Supplemental Figure 1. Genomes analyzed

Group	Subdivision	Species (Common Name)	*Klkb1*	*F11*	*F12*	*HGFAC*	*HK*
Jawless Fish (Agnatha)		*Eptatretus burgeri* (Inshore Hagfish)	-	-	-	?	-
		Eptatretus stoutii (Pacific Hagfish)	-	-	-	?	-
		Lethenteron camtschaticum (Arctic Lamprey)	-	-	-	?	-
		Petromyzon marinus (Sea Lamprey)	-	-	-	?	-
Cartilaginous Fish (Chondrichthyes)		*Callorhinchus mili* (Elephant Shark)	-	-	-	+	-
		Chiloscyllium punctatum (Brownbanded bamboo shark)	-	-	-	+	-
		Discopyge ommata (Ocellated electric Ray)	-	-	-	+	-
		Rhincodon typus (Whale Shark)	-	-	-	+	-
		Scyliorhinus canicula (Small-Spotted Catshark)	-	-	-	+	-
Ray-Finned Fish (Actinopterygii)		*Acanthochromis polyacanthus* (Spiny Chromis Damselfish)	-	-	-	+	-
		Amphiprion ocellaris (Ocellaris Clownfish)	-	-	-	+	-
		Anabas testudineus (Climbing Perch)	-	-	-	+	-
		Archocentrus centrarchus (a Cichlid)	-	-	-	+	-
		Astatotilapia calliptera (Eastern Happy)	-	-	-	+	-
		Astyanax mexicanus (Mexican tetra)	-	-	-	+	-
		Austrofundulus limnaeus (a Killifish)	-	-	-	+	-
		Betta splendens (Siamese Fighting Fish)	-	-	-	+	-
		Boleophthalmus pectinirostris (Mudskipper)	-	-	-	+	-
		Carassius auratus (Goldfish)	-	-	-	+	-
		Chanos chanos (Milkfish)	-	-	-	+	-
		Clupea harengus (Atlantic Herring)	-	-	-	+	-
		Cynoglossus semilaevis	-	-	-	+	-
		Cyprinodon variegatus (Sheepshead Minnow)	-	-	-	+	-
		Danio rerio (Zebrafish)	-	-	-	+	-
		Denticeps clupeoides (Denticle Herring)	-	-	-	+	-
		Echeneis naucrates (Live Sharksucker)	-	-	-	+	-
		Electrophorus electricus (Electric Eel)	-	-	-	+	-
		Erpetoichthys calabaricus (Reedfish)	-	-	-	+	-
		Fundulus heteroclitus (Mummichog)	-	-	-	+	-
		Fugu rubripes (Pufferfish)	-	-	-	+	-
		Gadus morhua (Atlantic Cod)	-	-	-	+	-
		Gouania willdenowi (Blunt-Snouted Clingfish)	-	-	-	+	-
		Haplochromis burtoni (Burton's Mouthbrooder)	-	-	-	+	-
		Hippocampus comes (Tiger Tail Seahorse)	-	-	-	+	-
		Ictalurus punctatus (Channel Catfish)	-	-	-	+	-
		Kryptolebias marmoratus (Mangrove Killifish)	-	-	-	+	-
		Labrus bergylta (Ballan Wrasse)	-	-	-	+	-
		Larimichthys crocea (Large Yellow Croaker)	-	-	-	+	-
		Lates calcarifer (Asian Sea Bass)	-	-	-	+	-
		Mastacembelus armatus (Zig-Zag Eel)	-	-	-	+	-
		Maylandia zebra (Zebra Mbuna Cichlid)	-	-	-	+	-
		Monopterus albus (Asian Swamp Eel)	-	-	-	+	-
		Myripristis murdjan (Pinecone Soldierfish)	-	-	-	+	-
		Neolamprologus brichardi (Fairy Cichlid)	-	-	-	+	-
		Nothobranchius furzeri (Turquoise Killifish)	-	-	-	+	-
		Oncorhynchus mykiss (Rainbow Trout)	-	-	-	+	-
		Oncorhynchus tshawytscha (Chinook Salmon)	-	-	-	+	-
		Oreochromis aureus (Blue Tilapia) *Oreochromis niloticus* (Nile Tilapia)	-	-	-	+	-
		Oryzias melastigma (a Ricefish)	-	-	-	+	-
		Pangasianodon hypophthalmus (Iridescent Shark)	-	-	-	+	-
		Paralichthys olivaceus (Olive Flounder)	-	-	-	+	-
		Parambassis ranga (Indian Glassy Perch)	-	-	-	+	-
		Paramormyrops kingsleyae (Old Calabar Mormyrid)	-	-	-	+	-
		Perca flavescens (Yellow Perch)	-	-	-	+	-
		Poecilia formosa (Amazon Molly)	-	-	-	+	-
		Poecilia latipinna (Sailfin Molly)	-	-	-	+	-
		Poecilia mexicana (Shortfin Molly)	-	-	-	+	-
		Poecilia reticulata (Guppy)	-	-	-	+	-
		Pundamilia nyerere (a Cichlid)	-	-	-	+	-

Group	Subdivision	Species (Common Name)	*Klkb1*	*F11*	*F12*	*HGFAC*	*HK*
Ray-Finned Fish		*Salmo salar* (Atlantic salmon)	-	-	-	+	-
		Salmo trutta (Brown trout)	-	-	-	+	-
		Salvelinus alpinus (Arctic Char)	-	-	-	+	-
		Sander lucioperca (Zander)	-	-	-	+	-
		Scleropages formosus (Asian Arowana)	-	-	-	+	-
		Seriola dumerili (Greater Amberjack)	-	-	-	+	-
		Seriola lalandi dorsalis (California Yellowtail)	-	-	-	+	-
		Sinocyclocheilus anshuiensis (a Cavefish)	-	-	-	+	-
		Sinocyclocheilus grahami (Golden-Line Barbel)	-	-	-	+	-
		Sinocyclocheilus rhinocerous (a Cavefish)	-	-	-	+	-
		Sparus aurata (Gilt-Head Bream)	-	-	-	+	-
		Sphaeramia orbicularis (Orbiculate Cardinalfish)	-	-	-	+	-
		Stegastes partitus (Dicolor Damselfish)	-	-	-	+	-
		Tachysurus fulvidraco (Yellowhead Catfish)	-	-	-	+	-
		Xiphophorus couchianus (Monterrey Platyfish)	-	-	-	+	-
		Xiphophorus maculatus (Southern Platyfish)	-	-	-	+	-
Lobe-finned Fish (Sarcoptyrigii)		*Latimeria chalumnae* (West Indian Ocean Coelacanth)	+	-	-	+	+
		Protopterus annectens (West African Lungsish)	+	-	+	+	+
Amphibians		*Microcaecilia unicolor* (a Cacecilian)	+	-	+	+	+
		Nanorana parkeri (High Himalya Frog)	+	-	+	+	+
		*Rhinatrema bivittatum (*Two-lined Caecilians*)*	+	-	+	+	+
		Xenopus laevis (African Clawed Frog)	+	-	+	+	+
		Xenopus tropicalis (Western Clawed Frog)	+	-	+	+	+
Reptiles	Turtles	*Chrysemys picta bellii* (Box Turtle)	+	-	+	+	+
		Chelonia mydas (Green Sea Turtle))	+	-	+	+	+
		Gopherus evgoodei (Gopher Tortoise)	+	-	+	+	+
		Pelodiscus sinensis (Chinese Softshell Turtle)	+	-	+	+	+
		Terrapene carolina triunguis (Three-Toed Box Turtle)	+	-	+	+	+
	Lizards	*Paroedura picta* (Ocelot Gecko)	+	-	+	+	+
		Anolis carolinensis (Green Anole)	+	-	+	+	+
		Gekko japonicus (Schlegel's Japanese Gecko)	+	-	+	+	?
		Podarcis muralis (Common Wall Lizard)	+	-	+	+	+
		Pogona vitticeps (Central Bearded Dragon)	+	-	+	+	+
	Snakes	*Daboia russelii* (Russell's Viper)	+	-	+	+	
		Notechis scutatus (Tiger Snake)	+	-	+	+	+
		Protobothrops mucrosquamatus (Brown-Spotted Pit Viper)	+	-	+	+	+
		Pseudonaja textilis (Eastern Brown Snake)	+	-	+	+	+
		Python bivittatus (Burmese Python)	+	-	+	+	+
		Thamnophis sirtalis (Common Garter Snake)	+	-	+	+	+
	Crocodylians	*Alligator mississippiensis* (American Alligator)	+	-	+	+	+
		Crocodylus porosus (Saltwater Crocodile)	+	-	+	+	+
		Gavialis gangeticus (Gharial)	+	-	+	+	?
Birds		*Amazona aestiva* (Turquoise-Fronted Amazon)	+	-	-	+	+
		Anas platyrhynchos (Mallard)	+	-	-	+	+
		Anser cygnoides domesticus (Domestic Goose)	+	-	-	+	+
		Antrostomus carolinensis (Chuck-Will's-Widow)	+	-	-	+	+
		Apaloderma vittatum (Bar-Tailed Trogon)	+	-	-	+	+
		Aptenodytes forsteri (Emperor Penguin)	+	-	-	+	+
		Apteryx australis mantelli (North Island Brown Kiwi)	+	-	-	+	+
		Apteryx rowi (Okarito Kiwi)	+	-	-	+	+
		Aquila chrysaetos canadensis (Golden Eagle)	+	-	-	+	+
		Buceros rhinoceros silvestris (Rhinoceros Hornbill)	+	-	-	+	+
		Calidris pugnax (Ruff)	+	-	-	+	+
		Calypte anna (Anna's Hummingbird)	+	-	-	+	+
		Cariama cristata (Red-legged Seriema)	+	-	-	+	+
		Cathartes aura (Turkey Vulture)	+	-	-	+	+
		Chaetura pelagica (Chimney Swift)	+	-	-	+	+
		Charadrius vociferous (Killdeer)	+	-	-	+	+

Group	Subdivision	Species (Common Name)	*Klkb1*	*F11*	*F12*	*HGFAC*	*HK*
Birds		*Columba livia* (Rock Dove)	+	-	-	+	+
		Corapipo altera (White-Ruffed Manakin)	+	-	-	+	+
		Corvus brachyrhynchos (American Crow)	+	-	-	+	+
		Coturnix japonica (Japanese Quail)	+	-	-	+	+
		Cuculus canorus (Common Cuckoo)	+	-	-	+	+
		Dromaius novaehollandiae (Emu)	+	-	-	+	+
		Egretta garzetta (Little Egret)	+	-	-	+	+
		Empidonax traillii (Willow Flycatcher)	+	-	-	+	+
		Erythrura gouldiae (Gouldian Finch)	+	-	-	+	+
		Eurypyga helias (Sunbittern)	+	-	-	+	+
		Falco cherrug (Saker Falcon)	+	-	-	+	+
		Falco peregrinus (Peregrine Falcon)	+	-	-	+	+
		Ficedula albicollis (Collared Flycatcher)	+	-	-	+	+
		Gavia stellata (Red-Throated Loon)	+	-	-	+	+
		Gallus gallus domesticus (Chicken)	+	-	-	+	+
		Geospiza fortis (Medium Ground Finch)	+	-	-	+	+
		Haliaeetus albicilla (White-Tailed eagle)	+	-	-	+	+
		Haliaeetus leucocephalus (Bald Eagle)	+	-	-	+	+
		Lepidothrix coronata (Blue-Crowned Manakin)	+	-	-	+	+
		Leptosomus discolor (Cuckoo Roller)	+	-	-	+	+
		Limosa lapponica baueri (Bar-Tailed Godwit)	+	-	-	+	+
		Lonchura striata domestica (Society Finch)	+	-	-	+	+
		Manacus vitellinus (Golden-Collared Manakin)	+	-	-	+	+
		Meleagris gallopavo (Wild Turkey)	+	-	-	+	+
		Merops nubicus (Northern Carmine Bee-Eater)	+	-	-	+	+
		Neopelma chrysocephalum (Saffron-Crested Tyrant-Manakin)	+	-	-	+	+
		Nestor notabilis (Kea)	+	-	-	+	+
		Nipponia nippon (Crested Ibis)	+	-	-	+	+
		Nothoprocta perdicaria (Chilean Tinamou)	+	-	-	+	+
		Numida Meleagris (Helmeted Guineafowl)	+	-	-	+	+
		Opisthocomus hoazin (Hoatzin)	+	-	-	+	+
		Pelecanus crispus (Dalmatian Pelican)	+	-	-	+	+
		Parus major (Great Tit)	+	-	-	+	+
		Patagioenas fasciata monilis (Band-Tailed Pegeon)	+	-	-	+	+
		Picoides pubescens (Downy Woodpecker)	+	-	-	+	+
		Pipra filicauda (Wire-Tailed Manakin)	+	-	-	+	+
		Phalacrocorax carbo (Great Cormorant)	+	-	-	+	+
		Phaethon lepturos (White-Tailed Tropicbird)	+	-	-	+	+
		Podiceps cristatus (Great Crested Grebe)	+	-	-	+	+
		Pseudopodoces humilis (Ground Tit)	+	-	-	+	+
		Pterocles gutturalis (Yellow-Throated Sandgrouse)	+	-	-	+	+
		Pygoscelis adeliae (Adélie Penguin)	+	-	-	+	+
		Serinus canaria (Atlantic Canary)	+	-	-	+	+
		Sturnus vulgaris (Common Starling)	+	-	-	+	+
		Struthio camelus australis (Southern Ostrich) *Taeniopygia guttata* (Zebra Finch)	+	-	-	+	+
		Tauraco erythrolophus (Red-Crested Turaco)	+	-	-	+	+
		Tinamus guttatus (White-Throated Tinamou)	+	-	-	+	+
		Tyto alba (Barn Owl)	+	-	-	+	+
		Zonotrichia albicollis (White-Throated Sparrow)	+	-	-	+	+

Group	Subdivision	Species (Common Name)	*Klkb1*	*F11*	*F12*	*HGFAC*	*HK*
Mammals	Monotremes	*Ornithorhynchus anatinus* (Duck-Billed Platypus)	+	+	+	+	+
		Tachyglossus aculeatus (Short-Beaked Echidna)	ND	+	ND	ND	ND
	Marsupials	*Monodelphis domestica* (Gray Short-Tailed Opossum)	+	+	+	+	+
		Phascolarctos cinereus (Koala)	+	+	+	+	+
		Sarcophilus harrisii (Tasmanian Devil)	+	+	+	+	+
		Vombatus ursinus (Common Wombat)	+	+	+	+	+
	Placental (Eutherian) Terrestrial	*Acinonyx jubatus* (Cheetah)	+	+	+	+	+
		Ailuropoda melanoleuca (Giant Panda)	+	+	+	+	+
		Bos mutus (Domestic Yak)	+	+	+	+	+
		Bos tarus (Domestic Cattle)	+	+	+	+	+
		Callorhinus ursinus (Northern Fur Seal)	+	+	+	+	+
		Camelus bactrianus (Bactrian Camel)	+	+	+	+	+
		Camelus dromedaries (Dromedary)	+	+	+	+	+
		Camelus ferus (Wild Bactrian Camel)	+	+	+	+	+
		Canis lupus familiaris (Dog)	+	+	+	+	+
		Castor canadensis (American Beaver)	+	+	+	+	+
		Cavia porcellus (Guinea Pig)	+	+	+	+	+
		Ceratotherium simum simum (Southern White Rhinoceros)	+	+	+	+	+
		Chinchilla lanigera (Long-Tailed Chinchilla)	+	+	+	+	+
		Chrysochloris asiatica (Cape Golden Mole)	+	+	+	+	+
		Condylura cristata (Star-Nosed Mole)	+	+	+	+	+
		Cricetulus griseus (Chinese Hamster)	+	+	+	+	+
		Dipodomys ordii (Ord's Kangaroo Rat)	+	+	+	+	+
		Enhydra lutris kenyoni (Sea Otter)	+	+	+	+	+
		Equus asinus (Donkey)	+	+	+	+	+
		Equus caballus (Horse)	+	+	+	+	+
		Equus przewalskii (Przewalski's Horse)	+	+	+	+	+
		Erinaceus europaeus (European Hedgehog)	+	+	+	+	+
		Eumetopias jubatus (Stellar Sea Lion)	+	+	+	+	+
		Felis catus (Cat)	+	+	+	+	+
		Fukomys damarensis (Damara Mole-Rat)	+	+	+	+	+
		Heterocephalus glaber (Naked Mole Rat)	+	+	+	+	+
		Hippopotamus amphibious (Hippopotamus)	+	+	+	+	+
		Homo sapiens (Human)	+	+	+	+	+
		Ictidomys tridecemlineatus (Thirteen-Lined Ground Squirrel)	+	+	+	+	+
		Jaculus jaculus (Lesser Egyptian Jerboa)	+	+	+	+	+
		Leptonychotes weddellii (Weddell Seal)	+	+	+	+	+
		Loxodonta Africana (African Elephant)	+	+	+	+	+
		Lynx canadensis (Canada Lynx)	+	+	+	+	+
		Manis javanica (Sunda Pangolin)	+	+	+	+	+
		Marmota flaviventris (Yellow-Bellied Marmot)	+	+	+	+	+
		Marmota marmota marmota (Alpine Marmot)	+	+	+	+	+
		Marmota monax (Groundhog)	+	+	+	+	+
		Mesocricetus auratus (Golden Hamster)	+	+	+	+	+
		Mustela putorius furo (Ferret)	+	+	+	+	+
		Nannospalax galili (Spalax)	+	+	+	+	+
		Neomonachus schauinslandi (Hawaiian Monk Seal)	+	+	+	+	+
		Octodon degus (Common Degu)	+	+	+	+	+
		Odobenus rosmarus divergens (Pacific Walrus)	+	+	+	+	+
		Odocoileus virginianus texanus (White-Tailed Deer)	+	+	+	+	+
		Orycteropus afer afer (Aardvark)	+	+	+	+	+
		Oryctolagus cuniculus (European Rabbit)	+	+	+	+	+
		Panthera pardus (Leopard)	+	+	+	+	+
		Panthera tigris altaica (Siberian Tiger)	+	+	+	+	+
		Peromyscus leucopus (White-Footed Mouse)	+	+	+	+	+
		Peromyscus maniculatus bairdii (Deer Mouse)	+	+	+	+	+
		Puma concolor (Cougar)	+	+	+	+	+
		Sorex araneus (Common Shrew)	+	+	+	+	+
		Suricata suricatta (Meerkat)	+	+	+	+	+
		Sus scrofa (Wild Boar)	+	+	+	+	+
		Tupaia chinensis (Northern Tree Shrew)	+	+	+	+	+
		Urocitellus parryii (Arctic Ground Squirrel)	+	+	+	+	+
		Ursus arctos horribilis (Grizzly Bear)	+	+	+	+	+

Group	Subdivision	Species (Common Name)	*Klkb1*	*F11*	*F12*	*HGFAC*	*HK*
Mammals	Placentals (Eutherian) Terrestrial	*Ursus maritimus* (Polar Bear)	+	+	+	+	+
		Vulpes Vulpes (Red Fox)	+	+	+	+	+
		Zalophus californianus (California Sea Lion)	+	+	+	+	+
	Placentals (Eutherian) Cetacean	*Balaenoptera acutorostrata scammoni* (Minke Whale)	-	+	-	+	+
		Balaenoptera physalus (Fin Whale)	-	+	-	+	+
		Delphinapterus leucas (Beluga Whale)	-	+	-	+	+
		Globicephala melas (Long-Finned Pilot Whale)	-	+	-	+	+
		Lagenorhynchus obliquidens (Pacific White-Sided Dolphin)	-	+	-	+	+
		Lipotes vexillifer (Baiji)	-	+	-	+	+
		Neophocaena asiaeorientalis (Finless Porpoise)	-	+	-	+	+
		Monodon Monoceros (Narwhal)	-	+	-	+	+
		Orcinus orca (Killer Whale)	-	+	-	+	+
		Physeter catodon (Sperm Whale)	-	+	-	+	+
		Sousa chinensis (Indo-Pacific Humpbacked Dolphin)	-	+	-	+	+
		Tursiops truncates (Common Bottlenose Dolphin)	-	+	-	+	+

ND – Not Done

? – Sequences may be present, but there is uncertainty.

Data bases analyzed in the current study included nucleotide collections, non-redundant protein sequences, whole-genome shotgun contigs (WGS) and the Sequence Read Archives (SRA) of DNA and RNA stored in the National Center for Biotechnology Information (NCBI). The Ensembl Genome Browser of the European Bioinformatics Institute (EMBL-EBI) was used for additional searches, localization of homologous genes, sequence verification, and for gene structure and synteny studies in available genomes. Basic Local Alignment Search Tool (BLAST) was used to conduct the surveys for prekallikrein (*KlKb1*), factor XII (*F12*), high-molecular-weight kininogen (*Kng1*), factor XI (*F11*) and pro-hepatocyte growth factor activator (*HGFAC*) orthologs and paralogs for the species under study in the listed data resources. Predicted protein sequences based on nucleotide collections were downloaded from NCBI BLAST results in FASTA format. Reconstructions for protein sequences for some species not present in these collections (listed below) were based on SRA sequences and manual assembly, assisted by BLAST and using multiple amino acid sequence alignments. Additional multiple sequence alignments and phylogenetic relations were made and analyzed with CLUSTAL. In some cases, additional multiple sequence alignments were made manually and checked by identifying conserved cysteine residues and other conserved amino acids.

SRA data sets were analyzed for the following organisms (SRA ID are provided for each species):

Protopterus annectens (West African Lungfish) SRX1016235, SRX1016236; *Protopterus sp*., ERX2256863, ERX2256864

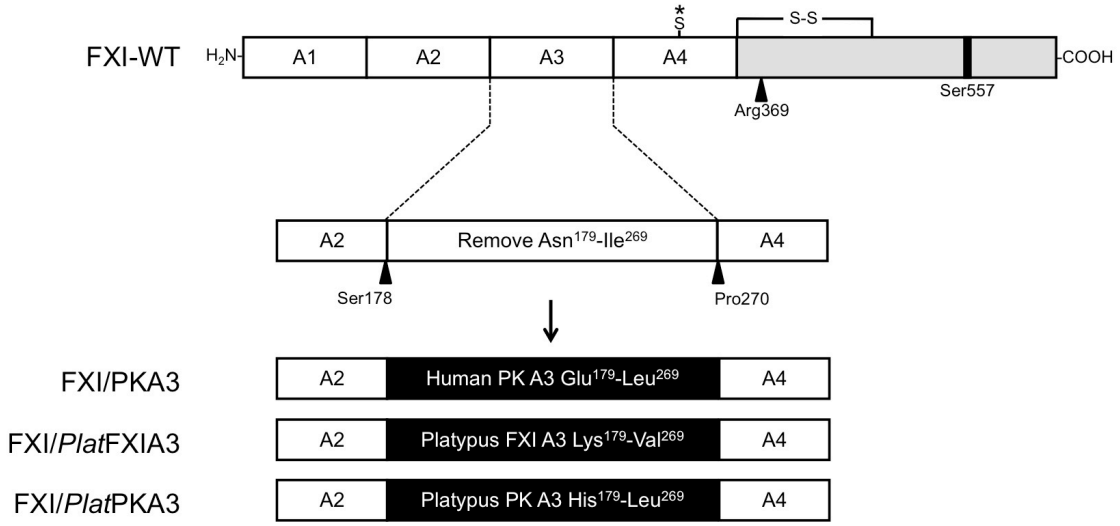
Latimeria chalumnae (West Indian Ocean Coelacanth) DRX001703, DRX001704, DRX002994, SRX110147, SRX110150, SRX112771

Hippopotamus_amphibius (Hippopotamus) SRX1164570, SRX2880553, SRX2899275, SRX2899276, SRX2899277, SRX2899278, SRX2899279

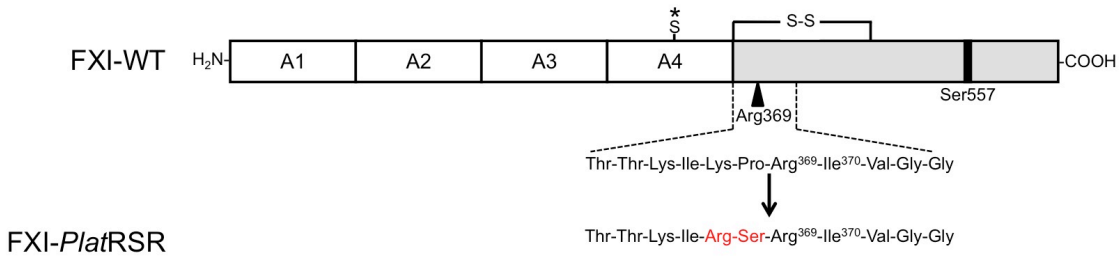
Tachyglossus aculeatus (Short-Beaked Echidna) SRX317056, SRX317058, SRX7214460, SRX7214453

Supplemental Figure 2. Recombinant Factor XI

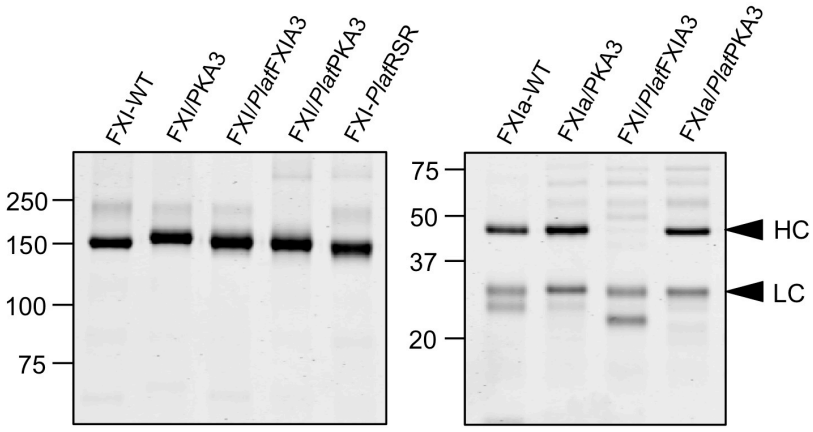
Factor XI A3 Domain variants. Nucleic acid sequence encoding the A3 domain (amino acids 179 through 269 in the cDNA for human wild type fXI (FXI-WT) were replaced by the corresponding sequence from the human PK-A3 to generate a cDNA for the chimera fXI/PKA3, from platypus fXI-A3 to generate fXI/*Plat*FXIA3 cDNA, and from platypus PK-A3 to generate fXI/*Plat*FXIA3 cDNA. In the schematic diagram of fXI below, A1 to A4 represent the four apple domains and the light gray box the catalytic protease domain. Arg369 is the activation cleavage site and Ser557 the active site serine residue. S* indicates the position of Cys321, which forms the interchain disulfide bond in the FXI dimer.



FXI with platypus activation cleavage site. Nucleic acid sequence encoding Lys367 and Pro368 in human fXI-WT were replaced with sequence encoding Arg367 and Ser368 from the platypus fXI sequence to form a cDNA for fXI-*Plat*RSR.

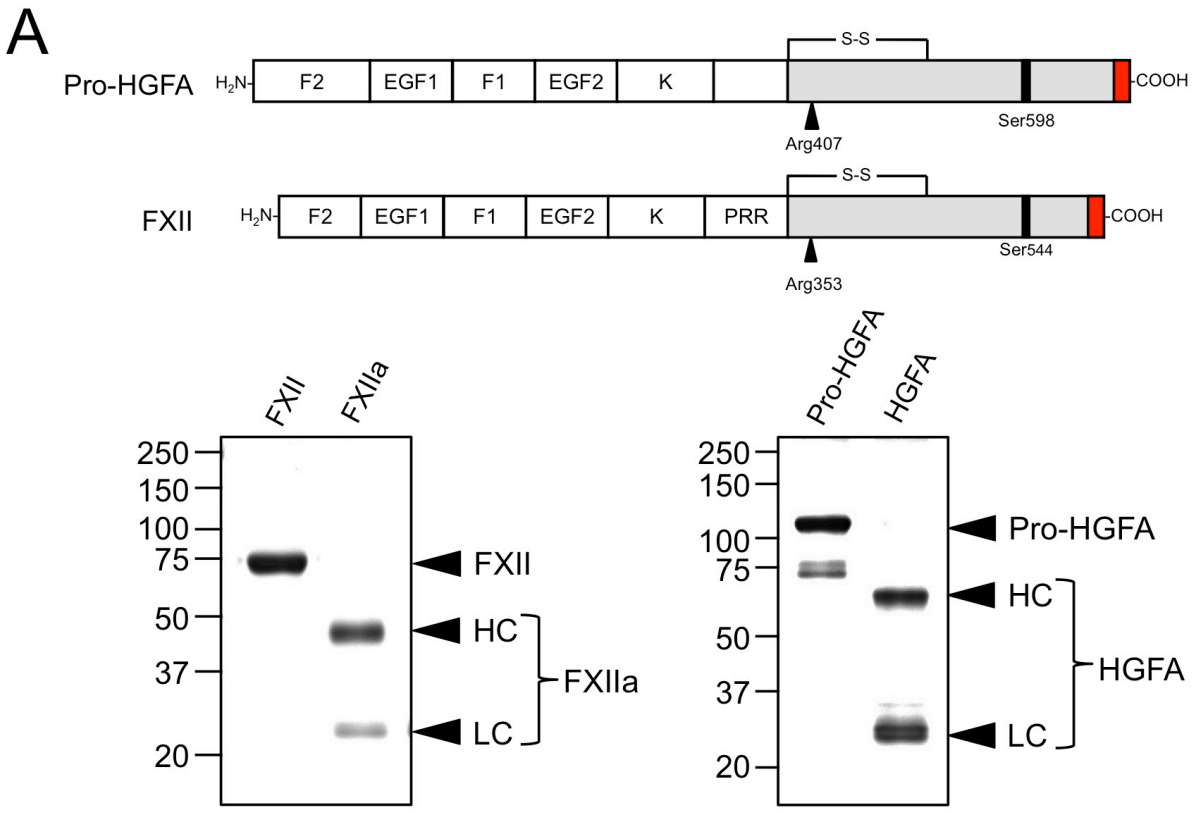


Recombinant FXI and FXIa. *Left Panel – FXI.* FXI variants described above were expressed in HEK293 cells and purified from conditioned media by monoclonal IgG chromatography. Samples (2 µg) were run on 7.5% polyacrylamide-SDS gels under non-reducing conditions and stained with Coomassie Blue. *Right Panel – FXIa*. Proteins shown in the left panel were incubated with fXIIa to generate the heavy chain (HC) and light chain (LC) of fXIa. Samples (2 µg) were run on 7.5% polyacrylamide-SDS gels under reducing conditions and stained with Coomassie Blue. The heavy chain of fXIa/*Plat*FXIA3 sustained an additional cleavage, dividing it between the A2 and A3 domains. This did not appear to impact activity in aPTT or factor IX activation assays. For both panels, positions of molecular mass markers are on the left.

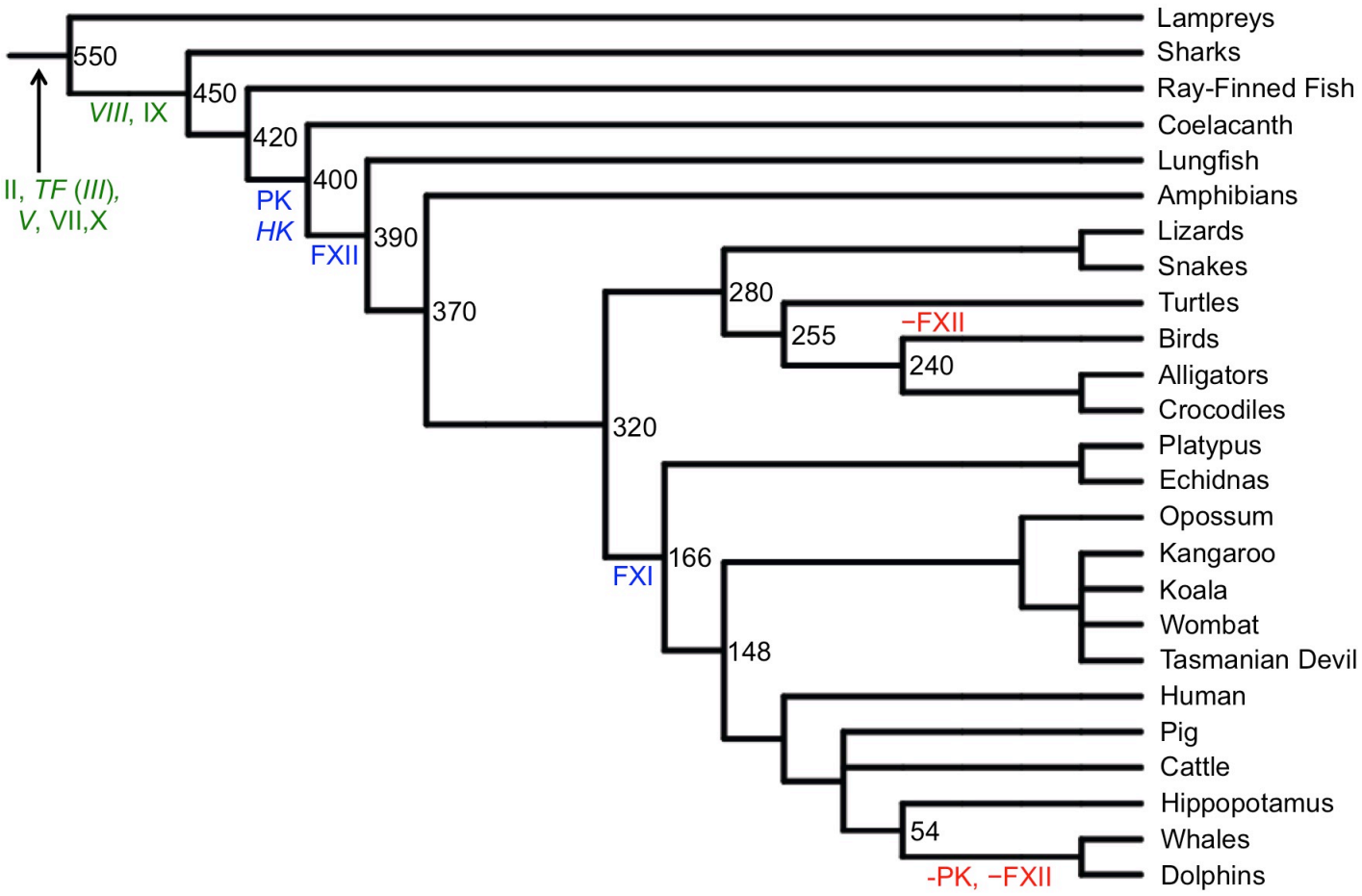


Supplemental Figure 3. Recombinant FXII and Pro-HGFA. The schematics show the domain organization for Pro-hepatocyte growth factor activator (Pro-HGFA) and its paralog factor XII (FXII). The non-catalytic heavy chains of both proteins contain fibronectin type II (F2), epidermal growth factor (EGF1 and EGF2), fibronectin type I (F1) and kringle (K) domains. FXII also has a proline-rich region (PRR), The catalytic domains are shown in grey. Arg407 and Arg353 are the activation cleavage sites, and Ser598 and Ser544 the active site serines. The cDNAs for human Pro-HGFA and fXII were modified to include sequence for an eight amino acid hemagglutinin tag at the C-termini (indicated in red). The cDNAs were expressed in HEK293 cells and purified by anti-HA IgG affinity chromatography.

FXII was converted to fXIIa by incubation with dextran sulfate. Pro-HGFA was converted to the active protease HGFA by incubation with dextran sulfate and thrombin. Reactions were stopped by addition of polybrene to dissociate the proteins from the dextran sulfate and hirudin to neutralize thrombin. The proteins were repurified by anti-HA IgG affinity chromatography. Samples (2 μg) of the unactivated and activated proteins were run on 10% polyacrylamide-SDS gels under reducing conditions and stained with Coomassie Blue. The heavy chains (HC) and light chains (LC) of the activated forms are indicated at the right of each panel. Molecular mass markers are indicated at the left of each panel.



Supplemental Figure 4. Cladogram depicting relationships of vertebrate organisms. Numbers indicate estimated ages of points of divergence in millions of years. Green lettering indicates estimated points of origin of protein components of the vitamin K-dependent plasma thrombin generation mechanism (factors II, V, VII, VIII, IX, and X and tissue factor [TF, factor III]). The kallikrein-kinin system (factor XII, PK and HK) and FXI are indicated in blue. Red lettering indicates loss of the FXII and PK genes (–FXII, –PK) in certain lineages. Plasma factors indicated in non-italicized lettering are plasma proteases, while factors indicated in italics are non-enzymatic cofactors.



Supplemental Figure 5. Prekallikrein Amino Acid Sequence Comparisons

Human (*Homo sapiens*)
Hippopotamus (*Hippopotamus amphibius*) (based on SRA SRR2183469)
Pigeon (*Columba livia domestica*)
Alligator (*Alligator mississippiensis*)
African Clawed Frog (*Xenopus laevis*)
West African Lungfish (*Protopterus annectens*, based on liver specimen 5 Accession: SRX1016236)
Coelacanth (*Latimeria chalumnae*)

Blue Highlight indicates residues of the catalytic triad
Residue 371 is the arginine immediately preceding the activation cleavage site.
X indicates presumed missing sequence
*Amino acid numbering is for human prekallikrein

```
             *1
Human         GCLTQLYENA FFRGGDVASM YTPNAQYCQM RCTFHPRCLL FSFLPASSIN DMEKRFGCFL KDSVTGTLPK
Hippopotamus  ECLTQLYNNI FFRGGDVSGV FTPNAKYCQI VCTHHPRCLL FTFLPASSTT DTDKRFGCFL KDSVTGTLPR
Pigeon        ECVTQTYENT YFQGGDLTVV FTPSANYCQV VCTYHPTCLL FTYLPATWTK DPAKRFSCYL KDSDTEMLPK
Alligator     ECMTQIYENT YFQGGDVMTV FTPNVNYCQI VCTYHPTCLL FTYLPPTGIH DPTKRFTCYL KDSKTEILPK
Frog          GCISELYQDS YWQGGDLRSV FAPDVEYCQL VCTFSPRCLM FSYLPASWPK E-NERFACYL KESATNMLPK
Lungfish      GCVTQIYQDT YFQGPDLATY YSPDAAFCQM VCTFHPRCLY YTXXXXXXXX XXXXXXXXXX XXXXXXXXXX
Coelacanth    ECITELHDNI YFQGGDVSAV YAPDVKYCQI VCTYHPQCLF FTFLTETWTT R-EQRYACFL KDGDKIGTPS

             71
Human         VH-RTGAVSG HSLKQCGHQI SACHRDIYKG VDMRGVNFNV SKVSSVEECQ KRCTNNIRCQ FFSYATQTFH
Hippopotamus  VS-RTSAISG HSLKQCGDRI SACHRGIHEG LDMRGVNFNV SKVKSVQECQ EKCTNSIHCQ FFTYATQSFF
Pigeon        VD-MEGAISG HSLKQCNIQI SACSPDVHIG LDMEGKIYDV TVADSYQQCQ KRCTNDNRCH FFTYASETFN
Alligator     VH-MDGAISG HSLKQCHAQI SGCSRDIHTG LDMQGINYNV TTEYSYQECQ KRCTNDNHCQ FFTYATGAFH
Frog          VT-LTGAISG HSLKTCKSKI NVCRDKNFHG IDMIGTNYNV TWATNVQQCK EGCTNDIHCQ YFTYVTEQFH
Lungfish      PKTFNGAVSG HSLKQCQSQI SACIENLYEG MDMXXXXXXX XXVDNFKECE KMCTNDPHCQ FFSYATSKFH
Coelacanth    KVTLQGVISG HSLKQCDSLI DACFEEVHNG LDLMGINYHI TTVDSYQQCQ KTCTNDPHCQ FFTYVTDRFH

            140
Human         KAEYRNNCLL KYSPGGTPTA IKVLSNVESG FSLKPCALSE IGCHMNIFQH LAFSDVDVAR VLTPDAFVCR
Hippopotamus  NAEYRNNCLL KNSPQGTPTS IKVLADVESG FSLKPCADSK IGCHMNIFQH RAFSDVNVAR II-PDAFVCR
Pigeon        NASFRKKCLL KQASVGTPTS IKVLDEVVSG FSLKTCQLSE MDCQMDIFED QEFSGINITS FFAPDISVCQ
Alligator     SAGFRNKCFL KYTKTGTPAS IRILNNAVSG FSLKLCQLSQ TDCRLDIFQD KEFSGNNIMS VFTPDTFVCR
Frog          SAPMRNRCYF KYSAKGMPTR IRLLDNVISG FSLKACGKSS LGCQNDLFQN MELPGETLTR VFAPDVHTCQ
Lungfish      SASYRSMCLL KYTQSGTPKQ VKILENVISG FSLKSCQRSE KGCNSDLFQE IDFSGDDVTS VLTPDAEVCR
Coelacanth    CADLRNRCYL KYTMKGTPAR IRQLPNVVSG FSLKACGYSE KGCRLDLFPN VEFSGGDITS FMAPDANVCR

            210
Human         TICTYHPNCL FFTFYTNVWK IESQRNVCLL KTSESGTPSS STPQENTISG YSLLTCKRTL PEPCHSKIYP
Hippopotamus  TICTYHPNCL FFTFYTNAWK TESQRNVCLL KTSQSGTPSS PTPQENAISG YSLLTCKQTL PEPCHSKIYS
Pigeon        TICTYFPKCL FFTFFTKEWQ IESQRNLCLL KTSTSGIPEA LTSRENAVSG FGLLNCRRSL PA-CNSRTYT
Alligator     TICTYHPNCL FFTFFTSEWE VETQRNLCLL KTSRSGIPSA YVERENAMSG FSLLNCRRLF PA-CHSHTYN
Frog          KICTFYPNCL FFTFYKKESK EPLQRNVCYI RTSTKGIPDE GINKEHTISG FSLLSCKFSP SV-CPLTILS
Lungfish      LACTYHPTCL FFTXXXXXXX XESQRNRCYL KTSKSGQPAA PTSHGHAISG FSMLTCKXXX XXXXXXXXXX
Coelacanth    LICTYYPKCL FFTFMTKKWN RESQKNVCYL KTSESGDPLA PRAQENVISG FSLLSCKRPL SD-CSLKYYF

            280                                         321   326
Human         GVDFGGEELN VTFVKGVNVC QETCTKMIRC QFFTYSLLPE DCKEEK-CKC FLRLSMDGSP TRIAYGTQGS
Hippopotamus  QVDFGGEELN VTFVEGANGC QETCTKMIRC QFFSYSLLPE DCRGER-CKC SLRLSLDGSP VRITYGTRAS
Pigeon        HMNFLGDELN VTYTKGHRAC QQVCTEVIRC QFFTYFSLQD SCNEEGKCEC HLRMSSNGSP VKIVHGPGRI
Alligator     DTNFLGDELN VTYVKGHKAC QLVCTDMVRC QFFTYFPLQE -CTQEGKCKC HLRMSSNGSP NRIVHEKGKI
Frog          DAEFLGDELL VEEVSGEKEC QQECTNNIRC QFFTYRPMQS GCSENK-CKC HMKISSNGLP TGIRHGNGEI
Lungfish      XXTFSGNDIG TIDVTSTKAC QDACTNTDRC QFFTYTARAG TCKNQI-CKC YLKMSSNGLP SGIQQSVGDI
Coelacanth    NYTFLGDDLQ RVDVLNHSVC RHQCNQDKRC QFFTYIPDSK AHDENK-FNC YLKRSKIGLP TEIQQGVEAT

            349                                 371
Human         SGYSLRLC-- ------NTGD NSVCTTKTST --RIVGGTNS SWGEWPWQVS LQVKLTA--Q RHLCGGSLIG
Hippopotamus  SGYSLRLC-- ------KSGE SSVCTTKNA- --RIVGGTNS SWGEWPWQVS LQVNLRP--Q SHLCGGSIIG
Pigeon        SGYSLRLC-- ------KKKA STVCMQHSAR TIRIVGGTDS APGEWPWQVS LHVKLSR--R RHVCGGSIIS
Alligator     SGYSLRLCQR KANTVFKS-I SIACMQPSKE SIRIVGGTDS SPGEWPWQVS LQVKLST--Q KHLCGGSIIS
Frog          SGFSLRLC-- ------KIKS VKGCGEPIEH ANRIVGGTDS VLGEWPWQVS MHLRLTASYK KHACGGSIIS
Lungfish      SGFSLRLCXX XXXXXXXXXX XXXXXXXXXX XXRIVGGVNA EIKEWPWQVS LQIKANT--I KHVCGGS--N
Coelacanth    SGFSLRLC-- -----RNKIP SVQCGQAVEF ATRIVGGSTS SVREWPWQVS LHGNVGS--Y RHMCGGSIIN
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Supplemental Figure 5. Prekallikrein Amino Acid Sequence Comparisons (continued)

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            407     415
Human         HQWVLTAAHC FDGLPLQDVW RIYSGILNLS DITKDTPFSQ IKEIIIHQNY KVSEGNHDIA LIKLQAPLNY
Hippopotamus  HQWVLTAAHC FDGLPLSDVW RIYGGILNLS EITKETPFSQ IKEIIIHQNY KISEGGHDIA LIKLETPLNY
Pigeon        NQWILTAAHC VMSLANPNIW HVYAGILKQS EINEDTPFFK VEEIIVHPQY KYARTGYDIA LMKLDKPMNF
Alligator     DRWILTAAHC TDGYESPNIW RVYTGILKQS EINEDTPFFR VQDIVIHPQY VIAETGYDIA LMKLDKPMNF
Frog          NQWIVTAAHC FAMHPLPQMW IIYSGVVKLS NITQSTPFSE TEQIIIHPHY TGAGNGTDIA LLKLKTPISF
Lungfish      SLWIITAAHC FDVIKKPETW RIYAGFFQXX XXXXXXXXXX XXXXXXXXXX XXXXXXXXVA LLELTTPINF
Coelacanth    KNWIVTAAHC FERLKNPDDW HIYSGFLKQS EMNDDAPFFS VHTIIVHPQY CAIEHVYDIA LLKLQEPMYY

            477
Human         TEFQKPICLP SKGDTSTIYT NCWVTGWGFS KEKGEIQNIL QKVNIPLVTN EECQKRYQDY KITQRMVCAG
Hippopotamus  TDSQKSICLP SKDDTKTIYT DCWITGWGFT EEKGKIQNTL RKAKVPLVSN EECQRSYRDY KITKQMICAG
Pigeon        TDLQLPICLP SKEDADILYT DCWVIGWGYR KEKGRVEDIL QKATVPLMSR EECQARYRKR RIGDKVICAG
Alligator     TDFQQPICLP SKEEANIVYT SCWVIGWGYA KERGQITDIL QKANIPLVSE EECQSRYQEQ TINNKVICAG
Frog          NDHQKAICLP PREPTFVLPN SCWITGWGFT EESGSLSNIL QKAEVPPIST EECQGNYEQT RIDKKILCAG
Lungfish      TECQMPICLP QKDVKMESYE SCYSTGWGYT QEQGSVSDIL QKALIPQI-- DVCQSKYKNY NISDLLLCAG
Coelacanth    TDYMLPICLP ETKFESN-YE DCWVTGWGYT SEAGHVNNIL QKASVPVITN EDCQSYYKSY NITESMLCAG

            547         559                                                         619
Human         YKEGGKDACK GDSGGPLVCK HNGMWRLVGI TSWGEGCARR EQPGVYTKVA EYMDWILEKT QSSDGKAQMQ SPA
Hippopotamus  YKEGGKDACK GDSGGPLVCK HDDVWHLVGI TSWGEGCARR DHPGVYTKVA EYMDWILEKT QDGGGPSSMK -PA
Pigeon        YDEGGRDACK GDSGGPLSCK HEEVWYLVGI TSWGEGCARP RQPGVYTKVA EYSDWIIEKT T
Alligator     YKEGGKDACK GDSGGPLSCK HEQIWYLVGI TSWGEGCARP ELPGVYTKVA EYVEWILEKT T
Frog          YKRGKIDSCK GDSGGPLACV VDEIWYLTGI TSWGEGCARP GKPGVYTRVS EFTDWIIEHT RV
Lungfish      FKEGGIDACK GDSGGPYVCK RLEAWHLMGI TSWGEGCAXX XXPGVYTKVA YYFDWISRHV
Coelacanth    YKEGGIDACK GDSGGPFVCQ YEQTWYLQGI TSWGEGCARP DRPGVYTRVG YFKDWILKHV
```

Supplemental Figure 6. Comparison of Prekallikrein and Kalliklectin.

Shown are alignments for the apple domains of human and coelacanth prekallikrein and kalliklectin from the goosefish (*Lophiomus setigerus*), The numbering system is for human prekallikrein. R indicates the arginine residue at teh activation cleavage site for prekallikrein.

```
Human PK        GCLTQLYENA FFRGGDVASM YTPNAQYCQM RCTFHPRCLL FSFLPASSIN DMEKRFGCFL KDSVTGTLPK
Coelacanth PK   ECITELHDNI YFQGGDVSAV YAPDVKYCQI VCTYHPQCLF FTFLTETWTT R-EQRYACFL KDGDKIGTPS
Kalliklectin    ECIPQLVKDM DFPGSDIENV FAPDAEHCQK LCTEHPKCLF FTFVEPEWTR D-SRNYYCYL KYTSTGKPNS

                71
Human PK          VH--RTGAVS GHSLKQCGHQ ISACHRDIYK GVDMRGVNFN VSKVSSVEEC QKRCTNNIRC QFFSYATQTF
Coelacanth PK     KVT-LQGVIS GHSLKQCDSL IDACFEEVHN GLDLMGINYH ITTVDSYQQC QKTCTNDPHC QFFTYVTDRF
Kalliklectin      QVVVVDATAG YSLKPSCPKQ -KSCVSKVYE DVDFNGADYE SLFVDNQNEC QKVCTNDPFC QFFTFVENGY

               139
Human PK          HKAEYRNNCL LKYSPGGTPT AIKVLSNVES GFSLKPCALS EIGCHMNIFQ HLAFSDVDVA RVLTPDAF--
Coelacanth PK     HCADLRNRCY LKYTMKGTPA RIRQLPNVVS GFSLKACGYS EKGCRLDLFP NVEFSGGDIT SFMAPDAN--
Kalliklectin      KQQNIRNKCH LKFSWTVPII PVVVANPGVI SGFSCNAKMS P-ACGEVCKS ELFPDTDIPG SDLLALPAAS

                 207
Human PK          ---VCRTICT YHPNCLFFTF YTNVWKIESQ RNVCLLKTSE SGTPSSSTPQ ENTISGYSLL TCKRTLPEPC
Coelacanth PK     ---VCRLICT YYPKCLFFTF MTKKWNRESQ KNVCYLKTSE SGDPLAPRAQ ENVISGFSLL SCKRPLSD-C
Kalliklectin      SPQHCQALCS AHPKCTFFSF DSNAFK---- ---CYLKNNP DYLEKTKKAG WT--SGLPAR NCQMDKKWLM

               274                                                 321   326
Human PK          HSKIYPGVDF GGEELNVTFV KGVNVCQETC TKMIRCQFFT YSLLPEDCKE EKCKCFLRLS MDGSPTRIAY
Coelacanth PK     SLKYYFNYTF LGDDLQRVDV LNHSVCRHQC NQDKRCQFFT YIPDSKAHDE NKFNCYLKRS KIGLPTEIQQ
Kalliklectin      IQYDGVD--F RGSDIRYVEM DDPDTCQKTC DEDSNCQFYT YVRNSSTAVV HRRRCYLKRV ITMPAPPRVS

               344                                 371
Human PK          GTQGSSG-YS LRLCNTGDNS V-CTTKTST- -R
Coelacanth PK     GVEATSG-FS LRLCRNKIPS VQCGQAVEFA TR
Kalliklectin      KLTNVVSGFS RRNCI
```

Supplemental Figure 7. High Molecular Weight Kininogens.

D5 domain His rich domain
D6 domain interacts with prekallikrein and Factor XI

Bradykinin-like motif is shown in red
*Amino acid numbering is for human factor XII.

```
                                    *1
Human               MKLITILFL---C SRLLLSLTQE -SQSEEIDCND KDLFKAVDAA LKKYNSQNQSN NQFVLYRITE
Dog                 MKLLAMLFL---C SRLLPSLTQE -SLSEEIDCND EDLFKAVDTA LKKYNSRNQSG NQFVLYRVTE
Siberian Tiger      MKLIAVLFL---C SRLLPSLTEE -SFSQDIDCND EDLFKAVDTA LKKYNSRNQSG NQFVLYRVTE
Grizzly Bear        MRLLALLFL---C SRLLPSVSQE -SSQE-IDCDD EDLFKAVDTA LKKYNSRNQIG NQFVLHRVTE
Hippopotamus        MKLITILFL---C SRLLPSLTQD -SLQE-IDCND QDVFEAVDTA LKKYNSGNKSG NQFVLYRVTE
BN Dolphin          MKLITILFL---C SRLLPSLTQD -SSQE-IDCND PDVFQAADTA LKKYNSGNKSG NQFVLYRVTE

Opossum             MKLAVVL-L-L-V TSQL-NVQGE -S--E-VSCQD NDVFRAMDAA LTEYNNQKTSG NQFVLHQIMA
Koala               MELAAIL-L-L-V A-IQLNVQVV -SQVKDVACDD NDVFQAVDVA LTKYNNQKSSG PQFVLYRIIT
Wombat              MELAVIL-L-L-V AS-QLNVQVE -SQVKDVACDD NDVFRAVDVA LTKYNNQKSSG PQFVLYRIIT

Platypus            MKLLGVL-L-F-L GSSLLPSRTA -PVPQDVDCND SDVFKAVDRA LRWYNEHLKDG NQFLLYRVTE

Kiwi                MKPFLAI--VL-C CSFFSSRATP -LPFEFSDCDD PDVLEAVDTA LKKYNGGRTTG NQFALYMVME
Pigeon              MKPFLVL--AL-C CSFLSSRATP -LPFEFLDCDD PDVFKAVDAA LQEYNGDRASG NQFALYTVVE
W-T Tropicbird      MKLFIVL--AL-C CSFFSSRATP -LPFEFSDCDD PDVFKAVDTA LKKYNGDRGTG NQFALYMVME

Alligator           M---------L-C CLFKSTND                                      DFMLL-----
Green Sea Turtle    MKLSTVL--VL-C CSFFSSRASP —LPTQDADCDD PDVFEAVDIA LRKYNGDKTDG NQFALYMVME
E Brown Snake       MEVFI-LLLLGIGFCQAARDKVDR ---------ND PEVVDAVAGA IAALNEDRSHG NKLALGAILH
Anolis Lizard       MELFILLVLTF-C C----KQAVP -LEGEDADCDS PDVFSAVDLA VKAHNEDQKHG NLFALRVILA

Xenopus leavis      MKHLFSF--IFF- LHLL--RG-S ASQTIEADCND HNIFYAVDEA LRHHNKELIDG NQFVLYRITE
Xenopus tropicalis  MQHLFTF  IVF- LHLL--RG-S ATQAIEADCND HNIFNAVDEA LRHHNRALTDG NKFVLYRITE
Nanorana parkeri    MVV------VVV- VGGDS AAVP V-PDIGVDCDD PNIFKAVDEA LRYYNDAKEDG NQFLLFRV--

Coelacanth          MKVLALV--LL-C TKLYVSWAK- -SLLQPADCGD PRVHQAVDAA IKKYNEELKDG HQFALYRITK

Zebrafish           MARDKILTVLA-M LWLYFCGGLA —QTDSSVPCDD RRVEKVVNLT LGTHNKMITEG AQLALYEIL-
Pufferfish          MRSGLGLCVLG-L LCLSSSVR-A -QEPVKVSCDD PSVEKAVSSA VEKFNEKLTTG NKLALFQIQ-
Elephant Shark      MKLFVVLLFSSQLLHSNARSVSDIDSVDPIPIDCDD PELLKAVDFT LRKFNGERRTT HQYALDRVS-

                   44
Human               ATKT-VGSDTF -Y-S-FKY-EIKEG -DCPVQSGKT WQDCEYKDAA KAATGECTAT VGKRSSTKFS
Dog                 GTRT-DDPDTF -Y-S-FKY-QIREG -NCSVQSGKT WQDCDYKEST QAATGECSAT VGKRGKTKFS
Siberian Tiger      VTRT-DDPETF -Y-S-FKY-QIREG -DCSVQSDKT WQDCDYKESA QAATGECSAT VGKRGNTKFS
Grizzly Bear        VVRT-DDPDTF -Y-S-FKY-QIREG -NCSAESGKT WQECDYKDAA QAATGECSAT VGKRGNTKFS
Hippopotamus        VTRM-DNPDTF -Y-S-FKY-QIKEG GDCPVQSNKT WQDCDYKDSA QAATGECTAT VAKRGNMKFS
BN Dolphin          VIRT-DDPDTF -Y-S-FKY-QIKEG -NCPVQSDKT WQDCDYNDSA QAATGECTAT VAKRGNMKFS

Opossum             VSLT-ESSQRT -F-T-VTY-NIQEG -DCHVRMGKN WKECGIKKDL NKERGQCTAI VKSHNENEFT
Koala               ASLT-DSSERT -F-T-ITY-EIRES -NCMIETGKN WKECSYKDSA EWKQGECTAI VKSQNGKEFK
Wombat              TASL-TDSNER -T-FTITYE-IRE- SNCMIETGKN WKECSYKDSA KWEQGECTAI LKSQNGKEFK

Platypus            ASMT-TDSDTF -Y-S-LKYQ-IREG -DCPVQKDKH WQDCDYREAA EAATGECTAT VKTKNKEKFT

Kiwi                GKKT-AGPDTQ -F-Y-VKYQ-IRET -SCAIEENKH WKDCDYKAPA EAKTGECTAR VHINKAEKTS
Pigeon              AKRT-VGPVTQ -F-H-VKYR-IRET -TCATEENKL WQDCDYKASA EAQTGECIAQ VHLNDAEKTS
W-T Tropicbird      AKKT-ASPDTQ -F-Y-VKYR-IQET -TCAIEENKL WQDCDYKVPS EAKTGECTAR VHMYNTEKTS

Alligator           FFSQ-AGPGAQ -F-F-VKYR-IRES -TCAIGEGKA WQDCDYNAAV EAETGECTAE VYIDKTQKIS
Green Sea Turtle    AKRI-EGSGKQ —F-F-VMYR-IRES -SCAVGGDKL WQDCDYRASA EAESGECTAQ VYVDKTEQIS
E Brown Snake       AYRI-ADPRKK —FLIIYHVR--ETV --CPIAVDKP WQKCELLRTS KAHSGKCTAN IDINESEQFT
Anolis Lizard       ARRT-AGPGKN -F-L-IKYQ-LAET -SCPLKGSVS WQNCEFLPPS EGDSGECTAE IHTDDSQVFS

Xenopus leavis      ARI-KTEN-GG -T-HNFVSYDIREG -SCGVKSGKV WQNCDFKQSD E-KVGKCSAH IVVNKELKTS
Xenopus tropicalis  AKI-KIENDSG -T-HNFVSYDIREG -SCGVKSGKV WQNCDFKQSD E-KVGKCSAH VLVNKELKSS
Nanorana parkeri    TDA-KQRNDEN GQIHYFLDYEIREG -SCTVKSMHS WQDCQFQAHT P-EQGKCSAH LLINTEKKIR

Coelacanth          AKTQ-LE-KE- ---THYFVTYEIRE STCSVHDNKI WQECNYVSPI SATTGTCIAE VYIDETVKTS

Zebrafish           ----EATKAQN ESGDVLLVRFSSRE TDCPAGGEKT WHECDYLQQA DKALRICHAK VQFTEAGEEL
Pufferfish          ----SASKTGS GADAVYSLQFTSRR SDCPAGGIKP WTDCDYLPRR —KSPVPCSAI VHVTATEVNT
Elephant Shark      -----FGTVQR KRGSRYFIKFDIQE SNCLVESEKT WTECDHRPPT VANIGHCESS VYIHRAGRIL
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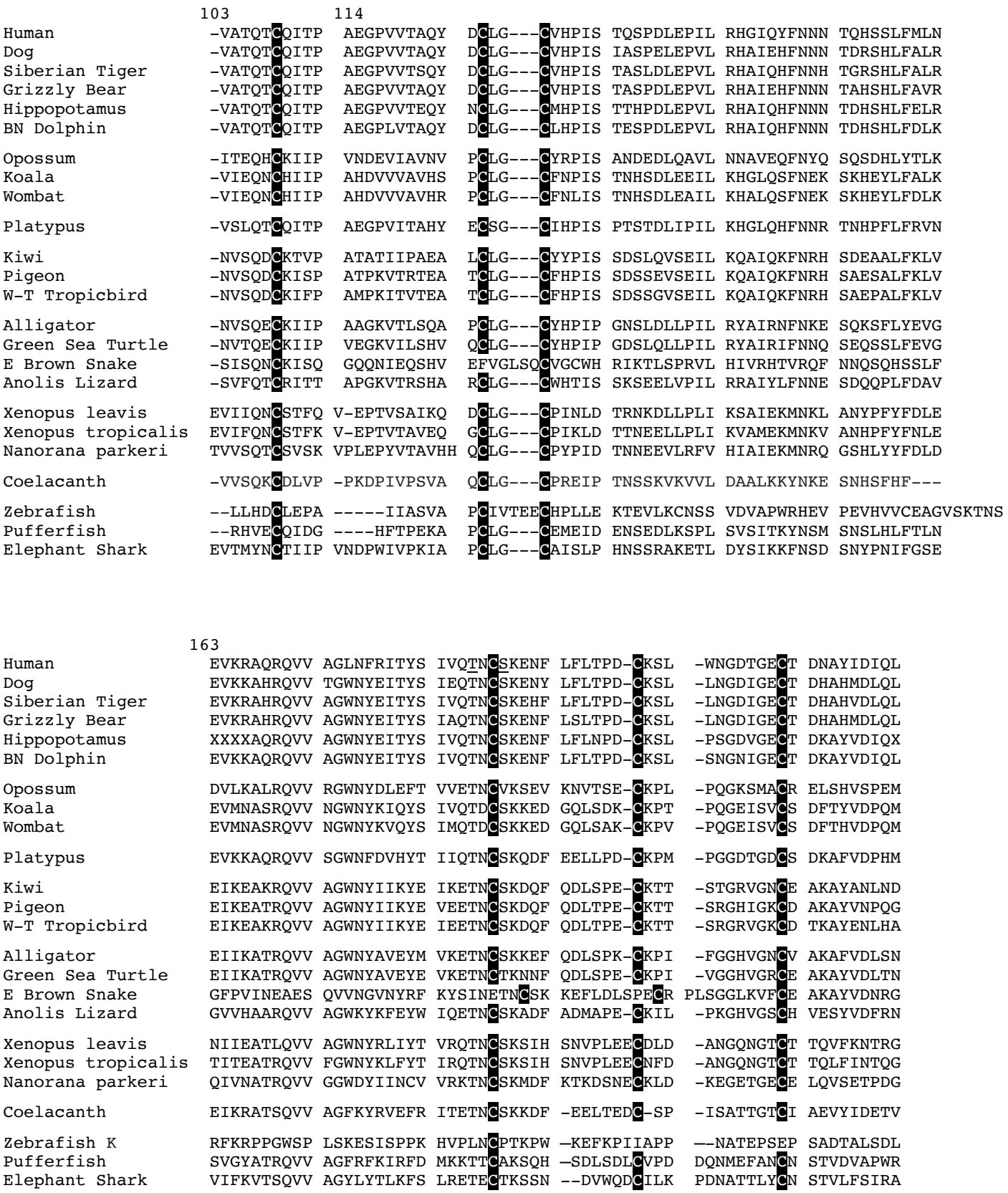
Supplemental Figure 7. High Molecular Weight Kininogens (continued).



Supplemental Figure 7. High Molecular Weight Kininogens (continued).

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                222            235
Human             R-IASFSQNCD IYPGKDFVQP PTKICVGCPRD IPTNSPELEE TLTHTITKL NAENNATFYF
Dog               R-IASFSQKCE LFPGEDFVQP PSRICLGCPKK IPVDSPELEV PLTHSIAKL NAENNGTFYF
Siberian Tiger    R-IASLSQKCE LYPGEDFIEP PPSICPGCPKE IPVNSPELEV ALNHSTAKL NAENNGTFYF
Grizzly Bear      R-IASFSQKCD LYPAEDFVQP -SKICSDCPKD IPVNSPELEV ALNHSIAKL NAENNGTFYF
Hippopotamus      R-IASFSQKCN VYPEEDFVAP PTRICAGCPIR IPVDSPELEE PLNHSIAKL NAENNGTFYF
BN Dolphin        R-IASFSQKCD LYPGEDFVQP PTRICAGCPIS IPVNSPELEE PLEHSIAKL NVENNGTFYF

Opossum           K-ISSHLQTCQ ——TEADS-QF     SD  MQY ISGYSPELKE SLRAALENF NSENESDFYF
Koala             K-ISIVSQACN --PGSDSASS VT-------QK MSVYSPELKE PLRHSLEKI NSENKNNFYF
Wombat            K-ISIVSQVCN ---------P RSDSGSSVTQK MSIYSPELNE PLRYSLEKI NSESKNNFYF

Platypus          K-ITGFVQNCE LFPGAEWIPP PDLMCAGCPQN LPVDSPELKE PLKHSLDKV NSADNYTFYF

Kiwi              Q-IIDTASQCK FPAEETVDPP -TLICAGCPRP IPKTSPELKE LLEVSMEKY NLETNDDFYY
Pigeon            Q-IVDIASQCK LPVEKTVNPD ---IRTGCTKT IPTDSPELKE LLKLSMEKY NSESNDDHYY
W-T Tropicbird    Q-IVDIASQCK LPVEDTVVPA -TR--TNCPKT IPKDSPELKE LLKVSMEKY NLESDDDFYY

Alligator         T-LVDVTQQCK FPVEETVPPP --QMCPGCPKR IPNDSPELKE VLKASMEKY NSESDDDFYY
Green Sea Turtle  T-IADVAQKCK FPVAETVSPH —ISICAGCPRP IPTNSTELEE PLRATLEKY NAESNDDFYY
E Brown Snake     T-LIHSEVECR PEAEDNMRIL —AQACPGCHSP LAPDSQELKR PLEAVVKLF NIKSSSDFYF
Anolis Lizard     T-IVNVEQKCE LEVDTKKN-- ----CPGCPRT IPSDSPQLKE PLAAIVENY NTKCSNGFLY

Xenopus leavis    E-IIDINLSCF SQ------KG FCLSCPDAVDV DDPELLDLLR QVMDEYNSY NNNTNLYNFV
Xenopus tropicalis E-IKDINLECF SQ------KG FCLSCPDAVDV DDPELLDLLR QVMDEYNSN NNNTNLYNFV
Nanorana parkeri  Q-VNDIILKCT SQAGVC-------LNCPLNVDS DDAELQNLLS QVIDEYNSN INVTNLHKLN

Coelacanth        KTSEVVSQKCD LVPPKDPIVP SVAQCLGCPRE IPTNSSKVKV VLDAALKKY NKESNHSFHF

Zebrafish         DLIR
Pufferfish        HELPQVQMECE EGMLIMPLIK RRPPGWTPLRK FEKPGSAAKE ESSEEDTAA AQPSASPVVD
Elephant Shark    NEIDTF-VSCS TDPIGFQMEG FRSQPTETIGI LAQQRQRHRQ HHFYQQQDS HEQHLINKSE



                282
Human             KIDNVKKARV QVVAGKKYFI DFVARETTCSK ESNEELTESC ETKKLGQSL DCNAEVYVVP
Dog               KIDSVQSATV QMVAGEKFFI QFVARETMCSK ESNEELAESC QINKYGEQL KCEAEVYVIP
Siberian Tiger    KIDHVKSATV QVVAGKKFSI EFTARETTCSK ESNEELTESC NTNKFGKIL DCKAEVYVIP
Grizzly Bear      KIDSVKSATV QMVAGKKFSI AFIARETTCSK ESNEELTESC QINKYGHTL ECKAEVFVIR
Hippopotamus      KIDMVQKATV QVVAGKKYSI AFTARETTCSK ESNEELTKSC EIHKHGRLL SCNADVYVVP
BN Dolphin        KIDTVEKATV QVVAGKKYSI VFTARETTCSK ESNEELTKSC EINKHSPIL NCKADVYVVP

Opossum           KPSILLKAHL -VEPGEKHSI EIQVQETECSK EKG-QFSEDC EFKTDGRVL QCIVQVPMGQ
Koala             KMETIEKAES PAGPGPKYII EFLIKETECSK EKD-KYSEDC AFKESGDGL KCIANVSVED
Wombat            KMDTIQKAES LVGPEPKYII EFLIKETECSK EKD-KYSEDC TFKELGDGL KCVANIPMED

Platypus          KVETIRKATF QLVAGQKFSI EFLVRQTRCSK EDNEKMPEDC EVDSNGKVL VCNAMVYMVP

Kiwi              KAGDIEKATV QVVAGKSYRI TFTVKKTNCSK KEFEKLNEDC EATPNSVRL KCEAQIYVIP
Pigeon            KSGDIEAAAV QVVSGKIYHL EFAVRKTNCSK KEFEKLHEDC EFTSDSAPL PCEAQIHVIP
W-T Tropicbird    K-GEIEEATV QVVAGQNYHL IFAVRKTNCSK KEFEKLNEDC EATSDSAPL PCEAHVHVIP

Alligator         KVESVFHSTV QVVAGKNYEI EFLIGKTNCSK SEVEKLNEDC KIVIPKISL QCTANIYVVP
Green Sea Turtle  KAEVILYATV QVVAGKNYNI RFKIRKTNCSK TDVKKLNEDC VTTTDSKPL LCTAQVYVIP
E Brown Snake     KIVDITKISG QMLVGHVYRI DFKAQRTNCSK AEVEKPDKNC HAVKGGELM TCHALIYVKP
Anolis Lizard     RITKVTKATV QIVSGIMYRI EFQITETNCSN AEVHELNEDC IAMENSESL QCYGSAWEKP

Xenopus leavis    SVDYASKKGV HEKTYDVTFN ---IKETNCSK SDYAILGEEC QFIETKNAL NCDAKVNVTD
Xenopus tropicalis SVNQASKKGT HEKTYDVKFN ---IQETNCSK SDYSILGEEC EFIETKEAL NCDAKVNITD
Nanorana parkeri  QVIKATKHGF QEQIYEVLFS ---MMPTVCSK PDHTILGDEC NNLENASPL SCDTTIKVTD

Coelacanth        GVVEIKRATS QVVAGFKYRV EFRITETNCSK KDFEELTEDC AISEK-NPH NCNSATTVVP

Zebrafish         VVPDDPLHCP SK
Pufferfish        DPLHCPSKVV PD
Elephant Shark    PAVITTSAPL ELPLSAIDQL ADLLGPEPPVN CPGKPWKPL QSN
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Supplemental Figure 7. High Molecular Weight Kininogens (continued).

```
                342                 358                         384
Human             -WEKKIYPTVN --CQPLGMIS-LM KRPPGFSPFRS SRIGEIKEET TVSPPHTSM --APAQDEERDS
Dog               -WEKKIYPTVN --CQSLGKVI-LM RRPPGFSPFRS SFMEKTEKGT TVSSPHNSM --VPVQDEEWDS
Siberian Tiger    -WEKKIYPTVH --CQSRGETT-LM KRPPGFSPFRS VQVEKTKEGT TVSPPHTSM --ASVQDEEQDS
Grizzly Bear      -WEEKIYPTVN --CQPLKKII-LM KRPPGFSPFRS VPLEKTEEGT TVSSPHISM --APVPDEERDS
Hippopotamus      -WEEKIYPTVN --CQPLGQTS-LM KRPPGFSPFRS VQVEKTKEGT TVSPPHPSM --APEQDEERDS
BN Dolphin        -WEKKIYPTVN --CQSLGQTT-LM KRPAGFSPFRS VPVEKTKEGT IVSPPHTFL --APVQDEERDS

Opossum           DGEVKPVI--D --CHEPPPELGLM KRPSGFSPFRS AL--RILEEK IIAAREPQN FNTTEQEEEQTP
Koala             --EGTFNPTVR --CEHPTEM--LM KRPPGFSPFRA AAVIPEMEGA EAPSEPQTS –DMTDQEEAQGP
Wombat            --EGTFNPTVH --CEQPTEM--LM KRPPGFSPFRA AALIPETNGA EAPSEPQTS –DTTDQEEAQ-P

Platypus          -WKNEVFPTVT --CQDLEMSSFL- KRPPGFSPFRS VQT-PAKEGS NVSPPQPPK –APDREEE-QA-

Kiwi              -WENKILPQVN --CTEELLPVFLA RRPPGFTPFRT AQYFAQSQPD TTS------ -SNKNETES
Pigeon            -WENKILPQVN --CSIERSAAVLL RRPPGFTPFRS --FVALGQPN ENSCSDQNE KEMQRPGEETG
W-T Tropicbird    -WENKIFPQVN --CSKERSMTVLL RRPPGFTPFRS FAMLSQPS-E IL-CSDKNE EERQTPGKEMR

Alligator         -WKQEIFPQVN --CSEVTPIIQAR RP-PGFTPFRS L-MLHEIYPQ TSPLQTAEE GKDPDKGPRENLGPGLENE
Green Sea Turtle  -WTKTIRPKVS --CAEEENLLMRR PPGFT--PFRS LAVEAKTVQY T-PQIKNEK GPREGQGRGKGSGK
E Brown Snake     -WEPSVVPEVT --CTDDQPFQAHE LEEPNILEDGF NIFHDYEEQK WFLWYLLGRY TLSVILKLF
Anolis Lizard     -WQPKSEVEVT FKCMEKAFNTALL RRPPGFTPFRS AAMATEEN-- TQVCGHRH- GHKNGHNKTKPKSSEDLQE

Xenopus leavis    -TKIIVASSPI --CNARARTMEFL NQFLAKMGDNT IFVVYSAMFT YKGLSPFRG VLPQGSPYRI
Xenopus tropicalis -TKITVASSPM --CIHRVRST--- -------PFFS ---------- YKGLSPFRM VLPQANPSNK
Nanorana parkeri  -KRINVHSGPV --CVEQQA----- ---------- ------LIMR LSGLSPLRM SKKPDQAETN

Coelacanth        -WKNTTTTDVN --CVL---EMALL RLPAGMSPFRV LQATPDSAK- --------- ----------

Zebrafish
Pufferfish
Elephant Shark
```

```
                402
Human             GKEQGHTRRH -DWGHEKQRKH NLGHGHKHE RDQGHGHQRG HGLGHGHEQQ HGLGHGHKFKL
Dog               GKEQGPTHGH -GRGHEKQIKH GHKYKHDQG YGHNRGHGLG HGHQKQHGLG HGGQRELDFDL
Siberian Tiger    GKEQGPTRGH -GWGHGKQIKH GFGHGHKHE HDQGHRHNRG RGLGHGHQKQ HGFAHGHQKQH GFGHGHQQ
Grizzly Bear      GKEQGPTRGH -GWGHEKQIKH GFGHGHKPE HDQGRGYHGG RGLGHGYQKQ HGLGHGHQREH
Hippopotamus      GKEQGPNRGH -GWGHGNQIKH GLGHGHKXX XXXXXXXXXX XXXXXXXXXX XXXXXXXXXX
BN Dolphin        GKEQGPTHRH -GWGHGKQIKH GLGHGHKHE HDQGHGHQKG HDLGHGHQRG HGHQGGHGLAH

Opossum           GK-AGYP--H -DHGHGWQRGR HPVHGTKNH PGSGLGHKHG HNHSHGRHRG HDLG-------
Koala             GKEQGLTRSF -GPGH--QKEH NSGHGHK-- ------DHRG LGLGHKHEHA H----------
Wombat            GKEKGLIR-- ---------- SFGHGHRKE HSSGHGHKDH PGLGLGHK---HGH--------

Platypus          GKRRGHPLGH -DSCRGRGPGQ GRGRGCKRA RD-------------------------------

Kiwi              QTPSTETR-- ---------- ---------- KDHGHGPEGE GEPGCKHRHK HGCKHGHGFKK
Pigeon            KDGGQEPE-- ---------- ---GEGEPE HKHGHKHEHK HGHKHEHKHE HKHEKDHKPDK
W-T Tropicbird    KDGGQEPEGK GEPEHKHRLMX GRKHRHGYI KDHESDKRHR HEIGCGHRTG YGHGHKKHSKN

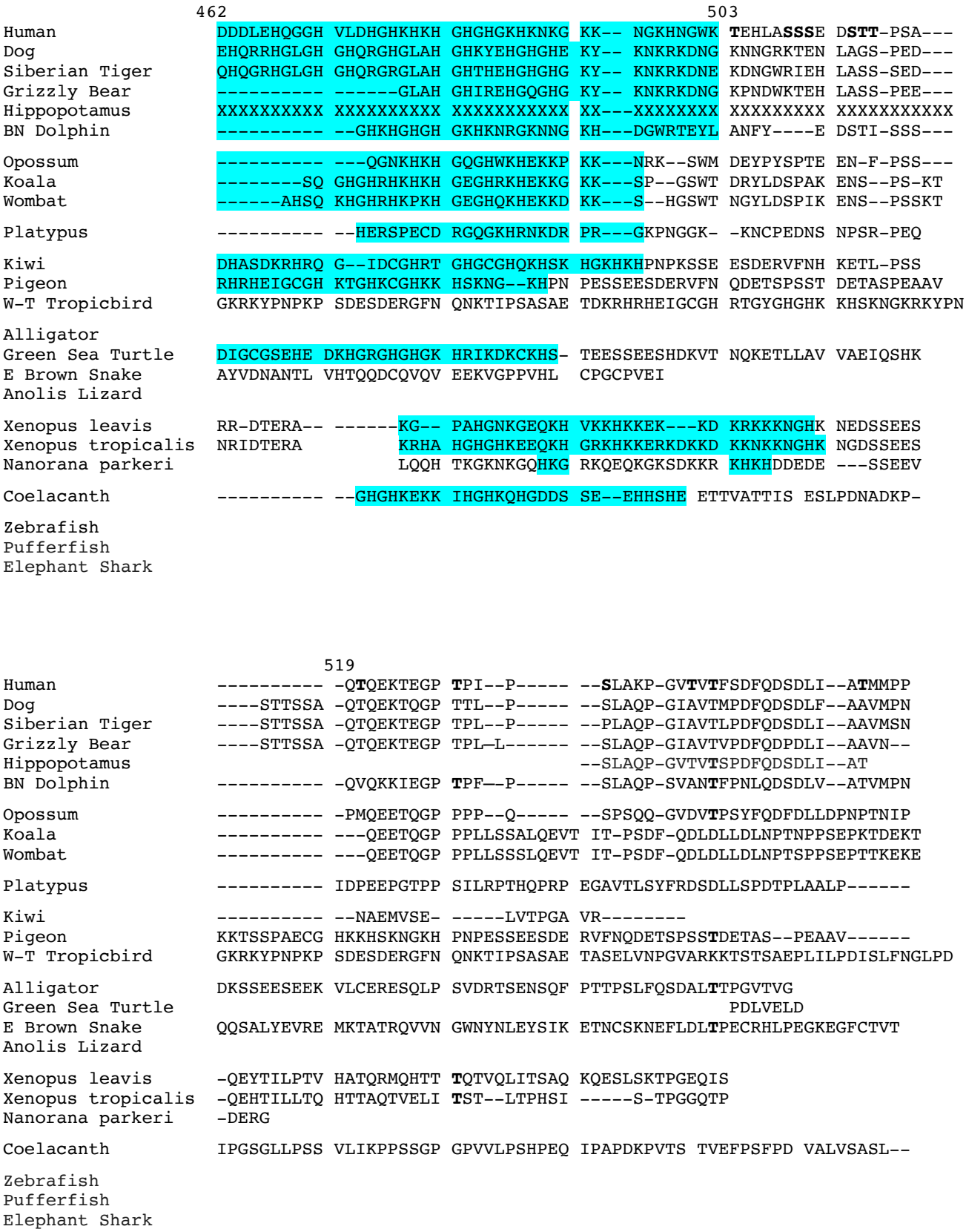
Alligator         DGQGDGCDHR VGHGNGHGHGQ GHRFNHKPG HGRGRGRGHD IGRGHKKHQK KDKHKDSK
Green Sea Turtle  DIRHEPA-HK QGHRHDIGHGP EHDHRRGHE DEHGCRHDIG HEPEHEHRRG HGHKDEHRCRH
E Brown Snake     LSFPCSSEAQ RSFSH
Anolis Lizard     DADKHDESLP TASTSNPLKLA MEVFILLVL SVGLCQAGPV QDDVSCDDPE XVFEAVARAIT

Xenopus leavis
Xenopus tropicalis
Nanorana parkeri

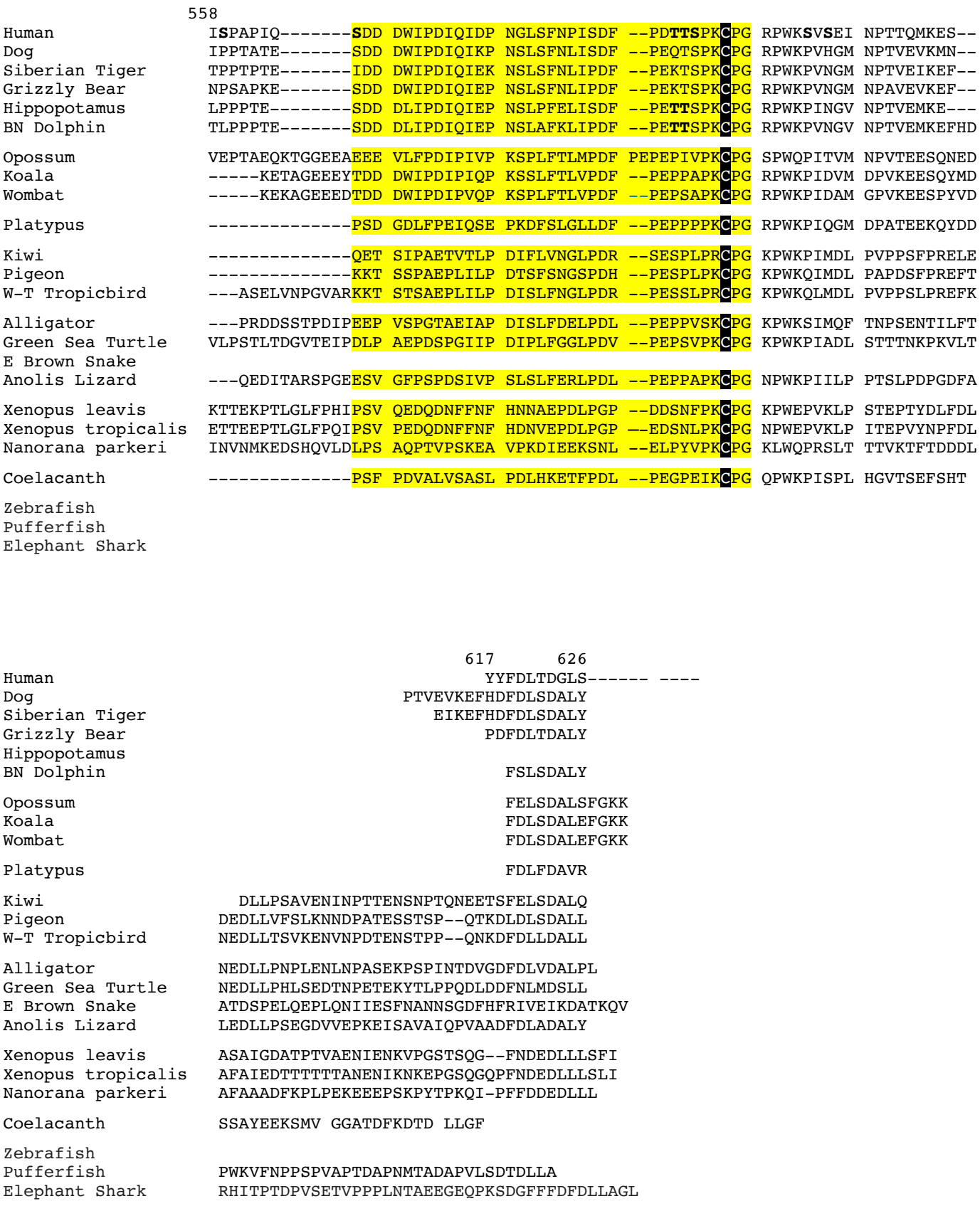
Coelacanth        -KEEKE---- ---TELRRT- ---------DD KPHGHERGRG RGRGQEHGR GPGHEH----

Zebrafish
Pufferfish
Elephant Shark
```

Supplemental Figure 7. High Molecular Weight Kininogens (continued).



Supplemental Figure 7. High Molecular Weight Kininogens (continued).



Supplemental Figure 8. Comparison of Human Factor XII and Human Pro-HGFA

Blue Highlight indicates residues of the catalytic triad
Residue 353 is the arginine immediately preceding the activation cleavage site of Factor XII.
*Amino acid numbering is for human factor XII

```
Factor XII                                -----  ----------  ----------  ----------  ----------
Pro-HGFA                                  QPGGN RTESPEPNAT ATPAIPTILV TSVTSETPAT SAPEAEGPQS

              *1
Factor XII     IPPWEAPKEH KYKAEEHTVV LTVTGEPCHF PFQYHRQLYH KCTHKGRPGP QPWCATTPNF DQDQRWGYCL
Pro-HGFA       GGLPPPPRAV PSSSSPQAQA LTEDGRPCRF PFRYGGRMLH ACTSEGSAHR K-WCATTHNY DRDRAWGYCV

              71
Factor XII     E--PKKVK-- --DHCSKHSP CQKGGTCVNM PSGPH--CLC PQHLTGNHCQ KEKCFEPQLL RFFHKNEIWY
Pro-HGFA       EATPPPGGPA ALDPCASG-P CLNGGSCSNT QDPQSYHCSC PRAFTGKDCG TEKCFDETRY EYLEGGDRWA

             133
Factor XII     RTEQAAVARC QCKGPDAHCQ RLASQACRTN PCLHGGRCLE VEGHRL--CH CPVGYTGAFC DVDTKASCYD
Pro-HGFA       RVRQGHVEQC ECFGGRTWCE GTRHTACLSS PCLNGGTCHL IVATGTTVCA CPPGFAGRLC NIEPDERCFL

             201
Factor XII     GRGLSYRGLA RTTLSGAPCQ PWASEATYRN VTAEQ--ARN W-GLGGHAFC RNPDNDIRPW CFVLNRDRLS
Pro-HGFA       GNGTGYRGVA STSASGLSCL AWNSDLLYQE LHVDSVGAAA LLGLGPHAYC RNPDNDERPW CYVVKDSALS

             268
Factor XII     WEYCDLAQCQ TPTQAAPPTP VSPRLHVPLM PAQPAPPKPQ PTTRTPPQSQ TPGALPAKRE QPPSLTRNGP
Pro-HGFA       WEYCRLEACE SLTRVQLSPD LLATLPEPAS PGRQA-----  ----------  ----------  ----------

             338             353                                       393
Factor XII     LSCGQRLRKS LSSMTRVVGG LVALRGAHPY IAALYWGHSF CAGSLIAPCW VLTAAHCLQD RPAPEDLTVV
Pro-HGFA       --CGRRHKKR TFLRPRIIGG SSSLPGSHPW LAAIYIGDSF CAGSLVHTCW VVSAAHCFSH SPPRDSVSVV

             408                                  442
Factor XII     LGQERRNHSC EPCQTLAVRS YRLHEAFSPV SYQHDLALLR LQEDADGSCA LLSPYVQPVC LPSGAARPSE
Pro-HGFA       LGQHFFNRTT DVTQTFGIEK YIPYTLYSVF NPSDHDLVLI RLKKKGDRCA TRSQFVQPIC LPEPGSTFPA

             478                                                                    544
Factor XII     TTLCQVAGWG HQFEGAEEYA SFLQEAQVPF LSLERCSAPD VHGSSILPGM LCAGFLEGGT DACQGDSGGP
Pro-HGFA       GHKCQIAGWG HLDENVSGYS SSLREALVPL VADHKCSSPE VYGADISPNM LCAGYFDCKS DACQGDSGGP

             548                                                 596
Factor XII     LVCEDQAAER RLTLQGIISW GSGCGDRNKP GVYTDVAYYL AWIREHTVS
Pro-HGFA       LACEKNGVAY ---LYGIISW GDGCGRLHKP GVYTRVANYV DWINDRIRPPRR LVAPS
```

Supplemental Figure 9. Comparison of Human Factor XII with Lungfish Factor XII and Lungfish Pro-HGFA

```
H-Factor XII - human factor XII
L-Factor XII - Lungfish factor XII
L-Pro-HGFA   - Lungfish Pro-HGFA

Blue Highlight indicates residues of the catalytic triad
Residue 353 is the arginine immediately preceding the activation cleavage site in human FXII.
X indicates missing sequence
*Amino acid numbering is for human factor XII.

                                    *1
H-Factor XII                         IPPWEAPKEH KYKAEEHTVV LTVTGEPCHF PFQYHRQLYH KCTHKGRPGP
L-Factor XII                         HNKWHKGEHE KHHIRSHFVV MTESGLPCKF PFRYQGRMVF TCLPARG---
L-Pro-HGFA    MLKICPYSVF LTKMDLWGKH QN-----MNN SEDMNSVYQV LTEGGQPCVF PFRYGGKLHY SCISNKV---

             51
H-Factor XII  QPWCATTPNF DQDQRWGYCL EPKKVKDHCS KHSPCQKGGT CVNMPSGPHC LCPQHLTGNH CQKEKCFEPQ
L-Factor XII  -PWCATTNNY DRDRQYSFCK DRQITTDFCA -SNPCQNGGT CQSNSKGYEC KCTPQYTGRD CQKEKCYEPR
L-Pro-HGFA    --WCATTANY DRDHKKGHCI IDPVIIDPCL -QNPCQNGGT CAYNEQTYFC LCTELFMGRN CEKEKCFDES

            121
H-Factor XII  LLRFFHKNEI WYRTEQAAVA RCQCKGPDAH CQRLASQACR TNPCLHGGRC LEVEGHRLCH CPVGYTGAFC
L-Factor XII  LLKYFDLQET WLRPGIVKHE ECQCKDSKIV CNPVPLTVCA RNPCMNGGIC FEGKKIQVCS CPKKFVGESC
L-Pro-HGFA    RYKYFDIGET WGRIWQMNVE QCTCNEKGIA CSRIKFTGCL ENVCSNNGTC RQITKEKVCS CRGNYAGHHC

            191
H-Factor XII  DVDTKASCYD G--RGLSYRG LARTTLSGAP CQPWASEATY RNVTAEQARN WGLGGHAFCR NPDNDIRPWC
L-Factor XII  GIDLSASCYE G--NGNLYRG TVKVTTSGNP CMSWDSNLLY NEIYSQEALQ QGLGRHSYCR NPDNDTKPWC
L-Pro-HGFA    NINLTHNCFD YDNNGTEYRG IVNKTVSGTD CIHWNSEILH EEFHLADAEK LGLGEHNFCR NPDADHQPWC

            259
H-Factor XII  FVLNRDRLSW EYCDLAQCQT PTQAAPPTPV SPRLH--VPL MPAQPAPPKP QPTTRTPPQS QTPGALPAKR
L-Factor XII  YTLKDNIVSW DYCAVSNCIP SVTLQPPVTT ASAIQSTLPP VPSELIPPQP SEVTSVMPEP ITTPSYSSTP
L-Pro-HGFA    YTMKENHLAW DYCAIPYCGR LTGRMVPSP- ---------- -PSLSALQKP QSTTQTPLRP LTSGWWCSPP

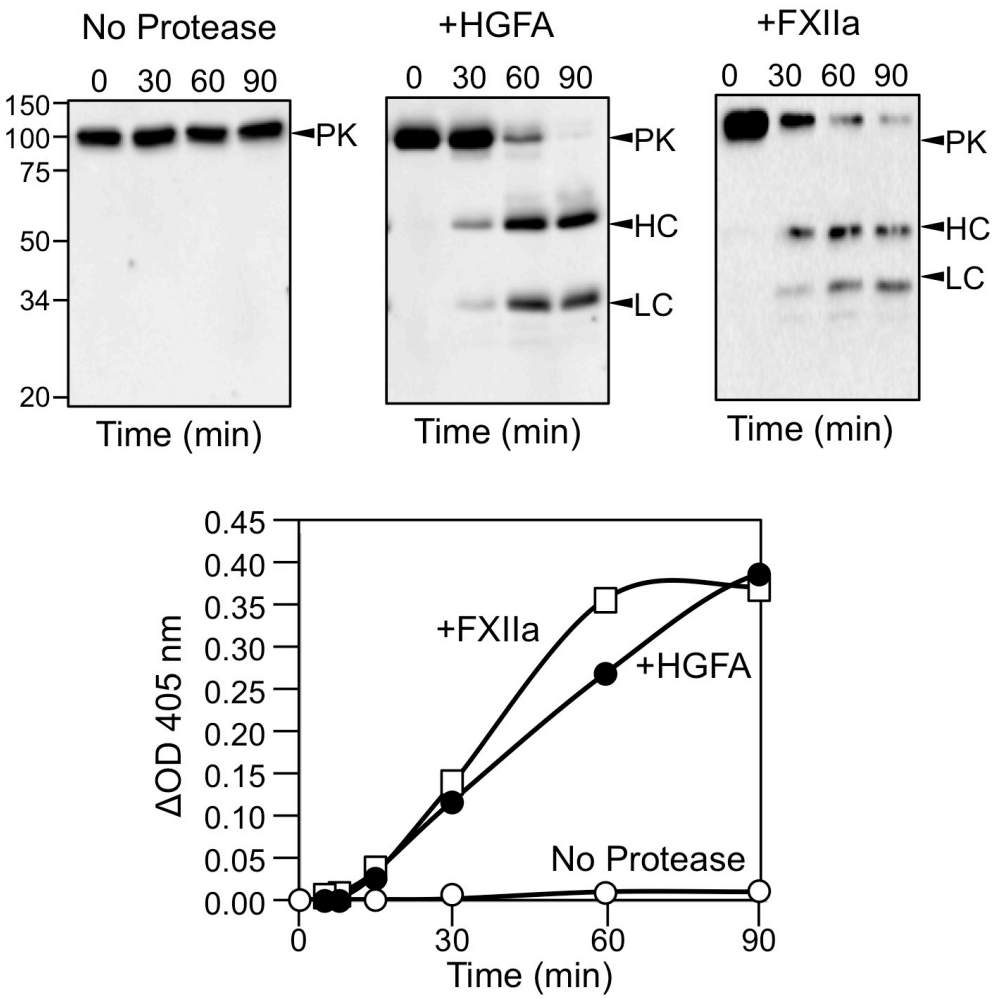
            327                         353                                 393
H-Factor XII  EQPPSLTRNG PLSCGQRLRK SLSSMTRVVG GLVALRGAHP YIAALYWGHS FCAGSLIAPC WVLTAAHCLQ
L-Factor XII  ----GEVSIL PDNCGVRHKK RFLLMSRIVG GMVALPASHP YIAAVYMATD FCGGSLISPC WVLTAAHCLA
L-Pro-HGFA    EQRTP-KHPS LANCGKRHQK R-KNKSRILG GISSLPGSHP WLAAIYIGKD FCAGSLIRPC WVLSAAHCFA

            397                                         442
H-Factor XII  DRPAPEDLTV VLGQERRNHS CEPCQTLAVR SYRLHEAFSP VSYQ-HDLAL LRLQEDADGS CALLSPYVQP
L-Factor XII  KRPLVSQMHV VLGQEQFNRS NENSITFEVQ SYIVHDNFDY STFN-NDIAL IQLRK-KDGR CAPVSNFIQP
L-Pro-HGFA    NSPLKSMIKV VLGQYLFNDT SSDTQSFEVE KYIFHDRFSA FNPTEYDIVL IRLKK-KNEK CVVASQFIRT

            466
H-Factor XII  VCLPSGAARP SETTLCQVAG WGHQFEGAEE YASFLQEAQV PFLSLERCSA PDVHGSSILP GMLCAGFLEG
L-Factor XII  ICLPEPYTNP PANINCEVAG WGHTHEEAEE YAQNLQEASI PIITEIECNA PEAYASKVTE NMICAGFLEG
L-Pro-HGFA    VCLPSSDMTF PEGQMCEIAG WXXXXXXXXX XXXXXXXXXX XXXXXXXXXX XXXXXXXXXX XXXXXXXXXX

            536     544                                                 596
H-Factor XII  GTDACQGDSG GPLVCEDQAA ERRLTLQGII SWGSGCGDRN KPGVYTDVAY YLAWIREHTV S
L-Factor XII  QTDSCQGDSG GPLVCEVN-- -GRMYLYGIV SWGDGCANIN RPGVYTKVSQ YIDWIKLKLK L
L-Pro-HGFA    XXXXXXXXXX XXXXXXXXXX XXXXXXXXXX XXXXXXXXXX XXXXXXXXXX XXXXXXXXXX X
```

Supplemental Figure 10. Prekallikrein Activation by Factor XIIa and Hepatocyte Growth Factor Activator.
Western blots (top row). PK (200nM) was incubated at 37ºC with vehicle, or with 25nM HGFA or fXIIa, in the presence of 10 μg/ml dextran sulfate (500 kDa). Aliquots were removed at various times into reducing SDS-sample buffer, size-fractionated by SDS-PAGE, and transferred to nitrocellulose. Western blots were developed with HRP-conjugated goat anti-human PK IgG. Markers at the left of each image show the locations of standard for PK and the heavy chain (HC) and light chain (LC) of PKa. Positions of molecular weight standards for the left and center panels are shown on the left of the image. The image on the right is of a gel that was run at a different time from gels in the other two panels.
Chromogenic assay (bottom row). Cleavage of the chromogenic substrate S-2302 (0.5 mM) by products from reactions similar to those in the western blots were followed by recording changes in ΔOD405 nm on a microplate spectrophotometer.



Supplemental Figure 11. Comparison of Human Prekallikrein and Factor XI

Blue Highlight indicates residues of the catalytic triad
Residues indicate sequence required to form the factor IX-binding exosite.
Residue 369 is the arginine immediately preceding the activation cleavage site in human FXI.
*Amino Acid numbering is for human factor XI

```
             *1
Prekallikrein GCLTQLYENA FFRGGDVASM YTPNAQYCQM RCTFHPRCLL FSFLPASSIN DMEKRFGCFL KDSVTGTLPK
Factor XI     ECVTQLLKDT CFEGGDITTV FTPSAKYCQV VCTYHPRCLL FTFTAESPSE DPTRWFTCVL KDSVTETLPR

             71
Prekallikrein VHRTGAVSGH SLKQCGHQIS ACHRDIYKGV DMRGVNFNVS KVSSVEECQK RCTNNIRCQF FSYATQTFHK
Factor XI     VNRTAAISGY SFKQCSHQIS ACNKDIYVDL DMKGINYNSS VAKSAQECQE RCTDDVHCHF FTYATRQFPS

            141
Prekallikrein AEYRNNCLLK YSPGGTPTAI KVLSNVESGF SLKPCALSEI GCHMNIFQHL AFSDVDVARV LTPDAFVCRT
Factor XI     LEHRNICLLK HTQTGTPTRI TKLDKVVSGF SLKSCALSNL ACIRDIFPNT VFADSNIDSV MAPDAFVCGR

            211
Prekallikrein ICTYHPNCLF FTFYTNVWKI ESQRNVCLLK TSESGTPSSS TPQENTISGY SLLTCKRTLP EPCHSKIYPG
Factor XI     ICTHHPGCLF FTFFSQEWPK ESQRNLCLLK TSESGLPSTR IKKSKALSGF SLQSCRHSIP VFCHSSFYHD

            281                                          321  326
Prekallikrein VDFGGEELNV TFVKGVNVCQ ETCTKMIRCQ FFTYSLLPED CKEEKCKCFL RLSMDGSPTR IAYGTQGSSG
Factor XI     TDFLGEELDI VAAKSHEACQ KLCTNAVRCQ FFTYTPAQAS CNEGKGKCYL KLSSNGSPTK ILHGRGGISG

            351                   371                                              415
Prekallikrein YSLRLCNTGD NSVCTTKTST RIVGGTNSSW GEWPWQVSLQ VKLTAQRHLC GGSLIGHQWV LTAAHCFDGL
Factor XI     YTLRLCKMD- -NECTTKIKP RIVGGTASVR GEWPWQVTLH TTSPTQRHLC GGSIIGNQWI LTAAHCFYGV

            421                                         464
Prekallikrein PLQDVWRIYS GILNLSDITK DTPFSQIKEI IIHQNYKVSE GNHDIALIKL QAPLNYTEFQ KPICLPSKGD
Factor XI     ESPKILRVYS GILNQSEIKE DTSFFGVQEI IIHDQYKMAE SGYDIALLKL ETTVNYTDSQ RPICLPSKGD

            491                                                                  559
Prekallikrein TSTIYTNCWV TGWGFSKEKG EIQNILQKVN IPLVTNEECQ KRYQDYKITQ RMVCAGYKEG GKDACKGDSG
Factor XI     RNVIYTDCWV TGWGYRKLRD KIQNTLQKAK IPLVTNEECQ KRYRGHKITH KMICAGYREG GKDACKGDSG

            561                                                        619
Prekallikrein GPLVCKHNGM WRLVGITSWG EGCARREQPG VYTKVAEYMD WILEKTQSSD GKAQMQSPA
Factor XI     GPLSCKHNEV WHLVGITSWG EGCAQRERPG VYTNVVEYVD WILEKTQAV
```

Supplemental Figure 12. Comparison of Human and Platypus Prekallikrein.

H-Prekallikrein – human prekallikrein
P-Prekallikrein – platypus prekallikrein

Blue Highlight indicates residues of the catalytic triad
Residue 371 is the arginine immediately preceding the activation cleavage site in human PK.
*Amino Acid numbering is for human prekallikrein.

```
                *1
H-Prekallikrein  GCLTQLYENA FFRGGDVASM YTPNAQYCQM RCTFHPRCLL FSFLPASSIN DMEKRFGCFL KDSVTGTLPK
P-Prekallikrein  DCVTRLQEDI FFKGGDLAAF FAPSADHCRL LCTFHPRCLL FSFLPAHTLQ DESRWFRCYL KDSVTETLPR

                71
H-Prekallikrein  VHRTGAVSGH SLKQCGHQIS ACHRDIYKGV DMRGVNFNVS KVSSVEECQK RCTNNIRCQF FSYATQTFHK
P-Prekallikrein  VTVAGAVSGH SFKHCGHLIS LCSREVHPGL DMRGTNHNRS WARSEDECQR RCTDDARCQF FTFATQRFHS

               141
H-Prekallikrein  AEYRNNCLLK YSPGGTPTAI KVLSNVESGF SLKPCALSEI GCHMNIFQHL AFSDVDVARV LTPDAFVCRT
P-Prekallikrein  AANRNACLLK HSSTGTPTTI KMMDGVVSGF SLKACALSHL GCTRDIFQDM AFSDDDVAKM VTPDAFVCQT

               211
H-Prekallikrein  ICTYHPNCLF FTFYTNVWKI ESQRNVCLLK TSESGTPSSS TPQENTISGY SLLTCKRTLP EPCHSKIYPG
P-Prekallikrein  ACTYHPSCLF FTFHTNAWTP EAQRNICLLK TSQSGSPSSS LPTPHAVSGY SLLACQGPLP ETCHRKVYPG

               281                                          321  326
H-Prekallikrein  VDFGGEELNV TFVKGVNVCQ ETCTKMIRCQ FFTYSLLPED CKEEKCKCFL RLSMDGSPTR IAYGTQGSSG
P-Prekallikrein  MAFEGDKLRQ VLVSGVDACQ KNCTDTLRCQ FFTYASLPTE CQGDRCECSL MMSSDGAPSK VVPGVGRASG

               351                   371                                            415
H-Prekallikrein  YSLRLCNTGD NSVCTTKTST RIVGGTNSSW GEWPWQVSLQ VKLTAQRHLC GGSLIGHQWV LTAAHCFDGL
P-Prekallikrein  YSLRLCRTGV GPVCSTKTNV RVVGGTKSAP GEWPWQVSLH VKKSTQHLLC GGSIIGPRWI LTAAHCFDGL

               421                                        464
H-Prekallikrein  PLQDVWRIYS GILNLSDITK DTPFSQIKEI IIHQNYKVSE GNHDIALIKL QAPLNYTEFQ KPICLPSKGD
P-Prekallikrein  NLPALWRVYG GILNQSTIDE NTPFSRVQEI IIHSQYKVLN SGHDIALMKL ESPLNFTDLQ RPICLPTPED

               491                                                                  559
H-Prekallikrein  TSTIYTNCWV TGWGFSKEKG EIQNILQKVN IPLVTNEECQ KRYQDYKITQ RMVCAGYKEG GKDACKGDSG
P-Prekallikrein  TGVTLANCWV TGWGYSRENG EVQAILQKAK IPVISNLECQ ERYPQHKVTS GMVCAGYKDG GKDACKGDSG

               561                                                  619
H-Prekallikrein  GPLVCKHNGM WRLVGITSWG EGCARREQPG VYTKVAEYMD WILEKTQSSD GKAQMQSPA
P-Prekallikrein  GPLACKHHGV WHLTGVTSWG EGCARKDHPG VYTRVAEYVA WIQENTQTRD EPASPELPT
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Supplemental Figure 13. Comparison of Human and Monotreme Factor XI.

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H-Factor XI - human factor XI
P-Factor XI - platypus factor XI
E-Factor XI - echidna factor XI

Blue Highlight indicates residues of the catalytic triad
Residues required for forming the factor IX-binding exosite
Residue 369 is the arginine immediately preceding the activation cleavage site in human FXI.
X indicates missing sequence
*Amino Acid numbering is for human factor XI

           *1
H-Factor XI ECVTQLLKDT CFEGGDITTV FTPSAKYCQV VCTYHPRCLL FTFTAESPSE DPTRWFTCVL KDSVTETLPR
P-Factor XI ECMTRMYNDT YFQGGYLRTV FSPNVQHCQL VCTLHPRCLL FSFLPGRLTP DPAKRFACFL KDSESEMLPK
E-Factor XI ECVTQIYNDT YFQGGDLRTV FSPNVQHCQL TCTLHPRCLL FSFLPGRLTP XXXXXXXXXX KDSKSETLPK

           71
H-Factor XI VNRTAAISGY SFKQCSHQIS -ACNKDIYVD LDMKGINYNS SVAKSAQECQ ERCTDDVHCH FFTYATRQFP
P-Factor XI VTVAGAVSGH SWKQCHHHIT -ACLKDVFPG LDMRGTNHDA GPAQNWRECQ ARCTNDAHCH FFTFATSAFH
E-Factor XI VTIAGAVSGH SWKQCRHHIS GACVKDVFPG LDMRGNNHDG GPAQSWQECQ AXXXXXXXXX XXXXXXXXX

          140
H-Factor XI SLEHRNICLL KHTQTGTPTR ITKLDKVVSG FSLKSCALSN LACIRDIFPN TVFADSNIDS VMAPDAFVCG
P-Factor XI STAHRNTCLL KHSATGAPTS ITILEHVLSG FSLKPCALSK MACLRDIFSE TAFADNDTAR AVAPDAFVCR
E-Factor XI XXXXRNTCLL KHSATGAPTS ITILEHVLSG FSLKPCGLSK XACLRDIFSE TAFADNETAR AVAPDAFVCR

          210
H-Factor XI RICTHHPGCL FFTFFSQEWP KESQRNLCLL KTSESGLPST RIKKSKALSG FSLQSCRHSI PVFCHSSFYH
P-Factor XI NLCTHHPSCL FFTFYSQEWP DPSQRNLCLL KSSASGIPTS RLSRERAYSG FSLRSCRHGV PIFCHPSLYS
E-Factor XI GLCTHHPACL FFTFYSQEWP DPSQRNLCLL KSSASGIPTA RLSRERAYSG RSLRSCRHGV PXXXXXXXXX

          280                                        321
H-Factor XI DTDFLGEELD IVAAKSHEAC QKLCTNAVRC QFFTYTPAQA SCNEGKGKCY LKLSSNGSPT KILHGRGGIS
P-Factor XI DTDFLGLELD VAYANGPAAC QKLCTDVARC QFFTHSPLHQ ADNPRRGKCS LKMSSNGSPS KIVYGRGGIS
E-Factor XI XTDFLGSELD VAYANGPAAC QKLCTDVDRC PFGIASACSR LQSPRRGKCS LKMSSDGSPS KIVYGRGGIS
E-FXI Var                                          ADNPGRGKC

          350                 369                                                413
H-Factor XI GYTLRLCKMD NECTTKIKPR IVGGTASVRG EWPWQVTLHT TSPTQRHLCG G--SIIGNQW ILTAAHCFYG
P-Factor XI GYTLRLCQMD NVCMTKIRSR VVGGVRSARG EWPWQVSLQV VQPRQKHLCG G--SIIGDSW ILTAAHCLDR
E-Factor XI GYTLRLCQMD NXCMTKIRSR VVGGEGSVRG EWPWQVSLQV VQPRQRHLCG GGESIIGNSW VLTAAHCFSR

          418                                        462
H-Factor XI VESPKILRVY SGILNQSEIK EDTSFFGVQE IIIHDQYKMA ESGYDIALLK LETTVNYTDS QRPICLPSKG
P-Factor XI VVTLEELRVY AGFLNQSEIR QGTPFSRVQK AIIHRQYQSA EFGFDIALLK LAAPISFTDI QRPICLPPEG
E-Factor XI LNLPALWRVY GGILNQSTID ENTPFSRVQE IIIHSQYKVL NSGHDIALMQ LESPLNFTGR QRPICLPPEG

          488                                                              557
H-Factor XI DRNVIYTDCW VTGWGYRKLR DKIQNTLQKA KIPLVTNEEC QKRYRGHKIT HKMICAGYRE GGKDACKGDS
P-Factor XI DPTLAFSNCW VTGWGYGRED GEIQNTLQKV SVPLVANEEC QAWYNPNRIT DHMVCAGSEE GDRDTCKGDS
E-Factor XI DPTLAFSNCW VTGWGYGKED GEVQAILQKA KIPVISNLEC QERYPQHKIT GGMVCAGYKD GGKDACKGDS

          558                                            607
H-Factor XI GGPLSCKHNE VWHLVGITSW GEGCAQRERP GVYTNVVEYV DWILEKTQAV
P-Factor XI GGPLACEDHG VWYLVGITSW GEGCGRRTRP GVYTRVSGFY NWILESTLA
E-Factor XI GGPLACEDHG VWYLVGVTSW GEGCARRDKP GVYTNVVEYV DWILEKTQ
```

Supplemental Figure 14. Comparisons of Factor XI A4 and Activation Cleavage Site Sequences.

Blue Highlight indicates residues of the catalytic triad
Residue 369 is the arginine preceding the activation cleavage site in human factor XI.
P Proline at residue 368 (human numbering system) consistent with thrombin cleavage site.
Residues of the hydrophobic interaction involved in FXI dimerization.
X indicates missing sequence
*Amino Acid numbering is for human factor XI.

```
                *273                                                  321     326
Human           CHSSFYHDTD FLGEELDIVA AKSHEACQKL CTNAVRCQFF TYTPAQASCN EG--KGKCYL KLSSNGSPTK
Mouse           CHPSFYNDTD FLGEELDIVD VKGQETCQKT CTNNARCQFF TYYPSHRLCN ERNRRGRCYL KLSSNGSPTR
White Foot Mouse CHPSFYNDTD FLGEELDIVD VRGHETCQKM CTDAIRCQFF TYSPSRGSCN EGNHRGRCYL KLSPNGSPTR
Deer Mouse      CHPSFYNDTD FLGEELDIVD VKGHETCQKM CTDAIRCQFF TYSPSRGSCN EG--KGRCYL KLSPNGSPTR
Rabbit          CHSSFYYDTD FLGEELDIVD VKGHEACQKM CTSAIRCQFF TYSSSQESHN KG--KGTCYL KLSSNGSPTK
Dog             CHSSFYHNTD FLGEELDIVD VKGHEACQKM CTDAIRCQFF TYSPSPESCH GG--KGKCYL KLSSNGSPTK
Red Fox         CHSSFYHNTD FLGEELDIVD AKGHEACQKM CTDAIRCQFF TYSPSLESCH GG--KGKCYL KLSSNGSPTK
Horse           CHPSFYHDTD FLGEELDIVD MKGHEACQKM CTDTSRCQFF TYSPPHESCN GG--KGKCYL KLSSNGSPTK
Prezwalski Horse CHPSFYHDTD FLGEELDIVD MKGHEACQKM CTDTSRCQFF TYSPPHESCN GG--KGKCYL KLSSNGSPTK
Yak             CHSSFYRNTD FLGEELDIVD ADSHEACQKT CTNSIRCQFF TYSPSQESCN GG--KGKCYL KLSANGSPTK
Cattle          CHSSFYRNTD FLGEELDIVD ADSHEACQKT CTNSIRCQFF TYSPSQESCN GG--KGKCYL KLSANGSPTK
Giant Panda     CHSSFYHNTD FLGEELDIVD AKGHEDCQKM CTDTIRCQFF TYSPSPESCN GG--KGKCYL KLSSNGSPTK
Grizzly Bear    CHSSFYHNTD FLGEELDIVD AKGHEDCQKM CTDTIRCQFF TYSPSPESCN GG--KGKCYL KLSSNGSPTK
Polar Bear      CHSSFYHNTD FLGEELDIVD AKGHEDCQKM CTDTIRCQFF TYSPSPESCN GG--KGKCYL KLSSNGSPTK
Cheetah         CHSSFYHDTD FLGEELDIVD VKGHEACQKM CTDSIRCQFF TYSPSPESCN GG--KGKCYL KLSSNGSPTK
Dromedary       CHSSFYHDTD FLGEELDIVD VPGHEACQKA CTNTIRCQFF TYSPSQESCN GE--GGKCYL KLSSNGSPTK
Beaver          CHSSFYHDTD FLGEELDIVD VKGHEVCQKL CTSSIRCQFF TYSQSQESYK ER--KGKCYL KLSSNGSPTK
Guinea Pig      CPSSFYRDTD FLGEELDIVD VQGHEACQKM CTNAIRCQFF TYLPPQESCN ER--KGKCYL KLSSNGSPTK
Rhinoceros      CHPSFYRDTD FLGEELDIVD TKGHEACQKM CTNTVRCQFF TYSPPQESCK AG--KGKCYL KLSSNGSPTK
Star Nosed Mole CHPSFYHDSD FLGEELDIVD VQGHEACQKM CTNTLRCQFF TYTPSQESCN GG--KGKCYL KLSSDGSPTK
Chinese Hamster CHPSFHNDTD FLGEELDIVE VKDHETCQKM CTNAVRCQFF TYSPSQGSCN EG--KGRCYL KLSSNGSPTR
Otter           CHSSFYHDTD FLGEELDIVD AKGHEACQKM CTDAIRCQFF TYSPSPESCN EG--KGKCYL KLSSNGSPTK
Naked Mole Rat  CHSSFYLDTD FLGEELDIAD VKGHEACQKM CTNAIRCQFF TYSPPQESCN ER--KGKCYL KLSSNGSPTK
Weddell Seal    CHSSFYHDTD FLGEELDIVD VKGHEACQKM CTDTIRCQFF TYSPSPESCN GG--KGKCYL KLSSNGSPTK
Sea Lion        CHSSFYHDTD FLGEELDIVD AKGHEACQKM CTDTIRCQFF TYSPSPESCN GG--KGKCYL KLSSNGSPTK
Elephant        CHSSFYPDTD FLGEELDIVD VEGREACQKV CTDTLRCQFF TYSPSQQPSN KG--KGKCYL KLSLNGSPTK
Lynx            CHSSFYHDTD FLGEELDIVD VKGHEACQKM CTDSIRCQFF TYSPSPESCN GG--KGKCYL KLSSNGSPTK
Pangolin        CHPSFYHNAD FLGEELDIVE VKGHEACQKA CTNTIRCQFF TYSPSQESCN GW--EGKCYL KLSANGSPTK
Groundhog       CHSSFYHDTD FLGEELDIVD VKGHEACQKM CTDVIRCQFF TYSPPQESCH EK--KGKCYL KLSSNGSPTK
Spalax          CHSSFYRDTD FLGEELDIID VKDHEACQKM CTNTIRCQFF TYSPSQDSCN ER--KGKCYL KLSSNGSPTR
Degu            CHSSFYRNTD FLGEELDIVD VEDHEACQKL CTKAIRCQFF TYLPPQESCK ER--KGRCYL KLSSNGSPTK
Walrus          CHSSFYHDTD FLGEELDIVD AKGHEACQKM CTDTIRCQFF TYSPSPESCN GG--KGKCYL KLSSNGSPTK
White Tail Deer CHSSFYRNAD FLGEELDIVH VDGHEACQKT CTNSIRCQFF SYSPSQESCN GG--KGKCYL KLSANGSPTK
Aardvark        CHSSFYHNID FLGEELDIVD VEGHEACQKM CTNAVRCQYF TYSPPQEPCN KR--KGKCYL KFSVNGSPTK
Tiger           CHSSFYHDTD FLGEELDIVD VKGHEVCQKM CTDSIRCQFF TYSPSPESCN GG--KGKCYL KLSSNGSPTK
Domestic Cat    CHSSFYHDTD FLGEELDIVD VKGHEVCQKM CTDSIRCQFF TYSPSPESCN GG--KGKCYL KLSSNGSPTK
Shrew           CHSSFYHDTD FLGEELDILD VKGHEACQKI CTNTVRCQFF TYSPIQKHCK EG--KGKCYL KLSSNGSPTK
Meerkat         CHPSFYHNTD FLGEELDIVD VKGHGACQKM CTSSIRCQFF TYSPSPESCN GG--KGKCYL KLSSNGSPMK
Wild Boar       CHSSFYHDTE FLGEELDIVG ENGHEACQKT CTNTIRCQFF TYSPSQESCN GG--KGKCYL KLSSNGSPTK
Squirrel        CHSSFYHDTD FLGEELDIVD VKGREACQKM CTDVIRCQFF TYSPPQESCH EK--KGKCYL KLSSNGSPTK

Mink Whale      CHSSFYRDTD FLGEELDIVD VDGHEACQKT CTNSIRCQFF TYSPSQESCN GG--KGKCYL KLSANGSPTK
Fin Whale       CHSSFYRDTD FLGEELDIVD VDGHEACQKT CTNSIRCQFF TYSPSQESCN GG--KGKCYL KLSANGSPTK
Beluga Whale    CHSSFYRDTD FLGEELDIVD VDGHEACQKT CTNSIRCQFF TYSPSQEPCN GG--KGKCYL KLSANGSPTK
Pilot Whale     CHSSFYRDTD FLGEELDIVD VDGHEACQKT CTNSIRCQFF TYSPSQEPCN GG--KGKCYL KLSANGSPTK
Pacif. WS-Dolphin CHSSFYRDTD FLGEELDIVD VDGHEACQKT CTNSIRCQFF TYSPSQEPCN GG--KGKCYL KLSANGSPTK
Baiji           CHSSFYRDTD FLGEELDIVD VDGHEACQKT CTNSIRCQFF TYSPSQESCN GG--KGKCYL KLSANGSPTK
F'less Porpoise CHSSFYRDTD FLGEELDIVD VDGHEACQKT CTNSIRCQFF TYSPSQEPSN GG--KGKCYL KLSANGSPTK
Killer Whale    CHSSFYRDTD FLGEELDIVD VDGHEACQKT CTNSIRCQFF TYSPSQEPCN GG--KGKCYL KLSANGSPTK
Sei Whale       CHSSFYRDTD FLGEELDIVD VDGHEACQKT CTNSIRCQFF TYSPSQESCN GG--KGKCYL KLSANGSPTK
IPH Dolphine    CHSSFYRDTD FLGEELDIVD VDGHEACQKT CTNSIRCQFF TYSPSQEPCN GG--KGKCYL KLSANGSPTK
Bot.Nose Dolphin CHSSFYHDTD FLGEELDIVD VDGHEACQKT CTNSIRCQFF TYSPSQEPCN GG--KGKCYL KLSANGSPTK

Opossum        CHASIYYNTD FLGEELDIFE VKDHSACQES CTNTIRCQFF TYSPPGETCN KE--KGKCYL KMSSNGSPSK
Koala          CHGTIYANTD FLGEELDIFE VEGHKACQER CTDTIXCQFF TYSPTKETYN KG--KGKCYL KMSLNGSPNK
Wombat         CHGSIYTNTD FLGEELDIFE VKGHKACQER CTDTIRCQFF TFSPTKETYN KG--KGKCYL KMSLNGSPTK
Tasmanian Devil CHGSVYSNTD FLGEELDIFE VKDHTTCQER CTDTIRCQFF TYSPSGETYN KG--KGKCYL KMSSNGSPIK

Platypus       CHPSLYSDTD FLGLELDVAY ANGPAACQKL CTDVARCQFF THSPLHQADN PR--RGKCSL KMSSNGSPSK
Echidna        XXXXXXXXTD FLGSELDVAY ANGPAACQKL CTDVDRCPFG IASACSRLQS PR--RGKCSL KMSSDGSPSK
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Supplemental Figure 14. Comparison of Factor XI A4 and Activation Cleavage Site Sequences (continued).

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                343                                 368                                 399
Human             ILHGRGGISG YTLRLCKMDN ---- ECTTKIKPRI VGGTASVRGE WPWQVTLHTT SPTQRHLCG
Mouse             ILHGRGGISG YSLRLCKMDN ---- VCTTKINPRV VGGAASVHGE WPWQVTLHIS ---QGHLCG
White Foot Mouse  ILHGRGGISG YTLRLCKMDN ---- VCTTKIKPRI VGGTASVHGE WPWQVTLHIS SPVQGHLCG
Deer Mouse        ILHGRGGISG YTLRLCKMDN ---- VCTTKIKPRI VGGTASVHGE WPWQVTLHIT SPVQGHLCG
Rabbit            ILHGRGGISG YTLRLCKMDN ---- VCTTKIKPRI VGGSASLPGE WPWQVTLHTV SPTQRHLCG
Dog               ILHGRGGISG YTLRLCKMDN ---- VCTTKIKPRI VGGTASVHGE WPWQITLHTT SPIRRHLCG
Red Fox           ILHGRGGISG YTLRLCKMDN ---- VCTTKIKPRI VGGTASVHGE WPWQITLHTT SPIRRHLCG
Horse             ILYGRGGISG YTLRLCKMDN ---- ACTTKIKPRV VGGRASVPGE WPWQITLHII SPTQKHLCG
Prezwalski Horse  ILYGRGGISG YTLRLCKMDN ---- ACTTKIKPRV VGGRASVPGE WPWQITLHII SPTQKHLCG
Yak               ILHGTGSISG YTLRLCKMDN ---- VCTTKIKTRI VGGTRSVHGE WPWQITLHVT SPTQRHLCG
Cattle            ILHGTGSISG YTLRLCKMDN ---- VCTTKIKTRI VGGTQSVHGE WPWQITLHVT SPTQRHLCG
Giant Panda       ILHGRGGISG YTLRLCKMDN ---- ECTTKIKPRI VGGTASVHGE WPWQITLHTT SPTQRHLCG
Grizzly Bear      ILHGRGGISG YTLRLCKMDN ---- ECTTKIKPRI VGGTASVHGE WPWQITLHTT SPTQRHLCG
Polar Bear        ILHGRGGISG YTLRLCKMDN ---- ECTTKIKPRI VGGTASVHGE WPWQITLHTT SPTQRHLCG
Cheetah           ILHGRGGISG YTLRLCKMDN ---- VCTTKIKPRI VGGEASVHGE WPWQITLHIT SPAQRHLCG
Dromedary         ILRGRGSISG YTLRLCKMDN ---- ACTTKIKPRI VGGTESVHGE WPWQITLHIT SPTQRHLCG
Beaver            ILHGRGGISG YTLRLCKMDN ---- VCTTKIKPRI IGGSPSVHGE WPWQVTLHIT SPMQRHLCG
Guinea Pig        ILRGRGSISG YTLRLCKMDN ---- VCTTKIKARI VGGTVSLRGE WPWQITLHIT KPIQRHLCG
Rhinoceros        ILHGRGGISG YTLRLCKMDN ---- ACTTKIKPRV VGGRASVSGE WPWQITLHVT SPTQRHLCG
Star Nosed Mole   ILHGRGGISG YTLRLCKMDN ---- VCTTKVKSRI VGGTASGFGE WPWQVTLHIS SPTQRHLCG
Chinese Hamster   ILHGQGGISG YTLRLCKMDN ---- VCTTKIKPRV VGGTASVHGE WPWQVSLHIT SPTQGHLCG
Otter             ILHGRGGISG YTLRLCKMDN ---- VCTTKIKPRI VGGTASVHGE WPWQITLQIT SPSQRHLCG
Naked Mole Rat    ILHGRGGISG YTLRLCKMDN ---- ICTTKIKPRI VGGTVSVRGE WPWQITLHIT APSRGHLCG
Weddell Seal      ILHGRGGISG YTLRLCKMDN ---- ECTTKIKPRI VGGTASVPGE WPWQITLHIT SPTQRHLCG
Sea Lion          ILHGRGGISG YTLRLCKMDN 18-- ECTTKIKPRI VGGTASVPGE WPWQITLHIT SPTQRHLCG
Elephant          ILHGRGGISG YTLRLCKMDN ---- VCTNRINARI VGGTASVYSE WPWQITLHTT SPTQRHLCG
Lynx              ILHGRGGISG YTLRLCKMDN ---- VCTTKIKPRI VGGEASVHGE WPWQITLHIT SPAQRHLCG
Pangolin          ILYGRGGISG YTLRLCKMDN ---- ECTTKIKPRI VGGTGSAPGE WPWQVTLHTM EPTQRHLCG
Groundhog         ILHGRGGISG YTLRLCKMDN ---- ECTTKIKPRI VGGTASVQGE WPWQVTLHVI SPIQRHLCG
Spalax            ILHGRGGISG YTLRLCKMDN ---- ECTTKIKSRI VGGTASVHGE WPWQVSLHTT SPTQRHLCG
Degu              ILHGTGSISG YTLRLCEMDN ---- ICTTKIKPRI VGGTLSVRGE WPWQITLHIT APIRGHLCG
Walrus            ILHGRGGISG YTLRLCKMDN ---- ECTTKIKPRI VGGTASVPGE WPWQITLHIT SPTQRHLCG
White Tail Deer   ILHGTGSISG YTLRLCKMDN ---- VCTTKIKARI VGGTQSVHGE WPWQITLHVI SPTQRHLCG
Aardvark          ILHGRGGISG YTLRLCKMDN ---- VCTTKINPRI VGGTASVYGE WPWQITLHIT SPTQRHLCG
Tiger             ILHGRGGISG YTLRLCKMDN ---- VCTTKIKPRI VGGEASVHGE WPWQITLHIT SPAQRHLCG
Domestic Cat      ILHGRGGISG YTLRLCKMDN ---- VCTTKIKPRI VGGEASVHGE WPWQITLHIT SPAQRHLCG
Shrew             ILHGRGGISG YTLRLCKMDN ---- ECTTKIKPRI VGGTASVSGE WPWQITLHIT SPTQRHLCG
Meerkat           ILHGRGGISG YTLRLCKMDN GDKS TCTTKIKPRI VGGEASVHGE WPWQVTLHIT SPTRRHLCG
Wild Boar         ILHGRGSISG YTLRLCKMDN ---- ACTTKIKPRI VGGTKSVLGE WPWQITVHIT SPTQRHLCG
Squirrel          ILHGRGGISG YTLRLCKMDN ---- ECTTKIKPRI VGGTASVQGE WPWQVTLHVM XPIQRHLCG

Mink Whale        ILNGRGNISG YTLRLCKMDN ---- ACTTKIKPRI VGGTKSVLGE WPWQITLHIT SPTQRHLCG
Fin Whale         ILHGRGNISG YTLRLCKMDN ---- ACTTKIKPRI VGGTKSVLGE WPWQITLHIT SPTQRHLCG
Beluga Whale      ILHGRGNISG YTLRLCKMDN ---- ACTTKIKPRI VGGTKSVLGE WPWQITLYIT SPTQRHLCG
Pilot Whale       ILHGRGNISG YTLRLCKMDN ---- ACTTKIKPRI VGGTKSVLGE WPWQITLYIT SPTQRHLCG
Pacif. WS-Dolphin ILHGRGNISG YTLRLCKMDN ---- ACTTKIKPRI VGGTKSVLGE WPWQITLYIT SPTQRHLCG
Baiji             ILHGRGNISG YTLRLCKMDN ---- ACTTKIKPRI VGGTKSVLGE WPWQITLYIT SPTQRHLCG
F'less Porpoise   ILHGRGNISG YTLRLCKMDN ---- ACTTKIKPRI VGGTKSVLGE WPWQITLYIT SPTQRHLCG
Killer Whale      ILHGRGNISG YTLRLCKMDN ---- ACTTKIKPRI VGGTKSVLGE WPWQITLYIT SPTQRHLCG
Sei Whale         ILHGRGNISG YTLRLCKMDN ---- ACTTKIKPRI VGGTKSVLGE WPWQITLHIT SPTQRHLCG
IPH Dolphine      ILHGRGNISG YTLRLCKMDN ---- ACTTKIKPRI VGGTKSVLGE WPWQITLYIT SPTQRHLCG
Bot.Nose Dolphin  ILHGRGNISG YTLRLCKMDN ---- ACTTKIKPRI VGGTKSVLGE WPWQITLYIT SPTQRHLCG

Opossum           IIHGKGGISG YTLRLCKMEN ---- ACTNKIKGKI VGGTKSVLAE WPWQVSLHIT SPIQKHLCG
Koala             ILHGRGGISD YTLRLCKMEN ---- ACTNKIKAKI VGGTNSVLAE WPWQVSLYVT SPIQKHLCG
Wombat            ILHGRGGISG YTLRLCKMEN ---- ACTNKIKAKI VGGTNSVLAE WPWQVSLHVT SPIQKHLCG
Tasmanian Devil   ILHGRGGVSG YTLRLCKMEN ---- ACTNKIKAKI VGGTNSVLAE WPWQISLHVT FPIQKHLCG

Platypus          IVYGRGGISG YTLRLCQMDN ---- VCMTKIRSRV VGGVRSARGE WPWQVSLQVV QPRQKHLCG
Echidna           IVYGRGGISG YTLRLCQMDN ---- XCMTKIRSRV VGGEGSVRGE WPWQVSLQVV QPRQRHLCG
```

Supplemental Figure 15. Specific Activities of Factor XI/Prekallikrein Chimeras. Human fXI-deficient plasma (30 µl) was mixed with 30 µl of Tris-buffered saline containing recombinant wild type fXI (FXI-WT, △), fXI with an A3 domain from platypus fXI (fXI-*Plat*XIA3, ◇), fXI with an A3 domain from human PK (fXI-PKA3, □), and fXI with an A3 domain from platypus PK (fXI-*Plat*PKA3, ○) at various concentrations (listed at the bottom of the graph), and 30 µl of silica-based aPTT reagent. After incubation for 5 min at 37 °C, 30 µl of 25 mM $CaCl_2$ was added and time to clot formation was recorded on an ST4 coagulation analyzer (Diagnostica Stago). Results of clotting time in seconds are plotted against recombinant fXI concentration on a log-log plot. The specific activity of fXI-WT (200 units/mg) was arbitrarily assigned a value of 100% for the purpose of comparison to the other recombinant proteins. Estimated specific activities were determined by drawing a line for the results for 1.25 µg/ml of recombinant protein horizontally to the point where it intersects the curve for fXI-WT.