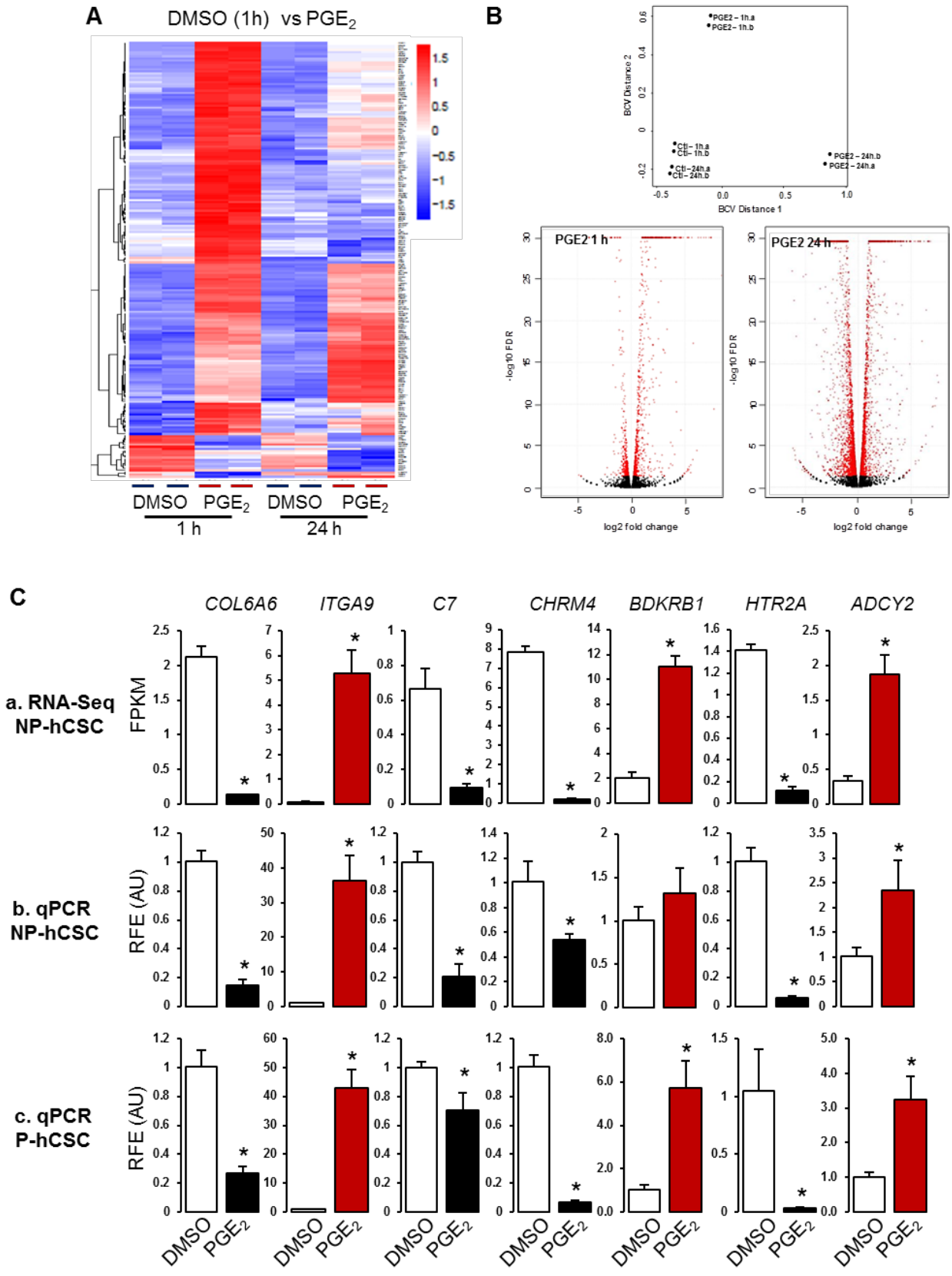
A
DMSO (1h) vs PGE2
1.5
1
0.5
0
−0.5
−1
−1.5
DMSO
PGE2
DMSO
PGE2
1 h
24 h
B
PGE2 − 1h.a
PGE2 − 1h.b
Ctl − 1h.a
Ctl − 1h.b
Ctl − 24h.a
Ctl − 24h.b
PGE2 − 24h.b
PGE2 − 24h.a
BCV Distance 2
BCV Distance 1
PGE2 1 h
PGE2 24 h
-log10 FDR
log2 fold change
C
COL6A6
ITGA9
C7
CHRM4
BDKRB1
HTR2A
ADCY2
a. RNA-Seq NP-hCSC
FPKM
b. qPCR NP-hCSC
RFE (AU)
c. qPCR P-hCSC
RFE (AU)
DMSO
PGE2

Fig. S1. RNA-Seq data analysis and validation in human CSCs. **A**. Heat map of data from cells treated with DMSO (0.1%) or PGE_2 (100 nM) for 1 or 24 h. Data represent hierarchical clustering of differentially expressed genes with False Discovery Rate (FDR) < 0.05, $\log_2$ fold change > 1.5, normalized to 1 h DMSO. **B.** Principal component analysis demonstrating Biological Coefficient of Variation (BCV) of different treatment groups. Volcano plots providing FDR values and fold change for all gene transcripts in PGE_2-treated hCSCs at 1 h (*Left*) or 24 h (*Right*). Differentially expressed genes with FDR< 0.05 are indicated in red. **C.** Validation of RNA-Seq data. Six different genes were selected by fold-change in different pathways significantly altered by PGE_2. (a) Data from RNA-Seq analysis at 24 h expressed as Fragments Per Kilobase of exon per Million fragments mapped (FPKM). (b) Relative quantification of mRNA in hCSCs prepared from non-pregnant (**qPCR NP-hCSC)** or (c) pregnant (**qPCR P-hCSC)** cervices treated with either DMSO or PGE_2 (100 nM) for 24. Data represent relative mean mRNA levels ± SD of triplicates normalized to GAPDH mRNA levels. $*P < 0.05$ compared with DMSO treated controls. RFE (AU), Relative Fold Expression (Arbitrary Units)

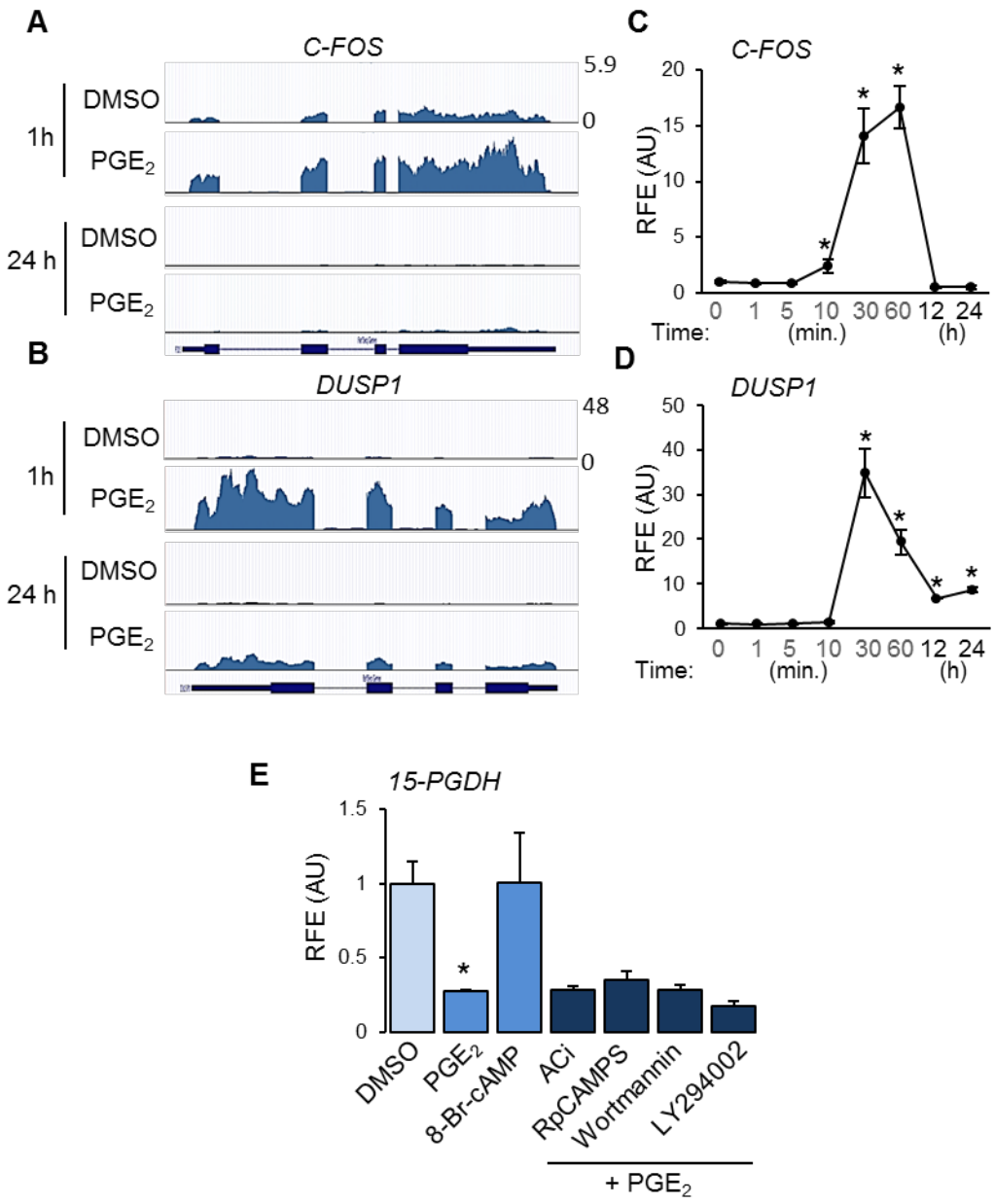

Fig. S2. PGE_2-mediated gene regulation is Ca^{2+}-dependent. Genomic browser snapshots of PGE_2-mediated upregulation of *C-FOS* (**A**) and *DUSP1* (**B**) from RNA-seq dataset. **C, D.** Relative expression of Ca^{2+}-responsive genes *C-FOS* and *DUSP1* in hCSCs treated with PGE_2 (100 nM) for different times quantified by RT-qPCR. Data represent relative mean mRNA levels ± SD of triplicates after normalizing to GAPDH. **P* < 0.01 compared with 0 h time point. ANOVA, RFE (AU), Relative Fold Expression (Arbitrary Units). **E**. 15-PGDH mRNA levels in

hCSCs after treatment with DMSO, PGE_2 (50 nM) or 8-Bromo-cAMP (2 µM), PGE_2 + adenylate cyclase inhibitor (ACi, 10 µM) or PKA inhibitor (RpCAMPS 5 µM), or PI3K inhibitors Wortmannin (1 µM) or LY94002 (1 µM). Data represent relative mean mRNA levels ± SD of triplicates after normalizing to *GAPDH* repeated in 3 cell preps. **P* < 0.01 compared to DMSO treated controls. Pathway inhibitors did not differ from PGE_2 alone, ANOVA followed by Tukey's test for multiple comparisons. RFE (AU), Relative Fold Expression (Arbitrary Units)

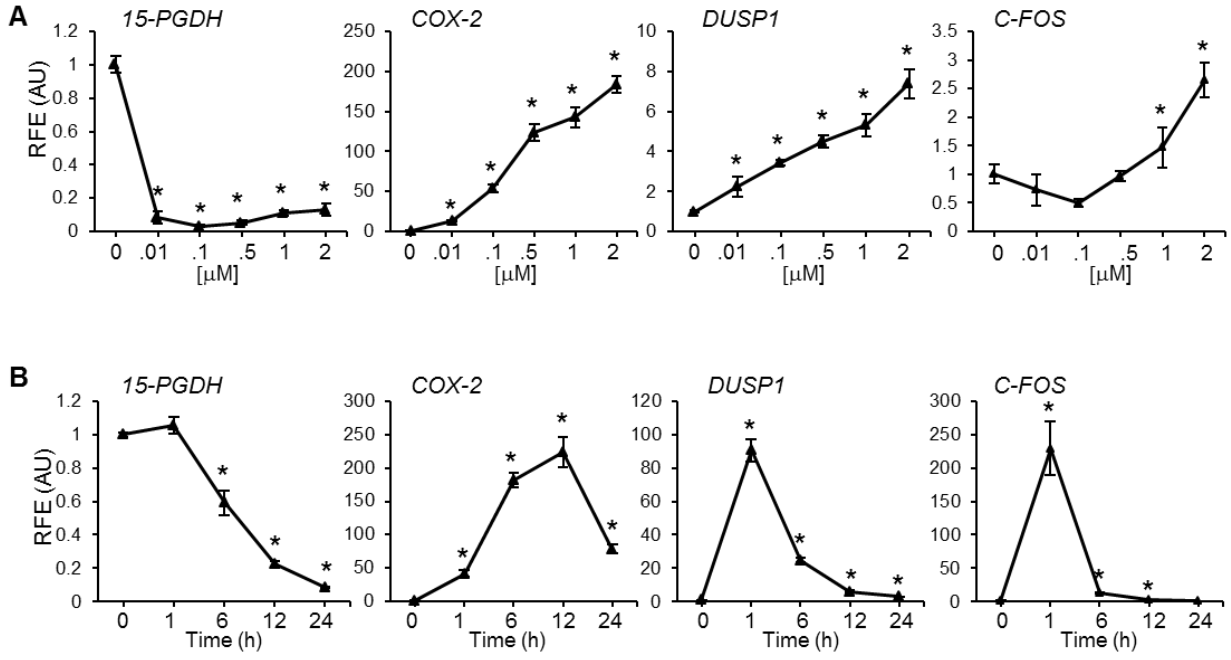

Fig. S3. Calcium ionophore A23187 mimics PGE_2 mediated effects in hCSCs. Relative levels of *15-PGDH*, *COX-2*, *DUSP1* and *C-FOS* mRNA in hCSCs treated with (**A**) increasing concentrations of the Ca^{2+} ionophore A23187 for 24 h, or (**B**) A23187 (1 µM) as a function of time. Data represent relative mean mRNA levels ± SD of triplicates after normalizing to GAPDH mRNA. **P* < 0.01 compared with 0 h time point. RFE (AU), Relative Fold Expression (Arbitrary Units).

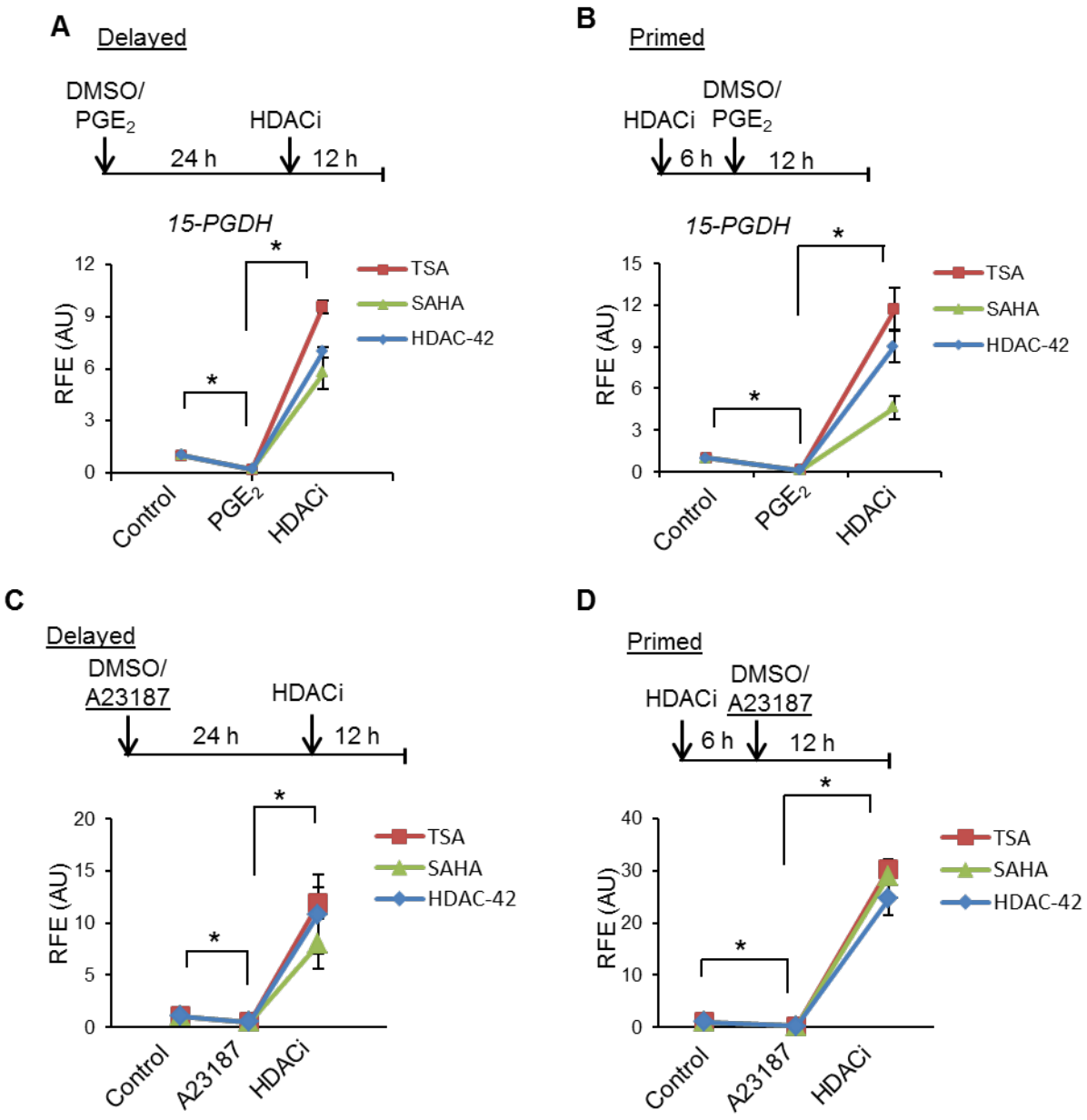

Fig. S4. HDACi treatment blocks PGE_2-mediated 15-PGDH gene repression both before and after PGE_2 treatment. 15-PGDH mRNA levels in hCSCs treated with DMSO or PGE_2 (**A**, 100 nM) or A23187 (**C**, 1 μM) for 24 h followed by treatment with HDACi [TSA (1 μM)/SAHA (2.5 μM)/HDAC-42 (1 μM)] for 12 h (**delayed** treatment), or with DMSO or HDACi [TSA (1 μM)/SAHA (2.5 μM)/HDAC-42 (1 μM)] for 6 h followed by treatment with PGE_2 (**B**, 100 nM) or A23187 (**D**, 1 μM) for 12 h (**primed** treatment). Data represent relative mean mRNA levels ±

SD of triplicates after normalizing to GAPDH mRNA levels. *P < 0.05 ANOVA, RFE (AU), Relative Fold Expression (Arbitrary Units)

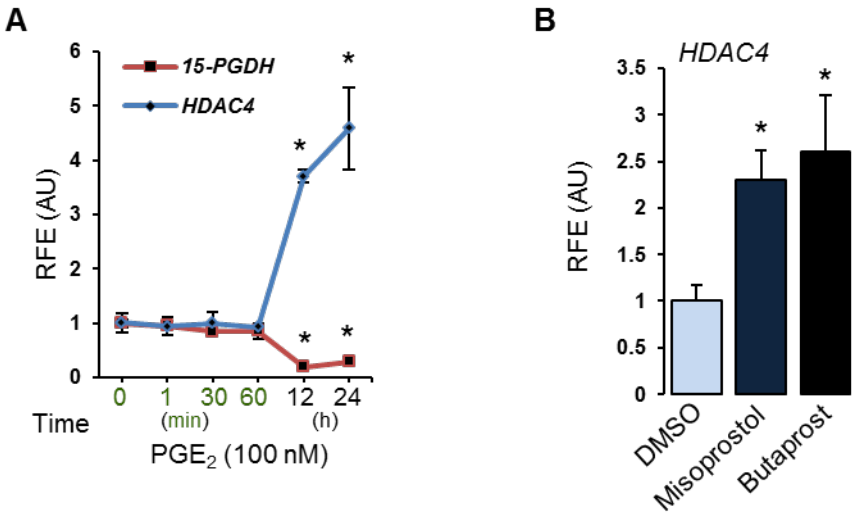

Fig. S5. Reciprocal regulation of 15-PGDH and HDAC4 by PGE_2. A. HDAC4 and 15-PGDH mRNA after treatment with PGE_2 (100 nm) as a function of time in minutes or hours as indicated. **B**. Misoprostol (100 nM) and butaprost (100 nM, EP2-selective agonist) increase HDAC4 mRNA (24h). Data represent relative mean mRNA levels ± SD of triplicates after normalizing to GAPDH mRNA levels in at least 3 cell preps. *P < 0.05 ANOVA, RFE (AU), Relative Fold Expression (Arbitrary Units)

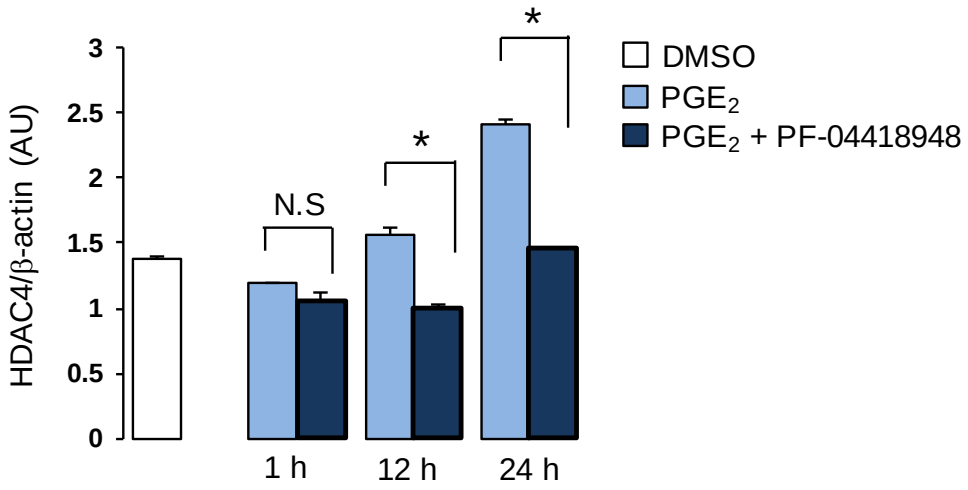

Fig. S6. PGE2 increases HDAC4 levels via EP2 receptor. Densitometric quantitation of immunoblots of HDAC4 protein in hCSCs pretreated with DMSO or PF-04418948 (2 μM) followed by DMSO or PGE_2. **P* < 0.05 compared with PGE_2 treatment alone, Student's *t* test. N = 3

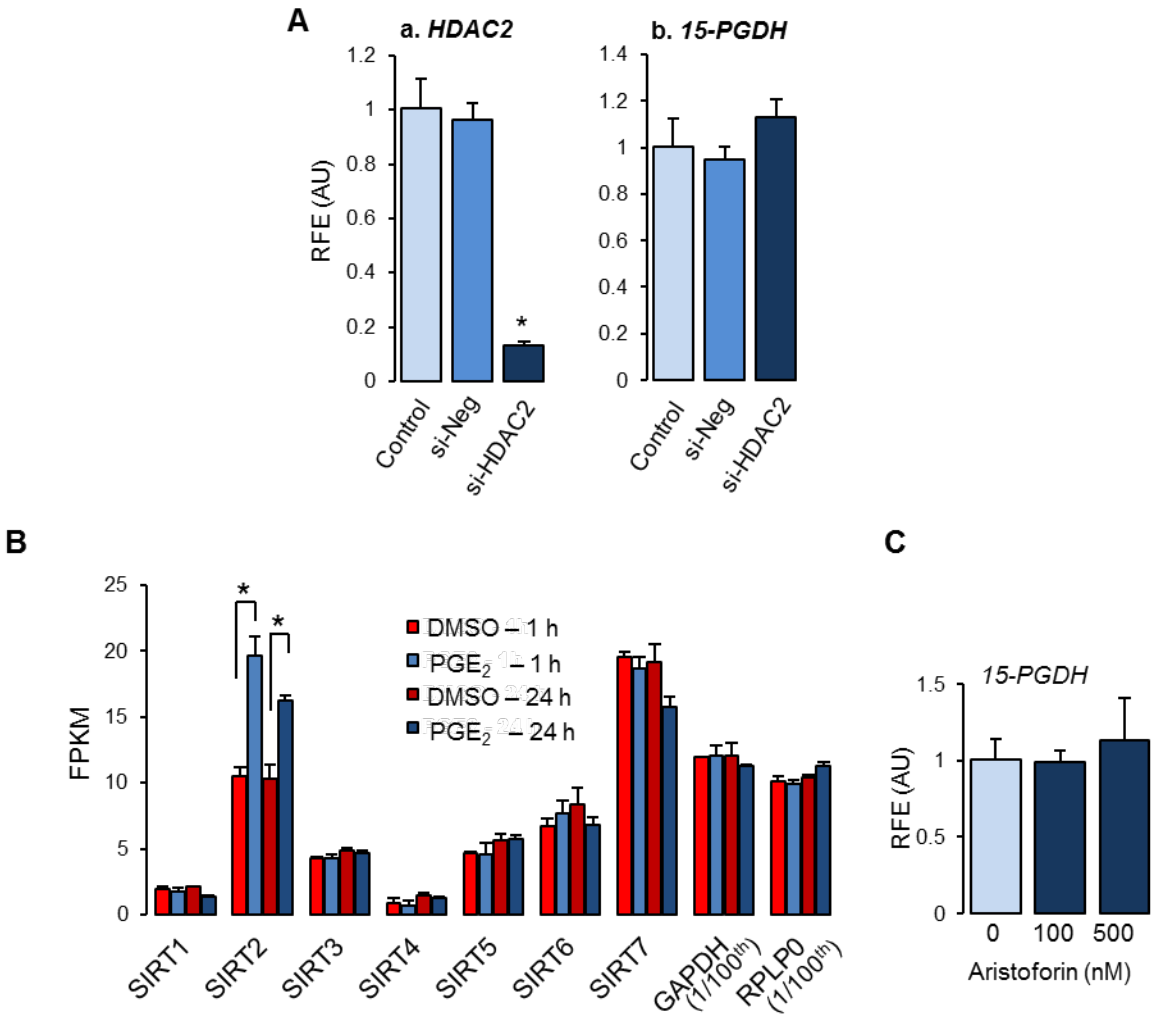

Fig. S7. HDAC2 and Sirtuins (Class III HDACS) do not regulate 15-PGDH gene in hCSCs. **A.** mRNA levels of *HDAC2* and *15-PGDH* in hCSCs transfected with negative siRNA control or HDAC2-specific siRNA. **B**. Data represents Fragments Per Kilobase of exon per Million fragments mapped (FPKM) of sirtuins expressed in hCSCs treated with DMSO or PGE_2 (100 nM) for 1 or 24 h mined from RNA-Seq dataset. GAPDH and RPLP0 are shown as controls ($1/100^{th}$ FPKM values). *$P < 0.05$ compared with corresponding DMSO control. **C.** 15-PGDH mRNA levels in hCSCs treated with indicated concentrations of sirtuin inhibitor Aristoforin for

24 h. Data represent relative mean mRNA levels ± SD of triplicates after normalizing to GAPDH mRNA levels in at least 3 cell preps. Relative Fold Expression (Arbitrary Units)

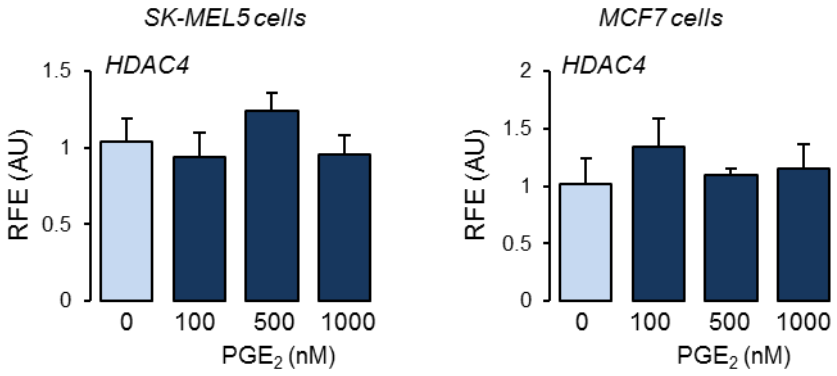

Fig. S8. PGE_2 does not alter HDAC4 gene expression in SK-MEL5 and MCF7 cells. HDAC4 mRNA levels quantified by RT-qPCR in SK-MEL5 melanoma cells or MCF7 breast cancer cells treated with increasing concentrations of PGE_2 as indicated for 24 h. Data represent relative mean mRNA levels ± SD of triplicates after normalizing to GAPDH mRNA levels. RFE(AU), Relative Fold Expression (Arbitrary Units)

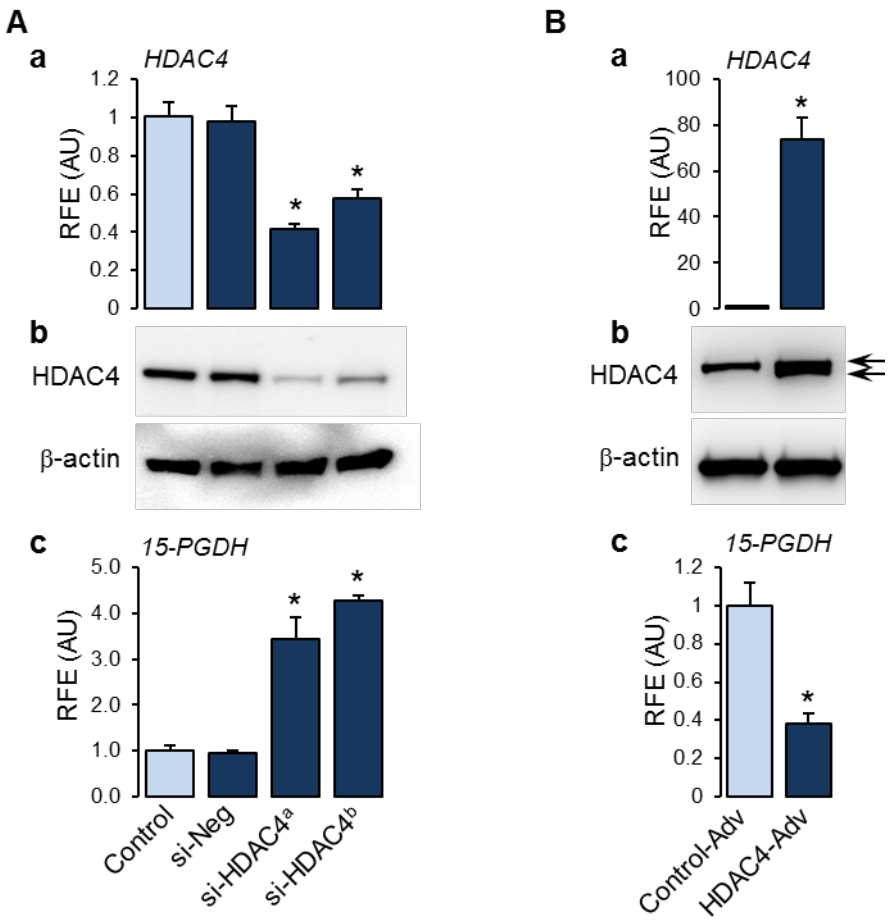

Fig. S9. 15-PGDH is an HDAC4 target gene in hCSCs. A. mRNA levels of HDAC4 (**Aa**) and 15-PGDH (**Ac**) and protein levels of HDAC4 (**Ab**) in hCSCs transfected with control negative siRNA or two different HDAC4 siRNAs ([a]Ambion; [b]Santa Cruz Biotechnologies) for 56 h. **P* < 0.001 compared to si-Neg. Student's *t* test. N = 3. **B**. mRNA levels of HDAC4 (**Ba**) and 15-PGDH (**Bc**) and protein levels of HDAC4 (**Bb**) in hCSCs infected with control adenovirus or HDAC4-expressing adenovirus for 48 h followed by incubation in serum-free medium for 24 h. Arrows indicate two immunoreactive proteins **P* < 0.001 compared with control adenovirus infected cells. Student's *t* test. RFE (AU), Relative Fold Expression (Arbitrary Units)

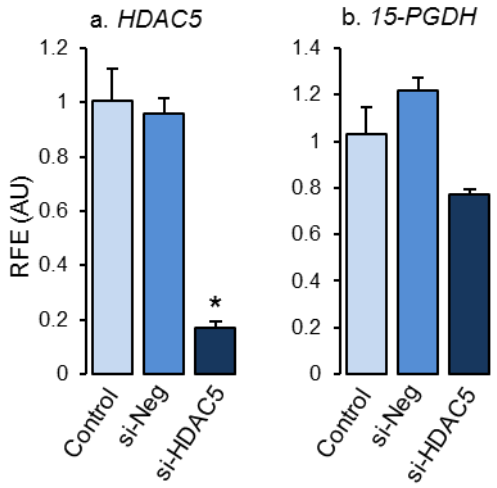

Fig. S10. HDAC5 does not regulate *15-PGDH* gene expression in hCSCs. mRNA levels of HDAC5 and 15-PGDH in hCSCs transfected with negative siRNA control or HDAC5 specific siRNA. Data represent relative mean mRNA levels ± SD of triplicates after normalizing to GAPDH mRNA levels. *P = 0.00002 compared with siNeg transfected cells. ANOVA, RFE(AU), Relative Fold Expression (Arbitrary Units)

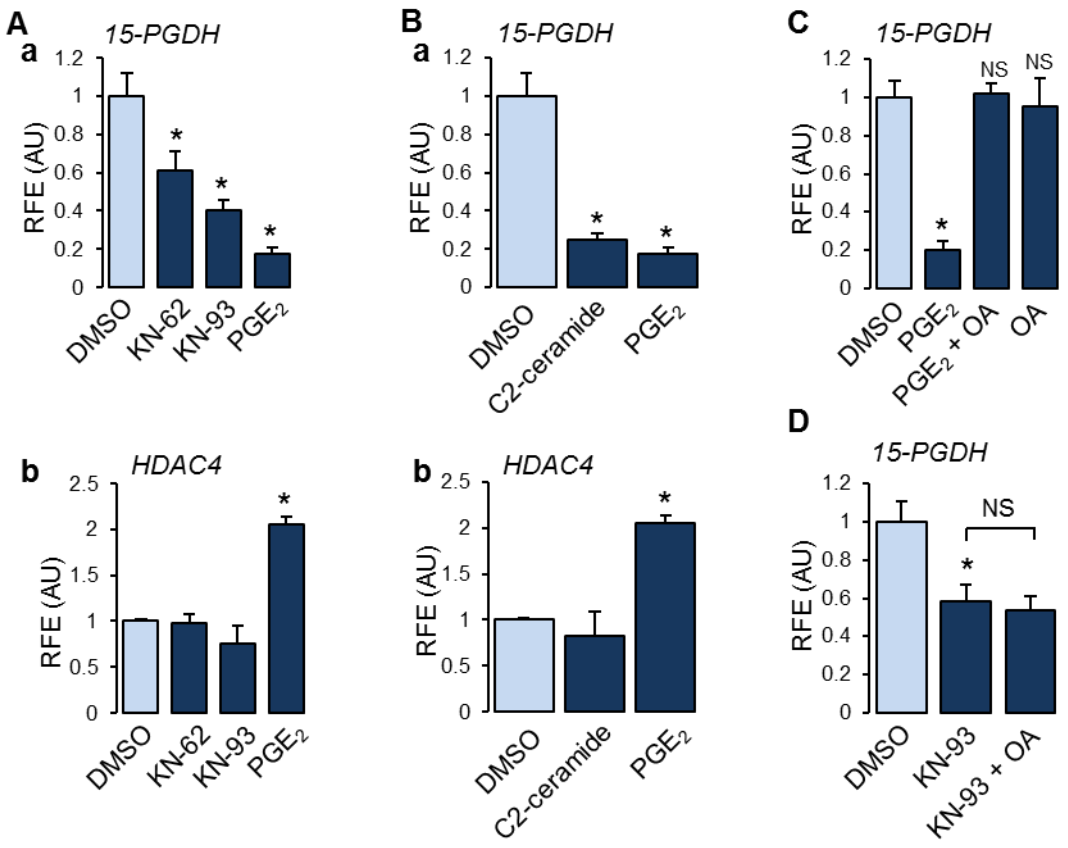

Fig. S11. HDAC4 mediated repression of *15-PGDH* is de-phosphorylation dependent. A. Relative mRNA levels of 15-PGDH (**a**) and HDAC4 (**b**) in hCSCs treated with DMSO, KN-62 (5 μM), KN-93 (5 μM), or PGE_2 (100 nM) for 24 h. **B**. Levels of *15-PGDH* (**a**) and *HDAC4* (**b**) mRNA in hCSCs treated with DMSO, C2 ceramide (50 μM) or PGE_2 (100 nM) for 24 h. **C, D**. 15-PGDH mRNA levels in hCSCs pretreated with DMSO or okadaic acid (OA, 100 nM) for 1 h followed by treatment with DMSO or KN-93 (5 μM) for 23 h. Data represent mean mRNA levels ± SD of triplicates after normalizing to GAPDH mRNA levels. **P* < 0.01 compared with DMSO treatment. Student's *t* test. N = 3 in at least 3 cell preps. RFE (AU), Relative Fold Expression (Arbitrary Units). **OA**, okadaic acid

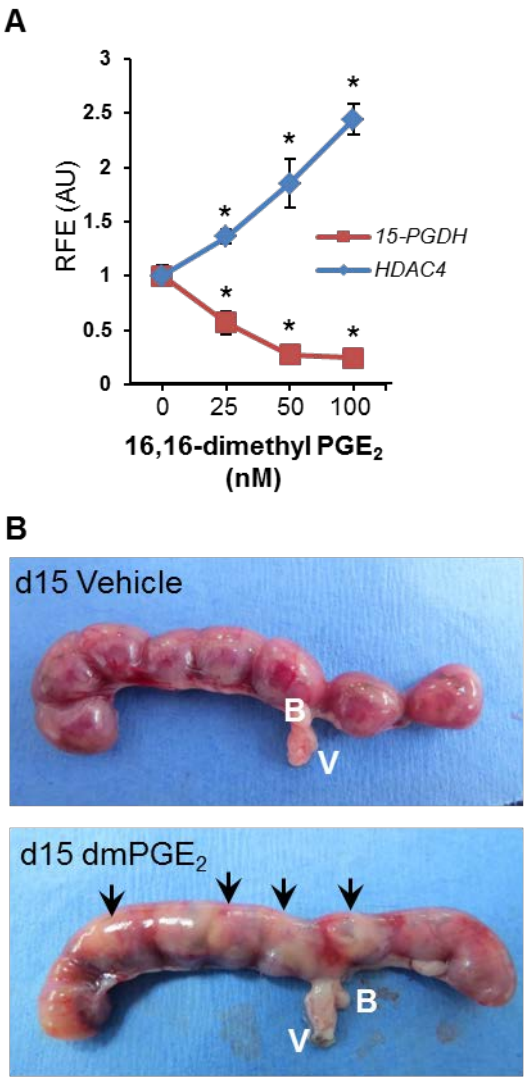

Fig. S12. 16,16-dimethyl PGE_2 induces HDAC4 and represses 15-PGDH gene expression and has adverse fetal effects in pregnant mice. A. HDAC4 and 15-PGDH mRNA levels in hCSCs treated with increasing concentrations of 16,16-dimethyl PGE_2 as indicated for 24 h. *P < 0.01 compared with DMSO treatment. ANOVA followed by Dunnett's with time 0 as control, n = 3. **B**. Female reproductive tract dissected on d15, 6 h after treatment for assessment of health of pups and gross morphological changes in cervix and uterus. Arrow indicates pale ischemic pups; **V**, vagina; **B**, bladder

Supplementary Table 1. Clinical characteristics of pregnant women from who samples were obtained for analysis.

	Early Gestation Nonripe cervix n = 4	**Not in labor Nonripe cervix n = 7**	**Not in labor Ripe cervix n = 8**	**Labor Dilated cervix n = 8**
Gestational age (mean ± SEM)	13 ± 0.8	35 ± 1.3	37.4 ± 0.82	38.9 ± 0.6
Parity, median [range]	3 [1,4]	2 [1-3]	2 [1,5]	3 [1-4]
Bishop score Median [range]	2 [1,2]	3 [2,4]	6.5 [5-8]	8 [7,10]
Surgical indications	1 fetal demise, failed attempt at dilation 1 molar pregnancy desiring hysterectomy 2 repeat C/S; fetal demise; placenta invading uterine scar	5 repeat C/S; placenta previa/accreta 1 primary C/S placenta previa 1 repeat C/S; uterine atony	4 repeat C/S; placenta previa/accreta 1 term molar pregnancy 3 repeat C/S; uterine atony	2 placenta percreta 2 fetal distress; uterine hemorrhage 1 failure to progress; hemorrhage 1 repeat C/S leiomyomas 1 abruptio placentae 1 primary C/S breech;

Supplementary Table 2: Sequences and Catalog numbers for primers used in RT-qPCR

S. No.	Gene	Sequence/Catalog #
1	COL6A6[a]	Hs01029204_m1
2	ITGA9[a]	Hs00979865_m1
3	C7[a]	Hs00940408_m1
4	CHRM4[a]	Hs00265219_s1
5	BDKRB1[a]	Hs00664201_s1
6	HTR2A[a]	Hs01033524_m1
7	ADCY2[a]	Hs01058848_m1
8	15-PGDH	5'GCCGGTTTATTGTGCTTCAAA3' & 5' TCTCACACCACTGTTCATAAGATTAG 3'
9	PTGES[a]	Hs01115610_m1
10	COX-2	GAATCATTCACCAGGCAAATT & TCTGTACTGCGGGTGGAACA Probe FAMTCCTACCACCAGCAACCCTGC
11	DUSP1[a]	Hs00610256_g1
12	C-FOS[b]	QHsaCED0046695
13	HDAC4[a]	Hs01041638_m1
14	HDAC5[a]	Hs00608366_m1
15	HDAC2[a]	Hs00231032_m1
16	GAPDH	5'GGAGTCAACGGATTTGGTCGTA3' & 5'CAACAATATCCACTTTACCAGAGTTA3'

[a]ThermoFisher Scientific; [b]BIO-RAD