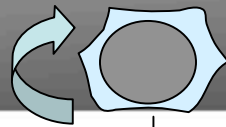
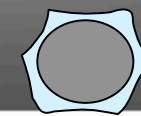


Hematopoiesis

Tpo
SCF
Flt3L
IL-3
IL-6

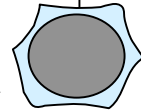


Hematopoietic stem cell



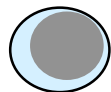
lymphoid

Bone marrow



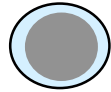
CFU-GEMM

BFU-E



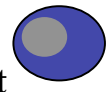
Epo

CFU-E

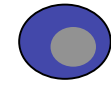


Epo

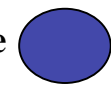
Pro erythroblast



Normoblast



Reticulocyte



peripheral blood

Erythrocyte



Platelets

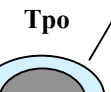


Tissue

Platelets



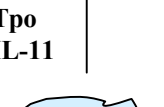
Macrophage



CFU-Mega megakaryoblast



Pro-megakaryocyte



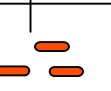
Meta-megakaryocyte



CFU-M monoblast



Pro-monocyte

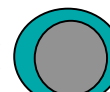


Monocyte



Macrophage

CFU-GM



M-CSF



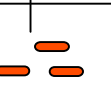
CFU-M monoblast

M-CSF



Pro-monocyte

M-CSF



Monocyte



Macrophage

CFU-G myeloblast

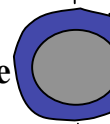


GM-CSF



Myelocyte

G-CSF



Meta-myelocyte

G-CSF



N./E./B. band

G-CSF



neutrophil



eosinophil



basophil



neutrophil

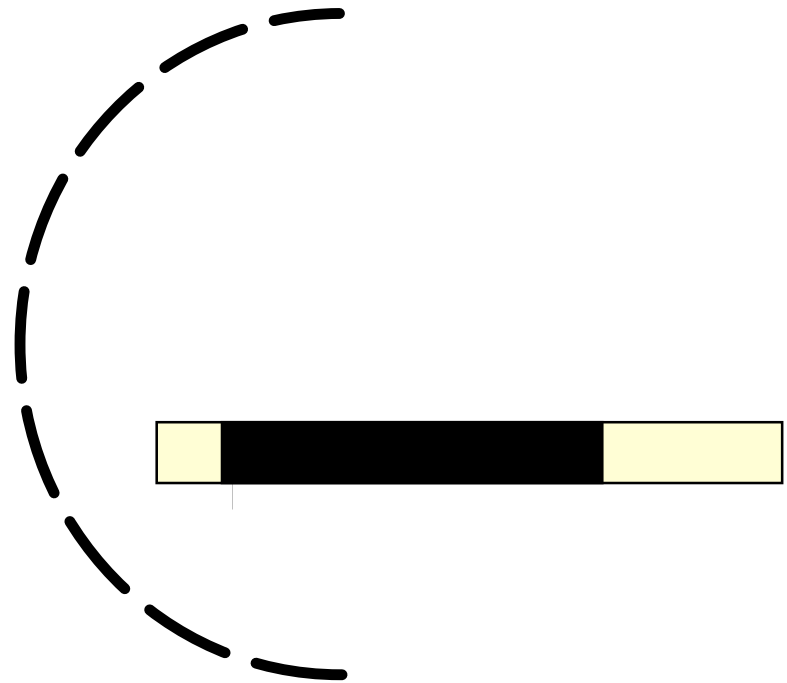
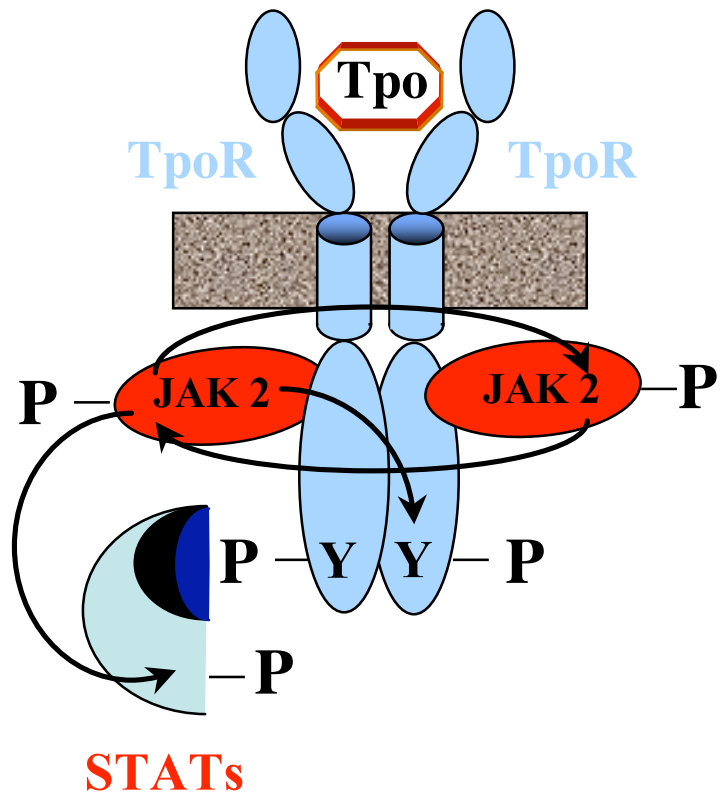


eosinophil



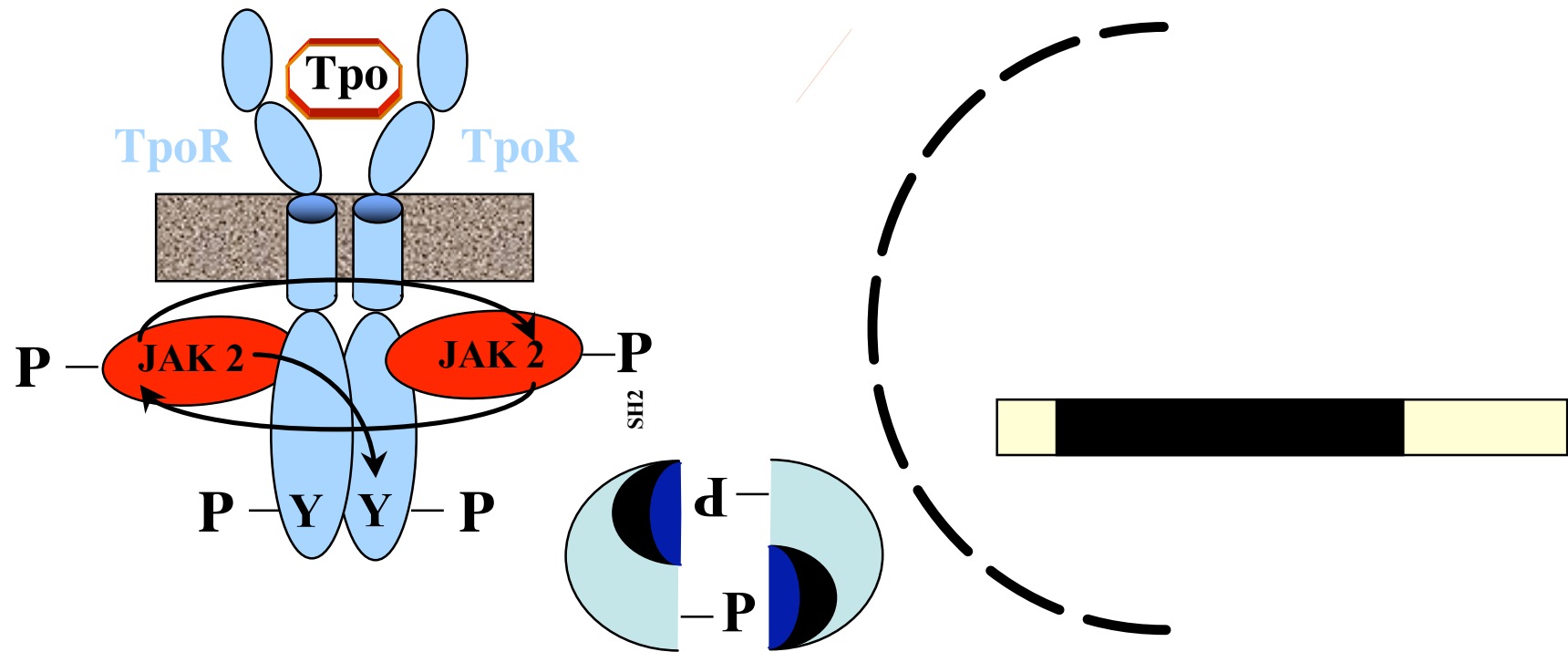
basophil

The JAK-STAT Pathway



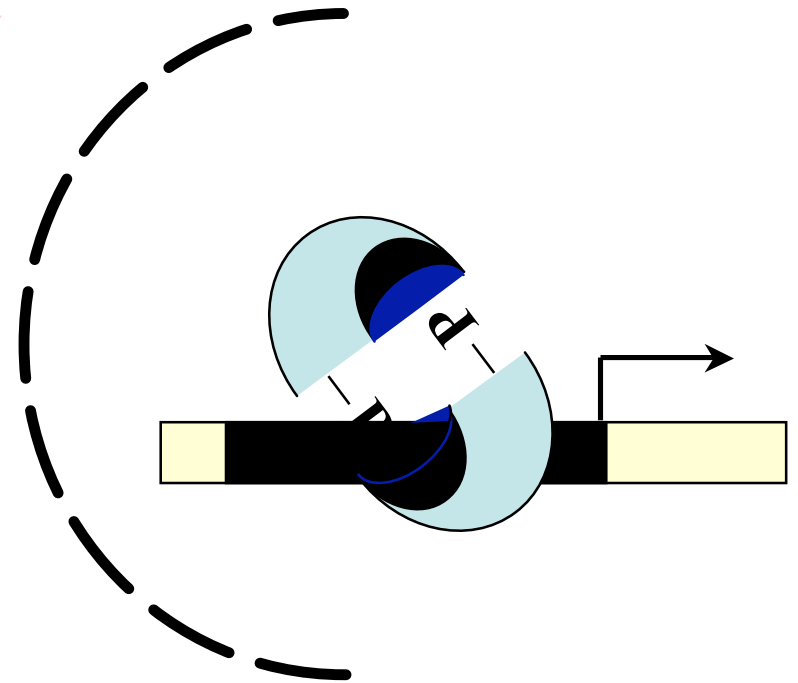
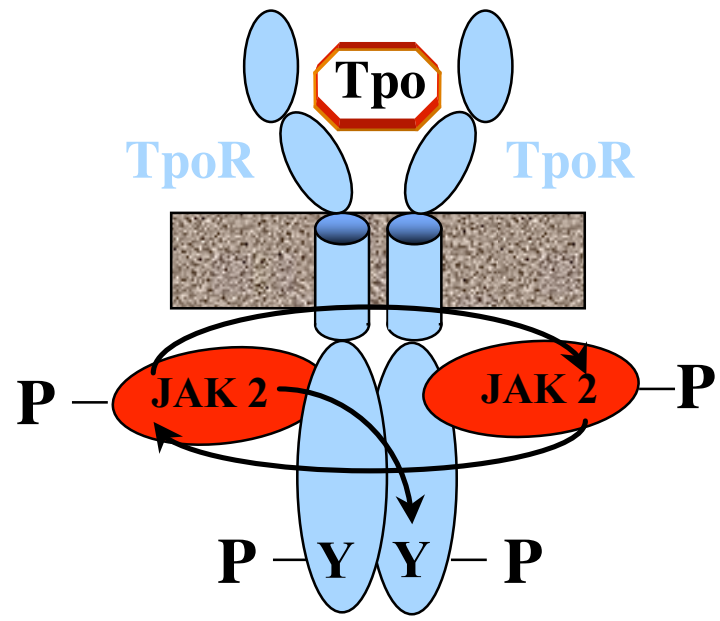
Courtesy of Y. Royer

The JAK-STAT Pathway



Courtesy of Y. Royer

The JAK-STAT Pathway



Courtesy of Y. Royer

Myeloproliferative Neoplasms

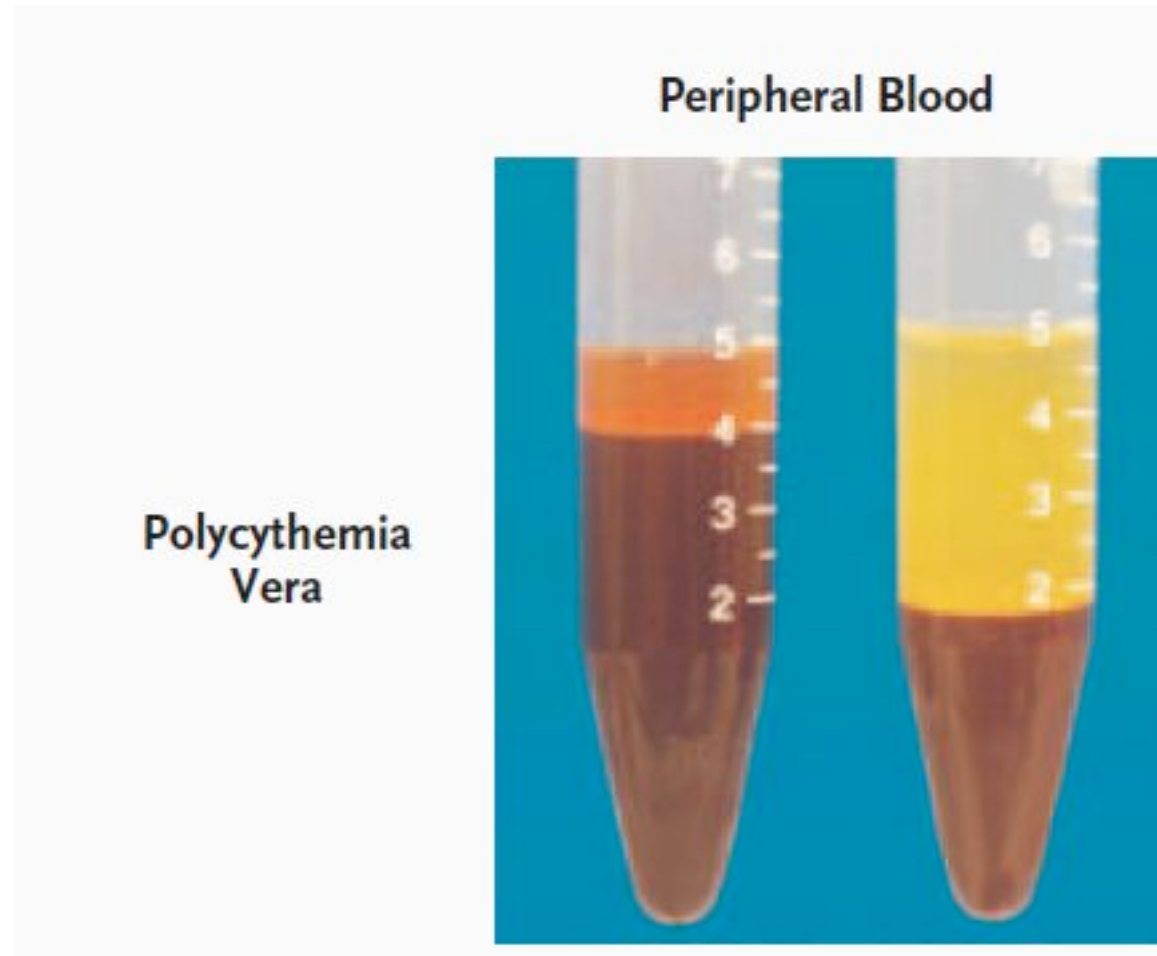
Polycythemia vera (PV)
(Vaquez 1892)

Essential thrombocythemia (ET)
(Epstein and Goedel, 1934)

Primary myelofibrosis (IMF)
(Heuck 1879)

PV, ET and PMF are 5 fold more prevalent than chronic myelogenous leukemia

Polycythemia Vera (Vaquez-Osler Disease)

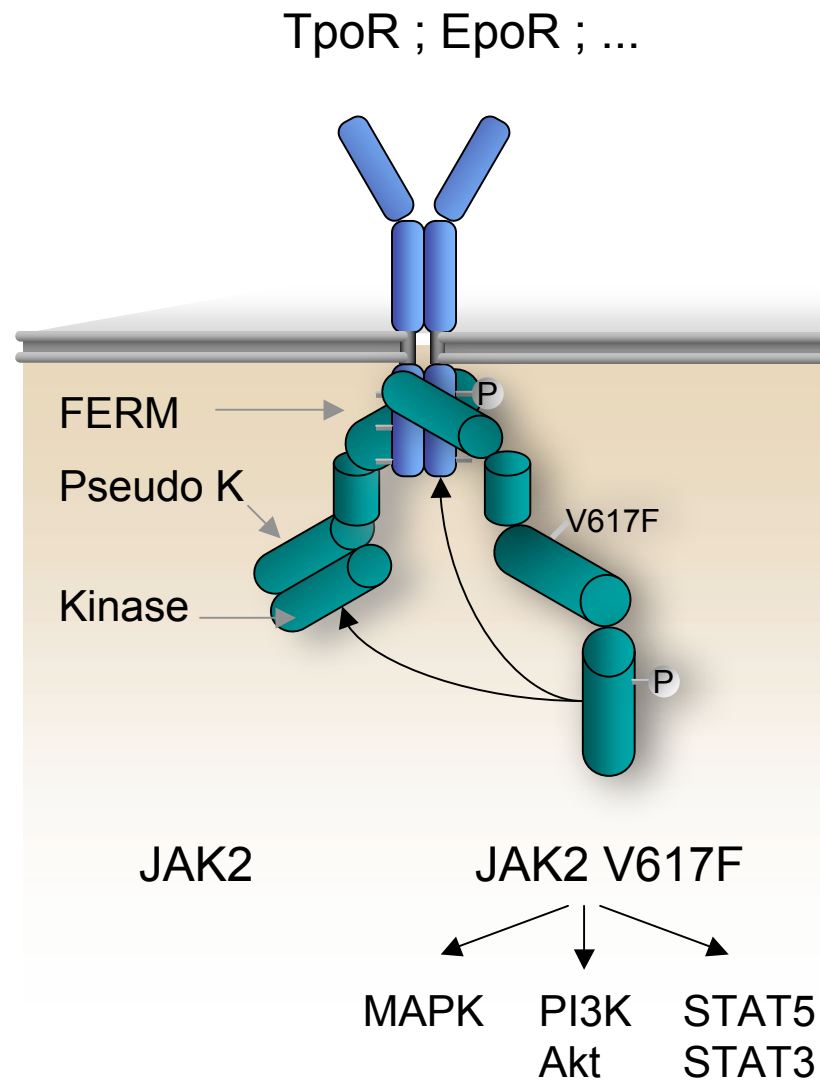
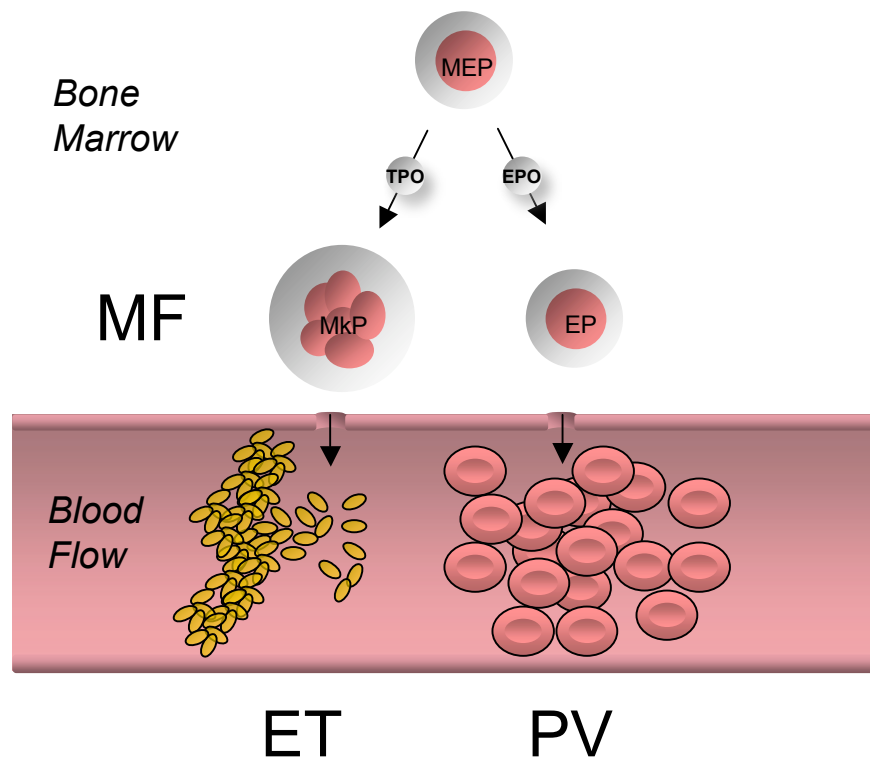


Myeloproliferative Neoplasms

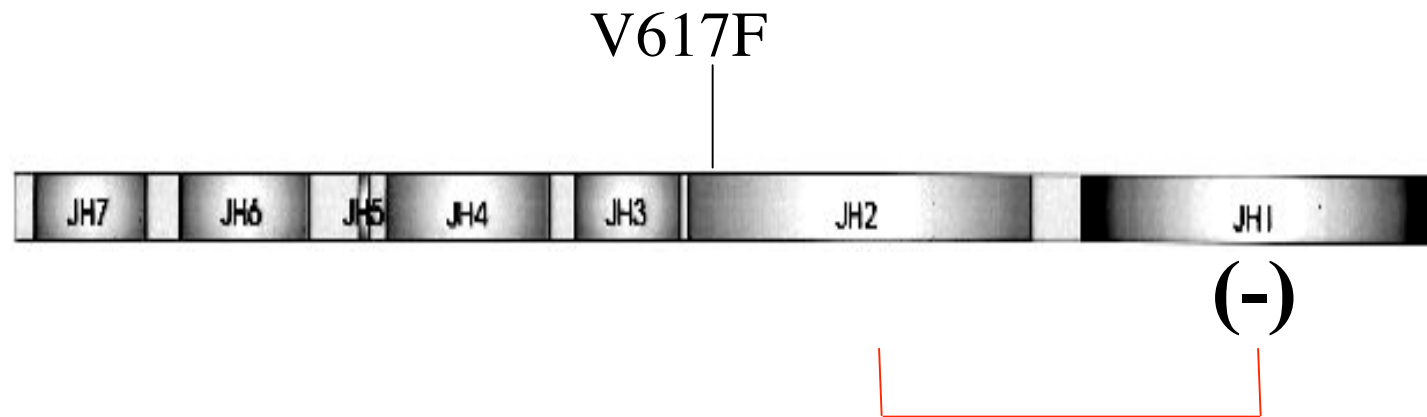
Polycythemia Vera

Essential Thrombocythemia

Myelofibrosis



Structure of Janus Kinases (Just Another Kinase)



JH1: Kinase domain

JH2: Pseudokinase domain

JH7-4: FERM domain

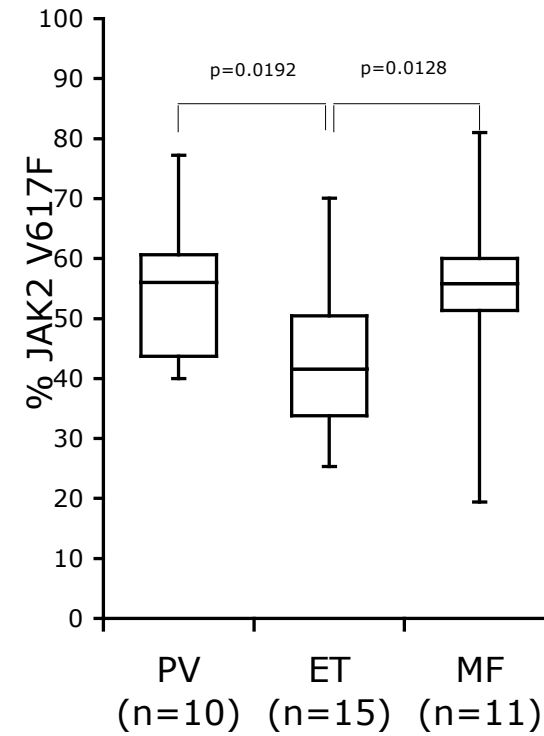
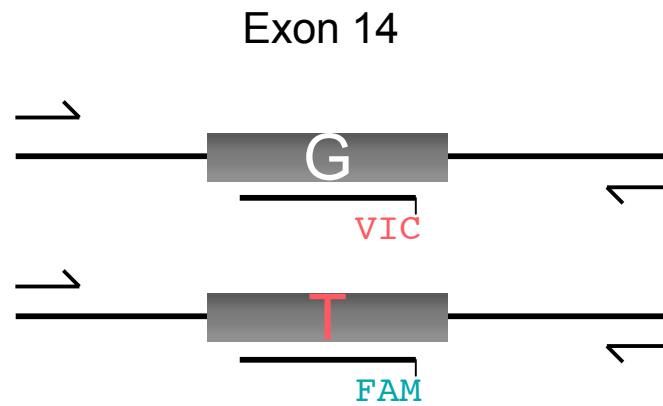
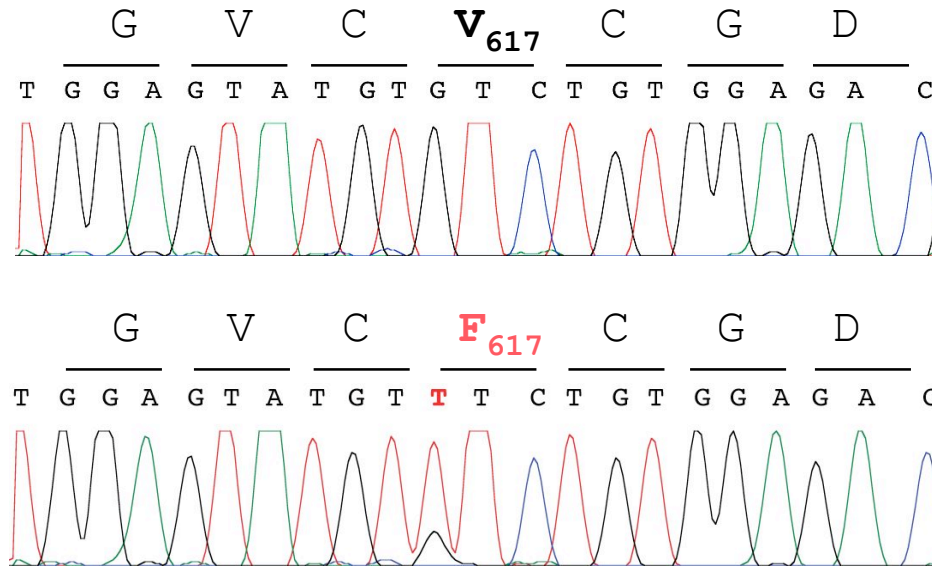
JH4-3: SH2 domain

V617F: Results in increased tyrosine kinase activity

JAK2 V617F is present in >95% of PV and >50% of ET and PMF patients



JAK2 V617F Genotyping



JAK2 V617F is the major molecular determinant of MPNs

Classical MPD

	<i>JAK2 mutant</i>
Polycythemia vera	> 95%
Essential thrombocythemia	50%
Idiopathic myelofibrosis	50%
Chronic myeloid leukemia	-

Rare MPD

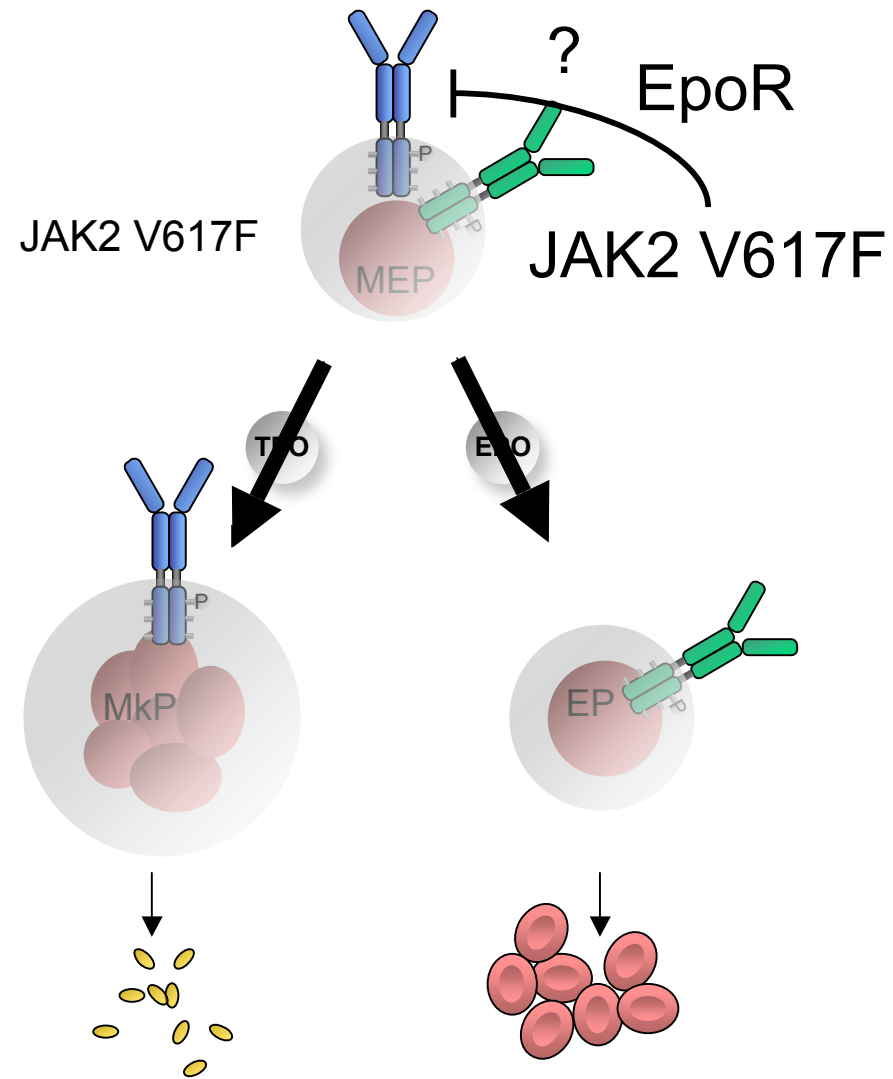
Chronic neutrophilic leukemia	rare
Chronic eosinophilic leukemia	-
Systematic mastocytosis	rare

Atypical MPD with myelodysplastic features, MPD/MDS

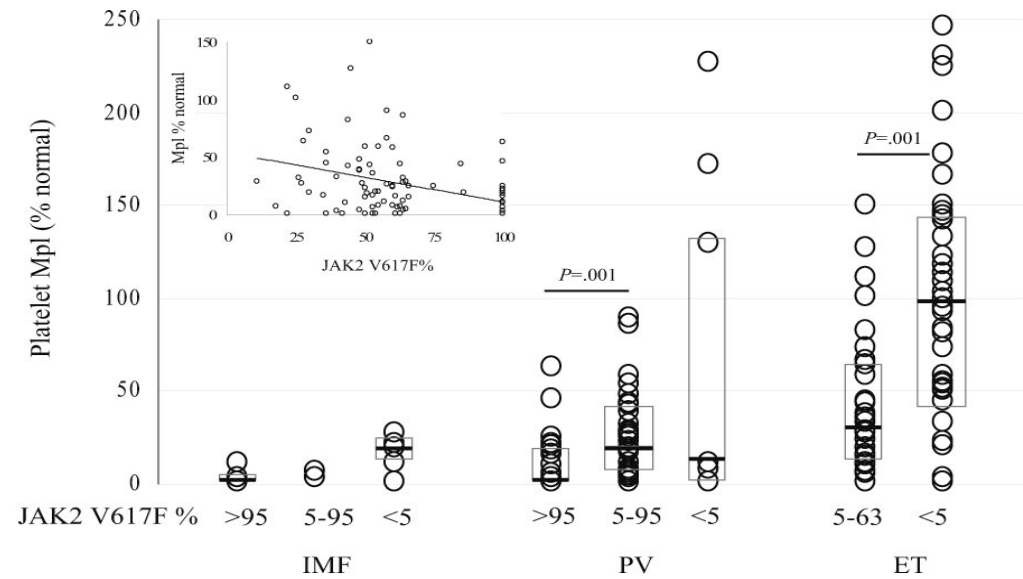
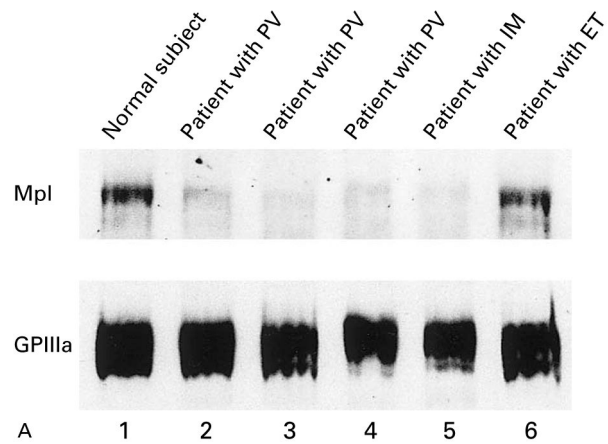
Chronic myelomonocytic leukemia	rare
Atypical chronic myeloid leukemia	rare

Hypothesis

TpoR



TpoR levels are inversely correlated with JAK2 V617F

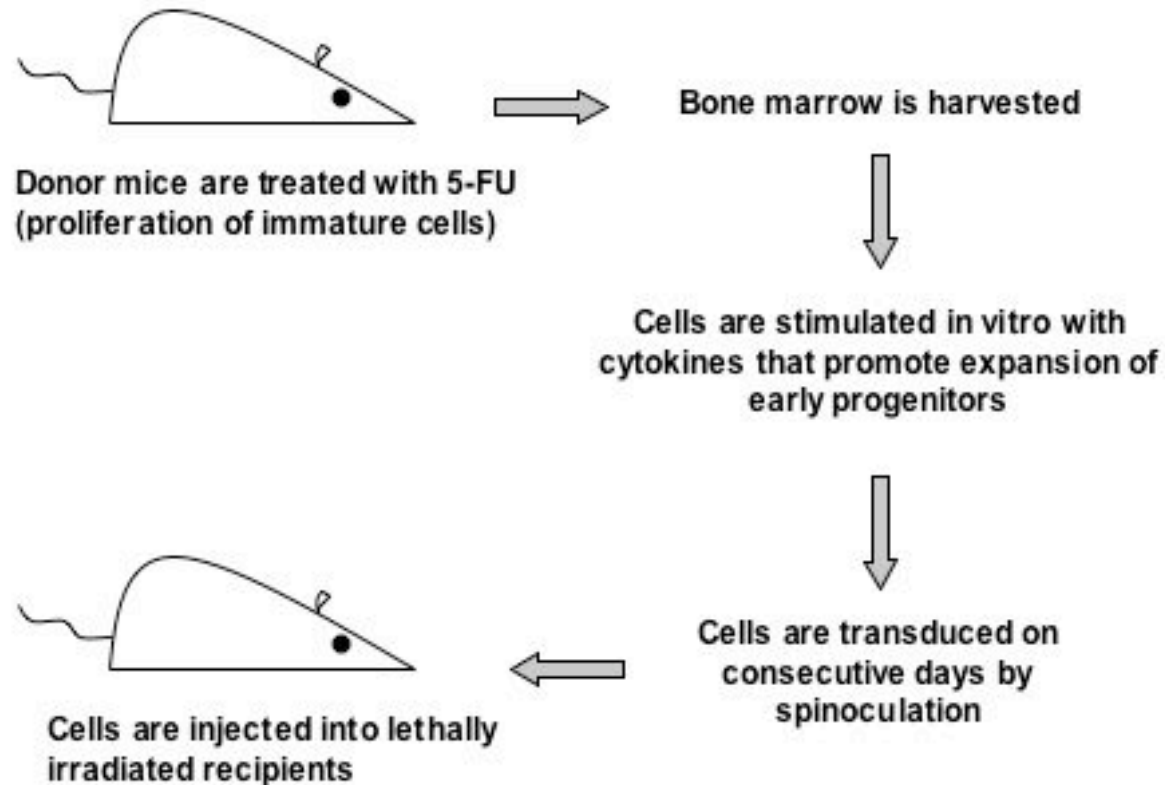


Moliterno A. , Hankins D. , Spivak J.L.,
New Eng. J. Med. 1998

Moliterno A. , Williams D.M. , Rogers O., Spivak J.L.,
Blood. 2006

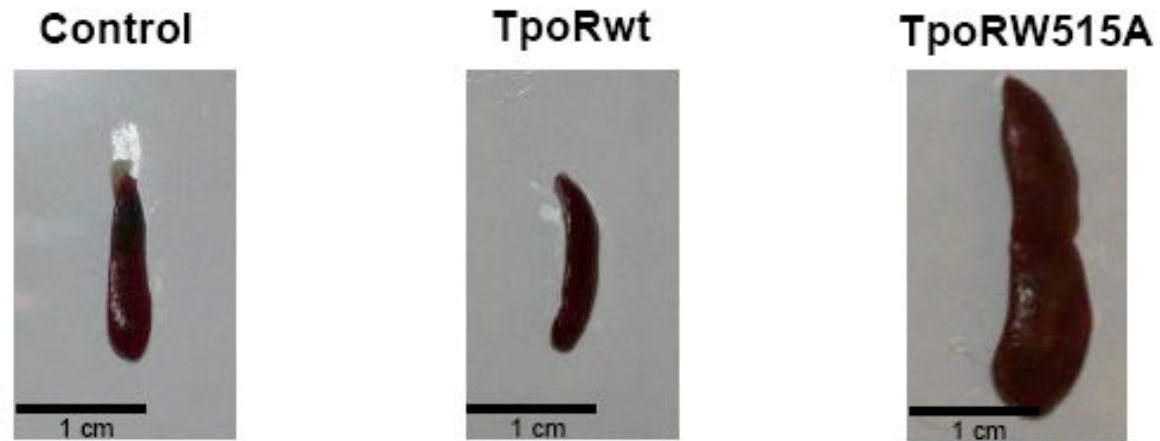
In vivo Adoptive Transfer of Transduced Bone Marrow

Bone marrow Hematopoietic reconstitution of 6-8 weeks old C57B6 mice

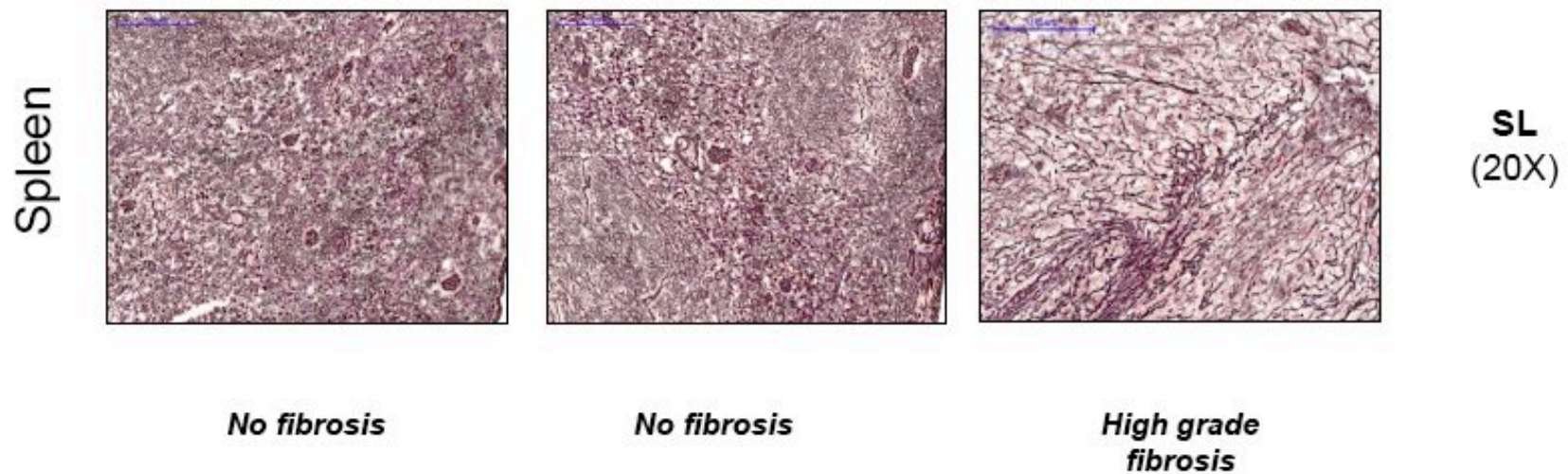


TpoR W515A Induces A Severe Myeloproliferative Disorder in Mice at Day 45 Post-Reconstitution

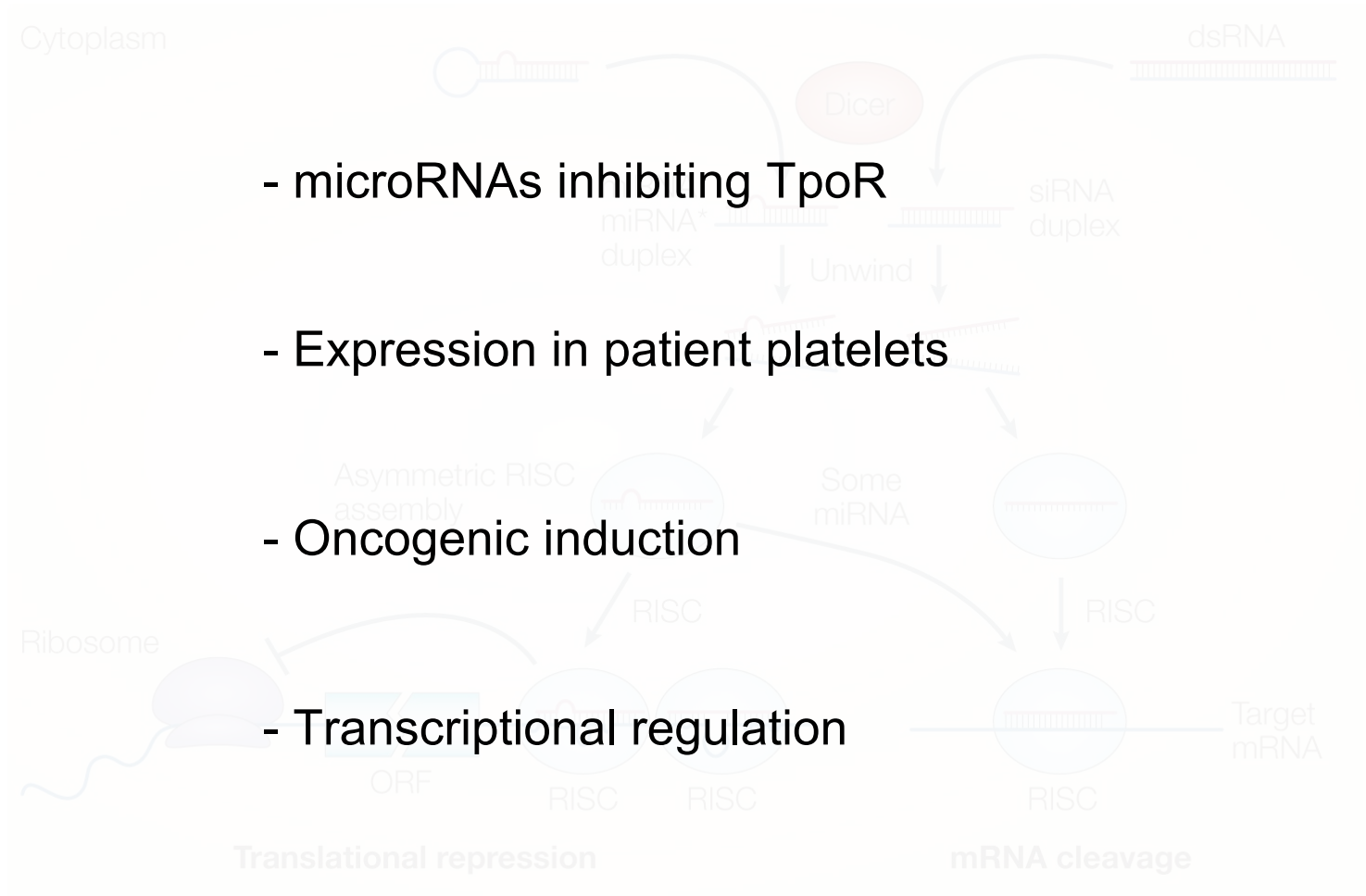
A



B



Searching for microRNAs targeting TpoR 3'UTR



He L. , Hannon G.,
Nat. Rev. Genet. 2004



miR-28: A Marker of Malignant Proliferation In Myeloproliferative Neoplasms

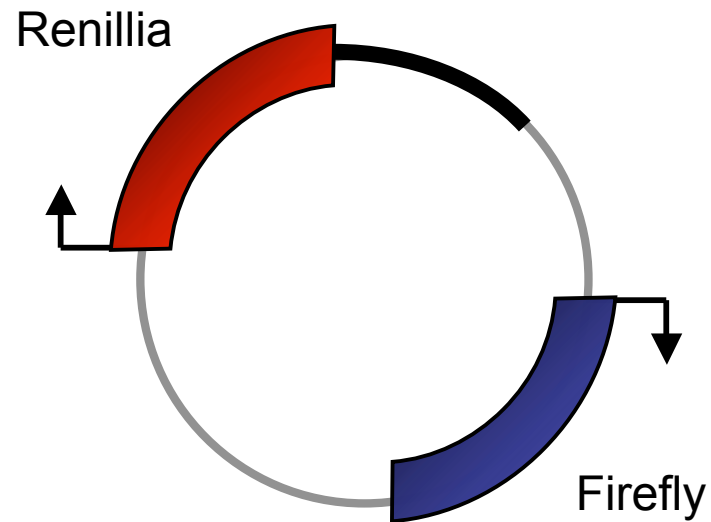
Michael Girardot

microRNAs targeting TpoR 3'UTR

```
5' AGGACAGGCUCCUCACUCCAGUUCCUGGACAGAGCUAAACUCUCGAGACUUCUCUGUGAACUCCCUACCCUAC
CCCCACAACAAGCACCCAGACCUCACCUCCAUCGCCUCUGCCUCACAAUAGGCUUCAUUGCAGUGAUUCU
                                     3' GAGUUAUCUGACACUCGAGGAA 5' hsa-miR-28 ΔG= -16.2 kCal/mol
                                     |||| | || | |||||
UACUCUACUGCUGCUGACAUAAAACCAGGACCCUUUCUCCACAGGCAGGCUCAUUUCAC--UAAGCUCUCCUUAUUUUUUC
UCUCUCCUUAUGAUGUCAAACGCCUUGAAAACAAGCCUCCACUUCUCCACACUUCUUAUUUACUCUUGAGACUACUUC
AAUUAGUCCUACUACACUUGCUAGUGAAACUGCCAGGCAAAGUGCACCUCAAUCUUAUUUCAAGAUCCAA
UAGGAUCUCGUAAAUCAUCAGUUCUUUGAUUCUCGUGAAAGAUUUGUCAAGGCUGACUACUACUUCUUAUUUUUU
UUUUCCUACCUUGGUCUCCUUAAGUUAUUAGUAGGUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUAG
                                     3' UUG-GU-AUGUUGGAUGAUGGAGU 5' hsa-let-7c ΔG= -20.5 kCal/mol
                                     |:| || |:||||| | ||||
                                     3' UGAUAUGUUGGAGGAUGGAGU 5' hsa-let-7e ΔG= -25.1 kCal/mol
                                     3' UGAUACGUUGGAUGAUGGAGA 5' hsa-let-7d ΔG= -20.3 kCal/mol
                                     | ||||| || ||||
CCCAGGCUGCAGUGCAAUGGCCGGAUCUCAGCUCACUGCAACCU-CCACCUCGGGUUCAAGCGAUUCUUGGCCUCGGC
CUCCCUAGUAGCUGGGAUUACAGGCGCACACCACCACACACAGCUAAUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUAG
                                     3' UGAUACGUUGGAUGAUGGAGA 5' hsa-let-7d ΔG= -24.6 kCal/mol
                                     3' UGAUAUGUUGGAGGAUGGAGU 5' hsa-let-7e ΔG= -22.6 kCal/mol
                                     3' UUGUUAUGUUGAUGAUGGAGU 5' hsa-miR-98 ΔG= -18.6 kCal/mol
                                     3' UUGAUUGUUGGAGGAUGGAGU 5' hsa-let-7a ΔG= -22.8 kCal/mol
                                     3' UUGAUUGUUGAUGAUGGAGU 5' hsa-let-7f ΔG= -15.9 kCal/mol
                                     | |:||| || |:||||
ACGGAGCCUUGCUCUGUUGCCAGACUGGAGUGCAGUGGCACGAUCUCGGUCACUGCAACCU-CUGCCUCCGGGUUCAA
GCCAUUCUGCCUCAGCCUCCCAAGUAGCUGGGAGUACAGGCCUCUGCCACCAUGCCUAAUUUUUUUUUUUUUUUUUUUUUUUUAGGAG
                                     3' UGA-UACGUUGGAUGAUGGAGA 5' hsa-let-7d ΔG= -19.4 kCal/mol
                                     || :|| | || |:||||
                                     3' UGAUAUG-UUGGAGGAUGGAGU 5' hsa-let-7e ΔG= -20.2 kCal/mol
                                     || | || |:||||
AGACCUGUUUACACCAGUUGGCCAGGAUGGUCUCGAUAUCCUGAUCUCGUG-AUCCGCCUGCCUCUGCCUCCCAAAGUG
CUGGGAUUACAGGUGUGACCCACUGCGCACAGCCCCAGCUAAUUUUUCAUAAUUUUUAGUAGACAGGGUUUUUGCCAUGUU
GCCAGGCUGGUCUUGAACUCCUAACCUCCGGGUGAUCCACCACCUUGGCCUCCCAAAGUGUUAAGAUUACAGGCAUGAG
CCACUGCGCCCGGUGAGUGUACUAGUAGUUAAGAGAAUAAACUAGAUUCUAGAAUCAGAGCUGGAUUAUUUCUGUCCU
UCACAUUUACUAGCUGUGCAACCUUGGGCACAAACUAAUUGUCUUUUGAGCCUUAGUUUUUUUAUCUGUAAAAC
AGGGAUAAUACAGCACCCCAUAGAGUUGUGACGAGGAUUGAGAUAAUCUAAAGUAAAGCACAGUCCCUAG
GACAUAGUAAAUGAUUCAUAUUAUCCGAACUACUGUUAUAUUUUUUUUUUUUUUCUCCUCUUCUAGCAU
UUUUCCAAUUAUUAACAGUCCUUAAGAUUCCAUUUCUAAACAGUCUCCAUAUCCAUUAUUCUUGCCU
UUACUUAUUGUUGACCAUCCAAAGUUCUUAUCUCUAGCUCAGACAUCUACUACAGCACUGUGAUGCUUU
                                     3' GAGUUAUCUGACACUCGAGGAA 5' hsa-miR-28 ΔG= -27.4 kcal/mol
                                     :| ||||| |||||
AUGCAACUAACUGUUUACAUUUCUGUCCUUGCUACUAGAUUGUGAGCUCCUUGAGGGAAGGAACAUGA
UUUUAUUUGCCUUUUCCCCAGCACCUAGAGUAGUGCUUGGUGCAUGAUAGUAGGCCUUCAUAAAUUUU
UUUUAUUUGAAUGA 3'
```

Luciferase assay

TpoR 3'UTR



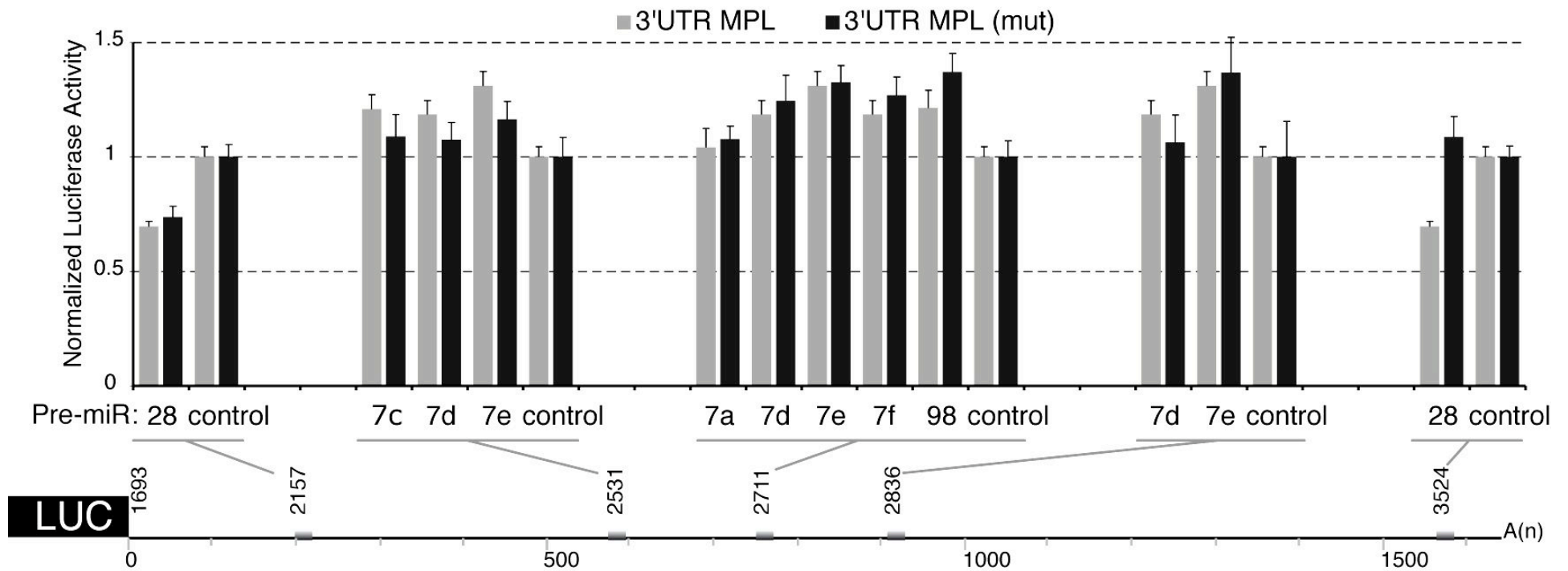
```
GAGUUAUCUGACACUCGAGGAA 5' hsa-miR-28  
:| |||||  
UGCUCUAGAUUGUGAGCUCCUUGAGG WT 3'UTR
```

$\Delta G = -27.4$ kcal/mol

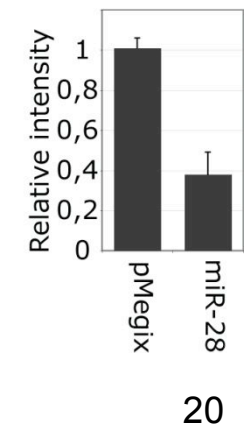
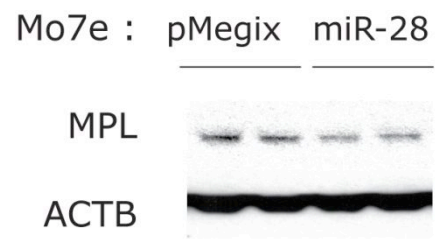
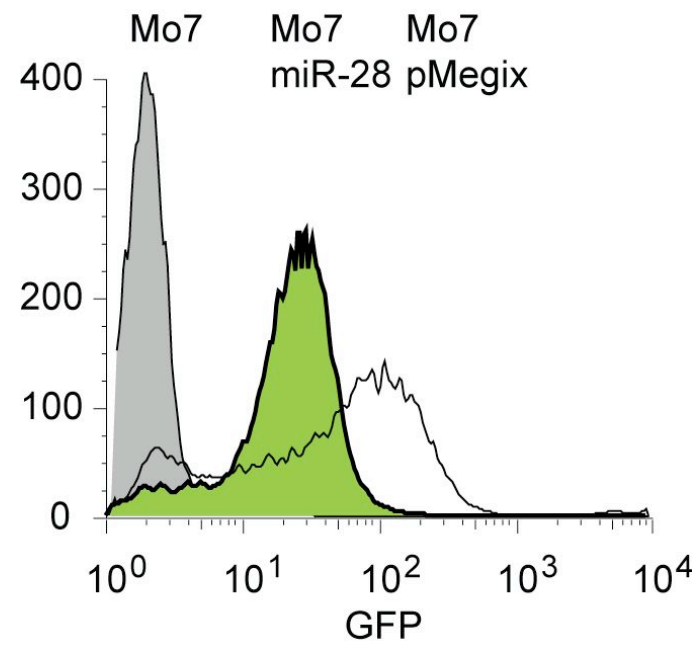
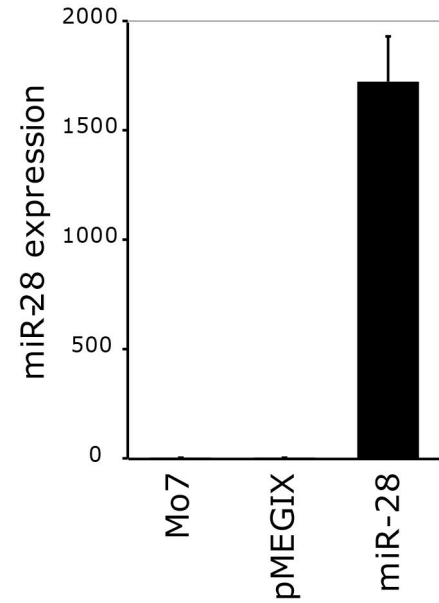
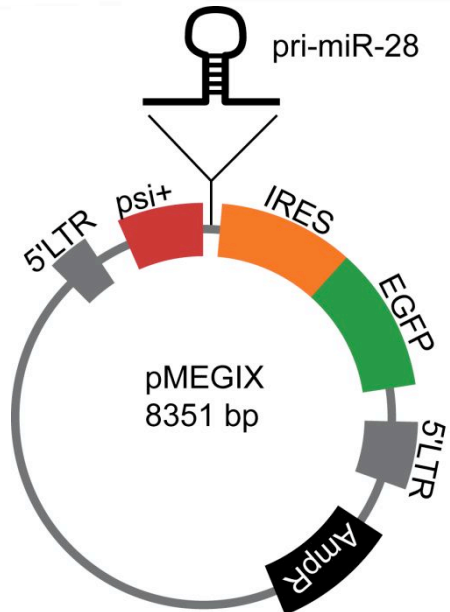
```
GAGUUAUCUGACACUCGAGGAA 5' hsa-miR-28  
:| |:| | | | |:| | |  
UGCUCACUgGAUccUuAaaUuCUUGAGG Mut 3'UTR
```

$\Delta G = -6.3$ kcal/mol

miR-28 binds TpoR 3'UTR



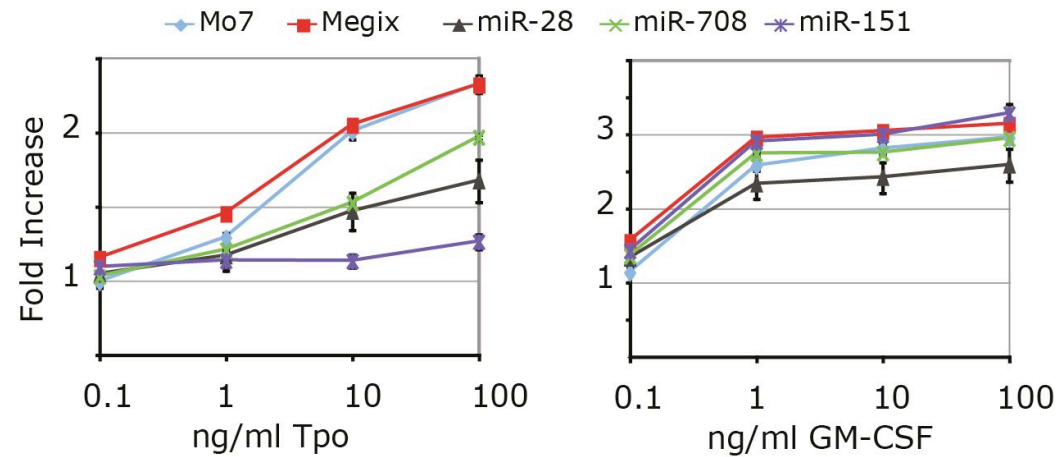
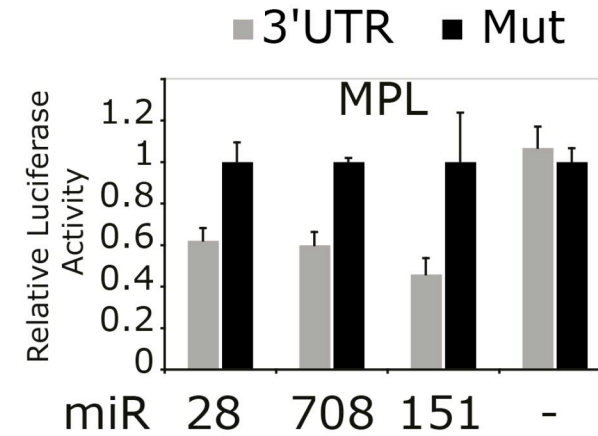
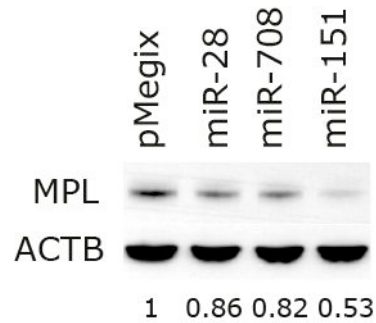
miR-28 inhibits TpoR translation



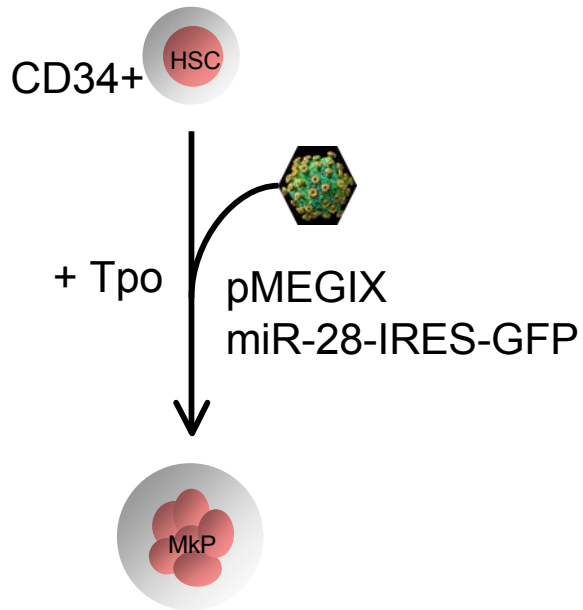
miR-28 inhibits Tpo dependent cell proliferation

```

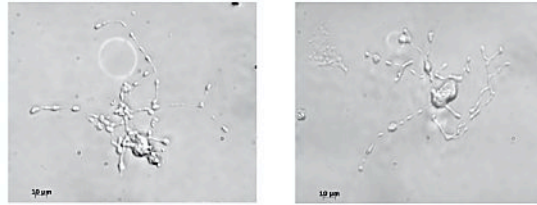
hsa-miR-28  --AAGGAGCUCACAGUCUAUUGAG-  22
hsa-miR-151 UCGAGGAGCUCACAGUCUAGU----  21  80%
hsa-miR-708 --AAGGAGCUUACAAUCUAGCUGGG  23  68%
          *****  ***  *****
    
```



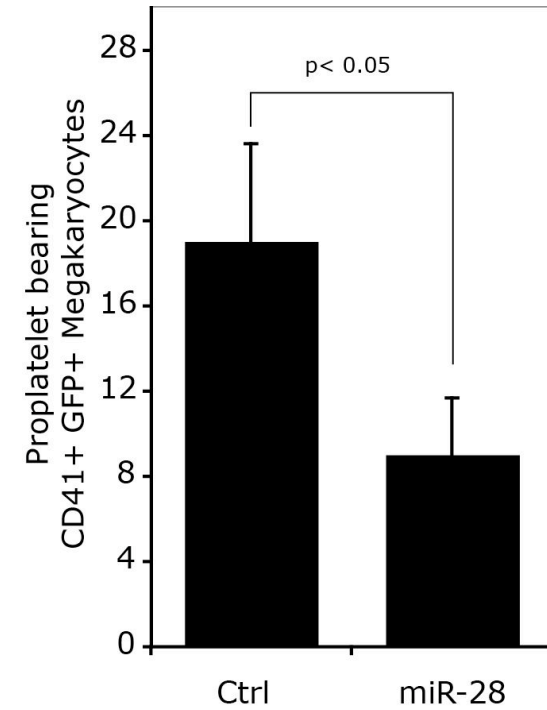
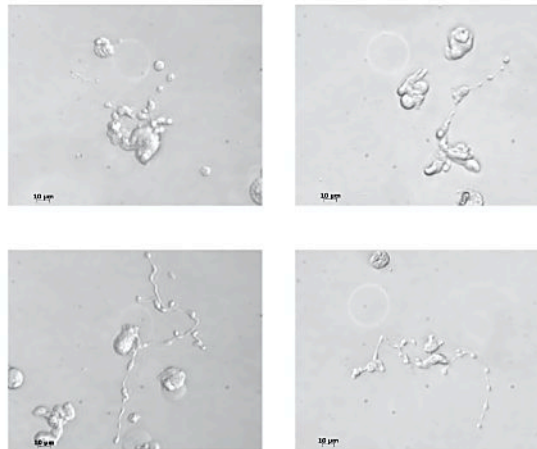
miR-28 inhibits Tpo-induced proplatelet formation



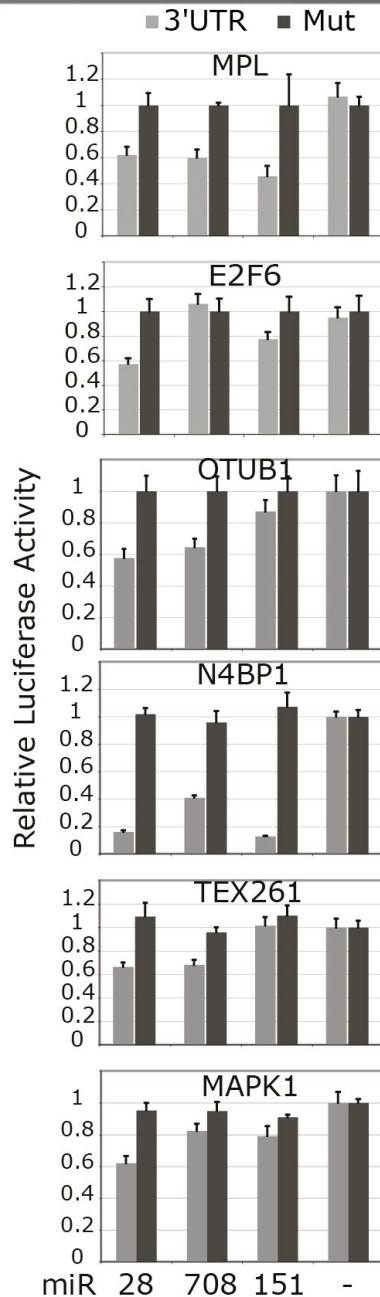
Control Megakaryocytes:



miR-28 Megakaryocytes:



miR-28 potential targets



- *MPL*: Thrombopoietin receptor (megakaryocyte differentiation)

- *E2F6*: negative regulator of E2F1 (cell cycle and apoptosis)

- *OTUB1*(Otubain1)

negative regulators

- *N4BP1*(NEDD4 binding protein 1)

of E3 Ub ligase

-*TEX261* (testis expressed 261): NMDA mediated

megakaryocyte differentiation

-*MAPK1* (mitogen-activated protein kinase 1/ERK2): Tpo dependent

megaK differentiation

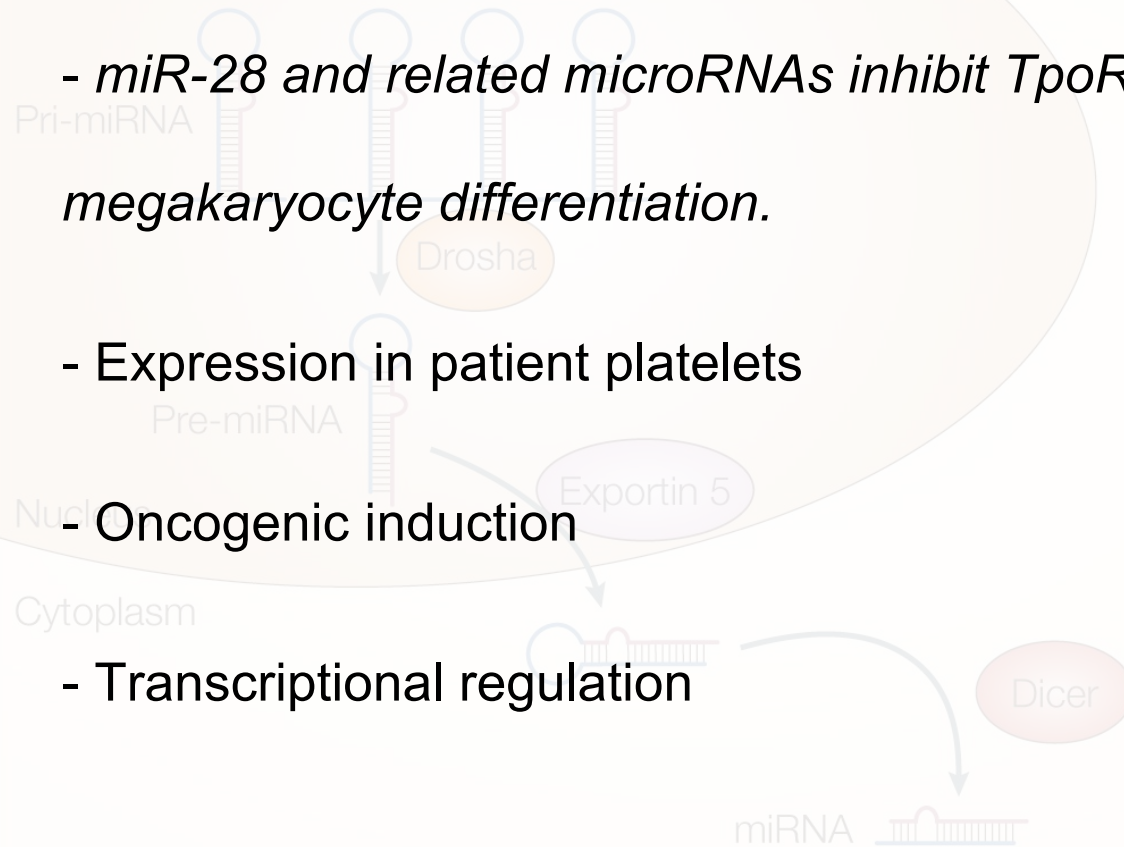
Searching for microRNAs targeting TpoR 3'UTR

- *miR-28 and related microRNAs inhibit TpoR translation and megakaryocyte differentiation.*

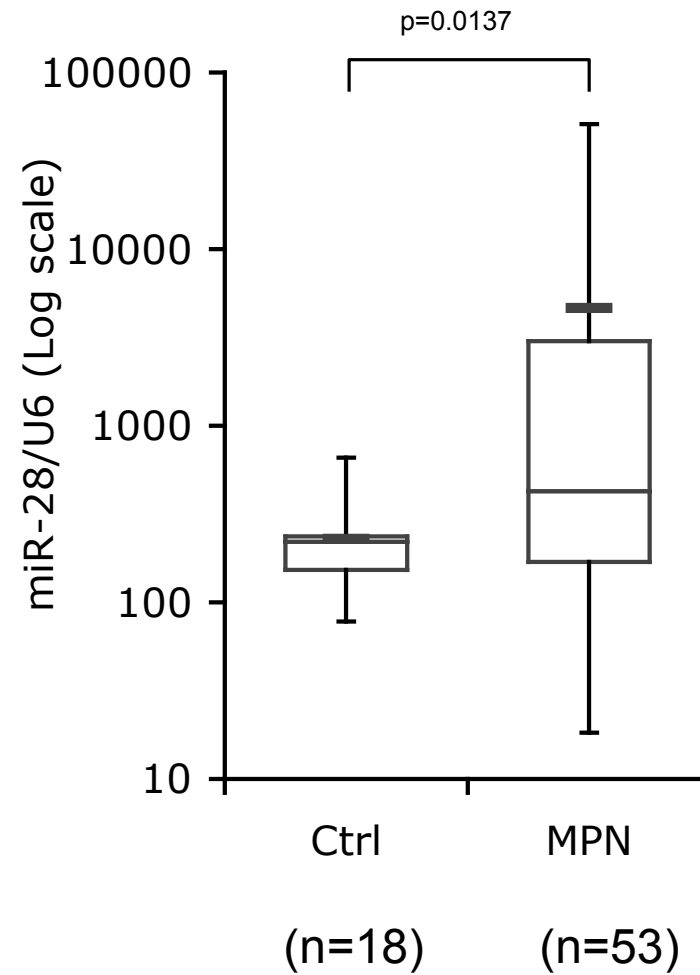
- Expression in patient platelets

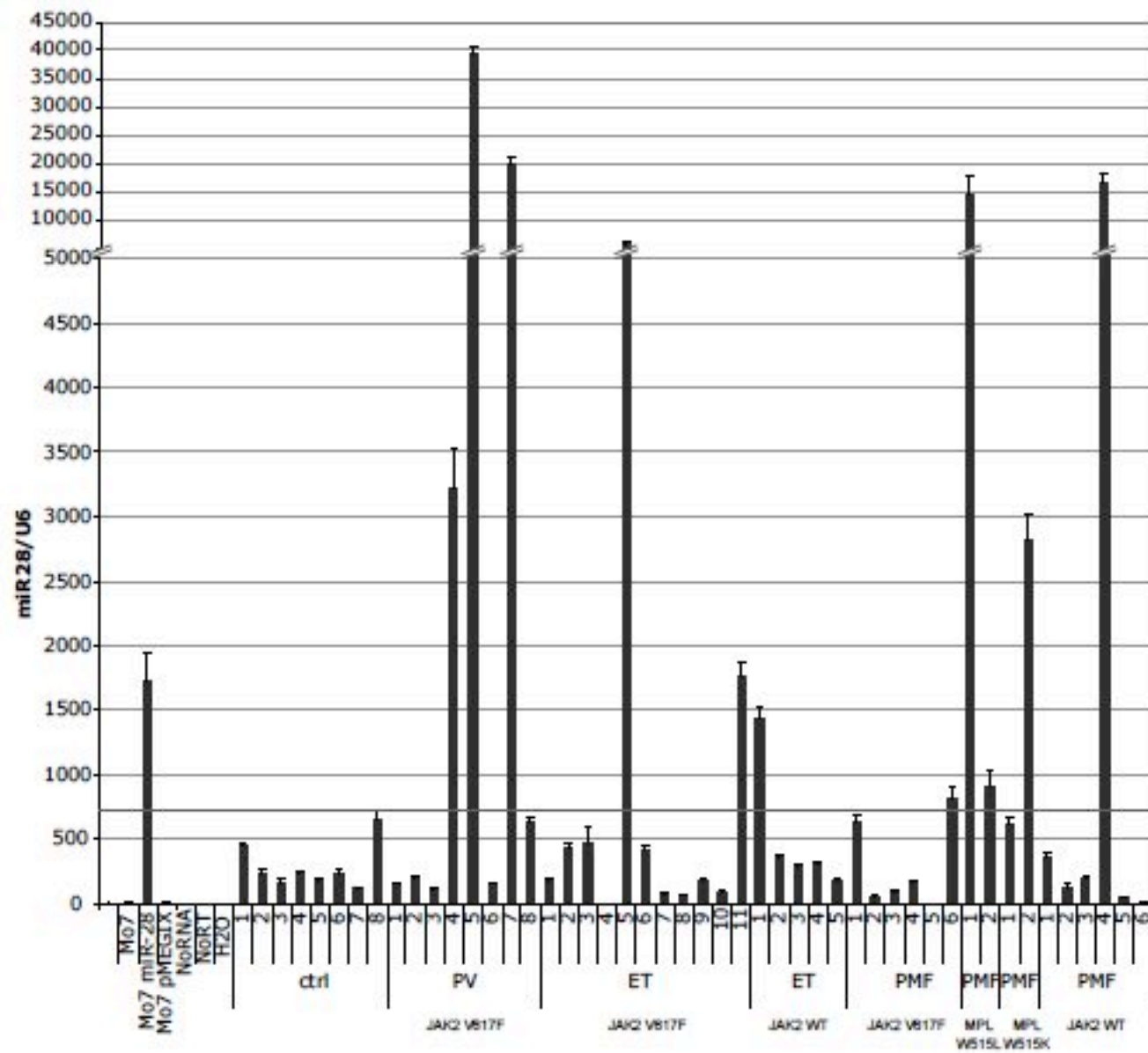
- Oncogenic induction

- Transcriptional regulation

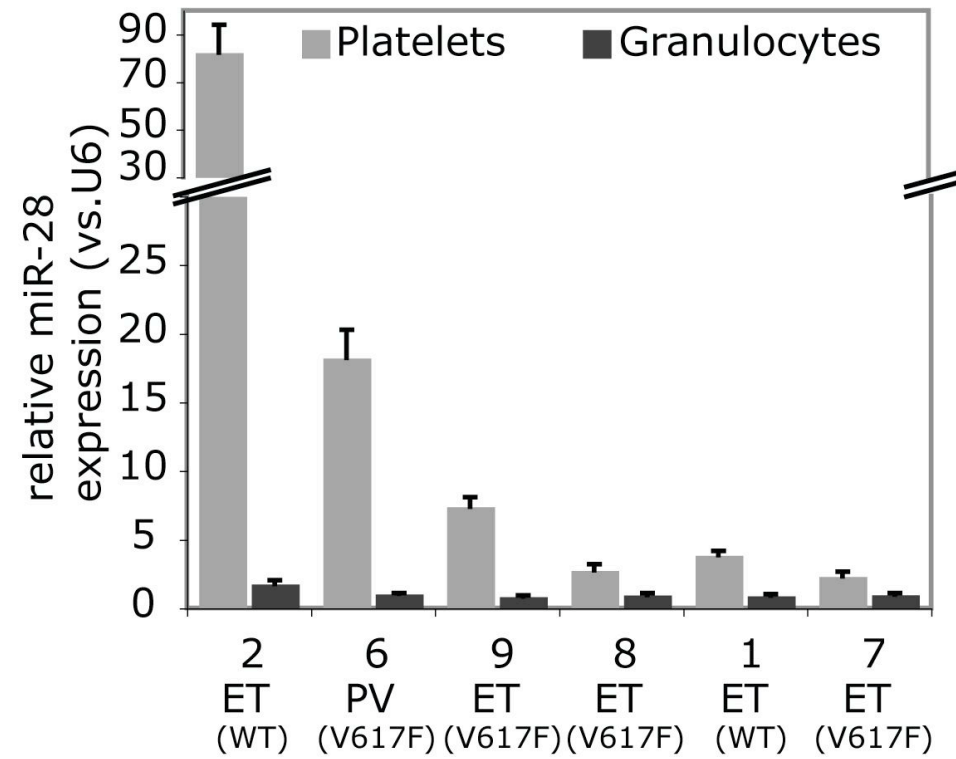
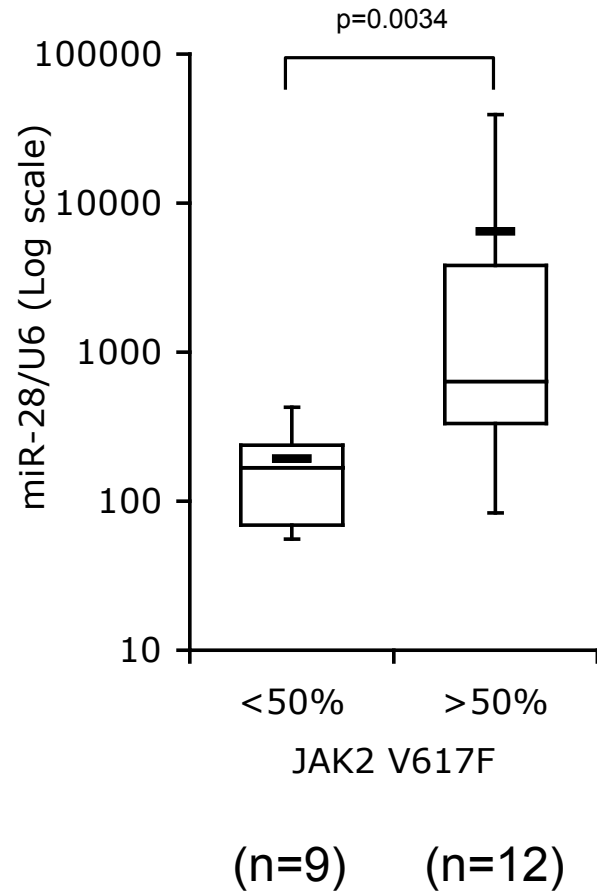


miR-28 expression in patients' platelets





miR-28 is correlated to JAK2 V617F allele burden



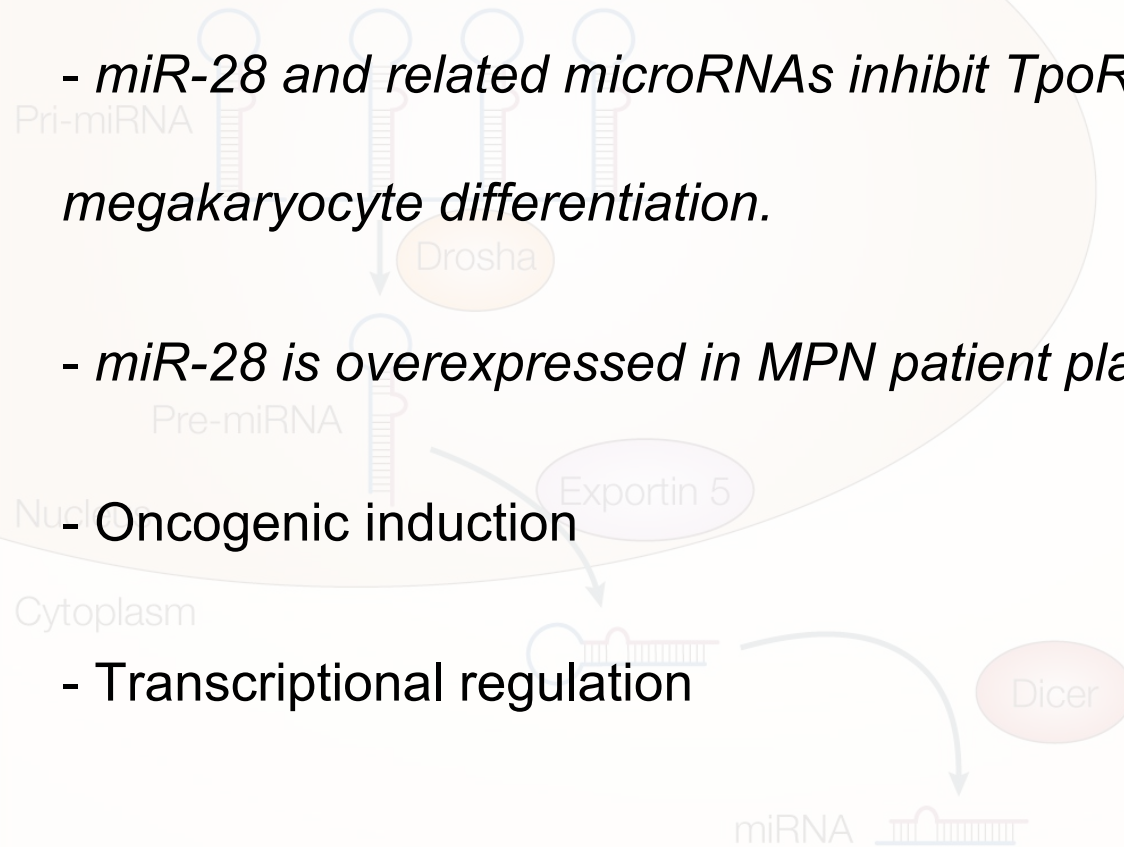
Searching for microRNAs targeting TpoR 3'UTR

- *miR-28 and related microRNAs inhibit TpoR translation and megakaryocyte differentiation.*

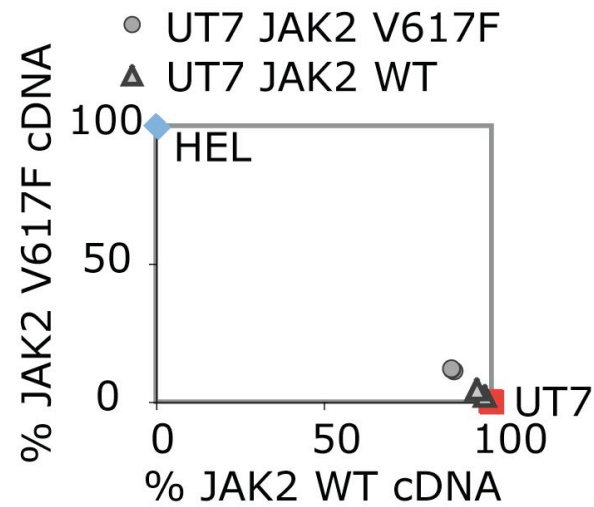
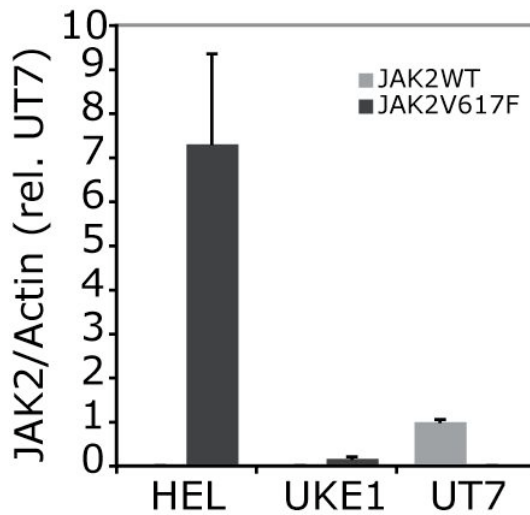
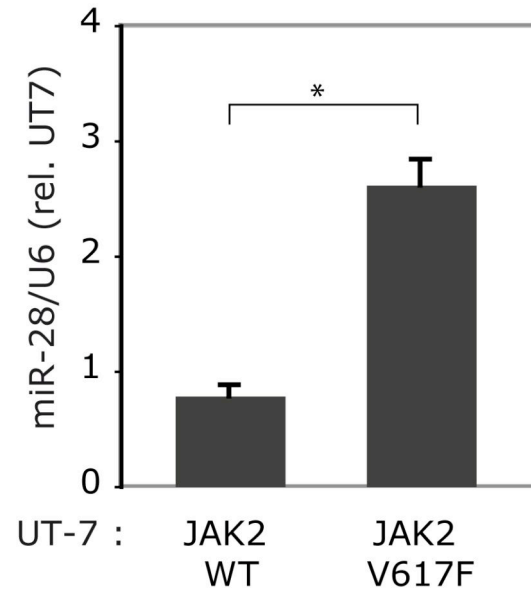
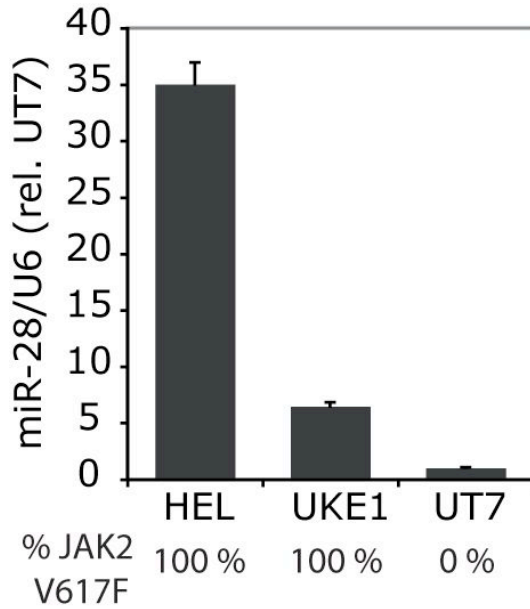
- *miR-28 is overexpressed in MPN patient platelets*

- **Oncogenic induction**

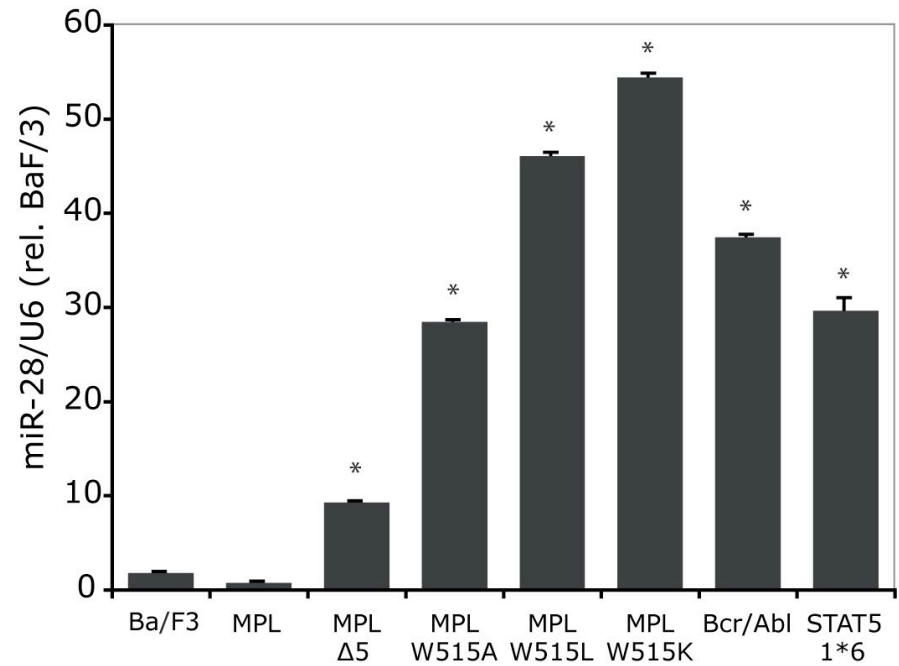
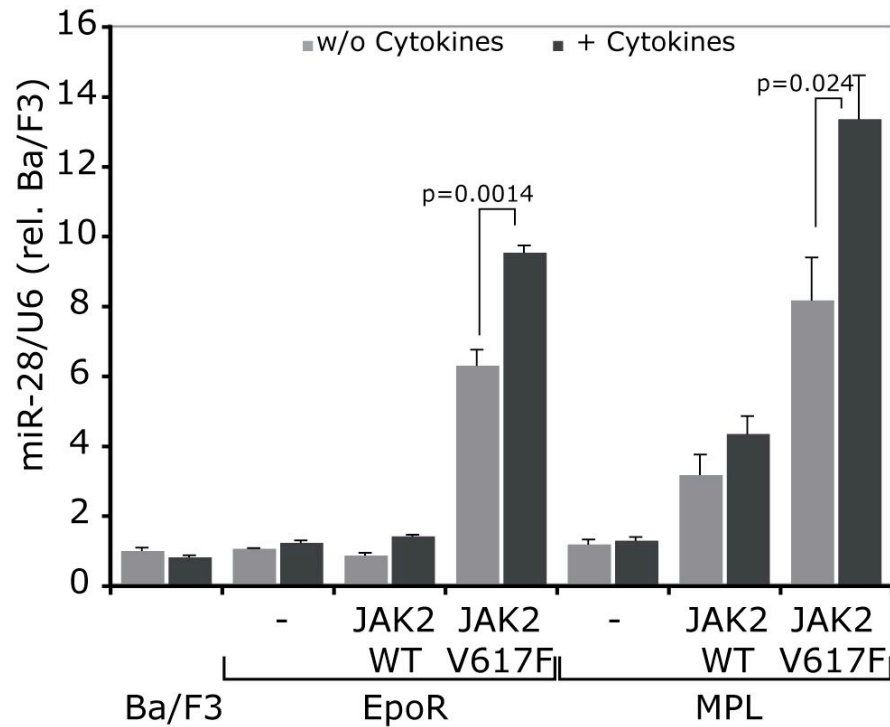
- **Transcriptional regulation**



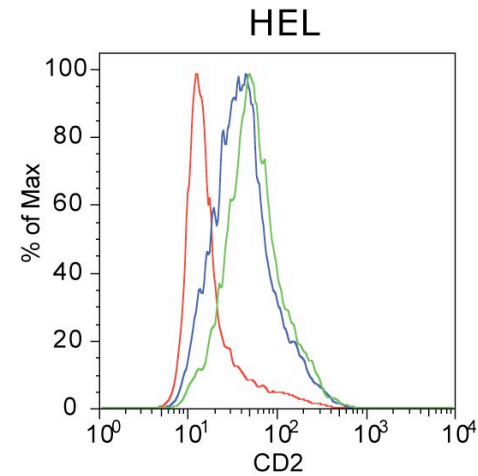
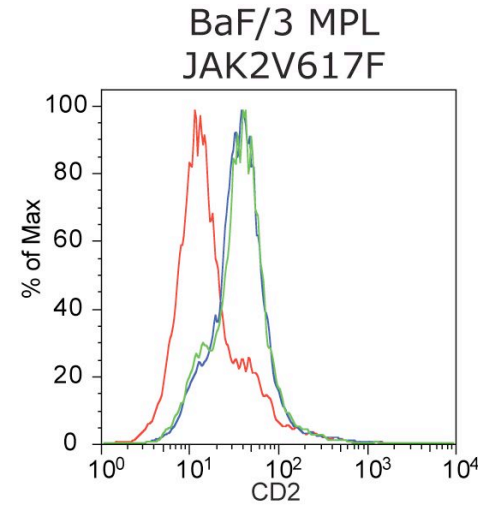
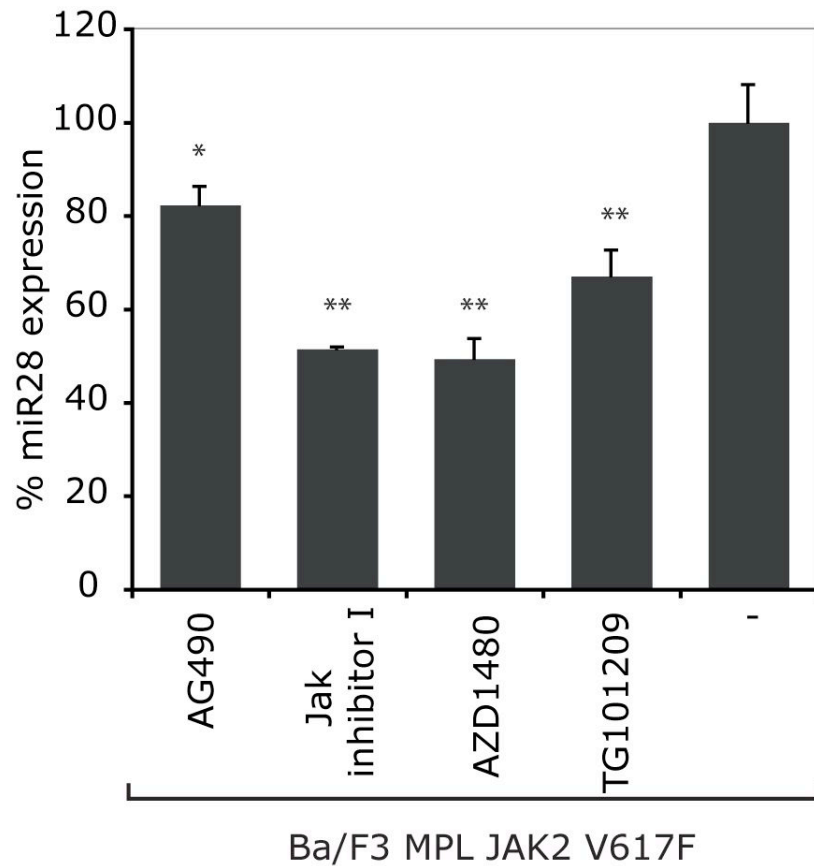
miR-28 is induced by JAK2 V617F



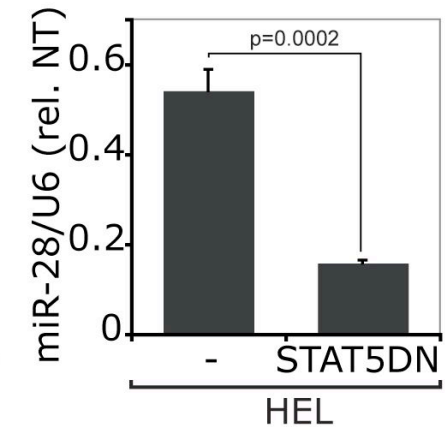
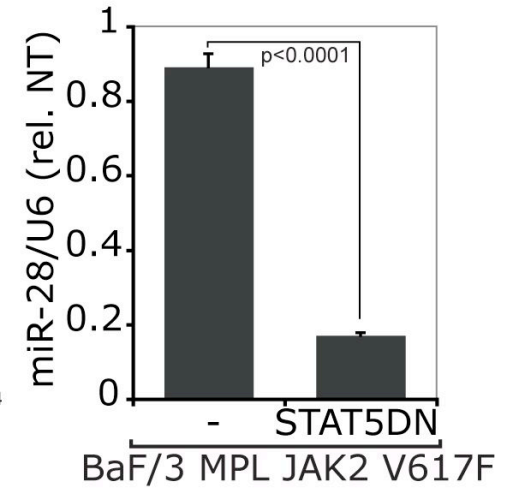
miR-28 is induced by STAT5 activating oncogenes



miR-28 expression in transformed cells is STAT5 dependent



■ pREX-STAT5DN-CD2
■ pREX-CD2
■ -



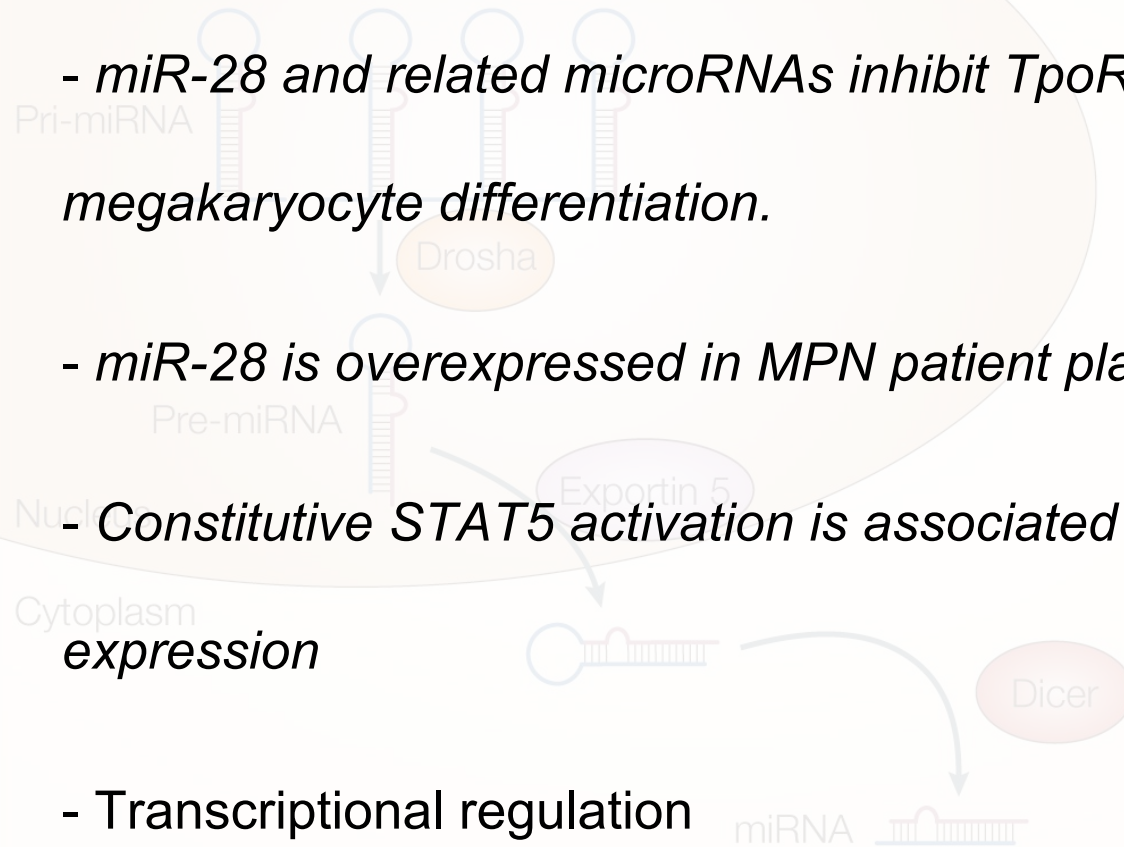
Searching for microRNAs targeting TpoR 3'UTR

- *miR-28 and related microRNAs inhibit TpoR translation and megakaryocyte differentiation.*

- *miR-28 is overexpressed in MPN patient platelets*

- *Constitutive STAT5 activation is associated with miR-28*

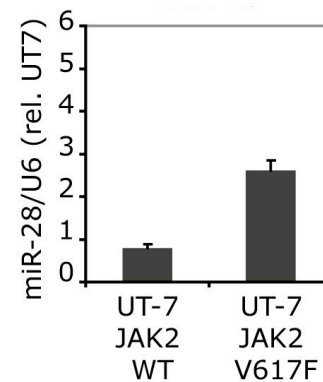
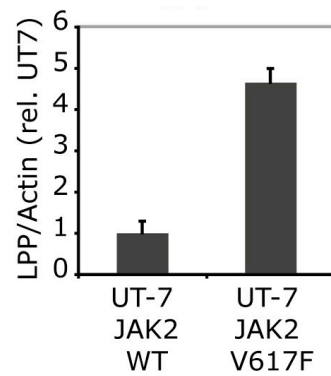
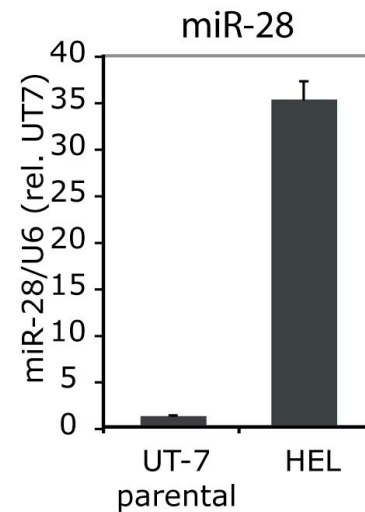
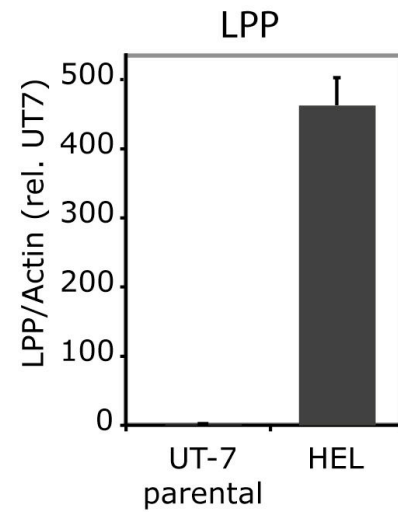
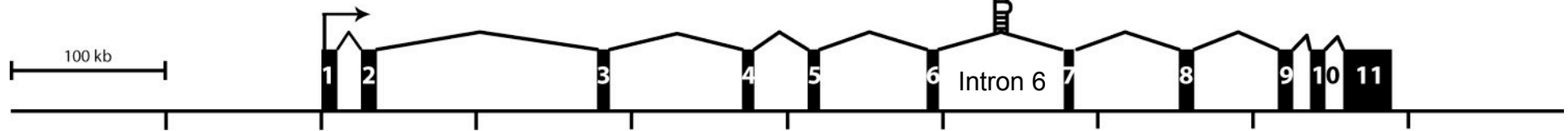
- *Transcriptional regulation*



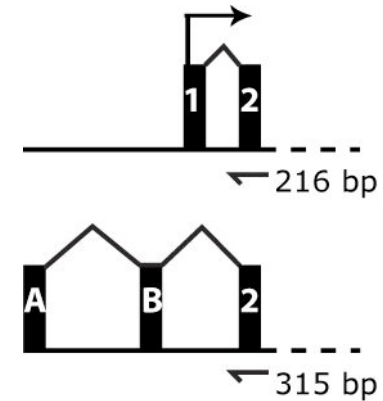
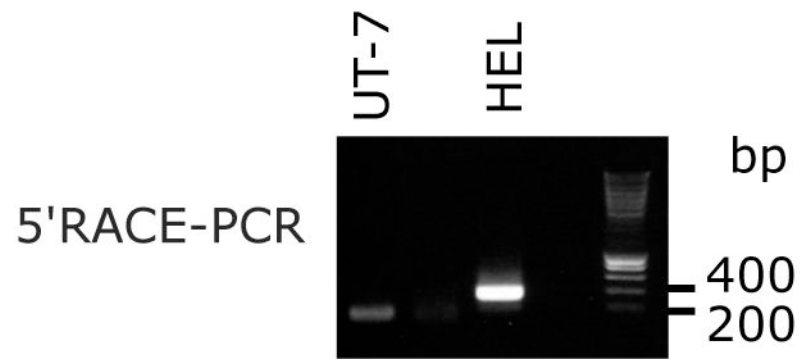
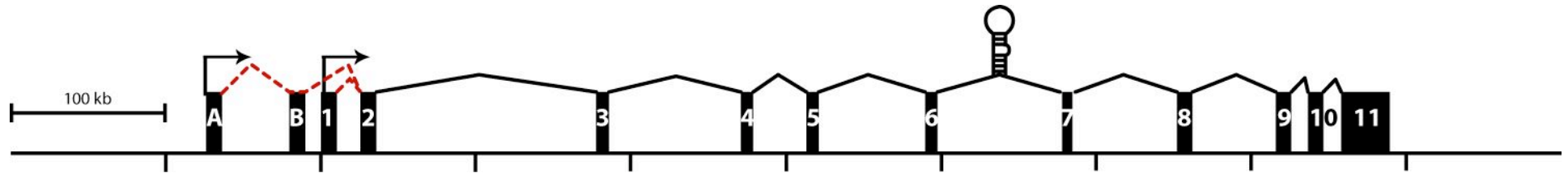
miR-28/LPP gene

LPP: LIM domain Lipoma-Preferred partner

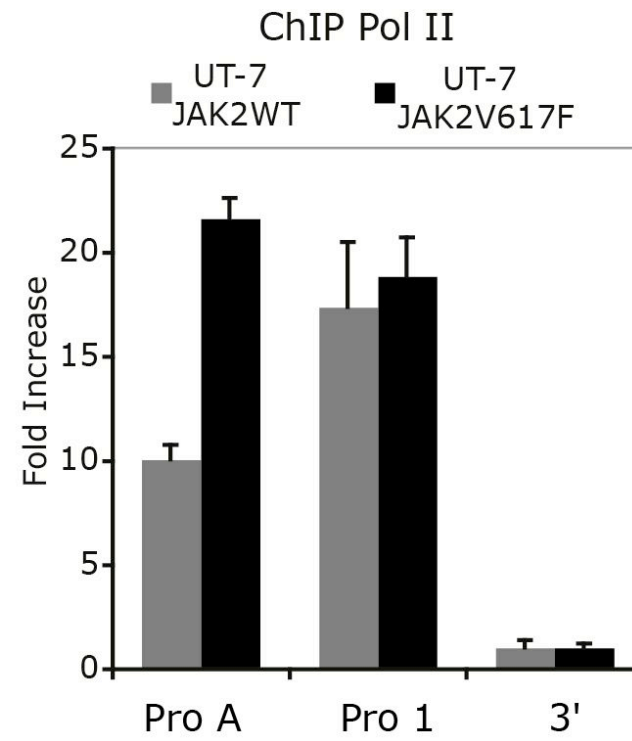
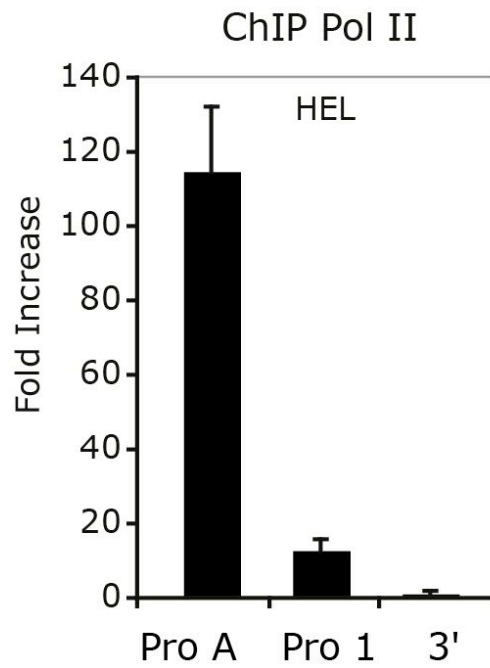
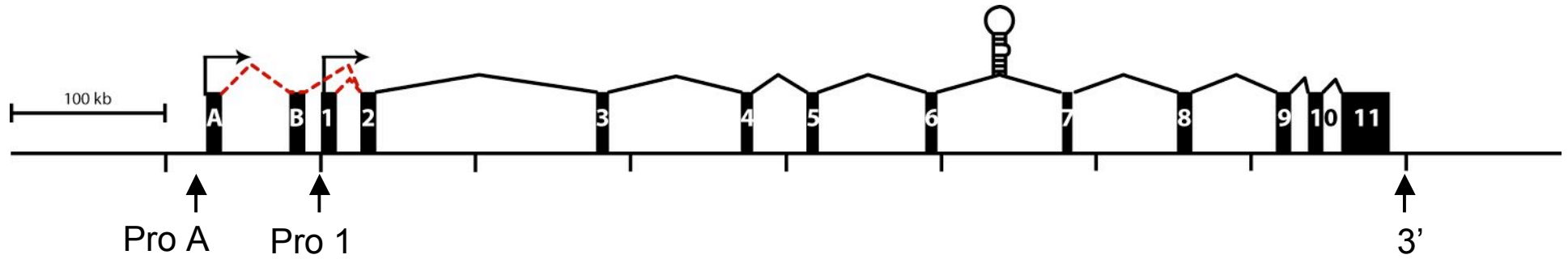
miR-28



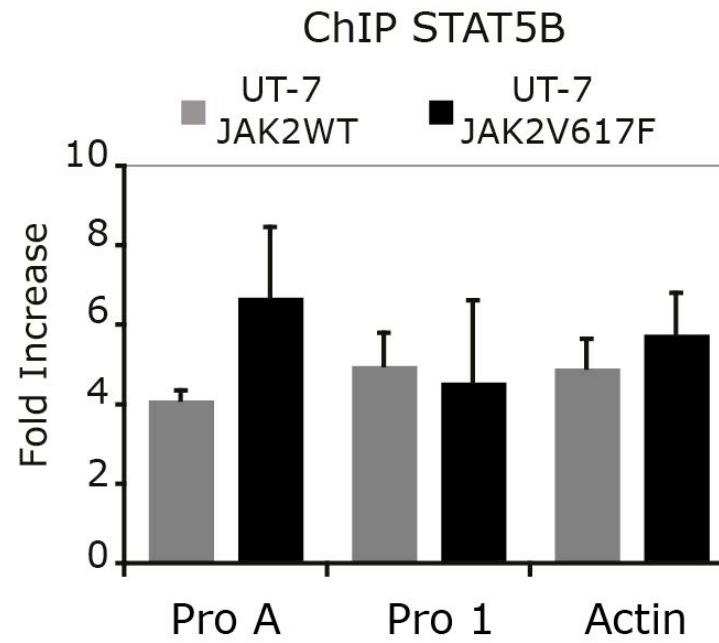
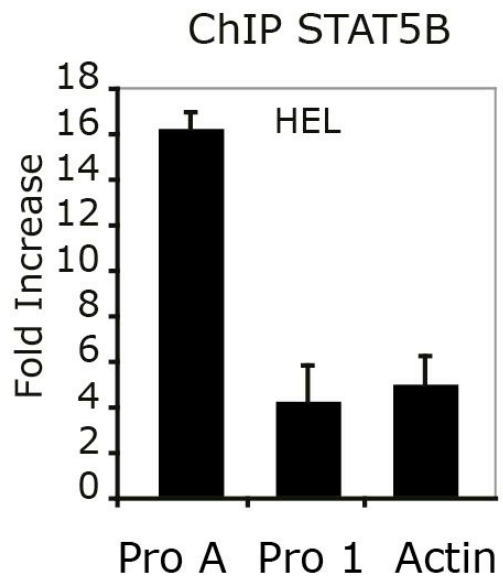
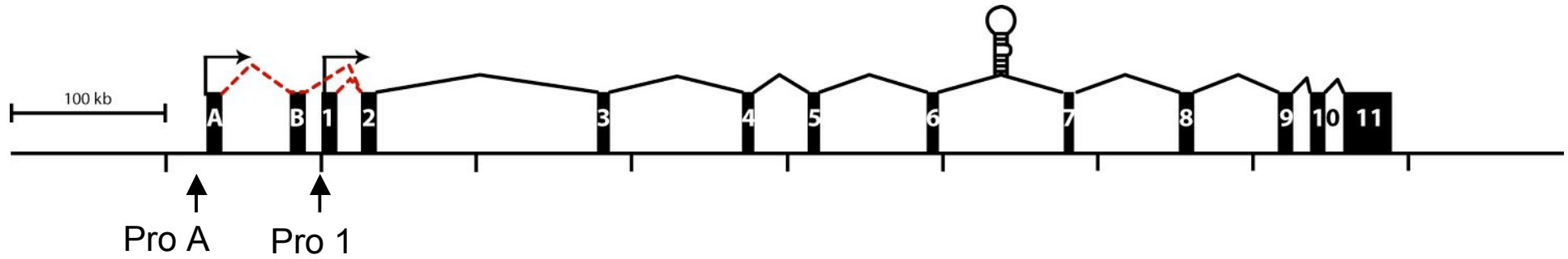
LPP alternative promoter



RNA pol II promoter binding



STAT5B promoter binding



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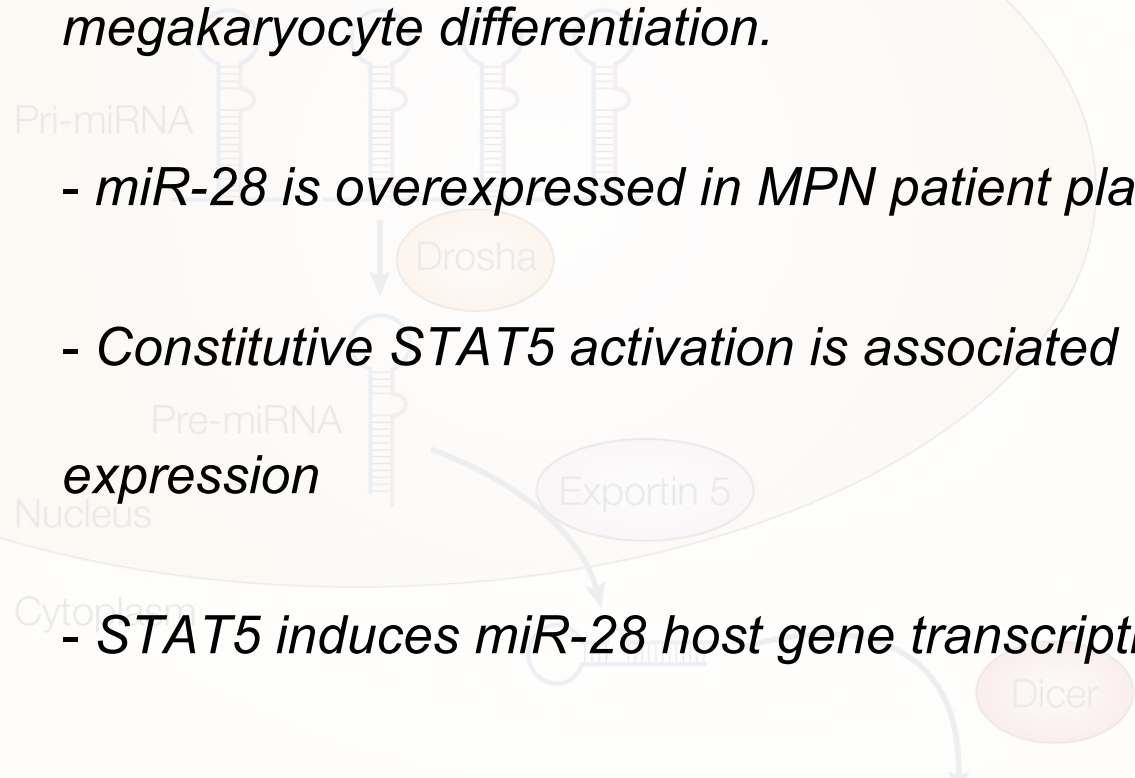
expression

- *STAT5 induces miR-28 host gene transcription*

miR-28 is a thrombopoietin receptor targeting microRNA detected in a fraction of myeloproliferative neoplasm patient platelets

Michael Girardot, Christian Pecquet, Siham Boukour, Laurent Knoops, Augustin Ferrant, William Vainchenker, Stéphane Giraudier and Stefan N. Constantinescu.

Blood. 2010 May 5. [Epub ahead of print]



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