Supplementary table legends

Table S1. Primers used for nested PCR. The first reaction used primers F1-R2 and the second (nested) reaction used F2-R1.

Table S2. Functionality of genes examined in this study. Genes are reported as "Functional", "Pseudogene", "no BLAST results", "no PCR results", "Functional?" or "Pseudogene?" "Functional?" refers to sequences that do not demonstrate evidence of inactivation, but other lines of evidence suggest that they are pseudogenes (e.g., gene inactivation timing estimates). "Pseudogene?" denotes sequences that that have putative inactivating mutations but other lines of evidence make these designations uncertain. Specificially, 1) *PDE6B* in *Choloepus hoffmanni* has a frameshift deletion, but this is near the 3' end of this long gene and all other rod genes are functional in this species, and 2) *Mylodon darwinii*'s *PDE6H* has a splice acceptor mutation shared with *C. hoffmanni*, but no other inactivating mutations were found. If reported as a pseudogene, the putative inactivating mutations were tabulated: SA = splice acceptor mutation; SD: splice donor mutation; INS = frameshift insertion; DEL = frameshift deletion; STOP = premature stop codon; Other = other putative inactivating mutations; Reference = previous study that determined gene functionality. Numbers correspond to the location of the mutations in dataset S1.

Table S3. Putative inactivating mutations shared by species of various clades represented in our study. Euphractinae = *Euphractus*, *Chaetophractus*, *Zaedyus*; Tolypeutinae = *Priodontes*, *Tolypeutes*; Myrmecophagidae = *Myrmecophaga*, *Tamandua*. Mutation types same as table S2. Position of mutation corresponds to the location of the mutations in dataset S1.

Table S4. Results from pseudogene dating calculations using dataset S2. Stem Xenarthra, *Dasypus, Choloepus* and *Manis* indicate the branch on which we assumed the gene was inactivated based on shared or unique inactivation mutations (table S3). CF 1, CF 2 and CF 3 correspond to codon models one, two and three in PAML respectively. mb age = mixed branch age (from [2]); mb ω = mixed branch ω; fb ω = functional branch ω; p ω = pseudogene branch ω; date 1 = pseudogenization date, one synonymous substitution rate assumption; date 2 = pseudogenization date, two synonymous substitution rates assumption (see [3] for details); average = average of all six dating estimates. The pseudogene branch ω was assumed to be 1 whenever a pseudogene branch category was not available. The stem Xenarthra estimate was added to Meredith et al.'s [2] average estimate for the crown Xenarthra divergence (65.43 Ma). In cases where the mixed branch ω exceeded the pseudogene branch ω, we set the inactivation date estimate at the earliest age of the mixed branch (e.g., *Dasypus LWS* and *GUCY2F*).

Table S5. Comparison of *SWS1* inactivation dates across Mammalia. Lineage refers to the branch that we assume *SWS1* was inactivated on. "Various" indicates multiple lineages within a clade that lost *SWS1* independently. Inactivation Date refers to inactivation dates in millions of years based on point estimates (Xenarthra, *Manis pentadactyla*, Chrysochloridae, *Spalax ehrenbergei*) and ranges derived from phylogenetic bracketing. Ranges derived from divergence time estimates [1,3,4]

Table S6. Accession numbers for all new gene sequences reported in this study. All sequences for *Mylodon darwinii* were downloaded from the NCBI Sequence Read Archive. Several *de novo* sequences were too short (<200 bp) to be submitted to GenBank, but can be found in dataset S1.

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Table S1

SWS1	
exon 1	
F1	CTTGGGKAMTTCTAATTCCAAACTYTGTC
R2	CCAACAGCCCAGTWCCTRGTTCCAAATCTGG
F2	GTGGCTTTGGAGGAGGRCYTCTGTGTKGGG
R1	GTTCCAAATCTGGAGCMGYGTWCTTTGCCTC
exon 2	
F1	GCAGCCCAGACCAGAAGTCTGACCCAAATAGG
R2	CTCTTSCYACACCCAGTCAACCTAAGCTCCTSGC
F2	CATCCTTTTTCCACTCTGCCTGCCAGGCTGG
R1	CCTRAGCTCCTSGCTGAGTYAGCACCACTG
exon 3	
F1	CTCCYTTCYCCAGTCKTTTGGCATTGAG
R2	GGYACATGTRTCTCTTGACATTCAGAATC
F2	GTCKTTTGGCATTGAGTTCYCAACWGAGAACAC
R1	CAGAGCACTMACCCAYTYCTCCCACCTAGAG
exon 4	
F1	GAGATCTGAGTTATGGGCCAGAGAGAAGAGG
R2	GTGGTAGACGGACGCCCTAACCACTGGGCC
F2	GGGCCAGAGAGAAGAGGGACATGGTGG
R1	CCACTGGGCCAAGTCCGCTTCTCTAGAG
exon 5	
F1	CCTGGTGTTCTCTCTTCTGCATTAGTCTGCC
R2	GTGGCCTTTGGGGTGGTCAAGTAGTCCAAGCTG
F2	GCATTAGTCTGCCAAGAGGGCTATCTCCAG
R1	CCAAGCTGGGAATAGTAGCTTGTGGGCAG
PDE6C	
exon 4	
F1	GAATAGATACWTGTTCAAAAATAGAAYTG
R2	GCAAAGCCAAMATTCAAGYCTAGATCTTTTTGYTC
F2	CWTGTTCAAAAATAGAATTGAATTAAGAMTGSC
R1 Cingulata	GATCTTTTTGYTCTRAAATATATACTTTTGCMTCTG
R1 Pilosa	GATCTTTTTGYTCTRAAATGCACACTTTTGCMTCTG

exon 5	
F1	GSTCCTTTCTAGCTCYRAAATTCCAG
R2	GGTCCCACAGARAGTTWCAGWACTAGAACCC
F2	CCAGAATTACATAAATTARMATRAAATGTGC
R1	GTTWCAGWACTAGAACCCAGGYCTCCC

Table S2

	Dasypus	***Choloepus hoffmanni***	***Mylodon***	
ARR3	**No BLAST results**	**No BLAST results**		
CNGA1	Functional	Functional		
CNGA3	Functional	Functional		
CNGB1	Functional	Functional		
CNGB3	**Pseudogene**	Functional		
SD	650			
INS	1873, 1990			
DEL	1291			
STOP	763-5, 1330-2, 1450-2, 2134-6			
GNAT1	Functional	Functional		
GNAT2	**Pseudogene**	Functional		
SD	163, 887			
SA	117			
INS	388-9, 478-9, 838, 850			
DEL	327-33, 396			
STOP	298-300, 949-51, 1051-3			
Other	Partial exon-intron deletion: 723-732			
GNB1	Functional	Functional		
GNB3	Functional	Functional		
GNGT1	Functional	Functional		
GNGT2	Functional	**Pseudogene**		
INS		183-660		
GRK1	Functional	Functional		
GRK7	**Pseudogene**	**Pseudogene**	**Pseudogene**	
SD	1060, 1338			

INS	937, 1495-9	1255, 1589-1629	1255, 1589-1619	
DEL	548-9, 1537	1301-2		
STOP	478-80, 718-20, 1294-6, 1363-5	1207-9, 1315-7, 1534-6, 612-4	612-4	
Other	Repetitive element insertion: 1-463 Partial exon-intron deletion: 947-1179	Partial exon-intron deletion: 947-1179	Partial exon-intron deletion: 947-1179	
GUCA1A	Functional	Functional		
GUCA1B	**Pseudogene**	Functional		
DEL	267-428, 558-9, 563, 568			
GUCY2D	Functional	Functional		
GUCY2F	**Pseudogene**	**Pseudogene**	**Pseudogene**	
SD	2291	1585, 2291, 3172		
SA		2439		
INS	496, 819-4572, 883, 2772	1318, 1351-4, 2839-40		
DEL	237, 306-39, 472-3, 682-98, 915, 955, 1321, 1346, 1410, 1661, 1902, 2031, 2123-38, 2567-8, 3069	163, 253-9, 882, 1324, 2724-805	253-9, 2724-805	
STOP	394-6, 613-615, 703-5, 796-8, 1144-6, 1162-4, 1198-1200, 1363-5, 1387-9, 1528-30, 1612-4, 1630-2, 2149-51, 2170-2, 2440-2, 2929-2931	181-3, 229-31, 829-31, 1372-4, 1396-8, 1759-61, 1897-9, 2410-2, 3055-7, 3118-3120, 3151-3	181-3	
LWS	**Pseudogene**	Functional		
SA	983			
DEL	119-25, 144-65, 303, 532, 884, 1007, 1051			
STOP	244-6			
PDE6A	Functional	Functional		

PDE6B	Functional	**Pseudogene?**		
DEL		2491-2		
PDE6C	**Pseudogene**	**Pseudogene**	**Pseudogene**	
SD	724, 868, 946, 1011, 2320	1126	1126	
SA	632, 723, 1418	1418, 1743		
INS	67-8, 727, 1933-40, 2161-5, 2194, 2200, 2545-8	919-20, 1851-8		
DEL	271, 392, 805-8, 843, 1356-60, 1480, 1488, 1632-3, 1702-9, 2003-4, 2223, 2376, 2447	278-91, 338, 479-94, 1279-82, 1414-7, 1630, 1954	479-94, 560, 1954	
STOP	289-91, 829-31, 943-5, 961-3, 1585-7	814-6, 829-31, 943-5	814-6, 829-31, 1111-3, 1915-7	
PDE6G	Functional	Functional		
PDE6H	**Pseudogene**	**Pseudogene**	**Pseudogene?**	
SD		136, 176		
SA	134	133	133	
DEL	160, 232-5			
STOP		235-7		
Other		Start codon mutation: 1-3		
RH1	Functional	Functional		
SAG	Functional	Functional		
SWS1	**Pseudogene**	**Pseudogene**	**Pseudogene**	
SD	731, 970			
INS	247-57, 580-1, 685-686	43-6, 398	398	
DEL	33-4, 320-1, 340, 345, 645, 692, 823-36, 848-76, 922	98-9, 139-40, 340-3, 613, 681-2, 1067-8	139-40	
STOP	82-4, 208-10, 313-5, 391-3, 445-7, 532-4	70-2, 316-8, 505-7, 700-2, 973-5	505-7	
Other	Pro23Leu: 67-9	Pro23Leu: 67-9 Partial exon-intron deletion: 550-91		

SLC24A2	Functional	Functional		
	Balaenoptera	***Physeter***	***Manis***	***Tursiops***
CNGA3	Functional	**Pseudogene**	Functional	Functional
SD		738		
SA			451-2	
INS		1171, 1936		
STOP		469-471		
CNGB3	**Pseudogene**	**Pseudogene**	Functional	Functional
SA	1183			
DEL		554, 1407		
STOP	1525-7			
GNAT2	Functional	**Pseudogene**	Functional	Functional
SA	726			
INS		1012		
GNB3	Functional	Functional		Functional
GNGT2	Functional	**Pseudogene**	Functional	Functional
STOP		115-7		
LWS	**Pseudogene**	**Pseudogene**	Functional	Functional
Reference	[12]	[12]		
PDE6C	Functional	Functional	Functional	Functional
PDE6H	**Pseudogene**	Functional	Functional	Functional
STOP	235-7			
SWS1	**Pseudogene**	**Pseudogene**	**Pseudogene**	**Pseudogene**
INS			1042	
DEL			613, 694-712, 1002-3, 1049	
Reference	[12]	[12]		[12]
	Chaetophractus	***Euphractus***	***Priodontes***	***Tolypeutes***
PDE6C	**Pseudogene**	**Pseudogene**	**Pseudogene**	**Pseudogene**
SD			868	868
SA				866
DEL	763-4, 805-8,	763-4, 805-8	780-3	
STOP				883-5

SWS1	**Pseudogene**	**Pseudogene**	**Pseudogene**	**Pseudogene**
SD		731		731
INS			1006	115-6, 418
DEL	33-4, 320-1, 340-1, 359	33-4, 263, 320-1, 340-1, 359	33-4, 284-5, 320-1, 340-1	31-4, 320-1, 340-1
STOP	208-10, 391-3	208-10, 391-3	208-10	208-10, 391-3
Other	Pro23Leu: 67-9	Pro23Leu: 67-9	Pro23Leu: 67-9	Pro23Leu: 67-9; Start codon mutation: 10-12
	Zaedyus	***Choloepus didactylus***	***Bradypus***	
PDE6C	Functional?	**Pseudogene**	**Pseudogene**	
SA			723	
INS		919-20	919-20	
STOP		814-6, 829-31, 943-5	814-6, 829-31	
SWS1	**Pseudogene**	**Pseudogene**	**Pseudogene**	
SD	731			
INS		43-6, 398	43-6, 398	
DEL	33-4, 199-200, 320-1, 340-1, 359	98-9, 139-40, 340-3, 417, 613, 681-2	98-9, 139-40, 199-200, 340-3	
STOP	208-10	70-2, 316-8, 505-7, 700-2	40-2, 70-2, 316-8, 505-7	
Other	Pro23Leu: 67-9	Pro23Leu: 67-9 Partial exon-intron deletion: 550-91	Pro23Leu: 67-9	
	Myrmecophaga	***Tamandua***	***Cyclopes***	
PDE6C	Functional?	No PCR results	Functional?	
SWS1	**Pseudogene**	**Pseudogene**	No PCR results	
INS	556, 983	556		
DEL	654, 700-24, 1073	654, 700-24		

Table S3

Clade	**Gene**	**Mutation**	**Position**
Xenarthra	*GRK7*	DEL	947-1179
Folivora	*GRK7*	INS	1255
Folivora	*GRK7*	INS	1589-629
Folivora	*GUCY2F*	STOP	181-3
Folivora	*GUCY2F*	DEL	253-9
Folivora	*GUCY2F*	DEL	2724-805
Euphractinae	*PDE6C*	DEL	763-4
Euphractinae	*PDE6C*	DEL	805-8
Tolypeutinae	*PDE6C*	SD	868
Folivora	*PDE6C*	DEL	479-94
Folivora	*PDE6C*	STOP	814-6
Folivora	*PDE6C*	STOP	829-31
Folivora	*PDE6C*	INS	919-20
Folivora	*PDE6C*	DEL	1954
Choloepus	*PDE6C*	STOP	943-5
Folivora	*PDE6H*	SA	133
Xenarthra	*SWS1*	missense P23L	67-9
Cingulata	*SWS1*	DEL	33-4
Cingulata	*SWS1*	STOP	208-10
Cingulata	*SWS1*	DEL	320-1
Cingulata	*SWS1*	STOP	391-3
Cingulata	*SWS1*	STOP	700-2
Cingulata	*SWS1*	SD	731
Euphractinae+Tolypeutinae	*SWS1*	DEL	340-1
Euphractinae	*SWS1*	DEL	359
Folivora	*SWS1*	INS	43-6
Folivora	*SWS1*	STOP	70-2
Folivora	*SWS1*	DEL	98-9
Folivora	*SWS1*	DEL	139-40
Folivora	*SWS1*	STOP	316-8
Folivora	*SWS1*	DEL	340-3
Folivora	*SWS1*	INS	398
Folivora	*SWS1*	STOP	505-7
Choloepus	*SWS1*	partial exon-intron deletion	550-591
Choloepus	*SWS1*	DEL	613
Choloepus	*SWS1*	DEL	681-2
Myrmecophagidae	*SWS1*	INS	556
Myrmecophagidae	*SWS1*	DEL	654
Myrmecophagidae	*SWS1*	DEL	700-24

Table S4

	mb age	mb ω	fb ω	p ω	date 1	date 2	average
stem Xenarthra	33.22						
SWS1							
CF 1		0.6017	0.1602	0.9677	83.59	80.64	80.06
CF 2		0.4947	0.1209	0.8161	83.29	80.34	
CF 3		0.3677	0.1102	0.8157	77.55	74.96	
GRK7							
CF 1		1.1605	0.1404	0.7049	98.65	98.65	94.73
CF 2		0.5556	0.1237	0.7528	88.24	85.54	
CF 3		0.7624	0.1204	0.758	98.65	98.65	
	mb age	**mb ω**	**fb ω**	**p ω**	**date 1**	**date 2**	**average**
Dasypus	65.43						
CNGB3							
CF 1		0.7814	0.2826		45.49	40.24	43.65
CF 2		0.7955	0.288		46.64	41.53	
CF 3		0.7927	0.2808		46.57	41.45	
GNAT2							
CF 1		0.9969	0.0524		65.22	65.12	59.55
CF 2		0.9252	0.0446		60.31	58.35	
CF 3		0.8592	0.0431		55.8	52.49	
GUCA1B							
CF 1		0.9496	0.1384		61.6	60.1	32.24
CF 2		0.3858	0.0749		21.99	17.12	
CF 3		0.327	0.0619		18.49	14.14	
GUCY2F							
CF 1		1.0547	0.1851		65.43	65.43	65.43
CF 2		1.0589	0.185		65.43	65.43	
CF 3		1.0129	0.1767		65.43	65.43	
LWS							
CF 1		1.1255	0.107	0.9784	65.43	65.43	65.43
CF 2		0.6981	0.0745	0.6898	65.43	65.43	
CF 3		0.7183	0.0666	0.6984	65.43	65.43	
PDE6C							
CF 1		0.7372	0.1266		45.74	40.52	45.7
CF 2		0.7901	0.1316		49.61	44.96	
CF 3		0.7805	0.1222		49.07	44.32	

PDE6H							
CF 1		1.6689	0.0752		65.43	65.43	65.43
CF 2		1.8902	0.0721		65.43	65.43	
CF 3		3.278	0.0957		65.43	65.43	
	mb age	**mb ω**	**fb ω**	**p ω**	**date 1**	**date 2**	**average**
Choloepus	65.43						
GNGT2							
CF 1		0.7316	0.1886		43.79	38.35	38.56
CF 2		0.7034	0.138		42.92	37.4	
CF 3		0.6297	0.1376		37.34	31.53	
GUCY2F							
CF 1		0.8563	0.1851		53.89	50.11	51.22
CF 2		0.8484	0.185		53.26	49.33	
CF 3		0.8362	0.1767		52.41	48.29	
PDE6C							
CF 1		0.9972	0.1266		65.22	65.13	65.35
CF 2		1.072	0.1316		65.43	65.43	
CF 3		1.0001	0.1222		65.43	65.43	
PDE6H							
CF 1		0.6567	0.0752		41.14	35.49	38.63
CF 2		0.6238	0.0721		38.9	33.14	
CF 3		0.7073	0.0957		44.25	38.86	
	mb age	**mb ω**	**fb ω**	**p ω**	**date 1**	**date 2**	**average**
Manis	82						
SWS1							
CF 1		0.8208	0.1602	0.9677	67.08	62.23	60.48
CF 2		0.6487	0.1209	0.8161	62.25	56.43	
CF 3		0.6307	0.1102	0.8157	60.5	54.39	

Table S5

Lineage	Inactivation Date (Ma)	Reference
Xenarthra	80.06	This study
Various Pteropodidae	<62.92	[1,5,6]
Manis pentadactyla	60.48	This study
Lorisiformes	54.23-34.74	[3,7]
Atherurus africanus	<49.03	[1,8]
Rhinolophidae	<42.24	[1,6]
Hipposideridae	<42.24	[1,6]
Pinnipedia	38.95-21.34	[1,9,10]
Phaner spp.	<30.65	[1,11]
Odontoceti	29.36-25.19	[1,12]
Mysticeti	29.36-17.77	[1,12]
Various Procyonidae	<28.8	[1,13]
Didelphis virginiana	<22.87	[1,14]
Pteromyinae	20.56-16.13	[4,15]
Mesocricetus spp.	20.15-5.53	[4,16,17]
Aotus spp.	19.73-3.31	[3,18]
Cricetomys spp.	19.62-0.795	[4,19]
Various Muridae	<18.33	[4,20,21]
Chrysochloridae	15.84	[22]
Spalax ehrenbergei	4.98	[22]

Table S6

	CNGA1	***CNGA3***	***CNGB1***	
Dasypus	PreEnsembl77	AAGV03048495 AAGV03048497	PreEnsembl77	
Choloepus	ABVD02324799 ABVD02324800	ABVD02209073 ABVD02209074	ABVD02313158 ABVD02313159 ABVD02313160 ABVD02313161	
Manis		JPTV01040328		
Balaenoptera		ATDI01097026		
Physter		AWZP01040480 AWZP01040481		
Tursiops		Ensembl77		
	CNGB3	***GNAT1***	***GNAT2***	***GNB1***
Dasypus	AAGV03211124 AAGV03211126 AAGV03211127 AAGV03211128 AAGV03211129 AAGV03211130	PreEnsembl77	PreEnsembl77 AAGV03021203	PreEnsembl77 AAGV03047165

Choloepus	ABVD02023726 ABVD02284123 ABVD02284124 ABVD02285446 ABVD02285447 ABVD02313196	ABVD02276772	ABVD02295506	ABVD02283083 ABVD02283084
Manis	JPTV01084724 JPTV01048282 JPTV01048283 JPTV01048285 JPTV01048287 JPTV01104307 JPTV01156557		JPTV01016472	
Balaenoptera	ATDI01074324 ATDI01074325 ATDI01074327 ATDI01074328 ATDI01074329 ATDI01074330 ATDI01074334 ATDI01074335		ATDI01126518	
Physeter	AWZP01071271 AWZP01071272 AWZP01070593 AWZP01070594 AWZP01070595		AWZP01019448 AWZP01019449	
Tursiops	Ensembl77 ABRN02336514 ABRN02336517 ABRN02336518		Ensembl77	
	GNB3	***GNGT1***	***GNGT2***	***GRK1***
Dasypus	AAGV03018811	PreEnsembl77	AAGV03072014	PreEnsembl77
Choloepus	ABVD02276487	ABVD02262436	ABVD02305635	ABVD02324237 ABVD02324238
Manis	JPTV01014604		JPTV01081741	
Balaenoptera	ATDI01107455		ATDI01043175	
Physeter	AWZP01024304		AWZP01027662	
Tursiops	Ensembl77 ABRN02026850		Ensembl77	
	GRK7	***GUCA1A***	***GUCA1B***	***GUCY2D***
Dasypus	AAGV03101898 AAGV03101900	PreEnsembl77	AAGV03123970	AAGV03093596 AAGV03093597 AAGV03093598
Choloepus	ABVD02351822 ABVD02351823	ABVD02358812 ABVD02358813	ABVD02358812	ABVD02338829
	GUCY2F	***LWS***	***PDE6A***	***PDE6B***

Dasypus	AAGV03190987 AAGV03190992 AAGV03190993 AAGV03190994 AAGV03190999 AAGV03191001 AAGV03191002 AAGV03191003 AAGV03191006	AAGV03294213 AAGV03294214	PreEnsembl77	PreEnsembl77 AAGV03252435 AAGV03252438
Choloepus	ABVD02035242 ABVD02035244 ABVD02097780 ABVD02260638 ABVD02260639 ABVD02298362	ABVD02349172 ABVD02349173	ABVD02099621	ABVD02334313 ABVD02334319 ABVD02334320
Manis		JPTV01114000		
	PDE6C	***PDE6G***	***PDE6H***	***RH1***
Dasypus	AAGV03030957 AAGV03030958 AAGV03030959 AAGV03030961 AAGV03030962 AAGV03030963 KP096706	AAGV03151984	AAGV03107798	AAGV03234874
Chaeophractus	KP096707			
Euphractus	KP096708			
Priodontes	KP096709			
Tolypeutes	KP096710			
Choloepus hoffmanni	ABVD02328607 KP096711	ABVD02278694	ABVD02275907	ABVD02265667
Choloepus didactylus	KP096712			
Bradypus	KP096713			
Manis	JPTV01024353 JPTV01024354		JPTV01007876	
Balaenoptera	ATDI01012999 ATDI01013000 ATDI01013002 ATDI01013004 ATDI01013005 ATDI01013006 ATDI01013007		ATDI01106906	
Physeter	AWZP01021317 AWZP01021318 AWZP01021320 AWZP01021321		AWZP01022345	

Tursiops	Ensembl77 ABRN02390512 ABRN02390513 ABRN02390518		Ensembl77	
	SAG	***SLC24A2***	***SWS1***	
Dasypus	PreEnsembl77	PreEnsembl77 AAGV03003191	AAGV03126450	
Chaeophractus			KP096697	
Euphractus			KP096698	
Priodontes			KP096699	
Tolypeutes			KP096700	
Zaedyus			KP096701	
Choloepus hoffmanni	ABVD02329548	ABVD02254892 ABVD02254894	ABVD02055170 KP096702	
Choloepus didactylus			KP096703	
Bradypus			KP096704	
Myrmecophaga			KP096705	
Manis			JPTV01022983	