

Supplementary materials for

Recurrent *DCC* gene losses during bird evolution

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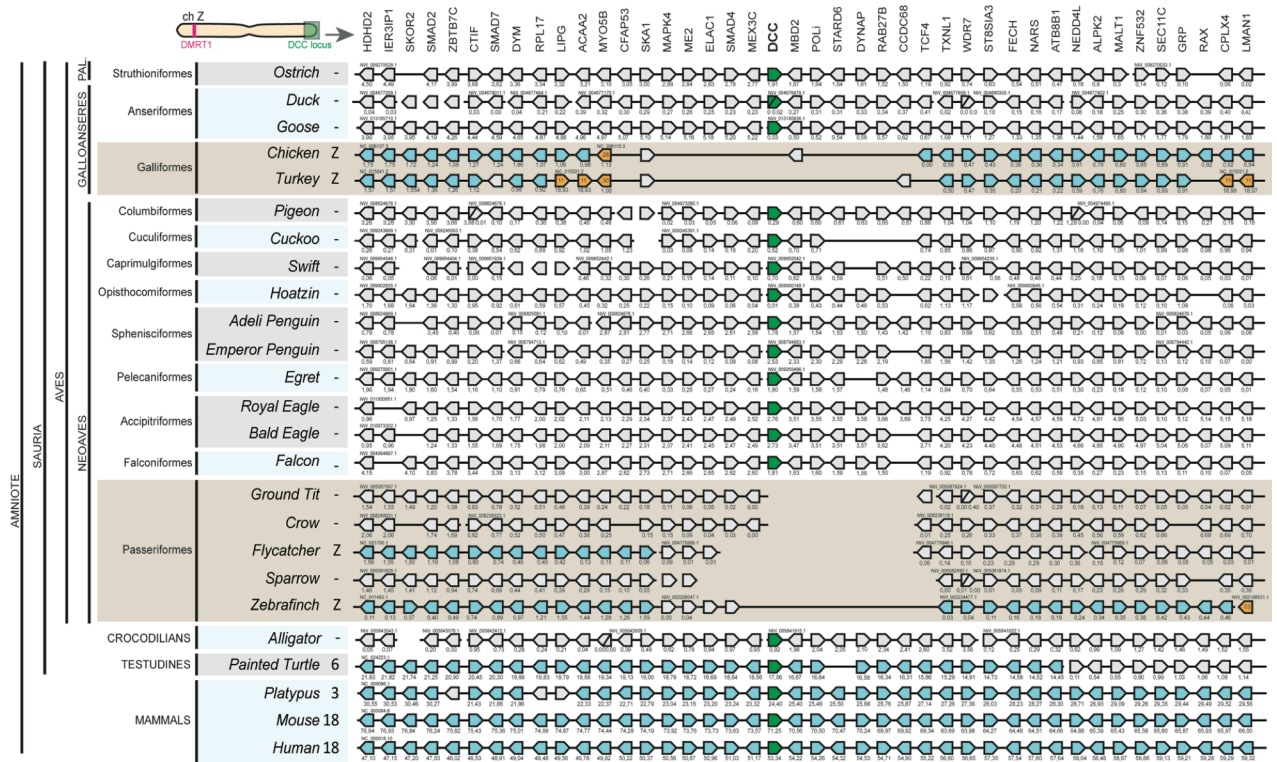


Figure S1 – Synteny analysis of *DCC* chromosomal region in amniotes.

Genomic synteny map comparing positions of *DCC* and its neighbouring genes in different amniote species, including a wide variety of birds. Orthologues of each gene are displayed in the same column. Genes from unplaced genomic scaffolds are represented in grey. Genes that have been positioned on chromosomes on annotated genomes are represented in blue if they are on the same chromosome as *DCC* or on bird dimorphic sexual Z chromosome, and in orange if they are located on another chromosome. Chromosome or scaffold references are indicated above each independent sequence.

The genes shown in this figure are not necessary presented in the same order as their actual locations on the chromosomes and scaffolds, which are indicated in 10^6 base pairs. A broken line symbolizes the location of a gene on different scaffolds or contigs. The detailed genomic locations of all the genes are given in Supplementary Table S3. Detailed gene names and species names are given in Supplementary Table S4 and S5 respectively.

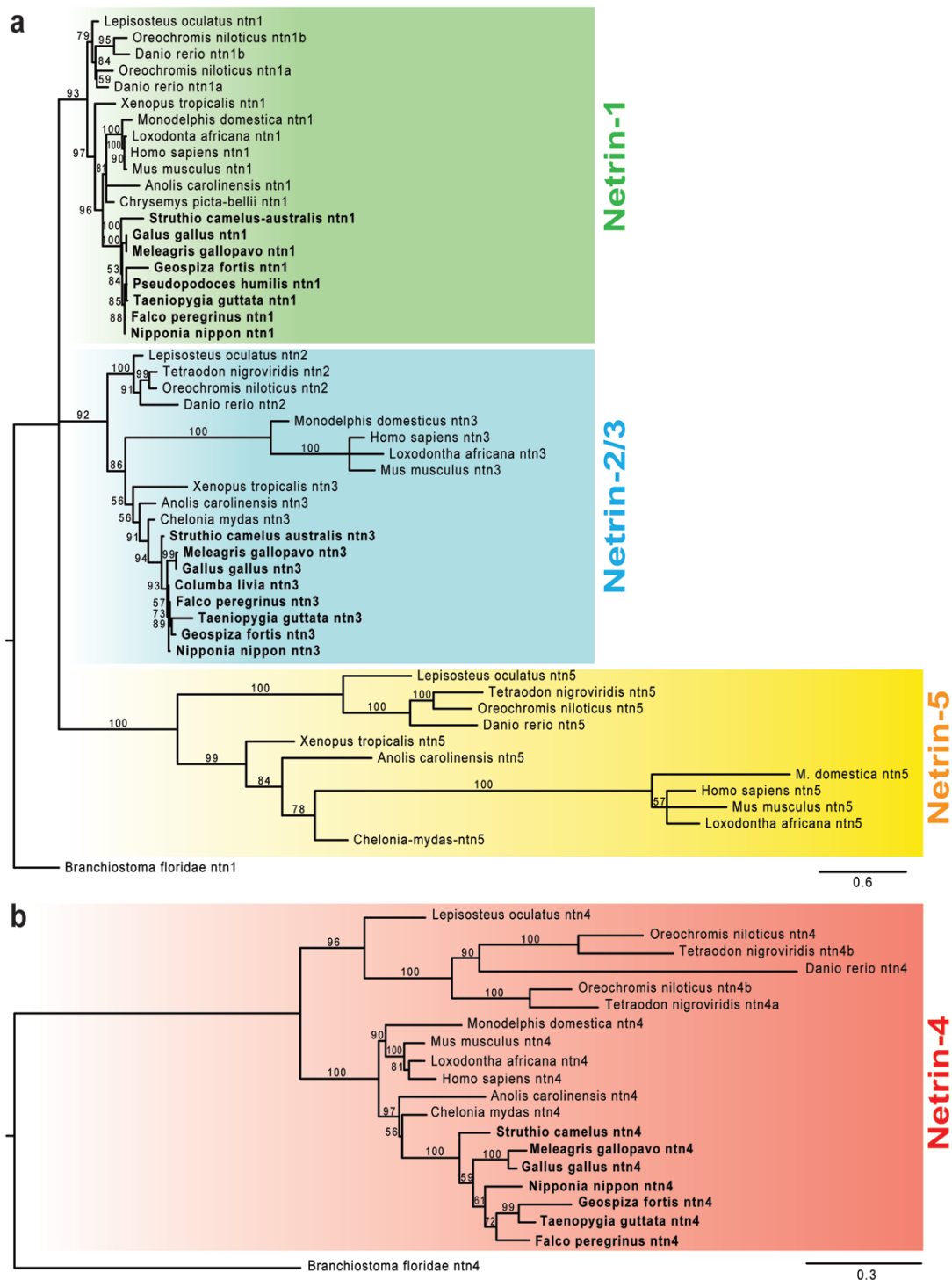


Figure S2 – Consensus phylogenetic tree of vertebrate NETRIN-1/2/3/5 and NETRIN-4. Phylogenetic analysis of 52 vertebrate NETRIN-1, NETRIN-2/3 and NETRIN-5 amino acid sequences (**a**) and 19 vertebrate NETRIN-4 amino acid sequences (**b**) were performed using the Maximum likelihood method, with 1000 bootstrap replicates (for the references of sequences, see Supplementary Table S1). The number displayed at each branch node indicates bootstrap value in percentage. Only values above 50% are indicated. The tree was rooted using *Branchiostoma floridae* NETRIN-1 and NETRIN-4 sequences as outgroups, respectively. Vertebrate NETRIN-1 group is indicated in red, NETRIN-2/3 in green, NETRIN-5 in yellow and NETRIN-4 in red. Bird protein sequences are presented in bold.

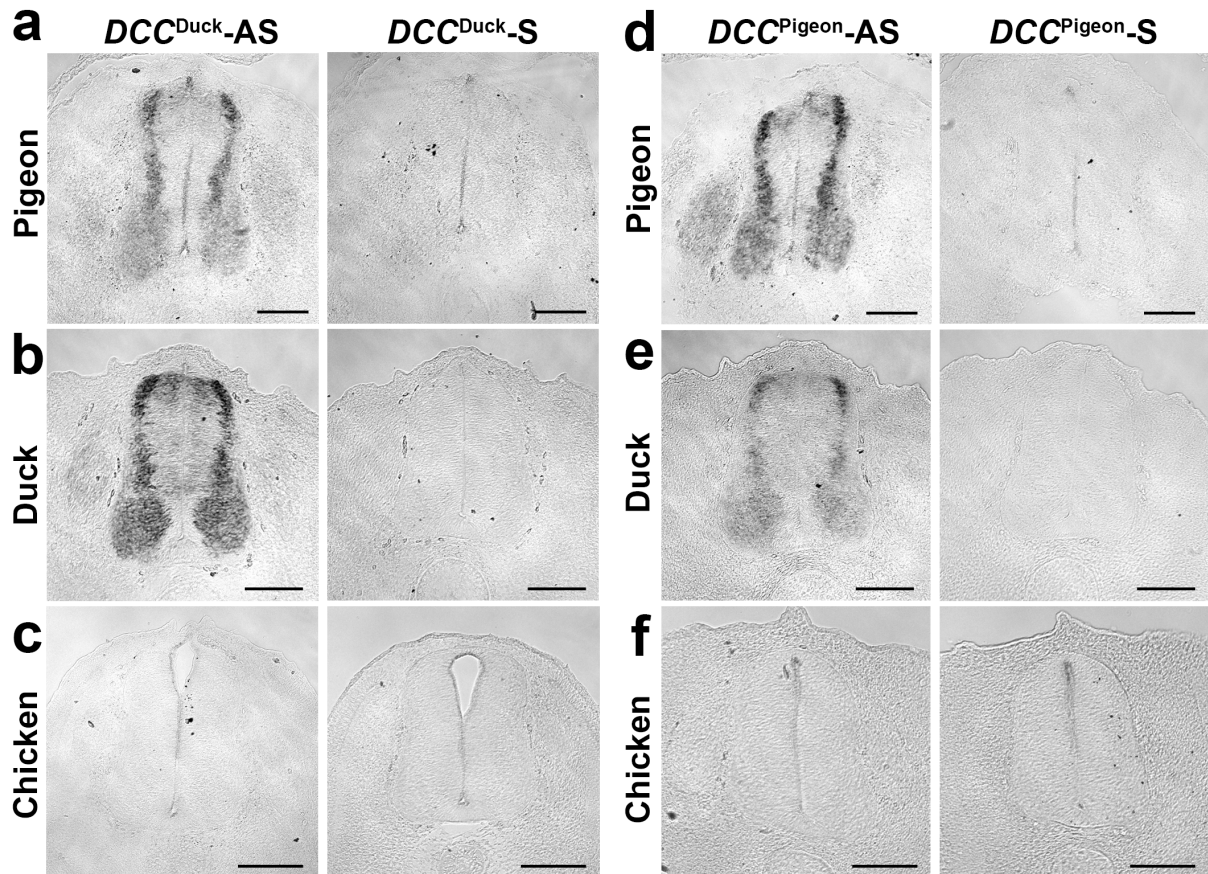


Figure S3. *In situ* hybridization for *DCC* in pigeon, duck and chicken spinal cord. a-c. *In situ* hybridization using *DCC* antisense (AS) riboprobe amplified from duck cDNAs shows labeling in spinal cord interneurons and motor neurons territory from pigeon (a), duck (b), but not chicken (c) embryos. No labeling is seen with sense (S) riboprobes. **d-f.** *In situ* hybridization using *DCC* antisense (AS) riboprobe from pigeon cDNA shows labeling in spinal cord from pigeon (d), duck (e), but not chicken (f) embryos. No staining is seen with sense (S) riboprobes. Scale bars: 50 μ m.

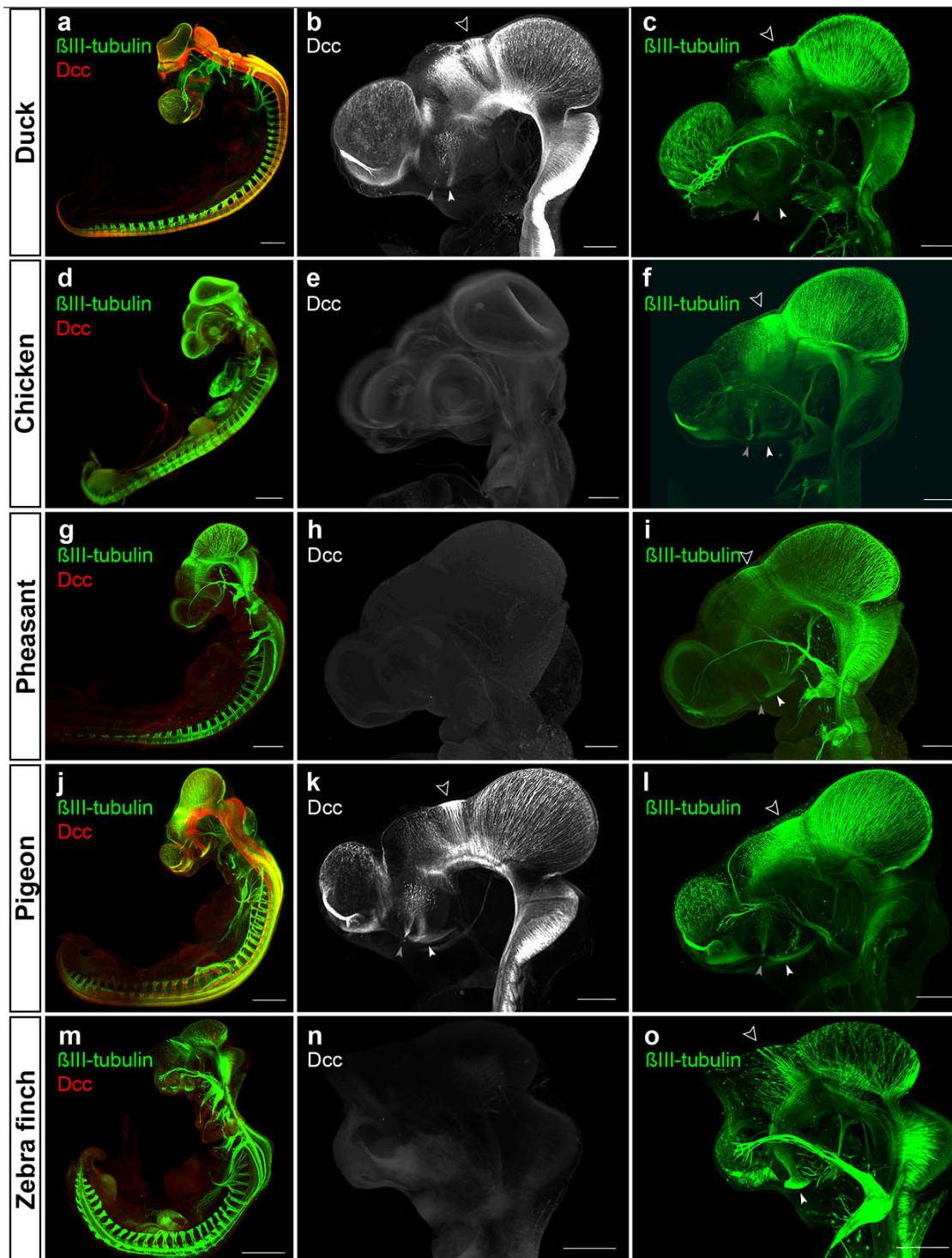


Figure S4. DCC expression in duck, chicken, pheasant, pigeon and zebra finch. Light sheet images of HH21-23 duck (**a-f-k**), chicken (**b-g-l**), pheasant (**c-h-m**), pigeon (**d-i-n**) and zebra finch (**e-j-o**) embryos after DCC and β III-tubulin whole-mount immunostaining and 3DISCO clearing. Open arrows indicate the posterior commissure, white arrowheads the post-optic commissure and grey arrowheads the optic nerve. Scale bars: 500 μ m (left); 200 μ m (right).

Table S1 – Names and references of DCC/NEOGENIN proteins identified and used in this study.

	Name	Latin name	Gene ID	
			DCC	Neogenin
	Spotted Gar	<i>Lepisosteus oculatus</i>	ENSLOCP00000003949	XP_006629128.1
	Zebrafish	<i>Danio rerio</i>	NP_001030157.1	AAL92552.1
	Tilapia	<i>Oreochromis niloticus</i>	XP_005470639.1	XP_005455737.1
	Medaka	<i>Oryzias latipes</i>	XP_011480439.1	XP_011485992.1
	Tetraodon	<i>Tetraodon nigroviridis</i>	ENSTNIP00000017207	ENSTNIT00000012640
	Xenopus	<i>Xenopus tropicalis</i>	XP_004910423.1	ABA02169.1
	Opossum	<i>Monodelphis domestica</i>	ENSMODP00000013810	XP_007478277.1
	Elephant	<i>Loxodonta africana</i>	XP_003406765.1	XP_010592181.1
	Mouse	<i>Mus musculus</i>	ENSMUSP000000110593	AAH54540.1
	Human	<i>Homo sapiens</i>	ENSP00000389140	AAC51287.1
	Lizard	<i>Anolis carolinensis</i>	ENSACAP00000008535	XP_003227756.1
	Gekko	<i>Gekko japonicus</i>	XP_015283785.1	XP_015281163.1
	American Alligator	<i>Alligator mississippiensis</i>	XP_014449591.1	XP_014459957.1
	Chinese Alligator	<i>Alligator sinensis</i>	XP_006018932.1	XP_006026332.1
	Painted turtle	<i>Chrysemys picta bellii</i>	XP_005296926.1	XP_005281067.1
	Chinese turtle	<i>Pelodiscus sinensis</i>	XP_006136853.2	XP_006122251.2
	Sea Turtle	<i>Chelonia mydas</i>	XP_007063909.1	XP_007059969.1
	Ostrich	<i>Struthio camelus australis</i>	XP_009664396.0	XP_009673998.0
	Kiwi	<i>Apteryx australis mantelli</i>	XP_013800074.1	XP_013800108.1
	Tinamu	<i>Tinamus guttatus</i>	XP_010215865.1	XP_010223404.1
Paleo.	Chicken	<i>Gallus gallus</i>	ND	AAC59662.1
	Quail	<i>Coturnix Japonicus</i>	ND	XP_015727843.1
Galli.	Turkey	<i>Meleagris gallopavo</i>	ND	XP_003209386.1
	Duck	<i>Anas platyrhynchos</i> (mallard)	XP_005011860.1	XP_005017275.1
Ans.	Goose	<i>Anser cygnoides domesticus</i>	XP_013050573.1	XP_013049547.1
	Hummingbird	<i>Calypte anna</i>	ND	XP_008500653.1
Striso.	Swift	<i>Chaetura pelagica</i>	XP_010001093.1	XP_010004197.1
	chuck-will's-widow	<i>Caprimulgus carolinensis</i>	ND	XP_010166263.1
Columbaves	Turaco	<i>Tauraco erythrolophus</i>	XP_009978908.1	XP_009991006.1
	Pigeon	<i>Columba livia</i>	XP_005503037.1	XP_005512248.1
	Sandgrouse	<i>Pterocles gutturalis</i>	ND	XP_010070753.1
	Cuckoo	<i>Cuculus canorus</i>	XP_009561651.1	XP_009567164.1
	Mesite	<i>Mesitornis unicolor</i>	Part. (XP_010181434.1)	Part. XP_010191234.1
Gru.	Crowned crane	<i>Balearica regulorum gibbericeps</i>	XP_010310977.1	XP_010299714.1
	Bustard	<i>Chlamydotis macqueenii</i>	Part. (XP_010126392.1)	XP_010125600.1
Aequoriformithes	Loon	<i>Gavia stellata</i>	XP_009818994.1	XP_009811795.1
	Fulmar	<i>Fulmarus glacialis</i>	XP_009583160.1	XP_009579568.1
	Cormorant	<i>Phalacrocorax carbo</i>	ND	XP_009501723.1
	Pelican	<i>Pelecanus crispus</i>	XP_009476861.1	XP_009483113.1
	Egret	<i>Egretta gazetta</i>	XP_009635714.1	XP_009642196.1
	Ibis	<i>Nipponia nippon</i>	XP_009461288.1	XP_009467911.1
	Killdeer	<i>Charadrius vociferus</i>	XP_009884696.1	XP_009893858.1
	Ruff	<i>Calidris pugnax</i>	XP_014795236.1	XP_014809326.1
	Tropicbird	<i>Phaethon lepturus</i>	ND	XP_010290237.1
	Sunbittern	<i>Eurypyga helias</i>	ND	XP_010145839.1
	Adeli Penguin	<i>Pygoscelis adeliae</i>	XP_009321339.1	XP_009318182.1
	Emperor Penguin	<i>Aptenodytes forsteri</i>	XP_009273020.1	XP_009277190.1
	Golden Eagle	<i>Aquila chrysaetos canadensis</i>	XP_011588980.1	XP_011588100.1
	White-tailed Eagle	<i>Haliaeetus albicilla</i>	XP_009927949.1	XP_009926840.1
	Bald Eagle	<i>Haliaeetus leucocephalus</i>	XP_010581336.1	XP_010569893.1
Rhinoceros hornbill	<i>Buceros rhinoceros silvestris</i>	XP_010140526.1	XP_010144031.1	
Coracii.	Cuckoo roller	<i>Leptosomus discolor</i>	Part. (XP_009950574.1)	XP_009955259.1
	Bee-eater	<i>Merops nubicus</i>	ND	XP_008942215.1
	Woodpecker	<i>Picoides pubescens</i>	ND	XP_009904177.1
	Trogon	<i>Apaloderma vittatum</i>	Part. (XP_009875528.1)	Part. (XP_009863463.1)
Hoatzin	<i>Opisthocomus hoazin</i>	XP_009943494.1	XP_009934163.1	
Seriema	<i>Cariama cristata</i>	ND	XP_009708043.1	
Falc.	Falcon	<i>Falco cherrug</i>	XP_005438019.1	XP_005437570.1
	Falcon	<i>Falco peregrinus</i>	XP_005242943.1	XP_005232662.1
Psit.	Kea	<i>Nestor notabilis</i>	ND	XP_010011546.1
	Budgerigar	<i>Melopsittacus undulatus</i>	XP_012983556.1	XM_005145789.2
Passeriforme	Ground tit	<i>Pseudopodoces humilis</i>	ND	XP_005521773.1
	Flycatcher	<i>Ficedula albicollis</i>	ND	XP_005051876.1
	Zebra Finch	<i>Taeniopygia guttata</i>	ND	XP_002190793.1
	Ground Finch	<i>Geospiza fortis</i>	ND	XP_005425990.1
	Sparrow	<i>Zonotrichia albicollis</i>	ND	XP_014124836.1
	Hooded crow	<i>Corvus cornix</i>	ND	XP_010410734.1
	Canary	<i>Serinus canaria</i>	ND	XP_009088347.1
	Manakin	<i>Manacus vitellinus</i>	ND	XP_008922371.1
	American crow	<i>Corvus brachyrhynchos</i>	ND	XP_008629530.1
	Starling	<i>Sturnus vulgaris</i>	ND	XP_014744736.1
	Rifleman	<i>Acanthisitta chloris</i>	ND	XP_009075656.1
	Name	Latin name	Frazzled	
	Fruit fly	<i>Drosophila melanogaster</i>	NP_523716.2	

Key	NCBI Genome Browser
	Ensembl Genome Browser
	Partial sequence
	ND : No Data

- Paleo. Paleognathes
- Galli. Galliformes
- Ans. Anseriformes
- Striso. Strisores
- Gru. Gruiformes
- Acci. Accipitriformes
- Coracii. Coraciiformes
- Falc. Falconiformes
- Psit. Psittaciformes

Table S2 – Names and references of NETRIN proteins identified and used in this study.

Name	Latin name	Gene ID			
		NTN1 (a/b)	NTN2/3	NTN5	NTN4 (a/b)
Spotted Gar	<i>Lepisosteus oculeatus</i>	ENSLTCP00000016355	ENSLTCP00000010931	ENSLTCP00000017788	ENSLTCP00000019382
Zebrafish	<i>Danio rerio</i>	ENSDARP00000024821 ENSDARP00000038885	ENSDARP000000127895	ENSDARP00000052908	ENSDARP000000132076
Tilapia	<i>Oreochromis niloticus</i>	ENSONIP00000022872 ENSONIP00000010389	ENSONIP00000025223	ENSONIP00000023890	ENSONIP00000010787 ENSONIP00000022484
Tetraodon	<i>Tetraodon nigroviridis</i>	ENSTNIP00000013104 ENSTNIP00000013104	ENSTNIP00000015771	ENSTNIP00000013058	ENSTNIP00000015647 ENSTNIP00000019104
Xenopus	<i>Xenopus tropicalis</i>	ENSXETP00000042263	ENSXETP00000043788	ENSXETP00000063547	XP_002941301.2
Opossum	<i>Monodelphis domestica</i>	ENSMODP00000010543	ENSMODP00000019264	ENSMODP00000040382	ENSMODP00000010079
Elephant	<i>Loxodonta africana</i>	XP_003416830.1	ENSLAFP00000010538	ENSLAFP00000001628	ENSLAFP00000003437
Mouse	<i>Mus musculus</i>	ENSMUSP000000104314	NP_035077.1	ENSMUSP000000138412	ENSMUSP00000020204
Human	<i>Homo sapiens</i>	ENSP000000173229	ENSP000000293973	ENSP000000270235	ENSP000000340998
Lizard	<i>Anolis carolinensis</i>	XP_008102492.1	ENSACAP00000001541	ENSACAP00000015606	ENSACAP00000013843
Painted turtle	<i>Chrysemys picta bellii</i>	XP_005309923.1	ND	XP_005312122.1	XP_005306562.1
Sea Turtle	<i>Chelonia mydas</i>	XP_007072692.1	XP_007054595.1	XP_007067254.1	XP_007069305.1
Ostrich	<i>Struthio camelus australis</i>	XP_009672469.1	XP_009670387.1	ND	XP_009672888.1
Chicken	<i>Gallus gallus</i>	ENSGALP000000038541	ENSGALP00000043056	ND	ENSGALP00000018590
Turkey	<i>Meleagris gallopavo</i>	XP_010719536.1	XP_010718184.1	ND	XP_010707623.1
Falcon	<i>Falco peregrinus</i>	XP_005236273.1	XP_005241062.1	ND	XP_005242758.2
Ibis	<i>Nipponia nippon</i>	XP_009461473.1	XP_009460387.1	ND	XP_009462272.1
Pigeon	<i>Columba livia</i>	XP_005515376.2	XP_005505712.1	ND	XP_013225601.1
Zebra Finch	<i>Taeniopygia guttata</i>	ENSTGUP00000005317	ENSTGUP00000003367	ND	ENSTGUP00000008736
Ground Finch	<i>Geospiza fortis</i>	XP_005427923.1	XP_005425254.1	ND	XP_014162721.1
Ground tit	<i>Pseudopodoces humilis</i>	XP_005531006.1	XP_005522839.1	ND	XP_014104309.1
Name	Latin name	NTN1	NTN4		
Amphioxus	<i>Branchiostoma floridae</i>	XP_002606106.1	XP_002209834.1		

Sauropsid
Aves
Galli
Pass.

Key : NCBI Genome Browser
Ensembl Genome Browser
Partial sequence
ND : No Data

Galli. Galliformes
Pass. Passeriformes

Table S4 – Names of the genes used in DCC synteny analysis.

Short name	Full name
HDHD2	haloacid dehalogenase-like hydrolase domain containing 2
IER3IP1	immediate early response 3 interacting protein 1
SKOR2	SKI family transcriptional corepressor 2
SMAD2	SMAD family member 2
ZBTB7C	zinc finger and BTB domain containing 7C
CTIF	CBP80/20-dependent translation initiation factor
SMAD7	SMAD family member 7
DYM	dymeclin
RPL17	60S ribosomal protein L17
LIPG	lipase, endothelial
ACAA2	acetyl-CoA acyltransferase 2
MyoVb	myosin VB
CFAP53	cilia and flagella associated protein 53
SKA1	spindle and kinetochore associated complex subunit 1
MAPK4	mitogen-activated protein kinase 4
ME2	malic enzyme 2, NAD(+)-dependent, mitochondrial
ELAC1	elaC ribonuclease Z 1
SMAD4	SMAD family member 4
MEX3C	mex-3 RNA binding family member C
DCC	Deleted in Colorectal Carcinoma
MBD2	methyl-CpG binding domain protein 2
POLi	polymerase (DNA directed) iota
STARD6	StAR-related lipid transfer (START) domain containing 6
DYNAP	dynactin associated protein
RAB27B	RAB27B, member RAS oncogene family
CCDC68	coiled-coil domain containing 68
TCF4	transcription factor 4
TXNL1	thioredoxin-like 1
WDR7	WD repeat domain 7
ST8SIA3	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 3
FECH	ferrochelataase
NARS	asparaginyl-tRNA synthetase
ATP8B1	ATPase, aminophospholipid transporter, class I, type 8B, member 1
NEDD4L	neural precursor cell expressed, developmentally downregulated 4-like
ALPK2	alpha-kinase 2
MALT1	mucosa associated lymphoid tissue lymphoma translocation gene 1
ZNF532	zinc finger protein 532
SEC11C	SEC11 homologue C (<i>S. cerevisiae</i>)
GRP	gastrin-releasing peptide
RAX	retina and anterior neural fold homeobox
CPLX4	complexin 4
LMAN1	lectin, mannose-binding, 1

Table S5 – List of species used in DCC synteny analysis.

Species	Latin name
Human	<i>Homo sapiens</i>
Mouse	<i>Mus musculus</i>
Platypus	<i>Ornithorhynchus anatinus</i>
Painted Turtle	<i>Chrysemys picta bellii</i>
Alligator S	<i>Alligator sinensis</i>
Duck	<i>Anas platyrhynchos</i> (mallard)
Goose	<i>Anser cygnoides domesticus</i>
Chicken	<i>Gallus gallus</i>
Turkey	<i>Meleagris gallopavo</i>
Quail	<i>Coturnix japonica</i>
Saker Falcon	<i>Falco cherrug</i>
Bald Eagle	<i>Haliaeetus leucocephalus</i>
Adeli Penguin	<i>Pygoscelis adeliae</i>
Royal Eagle	<i>Aquila chrysaetos canadensis</i>
Pigeon	<i>Columba livia</i>
Emperor Penguin	<i>Aptenodytes forsteri</i>
Ibis	<i>Nipponia nippon</i>
Cuckoo	<i>Cuculus canorus</i>
Ostrich	<i>Struthio camelus australis</i>
Egret	<i>Egretta garzetta</i>
Hoatzin	<i>Opisthocomus hoazin</i>
Chimney Swift	<i>Chaetura pelagica</i>
Tibetan ground Tit	<i>Pseudopodoces humilis</i>
Zebra finch	<i>Taeniopygia guttata</i>
Sparrow	<i>Zonotrichia albicollis</i>
Flycatcher	<i>Ficedula albicollis</i>
Crow	<i>Corvus brachyrhynchos</i>

Table S6 – Recapitulation of animal samples used in this study: names, stages and staining.

Species	Incubation time (days)	Hamburger-Hamilton stage or equivalent	Total number
Chicken	4	HH21-22	8
	6	HH27-28	8
	8	HH31	5
	10	HH36	3
Duck	4,5	21	5
	6	23	4
	8	27-28	7
	10	30	3
Pigeon	4	21	2
	7	25	1
	9	30	3
Zebra Finch	4	22	6
	7	31	3
Quail	4	22	2
Pheasant	4	21	3
Partridge	4	21	3

Movie S1

Triple whole-mount immunolabeling for DCC (blue), β III-Tubulin (yellow) and Doublecortin (red), followed by 3Disco clearing in embryos from 4 bird species (Duck, Chicken, Pigeon and Zebra finch).

Movie S2

ROBO3 whole-mount immunolabeling and 3Disco clearing in a HH22 chick embryo.

Movie S3

ROBO3 whole-mount immunolabeling and 3Disco clearing in a 4 day-old pigeon embryo.