**SI Appendix**

### Fig. S1: Identification of DBC1 as ELL interacting proteins

A. Mass Spectrometric analysis showing DBC1 peptides in our ELL.com preparation as discussed earlier (1).

B. Western blotting analysis showing generation of stable DBC1 cell line ectopically expressing DBC1 as FLAG-HA-tagged.

Lane C denotes control in this experiment.

C. Co-IP and subsequent western blotting analysis showing interaction between DBC1 and ELL through their co-expression in 293T cells.

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**Table:**

| Protein  | Coverage | No of Unique Peptides | Total No of Peptides | Representing Peptides                      |
|----------|----------|------------------------|----------------------|-------------------------------------------|
| K1A 1967 | 42.6     | 34                     | 62                   | AAAQPIAGAEQTEPQAEQTHQTELQAPDLAQETLQSLR    |
| K1A 1967 | 42.6     | 34                     | 62                   | KEEAVVYGGWGSQDSLEDPQAPQVLYR               |
| K1A 1967 | 42.6     | 34                     | 62                   | AAVPQGQAEPYNAK                             |
| K1A 1967 | 42.6     | 34                     | 62                   | SAPPPLHAALQGK                              |
| K1A 1967 | 42.6     | 34                     | 62                   | FAEQYTLQPGPR                               |
| K1A 1967 | 42.6     | 34                     | 62                   | EAFAQH06AEPTINDSHPVSSX                     |

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Fig. S2: Positive regulation of transcription of chromosomally integrated reporter gene by DBC1 and ELL

A. Effect of DBC1 on activator (GAL4-VP16)-dependent chromosomally integrated luciferase reporter gene expression. The inset panel shows expression of transfected DBC1 within Gal-luciferase cells.

B. Western blot analysis showing effect of increasing expression of DBC1 on expression of activator GAL4-VP16 within Gal-luciferase cells.

C. Effect of ELL on activator (GAL4-VP16)-dependent chromosomally integrated luciferase reporter gene expression. The inset panel shows expression of transfected ELL in the Gal-luciferase cells.

D. Effect of increasing expression of DBC1 with constant ELL expression on activator (GAL4-VP16)-dependent reporter gene expression. The inset panel shows expression of transfected ELL and DBC1 in the Gal-luciferase cells.

E. Effect of increasing expression of ELL with constant DBC1 expression on activator (GAL4-VP16)-dependent reporter gene expression. The inset panel shows expression of transfected ELL and DBC1 in the Gal-luciferase cells.

In all the experiments mentioned above, one-tailed student’s t test was used to calculate the statistical significance of the data. * denotes p≤0.05, ** denotes p≤0.01, *** denotes p≤0.001, and ns denotes not significant.
Fig. S3: Interaction of various deacetylases with ELL and their effect on regulating ELL stability

A. Western blot analysis showing the effect of DBC1 over-expression on ectopic expression of control GFP.

B. qRT-PCR analysis showing levels of DBC1 (top panel) and ELL (bottom panel) mRNA (normalized to 18s rRNA) when increasing amount of DBC1 is co-expressed with constant ELL (Fig. 2A). For showing relative RNA level, 18s rRNA normalized values were further normalized to the value in lane no. 1 for each of the DBC1 and ELL RNA levels.

C. Western blot analysis showing the effect of over-expression of DBC1 on endogenous ELL expression in HCT116 cells.

D. Co-IP and western blotting analysis showing absence of ELL and SIRT1 interaction in 293T cells.
E. qRT-PCR analysis showing ELL (lower panel) and HDAC3 (upper panel) mRNA (relative to 18s rRNA) level in presence of over-expressed HDAC3 (Fig. 2E).

F. Western blot analysis showing the effect of over-expression of HDAC3 on ectopic expression of ELL in HCT116 cells

G. Western blot analysis showing the effect of over-expression of SIRT1-7 on ELL stabilization in 293T cells. Red filled circles indicate target protein bands in our assay. Other bands present on the blot are either degradation or non-specific in nature. In presence of over-expressed SIRT6, we consistently observe the appearance of additional bands above the target SIRT6 band when immunoblotted with α-FLAG antibody.

H. Western blotting showing interaction of ELL and HDAC3 in 293T cells by immunoprecipitation of ELL.

I. Western blotting showing interaction of HDAC3 and ELL in 293T cells by immunoprecipitation of HDAC3. Presence of DBC1 was used as a positive control in this assay.

J. Effect of HDAC3 knockdown on ELL mRNA level as measured by qRT-PCR analysis in 293T cells.
Fig. S4: p300-mediated acetylation stabilizes ELL
A. Western blot analysis showing ELL acetylation through increased over-expression of p300 within 293T cells.
B. Purification of the HAT domain of p300 and GST-ELL (using bacterial expression system) that were used for in vitro acetylation of ELL by p300 (Fig. 4B).
C. Western blot analysis showing the effect of increasing over-expression of p300 on ectopic expression of ELL in 293T cells (upper panel) and quantitation of ELL protein relative to Actin (lower panel).
D. qRT-PCR analysis showing the effect of p300 over-expression on expression of endogenous ELL mRNA (Fig. 4C) normalized to 18s rRNA. The data obtained were further normalized with value in EV control lane.
E. Western blot analysis showing stable p300 knockdown by two different shRNAs within 293T cells.
F. RNA analysis showing mRNA levels of endogenous ELL in the two generated p300 knockdown cell lines when compared to 18s rRNA. The data obtained were further normalized with the control scramble sample.
G. Western blot analysis showing effect of stable p300 knockdown, by two different shRNA constructs, on endogenous ELL protein level within HCT116 cells.
H. Immunoprecipitation and western blotting analysis showing the effect of p300 knockdown on acetylation levels of endogenous ELL. The bottom panel shows the quantification of acetylation signal as compared to that of scramble control cells.
I. *In vitro* deacetylation assay to show deacetylation of ELL by purified HDAC3.
J. Immunoblots showing the acetylation status of endogenous ELL in HDAC3 knockdown cell lines.
K. Immunoprecipitation of endogenous ELL in control and DBC1 over-expressed 293T cells and subsequent western blotting analysis showing decreased association of endogenous ELL with HDAC3 upon its binding with ectopic DBC1. Interestingly, the reduced HDAC3 binding upon DBC1 over-expression also results in increased acetylation of endogenous ELL as can be seen in the IP panel.
**Fig. S5: Over-expression of DBC1 and p300 stabilizes ELL from ubiquitin-mediated degradation.**

A. Western blot analysis showing the rescue of ubiquitin-mediated ELL degradation through concomitant over-expression of DBC1.

B. Western blot analysis showing the rescue of ubiquitin-mediated ELL degradation through concomitant over-expression of p300.

C. Western blot analysis showing a strong correlation of increase in acetylation and decrease in ubiquitin mediated poly-ubiquitylation of ELL through concomitant over-expression of DBC1 in 293T cells.

D. Western blot analysis showing strong correlation of increase in acetylation and decrease in ubiquitin-mediated poly-ubiquitylation of ELL through concomitant over-expression of p300 in 293T cells.

E. Coomassie staining of purified GST-tagged full-length and deletion mutants of ELL (as indicated) (top panel). In vitro acetylation assay and subsequent immunoblotting showing acetylation of different ELL fragments by p300 HAT (bottom panel). The blots were also probed with α-GST antibody to show the amount of GST-ELL protein used in our *in vitro* assays.

F. Multiple sequence alignment of the N-terminal 60 amino acids of ELL showing conserved nature of Lysine 5 (K5) and Lysine 29 (K29) across various species.

G. *In vitro* acetylation assay showing p300-mediated acetylation of ELL (WT) and ELL(K5A, K29A) mutant.

H. Western blot analysis showing defective acetylation of ELL (K5A, K29A) mutant as compared to wild type in presence of over-expressed p300 within 293T cells.

I. Western blotting analysis showing HDAC3-mediated poly-ubiquitylation of ELL (WT) and ELL (K5A, K29A) mutant within 293T cells.

J. Western blotting analysis showing an effect of DBC1 over-expression on expression of ELL (WT) and K5A, K29A mutant in 293T cells (input panels). Further, consistent with a role of K5 and K29 sites ELL as targets for acetylation, DBC1 over-expression fails to show any increase in acetylation in ELL (K5A, K29A) mutant when compared to the wild type.
Fig. S6: Global co-regulation of gene expression by DBC1 and ELL are implicated in multiple cellular events

A. Reporter assay showing the effect of ELL (WT) and ELL (K5A, K29A) on activator (GAL4-VP16)-dependent chromosomally-integrated luciferase reporter gene (upper panel). The lower panel shows the expression levels of the ELL constructs used in the assay.

B. qRT-PCR showing Siah1 (lower panel) and ELL (upper panel) mRNA expression in the experiment as described in Fig. 5I. Interestingly, in presence of increased Siah1 expression, ELL mRNA level does not decrease thus indicating an effect of Siah1 on decreased ELL protein level is a result of its effect on ELL protein and not RNA.

C. Venn Diagram showing the number of overlapping genes that are down-regulated by knockdown of both DBC1 (GSE35480) and ELL (GSE34104) in 293T cells as obtained from two different publicly available datasets.

D. Pathway analysis predicting cellular processes that are affected by DBC1-ELL co-regulated genes.
E. Western blotting analysis showing stable knockdown of DBC1 in 293T cells by two different shRNAs. Decreased DBC1 also results in a concomitant decrease in ELL protein level.

F. Immunoblots showing stable knockdown of DBC1 in HCT116 cells by two different shRNAs. Here also, decreased DBC1 also results in concomitant decrease in ELL protein level.

G. ChIP analysis showing the recruitment of indicated target factors at the TSS of *NEDD9* and *PTPRF* genes, whose expressions are not impaired upon DBC1 knockdown as shown in Fig. 6A.
Fig. S7: Knockdown of DBC1 causes down-regulation of expression of TAF components of TFIIID as well as key SEC components.

A. Cell proliferation assay demonstrating an effect of DBC1 knockdown (by two different shRNAs) on proliferation of 293T cells. Equal number of cells were seeded and counting was done on the indicated days.

B. Western blot analysis showing the effect of DBC1 knockdown on the expression of target TAF subunits of TFIIID in 293T cells.

C. qRT-PCR analysis showing the effect of DBC1 knockdown on mRNA expression of indicated TAF subunits of TFIIID.

D. qRT-PCR analysis showing the effect of re-expression of ELL in DBC1 knockdown cells on the restoration of expression of TAF3 subunit of TFIIID.

E. Western blot analysis showing the effect of DBC1 knockdown on the expression of SEC subunits in 293T cells.

F. qRT-PCR analysis showing effect of DBC1 knockdown on the expression of indicated SEC subunits at the mRNA level.

In all of our statistical analyses, one-tailed student’s t test was used to calculate the statistical significance of the data, wherein, * denotes p≤0.05, **denotes p≤0.01, *** denotes p≤0.001, and ns denotes not significant.
**Supplemental Table 1: List of Plasmids used in this study**

| Name of plasmid | Plasmid details | Source |
|-----------------|-----------------|--------|
| M10             | pcDNA5/FRT/TO vector with cloned FLAG-HA tag | Biswas et al., 2011 |
| M15             | ELL cloned into M10 vector (Biswas et al., 2011) | Biswas et al., 2011 |
| M43             | pFASTBAC vector | Invitrogen |
| M44             | pFASTBAC vector with FLAG tag | Biswas et al., 2011 |
| M56             | pGEX vector with GST tag | Amersham |
| M61             | ELL cloned into M56 vector | This study |
| M90             | 6-His pET-11d vector | Kumari et al., 2019 |
| M296            | psPAX2 lentiviral packaging plasmid | Addgene #12260 |
| M297            | pMD2.G VSV-G envelope plasmid | Addgene #12259 |
| M336            | DBC1 cloned into M10 vector | This study |
| M337            | DBC1 cloned into M44 vector | This study |
| M340            | pM vector | Clontech |
| M378            | FLAG-PCAF cloned in pCI vector | Addgene #8941 |
| M379            | HA-CBP in pRc/RSV vector | Addgene #16701 |
| M380            | FLAG-HDAC1 in pcDNA3.1 | Addgene #13820 |
| M381            | FLAG-SIRT1 in pECF vector | Addgene #1791 |
| M442            | VP16 Activation domain cloned into M340 vector | Ghosh et al., 2018 |
| M447            | pcDNA5/FRT/TO vector with cloned FLAG tag | Yadav et al., 2019 |
| M451            | pcDNA5/FRT/TO vector with cloned myc tag | This study |
| M464            | DBC1 cloned into M447 vector | This study |
| M496            | pEGFP-N2 vector | Clontech |
| M532            | P300 cloned in M451 | Kumari et al., 2019 |
| M594            | Myc tagged Dbc1 in pcDNA3 | Addgene #35096 |
| M686            | p300 HAT domain (1284-1672 aa) cloned in M90 | Kumari et al., 2019 |
| M742            | ELL (1-500 aa) cloned into M447 vector | This study |
| M743            | ELL (1-373 aa) cloned into M447 vector | This study |
| M744            | ELL (45-621 aa) cloned into M447 vector | This study |
| M745            | ELL (374-621 aa) cloned into M447 vector | This study |
| M746            | ELL (45-373 aa) cloned into M447 vector | This study |
| M747            | ELL cloned into M447 vector | This study |
| M773            | MOF cloned in M449 vector | Kumari et al., 2019 |
| M787            | FLAG-HDAC4 in pcDNA3 vector | Addgene #30485 |
| M788            | FLAG-HDAC7 in pcDNA3.1 vector | Addgene 13824 |
| M789            | FLAG-HDAC8 in pcDNA3.1 vector | Addgene #13825 |
| M790            | FLAG-HDAC2 in pcDNA3 vector | Addgene #68117 |
| M791            | FLAG-HDAC6 in pcDNA3.1 vector | Addgene #13823 |
| M792            | FLAG-HDAC3 in pcDNA3.1 vector | Addgene #13819 |
| M794            | FLAG-GCN5 in pAdEasy vector | Addgene #14106 |
| M796            | FLAG-HDAC5 in pcDNA3.1 vector | Addgene #13822 |
| M912            | Ubiquitin cloned in EGFP-C1 vector | Addgene #11928 |
| Antibodies  | Source                                      |
|------------|---------------------------------------------|
| ELL        | Bethyl Laboratories and Cell Signaling Technology |
| EAF1       | Santa Cruz Biotechnology                    |
| EAF2       | Abcam                                       |
| CCNT1      | Santa Cruz Biotechnology                    |
| CDK9       | Santa Cruz Biotechnology                    |
| DBC1       | Bethyl Laboratories and Cell Signaling Technology |
| AF4        | Abcam                                       |
| TBP        | Cell Signaling Technology                   |
| AF9        | Bethyl Laboratories                         |
| β-Actin    | Santa Cruz Biotechnology                    |
| P300       | Cell Signaling Technology                   |
| Rpb1 CTD (4H8 clone) | Cell Signaling Technology        |
| Ubiquitin  | Cell Signaling Technology                   |
| Flag epitope | Sigma                                      |
| HA epitope | Santa Cruz Biotechnology                    |
| His epitope | Santa Cruz Biotechnology                    |
| Myc epitope | Cell Signaling Technology                   |
| GST        | Santa Cruz Biotechnology                    |
| GFP        | BioBharati Life Science                     |
| Acetylated Lysine | Cell Signaling Technology                  |
| Phospho Rpb1 CTD (Ser2) | Cell Signaling Technology                  |
| Phospho Rpb1 CTD (Ser5) | Cell Signaling Technology                  |
| Normal Rabbit IgG | Cell Signaling Technology                 |
| Normal Mouse IgG | Cell Signaling Technology                  |
| HRP conjugated secondary antibody (Rabbit) | Bio-Rad                                   |
| HRP conjugated secondary antibody (Mouse) | Cell Signaling Technology                  |

Supplemental Table 3: List of qRT-PCR primers used for RNA analysis in this study

| Gene   | Forward Sequence | Reverse Sequence               |
|--------|------------------|--------------------------------|
| AF9    | GAGAGAAAGACACATTCTGC | GGATGTTCCAGATGTTTCC          |
| APOE   | GCGGATGGAGGAGATG   | CTCGAACCAGCTCTTG             |
| ATF2   | GTACCAGGCCCATTTCTCTTC | GAACGAGTGGGACTGCAGCTG       |
| Gene | Upper Oligo | Lower Oligo |
|------|-------------|-------------|
| **DBC1** sh#1 | CCGGGCAAGGAAAGATCTTCTTCTG | AGAAAGAGATCTTCTTCTG |
| **DBC1** | CCGGGCATTTGAGCCTGACTTC | AATTCAAAAAGCCAAAGGAAAGATCTTCTTCTG |

**Supplemental Table 4: Oligo Sequences for shRNA construct generation for DBC1 knockdown**
| Gene  | 5’ Primer                  | 3’ Primer                  |
|-------|----------------------------|----------------------------|
| CCND1 | GCATCAGAGGATTCTACTGT       | CTCCTGTGTGAATTCCG          |
| MDM2  | GGAGTTAAGTCCGTGACTTTGTCT  | GATCAGCAGAGAAAAATG GCC     |
| SK1   | CAGAAGACGCTGGAGCAGTCCGTC  | CTTGGCGCTCCTCTTTGTAG       |
| KIF1B | TCCTCCGACAGCCGCTCAC        | CATCAGCAGCCAGCCCG          |
| BCL6  | CCTCTTATGTGCACCAGATTAGC   | GGTTCCGGTGTCAGGCTC         |
| GLUT1 | GGTCTCTAAACGCTACGGTCC     | CGTTTGGTCTCCTGCTCCCTCG     |
| NEDD9 | GAAGCAGACGAGAAGATTAGA     | CTAGAGCCAGCTGTAAGC         |
| PTPRF | TCCAGCTTCCGGCTCCGC        | TCCACCTGGTCCCGCTCC         |

**Table 5: qRT-PCR primer Sequences for ChIP analysis**

**Supplemental Experimental Methods**

**Cell Culture and Transfection**

All mammalian cells used in this study were cultured in DMEM (Invitrogen, USA), supplemented with 10% FBS (Gibco, USA) and 100 U/ml Penicillin-Streptomycin (Gibco, USA). Cells were maintained in a humidified incubator at 37°C and 5% CO₂. Sf9 cells were cultured in Grace’s Insect Media (Himedia, India), supplemented with 10% FBS (Gibco, USA) and 7 µg/mL gentamicin (Gold Biotechnology, USA). For transfection in mammalian cells, Lipofectamine2000 (Invitrogen, USA) was used as per manufacturer’s protocol. Sf9 transfections were performed using Cellfectin II reagent (Invitrogen, USA) as per manufacturer’s protocol. For all assays, unless otherwise mentioned, cells were harvested 48 hours post transfection.

**Creation of different plasmid constructs**

The cDNAs for ELL and DBC1 were purchased commercially (Open Biosystems, USA). The genes were cloned into different epitope tagged pcDNA5/FRT/TO plasmids for mammalian expression and generation of stable cell lines. Deletion constructs of ELL as well as DBC1 were also created in epitope tagged pcDNA5/FRT/TO vectors. For baculovirus-mediated expression in insect cells, genes were cloned in pFASTBAC vector as mentioned. For His-tagged and GST tagged protein expression in bacteria, genes were cloned in 6×His pET-11d and pGEX vectors respectively. Site directed mutagenesis was performed using the QuikChange II Site-Directed Mutagenesis Kit (Agilent technologies, USA). Details of all clones are available upon request from the corresponding author.

**Stable Cell line Generation**
Generation of stable mammalian cell line for FLAG-HA-ELL has been described previously (1). For creation of stable lines expressing FLAG-HA-DBC1, essentially the same protocol was followed. Briefly, after transfection of Flp-In T-REx 293 cells with the respective plasmid, along with pOG44, cells were subjected to 200 µg/ml Hygromycin (Invitrogen, USA) selection for several weeks until individual colonies were obtained. Individual colonies were further amplified and then screened for the expression of the protein of interest. Cells were harvested in 1X Phosphate buffered saline (PBS, 137 mM NaCl, 2.7 mM KCl, 8 mM Na₂HPO₄, and 2 mM KH₂PO₄) and lysed in RIPA Buffer (10 mM Tris-Cl (pH 8.0), 1 mM EDTA, 1% Triton X-100, 0.1% SDS, 150 mM NaCl). Expression of target proteins was checked via SDS-PAGE followed by probing with epitope tag-specific antibodies.

**Mass Spectrometry Analysis**

Mass spectrometry analyses of DBC1 associated proteins was performed as described previously (1). Purified DBC1.com was resolved on a 4-12% SDS-PAGE gel and proteins were stained using Coomassie Brilliant Blue-G. Protein bands were excised carefully and destained in 20% methanol for 6 hours. Cysteines were reduced and alkylated with IAA. Proteins were then digested overnight with modified Trypsin (sequencing grade). The peptides were resolved on a nanocapillary reverse phase column using a 1% acetic acid/acetonitrile gradient at 400nl/min rate flow. The separated peptides were injected into a linear ion-trap mass spectrometer (LTQ XL, ThermoFisher). MS/MS spectra of the most were collected and proteins identified by comparing the data against Human IPI database (v 3.41) using X!Tandem/Trans-Proteomic Pipeline (TPP) software suite.

**Nuclear Extract Preparation**

Nuclear extract preparation from cells was done as described earlier (1). Briefly, cells were harvested in PBS and centrifuged at 800xg for 5 minutes. The packed cell volume (PCV) was estimated and cells were resuspended in 2xPCV of Buffer NE1 (10 mM Tris-Cl pH 7.3, 1.5 mM MgCl₂, 100 mM NaCl, 0.7 µl/ml β-Mercaptoethanol. After leaving on ice for 15 minutes, the cells were drawn into a syringe with a 23 gauge needle, and repeatedly injected and aspirated 8 times in order to shear the cell membrane. The lysate was centrifuged at 6000 rpm for 5 minutes at 4°C to obtain the nuclear pellet. After estimation of the nuclear pellet volume (NPV), the pellet was resuspended in 0.5xNPV of Buffer NE2 (20 mM Tris-Cl pH 7.3, 1.5 mM MgCl₂, 20 mM NaCl, 0.2 mM EDTA, 25% Glycerol, 0.7 µl/ml β-Mercaptoethanol and Protease inhibitor Cocktails (PIC) (Roche, USA). An equal volume of NE3 Buffer (20 mM Tris-Cl pH 7.3, 1.5 mM MgCl₂, 1.2 M NaCl, 0.2 mM EDTA, 25% Glycerol, 0.7 µl/ml β-Mercaptoethanol and PIC) was then added and the cells were vortexed immediately to ensure uniform mixing. The resulting homogenate was incubated on ice for 45 minutes and vortexed every 3 minutes for mixing and subsequent extraction from nuclei. Finally, the nuclear lysate was centrifuged at 12000xg for 20 minutes at 4°C to obtain the supernatant containing the nuclear extract. The extract was either immediately utilized for experimental procedures or flash frozen and stored at -80°C for future use.

**Immunoprecipitation analyses**

For exogenously expressed epitope tagged proteins, cells were harvested 48 hours post transfection and lysed in BC300 buffer (20 mM Tris-Cl, pH 8.0, 300 mM KCl, 2 mM EDTA, 20% Glycerol). The cell
supernatant containing the total protein was subjected to immunoprecipitation using anti-epitope antibody-tagged magnetic/agarose beads. After incubation, the beads were washed thrice with BC300 + 0.1% NP40 to remove unbound proteins and non specific interactors. The beads were then boiled in 1X SDS loading dye at 95°C for 10 minutes to elute bead bound proteins, which were subsequently analyzed by Western blotting using tag/protein-specific antibodies.

For endogenous immunoprecipitation experiments, nuclear extract was prepared from 293T cells as described previously. The extract, containing approximately 500µg of proteins was subjected to pre-clearing using Protein-A agarose beads for 2hours at 4°C. The beads were then discarded and the pre-cleared cell lysate was incubated with 2µg of the indicated primary antibody overnight at 4°C. The antibody bound proteins were then immunoprecipitated using Protein G magnetic beads (BioRad, USA) at 4°C for 1 hour. The supernatant was discarded and the beads were washed 3X using the buffer used for nuclear extract preparation. Subsequently, the beads were boiled in 1X SDS loading dye to at 95°C for 10 minutes to elute bound proteins, which were analyzed by SDS-PAGE followed by Western blotting.

**DBC1.com complex purification from nuclear extract**

Flag-HA tagged DBC1 expressing stable cells were grown in a large volume (~ 6 litres) in a spinner flask. Nuclear extract was prepared as per the method described earlier. The extract was pre-cleared using Protein-A agarose beads for 2 hours at 4°C. The pre-cleared extract was incubated with Anti-FLAG M2 Magnetic Beads (Sigma, USA) overnight at 4°C. Bead bound proteins were subjected to extensive washing with BC300 buffer. Finally, bound proteins were eluted by incubating with 3X FLAG peptide (250ng/µl) for 1 hour at 4°C. The purified complexes were separated on a 4-12% gel and stained using ProteoSilver Silver Stain Kit(Sigma, USA).

**Luciferase Reporter Gene Assay**

Luciferase assays were carried out using the G-293T cell line (2). G-293T is a HEK-293T-based cell line containing a chromosomally integrated luciferase gene downstream of Gal4 binding sites. For the assay, cells were transfected with the mentioned amount of the respective constructs along with Gal4-VP16 activator. 48 hours post transfection, cells were harvested as per the protocol mentioned in Dual-Glo® Luciferase Assay System (Promega, USA) and luciferase activity measured using the GloMax 20/20 Luminometer (Promega, USA).

**Cycloheximide Chase Assay**

Cells were transfected with the indicated constructs in the combinations mentioned. 24 hours post transfection, cells were treated to 100 µg/ml cycloheximide (Sigma, USA). At the indicated time points, cells were harvested in ice-cold PBS and lysed using RIPA buffer. Lysates were analysed by western blotting with respective antibodies.
Recombinant protein purification

For the purpose of in vitro reactions and interaction analyses, proteins were purified either from bacterial or mammalian cells. For purification of proteins from bacteria, E. coli BL21-DE3 cells expressing the protein of interest were grown for 4 hours after induction with 1mM IPTG (Goldbio, USA). Harvested cells were lysed in appropriate buffer by sonication [60% amplitude with 10 cycles of alternating on/off phases]. The cell supernatant containing the protein of interest was incubated with Ni-NTA beads for His-tagged proteins and GST agarose beads for GST-tagged proteins. The beads were then washed with appropriate buffers, and eluted using GST elution buffer or His elution buffer.

In vitro acetylation assay

Purified ELL was incubated with purified p300 HAT domain and 0.5mM acetyl-CoA in HAT buffer (75mMTris-Cl (pH 8.0), 1.25mM EDTA, 12.5mM DTT, 0.25% Tween20, 25% glycerol). The reaction mix was incubated at 30°C for 2 hours with intermittent tapping. The reaction was stopped by adding SDS loading dye, followed by boiling at 95°C for 10 minutes. The products were analysed by SDS PAGE followed by immunoblotting using pan acetyl lysine-specific antibody.

In vitro deacetylation assay

Flag tagged HDAC3 was purified by immunoprecipitation from 293T cells. Purified acetylated ELL was incubated with HDAC3 in HDAC buffer (50mMTris [pH 8.0], 4mM MgCl₂, 0.2mM DTT) on a rotor for 3 hours at 37°C. The reaction was stopped by adding SDS loading dye and boiling the samples for 10 minutes at 95°C. Proteins were resolved by SDS-PAGE and analyzed by western blotting.

NGS data mining and GO analysis

Identification of target genes for both DBC1 and ELL was done using the publicly available datasets GSE35480 (3) and GSE34104 (4). Genes were sorted in MS Excel and overlapping genes identified using Venny. Initial gene ontology of overlapping genes was performed using Protein Annotation Through Evolutionary Relationship (PANTHER) software (5). Further functional classification and disease annotation of common genes was done using Database for Annotation, Visualization and Integrated Discovery (DAVID) (6).

Generation of stable knockdown cells

For the purpose of generating cells with DBC1 stably knocked down, DBC1 specific shRNAs were cloned into pLKO.1 vector. 375ng psPAX2 packaging plasmid, 125ng pMD2.G VSV-G envelope plasmid and 500ng of the respective shRNAs were co-transfected into 5x10⁵ cells in a 6 well plate. 72 hours post transfection, the supernatant containing virus particles was collected, centrifuged briefly and used to transduce fresh cells. Polybrene was added at a concentration of 8 µg/ml to aid viral transduction. Cells were then subjected to selection with 3 µg/ml puromycin. Puromycin resistant cells were amplified and screened to identify knock down cell lines via immunoblotting and qRT-PCR analysis.
qRT PCR for gene expression analysis

For qRT-PCR assay, total RNA was extracted from cells using TRizol as per manufacturer’s protocol. 1 µg of total RNA was used as a template for cDNA synthesis using Verso cDNA synthesis kit (Thermo Fisher Scientific, USA). cDNA was diluted 25 times using Ultrapure water (Life Technologies, USA). qRT-PCR was performed in a CFX96 Touch Real-Time PCR Detection System (BioRad, USA) using iTaq Universal SYBR Green Supermix (BioRad, USA) and specific primers for target genes. Relative expression was calculated by normalizing the gene expression to 18s rRNA expression as an internal control.

Chromatin Immunoprecipitation Assay

Chromatin immunoprecipitation (ChIP) assays were performed as described earlier (2). Cells were cross-linked using 1% formaldehyde (Sigma, USA) for 10 minutes at room temperature. After a brief wash with cold 1X PBS, quenching was done by addition of 125mM glycine. After another brief wash with cold PBS, cells were harvested with a scraper and centrifuged at 2500 RPM for 10 minutes. Nuclear fraction was prepared by lysing the cells in nuclear extraction buffer (0.5% NP40, 1% Triton X-100, 300mM NaCl, 20mM Tris (pH 7.5), 2mM EDTA and protease inhibitor cocktail (PIC)). The nuclear fraction thus obtained was resuspended in shearing buffer (1% SDS, 50mM Tris (pH 8.1), 10mM EDTA and PIC). Sonication was performed in a UCD-200 Bioruptor (Diagenode) for 30 min with alternating on/off pulses at 30 seconds interval. The sonicated chromatin was checked on a 1% agarose gel to ensure DNA fragmentation between 200-500 bp. Following sonication, the samples were pre-cleared by incubation with Protein A agarose beads at 4°C. The pre-cleared lysate was diluted 10 times with ChIP dilution buffer (0.01% SDS, 1.1% Triton X-100, 1.1mM EDTA, 20mM Tris-Cl (pH 8.0) and 167mM NaCl). Immunoprecipitations were performed overnight at 4°C using 2 µg of the respective primary antibodies. The antibody bound complexes were then incubated with Protein G magnetic beads (BioRad, USA) at 4°C for 1 hour. The bead bound complexes were then washed with low salt buffer (0.1% SDS, 1% TritonX-100, 2mM EDTA, 20mM Tris-Cl (pH 8.0), 150mM NaCl, and PIC), followed by high salt buffer (0.1% SDS, 1% Triton X-100, 2mM EDTA, 20mM Tris-Cl (pH 8.0), 500mM NaCl, and PIC) and LiCl buffer (0.5M LiCl, 1% NP40, 1% deoxycholate, 20mMTrisCl(pH8.0) and 1mM EDTA). DNA complexes were eluted by incubation with elution buffer (1% SDS, 0.1M NaHCO₃) and cross links reversed by incubation at 65°C for 4 hours. The DNA was then purified by QIAquick PCR purification kit (Qiagen, Germany) as per the recommended protocol. The purified DNA was used as the template for subsequent qRT-PCR reactions.

PBMC Isolation

For the purpose of downstream experiments, peripheral blood mononuclear cells (PBMCs) were isolated from whole blood using HiSep LSM1077 (HiMedia Laboratories, India) as per the manufacturer’s protocol. The HiSep Solution was carefully pipetted into sterile 15ml centrifuge tubes. Whole blood collected in EDTA coated vials was gently layered onto the HiSep solution. The ratio of HiSep: blood was maintained at 1:1. The tubes were centrifuged at 500g for 30 minutes at 25 °C. The interface of plasma and HiSep, containing the PBMCs was aspirated carefully and washed thrice with 1x PBS. They were then lysed using RIPA buffer for obtaining proteins, or used for RNA extraction with TRizol.
References:

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