Extracellular proteasome-osteopontin circuit regulates cell migration with implications in multiple sclerosis

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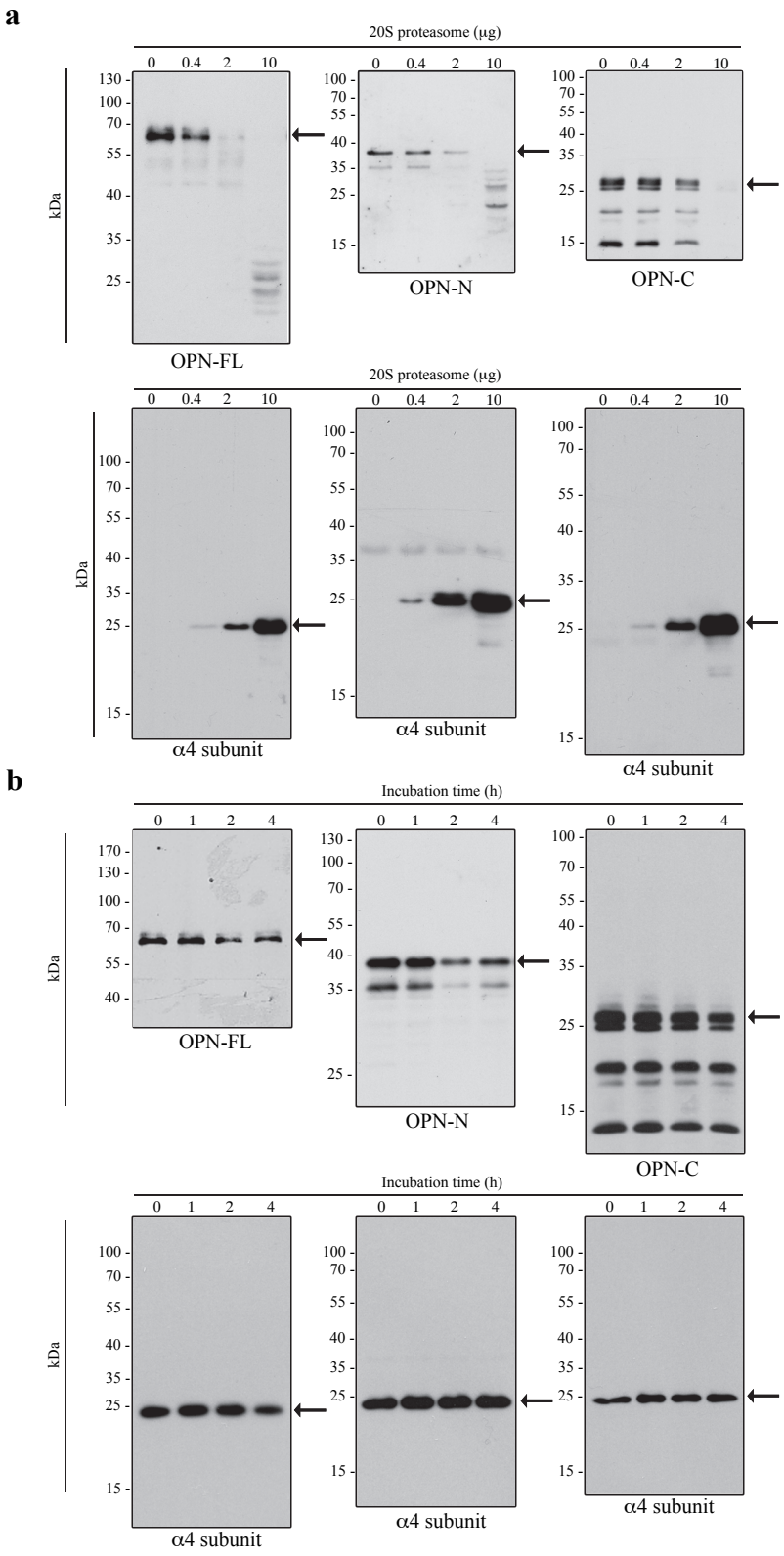
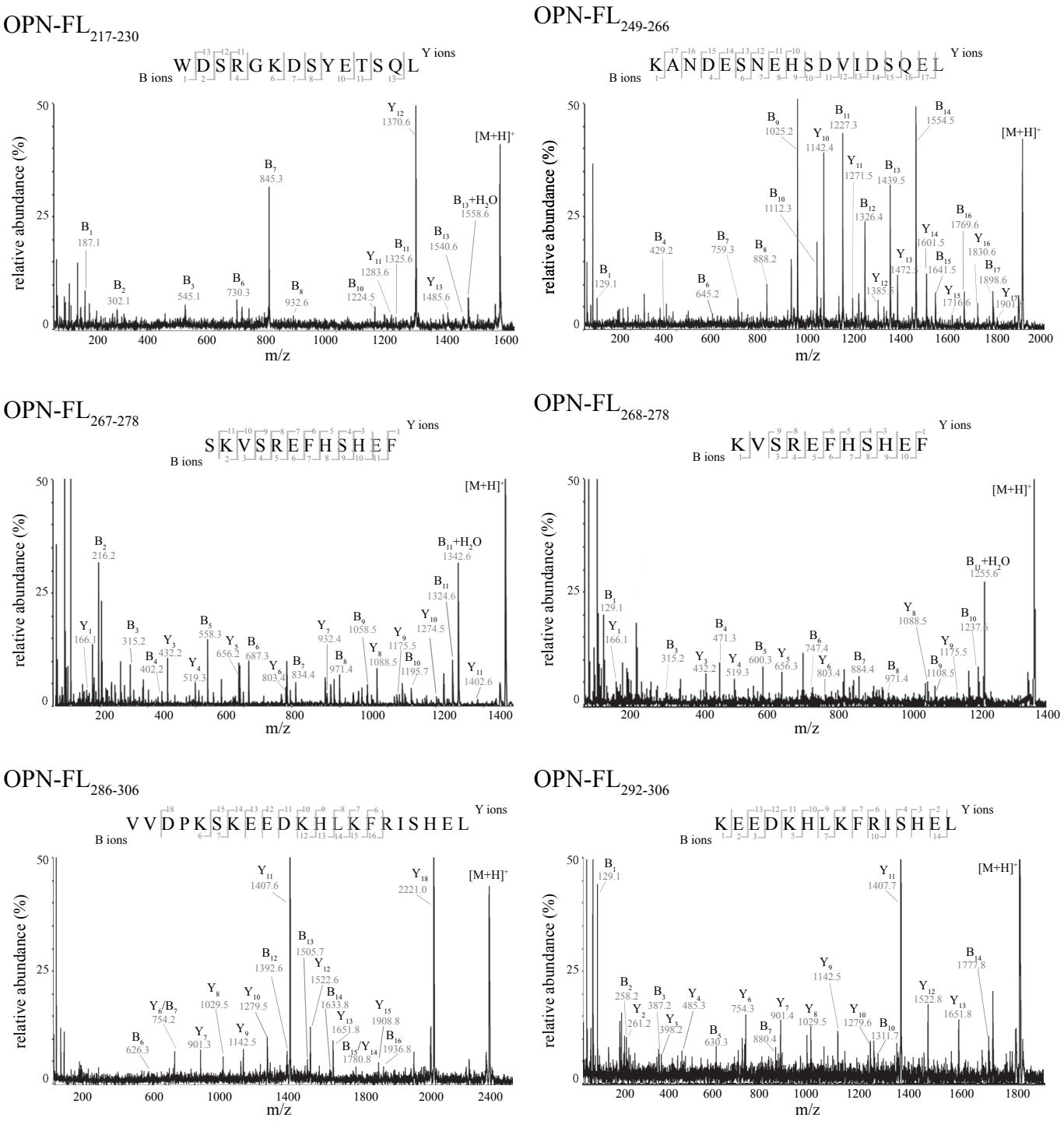
		Healthy Controls	RRMS Rem	RRMS Rel	CIS
Italian population	Age (y)	36.9 ± 14.8	42.3 ± 10.3	36 ± 10.4	
	Count (n)	62	50	25	
	Gender (M/F)	17/45	22/28	9/16	
	Disease onset (y)	-	41.4 ± 11.2	31.8 ± 9.4	
	Disease duration (y)	-	9.7 ± 7.4	5.0 ± 5.7	
	MSSS	-	3.0 ± 2.2	4.9 ± 2.5	
German population	Age (y)	27.2 ± 4.0	36.8 ± 11.9		32.8 ± 8.8
	Count (n)	50	13		37
	Gender (M/F)	23/27	5/8		15/22
	Disease onset (y)	-	33.1 ± 11.6		31.3 ± 8.8
	Disease duration (y)	-	2.3 ± 1.0		1.2 ± 0.6
	MSSS	-	4.7 ± 2.8		-

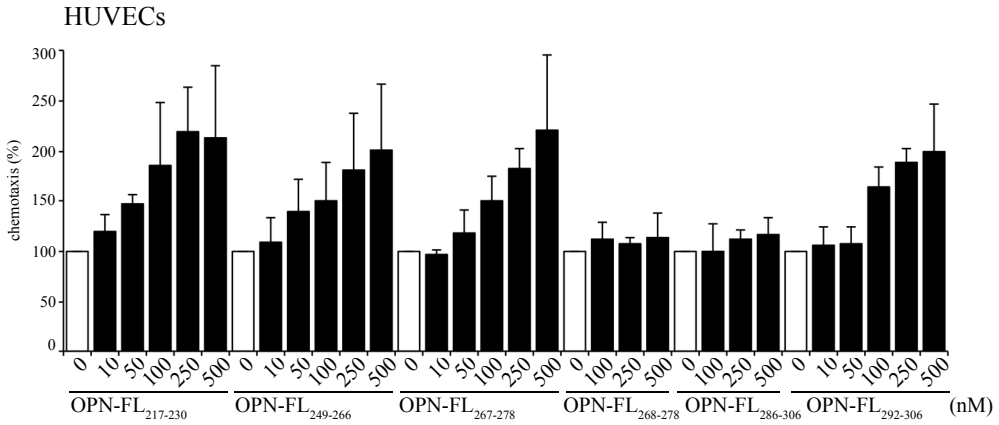
Supplementary table 1. Characteristics of healthy control and patient cohorts. Continuous variables are reported as mean ± SD. M = male, F = female, MSSS = Multiple Sclerosis Severity Scale.



Supplementary figure 1. Extracellular 20S proteasome breaks OPN molecules. (**a**) Representative *in vitro* digestion (n = 3) of recombinant OPN-FL, OPN-N, OPN-C by different amount of human erythrocyte 20S standard proteasome. The band pattern visible in the samples with 10 μg proteasome resembles that of the proteasome subunits and is thus likely the outcome of a cross-reactivity of the antibodies with such a large amount of proteasome. (**b**) Degradation kinetics of OPN-FL, OPN-N and OPN-C by T2 20S standard proteasomes are shown by representative Western Blot assay of 4-5 independent experiments. (**a-b**) The full Western blots are shown. The relevant bands of the OPNs (upper panels) or the proteasome α4 subunits (as controls; lower panels) are marked with an arrow and they are shown in Fig. 1.



Supplementary figure 2. Tandem mass spectrometry identification of the six studied fragments of OPN-C generated during *in vitro* digestion by 20S proteasomes. MALDI-tandem mass spectrometry spectra of the peptides WDSRGKDSY-ETSQL ($OPN_{217-230}$) [peptide score = 59, p = 0.097], KANDESNEHSDVIDSQEL ($OPN_{249-266}$) [peptide score = 101, p = 5.8*10-6], SKVSREFHSHEF ($OPN_{267-278}$) [peptide score = 96, p = 2.1*10-5], KVSREFHSHEF ($OPN_{268-278}$) [peptide score = 72, p = 0.0057], VVDPKSKEEDKHLKFRISHEL ($OPN_{286-306}$) [peptide score = 75, p = 0.0027], and KEEDKHLKFRISHEL ($OPN_{292-306}$) [peptide score = 69, p = 0.0099] identified in the *in vitro* digestion of the substrate OPN-C by erythrocyte 20S proteasome for 20 h. The identity of the peptide $OPN_{217-230}$ is verified by comparison with the fragment pattern of the synthetic analog.



Supplementary figure 3. Dose-dependent chemotactic effect of proteasome-generated OPN fragments. Effect of different concentrations of OPN fragments (10 - 500 nM) on the HUVEC chemotaxis is depicted. Values are reported as percentage of treated vs untreated cells that migrated after 20 h and they are expressed as the mean and the SD of independent experiments (n = 5 - 14). Cell migration is measured in the Boyden chamber migration assay.