Deregulated JAK3 mediates growth advantage and hemophagocytosis in extranodal nasal-type natural killer/T-cell lymphoma

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Supplemental material

Supplemental Table 1. Interferon-γ production by NKTCL cell lines according to JAK3, MEK and AKT activity.

	Control		**NKL**		**KHYG-1**	
	IL-2-	**IL-2+**	**IL-2-**	**IL-2+**	**IL-2-**	**IL-2+**
Vehicle	0	45 (17)	6,1 (1.4)	149 (14)	23 (7)	213 (18)
+ CP-690550	0	0	0	13.3 (2.3)	0	4.6 (2.7)
+ UO126	0	0	0	4 (5.4)	0	72 (3)
+ GDC-0941	0	0	0	0	0	0

Interferon-γ concentrations (expressed as mean (standard deviation) pg/mL/10^6 **viable** cells) were assessed by ELISA using Quantikine assay (R&D Systems, Abingdon, UK) according to the supplier's instructions, with the use of a Microplate Reader model 680 (Life Science, Bio-Rad, Marne la Coquette, France) and the Microplate Manager 5.2 software. Culture supernatants were collected 2 days following chemical inhibitors exposure targeting JAK3 (CP-690550, 50 nM), MEK1/MEK2 (UO126, 10 µM) or PI3K/AKT (GDC-0941, 5 µM). Results were obtained from at least three independent experiments. Interferon-γ concentrations were normalized according to the number of viable cells at time of supernatant collection. IL-2: interleukin-2. Control was a NK cell population from a healthy donor. Vehicle was dimethylsulfoxyde.

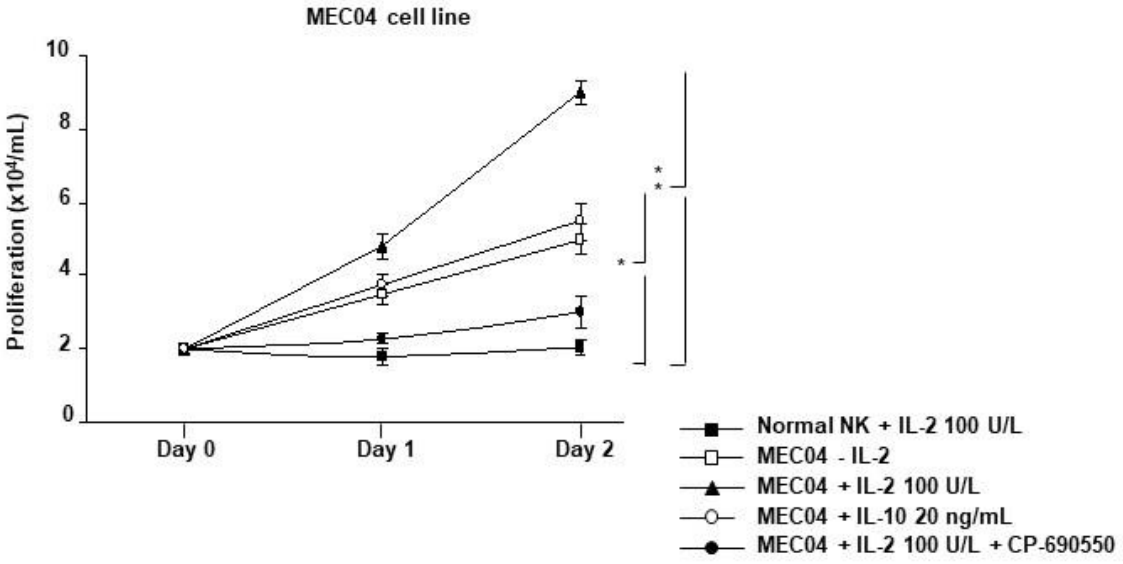
Supplemental Table 2. Blood cell count in mice with JAK3 A573V-induced lymphoproliferative disease.

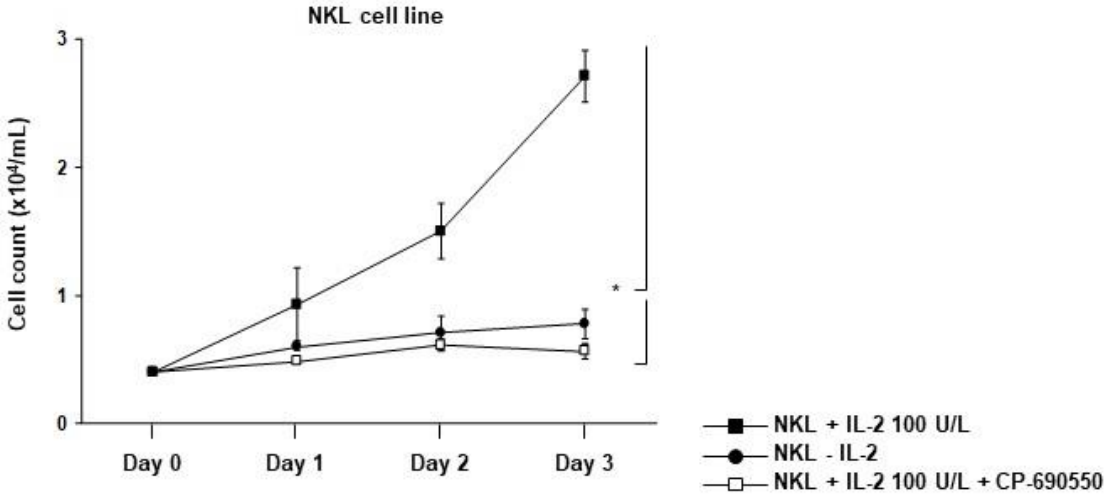
	Empty vector (N=2)	**JAK3 WT (N=2)**	**JAK3 A573V (N=2)**
White blood cells (10^9/L)	11.90 (4.8)	7.45 (0.04)	4.36 (0.4)
Monocytes (10^9/L)	0.18 (0.04)	0.34 (0.18)	0.25 (0.07)
Lymphocytes (10^9/L)	6.6 (0.09)	10.5 (3.65)	3.3 (0.28)
Neutrophils (10^9/L)	0.7 (0.02)	1.48 (0.85)	0.85 (0.21)
Platelets (10^9/L)	596 (201)	300 (276)	99 (17)
Hemoglobin (g/dL)	11.6 (0.57)	11.2 (0.71)	10.0 (0.42)

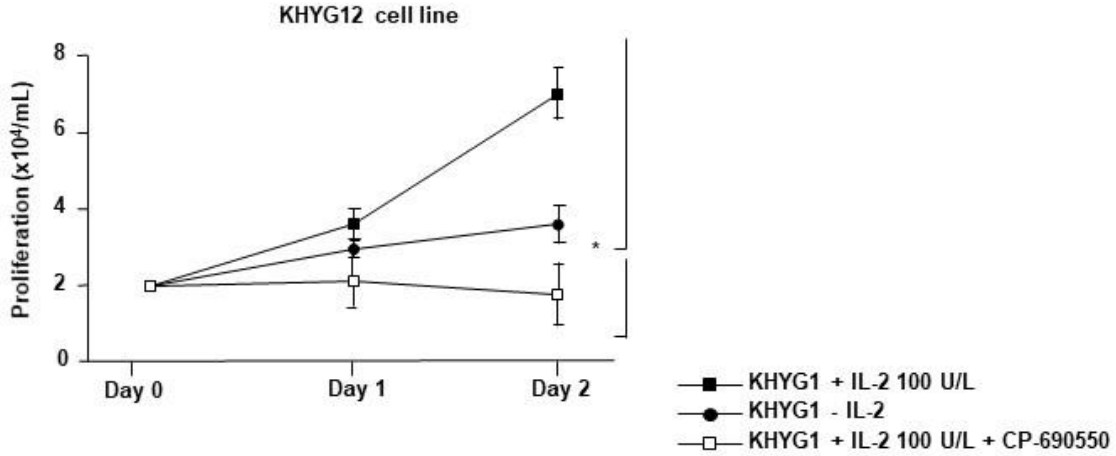
Mice were sampled before sacrifice for autopsy. Results are expressed as mean (standard deviation). WT: wild type.

Supplemental Figure 1

A.

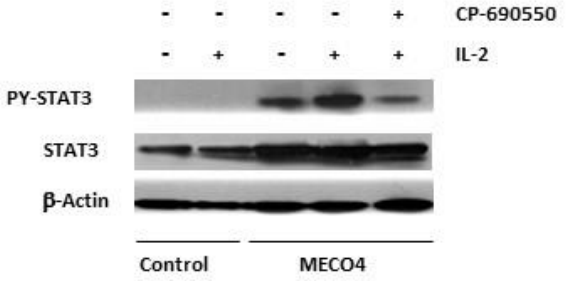


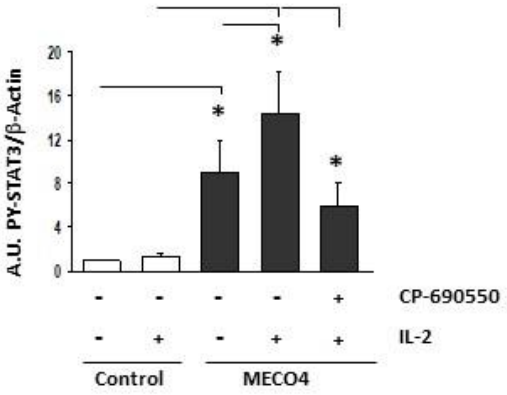




Supplemental Figure 1

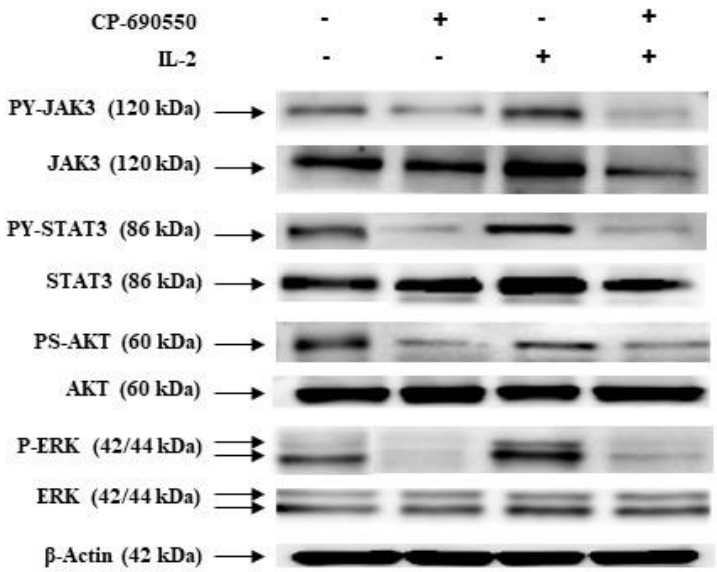
B.





Supplemental Figure 1

C



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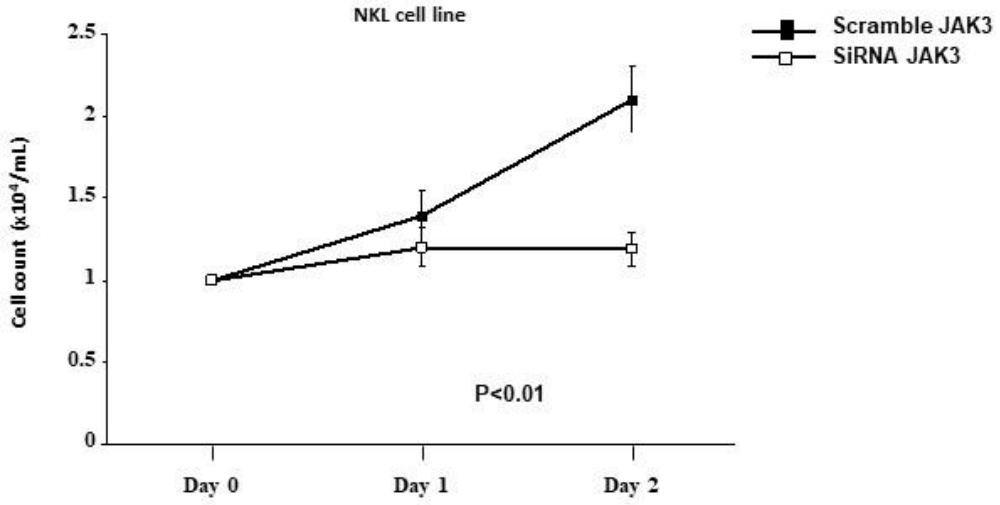
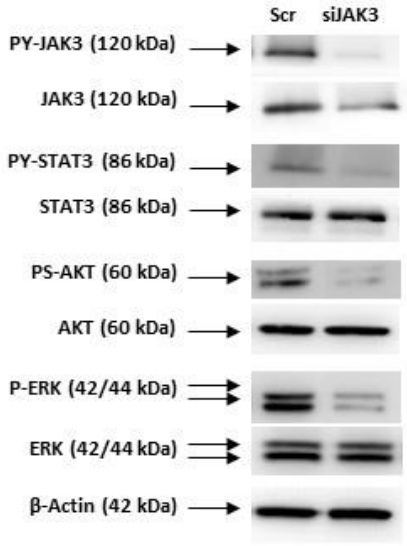


Supplemental Figure 1

E



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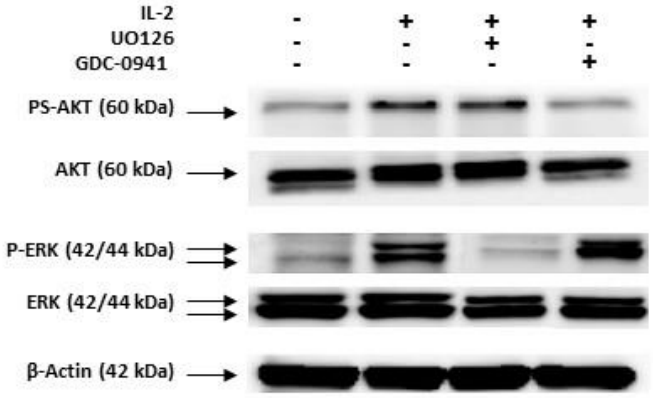

Supplemental Figure S1

Hyperresponsiveness of NKTCL cell lines to rIL-2 and interferon-γ hypersecretion are JAK3-dependent. (A) Proliferation of MEC04 cells in the presence or absence of rIL-2 100 U/L. MEC04 cells were cultured under rIL-2 100 U/L and in the presence of the JAK3 inhibitor CP-690550 50 nM. Normal NK cells obtained from healthy donors cultured with rIL-2 100 U/L, or in the presence of rIL-10 ng/mL (that does not activate JAK3) were used as **controls**[1]. The significance of differences between experimental conditions was determined by the use of Student t test. *, P<0.001; **, P<0.0001. **(B)** Western blot analysis of the expression level of total and phosphorylated STAT3 as a substrate of JAK3. MEC04 cells were cultured overnight without rIL-2 and subsequently stimulated with rIL-2 50 U/L for 30 minutes. MEC04 cells were also exposed to CP-690550 100 nM for 15 minutes before rIL-2 stimulation. Normal NK cells cultured with or without rIL-2 100 U/L were used for comparison, and β-Actin as loading control. The relative intensity of the immunoreactive bands (mean±SD of 3 independent experiments) was quantified by autoradiography (arbitrary units, AU), using the ImageJ Software. The statistical significance was calculated by the Student t test. *, p≤0.05. **(C)** Western blot analysis of the expression levels of total and phosphorylated JAK3, STAT3, AKT and ERK1/2 in NKL cell line following a 48-h exposition to the JAK3 inhibitor CP-690550 50 nM. β-Actin was used as loading control. **(D)** Specific JAK3 inhibition decreases NKL cell line growth and interferon-γ hypersecretion. NKL cells were transfected with *JAK3*-specific or scrambled control siRNAs (Scr), then cultured in the presence of rIL-2 for 2 days and analyzed by western blotting at day 2. After trypan blue coloration, the number of viable cells (i.e., not colored by trypan blue) was evaluated daily. **(E)** Western blot analysis of the expression levels of total and phosphorylated JAK3, STAT3, AKT and ERK1/2 in NKL cell line at day-2 following specific JAK3 inhibition. β-Actin was used as loading control. Abbreviations: Scr: scrambled; siJAK3: anti-*JAK3* siRNA. **(F)** Interferon-γ hypersecretion involves JAK3

substrates MEK1/2 and AKT. NKL cells were cultured for 2 days in the presence of rIL-2 100 U/L and exposed to the MEK1/MEK2 (UO126, 10 µM) or PI3K/AKT (GDC-0941, 5 µM) inhibitors. At day-2, supernatant was collected for interferon-γ concentration measurement for each condition. Day-2 western blot analysis of the expression levels of total and phosphorylated AKT and ERK1/2. β-Actin was used as loading control.

1. Wendt K, Wilk E, Buyny S, Schmidt RE, Jacobs R. Interleukin-21 differentially affects human natural killer cell subsets. Immunology 2007;122(4):486-95.