STEM CELL
EPIGENETICS

REGULATORS OF GENE TRANSCRIPTION

ACTIVE MOTIF®

STEM CELL EPIGENETICS

The two hallmark features of stem cells are pluripotency, the ability to differentiate into any mature cell type, and self-renewal, the capacity to undergo indefinite replicative cycles without losing stem cell identity. The general types of stem cells include embryonic stem cells (ESCs), adult stem cells and induced pluripotent stem cells (iPSCs). ESCs are derived from a population of cells in the blastocyst of a pre-implantation embryo called the inner cell mass that can differentiate into any cell type derivative of the three germ layers (endoderm, mesoderm and ectoderm). Adult stem cells can be found throughout the post-embryonic and adult organism, and function primarily in the maintenance, repair and regeneration of tissue and organs. iPSCs are pluripotent stem cells that are artificially derived when adult somatic cells are genetically reprogrammed to an embryonic stem cell phenotype.

A stem cell's decision whether to maintain its stem cell identity or differentiate into a specific cell type is ultimately determined by the outcome of the complex crosstalk that occurs between extracellular signaling pathways, transcriptional regulatory networks, chromatin remodeling complexes and non-coding RNAs. At the transcriptional level, pluripotency is largely controlled by the 'master regulators' OCT4, SOX2 and NANOG[1,2]. These transcription factors form the core of the ESC transcriptional network and are essential for induction and maintenance of the stem cell phenotype[1,3]. The primary cell signaling pathways involved in maintenance of pluripotency and self-renewal of ESCs are the WNT, TGFβ/ACTIVIN/NODAL and FGFR pathways[4,5]. These signaling pathways regulate the activity of OCT4, SOX2 and NANOG as well as auxiliary transcription factors and cofactors to drive the expression of stem cell-specific genes. During differentiation, other signaling pathways, such as BMP and NOTCH, signal the activation of the downstream expression of lineage-specific genes that promote the loss of pluripotency and diminish the proliferative potential of the cell[4,5,6].

For a comprehensive understanding of the mechanisms that control self-renewal and pluripotency, it is essential to look beyond transcriptional networks towards the post-translational epigenetic events that modulate gene expression. These stochastic events set the epigenetic landscape within the cell by creating global changes that define regulatory networks, chromatin rearrangements, and the positioning of nuclear domains that determine the accessibility and transcriptional potential of underlying genes[7,8,9]. Posttranslational epigenetic marks come in the form of acetylation, phosphorylation, methylation, citrullination and ubiquitination. By controlling the accessibility of DNA regulatory elements, these modifications modulate the interaction of transcription factor networks with other regulatory factors including transcriptional cofactors, chromatin remodeling proteins, histone modifiers, DNA methyltransferases and hydroxylases, and non-coding RNA regulators. This determines whether specific genes are actively transcribed, poised, or silenced at any given time[7,8,10]. Having a multilayered transcriptional control mechanism serves as a system of checks and balances that allows fine-tuning and adaptability of the gene expression profile of a stem cell. This flexibility is the key to pluripotency, endowing stem cells the versatility to quickly modify gene expression in response to developmental and environmental cues, and to differentiate into essentially any cell type in the adult.

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4. Boiani, M. and Schöler, H.R. (2005) *Nat Rev Mol Cell Biol.* **6**: 872-884.
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6. Varga, A.C. and Wrana, J.L. (2005) *Oncogene* **24**: 5713-5721.
7. Young, R.A. (2011) *Cell* **144:** 940-954.
8. Han, J.W. and Yoon, Y.S. (2012) *Antioxid Redox Signal* **17:** 205-223.
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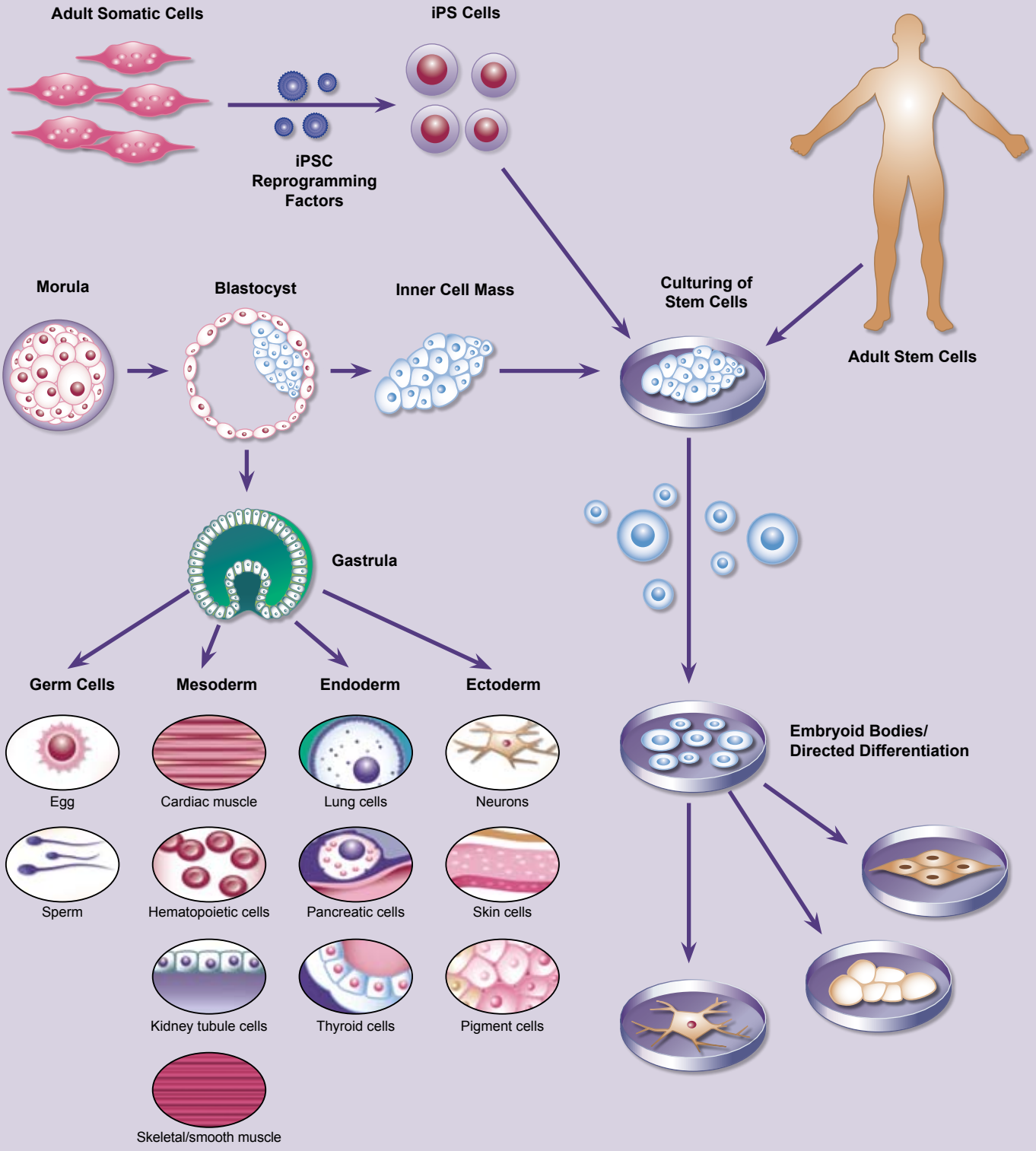
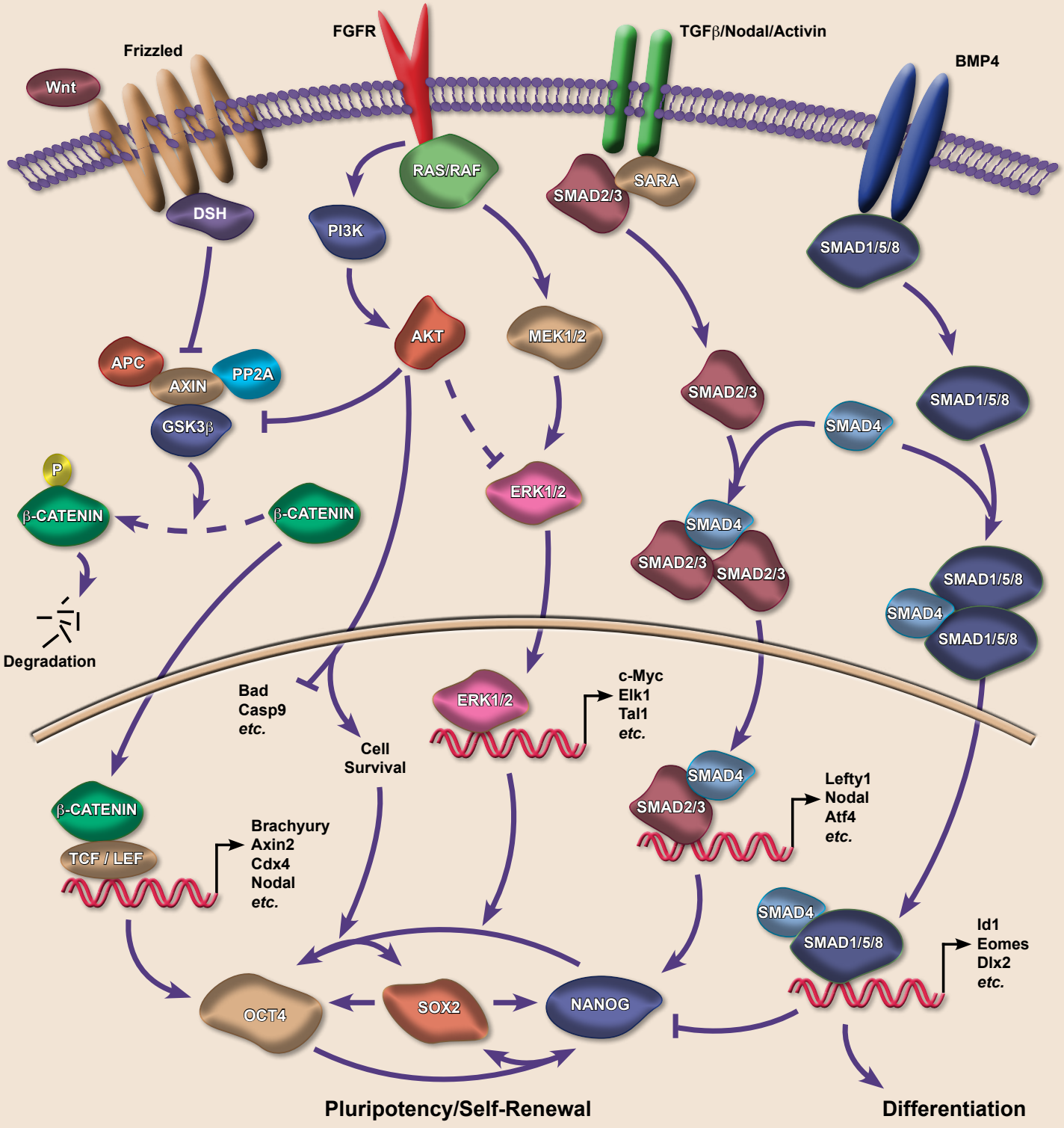
The Fate of Stem Cells



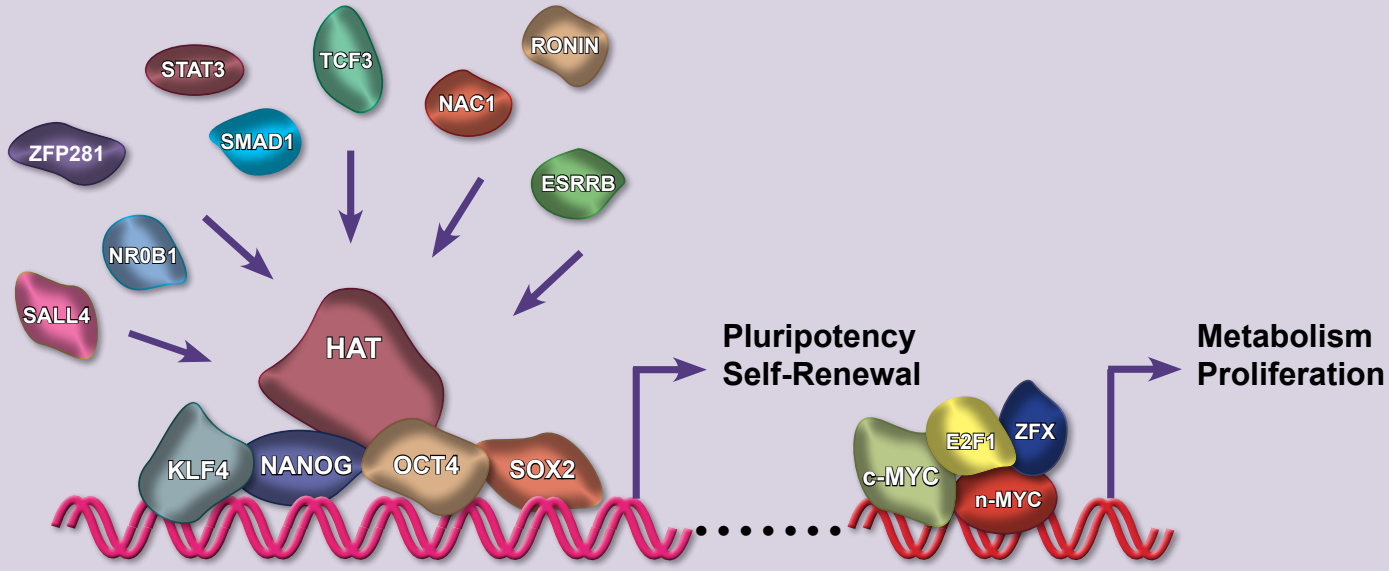
Cell Signaling Pathways Controlling Pluripotency and Self-Renewal



This diagram depicts the interaction between key intracellular signaling pathways regulating stem cell pluripotency and self-renewal.

Transcriptional Networks and Stem Cell Identity

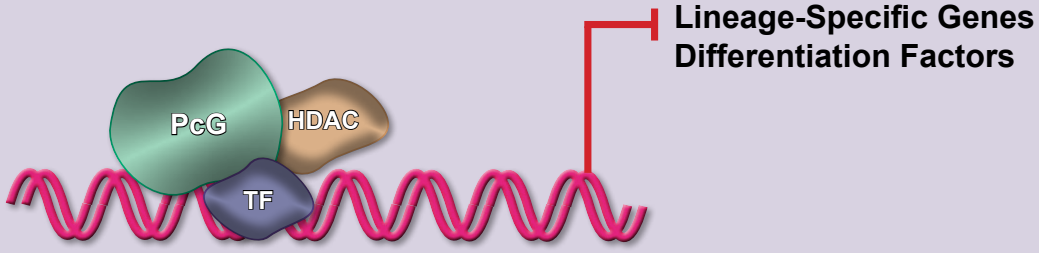
ACTIVATION



OCT4 and SOX2 Transcription Factor Network

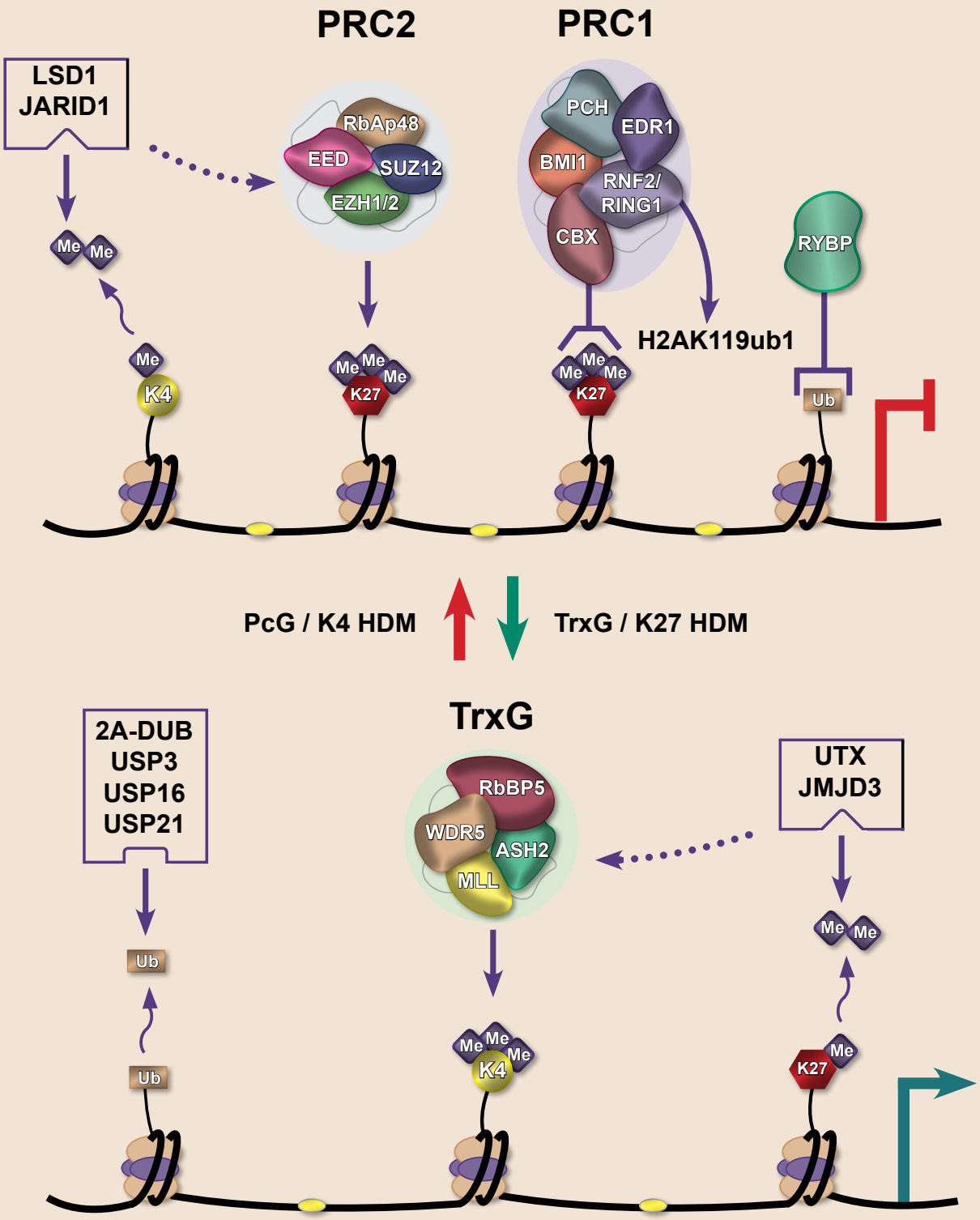
c-MYC Transcription Factor Network

REPRESSION



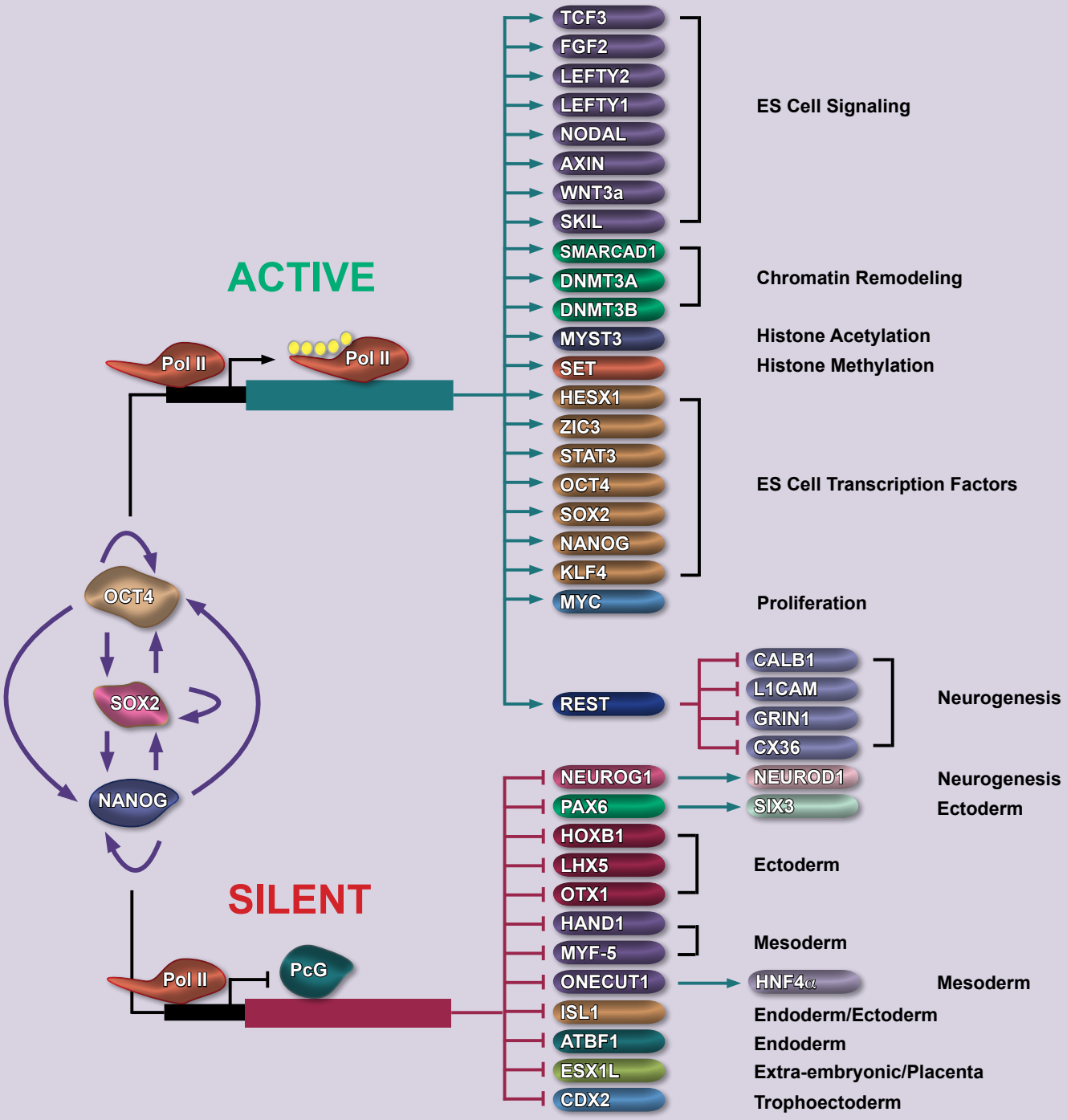
A transcriptional network built around the master regulators OCT4, SOX2 and NANOG co-occupies promoter regions of genes regulating pluripotency and self-renewal. Recruitment of coactivators, such as histone acetyl-transferases (HATs), signals transcriptional *activation.* In contrast, c-MYC functions to regulate the transcriptional efficiency of POLII, as well as to co-occupy and regulate genes involved in metabolism and proliferation. The subset of transcription factors (TF) co-occupying promoters varies in response to epigenetic and intracellular signals. Low-level occupancy of promoters by reprogramming factors signals transcriptional *repression* and is accompanied by recruitment of corepressors, including the histone deacetylase (HDACs) complexes NuRD, NCoR/SMRT, SIN3A and REST, as well as Polycomb Group (PcG) proteins. In stem cells, this leads to repression of the somatic cell program.

Transcriptional Regulation by Polycomb and Trithorax Groups



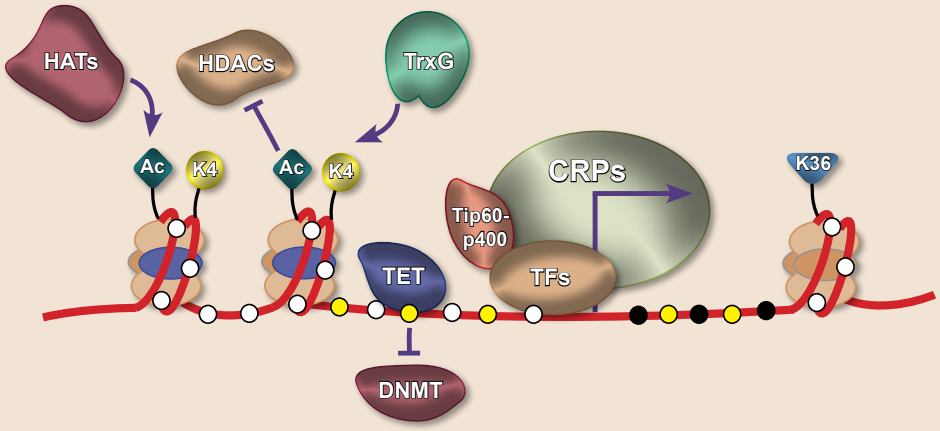
This diagram depicts the antagonistic relationship between the repressive Polycomb Group (PcG) and activating Trithorax Group (TrxG) proteins in regulating the transcriptional dynamics of embryonic stem cells. PcG gene silencing results from PRC2-mediated trimethylation of H3K27 by the histone methyltransferase (HMT) EZH1/2. H3K27me3 is recognized by the chromobox (CBX) subunit of PRC1, which leads to recruitment of PRC1 to the chromatin and the subsequent ubiquitination of H2AK119 via RNF2/RING1 ubiquitin ligase. The RYBP repressor protein recognizes H2A mono-ubiquitination, contributing to transcriptional repression. Opposing this activity, TrxG recruitment of HMTs, such as MLL, mediate trimethylation of H3K4, leading to transcriptional activation and inhibition of PcG binding. The respective histone demethylases (HDMs) and deubiquitinating enzymes (DUB) are also shown.

The Transcriptional Regulatory Circuitry and Nuclear Reprogramming



This transcriptional wiring diagram represents the core transcriptional regulatory circuitry in human embryonic stem cells based on expression data for OCT4, SOX2 and NANOG target genes. The core transcription factor interconnected autoregulatory loop is depicted on the left and the activation and silencing of specific gene promoters and corresponding gene products (middle) are also shown. (Permission for use of this image was kindly provided by Dr. Rudolf Jaenisch, Professor of Biology at the Whitehead Institute for Biomedical Research at MIT).

Epigenetic Control of Chromatin Remodeling In Stem Cells

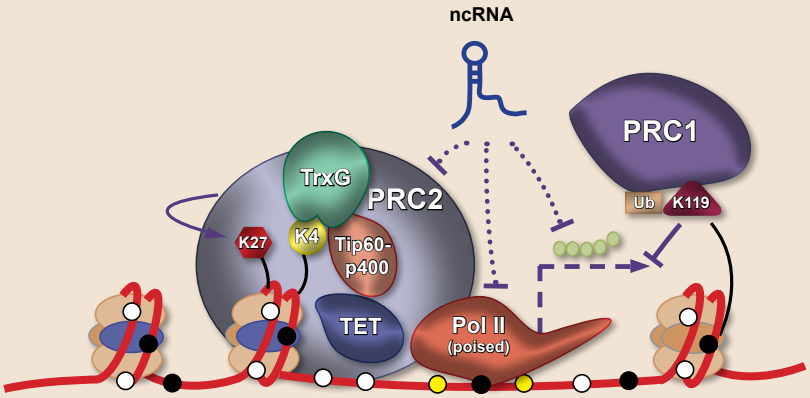


Active Chromatin
(Euchromatin)

Signaling and stem cell maintenance proteins
FGF8, FGFR3, Lefty1, Inhba, Ezh1, *etc.*

Transcription factors
Stat3, Tcf3, Sall4, Esrrb, *etc.*

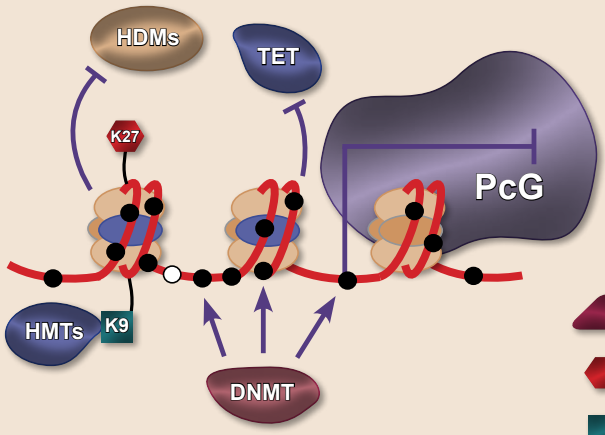
Regulators of proliferation/homeostasis
c-Myc, Tbx3, p53, GAPDH, Evi1, miR302-367, *etc.*



Poised Chromatin
(Permissive/Repressed)

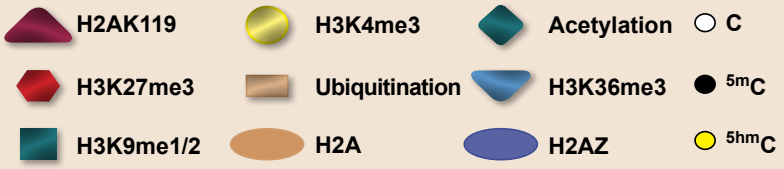
Core Transcription factors
Oct4, Sox2, Nanog, *etc.*

Early developmental regulators
Myf5, MyoD, Brachyury, Irx3, Pax6, *etc.*



Silent Chromatin
(Heterochromatin)

Lineage specification factors
hCG, Adcy4, Anxa1, Actn1, Ebf1, *etc.*



In embryonic stem cells, H3K4 & H3K36 methylation and H3 & H4 acetylation are characteristic, active marks exclusively found within euchromatin. In addition, DNA regulatory elements of active genes are characterized by DNA hypomethylation. In combination, these modifications function to neutralize histone charges and recruit chromatin remodeling proteins (CRPs) that lead to unraveling of the chromatin structure, allowing access to the basal transcriptional machinery. In contrast, silenced genes are associated with condensed heterochromatin and are characteristically marked by H3K27 & H3K9 methylation and DNA hypermethylation.

'Bivalent domains,' where both activating H3K4 and repressive H3K27 methylation are present, mark genomic loci of early developmental regulators and HOX genes. These opposing marks silence genes while keeping them 'poised' for activation. Together, the cofactors and regulatory proteins effecting these epigenetic modifications define the chromatin landscape that dictates the expression profile of the cell.

TrxG, Trithorax group; PcG, Polycomb group; HATs, Histone acetyltransferases; HDACs, Histone deacetylases; TFs, Transcription factors; HDMs, Histone demethylases; DNMT, DNA methyltransferase; TET, Ten-eleven translocation enzymes; HMTs, Histone methyltransferases; PRC, Polycomb Repressive Complex.

Stem Cell Epigenetics Antibodies

Description	Applications	Cat. No.
MASTER REGULATORS		
c-Myc pAb	WB	39012
KLF4 pAb	WB	39745
LIN28A pAb	WB	61191
Oct-4 pAb	WB	39811
Sox2 pAb	Ch, IF, IHC, IP, WB	39823
POLYCOMB GROUP		
BMI-1 mAb	Ch, IP	39993
CBX8 pAb	WB	61237
EED mAb	IHC, WB	61203
EZH2 mAb	Ch, IF, IP	39875
EZH2 phospho Thr345 pAb	DB, WB	61241
GCN5 mAb	ELISA, IF, WB	39975
PCL2 mAb	WB	61153
Phc1 mAb	IF, IP, WB	39723
Phc2 mAb	Ch, IF, IP	39661
Ring1B mAb	Ch, IF, IP, WB	39663
Suz12 pAb	Ch, WB	39357
TRITHORAX GROUP		
ASH2 pAb	IF, IP, WB	39099
MLL pAb	Ch, WB	61295
MLL1/HRX mAb	Ch, IP, WB	39829
TRANSCRIPTION & REPROGRAMMING		
AKT1 phospho Ser473 mAb	WB	40902
DAX-1 / NR0B1 mAb	ICC, IF, IHC, IP, WB	39983
FOXO1/FKHR pAb	WB	39629
GATA-1 pAb	Ch, WB	39025
GATA-4 pAb	WB	39893
GATA-6 pAb	WB	61063
GLI1 pAb	WB	61215
Goosecoid pAb	WB	61121
HNF-3β/ FOXA2 pAb	IHC, WB	39827
HNF4A pAb	WB	61189
HOXA9 pAb	WB	39825
KLF5 pAb	WB	61099
KLF6 mAb	IHC, WB	61297
Myf-5 mAb	IF, WB	39801
MyoD mAb	IF, WB	39991
N-Myc pAb	WB	61185
NKX2.5 pAb	WB	61267
Notch1 mAb	WB	61147
Notch3 mAb	WB	61149
NR2C2 pAb	WB	61279
p53 pAb	Ch, EMSA	39334
PAX7 mAb	IF, WB	39803
PBX1b mAb	Ch, IHC, IP, WB	61165
PDX1 pAb	WB	61289
PLZF mAb	Ch, IF, WB	39987
PP2A pAb	Ch, IP, WB	39192
RNA pol II mAb	Ch IF, IP, WB	39097
SALL4 pAb	WB	39957
SIP1 mAb	IF, IHC, IP, WB	61095
SMAD3 pAb	WB	61249
Sox11 pAb	WB	61181
Sp1 pAb	Ch, WB	39058
STAT3 phospho Ser727 pAb	DB, WB	39613
STAT3 phospho Tyr705 pAb	DB, WB	39595
TAL-1 mAb	EMSA, IHC, WB	61259
TAZ / WWTR1 pAb	WB	61265
TCF7L1 / TCF3 pAb	WB	61125
UTF1 pAb	WB	61253
YY1 pAb	Ch, WB	39071
EPIGENETICS & CHROMATIN REMODELING		
5-Carboxylcytosine (5-caC) pAb	DB	61229
5-Formylcytosine (5-fC) pAb	DB, IF	61223
5-Hydroxymethylcytosine (5-hmC) mAb	DB, MeDIP	39999
5-Hydroxymethylcytosine (5-hmC) pAb	DB, IF, IHC, MeDIP	39769
5-Methylcytosine (5-mC) mAb	DB, FACS, IHC, IP, MeDIP	39649
Ago1/2/3 mAb	Ch, IF, IHC, IP, WB	39937
BRG-1 mAb	IF, WB	39807
BRM mAb	Ch, IF, WB	39805
CGBP pAb	WB	39203
CHD1 pAb	Ch, WB	39729
CoREST pAb	WB	39955
Dicer mAb	WB	39817
DNMT1 mAb	Ch, IHC, IP, WB	39204

Description	Applications	Cat. No.
EPIGENETICS & CHROMATIN REMODELING, cont		
DNMT2 pAb	WB	39205
DNMT3A mAb	Ch, IF, IHC, WB	39206
DNMT3B mAb	Ch, IF, IP, WB	39207
Drosha pAb	WB	39783
HDAC1 pAb	Ch, ChC, ChS, WB	40967
Histone H2A pAb	WB	39209
Histone H2AX pAb	IF, WB	39689
Histone H2AX phospho Ser139 pAb	DB, IF, WB	39117
Histone H2A.Z pAb	Ch, WB	39113
Histone H3, C-terminal pAb	Ch, WB	61277
Histone H3 mAb	Ch, IF, WB	39763
Histone H3 monomethyl Lys4 pAb	Ch, ChC, ChS, DB, IF, WB	39297
Histone H3 dimethyl Lys4 pAb	Ch, ChC, ChS, DB, WB	39141
Histone H3 trimethyl Lys4 pAb	Ch, ChC, ChS, DB, IF, WB	39159
Histone H3 acetyl Lys9 pAb	Ch, ChC, ChS, DB, IF, WB	39917
Histone H3 dimethyl Lys9 pAb	Ch, DB, IF, WB	39239
Histone H3 pan-methyl Lys9 pAb	DB, IF, WB	39241
Histone H3 trimethyl Lys9 mAb	Ch, DB, IF, IP, WB	61013
Histone H3 trimethyl Lys9 pAb	Ch, ChC, ChS, DB, IF, WB	39161
Histone H3 acetyl Lys27 pAb	Ch, ChC, ChS, DB, IF, WB	39133
Histone H3 di/trimethyl Lys27 mAb	Ch, ChC, ChS, WB	39535
Histone H3 trimethyl Lys27 pAb	Ch, DB, ELISA, WB	39156
Histone H3 trimethyl Lys36 pAb	Ch, ChC, ChS, DB, WB	61101
HMGA1 pAb	IF, WB	39615
HP1α mAb	Ch, ELISA, ICC, IF, IHC	39977
LSD1 pAb	Ch, ChC, ChS, IP, WB	39186
MBD2 pAb	WB	39547
MBD3 mAb	WB	39216
MeCP2 mAb	Ch, IF, IHC, IP, WB	61291
Mili / PiwiL2 mAb	IF, IHC, IP, WB	61143
MMSET / WHSC1 mAb	Ch, IF, IP, WB	39879
PHF8 pAb	WB	39711
PRMT5 pAb	WB	61001
PRMT6 pAb	WB	61003
SATB1 pAb	WB	39839
SIN3A pAb	Ch, WB	39865
SIRT1 mAb	IF, IP, WB	39353
SMRT / NCoR2 mAb	WB	61105
SUV39H1 mAb	Ch, IP, WB	39785

Applications Key

Ch	Chromatin immunoprecipitation
ChC	ChIP-chip
ChS	ChIP-Seq
DB	Dot blot
ICC	Immunocytochemistry
IF	Immunofluorescence
IHC	Immunohistochemistry
IP	Immunoprecipitation
WB	Western blot

For an up-to-date list of available stem cell antibodies, please visit www.activemotif.com/stemcellabs.

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