Supplementary Figures and Tables for

Tissue-specific regulation of translational readthrough tunes functions of the traffic jam transcription factor

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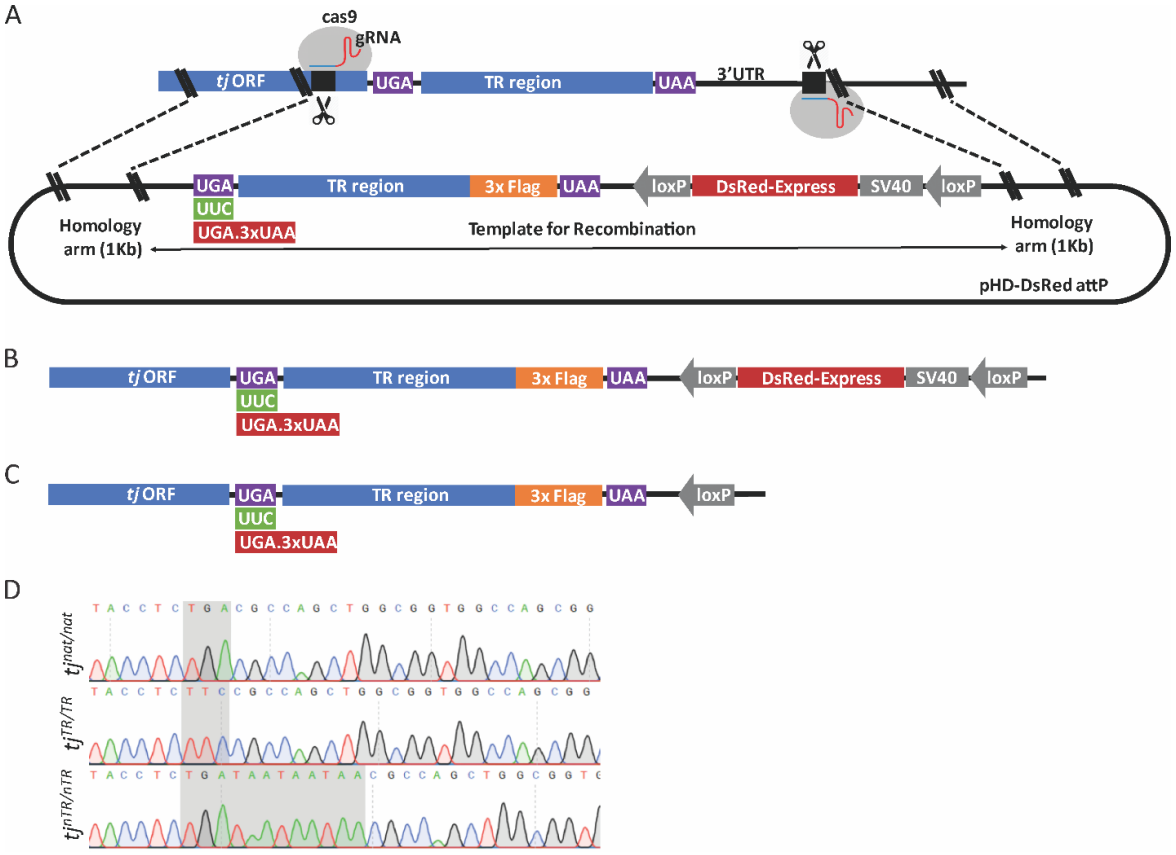

Figure S1. Construct design for CRISPR/Cas9-mediated genome editing to create *tj*-TR mutants, A. Gene locus surrounding the TR region of *tj* with proximal and distal PAM (protospacer adjacent motif) sites for guide RNA (gRNA)-directed Cas9 cleavage depicted above the modified pHD-DsRed attP vector containing the Template for Recombination (TfR) flanked by 1-Kb homology arms. Dotted lines represent the region of homology between the gene locus and modified vector. The TfR contains the modifications that introduce the desired mutations at the primary *tj* stop codon, 3xFlag upstream of the second stop codon and a loxP flanked *DsRed* marker. B. Sequence depicting the modifications introduced in the *tj* locus in *tj*-TR mutants post-CRISPR/Cas9 editing. C. Restoration of the native 3' UTR in *tj*-TR mutants via Cre recombinase-mediated removal of the loxP-flanked DsRed marker. D. Sequence verification of tj^{nat}, tj^{TR} *and* tj^{nTR} mutations in homozygous mutant flies.

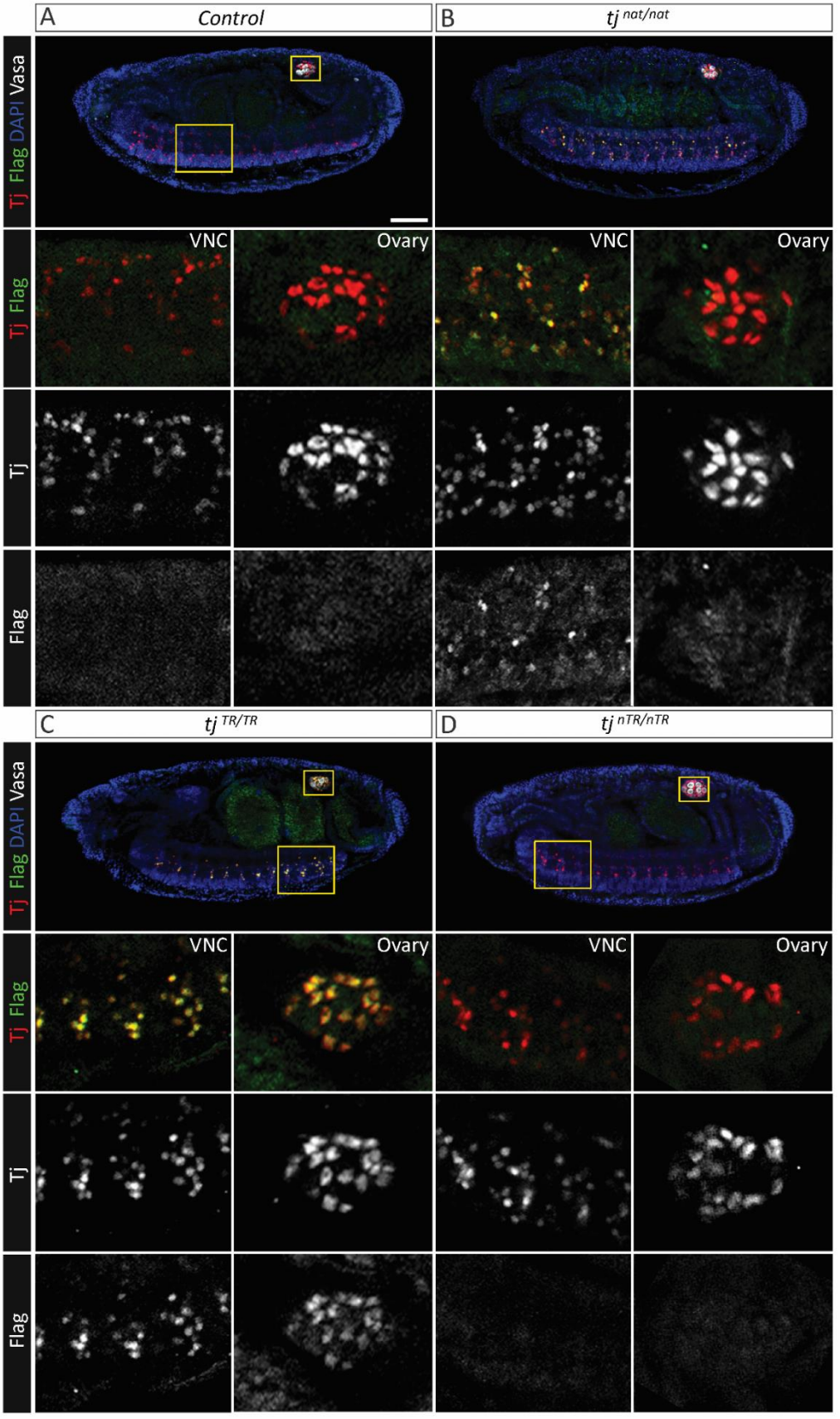
A
Control
B
tj nat/nat
C
tj TR/TR
D
tj nTR/nTR
Tj Flag DAPI Vasa
Tj Flag
Tj
Flag
VNC
Ovary

Figure S2. Tissue-specific regulation of TR in *tj* during embryogenesis. Stage 15-17 embryos are stained with the nuclear stain DAPI (blue), anti-Tj (red), and anti-Flag (green) antibodies, as well as anti-Vasa to mark the ovaries (greyscale). Anterior is to the left and dorsal is up in all images. Embryos of all genotypes express Tj in neural cells of the embryonic ventral nerve cord [VNC, lower yellow boxes in A-D] as well as in somatic gonadal precursor cells (SGPs) of embryonic ovaries [small upper yellow boxes in A-D]. Below each whole embryo image are enlargements of VNC and ovary tissues. Individual channels for anti-Tj and anti-Flag in greyscale as well as the overlapping channel are shown. A. Control embryos do not express Flag in any tissues. B. In $tj^{nat/nat}$ embryos, the Flag-tagged Tj-TR isoform is selectively expressed in the embryonic VNC and excluded from ovaries. C. $tj^{TR/TR}$ embryos exhibit constitutive expression of the Tj-TR isoform in both VNC and ovary. D. $tj^{nTR/nTR}$ embryos do not express the Tj-TR isoform in any tissues. Scale bar: 50 µm.

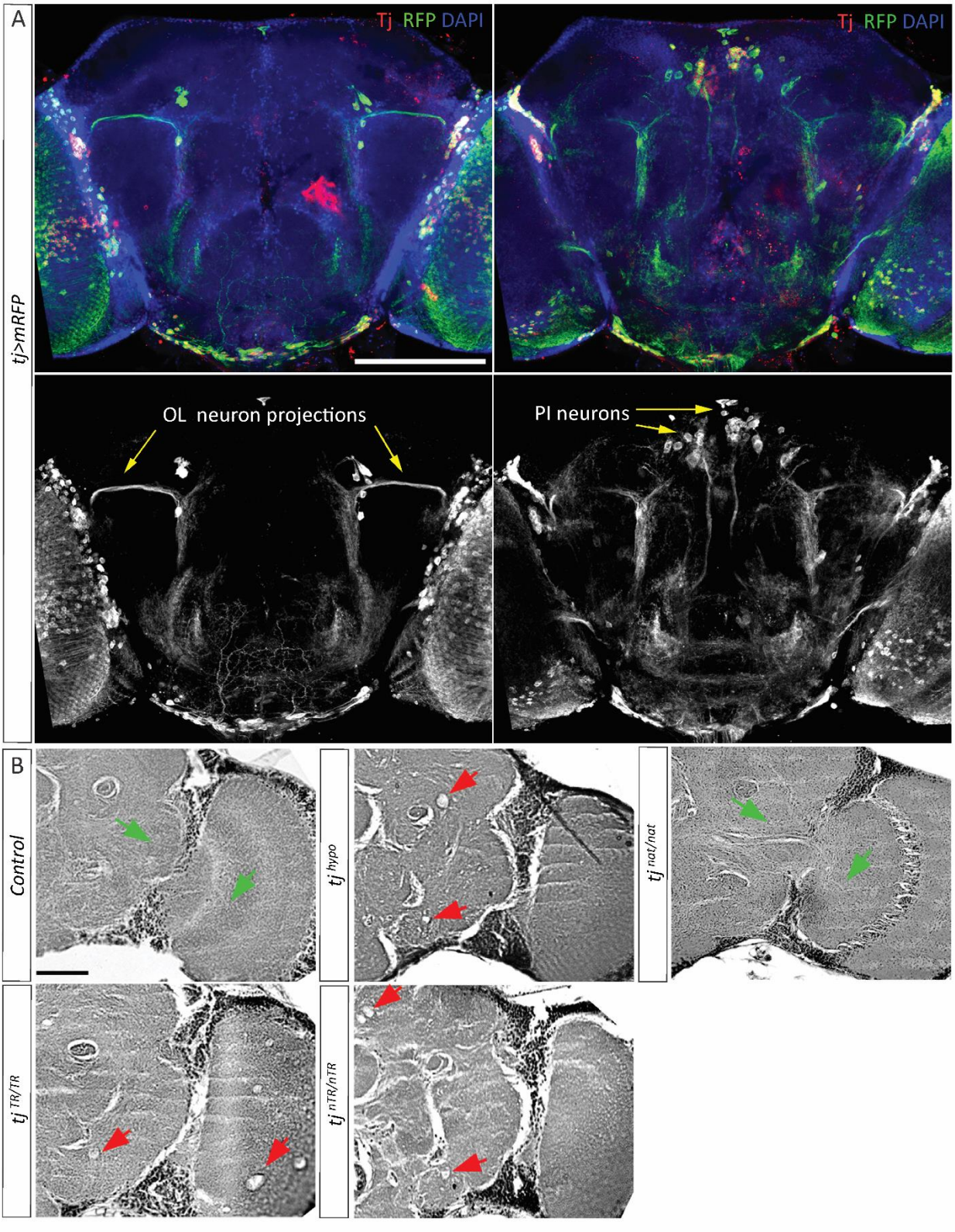
A
tj>mRFP
Tj RFP DAPI
Tj RFP DAPI
OL neuron projections
PI neurons
B
Control
tj hypo
tj nat/nat
tj TR/TR
tj nTR/nTR

Figure S3. Visualization of Tj and Tj-TR isoforms in adult brains from *tj*-TR mutants. A. Membrane-bound RFP was expressed under the control of *tj-Gal4*, an enhancer trap that closely mimics the endogenous expression pattern of *tj*. RFP marks cell bodies as well as neuronal projections of *tj*-expressing cells. Left panels depict projection patterns in an anterior slice from a z-stack of a whole brain, and the right panels depict a more posterior slice (RFP alone is shown in greyscale panels). Many cells of the retina and OL express RFP, as do a smaller number of CB neurons. In particular, OL neuronal projections extend into the CB (left panels), and a subset of *pars intercerebralis* (PI) neurons are visible near the brain midline (right panels). B. H&E-stained brain sections illustrate wild-type appearance with homogeneous staining (green arrows) in Control and $tj^{nat/nat}$ brains, as well as sporadic lesions (red arrows) in tj^{hypo}, $tj^{TR/TR}$, and $tj^{nTR/nTR}$ brains. Scale bar: 100 µm

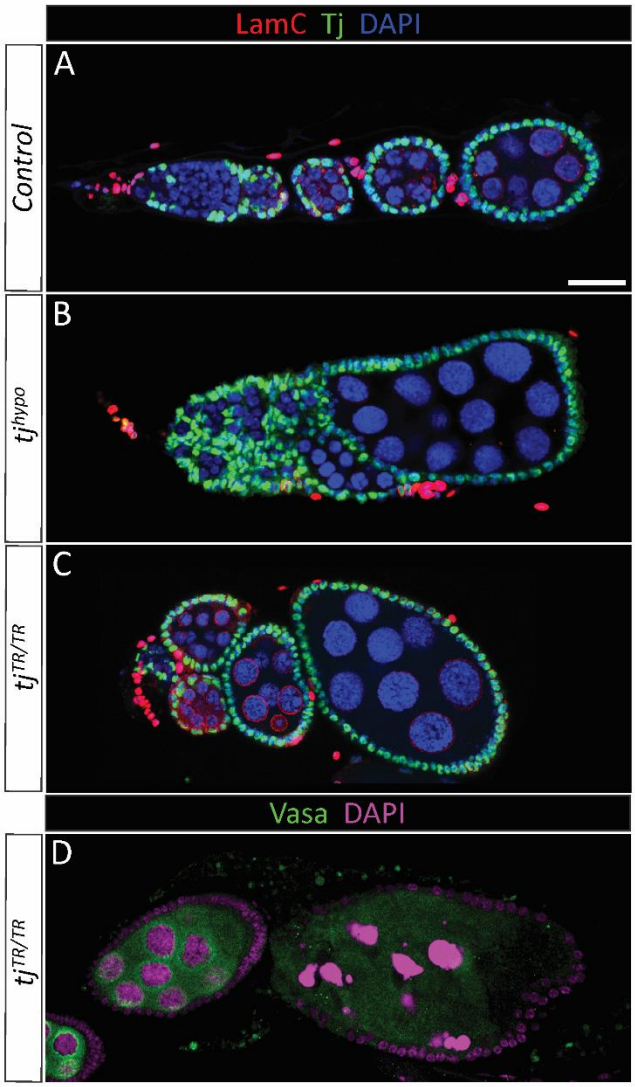

Figure S4. Forced *tj* TR causes various strong ovarian phenotypes similar to those in *tj* hypomorphic ovaries. a. Control ovariole stained for the nuclear marker DAPI as well as anti-Tj and anti-LamC. Anti-LamC is a nuclear envelope marker that strongly stains TFCs and CpCs as well as stalk cells (the cells that connect adjacent egg chambers) and more weakly marks nurse cells. It is also visible in the nuclei of the muscle cells that surround each ovariole. b. A *tj* hypomorphic ovariole exhibiting a strong disorganization phenotype. The egg chambers are not properly separated, and the germarium is very small or absent. Note that a LamC-positive TF is still visible at the anterior tip (to the left of the panel). c. A $tj^{TR/TR}$ germarium with a very small germarium. Similar to the hypomorph, the TF is still present. d. A $tj^{TR/TR}$ germarium exhibiting cell death in the germline of the larger egg chamber, evidenced by the strongly condensed and fragmented DAPI staining. Scale bar: 20 µm.

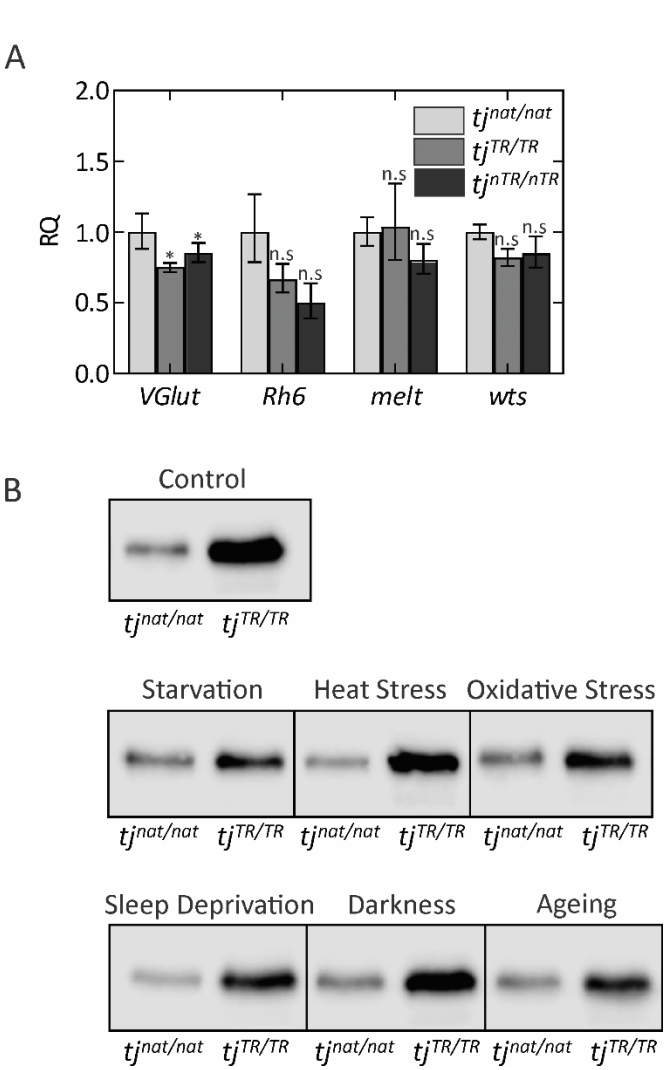

Figure S5. Effect of TR in tj on transcriptome profile and response to stress. A. RT-qPCR analysis of genes regulated by Tj in *tj*-TR mutants using cDNA prepared from adult heads. Error bars represent the upper and lower limits of RQ values defined by the standard deviation of $\Delta\Delta C_T$. The data were normalized against average ΔC_T of the housekeeping gene *αTub84B.* p-values were calculated using two-tailed Student's t-test from $\Delta\Delta C_T$ of $tj^{nat/nat}$ vs $tj^{TR/TR}$ and $tj^{nat/nat}$ vs $tj^{nTR/nTR}$ samples (*p<0.05, n.s., not significantly different) B. Western blot images used to quantify TR efficiency of Tj upon exposure to various stressors. The two bands in each box represent signal from anti-Flag antibodies that show the relative abundance of Flag-tagged Tj-TR isoform in adult heads from $tj^{nat/nat}$ and $tj^{TR/TR}$.

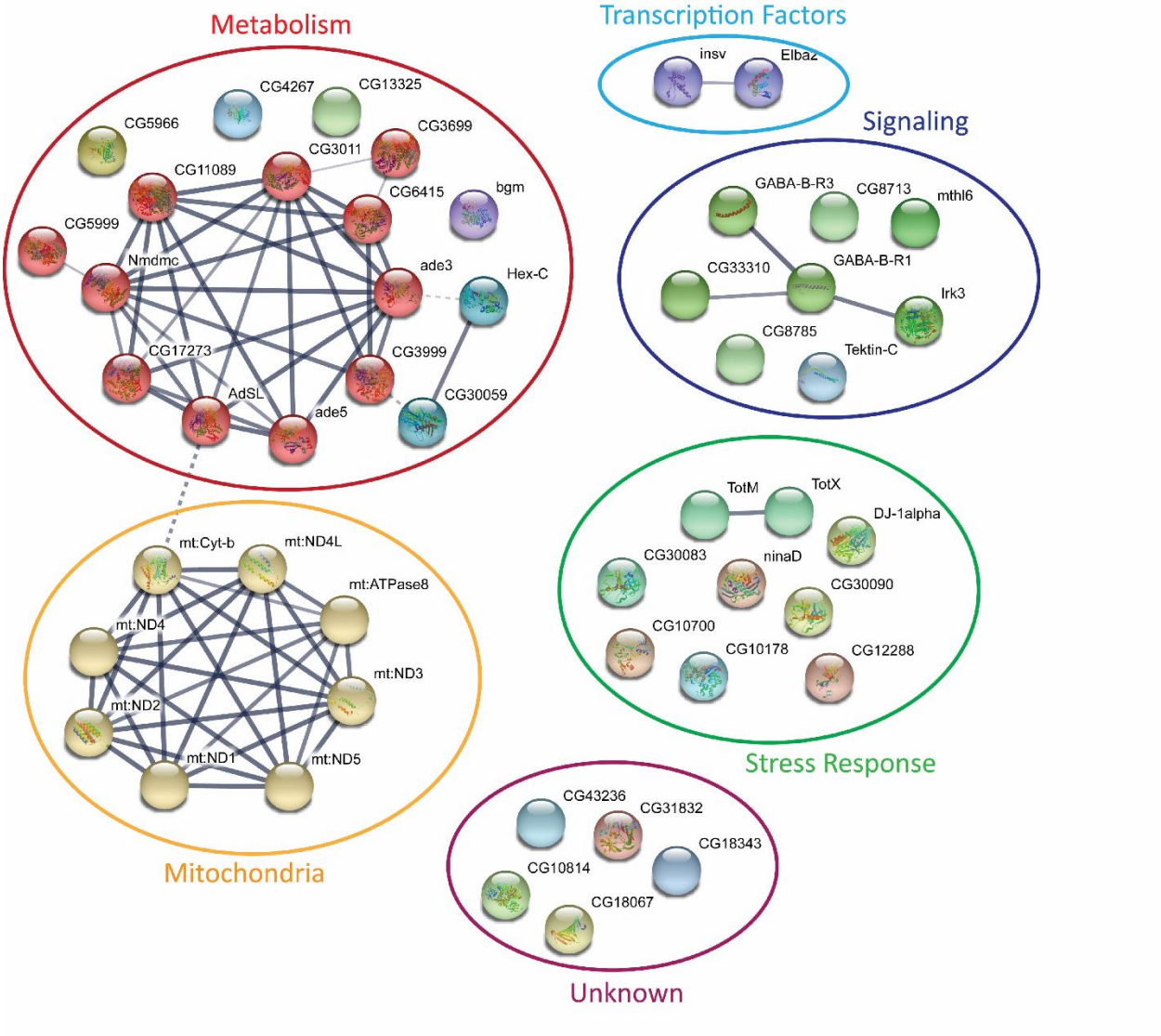

Figure S6. STRING interaction diagram of the genes deregulated in the *tj*-TR mutants. Each node represents a gene that was identified to be deregulated among the three *tj*-TR mutants in high-throughput RNA sequencing analysis of brain samples. The threshold for dysregulation, upon comparing the expression levels between each mutant was set to be >+1 or <-1 $\log_2$ fold change. Pairwise comparisons were made between $tj^{nat/nat}$, $tj^{TR/TR}$ and $tj^{nTR/nTR}$ in all permutations.

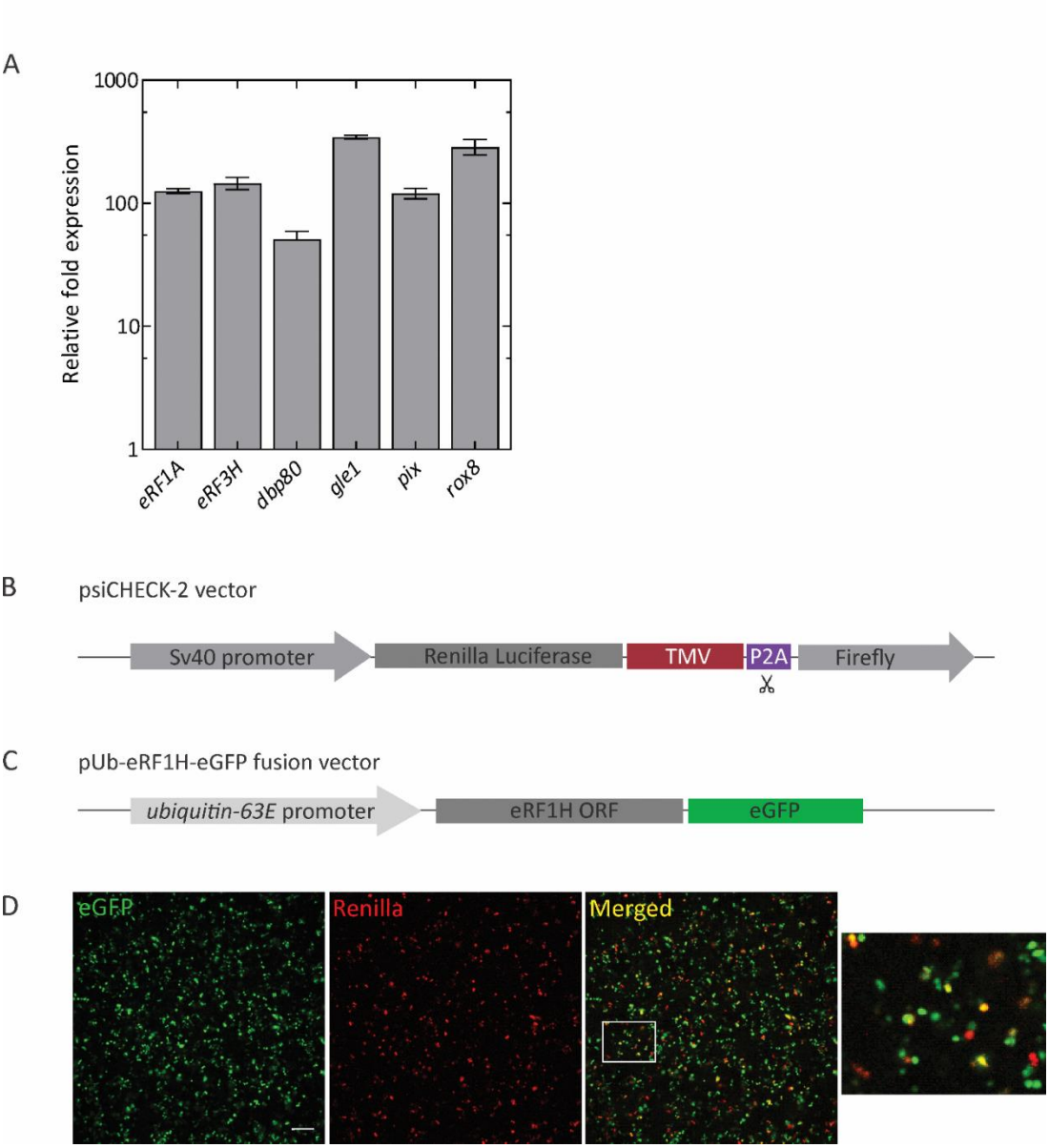

Figure S7. Expression of psiCHECK-2 and pUb vectors in S2 cells. A. RT-qPCR quantification of over-expression of various factors upon transfecting S2 cells with pUb expression vectors. The C_T values for each test transcript were normalized against the respective C_T values of *αTub84B*. The ΔC_T values obtained from transfected cells were then compared to the corresponding ΔC_T values obtained for each test transcript from untransfected cells to derive $\Delta\Delta C_T$. Error bars represent the upper and lower limit of RQ defined by the standard deviation of $\Delta\Delta C_T$ from three technical replicates. B. Construct design for psiCHECK-2 vector containing TMV test cassette. C. pUb vector expressing eRF1H-eGFP fusion product. D. S2 cells co-transfected with vectors B and C observed for eGFP fluorescence and *Renilla* luminescence. 45.4±6.3% of cells that are positive for *Renilla* luminescence express eRF1H-eGFP, while 21.7±1.6% of eGFP positive cells exhibit *Renilla* luminescence. Scale bar: 50 µm.

Table S1. List of candidates selected for TR validation

ene	Flybase ID	TR length, codons	Region Profile	Peptide feature	Expression
br-RP	FBtr0330406	131	Ala/Gly/His rich	Disordered	Embryonic/larval CNS
klu-RB	FBtr0330080	15	-	-	Embryonic neuroblasts, larval CNS
chinmo-RE	FBtr0303933	236	Thr rich	BTB domain, disordered	Embryonic/larval nervous system, eye disc, adult testes
wit-RB	FBtr0330072	10	-	-	Embryonic/larval/adult CNS, midgut, eye, salivary gland
dsx-RE	FBtr0330074	23	-	Non-cytoplasmic/ signal peptide	Embryonic gonad, embryonic/larval/adult CNS, testis
Khc-73-RE	FBtr0329957	58	-	-	Enriched in larval/pupal CNS, ubiquitous
fru-RO	FBtr0330040	187	Gln/Asn rich	Polar, disordered	Ubiquitous in embryos, larval/pupal/adult CNS
svp-RE	FBtr0331183	11	-		Embryonic neuroblasts, larval photoreceptor cells, fat body, adult optic lobe, photoreceptors
aPKC-RL	FBtr0329889	131	Asn/Gln rich	Polar, disordered	Ubiquitous in early embryos, larval/pupal/adult CNS
dlg1-RR	FBtr0330390	41	-	-	Embryonic/larval/adult CNS, salivary glands, fat bodies
tj-rC	FBtr0329891	44	-	Disordered	Gonadal somatic cells, embryonic/larval CNS

Table S2. Forced TR of Tj affects germarium and GSC niche morphology

Genotype	**Observed germaria phenotypes**			**p-value**	**Number of germaria analyzed**
	Normal	**Deformed**	**Small**		
$tj^{nat/nat}$	91%	5%	4%		128
$tj^{TR/TR}$	31%	18%	51%	$^{1}p=2.7e^{-21}$ $^{2}p=1.1e^{-15}$	146
$tj^{nTR/nTR}$	80%	10%	10%	$^{1}p=0.155$	153
Genotype	**Observed GSC niche phenotypes**			**p-value**	**Number of niches analyzed**
	Normal	**Small& Absent**	**Large&Fused**		
$tj^{nat/nat}$	96%	2%	2%		122
$tj^{TR/TR}$	33%	56%	11%	$^{1}p=8.7e^{-25}$ $^{2}p=1.5e^{-05}$	147
$tj^{nTR/nTR}$	88%	9%	3%	$^{1}p=0.215$	138

For comparison of the observed germaria phenotypes, two-way tables and χ^2-test were used.

[1] – compared to $tj^{nat/nat}$

[2] – compared to $tj^{nTR/nTR}$

Table S3. Genes identified to be dysregulated in *tj*-TR mutants

$tj^{nat/nat}$ vs $tj^{TR/TR}$			
Gene ID	Gene Name	Log2 fold change	p-value
FBgn0013673	mt:ATPase8	-2.12	4.44E-67
FBgn0013680	mt:ND2	-1.69	6.85E-47
FBgn0033792	CG13325	-1.43	2.58E-34
FBgn0029831	CG5966	-1.16	3.84E-21
FBgn0013683	mt:ND4L	-1.08	5.83E-19
FBgn0260446	GABA-B-R1	-1.03	2.58E-29
FBgn0033830	CG10814	-1.00	3.77E-20
FBgn0027348	bgm	1.02	2.72E-26
FBgn0033760	CG8785	1.03	3.57E-17
FBgn0031435	Elba2	1.08	9.90E-19
FBgn0033683	CG18343	1.31	2.24E-31
FBgn0032620	CG12288	1.33	2.17E-29
FBgn0032706	Irk3	1.71	4.87E-93
FBgn0033257	sand	1.84	3.42E-51

$tj^{nat/nat}$ vs $tj^{nTR/nTR}$			
Gene ID	Gene name	Log2 fold change	p-value
FBgn0037801	CG3999	-1.63	4.07E-36
FBgn0001187	Hex-C	-1.56	1.46E-74
FBgn0264979	CG4267	-1.46	9.77E-26
FBgn0033792	CG13325	-1.30	1.15E-23
FBgn0039241	CG11089	-1.27	1.18E-25
FBgn0038467	AdSL	-1.20	1.73E-26
FBgn0029831	CG5966	-1.16	1.57E-17
FBgn0029823	Shmt	-1.16	4.32E-28
FBgn0033885	DJ-1alpha	-1.03	1.77E-18

FBgn0035638	Tektin-C	1.04	9.79E-14
FBgn0031434	insv	1.06	6.34E-16
FBgn0260475	CG30059	1.23	6.62E-21
FBgn0035789	mthl6	1.26	6.91E-21
FBgn0031435	Elba2	1.41	7.74E-25
FBgn0000964	tj	1.43	1.85E-63
FBgn0267635	CR45973	1.64	4.59E-33
FBgn0267160	CR45600	2.12	4.80E-53

$tj^{TR/TR}$ vs $tj^{nTR/nTR}$			
Gene ID	Gene name	Log2 fold change	p-value
FBgn0033257	sand	-2.48	1.39E-53
FBgn0031701	TotM	-1.72	1.05E-26
FBgn0262881	CG43236	-1.70	8.72E-27
FBgn0032706	Irk3	-1.54	9.95E-39
FBgn0032620	CG12288	-1.52	2.91E-32
FBgn0038083	CG5999	-1.48	1.21E-26
FBgn0001187	Hex-C	-1.44	2.66E-55
FBgn0032754	CG10700	-1.42	5.76E-20
FBgn0032684	CG10178	-1.26	2.42E-23
FBgn0050090	CG30090	-1.13	1.80E-12
FBgn0034512	CG18067	-1.10	5.10E-36
FBgn0044810	TotX	-1.10	4.69E-12
FBgn0040349	CG3699	-1.09	5.51E-13
FBgn0002939	ninaD	-1.08	5.47E-12
FBgn0020513	ade5	-1.01	3.76E-20
FBgn0053310	CG33310	1.00	2.67E-10
FBgn0013688	mt:srRNA	1.00	3.25E-10
FBgn0260446	GABA-B-R1	1.09	3.45E-20

FBgn0051832	CG31832	1.10	2.44E-12
FBgn0050083	CG30083	1.31	4.84E-16
FBgn0000964	tj	1.61	6.37E-59
FBgn0013680	mt:ND2	1.74	1.17E-29
FBgn0013683	mt:ND4L	1.79	4.80E-29
FBgn0267635	CR45973	1.92	1.16E-32
FBgn0035638	Tektin-C	2.33	1.52E-51
FBgn0013673	mt:ATPase8	2.76	9.95E-76

Table S4. List of primers used for psiCHECK™-2 vector modification

Name	Primer Sequence 5' to 3'	Comments
CM135_F	ATGGCCGATGCTAAGAACATTAAG	Amplification of psiCHECK™-2 template at Fluc start
CM138_R	GTTGGTGGCGCCGGAGCCCTGCTCGTTCTTCAGCAC	Insertion of 1-18 bp of P2A after Rluc stop
CM141_F	GAGGAGAACCCCGGCCCCATGGCCGATGCTAAGAAC	Insertion of 49-66 bp of P2A before Fluc start
CM146_F	CAGGCCGGCGACGTGGAGGAGAACCCCGGCC	Insertion of 34-48 bp of P2A
CM147_R	CTTCAGCAGGGAGAAGTTGGTGGCGCCGGAGCC	Insertion of 19-33 bp of P2A
PK27_F	GCCGATGCTAAGAACATTAAGAAGGGC	Deletion of Fluc AUG from modified psiCHECK™-2 P2A constructs
PK28_R	GGGGCCGGGGTTCTCC	

Table S5. List of primers used for generating dual luciferase constructs for candidate TR genes

Name	Primer sequence 5' to 3'	Comments
PK96_F	GTGCTGAAGAACGAGCAGCTGAGCTTGTACGACG ATCGGATG	Insertion of *wit* TR motif into psiCHECK™ -2 vector with 18 bp overhang for Gibson cloning
PK97_R	GTTGGTGGCGCCGGAGCCGTTCTGCTGCATTCGA TTAGTTTATAGCTCC	
PK100_F	GTGCTGAAGAACGAGCAGAACGGAGCCTACCACC ACGG	Insertion of *dsx* TR motif into psiCHECK™ -2 vector with 18 bp overhang for Gibson cloning
PK101_R	GTTGGTGGCGCCGGAGCCGACAGCGGCCGCTGC	
PK102_F	GTGCTGAAGAACGAGCAGCAATTGCAGCCGCAAC AC	Insertion of *fru* TR motif into psiCHECK™ -2 vector with 18 bp overhang for Gibson cloning
PK103_R	GTTGGTGGCGCCGGAGCCGGGGTCATCGGGACG C	
PK104_F	GTGCTGAAGAACGAGCAGATGACACGCTCCAAGA GCC	Insertion of *khc-73* TR motif into psiCHECK™ -2 vector with 18 bp overhang for Gibson cloning
PK105_R	GTTGGTGGCGCCGGAGCCCCTGCAATTAGTCCAA CGCTGCAGC	
PK106_F	GTGCTGAAGAACGAGCAGGCAGCTGCAGCATCAG CAGCGG	Insertion of *chinmo* TR motif into psiCHECK™ -2 vector with 18 bp overhang for Gibson cloning
PK107_R	GTTGGTGGCGCCGGAGCCCTCCTTGTTGGCGTTC ATGACTACTGA	
PK108_F	GTGCTGAAGAACGAGCAGCTAACCCTGGGTGGAC CCATG	Insertion of *klu* TR motif into psiCHECK™ -2 vector with 18 bp overhang for Gibson cloning
PK109_R	GTTGGTGGCGCCGGAGCCACAGGTCATAAATGGT CTGGATGCTG	
PK110_F	GTGCTGAAGAACGAGCAGCAGCAGCAGCAACAGT C	Insertion of *br* TR motif into psiCHECK™ -2 vector with 18 bp overhang for Gibson cloning
PK111_R	GTTGGTGGCGCCGGAGCCGGAGTTGTTGAGCGCC AC	
PK114_F	GTGCTGAAGAACGAGCAGGATATGCTGCTGAGCG GCAAC	Insertion of *svp* TR motif into psiCHECK™ -2 vector with 18 bp overhang for Gibson cloning
PK115_R	GTTGGTGGCGCCGGAGCCAGTTGTTGTCAATTGG CGCCACATCGTG	
PK156_F	AATCAGCAGCAACTCTTGCAGC	UAAG to UAAA mutation in *br* TR motif using blunt end ligation
PK155_R	TTATAAGAAGTCCATGCACGGTTTGACAATGC	

PK158_F	TCGATCAGCAGCAACTCTTGCAGC	UAA to UUC mutation in *br* TR motif using blunt end ligation
PK157_R	ATAAGAAGTCCATGCACGGTTTGACAATGC	
PK160_F	AAAAGCAGCCGCAACAGC	UAGG to UAAA mutation in *chinmo* TR motif using blunt end ligation
PK159_R	TATGGTGAATGATTGCTGGCTGCC	
PK162_F	TCGAAGCAGCCGCAACAG	UAA to UUC mutation in *chinmo* TR motif using blunt end ligation
PK161_R	ATGGTGAATGATTGCTGGCTGC	
PK164_F	AAAGTATCGCAACGTTGCTGC	UAGC to UAAA mutation in *dsx* TR motif using blunt end ligation
PK163_R	TACGTGGCAGCCGTGGAG	
PK166_F	TCCAGTATCGCAACGTTGCTG	UAG to UUC mutation in *dsx* TR motif using blunt end ligation
PK165_R	ACGTGGCAGCCGTGGA	
PK168_F	AAAACAGTCAGTACCTGGGCTGGA	UGAU to UAAA mutation in *fru* TR motif using blunt end ligation
PK167_R	ATTCACTTGTGGCATTGTGCTGC	
CM183_F	TCTACAGTCAGTACCTGGGCTGGAACTACGGCG	UGA to UUC mutation in *fru* TR motif using blunt end ligation
CM184_R	ATTCACTTGTGGCATTGTGCTGCTGCTG	
PK176_F	AAATGTACCCAAAGTGTTCGCATCAG	UGAU to UAAA mutation in *khc-73* TR motif using blunt end ligation
PK175_R	ATTTACGCGCCGAAAGGTTTAGC	
PK178_F	TCTTGTACCCAAAGTGTTCGCATCAGC	UGA to UUC mutation in *khc-73* TR motif using blunt end ligation
PK177_R	ATTTACGCGCCGAAAGGTTTAGC	
PK180_F	AGGTGTCTGTATGCAGCAGC	UAAC to UAAA mutation in *klu* TR motif using blunt end ligation
PK179_R	TTAGGCGCTCTCCGTCTTGACAAC	
PK182_F	TCCGGTGTCTGTATGCAGCAGC	UAA to UUC mutation in *klu* TR motif using blunt end ligation
PK181_R	AGGCGCTCTCCGTCTTGAC	
PK188_F	AAATGCCTTCGATGTGACACACGA	UGAC to UAAA mutation in *svp* TR motif using blunt end ligation
PK187_R	AGGGCCAGGAGAAACTGTTGC	
PK190_F	TCCTGCCTTCGATGTGACACACG	UGA to UUC mutation in *svp* TR motif using blunt end ligation
PK189_R	AGGGCCAGGAGAAACTGTTGC	
PK192_F	AAAATGAGGAGGTTCTGCTGC	UAGC to UAAA mutation in *wit* TR motif using blunt end ligation
PK191_R	AGAGAATGTTGAGCAGGGAGGAGT	

PK194_F	TCCATGAGGAGGTTCTGCTGC	UAG to UUC mutation in *wit* TR motif using blunt end ligation
PK193_R	AGAGAATGTTGAGCAGGGAGGAGT	
PK545_F	TAGCAATTACAGATTGAGCTCGGCTCCGGCGCCACC	Insertion of TMV RT sequence into psiCHECK-P2A vector. Each forward primer contains the desired mutation.
PK546_F	TGACAATTACAGATTGAGCTCGGCTCCGGCGCCACC	
PK547_F	TAACAATTACAGATTGAGCTCGGCTCCGGCGCCACC	
PK548_F	TTCCAATTACAGATTGAGCTCGGCTCCGGCGCCACC	
PK549_R	TTGTGTTCCTGCGGATCCCTGCTCGTTCTTCAGCACGC	

Table S6. List of primers used for preparing constructs for CRISPR/Cas9 injections

Name	Primer sequence 5' to 3'	Comments
PK241_F	GAGAGCTTTGGCTATCGCCGCGTTTTAGAGCTAGAAATAGC	Insertion of proximal PAM site at 5' end of gRNA scaffold in pU6-BbsI-chiRNA vector
PK242_F	GACACAATGTATAAGGTAAATGTTTTAGAGCTAGAAATAGC	Insertion of distal PAM site at 5' end of gRNA scaffold in pU6-BbsI-chiRNA vector
PK243_R	GAAGTATTGAGGAAAACATA	Reverse amplification of pU6-BbsI-chiRNA for PAM insertion
PK77_F	ATATGCACACCTGCGATCGGTGAACACATCTTCGGG	Amplification of HA1+TfR from gDNA with 18 bp overhangs for Gibson assembly
PK135_R	TGAATTAGATCCCGTACGTACCTTATACATTGTGTCTAGGAAAAGC	
PK132_F	CGTACGGGATCTAATTCAATTAGAGACTAATTCAATTAGAG	Amplification of pHD-DsRed without loxP1 and attP site for HA1+TfR insertion
PK71_R	GATCGCAGGTGTGCATATGTCCG	
PK153_F	TAAGTAGAGAGCGTTCCGTGTTTAAGG	Amplifcation of pHD-DsRed for 3xFlag insertion
PK154_R	GTTGACCAGCTGCTGGGGATTC	
PK147_F	CCCCAGCAGCTGGTCAACGACTACAAGGACCACGACGGTGACTACAAGGACCACGACATCGACTACAAGGACGACGACGACAAGTAAGTAGAGAGCGTTCCG	Insertion of 3xFlag upstream of tj second stop codon by Gibson assembly
PK148_R	CGGAACGCTCTCTACTTACTTGTCGTCGTCCTTGTAGTCGATGTCGTGGTCCTTGTAGTCACCGTCGTGGTCCTTGTAGTCGTTGACCAGCTGCTGGGG	
PK207_F	TGTATGCTATACGAAGTTATAATTGGTTTGATTCCAAGAATGTTTTC	Amplification of HA2 from gDNA with 18 bp overhangs for Gibson assemby
PK208_R	ATCTTTACTAGTGCTCTTCTCGCGTGTGTTTCTTCTAG	
PK209_F	AGAAGAGCACTAGTAAAGATCTCCATGC	Amplification of pHD-DsRed for HA2 insertion
PK210_R	ATAACTTCGTATAGCATACATTATACGAAGTTATACCG	
PK83_F	CAACCGCGGGCGGAGATAGCCAAAG	Introduction of synonymous point mutations at proximal PAM site in the HA1+TfR cloned pHD-DsRed vector by QuikChange mutagenesis
PK84_R	CTTTGGCTATCTCCGCCCGCGGTTG	
PK21_F	GGAATTCTACCTCTTCCGCCAGCTGGCGG	

PK22_R	CCGCCAGCTGGCGGAAGAGGTAGAATTCC	Mutation of *tj* stop codon UGA to sense codon UUC coding for Phe by QuikChange mutagenesis
PK229_F	TAATAATAACGCCAGCTGGCGGTGG	Insertion of UAAUAAUAA after tj stop codon UGA by blunt-end ligation
PK230_R	TCAGAGGTAGAATTCCGGAGAGCTTTGGC	
PK277_F	CGAAATCTAAGAAACCGGCATCGAAG	Generation of gDNA amplicon for sequencing
PK278_R	GGTGGTAATGGGAATGCACTTCTCTTG	
PK279_F	GCGACGCACCCTGAAGAATCG	Sequencing primer for genotyping mutants

Table S7. List of primers used for qPCR

Flybase ID	Gene	Primer sequence (5' to 3')
FBgn0000964	*tj*	Forward: GGCGGTTAAATGGACGACAAT
		Reverse: AAGGACCTCAGCTTGATGTGC
FBgn0031424	*VGlut*	Forward: CCTTCGGCATGAGGTGCAATA
		Reverse: CGAGTCCACATGGCTCTCC
FBgn0003884	*αTub84B*	Forward: CACACCACCCTGGAGCATTC
		Reverse: CCAATCAGACGGTTCAGGTTG
FBgn0033257	*sand*	Forward: GGTTTATAGCACGGAACTTCAGT
		Reverse: GGTGGTCGAAGAAGCTGATGT
FBgn0027348	*bgm*	Forward: TGGACAAGATTCACGCCATTC
		Reverse: CGACCACCTGTAGTAGCCATC
FBgn0032706	*Irk3*	Forward: CTGCCACGGATTCCCTAACC
		Reverse: CCGTCTCCTTTTCGGAGGAAC
FBgn0002939	*ninaD*	Forward: TGTGGGGTGACCCAACAAAAG
		Reverse: CCCTGAGTCTATAAAGCCAGGC
FBgn0039678	*Obp99a*	Forward: TTGCCATCTGCGTGCTGATT
		Reverse: TTGGGGTACTCCCACTTCTGG
FBgn0260446	*GABA-B-R1*	Forward: AACCGCAAAAGCTGATGCTG
		Reverse: CCGTAGCAGAGCACAATTAGATT
FBgn0053310	*CG33310*	Forward: GAGCAACGCGAATCAACTAACG
		Reverse: ATCTTGGAACCCTTCACTTCATC
FBgn0053200	*VepD*	Forward: CCAGGAACATACACGCTCCAC
		Reverse: CAAGGGCCTCCCAGTGAAG
FBgn0029823	*Shmt*	Forward: CTTGACGCACGGTTTCTTCAC
		Reverse: TCTCCGGGTTCACTTTGTACG
FBgn0001187	*Hex-C*	Forward: CCCGGTGTGGACCTATTCG
		Reverse: GTGGCAGATATGCGGTCTTCA
FBgn0019940	*Rh6*	Forward: TACCTCGTCGAAGGGACTG

		Reverse: GGGAACATGGTGAACATCATCA
FBgn0023001	*melt*	Forward: CAAACGCGATCTCTCAAGAGC
		Reverse: ATCGGACTGATCTCGGAAAGC
FBgn0011739	*wts*	Forward: CAAGCAGGACCTAACCCGATT
		Reverse: CGTGTATCGCAGAGGTGTGTA
FBgn0036974	*eRF1 (*universal*)*	Forward: CACGGATAAGTCCCAGGAAGG
	eRF1A/B/C/E/F/G	Reverse: CCAATTCATCGAGCTGCATACTC
	eRF1H	Reverse: CTCATCGGCCTGTAGGGAT
	eRF1I	Reverse: GTCCACGTGCATTGCCAAC
FBgn0020443	*eRF3A/B/D*	Forward: TCAACCCCTCGGACAAAATCG
	eRF3C	Forward: CGGATCTCGCTACATTTACTCG
	*eRF3 (*universal*)*	Reverse: CCCTCCGTGAACTCAGCATC
FBgn0037249	*eIF3-S10*	Forward: CCCGCTATACGCAACGTCC
		Reverse: GGCATAGTTCCATCGCTTGTT
FBgn0034237	*eIF3-S9*	Forward: GAAGCTGAAGTTGGTCATCAACA
		Reverse: TCTGGCCTGCTTGTACTCCA
FBgn0265297	*pAbp*	Forward: GCTATGCCTACGTCAACTTCC
		Reverse: CTTGTTGCGAACCAGGTCAA
FBgn0086706	*pix*	Forward: CTGTGCATCGGTTGCGGTAT
		Reverse: TGCAGCTTGAAGGAGTTCTTG
FBgn0033316	*Gle1*	Forward: AGGATCGGGAACCTATATGGG
		Reverse: GAATCTCGTTATTGGGCTCTGG
FBgn0024804	*Dbp80*	Forward: TCTGGAGAAGAAACCGATTTCG
		Reverse: ACCAGCCCCTTTCCTAGTATTT
FBgn0005649	*Rox8*	Forward: CAGCTCTGACCGCCATGAATA
		Reverse: TGATGTCTGTCTTCGGCTGAT

Table S8. List of primers for tRNA quantification

Gene	Primer sequence (5' to 3')
Primers for cDNA preparation	
ArgUCG	GTCGTATCCAGAATTTGTTGCAACGAACAGGTCTGGATACGACTCGTGACAGG
ArgUCU	GTCGTATCCAGAATTTGTTGCAACGAACAGGTCTGGATACGACTCGAACCCGC
CysGCA1	GTCGTATCCAGAATTTGTTGCAACGAACAGGTCTGGATACGACTGACACCCGG
CysGCA2,3,4	GTCGTATCCAGAATTTGTTGCAACGAACAGGTCTGGATACGACTGGCACCCGG
TrpCCA	GTCGTATCCAGAATTTGTTGCAACGAACAGGTCTGGATACGACTACCCCGACG
PheGAA	GTCGTATCCAGAATTTGTTGCAACGAACAGGTCTGGATACGACTCGAAACCCG
18S rRNA	GTCGTATCCAGAATTTGTTGCAACGAACAGGTCTGGATACGACTCCTTCCGCA
Primers for qPCR	
ArgUCG	Forward: GGATAAGGCGTCGGACTTCG
ArgUCU1,2	Forward: GGATAGCGCGTTGGACTTCT
ArgUCU3	Forward: CTAATGGATAAGGCGTCGGATTTCT
CysGCA	Forward: GCTCAGGGGTAGAGCATTCGACTGCA
TrpCCA1	Forward: CGGTAGCGCGTCTGACTCCA
TrpCCA2	Forward: GGTAGCGCGTCCGACTCCA
PheGAA	Forward: CAGTTGGGAGAGCGTTAGACTGAA
18S rRNA	Forward: GGAAGTAAAAGTCGTAACAAGGTTTCCG
Universal	Reverse: GTTGCAACGAACAGGTCTGGATACG

Table S9. List of primers contruction of pUb expression vectors

Name	Primer sequence 5' to 3'	Comments	Flybase ID
PK528_F	GATCCACCGGTCGCCACCATG TCTGGCGAGGAAACGTC	Cloning of eRF1 isoforms into pUb vector. Forward (universal)	
PK529_R	AAAGATCCTCTAGACTAGCTA GTAATCATCTAGATCGAAGCC ATCATTG	Cloning of *eRF1-RA*	FBtr0078177
PK530_R	AAAGATCCTCTAGACTAGTTAA TAGTCATCGAGATCGACATCC TCCAG	Cloning of *eRF1-RH*	FBtr0331817
PK574_F	GATCCACCGGTCGCCACCATG GGTGATTGGGCTAAAAAAGC	Cloning of *Dbp80-RB* into pUb vector	FBtr0113715
PK575_R	AAAGATCCTCTAGACTAGTCA GAAAAGCTTCCTTTTGCC		
PK576_F	GATCCACCGGTCGCCACCATG GACGACATGATGCG	Cloning of *gle-RA* into pUb vector	FBtr0088695
PK577_R	AAAGATCCTCTAGACTAGCTAC CAAAATCCTGGAGGC		
PK578_F	GATCCACCGGTCGCCACCATG GCTTCTCTATACGTCGGTG	Cloning of *pAbp-RA* into pUb vector	FBtr0086738
PK579_R	AAAGATCCTCTAGACTAGTTAG TTGGCGGGCTCGG		
PK580_F	GATCCACCGGTCGCCACCATG TCGCGCAGAAAGGAGAAC	Cloning of *pix-RA* into pUb vector	FBtr0076557
PK581_R	AAAGATCCTCTAGACTAGTTAG TTGCAGGCTTCGTCCTC		
PK582_F	GATCCACCGGTCGCCACCATG GACGAGTCGCAAC	Cloning of *Rox8-RD* into pUb vector	FBtr0084591
PK583_R	AAAGATCCTCTAGACTAGTCAT TGGGTCTGGTATTGTGGCATC		