Proliferation is a central independent prognostic factor and target for personalized and risk-adapted treatment in multiple myeloma

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Citation: Hose D, Rème T, Hielscher T, Moreaux J, Messner T, Seckinger A, Benner A, Shaughnessy Jr. JD, Barlogie B, Zhou Y, Hillengass J, Bertsch U, Neben K, Möhler T, Rossi JF, Jauch A, Klein B, and Goldschmidt H. Proliferation is a central independent prognostic factor and target for personalized and risk-adapted treatment in multiple myeloma. Haematologica 2011;96(1):87-95. doi:10.3324/haematol.2010.030296

Supplementary Design and Methods

Statistical analysis

Gene-expression data were preprocessed by GC-RMA.[1,2] The use of a modified version of the "documentation by value" (docval)[1,2] package allowed the assessment of gene expression as if a new patient's *cel-file had been part of the initial GC-RMA preprocessed gene-expression dataset. This enables calculation of gene expression-based proliferation indices (see below) or risk scores and usage of previously validated thresholds for a patient not part of the initial analysis-set. An R-script allowing this calculation can be found in *Online Supplementary File S1*. To assess the presence or absence of gene expression, the "Presence-Absence calls with Negative Probesets (PANP)" algorithm[3] was used. *P* values were adjusted for multiple testing controlling the false discovery rate, as defined by Benjamini and Hochberg, at a level of 5%.[4] For myeloma cells, the association of chromosomal aberrations and clinical parameters with gene expression was assessed using two-sample t-statistics. Differences in clinical parameters between defined groups were investigated using the exact Wilcoxon's rank-sum test. Correlations were assessed using Pearson's correlation coefficient, or Kendall's tau coefficient (for categorical variables), the relationship between categorical variables by Fisher's exact test. The presence of a translocation t(4;14) within the LR group was assessed using a call-based predictor.[5] The indices by Bergsagel *et al.*[6] (GEP-B), Shaughnessy *et al.*[7] (GEP-SH) and Hose *et al.*[8] (GPI; see below and *Online Supplementary Table S6*) were calculated as published. The HM1 cohort was used as a reference myeloma dataset for the initial calculation of the GPI (see also below).

Gene expression profiling-based classifications

The group attribution according to the molecular classification of myeloma was assessed on the same dataset and using the same method as within the original series.[7] It can also be found under the accession number Series GSE4581. TC-class assessment was performed as described by Bergsagel *et al.*[9] Probe-sets for Affymetrix HGU95A arrays were shifted to the corresponding probe-sets on U133 2.0 and MAS5 normalized data used. High-risk scores by Shaughnessy *et al.* (Shaughnessy-HR)[10] and Decaux *et al.* (Decaux-HR)[11] were calculated as described by the respective authors, i.e. using normalization protocols (MAS5 in the case of the Shaughnessy-HR score) and cut-offs. An optimal threshold for LDH was calculated by maximum log-rank statistics, (R, maxstat-package). An effect was considered as statistically significant if the *P* value of its corresponding statistical test was not higher than 0.05. All statistical computations were performed using R[12] version 2.8.1, and Bioconductor,[13] version 2.3.

Calculation of the gene expression-based proliferation index

The gene expression-based proliferation index was calculated as follows. In brief, genes were selected from those over-expressed in proliferating cells [malignant: human myeloma cell lines (HMCL), benign: polyclonal plasmablastic cells (PPC)] compared to in non-proliferating cells [normal bone marrow plasma cells (BMPC) and memory B cells (MBC)]. Here, four comparisons between the groups were made, (i) HMCL *versus* MBC, (ii) HMCL *versus* BMPC, (iii) PPC *versus* BMPC and (iv) PPC *versus* MBC by a one-sided t-test, with the alternative hypothesis that expression values of HMCL and PPC are greater than those for BMPC and MBC in each comparison. *P* values were permutation-adjusted regarding a family wise error rate with an α-level of 0.025. To adjust for comparing each group twice, the α-level was halved to 0.0125. Only genes statistically significant in each of the four comparisons were retained for the index. To select biologically relevant genes (in terms of proliferation) only genes with the gene-ontology term "cell proliferation" or "cell cycle" were retained. Thus, 50 genes (57 probe-sets) were included in the final index. For genes with more than one probe-set per gene, the probe-set with the highest variance within the HM1 cohort was selected. The index was calculated as follows. Given that proliferation-genes determined as stated above are over-expressed by definition, the individual gene expression based-proliferation index for each sample was calculated as the sum of expression values of each of the 50 genes in an individual sample. For genes not expressed as judged by PANP, the expression level of the respective gene was defined as 0.

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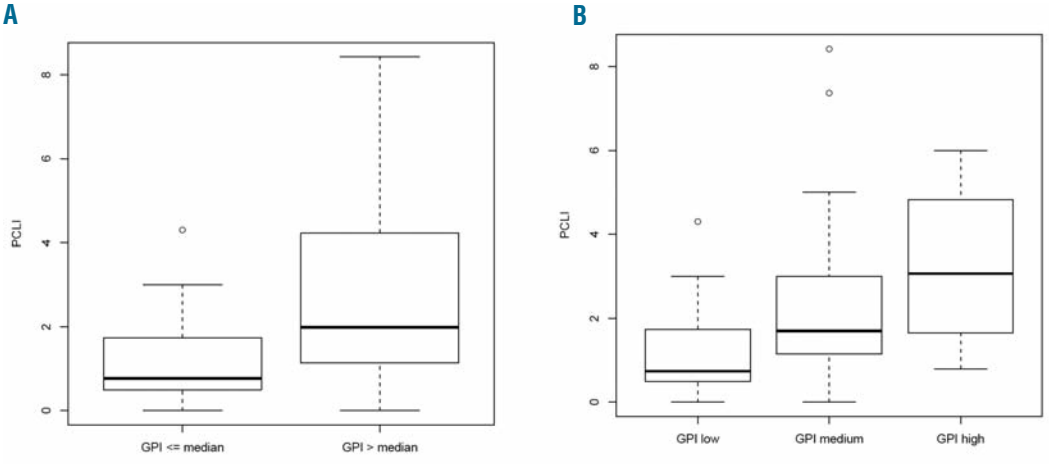
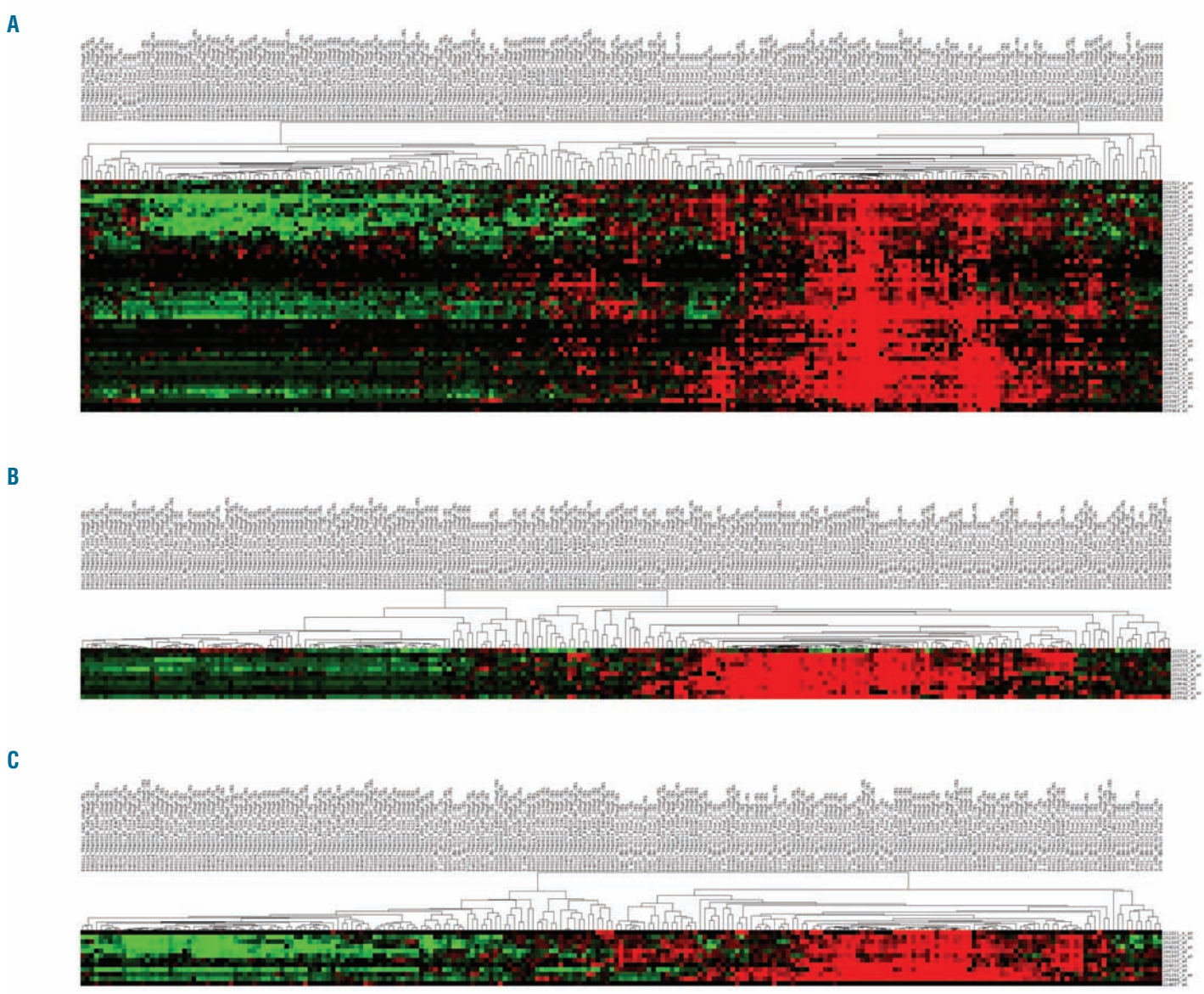
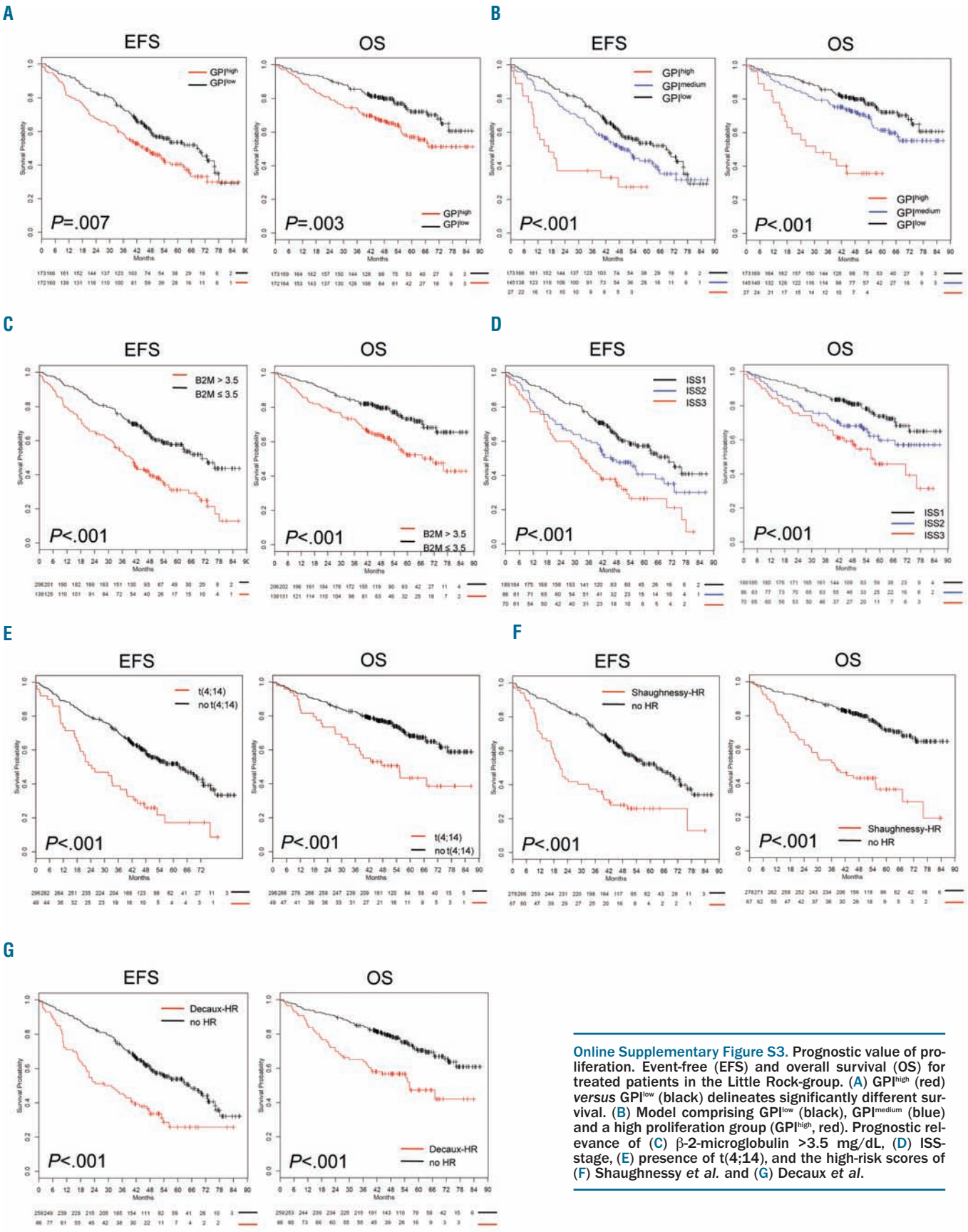


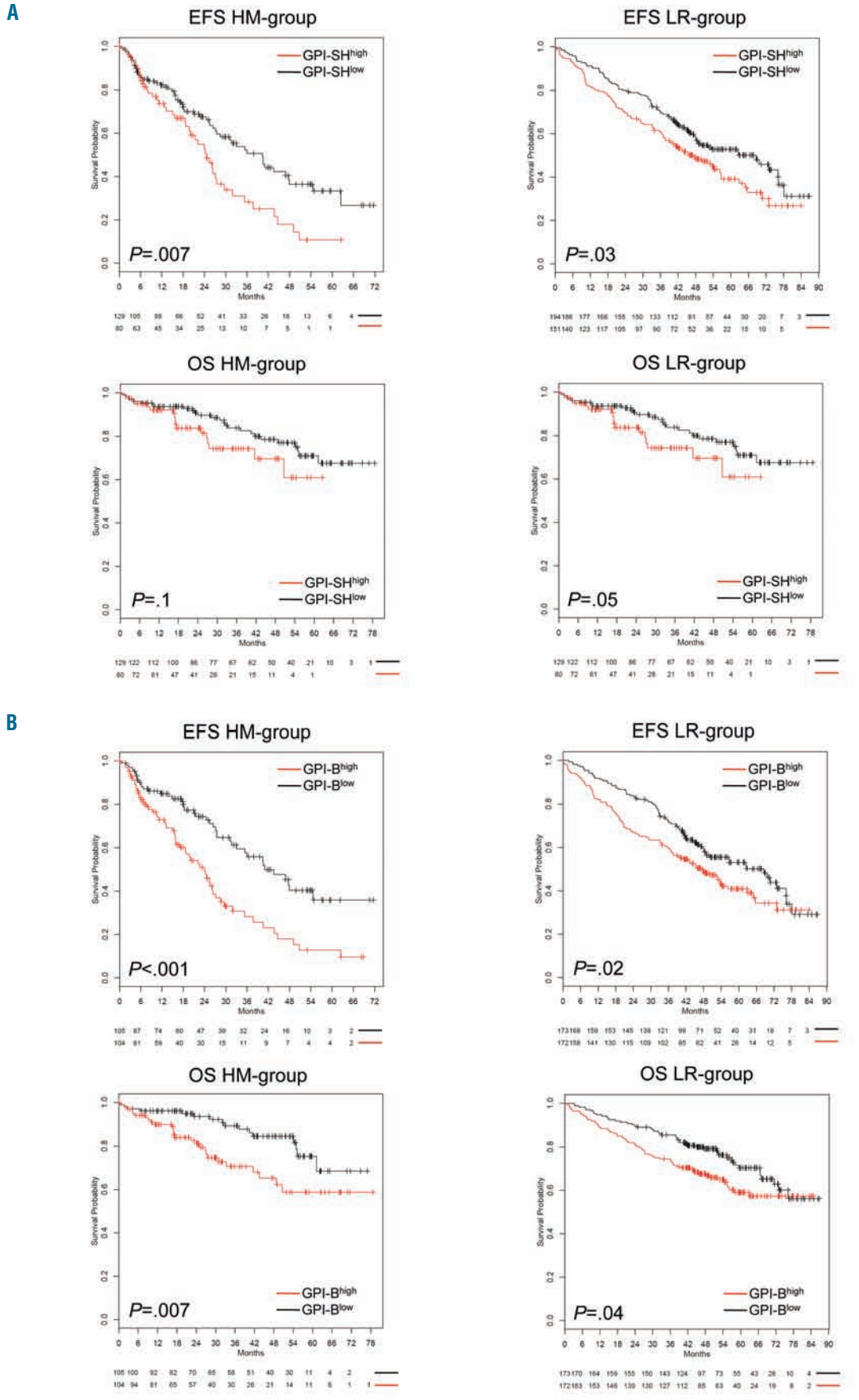
Online Supplementary Figure S1. The plasma cell labeling index (ordinate, PCLI) for the gene expression-based proliferation index (GPI) divided into (A) two groups according to values below or above the median (*P*=0.003, n=66) and (B) three groups high/median/low (low *versus* medium *P*=0.01, low or medium *versus* high *P*=n.s. due to low number (n=4) of measurements in the GPI "high" group; n=66).



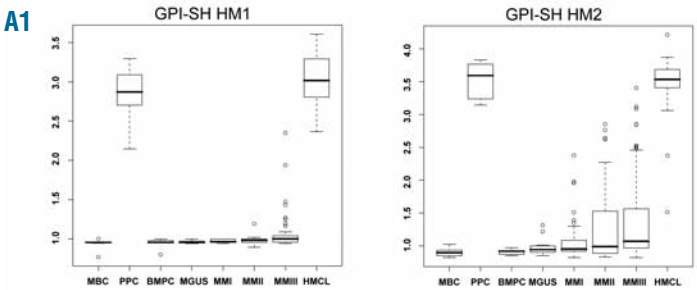
Online Supplementary Figure S2. Unsupervised hierarchical clustering of myeloma cells. Clustering based on (A) the GPI of Hose *et al.*, (B) the index of Shaughnessy *et al.*, and (C) the index of Bergsagel *et al.* The data for the HM2 group are shown (see *Online Supplementary Design and Methods* for details).

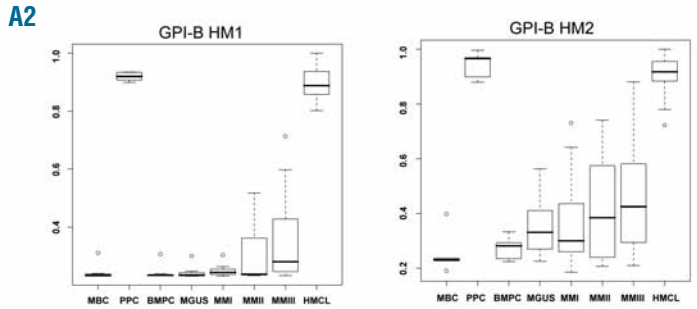


Online Supplementary Figure S3. Prognostic value of proliferation. Event-free (EFS) and overall survival (OS) for treated patients in the Little Rock-group. (A) GPI^{high} (red) *versus* GPI^{low} (black) delineates significantly different survival. (B) Model comprising GPI^{low} (black), GPI^{medium} (blue) and a high proliferation group (GPI^{high}, red). Prognostic relevance of (C) β-2-microglobulin >3.5 mg/dL, (D) ISS-stage, (E) presence of t(4;14), and the high-risk scores of (F) Shaughnessy *et al.* and (G) Decaux *et al.*

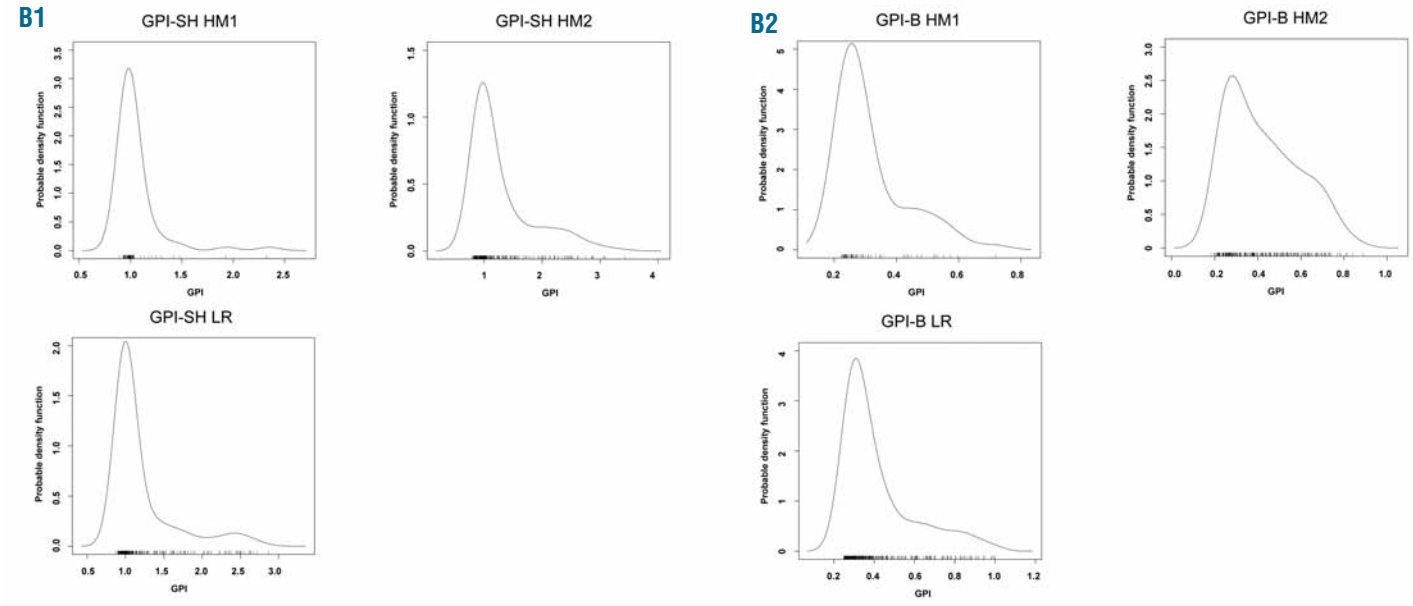


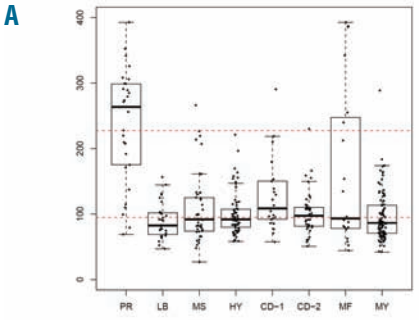
Online Supplementary Figure S4. Prognostic value of the GPI from Shaughnessy *et al.* (GPI-SH) and Bergsagel *et al.* (GPI-B). (A) Event-free (EFS) and (B) overall survival (OS) for patients treated with high-dose chemotherapy and autologous stem cell transplantation within our series (HM) (n=209) and the Little Rock (LR)-group (n=345). GPI above (GPI^{high}, black curve) *versus* below (GPI^{low}, red curve) the median delineate significantly different survival.

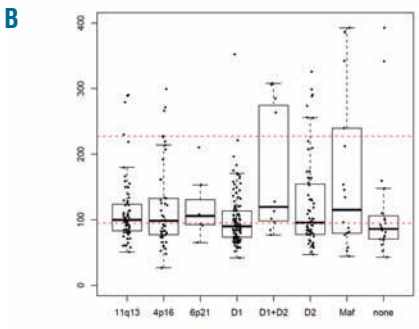


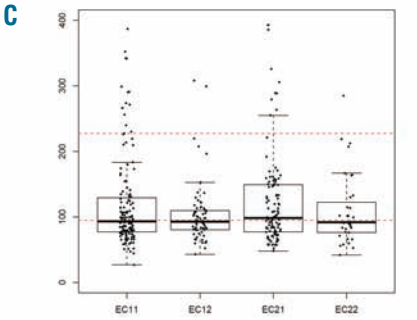


Online Supplementary Figure S5. (A) Gene-expression based proliferation index (GPI) of normal bone marrow plasma cells (BMPC), memory B cells (MBC), primary myeloma cells (MMC) and human myeloma cell lines (HMCL). MMC samples are subdivided in monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma (MM) stage I/II/III (MMI/MMII/MMIII) according to Durie and Salmon. Significant differences are indicated by an asterisk (*). (A1) Depicts the index of Shaughnessy *et al.* (GPI-SH), (A2) the index of Bergsagel *et al.* (GPI-B). Boxes extend from quartile 1 to 3 with a black horizontal line indicating the median. Whiskers extend to most extreme observed value within 1.5 times the interquartile range of quartile 1 minus 3. (B) Distribution of both GPI for newly-diagnosed patients within our cohorts (HM1 and HM2) (n=298) and the Little Rock (LR) group (n=345).









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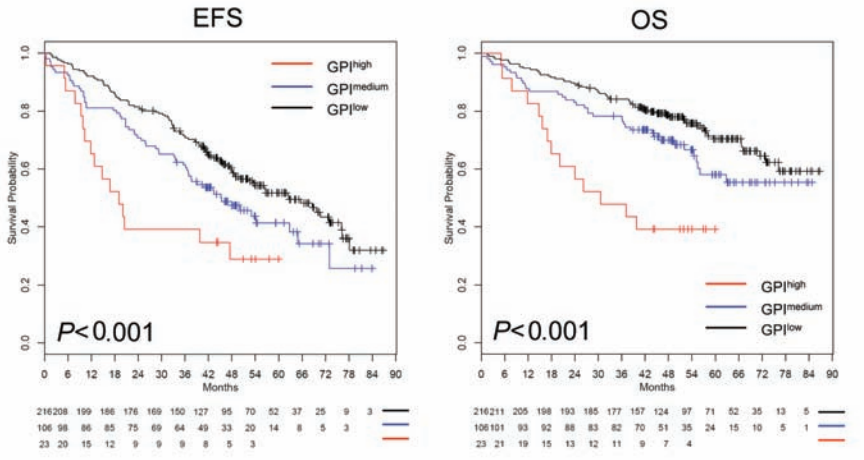
	n	GPI high	GPI median	GPI low
Molecular classification		%	%	%
PR	29	59	34	7
LB	31	0	35	65
MS	42	2	45	52
HY	65	0	45	55
CD-1	22	5	64	32
CD-2	41	2	51	46
MF	20	30	20	50
MY	95	1	39	60
TC-classification				
11q13	63	6	52	41
4p16	49	8	43	49
6p21	7	0	57	43
D1	114	1	42	57
D1+D2	11	36	36	27
D2	63	11	40	49
Maf	18	28	28	44
none	20	10	25	65
EC-classification				
EC1.1	138	10	39	51
EC1.2	65	3	43	54
EC2.1	107	9	45	46
EC2.2	35	3	43	54

Online Supplementary Figure S6. Distribution of proliferation within published gene expression-based classifications of myeloma. Myeloma cell samples are subdivided according to the classification of (A) Zhan *et al.* (molecular classification), (B) Bergsagel *et al.* (TC-classification), and (C) Hose *et al.* (EC-classification). The lower red dotted horizontal line depicts the median GPI (below: GPI^{low}), the upper the GPI^{high}-group. GPI^{median}-group between the two dotted lines. (D) Numbers (n) and percentages (%) of patients with $GPI^{high/median/low}$ in the respective molecular classifications.



Online Supplementary Figure S7. Documentation by value. Event-free (EFS) and overall survival (OS) for patients treated with high-dose chemotherapy and autologous stem cell transplantation within the Little Rock group (n=345). Model comprising GPI^{low} (black), GPI^{medium} (blue) and GPI^{high} (red) with cut-offs derived from the HM2-group after applying the documentation by value strategy (docval-package) on the Little Rock *cel-files.

Online Supplementary Table S1. Clinical data for the non-selected, previously untreated patients presenting at the university hospitals of Heidelberg and Montpellier undergoing high-dose chemotherapy with 2x100 mg/m² melphalan and autologous stem cell transplantation. Age, serum β2-microglobulin, and plasma cell infiltration in the Heidelberg/Montpellier-group 1 (HM1), -2 (HM2) and the Little Rock (LR) group.

Characteristic	HM1 n=48	HM2 n=161	LR n=345
Age	58,5 [37-72]	57 [27-73]	57 [25-77]
Monoclonal protein			
IgG	25	97	193
IgA	11	36	93
Bence Jones	10	25	47
Asecretory	2	2	6
IgD	0	1	3
NA	0	0	3
Myeloma in Durie and Salmon stage			
I	4	16	NA
II	5	27	NA
III	39	118	NA
Myeloma in ISS stage			
I	15	80	189
II	26	50	86
III	7	28	70
NA	0	3	0
Serum β2-microglobulin	3.55 [1.3-11.9]	3.0 [1.3-53.6]	2.9 [1.0-38.7]
Plasma cells in bone marrow	45 [5-100]	38 [1-100]	42 [4-98]

Median value and range are given. NA, not available; ISS, International Staging System. Induction treatment: VAD (vincristine, adriamycin, dexamethasone; n=139); TAD (thalidomide, adriamycin, dexamethasone; n=34); PAD (bortezomib, adriamycin, dexamethasone; n=26); other (e.g. bortezomib/dexamethasone; n=10).

Online Supplementary Table S2. Overview of the populations, subpopulations and samples used.

Analysis	Details						n
GEP	MMC	HM-group	HM1	U133A+B			65
			HM2	U133 Plus 2.0			233
							298
		LR-group		U133 Plus 2.0			**345**
	other populations	HM-group	HM1	U133A+B	MBC		7
					PPC		7
					BMPC		7
					MGUS		7
					HMCL		20
							48
			HM2	U133 Plus 2.0	MBC		6
					PPC		5
					BMPC		7
					MGUS		16
					HMCL		32
							66
							757
Survival (HDT)	MMC	HM-group	HM1				48
			HM2				161
		LR-group					345
							554
PCLI	MMC	HM-group	HM2				**66**
iFISH					HM1	HM2	
	MMC	HM-group		t(4;14)	65	175	**240**
				t(11;14)	65	177	**242**
				t(14;16)	1	68	**69**
				11q13	56	177	**233**
				1q21	53	170	**223**
				17p13	58	176	**234**
				13q14	65	180	**245**
				11q23	64	178	**242**
				9q34	44	114	**158**
				15q22	46	118	**164**
				19q13	46	139	**185**
				4p16	12	140	**152**
				8p21	50	130	**180**
				14q32	22	170	**192**
				22q11	45	89	**134**
		LR-group		1q21			**244**
				any			**344**

GEP, gene-expression profiling; MMC, multiple myeloma cells; HM-group, Heidelberg/Montpellier-group; LR-group, Little Rock group; MBC, memory B cells; PPC, polyclonal plasmablastic cells; BMPC, bone marrow plasma cells; MGUS, monoclonal gammopathy of unknown significance; HMCL, human myeloma cell line; HDT, high-dose chemotherapy, PCLI, plasma cell labeling index; iFISH, interphase fluorescence in situ hybridization.

Online Supplementary Table S3. Association of chromosomal aberrations and proliferation. Only aberrations significantly associated with the gene expression-based proliferation index in more than one cohort are further considered in the text.

		HM2	LR
IgH-translocations	t(4;14)	0.2	-
	t(11;14)	0.7	-
	t(14;16)	0.1	-
	any (IgH-split)	0.6	-
hyperdiploidy	score	0.06	-
	9q34	0.03	-
	15q22	0.04	-
	19q13	0.04	-
	11q23	0.003	-
progresssion associated chromosomal aberration	1q21 (all)	<0.001	0.001
	1q21 (without subclones)	<0.001	0.001
	17p13 (all)	0.8	-
	17p13 (without subclones)	0.6	-
	13q14 (all)	0.01	-
	13q14 (without subclones)	<0.001	-
other numerical aberrrations	4p16	0.7	-
	6q21	0.7	-
	8p21	0.8	-
	14q32	0.7	-
	22q11	0.6	-
subclones	presence vs. absence	0.2	-
conventional cytogenetics	any abnormality	-	<0.001

HM: Heidelberg/Montpellier group; LR: Little Rock group; score, copy number score according to Wuilleme et al., (see Design and Methods section for details).

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		EFS					
		HM			LR		
		months [95%CI]	HR [95%CI]	*P*-value	months [95%CI]	HR [95%CI]	*P*-value
GPI	median cut	24.6 [18.6;28.6] vs. 40.6 [31.2;54.6]	1.8 [1.2;2.7]	0.002	45.2 [36.6;54.1] vs. 68.6 [48.9;76.2]	1.5 [1.1;2.0]	0.007
	high vs. median vs. low	12.7 [6.1;26.3] vs. 26.2 [19.7;31.9] vs. 40.6 [31.2;54.6]	--	0.002	16.8 [7.9;47.5] vs. 50.1 [39;62.8] vs. 68.6 [48.9;76.2]	--	<0.001
	median vs. low	--	1.7 [1.1;2.6]	0.01	--	1.3 [1.0;1.8]	0.06
	high vs. median	--	1.7 [0.9;3.3]	0.1	--	2.0 [1.3;3.5]	0.004
ISS	III vs. II vs. I	18.6 [6.1;43.6] vs. 26.4 [17;35.3] vs. 37.7 [28.6;49.1]	1.3 [0.9;2.1] 2.1 [1.2;3.6]	0.02	32.6 [20.7;41.2] vs. 45.7 [34.8;64.9] vs. 70.7 [56.7;Inf]	1.6 [1.1;2.3] 2.5 [1.7;3.5]	<0.001
B2M35		24 [15.8;27.5] vs. 35.3 [27.1;46.9]	1.5 [1.0;2.3]	0.04	39.8 [32.4;47.5] vs. 70.7 [56.7;Inf]	2.1 [1.6;2.8]	<0.001
t(4;14) *		15.8 [7.8;24.0] vs. 33.1 [25.5;43.7]	2.9 [1.8;4.8]	<0.001	24.3 [19.0;39.9] vs. 62.6 [52.5;72.9]	2.4 [1.7;3.5]	<0.001
17p13		22.7 [11.6;43.7] vs. 26.4 [24;35.4]	1.2 [0.5;1.3]	0.4	--	--	--
Shaughnessy-HR		20.1 [12.7;27.1] vs. 35.4 [26.4;43.6]	1.9 [1.2;3.0]	0.009	20.8 [16.8;33.5] vs. 62.8 [53;72.8]	2.6 [1.9;3.6]	<0.001
Decaux-HR		17.6 [6.1;26.3] vs. 33.1 [26.4;41]	2.4 [1.4;4.2]	0.001	28.4 [20.1;41.3] vs. 64.9 [53.5;72.9]	2.2 [1.6;3.0]	<0.001

		OS					
		HM			LR		
		% survival at 60 months [95%CI]	HR [95%CI]	*P*-value	% survival at 60 months [95%CI]	HR [95%CI]	*P*-value
GPI	median cut	56.7 [39.7;70.6] vs. 75.6 [62.1;84.9]	1.8 [1.0;3.4]	0.05	57 [48;65] vs. 72 [63.4;79]	1.7 [1.1;2.5]	0.003
	high vs. median vs. low	39.4 [10.9;67.6] vs. 60.7 [41.4;75.3] vs. 75.4 [61.9;84.7]	--	0.002	35.7 [18.1;54.6] vs. 61.4 [51.4;69.9] vs. 72 [63.4;79]	--	<0.001
	median vs. low	--	1.5 [0.8;2.8]	0.3	--	1.5 [1.0;2.2]	0.06
	high vs. median	--	3.3 [1.3;8.0]	0.006	--	2.8 [1.6;4.8]	<0.001
ISS	III vs. II vs. I	43.2 [17.4;66.7] vs. 67.4 [52.9;78.2] vs. 75.9 [57.3;87.6]	2.3 [1.1;4.9] 4.3 [1.9;10.0]	0.002	45.8 [31.6;58.8] vs. 59.8 [47.1;70.3] vs. 73.8 [65.7;80.3]	1.6 [1.0;2.6] 2.6 [1.6;4.0]	<0.001
B2M35		55.5 [39.8;68.6] vs. 76.5 [62.3;85.9]	3.0 [1.6;5.7]	<0.001	52.2 [42.1;61.3] vs. 73.2 [65.5;79.4]	2.0 [1.4;2.9]	<0.001
t(4;14) *		51.6 [30.9;68.8] vs. 69.5 [57.2;79]	2.9 [1.5;5.6]	<0.001	43.3 [30.6;61.7] vs. 68.3 [62.3;74.8]	2.2 [1.4;3.4]	<0.001
17p13		63.5 [49.7;74.5] vs. 64.3 [42.3;79.8]	1.5 [0.3;1.3]	0.3	--	--	--
Shaughnessy-HR		n.a. [n.a.] vs. 71.7 [60.8;80.0]	2.5 [1.2;4.9]	0.008	36.4 [23.6;49.3] vs. 71.4 [64.6;77.1]	3.5 [2.4;5.2]	<0.001
Decaux-HR		n.a. [n.a.] vs. 69.5 [58.8;77.9]	2.5 [1.1;5.6]	0.03	47.2 [34.7;58.8] vs. 70.4 [63.4;76.3]	2.3 [1.6;3.3]	<0.001

B

		EFS		OS			EFS		OS	
		HM	LR	HM	LR		HM	LR	HM	LR
		P-value	*P*-value	*P*-value	*P*-value		*P*-value	*P*-value	*P*-value	*P*-value
GPI	cont.	<0.001	<0.001	<0.001	<0.001		--	--	--	--
B2M	cont.	<0.001	<0.001	<0.001	<0.001		--	--	--	--
GPI + B2M	GPI cont.	<0.001	<0.001	<0.001	<0.001	GPI high/low	0.004	0.02	0.2	0.007
	B2M cont.	0.09	<0.001	0.04	<0.001	B2M 3.5	0.1	<0.001	0.001	<0.001
	model (logrank)	<0.001	<0.001	<0.001	<0.001	model (logrank)	0.002	<0.001	<0.001	<0.001
GPI + ISS	GPI cont.	<0.001	<0.001	0.004	<0.001	GPI high/low	0.004	0.02	0.1	0.009
	ISS	0.06	<0.001	0.04	<0.001	ISS	0.02	<0.001	0.001	<0.001
	model (logrank)	<0.001	<0.001	<0.001	<0.001	model (logrank)	<0.001	<0.001	<0.001	<0.001
GPI + t(4;14) *	GPI cont.	<0.001	<0.001	<0.001	<0.001	GPI high/low	0.03	0.004	0.08	0.002
	t(4;14)	0.003	<0.001	0.004	<0.001	t(4;14)	<0.001	<0.001	0.001	<0.001
	model (logrank)	<0.001	<0.001	<0.001	<0.001	model (logrank)	<0.001	<0.001	0.001	<0.001
GPI + 17p13	GPI cont.	<0.001	--	<0.001	--	GPI high/low	0.04	--	0.2	--
	del17p	0.5	--	0.4	--	del17p	0.5	--	0.3	--
	model (logrank)	<0.001	--	<0.001	--	model (logrank)	0.08	--	0.2	--
GPI + Avet-Loiseau-HR	GPI cont.	<0.001	--	0.004	--	GPI high/low	0.01	--	0.2	--
	Avet-Loiseau-HR	1	--	0.6	--	Avet-Loiseau-HR	0.005	--	0.2	--
	model (logrank)	<0.001	--	<0.001	--	model (logrank)	<0.001	--	0.1	--
GPI + B2M + t(4;14) *	GPI cont.	<0.001	<0.001	<0.001	<0.001		--	--	--	--
	B2M cont.	0.005	<0.001	<0.001	<0.001		--	--	--	--
	t(4;14)	0.003	<0.001	0.003	<0.001		--	--	--	--
	model (logrank)	<0.001	<0.001	<0.001	<0.001		--	--	--	--
GPI + Shaughnessy-HR	GPI cont.	<0.001	0.04	0.003	0.09	GPI high/low	0.01	0.1	0.2	0.2
	Shaughnessy-HR	0.7	<0.001	0.3	<0.001	Shaughnessy-HR	0.05	<0.001	0.03	<0.001
	model (logrank)	<0.001	<0.001	<0.001	<0.001	model (logrank)	0.001	<0.001	0.01	<0.001
GPI + Decaux-HR	GPI cont.	0.001	0.01	<0.001	0.004	GPI high/low	0.02	0.09	0.2	0.05
	Decaux-HR	0.5	<0.001	0.8	0.009	Decaux-HR	0.02	<0.001	0.2	<0.001
	model (logrank)	<0.001	<0.001	<0.001	<0.001	model (logrank)	<0.001	<0.001	0.04	<0.001
GPI + B2M + t(4;14) * + Shaughnessy-HR	GPI cont.	<0.001	0.02	0.007	0.1		--	--	--	--
	B2M cont.	0.005	<0.001	<0.001	<0.001		--	--	--	--
	t(4;14)	0.07	<0.001	0.008	0.04		--	--	--	--
	Shaughnessy-HR	1	0.05	0.9	0.001		--	--	--	--
	model (logrank)	<0.001	<0.001	<0.001	<0.001		--	--	--	--
GPI + B2M + t(4;14) * + Decaux-HR	GPI cont.	0.001	0.008	<0.001	0.003		--	--	--	--
	B2M cont.	0.005	<0.001	<0.001	<0.001		--	--	--	--
	t(4;14)	0.003	<0.001	0.002	<0.001		--	--	--	--
	Decaux-HR	1	0.1	0.3	0.4		--	--	--	--
	model (logrank)	<0.001	<0.001	<0.001	<0.001		--	--	--	--

Online Supplementary Table S4. Prognostic factors tested (A) as single variables and (B) within different Cox-proportional hazard regression models and log-rank test. Explorative *P* values are shown. Significant values are depicted in red. EFS, event-free survival; OS, overall survival; HM, Heidelberg/ Montpellier group; LR, Little Rock group; CI, confidence interval; GPI, gene expression-based proliferation index; ISS, International Staging System; HR, high-risk score; logrank(model) *P* value (log-rank test) for the respective model including the factors detailed above in each row; n.a. not available. B2M and GPI are tested as continuous variables in the left two columns, all other variables including the HR-scores as di- or tri-chotomized variables.

A

HM		LR					
Correlation	P-value	Correlation	P-value	Probeset ID	Symbol	Chromosome	No.
0.95	0	**0.85**	0	204033_at	**TRIP13**	5p15.33	3
0.95	0	0.78	0	204092_s_at	**STK6**	20q13.2-q13.3	12
0.94	0	**0.86**	0	225834_at	**MGC57827**	1q21	19
0.91	0	**0.88**	0	219918_s_at	**ASPM**	1q31	9
0.91	0	**0.89**	0	206364_at	**KIF14**	1pter-q31.3	43
0.84	0	**0.83**	0	226936_at	**C6orf173**	6q22	30
0.84	0	0.73	0	205235_s_at	**MPHOSPH1**	10q23.31	51
0.82	0	0.73	0	204023_at	**RFC4**	3q27	16
0.81	0	0.72	0	201897_s_at	**CKS1B**	1q21.2	17
0.81	0	0.75	0	224200_s_at	**RAD18**	3p25-p24	39
0.78	0	0.48	0	201231_s_at	ENO1	1p36.3-p36.2	28
0.77	0	0.72	0	203432_at	TMPO	12q22	45
0.72	0	0.43	0	201614_s_at	RUVBL1	3q21	33
0.71	0	0.39	0	220789_s_at	TBRG4	7p14-p13	24
0.69	0	0.69	0	212533_at	WEE1	11p15.3-p15.1	47
0.68	0	0.58	0	201947_s_at	CCT2	12q15	10
0.67	0	0.53	0	216194_s_at	CKAP1	19q13.11-q13.12	18
0.67	0	0.48	0	221970_s_at	DKFZP586L0724	17q24.2	46
0.66	0	0.61	0	1555864_s_at	PDHA1	Xp22.2-p22.1	2
0.66	0	0.06	0.263	204016_at	LARS2	3p21.3	7
0.66	0	0.57	0	208117_s_at	FLJ12525	xq12-q13	14
0.66	0	0.39	0	200634_at	PFN1	17p13.3	21
0.66	0	0.3	0	213310_at	EIF2C2	8q24	26
0.65	0	0.57	0	225082_at	CPSF3	2p25.1	35
0.64	0	0.33	0	222417_s_at	SNX5	20p11	40
0.62	0	0.42	0	202345_s_at	FABP5	8q21.13	1
0.62	0	0.52	0	218947_s_at	PAPD1	10p11.23	25
0.62	0	0.52	0	200750_s_at	RAN	12q24.3	42
0.61	0	0.24	0	210334_x_at	BIRC5	17q25	15
0.6	0	0.35	0	58696_at	EXOSC4	8q24.3	31
0.59	0	0.44	0	211576_s_at	SLC19A1	21q22.3	6
0.59	0	0.49	0	210460_s_at	PSMD4	1q21.2	41
0.58	0	0.3	0	213535_s_at	UBE2I	16p13.3	11
0.56	0	0.49	0	224523_s_at	MGC4308	3q12.1	27
0.55	0	0.32	0	213607_x_at	FLJ13052	1p36.33-p36.21	13
0.54	0	0.47	0	1565951_s_at	OPN3	1q43	8
0.53	0	0.25	0	200966_x_at	ALDOA	16q22-q24	34
0.53	0	0.44	0	201091_s_at	CBX3	7p15.2	44
0.51	0	0.48	0	208931_s_at	ILF3	19p13.2	22
0.5	0	0.48	0	1555274_a_at	SELI	2p24.1	5
0.5	0	0.35	0	200916_at	TAGLN2	1q21-q25	32
0.5	0	0.36	0	242488_at	NA	1q43	36
0.36	0	0.14	0.01	200638_s_at	YWHAZ	8q23.1	50
0.35	0	0.08	0.12	213194_at	ROBO1	3p12	48
0.32	0	0.36	0	243011_at	MGC15606	3q12.3	37
0.25	0	0.01	0.827	200850_s_at	AHCYL1	1p13.2	57
0.24	0	0.23	0	201105_at	LGALS1	22q13.1	38
0.23	0	0.16	0.003	238952_x_at	DKFZp779O175	19q13.12	20
0.22	0	0.37	0	206332_s_at	IFI16	1q22	23
0.17	0.003	0.12	0.028	217901_at	DSG2	18q12.1	29
0.05	0.375	-0.09	0.079	230192_at	RFP2	13q14	66
0.05	0.427	-0.14	0.01	212435_at	TRIM33	1p13.1	70
0.04	0.454	0.1	0.068	227278_at	NA	1p13	53
-0.03	0.662	-0.03	0.568	1557277_a_at	NA	6p21.31	61
-0.07	0.215	-0.06	0.306	226954_at	UBE2R2	9p13.2	64
-0.08	0.158	0.17	0.002	206513_at	AIM2	1q22	4
-0.09	0.112	-0.08	0.142	218924_s_at	CTBS	1p22	63
-0.1	0.095	-0.11	0.035	201921_at	GNG10	9q31.3	52
-0.1	0.087	0.05	0.315	48106_at	FLJ20489	12q13.11	67
-0.11	0.063	-0.11	0.044	1554736_at	PARG1	1p22.1	62
-0.18	0.002	-0.14	0.012	237964_at	NA	11q13.1	68
-0.19	0.001	-0.1	0.074	202838_at	FUCA1	1p34	65
-0.22	0	-0.14	0.01	244686_at	TCOF1	5q32-q33.1	49
-0.22	0	-0.37	0	222495_at	AD-020	1p13.3	60
-0.26	0	-0.07	0.228	227547_at	NA	20q11.21	55
-0.27	0	-0.14	0.012	202729_s_at	LTBP1	2p22-p21	69
-0.31	0	-0.05	0.379	209740_s_at	PNPLA4	Xp22.3	54
-0.32	0	-0.18	0.001	209717_at	EVI5	1p22	59
-0.47	0	-0.2	0	213628_at	MCLC	1p13.3	58
-0.63	0	-0.28	0	225582_at	KIAA1754	10q25.1	56

Online Supplementary Table S5. Association of high-risk scores and proliferation. (A) UAMS high-risk score (Shaughnessy-HR), (B) IFM high-risk score (Decaux-HR). GPI, gene-expression based proliferation index HM, Heidelberg/Montpellier group; LR, Little Rock group. Pearson's correlation coefficients are given. *P* values below 0.0001 are reported as 0.

B

HM		LR					
Correlation	P-value	Correlation	P-value	Probeset ID	Symbol	Chromosome	No.
0.81	0	**0.86**	0	200783_s_at	**STMN1**	1	4
0.72	0	0.2	0	228737_at	**TOX2**	20	15
0.69	0	0.37	0	202486_at	**AFG3L2**	18	8
0.52	0	0.34	0	202470_s_at	**CPSF6**	12	2
0.51	0	0.22	0	212098_at	**NA**	NA	9
0.49	0	0.32	0	202951_at	**STK38**	6	3
0.44	0	0.39	0	208644_at	**PARP1**	1	1
0.43	0	0.31	0	231736_x_at	**MGST1**	12	6
0.32	0	0.44	0	217752_s_at	**CNDP2**	18	7
0.24	0	0.13	0.015	204072_s_at	**FRY**	13	14
0.21	0	0.22	0	228677_s_at	RASAL3	19	11
-0.16	0.005	-0.08	0.116	200779_at	ATF4	22	12
-0.31	0	0.02	0.78	203657_s_at	CTSF	11	13
-0.38	0	-0.13	0.012	209683_at	FAM49A	2	10
-0.48	0	-0.23	0	201425_at	ALDH2	12	5

	GPI	GEP-SH	GEP-B
Genes	50 Genes (see Figure 2)	TOP2A, BIRC5, CCNB2, NEK2, ANAPC7, STK6, BUB1, CDC2, C10orf3, ASPM, CDCA1	TYMS, TK1, CCNB1, MKI67, KIAA101, KIAA0186, CKS1B, TOP2A, UBE2C, ZWINT, TRIP13, KIF11
Gene Selection	Based on genes over-expressed in proliferating malignant (HMCL) as well as non-malignant cells (PPC) compared to non-proliferating, non-malignant cells (BMPC, MBC), carrying the GO-Term "cell proliferation" or "cell cycle".	"Genes associated with proliferation".	"Genes associated with proliferation".
Model	Sum of expression values of each of the 50 genes. For genes not expressed as judged by PANP, the expression level of the respective gene is defined 0.	Normalized value of 11 genes associated with proliferation scaled to the maximum value among plasma cell samples, myeloma samples and cell lines.	Median value of 12 genes associated with proliferation, scaled to the maximum value among all samples.
Published Validation	Correlation with plasma-cell labeling index.	None.	Correlation with plasma-cell labeling index.

Online Supplementary Table S6. Overview of the different gene expression-based proliferation indices used. GPI, gene expression based proliferation index; GEP-SH gene expression-based proliferation index from Shaughnessy's group; GEP-B gene expression-based proliferation index from Bergsagel *et al.*; HMCL, human myeloma cell line; PPC, polyclonal plasmablastic cells; BMPC, normal bone marrow plasma cells; MBC, memory B cells.