The role of embryonic stem (ES) cell factor SALL4 in MDS/AML

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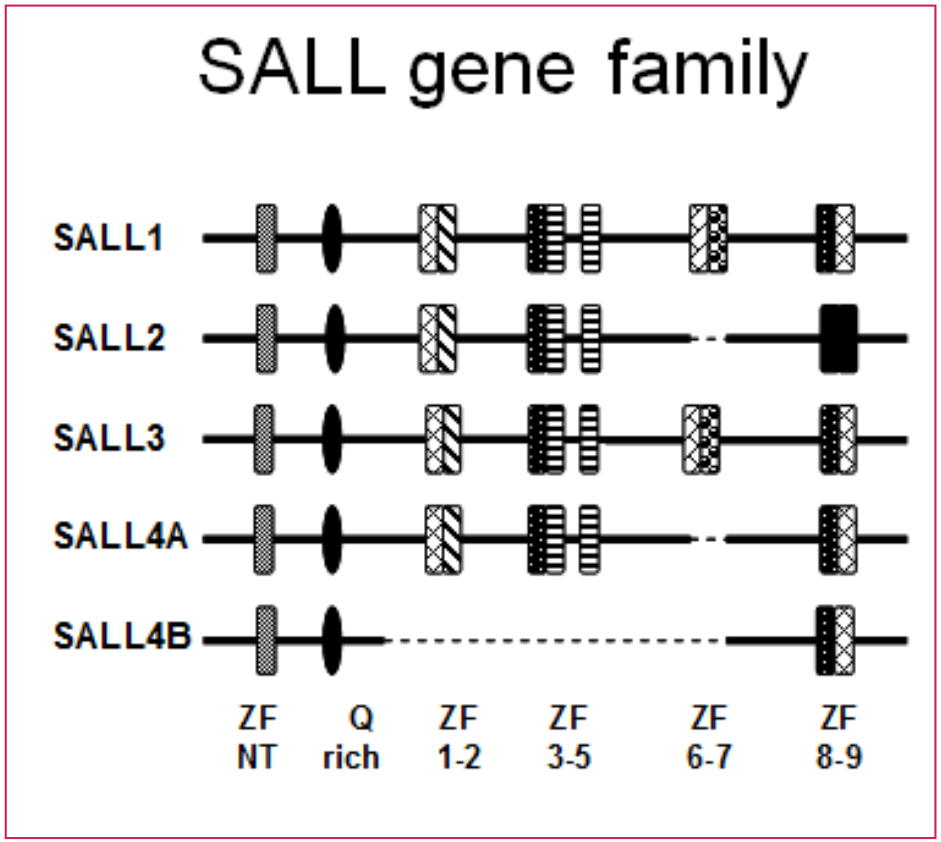
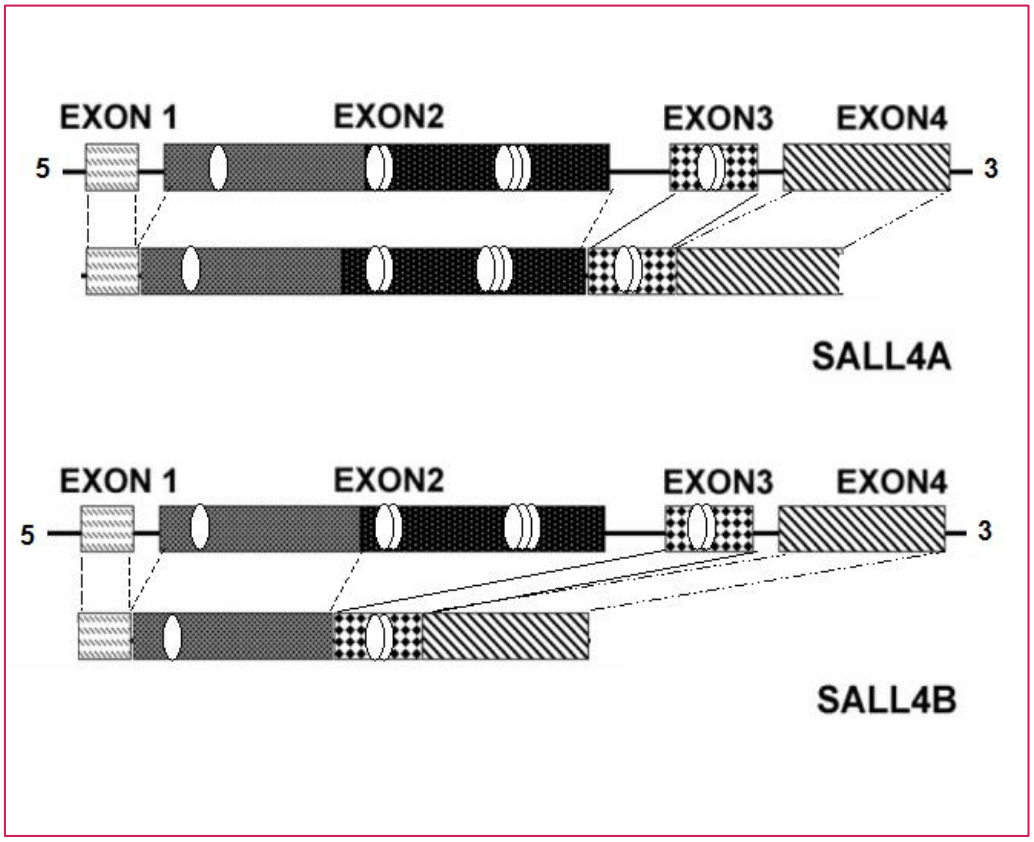
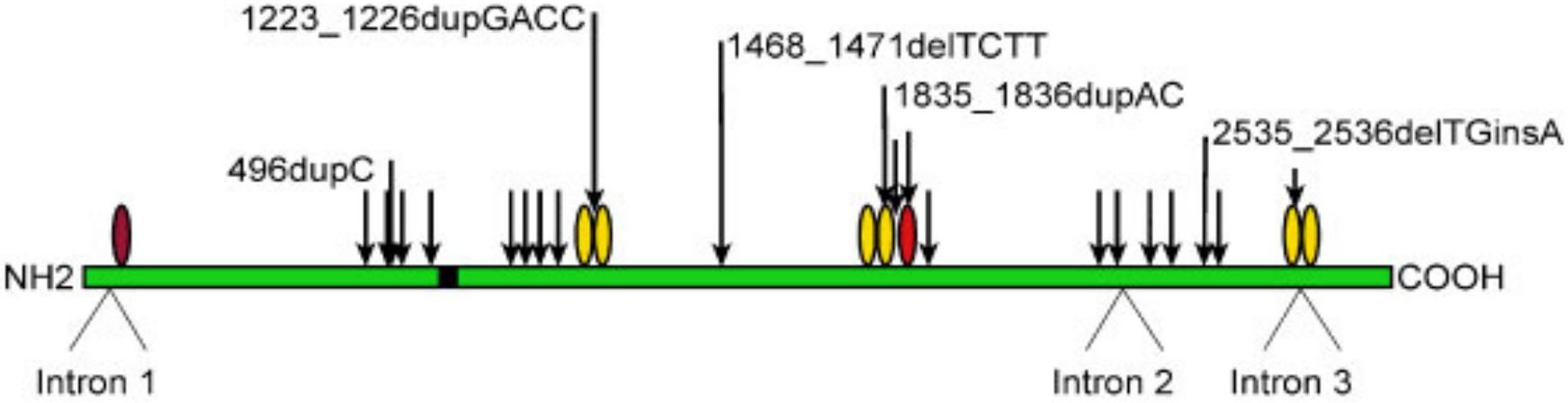
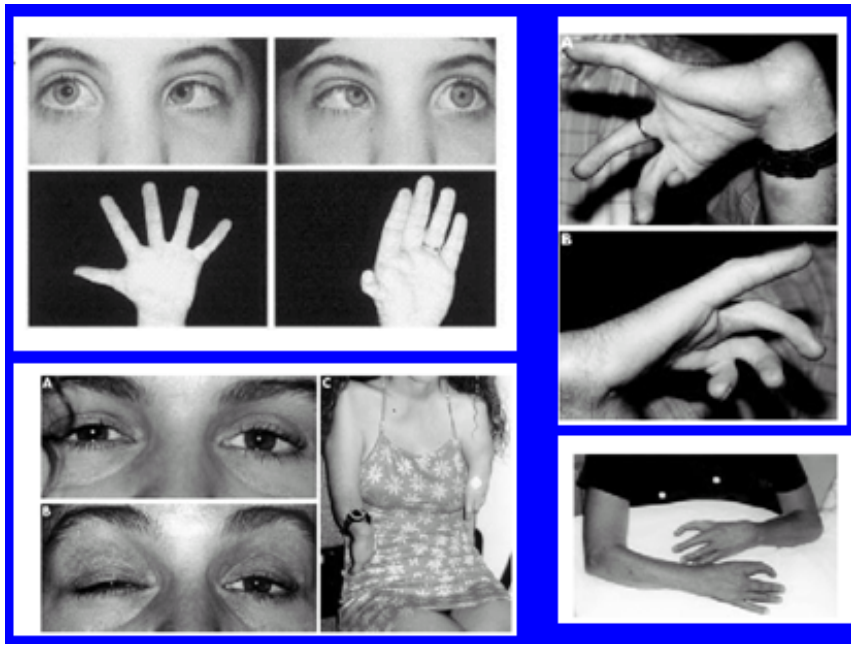
SALL4 has two isoforms



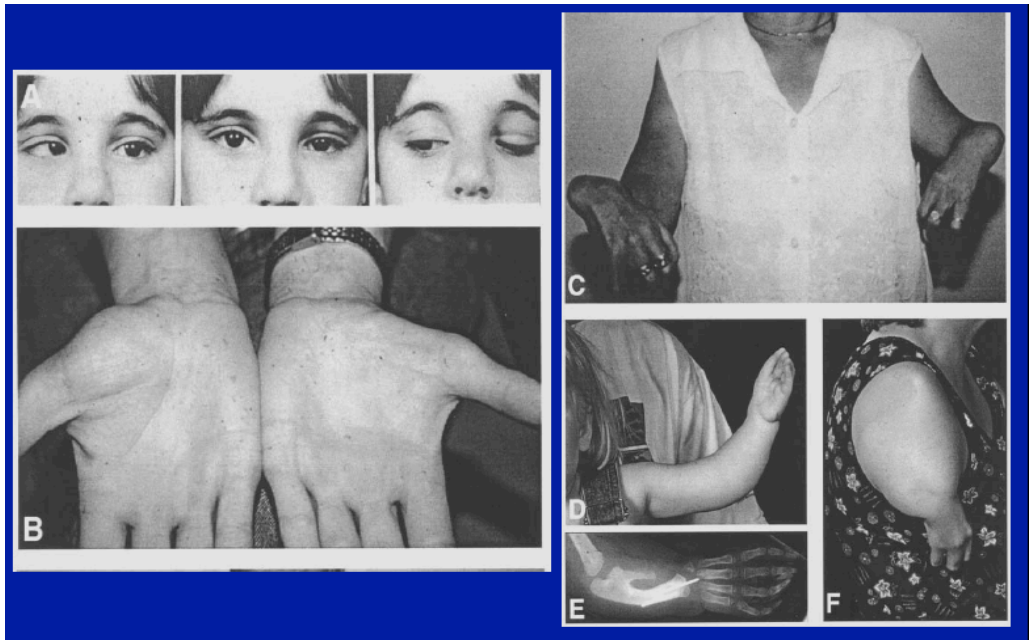


SALL4 and Duane Radial Ray Syndrome (Okihiro Syndrome)



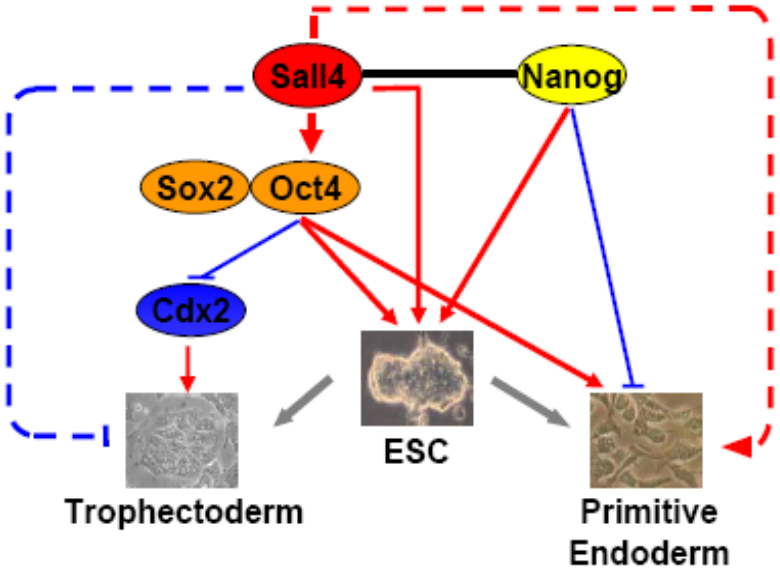


Al-Baradie et al . AJHG 2002, 71, 5, 1195-1199



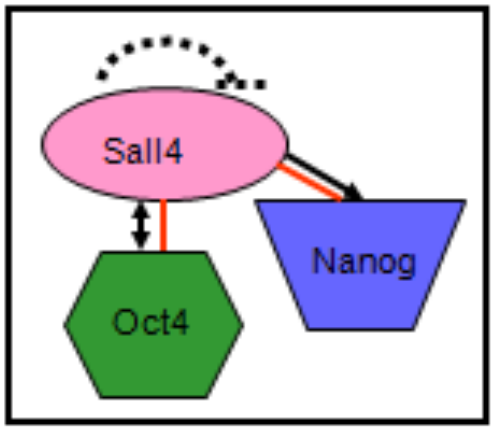
Jürgen Kohlhase et al. Hum. Mol. Genet. 2002;11:2979-2987

SALL4 in embryonic stem cells



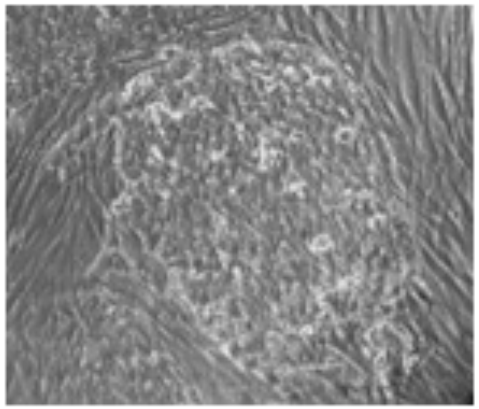
Zhang, et al, Nat Cell Biol. 2006

Sall4/Oct4/Nanog complex



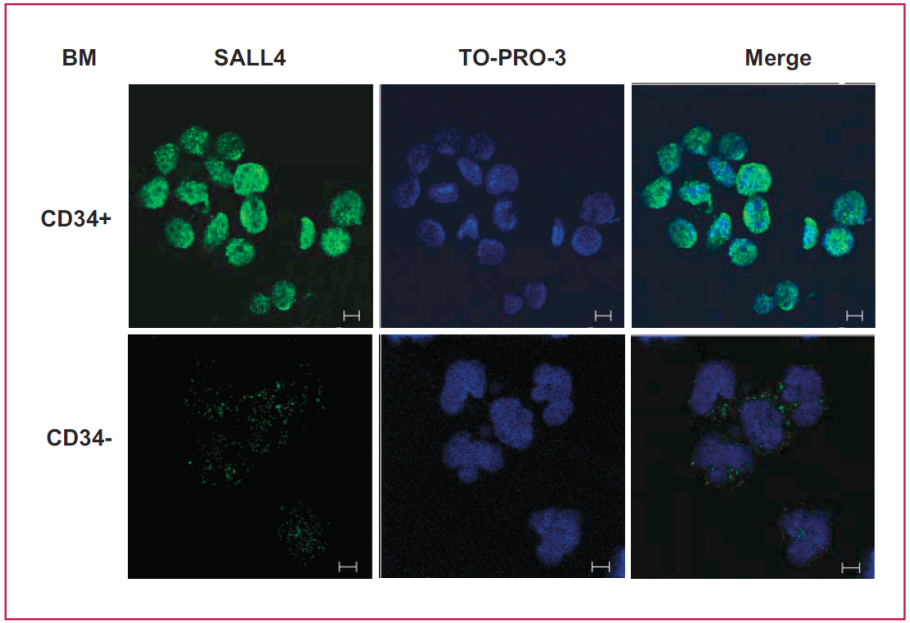
Maintain human or murine ESC properties

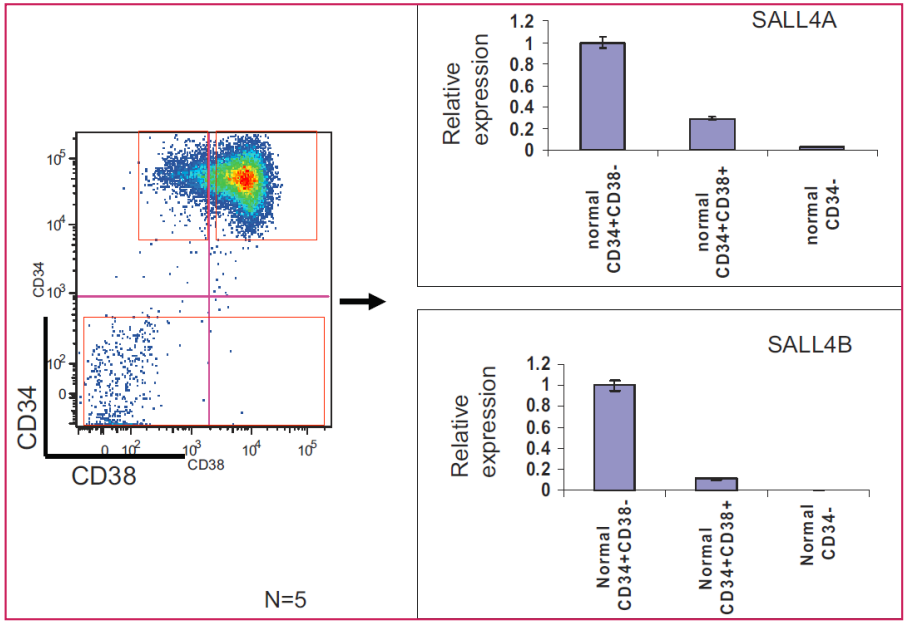
ESC



Yang, et al, Plos One ,2008

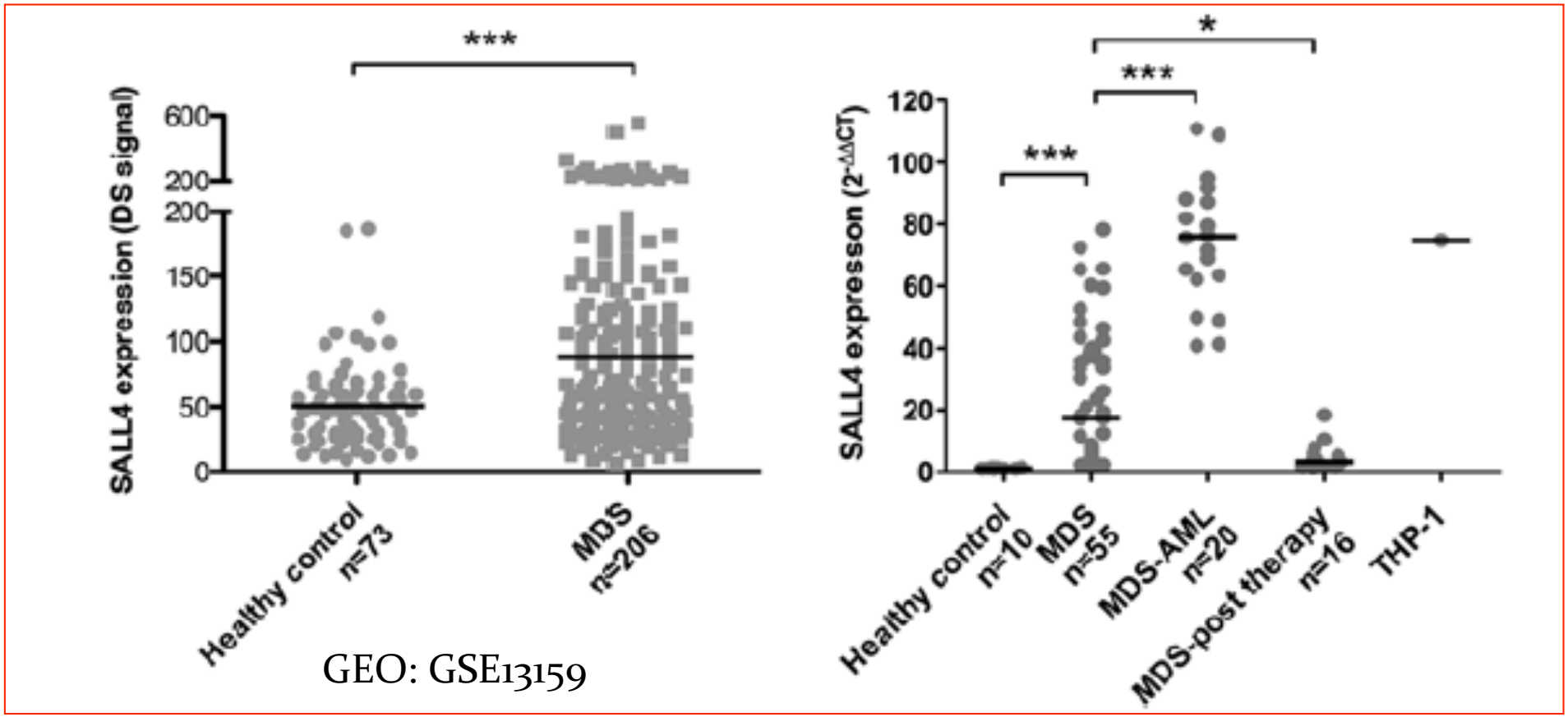
SALL4 expression in normal human hematopoietic cells





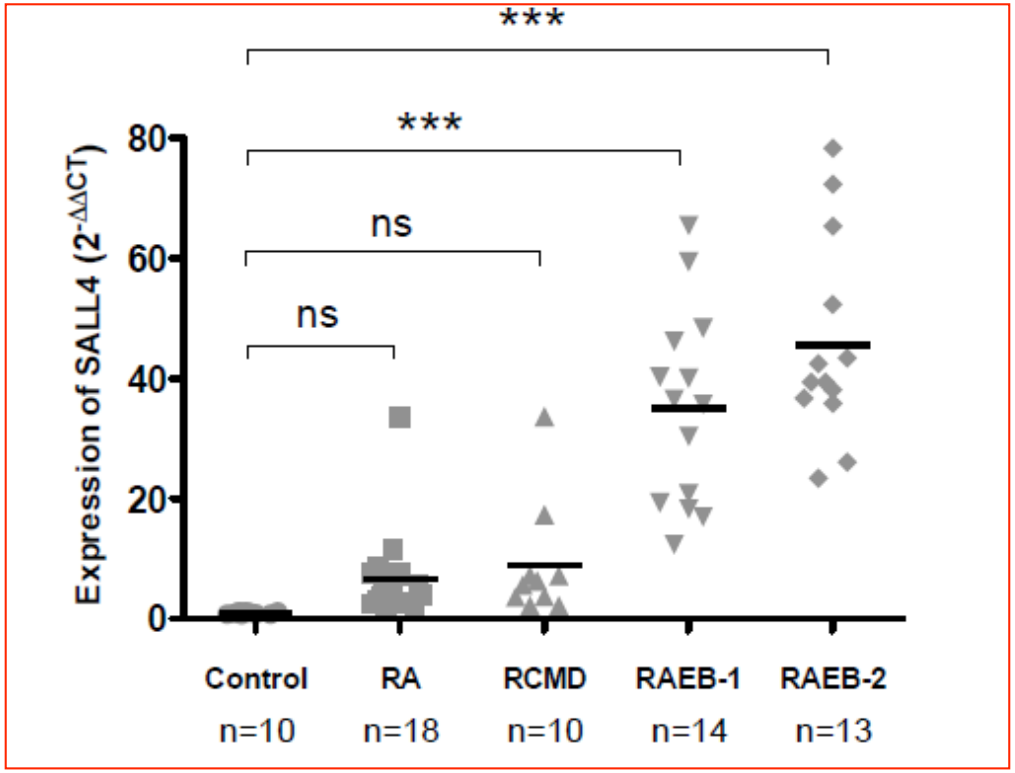
Gao, et al, Transfusion. 2012

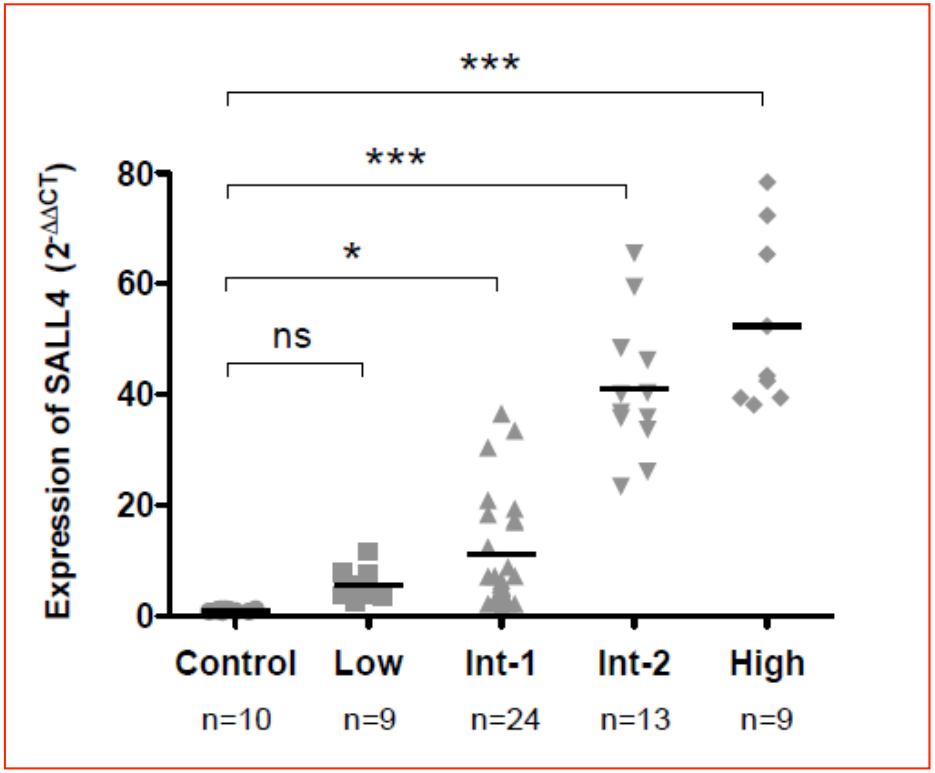
SALL4 expression in MDS



Wang, et al, 2013, Journal of Hematology & Oncology

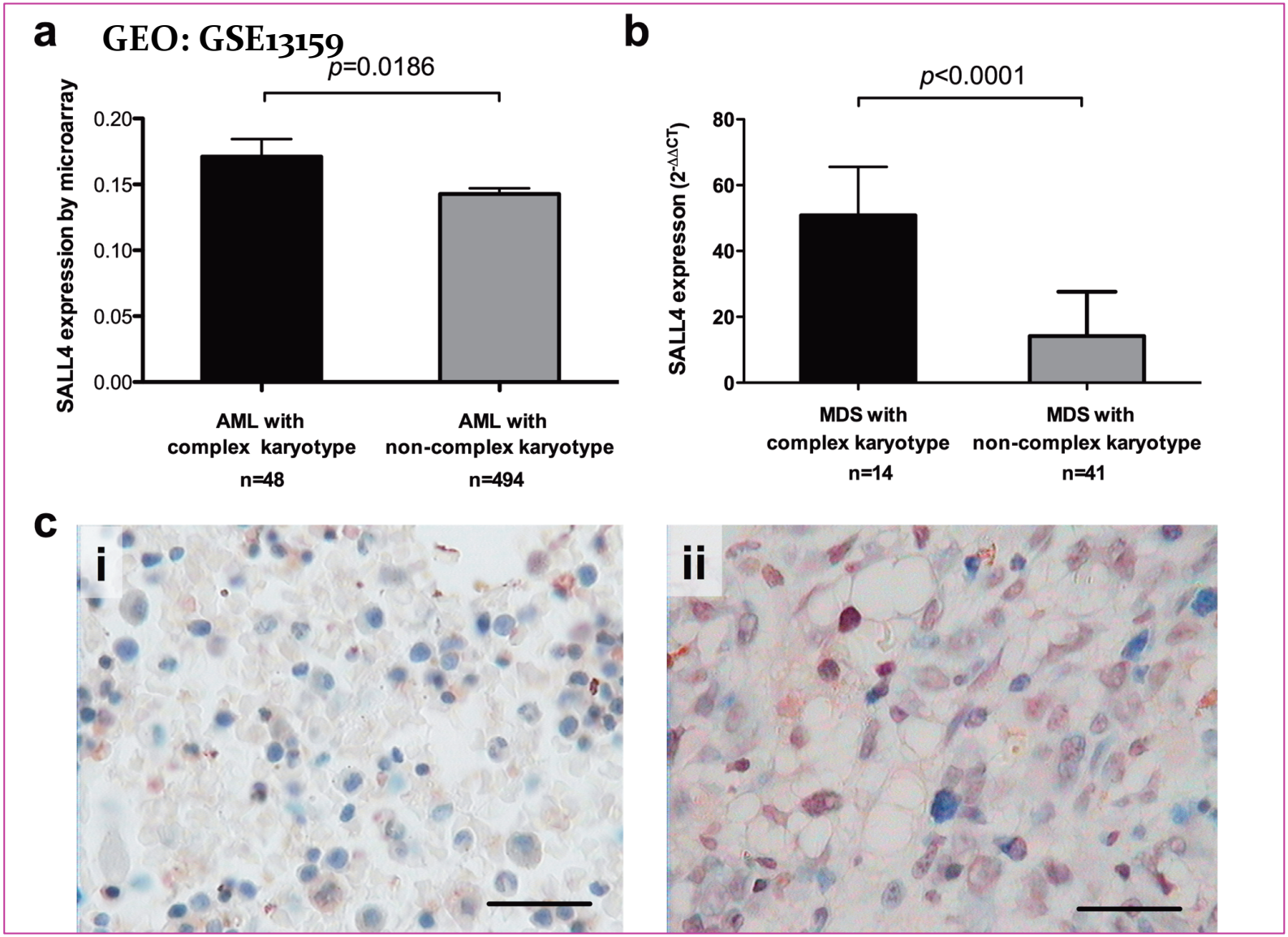
SALL4 expression in MDS sub-types





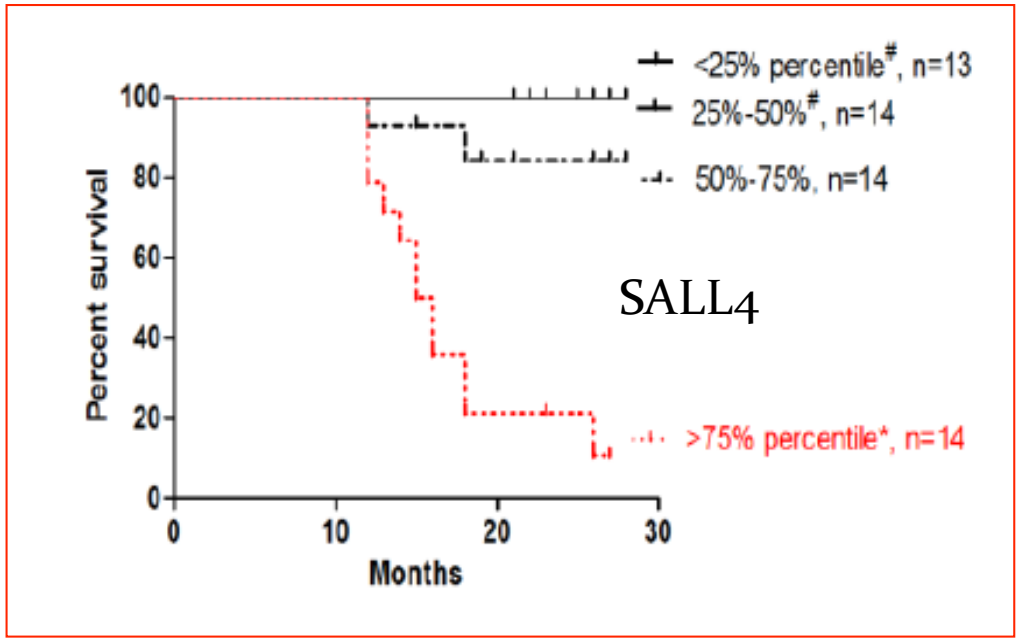
Wang, et al, 2013, Journal of Hematology & Oncology

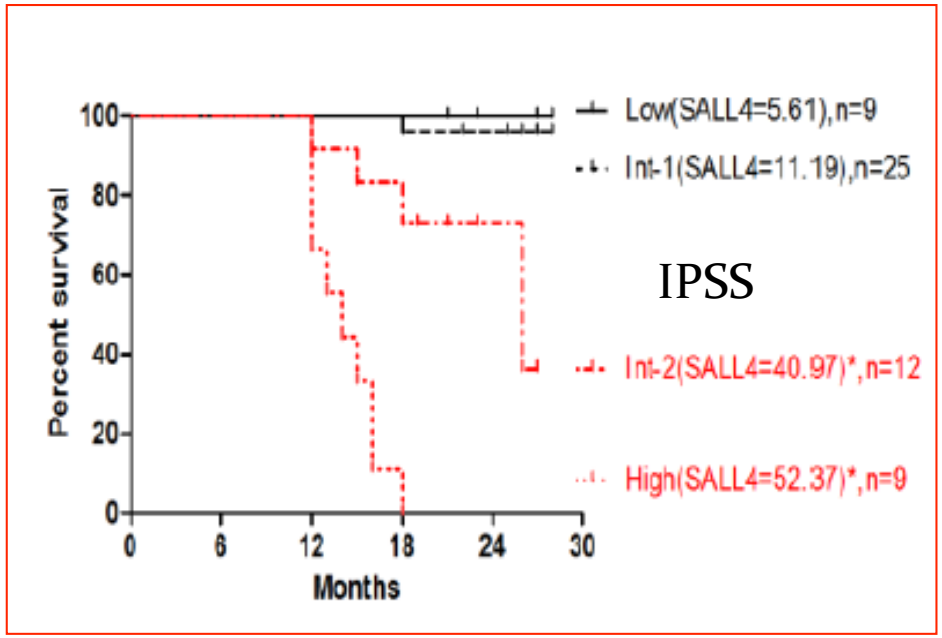
SALL4 expression in MDS sub-types

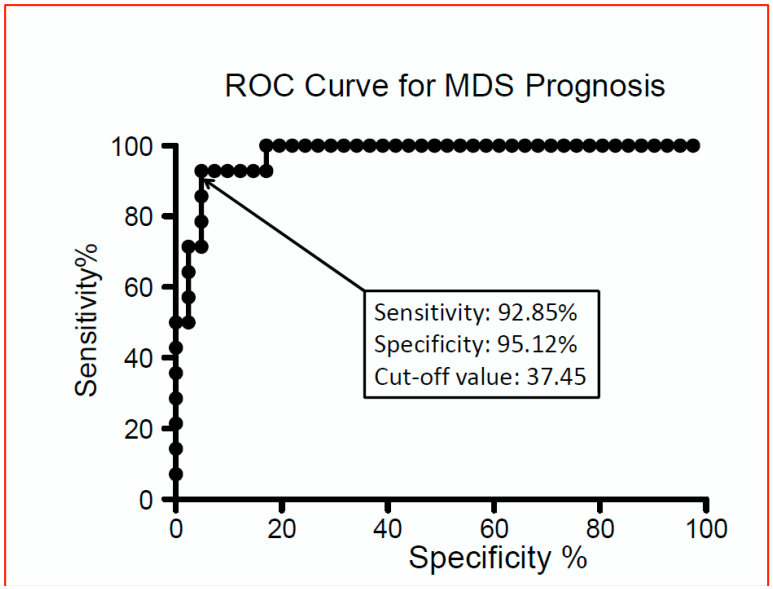


Wang, et al, Oncogene, 2016

SALL4: a potential prognostic biomarker for MDS







Wang, et al, 2013,

Journal of Hematology & Oncology

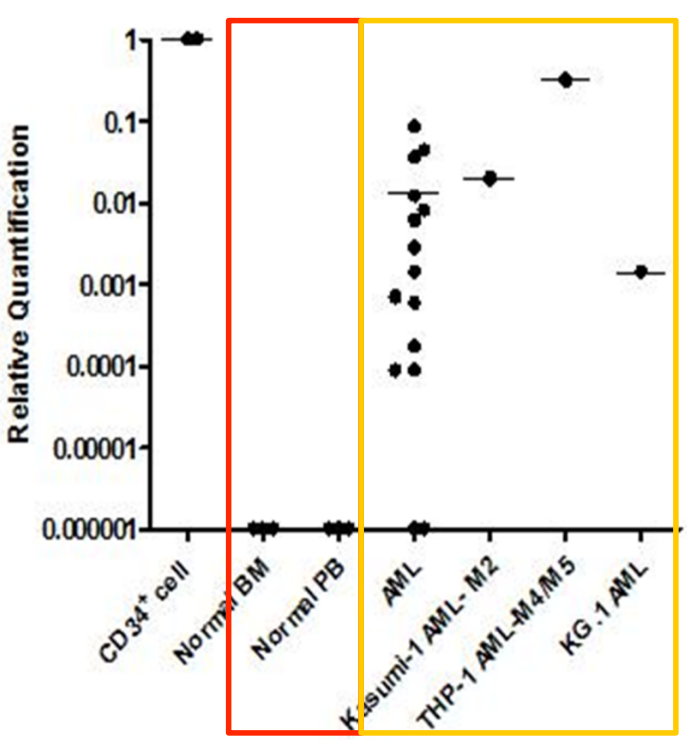
SALL4 expression in MDS

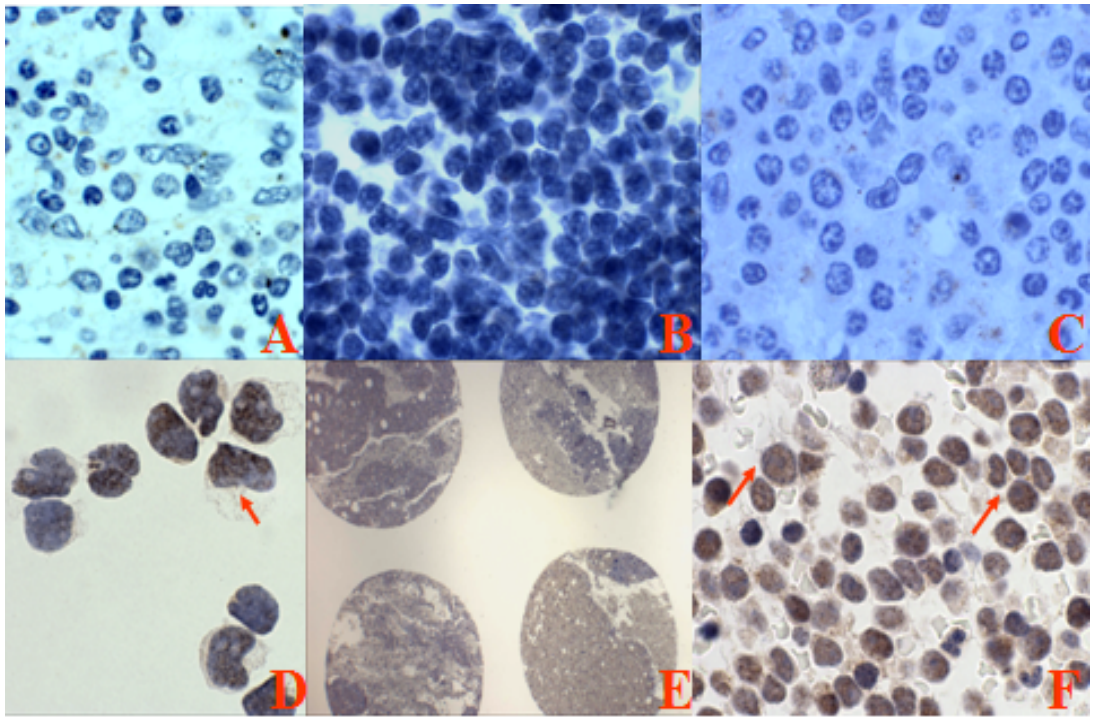
Table 1. Characteristics of newly diagnosed MDS patients

Case	WHO	Sex	Age(y)	Blasts(%)	Karyotype	IPSS	Risk	Outcome	Follow-up(mo)	Bmi-14$^{(2-\Delta\Delta CT)}$	SALL4$^{(2-\Delta\Delta CT)}$
1	RA	F	66	0.5	Normal	0	Low	Alive	28	7.75	3.99
2	RA	F	36	1.5	47,XX,+8	0.5	Int-1	Alive	27	2.28	2.33
3	RA	F	29	0.5	Normal	0	Low	Alive	27	3.43	3.65
4	RA	M	45	2	Normal	0	Low	Alive	27	6.31	2.54
5	RA	F	60	3.5	46,XX,20q-	0.5	Int-1	Alive	27	8.15	5.71
6	RA	F	30	2	Normal	0.5	Int-1	Alive	27	10.93	2.65
7	RA	F	56	0.5	Normal	0.5	Int-1	Alive	27	7.86	7.16
8	RA	F	72	1	Normal	0.5	Int-1	Dead	18	8.59	33.5
9	RA	M	25	1.5	Normal	0.5	Int-1	Alive	26	11.18	5.13
34	RAEB-1	F	73	6.5	Normal	1	Int-1	Alive	26	15.56	20.92
35	RAEB-1	M	73	5.5	Complex	1.5	Int-2	Dead	26	24.59	59.44
36	RAEB-1	F	70	7.5	Normal	1	Int-1	Alive	26	7.36	30.43
37	RAEB-1	F	59	8.5	Normal	1	Int-1	Alive	26	14.19	19.36
38	RAEB-1	M	69	5	45,XY,-7	2	Int-2	Alive	23	29.11	40.13
39	RAEB-1	M	79	7.5	Normal	1	Int-1	Alive	22	7.38	17.03
40	RAEB-1	F	63	5	46,XX,r(2),del(11q23)	1.5	Int-2	Dead	18	32.86	48.44
41	RAEB-1	M	78	5.5	Complex	2	Int-2	Alive	18	23.81	35.78
42	RAEB-1	F	81	8.5	Complex	2	Int-2	Dead	15	30.35	65.5
43	RAEB-2	F	54	11.5	Normal	2	Int-2	Alive	19	13.18	26.11
44	RAEB-2	F	65	13	Normal	2	Int-2	Alive	21	20.39	36.76
45	RAEB-2	M	45	15.5	Normal	2	Int-2	Alive	18	19.08	35.92
46	RAEB-2	F	77	11	46,XX,5q-	2	Int-2	Alive	15	57.28	23.43
47	RAEB-2	M	78	10.5	Complex	3	High	Dead	14	40.08	72.6
48	RAEB-2	F	69	12	Complex	3	High	Dead	12	29.12	39.4
49	RAEB-2	M	79	18	Complex	3	High	Dead	16	11.99	43.41
50	RAEB-2	M	82	13.5	Complex	3	High	Dead	18	35.87	39.43
51	RAEB-2	M	69	15.5	Complex	3	High	Dead	12	10.34	38.14
52	RAEB-2	M	25	15	Complex	3	High	Dead	15	46.71	52.38
53	RAEB-2	F	64	19.5	Complex	3	High	Dead	12	53.26	78.39
54	RAEB-2	F	60	17	Complex	3	High	Dead	13	27.96	42.51
55	RAEB-2	M	28	18.5	Complex	3	High	Dead	16	48.64	65.36

Wang, et al, 2013, Journal of Hematology & Oncology

SALL4 in AML

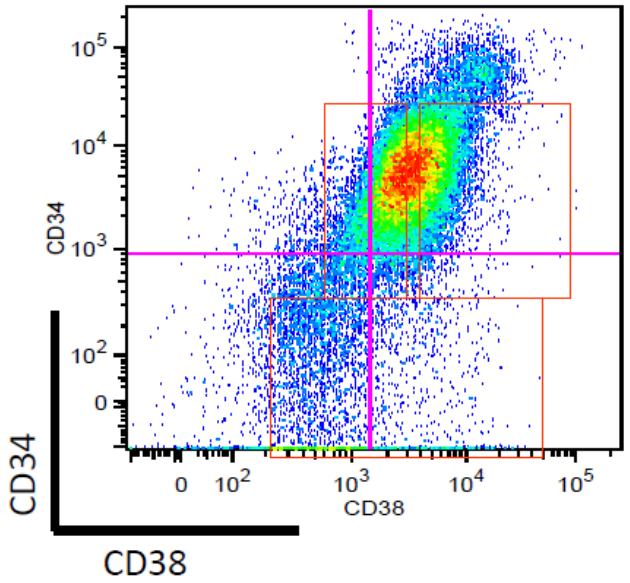


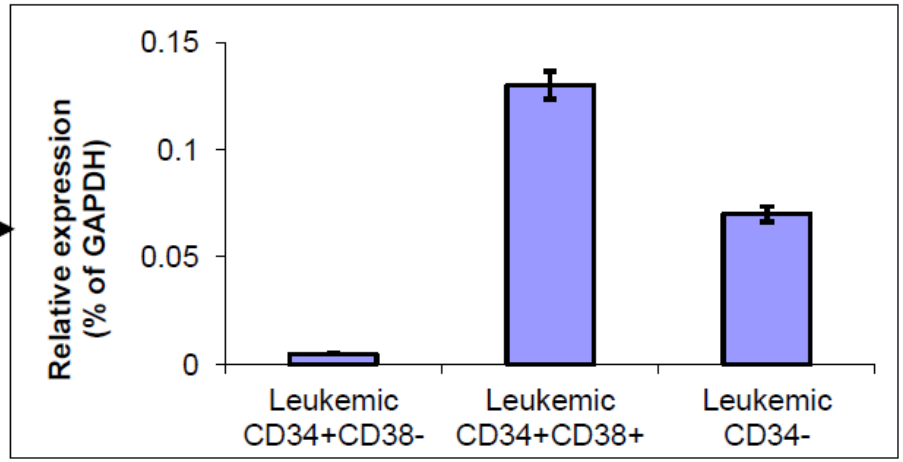


Ma, et al Blood. 2006

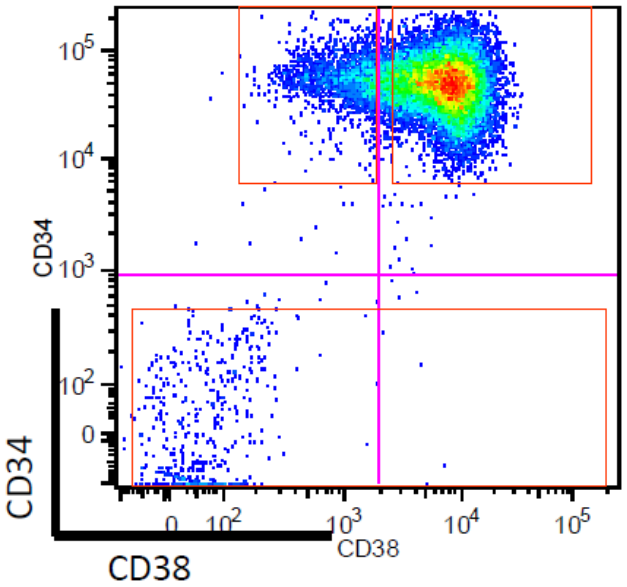
Aberrant SALL4 expression in leukemia

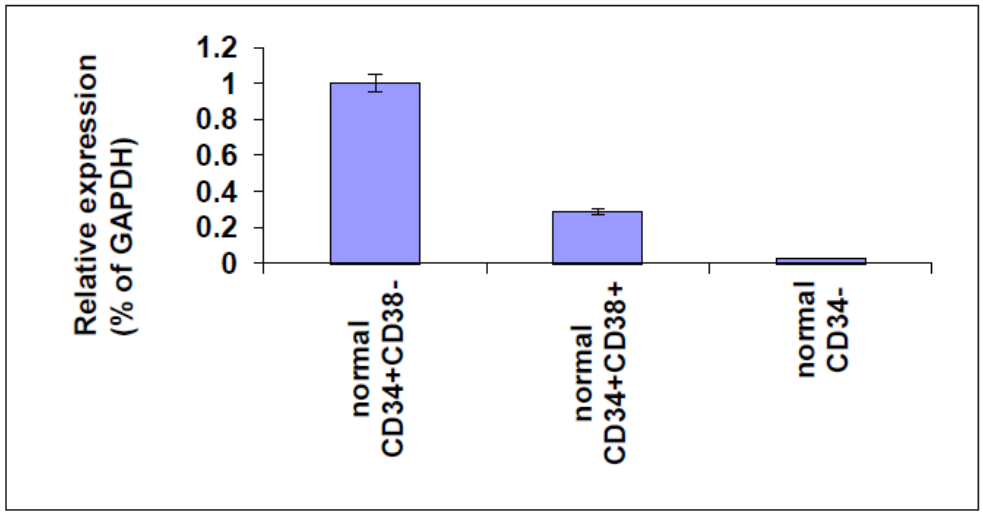
Leukemic CD34+ cells



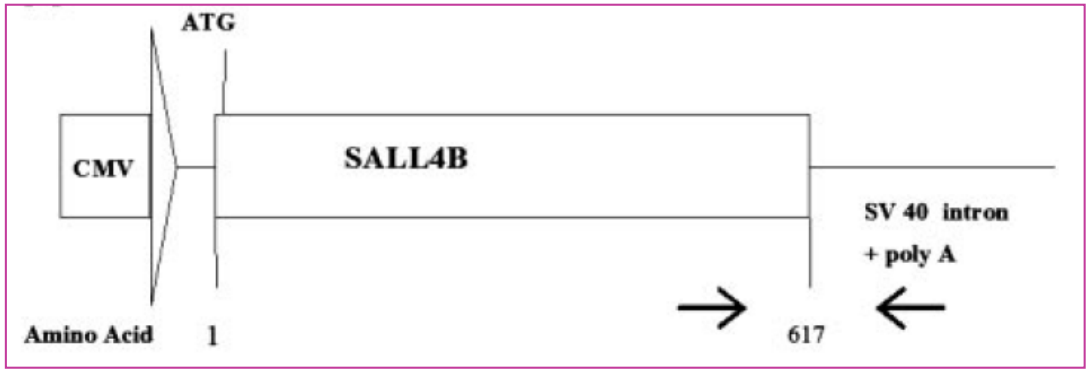


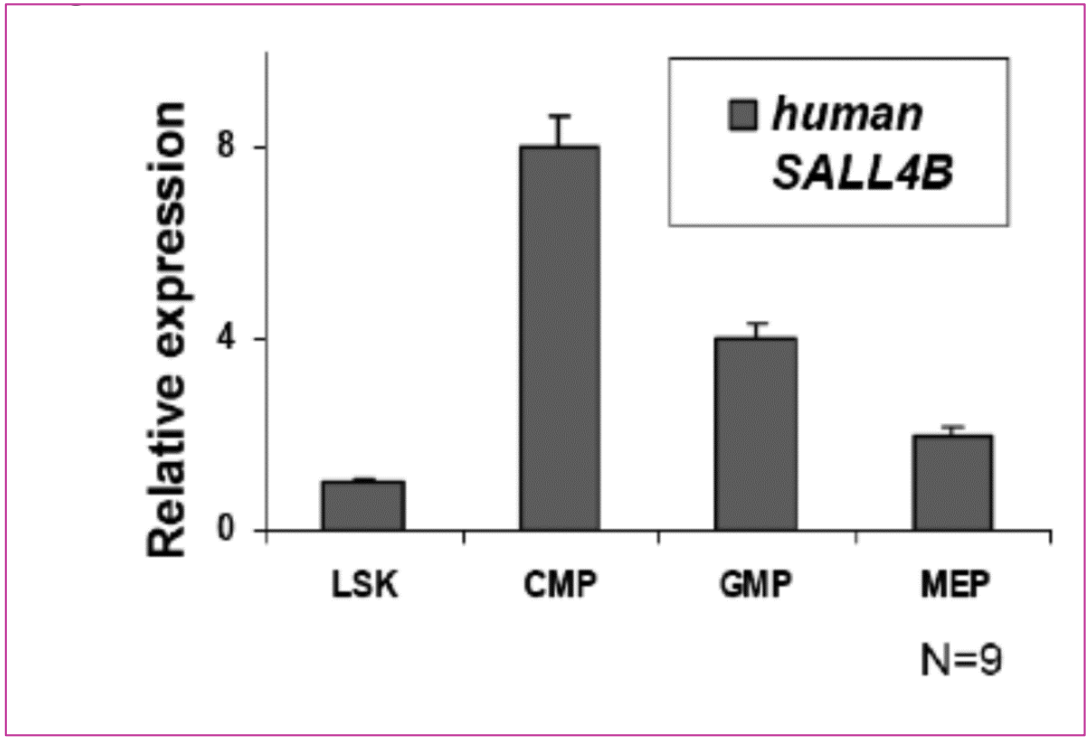
Normal CD34+ cells

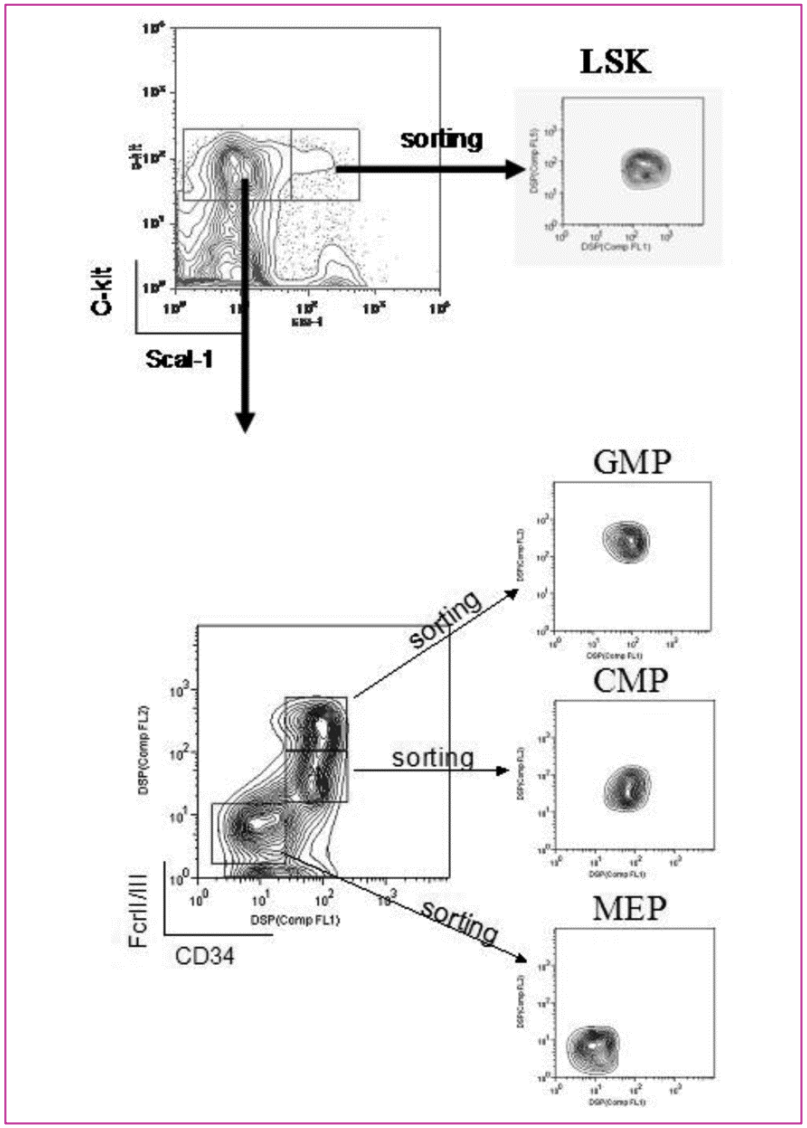




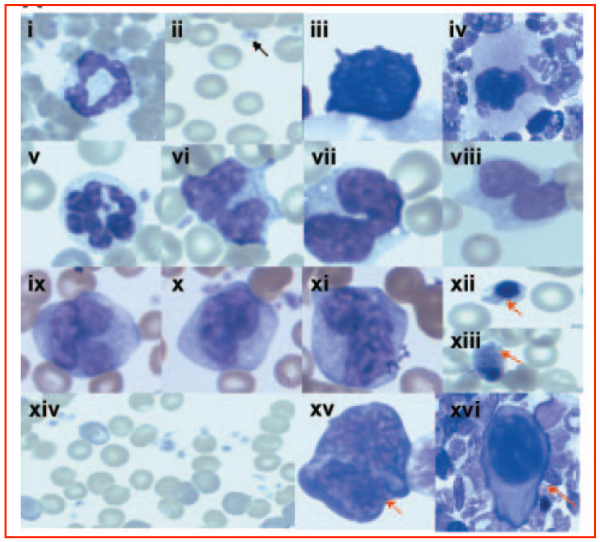
Generation of SALL4B Tg mice

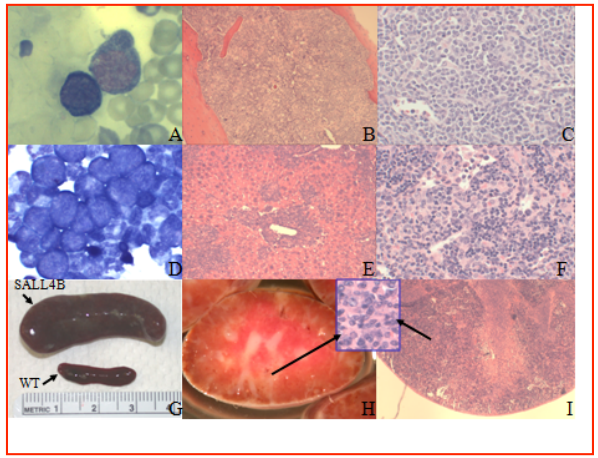




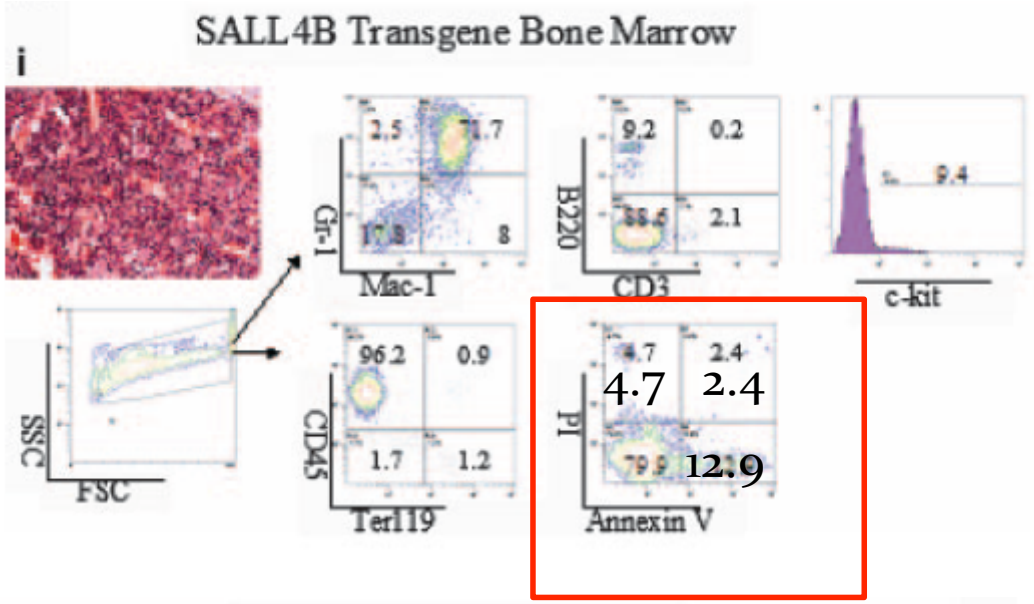


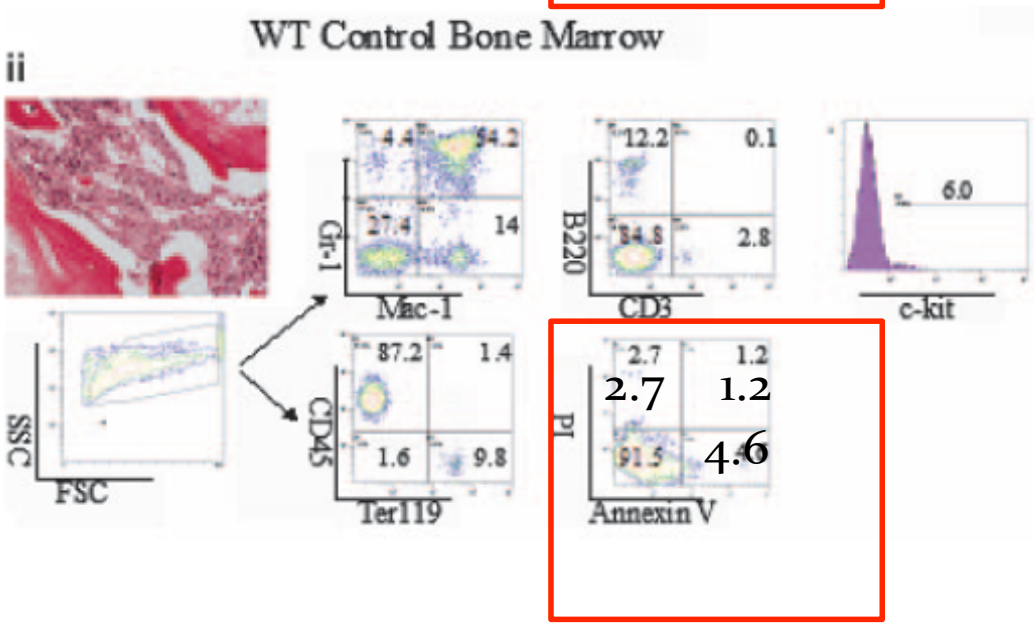
SALL4 Tg mice develop MDS/AML



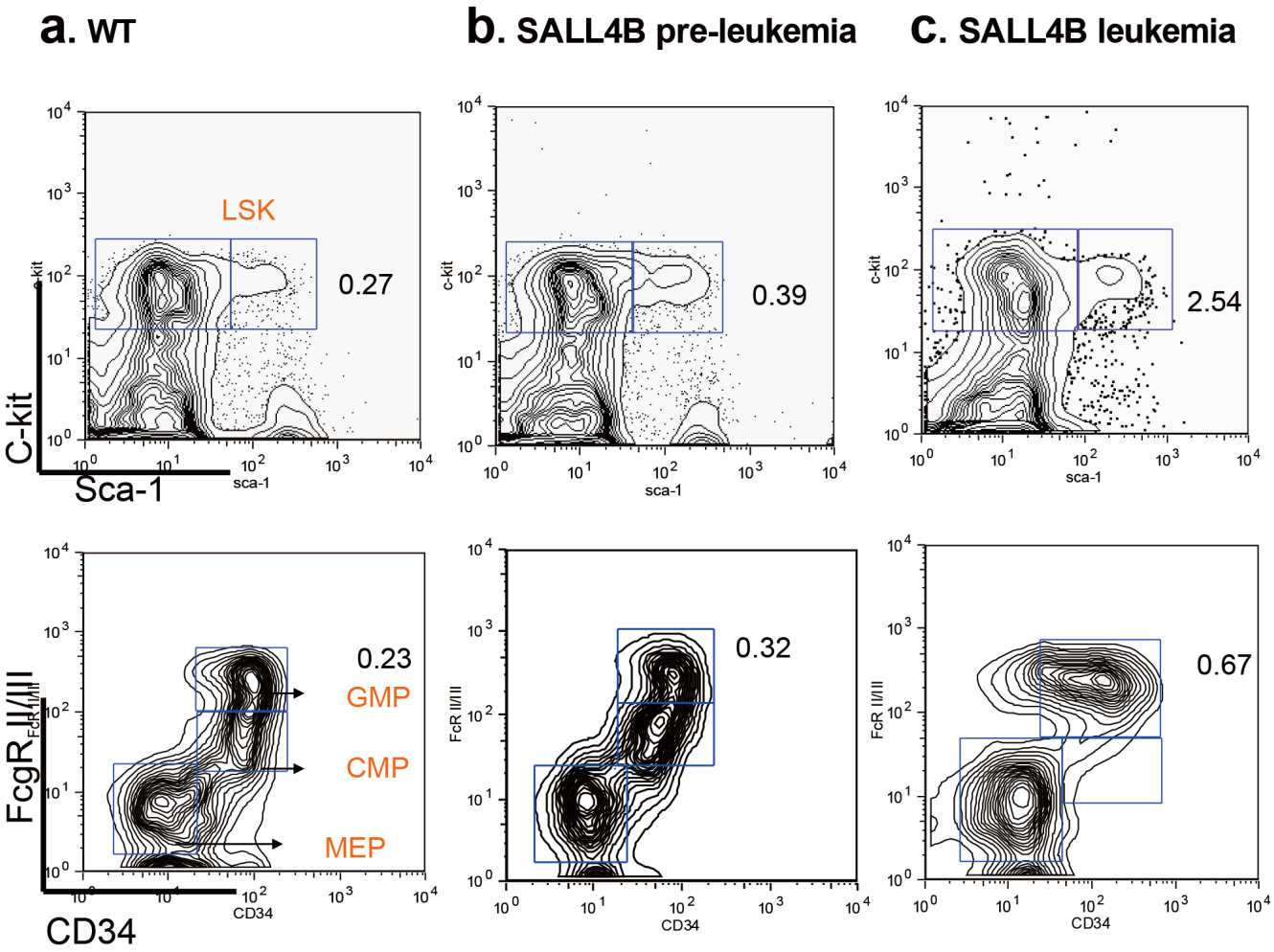


Ma, et al, 2006, Blood



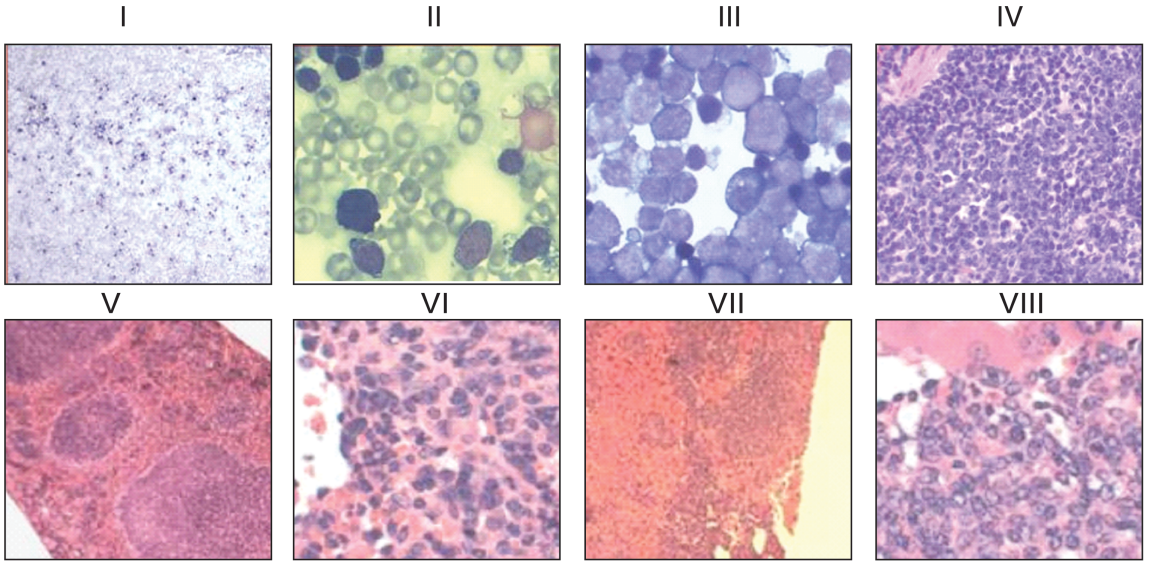


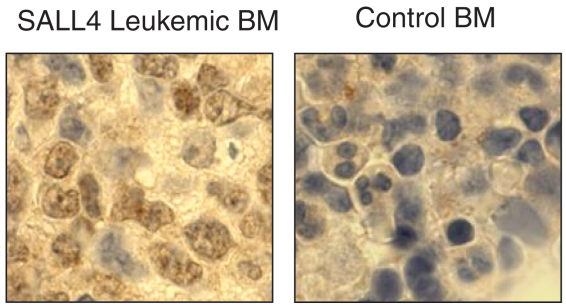
Monitoring of SALL4 Tg MDS/AML progression



Wang, et al, Oncogene, 2016

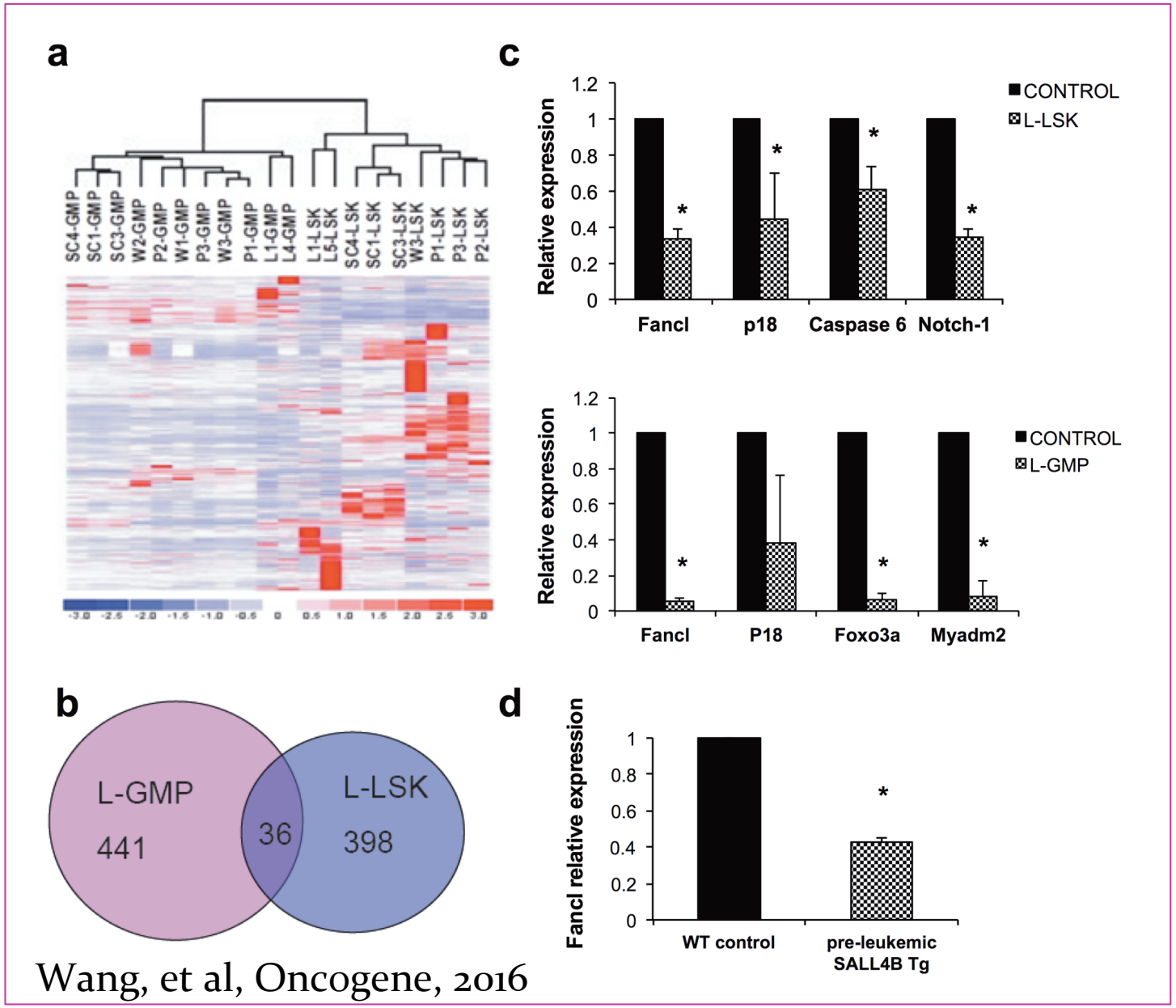
Serial leukemic transplants





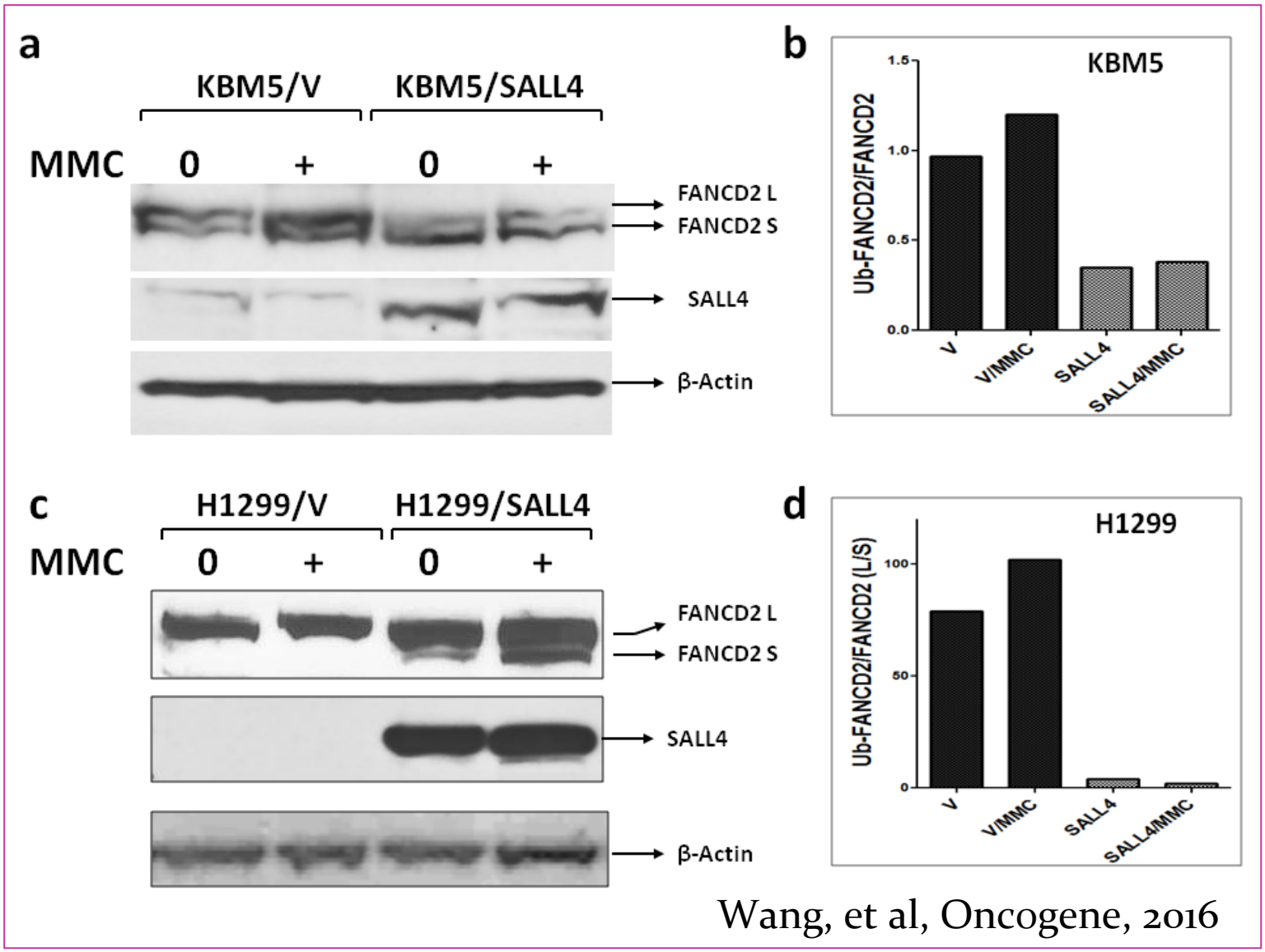
Li, et al, 2013 JCI

Fancl was down-regulated in SALL4B Tg leukemic and pre-leukemic bone marrow cells



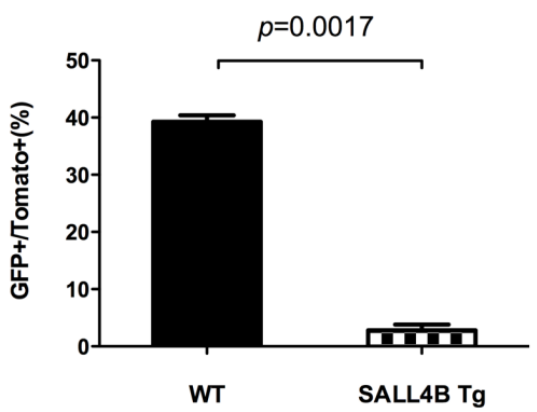
Wang, et al, Oncogene, 2016

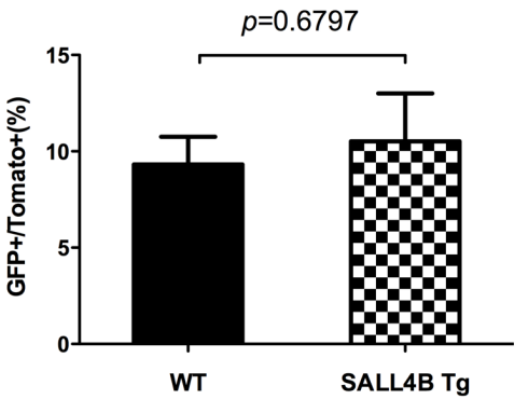
Overexpression of SALL4 leads to decreased monoubiquitination of FANCD2

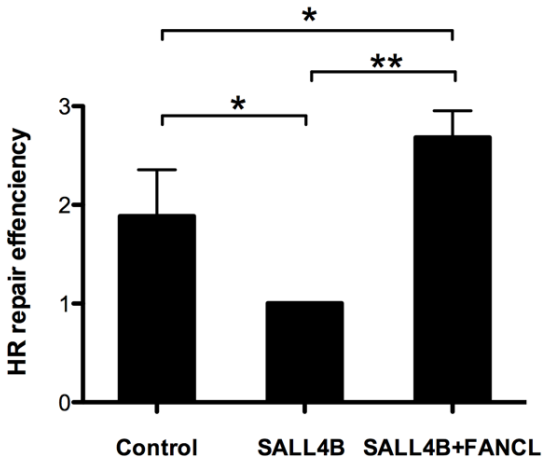


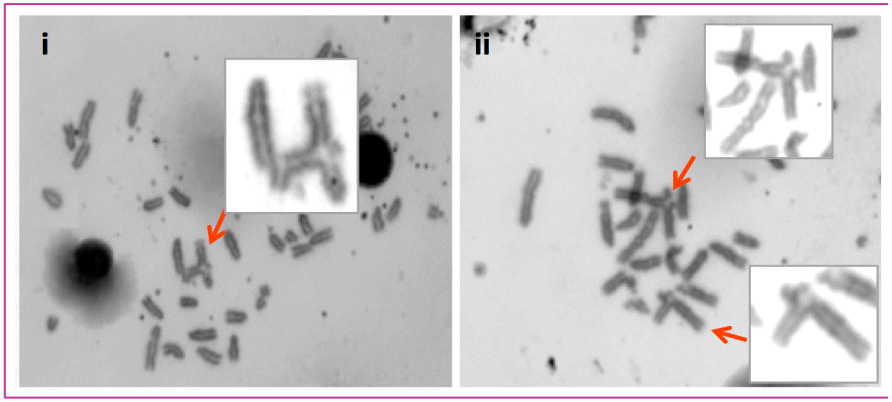
Wang, et al, Oncogene, 2016

SALL4B transgenic mice have HR not NHEJ DNA damage repair deficiency





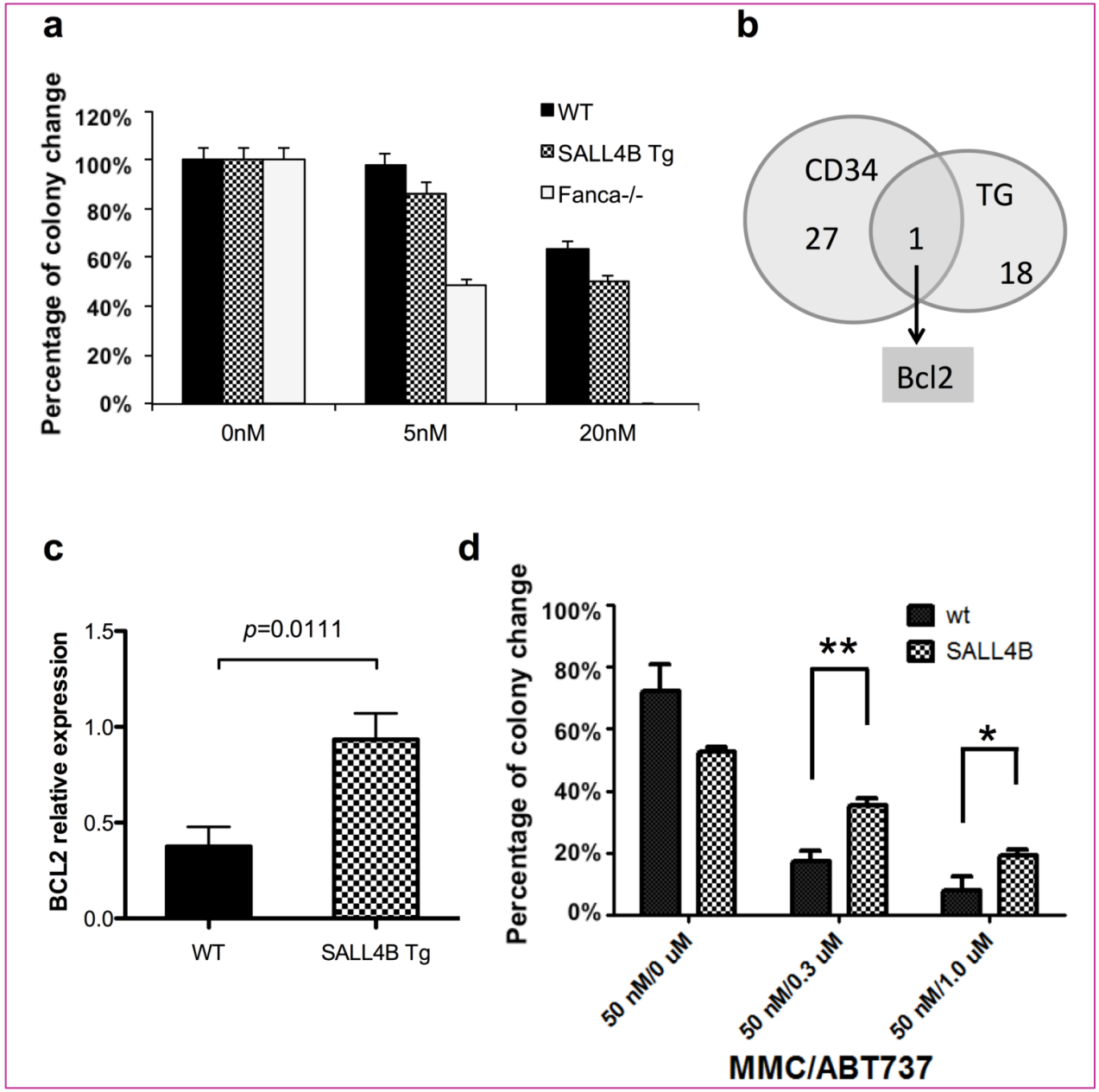




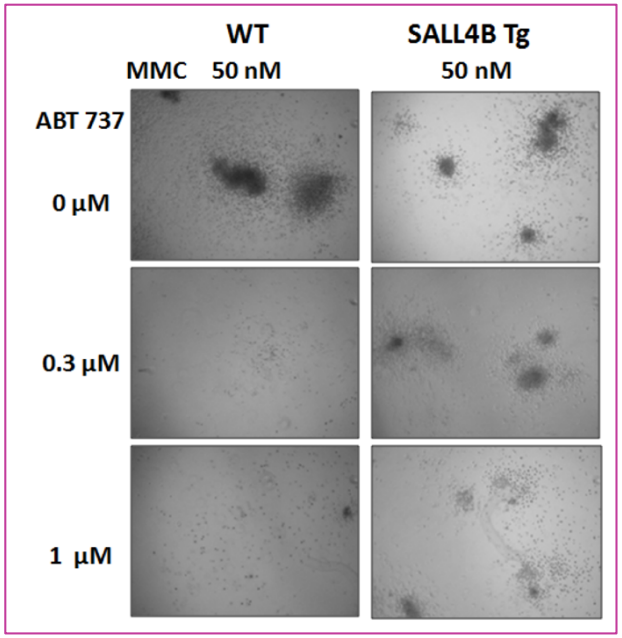
WT SALL4B TG SALL4B TG young SALL4B TG old

Wang, et al, Oncogene, 2016

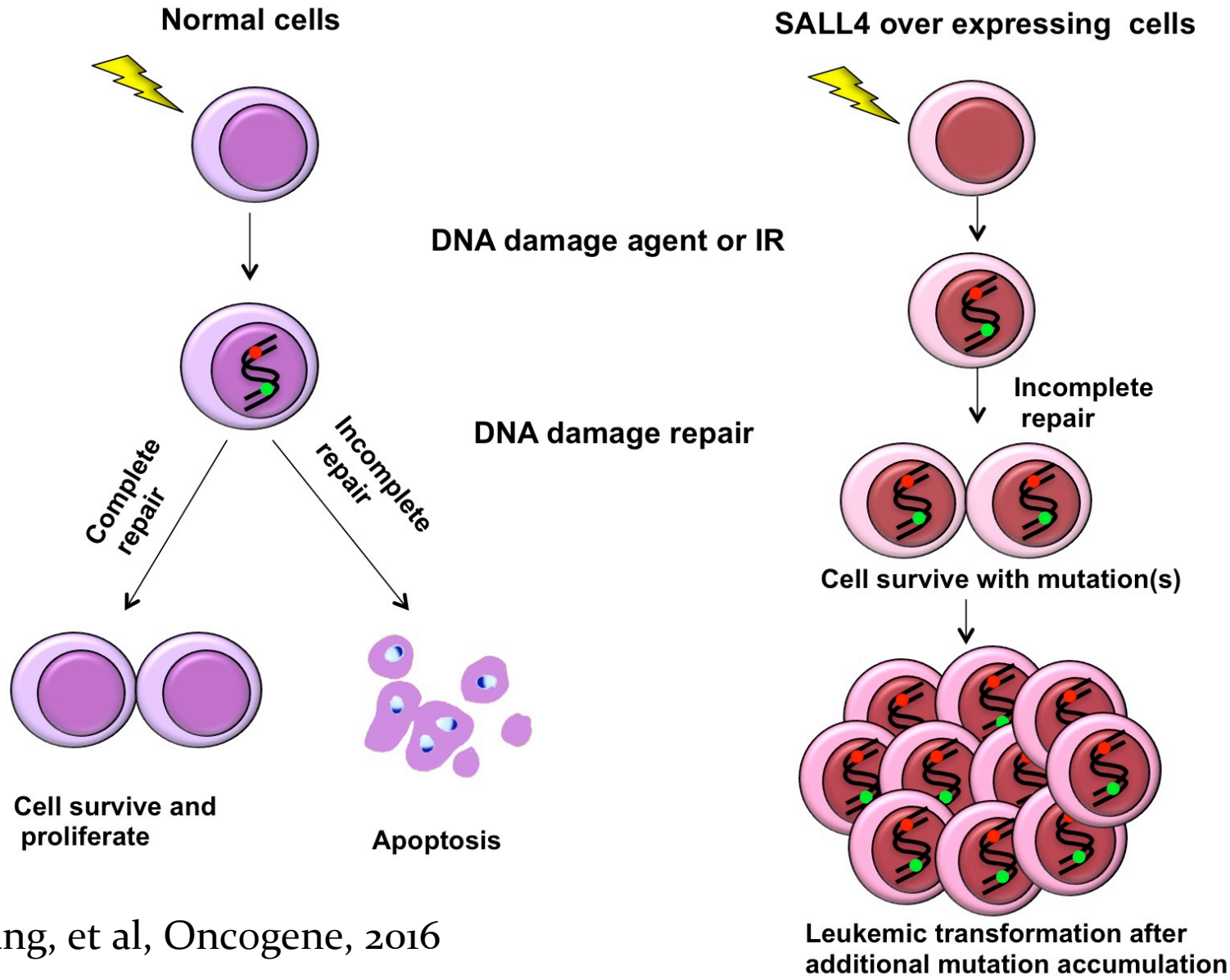
A SALL4/BCL2 pathway in SALL4B Tg mice



Wang, et al,
Oncogene, 2016

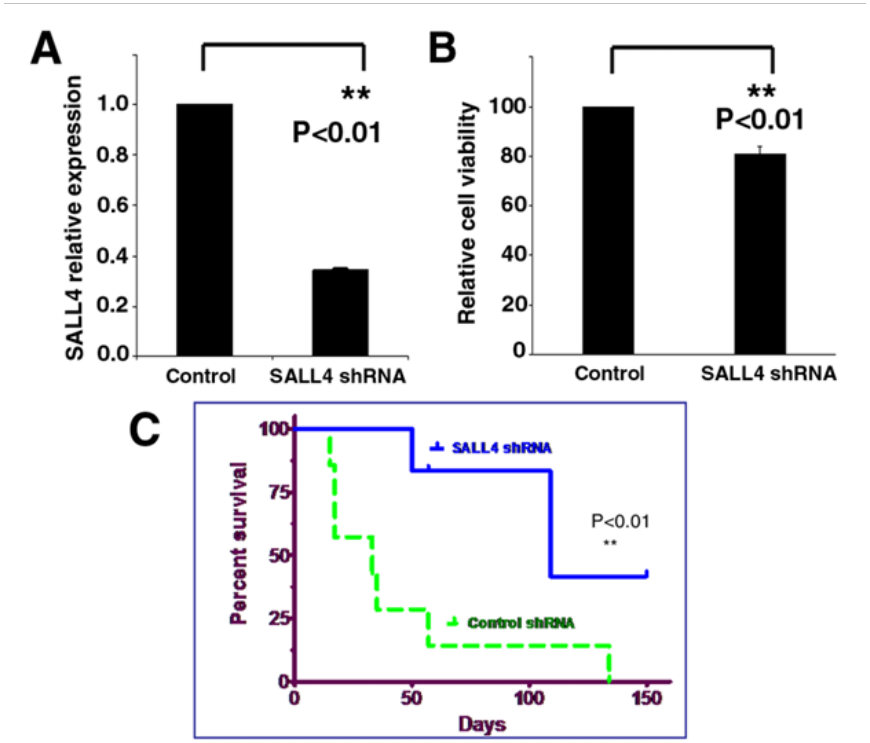


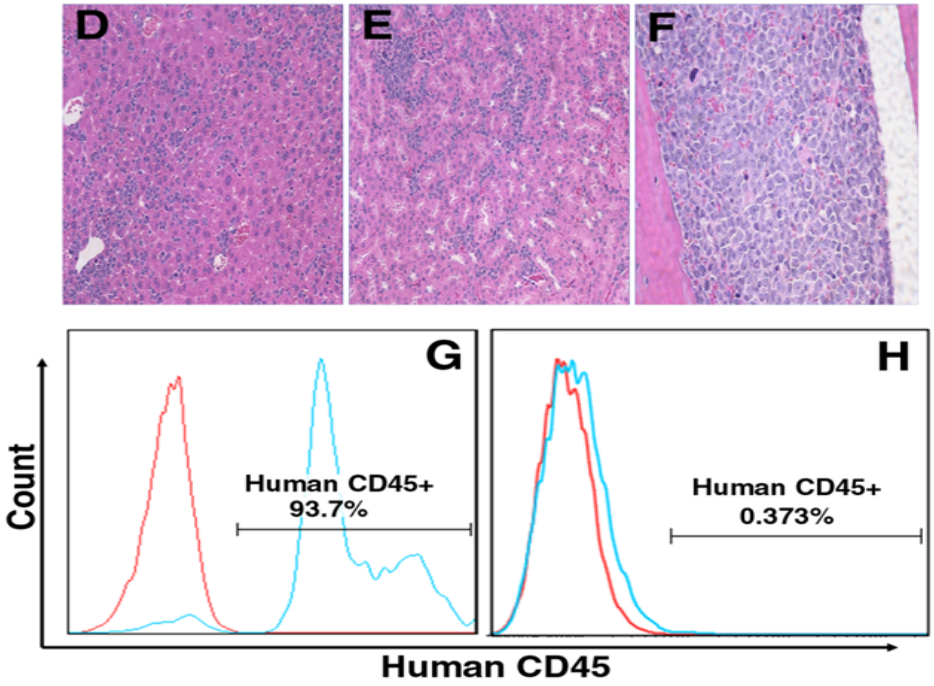
Hypothetic model on SALL4 -mediated MDS/ AML progression



Wang, et al, Oncogene, 2016

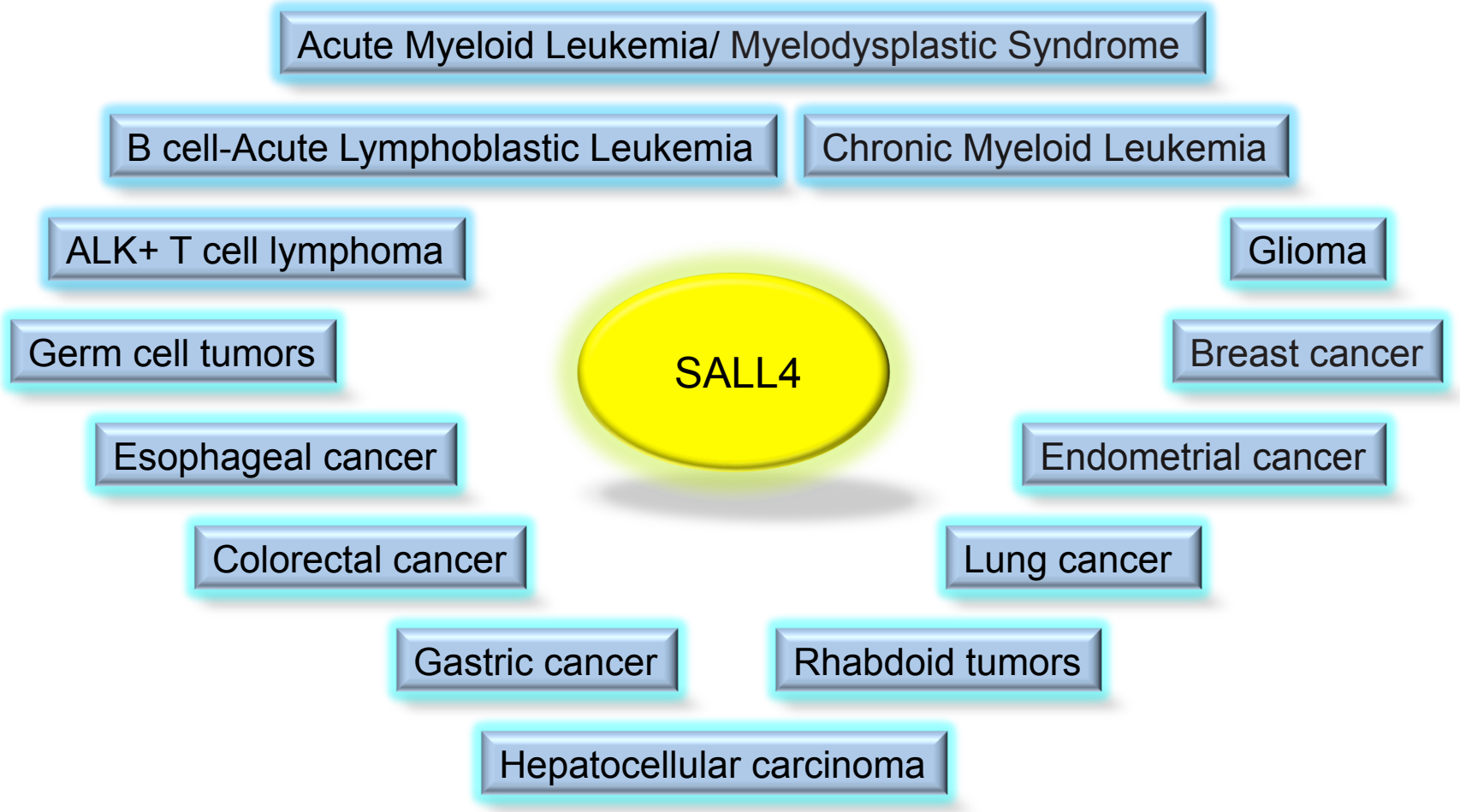
SALL4 is important for primary AML cell survival



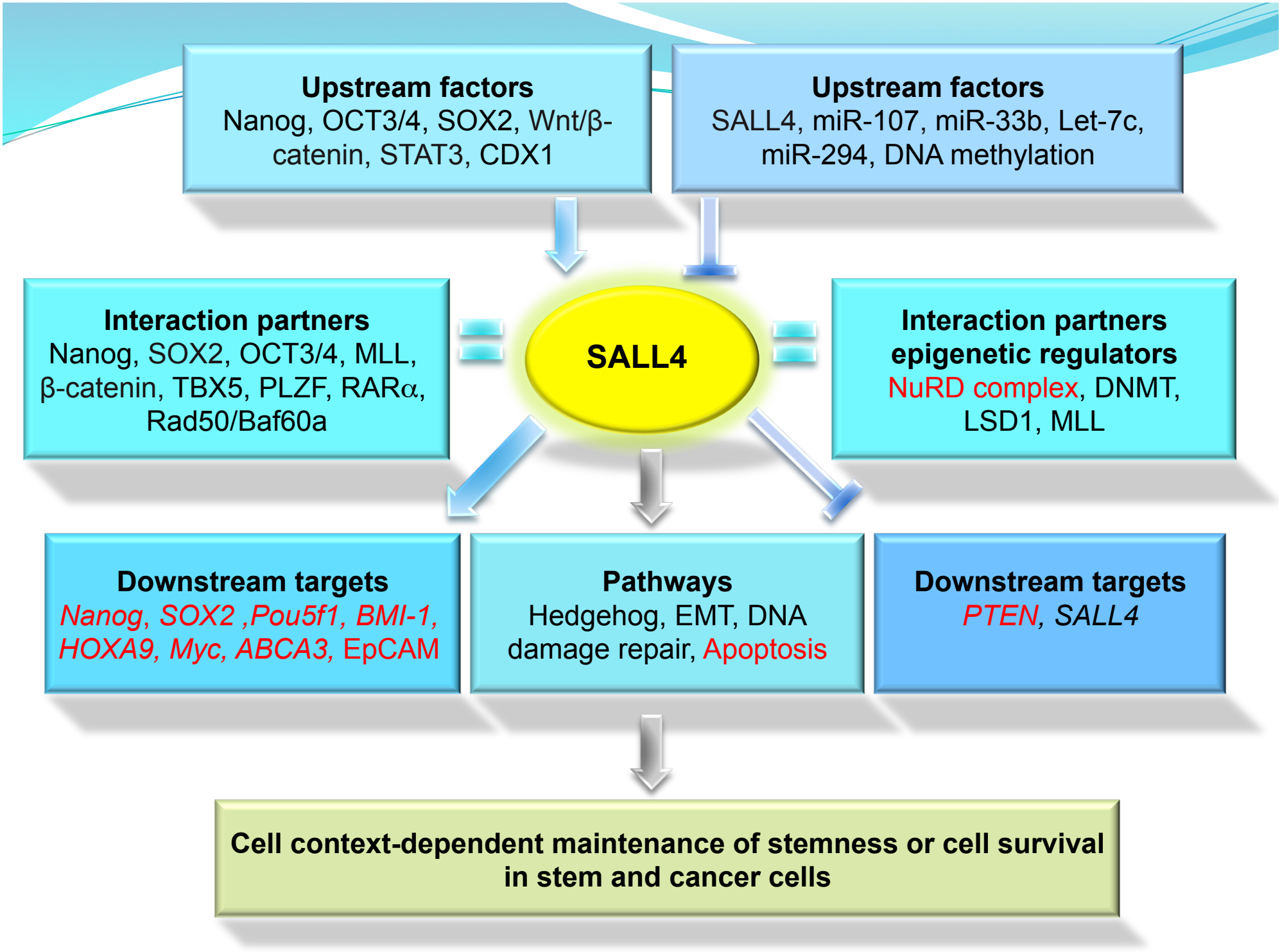


Ma, et al 2014, Blood

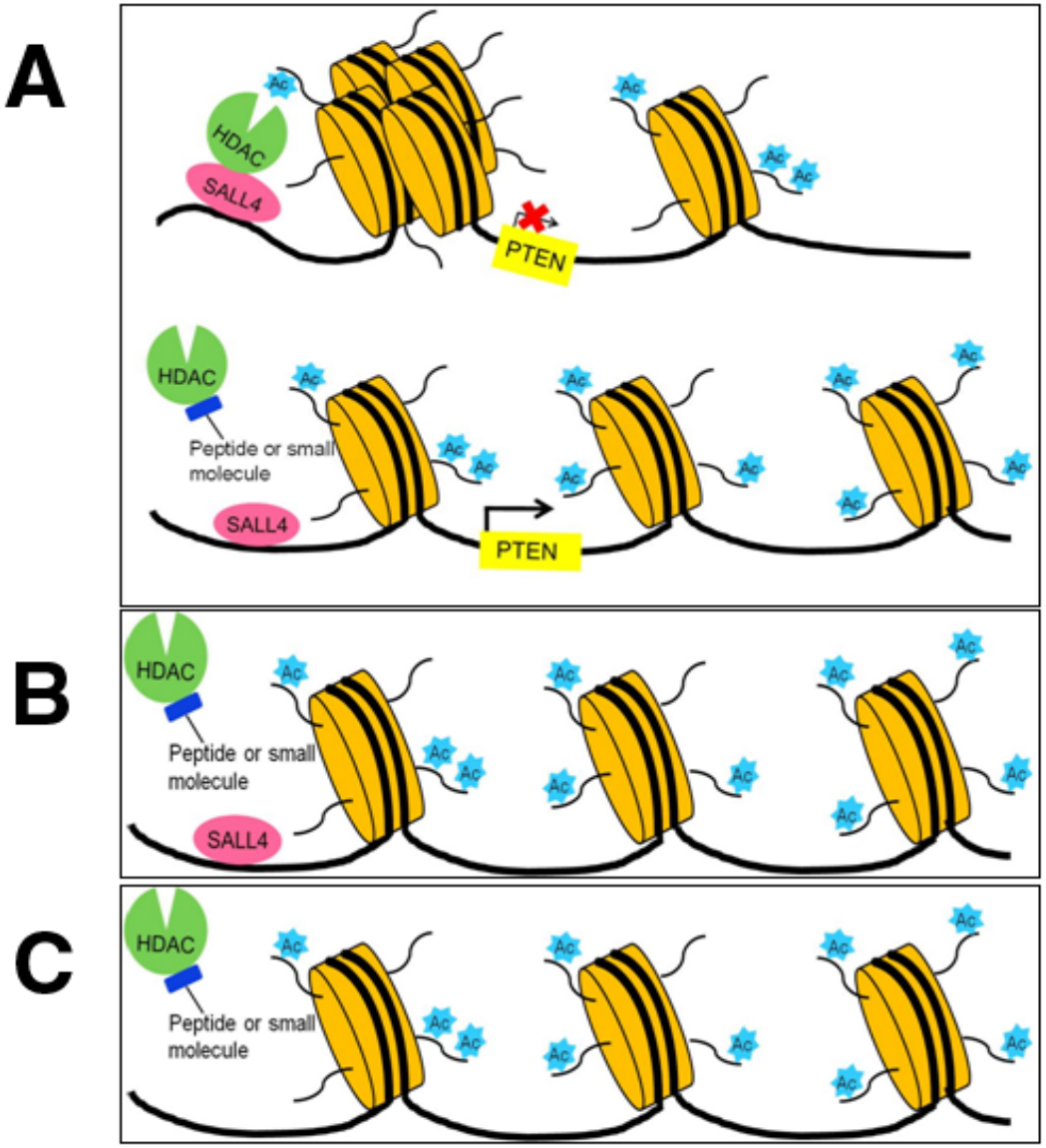
Summary of SALL4 in cancers



(Tatetsu et al, Gene. 2016 Jun 15;584(2):111-9.)


Upstream factors
Nanog, OCT3/4, SOX2, Wnt/β-catenin, STAT3, CDX1
Upstream factors
SALL4, miR-107, miR-33b, Let-7c, miR-294, DNA methylation
Interaction partners
Nanog, SOX2, OCT3/4, MLL, β-catenin, TBX5, PLZF, RARα, Rad50/Baf60a
SALL4
Interaction partners epigenetic regulators
NuRD complex, DNMT, LSD1, MLL
Downstream targets
Nanog, SOX2 ,Pou5f1, BMI-1, HOXA9, Myc, ABCA3, EpCAM
Pathways
Hedgehog, EMT, DNA damage repair, Apoptosis
Downstream targets
PTEN, SALL4
Cell context-dependent maintenance of stemness or cell survival in stem and cancer cells

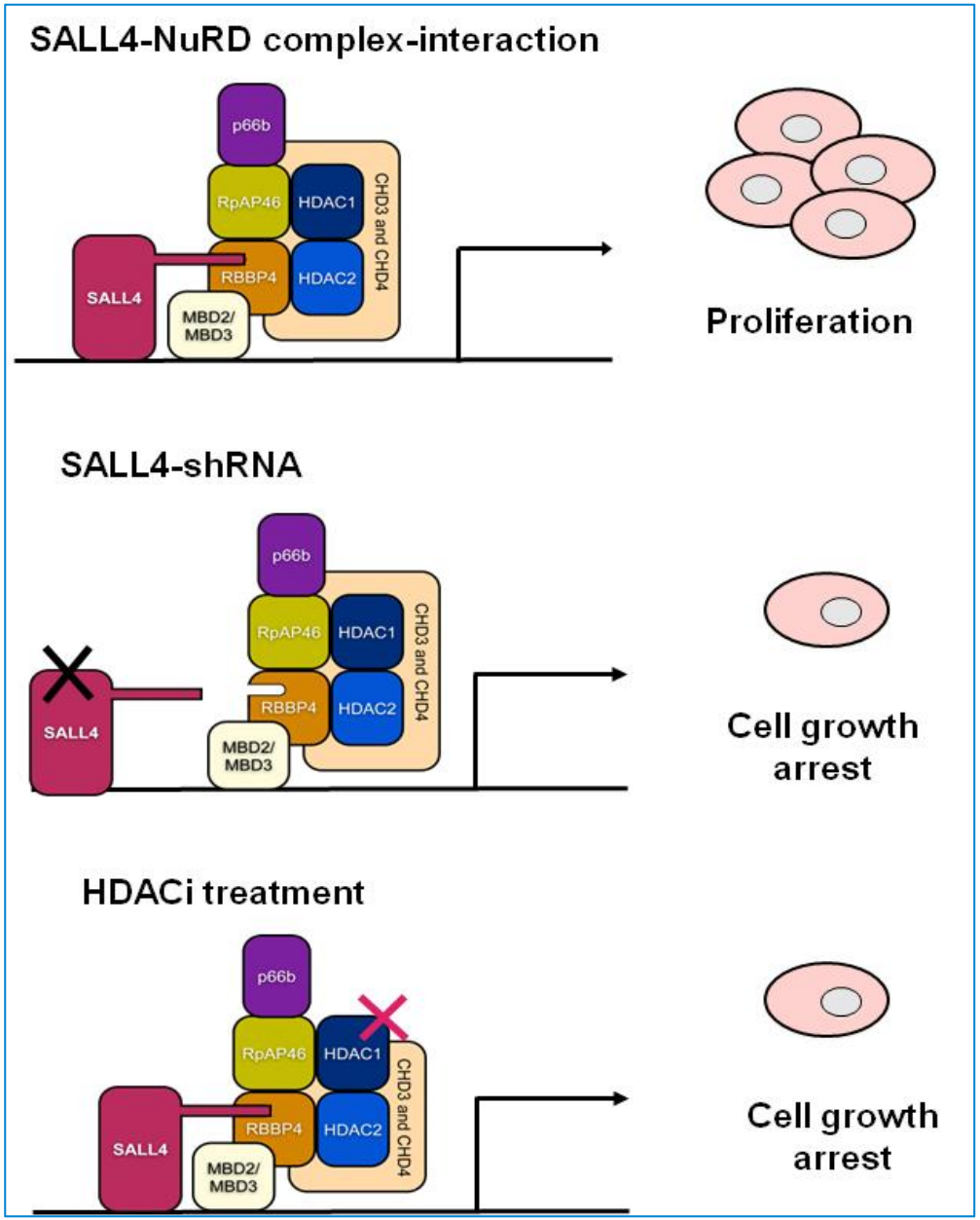
Targeting the SALL4/NuRD complex by SALL4 inhibitor(s)



Yong, et al
2013, NEJM

Gao, et al
2013, Blood

Targeting the SALL4/NuRD Complex by HDACi (MS-275, entinostat)



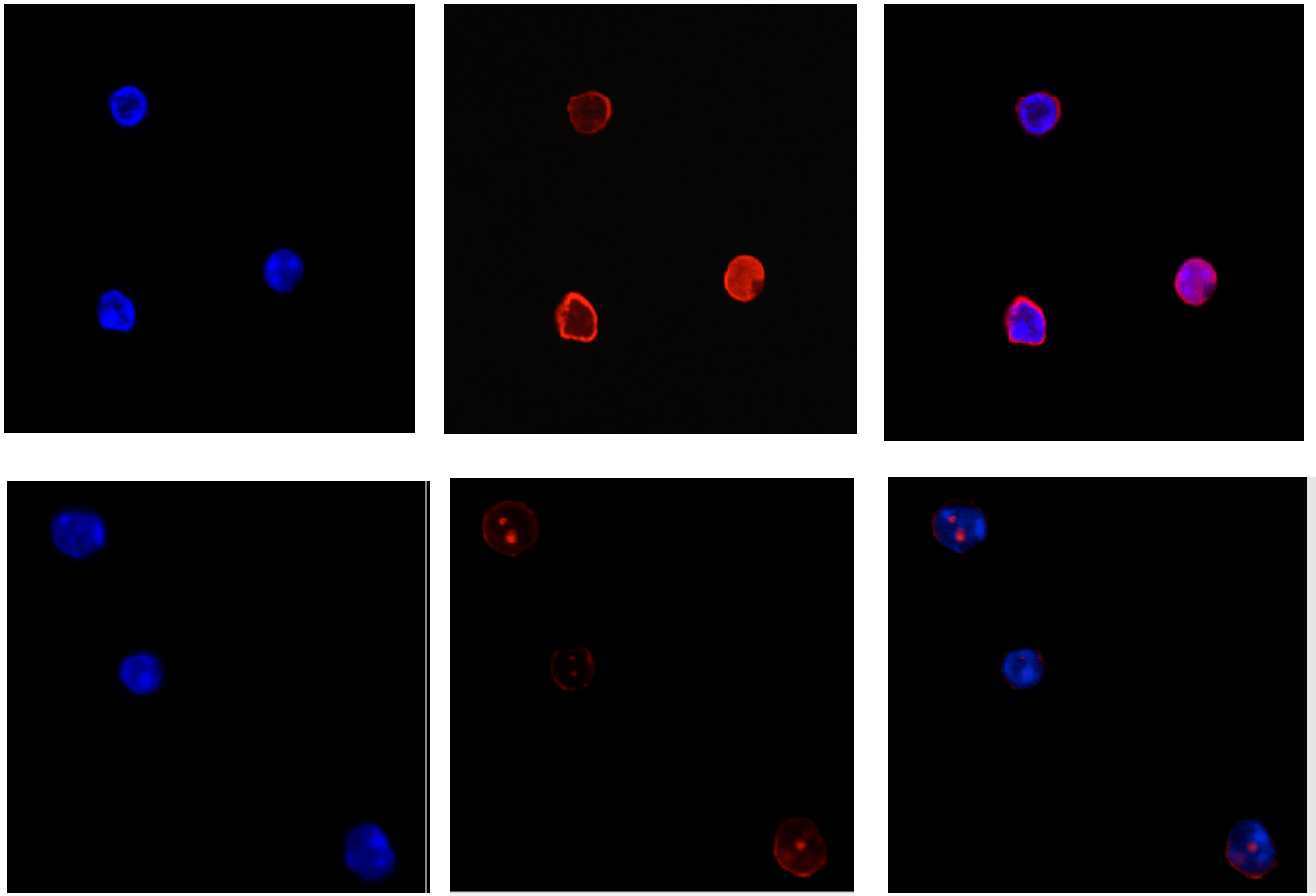
Yong, et al, Oncotarget, 2016

Remaining questions: what are the 2nd hits?

S4Tx-1-DAPI

S4Tx-1-NPM

S4Tx-1-Merge

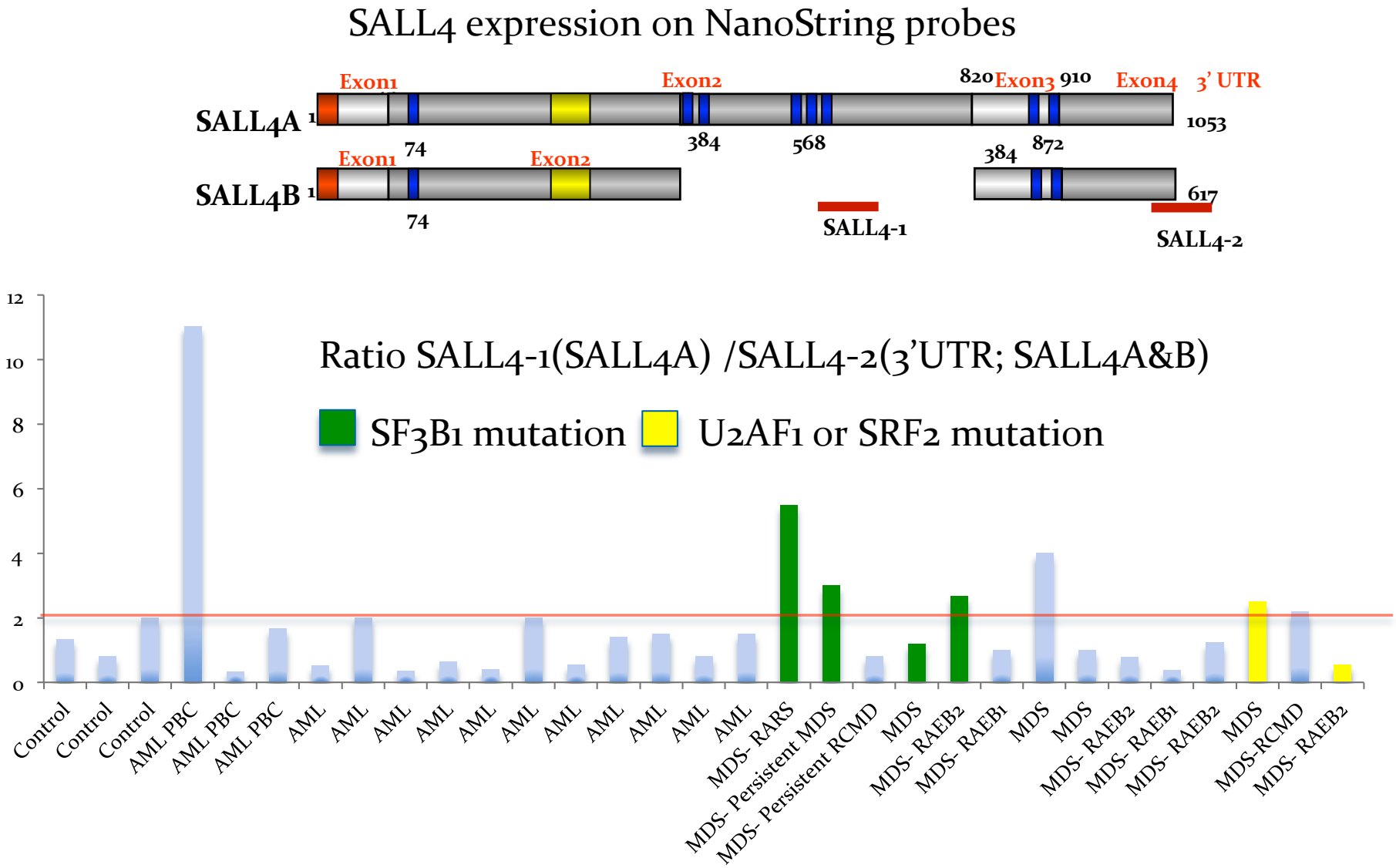


Wt-2-DAPI

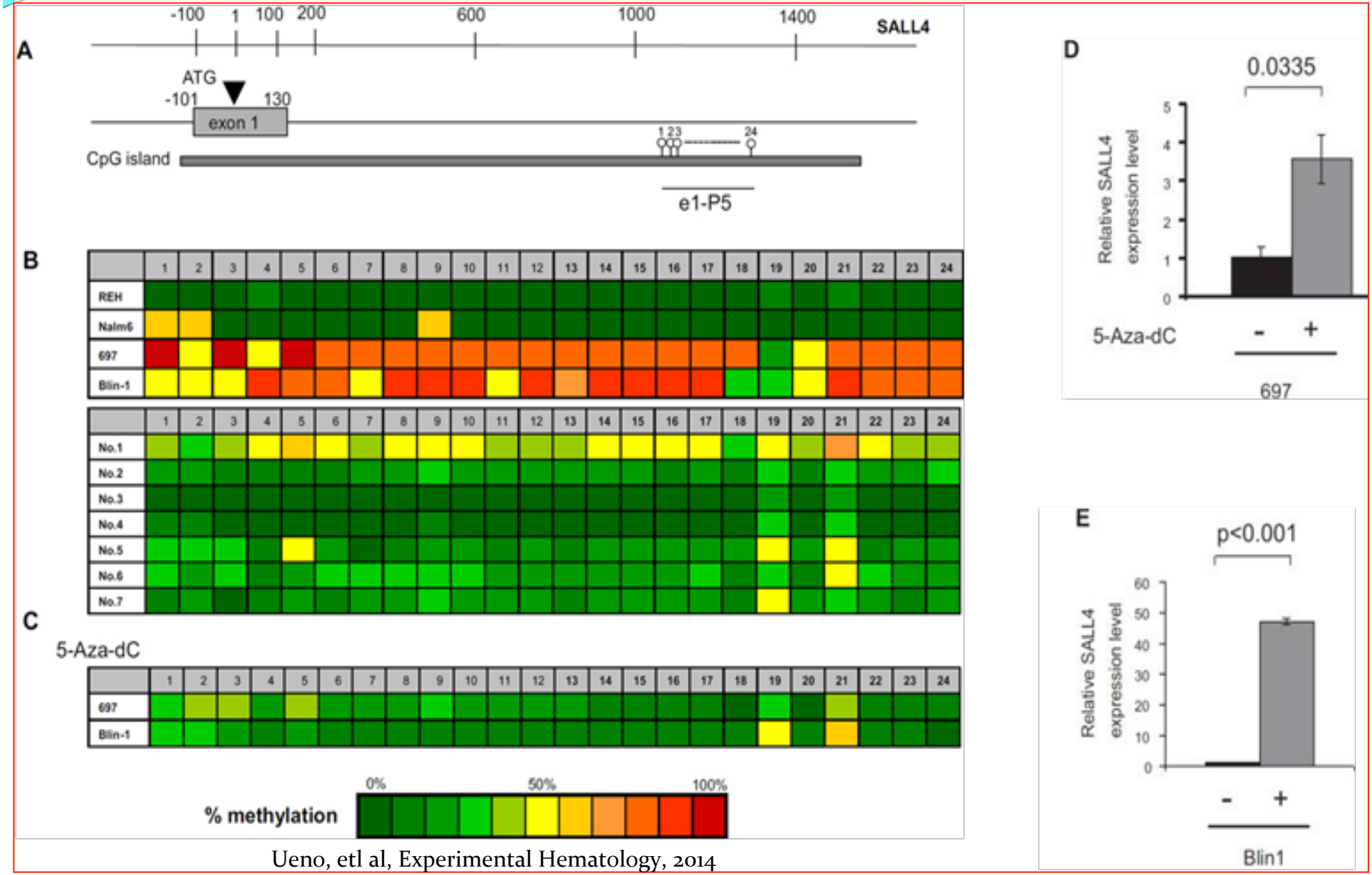
Wt-2-NPM

Wt-2-Merge

Aberrant SALL4A isoform ratio in human MDS cases with RNA splicing gene mutations



Hypomethylation of the CpG sites is correlated with *SALL4* expression



Ueno, etl al, Experimental Hematology, 2014

Summary

- SALL4 is expressed in ES cells and during organ development
- SALL4 is aberrantly expressed in MDS and AML
- SALL4 could be used as a companion biomarker for MDS/AML
- SALL4 Tg mice develop MDS/AML
- SALL4 is essential for leukemia and cancer survival
- Targeting SALL4 is a novel approach in treating MDS/AML

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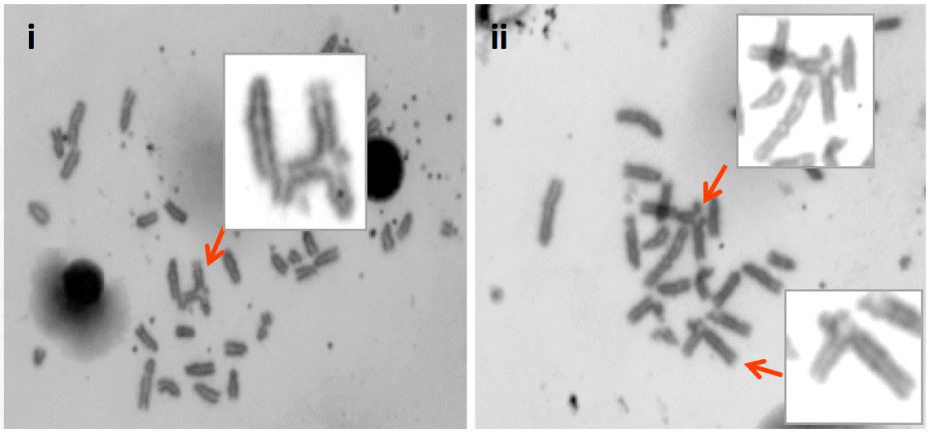
HR DNA damage repair defects in SALL4B Tg mice

Frequency of metaphase cells with chromatid interchange (radial) induced by MMC treatment in cultured mouse bone marrow cells

Mouse bone marrow	Treatment	No. scored metaphases	Aberrant metaphases (%)
Congenic control 1	None	50	0
	50 nM MMC	50	0
Congenic control 2	None	50	0
	50 nM MMC	50	0
Congenic control 3	None	50	0
	50 nM MMC	50	0
SALL4B Tg mouse 1	None	50	0
	50 nM MMC	50	10
SALL4B Tg mouse 2	None	50	0
	50 nM MMC	50	12
SALL4B Tg mouse 3	None	50	0
	50 nM MMC	50	15



Cells with ≤ 1 or > 1 radial / counted cells

Age	Mouse	Cell with Radial / Total cells MMC (50 nM) Radial(s) per cell 1	>1
≤8 months	1	5/50	3/50
	2	6/50	3/50
	3	9/50	2/50
>8 months	1	5/50	5/50
	2	5/50	7/50
	3	6/50	9/50

c

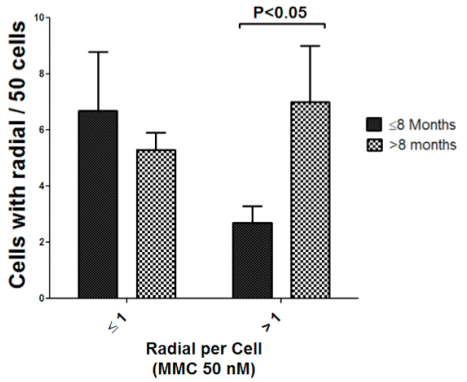


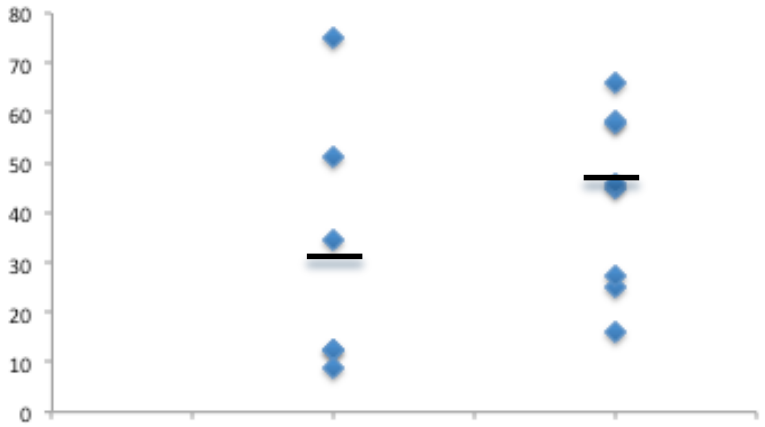
Table 2 IHC results for TP53 and SALL4 in 20 MDS patients

No	Karyotype	TP53	SALL4
1	-5q22,q35	-	-
2	Normal	-	-
3	Complex	2+	3+
4	Complex	3+	1+
5	Complex	3+	1+
6	Normal	-	-
7	Complex	-	-
8	+8	1+	4+
9	Complex	-	2+
10	+19p13	1+	-
11	+19p13	1+	1+
12	Normal	-	-
13	Normal	-	4+
14	Complex	2+	3+
15	Complex	3+	3+
16	Normal	-	-
17	Normal	-	-
18	Normal	-	3+
19	?-8q23,?22q11	-	-
20	+21	-	-

TP53 was positive in 71.4% (5/7) MDS patients with complex karyotype and 23.1% (3/13) in non-complex karyotype. SALL4 was positive in 85.7% (6/7) MDS patients with complex karyotype and 30.8% (4/13) in non-complex karyotype.
Consistency in terms of positive or negative for TP53 and SALL4 is 80%(16/20).

TET2 mutation and SALL4 expression

SALL4A



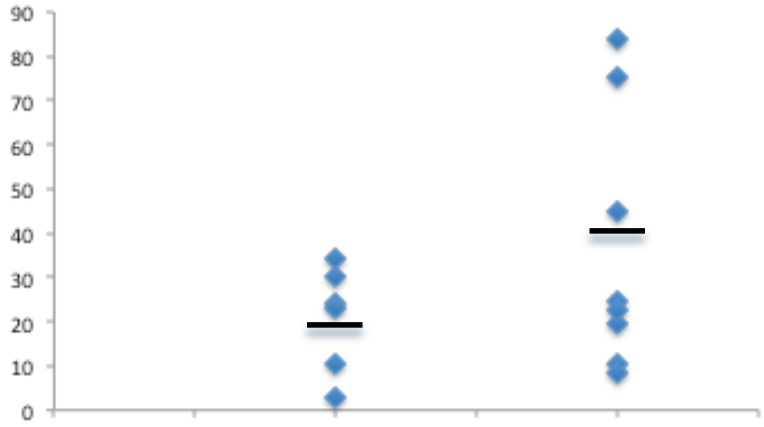
Ave 32.3 42.6

\+ -

TET2 mutation

P=0.405

SALL4 3'UTR



20.8 36.1

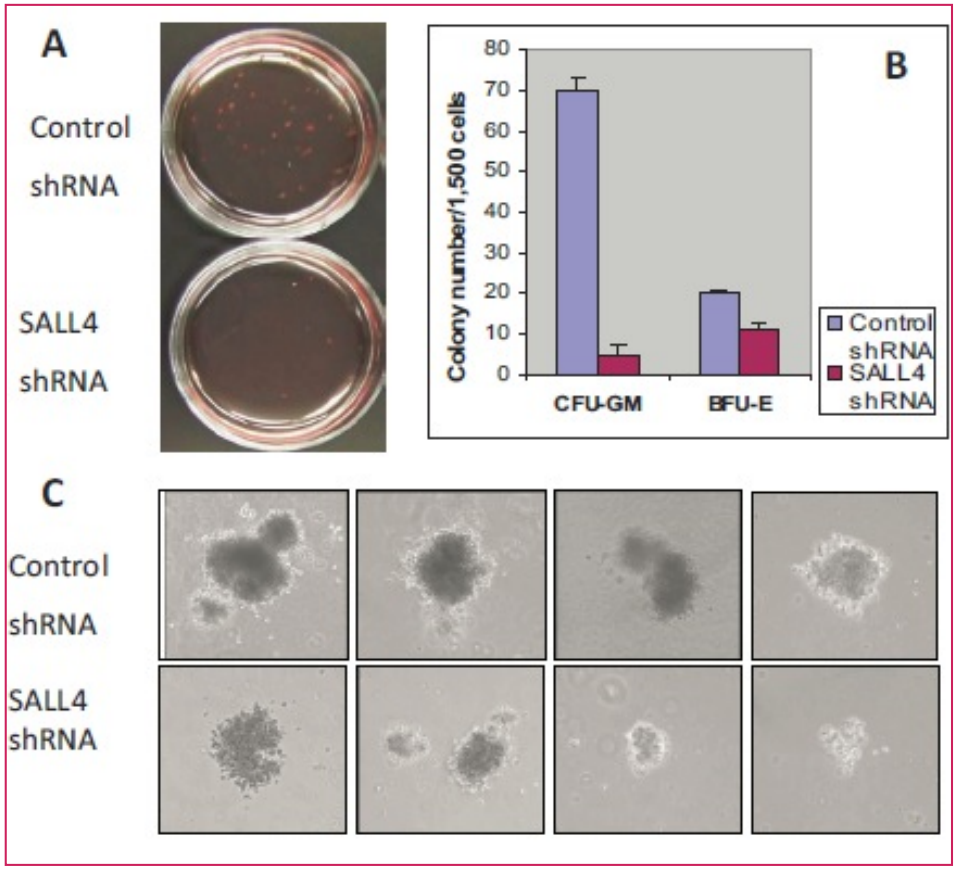
\+ -

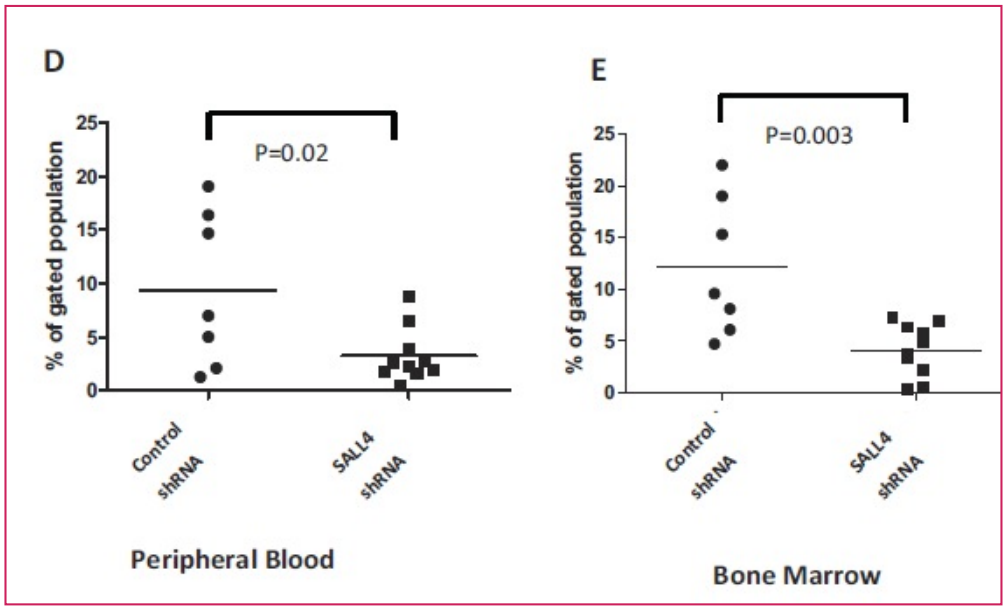
TET2 mutation

P=0.24

TET2 mutation was detected in 6 of 14 cases. 3'UTR SALL4 expression in the patients who have TET mutation looks lower SALL4 expression level than those who do not have TET mutation. But There no significant difference between them.

Impaired colony forming abilities *in vitro* and engraftment *in vivo* upon SALL4 knocking down



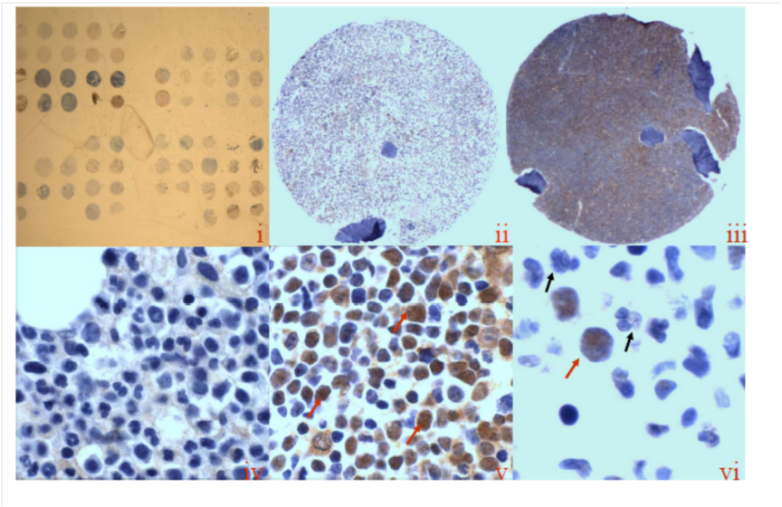


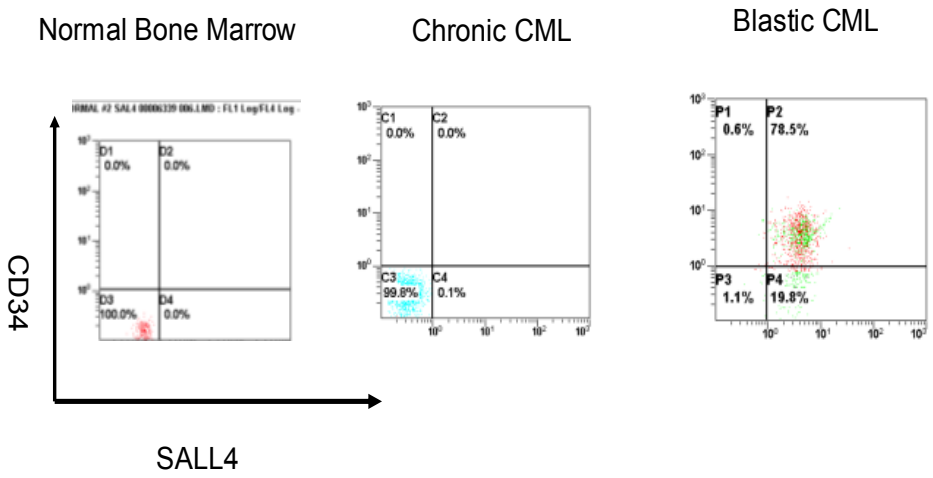
Gao, et al, Transfusion. 2012

Table S3. Correlation coefficient (r) of SALL4 and HOXA genes in 385 primary AML patients (GSE14468) [1]
* indicates p<0.05

FAB	M0 (n=16)	M1 (n=98)	M2 (n=110)	M3 (n=23)	M4 (n=86)	M5 (n=106)	M6 (n=6)
HOXA 1	-0.256	-0.105	-0.182	0.000	-0.133	**-0.245***	-0.883
HOXA 2	0.000	0.021	-0.114	0.000	-0.201	-0.031	0.041
HOXA 3	0.318	0.012	0.027	0.087	0.168	-0.101	-0.824
HOXA 4	0.166	-0.032	-0.097	0.315	**0.267***	-0.072	0.133
HOXA 5	0.185	-0.038	-0.038	0.458	**0.339***	-0.006	-0.491
HOXA 6	**0.577***	0.192	**0.271***	0.567	**0.357***	**0.222***	**0.203***
HOXA 7	-0.190	0.013	0.010	0.000	**0.297***	-0.013	-0.406
HOXA 9	0.223	-0.030	0.016	**0.458***	**0.326***	0.022	0.579
HOXA 10	0.310	-0.027	0.029	0.156	**0.238***	0.012	-0.391
HOXA 11	**0.651***	0.089	-0.072	0.000	-0.010	0.079	0.319
HOXA 13	0.225	0.144	-0.083	0.000	0.267*	0.046	0.722

SALL4 in myeloid leukemia





Lu, et al 2009, Leukemia

Summary

- SALL4 is important for maintaining cancer cell survival and metastasis
- SALL4 is an ideal target for cancer therapy

Outline

- SALL4 in normal and cancer tissues
  - ES cells, pre-malignant and malignant hematological diseases
- Functional role(s) of SALL4 in cancer
  - Loss-of-function
  - Gain-of-function
- Mechanism
  - A SALL4/NuRD/Pten pathway
- Target SALL4 in leukemia