Leibniz Institute on Aging –
Fritz Lipmann Institute

CMB
CENTER FOR MOLECULAR BIOMEDICINE

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The Emerging Tumor Suppressor RFX7 is a Potential Regulator for Differentiation Therapy in Acute Myeloid Leukemia

Setenay Gupse Özcan

40th TBI Winterseminar | 12.02.2025 | Bled

Computational Biology of Aging (**AG Hoffmann**) – Leibniz FLI
Institute of Molecular Cell Biology / Department of Hematology and Medical Oncology - **AG Schenk -** UKJ

Background: Myeloid Differentiation

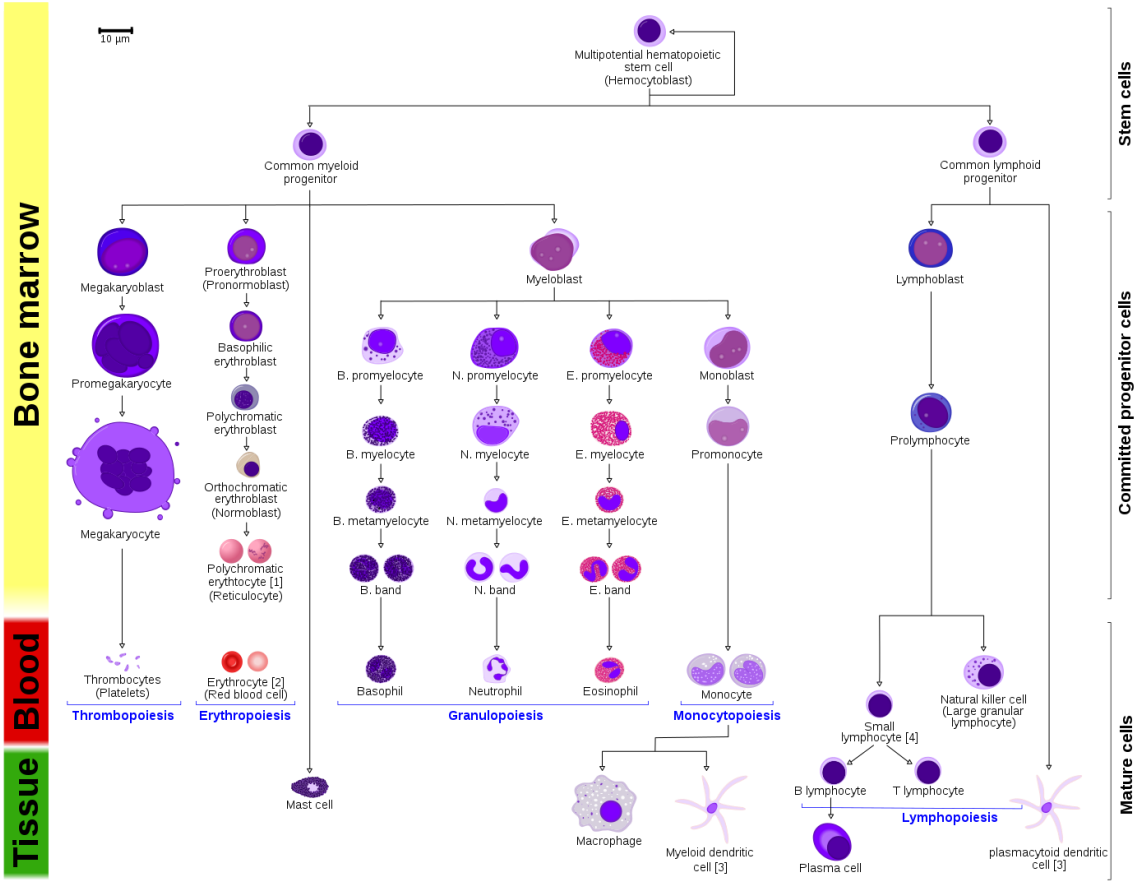

Figure by Rad A.

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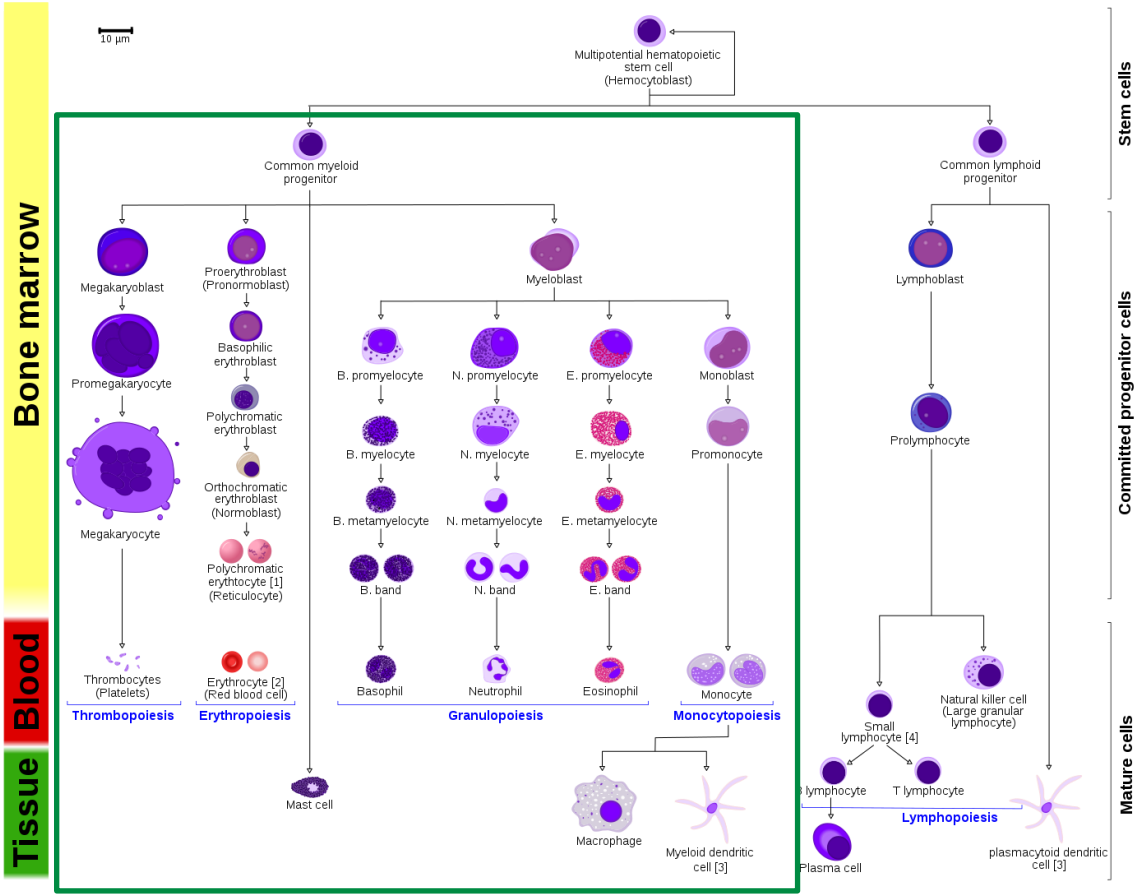

Figure by Rad A.

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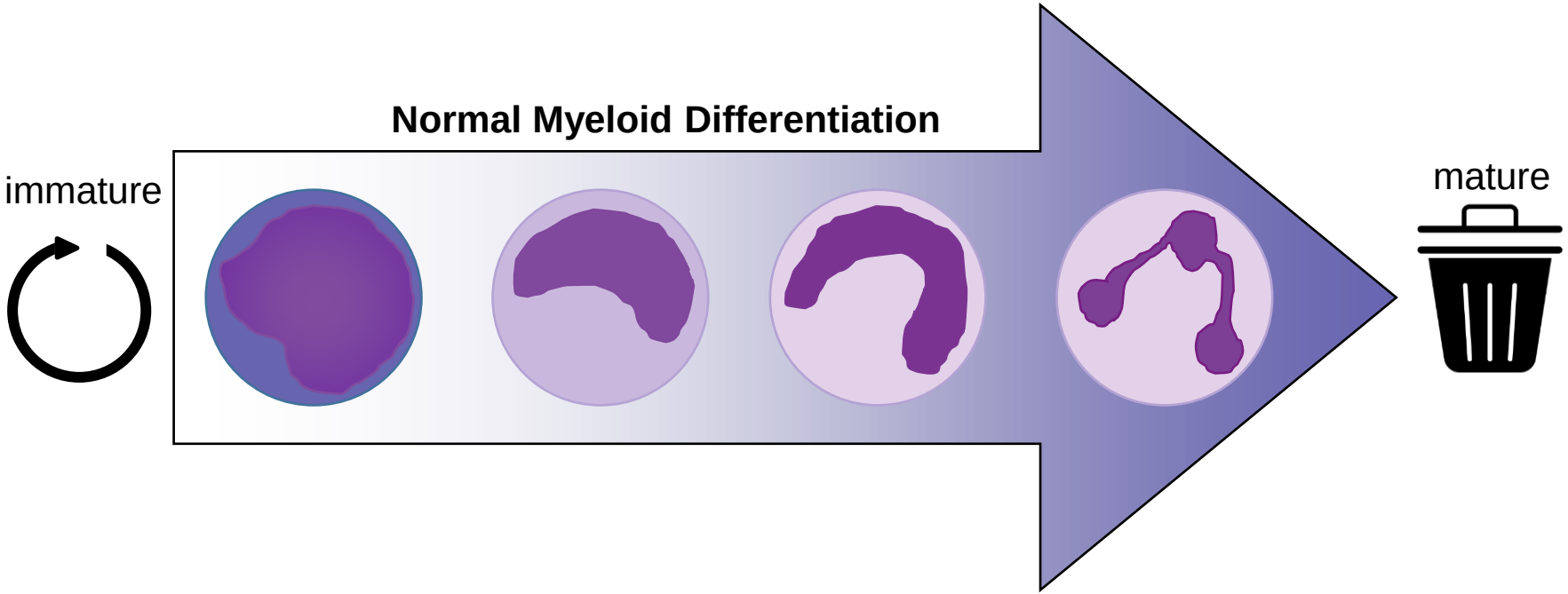

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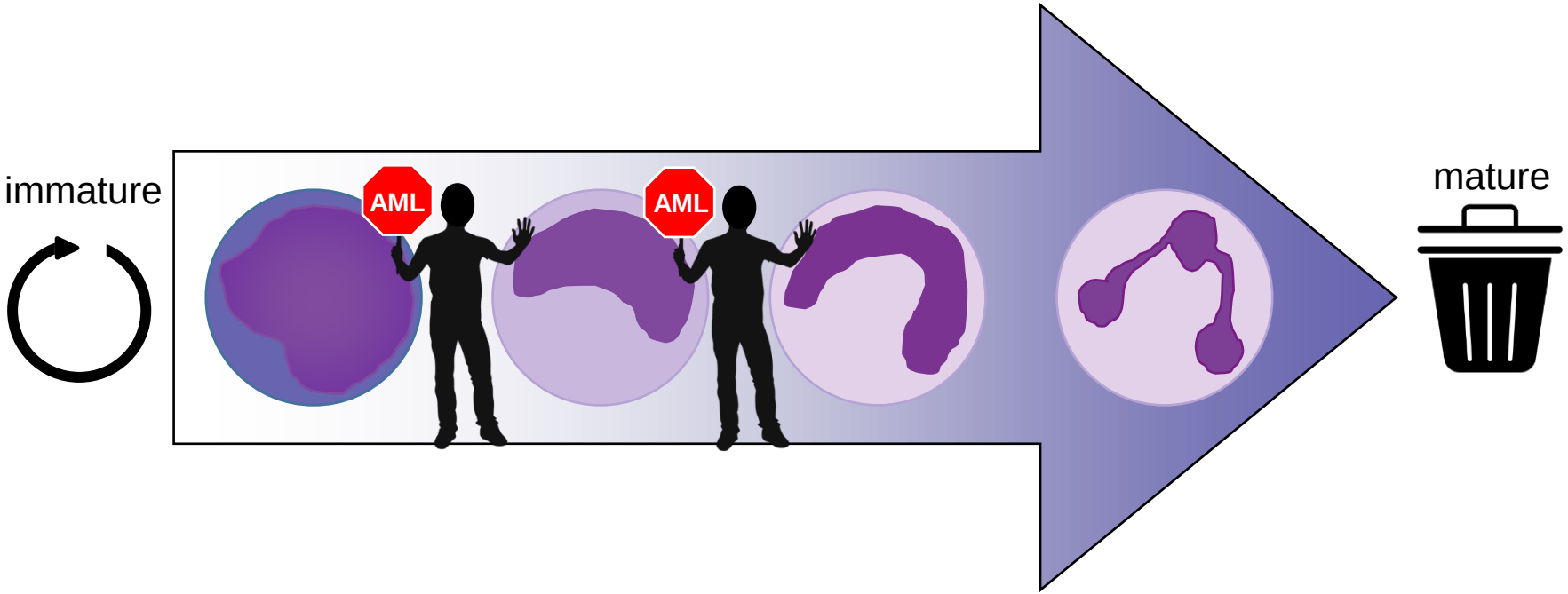

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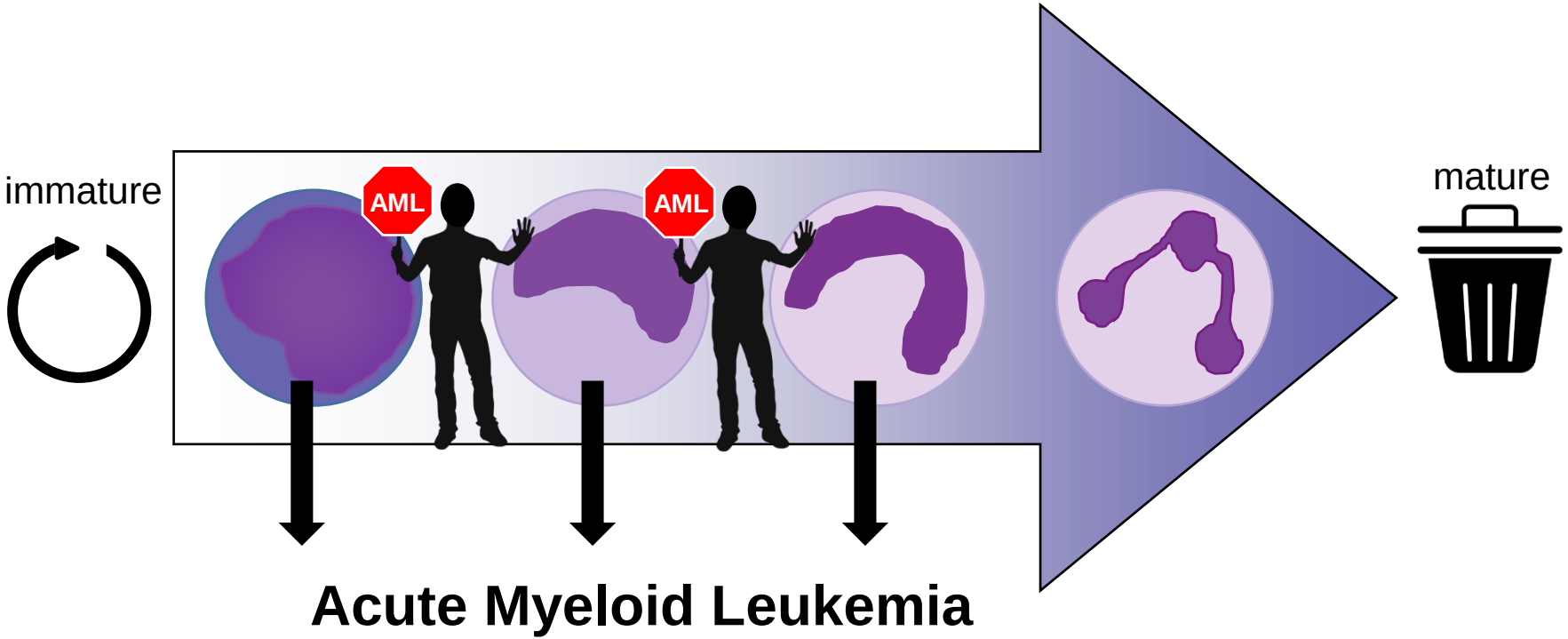
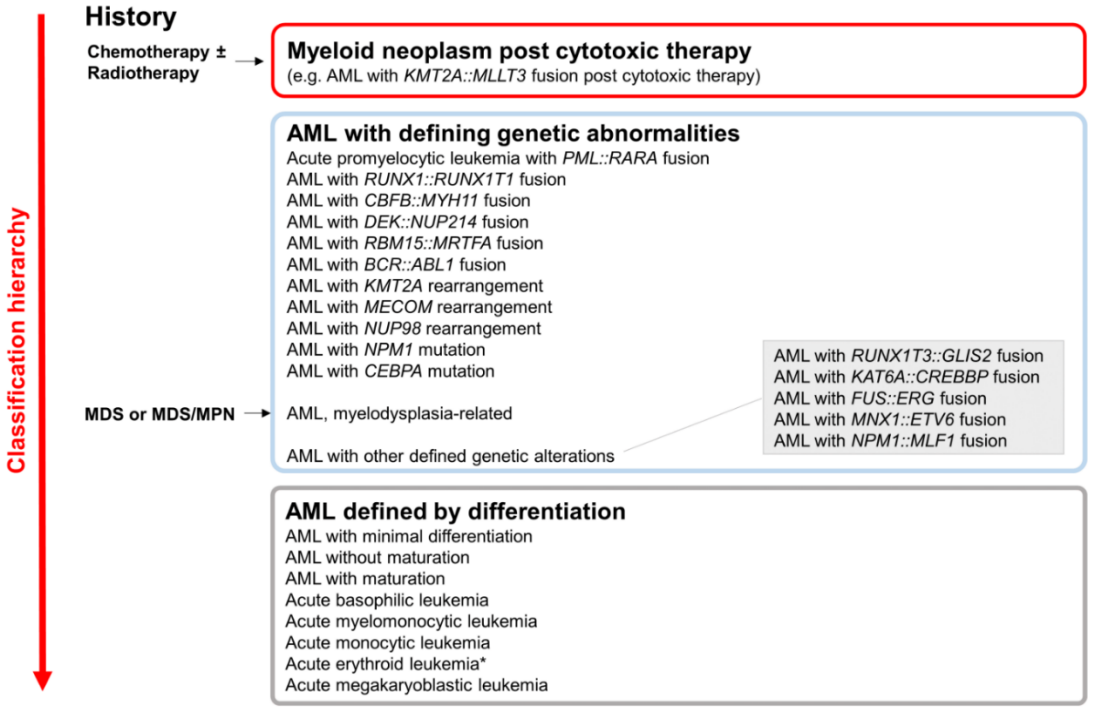
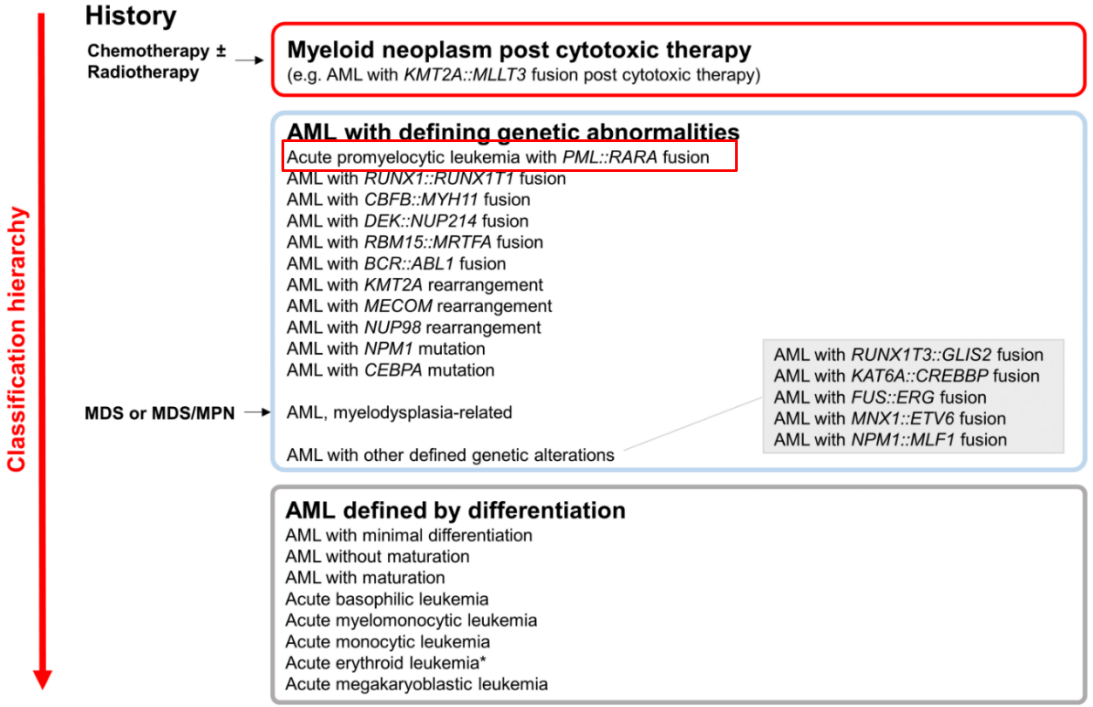


Acute Myeloid Leukemia



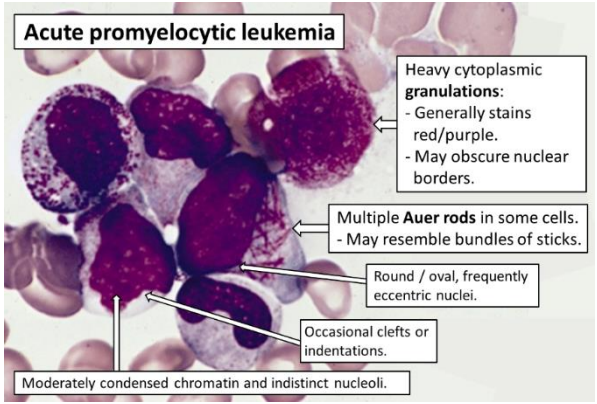
Khoury JD et al. *Leukemia* 2022

Acute Myeloid Leukemia



Khoury JD et al. *Leukemia* 2022

Acute Promyelocytic Leukemia (APL) as a model for differentiation therapy



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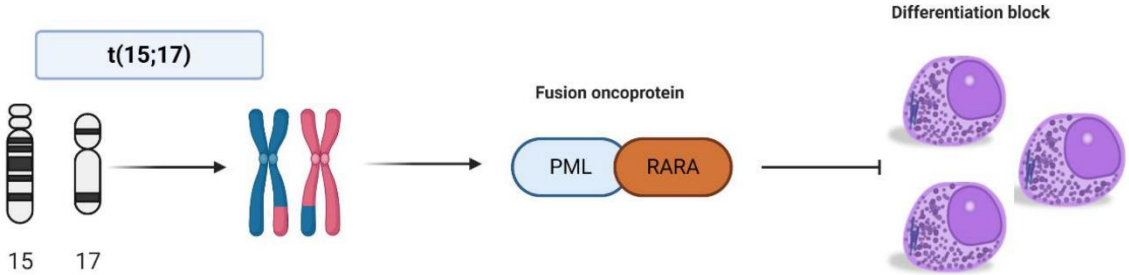
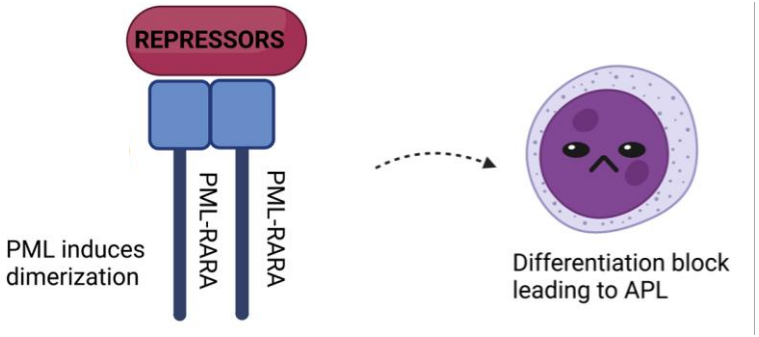


PML: Promyelocytic Leukemia
RARA: Retinoic Acid Receptor Alpha

de Almeida TD et al. *Future Pharmacol.* 2023

Acute Promyelocytic Leukemia as a model for differentiation therapy



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Korsos V et al. *J Mol Endocrinol*. 2022

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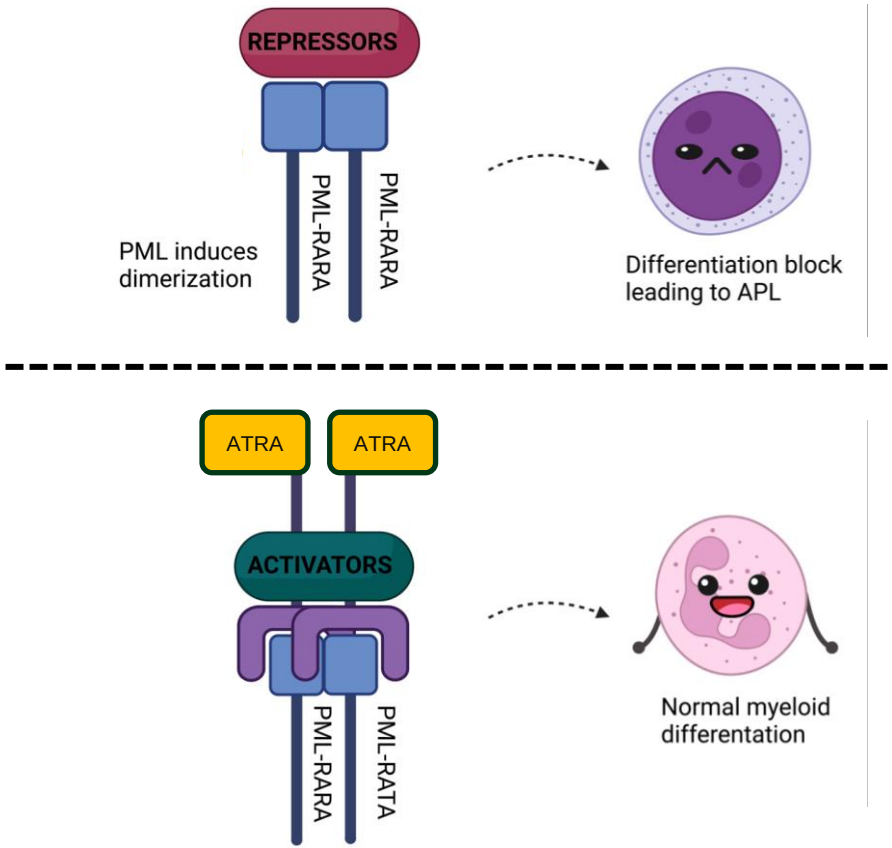

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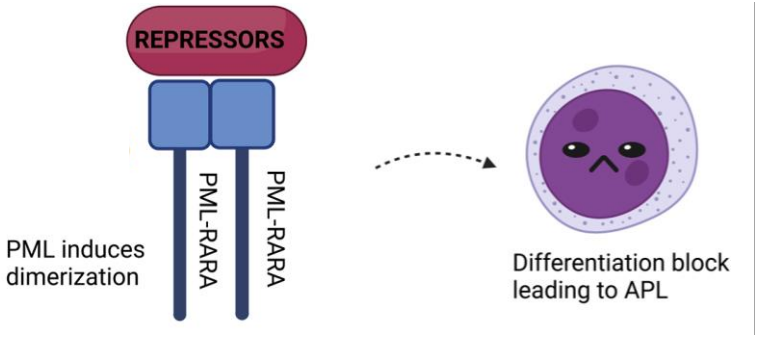

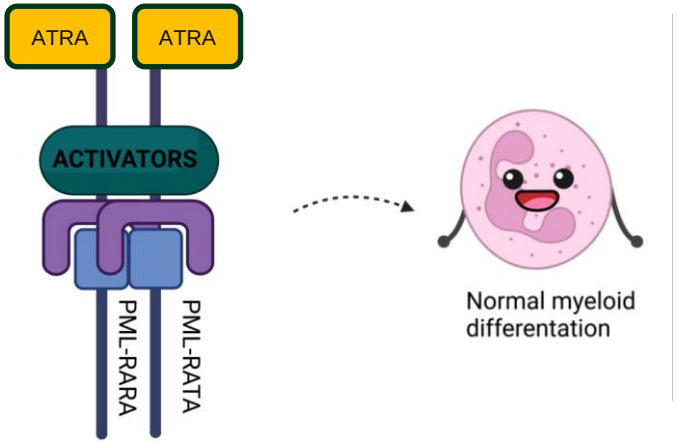

Differentiation is a therapeutic strategy

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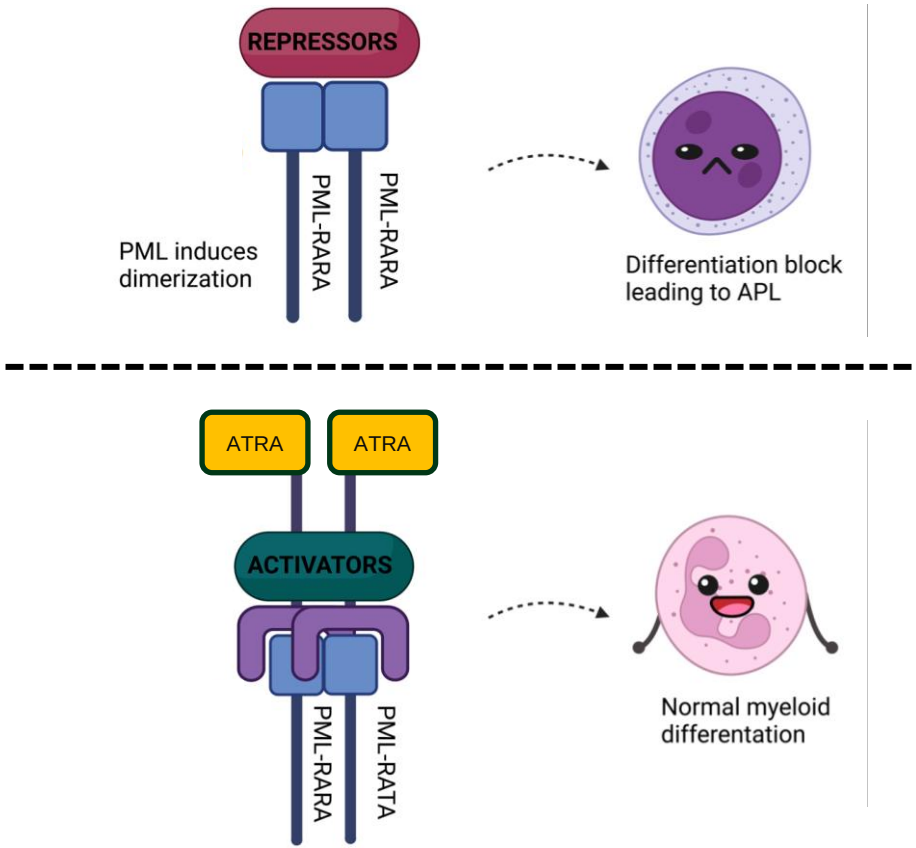

Differentiation is a therapeutic strategy

How can we transfer the success of ATRA to other types of AML?

PML: Promyelocytic Leukemia
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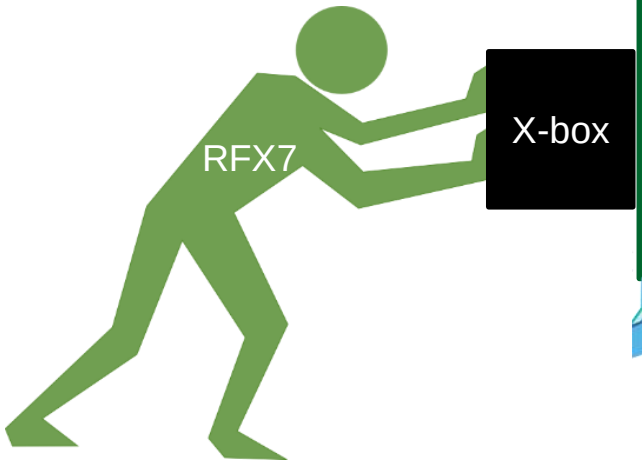
Korsos V et al. *J Mol Endocrinol*. 2022

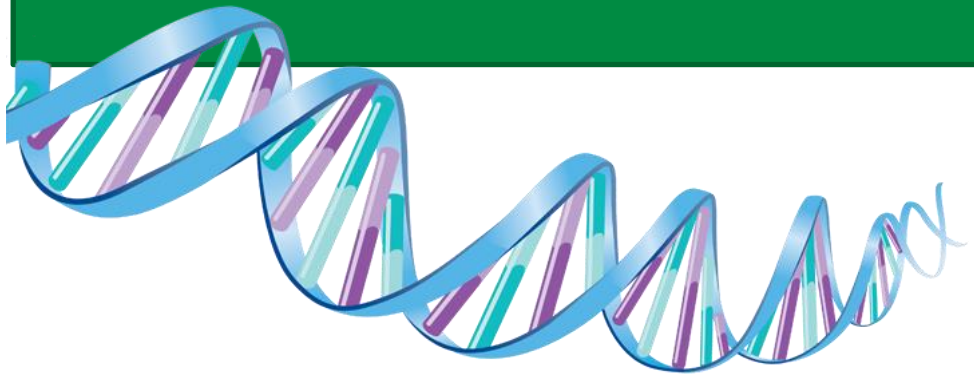
Regulatory Factor X7 (RFX7)



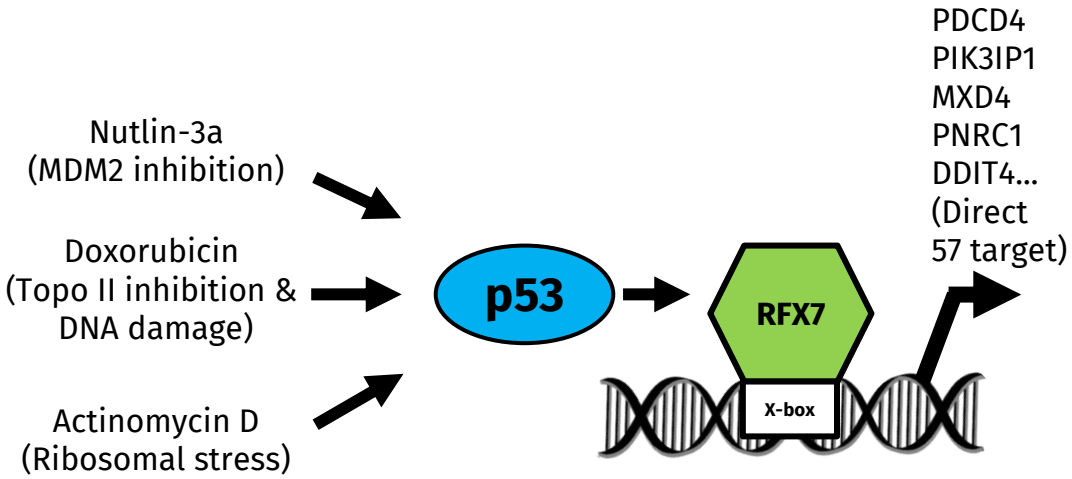
X-box

Regulatory Factor X7

- A transcription factor that binds DNA through **X-box** motif
- Activated by p53 (**Coronel et al. 2021**)
- Tumor suppressor (**Weber et al. 2019**, **Coronel et al. 2021**)
- Plays potential role in neuronal development (**Schwab et al. 2023**)



A look into RFX7 signaling



PDCD4: Programmed cell death 4
PIK3IP1: Phosphatidylinositol 3-kinase interacting protein 1
MXD4: Max interacting transcriptional repressor
PNRC1: Proline rich nuclear receptor coactivator 1
RFX5: Regulatory Factor X5

Coronel L [...] Hoffmann S, Fischer M. *Nucleic Acids Res*. 2021

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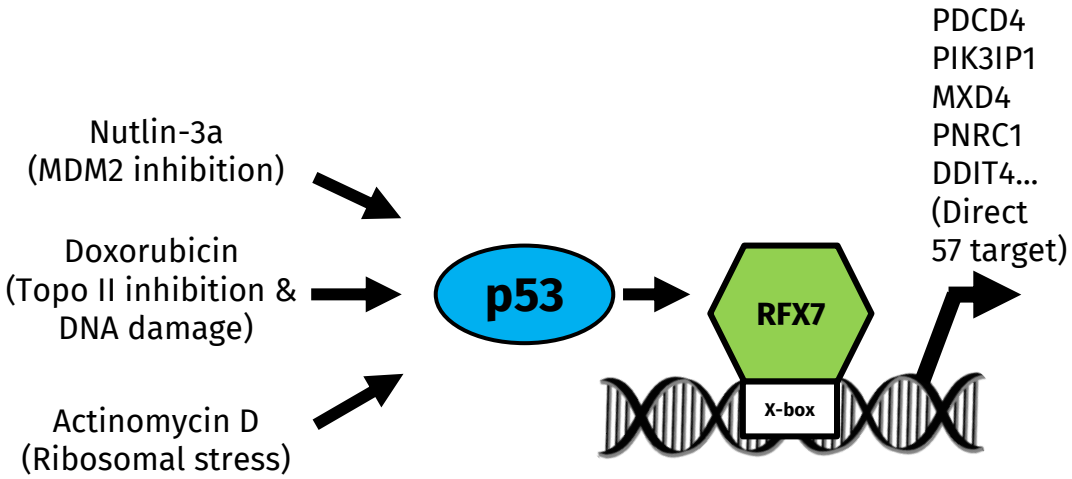

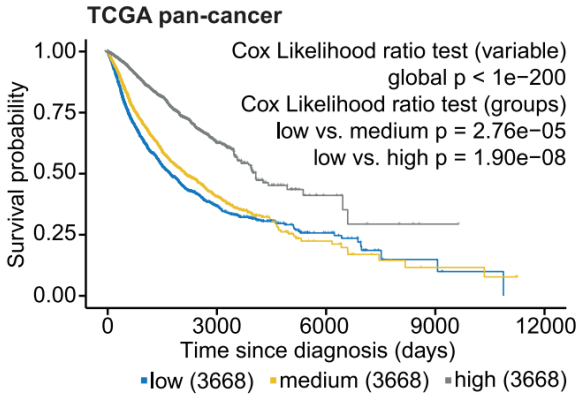

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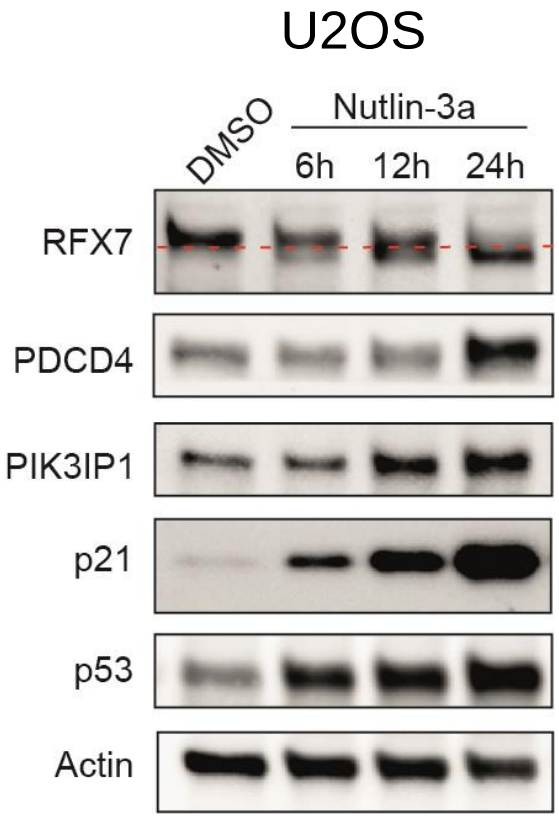

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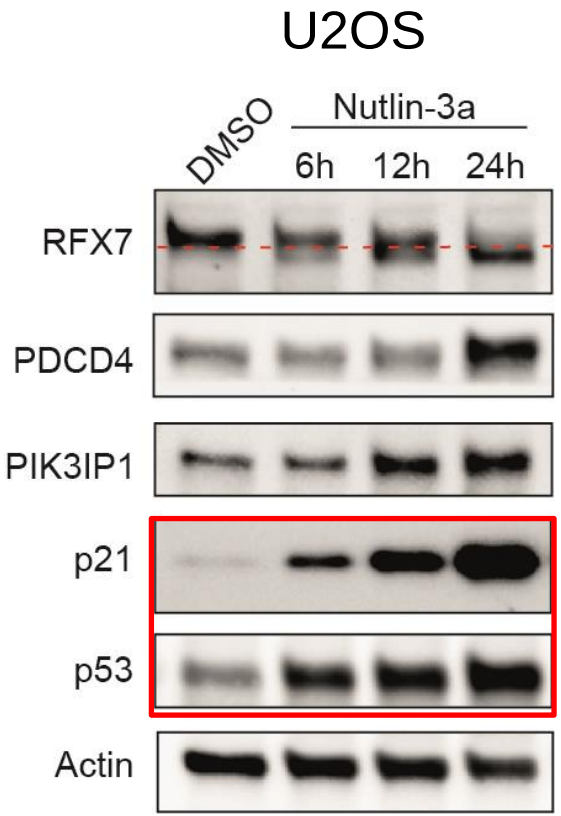

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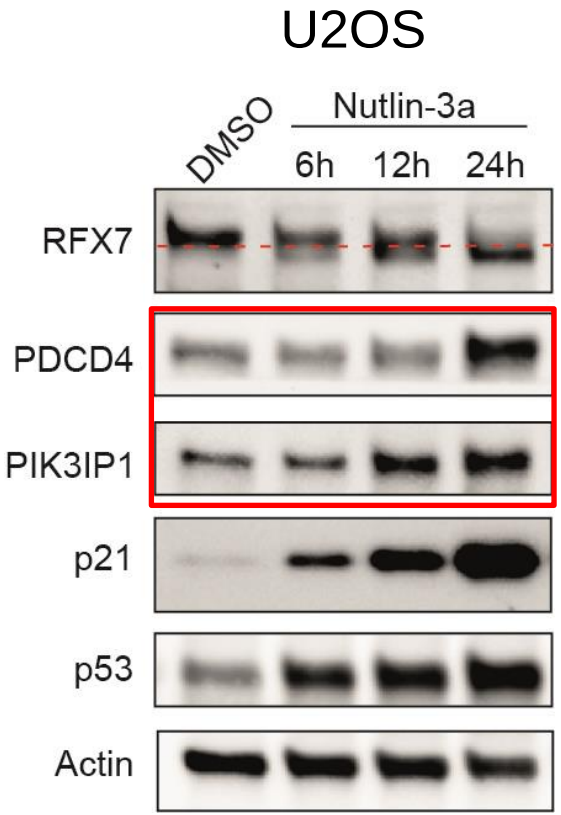

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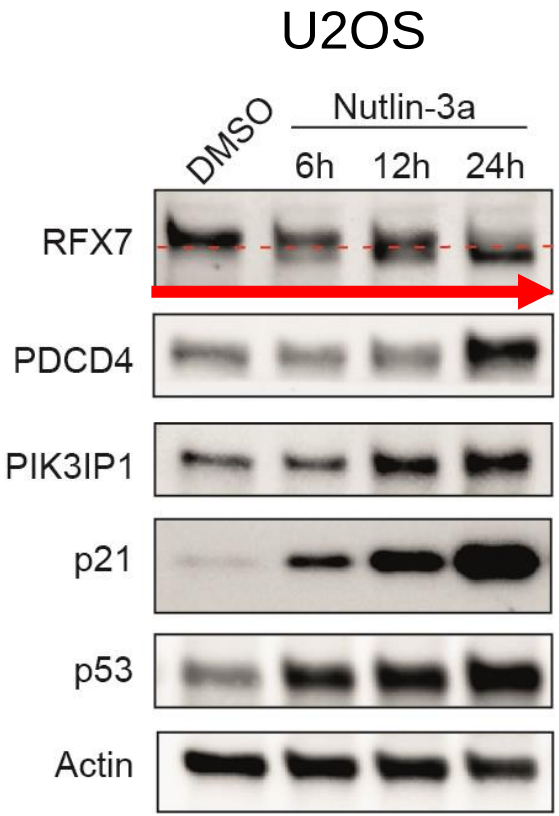

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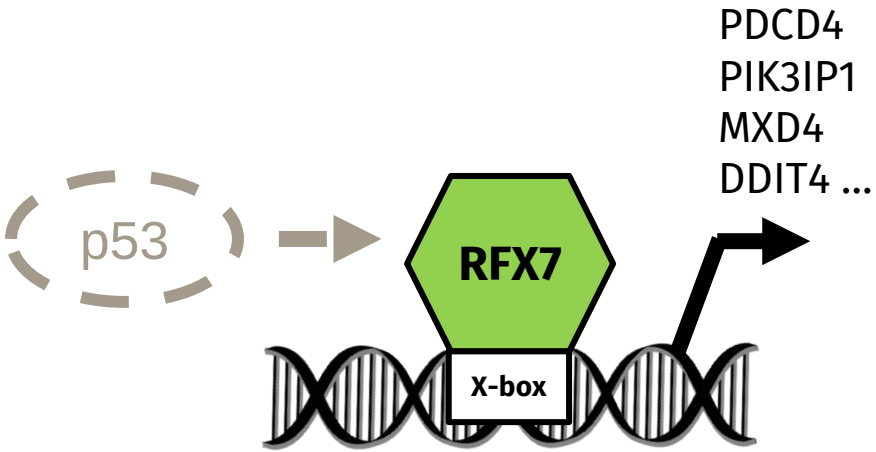
What about without p53?



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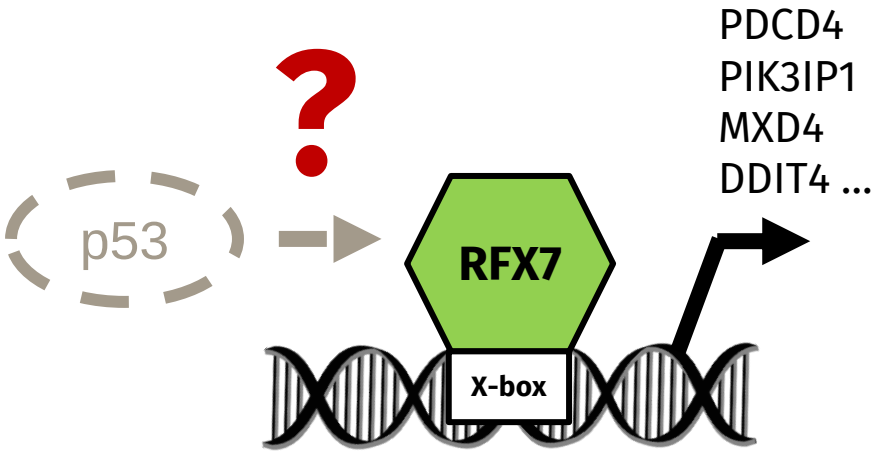

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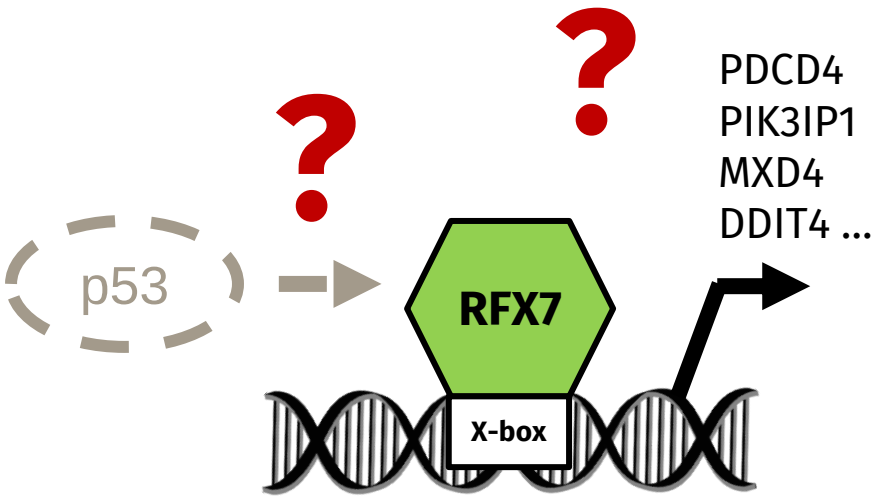

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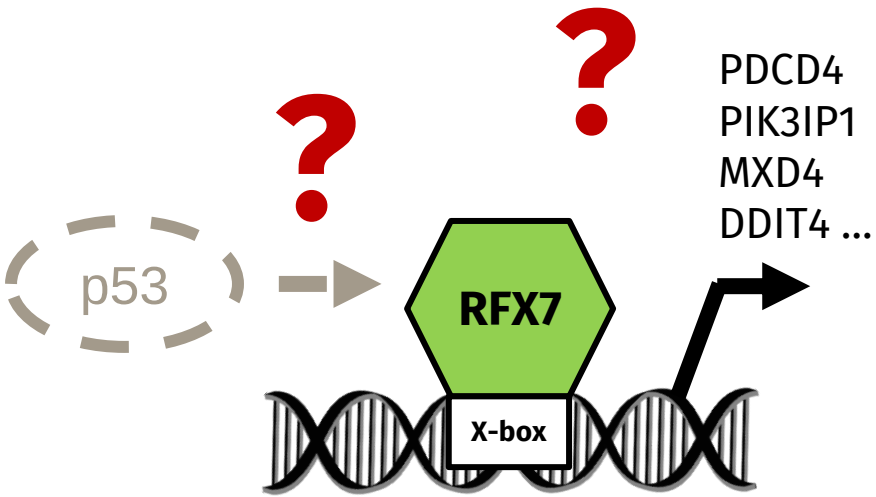

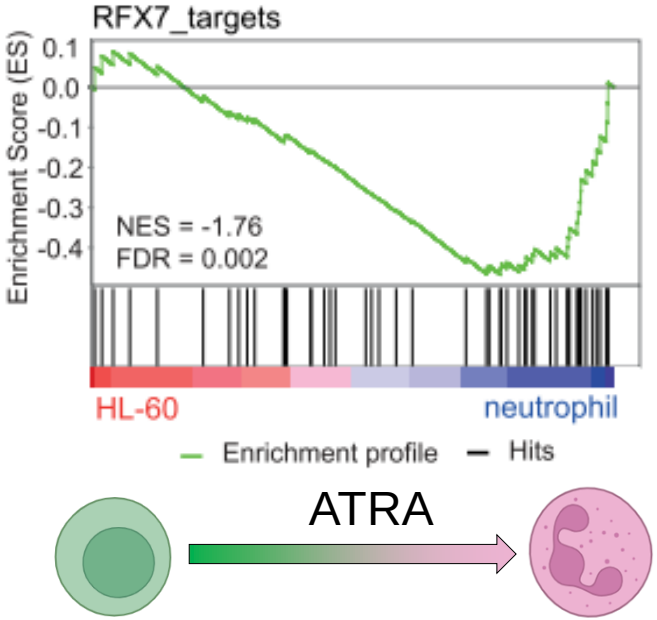

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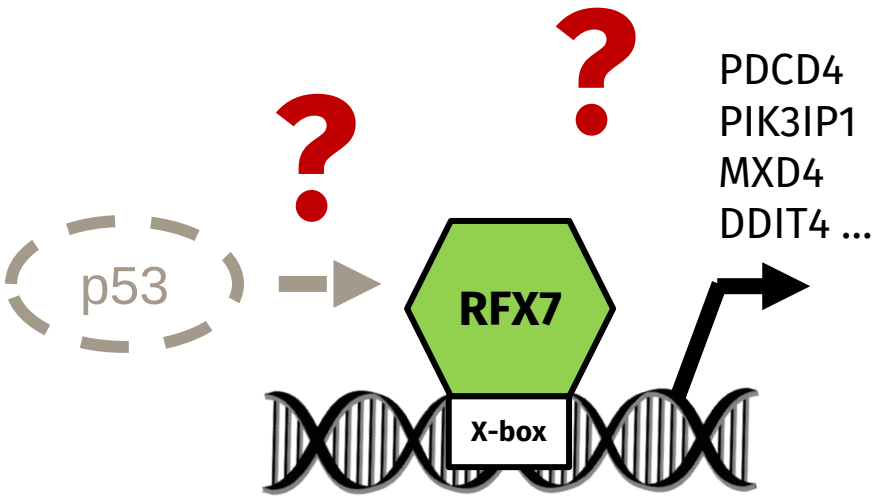

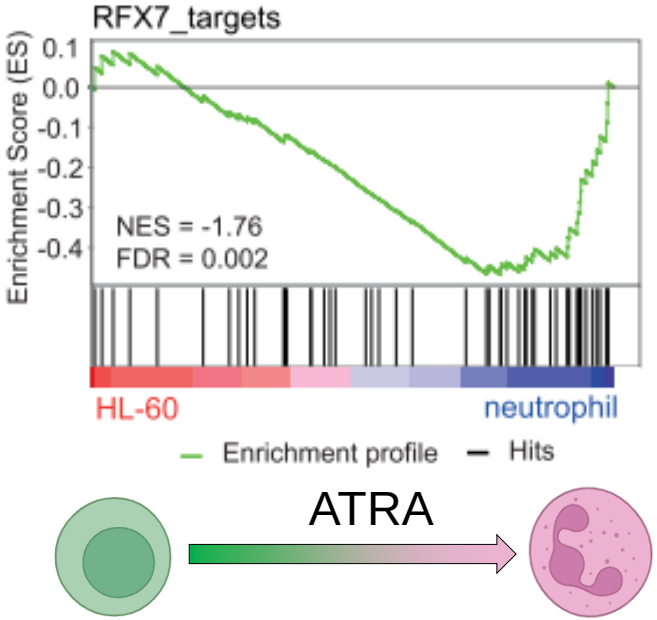

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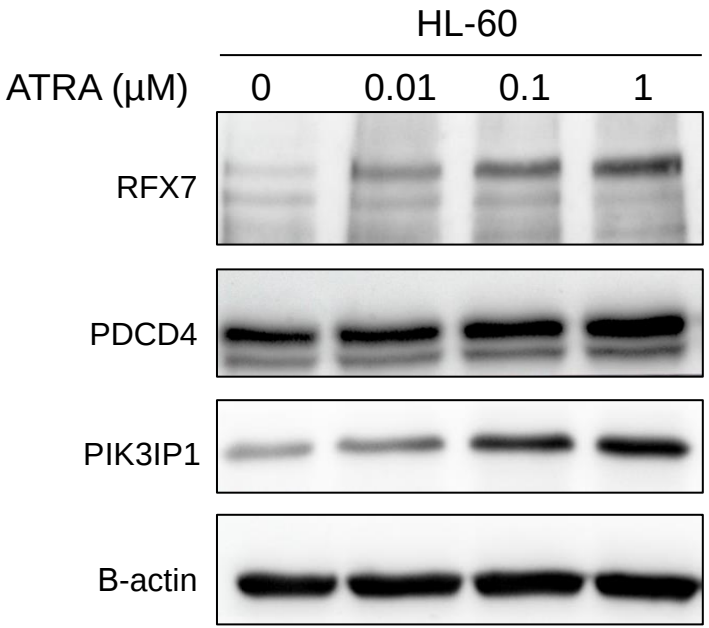
RFX7 signaling is activated in response to ATRA



HL-60 cells were treated with different doses of ATRA for 48h.

PDCD4: Programmed cell death 4
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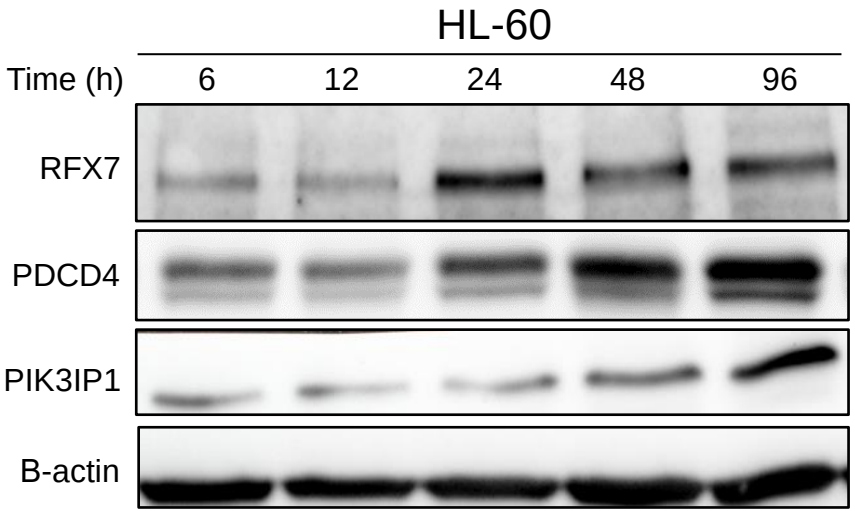
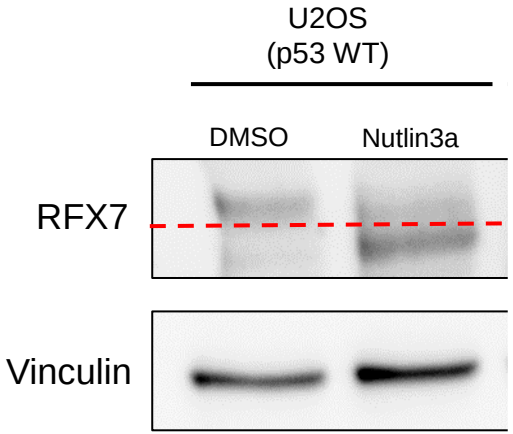
RFX7 Dynamics in HL-60: Time Course



HL-60 cells were treated with 1µM ATRA for different time points.

PDCD4: Programmed cell death 4
PIK3IP1: Phosphatidylinositol 3-kinase interacting protein 1

What about the size shift?



U2OS cells were treated with DMSO or Nutlin3a for 24h; HL-60 cells were treated with 0.05% DMSO or 1μM ATRA for 48h.

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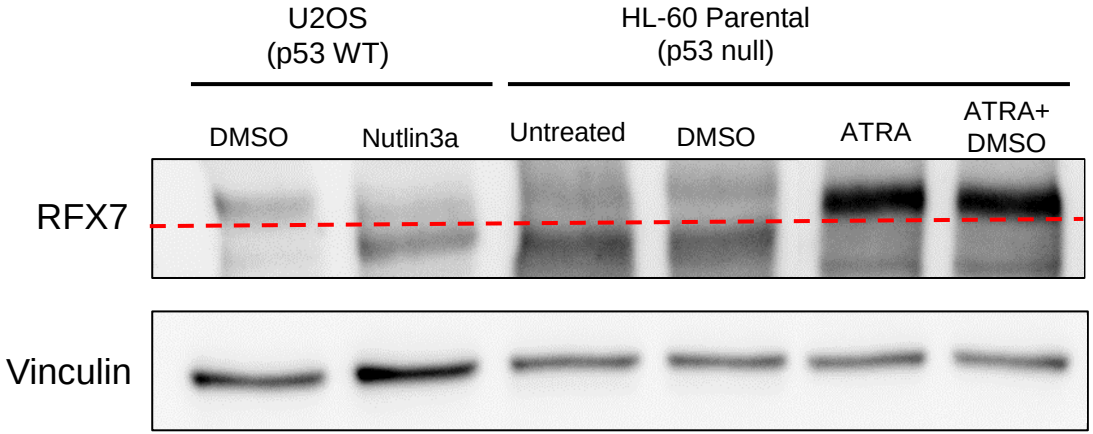

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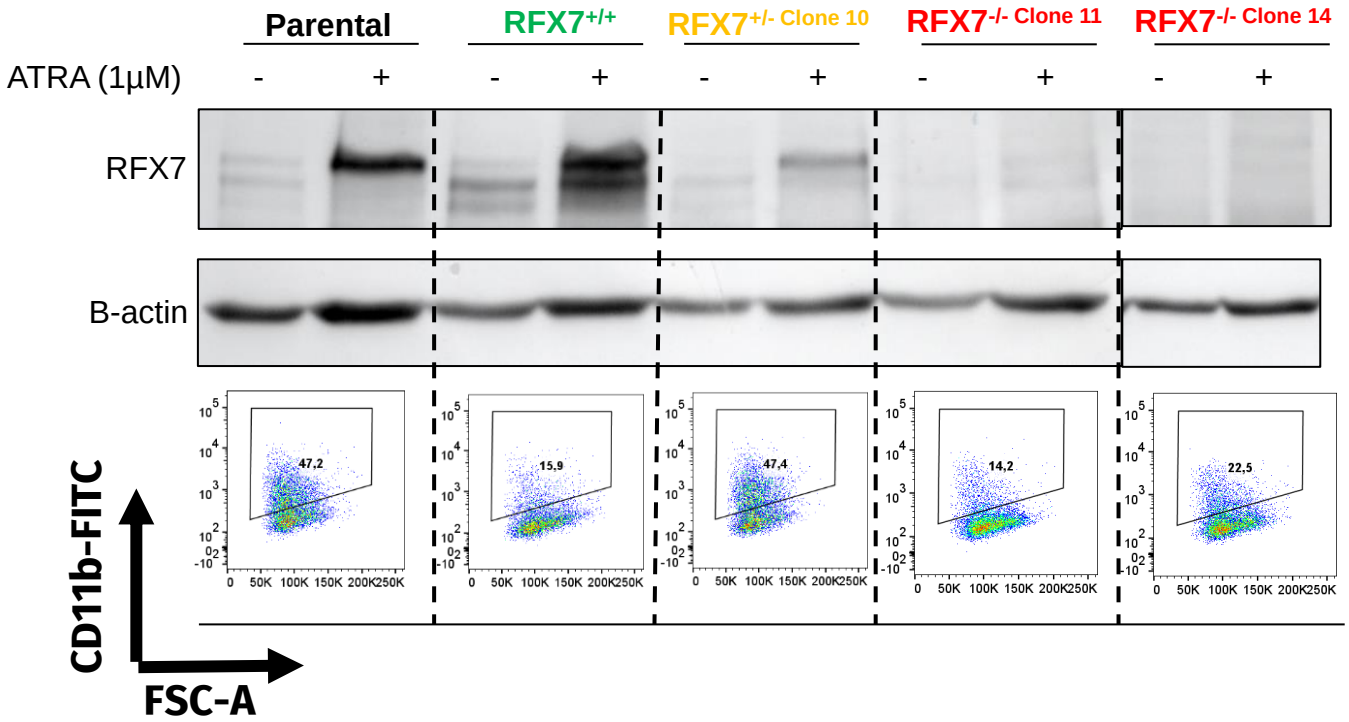
Assessment of ATRA reponse in RFX7 knockout Cells



CD11b: Integrin alpha M (Marker for myeloid differentiation)
Cells were treated with 1µM ATRA for t=72h for differentiation analysis and t=48h with for Western Blot

To overexpress or not to overexpress?

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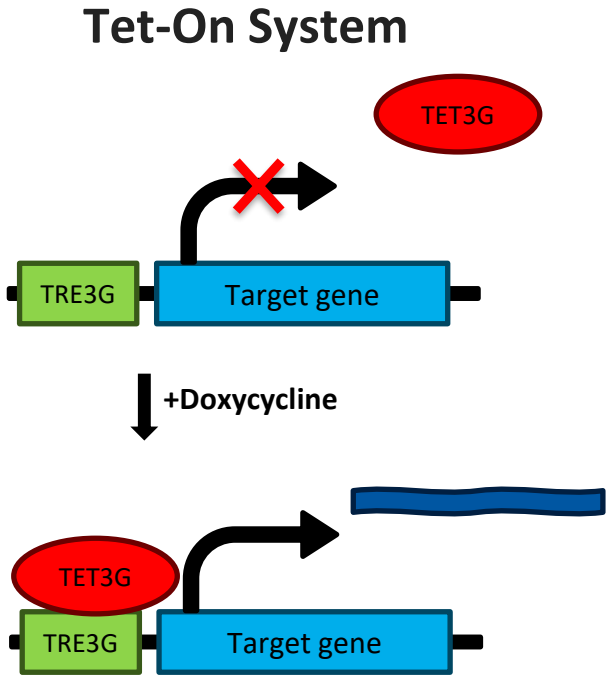
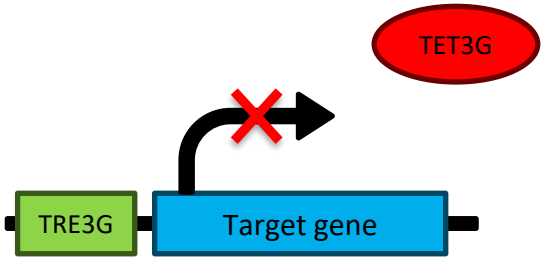


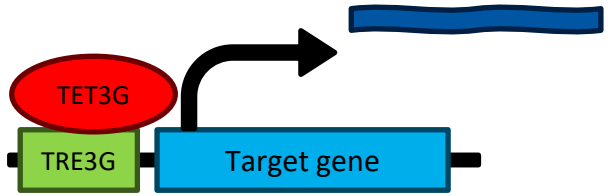
TET3G: Reverse tetracycline-controlled transactivator
TRE3G: Tetracycline responsive promoter

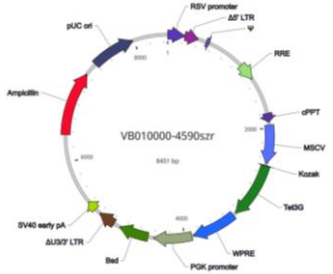
To overexpress or not to overexpress?

Tet-On System

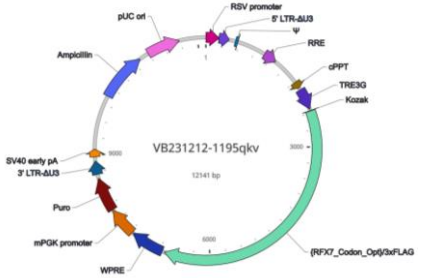


+Doxycycline





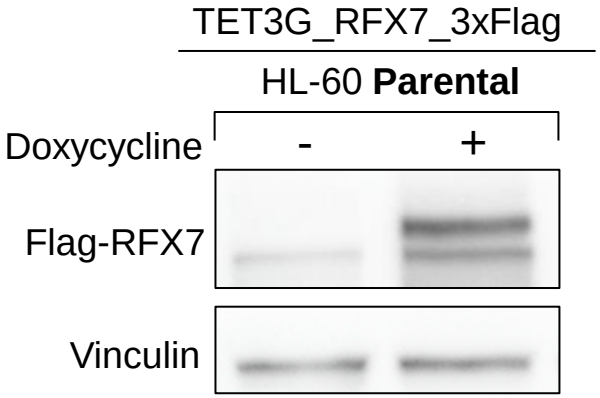
pLV_Bsd_MSCV_TET3G
8451 bp

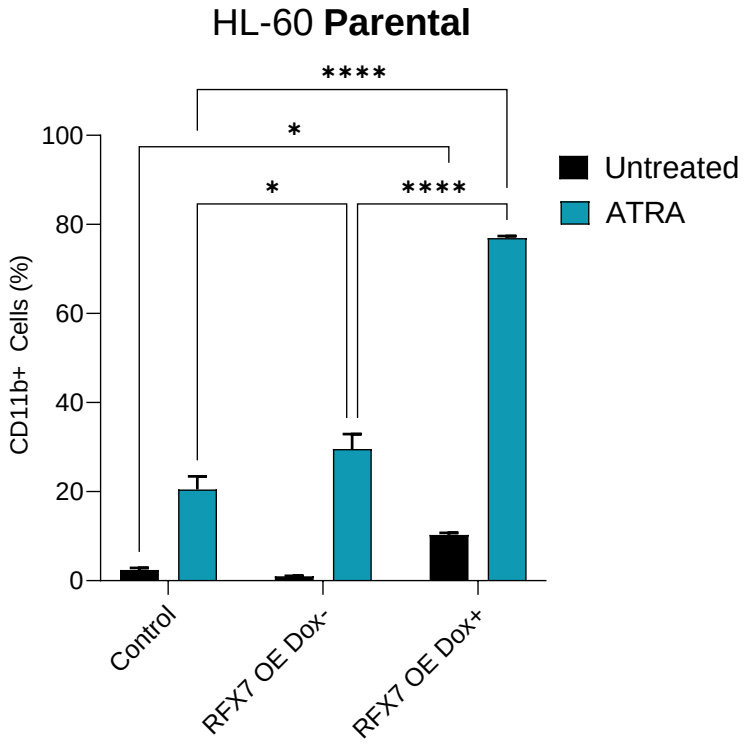


pLV_Puro_TRE3G_hRFX7[Codon Optimized]3xFlag
12141 bp

TET3G: Reverse tetracycline-controlled transactivator
TRE3G: Tetracycline responsive promoter

Over-expression of RFX7 augments the differentiation response of HL-60

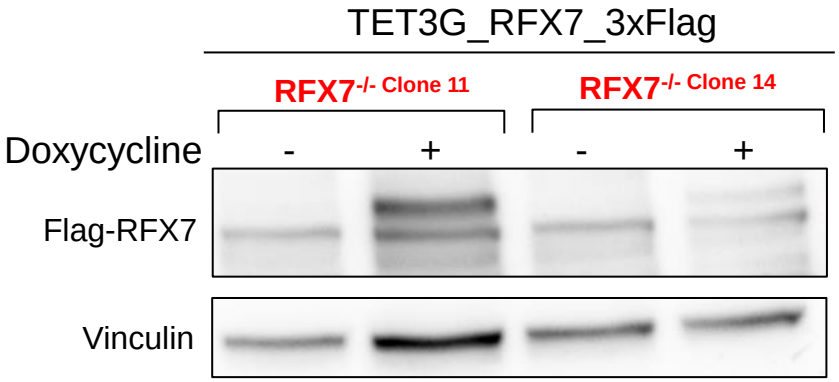


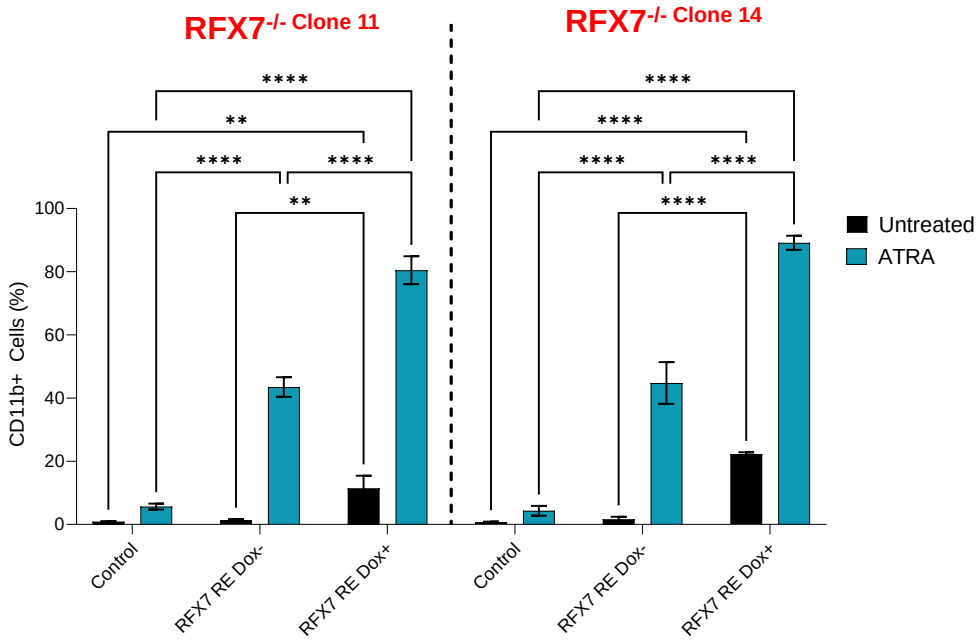


Cells were treated with 0.1μM ATRA for t=72h for differentiation analysis and t=24h with Doxycycline for Western Blot

Two-way ANOVA, 3 independent experiments
p values: * ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001, **** ≤0.0001

Re-introduction of RFX7 re-enables the differentiation response





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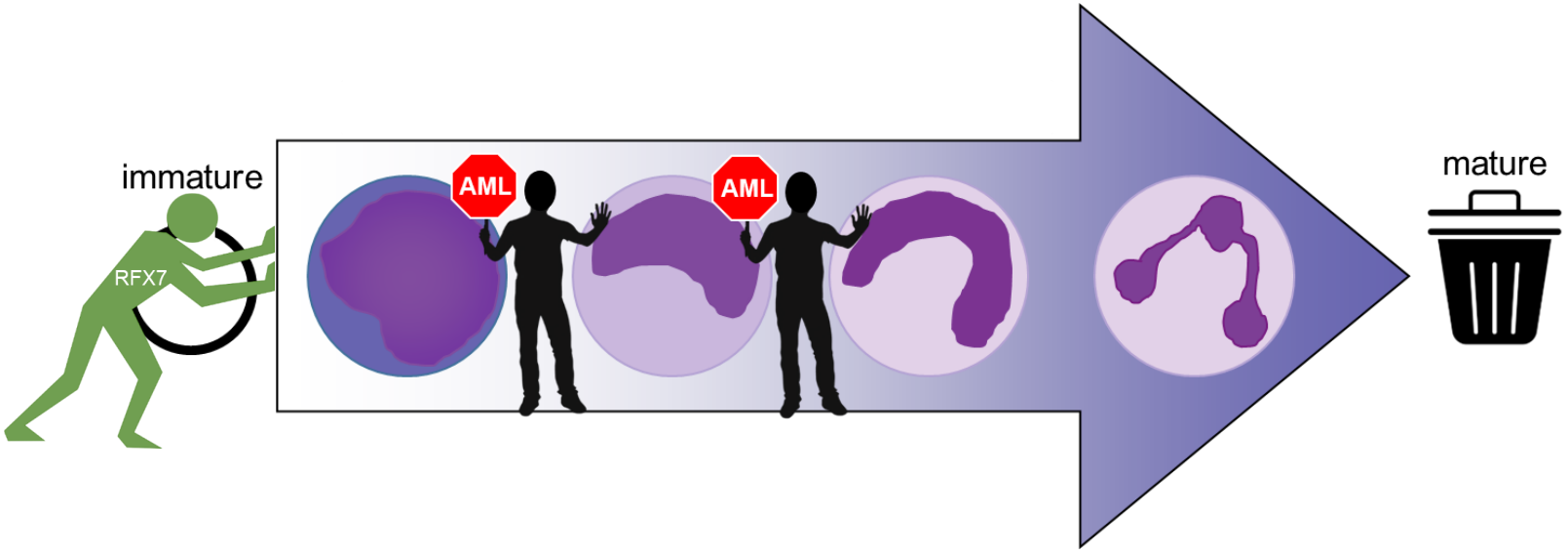
Summary



Our results suggest a role for p53-independent RFX7 signaling in ATRA induced differentiation response in AML.

Acknowledgements

Leibniz Institute on Aging – Fritz Lipmann Institute

Computational Biology of Aging

Steve Hoffmann
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Robert Schwarz
Omid Omrani
Maja Kinga Olecka
Aleksandr Cherkasov
Atakan Ayden
Tushar Patel
Tycho Kirchner
Silke Förste
Linus Blochinger
Lukas Grein
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Laura Burkhardt
Marianne Rosenthal

JSMM Jena School of Molecular Medicine

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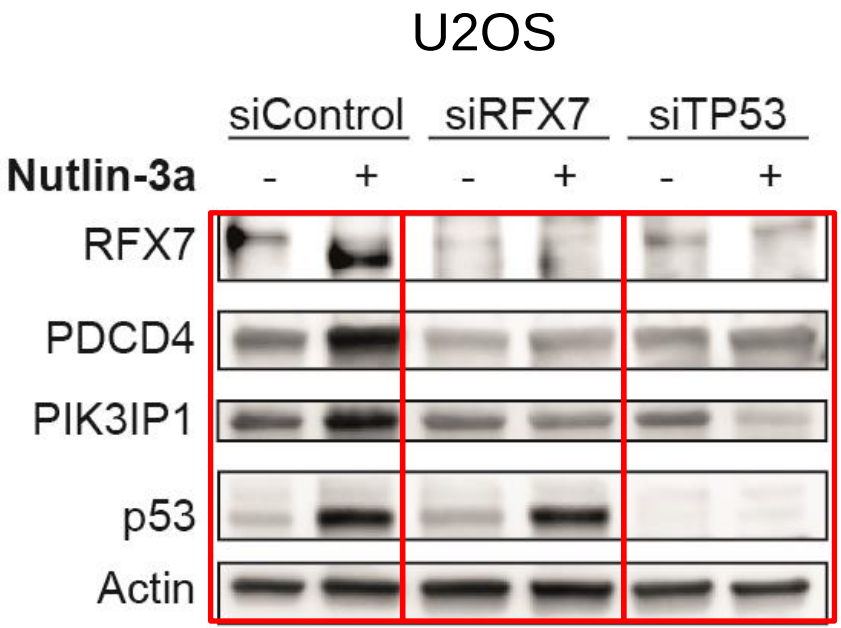
DFG Deutsche Forschungsgemeinschaft

Interdisziplinäres Zentrum für Klinische Forschung

GRADUIERTEN AKADEMIE

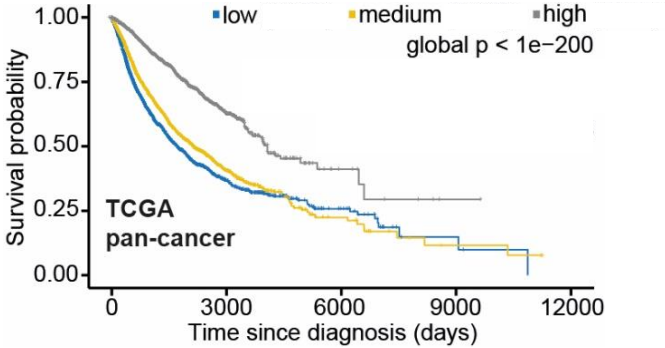
BACK UP SLIDES

RFX7 is at the downstream of p53

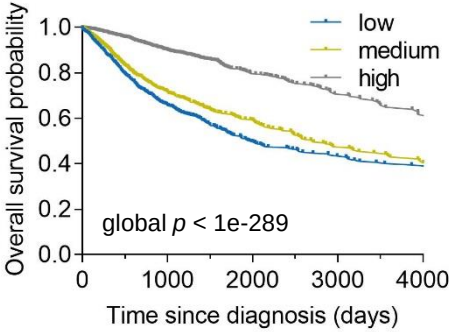


RFX7 target gene expression in cancer

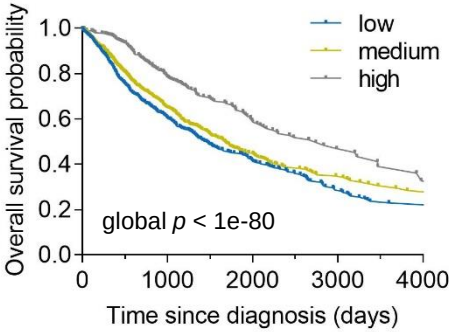
- RFX7 target gene expression correlates with good prognosis across the TCGA pan-cancer cohort and in 11 out of 33 individual cancer types



p53 wild-type

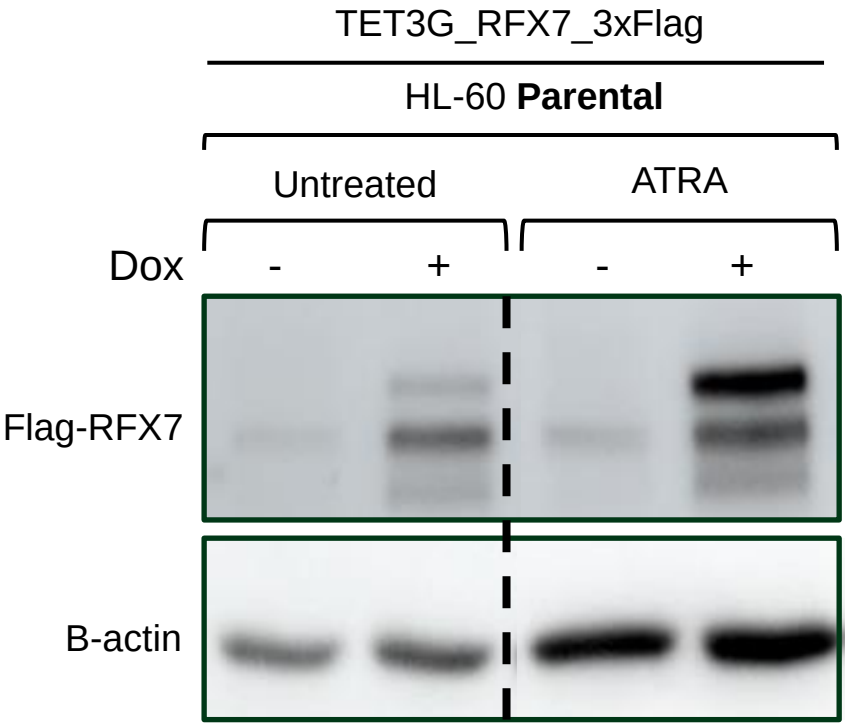


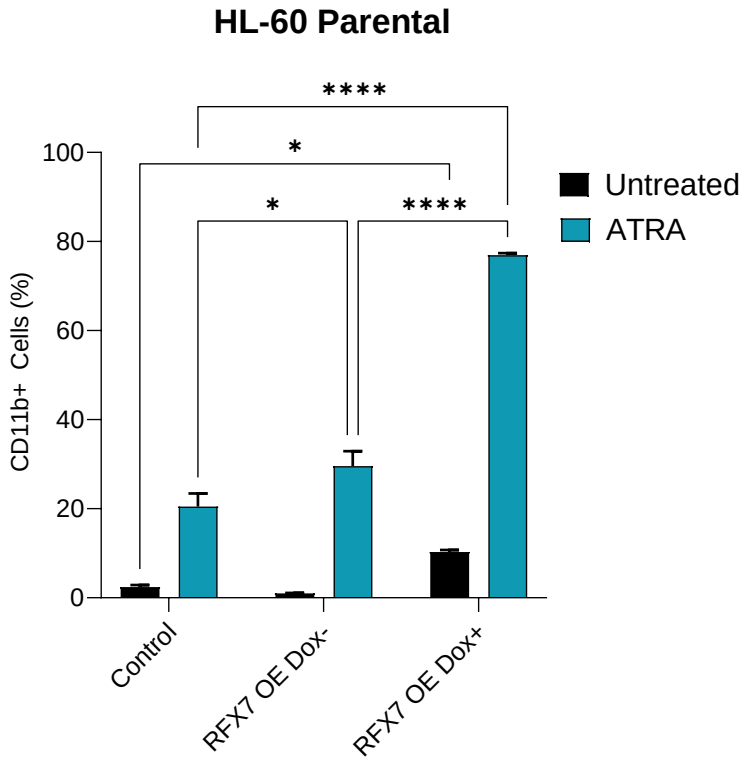
p53 mutant



Coronel L, […], Hoffmann** S, Fischer** M (2021) *Nucl. Acids Res.*

Over-expression of RFX7 augments the differentiation response of HL-60



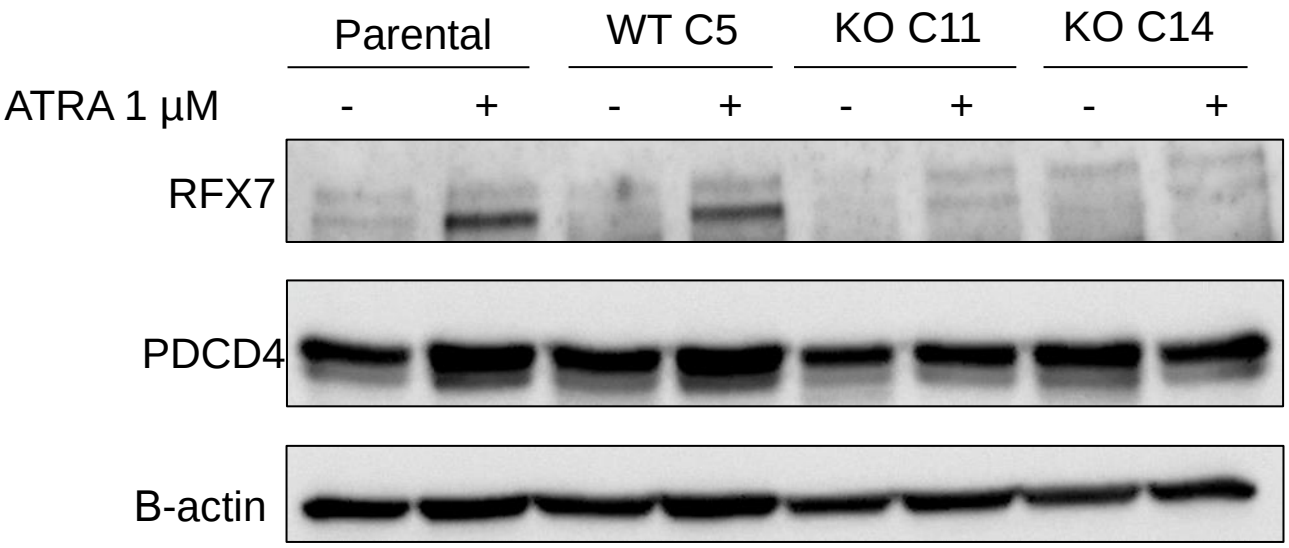


Cells were treated with 0.1μM ATRA for t=72h for differentiation analysis and t=24h with Doxycycline for Western Blot

Two-way ANOVA, 3 independent experiments
p values: * ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001, **** ≤0.0001

Re-introduction of RFX7 re-enables the differentiation response

Cells were treated with 0.1µM ATRA and Doxycycline for 24h Western Blot


Parental
WT C5
KO C11
KO C14
ATRA 1 µM
- + - + - + - +
RFX7
PDCD4
B-actin