

## **SUPPLEMENTARY DATA**

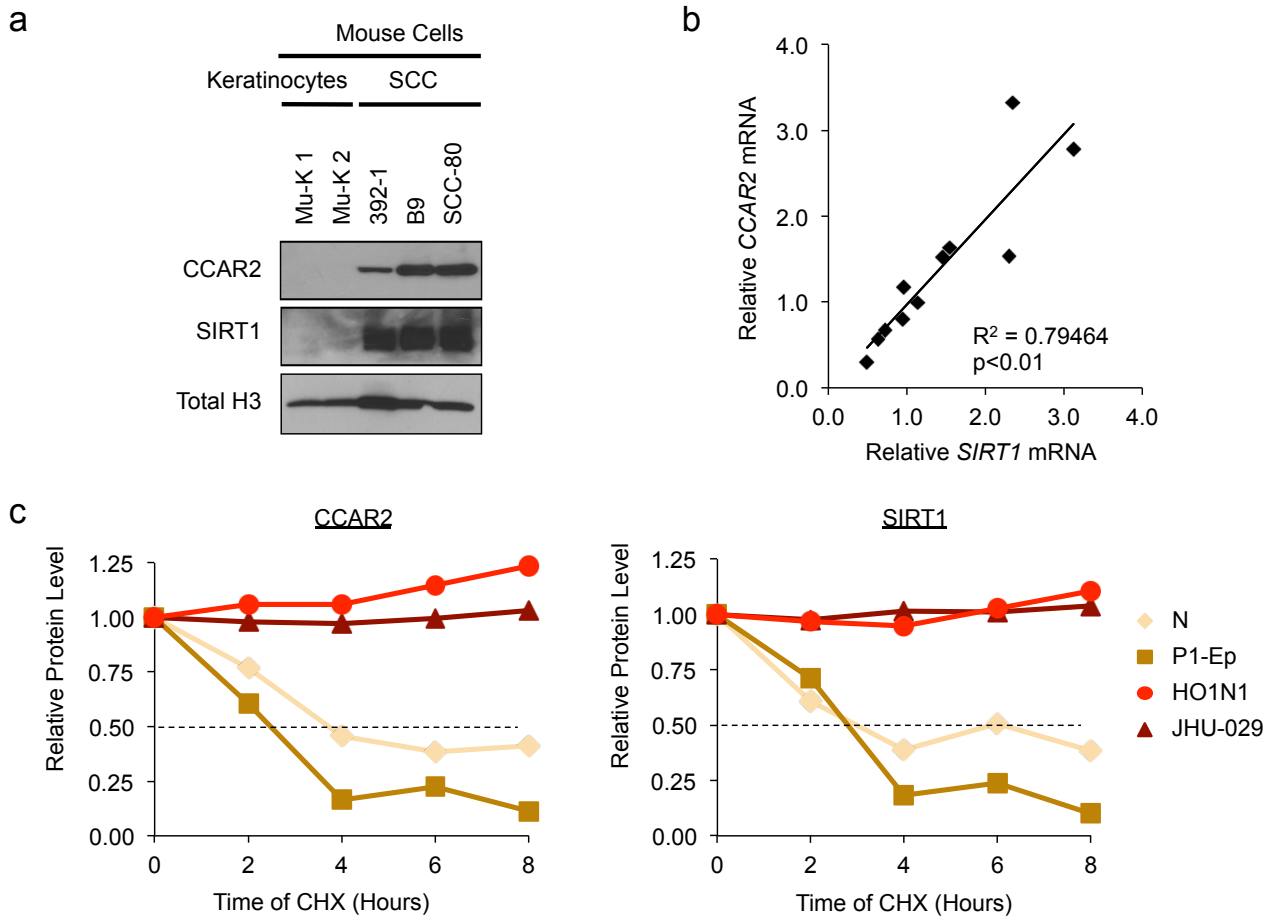
**4 Supplementary Figures**

**Supplementary Figure Legends**

**7 Supplementary Tables**

**Supplementary Materials and Methods**

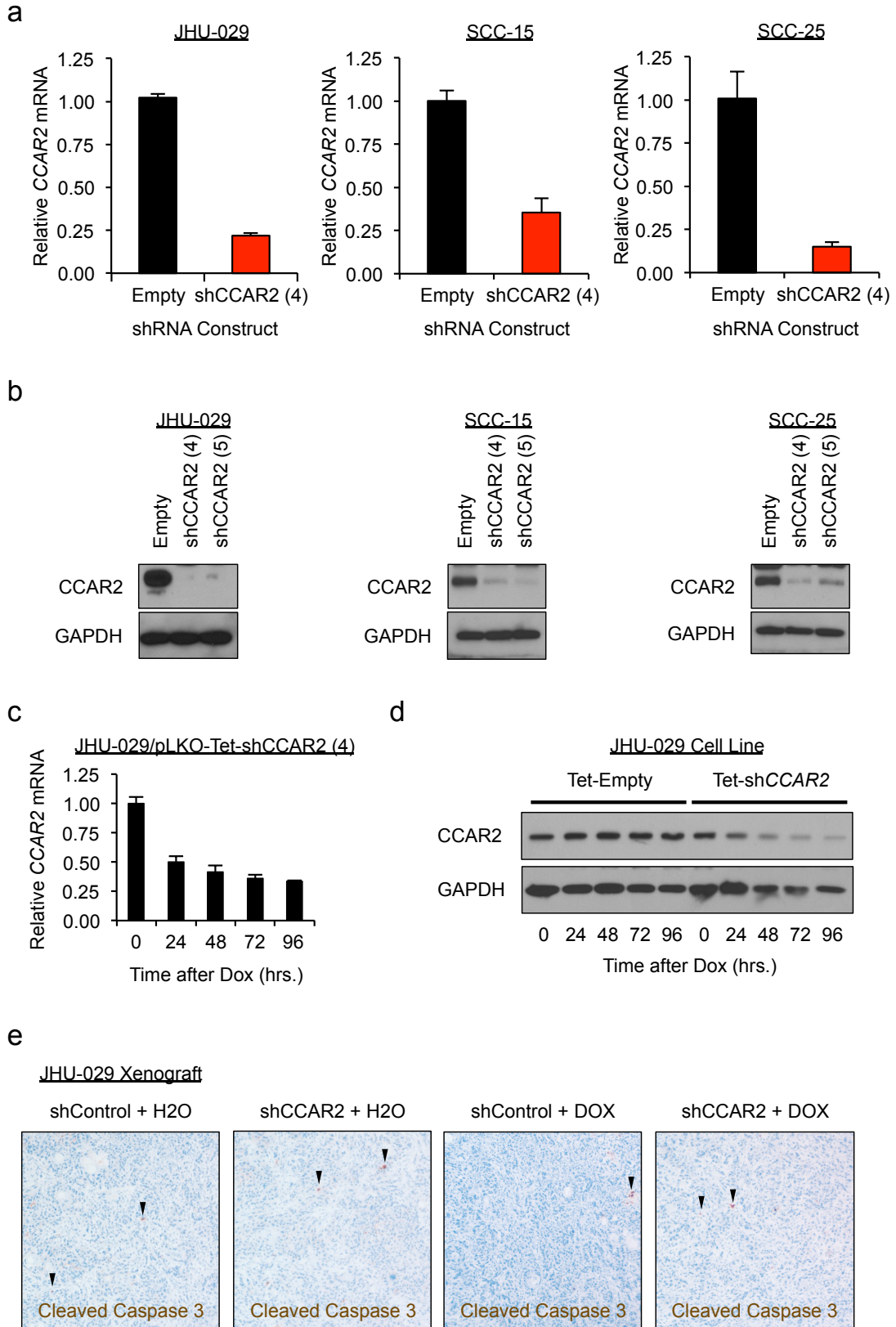
**Supplementary References**



Supplementary Figure S1: CCAR2 and SIRT1 expression in mouse and human cells

### **Supplementary Figure S1: CCAR2 and SIRT1 expression in mouse and human cells**

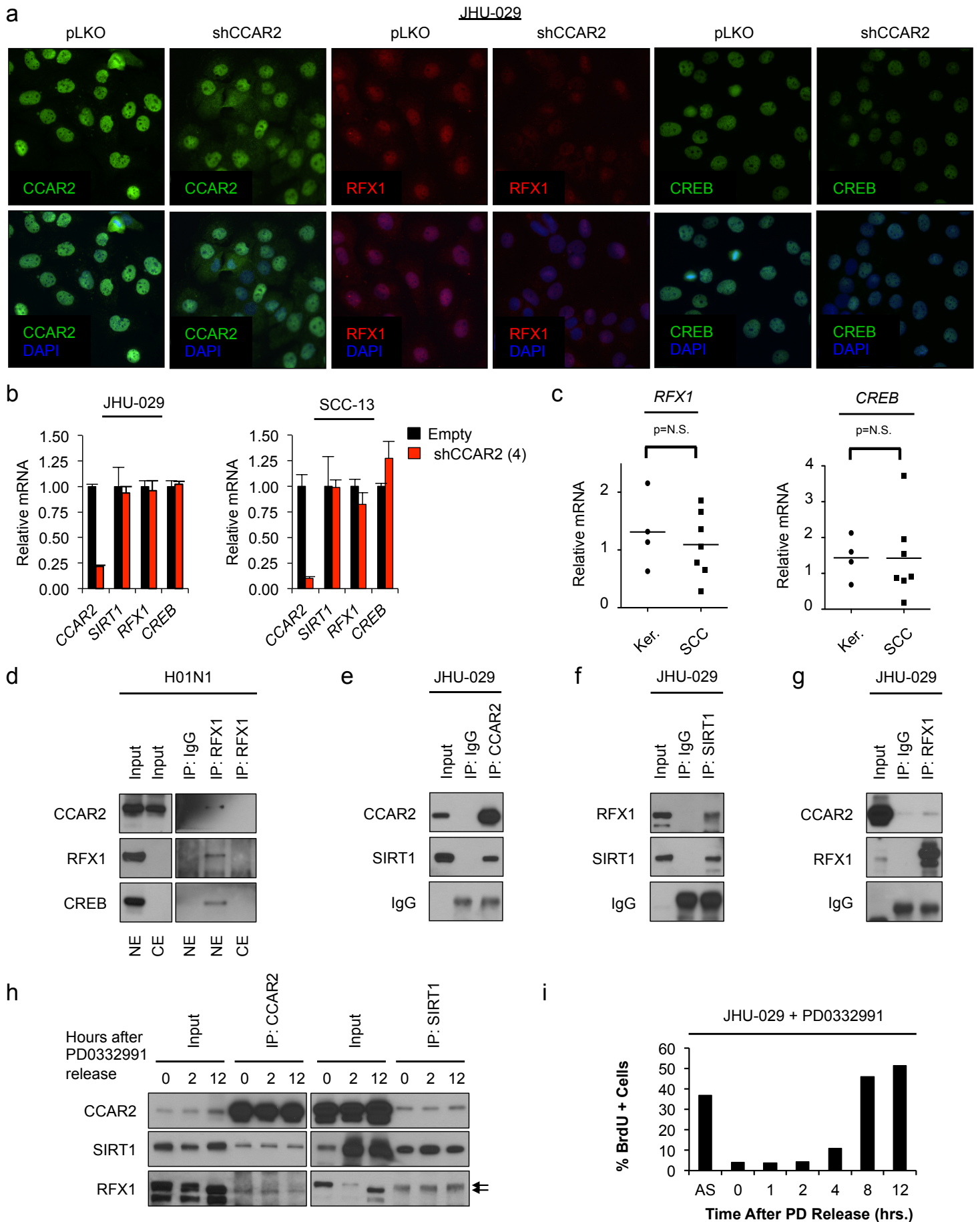
**(a)** Western blot of CCAR2 and SIRT1 protein expression in mouse primary keratinocytes (MuK 1 and MuK 2) and mouse SCC cell lines (392-1, B9 and SCC-80). Total Histone-3 (H3) provides the loading control. **(b)** Correlation between *SIRT1* and *CCAR2* mRNA levels in human keratinocytes and SCC cell lines from Figure 1c. Linear regression fits the data points with  $R^2 = 0.79$ ,  $p < 0.01$ . **(c)** Graphical representation of CCAR2 and SIRT1 protein levels from Figure 1d as quantified by Image J software, relative to expression of cells before cyclohexamide (CHX) treatment. Values were normalized to the GAPDH loading control for each sample.



Supplementary Figure S2: shRNA-mediated knock down of CCAR2

## **Supplementary Figure S2: shRNA-mediated knock down of CCAR2**

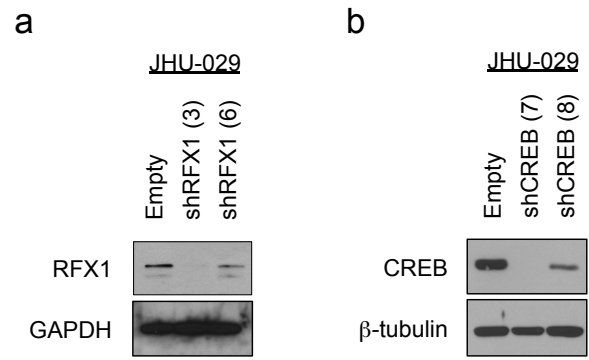
**(a)** Quantitative RT-PCR of *CCAR2* in JHU-029 (left), SCC-15 (middle) and SCC-25 (right) cells following shRNA mediated knockdown of *CCAR2* for cells used in Figure 2a. Values are normalized to Empty vector control. **(b)** Western blot analysis of *CCAR2* in JHU-029 (left) SCC-15 (middle) and SCC-25 (right) cells following shRNA mediated knockdown of *CCAR2* for cells used in Figure 2b. GAPDH serves as a loading control **(c)** Quantitative RT-PCR of *CCAR2* in JHU-029/pLKO-tet-shCCAR2 (4) cells at indicated times following addition of doxycycline (Dox) to culture media. Values are normalized to 0 hours with doxycycline. **(d)** Western blot analysis of *CCAR2* at indicated time following treatment with Doxycycline (Dox) in JHU-029 cells stably infected with inducible empty vector (tet-Empty) or shCCAR2 (tet-shCCAR2 (4)). **(f)** IHC staining of JHU-029 Xenograft tumors for Cleaved Caspase 3. Arrowheads indicate positive cells.



Supplementary Figure S3: CCAR2 physically interacts with SIRT1, RFX1, and CREB

### **Supplementary Figure S3: CCAR2 physically interacts with SIRT1, RFX1, and CREB**

**(a)** Immunofluorescent imaging of JHU-029 cells with indicated antibodies 48 hours following infection with shRNA directed against *CCAR2*. DAPI stains the nuclei. **(b)** Quantitative RT-PCR of *CCAR2*, *SIRT1*, *RFX1*, and *CREB* mRNA levels in SCC cell lines (JHU-029 and SCC-13) with *CCAR2* knockdown (shCCAR2; red bars) or control (Empty; black bars). **(c)** Quantitative RT-PCR of *RFX1* (left panel) and *CREB* (right panel) mRNA expression in keratinocytes (ker.) and SCC cell lines from Figure 4b. The line indicates the mean,  $p > 0.05$ , not significant (NS). **(d)** Co-immunoprecipitation of RFX1 protein with CREB and CCAR2 from nuclear fractions in HO1N1 cells. **(e)** Co-Immunoprecipitation of CCAR2 and SIRT1 proteins from whole cell extracts of JHU-029 cells. IgG serves as a loading control. **(f)** Co-Immunoprecipitation of RFX1 and SIRT1 proteins from whole cell extracts of JHU-029 cells. IgG serves as a loading control. **(g)** Co-Immunoprecipitation of CCAR2 and RFX1 proteins from whole cell extracts of JHU-029 cells. IgG serves as a loading control. **(h)** Co-immunoprecipitation of CCAR2, SIRT1, and RFX1 from whole cell lysates of JHU-029 cells following cell cycle block and release using CDK4/6 inhibitor PD0332991. Samples were taken at 0 hours to 12 hours post withdrawal of PD0332991. Arrows indicate differentially migrating bands of RFX1. **(i)** FACS analysis of BrdU incorporation of JHU-029 cells following arrest with PD0332991 and release for indicated time AS (asynchronous) provides the control.



Supplementary Figure S4: shRNA mediated knock down of RFX1 and CREB



**Supplementary Figure S4: shRNA mediated knock down of RFX1 and CREB**

**(a)** Western blot analysis of RFX1 in JHU-029 cells following shRNA mediated knockdown of *RFX1* for cells used in Figures 5a and 5c. GAPDH serves as a loading control. **(b)** Western blot analysis of CREB in JHU-029 cells following shRNA mediated knockdown of *CREB* for cells used in Figure 5b and 5d.  $\beta$ -tubulin serves as a loading control.

**Supplementary Table S1: p53 alterations in SCC cell lines used in this study**

| Cell line | Origin                            | p53 alteration     |
|-----------|-----------------------------------|--------------------|
| SCC-13    | cutaneous SCC                     | E258K              |
| SCC-15    | base of tongue SCC                | fs224              |
| SCC-25    | base of tongue SCC                | fs209 (no protein) |
| JHU-011   | Laryngeal squamous cell carcinoma | fs247 (no protein) |
| JHU-029   | Laryngeal squamous cell carcinoma | fs121 (no protein) |
| H01N1     | oral SCC                          | G266R              |

**Supplementary Table S2: Significant CCAR2-regulated genes from microarray**

| Fold Change | Gene Symbol | Gene Name   |
|-------------|-------------|---|
| -3.49       | SET         | SET nuclear oncogene; similar to SET translocation                                    |
| -2.67       | NCAPD2      | non-SMC condensin I complex, subunit D2   |
| -2.55       | INCENP      | inner centromere protein antigens 135/155kDa  |
| -2.44       | AHNAK       | AHNAK nucleoprotein   |
| -2.41       | DBI         | diazepam binding inhibitor (GABA receptor modulator, acyl-Coenzyme A binding protein) |
| -2.37       | SLC35A2     | solute carrier family 35 (UDP-galactose transporter), member A2                       |
| -2.35       | MAP7D1      | MAP7 domain containing 1  |
| -2.26       | CD151       | CD151 molecule (Raph blood group)   |
| -2.17       | BASP1       | brain abundant, membrane attached signal protein 1                                    |
| -2.16       | IMPDH1      | IMP (inosine monophosphate) dehydrogenase 1   |
| -2.11       | CREB3L2     | cAMP responsive element binding protein 3-like 2                                      |
| -2.11       | TMEM109     | transmembrane protein 109   |
| -2.10       | TRAM2       | translocation associated membrane protein 2   |
| -2.10       | SLC44A2     | solute carrier family 44, member 2  |
| -2.09       | RPS6KA4     | ribosomal protein S6 kinase, 90kDa, polypeptide 4                                     |
| -2.04       | RXRA        | retinoid X receptor, alpha  |
| -2.04       | AHNAK2      | AHNAK nucleoprotein 2   |
| -1.98       | ASPSCR1     | alveolar soft part sarcoma chromosome region, candidate 1                             |
| -1.97       | NSMCE4A     | non-SMC element 4 homolog A (S. cerevisiae)   |
| -1.95       | CDCA7       | cell division cycle associated 7  |
| -1.94       | PDXK        | pyridoxal (pyridoxine, vitamin B6) kinase   |
| -1.94       | PGD         | phosphogluconate dehydrogenase  |
| -1.93       | HK1         | hexokinase 1  |
| -1.93       | PTTG1       | pituitary tumor-transforming 1; pituitary tumor-transforming 2                        |
| -1.90       | PTTG3P      | pituitary tumor-transforming 3  |
| -1.87       | EGR1        | early growth response 1   |
| -1.87       | MAD2L2      | MAD2 mitotic arrest deficient-like 2 (yeast)  |
| -1.87       | GLT25D1     | glycosyltransferase 25 domain containing 1  |

|       |         |  |
|-------|---------|--|
| -1.86 | RPA1    | replication protein A1, 70kDa  |
| -1.85 | RNF167  | ring finger protein 167  |
| -1.85 | CDCA5   | cell division cycle associated 5   |
| -1.80 | ALDOA   | aldolase A, fructose-bisphosphate  |
| -1.79 | EIF1    | similar to eukaryotic translation initiation factor 1; eukaryotic translation initiation factor 1          |
| -1.78 | PTGS2   | prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)                      |
| -1.78 | TAF10   | TAF10 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 30kDa                           |
| -1.77 | MCM3    | minichromosome maintenance complex component 3   |
| -1.76 | AURKB   | aurora kinase B  |
| -1.76 | PFAS    | phosphoribosylformylglycinamide synthase   |
| -1.75 | FSCN1   | fascin homolog 1, actin-bundling protein ( <i>Strongylocentrotus purpuratus</i> )                          |
| -1.75 | TMEM62  | transmembrane protein 62   |
| -1.75 | MTP18   | mitochondrial protein 18 kDa   |
| -1.74 | TTC4    | tetratricopeptide repeat domain 4  |
| -1.74 | CTDSP2  | similar to hCG2013701; CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) small phosphatase 2 |
| -1.72 | RER1    | RER1 retention in endoplasmic reticulum 1 homolog ( <i>S. cerevisiae</i> )                                 |
| -1.72 | SH3BP4  | SH3-domain binding protein 4   |
| -1.72 | ICMT    | isoprenylcysteine carboxyl methyltransferase   |
| -1.72 | USP5    | ubiquitin specific peptidase 5 (isopeptidase T)  |
| -1.71 | DPH2    | DPH2 homolog ( <i>S. cerevisiae</i> )  |
| -1.71 | CNPY2   | canopy 2 homolog (zebrafish)   |
| -1.70 | DHRS4   | dehydrogenase/reductase (SDR family) member 4  |
| -1.70 | HEATR1  | HEAT repeat containing 1   |
| -1.68 | NCSTN   | nicastrin  |
| -1.68 | NDUFB7  | NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 7, 18kDa  |
| -1.66 | WASF2   | WAS protein family, member 2   |
| -1.66 | ITGA3   | integrin, alpha 3 (antigen CD49C, alpha 3 subunit of VLA-3 receptor)                                       |
| -1.66 | DLGAP5  | discs, large ( <i>Drosophila</i> ) homolog-associated protein 5  |
| -1.65 | MYH9    | myosin, heavy chain 9, non-muscle  |
| -1.64 | MKNK2   | MAP kinase interacting serine/threonine kinase 2   |
| -1.64 | TP53I13 | tumor protein p53 inducible protein 13   |
| -1.63 | LTA4H   | leukotriene A4 hydrolase   |
| -1.62 | XPO6    | exportin 6   |
| -1.61 | PCNA    | proliferating cell nuclear antigen   |
| -1.61 | LARS2   | leucyl-tRNA synthetase 2, mitochondrial  |
| -1.61 | YWHAH   | tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, eta polypeptide                    |
| -1.60 | CENPB   | centromere protein B, 80kDa  |
| -1.60 | MCM5    | minichromosome maintenance complex component 5   |
| -1.59 | MCM6    | minichromosome maintenance complex component 6   |

|       |         |   |
|-------|---------|---|
| -1.58 | NCOA6   | nuclear receptor coactivator 6  |
| -1.57 | NCOR2   | nuclear receptor co-repressor 2   |
| -1.57 | SCAMP3  | secretory carrier membrane protein 3  |
| -1.57 | DBNL    | drebrin-like  |
| -1.56 | TUBB    | tubulin, beta; similar to tubulin, beta 5; tubulin, beta pseudogene 2; tubulin, beta pseudogene 1   |
| -1.56 | ITPRIP  | inositol 1,4,5-triphosphate receptor interacting protein  |
| -1.56 | POFUT1  | protein O-fucosyltransferase 1  |
| -1.55 | BCKDK   | branched chain ketoacid dehydrogenase kinase  |
| -1.54 | EVI5L   | ecotropic viral integration site 5-like   |
| -1.54 | NOTCH1  | Notch homolog 1, translocation-associated (Drosophila)  |
| -1.53 | SIP1    | survival of motor neuron protein interacting protein 1  |
| -1.53 | HNRNPA0 | heterogeneous nuclear ribonucleoprotein A0  |
| -1.53 | CNNM3   | cyclin M3   |
| -1.53 | KIFC1   | kinesin family member C1  |
| -1.51 | DIAPH1  | diaphanous homolog 1 (Drosophila)   |
| -1.51 | IMPA2   | inositol(myo)-1(or 4)-monophosphatase 2   |
| -1.51 | MBNL1   | muscleblind-like (Drosophila)   |
| -1.50 | PMM2    | phosphomannomutase 2  |
| -1.49 | NUP62   | nucleoporin 62kDa   |
| -1.49 | NDE1    | nudE nuclear distribution gene E homolog 1 (A. nidulans)  |
| -1.49 | DGCR2   | DiGeorge syndrome critical region gene 2  |
| -1.49 | SRF     | serum response factor (c-fos serum response element-binding transcription factor)   |
| -1.49 | ZNF319  |   |
| -1.48 | INO80C  | INO80 complex subunit C   |
| -1.48 | MTA2    | metastasis associated 1 family, member 2  |
| -1.47 | UBL7    | ubiquitin-like 7 (bone marrow stromal cell-derived)   |
| -1.47 | GPR56   | G protein-coupled receptor 56   |
| -1.47 | TMEM214 | transmembrane protein 214   |
| -1.47 | SDHD    | similar to succinate dehydrogenase complex, subunit D, integral membrane protein; succinate dehydrogenase complex, subunit D, integral membrane protein |
| -1.46 | PTPRU   | protein tyrosine phosphatase, receptor type, U  |
| -1.46 | RCC2    | regulator of chromosome condensation 2  |
| -1.46 | AP1S2   | adaptor-related protein complex 1, sigma 2 subunit pseudogene; adaptor-related protein complex 1, sigma 2 subunit                                       |
| -1.45 | DHX37   | DEAH (Asp-Glu-Ala-His) box polypeptide 37   |
| -1.45 | TPI1    | TPI1 pseudogene; triosephosphate isomerase 1  |
| -1.45 | CCDC28A | coiled-coil domain containing 28A   |
| -1.45 | ASPM    | asp (abnormal spindle) homolog, microcephaly associated (Drosophila)  |
| -1.45 | GINS3   | GINS complex subunit 3 (Psf3 homolog)   |
| -1.45 | CHMP2A  | chromatin modifying protein 2A  |
| -1.44 | MFN2    | mitofusin 2   |
| -1.44 | ARPC2   | actin related protein 2/3 complex, subunit 2, 34kDa   |

|       |          |   |
|-------|----------|---|
| -1.43 | CDC42EP4 | CDC42 effector protein (Rho GTPase binding) 4   |
| -1.43 | TRMT5    | TRM5 tRNA methyltransferase 5 homolog ( <i>S. cerevisiae</i> )  |
| -1.43 | FANCE    | Fanconi anemia, complementation group E   |
| -1.43 | FOXK1    | forkhead box K1   |
| -1.42 | LPCAT3   | lysophosphatidylcholine acyltransferase 3   |
| -1.42 | PCSK9    | proprotein convertase subtilisin/kexin type 9   |
| 1.42  | WEE1     | WEE1 homolog ( <i>S. pombe</i> )  |
| 1.42  | ENTPD7   | ectonucleoside triphosphate diphosphohydrolase 7  |
| 1.43  | AP1S1    | adaptor-related protein complex 1, sigma 1 subunit  |
| 1.43  | GXYLT1   | glycosyltransferase 8 domain containing 3   |
| 1.43  | STRADB   | STE20-related kinase adaptor beta   |
| 1.44  | MAPK6    | mitogen-activated protein kinase 6  |
| 1.44  | BCYRN1   | brain cytoplasmic RNA 1 (non-protein coding)  |
| 1.45  | RBMS2    | RNA binding motif, single stranded interacting protein 2; RNA binding motif, single stranded interacting protein 2 pseudogene |
| 1.45  | TPRG1L   | tumor protein p63 regulated 1-like  |
| 1.46  | YOD1     | YOD1 OTU deubiquinating enzyme 1 homolog ( <i>S. cerevisiae</i> )   |
| 1.46  | NECAP2   | NECAP endocytosis associated 2  |
| 1.46  | SECISBP2 | SECIS binding protein 2   |
| 1.47  | RNF38    | ring finger protein 38  |
| 1.47  | TDG      | similar to G/T mismatch-specific thymine DNA glycosylase; thymine-DNA glycosylase   |
| 1.47  | CALD1    | caldesmon 1   |
| 1.48  | CLDN12   | claudin 12  |
| 1.50  | CHD7     | chromodomain helicase DNA binding protein 7   |
| 1.50  | MMD      | monocyte to macrophage differentiation-associated   |
| 1.51  | VPS25    | vacuolar protein sorting 25 homolog ( <i>S. cerevisiae</i> )  |
| 1.52  | DYNLL1   | dynein, light chain, LC8-type 1   |
| 1.52  | C15ORF57 | chromosome 15 open reading frame 57   |
| 1.52  | EHD1     | EH-domain containing 1  |
| 1.53  | KLHDC3   | kelch domain containing 3   |
| 1.56  | YRDC     | yrdC domain containing ( <i>E. coli</i> )   |
| 1.57  | AMMECR1  | Alport syndrome, mental retardation, midface hypoplasia and elliptocytosis chromosomal region gene 1                          |
| 1.65  | UHMK1    | U2AF homology motif (UHM) kinase 1  |
| 1.69  | CSF2     | colony stimulating factor 2 (granulocyte-macrophage)  |
| 1.69  | TRAK2    | trafficking protein, kinesin binding 2  |
| 1.70  | C3ORF52  | chromosome 3 open reading frame 52  |
| 1.76  | TXNRD1   | thioredoxin reductase 1; hypothetical LOC100130902  |
| 1.78  | NT5E     | 5'-nucleotidase, ecto (CD73)  |
| 1.86  | C9ORF5   | chromosome 9 open reading frame 5   |
| 1.92  | CDKN1A   | cyclin-dependent kinase inhibitor 1A (p21, Cip1)  |
| 2.03  | ANKRD46  | ankyrin repeat domain 46  |

**Supplementary Table S3: GO terms associated with CCAR2-regulated genes**

| Term  | PValue   | Genes  |
|---|----------|--|
| GO:0000279~M phase                                      | 1.34E-07 | KIFC1, DLGAP5, PTTG1, AURKB, MYH9, WEE1, KLHDC3, NCAPD2, RPA1, NDE1, TUBB, RCC2, INCENP, MAD2L2, CDCA5, ASPM   |
| GO:0007049~cell cycle                                   | 1.43E-07 | KIFC1, DLGAP5, AURKB, PTTG1, STRADB, MYH9, MCM3, UHMK1, WEE1, MCM6, KLHDC3, NCAPD2, MFN2, RPA1, SH3BP4, NDE1, TUBB, CDKN1A, MAPK6, RCC2, INCENP, MAD2L2, CDCA5, ASPM   |
| GO:0007067~mitosis                                      | 3.94E-07 | KIFC1, DLGAP5, PTTG1, AURKB, WEE1, NCAPD2, TUBB, NDE1, RCC2, INCENP, MAD2L2, CDCA5, ASPM   |
| GO:0000280~nuclear division                             | 3.94E-07 | KIFC1, DLGAP5, PTTG1, AURKB, WEE1, NCAPD2, TUBB, NDE1, RCC2, INCENP, MAD2L2, CDCA5, ASPM   |
| GO:0022403~cell cycle phase                             | 4.68E-07 | KIFC1, DLGAP5, PTTG1, AURKB, MYH9, WEE1, KLHDC3, NCAPD2, RPA1, NDE1, TUBB, CDKN1A, RCC2, INCENP, MAD2L2, CDCA5, ASPM   |
| GO:0000087~M phase of mitotic cell cycle                | 4.78E-07 | KIFC1, DLGAP5, PTTG1, AURKB, WEE1, NCAPD2, TUBB, NDE1, RCC2, INCENP, MAD2L2, CDCA5, ASPM   |
| GO:0048285~organelle fission                            | 6.05E-07 | KIFC1, DLGAP5, PTTG1, AURKB, WEE1, NCAPD2, TUBB, NDE1, RCC2, INCENP, MAD2L2, CDCA5, ASPM   |
| GO:0022402~cell cycle process                           | 1.41E-06 | KIFC1, DLGAP5, PTTG1, AURKB, MYH9, UHMK1, WEE1, KLHDC3, NCAPD2, MFN2, RPA1, NDE1, TUBB, CDKN1A, RCC2, INCENP, MAD2L2, CDCA5, ASPM  |
| GO:0051301~cell division                                | 1.43E-06 | KIFC1, PTTG1, AURKB, MYH9, WEE1, MCM5, NCAPD2, NOTCH1, NDE1, RCC2, INCENP, MAD2L2, CDCA5, ASPM   |
| GO:0000278~mitotic cell cycle                           | 1.68E-05 | KIFC1, DLGAP5, PTTG1, AURKB, WEE1, NCAPD2, NDE1, TUBB, CDKN1A, RCC2, INCENP, MAD2L2, CDCA5, ASPM   |
| GO:0005819~spindle                                      | 3.37E-05 | KIFC1, NDE1, RCC2, DLGAP5, INCENP, AURKB, MYH9, MAP7D1, ASPM   |
| GO:0046907~intracellular transport                      | 1.49E-04 | SCAMP3, XPO6, WASF2, RER1, ICMT, STRADB, MYH9, MFN2, NDE1, AP1S1, AP1S2, SET, YWHAH, TRAK2, PCNA, PCSK9, EHD1  |
| GO:0003682~chromatin binding                            | 1.99E-04 | RPA1, NOTCH1, CHD7, NUP62, CENPB, NCOA6, CDCA5, KLHDC3   |
| GO:0006259~DNA metabolic process                        | 3.92E-04 | PTTG3P, GINS3, PTTG1, MCM3, MCM5, KLHDC3, MCM6, RPA1, SET, NCOA6, PCNA, TDG, FANCE, NT5E   |
| GO:0044427~chromosomal part                             | 4.13E-04 | RPA1, NDE1, CHD7, RCC2, INCENP, CENPB, PCNA, AURKB, MCM3, CDCA5, NCAPD2, KLHDC3  |
| GO:0007059~chromosome segregation                       | 6.48E-04 | KIFC1, DLGAP5, INCENP, PTTG1, CDCA5, NCAPD2  |
| GO:0015630~microtubule cytoskeleton                     | 7.18E-04 | KIFC1, DLGAP5, DYNLT1, AURKB, MYH9, MCM3, MFN2, TUBB, NDE1, RCC2, NUP62, INCENP, MAP7D1, ASPM  |
| GO:0001701~in utero embryonic development               | 7.94E-04 | MFN2, RPA1, CHD7, NCOA6, ICMT, MBNL1, MYH9, SRF  |
| GO:0043228~non-membrane-bounded organelle               | 1.01E-03 | ALDOA, KIFC1, FOXK1, DIAPH1, CALD1, WASF2, AURKB, SRF, RPA1, TMEM109, NDE1, TUBB, CHD7, ARPC2, INCENP, CENPB, CDC42EP4, CDCA5, ASPM, DBNL, DLGAP5, MTA2, FSCN1, DYNLT1, HEATR1, BASP1, MYH9, MCM3, NCAPD2, KLHDC3, MFN2, NUP62, RCC2, NCOA6, PCNA, MAP7D1, NCOR2 |
| GO:0043232~intracellular non-membrane-bounded organelle | 1.01E-03 | ALDOA, KIFC1, FOXK1, DIAPH1, CALD1, WASF2, AURKB, SRF, RPA1, TMEM109, NDE1, TUBB, CHD7, ARPC2, INCENP, CENPB, CDC42EP4, CDCA5, ASPM, DBNL, DLGAP5, MTA2,   |

|  |          |   |
|--|----------|---|
|  |          | FSCN1, DYNLT1, HEATR1, BASP1, MYH9, MCM3, NCAPD2, KLHDC3, MFN2, NUP62, RCC2, NCOA6, PCNA, MAP7D1, NCOR2   |
| GO:0005856~cytoskeleton  | 1.05E-03 | ALDOA, KIFC1, DBNL, DIAPH1, DLGAP5, CALD1, FSCN1, WASF2, DYNLT1, AURKB, BASP1, MYH9, MCM3, MFN2, RPA1, NDE1, TUBB, NUP62, RCC2, ARPC2, INCENP, CDC42EP4, MAP7D1, ASPM |
| GO:0043566~structure-specific DNA binding                        | 1.08E-03 | RPA1, EGR1, NOTCH1, RXRA, TDG, PCNA, MCM6   |
| GO:0051640~organelle localization                                | 1.15E-03 | MFN2, NDE1, DLGAP5, MYH9, CDCA5, ASPM   |
| GO:0006260~DNA replication                                       | 1.24E-03 | RPA1, SET, GINS3, NCOA6, PCNA, MCM3, MCM5, MCM6   |
| GO:0008104~protein localization                                  | 1.35E-03 | CHMP2A, SCAMP3, XPO6, ICMT, PTPRU, STRADB, AURKB, MYH9, MFN2, TRAM2, AP1S1, AP1S2, YWHAH, NUP62, TRAK2, PCNA, NECAP2, VPS25   |
| GO:0005694~chromosome  | 1.73E-03 | RPA1, NDE1, CHD7, RCC2, INCENP, CENPB, PCNA, AURKB, MCM3, CDCA5, NCAPD2, KLHDC3   |
| GO:0015031~protein transport                                     | 2.15E-03 | CHMP2A, SCAMP3, XPO6, ICMT, STRADB, MYH9, MFN2, TRAM2, AP1S1, AP1S2, YWHAH, TRAK2, NUP62, PCNA, NECAP2, VPS25   |
| GO:0045184~establishment of protein localization                 | 2.35E-03 | CHMP2A, SCAMP3, XPO6, ICMT, STRADB, MYH9, MFN2, TRAM2, AP1S1, AP1S2, YWHAH, TRAK2, NUP62, PCNA, NECAP2, VPS25   |
| GO:0034613~cellular protein localization                         | 2.79E-03 | MFN2, AP1S1, AP1S2, YWHAH, XPO6, TRAK2, PCNA, ICMT, AURKB, STRADB, PTPRU  |
| GO:0070727~cellular macromolecule localization                   | 2.94E-03 | MFN2, AP1S1, AP1S2, YWHAH, XPO6, TRAK2, PCNA, ICMT, AURKB, STRADB, PTPRU  |
| GO:0035257~nuclear hormone receptor binding                      | 3.36E-03 | TAF10, YWHAH, NUP62, RXRA, NCOA6  |
| GO:0000070~mitotic sister chromatid segregation                  | 3.57E-03 | KIFC1, DLGAP5, CDCA5, NCAPD2  |
| GO:0000819~sister chromatid segregation                          | 3.86E-03 | KIFC1, DLGAP5, CDCA5, NCAPD2  |
| GO:0003779~actin binding   | 4.63E-03 | ALDOA, DBNL, YWHAH, DIAPH1, ARPC2, CALD1, WASF2, FSCN1, MYH9  |
| GO:0051427~hormone receptor binding                              | 5.41E-03 | TAF10, YWHAH, NUP62, RXRA, NCOA6  |
| GO:0005905~coated pit  | 6.04E-03 | SH3BP4, AP1S1, AP1S2, NECAP2  |
| GO:0019318~hexose metabolic process                              | 6.18E-03 | ALDOA, TPI1, PGD, HK1, POFUT1, SLC35A2, PMM2  |
| GO:0051303~establishment of chromosome localization              | 7.12E-03 | NDE1, DLGAP5, CDCA5   |
| GO:0050000~chromosome localization                               | 7.12E-03 | NDE1, DLGAP5, CDCA5   |
| GO:0043009~chordate embryonic development                        | 7.62E-03 | MFN2, RPA1, CHD7, NCOA6, ICMT, MBNL1, MYH9, POFUT1, SRF   |
| GO:0009792~embryonic development ending in birth or egg hatching | 8.03E-03 | MFN2, RPA1, CHD7, NCOA6, ICMT, MBNL1, MYH9, POFUT1, SRF   |
| GO:0006270~DNA replication initiation                            | 8.09E-03 | MCM3, MCM5, MCM6  |
| GO:0051276~chromosome organization                               | 8.79E-03 | RPA1, TAF10, KIFC1, SET, CHD7, PTTG3P, DLGAP5, MTA2, PTTG1, CDCA5, NCAPD2   |

**Supplementary Table S4: Pathways associated with CCAR2-regulated genes**

| Pathway Term                              | P-Value  | Genes  |
|---|----------|--|
| hsa04110: Cell cycle                      | 3.24E-05 | CDKN1A, YWHAH, PCNA, PTTG1, MAD2L2, MCM3, MCM5, WEE1, MCM6                         |
| REACT_152: Cell Cycle, Mitotic            | 9.04E-05 | PTTG1, AURKB, MCM3, WEE1, MCM5, MCM6, RPA1, TUBB, CDKN1A, NDE1, RCC2, INCENP, PCNA |
| hsa03030: DNA replication                 | 4.50E-04 | RPA1, PCNA, MCM3, MCM5, MCM6   |
| REACT_383: DNA Replication                | 4.56E-03 | RPA1, CDKN1A, PCNA, MCM3, MCM5, MCM6   |
| hsa00051: Fructose and mannose metabolism | 4.83E-03 | ALDOA, TPI1, HK1, PMM2   |
| REACT_1538: Cell Cycle Checkpoints        | 8.37E-03 | RPA1, CDKN1A, MCM3, MCM5, WEE1, MCM6   |

**Supplementary Table S5: Enrichment of Transcription Factor Binding Sites in CCAR2-Regulated Genes**

| Transcription Factor | Count | p-Value  | Benjamini Adj. p-Value |
|----------------------|-------|----------|------------------------|
| STAT3                | 86    | 3.75E-06 | 6.64E-04               |
| TFCP2                | 74    | 1.01E-05 | 8.94E-04               |
| HEN1                 | 99    | 1.38E-05 | 8.13E-04               |
| TAXCREB              | 93    | 1.69E-05 | 7.46E-04               |
| ELK1                 | 87    | 2.32E-05 | 8.21E-04               |
| NFKAPPAB             | 66    | 6.54E-05 | 1.93E-03               |
| ZIC2                 | 47    | 2.55E-04 | 6.42E-03               |
| PAX5                 | 92    | 2.80E-04 | 6.17E-03               |
| RFX1                 | 98    | 3.61E-04 | 7.07E-03               |
| ROAZ                 | 77    | 4.14E-04 | 7.30E-03               |
| ARNT                 | 79    | 5.30E-04 | 8.49E-03               |
| EGR3                 | 36    | 5.69E-04 | 8.36E-03               |
| HOX13                | 76    | 6.71E-04 | 9.10E-03               |

**Supplementary Table S6: shRNA target sequences**

| Gene targeted | Clone designation | Target sequence  |
|---------------|-------------------|--|
| CCAR2 (4)     | TRCN0000053724    | GCATTGATTTGAGCGGCTGTA  |
| CCAR2 (5)     | TRCN0000053725    | GCCAAAGGAAAGGATCTCTTT  |
| RFX1 (3)      | TRCN0000014813    | GCCTGTGAGATAGATGTTTAT  |
| RFX1 (6)      | TRCN0000014816    | GCAGGCACCTACGTGATCCAA  |
| CREB1 (7)     | TRCN0000226467    | CCGGACGGTGCCAACTCCAATTTACCTCGAGGTAAATTGGAGTT<br>GGCACCGTTTTTTT |
| CREB1 (8)     | TRCN0000226468    | CCGGACAGCACCCACTAGCACTATTCTCGAGAATAGTGCTAGT<br>GGGTGCTGTTTTTT  |



**Supplementary Table S7: Quantitative RT-PCR Primers**

| <b>Gene</b>                        | <b>Sequence</b>   | <b>Product size</b> |
|------------------------------------|---|---------------------|
| CCAR2                              | F: GAGGCCCGCTGAAGGAGGA<br>R: CGCCGGTGCAAGTAGCCACA       | 335 bp              |
| SIRT1                              | F: GAGCTGGGGTGTCTGTTTC<br>R: GCAAAGGAACCATGACACTG       | 350 bp              |
| Beta-2-Microglobulin ( $\beta$ 2M) | F: AGCTGTGCTCGCGCTACTCTC<br>R: CACACGGCAGGCATACTCATC    | 286 bp              |
| AURKB                              | F: TTAACCAGGCGGCACTTCAC<br>R: GAGACGCAGGATGTTGGGAT      | 222 bp              |
| INCENP                             | F: TGCCCCAGAAAGTTGGTTCT<br>R: AGGGGAGTGTTGCGCTTAAT      | 478 bp              |
| CDCA7                              | F: TGAGGTGCAAGATGTATTAGACCAT<br>R: CTTGATTGTGAGTCGGAGCC | 468 bp              |
| CDCA5                              | F: AAGTCAGGCGTTCCTACAGC<br>R: TCATCCAGCTCCGTTTTCAAG     | 274 bp              |
| ASPM                               | F: TCTCAAACGCCATCAGGAGA<br>R: TCCTAGATTGCAGGATGATTGAATA | 287 bp              |
| NCAPD2                             | F: CATGATGGCACGAGGAAAGC<br>R: GCAGTCGCTCAAACAACCTG      | 212 bp              |
| NDEL1                              | F: GTCACAGAATGGCCTCGGA<br>R: TCCATGATCAAGAAAGCCTCCA     | 207 bp              |
| RFX1                               | F: GCTGGCTGTACATGACGAGG<br>R: CACGTTGTCACAGTGCTTGG      | 106 bp              |
| CREB                               | F: ACAGATTGCCACATTAGCCC<br>R: TCCTTACAGGAAGACTGAACTGT   | 183 bp              |
| STAT3                              | F: GAGCTGGGGTGTCTGTTTC<br>R: GCAAAGGAACCATGACACTG       | 350 bp              |

## **SUPPLEMENTARY MATERIALS AND METHODS**

### **Human Cell Lines**

JHU-029 cells (Rocco *et al.*, 2006; Zhao *et al.*, 2011) were a gift of David Sidranski (Johns Hopkins University, Baltimore, MD, USA). HO1N1 cells (Shimizu *et al.*, 2007), were a gift of S. Michael Rothenberg (Massachusetts General Hospital, Boston, MA, USA). SCC-13, SCC-15, SCC-25, and G5-Ep were obtained from the BWH Department of Dermatology Cell Culture Core. P1-Ep (Rollins *et al.*, 1989), OKF6 (Dickson *et al.*, 2000), N, (Rheinwald *et al.*, 2002) were a gift of James Rheinwald. Mouse SCC lines SCC-80 (Gurumurthy *et al.*, 2008) and B9 (Burns *et al.*, 1991) were a gift of Nabeel Bardeesy (Massachusetts General Hospital, Boston, MA, USA). Mouse SCC line 392-1 (Ramsey *et al.*, 2013) was a gift of Leif Ellisen (Massachusetts General Hospital, Boston, MA, USA).

### **Generation of murine keratinocytes**

To generate murine keratinocytes, back hair was shaved and a 2 cm x 2 cm region of skin was removed and placed flat on filter paper with 5 mg/ml Dispase II (Sigma) and incubated at 37 °C for 1 hour. The epidermis was then pulled from the dermis, and incubated in pre-warmed 0.25 % trypsin/EDTA at 37 °C for 5 minutes with rotation. The solution was filtered through a 70 µm sieve and centrifuged at 1300 g for 5 minutes. Recovered cells were re-suspended in K-sfm Low Calcium Medium (Gibco), 12.5 µg/ml Bovine Pituitary Extract, 0.2 ng/ml Epidermal Growth Factor, Penicillin/Streptomycin and plated onto Collagen coated tissue culture dishes (4 hours incubation at 37 °C with 2.5 mg/ml Collagen (Sigma), 0.1 mg/ml Bovine Serum Albumin (Sigma), 0.02 M HEPES, HBSS). Media was changed every two days until the primary keratinocytes had propagated and could be harvested for protein.

### **Immunoprecipitation from nuclear and cytoplasmic fractions**

Nuclear and cytoplasmic extracts were prepared by suspending cells in hypotonic buffer (10 mM Tris-HCl pH 7.5, 1.5 mM MgCl<sub>2</sub>, 10 mM KCl) for 20 minutes, followed by douncing. Nuclei were pelleted, then supernatant was collected and cleared by centrifugation for cytoplasmic extract. Nuclei were suspended in 1 pellet volume 20 mM KCl nuclear buffer (20 mM Tris-HCl pH 7.5, 1.5 mM MgCl<sub>2</sub>, 0.2 mM EDTA, 25 % glycerol). One pellet volume 1.2 M KCl nuclear buffer was added dropwise then incubated for 30 minutes at 4 °C with rotation. Cleared supernatant was dialyzed against BC-100 buffer (100 mM KCl, 20 mM Tris-HCl pH 7.5, 0.2 mM EDTA, 20 % glycerol). Cleared nuclear lysates were incubated with antibody and protein A beads for 3.5 hours at 4 °C, and immunocomplexes were washed with 150 mM, 200 mM, 250 mM, 200 mM, 150 mM KCl wash buffer.

### **Immunofluorescence (IF) Staining**

Human skin or tumor sections were snap-frozen in HistoPrep (Fisher Scientific), and 5 µm sections were cut. Sections or cultured cells were fixed in 3.7 % Paraformaldehyde and permeabilized with 0.1 % Triton X-100 followed by block in 10% Normal Goat Serum, primary antibody staining and secondary antibody staining (AlexaFluor-488, AlexaFluor-568, Invitrogen). Nuclei were visualized by staining with Hoechst 33342 dye (Invitrogen). Samples were cover-slipped in Fluoromount mounting medium (Southern Biotech) and black and white images were obtained using a Nikon Eclipse E600 microscope and SPOT software. False color was applied to using Photoshop software (Adobe).

### **Immunohistochemical (IHC) Staining**

Assistance in processing of Xenograft samples was provided by the Dana-Farber/Harvard Cancer Center Specialized Histopathology Core Facility. Five micron sections were cut and stained for Ki67 using standard protocols. Quantification of Ki67 positive cells was performed by assessing number of positive cells in 1-3 independent fields for each sample. Staining for Cleaved Caspase 3 was performed using the SignalStain Cleaved Caspase-3 (Asp175) IHC detection kit (Cell Signaling) according to manufacturer's instructions.

### **Antibodies**

*Western Blot:* CCAR2 (A300-434A-1, Bethyl Laboratories), GAPDH (FL335, Santa Cruz), SIRT1 (1F3, Cell Signaling), RFX1 (A303-043A, Bethyl Laboratories), CREB (86B10, Cell Signaling), Histone H3 (9715, Cell Signaling).

*Immunoprecipitation:* CCAR2 (A300-434A-1, Bethyl Laboratories), SIRT1 (A300-687A, Bethyl Laboratories), Acetyl lysine (9441, Cell Signaling), RFX1 (A303-043A, Bethyl Laboratories).

*Immunofluorescence:* CCAR2 (A300-434A-1, Bethyl Laboratories), Keratin 14 (SIG-3476, Covance), RFX1 (A303-043A, Bethyl Laboratories), CREB (48H2, Cell Signaling).

*Immunohistochemistry:* Ki67 (NCL-Ki67p, Novacastra), Cleaved Caspase-3 (Asp175) (D3E9, Cell Signaling).

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