Supplementary Table

Supplementary Table 1A. The 6 standard proteins obtained for Sigma were spiked in 10 µg of kidney tissue lysates at various concentrations (fmol) spanning a wide dynamic range.

Protein name	1*	1/2	1/4	1/8	1/16	1/32	1/64	1/128
Lysozyme (chicken)	1,000	500	250	125	62.5	31.25	15.625	7.8125
Myoglobin (horse)	500	250	125	62.5	31.25	15.625	7.8125	3.90625
β-Lactoglobulin (bovine)	500	250	125	62.5	31.25	15.625	7.8125	3.90625
Fetuin (bovine)	50	25	12.5	6.25	3.125	1.5625	0.78125	0.390625
Ovalbumin (chicken)	50	25	12.5	6.25	3.125	1.5625	0.78125	0.390625
Transferrin (human)	5	2.5	1.25	0.625	0.3125	0.15625	0.078125	0.0390625

* The 6 standard proteins mix was serially diluted. The red column was the amount used for repeatability analysis. The grey cells were out of the range of good linearity.

Supplementary Table 1B. The 6 standard proteins obtained for Sigma were spiked in 10 µg of kidney tissue lysates at various concentrations (µg) spanning a wide dynamic range.

Protein name	MW	1*	1/8	1/16	1/32	1/128
Lysozyme (chicken)	16,239	0.016239	0.002030	0.001015	0.000507	0.000127
Myoglobin (horse)	17,083	0.008542	0.001068	0.000534	0.000267	0.000067
β-Lactoglobulin (bovine)	19,883	0.009942	0.001243	0.000621	0.000311	0.000078
Fetuin (bovine)	42,663	0.002133	0.000267	0.000133	0.000067	0.000017
Ovalbumin (chicken)	42,881	0.002144	0.000268	0.000134	0.000067	0.000017
Transferrin (human)	76,960	0.000385	0.000048	0.000024	0.000012	0.000003

* The 6 standard proteins mix was serially diluted. The red column was the amount used for repeatability analysis. The grey cells were out of the range of good linearity.

Supplementary Table 2. A summary of the study design in experimental procedures.

Sample	Study #1	Study #2	Study #3	Study #4
Standard peptide solution	**Yes**	**Yes**	n/a	n/a
Kidney tissue lysates	**Yes**	**Yes**	n/a	n/a
HT29-MTX cell lysates	**Yes**	**Yes**	n/a	n/a
Depleted human serum	**Yes**	**Yes**	n/a	n/a
Human serum albumin-bound proteins	**Yes**	**Yes**	n/a	n/a
6 standard proteins spiked in kidney tissue lysates	**Yes**	**Yes**	n/a	n/a
Lysozyme spiked human lymphocyte culture media	n/a	n/a	**Yes**	n/a
Pituitary tissue lysates	n/a	n/a	n/a	**Yes**

Study #1: Repeatability. Study #2: Linearity. Study #3: The quantification of an identical concentration (0.00125 µg/µL) of lysozyme spiked in human lymphocyte culture media containing secreted proteins in eight injections of four samples belonging to two groups. Study #4: A prototypical proteomics experiment that compared pituitary protein expression between wild-type and dwarfed mice. n/a, not applicable.

Supplementary Table 3A. The peptide sequences and protein names in Figure 4F, G.

Peptides and protein in Figure 4F		Peptides and protein in Figure 4G	
Aspartoacylase-2		Tubulin beta-3 chain	
1	2_AMVASILDFIELFNQGMEFPAFEMEVYK	1	2_AILVDLEPGTMDSVR
2	3_AMVASILDFIELFNQGMEFPAFEMEVYK	2	2_ALTVPELTQQMFDAK
3	2_AQELNQLLGPK	3	2_EVDEQMLAIQSK
4	3_LFSGEDVLYEGDSVVYPLFVNEAAYYEK	4	3_FWEVISDEHGIDPSGNYVGDSDLQLER
5	3_LQDHDFEPLRPGEPIFK	5	3_LATPTYGDLNHLVSATMSGVTTSLR
6	2_NGISLELGPQPQGVLR	6	2_LHFFMPGFAPLTAR
7	**2_NLGSVDFPR**	7	3_LHFFMPGFAPLTAR
8	3_TFTLTFLGSTATPDDPYEVKR	8	2_MSSTFIGNSTAIQELFK
		9	**2_MSSTFIGNSTAIQELFKR**
		10	3_MSSTFIGNSTAIQELFKR
		11	3_SGAFGHLFRPDNFIFGQSGAGNNWAK

Supplementary Table 3B. The correlation coefficient of peptide 7 in Figure 4F and peptide 9 in Figure 4G.

Peptides in Figure 2F		Peptides in Figure 2G	
1	0.932	1	0.974
2	0.926	2	0.974
3	0.944	3	0.974
4	0.946	4	0.964
5	0.941	5	0.955
6	0.936	6	0.901
7	**0.595**	7	0.971
8	0.944	8	0.971
		9	**0.814**
		10	0.973
		11	0.969

Supplementary Table 4. The fold differences from the linear regressions at different concentrations and their R^2 using raw data.

	Fold						R^2					
DF	10*	5	2.5	1.25	0.625	0.3125	10	5	2.5	1.25	0.625	0.3125
2	2.01	1.84	1.92	2.07	2.06	2.09	0.9912	0.9987	0.9981	0.9968	0.9956	0.9896
4	3.87	3.54	3.99	4.28	4.30		0.9887	0.9971	0.9942	0.9891	0.9794	
8	7.46	7.35	8.22	8.90			0.9911	0.9929	0.9861	0.9707		
16	15.49	15.14	17.10				0.9890	0.9848	0.9683			
32	31.92	31.49					0.9824	0.9659				
64	66.33						0.9608					

* 10 µg sample, 5 µg sample, etc.; DF = dilution factor.

Supplementary Table 5A. Fold differences of HT29-MTX cell lysate from linear regressions at different concentrations and their R^2 using log-transformed data.

	Fold (log2)						R^2					
DF	10*	5	2.5	1.25	0.625	0.3125	10	5	2.5	1.25	0.625	0.3125
1	1.27	0.94	1.10	1.29	1.23	1.22	0.9645	0.9861	0.9879	0.9914	0.9872	0.9662
2	2.05	2.00	2.42	2.56	2.50		0.9668	0.9778	0.9747	0.9731	0.9457	
3	2.98	3.31	3.74	3.83			0.9726	0.9650	0.9485	0.9305		
4	4.28	4.62	5.03				0.9619	0.9402	0.9030			
5	5.55	5.87					0.9431	0.9009				
6	6.79						0.9055					

* 10 µg sample, 5 µg sample, etc.; DF = dilution factor.

Supplementary Table 5B. Fold differences of HT29-MTX cell lysate from linear regressions at different concentrations and their R^2 using raw data.

	Fold						R^2					
DF	10*	5	2.5	1.25	0.625	0.3125	10	5	2.5	1.25	0.625	0.3125
2	2.12	1.82	2.00	2.24	2.08	2.06	0.9884	0.9962	0.9950	0.9964	0.9932	0.9832
4	3.85	3.63	4.47	4.66	4.28		0.9883	0.9916	0.9866	0.9861	0.9659	
8	7.70	8.12	9.27	9.56			0.9893	0.9822	0.9715	0.9545		
16	17.25	16.83	18.96				0.9806	0.9671	0.9353			
32	35.75	34.48					0.9655	0.9343				
64	73.32						0.9345					

* 10 µg sample, 5 µg sample, etc.; DF = dilution factor.

Supplementary Table 6A. Fold differences of depleted serum from linear regressions at different concentrations and their R^2 using log-transformed data.

	Fold (log2)						R^2					
DF	10*	5	2.5	1.25	0.625	0.3125	10	5	2.5	1.25	0.625	0.3125
1	1.00	0.76	1.18	1.72	1.35	0.52	0.9890	0.9983	0.9967	0.9953	0.9909	0.9797
2	1.80	1.96	2.92	3.07	1.96		0.9851	0.9933	0.9879	0.9819	0.9604	
3	3.03	3.72	4.26	3.69			0.9787	0.9840	0.9752	0.9482		
4	4.80	5.07	4.90				0.9713	0.9713	0.9391			
5	6.14	5.74					0.9607	0.9325				
6	6.81						0.9233					

* 10 µg sample, 5 µg sample, etc.; DF = dilution factor.

Supplementary Table 6B. Fold differences of depleted serum from linear regressions at different concentrations and their R^2 using raw data.

	Fold (log2)						R^2					
DF	10*	5	2.5	1.25	0.625	0.3125	10	5	2.5	1.25	0.625	0.3125
2	2.64	1.91	2.08	2.17	2.14	2.03	0.9978	0.9991	0.9989	0.9955	0.9941	0.9954
4	5.05	3.97	4.50	4.65	4.35		0.9962	0.9975	0.9913	0.9860	0.9897	
8	10.48	8.61	9.62	9.44			0.9943	0.9888	0.9789	0.9822		
16	22.70	18.41	19.55				0.9853	0.9760	0.9752			
32	48.49	37.41					0.9714	0.9735				
64	98.54						0.9683					

* 10 µg sample, 5 µg sample, etc.; DF = dilution factor.

Supplementary Table 7A. Fold differences of albumin-bound proteins from linear regressions at different concentrations and their R^2 using log-transformed data.

	Fold (log2)						R^2					
DF	10*	5	2.5	1.25	0.625	0.3125	10	5	2.5	1.25	0.625	0.3125
1	1.12	1.36	2.12	1.24	1.06	1.78	0.9930	0.9873	0.9769	0.9933	0.9741	0.9549
2	2.42	3.71	3.31	2.44	3.05		0.9844	0.9370	0.9681	0.9533	0.9070	
3	4.76	4.86	4.34	4.43			0.9348	0.9301	0.9408	0.8865		
4	5.92	5.76	6.08				0.9261	0.9149	0.8947			
5	6.79	7.35					0.9145	0.8838				
6	8.32						0.8896					

* 10 µg sample, 5 µg sample, etc.; DF = dilution factor.

Supplementary Table 7C. Fold differences of albumin-bound proteins from linear regressions at different concentrations and their R^2 using raw data.

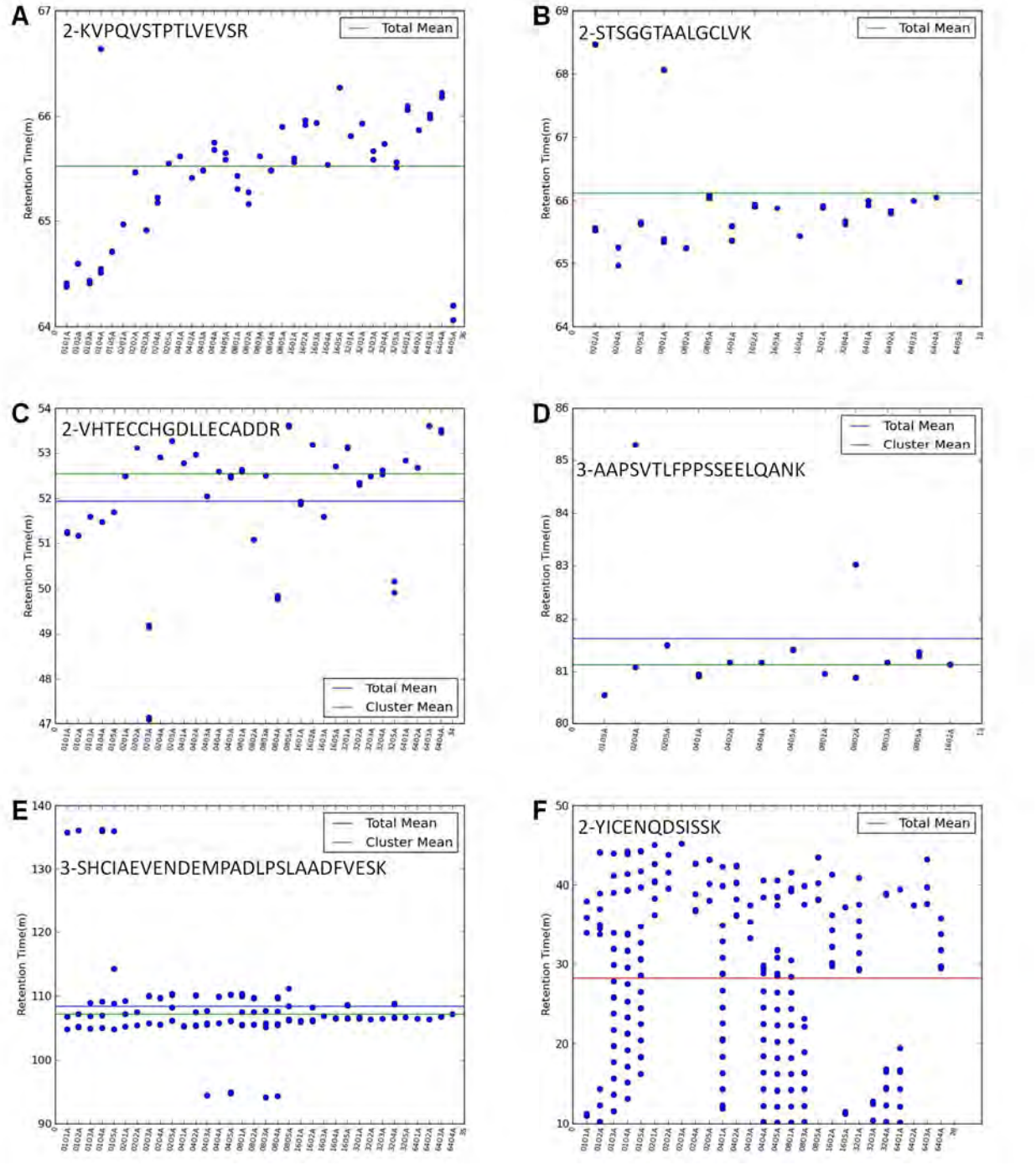
	Fold						R^2					
DF	10*	5	2.5	1.25	0.625	0.3125	10	5	2.5	1.25	0.625	0.3125
2	2.00	2.04	1.93	2.45	2.02	2.50	0.9998	0.9998	0.9999	0.9997	0.9995	0.9999
4	4.09	3.95	4.74	4.97	5.06		0.9996	0.9995	0.9991	0.9985	0.9994	
8	7.91	9.69	9.60	12.42			0.9992	0.9985	0.9975	0.9984		
16	19.39	19.60	24.00				0.9980	0.9966	0.9975			
32	39.22	49.02					0.9958	0.9967				
64	98.10						0.9958					

* 10 µg sample, 5 µg sample, etc.; DF = dilution factor.

Supplementary Table 8. A summary of the optimal filtering thresholds for peptide frequency, retention time, intensity CV, and correlation filters.

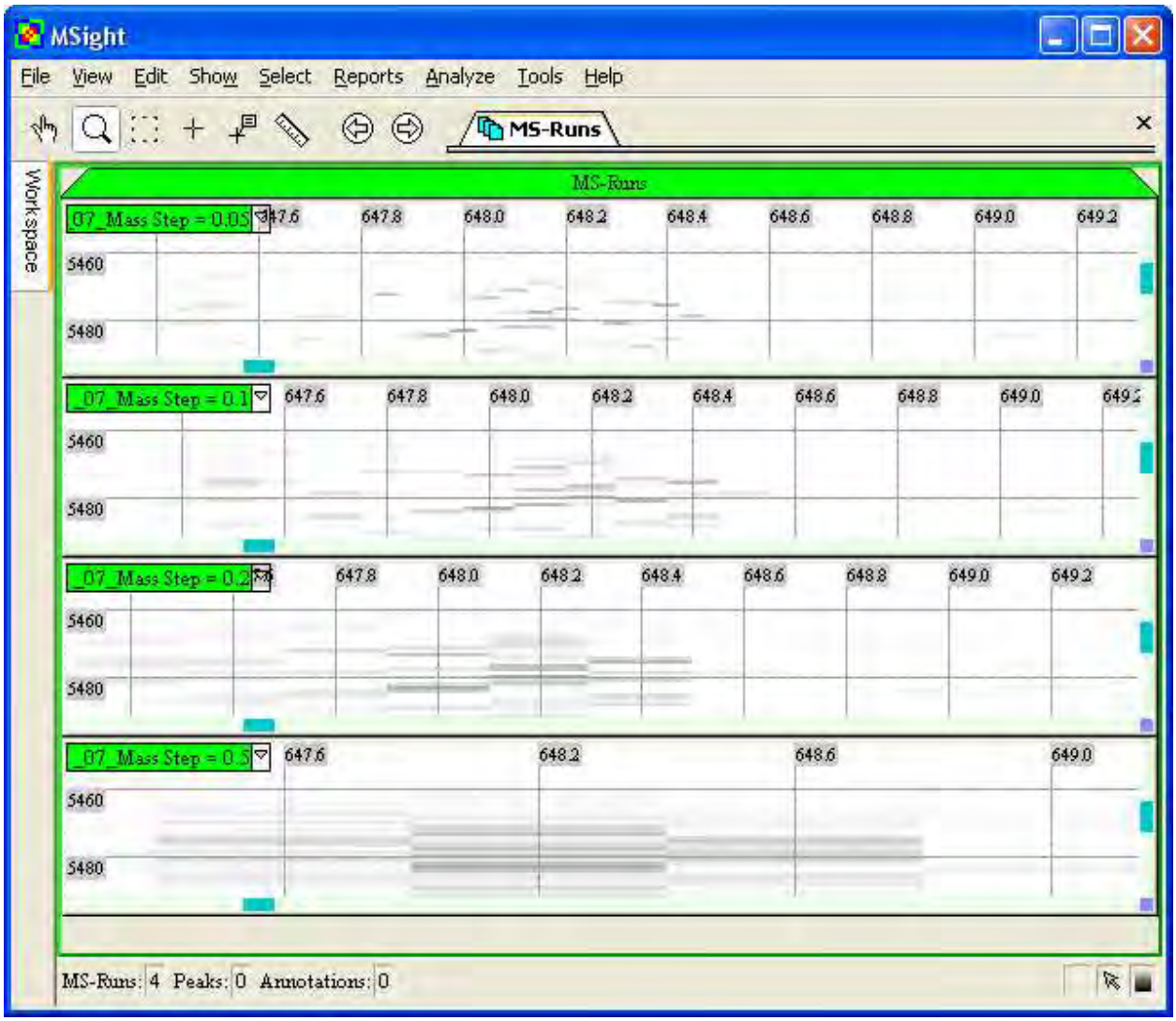
Filter	Filtering thresholds
Peptide Frequency	Identification from 2 or 3 injections (or number of identifications > 10 % of all injections) are suggested.
Retention Time	3 min is used as the basic cut-off. Combination of IQR with frequencies is applied.
Intensity CV	If a peptide has an unacceptable CV in one injection, it should be excluded from all injections; if a peptide has an excessively high CV, it should be excluded; if a peptide has too few low CV values, it is excluded.
Correlation	CC ≥ 0.9 for two groups. Normally set the threshold to filter out 10-15% of peptides.

Supplementary Figures Legends

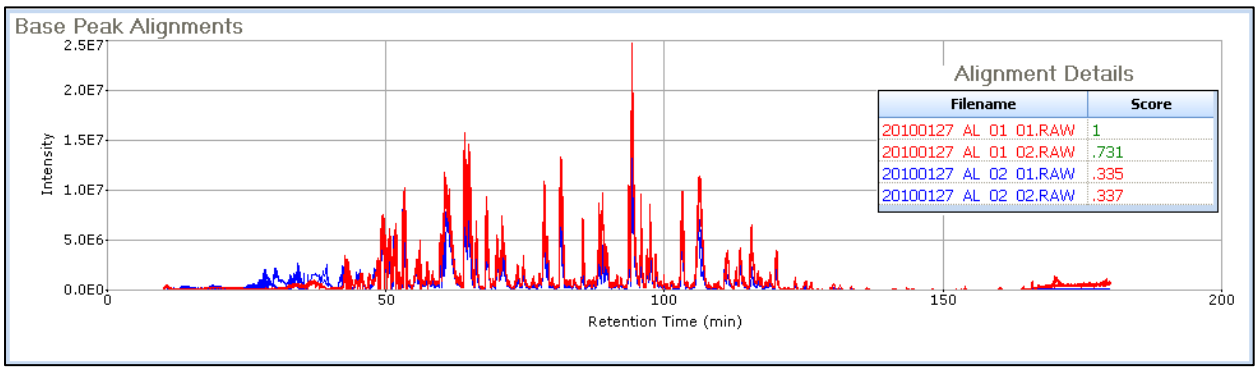


Supplementary Figure 1. Typical MS/MS spectrum distributions of six selected peptides across multiple injections. The x-axis is indicated according to different samples. (A) Multiple spectra are limited to a 3 min retention time range (B) Most spectra are limited

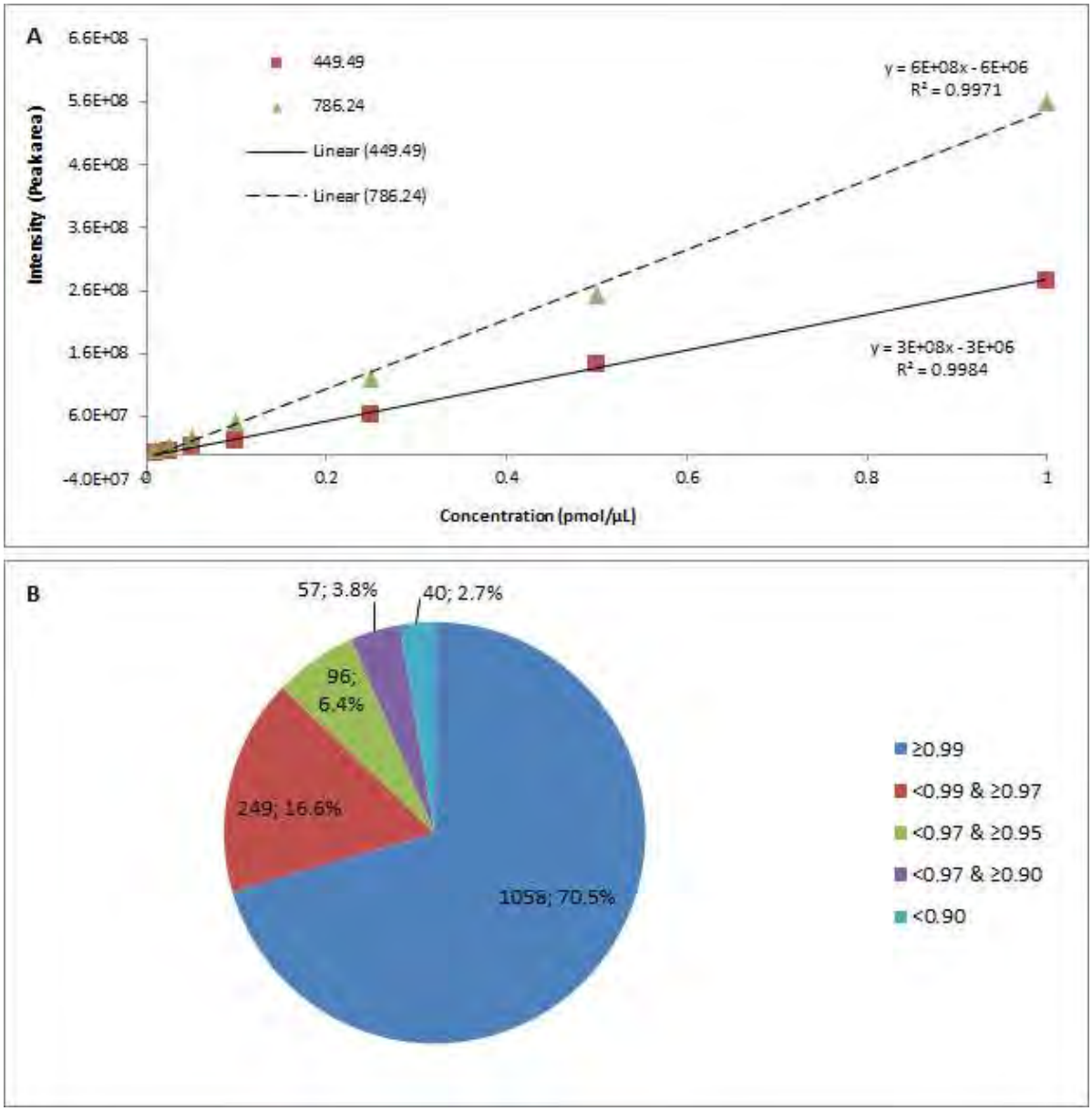
to a 3 min range; a few are scattered in a broad range. (C) Some spectra are concentrated in a narrow window of longer retention time, while other spectra are scattered in a wide range with a shorter retention time. (D) Some spectra are concentrated in a narrow window of shorter retention time, while other spectra are scattered in a wide range with a longer retention time. (E) Some spectra are concentrated in a narrow window of retention time, while other spectra are scattered in a wide range with a shorter and longer retention times. (F) All spectra are scattered in a wide range of retention time.



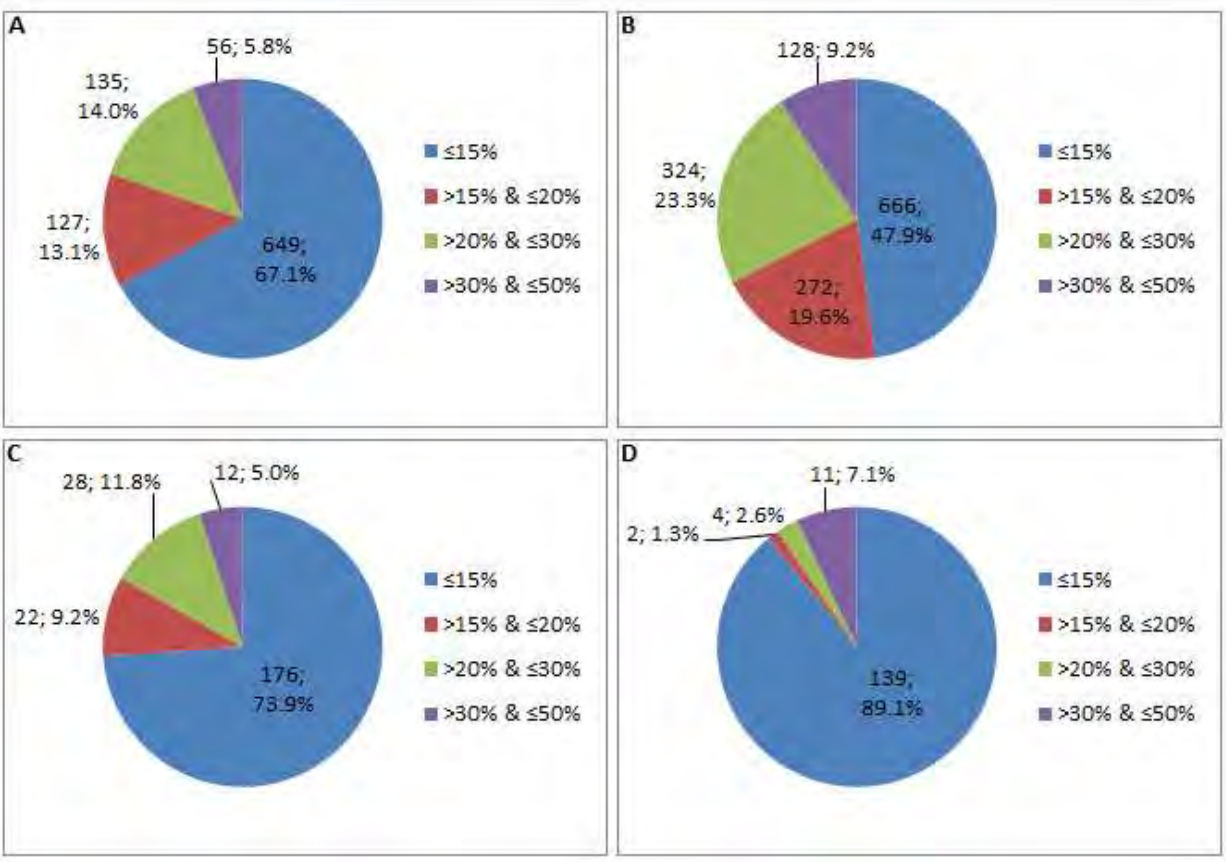
Supplementary Figure 2. MSight alignment of low resolution data at different mass steps. LC-MS data from tryptic peptides of a human serum albumin-bound protein sample used in this study were imported into MSight, the mass step was set up 0.05, 0.1, 0.2, and 0.5 each time. The result indicates that MSight was incapable of producing spots.



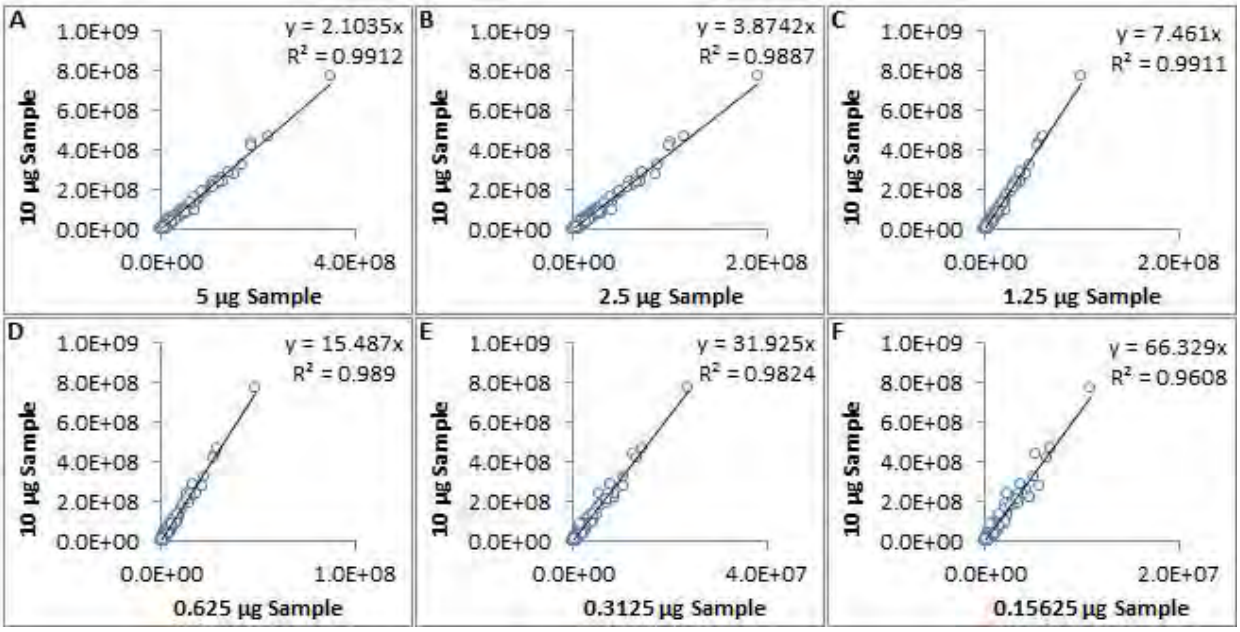
Supplementary Figure 3. Peak alignments performed by SIEVE. SIEVE software developed by Thermo Scientific is an automated software package for the label-free, semi-quantitative differential expression analysis of proteins, peptides and metabolites. Four injections' data from two concentrations including two injections in each concentration were submitted to SIEVE. The base peaks are significantly different before 50 min, rendering the accuracy of retention time determination by aligning only the base peak unreliable.



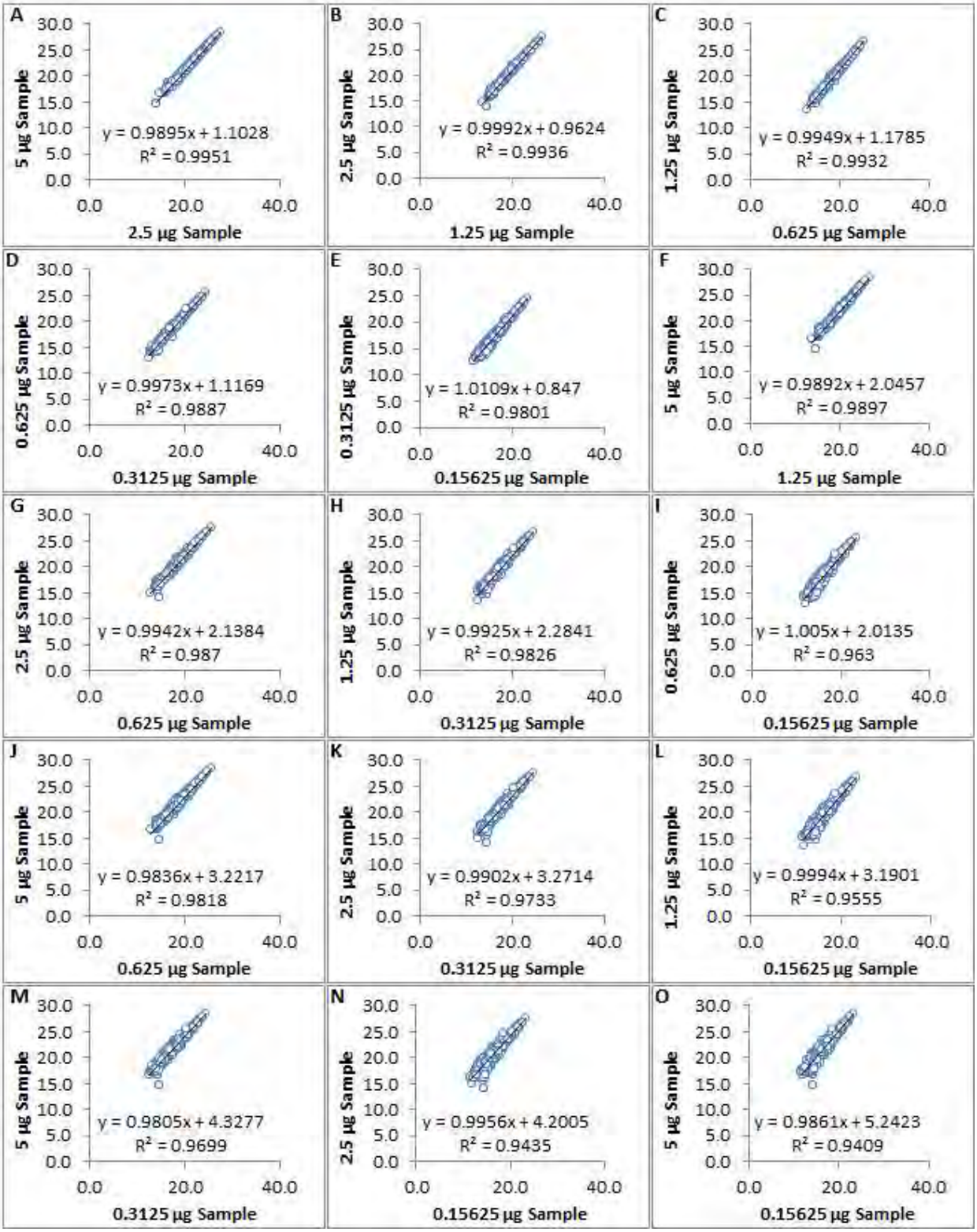
Supplementary Figure 4. The performance of the approach in protein quantification using raw data. The calibration curve of two standard peptides from 7 concentrations in 5 replicates each (Angiotensin III, 449.49; Fibrinopeptide B, 786.24). Extraordinary quantitative linearity was observed with R^2 of 0.9971 and 0.9984 across 7 concentrations with two orders of magnitude. (B) The repeatability analysis of 1,011 proteins in the current kidney tissue lysates indicates 96.2% of proteins had CVs ≤ 30%.



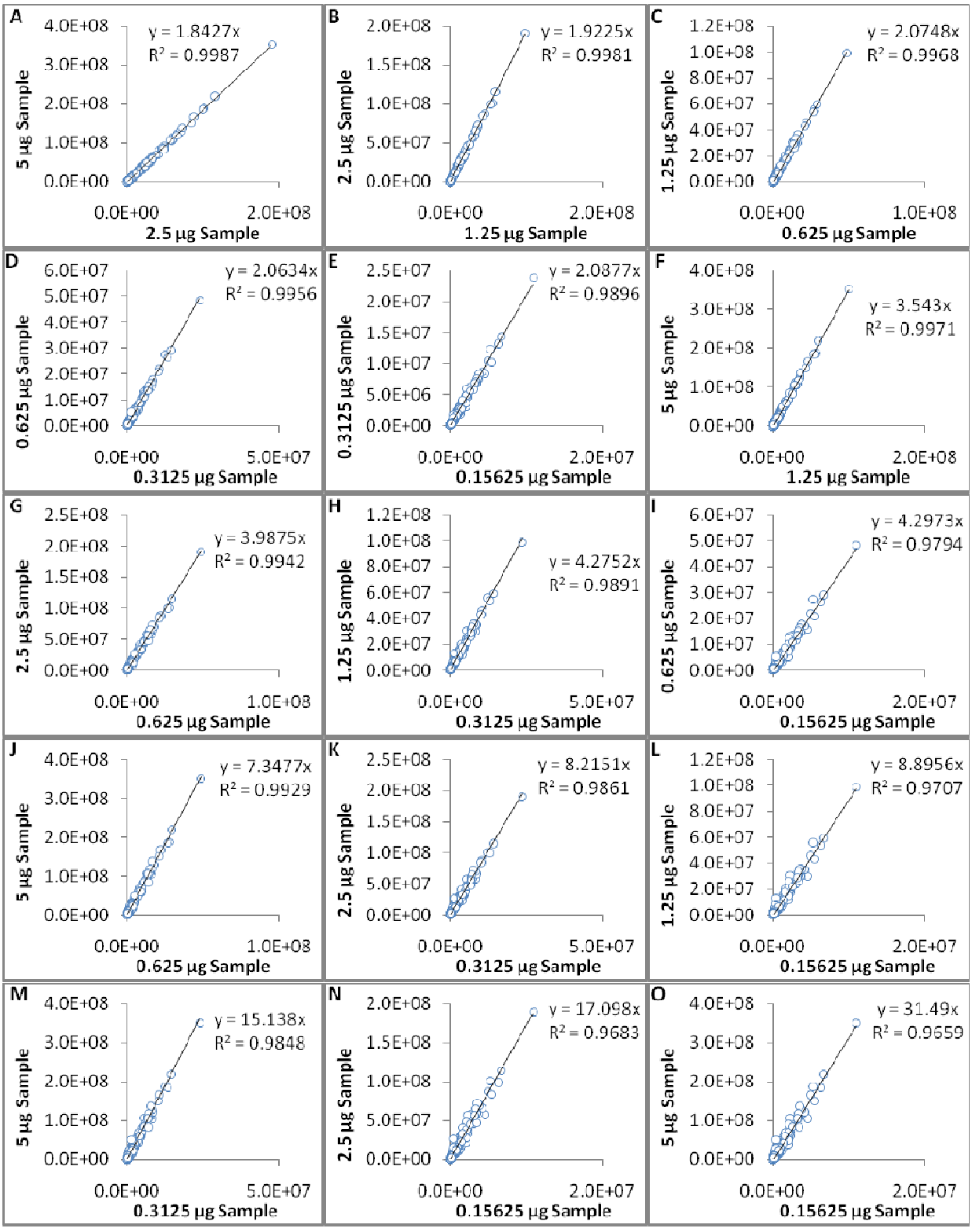
Supplementary Figure 5. Repeatability analysis of 10 µg of kidney tissue lysates spiked with 6 standard proteins (0.625-125 fmol, 0.000048-0.002030 µg), HT29-MTX cell lysate, depleted serum, and albumin-bound protein experiments. (A) 94.2% of 967 proteins in the kidney tissue lysates spiked with 6 standard proteins had CVs ≤ 30%. (B) 90.8% of 1,390 proteins in the HT29-MTX cell lysate had CVs ≤ 30%. (C) 95.0% of 238 proteins in the depleted serum had CVs ≤ 30%. (D) 92.9% of 156 proteins in the albumin-bound protein experiment cell lysate had CVs ≤ 30%.



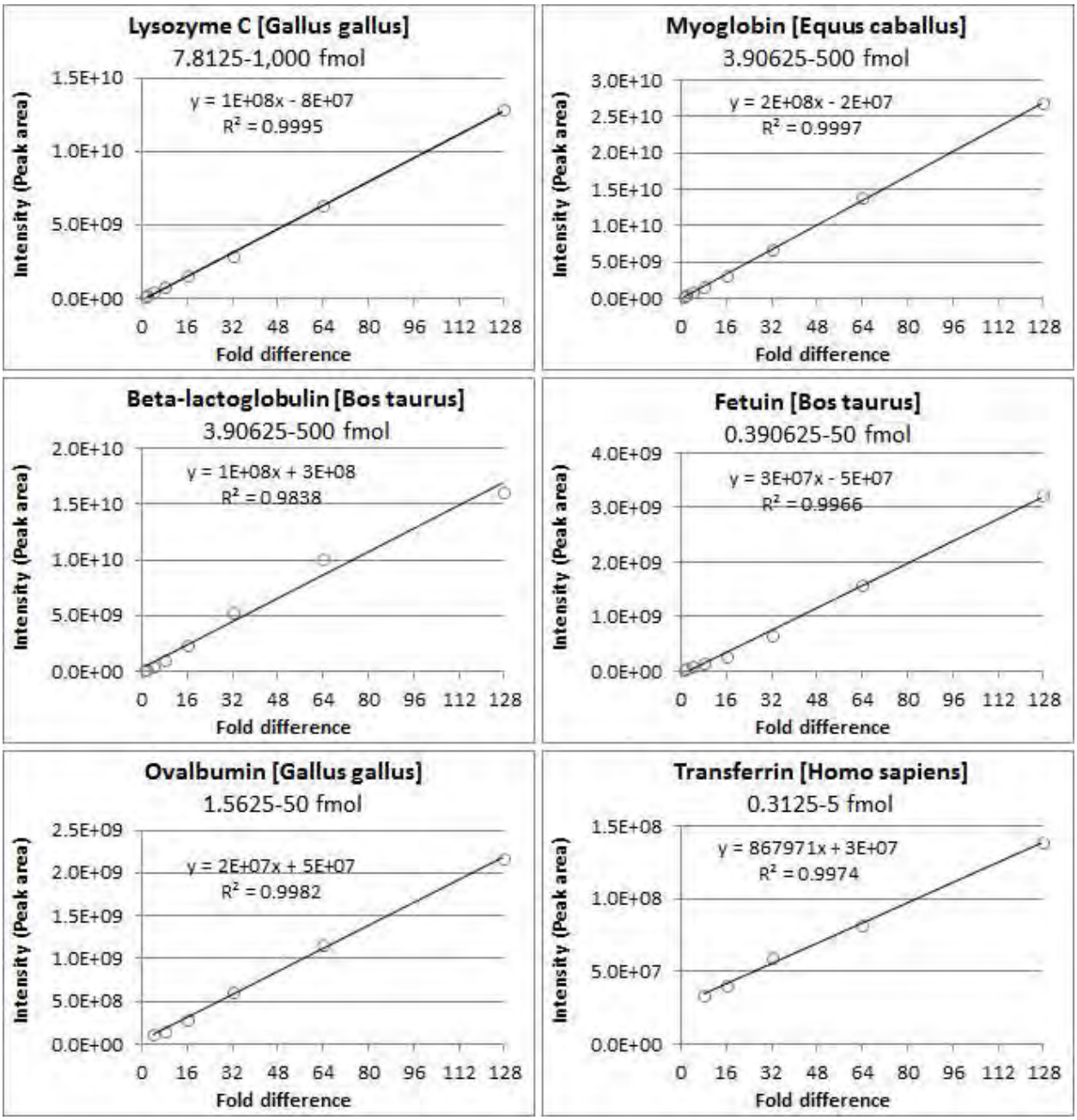
Supplementary Figure 6. Linear regression analysis of 1,500 proteins using raw data between 7 different concentrations of kidney tissue lysates. All fold differences fall in the ± 11.5% range, and the minimal R^2 is 0.9608. Even the largest fold difference (64 fold) was successfully determined as 66.33, an error of only 3.6%.



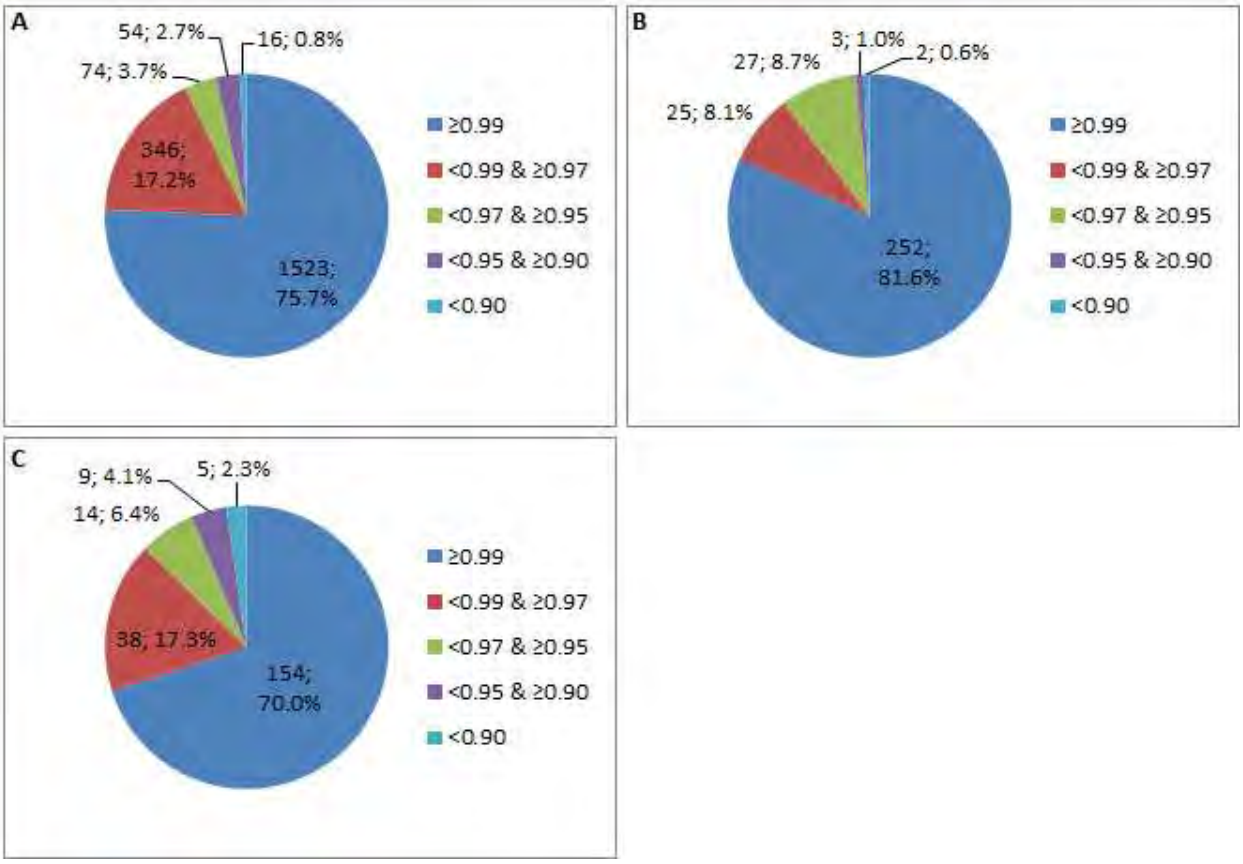
Supplementary Figure 7A. Linear regression analysis of 1,500 proteins using log-transformed data between different concentrations of kidney tissue lysates.



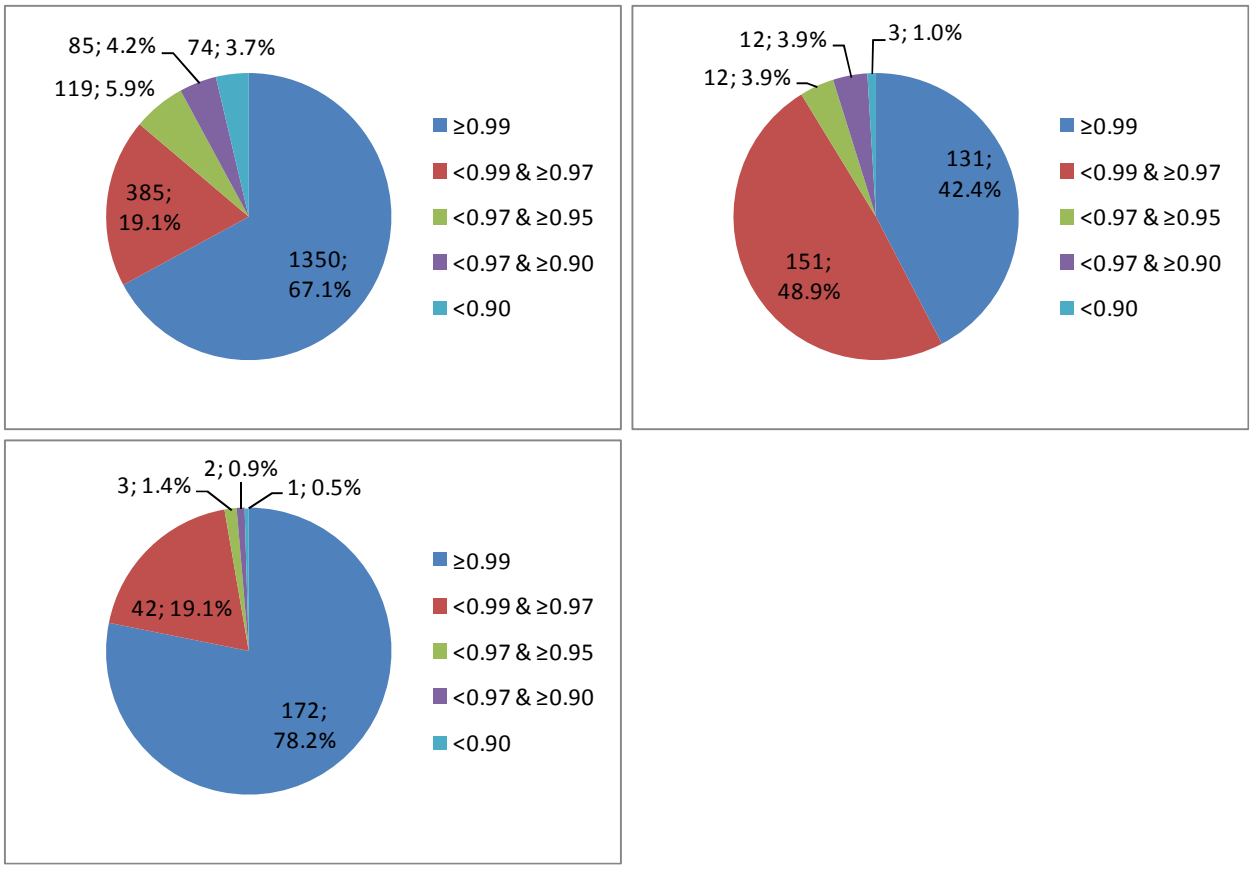
Supplementary Figure 7B. Linear regression analysis of 1,500 proteins using raw data between different concentrations of kidney tissue lysates.



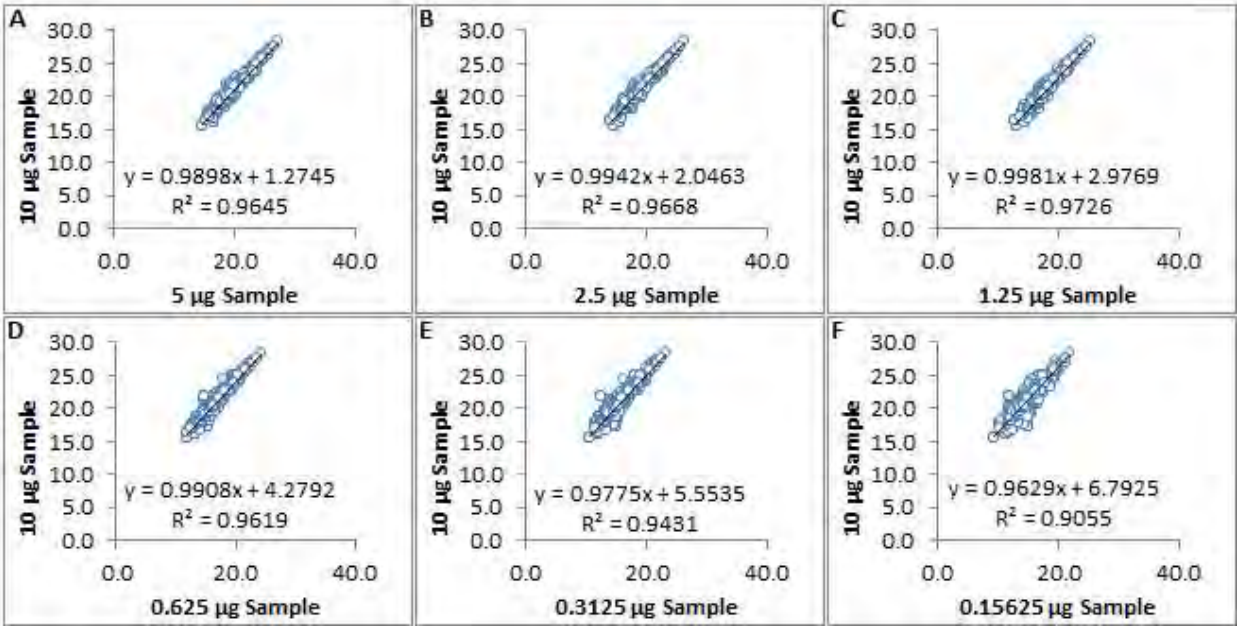
Supplementary Figure 8. Linear regression analysis using raw data between 8 different concentrations of 6 standard proteins (0.3125-1,000 fmol, 0.000017-0.016239 µg) spiked in 10 µg of kidney tissue lysates. All 6 proteins have an excellent correlation in the range, and the minimal R^2 is 0.9838.



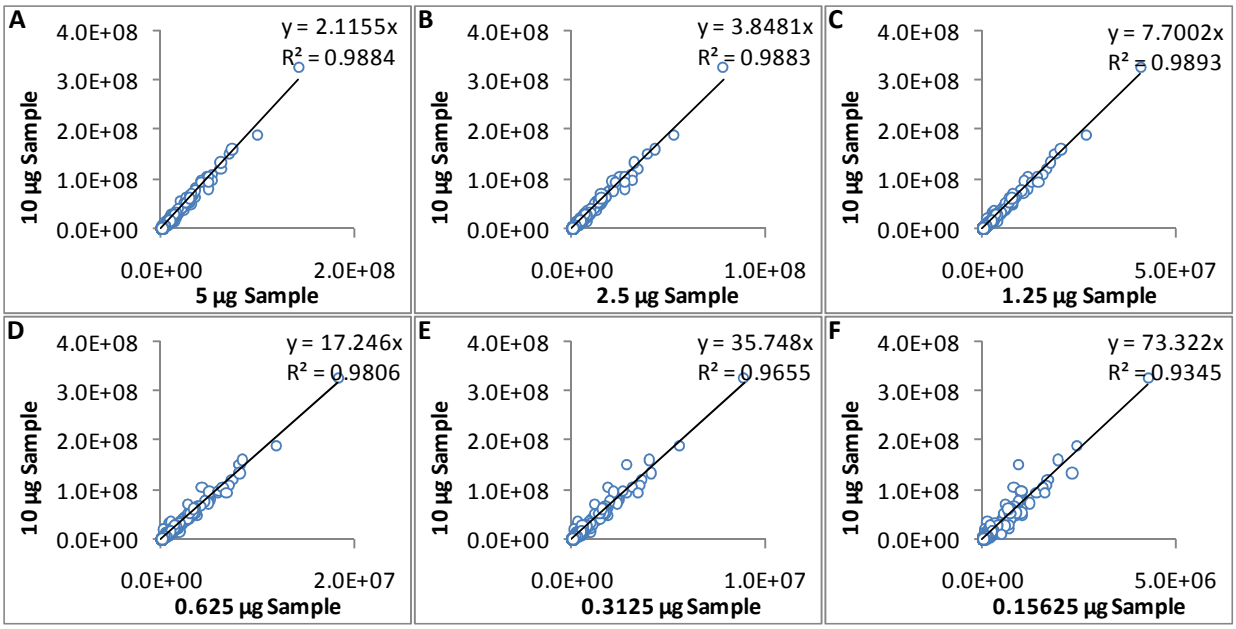
Supplementary Figure 9A. Linearity analysis using log-transformed data of HT29-MTX cell lysate, depleted serum, and albumin-bound protein experiments. (A) 96.5% of 2,013 proteins in the HT29-MTX cell lysate had $R^2 \geq 0.9500$. (B) 98.4% of 309 proteins in the depleted serum had $R^2 \geq 0.9500$. (C) 93.6% of 220 proteins in the albumin-bound protein experiment cell lysate had $R^2 \geq 0.9500$.



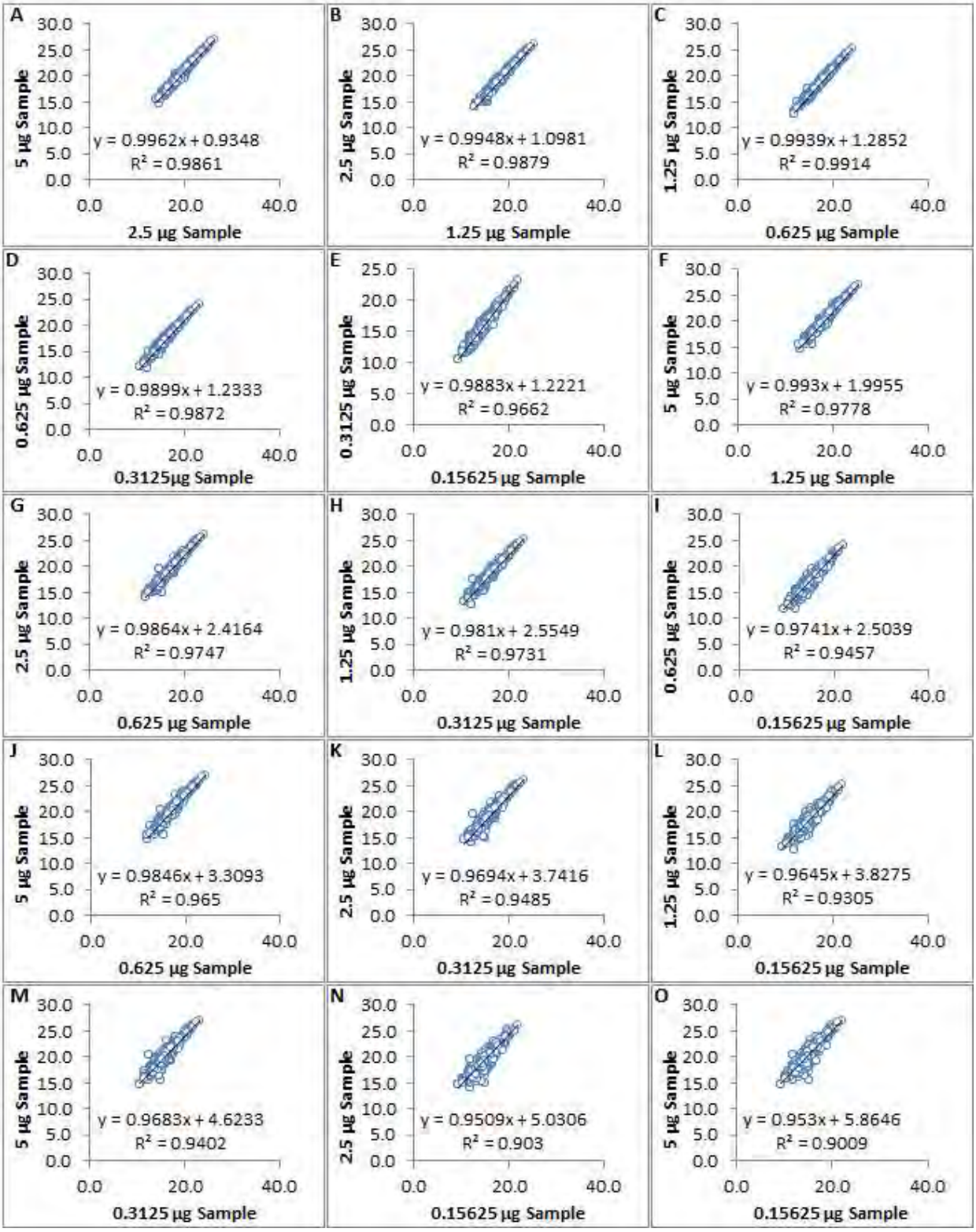
Supplementary Figure 9B. Linearity analysis using raw data of HT29-MTX cell lysate, depleted serum, and albumin-bound protein experiments. (A) 92.1% of 2,013 proteins in the HT29-MTX cell lysate had $R^2 \geq 0.9500$. (B) 95.1% of 309 proteins in the depleted serum had $R^2 \geq 0.9500$. (C) 98.6% of 220 proteins in the albumin-bound protein experiment cell lysate had $R^2 \geq 0.9500$.



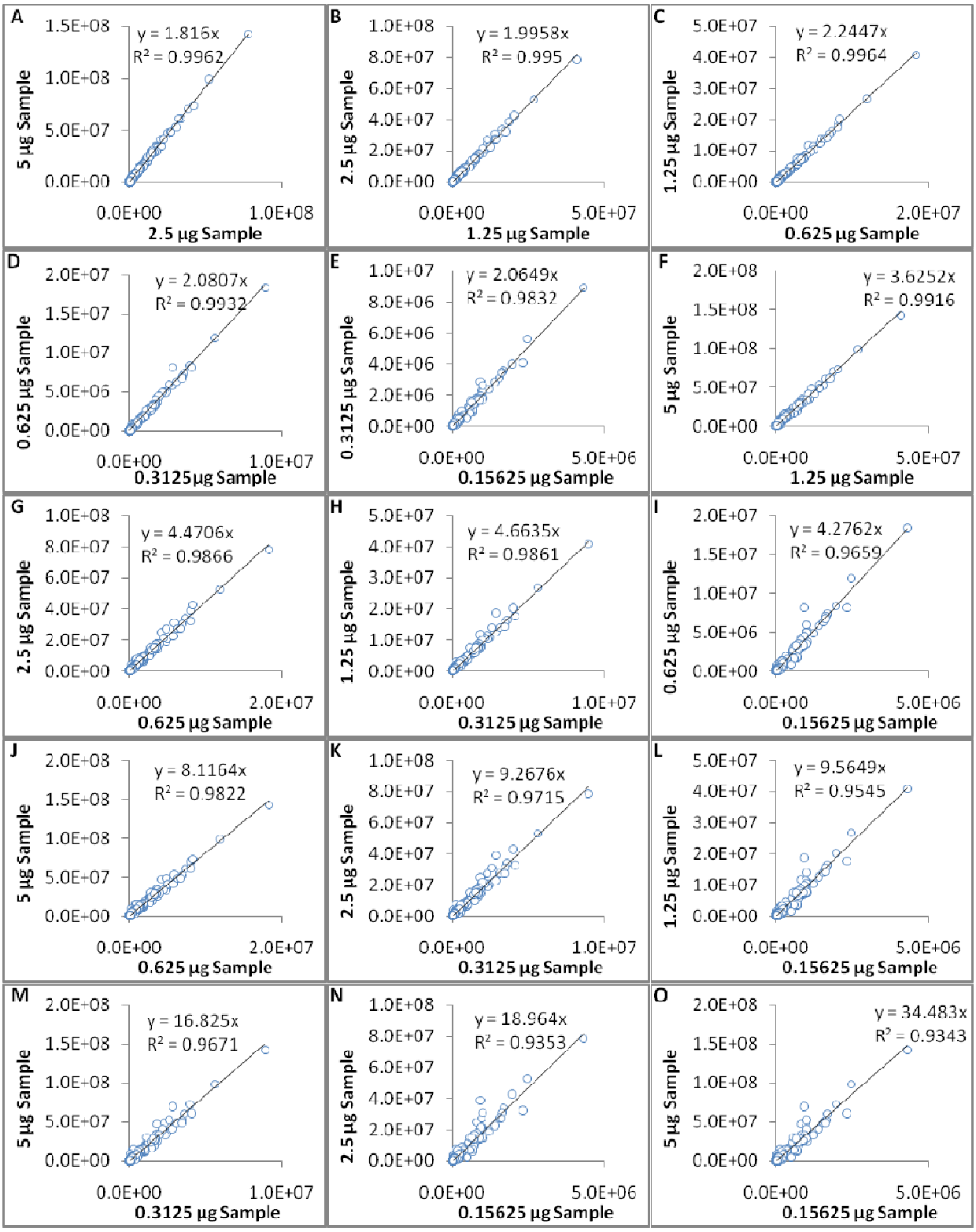
Supplementary Figure 10A. Linear regression analysis of 2,013 proteins using log-transformed data between 10 µg and various other concentrations of HT29-MXT cell lysate.



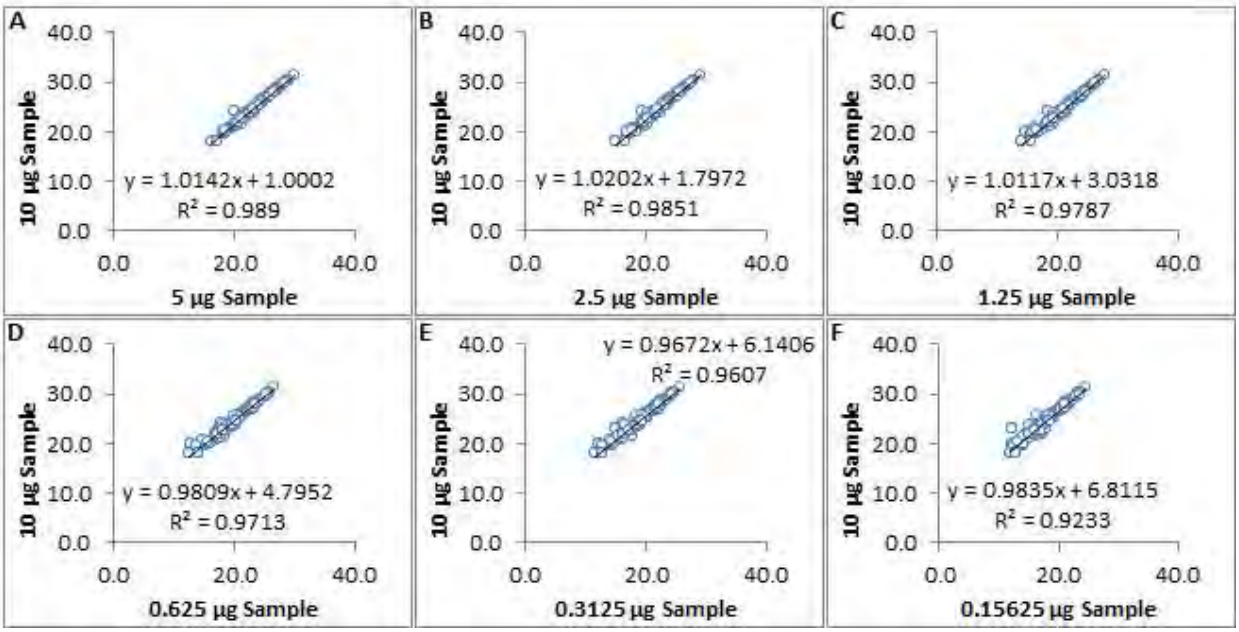
Supplementary Figure 10B. Linear regression analysis of 2,013 proteins using raw data between 10 µg and various other concentrations of HT29-MXT cell lysate.



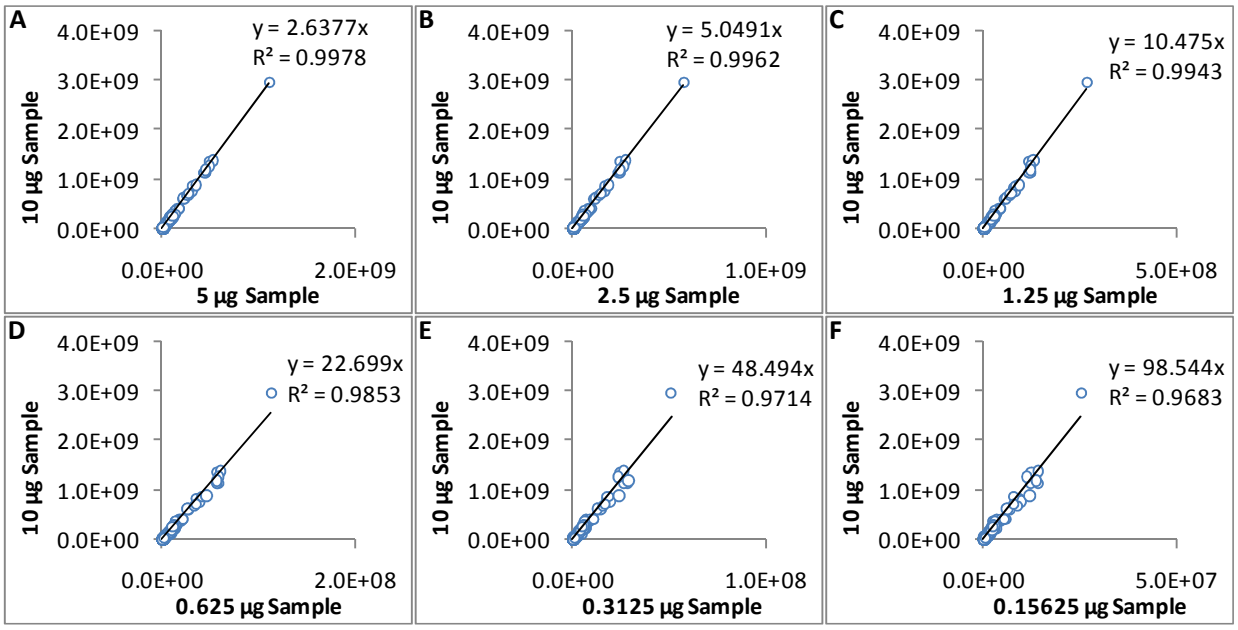
Supplementary Figure 11A. Linear regression analysis of 2,013 proteins using log-transformed data between different concentrations of HT29-MTX cell lysate.



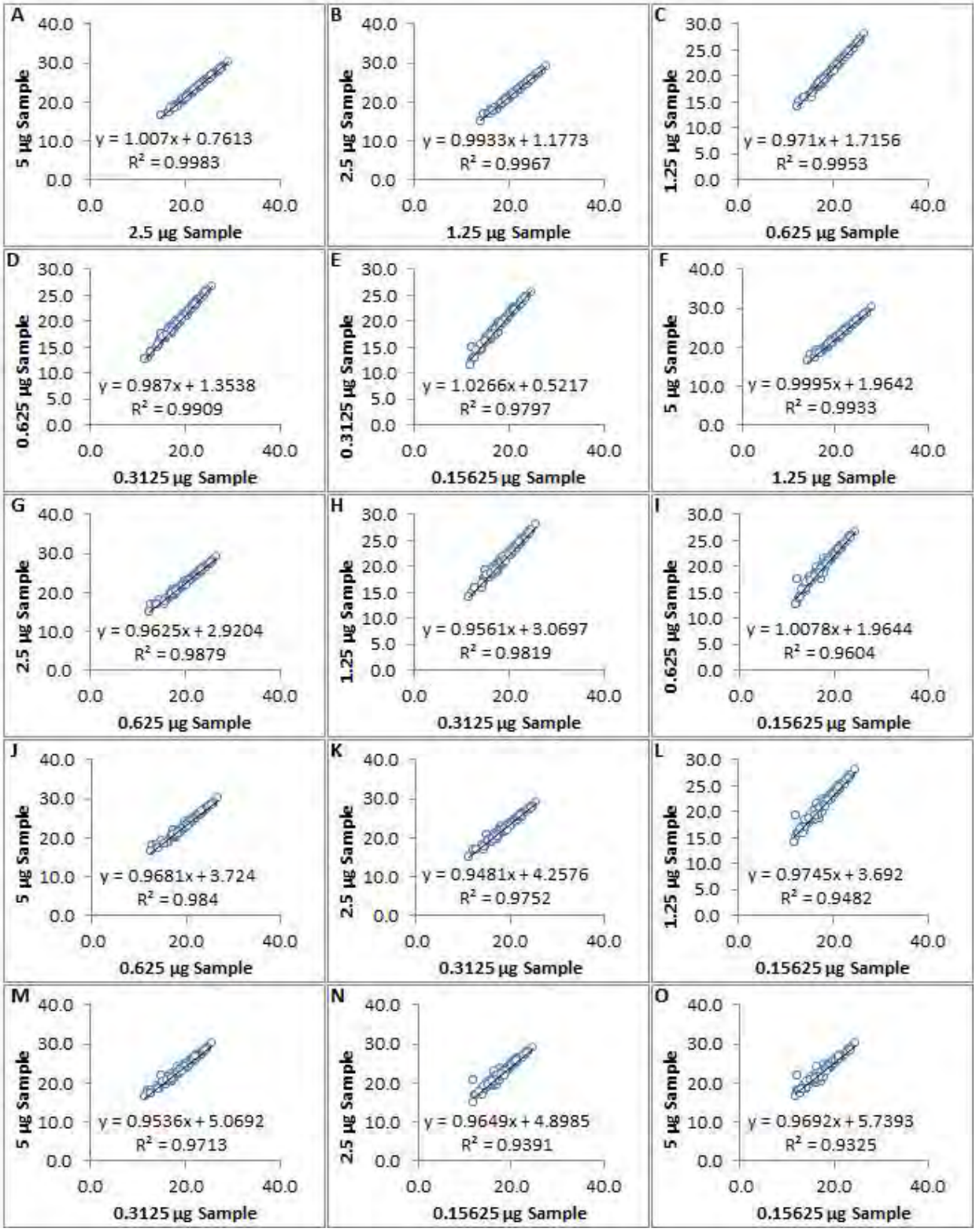
Supplementary Figure 11B. Linear regression analysis of 2,013 proteins using raw data between different concentrations of HT29-MTX cell lysate.



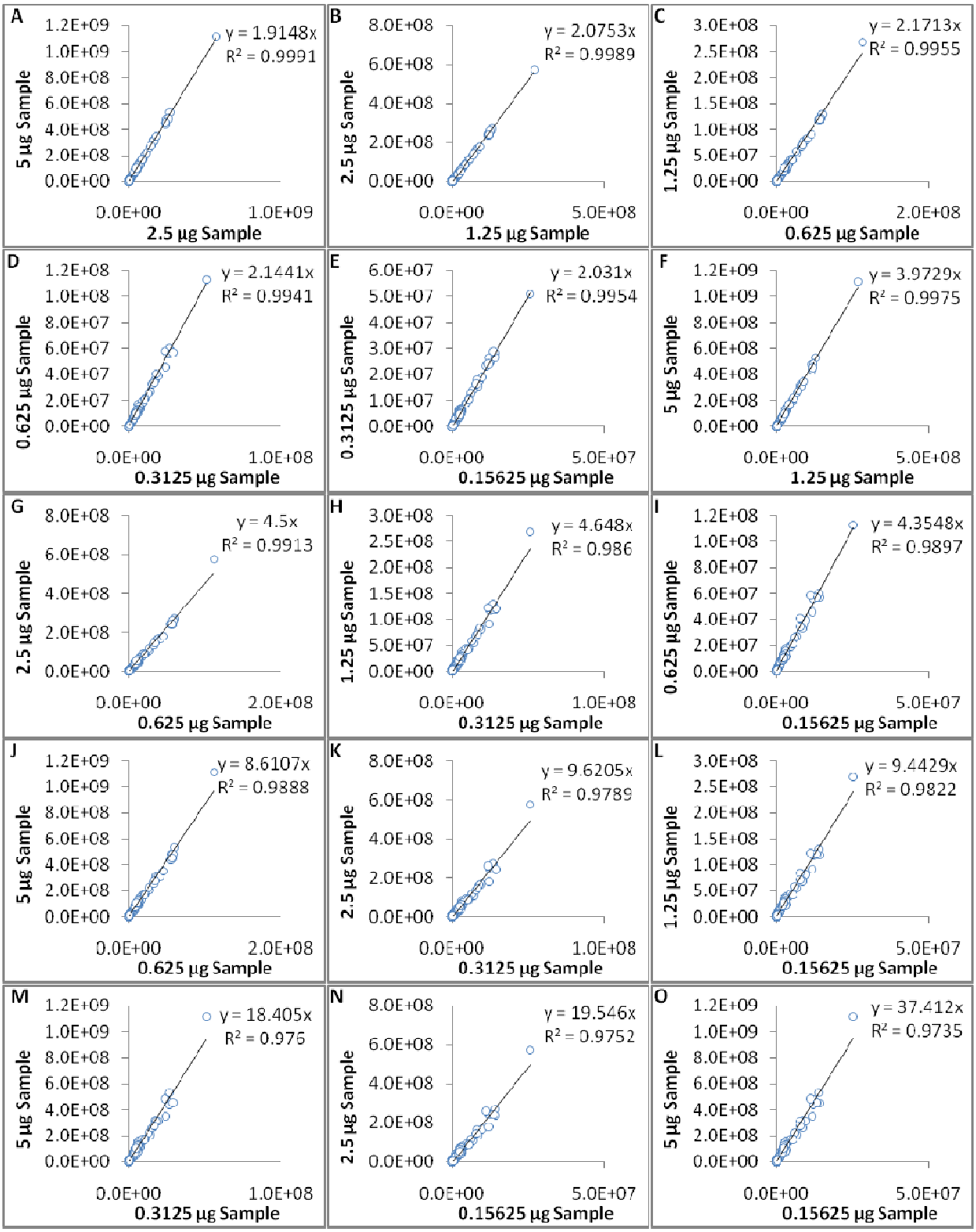
Supplementary Figure 12A. Linear regression analysis of 309 proteins using log-transformed data between 10 µg and various other concentrations of depleted serum.



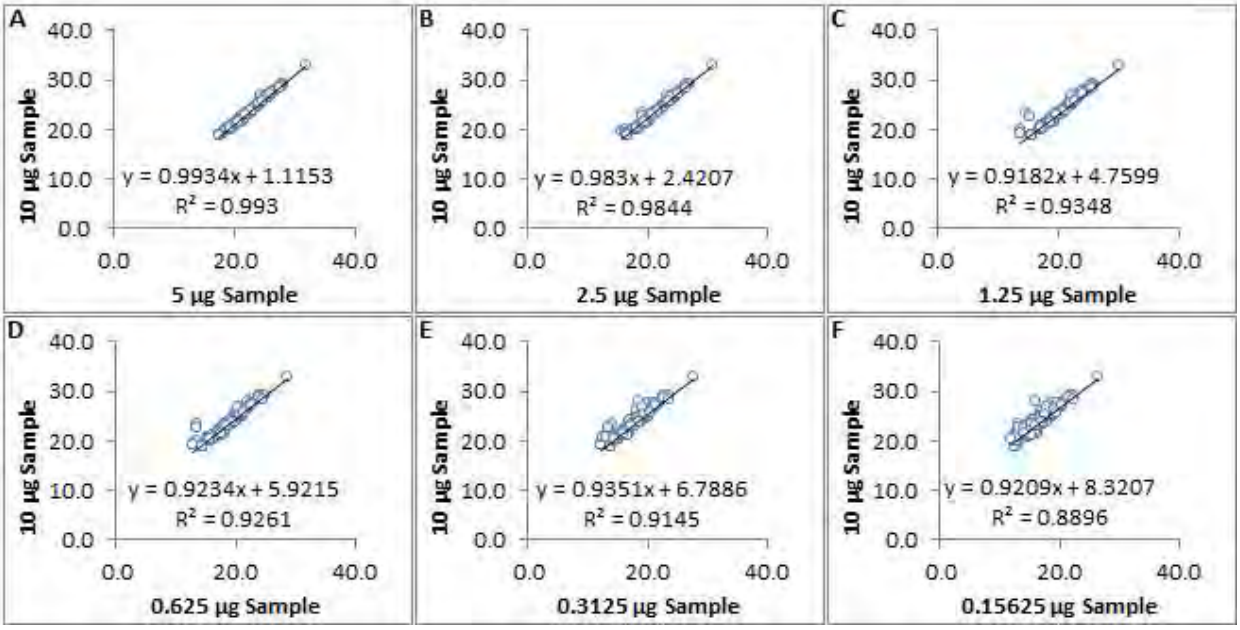
Supplementary Figure 12B. Linear regression analysis of 309 proteins using raw data between 10 µg and various other concentrations of depleted serum.



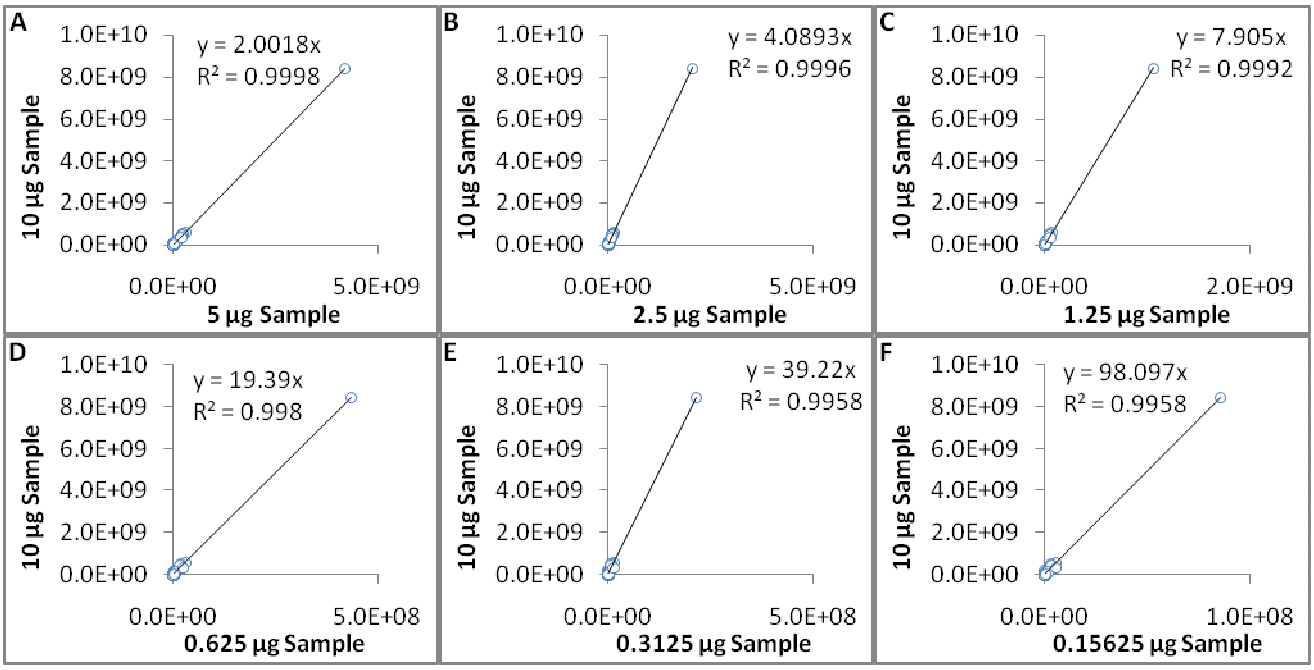
Supplementary Figure 13A. Linear regression analysis of 309 proteins using log-transformed data between different concentrations of depleted serum.



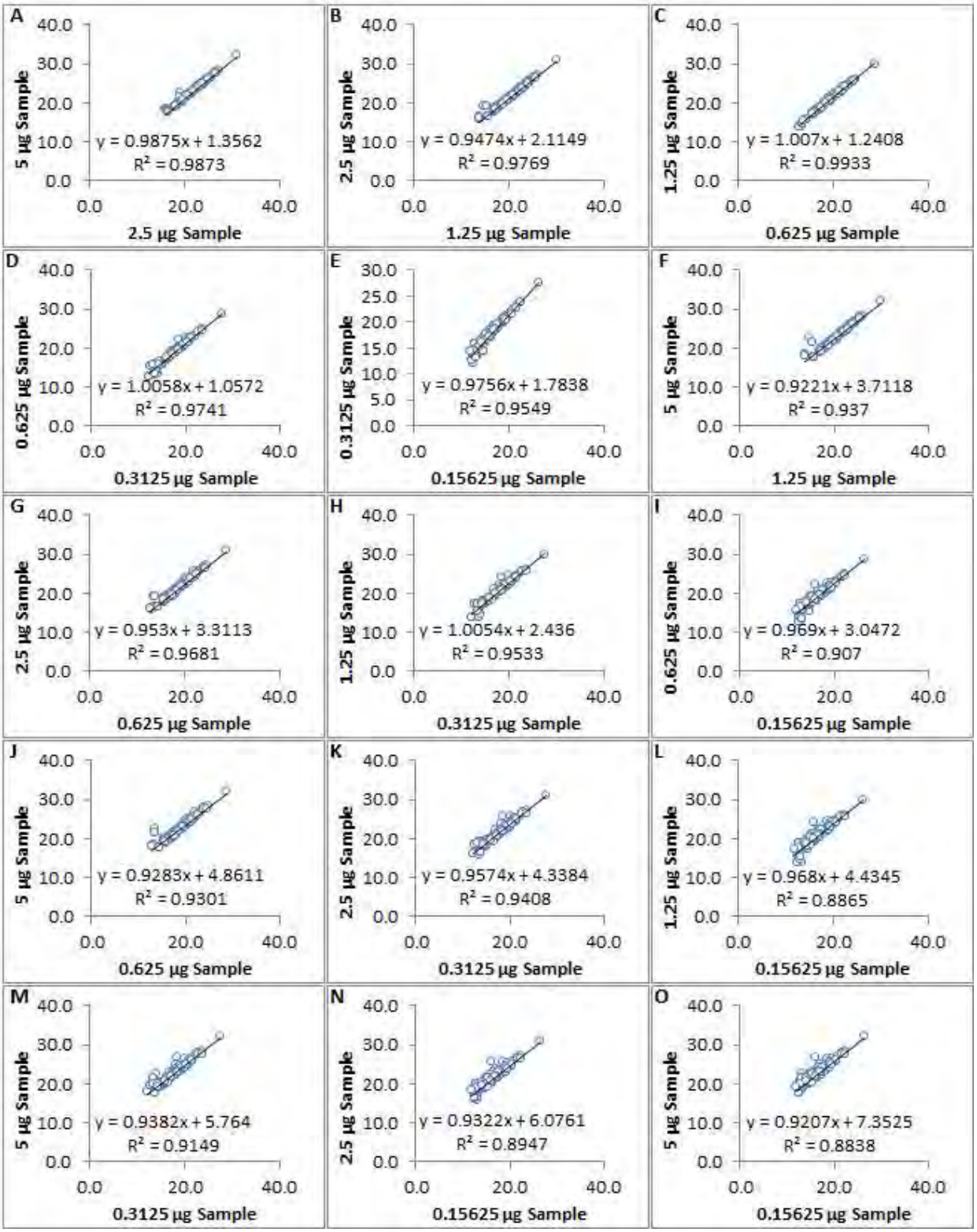
Supplementary Figure 13B. Linear regression analysis of 309 proteins using raw data between different concentrations of depleted serum.



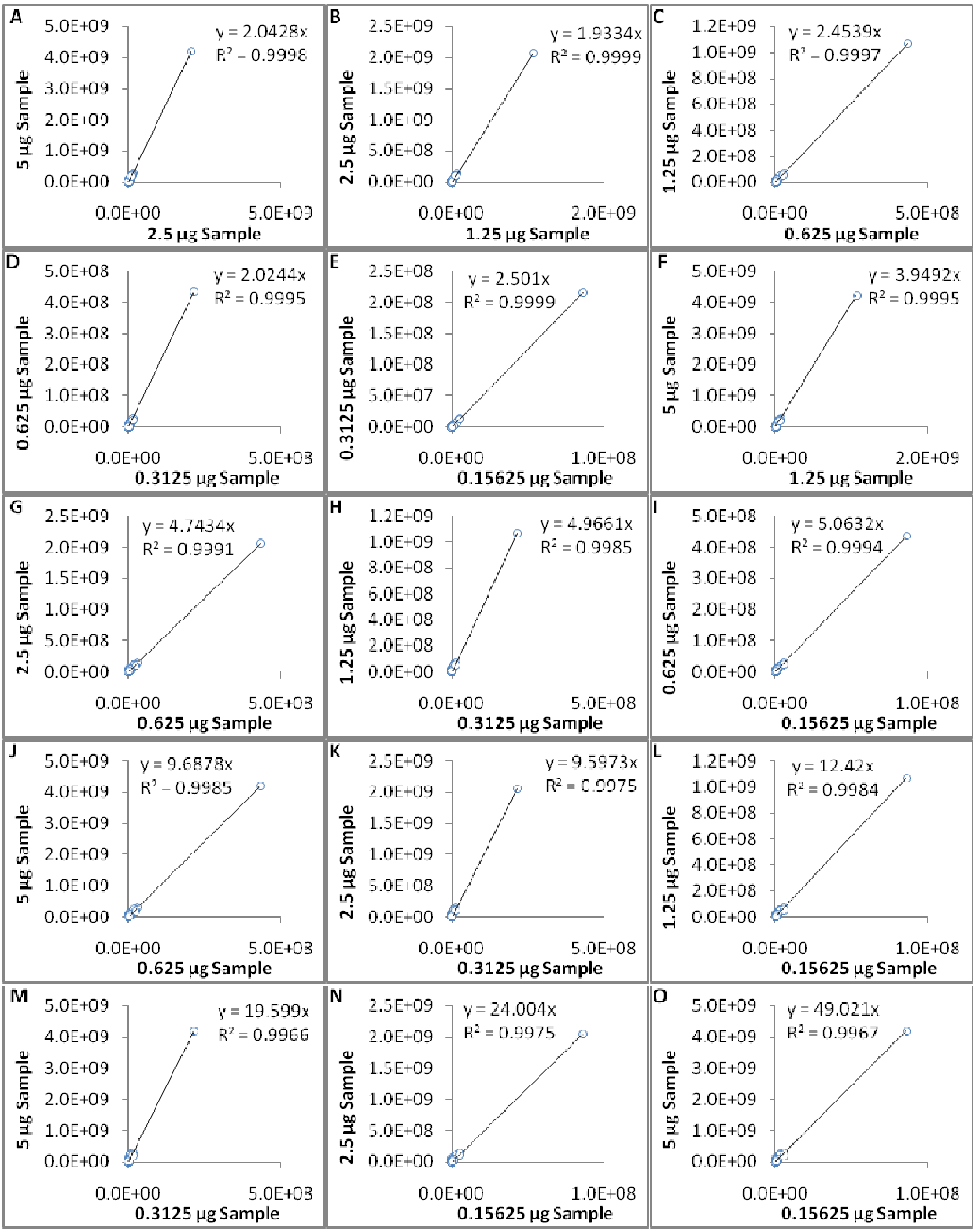
Supplementary Figure 14A. Linear regression analysis of 220 proteins using log-transformed data between 10 µg and various other concentrations of albumin-bound proteins.



Supplementary Figure 14B. Linear regression analysis of 220 proteins using raw data between 10 µg and various other concentrations of albumin-bound proteins.



Supplementary Figure 15A. Linear regression analysis of 220 proteins using log-transformed data between different concentrations of albumin-bound proteins.



Supplementary Figure 15B. Linear regression analysis of 220 proteins using raw data between different concentrations of albumin-bound proteins.