

Figure S1. Phosphatase treatment of GLI2 and GLI3. (A) Phosphatase treatment of control full-length GLI2-185 did not shift mobility to that of the *talpid*² GLI2-185. (B) Phosphatase treatment shifted GLI3-190 in both control and *talpid*² embryos.

GTGAAGCGGGAGATGCTCCCGTCGGGGCGGCTGTTGCCGGGCAGCGCGCGCCCTGAGG
 GAAGCGTTGAGGTGAAGCC
 1 ATGCACGACAGGAGGCGCGGGCCCTCAGCCCCAGCCCCGGGGGTACCGCGGGCGTCGA 60
 M Q Q R R P R A S A P A P G V S R R R R 20

Exon2
 61 GCTCCCGGTGACATCCCGGCTCCACCAGCCTGCCCGCGTGGTGGAGGGCCCTCCGCGC 120
 A P G D I P A S T S L P P L V E G P P R 40

121 TGCCTCCTCTGCTGCACCGTGGCAGAATCGTGTGGACGGCCGCAAAACCGCCCTCCGCC 180
 C V L C C T V G R I V W T A A K P P S A 60

181 GCCGTCTCTGCTCCGCTGAGGTGGTGGGGTGGAGCCTCGGACGGTACCGTGTCCACCC 240
 A V L V R L R W W G E T S D G T V F H P 80

241 TCCTCAAAGCGGGCAGCGCGGGCAGCAGCCGTACACCGTGCGGTGCGGCCCTCCG 300
 S S K P G Q A A G S S R Y T V R C G P R 100

Exon3
 301 CAGCTCACGGCTACCTCACAGATATGGGCATGCTTGTCTGGAGTCTGACCAAACCT 360
 Q L T A Y L T D M G M L V L E V L T K P 120

361 GACCGTCTTCCAGTTGGCAGAGTGCAGATCACCGAATTGTCCCAGCTCTCTCCAGCCAT 420
 D R L P V G R V Q I T E L S Q L S P S H 140

Exon4
 421 CCCATCAGTGGTTTTCACCATAGTTTACCACAGCTGATAAATGGGAGAGCTACAG 480
 P I S G F F T I V S P T A D K L G E L Q 160

481 GTTCACTTGTCTCGAACCTCTATCAGACACATAAAAACAGCAGCTCTGTCCCAACC 540
 V S L V L E P L S D T Y K T S S S V P T 180

541 ACAGCTACAAGTTCAGATATGACTCCTGCTCCATCGCAGCAAATGCAGACCCTCTCT 600
 T A T S S D M T P A P S Q Q M Q T T P P 200

Exon5
 601 GGCCAAGAGCCTGTTAACAAAAGCAGGGTCACGACTCCCACTGGGGAAAGGACCATTCA 660
 G Q E P V N K S R V T T P S W G K D H S 220

661 TTCTTTCAAGAGACTCTGAAAACATAAAGGATGTTTCTCTGCTTCCCACCACCGCTC 720
 F F Q E N S E N I K D V S S A S H H R L 240

721 ATTTTACCAGATGAACATTTGAAAGTGAATCCTTTGAGTACAGCAGTCAATATCCCC 780
 I L P D E H L K V N P L S Q Q S V S F P 260

781 AGCAGCACTGATCCTGAAGCACCCCTCAGTACAAACACACCGTGTCTCTTGCACAAC 840
 S S T D P E A P L S T N T H V F S L H N 280

Exon6
 841 CCAACTACAAAAGACCTGCTTTCAGTCTCTTTGGATCAAGGCAATAAATCCGTGATGCC 900
 P T T K D L L S A L L D Q G N K L R D A 300

901 ATGGTTGCTTCTGCAATGAAGTCCAGCCAGACATGGAGATTGAATTGAATGAAGTGCCT 960
 M V A S A M K S S P D M E I E L N E V P 320

Exon7
 961 CCTTTGTAAAGCCAGATGGTTTCAGAGCAGCAGAAAAGAGCCAAAAGGCTTGAACTCA 1020
 P F V K P D G F R A A A K S P K G L N S 340

1021 ACTTTTATGCCTCCTCCTGAGGATACCCATCTTTCACATCTGAAGTACAGGTCAGCAA 1080
 T F M P P P E D T H L F T S E V T G Q Q 360

Exon8
 1081 TTAGACCTCAACTCTGAGGAGAGCAATACAGTTACTGTGGGCAGTCTGATTATCT 1140
 L D L N S E E R A I Q L L L G S A D L S 380

1141 CCTGTGCATTTCTGGGATCAGCCAGGTTCCCTTCTGGATTCTCTCTGTTGGAAGTGAG 1200
 P V H F W D Q P G S L L D S L S V G S E 400

1201 GTGTATGACAGTGAATCAATGATCCCCACTATGACCAGAGTCTGCTAGAAAGCTTTTT 1260
 V Y D S E L N D P H Y D Q S L L E S L F 420

Exon9
 1261 TACACGACACCTAAATCAGATTCCAGTACAAGTATTCTCCTCAGTATGAAGATGTTAAC 1320
 Y T T P K S D S S T S D F L S D E D V N 440

1321 CCTCCAAAAAAGTAAAGCAAAACAAAAGTGAAGAAAGCTGATTCTGTGAATGCACAG 1380
 P S K K V S K T K T V K K A D S V N A Q 460

Exon10
 1381 AAGGATCCACTGCCTTAGATCAGGATCAGAAGGTGACAGAAATTAACCCAGTGTCTCT 1440

Figure S2. cDNA and amino acid sequence of chicken C2CD3.

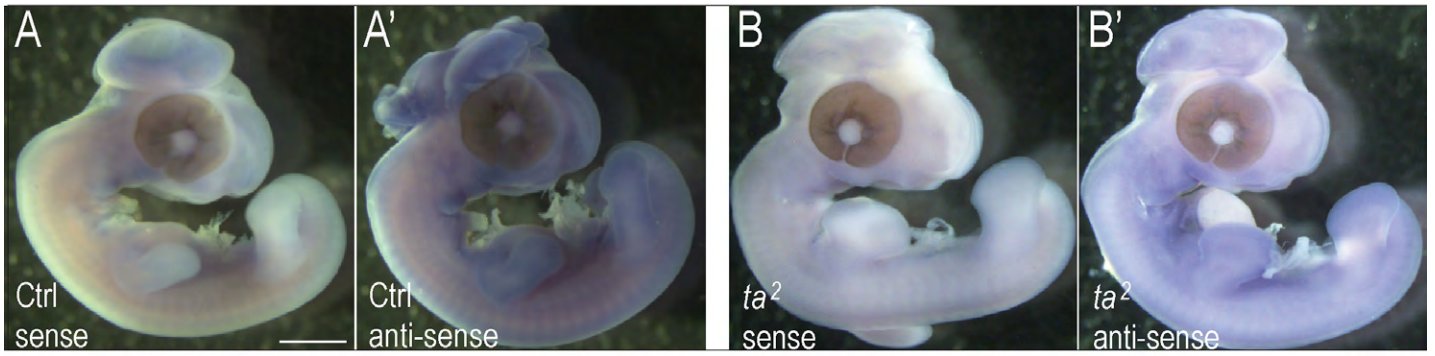


Figure S3. *C2CD3* is expressed in whole chicken embryos. (A-B') *In situ* hybridization for *C2CD3* in day 4 whole embryos. Scale bar represents 500 μ m.

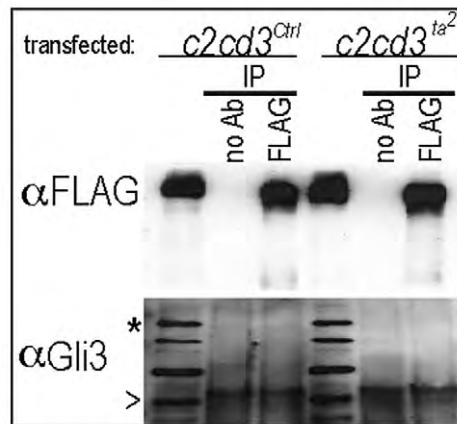


Figure S4. *C2CD3* does not interact with GLI3. CEFs were transfected with either p3XFLAG-*c2cd3*^{Ctrl} or p3XFLAG-*c2cd3*^{ta²} and immunoprecipitation was performed with anti-GLI3. * indicates GLI3-190, > indicates cleaved GLI3-83.

Table S1. Densitometry readings of GLI2 and GLI3 nuclear fraction

		GLI2		GLI3		LAMIN	
		raw #	normalized	raw#	normalized	raw #	normalized
FNP	ctrl	1926.882	1	3982.368	1	2948.882	1
	TA2	2688.489	1.395253575	16254.154	4.081529884	4076.276	1.382312348
MXP	ctrl	4366.518	1	6927.711	1	3294.225	1
	TA2	4703.43	1.077158047	19972.267	2.882953258	3812.296	1.157266428
MNP	ctrl	2385.305	1	3486.794	1	4547.205	1
	TA2	4756.903	1.994253565	12053.296	3.456842016	9019.539	1.983534721

Table S2.

[Download Table S2](#)

Table S3.

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Table S4. Variant frequency in ta2.003 candidate intervals

candidate ta-2 region Galgal4 coordinates	size Mb	UCD003 SNP	UCD003 DIP	tal2-specific SNP	tal2- specific DIP	UCD003 SNP/Mb	tal2- specific SNP/Mb	fixed tal2- specific SNP	fixed tal2-specific SNP/Mb	fixed ta- 2/UCD003 SNP ratio	tal2/UCD003 DIP ratio
chr1:115764249-118327843	2.56	25484	1407	2575	335	9955	1006	752	294	0.030	0.24
chr1:193748573-195187547	1.44	15300	658	2819	238	10625	1958	1550	1076	0.101	0.36
chr10:8513002-10170824	1.66	14620	815	1134	151	8807	683	314	189	0.021	0.19
chr10:11552743-12453463	0.90	11022	627	730	95	12247	811	89	99	0.008	0.15
chr15:10301074-11866661	1.57	17508	857	125	40	11152	80	59	38	0.003	0.05

Variant frequencies are shown (total and per Mb) for UCD003 and ta2.003-specific. Fixed refers to those variants found in all ta2.003 reads that likely include fewer false positives. DIP, deletion/insertion polymorphism.

Table S5. Coding variants in C2CD3

mRNA coord rel to ATG 89	Coordinate (Galgal4) 194883819	Reference C	GGA1 Reference		variant in ta2.003
			ta2.003 G	Amino Acid Change Ser30Thr	
167	194883741	T	C	Lys56Arg	ta2.003
957	194879146	C	T	synonymous	ta2.003
2178	194867809	G	A	synonymous	ta2.003
2325	194867662	G	A	synonymous	ta2.003
2344	194867643	C	G	Ala782Pro	ta2.003
2346	194867641	A	G	synonymous	ta2.003
4049	194858002	C	T	Gly1350Asp	ta2.003
4595	194856869	G	A	Pro1532Leu	ta2.003
4598	194856866	G	A	Thr1533Ile	ta2.003
4674	194856790	C	A	Gln1558His	ta2.003
5870	194844961	C	T	Gly1957Asp	ta2.003
6570	194844261	T	C	synonymous	ta2.003
6626-6644	194844184-194844202		19 bp deletion	deletes a.a. from 2210-2330	ta2.003
6648	194844180	A	G	synonymous after frameshift/stop	ta2.003
6767	194842820	A	G	Val2256Ala after frameshift/stop	ta2.003
1022	194877027	G	T	Thr341Asn	ta2.003 and UCD003
1194	194875767	T	C	synonymous	ta2.003 and UCD003

Exonic variants confirmed by whole genome sequencing and resequencing in C2CD3.