

Supplementary Table 1. MudPIT gradient elution profile

Step 1

Time	Flow (mL/min)	A%	B%	C%
0.00	0.2	100	0	0
0.99	0.2	100	0	0
1.00	0.2	100	0	0
5.00	0.2	100	0	0
5.01	0.2	100	0	0
10.00	0.2	90	10	0
40.00	0.2	50	50	0
45.00	0.2	0	100	0
50.00	0.2	0	100	0
51.00	0.2	100	0	0
60.00	0.2	100	0	0

Step 2-9

Time	Flow (mL/min)	A%	B%	C%
0.00	0.2	100	0	0
0.99	0.2	100	0	0
1.00	0.2	X	0	y
5.00	0.2	X	0	y
5.01	0.2	100	0	0
10.00	0.2	90	10	0
90.00	0.2	50	50	0
100.00	0.2	0	100	0
110.00	0.2	0	100	0
111.00	0.2	100	0	0
120.00	0.2	100	0	0

x = 90	y = 10
x = 80	y = 20
x = 70	y = 30
x = 60	y = 40
x = 50	y = 50
x = 40	y = 60
x = 30	y = 70
x = 20	y = 80
x = 0	y = 100

Step 10-11

Time	Flow (mL/min)	A%	B%	C%
0.00	0.2	100	0	0
0.99	0.2	100	0	0
1.00	0.2	0	10	90
5.00	0.2	0	10	90
5.01	0.2	100	0	0
10.00	0.2	90	10	0
90.00	0.2	50	50	0
100.00	0.2	0	100	0
110.00	0.2	0	100	0
111.00	0.2	100	0	0
120.00	0.2	100	0	0

Supplementary Figure 1. (a) Frequency distribution histograms of \log_2 protein ratios between p1 or p45 samples, each labeled by three different TMT isobaric tags in sixplex. p1/p1 ratios are observed from the tags, 128/126, 130/126, 130/128 whereas p45/p45 ratios are observed from the tags, 129/127, 131/127, 131/129. The median of p1/p1 ratios is 1.01 and ~93% of proteins fall within SD of ± 0.25 ; the median of p45/p45 ratios is 1.01 and about 95% of proteins fall within SD of ± 0.25 . (b) boxplot depicting the spread of the three p1/p1 and three p45/p45 ratios observed in the TMT sixplex experiment.

Supplementary Figure 2. Frequency distribution histograms of the \log_2 protein ratios for p45 compared against p1 sample in the (a) sixplex TMT experiment, and (b) duplex TMT experiments. For sixplex, the three p45/p1 ratios are observed from the tags 127/126, 129/128, and 131/130. For duplex, the p45/p1 ratios are observed from tags 127/126 run in triplicate. (c) boxplot depicting the spread of the three p45/p1 ratios observed in the sixplex experiment and three p45/p1 ratios observed in triplicate duplex experiments.

Supplementary Figure 3. Histogram depicting the standard deviation (SD) of the average p45/p1 ratios in sixplex and duplex TMT experiments. The x -axis denotes the SD, while the y -axis represents the number of observed proteins. (Binwidth = 0.05)

Twenty six proteins in sixplex and fifteen proteins in duplex have $SD > 2.0$ and are not included during histogram plotting.

Supplementary Figure 4. Heatmaps of significantly altered p45/p1 protein expression ratios commonly detected by sixplex or duplex TMT experiments when compared against SILAM-based quantification approach. The altered proteins have p -value < 0.05 in both TMT and SILAM-based quantitation, however, only in TMT quantification $-1.0 \leq \log_2 (\text{p45/p1 ratio}) \leq 1.0$

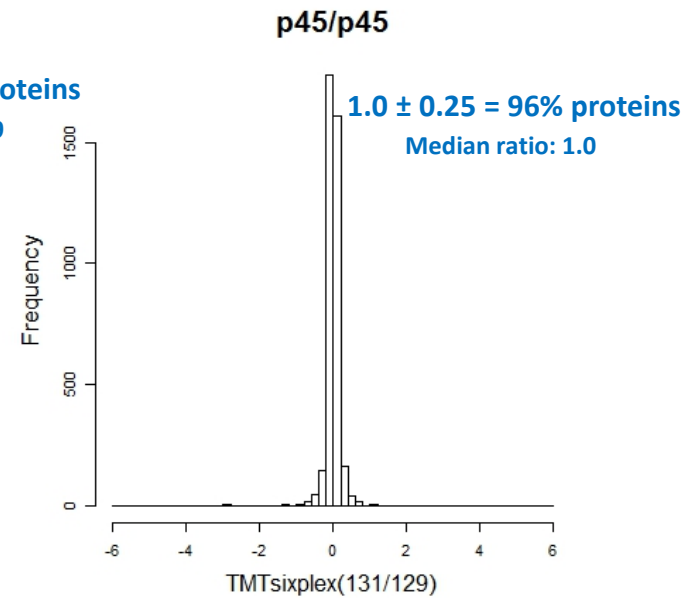
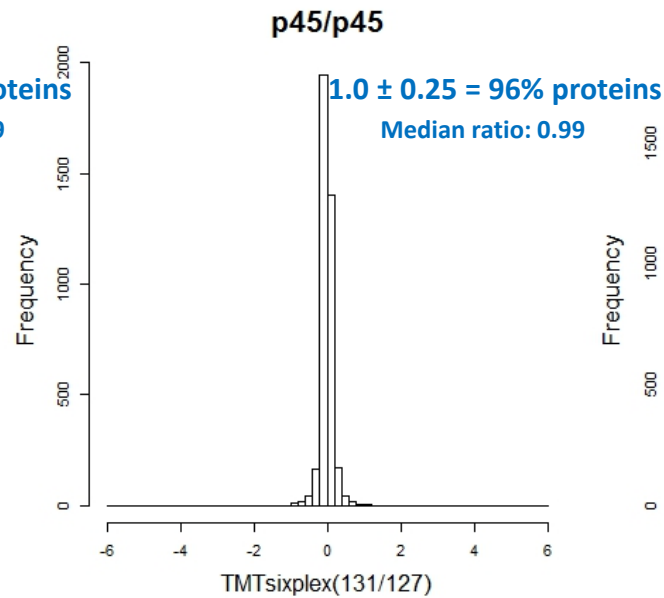
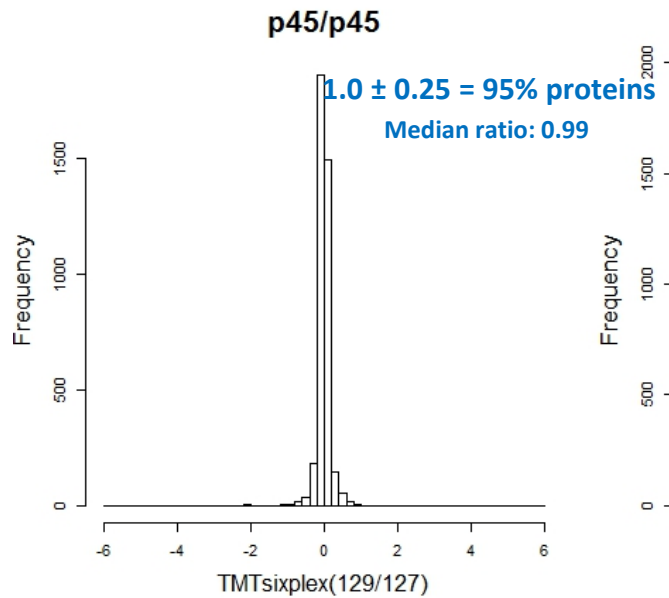
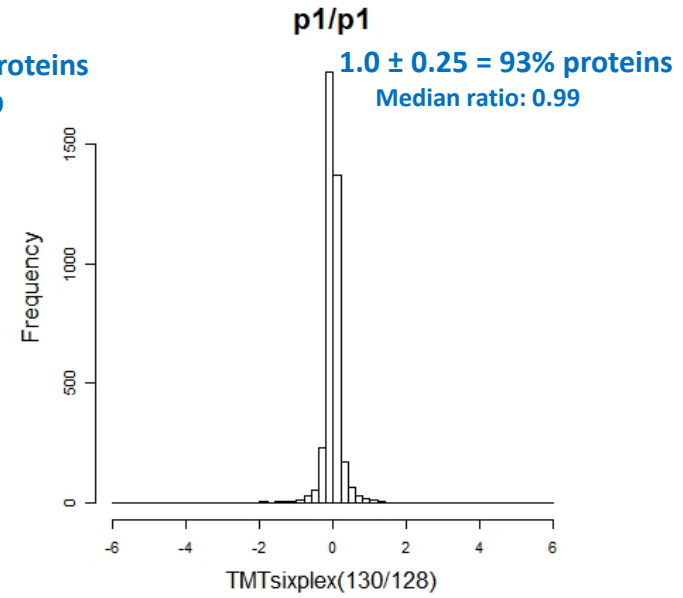
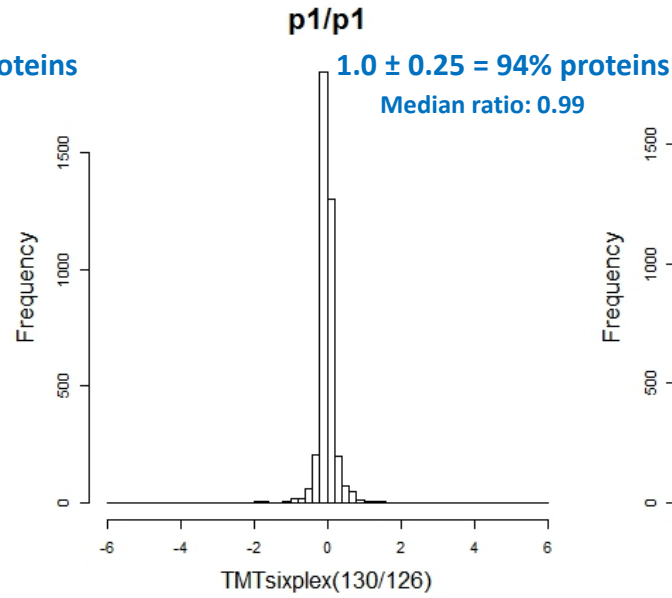
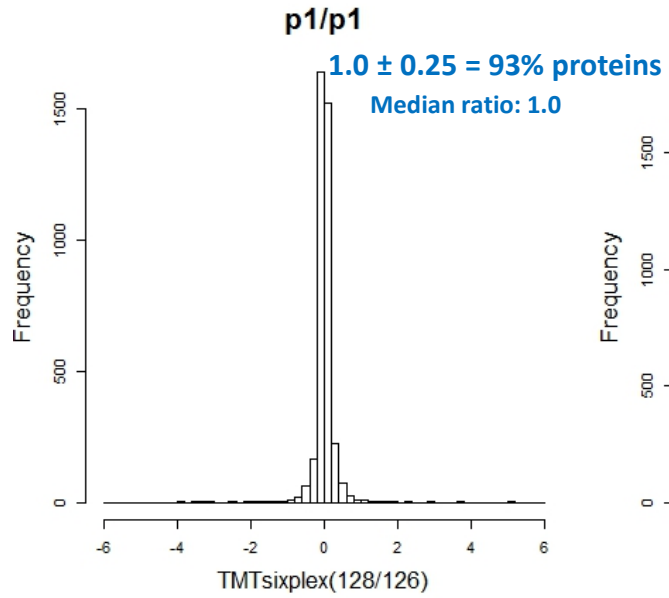
was considered. (No of proteins in sixplex and SILAM comparison (N) = 375, No of proteins in duplex and SILAM comparison (N) = 225)

Supplementary Figure 5. Distribution of p45/p1 ratios ($-1.0 \leq \log_2(\text{p45/p1 ratio}) \leq 1.0$ and p value of < 0.05) for TMT sixplex or duplex with matching p45/p1 ratios (p value < 0.05) by SILAM approach. (No of proteins in sixplex and SILAM comparison (N) = 375, No of proteins in duplex and SILAM comparison (N) = 225)

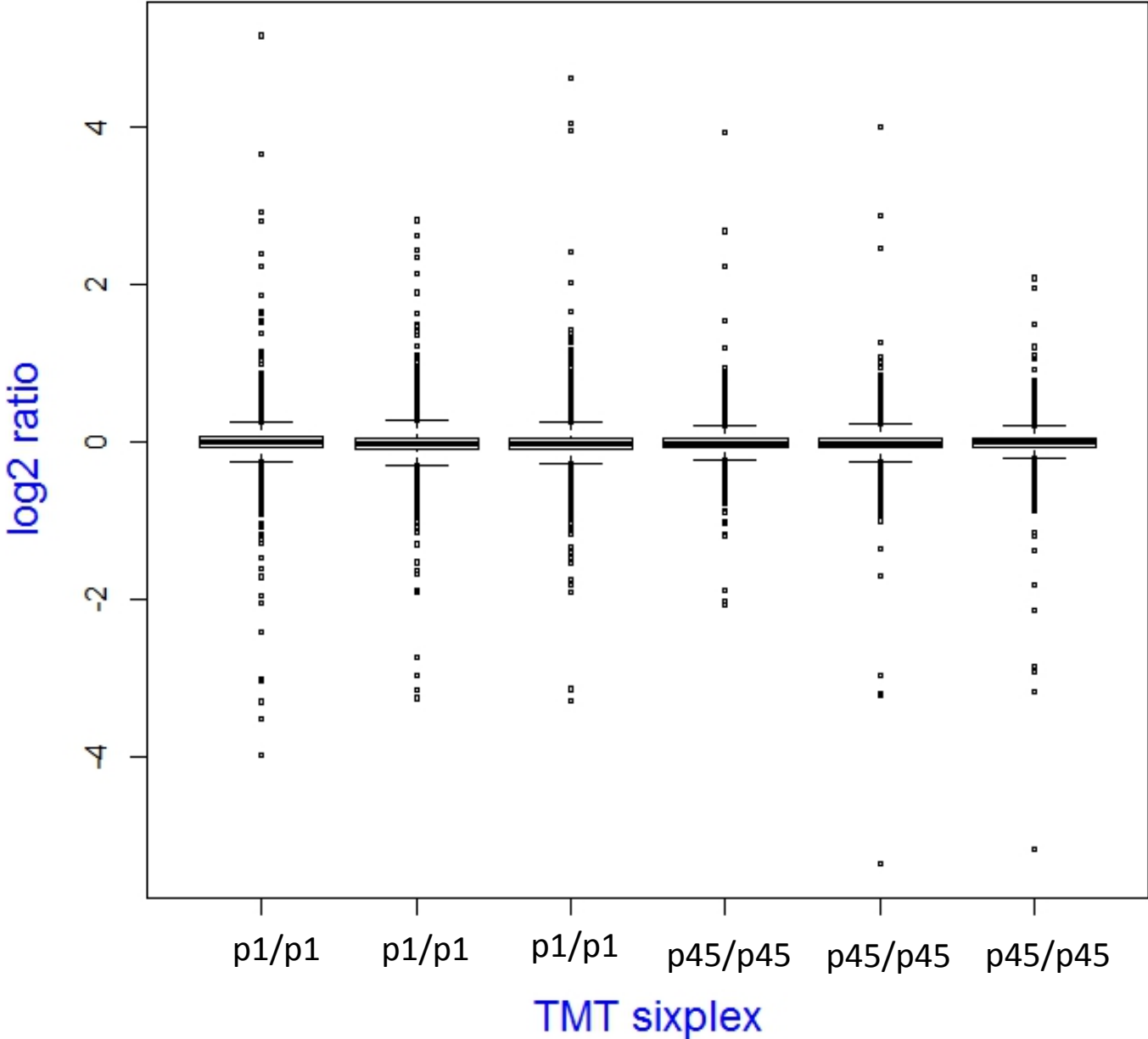
Supplementary Figure 6. Scatter plot examining the comparison of fold change (p45/p1) observed by the TMT-based quantitation using repeat count settings of 4 and one, respectively. A decent correlation ($R^2 = 0.86$) of the \log_2 -transformed p45/p1 protein ratios was observed between the two repeat count settings.

Supplementary Figure 7. Bar chart showing number of observed proteins (y -axis) and their spectral counts (x -axis) in (a) sixplex and (c) duplex experiments. The right vertical axis represents the percentage of counted proteins. Cumulative percentage of the proteins with these spectral counts are shown in (b) for sixplex, and (d) for duplex TMT experiments.

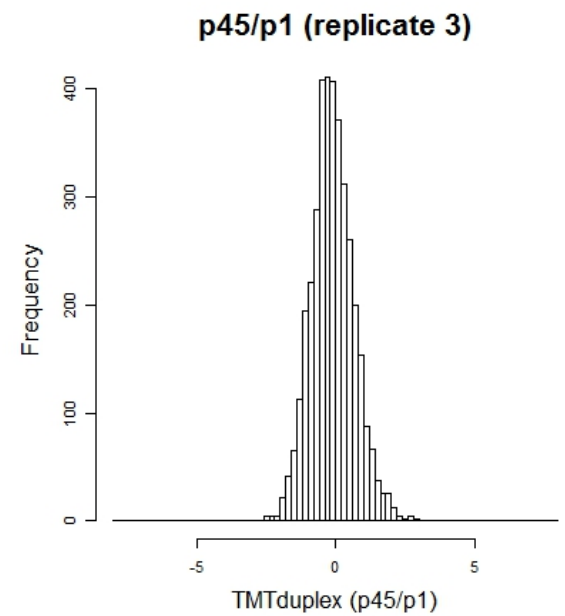
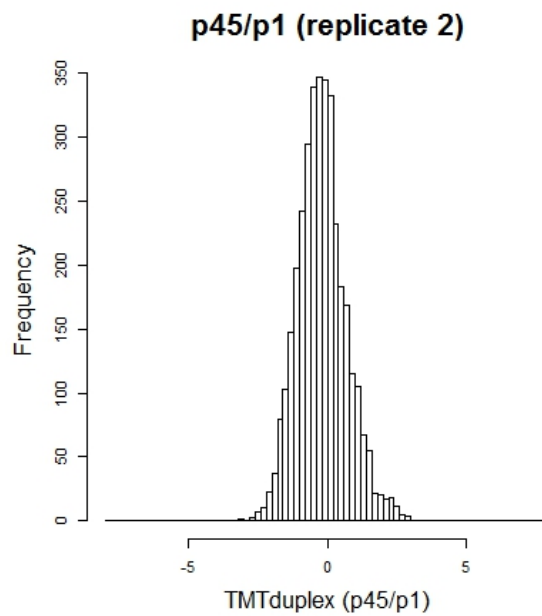
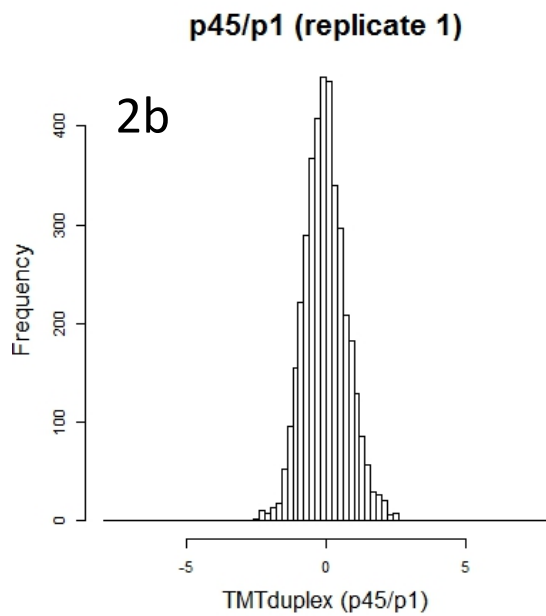
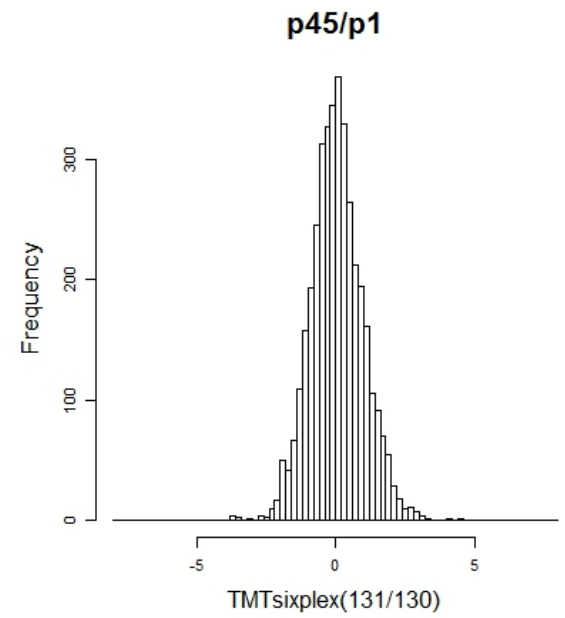
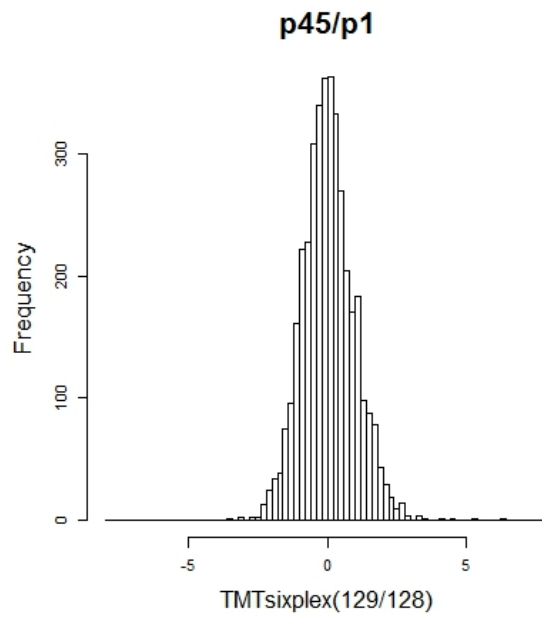
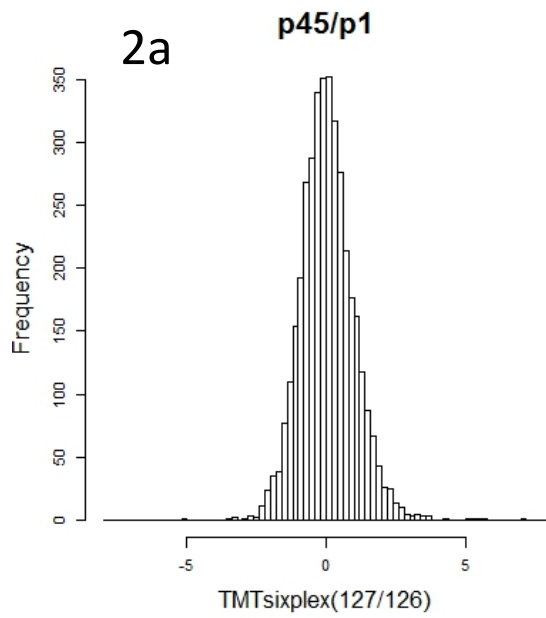
Supplementary Figure 1a



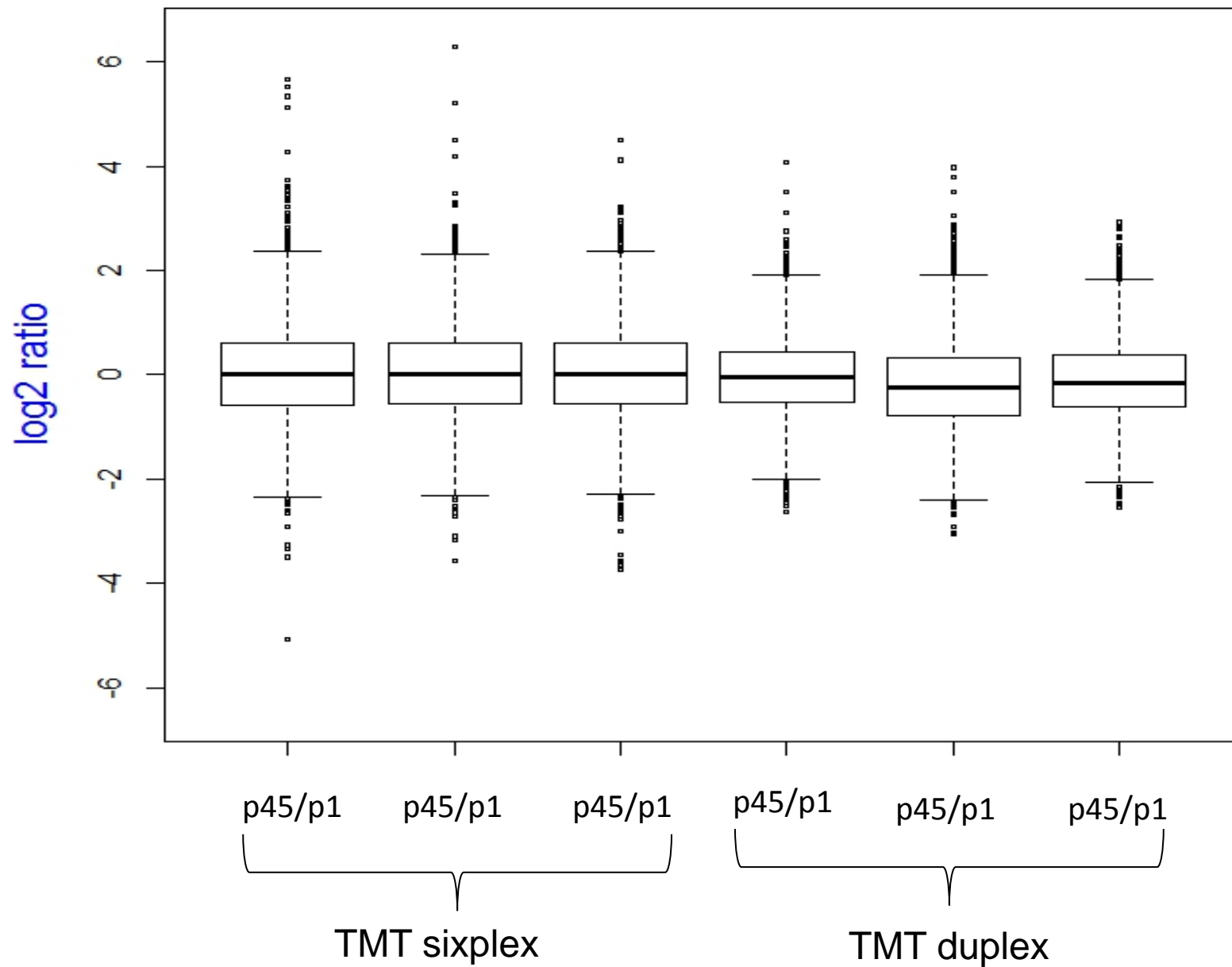
Supplementary Figure 1b



Supplementary Figure



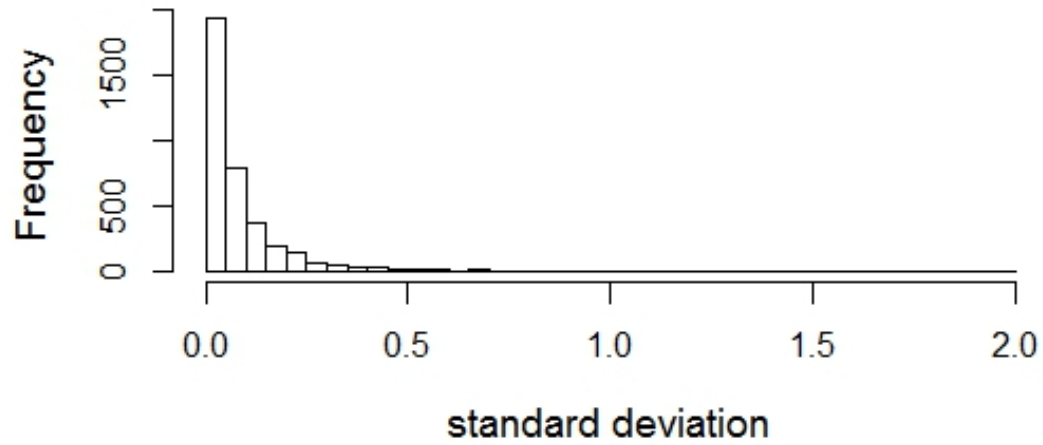
2c



Supplementary Figure 3

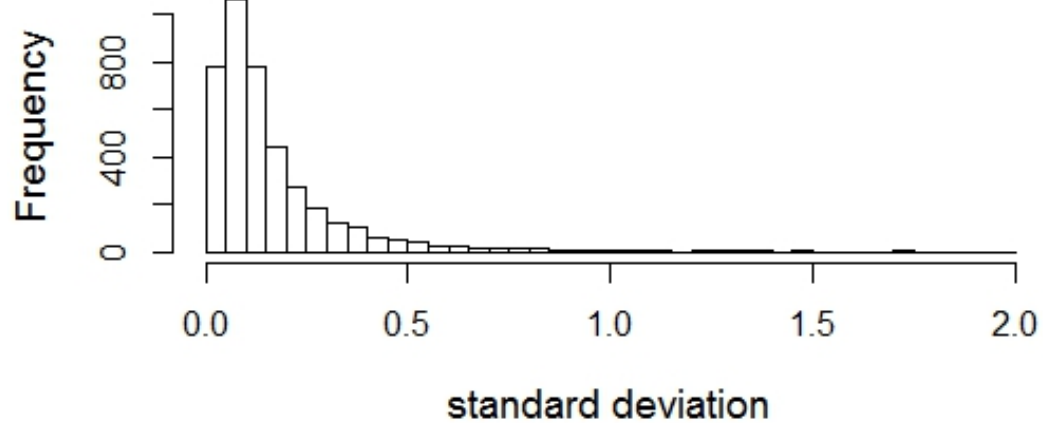
Standard Deviation: 6plex

N = 3806

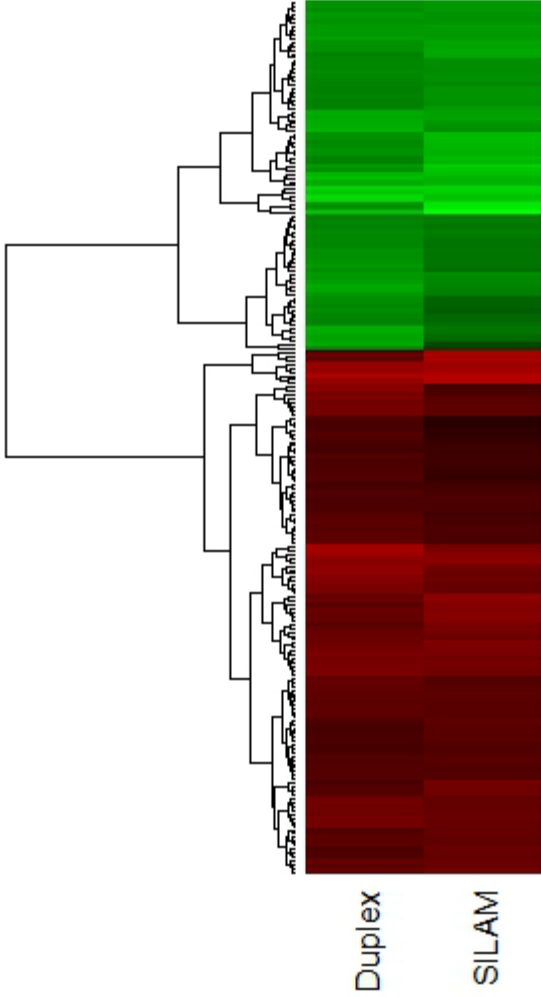
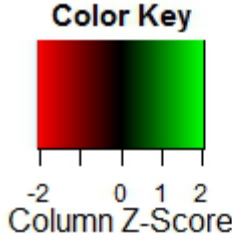
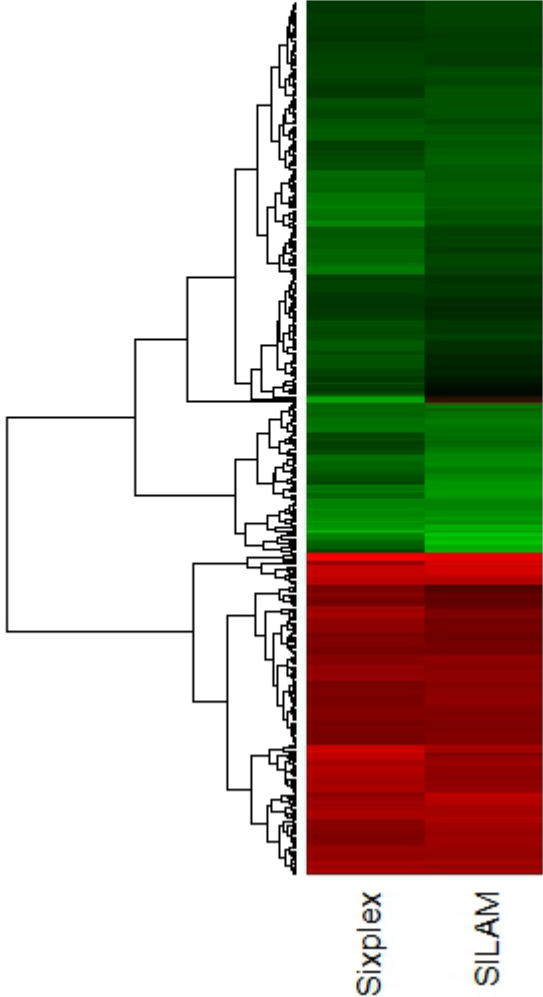
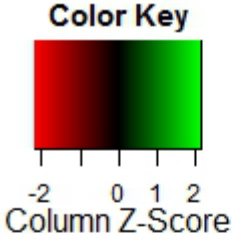


Standard Deviation: 2plex

N = 4091

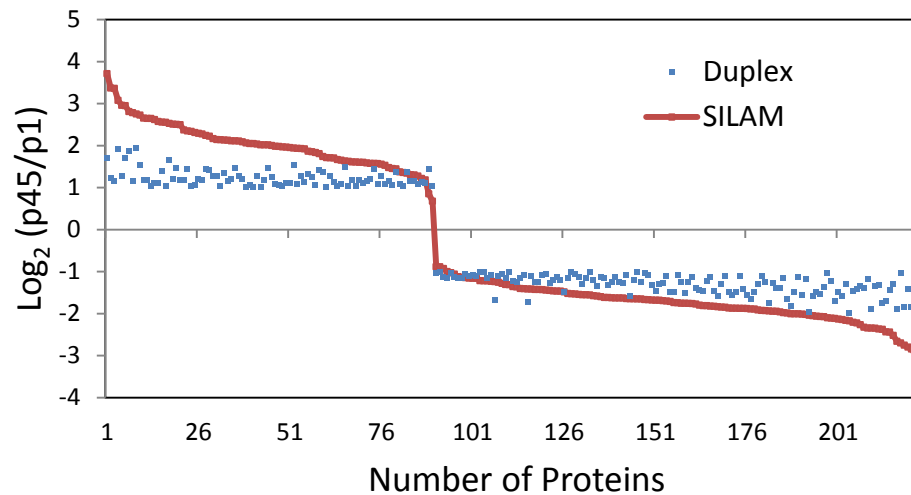
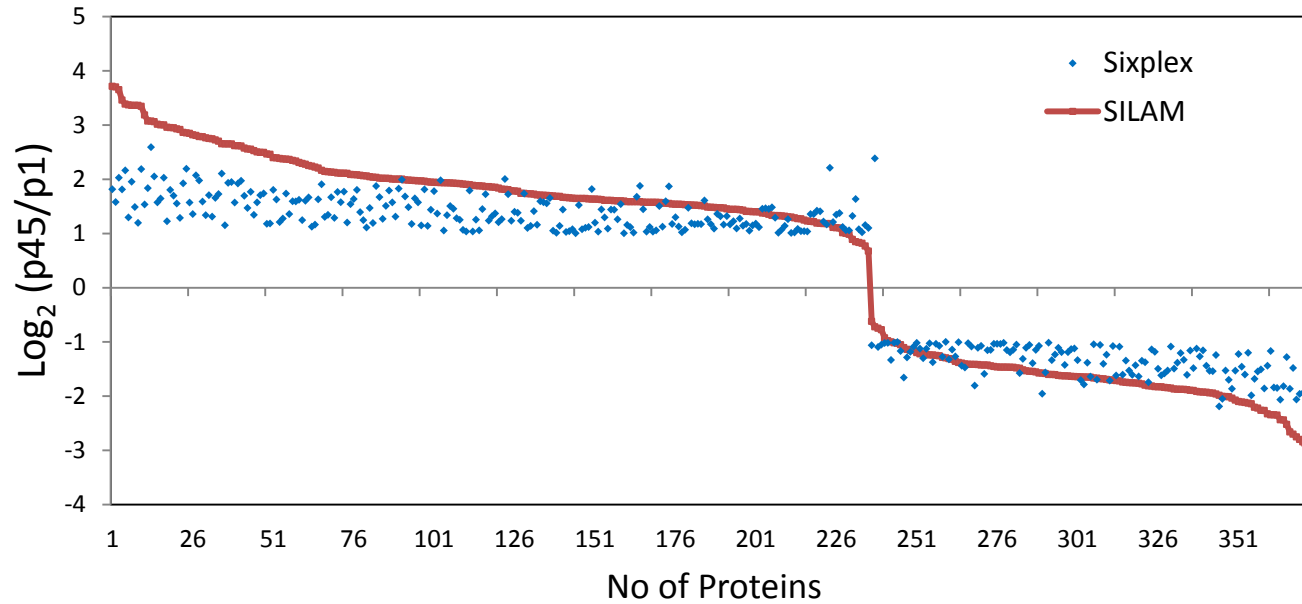


Supplementary Figure 4

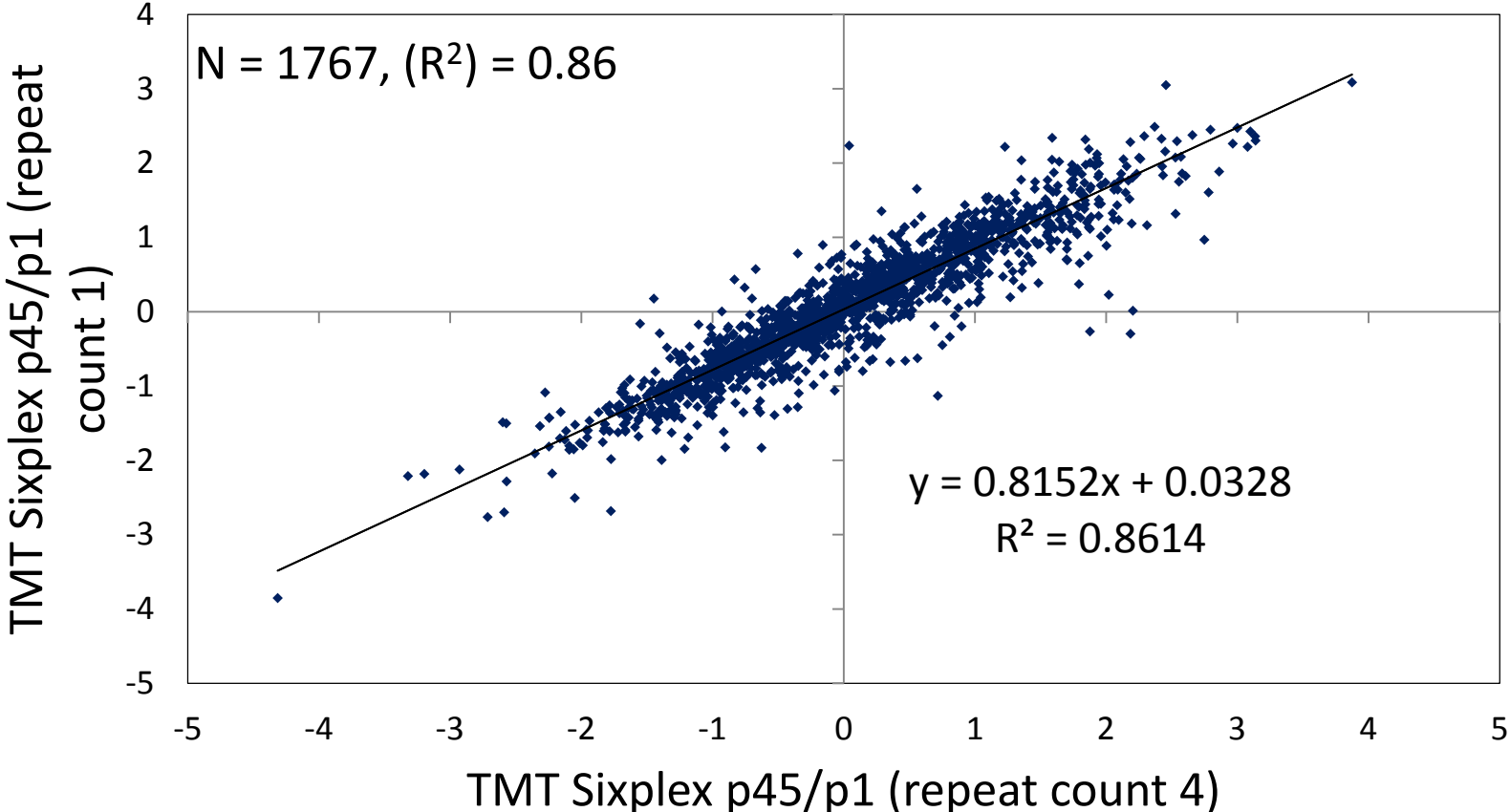


Supplementary Figure 5

Distribution of $p45/p1$ ratios ($-1.0 \leq \log_2(p45/p1) \leq +1.0$ and p value of < 0.05) for TMT sixplex or duplex with matching $p45/p1$ ratios (p value < 0.05) by SILAM approach



Supplementary Figure 6



Supplementary Figure 7

