Supplemental Materials for:

Parallel evolution of sperm hyper-activation Ca\(^{2+}\) channles

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Supplementary Figure 1: Every CatSper gene shows patterns of positive selection in the primate phylogeny. PAML model 0 dN/dS values are labeled on each branch, and branches where dN/dS>1 are in bold and colored. Branch color for each gene corresponds to the color code from Figure 1. In cases where dS=0 (causing dN/dS to be infinite), the number of non-synonymous (N*dN) and synonymous (S*dS) changes is reported instead. The values from this figure are summarized in Figure 1A.
| Species | Sequence |
|---------|----------|
| Hsap    | MESPLIYVSVLLLNIFEFSSGIVYNKDTEKRFACSNKGFPQENEIIKLY 50 |
| Caty    | MELPLIYVSVLLLNIFEFSSGIVYNKDTEKRFACSSKGYPQETEIIKLF |
| Panu    | MELPLIYVSVLLLNIFEFSSGIVYNKDTEKRFACSSKGYPQETEIIKLF |
| Mfas    | MELPLIYVSVLLLNIFEFSSGIVYNKDTEKRFACSSKGYPQETEIIKLF |
| Mmul    | MELPLIYVSVLLLNIFEFSSGIVYNKDTEKRFACSSKGYPQETEIIKLF |
| Ppan    | MESPLIYVSVLLLNIFEFSSGIVYNKDTEKRFACSSKGYPQETEIIKLF |
| Ggor    | MESPLIYVSVLLLNIFEFSSGIVYNKDTEKRFACSSKGYPQETEIIKLF |
| Ptro    | MESPLIYVSVLLLNIFEFSSGIVYNKDTEKRFACSSKGYPQETEIIKLF |
| Nleu    | MELPLIYVSVLLLNIFEFSSGIVYNKDTEKRFACSSKGYPQETEIIKLF |
| Cjac    | MELPLIYVSVLLLNIFEFSSGIVYNKDTEKRFACSSKGYPQETEIIKLF |
| Cang    | MELPLIYVSVLLLNIFEFSSGIVYNKDTEKRFACSSKGYPQETEIIKLF |
| Ccap    | MELPLIYVSVLLLNIFEFSSGIVYNKDTEKRFACSSKGYPQETEIIKLF |
| Mleu    | MELPLIYVSVLLLNIFEFSSGIVYNKDTEKRFACSSKGYPQETEIIKLF |
| Csab    | MELPLIYVSVLLLNIFEFSSGIVYNKDTEKRFACSSKGYPQETEIIKLF |
| Nlar    | MELPLIYVSVLLLNIFEFSSGIVYNKDTEKRFACSSKGYPQETEIIKLF |
| Pabe    | MELPLIYVSVLLLNIFEFSSGIVYNKDTEKRFACSSKGYPQETEIIKLF |

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| Species | Sequence |
|---------|----------|
| Hsap    | LFLENLKIQCFFQTENIEASKMLSVFTSGGLAPSLGIMNSTYNGIFHFN 100 |
| Caty    | LFSGNLKIQCFFQTENIEASKMLSVFTSGGLAPSLGIMNSTYNGIFHFN |
| Panu    | LFSGNLKIQCFFQTENIEASKMLSVFTSGGLAPSLGIMNSTYNGIFHFN |
| Mfas    | LFSGNLKIQCFFQTENIEASKMLSVFTSGGLAPSLGIMNSTYNGIFHFN |
| Mmul    | LFSGNLKIQCFFQTENIEASKMLSVFTSGGLAPSLGIMNSTYNGIFHFN |
| Ppan    | LFLENLKIQCFFQTENIEASKMLSVFTSGGLAPSLGIMNSTYNGIFHFN |
| Ggor    | LFLENLKIQCFFQTENIEASKMLSVFTSGGLAPSLGIMNSTYNGIFHFN |
| Ptro    | LFLENLKIQCFFQTENIEASKMLSVFTSGGLAPSLGIMNSTYNGIFHFN |
| Nleu    | LFLENLKIQCFFQTENIEASKMLSVFTSGGLAPSLGIMNSTYNGIFHFN |
| Cjac    | LFSENGLHIQCFFQTENELASKMLSVFTSGGLAPSLGIMNSTYNGIFHFN |
| Cang    | LFSGNLKIQCFFQTENELASKMLSVFTSGGLAPSLGIMNSTYNGIFHFN |
| Ccap    | LFSENLHIQCFFQTENELASKMLSVFTSGGLAPSLGIMNSTYNGIFHFN |
| Mleu    | LFSGNLKIQCFFQTENELASKMLSVFTSGGLAPSLGIMNSTYNGIFHFN |
| Csab    | LFSGNLKIQCFFQTENELASKMLSVFTSGGLAPSLGIMNSTYNGIFHFN |
| Nlar    | LFSGNLKIQCFFQTENELASKMLSVFTSGGLAPSLGIMNSTYNGIFHFN |
| Pabe    | LFSENLHIQCFFQTENELASKMLSVFTSGGLAPSLGIMNSTYNGIFHFN |

** **:***:  *.:***** ***** ***:****:.** **:.*:***.

| Species | Sequence |
|---------|----------|
| Hsap    | LTLFSDRILWVDIPRENTQSTDIAAVEEWLVRITLHHLNIYATEGTL 150 |
| Caty    | LTLFSDRILWVDIPRENTQSTDIAAVEEWLVRITLHHLNIYATEGTL |
| Panu    | LTLFSDRILWVDIPRENTQSTDIAAVEEWLVRITLHHLNIYATEGTL |
| Mfas    | LTLFSDRILWVDIPRENTQSTDIAAVEEWLVRITLHHLNIYATEGTL |
| Mmul    | LTLFSDRILWVDIPRENTQSTDIAAVEEWLVRITLHHLNIYATEGTL |
| Ppan    | LTLFSDRILWVDIPRENTQSTDIAAVEEWLVRITLHHLNIYATEGTL |
| Ggor    | LTLFSDRILWVDIPRENTQSTDIAAVEEWLVRITLHHLNIYATEGTL |
| Ptro    | LTLFSDRILWVDIPRENTQSTDIAAVEEWLVRITLHHLNIYATEGTL |
| Nleu    | LTLFSDRILWVDIPRENTQSTDIAAVEEWLVRITLHHLNIYATEGTL |
| Cjac    | LTLFSDRILWVDIPRENTQSTDIAAVEEWLVRITLHHLNIYATEGTL |
| Cang    | LTLFSDRILWVDIPRENTQSTDIAAVEEWLVRITLHHLNIYATEGTL |
| Ccap    | LTLFSDRILWVDIPRENTQSTDIAAVEEWLVRITLHHLNIYATEGTL |
| Mleu    | LTLFSDRILWVDIPRENTQSTDIAAVEEWLVRITLHHLNIYATEGTL |
| Csab    | LTLFSDRILWVDIPRENTQSTDIAAVEEWLVRITLHHLNIYATEGTL |
| Nlar    | LTLFSDRILWVDIPRENTQSTDIAAVEEWLVRITLHHLNIYATEGTL |
| Pabe    | LTLFSDRILWVDIPRENTQSTDIAAVEEWLVRITLHHLNIYATEGTL |

** **:***:  *.:***** ***** ***:****:.** **:.*:***.
|    | Hsap            | LDVIREPILQWTPGDVIPESEISKLYPHVDLKVTKCPACNDVALLGFIV | 200 |
|----|-----------------|-----------------------------------------------|-----|
| Caty | LDRIRPEPILQWAPGVEIPASEMGKIYPHVVDLKVTKCPACNDVALLGFVM |
| Panu | LDRIRPEPILQWALGEIPASEMGKIYPHVVDLKVTKCPACNDVALLGFVM |
| Mfas | LDRIRPEPILQWALGEIPASEMGKIYPHVVDLKVTKCPACNDVALLGFVM |
| Mmul | LDRIRPEPILQWALGEIPASEMGKIYPHVVDLKVTKCPACNDVALLGFVM |
| Ppan | LDRIRPEPILQWTPGDVIPESEISKLYPHVDLKVTKCPACNDVALLGFIV |
| Ggor | LDIIREPILQWTPGDVIPESEISKLYPHVDLKVTKCPACNDVALLGFIV |
| Ptro | LDVIREPILQWTPGDVIPESEISKLYPHVDLKVTKCPACNDVALLGFIV |
| Nleu | LDVIREPILQWTPGDVIPESEISKLYPHVDLKVTKCPACNDVALLGFIV |
| Cjac | LDVFIREPILQWTPGDVIPESEISKLYPHVDLKVTKCPACNDVALLGFIV |
| Cang | LDVFIREPILQWTPGDVIPESEISKLYPHVDLKVTKCPACNDVALLGFIV |
| Ccap | LDVFIREPILQWTPGDVIPESEISKLYPHVDLKVTKCPACNDVALLGFIV |
| Mleu | LDVFIREPILQWTPGDVIPESEISKLYPHVDLKVTKCPACNDVALLGFIV |
| Csab | LDVFIREPILQWTPGDVIPESEISKLYPHVDLKVTKCPACNDVALLGFIV |
| Nlar | LDVFIREPILQWTPGDVIPESEISKLYPHVDLKVTKCPACNDVALLGFIV |
| Pabe | LDVFIREPILQWTPGDVIPESEISKLYPHVDLKVTKCPACNDVALLGFIV |

|    | Hsap            | DTIVDGVYIGITFGGFWHDYDATTWFNMTQTIYSQLQEEYEDLSLVDMVLT | 250 |
| Caty | DALLDGVYIGITFGGFWHDYDATTWFNMTQTIYSQLQEEYEDLSLVDMVLT |
| Panu | DALLDGVYIGITFGGFWHDYDATTWFNMTQTIYSQLQEEYEDLSLVDMVLT |
| Mfas | DALLDGVYIGITFGGFWHDYDATTWFNMTQTIYSQLQEEYEDLSLVDMVLT |
| Mmul | DALLDGVYIGITFGGFWHDYDATTWFNMTQTIYSQLQEEYEDLSLVDMVLT |
| Ppan | DTIVDGVYIGITFGGFWHDYDATTWFNMTQTIYSQLQEEYEDLSLVDMVLT |
| Ggor | DTIVDGVYIGITFGGFWHDYDATTWFNMTQTIYSQLQEEYEDLSLVDMVLT |
| Ptro | DTIVDGVYIGITFGGFWHDYDATTWFNMTQTIYSQLQEEYEDLSLVDMVLT |
| Nleu | DTIVDGVYIGITFGGFWHDYDATTWFNMTQTIYSQLQEEYEDLSLVDMVLT |
| Cjac | DSVKGYVYMGMTFGGWYHAYTWINVTETIYSHLNEEHEGLSVDVMVLT |
| Cang | DSVKGYVYMGMTFGGWYHAYTWINVTETIYSHLNEEHEGLSVDVMVLT |
| Ccap | DSVKGYVYMGMTFGGWYHAYTWINVTETIYSHLNEEHEGLSVDVMVLT |
| Mleu | DSVKGYVYMGMTFGGWYHAYTWINVTETIYSHLNEEHEGLSVDVMVLT |
| Csab | DSVKGYVYMGMTFGGWYHAYTWINVTETIYSHLNEEHEGLSVDVMVLT |
| Nlar | DSVKGYVYMGMTFGGWYHAYTWINVTETIYSHLNEEHEGLSVDVMVLT |
| Pabe | DSVKGYVYMGMTFGGWYHAYTWINVTETIYSHLNEEHEGLSVDVMVLT |

|    | Hsap            | NHFLVILTSLGLFLFSEDLSRHPYRSHLFSRSRADFGFVERVYVGKGLWYNE | 300 |
| Caty | NHFLVILTSLGLFLFSEDLSRHPYRSHLFSRSRADFGFVERVYVGKGLWYSE |
| Panu | NHFLVILTSLGLFLFSEDLSRHPYRSHLFSRSRADFGFVERVYVGKGLWYSE |
| Mfas | NHFLVILTSLGLFLFSEDLSRHPYRSHLFSRSRADFGFVERVYVGKGLWYSE |
| Mmul | NHFLVILTSLGLFLFSEDLSRHPYRSHLFSRSRADFGFVERVYVGKGLWYSE |
| Ppan | NHFLVILTSLGLFLFSEDLSRHPYRSHLFSRSRADFGFVERVYVGKGLWYSE |
| Ggor | NHFLVILTSLGLFLFSEDLSRHPYRSHLFSRSRADFGFVERVYVGKGLWYSE |
| Ptro | NHFLVILTSLGLFLFSEDLSRHPYRSHLFSRSRADFGFVERVYVGKGLWYSE |
| Nleu | NHFLVILTSLGLFLFSEDLSRHPYRSHLFSRSRADFGFVERVYVGKGLWYSE |
| Cjac | NHFLVILTSLGLFLFSEDLSRHPYRSHLFSRSRADFGFVERVYVGKGLWYSE |
| Cang | NHFLVILTSLGLFLFSEDLSRHPYRSHLFSRSRADFGFVERVYVGKGLWYSE |
| Ccap | NHFLVILTSLGLFLFSEDLSRHPYRSHLFSRSRADFGFVERVYVGKGLWYSE |
| Mleu | NHFLVILTSLGLFLFSEDLSRHPYRSHLFSRSRADFGFVERVYVGKGLWYSE |
| Csab | NHFLVILTSLGLFLFSEDLSRHPYRSHLFSRSRADFGFVERVYVGKGLWYSE |
| Nlar | NHFLVILTSLGLFLFSEDLSRHPYRSHLFSRSRADFGFVERVYVGKGLWYSE |
| Pabe | NHFLVILTSLGLFLFSEDLSRHPYRSHLFSRSRADFGFVERVYVGKGLWYSE |
| Species | Sequence  | Length |
|---------|-----------|--------|
| Hsap   | RCFANREHFEVDYVTVTFERNRTLSESSSCFYSQEPLLEWVPCLPHIFKG | 350 |
| Caty   | RCFANREHFEVDYVTVTFERNRTLSESSSCFYSQEPLLEWVPCLPYIFKG | 350 |
| Panu   | RCFANREHFEVDYVTVTFERNRTLSESSSCFYSQEPLLEWVPCLPYVFKG | 350 |
| Mfas   | RCFANREHFEVDYVTVTFERNRTLSESSSCFYSQEPLLEWVPCLPYVFKG | 350 |
| Mmul   | RCFANREHFEVDYVTVTFERNRTLSESSSCFYSQEPLLEWVPCLPYVFKG | 350 |
| Ppan   | RCFANREHFEVDYVTVTFERNRTLSESSSCFYSQEPLLEWVPCLPHIFKG | 350 |
| Ggor   | RCFANREHFEVDYVTVTFERNRTLSESSSCFYSQEPLLEWVPCLPHIFKG | 350 |
| Ptro   | RCFANREHFEVDYVTVTFERNRTLSESSSCFYSQEPLLEWVPCLPHIFKG | 350 |
| Nleu   | RCFANREHFEVDYVTVTFERNRTLSESSSCFYSQEPLLEWVPCLPHIFKG | 350 |
| Cjac   | RCFANREHFEVDYVTVTFERNRTLSESSSCFYSQEPLLEWVPCLPHIFKG | 350 |
| Cang   | RCFANREHFEVDYVTVTFERNRTLSESSSCFYSQEPLLEWVPCLPHIFKG | 350 |
| Ccap   | RCFANREHFEVDYVTVTFERNRTLSESSSCFYSQEPLLEWVPCLPHIFKG | 350 |
| Mleu   | RCFANREHFEVDYVTVTFERNRTLSESSSCFYSQEPLLEWVPCLPHIFKG | 350 |
| Csab   | RCFANREHFEVDYVTVTFERNRTLSESSSCFYSQEPLLEWVPCLPHIFKG | 350 |
| Nlar   | RCFANREHFEVDYVTVTFERNRTLSESSSCFYSQEPLLEWVPCLPHIFKG | 350 |
| Pabe   | RCFANREHFEVDYVTVTFERNRTLSESSSCFYSQEPLLEWVPCLPHIFKG | 350 |

| Species | Sequence  | Length |
|---------|-----------|--------|
| Hsap   | IFRFPSSFSSPVGMVFHRPHSHFLYAYGNIWLSVDGGNTFQILANFHDDI | 450 |
| Caty   | IFRFPSSFSSPVGMVFHRPHSHFLYAYGNIWLSVDGGNTFQILANFHDDI | 450 |
| Panu   | IFRFPSSFSSPVGMVFHRPHSHFLYAYGNIWLSVDGGNTFQILANFHDDI | 450 |
| Mfas   | IFRFPSSFSSPVGMVFHRPHSHFLYAYGNIWLSVDGGNTFQILANFHDDI | 450 |
| Mmul   | IFRFPSSFSSPVGMVFHRPHSHFLYAYGNIWLSVDGGNTFQILANFHDDI | 450 |
| Ppan   | IFRFPSSFSSPVGMVFHRPHSHFLYAYGNIWLSVDGGNTFQILANFHDDI | 450 |
| Ggor   | IFRFPSSFSSPVGMVFHRPHSHFLYAYGNIWLSVDGGNTFQILANFHDDI | 450 |
| Ptro   | IFRFPSSFSSPVGMVFHRPHSHFLYAYGNIWLSVDGGNTFQILANFHDDI | 450 |
| Nleu   | IFRFPSSFSSPVGMVFHRPHSHFLYAYGNIWLSVDGGNTFQILANFHDDI | 450 |
| Cjac   | IFRFPSSFSSPVGMVFHRPHSHFLYAYGNIWLSVDGGNTFQILANFHDDI | 450 |
| Cang   | IFRFPSSFSSPVGMVFHRPHSHFLYAYGNIWLSVDGGNTFQILANFHDDI | 450 |
| Ccap   | IFRFPSSFSSPVGMVFHRPHSHFLYAYGNIWLSVDGGNTFQILANFHDDI | 450 |
| Mleu   | IFRFPSSFSSPVGMVFHRPHSHFLYAYGNIWLSVDGGNTFQILANFHDDI | 450 |
| Csab   | IFRFPSSFSSPVGMVFHRPHSHFLYAYGNIWLSVDGGNTFQILANFHDDI | 450 |
| Nlar   | IFRFPSSFSSPVGMVFHRPHSHFLYAYGNIWLSVDGGNTFQILANFHDDI | 450 |
| Pabe   | IFRFPSSFSSPVGMVFHRPHSHFLYAYGNIWLSVDGGNTFQILANFHDDI | 450 |
Hsap  IKKTFHSFYSAITFVSRQGKVYSTKAGMGRYSAVGSVTERIFTLYDHL
Caty  IKKSFHSFYSAITFVSRQGKVYTKGMRHGSAGTVKETIRFTLIFDYDHL
Panu  IKKTFHSFYSAITFVSRQGKVYTKGMRHGSAGTVKETIRFTLIFDYDHL
Mfas  IKKTFHSFYSAITFVSRQGKVYTKGMRHGSAGTVKETIRFTLIFDYDHL
Mmul  IKKTFHSFYSAITFVSRQGKVYTKGMRHGSAGTVKETIRFTLIFDYDHL
Ppan  IKKTFHSFYSAITFVSRQGKVYSTKAGMGRYSAVGSVTERIFTLYDHL
Ggor  IKKTFHSFYSAITFVSRQGKVYSTKAGMGRYSAVGSVTERIFTLYDHL
Ptro  IKKTFHSFYSAITFVSRQGKVYSTKAGMGRYSAVGSVTERIFTLYDHL
Nleu  IKKTFHSFYSAITFVSRQGKVYSTKAGMGRYSAVGSVTERIFTLYDHL
Cjac  ISRTFHSFYSAITFVSRQGKVYSTKAGLGRYAAAGSVNDRIFTLYDHL
Cang  IKKTFHSFYSAITFVSRQGKVYSTKAGMGRHAGTVKETIRFTLIFDYDHL
Ccap  ISKTFHSFYSAITFVSRQGKVYSTKAGLGRYTAAGSVNDRIFTLYDQL
Mleu  IKKSFHSFYSAITFVSRQGKVYSTKAGMGRHAGTVKETIRFTLIFDYDHL
Csab  IKKTFHSFYSAITFVSRQGKVYSTKAGMGRYSAVGSVTERIFTLYDHL
Nlar  IKKTFHSFYSAITFVSRQGKVYSTKAGMGRYSAVGSVTERIFTLYDHL
Pabe  IKKTFHSFYSAITFVSRQGKVYSTKAGMGRYSAVGSVTERIFTLYDHL

Hsap  GFLHKLTLGRFEASGPPTAFGNSRNLFQGPPDMGFETALAPQHTSDELII
Caty  GFLHKLTPGHFEASGPPTAFGNSRNLFQGPPDMGFETALAPQHTSDELII
Panu  GFLHKLTPGHFEASGPPTAFGNSRNLFQGPPDMGFETALAPQHTSDELII
Mfas  GFLHKLTPGHFEASGPPTAFGNSRNLFQGPPDMGFETALAPQHTSDELII
Mmul  GFLHKLTPGHFEASGPPTAFGNSRNLFQGPPDMGFETALAPQHTSDELII
Ppan  GFLHKLTPGHFEASGPPTAFGNSRNLFQGPPDMGFETALAPQHTSDELII
Ggor  GFLHKLTPGHFEASGPPTAFGNSRNLFQGPPDMGFETALAPQHTSDELII
Ptro  GFLHKLTPGHFEASGPPTAFGNSRNLFQGPPDMGFETALAPQHTSDELII
Nleu  GFLHKLTPGHFEASGPPTAFGNSRNLFQGPPDMGFETALAPQHTSDELII
Cjac  GFLHKLTPDNFEASGPPTAFGNSRNLFQGPPDMGFETALAPQHTSDELII
Cang  GFLHKLTPDNFEASGPPTAFGNSRNLFQGPPDMGFETALAPQHTSDELII
Ccap  GFLHKLTPDNFEASGPPTAFGNSRNLFQGPPDMGFETALAPQHTSDELII
Mleu  GFLHKLTPDNFEASGPPTAFGNSRNLFQGPPDMGFETALAPQHTSDELII
Csab  GFLHKLTPDNFEASGPPTAFGNSRNLFQGPPDMGFETALAPQHTSDELII
Nlar  GFLHKLTPDNFEASGPPTAFGNSRNLFQGPPDMGFETALAPQHTSDELII
Pabe  GFLHKLTPDNFEASGPPTAFGNSRNLFQGPPDMGFETALAPQHTSDELII

Hsap  FFAYVPENPQTIYSKFFGNIHYGKVIHSGKTGRAYIRKVLQHHTTPGKF
Caty  FFAYVPENPQTIYSKFFGNIHYGKVIHSGKTGRAYIRKVLQHHTTPGKF
Panu  FFAYVPENPQTIYSKFFGNIHYGKVIHSGKTGRAYIRKVLQHHTTPGKF
Mfas  FFAYVPENPQTIYSKFFGNIHYGKVIHSGKTGRAYIRKVLQHHTTPGKF
Mmul  FFAYVPENPQTIYSKFFGNIHYGKVIHSGKTGRAYIRKVLQHHTTPGKF
Ppan  FFAYVPENPQTIYSKFFGNIHYGKVIHSGKTGRAYIRKVLQHHTTPGKF
Ggor  FFAYVPENPQTIYSKFFGNIHYGKVIHSGKTGRAYIRKVLQHHTTPGKF
Ptro  FFAYVPENPQTIYSKFFGNIHYGKVIHSGKTGRAYIRKVLQHHTTPGKF
Nleu  FFAYVPENPQTIYSKFFGNIHYGKVIHSGKTGRAYIRKVLQHHTTPGKF
Cjac  FFAYVPENPQTIYSKFFGNIHYGKVIHSGKTGRAYIRKVLQHHTTPGKF
Cang  FFAYVPENPQTIYSKFFGNIHYGKVIHSGKTGRAYIRKVLQHHTTPGKF
Ccap  FFAYVPENPQTIYSKFFGNIHYGKVIHSGKTGRAYIRKVLQHHTTPGKF
Mleu  FFAYVPENPQTIYSKFFGNIHYGKVIHSGKTGRAYIRKVLQHHTTPGKF
Csab  FFAYVPENPQTIYSKFFGNIHYGKVIHSGKTGRAYIRKVLQHHTTPGKF
Nlar  FFAYVPENPQTIYSKFFGNIHYGKVIHSGKTGRAYIRKVLQHHTTPGKF
Pabe  FFAYVPENPQTIYSKFFGNIHYGKVIHSGKTGRAYIRKVLQHHTTPGKF
Hsap            RNAKGFRMLEIPLLTVFVGNPNLLEVTAEVTDFDDTSDYVITISAASKVLH
Caty            RNSKGFRMLEIPLLTVFVGNPNLLEVKAEAAFDDTDSYLIAISAASKVLQ
Panu            RNSKGFRMLEIPLLTVFVGNPNLLEVKAEAAFDDTDSYLIAISAASKVLQ
Mfas            RNSKGFRMLEIPLLTVFVGNPNLLEVKAEAAFDDTDSYLIAISAASKVLQ
Mmul            RNSKGFRMLEIPLLTVFVGNPNLLEVKAEAAFDDTDSYLIAISAASKVLQ
Ppan            RNAKGFRMLEIPLLTVFVGNPNLLEVTAEVTDFDDTSDYVITISAASKVLQ
Ggor            RNAKGFRMLEIPLLTVFVGNPNLLEVTAEVTDFDDTSDYVITISAASKVLQ
Ptro            RNAKGFRMLEIPLLTVFVGNPNLLEVTAEVTDFDDTSDYVITISAASKVLQ
Nleu            RNAKGFRMLEIPLLTVFVGNPNLLEVKAEAAFDDTDSYLIAISAASKVLQ
Cjac            RNAKGFRMLEIPLLTVFVGNPNLLEVKAEAAFDDTDSYLIAISAASKVLQ
Cang            RNAKGFRMLEIPLLTVFVGNPNLLEVKAEAAFDDTDSYLIAISAASKVLQ
Ccang            RNAKGIRKLEIPLLTVFVGNPNLLDVKAEVTFDDTDSYVVTISAASKLLH
Mleu            RNSKGFRMLEIPLLTVFVGNPNLLEVKAEAAFDDTDSYLIAISAASKVLQ
Csab            RNSKGFRMLEIPLLTVFVGNPNLLEVKAEAAFDDTDSYLIAISAASKVLQ
Nlar            RNAKGFRMLEIPLLTVFVGNPNLLEVKAEAAFDDTDSYLIAISAASKVLQ
Pabe            RNAKGFRMLEIPLLTVFVGNPNLLEVKAEAAFDDTDSYLIAISAASKVLQ

Hsap            QGSTSLAFIMWSASTECFVTTMVTLLKSSCYSLLRSMHHIPSFPFEDWI
Caty            QGSTSMAFIMWSASVECFVTTMVTLLKSSCYSLLRSMHHIPSFPFEDWI
Panu            QGSTSMAFIMWSASVECFVTTMVTLLKSSCYSLLRSMHHIPSFPFEDWI
Mfas            WGSTSVAFIMWSASAECFVTTMVTLLKSSCYSLLRSMHHIPSFPFEDWI
Mmul            WGSTSVAFIMWSASAECFVTTMVTLLKSSCYSLLRSMHHIPSFPFEDWI
Ppan            QGSTSLAFIMWSASTECFVTTMVTLLKSSCYSLLRSMHHIPSFPFEDWI
Ggor            QGSTSLAFIMWSASTECFVTTMVTLLKSSCYSLLRSMHHIPSFPFEDWI
Ptro            QGSTSLAFIMWSASTECFVTTMVTLLKSSCYSLLRSMHHIPSFPFEDWI
Nleu            QGSTSLAFIMWSASTECFVTTMVTLLKSSCYSLLRSMHHIPSFPFEDWI
Cjac            QGSTSLAFIMWSASTECFVTTMVTLLKSSCYSLLRSMHHIPSFPFEDWI
Cang            QGSTSLAFIMWSASTECFVTTMVTLLKSSCYSLLRSMHHIPSFPFEDWI
Ccang            QGSTSLAFIMWSASTECFVTTMVTLLKSSCYSLLRSMHHIPSFPFEDWI
Mleu            QGSTSLAFIMWSASTECFVTTMVTLLKSSCYSLLRSMHHIPSFPFEDWI
Csab            QGSTSLAFIMWSASTECFVTTMVTLLKSSCYSLLRSMHHIPSFPFEDWI
Nlar            QGSTSLAFIMWSASTECFVTTMVTLLKSSCYSLLRSMHHIPSFPFEDWI
Pabe            QGSTSLAFIMWSASTECFVTTMVTLLKSSCYSLLRSMHHIPSFPFEDWI

Hsap            SGVHKDSQGFNILKTLPINRPPSNMGIAIPLTDFNYHADPSKIPFPRNMF
Caty            SGVHKDSQGFNILKTLPINRPPSNMGIAIPLTDFNYHADPSKIPFPRNMF
Panu            SGVHKDSQGFNILKTLPINRPPSNMGIAIPLTDFNYHADPSKIPFPRNMF
Mfas            SGVHKDSQGFNILKTLPINRPPSNMGIAIPLTDFNYHADPSKIPFPRNMF
Mmul            SGVHKDSQGFNILKTLPINRPPSNMGIAIPLTDFNYHADPSKIPFPRNMF
Ppan            SGVHKDSQGFNILKTLPINRPPSNMGIAIPLTDFNYHADPSKIPFPRNMF
Ggor            SGVHKDSQGFNILKTLPINRPPSNMGIAIPLTDFNYHADPSKIPFPRNMF
Ptro            SGVHKDSQGFNILKTLPINRPPSNMGIAIPLTDFNYHADPSKIPFPRNMF
Nleu            SGVHKDSQGFNILKTLPINRPPSNMGIAIPLTDFNYHADPSKIPFPRNMF
Cjac            SGVHKDSQGFNILKTLPINRPPSNMGIAIPLTDFNYHADPSKIPFPRNMF
Cang            SGVHKDSQGFNILKTLPINRPPSNMGIAIPLTDFNYHADPSKIPFPRNMF
Ccang            SGVHKDSQGFNILKTLPINRPPSNMGIAIPLTDFNYHADPSKIPFPRNMF
Mleu            SGVHKDSQGFNILKTLPINRPPSNMGIAIPLTDFNYHADPSKIPFPRNMF
Csab            SGVHKDSQGFNILKTLPINRPPSNMGIAIPLTDFNYHADPSKIPFPRNMF
Nlar            SGVHKDSQGFNILKTLPINRPPSNMGIAIPLTDFNYHADPSKIPFPRNMF
Pabe            SGVHKDSQGFNILKTLPINRPPSNMGIAIPLTDFNYHADPSKIPFPRNMF

****:********:**.********* *****.***********:** ****

Hsap            SGVHKDSQGFNILKTLPINRPPSNMGIAIPLTDFNYHADPSKIPFPRNMF
Caty            SGVHKDSQGFNILKTLPINRPPSNMGIAIPLTDFNYHADPSKIPFPRNMF
Panu            SGVHKDSQGFNILKTLPINRPPSNMGIAIPLTDFNYHADPSKIPFPRNMF
Mfas            SGVHKDSQGFNILKTLPINRPPSNMGIAIPLTDFNYHADPSKIPFPRNMF
Mmul            SGVHKDSQGFNILKTLPINRPPSNMGIAIPLTDFNYHADPSKIPFPRNMF
Ppan            SGVHKDSQGFNILKTLPINRPPSNMGIAIPLTDFNYHADPSKIPFPRNMF
Ggor            SGVHKDSQGFNILKTLPINRPPSNMGIAIPLTDFNYHADPSKIPFPRNMF
Ptro            SGVHKDSQGFNILKTLPINRPPSNMGIAIPLTDFNYHADPSKIPFPRNMF
Nleu            SGVHKDSQGFNILKTLPINRPPSNMGIAIPLTDFNYHADPSKIPFPRNMF
Cjac            SGVHKDSQGFNILKTLPINRPPSNMGIAIPLTDFNYHADPSKIPFPRNMF
Cang            SGVHKDSQGFNILKTLPINRPPSNMGIAIPLTDFNYHADPSKIPFPRNMF
Ccang            SGVHKDSQGFNILKTLPINRPPSNMGIAIPLTDFNYHADPSKIPFPRNMF
Mleu            SGVHKDSQGFNILKTLPINRPPSNMGIAIPLTDFNYHADPSKIPFPRNMF
Csab            SGVHKDSQGFNILKTLPINRPPSNMGIAIPLTDFNYHADPSKIPFPRNMF
Nlar            SGVHKDSQGFNILKTLPINRPPSNMGIAIPLTDFNYHADPSKIPFPRNMF
Pabe            SGVHKDSQGFNILKTLPINRPPSNMGIAIPLTDFNYHADPSKIPFPRNMF

****:********:**.********* *****.***********:** ****
|    | Sequence                                                                 | Length |
|----|--------------------------------------------------------------------------|--------|
| Hsap | HMSKKGKFKQCANVSTREECNCTKDFKGFHAVAFSDCREKFPKRFPIT                      | 950    |
| Caty | HLSKKGKFKQCANVSTREECNCTKDFKGFHAVAFSDCREKFPKRFPIT                      | 1000   |
| Panu | HLSKKGKFKQCANVSTREECNCTKDFKGFHAVAFSDCREKFPKRFPIT                      | 1050   |
| Mfas | HLSKKGKFKQCANVSTREECNCTKDFKGFHAVAFSDCREKFPKRFPIT                      |        |
| Mmul | HLSKKGKFKQCANVSTREECNCTKDFKGFHAVAFSDCREKFPKRFPIT                      |        |
| Ppan | HLSKKGKFKQCANVSTREECNCTKDFKGFHAVAFSDCREKFPKRFPIT                      |        |
| Ggor | HLSKKGKFKQCANVSTREECNCTKDFKGFHAVAFSDCREKFPKRFPIT                      |        |
| Ptro | HLSKKGKFKQCANVSTREECNCTKDFKGFHAVAFSDCREKFPKRFPIT                      |        |
| Nleu | HLSKKGKFKQCANVSTREECNCTKDFKGFHAVAFSDCREKFPKRFPIT                      |        |
| Cjac | HLSKKGKFKQCANVSTREECNCTKDFKGFHAVAFSDCREKFPKRFPIT                      |        |
| Cang | HLSKKGKFKQCANVSTREECNCTKDFKGFHAVAFSDCREKFPKRFPIT                      |        |
| Ccap | HLSKKGKFKQCANVSTREECNCTKDFKGFHAVAFSDCREKFPKRFPIT                      |        |
| Mleu | HLSKKGKFKQCANVSTREECNCTKDFKGFHAVAFSDCREKFPKRFPIT                      |        |
| Csab | HLSKKGKFKQCANVSTREECNCTKDFKGFHAVAFSDCREKFPKRFPIT                      |        |
| Nlar | HLSKKGKFKQCANVSTREECNCTKDFKGFHAVAFSDCREKFPKRFPIT                      |        |
| Pabe | HLSKKGKFKQCANVSTREECNCTKDFKGFHAVAFSDCREKFPKRFPIT                      |        |

|    | Sequence                                                                 | Length |
|----|--------------------------------------------------------------------------|--------|
| Hsap | QYPVSIEINEDGRPQLQSLPVTLTVETVEMNRHNWLHTKEPIKMKKQFVDE                     | 950    |
| Caty | QYPVSIEINEDGRPQLQSLPVTLTVETVEMNRHNWLHTKEPIKMKKQFVDE                    | 1000   |
| Panu | QYPVSIEINEDGRPQLQSLPVTLTVETVEMNRHNWLHTKEPIKMKKQFVDE                    | 1050   |
| Mfas | QYPVSIEINEDGRPQLQSLPVTLTVETVEMNRHNWLHTKEPIKMKKQFVDE                    |        |
| Mmul | QYPVSIEINEDGRPQLQSLPVTLTVETVEMNRHNWLHTKEPIKMKKQFVDE                    |        |
| Ppan | QYPVSIEINEDGRPQLQSLPVTLTVETVEMNRHNWLHTKEPIKMKKQFVDE                    |        |
| Ggor | QYPVSIEINEDGRPQLQSLPVTLTVETVEMNRHNWLHTKEPIKMKKQFVDE                    |        |
| Ptro | QYPVSIEINEDGRPQLQSLPVTLTVETVEMNRHNWLHTKEPIKMKKQFVDE                    |        |
| Nleu | QYPVSIEINEDGRPQLQSLPVTLTVETVEMNRHNWLHTKEPIKMKKQFVDE                    |        |
| Cjac | QYPVSIEINEDGRPQLQSLPVTLTVETVEMNRHNWLHTKEPIKMKKQFVDE                    |        |
| Cang | QYPVSIEINEDGRPQLQSLPVTLTVETVEMNRHNWLHTKEPIKMKKQFVDE                    |        |
| Ccap | QYPVSIEINEDGRPQLQSLPVTLTVETVEMNRHNWLHTKEPIKMKKQFVDE                    |        |
| Mleu | QYPVSIEINEDGRPQLQSLPVTLTVETVEMNRHNWLHTKEPIKMKKQFVDE                    |        |
| Csab | QYPVSIEINEDGRPQLQSLPVTLTVETVEMNRHNWLHTKEPIKMKKQFVDE                    |        |
| Nlar | QYPVSIEINEDGRPQLQSLPVTLTVETVEMNRHNWLHTKEPIKMKKQFVDE                    |        |
| Pabe | QYPVSIEINEDGRPQLQSLPVTLTVETVEMNRHNWLHTKEPIKMKKQFVDE                    |        |

|    | Sequence                                                                 | Length |
|----|--------------------------------------------------------------------------|--------|
| Hsap | VEPILGAAYVPNGLSNSIKGSELHFHRVTISGVTFCNLIELFFQIYVDE                      | 1050   |
| Caty | VEPILGAAYVPNGLSNSIKGSELHFHRVTISGVTFCNLIELFFQIYVDE                      |        |
| Panu | VEPILGAAYVPNGLSNSIKGSELHFHRVTISGVTFCNLIELFFQIYVDE                      |        |
| Mfas | VEPILGAAYVPNGLSNSIKGSELHFHRVTISGVTFCNLIELFFQIYVDE                      |        |
| Mmul | VEPILGAAYVPNGLSNSIKGSELHFHRVTISGVTFCNLIELFFQIYVDE                      |        |
| Ppan | VEPILGAAYVPNGLSNSIKGSELHFHRVTISGVTFCNLIELFFQIYVDE                      |        |
| Ggor | VEPILGAAYVPNGLSNSIKGSELHFHRVTISGVTFCNLIELFFQIYVDE                      |        |
| Ptro | VEPILGAAYVPNGLSNSIKGSELHFHRVTISGVTFCNLIELFFQIYVDE                      |        |
| Nleu | VEPILGAAYVPNGLSNSIKGSELHFHRVTISGVTFCNLIELFFQIYVDE                      |        |
| Cjac | VEPILGAAYVPNGLSNSIKGSELHFHRVTISGVTFCNLIELFFQIYVDE                      |        |
| Cang | VEPILGAAYVPNGLSNSIKGSELHFHRVTISGVTFCNLIELFFQIYVDE                      |        |
| Ccap | VEPILGAAYVPNGLSNSIKGSELHFHRVTISGVTFCNLIELFFQIYVDE                      |        |
| Mleu | VEPILGAAYVPNGLSNSIKGSELHFHRVTISGVTFCNLIELFFQIYVDE                      |        |
| Csab | VEPILGAAYVPNGLSNSIKGSELHFHRVTISGVTFCNLIELFFQIYVDE                      |        |
| Nlar | VEPILGAAYVPNGLSNSIKGSELHFHRVTISGVTFCNLIELFFQIYVDE                      |        |
| Pabe | VEPILGAAYVPNGLSNSIKGSELHFHRVTISGVTFCNLIELFFQIYVDE                      |        |

|    | Sequence                                                                 | Length |
|----|--------------------------------------------------------------------------|--------|
| Hsap | LEPILGAAYVPNGLSNSIKGSELHFHRVTISGVTFCNLIELFFQIYVDE                      | 950    |
| Caty | LEPILGAAYVPNGLSNSIKGSELHFHRVTISGVTFCNLIELFFQIYVDE                      | 1000   |
| Panu | LEPILGAAYVPNGLSNSIKGSELHFHRVTISGVTFCNLIELFFQIYVDE                      | 1050   |
| Mfas | LEPILGAAYVPNGLSNSIKGSELHFHRVTISGVTFCNLIELFFQIYVDE                      |        |
| Mmul | LEPILGAAYVPNGLSNSIKGSELHFHRVTISGVTFCNLIELFFQIYVDE                      |        |
| Ppan | LEPILGAAYVPNGLSNSIKGSELHFHRVTISGVTFCNLIELFFQIYVDE                      |        |
| Ggor | LEPILGAAYVPNGLSNSIKGSELHFHRVTISGVTFCNLIELFFQIYVDE                      |        |
| Ptro | LEPILGAAYVPNGLSNSIKGSELHFHRVTISGVTFCNLIELFFQIYVDE                      |        |
| Nleu | LEPILGAAYVPNGLSNSIKGSELHFHRVTISGVTFCNLIELFFQIYVDE                      |        |
| Cjac | LEPILGAAYVPNGLSNSIKGSELHFHRVTISGVTFCNLIELFFQIYVDE                      |        |
| Cang | LEPILGAAYVPNGLSNSIKGSELHFHRVTISGVTFCNLIELFFQIYVDE                      |        |
| Ccap | LEPILGAAYVPNGLSNSIKGSELHFHRVTISGVTFCNLIELFFQIYVDE                      |        |
| Mleu | LEPILGAAYVPNGLSNSIKGSELHFHRVTISGVTFCNLIELFFQIYVDE                      |        |
| Csab | LEPILGAAYVPNGLSNSIKGSELHFHRVTISGVTFCNLIELFFQIYVDE                      |        |
| Nlar | LEPILGAAYVPNGLSNSIKGSELHFHRVTISGVTFCNLIELFFQIYVDE                      |        |
| Pabe | LEPILGAAYVPNGLSNSIKGSELHFHRVTISGVTFCNLIELFFQIYVDE                      |        |
| Species | Hsap | Caty | Panu | Mfas | Mmul | Ppan | Ggor | Ptro | Nleu | Cjac | Cang | Ccap | Mleu | Csab | Nlar | Pabe |
|---------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| Amino acids | APLFPFGHTLIAVAVVLGGLIFMAFMFQHLGIHPWKTQWIRRKQAK | APLFPFGHTLIAVAVVLGGLIFMAFMFQHLGIHPWKTQWIRRKQAK | APLFPFGHTLIAVAVVLGGLIFMAFMFQHLGIHPWKTQWIRRKQAK | APLFPFGHTLIAVAVVLGGLIFMAFMFQHLGIHPWKTQWIRRKQAK | APLFPFGHTLIAVAVVLGGLIFMAFMFQHLGIHPWKTQWIRRKQAK | APLFPFGHTLIAVAVVLGGLIFMAFMFQHLGIHPWKTQWIRRKQAK | APLFPFGHTLIAVAVVLGGLIFMAFMFQHLGIHPWKTQWIRRKQAK | APLFPFGHTLIAVAVVLGGLIFMAFMFQHLGIHPWKTQWIRRKQAK | APLFPFGHTLIAVAVVLGGLIFMAFMFQHLGIHPWKTQWIRRKQAK | APLFPFGHTLIAVAVVLGGLIFMAFMFQHLGIHPWKTQWIRRKQAK | APLFPFGHTLIAVAVVLGGLIFMAFMFQHLGIHPWKTQWIRRKQAK | APLFPFGHTLIAVAVVLGGLIFMAFMFQHLGIHPWKTQWIRRKQAK | APLFPFGHTLIAVAVVLGGLIFMAFMFQHLGIHPWKTQWIRRKQAK | APLFPFGHTLIAVAVVLGGLIFMAFMFQHLGIHPWKTQWIRRKQAK | APLFPFGHTLIAVAVVLGGLIFMAFMFQHLGIHPWKTQWIRRKQAK |
| | FSSISLSELIHRSKSEEX | FSSITLSELIRRKXSEEX | LSSITLSELIRRXKSEEX | LSSITLSELIRRXKSEEX | LSSITLSELIRRXKSEEX | FSSISLSELIHRSKSEEX | FSSISLSELIHRSKSEEX | FSSISLSELIHRSKSEEX | FSSISLSELIHRSKSEEX | FSSISLSELIHRSKSEEX | FSSISLSELIHRSKSEEX | FSSISLSELIHRSKSEEX | FSSISLSELIHRSKSEEX | FSSISLSELIHRSKSEEX | FSSISLSELIHRSKSEEX | FSSISLSELIHRSKSEEX |

**Supplementary Figure 2:** *CATSPERβ* protein sequences align well across primates despite high levels of amino-acid divergence. This alignment for 16 primate species was generated with CLUSTAL Omega with default settings.
Supplementary Figure 3: Branch model analysis of Dpkd2. dN/dS values are labeled on each branch.

Supplementary Figure 4: *Drosophila pkd2* has indel variation in the *D. melanogaster* sub-group. *pkd2* is represented as a linear bar. Light grey sections indicate intracellular segments, while dark grey represents extracellular segments. Blue boxes represent the transmembrane domains, and the green box represents the C-terminal coiled coil domain. We split the gene into four segments for these analyses, as indicated by the brackets. Measurements were taken from the melanogaster sub-group. Ratios represent the rate of change of each segment compared to the rate of change of the whole gene. A higher value means that a specific region of the gene changes in length more than the average change in length of the gene. The extracellular domain explains a disproportionate amount of the change in length compared to the other segments of *pkd2*. 
Supplementary Figure 5: Primate PKD2 does not show signs of recurrent selection. A) *Drosophila pkd2* is correctly grouped with the primate PKD2 genes in a maximum likelihood tree for the primate PKD and CatSper genes. Bootstrap values were calculated from 1000 iterations of the tree. B) Estimation of dN/dS for primate PKD2 along the primate phylogeny using the PAML branch model shows no branches with elevated dN/dS. C) The M7-M8 log likelihood test does not reject the null model of neutral or purifying selection for primate PKD2. Both *Drosophila pkd2* and primate PKD2 are represented as bars, with transmembrane domains in blue and the coiled coil domain in green. The red box in primate PKD2 is an EF-Hand domain that is not predicted to be present in *Drosophila pkd2*.
Supplementary Figure 6: Pipeline for homolog search and molecular evolutionary analyses. We used the first part of the pipeline to generate alignments for running the branch model of PAML. For the CatSper genes we ran the pipeline two ways: first without the 95% length filter to acquire sequences from every branch of the phylogeny for estimating dN/dS, and second with the 95% length filter for running the NSsites model tests.
| Gene     | species used | aligner | % aligned | Model 7 (β) | Model 8 (β+ω) | 2ΔlnL | M7/M8 P-value | Model 8a (β+ω=1) | 2ΔlnL (M8/M8a) | M8/M8a P-value |
|----------|--------------|---------|-----------|-------------|---------------|-------|---------------|-------------------|----------------|----------------|
| Catsper1 | 13           | T-Coffee| 92        | -5954.560347| -5951.450387  | 6.220 | 0.0446        | -5953.649166     | 4.40           | 0.0360         |
| Catsper2 | 13           | Clustal | 99        | -3960.619099| -3950.714800 | 19.81 | 4.996 E-5     | -3960.617504     | 19.85          | 8.574 E-7      |
| Catsper3 | 16           | Muscle  | 98        | -3354.750380| -3339.138875 | 31.22 | 1.660 E-7     | -3354.057504     | 29.84          | 4.69 E-8       |
| Catsper4 | 17           | Muscle  | 96        | -3947.619450| -3942.513649 | 10.21 | 0.0061        | -3946.599674     | 8.17           | 0.00425        |
| Catsperβ | 16           | T-Coffee| 99        | -8764.288301| -8743.208070 | 42.16 | 6.998 E-10    | -8764.112789     | 41.81          | 1.006 E-10     |
| CatsperΔ | 12           | T-Coffee| 77        | -6735.581784| -6678.633488 | 113.90| p < 1.0 E-21  | -6734.887825     | 112.90         | p < 1.0 E-21   |
| Catsperγ | 10           | Clustal | 99        | -7796.750878| -7784.410876 | 24.68 | 4.373 E-6     | -7796.674927     | 24.53          | 7.323 E-7      |
| Catsperε | 11           | Clustal | 98        | -7563.057369| -7536.058409 | 54.00 | 1.881 E-6     | -7561.730546     | 51.34          | 7.750 E-13     |
| Catsperζ | 17           | Muscle  | 86        | -1411.584002| -1408.224779 | 6.718 | 0.03476       | -1411.410441     | 6.37           | 0.0116         |

**Supplementary Table 1**: PAML NSites results for CatSper genes. The highest (least significant) p-value, and the corresponding aligner is reported, along with the proportion of the original alignment and the number of species used in these analyses.
### Supplementary Table 2: Sites under selection in each of the CatSper proteins. Each reported amino acid corresponds to the position and amino acid of the *H. sapeins* full amino acid sequence.

| Gene  | Length (aa) | Sites under selection*, BEB > 0.90 | Total sites | Extracellular Sites | Intracellular Sites |
|-------|-------------|------------------------------------|-------------|---------------------|---------------------|
| *Catsper1* | 780 | none | 0 | 0 | 0 |
| *Catsper2* | 530 | 63V, 65G, 134E, 175F, 310Q, 384M, 388A, 425E, 447S, 455S, 469I | 11 | 2 | 9 |
| *Catsper3* | 398 | 50R, 116L, 131L, 140T, 202P, 204N, 240E | 7 | 4 | 3 |
| *Catsper4* | 472 | 166I, 451V, 474Q | 3 | 0 | 3 |
| *Catsperβ* | 1116 | 57K, 130E, 141L1 153V 163P, 175L, 203I, 211I, 220Y, 229Q, 236Q, 273R, 274H, 275S, 277S, 351I, 353I, 354F, 378R, 382I, 395S, 443I, 448D, 483S, 487S, 510R, 570G, 651D, 686S, 724H, 756R, 790T, 799H, 800Q, 814T, 845F, 857Q, 898M, 901M, 925D, 922N, 1004L, 1081Q, 1100F | 44 | 42 | 2 |
| *CatsperΔ* | 798 | 48H, 589W, 590R, 591K | 4 | 4 | 0 |
| *Catsperγ* | 1159 | 158P, 212R, 392S, 397I, 415T, 441D, 617H | 7 | 7 | 0 |
| *Catsperɛ* | 968 | 66T, 94F, 95V, 96E, 109H, 110F, 111F, 161V, 268K | 9 | 9 | 0 |
| *Catsperζ* | 160 | 108R, 136K | 2 | NA | 2 |
| Species         | Strain       | Source / Location                  |
|-----------------|--------------|------------------------------------|
| D. melanogaster | w^{118}      | Bloomington Stock Center           |
| D. melanogaster | Iso female 95| Lake Kariba, Zimbabwe              |
| D. melanogaster | Iso female 159| Lake Kariba, Zimbabwe              |
| D. melanogaster | Iso female 216| Lake Kariba, Zimbabwe              |
| D. melanogaster | Iso female 346| Lake Kariba, Zimbabwe              |
| D. melanogaster | Iso female 13 | 70Km East of Wanie-Rukula, Congo   |
| D. melanogaster | Iso female 18 | 70Km East of Wanie-Rukula, Congo   |
| D. melanogaster | Iso female 26 | 70Km East of Wanie-Rukula, Congo   |
| D. simulans     | w^{501}      | UCSD Stock Center                  |
| D. simulans     | Iso female 15 | 70Km East of Wanie-Rukula, Congo   |
| D. simulans     | Iso female 17 | 70Km East of Wanie-Rukula, Congo   |
| D. simulans     | Iso female 20 | 70Km East of Wanie-Rukula, Congo   |
| D. simulans     | Iso female 23 | 70Km East of Wanie-Rukula, Congo   |
| D. simulans     | Iso female 24 | 70Km East of Wanie-Rukula, Congo   |
| D. simulans     | Iso female 4  | 70Km East of Wanie-Rukula, Congo   |
| D. simulans     | Iso female 5  | 70Km East of Wanie-Rukula, Congo   |

**Supplementary Table 3:** D. melanogaster and D. simulans lines used for the McDonald-Kreitman test.
**Supplementary Table 4:** Statistics for the tests of recurrent selection for *Drosophila pkd2*. The $2\Delta\ln L$ and highest $p$-value are reported in Figure 2.

| ME test | species used | aligner | % aligned | Model 7 ($\beta$) | Model 8 ($\beta+\omega$) | $2\Delta\ln L$ | $p$-value | Model 8a ($\beta+\omega=1$) | $2\Delta\ln L$ (M8/M8a) | M8/M8a P-value |
|---------|--------------|---------|-----------|-------------------|-------------------------|---------------|-----------|-----------------------------|---------------------|----------------------|
| PAML    | 17           | T-Coffee| 83        | -16893.966863     | -16886.458028           | 15.02         | 0.00027   | -16893.643337               | 14.37               | 0.00038              |
| BUSTED  | 17           | T-Coffee| 83        | -                  | -                       | -             | 0.0020    | -                           | -                   | -                    |

**Supplementary Table 5 -** PAML NSsite models for the primate PKD genes.

| Gene    | Model 7 ($\beta$) | Model 8 ($\beta+\omega$) | $2\Delta\ln L$ | Highest $p$-value | aligner | % aligned | species used |
|---------|-------------------|--------------------------|---------------|------------------|---------|-----------|--------------|
| PKD1    | -27906.531879     | -27906.532368            | 0.00          | 0.99             | Clustal | 98        | 7            |
| PKD2    | -5282.345595      | -5282.345601             | 0.00          | 0.99             | Clustal | 99        | 11           |
| PKD1L1  | -18036.543699     | -18031.994118            | 9.099         | 0.0106           | Muscle  | 99        | 7            |
| PKD1L2  | NA                | NA                       | NA            | NA               | Clustal | NA        | 1            |
| PKD1L3  | -10781.582277     | -10754.534889            | 54.09         | 1.79 E -12       | Clustal | 99        | 9            |
| PKD2L1  | -5286.140128      | -5285.175660             | 1.929         | 0.3811           | Clustal | 96        | 19           |
| PKD2L2  | -3766.462705      | -3764.291786             | 4.342         | 0.114            | Clustal | 96        | 17           |