Supplementary Table S1

Spectral multiplicity in HeLa triplicate experiment.

Multiplicity	*No. of Spectra*	*Frequency*
1	59380	36.82%
2	38637	23.96%
3	25646	15.90%
4	16400	10.17%
5	9915	6.15%
6	5632	3.49%
7	3033	1.88%
8	1466	0.91%
9	696	0.43%
10	280	0.17%
11	113	0.07%
12	37	0.02%
13	11	0.01%
14	7	0.00%
15	4	0.00%

Supplementary Table S2

Lists of DeMix identifications: unique peptides and protein groups (*in a separate Excel spreadsheet*)

Supplementary Table S3

Matching fragments and ion intensities in the primary and the secondary spectra in **Fig. S4**.

Fragment	*m/z*	*Secondary intensity*	*Primary intensity*
y1	147.113	1916274	2305851
b2	157.097	1590674	2601578
y2	204.135	3127053	641319
b3	270.182	6706185	7613953
y3	305.183	279984	438165
y4	362.203	237962	0
b4	383.266	946546	979980
y5	459.257	1592772	1526405
y6	556.310	5754106	5834155
y7	613.331	7312736	6658751
y8	776.394	5916213	6468980
y9	889.484	2739208	2045044
y10	1002.561	137281	401425

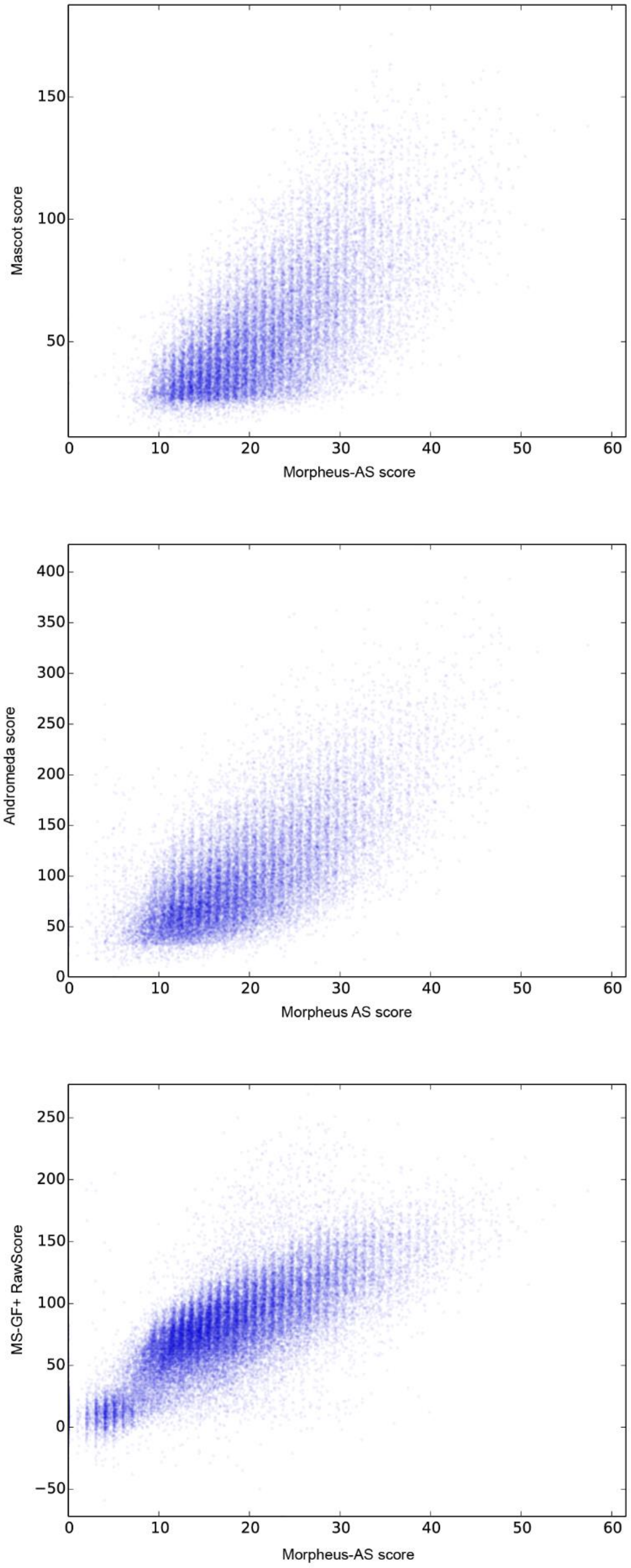
Supplementary Table S4

The effect of the input type, deisotoping, software lock mass and precursor mass shift on the number of peptides identified at 1% FDR

	Input type	*Deisotope*	*Lock-mass*	*MS1 tolerance*	*MS2 tolerance*	*Clone*	*PSM*	*Peptide*
Morpheus	Raw	No	No	10 ppm	20 ppm	No	78,333	20,594
Morpheus	Raw	No	No	6 ppm	16 ppm	No	90,307	22,065
Morpheus	mzML	Yes	No	20 ppm	20 ppm	No	99,278	23,264
Morpheus	mzML	Yes	Yes	10 ppm	20 ppm	No	106,826	28,303
Morpheus-AS	mzML	Yes	Yes	Posterior	Posterior	No	122,208	33,712
DeMix (Morpheus-AS)	mzML	Yes	Yes	Posterior	Posterior	Yes	200,292	41,628
Morpheus	artificial mzML (+30 ppm)	Yes	No	10 ppm	20 ppm	No	767	428
Morpheus	artificial mzML (+3 Da)	Yes	No	10 ppm	20 ppm	No	46	12
Morpheus	artificial mzML (random)	Yes	No	10 ppm	20 ppm	No	28	28
Morpheus	artificial mzML (merge)	Yes	No	10 ppm	20 ppm	No	603	311

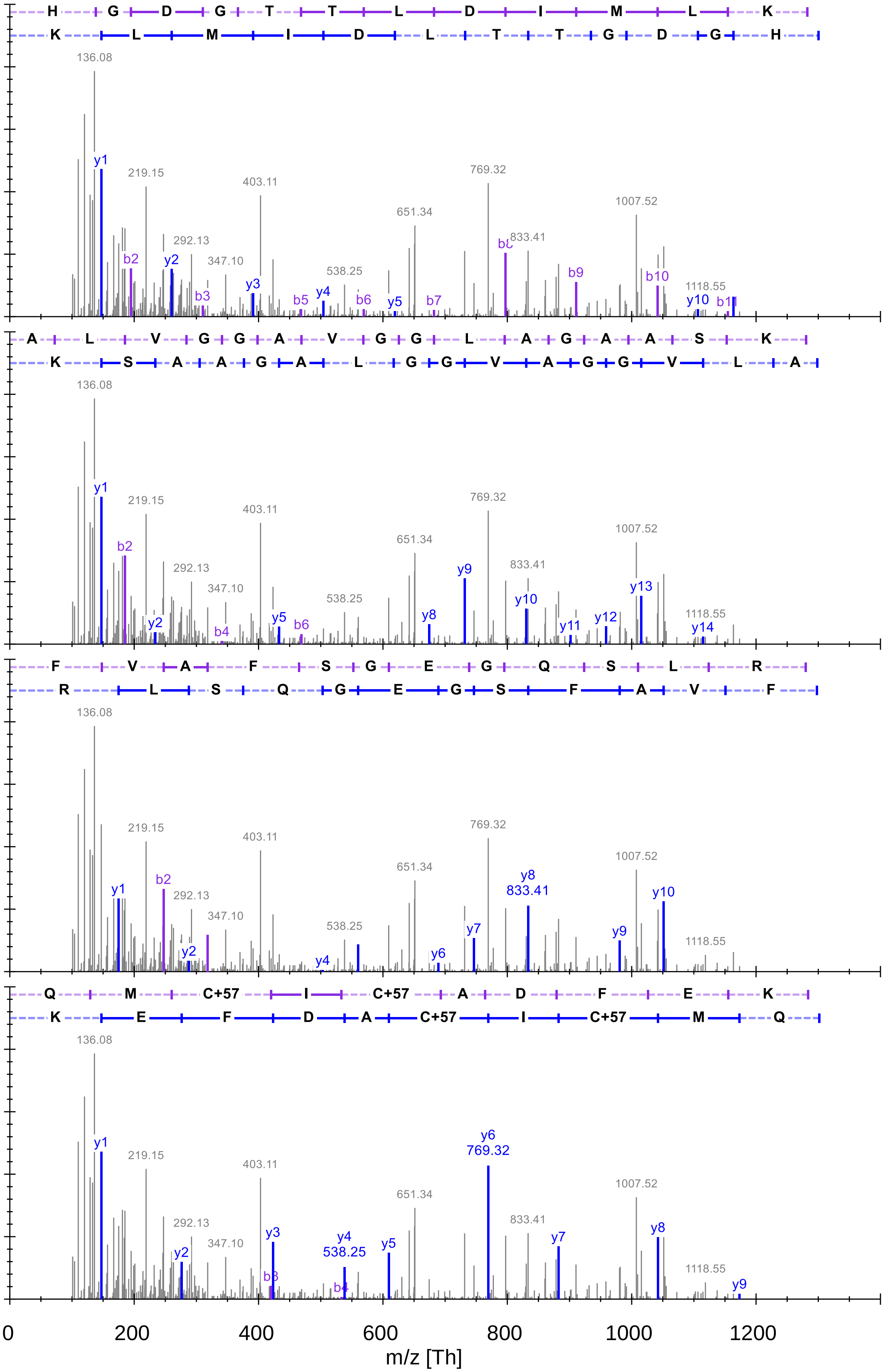
Supplementary Figure S1

Comparisons of Morpheus AS score to scores of Mascot, Andromeda and MS-GF+.



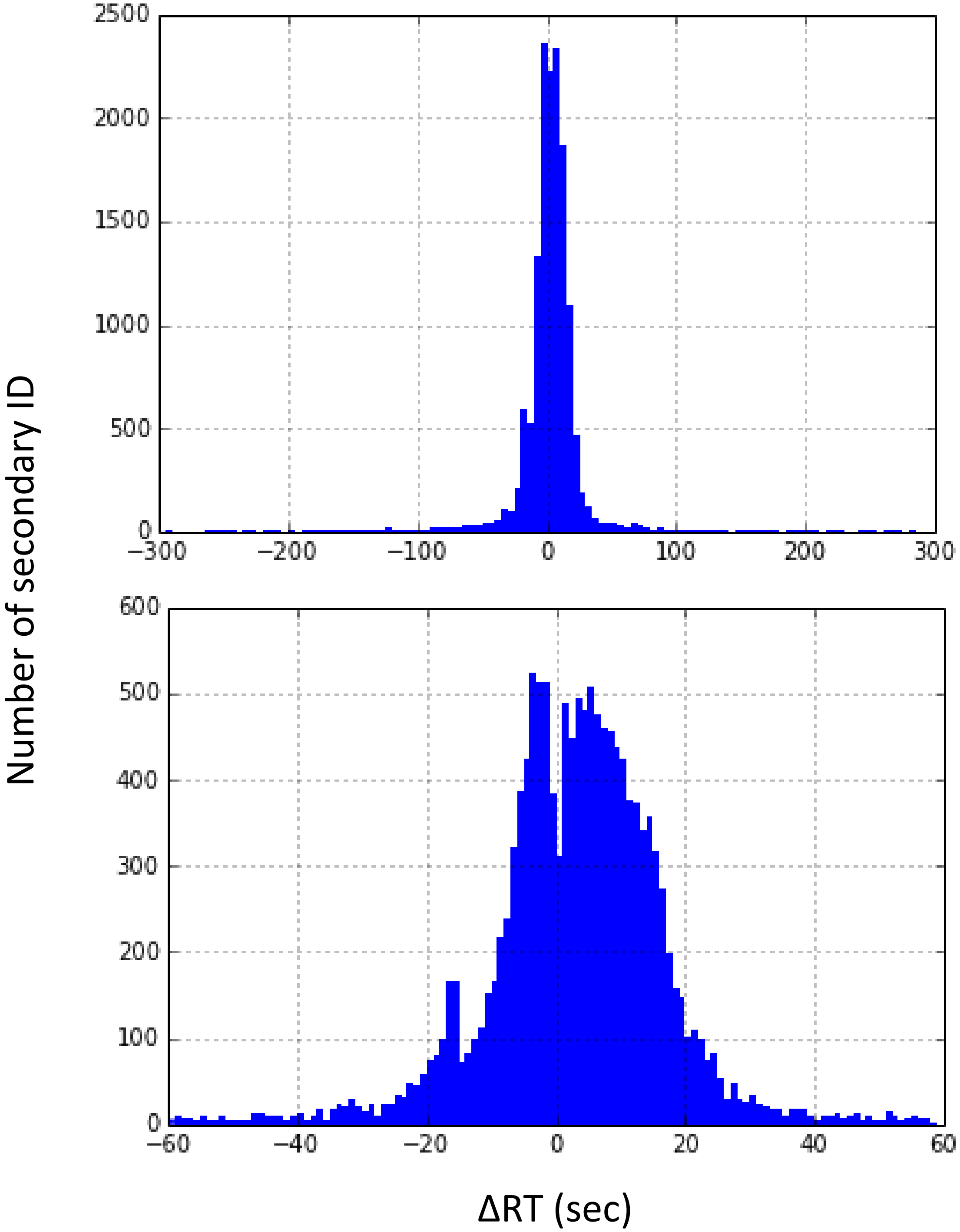
Supplementary Figure S2

Example of four peptides annotated in a chimeric MS/MS spectrum.



Supplementary Figure S3

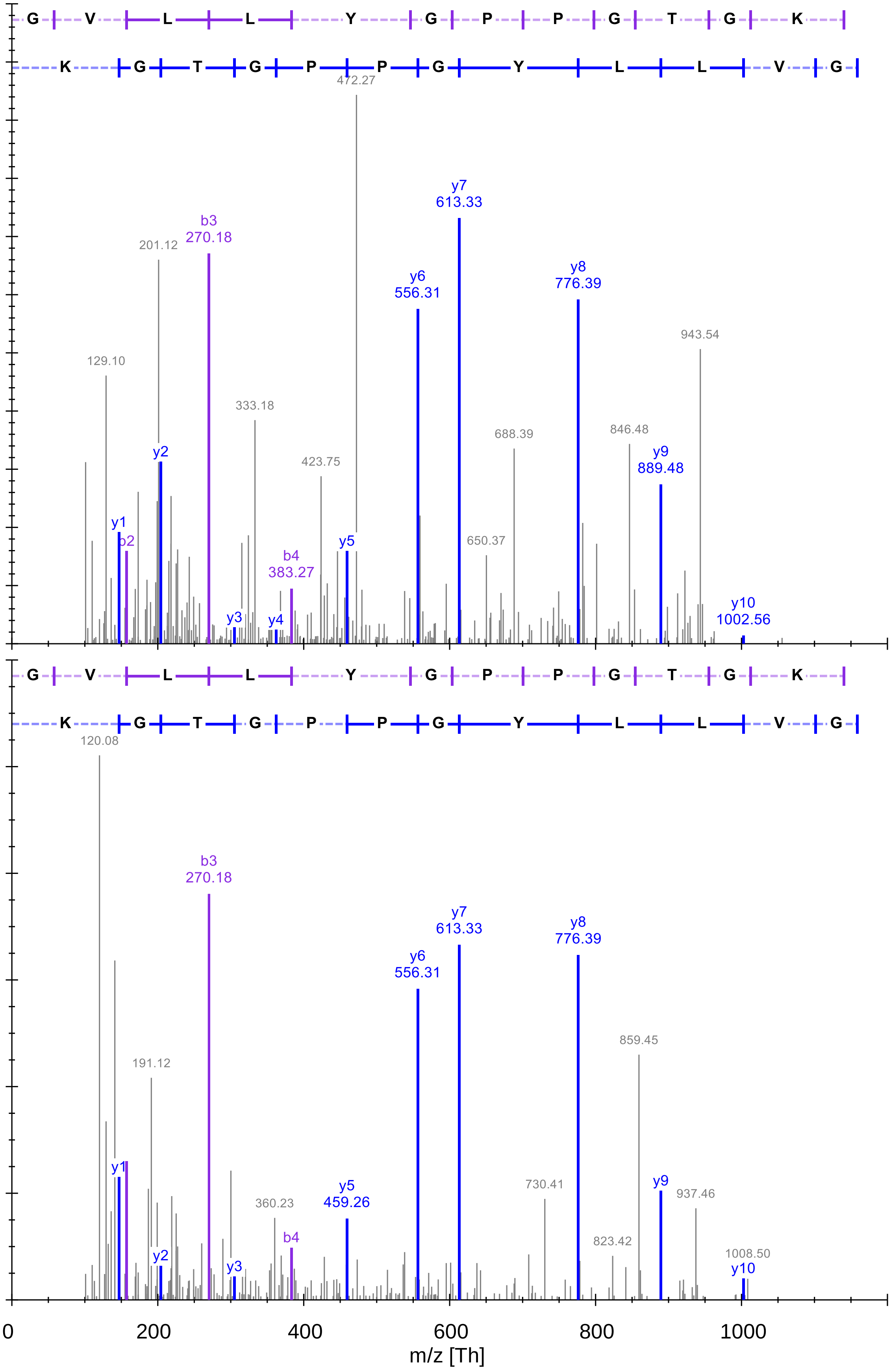
Distribution of retention time differences between peptide identifications from primary MS/MS spectra and secondary MS/MS spectra (clones). Top: in the range of 5 minutes; Bottom: in the range of 1 minute.



Supplementary Figure S4

Annotation for a peptide (GVLLYGPPGTGK) identified from two distant features.

Top: secondary (clone) spectrum, retention time 88 min. Bottom: primary spectrum, retention time 92.5 min;



Supplementary Figure S5

Intensities correlation from the same fragment peaks in primary and secondary spectra in **Table S3**.