HIF1α-mediated RelB/APOBEC3B downregulation allows Hepatitis B Virus persistence

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Supplementary Material and Methods

Immunoblotting

Cells for western blotting analysis were lysed in RIPA buffer (Cell Signalling Technologies), supplemented with Complete and PhosSTOP (both Roche). Protein concentration was measured by bicinchoninic acid (BCA) assay. The same amount of protein was then separated on an 8% SDS-PAGE, followed by transfer on a 0.2 µm PVDF membrane. Membranes were blocked in 5% non-fat dry milk, incubated over-night at 4°C with primary antibodies and one hour at room temperature with secondary antibodies. All used antibodies are listed in the ***Table S1*.**

Immunohistochemistry and *in-situ* hybridization

2 µM sections of human liver specimen were stained with antibodies against HIF1α or HBcAg using the BOND-MAX Automated IHC/ISH Stainer (Leica). All reagents were purchased from Leica. Secondary antibody-polymer (Leica) coupled to horseradish peroxidase was used for detection. All used antibodies are listed in the ***Table S1*.**

5 µM sections of human liver specimen were used for APOBEC3B *in-situ* hybridization. The probe was purchased from ACD, as well as all buffers and other reagents. *In-situ* hybridization was performed strictly according to the manufacturer's instructions.

Double immunohistochemistry and in-situ hybridization was conducted by first performing *in situ* hybridization and then immunohistochemistry. Shortly, for *in situ* hybridization manufacturer's instructions (ACD) were followed closely with one exception: suggested incubation time with protease of 30 minutes was reduced to 15 minutes to insure good protein detection by the subsequent immunohistochemistry. Immunohistochemistry was performed as described above and

signal was detected using Opal chemistry (Akoya Biosciences). Substrate that emits in the FITC channel was chosen to detect HIF1α (i.e. Opal520). Nuclei were stained with DAPI for 10 min.

Immunocytochemistry

Differentiated HepaRG were seeded into 4-chamber slides (ThermoFisher). Experiments were stopped by removing culture medium, washing once with PBS and then incubating cells with 4% paraformaldehyde (Carl Roth) for 15 minutes at room temperature. Cells were then washed with PBS twice, before permeabilisation with 0.2% Triton X-100 (Sigma Aldrich) for 10 minutes at room temperature. Cells were washed once in PBS, incubated 5 minutes in 70% ethanol and washed extensively in PBS again, before submitting to automated staining using the BOND-MAX Automated IHC/ISH Stainer (Leica). Secondary antibody-polymer (Leica) coupled to alkaline phosphatase was used for detection. All used antibodies are listed in the ***Table S1*****.**

RT-qPCR

RNAs were either prepared using the Monarch® Total RNA Miniprep Kit (New England BioLabs), according to the manufacturer's instructions. Eluted RNA was measured with a nanodrop spectrometer (ThermoFisher). For mRNA, 500 ng total RNA was reverse transcribed using the Quantitect Kit (Qiagen) according to the manufacturer's protocol. The cDNA was diluted 1:10. For the qPCR reaction, 3 µL cDNA was mixed with 6 µL FS Universal SYBR Green MasterRox (Roche), 0.12 µL reverse+forward primer mix, and 2.88 µL water (amounts given for one reaction). Samples were analysed in a QuantStudio 5 light cycler (ThermoFisher). Data was

analysed using the QuantStudio software (ThermoFisher) and the ΔΔCT method. RHOT2, PRNP, or HPRT were used as housekeeping genes for relative quantification.

All used primers are listed in ***Table S2***.

cccDNA clean-up and quantification

HBV covalently closed, circular DNA was extracted from infected dHepaRG using either the QiaAmp DNA Mini Kit (Qiagen; column based, non-selective for cccDNA over rcDNA) or the MasterPure™ Complete DNA and RNA Purification Kit (Epicentre; precipitation based, selective for cccDNA over rcDNA). In the case of column-based extraction, before qPCR, 300-500 ng total DNA were subjected to T5 Exonuclease digestion at 37°C for 30 minutes, followed by heat inactivation of the enzyme at 70°C for 20 minutes. T5 exonuclease digested and diluted samples were used for cccDNA quantification, undigested samples were used for gDNA quantification. cccDNA levels were normalized to HepaRG gDNA. In the case of precipitation-based extraction, DNA quantity was determined by Nanodrop measurement, and 40 ng DNA was used per qPCR reaction. qPCR was performed using the Luna Universal Probe qPCR Master Mix (New England Biolabs) and custom made, FAM-labelled and BHQ-quenched probes (Sigma). All used primers are listed in ***Table S2***.

Southern blot

cccDNA was extracted from HBV-infected dHepaRG using the KCl protein precipitation method, separated through 0.8 % agarose gel, blotted onto nylon membrane and hybridized with ^{32}P HBV-DNA probe, as previously described (1,2).

Immunoprecipitation

For chromatin immune-precipitation, cells were fixed and nucleic acids and proteins were cross-linked and processed as previously described (3). Immunoprecipitated DNA was measured with specific primers on a SYBR-based qPCR reaction on a QuantStudio 5 real time PCR instrument (ThermoFisher). All primers used are listed in ***Table S2.***

For co-immuno-precipitation, cells were washed with ice cold PBS, lysed with cell lysis buffer (Cell signalling; 9803S), and kept on ice at all time. Samples were pre-cleared with A/G beads for 1h at 4°C. Beads were discarded and the samples were incubated o/n at 4°C with the indicated antibody (**Table S1**). The next day, samples were incubated 2 hours with A/G beads at 4°C. Beads were washed afterwards 5 times, then beads with bound proteins were boiled in 4x loading dye containing 5% beta-mercapto ethanol (Carl Roth) at 95°C for 5 minutes. Before loading on an SDS-PAGE, beads were pelleted by centrifugation at 13.000 rpm for one minute. Same volumes of IPs were loaded.

Mass spectrometry

For mass spectrometry analysis, cultured cells were lysed in RIPA buffer, complemented with Complete and PhosStop (both Roche). Samples were cleared by centrifugation and protein concentrations were measured by BCA assay as for immunoblotting. Protein amounts were adjusted to be the same and then submitted to the proteomics core facility of the DKFZ. Proteins have been loaded on SDS-PAGE-gel, which ran only a short distance of 0.5 cm. After Commassie staining the total sample was cut out unfractionated and used for subsequent Trypsin digestion according to a slightly modified protocol described by Shevchenko et al. (4) on a DigestPro MSi

robotic system (INTAVIS Bioanalytical Instruments AG). Peptides have been loaded on a cartridge trap column, packed with Acclaim PepMap300 C18, 5µm, 300Å wide pore (Thermo Fisher Scientific) and separated in a three step, 180 min gradient from 3% to 40% ACN on a nanoEase MZ Peptide analytical column (300Å, 1.7 µm, 75 µm x 200 mm, Waters) carried out on a UltiMate 3000 UHPLC system. Eluting peptides have been analysed online by a coupled Q-Exactive-HF-X mass spectrometer (Thermo Fisher Scientific) running in data depend acquisition mode where one full scan at 120 k resolution (375-1500 m/z, maxIT 54 ms) was followed by up to 35 MSMS scans at 15 k resolution of eluting peptides at an isolation window of 1.6 m/z and a collision energy of 27 NCE. Ion injection time was set to a maximum of 22 ms or 1e5 ions (AGC target). Unassigned and singly charged peptides have been excluded from fragmentation and dynamic exclusion was set to 60 sec to prevent oversampling of same peptides. Data analysis was carried out by MaxQuant (version 1.6.3.3) (5) using an organism specific database extracted from Uniprot.org under default settings. Identification FDR cutoffs were 0.01 on peptide level and 0.01 on protein level. Match between runs option was enabled to transfer peptide identifications across Raw files based on accurate retention time and m/z. Quantification was done using a label free quantification approach based on the MaxLFQ algorithm (6). A minimum of 2 quantified peptides per protein was required for protein quantification. Data have been further processed by in-house compiled R-scripts to plot and filter data and the Perseus software package (version 1.6.7.0) using default settings for further imputation of missing values and statistical analysis (7). Proteins with three non-missing intensities in at least one condition are retained for the analysis. The remaining missing values are imputed either by half of the minimum measured intensity and then log2 transformed, or after log2 transformation via the regularized expectation maximization (REM) algorithm by Schneider *et al.* (8), regarded as superior in a variety of settings explored in (9). A

moderated t statistics (10) is obtained for each contrast of interest, and for each imputed data-set, with the R package Limma (11). The resulting p-values for each contrast are adjusted with the Benjamini-Hochberg (12) procedure to control for the false discovery rate (FDR). To enhance the robustness of the analysis, only proteins significant or non-significant at level 5% in both analyses, i.e. under each imputation approach, and the corresponding REM imputed analysis values, are retained for each contrast for plotting and pathway analysis purposes. Self-contained pathway analyses (KEGG annotation) are performed via the Limma function mroast, where p-values for each pathway are obtained via the rotation test method described in (13). The FDR is again controlled via the Benjamini-Hochberg adjustment. The regularized expectation maximization algorithm is run in MatLab v. R2019b, with code available at https://github.com/tapios/RegEM. The remaining analyses are performed in R, v. 3.6.1. Raw data and additional information is accessible via http://www.ebi.ac.uk/pride, project accession: PXD022925.

Flow cytometry

Cells cultured under normoxia or hypoxia were detached with Versene (Lonza), then collected by centrifugation and immediately fixed with 4% paraformaldehyde for 5 minutes at room temperature. After washing cells with PBS, incubation with the primary antibody and labelling of the primary antibody with a secondary, Alexa-647-linked, antibody was both done for 30 minutes at room temperature. Measurement was conducted on a Fortessa flow cytometer (BD). All antibodies are listed in ***Table S1.***

Viability assays

Cytotoxicity was assessed by Sulforhodamin B as previously described (14).

Mice

A total of 6 C57BL6/J mice (3 males and 3 females), 11 to 12 weeks old, were injected with 300 mg/kg of DMOG in i.p. 6h after injection, mice were sacrificed and liver were harvested for mRNA and protein extraction. Experiment on mice were approved by the Ethics Committee of ULiege (#1939).

Cytoplasm/nucleus extraction

The cells were washed with cold-PBS, scraped and centrifuged at 2000 rpm for 5 minutes at 4°C. Cytosolic fractions were obtained by lysis of the cell pellet in 1 ml Buffer (Hepes 10 mM pH7.9, KCl 10 mM, MgCl2 2 mM, EDTA 0.1 mM, NP40 0.02%, DTT 1 mM) supplemented with Complete and PhosSTOP. After 10 minutes on ice, cells were centrifuged and cytosolic fractions were collected. Nuclear pellets were washed (Hepes 10 mM pH7.9, KCl 20 mM, MgCl2 2 mM, EDTA 0.1 mM) then lysed on ice during 30 minutes in nuclear Buffer Lysis (Hepes 20 mM pH7.9, MgCl2 1.5 mM, EDTA 0.2 mM, NaCl 0.42 M, Glycerol 25%, DTT 0.5 mM) supplemented with Complete and PhosSTOP. Debris were removed by centrifugation.

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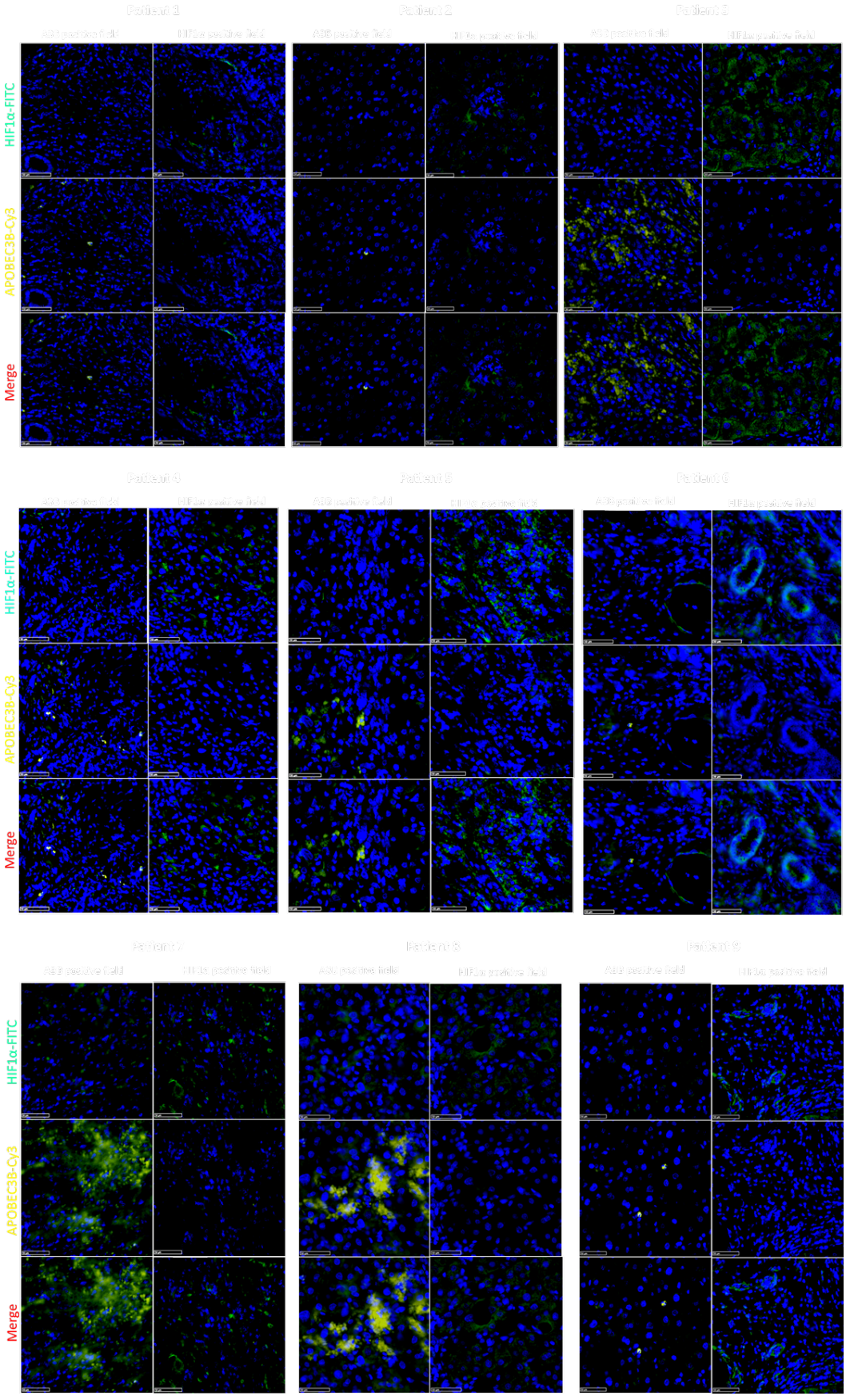
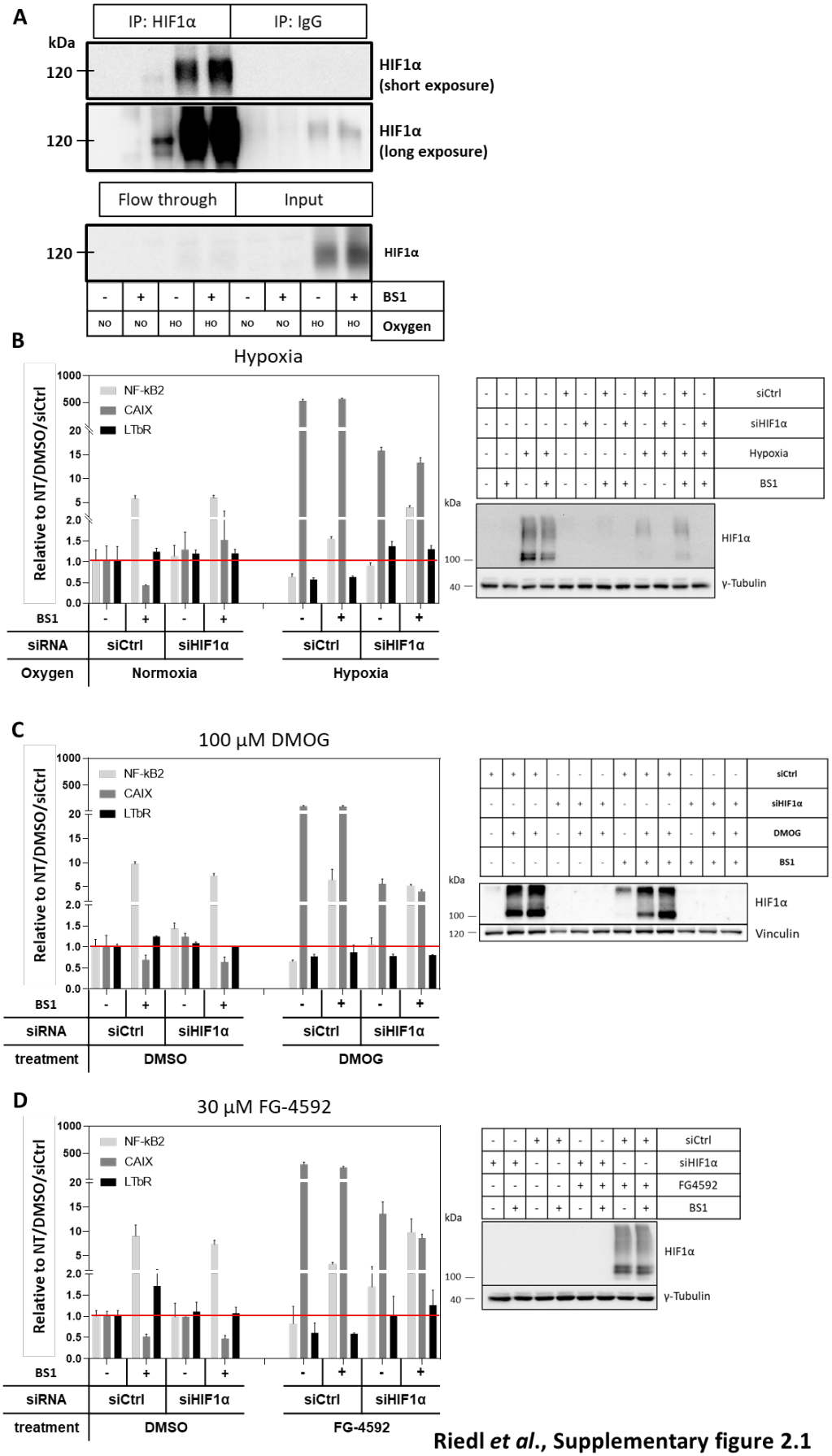
Supplementary figure 1 – In vivo, high HIF1α expression is associated with low APOBEC3B expression and vice versa. Paraffin sections of chronic HBV patients were stained for APOBEC3B mRNA by *in situ* hybridization (Cy3 in yellow, HIF1α by IHC (FITC in green), and nuclei were stained with DAPI (in blue). Photos were taken in one APOBEC3B high area and one HIF1α high area per patient.



Riedl *et al.*, Supplementary figure 1

Supplementary figure 2 – HIF1α stabilisation interferes with efficient NF-κB target gene induction upon BS1 treatment. (A) dHepaRG were incubated at 1% or 20% oxygen for 3 days and treated +/- 0.5 µg/mL of BS1. Proteins were immune-precipitated with the indicated antibody and analysed by immunoblotting. **(B)** dHepaRG were infected with HBV. 6 d.p.i., cells were transfected with either 10 nM HIF1α-targeting or control siRNAs. On the next day, cells were incubated under 1% (Hypoxia) or 20% (Normoxia) oxygen for 3 days, in presence or absence of 0.5 µg/mL BS1. Cells were transfected then again under normoxia, and incubated then again under 1% or 20% oxygen for 3 days, in presence or absence of 0.5 µg/mL BS1. Then, proteins and RNAs were extracted and analysed by immunoblotting with the indicated antibody and RT-qPCR, respectively. Bars represent the mean +/- SD of one experiment performed in quadruplicates. **(B)** dHepaRG were infected with HBV. At 10 and 13 d.p.i., cells were transfected with either 10 nM HIF1α-targeting or control siRNAs. One day after the second transfection, cells were treated or not with 0.5 µg/mL BS1, either under presence of 100 µM of DMOG or DMSO. 6 days post treatment start proteins and RNAs were extracted and analysed by immunoblotting with the indicated antibody and RT-qPCR, respectively. Bars represent the mean +/- SD of three independent experiments performed in triplicates. **(D)** dHepaRG were infected with HBV. At 10 and 13 d.p.i., cells were transfected either 10 nM HIF1α-targeting or control siRNAs. One day after the second transfection, cells were treated or not with 0.5 µg/mL BS1, either under presence of 30 µM of FG-4592 or DMSO. 6 days after the treatment start, proteins and RNAs were extracted and analysed by immunoblotting with the indicated antibody and RT-qPCR, respectively. Bars represent the mean +/- SD of three independent experiments performed in triplicates. (E) dHepaRG were infected with HBV. At 7 d.p.i., cells were treated or not with 0.5 µg/mL BS1, either with

DMOG 100 µM or DMSO. 6 days post treatment start DNA was extracted and analysed by qPCR. Bars represent the mean +/- SD of three independent experiments performed in triplicates.



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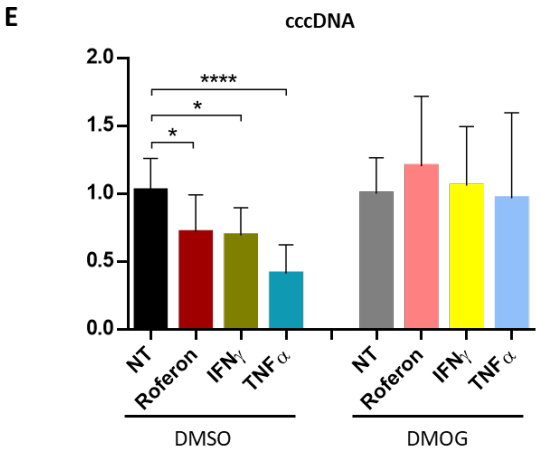

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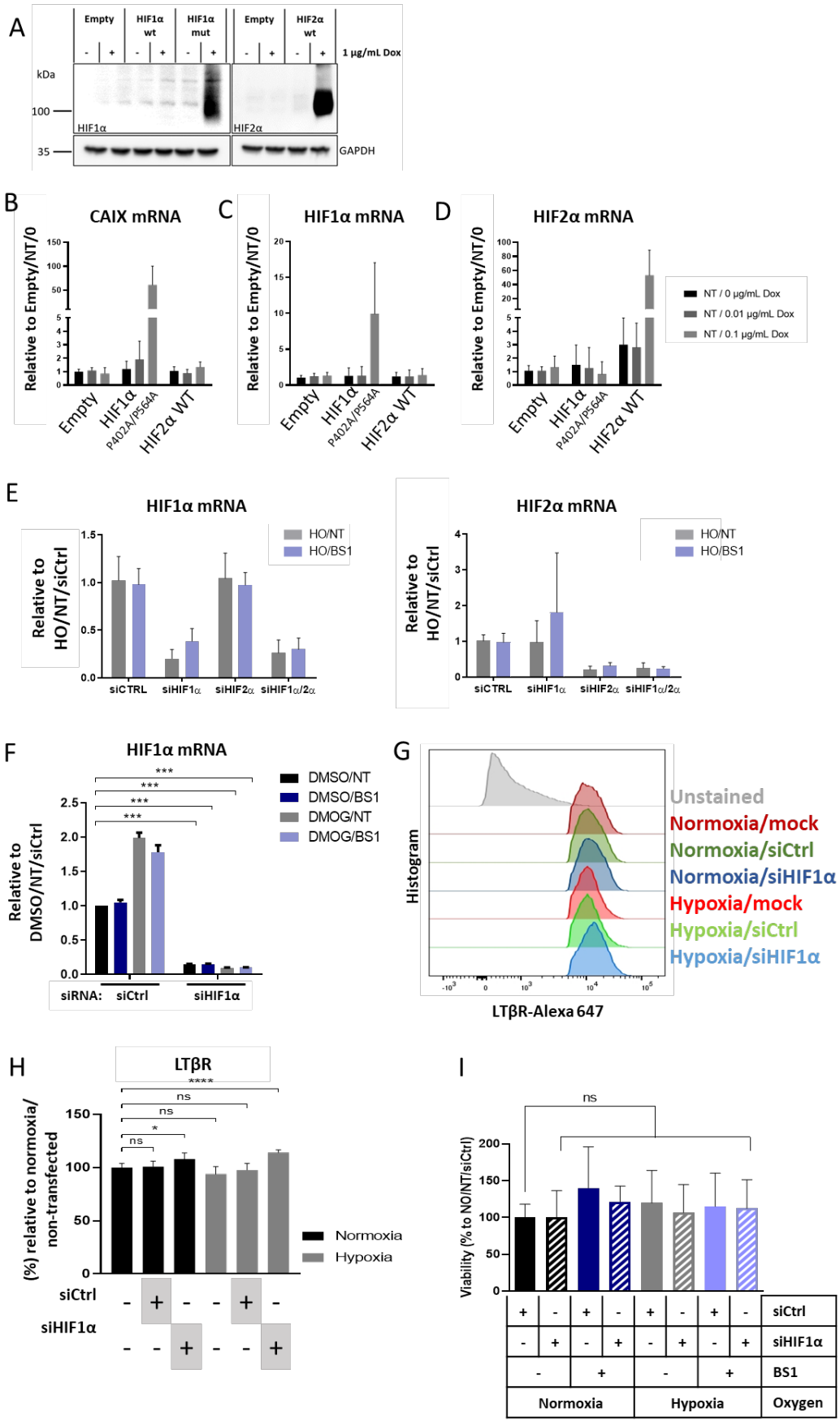
Supplementary figure 3 – HIF1α stabilisation, but not HIF2α, induces impairment of A3B induction. (A) HepaRG expressing inducible wild-type HIF1α, a degradation resistant mutated HIF1α (P402A/P564A, "mut") or wild-type HIF2α were exposed to 1 µg/mL Doxycycline for 3 days. Proteins were extracted and analysed via immunoblotting for the indicated proteins. **(B-D)** dHepaRG were treated with increasing doses of doxycycline as indicated. mRNAs were extracted and **(B)** CAIX expression, **(C)** HIF1α expression and **(D)** HIF2α were analysed by RT-qPCR. Bars represent the mean +/- SD of four independent experiments performed in triplicates. **(E)** dHepaRG were transfected with 10 nM of either HIF1α-targeting, HIF2α-targeting, or both siRNAs or control siRNAs. The next day cells were treated +/- 0.5 µg/mL of BS1 under 1% oxygen. mRNAs were extracted and analysed by RT-qPCR for the indicated genes. The bars represent the mean +/- SD of three independent experiments performed in triplicates. **(F)** dHepaRG were transfected with either 10 nM HIF1α-targeting or control siRNAs. One day after the second transfection, cells were treated or not with 0.5 µg/mL BS1, either under presence of 100 µM of DMOG or DMSO. 24h post treatment start RNAs were extracted and analysed by RT-qPCR. Bars represent the mean +/- SD of three independent experiments performed in triplicates. **(G-H)** dHepaRG were transfected with either 10 nM HIF1α-targeting or control siRNAs or left untransfected. From the following day on, cells were incubated under 1% (Hypoxia) or 20% oxygen (Normoxia) for 3 days. Then, cells were transfected under hypoxia or normoxia, and cultured for 3 more days. Cells were then detached, fixed with 4% paraformaldehyde, stained for the lymphotoxin beta receptor and analysed by flow cytometry. **(G)** Histograms of representative samples and **(H)** geometric mean of two independent experiments with 3 or 4 biological replicates. **(I)** dHepaRG were transfected with 10 nM HIF1α-targeting siRNAs or control siRNAs. One day post transfection, cells were cultured under 1% (Hypoxia: HO) or 20% (Normoxia: NO) oxygen for 6 days +/- 0.5 µg/mL of BS1.

297 Cytotoxicity was assessed by sulforhodamine B assay. Data represent the mean +/- SD of three
298 independent experiments performed in triplicates. Data were subjected **(F, H, I)** to one-way
299 ANOVA**.** *: $p < 0.05$; ***: $p < 0.001$; ****: $p < 0.0001$; ns: not significant.



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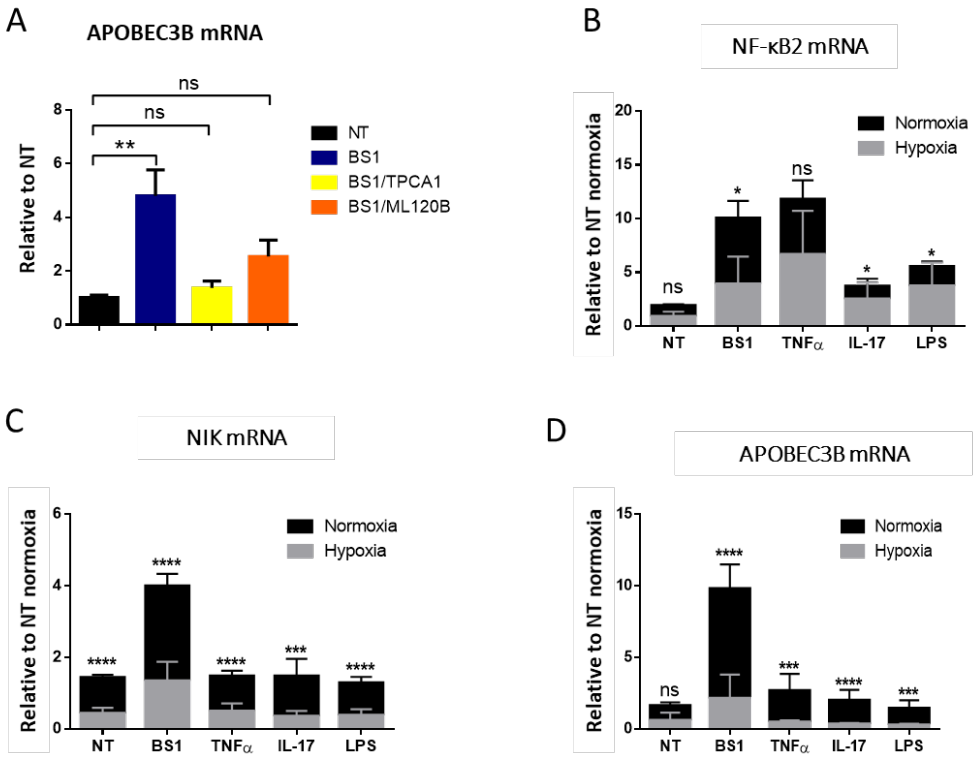
Riedl *et al.*, Supplementary figure 3

Supplementary figure 4: HIF1α stabilisation inhibits NF-κB target genes in an oxygen dependant manner. (A) dHepaRG were treated for 2h with 5µM TPCA1 or 10µM ML120B, before adding 0.5 µg/mL BS1. 3 days after treatment start, mRNA were extracted and analysed by RT-qPCR. Bars represent the mean +/-SD of three independent experiments performed in duplicates. **(B-D)** dHepaRG were cultured under 1% or 20% oxygen and treated with, either, 0.5 µg/mL BS1, 10 ng/mL of TNFα, 50 ng/mL of IL-17, or 100 ng/mL of LPS. 6 days post treatment, mRNAs were extracted and analysed by RT-qPCR. **(A-D)** Data represent the mean +/- SD of three independent experiments performed in duplicates or triplicates. Data were submitted to **(B-D)** unpaired student's t-test or **(A)** one-way ANOVA. *: $p < 0.05$; ***: $p < 0.001$; ****: $p < 0.0001$; ns: not significant.



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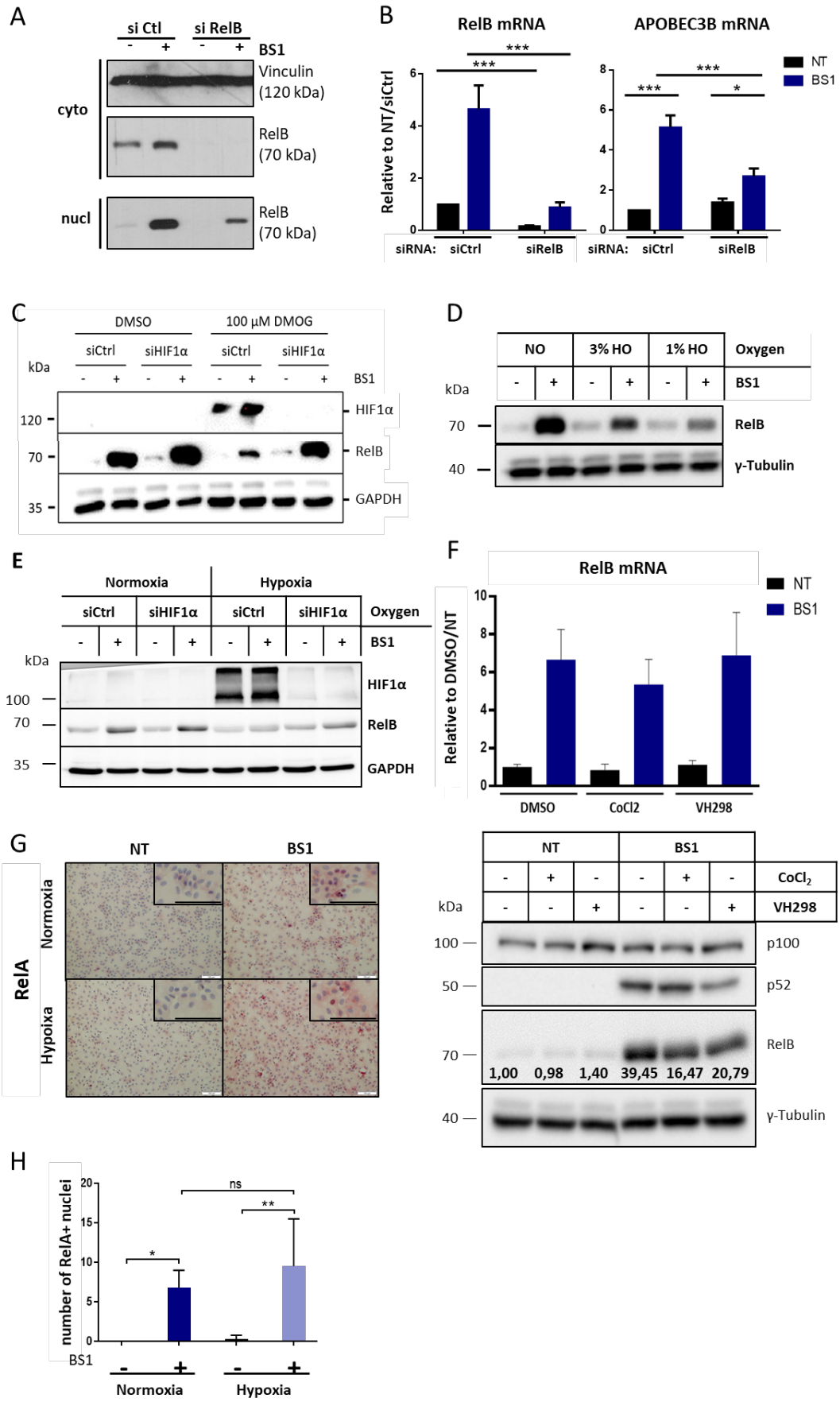
Supplementary figure 5 – HIF1α stabilisation modulates NF-κB signalling through RelB protein decrease. (A, B) dHepaRG were transfected with RelB-targeting or non-targeting control siRNAs, one day prior to treatment with 0.5 µg/mL BS1. After 48 hours, **(A)** proteins and **(B)** mRNAs were extracted and analysed by immunoblotting and RT-qPCR, respectively. Bars represent the mean +/-SD of three independent experiments performed in triplicates. **(C)** dHepaRG cells were transfected with either 10 nM HIF1α-targeting or control siRNAs. On the next day, cells were treated or not with 0.5 µg/mL BS1, either under presence of 100 µM of DMOG or DMSO. 3 days post treatment start proteins were extracted and analysed by immunoblotting. **(D)** Cells were cultured under 1%, 3%, or 20% oxygen and treated +/- 0.5 µg/mL of BS1. 3 days after treatment start proteins were extracted and analysed by immunoblotting. **(E)** dHepaRG were transfected with either 10 nM HIF1α-targeting or control siRNAs. On the next day, cells were incubated under 1% (Hypoxia) or 20% (Normoxia) oxygen for 3 days, in presence or absence of 0.5 µg/mL BS1. Proteins were extracted and analysed by immunoblotting with the indicated antibody. **(F)** dHepaRG were incubated for 3 days +/- 100 µM of CoCl2 or VH298 in presence or absence of 0.5 µg/mL of BS1. mRNAs and proteins were extracted and analysed by RT-qPCR and immunoblotting, respectively. Bars represents the mean +/- SD of four independent experiments performed in triplicates. **(G-H)** dHepaRG were seeded into 4-well chamber slides. 3 days after seeding, cells were cultured under either 1% (Hypoxia) or 20% (Normoxia) oxygen for 3 more days, either in the presence or absence of 0.5 µg/mL BS1. Cells were then prepared for immunocytochemistry and stained for RelA. **(G)** Representative pictures and **(H)** quantification of RelA positive nuclei**.** Data represent the mean of 5 pictures per condition form two experiments. Data were submitted to **(B, F, H)** one way ANOVA. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; ns: not significant.



Riedl *et al.*, Supplementary figure 5

Supplementary figure 6 – ARNT is not involved in HIF1α modulation of NF-κB signalling. **(A-C)** dHepaRG cells were transfected with either 10 nM ARNT-targeting or control siRNAs. Two days after transfection, the cells were treated for 24h with +/- 0.5 µg/mL of BS1 and 100 µM DMOG or the respective amount of DMSO. mRNAs were extracted and analysed by RT-qPCR. Bars represent the mean +/-SD of three experiments. Data were submitted to one way ANOVA. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; ns: not significant.

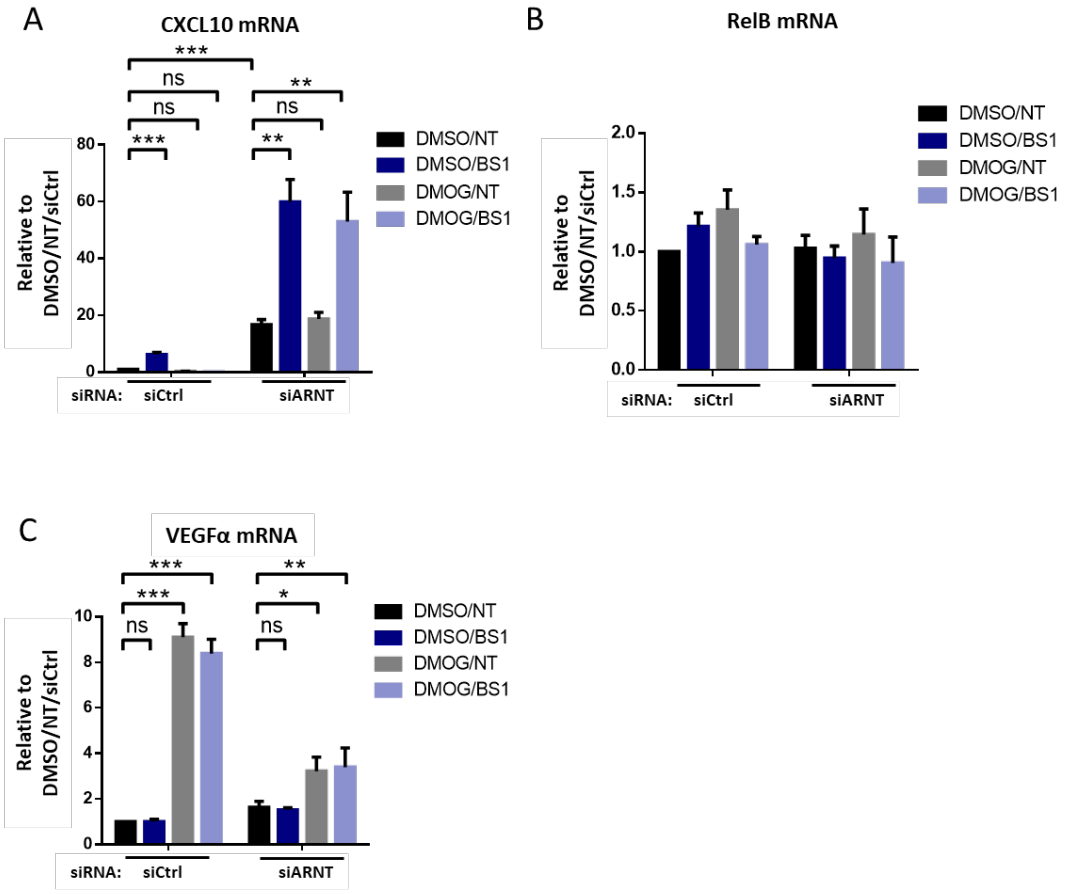


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Table S1. Antibodies and molecules

Type	Usage	Target	Supplier	Reference	Concentration
Antibodies	Western blot	GAPDH	Cell Signaling Technologies	2118	1:10.000
		HIF1α	Becton Dickinson	610959	1:500
		IKKβ	Cell Signaling Technologies	2678	1:1.000
		p100/p52	Merck Millipore	05-361	1:500
		p105/p50	Cell Signaling Technologies	3035	1:1.000
		pRelA	Cell Signaling Technologies	8242	1:1.000
		RelA	Cell Signaling Technologies	3033	1:1.000
		RelA	Cell Signaling Technologies	6956	1:1.000
		RelB	Cell Signaling Technologies	4922	1:5.000
		Vinculin	Sigma Aldrich	V9131	1:5.000
		p105/p50	Merck Millipore	06-886	1:2.000
		Tubulin	Sigma Aldrich	T6074	1:3.000
		Vinculin	Santa Cruz	sc-73614	1:2.000
		HIF-1a (D1S7W)	Cell Signaling Technologies	36169	1:1.000
		HIF1a	BD Biosciences	610958	1:1.000
		Histone H3	Abclonal	A2348	1:1.000
		HIF-1b/ARNT (D28F3) XP	Cell Signaling Technologies	5537	1:1.000
		Ikba	Cell Signaling Technologies	9242	1:2.000
		mouse IgG	Cell Signaling Technologies	7076	1:20.000
		rabbit IgG	Cell signaling Technologies	7074	1:20.000
	Flow cytometry	LTβR	Novus Biologicals	AF629	1:50
	ChIP	HIF1α	R and D systems	NB100-105	1:150
	Histology	HBcAg	DAKO	B0586	1:250
		HIF1α	R and D systems	NB100-105	1:40
		RelA	Novus Biologicals	NB100-2176	1:200
		RelB	Cell Signaling Technologies	4922	1:200
	ISH probe	BA-Hs-Apobec3B-1zz-st	ACD	#701271	
Treatments and inhibitors	HIF1α stabiliser	DMOG	Sigma Aldrich	D3695	100 µM
		CoCl2	Sigma Aldrich	C8661-25G	100 µM
		VH298	Biomol	Cay21133-5	100 µM
		FG-4592	Biomol	Cay15294	30 µM
	NF-κB inhibitor	ML120B	Sigma Aldrich	SML1174-5MG	10 µM
		TPCA-1	Sigma Aldrich	T1452	5 µM
	Immune stimulator	BS1	Biogen/Idec	N/A	0.5 µg/mL
		LPS (from S. minnesota R595)	InVivogen	tlrl-smlps	100 ng/mL
		TNFalpha	Novus Biologicals	210-TA	10 ng/mL
		Interleukin 17A	Novus Biologicals	7955-IL	50 ng/mL
		TNFalpha - anti-viral experiement	R and D systems	210-TA	800 IU
		Interferon gamma	R and D systems	285-IF	200 IU
		Roferon (PEG-interferon alpha)	Roche	Roferon	1000 IU

Table S2. Primers sequences

Type	Target	Forward	Reverse
qPCR	APOBEC3B	GACCCTTTGGTCCTTCGAC	GCACAGCCCCAGGAGAAG
	APOBEC3B-promoter	ACAGATAAAGACAGAGCAGCC	CCCAGGGCCTTATGAGTCATG
	CAIX	GTCTCGCTTGGAAGAAATCGC	CACAGGGCGGTGTAGTCAG
	CXCL10	TATTCCTGCAAGCCAATTTTGTC	TCTTGATGGCCTTCGATTCTG
	CXCL0	GTGGCATTCAAGGAGTACCTC	TGATGGCCTTCGATTCTGGATT
	GLUT1	TCTTCAGCCAGGGTCCACGTC	CGTAGGGACCACACAGTTGCTC
	HIF1α	TCATCAGTTGCCACTTCCACATA	CCATCATCTGTGAGAACCATAACAA
	HIF1α	ATAAAGTCTGCAACATGGAAGGT	ATTTGATGGGTGAGGAATGGGTT
	HIF1β/ARNT	CAAGCCATCTTTCCTCACTGATC	ACACCACCCGTCCAGTCTCA
	HIF2α	CAATGACAGCTGACAAGGAGAAG	CATGGGCCAGCTCATAGAAC
	HPRT	TCAGGCAGTATAATCCAAAGATGGT	AGTCTGGCTTATATCCAACACTTCG
	LTβR	GAGAACCAAGGTCTGGTGGA	GAGCAGAAAGAAGGCCAGTG
	NFκB2	GGGCCGAAAGACCTATCCC	CAGCTCCGAGCATTGCTTG
	NIK	AGCAGAAGGAACTCCCCAAA	ATCACGTCATTCAGGATCTCCC
	RelB	CATTGAGCGGAAGATTCAAC	GCAGCTCTGATGTGTTTGTG
	RelB	CAGCATCCTTGGGGAGAG	GACTCGGTGAGGCCAGTC
	mouseRelB	CATCGAGCTTCGAGACTGTG	CACGAGGCTATGTGGGTGTA
	RHOT2	CTGCGGACTATCTCTCCCCTC	AAAAGGCTTTGCAGCTCCAC
	VEGF	CAAGACAAGAAAATCCCTGTGG	GCTTGTCACATCTGCAAGTACG
	cccDNA	CCGTGTGCACTTCGCTTCA	GCACAGCTTGGAGGCTTGA
	PRNP	TGCTGGGAAGTGCCATGAG	CGGTGCATGTTTTCACGATAGTA
Taqman probes	RHOT2	[6FAM] GCTACGCCATCGACACGGTGCAGGT [BHQ1]	
	HBV cccDNA	[6FAM] CATGGAGACCACCGTGAACGCCC [BHQ1]	
siRNA	siHIF1α	1: GAACAAAUACAUGGGAUUA	
		2: AGAAUGAAGUGUACCUAA	
		3: GAUGGAAGCACUAGACAAA	
		4: CAAGUAGCCUCUUUGACAA	
	siAhR	CAAGCGGCATAGAGACCGACTTAATACAGAGTTGGACCGTTTGGCTAGCCTGCTGCCTTTCCCACAAGATGTTATTA ATAAGTTGGACAAACTTTCAGTTCTTAGGCTCAGCGTCAGTTACCTGAGAGCCAAGAGCTTCTTTGATGTTGCATTAAA ATCCTCCCCTACTGAAAGAAACGGAGGCCAGGATAACTGTAGAGCAGCAAATTTCAGAGAAGGCCTGAACTTACAAGA AGGAGAATTCTTATTACAGGCTCTGAATGGCTTTGTATTAGTTGTCACTACAGATGCTTTGGTCTTTTATGCTTCTTCT ACTATACAAGATTATCTAGGGTTTCAGCAGTCTGATGTCATACATCAGAGTGTATATGAACTTATCCATACCGAAGACC GAGCTGAATTTC	
	siARNT	TCCACTTAGTATTGCAGCCAAAGAATATTTAAATAAACGTCTTTGCTGCGCTTGCATCCATGCCCAGCCAATATACAAC TGTAAAGCAAATATAGAAAGTCGGCTGTTGATACGATTGTCTGTTATCGAACACATTCAGTGATAAAGCTGGGTTACTG CTGCTTTTGGTGCTCTCACCTTATCTGGAAGATCTGCAAACATTACCTAAATAGGCTGGCAAGATAAACACTTTCTGG AACCCGAGACTTGGCCATAAAGATAATGCTGCATTTTTCTGTCAGAATCACATATGATGTGTGTTCTGTAGAGGTTATT TCTGCATGGAAACTCAACTTCTTGGATTAGCCGTCCCAGTGAAAATCCTCATTGTTGGAGTGTAAACCAAATACGAAG CCCTCTTGCA	
	siRelB	1: CUGCGGAUUUGCCGAAUUA	
		2: GCACAUGAAUUGGAGAU	
		3: CCAUUGAGCGGAAGAUUCA	
		4: GCCCGUCUAUGACAAGAAA	