Table S1. Characterization of MT reference peptides. Eleven of twelve human MT reference peptides were custom-synthesized from a commercial source. Initial purity was assessed by rpHPLC. Each was alkylated with  $^{15}N$  iodoacetamide and further purified > 95% as described in Experimental Procedures. An equimolar solution of each purified, alkylated peptide ( $\sim$ 0.25 µg, or 110 pmol) was analyzed by rpHPLC and relative ionization intensities were compared.

Isoform	Initial Purity	Total	Normalized	
isolorm	(%)	Ionization	Ionization	
MT-1A	11.0	110517	0.34	
MT-1B	34.7	273032	0.84	
MT-1E	44.9	273032	0.84	
MT-1F	12.8	163048	0.50	
MT-1G1	failed			
MT-1G2	10.2	138968	0.43	
MT-1H	17.7	234697	0.72	
MT-1L	34.5	294156	0.90	
MT-1M	25.7	126377	0.39	
MT-1X	90.3	325968	1.00	
MT-2	73.3	154424	0.47	
MT-3	73.3	265430	0.81	

Table S2. Quantitative real time PCR measurements of MT isoform mRNA in control and  $Cd^{2+}$ -induced HK-2(MT-3) cells. Values are expressed as MT transcripts per  $10^6$  18S rRNA transcripts and represent the mean  $\pm$  S.D. of three biological replicates. N.D. indicates not detected.

Isoform	Control	Cd <sup>2+</sup> -treated	Fold Induction	
MT-1A	N.D.	N.D.		
MT-1B	N.D.	N.D.		
MT-1E	107.8 ± 25.9	326.5 ± 57.2	3	
MT-1F	F-1F 2.7±0.4 8.5±2.1		3.1	
MT-1G	2.7 ± 0.3	13.5 ± 0.8	4.9	
MT-1H	N.D.	N.D.		
MT-1M	2.1 ± 0.6	3.1 ± 0.1	1.5	
MT-1X	6.9 ± 2.3	35.1 ± 8.4	5.1	
MT-2	35.2 ± 9.1	109.0 ± 26.5	3.1	
MT-3	1.3 ± 0.2	2.2 ± 0.2	1.7	
MT-L	N.D.	N.D.		

Table S3. Mass spectrometry determination of absolute protein levels for MT isoforms in control and  $Cd^{2+}$ -induced HK-2(MT-3) cells. Values are expressed as ng of MT isoform per  $\mu g$  of cytosolic protein and represent the mean  $\pm$  S.D. of three biological replicates. N.D. indicates not detected.

Isoform	Control	Cd <sup>2+</sup> -treated	Fold Induction	
MT-1A	N.D.	N.D.		
MT-1B	N.D.	N.D.		
MT-1E	0.4150 ± .0181	2.4364 ± .1187	5.9	
MT-1F	0.1083 ± .0030	1.0742 ± .1912	9.9	
MT-1G1	N.D.	N.D.		
MT-1G2	0.1513 ± .0212	0.8253 ± .1717	5.5	
MT-1H	N.D.	N.D.		
MT-1L	N.D.	N.D.		
MT-1M	N.D.	0.3757 ± .1257		
MT-1X	0.3486 ± .0639	1.6396 ± .2841	4.7	
MT-2	1.6955 ± .2169	7.2757 ± .5457	4.3	
MT-3	0.6013 ± .0431	1.0544 ± .1550	1.8	

Table S4. Quantitative real time PCR measurements of MT isoform mRNA in human breast epithelial cell lines. Only transcripts detected in at least one cell line are shown. Values are expressed as MT transcripts per  $10^6$  18S rRNA transcripts and represent the mean  $\pm$  S.D. of three biological replicates. N.D. indicates not detected.

cell line	tumorogenic	ER status	MT-1X	MT-1E	MT-2A
MCF-7	yes	+	1.6 ± 0.6	N.D.	5.8 ± 3.0
T-47D	yes	+	0.3 ± 0.2	N.D.	1.2 ± 0.9
MCF-10A	no	-	6.5 ± 0.2	27.2 ± 6.8	22.0 ± 04.8
MDA-MB-231	yes	-	4.4 ± 0.9	51.3 ± 33.8	67.0 ± 17.0
Hs578T	yes	-	13.1 ± 4.0	53.5 ± 7.5	118.3 ± 13.8

Table S5. Mass spectrometry-based determination of absolute protein levels for MT isoforms in human breast epithelial cell lines. Only isoforms detected in at least one cell line are shown. Values are expressed as ng of MT isoform per  $\mu g$  of cytosolic protein and represent the mean  $\pm$  S.D. of three biological replicates. N.D. indicates not detected.

cell line	tumorogenic	tumorogenic ER status MT-1		MT-1E	MT-2A
MCF-7	yes	+	0.0754 ± .0079	N.D.	0.1354 ± .0520
T-47D	yes	+	0.0500 ± .0020	N.D.	0.1350 ± .0409
MCF-10A	yes	-	0.0663 ± .0188	0.0707 ± .0130	0.5022 ± .1556
MDA-MB-231	yes	-	0.1602 ± .0172	0.3387 ± .0956	1.4163 ± .2516
Hs578T	no	-	0.2888 ± .0564	0.2441 ± .1003	1.9436 ± .5578

## **Supplemental Figures**

Figure S1. MS/MS of synthetic, <sup>15</sup>N-iodoacetamide-labeled N-terminal MT peptides. All peptides are N-terminally acetylated. (A) MT-1A. (B) MT-1B. (C) MT-1E. (D) MT-1F. (E) MT-1G2. (F) MT-1H. (G) MT-1L. (H) MT-1M. (I) MT-1X. (J) MT-2. (K) MT-3.

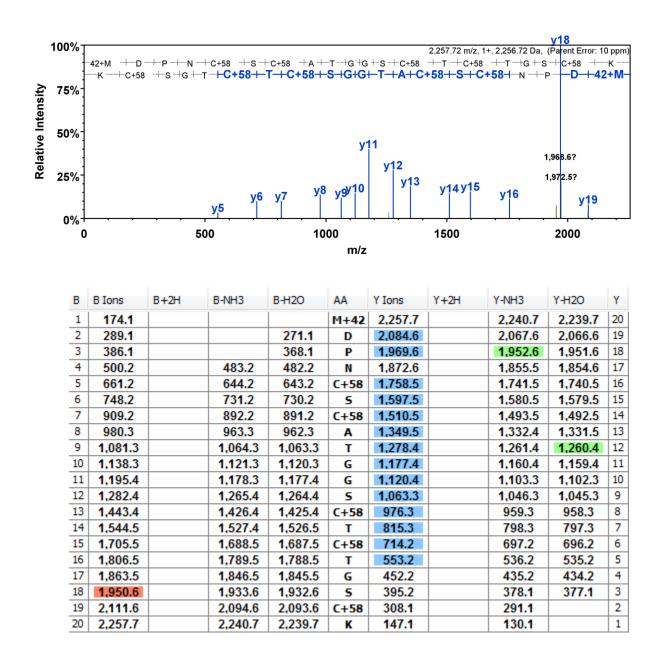
**Figure S2**. **MS/MS of endogenous N-terminal MT peptides**. All peptides are N-terminally acetylated and alkylated with <sup>14</sup>N-iodoacetamide. (A) MT-1E. (B) MT-1F. (C) MT-1G1. (D) MT-1G2. (E) MT-1H. (F) MT-1M. (G) MT-1X. (H) MT-2, (I) MT-3.

Figure S3. Enrichment of MT peptides by SCX chromatography. Trypsin-digested Hs578T cell lysate with ~100 pmol/isoform pure acetylated N-term MT peptide standards was subjected to SCX chromatography as described in Experimental Procedures. The bracketed region (7-21 min; ~20-25 mM NaCl) is enriched with the acetylated N-terminal tryptic MT peptides along with other weakly interacting peptides. The bulk of tryptic peptides elute at higher ionic strength.

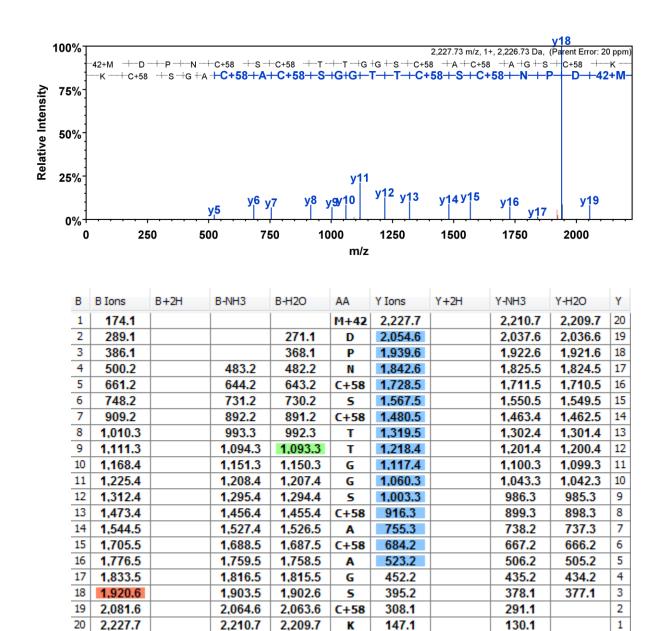
Figure S4. Example of conditions generating truncated MT peptides. The SCX fraction analyzed in Figure 2 was stored for six weeks at  $4^{\circ}$ C. The sample was treated with dimethyl sulfide to reverse methionine oxidation. Some residual oxidation (ox) was observed. The extracted ion chromatogram includes all precursor intensities between 1910 and 2310 m/z. Additional peaks appeared that were not present in the original assay including some MT peptides truncated at the Asp-Pro bond ( $\Delta$ ). This cleavage was attributed to storage in SCX buffer, which contained 0.1% formic acid. Dimethyl sulfide treatment did not generate these

truncations (see Figure 2). Samples in which SCX fractions were promptly treated with dimethyl sulfide and fractionated by rpHPLC showed very little formation of truncated peptides (see Figure 4). The remaining unlabeled peaks are predominantly isobaric forms of the parent MT peptide peaks and are attributed to slow isomerization of prolyl peptide bonds. Conditions for minimizing this additional source of ion complexity among N-terminal MT peptides has not been fully addressed yet. MT isoforms are color-coded as in Figure 4. (A) Endogenous <sup>14</sup>N-labeled peptides; maximum peak intensity 1.3 x 10<sup>6</sup>. (B) The <sup>15</sup>N-labeled MT-3 reference peptide; maximum peak intensity 6.1 x 10<sup>5</sup>.

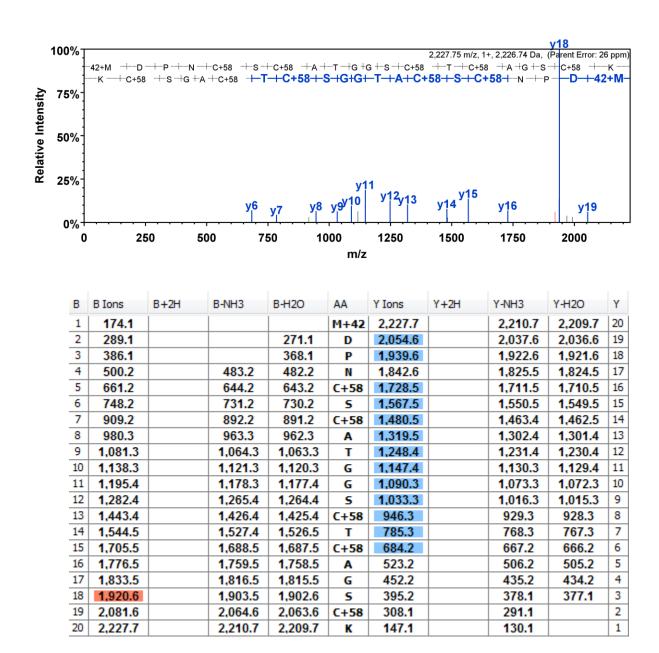
**Figure S5. Peptide mass fingerprints of RP-HPLC fractions containing N-terminal acetylated MT peptides.** Mass spectra of precursor ions described in Figure 4 are shown here in an expanded mass range. Asterisks indicated the endogenous <sup>14</sup>N-labeled MT peptides. The corresponding <sup>15</sup>N-labeled reference peptides are shifted by five Da. The MH<sup>1+</sup> masses for each peptide are listed in Table 1. Peptide mass fingerprints are from (A) untreated (B) or Cd<sup>2+</sup>-treated HK-2 cells as described in Figure 4.



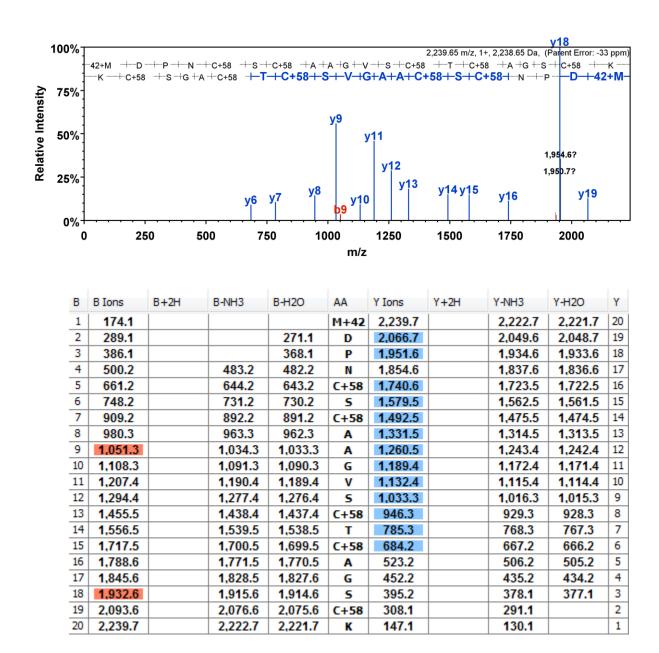
**Figure S1A**. MS/MS of synthetic acetylated (<sup>15</sup>N) MT-1A N-terminal peptide. Mascot ion score = 141.5.



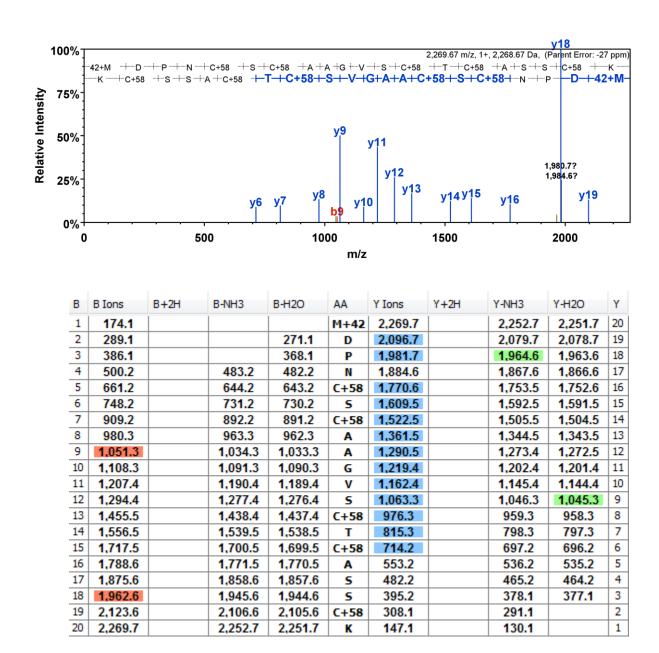
**Figure S1B**. MS/MS of synthetic acetylated (<sup>15</sup>N) MT-1B N-terminal peptide. Mascot ion score = 162.6.



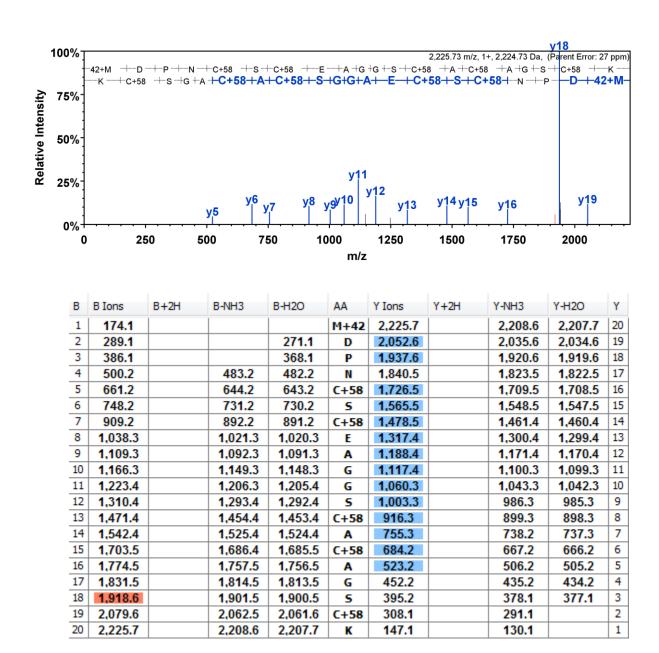
**Figure S1C**. MS/MS of synthetic acetylated (<sup>15</sup>N) MT-1E N-terminal peptide. Mascot ion score = 127.7.



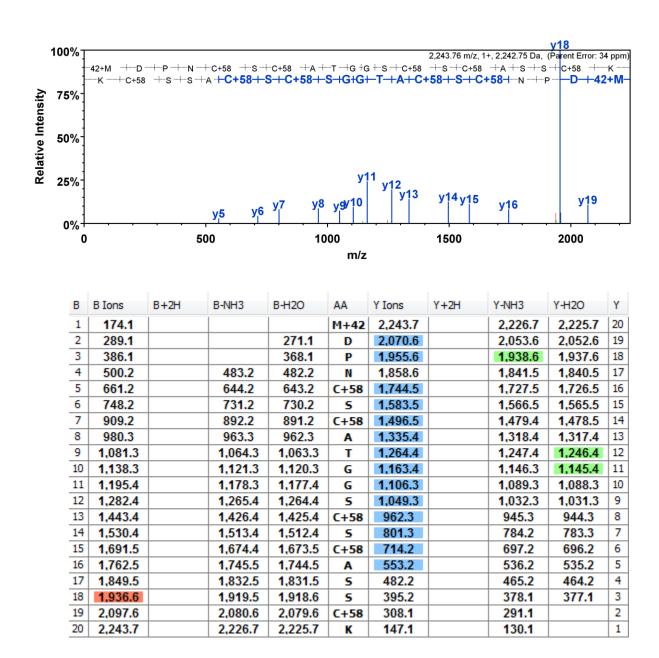
**Figure S1D**. MS/MS of synthetic acetylated (<sup>15</sup>N) MT-1F N-terminal peptide. Mascot ion score = 127.6.



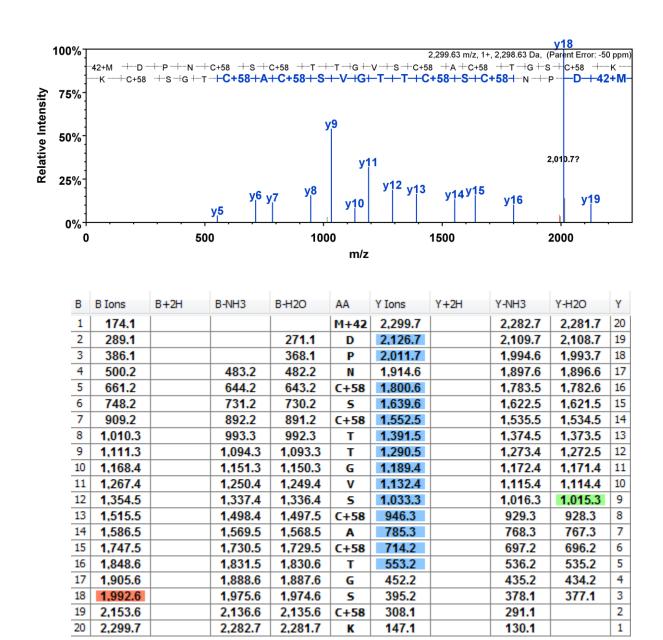
**Figure S1E**. MS/MS of synthetic acetylated (<sup>15</sup>N) MT-1G2 N-terminal peptide. Mascot ion score = 133.9.



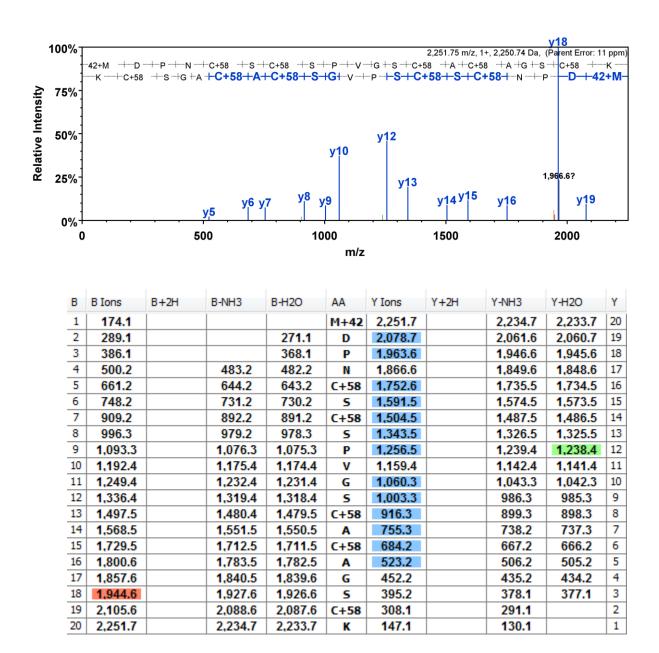
**Figure S1F**. MS/MS of synthetic acetylated (<sup>15</sup>N) MT-1H N-terminal peptide. Mascot ion score = 141.4.



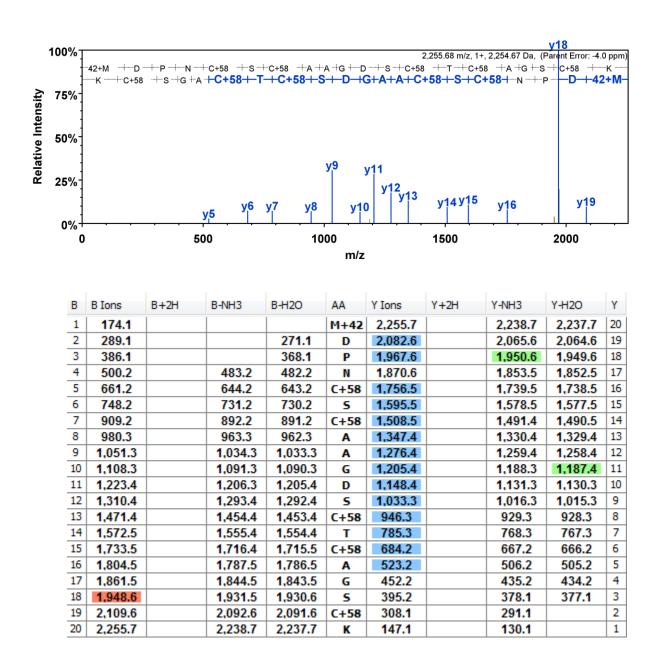
**Figure S1G**. MS/MS of synthetic acetylated (<sup>15</sup>N) MT-1L N-terminal peptide. Mascot ion score = 142.5.



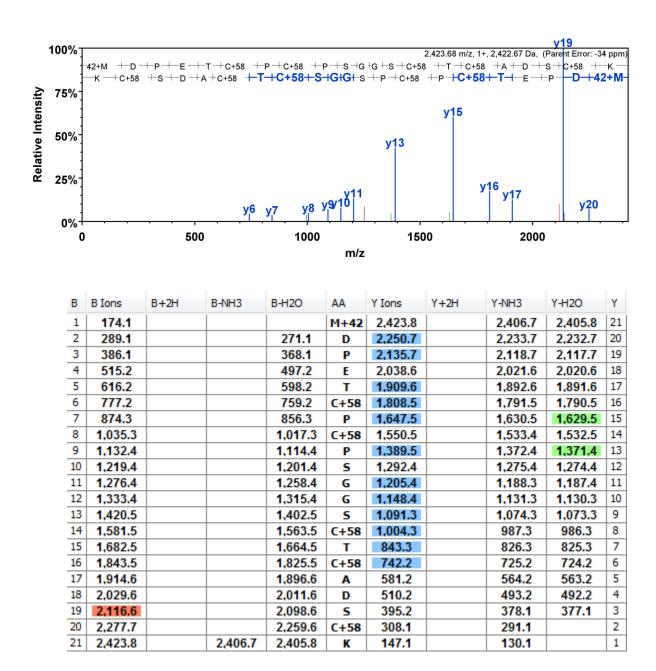
**Figure S1H**. MS/MS of synthetic acetylated (<sup>15</sup>N) MT-1M N-terminal peptide. Mascot ion score = 155.5.



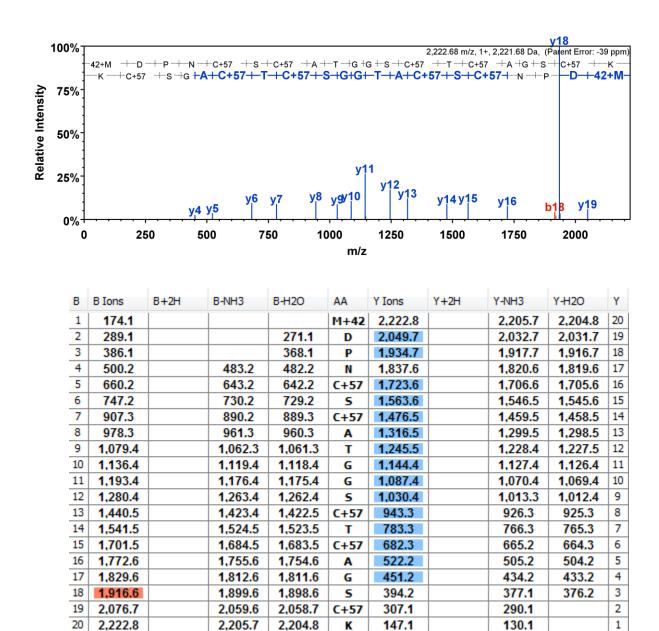
**Figure S1I**. MS/MS of synthetic acetylated (<sup>15</sup>N) MT-1X N-terminal peptide. Mascot ion score = 141.6.



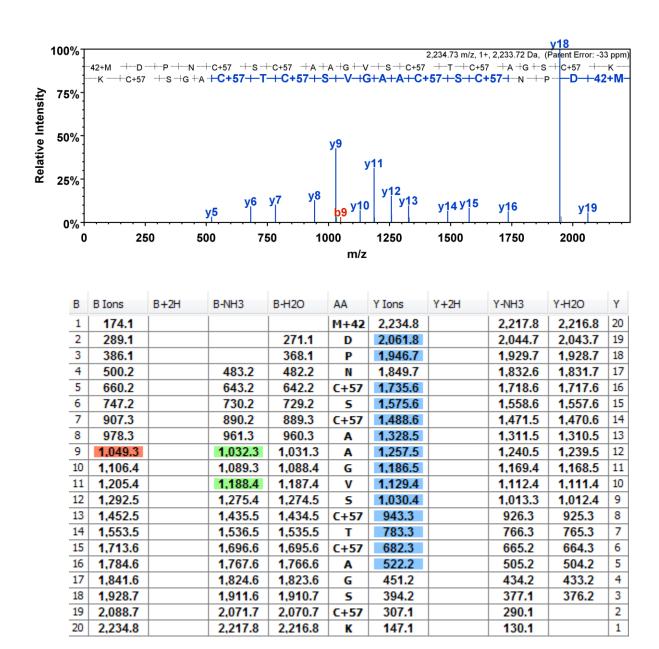
**Figure S1J**. MS/MS of synthetic acetylated (<sup>15</sup>N) MT-2 N-terminal peptide. Mascot ion score = 141.6.



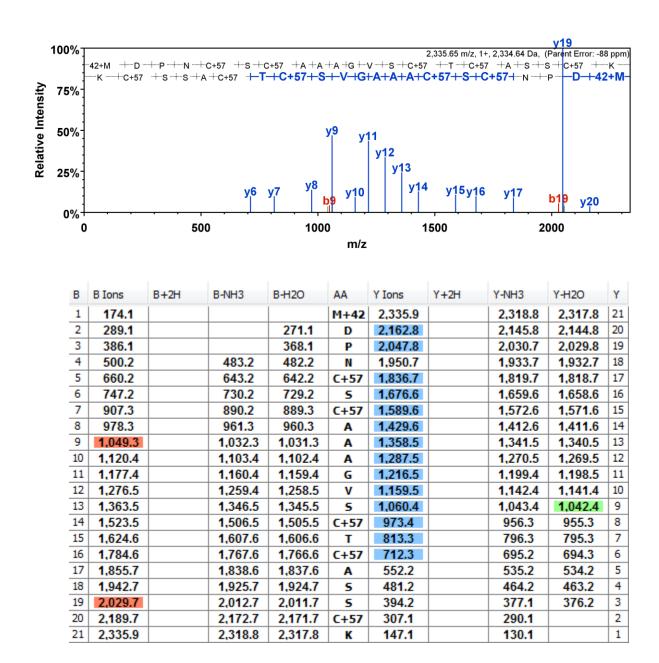
**Figure S1K**. MS/MS of synthetic acetylated (<sup>15</sup>N) MT-3 N-terminal peptide. Mascot ion score = 108.7.



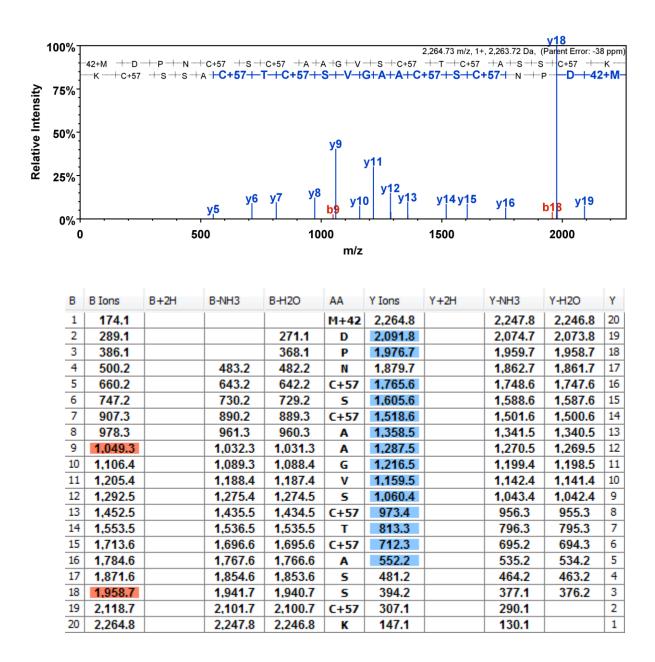
**Figure S2A**. MS/MS of endogenous acetylated ( $^{14}$ N) MT-1E N-terminal peptide. Mascot ion score = 162.6.



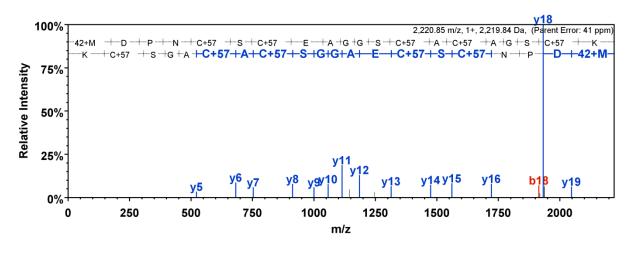
**Figure S2B**. MS/MS of endogenous acetylated (<sup>14</sup>N) MT-1F N-terminal peptide. Mascot ion score = 141.8.



**Figure S2C**. MS/MS of endogenous acetylated ( $^{14}$ N) MT-1G1 N-terminal peptide. Mascot ion score = 140.2.

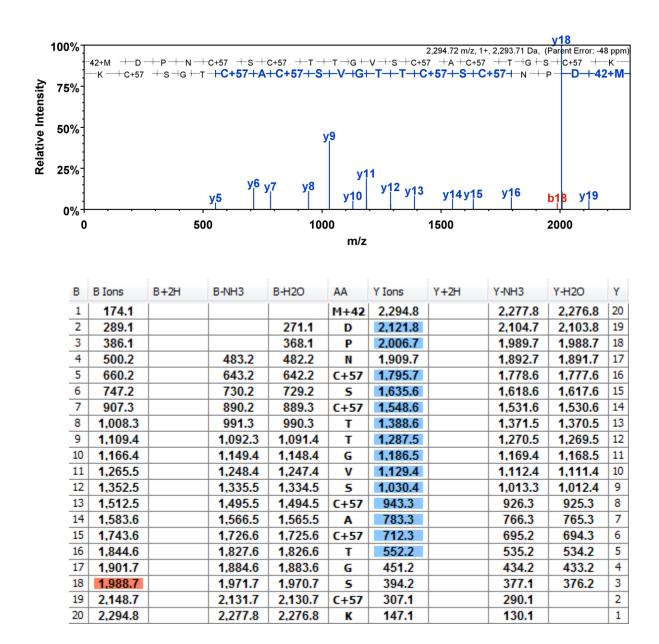


**Figure S2D**. MS/MS of endogenous acetylated (<sup>14</sup>N) MT-1G2 N-terminal peptide. Mascot ion score = 141.7.

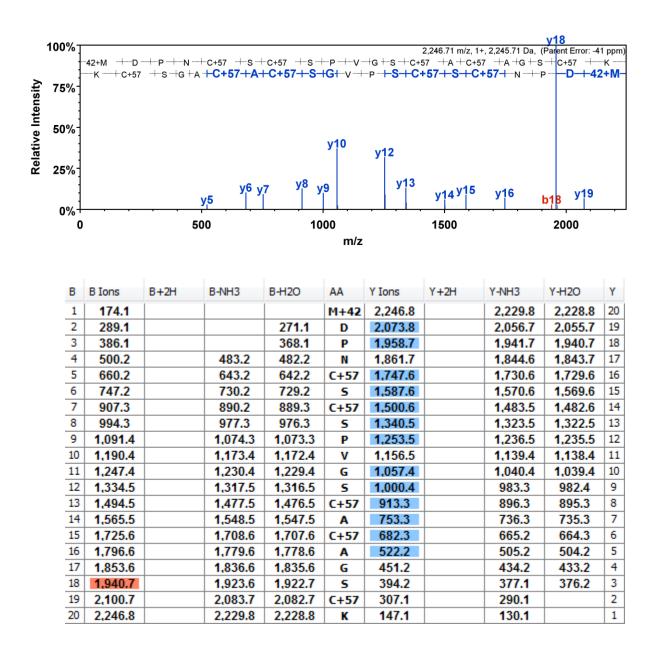


В	B Ions	B+2H	B-NH3	B-H2O	AA	Y Ions	Y+2H	Y-NH3	Y-H2O	Υ
1	174.1		1		M+42	2,220.8		2,203.7	2,202.7	20
2	289.1			271.1	D	2,047.7		2,030.7	2,029.7	19
3	386.1			368.1	P	1,932.7		1,915.6	1,914.7	18
4	500.2		483.2	482.2	N	1,835.6		1,818.6	1,817.6	17
5	660.2		643.2	642.2	C+57	1,721.6		1,704.6	1,703.6	16
6	747.2		730.2	729.2	5	1,561.5		1,544.5	1,543.5	15
7	907.3		890.2	889.3	C+57	1,474.5		1,457.5	1,456.5	14
8	1,036.3		1,019.3	1,018.3	E	1,314.5		1,297.5	1,296.5	13
9	1,107.4		1,090.3	1,089.3	Α	1,185.4		1,168.4	1,167.4	12
10	1,164.4		1,147.3	1,146.4	G	1,114.4		1,097.4	1,096.4	11
11	1,221.4		1,204.4	1,203.4	G	1,057.4		1,040.4	1,039.4	10
12	1,308.4		1,291.4	1,290.4	5	1,000.4		983.3	982.4	9
13	1,468.5		1,451.4	1,450.4	C+57	913.3		896.3	895.3	8
14	1,539.5		1,522.5	1,521.5	Α	753.3		736.3	735.3	7
15	1,699.5		1,682.5	1,681.5	C+57	682.3		665.2	664.3	6
16	1,770.6		1,753.5	1,752.6	A	522.2		505.2	504.2	5
17	1,827.6		1,810.6	1,809.6	G	451.2		434.2	433.2	4
18	1,914.6		1,897.6	1,896.6	5	394.2		377.1	376.2	3
19	2,074.6		2,057.6	2,056.6	C+57	307.1		290.1		2
20	2,220.8		2,203.7	2,202.7	K	147.1		130.1		1

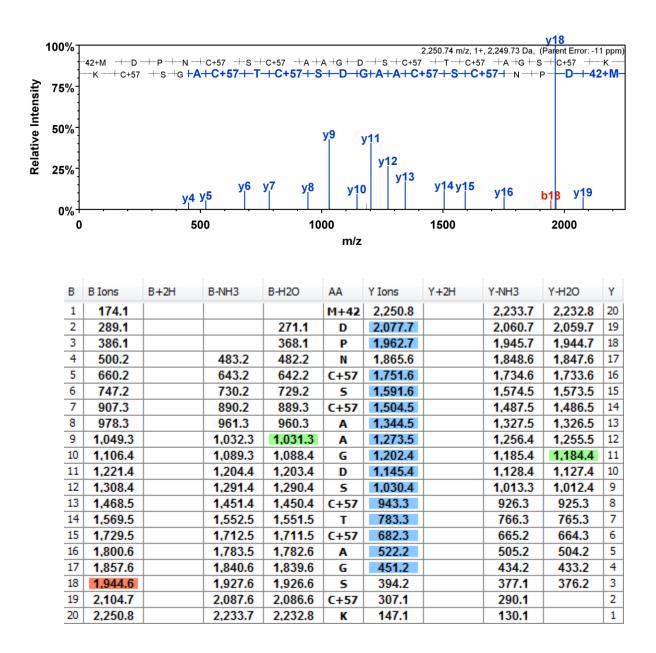
**Figure S2E**. MS/MS of endogenous acetylated (<sup>14</sup>N) MT-1H N-terminal peptide. Mascot ion score = 143.6.



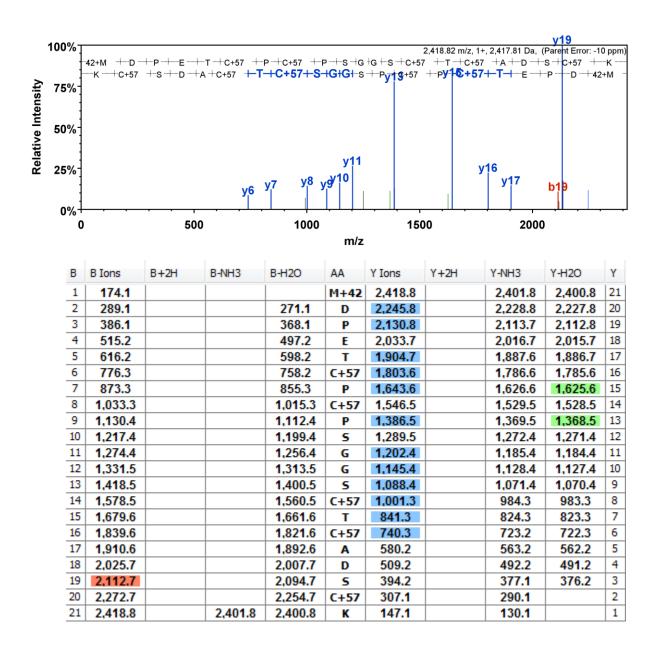
**Figure S2F**. MS/MS of endogenous acetylated (<sup>14</sup>N) MT-1M N-terminal peptide. Mascot ion score = 155.7.



**Figure S2G**. MS/MS of endogenous acetylated (<sup>14</sup>N) MT-1X N-terminal peptide. Mascot ion score = 141.8.



**Figure S2H**. MS/MS of endogenous acetylated (<sup>14</sup>N) MT-2 N-terminal peptide. Mascot ion score = 156.5.



**Figure S2I**. MS/MS of endogenous acetylated (<sup>14</sup>N) MT-3 N-terminal peptide. Mascot ion score = 88.9.

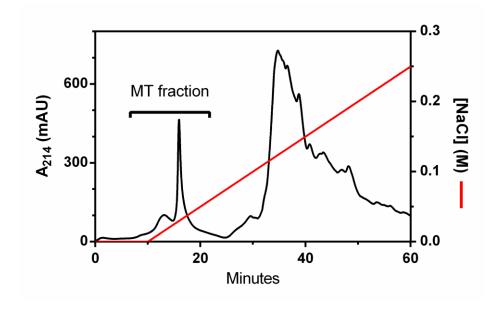
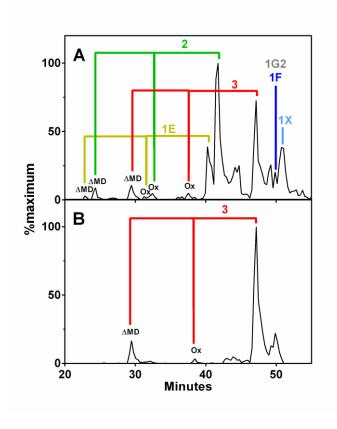


Figure S3. Enrichment of MT N-terminal peptides by SCX chromatography.



**Figure S4**. Generation of truncated N-terminal MT peptides by extended incubation in 0.1% formic acid.

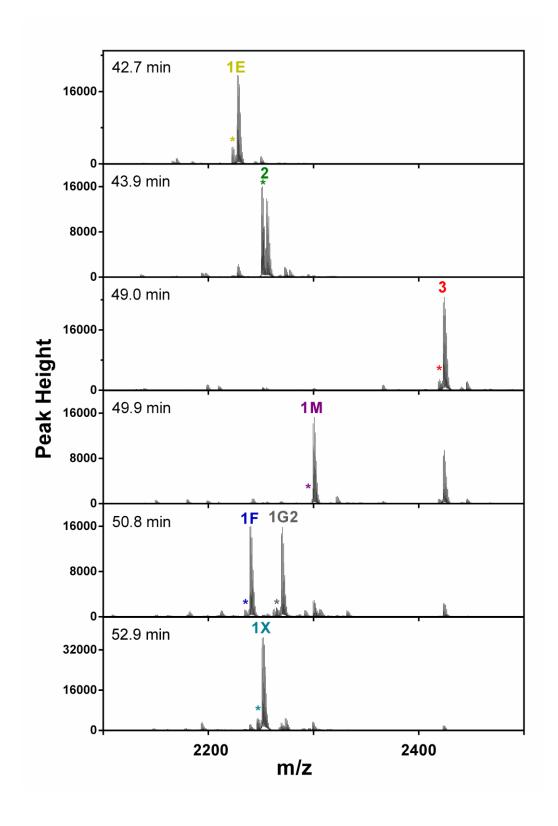
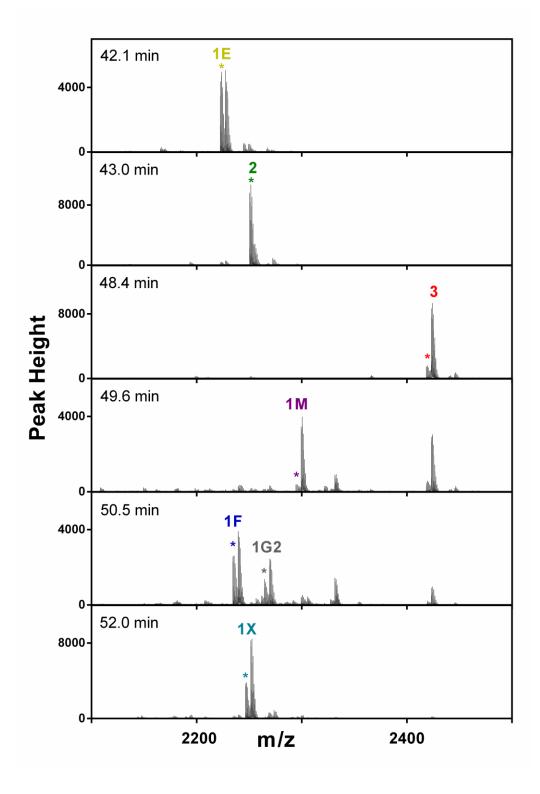


Figure S5A. Peptide mass fingerprints from untreated HK-2 cells.



**Figure S5B**. Peptide mass fingerprints from Cd<sup>2+</sup>-treated HK-2 cells.