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which should be cited to refer to this work.

Supplemental methods

Library preparation and Next-Generation sequencing

The TruSeq DNA LT Sample Prep Kit (Illumina, Inc, California, USA, Catalog No. FC-121-2001) was used in the succeeding steps for sequencing library preparation.The fragmented DNA samples were end-repaired and polyadenylated. TruSeq adapters containing the index for multiplexing were ligated to the fragmented DNA samples. No Size selection was performed after adaptor ligationas the representation of all the fragments in the final sequencing is important.PCR amplification was used to selectively enrich those DNA fragments that have adapter molecules on both ends and to amplify the amount of DNA in the library. The number of PCR cycles is limited 9 to avoid skewing the representation of the library. Library is validated using D1K tape on Agilent Tapestation and normalized to run on 1lane of IlluminaHiseq 2000. The sequencing was performed in single read mode generating reads with a length of 101bp.

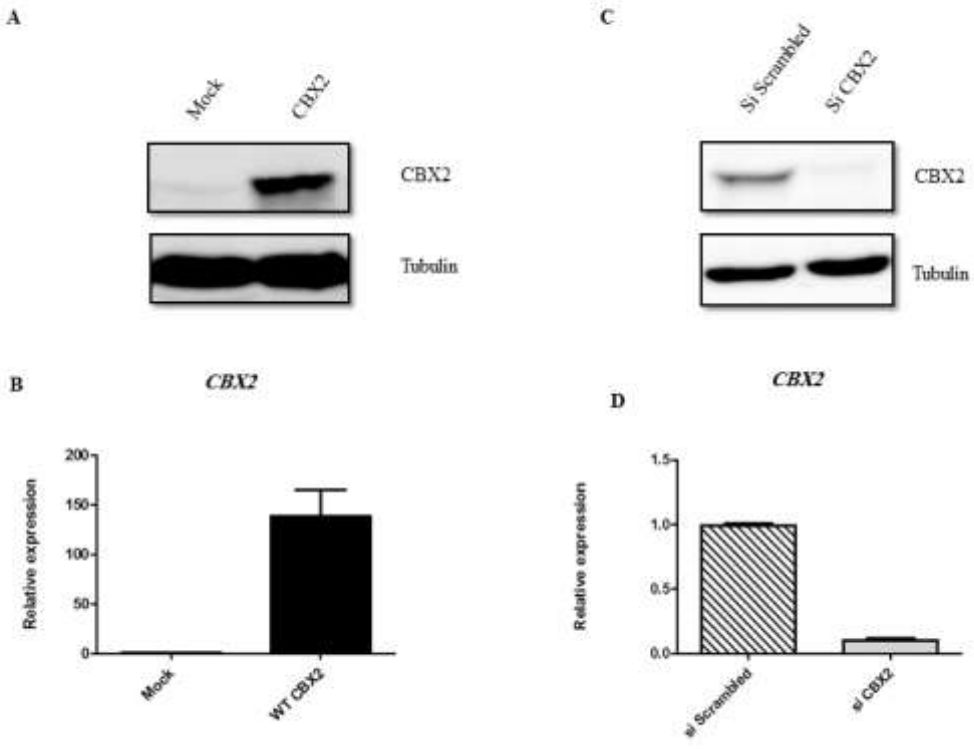
The reads were quality-checked with FastQC which computes various quality metrics for the raw reads. Before mapping the low quality ends of the reads were clipped (4 bases from the read start and 10 bases from the read end).

Plasmids and virus construction

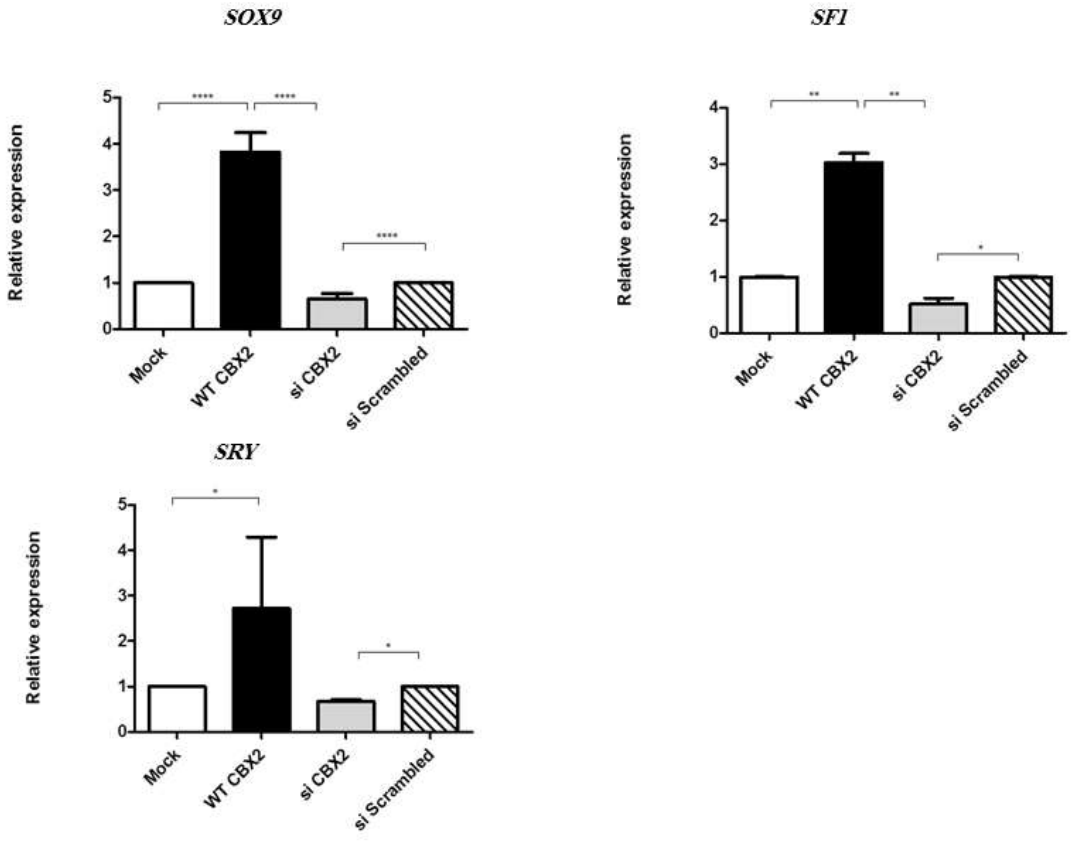
DamID lentiviral vectors, pLgw-V5-EcoDam and pLgw-V5-EcoDam-RFC1 were a kind gift from Bas van Steensel, Netherlands Cancer Institute. Human CBX2 cDNA (Origene, Australia) was amplified and cloned into pENTR11 (Invitrogen) and then recombined into the destination vector pLgw-V5-EcoDam-RFC1 (pLgw-V5-EcoDam-CBX2), using the Gateway LR Clonase II enzyme mix according to the manufacturer's directions (Invitrogen). Lentiviral packaging plasmids, pRSV-Rev (Addgene plasmid 12253), pCMV-dR8.2 (Addgene plasmid 8455) and pMD2.G (Addgene plasmid 12259) were obtained from Addgene. Mutated CBX2 cDNAs were constructed with the Quick-Change II site-directed mutagenesis kit (Stratagene); all constructs were verified by DNA sequencing.

Antibodies

Antibodies used are: α-tubulin (GTX11323, GeneTex), α-CBX2 (ab80044, Abcam), α-V5 (sc-81594, Santa Cruz Biotechnology). Secondary HRP-conjugated anti-mouse and anti-rabbit antibodies were from GE-Healthcare. Alexa Flour-488 and -594-conjugated secondary antibodies were from Invitrogen.

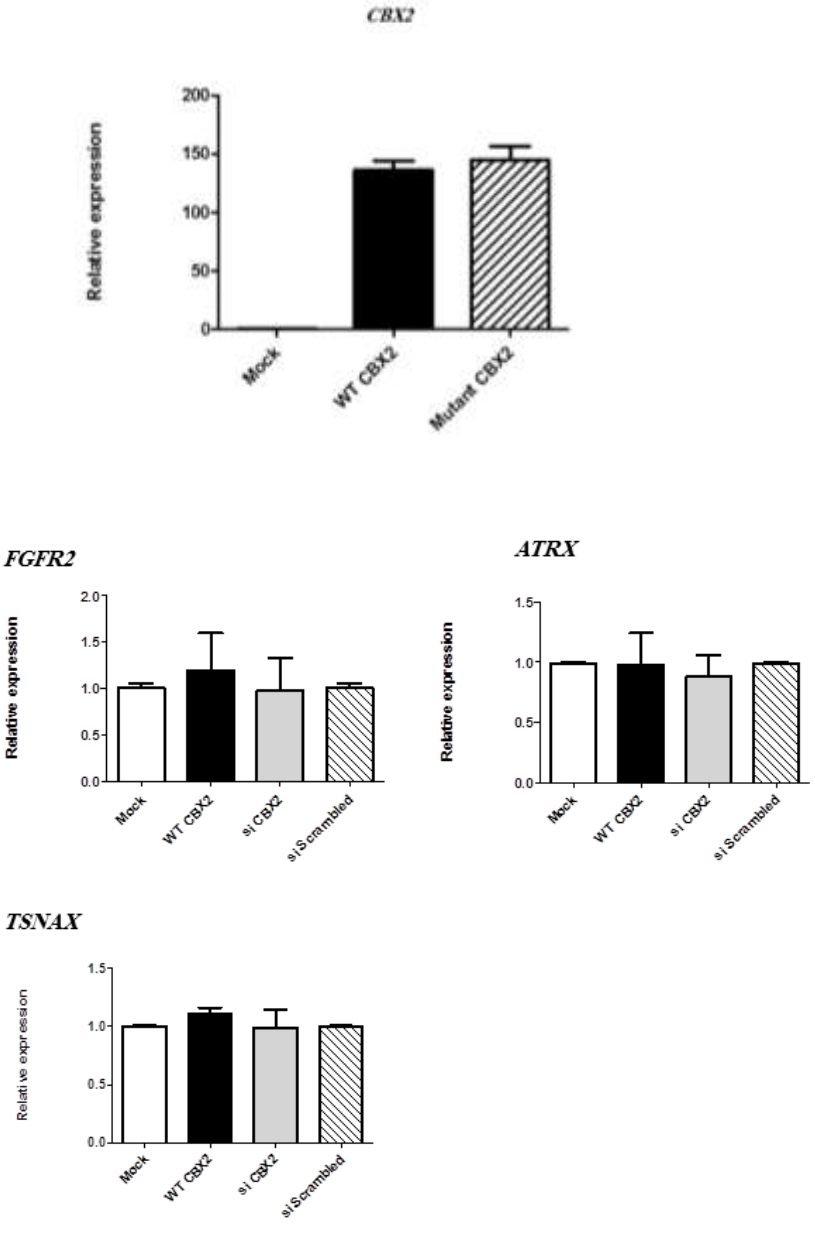


Supplemental Figure 1: CBX2 levels upon overexpression and downregulation (A) Immunoblot analysis of CBX2 expression in NT2-D1 cells transfected with empty vector (Mock) or wild-type (WT) CBX2. Whole cells extracts were prepared 48 hours post-transfection and were analyzed using the indicated antibodies. (B) Quantitative RT-PCR analysis of cells transfected as in A. Total RNA were extracted, 1 µg reverse-transcribed and used to analyze the relative expression of CBX2. (C) Immunoblot analysis of CBX2 expression in NT2-D1 cells in which endogenous CBX2 has been selectively knocked down using siRNA, whole cells extracts were prepared 72 hours post-transfection and were analyzed using the indicated antibodies. (D) qRT-PCR analysis of cells transfected as in C.

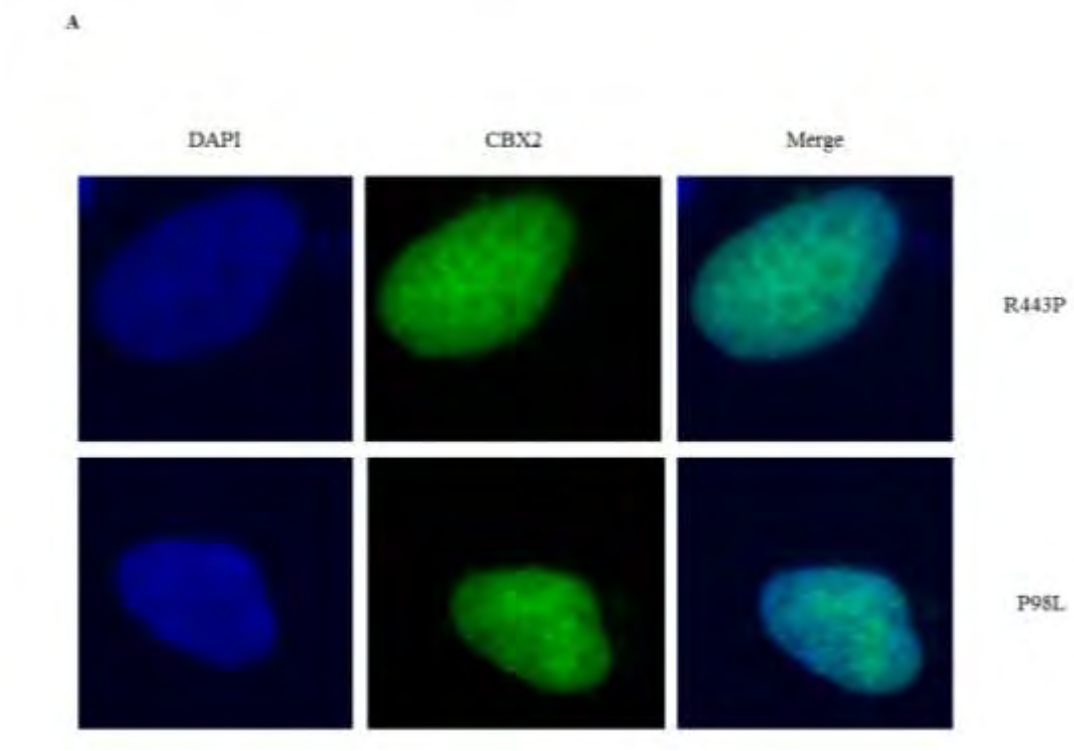


Supplemental Figure 2: CBX2- depenedent transcriptional regulation of *SRY*, *SOX9* and *SF1*. qRT-PCR expression analysis of *SRY*, *SOX9* and *SF1* in cells that were transfected with either WT-CBX2 or siRNA against CBX2. The data in all graphs are the average of three independent experiments, error bars represent standard deviation from the mean and values are expressed as relative to control = 1. (****) $P < 0.0001$; (***) $P < 0.001$; (**) $P < 0.01$; (*) $P < 0.05$.

B



Supplemental Figure 3: CBX2 does not affect the expression of *TSNAX*, *FGFR2*, *ATRX*. qRT-PCR expression analysis of *TSNAX*, *FGFR2* and *ATRX* upon overexpressing WT-CBX2 or upon knocking down endogenous CBX2 using siRNA.



Supplemental Figure 4: Mutated forms of CBX2 are expressed at levels comparable to the wild-type form and localize to the nucleus. (A) NT2-D1 cells were transfected with each of the mutated CBX2 forms (P98L or R443P), 48 h later cells were detergent-extracted and fixed. CBX2 was visualized by indirect immunofluorescence using a CBX2-specific antibody, nuclei were visualized by DAPI. (B) qRT-PCR expression analysis of CBX2 in cells transfected with empty vector (Mock), WT-CBX2 or the two mutated forms of CBX2 at equimolar ratio (Mutant CBX2).