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SUPPLEMENTARY INFORMATION

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# Bromodomain inhibition of the coactivators CBP/EP300 facilitate cellular reprogramming

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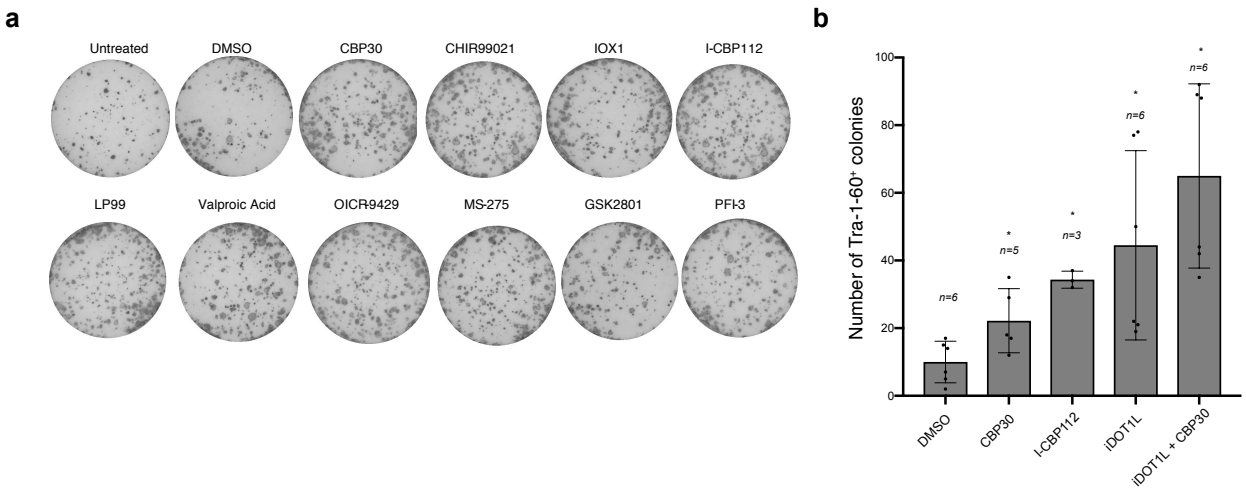
Supplementary Table 1

| Compound name | Target | Final Concentration (μM) |
|---|---|---|
| 3-Deazanoplanocin (3-DZNep) | Histone methyltransferase inhibitor; decreases global histone methylation. Inhibits EZH2 histone methyltransferase activity | 0.5 |
| 5-Azacitidine | DNA methyltransferase inhibitor | 1 |
| 5-Iodotubercidin | Potent adenosine kinase inhibitor | 0.1 |
| A-366 | Potent and selective G9a/GLP inhibitor | 0.1 |
| BAZ2-ICR | Selective BAZ2 bromodomain inhibitor | 1 |
| Belinostat | Hydroxamic acid-type histone deacetylase (HDAC) inhibitor | 0.5 |
| Bromosporin | Broad spectrum bromodomain inhibitor | 0.5 |
| CDX101 | Potent inhibitor of histone deacetylase | 0.2 |
| Chaetocin | Histone methyltransferase SUV39H1 inhibitor | 0.005 |
| CHIR99021 | Potent and highly selective inhibitor of glycogen synthase kinase 3 (GSK-3) | 1 |
| CI 994 | Orally active class I histone deacetylase (HDAC) inhibitor | 0.25 |
| MS-275 | Strongly inhibits HDAC1 and HDAC3 | 0.1 |
| EX 527 | Selective inhibitor of SIRT1 that does not inhibit histone deacetylase (HDAC) or other sirtuin deacetylase family members | 0.2 |
| Forskolin | Cell-permeable activator of adenylyl cyclase | 10 |
| GSK2801 | Selective BAZ2A and BAZ2B inhibitor | 0.5 |
| GSK343 | Potent, specific inhibitor of the histone H3-lysine 27 (H3K27) methyltransferase EZH2 | 0.6 |
| GSK-J4 | H3K27 Histone lysine demethylase (KDM) inhibitor | 1 |
| GSK-LSD1 | Potent and selective lysine specific demethylase 1 (LSD1) inhibitor | 1.25 |
| I-BET | BET bromodomain inhibitor | 0.5 |
| I-CBP112 | CBP/p300 BRD inhibitor | 1 |
| IOX1 | Histone demethylase JMJD inhibitor | 1 |
| IOX2 | Potent, selective HIF-1α prolyl hydroxylase-2 (PHD2) inhibitor and and histone demethylases JMJD2A, C, E and JMJD3 | 2.5 |
| JIB-04 | Pan Jumonji histone demethylase inhibitor | 0.01 |
| K00135 | Kinase inhibitor - ATP competitive - PIM | 0.5 |
| LLY-507 | Potent and selective SMYD2 inhibitor | 0.25 |
| LP99 | Potent and selective inhibitor of the BRD7 and BRD9 bromodomains | 2.5 |
| Methylstat | demethylases (JHDMs) | 0.1 |
| ML324 | Potent JMJD2 demethylase inhibitor | 1.25 |
| NI-57 | Potent and selective BRPF bromodomain inhibitor | 1 |
| OF-1 | Selective BRPF1B and BRPF2 bromodomain inhibitor | 1 |
| OICR 9429 | High affinity WDR5 antagonist; disrupts WDR5-MLL interaction | 1 |
| Olaparib | Poly ADP ribose polymerase (PARP) | 0.1 |
| PCI 34051 | Potent and selective histone deacetylase 8 (HDAC8) inhibitor | 0.5 |
| PD0325901 | selective and non ATP-competitive MEK inhibitor | 1 |
| PFI-1 | Potent BET bromodomain inhibitor | 1 |
| PFI-3 | Potent and selective SMARCA2/4 and polybromo 1 inhibitor | 0.5 |
| PFI-4 | Potent and selective BRPF1 bromodomain inhibitor | 1 |
| RGFP966 | Selective inhibitor of HDAC3 | 0.1 |
| Rocilinostat | Selective HDAC6 inhibitor | 0.1 |
| Rucaparib | Poly ADP ribose polymerase (PARP) | 1 |
| RVX-208 | Potent BET bromodomain antagonist | 2.5 |
| SAHA | Inhibits Class I and II histone deacetylases (HDACs) | 0.25 |
| SET7/9-1 | Potent and selective SETD7 histone lysine methyltransferase inhibitor | 0.25 |
| SET7/9-2 | Potent and selective SETD7 histone lysine methyltransferase inhibitor | 0.25 |
| SGC 0946 | Highly potent DOT1L methyltransferase inhibitor | 1.5 |
| SGC707 | Potent allosteric inhibitor of protein arginine methyltransferase 3 (PRMT3) | 1 |
| SGC-CBP30 | CBP/p300 bromodomain (BRD) inhibitor | 0.5 |
| SMARCA | Inhibitor of SMARCA bromodomains | 1.25 |
| SRT1720 | Activator of SIRT1 | 0.5 |
| Tranylcypromine hydrochloride | Irreversible inhibitor of lysine-specific demethylase 1 | 2 |
| TTNPB | Extremely potent analog of retinoic acid, selective for the retinoic acid receptor (RAR) subtype | 1 |
| Tubastatin A | potent and specific inhibitor of histone deacetylase (HDAC) | 0.5 |
| UNC 0638 | Selective inhibitor of G9a and GLP histone lysine methyltransferases | 0.5 |
| UNC0642 (G9a/GLP Probe) | inhibitor | 0.25 |
| UNC1215 | Potent inhibitor of L3MBTL3 methyllysine (Kme) reader domain | 0.5 |
| UNC1999 | Potent and selective EZH2/EZH1 inhibitor | 0.25 |
| Valproic acid | Histone deacetylase inhibitor | 500 |
| (-)-JQ1 (inactive stereoisomer) | Inactive control for (+)-JQ1 | 0.02 |
| (+)-JQ1 | Potent, high affinity, selective BET bromodomain inhibitor | 0.02 |
| (R)-PFI-2 | Potent and selective SETD7 histone lysine methyltransferase inhibitor | 0.5 |

Supplementary Table 2

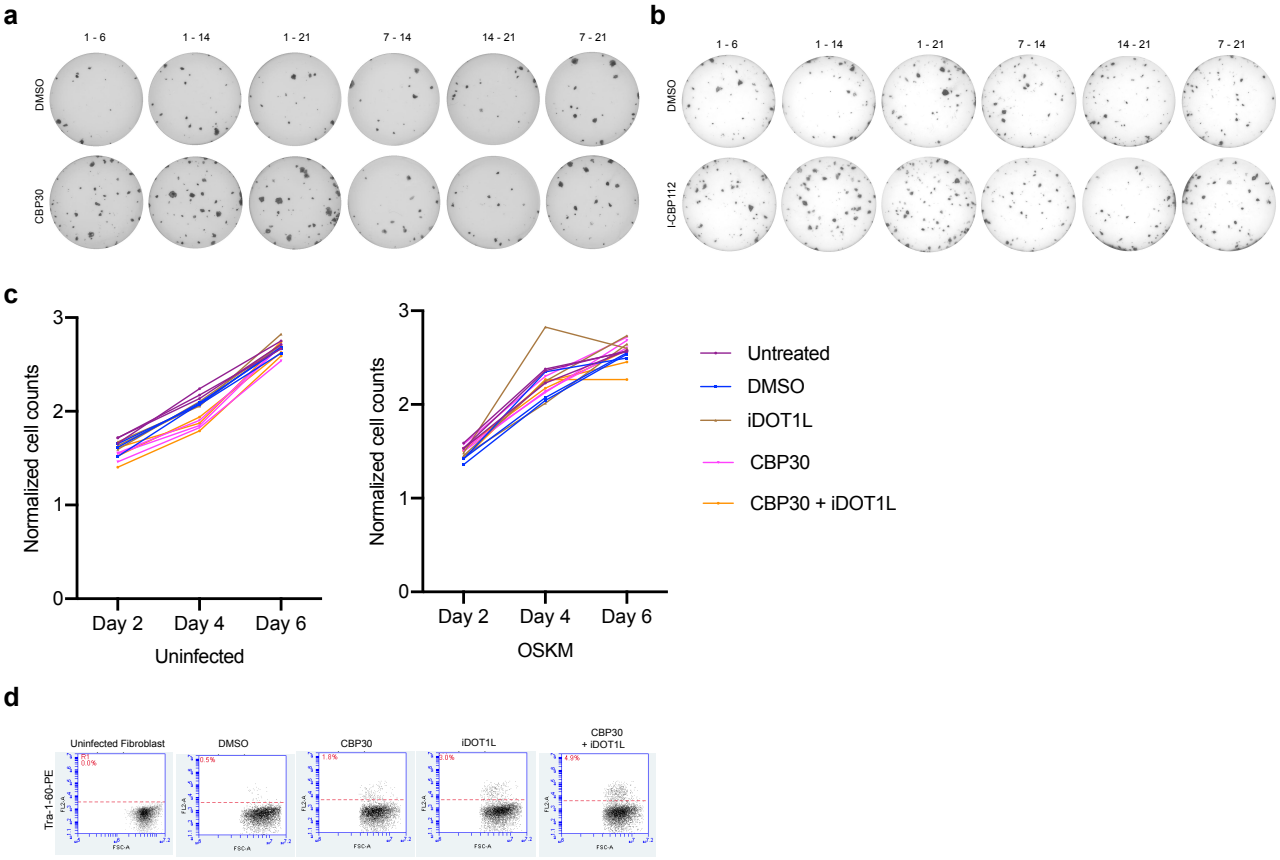
| Gene | Forward sequence (5'-3') | Reverse sequence (5'-3') |
|---|---|---|
| *BETA-ACTIN* | TGAAGTGTGACGTGGACATC | GGAGGAGCAATGATCTTGAT |
| *OCT4* | CCTCACTTCACTGCAACTGTA | CAGGTTTTCTTTCCCTACGT |
| *Trans-OCT4* | CCTCACTTCACTGCACTGTA | CCTTGAGGTACCAGAGATCT |
| *SOX2* | CCCAGCAGACTTCACATGT | CCTCCCATTTCCCTCGTTTT |
| *Trans-SOX2* | CCCAGCAGACTTCACATGT | CCTTGAGGTACCAGAGATCT |
| *KLF4* | GATGAACTGACCAGGCAGTA | GTGGGTCATATCCACTGTCT |
| *MYC* | TGCCTCAAATTGGACTTTGG | GATAATTCTGTGTAACTGC |
| *NANOG* | TGATTTGTGGGCCTGAAGAAA | TGGTGGTAGGAAGAGTAAAG |
| *LIN28A* | TGCGGGCATCTGTAAGTGG | GGAACCCTTCCATGTGCAG |
| *CDH1* | ATGGGCCCTTGGAGCCGCAGCCTCT | CTAGTCGTGCTCGCCGCCTCCGTA |
| *PRRX1* | ACACCCTGCAGGCGAAA | CTTCTGAGTTCAGCTGGTCA |
| *SALL4* | GCCCAGATATCCTGGAAACCA | TTCTCGGAGCTCTCTGCTTTG |




**Supplementary Figure 1**

**(a)** Representative Tra-1-60 stained wells from the chemical screen. All wells except the untreated condition were also treated with iDOT1L (EPZ004777 – 3μM). Images are from one of three replicates.

**(b)** Number of Tra-1-60 positive colonies generated upon episomal reprogramming in the presence of CBP30, I-CBP112, iDOT1L or in combination. Bar graphs show the mean and error bars represent standard deviation. *P* values were determined by a two-tailed Student's t-test; * $P<0.05$, *n* for each condition is indicated on the graph. *P* values were 0.0295, 0.0004, 0.0146, 0.0007 from left to right.



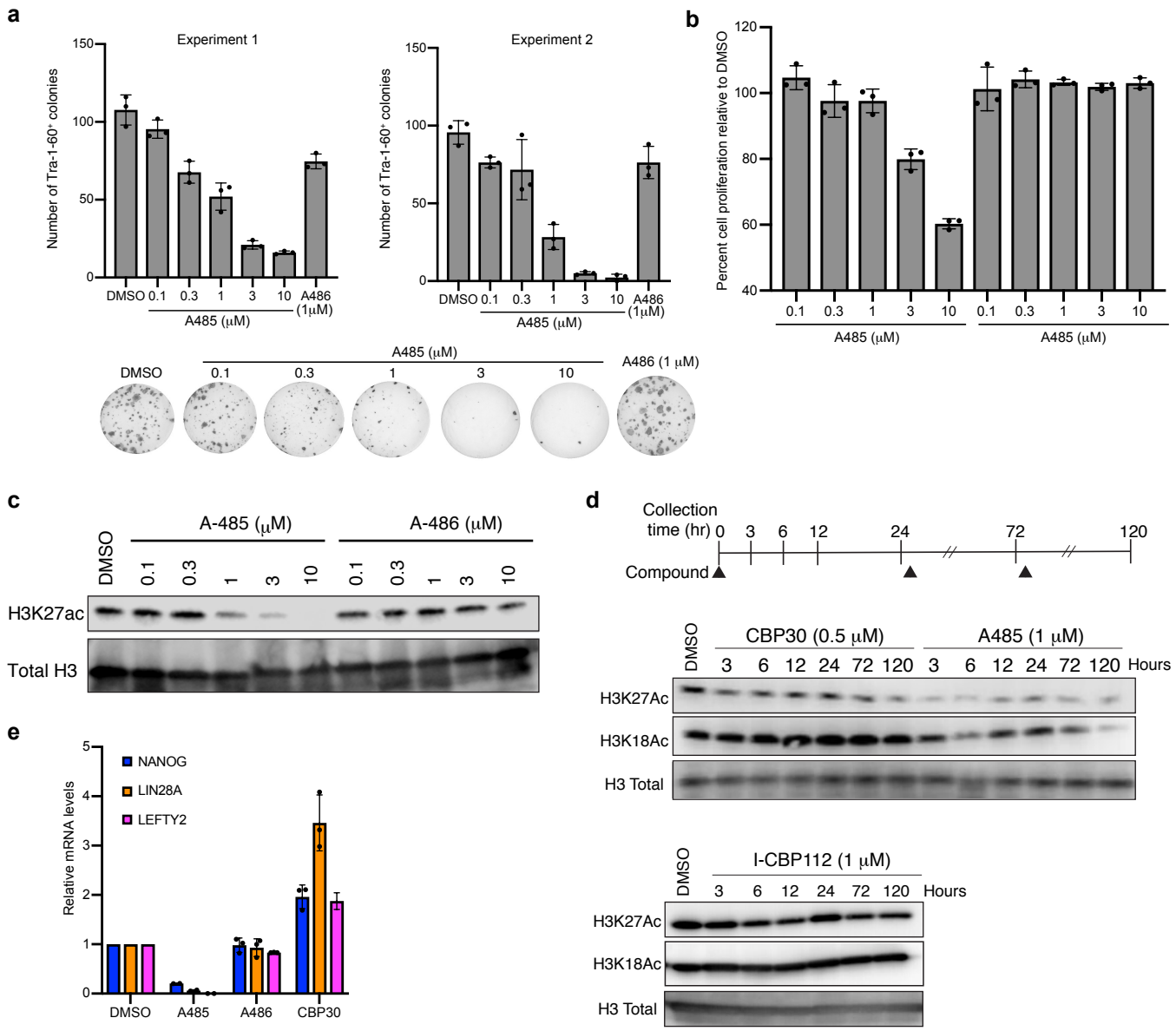

**Supplementary Figure 2**

**(a)** Representative images of Tra-1-60 stained wells from time-course experiment with CBP30 in *Fig. 2b*.

**(b)** Representative images of Tra-1-60 stained wells from time-course experiment with I-CBP112 in *Fig. 2b*.

**(c)** Cell proliferation analysis over 6 days in uninfected (left) or OSKM-transduced (right) fibroblasts treated with the indicated compounds. All values were normalized to initial seeding density (n=3; biological replicates)

**(d)** Flow cytometry plots of representative Tra-1-60-PE (FL2) staining of reprogramming cells on day 6.




**Supplementary Figure 3**

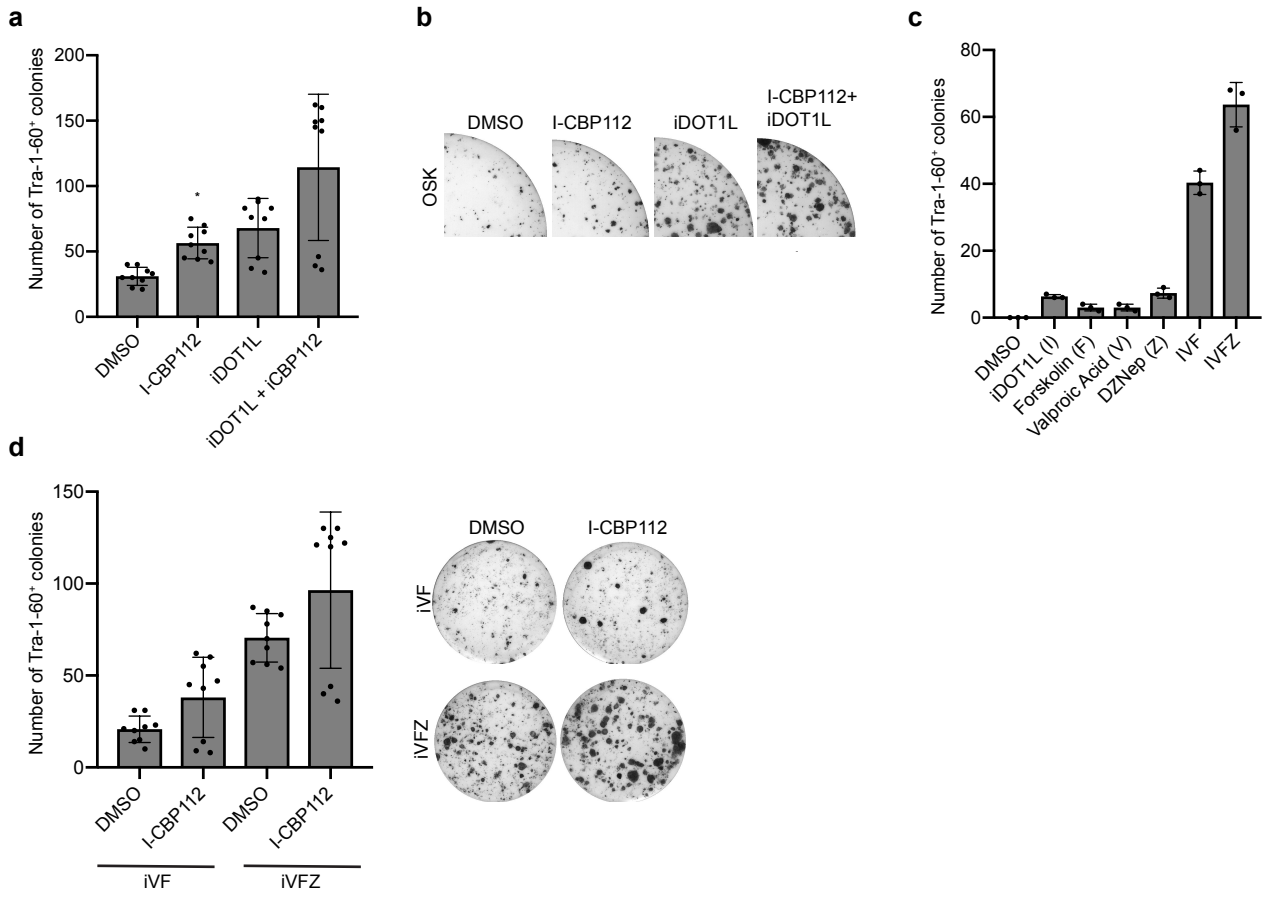
**(a)** Number of Tra-1-60 positive colonies generated upon treatment with A485 at indicated concentrations for 6 days. Bar graphs show mean and the error bars represent standard deviation. Two biologically independent experiments each with three technical replicates are shown. Representative well images are shown below the graphs.

**(b)** Proliferation rate of cells treated with A485 and A486 at the indicated concentrations relative to DMSO control after 5 days. Bar graphs show the mean and error bars represent standard deviation (n=3; biological replicates).

**(c)** Immunoblotting analysis of Histone H3 Lysine 27 Acetylation (H3K27Ac) in cells treated with indicated concentrations of A485 and A486 for 5 days. Total Histone H3 was used as a loading control. Two independent experiments were carried out with similar results. Uncropped gel images can be found in Supplementary Figure 7.

**(d)** Immunoblotting analysis of Histone H3 Lysine 27 Acetylation (H3K27Ac) in cells treated CBP30 (0.5μM), A485(1μM) or I-CBP112(1μM) for the indicated numbers of hours. Compound treatment over the time course was performed according to the schedule depicted above the graphs. Total Histone H3 was used as a loading control. Two independent experiments were carried out with similar results. Uncropped gel images can be found in Supplementary Figure 7.

**(e)** Relative mRNA expression of NANOG, LIN28A and LEFTY2 6 days after OSKM transduction in cells treated with DMSO, A-485(1μM), A-486(1μM) and CBP30 (0.5μM). Bar graphs show the mean and error bars represent standard deviation (n=3; biological replicates).



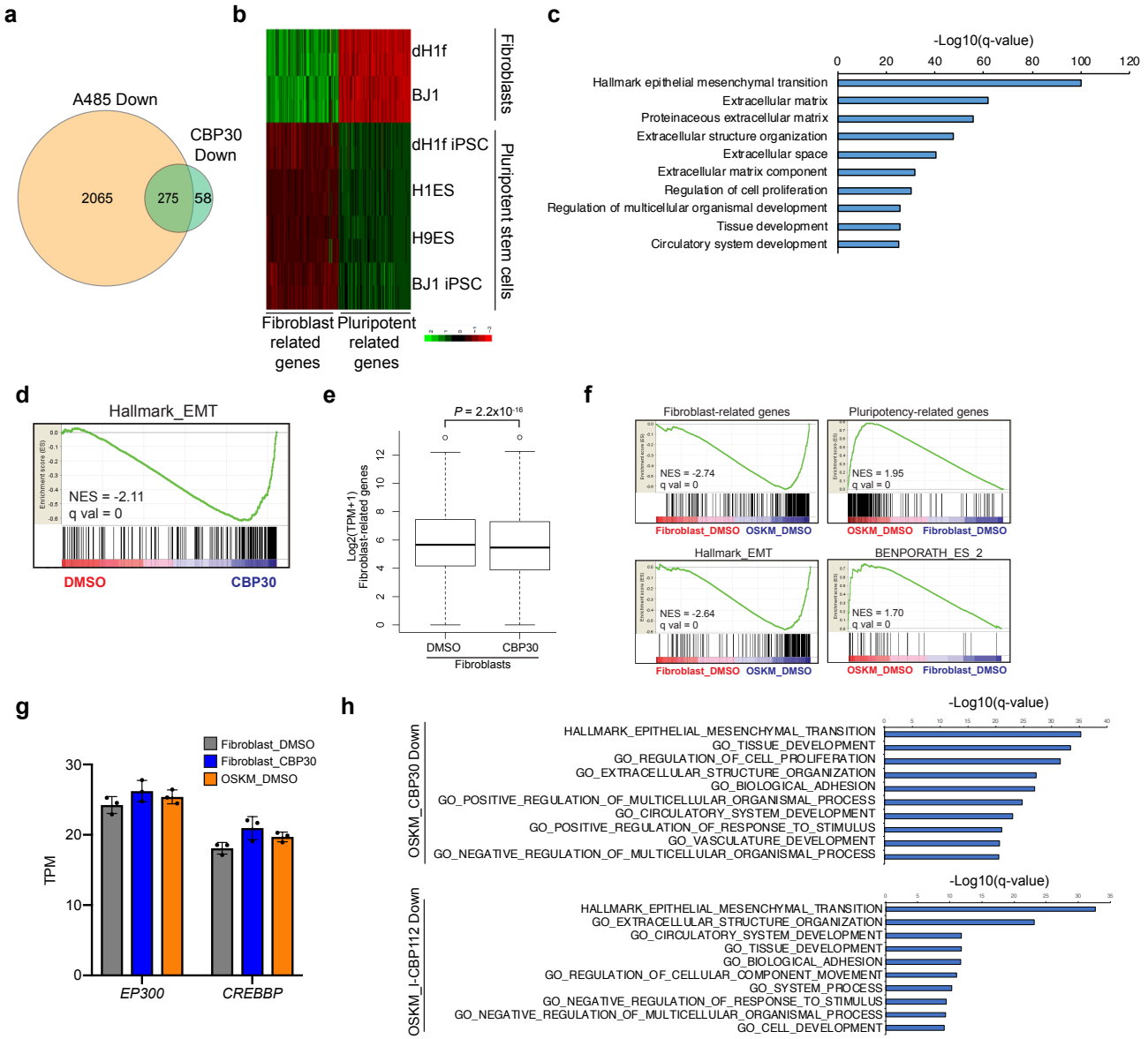

**Supplementary Figure 4**

**(a)** Number of Tra-1-60 positive colonies generated by OSK-infected fibroblasts treated with DMSO, I-CBP112, iDOT1L and combination of I-CBP112 and iDOT1L. Bar graph shows the mean and error bars represent s.e.m. *P* values were determined by a two-tailed Student's t-test; * P < 0.05. *n* = 3 biologically independent experiments each with three technical replicates.*P* values were 0.033, 0.067, 0.087 from left to right.

**(b)** Tra-1-60 stained plates of OSK-mediated reprogramming experiments carried out in the presence of DMSO, I-CBP112, iDOT1L or in combination.Images are from one of the three experiments shown in *a*.

**(c)** Number of Tra-1-60 positive colonies generated by OCT4 and SOX2 infected fibroblasts treated with iDOT1L(i), Valproic Acid (V), Forskolin(F), DZNep(Z) and in combination. Bar graphs shows the mean and error bars represent standard deviation (*n*=3; biological replicates).

**(d)** Number of Tra-1-60 positive colonies generated by OS-infected fibroblasts treated with iDOT1L(i), Valproic Acid (V), Forskolin(F) or DZNep(Z) in combination with I-CBP112. *n* = 3 biologically independent experiments each with three technical replicates. Representative well images are shown on the right.




**Supplementary Figure 5**

**(a)** Venn-diagram shows the number of differentially common downregulated genes upon CBP30 and A485 treatment of uninfected fibroblasts.

**(b)** Heatmap for highly differentially expressed genes between fibroblasts and pluripotent stem cell lines.

**(c)** Top 10 GO and Hallmark gene sets enriched in fibroblast-related gene set. Number of genes (n) in comparison were 286. *P* values were calculated by hypergeometric distribution.

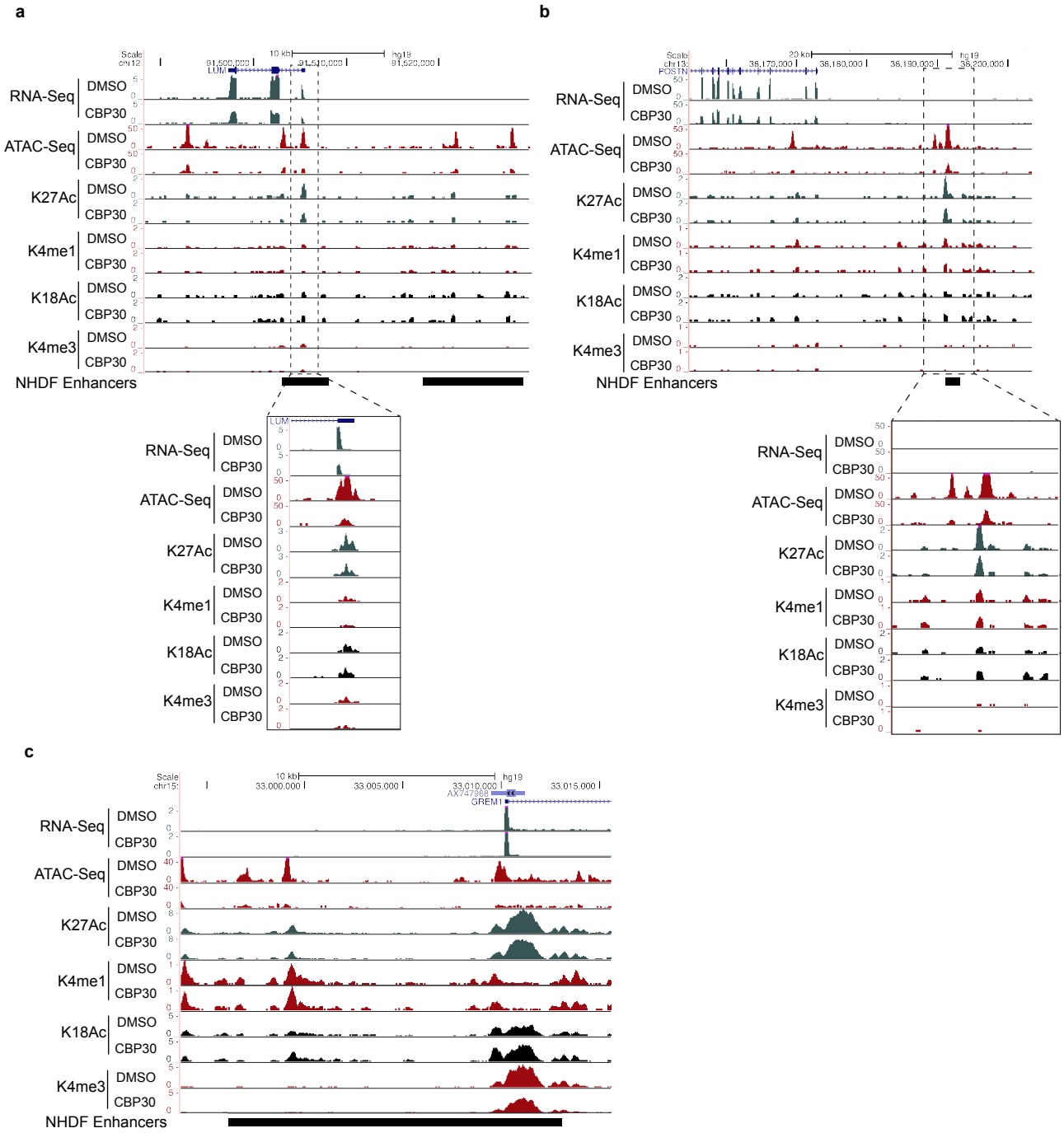
**(d)** GSEA analysis of CBP30-induced changes in uninfected fibroblasts with respect to the Hallmark_EMT gene set. *P* values for the significance of enrichment scores was calculated by permuting the gene sets (1000 permutations) and false discovery rate (q val) was used as an adjustment for multiple hypothesis testing.

**(e)** Average expression levels of the 286 genes in the fibroblast-related gene set in DMSO and CBP30 treated cells. Whiskers indicate 95% confidence interval. *P* values were calculated by a two-sided Wilcoxon signed-rank test.

**(f)** GSEA analysis of control DMSO-treated fibroblast upon OSKM expression on day 6 with respect to the indicated gene sets.

**(g)** Transcripts per million (TPM) values from RNA-sequencing data for *EP300* and *CREBBP (CBP)* genes. Bar graphs show the mean and error bars represent standard deviation (n=3; biological replicates).

**(h)** Top 10 GO and Hallmark gene sets associated with downregulated genes on day 6 of reprogramming with CBP30 (top) or I-CBP112 (bottom) treatment . There were 427 and 118 genes respectively in each group. *P* values were calculated by hypergeometric distribution.



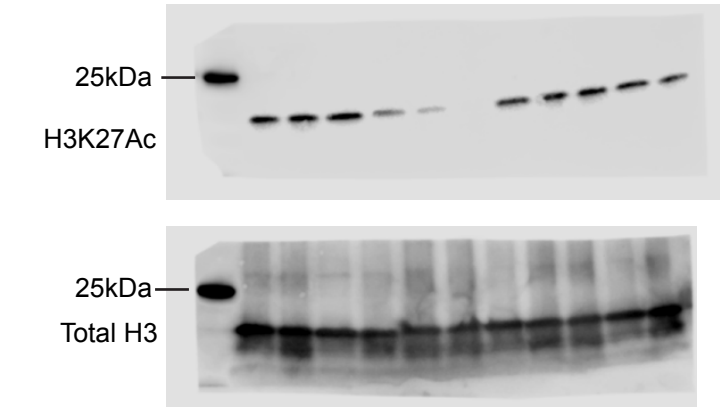

**Supplementary Figure 6**

**(a)** ChIP-seq and ATAC-seq profile changes associated with *LUM* in fibroblasts following treatment with DMSO or CBP30. The zoomed in section below shows region around the TSS. The UCSC browser view of the ChIP-seq intensity from one of three independent experiments is shown.

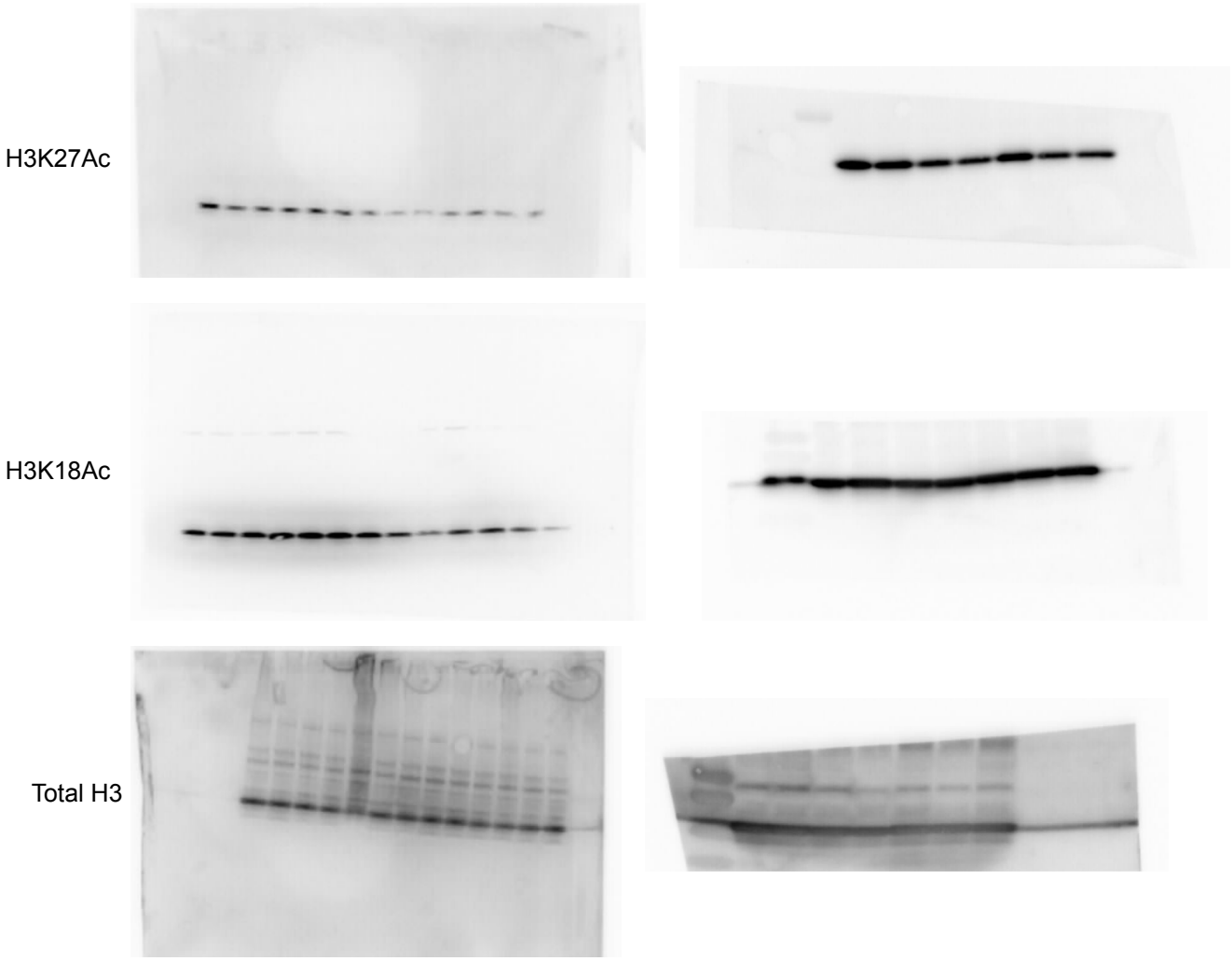
**(b)** ChIP-seq and ATAC-seq profile changes associated with *POSTN* in fibroblasts following treatment with DMSO or CBP30. The zoomed in section below shows the region around the enhancer in NHDFs as defined in ref 20.

**(c)** ChIP-seq and ATAC-seq profile changes associated with *GREM1* in fibroblasts following treatment with DMSO or CBP30. RNA-Seq and ChIP-seq tracks are from one of three independent samples with similar results. ATAC-seq tracks is from one of two independent samples with similar results.

Supplementary Figure 3C




Supplementary Figure 3D




**Supplementary Figure 7**. Uncropped western blot images from Supplementary Figure 3C and 3D.