

Analysis of the processing of seven human tumor antigens by intermediate proteasomes

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Figure S1. Identification of proteasome subunits of EB81-MEL by 2D gel electrophoresis and mass spectrometry (MS).

The gel was loaded with purified proteasomes from EB81-MEL tumor cell line (80 µg). Protein spots stained with colloidal blue on the 2D-gel were excised, digested with trypsin, and analysed by MS. The table shows the MS identification data for each numbered spot. The main isoform of catalytic subunit $\beta 2i$ should be localized in spot #7, but it was not found in the mass spectrometry analysis.

Figure S2. Fragments resulting from cleavage at the C-terminus and N-terminus of peptide MAGE-A3₁₁₄₋₁₂₂ (A) and peptide MAGE-C2₄₂₋₅₀ (B).

MS detection of the indicated peptide fragments in the digests shown in Figure 4.

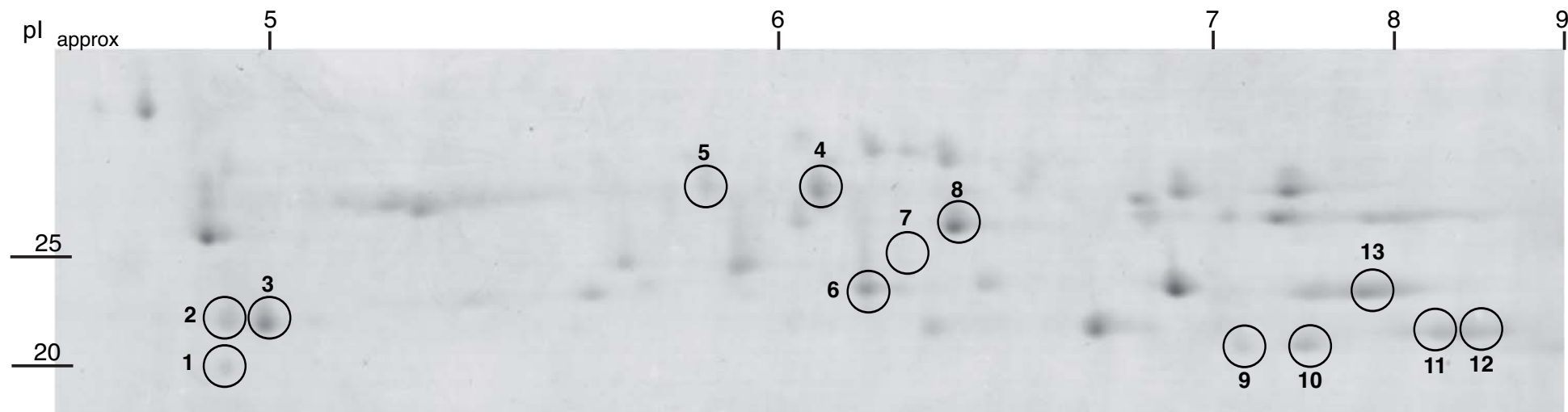
Figure S3. Fragments resulting from cleavage within and at the N-terminus of peptide Melan-A₂₆₋₃₅ (A) and from cleavage at the N-terminus of peptide gp100₂₀₉₋₂₁₇ (B) and peptide tyrosinase₃₆₉₋₃₇₇ (C).

MS detection of the indicated peptide fragments in the digests shown in Figure 4C and 6, monitoring production of the N-terminus and destruction of peptide Melan-A₂₆₋₃₅ (EAAGIGILTV) (A), and production of the N-terminus of peptide gp100₂₀₉₋₂₁₇ (ITDQVPFSV) (B) and of peptide tyrosinase₃₆₉₋₃₇₇ (YMDGTMSQV) (C). The peptide fragment SSAFT was detected by MS/MS.

Fig. S4. Additional fragments detected in the digest of the MAGE-C2₁₉₁₋₂₀₀ peptide precursor.

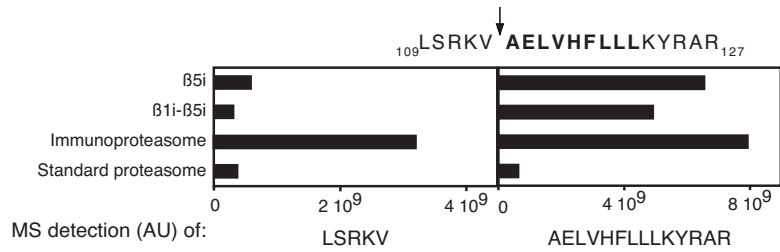
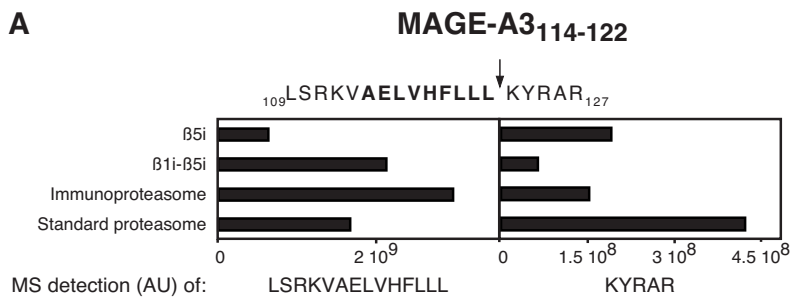
MS detection of the indicated peptide fragments in the digests shown in Figure 7D.

Fig. S1



Spot N°	Most abundant proteins	Mascot score	Accession N° (SwissProt)	Sequence coverage (%)	N° unique peptides (p ≤ 0.01)
1	Beta1i	423	P28065	30	9
2	Beta1i	831	P28065	42	13
	Beta1	744	P28072	19	6
3	Beta1	2641	P28072	28	9
4	Beta2	851	Q99436	36	22
	Alpha1	724	P60900	56	21
5	Beta2	502	Q99436	24	14
6	Beta3	1893	P49720	62	31
7	Beta7	346	P28070	35	8
	Beta3	330	P49720	37	10
	Alpha1	305	P60900	44	10
8	Alpha1	4479	P60900	69	33
9	Beta5i	4565	P28062	43	25
10	Beta5i	1617	P28062	44	25
11	Beta5	1213	P28074	46	20
12	Beta5	1740	P28074	49	24
13	Beta6	1938	P20618	58	28

Fig. S2 A



B

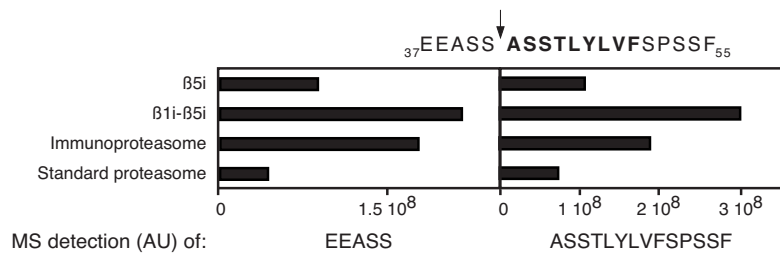
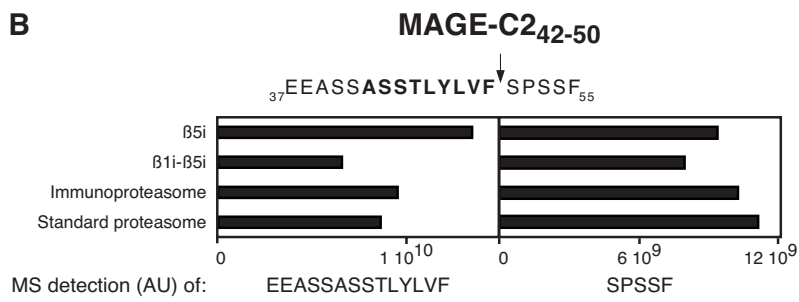
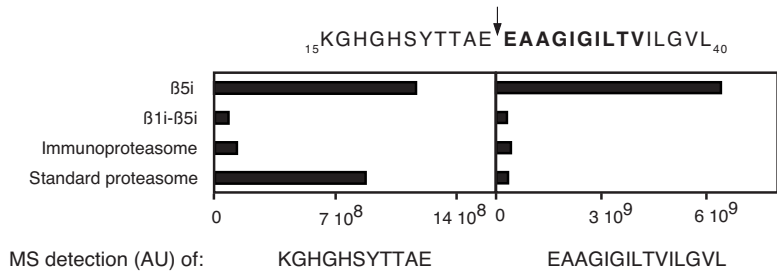
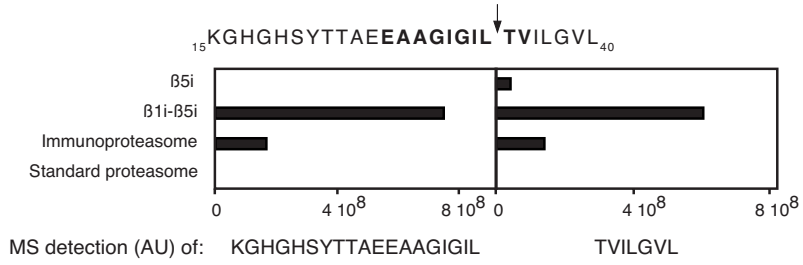
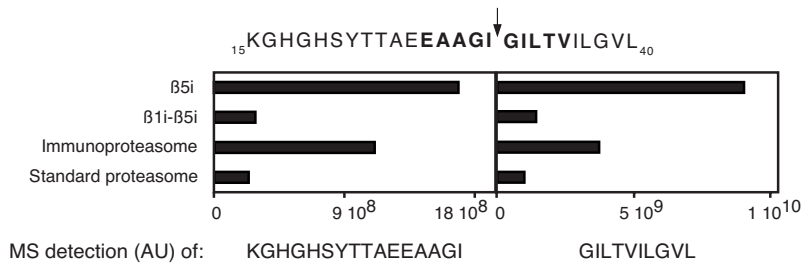


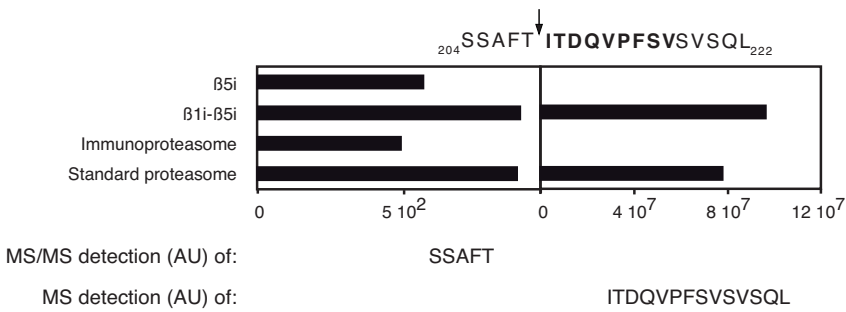
Fig. S3 A

Melan-A₂₆₋₃₅



B

gp100₂₀₉₋₂₁₇



C

Tyrosinase₃₆₉₋₃₇₇

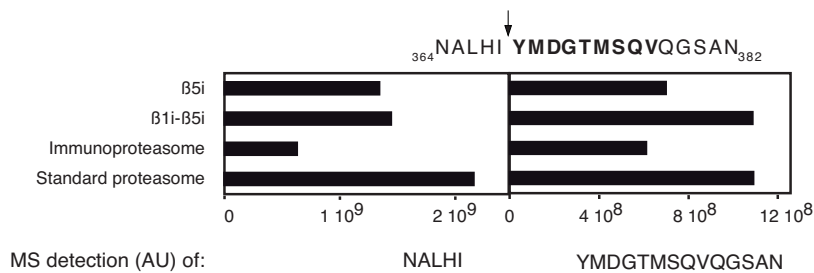


Fig. S4

MAGE-C2₁₉₁₋₂₀₀

