

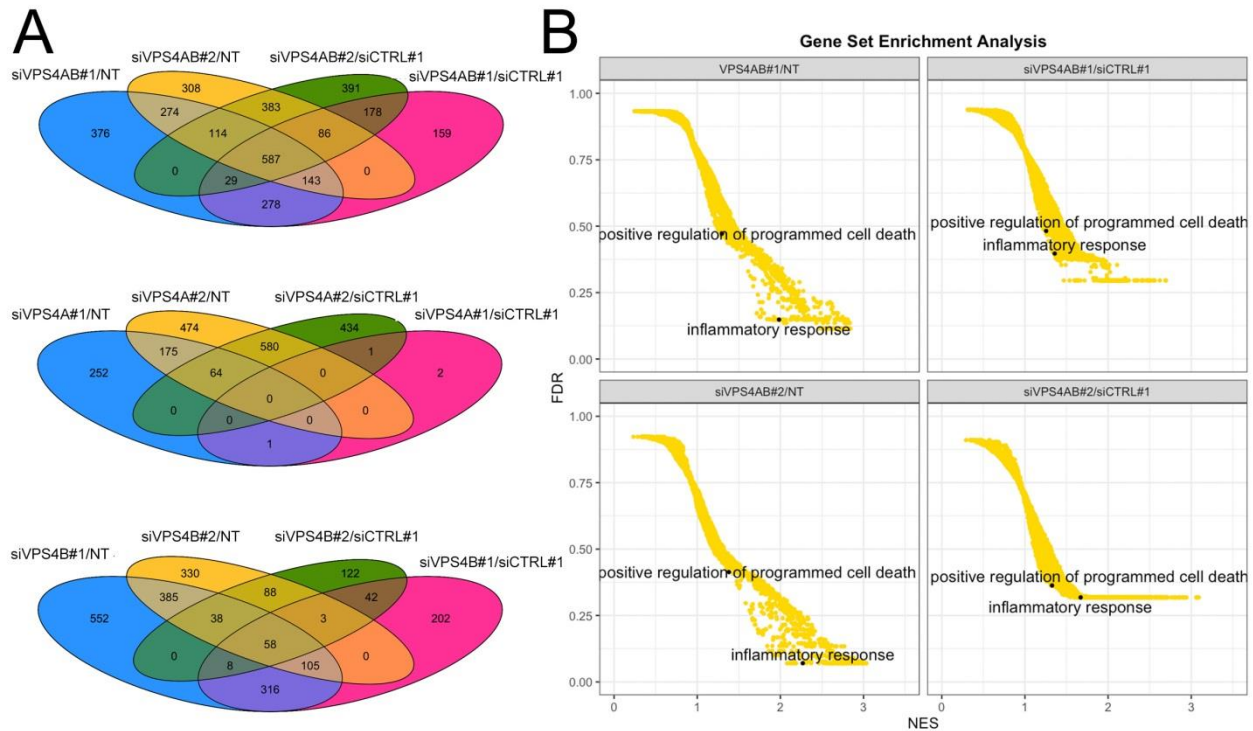
# **Synthetic lethality between VPS4A and VPS4B triggers an inflammatory response in colorectal cancer**

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## **APPENDIX FIGURES AND TABLES**

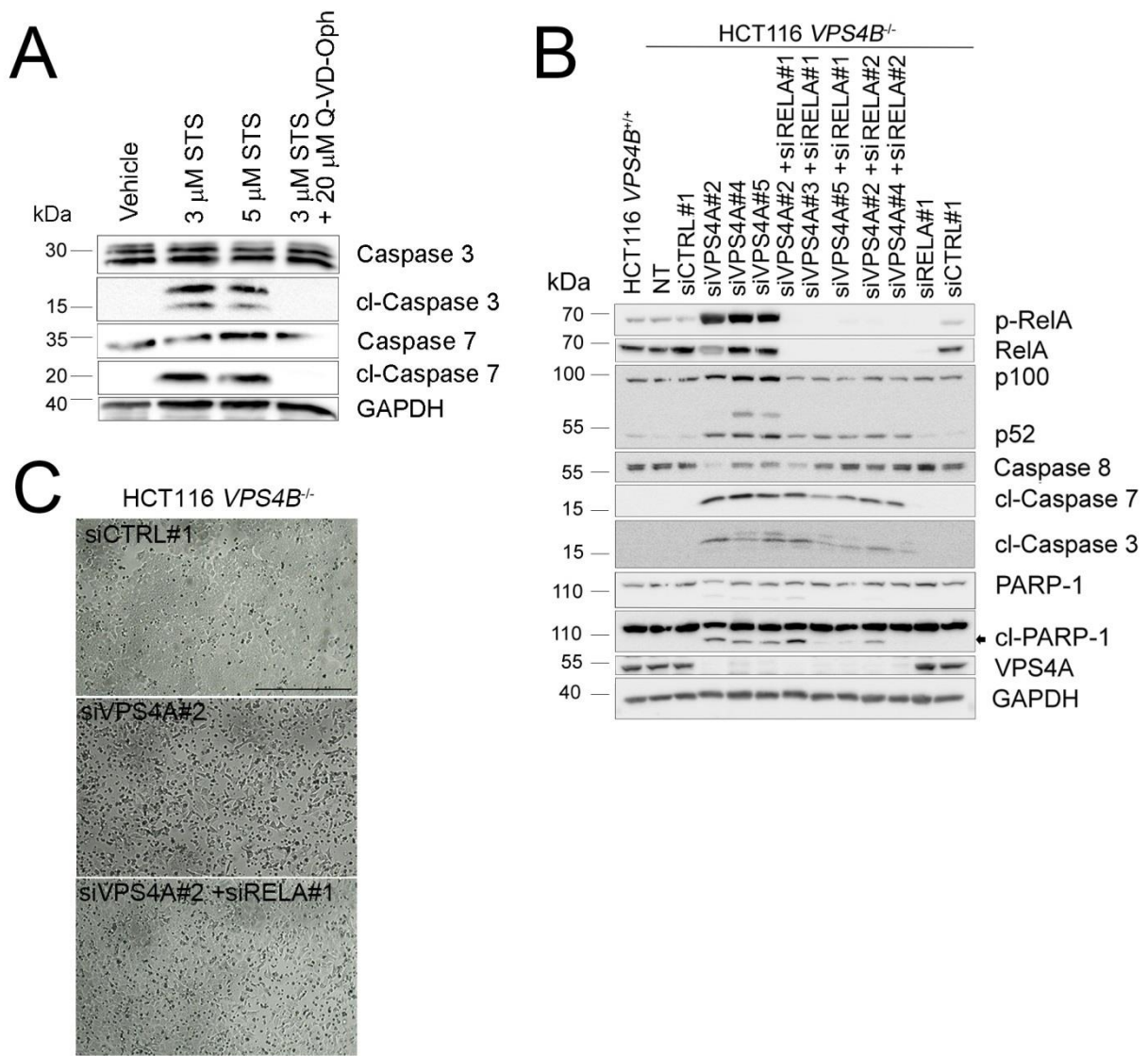
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**Appendix Figure S1. Transcriptional alterations after individual or combined knockdown of VPS4 paralogs in HCT116 cells.**

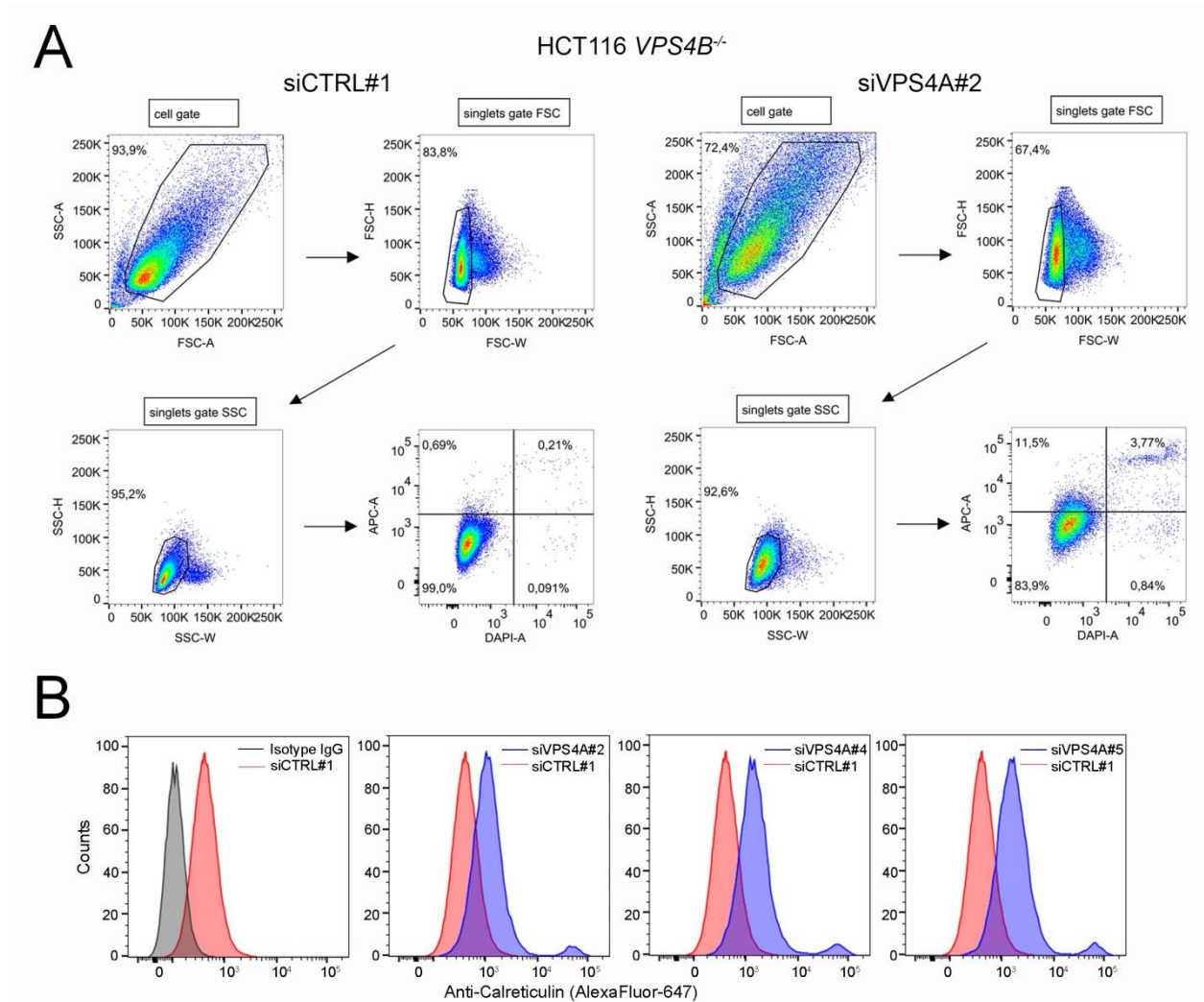
- A** Venn diagrams of transcriptionally upregulated genes ( $\geq 1.5$ -fold; adjusted  $p < 0.05$ ) after individual or combined *VPS4A* and/or *VPS4B* knockdown when normalized to non-transfected (NT) cells and cells transfected with non-targeting siRNA (siCTRL#1), as indicated. Transcriptionally upregulated genes were identified using DESeq2.
- B** Rank order based on Normalized Enrichment Score (NES) and False Discovery Rate (FDR) of enriched gene signatures for four different contrasts of combined *VPS4A+B* silencing and normalization conditions was prepared using the gseGO function from clusterProfiler.



**Appendix Figure S2. Analyses of caspase activation in dying HCT116 cells upon chemical treatment or RelA depletion.**

**A** Immunoblotting analysis of caspase activation in apoptotic HCT116 cells. To induce apoptosis cells were treated for 24 h with staurosporine (STS). To prevent caspase activation, cells were pretreated with Q-VD-Oph 30 min before STS administration and then cultured for 24 h in the presence of STS and Q-VD-Oph. Vehicle – lysates from DMSO-treated cells. cl - cleaved caspases. GAPDH served as a loading control.

- B** Immunoblotting analysis of caspase activation in lysates from cells with depletion of both *VPS4* paralogs (as in Fig 5A) with or without co-depletion of RelA. Two independent siRNA oligonucleotides targeting *RELA* (siRELA#1 or #2) alone or in combination with siVPS4A were used for transfections. Representative blot from 3 experiments is shown. GAPDH served as a loading control. NT- non-transfected; p-RelA - phospho-RelA; cl – cleaved caspases or PARP-1.
- C** Phase contrast microscopy images of HCT116 *VPS4B*<sup>-/-</sup> cells transfected as in (B) acquired 66 h after siRNA transfection. Scale bar, 500  $\mu$ m.



**Appendix Figure S3. Flow cytometry analysis of calreticulin cell surface exposure in *VPS4A*-depleted HCT116 *VPS4B*<sup>-/-</sup> cells.**

**A** Examples of gating strategy for flow cytometric analysis (Fig 6D), applied for siCTRL#1- (left) or siVPS4A#2-transfected (right) HCT116 *VPS4B*<sup>-/-</sup> cells, including cell gate (FSC vs SSC), singlets discrimination gates (FSC-H vs FSC-W, SSC-H vs SSC-W). DAPI staining was used to discriminate live (DAPI-) and dead cells (DAPI+). APC - AlexaFluor-647 fluorescence. This strategy was applied for all flow cytometry experiments.

**B** Overlay histograms showing fluorescence signal of AlexaFluor 647 indicating cell surface calreticulin exposure. One representative experiment from Fig 6D is shown. Grey histogram represents siCTRL#1-transfected cells stained with primary isotype IgG, followed by AlexaFluor 647-conjugated secondary IgG. Red histograms represent siCTRL#1-transfected cells and blue histograms represent cells transfected with siVPS4A (#2, #4, #5) which were stained with anti-calreticulin antibody, followed by AlexaFluor 647-conjugated secondary IgG.

<b>ID</b>	<b>Description</b>	<b>NES</b>	<b>FDR</b>	<b>Type of contrast</b>
GO:0006954	inflammatory response	1.356	0.387	VPS4AB#1/Ctrl
GO:0043068	positive regulation of programmed cell death	1.255	0.425	VPS4AB#1/Ctrl
GO:0006954	inflammatory response	1.669	0.320	VPS4AB#2/Ctrl
GO:0043068	positive regulation of programmed cell death	1.319	0.395	VPS4AB#2/Ctrl
GO:0006954	inflammatory response	1.984	0.148	VPS4AB#1/NT
GO:0043068	positive regulation of programmed cell death	1.297	0.472	VPS4AB#1/NT
GO:0006954	inflammatory response	2.271	0.100	VPS4AB#2/NT
GO:0043068	positive regulation of programmed cell death	1.389	0.408	VPS4AB#2/NT

**Appendix Table S1. List of selected terms after gene set enrichment analysis under conditions of combined silencing of *VPS4A+B*.**

Gene name	Forward primer	Reverse primer
<i>ACTB</i>	CAGGTCATCACCATTGGCAAT	TCTTTGCGGATGTCCACGT
<i>B2M</i>	GGAGGCTATCCAGCGTACTC	GAAACCCAGACACATAGCAATTC
<i>VPS4A</i>	CCATCAGGAGGAGGTTTGAA	CCGAGTAGCCTTCCGTCTTC
<i>VPS4B</i>	TCGTTAAATATGAAGCACAGGGTGA	TTCTCCTTCCCCATCACTGT
<i>VPS4B-2</i>	GAACTCCGCCATGTCATCC	TCGTAGTCCCAGCCTTGTC
<i>Nos2</i>	TCAACTGCAAGAGAACGGAGA	TCTTTCAGGTCACCTTGGTAGGA
<i>Cxcl9</i>	TCGGACTTCACTCCAACACAG	AGGGTTCCTCGAACTCCACA
<i>Arg1</i>	CGTAGACCCTGGGGAACTACTAT	TCCATCACCTTGCCAATCCC
<i>Ym1</i>	AGAAGCTCTCCAGAAGCAATCC	ATCAGCTGGTAGGAAGATCCCAG
<i>Il10</i>	GACTTTAAGGGTTACTTGGGTTGC	ATTTCTGGGCCATGCTTCTCT
<i>Ccl22</i>	AGGACTACATCCGTCACCTT	GACGGTTATCAAAAACAACGCCA
<i>Il18</i>	CGACTTCACTGTACAACCGCA	TGGGGTTCCTGACTGGCACTTTG
<i>Il6</i>	GATGGATGCTACCAAACCTGG	TCTGAAGGACTCTGGCTTTG
<i>Rpl19</i>	AGGCATATGGGCATAGGGAAGAG	TTGACCTTCAGGTACAGGCTGTG
<i>Vps4a</i>	CAATTGATGGGTGCTGTTGTGA	GGCCCAAAGAGGAGTATGCC
<i>Vps4b</i>	AAATGCAAGGAGTTGGTGTGG	TCTAAACATGGCTGCTCGGG

**Appendix Table S2. List of qRT-PCR primers used in this study.**



Figures	Groups	Symbol	p-value	n	statistic test	post-test
EV1A	normal colon vs. adenoma	ns	0.4259	24 vs. 42	Kruskal-Wallis	Dunn's test with Benjamini-Hochberg correction
	normal colon vs. adenocarcinoma	ns	0.0628	24 vs. 26	Kruskal-Wallis	Dunn's test with Benjamini-Hochberg correction
	adenoma vs. adenocarcinoma	ns	0.1071	42 vs. 26	Kruskal-Wallis	Dunn's test with Benjamini-Hochberg correction
EV2A	VPS4A - siCTRL (#1 + #2) group vs. siVPS4B (#1 + #2) group	ns	0.5176	4	two-tailed unpaired t-test	
	VPS4B - siCTRL (#1 + #2) group vs. siVPS4A (#1 + #2) group	ns	0.3525	4	two-tailed unpaired t-test	
EV2B	VPS4A protein abundance - normalized siCTRL#1 (set as 1) vs. siVPS4B#1	ns	0.8750	4	Wilcoxon signed rank test	
	VPS4A protein abundance - normalized siCTRL#1 (set as 1) vs. siVPS4B#2	ns	0.6250	4	Wilcoxon signed rank test	
	VPS4B protein abundance - normalized siCTRL#1 (set as 1) vs. siVPS4A#1	ns	0.2500	4	Wilcoxon signed rank test	
	VPS4B protein abundance - normalized siCTRL#1 (set as 1) vs. siVPS4A#2	ns	0.6250	4	Wilcoxon signed rank test	
	VPS4B protein abundance - normalized siCTRL#1 (set as 1) vs. siVPS4A#3	ns	0.8750	4	Wilcoxon signed rank test	
EV3B	HCT116 <i>VPS4B</i> <sup>+/+</sup> vs. HCT116 <i>VPS4B</i> <sup>-/-</sup> 1C5	ns	0.6831	5	one-sample t-test	
	HCT116 <i>VPS4B</i> <sup>+/+</sup> vs. HCT116 <i>VPS4B</i> <sup>-/-</sup> 2B3	ns	0.0681	5	one-sample t-test	
EV3D	siCTRL#1 vs. siVPS4A (#2, #4 and #5) group	****	8.79E-07	4	one-sample t-test	
EV3F	HCT116 <i>VPS4B</i> <sup>-/-</sup> Dox- vs. HCT116 <i>VPS4B</i> <sup>-/-</sup> Dox+	ns	0.4158	3	one-sample t-test	
	HCT116 <i>VPS4B</i> <sup>-/-</sup> shCTRL#1 Dox- vs. HCT116 <i>VPS4B</i> <sup>-/-</sup> shCTRL#1 Dox+	ns	0.5672	3	two-tailed unpaired t-test	
	HCT116 <i>VPS4B</i> <sup>-/-</sup> shCTRL#2 Dox- vs. HCT116 <i>VPS4B</i> <sup>-/-</sup> shCTRL#2 Dox+	ns	0.4244	3	two-tailed unpaired t-test	
	HCT116 <i>VPS4B</i> <sup>-/-</sup> shVPS4A#1 Dox- vs. HCT116 <i>VPS4B</i> <sup>-/-</sup> shVPS4A#2 Dox+	**	0.0097	3	two-tailed unpaired t-test	
	HCT116 <i>VPS4B</i> <sup>-/-</sup> shVPS4A#2 Dox- vs. HCT116 <i>VPS4B</i> <sup>-/-</sup> shVPS4A#2 Dox+	*	0.0208	3	two-tailed unpaired t-test	
EV4A	EEA1 NT vs. siVPS4A + VPS4B	**	0.0058		Welch t-test	
	Rab7 NT vs. siVPS4A + VPS4B	**	0.0100		Welch t-test	
	LAMP-1NT vs. siVPS4A + VPS4B	*	0.0430		the Mann-Whitney U test	
EV4B	(chart: % of Tf-A647 -positive cells) siCTRL#1 vs. siVPS4A#2 and #5 group	**	0.0034	4	two-tailed unpaired t-test	
	(chart: intensity of Tf-A647 -positive cells) siCTRL#1 vs. siVPS4A#2 and #5 group	***	0.0009	4	two-tailed unpaired t-test	
EV4C	G0/G1 siCTRL#1 vs. siVPS4A#2+siVPS4A5	**	0.0061	4	the Man -Whitney U test	
	S siCTRL#1 vs. siVPS4A#2+siVPS4A5	ns	0.1091	4	the Man -Whitney U test	
	G2/M siCTRL#1 vs. siVPS4A#2+siVPS4A5	**	0.0061	4	the Man -Whitney U test	

**Appendix Table S3. Exact p-values for data from EV figures 1 - 4.**