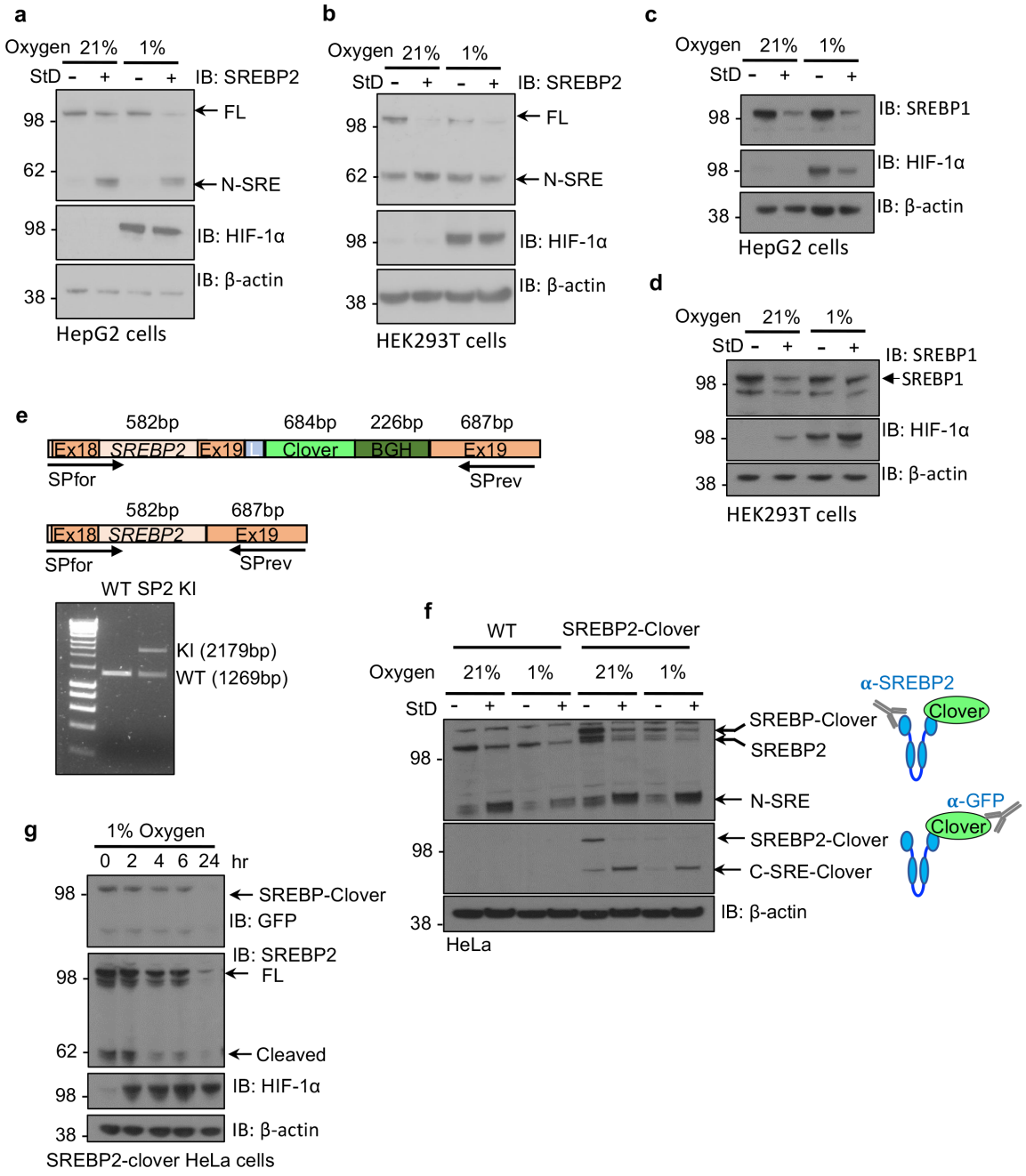
Extended Data Figures and Supplementary Information

Extended Data Figures

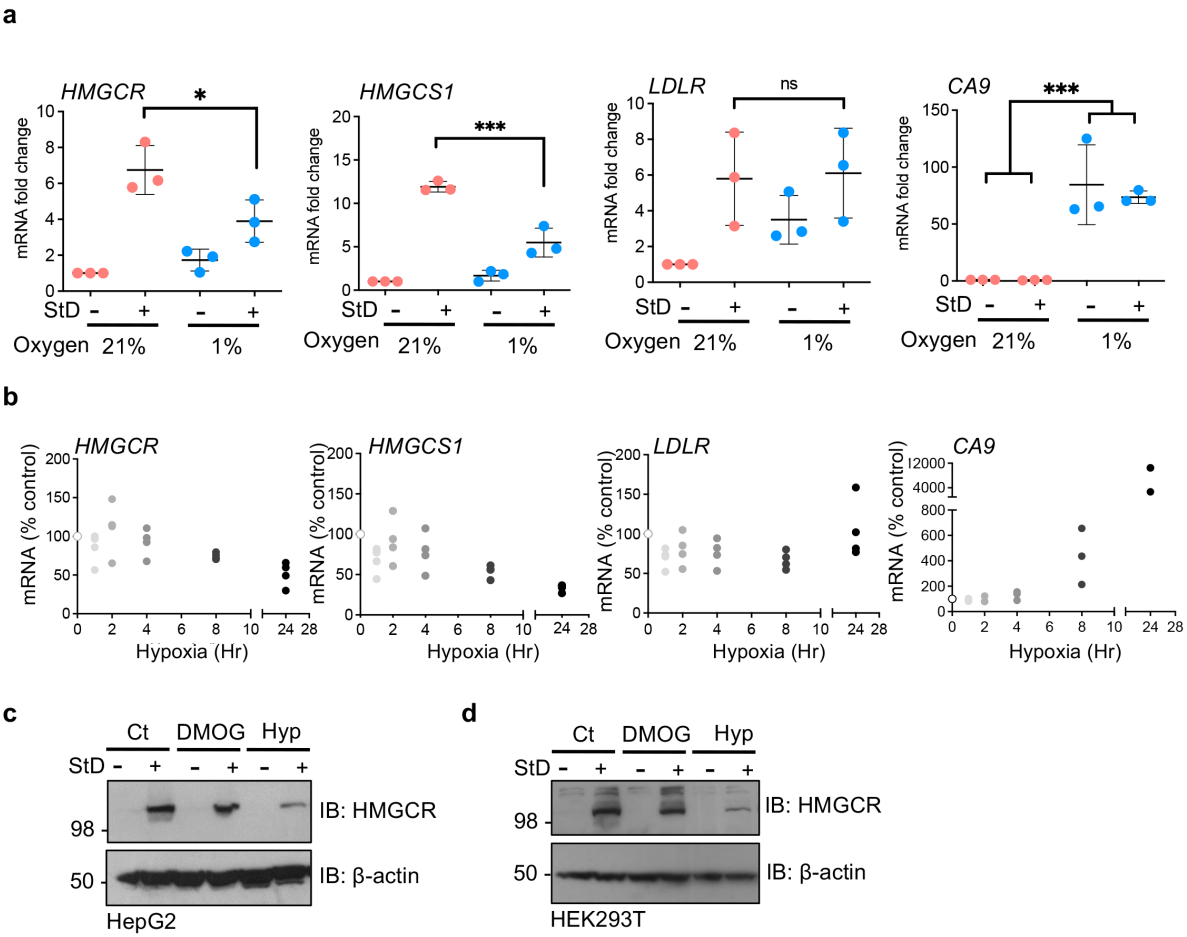
Extended Data Fig.1: Hypoxia promotes SREBP2 degradation.



(**a, b**) SREBP2 processing in HepG2 cells (**a**) or HEK23T cells (**b**) treated with or without StD in 21% and 1% oxygen for 40 hr. *Immunoblots representative of 3 independent experiments.* (**c, d**) SREBP1 levels in HepG2 cells (**c**) or HEK23T cells (**d**) treated with or without StD in 21% and 1% oxygen for 40

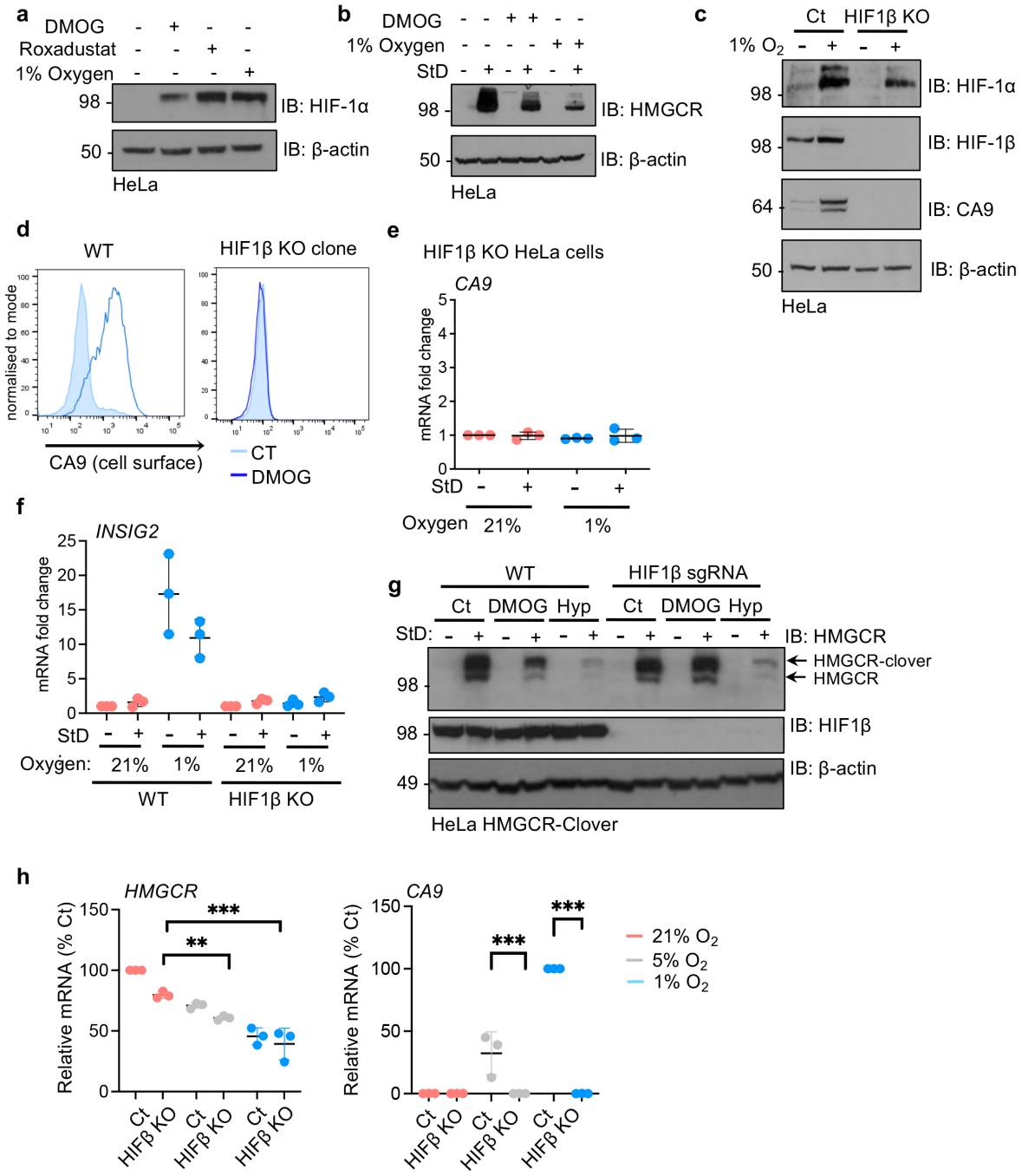
hr. *Immunoblots representative of 2 independent experiments.* (**e**) PCR confirmation of Clover knock-in at the C-terminus of SREBP2. Genomic DNA was extracted from parental HeLa cells and clonal SREBP2-Clover knock-in cells, and amplified using the primers indicated. The presence of the 2179bp fragment confirmed incorporation of Clover to the C-terminus in the knock-in cells (KI), and was not observed in the parental cells that only encoded WT SREBP2. (**f**) SREBP2 processing in HeLa (WT) or HeLa SREBP2-Clover cells treated with or without StD for 40 hr. Cells were exposed to 1% oxygen for the last 16 hr as indicated. SREBP2 antibody recognises the N-terminal region, whereas the GFP antibody detects the Clover tag. *C-SRE,* cleaved C-terminal region following processing in StD. (**g**) Time-course analysis of SREBP2 in HeLa SREBP2-Clover knock-in cells in 1% oxygen for up to 24 hr. *Immunoblots representative of 3 independent experiments.*

Extended Data Fig.2: Hypoxia overrides the canonical sterol-sensing response.



(**a**) mRNA expression of SREBP2 target genes (*HMGCR and HMGCS1*), the SREBP1 and 2 target *LDLR*, and the HIF-1 target gene *CA9* in HeLa cells treated with or without sterol depletion (StD) in 21% and 1% oxygen for 42 hr. *n=3 biological repeats, mean ± SD. *P<0.05, ***P<0.001 Two-way ANOVA*. (**b**) Time-course of mRNA expression of SREBP2 target genes (*HMGCR and HMGCS1*), the SREBP1 and 2 target *LDLR*, and the HIF-1 target gene *CA9* in HeLa cells incubated in StD conditions and 1% oxygen for up to 24 hr. *n=3 biological repeats, mean ± SD.* (**c, d**) Endogenous HMGCR levels in HepG2 cells (**c**) and HEK293T cells (**d**) following incubation in 21% (Ct), 1 mM DMOG treatment, or 1% oxygen (Hyp), with or without StD, for 24 hr. *Immunoblots representative of 2 independent experiments.*

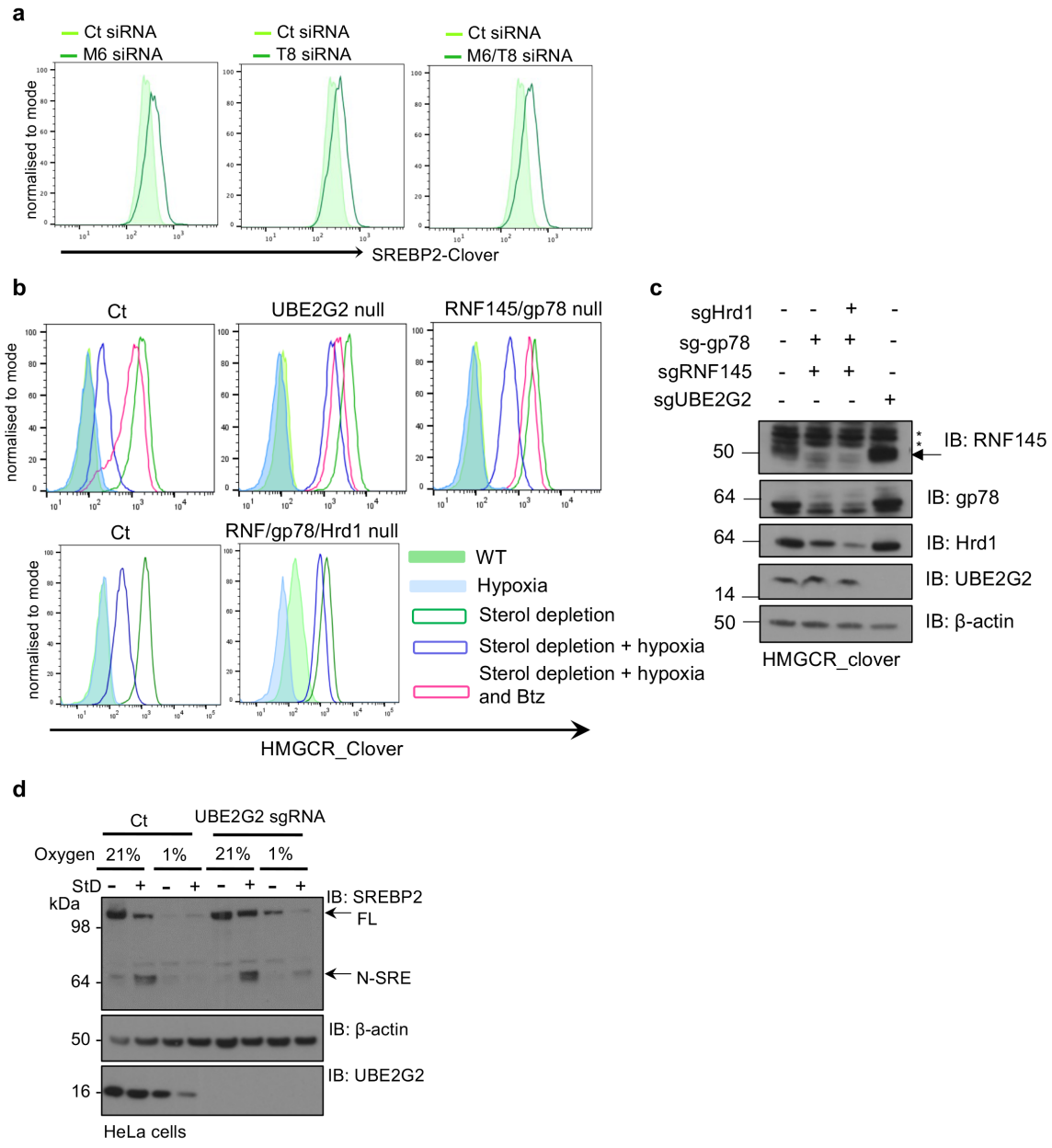
Extended Data Fig.3: ***Oxygen-mediated regulation of cholesterol synthesis is independent of HIFs***



(**a**) HIF-1α levels in HeLa cells following treatment with the 2-OG dependent dioxygenase inhibitor, DMOG 1 mM, the PHD inhibitor, Roxadustat 100μM, or incubation in 1% oxygen for 24 hr. (**b**) Endogenous HMGCR levels in HeLa cells with or without StD (40 hr) following incubation in 21%, 1 mM DMOG treatment, or 1% oxygen for the last 16 hr. (**c, d**) Confirmation of HIF1β deficient clone, with HIF-1α, HIF1β and CA9 levels after 24 hr exposure to 1% oxygen (**c**), or cell surface CA9 levels after 16 hr treatment with 1 mM DMOG (**d**). (**e**) mRNA levels of *CA9* (HIF-1 target gene) in HIF1β

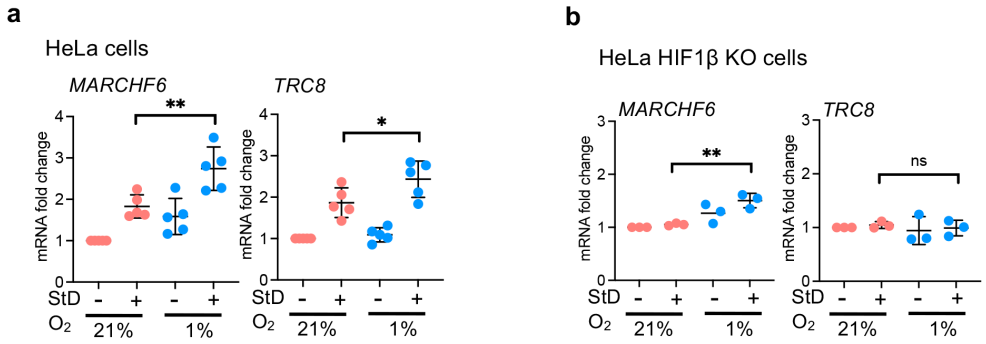
clonal KO HeLa cells following sterol depletion, with or without incubation in 1% oxygen. *n=3 biological repeats, mean ± SD.* (**f**) mRNA levels of *INSIG2* in HIF1β clonal KO or control HeLa cells with or without sterol depletion for 24 hr, followed by incubation in 21% or 1% oxygen for 16 hr. *n=3 biological repeats, mean ± SD.* (**g**) HMGCR levels in control HeLa cells or following depletion of HIF1β (HIF1β sgRNA) with or without sterol depletion for 24 hr, followed by incubation in 21% or 1% oxygen, or following 1 mM DMOG treatment for 16 hr. HMGCR levels measured by immunoblot. *Representative of 3 independent experiments.* (**h**) mRNA levels of *HMGCR* (left), and *CA9* (right) in sterol depleted (24 hr) HIF1β KO or control HeLa cells following, incubated in 21%, 5% or 1% oxygen (16 hr). *n=3 biological repeats, mean ± SD. **p<0.01, ***p<0.001 Two-way ANOVA*.

Extended Data Fig.4: The E3 ligases RNF145, GP78, Hrd1, and E2 enzyme UBE2G2 degrade HMGCR in hypoxia



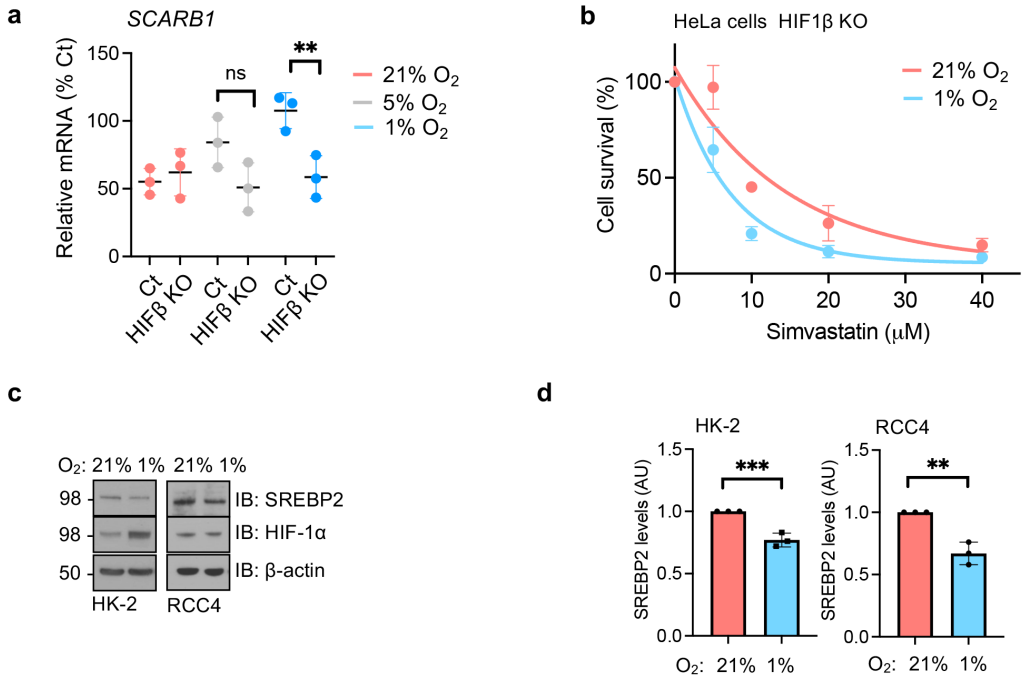
(**a**) SREBP2-Clover knock-in levels, measured by flow cytometry, in HeLa cells following siRNA-mediated depletion of MARCHF6 (M6), TRC8 (T8), or combined M6/T8 depletion. (**b, c**) HeLa HMGCR-Clover control, UBE2G2 deficient, RNF145/gp78 deficient, or RNF145/gp78/HRD1 deficient cells were generated by sgRNA. Cells were placed under StD conditions for 42 hr, with or without hypoxia 1% oxygen for the final 18 hr. StD cells were also treated with 20nM bortezomib (Btz) for the final 18 hr. HMGCR levels were analysed by flow cytometry (**b**) or immunoblot (**c**). (**d**) SREBP2 levels in control or UBE2G2 deficient (sgRNA) HeLa cells followed StD with or without incubation in 1% oxygen as described.

Extended Data Fig.5: Transcriptional regulation of *MARCHF6* and *TRC8*



(**a, b**) HeLa control (**a**) or HIF1β null clonal cells (**b**) were sterol depleted for 42 hr, with or without 1% oxygen for the final 18 hr and *HMGCR, MARCHF6,* and *HMGCR* mRNA levels measured by qPCR. *n=3 biological repeats, mean ± SD. *P<0.05, **P<0.01 Two-way ANOVA*.

Extended Data Fig.6: Hypoxia promotes cholesterol auxotrophy in tumour cells by supressing cholesterol synthesis.



(**a**) mRNA levels of *SCARB1* in HIF1β KO or control HeLa cells with or without StD for 24 hr, followed by incubation in 21% or 1% oxygen for 16 hr. *n=3 biological repeats, mean ± SD. **p<0.01, Two-way ANOVA*. (**b**) HeLa HIF1β null cells were incubated in 21% or 1% oxygen for 48 hr with simvastatin as indicated. Cell viability was measured by Cytotox staining at 48 hr. *n=3 biological repeats, mean ± SD*. (**c, d**) SREBP2 levels in HK2 or RCC4 cells incubated in 21% or 1% oxygen for 24 hr. SREBP2 levels were measured by immunoblot (**c**) and quantified using ImageJ (**d**). *n=3 biological repeats, mean ± SD. **p<0.01, ***p<0.001 Two-way ANOVA*.

Supplementary Tables

Supplementary Table 1: Reagents

Reagents	Source	Identifier
Antibodies		
β-actin	Sigma Aldrich	A2228
HA	Covance	16B12
HA	Roche	11867423001
UBE2G2	Santa Cruz Biotechnology	sc-100613
HIF1α	BD Transduction Laboratories	619959
HIF2α	Cell Signaling	7096S
HIF1β	Cell Signaling	5537S
CA9 (Carbonic Anhydrase 9) M75	Absolute Antibodies	AB00414-1
HMG CoA Reductase	Santa Cruz Biotechnology	sc-271595
Hrd1	Abgent	AP2184a
RNF145	ProteinTech	24524-1-AP
gp78	ProteinTech	16675-1-AP
Ubiquitin	Cell Signaling	3936
SREBP2	R&D Systems	AF7119
Anti-goat HRP	Jackson ImmunoResearch	115-35-146
Anti-mouse HRP	Jackson ImmunoResearch	115-035-045
Anti-rabbit HRP	Jackson ImmunoResearch	111-035-045
Anti-rat HRP	Jackson ImmunoResearch	112-35-003
Alexa Fluor Anti-mouse 647	Invirtogen	A11036
Alexa Fluor Anti-mouse 488	Invitrogen	A31553
NADK	Cell Signaling	55948
Chemicals, peptides, and recombinant proteins		
FuGENE	Promega	E5911
Trans-IT 293	Mirus	MIR-2705
Trans-IT HeLa Monster	Mirus	MIR-2900
Lipofectamine RNAiMAX	Invitrogen	13778150
MG132	Sigma Aldrich	M8699
Bortezomib (Velcade)	Gift from Alfred Goldberg	N/A
Lipoprotein-deficient serum (LPDS)	Biosera	FB-1001L/10

Digitonin	Calbiochem	300410
Mevastatin	Sigma Aldrich	M2537
Simvastatin	Sigma Aldrich	S6196
DMOG	Sigma Aldrich	D3695
Roxadustat (FG-4592)	Selleckchem	S1007
Blasticidin	Cambridge Bioscience	14499
Hygromycin	Cambridge Bioscience	2589-1000
Puromycin	Cambridge Bioscience	P097
Cycloheximide	Sigma Aldrich	01810
Hoechst	BioTechne	5117/50
Protoscript II reverse transcriptase	New England Biolabs	M0368
Power SYBR Green Master Mix	Thermo Fisher Scientific	4368577
cOmplete Protease Inhibitor Cocktail	Roche	11697498001
Denerase	c-Lecta	20804
Benzonase	Sigma Aldrich	E1014
NuPAGE 4 to 12%, Bis-Tris gel	Thermo Fisher	#NP0322BOX
Methanol	Sigma Aldrich	#34860
[C^{13}] glucose	Sigma Aldrich	389374
DMEM	Sigma Aldrich	D6429
RPMI-1640	Sigma Aldrich	R8758
MES Running Buffer	Thermo Fischer	NP0002
ECL Supersignal	Thermo Fischer	A38556
Supersignal West Pico PLUS	Thermo Fischer	34577
PureLink RNA mini kit	Thermo Fisher Scientific	#12183025
Gentra Puregene Core kit	Qiagen	#158722
NADP+/NAPDH Assay Kit	Abcam	Ab65349

Supplementary Table 2: Plasmids

Plasmids		
Bassik lab Human CRISPR-Cas9 deletion library	Morgens et al[1]	Addgene #101926-34
Toronto KnockOut (TKO) CRISPR Library- Version 3	Mair et al[2]	Addgene #90294
pKLV-U6gRNA-EF(BbsI) -PGKpuro2ABFP	Ochiai et al[3]	Addgene #62348
LentiCRISPRv2	Sanjana et al[4]	Addgene #52961
Lenti-Cas9-T2A-Puro	Hart et al[5]	Addgene #73310
LentiCas9-Blast	Sanjana et al[4]	Addgene #52962
pHRSIN-FLAG-NLS-Cas9-NLS-pGK-Hygro	Ortmann et al[6]	N/A
pHRSIN-pSFFV-HA-pPGK-Puro	Stefanovic-Barrett et al[7]	N/A
pMD.G (Lentiviral VSVG)	Burr et al[8]	N/A
pMD.GagPol (Lentiviral Gag/Pol)	Burr et al[8]	N/A
SREBF2 image clone 6169568	Source Bioscience	IRATp970B0781D
HA-SREBP2	This paper	N/A

Supplementary Table 3: sgRNA sequences

Gene	Sequence (5' → 3')
HIF1β	CAGTCCTCCGTCTCCTCACC
HRD1	GGTGTTCTTTGGGCAACTGA
MARCH6	TATCATCCTTGTGTATGTAC
TRC8	GCACGATGCAGAACCGGCTT
UBE2G2	CATGGGCTACGAGAGCAGCG
RNF145	TGTTAAATGTGGCCCTG
gp78	GTTAGCTGGTCCGGCTCGCC

Supplementary Table 4: Primers for generation of SREBP2 plasmids and knock-in

Primer name	Sequence (5' → 3')
HA_SREBP2_FL_fwd	GCTTATCCTTACGACGTGCCTGACTACGCCGGATCC GACGACAGCGGCGAGCTGGGTGG
FL_SREBP2 _notag_rev	GCCTGCAGGTCGACTCTAGAGTCGC TCAGGAGGCGGCAATGGCAGTG
SREBP2 5' arm for	CAGCGGCCGCGGTGCCAGGGCGTGCC CTTGGGCTCCCCGGGCGCGACTAG
SREBP2 5' arm rev	GCTCACCATGGATCCACCAGATCCGCCACCA GATCCGCCACCAGATCCGCCCGATCGG
SREBP2 3' arm for	CCAGCTGGGGCTCGAGATAA CTTCGTATAGCATACATTATACG
SREBP2 3' arm rev	GAGATCCACTAGAGTGTGGCGGCC GCATTCTTATAATCAGCATCATGATGTG
SREBP2 3' arm rev	GAGATCCACTAGAGTGTGGCGGCC GCATTCTTATAATCAGCATCATGATGTG
SREBP2_0_0 sgRNA (knock-in)	GGCTGAGCCTGGTGGTCAGG
SREBP2_1_0 sgRNA (knock-in)	GTGGGCTGAGCCTGGTGGTC
SREBP2_3_0 sgRNA (knock-in)	GTGGAGGGGTGGGCTGAGCC
SREBP2_4_0 sgRNA (knock-in)	GAAATCGAGAGAGAGGTGGA
SPfor	CACCTCTCGCCTCTCTGAGAATG
SPrev	GAGTGGGAAGGAACAGGACAATTA

Supplementary Table 5: Primers used in the mutagenesis screens

HMGCR_Clover Bassik Library screen Primer name	Sequence (5' → 3')
Outer_Fwd	AGGCTTGGATTTCTATAACTTCGTATAGCATACATTATAC
Outer_Rev	ACATGCATGGCGGTAATACGGTTATC
P5_inner_Fwd	AATGATACGGCGACCACCGAGATCTACACTCTCTTGTGGAAAGGACGAAACACCG
P5_index_inner_Rev	CAAGCAGAAGACGGCATACGAGAT*NNNNNNN*GTGACTGGAGTTCAGAC GTGTGCTCTTCCGATCCGACTCGGTGCCACTTTTTC
Illumina sequencing primer	AGACTATAAGTATCCCTTGGAGAACCACCTTGTTGG
SREBP2_Clover TKOv3 Primer name	Sequence (5' → 3')
Outer_Fwd	GAGGGCCTATTTCCCATGATTC
Outer_Rev	CAAACCCAGGGCTGCCTTGGAA

Inner_Fwd	AATGATACGGCGACCACCGAGATCTACACTCTCTTGTGGAAAGGACGAGGTACCG
PCR2_inner_Rev	CAAGCAGAAGACGGCATACGAGAT*NNNNNN*GTGACTGGAGTTCAGACGTGTGCTC TTCCGATCTATTTTAACTTGCTATTTCTAGCTCTAAAAC
Illumina sequencing primer	ACACTCTCTTGTGGAAAGGACGAGGTACCG

Supplementary Table 6: Quantitative PCR primers

Primer name	Sequence (5’ → 3’)
β-actin fw	CTGGGAGTGGGTGGAGGC
β-actin rv	TCAACTGGTCTCAAGTCAGTG
CA9 fw	GCCGCCTTTCTGGAGGA
CA9 rv	TCTTCCAAGCGAGACAGCAA
HMGCR fw	CGTGGAATGGCAATTTTAGGTCC
HMGCR rv	ATTTCAAGCTGACGTACCCCT
HMGCS1 fw	GATGTGGGAATTGTTGCCCTT
HMGCS1 rv	ATTGTCTCTGTTCCAACTTCCAG
INSIG2 fw	TAATGCGGTGTGTAGCAGTCT
INSIG2 rv	GTCCAATGGATAGTGCAGCCA
LDLR fw	ACCAACGAATGCTTGGACAAC
LDLR rv	ACAGGCACTCGTAGCCGAT
SREBP2 fw	AACGGTCATTCACCCAGGTC
SREBP2 rv	GGCTGAAGAATAGGAGTTGCC
SCARB1 fw	CCTATCCCCTTCTATCTCTCCG
SCARB1 rv	GGATGTTGGGCATGACGATGT
MARCHF6 fw	CACCTGAGAAACCGCTTTA
MARCHF6 rv	CCGTGAAGGCATATCTGGAGA
TRC8 fw	ATCGACGCCATCTTCAACTCC
TRC8 rv	AAGCTGACGTGTTGTAGGCAC

References.

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