Supplementary Material

Supplementary Methods, Site-directed mutagenesis

1. Primers used for site-directed mutagenesis of HSP90:

HSP90_K186R-s 5'-GTGATCCTCCATCTTAGAGAAGATCAGACAGAGTACC-3',

HSP90_K186R-as 5'-GGTACTCTGTCTGATCTTCTCTAAGATGGAGGATCAC-3',

HSP90_K438R-s 5'-GCATTCTCTAAAAATCTCAGGCTTGGAATCCACGAAGAC-3',

HSP90_K438R-as 5'-GTCTTCGTGGATTCCAAGCCTGAGATTTTTAGAGAATGC-3',

HSP90_K559R-s 5'-GAAGAGAGCAAGGCAAGGTTTGAGAACCTCTGC-3',

HSP90_K559R-as 5'-GCAGAGGTTCTCAAACCTTGCCTTGCTCTCTTC-3',

HSP90_K685R-s 5'-CGCATCTATCGCATGATCAGGCTAGGTCTAGGTATT-3',

HSP90_K685R-as 5'-AATACCTAGACCTAGCCTGATCATGCGATAGATGCG-3'

2. Primers used for knock-down of SENP proteins:

SENP1-shRNA1-S	5'-GGATCCCCAATCCTTCCTCAGACAGTTTTTTCAAGAGA-3'
SENP1-shRNA1-AS	5'-AAGCTTTTCCAAAAAAATCCTTCCTCAGACAGTTTTTCTCTTGAA-3'
SENP2-shRNA1-S	5'-GGATCCCCAACATGCTGAAACTGGGTAATTTCAAGAGA-3'
SENP2-shRNA1-AS	5'-AAGCTTTTCCAAAAAAACATGCTGAAACTGGGTAATTCTCTTGAA-3'
SENP3-shRNA1-S	5'-GGATCCCCAAACTCCGTACCAAGGGTTATTTCAAGAGA-3'
SENP3-shRNA1-AS	5'-AAGCTTTTCCAAAAAAAACTCCGTACCAAGGGTTATTCTCTTGAA-3'
SENP5-shRNA1-S	5'-GATCCCCAAGTCCACTGGTCTCTCATTATTCAAGAGA-3'
SENP5-shRNA1-AS	5'-AAGCTTTTCCAAAAAAAGTCCACTGGTCTCTCATTATCTCTTGAA-3'

Supplementary Table 1: Summary of paraproteins from patients positive for HSP90-SUMO reactivity

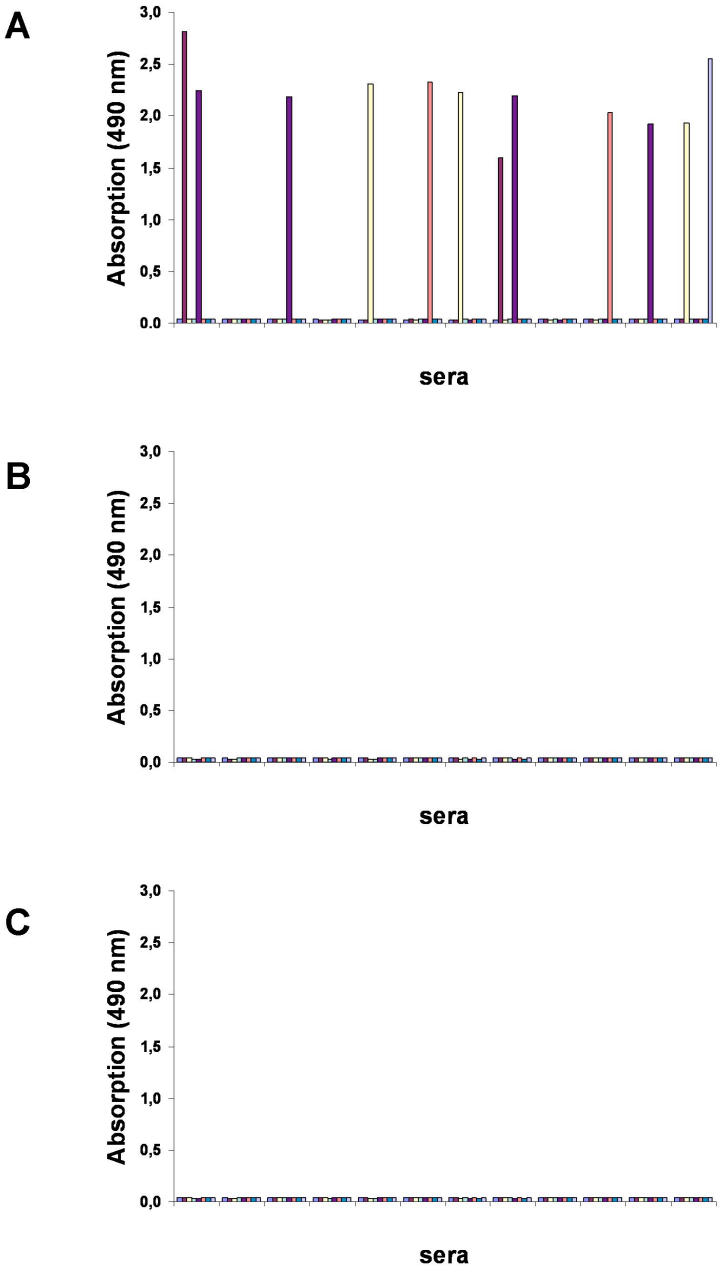
	Europeans		African-Americans		Japanese	
Paraprotein Type	n	Anti-HSP90-SUMO positive	n	Anti-HSP90-SUMO positive	n	Anti-HSP90-SUMO positive
IgA	50	5 (18.5%)	12	2 (22.2%)	36	3 (33.3%)
IgD	0	0	0	0	11	0
IgG	128	17 (62.9%)	68	7 (77.7%)	129	6 (66.6%)
IgG_1		2		3		2
IgG_2		1		0		1
IgG_3		14		4		3
IgG_4		0		0		0
IgM	48	5 (18.5%)	0	0	0	0
Total	**226**	**27**	**80**	**9**	**176**	**9**

Supplementary Table 2: Detection of HSP90-SUMO carrier state in other malignancies of European patients.

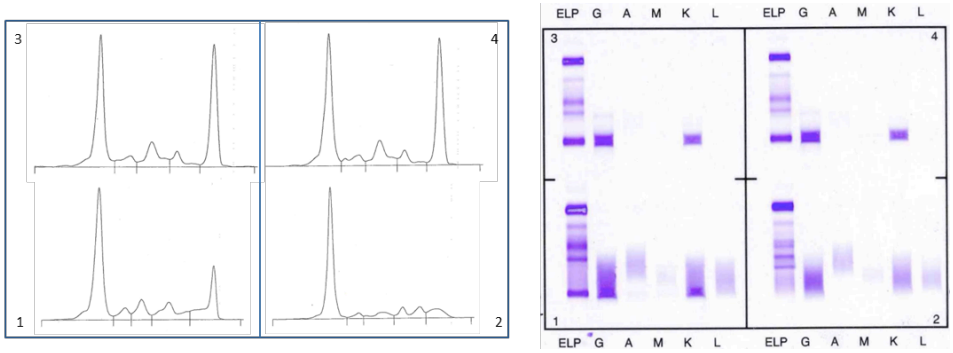
Malignancy	
AML/ALL	0/64
CLL	0/50
Follicular Lymphoma	0/50
CML	1/50
Other hematological neoplasms	0/112
Colon-Ca	1/50
Mamma-Ca	1/50
Melanoma	1/66
Urological cancers	0/95
(European healthy controls)	(5/550; 0.9%)

Supplementary Table 3: Detection of HSP90-SUMO carrier state in autoimmune diseases.

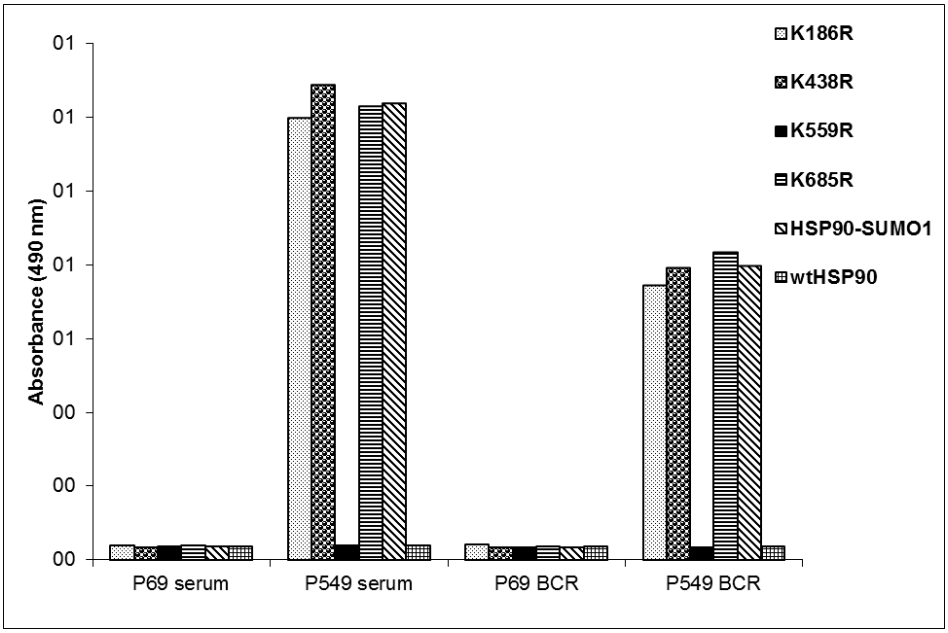
Autoimmune disorder	
Rheumatoid Arthritis	0/100
Systemic lupus erythematosus	0/30
Polymyalgia rheumatica	1/15
Granulomatosis with polyangiitis	0/10
Multiple sclerosis	0/10
Crohn`s disease	0/10
Churg-Strauss syndrome	0/5
SAPHO syndrome	0/5
Sjögren´s syndrome	0/5
Total autoimmune disorders	**1/190 (0.5%)**



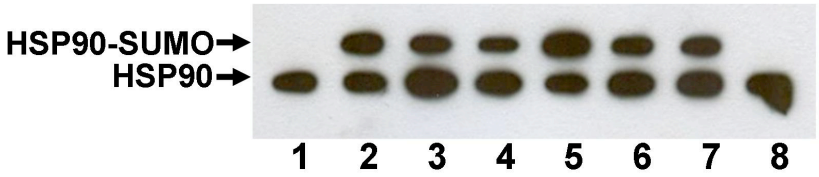
Supplementary Figure 1: Reactivity of HSP90-SUMO, wtHSP90 or wtSUMO1 with paraproteins by ELISA. Twelve of 96 paraprotein-containing sera reacted with (A) HSP90-SUMO at a dilution of $1{:}10^6$, but not with (B) wt-HSP90, or (C) wtSUMO1 (represented by VCP-SUMO1), respectively, at a dilution of $1{:}10^2$.



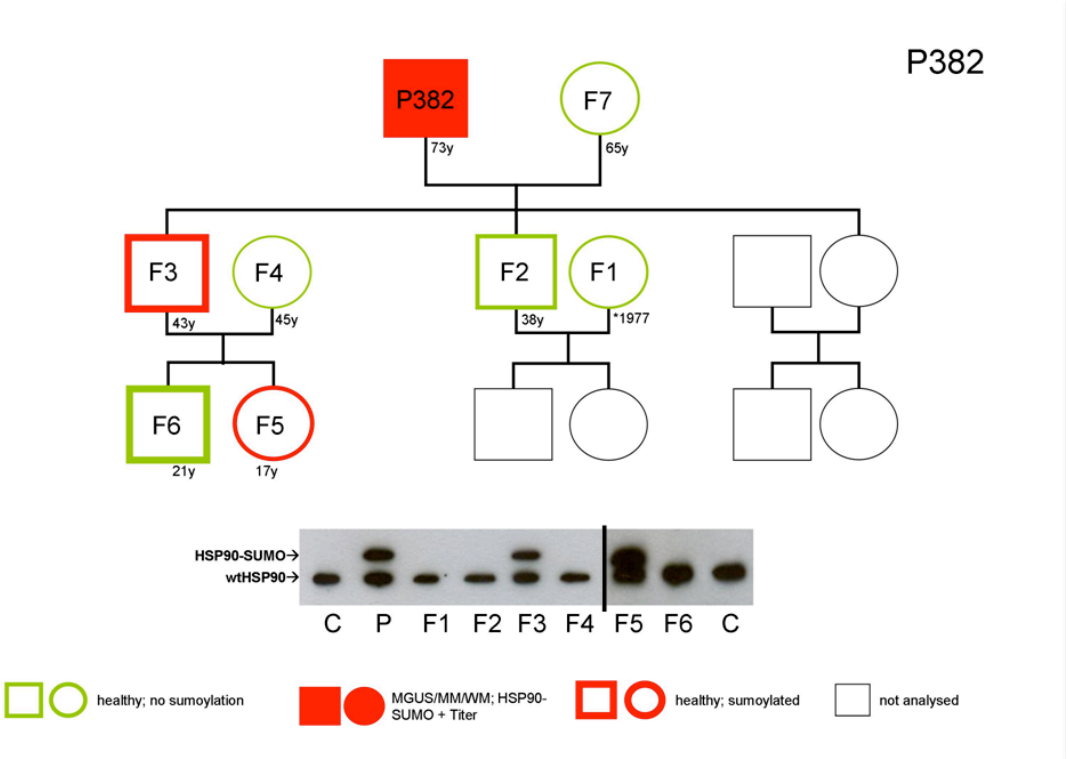
Supplementary Figure 2: Depletion of paraprotein containing sera by a HSP90-SUMO affinity column. Analysis was done by serum electrophoresis (left) and immunofixation (right). (1) Serum containing an IgG-κ paraprotein with HSP90-SUMO reactivity and (2) absorption by a HSP90-SUMO column. (3) Serum containing an IgG-κ paraprotein with non-HSP90-SUMO reactivity and (4) absorption by a HSP90-SUMO column (control). ELP: reference track.



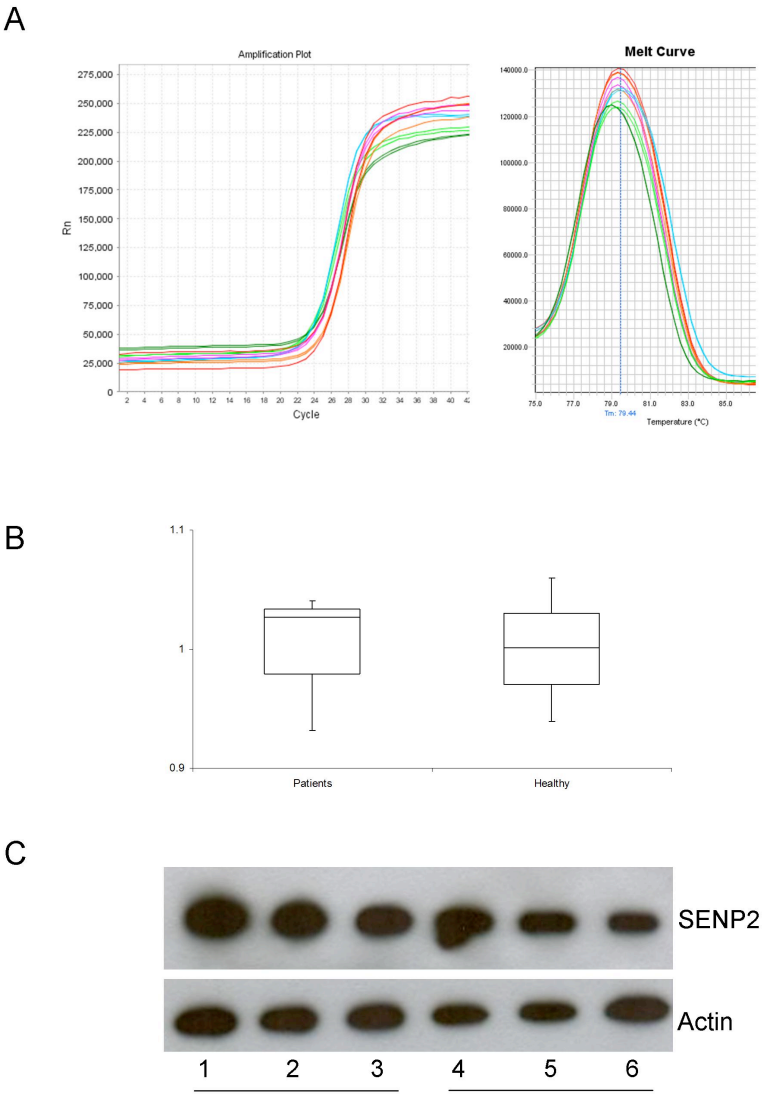
Supplementary Figure 3: Patients paraprotein and BCR recognize the same HSP90 sumoylation site. Elisa: Immunoreactivity of patient's serum or BCR versus mutagenized and sumoylated HSP90. Serum and BCR did not react with wtHSP90 or HSP90-SUMO (K559R), while all other mutations did not affect the immunoreactions. P549 represents a patient with a HSP90-SUMO specific paraprotein while P69 represents a patient having a paratarg-7 specific paraprotein.



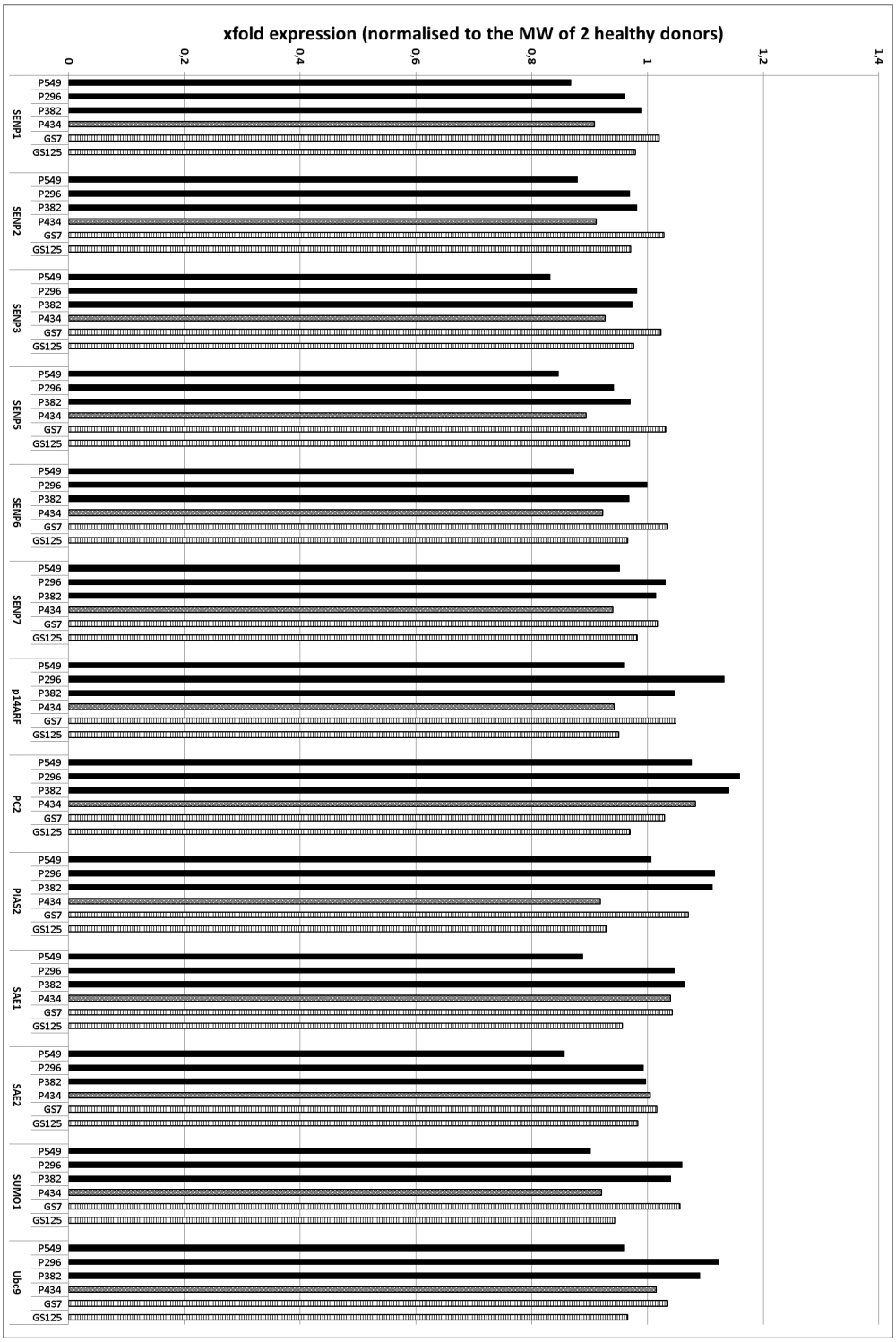
Supplementary Figure 4: Expression of HSP90-SUMO in human blood cell fractions and mouth swabs. Freshly drawn human blood was subjected to Ficoll centrifugation followed by MACS cell fractionation and lysed. Mouth line scrapings were lysed in SDS lysis buffer. Analysis was done by SDS-PAGE and Western blot analysis using anti-HSP90. 1: wtHSP90, 2: total blood, 3: erythrocytes, 4: granulocytes, 5: B cells, 6: T cells, 7: macrophages, 8: mouth swap.



Supplementary Figure 5: Pedigree of a family with an MM patient carrying HSP90-SUMO as paraprotein target. Shown are the pedigree of the family and a Western blot analysis of HSP90 state. Sumoylated HSP90 was detected in one of his two sons (F3), while the other one (F2) carried wtHSP90; both being healthy. The son with HSP90-SUMO (F3) had two children (F5, F6), both healthy, but the daughter (F5) was carrier of HSP90-SUMO, while the son (F6) was not. P: patient #382, C: healthy blood donor as control.



Supplementary Figure 6: SENP2 expression in PBMCs derived from HSP90-SUMO carrying patients and healthy donors. A) qRT-PCR of SENP2 in PBMCs (in replicates) derived from patients (n=3, red), healthy donors (n=3, green) and from a MM-patient with a paraprotein of other specificity (n=1, blue). Shown are raw amplification data and melting point analyses. **B)** Box plot of qRT-PCR data normalized to 18s RNA; mean value of the expression in healthy people is set to 1. Patients: HSP90-SUMO carrying patients, Healthy: 2 healthy donors and 1 MM patient with a paraprotein of different specificity. **C)** Western blot analysis followed by immunodetection with anti-SENP2 or anti-actin. 1-3: healthy donors, 4-6: HSP90-SUMO positive patients.



Supplementary Figure 7: Relative expression of enzymes involved in sumoylation. Data were obtained by qRT-PCR. Gene-specific amplification was performed on cDNA derived from peripheral blood of individual donors. Gene specific expression was normalized to the MW of specific gene expression in 2 healthy donors. P296, P382, P549: MM patients with a

HSP90-SUMO specific paraprotein, P434: MGUS patient with a paraprotein of unknown specificity, GS7, GS125: healthy donors.