Supporting Information

Block design with common reference samples enables robust large-scale label-free quantitative proteome profiling

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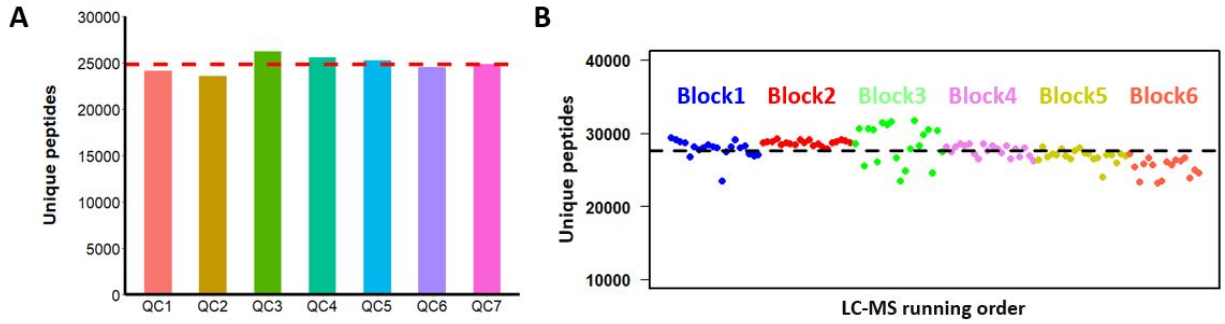
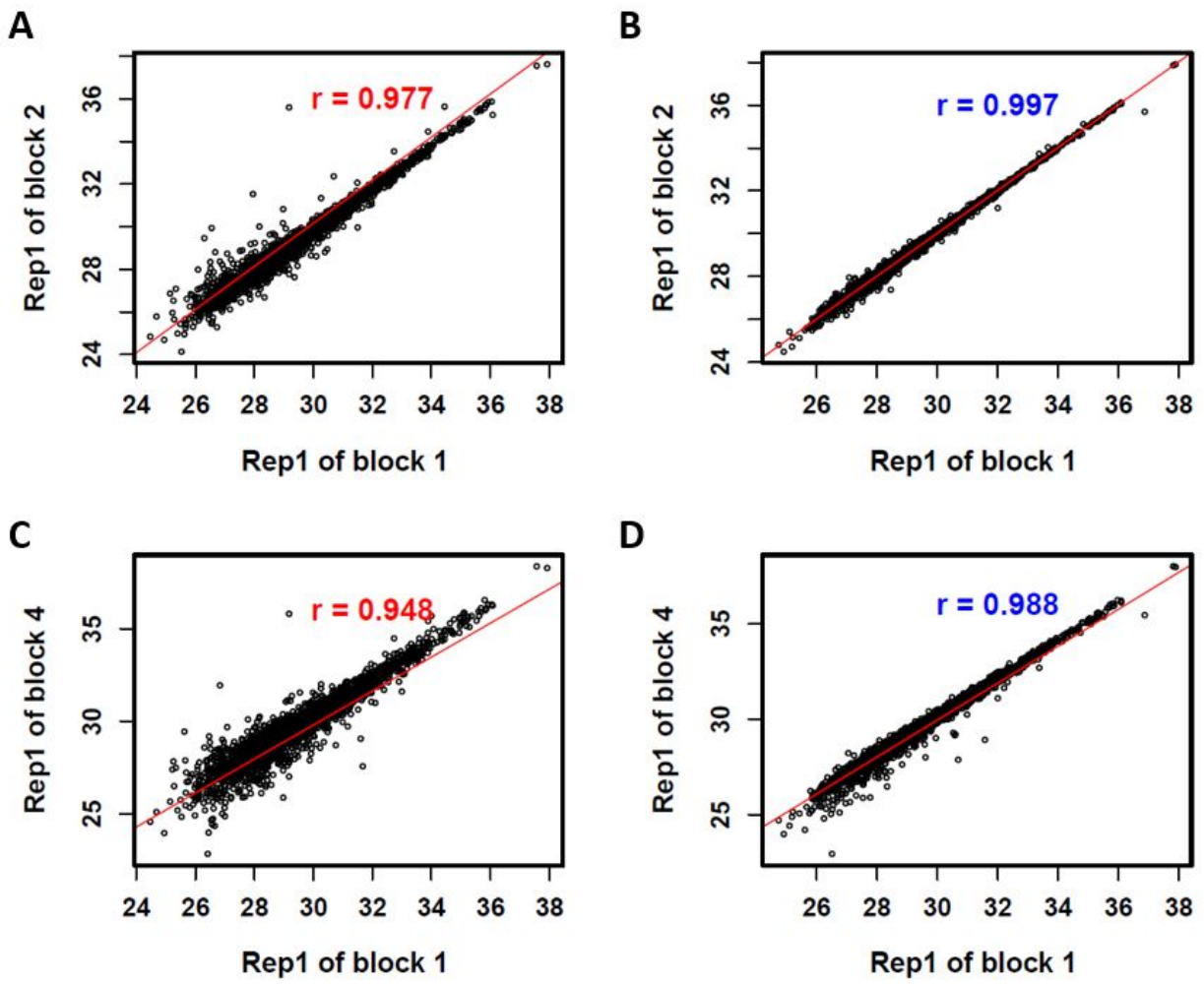
List of supplemental information

Supplemental Figure 1



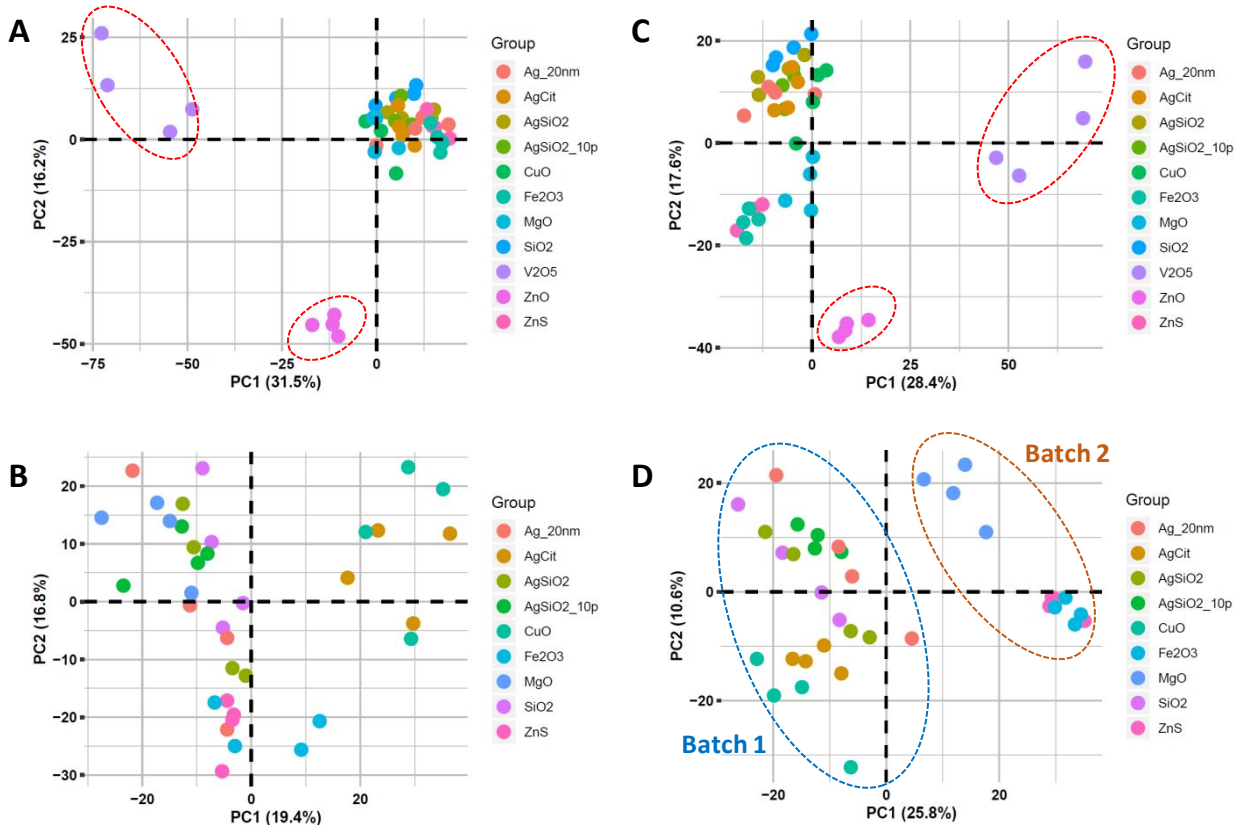
Supplemental Figure 1. Quality control metrics. (**A**) The number of unique peptides identified from quality control (QC) samples between each analytical block. (**B**) The number of unique peptides identified from six blocks according to the LC-MS running order.

Supplemental Figure 2



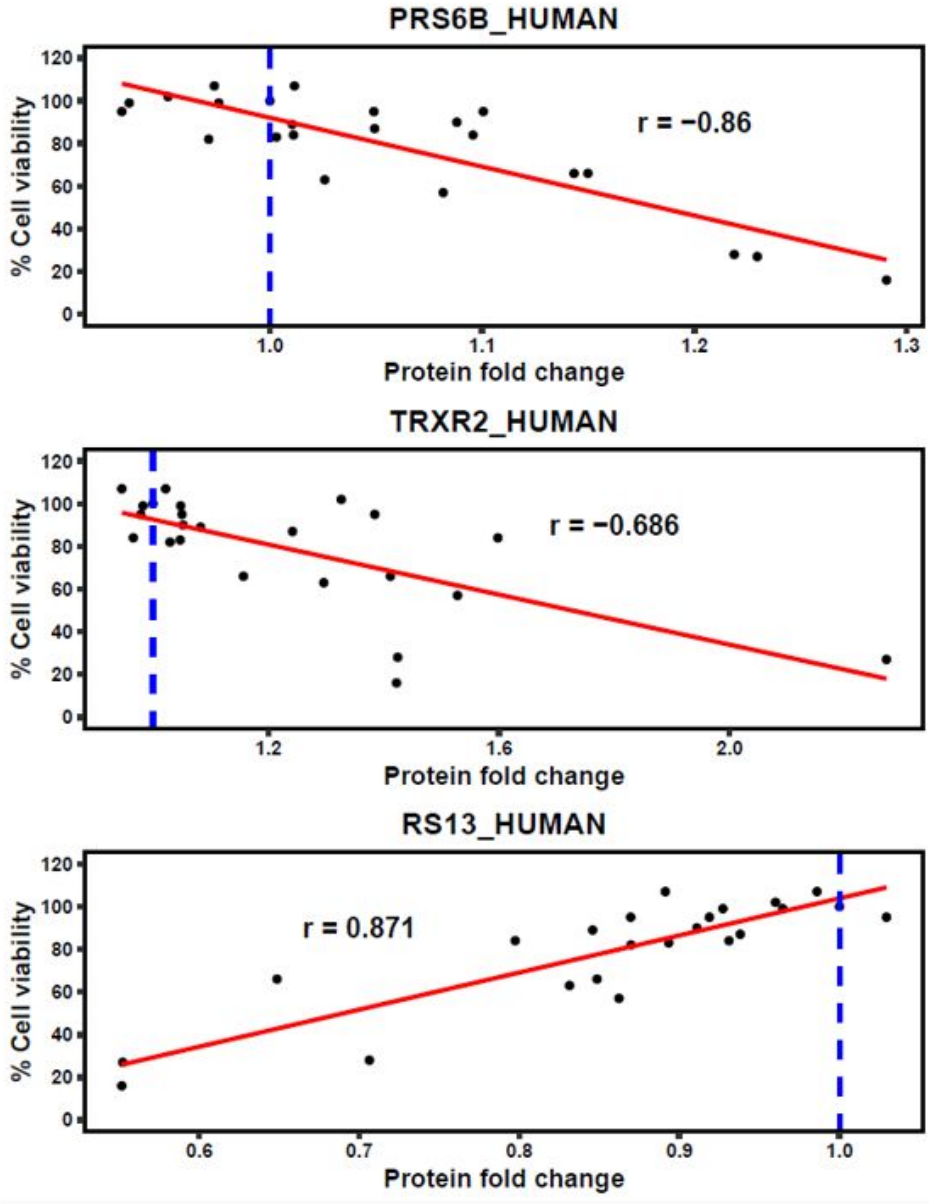
Supplemental Figure 2. Block correction improves quantification correlation among biological replicates. Correction of common reference (control) samples, either from the same processing batch but different blocks (A and B), or from different processing batch (C and D), were shown. Left panels (A and C) show the correlation before block correction, and right panels (B and D) show the correlation after block correction.

Supplemental Figure 3



Supplemental Figure 3. Quantification of all ENM-treated samples across six blocks readily distinguish non-cytotoxic and cytotoxic ENMs. (A) PCA plot of ENM-treated proteomes obtained from six blocks after common reference-based block correction. Note that the most cytotoxic ENMs, ZnO and V_2O_5 (highlighted in dashed red ovals) are readily separated from other samples. The remaining of the plot was zoomed in (B). (B) Moderately cytotoxic ENMs (CuO and AgCit, highlighted in dashed red ovals) are separated from the remaining ENMs. (C) PCA plot using dataset without common reference-based block correction. Note that highly toxic ENMs such as ZnO and V_2O_5 can still be separated from others, while the remaining ENMs are separated by processing batches, as shown in (D).

Supplemental Figure 4



Supplemental Figure 4. Reproducible quantification reveals potential protein markers for predicting biological impact of ENMs. Pearson correlations of control samples in six blocks before and after normalization. Three proteins whose protein abundance changes are highly correlated with cell viability following exposure to ENMs were shown. See **Supporting Data 1** for the full list. The methods and data on cell viability has been previously described.[1]

Supplemental Table 1

Table S1. Block assignment of sample groups for LC-MS

Processing batch	Block	Experimental Group[a] (n = 4 per group)				
1	1	Control	SiO2_L	SiO2_H	1%Ag_SiO_2_L	1%Ag_SiO_2_H
	2	Control	10%Ag_SiO_2_L	10%Ag_SiO_2_H	Ag_L	Ag_H
	3	Control	Ag_Cit_L	Ag_Cit_H	CuO_L	CuO_H
2	4	Control	V_2O_5_L	V_2O_5_H	ZnS_L	ZnS_H
	5	Control	Fe_2O_3_L	Fe_2O_3_H	ZnO_L	ZnO_H
	6[b]	Control	MgO_L	MgO_H	CuO_H	CuO

[a] L = 12.5 µg/ml; H = 25 µg/ml;

[b] Only one ENM at both dosages was tested; Four samples of CuO with high dosage (CuO_H) were included as a positive control for processing batch 2.

Supplemental Table 2

Table S2. Number of significant proteins using different controls

Block	ENM	Dosage	Controls from individual block[a]	Controls from Block 1[b]	Random Controls_run1 (B1_C3;B3_C2;B3_C4;B5_C4)[c]	Random Controls_run2 (B2_C1;B3_C1;B4_C1;B5_C3)	Random Controls_run3 (B2_C3;B3_C3;B5_C1;B5_C2)	Random Controls_run4 (B1_C1;B1_C3;B1_C4;B6_C4)	Random Controls_run5 (B1_C3;B1_C4;B2_C2;B2_C4)	Random Controls_run6 (B1_C1;B2_C2;B3_C3;B5_C3)
1	SiO2	Low	68	68	93	85	164	37	72	54
1	SiO2	High	106	106	126	107	203	82	133	93
1	1%Ag_SiO_2	Low	49	49	74	81	135	40	68	48
1	1%Ag_SiO_2	High	91	91	114	106	168	58	110	65
2	10%Ag_SiO_2	Low	51	174	79	38	67	100	42	37
2	10%Ag_SiO_2	High	185	266	127	87	140	180	150	119
2	Ag	Low	51	185	116	41	84	86	47	29
2	Ag	High	65	182	107	38	94	98	58	46
3	Ag_Cit	Low	167	289	121	101	217	206	223	173
3	Ag_Cit	High	249	248	155	136	204	172	204	141
3	CuO	Low	139	169	92	81	122	123	139	93
3	CuO	High	287	296	215	211	255	215	264	211
4	V_2O_5	Low	784	901	630	508	575	679	806	602
4	V_2O_5	High	1095	1126	966	862	913	966	1091	947
4	ZnS	Low	46	513	132	65	85	111	397	101
4	ZnS	High	16	560	155	33	32	105	437	126
5	Fe_2O_3	Low	186	532	217	89	136	167	428	140
5	Fe_2O_3	High	27	480	120	44	33	81	379	95
5	ZnO	Low	640	738	462	358	396	493	642	457
5	ZnO	High	859	929	713	613	594	743	866	695
6	MgO	Low	182	535	219	111	110	167	423	181
6	MgO	High	262	499	235	121	177	208	446	190

[a]: Control samples in individual block was used for student t test; significant proteins were defined as these with $p < 0.01$. The same criteria were applied for other comparisons.

[b]: Control samples from Block 1 were used for all comparisons. Note that if control samples are only presented in the first block, systematic variation among blocks could result in false positive results.

[c]: Four control samples (indicated in parenthesis) were randomly selected from 24 controls across all six blocks. Six runs were performed and the results showed that variations among controls could either overestimate or underestimate the proteome responses.

Supporting Data 1

See *Supporting Data 1.xlsx* for detail.

Supporting Data 2

See *Supporting Data 2.xlsx* for detail.

Supporting Data 3

See *Supporting Data 3.xlsx* for detail.

References:

[1] Zhang T, Gaffrey MJ, Thomas DG, Weber TJ, Hess BM, Weitz KK, Piehowski PD, Petyuk VA, Moore RJ, Qian W-J, Thrall BD (2020) A proteome-wide assessment of the oxidative stress paradigm for metal and metal-oxide nanomaterials in human macrophages. *NanoImpact* **17:** 100194. .