Boolean Implications Identify Wilms' Tumor 1 Mutation as a Driver of DNA Hypermethylation in Acute Myeloid Leukemia

Subarna Sinha PhD
Department of Computer Science

Principal Investigator: David Dill

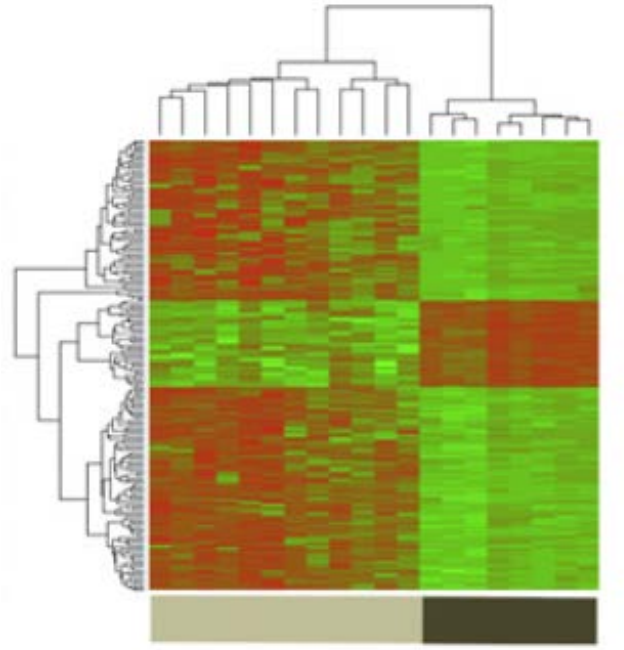
Daniel Thomas MD PhD
Department of Medicine, Hematology Division
Stem Cell & Regenerative Medicine Institute

Principal Investigator: Ravi Majeti

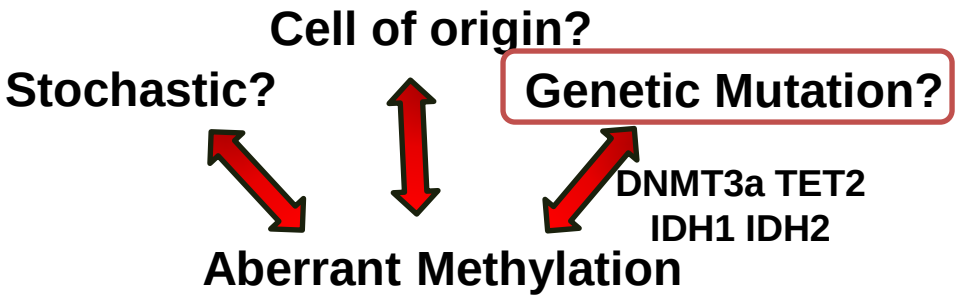
Poster # 13

Aberrant Methylation in Acute Myeloid Leukemia

- Acute Myeloid Leukemia (AML) is a disease characterised by the accumulation myeloid precursor cells in the bone marrow that are blocked in their ability to differentiate into mature blood cells
- AML is associated with widespread deregulation of DNA methylation.

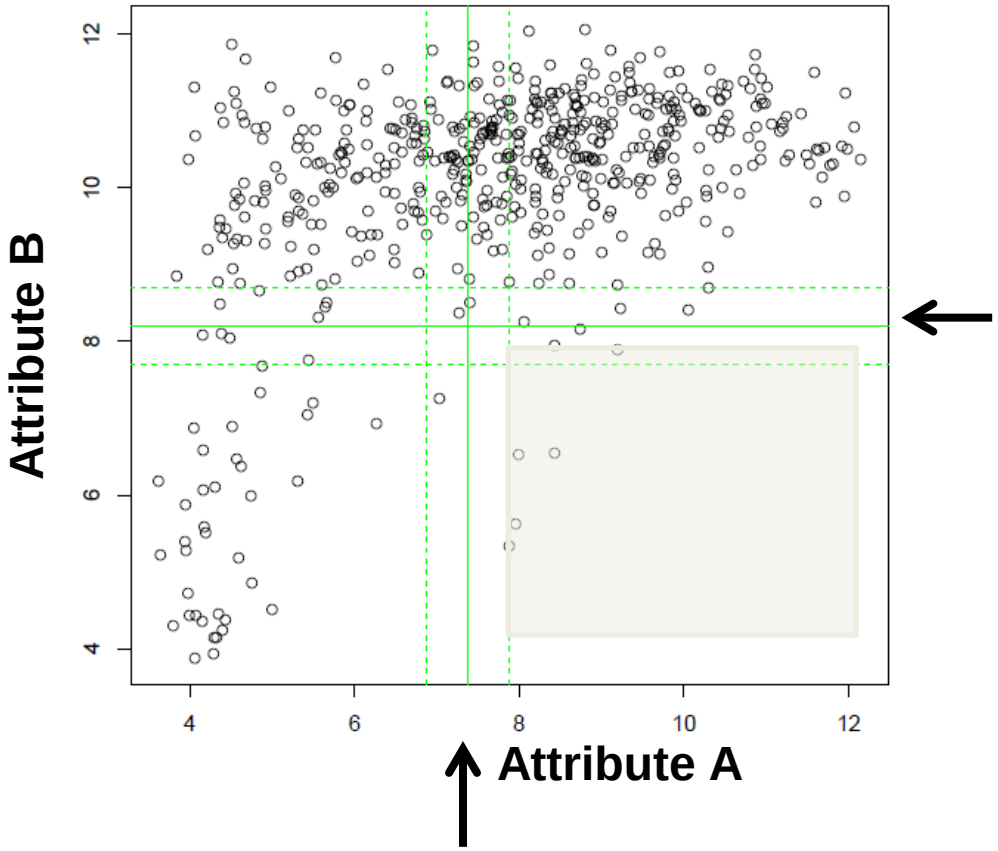


Methylation
Red=hyper
Green=hypo
Figueroa et al 2010



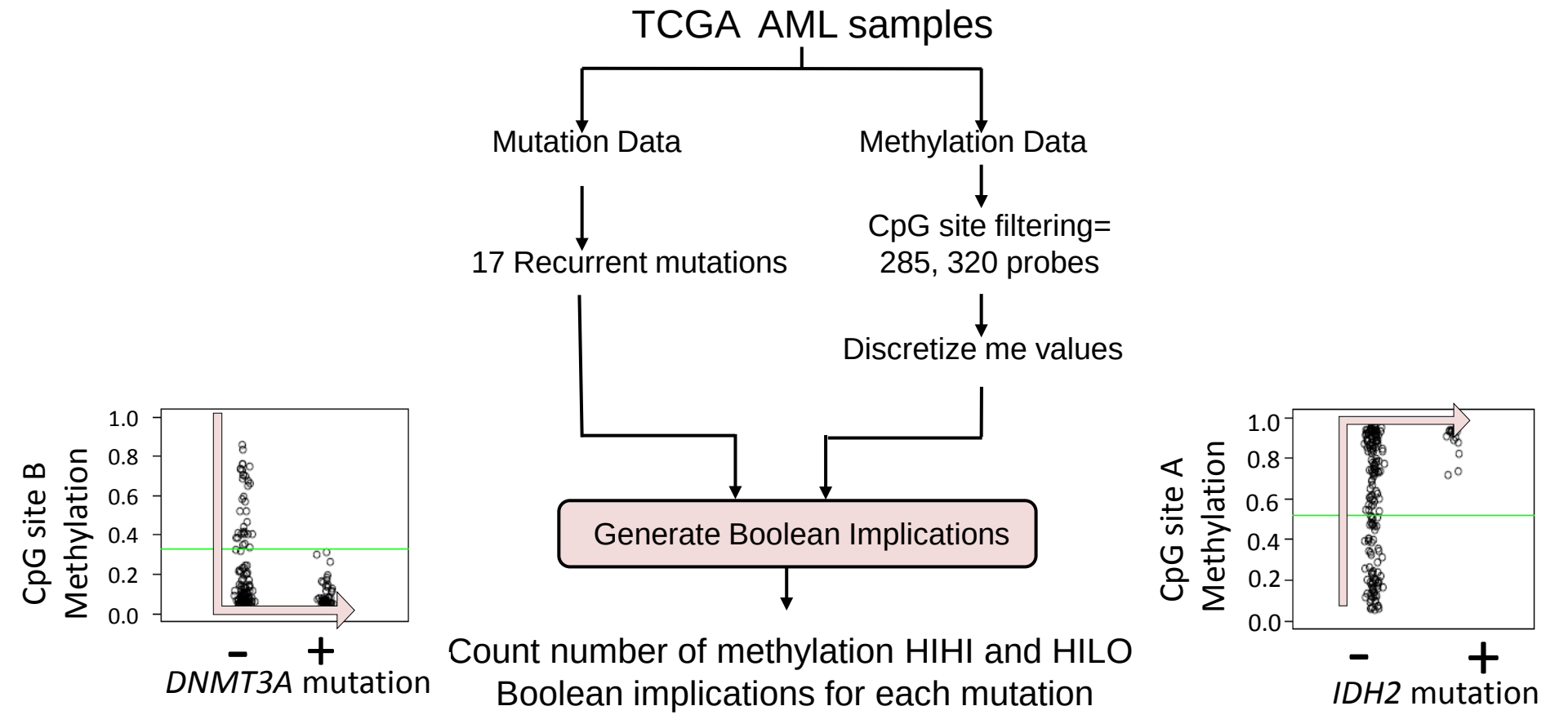
1. **Identify genetic drivers of aberrant methylation.**
2. **Find leads for a mutation-specific therapy.**

Boolean Implications (IF –THEN Rules)



- Four different implications:
  - HIHI: IF A high, THEN B high
  - HILO: IF A high, THEN B low
  - LOHI: IF A low, THEN B high
  - LOLO: if A low, THEN B low

Computational Pipeline



WT1 mutation AML is linked to hypermethylation

Hyper-		
IDH2	12950	36
WT-1	2028	13
CEBPA	7839	42

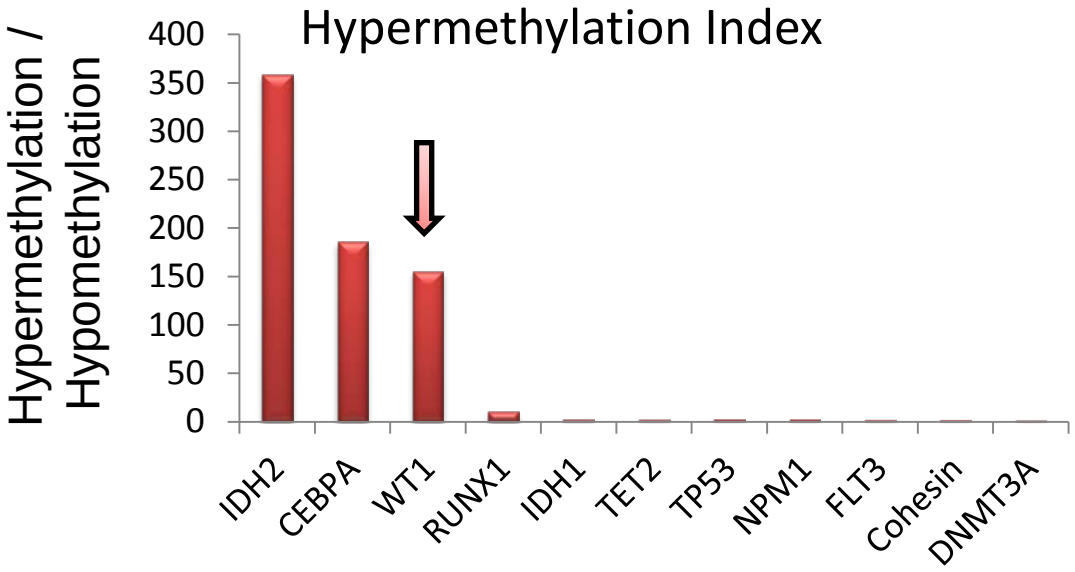
Hypo-		
DNMT3A	325	3469
Cohesin	185	852

Mixed		
RUNX1	4384	399
IDH1	4074	1345
TET2	1314	894
FLT3	614	1350
NPM1	2145	3683
TP53	4175	4870

Very few (<500)		
KIT	54	281
KRAS	9	23
MT-CO2	105	15
NRAS	182	53
PTPN11	107	8
U2AF1	60	108

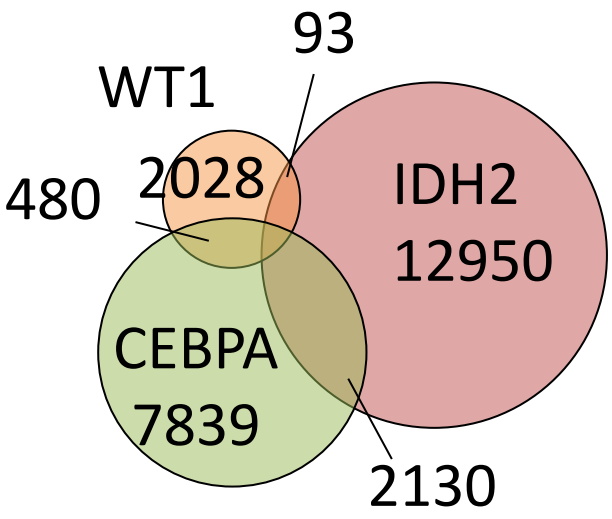
No. of hypermethylation implications

No. of hypomethylation implications

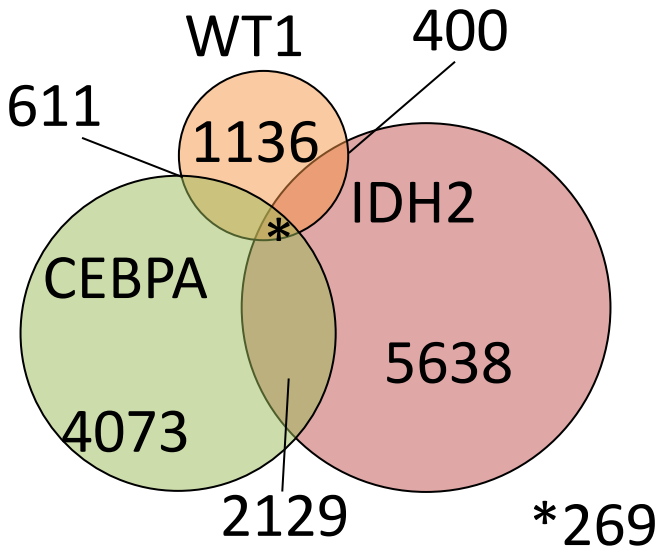


Distinct CpG sites and associated genes linked to hypermethylating mutations

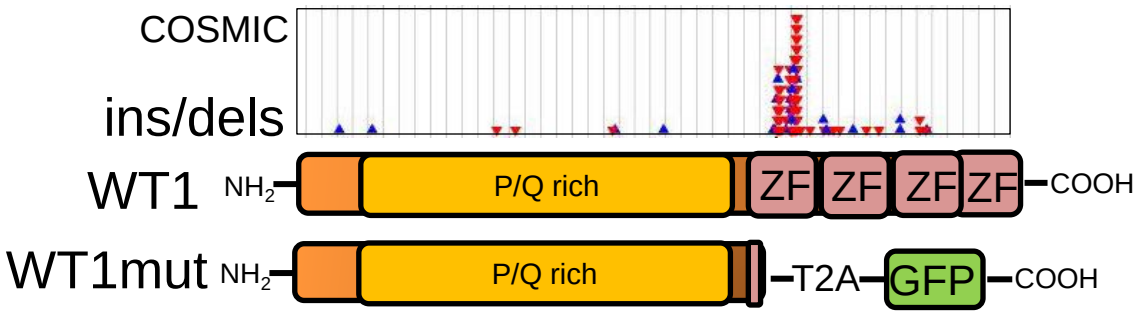
CpG sites



Methylated Genes



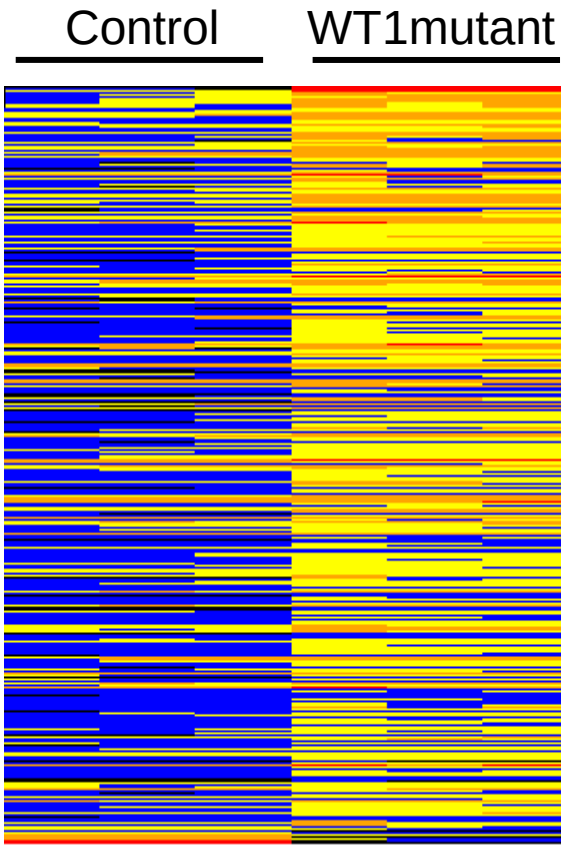
WT1 mutation induces hypermethylation in AML cells



transduced THP-1
stable cell-lines

10 passages

450K Beadchip array



CpG me
(β value)

- 0.0 to 0.2
- 0.2 to 0.4
- 0.4 to 0.6
- 0.6 to 0.8
- 0.8 to 1.0

Overlap with patients: 8.5E-34
Fisher's exact test

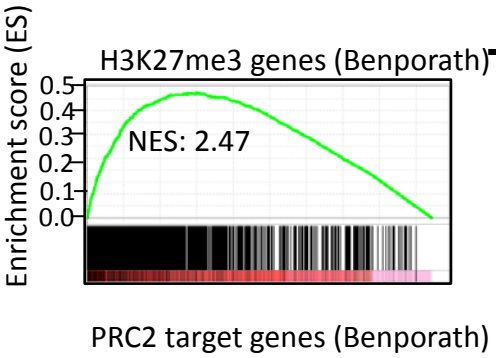
Mutant WT1 methylation signature is enriched for PRC2 target genes

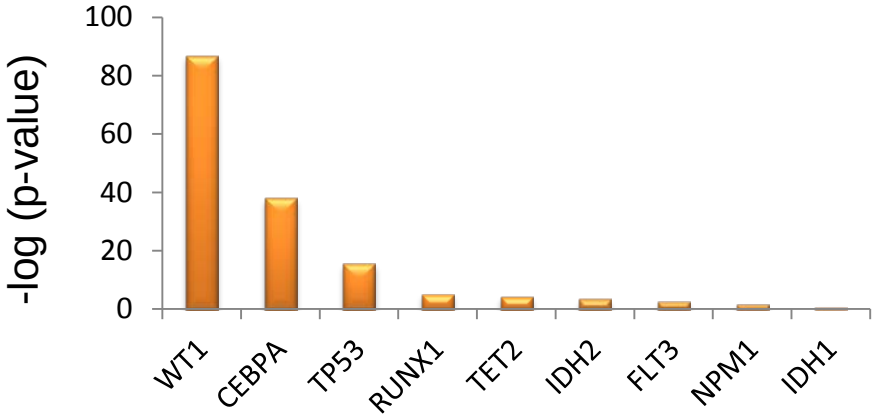
Patient samples with WT1 mut

Gene Sets	P-value
Benporath ES with H3K27ME3	1.6E-87
Benporath EED targets	2.88E-84
Benporath Suz12 targets	1.65E-81
Benporath PRC2 targets	8.13E-63
Mikkelsen MEF HCP with H3K27ME3	1.58E-51
Mikkelsen Brain HCP with H3K4ME3 and H3K27ME3	2.64E-41
Mikkelsen MCV6 HCP with H3K27ME3	2.73E-40
Meissner Brain HCP with H3K27ME3	9.42E-37
Mikkelsen NPC HCP with H3K27ME3	1.09E-27
Meissner NPC HCP with H3K4ME3 and H3K27ME3	8.03E-25

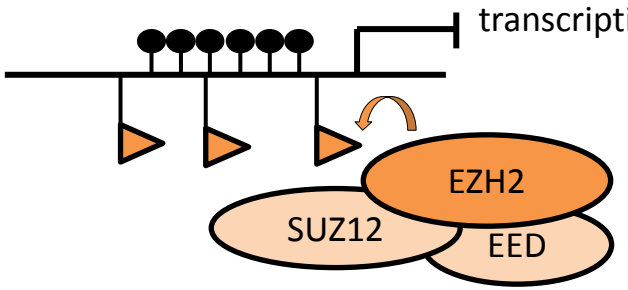
THP1 cell-line with WT1mut

Gene Sets	P-value
Benporath ES with H3K27ME3	9.06E-68
Mikkelsen MEF HCP with H3K27ME3	5.12E-56
Benporath EED targets	1.59E-46
Benporath SUZ12 targets	7.31E-46
Benporath PRC2 targets	2.24E-42
Mikkelsen Brain HCP with H3K4ME3 and H3K27ME3	1.84E-40
Mikkelsen NPC HCP with H3K27ME3	4.52E-37
Mikkelsen MCV6 HCP with H3K27ME3	8.61E-36
Meissner NPC HCP with H3K4ME3 and H3K27ME3	1.65E-30
Mikkelsen MEF HCP with H3K27ME3	9.65E-21





WT1 mutant AML shows aberrant repression of Polycomb repressor complex 2 targets



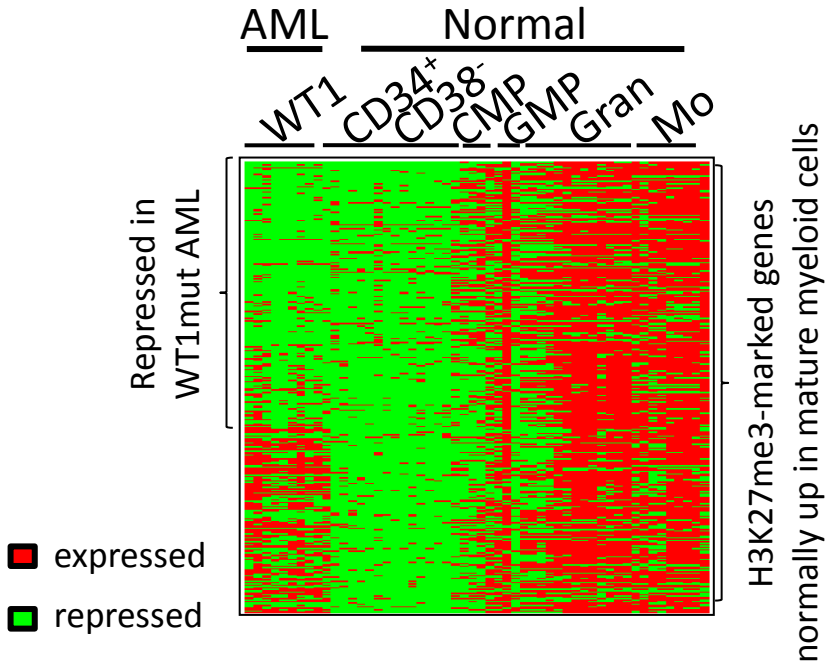
K562 cells

↓

H3K27me3 CHIP (ENCODE)

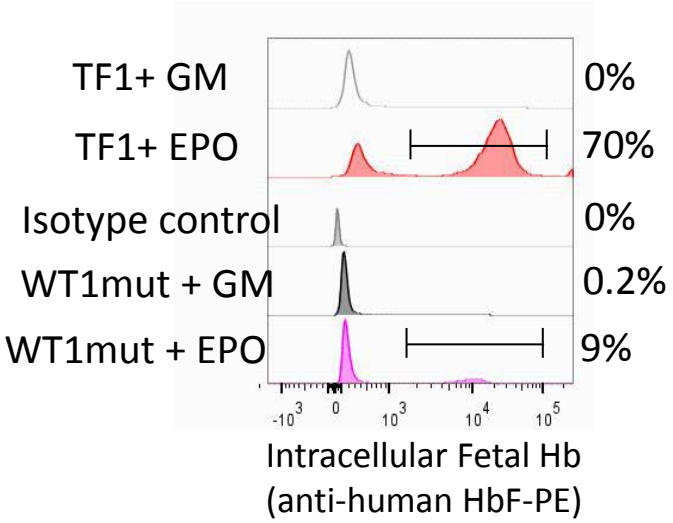
↓

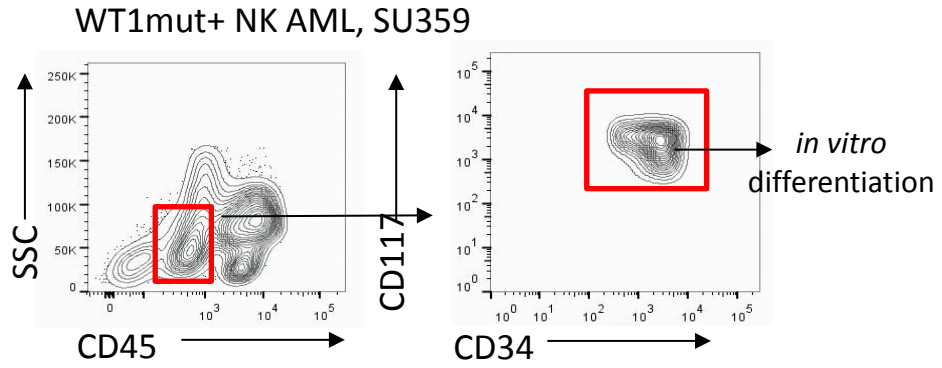
PRC2 marked genes in adult hematopoiesis

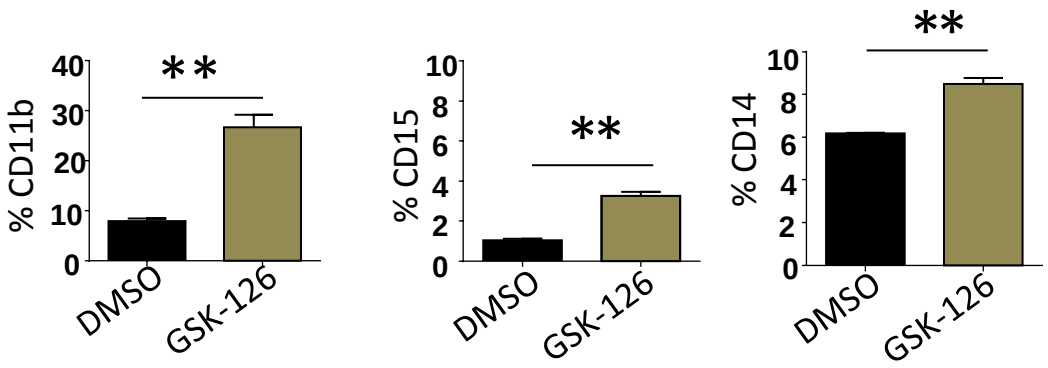


Does WT1 mutation block myeloid differentiation?

Inhibition of PRC2 promotes differentiation in AML with WT1 mutation



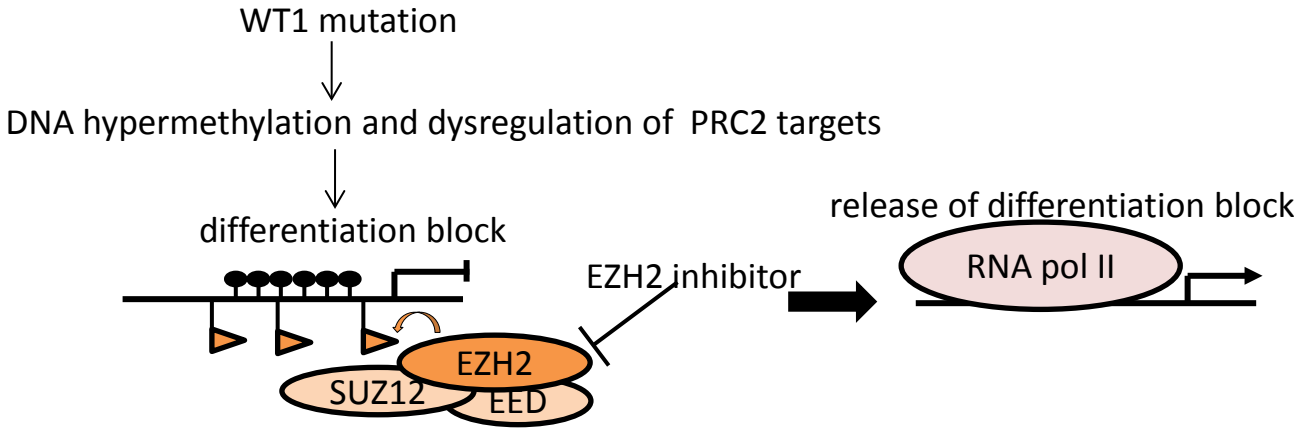




* p<0.01

Conclusions

- Mutation in WT1 is strongly linked to DNA hypermethylation in AML
- Introduction of mutant WT1 into wildtype cells induced the same pattern of DNA hypermethylation
- The pattern of methylation and gene expression is consistent with a differentiation block caused by WT1mut through dysregulated silencing of PRC2 targets
- Differentiation block in WT1mut AML can be overcome by EZH2 inhibition
- **EZH2 inhibitors have activity in WT1mut AML**
- **Boolean implications are a useful data mining tool for large, heterogeneous cancer data sets**



Acknowledgements

The Cancer Genome Atlas

Understanding genomics to improve cancer care

Dan Thomas

David Dill

Stanford Centre for Cancer Systems Biology (CCB, NCI)
Progenitor Cell Biology Consortium (PCBC, NHLBI)

Ravindra Majeti

NHMRC CJ Martin Fellowship
Hem/Oncology HSANZ Targetted Therapy Fellowship

Sylvia Plevritis

Andrew Gentles

Andrew Feinberg

Namyoung Jung