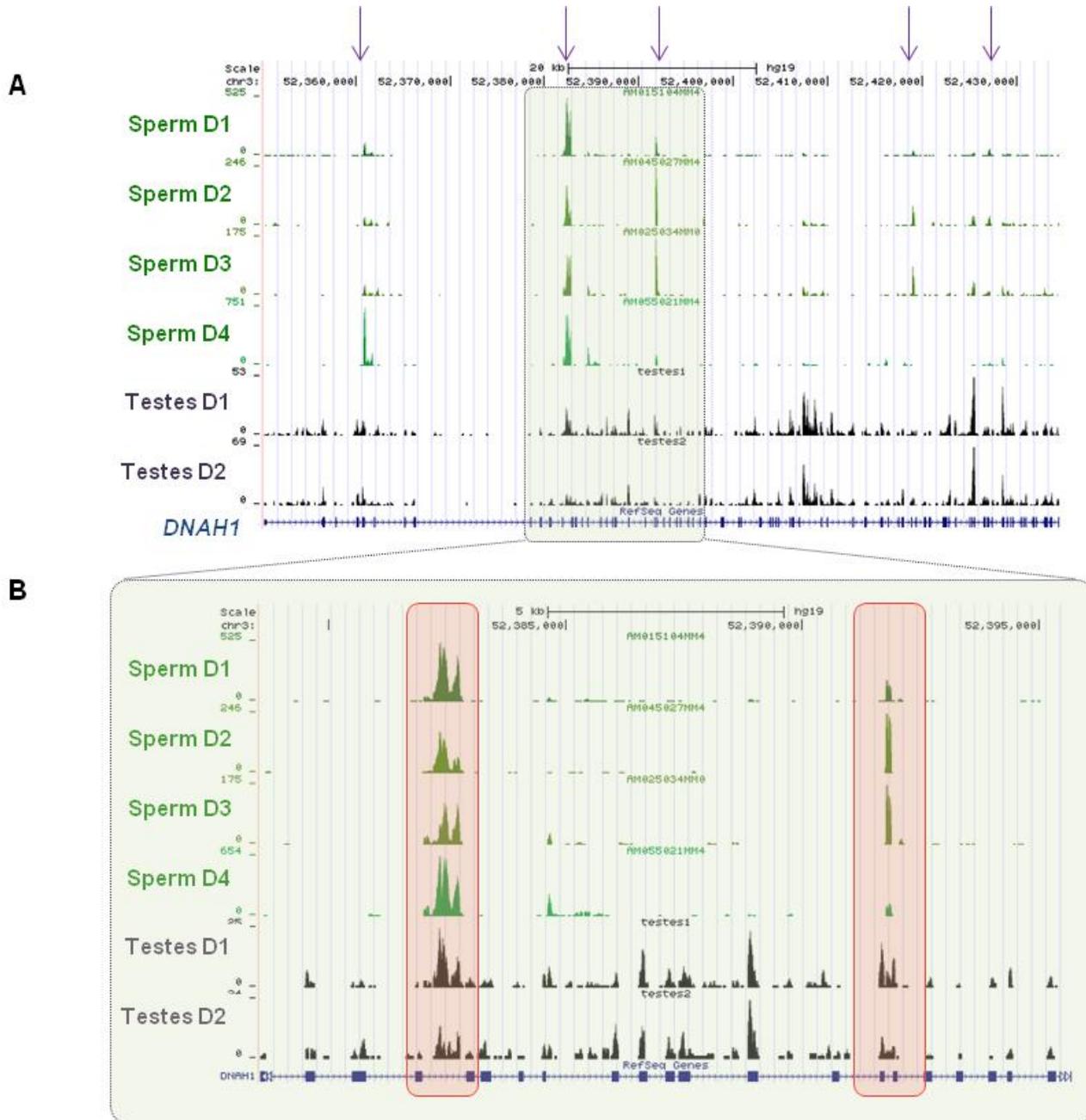
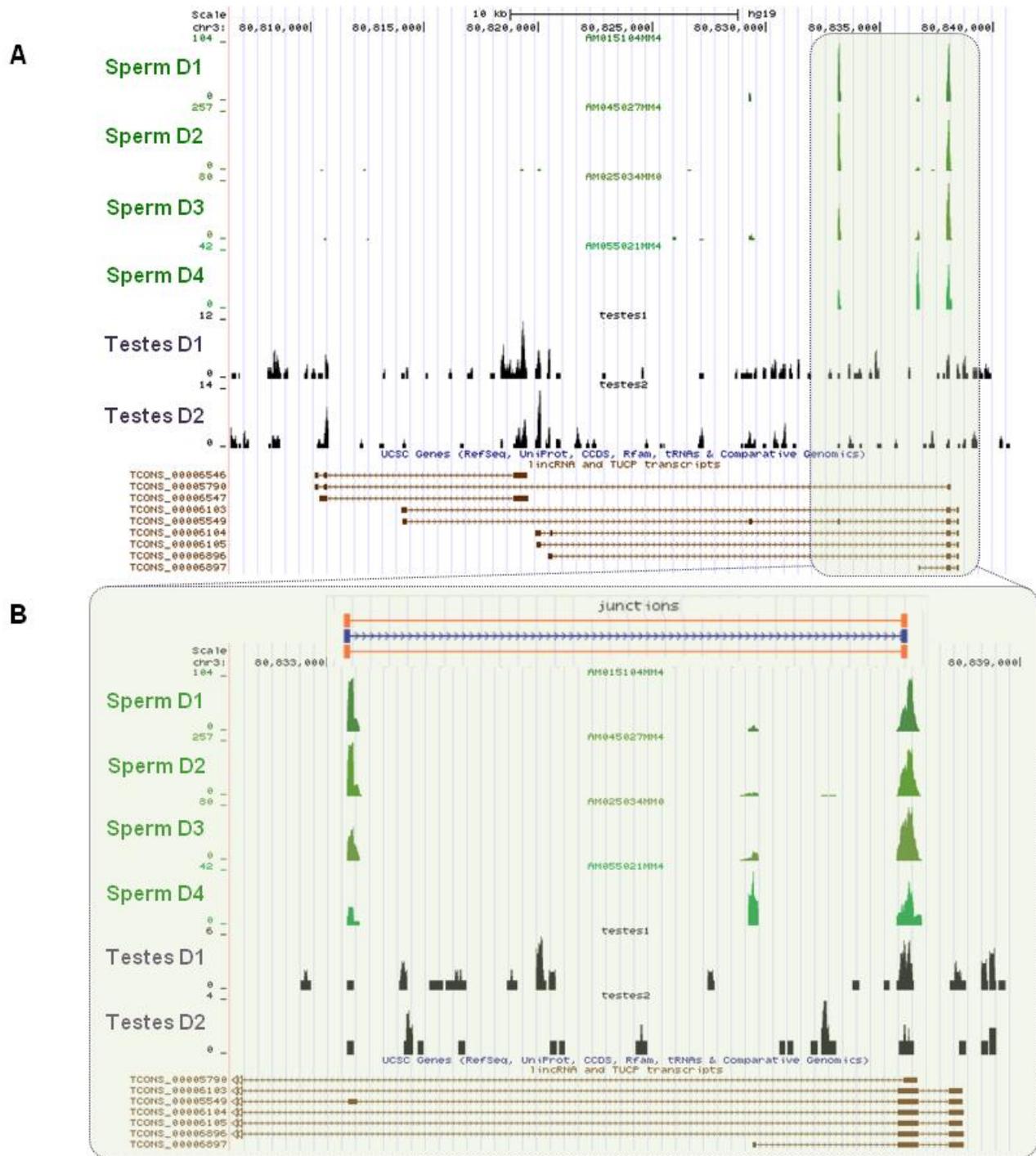


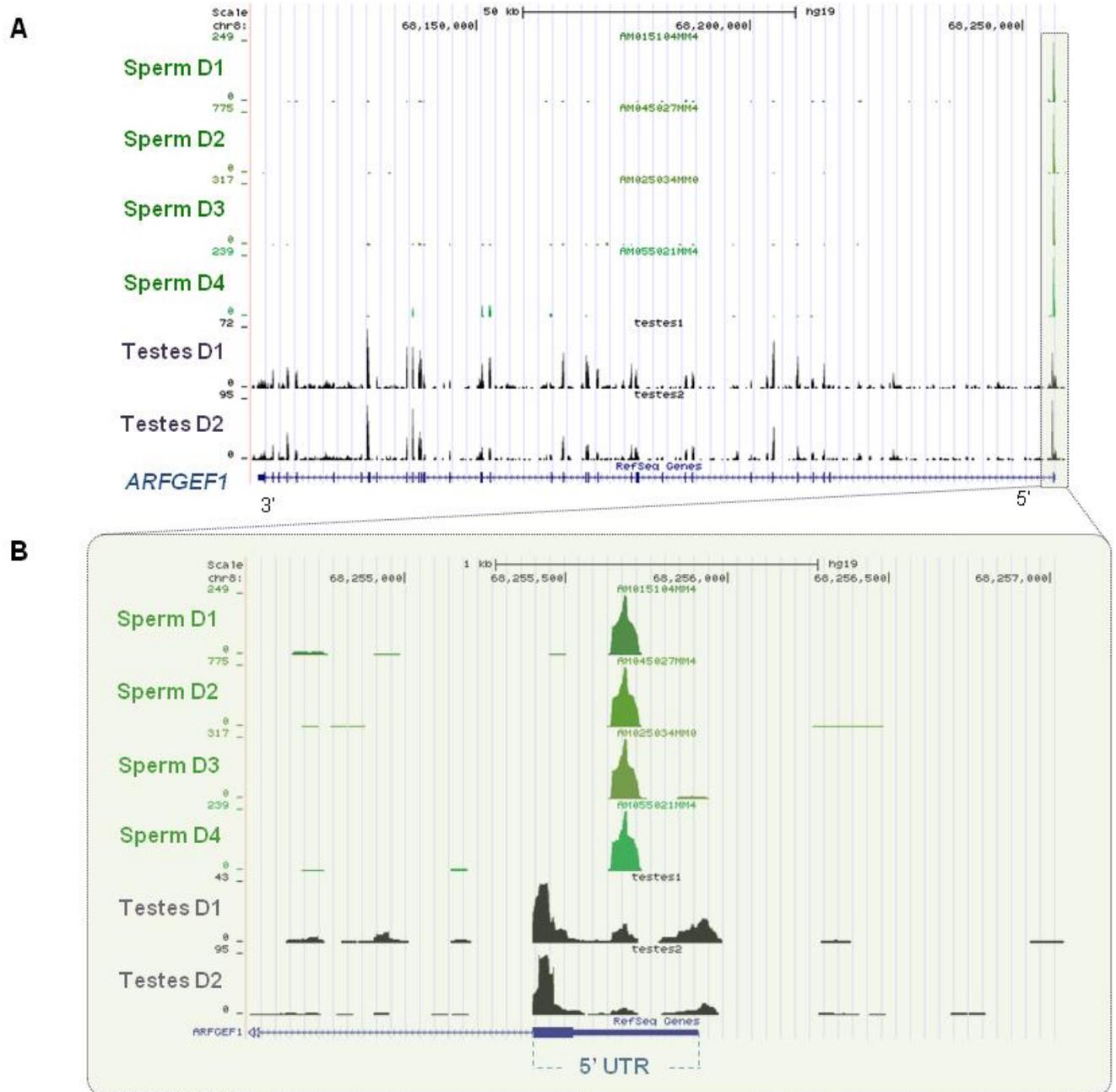
**Supplementary Figure 1:** Alternative polyadenylation of sperm transcripts. Corresponding to and expanding upon Figure 3 in main body of paper, transcript expression from four normal sperm samples and two testes samples is shown, revealing distinct expression of this sperm characteristic in all samples surveyed. (A) Example transcript *GIGYF2* in sperm and testes samples. (B) Magnified view of 3' UTR region shows a truncated 3' UTR in sperm (green) in contrasts with coverage extending over the majority of the UTR observed in testes (black).



**Supplementary Figure 2:** Intronic Retained Elements in Sperm. Corresponding to and expanding upon Figure 5 in main body of paper, transcript expression from four normal sperm samples and two testes samples is shown, revealing distinct expression of this sperm characteristic in all samples surveyed. (A) Example transcript *DNAH1* shows five introns (purple arrows) in sperm samples showing significant expression, while the coding regions of this RNA are absent. In equivalently sequenced testes samples these intronic regions are underrepresented and resemble levels observed across the complete transcript (note y-axis). (B) Magnified view of two such introns showing that coverage in sperm is strictly restricted to the intronic sequences.



**Supplementary Figure 3:** Unique sperm lincRNAs isoforms. Corresponding to and expanding upon Figure 6 in main body of paper, transcript expression from four normal sperm samples and two testes samples is shown, revealing distinct expression of this sperm characteristic in all samples surveyed. (A) Example of a 30 kb region of chromosome 3 containing a series of putative lincRNAs. (B) Magnified view of a part of this region shows the presence of two exons is part of a single spliced transcript in sperm samples, which was not previously identified.



**Supplementary Figure 4: Exonic sperm elements.** Corresponding to and expanding upon Figure 7 in main body of paper, transcript expression from four normal sperm samples and two testes samples is shown, revealing distinct expression of this sperm characteristic in all samples surveyed. (A) Example transcript *ARFGEF1*. In testes, significant coverage of the complete transcript is observed. In contrast, sperm shows virtually no expression within the length of the coding transcript region. (B) Magnified view of 5'UTR region shows a sperm specific element virtually absent in equivalently prepared testes samples.

Supplementary Table 1: Transcripts identified between human and bovine spermatozoa

Transcript (Percentile Ranking in Human)	Transcript Name	Reported function #	References
SMCP (0.99)	Sperm mitochondria-associated cysteine-rich protein	Sperm motility	(Nayernia, et al., 2002)
GLUL (0.99)	Glutamate-ammonia ligase	Functional role in sperm not reported	
GPX4 (0.99)	Glutathione peroxidase 4 (phospholipid hydroperoxidase)	Sperm motility and tail morphology.	(Imai, et al., 2009)
DNAJB7 (0.99)	Dnaj (Hsp40) homolog, subfamily B, member 7	Functional role in sperm not reported Probably acts as a co-chaperone, Evidence at transcript level	
TPPP2 (0.99)	Tubulin polymerization-promoting protein family member 2	Essential for progression of germ cells past the pachytene stage of spermatogenesis May regulate sperm motility	(Zindy, et al., 2001)
SPATA3 (0.99)	Spermatogenesis associated 3	Functional role in sperm not reported	
GTSF1L (0.99)	Gametocyte specific factor 1-like	Functional role in sperm not reported	
TCEB2 (0.99)	Transcription elongation factor B polypeptide 2	Functional role in sperm not reported	
TAF10 (0.99)	Transcription initiation factor TFIID subunit 10	Functional role in sperm not reported May play a role in sperm morphogenesis, cell cycle progression and embryogenesis.	(Mohan, et al., 2003)

CCDC91 (0.99)	Coiled-coil domain containing protein 91	Functional role in sperm not reported	
ZNF706 (0.99)	Zinc finger protein 706	Functional role in sperm not reported	
CCDC54 (0.98)	Coiled-coil domain-containing protein 54	Functional role in sperm not reported	
PAFAH1B1 (0.98)	Platelet-activating factor acetylhydrolase IB subunit alpha	Role in spermiogenesis and sperm motility Role in neuronal migration and early embryo development	(Hirotsume, et al., 1998, Nayernia, et al., 2003)
FXR1 (0.98)	Fragile X mental retardation syndrome-related protein 1 RNA-binding protein	Involved in movement/motility that are energy dependent.	(Huot, et al., 2001)
SPATA6 (0.98)	Spermatogenesis-associated protein 6	Functional role in sperm not reported May play a role in spermatid maturation or sperm function	
UBE2N (0.98)	Ubiquitin-conjugating enzyme E2 N	Functional role in sperm not reported	
SRPK2 (0.98)	SRSF protein kinase 2 Serine/arginine-rich protein-specific kinase	Condensation of sperm chromatin	(Kuroyanagi, et al., 1998, Papoutsopoulou, et al., 1999)
TSPAN6 (0.98)	Tetraspanin-6	Functional role in sperm not reported	
CAPZB (0.98)	CAPZB protein F-actin capping protein subunit beta 2	Proposed to have a role on capacitation and acrosome reaction	(Chen, et al., 2012)

DDX4 (0.98)	Probable ATP-dependent RNA helicase DDX4 DEAD Box Protein 4	Germ cell development. sperm motility	(Li, et al., 2010)
MLF1 (0.97)	Myeloid leukemia factor 1	Functional role in sperm not reported	
C15orf23 (0.97)	Small kinetochore-associated protein	Functional role in sperm not reported Involved in mitosis, cell division from metaphase to anaphase	(Fang, et al., 2009)
C6orf225 (0.97)	Putative uncharacterized protein	Functional role in sperm not reported	
FILIP1L (0.97)	Filamin-A-interacting protein 1	Functional role in sperm not reported	
HEMGN (0.97)	Hemogen	Functional role in sperm not reported Expressed in round spermatids Testis-specific isoform of <i>HEMGN</i> proposed to have a role in spermatogenesis, first meiosis	(Yang, et al., 2003) (Liu, et al., 2013)
TMBIM6 (0.97)	Bax inhibitor 1 Testis-enhanced gene transcript protein	Functional role in sperm not reported Suppressor of apoptosis Highly abundant in testis.	(Walter, et al., 1995)
C12orf54 (0.97)	Uncharacterized protein C12orf54 (HSD 29)	Functional role in sperm not reported May regulate hormone activity?	(Oppermann and Maser, 1996)
GKAP1 (0.96)	G kinase-anchoring protein 1	Functional role in sperm not reported May have a role through the interaction with Golgi-associated proteins during spermatogenesis.	(Yuasa, et al., 2000)
CHMP5 (0.96)	Charged multivesicular body protein 5	Functional role in sperm not reported	

ARL4A (0.96)	ADP-ribosylation factor-like protein 4A	Spermatid maturation and sperm production	(Jacobs, et al., 1998, Schurmann, et al., 2002)
SPATA19 (0.95)	Spermatogenesis-associated protein 19, mitochondrial Spermatogenic cell-specific gene 1 protein	Functional role in sperm not reported Act as a binding molecule between adjacent mitochondria in the sheath	(Doiguchi, et al., 2002)
GSTM3 (0.95)	Glutathione S-transferase Mu 3	Functional role in sperm not reported Sperm fibrous sheath function	(Fulcher, et al., 1995)
POLR21 (0.95)	DNA-directed RNA polymerase 21 kDa subunit	Functional role in sperm not reported	
TNP1 (0.99)	Spermatid nuclear transition protein 1 *	Chromatin condensation	(Yu, et al., 2000)
HMGB4 (0.99)	High mobility group protein B4 *	Chromatin-associated protein, present in the euchromatin of late pachytene spermatocytes and haploid round spermatids. Stronger expression is observed during the elongation phase of spermatid	(Catena, et al., 2009)
TMCO2 (0.99)	Transmembrane and coiled-coil domain-containing protein 2 *	Functional role in sperm not reported	
H3F3C (0.99)	Histone H3.3C *	Sperm nucleosome assembly	(Henikoff, et al., 2004, Loppin, et al., 2005)
UBB (0.99)	Polyubiquitin-B *	Functional role in sperm not reported May be important for acrosomal reaction during fertilization process.	(Hou and Yang, 2012)
DCUN1D1 (0.99)	DCN1-like protein 1 *	Functional role in sperm not reported	

CA2 (0.99)	Carbonic anhydrase 2 *	Associated with motility The CA2 protein is expressed in the spermatogenic stage of elongating spermatids and localized in the sperm tail. The expression of CA2 is high asthenozoospermic men	(Zhao, et al., 2010)
UQCRB (0.98)	Cytochrome b-c1 complex subunit 7 *	Functional role in sperm not reported Mitochondrial respiration	
CAPZA3 (0.97)	F-actin-capping protein subunit alpha-3/ Germ cell-specific protein 3 *	Functional role in sperm not reported Capacitation reaction	(Sosnik, et al., 2010)
SERF2 (0.97)	Small EDRK-rich factor 2 *	Functional role in sperm not reported	
GABARAP (0.96)	GABA(A) receptor-associated protein *	Functional role in sperm not reported	
RPL35 (0.96)	60S ribosomal protein L35*	Functional role in sperm not reported	
DNAJB3 (0.95)	DNAJ homolog subfamily B member 3*	Acrosomal reaction and sperm-oocyte interaction	(Berruti and Martegani, 2005, Meccariello, et al., 2008)

The transcripts were selected and compared based on the RNA seq data from human (Sendler et al., 2013) and bovine (Card, et al., 2013). The top 5% of the transcripts of the RNA seq data from human were compared with bovine (FPKM>100).

Orthologous genes were identified with Genomatix RegionMiner: Search for orthologous GeneIDs module

Only stallion, bovine were compared for abundance, as these are the only species for which equivalent RNA-seq data is available

\* represents genes which do not show required level of similarity to be classified as orthologous by this module, but which nevertheless share similar name to human transcript

# The functions were checked in [www.gpubmed.com](http://www.gpubmed.com), [www.iHop-net.com](http://www.iHop-net.com) and [www.uniprot.org](http://www.uniprot.org)

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Supplementary Table 2: Transcripts identified between human and stallion spermatozoa

Transcript (Percentile Ranking in Human)	Transcript Name	Reported function #	References
HNRNPH1 (0.99)	Heterogeneous nuclear ribonucleoprotein H	Functional role in sperm not reported It is an anti-apoptosis protein, expressed in the nucleus of spermatocytes.	(Zhu, et al., 2011)
HDAC11 (0.99)	Histone deacetylase 11	Functional role in sperm not reported Highly conserved from C.elegans to man.	(Doiguchi, et al., 2002)
ODF2 (0.99)	Outer dense fiber protein 2 (ODF of sperm tails protein 2)	Sperm motility	(Tarnasky, et al., 2010)
ACE (0.99)	Angiotensin-converting enzyme 2	Sperm transport in oviduct	(Chen, et al., 2012)
REEP6 (0.99)	Receptor expression-enhancing protein	Localized to the posterior head, may involve in sperm-egg fusion	(ZHANG Mian-qiu, 2011)
AKAP1 (0.99)	A kinase-anchoring proteins	pKa signalling in spermatozoa, various functions including motility	(Lin, et al., 1995)
PICK1 (0.99)	Protein interacting with C kinase 1	Sperm morphogenesis, acrosome formation and sperm motility	(Xiao, et al., 2009)
UBXN6 (0.99)	UBX domain-containing protein 6	Functional role in sperm not reported May enhance the expression of genes involved in apoptosis, cell cycling or targeting of proteins for degradation	(Carim-Todd, et al., 2001)
HGS (0.99)	Hepatocyte growth factor-regulated tyrosine kinase substrate	Functional role in sperm not reported Involved in intracellular signal transduction mediated by cytokines and growth factors	(Itman, et al., 2011)
CLK3 (0.99)	Dual specificity protein kinase CLK3	Functional role in sperm not reported Proposed to regulate capacitation and acrosomal reaction	(Menegay, et al., 1999)
CALCOCO2 (0.99)	Calcium-binding and coiled-coil domain-	Functional role in sperm not reported	

	containing protein 2		
LPIN1 (0.99)	Phosphatidate phosphatase LPIN1	Functional role in sperm not reported	
TCP11 (0.99)	T-complex protein 1 subunit alpha	Sperm capacitation and promote fertilizing ability	(Fraser, et al., 1997)
OAZ3 (0.99)	Ornithine decarboxylase antizyme 3	Involved in spermiogenesis male mice are infertile due to the separation of sperm heads and tails,	(Tokuhiro, et al., 2009)
CARM1 (0.99)	Coactivator-associated arginine methyltransferase 1 (Histone-arginine methyltransferase)	Functional role in sperm not reported Plays Complex role in controlling gene expression, regulating transcription, DNA packaging, mRNA stability and translation. Mainly involved in estrogen dependent actions?	(Yadav, et al., 2003)
ERGIC3 (0.99)	Endoplasmic reticulum-Golgi intermediate compartment protein 3	Functional role in sperm not reported Possible role in transport between endoplasmic reticulum and Golgi	
NSUN4 (0.99)	5-methylcytosine rRNA methyltransferase NSUN4	Functional role in sperm not reported Involved in mitochondrial ribosome large subunit biogenesis.	
FUS (0.99)	RNA-binding protein FUS	Functional role in sperm not reported Spermatogenic RNA-binding proteins Involved in meiotic stage of spermatogenesis	(Kuroda, et al., 2000)
IQCE (0.99)	IQ domain-containing protein E	Functional role in sperm not reported	
HABP4 (0.99)	Intracellular hyaluronan-binding protein 4	Functional role in sperm not reported Hyaluronan binding proteins are involved in sperm maturation, motility	(Ranganathan, et al., 1994)
GPI (0.99)	Glucose-6-phosphate isomerase SA-36 (Sperm antigen 36)	Sperm agglutination	(Ronfani, et al., 2001)
RERE (0.99)	Arginine-glutamic acid dipeptide repeats protein	Functional role in sperm not reported	

DDX5 (0.99)	Probable ATP-dependent RNA helicase DDX5/DEAD box protein 5	Functional role in sperm not reported May regulate late pachytene spermatocytes and haploid spermatids development. Play an important roles in diverse biological processes such as development, regulation of transcription, RNA processing, and ribosome biogenesis, miRNA pathway and signaling events	(Arun, et al., 2012) Reviewed in (Cordin, et al., 2006)
GAPDHS (0.99)	glyceraldehyde-3-phosphate dehydrogenase-S	Sperm Motility May play an important role in regulating the switch between different pathways for energy production during spermiogenesis and in the spermatozoon. Required for sperm motility and male fertility	(Li and Zhang, 2013, Miki, et al., 2004)
VPRBP (0.99)	Protein VPRBP	Functional role in sperm not reported Involved in spermiogenesis	(Nagamori, et al., 2005)
PDE4A (0.99)	cAMP-specific 3',5'-cyclic phosphor-diesterase 4A	cAMP specific involved in regulating sperm motility (PDE4 inhibitors enhanced sperm Motility)	(Conti, et al., 1995, Fisch, et al., 1999)
CIB1 (0.99)	Calcium and integrin-binding protein 1	Functional role in sperm not reported Regulate cell cycle and spermatogenesis	(Yuan, et al., 2006)
NRD1 (0.98)	Nardilysin	Functional role in sperm not reported Associated with the morphological transformations during final steps of spermiogenesis.	(Chesneau, et al., 1996)
CALR3 (0.98)	Calreticulin-3	Functional role in sperm not reported This gene is specifically expressed in elongated spermatid May regulate sperm transport in the female reproductive tract and spem-zona binding	(Hayashi, et al., 2007, Ikawa, et al., 1997, Yamagata, et al., 2002)
C17orf105 (0.98 )		Functional role in sperm not reported	
NBR1 (0.98)	Next to BRCA1 gene 1 protein	Functional role in sperm not reported Probably involved in spermiogenesis Receptor for selective autophagosomal degradation of ubiquitinated targets	(Dimitrov, et al., 2001)
FGFR1OP	FGFR1 oncogene partner	Functional role in sperm not reported	

(0.98)		Required for anchoring microtubules to the centrosomes	
PPP6R1 (0.98)	Serine/threonine-protein phosphatase 6 regulatory subunit 1	Functional role in sperm not reported Ubiquitous with higher expression in testis	(Stefansson and Brautigan, 2006)
NDEL1 (0.98)	Nuclear distribution protein nudE-like 1	Functional role in sperm not reported NUDEL plays an important role in germ cell formation, including nucleoplasmic transport and nucleus shaping by manchette microtubules	(Yamaguchi, et al., 2004)
CLIP3 (0.98)	CAP-Gly domain-containing linker protein 3	Functional role in sperm not reported Higher affinity to bind to Akt2 and may regulate glucose transport	(Couesnon, et al., 2013)
CHD5 (0.98)	Chromodomain-helicase-DNA-binding protein 5	Functional role in sperm not reported	
DNAJA4 (0.98)	DnaJ (Hsp40) homolog, subfamily A, member 4	Functional role in sperm not reported Expression is abundant in testis and elongated spermatids	(Hafizur, et al., 2004)
REXO1 (0.98)	REXO1	Associated to sperm concentration and sperm morphology, and mild testicular atrophy.	(Rezende, et al., 2011)
MORC2 (0.98 )	MORC family CW-type zinc finger protein 2	Functional role in sperm not reported Regulate chromatin remodeling	(Li, et al., 2012)
TRA2A (0.98)	Transformer-2 protein homolog alpha	Functional role in sperm not reported Sequence-specific RNA-binding protein which participates in the control of pre-mRNA splicing	
PDXDC1 (0.97)	Pyridoxal-dependent decarboxylase domain-containing protein 1	Functional role in sperm not reported	
GGNBP2 (0.97)	Gametogenetin-binding protein 2	Functional role in sperm not reported Germ cell development. Suggested to have a role in spermatocytogenesis and spermiogenesis	(Zhang, et al., 2005)
RNF168	E3 ubiquitin-protein ligase	Functional role in sperm not reported	(Bohgaki, et al., 2011)

(0.97)	RNF168	Suggested function in Spermatogenesis	
ARHGAP33 (0.97)	Rho GTPase-activating protein 33	Functional role in sperm not reported	
RBBP6 (0.97)	E3 ubiquitin-protein ligase RBBP6 p53-associated cellular protein of testis	Functional role in sperm not reported Highly expressed in the placenta and testis Ubl conjugation pathway	
KIF17 (0.97)	Kinesin-like protein KIF17	Functional role in sperm not reported Microtubule-dependent mobility of chromatoid bodies, as well as for the transport of the specific components in and out of the chromatoid body Spermatogenesis	(Kotaja, et al., 2006, Kotaja, et al., 2005)
ZRANB1 (0.97 )	Ubiquitin thioesterase ZRANB1	Functional role in sperm not reported	
ACIN1 (0.97 )		Functional role in sperm not reported	
CKAP5 (0.97 )	CKAP5 protein	Functional role in sperm not reported	
CEP152 (0.97)	Centrosomal protein of 152 kDa	Functional role in sperm not reported Centrosomal protein; maintaining genomic stability through regulation of the DNA damage response	
LRRC27 (0.97)	Leucine-rich repeat-containing protein 27	Functional role in sperm not reported	
HIP1 (0.97)	Huntingtin-interacting protein 1	Stabilizing actin and microtubules, which are important cytoskeletal elements enabling normal spermatid and Sertoli cell morphology and function. Regulates sperm motility?	(Khatchadourian, et al., 2007)
SPATA24	Spermatogenesis-	Functional role in sperm not reported	

(0.97)	associated protein 24	Cytoplasm movement and removal during spermiogenesis??	
IPO5 (0.96)	IPO5 protein	Functional role in sperm not reported Functions related to germ cell development Transport factors between the nucleus and cytoplasm at specific stages of spermatogenesis	(Loveland, et al., 2006)
INTS1 (0.96)	Integrator complex subunit 1	Functional role in sperm not reported Involved in the small nuclear RNAs (snRNA) U1 and U2 transcription and processing. May have a role in spermiogenesis Essential for development of central nervous system in early stage embryos	(Shackleford and Varmus, 1987) (Ariel, et al., 1991)
SLC25A39 (0.96)	Solute carrier family 25 member 39	Functional role in sperm not reported Abundantly expressed in testis.	
CAMKV (0.96)	CaM kinase-like vesicle-associated protein	Functional role in sperm not reported	
RAB3IL1 (0.96)	Guanine nucleotide exchange factor for Rab-3A	Functional role in sperm not reported Guanine nucleotide exchange factor (GEF) which may activate RAB3A, a GTPase that regulates synaptic vesicle exocytosis. Promotes the exchange of GDP to GTP	
VAV2 (0.95)	Guanine nucleotide exchange factor VAV2	Functional role in sperm not reported Plays an important role in angiogenesis. Its recruitment by phosphorylated EPHA2 is critical for EFNA1-induced RAC1 GTPase activation and vascular endothelial cell migration and assembly	
RFX3 (0.95)	Transcription factor RFX3	Functional role in sperm not reported Required for the biogenesis of motile cilia by governing growth and beating efficiency of motile cells	(Bonnafe, et al., 2004)
GOLGA2 (0.95)	Golgin subfamily A member 2	Functional role in sperm not reported Involved in spermatogenesis: acrosome biogenesis	(Moreno, et al., 2000)
FBL (0.95)	F Box protein	Functional role in sperm not reported	

GIGYF2 (0.99)	PERQ amino acid-rich with GYF domain-containing protein 2 *	Functional role in sperm not reported Present in testis May involved in receptor signaling	(Giovannone, et al., 2003)
UBC (0.99)	Polyubiquitin-C *	Sperm motility Ubiquitin, a house-keeping protein that marks other proteins for proteasomal degradation, tags defective sperm during epididymal passage	(Rawe, et al., 2002) (Sutovsky, et al., 2004)
TOB1 (0.98)	Protein Tob1 *	Functional role in sperm not reported ? Tob genes are expressed in oocytes, sperm, early embryos, and various adult tissues, depending on the species. Tob proteins play important roles in spermatogenesis, embryonic dorsoventral patterning.	Reviewed in (Jia and Meng, 2007)
ACRBP (0.98)	Acrosin-binding protein *	Mediates acrosin activation and zona binding. Involved in packaging and condensation of the acrosin zymogen in the acrosomal matrix via its association with proacrosin	(Veaute, et al., 2010) (Tardif, et al., 2012)
BSCL2 (0.97)	Seipin *	Functional role in sperm not reported Seipin localization is specifically observed in spermatids and absent in spermatozoa Highly expressed in brain, testis and adipose tissue	(Ito, et al., 2008)

The transcripts were selected and compared based on the RNA seq data from human (Sendler et al., 2013) and stallion (Das, et al., 2013). The top 5% of the transcripts of the RNA seq data from human were compared with stallion (FPKM >40 )]

Orthologous genes were identified with Genomatix RegionMiner: Search for orthologous GeneIDs module

Only stallion, bovine were compared for abundance, as these are the only species for which equivalent RNA-seq data is available

\* represents genes which do not show required level of similarity to be classified as orthologous by this module, but which nevertheless share similar name to human transcript

# The functions were checked in [www.pubmed.com](http://www.pubmed.com), [www.iHop-net.com](http://www.iHop-net.com) and [www.uniprot.org](http://www.uniprot.org)

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