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Appendix Supplementary Methods

Cell Culture

293FT and MCF-7 cells were cultured in DMEM containing 10% fetal bovine serum (FBS) plus 1% penicillin streptomycin (Pen Strep). T47D cells were maintained in RPMI medium containing 10% FBS plus 1% Pen Strep. Following lentivirus infection, cells were maintained in the presence of hygromycin (200 μg/ml) or puromycin (2 μg/ml) depending on the vector. All cells were maintained at 37°C in 5% CO_2 incubator.

Western Blot Analysis and Antibodies

EBC buffer (50 mM Tris pH8.0, 120 mM NaCl, 0.5% NP40, 0.1 mM EDTA and 10% Glycerol) supplemented with complete protease inhibitor (Roche Applied Biosciences) was used to harvest whole cell lysates from 5×10^5 indicated cells for immunoblots. Subcellular protein Fractionation kits were obtained from Thermo Scientific. Cell lysate concentrations were measured by Bradford assay. Equal amount of cell lysates were resolved by SDS-PAGE. The experiments were repeated for three times with similar results. Rabbit EglN2 antibody (NB100-310), HIF2α (NB100-122) and HIF1α (NB100-479) were from Novus Biological. Rabbit anti Cyclin D1 was from Neomarker. Mouse anti-HIF1α (610958), anti-ARNT (611079) and anti-Cytochrome C (556433) were from BD Bioscience. Antibodies against Vinculin (V9131), α-Tubulin (T9026) and MCU (HPA016480) were from Sigma. Mouse antibody against hemagglutinin (HA, MMS-101P) was obtained from Covance. Mouse anti-PGC1α antibody (St1202) and rabbit anti-PDH Ser293 (AP1062) were from Calbiochem. Sheep anti-EglN2 antibody (AF6394) was from R&D. Mouse anti-NRF1 (ab55744), anti-CLPP (ab124822), anti-Mitofusin 1 (ab57602) and anti-Mitofusin 2 (ab56889) were obtained from Abcam. Antibodies against AIF (#4642), Bcl-XL (#2762), PDH (#2784) and VDAC (#4866) were obtained from Cell Signaling. Anti-COX3

(G2413) was from Santa Cruz Biotechnology. Antibodies against ND1 (19703-1-AP), ND2 (19704-1-AP), ND5 (55410-1-AP), COX1 (55071-1-AP), COX2 (55070-1-AP), NDUFA9 (20312-1-AP), SDHA (14865-1-AP) and UQCRFS1 (18443-1-AP) were from Proteintech. Peroxidase conjugated goat anti-mouse secondary antibody (31430) and peroxidase conjugated goat anti-rabbit secondary antibody (31460) were purchased from Thermo Scientific.

Plasmids

Full length FLAG and HA double tagged EglN2 was amplified by PCR with a 5' primer that introduced a FLAG tag and an HA tag with a BamHI site and a 3' primer that introduced an EcoRI site. The PCR product was digested with BamHI and EcoRI and cloned into the pBABE-puro vector cut with these two enzymes. pLenti6 FLAG-EglN2 was described previously (Koivunen et al., 2012). pSG5-TETr-EglN2 fusion was created by ligating the full length EglN2 ORF (amplified with 5' BamHI site and 3' EcoRI site) into the pSG5-TETr empty vector cut with BamHI/ EcoRI. pSG5-TETr-CDK2, pSG5-TETr-E2F1 and pUHC 13-3 reporter were described previously (Kim and Kaelin, 2001). GST-NRF1 and HA-NRF1 were described previously (Wang et al., 2006). The full-length FOXO3a insert was cut with BamHI and NotI and ligated into pGex 4T.2 vector cut with these two enzymes to make the construct for GST-FOXO3a. Full length HA tagged NRF1 was amplified by PCR with a 5' primer that introduced a BamHI site and an HA tag and a 3' primer that introduced a NotI site. The PCR product was digested with BamHI and NotI and cloned into pLenti-UBC-pGK-Hyg (a modified version of pLL3.7) vector cut with these two enzymes. Full length HA tagged FDXR was amplified by PCR with a 5' primer that introduced an XbaI site and an HA tag and a 3' primer that introduced an XhoI site. The PCR product was digested with XbaI and XhoI and cloned into pLenti CMV GFP vector (addgene) digested with XbaI and SalI.

Virus Production and Infection

293FT packaging cell lines was used for lentiviral amplification. Lentiviral infection was carried out similarly as previously described (Zhang et al., 2009). Briefly, post-transfection with lipofectamine 2000, viruses were collected twice after 48 and 72 hours. After passing through 0.45 μM filters, appropriate amount of viruses were used to infect target cells in the presence of 8 μg/ml polybrene. Subsequently, target cell lines underwent appropriate antibiotic selection.

siRNAs and Lentiviral shRNA Vectors

Non-Targeting siRNA no. 2 was obtained from Dharmacon (catalogue number: D0012100220). EglN2 (1) and EglN2 (4) siRNAs were described before (Zhang et al., 2009). NRF1 smart pool siRNA was obtained from Dharmacon (catalogue number: L-017924). PGC1α siRNAs were obtained from Dharmacon with the following targeting sequences:

PGC1α (#1): GCAGGUAACAUGUUCCCUA

PGC1α (#2): ACTCUCAGCUAAGUUAUAA

PGC1α (#3): GAAGAGCGCCGUGUGAUUU

PGC1α (#4): GAGAAUUCAUGGAGCAAUA

Lentiviral EglN2, HIF1α, ARNT and FDXR shRNAs were obtained from Broad Institute TRC shRNA library. Target sequences are listed as follows:

Ctrl shRNA: AACAGTCGCGTTTGCGACTGG

EglN2 (325): GCTGCATCACCTGTATCTATT

EglN2 (326): GCCACTCTTTGACCGGTTGCT

EglN2 (327): ACTGGGACGTTAAGGTGCATG

EglN2 (328): CTGGGACGTTAAGGTGCATGG

HIF1α (3809): CCAGTTATGATTGTGAAGTTA

HIF1α (3810): GTGATGAAAGAATTACCGAAT

HIF2α (3804): CGACCTGAAGATTGAAGTGAT

ARNT (1770): GAGAAGTCAGATGGTTTATTT

FDXR (434): GCTCAGCAGCATTGGGTATAA

FDXR (435): CCATTTCTCCACACAGGAGAA

Real-Time RT-PCR

Total RNA was isolated with RNeasy mini kit (Qiagen) from 2×10^5 indicated cells. First strand cDNA was generated with the iScript cDNA synthesis kit (Biorad). Real time PCR was performed in triplicate as described before (Zhang et al., 2009). Real-Time RT-PCR primers used in this study are included in the Table EV6. The experiments were repeated for three times with similar results.

GST Protein Purification and GST Pull Down

GST plasmids were transformed with BL21 competent cells. Single colonies were picked from above and cultured in 50 ml LB medium containing ampicillin. After overnight culture, 5 ml LB medium were diluted in 500 ml LB medium for shaking at 37°C for 2-3 hours until OD600 was reached then 0.8-1.0. 0.2 mM IPTG was added to induce GST protein production for 4 hours before harvesting pellets. Bacteria lysates were disrupted by the nanodebee homogenizer. Cleared bacteria lysates were purified by using Glutathione-sepharose 4B beads. 20 μL GST suspension proteins were incubated with either *in vitro* translated protein in 500 μl NETN buffer or cell lysates. After overnight incubation, bound complexes were washed with NETN buffer 8 times followed by boiling in SDS loading buffer and SDS-PAGE.

Immunoprecipitation

Cells were lysed in EBC lysis buffer supplemented with complete protease inhibitors (Roche Applied Bioscience). The lysates were clarified by centrifugation and then mixed with primary

antibodies or 3F10 HA conjugated beads (Roche Applied Bioscience) overnight. For primary antibody incubation overnight, cell lysates were incubated further with protein G sepharose beads (Roche Applied Bioscience) for 2 hours. The bound complexes were washed with NETN buffer for 8 times and were eluted by boiling in SDS loading buffer. Bound proteins were resolved in SDS-PAGE followed by western blot analysis. The experiments were repeated for three times with similar results.

Luciferase Reporter Assay

For TETr-fusion protein transcription assay, subconfluent 293FT or T47D cells (200,000 cells/24 well plate) were transiently transfected with pCMV-renilla (30 ng), 100 ng of pUHC13-3 reporter plasmid and indicated plasmids encoding TETr-fusion plasmids or empty vector (100 ng unless indicated otherwise). 48 hours after transfection, luciferase assay were performed as described previously (Kim and Kaelin, 2001). The experiments were repeated for three times with similar results.

Cell Proliferation Assays

T47D cells were plated, in triplicate, in 96 well plates (3000 cells/well) in appropriate growth medium. At indicated time points, cells were replaced with 90 μl fresh growth medium supplemented with 10 μl MTS reagents (Promega) followed by incubation at 37°C for 2 hr. The OD absorbance value was measured at 490 nm using a 96-well plate reader. The experiments were repeated for three times with similar results.

Anchorage Independent Growth Assay

Cells were plated at a density of 5000 cells per ml in complete medium with 0.4% agarose, onto bottom layers composed of medium with 1% agarose followed by incubation at 4^0C for 10 minutes. Afterwards, cells were moved to 37^0C incubator. For every 4 days, three drops of complete media were added onto the plate. After two weeks, the extra liquid on the plate was aspirated and 1 ml medium was added into each well, colonies were stained by 100 μg/ml iodonitrotetrazoliuim chloride solution. Cell culture plates were put back in the incubator overnight followed by counting of foci number. The experiments were repeated for three times with similar results.

Orthotopic Tumor Growth

Six-week-old female NOD SCID Gamma mice (NSG, Jackson lab) were randomly distributed for xenograft studies. FDXR knockdown (sh434 or sh435) and control cells were injected bilaterally in order to have fair comparison. Approximately 4 $x10^6$ viable T47D breast cancer cells were resuspended in 100 μl growth factor reduced matrigel (BD biosciences) and injected orthotopically into the mammary gland as described previously (Zhang et al., 2009). Bioluminescence imaging was performed as described previously (Zhang et al., 2009). Seven mice were included in the group (FDXR sh434/Ctrl) and six mice were included in the group (FDXR sh435/Ctrl). For each mouse, total photons from mammary fat pad injected with cells expressing FDXR shRNA were divided by total photons from the contralateral fat pad with cells expressing control shRNA and normalized to the ratio for that mouse on the day 3 post-implantation of cancer cells. Mice were sacrificed 4 weeks after the first imaging, as specified in the figure legends. The total mass of tumors were presented as mean ± SEM and evaluated statistically using the unpaired two-tail student's t test. Data represent mean ± SEM. All animal

experiments complied with National Institutes of Health guidelines and were approved by the University of North Carolina at Chapel Hill Animal Care and Use Committee.

Appendix References

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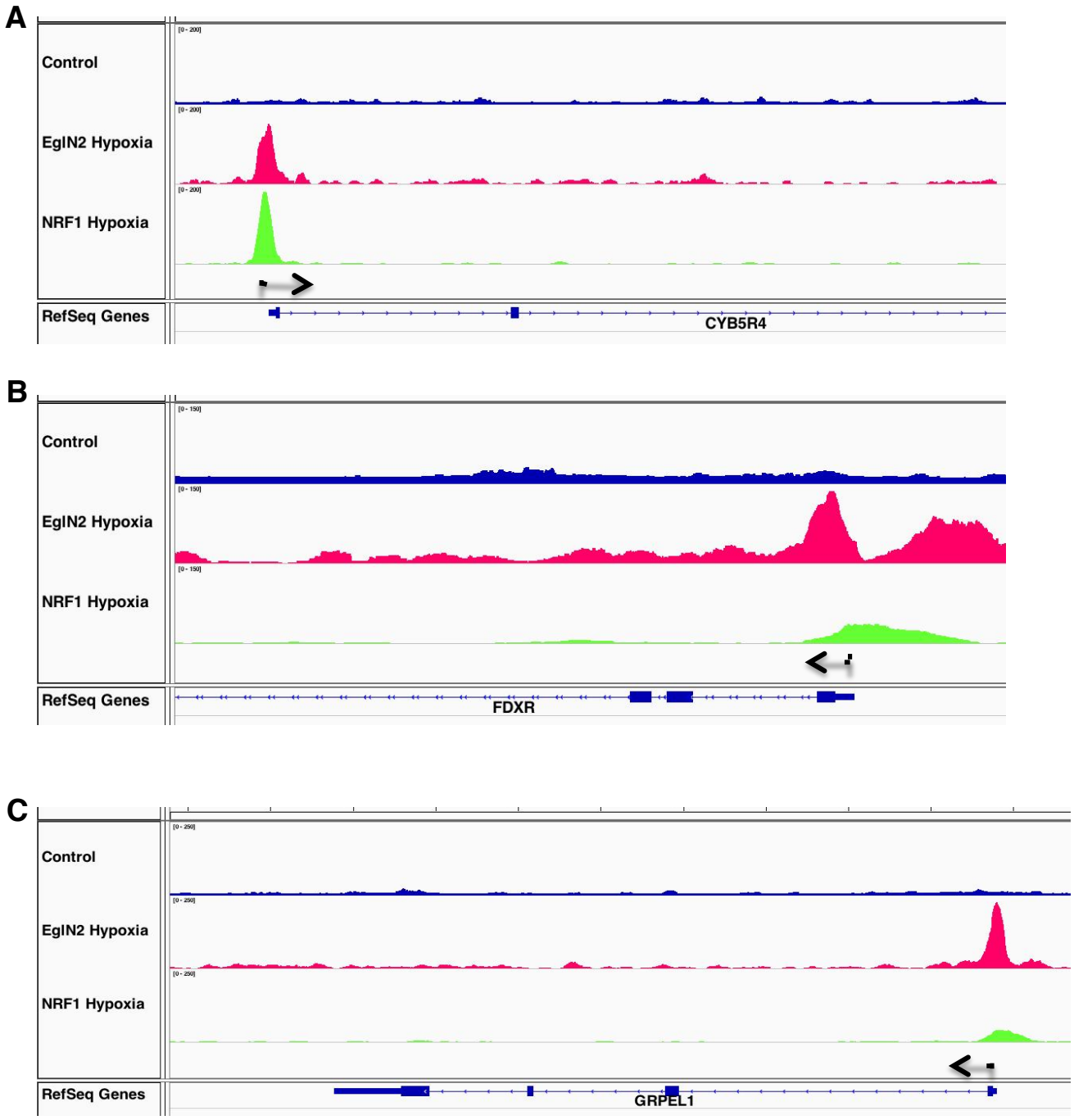
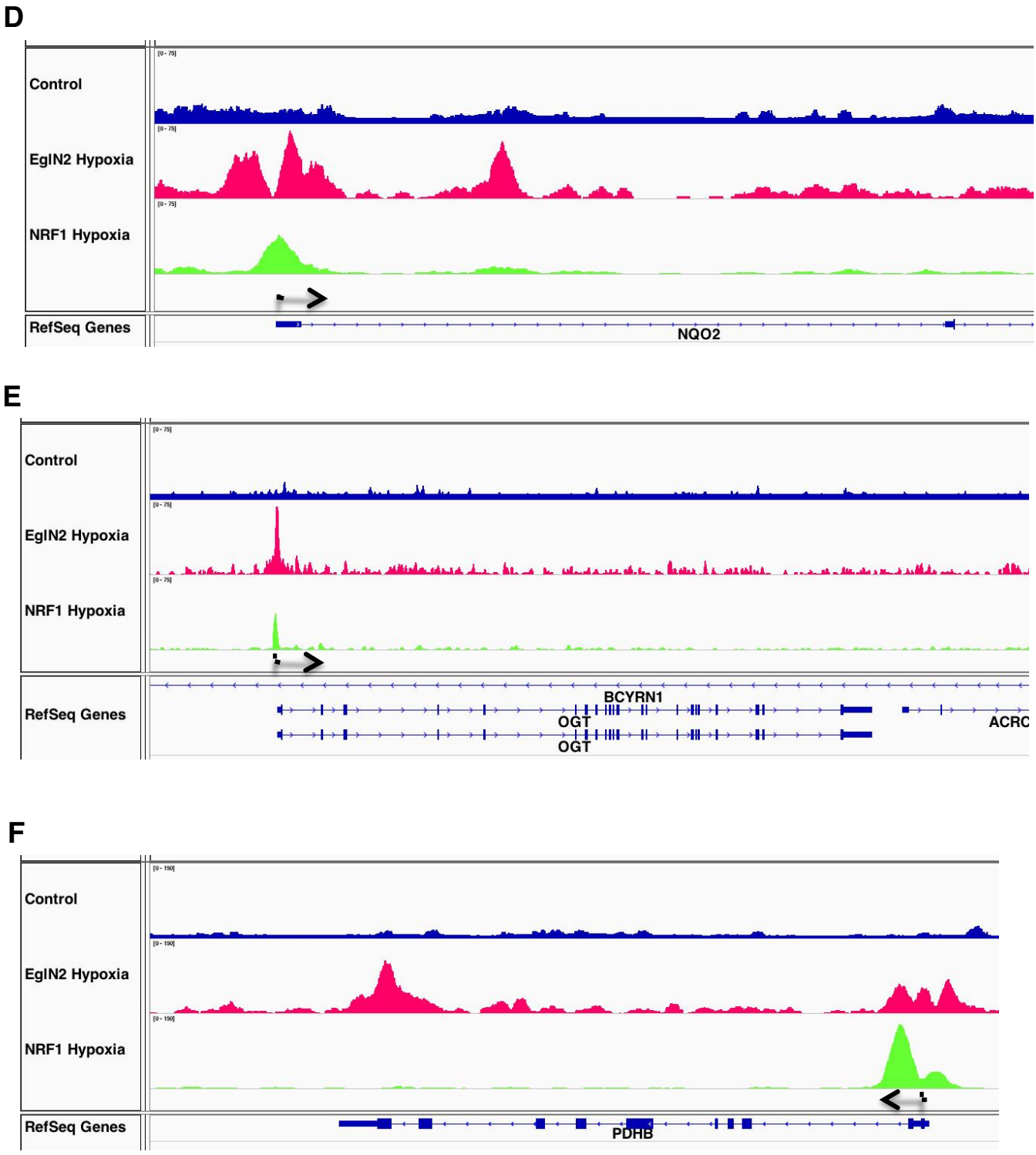
Appendix Figure S1



Appendix Figure S1. Representative ChIP-Seq binding peaks of EglN2 and NRF1 on target gene promoters, including: CYB5R4 (A), FDXR (B) and GRPEL1 (C).

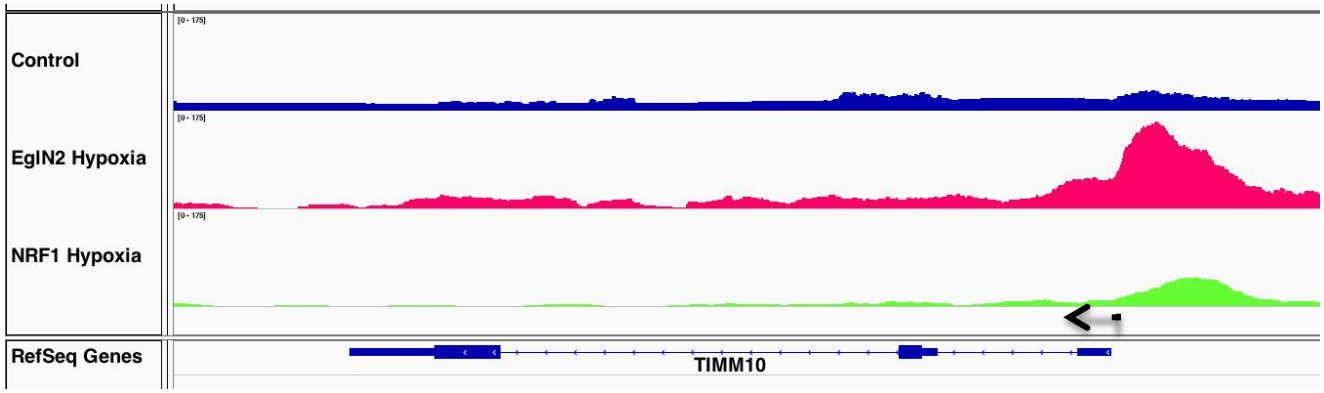
Appendix Figure S1-Continued



Appendix Figure S1 (Continued). Representative ChIP-Seq binding peaks of EglN2 and NRF1 on target gene promoters, including: NQO2 (D), OGT (E) and PDHB (F)**.**

Appendix Figure S1-Continued

G



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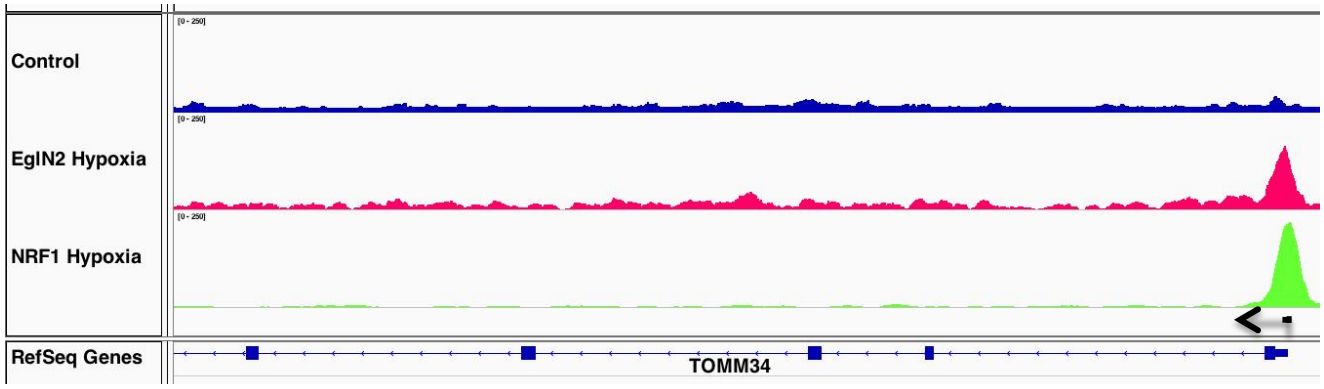

Appendix Figure S1 (Continued). Representative ChIP-Seq binding peaks of EglN2 and NRF1 on target gene promoters, including: TIMM10 (G) and TOMM34 (H)**.**