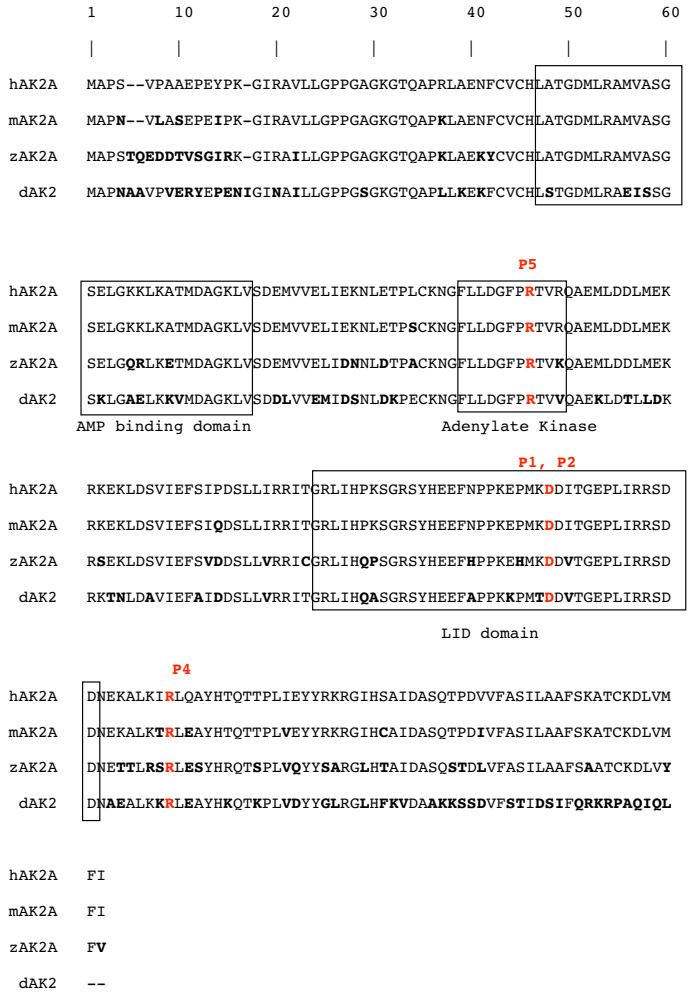
Supplementary Figure 1

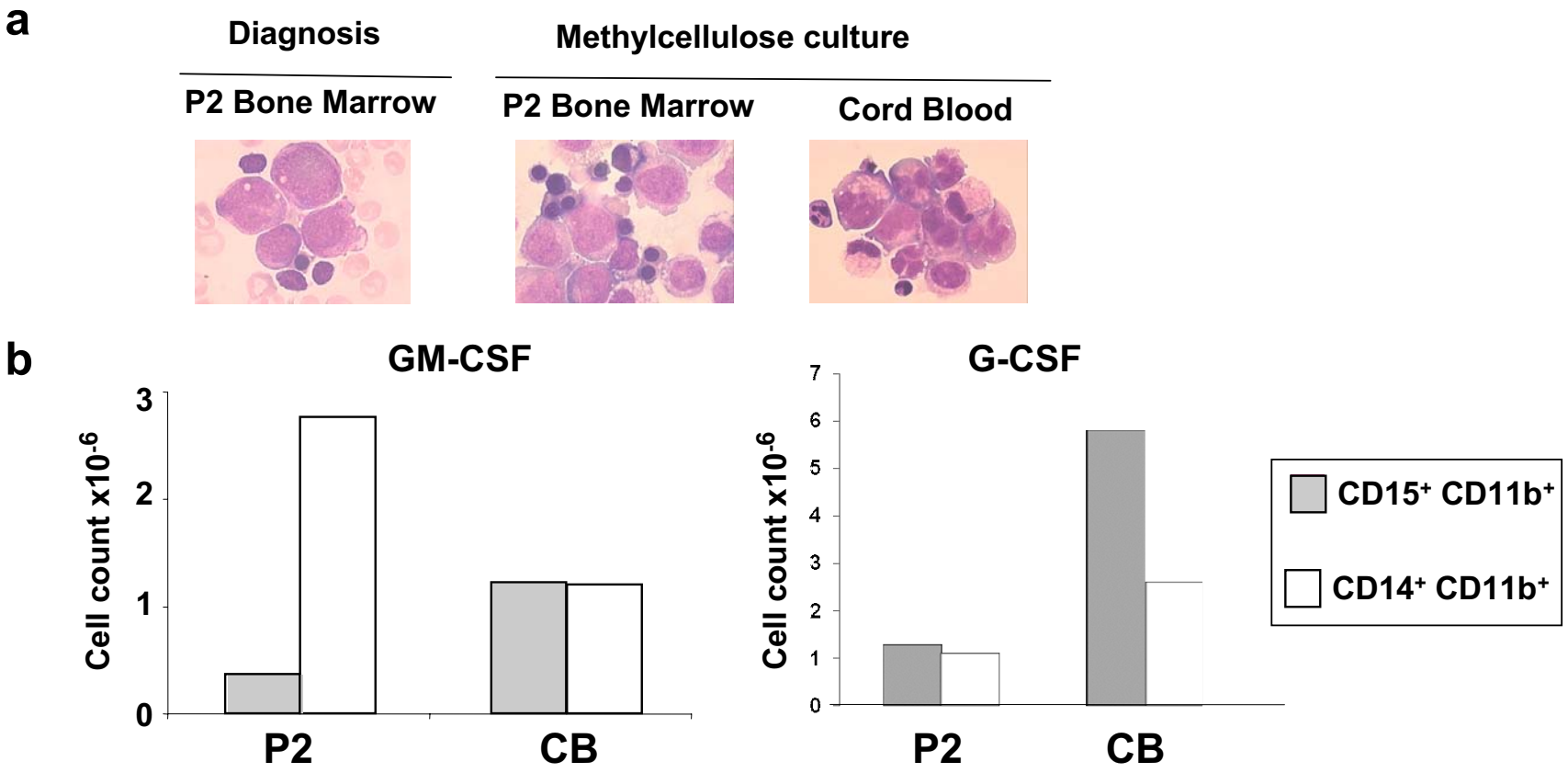
Conservation of the AK2 protein sequence in various orthologs



Alignment of human AK2 (hAK2), murine AK2 (mAK2), zebrafish AK2 (zAK2) and drosophila AK2 (dAK2) protein sequences. Amino acids affected by a mutation are denoted in red.

Supplementary Figure 2

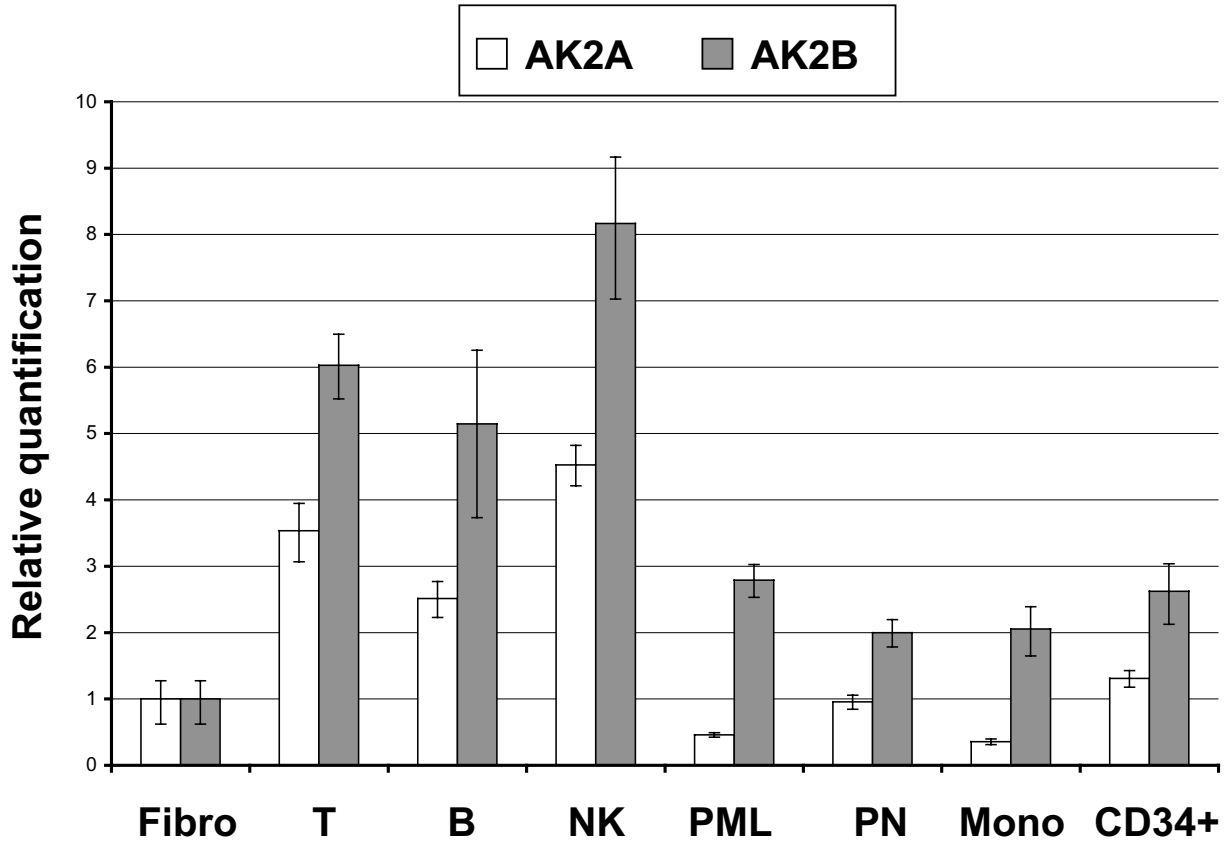
Phenotype of bone marrow cells from an RD patient



(**a**) May-Grünwald/Giemsa staining of BM cells from patient P2 at diagnosis (left panel) and after methylcellulose culture (right panel). In both conditions, only a few myeloblast cells could be identified. In the control cord blood (CB) culture, myeloid precursors and polynuclear cells were detected.
(**b**) Granulocyte and monocyte differentiation of patient P2's BM cells. CD34+ cells isolated from patient P2's BM or from control cord blood (CB) cells were cultured in the presence of SCF, FLT-3L and GM-CSF (all at 100ng/ml) or G-CSF (100ng/ml) for 2 weeks. At the end of the culture, flow cytometry analysis was performed to determine the proportion of neutrophils ($CD15^+$ $CD11b^+$) and monocytes ($CD14^+$ $CD11b^+$).

Supplementary Figure 3

AK2 gene expression profile in bone marrow haematopoietic subpopulations

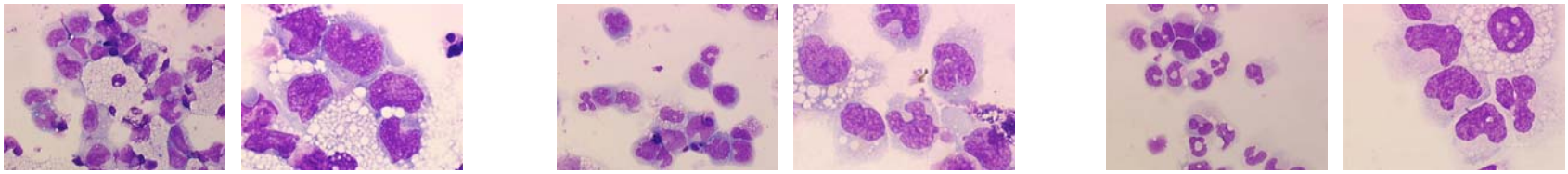


AK2A and *AK2B* expression were quantified by real-time RT-PCR in various bone marrow haematopoietic populations as compared to a SV40-transformed fibroblast cell line (Fibro). The cell populations analyzed were sorted by flow cytometry to isolate : $CD3^+$ T-cell (T), $CD19^+$ B-cell (B), $CD16^+CD56^+$ NK-cell (NK), $CD34^+$ progenitors (CD34), $CD15^+CD11b^-$ promyelocytes (PML), $CD15^+CD11b^+$ polynucleated neutrophils (PN) and $CD14^+$ monocytes (Mono). Data correspond to the mean of two independent adult bone marrow-derived populations.

Supplementary Figure 4

Complementation of the neutrophil differentiation defect by restoration of *AK2* expression in P6 BM cells

a



b

Enumeration of myeloid subpopulations among CFU-G/GM

		P6 BM Mock	P6 BM AK2A+B+GFP	CB Mock
Myeloid precursors	%	99	80	35
	Absolute cell number	127,700	494,600	51,400
Differentiated myeloid cells	%	1	20	65
	Absolute cell number	1,300	123,700	95,500

Mononuclear cells isolated from the bone marrow (BM) of patient P6 were transduced with either two bicistronic lentivirus vectors (encoding *AK2*A and *AK2*B, together with GFP : AK2A+B+GFP) or mock transduced. Mock transduced cord-blood-derived $CD34^+$ cells were used as a positive control. After lentiviral transduction, 250,000 mononuclear cells from P6's BM were seeded in semi-solid medium for each condition. As a control experiment, 1,000 CD34+ cord blood cells were seeded in semi-solid medium.

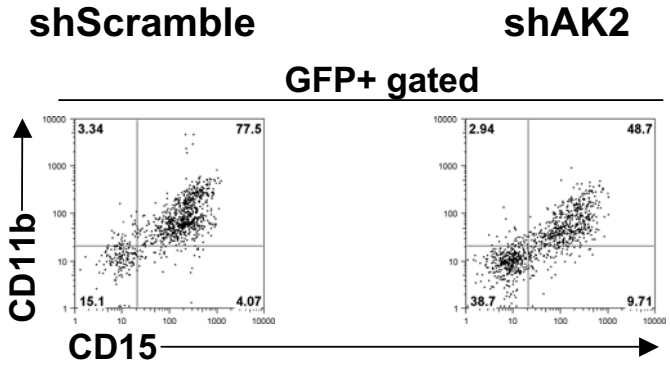
(**a**) After 13 days, CFU-G/GM colonies were collected and cytospin preparations were analyzed by May-Grunwald/Giemsa staining. Representative pictures of various stages of neutrophil maturation are shown at magnifications of 50x (left panel) or 100x (right panel).

(**b**) Enumeration of myeloid subpopulations among CFU-G/GM colonies. Myeloid precursors (blasts, myeloblasts and promyelocytes) and differentiated myeloid cells (myelocytes, metamyelocytes and granulocytes) are represented in pourcentage and in absolute cell numbers.

Supplementary Figure 5

Inhibition of neutrophil differentiation after AK2 knock-down

a



b

		shScramble		shAK2		Fold difference
	Population	**%**	**Cell number**	**%**	**Cell Number**	**shScramble/shAK2**
d0	**Total**		500,000		500,000	
d3	**Total**		634,000		295,000	
	GFP+	25	158,500	15	44,300	
d3+8	**Total**		152,200		14,500	
	$CD15^+CD11b^+$	78	118,700	49	7,100	**x17**
	Differentiated myeloid cells	30	45,700	12	1,700	**x27**

$CD34^+$ CB progenitors were transduced three times with a lentivirus encoding GFP together with a shRNA directed against AK2 (shAK2) or with a shRNA corresponding to a scramble version as a control (shScramble). At day 3 (d3), the transduced GFP positive cells were sorted and culture in the presence of G-CSF. Eight days later (d3+8), we evaluated neutrophil differentiation ($CD15^+CD11b^+$ count) by flow cytometry analysis (**a**). These results were combined with the analysis of May-Grunwald/Giemsa cytospin preparations. Using both flow cytometry and morphological analysis, we enumerated respectively the absolute numbers of mature granulocytes $CD15^+CD11b^+$ as well as differentiated myeloid cells (myelocytes, metamyelocytes, granulocytes) obtained in both shScramble and shAK2 conditions (**b**).