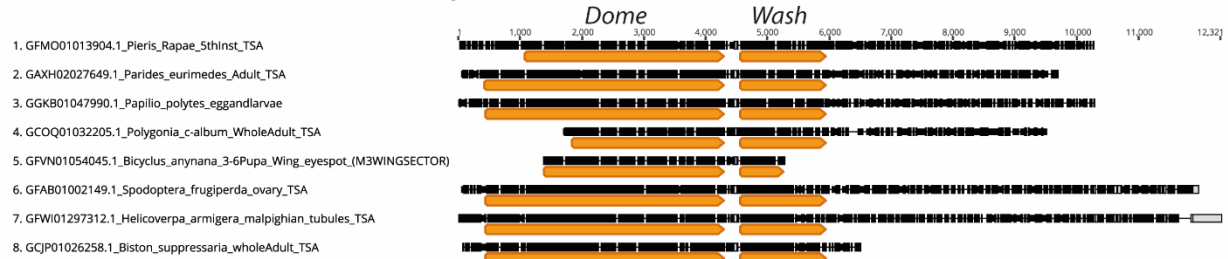
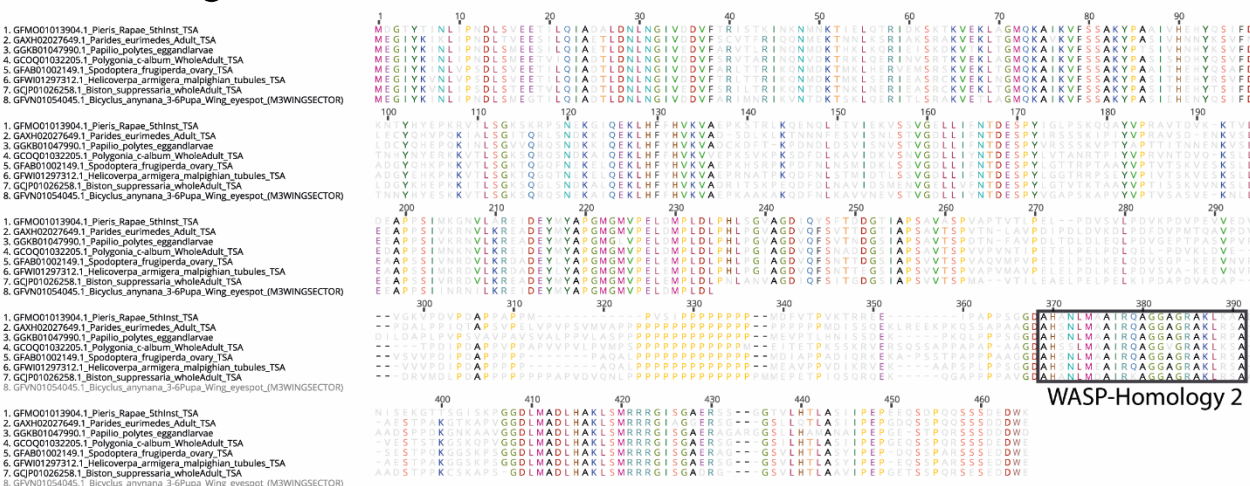


ORFs found in TSA transcripts



Washout Alignment



Domeless Alignment

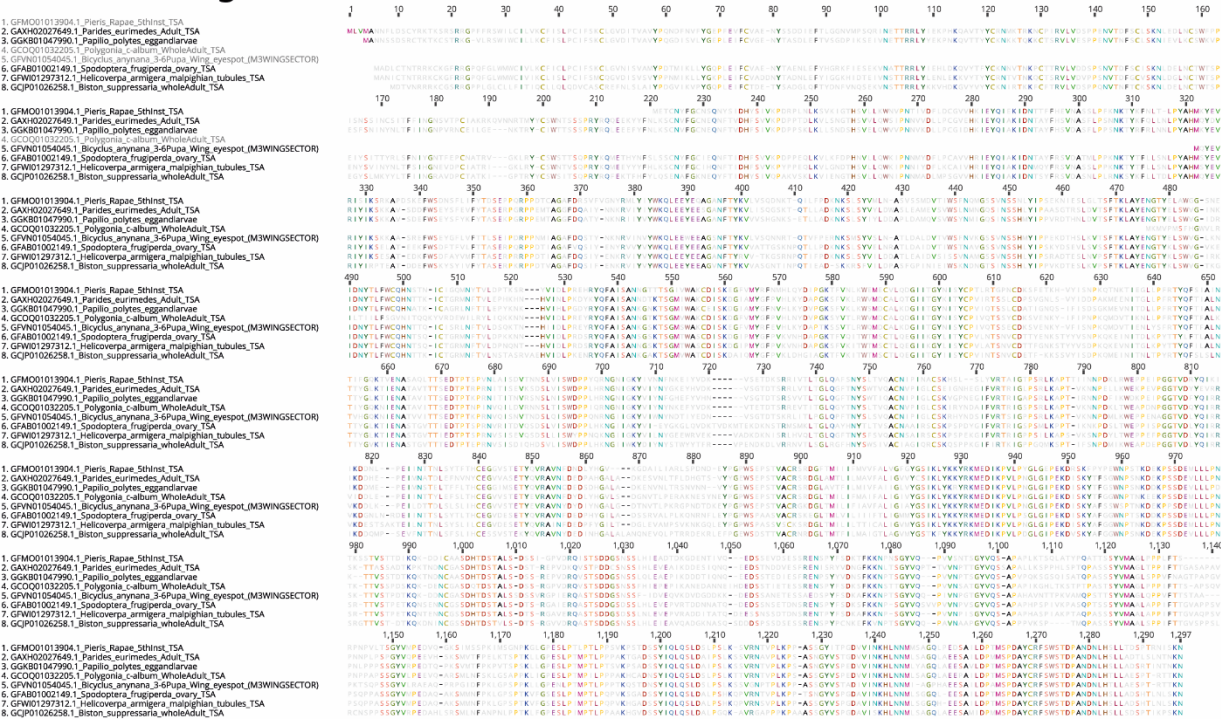


Figure S2.1: To examine the conservation of *dome/wash* bi-cistronic transcription in the Lepidoptera we performed BLASTn searches using the previously annotated *dome* transcripts from the *H. melpomene* genome (Hmel2) found on Lepbase (Challi et al., 2016), against the Transcription Shotgun Assembly (TSA) sequence archive on NCBI. We recovered several assembled transcripts containing both the *dome* and *wash* ORFs in various divergent lepidoptera. The positions of *dome* and *wash* ORFs are shown (arrows blocks in TSA transcript) as well as the encoded Wash and Dome proteins below.

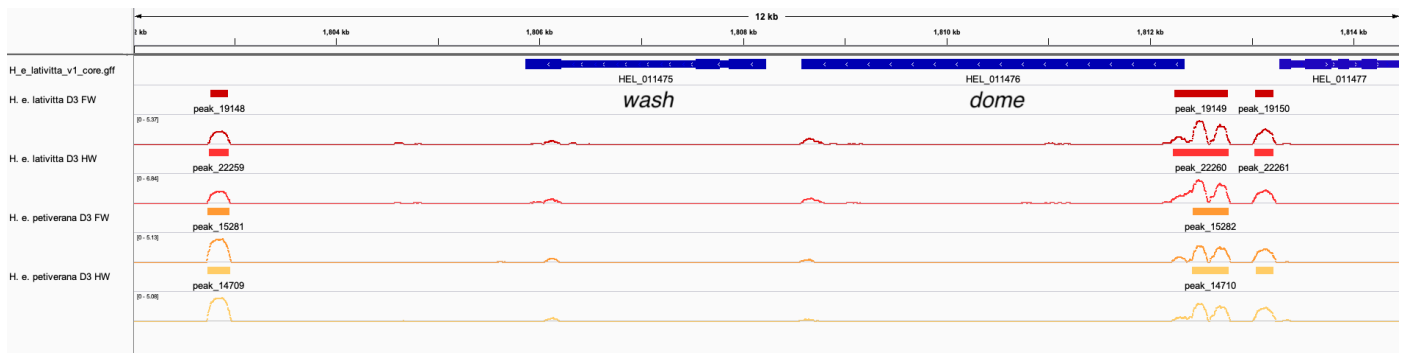


Figure S2.2: ATAC-seq analysis supports *dome/wash* bi-cistronic transcription in *Heliconius erato*. We explored whether *dome/wash* share a promoter by analysing published ATAC-seq data from (Lewis *et al*, 2019). We analysed samples corresponding to the forewings and hindwings of day three pupa of *H. e. lativitta* and *H. e. petiverana*. Peaks were called using Genrich (<http://github.com/jsh58/Genrich/>), using the parameter -j (ATAC-seq mode) with a cutoff of $q < 0.05$ ($q = \text{FDR adjusted } p\text{-value}$). Peaks were visualised in IGV. Coloured blocks correspond to significant peaks and lines represent $-\log(q)$. Peaks, indicating *cis*-regulatory activity, were observed upstream of *dome* for all samples whereas no peaks were present upstream of the start of *wash*, indicating a shared promoter for *dome/wash*.