Supplemental Methods

Phylogenetic tree building of final consensus sequences

We built Gag- and RT- based phylogenetic trees of the retained consensus non-LTR retrotransposons using MrBayes 3.2.6 (Ronquist et al. 2012), implemented in Geneious (Kearse et al. 2012). We determined the amino acid substitution model using ProTest 3.4.2 (Darriba et al. 2011). We performed MCMC analysis over 1,100,000 generations. We estimated convergence of the joint posterior probability density each 200 generations with a 25% burn-in of the sampled chains. We stopped the analysis once the maximum standard deviation of split frequencies was < 0.001. The *D. melanogaster* reference jockey element served as the outgroup. We built phylogenies of Gag and RT domains defined by our pipeline using the majority-rule consensus sequences only (Fig. 1, Supplemental Table S1, Supplemental Fig. S3).

Cytogenetics of telomeric and non-telomeric mobile elements

We first dissected salivary glands from third-instar larvae in a PBS-Tween 0.1% solution. We then transferred salivary glands into fresh 45% acetic acid and 2% paraformaldehyde for one minute on a coverslip treated with Sigmacote® (Sigma-Aldrich). We lowered Poly-L-lysine (Sigma-Aldrich) treated slides onto the coverslip and spread the polytene chromosomes by tapping the slide with a rubber hammer. We flash-froze the slides in liquid nitrogen and transferred into 100% ethanol (-20^{o}C) for 10 minutes. To prepare chromosomes for probe hybridization, we washed twice in PBS-Tween 0.1% for 5 minutes (min) and then transferred slides in successive pre-hybridization solutions (5 minutes in 2X SSC plus 0.1% Tween, 5 minutes in 2X SSC plus 0.1% Tween and 50% formamide, 2.5 min at 92 ^{o}C in 2X SSC plus 0.1% Tween and 50% formamide, and then 20 min at 60 ^{o}C in 2X SSC plus 0.1% Tween and 50% formamide).

We labeled DNA FISH probes either with DIG dUTP (Sigma-Aldrich cat#11093274910, HeT-A, TART, TARTAHRE) or used Oligopaints (TR2, Supplemental Table S9). We diluted probes (15μg/ml for PCR DIG probes and 2μg/ml for Oligopaints) in a hybridization buffer (50% formamide, 25% Dextran Sulfate, 10μg of RNase A and 12.5% of ddH2O), denatured probes for 2.5 min at 95°C and then incubated on ice for 2 minutes. We performed hybridization overnight at 50°C for PCR-DIG probes and 37°C for Oligopaints. We removed unbound probes with two 10 minute washes in 2X SSC at 60°C and three 10 minute washes in PBS at room temperature. We stained PCR-DIG probes for 1 hour with anti-Dioxigenin-AP Fab fragments (Sigma-Aldrich, cat# A-21436, diluted 1:250 in PBST+BSA 3%) and then anti-Sheep IgG (H+L) Alexa Fluor 555 conjugated secondary antibody (Invitrogen™, diluted 1:500 in PBST+BSA 3%) for 1 hour. For Oligopaints, we followed the (Beliveau et al. 2015) protocol for secondary incubation. We visualized hybridization experiments on a Leica SP8 confocal microscope.

DNA preparation for single molecule-based sequencing

We detected no evidence of retrotransposons related to TAHRE, TART, TARTAHRE, or TR2 in the (short) raw reads of *D. biarmipes.* To define the set of telomeric genetic elements found in this unusual species, we conducted single-molecule sequencing using the Pacific Biosciences platform. We prepared high quality DNA using Qiagen Blood and Cell culture DNA MIDI Kit according to (Chakraborty et al. 2018) with some modifications. Specifically, we collected 150mg of tissue from 200 zero to two-day old, whole females. We held the females in empty vials for two hours prior flash-freezing in liquid nitrogen and grinding to a fine powder. We transferred the sample into 9.5ml of buffer G2 with addition of 38μl of RNase-A (100mg/ml) and 250μl of protease (0.75AU). We homogenized the sample and then incubated at 50°C overnight with gentle shaking. The next day, we centrifuged the sample at 5000×g for 10 min at 4°C. We decanted the supernatant into a fresh 15ml tube, vortexed, and applied it to the anion exchange

column. We washed the column and precipitated genomic DNA with 0.7 volume of isopropanol resuspended in Tris buffer (pH 8) and stored at 4^{o}C overnight. Genewiz (South Plainfield, NJ) prepared a 20 kb PacBio SMRTbell library with Blue Pippin size selection per manufacturer's instruction.