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Supplemental Data

Identification of SUMO-Dependent

Chromatin-Associated Transcriptional Repression

Components by a Genome-wide RNA Interference Screen

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SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Plasmids

Expression vectors for *D. melanogaster* cells and reporters

Expression vectors for the long and the short isoforms of Sp3 (pPacUSp3 and pPacSp3) and corresponding SUMOylation-deficient mutants (pPacUSp3 K551R and pPacSp3 K551D) have been previously described (Dennig et al., 1996; Hagen et al., 1994; Sapetschnig et al., 2004). The Sp3-dependent firefly reporter gene plasmid (GC)2-FLuc was obtained by replacing the chloramphenicol acetyl transferase gene in BCAT-2 (Pascal and Tjian, 1991) by the firefly luciferase gene obtained as EcoRI/HpaI fragment from the pGAW plasmid, a self-made derivative of the pGL3-Basic vector (Promega). The SV40 promoter-driven firefly reporter gene construct pGL3-promoter is commercially available (Promega). The actin promoter-driven *Renilla* coreporter gene construct pPac-RLuc was a kind gift of C. Thomas. Vectors for expression of *Drosophila* Dorsal, the SUMOylation-deficient Dorsal K382R mutant and Twist as well as the Dorsal-dependent reporter gene construct DE5 (Bhaskar et al., 2002; Shirokawa and Courey, 1997) were kindly provided by A. Courey. Expression vectors for C-terminal FLAG-tagged *Drosophila* Mi-2 (pPac-dMi-2-FLAG and pPac-dMi-2ΔATPase-FLAG) were described (Bouazoune and Brehm, 2005). The expression plasmids for double-epitope-tagged *Drosophila* Sfmbt and MEP-1 (pPac-HA-FLAG-dSfmbt and pPac-HA-FLAG-dMEP-1) were generated by PCR-cloning of the respective ORFs into the pPac-HA-FLAG vector (Braun et al., 2001) using XbaI- (Sfmbt) and XbaI/SalI- (MEP-1) tailed gene-specific primers. The plasmid pBS-KS(-)-dSfmbt used as PCR template were kindly provided by J. Müller.

Vectors for *in vitro* transcription (IVT)

Vectors suitable for IVT were pT7L-dMi-2 and pSPT18-Sp3 (Sapetschnig et al., 2004). The plasmid pSPT18-Sfmbt was obtained by ligating a XbaI-restricted PCR fragment encompassing the entire ORF into the XbaI site of pSPT18. pSPT18-MEP-1 was constructed by introducing the *Drosophila* MEP-1 cDNA obtained as BamHI fragment from pFlc1-dMEP-1 into BamHI-restricted pSPT18.

Vectors for protein expression in *E. coli*

The *E. coli* expression vector pGEX-hSUMO-1 was generously provided by T. Stamminger. The plasmid pGEX-2TK-hPIAS1 has been previously described (Sapetschnig et al., 2002). The vector for full-length *Drosophila* SUMO (pGEX-2TK-dSmt3-full) was constructed by cloning of a PCR fragment obtained with BamHI/EcoRI-tailed gene-specific primers from the intronless dSmt3 ORF into the BamHI/EcoRI restricted pGEX-2TK-P vector. The pGEX-4T1-dMi-2 vector was a kind gift from A. Brehm. The vector pGEX-Sfmbt was constructed by ligating the ORF of Sfmbt obtained by PCR with XbaI-tailed primers into pGEX-2TK-P. pGEX-dMEP-1 was generated by PCR cloning of the dMEP-1 ORF from pFlc1-dMEP-1 using XbaI/SalI-tailed gene-specific primers. Primer sequences and further details on cloning procedures will be provided upon request (Suske@imt.uni-marburg.de).

Cell lines

D. melanogaster Kc_{167} (Echalier and Ohanessian, 1970) and SL2 cells (Schneider, 1972) were maintained at 25 °C in Schneider's *Drosophila* Medium (Invitrogen) supplemented with 10% fetal bovine serum (PAA Gold), 2 mM L-glutamine (Invitrogen), 100 U/ml Penicillin and 100 μg/ml Streptomycin (Cambrex). SL2 cells stable transfected with HA-FLAG-epitope-tagged expression vectors for either wild-type Sp3 or the SUMOylation-deficient Sp3 SD mutant lacking 13 amino acids (Braun and Suske, 1999) were transfected with (GC)2-FLuc, pPac-RLuc and pBS-PURO plasmids. The pBS-PURO plasmid contains the puromycin-resistance gene under the control of the *Drosophila* heat-shock promoter (Benting et al., 2000). Cells were selected with puromycin (2 μg/ml) and single clones were analyzed for *Renilla* luciferase activity and for Sp3 responsiveness of the (GC)2-FLuc reporter gene (Figure S5).

Establishment of conditions for the RNAi screen

To establish conditions that allow for a high-throughput RNA interference screen, we tested different *D. melanogaster* cell lines (SL2, S2R+ and Kc_{167} cells), transfection conditions (batch transfection versus direct

transfection) and a number of commercially available transfection reagents. *Drosophila* Kc_{167} cells exhibited highest transfection efficiency, and a robust activation of the (GC)2-FLuc reporter by the Sp3 K551R mutant but not by wild-type Sp3 was observed when transfected on 384-well plates directly. Moreover, the activity of a co-transfected actin promoter-driven *Renilla* luciferase expression vector (pPac-RLuc) was unaffected by wild-type Sp3 and the Sp3 K551R mutant.

Primary rescreen on 384-well plates

For the primary rescreen of 265 top candidates, PCR fragments were reamplified using tag-specific oligonucleotides containing T7 polymerase binding sites (Hild et al., 2003) and subsequently subjected to *in vitro* transcription (Boutros et al., 2004; Müller et al., 2005). The rescreen was performed in triplicate on 384-well plates containing 0.3 to 0.5 μg dsRNA per well. For normalization, a total of 25 wells received 0.5 μg dsRNA targeting GFP. Additional 12 wells contained either 0.5 μg of dsRNAs against SUMO or FLuc, respectively. Plating of Kc_{167} cells and transfections of the pPacUSp3 WT plasmid and reporters were performed as described for the primary screen. Firefly and *Renilla* luciferase values were normalized to the median of the 25 GFP-targeting wells for each given plate. One-hundred-eighty-five genes (70%) identified in the primary screen activated the (GC)2-FLuc reporter again by more than 3-fold and were chosen for further secondary rescreens.

Secondary rescreens and additional reporter gene experiments

Kc_{167} cells were plated at 50% confluence in 24-well or 96-well cell culture plates (Greiner/Nunc) containing 6 μg of dsRNA for 24-well and 1 μg of dsRNA for 96-well plates, respectively. Transfections were performed with a total of 550 ng DNA per well for 24-well plates (500 ng pGL3-promoter vector, 0,5 ng pPac-RLuc, 50 ng pPacSp3si WT or pPacSp3si K551D), or with a total of 110 ng DNA per well for 96-well plates (100 ng pGL3-promoter vector, 0,1 ng pPac-RLuc, 10 ng pPacSp3si WT or pPacSp3si K551D).

Reporter gene assays for Dorsal activation were performed on 24-well plates using a total of 570 ng DNA per well (500 ng DE5 reporter, 0,5 ng pPac-RLuc, 20 ng pPacTwist and 50 ng pPacDorsal or the pPacDorsal K382R mutant, respectively.)

Cells were harvested 5 days post-transfection, and luciferase reporter gene assays were performed using the Dual Luciferase kit (Promega) according to the manufacturer's instructions. Luciferase was measured with an autoinjection luminometer (Berthold Technologies). Relative firefly luminescence was obtained by calculating the ratio of firefly and *Renilla* luciferase activity. Fold activation values were calculated by dividing each

relative luminescence value by the relative luminescence value obtained with dsRNA against GFP, which was set to 1.

Generation of dsRNAs for rescreens and reporter gene experiments

For secondary rescreens in 24- and 96-well plates or for generation of additional dsRNAs not present in the RNAi library, fragments of genes were amplified by PCR from SL2 cell genomic DNA using T7-tailed gene-specific primers (Table S2). PCR products were transcribed using the T7 MEGAscript High Yield Transcription Kit (Ambion) according to the manufacturer's instructions. Double-stranded RNAs were treated with DNase I, precipitated with ammonium acetate/ethanol, resolved in RNase-free water and quantified by spectrophotometry. The integrity of dsRNAs was controlled by agarose gel electrophoresis (Figure S6).

HDAC inhibitor experiments

SL2 cells were transfected by the calcium-phosphate method (Suske, 2000) on 6 cm plates with 4 μg BCAT-2 reporter, 2 μg p97b coreporter and 20 ng pPacUSp3 or pPacUSp3 K551R, respectively. Thirty-six hours post-transfection cells were incubated with 1 μM trichostatin A (TSA, Sigma) or 5 mM nicotine amide (NAM, Sigma) or equal volume of the vehicle for 12 hours. Chloramphenicol acetyl transferase reporter gene assays were performed with the CAT ELISA Kit (Roche) according to the manufacturer's instructions.

Western blot analyses and immunodetection

For analyses of RNAi efficiencies, Kc_{167} cells on 24-well plates were incubated with 6 μg of appropriate dsRNAs for 6 days. For analysis of relative Sp3 SUMOylation, cells were incubated for 24 hours with dsRNA, transfected with 500 ng of pPacUSp3 WT, and harvested 5 days post-transfection in SDS-containing lysis buffer (Sapetschnig et al., 2002). Equal amounts of protein were separated by SDS-PAGE and subsequently transferred to Immobilon-P membrane (Millipore) according to the manufacturer's instructions. Membranes were blocked in 5% skimmed milk in TBST (20 mM Tris, pH 7.6, 137 mM NaCl, 0.1% Tween 20). Antibody incubations were carried out in 1% skimmed milk in TBST for 1 hour at room temperature. Horseradish peroxidase-coupled secondary antibodies were detected using the Immobilon detection system (Millipore).

For fluorescence Western blot imaging proteins were transferred to nitrocellulose membranes (Hybond, Amersham). Membranes were blocked with 5% skimmed milk in TBS and subsequently incubated at room temperature for 1 hour with antibodies diluted in 1% skimmed milk in TBS. Membranes were washed twice for 10 minutes in TBST and twice in

TBS. The LI-COR Odyssey Infrared Imaging System was used for quantification.

Immunoprecipitation

For immunoprecipitation of tagged Mi-2, MEP-1 and Sfmbt, Kc_{167} cells were plated on 10 cm dishes at 70% confluence and transfected with 5 µg of the appropriate expression construct (pPac-dMi-2-FLAG, pPac-dMi-2ΔATPase-FLAG, pPac-HA-FLAG-dMEP-1 or pPac-HA-FLAG-dSfmbt, respectively) using the FuGENE6 transfection reagent (Roche) according to manufacturer's instructions. Thirty-six hours post transfection nuclear extracts were prepared according to (Andrew and Faller, 1991). High salt nuclear extracts were diluted to 150 mM NaCl and precleared with Protein A/G sepharose FF (Amersham Biosciences). Cleared extracts were incubated with 1 to 2 µg antibody per 200 µl extract (0,5 mg total protein) for 2 hours at 4 °C and antigen-antibody complexes were precipitated with protein A/G sepharose FF. After washing (twice with TBS, 0.5% NP-40, 0.5 mM PMSF, 0.5 x protease inhibitor cocktail (Roche), and twice with 50 mM Tris/Cl, pH 7.4, 250 mM NaCl, 0.5% NP-40, 0.5 mM PMSF, 0.5 x protease inhibitor cocktail for 5 minutes at 4°C each), beads were suspended in equal volume of 2x SDS sample buffer and proteins were subjected to SDS-PAGE and Western blot analysis.

Coimmunoprecipitation of endogenous proteins

Kc_{167} cells grown on 75 cm^2 flasks to confluence were washed twice in 1x PBS, collected by centrifugation, and the cell pellets were resuspended in 1 ml buffer IPH (50 mM Tris/Cl, pH 8.0, 150 mM NaCl, 0.5 mM EDTA, 12.5 mM $MgCl_2$, 0.5 % NP-40, 0.1 mM PMSF, 0.5 x PIC). After a 20 min incubation on ice, extracts were cleared by centrifugation, 20 % glycerol was added and WCEs were stored at -80 °C.

WCEs (approximately 4 mg of protein per IP) were precleared for 2 hours at 4 °C using 25 µl of a 1:1 mix of Protein A/G-sepharose equilibrated in buffer IPH, 10 % glycerol. The cleared supernatant was incubated with 1 µl of rabbit polyclonal serum (anti-MEP-1, anti-Mi-2, anti-Sfmbt or control serum) for 2 hours at 4 °C. Subsequently, 25 µl of a 1:1 mix of Protein A/G-Sepharose equilibrated in buffer IPH, 10 % glycerol was added and extracts were incubated for addtional 2 hours. Beads were collected by centrifugation and washed 5 times in buffer IPH, 250 mM NaCl. Protein was eluted with 2x Laemmli sample buffer, boiled and subjected to Western Blotting.

***In vitro* Sumoylation assays**

Expression and purification of recombinant heterodimeric E1 enzyme (His-Aos1 and Uba2), Ubc9, SUMO-1 for *in vitro* SUMOylation assays were

previously described (Pichler et al., 2002; Sapetschnig et al., 2002). *In vitro* modification of 50 µl [^{35}S]-labeled Sp3 with SUMO-1 was performed in 100 µl reactions containing 750 ng Aos1/Uba2, 1.25 µg Ubc9 and 250 ng SUMO-1 in SUMO reaction buffer (Pichler et al., 2002) for 75 min at 30 °C.

GST pulldown experiments

[^{35}S]-labeled proteins were produced by coupled *in vitro* transcription/translation using the TNT Coupled Reticulocyte Lysate System (Promega) according to manufacturer's instructions. L-[^{35}S]-methionine (SJ1515) and Rainbow [^{14}C]-methylated protein marker (CFA 756) were obtained from Amersham Biosciences.

GST fusion proteins were expressed from pGEX vectors (Amersham Biosciences) in *E.coli* strain BL21 after induction with 1 mM IPTG. Bacteria were lysed in PBS supplemented with 0.5% TritonX-100, 1 mM DTT, 0.5 mM PMSF, 0.5 x protease inhibitor cocktail and 0.5 mg/ml lysozyme, sonicated and incubated for 30 minutes at 4 °C on a rotation wheel. Extracts were cleared by centrifugation and 2 µg of GST-fusion proteins were bound to 20 µl glutathione sepharose 4B. Beads were incubated in 300 µl binding buffer (25 mM Hepes, pH 7.6, 150 mM KCl, 12.5 mM $MgCl_2$, 20% glycerol, 0,1% NP-40, 1 mM DTT, 0.5 x protease inhibitor cocktail, 1 mg/ml BSA) with 10 µl of [^{35}S]-labeled proteins at 4 °C for 4 hours. After washing 6x at 4 °C in washing buffer (25 mM HEPES, pH 7.6, 300 mM KCl, 12.5 mM $MgCl_2$, 20% glycerol, 0.5% NP-40, 1 mM DTT, 0.5 x protease inhibitor cocktail), 2x SDS sample buffer was added and proteins were separated by SDS-PAGE. Gels were subsequently treated with fixing solution (10% acetic acid, 25% 2-propanol) and Amplify reagent (Amersham Biosciences) prior to drying and exposure to Hyperfilm MP (Amersham)**.**

SUPPLEMENTAL TABLES

Table S1

	Name	dsRNA probe	Screen Fold change (GC)2-FLuc Sp3li	Rescreen Fold change (GC)2-FLuc Sp3li	Rescreen Fold change SV40-FLuc Sp3si	Rescreen Fold change SV40-FLuc Sp3si-K/D	Ratio Sp3si WT/KD SV40-FLuc	Molecular and Biological Function	Protein domains	Mammalian Ortholog	Classification	Presence in other genome-wide screens	Potential Off-targets >18 nt
1	His4R (CG3379)	HFA16703	12.88	29.34	24.15	0.53	45.72	Nucleosome assembly	Histone	Histone H4	Transcription Regulation	Viability Cell division Listeria infection Protein secretion MAPK signaling Ca2+ entry	0
2	His3	HFA21267	40.69	62.40	27.64	0.62	44.93	Nucleosome assembly	Histone	Histone H3	Transcription Regulation	MAPK Signaling	1
3	His3.3A	HFA03343	33.42	38.03	23.78	0.65	36.79	Nucleosome assembly	Histone	Histone H3.3	Transcription Regulation	Viability MAPK signaling Ca2+ entry	1
4	CG31302	HFA15724	13.75	19.86	13.11	0.66	19.88	Signal transduction	SH3, Fibronectin, type III	RIMBP2	Signaling	Viability Listeria infection Protein secretion MAPK signaling	0
5	His3.3B	HFA18673	29.73	40.81	13.81	0.87	15.84	Nucleosome assembly	Histone	Histone H3.3	Transcription Regulation	NA	1
6	Kay	HFA16977	4.94	4.48	4.39	0.28	15.40	Transcription factor	Basic-leucine zipper (bZIP)	Fos	Transcription Regulation	Viability JAK/STAT signaling Mycobacterium infection MAPK signaling	0
7	His4 (CG33885)	HFA21268	7.11	12.90	12.27	0.83	14.70	Chromatin assembly	Histone	Histone H4	Transcription Regulation	Listeria infection MAPK signaling Ca2+ entry	0
8	Mi-2	HFA11222	6.59	7.87	10.14	1.39	7.30	ATP-dependent DNA helicase	SNF2-related Chromo Helicase, Zn-finger, PHD finger	Mi-2beta	Transcription Regulation	Listeria infection Wg signaling MAPK signaling	2
9	Su(var)2-10	HFA07721	4.66	3.01	7.89	1.09	7.24	DNA binding, DEAD/H-box RNA helicase binding, zinc ion binding	DNA-binding SAP Zn-finger, MIZ type	PIAS1	Signaling	Wg signaling	1
10	Jra	HFA07447	5.63	4.34	1.97	0.29	6.80	Transcription factor, JNK cascade	Basic-leucine zipper (bZIP)	Jun-D	Transcription Regulation	MAPK signaling	0
11	Lwr	HFA00828	13.27	5.75	5.28	0.78	6.73	SUMO conjugating	Ubiquitin-conjugating enzymes	Ubc9	Signaling	NA	1

								enzyme activity					
12	CAP	HFA06744	3.73	8.81	7.26	1.10	6.61	MAPKKK cascade, cytoskeleton organization	SH3, Sorbin-like	Unknown	Signaling	NA	1
13	CG2865	HFA18528	3.77	6.42	4.34	0.72	6.00	Unknown	SERTA domain	Unknown	Unknown	Wg signaling	9
14	Sbb	HFA07676	7.92	8.39	5.51	1.06	5.18	Transcription factor	Zn-finger, C2H2 type	ZNF608	Transcription Regulation	MAPK signaling	1
15	CG1244	HFA08274	13.25	18.18	5.58	1.15	4.84	Nucleic acid binding, zinc ion binding	Zn-finger, C2H2 type	Unknown (ortholog of C.elegans MEP-1)	Transcription Regulation	Wg signaling MAPK signaling Ca2+ entry	3
16	CG30387	HFA04256	17.67	11.46	10.51	2.19	4.79	Receptor signaling	Ankyrin repeats, KAP P-loop	Kidins220	Signaling	Viability Protein secretion MAPK signaling	40, CAR repeats
17	CG15654	HFA04245	4.33	5.38	6.68	1.42	4.70	Unknown	Unknown	Unknown	Unknown	NA	2
18	CG14972	HFA08431	9.07	15.29	4.14	0.90	4.61	Unknown	Unknown	Unknown	Unknown	NA	0
19	CG10616	HFA09750	11.39	18.99	4.86	1.09	4.46	Unknown	Unknown	C1orf27	Unknown	NA	0
20	CG15422	HFA00436	4.87	4.33	4.45	1.04	4.30	Unknown	Unknown	Unknown	Unknown	Ca2+ entry	33
21	Hoe1	HFA00333	8.59	18.79	3.60	0.86	4.17	Transporter activity	Arsenical pump membrane protein, Citrate transporter	OCA2	Transport	MAPK signaling	1
22	Rep3	HFA07529	7.47	8.78	4.05	1.04	3.90	Protein binding, apoptosis	Caspase-activated nuclease CIDE-N	CIDEC	Signaling	NA	6
23	Pdm-2	HFA03587	7.95	9.50	3.18	0.82	3.89	Transcription factor	POU homeobox	Oct2	Transcription Regulation	Ca2+ entry	0
24	l(2)01289 (CG9432)	HFA05021	6.52	7.30	3.19	0.83	3.84	Electron transport	Thioredoxin domain 2, SGA1	Unknown	Transport	NA	1
25	CG3996	HFA15533	9.52	13.32	2.37	0.63	3.75	DNA binding, cell cycle	RabGAP/TBC domain	Unknown	Signaling	Ca2+ entry	5
26	MAP205	HFA16732	8.44	9.97	3.72	1.00	3.72	microtubule binding	Ataxin-2, C-terminal	Unknown	Signaling	DCV screen	1
27	GstD3	HFA15571	9.07	15.13	3.73	1.02	3.66	Glutathione transferase activity	Glutathione S-transferase	GSTT2	Signaling	NA	2
28	Pcp	HFA03395	5.43	12.62	4.87	1.37	3.54	Structural protein	Insect cuticle protein	Unknown	Structural Protein	NA	0
29	CG5554	HFA04513	3.47	2.89	2.55	0.74	3.46	Electron transport	Thioredoxin domain 2	TXNDC	Signaling	NA	4
30	CG1513	HFA06553	6.65	4.32	3.98	1.17	3.40	Oxysterol binding	Pleckstrin-like	ORP-9	Signaling	MAPK signaling Ca2+ entry	2
31	CG30463	HFA07185	3.77	3.49	3.62	1.08	3.34	Polypeptide N-acetylgalactos-aminyltransferase	Ricin B-related lectin	GALNT11	Metabolism	NA	0
32	CG15269	HFA01942	5.24	7.16	3.75	1.13	3.33	Transcription factor	Zn-finger, C2H2 type	ZNF658B	Transcription Regulation	JAK/STAT signaling	14
33	CG9067	HFA07328	4.38	6.46	2.28	0.71	3.20	Unknown	Sybindin domain, TRS20	HSPC176	Unknown	NA	0
34	CG7945 (CG17014)	HFA10934	8.87	14.84	3.88	1.22	3.17	Protein folding (Chaperone)	Unknown	BAG2	Metabolism	Ca2+ entry	0
35	CG12379	HFA19451	9.17	11.32	2.55	0.82	3.12	Unkown	Zn-finger	RP11-413M3.2	Unknown	NA	2

36	CG1814	HFA06711	9.50	21.52	5.85	1.88	3.10	Nucleic acid metabolism	5'-Nucleotidase	5'-nucleotidase domain containing 3	Metabolism	MAPK signaling DCV screen	0
37	CG14852	HFA14976	4.17	4.68	1.68	0.55	3.08	Unknown	Unknown	Unknown	Unknown	NA	8
38	CG3964	HFA00636	4.68	7.02	2.97	1.00	2.97	Tubulin-tyrosine ligase activity	TTL	TTLL4	Metabolism	NA	2
39	CG3213	HFA00576	3.42	4.11	4.32	1.49	2.89	Ribosomal protein	Smc domain	Unknown	Metabolism	MAPK signaling	2
40	CG13088	HFA02192	4.05	7.12	1.95	0.69	2.84	Unknown	Unknown	Unknown	Unknown	NFAT signaling	0
41	MTA1-like	HFA12382	5.14	6.83	2.93	1.04	2.82	Unknown	BAH, ELM2, SANT, GATA Zn finger, Myb-like	MTA2	Transcription Regulation	NA	121, CAR repeats
42	CG31814	HFA01336	3.54	4.02	5.25	1.87	2.81	Signal transduction	Immunoglobulin-like	Unknown	Signaling	Protein secretion MAPK signaling	68, CAR repeats
43	CG6969	HFA16157	4.50	6.26	2.51	0.93	2.69	Peroxidase activity	Haem peroxidase	PXDN	Signaling	NA	0
44	EIF4AIII	HFA16249	7.81	3.09	1.16	0.43	2.69	ATP-dependent RNA helicase activity	DEAD/DEAH box helicase	DDX48	Translation Regulation	Ca2+ entry	2
45	HSP60D (CG16954)	HFA02543	4.99	5.57	2.98	1.12	2.65	Protein folding (Chaperone)	Chaperonin Cpn60/TCP-1	HSP60	Metabolism	NA	2
46	Ocho	HFA11239	4.60	10.00	3.59	1.37	2.62	Unknown	Unknown	Unknown	Unknown	NA	2
47	PGRP-SC2	HFA06481	5.00	5.12	2.68	1.03	2.60	Peptidoglycan binding	N-acetylmuramoyl-L-alanine amidase family 2	PGRP-S	Signaling	NA	2
48	Patj	HFA08712	5.78	17.15	3.21	1.26	2.55	Protein binding	PDZ/DHR/GLGF L27	MPDZ	Signaling	Protein secretion	2
49	Ef1gamma	HFA16659	6.61	18.96	3.07	1.20	2.55	Translation elongation factor	Glutathione S-transferase	EF-1-gamma	Translation Regulation	Protein secretion Ca2+ entry	4
50	CG2010	HFA15413	6.90	13.07	2.58	1.01	2.54	Unknown	F-box	FBXL7	Signaling	NA	34
51	Taf5	HFA07562	3.66	3.19	3.37	1.34	2.51	General transcription factor	WD-40 repeat	Taf5	Transcription Regulation	MAPK signaling	1
52	CG13966	HFA02308	6.70	11.68	4.40	1.77	2.49	Unknown	Unknown	Unknown	Unknown	NA	0
53	Yem-alpha	HFA17097	3.71	3.68	2.53	1.02	2.48	DNA binding	Unknown	Ubinuclein	Transcription Regulation	NA	1
54	CG14145	HFA10147	6.46	12.01	1.70	0.69	2.47	Unknown	Unknown	BLOC1S2	Unknown	NA	3
55	CG30053	HFA07260	3.68	5.94	5.98	2.43	2.47	Unknown	Unknown	Unknown	Unknown	Hh signaling	0
56	Sh	HFA19785	6.35	6.65	2.26	0.92	2.45	Voltage-gated potassium channel	BTB/POZ, K+ channel	KCNA2	Transport	Viability Hh signaling Protein secretion Ca2+ entry	9
57	CG8515	HFA07204	8.72	5.73	2.18	0.89	2.45	Structural protein	Unknown	Unknown	Structural Protein	NA	3, CAN repeats, no CAR
58	MRpL40	HFA15749	4.86	6.93	2.30	0.94	2.44	Ribosomal protein	Unknown	MRPL40	Metabolism	NA	2
59	CG17189	HFA15183	5.84	7.33	2.48	1.02	2.43	Unknown	DUF233 domain	Unknown	Unknown	NA	1
60	Zfh-1	HFA17098	4.05	3.60	9.32	3.84	2.43	Transcription factor	Homeobox Zn-finger, C2H2 type	TCF8	Transcription Regulation	Listeria infection Hh signaling MAPK signaling Ca2+ entry	6
61	CG12856	HFA06196	3.92	7.11	2.56	1.07	2.40	Unknown	Unknown	Unknown	Unknown	NA	11

62	CG9426	HFA03219	5.89	6.83	3.60	1.53	2.35	Actin binding	BTB/POZ, Kelch motif	MIPP protein	Signaling	NA	0
63	Toll-9	HFA11776	3.64	3.56	1.65	0.71	2.34	Transmembrane receptor	TIR, Leucine-rich repeat	TLR1	Signaling	NA	0
64	EEF1delta	HFA02790	7.77	9.81	3.94	1.69	2.33	Translation elongation factor	Elongation factor 1, beta/beta'/delta chain	EF-1-delta	Translation Regulation	Hh signaling	3
65	LysC	HFA21249	5.44	10.05	1.66	0.72	2.30	Lysozyme activity	LYZ1, C-type lysozyme	Lysozyme C precursor	Signaling	Ca2+ entry	3
66	CG14656	HFA12242	4.49	6.00	1.54	0.67	2.29	Unknown	Unknown	Unknown	Unknown	Hh signaling	19
67	CG10659	HFA02089	4.77	3.86	1.91	0.84	2.27	Unknown	Acetyltransferase (GNAT)	Unknown	Unknown	NA	0
68	CG7685	HFA16285	5.44	9.12	2.05	0.91	2.25	Alpha-glucosidase activity	Low density lipoprotein-receptor, class A	Unknown	Signaling	NA	1
69	Pros26	HFA11256	3.82	3.39	1.81	0.81	2.22	Ubiquitin-dependent protein catabolism	20S proteasome, A and B subunits	PSMB1	Metabolism	Viability Listeria infection Protein secretion Hh signaling MAPK signaling Ca2+ entry	0
70	Tsp42Eo	HFA06184	4.71	8.46	2.39	1.09	2.20	Unknown	CD9/CD37/CD63 antigen, Tetraspanin	Unknown	Signaling	NA	0
71	Gukh	HFA14768	3.79	4.16	1.53	0.71	2.16	Protein binding	Unknown	NHS	Unknown	NA	2
72	CG13084	HFA02188	3.82	6.04	1.51	0.73	2.08	Unknown	Unknown	Unknown	Unknown	NA	0
73	CG4567	HFA02758	5.36	6.80	2.04	1.00	2.03	Translation elongation	Elongation factor G	Elongation factor G1 (GFM1)	Translation Regulation	Ca2+ entry	0
74	Pkg21D	HFA00772	14.96	17.10	2.86	1.46	1.97	cGMP-dependent protein kinase	Protein kinase	cGKII	Signaling	NA	5
75	CG7056	HFA16186	7.03	9.91	1.79	0.92	1.94	Transcription factor	Homeodomain	Unknown	Transcription Regulation	NA	2
76	CG16975 (Sfmbt)	HFA02552	5.14	3.30	1.65	0.85	1.94	Chromatin binding	Sterile alpha motif SAM, Mbt repeat	L3MBTL2	Transcription Regulation	JAK/STAT signaling	1
77	CG6762	HFA20005	5.32	8.41	2.16	1.12	1.93	DNA binding	ParB-like nuclease	SRXN1	Metabolism	Ca2+ entry	0
78	CG13033	HFA09913	4.16	6.02	1.31	0.68	1.91	Unknown	Unknown	Unknown	Unknown	NA	1
79	GlyP	HFA00752	4.28	3.09	2.71	1.42	1.91	Phosphorylase activity	Phosphorylase	PYGM	Metabolism	NA	0
80	Rpn5	HFA12367	7.70	3.71	1.96	1.04	1.89	Ubiquitin-dependent protein catabolism	Proteasome component region PCI	PSMD12	Metabolism	Protein secretion MAPK signaling	1
81	CG14770	HFA17947	10.09	13.78	1.88	1.03	1.83	Unknown	Unknown	Unknown	Unknown	Ca2+ entry	0
82	CG32686	HFA17645	4.47	4.38	2.74	1.50	1.83	ATP synthesis coupled proton transport	H+-transporting two-sector ATPase	Unknown	Transport	NA	0
83	Vir-1	HFA02863	4.35	5.72	1.21	0.66	1.82	Unknown	Amiloride-sensitive sodium channel	Unknown	Transport	NA	7
84	Tsp29Fb	HFA03233	4.66	4.80	1.57	0.87	1.81	Receptor binding	IQ calmodulin-binding region, CD9/CD37/CD63 antigen, Tetraspanin	TSPAN6	Signaling	NA	1

85	RdgA	HFA18827	6.78	34.43	2.58	1.43	1.80	Diacylglycerol kinase	Diacylglycerol kinase Ankyrin, Protein kinase C	DGKI	Signaling	NA	2
86	CG6020	HFA11791	5.33	5.61	2.17	1.20	1.80	NADH dehydrogenase activity	Epimerase domain	NDUFA9	Transport	NA	1
87	CG4500	HFA01944	5.91	15.03	3.33	1.85	1.80	Long-chain-fatty-acid-CoA ligase activity	AMP-binding	ACSBG2	Metabolism	Ca2+ entry	0
88	ast	HFA00803	7.78	14.09	1.78	1.00	1.78	Epidermal growth factor receptor signaling pathway	XPG	ASTE1	Signaling	NA	2
89	sd	HFA20370	5.23	3.14	1.86	1.05	1.78	Transcription factor	TEA/ATTS Homeodomain-like	TEAD4 (TEF3)	Transcription Regulation	NA	0
90	Chd64	HFA08649	13.26	15.15	2.16	1.23	1.76	Actin binding	SM22/calponin	TAGLN3	Signaling	NA	1
91	TRAF1	HFA00794	8.03	5.60	1.73	0.99	1.75	Receptor binding, Toll signaling pathway	Zn-finger, TRAF type MATH. Ankyrin, TRAF-like	TRAF4	Signaling	NA	0
92	CG11050	HFA02125	3.73	3.64	4.40	2.52	1.75	Unknown	HD domain	HDDC2	Signaling		12, CAN repeats, no CAR
93	CG32150	HFA10215	4.32	5.49	1.49	0.85	1.74	Unknown	Unknown	Unknown	Unknown	Hh signaling Ca2+ entry	0
94	And	HFA14110	6.66	9.85	1.94	1.12	1.74	Calmodulin binding, calcium-mediated signaling	Efh	CALM2	Signaling	NA	1
95	CG5414	HFA10506	3.84	3.95	0.93	0.54	1.74	Isoleucine-tRNA ligase activity	Aminoacyl-tRNA synthetase, class I	IARS2	Translation Regulation	NA	0
96	Chd3	HFA11114	4.06	3.65	1.82	1.05	1.74	ATP-dependent DNA helicase	SNF2-related Chromo, Helicase, Zn-finger, PHD finger	CHD3	Transcription Regulation	Wg signaling	2
97	Hsp67Ba	HFA11191	6.07	12.32	2.84	1.64	1.73	Protein folding, response to stress	HSP20-like chaperone	HSPB1	Metabolism	NA	21
98	CG5641	HFA15852	9.88	5.28	1.69	0.98	1.72	Transcription cofactor activity	DZF	ILF2 (NFAT subunit)	Transcription Regulation	NA	0
99	CG14958	HFA08417	3.67	4.43	2.00	1.17	1.70	Unknown	Unknown	Unknown	Unknown	NA	0
100	CG31872	HFA02568	3.46	4.97	1.63	0.96	1.70	Triacylglycerol lipase activity	Esterase/lipase/ thioesterase	Unknown	Metabolism	NA	0
101	CG15636	HFA00471	3.78	4.26	1.86	1.10	1.69	Chromatin binding	Chromo, Chromo shadow	CBX5 (HP1)	Transcription Regulation	NA	0
102	Esg	HFA03530	4.29	3.78	1.99	1.19	1.67	Transcription factor	Zn-finger, C2H2 type	SNAI2 (SLUG)	Transcription Regulation	NA	0
103	CG7408	HFA10841	4.76	4.24	1.40	0.84	1.67	N-acetylgalactos-amine-4-sulfatase	Sulfatase	ARSB	Metabolism	NA	0
104	CG10710	HFA09784	3.92	3.30	1.14	0.69	1.66	Unknown	Unknown	Unknown	Unknown	NA	0
105	CG32066	HFA10652	8.72	6.30	1.95	1.17	1.66	Unknown	Protein of unknown function DUF1394	FAM49B	Unknown		1
106	CG14053	HFA17908	4.00	3.29	1.19	0.72	1.65	Unknown	Unknown	Unknown	Unknown	NA	0
107	CG6962	HFA16153	8.34	22.60	1.68	1.02	1.64	Unknown	Unknown	FLJ20297 (KIAA1418 protein)	Unknown	Hh signaling DCV screen Ca2+ entry	2

108	Nrx-1	HFA16184	7.62	5.45	6.17	3.77	1.64	Receptor activity, signal transduction	EGF-like, Laminin G	NRXN3	Signaling	JAK/STAT signaling	0
109	Or7a	HFA18691	5.67	4.83	1.51	0.94	1.60	Odorant binding, G-protein coupled receptor protein signaling pathway	7tm Odorant receptor	Unknown	Signaling	NA	0
110	VhaSFD	HFA03471	5.92	3.65	2.17	1.37	1.59	Hydrogen-transporting ATPase activity	V-ATPase subunit H, Armadillo-like helical	ATP6V1H	Transport	Listeria infection	0
111	CG13027	HFA09907	4.28	3.61	1.11	0.71	1.58	Unknown	Unknown	Unknown	Unknown	NA	0
112	Dlp	HFA10472	4.90	5.47	2.14	1.36	1.57	Wnt receptor signaling pathway	Glypican	GPC4	Signaling	Wg signaling MAPK signaling	6
113	CG13937	HFA08406	5.55	9.20	1.99	1.27	1.57	HNK-1 sulfotransferase activity	Sulfotransferase	CHST11	Metabolism	NA	0
114	Osi5	HFA12276	5.09	3.97	1.31	0.85	1.54	Unknown	Unknown	Unknown	Unknown	Ca2+ entry	0
115	CG1973	HFA15408	6.69	6.88	1.64	1.07	1.53	Protein kinase	HEAT, Protein kinase Armadillo-like helical	SCYL1	Signaling	NA	0
116	ERCC1	HFA07424	4.75	9.72	1.83	1.20	1.52	Endodeoxyribo-nuclease activity, nucleotide-excision repair	Helix-hairpin-helix motif, DNA repair protein rad10, RuvA domain 2-like	ERCC1	Metabolism	MAPK signaling DCV screen	0
117	Lk6	HFA12867	3.92	3.77	2.86	1.88	1.52	Protein kinase	Serine/threonine protein kinase	MKNK2	Signaling	NA	
118	Hsp70Bbb	HFA15380	4.24	7.02	1.63	1.08	1.51	Protein folding (chaperone)	Heat shock protein Hsp70	Hsp70	Metabolism	Listeria infection Protein secetion MAPK signaling Ca2+ entry	4
119	CG14365	HFA14827	4.85	5.13	1.81	1.20	1.51	Unknown	Unknown	Unknown	Unknown	NA	3
120	CG12753	HFA14536	6.06	6.18	2.11	1.40	1.51	Unknown	Unknown	KIAA0350	Unknown	NA	0

Table S1. Summary of the screening results. The original screen and the rescreen with new synthesized dsRNAs was performed in duplicate and triplicate, respectively, with the long isoform of Sp3 (Sp3li) and the (GC)2-FLuc reporter. DsRNAs that activated the (GC)2-FLuc reporter greater than 3-fold were rescreened in duplicate with the small isoform Sp3 (Sp3si) and the corresponding SUMOylation-deficient mutant Sp3 K551D using the SV40-FLuc reporter construct. Sp3si WT/KD values greater than 2 are based on two independent experiments, and Sp3si WT/KD values between 1.5 and 2 are derived form at least four independent experiments. Information on molecular and biological function as well as on protein domains was obtained from FlyBase (http://www.flybase.org) and/or Blast searches (http://www.ncbi.nlm.nih.gov/blast). The sequences of the dsRNA probes (HFA numbers) can be obtained at the German Cancer Research Center, Heidelberg (http://www.dkfz.de/signaling2/rnai/ernai_probes.php). Mammalian orthologs to genes identified in the screen were determined by orthology calls from the FlyBase reports and/or by Blast search. Names refer to gene name from UniProt (http://www.ebi.uniprot.org). Predicted proteins were classified into functional groups according to their molecular function, predicted protein domains and literature search. Information for the identification of genes in other screens was obtained from databases at the German Cancer Research Center in Heidelberg (http://www.dkfz.de/signaling2/rnai/) and the *Drosophila* RNAi Screening Center at Harvard Medical School (http://flyrnai.org/RNAi_index.html) and refers to the following publications: Viability (Boutros et al., 2004); Cell division (Eggert et al., 2004); Listeria infection (Agaisse et al., 2005); Protein secretion (Bard et al., 2006); Ca^{2+} entry (Vig et al., 2006); DCV screen (Cherry et al., 2005; Cherry et al., 2006); MAPK Signaling (Friedman and Perrimon, 2006); JAK/STAT signaling (Baeg et al., 2005; Müller et al., 2005); Mycobacterium infection (Philips et al., 2005); Wg signaling (DasGupta et al., 2005); Hh signaling (Nybakken et al., 2005). Potential off-targets >18nt and CAN/CAR repeat information were obtained from the *Drosophila* RNAi Screening Center at Harvard Medical School (http://flyrnai.org/cgi-bin/RNAi_gene_lookup_public.pl?).

Table S2

Name	Primer	Sequence
Smt3 (SUMO)	5'-T7-Smt3 (HFA)	gaattaatacgactcactatagggGTCTGACGAAAAGAAGGGAG
	3'-T7-Smt3 (HFA)	gaattaatacgactcactatagggATGGAGCGCCACCAGTC
	5'-T7-Smt3(2)	gaattaatacgactcactatagggAGACCACTTAGCAGCTTCAAC
	3'-T7-Smt3(2)	gaattaatacgactcactatagggAGACGCATTTAGAATCTACAGT
lwr (Ubc9)	5'-T7-lwr	gaattaatacgactcactatagggAGAAATCCAGGACTCGAGGAC
	3'-T7-lwr	gaattaatacgactcactatagggAGATCGTACTCCAGTCGGTTCTG
Su(var)2-10 (PIAS)	5'-T7-Su(var)2-10 (HFA)	gaattaatacgactcactatagggCATGCCATTTCCGTCTCTT
	3'-T7-Su(var)2-10 (HFA)	gaattaatacgactcactatagggGTAGAAAGGCACCTCTTGGA
GFP	5'-T7-EGFP	gaattaatacgactcactatagggaGAGCTGGACGGCGACGTAA
	3'-T7-EGFP	gaattaatacgactcactatagggagACTTGTACAGCTCGTCCATG
FLuc	5'-T7-Fluc	taatacgactcactatagggGGAAGAACGCCAAAAAC
	3'-T7-Fluc	taatacgactcactatagggCTCTGGCACAAAATCG
RLuc	5'-T7-Rluc	taatacgactcactatagggATGACTTCGAAAGTTT
	3'-T7-Rluc	taatacgactcactatagggGATGCTCATAGCTATA
CAP	5'-T7-CAP(2)	taatacgactcactatagggCCGCTAATTCTGCTGCTTTC
	3'-T7-CAP(2)	taatacgactcactatagggTTGGTTCGGACACGACAATA
CG10616	5'-T7-CG10616(2)	taatacgactcactatagggGTGTTCTTCAGCCTGCCTTC
	3'-T7-CG10616(2)	taatacgactcactatagggTGGCATCGAACTGGTCATAA
CG1244	5'-T7-CG1244(2)	taatacgactcactatagggAGCAGAGAACGGACCTCAAA
	3'-T7-CG1244(2)	taatacgactcactatagggCCTGGGCAACATCCTCTTTA
	5'-T7-CG1244(5U)	gaattaatacgactcactatagggCTTATCGACATTTGTGG
	3'-T7-CG1244(5U)	gaattaatacgactcactatagggAATCGCTGACGCTTGG
	5'-T7-CG1244(3U)	gaattaatacgactcactatagggGTGAGGGAAAAGCAGAGTGG
	3'-T7-CG1244(3U)	gaattaatacgactcactatagggAAGCGAGGCACGTCATCTAT
CG13088	5'-T7-CG13088(2)	taatacgactcactatagggTGCCTCCTTGAAAATCTCCT
	3'-T7-CG13088(2)	taatacgactcactatagggCCCTTGCAAAGGGTACAACA
CG14852	5'-T7-CG14852(2)	taatacgactcactatagggTTCTGGTCCTGTGCTTTTCC
	3'-T7-CG14852(2)	taatacgactcactatagggGTGCTATCTGGCGATCCTGT
CG14972	5'-T7-CG14972(2)	taatacgactcactatagggCCTGTTTGAGGGAAACGG
	3'-T7-CG14972(2)	taatacgactcactatagggGCAGCTTGGCGATGTTGAAG
CG1513	5'-T7-CG1513(2)	taatacgactcactatagggACCACCACTTACGCGTTTTC
	3'-T7-CG1513(2)	taatacgactcactatagggAGAGCTAGTCACCTCTGCCG
CG15269	5'-T7-CG15269(2)	taatacgactcactatagggACCGAGGAACCAAGGAGTTT
	3'-T7-CG15269(2)	taatacgactcactatagggTTTTCCCGGTACTGGAAGTG
CG15422	5'-T7-CG15422(2)	taatacgactcactatagggATGCGGTACTATGAAGATCC
	3'-T7-CG15422(2)	taatacgactcactatagggCCGTAGGGTTGCGGCATTAT
CG15654	5'-T7-CG15654(2)	taatacgactcactatagggACTTGAATTCGAGGGTCAGG
	3'-T7-CG15654(2)	taatacgactcactatagggTCACCTATGGTCGTGTAAATCG
CG16954	5'-T7-CG16954(2)	taatacgactcactatagggGCTTTCACCGATGTGGAACT
	3'-T7-CG16954(2)	taatacgactcactatagggTTTTCGGAATCTTCGGTTTG
CG1814	5'-T7-CG1814(2)	taatacgactcactatagggGAACTGGCTGTGGGTGTTTT
	3'-T7-CG1814(2)	taatacgactcactatagggAAGTGGGAGGCATATTCGTG
CG2865	5'-T7-CG2865(2)	taatacgactcactatagggACGGATGAAGCTGAATGACC
	3'-T7-CG2865(2)	taatacgactcactatagggAATCTTTGTCTGCTGCTCGG
CG30387	5'-T7-CG30387(2)	taatacgactcactatagggACAAGGATGGAATGACTGCC
	3'-T7-CG30387(2)	taatacgactcactatagggTTTCTCCAGCTTTGTTGGCT

CG31302	5'-T7-CG31302(2)	taatacgactcactatagggTCACTGGAGCCACATCTCTG
	3'-T7-CG31302(2)	taatacgactcactatagggCGGTTATGCATCATGGAGAC
CG31814	5'-T7-CG31814(2)	taatacgactcactatagggAGGAAGGGCTATTTGCAGGT
	3'-T7-CG31814(2)	taatacgactcactatagggTTGCTAATCGAAGGTGGGAC
CG3213	5'-T7-CG3213(2)	taatacgactcactatagggGCTGGAGTTCAAGTGCATCA
	3'-T7-CG3213(2)	taatacgactcactatagggAGACCACCACCAGTTTCGTC
CG3964	5'-T7-CG3964(2)	taatacgactcactatagggATCTTCACCACCTGCCTGAC
	3'-T7-CG3964(2)	taatacgactcactatagggTGCCCATTTCCCTATTCTTG
CG3996	5'-T7-CG3996(2)	taatacgactcactatagggAACTGCGGAAACCAAAAATG
	3'-T7-CG3996(2)	taatacgactcactatagggCCTCCACCTCAACTCTGCTC
CG5554	5'-T7-CG5554(2)	taatacgactcactatagggCTGCGGATCTTCAGCCTTAG
	3'-T7-CG5554(2)	taatacgactcactatagggCAAAAATGCTTGGCAAAAGG
CG6969	5'-T7-CG6969(2)	taatacgactcactatagggGGTTGGGGACCCAAGATAGT
	3'-T7-CG6969(2)	taatacgactcactatagggGTGGCTGTGATGTCATGGTC
CG7945 (CG17014)	5'-T7-CG7945(2)	taatacgactcactatagggAACCGACATGTGACAGACCA
	3'-T7-CG7945(2)	taatacgactcactatagggTCTGCATCATTTCGTTGCTC
CG8191	5'-T7-CG8191(2)	taatacgactcactatagggTGCCTACCTAATCGACCATTG
	3'-T7-CG8191(2)	taatacgactcactatagggAAATGTGTCCAGGAATTGGG
CG9067	5'-T7-CG9067(2)	taatacgactcactatagggGACCAACACTCGGGTGAAAT
	3'-T7-CG9067(2)	taatacgactcactatagggCCGGGAATGTAAAAGGGATT
CG9432	5'-T7-CG9432(2)	taatacgactcactatagggAGAGTACAGTCCGCGGAAAA
	3'-T7-CG9432(2)	taatacgactcactatagggCGATCGGTTACGGTTCTGAT
CHD3	5'-T7-CHD3(2)	taatacgactcactatagggGATTTACGTCAGAAGGCCATTGACA
	3'-T7-CHD3(2)	taatacgactcactatagggAGCGACCTTAAAGGACGAAAGATAC
eIF4AIII	5'-T7-eIF4AIII(2)	taatacgactcactatagggGACACTGGAAGGCATCAAGC
	3'-T7-eIF4AIII(2)	taatacgactcactatagggATGGGCATCTCGTCGATTTG
GstD3	5'-T7-GstD3(2)	taatacgactcactatagggACACATTCCTGGAGGGTCAG
	3'-T7-GstD3(2)	taatacgactcactatagggCAGCATTCTGTTTCTCCTCG
hoe1	5'-T7-hoe1(2)	taatacgactcactatagggTGATGTCCAACAACGAGCAT
	3'-T7-hoe1(2)	taatacgactcactatagggGGACTCAGCTCGAAGGTACG
Jra	5'-T7-Jra(2)	taatacgactcactatagggAGACTGAAACCCCCTCGAAT
	3'-T7-Jra(2)	taatacgactcactatagggACCAAAGAAAGGCACAAAGC
Kay	5'-T7-Kay(2)	taatacgactcactatagggAACCGCGAAGCACTTTTCTA
	3'-T7-Kay(2)	taatacgactcactatagggATCCTGCAAATCTACACGCC
MAP205	5'-T7-MAP205(2)	taatacgactcactatagggCGGCTTCAAAGGAGAAACTG
	3'-T7-MAP205(2)	taatacgactcactatagggGGCCCCTAAAGTTACCTTGC
Mi-2	5'-T7-Mi-2(2)	taatacgactcactatagggTTAACTCGCTGACCAAGGCT
	3'-T7-Mi-2(2)	taatacgactcactatagggATATCGTTGTGGGGATTCCA
	5'-T7-Mi-2(3U)	gaattaatacgactcactatagggGATATCAAGAAACAAAAAATGG
	3'-T7-Mi-2(3U)	gaattaatacgactcactatagggTCCTTTGCAATGGAATTAAATAC
MTA1-like	5'-T7-MTA1-like(2)	taatacgactcactatagggCAGAACGCGAGACAACAAAA
	3'-T7-MTA1-like(2)	taatacgactcactatagggTGGAACTTTAGAGCGCGATT
Ocho	5'-T7-Ocho(2)	taatacgactcactatagggAACTGGCAAAACAAACCCAG
	3'-T7-Ocho(2)	taatacgactcactatagggGTTGAGGGTCTTCTGCTTGC
Patj	5'-T7-Patj(2)	taatacgactcactatagggGACTACGCTCAGATCCAGGC
	3'-T7-Patj(2)	taatacgactcactatagggGCATCCTTCTTCAGCTCCAC
Pcp	5'-T7-Pcp(2)	taatacgactcactatagggTTAGGACGCATCCCTACCAG
	3'-T7-Pcp(2)	taatacgactcactatagggGCCAAAGAATCACGTCCATC
Pdm-2	5'-T7-Pdm-2(2)	taatacgactcactatagggCAACATTCCACATGCCAGAC
	3'-T7-Pdm-2(2)	taatacgactcactatagggGGGCACAACAGATACACACG

Rep3	5'-T7-Rep3(2)	taatacgactcactatagggAATGCATTTTTCCCTCAACG
	3'-T7-Rep3(2)	taatacgactcactatagggTCCGCTGAGTGAGGTTAGGT
Rpd3	5'-T7-Rpd3(2)	taatacgactcactatagggCGACGGCGTCTAATACCAAT
	3'-T7-Rpd3(2)	taatacgactcactatagggCCGCCCACTGATTACTGATT
Sbb	5'-T7-Sbb(2)	taatacgactcactatagggATATTGGCGGCATACCAGAG
	3'-T7-Sbb(2)	taatacgactcactatagggCGATTTATGCGACGATGATG
Sfmbt	5'-T7-dSfmbt(2)	taatacgactcactatagggTTCTACACAAAATCGCGACG
	3'-T7-dSfmbt(2)	taatacgactcactatagggTTCGCCGAAGCTATTCAACT
	5'-T7-dSfmbt(3U)	taatacgactcactatagggCGAAACACAAACGTTGCCTA
	3'-T7-dSfmbt(3U)	taatacgactcactatagggGAGCGGCTAGTTAATCGTGG

Table S2. Gene-specific primers with tagged T7 promoter sequence for generating dsRNA templates

Sequence of primers used for PCR amplification of gene fragments for the generation of control dsRNAs (SUMO, Ubc9, PIAS, GFP, FLuc, RLuc) and alternative dsRNAs not present in the HFA RNAi library. T7 RNA polymerase binding sites are in lower case, gene-specific sequences in upper case. Gene names are according to FlyBase release 2006_01.

SUPPLEMENTAL FIGURES

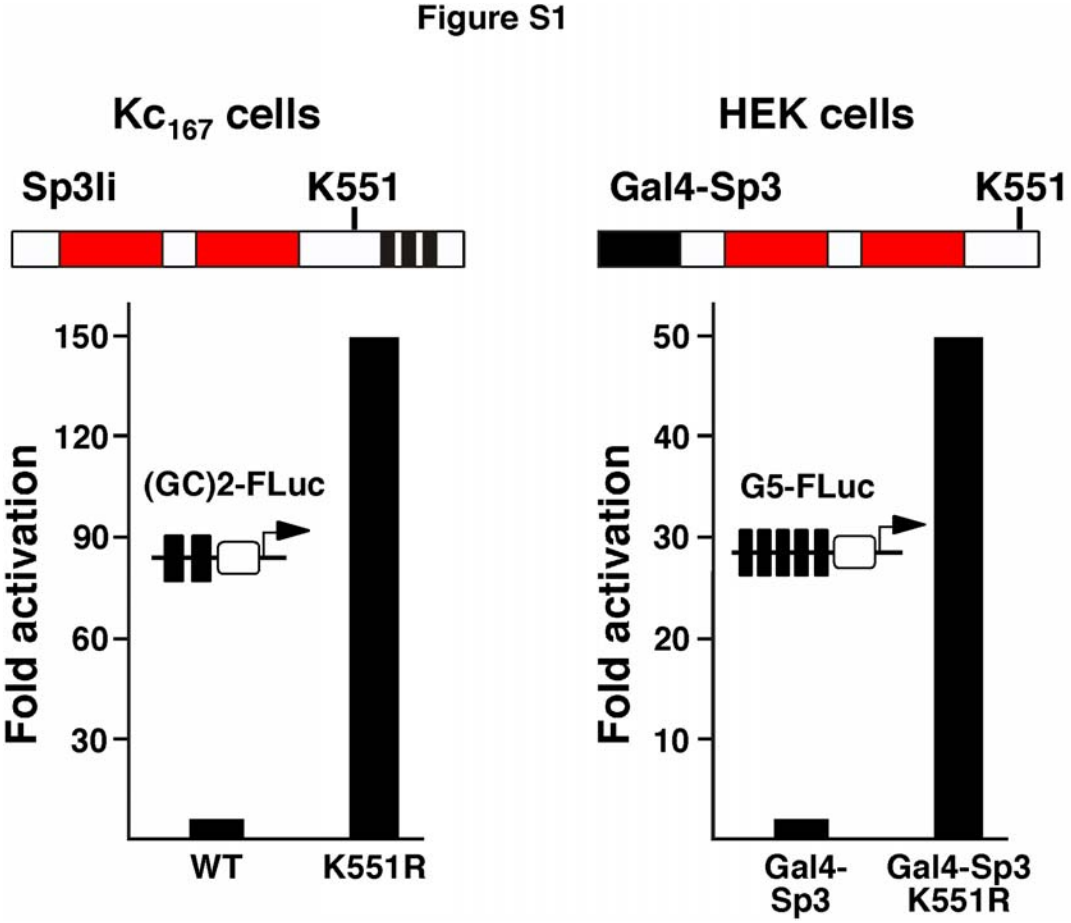

Figure S1. Repression of Sp3 by SUMO modification is conserved between mammalian and insect cells

Left: KC_{167} cells on 24-well plates were transfected with 50 ng of an expression plasmids for the long Sp3 isoform (Sp3li) or the corresponding lysine 551 mutant (K551R) along with 1 μg of the (GC)2-FLuc reporter. Firefly reporter activity was normalized to a cotransfected *copia* promoter-driven lacZ reporter.

Right: HEK cells were transfected with 50 ng of an expression plasmids for Gal4-Sp3 or the corresponding lysine 551 mutant (G4-Sp3 K551R) along with 1 μg of a Gal4-firefly luciferase reporter (G5-FLuc). Firefly reporter activity was normalized to a cotransfected *RSV* promoter-driven lacZ reporter.

Figure S2

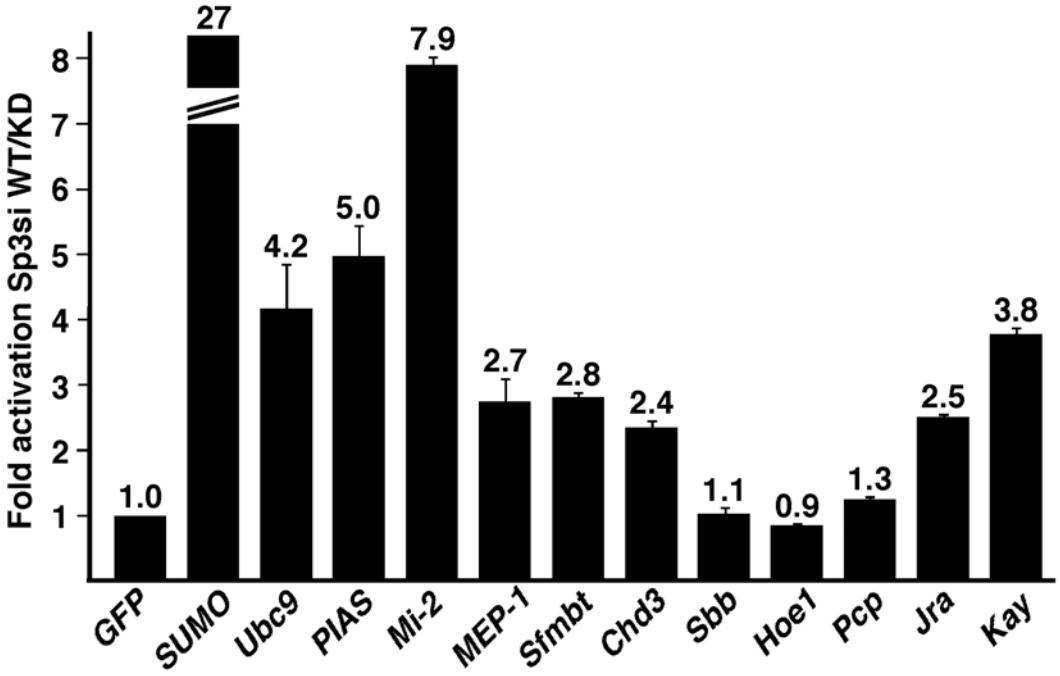

Figure S2. RNAi in SL2 cells

DsRNAs (1 μg) on 96-well plates targeting the genes indicated were incubated with *D. melanogaster* SL2 cells. After 24 hours cells were transfected with the SV40-FLuc reporter, the RLuc control reporter and an expression plasmid for wild-type Sp3si or for the Sp3si K551D mutant, respectively. Fold activation values (Sp3si WT/KD) were obtained by dividing the normalized fold activation values for Sp3si WT by the normalized fold activation values for the Sp3si K551D mutant. Bars represent means +/- SD of at least two independent experiments performed in duplicate.

Figure S3

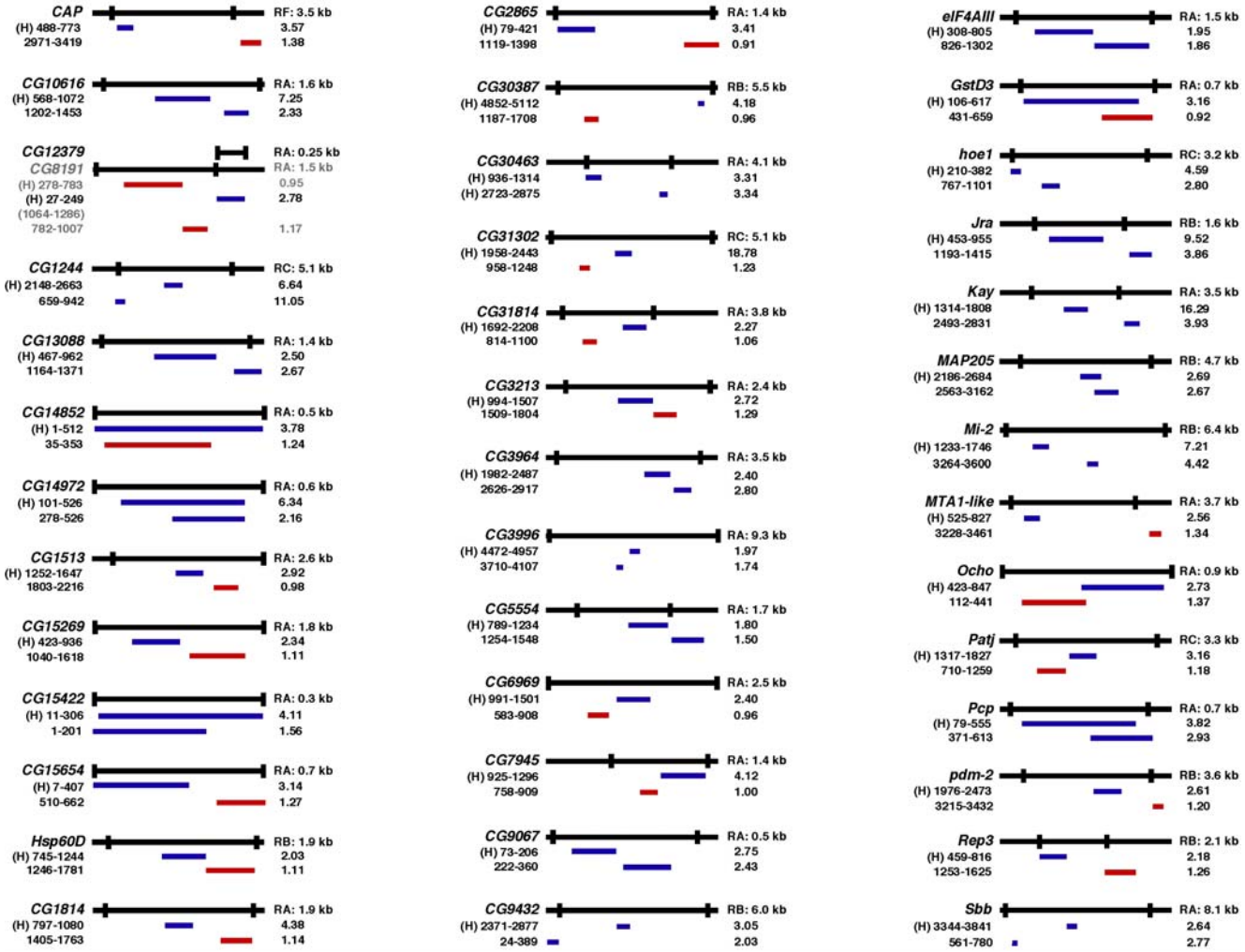
CAP
(H) 488-773
2971-3419
RF: 3.5 kb
3.57
1.38
CG10616
(H) 568-1072
1202-1453
RA: 1.6 kb
7.25
2.33
CG12379
CG8191
(H) 278-783
(H) 27-249
(1064-1286)
782-1007
RA: 0.25 kb
RA: 1.5 kb
0.95
2.78
1.17
CG1244
(H) 2148-2663
659-942
RC: 5.1 kb
6.64
11.05
CG13088
(H) 467-962
1164-1371
RA: 1.4 kb
2.50
2.67
CG14852
(H) 1-512
35-353
RA: 0.5 kb
3.78
1.24
CG14972
(H) 101-526
278-526
RA: 0.6 kb
6.34
2.16
CG1513
(H) 1252-1647
1803-2216
RA: 2.6 kb
2.92
0.98
CG15269
(H) 423-936
1040-1618
RA: 1.8 kb
2.34
1.11
CG15422
(H) 11-306
1-201
RA: 0.3 kb
4.11
1.56
CG15654
(H) 7-407
510-662
RA: 0.7 kb
3.14
1.27
Hsp60D
(H) 745-1244
1246-1781
RB: 1.9 kb
2.03
1.11
CG1814
(H) 797-1080
1405-1763
RA: 1.9 kb
4.38
1.14
CG2865
(H) 79-421
1119-1398
RA: 1.4 kb
3.41
0.91
CG30387
(H) 4852-5112
1187-1708
RB: 5.5 kb
4.18
0.96
CG30463
(H) 936-1314
(H) 2723-2875
RA: 4.1 kb
3.31
3.34
CG31302
(H) 1958-2443
958-1248
RC: 5.1 kb
18.78
1.23
CG31814
(H) 1692-2208
814-1100
RA: 3.8 kb
2.27
1.06
CG3213
(H) 994-1507
1509-1804
RA: 2.4 kb
2.72
1.29
CG3964
(H) 1982-2487
2626-2917
RA: 3.5 kb
2.40
2.80
CG3996
(H) 4472-4957
3710-4107
RA: 9.3 kb
1.97
1.74
CG5554
(H) 789-1234
1254-1548
RA: 1.7 kb
1.80
1.50
CG6969
(H) 991-1501
583-908
RA: 2.5 kb
2.40
0.96
CG7945
(H) 925-1296
758-909
RA: 1.4 kb
4.12
1.00
CG9067
(H) 73-206
222-360
RA: 0.5 kb
2.75
2.43
CG9432
(H) 2371-2877
24-389
RB: 6.0 kb
3.05
2.03
eIF4AIII
(H) 308-805
826-1302
RA: 1.5 kb
1.95
1.86
GstD3
(H) 106-617
431-659
RA: 0.7 kb
3.16
0.92
hoe1
(H) 210-382
767-1101
RC: 3.2 kb
4.59
2.80
Jra
(H) 453-955
1193-1415
RB: 1.6 kb
9.52
3.86
Kay
(H) 1314-1808
2493-2831
RA: 3.5 kb
16.29
3.93
MAP205
(H) 2186-2684
2563-3162
RB: 4.7 kb
2.69
2.67
Mi-2
(H) 1233-1746
3264-3600
RB: 6.4 kb
7.21
4.42
MTA1-like
(H) 525-827
3228-3461
RA: 3.7 kb
2.56
1.34
Ocho
(H) 423-847
112-441
RA: 0.9 kb
2.73
1.37
Patj
(H) 1317-1827
710-1259
RC: 3.3 kb
3.16
1.18
Pcp
(H) 79-555
371-613
RA: 0.7 kb
3.82
2.93
pdm-2
(H) 1976-2473
3215-3432
RB: 3.6 kb
2.61
1.20
Rep3
(H) 459-816
1253-1625
RB: 2.1 kb
2.18
1.26
Sbb
(H) 3344-3841
561-780
RA: 8.1 kb
2.64
2.77

Figure S3. Targeting different mRNA regions of forty candidate genes by alternative dsRNA probes

Target gene mRNAs (black lines) are presented schematically according to FlyBase release 2006_01. Black bars mark start and stop codons, respectively. The longest mRNA was chosen for representation (indicated at the right side) when more than one transcript is annotated in FlyBase. Blue and red lines indicate size and position of the dsRNA probes used in relation to the corresponding mRNA (numbers on the left in bp). The first line below the mRNA represents the HFA dsRNA probe (H) present in the RNAi library. Additional lines represent alternative, newly designed dsRNAs. Numbers right to the dsRNA probes are the normalized average fold activation values (Sp3si WT/K551D ratio) of the SV40-FLuc reporter gene obtained from at least two independent experiments performed in duplicate. For better survey, dsRNA probes that caused normalized fold activation values >1.5 are presented in blue, dsRNA probes that caused normalized fold activation values <1.5 are presented in red.

Note: *CG30463* was already represented by two alternative dsRNAs (HFA probes) in the library. One HFA probe targeted *CG12379* and *CG8191* (grey). An alternative HFA probe present in the library as well as a second dsRNA, both targeting specifically *CG8191*, did not activate wild-type Sp3.

All together, nineteen alternative dsRNAs (48%) enhanced again the activity of wild-type Sp3 by more than 1.5-fold confirming a role of these genes in SUMO conjugation or SUMO-mediated repression. Whether the inability of the remaining dsRNAs to activate the reporter is due to ineffective targeting their cognate genes or whether it indicates that the original dsRNAs used in the screen have off-target effects is not known at this stage.

Figure S4

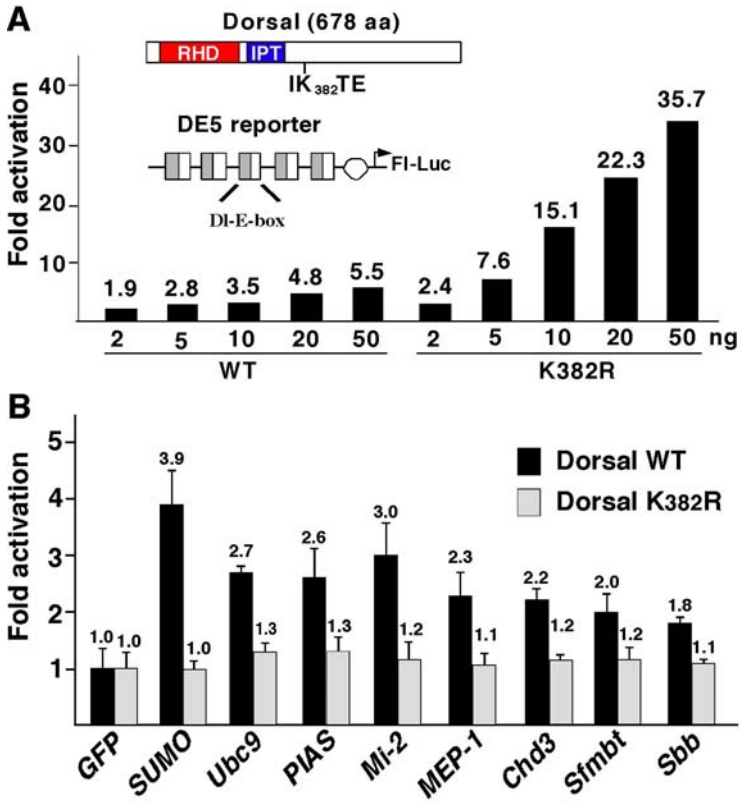

Figure S4. Knockdown of corepressors activate Dorsal-dependent transcription

(A) Kc_{167} cells were transfected with the Dorsal responsive reporter construct DE5, an expression construct for Twist, and increasing amounts of expression constructs for wild-type Dorsal or the SUMOylation-deficient Dorsal K382R mutant, respectively. Firefly reporter activity was normalized to a cotransfected actin promoter-driven *Renilla* coreporter and fold activation calculated.

(B) DsRNAs targeting the indicated genes were incubated with Kc_{167} cells and transfected with a Dorsal-responsive reporter construct, an actin promoter-driven *Renilla* coreporter, an expression construct for Twist, and 50 ng of expression constructs for wild-type Dorsal or the SUMOylation-deficient Dorsal K382R mutant, respectively. At least two independent experiments were performed in duplicate. Data are expressed as mean +/- SD.

It has to be mentioned that (Bhaskar et al., 2002) previously reported activation of Dorsal by coexpression of SUMO and Ubc9. This may be interpreted in view of the present RNAi experiments as sequestration of the SUMOylation-dependent corepressors due to SUMO overexpression.

Figure S5

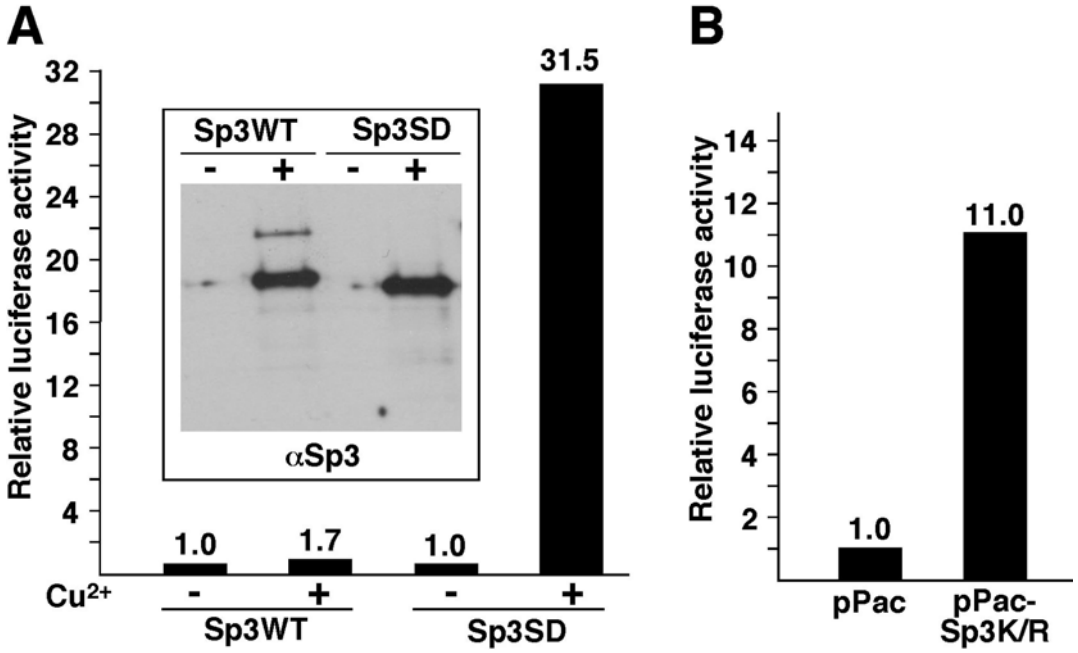

Figure S5. Inducible activation of an Sp3-responsive reporter in stable transfected insect cells

(A) Stable transfected SL2 cells containing the (GC)2-FLuc reporter, the actin promoter-driven *Renilla* coreporter and either a Cu^{2+}-inducible expression construct for epitope-tagged wild-type Sp3 (pMET-HA-FLAG-Sp3) or a SUMOylation-deficient Sp3 mutant (pMET-HA-FLAG-Sp3SD) (Braun and Suske, 1999) were induced with Cu^{2+} for 24 hours. Strong luciferase reporter gene induction occurs in cells containing the Sp3 SD mutant but not in cells containing wild-type Sp3. The insert presents immunoblots demonstrating similar expression of wild-type Sp3 and of the Sp3 SD mutant after Cu^{2+}-induction.

(B) SL2 cells containing the wild-type Sp3 expression vector and the luciferase reporters were transiently transfected with pPacSp3 K551R in the absence of Cu^{2+}. Induction of the firefly reporter gene demonstrates that the (GC)2-promoter driving firefly luciferase expression is accessible for the Sp3 protein.

Figure S6

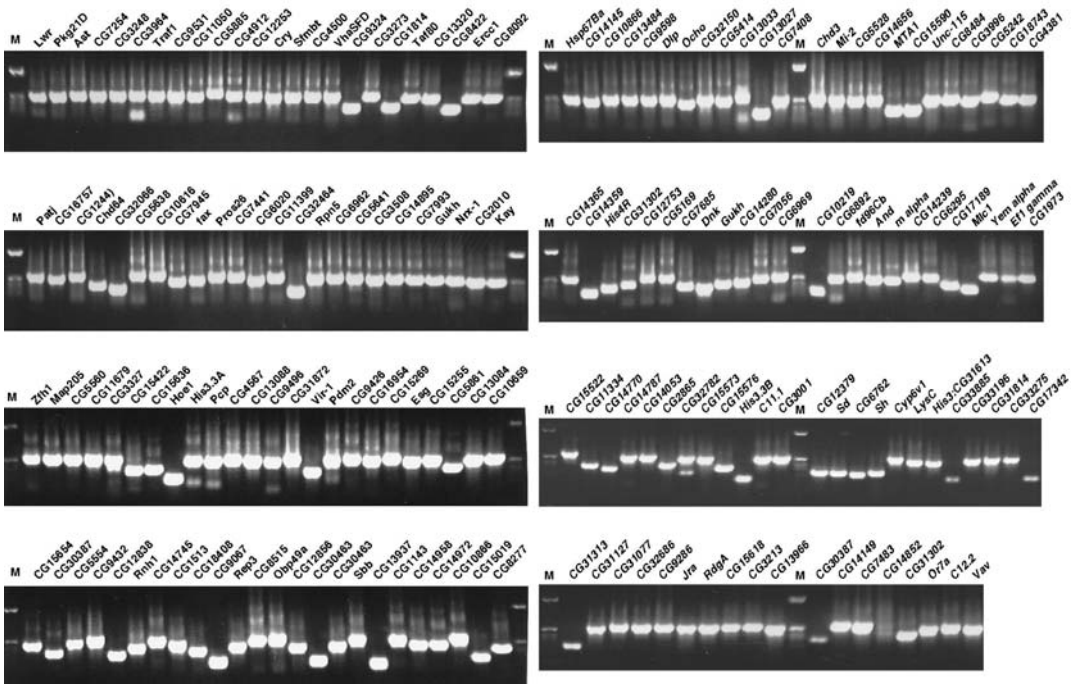

Figure S6. Quality control of dsRNAs for secondary screens
Re-amplified PCR fragments were transcribed *in vitro*, purified and dsRNA controlled by agarose electrophoresis.

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