

Award Number: W81XWH-07-1-0422

TITLE: Proteomic Analysis of Prostate Cancer Field Effect

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REPORT DATE: February 2011

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

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# REPORT DOCUMENTATION PAGE

*Form Approved*  
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<b>1. REPORT DATE</b> February 2011		<b>2. REPORT TYPE</b> Revised Final		<b>3. DATES COVERED</b> 15 May 2007 – 31 January 2011	
<b>4. TITLE AND SUBTITLE</b>  Proteomic Analysis of Prostate Cancer Field Effect				<b>5a. CONTRACT NUMBER</b>	
				<b>5b. GRANT NUMBER</b> W81XWH-07-1-0422	
				<b>5c. PROGRAM ELEMENT NUMBER</b>	
<b>6. AUTHOR(S)</b>  Michael J. Wilson, Ph.D.  <b>E-Mail:</b> wilso042@umn.edu				<b>5d. PROJECT NUMBER</b>	
				<b>5e. TASK NUMBER</b>	
				<b>5f. WORK UNIT NUMBER</b>	
<b>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</b>  University of Minnesota Minneapolis, MN 55455-2070				<b>8. PERFORMING ORGANIZATION REPORT NUMBER</b>	
<b>9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)</b> U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				<b>10. SPONSOR/MONITOR'S ACRONYM(S)</b>	
				<b>11. SPONSOR/MONITOR'S REPORT NUMBER(S)</b>	
<b>12. DISTRIBUTION / AVAILABILITY STATEMENT</b> Approved for Public Release; Distribution Unlimited					
<b>13. SUPPLEMENTARY NOTES</b>					
<b>14. ABSTRACT</b>  We have undertaken a novel proteomic approach to identify proteins altered in both cancer and benign cells near the cancers. This is to take advantage of the "field effect" of the cancer, the malignancy associated changes in normal cells, to discover cancer altered protein that could be more abundant in serum or urine since they would come from both benign and cancer cells. We have started statistical evaluation of proteins comparing proteins in cancer (CaP), benign near (BN), benign distant (BD), and benign prostatic hyperplasia (BPH) tissue areas of 7 prostates. In the evaluation of proteins found in 5 of at least 7 prostate specimens, 3 were changed in levels of expression comparing CaP vs BN (calreticulin, peptidylprolyl isomerase B, transferin), 4 comparing CaP vs BD (beta-microseminoprotein, filamin A, histone cluster 2, four and half LIM domains 1). However, 29 proteins were significantly varied in expression in CaP vs BPH comparisons. Calculations using missing value imputation is now being carried out to seek possible other biomarker candidate proteins.					
<b>15. SUBJECT TERMS</b> mass spectroscopy, prostate cancer, benign prostatic hyperplasia, tumor field effect, benign/normal cells, proteomics					
<b>16. SECURITY CLASSIFICATION OF:</b>			<b>17. LIMITATION OF ABSTRACT</b>	<b>18. NUMBER OF PAGES</b>	<b>19a. NAME OF RESPONSIBLE PERSON</b>
<b>a. REPORT</b>	<b>b. ABSTRACT</b>	<b>c. THIS PAGE</b>			USAMRMC
U	U	U	UU	43	<b>19b. TELEPHONE NUMBER</b> (include area code)

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### Introduction:

Three major challenges in treatment of prostate cancer (CaP) are the lack of specificity in diagnosis using prostate specific antigen (PSA), cancer missed in biopsy negative patients, and inability to distinguish aggressive and latent prostate cancers in biopsies. Success in addressing these needs is dependent upon discovery of new biomarkers that are specific for diagnosis of cancer and classification of the tumor. The search for new biomarkers has been extensive and although a number of promising proteins have been identified, they have not yet proven acceptable. We undertook a different focus in this search, and that is a proteomic approach to identify proteins altered in both cancer and benign glands near the cancers. This is to take advantage of the “field effect” of the cancer, the malignancy associated changes in normal cells, to discover cancer altered proteins that could be more abundant in serum or urine since they would come from both benign and cancer cells, and/or be Gleason grade related. Our study design was that we collected prostate tissues (cross section of the gland) from men undergoing radical prostatectomy in which we selected areas that were cancer (CaP), benign close to the cancer (BN), benign distant to the cancer (BD), and benign prostatic hyperplastic (BPH). In brief, frozen sections from these specific areas are extracted, cleaved with trypsin, differentially iTRAQ<sup>TM</sup> labeled, subjected to two dimensional liquid chromatography (2DLC), and peptides identified through mass spectroscopy (MS/MS). There were 8 different iTRAQ<sup>TM</sup> labels within a 2DLC-MS run so that we could label 8 different tissue area samples (e.g., CaP, BN, BD, and BPH from 2 prostates). The ratios of the iTRAQ<sup>TM</sup> labels for a given protein allowed us to determine if that protein was greater in CaP and/or BN vs BD and/or BPH. Thus, through this approach we expected to identify proteins elevated in cancer and benign glands near cancer that would warrant further analysis as cancer biomarkers.

### Body:

With the initiation of funding 15/05/2007, we contacted the Pathology Service of the Minneapolis VA Medical Center (MVAMC) to begin collecting deidentified prostate tissue samples from prostatectomy specimens (for Tasks 1 and 2, and Task 3, item 1). Our initial goal had been to collect tissues from cystoprostatectomy specimens for establishing laser capture microdissection (LCM) parameters and to use as control tissues in our studies (Tasks 1 and 2). The prostates of cystoprostatectomy specimens in the first 8 months were small and the Pathology Service would not give us tissues from them for fear of compromising their clinical evaluations. However, from radical prostatectomy specimens, rather than receiving a tissue piece from the posterior region of the prostate and one more anterior to it as described in our original proposal, we were given cross sections of prostates. This was a change for our initial plan and permitted us to examine areas of cancer, benign glands near the cancer, benign glands distant to the cancer (often on opposite side of the prostate), and benign prostate hyperplasia (BPH) in the same patient specimen. Thus, benign distant and BPH tissue could serve as control tissues within the same prostate for comparison to proteins from cancer and benign near to cancer areas (a decided advantage over our initial approach which was to use a pool of cytoprostatectomy tissue [which we could not obtain] in each iTRAQ<sup>TM</sup> 2DLC-MS run as a control). This changed our use of control tissue extracts as listed in Task 2 and Task 3, item 2. Note that our expectations when writing the proposal was that we would get small pieces of tissue from two areas within the prostate and LCM would be the only practical approach to collect cancer and benign near tissue epithelia. This change allowed us to use frozen sections from each of these tissue areas for extraction for proteomic analysis and for comparison to LCM of epithelia (much more time consuming) from the same areas. The former gives a more comprehensive (epithelia plus stroma) and more speedy examination of the tumor field effect, whereas the latter a more epithelium specific analysis.

The control studies outlined in Task 1 were undertaken with the first tissue specimen available. The thickness of frozen sections and the effect of the Arcturus stain were examined with respect to quality of LCM isolated tissues. One persistent problem with benign glands was attachment of strands of connective tissue from the stroma with the epithelia when the LCM capture caps were lifted from the slide. Use of frozen sections at 7 micron thickness decreased this, but did not eliminate it. Thus, use of LCM tissues became problematic and with the availability of tissues from distinct areas of the prostate (i.e., CaP, BPH, BN, BD), we changed to use of frozen sections for protein extraction. Different means of extracting proteins from the LCM captured cells on the caps, as well as from total frozen sections, were compared. It was determined that extraction with a triethylammonium, bicarbonate, SDS (sodium dodecylsulfate), Triton X-100, and protease inhibitor buffer gave a similar yield of protein as PPS Silent Surfactant (3-[3-(1,1-bisalkyloxyethyl)pyridin-1-yl]propane-1-

sulfonate); the former was chosen because it was much less expensive and was not time sensitive as PPS Silent Surfactant (must be used within 12 hrs).

The lack of availability of cystoprostatectomy prostate tissue samples also affected our progress in Task 2. Specifically, the comparison of proteins in secretion with the epithelium was problematic. The secretion tended to contract to the epithelium when the frozen section dried on the slides as evidenced in hematoxylin and eosin staining, and this made capturing secretion separately difficult. Although information on proteins in prostate secretion (Task 2) and of normal prostate function is desirable, it was not the major thrust of this proposal. The variability in protein expression of benign tissues (Task 2, item 1) was now directly obtained through the introduction of the 8 iTRAQ system (see below). The protein composition of prostate secretion (Task 2, item 2) can be obtained by proteomic study of expressed prostate secretions of men without evidence of cancer (a goal of a different project in our laboratory).

Two developments that considerably improved technical aspects of our project occurred but delayed initiation of MS analysis of samples. These were the introduction of new state of the art equipment [a Tempo LCMALDI powered by Eksigent from Applied Biosystems/MDS SCIEX to apply fraction drops from a C-18 reverse phase column to MALDI targets (384 or more fractions) and a 4800 TOF/TOF Analyzer (TOF, time of flight)] in the Mass Spectroscopy and Proteomics core facility at the University of Minnesota and availability of the 8-iTRAQ<sup>TM</sup> labeling system (in contrast to the 4-iTRAQ<sup>TM</sup> labeling system used previously by us and included in the original proposal). However, this delay in MS analysis of samples was less problematic because it coincided with an extended personal leave of Ms Betre.

In this study, we found 7 prostates of the deidentified prostate tissue samples from prostatectomy specimens collected at the Minneapolis VAMC (MVAMC), that were appropriate to collect CaP, BN, BD and BPH samples for proteomic analysis (for Tasks 1 and 2, and Task 3, item 1). We also used CaP and BPH tissue samples in which BN and BD were deemed unsatisfactory for use. These samples were selected from a total of 41 prostatectomy specimens, from frozen sections cut by Ms Konjit Betre and stained with hematoxylin and eosin, and then reviewed by Dr. Stephen Ewing, Chief of the Pathology Service at the MVAMC. Areas of cancer and their Gleason grade, high grade prostatic intraepithelial neoplasia (PIN), BPH, and other histologic characteristics were noted and recorded. In undertaking Task 3, the frozen tissue blocks of the 7 prostates with clearly distinguishable areas of CaP, BN, BD, and BPH were reexamined and areas of tissue were trimmed to yield cancer (>75%) and BN and BD to the CaP in the peripheral zone and BPH tissues. Multiple 10 micron sections were made and pooled of each area. A subsequent frozen section was made and stained with hematoxylin and eosin to verify the histology of the tissue remaining and that the nature of the tissue collected was that in the beginning sections. The frozen sections were extracted with 0.5 M triethylammonium bicarbonate, 0.05% SDS, 0.1% Triton X-100, and protease inhibitors, and 40 micrograms of protein were processed for iTRAQ labeling and 2D LC-MS.

We completed Task 3 to identify proteins which have altered expression in CaP and BN tissues vs BD and BPH and addressed Task 4 in compiling the results upon concluding the study. In total 612 proteins were identified with 95% confidence in the prostate tissues (Table 1 in appendix). Statistical analysis of proteins found in at least 5 of the 7 prostates (98 proteins of the total of 612 proteins identified) indicated 4 proteins that were altered in expression CaP vs BD comparisons (beta-microseminoprotein, filamin A, histone cluster 2, four and a half LIM domains 1), 3 proteins differing in CaP vs BN (calreticulin, peptidylprolyl isomerase B, and transferrin), and 29 proteins in CaP vs BPH comparisons (Tables 1 and 2 in Supporting Data, proteins increased or decreased in abundance). It should be noted that the CaP vs BN or BD comparisons were made within the peripheral zone of the prostate, whereas the CaP vs BPH comparison was across peripheral vs transition zones. This latter comparison may indicate differences in peripheral zone vs transition zone expression of these proteins, as well as differences in expression in cancer. The statistical analyses were also done using missing value imputation since this approach can identify possible additional marker proteins in samples in which more than 2 samples may be missing identification of a particular protein.

An increased expression of calreticulin in CaP was noted in comparison to both BN and BPH tissue areas. This increased level of calreticulin in CaP tissues was substantiated by western blotting (Figure 1, Supporting Data). Idmapping using IPA analysis of our data showed calreticulin to be an important signaling hub (Network 1 - Appendix). A second network determined through Idmapping implicated VEGF and PDGF signaling hubs (Network 2 - Appendix). Calreticulin functions as a chaperone in the endoplasmic reticulum and is found in the nucleus, functioning in transcription regulation in which it can inhibit binding of androgen receptor to its hormone-response elements. Calreticulin may potentially serve as a biomarker in cancer detection and/or progression status. However, elevation of this protein was not noted in advanced prostate cancer (Alur M, et al., Am J Pathol 175:882-890, 2009). It is possible that calreticulin could be used in evaluation of tumor-stromal relationships.

We completed studies to verify select proteins identified in the MS studies and their altered level of expression in CaP. We accomplished Task 4 to conclude data analysis of the data in the study and presented a poster at the DOD sponsored IMPaCT meeting in Orlando, FL, in March, 2011. An outline of a manuscript using these data was prepared.

#### Milestones in the study:

- Collection of prostatectomy tissue specimens.
- Preparation of frozen sections from multiple blocks of tissue (8-16/ prostate) from 41 prostatectomy specimens, which were stained with hematoxylin and eosin, and reviewed for their histology.
- Preparation of frozen section tissue extracts of CaP, BN, BD, and BPH tissues for 8 plex iTRAQ 2DLC-MS studies.
- Identification of proteins significantly altered in abundance in CaP vs BN, BD and BPH tissues.
- Determination of network implications through Idmapping using IPA indicated calreticulin, as well as PDGF and VEGF signaling hubs in cancer.
- Calreticulin identity and level changes in CaP vs BN and BPH verified by western blotting.
- Preparation of abstract entitled “Proteomic analysis of prostate cancer field effect” and its presentation at the DOD sponsored IMPaCT meeting in Orlando, FL, March, 2011.

#### Key Research Accomplishments:

Increased and decreased abundance of select proteins was demonstrated in cancer as compared with benign and BPH tissue areas in the prostate. The increased expression of calreticulin in cancer vs BPH or benign tissues was substantiated by immunoblotting and identification of calreticulin as a signaling hub in cancer was demonstrated by Idmapping using IPA analysis.

#### Reportable Outcomes:

The poster of the abstract entitled, “Proteomic analysis of prostate cancer field effect,” was presented at the DOD sponsored IMPaCT meeting in Orlando, FL, March, 2011.

The authors and collaborators in this study were Michael J. Wilson<sup>1,2,3,6,7</sup>, Sanjoy Dey<sup>4</sup>, Michael Steinbach<sup>4</sup>, Konjit Betre<sup>1,2</sup>, Stephen L. Ewing<sup>1,2</sup>, LeeAnn Higgins<sup>5</sup>, Akhouri A. Sinha<sup>1,6</sup>, Loraine B. Anderson<sup>5</sup>. With the affiliations of the Minneapolis VA Medical Center<sup>1</sup>, Departments of Laboratory Medicine and Pathology<sup>2</sup>, Pharmacology<sup>3</sup>, Computer Science and Electrical Engineering<sup>4</sup>, Biochemistry Molecular Biology Biophysics<sup>5</sup>, Genetics Cell Biology and Development<sup>6</sup>, and Masonic Cancer Center<sup>7</sup>, University of Minnesota. Minneapolis, MN 55417.

The text of the abstract is given in supporting data.

**Conclusions:**

We processed tissue extracts of CaP, BN, BD, and BPH tissue areas from 7 prostates for comparative studies by iTRAQ 2DLC-MS. Abundance of 4 proteins were found different in CaP vs BD comparison, and 3 in CaP vs BN and 29 in CaP vs BPH comparison. Calreticulin levels were increased in CaP compared to both BN and BPH in both MS/MS and western blotting analyses, and it was found as a significant hub of regulation by Idmapping using IPA. These studies indicated that our approach to look at the tumor field effect for a source of new biomarkers will be successful.

**References:**

Michael J. Wilson, Sanjoy Dey, Michael S. Steinbach, Konjit Betre, Stephen L. Ewing, LeeAnn Higgins, Akhouri A. Sinha, and Lorraine B. Anderson. Proteomic Analysis of Prostate Cancer Field Effect. IMPACT-Innovative Minds in Prostate Cancer Today. Orlando, FL, March 9-12, poster P8-17 (2011).

**Appendices:**

1. Table 1A. All proteins identified with 95% confidence in iTRAQ MS/MS studies of human prostate tissue extracts.
2. Network 1-calreticulin signaling hub.
3. Network 2- VEGF and PDFF signaling hubs.

**Supporting Data:**

Text of abstract:

**Background and Objectives:** Three major challenges in treatment of prostate cancer (CaP) are 1) the lack of specificity in diagnosis using prostate specific antigen (PSA), 2) determining which patients with initial negative biopsies should be re-biopsied, and 3) inability to distinguish aggressive and latent prostate cancers in biopsies. To discover new biomarkers to address these challenges (i.e., specific for diagnosis of cancer and classification of the tumor), we undertook a proteomic approach to identify proteins altered in both cancer and benign glands near the cancers.

**Methodologies:** We collected cross sections of prostatectomy tissues and isolated areas of cancer (CaP), benign near to the cancer (BN), benign distant to the cancer (BD), and benign prostatic hyperplasia (BPH). Frozen sections from these specific areas were extracted, cleaved with trypsin, peptides differentially iTRAQ labeled (8 different iTRAQ labels), subjected to 2 dimensional liquid chromatography, and peptide sequences identified through mass spectroscopy (MS/MS) were used to identify proteins. Ratios of iTRAQ labels for a given protein were used to determine if its level was greater in CaP and/or BN vs BD and/or BPH.

**Results:** Statistical analysis of proteins found in at least 5 of 7 prostates (98 of 612 proteins identified with 95% confidence) showed 4 proteins altered in expression comparing CaP vs BD (histone cluster 2, H4b was increased and beta-microseminoprotein isoform a, filamin A and four and a half LIM domains 1 were decreased in abundance). Three proteins differed in CaP vs BN comparisons (peptidylprolyl isomerase B [cylcophilin B] and calreticulin precursor were increased and transferin was decreased in abundance). These protein comparisons were made within the peripheral zone of the prostate. When comparisons were made of CaP vs BPH, peripheral to transition zones, 11 proteins were found increased in cancer including peptidylprolyl isomerase B and calreticulin precursor, and 18 proteins were decreased in expression including beta-microseminoprotein isoform a and four and a half LIM domains 1. The proteins increased in CaP participate in chaperone, calcium signaling, collagen synthesis, and protein translation functions. Those decreased in abundance in CaP were related to cytoskeleton and intermediate filaments, regulation gene transcription, protein trafficking, and extracellular proteins.

**Conclusions:** Differences in level of abundance of proteins were found in peripheral zone tissues of CaP compared with BD and BN tissues, however, the number of altered proteins was greater in the comparison of CaP and BPH of the transition zone. These proteins may represent new biomarkers of CaP.

**Impact Statement:** Proteins identified in our proteomic approach which are altered in expression level in CaP can be useful in detection of prostate cancer and in discerning aggressive from indolent disease. Proteins with altered levels in BN as well as CaP tissues could be used to determine which patients need to be re-biopsied

upon a negative biopsy, since the marker would be detected in benign tissue of the negative biopsy. We have now identified several potential protein biomarkers that need to be tested by other methods to verify if differences in their expression levels can be used for these diagnostic purposes.

Table 1. Proteins significantly increased in abundance in cancer tissues in comparison to tissue areas BD and BD, and in BPH.

Comparison	qi number	Protein	p value
CaP:BD*	4504321	histone cluster 2, H4b	0.009
CaP:BN	4758950	peptidylprolyl isomerase B precursor	0.00005
CaP:BN	4757900	calreticulin precursor	0.0003
CaP:BPH	4757900	calreticulin precursor	0.00001
CaP:BPH	4758950	peptidylprolyl isomerase B precursor	0.0002
CaP:BPH	20070125	prolyl 4-hydroxylase, beta subunit precur	0.0008
CaP;BPH	4507677	tumor rejection antigen (gp96) 1	0.001
CaP:BPH	21361657	protein disulfide isomerase-assoc 3 precur	0.001
CaP:BPH	16507237	heat shock 70 kDa protein 5	0.001
CaP:BPH	4504447	heterogeneous nuclear ribonucleoprotein A2/B1 isoform A2	0.002
CaP:BPH	31542947	chaperonin	0.005
CaP:BPH	113420086	keratin 8	0.008
CaP:BPH	4503483	eukaryotic translation elongation factor 2	0.01
CaP:BPH	5453860	calnexin precursor	0.01

\*The CaP:BD and CaP:BN were determined with tissue from 7 prostate specimens. The CaP:BPH comparison was done with tissue from 9 prostate specimens (The 7 above plus tissue from 2 additional prostate specimens).

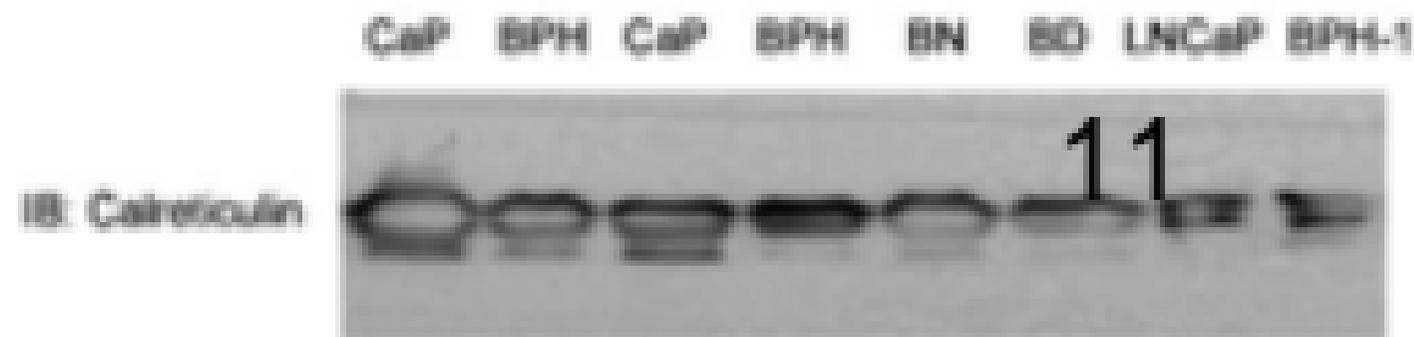
Table 2. Proteins significantly decreased in abundance in cancer tissues in comparison to tissue areas BD and BN, and in BPH.

Comparison	gi number	Protein	p value
CaP:BD*	4557036	beta-microseminoprotein isoform a precur	0.00008
CaP:BD	116063573	filamin A, alpha isoform 1	0.0003
CaP:BD	21361122	four and a half LLIM domains 1	0.0004
CaP:BN	136191	transferin	0.009
CaP:BPH	55743096	collagen, type XIV, alpha 1	0.000004
CaP:BPH	7669550	vinculin isoform meta-VCL	0.00001
CaP:BPH	62414289	vimentin	0.00007
CaP:BPH	4505047	lumican precursor	0.0003
CaP:BPH	42734430	polymerase I and transcript release fact	0.001
CaP:BPH	27436948	lamin A/C isoform 1 precursor	0.001
CaP:BPH	27436948	lamin A/C isoform 2	0.001
CaP:BPH	21361122	four and a half LIM domains 1	0.002
CaP:BPH	4557036	beta-microseminoprotein isoform a precur	0.002
CaP:BPH	4501891	actinin, alpha 1	0.003
CaP:BPH	4501897	actin, gamma 1 propeptide	0.003
CaP:BPH	21361120	calponin 1, basic, smooth muscle	0.004
CaP:BPH	21361462	EH-domain containing 2	0.004
CaP:BPH	4504517	heat shock 27 kDa protein 1	0.004
CaP:BPH	112382239	desmuslin isoform A	0.008
CaP:BPH	112382239	desmuslin isoform B	0.008
CaP:BPH	48255907	transgelin	0.008
CaP:BPH	34734066	fibulin 1 isoform D	0.009

\*The CaP:BD and CaP:BN were determined with tissue from 7 prostate specimens. The CaP:BPH comparison was done with tissue from 9 prostate specimens (The 7 above plus tissue from 2 additional prostate specimens).

Figure 1. Calreticulin protein identity verification and level of expression as determined by western blotting. Fifty micrograms of protein from tissue extracts of each tissue area were electrophoresed into SDS polyacrylamide gels, blotted to pvd membranes, and probed with a goat anti-human calreticulin antibody (R&D, Minneapolis, MN). The relative expression of calreticulin levels was done by densitometry and comparison to the level of calreticulin in BPH-1 cells. Extracts from 2 prostates were compared, one with CaP and BPH areas, and the second with CaP, BPH, BN, and BD tissues. The western blot data supported that of the iTRAQ MS/MS studies: 1) the molecular weight of tissue calreticulin was that reported in the literature and as expressed in 2 prostate cell lines, and 2) calreticulin levels were higher in CaP tissue areas than in BPH or in BN and BD.

The western-blot of the extracts from prostate tissues and from several cell lines:



	CaP	BPH	CaP	BPH	BN	BO	LNCaP	BPH-1
Area (µg)	79007, 79	43393, 43	67706, 28	54007, 79	35426, 79	33395, 24	15335, 45	14394, 28
Ratio	5, 29	3, 08	4, 71	4, 13	2, 48	2, 32	1, 07	1, 00

**1. Appendix – Table 1A. All proteins identified with 95% confidence in iTRAQ MS/MS studies of human prostate tissue extracts.**

## Sheet 1

albumin precursor [Homo sapiens]  
 smooth muscle myosin heavy chain 11 isoform SM1B [Homo sapiens]  
 filamin A, alpha isoform 2 [Homo sapiens]  
 cardiac muscle alpha actin 1 proprotein [Homo sapiens]  
 tropomyosin 1 alpha chain isoform 2 [Homo sapiens]  
 keratin 8 [Homo sapiens]  
 transferrin [Homo sapiens]  
 Cationic trypsin precursor (Beta-trypsin) [Contains: Alpha-trypsin chain 1; Alpha-trypsin chain 2]  
 transgelin [Homo sapiens]  
 desmin [Homo sapiens]  
 actinin, alpha 1 [Homo sapiens]  
 Hemoglobin subunit beta (Hemoglobin beta chain) (Beta-globin) [Contains: LVV-hemorphin-7]  
 apolipoprotein A-I preproprotein [Homo sapiens]  
 tropomyosin 2 (beta) isoform 2 [Homo sapiens]  
 alpha-2-glycoprotein 1, zinc [Homo sapiens]  
 calponin 1, basic, smooth muscle [Homo sapiens]  
 brain creatine kinase [Homo sapiens]  
 prostate specific antigen isoform 1 preproprotein [Homo sapiens]  
 prostatic acid phosphatase precursor [Homo sapiens]  
 actin, gamma 1 propeptide [Homo sapiens]  
 heat shock 70kDa protein 1A [Homo sapiens]  
 glyceraldehyde-3-phosphate dehydrogenase [Homo sapiens]  
 haptoglobin [Homo sapiens]  
 histone cluster 2, H2bf [Homo sapiens]  
 Hemoglobin subunit alpha (Hemoglobin alpha chain) (Alpha-globin)  
 annexin VI isoform 2 [Homo sapiens]  
 caldesmon 1 isoform 1 [Homo sapiens]  
 tubulin, beta [Homo sapiens]  
 histone cluster 2, H4b [Homo sapiens]  
 calreticulin precursor [Homo sapiens]  
 myosin light chain kinase isoform 3A [Homo sapiens]  
 vinculin isoform meta-VCL [Homo sapiens]  
 four and a half LIM domains 1 [Homo sapiens]  
 myosin regulatory light chain 9 isoform a [Homo sapiens]  
 brain glycogen phosphorylase [Homo sapiens]  
 myosin, light chain 6, alkali, smooth muscle and non-muscle isoform 2 [Homo sapiens]  
 prolyl 4-hydroxylase, beta subunit precursor [Homo sapiens]  
 keratin 18 [Homo sapiens]  
 eukaryotic translation elongation factor 1 alpha 1 [Homo sapiens]  
 heat shock 27kDa protein 1 [Homo sapiens]  
 annexin 5 [Homo sapiens]  
 tubulin, alpha, ubiquitous [Homo sapiens]  
 serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1 [Homo sapiens]  
 heat shock protein 90kDa alpha (cytosolic), class A member 1 isoform 2 [Homo sapiens]  
 cysteine and glycine-rich protein 1 [Homo sapiens]  
 vimentin [Homo sapiens]  
 histone cluster 1, H1e [Homo sapiens]  
 triosephosphate isomerase 1 [Homo sapiens]  
 heat shock 70kDa protein 8 isoform 1 [Homo sapiens]  
 lumican precursor [Homo sapiens]  
 orosomuroid 1 precursor [Homo sapiens]

## Sheet1

peroxiredoxin 2 isoform a [Homo sapiens]  
 lamin A/C isoform 2 [Homo sapiens]  
 H1 histone family, member 0 [Homo sapiens]  
 Peptidyl-prolyl cis-trans isomerase A (PPIase A) (Rotamase A) (Cyclophilin A) (Cyclosporin A-binding protein)  
 S100 calcium-binding protein A8 [Homo sapiens]  
 Peroxiredoxin-1 (Thioredoxin peroxidase 2) (Thioredoxin-dependent peroxide reductase 2) (Proliferation-associated  
 EH-domain containing 2 [Homo sapiens]  
 isocitrate dehydrogenase 2 (NADP+), mitochondrial precursor [Homo sapiens]  
 myosin, heavy polypeptide 9, non-muscle [Homo sapiens]  
 H2A histone family, member V isoform 1 [Homo sapiens]  
 dextrin isoform a [Homo sapiens]  
 protein disulfide isomerase-associated 3 precursor [Homo sapiens]  
 moesin [Homo sapiens]  
 PDZ and LIM domain 7 isoform 2 [Homo sapiens]  
 tyrosine 3/tryptophan 5 -monooxygenase activation protein, zeta polypeptide [Homo sapiens]  
 heterogeneous nuclear ribonucleoprotein A2/B1 isoform A2 [Homo sapiens]  
 heat shock 70kDa protein 5 [Homo sapiens]  
 thioredoxin [Homo sapiens]  
 tumor rejection antigen (gp96) 1 [Homo sapiens]  
 phosphoglycerate kinase 1 [Homo sapiens]  
 prostatic binding protein [Homo sapiens]  
 profilin 1 [Homo sapiens]  
 enolase 1 [Homo sapiens]  
 sorbitol dehydrogenase [Homo sapiens]  
 calmodulin 1 [Homo sapiens]  
 apolipoprotein A-II preproprotein [Homo sapiens]  
 high-mobility group box 1 [Homo sapiens]  
 retinol dehydrogenase 11 [Homo sapiens]  
 niban protein isoform 2 [Homo sapiens]  
 beta-microseminoprotein isoform a precursor [Homo sapiens]  
 clathrin heavy chain 1 [Homo sapiens]  
 cofilin 1 (non-muscle) [Homo sapiens]  
 S100 calcium-binding protein A9 [Homo sapiens]  
 L-plastin [Homo sapiens]  
 CNDP dipeptidase 2 [Homo sapiens]  
 high-mobility group nucleosomal binding domain 2 [Homo sapiens]  
 histone cluster 2, H3a [Homo sapiens]  
 collagen, type XIV, alpha 1 [Homo sapiens]  
 gamma filamin isoform b [Homo sapiens]  
 ribosomal protein L17 [Homo sapiens]  
 ATP synthase, H+ transporting, mitochondrial F1 complex, beta subunit precursor [Homo sapiens]  
 membrane metallo-endopeptidase [Homo sapiens]  
 biglycan preproprotein [Homo sapiens]  
 phosphoglucomutase 5 [Homo sapiens]  
 phosphoglycerate mutase 1 (brain) [Homo sapiens]  
 histone cluster 1, H2bo [Homo sapiens]  
 histone cluster 1, H2ba [Homo sapiens]  
 reticulon 4 isoform E [Homo sapiens]  
 H2A histone family, member Y isoform 2 [Homo sapiens]  
 chaperonin [Homo sapiens]  
 desmuslin isoform A [Homo sapiens]

## Sheet1

eukaryotic translation elongation factor 2 [Homo sapiens]  
 calnexin precursor [Homo sapiens]  
 nucleobindin 1 [Homo sapiens]  
 valosin-containing protein [Homo sapiens]  
 annexin A3 [Homo sapiens]  
 REVERSED ephrin receptor EphB4 precursor [Homo sapiens]  
 superoxide dismutase 1, soluble [Homo sapiens]  
 reticulocalbin 1 precursor [Homo sapiens]  
 amine oxidase, copper containing 3 precursor [Homo sapiens]  
 phosphoglycerate dehydrogenase [Homo sapiens]  
 chloride intracellular channel 1 [Homo sapiens]  
 ribosomal protein S5 [Homo sapiens]  
 diazepam binding inhibitor isoform 2 [Homo sapiens]  
 S100 calcium-binding protein A6 [Homo sapiens]  
 fibrinogen, beta chain preproprotein [Homo sapiens]  
 tryptase beta 2 precursor [Homo sapiens]  
 protein phosphatase 1, catalytic subunit, alpha isoform 3 [Homo sapiens]  
 serpin peptidase inhibitor, clade A, member 3 precursor [Homo sapiens]  
 trefoil factor 3 precursor [Homo sapiens]  
 lactate dehydrogenase B [Homo sapiens]  
 carbonyl reductase 1 [Homo sapiens]  
 glucose phosphate isomerase [Homo sapiens]  
 PREDICTED: similar to thymosin, beta 10 [Homo sapiens]  
 glutathione S-transferase theta 1 [Homo sapiens]  
 aldehyde dehydrogenase 1A3 [Homo sapiens]  
 RAB1B, member RAS oncogene family [Homo sapiens]  
 cytochrome b5 outer mitochondrial membrane precursor [Homo sapiens]  
 S100 calcium binding protein A11 [Homo sapiens]  
 LIM domain containing preferred translocation partner in lipoma [Homo sapiens]  
 ribosomal protein P1 isoform 1 [Homo sapiens]  
 platelet-activating factor acetylhydrolase, isoform 1b, beta subunit 30kDa [Homo sapiens]  
 macrophage migration inhibitory factor (glycosylation-inhibiting factor) [Homo sapiens]  
 cystatin B [Homo sapiens]  
 ras homolog gene family, member C precursor [Homo sapiens]  
 unactive progesterone receptor, 23 kD [Homo sapiens]  
 growth differentiation factor 15 [Homo sapiens]  
 MARCKS-like 1 [Homo sapiens]  
 heat shock 90kDa protein 1, beta [Homo sapiens]  
 leucine aminopeptidase 3 [Homo sapiens]  
 ribosomal protein L32 [Homo sapiens]  
 fibulin 1 isoform D [Homo sapiens]  
 REVERSED nebulin [Homo sapiens]  
 peptidylprolyl isomerase B precursor [Homo sapiens]  
 proteasome activator subunit 2 [Homo sapiens]  
 Lactotransferrin precursor (Lactoferrin) (Talaktoferrin) [Contains: Kaliocin-1; Lactoferroxin A; Lactoferroxin B; I  
 ribosomal protein L19 [Homo sapiens]  
 PREDICTED: hypothetical protein [Homo sapiens]  
 glyoxalase I [Homo sapiens]  
 aldolase A [Homo sapiens]  
 DEAH (Asp-Glu-Ala-His) box polypeptide 9 [Homo sapiens]  
 fibrinogen, alpha polypeptide isoform alpha-E preproprotein [Homo sapiens]

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REVERSED carboxypeptidase 2, cytosolic [Homo sapiens]  
 ankyrin repeat domain 34B [Homo sapiens]  
 glutathione transferase [Homo sapiens]  
 nucleophosmin 1 isoform 3 [Homo sapiens]  
 polymerase I and transcript release factor [Homo sapiens]  
 aminoacylase 1-like 2 [Homo sapiens]  
 tubulin, alpha 1a [Homo sapiens]  
 EF-hand domain family, member D2 [Homo sapiens]  
 pentraxin 3 [Homo sapiens]  
 poly(rC) binding protein 1 [Homo sapiens]  
 radixin [Homo sapiens]  
 heterogeneous nuclear ribonucleoprotein D isoform d [Homo sapiens]  
 protocadherin beta 9 precursor [Homo sapiens]  
 RAP1B, member of RAS oncogene family [Homo sapiens]  
 REVERSED polymerase (DNA directed), alpha 2 (70kD subunit) [Homo sapiens]  
 REVERSED Cdc42 effector protein 2 [Homo sapiens]  
 REVERSED WD repeat domain 61 [Homo sapiens]  
 guanine nucleotide binding protein (G protein) alpha 12 [Homo sapiens]  
 protein kinase C, delta binding protein [Homo sapiens]  
 DnaJ (Hsp40) homolog, subfamily C, member 13 [Homo sapiens]  
 HP1-BP74 [Homo sapiens]  
 REVERSED dystonin isoform 1 [Homo sapiens]  
 N-myc downstream regulated gene 1 [Homo sapiens]  
 IQ motif containing GTPase activating protein 1 [Homo sapiens]  
 DENN/MADD domain containing 2D [Homo sapiens]  
 ras-related GTP-binding protein RAB10 [Homo sapiens]  
 exocyst complex component 7 isoform a [Homo sapiens]  
 nuclear fragile X mental retardation protein interacting protein 1 [Homo sapiens]  
 alpha-2-macroglobulin precursor [Homo sapiens]  
 filamin A, alpha isoform 1 [Homo sapiens]  
 actin, gamma 2 propeptide [Homo sapiens]  
 AHNAK nucleoprotein isoform 1 [Homo sapiens]  
 heat shock protein 90kDa alpha (cytosolic), class A member 1 isoform 1 [Homo sapiens]  
 gamma filamin [Homo sapiens]  
 talin 1 [Homo sapiens]  
 heterogeneous nuclear ribonucleoprotein A1 isoform a [Homo sapiens]  
 annexin A2 isoform 1 [Homo sapiens]  
 actinin, alpha 4 [Homo sapiens]  
 PREDICTED: similar to Synaptopodin-2 (Myopodin) (Genethonin 2) isoform 4 [Homo sapiens]  
 gelsolin isoform a precursor [Homo sapiens]  
 epoxide hydrolase 1, microsomal (xenobiotic) [Homo sapiens]  
 pyruvate kinase, muscle isoform 1 [Homo sapiens]  
 nucleolin [Homo sapiens]  
 aldehyde dehydrogenase 9A1 [Homo sapiens]  
 plectin 1 isoform 1 [Homo sapiens]  
 dihydropyrimidinase-like 3 [Homo sapiens]  
 isocitrate dehydrogenase 1 (NADP+), soluble [Homo sapiens]  
 catenin (cadherin-associated protein), beta 1, 88kDa [Homo sapiens]  
 carboxypeptidase E preproprotein [Homo sapiens]  
 aldehyde dehydrogenase 1A1 [Homo sapiens]  
 non-metastatic cells 2, protein (NM23B) expressed in [Homo sapiens]

## Sheet 1

annexin I [Homo sapiens]  
 PREDICTED: similar to Phosphoglycerate mutase 1 (Phosphoglycerate mutase isozyme B) (PGAM-B) (BPG-de tyrosine 3/tryptophan 5 -monooxygenase activation protein, epsilon polypeptide [Homo sapiens]  
 proline arginine-rich end leucine-rich repeat protein precursor [Homo sapiens]  
 Serum albumin precursor (Allergen Bos d 6) (BSA)  
 N-acylsphingosine amidohydrolase (acid ceramidase) 1 isoform b [Homo sapiens]  
 GDP dissociation inhibitor 2 [Homo sapiens]  
 protein phosphatase 1, catalytic subunit, beta isoform 1 [Homo sapiens]  
 sorbin and SH3 domain containing 1 isoform 3 [Homo sapiens]  
 fermitin family homolog 2 [Homo sapiens]  
 ribophorin I precursor [Homo sapiens]  
 peroxiredoxin 5 precursor, isoform a [Homo sapiens]  
 heat shock 10kDa protein 1 [Homo sapiens]  
 glutamate dehydrogenase 1 [Homo sapiens]  
 fibrinogen, alpha polypeptide isoform alpha preproprotein [Homo sapiens]  
 peroxiredoxin 6 [Homo sapiens]  
 galectin 3 [Homo sapiens]  
 ubiquitin and ribosomal protein L40 precursor [Homo sapiens]  
 H2A histone family, member J [Homo sapiens]  
 beta-galactoside-binding lectin precursor [Homo sapiens]  
 PREDICTED: similar to sorbitol dehydrogenase [Homo sapiens]  
 solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 4 [Homo sapiens]  
 DJ-1 protein [Homo sapiens]  
 filamin B, beta (actin binding protein 278) [Homo sapiens]  
 acidic (leucine-rich) nuclear phosphoprotein 32 family, member A [Homo sapiens]  
 monoamine oxidase B [Homo sapiens]  
 heterogeneous nuclear ribonucleoprotein A3 [Homo sapiens]  
 complement component 1, q subcomponent binding protein precursor [Homo sapiens]  
 alpha glucosidase II alpha subunit isoform 3 [Homo sapiens]  
 proteasome activator subunit 1 isoform 1 [Homo sapiens]  
 aconitase 2 precursor [Homo sapiens]  
 nucleophosmin 1 isoform 1 [Homo sapiens]  
 cytosolic malate dehydrogenase [Homo sapiens]  
 aspartate aminotransferase 2 precursor [Homo sapiens]  
 transaldolase 1 [Homo sapiens]  
 related RAS viral (r-ras) oncogene homolog [Homo sapiens]  
 histidine triad nucleotide binding protein 1 [Homo sapiens]  
 complement component 3 precursor [Homo sapiens]  
 keratin 6B [Homo sapiens]  
 L-plastin [Homo sapiens]  
 glutathione-S-transferase omega 1 [Homo sapiens]  
 heparan sulfate proteoglycan 2 [Homo sapiens]  
 catenin, delta 1 isoform 1A [Homo sapiens]  
 far upstream element-binding protein [Homo sapiens]  
 DnaJ (Hsp40) homolog, subfamily A, member 1 [Homo sapiens]  
 integrin beta 1 isoform 1C-2 precursor [Homo sapiens]  
 fatty acid synthase [Homo sapiens]  
 phosphofructokinase, platelet [Homo sapiens]  
 ATP-dependent DNA helicase II, 70 kDa subunit [Homo sapiens]  
 oxoglutarate (alpha-ketoglutarate) dehydrogenase (lipoamide) isoform 1 precursor [Homo sapiens]  
 LUC7-like 2 [Homo sapiens]

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smoothelin isoform a [Homo sapiens]  
 transketolase [Homo sapiens]  
 histone cluster 3, H2bb [Homo sapiens]  
 allograft inflammatory factor 1 isoform 3 [Homo sapiens]  
 ATP-dependent DNA helicase II [Homo sapiens]  
 tropomyosin 1 alpha chain isoform 3 [Homo sapiens]  
 delta globin [Homo sapiens]  
 REVERSED solute carrier family 19, member 2 [Homo sapiens]  
 nucleosome assembly protein 1-like 1 [Homo sapiens]  
 ribosomal protein L3 isoform a [Homo sapiens]  
 orosomucoid 1 precursor [Homo sapiens]  
 oxygen regulated protein precursor [Homo sapiens]  
 acid alpha-glucosidase preproprotein [Homo sapiens]  
 inorganic pyrophosphatase 2 isoform 5 precursor [Homo sapiens]  
 serine (or cysteine) proteinase inhibitor, clade C (antithrombin), member 1 [Homo sapiens]  
 lamin B2 [Homo sapiens]  
 mitochondrial malate dehydrogenase precursor [Homo sapiens]  
 PREDICTED: similar to ribosomal protein S2 isoform 3 [Homo sapiens]  
 dipeptidyl peptidase 7 preproprotein [Homo sapiens]  
 ribosomal protein L4 [Homo sapiens]  
 heterogeneous nuclear ribonucleoprotein U isoform a [Homo sapiens]  
 interleukin enhancer binding factor 3 isoform c [Homo sapiens]  
 dystroglycan 1 preproprotein [Homo sapiens]  
 dihydrolipoamide dehydrogenase precursor [Homo sapiens]  
 class III alcohol dehydrogenase 5 chi subunit [Homo sapiens]  
 SH3-domain GRB2-like endophilin B2 [Homo sapiens]  
 proteasome (prosome, macropain) 26S subunit, non-ATPase, 6 [Homo sapiens]  
 polypyrimidine tract-binding protein 1 isoform a [Homo sapiens]  
 vitamin D-binding protein precursor [Homo sapiens]  
 PREDICTED: similar to 40S ribosomal protein S17 isoform 2 [Homo sapiens]  
 putative c-Myc-responsive isoform 1 [Homo sapiens]  
 CD44 antigen isoform 4 precursor [Homo sapiens]  
 monoamine oxidase A [Homo sapiens]  
 transgelin 2 [Homo sapiens]  
 NADH dehydrogenase (ubiquinone) Fe-S protein 8, 23kDa (NADH-coenzyme Q reductase) [Homo sapiens]  
 RAB2B protein [Homo sapiens]  
 carboxymethylenebutenolidase homolog [Homo sapiens]  
 lambda-crystallin [Homo sapiens]  
 dicarbonyl/L-xylulose reductase [Homo sapiens]  
 anterior gradient 2 homolog [Homo sapiens]  
 hepatoma-derived growth factor (high-mobility group protein 1-like) [Homo sapiens]  
 ribosomal protein S19 [Homo sapiens]  
 ribosomal protein L24 [Homo sapiens]  
 ribosomal protein L12 [Homo sapiens]  
 Na<sup>+</sup>/K<sup>+</sup> -ATPase beta 3 subunit [Homo sapiens]  
 cytochrome b-5 isoform 1 [Homo sapiens]  
 dihydroxyacetone kinase 2 [Homo sapiens]  
 succinate-CoA ligase, GDP-forming, alpha subunit [Homo sapiens]  
 otubain 1 [Homo sapiens]  
 transmembrane emp24 domain-containing protein 10 precursor [Homo sapiens]  
 homeobox prox 1 [Homo sapiens]

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ubiquitin-conjugating enzyme E2L 3 isoform 1 [Homo sapiens]  
 crystallin, mu isoform 1 [Homo sapiens]  
 Cytochrome c  
 Keratin, type I cytoskeletal 9 (Cytokeratin-9) (CK-9) (Keratin-9) (K9)  
 REVERSED orthodenticle homeobox 1 [Homo sapiens]  
 protein phosphatase 2A, regulatory subunit B' isoform d [Homo sapiens]  
 REVERSED taste receptor T2R4 [Homo sapiens]  
 PREDICTED: similar to retrotransposon-like 1 [Homo sapiens]  
 ezrin [Homo sapiens]  
 REVERSED EF-hand calcium binding domain 4A [Homo sapiens]  
 UV excision repair protein RAD23 homolog B [Homo sapiens]  
 high mobility group AT-hook 1 isoform a [Homo sapiens]  
 chromosome 9 open reading frame 19 [Homo sapiens]  
 CSE1 chromosome segregation 1-like protein [Homo sapiens]  
 autoantigen La [Homo sapiens]  
 RAB7, member RAS oncogene family [Homo sapiens]  
 EH-domain containing 3 [Homo sapiens]  
 REVERSED progesterone receptor [Homo sapiens]  
 junctophilin 3 [Homo sapiens]  
 X-box binding protein 1 isoform XBP1(U) [Homo sapiens]  
 biliverdin reductase B (flavin reductase (NADPH)) [Homo sapiens]  
 spectrin, beta, non-erythrocytic 2 [Homo sapiens]  
 sarco/endoplasmic reticulum Ca<sup>2+</sup> -ATPase isoform e [Homo sapiens]  
 REVERSED vacuolar protein sorting 33A [Homo sapiens]  
 REVERSED FtsJ homolog 2 [Homo sapiens]  
 KIAA1009 protein [Homo sapiens]  
 transmembrane protease, serine 11E [Homo sapiens]  
 enhancer of rudimentary homolog [Homo sapiens]  
 palladin [Homo sapiens]  
 REVERSED G protein-coupled receptor 51 [Homo sapiens]  
 REVERSED cell division cycle 25A isoform b [Homo sapiens]  
 hypothetical protein LOC127254 [Homo sapiens]  
 PREDICTED: similar to 60S ribosomal protein L7 isoform 3 [Homo sapiens]  
 thioredoxin-like 1 [Homo sapiens]  
 protein kinase, cGMP-dependent, type I isoform 1 [Homo sapiens]  
 REVERSED acidic alpha 1 syntrophin [Homo sapiens]  
 REVERSED PREDICTED: similar to 40S ribosomal protein S16 [Homo sapiens]  
 REVERSED ring finger protein 113A [Homo sapiens]  
 hemopexin [Homo sapiens]  
 small nuclear ribonucleoprotein polypeptide N [Homo sapiens]  
 ring finger protein 123 [Homo sapiens]  
 chaperonin containing TCP1, subunit 3 isoform a [Homo sapiens]  
 REVERSED titin isoform N2-A [Homo sapiens]  
 tensin [Homo sapiens]  
 REVERSED ADAM metalloproteinase with thrombospondin type 1 motif, 9 preproprotein [Homo sapiens]  
 2-aminoadipic 6-semialdehyde dehydrogenase [Homo sapiens]  
 pyruvate kinase, muscle isoform 2 [Homo sapiens]  
 REVERSED glutathione transferase zeta 1 isoform 1 [Homo sapiens]  
 actin related protein 2/3 complex subunit 2 [Homo sapiens]  
 2,4-dienoyl CoA reductase 1 precursor [Homo sapiens]  
 lysosomal trafficking regulator [Homo sapiens]

## Sheet 1

N-deacetylase/N-sulfotransferase (heparan glucosaminyl) 3 [Homo sapiens]  
 phosphoglucomutase 1 [Homo sapiens]  
 heterogeneous nuclear ribonucleoprotein H2 [Homo sapiens]  
 IQ motif containing GTPase activating protein 2 [Homo sapiens]  
 myosin, heavy polypeptide 7B, cardiac muscle, beta [Homo sapiens]  
 PREDICTED: similar to ribosomal protein L15 [Homo sapiens]  
 low density lipoprotein receptor-related protein associated protein 1 [Homo sapiens]  
 STRA8 [Homo sapiens]  
 zyxin [Homo sapiens]  
 eukaryotic translation initiation factor 4A isoform 1 [Homo sapiens]  
 centaurin delta 1 isoform a [Homo sapiens]  
 REVERSED zinc finger, FYVE domain containing 26 [Homo sapiens]  
 catenin, alpha 1 [Homo sapiens]  
 homogentisate 1,2-dioxygenase [Homo sapiens]  
 stonin 2 [Homo sapiens]  
 hypothetical protein LOC392979 [Homo sapiens]  
 coronin, actin binding protein, 1B [Homo sapiens]  
 cytochrome c oxidase subunit VIc proprotein [Homo sapiens]  
 dystonin isoform 1 [Homo sapiens]  
 REVERSED aldo-keto reductase family 1, member C3 [Homo sapiens]  
 chromosome 13 open reading frame 1 [Homo sapiens]  
 component of oligomeric golgi complex 5 isoform 2 [Homo sapiens]  
 REVERSED centrosomal protein 110kDa [Homo sapiens]  
 kalirin, RhoGEF kinase isoform 1 [Homo sapiens]  
 TAO kinase 2 isoform 1 [Homo sapiens]  
 fructose-1,6-bisphosphatase 1 [Homo sapiens]  
 heterogeneous nuclear ribonucleoprotein U-like 2 [Homo sapiens]  
 REVERSED neurotrophin 3 isoform 2 preproprotein [Homo sapiens]  
 galectin 3 binding protein [Homo sapiens]  
 REVERSED microfilament and actin filament cross-linker protein isoform b [Homo sapiens]  
 hypothetical protein LOC65250 [Homo sapiens]  
 PREDICTED: similar to melanoma inhibitory activity 3 isoform 1 [Homo sapiens]  
 REVERSED vacuolar protein sorting 39 [Homo sapiens]  
 REVERSED COP9 constitutive photomorphogenic homolog subunit 3 [Homo sapiens]  
 cingulin-like 1 [Homo sapiens]  
 crystallin, beta B1 [Homo sapiens]  
 REVERSED ankyrin repeat domain 35 [Homo sapiens]  
 REVERSED glycerol-3-phosphate dehydrogenase 1-like [Homo sapiens]  
 teashirt family zinc finger 1 [Homo sapiens]  
 interferon stimulated exonuclease gene 20kDa-like 2 [Homo sapiens]  
 profilin 2 isoform a [Homo sapiens]  
 REVERSED complement component 1, q subcomponent binding protein precursor [Homo sapiens]  
 REVERSED retinitis pigmentosa 1-like 1 [Homo sapiens]  
 REVERSED minor histocompatibility antigen HA-1 [Homo sapiens]  
 REVERSED methyltransferase like 4 [Homo sapiens]  
 zinc finger, CCHC domain containing 11 isoform c [Homo sapiens]  
 disabled homolog 2 interacting protein isoform 1 [Homo sapiens]  
 REVERSED aconitase 2 precursor [Homo sapiens]  
 REVERSED WD repeat domain 21C [Homo sapiens]  
 SH3 domain binding glutamic acid-rich protein like [Homo sapiens]  
 hepsin [Homo sapiens]

## Sheet 1

rapamycin-insensitive companion of mTOR [Homo sapiens]  
 troponin I, skeletal, slow [Homo sapiens]  
 smooth muscle myosin heavy chain 11 isoform SM2B [Homo sapiens]  
 histone cluster 2, H2ac [Homo sapiens]  
 PREDICTED: similar to H3 histone, family 2 isoform 2 [Homo sapiens]  
 PREDICTED: similar to actin-like protein [Homo sapiens]  
 histone cluster 1, H2bm [Homo sapiens]  
 PREDICTED: similar to synaptopodin 2 isoform 3 [Homo sapiens]  
 haptoglobin-related protein [Homo sapiens]  
 alpha 3 type VI collagen isoform 4 precursor [Homo sapiens]  
 sorbin and SH3 domain containing 1 isoform 4 [Homo sapiens]  
 thymosin-like 3 [Homo sapiens]  
 myoglobin [Homo sapiens]  
 PREDICTED: similar to ribosomal protein S27a [Homo sapiens]  
 nucleophosmin 1 isoform 2 [Homo sapiens]  
 A-kinase anchor protein 13 isoform 1 [Homo sapiens]  
 desmuslin isoform B [Homo sapiens]  
 integrin-linked kinase [Homo sapiens]  
 ubiquitin specific protease 34 [Homo sapiens]  
 NME1-NME2 protein [Homo sapiens]  
 REVERSED PREDICTED: hypothetical protein [Homo sapiens]  
 REVERSED spleen tyrosine kinase [Homo sapiens]  
 apolipoprotein H precursor [Homo sapiens]  
 PREDICTED: similar to ribosomal protein L5 isoform 2 [Homo sapiens]  
 hypothetical protein LOC345651 [Homo sapiens]  
 bromodomain PHD finger transcription factor isoform 1 [Homo sapiens]  
 collagen, type VI, alpha 1 precursor [Homo sapiens]  
 scavenger receptor class B, member 2 [Homo sapiens]  
 PREDICTED: similar to cytoplasmic beta-actin [Homo sapiens]  
 PREDICTED: similar to High mobility group protein B1 (High mobility group protein 1) (HMG-1) (Amphoterin) (H  
 eukaryotic translation initiation factor 4A2 [Homo sapiens]  
 FK506-binding protein 3 [Homo sapiens]  
 testin isoform 1 [Homo sapiens]  
 heterogeneous nuclear ribonucleoprotein D-like [Homo sapiens]  
 superoxide dismutase 3, extracellular precursor [Homo sapiens]  
 apolipoprotein A-IV precursor [Homo sapiens]  
 G antigen, family C, 1 [Homo sapiens]  
 caveolin 3 [Homo sapiens]  
 Glutamate dehydrogenase 1, mitochondrial precursor (GDH)  
 tumor protein, translationally-controlled 1 [Homo sapiens]  
 REVERSED neuro-oncological ventral antigen 2 [Homo sapiens]  
 REVERSED coatomer protein complex, subunit gamma 2 [Homo sapiens]  
 transportin 2 (importin 3, karyopherin beta 2b) [Homo sapiens]  
 PREDICTED: similar to 60S ribosomal protein L7 [Homo sapiens]  
 Beta-2-microglobulin precursor [Contains: Beta-2-microglobulin form pl 5.3]  
 REVERSED phosphoinositide-3-kinase, class 2, beta polypeptide [Homo sapiens]  
 REVERSED PREDICTED: hypothetical protein [Homo sapiens]  
 eukaryotic translation initiation factor 3, subunit 10 theta, 150/170kDa [Homo sapiens]  
 REVERSED FK506 binding protein 5 [Homo sapiens]  
 PREDICTED: hypothetical protein [Homo sapiens]  
 serum amyloid P component precursor [Homo sapiens]

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REVERSED sex comb on midleg 1 isoform 1 [Homo sapiens]  
 REVERSED NLR family, pyrin domain containing 3 isoform a [Homo sapiens]  
 heterogeneous nuclear ribonucleoprotein K isoform a [Homo sapiens]  
 MDN1, midasin homolog [Homo sapiens]  
 LON peptidase N-terminal domain and ring finger 2 [Homo sapiens]  
 ATP-binding cassette, sub-family A member 3 [Homo sapiens]  
 REVERSED clathrin, heavy polypeptide-like 1 [Homo sapiens]  
 PREDICTED: similar to Nonhistone chromosomal protein HMG-17 (High-mobility group nucleosome-binding do  
 BTB/POZ KELCH domain protein [Homo sapiens]  
 REVERSED WD repeat domain 55 [Homo sapiens]  
 beta 1,3-galactosyltransferase-like [Homo sapiens]  
 UTP14, U3 small nucleolar ribonucleoprotein, homolog A [Homo sapiens]  
 chaperonin containing TCP1, subunit 7 isoform a [Homo sapiens]  
 DNA polymerase theta [Homo sapiens]  
 REVERSED dihydrouridine synthase 2-like, SMM1 homolog [Homo sapiens]  
 OMA1 homolog, zinc metallopeptidase [Homo sapiens]  
 death inducer-obliterator 1 isoform c [Homo sapiens]  
 beta-defensin 115 [Homo sapiens]  
 MAS-related GPR, member F [Homo sapiens]  
 REVERSED pecanex-like 1 [Homo sapiens]  
 REVERSED dynein, axonemal, heavy chain 9 isoform 2 [Homo sapiens]  
 keratin 4 [Homo sapiens]  
 REVERSED a disintegrin and metalloproteinase domain 7 [Homo sapiens]  
 REVERSED cryptochrome 1 (photolyase-like) [Homo sapiens]  
 neuroblastoma-amplified protein [Homo sapiens]  
 colonic and hepatic tumor over-expressed protein isoform a [Homo sapiens]  
 lamin A/C isoform 1 precursor [Homo sapiens]  
 histone cluster 1, H1c [Homo sapiens]  
 histone cluster 2, H2aa3 [Homo sapiens]  
 proteasome activator subunit 1 isoform 2 [Homo sapiens]  
 keratin 7 [Homo sapiens]  
 keratin 19 [Homo sapiens]  
 keratin 1 [Homo sapiens]  
 manganese superoxide dismutase isoform A precursor [Homo sapiens]  
 eukaryotic translation elongation factor 1 gamma [Homo sapiens]  
 HLA-B associated transcript 1 [Homo sapiens]  
 ubiquitin-activating enzyme E1 [Homo sapiens]  
 PREDICTED: similar to ATP-dependent DNA helicase 2 subunit 1 (ATP-dependent DNA helicase II 70 kDa sub  
 ATP synthase, H<sup>+</sup> transporting, mitochondrial F1 complex, alpha subunit precursor [Homo sapiens]  
 apolipoprotein E precursor [Homo sapiens]  
 PREDICTED: similar to Nonhistone chromosomal protein HMG-17 (High-mobility group nucleosome-binding do  
 zinc finger protein 350 [Homo sapiens]  
 complement factor H isoform a precursor [Homo sapiens]  
 REVERSED apolipoprotein B precursor [Homo sapiens]  
 REVERSED calmodulin-binding transcription activator 1 [Homo sapiens]  
 REVERSED desmoplakin isoform I [Homo sapiens]  
 synaptopodin 2 [Homo sapiens]  
 ARP3 actin-related protein 3 homolog [Homo sapiens]  
 PREDICTED: similar to ribosomal protein S19 [Homo sapiens]  
 complement component 4A preproprotein [Homo sapiens]  
 heterogeneous nuclear ribonucleoprotein M isoform a [Homo sapiens]

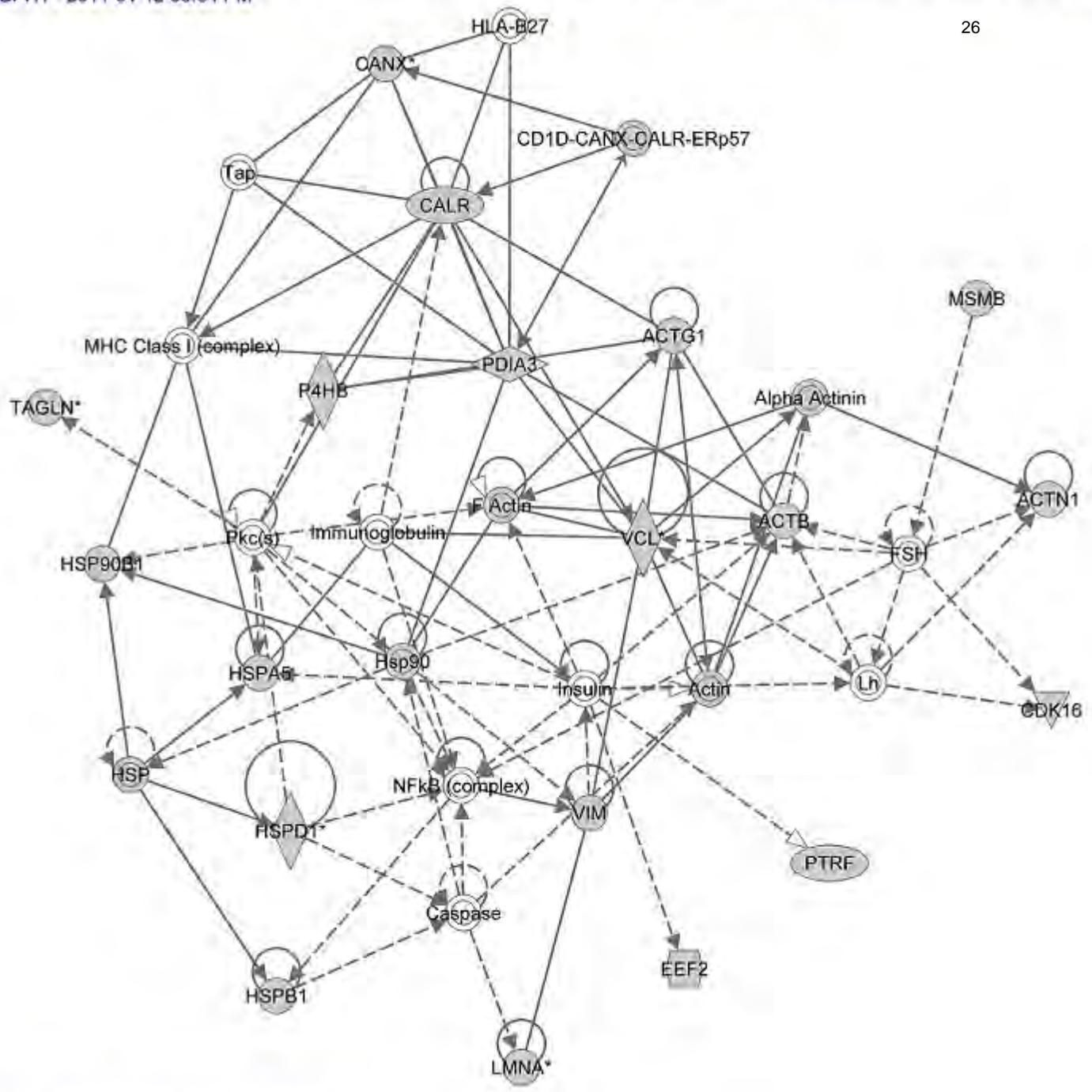
## Sheet 1

cytidine monophosphate (UMP-CMP) kinase 1, cytosolic [Homo sapiens]  
 aldo-keto reductase family 1, member A1 [Homo sapiens]  
 selenium binding protein 1 [Homo sapiens]  
 tropomyosin 3 isoform 1 [Homo sapiens]  
 H2A histone family, member Z [Homo sapiens]  
 ribosomal protein L10a [Homo sapiens]  
 synaptotagmin binding, cytoplasmic RNA interacting protein [Homo sapiens]  
 decorin isoform a preproprotein [Homo sapiens]  
 COP9 constitutive photomorphogenic homolog subunit 2 [Homo sapiens]  
 discoidin domain receptor family, member 1 isoform c [Homo sapiens]  
 PREDICTED: similar to Serine/threonine-protein kinase PRKX (Protein kinase PKX1) [Homo sapiens]  
 dehydrogenase/reductase (SDR family) member 7 [Homo sapiens]  
 aldehyde dehydrogenase 6A1 precursor [Homo sapiens]  
 gelsolin-like capping protein [Homo sapiens]  
 drebrin-like isoform b [Homo sapiens]  
 tumor protein D52-like 2 isoform d [Homo sapiens]  
 CD38 antigen [Homo sapiens]  
 eIF-5A2 protein [Homo sapiens]  
 L-3-hydroxyacyl-Coenzyme A dehydrogenase precursor [Homo sapiens]  
 thioredoxin domain containing 4 (endoplasmic reticulum) [Homo sapiens]  
 mitochondrial ATP synthase, O subunit precursor [Homo sapiens]  
 thioredoxin domain containing 5 isoform 2 [Homo sapiens]  
 CD59 antigen p18-20 [Homo sapiens]  
 ribosomal protein L15 [Homo sapiens]  
 S100 calcium-binding protein A4 [Homo sapiens]  
 capping protein (actin filament) muscle Z-line, alpha 2 [Homo sapiens]  
 defensin, alpha 3 preproprotein [Homo sapiens]  
 RAB2A, member RAS oncogene family [Homo sapiens]  
 high mobility group AT-hook 1 isoform b [Homo sapiens]  
 apolipoprotein C-I precursor [Homo sapiens]  
 alpha 1 type I collagen preproprotein [Homo sapiens]  
 carbonic anhydrase I [Homo sapiens]  
 chaperonin containing TCP1, subunit 8 (theta) [Homo sapiens]  
 REVERSED orthodenticle homeobox 2 isoform b [Homo sapiens]  
 ubiquilin 2 [Homo sapiens]  
 REVERSED ryanodine receptor 3 [Homo sapiens]  
 zinc finger protein 510 [Homo sapiens]  
 PREDICTED: similar to Phosphoglycerate mutase 1 (Phosphoglycerate mutase isozyme B) (PGAM-B) (BPG-de  
 calpain 13 [Homo sapiens]  
 adenylyl cyclase-associated protein [Homo sapiens]  
 REVERSED hypothetical protein LOC57703 [Homo sapiens]  
 REVERSED vacuolar protein sorting 33B (yeast homolog) [Homo sapiens]  
 complement factor B preproprotein [Homo sapiens]  
 nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor-like 1 [Homo sapiens]  
 XRP2 protein [Homo sapiens]  
 jumonji, AT rich interactive domain 1C [Homo sapiens]  
 REVERSED desert hedgehog preproprotein [Homo sapiens]  
 REVERSED zinc finger protein 615 [Homo sapiens]  
 Fraser syndrome 1 [Homo sapiens]  
 ferritin, heavy polypeptide 1 [Homo sapiens]  
 REVERSED 5-hydroxytryptamine receptor 5A [Homo sapiens]

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REVERSED thyroid hormone receptor interactor 11 [Homo sapiens]  
 KRI1 homolog [Homo sapiens]  
 ninein isoform 2 [Homo sapiens]  
 REVERSED WD repeat domain 47 [Homo sapiens]  
 protein expressed in prostate, ovary, testis, and placenta 2 [Homo sapiens]  
 REVERSED transformation/transcription domain-associated protein [Homo sapiens]  
 REVERSED vacuolar protein sorting 13C protein isoform 2A [Homo sapiens]  
 REVERSED mitofusin 2 [Homo sapiens]  
 citrate synthase precursor, isoform a [Homo sapiens]  
 guanine nucleotide-binding protein, beta-1 subunit [Homo sapiens]  
 Rho guanine nucleotide exchange factor (GEF) 17 [Homo sapiens]  
 acyl-Coenzyme A thioesterase 2, mitochondrial isoform a [Homo sapiens]  
 platelet-activating factor acetylhydrolase, isoform 1b, alpha subunit [Homo sapiens]  
 CD1E antigen isoform a precursor [Homo sapiens]  
 REVERSED mucin 16 [Homo sapiens]  
 plasma glutathione peroxidase 3 precursor [Homo sapiens]  
 REVERSED PREDICTED: hypothetical protein [Homo sapiens]  
 REVERSED bromodomain adjacent to zinc finger domain, 2A [Homo sapiens]  
 REVERSED asparagine-linked glycosylation 9 protein isoform b [Homo sapiens]  
 REVERSED dynein, axonemal, heavy chain 7 [Homo sapiens]  
 REVERSED epiplakin 1 [Homo sapiens]  
 REVERSED zinc finger protein, multitype 2 [Homo sapiens]  
 heat shock protein, alpha-crystallin-related, B6 [Homo sapiens]  
 REVERSED PREDICTED: hypothetical protein [Homo sapiens]  
 PREDICTED: hypothetical protein [Homo sapiens]  
 hypothetical protein LOC79632 isoform 1 [Homo sapiens]  
 REVERSED zinc finger, NFX1-type containing 1 [Homo sapiens]  
 toll-like receptor adaptor molecule 1 isoform 2 [Homo sapiens]  
 solute carrier family 27 (fatty acid transporter), member 5 [Homo sapiens]  
 REVERSED nucleoporin 155kDa isoform 2 [Homo sapiens]  
 Rho-associated, coiled-coil containing protein kinase 2 [Homo sapiens]  
 nucleoporin 188kDa [Homo sapiens]  
 ring finger protein 157 [Homo sapiens]  
 REVERSED DEAD (Asp-Glu-Ala-Asp) box polypeptide 46 [Homo sapiens]  
 microtubule-associated protein 4 isoform 2 [Homo sapiens]  
 REVERSED spectrin repeat containing, nuclear envelope 1 isoform 1 [Homo sapiens]  
 solute carrier family 22 member 11 [Homo sapiens]  
 REVERSED PI-3-kinase-related kinase SMG-1 [Homo sapiens]  
 REVERSED HECT domain containing 1 [Homo sapiens]  
 myeloid/lymphoid or mixed-lineage leukemia 3 [Homo sapiens]  
 REVERSED periostin, osteoblast specific factor [Homo sapiens]  
 REVERSED ring finger protein 20 [Homo sapiens]  
 ATPase, Ca<sup>++</sup> transporting, fast twitch 1 isoform a [Homo sapiens]  
 REVERSED DEAH (Asp-Glu-Ala-His) box polypeptide 8 [Homo sapiens]  
 splicing factor, arginine/serine-rich 10 [Homo sapiens]  
 REVERSED inositol 1,4,5-triphosphate receptor, type 2 [Homo sapiens]  
 HBxAg transactivated protein 2 [Homo sapiens]  
 hypothetical protein LOC401024 [Homo sapiens]  
 epiplakin 1 [Homo sapiens]  
 REVERSED transgelin 2 [Homo sapiens]  
 alanyl-tRNA synthetase [Homo sapiens]

**Appendix - Network 1-calreticulin signaling hub.**



**Appendix - Network 2- VEGF and PDFF signaling hubs.**

