

SACO (Serial Analysis of Chromatin Occupancy)

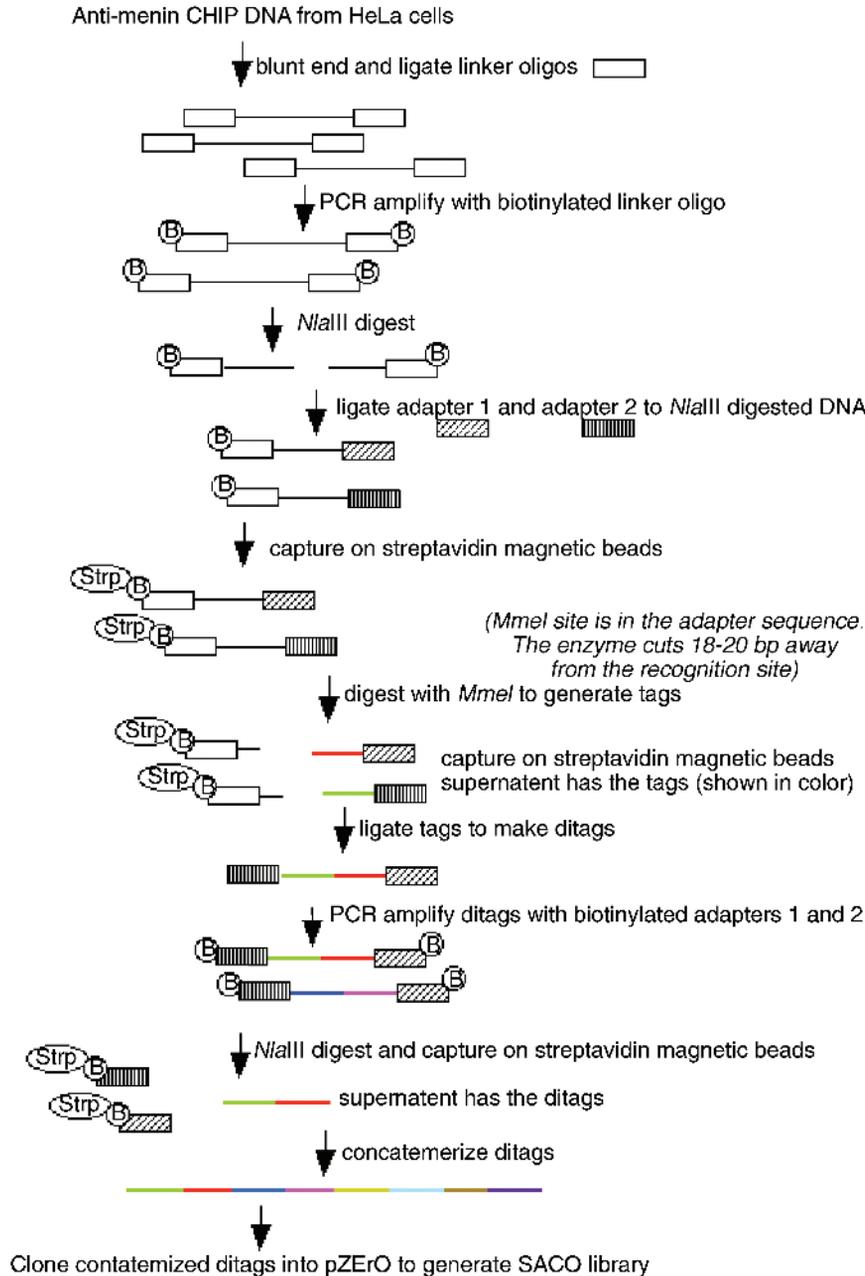


Figure W1. Scheme for the construction of the menin-SACO library. A SACO library was prepared using antimenin ChIP DNA obtained from 6×10^7 HeLa cells [18]. Duplex DNA adapters were ligated to blunt-ended ChIP DNA using T4 DNA ligase (NEB, Beverly, MA) and PCR-amplified using biotinylated adapter primers. Approximately 10 μ g of amplified ChIP DNA was digested with *NlaIII* (NEB) at 37°C for 2 hours. The digest was purified by phenol/chloroform extraction and EtOH precipitation. A modified version of the Long-SAGE protocol [22] was used to create ditags. Half of *NlaIII*-digested ChIP DNA was ligated to duplex Long-SAGE adaptor A, and the other half was ligated to duplex Long-SAGE adaptor B for 12 hours at 16°C. Ligations were purified over a QIAquick PCR column and bound to streptavidin-coated magnetic beads (DynaM M280, Oslo, Norway). After extensive washing in BW buffer (5 mM Tris pH 8.0, 1 M NaCl, 0.2 mg/ml BSA, and 0.5 mM EDTA), the tags were released from the beads by digesting with *MmeI* (NEB) for 2.5 hours at 37°C (Long-SAGE adaptors possess *MmeI* recognition site). Supernatants corresponding to Long-SAGE adaptor A and adaptor B tubes were pooled, the tags were purified by phenol/chloroform extraction and EtOH precipitation, and the pellets were air-dried. To obtain ditags, the air-dried pellet consisting of *MmeI*-digested tags was incubated with T4 DNA ligase. The ditags were amplified by PCR using biotinylated Long-SAGE adaptor primers. The amplified ditags were bound to streptavidin-coated magnetic beads (DynaM M280). After extensive washing in BW buffer (5 mM Tris pH 8.0, 1 M NaCl, 0.2 mg/ml BSA, and 0.5 mM EDTA), the ditags were released from the beads by digesting with *NlaIII* (NEB). The ditags contained in the supernatant were purified by phenol/chloroform extraction and EtOH precipitation, the ditags were separated by polyacrylamide gel electrophoresis (PAGE), and the band corresponding to the ditags was excised and purified. To generate concatemers of the ditags, PAGE-purified ditags were ligated using T4 DNA ligase for 2 to 3 hours at 16°C. Ditag concatemers were isolated by running on an agarose gel and purification from the gel (Qiagen). Concatemers were cloned into pZER0 (Invitrogen) kanamycin vector and transformed by electroporation into E. coli 10G electrocompetent cells (Lucigen). This antimenin plasmid SACO library was titered, and glycerol stocks were prepared from transformed bacteria. The average number of ditags in the plasmids was analyzed by PCR using vector primers flanking the insert.

Table W1. Menin-Related Expression of Target Genes Identified By Menin–SACO Analysis Compared with Previously Reported Menin Targets.

Gene Name	Description	Expression*
<i>ABL1</i>	V-abl Abelson murine leukemia viral oncogene homolog 1	Islet tumor down
<i>BTN2A1</i>	Butyrophilin, subfamily 2, member A1	Islet tumor down
<i>CD47</i>	CD47 antigen (Rh-related antigen, integrin-associated signal transducer)	Islet tumor down
<i>GAB2</i>	GRB2-associated binding protein 2	Islet tumor down
<i>MUC1</i>	Mucin 1, transmembrane	Islet tumor down
<i>NFIX</i>	Nuclear factor I/X (CCAAT-binding transcription factor)	Islet tumor down
<i>PVT1</i>	Pvt1 oncogene homolog, MYC activator (mouse)	Islet tumor down
<i>SART3</i>	Squamous cell carcinoma antigen recognized by T cells 3	Islet tumor down
<i>LDHB</i>	Lactate dehydrogenase B	Islet tumor up
<i>PRKCBP1</i>	Protein kinase C–binding protein 1	Islet tumor up
<i>SLC23A1</i>	Solute carrier family 23 (nucleobase transporters), member 1	PT adenoma up
<i>ANP32A</i>	Acidic (leucine-rich) nuclear phosphoprotein 32 family, member A	Null MEF (+menin) up
<i>CASP8</i>	Caspase 8, apoptosis-related cysteine protease	Null MEF (+menin) up
<i>DDR2</i>	Discoidin domain receptor family member 2	Null MEF (+menin) up
<i>SORBS1</i>	Sorbin and SH3 domain–containing protein 1	Null MEF (+menin) up

Islet tumor up or down = upregulated or downregulated in human MEN1 islet tumor *versus* normal human islets [15]; PT adenoma up = upregulated in human MEN1 parathyroid adenoma *versus* normal human parathyroids [16]; null MEF (+menin) up = upregulated in menin-null MEFs *versus* menin-transfected menin-null MEFs [7,13].

*Expression data from the literature. Three hundred nineteen differentially regulated genes were considered for comparison: 18 from vector-transfected *versus* MEN1-transfected human endocrine pancreatic cell line BON1 [14]; 49 from vector-transfected *versus* MEN1-transfected menin-null MEFs [7,12,13]; 5 from *Men1^{+/+}* *versus* *Men1^{-/-}* mouse embryos [5]; 63 from normal human parathyroids *versus* parathyroid adenomas [16]; and 189 from normal human islets *versus* MEN1 neuroendocrine tumors [15].

Table W2. AP1-Regulated* Menin Target Genes Identified By Previously Published Literature and Menin–SACO Analysis.

Gene	Description
<i>APP</i>	Amyloid beta (A4) precursor protein (protease nexin II, Alzheimer's disease)
<i>DDR2</i>	Discoidin domain receptor family, member 2
<i>DSCR1L1</i>	Down syndrome critical region gene 1–like 1
<i>Hoxa10</i>	Homeobox A10
<i>PRDX4</i>	Peroxiredoxin 4
<i>SEC23A</i>	Sec23 homolog A (<i>Saccharomyces cerevisiae</i>)
<i>SERPINH1</i>	Serine (or cysteine) proteinase inhibitor, clade H (heat shock protein 47), member 1 (collagen-binding protein 1)
<i>SKI</i>	v-Ski sarcoma viral oncogene homolog (avian)
<i>ST5</i>	Suppression of tumorigenicity 5
<i>TRAP1</i>	Tumor necrosis factor receptor–associated protein 1

*AP1-regulated genes [34]; information on the direction of regulation is not known.

Table W3. Chromosome Number: Nucleotide Position of GSTs with Gene Names and Controls as Analyzed By ChIP-PCR in Figure 1.

Location of fifty-one 5' end-associated GSTs	Fold over IgG	Location of 26 GSTs "inside" genes	Fold over IgG
<i>Median GST location</i>		<i>Median GST location</i>	
ch1:100426847 (DBT)	5.8	ch12:52651775 (Hoxc11)	4.8
ch1:101202267 (CGI-30)	7.9	ch12:52667658 (Hoxc10)	7.5
ch1:10465668 (DFFA)	6.1	ch12:52673762 (Hoxc10)	14
ch1:143006465 (PEX11B)	5.5	ch12:52690613 (Hoxc8)	2.6
ch1:147949837 (SB145)	6.7	ch12:52692977 (Hoxc8)	2.3
ch1:170416438 (KLHL20)	7.2	ch12:52695701 (Hoxc4)	5
ch1:227422719 (ARV1)	6.7	ch12:52725216 (Hoxc4)	2.5
ch1:26910232 (GPATC3)	10	ch7:26984196 (Hoxa10)	12
ch1:59029955 (FLJ30588)	3.2	ch6:1616101 (GMDS)	2.5
ch1:85435979 (GM117)	4.6	ch6:2046356 (GMDS)	7.7
ch1:85759792 (CYR61)	6.5	ch6:2102074 (GMDS)	10
ch3:196644888 (CENTB2)	5.2	ch6:2157361 (GMDS)	4.8
ch3:197552450 (BC013113)	2	ch6:15357484 (JARID)	4.9
ch4:101223598 (EGNR9427)	7.9	ch6:15398404 (JARID)	3.4
ch4:57176486 (SRP72)	5.4	ch6:15518265 (JARID)	2
ch5:107032622 (EFNA5)	5.9	ch6:15562026 (JARID)	2.6
ch5:134102719 (CAMLG)	5.3	ch5:58338386 (PDE4D)	4.2
ch5:180166440 (MGAT1)	3.1	ch5:58371112 (PDE4D)	5.2
ch5:947062 (BRD9)	6.1	ch5:58423248 (PDE4D)	7
ch6:27547990 (ZNF184)	5.8	ch5:59097976 (PDE4D)	16.5
ch6:33274120 (RXRB)	4.2	ch1:63975209 (ROR1)	1.8
ch6:37896675 (TEX27)	4.4	ch1:64020745 (ROR1)	1.7
ch6:46427484 (DSCR1L1)	0.4	ch1:64182024 (ROR1)	2.1
ch7:32308834 (KIAA0241)	7.7	ch1:107859935 (VAV3)	2.7
ch7:50632954 (GRB10)	6.7	ch1:107925042 (VAV3)	2.3
ch7:93181716 (NGT1)	2.6	ch1:107933694 (VAV3)	6.4
ch8:126173561 (FLJ32440)	6.7		
ch9:122105687 (RBM18)	6.8	<i>Negative control</i>	
ch9:26935646 (PLAA)	8	SI ch12:2997	1.1
ch9:74934923 (OSTF1)	7.2	SI H2A	1.3
ch10:115430829 (CASP7)	8.7	SI H2B	1.6
ch10:27026598 (TPRT)	0.1	SI ch12:6151	1.6
ch10:96111874 (FAD24)	5	SI LRP	1.3
ch11:116555642 (SIDT2)	6.4	SI MycP2	1.6
ch11:27484023 (LIN7C)	7.5	SI Tubulin	1.1
ch11:65868999 (BRMS1)	5.7		
ch11:8940803 (c11orf15)	6.5	<i>Positive control</i>	
ch12:119556407 (CABP1)	3	Hoxc8	3.1
ch12:54995030 (TMEM4)	6	Hoxa9	5.2
ch12:55127728 (TIMELESS)	5.5		
ch12:92275120 (NUDT4)	6.9		
ch15:38887917 (ZFYVE19)	6		
ch16:8798969 (PMM2)	3.9		
ch17:59931148 (DDX5)	5.3		
ch17:75623008 (TBC1D16)	6.1		
ch18:469846 (COLEC12)	3		
ch19:12998738 (NFIX)	4.4		
ch20:36129352 (C20orf77)	3.7		
ch20:6050653 (KIND1)	7.7		
ch21:43171577 (WDR4)	7.4		
M:11300 (mitochondrial)	1.4		