Functional analysis of the NUP98-CCDC28A fusion protein

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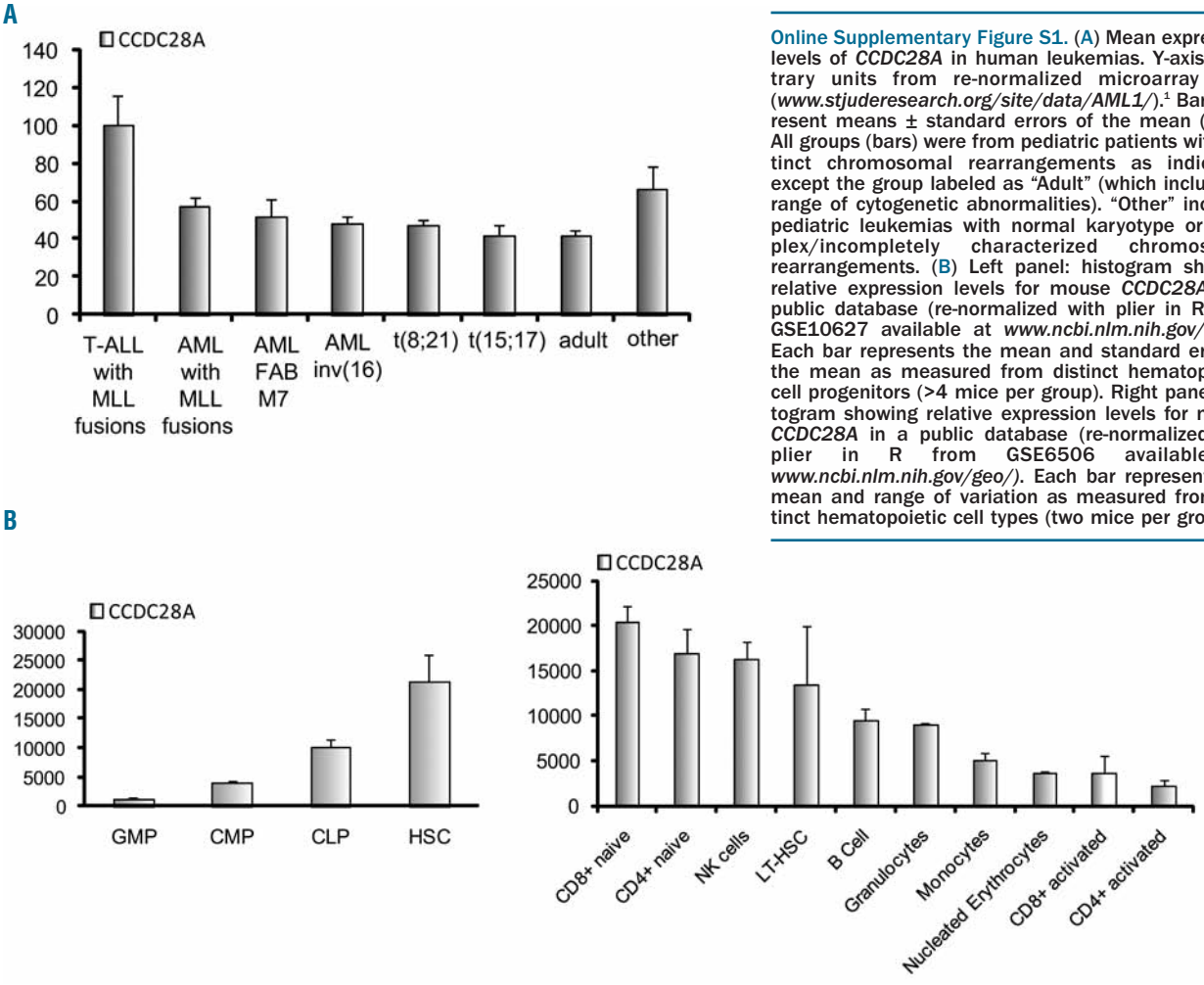
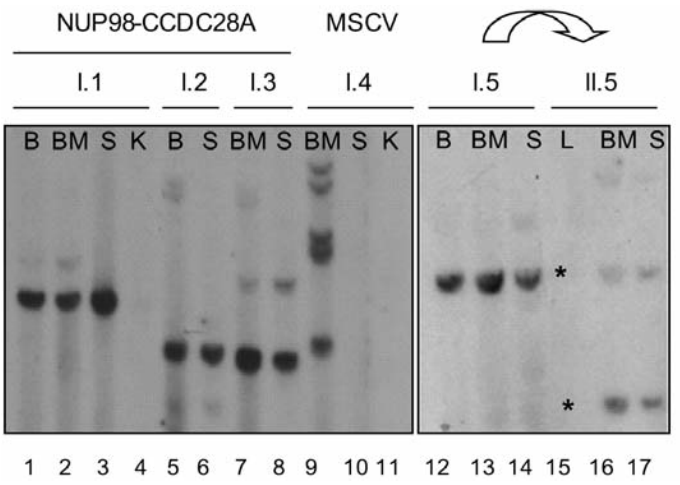
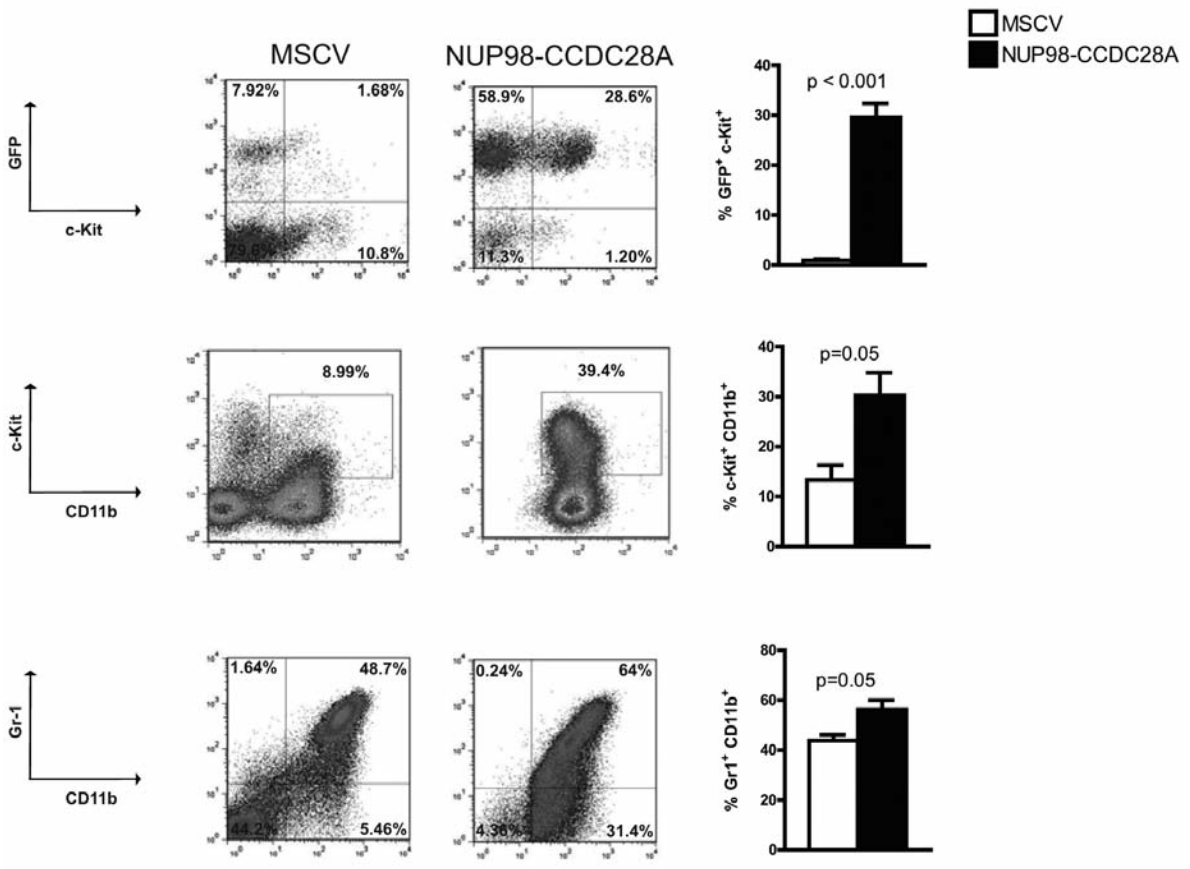


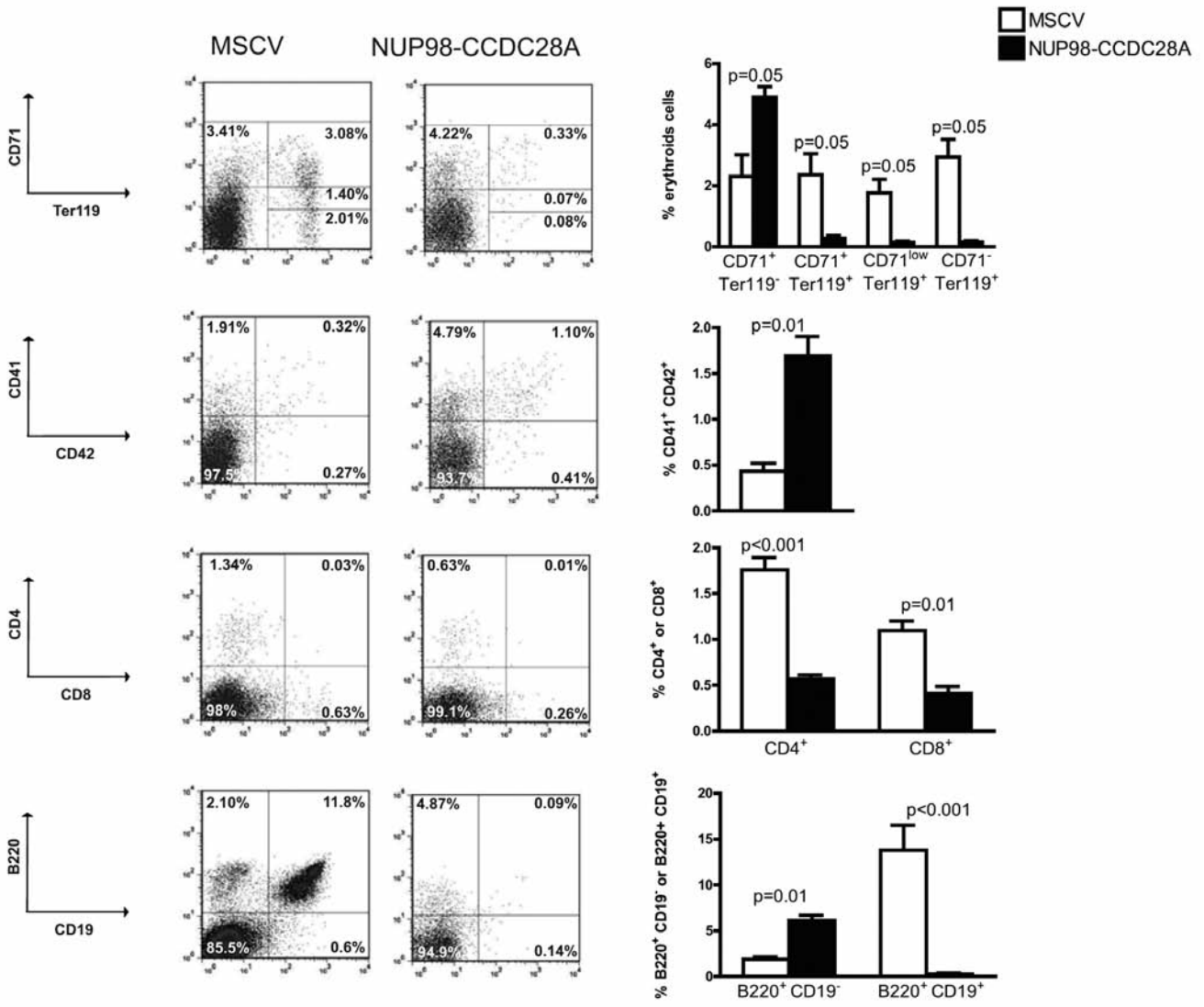
Online Supplementary Figure S1. (A) Mean expression levels of *CCDC28A* in human leukemias. Y-axis: arbitrary units from re-normalized microarray data (*www.stjuderesearch.org/site/data/AML1/*).[1] Bars represent means ± standard errors of the mean (SEM). All groups (bars) were from pediatric patients with distinct chromosomal rearrangements as indicated, except the group labeled as "Adult" (which included a range of cytogenetic abnormalities). "Other" includes pediatric leukemias with normal karyotype or complex/incompletely characterized chromosomal rearrangements. (B) Left panel: histogram showing relative expression levels for mouse *CCDC28A* in a public database (re-normalized with plier in R from GSE10627 available at *www.ncbi.nlm.nih.gov/geo/*). Each bar represents the mean and standard error of the mean as measured from distinct hematopoietic cell progenitors (>4 mice per group). Right panel: histogram showing relative expression levels for mouse *CCDC28A* in a public database (re-normalized with plier in R from GSE6506 available at *www.ncbi.nlm.nih.gov/geo/*). Each bar represents the mean and range of variation as measured from distinct hematopoietic cell types (two mice per group).



Online Supplementary Figure S2. Clonal analysis by Southern blot of DNA from four transduced mice. Membranes were hybridized to a probe specific for the GFP sequences. Lanes 1-8 and 12-14 correspond to hematopoietic (B, blood; BM, bone marrow; S, spleen) and non-hematopoietic (K, kidney; L, lung) organs of *NUP98-CCDC28A* primary mice; lanes 9-11 correspond to organs from MSCV-transduced mice. Asterisks indicate clones from BM of primary mice (mice I.5) that contribute to myeloid leukemias in the secondary transplant (mice II.5). While multiple clones persist in the BM cells transduced by the empty vector alone (lane 9), *NUP98-CCDC28A*-induced myeloproliferative neoplasms were predominantly mono-or bi-clonal (lanes 2, 7 and 13). An example of clonal transmission of the disease is shown [right panel, see secondary mouse II.5 (lanes 15-17) of primary mouse I.5 (lanes 12-14)].



Online Supplementary Figure S3. Representative fluorescent-activated cell sorting (FACS) analysis of bone marrow (BM) cells from primary control (MSCV)- and *NUP98-CCDC28A*-engrafted mice (left panels). Analyses reveal an expansion of Gr1-, Mac1/CD11b- and c-Kit-positive cells in the BM of the diseased animals. Histograms show the percentages of indicated cells in the BM from leukemic and control mice (right panels). Values shown are mean ± SEM (n=5 mice per group, Mann-Whitney test).



Online Supplementary Figure S4. Representative FACS profile of bone marrow cells from primary MSCV- and *NUP98-CCDC28A*-engrafted mice. Analyses show a decrease in the number of progenitors from B ($B220^+CD19^+$) and erythroid ($Ter119^+CD71^+$) lineages, a decrease in T cells ($CD4^+$ or $CD8^+$) and an increase in megakarocytes number ($CD41^+CD42^+$). Values shown are mean ± SEM (n=5 mice per group, Mann-Whitney test).

Online Supplementary Table S1. Fold-ratio between average levels of *CCDC28A* (Affymetrix probe 209479_at) for each FAB group *versus* the other groups ("fold"). Oncomine provided *P*-values. Asterisk (*): significant or nearly significant fold increases. Nd: not determined.

		M0	M1	M2	M3	M4	M5	M6
Valk PJ[2]	fold	-1.1	**1.1***	1	-1.1	-1.1	1	**1.8***
	P-value	0.88	**0,01**	0.77	0.88	0.97	0.36	**0.04**
Metzeler KH[3]	fold	nd	-1.1	1.2	nd	1	-1.1	**1.5***
	P-value	nd	0.87	0.16	nd	0.58	0.79	**0.06**
Wouters BJ[4]	fold	1	1.1	-1.1	-1.1	-1.1	1	**1.4***
	P-value	0.35	0.1	0.91	0.85	0.88	0.17	**0.01**

References

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