## Supplementary Information

## Supplementary Methods

### DNA cloning

For the cloning of the FOXO1 and FOXO3a inducible shRNA, the pH1tet-flex/FH1t(INSR)UTG vector system (Taconic Artemis) was utilized. The short hairpin targeting FOXO1 and FOXO3a, flanked by BbsI and XhoI recognition sequences (sequence: 5'-tcccgtgccctacttcaaggataagttcaagagacttatccttgaagtagggcacttttttctcgag-3') was first inserted in the pH1tet-flex vector digested with BbsI/XhoI. Subsequently, the H1 tetO-shRNA cassette was amplified with primers introducing PacI sites. After digestion with PacI, the H1tet-shRNA was inserted in the FH1t(INSR)UTG vector.

### Quantitative PCR (Q-PCR)

The oligomers used for the quantitative analysis of IDH expression were

IDH1_F: TTGTCCAGATGGCAAGACAG, IDH1_R: CAGGCAAAAATGGAAGCAAT, IDH2_F: CTCATCAGGTTTGCCCAGAT, IDH2_R: GTCCGTGGTGTTCAGGAAGT, IDH3A_F: GAGATGGTATTGGCCCAGAA, IDH3A_R: TTCCTCCAGGTCCTTGAATG, IDH3B_F: TGGTGATCATTCGAGAGCAG, IDH3B_R: TGAGACTTGGCTCGTGTGAC, IDH3G_F: ATGTGTACGCGGTGTTTGAA, IDH3G_R: ATGGAGGTGGCATAGGAGTG, Tubulin_F: TACACCATTGGCAAGGAGAT, Tubulin_R: AACCAAGAAGCCCTGAAGAC, mIDH1_F: TCACCAAAGATGCTGCAGAG, mIDH1_R: TGGGGATTTCCACATTTGTT, mPBDG_F: GCCTACCATACTACCTCCTGGCT, mPBDG_R: AAGACAACAGCATCACAAGGGTT.

The oligomers used for the amplification of the areas within *IDH1* after ChIP analysis were:

IDH1 F: CATTCATCTGGGGAGATTCAA, IDH1 R: GTTTCATGCATGGGGTAAGG, IDH1 neg F: CTTCCATCTGGTGATGTGGTT, IDH1 neg R: TTCCCCACTATCTTTGCTCCT

### Microarray analysis

HeLashFOXO cells were cultured in the presence or absence of doxycycline for 72 hrs. Cells were washed twice with PBS and RNA was extracted with the RNeasy kit (Qiagen), according to the manufacturer's instructions. Quality of the isolated RNA was tested by an Agilent 2100 Bioanalyzer

digital gel run of total RNA. For the microarray analysis, cDNA made from the isolated mRNA was hybridized to a pool of cDNAs from the parental HeLa cells (common reference sample). All RNA amplification and labeling procedures were performed in 96-well plates (Abgene) on a customized Sciclone ALH 3000 workstation (Caliper LifeSciences), supplemented with a PCR PTC-200 (Bio-Rad Laboratories), SpectraMax 190 spectrophotometer (Molecular Devices) and a magnetic bead locator (Beckman). Labeled cRNA product was purified with RNAClean (Agencourt, GC biotech) according to the manufacturer's protocol. Hybridizations of spotted oligo-arrays (Human Operon version2 onto Codelink glass) were performed on an HS4800Pro Hybstation (Tecan). Hybridized slides were scanned on an Agilent scanner (G2565AA) at 100% laser power, 30% photo multiplier tube. After data extraction using Imagene 7.5 (BioDiscovery), print tip Loess normalization was performed44 on mean spot intensities.

Statistical analysis was performed using the LIMMAS (linear models for microarray data) files, with the following criteria: (i) $p \leq 0.01$ (ii) fold induction: ≤0.8 fold (downregulated genes) and ≥1.3 fold (upregulated genes). GO term analysis was performed by the Database for Annotation, Visualization and Integrated Discovery (DAVID).

**Chromatin Immunoprecipitation (ChIP)**

To perform ChIP, FOXO3A3 in DL23 cells was activated by addition of 4-OH-tamoxifen for 4 hrs. 40 million cells per condition were fixed with 1% formaldehyde for 10 min at room temperature and the reaction was quenced by 0.125 M Glycine for 5 min. Subsequently cells were lysed in Darnham lysis buffer (5 mM PIPES, 85 mM KCl, 0.5% NP-40, protease inhibitor cocktail Roche) and nuclear extracts were collected by low speed centrifugation and re-suspended in RIPA buffer (1x PBS, 1% NP-40, 0.5% Na-deoxycholate, 0.1% SDS, supplemented with Roche protease inhibitor cocktail). Chromatin was then sonicated to acquire DNA fragments of about 500 bp which were then used for immunoprecipitations. Antibodies used were: rabbit IgG (Santa Cruz) and rabbit anti-FOXO3a (Santa-Cruz).

**Immunostaining**

HT1080shFOXO cells were grown during 72hs with or without doxycycline. When indicated cells were treated with the inhibitor PI3K/PKB(AKT) (VIII). Immunofluorescence was performed using FKHRL1 Antibody (H-144) (Santa Cruz sc-11351) and was performed using standard methods.

## Supplementary Figure and Table Legends

**Supplementary Figure 1 (related to Figure 1). (A)** Relative mRNA and protein levels of FOXO1, FOXO3, FOXO4 and IDH1 in HeLashFOXO cells. HeLashFOXO cells were cultured in the presence or absence of doxycycline for 72 hrs before sample collection (n=3). Protein levels were detected by immunoblotting of whole cell extracts (WCE) or in case of FOXO4 after immunoprecipitation of FOXO4 (ip) **(B)** Relative mRNA levels of IDH1 in A14 cells after insulin stimulation. A14 cells were cultured in serum free medium for 16 hrs before insulin stimulation for additional 4 hrs. Where indicated, cells were pre-treated with LY for 1 hr before insulin stimulation (n=3) **(C)** Relative mRNA levels of IDH2, IDH3a, IDH3b and IDH3g in RPEshFOXO cells, RPEshFOXO cells were cultured in the presence or absence of doxycycline for 72 hrs before sample collection (n=3). Unless stated otherwise error bars represent standard deviation.

**Supplementary Figure 2 (related to Figure 2). (A)** α-KG levels were measured in RPEshFOXO with and without doxycycline or transfected with IDH1 siRNA. Cells were cultured in the presence or absence of doxycycline for 96 hrs before sample collection and analysis by LC-MS (n=3). **(B)** NADPH/NADP+ ratio in RPEshFOXO with and without doxy or transfected with IDH1 siRNA. Cells were cultured in the presence or absence of doxycycline for 48 hrs and thereafter the medium was changed with medium without glucose and supplemented with pyruvate and glutamine for another 48 hrs before sample collection (n=3). **(C)** NADPH/NADP+ ratio in RPEshFOXO with and without doxy. Cells were cultured in the presence or absence of doxycycline for 48 hrs and thereafter the medium was changed with same medium (with glucose) and cultured for another 48 hrs before sample collection (n=3). **(D)** GSH/GSSG ratio in RPEshFOXO cells. RPEshFOXO cells were cultured in the presence or absence of doxycycline for 48 hrs and thereafter the medium was changed to medium with no glucose, supplemented with pyruvate and glutamine for another 48 hrs before sample collection. IDH1 silencing by siRNA served as a positive control. Experiments were repeated at least 3 times (n=3). *$p<0.001$, **$p<0.005$ p values were obtained from T-Test. Unless stated otherwise error bars represent standard deviation.

**Supplementary Figure 3 (related to Figure 3). (A)** Analysis of FOXO3 cellular localization by immunostaining. Cells were grown for 72 hrs with or without doxycycline. Where indicated cells were treated with the PI3K/PKB inhibitor VIII. Cells were fixed with PFA and incubated with anti-FKHRL1

and Alexa Fluor 2ry antibody. Negative control was performed with secondary antibody only. DAPI staining was used to stain nuclear DNA. **(B)** 2-HG levels were measured in HTshFOXO with and without doxycycline or transfected with IDH1 siRNA. Cells were cultured in the presence or absence of doxycycline for 96 hrs before sample collection and analysis by LC-MS (n=3). Unless stated otherwise error bars represent standard deviation.

**Supplementary Figure 4 (related to Figure 4). (A)** Left panel: Analysis of DNA methylation and hydroxymethylation levels in HT1080shFOXO cells grown in the presence or absence of doxycycline for 72 hrs before sample collection. Cells were transiently transfected with a plasmid containing the coding sequence of TET1 enzyme. Where indicated cells were also grown in the presence of 500 uM of 2-HG. Right panel: total protein lysates of cells treated as indicated were analyzed for expression of HA-TET1 and endogenous FOXO3, to control for TET1 expression and efficiency of knockdown.

**(B)** Effect of FOXO on apoptotic markers and p27 in RPEshLuc and RPEshFOXO during culture growth. HT1080shLuc and HT1080shFOXO cells were grown with or without doxy during 5 days. Samples were taken from day 3 and every day. Detection of apoptotic markers was done by western blot using cleaved caspase, cleaved PARP, p27, and GAPDH as a loading control antibody (Cleaved Caspase-3 (Asp175) (5A1E) Cell Signaling, Cleaved PARP (Asp214) (D64E10) Cell Signaling, p27 Kip1 Antibody #2552 and GAPDH (14C10) #2118). Results in A and B are representative of 3 independent experiments.

**Supplementary Table 1.** List of genes with significant ($p \leq 0.01$) changes in FOXO-depleted HeLa cells, compared to control cells (≤0.8 and ≥1.3 fold change). In bold the genes whose expression levels are depicted in Figure 1A.

**Supplementary Table 2 (related to figure 4F)**. Raw data of AnnexinV staining of siDH1 samples compared to other samples treated as indicated

| | % AnnexinV positive cells | | |
|---|---|---|---|
| | #1 | #2 | average |
| shLuc | 5.67 | 2.85 | 4.26 |
| shluc + doxy | 5.07 | 3.00 | 4.04 |
| shFOXO | 4.24 | 4.78 | 4.51 |
| shFOXO + doxy | 75.06 | 76.18 | 75.62 |
| shFOXO + 2HG | 4.67 | 7.43 | 6.05 |
| shFOXO + 2HG + doxy | 75.08 | 75.88 | 75.48 |
| shFOXO + siIDH1 | 6.59 | 7.55 | 7.07 |
| shFOXO + siIDH1 + doxy | 66.04 | 72.03 | 69.04 |