo	increased	3.3	>2.5	Zn-finger, ARF-GAP	ND
HFA07408	DRSC07408	E(Pa)	increased	3.7	NC	ND	Polytene chromosome maintenance
HFA02036	DRSC02036	e(y)1	increased	12.3	>2	Histone-fold, TFIID	Transcription factor activity
HFA00816	DRSC00816	ebi	decreased	-1.1	NC	WD-repeats	GTP-binding, cell cycle
HFA16937	DRSC16937	eIF-1A	increased	12.02	>3	Nucleic acid-binding protein	translation initiation factor activity
HFA10613	DRSC10613	Eip74EF	increased	7.5	>3	Ets domain, HSF/ETS DNA binding nuclear hormone receptor, VitD receptor	Transcription factor activity ligand-dependent nuclear receptor activity, transcription factor activity
HFA11864	DRSC11864	Eip78C	increased	6.5	>3	Importin	Nuclear Export
HFA03528	DRSC03528	emb	increased	4.3	>2.5	FKBP-like	FK506 binding,peptidyl-prolyl cis-trans isomerase activity
HFA16667	DRSC16667	FK506-bp1	decreased	-1.1	NC	BTB/POZ, Zn finger, C2H2 type	Transcription factor activity
HFA16951	DRSC16951	fru	increased	3.6	>3	Importin, Arm repeat	Nuclear import/protein carrier activity
HFA03328	DRSC03328	Fs(2)Ket	increased	19.61	>3	G-protein-like, Fz TM	Wnt-protein binding, TM receptor activity
HFA11348	DRSC11348	fz	decreased	-1.5	>2	G-protein-like, Fz TM	Wnt-protein binding, TM receptor activity
HFA18780	DRSC18780	fz4	decreased	-0.7	>2	ND	apoptosis activator activity
HFA11352	DRSC11352	grim	increased	6.5	>3	ND	Specific Transcriptional repressor activity, DNA binding
HFA18782	DRSC18782	gt	increased	10.2	>3	bZIP transcription family	Transcriptional repressor activity
HFA11353	DRSC11353	h	increased	5.1	>3	HLH Dimerization, DNA binding	ND
HFA16964	DRSC16964	hdc	increased	2.92	>3	nuclear hormone receptor, VitD receptor	ligand-dependent nuclear receptor activity
HFA03345	DRSC03345	Hr38	increased	3.8	>3	nuclear hormone receptor, VitD receptor	ligand-dependent nuclear receptor activity
HFA18495	DRSC18495	Hr4	increased	2.3	>2	Arm repeats, Bcl2, eLF	Transcription factor activity
HFA06854	DRSC06854	(2)01424	increased	12.9	>3	ND	Transcription factor activity
HFA17152	DRSC17152	igs	decreased	-1.5	>2	HMG box	Transcription factor activity
HFA00708	DRSC00708	illu	decreased	-1.6	>2	Homeobox, glucocorticoid receptor-like	Transcription factor activity
HFA03363	DRSC03363	Lim3	increased	5.6	>2	ND	ND
HFA07648	DRSC07648	man	increased	6.6	>1.5	Zinc finger C-x8-C-x5-C-x3-H type	DNA binding, transcription factor?
HFA06449	DRSC06449	mbl	increased	3.01	>1.5	Zinc finger, C2H2 type	transcription factor activity
HFA11413	DRSC11413	Meics	decreased	-1.1	>2	B-box, Zn finger, RING finger	ND
HFA17735	DRSC17735	mei-P26	increased	11.6	>3	Chromo, DEAD,Helicase,SNF2	Chromatin-binding, helicase activity, ATPase activity
HFA11222	DRSC11222	Mi-2	increased	5.9	>3	Histone-fold	Transcriptional repressor activity
HFA04059	DRSC04059	NC2alpha	increased	12.47	NC	KIX domain of CBP	Co-activator, creb-protein binding
HFA18801	DRSC18801	nej	increased	6.7	>3	ND	ND
HFA11376	DRSC11376	nikd	increased	6.9	>3	ND	histone binding, nucleoplasm ATPase activity
HFA16759	DRSC16759	Nip	decreased	-1	>1.5	ND	specific transcription factor activity
HFA00832	DRSC00832	odd	increased	8.8	>3	Zn finger C2H2 type	DNA binding
HFA17022	DRSC17022	osa	increased	3.7	>3	ARM repeat, AT-rich intron domain	Transcription factor activity, DNA binding
HFA04050	DRSC04050	cpo	increased	3.8	>2.5	Homeobox, Helix-turn-helix/repressor	Transcription factor activity, DNA binding
HFA17176	DRSC17176	pan	decreased	-1.5	>2	HMG box	Transcription factor activity
HFA05792	DRSC05792	par-1	increased	3.4	>3	protein ser/thr kinase	Protein kinase activity/Wq signaling
HFA12629	DRSC12629	pb	increased	4.41	>2.5	HLH, homeo domain repressor	Transcription regulator activity
HFA18819	DRSC18819	ph-d	increased	6.3	>3	ND	chromatin silencing
HFA17028	DRSC17028	pnt	increased	5.9	>3	Ets, DNA binding domain	Transcription factor activity
HFA03593	DRSC03593	poe	decreased	-1.1	NC	Zn finger recognition domain	calmodulin binding
HFA03080	DRSC03080	Pvr	increased	3.1	>1.5	Protein Kinase	Tyr Kinase activity
HFA14322	DRSC14322	pvoo	decreased	-1.2	>2	RHD-finger	Transcription regulator activity, RAB small monomeric GTPase activity, GTP binding
HFA17827	DRSC17827	Rab39	decreased	-0.9	>2	Ras GTPase superfamily, Rab family	GTPase activity
HFA00777	DRSC00777	Rab5	increased	4.4	>3	Ras GTPase superfamily	GTPase activity
HFA00779	DRSC00779	Rbp9	increased	4.7	NC	RNP-1 motif	RNA binding, 3'-UTR
HFA17045	DRSC17045	rin	increased	3.9	>3	RNA-binding, RNP/Ras signal transduction	RNA binding, SH3 domain binding, protein binding
HFA12365	DRSC12365	Rm62	increased	3.1	>2.5	RNA binding	RNA interference
HFA02401	DRSC02401	Samuel	increased	3.9	>3	ND	ND
HFA16845	DRSC16845	SF2	decreased	-1.2	>2	RNA-binding, RNP	RNA binding, pre-splicing factor activity
HFA17055	DRSC17055	sima	increased	7.4	>3	PAS,FAD,HLH,Myc,PYP-sensor	Transcription activator activity
HFA18833	DRSC18833	skpA	increased	7.9	>3	Skp1-Skp2 dimerization, POZ	ND
HFA17056	DRSC17056	slmb	increased	10.9	>2	Skp1-Skp2 dimerization, WD repeat	ubiquitin-protein ligase activity
HFA19495	DRSC19495	Smr	increased	4.4	>3	Myb DNA binding, homeo-domain	Transcriptional co-repressor activity
HFA03439	DRSC03439	Sos	increased	3.7	>2	Histone-fold, TFIID, rho-GEF	Ras GNEF activity, Ras signaling
HFA07545	DRSC07545	SP2353	increased	7.7	>3	ConA, Lecthins, EGF/laminins	ND
HFA17071	DRSC17071	stg	increased	-0.68	NC	protein phosphatase	protein Tyr/Ser/Thr phosphatase activity
HFA07721	DRSC07721	Su(var)2-10	increased	4	>2.5	SAP, DEAD	DNA, helicase binding
HFA15311	DRSC15311	svp	increased	4.4	>2	ligand binding domain of nuclear hormone receptor	ligand-dependent nuclear receptor activity, transcription factor activity
HFA12607	DRSC12607	Taf1	increased	7.7	NC	HMG, Zn-finger	Transcription factor activity, protein kinase activity
HFA16879	DRSC16879	Taf12	increased	7.2	>3	Histone-fold, TFIID	Transcription factor activity
HFA11298	DRSC11298	Taf2	increased	4.8	>1.5	Tbp-associated factor	Transcription factor activity
HFA11297	DRSC11297	Taf4	increased	5.9	>3	Taf homology domain	Transcription factor activity
HFA11299	DRSC11299	Taf6	increased	3.6	>2	Arm repeat, Histone fold found in TFIID complex	Transcription factor activity
HFA15445	DRSC15445	Taf7	increased	4.2	>1.5	histone fold	cell growth, maintenance
HFA02063	DRSC02063	Taf8	increased	3.1	>1.5	TFIID, TATA-binding	Transcription factor activity, DNA binding
HFA04662	DRSC04662	Tbp	increased	1.4	NC	ND	Transcription factor activity
HFA07082	DRSC07082	Tb1	increased	3.3	>1.5	ND	Transcription factor activity
HFA16883	DRSC16883	TFIIA-S	decreased	-1.41	>2	TFIIA	Transcription factor activity
HFA10937	DRSC10937	tna	increased	2.52	>2	ND	nucleic acid, protein metabolism
HFA12142	DRSC12142	Trap18	increased	1.6	>1.5	ND	general transcription factor activity
HFA18727	DRSC18727	Trf2	increased	10.4	>3	TFIID	Transcription factor activity
HFA11308	DRSC11308	Trl	increased	5.5	>2	BTB/POZ, Zn finger,homeodomain	Transcription factor activity, DNA binding
HFA04720	DRSC04720	twi	increased	3.7	>3	HLH Dimerization, DNA binding	Transcription factor activity, DNA binding
HFA11311	DRSC11311	Uba2	increased	3.2	NC	Ubiquitin-activating enzyme repeat domain	ubiquitin/SUMO activating enzyme activity
HFA11408	DRSC11408	vn	increased	2.68	>3	ND	EGFr binding, EGF signaling
HFA03636	DRSC03636	wq	decreased	-1.3	>2	glycoprotein	Wq signaling
HFA17096	DRSC17096	wts	increased	7.8	>3	protein kinase C-terminal domain	Protein Ser/Thr kinase activity
HFA18855	DRSC18855	z	increased	10.1	>3	ND	DNA binding, regulation of transcription
HFA03867	DRSC03867		decreased	-2.01	>3	ND	ND
HFA09080	DRSC09080		decreased	-1.5	>3	ND	ND
HFA11943	DRSC11943		decreased	-2.2	>3	ND	ND
HFA05088	DRSC05088		decreased	-1	>3	ND	ND
HFA00701	DRSC00701		increased	2.9	>3	ND	ND
HFA01970	DRSC01970		increased	8.8	>3	ND	ND
HFA06353	DRSC06353		increased	3.1	>3	ND	ND
HFA08435	DRSC08435		increased	1.4	>3	ND	ND
HFA10152	DRSC10152		increased	6.1	>3	ND	ND
HFA14333	DRSC14333		increased	7.4	>3	ND	ND
HFA14730	DRSC14730		increased	10.9	>3	ND	ND
HFA00125	DRSC00125		increased	5.25	>3	ND	ND
HFA00182	DRSC00182		increased	3.8	>3	ND	ND
HFA01088	DRSC01088		increased	6.8	>3	ND	ND
HFA1284	DRSC01284		increased	5.9	>3	ND	ND
HFA01191	DRSC01191		increased	4	>3	ND	ND
HFA05096	DRSC05096		increased	5	>2	ND	ND
HFA05818	DRSC05818		increased	5.5	>2	ND	ND
HFA07930	DRSC07930		increased	7.9	>1.5	ND	ND
HFA08770	DRSC08770		increased	5.3	>1.5	ND	ND
HFA08923	DRSC08923		increased	4.2	>1.5	ND	ND
HFA09080	DRSC09080		increased	3.2	>2	ND	ND
HFA09433	DRSC09433		increased	3.3	>3	ND	ND
HFA09463	DRSC09463		increased	3.2	NC	ND	ND
HFA11443	DRSC11443		increased	3.4	NC	ND	ND
HFA11579	DRSC11579		increased	5	NC	ND	ND
HFA12723	DRSC12723		increased	7.1	NC	ND	ND
HFA20883	DRSC20883		increased	2.9	NC	ND	ND
HFA21005	DRSC21005		increased	1.5	NC	ND	ND
HFA01970	DRSC01970		increased	5.3	>1.5	ND	ND

Table S1B

Unique amplicons		Unique amplicons		Amplicons sharing 21bp overlaps	
Phenotype	Gene	Phenotype	Gene	Phenotype	Gene
increased	Rab5	decreased	lilli	increased	Rbp9
increased	dronqo	decreased	cdc2	increased	CG14030
increased	Pvr	decreased	Dp	increased	bun
increased	emb	decreased	CG8580	increased	CG33147
increased	CG12299	decreased	stg	increased	h
increased	croI	decreased	pygo	increased	CG14995
increased	dom	decreased	arm	increased	CG32043
increased	Fs(2)Ket	decreased	pan	increased	Eip74EF
increased	NC2alpha	decreased	CG7837	increased	CG12207
increased	Tfb1	decreased	las	increased	Antp
increased	I(2)01424	decreased	[(1)G0003	increased	aop
increased	Su(var)2-10	decreased	wg	increased	CG31475
increased	CG8092	decreased	arr	increased	CG13847
increased	CG6546/Bap5	decreased	CG17680	increased	CG12054
increased	CG8192	decreased	CG15709	increased	z
increased	CG11132	decreased	CG14107	increased	CG12236
increased	CG10107	decreased	fz	increased	mei-P26
increased	Taf2	decreased	CG32465	increased	Smr
increased	Taf6	decreased	CG2023	increased	CG8300
increased	dIp	decreased	CG11983	increased	Samuel
increased	TrI	decreased	CG31374	increased	CG5674
increased	Taf4	decreased	CG6834	increased	CG5953
increased	Taf12	decreased	SF2	increased	Lim3
increased	Rm62	decreased	cic	decreased	Tbp
increased	CG4699	decreased	TfIIA-S	increased	Hr38
increased	osa	decreased	CG5402	increased	twi
increased	slmb	decreased	fz4	increased	mam
increased	Arc70	decreased	CG32767	increased	CG14496
increased	Axn	decreased	Rab39	increased	CG13894
increased	skpA	decreased	CG12993	increased	CG3891
increased	CG6121	decreased	CG33136	increased	CG7628
increased	nej	decreased	lilli	increased	CG33205
increased	CG17255	decreased	ebi	increased	CG17689
increased	CG11943	decreased	poe	increased	CG4328
increased	e(y)1	decreased	FK506-bp1	increased	CG32105
increased	CG6842	decreased	Nlp	increased	comm2
increased	Sos	decreased	CG3753	increased	TORC
increased	E(Pc)	decreased	CG13783	increased	CG5151
increased	CG33456	decreased	CG8888	increased	grim
increased	SP2353	decreased	CG17048	increased	Eip78C
increased	Uba2	decreased	CG13310	increased	CG12768
increased	dIp	decreased	Meics	increased	CG32432
increased	CG13467	decreased	CG11966	increased	CG12977
increased	CG5830	decreased	CG17665	increased	CG14459
increased	nkd			increased	CG16777
increased	CG7177			increased	svp
increased	Chd3			increased	CG9381
increased	CSN3			increased	CG17304
increased	Mi-2			increased	rin
increased	Taf1			increased	fru
increased	pb			increased	CG7031
increased	Taf7			increased	pnt
increased	bon			increased	CG5794
increased	eIF-1A			increased	CG14509
increased	CG11873			increased	hdc
increased	CG32737			increased	CG12425
increased	Trf2			increased	CG16918
increased	Klalpha			increased	wts
increased	CG2577			increased	CG12071
increased	par-1			increased	sima
increased	CG17514			increased	gt
increased	Cdp84Ad			increased	CG4325
increased	Axn			increased	ph-d
increased	CG2865			increased	CG11380
increased	Trap18			increased	CG12454
increased	CG18787			increased	CG32560
increased	CG8786			increased	B-H2
increased	bel			increased	CG15628
increased	CG32705			increased	cpo
increased	Taf8			increased	CG8179
increased	CG4136			increased	bs
increased	otp			increased	bab1
				increased	CG16918
				increased	CG32772
				increased	CG4136
				increased	Hr4
				increased	CG11769
				increased	CG4841
				increased	mbl
				increased	CG32245
				increased	Atx2
				increased	CG9216
				increased	CG32575
				increased	CG7990
				increased	CG4133
				increased	odd
				increased	bru-2
				increased	CG6043
				increased	vn
				increased	tna
				increased	CG11676
				increased	CG5060

Table S2

Phenotype	Gene	Protein Domains	Orthologs/RBB (+/-)
increased	Antp	homeobox, homeodomain	KARP-1-binding protein GI:7662142 (-)
increased	Arc70	TFIIIS, elonginA	cofactor required for Sp1 GI:28558977 (+)
decreased	arm	Arm repeat	beta-catenin GI:4503131 (+)
decrease	arr	Ligand binding dom. of LDL receptors	LRP6 GI:4505017 (+)
increased	Axn	RGS(regulator of G-prot. Signaling)	Axin1 GI:31083144 (+)
increased	Axn	RGS(regulator of G-prot. Signaling)	Axin1 GI:31083144 (+)
increased	bab1	BTB/POZ, Homeo-domain	zinc finger protein 151GI:7378713 (-)
increased	B-H2	homeobox, homeodomain	BarH-like 1 GI:14149728 (+)
increased	bon	B-box, Zn finger, PHD	tripartite motif-containing 33 GI:14971411 (+)
increased	bs	MADS-Box domain, SRF-like	serum response factor GI:4507205 (+)
increased	bun	TSC-22/djp/Bun family	TSC-22 related GI:37622903 (+)
increased	Ccp84Ad	ND	SIM1 GI:56208197 (-)
decreased	cdc2	Protein Kinase	cdc2 GI:4502709 (+)
increased	CG10107	Cysteine proteinases	SUSP1 GI:20306786 (-)
increased	CG11132	Myb DNA binding	KIAA1425 GI:7243231 (+)
increased	CG11380	ND	None
increased	CG11769	ND	mucin5 GI:17384256 (-)
increased	CG11873	ND	glioma tumor suppressor candidate 1 Q9NZM4
increased	CG11943	ND	similar to Nup205 GI:41148144 (+)
decreased	CG11983	ND	tropomyosin 2 (beta) isoform 1 GI:42476296 (-)
increased	CG12054	Zn finger, C2H2&C2HC Zn fingers	juxtaposed with another zinc finger gene 1 GI:51094968 (+)
increased	CG12071	Zn finger, C2H2&C2HC Zn fingers	zinc finger protein 559 GI:23618926 (-)
increased	CG12207	LysM domain	peptidolysin binding-like GI:32394698(+)
increased	CG12236	BTB/POZ, Zn finger	zinc finger protein 161 GI:19923242 (-)
increased	CG12299	ZINC_FINGER_C2H2	hypothetical protein GI:34364774 (-)
increased	CG12425	ND	None
increased	CG12454	ND	None
increased	CG12768	ND	KIAA0754 protein GI:20521149 (-)
increased	CG12977	ND	mastermind-like 2 GI:33286444 (-)
decreased	CG12993	ND	unnamed protein product GI:16550237 (-)
increased	CG13467	Doublecortin	Doublecortin homolog GI:30181242 (+)
increased	CG13847	ND	CCAAT/enhancer binding protein alpha GI:28872794 (-)
increased	CG13894	ND	HAP domain containing 11 GI:40354197 (-)
increased	CG14030	Protein Kinase	hBUB1=GI:4757878 (-)
decreased	CG14107	ND	hypothetical protein GI:348511864 (-)
increased	CG14459	ND	None
increased	CG14496	ND	none
increased	CG14509	ND	None
increased	CG14995	Outer Arm of Dynein light chain	C21orf2 GI:21411470 (+)
increased	CG15610	PAH2 domain	novel protein GI:55662988 (+)
increased	CG15628	Acyl-CoA N-acyltransferases,GCN5-related N-acyltransferase	BXMAS2-10 GI:41197145 (+)
decreased	CG15709	ND	polycystin-1L1 GI:38091303 (-)
increased	CG16777	ND	None
increased	CG16918	serine protease, trypsin family	ECHOS1 GI:37182040 (-)
increased	CG16918	serine protease, trypsin family	ECHOS1 GI:37182040 (-)
increased	CG17255	ND	BAT2 GI:38173707 (+)
increased	CG17304	ND	homeobox protein NKX2 GI:3473847 (+)
increased	CG17514	Arm repeat	GCN1GI:54607053 (+)
decreased	CG17680	ND	hypothetical protein GI:21314769 (+)
increased	CG17689	ND	p38 interacting protein GI:8923735 (+)
decreased	CG2023	ND	BNIP1 GI:15012107 (+)
increased	CG2577	Protein kinase-like (PK-like)	CKI-alpha GI:31077177 (+)
increased	CG2865	ND	unnamed protein product GI:28375479 (-)
decreased	CG31374	ND	golgi autoantigen, golgin subfamily a GI:55959808 (-)
increased	CG31475	EF-hand	unnamed protein GI:14041853 (+)
increased	CG32043	ND	calcitonin receptor GI:117795 (-)
increased	CG32105	Homeobox, glucocorticoid receptor-like	LIM homeobox transcription factor 1 GI:28893581 (+)
increased	CG32432	Ligand binding dom. of LDL receptors	megalyn GI:32816597 (-)
decreased	CG32465	thioesterase, hydrolase	neuroigin 3 GI:51593088 (-)
increased	CG32580	ND	KIAA1743 DOC-2/DAB2 interactive GI:20521984 (+)
increased	CG32737	ND	None
decreased	CG32767	ND	None
increased	CG32772	HMG box	unnamed protein product GI:21751894 (-)
decreased	CG33136	ND	similar to phospho-kinase-like 3 GI:51458637 (-)
increased	CG33147	Nucleotide 3-Phosphate hydrolases	heparan sulfate 3-OST-5 GI:23506319(-)
increased	CG33205	ND	ataxin-2 GI:1679684 (-)
increased	CG3891	CCAAT-binding transcription factor	nuclear transcription factor Y GI:56417679 (+)
increased	CG4136	homeobox, homeodomain	ceh-10 homeodomain containing protein GI:33285958 (+)
increased	CG4325	RING finger, C3HC4	hypothetical protein GI:51476246 (-)
increased	CG4328	Homeobox, glucocorticoid receptor-like	LIM homeobox transcription factor 1 beta GI:2883993 (+)
increased	CG4699	ND	LOC284059 protein GI:13623334 (+)
increased	CG5151	ND	C18orf1 protein GI:44890592 (+)
decreased	CG5402	ND	glypican 1 precursor GI:106224 (-)
increased	CG5674	ND	None
increased	CG5794	Arm repeat	None
increased	CG5830	HAD-like	small CTD phosphatase 1 GI:31074175 (+)
increased	CG5953	ND	alpha-kinase 1 GI:21361969 (-)
increased	CG6064	ND	mucoepidermoid carcinoma translocated 1 GI:34732709 (+)
increased	CG6121	Chromo domain, Acyl-CoA N-acyltransferase	Tat interactive protein GI:36287069 (+)
increased	CG6546/Bap55	Actin-like ATPase domain	BAF53a GI:4757718 (+)
decreased	CG6606	Calcium-lipid binding, C2 domain	rab-coupling protein GI:20127404 (+)
decreased	CG6834	ND	MARVELD2 protein GI:21707225 (-)
increased	CG6842	AAA ATPase superfamily	SKD1-homolog, vacuolar sorting GI:7019569 (+)
increased	CG7031	ND	None
increased	CG7177	protein kinase	protein kinase, lysine deficient 3 GI:50845418 (+)
increased	CG7337	WD repeat	KIAA0596 GI:20521099 (+)
increased	CG7628	Phosphate transporter family	solute carrier family 20 GI:31543630 (+)
decreased	CG7837	BTB/POZ, ARM repeat	FLJ13063 protein GI:15928953 (+)
increased	CG8092	HMG	None
increased	CG8179	SH3 domain	None
increased	CG8192	Chitin binding, Tachycitin	None
increased	CG8300	ND	Chromosome 14 open reading frame 24 GI:20810592 (+)
decreased	CG8580	ND	FLJ12686 GI:13375791 (+)
increased	CG9381	RING finger	hypothetical protein GI:21740275 (+)
increased	Chd3	Chromo, DEAD, Helicase, SNF2	CHD4 protein GI:24047226 (-)
increased	Cklalpha	protein ser/thr kinase	CKI-alpha GI:31077177 (+)
increased	comm2	ND	Unknown protein for IMAGE:4825300 GI:28278130 (-)
increased	cpo	RNA-binding, RNP	RNA-binding protein GI:34485858 (+)
increased	CR31615,	pseudogene	None
increased	CR31616	Zn-finger	inc. finger protein 84 GI:4508037 (+)
increased	CSN3	ND	COP9 constitutive photomorphogenic 3 GI:23238222 (+)
increased	dlp	ND	glypica-4 GI:3831547 (+)

increased	djp	ND	qivpican 4 GI:3420277 (+)
increased	dom	SNF2, helicase	KIAA0309 GI:34327954 (+)
decreased	Dp	E2F dimerization domain	Dp-1 GI:24079969 (+)
increased	drongo	Zn-finger, ARF-GAP	nucleoporin-like protein GI:950051 (+)
increased	DRSC00125	ND	None
decreased	DRSC00182	ND	None
increased	DRSC00701	ND	None
increased	DRSC01088	ND	None
increased	DRSC01191	ND	None
increased	DRSC01284	ND	None
decreased	DRSC01970	ND	None
decreased	DRSC01970	ND	None
increased	DRSC03867	ND	None
increased	DRSC05088	ND	None
increased	DRSC05096	ND	None
decreased	DRSC05818	ND	None
increased	DRSC06353	ND	None
increased	DRSC07930	ND	None
increased	DRSC08435	ND	None
increased	DRSC08770	ND	None
increased	DRSC08923	ND	None
increased	DRSC09080	ND	None
decreased	DRSC09080	ND	None
increased	DRSC09433	ND	None
increased	DRSC09463	ND	None
increased	DRSC10152	ND	None
increased	DRSC11443	ND	None
increased	DRSC11579	ND	None
increased	DRSC11943	ND	None
increased	DRSC12723	ND	None
increased	DRSC14333	ND	None
increased	DRSC14730	ND	None
increased	DRSC20883	ND	None
increased	DRSC21005	ND	None
increased	E(Pc)	ND	enhancer of polycomb 1 GI:13376810 (+)
increased	e(y)1	Histone-fold, TFIID	TBP-associated factor 9L GI:20070280 (+)
increased	eIF-1A	Nucleic acid-binding protein	Eukaryotic translation initiation factor 1A GI:51316213 (+)
increased	Eip74EF	Ets domain, HSF/ETS DNA binding	E74-like factor 2 GI:6857816 (+)
increased	Eip78C	nuclear hormone receptor, VitD receptor	nuclear receptor subfamily 1, group D2 GI:40254810 (+)
increased	emb	Importin	CRM1 GI:5541867 (+)
increased	fru	BTB/POZ, Zn finger, C2H2 type	Kelch-like protein 3 GI:13431657 (-)
increased	Fs(2)Ket	Importin, Arm repeat	karvopherin beta 1 GI:19923142 (+)
decreased	fz	G-protein-like, Fz TM	Frizzled 1 homolog GI:4503825 (+)
decreased	fz4	G-protein-like, Fz TM	Frizzled 4 GI:22547161 (-)
increased	grim	ND	Grim reaper homolog GI:394399 (+)
increased	gt	bZIP transcription family	hepatic leukemia factor GI:45044219 )
increased	h	HLH Dimerization, DNA binding	hairly and enhancer of split 1 GI:5031763 (-)
increased	hdc	ND	Headcase homolog 1 GI:3493748(+)
increased	Hr38	nuclear hormone receptor, VitD receptor	nuclear receptor subfamily 4 GI:27894353 (+)
increased	Hr4	nuclear hormone receptor, VitD receptor	nuclear receptor subfamily 6 GI:53729325 (-)
increased	l(2)01424	Arm repeats, Bcl2, eLF	EIF4G2 GI:34782924 (+)
decreased	lgs	β-catenin binding, protein binding	Bcl-9 GI:23396460 (+)
decreased	lilli	HMG, AF4	HUMFMR2R GI:1311524 (+)
decreased	lilli	HMG, AF4	HUMFMR2R GI:1311524 (+)
increased	Lim3	Homeobox, glucocorticoid receptor-like	LIM homeobox protein 3 GI:7657303 (+)
increase	mam	ND	mastermind-like 1 GI:7661990 (+)
increased	mei-P26	B-box, Zn finger, RING finger	transcription intermediary factor 1 GI:2267585 (-)
increased	mei-W68	Type II DNA topoisomerase	meiotic recombination protein GI:6912680 (+)
increased	Mi-2	Chromo, DEAD,Helicase,SNF2	Mi-2 protein homolog GI:1107696 (+)
increased	NC2alpha	Histone-fold	Dr1-associated corep. GI:1244714 (+)
increased	nej	KIX domain of CBP	CREB-binding protein GI:4321116 (+)
increased	nkd	ND	Naked cuticle homolog 1 GI:30410966 (+)
increased	osa	ARM repeat, AT-rich intron domain	SWI1-like GI:40068466 (+)
increased	otp	Homeobox, Helix-turn-helix/repressor	orthopedia GI:14149760 (+)
decreased	pan	HMG box	Tcf-3 GI:13786123 (+)
increased	pb	HLH, homeo domain repressor	homeo box A2 GI:51094983 (+)
increased	ph-d	ND	polyhomeotic like 3 GI:21359978 (+)
increased	pnt	Ets, DNA binding domain	ets protein GI:182271 (+)
increased	Pvr	Protein Kinase	PDGFbeta GI:21594833 (+)
decreased	pygo	PHD-finger	Pvgoop GI:23396828(+)
decreased	Rab39	Ras GTPase superfamily, Rab family	as-related protein Rab-39A GI:46577701 (+)
increased	Rab5	Ras GTPase superfamily	RAB5C GI:4759020 (+)
increased	Rbp9	RNP-1 motif	Hu antioen C GI:4503553 (-)
increased	rin	RNA-binding, RNP	Ras-GTPase-activating protein GI:54695638 (+)
increased	Rm62	RNA binding	DEAD/H box polypeptide 5 GI:30582725 (+)
increased	Samuel	ND	sterile alpha motif domain containing 11 GI:22749013 (-)
decreased	SF2	RNA-binding, RNP	splicing factor, arginine/serine-rich 1 GI:5902076 (+)
increased	sima	PAS,FAD,HLH,Myc,PYP-sensor	hypoxia-inducible factor 1, alpha (+)
increased	skpA	Skp1-Skp2 dimerization, POZ	S-phase kinase-associated protein 1A GI:25777713 (+)
increased	slmb	Skp1-Skp2 dimerization, WD repeat	beta-TRCP GI:16117783 (+)
increased	Smr	Myb DNA binding, homeo-domain	Smr-like GI:4559298 (+)
increased	Sos	Histone-fold, TFIID, rho-GEF	son of sevenless homolog 1 GI:15529996 (+)
increased	SP2353	ConA, Lecthins, EGF/aminins	hypothetical protein FLJ39155 GI:33469931
decreased	stg	protein phosphatase	cdc25 GI:12408658 (+)
increased	Su(var)2-10	SAP, DEAD	inhibitor of activated STAT GI:7706637 (+)
increased	svp	ligand binding domain of nuclear hormone receptor	nuclear receptor subfamily 2 GI:5032173 (+)
increased	Taf1	HMG, Zn-finger	TBP-associated factor 1 GI:20357588 (+)
increased	Taf12	Histone-fold, TFIID	TAF12 GI:56206092 (+)
increased	Taf2	Tbp-associated factor	TAF2 GI:4507347 (+)
increased	Taf4	Taf homology domain	TAF4 GI:55859696 (+)
increased	Taf6	Arm repeat, Histone fold	TAF6 GI:21536355 (+)
increased	Taf7	found in TFIID complex	TBP-associated factor TAFII55 GI: 2136248 (+)
decreased	Tbp	TFIID, TATA-binding	Tbp GI:56405299 (+)
increased	Tfb1	ND	TFIIH GI:4885365 (+)
decreased	TFIIA-S	TFIIA	TFIIIA GI:364377 (+)
increased	Trf2	TFIID	TAF 270 GI:394304(+)
increased	Tri	BTB/POZ, Zn finger,homeodomain	kelch-like 18 GI:55925604 (-)
increased	twi	HLH Dimerization, DNA binding	twist GI:1924948 (+)
increased	Uba2	Ubiquitin-activating enzyme repeat domain	sentrin activating enzyme GI:4574149 (+)
decreased	wg	Developmental signaling protein	Wnt-1 GI:4885655 (+)
increased	wts	protein kinase C-terminal domain	LATS homolog 1 GI:4758666 (+)
increased	z	ND	hypothetical protein GI:18087819 (-)



Table S3

Scores as strong hit = "++"  
 Scores as weak hit = "+"  
 Does not score = "-"  
 Note: Potential 21bp overlaps = "

Gene	Kc-Wa	Ci8-Wa	S2R-Wa
cic	++	++	++
Antp*	++	++	++
arm	++	++	++
arf	++	++	++
Axn	++	++	++
Axn1	++	++	+
bab1*	++	++	++
B-H2	++	++	++
bs*	++	++	++
bun	++	++	++
CG11132	-	++	++
CG11380	++	++	++
CG11769*	++	++	++
CG11873	-	++	++
CG11983	++	++	++
CG12054	++	++	++
CG12071	++	++	++
CG12207*	++	++	++
CG12236	++	++	++
CG12454	++	++	++
CG12768	++	++	++
CG12977	++	++	++
CG12993	++	++	++
CG13467	++	++	++
CG13847	++	++	++
CG13894	++	++	++
CG14030	++	++	++
CG14107	++	++	++
CG14459	++	++	++
CG14496	++	++	++
CG14509	++	++	++
CG14995	++	++	++
CG15010	++	++	++
CG15028	++	++	++
CG15709	++	++	-
CG16777	++	++	++
CG16918*	++	++	++
CG16918	++	++	++
CG17255	-	++	++
CG17304	++	++	++
CG17630	++	++	++
CG17689	++	++	++
CG2023	++	++	-
CG2577	-	++	++
CG2865	++	++	++
CG31374	++	++	++
CG31475	++	++	++
CG32043	++	++	++
CG32105	++	++	++
CG32432	++	++	++
CG32465	++	++	++
CG32560	++	++	++
CG32767	++	++	-
CG32772*	++	++	++
CG33136	++	++	++
CG33147	++	++	++
CG33205	++	++	++
CG33891	++	++	++
CG41381	++	++	++
CG4325	++	++	++
CG4328	++	++	++
CG4689	++	++	++
CG5151	++	++	++
CG5402	++	++	-
CG5674	++	++	++
CG5794	++	++	++
CG5830	++	++	++
CG5953	++	++	++
CG6054	++	++	++
CG6121	-	++	++
CG6634	++	++	++
CG6842	++	++	++
CG7011	++	++	++
CG7177	++	++	++
CG7628	++	++	++
CG7837	++	++	++
CG8002	-	++	++
CG8179*	++	++	++
CG8182	++	++	++
CG8300	++	++	++
CG9381*	++	++	++
Ckialoha	+	++	++
comm2	++	++	++
cpo	++	++	++
CR31615,			
CR31616	++	++	+
CSN3	++	++	++
dlo	+	++	++
dom	++	++	++
dronao	++	++	++
Eio74EF	++	++	++
Eio78C	++	++	++
emb	+	++	++
fru	++	++	++
Fsf2Ket	+	++	++
frA	++	++	-
grim	++	++	++
gt	++	++	++
h	++	++	++
hdc	++	++	++
Hr38	++	++	++
Hrs*	++	++	++
IJ2101424	++	++	++
lgs	++	++	++
lilli	++	++	++
Lim3	+	++	++
mam	++	++	++
mei-P26	++	++	++
Mis2	++	++	++
nei	-	++	++
nkd	++	++	++
osa	-	++	++
oto	++	++	++
oan	++	++	++
dar-1	++	++	++
ob	++	++	++
ont	++	++	+
Pvr	+	++	++
pvc0	++	++	++
Rbp9	++	++	++
rin	++	++	++
Rm62	-	++	++
Sarwal	++	++	++
simA	++	++	++
slmh	++	++	++
Smr	++	++	++
Srs	++	++	++
SP2353	++	++	++
svd	++	++	++
Taf12	++	++	++
Taf4	-	++	++
Taf7	+	++	++
Tbo	++	++	++
Trf2	-	++	++
twi	++	++	++
wg	++	++	++
wis	++	++	++
Z	++	++	++

**Table S4**

Wg induction-independent Negative Regulators	Wg induction-dependent Negative Regulators
Axn	Antp
bab1	aop
B-H2	Arc70
bs	CG11380
bun	CG11873
CG11132	CG12207
CG11769	CG12768
CG11943	CG13467
CG12054	CG13847
CG12071	CG14030
CG12236	CG17304
CG12454	CG17514
CG12977	CG2865
CG13894	CG5953
CG14459	CG6121
CG14496	CG6546/Bap55
CG14509	CG7628
CG14995	CG8092
CG15610	comm2
CG15628	crol
CG16777	CSN3
CG16918	dip
CG16918	drongo
CG17255	e(y)1
CG17689	eIF-1A
CG2577	emb
CG31475	grim
CG32043	h
CG32105	hdc
CG32432	Lim3
CG32560	nkd
CG32772	osa
CG33147	otp
CG33205	pb
CG3891	Rab5
CG4136	Rbp9
CG4325	sima
CG4328	Sos
CG4699	SP2353
CG5151	Su(var)2-10
CG5674	Taf12
CG5794	Taf2
CG5830	Taf6
CG6064	Taf7
CG6842	Tbp
CG7031	Trl
CG7177	
CG8179	
CG8192	
CG8300	
CG9381	
Cklalpha	
cpo	
CR31615, CR31616	
dom	
Eip74EF	
Eip78C	
fru	
Fs(2)Ket	
gt	
Hr38	
Hr4	
l(2)01424	
mam	
mei-P26	
Mi-2	
nej	
par-1	
ph-d	
pnt	
Pvr	
rin	
Rm62	
Samuel	
skpA	
slmb	
Smr	
svp	
Taf4	
Trf2	
twi	
wts	
z	

**Table S5**

Gene	FBgn	Disease	Related genes or Homologs based on sequence homologies: "Homophila"
Rab5	FBgn0014010	<b>GRISCELLI SYNDROME, TYPE 2</b> [RAB27A, TRP73GLY ], RAB27A, INV3DS, A-G, +3 ], [RAB27A, 550C-T ], [RAB27A, 67.5-kb DEL ], [RAB27A, LEU130PRO ], [RAB27A, ALA152PRO ], [RAB27A, 2-BP DEL, 51CT ]	(OMIM: 603868 Gene: GS2/Rab27A Score: 8.9e-23)
ebi	FBgn0023444	<b>LISSENCEPHALY HETEROTOPIA</b> [PAFAH1B1, PHE31SER ], [PAFAH1B1, GLY162SER ]; <b>SUBCORTICAL LAMINAR</b> [PAFAH1B1, ARG241PRO], [PAFAH1B1, ARG8TER]	(OMIM: 601545 Gene: LIS1 Score: 7.9e-25)
CG14030	FBgn0031696	<b>COLORECTAL CANCER WITH CHROMOSOMAL INSTABILITY</b> [BUB1, SER492TYR] <b>GASTROINTESTINAL STROMAL TUMOR, SOMATIC</b> [PDGFRA, 2-CODON INS, 561ER], [PDGFRA, 5-CODON DEL, 560RVIES], [PDGFRA, 6-CODON DEL, 566SPDGHE];	(OMIM: 602452 Gene: BUB1 Score: 7.9e-39)
Pvr	FBgn0032006	<b>HYPEREOSINOPHILIC SYNDROME, IDIOPATHIC, RESISTANT TO IMATINIB</b> [PDGFRA, THR674ILE ] <b>HETEROTAXY, VISCERAL, X-LINKED</b> [ZIC3, GLN249TER ]	(OMIM: 173490 Gene: PDGFRA Score: 3.9e-78)
CG12299	FBgn0032295	<b>MELANOMA, CUTANEOUS MALIGNANT, 3</b> [CDK4, ARG24CYS], [CDK4, ARG24HIS]	(OMIM: 300265 Gene: HTX Score: 3.9e-23)
cdc2	FBgn0004106	<b>Cerebrooculofacioskeletal syndrome</b> [ERCC6, 2-BP DEL, 3794AA ]; <b>COCKAYNE SYNDROME, TYPE B</b> [ERCC6, PRO1095ARG ], [ERCC6, 4-BP INS, 1053TGTC ], [ERCC6, 1-BP DEL, 1597G]	(OMIM: 123829 Gene: CDK4 Score: 7.8e-53)
dom	FBgn0020306	<b>DEAFNESS, AUTOSOMAL DOMINANT 20</b> [ACTG1, THR89ILE ], ACTG1, PRO332ALA], [ACTG1, PRO264LEU ], [ACTG1, THR278ILE]	(OMIM: 133540 Gene: CKN2 Score: 3e-44)
Bap55	FBgn0025716	<b>SPINOCEREBELLAR ATAXIA 2</b> [ATXN2, (CAG) <sup>n</sup> EXPANSION ]	(OMIM: 102560 Gene: ACTG1 Score: 3.9e-60)
Atx2	FBgn0041188	<b>LISSENCEPHALY SEQUENCE, ISOLATED</b> [PAFAH1B1, GLY162SER ]; <b>SUBCORTICAL LAMINAR HETEROTOPIA</b> [PAFAH1B1, AGAMMAGLOBULINEMIA, NON-BRUTON TYPE, AUTOSOMAL DOMINANT [LRR8, 2.5-LRR DEL] <b>COLORECTAL CANCER</b> [CTNNB1, 3-BP DEL, SER45DEL ], [CTNNB1, SER33TYR ];	(OMIM: 601517 Gene: ATX2 Score: 2.2e-43)
slmb	FBgn0023423	<b>HEPATOBLASTOMA, SOMATIC</b> [CTNNB1, THR41ALA ], [CTNNB1, ASP32TYR ], [CTNNB1, GLY34VAL ]; <b>PILOMATRICOMA, SOMATIC</b> [CTNNB1, ASP32GLY ], [CTNNB1, SER33PHE ], [CTNNB1, GLY34GLU ], [CTNNB1, SER37CYS], [CTNNB1, SER37PHE ], [CTNNB1, THR41ILE ];	(OMIM: 601545 Gene: LIS1 Score: 2.4e-29)
stq	FBgn0003525	<b>OVARIAN CARCINOMA, ENDOMETRIOID TYPE, SOMATIC</b> [CTNNB1, SER37CYS ]; <b>HEPATOCELLULAR CARCINOMA, SOMATIC</b> [CTNNB1, SER45PHE ], [CTNNB1, SER45PRO]; <b>MEDULLOBLASTOMA, SOMATIC</b> [CTNNB1,	(OMIM: 608360 Gene: KIAA1437 Score: 3.3e-24)
arm	FBgn0000117	<b>HEPATOCELLULAR CARCINOMA, SOMATIC</b> [AXIN1, 33-BP DEL]	(OMIM: 116806 Gene: CTNNB1 Score: 0) (OMIM: 603816 Gene: AXIN Score: 5.3e-16), (OMIM: 603816 Gene: AXIN1 Score: 5.3e-16), (OMIM: 604025 Gene: AXIN2 Score: 8.1e-12)
Axn	FBgn0026597	<b>COLORECTAL CANCER</b> [EP300, ARG580TER], [EP300, PRO2221GLN ]	(OMIM: 600140 Gene: CBP Score: 0)
nej	FBgn0015624	<b>SPASTIC PARAPLEGIA 4</b> [SPG4, CYS448TYR], [SPG4, 1-BP DEL, 1520T], [SPG4, ARG499CYS], [SPG4, 1813, A-G, -2]	(OMIM: 604277 Gene: SPAST Score: 2.2e-58)
CG6842	FBgn0027605	<b>NEUTROPENIA, SEVERE CONGENITAL</b> [GFI1, ASN382SER]	(OMIM: 603023 Gene: IK1 Score: 6.7e-19)
odd	FBgn0002985	<b>SCHIMKE IMMUNOSSEOUS DYSPLASIA</b> [SMARCAL1, ARG645CYS ], [SMARCAL1, ILE548ASN ], [SMARCAL1, GLN34TER ], [SMARCAL1, ARG17TER ], [SMARCAL1, 4-BP DEL,	(OMIM: 606622 Gene: HARP Score: 1.5e-99) (OMIM: 182530 Gene: GF1 Score: 0)
Marcal1	FBgn0031655	<b>FIBROMATOSIS, GINGIVAL, 1</b> [SOS1, 1-BP INS]	
Sos	FBgn0001965	<b>PARKINSON DISEASE, SUSCEPTIBILITY TO</b> [NR4A2, 1-BP DEL, -291T], [NR4A2, -245T-G]	(OMIM: 601828 Gene: NOT Score: 1e-125)
Hr38	FBgn0014859	<b>SHORT STATURE, PITUITARY AND CEREBELLAR DEFECTS, AND SMALL SELLA TURCICA</b> [LHX4, IVS5, G-C, -1 ]	(OMIM: 602146 Gene: LHX4 Score: 2.4e-92)
Lim3	FBgn0002023	<b>SPINOCEREBELLAR ATAXIA 17</b> [TBP, (CAG) <sup>n</sup> EXPANSION]	(OMIM: 600075 Gene: SCA17 Score: 1.9e-87)
Tbp	FBgn0003687		

twi	FBgn0003900	<b>SAETHRE-CHOTZEN SYNDROME</b> [TWIST, 21-BP DUP ], [TWIST, LEU135PRO], [TWIST, GLU130TER]; <b>CRANIOSYNOSTOSIS WITH RADIAL DEFECTS</b> [TWIST, GLU181TER]; <b>ROBINOW-SORAUFG SYNDROME</b> [TWIST, 1-BP <b>FUNDUS ALBIPUNCTATUS, AUTOSOMAL RECESSIVE</b> [RDH5, ALA294PRO], [RDH5, VAL177GLY], [RDH5, TYR281HIS], [RDH5, SER73PHE	(OMIM: 601622 Gene: ACS3 Score: 5.9e-23)
CG8888	FBgn0033679	<b>OSTEOPOROSIS-PSEUDOGLIOMA SYNDROME</b> [LRP5, TRP10TER ], [LRP5, ARG428TER ], LRP5, 1-BP DEL, 1467G ]; <b>HIGH BONE MASS</b> [LRP5, GLY171VAL ]; <b>OSTEOPETROSIS, AUTOSOMAL DOMINANT, TYPE I</b> [LRP5, GLY171ARG]; <b>ENDOSTEAL HYPEROSTOSIS, AUTOSOMAL DOMINANT</b> [LRP5, ALA214THR]; <b>EXUDATIVE VITREORETINOPATHY, FAMILIAL, AUTOSOMAL DOMINANT</b> [LRP5, 1-BP INS, 4119C], [LRP5, GLU1367LYS], [LRP5, ARG570GLN]	(OMIM: 601617 Gene: RDH5 Score: 7.9e-19)
arr	FBgn0000119	<b>SCHWARTZ-JAMPEL SYNDROME, TYPE 1</b> [HSPG2, EX60/61 FUS ]	(OMIM: 603506 Gene: BMND1 Score: 0), (OMIM: 603506 Gene: LRP5 Score: 0), (OMIM: 603506 Gene: LRP7 Score: 0)
SP2353	FBgn0034070	<b>NAIL-PATELLA SYNDROME</b> [LMX1B, ARG208TER], [LMX1B, ARG200GLN], [LMX1B, 672, ARG208TER], [LMX1B, ARG200GLN], [LMX1B, 672, EXUDATIVE VITREORETINOPATHY 1 [FZD4, 6-BP DEL, NT1479], [FZD4, 2-BP DEL, 1501CT]	(OMIM: 142461 Gene: HSPG2 Score: 1.7e-45)
CG4328	FBgn0036274	<b>Cerebrooculofacioskeletal syndrome</b> [ERCC6, 2-BP DEL, 3794AA ]; <b>COCKAYNE SYNDROME, TYPE B</b> [ERCC6, PRO1095ARG ], [ERCC6, 4-BP INS, 1053TGTC ], [ERCC6, 1-BP DEL, 1597G]	(OMIM: 602575 Gene: LMX1B Score: 3e-52)
CG32105	FBgn0052105	<b>PSEUDOHYPOALDOSTERONISM, TYPE II</b> [WNK1, 41-KB DEL, IVS1 ], [WNK1, 22-KB DEL, IVS1 ]	(OMIM: 602575 Gene: LMX1B Score: 1e-52)
fz	FBgn0001085	<b>Cerebrooculofacioskeletal syndrome</b> [ERCC6, 2-BP DEL, 3794AA ]; <b>COCKAYNE SYNDROME, TYPE B</b> [ERCC6, PRO1095ARG ], [ERCC6, 4-BP INS, 1053TGTC ], [ERCC6, 1-BP DEL, 1597G]	(OMIM: 601500 Gene: SMO Score: 5.7e-44)
Mi-2	FBgn0013591	<b>MENTAL RETARDATION, X-LINKED</b> [NLGN4, 2-BP DEL, 1253AG ]	(OMIM: 133540 Gene: CKN2 Score: 2.5e-67)
CG7177	FBgn0037098	<b>ENHANCED S-CONE SYNDROME</b> [NR2E3, IVS1AS, A-C], [NR2E3, ARG76TRP]; <b>RETINITIS PIGMENTOSA, AUTOSOMAL RECESSIVE</b>	(OMIM: 605232 Gene: PHA2C Score: 2.5e-88)
Chd3	FBgn0023395	<b>MYOTONIC DYSTROPHY</b> [DM, (CTG) <sub>n</sub>	(OMIM: 133540 Gene: CKN2 Score: 5.6e-67)
CG32465	FBgn0052465	<b>SPINOCEREBELLAR ATAXIA 17</b> [TBP, (CAG) <sub>n</sub> EXPANSION]	(OMIM: 300427 Gene: KIAA1260 Score: 4.4e-71)
svp	FBgn0003651	<b>EXUDATIVE VITREORETINOPATHY 1</b> [FZD4, 6-BP DEL, NT1479], [FZD4, 2-BP DEL, 1501CT]	(OMIM: 604485 Gene: ESCS Score: 9.2e-74)
wts	FBgn0011739	<b>HETEROTAXY, VISCERAL, X-LINKED</b> [ZIC3, THR325MET], [ZIC3, CYS270TER]	(OMIM: 605377 Gene: DM Score: 3.5e-65)
Trf2	FBgn0026758	<b>GRISCELLI SYNDROME, TYPE 2</b> [RAB27A, TRP73GLY ], [RAB27A, INV3DS, A-G, +3 ], [RAB27A, 550C-T ], [RAB27A, 67.5-kb DEL ], [RAB27A, LEU130PRO ], [RAB27A, ALA152PRO ], [RAB27A, 2-BP DEL, 51CT ]	(OMIM: 600075 Gene: SCA17 Score: 5.9e-36)
fz4	FBgn0027342	<b>WAARDENBURG SYNDROME, TYPE III</b> [PAX3, ASN47HIS]	(OMIM: 601500 Gene: SMO Score: 2.1e-14)
CG32767	FBgn0052767	<b>BASAL CELL CARCINOMA</b> [RASA1, ILE401VAL]; <b>CAPILLARY MALFORMATION-ARTERIOVENOUS MALFORMATION</b> [RASA1, 2-BP DEL, 475CT]	(OMIM: 300265 Gene: HTX Score: 2.3e-15)
Rab39	FBgn0029959	<b>HOLOPROSENCEPHALY 5</b> [ZIC2, 30-BP INS ], [ZIC2, 7-BP DEL ], [ZIC2, 2-BP DEL, 180AC]	(OMIM: 603868 Gene: GS2 Score: 3.1e-31)
B-H2	FBgn0004854	<b>MICROPHthalmia, CATARACTS, AND IRIS ABNORMALITIES</b> [CHX10, ARG200GLN], [CHX10, ARG200PRO]	(OMIM: 606597 Gene: CDHS Score: 2.8e-14)
CG32560	FBgn0052560	<b>ADRENOCORTICAL INSUFFICIENCY WITHOUT OVARIAN DEFECT</b> [NR5A1, ARG255LEU]; <b>SEX REVERSAL, XY, WITH ADRENAL FAILURE</b>	(OMIM: 139150 Gene: CMAVM Score: 3.7e-36)
CG32772	FBgn0052772	[NR5A1, ARG92GLN ]	(OMIM: 603073 Gene: HPE5 Score: 5.6e-21)
CG4136	FBgn0029775		(OMIM: 142993 Gene: CHX10 Score: 1.6e-47)
Hr4	FBgn0023546		(OMIM: 184757 Gene: FTZ1 Score: 1.4e-28)

## **Figure legends for Supplementary Tables**

### **Supplementary Table S1A**

List of “hits” identified as potential regulators of the Wnt-Wg-pathway. The identifiers are listed by DRSC (Drosophila RNAi Screening Center) amplicons, HFA or gene names. Detailed information can be found at the DRSC website: [www.flyrnai.org](http://www.flyrnai.org). The Z-scores corresponding to each dsRNA from the primary screen are listed. Secondary screens (in 96-well plate format) of the “hits” have been depicted as “fold change of reporter activity” with respect to (wrt) GFP dsRNA knockdown. Hits were considered significant and reproducible only if they displayed a fold change of  $\geq 1.5$  for both positive and negative regulators. Using this method, ~90% of the hits obtained from the primary screen could be reproduced in the secondary screens. “Phenotype” corresponds to the effect of dsRNA-mediated knockdown of the corresponding gene on the Wnt-reporter activity. RNAi towards negative regulators result in an “increased” (superactivation of reporter gene) phenotype whereas those towards positive effectors lead to a “decreased” (repression of reporter gene) phenotype. Gene ontology information was obtained from InterPro (<http://www.ebi.ac.uk/interpro>) and Flybase (<http://flybase.bio.indiana.edu>). Note: ND=Not Determined or unknown; NC=No Change.

### **Supplementary Table S1B**

List of all “hits” identified separated by their phenotypes and dsRNA amplicons containing 21bp overlaps with other genes in the genome. We identified 91 dsRNAs that share 21bp overlaps with other genes. Initial studies on selected genes (that share 21bp overlap) using alternative and unique dsRNAs have revealed that the effect of their knockdown on the Wnt-reporter can be reproduced (data not shown). However follow-up studies on any of the genes sharing 21bp overlaps would require independent confirmation of all 91 “hits” using unique dsRNAs.

### **Supplementary Table S2**

Human orthologs to genes identified in the screen. All of the protein coding sequences for genes targeted by dsRNA were subjected to Blast searches using [www.ncbi.nih.gov/BLAST](http://www.ncbi.nih.gov/BLAST) against the *Homo sapien* non-redundant database. GI numbers are given for the human ortholog with the best E-score. Reciprocal Best Blast (RBB) were performed with Human orthologs by blasting them against the *Drosophila* non-redundant database. All orthologs that returned the starting *Drosophila* ortholog were considered to be the RBB(+). Orthologs that are not the RBB(-) are also shown and are the ortholog with the best E-score. Note: ND=Not Determined or unknown.

### **Supplementary Table S3**

Subset of the list of genes from Table S1A that scored positive in the reporter assay in at least 2 or more *Drosophila* cell types. The cell lines used were clone8 (epithelial in origin), kc167 and S2R+ (haemocytic lineage) cells. “Positive hits” were determined by genes that affected the Wnt-reporter activity by  $\geq 2$  folds with respect to gfp dsRNA, upon their RNAi knockdown. The reporter assay was scaled up to a 96-well plate format.

### **Supplementary Table S4**

Categorization of the negative regulators identified in the screen. Secondary screens were performed on the negative regulators in a 96-well plate format to assess the effect of their dsRNA-mediated knockdown on TOP-Flash reporter activity in the absence or presence of the Wg-stimulus. 83/129 genes increased baseline reporter activity (by  $\geq 2$  folds with respect to gfp dsRNA) upon RNAi in the absence of Wg-induction whereas 46/129 super-activated the reporter ( $\geq 2$  folds with respect to gfp dsRNA ) over and above Wg-mediated activation of the Wnt-reporter.

## Supplementary Table S5

List of candidate genes that are “highly-related” to human genes associated with genetic diseases (as curated from the homophila website : <http://superfly.ucsd.edu/homophila/>).

The fly genes are listed together with the corresponding Fbgns (FlyBase genome annotations) and the disease-related human orthologs or “highly-related” genes as well as the mutations that are associated with the specific diseases. The human homologs are purely based on sequence homologies using a strict criteria of E value  $< 10^{-20}$  to identify potential homologs; hence referred as "highly-related" genes. This is in accordance with the primary paper (Reiter LT, Potocki L, Chien S, Gribskov M, Bier E., "A Systematic Analysis of Human Disease-Associated Gene Sequences In *Drosophila melanogaster*," Genome Research 11, 1114–1125, Cold Spring Harbor Laboratory Press), where sequence homologies of less than  $E < 10^{-10}$  were considered highly-related.

## References and Notes

49. J. Y. Yu, S. L. DeRuiter, D. L. Turner, *Proc Natl Acad Sci U S A* **99**, 6047 (2002).
50. A. Kaykas, R. T. Moon, *BMC Cell Biol* **5**, 16 (2004).
51. K. M. Cadigan, R. Nusse, *Development* **122**, 2801 (1996).
52. G. H. Baeg, X. Lin, N. Khare, S. Baumgartner, N. Perrimon, *Development* **128**, 87 (2001).
53. A. Kaykas et al., *Nat Cell Biol* **6**, 52 (2004).
54. A. C. Lekven, C. J. Thorpe, J. S. Waxman, R. T. Moon, *Dev Cell* **1**, 103 (2001).