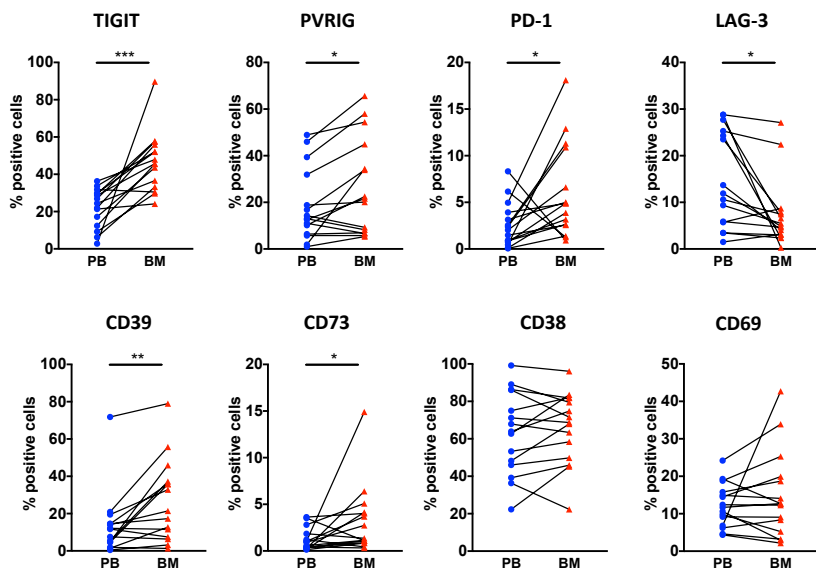


Supplementary Materials Figure S1. Gating strategy

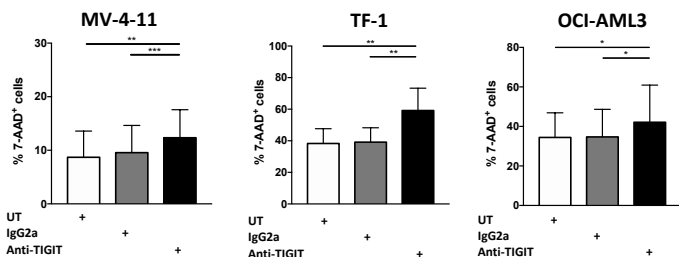
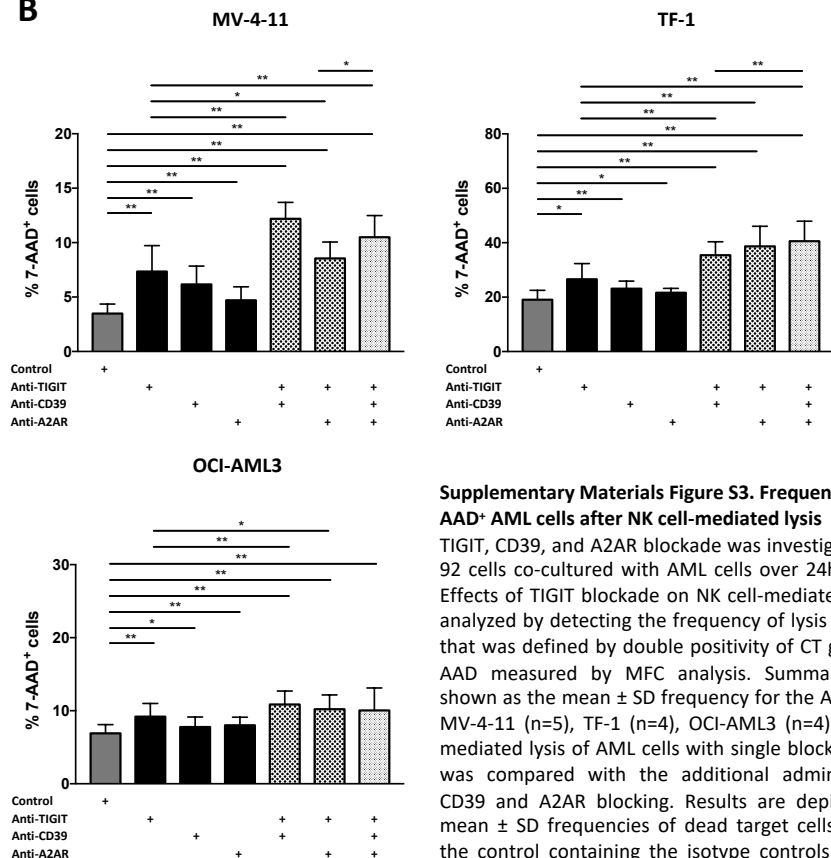
Gating strategy used to identify CD56⁺CD16⁺ natural killer (NK) cells and their CD56^{bright}CD16⁻, CD56^{dim}CD16⁻, CD56^{dim}CD16⁺, CD56⁻CD16⁺ subpopulations from the bone marrow (BM) and peripheral blood (PB). The same gating strategy was used to analyze samples from PB and BM: after elimination of doublets (1) and exclusion of cell debris, B cells and monocytes/macrophages via the *DUMP* channel (2), the T cells were defined as CD3⁺ (3). Next, AML cells were identified on the basis of their expression of CD117, CD34, and CD33 on the CD3⁺ cell subset (4). Using CD56 and CD16, NK cells were identified within the remaining cells (5). Within this population, NK cell subpopulations were defined on the basis of the co-expression pattern of CD56 and CD16 (6).



Supplementary Materials Figure S2. Checkpoint expression on NK cells in corresponding PB- and BM-derived aspirates of AML patients

The surface expression of TIGIT, PVRIG, PD-1, LAG-3, CD39, CD73, CD38, and CD69 was analyzed on NK cells derived from the peripheral blood (PB) and corresponding bone marrow (BM) from patients with newly diagnosed AML (n=15).

Summary data illustrating the frequency of positive cells on paired PB- and BM-derived NK cells in AML. P values were obtained by the Wilcoxon test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

A**B**

Supplementary Materials Figure S3. Frequencies of 7-AAD⁺ AML cells after NK cell-mediated lysis

TIGIT, CD39, and A2AR blockade was investigated for NK-92 cells co-cultured with AML cells over 24h *in vitro*. **A)** Effects of TIGIT blockade on NK cell-mediated lysis were analyzed by detecting the frequency of lysis of AML cells that was defined by double positivity of CT green and 7-AAD measured by MFC analysis. Summary data are shown as the mean \pm SD frequency for the AML cell lines MV-4-11 (n=5), TF-1 (n=4), OCI-AML3 (n=4). **B)** NK cell-mediated lysis of AML cells with single blockade of TIGIT was compared with the additional administration of CD39 and A2AR blocking. Results are depicted as the mean \pm SD frequencies of dead target cells, relative to the control containing the isotype controls and DMSO. MV-4-11 (n=3), TF-1 (n=3), OCI-AML3 (n=3). All measurements were performed in technical triplicates. P values were obtained by the Wilcoxon test. *P<0.05, **P<0.01, ***P<0.001.

p-value	Anti-TIGIT	Anti-CD39	Anti-A2AR	Anti-TIGIT + Anti-CD39	Anti-TIGIT + Anti-A2AR	Anti-TIGIT + Anti-CD39 + Anti-A2AR
Control	0.0039 0.0078 0.0039	0.0039 0.0039 0.0039	0.0039 0.0195 0.0039	0.0039 0.0039 0.0039	0.0039 0.0039 0.0039	0.0039 0.0039 0.0039
Anti-TIGIT	-	-	-	0.0039 0.0039 0.0039	0.0195 0.0039 n.s.	0.0078 0.0039 n.s.
Anti-TIGIT + Anti-CD39	-	-	-	-	-	0.0039 0.0078 n.s.
Anti-TIGIT + Anti-A2AR	-	-	-	-	-	0.0195 n.s. n.s.

- MV-4-11
- TF-1
- OCI-AML3

- : not analyzed

Supplementary Materials Table S1. P-values for the differences in the fold change of 7-AAD⁺ AML cells

This table gives further information about the p-values for the significant differences in the fold change of 7-AAD⁺ AML cells (MV-4-11 (orange), TF-1 (green), and OCI-AML3 (blue)) between the various checkpoint blockades relatively to the control, depicted in **Figure 5C**. P-values smaller than 0.05 were considered significant.

p-value	Anti-TIGIT	Anti-CD39	Anti-A2AR	Anti-TIGIT + Anti-CD39	Anti-TIGIT + Anti-A2AR	Anti-TIGIT + Anti-CD39 + Anti-A2AR
Control	0.0039 0.0117 0.0039	0.0039 0.0039 0.0117	0.0039 0.0391 0.0039	0.0039 0.0039 0.0039	0.0039 0.0039 0.0039	0.0039 0.0039 0.0039
Anti-TIGIT	-	-	-	0.0039 0.0039 0.0039	0.0195 0.0039 0.0391	0.0078 0.0039 n.s.
Anti-TIGIT + Anti-CD39	-	-	-	-	-	0.0039 0.0078 n.s.
Anti-TIGIT + Anti-A2AR	-	-	-	-	-	0.0117 n.s. n.s.

- MV-4-11
- TF-1
- OCI-AML3

- : not analyzed

Supplementary Materials Table S2. P-values for the differences in the frequency of 7-AAD⁺ AML cells

This table shows the p-values for the significant differences in the frequency (%) of 7-AAD⁺ AML cells (MV-4-11 (orange), TF-1 (green), and OCI-AML3 (blue)) between the various checkpoint blockades or the control, depicted in **Supplementary Materials Figure S3B**. P-values smaller than 0.05 were considered significant.

Patients for protein analyses	AML N _{total} = 25	HD N _{total} = 12
Age		
median	66.0 (25-84)	59.0 (41-70)
Sex		
female	13 (52%)	6 (50%)
male	12 (48%)	6 (50%)
FAB*	N_{total} = 25 (%)	
M0	1 (4)	
M1	10 (40)	
M2	5 (20)	
M3	0 (0)	
M4/M4Eo	5 (20)	
M5	3 (12)	
M6	0 (0)	
sAML: tAML	1 (4)	
ELN*	N_{total} = 25 (%)	
favorable	9 (36)	
intermediate	8 (32)	
adverse	8 (32)	
Molecular genetic*	N_{total} = 25 (%)	
FLT 3 ITD	9 (36)	
FLT 3 TKD	4 (16)	
NPM1 mut	10 (40)	
DNMT3A mut**	11 (44)	

*Data of 1 patient unknown

**Data of 6 patients unknown

Supplementary Materials Table S3. Patient characteristics

Age, sex, French American British classification (FAB), European LeukemiaNet classification (ELN) and the molecular aberrations: FMS like tyrosine kinase 3 internal tandem duplication or the tyrosine kinase domain mutation (FLT3 ITD or FLT 3 TKD), nucleophosmin 1 mutation (NPM1) and the DNA (cytosine-5)-methyltransferase 3A mutation (DNMT3A) of the donors who donated bone marrow (BM) aspirates or peripheral blood (PB) specimens are depicted in Table S3.