

File S1. Primer sets used in this study

Paralog	Fwd primer	Primer set	RC primer (5'-3')	Primer set	genomic size	Specific in genomic DNA?
a1p	CATGATGTCCAACATATTTCTTTATG	2004F	GCAAGTAACCGCAAGTGGC	2004R	1027 bp	Y
a2 (Abph)	CAGAAGAGTATGTTAATTATGTGGAG	2008F	AAAGATGTAGCCATCAACATAACGG	2008R	934 bp	Y
a3	CTGCAAAAATCAAGCAATGTG	2009q2 F	TAGCGGGCTGGCTTCTATT	2009q2 R	876 bp	N
a4p	GCTTGCTGGTCTGTGGTTC	2008F	CTTAGACACCTGGAGGGAAGGGA	2008R	1269 bp	-
a5p	GCTGTGGTATTCTAGGTGCT	2009q2 F	CTGGGCAATGGCACAAT	2009q2 R	184 bp	N
a6p	TTGCCAGCTATAAAAAGAGGATA	2008F	GGCTTCTATTTTTCTGCGT	2008R	981 bp	Y
a7_29	ACCTTGACAGAGGAAGACAAGATAC	2008q1 F	CAAAGTTGTAGCCATCAACATAGC	2008q1 R	867 bp	Y
a8_13p	TCCTCGGGCTGCCCTGT	2008F	CATAGTCTTATGAAAAAGAATAATAC	2008R	1235 bp	-
a9_14_16p	CAGCTATAAAAAGAGGATGTTTGCTC	2008q1 F	CCATGACATAGCAGCCTGT	2008q1 R	992 bp	Y
a10_15_17	CCTGCTCCTCTCTTTCAT	2008F	AAATGGCTTCTTGAGAGCAG	2008R	1176 bp	Y
a11_18	GGTTGTTATTACCGTGTGGGT		CACCTGGAGAGAAGGACTGT	2008R	1200 bp	Y
a12	GAAACAATACAAGATGACCCTGTAAC	2008q1 F	CCTAGAGGAAAAGACTATAATCTGCC	2013R	1002 bp	Y
a19	ATGAAGCTTGTGTGTGCTG	2013F	CATAGTGGGCTGGCTTCTATC	2013R	1228 bp	N
a20	ACCTTGACAGAGGAAGACAAGATAC	2008q1 F	CCAAAATTGTAGCCATCAACATATC	2008q1 R	873 bp	Y
a21p	AGGATGTTACCTATTTTTAAACAG	2008F	GCTGGCTGGCTTCTATTTTTTC	2008R	968 bp	Y
a22p	GCTGTGGTATCCTCAGGC	2008F	TCATCTATGCAGATGAGCAGAA	2008R	1225 bp	Y
a23p	CTGGTGCTTTGATGATCCTTGA	2008F	GTGGCCATCAACATGCCGTG	2008R	1171 bp	Y
a24	GATGTTACATATTCTTTCACAGG	2004F	AAGATGCAACATCAACATGCTGGT	2004R	987 bp	Y
a25p	GCTGCACTGCTCCTGACTG	2008F	TGACTTCTTCTAGAGCTTCT	2008R	1135 bp	Y
a26p	AAGATATGTTGAGTACCTGAAAC	2008F	CTGGTGAGCAGGCAATGG	2008R	988 bp	Y
a27	ACAGAGAAAAGTTGATTTTTGAATG	2004F	GGAGGCAATTGGTTTCCG	2004R	1019 bp	Y
a28p	GAGGAGGTTCTGCTATTTTTAAACG	2004F	AAGTTAGAGCCATCAACATAGTGT	2004R	981 bp	Y
a30p	AACAATAAATAATGACCCTCTCG	2004F	ACTGGTGAGCAGTAAGTGGC	2004R	968 bp	Y

Paralog	Fwd primer	Primer set	RC primer (5'-3')	Primer set	genomic size	Specific in genomic DNA?
bg1	GGGTGCTCTCTGGATACA	2008F (old bg1)	CAAGACTTCTTGGTATAATGACT	2008R (old bg1)	1203 bp	Y
bg2 (Abpe)	TTTGGGTATTCTCTGGAAAA	2008F (old bg2)	AGAATGTTCTTCAAGACTTCTTTG	2008R (old bg2)	1213 bp	Y
bg3p	TTGAGCACTGTCTGGAAAA	2008F (old bg12)	CATCCATTCTTGGTAATCACAC	2008R (old bg12)	1211 bp	Y
bg4p	AAACATACCTTGGAAACGCAAAA	2009q2 F	GCCCTTCTATAGTGTGACTGG	2009q2 R	1065 bp	N
bg5p	GGGCTGCTATAGAGAGGAAAGAT	2009q2 F	CGGTGTCCTTCTATAGTGTGC	2009q2 R	1092 bp	N
bg6p	GGACACTTCTTCTGCTGACCTT	2008q1 F	GTCTGGAGTTCTGATACAATCTTTTC	2008q1 R	594 bp	Y
bg7	GCAATTTTTCTTTCTTTGAAGC	2013F	CCATAGTATGACAGGCATTGAGG	2009q2 R	1212 bp	Y
bg8_13p	TTAATGGTTTATAAAGAAGAGGAGCTT	2008q1 F	TCCTCTGTAGCAGCCACAGA	2008q1 R	712 bp	N
bg9_14_16p	GGGCTGCTATAGAGAGGAAAGAT	2009q2 F	CGGTGTCCTTCTATAGTGTGC	2009q2 R	1103 bp	N
bg10_15_17p	TAAAGGTGGCCTTGGAAAA	2009q2 F	GCTGGCAATCATGGCTTC	2009q2 R	1089 bp	Y
bg11_18	GGAGAAGTGGCTTCCAGACAT	2008q1 F	CTTGATAGCCTTCAAAGAAAAGA	2008q1 R	523 bp	Y
bg12	ACTCTTACCATGAAGGGGAT	2013F	GAAATAAAGTCTAAAATGGATCTAAAATTGTC	2013R	1779 bp	Y
bg19	CAGGGTTGCTACAGAGAGGAA	bg12/19 2009q2F	AGGTTGGAAAATAAAGTCTAAAATGGA	bg12/19 2009q2R	1125 bp	Y
bg20 (Abpd)	TGTCTTCTTTCTCGAAGGC	2013F	CCATAGTATGACAGGCATTGAGG	bg7 2009q2R	1207 bp	Y
bg21	TGCAAGTGTGCTCTGGAAGT	2008F (old bg5)	GTCCTAATAGCTTCAAAGAAGG	2008R (old bg5)	1237 bp	Y
bg22p		-		-	-	-
bg23p		-		-	-	-
bg24 (Abpz)	GCTGGTGTATCTCAGGAAGCA	2008F (old bg8)	CGTAAAGGTTTCTCGGAATAGT	2008R (old bg8)	1202 bp	Y
bg25p	GCAGTACTCTGGACTTAAGAT	2008F (old bg9)	CCATGCTTGATAATAGAAGCC	2008R (old bg9)	didn't show up	Y
bg26	CGGAGCAATACTACTAAGG	2008F (old bg10)	CTTTAAGAGGTCATTGCCATAGT	2008R (old bg10)	1217 bp	Y
bg27	TAAAATACTGGGTGAAAATAGGC	2008F (old bg11)	CCGCCATTTTGTACAGAAATCT	2008R (old bg11)	1208 bp	Y
bg28	CAAACCTTGGAAACCCAAAATTA	2009q2 F	AGTCCCTTTATAGTGTGACTG	2009q2 R	1041 bp	Y
bg29_31_32p	CACTGTGAGGAGCATTTAGCACT	2008q1 F	ATAGCCTTTGAAGTAAGGAACACATT	2008q1 R	588 bp	Y
bg30_33p	GCTACAGAGAGAAAAATTAAGAAACA	2009q2 F	GGTTGGAAAATAAAGTCTAAAATGGAT	2009q2 R	1126 bp	Y
bg34p		-		-	-	-

The symbol "-" means that the primer did not amplify anything
Primer sets dated 2004 were published in ref. 9

File S2. Supplementary Materials and Methods

Mass spectrometry and proteome data were acquired by the Arizona Proteomics Consortium supported by NIEHS grant ES06694 to the SWEHSC, NIH/NCI grant CA023074 to the AZCC and by the BIO5 Institute of the University of Arizona. The Thermo Fisher LTQ Orbitrap Velos mass spectrometer was provided by grant 1S10 RR028868-01 from NIH/NCRR.

Transcript analyses

RNA isolation was performed with a GenElute Total RNA mini-prep kit (Sigma). PCR was performed with Dream Taq (Fermentas) and primers provided by Bioneer. Primer sequences are available from RCK on request. Dye-terminator DNA sequencing was done on Applied Biosystems 3730 DNA Analyzers by the University of Arizona UAGC laboratory using the BigDye® Terminator v3.1 Cycle Sequencing Kit.

Preparation of samples for mass spectrometric analysis and two-dimensional gel separation

Tear fluids were provided to the Proteomics Core Facility of the University of Arizona where personnel determined the protein quantity in the samples using the Pierce 660 nm assay (<http://www.piercenet.com/>). A total of 175 µg of sample was used. The sample was mixed into a solution with a final concentration of 7 M Urea/2 M Thiourea/ 4% CHAPS. No reducing agent was used. The samples were then introduced to an 11 cm pH 3-10 linear IPG strip (Biorad, Hercules CA) using the Protean IEF Cell (Biorad). The IEF strips were rehydrated and focused using a linear method with a 16h active rehydration. The focusing was considered complete when 35000 v/hours were reached. Electrode Wicks (Biorad) were placed between the strip and the actual electrode wire during the first step of the focusing procedure to absorb excess salts. The focused IEF strips were equilibrated in a solution of 6 M urea/0.375 M Tris/2% SDS/ 20% Glycerol for 15 minutes, no reduction or alkylation was done on the strips. The strips were then individually placed onto their own 12.5% Criterion IPG +1 well gel (Biorad) and overlaid with a 1% agarose solution containing bromophenol blue dye to track the progress of the run. 4 µl of Precision Plus Standard (Biorad) was added to the standard well. The gels were run together on a Criterion gel cell (Biorad) for 15 min at 100 V and then 150V until the tracking dye reached the bottom of

each gel. The gels were then rinsed 3 times with deionized water and then stained with Biorad Bio-Safe Coomassie stain per the manufacturer's instructions (Biorad). The gels were imaged and individual spots or sections of spots were cut out and then digested as described previously with trypsin (Karn & Laukaitis, 2011).

Tandem mass spectrometry coupled to liquid chromatography (LC-MS/MS) of 2D separated proteins

LC-MS/MS analysis of trypsin digested 2D-SDS-PAGE gel pieces (Shevchenko et al., 1996) was carried out using a LTQ Orbitrap Velos mass spectrometer (Thermo Fisher Scientific, San Jose, CA) equipped with an Advion nanomate ESI source (Advion, Ithaca, NY), following ZipTip (Millipore, Billerica, MA) C18 sample clean-up according to the manufacturer's instructions. Peptides were eluted from a C18 precolumn (100- μ m id \times 2 cm, Thermo Fisher Scientific) onto an analytical column (75- μ m ID \times 10 cm, C18, Thermo Fisher Scientific) using a 5-10% gradient of solvent B (acetonitrile, 0.1% formic acid) over 5 minutes, followed by a 10-35% gradient of solvent B over 35 minutes, 35-50% gradient of solvent B over 20 minutes, 50-95% gradient of solvent B over 5 minutes, and finally by a 95% solvent B hold for another 5 minutes. Solvent A consisted of water and 0.1% formic acid. Data dependent scanning was performed by the Xcalibur v 2.1.0 software (Andon et al., 2002) using a survey mass scan at 60,000 resolution in the Orbitrap analyzer scanning m/z 400-1600, followed by collision-induced dissociation (CID) tandem mass spectrometry (MS/MS) of the fourteen most intense ions in the linear ion trap analyzer. Precursor ions were selected by the monoisotopic precursor selection (MIPS) setting with selection or rejection of ions held to a \pm 10 ppm window. Dynamic exclusion was set to place any selected m/z on an exclusion list for 45 seconds after a single MS/MS. All MS/MS spectra were searched against Uniprot *Mus musculus* downloaded January 11, 2012 (<http://www.uniprot.org/taxonomy/10090>), appended with ABP sequences provided by Robert Karn, using Thermo Proteome Discoverer 1.3 (Thermo Fisher Scientific). At the time of the search, this combined protein database contained 50,565 entries. Proteins were identified at 99% confidence with XCorr score cut-offs (Qian et al., 2005) as determined by a reversed database search. The results were displayed with Scaffold v 3.6.1 (Proteome Software Inc., Portland OR), a program that relies on various search engine results (ie: Sequest,

X!Tandem, MASCOT) and which uses Bayesian statistics to reliably identify more spectra (Keller et al., 2002, Nesvizhskii et al., 2003).

Criteria for protein identification

Scaffold was used to validate MS/MS based peptide and protein identifications. Peptide identifications were accepted if they exceeded specific database search engine thresholds. Sequest identifications required at least deltaCn scores of greater than 0.08 and XCorr scores of greater than 1.8, 2.5, 3.5 for singly, doubly, triply charged peptides. Protein identifications were accepted if they contained at least 2 identified peptides. Proteins that contained similar peptides and could not be differentiated based on MS/MS analysis alone were grouped to satisfy the principles of parsimony.

Data analysis

The program DNAsp was used to obtain the rates of nonsynonymous (Ka) and synonymous (Ks) nucleotide substitutions (Librado & Rozas, 2009). Mouse *Abp* transcript cDNA sequences were modified to contain only the coding regions for their respective secreted protein products (i.e. signal peptides were removed) and these were aligned using CLUSTALX (Jeanmougin et al., 1998, Thompson et al., 1997).

Phylogenetic trees were constructed from the alignments using the program PAUP* (Swofford, 1998) using neighbor joining (NJ) distance parameters with Jukes-Cantor correction and random-seeding. These phylogenies were used as guide trees for CODEML analysis.

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Jeanmougin, F., Thompson, J. D., Gouy, M., Higgins, D. G. & Gibson, T. J. 1998. Multiple sequence alignment with Clustal X. *Trends Biochem Sci* **23**: 403-5.

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- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**: 4876-82.

File S3. Guide Trees

Expressed Abpa's

1

((1,2),3,4);

// end of file

1: a12

2: a20

3: a3

4: a2

Non-expressed Abpa's

1

((((1,2),3),4),5,6);

// end of file

1: a15

2: a17

3: a24

4: a19

5: a7

6: a11

Expressed Abpbg's

1

((1,3),2),(4,5),6);

// end of file

1: bg2

2: bg3p

3: bg24

4: bg20

5: bg7

6: bg12

Non-expressed Abpbg's

1

((1,2),3),4,5);

// end of file

- 1: bg11
- 2: bg18
- 3: bg19
- 4: bg21
- 5: bg1

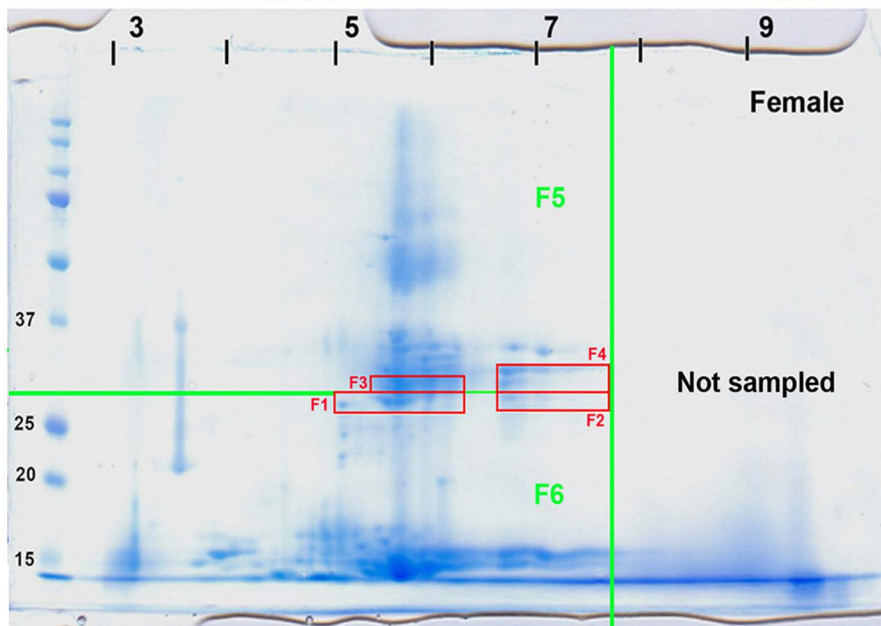
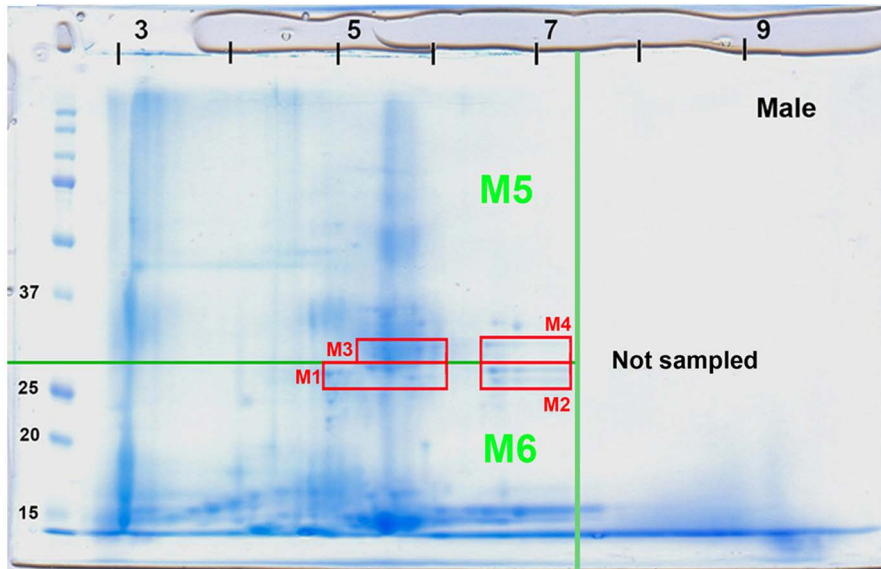
File S4. List of all 64 Abp paralogs with GenBank accession numbers and coordinates

GENE	GenBank Acc #	CHR	STRAND	mm9 coordinates		mm10 coordinates		LENGTH
B6_a1ψ	KM014069	7	n	32039982	32041416	31254963	31256397	1435
B6_bg1	KM014083	7	p	32048657	32050908	31263638	31265889	2252
B6_a2	KM014043	7	n	32075543	32076939	31290524	31291920	1397
B6_bg2	KM014055	7	p	32087682	32089952	31302663	31304933	2271
B6_bg3ψ	KM014056	7	n	32143937	32147206	31358918	31362187	3270
B6_a3	KM014044	7	p	32160507	32161931	31375488	31376912	1425
B6_bg4ψ	KM014084	7	n	32185931	32188316	31400912	31403297	2386
B6_a4ψ	KM014070	7	p	32194574	32195996	31409555	31410977	1423
B6_a28ψ	KM014081	7	p	32228722	32230136	31443703	31445117	1415
B6_bg5ψ	KM014085	7	n	32285081	32286475	31500062	31501456	1395
B6_a5ψ	KM014071	7	p	32292340	32293744	31507321	31508725	1405
B6_bg28ψ	KM014094	7	n	32329941	32331947	31544922	31546928	2007
B6_bg6ψ	KM014086	7	n	32402639	32404664	31617620	31619645	2026
B6_a6ψ	KM014072	7	p	32410499	32411928	31625480	31626909	1430
B6_bg7	KM014057	7	n	32488842	32490875	31703823	31705856	2034
B6_a7	KM014045	7	p	32497586	32498993	31712567	31713974	1408
B6_bg8ψ	KM014087	7	n	32563678	32565696	31778659	31780677	2019
B6_a8ψ	KM014073	7	p	32573178	32574586	31788159	31789567	1409
B6_bg9ψ	KM014088	7	n	32700132	32702523	31915113	31917504	2392
B6_a9ψ	KM014074	7	p	32709242	32710665	31924223	31925646	1424
B6_bg29ψ	KM014095	7	n	32797931	32799937	32012912	32014918	2007
B6_bg10ψ	KM014058	7	n	32877046	32879077	32092027	32094058	2032
B6_a10	KM014046	7	p	32885768	32887186	32100749	32102167	1419
B6_bg30ψ	KM014096	7	n	32931141	32933170	32146122	32148151	2030
B6_bg11	KM014059	7	n	32994236	32996260	32209217	32211241	2025
B6_a11	KM014047	7	p	33007756	33009172	32222737	32224153	1417
B6_bg12	KM014060	7	n	33110343	33112366	32325324	32327347	2024
B6_a12	KM014048	7	p	33119097	33120516	32334078	32335497	1420
B6_a29	KM014045	7	p	33226445	33227857	32441426	32442838	1413
B6_bg13ψ	KM014089	7	n	33292401	33294418	32507382	32509399	2018
B6_a13ψ	KM014075	7	p	33301910	33303318	32516891	32518299	1409
B6_bg14ψ	KM014090	7	n	33432547	33434939	32647528	32649920	2393
B6_a14ψ	KM014076	7	p	33441664	33443088	32656645	32658069	1425
B6_bg31ψ	KM014097	7	n	33530194	33532200	32745175	32747181	2007
B6_bg15ψ	KM014061	7	n	33612747	33614778	32827728	32829759	2032
B6_a15	KM014049	7	p	33621469	33622887	32836450	32837868	1419
B6_bg16ψ	KM014090	7	n	33659437	33661829	32874418	32876810	2393
B6_a16ψ	KM014076	7	p	33668554	33669978	32883535	32884959	1425
B6_bg32ψ	KM014097	7	n	33757093	33759099	32972074	32974080	2007
B6_bg17ψ	KM014061	7	n	33839646	33841677	33054627	33056658	2032
B6_a17	KM014049	7	p	33848364	33849782	33063345	33064763	1419
B6_bg33ψ	KM014098	7	n	33896570	33898596	33111551	33113577	2027
B6_bg18	KM014062	7	n	33956955	33958979	33171936	33173960	2025
B6_a18	KM014047	7	p	33970467	33971883	33185448	33186864	1417

B6_bg19	KM014063	7 n	34063433	34065457	33278414	33280438	2025
B6_a19	KM014050	7 p	34072206	34073626	33287187	33288607	1421
B6_bg20	KM014064	7 n	34149406	34151437	33364387	33366418	2032
B6_a20	KM014051	7 p	34158154	34159571	33373135	33374552	1418
B6_bg34ψ	KM014099	7 n	34227925	34229954	33442906	33444935	2030
B6_bg21	KM014065	7 n	34303544	34305567	33518525	33520548	2024
B6_a21ψ	KM014077	7 p	34312275	34313697	33527256	33528678	1423
B6_bg22ψ	KM014091	7 n	34389169	34391191	33604150	33606172	2023
B6_a22ψ	KM014078	7 p	34399532	34400942	33614513	33615923	1411
B6_bg23ψ	KM014092	7 n	34410209	34412238	33625190	33627219	2030
B6_a23ψ	KM014079	7 p	34420061	34421471	33635042	33636452	1411
B6_bg24	KM014066	7 n	34522246	34524428	33737227	33739409	2183
B6_a24	KM014052	7 p	34528700	34530118	33743681	33745099	1419
B6_bg25ψ	KM014093	7 n	34637923	34639956	33852904	33854937	2034
B6_a25ψ	KM014080	7 p	34652853	34654216	33867834	33869197	1364
B6_bg26	KM014067	7 n	34728065	34730103	33943046	33945084	2039
B6_a26ψ	KM014053	7 p	34743704	34745121	33958685	33960102	1418
B6_bg27	KM014068	7 n	34796983	34799005	34011964	34013986	2023
B6_a27	KM014054	7 p	34806467	34807888	34021448	34022869	1422
B6_a30ψ	KM014082	7 p	34884647	34885844	34099628	34100825	1198

SDS Gel
Electrophoresis

Isoelectric focusing



a2 **gicpaikedvhlflfgtpeeyvnyvekykddpetlenteklkicvdrtltkenkehaaafiekiessplc** M & F transcript
 hlflfgtpeeyvnyvekykddpetlenteklkicvdrtltkenkehaaafiekiessplc
GICPAIKedvhlflfgtpeeyvnyvekykddpetlenteklkicvdr**TLTKENKEHAAAFIEKIESSPLC** M1 (gel piece)
 gicpaikedvhlflfgtpeeyvnyvekykddpetlenteklkicvdrtltkenkehaaafiekiessplc M2 " "
 gicpaikedvhlflfgtpeeyvnyvekykddpetlenteklkicvdrTLTKENKEHAAAFIEKIESSPLC M3 " "
 gicpaikedvhlflfgtpeeyvnyvekykddpetlenteklkicvdrtltkenkehaaafiekiessplc M4 " "
 gicpaikedvhlflfgtpeeyvnyvekykddpetlenteklkicvdrtltkenkehaaafiekiessplc M5 " "
GICPAIKEDVHFLFLFGTPEEYVNYVEKYKDDPETLENTEKlkicvdr**TLTKENKEHAAAFIEKIESSPLC** M6 " "
 gicpaikedvhlflfgtpeeyvnyvekykddpetlenteklkicvdrtltkenkehaaafiekiessplc F1 " "
 gicpaikedvhlflfgtpeeyvnyvekykddpetlenteklkicvdrtltkenkehaaafiekiessplc F2 " "
GICPAIKEDVHFLFLFGTPEEYVNYVEKYKDDPETLENTEKlkicvdrtltkenkehaaafiekiessplc F3 " "
 gicpaikedvhlflfgtpeeyvnyvekykddpetlenteklkicvdrtltkenkehaaafiekiessplc F4 " "

a3 **gicpaikedvrlflngtseeyveyvnyvekykddpeilentakikqcvds** M transcript
GICPAIKEDVRLFLNGTSEEYVEYVNYVEKYKDDPEILENTAKIKQCVDSLTEEDKAHATAFIEKIEASPLC M1 (gel piece)
 GICPAIKEDVRLflngtseeyveyvnyvekykddpeilentakikqcvdstlteedkahatafiekieasplc M3 " "
 GICPAIKEDVRLflngtseeyveyvnyvekykddpeilentakIKQCVDSLTEEDKAHATAFIEKIEASPLC M4 " "
 GICPAIKEDVRLFLNGTSEEYVEYVNYVEKYKDDPEILENTAKIKQCVDSLTEEDKAHATAFIEKIEASPLC M5 " "
 GICPAIKEDVRLFLNGTSEEYVEYVNYVEKYKDDPEILENTAKIKQCVDSLTEEDKAHATAFIEKIEASPLC M6 " "

m in a12
 mklagamvilgaalllltsggdc t in 12 d in 12 M transcript
 v in a19 v in 19 n in 19

a12 **gicpaikedvrlflngtseeyveyvnyvekykddpvtlentakikqcvdstlsteedrahattfiekieasplc** M transcript
GICPAIKEDVRLflngtseeyveyvnyvekykddpvtlentakIKQCVDSLTEEDRAHATTFIEKIEASPLC M5 (gel piece)
 [continued]

a20 **gicpaikedvrlflngtseeyveyvnyvekykddpvtlentakikqcvdstlsteedkihattfiekieasplc** M & F transcript
GICPAIKEDVRLFLNGTSEEYVEYVNYVEKYKDDPVILENTAKIKQCVDSLTEEDKIHATTFIEKIEASPLC M1 (gel piece)
 gicpaikedvrlflngtseeyveyvnyvekykddpvtlentakikqcvdstlsteedkihattfiekieasplc M2 " "
 GICPAIKEDVRLflngtseeyveyvnyvekykddpvtlentakIKQCVDSLTEEDKIHATTFIEKIEASPLC M3 " "
 GICPAIKEDVRLflngtseeyveyvnyvekykddpvtlentakIKQCVDSLTEEDKIHATTFIEKIEASPLC M4 " "
 GICPAIKEDVRLFLNGTSEEYVEYVNYVEKYKDDPVILENTAKIKQCVDSLTEEDKIHATTFIEKIEASPLC M5 " "
 GICPAIKEDVRLFLNGTSEEYVEYVNYVEKYKDDPVILENTAKIKQCVDSLTEEDKIHATTFIEKIEASPLC M6 " "
 GICPAIKEDVRLflngtseeyveyvnyvekykddpvtlentakIKQCVDSLTEEDKIHATTFIEKIEASPLC F1 " "
 GICPAIKEDVRLflngtseeyveyvnyvekykddpvtlentakIKQCVDSLTEEDKIHATTFIEKIEASPLC F3 " "
 not a7 because of the ATA codon for I

a24 **vhlffhrtseeyveyvnyvekykddpeilentekikkcvdstltdedkthatafiekiearpac** M & F transcript
a27 **grkvdlflngtseeyveyvnyvekykddpeilentekikkcvdstltdedkthatafiekiearpac** M & F transcript

File S7

lg2 *lglsgrnrlglsgrnrlgltelapfdptveekeafeqkdcyeeaglkaksgvdqvmasmffsseclykysndtmtkilsvitkkwm* M transcript
 cipffgvyllglsgrnrlgltelapfdptveekeafeqkdcyeeaglkaksgvdqvmasmffsseclykysndtmtkilsvitkkwm M1 gel piece
 cipffgvyllglsgrnrlgltelapfdptveekeafeqkdcyeeaglkaksgvdqvmasmffsseclykysndtmtkilsvitkkwm M2 " "
 cipffgvyllglsgrnrlgltelapfdptveekeafeqkdcyeeaglkaksgvdqvmasmffsseclykysndtmtkilsvitkkwm M3 " "
 cipffgvyllglsgrnrlgltelapfdptveekeafeqkdcyeeaglkaksgvdqvmasmffsseclykysndtmtkilsvitkkwm M4 " "
 cipffgvyllglsgrnrlgltelapfdptveekeafeqkdcyeeaglkaksgvdqvmasmffsseclykysndtmtkilsvitkkwm M5 " "
CIPFFGVYLLGILSGNRIGLHTELAPDFDPTVEEKEAFEKIQDCYEEBGLKAKTEDEMKIMTTLILFSSSECRSYTTEVKLNILVKFSKLLT M6 " "
CIPFFGVYLLGILSGNRIGLHTELAPDFDPTVEEKEAFEKIQDCYEEBGLKAKTEDEMKIMTTLILFSSSECRSYTTEVKLNILVKFSKLLT F1 " "
 cipffgvyllglsgrnrlgltelapfdptveekeafeqkdcyeeaglkaksgvdqvmasmffsseclykysndtmtkilsvitkkwm F2 " "
CIPFFGVYLLGILSGNRIGLHTELAPDFDPTVEEKEAFEKIQDCYEEBGLKAKTEDEMKIMTTLILFSSSECRSYTTEVKLNILVKFSKLLT F3 " "
 cipffgvyllglsgrnrlgltelapfdptveekeafeqkdcyeeaglkaksgvdqvmasmffsseclykysndtmtkilsvitkkwm F4 " "
 cipffgvyllglsgrnrlgltelapfdptveekeafeqkdcyeeaglkaksgvdqvmasmffsseclykysndtmtkilsvitkkwm F5 " "
 cipffgvyllglsgrnrlgltelapfdptveekeafeqkdcyeeaglkaksgvdqvmasmffsseclykysndtmtkilsvitkkwm F6 " "

bg3 *lstvsgkrllwhhelsyfnptdgetksfkkiqdcyeeaglkaksgvdqvmasmffsseclykysndtmtkilsvitkkwm* M transcript
 casffgvyllstvsgkrllwhhelsyfnptdgetksfkkiqdcyeeaglkaksgvdqvmasmffsseclykysndtmtkilsvitkkwm M1 gel piece
 casffgvyllstvsgkrllwhhelsyfnptdgetksfkkiqdcyeeaglkaksgvdqvmasmffsseclykysndtmtkilsvitkkwm M2 " "
 casffgvyllstvsgkrllwhhelsyfnptdgetksfkkiqdcyeeaglkaksgvdqvmasmffsseclykysndtmtkilsvitkkwm M3 " "
 casffgvyllstvsgkrllwhhelsyfnptdgetksfkkiqdcyeeaglkaksgvdqvmasmffsseclykysndtmtkilsvitkkwm M5 " "
 casffgvyllstvsgkrllwhhelsyfnptdgetksfkkiqdcyeeaglkaksgvdqvmasmffsseclykysndtmtkilsvitkkwm M6 " "

bg7

			n (20/29)		r (20)	
l (20)	v (29)	m (29)	*		(29 disrupted from here)	*

acpfefayasvlgsvrvlyhelqafdataeekvalekiqdcyeeserirnillepikameavspceclsyyg M & F transcript
 cfpffeyasvlgsvrvlyhelqafdataeekvalekiqdcyeeserirnillepikameavspceclsyygldnirsildyiskllge M1 gel piece
 cfpffeyasvlgsvrvlyhelqafdataeekvalekiqdcyeeserirnillepikameavspceclsyygldnirsildyiskllge M2 " "
 cfpffeyasvlgsvrvlyhelqafdataeekvalekiqdcyeeserirnillepikameavspceclsyygldnirsildyiskllge M3 " "
 cfpffeyasvlgsvrvlyhelqafdataeekvalekiqdcyeeserirnillepikameavspceclsyygldnirsildyiskllge M4 " "
 cfpffeyasvlgsvrvlyhelqafdataeekvalekiqdcyeeserirnillepikameavspceclsyygldnirsildyiskllge M5 " "
CFPFEEAYASVLSGSRVWLYHELQAFDATAEKEVALEKIQCDCYSEERIRNILLEPKIMEAMVASPECLSYGLDNIRSIILDYISKLLGE M6 " "
 cfpffeyasvlgsvrvlyhelqafdataeekvalekiqdcyeeserirnillepikameavspceclsyygldnirsildyiskllge F1 " "
 cfpffeyasvlgsvrvlyhelqafdataeekvalekiqdcyeeserirnillepikameavspceclsyygldnirsildyiskllge F5 " "
 cfpffeyasvlgsvrvlyhelqafdataeekvalekiqdcyeeserirnillepikameavspceclsyygldnirsildyiskllge F6 " "

bg10 *mkgtlllallvtgelsfqtttes* M transcript
 lvpffnyasvlgskrylhelqafdataeekvalekiqdcyeeserirnillepikameavspceclsyygldnirsildyiskllge M6 gel piece
LVPFFNYASVLSGSRRLYHELQAFDATAEKEVALEKIQCDCYSEERIRNILLEPKIMEAMVASPECLSYGLDNIRSIILDYISKLLGE
 r (8/13) i (30/33) g (30/33)

bg12 *mkgillllgllitgelsfqtttes* M transcript
 signal peptide part of the M transcript
 t (11/18/19)
 g (19) v (19) n (19)

bg12 *Cvpffeyasvlgsvrvlyhelqsfdataeekvalekiqdcyereerirnillepikameavspceclsyygldnirsildyiskllge* M transcript
 [continued]
 cvpffeyasvlgsvrvlyhelqsfdataeekvalekiqdcyereerirnillepikameavspceclsyygldnirsildyiskllge M1 gel piece
 cvpffeyasvlgsvrvlyhelqsfdataeekvalekiqdcyereerirnillepikameavspceclsyygldnirsildyiskllge M3 " "
 cvpffeyasvlgsvrvlyhelqsfdataeekvalekiqdcyereerirnillepikameavspceclsyygldnirsildyiskllge M4 " "
 cvpffeyasvlgsvrvlyhelqsfdataeekvalekiqdcyereerirnillepikameavspceclsyygldnirsildyiskllge M5 " "
CVPFEEYASVLSGSRVWLYHELQSFDATAEKEVALEKIQCDCYREERIRNILLEPKIMEAMVASPECLSYGLDNIRSIILDYISKLLGE M6 " "

bg20 *lvtgelsfqtttes (signal)* F transcript
 Cfpffeyasvlgsvrvlyhelqafnataeekvalekiqdcyeeserirnillepikameavspceclsyygldnirsildyiskllge M transcript
 asvlgsvrvlyhelqafnataeekvalekiqdcyeeserirnillepikameavspceclsyygldnirsildyiskllge M4 gel piece
CIPFFEGYAGVLSGSRRLWLYHELQAFNATAEKEVALEKIQCDCYSEERIRNILLEPKIMEAMVASPECLSYGLDNIRSIILDYISKLLGE M5 " "
CIPFFEGYAGVLSGSRRLWLYHELQAFNATAEKEVALEKIQCDCYSEERIRNILLEPKIMEAMVASPECLSYGLDNIRSIILDYISKLLGE M6 " "
CIPFFEGYAGVLSGSRRLWLYHELQAFNATAEKEVALEKIQCDCYSEERIRNILLEPKIMEAMVASPECLSYGLDNIRSIILDYISKLLGE F6 " "

bg24 *lallmigelghfhttea (signal peptide)* M transcript
 cvpffagygvisgsvrvlyhelqafnataeekvalekiqdcyeeqgvrksqtlepqlilasiltvpeol M transcript
 afngtpketvayekiqdcyeeqgvrksqtlepqlilasiltvpeol F transcript
CVPFYAGYAGVISGSRRLWLYHELQAFNATAEKEVALEKIQCDCYEEQGVKSKTLEPQLILASILTVPOLGYSEETF M1
 cvpffagygvisgsvrvlyhelqafnataeekvalekiqdcyeeqgvrksqtlepqlilasiltvpeolgyseetftkikdalkkiasg M2
 cvpffagygvisgsvrvlyhelqafnataeekvalekiqdcyeeqgvrksqtlepqlilasiltvpeolgyseetftkikdalkkiasg M3
 cvpffagygvisgsvrvlyhelqafnataeekvalekiqdcyeeqgvrksqtlepqlilasiltvpeolgyseetftkikdalkkiasg M4
 CVPFYAGYAGVISGSRRLWLYHELQAFNATAEKEVALEKIQCDCYSEERIRNILLEPKIMEAMVASPECLSYGLDNIRSIILDYISKLLGE M5
CVPFYAGYAGVISGSRRLWLYHELQAFNATAEKEVALEKIQCDCYEEQGVKSKTLEPQLILASILTVPOLGYSEETF M6
CVPFYAGYAGVISGSRRLWLYHELQAFNATAEKEVALEKIQCDCYEEQGVKSKTLEPQLILASILTVPOLGYSEETF F1
CVPFYAGYAGVISGSRRLWLYHELQAFNATAEKEVALEKIQCDCYEEQGVKSKTLEPQLILASILTVPOLGYSEETF F2
CVPFYAGYAGVISGSRRLWLYHELQAFNATAEKEVALEKIQCDCYEEQGVKSKTLEPQLILASILTVPOLGYSEETF F3

bg26 *gailtllrrtflhgdlsqfyatvaervafekiqdcfreenqgktilnpg* M & F transcript
bg27 *kilggnrlalnaylsmfqataervafekiqdcfreenqgktilnpg* M & F transcript

File S8. *Abp* putative pseudogenes

	<u>Noncanonical splice site</u>	<u>Coding region coding disruption</u>	<u>both</u>
Clade 1			a1p
Clade 2	bg3*, a5p, a28p	bg4p, bg5p, bg28p, a6p, a8p, bg9p, bg29p, a13p, bg14p, bg31p, bg16p, bg32p, a21p, bg22p, bg23p, bg34p	a4p, bg6p, bg8p, a9p, bg30p, bg13p, bg10*, a14p, bg15*, a16p, bg17*, bg33p, a22p, a23p
Clade 3			
Clade 4		bg25p	a25p
Clade 5	a26p	a30p	

*bg3 and bg10 are expressed inspite of noncanonical splice sites and/or coding region disrutions

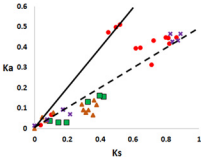
File S9. Data from qPCR analyses

Gland	Paralog	Avg Male CT value	ng total RNA	Male Protein Identification?	Paralog	Avg Female CT value	ng total RNA	Female Protein Identification ?	Male to Female Ratio ng total RNA	Sex specific (as per previous transcript data)
Lacrimal	Abpa2	15.81	0.21	Y	Abpa2	16.48	0.14	Y	1.5	N
Submax	Abpa2	*	n/a	N	Abpa2	37	7.94E-07	N	n/a	
Lacrimal	Abpbg2	18.08	0.035	Y	Abpbg2	18.9	0.022	Y	1.59	N
Lacrimal	Abpa3	16.43	0.026	Y	Abpa3	24.6	2.05E-05	N	1.27E+03	Y
Lacrimal	Abpbg3	22.5	0.096	Y	Abpbg3	*	n/a	N	n/a	Y
Lacrimal	Abpbg7	16.24	0.05	Y	Abpbg7	22.46	1.44E-03	Y	37.90	N
Lacrimal	Abpbg10	17.82	0.01	Y	Abpbg10	24.15	1.22E-04	N	85.55	Y
Lacrimal	Abpbg12	20.76	0.13	Y	Abpbg12	37.15	2.05E-05	N	6.48E+03	Y
Lacrimal	Abpbg20 ^b	25.42	0.09	Y	Abpbg20 ^b	29.42	3.49E-03	Y	26.26	N
Lacrimal	Abpa24 ^a	19.26	0.02	N	Abpa24 ^a	19.02	0.02	N	0.86	N
Lacrimal	Abpbg24 ^b	14.61	0.02	Y	Abpbg24 ^b	14.59	0.02	Y	0.93	N
Submax	Abpbg24	31.87	5.01E-09	N	Abpbg24	30.06	7.24E-09	N	0.69	
Lacrimal	Abpbg26 ^b	*		N	Abpbg26	*		N		N
Submax	Abpbg26 ^b	16.84	0.04	Y	Abpbg26 ^b	16.22	0.06	Y	0.67	N
Lacrimal	Abpa27	38.05	7.76E-07	N	Abpa27	37.08	1.49E-06	N	0.52	N
Submax	Abpa27	20.59	0.09	Y	Abpa27	21.16	0.06	Y	1.47	N
Lacrimal	Abpbg27	32.39	7.26E-07	N	Abpbg27	30.85	2.27E-06	N	0.32	N
Submax	Abpbg27	18.48	0.02	Y	Abpbg27	19.03	1.43E-02	Y	1.50	N
Lacrimal	GAPDH ^b	24.13	0.27		GAPDH ^b	21.85	1.21		0.22	N
Submax	GAPDH ^b	22.22	1.05		GAPDH ^b	25.49	0.11		9.81	N

*undetectable Ct value

^a Run at 60°

^b Run at 63°



- Abpa
- Abpbg
- ▲ Non-expr Abpa
- ✕ Non-expr Abpbg

File
S10.