Figure S1. The TRiP Valium series vectors

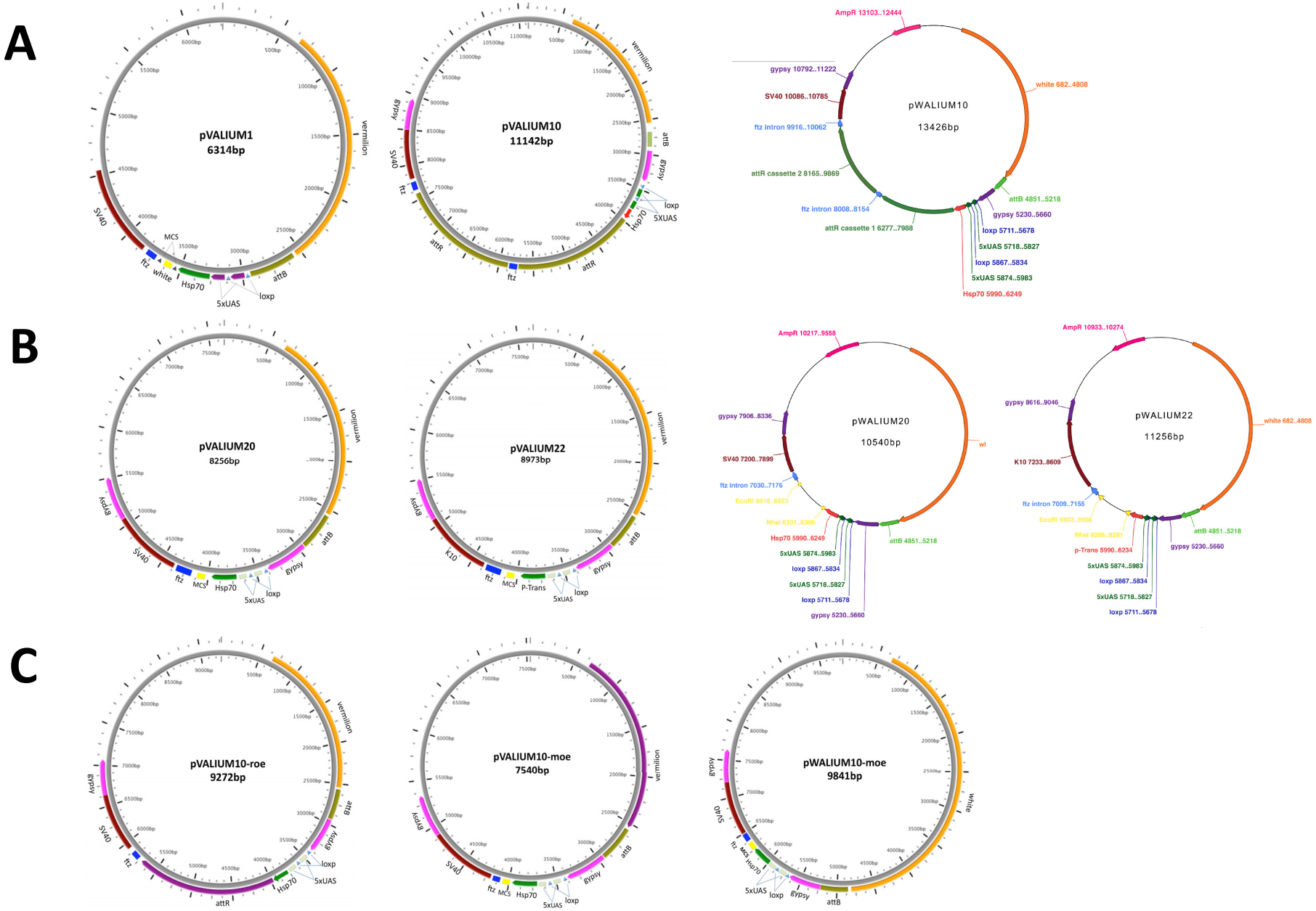

Figure S1 The TRiP vectors.

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^{o}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 *vermilion* is replaced with *white*. Except for the selectable marker, the WALIUM vector has all of the same attributes of their *vermilion* 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 mis-matches 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”).