SUPPLEMENTAL FIGURE LEGENDS

Figure S1. CHD1-KO RPE1 cells show normal growth and checkpoint activation after irradiation. Related to Figures 1 and 2. (A) The number of viable cells were measured in 5 consecutive days using the CellTiter-Glo® Luminescent Cell Viability Assay kit (Promega). The number of cells on each day was normalized to day 1. (B). Cell cycle analysis to determine whether CHD1-KO cells are proficient in DNA damage checkpoint. Cells were treated with 5 Gy IR. To monitor DNA synthesis, EdU was added to medium 1 hour before fixing cells. DNA content was visualized by PI. (C) Cell cycle profiles of WT and CHD1-KO cells after IR (5 Gy). The cell cycle profiles were analyzed using CytoFlex based on the EdU and PI staining. (D) CHK2 phosphorylation on Thr68 was detected in both WT and CHD1-KO cells after IR (5 Gy) of irradiation. Western blotting was performed using antibodies against phospho-CHK2 (Thr68), actin, or CHD1.

Figure S2. The gating schemes of GFP-based repair assays, schematics of the GFP repair assays, and representative raw FACS data. Related to Figure 1 and 2. (A) Profiles of GFP positive cells were acquired on CytoFLEX flow cytometer (Beckman Coulter). The acquired data were analyzed by the CytExpert software (Beckman Coulter), by subsequently defining cells (P1), live cells (P2), single live cells (P3), and GFP-positive single cells (P4). (B) An illustration of the NHEJ repair substrate (EJ5), related to Figure 1. (C) An illustration of the DR-GFP repair assay substrate. (D) Representative flow cytometry profile of GFP-positive cells after I-SceI cleavage in U2OS cells treated with siControl or siCHD1.

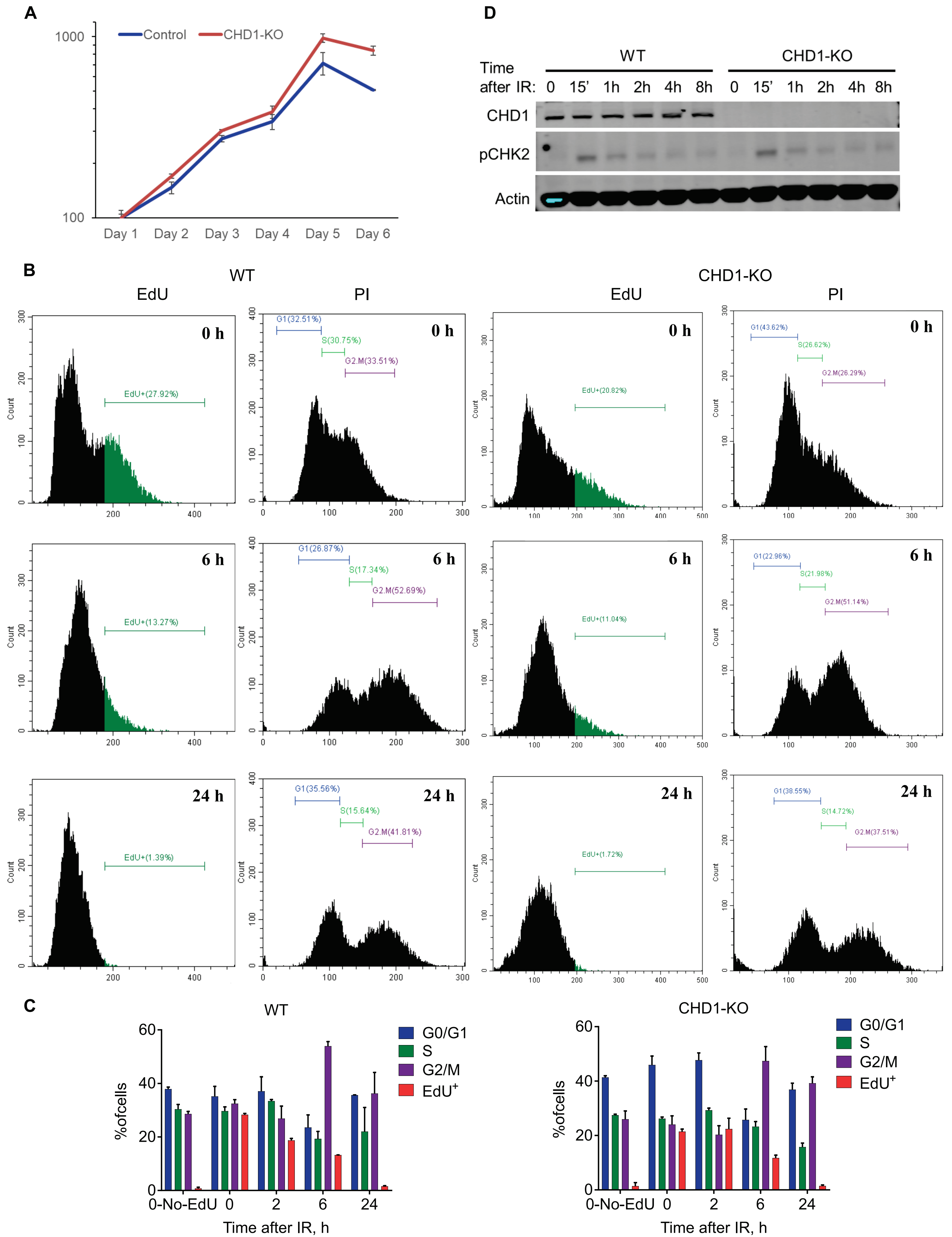
Figure S3. Additional ChIP data showing reduced γH2AX and H2AX accumulation at the induced DSB site. Related to Figure 2**.** (A) γH2AX formation at the DSB created by the ZFN nuclease in control siRNA- or siCHD1-treated T98G cells. The percentage of DNA immunoprecipitated by ChIP was normalized to input, and the fold enrichment of γH2AX before

and after ZFN cleavage was calculated and shown. (B) An independent repeat of the same experiment as shown in (A). (C) H2AX deposition before and after ZFN cleavage at the DSB site in siControl- or siCHD1- treated T98G cells. H2AX ChIP with ZFN was normalized to without ZFN. (D) An independent repeat of the same experiment as shown in (C). At least two qPCR reactions were performed for each data point.

Figure S4. γH2AX formation, MRE11 recruitment and checkpoint proficiency in CHD1-KO cells. Related to Figure 2. (A) Western blot of γH2AX after irradiation (5 Gy) in WT and CHD1-KO cells. γH2AX levels in all samples were normalized to time-0 (No IR) of WT cells (black bars); and γH2AX levels were normalized to their own time-0 (No IR) for either WT or CHD1-KO cells (blue bars). (B) Representative images of laser striping experiments showing MRE11 recruitment to laser tracts in WT and CHD1-KO cells. Cells were fixed 5 min after laser striping and stained for MRE11 and γH2AX. (C) Quantification of MRE11 recruitment in the laser tracts in WT and CHD1-KO cells. N > 100 of stripes from two experiments were counted.

Figure S5. Alignment of the full-length human CHD1 and CHD2 proteins. The gray boxes highlight the identical amino acids. The N terminus of CHD1, as specified in this study (*i.e.,* amino acids 1-271), is outlined in a box. The red arrowheads indicate the sites where the deletions were made for the experiments shown in Figure 5. The deletion of the whole N terminus is referred to as ΔN.

Zhou_Fig S1



Zhou_Fig S2

A

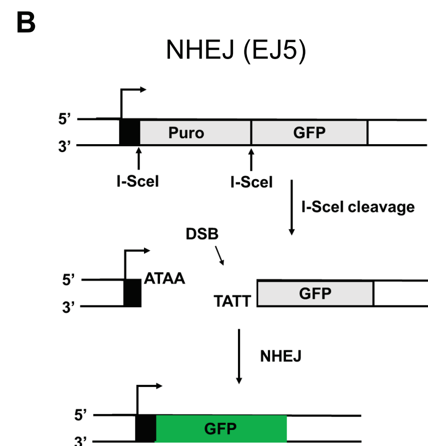
siCHD1_2 1: All Events
P1-Cells(47.40 %)
SSC-H (x 10^4) 0 200 400
FSC-H (x 10^4) 0 200 400
1

siCHD1_2 1: P1-Cells
P2-live cells(98.78 %)
SSC-A (x 10^5) 0 50 100
FSC-A (x 10^5) 0 50 100
2

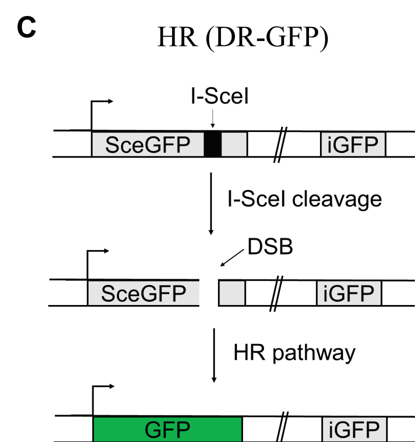
siCHD1_2 1: P2-live cells
P3-single cells(95.61 %)
FSC-A (x 10^5) 0 50 100
FSC-H (x 10^4) 0 200 400
3

siCHD1_2 1: P3-single cells
GFP+ cells(6.45 %)
GFP FITC-H 10^3 10^4 10^5 10^6 10^7
ECD-H 10^2 10^3 10^4 10^5 10^6
4

B



C

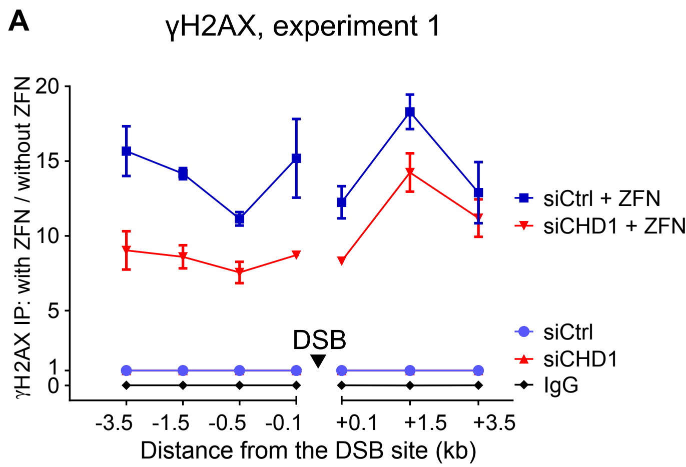


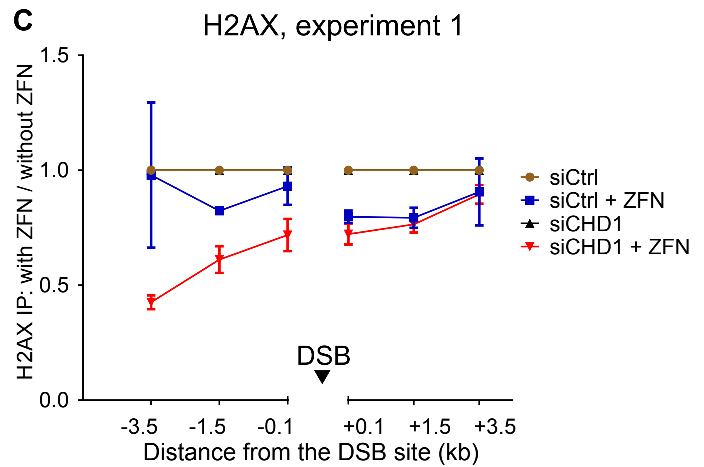
D

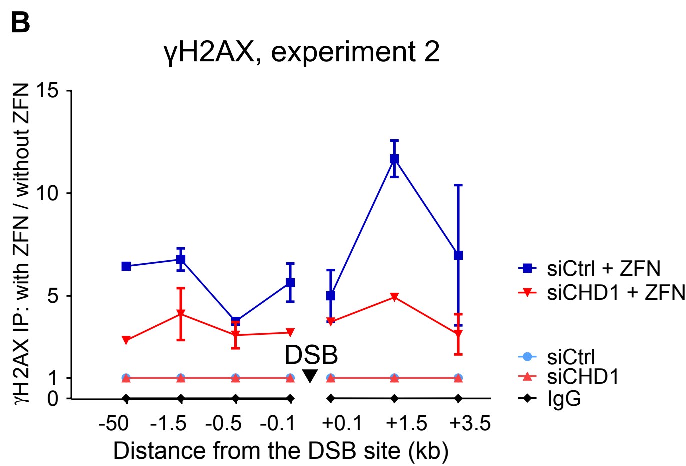
siControl
GFP+ cells(6.25 %)
GFP FITC-H 10^3 10^4 10^5 10^6 10^7
ECD-H 10^2 10^3 10^4 10^5
10000 Show

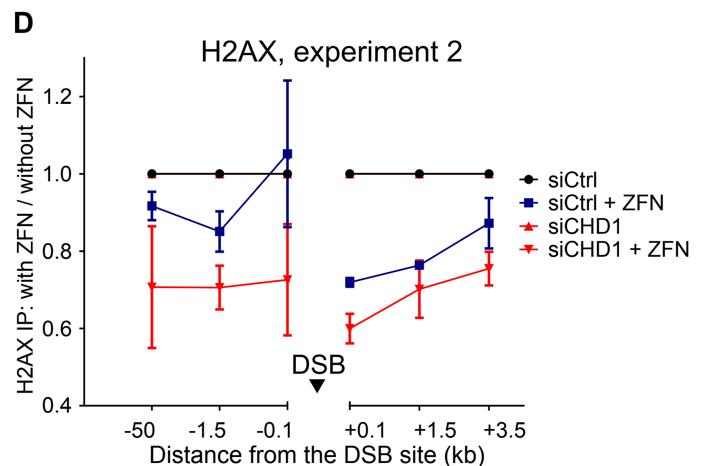
siCHD1
GFP+ cells(1.77 %)
GFP FITC-H 10^3 10^4 10^5 10^6 10^7
ECD-H 10^2 10^3 10^4 10^5
10000 Show

Zhou_Fig S3



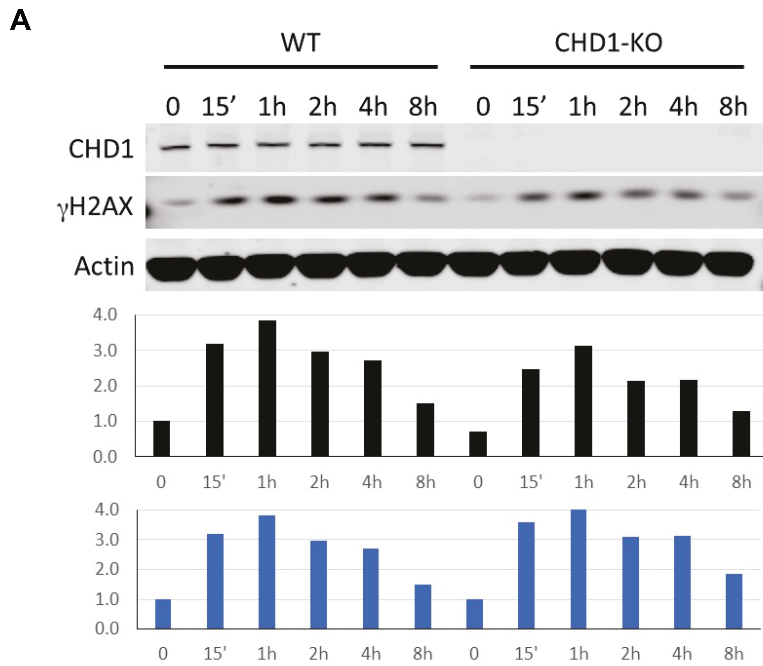






Zhou_Fig S4

A



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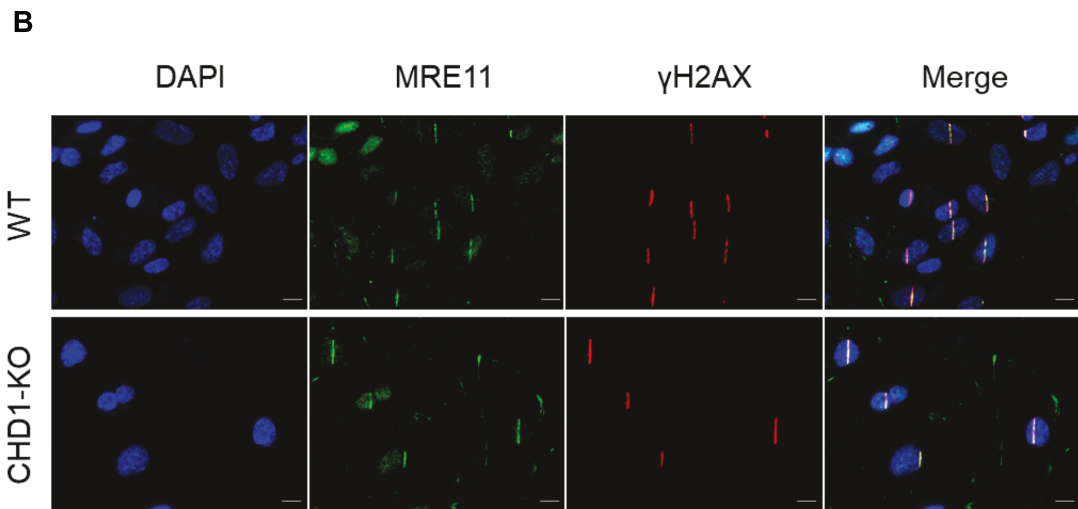

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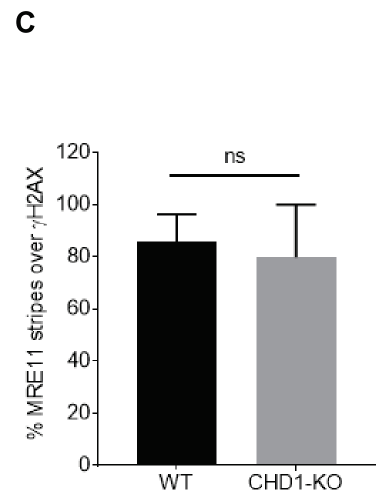

Zhou_Fig S5

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                  ▼(69)
hCHD1 MNGHSD---EESVRNSSGESSQSDDDSGSASGSGSGSSSGSSSD-GSSSQSGSSDSDSGSESGSQSESESDTSREN----KVQAKPPK-----VDGAEFWKSSPSILA 95
hCHD2 MMRNKDKSQEEDSSLHSNASSHSASEEASGSDSGSQSESEQGSDPGSGHGSESNSSSESSESQSESESESAGSKSQPVLPEAKEKPASKKERIADVKKMWEEYPDVYG 108
                                          ▼(137)
      VQRSAILKKQQQQQQQQQHQASSNSGSEEDSSSSEDSDDSSSEVKRKKHKDEDWQMSGSGSPSQSGSDSESE-EEREKSSCDETESDYEPKNKVKSRKPQNRSKSKNG 202
      VRRSNRSR-QEPSRFNIKEEAS--SGSE---SGSPKRRGQ-----RQLKKQEKWKQEPSEDEQEQGTSAESEPEQKKVKARRPVPRRTVPKPRVK-KQP----KTQRG 200
          ▼(209)                                                      (271)▼
      KKILGQKKRQIDSSEEDDDEEDYDNDKRSSRRQATVNVSYKEDEEMKTDSDDLLEVCGEDVPQPEEEEFETIERFMDCRIGRKGATGATTTIYAVEADGDPNAGFEKN 310
      K----RKKQ--DSSDEDDD--DDEAPKRQTRRRAAKNVSYKEDDDFETDSDDLIEMTGEGVDE-QQDNSETIEKVLDSRLGKKGATGASTTVYAIEANGDPSGDFDTE 299

      KEPGEIQYLIKWKGWSHIHNTWETEETLKQQNVRGMKKLDNYKKKDQETKRWLKNASPEDVEYYNCQQELTDDLHKQYQIVERIIAHSNQKSAAG------------- 405
      KDEGEIQYLIKWKGWSYIHSTWESEESLQQQKVKGLKKLENFKKKEDEIKQWLGKVSPEDVEYFNCQQELASELNKQYQIVERVIAVKTSKSTLGQTDFPAHSRKPAP 407

      --YPDYYCKWQGLPYSECSWEDGALISKKFQACIDEYFSRNQSKTTPFKDCKVLKQRPRFVALKKQPSYIGGHEGLELRDYQLNGLNWLAHSWCKGNSCILADEMGLG 511
      SNEPEYLCKWMGLPYSECSWEDEALIGKKFQNCIDSFHSRNNSKTIPTRECKALKQRPRFVALKKQPAYLGG-ENLELRDYQLEGLNWLAHSWCKNNSVILADEMGLG 514

      KTIQTISFLNYLFHEHQLYGPFLLVVPLSTLTSWQREIQTWASQMNAVVYLGDINSRNMIRTHEWTHHQTKRLKFNILLTTYEILLKDKAFLGGLNWAFIGVDEAHRL 619
      KTIQTISFLSYLFHQHQLYGPFLIVVPLSTLTSWQREFEIWAPEINVVVYIGDLMSRNTIREYEWIHSQTKRLKFNALITTYEILLKDKTVLGSINWAFLGVDEAHRL 622

      KNDDSLLYKTLIDFKSNHRLLITGTPLQNSLKELWSLLHFIMPEKFSSWEDFEEEHGKGREYGYASLHKELEPFLLRRVKKDVEKSLPAKVEQILRMEMSALQKQYYK 727
      KNDDSLLYKTLIDFKSNHRLLITGTPLQNSLKELWSLLHFIMPEKFEFWEDFEEDHGKGRENGYQSLHKVLEPFLLRRVKKDVEKSLPAKVEQILRVEMSALQKQYYK 730

      WILTRNYKALSKGSKGSTSGFLNIMMELKKCCNHCYLIKPPDNNEFYNKQEALQHLIRSSGKLILLDKLLIRLRERGNRVLIFSQMVRMLDILAEYLKYRQFPFQRLD 835
      WILTRNYKALAKGTRGSTSGFLNIVMELKKCCNHCYLIKPPEENERENGQEILLSLIRSSGKLILLDKLLTRLRERGNRVLIFSQMVRMLDILAEYLTIKHYPFQRLD 838

      GSIKGELRKQALDHFNAEGSEDFCFLLSTRAGGLGINLASADTVVIFDSDWNPQNDLQAQARAHRIGQKKQVNIYRLVTKGSVEEDILERAKKKMVLDHLVIQRMDTT 943
      GSIKGEIRKQALDHFNADGSEDFCFLLSTRAGGLGINLASADTVVIFDSDWNPQNDLQAQARAHRIGQKKQVNIYRLVTKGTVEEEIIERAKKKMVLDHLVIQRMDTT 946

      GKTVLHTGSAPSSSTPFNKEELSAILKFGAEELFKEPEGEEQEPQEMDIDEILKRAETHENEPGPLTVGDELLSQFKVANFSNMDEDDIELEPERNSKNWEEIIPEDQ 1051
      GRTILENNSGRSNSNPFNKEELTAILKFGAEDLFKELEGEESEPQEMDIDEILRLAETRENE-VSTSATDELLSQFKVANFATME-DEEELE-ERPHKDWDEIIPEEQ 1051

      RRRLEEEERQKELEEIYMLPRMRNCAKQISFNGS----EGRRSRSRRYSGSDSDS--ISEGKRPKKRGRPRTIPRENIKGFSDAEIRRFIKSYKKFGGPLERLDAIAR 1153
      RKKVEEEERQKELEEIYMLPRIRSSTKKAQTNDSDSDTESKR-QAQRSSASESETEDSDDDKKPKRRGRPRSVRKDLVEGFTDAEIRRFIKAYKKFGLPLERLECIAR 1158

      DAELVDKSETDLRRLGELVHNGCIKALKDSSSGTERTG--GR-LGKVKGPTFRISGVQVNAKLVISHEEELIPLHKSIPSDPEERKQYTIPCHTKAAHFDIDWGKEDD 1258
      DAELVDKSVADLKRLGELIHNSCVSAMQEYEEQLKENASEGKGPGKRRGPTIKISGVQVNVKSIIQHEEEFEMLHKSIPVDPEEKKKYCLTCRVKAAHFDVEWGVEDD 1266

      SNLLIGIYEYGYGSWEMIKMDPDLSLTHKILPDDPDKKPQAKQLQTRADYLIKLLSRDLAKKEALSGAGSSKRRKARAKKNKAMKSIKVKEEIKSDSS-P----LPSE 1361
      SRLLLGIYEHGYGNWELIKTDPELKLTDKILPVETDKKPQGKQLQTRADYLLKLLRKGLEKKGAVTGGEEAKLKKRKPRVKKENKVPRLKEEHGIELSSPRHSDNPSE 1374

      KSDEDDDKLSES---------------------KSD--G-RERSK-----------------KSSVSDAPVHITASGEPVPISE-ESEELDQKTFSICKERMRPVKAA 1427
      EGEVKDDGLEKSPMKKKQKKKENKENKEKQMSSRKDKEGDKERKKSKDKKEKPKSGDAKSSSKSKRSQGPVHITAGSEPVPIGEDEDDDLDQETFSICKERMRPVKKA 1482

      LKQLDRPEKGLSEREQLEHTRQCLIKIGDHITECLKEYTNPEQIKQWRKNLWIFVSKFTEFDARKLHKLYKHAIKKR---QESQQNSDQNSN----LNPHVIRNP-DV 1527
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      ERLKENTNHDDSSRDSYSSDRHLTQYHDHHKDRHQGDS---YKKSD----SRKRPYSSFSNGKDHRDWD---HYKQDSRYYSDREK--HRKLDDHRSRDHRSNLEGSL 1623
      LISQSHTSHNLHPQKPHLPASHGPQMHGHPRDNYNHPNKRHFSNADRGDWQRER-KFNYGGGNNNPPWGSDRHHQYEQHWYKDHHYGDRRHMDAHRSGSYRPNNMSRK 1697

      K-------DRSHSDHRSHSDHRLHSDHRSSSE-----YTHHKSSR---DYR----YHSDWQMDHRASS-------------------SGPRSPLDQRSPYGS------ 1687
      RPYDQYSSDRDHRGHRDYYDRHHHDSKRRRSDEFRPQNYHQQDFRRMSDHRPAMGYHGQGPSDHYRSFHTDKLGEYKQPLPPLHPAVSDPRSPPSQKSPHDSKSPLDH 1805

      RSPFEHSVEHKSTPEHTWSSRKT 1710
      RSPLERSLEQKNNPDYNWNVRKT 1828
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Supplemental table 1. Sequences of primers used in ChIP-qPCR. Related to Figure 2 and Figure S3.

Name	Sequence (5' to 3')
F -50kb	GCTCTCTCCTTCCACACACC
R -50kb	CTCAGGTCCAGGAATGAAGG
F -3.5kb	CCGAGATCACATCACTGCAC
R -3.5kb	GGAAAGAGGAGGGAAGAGGA
F -1.5kb	GGGGCAGTCTGCTATTCATC
R -1.5kb	CGATGCACACTGGGAAGTC
F -0.5kb	TGGGTTCCCTTTTCCTTCTC
R -0.5kb	GTCCAGGCAAAGAAAGCAAG
F -0.1kb	CTTAGAGGTTCTGGCAAGGAG
R -0.1kb	ACAGAAAAGCCCCATCCTTAG)
F +0.1kb	AGAACCAGAGCCACATTAACC
R +0.1kb	CACTTCAGGACAGCATGTTTG
F +1.5kb	CCTCAGCTCCAGTTCAGGTC
R +1.5kb	GGCTGTCACACTCCAGTTCA
F +3.5kb	TCGCCAGTGCTTTTTCTTTT
R +3.5kb	GTTGGGGGATGATGAAAATG
DSB-For	CGGTTAATGTGGCTCTGGTT
DSB-Rev	ACAGGAGGTGGGGGTTAGAC