SUPPLEMENTAL DATA – GFP-PSMA1 PIG

EMBRYO TRANSFER OUTCOMES

Six embryo transfers were performed, transferring 881 embryos. Eight piglets were recovered at C-Section (six breathed). Five of the eight did not express and one healthy expressing male founder was identified.

Surrogate #	# Transferred	Day of Surrogate	Status
O727	214	1	Cycled day 20
O709	142	1	Cycled day 28
O731	140	0	Delivered 5 (4 live) pigs 6/7/10 - none expressed
O762*	140	1	Delivered 2 piglets 6/14/10 - one never breathed
O753	120	0	Cycled day 25
O754	125	0	2 piglets delivered 6/29/10 - one never breathed and the other died on day 3

*Surviving piglet expressed the transgene.

TRANSGENIC OFFSPRING SEMEN ANALYSIS AND IVF

The sperm concentration of GFP offspring was significantly lower than wild type boar (0.4 *vs.* 1.5×10^9 spermatozoa/ml, $p<0.05$). Also the semen of GFP offspring showed high agglutination (head to head binding; **Suppl. Table 1**). Collected semen was diluted with X-Cell extender and sperm motility was measured during storage. The semen of GFP offspring showed 60-75% motility during storage, and the motility was significantly reduced on day 3 compared to the control wild type boar (87.5%, $p<0.05$; **Suppl .Table 2**).

There was no significant difference in monospermic fertilization between GFP offspring and wild type boar, however, the percentage of polyspermy was dramatically different (0.0% in GFP offspring *vs.* 63.9% in wild type boar, $p<0.05$; **Suppl. Table 3).** The percentage of cleaving embryos was significantly higher in wild type boar (68.8%) than in the GFP offspring (33.5%, $p<0.05$), but there was no significant difference in blastocyst formation rate (3.9% in GFP offspring vs. 3.4% in wild type boar). Additionally, mean cell number per blastocyst was higher in GFP offspring than in that of wild type boar (31.5 vs. 18.5; $p<0.05$; **Suppl. Table 4**). The expression of GFP was first detected in late 2 cell embryos and increased from 4-cell to blastocyst (56.0%; see **Fig. 3 B** in manuscript). We concluded that the male of GFP offspring was fertile *in vitro*.

Supplemental Table 1: Comparison of semen characteristics between GFP offspring and wild type boars

Boar	*Breed*	*Boar No.*	*Collected semen volume (ml)*	*Sperm concentration ($x10^9$/ml)*	*% motile spermatozoa*	*Notes*
GFP offspring	Minnesota mini	G137	117.5±7.5[a]	0.4±0.1[b]	75±5.0	High agglutinations
Wild type	Duroc	89-10	50±0.0[b]	1.5±0.1[a]	90±5.0	None

FOOTNOTES:

- Semen was collected twice from each boar.
- Mean±SEM for 2 ejaculates from each boar are shown.
- [a,b] Means in the same column with different superscripts differ significantly ($p<0.05$).

Supplemental Table 2: Comparison of sperm motility (%) during storage

Boar	*Storage period (day)*			
	1	*2*	*3*	*4*
GFP offspring	75±5.0	75±5.0	65±5.0[b]	60±5.0
Wild type	92.5±2.5	92.5±2.5	87.5±2.5[a]	87.5±2.5

FOOTNOTES:

- Boar semen was collected twice from each boar, diluted with X-Cell extender and stored at room temperature; sperm motility was assessed under stereomicroscope at 37.5°C.
- Mean±SEM for 2 ejaculates from each boar.
- [a,b] Means in the same column with different superscripts differ significantly ($p<0.05$).

Supplemental Table 3: Comparison of fertilization parameters on porcine IVF

Boar	*No. oocyte inseminated*	*% monospermic oocyte*	*% polyspermic oocyte*	*% total fertilization*
GFP offspring	49	22.3±6.5	0.0±0.0[b]	22.3±6.5[b]
Wild type	51	32.6±7.5	63.9±9.7[a]	96.5±3.6[a]

FOOTNOTES:

- Experiment was repeated four times, and data indicate Mean±SEM.
- [a,b] Means in the same column with different superscripts differ significantly ($p<0.05$).

Supplemental Table 4: Comparison of embryo development after IVF

Boar	*No. oocytes inseminated*	*% cleaving oocytes*	*% blastocyst*	*Mean cell no. per blastocyst*	*% GFP expression from embryos*
GFP offspring	56	33.5±3.7[b]	3.9±2.2	31.5±10.5	56.0±9.7
Wild type	55	68.8±8.1[a]	3.4±2.0	18.5±1.5	None

FOOTNOTES:

- Experiment was repeated four times, and data indicate Mean±SEM.
- [a,b] Means in the same column with different superscripts differ significantly ($p<0.05$).

METHODOLGY FOR SEMEN COLLECTION, IVM, IVF, IMMUNOLFUORESCENCE AND WESTERN BLOTTING

Semen collection and processing

Ejaculates from wild type boar and the *PSMA1-GFP* transgenic Minnesota Mini boars were collected under the guidance of approved Animal Care and Use (ACUC) protocols of the University of Missouri, Columbia (UM). The wild-type boar was placed on a routine weekly collection while the transgenic boars were collected less frequently, as needed. Sperm-rich

fractions of the ejaculates with greater than 85% sperm motility and normal sperm acrosomes were used. Sperm concentrations were estimated using a hemacytometer (Fisher Scientific, Houston, TX, USA). The percentage of motile sperm was estimated at 38.5°C by light microscope at 250 x magnification. Semen was slowly cooled to room temperature (20°C) within 2 hrs after collection. Semen was then transferred into 15 ml tubes, centrifuged at room temperature for 10 min at 800 x *g*, and the supernatant was removed. The spermatozoa were processed according to each experiment's requirements and stored at -80°C. In order to use the semen for *in vitro* fertilization (IVF), the semen was diluted with X-Cell Extender (Cat. #USA851X, IMV Technologies, Maple Grove, MN; final concentration of 1×10^8 spermatozoa/ml). The diluted semen was stored in a styrofoam box at room temperature for 5 days. Unless otherwise noted, all chemicals used in this study were purchased from Sigma Chemical Co. (St. Louis, MO).

Collection and *In Vitro* Maturation (IVM) of Porcine Oocyte

Ovaries were collected from prepubertal gilts at a local slaughterhouse and transported to the laboratory in a warm box (25-30°C). Cumulus-oocyte complexes (COCs) were aspirated from antral follicles (3-6 mm in diameter), washed three times in HEPES-buffered Tyrode lactate (TL-HEPES-PVA) medium containing 0.01% (w/v) polyvinyl alcohol (PVA), then washed three times with the maturation medium [1]. Each time, a total of 50 COCs were transferred to 500 µl of the maturation medium that had been covered with mineral oil in a 4-well multidish (Nunc, Roskilde, Denmark) and equilibrated at 38.5°C, with 5% CO_2 in air. The medium used for oocyte maturation was tissue culture medium (TCM) 199 (Gibco, Grand Island, NY) supplemented with 0.1% PVA, 3.05 mM D-glucose, 0.91 mM sodium pyruvate, 0.57 mM cysteine, 0.5 µg/ml LH (L5269, Sigma), 0.5 µg/ml FSH (F2293, Sigma), 10 ng/ml epidermal growth factor (E4127, Sigma), 10% porcine follicular fluid, 75 µg/ml penicillin G, and 50 µg/ml streptomycin. After 22 h of culture, the oocytes were washed twice and cultured in TCM199 without LH and FSH for 22 h at 38.5°C, 5% CO_2 in air.

***In Vitro* Fertilization (IVF) and Culture (IVC) of Porcine Oocytes**

After IVM, cumulus cells were removed with 0.1% hyaluronidase in TL-HEPES-PVA medium and ova were washed three times with TL-HEPES-PVA medium and three times with Tris-buffered (mTBM) medium [1] containing 0.2% BSA (A7888, Sigma). Thereafter, 20 oocytes were placed into each of four 100 µl drops of the mTBM medium, which had been covered with mineral oil in a 35 mm polystyrene culture dish. The dishes were allowed to equilibrate in the incubator for 30 min until spermatozoa were added for fertilization. One ml liquid semen preserved in X-Cell Extender was washed twice in PBS containing 0.1% PVA (PBS-PVA) at 800×*g* for 5 min, respectively. At the end of the washing procedure, the spermatozoa were resuspended in mTBM medium. After appropriate dilution, 1 µl of this sperm suspension was added to the medium that contained oocytes to give a final sperm concentration of 5×10^5 sperm/ml. Oocytes were co-incubated with spermatozoa for 6 hrs at 38.5°C, 5% CO_2 in air. At 6

hrs after IVF, oocytes were transferred into 500 µl PZM-3 medium [2] containing 0.4% BSA (A6003, Sigma) for further culture during 16-19 hrs or 144 hrs.

Evaluation of Oocyte Fertilization and Embryo Culture

Semen collection, in vitro oocyte maturation and in vitro fertilization were performed using standard methods described in **Supplemental Data** File. For evaluation of fertilization, oocytes/zygotes or embryos were fixed with 2% formaldehyde for 40 min at room temperature, washed with PBS three times, permeabilized with PBS-TX for 40 min at room temperature, and stained with 2.5 µg/ml DAPI (Molecular Probes, Eugene, OR) for 40 min. Sperm penetration and fertilization status of the zygotes (unfertilized, fertilized-monospermic or fertilized-polyspermic) or the number of nuclei in embryos/blastocyst were assessed under epifluorescence microscope. Image acquisition was performed on a Nikon Eclipse 800 microscope (Nikon Instruments Inc., Melville, NY) with Cool Snap camera (Roper Scientific, Tucson, AZ) and MetaMorph software (Universal Imaging Corp., Downington, PA). The same imaging system was used for the analysis of tissue fragments collected from stillborn transgenic siblings of the founder boar; microscopic tissue fragments were whole-mounted on ceroscopy slides in TL-HEPES medium with 10% PVP and directly imaged under epifluorescence illumination at the excitation wave length corresponding to peak excitation wavelength of GFP. To assure that the resultant signals were not due to autofluorescence, control acquisitions were also made in the UV and red excitation & emission bands. None of the GFP-patterns described in this study were observed in tissues of non-transgenic offspring.

Immunofluorescence of Boar Spermatozoa

Spermatozoa were affixed to poly-lysine treated microscopy coverslips and fixed in 2% formaldehyde, washed, permeablized in PBS with 0.1% Triton-X-100 (PBS-TX) and and blocked in PBS-TX containing 5% normal goat serum. Spermatozoa were incubated with mouse monoclonal antibody raised against the green fluorescent protein (GFP) from the jellyfish *Aequorea victoria* (1:100 dilution, cat #33-2600; Zymed Laboratories Inc., South San Francisco, CA, USA) overnight. Then they were incubated in PBS-TX containing 1% normal goat serum with goat-anti-mouse (GAM)-IgG-FITC (1:100 dilution; Zymed – Invitrogen) and DAPI (1:100; Molecular Probes - Invitrogen) for 40 min. Image acquisition was performed as described for oocytes.

Western blotting

Sperm were washed in protein-free PBS and sperm concentration was determined using a hemocytometer so that approximately 1 x 10^9 spermatozoa/ml were loaded per lane after extraction. Spermatozoa were washed again in PBS and boiled for 5 min with loading buffer (50 mM Tris (pH 6.8), 150 mM NaCl, 2% SDS, 20% glycerol, 5% β-mercaptoethanol, 0.02% bromophenol blue). Gel electrophoresis of 10 µl total protein/lane was performed on 4-20% gradient gels (PAGEr Gels, Lonza, Rockland, ME), followed by protein transfer to PVDF

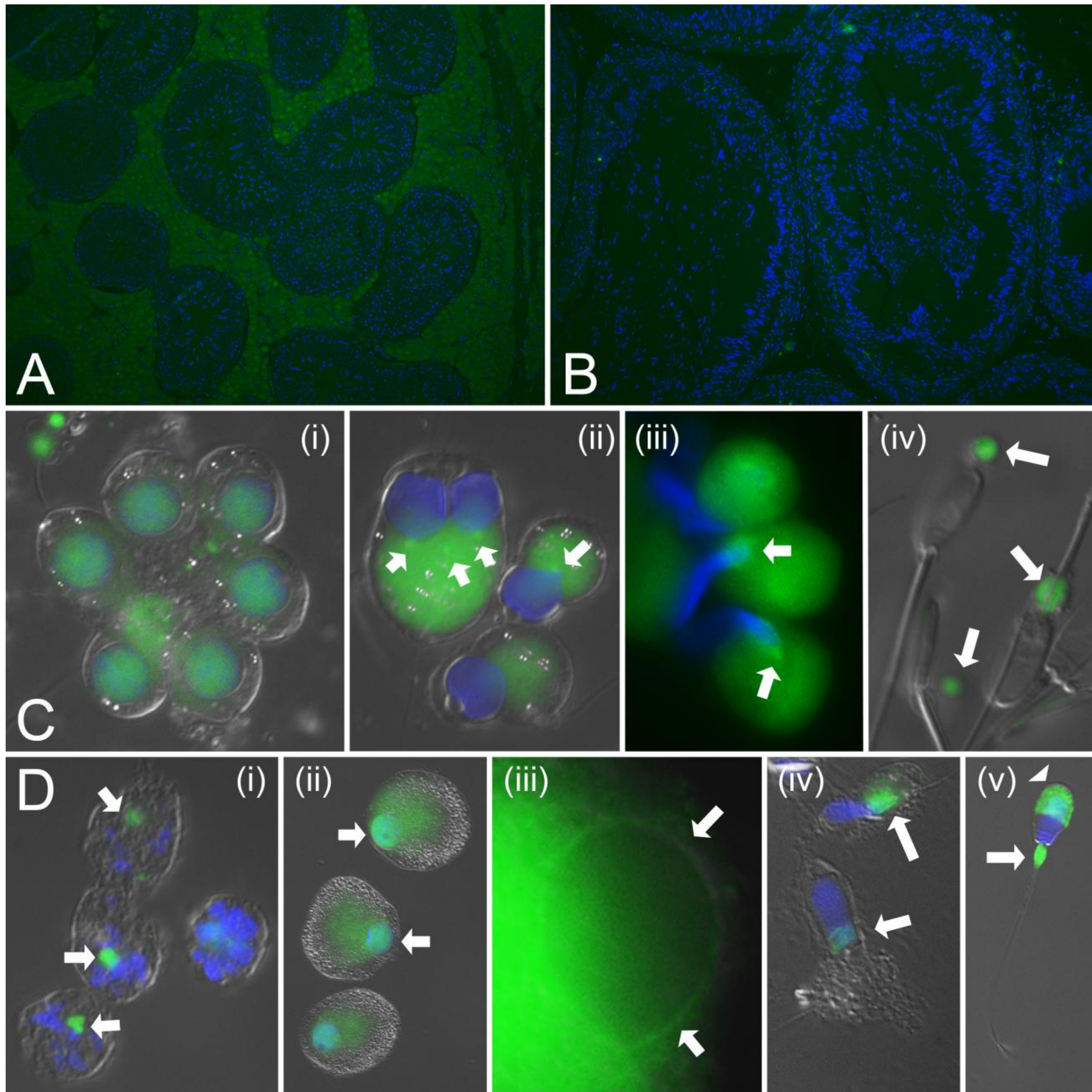
membranes (Immobilon P, Millipore Corp., Billerica, MA) using an Owl wet transfer system (Fisher Scientific, Houston, TX) at a constant 50 V for 4 hrs. The membranes were sequentially incubated with 10% non-fat milk for 1 hr and with one of the following antibodies: mouse monoclonal anti-GFP antibody (1:2000 dilution, cat. # 33-2600; Zymed - Invitrogen), mouse monoclonal anti-GFP antibody (1:2000 dilution, cat. #A11120; Invitrogen), mouse monoclonal anti-PSMA1/α-6 proteasome subunit (1:2000 dilution, cat. #PW9390; Enzo), mouse monoclonal anti-proteasome 20S core subunits alpha-type 1,2,3,5,6, & 7 (1:2,000 dilution, cat. #PW8195; Enzo), mouse monoclonal anti-proteasome 20S core antibodies (1:2,000 dilution, cat. #PW8155; Enzo), or mouse monoclonal anti-MFGE8 antibody (1:1,000 dilution, cat. #D199-3; MBL) overnight. The membranes were washed and incubated with an appropriate species-specific secondary antibody such as the HRP-conjugated goat-anti-mouse (GAM-IgG-HRP), HRP-goat-anti-rabbit IgG or goat-anti-Armenian hamster IgG-HRP antibodies (1:10,000 dilution; used to detect anti-MFGE8 antibody) for 1 hr at room temperature in 1% nonfat dry milk in TBS/Tween. The membranes were washed and reacted with 1.5 mL of chemiluminescent substrate (Illuminata Crescendo, Millipore Corp., Billerica, MA) for 5 min prior to being exposed to Kodak BioMax Light film (Kodak, Rochester, NY, USA).

Immunoprecipitation and MALDI-TOF Mass Spectroscopy

Boar sperm extracts were immunoprecipitated with the anti-GFP antibody (catalog no. A11120; Invitrogen) by using the Seize X Protein G Immunoprecipitation Kit (Pierce), separated on 4–20% gradient gels (PAGEr Gels; Lonza) and stained with Coomassie blue. The immunoprecipitated bands were excised carefully from the Coomassie blue-stained gel, destained, reduced with DTT, alkylated with iodoacetamide, and then trypsinized overnight. The digest solutions were recovered from the gel pieces and transferred to Axygen MAXYMum Recovery microtubes. The gel pieces were extracted further, pooled, and lyophilized dry. The dried digests were reconstituted and analyzed by Nano-LCNanospray quadrupole time-of-flight MS plus MS/MS on an Agilent 6520A mass spectrometer. The "MS plus MS/MS" data were analyzed with the "Find Compounds by Auto MS/MS" program in the Agilent Mass Hunter software (version B.04.00) suite. The MALDI-TOF MS spectra peak lists were obtained for the spectra after internal recalibration using trypsin autolysis fragment masses, computer baseline correction, noise removal, and peak de-isotoping. The threshold for generating peak lists was set to 2% of the maximum observed peak area. Data were exported in the Mascot Generic Format (.mgf) for submission to an in-house copy of Matrix Science's Mascot program (www.matrixscience.com). Database searches were performed against the NCBInr Mammalian protein databases (last updated September 19, 2011) and were adjusted for trypsin digestion with no missed cleavage, fixed modification by carbamidomethylation, and variable modification by methionine oxidation. Mowse and Mascott ion scores were used to identify highly probable matches with known amino acid sequences.

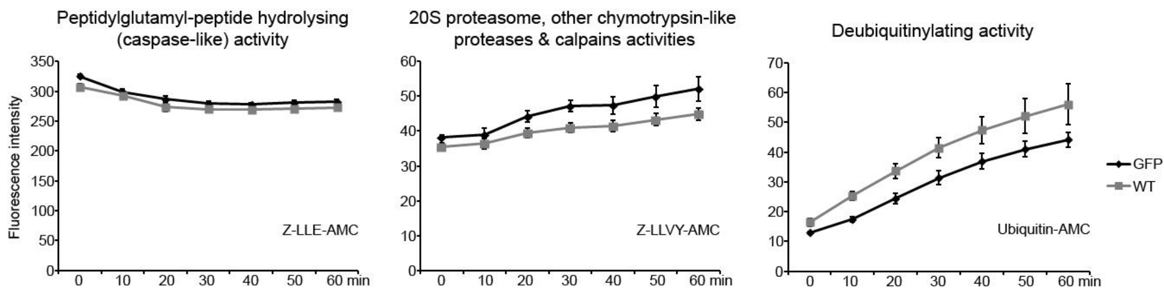
Statistical Analysis

Analyses of variance were carried out using the Statistical Analysis Software package in a completely randomized design. Duncan's multiple range test was used to compare values of individual treatment when the F-value was significant ($P < 0.05$).



Supplemental Figure 1. Histological and immunocytochemical analysis of the gonads and testicular germ cells of the founder PSMA1-GFP boar. (**A, B**) Histology of adult transgenic male gonads reveals normal testicular (A) and epididymal (B) tissue architecture, normal spermatogenesis in the testis (A) and abundant spermatozoa within the epididymal tubule lumen (B). (**C**) Live cell imaging of PSMA1-GFP fluorescence in the germ cells of founder boar. Green fluorescence is visible in the nuclei of round spermatids (i), at the base of the nucleus, probably the chromatoid body or the site of flagellum biogenesis (arrows) in the early step elongating spermatids (ii), in the caudal manchette (arrows) of elongated spermatids (iii), and in the cytoplasmic droplets (arrows) of fully differentiated spermatozoa (iv). Green color channel was

contrast-enhanced due to its low intensity. (**D**) Amplification of PSMA1-GFP1 fluorescence by anti-GFP antibody in the fixed, permeabilized testicular cells of the founder boar. Fluorescence is concentrated in the chromatoid bodies (arrows) of secondary spermatocytes (i), in the nuclei (arrows) of round spermatids, in the subacrosomal/inner acrosomal membrane layer (arrows) of an early step elongating spermatid (iii), in the caudal manchette (iii) of the elongated spermatids (iv), and in the acrosomal cap (arrowhead) and cytoplasmic droplet (arrow) of a fully differentiated testicular spermatozoon. The localization of PSMA1-GFP in panels C and D corroborates previous reports of proteasomal subunit localization in the hotspots of spermatid protein recycling such as the nucleus, redundant nuclear envelopes, caudal manchette, acrosomal cap and cytoplasmic droplet [3-6]. Localization of a proteasomal subunit to the chromatoid body is reported for the first time.



Supplemental Figure 2. Comparisons of proteasomal-proteolytic activities in spermatozoa of PSMA1-GFP offspring (GFP) and wild type boars (WT). Proteasomal proteolytic and deubiquitinating activities were measured using specific fluorometric substrates Z-LLE-AMC, Z-LLVY-AMC, and ubiquitin-AMC, respectively. The relative emitted fluorescence (no units) was measured at multiple time points to follow the kinetics of the reaction (ex: 380 nm, em: 460 nm). Experiments were repeated six times (with two different WT boars as a control). Values are expressed as the mean of fluorescence intensity±SEM.

Methodology: Proteasomal-proteolytic activity of boar spermatozoa was measured by a standard fluorometric proteasomal substrate assay as described [7]. Spermatozoa preserved in X-cell Extender were loaded onto a 96-well black plate (final sperm con. $1x10^6$ spermatozoa/ml), and incubated at 37.5°C with Z-LLE-AMC (a specific substrate for 20S chymotrypsin-like peptidyl-glutamylpeptide hydrolyzing [PGPH] activity, final conc. 100 μM; Enzo Life Sciences, Plymouth, PA), Z-LLVY-AMC (a specific substrate for 20S proteasome other chymotrypsin-like proteases and calpains; final con. 100 μM; Enzo), or ubiquitin-AMC (a specific substrate for deubiquitinylating activity; final conc. 300 nM; Enzo). The emitted fluorescence (no units) was measured every 10 min for a period of 1 hr, yielding a curve of relative fluorescence (excitation: 355 nm, emission: 460, Thermo Fluoroskan, ThermoFisher Scientific).

Supplemental Table 5 A: Proteasomal Subunits and Putative Proteasome-Interacting Proteins Co-immunoprecipitated with Anti-GFP-Antibody

Protein annotation	NCBI gi #	Score	% Coverage	MW (kDa)
A- PROTEASOMAL SUBUNITS				
Proteasome subunit alpha type-1 like	311248177	440	41	29.5
Proteasome subunit alpha type-3 isoform 1	194034201	985	39	28.4
Proteasome subunit alpha type-3 isoform 2	194034199	985	40	27.6
Proteasome subunit alpha type-4	347300165	1171	59	29.5
Proteasome subunit alpha type-4 isoform 1	347300165	669	64	29.5
Proteasome subunit alpha type-5	222136590	654	55	26.4
Proteasome subunit alpha type-6	8394076	1092	41	27.4
Proteasome subunit alpha type-7-like isoform 1	311259068	601	65	27.9
Proteasome (prosome, macropain) subunit, alpha type (alpha 7)	343887360	467	58	27.8
Proteasome subunit beta type-2 isoform 1	4506195	638	61	22.8
Proteasome subunit beta type-2 isoform 2	315139006	638	69	20.2
Proteasome subunit beta type-5	335292522	2006	59	30.1
Proteasome subunit beta type-6	344259274	566	61	25.4
Full-proteasome subunit beta type-7	194034199	275	39	30
Chain H, Crystal Structure of the Mammalian 20s Proteasome at 2.75 A Resolution (Proteasome subunit beta type-6)	21465649	607	65	21.9
B-OTHER SPERM PROTEINS				
Spermadhesins & Acrosome Associated Proteins				
Spermadhesin AWN	66990208	761	74	16.9
Major Seminal Plasma Glycoprotein PSP-I precursor	47523176	275	46	14.5
Seminal Plasma Sperm Motility Inhibitor	72535165	419	69	15
Seminal Plasma Protein pB1 precursor	47523184	178	43	15.4
Acrosin-binding protein (degradation product)	75052483	940	26	60.5
Seminal Plasma Acrosin Inhibitor A1	123986	177	67	7.6
Disintegrin/ADAM-Family Proteins				
Disintegrin and metalloproteinase domain-containing protein 5	323276507	443	23	45.1
Disintegrin and metalloproteinase domain-containing protein 20-like	311261282	322	26	82.5
Other Sperm Proteins & GFP				
Lactadherin	172072653	1264	49	47.8
Enhanced Green Fluorescent Protein	13194618	713	42	27
Ropporin-1-like	301783203	404	33	23.9

Supplemental Table 5 B: Immunoprecipitation & MS/MS Identification of Proteasome Interacting Sperm Proteins. Identified peptides are shown in red.

Proteasomal Subunits:

Protein	NCBI gi #	Score	% Coverage	MW (kDa)
Proteasome subunit alpha type-1 like	311248177	440	41	29.5

1 mfr**nqydndv tvwspqgrih qieyameavk** qgsatvglks kthavlvalk raqselaahq

61 k**kilhvdnhi gisiagltad arllcnfmr**q ecldsr**fvfd rplpvsrlvs ligsk**tqipt

121 qrygrrpygv glliagyddm gphifqtcps anyfdcrams igarsqsart yler**hmsefm**

181 **ecnlnelvk**h glralr**etlp aeqdlttknv sigivgk**dle ftiyddddvs pflegleerp

241 qrkaqpaqpa depaekadep meh

Protein	NCBI gi #	Score	% Coverage	MW (kDa)
Proteasome subunit alpha type-3 isoform 1	194034201	985	39	28.4

1 **mssigtgydl sastfspdgr vfqveyamka vensstaigi rckdgvvfgv ek**lvlsklye

61 egsnkrlfnv dr**hvgmavag lladar**slad iareeasnfr **snfgyniplk** hladrvamyv

121 haytlysavr pfgcsfmlgs ysvndgaqly midpsgvsyg ywgcaigkar qaakteiekl

181 qmkemtcrdv vkevak**iiyi vhdevkdkaf elelswvgei tk**grheivpk direeaekya

241 keslkeedes dddnm

Protein	NCBI gi #	Score	% Coverage	MW (kDa)
Proteasome subunit alpha type-3 isoform 2	194034199	985	40	27.6

1 **mssigtgydl sastfspdgr vfqveyamka vensstaigi rckdgvvfgv ek**lvlsklye

61 egsnkrlfnv dr**hvgmavag lladar**slad iareeasnfr **snfgyniplk** hladrvamyv

121 haytlysavr pfgcsvndga qlymidpsgv sygywgcaig karqaaktei eklqmkemtc

181 rdvvkevak**i iyivhdevkd kafelelswv geitk**grhei vpkdireeae kyakeslkee

241 desdddnm

Protein	NCBI gi #	Score	% Coverage	MW (kDa)
Proteasome subunit alpha type-4	347300165	1171	59	29.5

1 msrrydsr**tt ifspegr**lyq veyameaigh agtclgilan dgvllaaerr nihk**lldevf**

61 **fsek**iyk**lne dmacsvagit sdanvltnel r**liaqr**yllq yqepipceql vtalcdik**qa

121 ytqfggk**rpf gvsllyigwd khygfqlyqs dpsgnyggwk atcignnsaa avsmlkqdyk**

181 **egemtlksal alaik**vlnkt mdvsk**lsaek veiatltr**en gktvirvlk**q keveqlikk**h

241 eeeeakaere kkekeqkekd k

Protein	NCBI gi #	Score	% Coverage	MW (kDa)
Proteasome subunit alpha type-4 isoform 1	347300165	669	64	29.5

1 msrrydsrtt ifspegr**lyq veyameaigh agtclgilan dgvllaaer**r nihk**lldevf**

61 **fsek**iyk**lne dmacsvagit sdanvltnel r**liaqr**yllq yqepipceql vtalcdik**qa

121 ytqfggk**rpf gvsllyigwd khygfqlyqs dpsgnyggwk atcignnsaa avsmlkqdyk**

181 **egemtlksal alaik**vlnkt mdvsk**lsaek veiatltr**en gktvirvlkq keveqlikkh

241 eeeeakaere kkekeqkekd k

Proteasome subunit alpha type-5	222136590	654	55	26.4

1 **mfltr**seydr gvntfspegr **lfqveyaiea iklgstaigi qtsegvclav ekritsplme**
61 **pssiek**ivei dahigcamsg liadaktlid karvetqnhw ftynetmtve svtqavsnla
121 lqfgeedadp gamsrpfgva llfggvdek**g pqlfhmdpsg tfvqcdarai gsasegaqss**
181 **lqevyhksmt lk**eaik**ssli ilk**qvmeek**l natnielatv qpgqnfhmft keeleevikd**
241 **i**

Proteasome subunit alpha type-6	8394076	1092	41	27.4

1 msrgssagfd r**hitifspeg rlyqveyafk ainqggltsv avr**gkdcavi vtqkkvpdkl
61 ldsstvthlf k**itenigcvm tgmtadsr**sq vqraryeaan wkyk**ygyeip vdmlckr**iad
121 isqvytqnae mrplgccmil igideeqgpq vyk**cdpagyy cgfk**ataagv k**qtestsfle**
181 **kk**vkkkfdwt feqtvetait clstvlsidf kpseievgvv tvenpkfr**il teaeidahlv**
241 **alaerd**

Proteasome subunit alpha type-7-like isoform 1	311259068	601	65	27.9

1 masrydr**ait vfspdghlfq veyaqeavkk** gstavair**gt divvlgvekk** svaklqdert
61 vr**kicalddh vcmafaglta dar**vvinrar vecqshk**ltv edpvtveyit r**fiatlkqk**y**
121 **tqsngrrpfg isalivgfdd dgiprlyqtd psgtyhawk**a naigrsaktv reflek**nyte**
181 **daiandneai k**lair**allev vqsggkniel aiirr**nqplk mfsak**eielq vneiekekee**
241 **aek**kkskkta

Proteasome (prosome, macropain) subunit, alpha type	343887360	467	58	27.8

1 msydr**aitvf spdghlfqve yaqeavkk**gs tavgvr**grdi vvlgvekk**sv arlqdertvr
61 k**icalddnvc mafagltada r**ivinrarve cqshr**ltved pvtveyitr**y iaslkqrytq
121 sngr**rpfgis alivgfdfdg tprlyqtdps gtyhawk**ana igrgaksvre flek**nytdea**
181 **ietdgltik**l vik**allevvq sggk**nielav mrrdqplk**il npeeiekyva eiek**ekeene
241 kkkqkkas

Proteasome subunit beta type-2 isoform 1	4506195	638	61	22.8

1 meyligiqgp dyvlvasdrv aasnivqmkd dhdkmfkmse k**illlcvgea gdtvqfaeyi**
61 **qk**nvqlykmr **ngyelsptaa anftr**rnlad clrsr**tpyhv nlllagydeh egpalyymdy**
121 **laalakapfa ahgygafltl sildryytpt isr**eravell rkcleelqk**r filnlptfsv**
181 **riidkngihd ldnisfpk**qg s

Proteasome subunit beta type-2 isoform 2	315139006	638	69	20.2

1 meylidhdkm fkmsek**illl cvgeagdtvq faeyiqk**nvq lykmr**ngyel sptaaanftr**
61 rnladclrsr **tpyhvnllla gydehegpal yymdylaala kapfaahgyg afltlsildr**
121 **yytptisr**er avellrkcle elqk**rfilnl ptfsvriidk ngihdldnis fpk**qgs

Proteasome subunit beta type-5	335292522	2006	59	30.1

1 mfwrvvpfpl ldmalasvle rplavnrrgf fgfggradll dlgpgspgdg lslvapswgv
61 peepriemlh gtttlafk**fl hgvivaadsr atagayiasq tvkkvieinp yllgtmagga**
121 **adcsfwer**ll arqcriyelr nkerisvaaa sk**llanmvyq ykgmglsmgt micgwdkrgp**
181 **glyyvdsegn risgatfsvg sgsvyaygvm drgysydlev eqaydlar**ra iyqatyr**day**
241 **sggsvnlyhv redgwirvss dnvadlhdky sestp**

Proteasome subunit beta type-6	344259274	553	61	25.4

1 maatlvaarg aglapawghe aitpdwenre vstgttimav qfdggvvlga dsrtttgsyi
61 anrvtdkltp ihdrifccr**s gsaadtqava davtyqlgfh sielnepplv htaaslfk**em
121 cyr**yredlma giivagwdpq eggqvysvpm ggmmvrqafa iggsgssyiy gyvdatyreg**
181 **mtkeeclqft analalamer** dgssggvir**l aaiaesgver qvllgdqipk ftiatlppp**

Full-proteasome subunit beta type-7	160419232	275	39	30

1 maavsvyerp vggfsfdncr rnaileadfa kkgyklptar ktgttiagvv yk**dgivlgad**
61 **tr**ategmvva dkncskihfi spniyccgag taadtdmttq lissnlelhs lstgrlprvv
121 tanrmlk**qml fryqgyigaa lvlggvdvtg phlysiyphg stdklpyvtm gsgslaamav**
181 **fedkfrpeme eeeak**qlvse aiaagifndl gsgsnidlcv isksk**ldflr pysvpnkk**gt
241 rfgryrcekg ttavltek**vt aldievleet vqtmdts**

Chain H, Crystal Structure of the Mammalian 20s Proteasome at 2.75 A Resolution	21465649	607	65	21.9

1 **ttimavqfdg gvvlgadsr**t ttgsyianrv tdkltpihdr ifccr**sgsaa dtqavadavt**
61 **yqlgfhsiel nepplvhtaa slfk**emcyr**y redlmagiii agwdpqeggq vysvpmggmm**
121 **vr**qsfaiggs gssyiygyvd atyr**egmtke eclqftanal alamer**dgss ggvir**laaia**
181 **esgverqvll gdqipk**fava tlppa

Spermadhesins & Other Acrosome-Associated Proteins:

Spermadhesin AWN	66990208	761	74	16.9

1 mklgsailwa lllstatlvs gawnrrsrsc ggvlr**dppgk ifnsdgpqkd cvwtikvkph**
61 **fhvvlaippl nlscgkeyve lldgppgsei igkicggisl vfrsssniat ikylr**tsgqr
121 **aspfhiyyya dpegplpfpy ferqtiiate knip**

Major Seminal Plasma Glycoprotein PSP-I precursor	47523176	275	46	14.5

1 mklgsaipwa llfstatlis tgwgldyhac ggr**ltddygt iftyk**gpk**te cvwtlqvdpk**
61 ykllvsiptl nltcgk**eyve ilegapgsk**s lgk**fceglsi lnr**gssgmtv kykr**dsghpa**
121 **spyeiiflr**d sqg

Seminal Plasma Sperm Motility Inhibitor	72535165	419	69	15

1 mklgsaipwa lllstatlvs taqnkgsddc ggflk**nysgw isyykalttn cvwtiemkpg**

61 **hk**iilqilpl nltcgkeyle vrdqr**agpdn flkvcggttf vyqsssnvat vk**ysr**dshhp**

121 **assfnvyfyg ipqgak**a

Sp32 Acrosin-binding protein (degradation product)	75052483	940	26	60.5

1 qlaagsllsl lkvlllplap apaqdansas tpgsplspte yerffalltp twkaettcrl

61 rathgcrnpt lvqldqyenh glvpdgavcs dlpyaswfes fcqftqyrcs nhvyyakrvr

121 csqpvsilsp nslkevdtss evpittmtsp vsshitatgr qvfqpwperl nnnveellqs

181 slslggqeqg qehkqehkqe qgqehkqdeg qeqeeqeeeq eeegkqeegq gteesleams

241 glqadsepkf qsefvssnpf sftprvreve stpmmmeniq elirsaqemd emgdvyeeen

301 iwr**aqspgsl lqlphvdall vlcysivent cvitptakaw qyledetlgf gk**svcdslgr

361 **rhlaacslcd fcslk**leqch setnlqrqqc dnshk**tpfis pllasqsmsi gtqigtlk**sg

421 r**fygldlygg lrmdfwcar**l atkgcednr**v aswlqtefls fqdgdfptki cdteyvqypn**

481 **ycafk**sqqcm mrnrdrkvsr mrclqnetyt vltqaksedl vlrwsqefst ltlgqag

Seminal Plasma Acrosin Inhibitor A1	123986	177	67	7.6

1 trkqpncnvy r**shlffctrq mdpicgtngk syanpcifcs ek**glr**nqkfd fghwghcr**ey

61 tsars

Seminal Plasma Protein pB1 precursor	47523184	178	43	15.4

1 maprlgifll wagvsvflpl dpvngdqhlp gr**fltpaits ddkcvfpfiy k**gnlyfdctl

61 hdstyywcsv ttyymkrwry crstdyar**ca lpfifrgkey dscikegsvf skywcpvtpn**

121 **ydqdr**awryc

Disintergins (ADAM Family) Proteins:

Disintegrin and metalloproteinase domain-containing protein 5	323276507	443	23	45.1

1 mhsggvkdfs tcslddfkyf aahsgltclh silldepvyk qrrricgngi leqgeqcdcg

61 tlencthkhc cdprtcrrkr nkqcgsgecc tqdck**irpan vicr**ksadec dfieycngty

121 shcvadtfar ngqscesgsa ycyggrcrsf tkqcr**nligg estgasfscf deinsr**kdrf

181 gncgr**eycny phllcgk**lvc nwphkylisr anlsviyshv r**eqmcvstfl naek**ipr**dti**

241 **ttvqfpgdrd r**tfvqdgtvc gpemfclnfs cveikyrvny gecnssrhcn angvcnnfnh

301 chck**kgfvpp dcnvgngfgs iddghqsk**vg prrlwegkvl pskhrfqlif yislpvliia

361 ttaiiikqnk irelcyrget esegsvsees sssklsptv snsl

Disintegrin and metalloproteinase domain-containing protein 20-like	311261282	322	26	82.5

1 mgpasaqaql rgdpclpllw lflgpiccsy appgwrftas eiviprkvsh rvstaeiqgq
61 lsykirfggq rhvvhmrvkk sllprhfpvi tdndqgamqe dypfvprdcy yygylegvpg
121 smgtldtchg glrgmlqvdd ftyeikplea sskfehvisl lvtqktpged ekckiggedt
181 nqadeealla empr**agpvym wwphr**kyikl lytvahsyfl lnpnqtsvie nvvimnnilh
241 siyfqaglev cir**vlciwna gdgmrldiwr** dggslvtr**fg lwk**mqr**wqgm iphdtavllt**
301 **gr**rfgndryy ahrggicnpn wgasfvcvgn nhiflastla ahtlghmigc rhdgpgcrcf
361 rrdkcvmape tglldmlsnc syvtlhevvh rwdpclstsn vpynnfpyva nrcgdkklda
421 reecdcgtmk dcaedpccen sciltlgstc segsccvgcn yaqpgrmcrd vlgicdlpey
481 ctglthtcpd dsyiqdgtpc splavcvkgn csdr**dmqcqa lfgfnvkeaa picyr**tlnmr
541 gdrfgncgvr virgggkpvk **ceeddimcgm lhcanvqk**ip gggehttfrh ivvhdvtpk**t**
601 **cfgfdahfgt ltpqlglvvd gascgpgqfc k**dqnctfypd lnfscdvstc nfrgvcnnrr
661 **hchcqqgwkp pncdvegggg svdsgpppdk r**ketrakir**m svnievalll arfallcisg**
721 **iigslfhlre vvdqryeeta sekl**

Other Sperm Proteins & eGFP:

Lactadherin	172072653	1264	49	47.8

1 mpgprlltai cgallcasgl fafsgdfcds sqclnggtcl ldqdpqnpfh clcpegftgl
61 icnetekgpc fpnpchndae ceviddahr**g dvftqyick**c phgytgihce iicnaplgme
121 tgaiadfqis assmhlgfmg lqrwapelar lhr**agivnaw tasnydrnpw iqvnllr**rmr
181 vtgvvtqgas ragsaeyikt fkvayssdgr **kfqfiqgaee sgdkifmgnl dnsglkvnlf**
241 **evplevqyvr lvpiichr**gc tlr**fellgce lsgcaeplgl kdntipnkqi tassfyrtwg**
301 **lsafswypfy ar**ldnqgk**fn awtaqsnsas ewlqidlgsq r**rvtgiitqg ar**dfghiqyv**
361 **aaykvaysdd gvswteyr**dq galegk**ifpg nldnnshkkn mfetpfltr**f vr**ilpvawhn**
421 **r**itlrvellg c

Enhanced Green Fluorescent Protein	13194618 GenBank: AAK15492.1	713	42	27

1 mvsk**geelft gvvpilveld gdvnghkfsv sgegegdaty gk**ltlkfict tgklpvpwpt
61 lvttltygvq cfsrypdhmk qhdffk**samp egyvqertif fkddgnyk**tr aevk**fegdtl**
121 **vnr**ielk**gid fkedgnilgh kleynynshn vyimadkqk**n gikvnfkirh niedgsvqla
181 dhyqqntpig dgpvllpdnh ylstqsalsk dpnekrdhmv llefvtaagi tlgmdelyk

Ropporin-1-like	301783203	404	33	23.9

1 mpqtdk**qici ppelpellk**q ftkaairtqp qdliqwaady fgamshgeip pvrerser**va**
61 **lsnwaeltpe llk**ilhsrva grliihadel aqmwkvlslp tdlfnsvmnv gr**fteeiewl**
121 **k**flalacssl gvtiaktlk**i vcevlssdhd ggppr**ipfst fqflytyiae vdgeisashv
181 sr**mlnyieqe vigpdglik**v ndftqnprvr le

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