Table S1: Gene sequences	used in this stud	y. Related to Figure 1
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Human         Mul 003883.4         Mul 003883.4         Mul 003885.4		Species (Common)	Species (Scientific)	GC-C Acession	NPR1	NPR2	Uroguanylin	Guanylin
Process Macaque         Macaca mulating         XM_0201080811         XM_0202082828.3         MM_0112083411.4         XP_0112020511.4         XP_011202017.1         XP_012020201.1         XP_001202001.1         XP_001202001.1         XP_001202001.1         XP_001202001.1         XP_001202001.1         XP_001202001.1         XP_001202001.1         XP_001200201.1         XP_		Human	Homo sapiens	NM 004963.4	NM 000906.4	NM 003995.4	NP 009033.1	NP 291031.2
Plant Marcau         Maccau memethine         MML_01172189012         XML_0117289012         XML_0128228972         XML_0117289012         XML_0028228972         XML_0117289012         XML_0028228972         XML_0117289012         XML_0028228972         XML_0117289012         XML_0028239011202         XML_0028239011202         XML_0028239011202         XML_0028239011202         XML_0028014933         XML_0028014933         XML_0000448933         XML         CP000281131         XPL004028131         XML_0000448933         XML         XML_0000498333 </td <td></td> <td>Rhesus Macaque</td> <td>Macaca mulatta</td> <td>XM_001089601.4</td> <td>XM_002808236.3</td> <td>NM 001205340.1</td> <td>XP_001087987.1</td> <td>XP_001085421.1</td>		Rhesus Macaque	Macaca mulatta	XM_001089601.4	XM_002808236.3	NM 001205340.1	XP_001087987.1	XP_001085421.1
Chromazze mode         Pain toglogical model         VML 268746.8         VML 27682744.2         VML 27682744.2         VML 27682744.2         VML 27682744.2         VML 27682744.2         VML 27682744.2         VML 2768274.2		Pigtailed Macaque	Macaca nemestrina	XM_011741690.2	XM_011769501.2	XM_011748195.2	XP 011762016 1	XP 011762017 1
Oraginan         Proposedii         XMI 002822922.2         XMI 002822922.2         XMI 002829533         XMI 002829532         XMI 002829532         XMI 002829532         XMI 002829532         XMI 002829532         XMI 002829533         XMI 002829533         XMI 002829532         XMI 002839333         XMI 002839333         XMI 002839333         XMI 002839333         XMI 0028393333         XMI 0018933333         XMI 0018933333         XMI 0018933333         XMI 0018933333         XMI 00189333333         XMI 0018933333         XMI 0018933333         XMI 0018933333         XMI 0018933333         XMI 00189333333         XMI 00189333333         XMI 00189333333         XMI 00189333333         XMI 00189333333         XMI 00189333333         XMI 0018933333333333333333333333333333333333		Chimpanzee	Pan troglodytes	XM_528746.6	XM_016927544.2	XM_001168194.5	XP 524686.2	XP_001172840.1
Biochom         Pape anubis         XML 021922088         XML 0039312024         XML 0039112024         XPL 0039173224         XPL 0039173224         XPL 003917324         XPL 004265131         XPL 004265131         XPL 004265131         XPL 004256131         XPL 0012511         XPL 004256131         XPL 0012511         XPL 0013711         XPL 00137113         XPL 001371131		Orangutan	Pongo abelii	XM_002822972.2	XM_024245905.1	XM_024252690.1	XP_0028110141	XP_0028110131
Nombody Biological Continue         Action anarymane (MU 012447637)         XM 0124476321         XM 0124761222         XM 0122400232         XM 0122400232         XM 0122400232         XM 0122400233         XM 002456131         XP 002250134           Biological Crab Eliming Macaque (Cata Eliming Macaque (Cat		Baboon	Panio anubis	XM_021022012.2	XM_0038026807	XM_003011520.4	XP 0038017321	XP_003801733.1
Section         Contrage and a genuine         XML 019037841         XML 0190229031         XML 0040830043         XP_0040286131         XP_0040286131         XP_0040286131         XP_00502976361           Big Borns         Marmoset         Bucconstructure         XML 01903796341         XML 01968001         XP_005243815.1         XP_005243815.1         XP_005243815.1         XP_005243815.1         XP_005263811.1         XP_00526381.1         XP_011934823.1         XP_01193483.1         XP_01193483.1 </td <td></td> <td>Night Monkey</td> <td>Actus nancymaae</td> <td>XM_021322000.1 XM_012447637.1</td> <td>XM_012476152.2</td> <td>XM_012/63327.2</td> <td>XP 012280025 1</td> <td>XP 012280880 1</td>		Night Monkey	Actus nancymaae	XM_021322000.1 XM_012447637.1	XM_012476152.2	XM_012/63327.2	XP 012280025 1	XP 012280880 1
Bit Namoset         Californi production         XM_0179765301         XM_002654082         NA         XP_002780783           Chab Earling Macaque         Normacus Buscogenya         XM_0026507232         XM_0005617322         XM_00126507831         XP_00273058115         XP_002730581         XP_0012730581         XP_00137058241         XP_011380581         XP_01189211         XP_001370153         XP_001370153         XP_0013701531		Gorilla	Gorilla gorilla gorilla	XM_019037844 1	XM_019022930.1	XM_012403327.2 XM_004048004.3	XP_004025613.1	XP_004025614.1
Park         Macros Bascularia         XM_00551232         XM_005517522         XM_005517522         XM_005517522         XM_0015515332         XM_0015515332         XM_0015515323         XM_0015515323         XM_0015515323         XM_0015515323         XM_0015555333         XM_0015555333         XM_0015555333         XM_0015555333         XM_0015555333         XM_001555553         XM_001555553         XM_001555553         XM_001555553         XM_001555553         XM_001555555         XM_001555555         XM_00155555         XM_001555555         XM_0015555555         XM_00155555555         XM_001555555555555555555555555555         XM_00155555555555555555555555555555555555	es	Marmoset	Callithrix jacchus	XM_017976534.1	XM_017966300.1	XM_002806498.3	NA _004020010.1	XP_002750736.1
Big Dobum         Moneya         MA         D02265282         NA         MA         D02373331         XP         D023733311         XP         D033733311         XP         D033733311         XP         D033733311         XP         D033733311         XP         D033733311         XP         D033733311         XP         D033733111         XP         D03373111         XP         D013342311         XP         D013342311         XP         D013342311         XP         D0133423111         XP         D013343111         XP         D0133431111         XP         D0133431111 </td <td>lat</td> <td>Crab Eating Macaque</td> <td>Macaca fascicularis</td> <td>XM_005570213.2</td> <td>XM_005541752.2</td> <td>XM_005581348.2</td> <td>XP 0055438151</td> <td>XP_005543814.1</td>	lat	Crab Eating Macaque	Macaca fascicularis	XM_005570213.2	XM_005541752.2	XM_005581348.2	XP 0055438151	XP_005543814.1
C         Composition and Dist	Ë	Gibbon	Nomascus leucogenys	XM_003265528.2	NA	XM_011939383.1	XP_003273395.1	XP_012366311.1
Dim         Mandhille baropheeus         XM_011993831         NA         XM_011993831         NA           Back and White Colubus         Spaint Bioliversis bulliversis bulliversis         XM_011939381         XM_011939381         XM_011929381         XM_011929812         XM_0098151202         XM_008512981         XM_00182351251         XM_00182351251<	۵.	Sooty Mangabey	Cercocebus atvs	XM_012058487.1	XM 012076614 1	XM_012087083.1	XP 011934624 1	XP_011934623.1
Back and While Colobus Squirel Monkey Gold Snub Nosed Monkey Colobus angolemis politanus Squirel Monkey Squirel Monkey Squirel Monkey Mouse Lemur Tarsier         Colobus angolemis politanus Squirel Monkey Colobus Squirel Monkey Colobus Squirel Monkey Mouse Lemur Tarsier         XP_011800271.1         XP_011800271.1         XP_011800271.1         XP_01037013.1         XP_0103220583.1         XP_02080583.1		Drill	Mandrillus leucophaeus	XM_011990839.1	NA	XM_0119801421	XP_011857110.1	XP_0118571111
Spinitre Monkey         Samitr bollwenses bollwense;         XM_003941876_2         XM_003941876_2         XM_003947892_2         XM_003937037_1         XP_003970371_1         XP_003970371_1         XP_003970371_1         XP_003970371_1         XP_003970371_1         XP_003970371_1         XP_003970371_1         XP_00777381_1         XM_0078777481_1         XM_007877041_1         XM_007877041_1         XM_007877381_1         XP_007877381_1         XM_0028511917_1         XP_008065362_1         XP_008065362_1         XP_008065362_1         XP_008065362_1         XP_008065362_1         XP_008065362_1         XP_008065362_1         XM_008161262_2         XM_0016161262_2         XM_0026161262_2		Black and White Colobus	Colobus angolensis palliatus	XM_011954200.1	NA	XM_011939383.1	XP 011800278 1	XP_011800291.1
Control         State         <		Squirrel Monkey	Saimiri boliviensis boliviensis	XM 003934548.2	XM 003941876.2	XM 003943789.2	XP 003937037.1	XP 003937036.1
Amount Lemma         Charonechus sabaeus         XM_007977041         XM_0079787241         XM_007987738.1         XM_00797738.1         XM_007987738.1         XM_00797738.1         XM_00797738.1         XM_007987878.1         XM_007987878.1         XM_007987738.1         XM_00797738.1         XM_007751738.1         XM_007751738.1         <		Gold Snub Nosed Monkey	Rhinopithecus roxellana	XM 010368479.1	XM 010364757.1	XM 010376182.2	XP 010370113.1	XP_010370115.1
Mccose Lemur         Mccosebus murinus         XM_012753729.2         XM_0102701228.1         XM_012750299.2         XM_02851197.1         XM_028507367.1         XM_02851197.1         XM_02851292.2         XM_00151292.2         XM_00151292.2         XM_00151292.2         XM_00157362.2         XM_00157362.2         XM_00157362.2         XM_00157362.2         XM_00151292.2         XM_00153621.1         XM_00153621.3         XM_00153621.2		African Green Monkey	Chlorocebus sabaeus	XM_007967738.1	XM_007977049.1	XM_007968782.1	XP_007977383.1	XP_007977382.1
Tansier         Carllo synichia         XM_008071877.1         NA         XM_008063120.2         XP_008065382.1         XP_008065382.1           Pale spear-nosed bat         Phyliostomus discolor         XM_008110435.2         XM_00811280.2         XM_008151280.2         XM_00805923.3         XM_00805923.3         XM_00805923.3         XM_00805923.3         XM_00805923.3         XM_00805728.3         XM_018205154.1         XM_005666652.2         XM_006765257		Mouse Lemur	Microcebus murinus	XM_012753729.2	XM_012791226.1	XM_012769991.2	XP_012620637.1	XP_012620638.1
Pale spear-nosed bat Big brown bat         Phyllostomus discolor Explosions viscuis         XM_028532971.1         XM_028507867.1         XM_028512623.1         XM_0285112623.1         XM_0081512802.2         XM_0081512802.2         XM_0081512802.2         XM_0081512802.2         XM_002856673.2         XM_00143556112.1         XM_002856673.2         XM_0014379362.2         XM_0014379362.2         XM_00160725742.3         XM_00160725742.3         XM_0016072574.3         XM_0016072574.3         XM_0016072574.3         XM_0016072574.3         XM_0016072574.3         XM_0016725754.3         XM_006686053.2         XM_0016725754.3         XM_0016725754.3         XM_0016725754.3         XM_006686053.2         XM_006686053.2         XM_006686053.2         XM_001633661.3         XM_0276514789.1         XM_0025666053.2         XM_006686053.2         XM_001633661.1         XM_02765		Tarsier	Carlito syrichta	XM_008071877.1	NA	XM_008063120.2	XP_008065362.1	XP_008065363.1
Pale spear-nosed bat Big forwn bat Biack flying fox Yampie bat Little borwn bat Biack flying fox Paropus ampying Biack flying fox Paropus ampying Sing Biack flying Sing Biack flying Sing Biach Biach flying Sing Biach flying Sing Biack flying S								
Big brown bat         Epseizus fuscus         XM_008140435.2         XM_028146098.1         XM_008151290.2         XM_008151290.2         XM_008151290.2         XM_008151290.2         XM_008151290.2         XM_008151290.2         XM_00807992.3         XM_00807992.3           Black flying fox         Pteropus andors othindus         XM_0024575708.1         XM_02456018.1         XM_02456018.1         XM_024554672.1         XM_00807230.3         XM_00807230.3         XM_00807230.3         XM_00807230.3         XM_00807230.3         XM_001877362.2         XM_00807230.3         XM_001877362.2         XM_001873730.6         XM_00187372.1         XM_01963310.1         XM_01963310.1         XM_01963725.1         XM_0184372.1         XM_0184205.1         XM_0184372.1         XM_00676870.2         XM_00676870.2         XM_007676870.1         XM_02566645.1         XM_00266051.2         XM_00260767.1         XM_025607645.1         XM_02566748.1<		Pale spear-nosed bat	Phyllostomus discolor	XM_028532971.1		XM_028507867.1	XM_028512623.1	XM_028511197.1
Black hying fox         Pheropus alecto         XM_006912643.3         XM_00691600.22         XM_00803991.3         XM_006080392.3           Uampie bat         Desmodus rotinduis         XM_0024556721         XM_024556721.4         XM_024556721.4         XM_024556721.4         XM_024556721.4         XM_024556721.4         XM_024566721.4         XM_0026087238.3         XM_006087238.3         XM_006087238.3         XM_006087238.3         XM_006087238.3         XM_006087238.3         XM_006087238.3         XM_006087238.3         XM_006087238.3         XM_006087238.3         XM_0060512.1         XM_019643726.1         XM_019643726.1         XM_016050512.1         XM_01605152.1         XM_016020165.1         XM_016020165.1         XM_016139667.1         XM_016139667.1         XM_016139667.1         XM_016139667.1         XM_006786202.2         XM_006786202.4         XM_006786673.2         XM_016139667.1         XM_005866051.1         XM_005866051.1         XM_005866052.2         XM_00758701.1         XM_002586288.1         XM_005866051.1         XM_002585288.1         XM_0026861352.1		Big brown bat	Eptesicus fuscus	XM_008140435.2		XM_028146098.1	XM_008151290.2	XM_008151289.2
Verture         Verture <t< td=""><td></td><td>Black flying fox</td><td>Pteropus alecto</td><td>XM_006912643.3</td><td></td><td>XM_006916002.2</td><td>XM_006903991.3</td><td>XM_006903992.3</td></t<>		Black flying fox	Pteropus alecto	XM_006912643.3		XM_006916002.2	XM_006903991.3	XM_006903992.3
Late         Under Specifies         XM_006084275.3         XM_014460804.2         XM_01078081238.3         XM_006087239.3         XM_006087239.3         XM_006087239.3         XM_006087239.3         XM_006087239.3         XM_006087239.3         XM_006087239.3         XM_001737934.2           Greatroundleaf bat         Hipposideros armiger         XM_01975991.1         XM_016222895.1         XM_0161205152.1         XM_005066051.1         XM_005866051.1         XM_005866051.1         XM_005866052.2         XM_0075862205         XM_0075862205         XM_0075862205         XM_007586212.0         XM_005866051.1         XM_005866051.1         XM_005866052.2         XM_0075852205         XM_0075852205         XM_0075852205         XM_0075852205         XM_0075852205         XM_0075852051.0         XM_0075852037.1         XM_005866051.1         XM_005866051.1         XM_005866052.2         XM_007585202.0         XM_0075852		Vampire bat	Desmodus rotundus	XM_024575708.1		XM_024560188.1	XM_024554679.1	XM_024554672.1
Bit         Large fying fox         Pieropus vampyrus         XM_01375981.2         XM_023524003.1         XM_011377932.2         XM_014377934.2         XM_01437934.2         XM_014375261.1         XM_00576257.1         XM_00576257.1         XM_00576257.1         XM_005766072.2         XM_01437524.1         XM_005766072.2         XM_01437524.1         XM_005860051.1         XM_005860051.1         XM_005860051.1         XM_005860051.1         XM_005860052.2         XM_01437524.1         XM_025650242.1         XM_005860051.1         XM_005860051.1         XM_005860051.1         XM_005860051.1         XM_0258288.1         XM_02583288.1         XM_02583282.1         XM_00474		Little brown bat	Myotis lucifugus	XM_006084275.3		XM_014468054.2	XM_006087239.3	XM_006087238.3
Call Great roundleat bat         Hippositeros amiger         XM_019643226.1         XM_019643227.1           Natal long-fingered bat         Minoplexus natalensis         XM_016140561.1         XM_01620515.41         XM_01620515.41           Expptian fruit bat         Rousettus aegyptiacus         XM_016140541.1         XM_01133151.2         XM_016139967.1         XM_01620515.41         XM_00676257.22           Brand's bat         Myotis brandtii         XM_005666793.2         XM_014531524.1         XM_00576257.42         XM_00676257.51           California sea lion         Zalophus californianus         XM_025692374.1         XM_026666254.1         XM_005866051.1         XM_005866052.2           Northem fur seal         Ursus arctos hombilis         XM_025692364.1.1         XM_02686288.1         XM_02686288.1           Bingo         California us ursitus         XM_02565287990.0.1         XM_025852888.1         XM_027614789.1           Bingo         Canis lupus familiariadi         XM_025455488.1         XM_02646288.1         XM_027614789.1           Bingo         California us rakus         XM_025455488.1         XM_027614789.1         XM_0268602842.1           Bingo         California us rakus         XM_025455488.1         XM_02761478.1         XM_02761478.1           Bingo         Califorinis us rakus         XM_0226502571.1	ats	Large flying fox	Pteropus vampyrus	XM_011375981.2		XM_023524003.1	XM_011377936.2	XM_011377934.2
Name         Number of the second	ш	Great roundleaf bat	Hipposideros armiger	XM_019643276.1		XM_019663310.1	XM_019643726.1	XM_019643727.1
Legpplant multicatAddustatis aegypalacusXM_01614041.1XM_001361514.2XM_01613996.1XM_01613996.1XM_01613996.1XM_01613996.1XM_006725272.2XM_01673286.2XM_006762572.3XM_006762572.4XM_006762572.4XM_006762572.4XM_006762572.4XM_006762572.4XM_006762572.4XM_006762572.4XM_006762572.4XM_006762572.4XM_006762572.4XM_006762572.4XM_006762572.4XM_006762572.4XM_006762572.4XM_005666052.2XM_005725617.1XM_005766703.2XM_005762572.4XM_0057666052.2XM_006762572.4XM_0057666052.2XM_006762572.4XM_0057666052.2XM_006762572.4XM_0057666052.2XM_006762572.4XM_0057666052.2XM_002560245.1XM_002560245.1XM_002560245.1XM_002560246.1XM_002560246.1XM_002560246.1XM_002560246.1XM_002560246.1XM_002560246.1XM_002560246.1XM_002560246.1XM_002560246.1XM_002560246.1XM_002560246.1XM_002560246.1XM_002560246.1XM_002560246.1XM_002560246.1XM_002560246.1XM_002560246.1XM_002560246.1XM_00256024.1XM_00266022.1XM_001272370.1XM_001272370.1XM_001272370.1 <th< td=""><td></td><td>Natal long-fingered bat</td><td>Miniopterus nataiensis</td><td>XM_016197509.1</td><td></td><td>XM_016222895.1</td><td>XM_016205154.1</td><td>XM_016205152.1</td></th<>		Natal long-fingered bat	Miniopterus nataiensis	XM_016197509.1		XM_016222895.1	XM_016205154.1	XM_016205152.1
Under Singular       Myous Bandii       XM_0007/322/2       XM_0007/322/1       XM_0017/322/1       XM_0017/322/1       XM_0017/322/1       XM_0017/322/1       XM_0017/322/1       XM_0017/32/1       XM_002/12/32/1       XM_002/12/31/1       XM_002/12/31/2       XM_002/12/31/2       XM_002/2/32/2       XM_004/2/3/2/2       XM_004/2/3/2/2       XM_004/2/3/2/2       XM_004/2/3/2/2       XM_004/2/2/3/2       XM_004/2/2/3/2       XM_004/2/2/3/2       XM_004/2/2/3/2       XM_004/2/2/3/2       XM_004/2/2/3/2       XM_004/2/2/3/2       XM_004/2/2/3/2       XM_004/2/2/3/2       XM_004/2/2/3		Egyptian truit bat	Rousettus aegyptiacus	XM_016140541.1		XM_011381514.2	XM_016139967.1	XM_016139966.1
Particles Dat     Myous Diahum     XM_003000193.2     XM_01431324.1     XM_0125508245.1     XM_0125508245.1     XM_0125508245.1     XM_0025652888.1     XM_0025652888.1     XM_0025652888.1     XM_0025652888.1     XM_0025652888.1     XM_0025695248.1     XM_003695151.1     XM_003695151.1     XM_0056950131.1     XM_0013656171.1     XM_0013656171.1     XM_0013656171.1     XM_00136561751.1<		Brandt's hat	Myous davidii Myotis brandtii	XM_005966702.2		XW_000756020.2	XW_0059660511	XWI_000702373.1
California sea lion Grizzly bear Grizzly bear Rea fox Vulpes vulpesXM_027591714.1 XM_025602374.1 XM_025602374.1 XM_025602374.1 XM_025602386.1 XM_025852888.1 XM_025852888.1 XM_025852888.1 XM_025852888.1 XM_025852888.1 XM_025852888.1 XM_025852888.1 XM_025852888.1 XM_025852888.1 XM_025852888.1 XM_025852888.1 XM_025852888.1 XM_025852888.1 XM_02243262.1 XM_025852888.1 XM_02243262.1 XM_021691775.1 XM_002918884.3 XM_011025870.1 XM_002918884.3 XM_004392264.2 XM_004392264.2 XM_004392264.2 XM_004392264.2 XM_004392264.2 XM_004392264.2 XM_004392264.2 XM_004392264.2 XM_004392264.2 XM_004392264.2 XM_004392264.2 XM_004392781.1 XM_008695194.1Topode Topode Topode Zabu Yak Bos indicus Sindicus Sindicus Sindicus Sindicus Sindicus Sindicus Sindicus Sindicus XM_01961517.1 XM_008695194.1XM_01966151.1 XM_008695194.1 XM_008695194.1Topode Topode Zabu Yak Bos indicus Sindicus 		Chinese rufous horseshoe bat	Rhinolophus Sinicus	(Sequence recovered	d from BLAST)	<u>NM_014551524.1</u>	XIVI_005606051.1	XIVI_005666052.2
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Omega       Bubbaius bubais       XM_00605135.2       XM_006064377.2         Goat       Capra hircus       XM_018064849.1       NM_001285674.1         Sheep       Ovis aries       XM_012150903.1       NA         Mouflon       Ovis aries musimon       XM_012150903.1       NA         Image: Muse mouse       Mus musculus       NM_001127318.1       NM_0113584.4         Ryukyu mouse       Mus caroli       XM_021165238.1       XM_021159499.2         Gairdner's shrewmouse       Mus pahari       XM_021190835.1       XM_021222144.2         Norway Rat       Ratus norvegicus       NM_013170.1       NM_053838.1         Mongolian gerbil       Meriones unguiculatus       XM_021644291.1       XM_021658450.1         Prairie vole       Microtus ochrogaster       XM_005364517.2       XM_0026786619.1         Deer Mouse       Peromyscus maniculatus bairdii       XM_0076515.1       XM_007403228.1         Chinese hamster       Cricetulus griseus       XM_00742999.1       XM_00730228.1         Chinese regyptian jerboa       Jaculus jaculus       XM_00671850.1       XM_004658329.2	ž	Bison	Bison bison bison	XM_010858720.1		XM_010846069.1		
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Construction         Infestion		Golden hamster	Mesocricetus auratus	ANI_U2/429909.1		AIVI_UZ/4U3ZZ0.1		
		Lesser Egyptian jerboa	Jaculus iaculus	XM_004671850_1		XM_004658329.2		

## Table S2: Likelihood ratio tests for positive selection in mammalian GC-C and NPR genes.Related to Figure 1.

Group	Gene	# species	BUSTED p-value	PAML M1- M2 p-value	PAML M7- M8 p-value	PAML M0 dN/dS	PAML M8 tree length	Sites BEB > 95%
Primates	GC-C	19	0.001	0.0004	1.53E-05	0.243	1.067	6
Primates	GC-C (extracellular)	19	1.77E-04	0.0023	0.0012	0.435	1.595	6
Primates	GC-C (Intracellular)	19	1.000	1.0000	0.6974	0.084	0.703	0
Primates	NPR1	14	1.000	1.0000	0.0624	0.066	0.561	1
Primates	NPR2	19	1.000	0.5576	0.9996	0.043	0.399	0
Bats	GC-C	12	<1.0E-18	1.69E-19	7.27E-21	0.429	1.774	35
Bats	GC-C (extracellular)	12	2.22E-16	5.99E-24	7.65E-25	0.954	2.626	41
Bats	GC-C (Intracellular)	12	0.1	1.0000	0.8561	0.128	1.158	0
Bats	NPR2	11	0.994	1.0000	0.9999	0.042	0.582	0
Myomorpha	GC-C	10	2.36E-06	1.0000	1.60E-05	0.133	1.877	5
Myomorpha	NPR2	10	1.000	1.0000	0.9971	0.013	0.880	0
Caniformia	GC-C	11	0.065	0.9926	0.9052	0.189	0.543	0
Bovids	GC-C	8	1.000	0.2218	0.2017	0.117	0.172	1

## Table S3: Positively selected amino acid positions in primate and bat GC-C identified by PAML. Related to Figure 1.

	Sites with dN/dS > 1 *	Probability
	S21	0.909
	F22	0.966
	\$2 <b>3</b>	0.908
	Q25	0.975
	V224	0.983
	G251	0.939
Primate GC-C	Q279	0.965
i i i i i i i i i i i i i i i i i i i	R312	0.923
	T317	0.93
	D364	0.99
	W372	0.927
	Y406	0.985
	\$419	0.928
	R688	0.915
	121	0.99
	W 22	0.981
	123	0.997
	5 24	0.996
	F 25	0.977
	5 27	0.997
	134	0.971
	0.48	0.995
	E 52	0.997
	E 60	0.966
	R 66	0.964
	E 67	0.996
	170	0.989
	N 71	0.917
	S 72	0.901
	S 79	0.901
	S 81	0.955
	Y 84	0.909
	P 88	0.998
	V 93	0.929
	1111	0.98
	K 114	0.986
	\$ 186	0.913
	N219	0.941
	N 221	0.992
Bat GC-C	K 230	0.944
	H 239	0.913
	N 240	0.944
	T 251	0.989
	T 254	0.937
	S 256	0.998
	1257	0.914
	Q 282	0.929
	S 300	0.927
	T 304	0.972
	1 308	0.977
	s 309	0.915
	M 318	0.951
	0 345	0.933
	L 349	0.989
	E 402	0.992
	F 404	0.396
	A 407	0.953
	T 410	0.99
	N 419	0.942
	N 423	0.932
	A 425	0.937
	E 427	0.993
	0.430	0.958
	K 428	0.550
	N 744	0.944

\*Posterior probabilities >90% by PAML Model 8 Bayes Emperical Bayes (BEB) analysis, probabilities >95% are bolded amino acididentities are shown for *H. sapiens* and *M. lucifugus* in primates and bats, respectively

## Table S4: GC-C dose-response values and statistics. Related to Figure 2.

	Hur	man	Orangutan		M. lucifugus		P. vampyrus*	
Agonist	Log EC50	Maximum	Log EC50	Maxiumum	Log EC50	Maximum	Log EC50	Maximum
STp	-6.345 ± 0.113	142.7 ± 8.437	-6.382 ± 0.196	372.1 ± 38.36	-7.028 ± 0.200	598.1 ± 48.08	-6.925 ± 0.183	646.4 ± 54.4
STh	-7.143 ± 0.127	117.5 ± 6.387	-5.82 ± 0.098	250.1 ± 15.650	-5.711 ± 0.116	560.5 ± 43.75	-5.528 ± 0.118	325.7 ± 26.77
Y-ST	-4.537 ± 0.635	279.9 ± 170.6	-5.126 ± 0.066	195.7 ± 12.67	-5.311 ± 7.455	434.2 ± 20.07	N/A	N/A
V-ST	-5.676 ± 0.139	131.2 ± 11.37	-5.736 ± 0.101	103.2 ± 5.663	-5.079 ± 0.1037	304.4 ± 33.31	-5.251 ± 0.061	351.0 ± 22.45
P value (Dunnett's T3 multiple comparison test)								
STp vs. STh	0.0001	0.1184	0.078	0.0306	<0.0001	0.9926	<0.0001	<0.0001
STp vs. Y-ST	0.0519	0.9587	<0.0001	0.0005	>0.9999	0.0168	N/A	N/A
STp vs. V-ST	0.0029	0.9584	0.0306	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
STh vs. Y-ST	0.0023	0.9128	<0.0001	0.0517	>0.9999	0.0656	N/A	N/A
STh vs. V-ST	<0.0001	0.8718	0.9914	<0.0001	0.0007	<0.0001	0.1176	0.8501
Y-ST vs. V-ST	0.4165	0.9408	<0.0001	<0.0001	>0.9999	0.0087	N/A	N/A
					* F	vampyrus Y-ST dos	e/response parameter	s outside of tested





**Figure S2: Synthesis and functional verification of STa variants. Related to Figure 2.** (A) HEK293T cells transduced with lentivirus carrying Human GC-C or and empty vector were treated with the indicated peptides at a concentration of 5µM followed by measurement of intracellular cGMP by ELISA. (B) HPLC traces of each synthesized peptide shows evidence of ~8 peaks following air oxidation. Each peak was then collected for downstream analysis. (C) HEK293T cells expressing human GC-C were stimulated with the major purification products to identify the most potent fractions by measuring intracellular cGMP. For each peptide, the predominant oxidation product represented the most potent GC-C ligand. Some peaks were unable to be purified in isolation. (D) Final HPLC purification of toxin peptides used for analysis.



Uroguanylin secreted peptide sequences were downloaded from genbank for the indicated species and aligned using clustalW (B) HPLC traces of synthesized uroguanylin from the indicated species. Two distinct topoisomers were observed following oxidation of synthesized uroguanylin from each species. (C) cGMP generation assays in HEK293T cells expressing GC-C from the indicated species were performed to identify the most active topoisomer. Human isomer #1 is the known active peak. *E. fuscus* peak #1, *M. lucifugus* peak #1, and *P. vampyrus* peak #2 were used in in experimental assays.