**SUPPLEMENTAL DATA**

# *STAT3* mutations identified in human hematological neoplasms induce myeloid malignancies in a mouse bone marrow transplantation model.

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## Supplemental data legends

**Figure S1:** Percentages of GFP-positive cells at different time-points after transduction.
A. Transduction efficiencies in the bone marrow cells right after the second spinfection and just before the injection into the primary recipients.
B. Representative percentages of GFP in the blood leukocytes of primary recipients at 1 month post-transplantation.

C. Percentage of GFP-positive cells in the bone marrow and spleen cells from three independent STAT3Y640F primary recipients at the time of transplantation into secondary recipients.

**Figure S2:** *STAT3* mutations in patients, proliferation assays and hematological follow-up of *in vivo* murine models.
A. *STAT3* sequences in tumor samples.
Vertical arrows indicate the sequence variations. Horizontal arrows indicate the origin of the indel mutations.
B. Proliferation assay of transduced murine BAF3 cells with empty, wild-type (WT), *STAT3* or *STAT3 Y640F* MSCV retroviruses in presence of IL3, IL4 or FLT3 ligand, as compared to a condition without cytokines (control).
C. White blood cells (WBC) count, hemoglobin level, platelets count and percentage of GFP-positive cells follow up of the individual 395 (STAT3 Y640F mice with thrombocytosis disease). The x-axis values represent the number of months after bone marrow transplantation.

**Table S1:** List of primers used for *STAT3* PCR and sequencing

**Table S2:** Antibodies combinations used for flow cytometry

**Table S3:** Clinical data of *STAT3* mutated patients

**Figure S1**

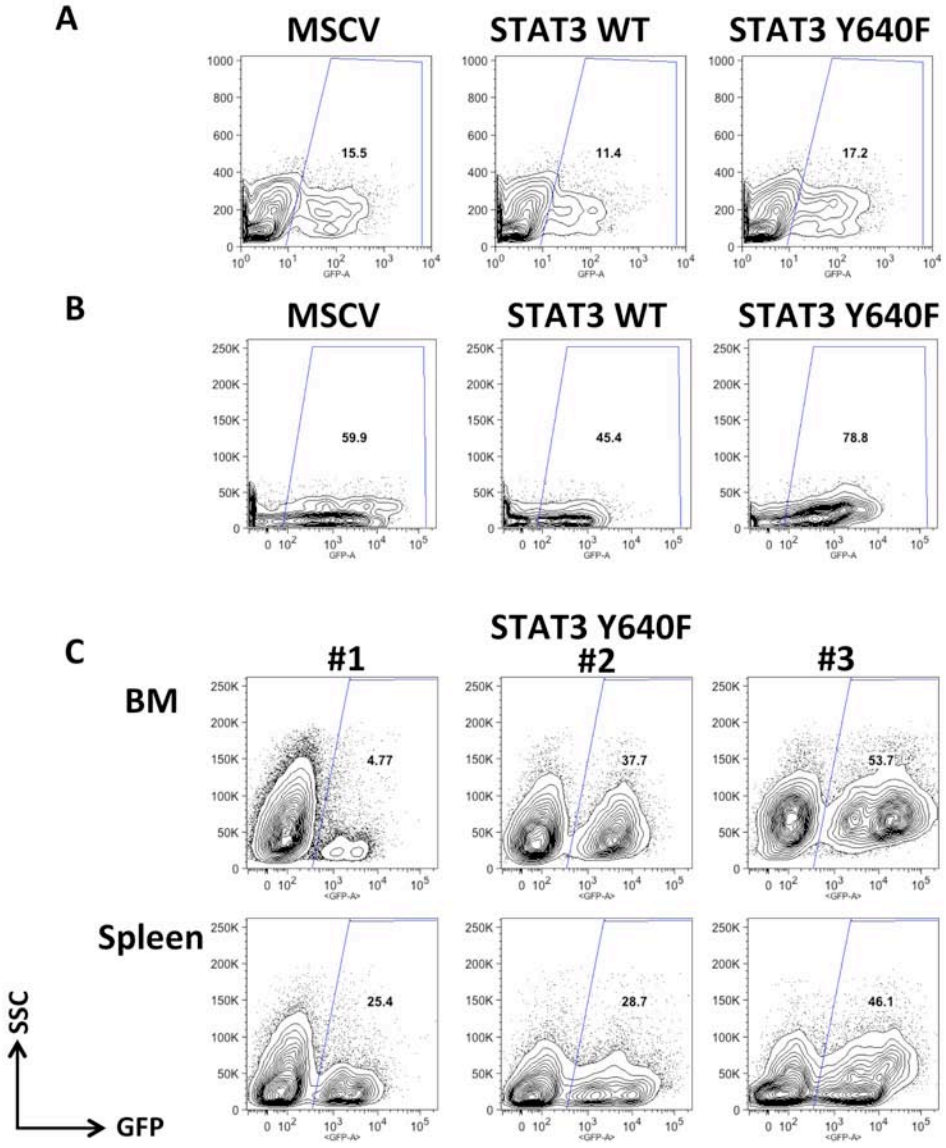
A
MSCV
STAT3 WT
STAT3 Y640F
15.5
11.4
17.2
B
MSCV
STAT3 WT
STAT3 Y640F
59.9
45.4
78.8
C
STAT3 Y640F
#1
#2
#3
BM
4.77
37.7
53.7
Spleen
25.4
28.7
46.1
SSC
GFP
GFP-A

**Figure S2**

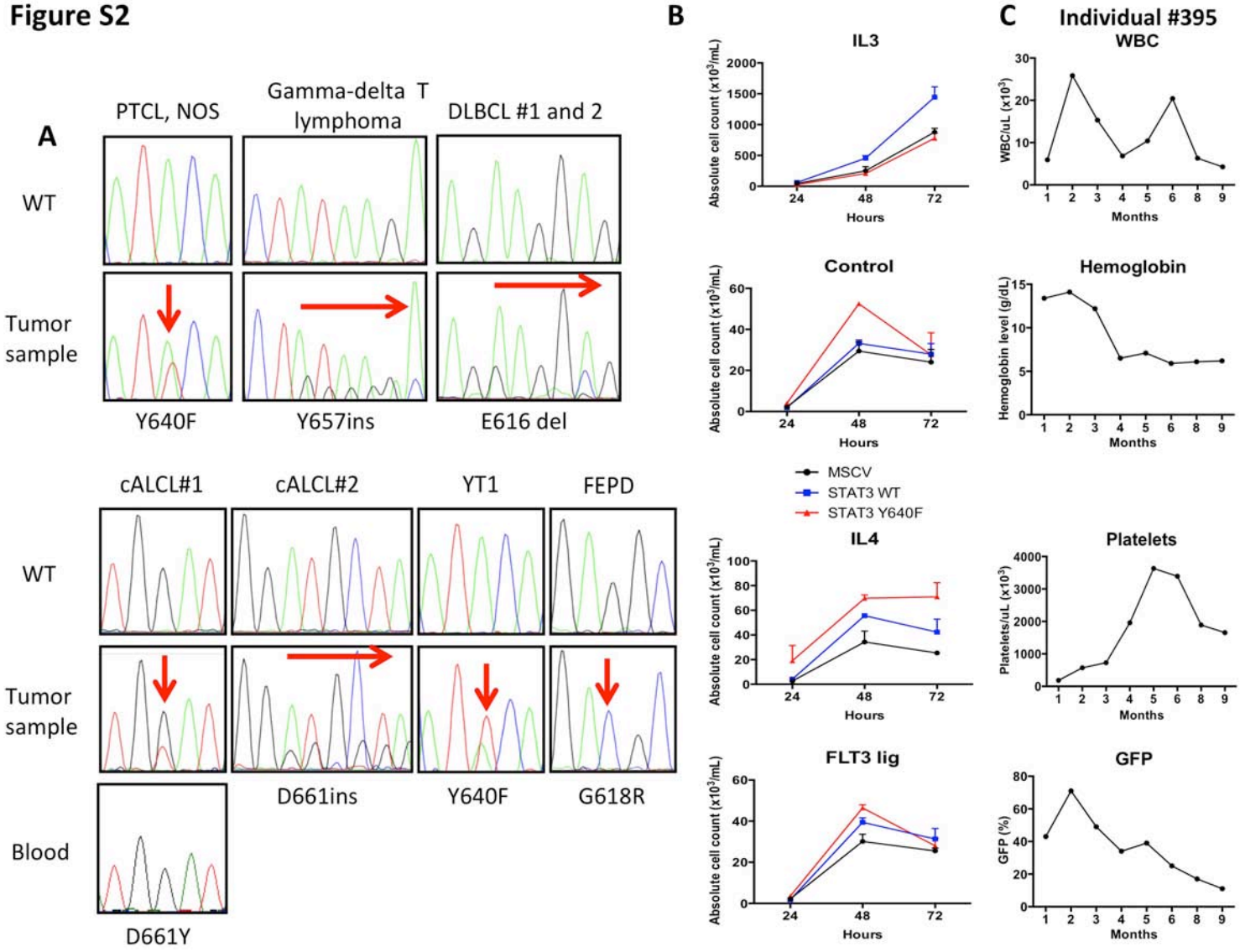
A
PTCL, NOS
Gamma-delta T lymphoma
DLBCL #1 and 2
WT
Tumor sample
Y640F
Y657ins
E616 del
cALCL#1
cALCL#2
YT1
FEPD
WT
Tumor sample
D661ins
Y640F
G618R
Blood
D661Y
B
IL3
Control
IL4
FLT3 lig
Absolute cell count (x10³/mL)
Hours
MSCV
STAT3 WT
STAT3 Y640F
C
Individual #395
WBC
WBC/uL (x10³)
Hemoglobin
Hemoglobin level (g/dL)
Platelets
Platelets/uL (x10³)
GFP
GFP (%)
Months

Table S1: List of primers used for *STAT3* PCR and sequencing

| | |
|---|---|
| *STAT3*exon3F | GAATGGGTTATAGCATCAGG |
| *STAT3*exon3F | CTCAGGTAATGATGCTCACG |
| *STAT3*exon6F | TTTTCTGTTCCCAAGGAAAT |
| *STAT3*exon6R | CCCGCCTTAAGATCTAAACAGA |
| *STAT3*exon17F | GACCCACTCCTTGCCAGTT |
| *STAT3*exon17R | CAGTAGACATGGCCCAAATG |
| *STAT3*exon20F | TGTAACCAAGTCCCCTGCTC |
| *STAT3*exon20R | CAGGGGGCAGTAGGTGCT |
| *STAT3*exon21F | AGTCTTTTCCCCTTCGAGGA |
| *STAT3*exon21R | CAAGGATCCCAAAATTTCCA |

**Table S2: Antibodies combinations used for flow cytometry**

| Antibody | Clone | Fluorochrome | Source |
|---|---|---|---|
| CD11b (Mac) | M1/70 | PE | eBioscience |
| Ly-6G (Gr-1) | RB6-8C5 | PE-Cy7 | eBioscience |
| CD41 | MWReg30 | APC | eBioscience |
| CD42b (GPIbα) | Xia.G5 | PE | Emfret Analytics |
| CD71 | RI7 217.1.4 | PE | eBioscience |
| Ter119 | TER-119 | APC | eBioscience |

PE: phycoerythrin, APC : allophycocyanin, PE-Cy7: phycoerythrin-cyanine 7

## Table S3: Clinical data of *STAT3* mutated patients

| | |
|---|---|
| PTCL, NOS Y640F | 60-year-old man<br>Diagnosed in 2004, with a PTCL, NOS stage IV (bone marrow, spleen and liver involvement)<br>CD8+ CD3-CD5-CD4-<br>Normal karyotype<br>CD4 lymphopenia and paraneoplastic dysautonomia syndrom<br>Therapy: 4 ACVBP, 2 MTX, 4 Holoxan/Vp16 and 2 Aracytine cycles<br>Currently in complete remission (8,5 years of follow up) |
| Gamma-delta T lymphoma Y657ins | 38-year-old man<br>Common variable immunodeficiency (CVID) with hypogammaglobulinemia, B and T-cell lymphopenia diagnosed in 1995<br>Diagnosed in May 1998 with a circulating CD3+ gamma/delta T-cell clone that may correspond with the leukemic phase of a gamma/delta T-cell lymphoma<br>Therapy: methotrexate per os (during 6 months, stopped because of digestive intolerance)<br>JC virus-positive progressive multifocal leukoencephalopathy (PML) in January 2000<br>Death on April 2000 (PML in the context of CVID and gamma/delta T-cell lymphoma) |
| DLBCL E616del #1 | 82-year-old man<br>Diagnosed in November 2008 with a DLBCL stage II (cervical lymph nodes)<br>GC subtype CD10+<br>Complex karyotype (hyperdiploidy)<br>Autoimmune hepatitis<br>Therapy: 4 R-miniCHOP cycles (did not get the two last ones because of a E. Coli septicemia)<br>In complete remission in August 2009 (9 months of follow-up)<br>No follow up since August 2009 |
| DLBCL E616del #2 | 56-year-old man<br>Diagnosed in March 2006 with a DLBCL stage III (cervical lymph nodes, spleen involvement)<br>GC subtype<br>Therapy:4 R-ACVBP, 2 high dose Methotrexate cycles and an high dose conditioning regimen (BEAM) with autologous bone marrow transplantation<br>In complete remission in July 2012 (6 years of follow up) |
| cALCL D661ins | 72-year-old man<br>Stage T1bN0M0<br>Relapse 6 months after remission (Global survival: 15 months) |
| cALCL D661Y | 82-year-old woman<br>Stage T3cN0M0<br>No remission (Global survival: 6 months) |