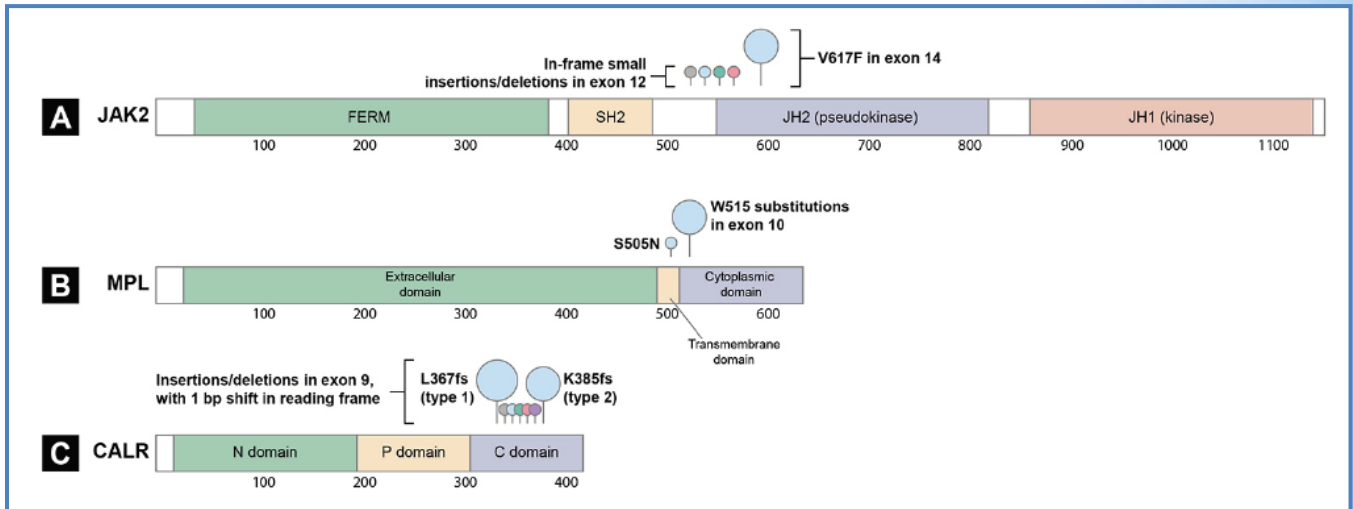


**First-hit SETBP1 mutations cause a  
myeloproliferative  
disorder with bone marrow fibrosis**

# BONE MARROW FIBROSIS

**Primary myelofibrosis: myeloproliferative neoplasm affecting a hematopoietic clone, resulting in marrow fibrosis, extramedullary hematopoiesis (spleen, liver), anemia, thrombocytopenia and myeloid leukocytosis/leukopenia**

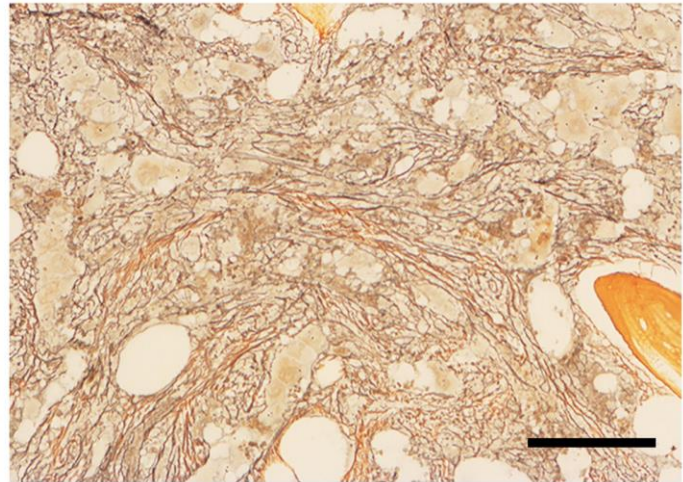
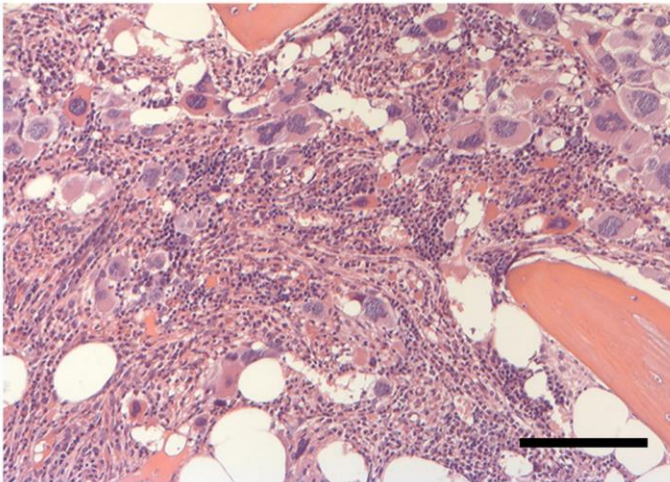
**Somatic mutations in JAK2, MPL and CALR explain ~80% of primary myelofibrosis**



## AIM OF THE STUDY

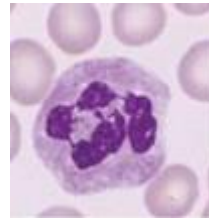
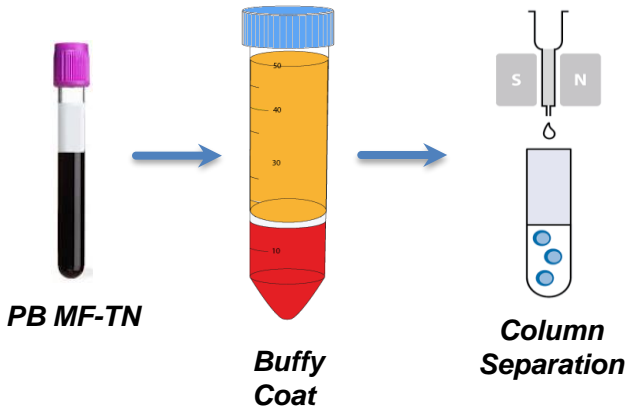
**JAK2, MPL and CALR negative myelofibrosis are defined as 'Triple-Negative Myelofibrosis', they tend to have an aggressive course and their origin is currently largely unknown**

**Aim:** Identify and characterize the molecular mechanisms responsible for the onset of Triple-Negative Myelofibrosis (MF-TN)

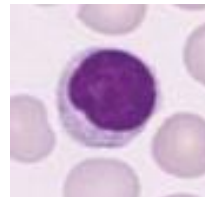


# METHODS

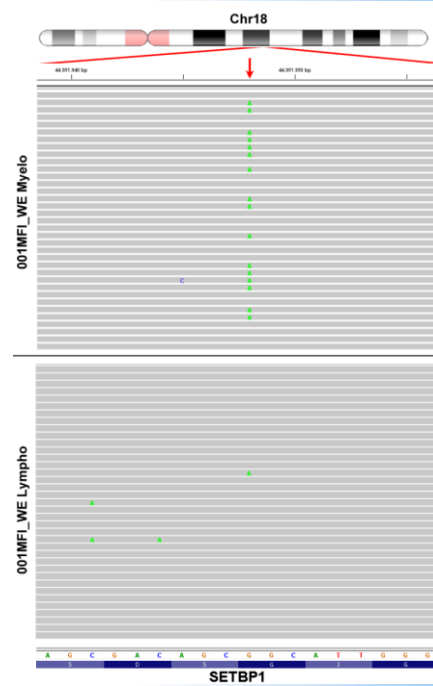
## Methods: Whole-Exome and Targeted sequencing



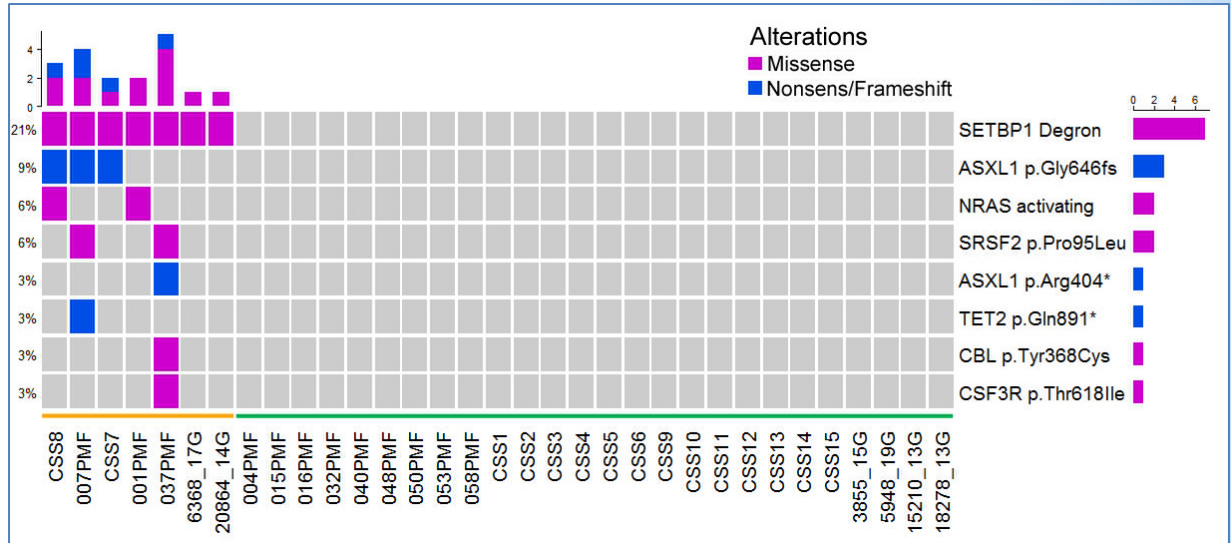
**Myeloid**



**Lymphoid**

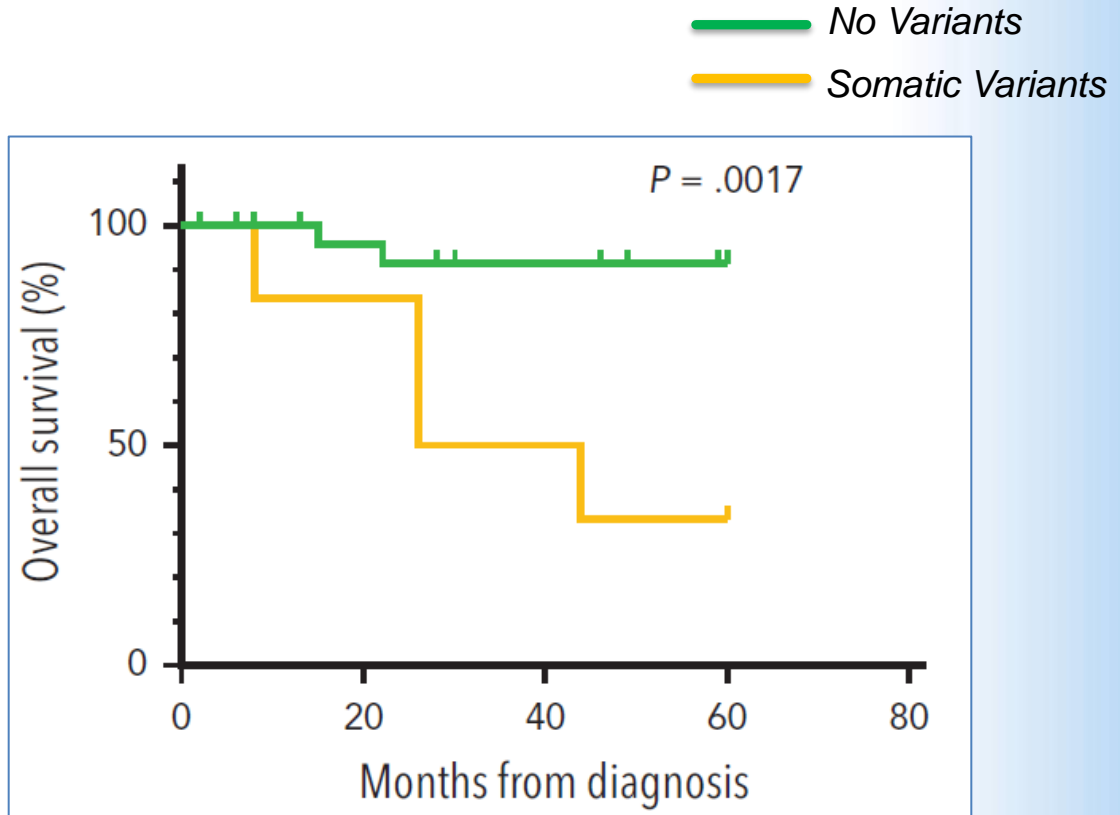


# RESULTS – SOMATIC VARIANTS



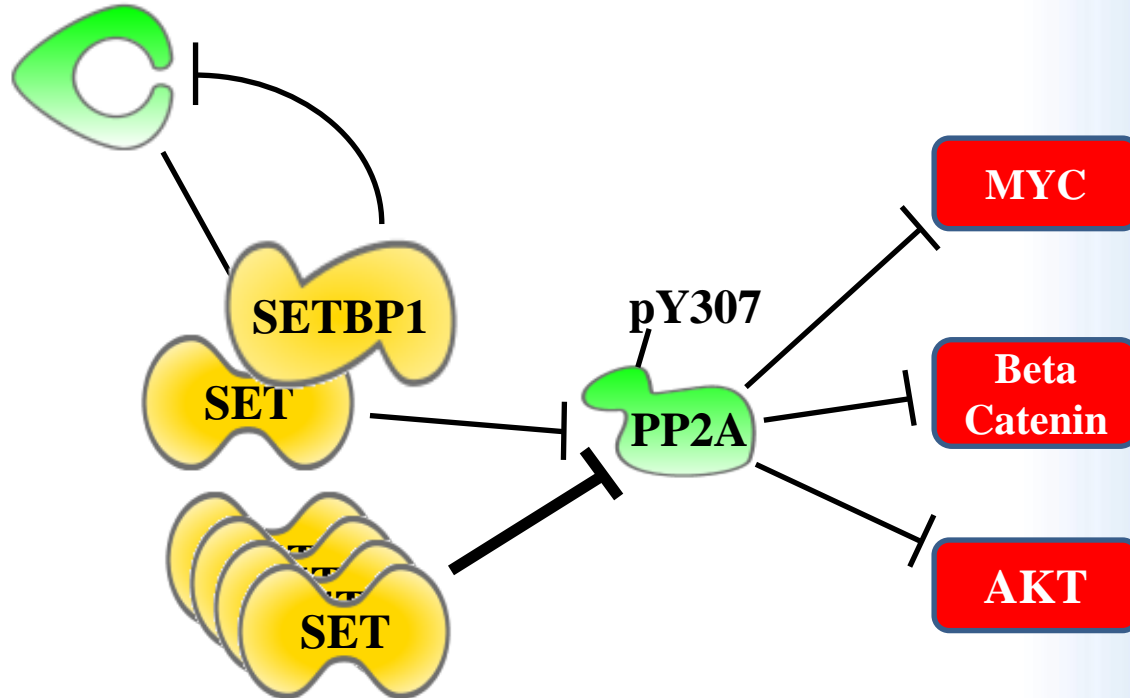
*In 7/36 cases (19.4%) we identified somatic mutations affecting SETBP1*

## RESULTS – Somatic variants

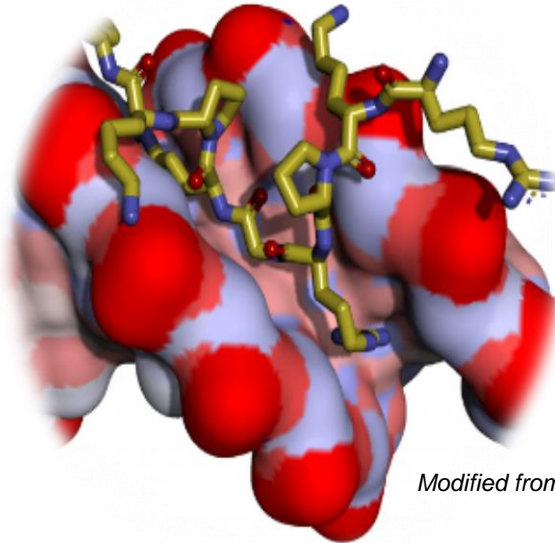
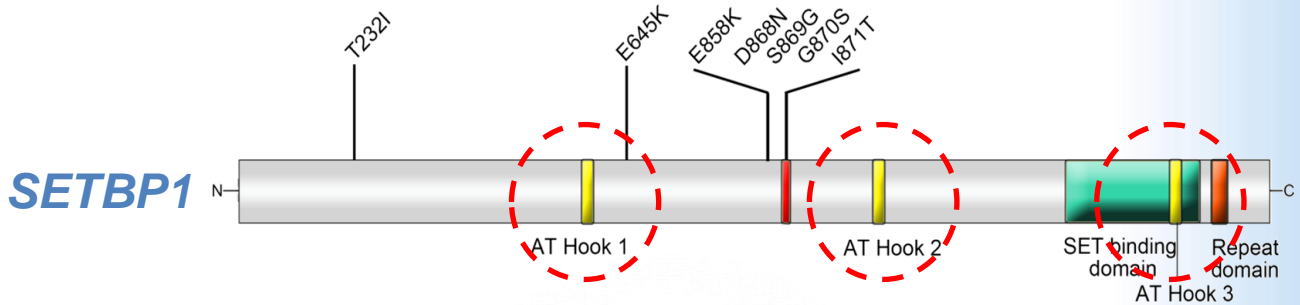


## SETBP1 - BACKGROUND

Proteases



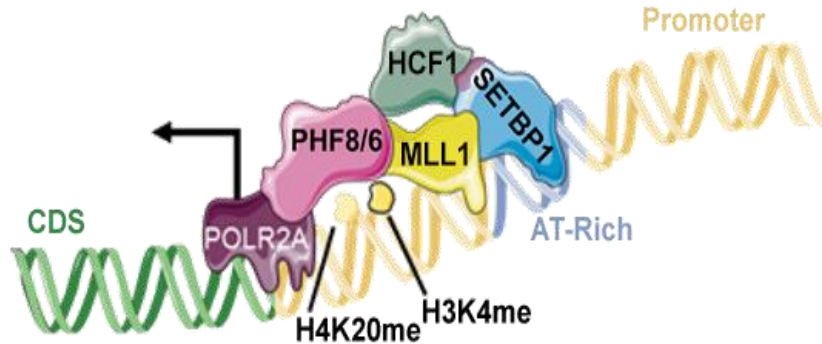
## *SETBP1 as an epigenetic modulator*



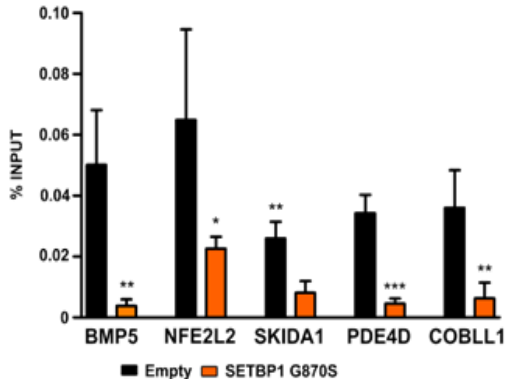
Modified from PLoS One. 2012;7(5):e3712



# SETBP1 Epigenetic Complex

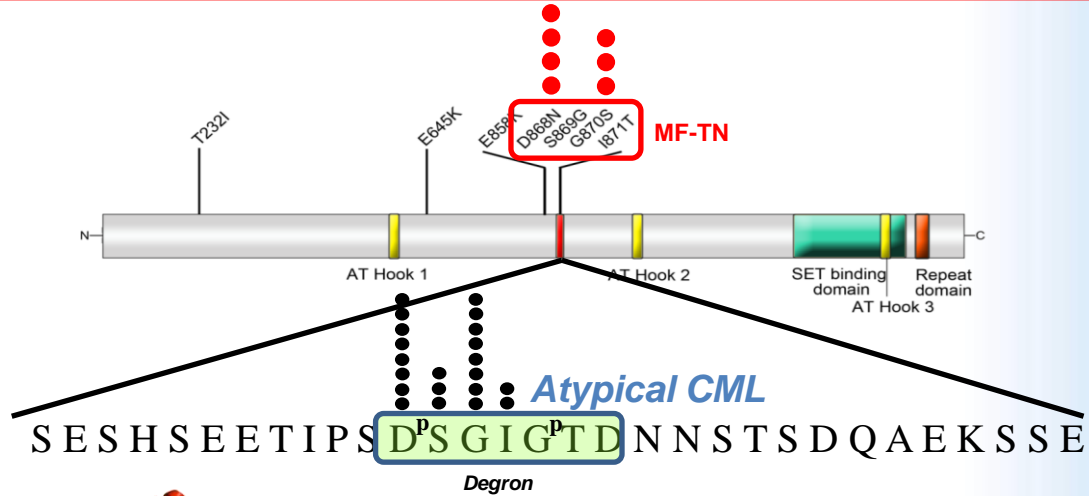


## H4K20me1

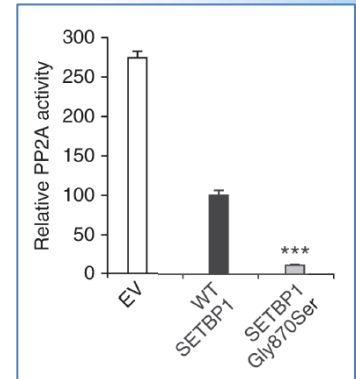
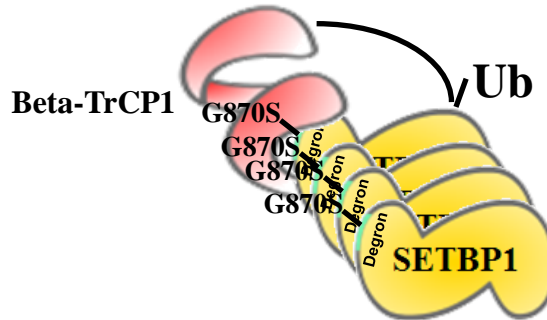


**KMT2A (MLL1): H3K4 METHYLASE**  
**PHF8: H4K20 DEMETHYLASE**

# SETBP1 - BACKGROUND



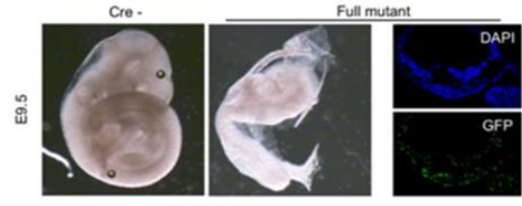
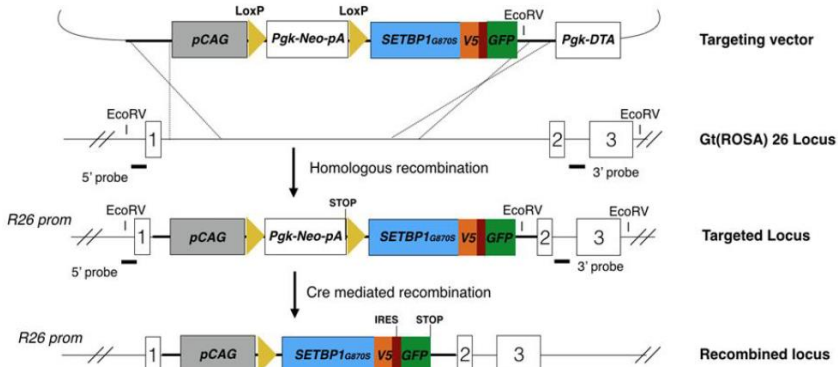
*Beta-TrCP1  
SCF complex*



Piazza R. et al. Nat Genet. 2013 Jan;45(1):18-24

Piazza R. et al. Nat Commun. 2018 Jun 6;9(1):2192

# SETBP1 mouse model – CMV::Cre and VAV::iCRE



R26::LSL-SETBP1<sup>G870S</sup>

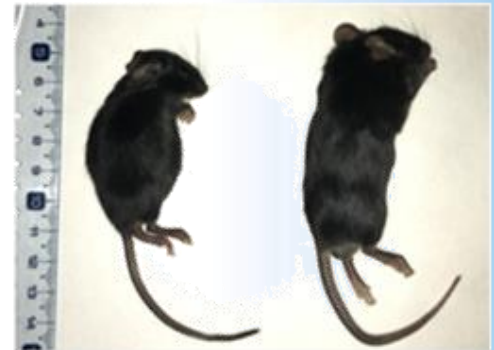
VAV-iCRE

X

R26::SETBP1<sup>G870S</sup>::VAV-iCRE

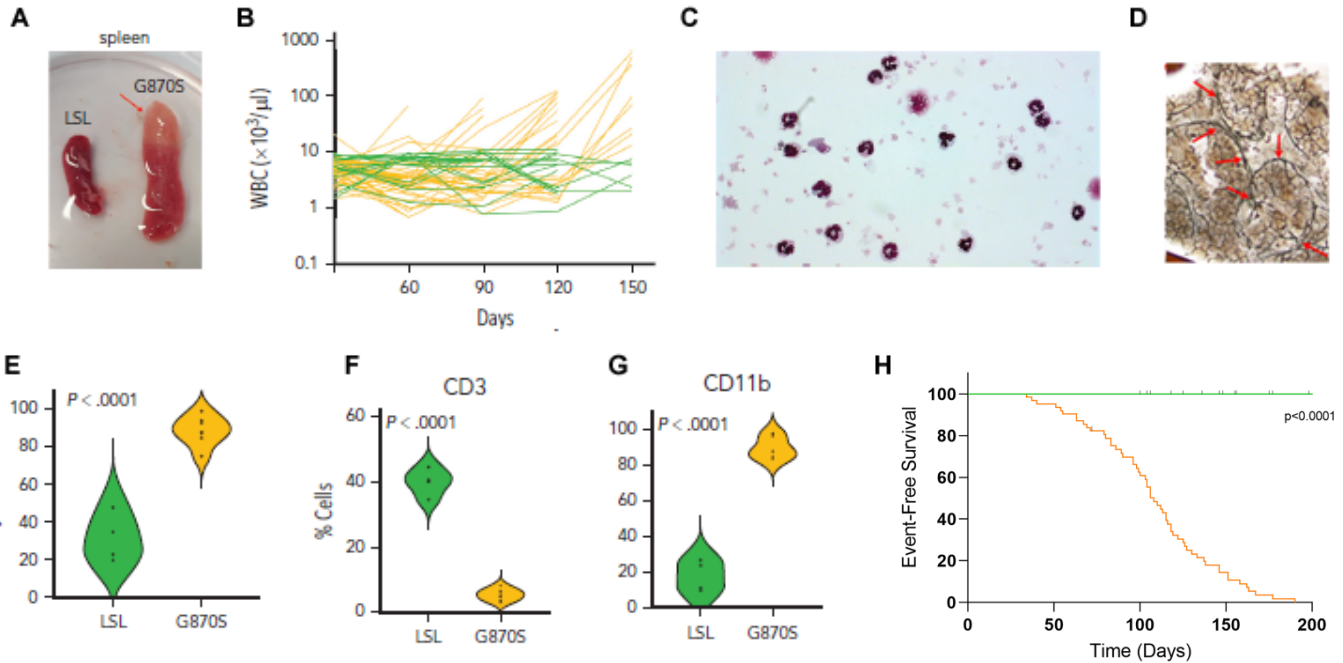
R26::VAV-SETBP1

WT

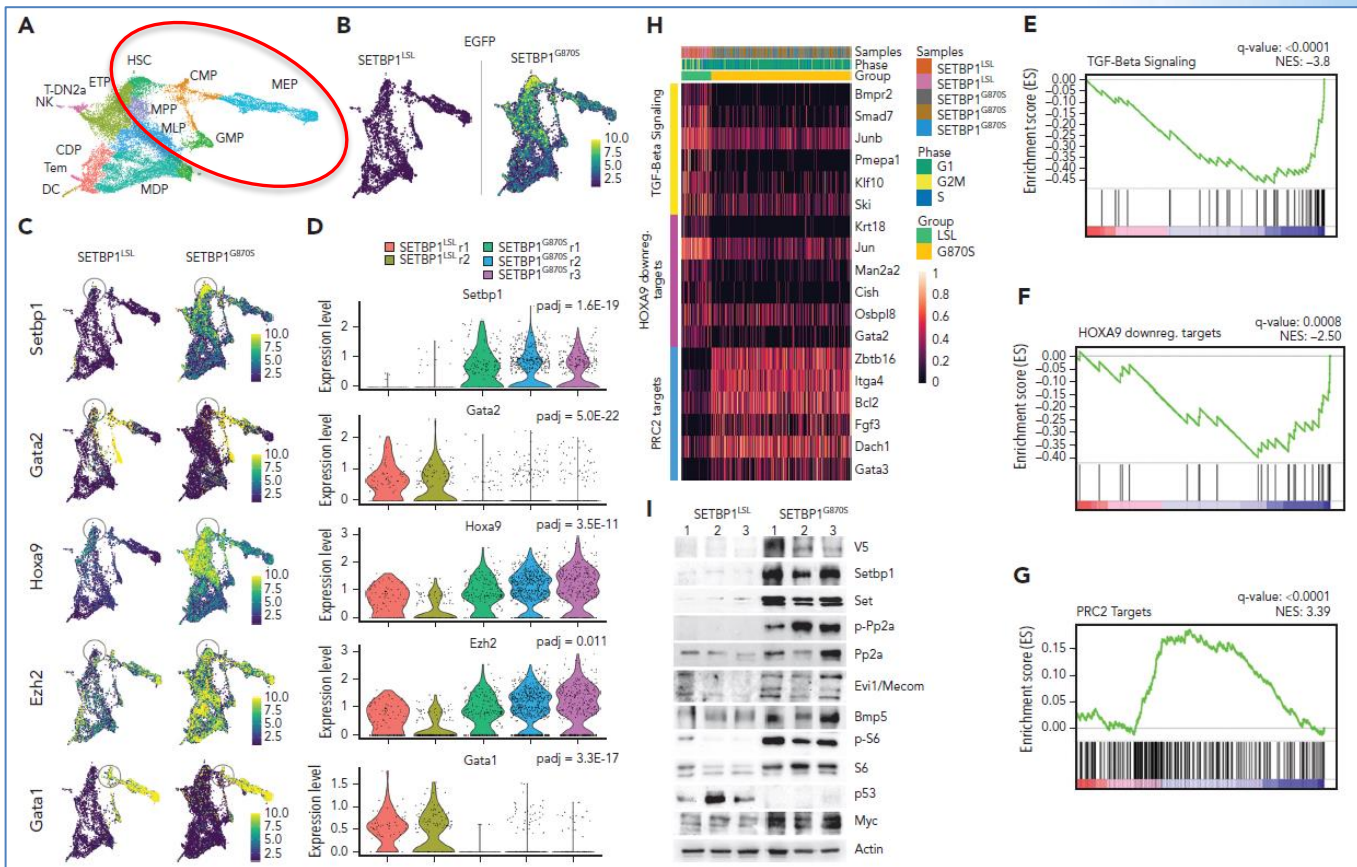


*Hematopoietic lineage restricted model*

# SETBP1 mouse model – VAV::iCRE

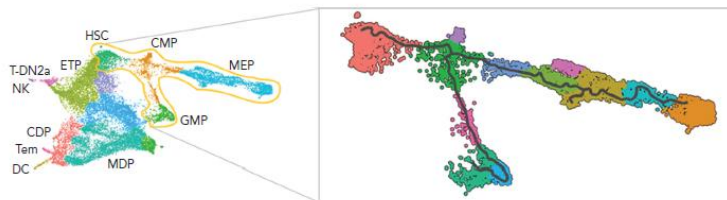


# scRNA on Bone Marrow Lin- cells

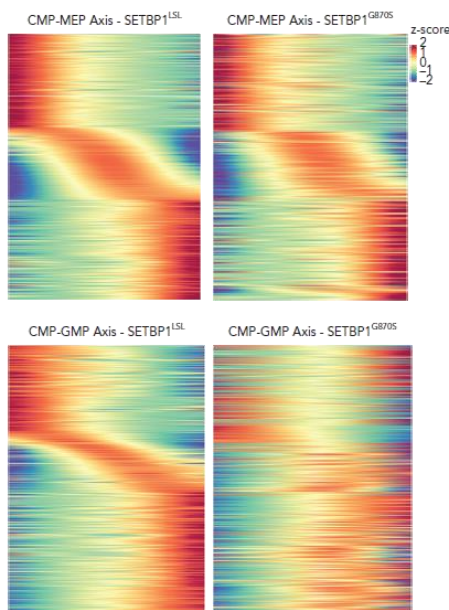


# scRNA on Bone Marrow Lin- cells

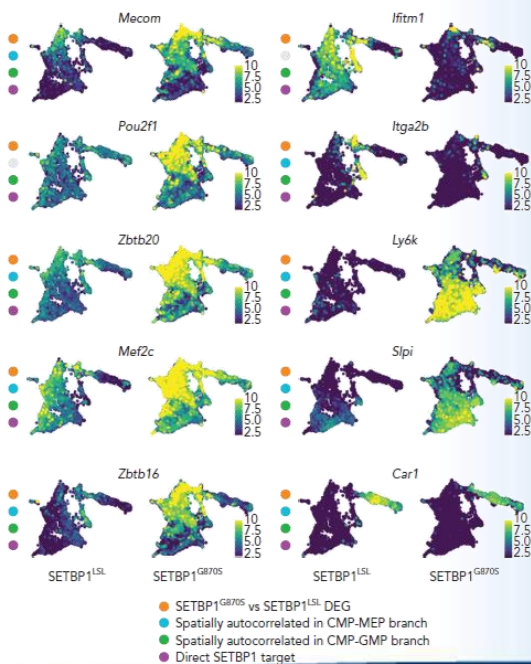
A



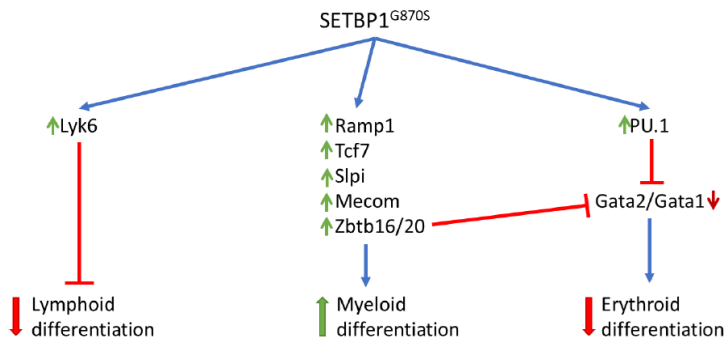
B



C

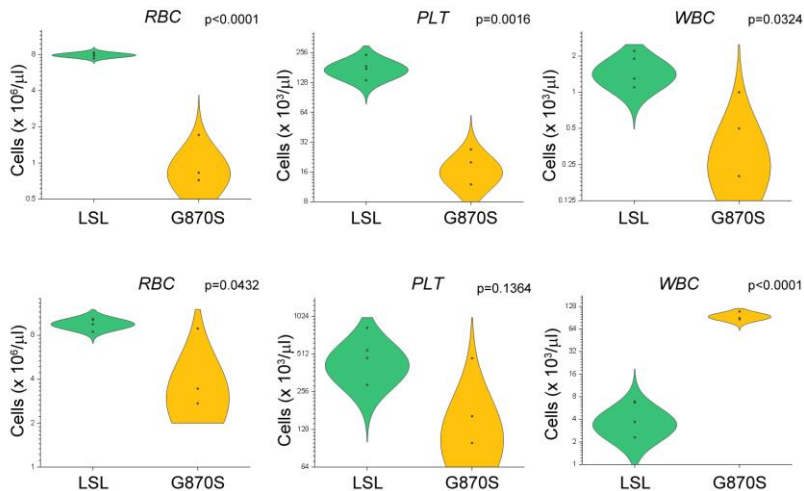


# BM cells transplantation



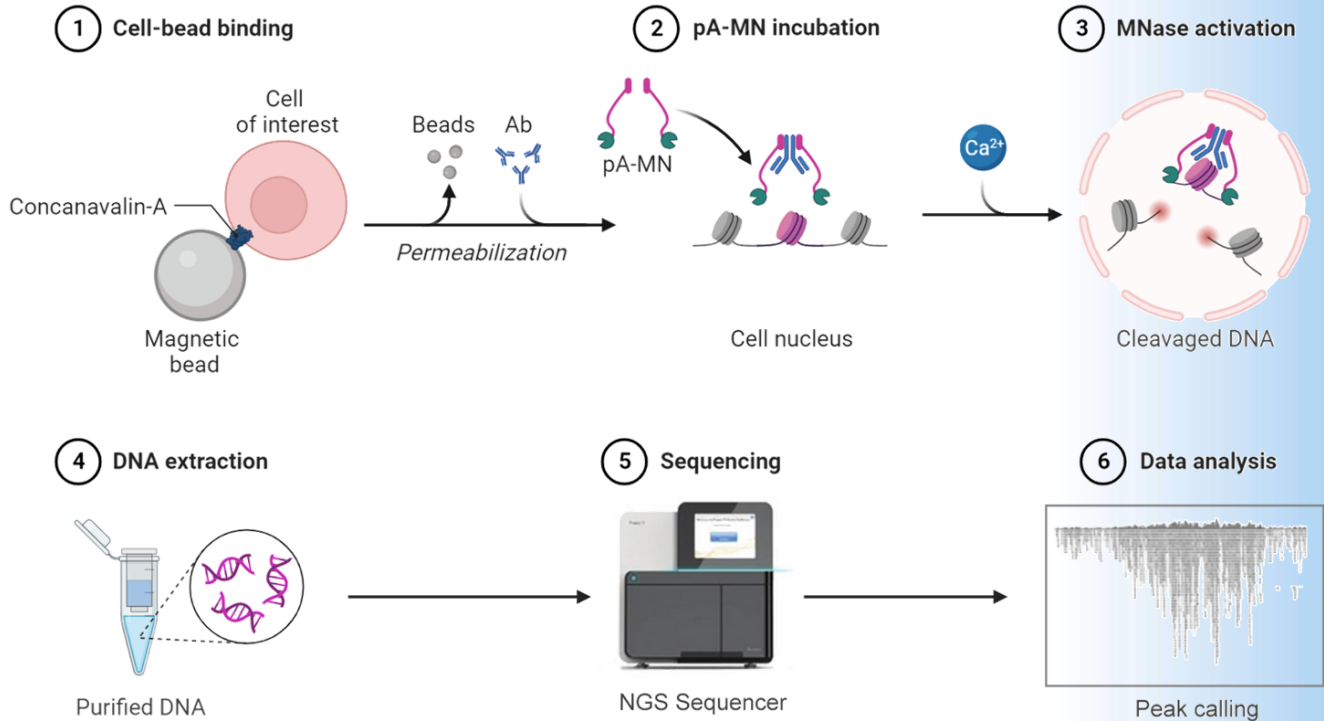
*Lethally irradiated recipients*

*Sublethally irradiated recipients*



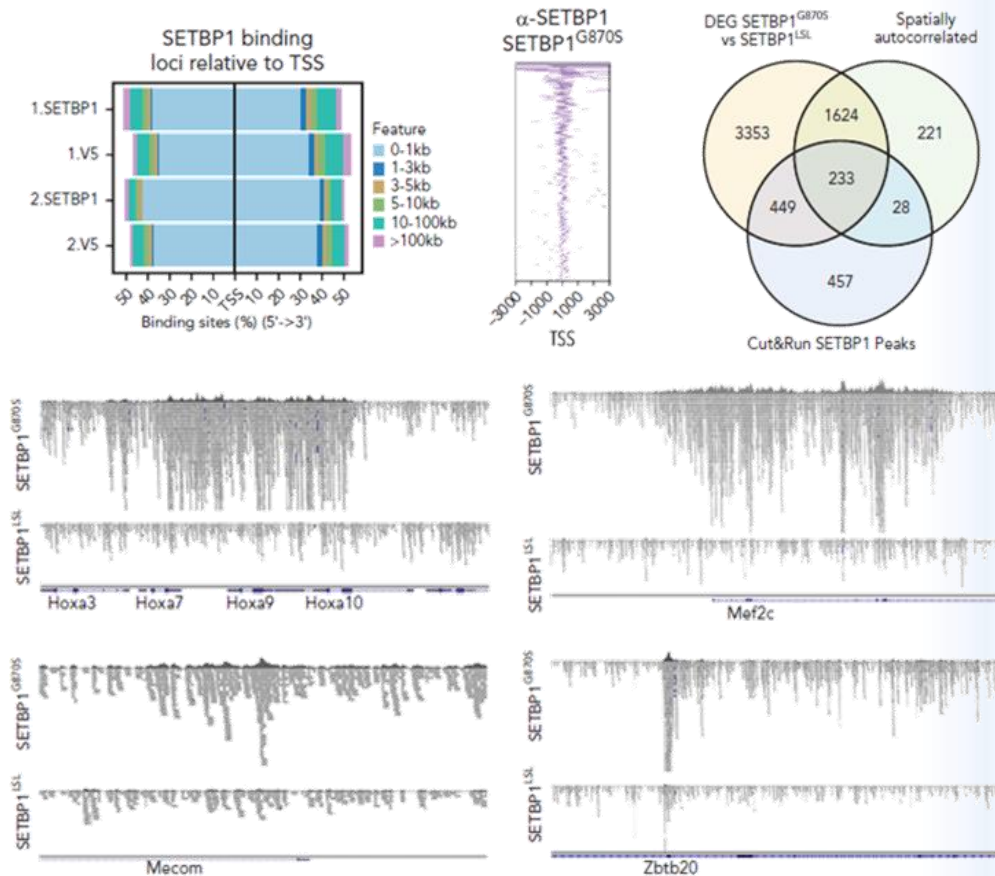


# Cut and Run on Bone Marrow Lin- cells

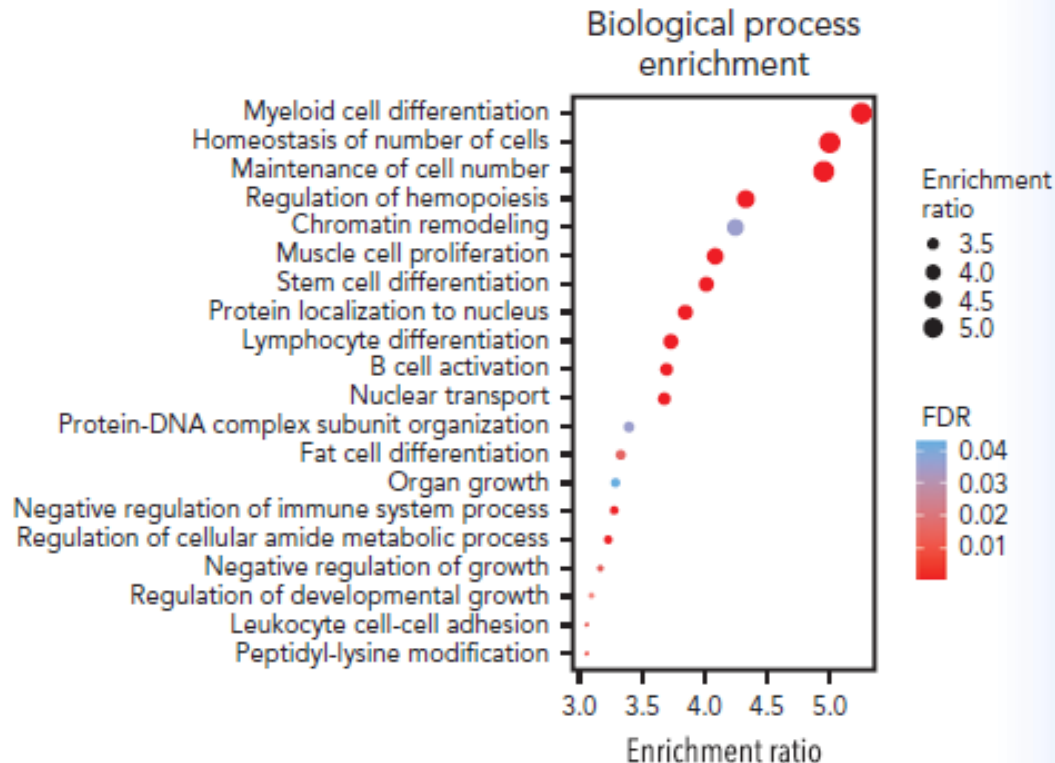




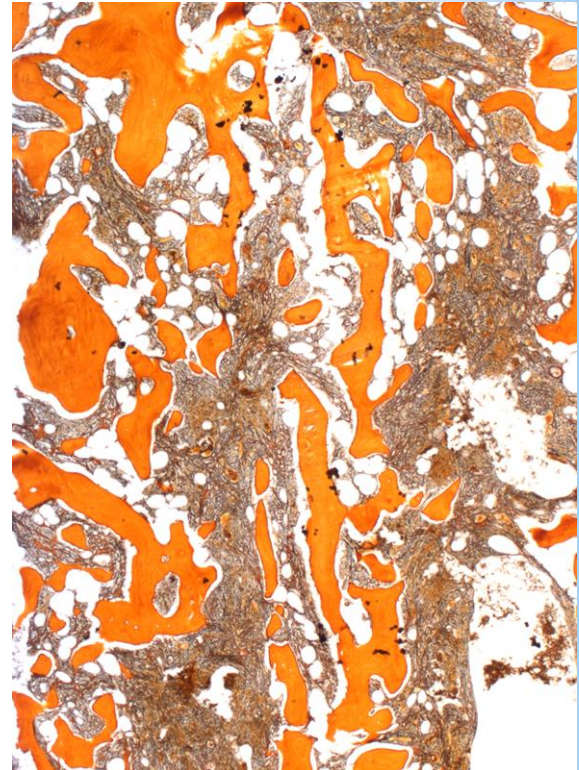
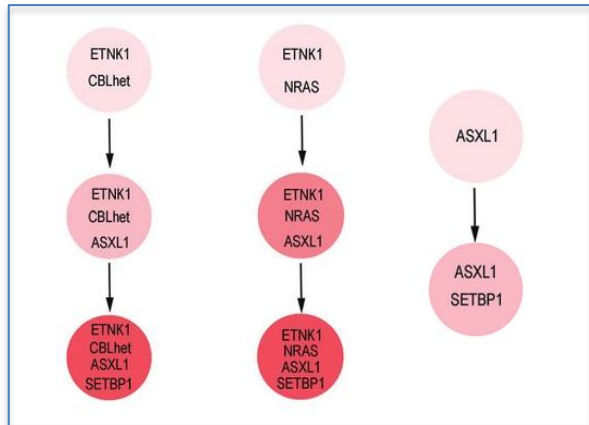
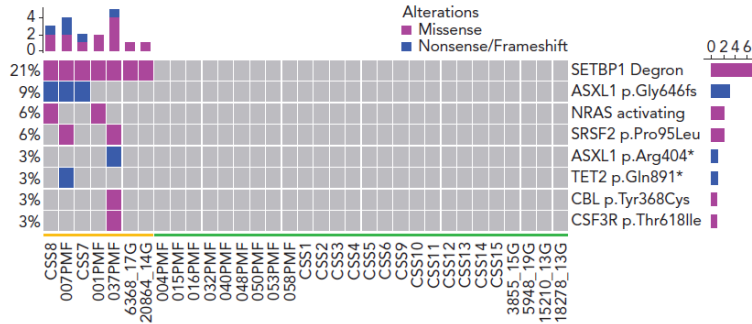
# Cut and Run on Bone Marrow Lin- cells - Results



## Cut and Run on Bone Marrow Lin- cells - Results



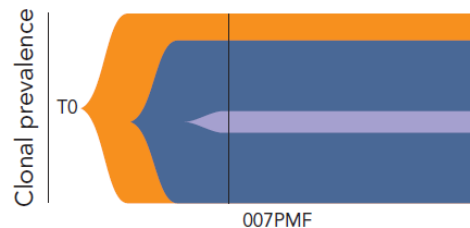
# About Clonal Hierarchy



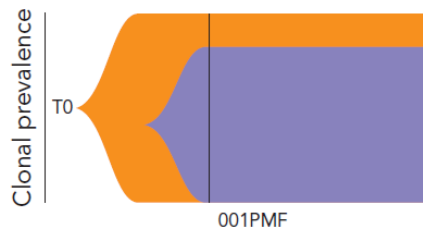
Crespiatico i. et al. *Blood*. 2024 Apr 4;143(14):1399-1413

Gambacorti C. et al. *Blood*. 2015 Jan 15;125(3):499-503

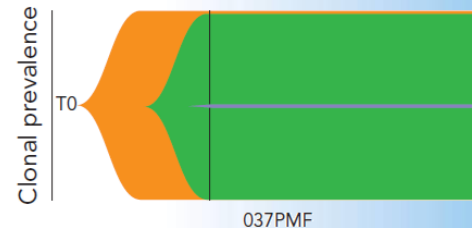
# SETBP1 degron variants as an early event?



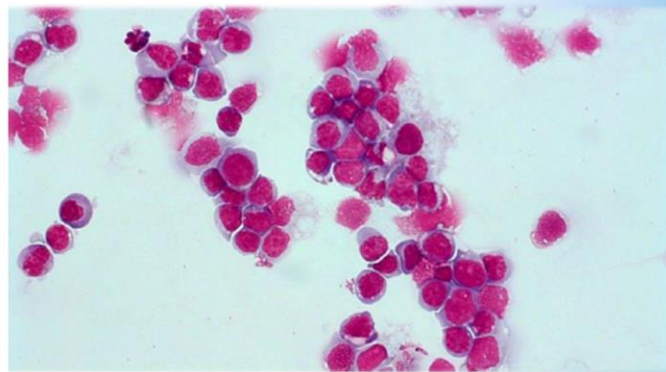
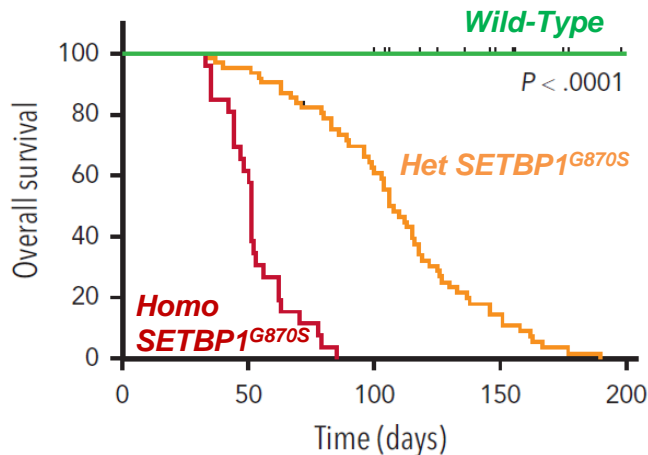
- SETBP1 p.Asp868Asn Het TET2 Wild Type
- SETBP1 p.Asp868Asn Homo TET2 p.Gln891\*
- Clonal Phylogeny C1 C2 C3

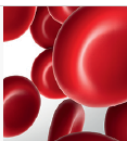


- SETBP1 p.Gly870Ser NRAS Wild Type
- SETBP1 p.Gly870Ser NRAS p.Gly12Ser
- Clonal Phylogeny C1 C2



- SETBP1 p.Asp868Asn CBL Wild Type
- SETBP1 p.Asp868Asn CBL p.Tyr368Cys
- Clonal Phylogeny C1 C2 C3





### MYELOID NEOPLASIA

# First-hit *SETBP1* mutations cause a myeloproliferative disorder with bone marrow fibrosis

Ilaria Crespiatico,<sup>1,\*</sup> Mattia Zaghi,<sup>2,\*</sup> Cristina Mastini,<sup>1</sup> Deborah D'Aliberti,<sup>1</sup> Mario Mauri,<sup>1</sup> Carl Mirko Mercado,<sup>1</sup> Diletta Fontana,<sup>1</sup> Silvia Spinelli,<sup>1</sup> Valentina Crippa,<sup>1</sup> Elena Inzoli,<sup>3</sup> Beatrice Manghisi,<sup>3</sup> Ivan Civettini,<sup>3</sup> Daniele Ramazzotti,<sup>1</sup> Valentina Sangiorgio,<sup>3</sup> Michele Gengotti,<sup>1</sup> Virginia Brambilla,<sup>4</sup> Andrea Aroldi,<sup>1,3</sup> Federica Banfi,<sup>2</sup> Cristiana Barone,<sup>1</sup> Roberto Orsenigo,<sup>5</sup> Ludovica Riera,<sup>6</sup> Mara Riminucci,<sup>7</sup> Alessandro Corsi,<sup>7</sup> Massimo Breccia,<sup>8</sup> Alessandro Morotti,<sup>9</sup> Daniela Cilloni,<sup>9</sup> Aldo Roccaro,<sup>10</sup> Antonio Sacco,<sup>10</sup> Fabio Stagno,<sup>11</sup> Marta Serafini,<sup>12</sup> Federica Mottadelli,<sup>12</sup> Giovanni Cazzaniga,<sup>1,12</sup> Fabio Pagni,<sup>4</sup> Roberto Chiarle,<sup>13-15</sup> Emanuele Azzoni,<sup>1</sup> Alessandro Sessa,<sup>2</sup> Carlo Gambacorti-Passerini,<sup>1,3</sup> Elena Maria Eli,<sup>3,†</sup> Luca Mologni,<sup>1,†</sup> and Rocco Piazza<sup>1,3,†</sup>

<sup>1</sup>Department of Medicine and Surgery, University of Milan-Bicocca, Monza, Italy; <sup>2</sup>Neuroepigenetics Unit, Division of Neuroscience, Istituto di Ricovero e Cura a Carattere Scientifico San Raffaele Scientific Institute, Milan, Italy; <sup>3</sup>Hematology Division and Bone Marrow Unit, Fondazione Istituto di Ricovero e Cura a Carattere Scientifico San Gerardo dei Tintori, Monza, Italy; <sup>4</sup>Department of Pathology, University of Milan-Bicocca, Monza, Italy; <sup>5</sup>Biomedical Research in Melanoma-Animal Models and Cancer Laboratory, Vall d'Hebron Research Institute, Vall d'Hebron Hospital Barcelona UAB, Barcelona, Spain; <sup>6</sup>Department of Pathology, Azienda Ospedaliero Universitaria Città della Salute e della Scienza, Turin, Italy; <sup>7</sup>Department of Molecular Medicine and <sup>8</sup>Department of Translational and Precision Medicine, Azienda Policlinico Umberto I, Sapienza University of Rome, Rome, Italy; <sup>9</sup>Department of Clinical and Biological Sciences, University of Turin, Orbassano, Turin, Italy; <sup>10</sup>Clinical Trial Center, Translational Research and Phase I Unit, Azienda Socio Sanitaria Territoriale Spedali Civili di Brescia, Brescia, Italy; <sup>11</sup>Division of Hematology, Azienda Ospedaliero Universitaria Policlinico G. Rodolico-S. Marco, Catania, Italy; <sup>12</sup>Centro Tettamanti, Istituto di Ricovero e Cura a Carattere Scientifico San Gerardo dei Tintori, Monza, Italy; <sup>13</sup>Department of Molecular Biotechnology and Health Sciences, University of Torino, Italy; <sup>14</sup>Department of Pathology, Children's Hospital and Harvard Medical School, Boston, MA; and <sup>15</sup>European Institute of Oncology Istituto di Ricovero e Cura a Carattere Scientifico, Division of Haematopathology, Milan, Italy