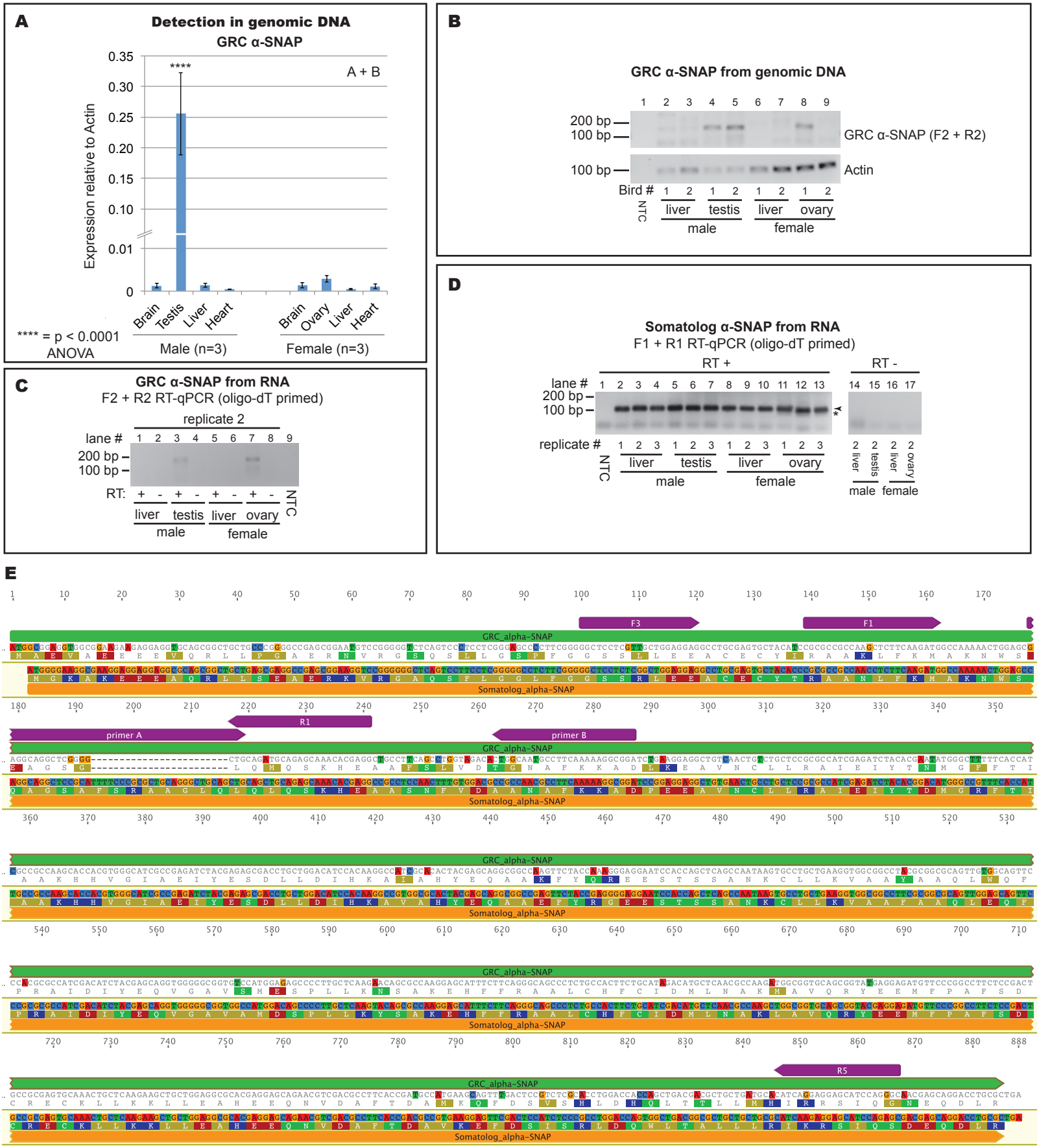
A
Detection in genomic DNA
GRC α-SNAP
A + B
Expression relative to Actin
0.35
0.30
0.25
0.20
0.15
0.10
0.01
0
****
Brain
Testis
Liver
Heart
Brain
Ovary
Liver
Heart
**** = p < 0.0001
ANOVA
Male (n=3)
Female (n=3)
B
GRC α-SNAP from genomic DNA
1 2 3 4 5 6 7 8 9
200 bp
100 bp
GRC α-SNAP (F2 + R2)
100 bp
Actin
Bird #
NTC
1 2 1 2 1 2 1 2
liver testis liver ovary
male female
C
GRC α-SNAP from RNA
F2 + R2 RT-qPCR (oligo-dT primed)
replicate 2
lane #
1 2 3 4 5 6 7 8 9
200 bp
100 bp
RT:
+ - + - + - + -
NTC
liver testis liver ovary
male female
D
Somatolog α-SNAP from RNA
F1 + R1 RT-qPCR (oligo-dT primed)
RT +
RT -
lane #
1 2 3 4 5 6 7 8 9 10 11 12 13
14 15 16 17
200 bp
100 bp
replicate #
1 2 3 1 2 3 1 2 3 1 2 3
NTC
liver testis liver ovary
male female
2 2 2 2
liver testis liver ovary
male female
E
F3
F1
GRC_alpha-SNAP
Somatolog_alpha-SNAP
R1
primer A
primer B
R5

Figure S1. Molecular analysis of zebra finch α-SNAP sequences. Related to Figure 1. **A.** Detection of GRC α-SNAP with paralog-specific primers A + B (see panel E). Error bars represent standard error of the mean; **** = $p < 0.0001$ by 2-way ANOVA. **B.** End-point gel electrophoresis confirming detection of GRC α-SNAP only from germline DNA. Top, F2+R2, bottom, Actin load control. Cloning was used to confirm the abundant band in testis is the expected product. NTC, no-template control. **C.** End-point gel electrophoresis of F2 + R2 RT-qPCR from Figure 1D (oligo-dT primed). Only replicate 2 shown (all qPCR performed in triplicate measurements). NTC, no-template control. **D.** End-point gel electrophoresis of F1 + R1 RT-qPCR from Figure 1E verifying that detected product was somatolog α-SNAP rather than the GRC. The 24-bp deletion in the GRC yields a 79bp product (asterisk) only detected faintly in ovary; the predominant 103bp product (arrowhead) comes from the somatolog (verified by cloning and sequencing). NTC, no-template control. **E.** Translation alignment of nucleic acid and amino acid sequences of GRC and somatolog α-SNAP. Color coding: green, GRC α-SNAP coding sequence, orange, somatolog α-SNAP coding sequence. Purple, primers used in this study. The somatolog α-SNAP is set as reference sequence and GRC differences are highlighted.

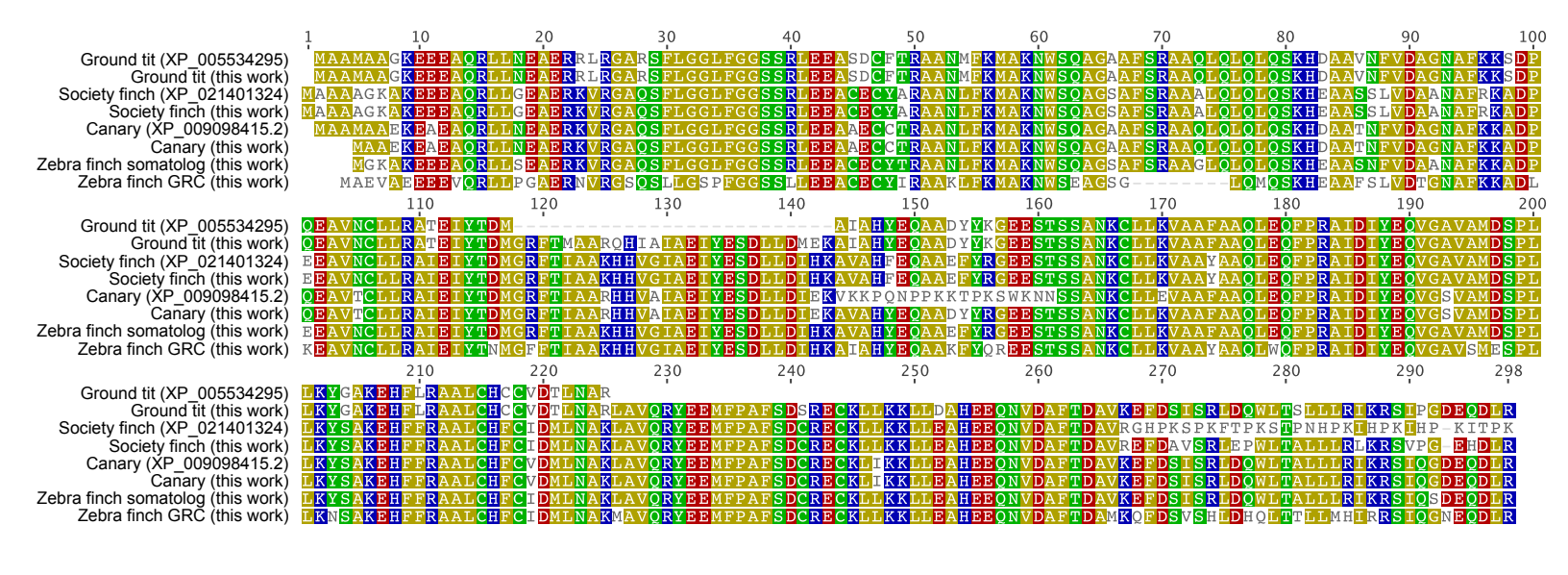
Ground tit (XP_005534295)
Ground tit (this work)
Society finch (XP_021401324)
Society finch (this work)
Canary (XP_009098415.2)
Canary (this work)
Zebra finch somatolog (this work)
Zebra finch GRC (this work)

Figure S2. Protein alignment of Genbank α-SNAP genes from ground tit, canary, and society finch, compared to *de novo* assembled sequences from this study. Related to Figure 3.

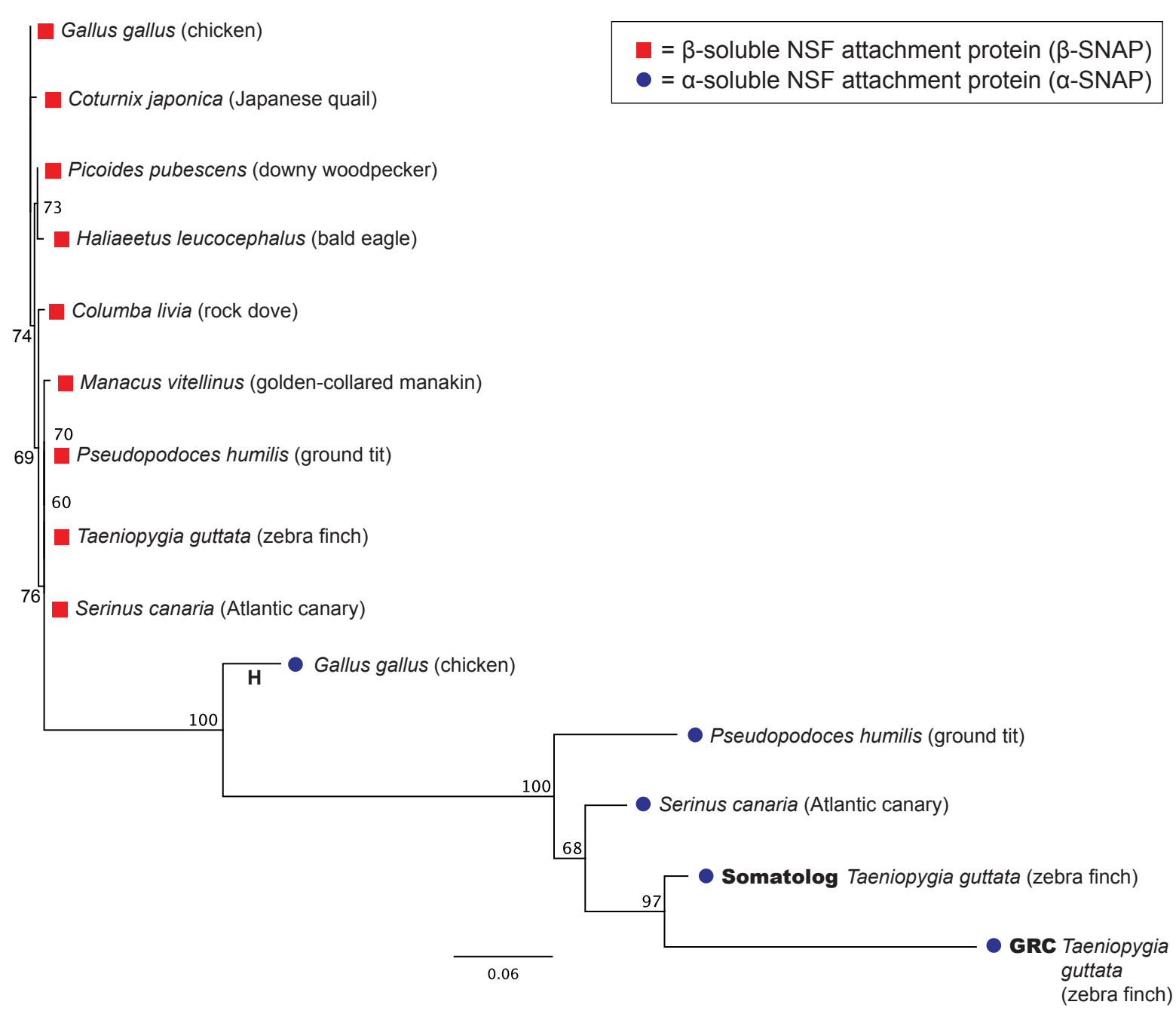
■ = β-soluble NSF attachment protein (β-SNAP)
● = α-soluble NSF attachment protein (α-SNAP)
Gallus gallus (chicken)
Coturnix japonica (Japanese quail)
Picoides pubescens (downy woodpecker)
73
Haliaeetus leucocephalus (bald eagle)
Columba livia (rock dove)
74
Manacus vitellinus (golden-collared manakin)
70
69
Pseudopodoces humilis (ground tit)
60
Taeniopygia guttata (zebra finch)
76
Serinus canaria (Atlantic canary)
Gallus gallus (chicken)
H
100
Pseudopodoces humilis (ground tit)
100
Serinus canaria (Atlantic canary)
68
Somatolog Taeniopygia guttata (zebra finch)
97
GRC Taeniopygia guttata (zebra finch)
0.06

Figure S3. Maximum likelihood phylogenetic tree of birds. Related to Figure 2A. Red boxes represent β-SNAP; blue dots represent α-SNAP proteins. Scale bar represents substitutions per site. Produced using RAxML.