Eight Supplemental Tables

Table S1. Accession numbers.

Symbol	GI number	Protein number	Species[a]
Ac LOC101846096	524907846	XP_005109034	Aplysia californica
Bf BRAFLDRAFT_240325	26081752	XP_002603636	Branchiostoma floridae
Bf BRAFLDRAFT_85511	26082794	XP_002608924	Branchiostoma floridae
Ce NCL-1	17554338	NP_498684.1	Caenorhabditis elegans
Ce NHL-2	17553200	NP_498026.1	Caenorhabditis elegans
Ci_zf(bbox/ring)-1	19308307	NP_001122371	Ciona intestinalis
Ci_zf(bbox/ring/phd)-1	11834420	NP_001071923	Ciona intestinalis
Ct CAPTEDRAFT_102254	44372503	ELU12781.1	Capitella teleta
Ct CAPTEDRAFT_90967	44369524	ELT96190.1	Capitella teleta
Dm Bonus	24648457	NP_524724.2	Drosophila melanogaster
Dm BRAT	17136846	NP_476945.1	Drosophila melanogaster
Dm MeiP26	21355671	NP_652022.1	Drosophila melanogaster
Dr TIF1-δ/TRIM66	52848502	XP_005166454	Danio rerio
Dr TIF1-α/TRIM24	67514537	NP_001002870	Danio rerio
Dr TIF1-γ/TRIM33	34730025	NP_001002871	Danio rerio
Dr TRIM71	32667466	XP_690252.5	Danio rerio
Gg TIF1-α/TRIM24	51315978	XP_416340.4	Gallus gallus
Gg TIF1-β/TRIM28	51323955	XP_428095.4	Gallus gallus
Gg TIF1-δ/TRIM66	51318634	XP_004941449	Gallus gallus
Gg TIF1-γ/TRIM33	51322520	XP_418009.4	Gallus gallus
Gg TRIM71	16783040	NP_001032352	Gallus gallus
Hr HELRODRAFT_130735	55569546	ESN98699.1	Helobdella robusta
Hs TIF1-α/TRIM24	47419911	NP_056989.2	Homo sapiens
Hs TIF1-β/TRIM28	5032179	NP_005753.1	Homo sapiens
Hs TIF1-δ*/TRIM66	20997709[b]	NP_055633[b]	Homo sapiens
Hs TIF1-γ TRIM33	74027249	NP_056990.3	Homo sapiens
Hs TRIM37	52487176	NP_001005207	Homo sapiens
Hs TRIM71	84993742	NP_001034200	Homo sapiens
Mm TIF1-α/TRIM24	94420998	NP_659542.3	Mus musculus
Mm TIF1-β/TRIM28	17029584	NP_035718.2	Mus musculus
Mm TIF1-δ/TRIM66	45861756	AAS78676.1	Mus musculus
Mm TIF1-γ/TRIM33	56404945	Q99PP7.2	Mus musculus
Mm TRIM71	10994830	NP_001035968	Mus musculus
Sk LOC100367035	29124261	XP_002741201	Saccoglossus kowalevskii
Sk LOC100372618	29123009	XP_002735005	Saccoglossus kowalevskii
Sp LOC580275	39035004	XP_785438.3	Strongylocentrotus purpuratus
Tr LOC101061951	41090836	XP_003967661	Takifugu rubripes
Tr LOC101064561	41091315	XP_003970052	Takifugu rubripes
Tr LOC101073124	41089906	XP_003963016	Takifugu rubripes
Tr LOC101076050	41091117	XP_003969064	Takifugu rubripes
Tr LOC101078035	41091901	XP_003972979	Takifugu rubripes
Xt TIF1-α/TRIM24	18923024	NP_001121448	Xenopus tropicalis
Xt TIF1-β/TRIM28	30161644	XP_002937648	Xenopus tropicalis
Xt TIF1-δ/TRIM66	51283299	XP_002942909	Xenopus tropicalis
Xt TIF1-γ/TRIM33	11860117	NP_001073032	Xenopus tropicalis
Xt TRIM71	42593626	F6QEU4.1	Xenopus tropicalis

a. Taxonomy and nomenclature. Vertebrates: Human Hs, Mouse Mm, Checken Gg, Frog Xt, Sebrafish Dr and PufferfishTr. Vertebrate closest relatives: Cephalochordate Bf, Hemichordate Sk, Urochordate Ci and Echinoderm Sp. Lophotrocozoans: Annelid Ct, Leech Hr and Mollusc Ac. Arthropod: Dm. Nematode: Ce. In the TRIM family naming is highly variable with the mouse names utilizing the TIF1 prefix and human names employing TRIM. We consistently apply both names while excluding unique names such as Ectodermin for TIF1-γ/TRIM33.

b. The 5' extended HsTIF1-δ*/TRIM66 was employed to generate the alignment underlying all trees. The existing prediction for HsTIF1-δ/TRIM66 does not contain a RING domain but the extended isoform we identified from genomic sequence conforms to the RING domain bearing mouse TIF1-δ/TRIM66 isoform1 (see Material and Methods and Figs. S1-4). Note the most recent release of Genbank contains a new prediction for a HsTIF1-δ/TRIM66 isoform that contains a nearly identical 5' extension (GI 530396083 and Protein XP_005253327.1).

Table S2. Location and probability of known domains in the TRIM family.

Domain Name	Start	End	E-value
Bonus			
RING	83	130	0.00121
low complexity	152	167	0.00121
BBOX	173	220	2.91E-06
BBOX	235	276	1.84E-08
BBC	283	409	2.15E-40
low complexity	448	491	2.15E-40
low complexity	583	605	2.15E-40
low complexity	612	627	2.15E-40
low complexity	650	669	2.15E-40
low complexity	777	794	2.15E-40
low complexity	810	824	2.15E-40
PHD	899	944	8.18E-10
BROMO	964	1071	7.17E-18
HsTIF1-β/TRIM28			
RING	65	120	1.58E-07
BBOX	148	195	2.97E-12
BBOX	204	245	1.11E-11
BBC	252	378	1.98E-39
low complexity	419	431	1.98E-39
low complexity	525	551	1.98E-39
PHD	627	670	2.16E-09
BROMO	697	801	1.34E-08
HsTIF1-α/TRIM24			
RING	76	130	6.00E-08
BBOX	158	205	6.27E-11
BBOX	218	259	2.22E-11
BBC	266	392	5.55E-43
low complexity	473	491	5.55E-43
low complexity	499	514	5.55E-43
low complexity	577	595	5.55E-43
low complexity	685	708	5.55E-43
low complexity	758	773	5.55E-43
PHD	828	871	3.15E-11
BROMO	901	1006	4.93E-39
low complexity	1017	1032	2.00E-17
HsTIF1-γ/TRIM33			
RING	125	184	2.61E-08
BBOX	212	259	1.24E-09
BBOX	271	312	1.54E-10
BBC	319	445	7.55E-45
low complexity	526	569	7.55E-45
low complexity	637	649	7.55E-45
low complexity	718	760	7.55E-45
low complexity	807	835	7.55E-45
low complexity	875	888	7.55E-45
PHD	889	932	4.15E-11
BROMO	959	1082	1.06E-29
Hs TIF1-δ*/TRIM66			
RING	33	86	0.9
BBOX	110	142	1.16
BBOX	170	211	0.0238
BBC	218	344	2.80E-39
PHD	1082	1125	1.35E-10
BROMO	1153	1259	3.58E-27

Table S3. Domain comparison between Bonus and TRIM proteins.

	HsTIF1-α/TRIM24	HsTIF1-β/TRIM28	HsTIF1-δ*/TRIM66	HsTIF1-γ/TRIM33
RING	Length: 54 Id: 21/54 (44%)[a] Sim: 25/54 (46%)[b] Gap: 19/54 (35%) Score: 94.0	Length: 56 Id: 17/56 (30%) Sim: 23/56 (41%) Gap: 08/56 (14%) Score: 78.5	Length: 58 Id: 18/58 (31%) Sim: 21/58 (36%) Gap: 14/58 (24%) Score: 52.5	Length: 60 Id: 21/60 (35%) Sim: 26/60 (43%) Gap: 12/60 (20%) Score: 89.0
BBOX	Length: 48 Id: 23/48 (48%) Sim: 36/48 (75%) Gap: 00/48 (00%) Score: 151.0	Length: 48 Id: 27/48 (56%) Sim: 38/48 (79%) Gap: 00/48 (00%) Score: 161.0	Length: 48 Id: 10/48 (21%) Sim: 08/48 (38%) Gap: 09/48 (19%) Score: 67.0	Length: 48 Id: 23/48 (48%) Sim: 34/48 (71%) Gap: 00/48 (00%) Score: 148.0
BBOX	Length: 42 Id: 24/42 (57%) Sim: 33/42 (79%) Gap: 00/42 (00%) Score: 155	Length: 42 Id: 23/42 (55%) Sim: 31/42 (74%) Gap: 00/42 (00%) Score: 145.0	Length: 42 Id: 20/42 (48%) Sim: 26/42 (62%) Gap: 00/42 (00%) Score: 119.0	Length: 42 Id: 23/42 (55%) Sim: 32/42 (76%) Gap: 00/42 (00%) Score: 150.5
BBC	Length: 130 Id: 29/130 (22%) Sim: 65/130 (50%) Gap: 08/130 (06%) Score: 113.0	Length: 128 Id: 31/128 (24%) Sim: 63/128 (49%) Gap: 02/128 (02%) Score: 119.0	Length: 127 Id: 25/127 (20%) Sim: 63/127 (50%) Gap: 00/127 (00%) Score: 133.0	Length: 131 Id: 30/131 (23%) Sim: 65/131 (50%) Gap: 06/131 (05%) Score: 103.0
PHD	Length: 46 Id: 26/46 (57%) Sim: 34/46 (74%) Gap: 02/46 (04%) Score: 173.5	Length: 46 Id: 19/46 (41%) Sim: 27/46 (59%) Gap: 02/46 (04%) Score: 115.5	Length: 46 Id: 28/46 (61%) Sim: 35/46 (76%) Gap: 02/46 (04%) Score: 175.5	Length: 46 Id: 26/46 (57%) Sim: 32/46 (70%) Gap: 02/46 (04%) Score: 169.5
BROMO	Length: 108 Id: 39/108 (36%) Sim: 58/108 (54%) Gap: 02/108 (02%) Score: 160.0	Length: 114 Id: 27/114 (24%) Sim: 41/114 (36%) Gap: 15/114 (13%) Score: 55.5	Length: 108 Id: 40/108 (37%) Sim: 54/108 (50%) Gap: 01/108 (01%) Score: 165.0	Length: 125 Id: 38/125 (30%) Sim: 61/125 (49%) Gap: 18/125 (14%) Score: 151.5
ALL	Length: 1251 Id: 316/1251 (25%) Sim: 488/1251 (39%) Gap: 319/1251 (26%) Score: 1000	Length: 1171 Id: 269/1171 (23%) Sim: 407/1171 (35%) Gap: 374/1171 (32%) Score: 725.0	Length: 1448 Id: 311/1448 (22%) Sim: 490/1448 (34%) Gap: 437/1448 (30%) Score: 718.0	Length: 1302 Id: 323/1302 (24%) Sim: 502/1302 (38%) Gap: 344/1302 (27%) Score: 972.5

a - red indicates the protein with the greatest percent identity to Bonus for that domain

b - blue indicates the protein with the greatest percent similarity to Bonus for that domain (identical amino acids plus conservative substitutions as defined biochemically)

Table S4. Cuticle defects in *bonus* zygotic mutants.

ventralized phenotypes	*bonus*^{487} / *bonus*^{21B}	*bonus*^{21B} / *bonus*^{EY1763}
wt (expect 100%)	82	86
herniated head	04	06
denticles extended	00	02
Filzkorper defects	10	04
partial U-shape	04	02

At least 50 cuticles were examined for each cross.

Table S5. *bonus* zygotic suppression of *sog*^{y506}.

paternal genotype	wt	*dpp*^{hr4} / +	*Medea*^{15}	*bonus*^{21B} / +	*faf*^{B6}/+
wt (expect 50%)	62	56	52	56	58
no denticles	30	12	08	16	18
denticles present/rescued	08	32	40	28	24

At least 50 cuticles were examined for each cross.

Table S6. Maternal heterozygosity for *nedd4* partially rescues *dpp*^{hr27} haploinsufficiency

maternal genotype	wt	*Medea*^{17}	*smurf*^{KG07014}/+	*nedd4*^{DG05310}/+	*nedd4*^{T119FS}/+	*hiw*^{BG02015}/*hiw*^{EP1308}
adult % expected	58[a]	01	84	80	78	61[b]

At least 200 adults were scored for each cross.

a. *dpp*^{hr27} haploinsufficiency is consistent with a previous report (Wharton et al. 1993).

b. This result is not statistically different from wild type.

Wharton K, Ray R, Gelbart W. 1993. An activity gradient of *dpp* is necessary for the specification of dorsal pattern in the *Drosophila* embryo. *Development* 117:807-822.

Table S7. Domain comparison between fly Nedd4 and two human Nedd4 proteins.

	HsNedd4	HsNedd4L
C2	Length: 108 Id: 62/108 (57%) Sim: 77/108 (71%) Gap: 09/108 (08%) Score: 294.5	Length: 106 Id: 63/106 (59%) Sim: 78/106 (74%) Gap: 04/106 (04%) Score: 312.5
WW	Length: 33 Id: 24/33 (73%) Sim: 25/33 (76%) Gap: 00/33 (00%) Score: 135.0	Length: 33 Id: 33/33 (100%) Sim: 33/33 (100%) Gap: 00/33 (00%) Score: 187.0
WW	Length: 33 Id: 14/33 (42%) Sim: 19/33 (58%) Gap: 00/33 (00%) Score: 82.0	Length: 33 Id: 33/33 (100%) Sim: 33/33 (100%) Gap: 00/33 (00%) Score: 189.0
WW	Length: 33 Id: 26/33 (79%) Sim: 29/33 (88%) Gap: 00/33 (00%) Score: 162.0	Length: 33 Id: 26/33 (79%) Sim: 29/33 (88%) Gap: 00/33 (00%) Score: 163.0
HECT	Length: 337 Id: 244/337 (72%) Sim: 282/337 (84%) Gap: 00/337 (00%) Score: 1351.0	Length: 337 Id: 238/337 (71%) Sim: 283/337 (84%) Gap: 00/337 (00%) Score: 1328.0
ALL	Length: 1043 Id: 488/1043 (47%) Sim: 606/1043 (58%) Gap: 179/1043 (17%) Score: 2299.5	Length: 1079 Id: 492/1079 (46%) Sim: 633/1079 (59%) Gap: 176/1079 (16%) Score: 2348.0

a - red as in Table S3

b - blue as in Table S3

Table S8. Domain comparison between fly Dad and two human Smad proteins.

	HsSmad6	HsSmad7
MH1	Length: 129 Id: 35/129 (27%) Sim: 53/129 (41%) Gap: 33/129 (26%) Score: 105.5	Length: 146 Id: 36/146 (25%) Sim: 53/146 (36%) Gap: 55/146 (38%) Score: 89.5
MH2	Length: 174 Id: 71/174 (41%) Sim: 86/174 (49%) Gap: 15/174 (09%) Score: 302.0	Length: 173 Id: 66/173 (38%) Sim: 94/173 (54%) Gap: 12/173 (07%) Score: 283.0
ALL	Length: 622 Id: 171/622 (28%) Sim: 227/622 (37%) Gap: 180/622 (29%) Score: 449.0	Length: 650 Id: 138/650 (21%) Sim: 193/650 (30%) Gap: 306/650 (47%) Score: 398.0

a - red as in Table S3 and S7

b - blue as in Table S3 and S7

Fourteen Supplemental Figures

```
MmTIF1-δ_isoform1     MSPGLPVSIPSQPHCSTDERVEALAPTCSMCGRDLQAEGSRLLPCQHLLCKDCYQ  55
MmTIF1-δ_isoform3     ---------------------------------------------------------
MmTIF1-δ_isoform2     ---------------------------------------------------------
HsTIF1-δ current      ---------------------------------------------------------

MmTIF1-δ_isoform1     GFMQELGHATRAYPGKLISCPGCQRVYLTRDVTEHIFLQCFSPVKPTMARNCSECKE 112
MmTIF1-δ_isoform2     ----------------------------------------------MARNCSECKE  10
MmTIF1-δ_isoform3     ----------------------------------------------MARNCSECKE  10
HsTIF1-δ current       ----------------------------------------------MARNCSECKE  10
```

Fig. S1. Alignment of human and mouse TIF1-δ/TRIM66 5' ends. Three experimentally identified isoforms of mouse TIF1-δ/TRIM66 are shown: isoform1 NP_001164383, isoform2 NP_862901 and isoform3 NP_001164384 (Khetchoumian et al., 2004). These are aligned with the current prediction for human TIF1-δ/TRIM66 (from the genomic scaffold of chromosome 11 corresponding to 11p15.4). Note that only mouse isoform1 contains the RING domain (Red) found in at least one isoform of all TIF1/TRIM family members and Bonus. Sequences identical in all TIF1-δ/TRIM66 isoforms are shown in green.

Khetchoumian K, Teletin M, Mark M, Lerouge T, Cerviño M, Oulad-Abdelghani M, Chambon P, Losson R. 2004. TIF1δ a novel HP1-interacting member of the TIF1 family expressed by elongating spermatids. *J Biol Chem*. 279:48329-48341.

A

```
     Score      Expect            Method             Identities   Positives   Gaps
68.2 bits(165) 2e-08  Compositional matrix adjust   33/65(51%)   44/65(67%)  2/65(3%)
Features: 19994bp at 5' side of TIF1-δ/TRIM66

Query  4         GLPVSIPSQPHCSTDERVEALAPTCSMCGRDLQAEGSRLLPCQHLLCKDCYQGFMQELGH  63
                 GLP++   Q HC    ++EA+  TCS+C +DL   GS LL CQHLL KDC+QG +QELG
Sbjct  8633537   GLPLA--GQKHCPKSGQMEAMVMTCSLCHQDLPGMGSHLLSCQHLLRKDCFQGLIQELGQ  8633364

Query  64        ATRAY  68
                   +A+
Sbjct  8633363   IAKAH  8633349
```

B

```
     Score     Expect            Method           Identities   Positives   Gaps
57.4 bits(137) 4e-05  Compositional matrix adjust   25/35(71%)   28/35(80%) 0/35(0%)
Features:  TIF1-δ/TRIM66

Query  71        KLISCPGCQRVYLTRDVTEHIFLQCFSPVKPTMAR  105
                 +LISCPGC+RVYLTRDVTEH FL C    +P MAR
Sbjct  8613451   ELISCPGCERVYLTRDVTEHFFLHCVPTEQPKMAR  8613347
```

Fig. S2. Identification of the RING domain in human TIF1-δ/TRIM66. Results of a TblastN query employing the N-terminal region of mouse TIF1-δ/TRIM66 isoform1 against the human RefSeq Genomic database. A) Region of significant similarity is identified roughly 20kb upstream of the current translation start codon. B) Region of significant similarity is identified immediately upstream and in-frame with the current translation start codon (underlined M).

A

```
MmTIF1-δ isoform1     -----MSPGLPVSIPSQPHCSTDERVEALAPTCSMCGRDLQAEGSRLLPCQHLLCKDCYQ  55
MmTIF1-δ isoform3     ------------------------------------------------------------
MmTIF1-δ isoform2     ------------------------------------------------------------
HsTIF1-δ current       ------------------------------------------------------------
HsTIF1-δ_new          MAVDMGMSFMGLPLAGQKHCPKSGQMEAMVMTCSLCHQDLPGMGSHLLSCQHLLSKDCFQ  60

MmTIF1-δ isoform1     GFMQELGHATRAYP---GKLISCPGCQRVYLTRDVTEHIFLQCFSPVKPTMARNCSECKE 112
MmTIF1-δ isoform2     ----------------------------------------------------MARNCSECKE  10
MmTIF1-δ isoform3     ----------------------------------------------------MARNCSECKE  10
HsTIF1-δ current       ----------------------------------------------------MARNCSECKE  10
HsTIF1-δ_new          GLIQELGQIAKAHETVADELISCPGCERVYLTRDVTEHFFLHCVPTEQPKMARNCSECKE 120
```

B

```
    Score      Expect          Method               Identities    Positives   Gaps
160 bits(404)  4e-37   Compositional matrix adjust  76/78(97%)   76/78(97%)   0/78(0%)
Features: 20167bp at 5' side of TIF1-δ/TRIM66

Query  1        MAVDMGMSFMGLPLAGQKHCPKSGQMEAMVMTCSLCHQDLPGMGSHLLSCQHLLSKDCFQ  60
                MAVDMGMSFMGLPLAGQKHCPKSGQMEAMVMTCSLCHQDLPGMG HLLSCQHLL KDCFQ
Sbjct  4346862  MAVDMGMSFMGLPLAGQKHCPKSGQMEAMVMTCSLCHQDLPGMGCHLLSCQHLLCKDCFQ  4346683

Query  61       GLIQELGQIAKAHETVAD  78
                GLIQELGQIAKAHETVAD
Sbjct  4346682  GLIQELGQIAKAHETVAD  4346629


     Score     Expect          Method               Identities    Positives   Gaps
79.3 bits(194) 1e-12  Compositional matrix adjust   35/35(100%)   35/35(100%) 0/35(0%)
Features: TIF1-δ/TRIM66

Query  79       ELISCPGCERVYLTRDVTEHFFLHCVPTEQPKMAR  113
                ELISCPGCERVYLTRDVTEHFFLHCVPTEQPKMAR
Sbjct  4326558  ELISCPGCERVYLTRDVTEHFFLHCVPTEQPKMAR  4326454
```

Fig. S3. Alignment of the new and current human 5' ends with mouse TIF1-δ/TRIM66. A) The new open reading frame created by connecting the distant upstream region, the immediately upstream region and the current sequence of human TIF1-δ/TRIM66 is shown. The presence of a well-defined RING domain in the new sequence that is 60% identical to the RING domain in mouse TIF1-δ/TRIM66 isoform1 is indicated (red). Sequences identical in all TIF1-δ/TRIM66 isoforms are shown in green. B) The 5' extension of the human TIF1-δ/TRIM66 open reading frame is 98% identical to sequences encoded by two regions of the *Pan troglodytes* chromosome 11 genomic scaffold (current translation start codon for human TIF1-δ/TRIM66 is the underlined M).

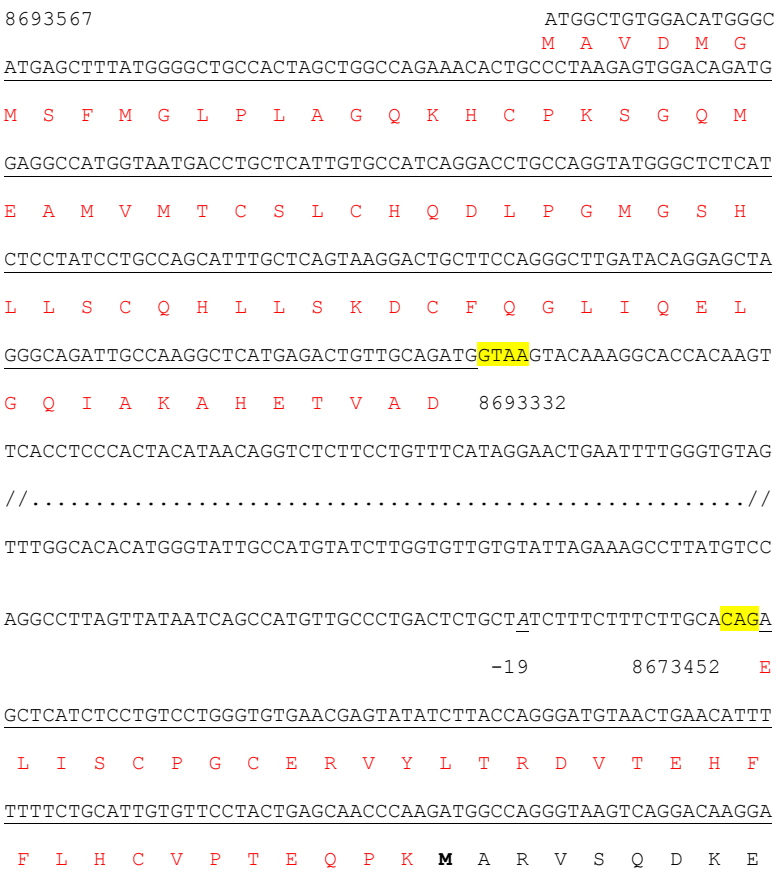
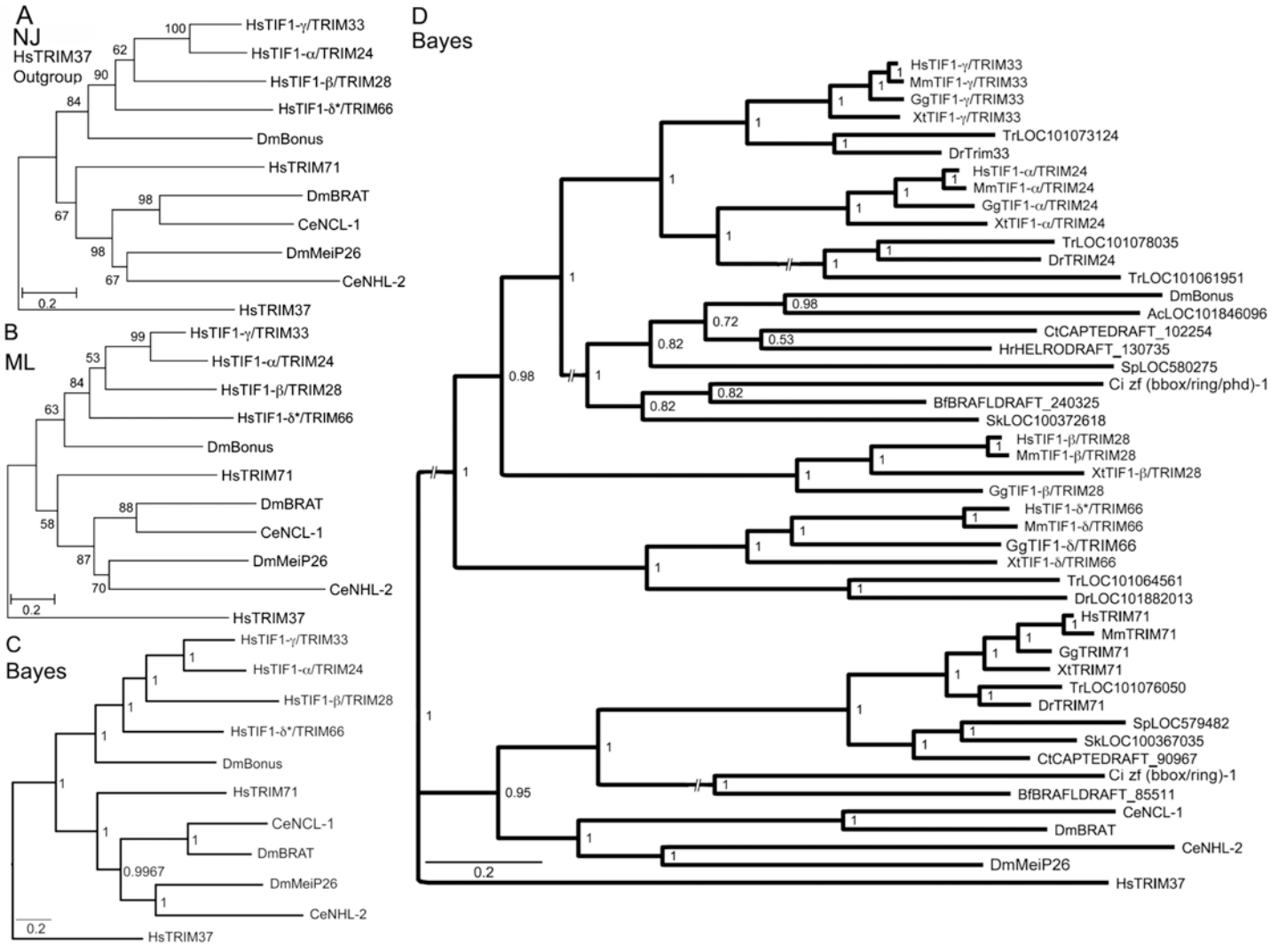


Fig. S4. Locations of new initiator methionine and splice sites for human TIF1-δ/TRIM66. Genomic sequence derived from GRCh37.p10 the primary assembly of human chromosome 11 is shown (NC_000011.9). Similarity to mouse TIF1-δ/TRIM66 isoform1 identifies the proposed new methionine at 8693567. An EST (TESTI4024751) identifies a splice donor site at 8693331 and a splice acceptor site at 8673452. The 5' and 3' ends of the intron are highlighted in yellow and the intervening 19880 nucleotides are shown as dots between pairs of forward slashes. A potential branch site at -19 from the splice acceptor site is underlined and italicized. The upstream extension of the current human TIF1-δ/TRIM66 open reading frame is in red with the corresponding nucleotide sequence underlined. The initiator methionine of the current human TIF1-δ/TRIM66 open reading frame is in bold.


A
NJ
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HsTIF1-γ/TRIM33
HsTIF1-α/TRIM24
HsTIF1-β/TRIM28
HsTIF1-δ*/TRIM66
DmBonus
HsTRIM71
DmBRAT
CeNCL-1
DmMeiP26
CeNHL-2
HsTRIM37
0.2
B
ML
HsTIF1-γ/TRIM33
HsTIF1-α/TRIM24
HsTIF1-β/TRIM28
HsTIF1-δ*/TRIM66
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HsTRIM71
DmBRAT
CeNCL-1
DmMeiP26
CeNHL-2
HsTRIM37
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Bayes
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HsTRIM71
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DmBRAT
DmMeiP26
CeNHL-2
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0.2
D
Bayes
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XtTIF1-γ/TRIM33
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Ci zf (bbox/ring/phd)-1
BfBRAFLDRAFT_240325
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BfBRAFLDRAFT_85511
CeNCL-1
DmBRAT
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DmMeiP26
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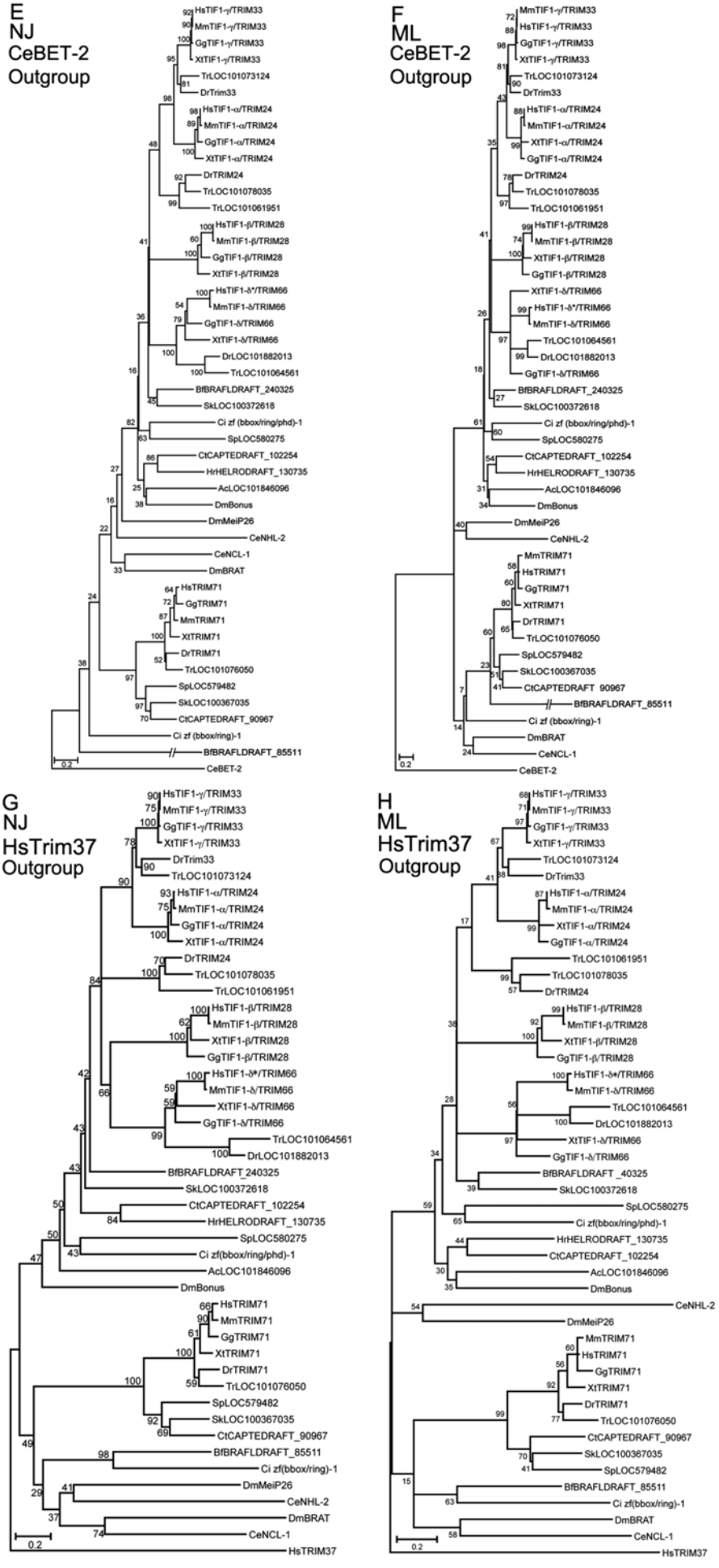
0.82
0.82
0.95
0.2


E
NJ
CeBET-2
Outgroup
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XtTIF1-γ/TRIM33
TrLOC101073124
DrTrim33
HsTIF1-α/TRIM24
MmTIF1-α/TRIM24
GgTIF1-α/TRIM24
XtTIF1-α/TRIM24
DrTRIM24
TrLOC101078035
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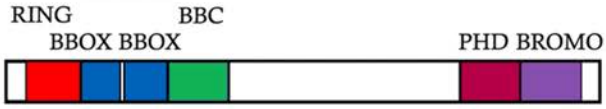
Fig. S5. Evolutionary relationships within the Bonus-Tif1-γ/TRIM33 subfamily are topologically consistent in multiple trees. A) Neighbor Joining (NJ), B) Maximum Likelihood (ML) and C) Bayesian trees of eleven human (Hs), fly (Dm) and nematode (Ce) TRIM family sequences from Table S1 related to Bonus. HsTRIM71 was chosen to anchor the non-Bonus fly and nematode sequences into a single subfamily. HsTRIM37 was chosen as the outgroup based Sardiello et al. (2008) indicating that HsTRIM37 did not cluster with any other human TRIM proteins. The alignment contained 517 informative positions. A scale bar showing amino acid substitutions per site is present. Bootstrap values (NJ tree and ML tree) above 40% or posterior probabilities above 0.5 (Bayesian tree) are shown. Bootstrap values above 70% and posterior probabilities above 0.95 are considered statistically significant. Each tree contains the same two distinct subfamilies. Within one subfamily Bonus is consistently the most distant sequence, either TIF1-β/TRIM28 or TIF1-δ/TRIM61 are the oldest vertebrate and TIF1-γ/TRIM33 is the youngest vertebrate sequence. D) Expanded Bayesian tree containing all 47 sequences in Table S1 derived from an alignment with 154 informative positions. The same two statistically supported subfamilies are visible. The topology of the Bonus-TIF1-γ/TRIM33 subfamily shows a slightly different topology than the other trees with Bonus midway between the four vertebrate sequences rather than as an outlier. E, F) Expanded NJ and ML trees employing CeBET-2 as an outgroup derived from an alignment with 173 informative positions and displaying all bootstrap values. G,H) Expanded NJ and ML trees employing HsTRIM37 as an outgroup derived from an alignment with 154 informative positions and displaying all bootstrap values. Each of the four large trees contains the same two distinct subfamilies with occasional non-significant outliers (e.g. CeNHL-2/DmMeiP26 in panel H). In the TIF1-γ/TRIM33 subfamily Bonus consistently maps as the most divergent sequence. Based on topology and branch lengths, either TIF1-β/TRIM28 or TIF1-δ/TRIM61 is the oldest and TIF1-γ/TRIM33 the youngest vertebrate sequence in this subfamily.

Sardiello M, Cairo S, Fontanella B, Ballabio A, Meroni G. 2008. Genomic analysis of the TRIM family reveals two groups with distinct evolutionary properties. *BMC Evol Biol*. 8:225

A



B

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HsTIF1-δ*/TRIM66 1   ------------------------------------------------MAVDMGMSFMG
HsTIF1-α/TRIM24  1   ------------------------------------------MEVAVEKAVAAAAAASAA
HsTIF1-γ/TRIM33  1   MAENKGGGEAESGGGGSGSAPVTAGAAGPAAQEAEPPLTAVLVEEEEEEGGRAGAEGGAA

DmBonus         37   TENTASGAPTTSSLSSSSLSLSNPCDSAEKRSESNSS------------------ASAKF
HsTIF1-β/TRIM28 19   SPGPGEGSAGGEKRSTAPSAAASASASAAASSPAGGG------------------PEALE
HsTIF1-δ*/TRIM66 12  LPLAGQKHCPKSGQMEA-----------------------------------------------
HsTIF1-α/TRIM24  19  AS--GGPSAAPSGENEAESRQGPDSER---GGEAAR----------------------LN
HsTIF1-γ/TRIM33  61  GEDDGGVAAASSGSAQAASSPAASVGTGVAGGAVSTPAPAPASAPAPGPSAGPPPGPPAS

DmBonus         79   TLFKGVYGAQLGGND--RPKLLECLHVAGAQGVSTKFSELDRSLP-------------P
HsTIF1-β/TRIM28 61   LLEHGGVGRERGRPER--EPRLLPCLHSAGSAGLGPAAPAAANSS---------------
HsTIF1-δ*/TRIM66 29  MVMTGGSLGHQDLPGMG---SHLLSPQHLLSKDGFQGLIQEIGQIAK--------------
HsTIF1-α/TRIM24  52  LLDTGAVGHQNIQSR---APKLLPCLHSFGQRGLPAPQRYEMLPAPMLGSAETPPPVPAP
HsTIF1-γ/TRIM33  121 LLDTGAVGQQSHQSRREAEPKLLPCLHSFGLRGLPEPERQESVPIP--------------

DmBonus         124  LIHCPVCDNASQQEFIVDNQFLIEQGTAGDSGDGVGLLGLHGEGQKSSAAASIQGSSCSD
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HsTIF1-δ*/TRIM66 72  -------AHETVADELISCPGCERVYLTRDVTEHFFLHCVPTEQPKMARN----CSECK-
HsTIF1-α/TRIM24  109 GSPVSGSSPFATTVGVIRCPVCSQEGAERHIIDNFFVKDTTEVPSSTVEKSNQVCTSCED
HsTIF1-γ/TRIM33  167 ----GESNGDIQLVGVIRCPVCRQEGRQIDLVDNYFVKDTSEAPSSSDEKSEQVCTSCED

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HsTIF1-α/TRIM24  169 NAEANGPCVEGVEWLGKTCERAHQRVKFTKDHTVRQKEEVSP--EAVGVTSQRPVGCPEH
HsTIF1-γ/TRIM33  223 NASAVGPCVECGEWLGKTCIEAHQRVKFTKDHLIRRKEDVS---ESVGASGQRPVGCPVH

DmBonus         244  PQEKLSLFCETCDKLTCRDCQLSDHDDHKYKFAHEIATESRQALSDLVEELNYKRFLSS
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HsTIF1-γ/TRIM33  280 KQEQLKLFCETCDRLTCRDCQLLEHKEHRYQFVEEAFQNQHGALENLLAKLLEKLNYHHF

DmBonus         304  ATKVLDDRQQQLEHLKKKRDEIKRLTAMAMKVINTRNDKGKQLMREVEDCDSRLKVLEKK
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HsTIF1-δ*/TRIM66 239 IPKQIEDRIFEVKHQHEVEMQIKMAKSVLMNEMNKQRNGLEELEGITNSLKRKLEQQL
HsTIF1-α/TRIM24  287 IQNQIQNRIIEVNQNGKQVEGQIKVAIFTLMVEMNKRGKALHQLESLAKDMRMKLQQQQ
HsTIF1-γ/TRIM33  340 AITQLQNRIKEVNQTGKVEGQIKVAIFTLMNEMNKRGKSLQQLENVTKDLQMKLQQQQ

DmBonus         364  EETQLLSDMTHCADGMLNALEKGSDFALGSSKRGSLVRHLQKLKCQRADIPNPEIPVRIQ
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HsTIF1-δ*/TRIM66 299 GEIMVRNGQFEHVQNGIEWAVCSKGSVPFLSKELIVFQDQLLETSCNTD-PGSPWSIR
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HsTIF1-γ/TRIM33  400 EDITGLSSQVKHVHNGTWAVASGSSTALLPSKGLITFQLHILLAARCEPV-PAANGAIR

DmBonus         424  VQLNQVSDLQKVLSQLGIIIVDGKPYPPTPSP----------------------------
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HsTIF1-δ*/TRIM66 358 ETWEPN-FWTKQLASLGCLLTEGGQMSRADAPAYGGLQGSSPFYQSHQSPVAQQEALS//
HsTIF1-α/TRIM24  406 EHCDPS-FWAQNIINLGSLVIEDKESQ-------------------------------------
HsTIF1-γ/TRIM33  459 EHCDPT-FWAKNVVNLGNLVIESK---------------------------------------

DmBonus         456  ----------------------NGTPQFQHPPRQPPSPNMAPPLRPGLPPGMPAGLSPNG
HsTIF1-β/TRIM28 414  ------------------------------GTNSTGPAP--------------------
HsTIF1-δ*/TRIM66 477 LPREKELACSPHPPKLLQPWLETQPPVEQESTSQRLGQQLTSQPVCIVPPQDVQQGAHAQ
HsTIF1-α/TRIM24  432 ------------------------PQMPKQNPVVEQNSQPPSGLSSNQLSKFPTQISLAQ
HsTIF1-γ/TRIM33  482 ------------------------PAPGYTPNVVVGQVPPG---TNHISKTPGQINLAQ

DmBonus         494  PPVNFGPQNGPPLYSNAAQQQFNNLSMSRSFPGDGSGKVRFGGMPPVGMQRHGQPHVSSS
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HsTIF1-δ*/TRIM66 537 PTLQTPSIQVQFGHHQKLKLSHFQQQPQQQLPPPPPPLPHPPPPLPPPPQQPHPPLPPSQ
HsTIF1-α/TRIM24  468 LRLQHMQQQVMAQRQQ------VQRRPAPVG-LPNPRMQGPIQQPSISHQQPPRLINPQ
HsTIF1-γ/TRIM33  514 LRLQHMQQQVYAQKHQQLQQMRMQQPPAPVPTTTTTTQQHPRQAAPQMLQQQPPRLISVQ

DmBonus         554  THPQN-----------------------------------MDISLRGLLNNQAAQSPNAH
HsTIF1-β/TRIM28 443  ------------------------------------------------------------
HsTIF1-δ*/TRIM66 597 HLASSQHESPPGPACSQNMDIMHHKFELEEMQKDLELLLQAQQPSLQLSQTKSPQHLQQT
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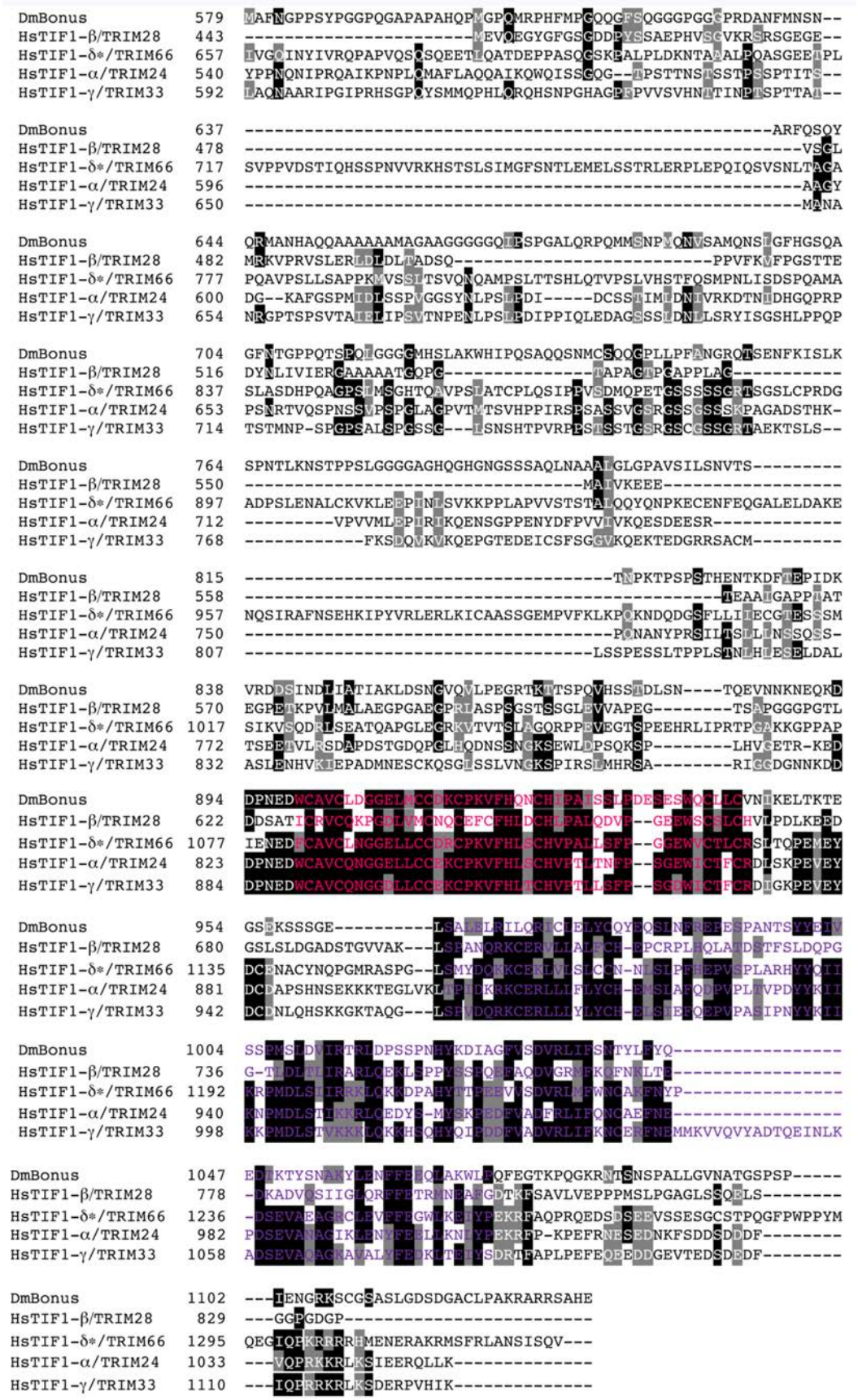
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Fig. S6. Alignment of Bonus-TIF1-γ/TRIM33 subfamily proteins. A) Color-coded schematic of domains and their location in Bonus-TIF1-γ/TRIM33 subfamily proteins (RING - orange, BBOX - blue, BBC - green, PHD - crimson and BROMO - purple). B) Alignment of Bonus-TIF1-γ/TRIM33 subfamily proteins. The background of an amino acid is shaded if the residue is identical (black) or similar (grey) at that position in at least three sequences. The color of an amino acid indicates the location of a functional domain following the coordinates shown in Table S2 and the color-scheme above.

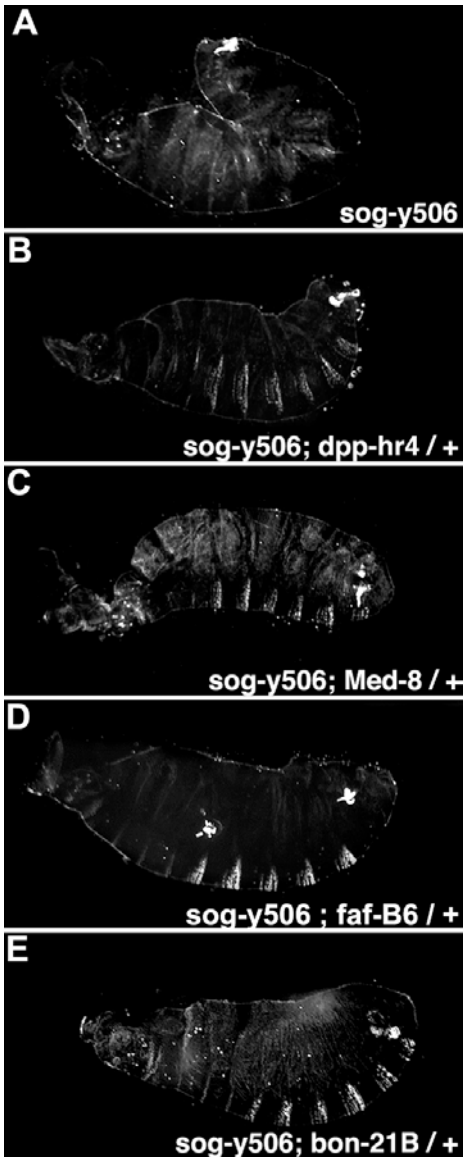

Fig. S7. *bonus* zygotic heterozygosity partially suppresses *sog* hemizygous mutant phenotypes. Cuticles in lateral view with anterior to the left and dorsal up. The maternally contributed allele is listed first. A) sog^{y506} hemizygous dorsalized cuticle containing no denticles, U-shaped body, herniated head and Filzkorper defects. B) sog^{y506} hemizygous and dpp^{hr4} heterozygous cuticle with head and Filzkorper defects but partially restored denticles and body shape. C) sog^{y506} hemizygous and $Medea^{8}$ heterozygous cuticle shows partial rescue. D) sog^{y506} hemizygous and $fat\ facets^{B6}$ heterozygous cuticle shows partial rescue. E) sog^{y506} hemizygous and $bonus^{21B}$ heterozygous cuticle shows partial rescue.

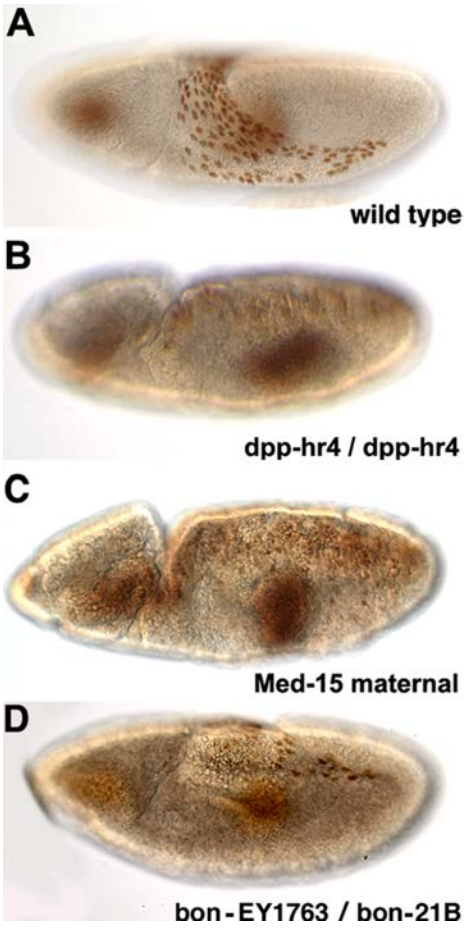

Fig. S8. Loss of Hindsight amnioserosa but not foregut or hindgut expression in *bonus* zygotic mutants. Stage 10 embryos in lateral view revealing Dpp-dependent Hindsight expression in amnioserosa cells. Dpp-independent Hindsight expression in the foregut and hindgut is visible below the plane of focus. The latter staining acts as an internal control and is observed in all genotypes. The maternally contributed allele is listed first. A) Wild type embryo. B) dpp^{hr4} homozygous ventralized embryo with a few scattered amnioserosa cells. C) Ventralized maternal $Medea^{15}$ embryo is similar to the dpp^{hr4} embryo with a few scattered amnioserosa cells. D) $bonus^{EY1763}/bonus^{21B}$ transheterozygous embryo with a reduced number of amnioserosa cells

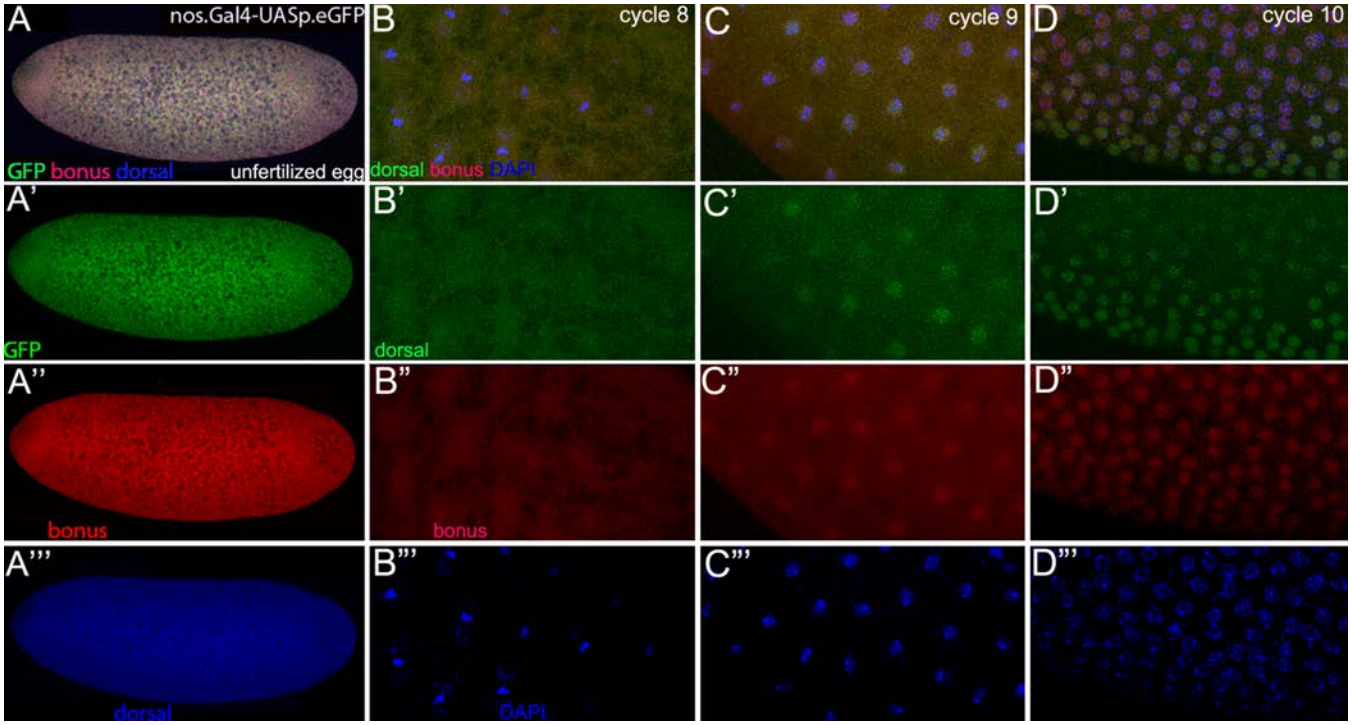

Fig. S9. Bonus and Dorsal are maternally loaded and cytoplasmic in eggs then translocate synchronously into nuclei at cycle 9. A) Unfertilized egg in lateral view from a female expressing UAS.eGFP under the control of the maternal driver nos.Gal4. Low magnification three-color and single channel images of eGFP (green), Bonus (red) and Dorsal (blue). All proteins are present and cytoplasmic. B-D) Wild type embryos in lateral view with ventral at the bottom. High magnification three color and single channel images of Dorsal (green), Bonus (red) and DAPI (blue). B) Cycle 8 embryo similar to Fig. 4A with uniformly cytoplasmic Dorsal and Bonus and uniformly nuclear DAPI. C) Cycle 9 embryo similar to Fig. 4B with cytoplasmic Dorsal toward the top (dorsal region) and a mix of nuclear and cytoplasmic Dorsal toward the bottom (ventral region) as well as a uniform mix of nuclear and cytoplasmic Bonus and uniformly nuclear DAPI. D) Cycle 10 embryo similar to Fig. 4C with cytoplasmic Dorsal toward the top (dorsal region) and nuclear Dorsal toward the bottom (ventral region) as well as uniformly nuclear Bonus and DAPI.

bonus exon1

A. nt16418861-16419766 Chromosome 3 sequence NT_033777.2

⮟ *bonus*^{21B} deleted from +12 to past exon1 splice donor

AAA**ATG**GCTGAACATTTTTTGTACAGAAGAAGCGTCCAACGAGAGCAAACGCCGTGAGACCGTG
TGTTTTCGCAGCGAATTACGCCAATAAAACCGCACTAAGAGCGCGCTCGCAGCGCGAATAAAGA
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GAGCAGCTGGATGCCGTGCCCACGGATGCCCTGCCCCAGATGAGCACACCAAACACGGCCAGCG
GTGCGCCCACAACCTCGTCATTGAGCTCCTCTTCGCTGAGTCTGAGCAACCCCTGCGACAGTGC
CGAGAAAAG

B. translated

⮟ *bonus*^{21B} deleted from +12 to past exon 1 splice donor

K **M** A E H F L Y R R S V Q R E Q T P * D R V F S Q R I T P I K P
H * E R A R S A N K E * Q P R M D E M R H E S L E K S T A R P K
N S S E I R A N V V Y K * N E S C V A L C E R E R E N P I F L Q
A R S R R * N * N A L A T Q L K R S Q L R T K L * E S A K N R L
* T S I Q A Q I V V F A C S R V V P V Y W C A R P V C V C A C A
C V * A L E W I N Y P T N S K T I I P Q H N R N S H A V S R R Q
P L G K H S V G R S V N Q K S V A I I S T P T F * F Q R S P A T
A Y A V K S Q D F A N M D M D L E Q L K N D F L P L I A G I K Q
E Q L D A V P T D A L P Q M S T P N T A S G A P T T S S L S S S
S L S L S N P C D S A E K

Fig. S10. Cryptic methionine in *bonus*^{21B} may yield a nearly full-length protein. A) Complete DNA sequence of *bonus* exon1 indicating the start of the deletion in *bonus*^{21B} and the presence of a start codon (bold) in the remaining transcribed sequence. Sequences encoding the Bonus open reading frame are in red. B) Translation of *bonus* exon1 revealing the cryptic Methionine (bold) and the 66 amino acids of the Bonus protein (red) that would be missing if the cryptic Methionine were able to splice into exon2 in frame. Previously Table S2 showed that the Ring domain of Bonus begins at amino acid 83 and thus none of the defined domains are located in the deleted region of exon1.

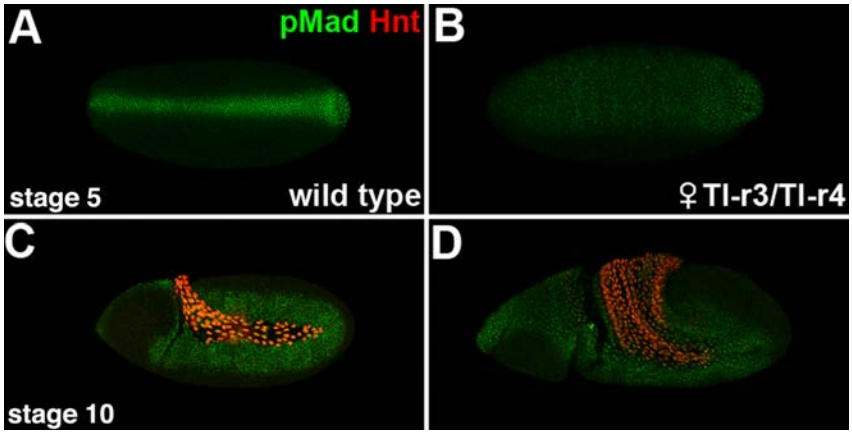

Fig. S11. Reduction in maternal Toll signaling leads to expanded pMad and Hindsight expression. A) Stage 5 wild type embryo in dorsal view displaying a sharp dorsal nuclear stripe of pMad expression. B) Stage 5 embryo in dorsal view from a $Toll^{r3}/Toll^{r4}$ female mated to a wild type male. The reduction in maternal Toll signaling associated with these loss of function alleles is reflected in a broad, ventrally expanded pMad stripe. C) Stage 10 wild type embryo in lateral view displaying Hindsight in the amnioserosa and a wide, lateral ectoderm stripe of pMad. D) Stage 10 embryo in lateral view from a $Toll^{r3}/Toll^{r4}$ female mated to a wild type male. The reduction in maternal Toll signaling is reflected in the ventral expansion of both Hindsight and pMad.

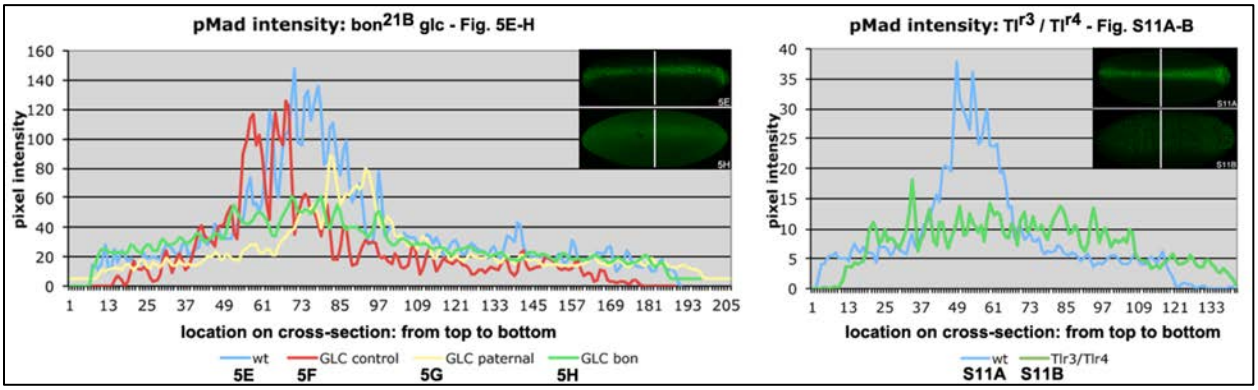

Fig. S12. *Bonus*^{21B} germline clone and maternal *Toll*^{r3}/*Toll*^{r4} similarly effect pMad expression. Pixel intensities along a cross section from top to bottom on each image are shown from left to right in each graph employing arbitrary units. Left: Intensities for the green (pMad) channel from each of the four genotypes shown in Fig. 5E-H are graphed. The wild type and *bonus*^{21B} germline clone embryos with the location of their cross section lines are shown as insets. The legend indicates the color associated with each genotype. Right: Intensities for the green (pMad) channel from the two genotypes shown in Fig. S11A,B are graphed. The wild type and maternal *Toll*^{r3}/*Toll*^{r4} embryos with the location of their cross section lines are shown as insets. Note that in both graphs the blue peak (wild type) is considerably narrower and sharper than the green peak (*bonus*^{21B} germline clones or maternal *Toll*^{r3}/*Toll*^{r4}).

NP_724052.1 Dorsal, isoform A [Drosophila melanogaster]

MFPNQNNGAAPGQGPAVDGQQSLNYNGLPAQQQQQLAQSTKNVRKKPYVKITEQPAGKALRFRY
ECEGRSAGSIPGVNSTPENKTYPTIEIVGYKGRAVVVVSCVTKDTPYRPHPHNLVGKEGCKKGV
CTLEINSETMRAVFSNLGIQCVKKKDIEAALKAREEIRVDPFKTGFSHRFQPSSIDLNSVRLCF
QVFMESEQKGRFTSPLPPVVSEPIFDKKAMSDLVICRLCSCSATVFGNTQIILLCEKVAKEDIS
VRFFEEKNGQSVWEAFGDFQHTDVHKQTAITFKTPRYHTLDITEPAKVFIQLRRPSDGVTSEAL
PFEYVPMDSDPAHLRRKRQKTGGDPMHLLLQQQQKQQLQNDHQDGRQTNMNCWNTQNIPPIKTE
PRDTSPQPFGLSYRAPPELTPSPQPLSPSSNYNHNSTPSPYNMASAVTPTNGQQQLMSPNHPQQ
QQQQQQYGATDLGSNYNPFAQQVLAQQQQHQQQQQQHQHQHQQQHQQQQQQQQQQQQQSLQFHA
NPFGNPGGNSWESKFSAAAVAAAAATATGAAPANGNSNNLSNLNNPFTMHNLLTSGGGPGNANN
LQWNLTTNHLHNQHTLHQQQQLQQQQQQQYDNTAPTNNNANLNNNNNNNNTAGNQADNNGPTLS
NLLSFDSGQLVHINSEDQQILRLNSEDLQISNLSIST

Fig. S13. Dorsal contains a candidate Bonus-interacting AF-2 AD domain. Bonus physically interacts with the AF-2 AD domain in βFTz-F1 (Beckstead et al., 2001). This domain has the consensus hhXEhh (h = hydrophobic amino acid, X = any amino acid, E = glutamic acid). Dorsal (red residues 211-216) contains a near-perfect match to this consensus with a weakly hydrophobic proline in the penultimate position.

Beckstead R, Ortiz J, Sanchez C, Prokopenko S, Chambon P, Losson R, Bellen HJ. 2001. Bonus, a *Drosophila* homolog of TIF1 proteins, interacts with nuclear receptors and can inhibit FTZ-F1-dependent transcription. *Mol Cell* 7:753-765.

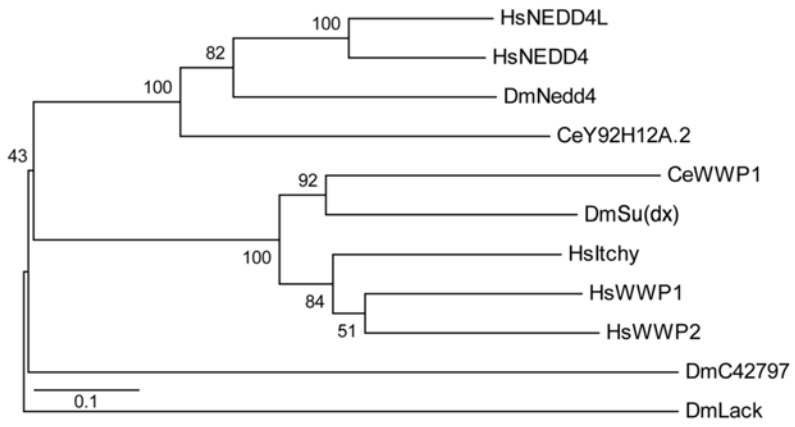

Fig. S14. Maximum likelihood tree of fly Nedd4 and closely related proteins. An unrooted tree generated from an alignment of the eleven sequences from human (Hs), fly (Dm) and nematode (Ce) most closely related to *Drosophila* Nedd4. These are all C2 and WW domain containing HECT class E-3 ubiquitin ligases (Grau-Bové et al., 2013). The statistically supported Nedd4 cluster contains a single member in flies and nematodes with two in humans.

Accession numbers (we employed the longest isoform as noted):

CeWWP1-NP_740775 (isoformA)

CeY92H12A.2-NP_490865

DmC42797-NP_001188572 (isoformE)

DmLack/dSmurf-NP_523779 (isoformA)

DmNedd4-NP_648993 (isoformJ)

DmSu(dx)-NP_722753 (isoformA)

HsItchy-NP_001244066 (isoform1)

HsNEDD4-NP_006145 (isoform1)

HsNEDD4L-NP_001138439 (isoform1)

HsWWP1-NP_008944

HsWWP2-NP_001257383 (isoformFL)

Grau-Bové X, Sebé-Pedrós A, Ruiz-Trillo I. 2013. A genomic survey of HECT ubiquitin ligases in eukaryotes reveals independent expansions of the HECT system in several lineages. *Genome Biol Evol*. 5:833-847.