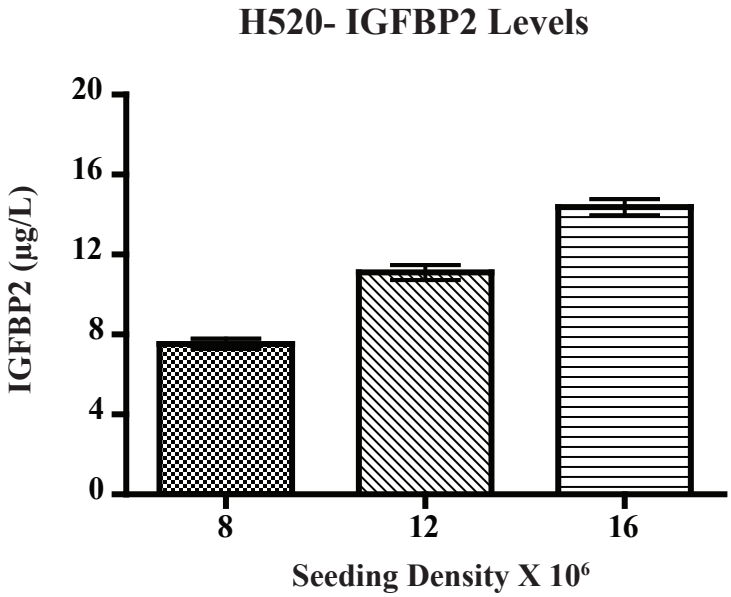
Supplementary Figure 1

A



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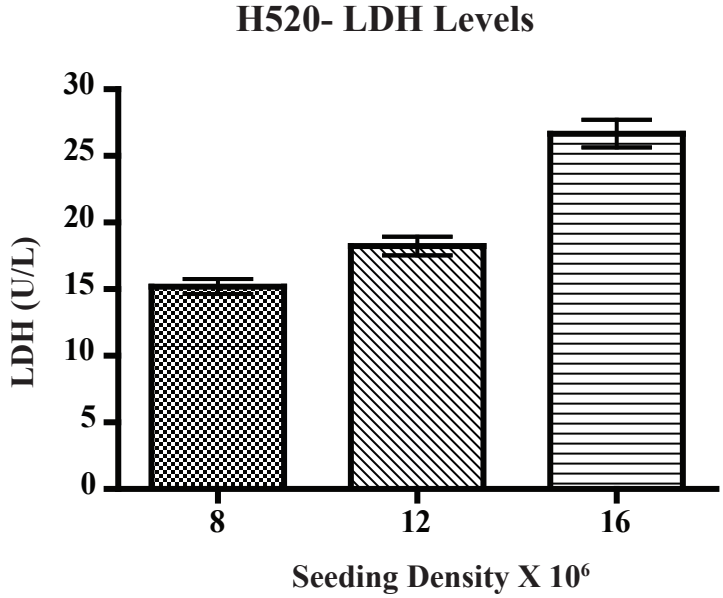

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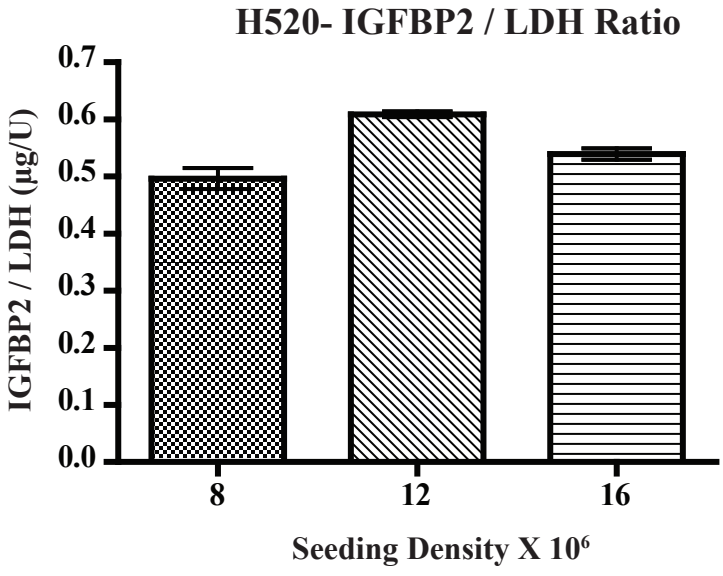
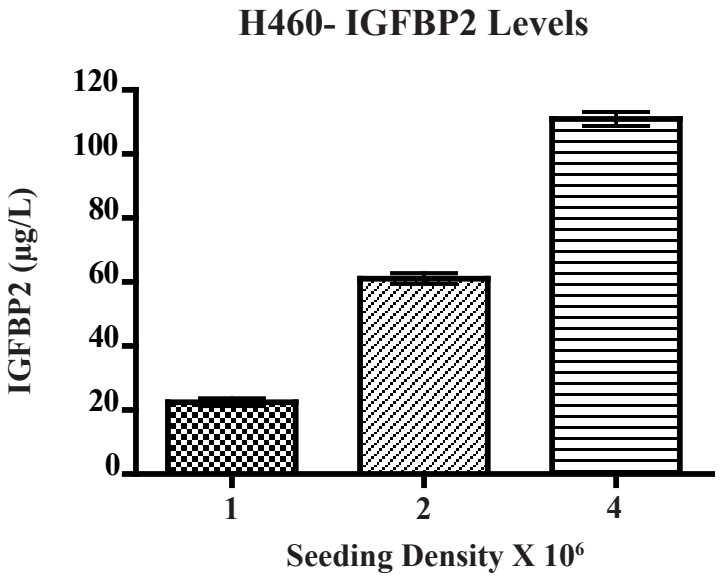


Supplementary Figure 2

A



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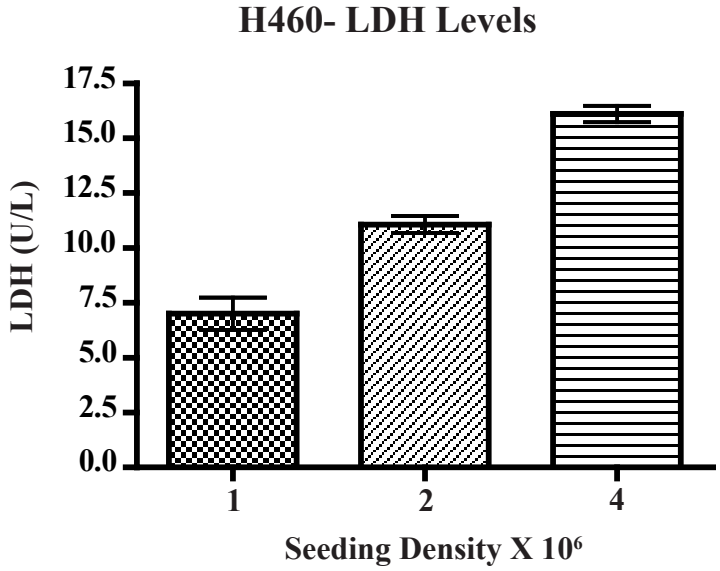

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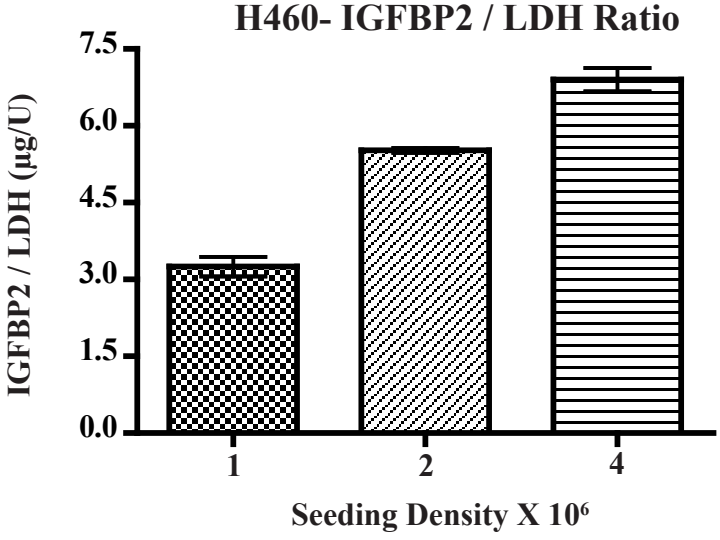
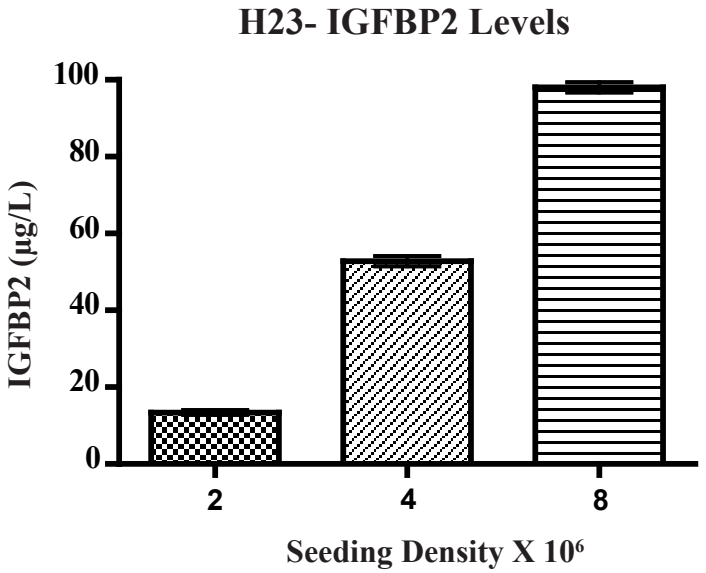


Supplementary Figure 3

A



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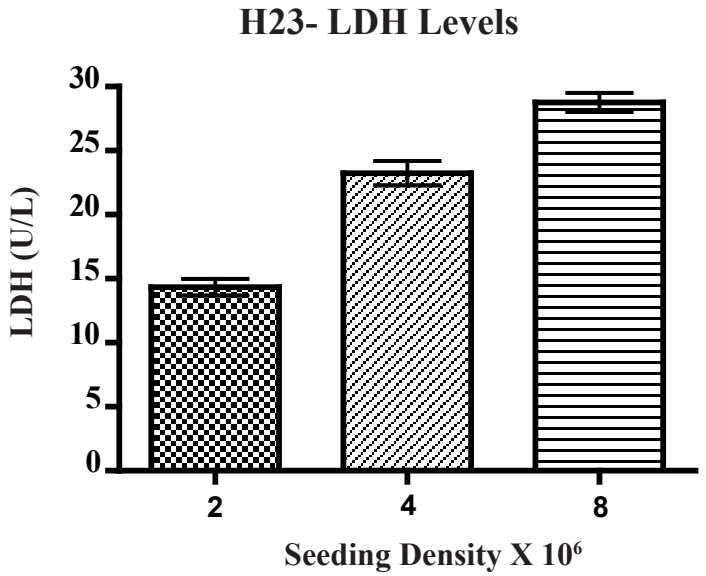

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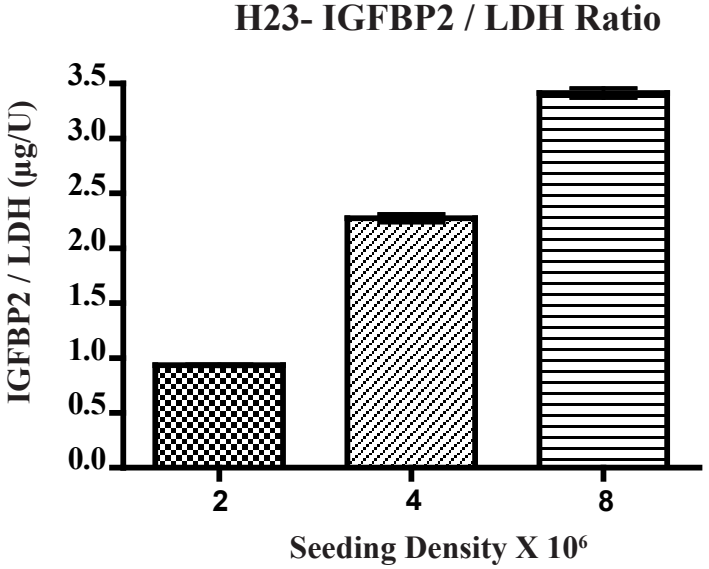
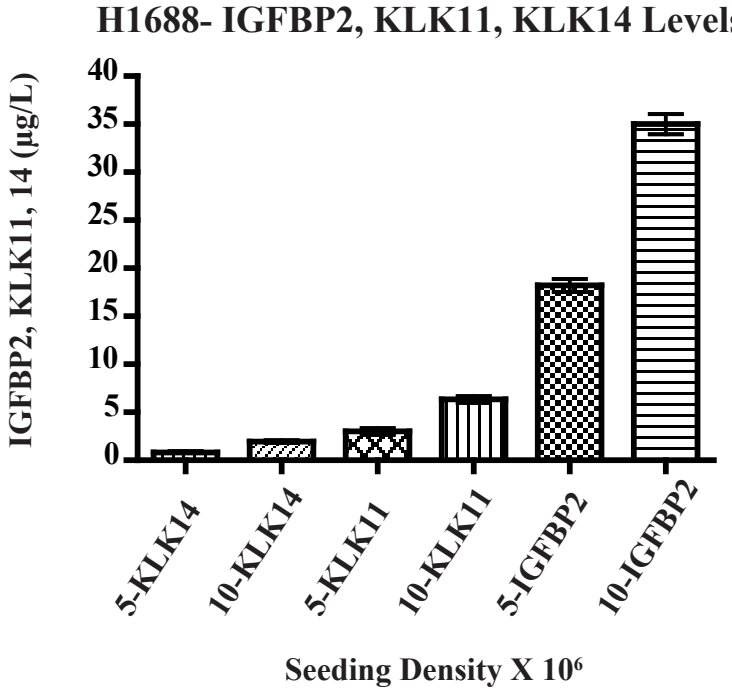


Supplementary Figure 4

A



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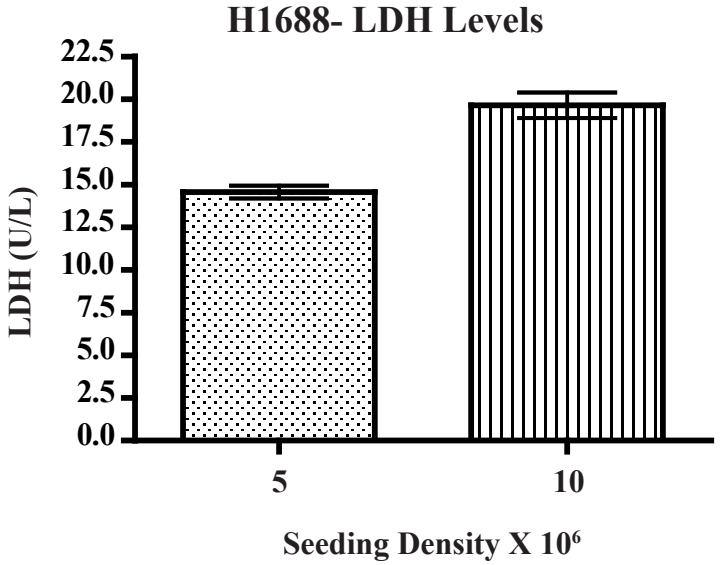

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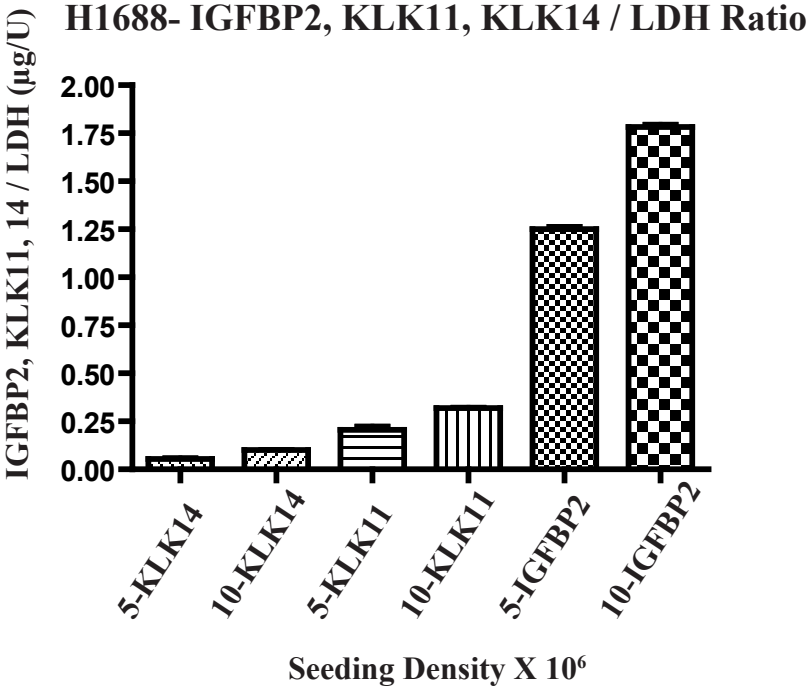


Supplementary Figure 5

A)

IPI00297284 (100%), 35137.1 Da
Gene_Symbol=IGFBP2 Insulin-like growth factor-binding protein 2 precursor
13 unique peptides, 48 unique spectra, 80 total spectra, 146/328 amino acids (45% coverage)

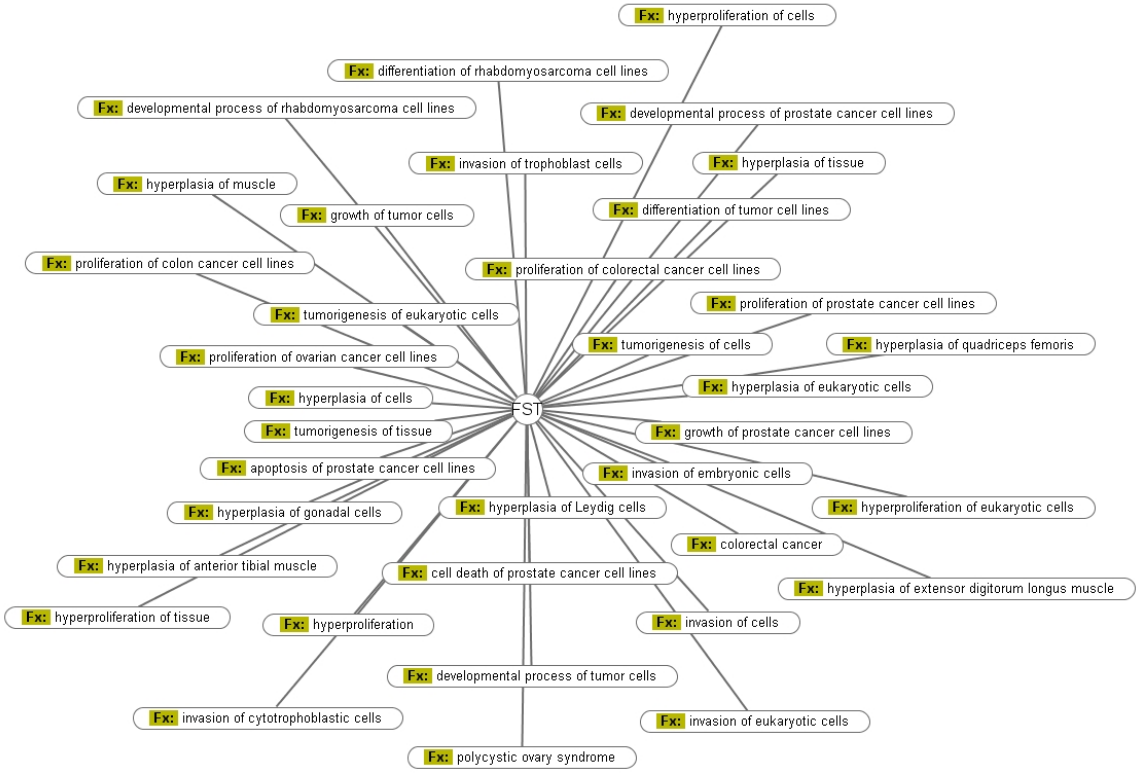
B)

IPI00002818 (100%), 27465.9 Da
Gene_Symbol=KLK11 Isoform 1 of Kallikrein-11 precursor
7 unique peptides, 14 unique spectra, 14 total spectra, 101/250 amino acids (40% coverage)

C)

IPI00793215 (100%), 29121.3 Da
Gene_Symbol=KLK14 kallikrein 14 preproprotein
3 unique peptides, 3 unique spectra, 3 total spectra, 46/267 amino acids (17% coverage)

Follistatin



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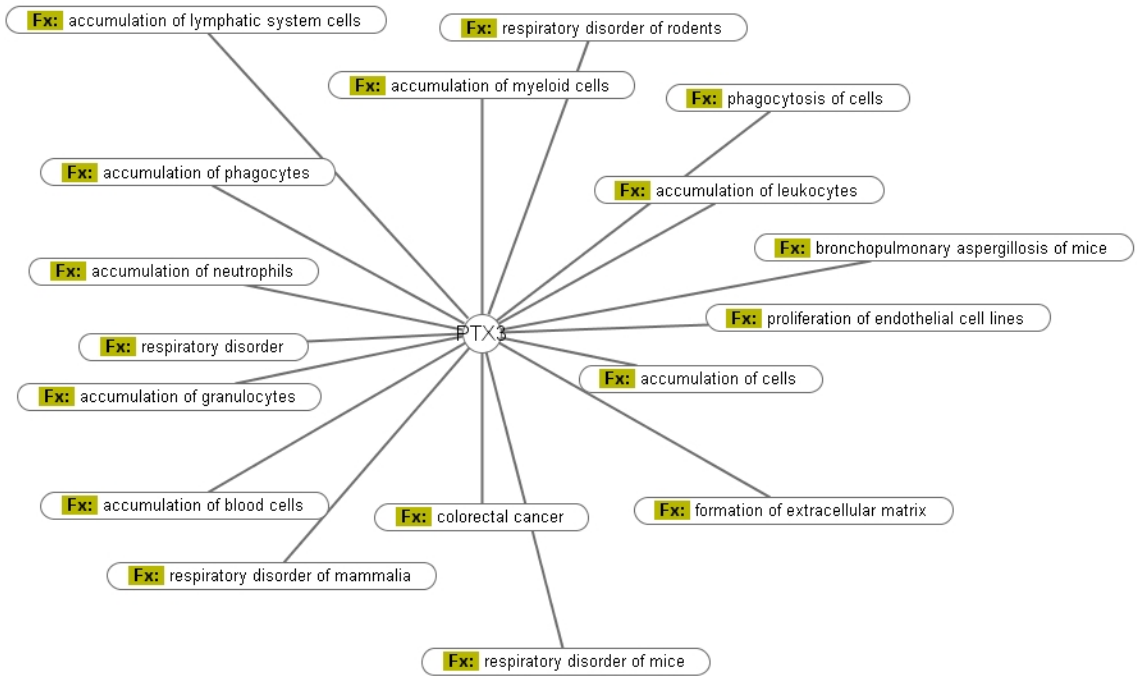
Supplementary figure 6

PTX3



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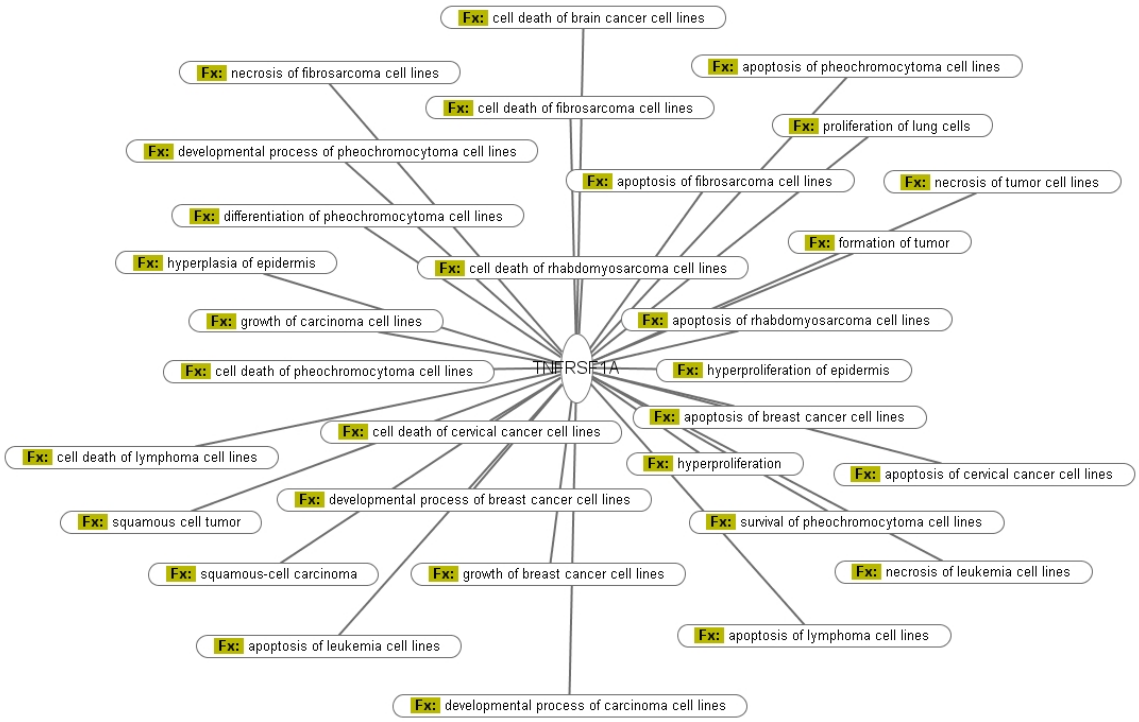
Supplementary figure 7

TNFRSF1A



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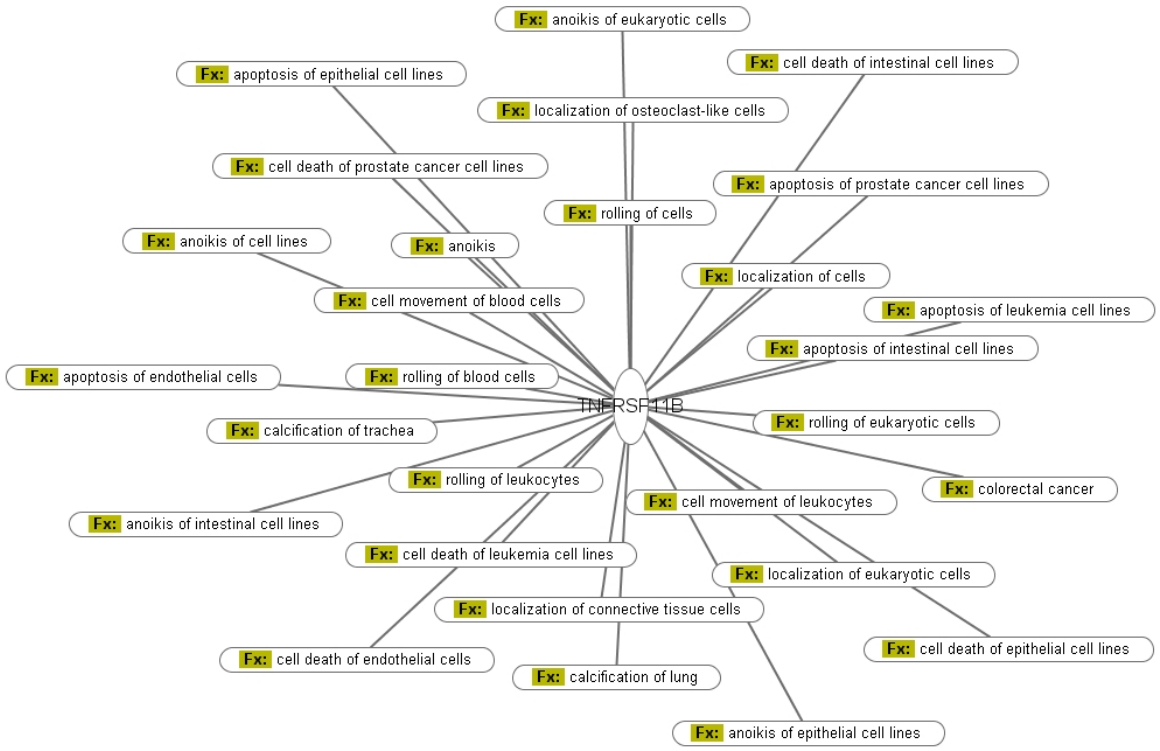
Supplementary Figure 8

New Pathway 3



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Supplementary Figure 9

Supplementary Table 1. Clinical and pathological characteristics of lung cancer patients for Osteoprotegerin measurement by ELISA

	Controls	Cases
Smoking status		
Yes	07	17
No[1]	17	08
x[2]	01	
Gender		
Female	15	10
Male	10	15
Age		
Mean	42	62
SD	09	14
Stage		
III		03
IV		22
Histology[3]		
ADC		08
SCC		06
BAC		01
LCC		01
Unspecified NSCLC		09

All patients had unresectable stage III or IV NSCLC at the time of sample collection. Surgical procedures occured >1 year before specimen collection.

[1].< 100 cigarettes/lifetime.

[2]. x, unknown.

[3]. ADC, adenocarcinoma; SCC, squamous cell carcinoma; BAC, bronchioloalveolar carcinoma; LCC, large cell carcinoma; NSCLC, non-small cell lung carcinoma.

Supplementary Table 2. Clinical and pathological characteristics of lung cancer patients for sTNF RI measurement by ELISA

	Controls	Cases
Smoking status		
Yes	07	16
No[1]	14	09
x[2]	04	
Gender		
Female	08	11
Male	17	14
Age		
Mean	36	62
SD	08	11
Stage		
III		04
IV		20
x[2]		01
Histology[3]		
ADC		14
SCC		04
BAC		01
Unspecified NSCLC		06

All patients had unresectable stage III or IV NSCLC at the time of sample collection. Surgical procedures occured >1 year before specimen collection.

[1].< 100 cigarettes/lifetime.

[2]. x, unknown.

[3]. ADC, adenocarcinoma; SCC, squamous cell carcinoma; BAC, bronchioloalveolar carcinoma; NSCLC, non-small cell lung carcinoma.

Supplementary Table 3. Clinical and pathological characteristics of lung cancer patients for Follistatin measurement by ELISA

	Controls	Cases
Smoking status		
Yes	07	17
No[1]	17	08
x[2]	01	
Gender		
Female	15	10
Male	10	15
Age		
Mean	42	62
SD	09	14
Stage		
III		02
IV		23
Histology[3]		
ADC		08
SCC		06
LCC		01
Unspecified NSCLC		10

All patients had unresectable stage III or IV NSCLC at the time of sample collection. Surgical procedures occured >1 year before specimen collection.

[1].< 100 cigarettes/lifetime.

[2]. x, unknown.

[3]. ADC, adenocarcinoma; SCC, squamous cell carcinoma; LCC, large cell carcinoma; NSCLC, non-small cell lung carcinoma.

Supplementary Table 4. Clinical and pathological characteristics of lung cancer patients for ADAM-17 measurement by ELISA

	Controls	Cases
Smoking status		
Yes	04	15
No[1]	16	06
x[2]	01	
Gender		
Female	14	08
Male	07	13
Age		
Mean	41	61
SD	09	13
Stage		
III		03
IV		18
Histology[3]		
ADC		07
SCC		06
BAC		01
LCC		01
Unspecified NSCLC		06

All patients had unresectable stage III or IV NSCLC at the time of sample collection. Surgical procedures occured >1 year before specimen collection.

[1].< 100 cigarettes/lifetime.

[2]. x, unknown.

[3]. ADC, adenocarcinoma; SCC, squamous cell carcinoma; BAC, bronchioloalveolar carcinoma; LCC, large cell carcinoma; NSCLC, non-small cell lung carcinoma.

Supplementary Table 5. Clinical and pathological characteristics of lung cancer patients for Pentraxin 3 by ELISA

	Controls	Cases
Smoking status		
Yes	08	19
No[1]	15	06
x[2]	02	
Gender		
Female	08	12
Male	17	13
Age		
Mean	34	64
SD	09	10
Stage		
III		05
IV		19
x[2]		01
Histology[3]		
ADC		10
SCC		06
Unspecified NSCLC		09

All patients had unresectable stage III or IV NSCLC at the time of sample collection. Surgical procedures occured >1 year before specimen collection.

[1].< 100 cigarettes/lifetime.

[2]. x, unknown.

[3]. ADC, adenocarcinoma; SCC, squamous cell carcinoma; NSCLC, non-small cell lung carcinoma.

Supplementary Figures

Supplementary Figure 1: Optimization of seeding density for H520.

A, IGFBP2 levels measured in CM at different seeding densities (8, 12 and 16 million cells); B, LDH levels measured in CM at different seeding densities (8, 12 and 16 million cells); C, IGFBP2 / LDH ratio calculated at different seeding densities (8, 12 and 16 million cells).

Supplementary Figure 2: Optimization of seeding density for H460.

A, IGFBP2 levels measured in CM at different seeding densities (1, 2 and 4 million cells); B, LDH levels measured in CM at different seeding densities (1, 2 and 4 million cells); C, IGFBP2 / LDH ratio calculated at different seeding densities (1, 2 and 4 million cells).

Supplementary Figure 3: Optimization of seeding density for H23.

A, IGFBP2 levels measured in CM at different seeding densities (2, 4 and 8 million cells); B, LDH levels measured in CM at different seeding densities (2, 4 and 8 million cells); C, IGFBP2 / LDH ratio calculated at different seeding densities (2, 4 and 8 million cells).

Supplementary Figure 4: Optimization of seeding density for H1688.

A, IGFBP2, KLK11 and KLK14 levels measured in CM at different seeding densities (5 and 10 million cells); B, LDH levels measured in CM at different seeding densities (5 and 10 million cells); C, IGFBP2, KLK11, KLK14 / LDH ratio calculated at different seeding densities (5 and 10 million cells).

Supplementary Figure 5: Identification of internal control proteins by LC MS/MS.

H1688 expresses IGFBP2 and kallikrein-related peptidases 11 and 14 (KLK11 and KLK14) in concentrations ranging from approximately 2-35 µg/L, as measured by ELISA. The sequences of the respective proteins are indicated (A) IGFBP2, (B) KLK11, (C) KLK14. The peptides identified by MS in the CM of H1688 are highlighted in yellow.

Supplementary Figure 6: Molecular functions related to diseases associated with Follistatin.

The web diagram generated through IPA software depicts the biological functions that Follistatin is associated with, in the context of disease.

Supplementary Figure 7: Molecular functions related to diseases associated with Pentraxin 3 (PTX3).

The web diagram generated through IPA software depicts the biological functions that PTX3 is associated with, in the context of disease.

Supplementary Figure 8: Molecular functions related to diseases associated with TNFRSF1A.

The web diagram generated through IPA software depicts the biological functions that TNFRSF1A is associated with, in the context of disease.

Supplementary Figure 9: Molecular functions related to diseases associated with Osteoprotegerin (TNFRSF11B).

The web diagram generated through IPA software depicts the biological functions that Osteoprotegerin is associated with, in the context of disease.