Δ Np63 is a pioneer factor that binds inaccessible chromatin and elicit chromatin remodeling

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Research

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Abstract

Background: Δ Np63 is a master transcriptional regulator playing critical roles in epidermal development and other cellular processes. Recent studies suggest that Δ Np63 functions as a pioneer factor that can target its binding sites within inaccessible chromatin and induce chromatin remodeling.

Methods: In order to examine if Δ Np63 can bind to inaccessible chromatin and to determine if specific histone modifications are required for binding we induced Δ Np63 expression in two p63 naive cell line. Δ Np63 binding was then examined by ChIP-seq and the chromatin at Δ Np63 targets sites was examined before and after binding. Further analysis with competitive nucleosome binding assays was used to determine how Δ Np63 directly interacts with nucleosomes.

Results: Our results show that before Δ Np63 binding, targeted sites lack histone modifications, indicating Δ Np63's capability to bind at unmodified chromatin. Moreover, the majority of the sites that are bound by ectopic Δ Np63 expression exist in an inaccessible state. Once bound Δ Np63 induces acetylation of the histone and the repositioning of nucleosomes at its binding sites. Further analysis with competitive nucleosome binding assays reveal that Δ Np63 can bind directly to nucleosome edges with significant binding inhibition occurring within 50 bp of the nucleosome dyad.

Conclusion: Overall, our results demonstrate that Δ Np63 is a pioneer factor that binds nucleosome edges at inaccessible un-modified chromatin sites and induces histone acetylation and nucleosome repositioning.

Introduction

Gene regulation is controlled by transcription factors (TFs), which switch genes on and off in a spatial and temporal manner. TFs are sequence-specific DNA binding proteins that recognize and bind to evolutionally conserved but often degenerative DNA sequences known as TF motifs [1, 2]. These degenerative TF motifs can occur millions of times across the human genome, but only a small proportion of these sites are actual bound in vivo [3].

The transcription factor p63 plays a pivotal role in maintaining epidermal lineage and in the epidermal commitment steps during skin development [4-7]. The critical role of p63 in epidermal morphogenesis is evident from the p63-null mice who exhibit distinct phenotype that includes defects of limbs, craniofacial region, total loss of squamous epithelia and agenesis of other epithelial-rich tissues [4, 5]. *TP63* mutations in human also cause severe developmental diseases, such as Ectrodactyly Ectodermal dysplasia–Clefting syndrome (EEC), Limb–Mammary Syndrome (LMS), Ankyloblepharon and Ectodermal dysplasia-Clefting Syndrome (AEC), Split-hand/foot malformations (SHFM) and Rapp–Hodgkin Syndrome [8]. p63 plays a versatile role as a master regulatory factor and affects a myriad of cellular processes, from basement membrane formation, barrier formation, terminal differentiation, to epithelial cell adhesion and proliferation. In humans and mice, p63 protein has multiple isoforms due to

alternative mRNA splicing and usage of different promoters [9, 10]. The Δ Np63 α isoform is the most predominant in epithelia cells and possess the majority of p63 biological functions [11, 12].

While the role of TP63 in regulating epidermal morphogenesis have been extensively investigated, the mechanism of how Δ Np63 modulates chromatin still remains elusive. Δ Np63 has been proposed to be both an activator and a repressor of transcription. As an example while Δ Np63 can activate important epidermal genes such as the keratin genes K5 and K14 [13], it can also act to repress expression of genes for non-epidermal lineages [14]. In its repressive role Δ Np63 has been demonstrated to physically interact with the histone deacetylase HDAC1 and HDAC2 [15]. As an activator of transcription Δ Np63 has been shown to require the Swi/Snf chromatin remodeling complex, BAF, to maintain nucleosome displacement at Δ Np63 binding sites [16]. Furthermore, a recent study has found an interaction between H3K4 histone methyl-transferase, KMT2D, and Δ Np63 in epidermal keratinocytes [17]. These studies suggest that one potential function of DNA-bound Δ Np63 could be to remodel the neighboring chromatin, thereby creating an active or repressive chromatin state.

Our previous studies have revealed that Δ Np63 bound sites across the genome in human keratinocytes are associated with an accessible and active chromatin environment. However we found that those sequences were predicted to have a higher chance of nucleosome deposition, suggesting that the accessible chromatin landscape at Δ Np63 bound sites might be driven by the pioneering activity of Δ Np63 [18]. To directly test if Δ Np63 is a pioneer factor capable of binding inaccessible chromatin and remodeling the neighboring chromatin, here we have performed studies with ectopically expressed Δ Np63 in cell lines that are Δ Np63 naïve. This has allowed us to directly determine the global state of histone modifications and chromatin accessibility required for Δ Np63 binding and the consequences of binding. By combining our analysis with *in vitro* nucleosome binding assays, we demonstrate that Δ Np63 is a pioneer factor capable of binding directly to nucleosome edges at chromatin inaccessible regions in a histone modification independent manner.

Results

Our previous examination of Δ Np63 binding in NHEK cells demonstrated an active chromatin signature at many of its binding sites consisting of high H3K27ac, H3K9ac, H3K4me1, H3K4me2, and H3K4me3 along with increased chromatin accessibility [18]. These sites while depleted of nucleosomes in NHEK contained nucleosome preferring sequences. However, it is unclear from this analysis if Δ Np63 α played an active role in establishing this chromatin architecture or required an *a priori* permissive chromatin environment to bind. Therefore, to directly test Δ Np63 pioneering capabilities we established an ectopic inducible Δ Np63 in a p63 naïve cell line, K562. K562 is a widely used immortalized myelogenous [leukemia cell line](#) and is an advantageous choice for these experiments because no p63 isoforms are expressed and there are extensive genomic and epigenomic datasets available from the ENCODE project [19]. Two doxycycline (Dox) inducible cell lines were generated containing the wildtype (WT) HA-tagged Δ Np63 α and DNA binding mutant Δ Np63 α (R304W). Western blot analysis showed that

both WT and mutant Δ Np63 α were expressed after Dox induction and the levels comparable to those observed in epithelial cells (Supplementary Figure S1).

Δ Np63 α (WT) and Δ Np63 α (R304W) were induced in K562 cells with Dox and ChIP-seq experiments performed on two biological replicates with Δ Np63-specific and anti-HA antibodies. Both ChIP-seq experiments for Δ Np63 α enriched for DNA sequences containing the p63 binding motif (Supplementary Figure S2). In contrast, ChIP-seq experiments for Δ Np63 α (R304W) show limited enrichment and revealed only a few background sites. In total 1980 common sites were identified in experimental replicates using WT Δ Np63 α ChIP which were not enriched in the control cells expressing Δ Np63 α (R304W) and were chosen for further analysis.

To understand the chromatin characteristics required for Δ Np63 α binding we examined the robust dataset of chromatin modification marks and accessibility for K562 that have been generated by the ENCODE project. This provided us with the chromatin characteristics before Δ Np63 was induced in our experiments. Ten histone modifications H3K4me1, H3K4me2, H3K4me3, H3K9ac, H3K9me1, H3K9me3, H3K27ac, H3K27me3, H3K36me3, H3K79me2 with DNase-seq and H2AFZ profiles were examined and clustered into 4 groups (Figure 1A). Groups a, b, c contain a total of 374 sites and appear to represent binding locations occurring in already active chromatin environments with high levels of H3K27ac and H3K4me1 or H3K4me3. The majority of the Δ Np63 α bound sites (1606 out of 1980) are in group d and represented genomic segments that were bereft of signal for any histone modification. The average chromatin architecture further demonstrates the absence of histone modifications and is shown in comparison to the active chromatin state at transcriptional start sites (TSS; Figure 1B). Examination of chromatin accessibility by DNase-seq show that the majority of these (1429 of 1980) regions are located at inaccessible sites in K562 as defined by DNase-seq (Figure 1C). Nucleosome occupancy at these binding sites is also enriched before Δ Np63 α binding (Figure 1D). Overall these results demonstrate that Δ Np63 α can bind at chromatin inaccessible, unmodified, and nucleosome occluded sites.

Comparison of Δ Np63 binding sites between NHEK and ectopic induced binding in K562

To further examine the chromatin characteristics at Δ Np63 α binding sites, we compared our K562 targets to the binding sites in a normal Δ Np63 α expressing cell type NHEK [18, 20]. Sites were classified as jointly bound, or bound only in each specific cell type. The consequence of Δ Np63 binding can be discerned by comparing the chromatin at sites bound in a Δ Np63 expressing cell line, NHEK, with the chromatin seen at the bound sites in K562. Three binding sites near the TP73 gene exemplify the chromatin differences in the two cell lines (Figure 2A). The sites bound in K562 are often within heterochromatin chromatin states (Gray) or weak-TX (Green). In NHEK, these same sites are shown to be active as Strong Enhancers (Red) or weak Enhancers (yellow). The DNase signal is also higher in 2 of 3 binding sites in NHEK.

Sites bound by Δ Np63 in both cell lines show active histone modifications in NHEK and the absence of similar modifications in K562 (Figure 2B and C). Sites in NHEK are flanked by transcriptional active histone modifications (H3K9ac, H3K27ac, H3K4me1, H3K4me2, H3K4me3), while in K562 these same sites show a very reduced signal. Sites bound only in NHEK (Figure 2B middle, and 2D) have high levels

of active histone modifications, while all modifications display low signal in K562. K562 specific sites (Figure 2B bottom and 2E) display relatively low levels of active histone modifications in both NHEK and K562.

Sites bound only in NHEK allow us to address the role of repressive histone modifications (H3K27me3 and H3K9me3). These 8195 sites are all bound in NHEK and thus represent bona fide targets of Δ Np63. These 8195 sites are unbound in K562, but are not enriched for repressive histone modifications, suggesting that active repression is not a strong driving force blocking Δ Np63 binding to these sites *in vivo*.

Δ Np63 α can bind inaccessible, inactive sites in HepG2

To validate that Δ Np63 α can target inactive and inaccessible chromatin in any cell type, we developed a Δ Np63 α -expressing HepG2 cell line. HepG2 is p63 naïve and is a widely used cell line in the endoderm lineage that has been extensively characterized. In total Δ Np63 bound 2939 targets in HepG2 (Supplementary Figure S3). Most sites bound in HepG2 have low levels of histone modifications and accessibility, which is consistent with what is observed in K562 (Figure 3A). In addition, the majority, 65%, are classified as inaccessible (Figure 3B). The chromatin architecture was further examined at the inaccessible bound sites and displayed a low signal compared with the chromatin modifications at TSS (Figure 3C).

Δ Np63 binds at nucleosomes and leads to H3K27ac and nucleosome depletion

Our comparison between K562 and NHEK binding sites suggest that Δ Np63 α binding leads to an active histone architecture. To test the consequences of Δ Np63 α binding directly we determined if H3K27ac increased at sites bound by Δ Np63 α . ChIP-seq for H3K27ac was performed in Δ Np63 α and Δ Np63 α (R304W) expressing K562 cell lines (Figure 4). Binding of Δ Np63 α leads to an increased in H3K27ac at Δ Np63 α bound sites while induction of DNA-binding mutant Δ Np63 α (R304W) does not change the H3K27ac (Figure 4A). In comparison H3K27ac does not change at TSS in K562 in either cell line (Figure 4B). In addition, these results show the characteristic peak-valley-peak for the H3K27ac surrounding the Δ Np63 binding sites, suggestive of nucleosome depletion at the binding site. Examination of single sites further highlights the starting structure and consequences of Δ Np63 α (Figure 4C&D). At these sites the p63BS is located within a well-positioned nucleosome. After induction of Δ Np63 α the flanking nucleosomes are acetylated with a dip in signal at the site of binding, suggesting remodeling of the centrally located nucleosome.

Δ Np63 binds nucleosome edges

To further understand the ability of Δ Np63 to bind to inaccessible chromatin we tested Δ Np63 binding to nucleosome DNA with a competitive nucleosome binding assay [21]. 16 templates derived from Widom 601 nucleosome positioning sequence were obtained: 14 nucleosome templates with a high-affinity or intermediate-affinity p63 binding site (p63BS) placed at increasing distance to the nucleosome dyad axis,

in an exposed or concealed rotational orientation. Each experiment is internally controlled with 2 nucleosome sequences lacking p63BS. Nucleosome population were obtained after *in vitro* reconstitution with unmodified histones via salt gradient dialysis on all nucleosome sequences simultaneously, and purified from free DNA with a sucrose gradient.

Δ Np63 was added to 0.25 pmol purified nucleosome at increasing concentration (0 to 2 pmol, 0 to 286 nM). The binding reactions were then separated on a native polyacrylamide gel to detect the Δ Np63-nucleosome complex (Figure 5A). The first lane contained only nucleosomes and was used to measure background and input levels for each experimental replicate. As Δ Np63 concentration increased a supershifted band appears and intensifies at higher amounts of Δ Np63. Meanwhile the nucleosome-only band intensity significantly decreased at higher concentrations of Δ Np63, similar to findings for p53 [21]. DNA was then extracted and purified from the supershifted and nucleosome band and sequenced. The sequencing results were then mapped back to the original 16 nucleosome sequences and compared to the control non-specific sequence in the same lane. This analysis method controls for non-specific binding, gel loading, PCR amplification, and next-generation sequencing. This approach is performed on each non-shifted nucleosome band independently to see what types of nucleosomes are bound by Δ Np63 and shifted, two replicates were conducted (Figure 5B and C). Our analysis demonstrates that nucleosomes containing a high-affinity or intermediate-affinity p63BS located around the nucleosomes boundaries at super-helix location (SHL) 6.5, 7, and in the linker SHL 8 are bound first at the lowest concentrations. While the nucleosomes containing a p63BS located near the dyad are not specifically bound as compared to the control nucleosomes. Examination of the supershifted fragments show similar results (Supplementary Figure S4), and is consistent with Δ Np63 binding only at nucleosome edges.

Discussion

Several studies have examined chromatin environment at transcription factor binding sites [22, 23]. These studies often cannot address cause and effect, due to the fact that the transcription factor is bound in the cell type at the time the chromatin environment is measured. In our previous studies on Δ Np63 binding in human and mouse keratinocytes we found that Δ Np63 binding occurred at predominately active chromatin regions containing active histone modifications (H3K27ac, H3K4me1, and H3K4me3) [18, 24]. These findings are confounded by the fact that Δ Np63 is required for the development and differentiation of keratinocytes. In human keratinocytes two pieces of evidence suggested that Δ Np63 was pivotal in creating the chromatin environment. First, Δ Np63 bound sites have higher sequence defined nucleosome occupancy, suggestive of a positioned nucleosome over the binding site in other cell types. Second, at chromatin open/accessible locations there is a further positive association between DNase signal and the quality of the p63BS [18]. These finding suggested that Δ Np63 functions as pioneer factor that can bind directly to nucleosomes and regulate epithelial differentiation.

To directly test Δ Np63 pioneer ability we used *in vivo* and an *in vitro* assays to determine the chromatin environment at Δ Np63 binding sites before Δ Np63 bound, and the Δ Np63 ability to bind directly to

nucleosomal DNA. K562 and HepG2 cells do not express any of the known p63-isoforms making their chromatin p63-naïve. Thus by activating ectopic Δ Np63 and measuring its binding sites, we can determine the chromatin environment conducive for Δ Np63 binding. In both cells lines, we found Δ Np63 is able to bind its binding sites located in inaccessible chromatin without active histone modifications.

To understand the mechanism for Δ Np63 binding to inaccessible chromatin we examined Δ Np63 binding to nucleosomal DNA with a competitive nucleosome binding assay [21]. In these experiments Δ Np63 can bind to its binding sites when they are located near the nucleosome edge and binding is inhibited within 50bp of the nucleosome dyad. These results are similar to TP53 protein which also preferentially binds at nucleosome edges [21, 25]. These results are consistent with a dynamic partial unwrapping near nucleosome edges where DNA near the entry-exit region is unwrapped from the histone proteins exposing the DNA to TF binding [26, 27]. Our results for Δ Np63 suggest that Δ Np63 can access the partially unwrapped nucleosome and remain stably bound.

To understand the consequences of Δ Np63 binding we examined the chromatin states at sites bound in both K562 and NHEK. In K562 the chromatin measurements all occurred in the absence of the expression of Δ Np63, and show that many of the sites are repressive chromatin states. In NHEK the same sites are characterized by a more active chromatin state, suggesting a direct role for Δ Np63 in activating chromatin. To validate Δ Np63 ability to activate chromatin domains we found a strong increase in H3K27ac at sites after Δ Np63 bind on nucleosomes flanking the binding site. This peak-valley-peak signal is characteristic of active regulatory regions in many cell types and is showing the formation of a nucleosome depleted region occurring at the binding site. Recent studies expressing Δ Np63 in dermal fibroblasts also showed increased chromatin accessibility and H3K27ac at binding sites [28]. How nucleosome remodeling is occurring is likely due to the recruitment of co-activators. In epithelium, Δ Np63 has been shown to require the BAF complex to maintain a nucleosome-depleted region at its binding sites [16], and has been shown to interact with H3K4 methyl-transferase, KMT2D [17]. Our findings show that Δ Np63 ability to remodeling chromatin after binding is not a cell type specific function, and suggests that Δ Np63 is directly recruiting general chromatin remodeling co-activators.

Our findings demonstrate that Δ Np63 is a pioneer factor that can bind to nucleosomal DNA in a histone modification independent manner and elicit chromatin remodeling. Δ Np63 differs from other well-characterized pioneer factors. For example, the canonical pioneer factor FOXA [29, 30] can bind the nucleosome dyad and displace the linker histone to maintain nucleosome accessibility [31]. The Yamanaka factors SOX2 and OCT4 [32] can bind heterochromatin domains, though heterochromatin impedes their binding [33]. Overall, as more pioneer factors are characterized it is becoming clear that they interact with nucleosomes and histone modifications in varied manners.

Materials And Methods

Cell culture and treatment

Human K562 cell line was grown in Roswell Park Memorial Institute (RPMI) 1640 medium (Hyclone SH30027.LS) supplemented with 1% penicillin/streptomycin (Gibco 15140-163), 4 mM L-Glutamin (Gibco 25030-164) and 10% fetal bovine serum (Gibco SH30071.03). HepG2 cells were grown in Dulbecco's modified eagle's medium (DMEM) supplemented with 1% penicillin/streptomycin (Gibco) and 10% fetal bovine serum (Gibco). The identities of all cell lines were confirmed via STR profiling and cultures are routinely checked for mycoplasma contamination. All cell lines were incubated at 37 °C and 5% CO₂. The cDNAs corresponding to human Δ Np63 α and Δ Np63 α (R304W) (Δ Np63 α with an amino acid substitution in DBD thus losing specific sequence binding ability) were cloned into the pINDUCER21 vector respectively. Stable cell lines expressing corresponding cDNAs were generated according to the pINDUCER lentivirus toolkit [34], which was followed by cell sorting to select the high-GFP population indicating stable expression of introduced Δ Np63 α . FACSsorting was performed on a BD Biosystems Arianal by Roswell Park Cancer Institute, Department of Flow and Image Cytometry. After cell selection, 300ng/ml doxycycline were added individually to different cell lines after they were grown to > 60% confluency in order to induce p63 expression. Expression of Δ Np63 α was validated by western blot with Δ Np63-specific in-house generated antibody, Δ Np63 α original expressing epithelial cell line A253 was applied as positive control to confirm Δ Np63 α ectopic expression in engineered cell lines.

Chromatin immunoprecipitation (ChIP) and library preparation

Cell pellets were then collected after 18-hours of Dox induction, and then fixed with 1% formaldehyde for 10 min, approximately 1 to 4 million cells were prepared for each ChIP-seq experiment. ChIP experiments were performed as described previously [24, 35], with sheared chromatin from 1 million cells for H3K27ac or 4 million cells for Δ Np63 α using the iDeal ChIP-seq kit for TFs (Diagenode: C01010055). ChIP for Δ Np63 α was carried out using ~3 ug each of 4A4 mouse monoclonal anti- Δ Np63 antibody or anti-HA antibody. ChIP for histone mark H3K27ac was performed using ~2 ug of H3K27ac (Diagenode: C15410174) antibody. Sequencing libraries were prepared using ThruPLEX DNA-seq kit from Rubicon Genomics. Samples were submitted to University at Buffalo Genomics and Bioinformatics Core (University at Buffalo, State University of New York; Buffalo, New York) and sequenced on a HiSeq using Standard 50-Cycle Single Read Sequencing. Sequencing and quality control were also performed at the University at Buffalo Genomics and Bioinformatics Core.

Data analysis

Raw sequencing reads from K562 (4A4, HA, H3K27ac and inputs) and HepG2 (HA and input) were analyzed through an identical pipeline as performed before [18]. Except that datasets were aligned to either hg19 or hg38 dependent on the downstream data analysis. All available Histone ChIP-seq and DNase-seq conducted in K562 and HepG2 were downloaded from the ENCODE repository: <http://genome.ucsc.edu/ENCODE/>. Chromatin State Segmentation files K562ChromHMM, NHEKChromHMM and HepG2ChromHMM were downloaded from GEO GSM936088, GEO GSM936087, and GEO GSM936090. K562 and HepG2 DNase/FAIRE/ChIP Synthesis file was downloaded from GEO

GSM1002657, and GEO GSM1002654. MNase-seq data was from Mieczkowski *et al*/2016 sample GSM2083140 [36], and analyzed as previously described [37, 38].

Identifying the chromatin profile at p63 bound sites

Histone mark ChIP-seq data in K562, NHEK, and HepG2 cell lines were obtained from ENCODE consortium [19]. The coordinates for p63 bound sites in NHEK were obtained from previous study [18]. Using ArchTex histone modifications and MNase-seq data were plotted for a 2 kb window at 10 bp resolution with a standardized tag count of 100 million or 1 billion, respectively [39]. This analysis was performed in each 3 cell lines respectively. An average signal across a 2 kb window centered at the Δ Np63 α binding site was plotted for corresponding histone marks described in the main text in 3 different cell lines respectively. k-means clustering algorithm implemented in Cluster 3.0 software was applied [40]. The heatmap was generated via Java TreeView software [41].

p63-nucleosome *in vitro* binding assay

Expression and purification of His-tagged Δ Np63 γ protein were performed as described previously [42]. Protein-nucleosome binding assays were carried out in duplicate with purified nucleosomes and Δ Np63 γ protein [21]. Two p63BS were used, an adapted high-affinity ideal sequence: 5'-GGGCATGTCCGGGCATGTCC-3' [43, 44] and a natural intermediate-affinity sequence from the *CDKN1A* promoter: 5'-AGACTGGGCATGTCTGGGCA-3'[45]. 14 nucleosome sequences were designed starting from the 217-bp Widom 601 sequence and compared to non-specific binding to 2 control sequences (Table S1). Protein binding was detected by mobility shift assay on 4% (w/v) native polyacrylamide gels. All visual bands were excised from the gel, as well as the bands at the same locations in the other lanes. DNA from each band was extracted and purified and quantified by qPCR. All samples were multiplexed and sequenced on a MiSeq using 2 $\times$ 150-bp paired-end sequencing. Sequencing and quality control were performed at the University at Buffalo Genomics and Bioinformatics Core. Quality sequence reads were mapped to each specific starting sequence using Blat [46]. The results were then analyzed relative to control/non-specific binding (relative supershift). Relative supershift is determined from the supershift bands and controls technical variability introduced by gel-excision, PCR, NGS-library construction, or NGS sequencing. In this method each specific nucleosome sequence is measured relative to non-specific binding (control 601 fragment).

$$(Eq. 1) \quad Relative\ shift = -\log_2 \left(\frac{\frac{reads\ nucleosome_N}{reads\ nucleosome_{601}}}{\frac{reads\ nucleosome\ input_N}{reads\ nucleosome\ input_{601}}} \right)$$

Where *N* is one of the 16 nucleosome sequences, *601* is the control nucleosome sequence, *reads nucleosome* is the nucleosome band at specific concentration of Δ Np63, *reads nucleosome input* is the nucleosome band in input lane without any Δ Np63 added.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

ChIP-seq datasets are available at NCBI GEO GSE140329 and *in vitro* nucleosome binding data available at NCBI SRA PRJNA588790.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

XY and PS performed the majority of the experiments and analysis. ST analyzed the MNase-seq data. MJB and SS designed and supervised the experiments. XY and MJB wrote the manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1

Δ Np63 binds inaccessible unmodified chromatin in K562 (A) 1980 high-confidence Δ Np63 bound sites in K562 were Kmeans clustered into 4 groups by DNase, H2AFZ, 5 active (H3K4me1, H3K4me2, H3K4me3, H3K9ac, H3K27ac), 2 repressive (H3K9me3, H3K27me3) and other (H3K79me2, H3K9me1, H3K36me3). Chromatin datasets are from K562 in the absence of Δ Np63. (B) Average chromatin architecture in K562 at 1980 Δ Np63 ChIP-seq summits compared to 72291 transcriptional start site (TSS). Data is plotted for 2 kb flanking the summit or TSS. (C) Chromatin accessibility for the 1980 Δ Np63 bound sites in K562.

Accessibility is defined from the synthesis track of DNase and FAIRE from ENCODE (GEO GSE40833 GSM1002657) [47]. (D) Average nucleosome occupancy at Δ Np63 ChIP-seq peaks centered on p63BS determined from MNase-seq, from Mieczkowski et al 2016 [36].

Figure 2

Chromatin changes after Δ Np63 binds. (A) UCSC Genome Browser image showing Δ Np63 binding sites shared in both NHEK and K562 near the TP73 gene. Chromatin State Segmentation (ChromHMM) and

DNase is shown for both K562 and NHEK. Chromatin states are defined by the composite track of Broad ChromHMM from ENCODE (GEO GSE38163 GSM936088, GEO GSE38163 GSM936087) [47]. Fifteen HMM states are associated with different segment colors: state 1-bright red-active promoter, state 2-light red-weak promoter, state 3-purple-inactive/poised promoter, state 4-orange-strong enhancer, state 5-orange-strong enhancer, state 6-yellow-weak/poised enhancer, state 7-yellow-weak/poised enhancer, state 8-blue-insulator, state 9-dark green-transcriptional transition, state 10-dark green-transcriptional elongation, state 11-light green-weak transcribed, state 12-gray-polycomb repressed, state 13-light gray-heterochromatin; low signal, state 14-light gray-repetitive/copy number variation, state 15-light gray-repetitive/copy number variation. (B) Δ Np63 sites bound in K562 and NHEK are grouped into K562 & NHEK (1037 sites), NHEK only (8195 sites), and K562 only (943 sites). (C-E) Average chromatin architecture in the specified cell line for Δ Np63 bound site in (C) both K562 and NHEK, (D) NHEK only, or (E) K562 only.

Figure 3

Δ Np63 expression in HepG2 leads to binding at inaccessible and unmodified chromatin regions. (A) The 2939 Δ Np63 bound sites in HepG2 cells with their histone modifications and DNase measurements. Sites are sorted by DNase signal. (B) Chromatin accessibility for the 2939 Δ Np63 bound sites in HepG2. Accessibility is defined from the synthesis track of DNase and FAIRE from ENCODE (GEO GSE40833

GSM1002654) [47]. (C) Average chromatin architecture in HepG2 at inaccessible Δ Np63 ChIP-seq summits compared to transcriptional start site (TSS). Data is plotted for 2 kb flanking the summit or TSS.

Figure 4

Δ Np63 binding causes H3K27ac of flanking nucleosomes. (A) Average chromatin architecture for H3K27ac at Δ Np63 ChIP-seq peaks centered on p63BS. Data is shown for each H3K27ac ChIP-seq experiment from K562 + Δ Np63 and K562 + Δ Np63(R304W) control experiments. (B) H3K27ac at transcriptional start site (TSS). (C, D) H3K27ac at two p63BS. Average H3K27ac from two replicate experiments for K562 + Δ Np63 and K562 + Δ Np63(R304W). Nucleosome occupancy as determined from MNase-seq from non-p63 expressing K562.

Figure 5

Δ Np63 binds to nucleosome edges. (A) A 217-bp dsDNA library was designed containing p3BS in various nucleosomal positions. A 20-bp-long p3BS (intermediate and high affinity) was placed at different positions 0, 5, 41, 46, 66, 71, and 81 bp away from the dyad. The superhelix location (SHL) is designated for each nucleosome sequence. Within the nucleosome, three different positions (dyad, intermediate, and edge) were chosen with increasing distance to nucleosome dyad. (B) Nucleosomes containing 16 different sequences were bound to increasing amounts of Δ Np63 and separated by native PAGE. Lanes contain the following: 1 – 0.25 pmol nucleosomes, 2-5 contain 0.25 pmol of nucleosomes with 18, 36, 71, 142, or 286 nM of Δ Np63 (0.125, 0.25, 0.5, 1, or 2 pmol). Nucleosome and the supershift (Ss) bands are indicated. (C-D) Relative shift for each nucleosome is determined by counting the frequency of each sequence within the nucleosome band and comparing it to non-specific binding to the 601 sequence. This value is then normalized to the input ratios of nucleosomes. Results for each experimental replication is shown in (C) and (D).

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