SUPPLEMENTAL FIGURES

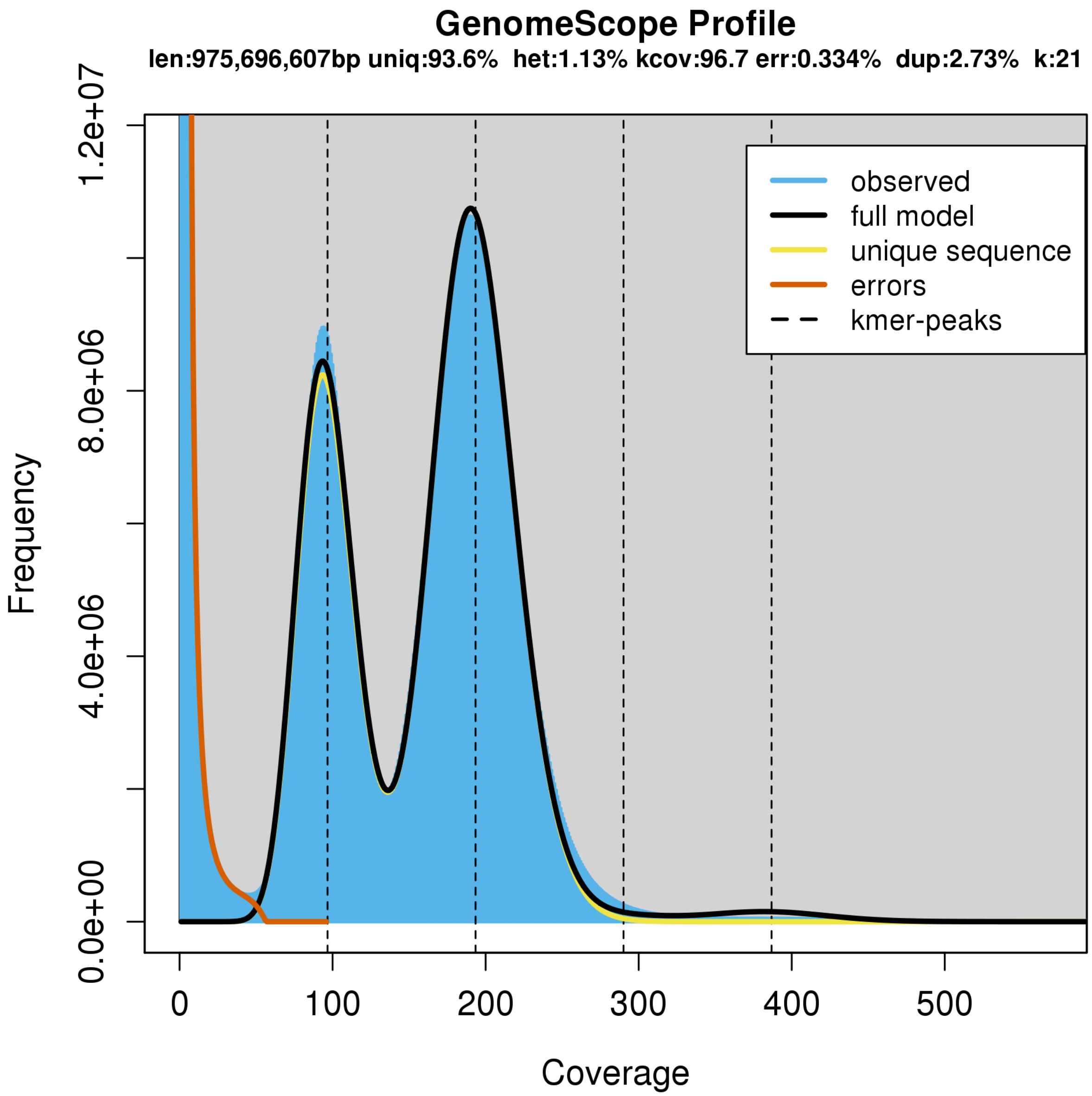

Figure S1. ***GenomeScope plot.*** *Coverage and k-mer frequency plot using the Illumina gDNA reads and the MaSuRCA assembly of the ruby-throated hummingbird.*

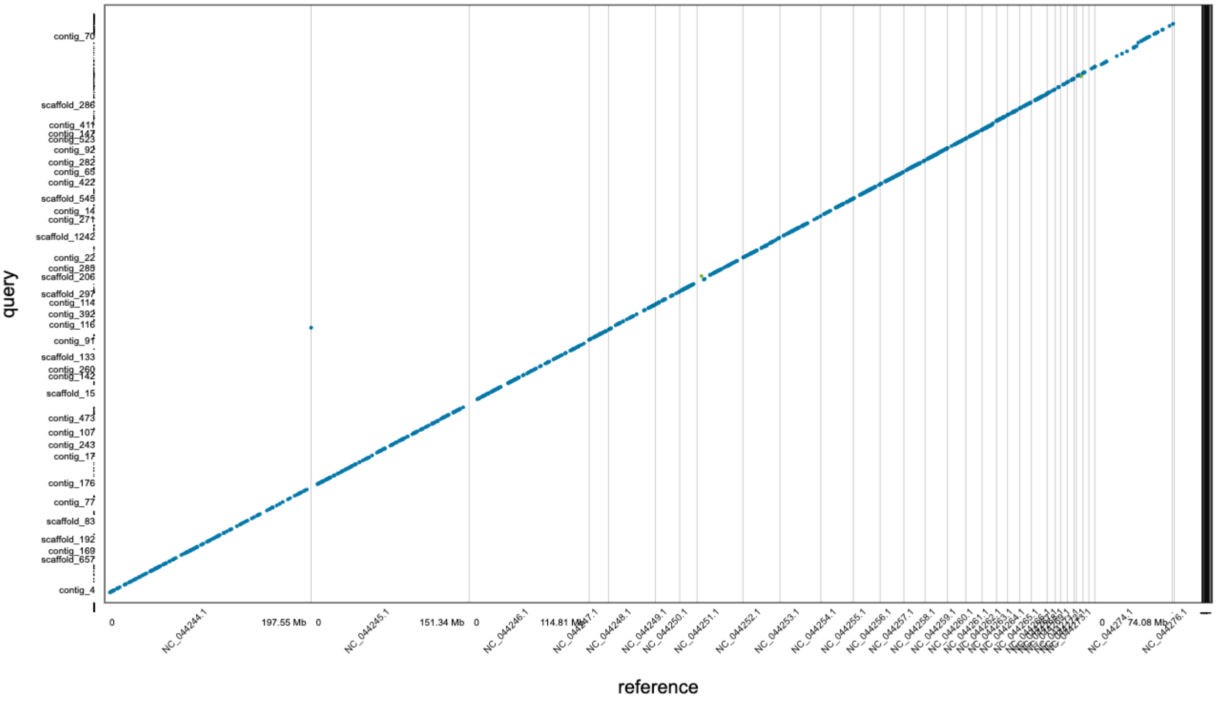

Figure S2. ***Assembly alignment.*** *Whole genome alignment of the unscaffolded ruby-throated hummingbird MaSuRCA assembly to the Anna's hummingbird assembly (GCA_003957555.2).*

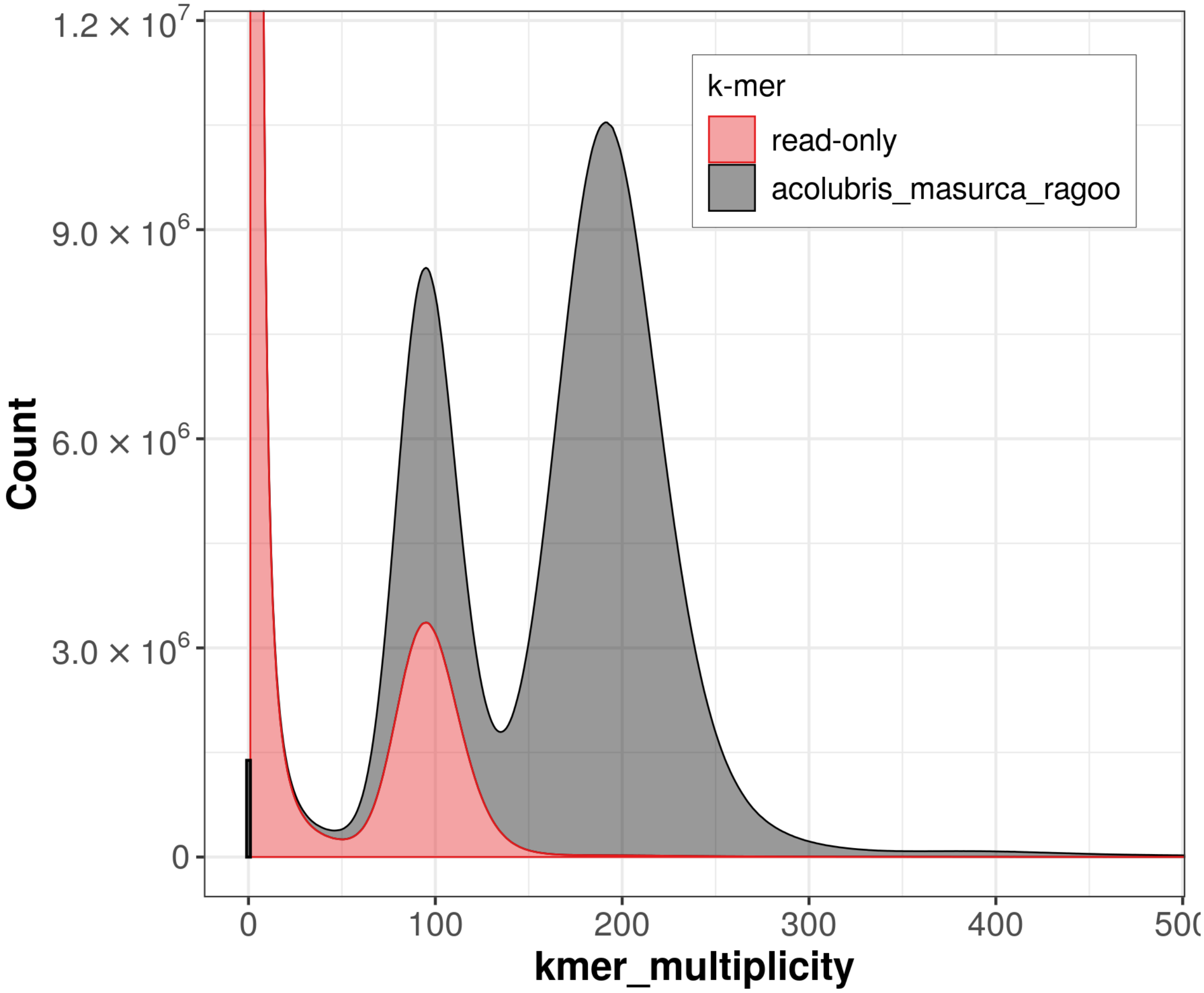

Figure S3. ***Merqury k-mer multiplicity plot.*** *Histogram of k-mer multiplicity collected from Illumina reads. The first peak represents 1-copy (heterozygous) k-mers in the genome, and the second peak represents 2-copy k-mers originating from homozygous sequence or haplotype-specific duplications. Depth of sequencing coverage determines where these peaks appear. K-mers missing from the assembly but present in the reads are heterozygous, expected from a haploid representation of the genome.*

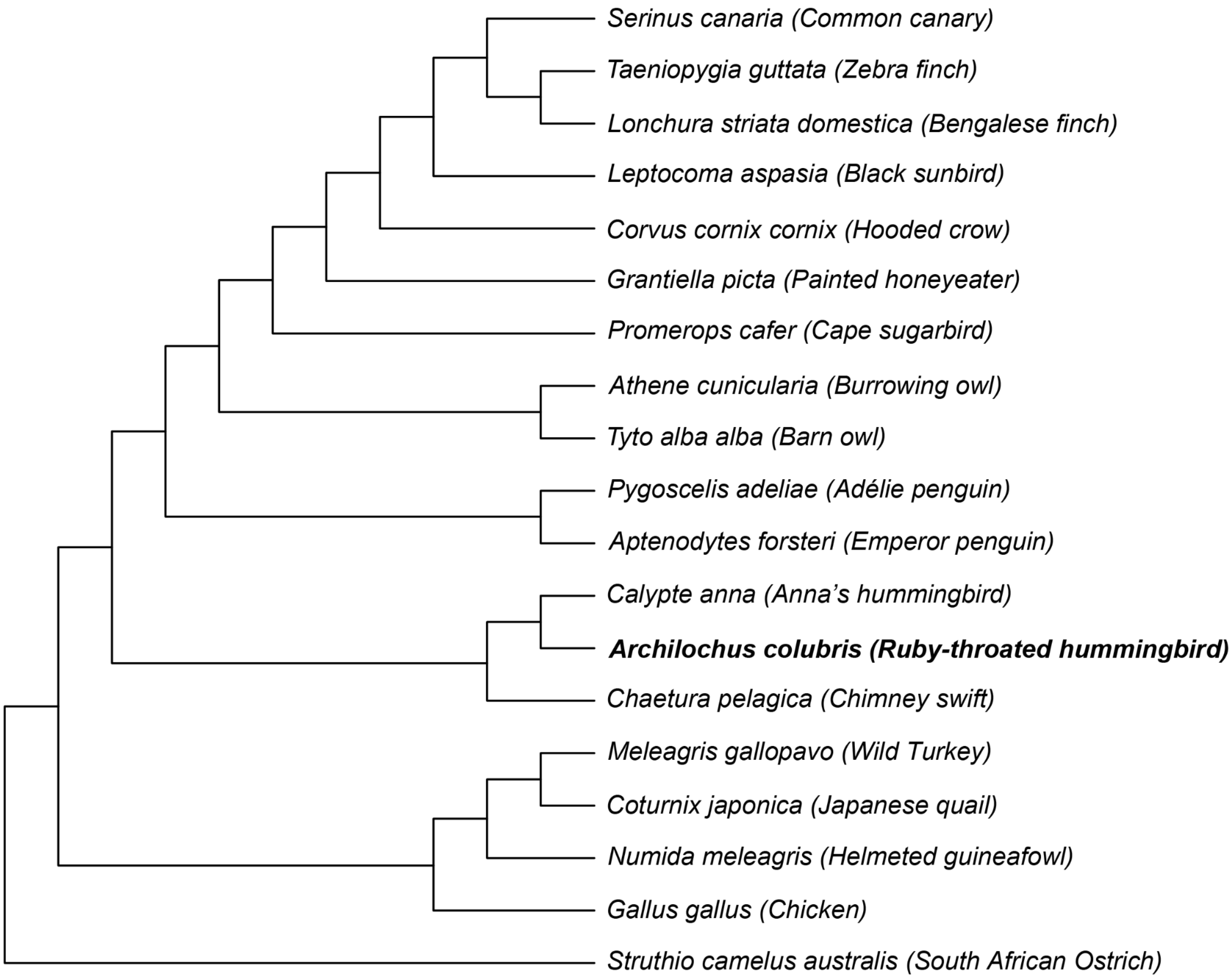

Figure S4. ***Phylogenetic tree.*** *Bird phylogeny for positive selection analysis, adapted from a consensus tree topology based on molecular phylogenies generated by Hacket et al., Oliveros et al., and Prum et al (Hackett et al. 2008; Oliveros et al. 2019; Prum et al. 2015).*

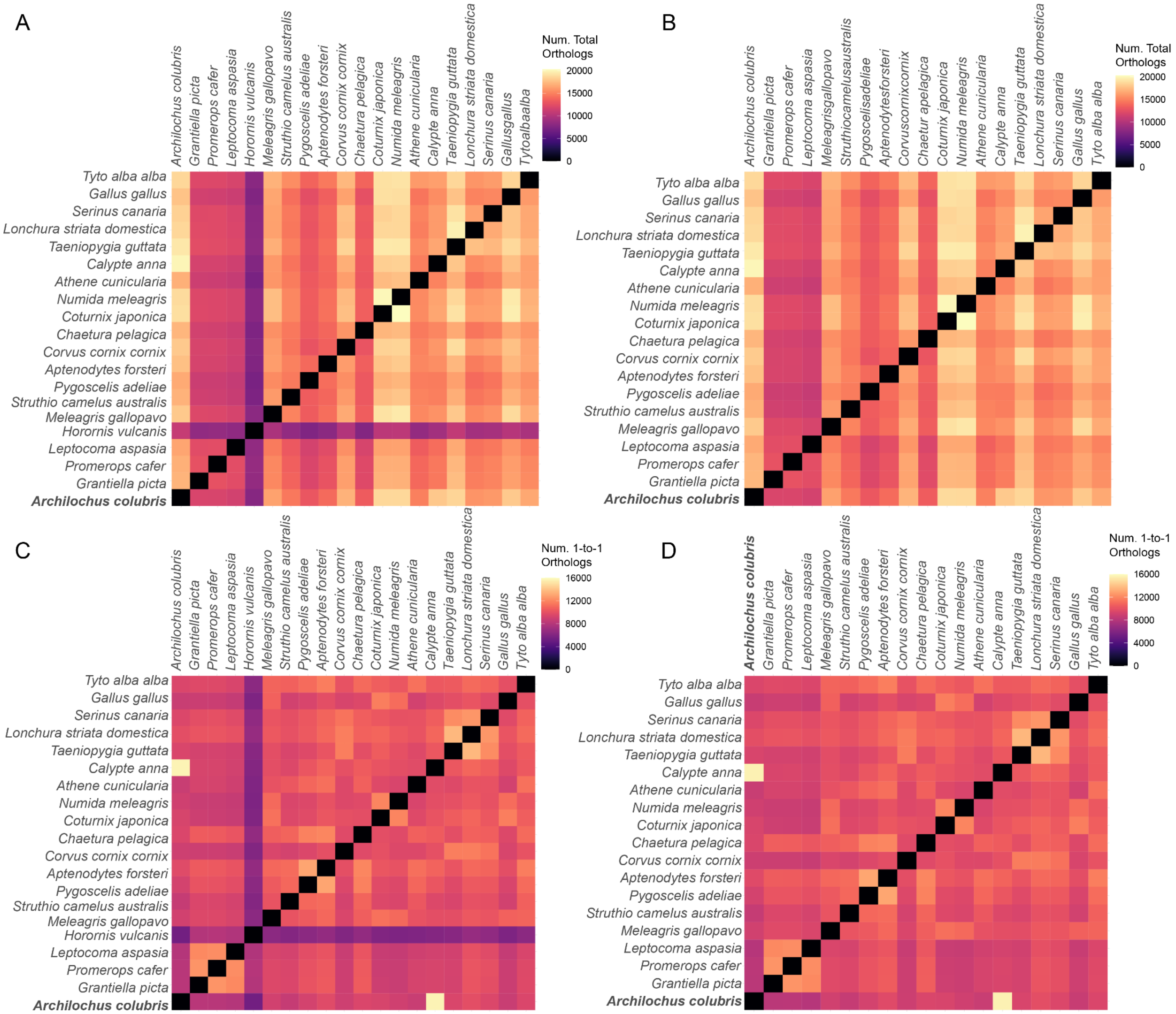

Figure S5. ***A)*** *Shared total orthologs with the Horornis vulcanis included.* ***B)*** *Shared total orthologs without the Horornis vulcanis.* ***C)*** *Shared 1-to-1 orthologs with the Horornis vulcanis included.* ***D)*** *Shared 1-to-1 orthologs without the Horornis vulcanis.*

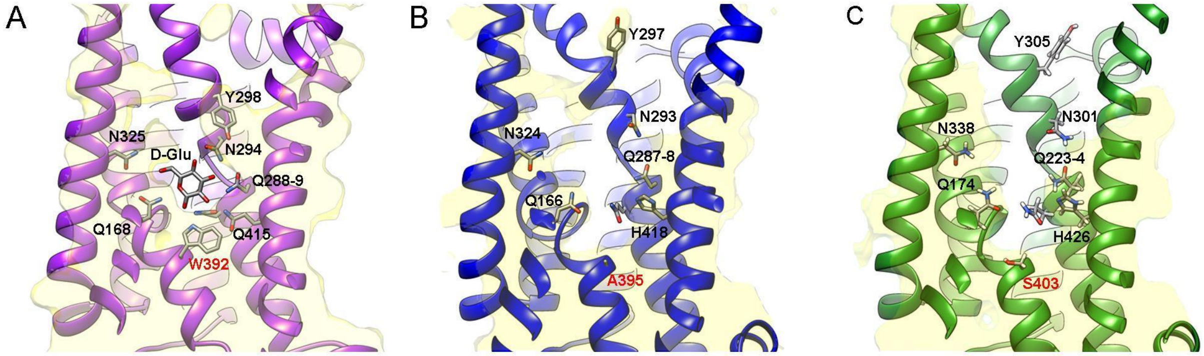

Figure S6***. Sugar binding site in GLUT structures.*** *Ribbon representations of the sugar binding pocket of atomic structures for:* ***A)*** *bacterial XylE in complex with D-Glucose (pdb code 4bgz, Sun et al., 2012),* ***B)*** *rat GLUT5 (pdb code 4ybq, Nomura et al., 2015), and* ***C)*** *an atomic model for ruby-throated hummingbird GLUT5 generated with AlphaFold2. Residues involved in hydrogen bonding interactions with the glucose in XylE are labeled on allthe three structures. The semitransparent overlays in yellow depict central sections of the volumes for the atomic models and show the changes at the bottom of the binding pocket where different amino acids are found in GLUT transporters (residues labeled in red).*

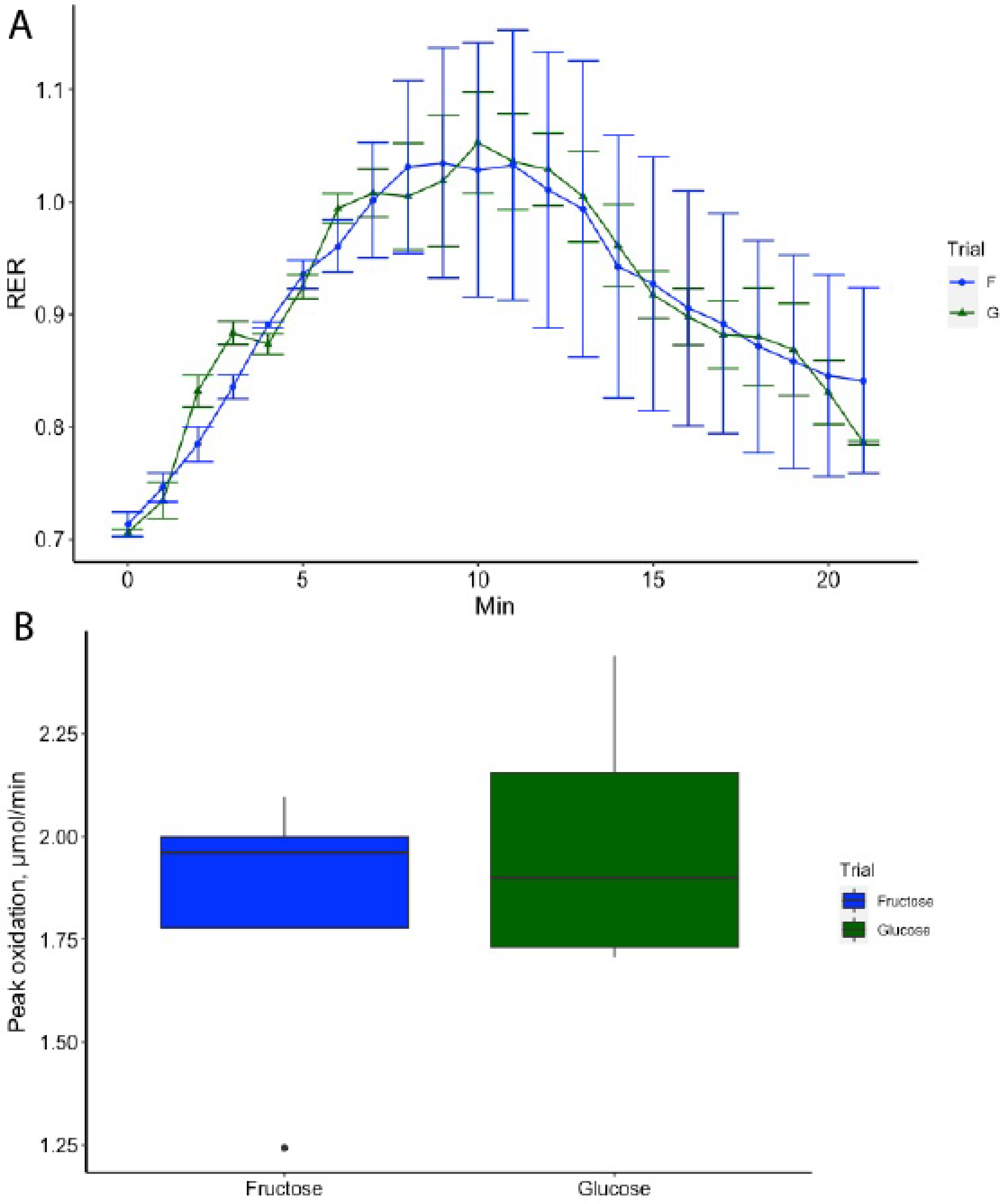

Figure S7. ***Tracer oxidation. A)*** *Respiratory exchange ratio ($RER=\dot{V}_{CO2}/\dot{V}_{O2}$) (RER) of glucose and fructose over a twenty minute time-course.* ***B)*** *Peak tracer oxidation between the enriched sucrose solutions (p = 0.66, paired t-test)*

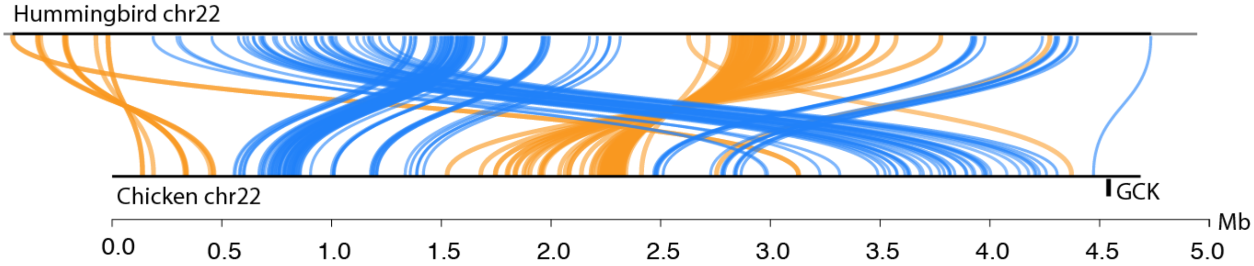

Figure S8 ***Chicken and hummingbird synteny.*** *Synteny plot showing syntenic sequence between the chicken and ruby-throated hummingbird in the forward direction (blue) and syntenic sequence that is reserved (orange). The alignment output file was processed with rustybam (https://github.com/mrvollger/rustybam) and plotted with SafFire (https://mrvollger.github.io/SafFire/).*