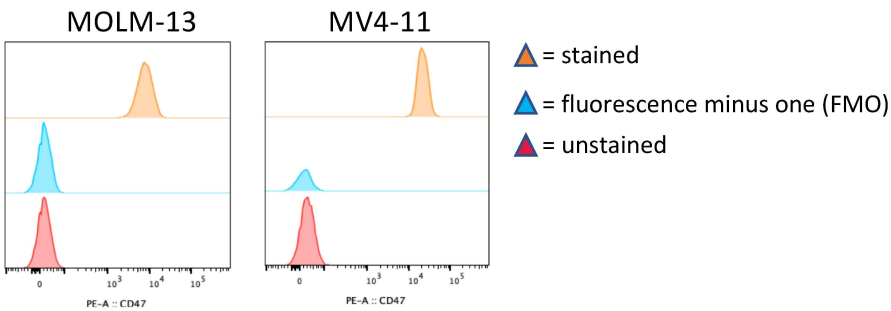
**Supplementary Table 1 Fluorochrom-conjugated antibodies.**

| Antibody | Clone | |
|---|---|---|
| anti-CD3 | OKT3 | Biolegend |
| anti-CD14 | M5E2 | Biolegend |
| anti-CD16 | 3G8 | Biolegend |
| anti-CD68 | 82A | Biolegend |
| anti-CD163 | GHI/61 | Biolegend |
| anti-CD86 | IT2.2 | Biolegend |
| anti-CD34 | 8G12 | BD Bioscience |
| anti-CD117 | 104D2 | Biolegend |
| anti-CD19 | HIB19 | Biolegend |
| anti-CD56 | HCD56 | Biolegend |
| anti-TIGIT | A15153G | Biolegend |
| anti-CD226 | 11A8 | Biolegend |
| anti-HLA DR | L243 | Biolegend |
| anti-CD39 | A1 | BD Bioscience |
| anti-CD73 | AD2 | Biolegend |
| anti-LAG-3 | 7H2C65 | Biolegend |
| anti-TIM-3 | F38-2E2 | Biolegend |
| anti-CD204 | 7C9C20 | Biolegend |
| anti-CD206 | 15-2 | Biolegend |

**Supplementary Methods 1 Cell lines.**

The MOLM-13 and MV4-11 cell lines were purchased from the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Germany). Both AML cell lines were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS, Gibco, Thermo Fisher Scientific). MOLM-13 and MV4-11 express CD47 which was regularly evaluated.



**Supplementary Methods 2 Patient cohorts and analyses.**

To evaluate the prognostic impact of CD163 three AML patient cohorts (TCGA cohort, GSE37642 and GSE12417) were analyzed. The TCGA AML cohort containing RNA sequencing data of 161 AML patients was analyzed using the software python, version 3.9.7, with the libraries lifelines version 0.26.3, pandas version 1.3.4, numpy version 1.19.5, scipy version 1.7.2, seaborn version 0.11.2, and matplotlib version 3.4.3. The Shapiro-Wilk test of normality, one-way ANOVA, Pearson correlation, and Spearman correlation were done with the scipy library. Logrank tests, Cox-PH models (using breslow estimation, with p values for individual variables and concordance indexes for the estimator), and Kaplan-Meier estimators were built with the lifelines package.
Furthermore, we extracted the microarray-based gene expression and survival data from GEO databases GSE37642 and GSE12417 (n=137 and n=240 patients, respectively) and performed a survival analyses based on CD163 expression below or above the median using Prism 9.0 software (GraphPad Software).

**Supplementary Methods 3 Statistical analyses.**

All MFC data were analyzed using FlowJo 10.5.2. software (Treestar). Statistical analyses were performed using Prism 9.0 software (GraphPad Software). The data sets were analyzed by the Mann-Whitney test for two unpaired groups, the Wilcoxon matched-pairs signed rank test for two paired groups, the Kruskal-Wallis or Friedmann tests for more than two groups, respectively. Pearson's correlation and Spearman's rank correlation coefficient were applied for bivariate correlation analysis. P-values <0.05 were considered significant, where *, ** and *** indicate p-values <0.05, <0.01, and <0.001, respectively.

**Supplementary Methods 4 t-distributed stochastic neighbor embedding (tSNE) analyses.**

As previously described [17], a subset of 3000 cells were selected for each donor at random and merged into a single expression matrix prior to tSNE analysis. The following channels were removed from the expression matrix to only include protein markers in tSNE analysis: viability, CD19, CD56, AML lineage markers (CD34, CD117), HLA DR, CD3 offset, residual and time. A total of 15000 cells and 12 markers were used to create a tSNE map. A perplexity parameter of 30 and iteration number of 550 was used for applying the dimensionality reduction algorithm. tSNE maps were generated by plotting each event by its tSNE dimensions in a dot-plot. Intensities for markers of interest were overlaid on the dot-plot to show the expression of markers on different cell islands.

| ***Patient group*** | **pAML** $N_{total}$ **= 59** | **lrAML** $N_{total}$ **= 8** | **rAML** $N_{total}$ **= 7** | **HD** $N_{total}$ **= 17** |
|---|---|---|---|---|
| Age | | | | |
| median | 59 (25-86) | 57 (47-66) | 57 (28-74) | 55 (27-78) |
| Sex | | | | |
| female | 24 (40,67%) | 6 (75%) | 2 (28.6%) | 8 (47.05%) |
| male | 35 (59,33%) | 2 (25%) | 5 (71.4%) | 9 (52.95%) |

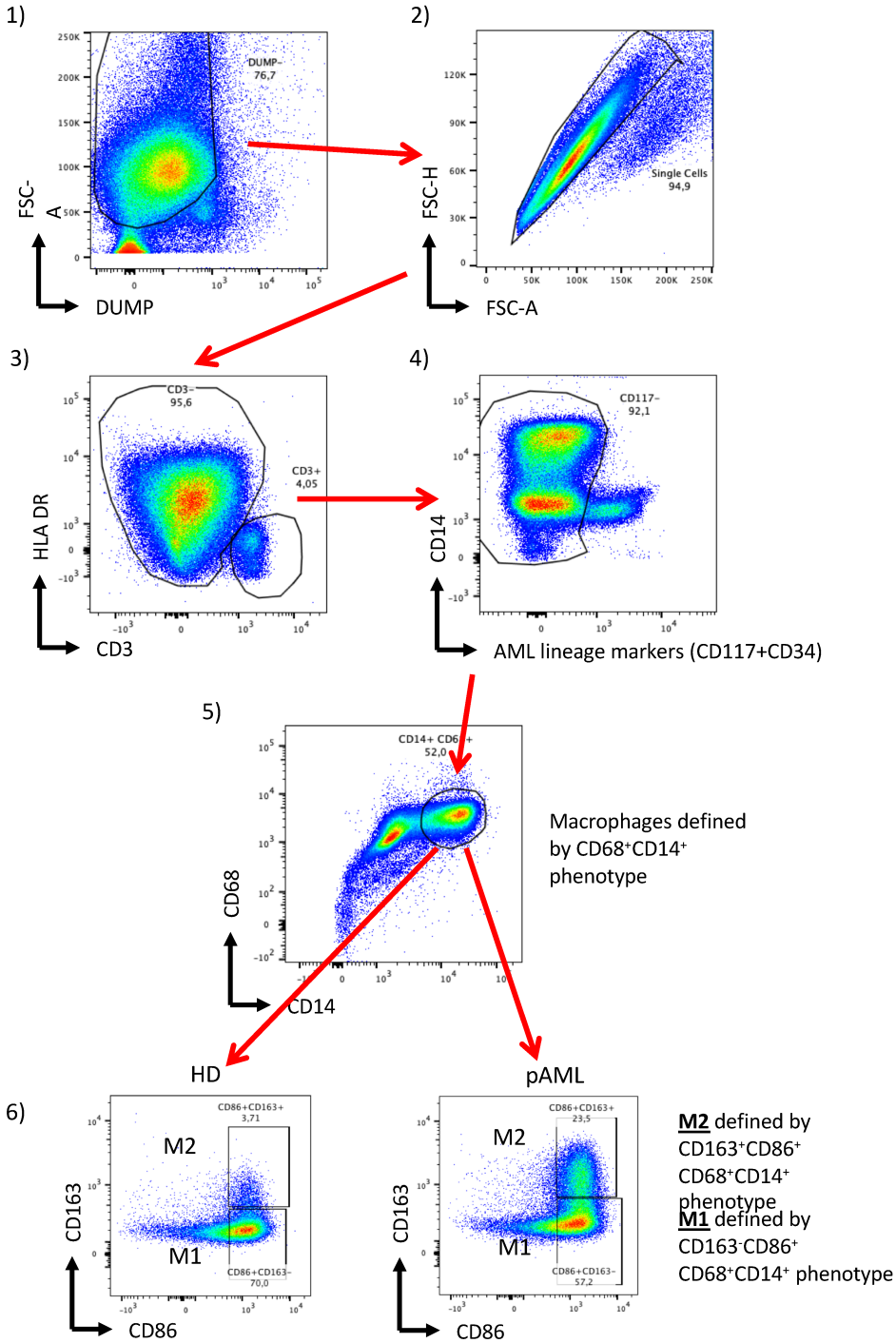
| **ELN*** | $N_{pAML}$ **= 50 (%)** | $N_{lrAML}$ **= 8 (%)** | $N_{rAML}$ **= 7 (%)** |
|---|---|---|---|
| favorable | 10 (20.00) | 8 (100) | 3 (42.86) |
| intermediate | 27 (54.00) | 0 (0) | 3 (42.86) |
| adverse | 13 (26.00) | 0 (0) | 1 (14.28) |

| **Molecular abberations*** | $N_{pAML}$ **= 50 (%)** | $N_{lrAML}$ **= 8 (%)** | $N_{rAML}$ **= 7 (%)** |
|---|---|---|---|
| FLT 3 ITD | 13 (23.9) | 0 (0) | 2 (28.6) |
| NPM1 mut | 20 (41.3) | 4 (50) | 5 (71.4) |

* Data of 9 patients unknown

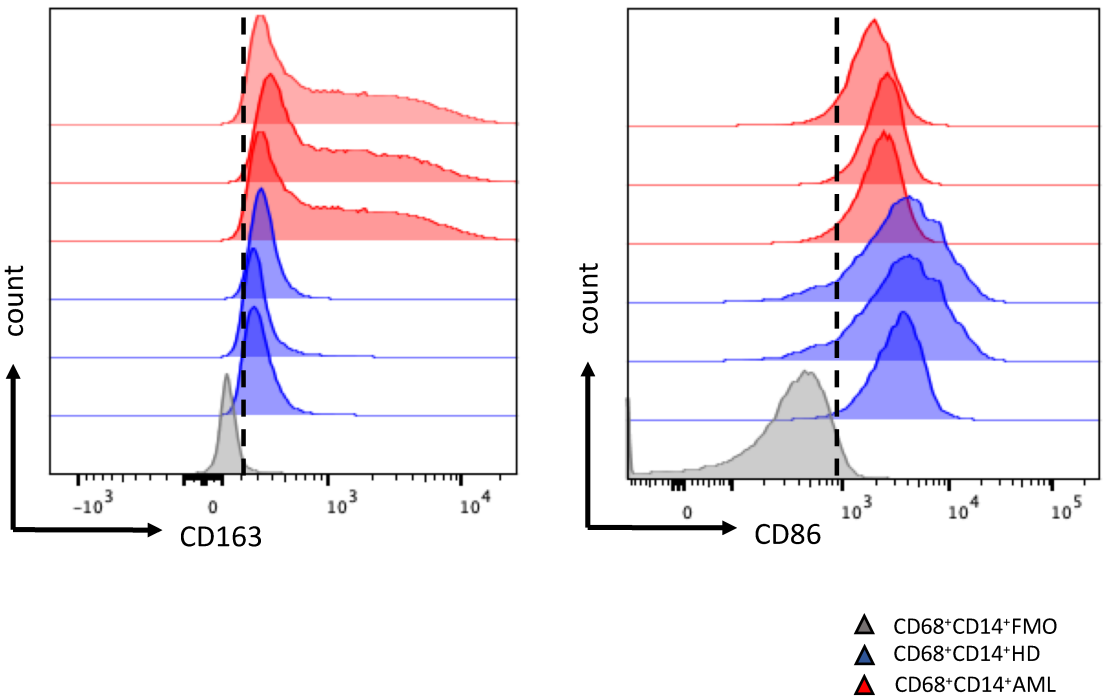
**Supplementary Table 2 Patient characteristics.**

Age, sex, ELN=European LeukemiaNet classification; FLT3 ITD=FMS like tyrosine kinase 1 intern tandem mutation; NPM1=nucleophosmin 1 mutation of the AML patients who donated bone marrow (BM) aspirates or peripheral blood (PB) specimens.



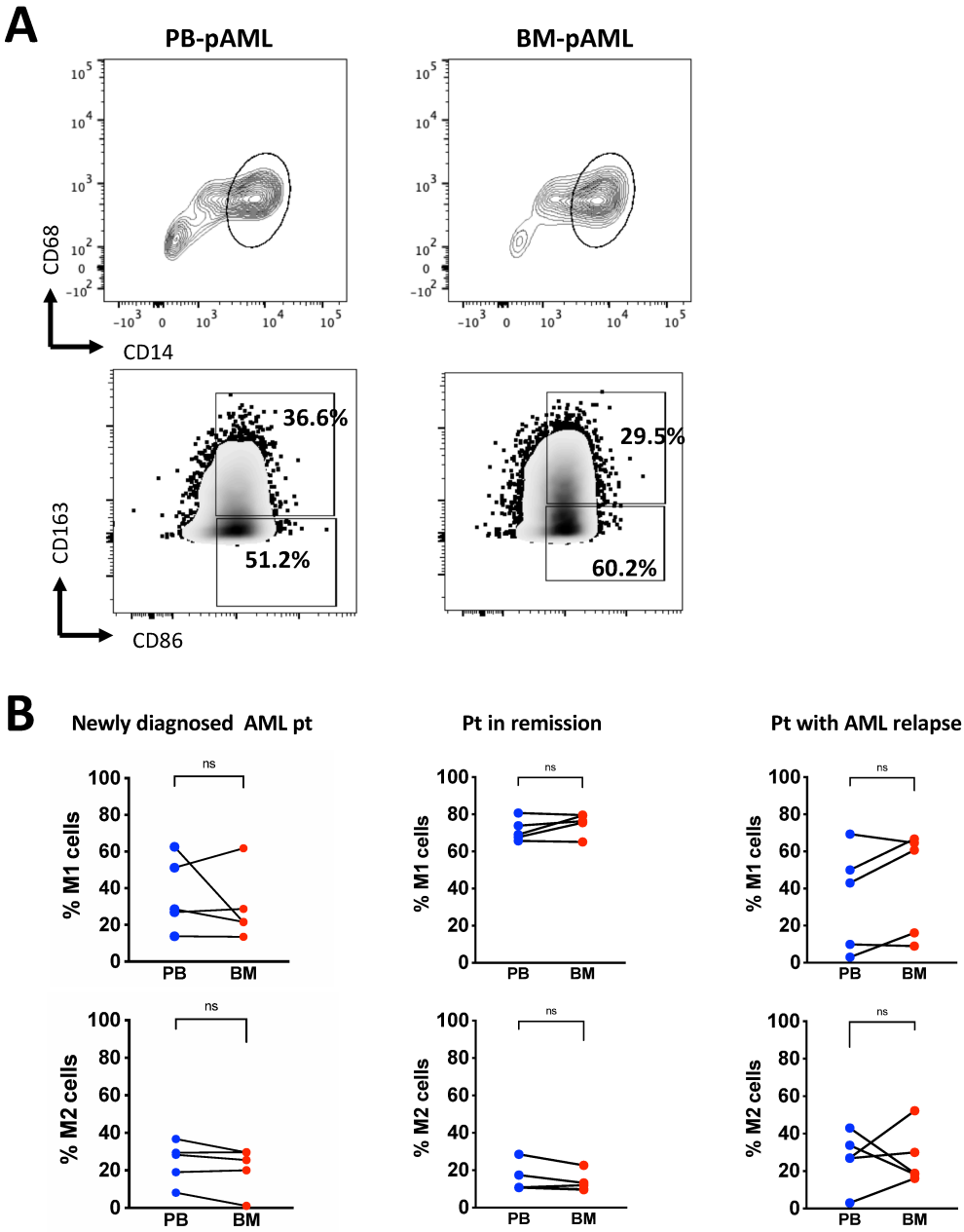

**Supplementary Figure 1 Gating Strategy.**

Gating strategy used to identify $CD68^+CD14^+$ macrophages/monocyte-derived macrophages and their $CD163^+CD86^+$ M2 and $CD163^-CD86^+$ M1 subpopulations from the bone marrow (BM) and peripheral blood (PB). The same gating strategy was used to analyze samples from PB and BM: after exclusion of cell debris, B cells and NK cells via the *DUMP channel* (1) and elimination of doublets (2), the T cells were defined as $CD3^+$/HLA-$DR^-$ (3). Next, AML cells were identified on the basis of their expression of CD117 and CD34 (4). $CD14^+CD68^+$ cells were identified within the remaining cells (5). Within this population, M2 and M1 subpopulations were defined on the basis of their expression of CD163 and CD86 (6).



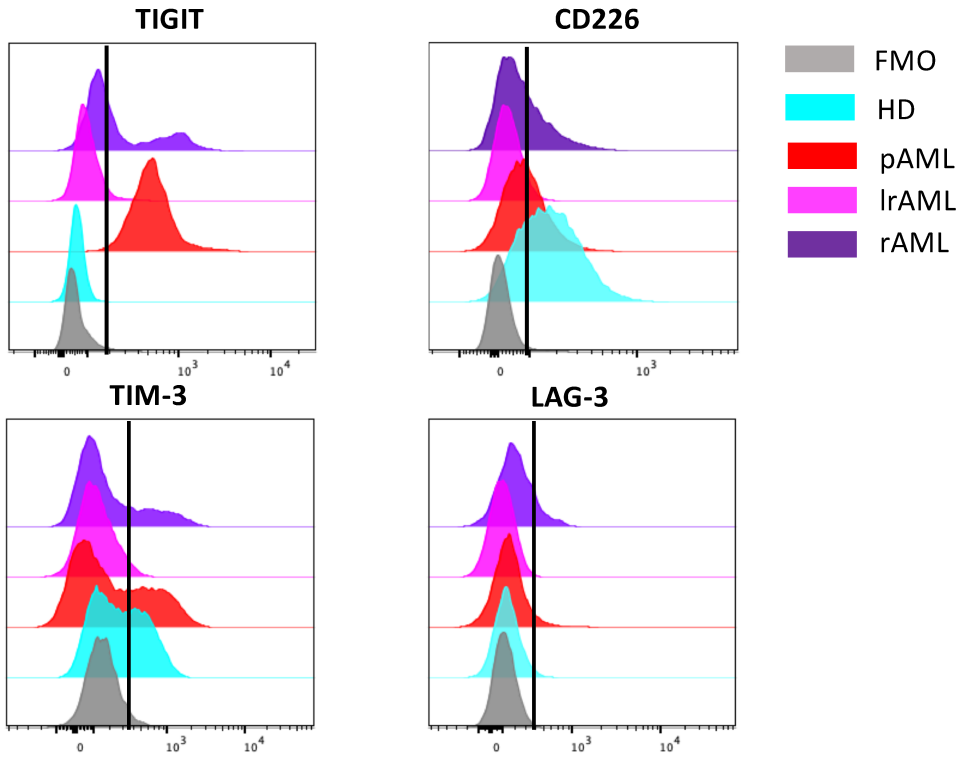

**Supplementary Figure 2 Expression of CD86 and CD163 on BM and monocyte-derived macrophages.**

The surface expression of CD163 and CD86 was analyzed in bone marrow (BM) aspirates from three patients with diagnosed AML (red histograms) and in monocyte-derived macrophages from three healthy donors (HDs, blue histograms). The histograms were gated on $CD14^+$ $CD68^+$ macrophages. The grey histograms represent the fluorescence minus one (FMO) control, respectively.

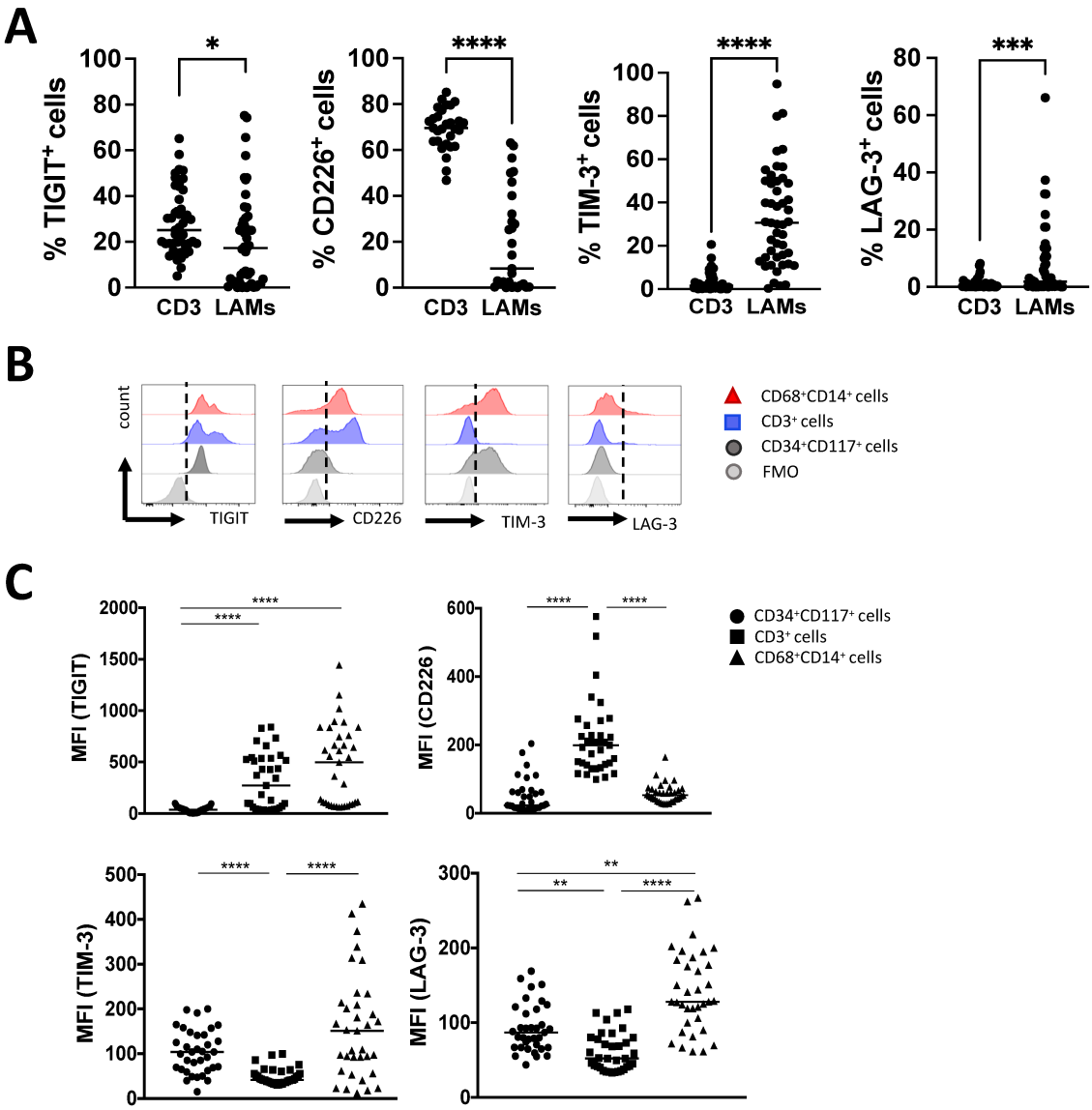



**Supplementary Figure 3 Comparison of M1 and M2 phenotypes in macrophages derived from BM or PB in patients with AML.**

The phenotype and frequency of M1 and M2 macrophages was compared between macrophages isolated directly from the bone marrow (BM) or derived from peripheral blood (PB) monocytes of n=5 patients with newly diagnosed AML (pAML), relpased AML (rAML), or AML in remission (lrAML), respectively. **(A)** Representative flow cytometry plots showing the population of $CD68^{+}CD14^{+}$ LAMs (upper row) and their M1 and M2 subpopulations (lower row) in PB (left plots) and BM (right plots) -derived macrophages. **(B)** Summary data illustrating the frequency of the M1 (upper row) or M2 (lower row) subpopulations. P values were obtained by the Wilcoxon test. *P<0.05, **P<0.01, ***P<0.001.



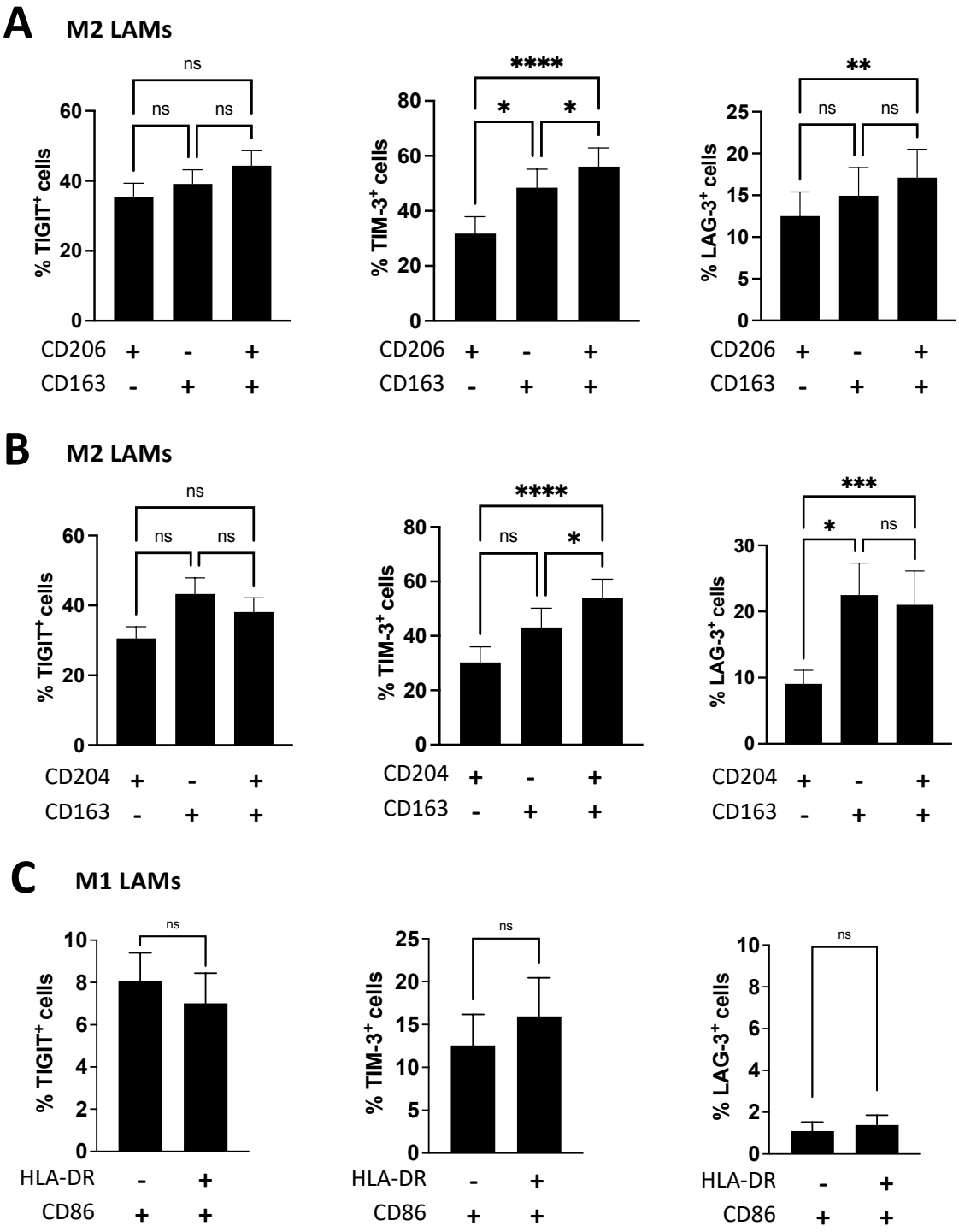

**Supplementary Figure 4 Expression of regulatory receptors on CD14⁺CD68⁺ macrophages from AML patients in comparison to FMO.**
The surface expression of TIGIT, CD226, TIM-3 and LAG-3 was compared in bone marrow (BM) aspirates from patients with newly diagnosed AML (red histograms, pAML), patients in remission (pink histograms, lrAML), patients with relapsed AML (violet histograms, rAML), monocyte-derived macrophages from healthy donors (blue histograms, HD). The histograms were gated on $CD14^+$ $CD68^+$ macrophages. The grey histograms represent the fluorescence minus one (FMO) control, respectively.




**Supplementary Figure 5 Comparison of co-regulatory receptor expression on LAMs vs. CD3⁺ T cells vs. AML blasts in newly diagnosed AML.**

**(A)** The expression of TIGIT, CD226, TIM-3 and LAG-3 was compared between leukemia-associated macrophages (LAMs) and corresponding CD3⁺ T cells from the bone marrow (BM) of patients newly diagnosed AML. **(B)** Representative flow cytometry plots show the expression of the co-regulatory receptors on CD14⁺CD68⁺ leukemia-associated macrophages (LAMs, red histograms), CD3⁺ T cells (blue histograms), and CD34⁺CD117⁺ AML cells (dark gray histograms) in comparison to the fluorescence minus one (FMO) controls (light gray histograms).

**(C)** Summary data showing the median fluorescence intensity (MFI) of co-regulatory molecules on CD14⁺CD68⁺ macrophages, CD3⁺ T cells and CD117⁺CD34⁺ AML cells from patients with pAML (n=35), lrAML (n=8), rAML (n=7) and HDs (n=16). P values were obtained by the ANOVA and Friedmann test, and by the Wilcoxon test. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.




**Supplementary Figure 6 Comparison of co-inhibitory receptor expression on CD163$^{+}$ CD206$^{+/-}$ and CD204$^{+/-}$ LAMs in newly diagnosed AML.**

The expression of co-inhibitory receptors was compared between leukemia associated macrophages (LAM) co-expressing either CD163 with or without CD206 or CD204 (defining M2 LAMS). (**A-C)** Summary data showing the median frequency of TIGIT, TIM-3 and LAG-3 on (**A)** CD163+/-CD206+/- M2 LAMs, (**B)** CD163 +/- CD204+/- M2 LAMs from 27 patients with pAML and (**C)** HLA-DR+/-CD86+ M1 LAMs from 16 patients with pAML**.** P values were obtained by the ANOVA and Friedmann test.*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

**Supplementary 7 CD163 is associated with patients risk profile.**

The association between TIGIT expressed by M2 LAMs and the patients' risk profile could be confirmed in three independent AML patient cohorts. High expression of CD163 was associated with a poor overall survival in the GSE37642, GSE12417 and TCGA LAML databases (refer to Figure 4). For datasets GSE37642 and GSE12417, correlation with clinicopathological parameters or multivariate analyses were not possible as the required information was not publicly available. But further analyses were performed for the TCGA LAML cohort containing RNA sequencing data of 157 AML patients. As observed in our cohort, the expression of CD163 correlated with the cytogenetic risk (Spearman's rho r=0.250, p<0.01). Furthermore, the concordance indices for CD163 and the cytogenetic risk score were very similar (c-index of 0.58 and 0.60) underlining their association. When the impact on survival of CD163 and cytogenetic risk score were analyzed, the significant prognostic influence of both variables was maintained (p=0.02 for CD163 and p<0.005 for cytogenetic risk score; **Supplementary Table 3A).** The age had the highest concordance index of 0.69 in the univariate analysis. Consistent with these data, the age emerged as strongest predictor of a poor overall survival in the multivariate analysis, whereas CD163 failed to reach significance **(Supplementary Table 3B)**, presumably because of its correlation with the prominent risk factors age and cytogenetic risk score (Spearman's rho r=0.292, p<0.01 for Spearman's rho r=0.250, p<0.01 for cytogenetic risk score, respectively).

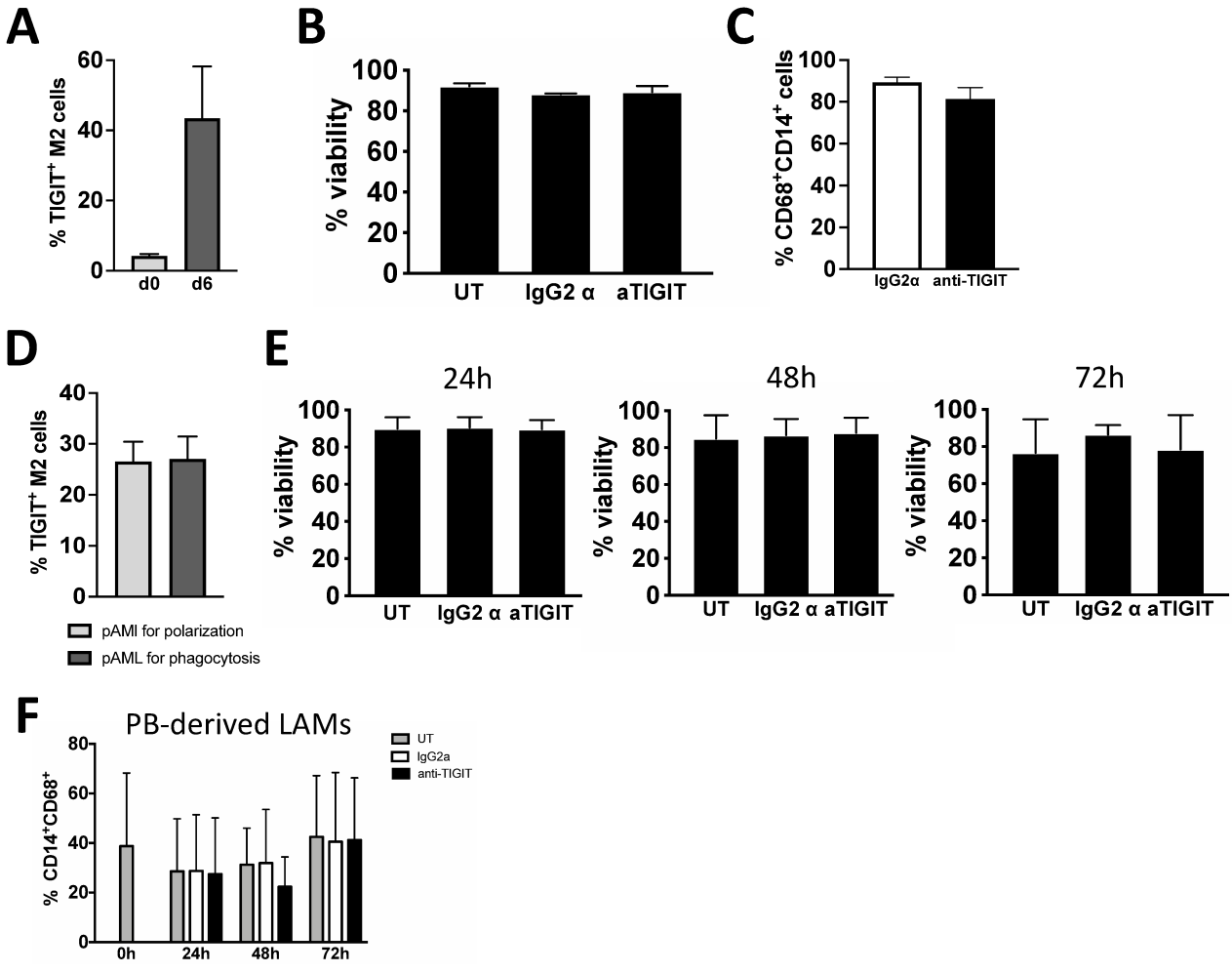
**Supplementary Table 3. Multivariate analysis of TCGA LAML cohort for overall survival.** CI=Confidence interval.

**A**

| Variable | Hazard Ratio | 95% CI | P-value |
|---|---|---|---|
| CD163 | 1.08 | 1.01 - 1.15 | 0.02 |
| Cytogenetic risk score | 1.74 | 1.24 - 2.42 | <0.005 |

**B**

| Variable | Hazard Ratio | 95% CI | P-value |
|---|---|---|---|
| CD163 | 1.05 | 0.98 - 1.12 | 0.18 |
| Cytogenetic risk score | 1.60 | 1.13 - 2.25 | 0.01 |
| FLT3 mutational status | 1.76 | 1.12 - 2.77 | 0.01 |
| Age | 1.04 | 1.02 - 1.06 | <0.005 |




**Supplementary Figure 8 Blockade of TIGIT reprograms TIGIT⁺ M2 like-macrophages into M1 phenotype *in vitro.***

M2-like macrophages gained by 6 days *in vitro* differentiation from CD14⁺ monocytes of healthy donors (n=4) were treated with anti-TIGIT antibodies or controls for 24h. **(A)** Expression of TIGIT is depicted by day 0 and after differentiation at day 6**. (B)** The viability was assessed after 24h treatment with anti-TIGIT or respective isotype control**. (C)** The percentages of CD68⁺CD14⁺ macrophages are depicted as the median ± SD. **(D)** Expression of TIGIT on primary M2 LAMS that were used for polarization experiments (left) and phagocytosis (right). **(E)** Fresh PBMCs from patients with CD117⁺CD34⁺ AML (n=7) were left untreated (UT) or incubated with an anti-TIGIT antibody or the IgG2α isotype control. The viability of leukemia-associated macrophages (LAMs) was analyzed over 72h by multiparametric flow cytometry. **(F)** Summary data of the overall frequency of LAMs. Measurements were performed in technical triplicates. All results are depicted as the median ± SD frequency. P values were obtained by the Wilcoxon or Anova and Friedman test. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001