

Figure S1. Functionality of the viral P2A peptide in *Tribolium castaneum*

Bicistronic expression of membrane-localized YFP (in green) and Histone2B-mCherry, mediated by the viral P2A peptide in blastoderm and early germband stage embryos of *Tribolium castaneum*. The two proteins are expressed from the same open reading frame, separated by the PTV1 peptide (Szymczak-Workman et al., 2012). The transgene was stably integrated in the genome. The distinct localization of membrane-YFP and Histone2B-mCherry proteins in the plasma membrane and chromatin, respectively, indicates that the P2A peptide is functional (mediates ribosomal skipping) in *Tribolium*.

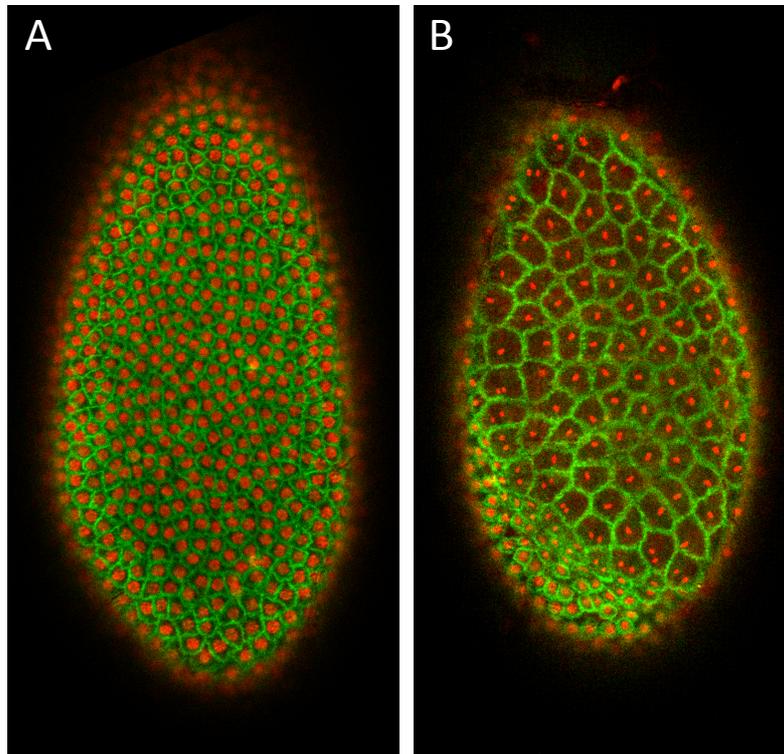


Table S1. Frequency of marked cell clones. The number of distinct clusters of H2B-mCherry-expressing cells was scored per embryo. We classified embryos in four categories: embryos with more than 10 clusters per embryo, 2-10 clusters, one cluster, or none. Each cluster was taken to represent a cell clone.

| | | >10 clones | 2-10 clones | 1 clone | no clones |
|---|--------|------------|-------------|---------|-----------|
| A. Embryos with marked clones after 10 min heat shock at 46°C | | | | | |
| <i>Valcyrie.LR #22</i> | (n=19) | 5% | 68% | 11% | 16% |
| <i>Valcyrie.LR #39*</i> | (n=15) | 60% | 0% | 0% | 40% |
| <i>Valcyrie.Uni #6</i> | (n=22) | 0% | 5% | 18% | 77% |
| <i>Valcyrie.Uni #11</i> | (n=17) | 0% | 59% | 12% | 29% |
| B. Embryos with marked clones after 10 min heat shock at 44°C | | | | | |
| <i>Valcyrie.LR #22</i> | (n=21) | 0% | 10% | 19% | 71% |
| <i>Valcyrie.LR #39*</i> | (n=11) | 9% | 18% | 9% | 64% |
| <i>Valcyrie.Uni #6</i> | (n=19) | 0% | 0% | 0% | 100% |
| <i>Valcyrie.Uni #11</i> | (n=20) | 0% | 0% | 10% | 90% |
| C. Effect of post-heat-shock temperature (after 10 min heat shock at 46°C, <i>Valcyrie.LR #22</i>) | | | | | |
| 32°C | (n=20) | 15% | 40% | 10% | 35% |
| 25°C | (n=20) | 0% | 25% | 15% | 60% |

* The *Valcyrie.LR #39* line is heterozygous, therefore approximately 50% of the embryos in these experiments did not carry the *Valcyrie.LR* construct.