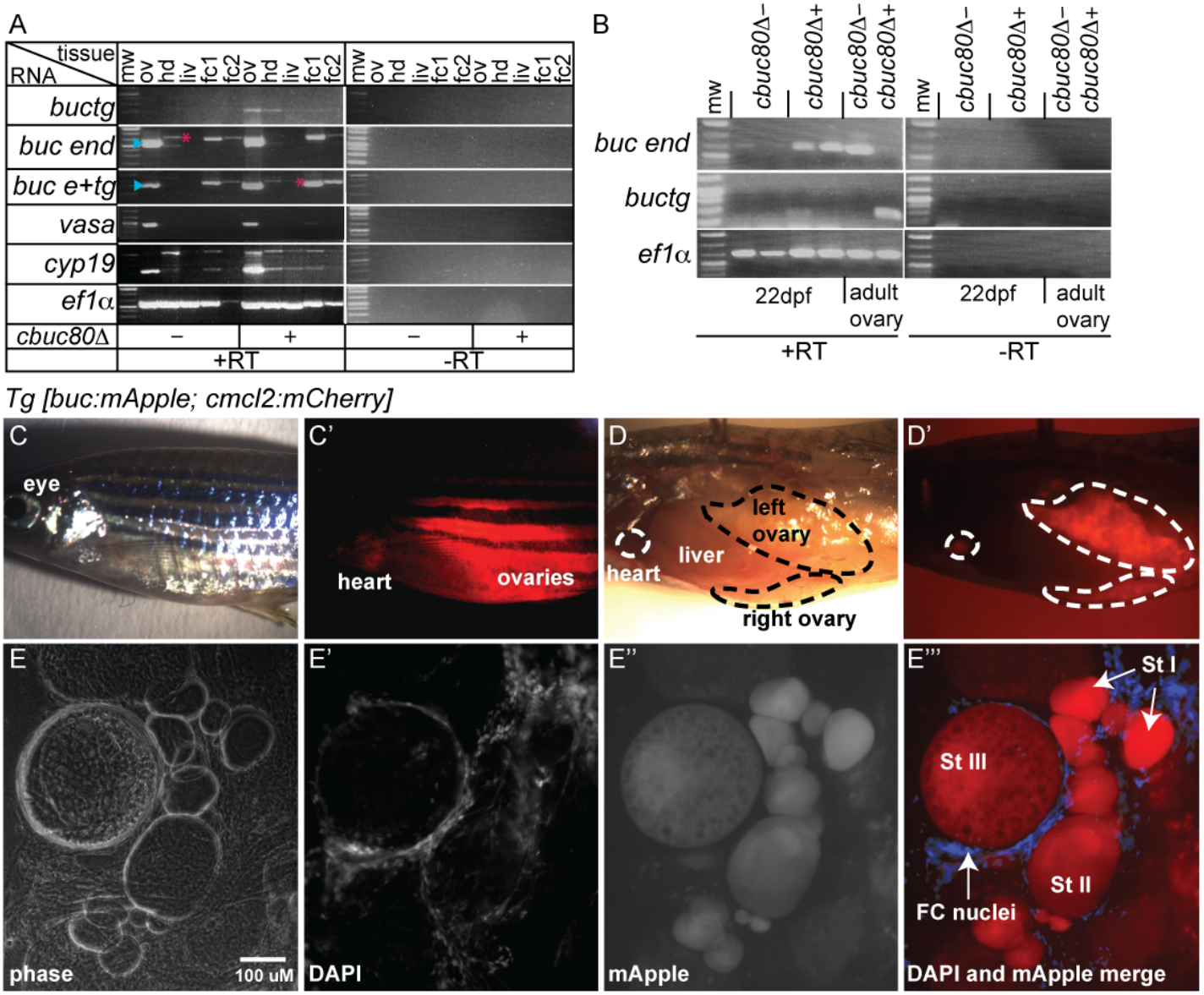

Figure S1. *bucky ball* promoter drives expression in the germline and transgenic *bucky ball* transcripts show similar expression profiles to endogenous *buc* transcripts. A) Images of DNA gels show RTPCR products amplified from cDNA (+RT) produced from various tissues of *cbuc80* transgene negative (*cbuc80-)* and positive (*cbuc80+)* F1 females. –RT indicates negative control for genomic contamination. Molecular weight ladder (mw), ovary (ov), head (hd), liver (liv), follicle cell (fc). *buctg* primers amplify transgenic transcripts, *buc end* primers amplify only *endogenous buc, buc e+tg* primers amplify both endogenous and transgenic transcripts. Aqua arrowhead indicates *buc* and pink asterisks indicate a nonspecific band amplified in some tissues (both were verified by sequencing). B) Images of DNA gels show RTPCR products amplified from cDNA (+RT) produced from juvenile and adult ovary of transgene negative (*cbuc80-)* and positive (*cbuc80+)* F1 females. C-E''') Transgenic F1 female expressing mApple under the control of the *buc* promoter. C-C′) Red fluorescent proteins mCherry (heart) and mApple (ovary) detected through the body wall. D-D′) *In situ* dissection of the *Tg* female revealed broad expression of mApple in early-stage oocytes throughout the ovaries. (E-E''') Whole-mount microscopy of dissected ovary demonstrates expression of *buc* promoter in early oocytes (stages I-III) (E"-E'''), but not in adjacent follicle cells (FC) labeled by DAPI (E′ and E''').

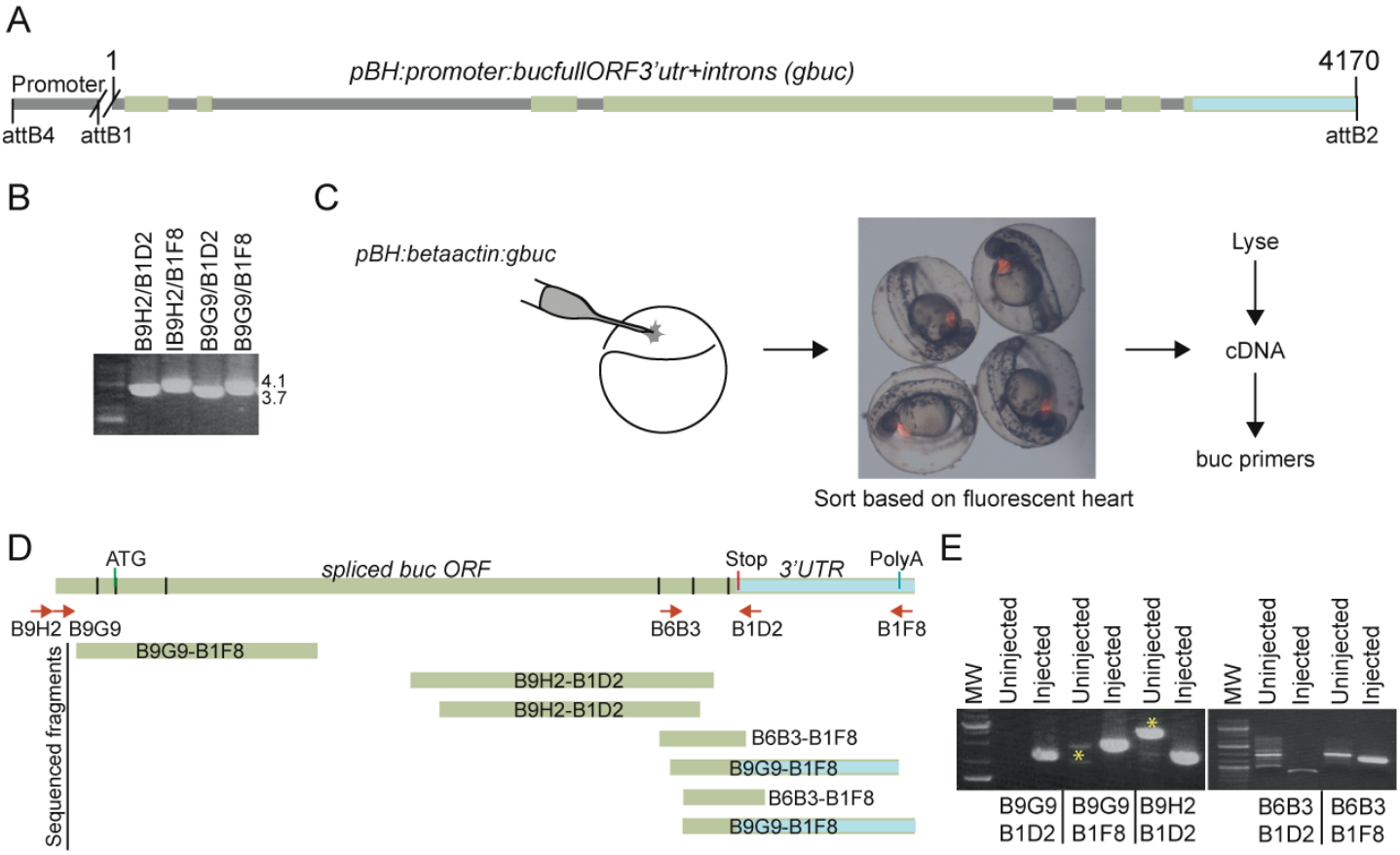

Figure S2. *bucky ball* constructs comprised of exons and introns are spliced correctly. A) Schematic of constructs used to generate *bucky ball* transgenics with introns and exons of *bucky ball*. Promoters used were *beta-actin* and *bucky ball*. B) Gels showing PCR products amplified from genomic DNA using primers within the 5′UTR and *3′UTR* of the *bucky ball* gene to obtain versions of *buc* with the introns intact and either full length or a truncated *3′UTR*. C) Assay for splicing of *buc* plasmids including exons and introns. The *pBH: β-actin:gbuc* construct was injected into single cell embryos along with transposase RNA. At 2dpf (a stage when endogenous *buc* transcripts are not detected), larvae with the integrated transgene were selected based on their fluorescent hearts and used to generate cDNA to examine the transcripts produced from the *buc+introns* construct. D) Schematic of spliced *buc* obtained from *pBH: β-actin:gbuc*. E) PCR fragments amplified from cDNA of *pBH:β-actin:gbuc* expressing embryos were as expected for properly spliced *buc*. Yellow asterisks indicate non-specific products amplified in the absense of *buc* as determined by sequencing.

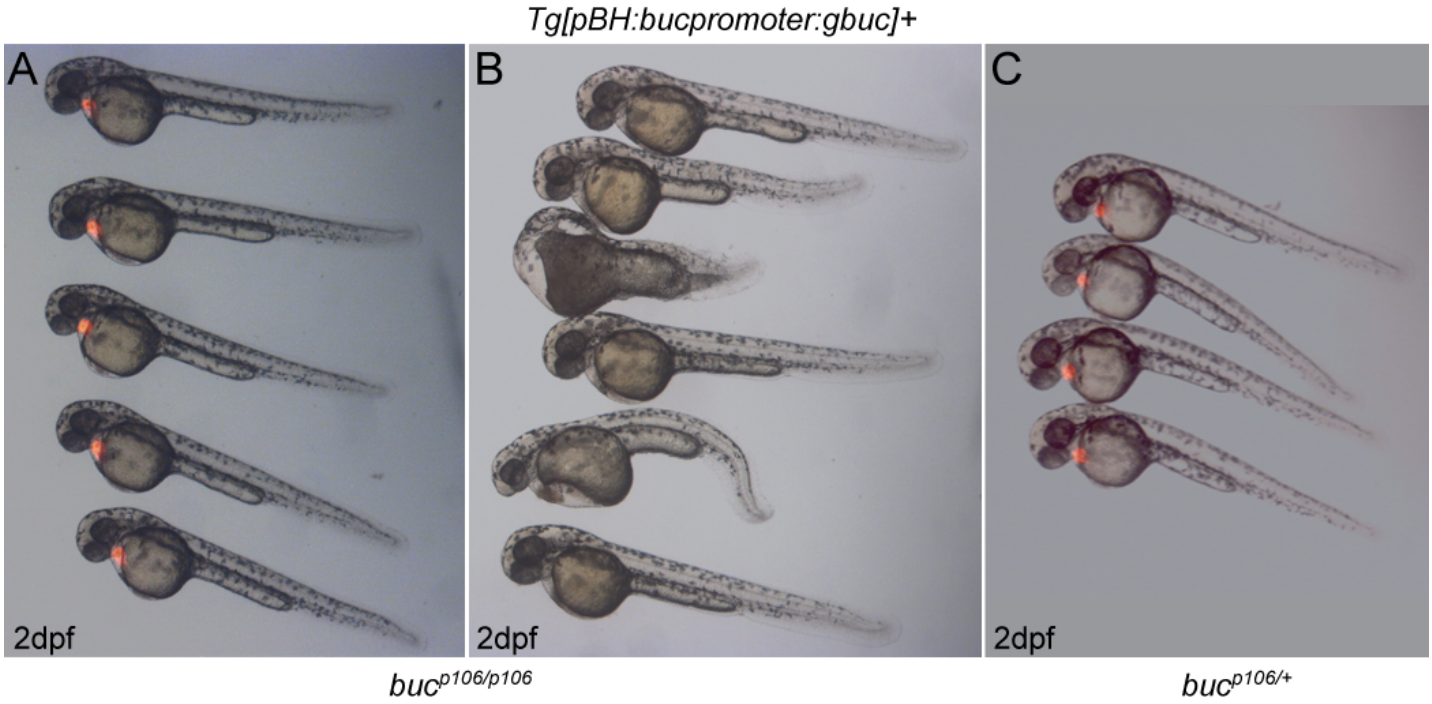

Figure S3. Progeny of *buc* mutant females are rescued by maternally supplied *buc* with introns. A-B) F2 embryos of a *bucky ball* mutant female bearing a transgene with the full *buc* coding sequence and introns. A) Half of the rescued progeny express the zygotic bleeding heart (red fluorescent protein in the heart) reporter; B) whereas, the other half do not express the zygotic reporter. C) Progeny of a F1 transgene positive *bucky ball* heterozygote expressing the bleeding heart reporter at 2 days post fertilization (dpf).

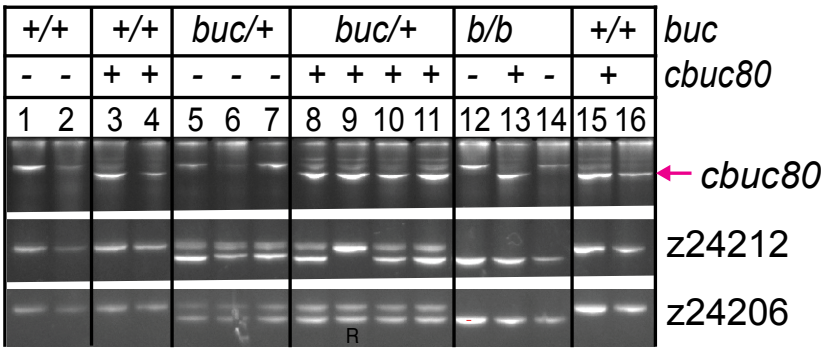

Figure S4. Representative PCR based genotyping assays. F1 females were genotyped for the presence of the transgene (arrow), and their genotype at the *buc* locus (z24212 and z24206). The numbers above the gel images correspond to the numbers above the bars in Fig. 3L. R denotes a recombinant fish.

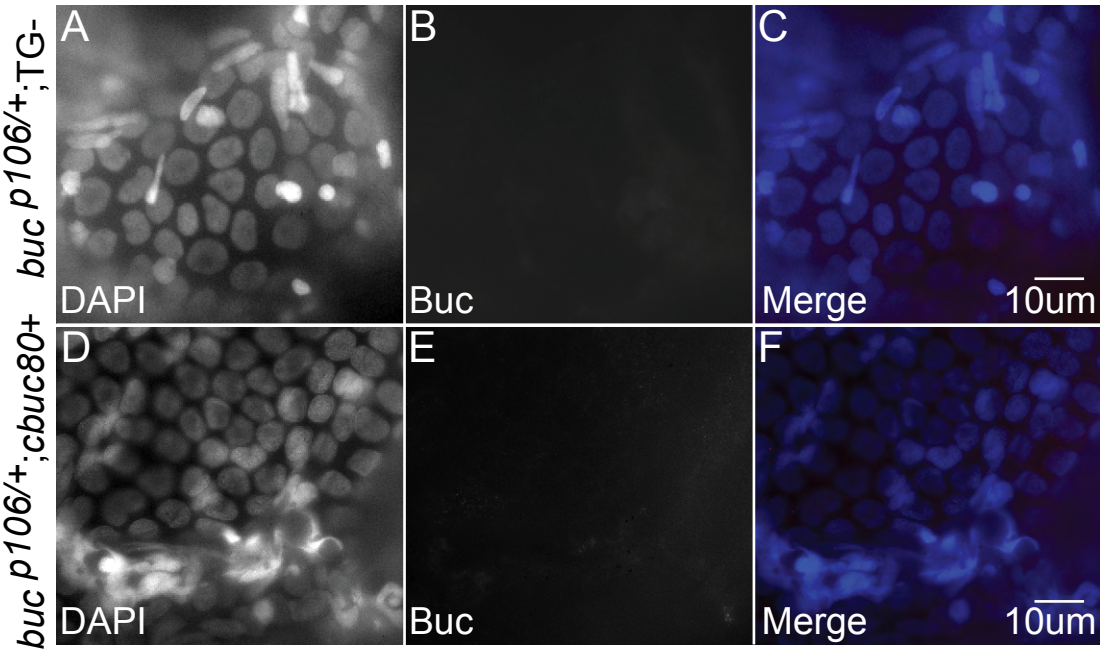

Figure S5. Buc protein is not detected in somatic follicle cells. Images of oocytes from *cbuc80* transgenic founder mothers. A-C) transgene negative or D-F) *cbuc80* positive females. 40x apotome images.

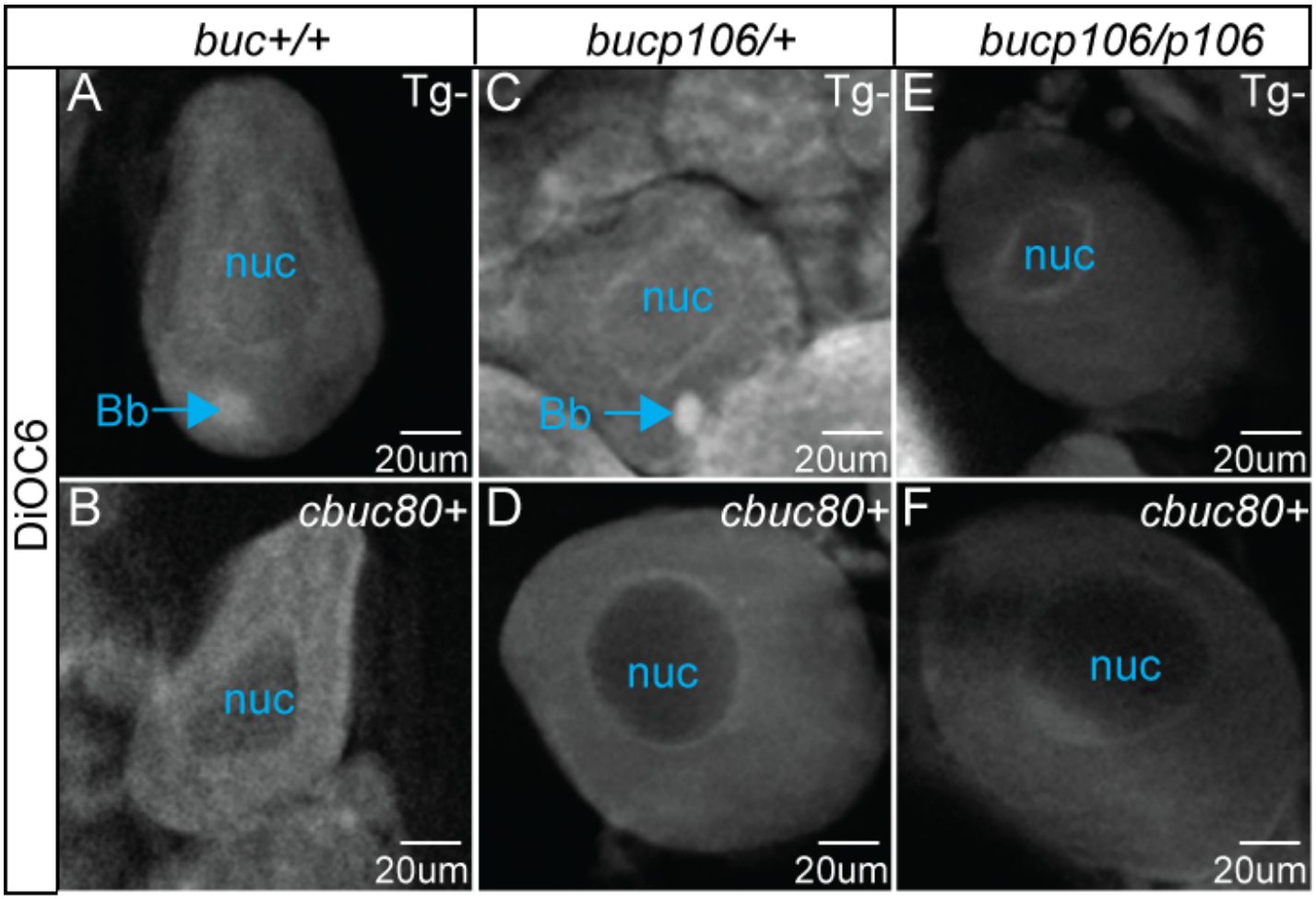

Figure S6. Ectopic Balbiani body formation requires the 3′UTR. Images of DiOC6 labeled oocytes from *cbuc80* transgenic F1 mothers. The animal (An) and vegetal (Vg) axes of eggs with normal polarity are marked accordingly. A-F) DiOC6 labels the Balbiani bodies (Bb) of A) homozygous WT and C) $buc^{p106/+}$ heterozygous females. Bb formation is disrupted in both B) homozygous WT and D) $buc^{p106/+}$ heterozygous genotypes when the *cbuc80* transgene (Tg) is present and E, F) in $buc^{p106/p106}$ homozygous mutants regardless of transgene status. Nuc denotes the nucleus. 40x apotome images.

Table S1. Primers

Cloning primers	**Sequence**
bucky ball ATG	5′-ATGGAAGGAATAAATAACAATTCA-3′
bucky ball 80bp reverse	5′-CCATGTGTACATTTATAGTGAAGTGC-3′
bucky ball Promoter forward	5′-CAACATGACTTCGGCAGCTA-3′
bucky ball Promoter reverse	5′-CTGAACCGTACACACAGAATCC-3′
buc 2K prom attB4 F	5′-GGGGACAACTTTGTATAGAAAAGTTGATGCTATCCCTGCATTGGTC-3′
buc prom attB1R	5′-GGGGACTGCTTTTTTGTACAAACTTGCTGAACCGTACACACAGAATCC-3′
bucky ball 3′UTR forward	5′-GAGGGGCAGAGGTTCTATGA-3′
bucky ball 3′UTR reverse	5′-GCAATGCGGAAACTTTAATGA-3′
bucky ball full3′utr transgene reverse (diagnostic and RT)	5′-CGAATTCGCCCTTGCAATGCG-3′
buc80bpΔ transgene diagnostic (for genomic DNA and RTPCR)	5′-AGAAGGTCGACTTGAAAATCAATCCACCAGCAAAGGC CAAGAAGTGAA-3′
bucky ball 5′UTR forward	5′-CGATAGGCCTGTTGGGTAGA −3′
bucky ball exon 1 forward	5′-TGGATCTCTGGAAACAGACG-3′
bucky ball exon 5 forward	5′-GAGAGAGGGGGTTGTGTCTG-3′
R4/R2 cassette F	5′-GTTTAAACGGGCCCTATCAACTTTGTATAG-3′
R4/R2 cassette R	5′-TTATCGATAAGCTTACATCAACTTTGTACA-3′
p106 genotyping- for sequencing (bucintron5/6 F)	5′-TGTGTGTGTCTCTGTGTGAAG-3′
QRT-PCR primers	**Sequence**
bucky ball both forward	5′-CAAGAGCGCCAGAGTGTCTT-3′
bucky ball both reverse	5′-CCATGTGTACATTTATAGTGAAGTGC-3′
bucky ball endogenous forward	5′AGAAGGTCGACTTGAAAATCAATCCACCAGCAAAGGCCAAGAAGTGAA-3′
bucky ball endogenous reverse	5′-GCAATGCGGAAACTTTAATGA-3′
bucky ball80Δ transgene reverse	5′-GAATTCGCCCTTTCCATGT-3′
EF1alpha forward	5′-AGCCTGGTATGGTTGTGACCTTCG-3′
EF1alpha reverse	5′-CCAAGTTGTTTTCCTTTCCTGCG-3′
vasa forward	5′-GTGGAAAGATTGGCCTGAGTAA-3′

vasa reverse	5′-CAAGGCGAAAATCACTGAGAG −3′
RNA IP primers	
dazl forward	5′-ATGGTTCAGGGGGTTCAGTTACC-3′
dazl reverse	5′-CTACATAAGGGTTAGCAAAGTCTGCAC-3′
bucky ball forward	5′-GAGGGGCAGAGGTTCTATGA-3′
bucky ball reverse	5′-GCAATGCGGAAACTTTAATGA-3′
vasa forward	5′-GTGGAAAGATTGGCCTGAGTAA-3′
vasa reverse	5′-CAAGGCGAAAATCACTGAGAG −3′
nanos forward	5′-AGCGGACATTGATGCTCCG-3′
nanos reverse	5′-CACAGGAAACAGCTATGACCATGA-3′
Y2H baits and prey	**Sequence**
bucforBaitF	5′-ATGGAAGGAATAAATAACAATTCA-3′
bucforBaitΔ1-252F	5′-AGCCAAAATGAAATGTCTGTCTGC-3′
bucforBaitR	5′-CCATGTGTACATTTATAGTGAAGTGC-3′
bucforBaitΔ1-386F	5′-CAAGAGCGCCAGAGTGTCTT-3′
bucAccI_F	5′- CGAGAGATTGACCCACTTGTCTCGACTTGTAAATCAA TCCA CC-3′
bucAccI_R	5′- GGTGGATTGATTTACAAGTCGAGACAAGTGGGTCAAT C TCTCG-3′
bucSmaDra_F	5′- CCAAATTAGAGTCTTCGTTTCCGGGAAAACCGTAATG GCC-3′
bucSmaDra_R	5′- GGCCATTACGGTTTTCCCGGAAACGAAGACTCTAATT TGGA GG-3′
rbpmsGWY2Hatg	5′-ATGAGTGTCAAGTCCGACTC-3′
rbpmsGWY2Hstop	5′-TTAACAGAACTGTCGGGATTTCC-3′