MOHITO, a novel mouse cytokine-dependent T-cell line, enables studies of oncogenic signaling in the T-cell context

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Supplementary Appendix

DNA and RNA isolation and PCR

DNA was extracted using the Wizard® Genomic DNA Purification Kit (Promega). RNA was extracted using a combined approach of TRIzol reagent (Invitrogen) and the RNeasy Mini kit (Qiagen). Analysis of the T-cell receptor (TCR) status in primary and cultured cells was performed by RT-PCR, as described by Baker *et al.*[1]

Karyotyping and m-FISH

MOHITO cells were collected at 0, 2 and 4 months of culture. Cytogenetic analysis and FISH followed routine protocols. Multicolor FISH (m-FISH) was performed using a 21XMouse kit from MetaSystems (MetaSystems, Altlussheim, Germany). FISH images were acquired using a 63x/1.40 oil-immersion objective in an Axioplan 2 fluorescence microscope equipped with an Axiophot 2 camera (Carl Zeiss Microscopy, Jena, Germany) and a MetaSystems Isis imaging system. FISH probes are listed in *Online Supplementary Table S2*.

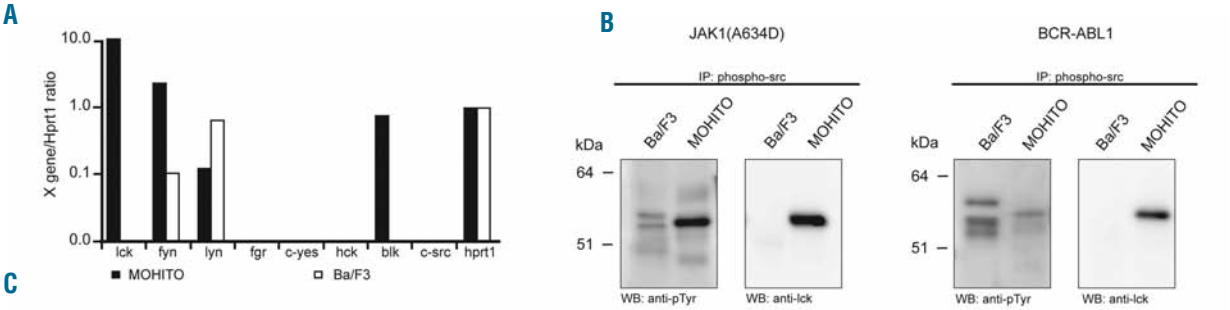
Western blotting and immunoprecipitation

Cells were lysed in a commercial cell lysis buffer (Cell Signaling) supplemented with complete protease inhibitors (Roche) and 5 mM Na3VO4. Equal amounts of protein (30 µg) were loaded onto NuPAGE® gels (Invitrogen) and subsequently transferred to an activated polyvinylidene difluoride (PVDF) membrane for detection. Membranes were blocked in 5% non-fat dry milk in TBS-T. Primary antibodies were applied either for 1 h or overnight (phospho-antibodies) diluted in blocking solution.

References

1. Baker FJ, Lee M, Chien YH, Davis MM. Restricted islet-cell reactive T cell repertoire of early pancreatic islet infiltrates in NOD mice. Proc Natl Acad Sci USA. 2002;99(14): 9374-9.

Online Supplementary Table and Figure. SEE PDF.



Online Supplementary Figure S9. Downstream signaling of oncogenic kinases depends on the cell context. (A) qRT-PCR expression analysis of Src-family kinase (SFK) in MOHITO (closed bars) and Ba/F3 cells (open bars). Relative expression of mRNA for each SFK was normalized for Hprt1 gene expression and is blotted at the Y-axis in logarithmic scale. (B) Activated SFKs were immunoprecipitated with a phospho-src antibody from either JAK1(A634D) (left panel) or BCR-ABL1 (right panel) dependent MOHITO and Ba/F3 cells. IP samples were separated by SDS-Page and protein bands were detected with a phospho-tyrosine specific antibody (anti-pTyr, clone 4G10). Blots were stripped and probed with anti-lck (right panel). Molecular weights (kD) are indicated on the left. (C) Western blot showing strong constitutive activation of the Mapk/Erk pathway in both JAK1(A634D) and BCR-ABL1 dependent Ba/F3 cells under baseline conditions. Whole cell lysates from both JAK1(A634D) (left panel) and BCR-ABL1 (right panel) and transformed MOHITO and Ba/F3 cells were analyzed. The membrane was stripped and re-probed in between to allow identification of proteins with similar molecular weight. β-actin is shown as loading control.