

Table S1 The TRiP Toolbox Stocks

TRiP Toolbox Stocks	Genotype	BL Stock#
Injection Stocks		
y sc v nanos-integrase; attP40	y[1] sc[1] v[1] P{y[+t7.7]=nos-phiC31\int.NLS}X; P{y[+t7.7]=CaryP}attP40	
y v nanos-integrase; attP40	y[1] v[1] P{y[+t7.7]=nos-phiC31\int.NLS}X; P{y[+t7.7]=CaryP}attP40	25709
y sc v nanos-integrase; attP2	y[1] sc[1] v[1] P{y[+t7.7]=nos-phiC31\int.NLS}X; P{y[+t7.7]=CaryP}attP2	25710
Gal4, UAS dcr2 Stocks		
w, elav-Gal4; UAS-dcr2	w[1118], P{w[+mC]=GAL4-elav.L}; P{w[+mC]=UAS-Dcr-2.D}2	25750
w, ms1096-Gal4; UAS-dcr2	w[1118], P{w[+mW.hs]=GawB}Bx[MS1096]; P{w[+mC]=UAS-Dcr-2.D}2	25706
w, UAS-dcr2; twist-Gal4	P{w[+mC]=UAS-Dcr-2.D}1, w[1118]; P{w[+mC]=GAL4-twi.2xPE}1	25707
w, UAS-dcr2; actin-Gal4/CyO	P{w[+mC]=UAS-Dcr-2.D}1, w[1118]; P{w[+mC]=Act5C-GAL4}25FO1 / CyO, Cy[1]	25708
w, UAS-dcr2; nanos-Gal4	P{w[+mC]=UAS-Dcr-2.D}1, w[1118]; P{w[+mC]=GAL4-nos.NGT}40	25751
w, UAS-dcr2; engrailed-Gal4, UAS-GFP	P{w[+mC]=UAS-Dcr-2.D}1, w[1118]; P{w[+mW.hs]=en2.4-GAL4}e16E, P{w[+mC]=UAS-2xEGFP}AH2	25752
w, UAS-dcr2; blistered-Gal4/CyO	P{w[+mC]=UAS-Dcr-2.D}1, w[1118]; P{w[+mC]=bs-GAL4.Term}G1	25753
w, UAS-dcr2; nubbin-Gal4	P{w[+mC]=UAS-Dcr-2.D}1, w[1118]; P{w[+mW.hs]=GawB}nubbin-AC-62	25754
w, UAS-dcr2; spalt-Gal4	P{w[+mC]=UAS-Dcr-2.D}1, w[1118]; P{w[+mW.hs]=GawB}salm[LP39]	25755
w, UAS-dcr2; Dmef2-Gal4	P{w[+mC]=UAS-Dcr-2.D}1, w[1118]; P{w[+mC]=GAL4-Mef2.R}R1	25756
w, UAS-dcr2; C96-Gal4	P{w[+mC]=UAS-Dcr-2.D}1, w[1118]; P{w[+mW.hs]=GawB}bbg[C96]	25757
w, UAS-dcr2; pannier-Gal4/TM3, Ser	P{w[+mC]=UAS-Dcr-2.D}1, w[1118]; P{w[+mW.hs]=GawB}pnr[MD237] /TM3, Ser[1]	25758

Gal4 Stocks		
MTD-Gal4	P{otu-GAL4::VP16.R}1, w[*]; P{GAL4-nos.NGT}40; P{GAL4::VP16-nos.UTR}CG6325[MVD1] (also known as MTD-GAL4)	31777
Mapping Stocks		
y sc v; Gla Bc/CyO	y[1] sc[1] v[1]; wg[Gla-1], Bc[1] / CyO, Cy[1]	
y v; Sco/CyO	y[1] v[1]; noc[Sco] / CyO, Cy[1]	
y v; TM3, Sb/TM6, Tb	y[1] v[1]; TM3, Sb[1] / TM6, Tb[1]	
y v; Ly/TM3, Sb	y[1] v[1]; sens[Ly-1] / TM3, Sb[1]	
y v; Sb/TM3, Ser	y[1] v[1]; Sb[1] / TM3, Ser[1]	
y v; Dr, e/ TM3, Sb	y[1] v[1]; Dr[1] e[1] / TM3, Sb[1]	
y sc v; Dr, e/ TM3, Sb	y[1] sc[1] v[1]; Dr[1] e[1] / TM3, Sb[1]	32261

Table S2 *D. persimilis* rescue fosmids

Fosmid clone ID	<i>D. melanogaster</i> genes covered	FBgn	Construct	Stock	Chromosome Location(s)	Rescue Observed
G727P8862D2	esg	FBgn0001981	Yes			
	CG15258	FBgn0032563				
G727P8482E4	E(spl)mdelta-HLH	FBgn0002734	Yes			
	E(spl)mgamma-HLH	FBgn0002735				
	Nf1	FBgn0015269				
	CG42261	FBgn0259146				
	CG42261	FBgn0259146				
G727P8542A9	B52	FBgn0004587	Yes	Yes		
	Task6	FBgn0038165				
	CG9588	FBgn0038166				
	lkb1	FBgn0038167				
	omd	FBgn0038168				
G727P8957F10	Sep5	FBgn0026361	No	Yes	on III	
	CSN4	FBgn0027054				
	nito	FBgn0027548				
	CG2906	FBgn0033240				
	CG2915	FBgn0033241				
	CG14763	FBgn0033243				

	CG8726	FBgn0033244				
	pcs	FBgn0033988				
	CG7639	FBgn0033989				
	CG7544	FBgn0033994				
G727P8116G11	ND75	FBgn0017566	No	Yes	on II	
	CG2147	FBgn0030025				
	sni	FBgn0030026				
G727P8171G2	CG14694	FBgn0037845	Yes			
	CG6574	FBgn0037846				
	CG14866	FBgn0038315				
	CG6276	FBgn0038316				
	CG6236	FBgn0038318				
G727P8220G11	zfh1	FBgn0004606	No	Yes		
G727P8229F12	Cbp53E	FBgn0004580	No	Yes	on III	
	CG12917	FBgn0033490				
	ste24b	FBgn0034175				
	ste24a	FBgn0034176				
	gem	FBgn0050011				
	CR30461	FBgn0050461				
	ste24c	FBgn0050462				
G727P8237E7	BBS4	FBgn0033578	Yes	Yes		
	CG13229	FBgn0033579				

	CG13231	FBgn0033580				
	CG12391	FBgn0033581				
	CG13230	FBgn0040764				
	Cyp12d1-p	FBgn0050489				
	Cyp12d1-d	FBgn0053503				
G727P8252D3	sd	FBgn0003345	Yes			
G727P8310A11	unk	FBgn0004395	No	Yes	on III	
	Hmu	FBgn0015737				
	CG3368	FBgn0039508				
	bigmax	FBgn0039509				
	CG3348	FBgn0040609				
G727P8508C2	G9a	FBgn0040372	Yes			
	CG3038	FBgn0040373				
G727P8552G3	Mipp1	FBgn0026061	Yes	No		
	CG13033	FBgn0036638				
	nxf2	FBgn0036640				
	Smn	FBgn0036641				
	mbf1	FBgn0262732				
G727P8133D3	mei-W68	FBgn0002716	Yes	Yes	on II	
	Spn55B	FBgn0028983				
	CG2064	FBgn0033205				
	CG12042	FBgn0033206				

	TBCB	FBgn0034451				
G727P8149E1	cno	FBgn0259212	No	Yes	on III	
G727P8209C9	baz	FBgn0000163	Yes	Yes	on II	Yes
	CG8918	FBgn0030823				
	CG32563	FBgn0052563				
G727P8331F10	wbl	FBgn0004003	No	Yes	on II & III	
	cora	FBgn0010434				
	CG7137	FBgn0034422				
G727P8333C5	CG6788	FBgn0030880	No	Yes	on II	
	CG15629	FBgn0031630				
	CG3225	FBgn0031631				
	CG5043	FBgn0032636				
	CG5050	FBgn0032637				
	CG5050	FBgn0032637				
	dl	FBgn0260632				
G727P8345D6	shg	FBgn0003391	Yes	Yes	on II	
	CG9350	FBgn0034576				
	cpa	FBgn0034577				
	RIC-3	FBgn0050296				
G727P8359B3	l(2)gl	FBgn0002121	Yes	Yes	on II	Yes
	CG3164	FBgn0025683				
	lr21a	FBgn0031209				

	CG4822	FBgn0031220				
G727P8401F10	CG8311	FBgn0034141	No	Yes	on II	
	CG3829	FBgn0035091				
	zip	FBgn0265434				
G727P8423G11	B52	FBgn0004587	No	Yes	on III	
	Task6	FBgn0038165				
	CG9588	FBgn0038166				
	lkb1	FBgn0038167				
	omd	FBgn0038168				
G727P8446F2	Arpc1	FBgn0001961	Yes			
	DNApol-gamma35	FBgn0004407				
	Orc5	FBgn0015271				
	RplI33	FBgn0026373				
	CG7968	FBgn0028532				
	CG7953	FBgn0028533				
	CG7916	FBgn0028534				
	CG9008	FBgn0028540				
	TM9SF4	FBgn0028541				
	CG8997	FBgn0028920				
	CG13083	FBgn0032789				
	CG10194	FBgn0032790				
	CG18094	FBgn0032791				

	CG10189	FBgn0032793				
	CG10188	FBgn0032796				
	Top3alpha	FBgn0040268				
	CG33306	FBgn0053306				
	CG33307	FBgn0053307				
	CG33649	FBgn0064115				
	mRpS23	FBgn0260407				
G727P8461B1	Tim8	FBgn0027359	No	Yes	on II	
G727P8479H2	Nab2	FBgn0028471	No		Yes	
	CG9173	FBgn0035218				
	CG5715	FBgn0039180				
	crb	FBgn0259685				Yes
G727P8481B7	yrt	FBgn0004049	No	Yes	on II	
	yellow-e3	FBgn0038150				
	yellow-e2	FBgn0038151				
	lr87a	FBgn0038153				
	yellow-e	FBgn0041711				
G727P8730B8	par-6	FBgn0026192	No	Yes	on II	Yes
	CG8188	FBgn0030863				
	CG8173	FBgn0030864				
	gce	FBgn0261703				
	chas	FBgn0263258				

G727P8733H3	alpha-Cat	FBgn0010215	Yes	Yes	on II	
G727P8776E4	CG10257	FBgn0033985	Yes	Yes	on II	
	ckn	FBgn0033987				
	aPKC	FBgn0261854				
G727P8779F6			No	Yes	on III	
G727P880D1	nrv2	FBgn0015777	No	Yes		
G727P883B7	l(3)neo18	FBgn0011455	No	Yes	on III	
	Nrx-IV	FBgn0013997				
	CG5645	FBgn0036254				
	Atg12	FBgn0036255				
	RhoGAP68F	FBgn0036257				
	CG5642	FBgn0036258				
	CG9760	FBgn0036259				
	Rh7	FBgn0036260				
	CG11534	FBgn0046296				
G727P8137E6	ref(2)P	FBgn0003231	Yes			
	osm-6	FBgn0031829				
	CoVb	FBgn0031830				
	CG11043	FBgn0031831				
	CG9596	FBgn0031832				
	CG13082	FBgn0032803				
	CG13081	FBgn0032804				

	CG10337	FBgn0032805				
	Tep4	FBgn0041180				
	CG33116	FBgn0053116				
	Ent2	FBgn0263916				
G727P8197B12	mys	FBgn0004657	No	Yes	on III	
	Upf2	FBgn0029992				
	CG1571	FBgn0029993				
	CG2254	FBgn0029994				
	Rph	FBgn0030230				
	Atg8a	FBgn0052672				
	Tango5	FBgn0052675				
G727P8470C11	CG17666	FBgn0036311	No	Yes	on III	
	CG10754	FBgn0036314				
	Atg1	FBgn0260945				
	CG42588	FBgn0260965				
	CG42709	FBgn0261674				
	Sap130	FBgn0262714				
G727P8823D9	CG6912	FBgn0038290	Yes			
	CG3984	FBgn0038291				
	CG3984	FBgn0038291				
	CG3987	FBgn0038292				
	CG6904	FBgn0038293				

G727P8387G2	CG31609	FBgn0051609	Yes			
	dm	FBgn0262656				
G727P8475E8	His3.3B	FBgn0004828	Yes			
	His3.3A	FBgn0014857				
	Ost48	FBgn0014868				
	CG7065	FBgn0030091				
	fh	FBgn0030092				
	dalao	FBgn0030093				
	Zpr1	FBgn0030096				
	CG9034	FBgn0040931				
	His3:CG31613	FBgn0051613				
	His3:CG33803	FBgn0053803				
	His3:CG33806	FBgn0053806				
	His3:CG33809	FBgn0053809				
	His3:CG33812	FBgn0053812				
	His3:CG33815	FBgn0053815				
	His3:CG33818	FBgn0053818				
	His3:CG33821	FBgn0053821				
	His3:CG33824	FBgn0053824				
	His3:CG33827	FBgn0053827				
	His3:CG33830	FBgn0053830				
His3:CG33833	FBgn0053833					

	His3:CG33836	FBgn0053836				
	His3:CG33839	FBgn0053839				
	His3:CG33842	FBgn0053842				
	His3:CG33845	FBgn0053845				
	His3:CG33848	FBgn0053848				
	His3:CG33851	FBgn0053851				
	His3:CG33854	FBgn0053854				
	His3:CG33857	FBgn0053857				
	His3:CG33860	FBgn0053860				
	His3:CG33863	FBgn0053863				
	His3:CG33866	FBgn0053866				
G727P8550H9	ash1	FBgn0005386	Yes			
	Max	FBgn0017578				
	nes	FBgn0026630				
	CG6888	FBgn0036490				
	mRpL21	FBgn0036853				
	CG9666	FBgn0036856				
	CG9629	FBgn0036857				
	CG14085	FBgn0036859				
	CG14086	FBgn0036860				
	CG14089	FBgn0036861				
	Gbs-76A	FBgn0036862				

	rept	FBgn0040075				
	CG42374	FBgn0259720				
	Bet1	FBgn0260857				
G727P8896D3	Arl1	FBgn0000115	Yes			
	brm	FBgn0000212				
	CG10516	FBgn0036549				
	CG10516	FBgn0036549				
	CG17026	FBgn0036550				
	CG17029	FBgn0036551				
	CG17028	FBgn0036552				
	CG17027	FBgn0036553				
	CG5830	FBgn0036556				
	Hip14	FBgn0259824				
	DNApol-delta	FBgn0263600				
G727P8254G11	His2Av	FBgn0001197	No	Yes	on III	
	ro	FBgn0003267				
	Rb97D	FBgn0004903				
	DIP1	FBgn0024807				
	BM-40-SPARC	FBgn0026562				
	ball	FBgn0027889				
	IntS12	FBgn0039459				
	CG5500	FBgn0039461				

G727P8779A12	os	FBgn0004956	No	Yes	on III	
	upd3	FBgn0053542				
G727P8106D5	sei	FBgn0003353	Yes			
	tsr	FBgn0011726				
	RpL39	FBgn0023170				
	Rap2I	FBgn0025806				
	gammaSnap	FBgn0028552				
	ppk29	FBgn0034965				
	CG13563	FBgn0034966				
	eIF-5A	FBgn0034967				
	RpL12	FBgn0034968				
	yki	FBgn0034970				
	CG3209	FBgn0034971				
G727P8372D3	Poc1	FBgn0036354	Yes			
	skap	FBgn0037643				
	CG11964	FBgn0037644				
	CG10038	FBgn0038013				
	CG10041	FBgn0038014				
	MBD-R2	FBgn0038016				
	CG4115	FBgn0038017				
G727P8486C3	ImpL2	FBgn0001257	Yes	Yes	on II & III	
	pav	FBgn0011692				

	CG14997	FBgn0035515				
	CG1265	FBgn0035517				
	CG15011	FBgn0035518				
	CG1309	FBgn0035519				
	CG11586	FBgn0035520				
	VhaM9.7-a	FBgn0035521				
	ago	FBgn0041171				
G727P8127C5	car	FBgn0000257	No	Yes	on II	
	car	FBgn0000257				
	Tao	FBgn0031030				Yes
G727P8143G8	cdc2c	FBgn0004107	No	Yes	on II	Yes
	CG17267	FBgn0038821				
	CG31199	FBgn0051199				
G727P8228G2	mei-41	FBgn0004367	Yes	Yes	on III	Yes
	CG1434	FBgn0030554				
	CG6847	FBgn0030884				
G727P8383H8	wee	FBgn0011737	No	Yes	on II	Yes
	nop5	FBgn0026196				
	neuroligin	FBgn0031866				
	CG13773	FBgn0042092				
G727P871G11	Zip3	FBgn0038412	No	Yes	on II	
	gish	FBgn0250823				Yes

G727P88F12	Cdk8	FBgn0015618	No	Yes	on II	
	CG1271	FBgn0035392				
	CG16753	FBgn0035393				
	CG10566	FBgn0037050				
	CG10565	FBgn0037051				
	Sk2	FBgn0052484				
	CG32485	FBgn0052485				
G727P8275B7	cora	FBgn0010434	No	Yes	on III	
	CG7137	FBgn0034422				
G727P8543F8	Atpalpha	FBgn0002921	No	Yes	on III	
G727P8822E6	CG18577	FBgn0037870	Yes	Yes		
	SdhC	FBgn0037873				
	Tctp	FBgn0037874				
	CG6672	FBgn0037875				
	CG4820	FBgn0037876				
	RpS25	FBgn0086472				

Table S3 C911 short hairpin designs

CG	FBgn	Gene	Hairpin ID	TRiP Stock ID	sense_C911	antisense_C911
CG3068	FBgn0000147	aurora	C911_SH01244.N2	C911_GL00047	ATGACGAGACGATCAAGAAG A	TCTTCTTGATCGTCTCGTCAT
CG14217	FBgn0031030	Tao-1	C911_SH03285.N	C911_HMS02205	CAGAGCTACATTGTAGACGAA	TTCGTCTACAATGTAGCTCTG
CG4488	FBgn0011737	wee	C911_SH01410.N2	C911_GL00231	CAAGAACGAGTATCTGATGAA	TTCATCAGATACTCGTTCTTG
CG17161	FBgn0261278	grapes	C911_SH01605.N2	C911_GL00220	ACGATGGGATCTGACTATCAA	TTGATAGTCAGATCCCATCGT
CG10295	FBgn0014001	PAK-kinase	C911_SH01885.N	C911_HMS01609	CACGACGACGGACGACAAGA A	TTCTTGTCGTCCGTCGTCGTG
CG7186	FBgn0026371	Sak kinase	C911_SH01169.N2	C911_GL00067	AAGGCTAGCTTACTTATCCAA	TTGGATAAGTAAGCTAGCCTT
CG14217	FBgn0031030	Tao-1	C911_SH00709.N	C911_HMS00761	CACGAGAAGCTATCTAAAGAA	TTCTTTAGATAGCTTCTCGTG
CG4488	FBgn0011737	wee	C911_SH01544.N2	C911_GL00305	CAGCAGCTGGTGTGTAAGATA	TATCTTACACACCAGCTGCTG
CG10572	FBgn0015618	Cyclin-dependent kinase 8	C911_SH01168.N2	C911_GL00020	CAAGGTGTTTGACTTGATCGA	TCGATCAAGTCGAACACCTTG
CG10895	FBgn0019686	loki	C911_SH01221.N2	C911_GL00008	CAGGATGCGATAGCTAAAGAA	TTCTTTAGCTATCGCATCCTG
CG1594	FBgn0004864	hopscotch	C911_SH01406.N2	C911_GL00244	TCCGAACATACAGAAGTTCAA	TTGAACTTCTGTATGTTGCGGA
CG1594	FBgn0004864	hopscotch	C911_SH01252.N2	C911_GL00051	CAGCGAATGTGCTGTGATCTA	TAGATCACAGCACATTGCTG
CG6498	FBgn0036511	dropout	C911_SH04743.N	C911_HMS02333	CACGATGATGTATCCAGTTAT	ATAACTGGATACATCATCGTG
CG6963	FBgn0250823	gilgamesh	C911_SH01171.N2	C911_GL00015	CGAAGACTGTAAGAAGACTTA	TAAGTCTTCTTACAGTCTTCG
CG2272	FBgn0030018	slipper	C911_SH05304.N	C911_HMC03331	ACCACCCGATCATCTAGTCAA	TTGACTAGATGATCGGGTGGT

Figure S1 The TRiP vectors.

A. First generation of TRiP RNAi vectors. The first generation of commonly used TRiP RNAi knockdown vectors commonly used, VALIUM1 and VALIUM10, are based on long dsRNAs.

VALIUM1 contains a multiple cloning site (MCS) that allows a single PCR product to be cloned in both orientations to generate the hairpin construct. Additionally, VALIUM1 contains two introns: the *white* intron, located between the inverted DNA repeats, which has been shown to reduce toxicity in bacteria; and the *ftz* intron, followed by the SV40 polyA tail to facilitate hairpin-RNA processing and export from the nucleus. VALIUM1 is an effective vector for RNAi knockdown, however, its strength can be weak but knockdown phenotypes can be boosted by using a higher experimental temperature (27-29°C) and having *UAS-Dicer2* in the genetic background (Ni et al., 2008). Based on the results with VALIUM1, we generated VALIUM10, the best performing vector from among 12 first generation vectors (Ni et al., 2009).

VALIUM10 differs from VALIUM1 in a number of ways: 1. it contains insulator sequences that increase significantly the level of expression of the hairpins; 2. instead of the MCS sites of VALIUM1, VALIUM10 contains a recombination system that facilitates the cloning of the hairpins, and 3. VALIUM10 contains two *ftz* introns. While increased temperature can increase the effectiveness of knockdown with VALIUM10, the presence of *UAS-Dicer2* makes less of a difference than with VALIUM1.

We generated versions of VALIUM10, pWALIUM10, in which *vermillion* is replaced with *white*. Except for the selectable marker, the WALIUM vector has all of the same attributes of their *vermillion* containing counterparts.

B. Second generation TRiP RNAi vectors. The second generation knockdown vectors used by the TRiP for RNAi stock production, VALIUM20 and VALIUM22 (variant: VALIUM21), carries short interfering RNA (siRNAs) hairpins embedded in a modified

scaffold of the microRNA *miR-1* that uses the endogenous microRNA pathway to deliver the short hairpin into the genome (Haley et al., 2008; 2010; Ni et al., 2011). Note, that in our design (Ni et al., 2011), unlike in Haley et al. (2008), the siRNAs do not include mismatches at positions 2 and 11. These vectors were used for generating most of the TRiP lines as they work effectively both in the germline and the soma.

VALIUM20, contains *vermilion* as a selectable marker; an attB sequence to allow phiC31-targeted integration at genomic attP landing sites; two gypsy sequences to enhance hairpin DNA transcription; two pentamers of UAS, one of which can be excised using the Cre/loxP system to generate a 5XUAS derivative; the *hsp70* basal promoter; a multiple cloning site (MCS) for cloning the short hairpins in the microRNA scaffold, and a *ftz* 3'UTR intron followed by a SV40 3'UTR as a source for a polyA signal sequence. Data from the TRiP and others show that VALIUM20 produces a more effective knockdown than VALIUM10 in the soma, and works well in the female germline (Ni et al., 2011).

VALIUM22 has each of the attributes of VALIUM20 but differs in having the *P-transposase* core promoter instead of the *hsp70* basal promoter and the *ftz* 3'UTR intron is followed by a *K10* polyA instead of the SV40 3'UTR. These unique attributes make VALIUM22 particularly effective for RNA knock down in the female germline. However, the P-element transposase promoter is less effective than the *hsp70* basal promoter to drive expression in somatic cells. VALIUM21, a variant of VALIUM22, differs only in that it lacks the *ftz* intron and gypsy sequences found in VALIUM22, however it is still highly effective in the germ line.

As for VALIUM10, we generated versions of VALIUM20 and VALIUM22, where *vermilion* is replaced with *white*, pWALIUM20 and pWALIUM22.

C. Overexpression vectors: We generated pVALIUM10-roe, pVALIUM10-moe, pWALIUM20-roe and pWALIUM10-moe. With

the latter vectors, researchers have the option to clone their genes for over-expression by recombination ("roe" versions) or in a multi-cloning site ("moe").