SUPPLEMENTARY APPENDIX

N-terminally truncated FOXP1 protein expression and alternate internal FOXP1 promoter usage in normal and malignant B cells

Philip J. Brown,[1*†] Duncan M. Gascoyne,[1*] Linden Lyne,[1] Hayley Spearman,[1] Suet Ling Felce,[1] Nora McFadden,[2] Probir Chakravarty,[3] Sharon Barrans,[4] Steven Lynham,[5] Dinis P. Calado,[2,6] Malcolm Ward,[5] and Alison H. Banham[1]

[1]Nuffield Division of Clinical Laboratory Sciences, Radcliffe Department of Medicine, Oxford University; [2]Immunity and Cancer Laboratory, The Francis Crick Institute, Lincoln's Inn Fields Laboratory, Lincoln's Inn Fields, London [3]Computational Biology Laboratory, The Francis Crick Institute, Lincoln's Inn Fields Laboratory, Lincoln's Inn Fields, London; [4]Leeds Teaching Hospitals NHS Trust, HMDS, Leeds Cancer Centre; [5]Centre of Excellence for Mass Spectrometry, Institute of Psychiatry, Psychology and Neuroscience, Kings College London; and [6]Peter Gorer Department of Immunobiology, Kings College London, UK

**PJB and DMG contributed equally to this work. †Deceased, 2nd March 2014*

 doi:10.3324/haematol.2016.142141

Received: January 15, 2016.

Accepted: April 4, 2016.

Pre-published: April 7, 2016.

Correspondence: alison.banham@ndcls.ox.ac.uk

Supplemental Table 1

Oligonucleotide primers and probes

Gene	Exon	Direction	5' to 3' Sequence or assay	application
hFOXP1	20-21	n/a	Hs00212860_m1	Taqman
hFOXP1	6-7	n/a	Hs00908896_m1	Taqman
hFOXP1	6b-7	n/a	Hs00909412_m1	Taqman
hFOXP1	7b	forward	GCTAACTCAACTGTCAGAACTGCAT	Taqman
hFOXP1	7b	reverse	TGAGGTGTCATCATAGCCACTGA	Taqman
hFOXP1	7b	probe	ACGGGAACCGTCCTTC	Taqman
hFOXP1	7c	forward	CGGCTACATGGAGAGAGTGTCA	Sybr
hFOXP1	7c	reverse	AGCACTTGTTGCTGGAGGAT	Sybr
mFoxp1	18	forward	CACCAACAGCAACGAGAGTG	Sybr
mFoxp1	19	reverse	GGTCGAGGGGTTCTTCTTTG	Sybr
mFoxp1	2	forward	TCTGTGTGCTGCAGTCTTGT	Sybr
mFoxp1	3	reverse (use with 2)	TCACCTCCAAAGGTCACGTC	Sybr
mFoxp1	4	forward	ACGGATCAGCCATCCAGAAC	Sybr
mFoxp1	5	reverse (use with 4F)	AGCTGCCTTGCCACCTGCAG	Sybr
mFoxp1	2b	forward	CTCACTGCCTGTAGTGCCTC	Sybr
mFoxp1	4b	forward	AAACCCCCGCTTCACAGAAA	Sybr
mFoxp1	5	reverse (use with 4bF)	GCTGGTTGCTTGTCATTCCTC	Sybr
mFoxp1	5b	forward	CTTGCGCGTGCAGTCC	Sybr
mFoxp1	6	reverse	TCATCATAGCCACTGACACGG	Sybr
hFOXP1	6	forward	GTCGGGCGGCAGCAACCACTTACTAG	RT-PCR
hFOXP1	6b(long)	forward (primer 1)	GTGAGAGCCCAGCCAGCTACTGTG	RT-PCR
hFOXP1	6b(long)	forward (primer 2)	ATGTTCCAGTGTGTTTTCTCTTCGAGT	RT-PCR
hFOXP1	6b(short)	forward	ATCCAAAGGCAGACAGTACGGGCTCC	RT-PCR
hFOXP1	8	forward	ATGATGACACCTCAAGTTATCACTCCCCAG	RT-PCR
hFOXP1	10	reverse	AAGGCCTTGGCGCTGCAAAGACAGGA	RT-PCR
m18s		forward	CGAACGTCTGCCCTATCAAC	Sybr
m18s		reverse	TGTGGTAGCCGTTTCTCAGG	Sybr

siRNA target sequences

Gene	Exon	target sequence
hFOXP1	Spans 1-2	CAGCGGGAACCCGAAAGTTTGTAAG
hFOXP1	5	GAGGTGACTATAACTGAAGATTGCT
hFOXP1	6	AAAGTAACGGTTCAGCCATCCAGAA
hFOXP1	7 (si#1)	GCAACAGCAGCAGCAAGTTAGTGGA
hFOXP1	7 (si#2)	CAAGACAGCTCCTTCTTCAGCAGCA
hFOXP1	8	TGGCTATGATGACACCTCAAGTTAT
hFOXP1	9	AACAGGAACAGTTGCAGCTTCAACT
hFOXP1	13	CAGCCCAATGTAGAGTACAAATGCA
hFOXP1	18	CAGTGCGTCATAATCTTAGTCTTCA
hFOXP1	20	TGGCTGAGAATAGTATACCTCTATA

Antibodies

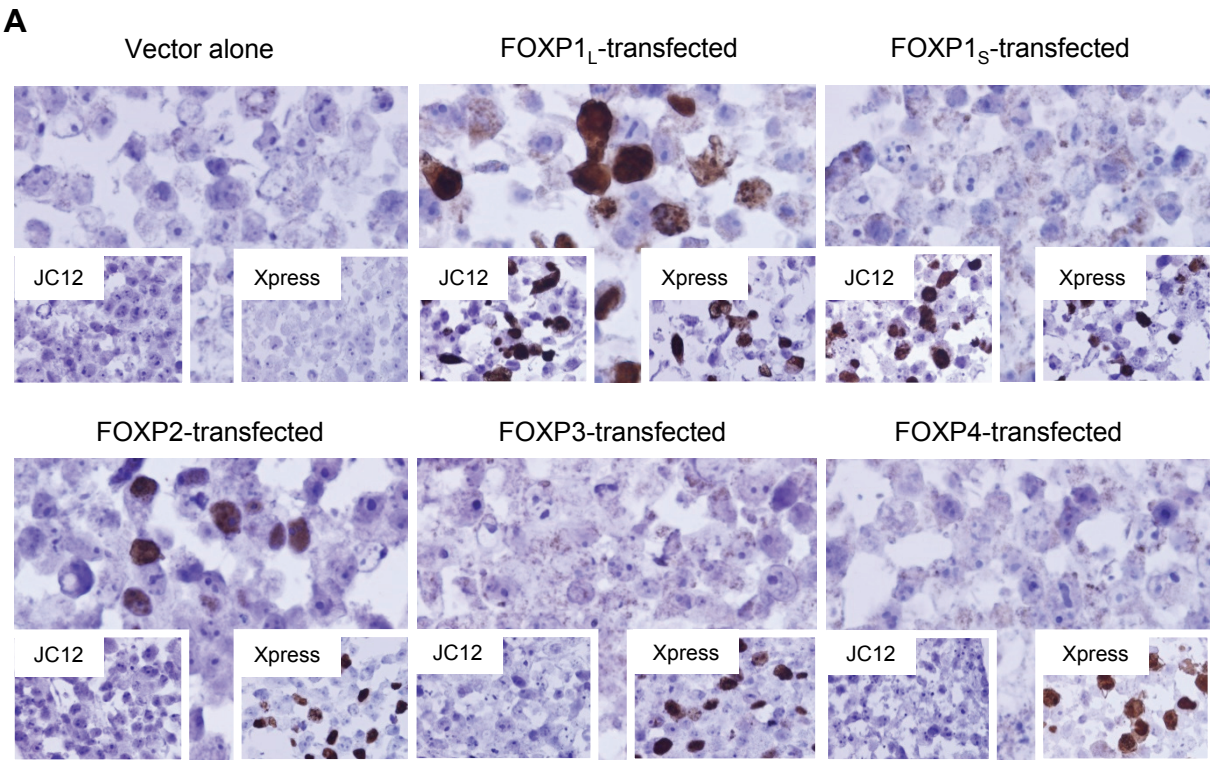
Target	Cat. number	supplier	Clone/species	Dilution/Application
FOXP1	n/a *	in house *	JC12/mouse monoclonal	1:30 for IB, 1:80 for IHC
FOXP1	LS-C6969	LS Bio	Rabbit polyclonal	1:2,000 for IB, 1:200 for IHC
Xpress tag	R910-25	Life Technologies	Mouse monoclonal	1:5,000 for IB, 1:5000 for IHC
CD20	Ab9475	in house/Abcam	L26/mouse monoclonal	1:20 for IHC
TBP	sc56795	Santa Cruz	1TBP18/mouse monoclonal	1:10,000 for IB
β-actin	A5441	Sigma-Aldrich	AC15/mouse monoclonal	1:40,000 for IB
FOXP4	HPA000382	Sigma-Aldrich	Rabbit polyclonal	1:2,000 for IB
IkBα	#9246	Cell Signalling	5A5/mouse monoclonal	1:1000 for IB
CD19	#13-0199	eBioscience	HIB19 / mouse monoclonal	1:200 for Flow
CD45	#13-0452	eBioscience	RA3-6B2 / rat monoclonal	1:200 for Flow
CD20	#17-0209	eBioscience	2H7 / mouse monoclonal	1:100 for Flow
CD27	#17-0279	eBioscience	O323 / mouse monoclonal	1:100 for Flow
CD38	#17-0389	eBioscience	HIT2 / mouse monoclonal	1:100 for Flow
CD138	#12-1389	eBioscience	DL-101 / mouse monoclonal	1:100 for Flow
CD30	FAB229P	RnD Systems	#81337/ mouse monoclonal	1:100 for Flow

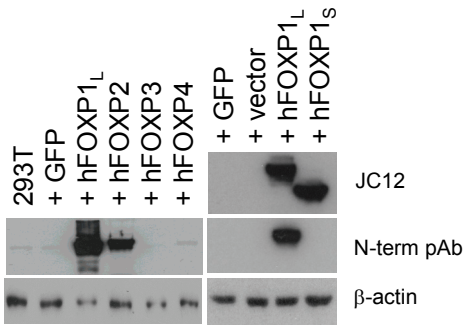
* JC12 antibody is also available as #MA1-84005 from Life Technologies

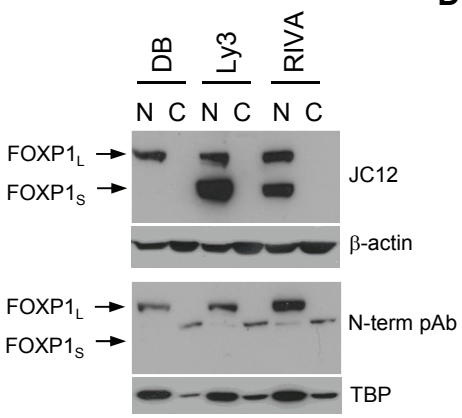
Supplemental Table 2

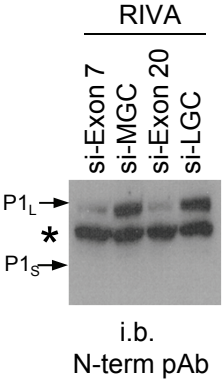
			ACB-DLBCL				GCB-DLBCL									
transcript_id	gene_id	gene	OCI_LY3	OCI_LY10	SU_DHL2	U2932	OCI_LY7	SU_DHL4	SU_DHL6	SU_DHL10	OCI_LY19	logFC	logCPM	LR	PValue	FDR
ENST00000491238	ENSG00000114861	FOXP1	555.23	1208.61	185.35	186.35	40.70	32.36	8.65	0.00	81.64	4.03	2.69	10.55	0.00	0.03
ENST00000497553	ENSG00000114861	FOXP1	22.05	25.48	11.51	20.36	2.54	0.10	0.00	0.00	7.09	3.24	-1.76	6.85	0.01	0.15
ENST00000485326	ENSG00000114861	FOXP1	32.60	82.38	56.75	84.60	4.21	0.14	4.30	0.00	34.87	2.85	-0.18	5.71	0.02	0.24
ENST00000497355	ENSG00000114861	FOXP1	883.20	1003.01	590.70	705.85	178.08	57.59	101.75	45.19	261.53	2.63	3.42	5.00	0.03	0.32
ENST00000484350	ENSG00000114861	FOXP1	27.81	36.55	0.00	15.34	0.00	22.07	0.00	0.00	0.00	2.22	-1.59	3.36	0.07	0.62
ENST00000318779	ENSG00000114861	FOXP1	6.71	4.22	2.64	11.70	2.51	131.64	1.39	12.49	5.32	-2.26	-0.89	3.27	0.07	0.64
ENST00000460805	ENSG00000114861	FOXP1	168.78	144.39	34.56	35.72	40.71	27.00	7.20	11.53	63.91	1.67	0.62	2.11	0.15	0.95
ENST00000475937	ENSG00000114861	FOXP1	1899.69	3299.52	1031.51	1442.10	463.82	731.88	127.17	106.74	1625.04	1.65	4.90	2.08	0.15	0.96
ENST00000498215	ENSG00000114861	FOXP1	39.32	34.83	7.95	13.68	21.25	9.06	0.00	0.00	20.06	1.24	-1.14	1.16	0.28	1.00
ENST00000468577	ENSG00000114861	FOXP1	0.00	9.32	0.00	43.40	136.74	9.17	0.00	11.53	0.00	-1.26	-0.66	1.12	0.29	1.00
ENST00000318789	ENSG00000114861	FOXP1	3236.48	3029.43	1248.82	1110.42	423.10	1140.86	167.88	141.37	2917.00	1.17	5.22	1.06	0.30	1.00
ENST00000498154	ENSG00000114861	FOXP1	6.70	15.28	9.74	21.90	67.07	25.19	2.11	0.00	36.93	-0.97	-0.83	0.67	0.41	1.00
ENST00000470112	ENSG00000114861	FOXP1	47.95	62.01	14.16	49.11	1.66	92.49	5.02	16.34	16.87	0.72	-0.16	0.39	0.53	1.00
ENST00000493089	ENSG00000114861	FOXP1	78.63	134.17	104.64	223.29	263.81	116.62	18.11	18.26	84.56	0.43	1.56	0.14	0.70	1.00
ENST00000327590	ENSG00000114861	FOXP1	779.65	205.50	178.25	269.22	252.68	326.51	271.17	206.77	409.12	0.29	3.02	0.06	0.80	1.00
ENST00000498714	ENSG00000242094	FOXP1-IT1	34.52	22.90	32.80	55.78	13.53	130.31	2.12	1.91	63.08	-0.21	0.06	0.03	0.86	1.00
ENST00000465742	ENSG00000244203	FOXP1-AS1	187.01	84.06	27.47	72.88	82.23	127.33	90.13	59.62	121.50	-0.05	1.27	0.00	0.96	1.00

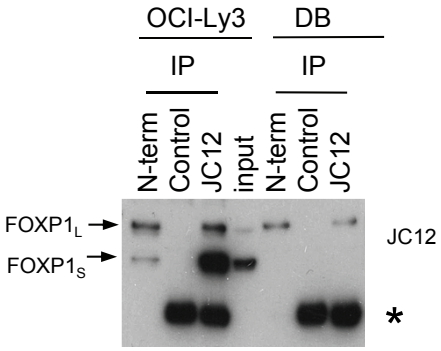
Supplemental Figure 1



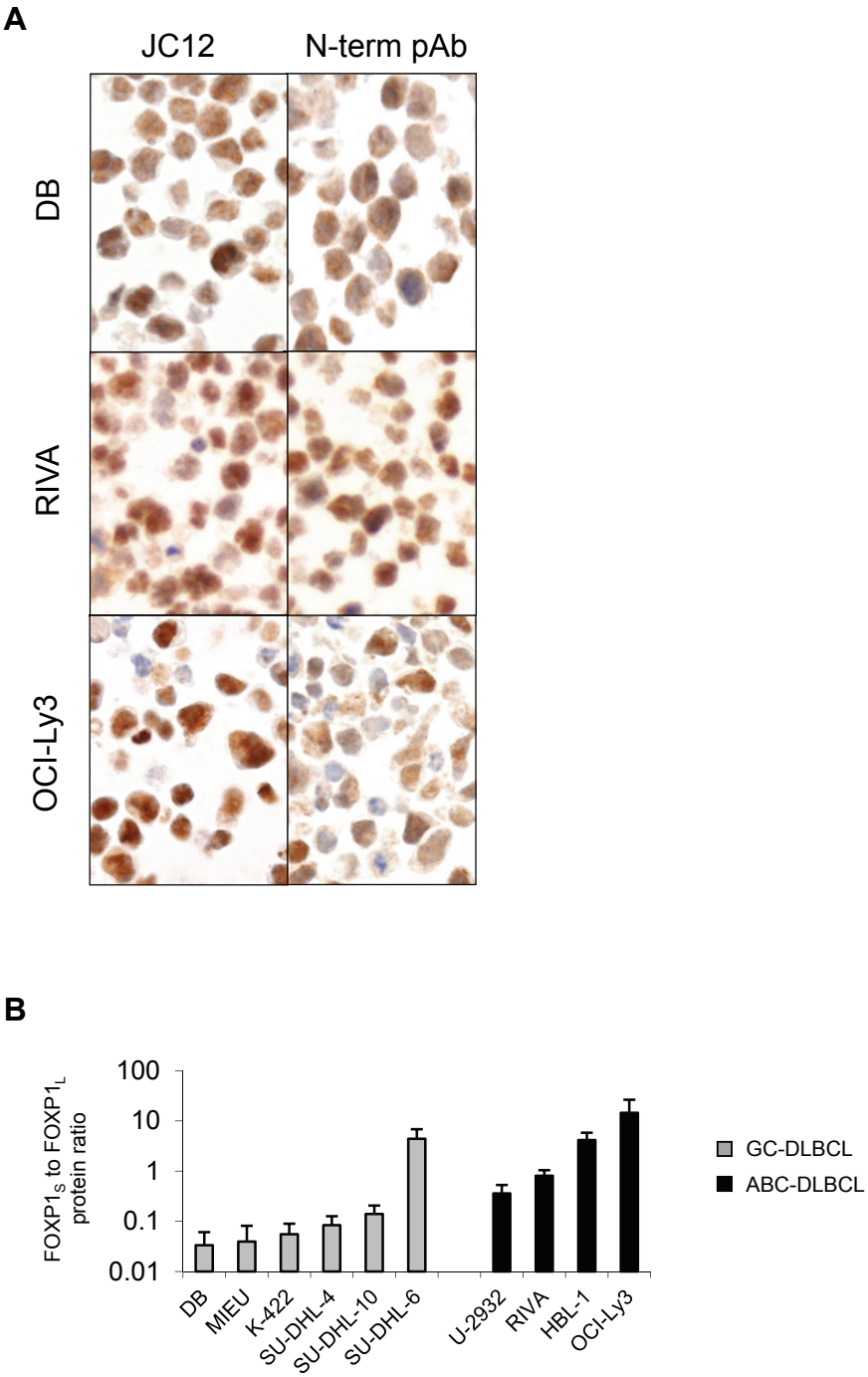




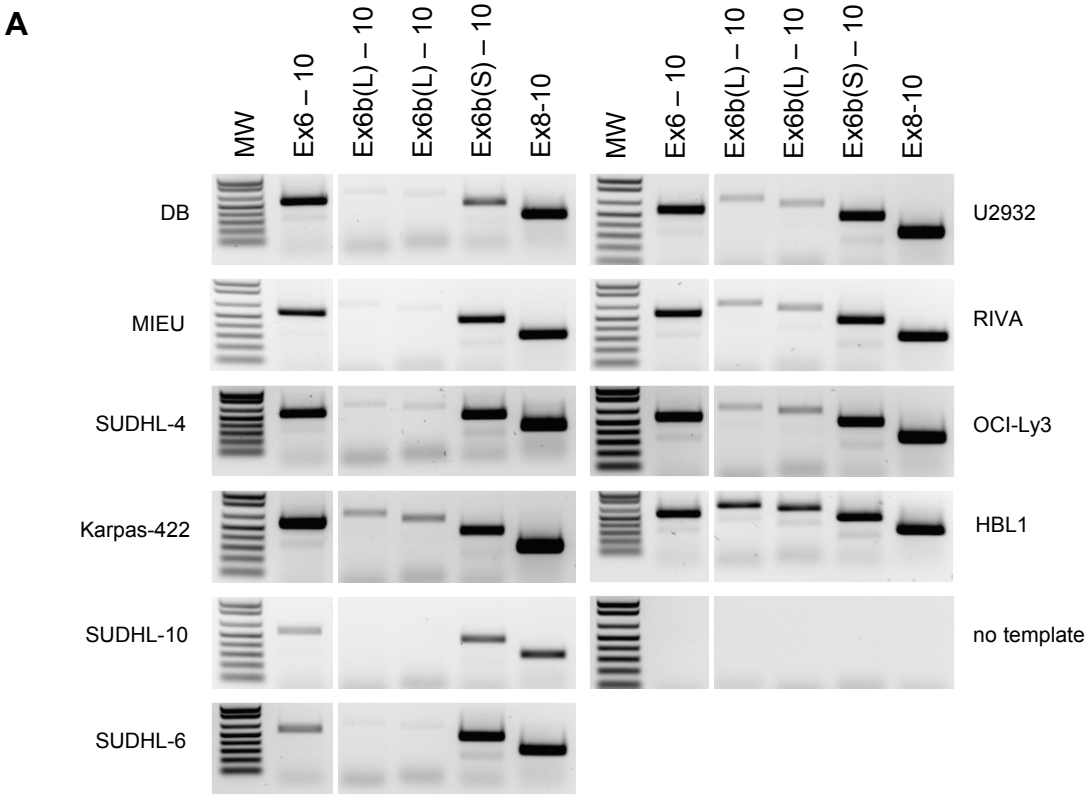


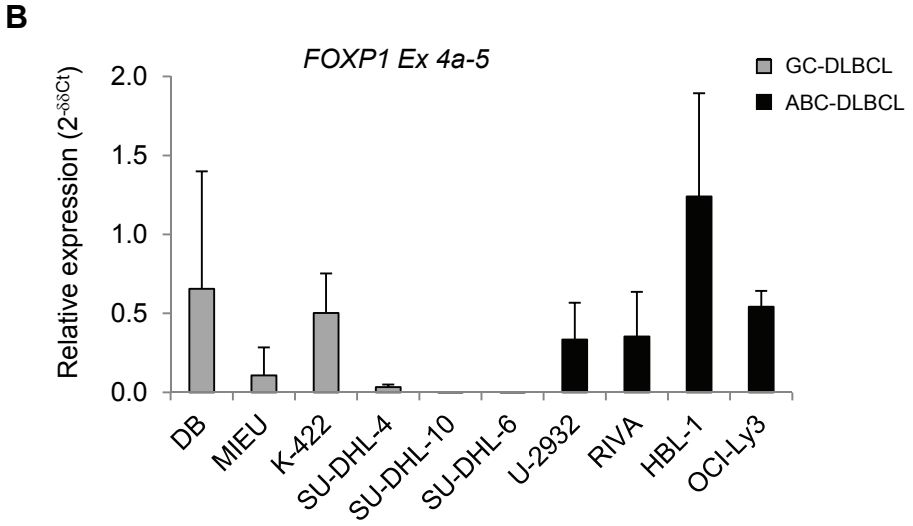


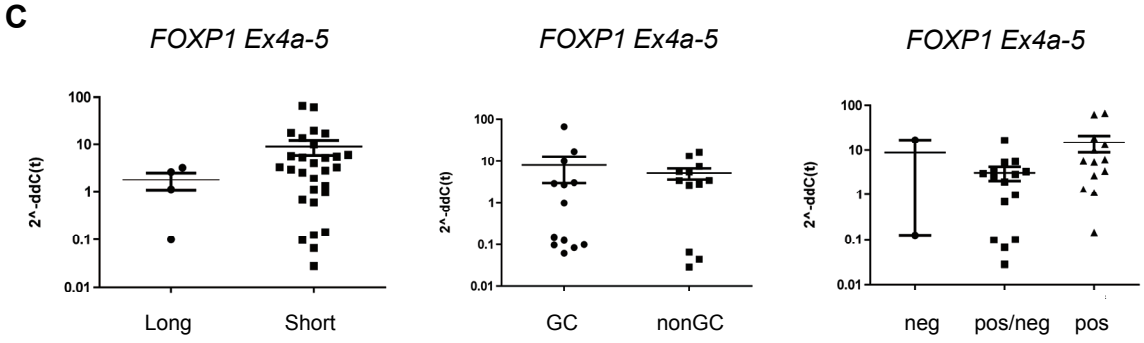
Supplemental Figure 2



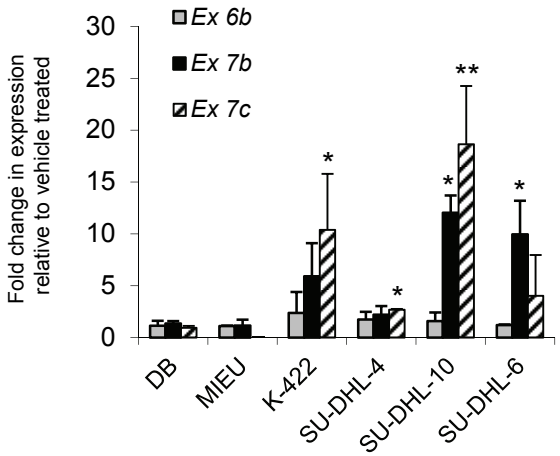
Supplemental Figure 3



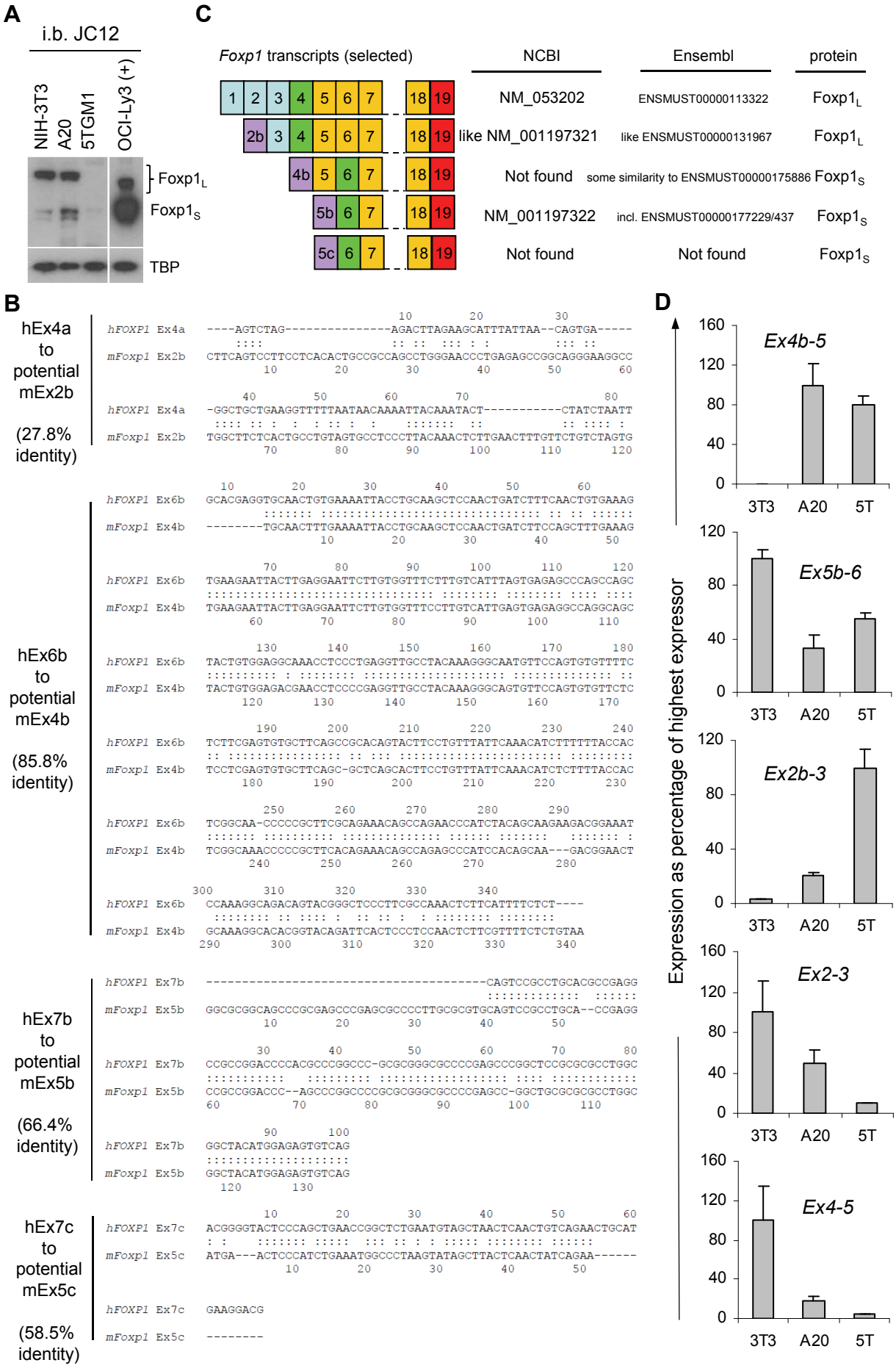




Supplemental Figure 4

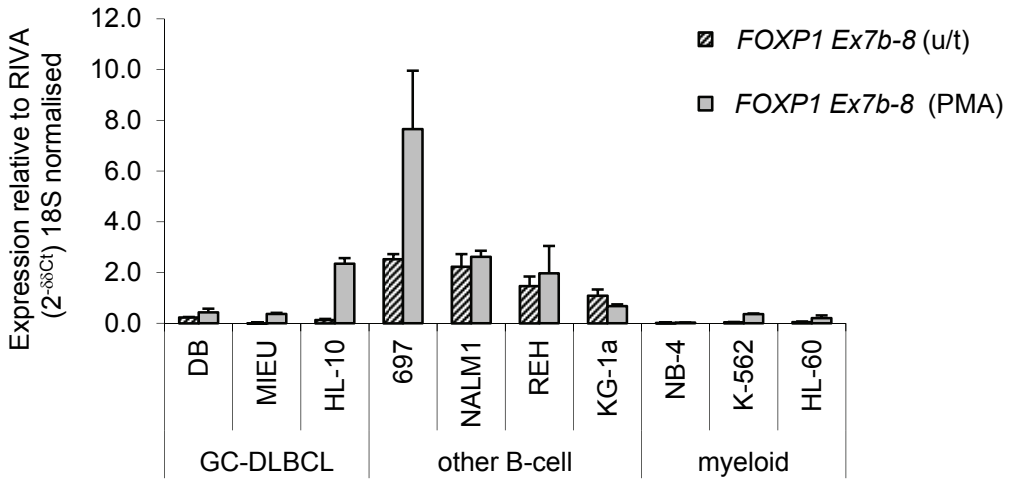


Supplemental Figure 5



Supplemental Figure 6

A



B