

Title: Molecular Therapy for Degenerative Disc Disease: Clues from Secretome Analysis of the Notochordal Cell-Rich Nucleus Pulposus

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SUPPLEMENTARY DATA

MATERIALS AND METHODS

Antibodies and other reagents - Human recombinant interleukin-1 β (IL-1 β), tumor necrosis factor-alpha (TNF α), connective tissue growth factor (CTGF), Wnt-inducible soluble protein 2 (WISP2) and transforming growth factor beta 1 (TGF β 1) proteins were purchased from Peprotech Inc. (Quebec, Canada). We obtained rabbit polyclonal antibodies for Collagen 2 (ab34712), MMP-13 (ab39012), Cox2 (ab15191), Oct4 (ab18976), Nanog (ab106465), CTGF (ab6995), rabbit monoclonal for MMP-3 (ab52915) and mouse monoclonal antibodies for Galectin 3 (ab2785) and β -actin (ab6276) were purchased from Abcam Inc. (CA, USA). Goat polyclonal Brachyury antibody (sc-17743) and rabbit polyclonal antibodies for Aggrecan (sc-25674), TIMP-1 (sc-5538), ADAMTS-4 (sc-25582), TNF α (sc-8301), TGF β 1 (sc-146) and mouse monoclonal WISP2 (sc-514070) were obtained from Santa Cruz Biotechnology Inc. (CA, USA).

RNA isolation and Real Time PCR- Total RNA was isolated from human degenerative disc NP cells (H1 / H2 / H3) treated with IL-1 β (10 ng/mL), TNF α (50 ng/mL), CTGF (100ng/mL), TGF β 1 (10 ng/mL) or a combination of CTGF (100 ng/mL) and TGF β 1 (10 ng/mL) using RNAeasy extraction kit (Cat#74134, Qiagen) and quantified using a Nanodrop spectrophotometer. Total RNA (~ 400ng) was reverse-transcribed using RT² First Strand Kit (Cat#330401, Qiagen) following the manufacturer's instructions for preparing cDNA. Effect of treatment on ECM genes including collagen 2, HAPLN1, versican, thrombospondin 1 (Thbs1), MMP-13 and Cox2 was evaluated using real time PCR performed on ABI 7900HT 384-well Fast block machine.

Cell viability and cell proliferation assays - Nucleus pulposus cells (rat, bovine and human) in passage (P2), were plated in 96-well flat bottom plates in order to evaluate the effect of treatment with growth factors in cell viability and proliferation assays. Rat NP cells (passage, P2) were treated with CTGF, WISP-2 and TGF β 1 in a dose (1 ng/mL – 100 ng/mL) and time dependent manner (24 hrs – 96 hrs) to determine the optimum dose and time for the assessment of these growth factors on viability. Cell viability was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich, USA) as described earlier⁴⁹. We used a BrdU - ELISA (colorimetric) assay (Cat# ab126556, Abcam) for determining the effect of treatment with CTGF (100 ng/mL) or TGF β 1 (10 ng/mL) on human and rat NP cells (healthy / injured) following the manufacturer's instructions. Briefly, NP cells were treated with CTGF (100 ng/mL) or TGF β 1 (10 ng/mL) alone or in combination for 48 hrs following addition of BrdU reagent O/N in each well. Incorporated BrdU in DNA of proliferating cells was determined using anti-BrdU antibody and quantified by ELISA as per the manufacturer's instructions.

Apoptosis assays - Inflammation induced apoptosis in NP cells (rat and human) was determined using Caspase 3/7 and Caspase 9 specific Lumi-Glo assays (Promega, Madison). Briefly, NP cells were plated and treated with either IL-1 β (10 ng/mL) alone or in combination with TNF α (50 ng/mL) only or in presence of CTGF (100 ng/mL) and TGF β 1 (10 ng/mL) for 48 hrs, followed by addition of specific reagents for Caspase 3/7 and Caspase 9 as per the manufacturer's instructions. Cells were incubated for another 4 hrs at 37⁰C, and plates were read in a multi-well luminescence plate reader.

RESULTS

Histological analysis - Histology of the degenerative disc NPs obtained from human and bovine was compared to healthy NPs obtained from NCD canine and Wistar rat IVDs. Both human and bovine degenerative IVDs showed fibrocartilaginous matrix with strong Safranin-O staining in comparison to healthy, highly cellular NC - rich NP (>90%) with faint Safranin-O staining in NCD – canines and Wistar rat (Supplementary Fig. 2A). Immunohistochemical analysis revealed no detectable expression of Brachyury (NC-specific marker) or Oct4 (stem cell marker) in human and bovine degenerative disc NPs (Supplementary Fig.1A). In contrast, strong nuclear expression of brachyury and Oct4 was observed in healthy, NCD canine and Wistar rat discs demonstrating the NC- and stem cell-rich NP (Supplementary Fig. 2A). However, negative control tissue sections showed no immunostaining demonstrating the specificity of the antibodies (Supplementary Fig. 2B).

FIGURE LEGENDS

Supplementary Fig. 1. (A) Histological changes in rat disc NP in a time dependent manner (healthy – 10 weeks p.i.) with hematoxylin and eosin (H&E) staining showing loss of NCs and Safranin-O (Saf-O) staining showing development of fibrocartilagenous matrix (Scale bar 50 μ). Panel (B) represents histograms showing fold changes in protein expression of IL1 β , TNF α , Cox2, MMP-3, MMP-13, TIMP1 and ADAMTS-4. Panel (C) represents histograms showing fold changes in protein expression of Brachyury, Galectin3, Oct4 and Nanog. Each bar in the histogram represents mean \pm S.D. of 3 independent experiments. ***p<0.05; *p-values are w.r.t No treatment controls; **p-values are w.r.t 10 weeks p.i.

Supplementary Fig. 2. Panel (A) Safranin O staining and immunohistochemical analysis of brachyury and Oct4 proteins in paraffin embedded sections of nucleus pulposus obtained from human degenerative disc, bovine degenerative disc, non-chondrodystrophic canine and healthy rat discs (Scale bar 10 μ). Arrows represent CLC, chondrocyte like cells and NC, notochordal cells. Panel (B) shows representative negative controls showing no immunostaining in tissue sections (rat healthy or mongrel IVD-NP), wherein specific rabbit or goat polyclonal antibodies were replaced by isotype controls (Scale bar 50 μ). Panel (C) represents histograms showing fold changes in protein expression of Collagen2, Oct4 and Nanog in rat IVD-NP injected with PBS, control media or NCCM. Each bar in the histogram represents mean \pm S.D. of 3 independent experiments. ***p<0.05; *p-values are w.r.t No treatment controls; **p-values are w.r.t 10 weeks p.i.

Supplementary Fig. 3. Evaluation of bioactivity in protein containing fractions (PF) collected after size exclusion chromatography. Histograms showing (A) cell viability, (B) caspase-3/7

activity in bovine NP cells treated with NCCM, the cytotoxic drug, etoposide (300 μ M, used as a positive control) in presence of NCCM or control medium (serum, phenol red and protein free Hybridoma Media). Each bar represents mean \pm S.D. of 2 independent experiments done in triplicates (n=6). (C) Histograms showing caspase-3/7 activity in bovine NP cells treated with etoposide in control medium (serum free, phenol red free Hybridoma Media) or in presence of protein containing fractions (PFs). Each bar represents mean \pm S.D. of 2 independent experiments done in triplicates (n=6). For these experiments, protein containing fractions (PFs) were mixed (1:1) with control medium (serum free, phenol red free Hybridoma Media) and compared to no treatment controls (i.e. elution buffer + control medium (1:1)).

Supplementary Fig. 4. Ingenuity Pathway Analysis showing (A) Canonical signaling pathways identified. (B) Radial model showing CTGF as important hub proteins regulating / interacting with several other growth factors and ECM proteins. (C) Protein network involved in cartilage development and degradation; (D) and (E) Cell signaling involved in axonal growth. Proteins highlighted were identified in the secretome analysis.

Supplementary Fig. 5. Dose (5 ng/mL – 100 ng/mL) and time dependent (24 hrs - 96 hrs) effects of (A) CTGF, (B) WISP2 and (C) TGF β 1 treatment alone or (D) combination of CTGF with WISP2, and TGF β 1 on cell viability determined using MTT assays in NP cells obtained from healthy rat IVDs. Each bar represents mean \pm S.D. of 3 independent experiments done in quadruplicates (n=12).

Supplementary Fig. 6. Effect of CTGF, WISP2 and TGF β 1 treatment alone or in combination on cell viability as determined using MTT assays in NP cells obtained from (A) rat (healthy / injured) disc, *p \leq 0.02, (B) bovine degenerative disc, *p<0.01 and (C) human (H1, H2)

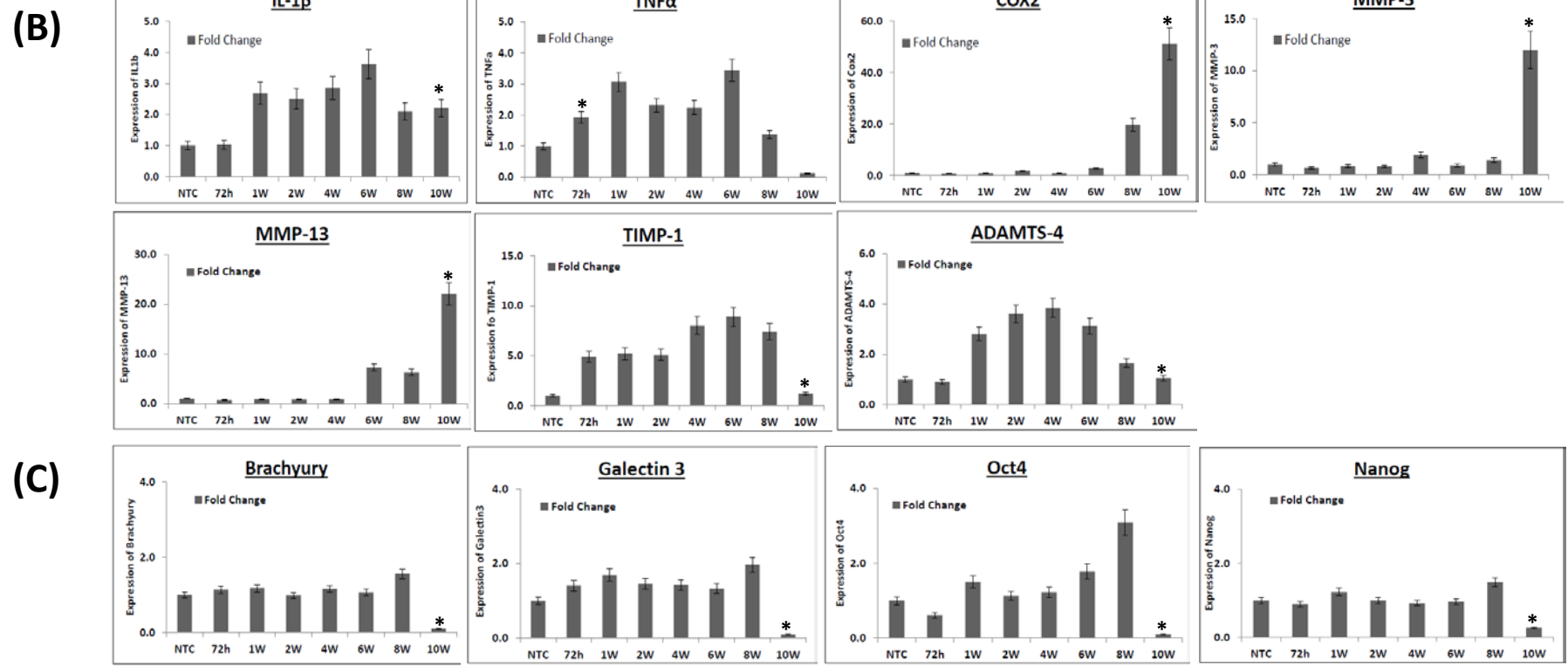
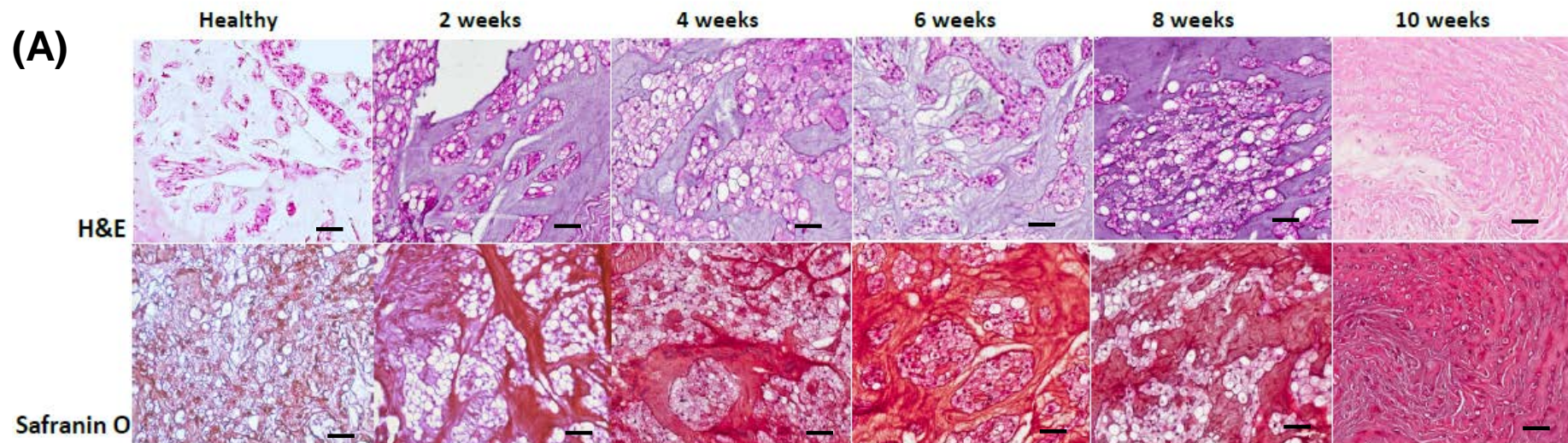
degenerative NP cells in 48 hrs (*p<0.005,**p=0.02). Each bar represents mean \pm S.D. of 3 independent experiments done in triplicates (n=9).

Supplementary Fig. 7. Panel (A) shows a representative Western blot panel verifying increased collagen 2 expression in human degenerative disc NP cells(#H2) on treatment with CTGF and TGF β 1. Panel (B) represents histograms showing fold changes in protein expression of Collagen2 in human NP cells treated with CTGF, TGF β 1 alone or in combination as compared to no treatment controls (NTC).

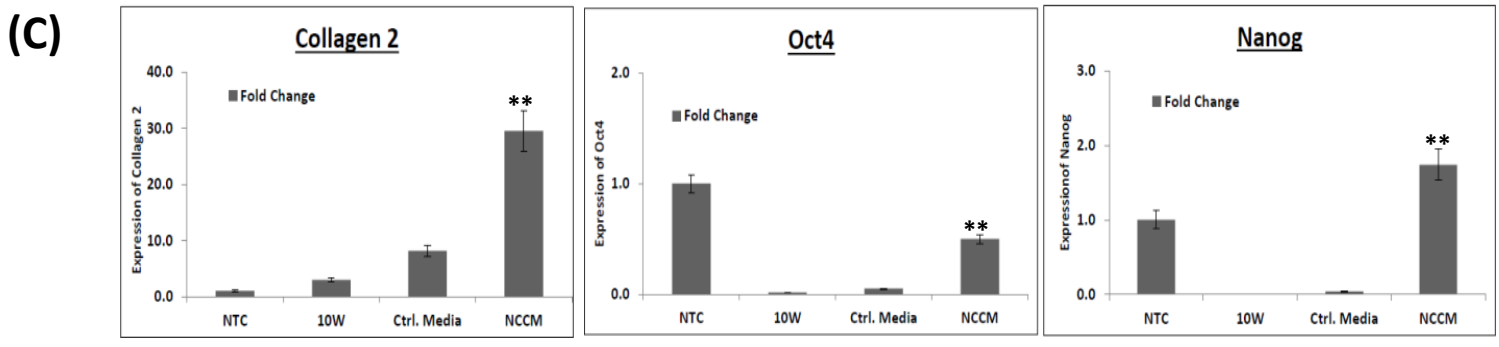
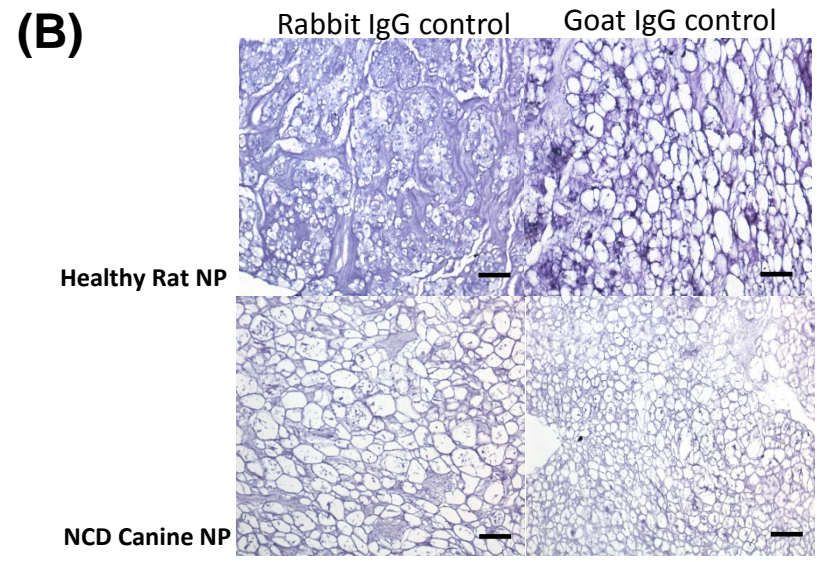
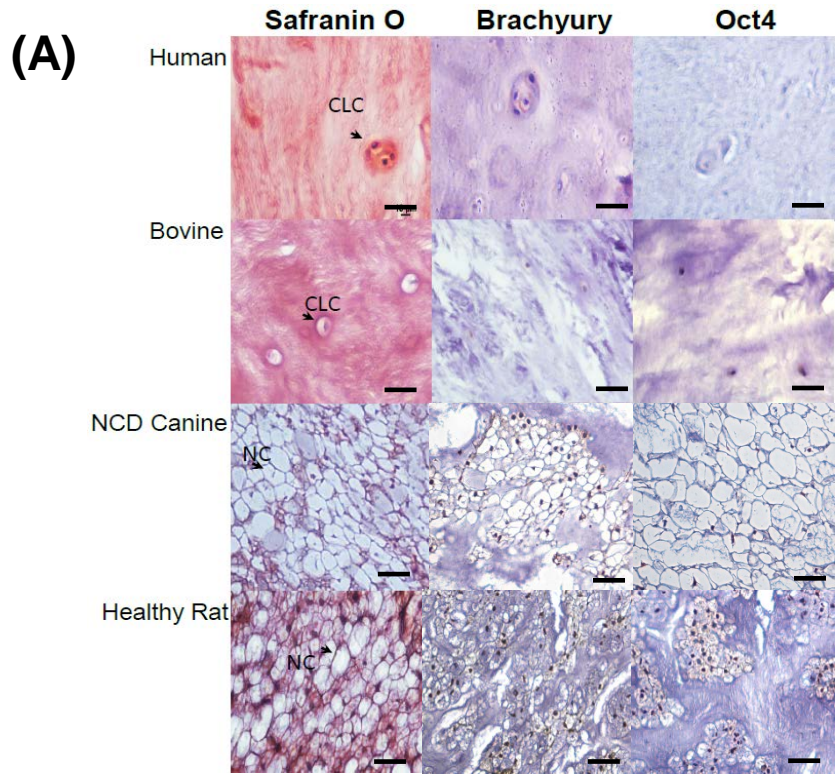
Supplementary Fig. 8. Panel (A) represents histograms showing fold changes in protein expression of MMP-3, MMP-13 and Cox2 on treatment with IL1 β alone or in presence of CTGF, WISP2 or TGF β 1. Panel (B) represents histograms showing fold changes in protein expression of MMP-3, MMP-13 and Cox2 on treatment with IL1 β and TNF α alone or in presence of CTGF, WISP2 or TGF β 1. Each bar in the histogram represents mean \pm S.D. of 5 independent experiments. ***p<0.05; *p-values are w.r.t No treatment controls (NTC); **p-values are w.r.t IL1 β or a combination of IL1 β and TNF α .

Supplementary Fig. 9. Panel represents histograms showing fold change in protein expression of MMP-13, Cox2, Brachyury and Oct4 in rat IVD-NP injected with PBS, CTGF, TGF β 1 alone or in combination. **p<0.05; p-values are w.r.t PBS controls. Each bar in the histogram represents mean \pm S.D. of 3 independent experiments.

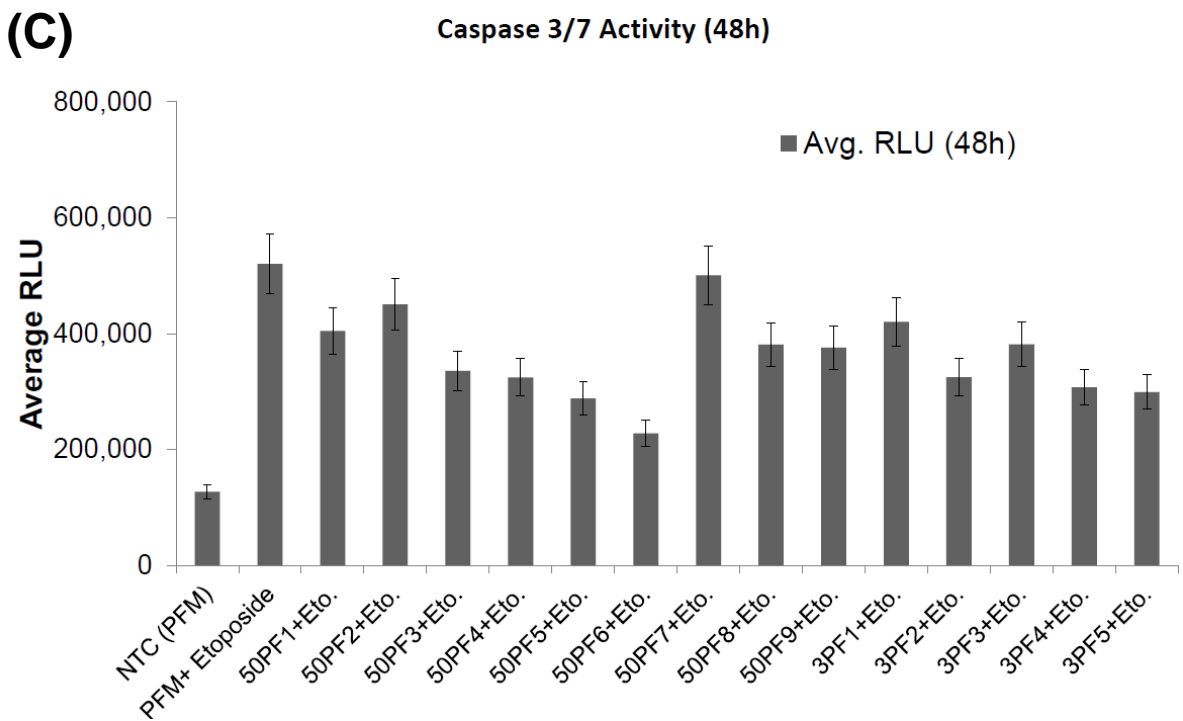
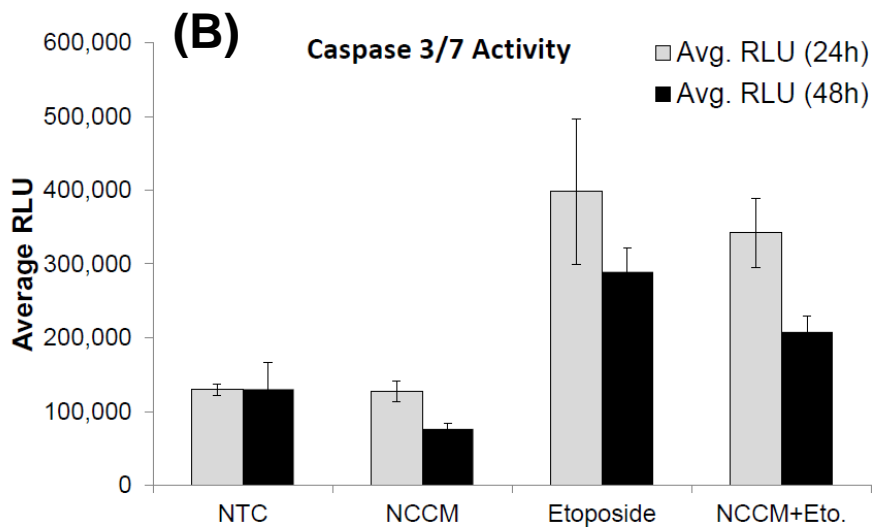
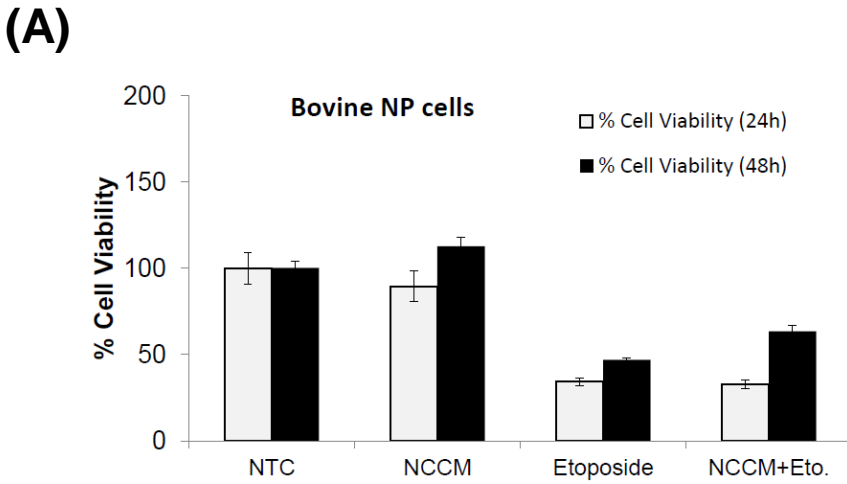
Supplementary Fig. 1.



Supplementary Fig. 2.

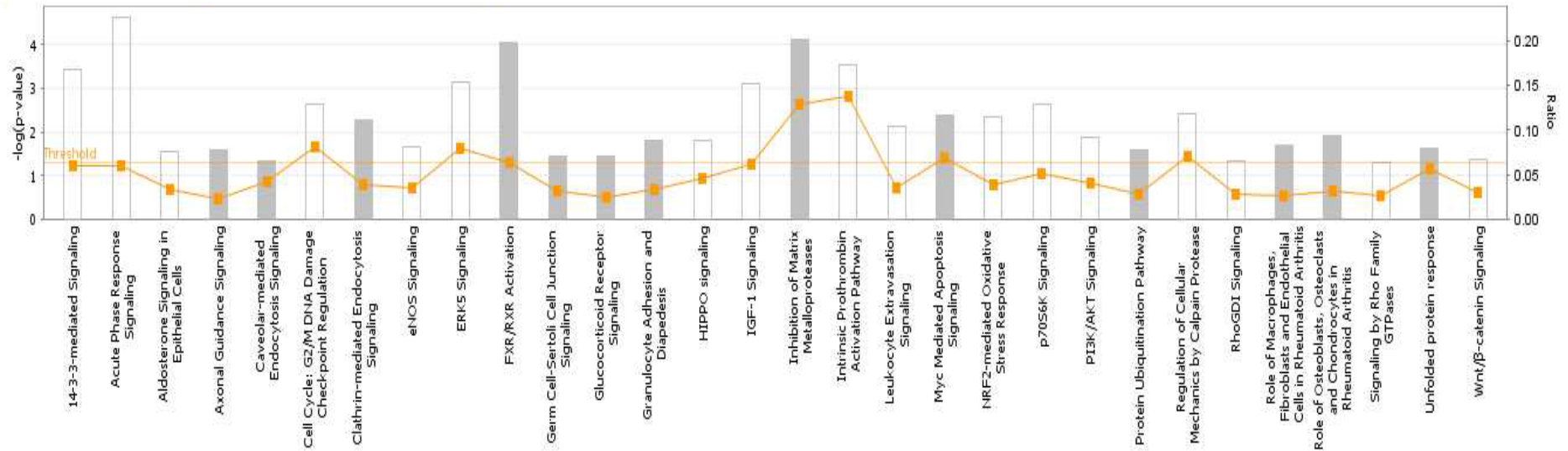


Supplementary Fig. 3.



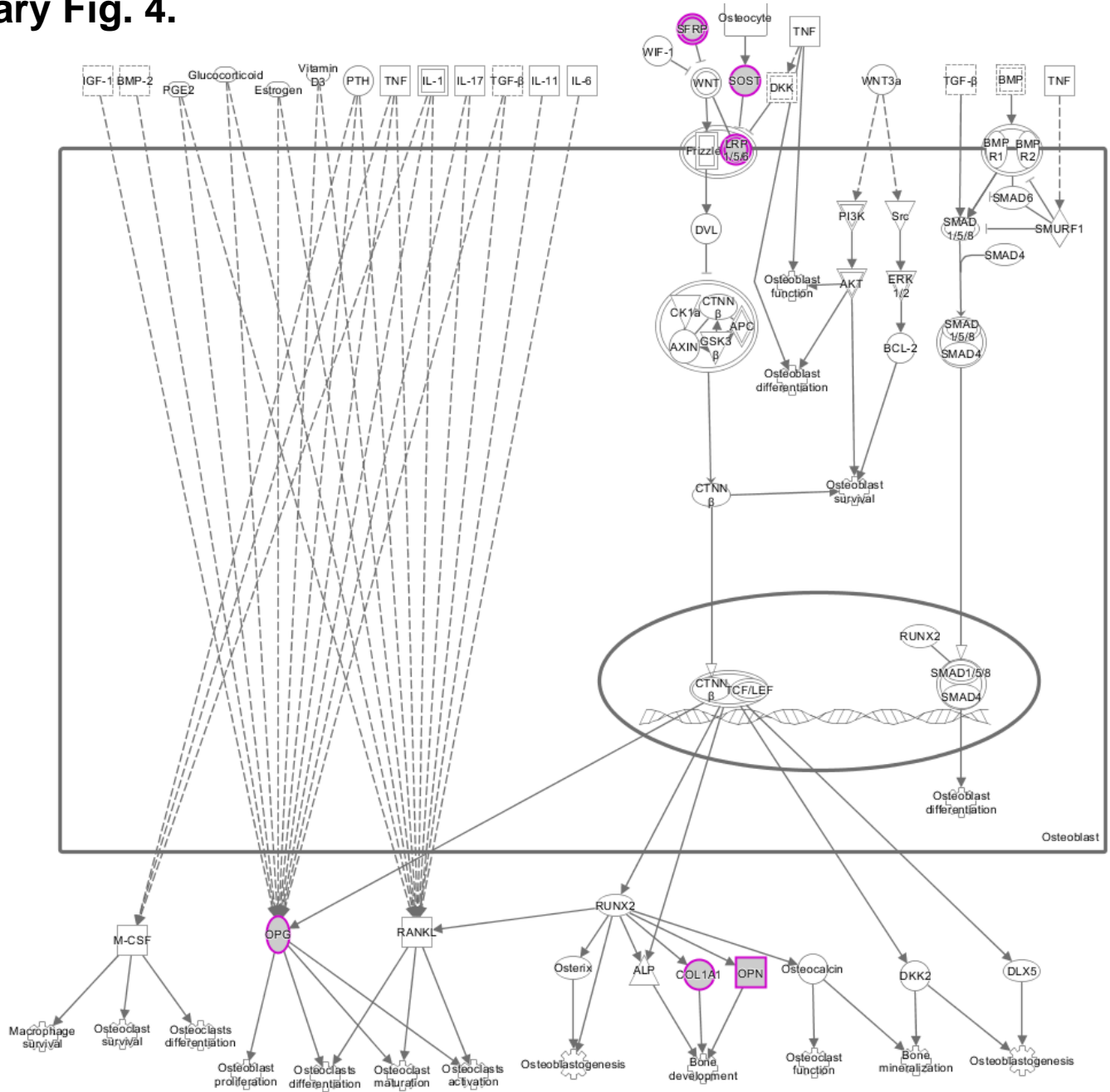
Supplementary Fig. 4.

(A)

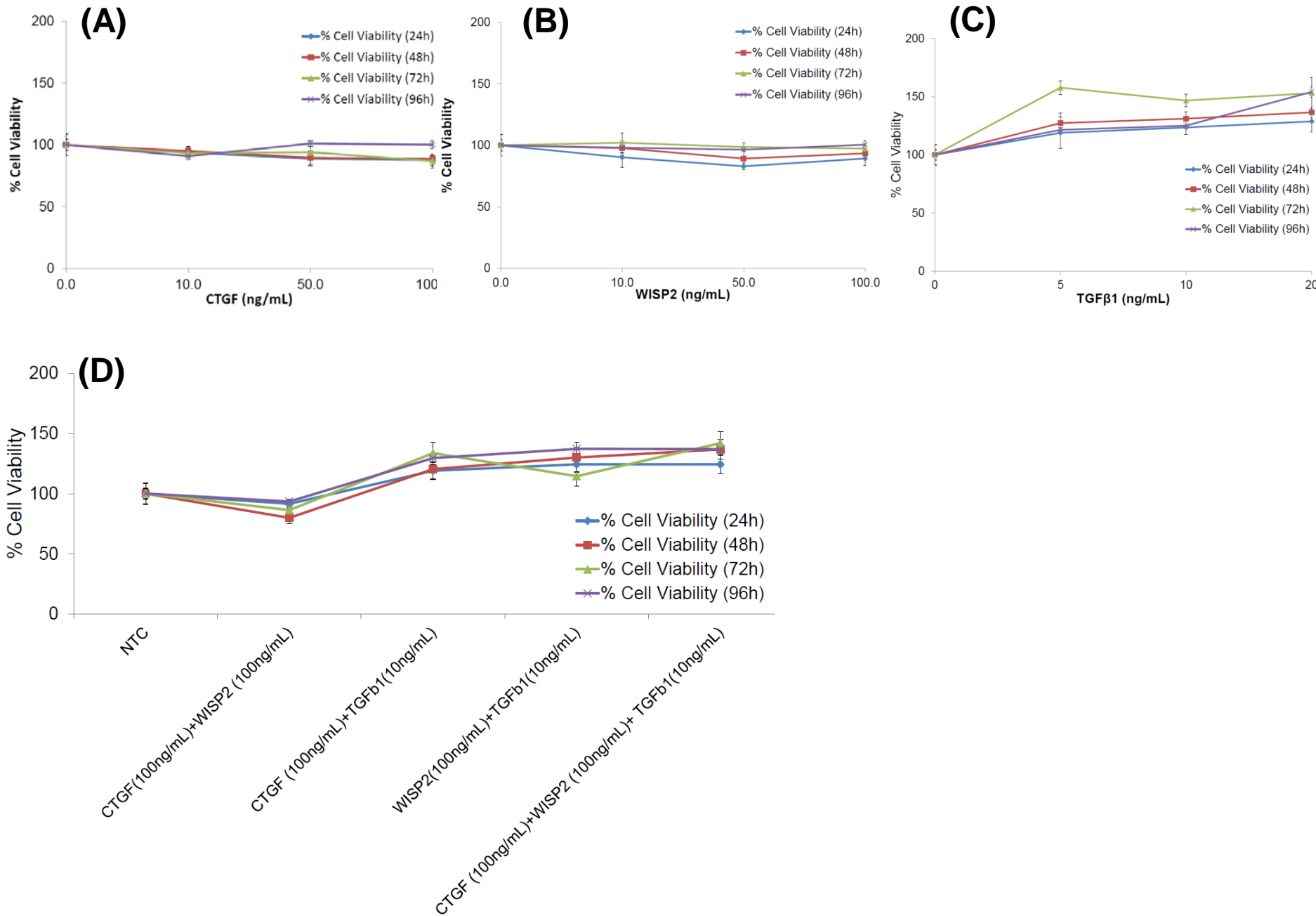


Supplementary Fig. 4.

(C)

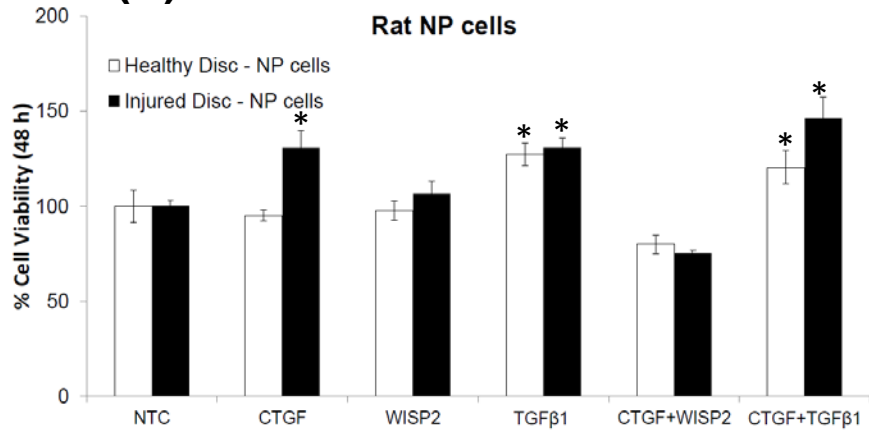


Supplementary Fig. 5.

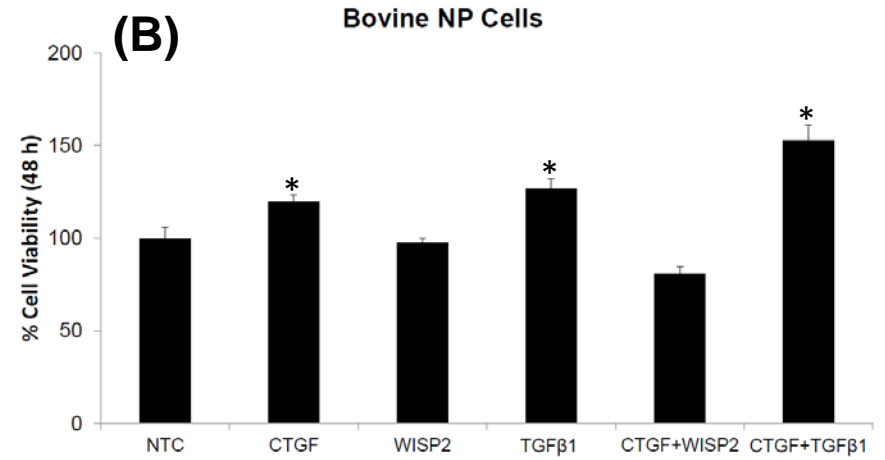


Supplementary Fig. 6.

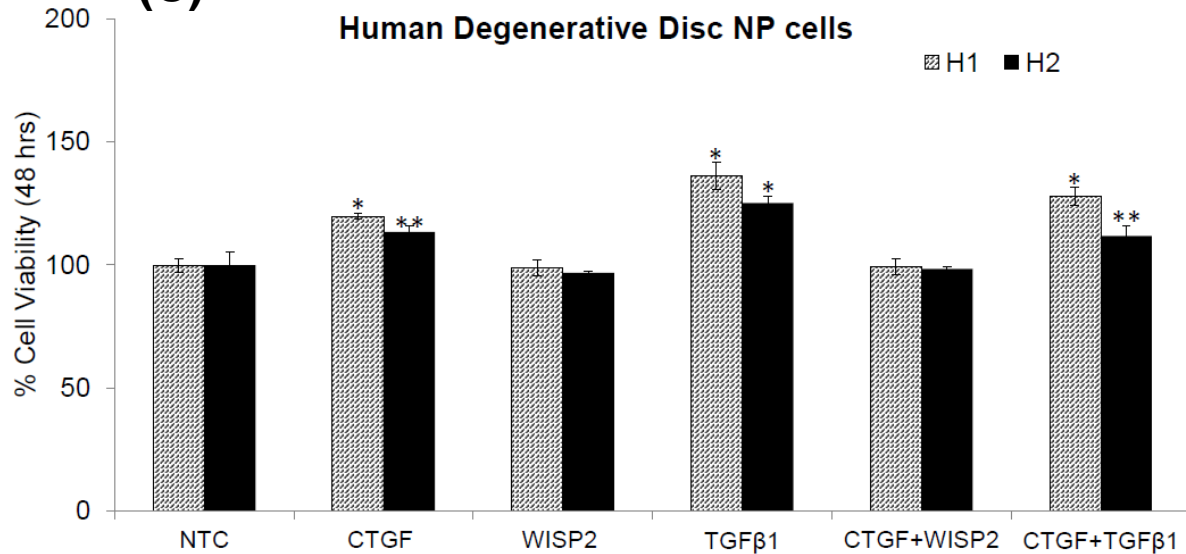
(A)



(B)

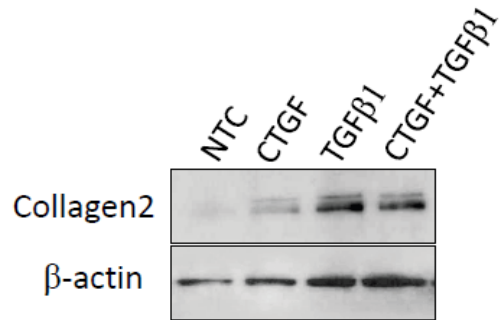


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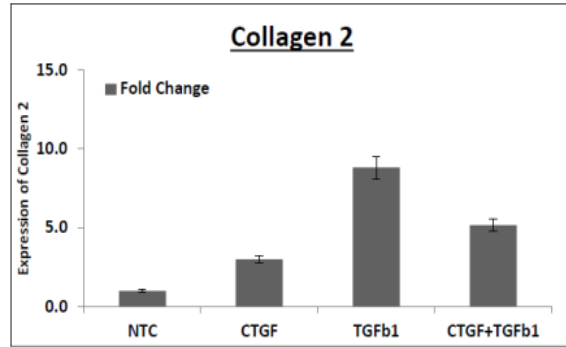


Supplementary Fig. 7.

(A)

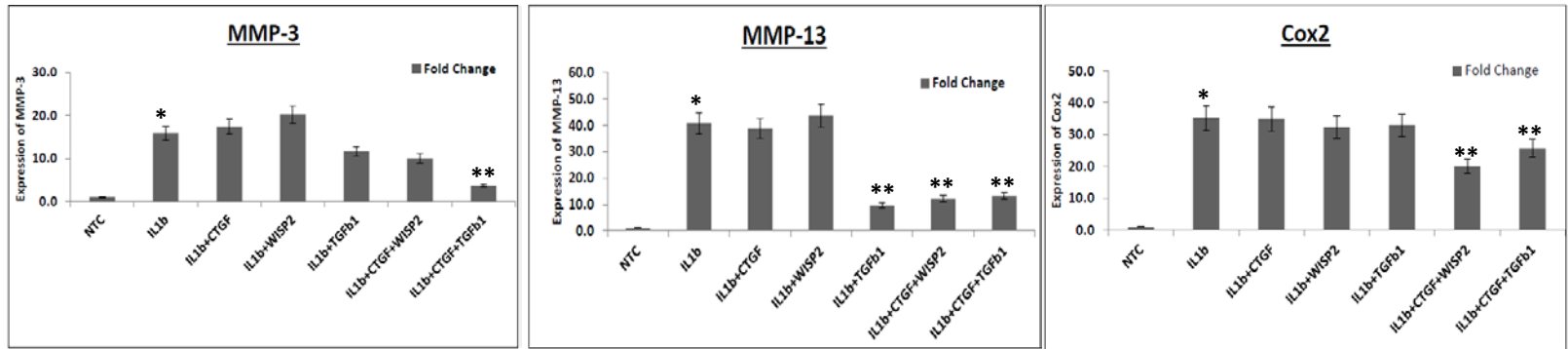


(B)

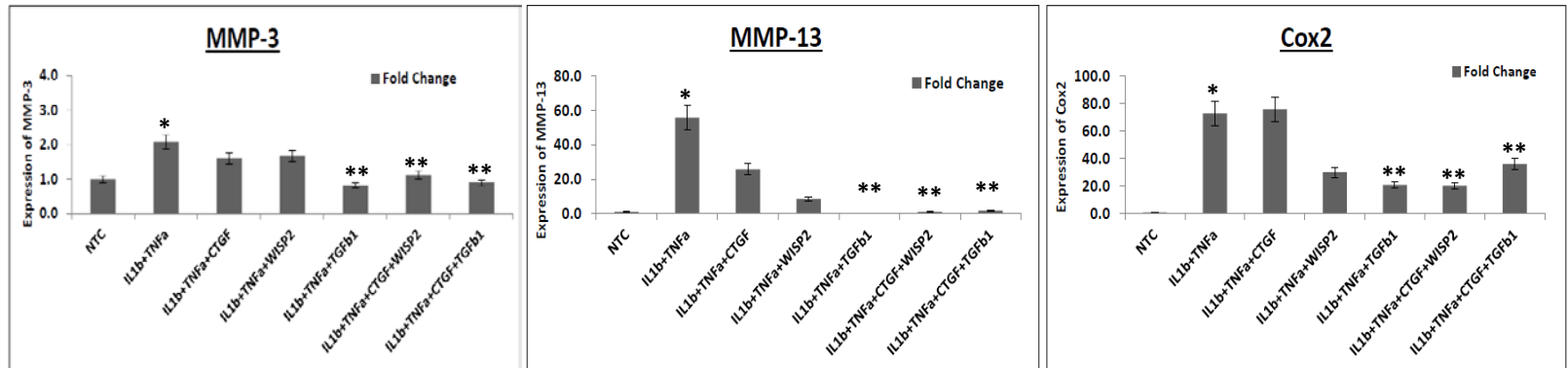


Supplementary Fig. 8.

(A)

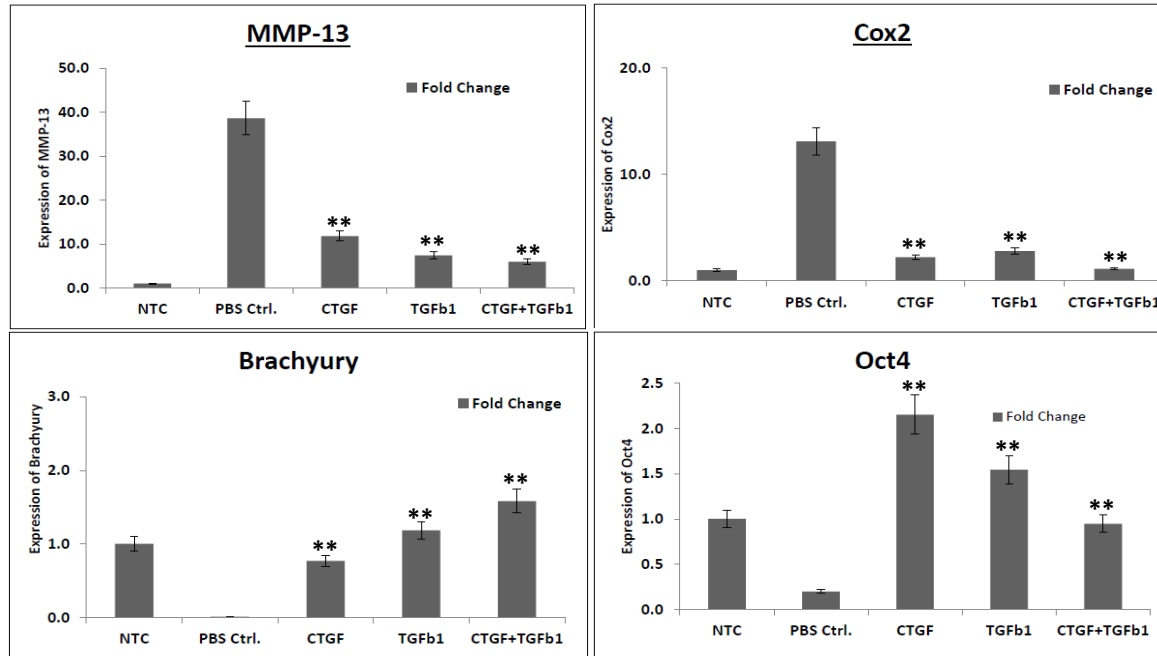


(B)



Supplementary Fig. 9.

(A)



Supplementary Table S1. List of proteins identified in NCCM

S. No.	Identified Proteins	Accession Number	Protein Name-FASTA
1	Uncharacterized protein (Fragment) OS=Canis familiaris GN=TGFBI PE=4 SV=2	F1PW10_CANFA (+1)	Transforming Growth Factor Beta-1
2	Uncharacterized protein OS=Canis familiaris GN=CTGF PE=4 SV=1	J9NTX8_CANFA	Connective Tissue Growth Factor
3	Uncharacterized protein OS=Canis familiaris GN=WISP2 PE=4 SV=2	E2RJ75_CANFA	WNT1 Inducible Signaling Pathway Protein 2(CCN5)
4	Uncharacterized protein OS=Canis familiaris GN=SOST PE=4 SV=1	J9P9E4_CANFA	sclerostin (BMP antagonist)
5	Chordin OS=Canis familiaris GN=CHRD PE=3 SV=2 Uncharacterized protein (Fragment) OS=Canis familiaris	E2RBE4_CANFA (+1)	Chordin
6	GN=FSTL1 PE=4 SV=2 Uncharacterized protein OS=Canis familiaris GN=CD109	F1PY69_CANFA (+1)	Follistatin-Like Protein 1
7	PE=4 SV=2 Uncharacterized protein (Fragment) OS=Canis familiaris	F1PM26_CANFA	CD109
8	GN=FRZB PE=4 SV=2 Uncharacterized protein OS=Canis familiaris GN=VWA1	F1PN55_CANFA	Frizzled-Related Protein 1 Von Willebrand Factor A Domain-Containing Protein 1
9	PE=4 SV=1 Uncharacterized protein OS=Canis familiaris GN=IGFBP7	F6XL96_CANFA	Insulin-Like Growth Factor Binding Protein 7
10	PE=4 SV=1 Uncharacterized protein OS=Canis familiaris GN=IGFL3	E2RNL2_CANFA	Insulin Growth Factor-Like Family Member 3
11	PE=4 SV=1 Cation-independent mannose-6-phosphate/insulin-like growth factor 2 receptor protein OS=Canis familiaris GN=CI-	E2QTE1_CANFA	Cation-independent mannose-6-phosphate/insulin- like growth factor 2 receptor protein
12	MPR/IGF2R PE=2 SV=1 Uncharacterized protein (Fragment) OS=Canis familiaris	B1H0W0_CANFA (+1)	EGFR
13	GN=EGFR PE=3 SV=2 Uncharacterized protein OS=Canis familiaris GN=EFEMP1	F1PF03_CANFA	EGF-Containing Fibulin-Like Extracellular Matrix Protein
14	PE=4 SV=1 Uncharacterized protein OS=Canis familiaris GN=EDIL3	E2R612_CANFA	EGF-Like Repeat And Discoidin I-Like Domain- Containing Protein 32
15	PE=4 SV=2 Uncharacterized protein OS=Canis familiaris GN=EFEMP2	F1P8D5_CANFA	EGF-Containing Fibulin-Like Extracellular Matrix Protein 2
16	PE=4 SV=1 Uncharacterized protein (Fragment) OS=Canis familiaris	E2RNX7_CANFA	Olfactomedin-Like Protein 2B
17	GN=OLFML2B PE=4 SV=2 Uncharacterized protein OS=Canis familiaris GN=OLFML3	F1PAD2_CANFA	Olfactomedin-Like 3
18	PE=4 SV=2 Uncharacterized protein OS=Canis familiaris GN=OGN PE=4	F1PQV4_CANFA	Osteoglycin
19	SV=2 Uncharacterized protein OS=Canis familiaris GN=SPARC	E2RNR0_CANFA	Secreted Protein, Acidic, Cysteine-Rich (Osteonectin)
20	PE=4 SV=1 Uncharacterized protein OS=Canis familiaris GN=SPP1 PE=4	E2RMA3_CANFA	Secreted Phosphoprotein 1 (Osteopontin)
21	SV=1 Uncharacterized protein (Fragment) OS=Canis familiaris	E2R161_CANFA (+1)	Peripherin
22	GN=PRPH PE=3 SV=2 Uncharacterized protein OS=Canis familiaris GN=GFAP	F1PGH6_CANFA	Glial Fibrillary Acidic Protein1
23	PE=3 SV=1 Uncharacterized protein OS=Canis familiaris GN=ANGPTL7	E2RS09_CANFA	Angiopoietin-Like 7
24	PE=4 SV=1 Uncharacterized protein OS=Canis familiaris GN=SLIT3 PE=3	E2R2G1_CANFA	Slit Homolog 3 (Drosophila)
25	SV=2 Uncharacterized protein (Fragment) OS=Canis familiaris	F1Q3G2_CANFA	Receptor Tyrosine Kinase-Like Orphan Receptor 2
26	GN=ROR2 PE=4 SV=2 Uncharacterized protein OS=Canis familiaris GN=PTN PE=4	F1PSD7_CANFA	pleiotrophin, Neurite Growth-Promoting Factor 11
27	SV=1 Uncharacterized protein OS=Canis familiaris GN=LRP1 PE=4	E2R8H9_CANFA (+1)	Low Density Lipoprotein Receptor-Related Protein 1
28	SV=2 Uncharacterized protein OS=Canis familiaris GN=VLDLR	E2R4Q1_CANFA (+1)	Very Low Density Lipoprotein Receptor
29	PE=4 SV=2 Uncharacterized protein (Fragment) OS=Canis familiaris	F1P787_CANFA	Low Density Lipoprotein Receptor-Related Protein 1B
30	GN=LRP1B PE=4 SV=2 Uncharacterized protein (Fragment) OS=Canis familiaris	F1PNZ9_CANFA (+1)	Low Density Lipoprotein Receptor-Related Protein 2
31	GN=LRP2 PE=4 SV=2 Uncharacterized protein OS=Canis familiaris GN=THY1 PE=4	F1PIB4_CANFA	CD90
32	SV=1 Metalloproteinase inhibitor 1 OS=Canis familiaris GN=TIMP1	J9P7X2_CANFA	TIMP1
33	PE=4 SV=2 Metalloproteinase inhibitor 2 (Fragment) OS=Canis familiaris	F1PQS2_CANFA (+1)	TIMP2
34	GN=TIMP2 PE=4 SV=2 Timp3 OS=Canis familiaris GN=timp3 PE=2 SV=1	F1Q4F9_CANFA	Timp3
35	Uncharacterized protein (Fragment) OS=Canis familiaris	F5CBP3_CANFA (+1)	COL15A1
36	GN=COL15A1 PE=4 SV=1	F1PS66_CANFA (+1)	

Supplementary Table S1 contd.

S. No.	Identified Proteins	Accession Number	Protein Name-FASTA
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37	GN=COL6A3 PE=4 SV=2 (F1PR31_CANFA)	F1PR31_CANFA [3]	COL6A3
	Uncharacterized protein OS=Canis familiaris GN=COL6A1		
38	PE=4 SV=2	F1PFM6_CANFA	COL6A1
	Uncharacterized protein OS=Canis familiaris GN=COL2A1		
39	PE=4 SV=1	F1PS24_CANFA (+1)	COL2A1
	Cluster of Uncharacterized protein OS=Canis familiaris		
40	GN=COL11A2 PE=4 SV=1 (F1PQD5_CANFA)	F1PQD5_CANFA [2]	COL11A2
	Uncharacterized protein OS=Canis familiaris GN=COL12A1		
41	PE=4 SV=1	F6XWS5_CANFA (+1)	COL12A1
	Uncharacterized protein OS=Canis familiaris GN=COL3A1		
42	PE=4 SV=2	F1PG69_CANFA (+1)	COL3A1
43	PE=1 SV=1	CO1A1_CANFA (+1)	Collagen alpha-1
	Uncharacterized protein (Fragment) OS=Canis familiaris		
44	GN=COL11A1 PE=4 SV=2	F1PD36_CANFA (+1)	COL11A1
	Uncharacterized protein OS=Canis familiaris GN=COL5A2		
45	PE=4 SV=2	F1PG08_CANFA	COL5A2
	Uncharacterized protein OS=Canis familiaris GN=PCOLCE2		
46	PE=4 SV=1	E2R7L9_CANFA (+2)	Procollagen C-Proteinase Enhancer 1
	Aggrecan core protein OS=Canis familiaris GN=ACAN PE=4		
47	SV=1	F1PWT9_CANFA	Aggrecan core protein
48	Fibronectin OS=Canis familiaris GN=FN1 PE=4 SV=1	J9P8M2_CANFA	Fibronectin
	Uncharacterized protein (Fragment) OS=Canis familiaris		
49	GN=HSPG2 PE=4 SV=1	J9NRJ0_CANFA	Heparan Sulfate Proteoglycan Core Protein, Perlecan
	Uncharacterized protein OS=Canis familiaris GN=LUM PE=4		
50	SV=1	E2R416_CANFA	Lumican Proteoglycan
	Uncharacterized protein OS=Canis familiaris GN=PRG4 PE=4		
51	SV=2	F1PXD6_CANFA	Proteoglycan 4
	Uncharacterized protein OS=Canis familiaris GN=CHAD		
52	PE=4 SV=1	E2QRS3_CANFA	Chondroadherin Proteoglycan, Cartilage Leucine-Rich Protein
	Uncharacterized protein OS=Canis familiaris GN=VCAN		
53	PE=4 SV=2	F1PGL1_CANFA	Versican
54	Decorin OS=Canis familiaris GN=DCN PE=2 SV=2	PGS2_CANFA	Decorin
	Uncharacterized protein OS=Canis familiaris GN=HAPLN1		
55	PE=4 SV=1	J9PAT9_CANFA	HAPLN1
56	Biglycan OS=Canis familiaris GN=BGN PE=4 SV=1	G1K2D8_CANFA (+1)	Biglycan
	Uncharacterized protein OS=Canis familiaris GN=FMOD		
57	PE=4 SV=2	F1PLN5_CANFA	Fibromodulin
	Uncharacterized protein (Fragment) OS=Canis familiaris		
58	GN=TNC PE=4 SV=1	F1PF31_CANFA (+1)	TNC
	Uncharacterized protein OS=Canis familiaris GN=GSN PE=4		
59	SV=1	F6Y3P9_CANFA	Gelsolin
	Uncharacterized protein OS=Canis familiaris GN=CSPG4		
60	PE=4 SV=1	J9P6X3_CANFA	Chondroitin Sulfate Proteoglycan 4
	Uncharacterized protein OS=Canis familiaris GN=THBS4		
61	PE=4 SV=2	F1PSS2_CANFA	Thrombospondin 4
	Uncharacterized protein OS=Canis familiaris GN=PRELP		
62	PE=4 SV=1	E2RL80_CANFA	Proline-Arginine-Rich End Leucine-Rich Repeat Protein2,Prolargin Proteoglycan1
	Uncharacterized protein OS=Canis familiaris GN=OMD PE=4		
63	SV=2	E2RNR9_CANFA (+1)	osteomodulin, Keratan Sulfate Proteoglycan Osteomodulin2
	Cluster of Uncharacterized protein OS=Canis familiaris		
64	GN=NEBL PE=4 SV=2 (F1PQ88_CANFA)	F1PQ88_CANFA [2]	Nebulette
	Uncharacterized protein OS=Canis familiaris GN=PSAP PE=4		
65	SV=1	E2RLF1_CANFA	prosaposin
66	Fibulin-1 OS=Canis familiaris GN=FBLN1 PE=3 SV=2	F1PLV6_CANFA	Fibulin-1
	Uncharacterized protein OS=Canis familiaris GN=ABI3BP		
67	PE=4 SV=2	F1PKX2_CANFA	ABI Gene Family, Member 3 (NESH) Binding Protein2
	Uncharacterized protein OS=Canis familiaris GN=NID2 PE=4		
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	Uncharacterized protein OS=Canis familiaris GN=SEMA3C		
69	PE=4 SV=2	E2R0R3_CANFA	Semaphorin
	Uncharacterized protein OS=Canis familiaris GN=SEMA3E		
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	Cluster of Uncharacterized protein OS=Canis familiaris		
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	Uncharacterized protein OS=Canis familiaris GN=CILP2		
72	PE=4 SV=2	E2RCP2_CANFA	Cartilage Intermediate Layer Protein-Like Protein 2
	Uncharacterized protein OS=Canis familiaris GN=CILP PE=4		
73	SV=1	F6V790_CANFA	CILP

Supplementary Table 1 contd.

S. No.	Identified Proteins	Accession Number	Protein Name-FASTA
74	Galectin OS=Canis familiaris GN=LGALS3 PE=2 SV=1 Uncharacterized protein (Fragment) OS=Canis familiaris GN=CLSTN1 PE=4 SV=1	E5Q8W5_CANFA (+2)	Galectin
75	Uncharacterized protein OS=Canis familiaris GN=LOXL3 PE=4 SV=1	F1PE64_CANFA	Calsyntenin 1
76	Uncharacterized protein OS=Canis familiaris GN=SMOC1 PE=4 SV=2	E2QYZ4_CANFA	LOXL3
77	Uncharacterized protein OS=Canis familiaris GN=SDCBP PE=4 SV=2	F1P8D9_CANFA	SPARC-Related Modular Calcium-Binding Protein 1
78	Dystroglycan OS=Canis familiaris GN=DAG1 PE=3 SV=1	F1PKR0_CANFA	Syndecan Binding Protein (Syntenin)
79	Clusterin OS=Canis familiaris GN=CLU PE=2 SV=1	DAG1_CANFA	Dystroglycan
80	Desmin OS=Canis familiaris GN=DES PE=3 SV=3 Uncharacterized protein (Fragment) OS=Canis familiaris GN=GPC1 PE=3 SV=1	CLUS_CANFA	Clusterin
81	Cluster of Uncharacterized protein (Fragment) OS=Canis familiaris GN=VCL PE=4 SV=2 (F1PKR3_CANFA)	DESM_CANFA	Desmin
82	Uncharacterized protein OS=Canis familiaris GN=FBN1 PE=4 SV=2	F1PPA1_CANFA	Glypican Proteoglycan 1
83	Uncharacterized protein OS=Canis familiaris GN=MATN2 PE=4 SV=2	F1PKR3_CANFA [2]	vinculin
84	Uncharacterized protein OS=Canis familiaris GN=VIT PE=4 SV=2	F1PIA3_CANFA	Fibrillin 1
85	Tumor necrosis factor receptor superfamily member 11B OS=Canis familiaris GN=TNFRSF11B PE=3 SV=1	F1PGH3_CANFA (+1)	Matrilin 2
86	Cluster of Uncharacterized protein OS=Canis familiaris GN=LYPD8 PE=4 SV=1 (F6XCE8_CANFA)	E2QY57_CANFA (+1)	VIT Tumor necrosis factor receptor superfamily member 11B
87	Uncharacterized protein OS=Canis familiaris GN=CLEC3A PE=4 SV=1	F6V4E0_CANFA	Ly6/PLAUR Domain-Containing Protein 8 C-Type (Calcium Dependent, Carbohydrate- Recognition Domain) Lectin, Superfamily Member 1 (Cartilage-Derived)1
88	Uncharacterized protein OS=Canis familiaris GN=LGALS3BP PE=4 SV=1	F6XCE8_CANFA [2]	Lectin Galactoside-Binding Soluble 3-Binding Protein2
89	Uncharacterized protein OS=Canis familiaris GN=CCL16 PE=4 SV=1	E2RQC0_CANFA	IL-10-Inducible Chemokine
90	Uncharacterized protein (Fragment) OS=Canis familiaris GN=MFGE8 PE=4 SV=2	J9NV49_CANFA	Milk Fat Globule-EGF Factor 8
91	Uncharacterized protein OS=Canis familiaris GN=CCDC80 PE=4 SV=2	F1PFZ5_CANFA	Coiled-Coil Domain-Containing Protein 80
92	Rab GDP dissociation inhibitor beta (Fragment) OS=Canis familiaris GN=GDI2 PE=4 SV=1	E2RGR7_CANFA	Rab GDP dissociation inhibitor beta
93	Uncharacterized protein (Fragment) OS=Canis familiaris GN=PTGFRN PE=4 SV=2	F1P8L7_CANFA	Prostaglandin F2 Receptor Inhibitor
94	Uncharacterized protein (Fragment) OS=Canis familiaris GN=HTRA1 PE=4 SV=2	F1PR26_CANFA	HtrA Serine Peptidase 1
95	Uncharacterized protein OS=Canis familiaris GN=KAL1 PE=4 SV=2	F1PU95_CANFA	Kallmann Syndrome 1 Sequence
96	Uncharacterized protein OS=Canis familiaris GN=TSPAN8 PE=4 SV=1	F1Q1G0_CANFA	tetraspanin-8
97	Uncharacterized protein OS=Canis familiaris GN=PARK7 PE=4 SV=1	E2QSC8_CANFA	Parkinson Protein 7
98	Sulfhydryl oxidase (Fragment) OS=Canis familiaris GN=QSOX1 PE=3 SV=2	E2QS13_CANFA	Sulfhydryl oxidase
99	CC chemokine ligand 21 OS=Canis familiaris GN=CCL21 PE=2 SV=1	F1PLT8_CANFA	CCL21
100	Uncharacterized protein OS=Canis familiaris GN=WDR1 PE=2 SV=2	Q68AM9_CANFA	CCL21
101	Uncharacterized protein OS=Canis familiaris GN=CDH2 PE=3 SV=2	F1PR93_CANFA	WD Repeat-Containing Protein 1
102	Cadherin-1 OS=Canis familiaris GN=CDH1 PE=1 SV=2 Uncharacterized protein (Fragment) OS=Canis familiaris GN=NCAM1 PE=4 SV=2	F1PEC4_CANFA	CDH2
103	Uncharacterized protein OS=Canis familiaris GN=BCAM PE=4 SV=2	CADH1_CANFA (+1)	Cadherin-1
104	Uncharacterized protein (Fragment) OS=Canis familiaris GN=NCAN PE=4 SV=2	F1PMU2_CANFA (+3)	Neural Cell Adhesion Molecule 1
105	Uncharacterized protein (Fragment) OS=Canis familiaris GN=NCAM1 PE=4 SV=2	F1PWJ7_CANFA (+1)	Basal Cell Adhesion Molecule
106	Trappin-2 OS=Canis familiaris GN=PI3 PE=4 SV=1	E2RBU8_CANFA	neurocan (Chondroitin Sulfate Proteoglycan 3)
107	Galectin OS=Canis familiaris GN=LGALS1 PE=2 SV=1 Uncharacterized protein OS=Canis familiaris GN=XIRP2 PE=4 SV=2	D3DMB4_CANFA	Trappin
108	Uncharacterized protein OS=Canis familiaris GN=CD55 PE=4 SV=1	E2RJL1_CANFA (+1)	Galectin
109	Uncharacterized protein OS=Canis familiaris GN=CD55 PE=4 SV=1	F1PCJ4_CANFA-DECOY (- Xin Actin-Binding Repeat-Containing Protein 2	Decay Accelerating Factor For Complement (CD55,
110		J9NTG7_CANFA	
111			

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S. No.	Identified Proteins	Accession Number	Protein Name-FASTA
	Uncharacterized protein OS=Canis familiaris GN=GM2A		
112	PE=4 SV=2	F1P8U3_CANFA	GM2 Ganglioside Activator
	Uncharacterized protein (Fragment) OS=Canis familiaris		
113	GN=CXCL12 PE=4 SV=2	F1PNB2_CANFA	CXCL12
	Uncharacterized protein OS=Canis familiaris GN=LECT2		
114	PE=4 SV=1	E2RCY6_CANFA	Leukocyte Cell-Derived Chemotaxin-2
	Uncharacterized protein OS=Canis familiaris GN=EHD3 PE=4		
115	SV=2	F1PXX1_CANFA	EH-Domain Containing 3
	Uncharacterized protein OS=Canis familiaris GN=FOLR2		
116	PE=4 SV=1	E2QXF0_CANFA	Folate Receptor 2 (Fetal)
	Uncharacterized protein OS=Canis familiaris GN=PDCD6		
117	PE=4 SV=2	F1Q1H3_CANFA	Programmed Cell Death 6
	Uncharacterized protein OS=Canis familiaris GN=SSC5D		
118	PE=4 SV=2	E2R0D2_CANFA	Scavenger Receptor Cysteine Rich Domain Containing (5 Domains)
	Uncharacterized protein OS=Canis familiaris GN=EML2 PE=4		
119	SV=2	E2RII4_CANFA (+1)	Echinoderm Microtubule Associated Protein Like 2
	Uncharacterized protein OS=Canis familiaris GN=FAM3C		
120	PE=4 SV=2	F1PGP3_CANFA	Family With Sequence Similarity 3, Member C
	Uncharacterized protein OS=Canis familiaris GN=CD63 PE=4		
121	SV=2	F1PIT4_CANFA	Tetraspanin-30
	Uncharacterized protein OS=Canis familiaris GN=S100A6		
122	PE=4 SV=1	E2R5P5_CANFA	S100A6
	Uncharacterized protein OS=Canis familiaris GN=S100A13		
123	PE=4 SV=1	E2R9P2_CANFA	S100A13
	Uncharacterized protein OS=Canis familiaris GN=LAMP1		
124	PE=4 SV=2	F1Q260_CANFA	Lysosome-Associated Membrane Protein 1
	Uncharacterized protein OS=Canis familiaris GN=GAS1 PE=4		
125	SV=1	E2R2S1_CANFA	Growth Arrest-Specific 1
	Uncharacterized protein OS=Canis familiaris GN=CTSL PE=2 SV=1		
126	Cathepsin L1 OS=Canis familiaris GN=CTSL PE=2 SV=1	CATL1_CANFA	Cathepsin L1
	Uncharacterized protein OS=Canis familiaris GN=SCRN2		
127	PE=4 SV=1	E2QTH4_CANFA	Secernin 2
	Uncharacterized protein OS=Canis familiaris GN=UNC5C		
128	PE=4 SV=2	F1PUV2_CANFA (+1)	Netrin Receptor UNC5C
	Uncharacterized protein (Fragment) OS=Canis familiaris		
129	GN=AEBP1 PE=4 SV=2	H9GW59_CANFA	AE Binding Protein 1
	Epididymal secretory protein E1 (Fragment) OS=Canis		
130	familiaris GN=NPC2 PE=4 SV=2	F1PAR9_CANFA (+1)	Epididymal secretory protein E1
	Uncharacterized protein OS=Canis familiaris GN=LAMP2		
131	PE=4 SV=2	E2RNJ1_CANFA (+1)	Lysosome-Associated Membrane Protein 2
	Cluster of Uncharacterized protein OS=Canis familiaris		
132	GN=PLXNA3 PE=4 SV=2 (F1PWQ0_CANFA)	F1PWQ0_CANFA [3]	plexin-A3
	Uncharacterized protein OS=Canis familiaris GN=PLXNB2		
133	PE=4 SV=2	F1P9S5_CANFA	Plexin B2
	Uncharacterized protein OS=Canis familiaris GN=PLXDC2		
134	PE=4 SV=2	F1PNK7_CANFA	Plexin Domain Containing 2
	Uncharacterized protein OS=Canis familiaris GN=EHD2 PE=4		
135	SV=2	F1PNP7_CANFA	EH-Domain Containing 2
	Uncharacterized protein OS=Canis familiaris GN=AHSG		
136	PE=4 SV=2	E2QUV3_CANFA	alpha-2-HS-glycoprotein
	Uncharacterized protein OS=Canis familiaris GN=CAPG		
137	PE=4 SV=2	E2R413_CANFA	Gelsolin-Like Capping Protein
	Uncharacterized protein OS=Canis familiaris GN=SLC29A1		
138	Mucin OS=Canis familiaris PE=2 SV=1	Q28226_CANFA	Mucin
	Uncharacterized protein OS=Canis familiaris GN=SLC29A1		
139	PE=3 SV=2	E2RLS3_CANFA	Solute Carrier Family 29 (Nucleoside Transporters), Member 1
	Uncharacterized protein OS=Canis familiaris GN=CA2 PE=4		
140	SV=2	F1PDY8_CANFA	CA2
	Uncharacterized protein OS=Canis familiaris GN=CA3 PE=4		
141	SV=2	F1PDZ7_CANFA	Carbonic Anhydrase 3
	Uncharacterized protein OS=Canis familiaris GN=PLOD1		
142	PE=4 SV=2	F1P9Z8_CANFA (+1)	Procollagen-Lysine, 2-Oxoglutarate 5-Dioxygenase 1
	Uncharacterized protein (Fragment) OS=Canis familiaris		
143	GN=PAM PE=4 SV=1	F1Q4G4_CANFA	Peptidylglycine Alpha-Amidating Monooxygenase
	Uncharacterized protein OS=Canis familiaris GN=ECHDC1		
144	PE=3 SV=2	F1PW22_CANFA	Enoyl-CoA Hydratase Domain-Containing Protein 1
	Uncharacterized protein (Fragment) OS=Canis familiaris		
145	GN=PCSK5 PE=3 SV=2	F1P9I5_CANFA (+2)	Proprotein Convertase Subtilisin/Kexin Type 5
	Uncharacterized protein (Fragment) OS=Canis familiaris		
146	GN=CHST6 PE=4 SV=2	E2RSN9_CANFA	Carbohydrate (N-Acetylglucosamine 6-O) Sulfotransferase 6
	Uncharacterized protein OS=Canis familiaris GN=APLP2		
147	PE=4 SV=1	E2RPM5_CANFA (+1)	Amyloid Beta (A4) Precursor-Like Protein 2

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S. No.	Identified Proteins	Accession Number	Protein Name-FASTA
	Uncharacterized protein OS=Canis familiaris GN=GBE1 PE=4		
148	SV=2 Beta-glucuronidase OS=Canis familiaris GN=GUSB PE=1	F1PX32_CANFA	Glucan (1,4-Alpha-), Branching Enzyme 1
149	SV=1 Xylosyltransferase 1 (Fragment) OS=Canis familiaris	BGLR_CANFA (+1)	Beta-glucuronidase
150	GN=XYLT1 PE=4 SV=1 Uncharacterized protein OS=Canis familiaris GN=GPLD1	F1PB62_CANFA (+1)	Xylosyltransferase 1 Glycosylphosphatidylinositol Specific
151	PE=4 SV=2 Uncharacterized protein OS=Canis familiaris GN=SIAE PE=4	E2RK02_CANFA	Phospholipase D11
152	SV=2 Carbohydrate sulfotransferase-like protein OS=Canis	F1PXN3_CANFA	Sialic Acid Acetyltransferase1
153	familiaris GN=CHST3 PE=4 SV=2 N-acetylgalactosamine-6-sulfatase (Fragment) OS=Canis	E2RLE9_CANFA	Carbohydrate sulfotransferase-like protein
154	familiaris GN=GALNS PE=4 SV=2 Uncharacterized protein OS=Canis familiaris GN=MLXIPL	F1PHF0_CANFA (+1)	N-acetylgalactosamine-6-sulfatase
155	PE=4 SV=1 Uncharacterized protein OS=Canis familiaris GN=LYZ PE=3	E2RAQ3_CANFA	MLX Interacting Protein-Like
156	SV=1 Serum albumin OS=Canis familiaris GN=ALB PE=1 SV=3	G1K265_CANFA (+1)	Lysozyme
157	Uncharacterized protein OS=Canis familiaris GN=SPTBN1	ALBU_CANFA	Serum albumin
158	PE=4 SV=2 Histone H4 OS=Canis familiaris GN=HIST1H4L PE=3 SV=1	F1PXT8_CANFA (+1)	Spectrin, Beta, Non-Erythrocytic 1
159	Uncharacterized protein OS=Canis familiaris GN=HPX PE=4	F2Z4N2_CANFA (+3)	Histone H4
160	SV=2 Uncharacterized protein OS=Canis familiaris GN=TF PE=4	F1PZR4_CANFA	hemopexin
161	SV=1 Apolipoprotein E OS=Canis familiaris GN=APOE PE=3 SV=1	J9P430_CANFA	transferrin
162	Uncharacterized protein OS=Canis familiaris GN=A2M PE=4	F1PJ74_CANFA	Apolipoprotein E
163	SV=1 Hemoglobin subunit alpha OS=Canis familiaris GN=HBA	F6UME0_CANFA	alpha-2-macroglobulin
164	PE=1 SV=1 Protein disulfide-isomerase OS=Canis familiaris GN=PDIA3	HBA_CANFA (+3)	Hemoglobin subunit alpha
165	PE=3 SV=1 Uncharacterized protein OS=Canis familiaris GN=NPEPPS	E2RD86_CANFA	Protein disulfide-isomerase
166	PE=4 SV=2 Uncharacterized protein OS=Canis familiaris GN=PGAM4	F1P7M0_CANFA	Aminopeptidase Puromycin Sensitive
167	PE=3 SV=2 Cyclic nucleotide gated channel beta 1 OS=Canis familiaris	E2RT65_CANFA	Phosphoglycerate Mutase Processed Protein
168	GN=CNGB1 PE=2 SV=1 Uncharacterized protein OS=Canis familiaris GN=UBB PE=4	S4V0G9_CANFA (+1)	Cyclic nucleotide gated channel beta 1
169	SV=2 Cluster of Superoxide dismutase [Cu-Zn] (Fragment)	F1PDG4_CANFA (+3)	Ubiquitin B1
170	OS=Canis familiaris GN=SOD1 PE=3 SV=2 (F1Q462_CANFA)	F1Q462_CANFA [2]	Superoxide dismutase [Cu-Zn]
171	Purine nucleoside phosphorylase OS=Canis familiaris	F1PQM1_CANFA	Purine nucleoside phosphorylase
172	GN=PNP PE=3 SV=2 Uncharacterized protein (Fragment) OS=Canis familiaris	F1PUL4_CANFA	Phosphoglucomutase 1
173	GN=PGM1 PE=3 SV=2 Triosephosphate isomerase OS=Canis familiaris GN=TPI1	TPIS_CANFA	Triosephosphate Isomerase
174	PE=1 SV=3 Aldose 1-epimerase OS=Canis familiaris GN=GALM PE=3	E2QWA2_CANFA (+1)	Aldose 1-epimerase
175	SV=1 Proteasome subunit alpha type OS=Canis familiaris	E2RMN2_CANFA	Proteasome subunit alpha
176	GN=PSMA6 PE=3 SV=1 Fructose-bisphosphate aldolase OS=Canis familiaris	F1PL63_CANFA (+1)	Fructose-bisphosphate aldolase
177	GN=ALDOC PE=3 SV=1 Uncharacterized protein OS=Canis familiaris GN=PGLS PE=4	E2RLQ6_CANFA	6-phosphogluconolactonase1
178	SV=1 Uncharacterized protein (Fragment) OS=Canis familiaris	E2RMJ8_CANFA	DNAH10
179	GN=DNAH10 PE=4 SV=2 Uncharacterized protein OS=Canis familiaris GN=BLVRB	E2QVU9_CANFA	Biliverdin Reductase B (Flavin Reductase (NADPH))
180	PE=4 SV=1 Uncharacterized protein OS=Canis familiaris GN=GPI PE=3	E2R2C3_CANFA	Glucose Phosphate Isomerase
181	SV=1 Fructose-bisphosphate aldolase (Fragment) OS=Canis	F1PBT3_CANFA	Fructose-bisphosphate aldolase
182	familiaris GN=ALDOA PE=3 SV=2 Uncharacterized protein (Fragment) OS=Canis familiaris	F1PGY1_CANFA	HSP90AA1
	GN=HSP90AA1 PE=4 SV=2		

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S. No.	Identified Proteins	Accession Number	Protein Name-FASTA
	Cluster of Uncharacterized protein OS=Canis familiaris		
183	GN=HSPA8 PE=3 SV=1 (E2R0T6_CANFA)	E2R0T6_CANFA [3]	HSPA8
	Uncharacterized protein OS=Canis familiaris GN=HSPA8		
184	PE=3 SV=1	E2R0T6_CANFA (+1)	HSPA8
	Uncharacterized protein OS=Canis familiaris GN=HSPA2		
185	PE=3 SV=1	E2QX84_CANFA	HSPA2
186	Annexin OS=Canis familiaris GN=ANXA5 PE=3 SV=2	E2RQ14_CANFA	ANXA5
	Cluster of Uncharacterized protein OS=Canis familiaris		
187	GN=ACTN4 PE=4 SV=1 (E2R5T9_CANFA)	E2R5T9_CANFA [3]	ACTN4
	Uncharacterized protein OS=Canis familiaris GN=ACTN4		
188	PE=4 SV=1	E2R5T9_CANFA (+1)	ACTN4
	Uncharacterized protein OS=Canis familiaris GN=ACTN1		
189	PE=4 SV=2	E2QY08_CANFA	ACTN1
190	Annexin A2 OS=Canis familiaris GN=ANXA2 PE=1 SV=1	ANXA2_CANFA	ANXA2
191	Annexin OS=Canis familiaris GN=ANXA4 PE=3 SV=2	F1PXG4_CANFA	ANXA4
192	Annexin OS=Canis familiaris GN=ANXA8L1 PE=3 SV=2	E2R0S6_CANFA	Annexin
	Glyceraldehyde-3-phosphate dehydrogenase (Fragment)		
193	OS=Canis familiaris GN=GAPDH PE=3 SV=2	F1PTZ9_CANFA	Glyceraldehyde-3-phosphate dehydrogenase
	Uncharacterized protein OS=Canis familiaris GN=LAMB2		
194	PE=4 SV=1	E2R4J1_CANFA	Laminin, Beta 2
	Uncharacterized protein OS=Canis familiaris GN=LAMC1		
195	PE=4 SV=2	F1PHK9_CANFA	Laminin B2 Chain
	Uncharacterized protein OS=Canis familiaris GN=LAMA4		
196	PE=4 SV=2	F1PR66_CANFA	Laminin, Alpha 4
	L-lactate dehydrogenase OS=Canis familiaris GN=LDHA		
197	PE=3 SV=2	F1PVW0_CANFA	LDHA
	Uncharacterized protein (Fragment) OS=Canis familiaris		
198	GN=MFI2 PE=4 SV=2	F1P7Y8_CANFA	melanotransferrin
	Superoxide dismutase [Cu-Zn] OS=Canis familiaris		
199	GN=SOD3 PE=3 SV=2	F1PAS8_CANFA	Superoxide dismutase [Cu-Zn]
	Uncharacterized protein OS=Canis familiaris GN=CP PE=4		
200	SV=2	F1PAX2_CANFA	Ceruloplasmin (Ferroxidase)
	Cluster of Uncharacterized protein OS=Canis familiaris		
201	GN=YWHAZ PE=3 SV=2 (F1PBL1_CANFA)	F1PBL1_CANFA [6]	YWHAZ
	Amyloid beta A4 protein OS=Canis familiaris GN=APP PE=4		
202	SV=2	F1P603_CANFA (+5)	Amyloid beta A4 protein
	Uncharacterized protein OS=Canis familiaris GN=YWHAZ		
203	PE=3 SV=2	F1PBL1_CANFA	YWHAZ
	Uncharacterized protein OS=Canis familiaris GN=YWHAB		
204	PE=3 SV=2	F1PKW7_CANFA	YWHAB
	Uncharacterized protein (Fragment) OS=Canis familiaris		
205	GN=YWHAG PE=3 SV=1	J9NRH5_CANFA	YWHAG
	Uncharacterized protein OS=Canis familiaris GN=YWHAH		
206	PE=3 SV=1	J9P6N4_CANFA	YWHAH
	Uncharacterized protein OS=Canis familiaris GN=YWHAG		
207	PE=4 SV=1	E2QVP6_CANFA	YWHAG
	Cluster of Actin, cytoplasmic 1 OS=Canis familiaris GN=ACTB		
208	PE=2 SV=3 (ACTB_CANFA)	ACTB_CANFA [2]	ACTB
	Actin, cytoplasmic 1 OS=Canis familiaris GN=ACTB PE=2		
209	SV=3	ACTB_CANFA	ACTB
	Uncharacterized protein OS=Canis familiaris GN=ACTG1		
210	PE=3 SV=2	F1PQL8_CANFA	ACTG1
	Uncharacterized protein (Fragment) OS=Canis familiaris		
211	GN=TUBA1C PE=3 SV=2	E2RNQ2_CANFA (+1)	Tubulin, Alpha 1c
	Uncharacterized protein OS=Canis familiaris GN=LTF PE=4		
212	SV=1	F1PR54_CANFA	lactotransferrin
	Phosphatidylethanolamine-binding protein 1 OS=Canis		
213	familiaris GN=PEBP1 PE=1 SV=1	PEBP1_CANFA	Phosphatidylethanolamine-binding protein 1
214	Alpha 1 antitrypsin OS=Canis familiaris PE=2 SV=1	A1ILJ0_CANFA (+1)	Alpha 1 antitrypsin
	Uncharacterized protein OS=Canis familiaris GN=MRC2		
215	PE=4 SV=2	F1PCR2_CANFA (+1)	Mannose Receptor, C Type 2
216	Malic enzyme OS=Canis familiaris GN=ME1 PE=3 SV=1	J9NZX7_CANFA	Malic enzyme
	Uncharacterized protein OS=Canis familiaris GN=PRDX2		
217	PE=4 SV=2	F1PCG4_CANFA	PRDX2
	Cluster of Uncharacterized protein OS=Canis familiaris		
218	GN=GSTM4 PE=3 SV=2 (F1P6R7_CANFA)	F1P6R7_CANFA [3]	GSTM4

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S. No.	Identified Proteins	Accession Number	Protein Name-FASTA
219	Uncharacterized protein OS=Canis familiaris GN=GSTM4 PE=3 SV=2	F1P6R7_CANFA (+1)	GSTM4
220	Uncharacterized protein OS=Canis familiaris GN=GSTM3 PE=3 SV=1	E2R5B9_CANFA	GSTM3
221	Uncharacterized protein OS=Canis familiaris GN=DPYSL2 PE=4 SV=2	F1P9U4_CANFA	Dihydropyrimidinase-Like 2
222	Uncharacterized protein OS=Canis familiaris GN=PRDX1 PE=4 SV=1	E2RHG2_CANFA	PRDX1
223	Uncharacterized protein OS=Canis familiaris GN=HSPA5 PE=3 SV=2	F1PIC7_CANFA	HSPA5
224	Annexin OS=Canis familiaris GN=ANXA1 PE=3 SV=1	F1P6B7_CANFA	Annexin
225	L-lactate dehydrogenase OS=Canis familiaris GN=LDHB PE=3 SV=1	E2R761_CANFA (+1)	L-lactate dehydrogenase
226	Phosphoglycerate kinase OS=Canis familiaris GN=PGK1 PE=3 SV=1	E2RRC9_CANFA	PGK1
227	Malate dehydrogenase (Fragment) OS=Canis familiaris GN=MDH1 PE=3 SV=2	E2QV08_CANFA (+1)	Malate dehydrogenase
228	Pyruvate kinase OS=Canis familiaris GN=PKM PE=3 SV=2	F1PHR2_CANFA	Pyruvate kinase
229	Uncharacterized protein OS=Canis familiaris GN=CALM1 PE=4 SV=1	E2REK6_CANFA	CALM1
230	Cluster of Uncharacterized protein OS=Canis familiaris GN=VIM PE=3 SV=1 (F1PLS4_CANFA)	F1PLS4_CANFA [17]	Vimentin
231	Cluster of Uncharacterized protein OS=Canis familiaris GN=EZR PE=4 SV=1 (E2RSI6_CANFA)	E2RSI6_CANFA	Ezrin
232	Uncharacterized protein OS=Canis familiaris GN=PLEC PE=4 SV=2	F1PHS5_CANFA	plectin
233	Uncharacterized protein (Fragment) OS=Canis familiaris GN=GALNT2 PE=3 SV=2	F1P835_CANFA (+1)	Polypeptide N-Acetylgalactosaminyltransferase 2
234	Cluster of Nucleoside diphosphate kinase (Fragment) OS=Canis familiaris GN=NME2 PE=3 SV=2 (E2RC20_CANFA)	E2RC20_CANFA [3]	Nucleoside diphosphate kinase
235	Uncharacterized protein (Fragment) OS=Canis familiaris GN=HSP70 PE=3 SV=1	G1K268_CANFA (+1)	HSP70
236	Annexin OS=Canis familiaris GN=ANXA11 PE=3 SV=1	E2QXN8_CANFA	Annexin
237	Ribonuclease A B1 OS=Canis familiaris GN=RNASE4 PE=3 SV=2	F1PPZ2_CANFA	Ribonuclease A B1
238	Uncharacterized protein OS=Canis familiaris GN=GSTP1 PE=3 SV=2	F1Q0J0_CANFA	GSTP1
239	Uncharacterized protein (Fragment) OS=Canis familiaris GN=UGP2 PE=4 SV=2	E2R5R9_CANFA (+1)	Uridyl Diphosphate Glucose Pyrophosphorylase 2
240	Uncharacterized protein (Fragment) OS=Canis familiaris GN=PRDX6 PE=4 SV=1	F1PC59_CANFA	PRDX6
241	Anionic trypsinogen (Fragment) OS=Canis familiaris PE=3 SV=1	A0FGS8_CANFA (+1)	Anionic trypsinogen
242	Transaldolase OS=Canis familiaris GN=LOC100855463 PE=3 SV=1	H9GW87_CANFA	Transaldolase
243	Elongation factor 1-alpha OS=Canis familiaris PE=3 SV=1	J9NU04_CANFA	Elongation factor 1-alpha
244	Uncharacterized protein OS=Canis familiaris GN=LECT1 PE=4 SV=2	F1PB82_CANFA	Leukocyte Cell Derived Chemotaxin 1
245	Uncharacterized protein OS=Canis familiaris GN=AKR1A1 PE=4 SV=2	F1PK43_CANFA	Aldo-Keto Reductase Family 1, Member A1 (Aldehyde Reductase)
246	Uncharacterized protein OS=Canis familiaris GN=SMPDL3A PE=4 SV=2	E2RRU2_CANFA (+1)	Sphingomyelin Phosphodiesterase, Acid-Like 3A
247	Uncharacterized protein OS=Canis familiaris GN=LMNA PE=3 SV=1	F1PBJ3_CANFA (+1)	Lamin A/C
248	Peptidyl-prolyl cis-trans isomerase OS=Canis familiaris GN=LOC100688474 PE=3 SV=1	F1PK62_CANFA (+1)	Peptidyl-prolyl cis-trans isomerase
249	Uncharacterized protein OS=Canis familiaris GN=ENO2 PE=3 SV=2	E2RAS8_CANFA	Enolase 2
250	Uncharacterized protein OS=Canis familiaris GN=TKT PE=4 SV=2	F1PE28_CANFA	transketolase
251	Creatine kinase B-type OS=Canis familiaris GN=CKB PE=1 SV=1	KCRB_CANFA	Creatine kinase B
252	Uncharacterized protein (Fragment) OS=Canis familiaris GN=ARSA PE=4 SV=1	F6PKZ1_CANFA (+1)	Arylsulfatase A
253	Haptoglobin OS=Canis familiaris GN=HP PE=3 SV=1	G1K2D9_CANFA (+1)	Haptoglobin
254	Uncharacterized protein OS=Canis familiaris GN=ATP6V1A PE=3 SV=1	E2QYG6_CANFA	ATPase, H+ Transporting, Lysosomal 70kDa, V1 Subunit A

Supplementary Table 1 contd.

S. No.	Identified Proteins	Accession Number	Protein Name-FASTA
	Uncharacterized protein OS=Canis familiaris GN=ARHGDI4		
255	PE=4 SV=2 Proteasome subunit beta type OS=Canis familiaris	F1PL93_CANFA	Rho GDP Dissociation Inhibitor (GDI) Alpha
256	GN=PSMB6 PE=3 SV=1 Uncharacterized protein (Fragment) OS=Canis familiaris	E2R0B6_CANFA	Proteasome subunit beta Inter-Alpha-Trypsin Inhibitor Heavy Chain Family, Member 5
257	GN=ITIH5 PE=4 SV=2 Uncharacterized protein OS=Canis familiaris GN=IMPA1	F1PDN8_CANFA (+1)	
258	PE=4 SV=1 Uncharacterized protein (Fragment) OS=Canis familiaris	J9P7W8_CANFA	Inositol(Myo)-1(Or 4)-Monophosphatase 1 Serpine Peptidase Inhibitor, Clade G (C1 Inhibitor), Member 1
259	GN=SERPING1 PE=3 SV=2	F1PYX9_CANFA	Ectonucleotide Pyrophosphatase/Phosphodiesterase Family Member 2, Extracellular Lysophospholipase D
	Uncharacterized protein OS=Canis familiaris GN=ENPP2		
260	PE=4 SV=1	F6UVK4_CANFA	
	Uncharacterized protein OS=Canis familiaris GN=SERPINA3		
261	PE=3 SV=2	F1PH86_CANFA (+1)	Serine (Or Cysteine) Proteinase Inhibitor, Clade A (Alpha-1 Antitrypsin, Antitrypsin), Member 31
	Uncharacterized protein OS=Canis familiaris GN=SERPINE2		
262	PE=3 SV=1	F6Y2H4_CANFA	Serine (Or Cysteine) Proteinase Inhibitor, Clade E (Nexin)
	Uncharacterized protein OS=Canis familiaris GN=SLC44A1		
263	PE=4 SV=2	E2R949_CANFA	Solute Carrier Family 44 (Choline Transporter), Member 1
	Uncharacterized protein OS=Canis familiaris GN=ABHD14B		
264	PE=4 SV=1	E2RLZ9_CANFA	Abhydrolase Domain Containing 14B
	Uncharacterized protein OS=Canis familiaris GN=PEPD PE=3 SV=2		
265		F1PNS6_CANFA	Peptidase D
	Keratin, type II cytoskeletal 2 epidermal OS=Canis familiaris GN=KRT2 PE=3 SV=2		
266		F1PTX4_CANFA (+1)	Keratin, type II cytoskeletal 2
	Uncharacterized protein OS=Canis familiaris GN=KRT72		
267	PE=3 SV=2	E2QUT7_CANFA	KRT72
	Uncharacterized protein OS=Canis familiaris GN=KRT8		
268	PE=3 SV=2	F1PW98_CANFA	KRT8
	Keratin, type II cytoskeletal 1 OS=Canis familiaris GN=KRT1 PE=3 SV=1		
269		F1PTY1_CANFA (+1)	KRT1
	Uncharacterized protein (Fragment) OS=Canis familiaris GN=KRT5 PE=3 SV=1		
270		L7N095_CANFA	KRT5
	Uncharacterized protein OS=Canis familiaris GN=KRT3		
271	PE=3 SV=1	L7N094_CANFA	KRT3
	Uncharacterized protein OS=Canis familiaris GN=KRT5		
272	PE=3 SV=2	E2R8Z5_CANFA	KRT5
	Uncharacterized protein OS=Canis familiaris GN=KRT74		
273	PE=3 SV=1	L7N0P4_CANFA	KRT74
	Uncharacterized protein (Fragment) OS=Canis familiaris GN=KRT7 PE=3 SV=2		
274		F1PRB0_CANFA	KRT7
	Cluster of Uncharacterized protein OS=Canis familiaris GN=KRT19 PE=3 SV=1 (F1Q0N9_CANFA)		
275		F1Q0N9_CANFA [6]	KRT19
	Uncharacterized protein OS=Canis familiaris GN=KRT19		
276	PE=3 SV=1	F1Q0N9_CANFA	KRT19
	Keratin, type I cytoskeletal 10 OS=Canis familiaris GN=KRT10 PE=3 SV=2		
277		F1PYU9_CANFA (+1)	KRT10
	Uncharacterized protein OS=Canis familiaris GN=KRT42		
278	PE=3 SV=1	E2R7W6_CANFA	KRT42
	Uncharacterized protein OS=Canis familiaris GN=KRT14		
279	PE=3 SV=2	F1Q0R0_CANFA	KRT14
	Uncharacterized protein OS=Canis familiaris GN=KRT17		
280	PE=3 SV=2	F1Q0Q9_CANFA	KRT17
	Cluster of Uncharacterized protein (Fragment) OS=Canis familiaris GN=KRT18 PE=3 SV=2 (E2REU6_CANFA)		
281		E2REU6_CANFA [2]	KRT18
	Uncharacterized protein (Fragment) OS=Canis familiaris GN=KRT18 PE=3 SV=2		
282		E2REU6_CANFA	KRT18
	Uncharacterized protein OS=Canis familiaris GN=KRT32		
283	PE=3 SV=1	L7N0G9_CANFA	KRT32
	Keratin, type I cytoskeletal 9 OS=Canis familiaris GN=KRT9 PE=4 SV=1		
284		F1Q0N7_CANFA (+3)	KRT9
	Uncharacterized protein OS=Canis familiaris GN=KRT82		
285	PE=3 SV=1	E2R831_CANFA	KRT82
	Uncharacterized protein (Fragment) OS=Canis familiaris PE=4 SV=2		
286		F1PQI2_CANFA	
	Uncharacterized protein OS=Canis familiaris PE=4 SV=2		
287		F1PCM6_CANFA	
	Uncharacterized protein OS=Canis familiaris GN=LOC100856711 PE=4 SV=1		
288		J9P1I7_CANFA (+1)	LOC100856711

Supplementary Table S1 contd.

S. No.	Identified Proteins	Accession Number	Protein Name-FASTA
289	Uncharacterized protein OS=Canis familiaris PE=3 SV=1	J9NTU0_CANFA	
290	Uncharacterized protein OS=Canis familiaris PE=3 SV=2	F1PCH3_CANFA	
291	Uncharacterized protein OS=Canis familiaris PE=4 SV=1	F6Y478_CANFA	
292	Cluster of Uncharacterized protein OS=Canis familiaris PE=4 SV=2 (E2RCC8_CANFA)	E2RCC8_CANFA	
293	Uncharacterized protein OS=Canis familiaris PE=4 SV=2	E2RCC8_CANFA	
294	Uncharacterized protein (Fragment) OS=Canis familiaris PE=3 SV=1	J9P219_CANFA	
295	Uncharacterized protein OS=Canis familiaris PE=4 SV=1	J9P9V0_CANFA	
296	Cluster of Uncharacterized protein OS=Canis familiaris GN=LOC100687814 PE=3 SV=1 (J9NW97_CANFA)	J9NW97_CANFA [4]	LOC100687814
297	Uncharacterized protein OS=Canis familiaris GN=LOC100687814 PE=3 SV=1	J9NW97_CANFA	LOC100687814
298	Uncharacterized protein OS=Canis familiaris GN=LOC100856491 PE=4 SV=1	J9P889_CANFA	LOC100856491
299	Uncharacterized protein OS=Canis familiaris GN=LOC612122 PE=4 SV=2	H9GWR0_CANFA (+4)	LOC612122
300	Uncharacterized protein OS=Canis familiaris GN=LOC609095 PE=3 SV=1	E2RQN4_CANFA	LOC609095
301	Uncharacterized protein (Fragment) OS=Canis familiaris GN=LOC609010 PE=4 SV=1	J9P0V2_CANFA	LOC609010
302	Uncharacterized protein (Fragment) OS=Canis familiaris GN=LOC609402 PE=3 SV=2	E2RLH6_CANFA (+1)	LOC609402
303	Uncharacterized protein (Fragment) OS=Canis familiaris GN=LOC100856547 PE=4 SV=2	H9GWE7_CANFA (+2)	LOC100856547