Figure S1

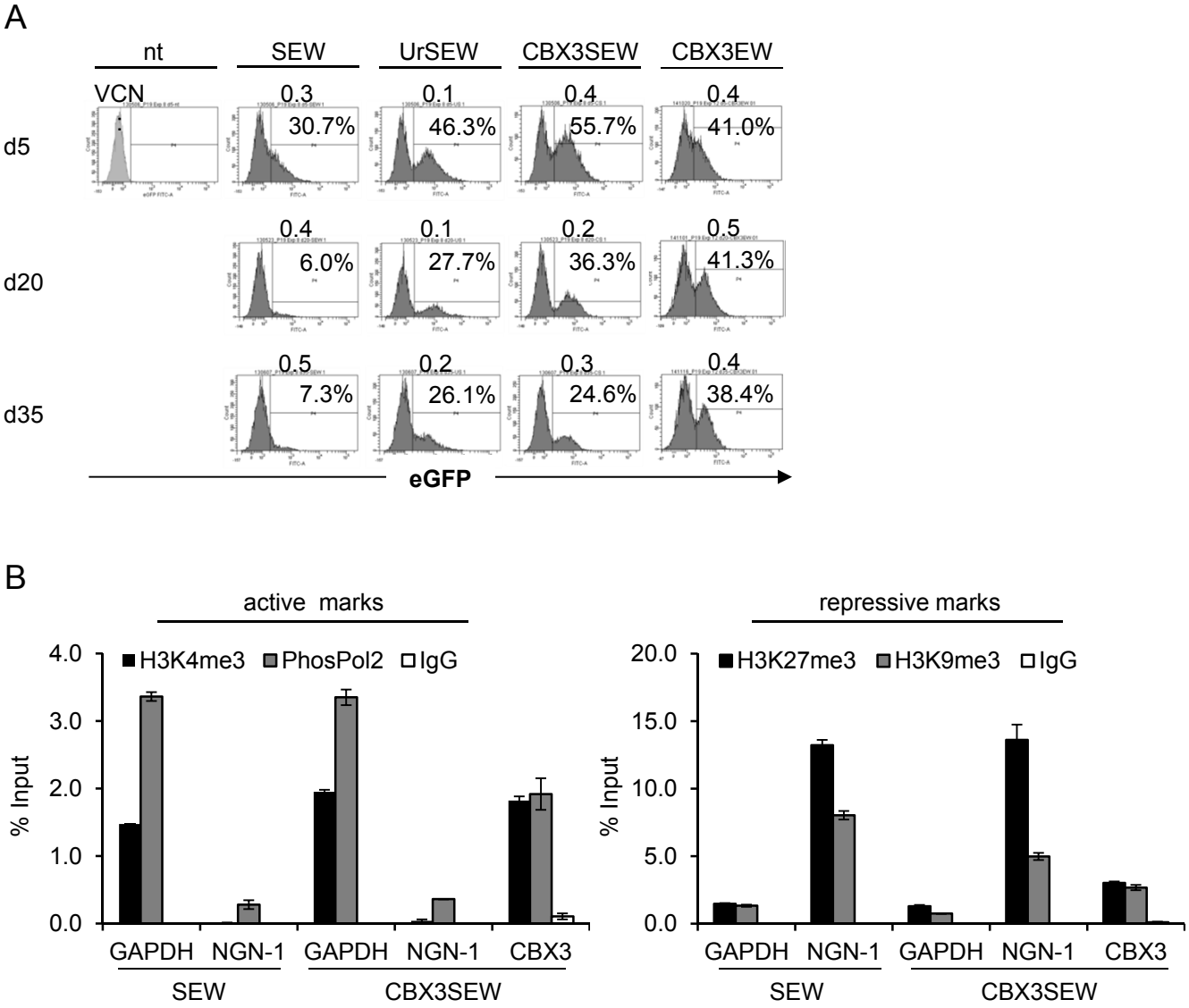


Figure S1. The CBX3-UCOE stabilizes transgene expression in the murine embryonic carcinoma cell line P19. (A) P19 cells were transduced with SEW, UrSEW, CBX3SEW and CBX3EW. EGFP expression was monitored over time by flow cytometry and VCN was determined in unsorted cells by qRT-PCR. **(B)** Active and repressive chromatin marks were quantified 35 days after transduction by ChIP experiments at control regions within the actively transcribed GAPDH promoter and the repressed NGN-1 locus in P19 cells. These experiments served as control for the data shown in Figure 2C. In CBX3SEW transduced cells also the chromatin status of the CBX3-UCOE within the vector was analyzed in parallel (representative results shown, n=2, mean ± S.D).

Figure S2

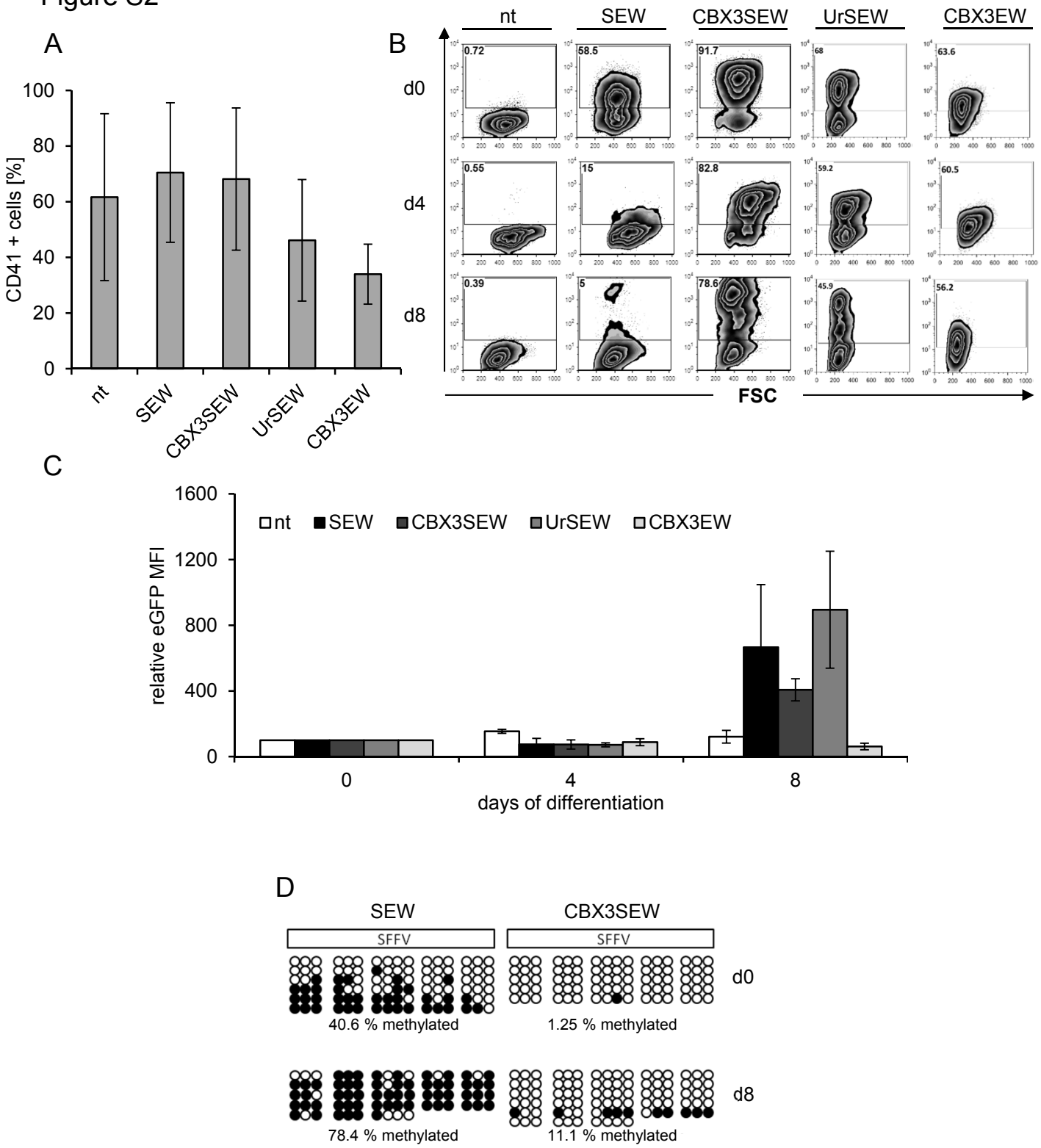


Figure S2. The CBX3-UCOE mediates stable transgene expression during hematopoietic differentiation of murine ES-cells (CCE). Murine ESCs were transduced with SEW, CBX3SEW, URSEW and CBX3EW lentiviral vectors and differentiated towards hematopoietic stem/progenitor cells following an EB-based protocol. **(A)** Expression of the early hematopoietic marker CD41 was analyzed at day 8 after transduction and differentiation (n=4, mean ± S.D.). **(B)** Transgene expression was analyzed by flow cytometry at day 0, 4 and 8 of differentiation (n=3, representative experiment shown). **(C)** MFI of eGFP positive cells determined by flow cytometry. Values at days 4 and 8 are given in comparison to the MFI at day 0 (n=3, mean ± S.D.). **(D)** DNA methylation at the SFFV promoter was analyzed using bisulfite PCR in non-differentiated cells (d0) and their hematopoietic progeny (d8).

Figure S3

Sequence alignment of wt CBX3 and CBX3*

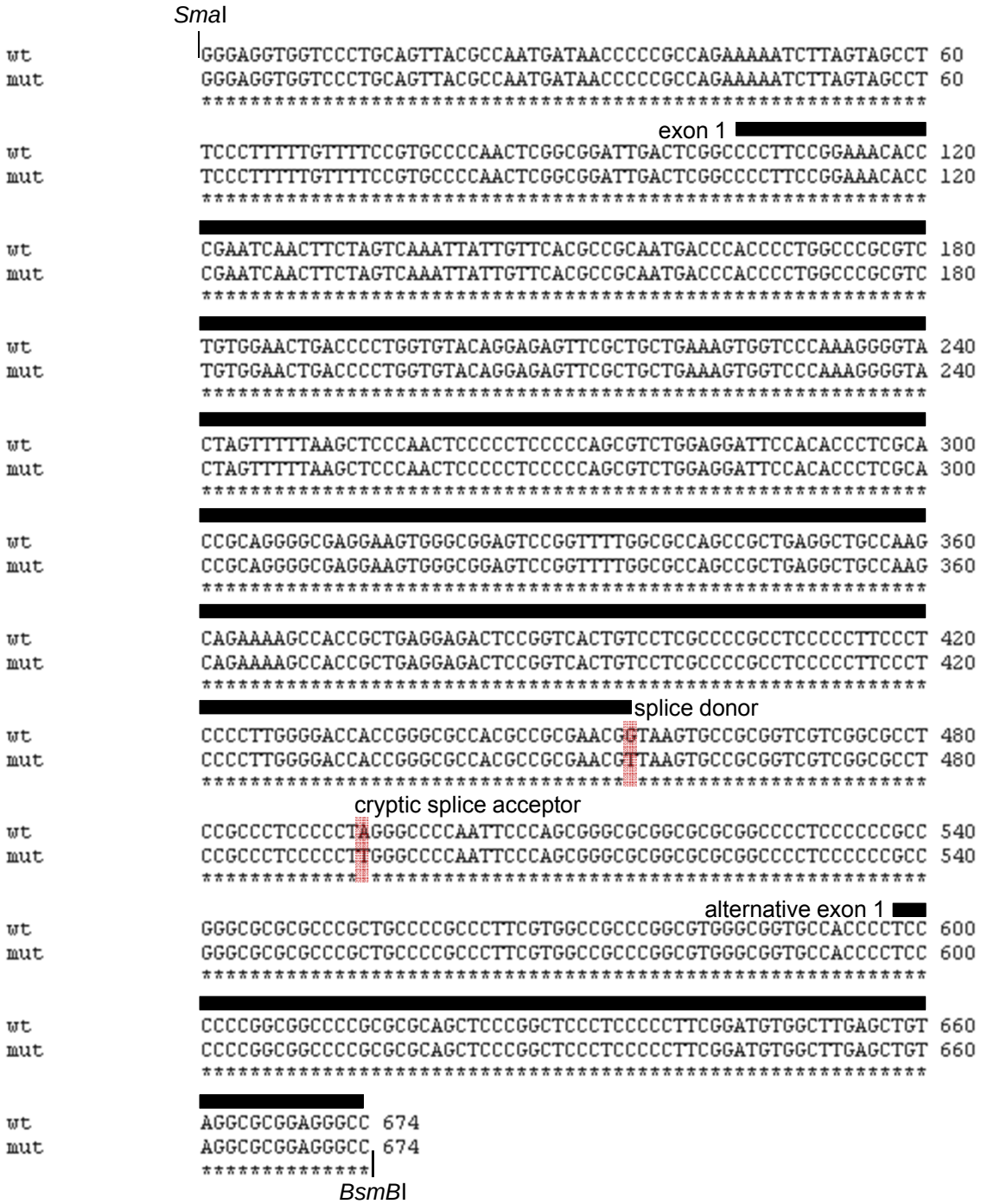
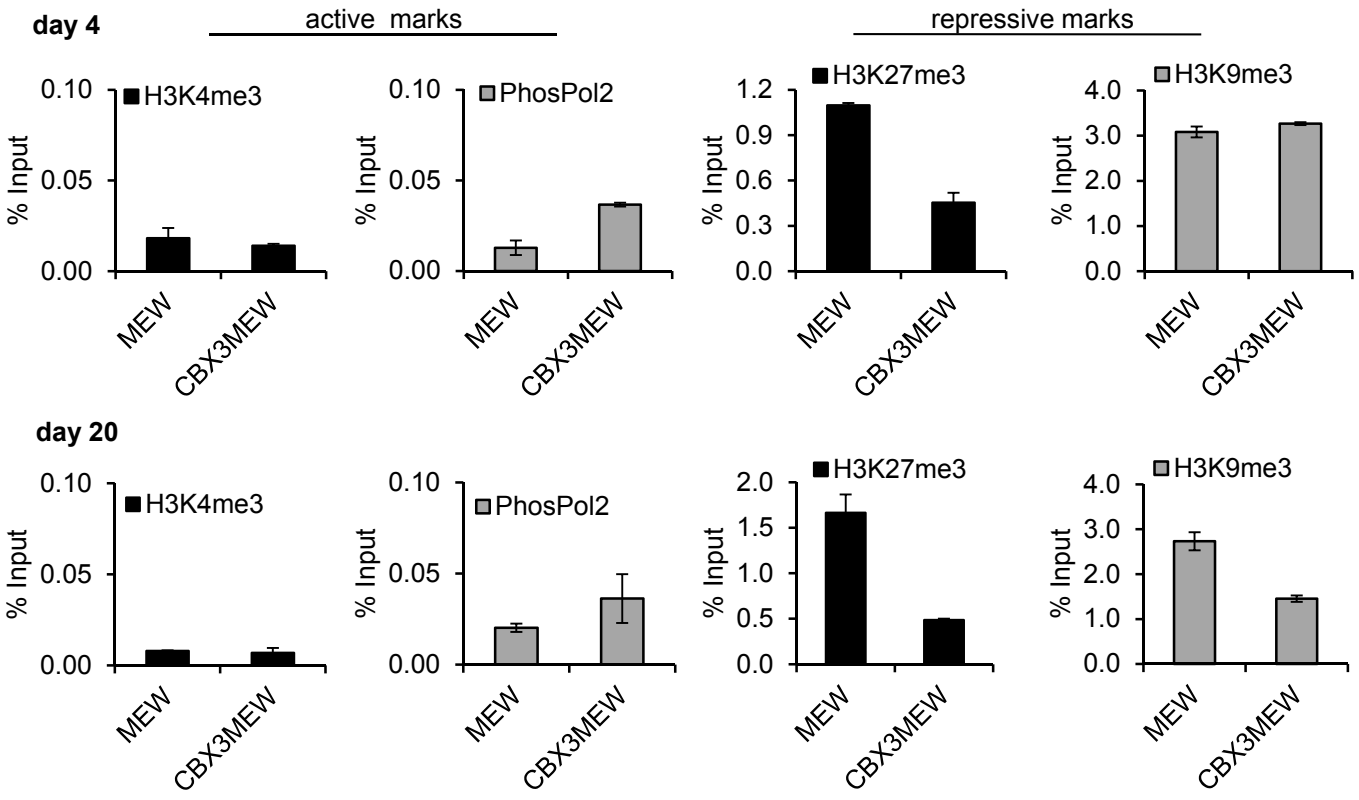


Figure S4

A



B

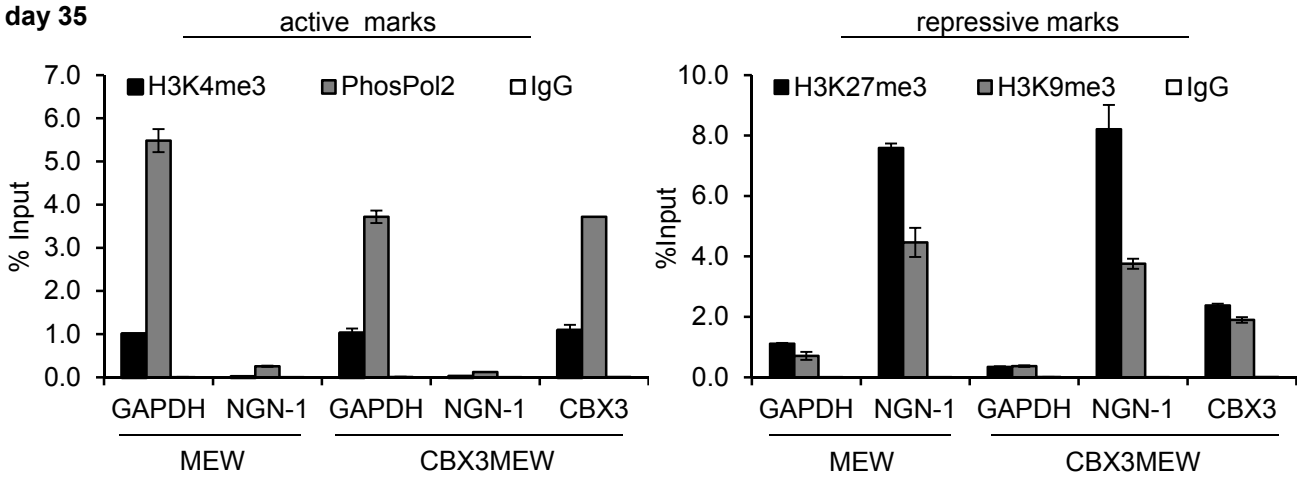


Figure S4. Chromatin structure at the MRP8 promoter in MEW and CBX3MEW transduced P19 cells. **(A)** Active and repressive histone marks at the MRP8 promoter were quantified 4 and 20 days after transduction by chromatin immunoprecipitation (representative results, mean ± S.D). **(B)** Active and repressive histone marks were quantified 35 days after transduction by ChIP experiments at control regions within the actively transcribed GAPDH promoter and the repressed NGN-1 locus in P19 cells. In CBX3MEW transduced cells also the chromatin status of the vector-encoded CBX3 promoter was analyzed (representative results shown, n=2, mean ± S.D).

Figure S5

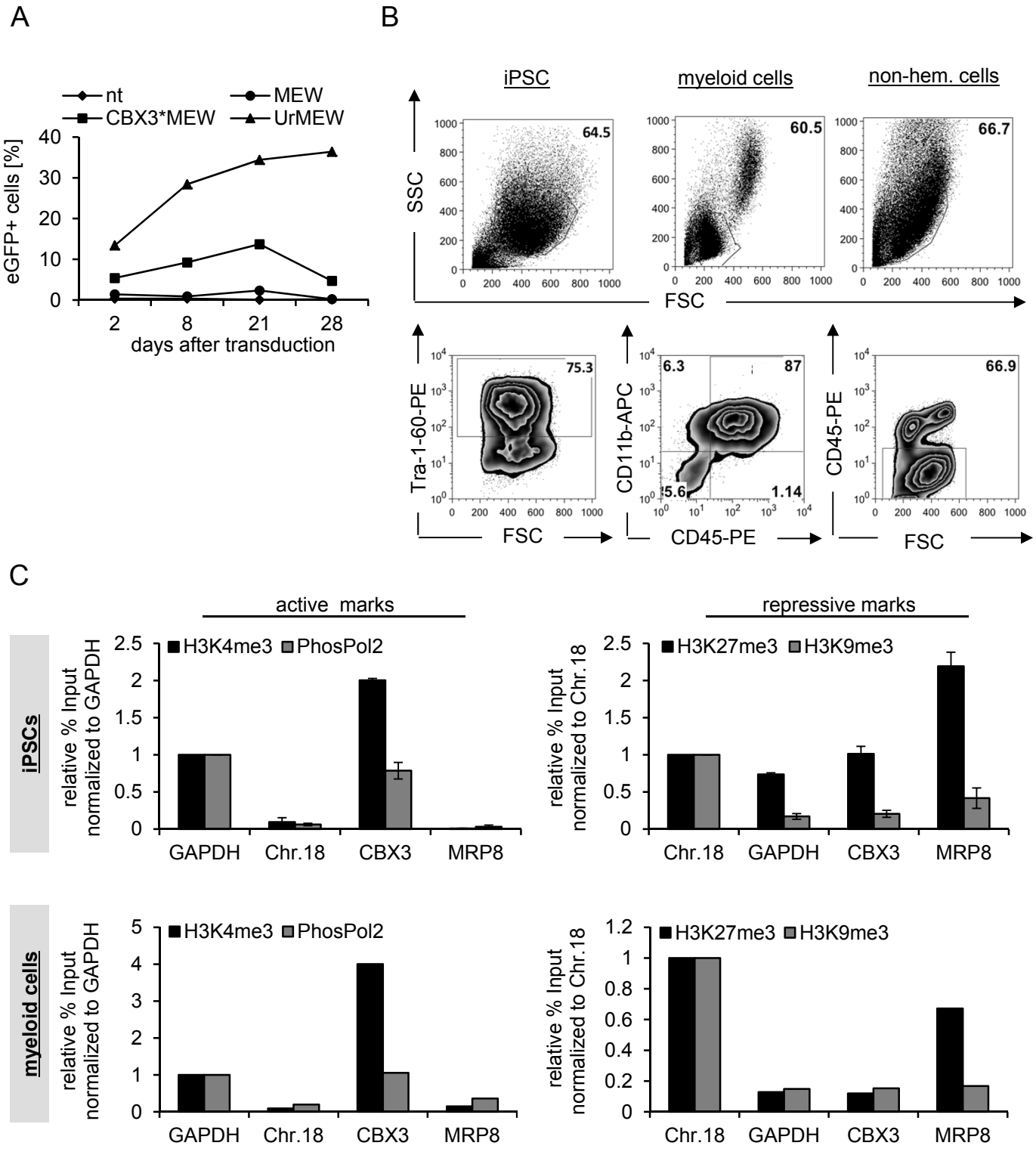


Figure S5. The CBX3-UCOE stabilizes transgene expression while maintaining tissue-specificity of the MRP8 promoter during myeloid differentiation of hiPSC. (A) hiPSC were transduced with MEW, CBX3*MEW and UrMEW and eGFP expression in Tra-1-60$^+$ cells was analyzed over 25 days by flow cytometry. **(B)** Representative gating strategy for analysis of eGFP$^+$ positive cells in hiPSC (Tra-1-60$^+$), myeloid cells (CD45$^+$/CD11b$^+$) and non-hematopoietic cells (CD45$^-$). **(C)** The chromatin structure of defined regions within the endogenous GAPDH, Chr18, CBX3 and MRP8 loci was analyzed by ChIP experiments in non-transduced hIPSc and differentiated myeloid cells (n=1-2, mean ± S.D). Values are given as percentage of input and normalized to the percentage of input for GAPDH (active chromatin) or Chr.18 (repressive chromatin marks).

Table S1

List of primers used in this study.

Oligonucleotide	Application	Sequence
m b-act F	qPCR	AGAGGGAAATCGTGCGTGAC
m b-act R	qPCR	CAATAGTGATGACCTGGCCGT
m b-act probe	qPCR	TexasRed-CACTGCCGCATCCTCTTCCTCCC-3`-BHQ2
h EPOR F	qPCR	ATGCCAGACTAGACCCAGAC
h EPOR R	qPCR	GGAAAGGAACTAACAAAGGGAC
h EPOR probe	qPCR	TexasRed-TCTTGGGGACTTTCACCTGATTTTCCTTCTAC-3`-BHQ2
CBX3 LAM1	cLAM PCR	CCAACTCCCCCTCCCCCAG
CBX3 LAM2	cLAM PCR	ATTCCACACCCTCGCACC
CBX3 LAM3	cLAM PCR	CGAGGAAGTGGGCGGAGT
LC1	cLAM PCR	GACCCGGGAGATCTGAATTC
LC2	cLAM PCR	GATCTGAATTCAGTGGCACAG
BS-SFFV F1	BS PCR	GTTATTTTGTAAGGTATGGAAAAATA
BS-SFFV R1	BS PCR	AAAAAATTATAAACTCTTTTATAAAA
BS-SFFV F2	BS PCR	AGGTATGGAAAAATATTAAATTAAGA
BS-CBX3 F1	BS PCR	AATTGATTTTTGGTGTATAGGA
BS-CBX3 R1	BS PCR	CTAAAAATTAAAACCCTAAAAAAAA
BS-CBX3 F2	BS PCR	TGATTTTTGGTGTATAGGAGAG
m gapdh F	ChIP	TCCCCTCCCCCTATCAGTTC
m gapdh R	ChIP	GACCCGCCTCATTTTTGAAA
m ngn-1 F	ChIP	TCCGTTTCCTGCGTTTCAA
m ngn-1 R	ChIP	TGCTCTGGGCTGGCTGTC
h GAPDH F	ChIP	GCTACTAGCGGTTTTACGGGCG
h GAPDH R	ChIP	TGCGGCTGACTGTCGAACAGG
h Chr.18 F	ChIP	ACTCCCCTTTCATGCTTCTG
h Chr.18 R	ChIP	AGGTCCCAGGACATATCCATT
SFFV F	ChIP	AATCAGCCTGCTTCTCGCTTCT
SFFV R	ChIP	TGAACAGCTCCTCGCCCTT
h MRP8 endogenous F	ChIP	GGTTGAGAAACCAGAGACTGTAGC
h MRP8 endogenous R	ChIP	GCTGCCCACAGCTTCAG
h MRP8 exogenous F	ChIP	CAGCTGGCCAAGCCTAAC
h MRP8 exogenous R	ChIP	AACAGCTCCTCGCCCTT
h CBX3 endogenous F	ChIP	AGTTCGCTGCTGAAAGTGGT
h CBX3 endogenous R	ChIP	GGGGAGTTGGGAGCTTAAAA

Table S2

Table S2: Relative MFI of SEW, UrSEW, CBX3SEW and CBX3EW transduced P19 cells. P19 cells were transduced with the indicated vectors and the MFI of eGFP positive cells was monitored over time by flow cytometry and normalized to day 3 after transduction. Examples of the corresponding FACS data are shown in Figure S1. (n=6, mean ± s.e.m.)

days after transduction	**3**	**7**	**15**	**17**	**21**	**32**	**35**	**42**
SEW [MFI ± s.e.m.]	100.0	107.5±9.3	59.7±4.8	77.0±8.8	61.0±7.1	49.6±5.6	68.0±9.6	62.1±7.7
UrSEW [MFI ± s.e.m.]	100.0	77.9±5.6	67.0±5.7	69.1±7.3	71.8±13.6	62.4±7.0	72.5±10.5	63.8±5.2
CBX3SEW [MFI ± s.e.m.]	100.0	102.3±12.9	50.7±5.8	83.8±13.4	60.4±9.3	48.4±6.7	62.7±8.5	54.3±5.7
CBX3EW [MFI ± s.e.m.]	100.0	113.5±13.2	88.8±16.1	121.6±17.2	88.4±16.4	75.5±12.2	78.6±12.4	63.9±10.5