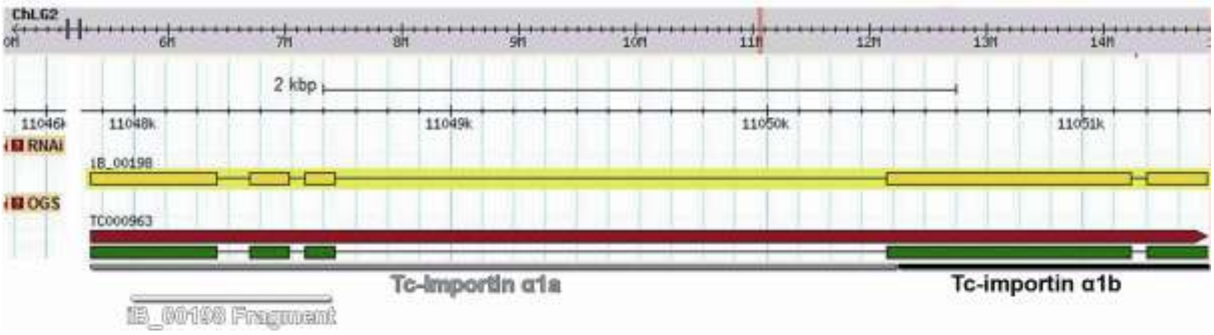
Additional file 1

Kitzmann et al.

A) Genomic structure of *Tc-importin α1 (Tc000963*) and location of the dsRNA fragments



TC000963 (NCBI LOC656915) is located on linkage group 2. The iB_00198 dsRNA targets the first three exons (white bar). The *Tc-importin α1*a dsRNA fragment targets in addition also a part of the fourth exon sequence (grey bar) and the *Tc-importin α1*b dsRNA fragment targets the fourth and fifth exon (black bar).

B) Tc000963 **open reading frame and dsRNA fragments used in RNAi experiments**

The iB_00198 fragment is underlined, the non-overlapping dsRNA fragments *Tc-importin α1*a and *-importin α1*b are highlighted in italic and regular fonts, respectively.

ATGTCGGGCTCCGCTCACAAACACCGCTACAAAAACGCGGCGCTGGACAGCGTCGAGTTGCGACGGC
GGCGAGAGGAAGAAGGCCTCCAGTTAAGGAAACAGAAACGGGAACAGCAGCTGAACAAGCGGCGG
AATGTTAACGTCAACCAATTGGCCGAGGACAATGACCATCTGCAAGTGGACAGCGAGATCCTCTCGC
CTGTCTCGCCCATGATCACCCCCGAAATGGTCCAGGCGTTGTACAGCCCCGACGTGGAGCAGCAAATC
AGCGCCACGCAAAAATTCAGACAACTGCTGAGCTACGAACCCAACCCACCCATTGATGAGGTGGTCC
AGACCGGGATCATCCCACGATTTGTAGAGTTTTTGCAGAATTCAAACAATTGTAGCCTTCAGTTTGAA
GCAGCATGGGCTTTGACCAACGTTGCCTCTGGTACCTCTCAGCAAACCAGGATGGTCATTGAAGCTG
GTGCTGTTCCTATTTTTATACGTTTGTTGAGCAGTCAATATGAGGATGTTCAGGAGCAAGCTGTTTGG
GCATTAGGGAATATAGCAGGGGATTCACCCGAATGTAGAGATCACGTCCTGGATTCGGGTATTTTGG
TTCCACTCTTACAACTCCTTAGTAAATCGACGCGTTTATCGATGACTCGCAATGCCGTGTGGGCGCTTT
*CGAACCTCTGCAGGGGCAAGAACCCTCCTCCAGACT*TGCGAAAGTCTCCCCAGCTCTGCCCGTACTC
GCTCGTCTCTTGTTTCACTCCGACCCTGACGTGCTCTCTGACACTTGCTGGGCCTTGAGCTACCTATCC
GACGGTCCTAACGAAAAAATCCAGGCGGTCATAGACGCCGGCGTTTGTCGGAAACTGGTCGAATTG
CTAATGCACCAACAGCCCAACGTAGTCTCGGCAGCTCTGCGCGCTGTCGGCAACATAGTGACTGGTG
ACGACGTCCAAACTCAGGTCATACTAAACTGCTCCGCTCTACATTGCTTGCACCACTTGTTGTCATCCT
CGAAGGAGTCCGTGAGGAAGGAGGCGTGTTGGACGATCAGCAACATCACGGCTGGAAACCGGCAG
CAAATCCAGGCGGTCATAGACGCGAACATTTTCCCGGTTTTGATAGAGATTTTGAGCAAGGCGGAGT

TTAAGACGCGGAAGGAGGCGGCCTGGGCTATCACGAATGCCACCAGCGGGGGCACACCCGACCAG
ATCAGGTATCTAGTGAACCAGGCTTGCATTGGGCCGCTTTGTGACCTTTTGACCGTCATGGACGCGA
AAATCGTGCAAGTGGCCCTCAACGGCCTGGAGAATATTCTGAGATTAGGGGAGCAAGACGCCAAGA
ATCACTCGGGGACCAACCCGTATGCTGTGCTAATCGAGCAGTGCTATGGGTTGGATAAAATCGAATT
TTTGCAATCGCATGTCAATATGGAGATTTATCAGAAGGCCTTCGACATTATTGAACACTTCTTCGGGA
CGGAGGAGGAGGACACGACTGTGGCGCCCTCGGTGAATCCCGACCAACAGCAGTACCATTTCAGCT
CGGATCAGTCGGTGCCGATGGGAGGATTCCATTTTTAA

C) Fasta sequences of the aligned amino acid sequences of Importin α1 orthologs

>*D.m.*/Importin α1

MSA-
HKKNAALDSTEMRRRREEVGIQLRKNKREQQLFKRRNVVLEPNEQQAADMHMADSNEEMIQMLFSGRENEQLEATQKFRKLLSRDPNPPIE
EVIQKGIVPQFVTFLRNSANATLQFEAAWTLTNIASGTSQQTKVVIEAGAVPIFIDLLSSPHDDVQEQAVWALGNIAGDSPMCRDHLLGSGILE
PLLHVLSNSDRITMIRNAVWTLSNLCRGKSPPADFAKISHGLPILARLLKYTDADVLSDTCWAIGYLSDGPNDKIQAVIDAGVCRRLVELLLHPQ
QNVSTAALRAVGNIVTGDDQQTQVILGYNALTCISHLLHSTAETIKKESCWTISNIAAGNREQIQALINANIFPQLMVIMQTAEFKTRKEAAWAI
TNATSSGTHEQIHYLVQVGCVPPMCDFLTVVDSDIVQVALNALENILKAGEKFQTPYAITIEECGGLDKIEYLQAHENRDIYHKSFYIIEQYFGEEE
DSRVAPVAG--TQQFEFDPDN-MPSTGFNF

>*D.m.*/Importin α2

MSKDSNKANSINTQDSRMRRHEVTIELRKSKKEDQMFKRRNIN-
DEPLKELNGQSPVQLVDEIVAAMNSEDQERQFLGMQSARKMLSRERNPPIDLMIGHGIVPICIRFLQNTNNSMLQFEAAWALTNIASGTSDQ
TRCVIEHNAVPHFVALLQSKSMNLAEQAVWALGNIAGDGAAARDIVIHHNVIDGILPLINNETPLSFLRNIVWLMSNLCRNKNPSPPFDQVKRL
LPVLSQLLLSQDIQVLADACWALSYVTDDDNTKIQAVVDSDAVPRLVKLLQMDEPSIIVPALRSVGNIVTGTDQQTDVVIASGGLPRLGLLLQH
NKSNIVKEAAWTVSNITAGNQKQIQAVIQAGIFQQLRTVLEKGDFKAQKEAAWAVTNTTTSGTPEQIVDLIEKYILKPFIDLLDTKDPRTIKVVQT
GLSNLFALAEKLGGNLCLMVEEMGGLDKLETLQQHENEEVYKKAYAIIDTYFSGDDEAEQELAPQEVNGALEFNATQPAPEGGYTF

>*D.m.*/Importin α3

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MSMEKNKGKDQDEMRRRRNEVTVELRKNKREETILKRRNVPNLDTDEEEQLSSSIDLKKLAKAAADATKPEQQLAAVQAARKLLSSDKNPPIN
DLIQSDILPILVECLKQHNHTMLQFEAAWALTNIASGTSAQTNEVVAAGAVPLFLQLLNSPAPNVCEQAVWALGNIIGDGPLLRDFVIKHGVVQ
PLLSFIKPDIPITFLRNVTWVIVNLCRNKDPAPPTATIHEILPALNVLIHHTDTNILVDTVWAISYLTDGGNDQIQMVIESGVVPKLIPLLGNSEVKV
QTAALRAVGNIVTGSDEQTQVVLNYDALSYFPGLLSHPKEKIRKEAVWFLSNITAGNQSQVQAVINVGLLPKIIENLSKGEFQTQKEAAWAISNL
TISGNREQVFTLIKEGVIPPFCDLLSCQDTQVINVVLDGLNNMLKVADSHVEAVANCIEECEGLAKIERLQSHENVEIYKLAYEIIDQYFTEGEQTN
MAPTSD-GAQ-YNFDPHADLTMNSFNF

>*M.m.*/Karyopherin α1

MSTGKEKNKSLNPDEMRRRREEEGLQLRKQKREEQLFKRRNVATAEEEVMSDGFEAINNTSDMTDMIFSNSPEQQLSATQKFRKLLSKEPNP
PIDEVINTGVVARFVEFLKRKENCTLQFESAWVLTNIASGNSLQTRNVIQAGAVPIFIELLSSEFEDVQEQAVWALGNIAGDSTMCRDYVLNCNI
LPPLLQLFSKQNRLTMTRNAVWALSNLCRGKSPPPEFAKVSPCLNVLSWLLFVSDTDVLADACWALSYLSDGPNDKIQAVIDAGVCRRLVELL
MHNDYKVVSPALRAVGNIVTGDDIQTQVILNCSALQSLLHLLSSPKESIKKEACWTISNITAGNRAQIQTVIDANMFPALISILQTAEFRTRKEAA
WAITNATSGGSAEQIKYLVELGCIKPLCDLLTVMDAKIVQVALNGLENILRLGEQEAKPYCALIEEAYGLDKIEFLQSHENQEIYQKAFDLIEHYFG
EDEDSSIAPQVDLSQQQYIFQQCE-APMEGFQL

>*M.m.*/Importin α2

MATKAPKYRGKEMSLPRQQRIASSLQLRKTRKDEQVLKRRNIDLFSDMVSQALVKEVNFLDDIIQAVNSSDPILHFRATRAAREMISQENTPPL
NLIIEAGLIPKLVDFLKATPHPKLQFEAAWVLTNIASGTSEQTRAVVKEGAIQPLIELLCSPHLTVSEQAVWALGNIAGDCAEFRDCVISNNAIPHL
INLISKGIPITFLRNISWTLSNLCRNKDPYPSESAVRQMLPPLCQLLLHRDNEILADTCWALSYLTKGGKEYIHHVVTTGILPRLVELMTSSELSISIP

CLHTIGNIVAGTDEQTQMAIDAGMLKVLGQVLKHPKTSIQVLAAWTMSNVAAGPRHQVEQLLCN-
LLPILVDLLRNAELKVQKEVVCTVINIATGASQDQLTLLAHSGILEPMLSLLSAPDLEVVIIVLDIISYLLQHIDNLQERLYFQIEKFGGFEKIECLQHH
HNISISNSALDIIEKYFC-DGDGDSLPGPGLRV------------------

>*M.m.*/Karyopherin α3

AENGLEKNKGRDVETMRRHRNEVTVELRKNKRDEHLLKKRNVPQEEDSDVDADFKAQNVLEAILQNATSDNPVVQLSAVQAARKLLSSDRN
PPIDDLIKSGILPILVKCLERDDNPSLQFEAAWALTNIASGTSAQTQAVVQSNAVPLFLRLLHSPHQNVCEQAVWALGNIIGDGPQCRDYVISLG
VVKPLLSFINPSIPITFLRNVTWVIVNLCRNKDPPPPMETVQEILPALCVLIYHTDINILVDTVWALSYLTDGGNEQIQMVIDSGVVPFLVPLLSHQ
EVKVQTAALRAVGNIVTGTDEQTQVVLNCDVLSHFPNLLSHPKEKINKEAVWFLSNITAGNQQQVQAVIDAGLIPMIIHQLAKGDFGTQKEAA
WAISNLTISGRKDQVEYLVQQNVIPPFCNLLSVKDSQVVQVVLDGLKNILIMAGDEASTIAEIIEECGGLEKIEVLQQHENEDIYKLAFEIIDQYFSI
DEDPSLIPEAT-QGGTYNFDPTANLQTKEFNF

>*M.m.*/Karyopherin α4

ADNKLDKNKGRDLETMRRQRNEVVVELRKNKRDEHLLKRRNVPQEDDSDIDGDYRVQNTLEAIVQNASSDNQGIQLSAVQAARKLLSSDRN
PPIDDLIKSGILPILVHCLERDDNPSLQFEAAWALTNIASGTSEQTQAVVQSNAVPLFLRLLHSPHQNVCEQAVWALGNIIGDGPQCRDYVISLG
VVKPLLSFISPSIPITFLRNVTWVMVNLCRHKDPPPPMETIQEILPALCVLIHHTDVNILVDTVWALSYLTDAGNEQIQMVIDSGIVPHLVPLLSH
QEVKVQTAALRAVGNIVTGTDEQTQVVLNCDALSHFPALLTHPKEKINKEAVWFLSNITAGNQQQVQAVIDANLVPMIIHLLDKGDFGTQKE
AAWAISNLTISGRKDQVAYLIQQNVIPPFCNLLTVKDAQVVQVVLDGLSNILKMAEDQAETIANLIEECGGLEKIEQLQNHENEDIYKLAYEIIDQ
FFSIDEDPSLVPESV-QGGTFGFNSSTNVPTEGFQF

>*M.m.*/Karyopherin α2

MSTENAKNKGKDSTEMRRRRIEVNVELRKAKKDEQMLKRRNVSSFPPLQENNNQGTVNWVEDIVKGINSNNLESQLQATQAARKLLSREKQ
PPIDNIIRAGLIPKFVSFLGKTDCSPIQFESAWALTNIASGTSEQTKAVVDGGAIPAFISLLASPHAHISEQAVWALGNIAGDGSAFRDLVIKHGAI
DPLLALLAVPLACGYLRNLTWTLSNLCRNKNPAPPLDAVEQILPTLVRLLHHNDPEVLADSCWAISYLTDGPNERIEMVVKKGVVPQLVKLLGA
TELPIVTPALRAIGNIVTGTDEQTQKVIDAGALAVFPSLLTNPKTNIQKEATWTMSNITAGRQDQIQQVVNHGLVPFLVGVLSKADFKTQKEAA
WAITNYTSGGTVEQIVYLVHCGIIEPLMNLLSAKDTKIIQVILDAISNIFQAAEKLGEKLSIMIEECGGLDKIEALQRHENESVYKASLNLIEKYFSEEE
DQNVVPETTSEGFAFQVQDGAP---GTFNF

>*M.m.*/Karyopherin α6

MASGKDKNNALNPEEMRRRREEEGIQLRKQKREQQLFKRRNVELINEAAMFDLLDSVSSTREMVEMLFSDDSDLQLATTQKFRKLLSKEPSPP
IDEVINTGVVDRFVEFLKRNENCTLQFEAAWALTNIASGTSQQTKIVIEAGAVPIFIELLNSDFEDVQEQAVWALGNIAGDSSLCRDYVLNCSILN
PLLTLLTKSTRLTMTRNAVWALSNLCRGKNPPPEFAKVSPCLPVLSRLLFSSDSDLLADACWALSYLSDGPNEKIQAVIDSGVCRRLVELLMHND
YKVASPALRAVGNIVTGDDIQTQVILNCSALPCLLHLLSSSKESIRKEACWTISNITAGNRAQIQAVIDANIFPVLIEILQKAEFRTRKEAAWAITNA
TSGGTPEQIRYLVSLGCIKPLCDLLTVMDSKIVQVALNGLENILRLGEQESKPYCGLIEEAYGLDKIEFLQSHENQEIYQKAFDLIEHYFGEDDDSSL
APQVDETQQQFIFQQPE-APMEGFQL

>*T.c.*/Importin α1

MSGAHKKNAALDSVELRRRREEEGLQLRKQKREQQLNKRRNVNVNQNDLQDSILSPVSPTPEMVQALYSPDVEQQISATQKFRQLLSYEPNP
PIDEVVQTGIIPRFVEFLQNSNNCSLQFEAAWALTNVASGTSQQTRMVIEAGAVPIFIRLLSSQYEDVQEQAVWALGNIAGDSPECRDHVLDS
GILVPLLQLLSKSTRLSMTRNAVWALSNLCRGKNPPPDFAKVSPALPVLARLLFHSDPDVLSDTCWALSYLSDGPNEKIQAVIDAGVCRKLVELL
MHQQPNVVSAALRAVGNIVTGDDVQTQVILNCSALHCLHHLLSSSKESVRKEACWTISNITAGNRQQIQAVIDANIFPVLIEILSKAEFKTRKEA
AWAITNATSGGTPDQIRYLVNQACIGPLCDLLTVMDAKIVQVALNGLENILRLGEQDAKPYAVLIEQCYGLDKIEFLQSHVNMEIYQKAFDIIEH
FFGEEEDTTVAPSVNPDQQQYHFSSDQSVPMGGFHF

>*T.c.*/Importin α2

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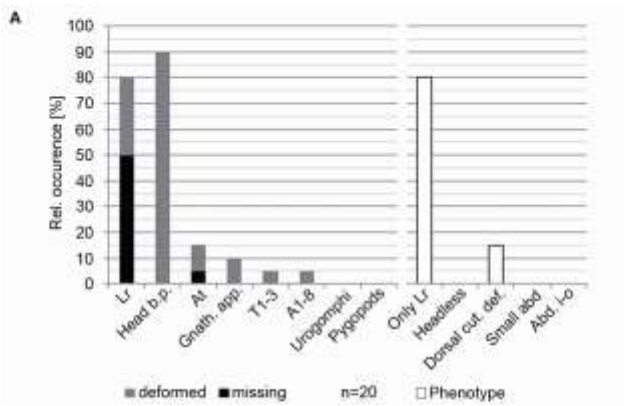
MDENKNKGKDCEEMRRRRAGQTIELRKAKKEDQLLKRRNISLQEPLQENNSVSLITMAEEILMGMMSPDENLQFKATQACRKMLSRERNPPI
DHMIRLGVVPRCVEFLGKTHNPSLVFEACWALTNIASGTSEQTAAVVQEGALPKLQQLLSSNRIDVVEQAIWAIGNIAGDGPESRDLVLNYGVL
PSLINLIKPNTTLSLLRNTVWVISNLCRNKNPYPDFELVKPALPVLARLLSHDDKDVLADTCWALSYLTDGSNEKIQAVLDTGLIDRLVMLLYSEES
TVLTPALRAVGNIVTGNDHQTDMVINAGALDCMIRLLQHSRLNIVKEAAWTVSNITAGNSEQIQKVLDAGILPYLLHVLQTGDFKSQKEAAWA
VTNFTSGGNSAQLAQLVEMGALKPMCNLLNSKDSKTIIVVLEGLNNILSAAAKLNQKVAIMIEECGGLDGIEALQSHDNEKVYERALHIIENYFA
GEEGVTVTVANGEIQFATPNMADQQSP---FSF

>*T.c.*/Importin α3

GQVGINKNKGKDQDEMRRRRNEVTVELRKNKREETLQKRRNVPVVDEDDIEKSLSTTNLEELVAQAAAIDNPPLQLMAIQTARKLLSSDRNPP
IDALISSGILPVLVMCLERYDEPSLQFEAAWALTNIASGTSAQTNRVVQAGAVPLFLKLLHSPQQNVCEQAVWALGNIIGDGPQLRDYVIELGV
VNPLLSFIKPDVPISFLRNVTWVIVNLCRNKDPPPPIQTIRELLPALNALIHHTDTNILVDTVWALSYLTDGGNEQIQMVIDSGVVPKLIPLLSHKE
VKVQTAALRAVGNIVTGTDEQTQVVLNCDALSHFPALLSHSKEKICKEAVWFLSNITAGNQMQVQAVIDAGLLPNIINNLSKGDFQTQKEAA
WAISNLTIGGNKEQVATLIREGVIPPFCDLLSCKDAQVIQVVLDGINNMLKMAGAEVDQLCTMIEECNGLDKIEMLQNHDNIEIYKIAYDIIEQY
FSADDDANLAPTAA-ETG-FQFDPNTNVPNEGFKF

D) Cuticle defects after *Tc-importin α1*a RNAi in *black* female pupae mated to *pig19* males



The *Tc-importin α1a* fragment was used for RNAi (1 µg/µl) in the strain combination, which was used in the iBeetle screen. This resulted in 80.0% with exclusive labrum defects which are either marked by a complete loss (Fig 2I, J) or deformation (Fig 2H) of the labrum. The next most frequently affected structure is the antenna, which shows defects in 15% of all cuticles. *Lr* labrum, *Head b.p.* Head bristle pattern, *At* antennae, *Gnath. app.* gnathal appendages, *T1-3* thoracic segment 1-3, *A1-8* abdominal segments 1-8, *Dorsal cl.* dorsal closure, *Abd. i-o* abdominal segments.

E) Tables summarizing the defects found in the *Tc-importin α1* RNAi experiments

Cuticle defects after injection of *Tc-importin α1*a dsRNA (1 µg/µl) in *black* female pupae which were crossed to *pig19* males.

ID	impα1a Blxpig
dsRNA[µg/µl]	1
n	21
wt	1
pt	20
% affected	95.2

	Lr	Head b.p.	At	Gnath. app.	T1-3	A1-8	Urogomphi	Pygopods
missing	10	0	1	0	0	0	0	0
deformed	6	18	2	2	1	1	0	0
∑	16	18	3	2	1	1	0	0
% ∑	80.0	90.0	15.0	10.0	5.0	5.0	0	0
% missing	50.0	0	5.0	0	0	0	0	0
% deformed	30.0	90.0	10.0	10.0	5.0	5.0	0	0

	Only Lr	Headless	Dorsal closure	Small abd	Abd i-o
n	16	0	3	0	0
%	80.0	0	15.0	0	0

Cuticle defects after injection of *Tc-importin α1*a dsRNA (1 µg/µl) in *SB* female pupae which were crossed to *SB* males.

ID	impα1a SBxSB
dsRNA[µg/µl]	1
n	30
wt	0
pt	30
% affected	100

	Lr	Head b.p.	At	Gnath. app.	T1-3	A1-8	Urogomphi	Pygopods
missing	4	4	8	12	5	17	15	15
deformed	10	19	4	8	18	11	3	5
∑	14	23	12	20	23	28	18	20
% ∑	46.7	76.7	40.0	66.7	76.7	93.3	60.0	66.7
% missing	13.3	13.3	26.7	40.0	16.7	56.7	50.0	50.0
% deformed	33.3	63.3	13.3	26.7	60.0	36.7	10.0	16.7

	Only Lr	Headless	Dorsal closure	Small abd	Abd i-o
n	0	4	7	4	11
%	0	13.3	23.3	13.3	36.6

Cuticle defects after injection of *Tc-importin α1*b dsRNA (1 µg/µl) in *SB* female pupae which were crossed to *SB* males.

ID	impα1b SBxSB
dsRNA[µg/µl]	1
n	13
wt	0
pt	13
% affected	100

	Lr	Head b.p.	At	Gnath. app.	T1-3	A1-8	Urogomphi	Pygopods
missing	0	0	1	0	1	9	8	8
deformed	3	9	3	4	7	4	0	0
∑	3	9	4	4	8	13	8	8
% ∑	23.1	69.2	30.8	30.8	61.5	100.0	61.5	61.5
% missing	0	0	7.7	0	7.7	69.2	61.5	61.5
% deformed	23.1	69.2	23.1	30.8	53.8	30.8	0	0

	Onyl Lr	Headless	Dorsal closure	Small abd	Abd i-o
n	0	0	3	2	6
%	0	0	23.1	15.4	46.2

Cuticle defects after injection of *Tc-importin α1*a dsRNA (1 µg/µl) in *black* female pupae which were crossed to *pBa19* males.

ID	impα1a BlxBl
dsRNA[µg/µl]	1
n	26
wt	4
pt	22
% affected	84.6

	Lr	Head b.p.	At	Gnath. app.	T1-3	A1-8	Urogomphi	Pygopods
missing	11	0	2	1	0	1	1	1
deformed	8	17	3	4	0	0	0	0
∑	19	17	5	5	0	1	1	1
% ∑	86.4	77.3	22.7	22.7	0	4.5	4.5	4.5
% missing	50.0	0	9.1	4.5	0	4.5	4.5	4.5
% deformed	36.4	77.3	13.6	18.2	0	0	0	0

	Only Lr	Headless	Dorsal closure	Small abd	Abd i-o
n	14	0	0	0	1
%	63.6	0	0	0	4.5

Cuticle defects after injection of *Tc-importin α1*b dsRNA (1 µg/µl) in *black* female pupae which were crossed to *black* males.

ID	impα1b BlxBl
dsRNA[µg/µl]	1
n	9
wt	2
pt	7
% affected	77.8

	Lr	Head b.p.	At	Gnath. app.	T1-3	A1-8	Urogomphi	Pygopods
missing	3	0	0	0	0	0	0	0
deformed	3	5	2	1	0	0	0	0
∑	6	5	2	1	0	0	0	0
% ∑	85.7	71.4	28.6	14.3	0	0	0	0
% missing	42.9	0	0	0	0	0	0	0
% deformed	42.9	71.4	28.6	14.3	0	0	0	0

	Only Lr	Headless	Dorsal closure	Small abd	Abd i-o
n	4	0	2	0	0
%	57.1	0	28.6	0	0

Cuticle defects after injection of *Tc-importin α1*a dsRNA (0.3 µg/µl) in *SB* female pupae which were crossed to *SB* males.

ID	impα1a SBxSB
dsRNA[µg/µl]	0.3
n	18
wt	2
pt	16
% affected	88.9

	Lr	Head b.p.	At	Gnath. app.	T1-3	A1-8	Urogomphi	Pygopods
missing	0	0	0	1	0	8	8	8
deformed	2	13	0	1	4	5	0	0
∑	2	13	0	2	4	13	8	8
% ∑	12.5	81.3	0	12.5	25.0	81.3	50.0	50.0
% missing	0	0	0	6.3	0	50.0	50.0	50.0
% deformed	12.5	81.3	0	6.3	25.0	31.3	0	0

	Onyl Lr	Headless	Dorsal closure	Small abd	Abd i-o
n	0	0	1	0	8
%	0	0	6.3	0	50.0

Cuticle defects after injection of *Tc-importin α1*a dsRNA (0.3 µg/µl) in *black* female pupae which were crossed to *black* males.

ID	impα1a BlxBl
dsRNA[µg/µl]	0.3
n	18
wt	1
pt	17
% affected	94.4

	Lr	Head b.p.	At	Gnath. app.	T1-3	A1-8	Urogomphi	Pygopods
missing	7	0	1	0	0	0	0	0
deformed	7	14	1	0	3	3	0	0
∑	14	14	2	0	3	3	0	0
% ∑	82.4	82.4	11.8	0	17.6	17.6	0	0
% missing	41.2	0	5.9	0	0	0	0	0
% deformed	41.2	82.4	5.9	0	17.6	17.6	0	0

	Only Lr	Headless	Dorsal closure	Small abd	Abd i-o
n	14	0	2	0	0
%	82.4	0	11.8	0	0

Cuticle defects after injection of *Tc-importin α1*a dsRNA (3 µg/µl) in *black* female pupae which were crossed to *black* males.

ID	impα1a BlxBl
dsRNA[µg/µl]	3
n	20
wt	0
pt	20
% affected	100

	Lr	Head b.p.	At	Gnath. app.	T1-3	A1-8	Urogomphi	Pygopods
missing	8	0	2	4	0	2	2	2
deformed	4	19	4	3	1	1	0	0
∑	12	19	6	7	1	3	2	2
% ∑	60.0	95.0	30.0	35.0	5.0	15.0	10.0	10.0
% missing	40.0	0	10.0	20.0	0	10.0	10.0	10.0
% deformed	20.0	95.0	20.0	15.0	5.0	5.0	0	0

	Only Lr	Headless	Dorsal closure	Small abd	Abd i-o
n	6	0	3	0	1
%	30.0	0	15.0	0	5.0

Cuticle defects after injection of *Tc-importin α1*a dsRNA (1 µg/µl) in *SB* female pupae which were crossed to *black* males.

ID	impα1a SBxBl
dsRNA[µg/µl]	1
n	25
wt	2
pt	23
% affected	92.0

	Lr	Head b.p.	At	Gnath. app.	T1-3	A1-8	Urogomphi	Pygopods
missing	2	2	4	4	2	13	13	13
deformed	4	13	2	2	9	1	0	0
∑	6	15	6	6	11	14	13	13
% ∑	26.1	65.2	26.1	26.1	47.8	60.9	56.5	56.5
% missing	8.7	8.7	17.4	17.4	8.7	56.5	56.5	56.5
% deformed	17.4	56.5	8.7	8.7	39.1	4.3	0	0

	Only Lr	Headless	Dorsal closure	Small abd	Abd i-o
n	1	2	4	3	10
%	4.3	8.7	17.4	13.0	43.5

Cuticle defects after injection of *Tc-importin α1*a dsRNA (1 µg/µl) in *black* female pupae which were crossed to *SB* males.

ID	impα1a BlxSB
dsRNA[µg/µl]	1
n	27
wt	3
pt	24
% affected	88.9

	Lr	Head b.p.	At	Gnath. app.	T1-3	A1-8	Urogomphi	Pygopods
missing	12	1	3	1	0	2	3	3
deformed	7	20	2	3	2	2	0	0
∑	19	21	5	4	2	4	3	3
% ∑	79.2	87.5	20.8	16.7	8.3	16.7	12.5	12.5
% missing	50.0	4.2	12.5	4.2	0	8.3	12.5	12.5
% deformed	29.2	83.3	8.3	12.5	8.3	8.3	0	0

	Only Lr	Headless	Dorsal closure	Small abd	Abd i-o
n	12	0	2	1	0
%	50.0	0	8.3	4.2	0

F) Tables summarizing the defects found in the other RNAi experiments

Quantification of cuticle phenotypes after *Tc-dll* RNAi in *black* female pupae crossed to *black* males.

ID	*Tc-dll* BlxBl
dsRNA[µg/µl]	1
n	50

Phenotype	**Level 4**	**Level 3**	**Level 2**	**Level 1**
∑	6	16	21	7
%	12	32	42	14

Quantification of cuticle phenotypes after *Tc-dll* RNAi in *SB* female pupae crossed to *SB* males.

ID	*Tc-dll* SBxSB
dsRNA[µg/µl]	1
n	50

Phenotype	**Level 4**	**Level 3**	**Level 2**	**Level 1**
∑	0	14	33	3
%	0	28	66	6

Quantification of cuticle phenotypes after *Tc-gt* RNAi in *SB* female pupae crossed to *SB* males.

ID	*Tc-gt* SBxSB
dsRNA[µg/µl]	1
n	50

Del. segm. n	**0**	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**	**3.5**	**4**	**4.5**	**5**	**5.5**	**6**	**6.5**	**7**
∑	1	4	3	9	7	11	5	4	3	2	0	0	1	0	0
%	2	8	6	18	14	22	10	8	6	4	0	0	2	0	0

Quantification of cuticle phenotypes after *Tc-gt* RNAi in *black* female pupae crossed to *black* males.

ID	*Tc-gt* BlxBl
dsRNA[µg/µl]	1
n	50

Del. segm. n	**0**	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**	**3.5**	**4**	**4.5**	**5**	**5.5**	**6**	**6.5**	**7**
∑	0	1	6	6	11	10	8	7	1	0	0	0	0	0	0
%	0	2	12	12	22	20	16	14	2	0	0	0	0	0	0

Quantification of pupal phenotypes after *Tc-wg* lRNAi in *SB* females.

ID	*Tc-wg* SB
dsRNA[µg/µl]	1
n	10

Phenotype	Level 4	Level 3	Level 2	Level 1
Mx ø [in]	0,16-0,21	0,22-0,27	0,28-0,33	>0,33
∑	5	1	1	3
%	45.5	9.1	9.1	27.3

Quantification of pupal phenotypes after *Tc-wg* lRNAi in *black* females.

ID	*Tc-wg* Bl
dsRNA[µg/µl]	1
n	12

Phenotype	Level 4	Level 3	Level 2	Level 1
Mx ø [in]	0,16-0,21	0,22-0,27	0,28-0,33	>0,33
∑	3	6	2	1
%	25	50	16.7	8.3

Quantification of pupal phenotypes after *Tc-six3* lRNAi in *black* females.

ID	*Tc-six3* Bl
dsRNA[µg/µl]	0.5
n	10

Phenotype	Level 4	Level 3	Level 2	Level 1
Eyefield [in²]	<0.16	0.17-0.23	0.24-0.30	>0.31
∑	3	4	1	2
%	30	40	10	20

Quantification of pupal phenotypes after *Tc-six3* lRNAi in *SB* females.

ID	*Tc-six3* SB
dsRNA[µg/µl]	0.5
n	11

Phenotype	Level 4	Level 3	Level 2	Level 1
Eyefield [in²]	<0.16	0.17-0.23	0.24-0.30	>0.31
∑	2	8	1	0
%	18.2	72.7	9.1	0

G) Measurements of maxillary palpus diameter and eyefield

SB wild-type pupae

ID	wt *SB*
n	10

	Mx ø [in]	**Eyefield [in²]**
1	0.35	0.41
2	0.35	0.41
3	0.35	0.43
4	0.33	0.41
5	0.36	0.4
6	0.35	
7	0.36	
8	0.35	
9	0.34	
10	0.36	

black wild-type pupae.

ID	wt *black*
n	10

	Mx ø [in]	**Eyefield [in²]**
1	0.35	0.46
2	0.35	0.45
3	0.35	0.45
4	0.35	0.46
5	0.37	0.41
6	0.35	
7	0.37	
8	0.35	
9	0.35	
10	0.36	

H) Alignment of *Tc-Importin α1* amino acid sequence in different Tribolium strains and metazoans

>*S. cerevisae* SRP1
MDNGTDSSTSKFVPEYRRTNFKNKGRFSADELRRRRDTQQVELRKAKRDEALAKRRNFIPPTDGADSDEEDESSVSADQQFYSQLQQELPQM
TQQLNSDDMQEQLSATVKFRQILSREHRPPIDVVIQA-
GVVPRLVEFMRENQPEMLQLEAAWALTNIASGTSAQTKVVVDADAVPLFIQLLYTGSVEVKEQAIWALGNVAGDSTDYRDYVLQCNAMEPIL
GLFN-
SNKPSLIRTATWTLSNLCRGKKPQPDWSVVSQALPTLAKLIYSMDTETLVDACWAISYLSDGPQEAIQAVIDVRIPKRLVELLSHESTLVQTPALR
AVGNIVTGNDLQTQVVINAGVLPALRLLLSSPKENIKKEACWTISNITAGNTEQIQAVIDANLIPPLVKLLEVAEYKTKKEACWAISNASSGGLQR
PDIIRYLVSQGCIKPLCDLLEIADNRIIEVTLDALENILKMGEADKEARGLNINENADFIEKAGGMEKIFNCQQNENDKIYEKAYKIIETYFGEEEDA
VDETMAPQNAGNTFGFGSNVN-----QQFNFN_

>*T. castaneum SB*
-------MS-GSAHKHR---YKNAA-LDSVELRRRREEEGLQLRKQKREQQLNKRRNVN--VNQLAEDNDHLQVDSEILSPVS--
PMITPEMVQALYSPDVEQQISATQKFRQLLSYEPNPPIDEVVQT-
GIIPRFVEFLQNSNNCNLQFEAAWALTNVASGTSQQTRMVIEAGAVPIFIRLLSSQYEDVQEQAVWALGNIAGDSPECRDHVLDSGILVPLLQL
LSKSTRLSMTRNAVWALSNLCRGKNPPPDFAKVSPALPVLARLLFHSDPDVLSDTCWALSYLSDGPNEKIQAVIDAGVCRKLVELLMHQQPNV
VSAALRAVGNIVTGDDVQTQVILNCSALHCLHHLLSSSKESVRKEACWTISNITAGNRLQIQAVIDANIFPVLIEILSKAEFKTRKEAAWAITNATS
GGT--PDQIRYLVNQACIGPLCDLLTVMDAKIVQVALNGLENILRLGEQDAKN-
HSGTNPYAVLIEQCYGLDKIEFLQSHVNMEIYQKAFDIIEHFFGTEEEDTTVAPSVNPDQQQYHFSSDQS--VPMGGFHF_-

>*T. castaneum Black*
-------MS-GSAHKHR---YKNAA-LDSVELRRRREEEGLQLRKQKREQQLNKRRNVN--VNQLAEDNDHLQVDSEILSPVS--
PMITPEMVQALYSPDVEQQISATQKFRQLLSYEPNPPIDEVVQT-
GIIPRFVEFLQNSNNCSLQFEAAWALTNVASGTSQQTRMVIEAGAVPIFIRLLSSQYEDVQEQAVWALGNIAGDSPECRDHVLDSGILVPLLQLL
SKSTRLSMTRNAVWALSNLCRGKNPPPDFAKVSPALPVLARLLFHSDPDVLSDTCWALSYLSDGPNEKIQAVIDAGVCRKLVELLMHQQPNVV
SAALRAVGNIVTGDDVQTQVILNCSALHCLHHLLSSSKESVRKEACWTISNITAGNRQQIQAVIDANIFPVLIEILSKAEFKTRKEAAWAITNATS
GGT--PDQIRYLVNQACIGPLCDLLTVMDAKIVQVALNGLENILRLGEQDAKN-
HSGTNPYAVLIEQCYGLDKIEFLQSHVNMEIYQKAFDIIEHFFGTEEEDTTVAPSVNPDQQQYHFSSDQS--VPMGGFHF_-

>*T. castaneum GA2*
-------MS-GSAHKHR---YKNAA-LDSVELRRRREEEGLQLRKQKREQQLNKRRNVN--VNQLAEDNDHLQVDSEILSPVS--
PMITPEMVQALYSPDVEQQISATQKFRQLLSYEPNPPIDEVVQT-
GIIPRFVEFLQNSNNCSLQFEAAWALTNVASGTSQQTRMVIEAGAVPIFIRLLSSQYEDVQEQAVWALGNIAGDSPECRDHVLDSGILVPLLQLL
SKSTRLSMTRNAVWALSNLCRGKNPPPDFAKVSPALPVLARLLFHSDPDVLSDTCWALSYLSDGPNEKIQAVIDAGVCRKLVELLMHQQPNVV
SAALRAVGNIVTGDDVQTQVILNCSALHCLHHLLSSSKESVRKEACWTISNITAGNRQQIQAVIDANIFPVLIEILSKAEFKTRKEAAWAITNATS
GGT--PDQIRYLVNQACIGPLCDLLTVMDAKIVQVALNGLENILRLGEQDAKN-
HSGTNPYAVLIEQCYGLDKIEFLQSHVNMEIYQKAFDIIEHFFGTEEEDTTVAPSVNPDQQQYHFSSDQS--VPMGGFHF_-

>*M .musculus*
----METMASPGKDNYRMKSYKNNA-LNPEEMRRRREEEGIQLRKQKREQQLFKRRNVE--
LINEEAAMFDSLLMDSYVSSTTGESVITREMVEMLFSDDSDLQLATTQKFRKLLSKEPSPPIDEVINTPGVVDRFVEFLKRNENCTLQFEAAWAL
TNIASGTSQQTKIVIEAGAVPIFIELLNSDFEDVQEQAVWALGNIAGDSSLCRDYVLNCSILNPLLTLLTKSTRLTMTRNAVWALSNLCRGKNPPP
EFAKVSPCLPVLSRLLFSSDSDLLADACWALSYLSDGPNEKIQAVIDSGVCRRLVELLMHNDYKVASPALRAVGNIVTGDDIQTQVILNCSALPCL
LHLLSSSKESIRKEACWTISNITAGNRAQIQAVIDANIFPVLIEILQKAEFRTRKEAAWAITNATSGGT--
PEQIRYLVSLGCIKPLCDLLTVMDSKIVQVALNGLENILRLGEQESKRSGSGVNPYCGLIEEAYGLDKIEFLQSHENQEIYQKAFDLIEHYFGVEDD
DSSLAPQVDETQQQFIFQQPEA---PMEGFQL_-

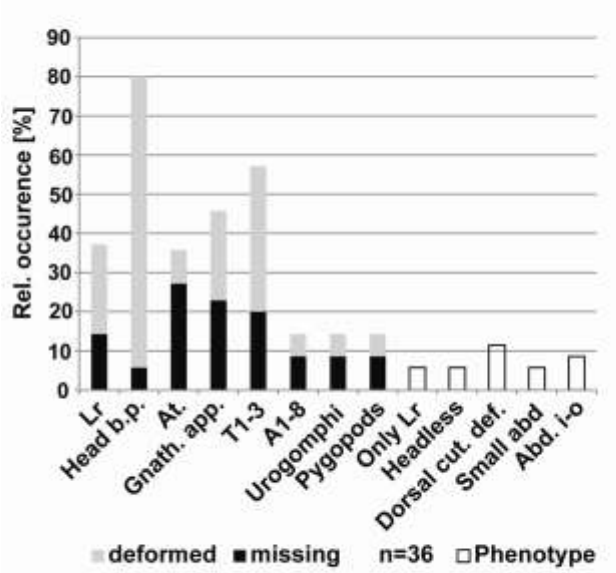
>*D. melanogaster*
--VINEEMIQMLFSGRENEQLEATQKFRKLLSRDPNPPIEEVIQK-
GIVPQFVTFLRNSANATLQFEAAWTLTNIASGTSQQTKVVIEAGAVPIFIDLLSSPHDDVQEQAVWALGNIAGDSPMCRDHLLGSGILEPLLHV
LSNSDRITMIRNAVWTLSNLCRGKSPPADFAKISHGLPILARLLKYTDADVLSDTCWAIGYLSDGPNDKIQAVIDAGVCRRLVELLLHPQQNVST
AALRAVGNIVTGDDQQTQVILGYNALTCISHLLHSTAETIKKESCWTISNIAAGNREQIQALINANIFPQLMVIMQTAEFKTRKEAAWAITNATS
SGT--HEQIHYLVQVGCVPPMCDFLTVVDSDIVQVALNALENILKAGEKFQTR----
PNPYAITIEECGGLDKIEYLQAHENRDIYHKSFYIIEQYFGNEEEDSRVAPVAG--TQQFEFD-PDN--MPSTGFNF_-

>*A. mellifera*
-------MSAATTHKYR---YKNVG-LDSQELRRRREEEGVQLRKQKREQQLSKRRNVP--NMVADDDNVTGNES-
AYPAPQSKTSIIVLEMVQELYSPNPEAQLAATQKFRKMLSREPNPPIDEVVKT-
GIVPKFVEFLENNANCTLQFEAAWALTNIASGTSQQTRVVVDAGAVPIFISLLGSEYEDVQEQAVWALGNIAGDSPECRDHVLDRGILTPLLQLL
SKATRLSMTRNAVWALSNLCRGKTPPPEFTKVAPCLPVLAHFLNHTDSDVLADACWALSYLSDGPNEKIQAVIDAGVCRRLVELLMHQQENV
VSAALRAVGNIVTGDDVQTQIVLNCSALHCLYHLLNSSQESIRKEACWTISNITAGNPQQIQAVIDAGIFPVLIDILAKAEFKIRKEAAWAITNATS

GGA--PEQIRYIVVEGCIPPLCNLLTVMDPKIVQVALSGLENILRVGEQDAAS-
HNGINHYAVLIEECFGLNKIEFLQSHQNVEIYQKAFDIIERYFGSEEEDTRVVPTIDAQGQQFQFRAPDSSQLPVQGFEF_-

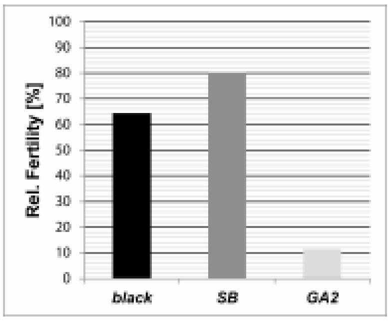
>*N. vitripennis*
-------MSSGPAHKYR---YKNVG-LDSQELRRRREEEGVQLRKQKREQQLSKRRNVP--
NIVTDDDGVSITEDYTVPTPIMQAPGITQDMVEALYSQDVNEQLTATQKFRKLLSKEPNPPIDEVIQT-
GIVPRFVEFLKNDTNCTLQFEAAWALTNIASGTSLQTRMVVDAGAVPMFIALLSSEYEDVQEQAVWALGNIAGDSPACRDHVLSSGILPPLLVL
LSKPARLTMTRNAVWALSNLCRGKEPAPDFQKVAPCLPVLAYLLNHQDADVLADACWALSYISDGPNEKIQAVIDAGVCRRLVELLMHEQHN
VTSAALRAVGNIVTGDDMQTQVVLNCSALTCLLHLLGQSRESIRKEACWTVSNITAGNPQQIQAVIDAKIFPVIIDILGKAEFKTRKEAAWAITN
ATSGGT--ADQIRYLADQNCIPPLCELLTVMDATIIQVALNGLENILRLGEQDAVH-
HNGVNPYCVLIEQCFGLDKIEFLQSHQNLEIYQKAFDIIERYFGSEEEDSKIAPSVDSQGQQFQFPAPDSSQLPVHSFQF_-

I) Phenotype of pig19 injected females mated to black males



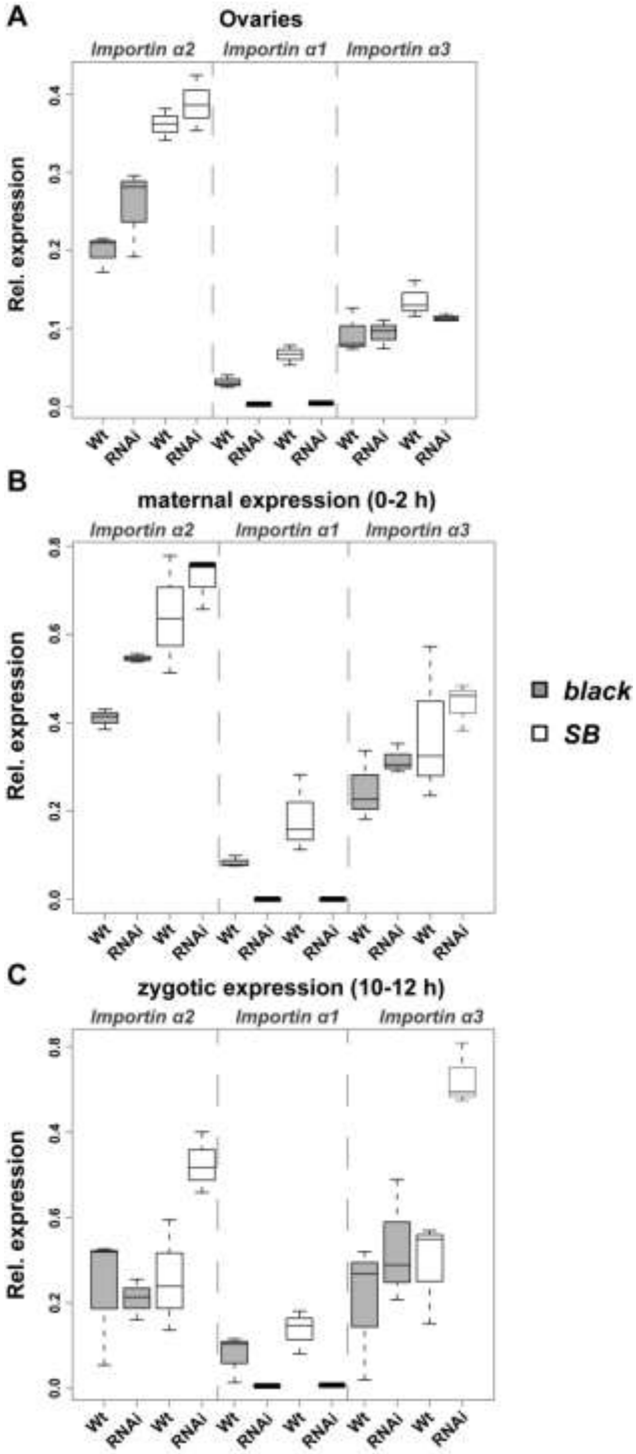
The phenotype of the offspring is an intermediate between black and SB in that “only labrum” phenotypes do occur (6%) but more structures are affected than in black (compare to Fig. A and D)

J) Effect of *Tc*-I*mportin α1*RNAi on fertility of different strains



Tc-dll (control) and *Tc-importin α1*a dsRNA (both 1 µg/µl) were injected into female pupae of *Black*, *SB* and *Georgia-2* and mated to untreated males of the same strain. The eggs laid per female were documented and the *Tc-importin α 1*a RNAi results were normalized to the control RNAi. *Tc-importin α1*a RNAi in the *Black* and *SB* background resulted in a reduction of the egglay of about 20% (*SB*) to 35% (*black*). The egglay of *GA2* was reduced by about 90%.

K) Upregulation of maternal *Tc-importin α2* after *Tc-importin α1* RNAi in the *black* strain



The level of expression was determined by qPCR in ovaries (A), freshly laid eggs (0-2h; maternal message; B) and 10-12 h embryos (zygotic expression; C) in wild-type and in *Tc-importin α1* knockdown animals. Results from the black strain are depicted with grey boxes, *SB* expression levels are shown as open boxes. wt and RNAi values are shown side by side, respectively. B) In *Tc-importin α1* knockdown embryos, the maternal load of *Tc-importin α2* is strongly elevated in *black* (compare first with second column) but less so in *SB*. C) Zygotic expression of *Tc-importin α2* and *3* are elevated in *SB* but less so in *black* (compare fourth with third and 12th with 11th column). Note that in contrast to figure 8, the values of the two strains were not normalized to each other.