SUPPLEMENT

METHODS

GVHD prophylaxis, diagnosis, and treatment
GVHD was clinically and histologically diagnosed and graded using standard criteria. Prophylaxis and treatment of GVHD were performed as reported previously [1]. In both centres, recipients of allografts from unrelated donors (UD) usually received antithymocyte globulin (ATG) treatment. However, in both centres there are also exceptions from this institutional policy which are reflected by the fact that ATG use does not match the proportion of UD allografts.

Assessment of serum levels of total IL-18, IL-18-binding protein A, CXCL9/MIG, ST2 and calculation of free IL-18 serum levels
Serum levels of IL-18, IL-18-binding protein A (IL-18BPa), C-X-C motif ligand 9 (CXCL9)/monokine induced by gamma-interferon (MIG) and soluble suppression of tumorigenecity 2 (ST2) were assessed by ELISA using commercial kits (DuoSet, R&D Systems, Wiesbaden, Germany) according to the manufacturer's instructions. All measurements were performed centrally in the Heidelberg centre. The concentration of free IL-18 was calculated on the basis of the concentrations of total IL-18 and total IL-18BPa as determined by ELISA applying the law of mass action, a 1:1 stoichiometry in the complex of IL-18 and IL-18BPa and a dissociation constant (Kd) of 400 pM [2]. All values were given in pg/mL.

Single nucleotide polymorphism (SNP) analyses
A total of 9 polymorphisms for IL-18 and IL18-BP genes were identified by tagging approach from 1000 Genomes database for the Caucasian population. A region of 40 kb (hg19:11:112,003,974-112,044,840) flanking IL-18 was screened and 5 SNPs (rs360718, rs544354, rs5744256, rs7106524, rs5744276) with minor allele frequency ≥0.05 were selected. A region of 27 kb (hg19:11:71,699,587-71,726,761) containing IL-18BP was screened and 4 SNPs (rs2298455, rs3814721, rs61890895, rs3793941) that have minor allele frequency ≥0.05 were selected. Genomic DNA from patients and controls was genotyped using an allele-specific method (TaqMan technology, Applied Biosystems, Foster City, CA). For quality control purpose, 10% of the samples were blindly replicated. PCR plates were read on a ViiA7 real time instrument (Applied Biosystems) and QuantStudio Real-Time PCR Software was used to call genotypes. The 5 polymorphisms for IL-18 (rs5744276, rs7106524, rs5744256, rs544354, rs360718) and the 4 polymorphisms for IL-18BP (rs61890895, rs3814721, rs2298455, rs3793941) tag an additional 60 SNPs (r^2 ≥0.8) based on 1000 genome database for CEU population.

Supplemental statistical methods
For definition of relapse standard disease corresponding criteria were applied. All patients were followed regularly post-transplant. Besides assessment of clinical signs of disease progression (including blood differential), the remission status post-transplant was assessed on regular basis on day +28 for patients with myeloproliferative neoplasms, myelodysplastic syndrome, multiple myeloma and acute leukaemia (bone marrow, MRD, donor chimerism). Remission status for lymphoma patients, and in cases when imaging techniques were necessary, was usually assessed on day+100. In patients with progressive disease prior to transplant, time to relapse was defined as time from day of transplant to first signs of relapsing disease including both standardised criteria (bone marrow, serology, and imaging) and clinical signs of disease progression/relapse.
To assess the relation of pre-transplant free IL-18 on age and hematopoietic cell transplantation-specific comorbidity index (HCT-CI according to Sorror et al. [3]), the Jonckheere trend test was applied [4]. The Kaplan-Meier method was used to estimate distributions of survival times. Follow-up times were calculated by the reverse Kaplan-Meier estimate [5].
Cause-specific Cox regression was applied for non-relapse mortality (NRM). Since the primary endpoint in our study was overall survival (OS) after alloSCT, the cause-specific approach, which allows for separate assessment of the relationship between each covariate and each hazard of interest (in our case, NRM and disease relapse) was chosen over the subdistribution hazards regression model, as it may provide more insight into the mechanisms leading to treatment failure (i.e. death post-transplant). The advantage of cause-specific Cox regression is that it gives detailed insights into the relationship between a risk factor and each separate outcome. Its major shortcoming of is represented by the fact that the cumulative incidence of a specific event (for example NRM) is dependent on the cause-specific hazards of all events. In contrast, the coefficients resulting from the subdistribution hazards regression model do have a direct relationship with the cumulative incidence, but the insight into the effect of a covariate on a cause-specific hazard instead of a probability is lost.
Violation of proportional hazards assumption was tested for by the cox.zph plots, which give an estimate of the time-dependent coefficient $\beta(t)$. Since the proportional hazards assumption for pre-transplant free IL-18 was

violated for OS after the 5th post-transplant year, the data set was restricted to the first 5-year epoch post-transplant. Since mortality following the onset of acute GVHD is most pronounced in the first year after alloSCT the observation period for the associations of pre-transplant free IL-18 with OS and NRM after acute GVHD was confined to the first year after onset of acute GVHD.
To illustrate the continuous effects on the different endpoints, patients were grouped according to the quartiles of free IL-18 and the observation period was restricted to the first 5 years post-transplant for pre-transplant free IL-18 and the first post-transplant year for day+28 (d+28) free IL-18.
Prediction error curves and concordance indices were generated to evaluate the uni- and multivariable effects applying Cox models on OS and cause-specific Cox models on NRM based on the effect of pre-transplant free IL-18. To validate the effect of pre-transplant free IL-18 the model was fitted with an offset which was equal to the effect in the model from the training cohort. Through the offset, the effect was transferred to the model for the validation cohort. The models included the same covariates: age, disease stage, diagnosis, MMUD *versus* RD/MUD, conditioning intensity (reduced-intensity conditioning *versus* others), ATG treatment, and sex of donor and recipient re-estimated on the validation cohort to consider possible differences between the cohorts. The 632+ bootstrap method was applied for the estimation of the prediction error [6,7]. In all, 1,000 bootstrap subsamples were drawn from the validation data set, and the two models explained above were fitted in each subsample. The resulting predictive model was then fitted to the out-of-bag sample (patients not contained in the respective subsample) to estimate the survival probabilities and the time-dependent prediction error for all event times in the validation data set. Prediction error was estimated by using the Brier score and calculated as a weighted combination of the apparent estimate and the bootstrap estimate [6,8].
Comparison of the predictive performance of pre-transplant free IL-18 and d+28 free IL-18 was done by receiver operating characteristics (ROC) analysis using the DeLong's test [9] and by calculating the prediction error (integrated Brier score).
The relationship between pre-transplant serum levels of total and free IL-18, IL-18BPa and CXCL9/MIG and pre-transplant free IL-18 and absolute neutrophil counts on d+28 post-transplant were assessed by Spearman's rank correlation coefficients.
Genotype frequencies of SNPs were tested in patients for deviation from Hardy-Weinberg equilibrium (HWE) using Pearson's $\chi 2$ test.
In order to provide guidance for possible interventions in the setting of alloSCT, an optimal cut-off determination with respect to NRM was performed in the training set. All pre-transplant free IL-18 levels were evaluated as cut-points and plotted against the corresponding Gray's test statistics in cumulative incidence function [10]. The resulting curve is a step function with jumps at every cut-point. The cut-off with highest Gray's test statistic was used as the optimal cut-off with clinical relevance also considered.
All statistical analyses were carried out in statistical software R, version 3.4.3, together with the R packages 'survival', version 2.43.3, 'cmprsk', version 2.2-7, 'maxstat', version 0.7-25, 'pec' version 2.5.4, 'DescTools' version 0.99.24, 'prodlim', version 2018.04.18, 'pec', version 2018.7.26, and 'riskRegression', version 1.43.

RESULTS

Correlations of pre-transplant serum levels of free IL-18 with additional patient characteristics in the training cohort

Pre-transplant free IL-18 levels were correlated to patient characteristics that reflect health and performance status, inflammatory states and comorbidities prior to transplant: age, sex, diagnosis, body-mass index (BMI), levels of C-reactive protein (CRP), Karnofsky performance status (KPS), HCT-CI and specific comorbidities prior to alloSCT (presence of diabetes, cardiovascular disease and infection as defined by Sorror et al. [3]). Information on BMI, CRP, KPS, HCT-CI and specific comorbidities were only available for the training cohort (supplemental **Table S1**).
Serum levels of pre-transplant free IL-18 were significantly lower in patients allografted for multiple myeloma (supplemental **Figure S1A**). However, in univariable analysis, the associations of higher pre-transplant free IL-18 levels with worse OS were similar in patients allografted for multiple myeloma and other malignancies (per 1-log2 increase: HR 1.92, 95% CI 1.24-2.97, P=0.004 and HR 1.24 95% CI 1.05-1.47, P=0.01, respectively). Median serum levels of pre-transplant free IL-18 were higher in patients with increased (>5 mg/L) pre-transplant CRP levels and lower (≤80%) KPS (supplemental **Figure S1C** and **S1G**). Further, there was a trend towards higher pre-transplant free IL-18 levels in patients with more advanced age and higher hematopoietic cell transplantation-specific comorbidity index (HCT-CI) (Jonckheere trend test, supplemental **Figure S1B** and **S1D**). No significant associations were found for patient sex, obesity (BMI ≥30 kg/m^2), presence of cardiovascular disease, diabetes or infection prior to alloSCT and immunosuppression regimen and pre-transplant serum levels of ST2 (supplemental **Figure S1E, S1F, S1H–L**).

Predictive value of pre-transplant free IL-18

The predictive performance of pre-transplant free IL-18 was assessed by calculation of the time-dependent concordance index and prediction error curves at all event times of the Cox models on OS and the cause-specific Cox models on NRM. In both cohorts, the univariable pre-transplant free IL-18 models showed a slightly improved prediction performance as compared to the respective references (supplemental **Figure S2**). In the context of the multivariable analyses, in both the training and the validation cohort prediction errors were lower and concordance indices were higher for the models that included pre-transplant free IL-18. Thus, a predictive value of pre-transplant free IL-18 for non-relapse and overall mortality can be assumed (supplemental **Figure S3**).

Table S1. Additional patient characteristics of the training cohort.

	Training cohort n=589
Parameter	
Karnofsky performance status, n (%)	
>80%	481 (84)
≤80%	95 (16)
Unknown	13
Pre-transplant CRP [mg/L], median (IQR)	4.8 (1-12.0)
Pre-transplant CRP, n (%)	
≤5 mg/L	299 (51)
>5 mg/L	284 (49)
Unknown	6
Pre-transplant BMI [kg/m^2], median (IQR)	24.9 (22.6-28.1)
Pre-transplant BMI, n (%)	
<18.5 kg/m^2	11 (2)
18.5-29.9 kg/m^2	445 (81)
≥30 kg/m^2	98 (17)
Unknown	35
HCT-CI[a], n (%)	
0	93 (18)
1+2	148 (28)
≥3	286 (54)
Unknown	62
Diabetes mellitus[b], n (%)	
Present	23 (4)
Absent	563 (96)
Unknown	3
Cardiovascular disease[c], n (%)	
Present	68 (12)
Absent	518 (88)
Unknown	3
Infection[d], n (%)	
Present	47 (8)
Absent	539 (92)
Unknown	3
Immunosuppression post-transplant, n (%)	
CsA + MTX	163 (28)
CsA + MMF	315 (53)
FK + MTX	14 (2)
FK + MMF	87 (15)
Post-transplant cyclophosphamide	10 (2)
Pre-transplant ST2 serum level [pg/mL][e], median (IQR)	542 (308-1007)

Abbreviations: BMI, body-mass index; CRP, C-reactive protein; CsA, cyclosporine A; FK, FK506 (tacrolimus); HCT-CI, hematopoietic cell transplantation-specific comorbidity index; IQR, interquartile range, MMF, mycophenolate mofetil; MTX, methotrexate; ST2, soluble suppression of tumorigenecity 2.
[a]HCT-CI according to Sorror et al. [3].
[b]Requiring treatment with insulin or oral hypoglycaemic agents but not diet alone.
[c]Congestive heart failure, myocardial infarction, or ejection fraction <50%, one or more vessel-coronary artery stenosis requiring medical treatment, stent, or bypass.
[d]Infection by date of HCT-CI assessment requiring continuation of antimicrobial treatment after day 0.
[e]Pre-transplant ST2 serum levels were available for 345 patients of the training cohort.

Table S2. Association of pre-transplant free interleukin-18 with overall survival (OS), non-relapse mortality (NRM) and relapse after onset of acute GVHD in the training and validation cohorts (univariable analysis).

	Training cohort[a] n=225		**Validation cohort[b] n=537**	
	Free IL-18 per 1-log2 increase[c]		**Free IL-18 per 1-log2 increase[c]**	
	HR 95%CI	***P***	**HR 95%CI**	***P***
Endpoint				
OS after onset of aGVHD	1.31 (0.97-1.77)	0.08	1.32 (1.10-1.60)	0.003
NRM* after onset of aGVHD	1.36 (0.92-2.01)	0.13	1.37 (1.08-1.77)	0.01
Relapse* after onset of aGVHD	1.03 (0.75-1.43)	0.84	1.12 (0.88-1.42)	0.36

[a]Number of events: OS after acute GVHD, n=78; NRM after acute GVHD, n=46; relapse after onset of acute GVHD, n=47.
[b]Number of events: OS after acute GVHD, n=208; NRM after acute GVHD, n=123; relapse after onset of acute GVHD, n=118.
[c]Doubling of pre-transplant free IL-18 serum level.
*Cause-specific hazard ratio.
Abbreviations: aGVHD, acute graft-*versus*-host disease; HR, hazard ratio; CI, confidence interval; IL-18, interleukin-18; HR, hazard ratio; OS, overall survival; NRM, non-relapse mortality.

Table S3. Multivariable analysis of the training cohort with the endpoints overall survival (OS), non-relapse mortality (NRM) and relapse after allogeneic stem cell transplantation considering pre-transplant free IL-18 and additional patients characteristics as confounding variables (complete case analysis).

	Training cohort n=484					
	OS		**NRM***		**Relapse***	
	HR 95% CI	***P***	**HR 95% CI**	***P***	**HR 95% CI**	***P***
Free IL-18 per 1-log2 increase[a]	1.22 (1.02-1.46)	0.03	1.35 (1.01-1.81)	0.04	1.06 (0.88-1.29)	0.53
Age per 1-year increase	1.01 (0.99-1.02)	0.37	1.03 (1.01-1.05)	0.01	0.99 (0.98-1.01)	0.43
KPS						
>80%	Ref		Ref		Ref	
≤80%	1.69 (1.19-2.40)	0.003	1.94 (1.12-3.35)	0.02	1.84 (1.24-2.73)	0.002
BMI						
<30 kg/m^2	Ref		Ref		Ref	
≥30 kg/m^2	1.08 (0.76-1.54)	0.65	1.20 (0.70-2.06)	0.51	1.24 (0.83-1.84)	0.29
CRP						
≤5 mg/L	Ref		Ref		Ref	
>5 mg/L	1.12 (0.84-1.49)	0.45	1.38 (0.89-2.16)	0.16	0.90 (0.65-1.25)	0.54
HCT-CI[b]						
<3	Ref		Ref		Ref	
≥3	1.11 (0.83-1.47)	0.48	0.92 (0.59-1.44)	0.73	1.38 (0.99-1.92)	0.06

Number of events: OS, n=213, NRM, n=86; relapse, n=163.
Abbreviations: BMI, body-mass index; CRP, C-reactive protein; HCT-CI, hematopoietic cell transplantation-specific comorbidity index; KPS, Karnofsky performance status; IQR, interquartile range.
*Cause-specific hazards from a competing risks analysis for relapse and NRM.
[a]Doubling of pre-transplant free IL-18 serum level.
[b]HCT-CI according to Sorror et al. [3].

Table S4. Pre-transplant serum levels of total IL-18, IL-18BPa and free IL-18 according to different single nucleotide polymorphisms (SNP) in the IL-18 (A) and IL-18BP (B) gene (training cohort).

A	**Total IL-18**			**IL-18BPa**			**Free IL-18**		
	Median	**IQR**	***P***	**Median**	**IQR**	***P***	**Median**	**IQR**	***P***
IL-18 SNP									
rs5744276			*0.688			*0.064			*0.582
GG	624	448-672	**0.540	5837	5484-11152	**0.030	399	292-518	**0.298
GC	616	433-899		5279	4624-11147		463	328-628	
CC	629	439-943		5377	4642-11879		466	327-703	
rs7106524			*0.281			*0.546			*0.202
AA	645	370-1073	**0.014	5527	4674-11564	**0.978	419	293-816	**0.504
AG	634	451-939		5330	4613-10354		475	330-646	
GG	607	428-788		5413	4690-11475		411	304-586	
rs5744256			*0.733			*0.138			*0.568
GG	660	442-728	**0.669	5837	5388-10472	**0.060	399	272-555	**0.381
AG	614	421-846		5355	4629-11245		461	314-611	
AA	622	437-931		5376	4642-11260		464	324-693	
rs544354			*0.942			*0.896			*0.736
AA	551	520-917	**0.827	5668	5420-5805	**0.709	407	386-702	**0.625
AG	612	542-934		5366	4654-7248		470	337-630	
GG	629	418-927		5388	4645-10576		460	310-655	
rs360718			*0.454			*0.894			*0.475
CC	616	499-934	**0.658	5388	4692-7014	**0.821	443	367-691	**0.644
AC	611	424-820		5305	4640-10476		458	297-592	
AA	632	433-970		5450	4659-11147		460	316-705	
B	**Total IL-18**			**IL-18BPa**			**Free IL-18**		
	Median	**IQR**	***P***	**Median**	**IQR**	***P***	**Median**	**IQR**	***P***
IL-18BP SNP									
rs61890895			*0.960			*0.515			*0.898
AA	692	612-700	**0.781	5077	4900-5233	**0.701	480	419-539	**0.807
AC	625	437-884		5580	4642-11354		438	315-606	
CC	622	435-914		5376	4652-11645		462	318-644	
rs3814721			*0.413			*0.692			*0.427
TT	334	334-334	**0.187	5233	5233-5233	**0.796	254	254-254	**0.199
CT	635	439-905		5580	4306-11147		471	315-615	
CC	618	430-916		5376	4674-11245		461	313-655	
rs2298455			*0.604			*0.918			*0.672
CC	437	381-762	**0.452	5464	4737-5765	**0.780	340	289-573	**0.562
AC	594	432-856		5388	4808-10985		420	314-622	
AA	629	446-929		5400	4649-11544		463	323-655	
rs3793941			*0.187			*0.299			*0.259
AA	629	443-995	**0.995	5356	4715-11564	**0.535	466	329-697	**0.890
AG	602	419-807		5363	4502-11261		419	295-580	
GG	637	450-931		5478	4905-11561		481	338-711	

Abbreviations: IL-18, interleukin-18; IQR, interquartile range.

**P* value for all (Kruskal-Wallis test: heterozygous genotype included); ***P* value for homozygous *versus* homozygous genotype.

Note: Hardy-Weinberg equilibrium was observed for all SNPs with the exception of the IL-18BP SNP rs3793941.

Table S5. Day +28 serum levels of total IL-18, IL-18BPa and free IL-18 according to different single nucleotide polymorphisms (SNP) in the IL-18 (A) and IL-18BP (B) gene (training cohort).

A	**Total IL-18**			**IL-18BPa**			**Free IL-18**		
	Median	**IQR**	***P***	**Median**	**IQR**	***P***	**Median**	**IQR**	***P***
IL-18 SNP									
rs5744276			*0.351			*0.663			*0.311
GG	710	572-1203	**0.266	17892	15428-20098	**0.752	353	267-580	**0.195
GC	943	693-1265		18297	15596-20691		452	344-606	
CC	890	642-1282		17632	14356-21091		443	330-583	
rs7106524			*0.438			*0.249			*0.420
AA	998	739-1269	**0.415	17724	14329-20082	**0.714	496	339-626	**0.256
AG	949	670-1283		18622	15335-21320		459	332-595	
GG	876	645-1260		17457	15011-2061		419	329-583	
rs5744256			*0.918			*0.579			*0.827
GG	937	583-1258	**0.845	19277	15537-22545	**0.306	410	276-627	**0.596
AG	933	678-1273		17788	15472-20617		448	336-605	
AA	928	649-1282		17752	14570-21077		447	337-583	
rs544354			*0.422			*0.206			*0.614
AA	1201	1028-1361	**0.182	21793	20693-22114	**0.100	549	486-595	**0.317
AG	931	653-1283		17722	14603-20821		459	323-627	
GG	933	673-1276		17768	15164-20865		446	338-588	
rs360718			*0.220			*0.033			*0.287
CC	791	636-1106	**0.194	16900	13564-17993	**0.020	406	330-530	**0.244
AC	884	653-1239		18123	15516-21069		435	336-564	
AA	944	675-1283		18092	14985-21209		482	325-627	
B	**Total IL-18**			**IL-18BPa**			**Free IL-18**		
	Median	**IQR**	***P***	**Median**	**IQR**	***P***	**Median**	**IQR**	***P***
IL-18BP SNP									
rs61890895			*0.738			*0.325			*0.938
AA	1255	849-1495	**0.481	22149	19758-23108	**0.156	530	370-637	**0.770
AC	864	668-1245		17659	13935-20377		429	336-618	
CC	933	656-1277		17817	15040-21092		445	332-591	
rs3814721			*0.309			*0.732			*0.225
TT	479	479-479	**0.154	17400	17400-17400	**0.936	236	236-236	**0.141
CT	1015	685-1269		17470	14043-20853		449	357-626	
CC	921	656-1277		18026	15321-21183		442	331-583	
rs2298455			*0.798			*0.910			*0.745
CC	1089	656-1230	**0.758	17702	14134-20047	**0.771	495	363-632	**0.618
AC	876	643-1246		17596	14086-21287		418	326-585	
AA	930	661-1290		17811	15250-20865		451	334-592	
rs3793941			*0.510			*0.560			*0.471
AA	986	665-1277	**0.301	18258	14268-21486	**0.960	446	345-605	**0.291
AG	886	664-1290		17307	15103-20705		443	329-627	
GG	896	642-1180		18393	15506-21091		435	325-549	

Abbreviations: IL-18, interleukin-18; IQR, interquartile range.

**P* value for all (Kruskal-Wallis test: heterozygous genotype included); ***P* value for homozygous *versus* homozygous genotype.

Note: Hardy-Weinberg equilibrium was observed for all SNPs with the exception of the IL-18BP SNP rs3793941.

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LEGEND TO SUPPLEMENTAL FIGURES

Figure S1. Analysis of median pre-transplant serum levels of free IL-18 according to additional patient characteristics in the training cohort.
Box plots are depicted. *P* values were derived by the Kruskal-Wallis Test (in A and K) and the Mann-Whitney *U* test (in C, E-J). For trend analysis in B, D and L the Jonckheere trend test was applied.
Abbreviations: ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; BMI, body-mass index; CRP, C-reactive protein; CsA, cyclosporine A; CVD, cardiovascular disease; FK, FK506 (tacrolimus); HCT-CI, hematopoietic cell transplantation-specific comorbidity index; IL-18, interleukin-18; KPS, Karnofsky performance status; MDS, myelodysplastic syndrome; MM, multiple myeloma; MMF, mycophenolate mofetil; MPN, myeloproliferative neoplasm; MTX, methotrexate; NHL, Non-Hodgkin lymphoma; PTCy, post-transplant cyclophosphamide; Q1-4, quartiles of the age distribution; ST2, soluble suppression of tumorigenecity 2.

Figure S2. Prediction performance of the univariable pre-transplant free IL-18 models with regard to overall survival (OS) and non-relapse mortality (NRM).
The prediction error curves (left panel) representing the Brier score over time for the univariable pre-transplant free IL-18 models (red curves) with regard to OS and NRM in the training cohort (A and B, respectively), and in the validation cohort (C and D, respectively) show better, albeit slightly improved, performance in comparison to the respective references (Kaplan-Meier survival estimates for OS, Aalen-Johansen estimator for NRM) (black curves). Corresponding concordance indices are given in the right panel.

Figure S3. Prediction performance of the multivariable pre-transplant free IL-18 models with regard to overall survival (OS) and non-relapse mortality (NRM).
The prediction error curves representing the Brier score over time for the univariable pre-transplant free IL-18 models (red curves) with regard to OS and NRM in the training cohort (A and B, respectively), and in the validation cohort (C and D, respectively) are depicted in the left panel. The models analyzed were cause-specific Cox models for OS and cause-specific Cox models for NRM. Covariates included age, disease stage, diagnosis, MMUD *versus* RD/MUD, conditioning intensity (reduced-intensity conditioning *versus* others), ATG treatment, and sex of donor and recipient.
"multiref" refers to the model that comprises only the confounding variables; no effect for pre-transplant free IL-18 has been estimated (blue curves). "multi+IL-18" refers to a multivariate model that takes into account the effect of pre-transplant free IL-18 (brown curves). The black curves represent the respective references (Kaplan-Meier survival estimates for OS, Aalen-Johansen estimator for NRM). Corresponding concordance indices are given in the right panel. Similar trends could be seen in both the training and the validation data set.

Figure S4. Validation of the effect of pre-transplant free IL-18 with regard to overall survival (OS) and non-relapse mortality (NRM).
Prediction error analysis showing superimposition of the prediction error curves for OS and NRM. "offset" refers to a model that includes the effect of pre-transplant free IL-18 on OS and NRM as an offset. The parameter estimate for the effect results from the model based on the training data set. "Validation cohort" refers to the model for which the effect of pre-transplant free IL-18 on OS and NRM was estimated directly on the basis of the validation data set. For the estimation of the prediction error, the 632+ bootstrap method was applied. The resulting prediction error curve is a step function with jumps at every event time in the validation data set. For a detailed description of the validation method see "supplemental Statistical Methods".

Figure S5. Relationship between pre-transplant serum levels of total and free IL-18, IL-18BPa and CXCL9/MIG.
(A) IL-18 is known to induce IFNγ. IFNγ conversely is known to increase the gene expression and synthesis of both IL-18BP and CXCL9/MIG.
(B) In the training cohort, IL-18 (total and free) and CXCL9/MIG and total IL-18 and IL-18BPa were positively (weakly) correlated, whereas IL-18BPa was moderately correlated to CXCL9/MIG. In the validation cohort, weak positive correlations between IL-18 (total and free) and CXCL9/MIG, and moderate positive correlations between total IL-18 and IL-18BPa, and IL-18BPa and CXCL9/MIG were observed.

Figure S6. Alluvial diagram showing the changes in the composition of the quartiles of the free IL-18 distribution between the time-points pre-transplant and d+28 (n=395).
When comparing the distribution of the individual patients according to the quartiles of pre-transplant free IL-18 and d+28 free IL-18, the majority of the patients remained in their respective quartiles (i.e. the majority of patients who constituted the upper two quartiles pre-transplant remained in the upper two quartiles of the free IL-

18 distribution on d+28 post-transplant). The median level of pre-transplant free IL-18 was 471 pg/mL (IQR 335-704); the median level of d+28 free IL-18 was 443 pg/mL (IQR 322-614).

Figure S7. Comparison of the predictive performance between pre-transplant free IL-18 and d+28 free IL-18 (as continuous variables) with regard to overall survival (OS) and non-relapse mortality (NRM) in the first post-transplant year.
(A) ROC analysis indicated similar prognostic associations of pre-transplant and d+28 free IL-18 with both post-transplant OS and NRM (Delong's test for OS and NRM: P=0.18 and P=0.81, respectively).
(B) Prediction error analysis showed superimposition of the prediction error curves for OS and NRM, again indicating similar prognostic associations of pre-transplant and d+28 free IL-18 with both post-transplant OS and NRM.

Figure S8. Illustration of the univariable associations of d+28 free IL-18 with overall survival (OS), non-relapse mortality (NRM) and relapse.
Higher levels of d+28 free IL-18 were associated with worse OS, due to a higher hazard of NRM rather than relapse. Hazard ratios per 1-log2 increase are given.

Figure S9. Heat map from Haploview.
Hardy-Weinberg equilibrium was observed for 4 polymorphisms of the IL-18 gene (rs5744276, rs7106524, rs5744256, rs544354, rs360718) and 3 polymorphisms of the IL-18BP gene (rs61890895, rs3814721, rs2298455).

Figure S10. Optimised cut-off of pre-transplant free IL-18 and associations with overall survival (OS) and non-relapse mortality (NRM) in the training and the validation cohorts.
(A) An optimal cut-off determination with regard to post-transplant NRM was conducted in the training set yielding multiple cut-points (maxima). The maximum at 470 pg/mL ($=2^{8.876}$) which also represents the median of the pre-transplant free IL-18 distribution in the training set, was chosen to stratify patients of both cohorts in high (≥470 pg/mL) and low (<470 pg/mL) pre-transplant free IL-18 groups.
(B, C) In the training cohort, high pre-transplant free IL-18 status was correlated with worse OS and increased NRM.
(D, E) Similar associations were also observed in the validation cohort.
Hazard ratios are given for high (≥470 pg/mL) *vs.* low (<470 pg/mL) pre-transplant free IL-18 group.

Figure S1

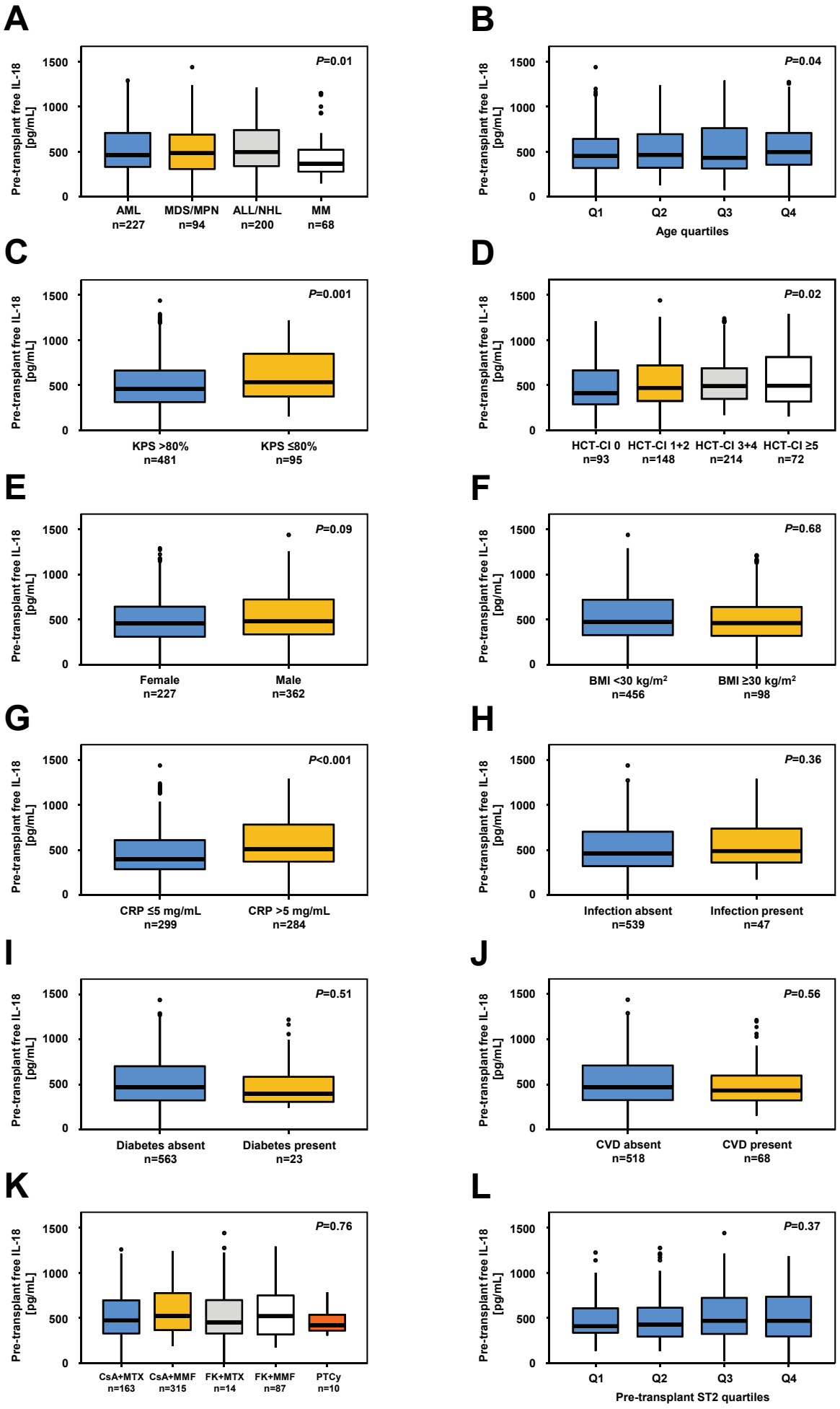

A
Pre-transplant free IL-18 [pg/mL]
1500
1000
500
0
P=0.01
AML n=227
MDS/MPN n=94
ALL/NHL n=200
MM n=68
B
P=0.04
Q1
Q2
Q3
Q4
Age quartiles
C
P=0.001
KPS >80% n=481
KPS ≤80% n=95
D
P=0.02
HCT-CI 0 n=93
HCT-CI 1+2 n=148
HCT-CI 3+4 n=214
HCT-CI ≥5 n=72
E
P=0.09
Female n=227
Male n=362
F
P=0.68
BMI <30 kg/m² n=456
BMI ≥30 kg/m² n=98
G
P<0.001
CRP ≤5 mg/mL n=299
CRP >5 mg/mL n=284
H
P=0.36
Infection absent n=539
Infection present n=47
I
P=0.51
Diabetes absent n=563
Diabetes present n=23
J
P=0.56
CVD absent n=518
CVD present n=68
K
P=0.76
CsA+MTX n=163
CsA+MMF n=315
FK+MTX n=14
FK+MMF n=87
PTCy n=10
L
P=0.37
Q1
Q2
Q3
Q4
Pre-transplant ST2 quartiles

Figure S2

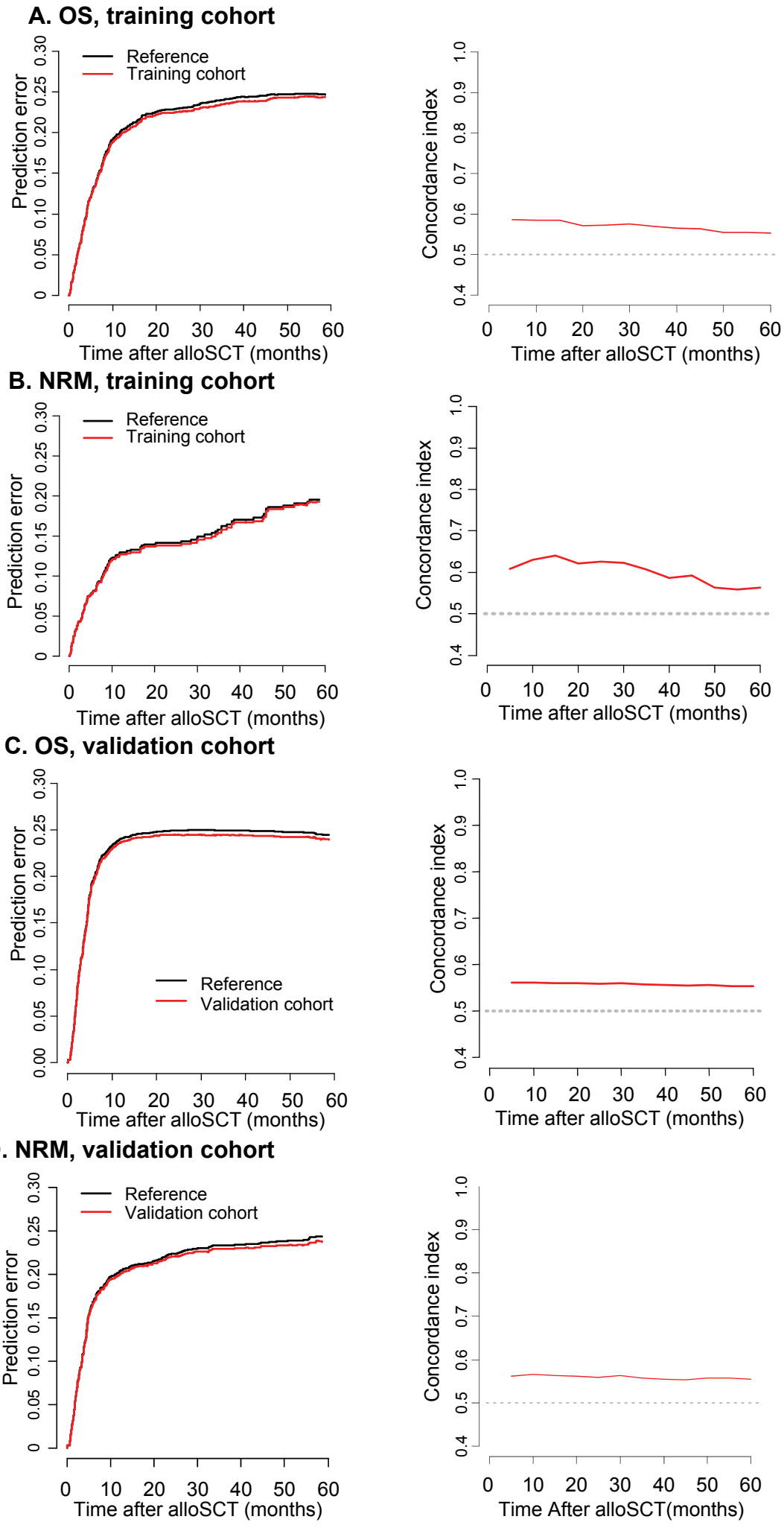

A. OS, training cohort
Reference
Training cohort
Prediction error
0 0.05 0.10 0.15 0.20 0.25 0.30
0 10 20 30 40 50 60
Time after alloSCT (months)
Concordance index
0.4 0.5 0.6 0.7 0.8 0.9 1.0
0 10 20 30 40 50 60
Time after alloSCT (months)
B. NRM, training cohort
Reference
Training cohort
Prediction error
0 0.05 0.10 0.15 0.20 0.25 0.30
0 10 20 30 40 50 60
Time after alloSCT (months)
Concordance index
0.4 0.5 0.6 0.7 0.8 0.9 1.0
0 10 20 30 40 50 60
Time after alloSCT (months)
C. OS, validation cohort
Reference
Validation cohort
Prediction error
0.00 0.05 0.10 0.15 0.20 0.25 0.30
0 10 20 30 40 50 60
Time after alloSCT (months)
Concordance index
0.4 0.5 0.6 0.7 0.8 0.9 1.0
0 10 20 30 40 50 60
Time after alloSCT (months)
D. NRM, validation cohort
Reference
Validation cohort
Prediction error
0 0.05 0.10 0.15 0.20 0.25 0.30
0 10 20 30 40 50 60
Time after alloSCT (months)
Concordance index
0.4 0.5 0.6 0.7 0.8 0.9 1.0
0 10 20 30 40 50 60
Time After alloSCT(months)

Figure S3

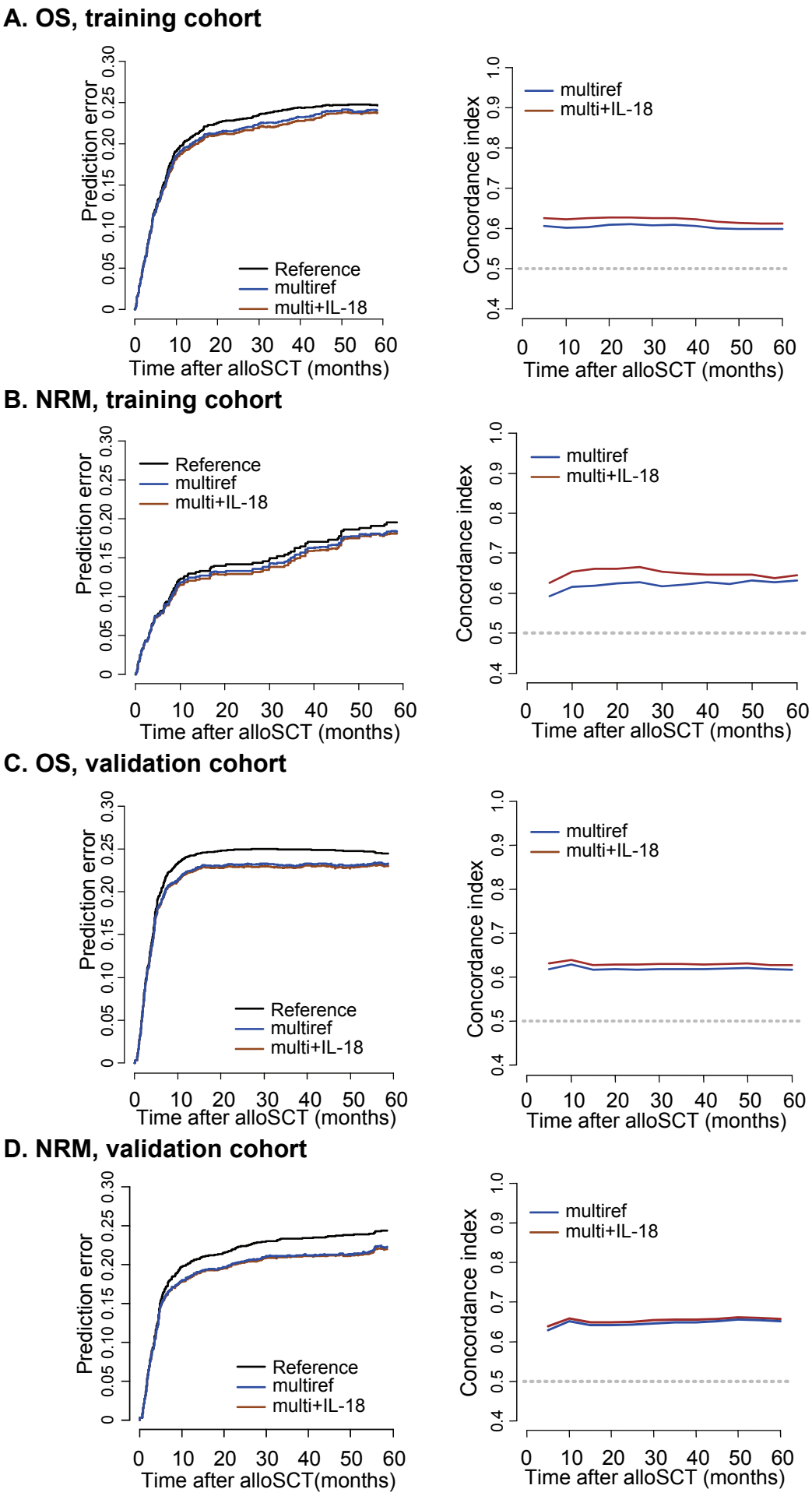

A. OS, training cohort
Prediction error
Reference
multiref
multi+IL-18
Time after alloSCT (months)
Concordance index
multiref
multi+IL-18
Time after alloSCT (months)
B. NRM, training cohort
Prediction error
Reference
multiref
multi+IL-18
Time after alloSCT (months)
Concordance index
multiref
multi+IL-18
Time after alloSCT (months)
C. OS, validation cohort
Prediction error
Reference
multiref
multi+IL-18
Time after alloSCT (months)
Concordance index
multiref
multi+IL-18
Time after alloSCT (months)
D. NRM, validation cohort
Prediction error
Reference
multiref
multi+IL-18
Time after alloSCT(months)
Concordance index
multiref
multi+IL-18
Time after alloSCT (months)

Figure S4

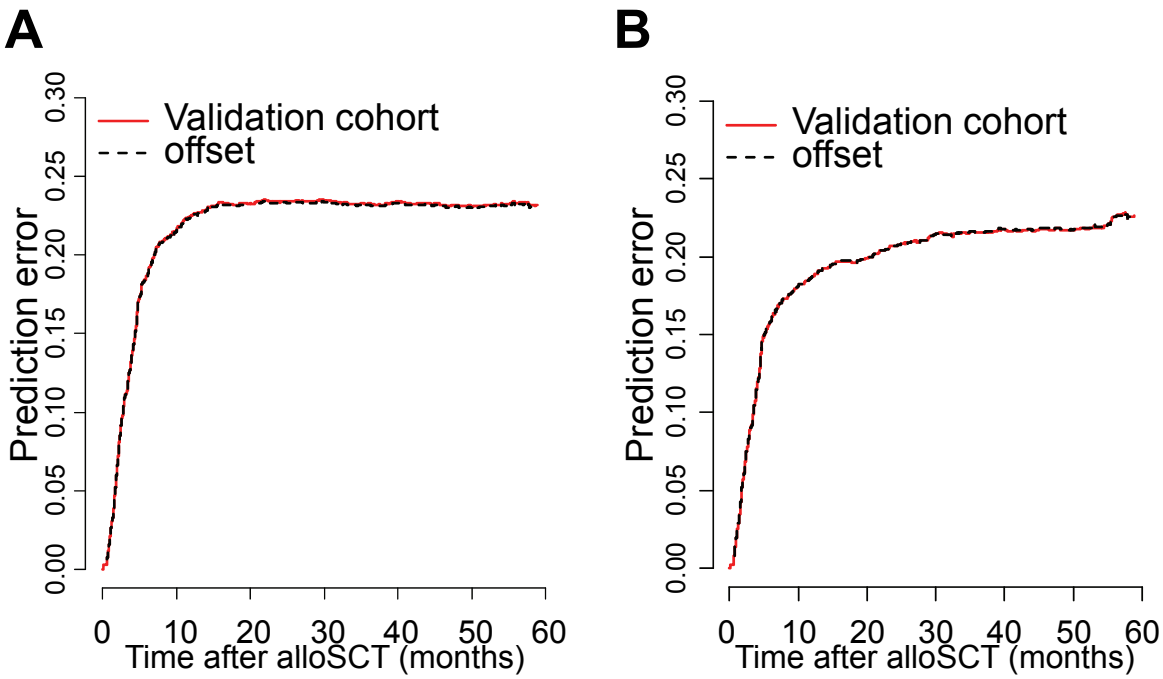
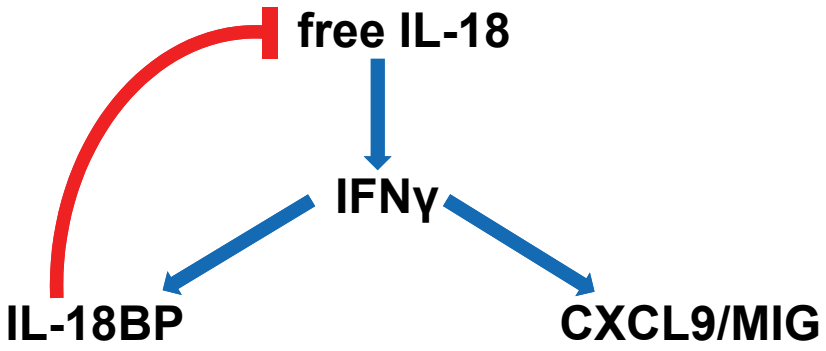

A
Validation cohort
offset
Prediction error
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0
10
20
30
40
50
60
Time after alloSCT (months)
B
Validation cohort
offset
Prediction error
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0
10
20
30
40
50
60
Time after alloSCT (months)

Figure S5

A



B

	Training cohort (n=589)				Validation cohort (n=399)			
	CXCL9/MIG		IL-18BPa		CXCL9/MIG		IL-18BPa	
	Spearman's rho	*P*	Spearman's rho	*P*	Spearman's rho	*P*	Spearman's rho	*P*
Total IL-18	0.34	<0.0001	0.21	<0.0001	0.28	<0.0001	0.39	<0.0001
Free IL-18	0.28	<0.0001			0.24	<0.0001		
IL-18BPa	0.53	<0.0001			0.41	<0.0001		

Figure S6

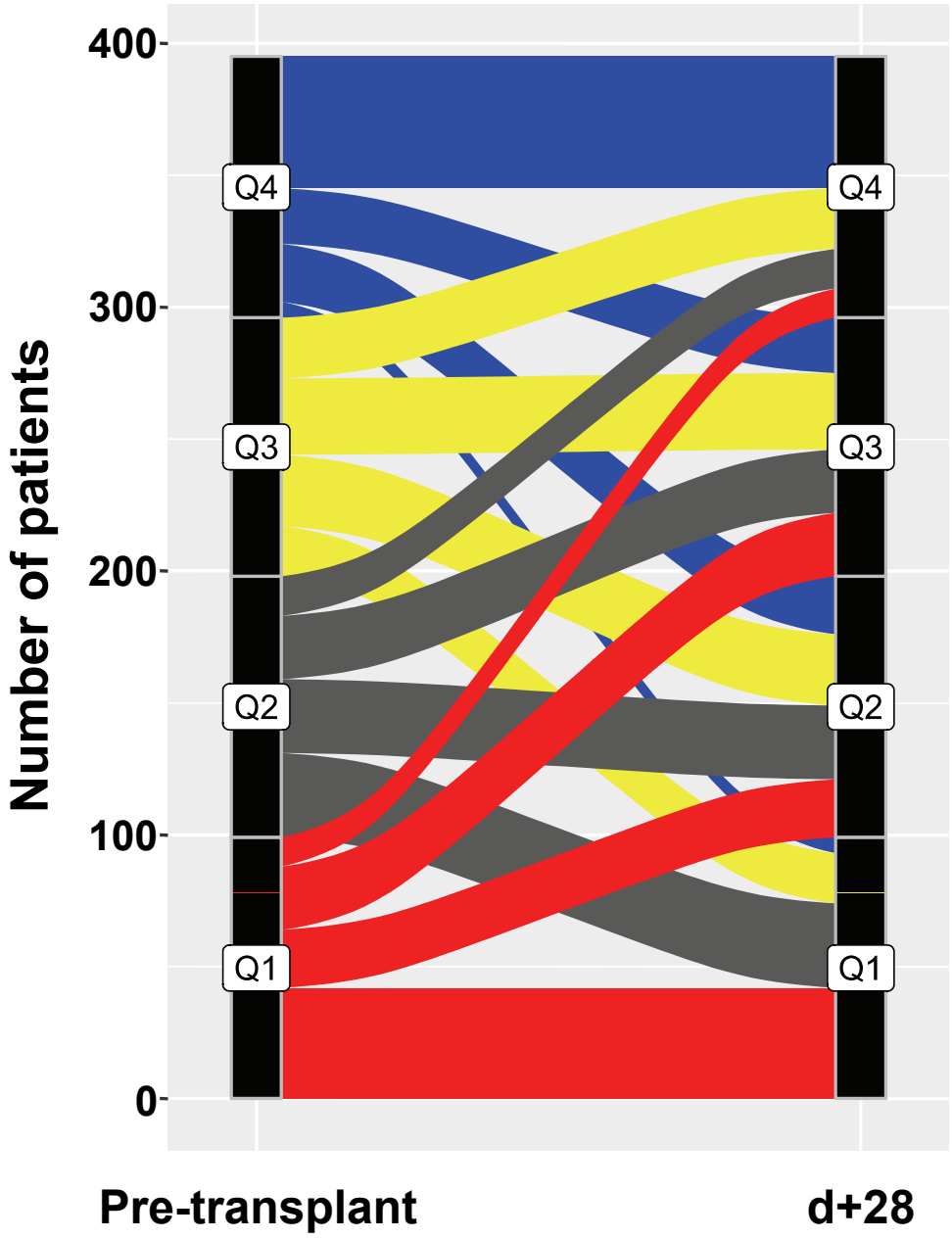

400
300
200
100
0
Number of patients
Q4
Q3
Q2
Q1
Q4
Q3
Q2
Q1
Pre-transplant
d+28

Figure S7

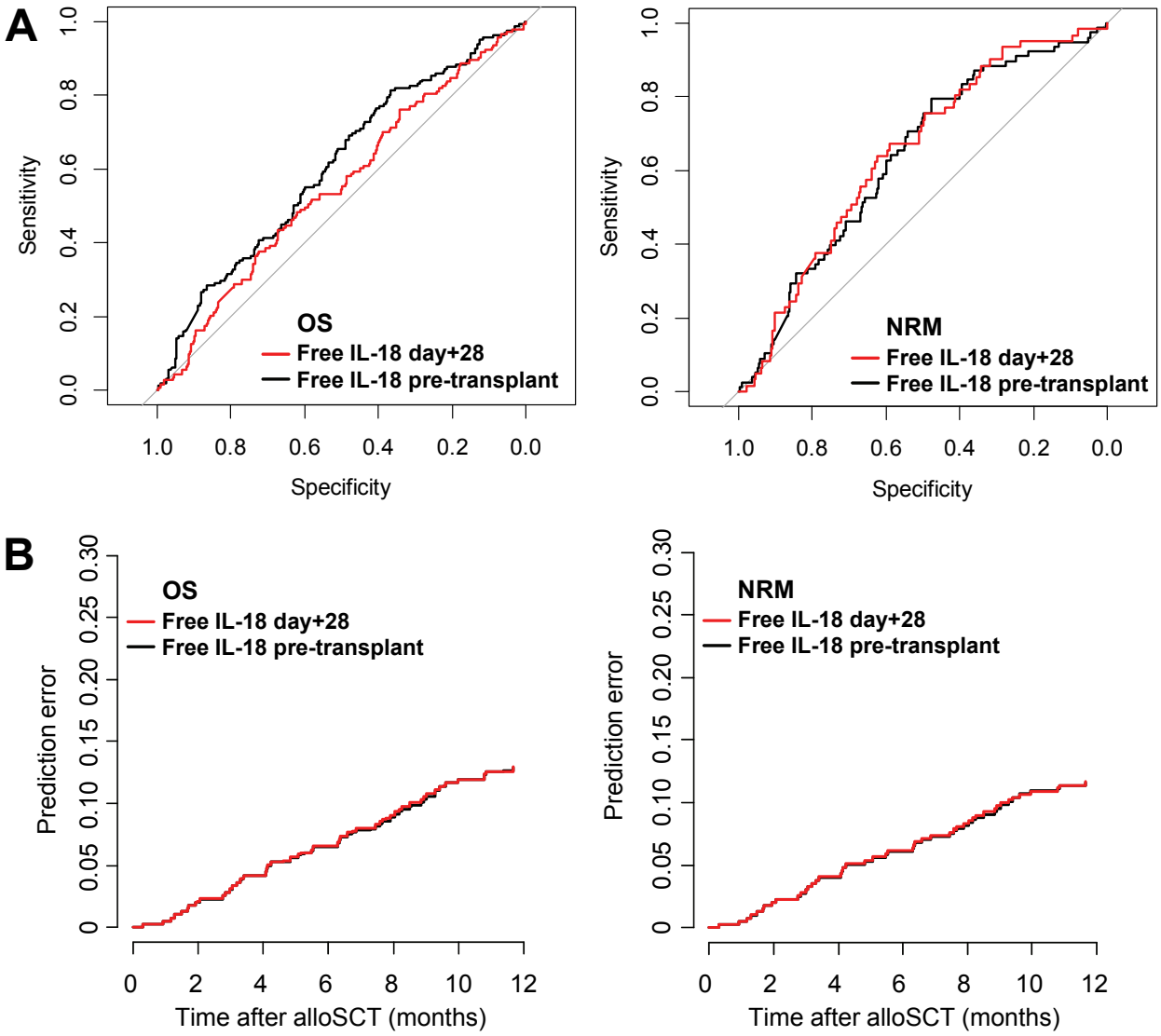

A
Sensitivity
Specificity
OS
Free IL-18 day+28
Free IL-18 pre-transplant
NRM
Free IL-18 day+28
Free IL-18 pre-transplant
B
Prediction error
Time after alloSCT (months)
OS
Free IL-18 day+28
Free IL-18 pre-transplant
NRM
Free IL-18 day+28
Free IL-18 pre-transplant

Figure S8

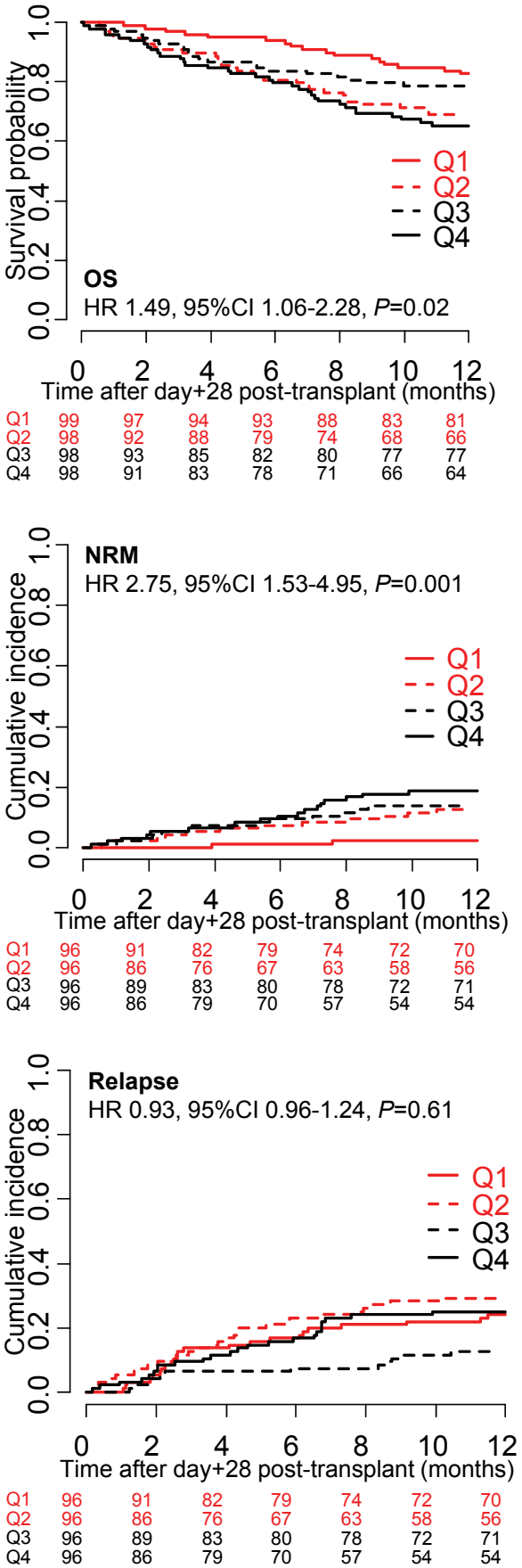

Survival probability
0.0
0.2
0.4
0.6
0.8
1.0
Q1
Q2
Q3
Q4
OS
HR 1.49, 95%CI 1.06-2.28, P=0.02
0 2 4 6 8 10 12
Time after day+28 post-transplant (months)
Q1 99 97 94 93 88 83 81
Q2 98 92 88 79 74 68 66
Q3 98 93 85 82 80 77 77
Q4 98 91 83 78 71 66 64
Cumulative incidence
NRM
HR 2.75, 95%CI 1.53-4.95, P=0.001
Time after day+28 post-transplant (months)
Q1 96 91 82 79 74 72 70
Q2 96 86 76 67 63 58 56
Q3 96 89 83 80 78 72 71
Q4 96 86 79 70 57 54 54
Relapse
HR 0.93, 95%CI 0.96-1.24, P=0.61
Time after day+28 post-transplant (months)
Q1 96 91 82 79 74 72 70
Q2 96 86 76 67 63 58 56
Q3 96 89 83 80 78 72 71
Q4 96 86 79 70 57 54 54

Figure S9

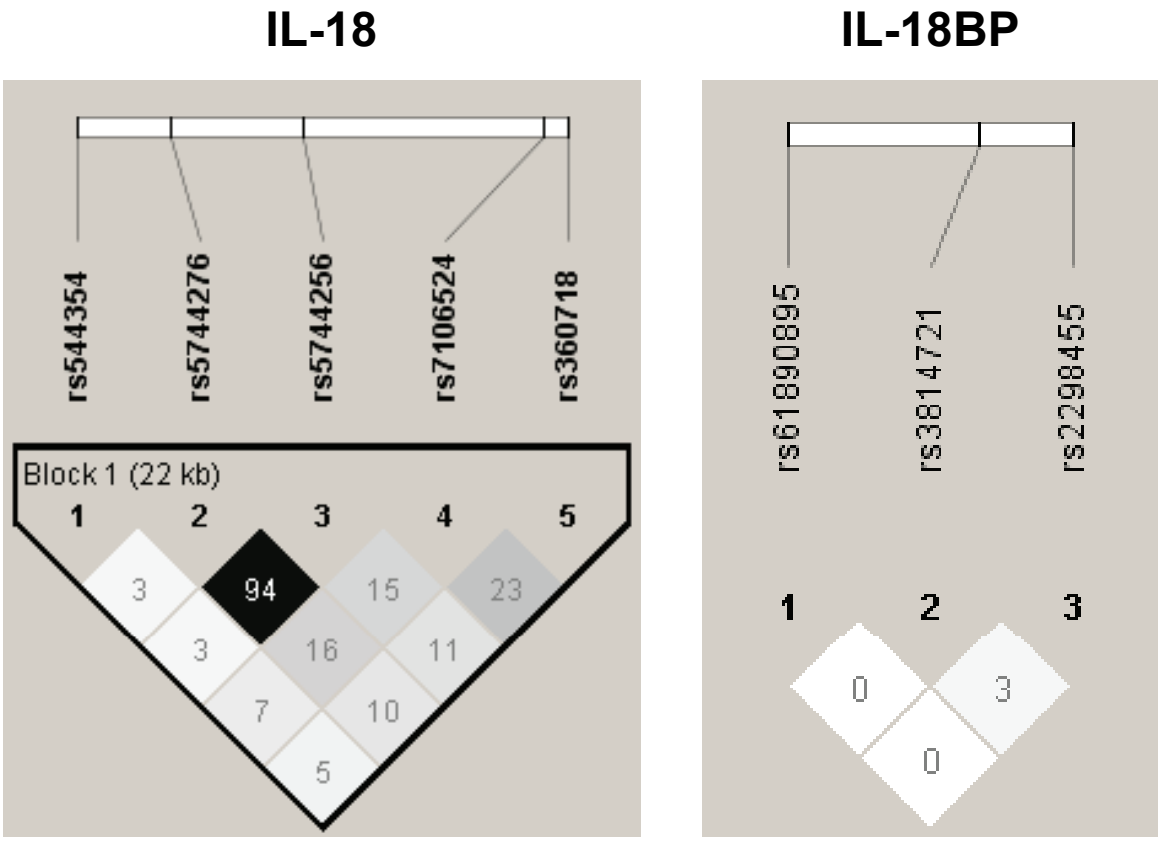
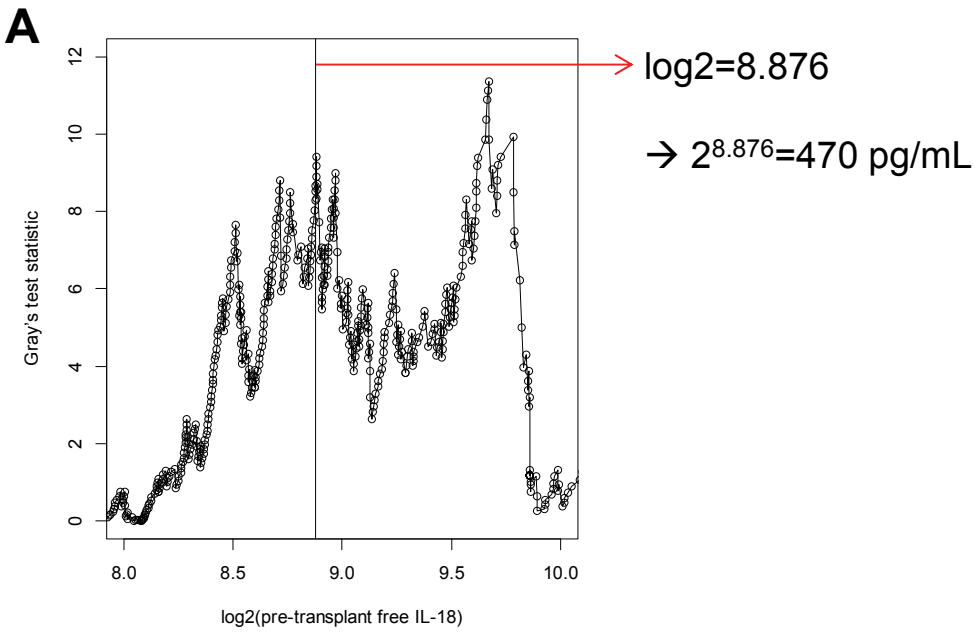
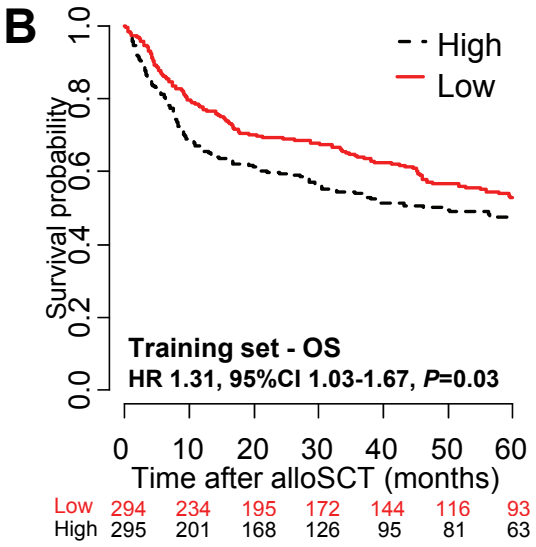
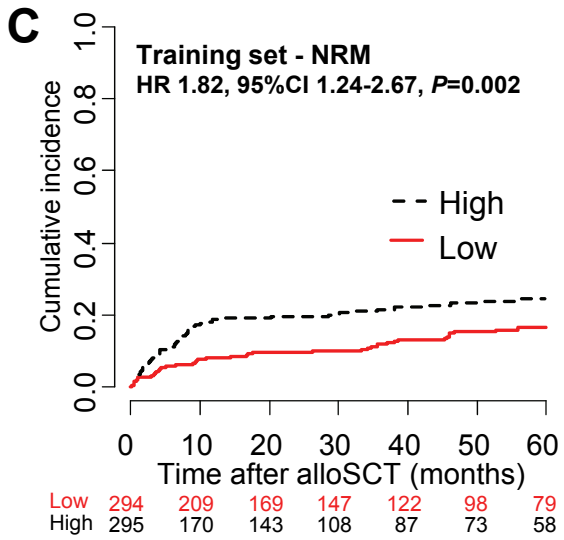
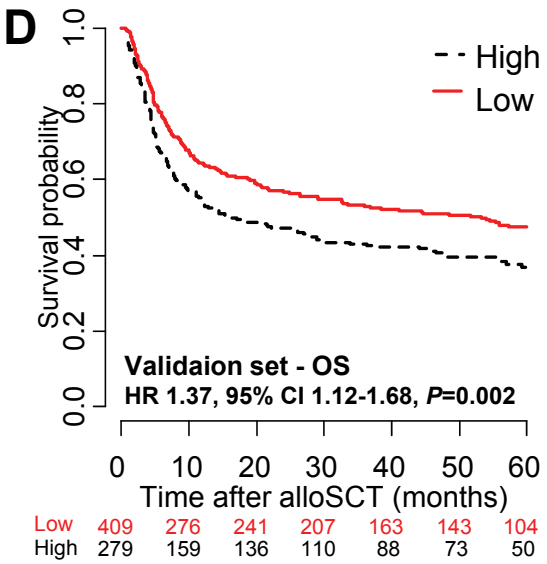

IL-18
rs544354
rs5744276
rs5744256
rs7106524
rs360718
Block 1 (22 kb)
1
2
3
4
5
3
94
15
23
3
16
11
7
10
5
IL-18BP
rs61890895
rs3814721
rs2298455
1
2
3
0
3
0

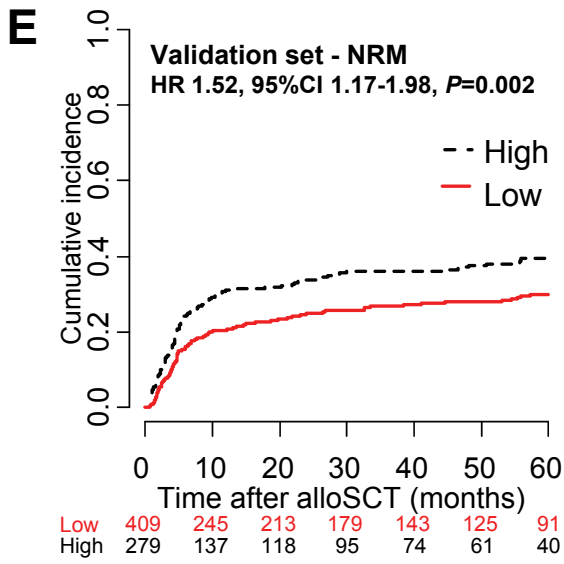
Figure S10


A
log2=8.876
→ 2^8.876=470 pg/mL
Gray's test statistic
log2(pre-transplant free IL-18)


B
High
Low
Survival probability
Training set - OS
HR 1.31, 95%CI 1.03-1.67, P=0.03
Time after alloSCT (months)
Low 294 234 195 172 144 116 93
High 295 201 168 126 95 81 63


C
Training set - NRM
HR 1.82, 95%CI 1.24-2.67, P=0.002
High
Low
Cumulative incidence
Time after alloSCT (months)
Low 294 209 169 147 122 98 79
High 295 170 143 108 87 73 58


D
High
Low
Survival probability
Validaion set - OS
HR 1.37, 95% CI 1.12-1.68, P=0.002
Time after alloSCT (months)
Low 409 276 241 207 163 143 104
High 279 159 136 110 88 73 50


E
Validation set - NRM
HR 1.52, 95%CI 1.17-1.98, P=0.002
High
Low
Cumulative incidence
Time after alloSCT (months)
Low 409 245 213 179 143 125 91
High 279 137 118 95 74 61 40