

**Combined transcriptome and proteome profiling of SRC kinase activity in healthy and E527K defective megakaryocytes**

Lore De Kock,<sup>1</sup> Fabienne Ver Donck,<sup>1</sup> Chantal Thys,<sup>1</sup> Anouck Wijgaerts,<sup>1</sup> Koji Eto,<sup>2,3</sup> Chris Van Geet<sup>1</sup> and Kathleen Freson<sup>1</sup>

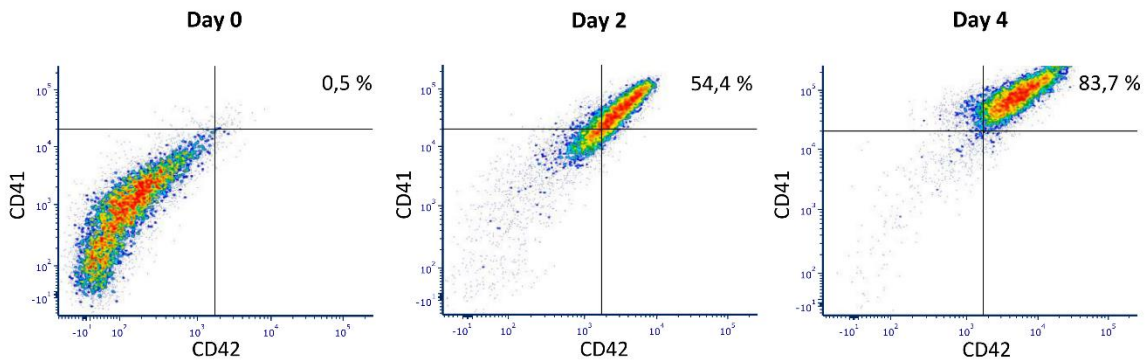
<sup>1</sup>Center for Molecular and Vascular Biology, Department of Cardiovascular Sciences, University of Leuven, Leuven, Belgium; <sup>2</sup>Department of Clinical Application, Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan and <sup>3</sup>Department of Regenerative Medicine, Chiba University Graduate School of Medicine, Chiba, Japan

Correspondence: KATHLEEN FRESON - [kathleen.freson@kuleuven.be](mailto:kathleen.freson@kuleuven.be)

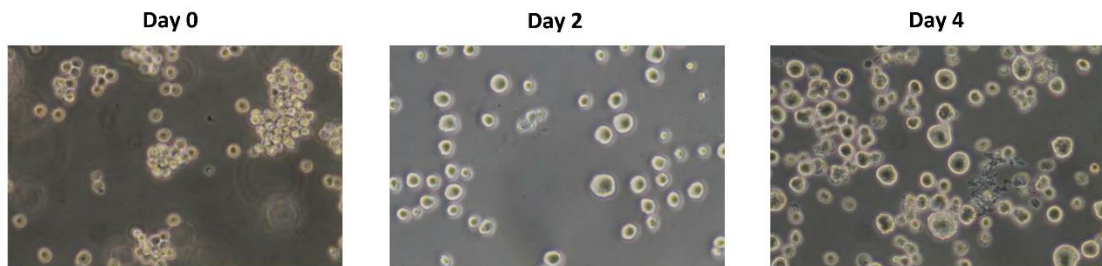
doi:10.3324/haematol.2021.279248

## Supplementary Figures

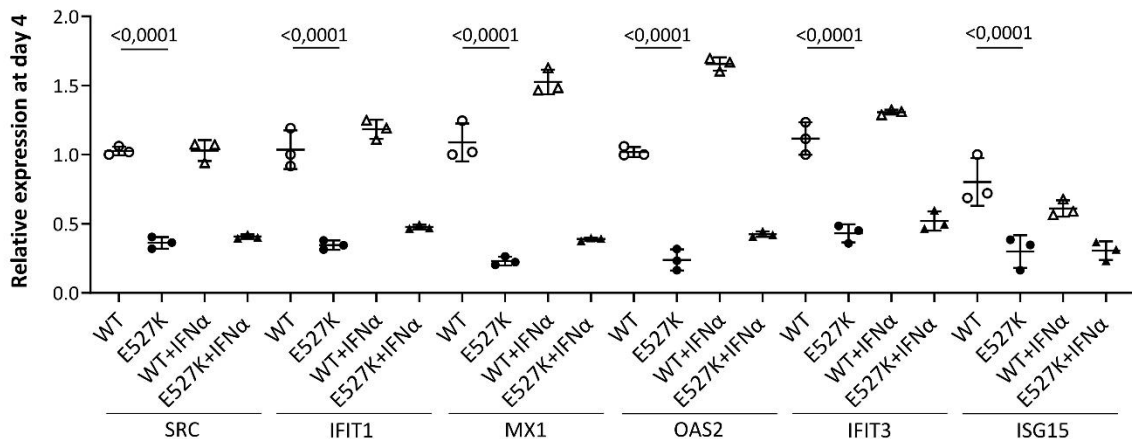
**A**



**B**



**C**



**Supplementary Figure 1: Differentiation of the immortalized megakaryocyte cell line (imMKCL) and expression analysis using quantitative RT-PCR for *SRC* and five interferon-stimulated genes in lentiviral transduced WT-SRC and E527K-SRC imMKCL. **A.** Flow cytometry analysis of day 0 (undifferentiated), day 2 and day 4 immortalized megakaryocyte cell lines (imMKCL). Cells were stained for CD41 and CD42 markers during different timepoints of differentiation. Percentages for the number of CD41+ and CD41/42+ megakaryocytes (MK) are shown. **B.** Images of the imMKCL during different timepoints of differentiation. The asterisk depicts proplatelet formation at day 4. Day 2 and 4 cultures contain larger MK. **C.** WT-SRC and E527K-SRC megakaryocyte samples were differentiated from immortalized megakaryocyte cell lines for four days, with and without the addition of interferon  $\alpha$  (IFN $\alpha$ ) to the medium. Relative expression of *SRC*, *IFIT1*, *MX1*, *IFIT3*, *ISG15* and *OAS2* was analyzed using *GAPDH* and *RUNX1* for normalization ( $\Delta\Delta C_t$  method). Bars represent the mean and standard deviation of three biological replicates (triplicated transduction experiment). P-values were measured by one-way ANOVA with multiple comparisons. Primer sequences and PCR conditions available upon request.**

### **Supplementary Table Legends**

**Supplementary Table 1:** Overview of all differentially expressed genes obtained after RNAsequencing on Illumina HiSeq4000 of total RNA from megakaryocytes from lentiviral transduced hematopoietic stem cells with SRC-WT and SCR-E527K (VIB Nucleomics Core facility, KULeuven).

**Supplementary Table 2:** Overview of all differentially expressed proteins obtained after shotgun proteomics (LC-MS/MS) and using the MaxQuant algorithm (version 1.6.10.43) for identification of protein extracts from megakaryocytes from lentiviral transduced hematopoietic stem cells with SRC-WT and SCR-E527K (VIB Proteomics Core facility, UGent).