

Supplementary Information

miR-34a and IRE1A/XBP-1(S) Form a Double-Negative Feedback Loop to Regulate Hypoxia-Induced EMT, Metastasis, Chemo-Resistance and Autophagy

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Inventory of Supplementary Information

- **Supplemental Figure S1**
- **Supplemental Figure S2**
- **Supplemental Figure S3:** Uncropped Western blot membranes
- **Supplemental Table S1:** Oligonucleotides used for qPCR
- **Supplemental Table S2:** List of antibodies
- **Supplemental Table S3:** Oligonucleotides used for qChIP
- **Supplemental Table S4:** Oligonucleotides used for cloning and mutagenesis of *XBP-1* and *IRE1A* 3'-UTRs
- **Supplemental Table S5:** Vectors used in this study

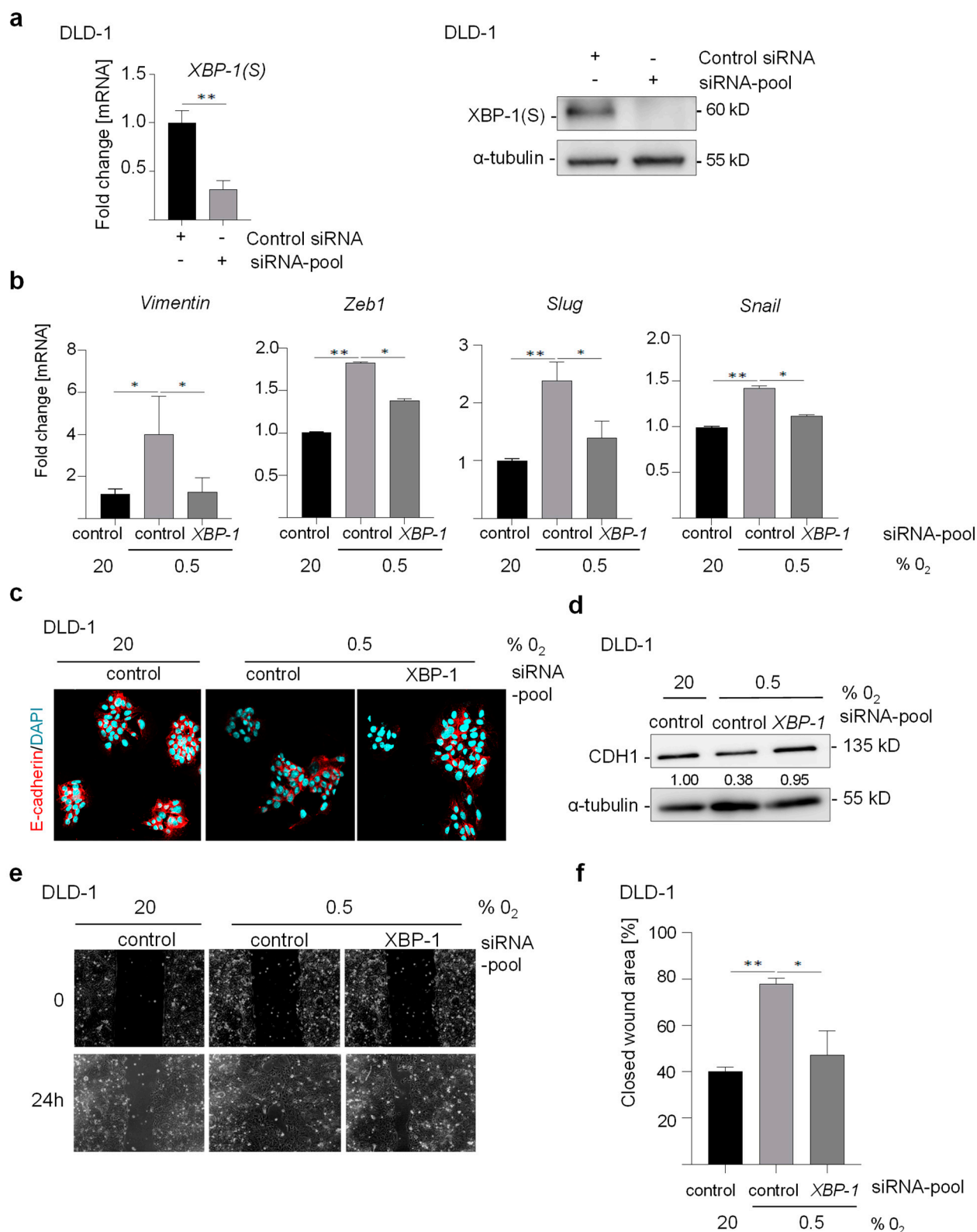


Figure S1. (a) qPCR analysis in DLD-1 cells transfected with *XBP-1* or control siRNAs Pool. (b) qPCR analysis in DLD-1 cells transfected with *XBP-1* or control siRNAs Pool and then cultured at 20% O₂ or 0.5% O₂ for 72 hours. (c-d) Indirect immunofluorescence detection and western blot analysis of *E-cadherin*/CDH1 in DLD-1 cells transfected with *XBP-1* or control siRNAs Pool and then cultured at 20% O₂ or 0.5% O₂. (e-f) Densitometric representation and of the wound-healing assay done in DLD-1 cells transfected with *XBP-1* or control siRNAs Pool and then cultured at 20% O₂ or 0.5% O₂. The normalized wound area was calculated using Image J software. In panel (a), (b), and (f) mean values \pm SD (n=3) are provided. (***) p < 0.001, (**) p < 0.01, (*) p < 0.05.

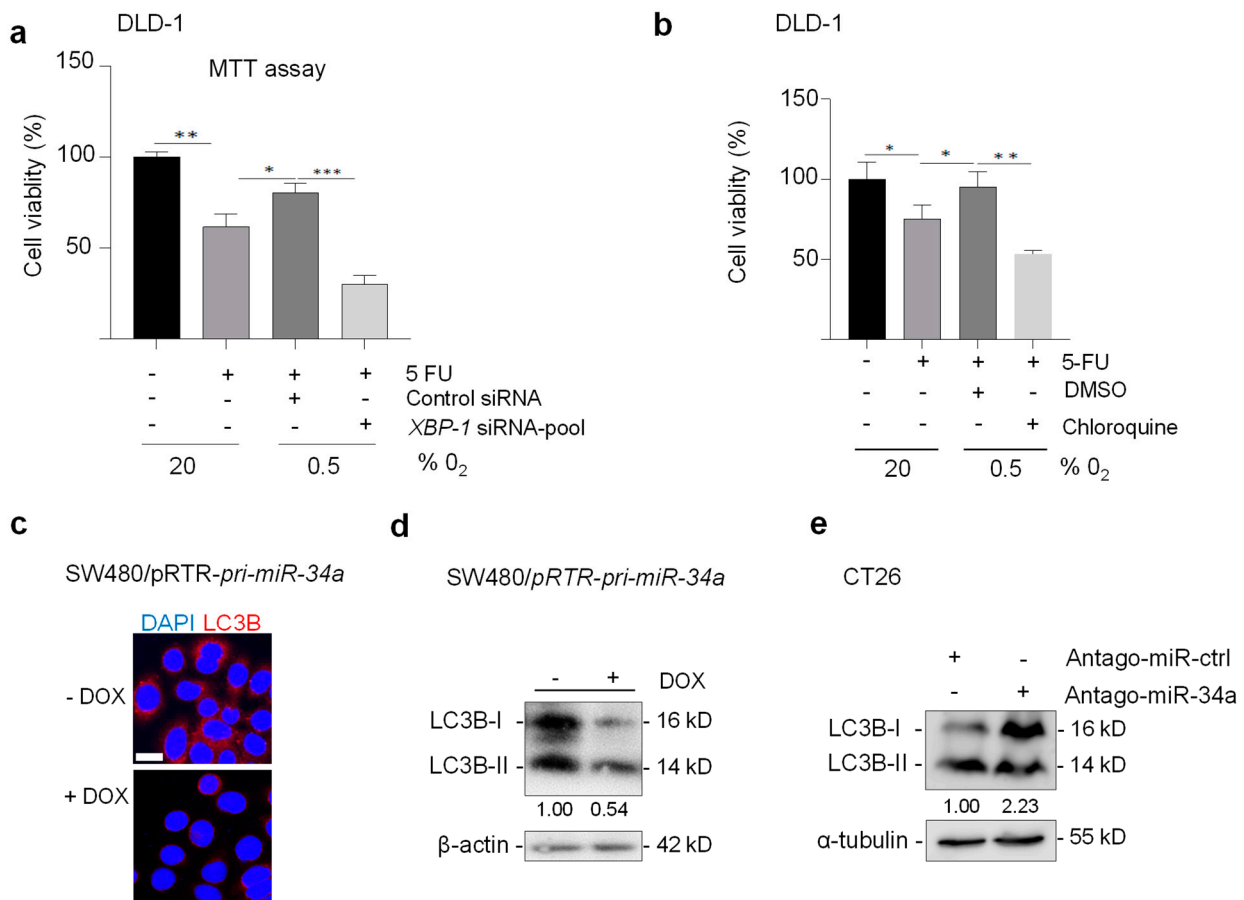
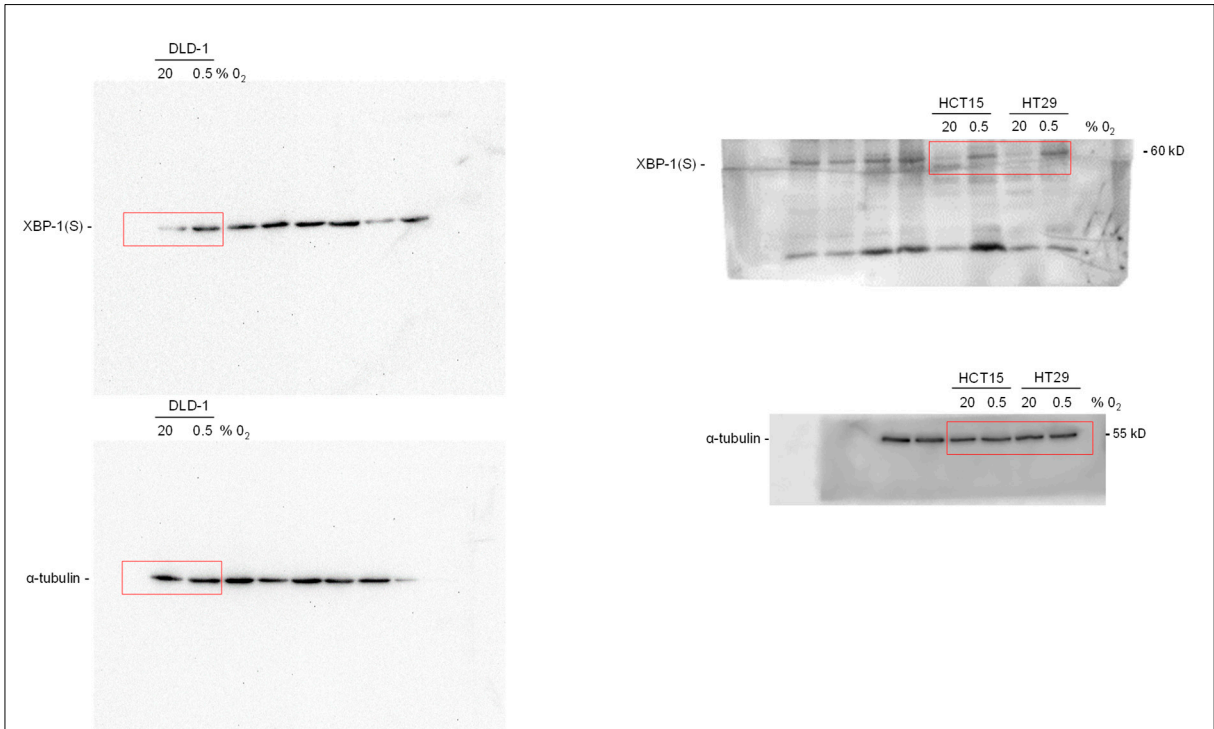


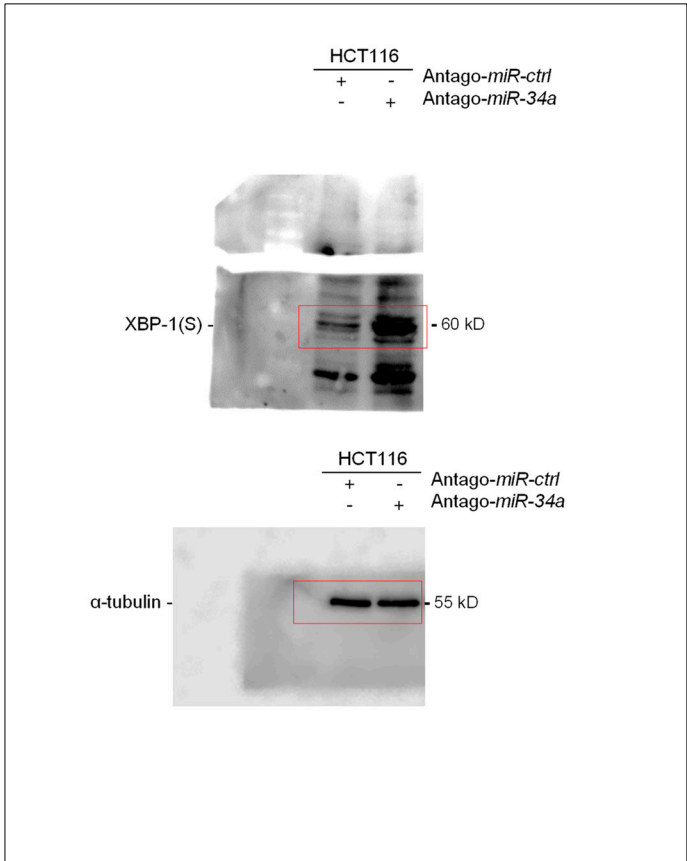
Figure S2. (a) MTT assay of DLD-1 cells transfected with *XBP-1* or control siRNAs Pool for 24 hours at 20.0% O₂, then exposed to 0.5% O₂ for 48 hours, and subsequently treated with or without 5-FU for 72 hours. **(b)** MTT assay of DLD-1 cells treated with DMSO or chloroquine (20 μM) for 24 hours at 20.0% O₂, then exposed to 0.5% O₂ for 48 hours, and subsequently treated with or without 5-FU for 72 hours. **(c-d)** Indirect immunofluorescence detection and western blot analysis of LC3B in SW480 cells harboring a pRTR/pri-miR-34a vector exposed to Doxycycline (DOX) for 48h. **(e)** Western blot analysis of LC3B in CT26 cells transfected with antago-miR-34a or antago-miR control oligonucleotides for 48 hours. In panel **(a)**, **(b)** mean values ± SD (n=3) are provided. (***) p < 0.001, (**) p < 0.01, (*) p < 0.05.

Figure S3. Source data of Western blot analyses

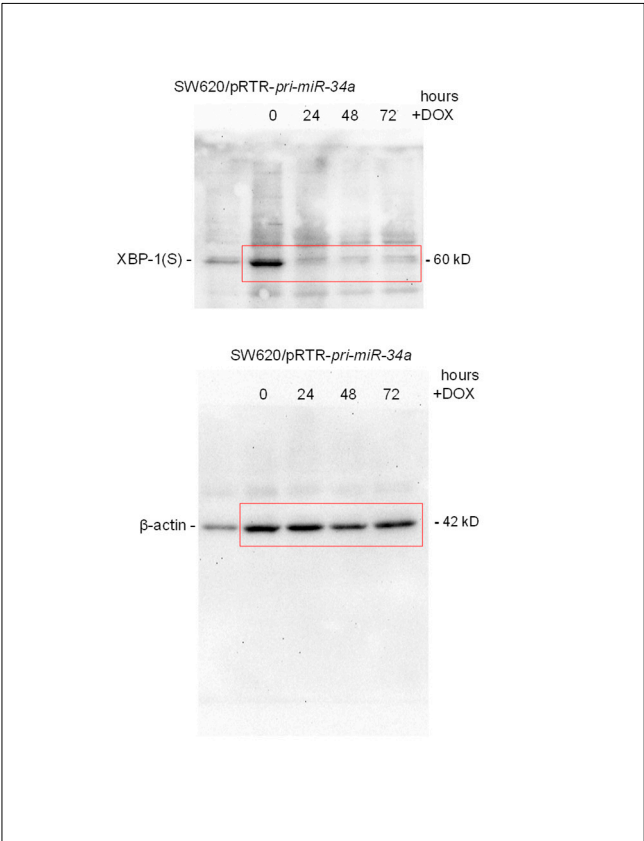
Related to Figure 1b



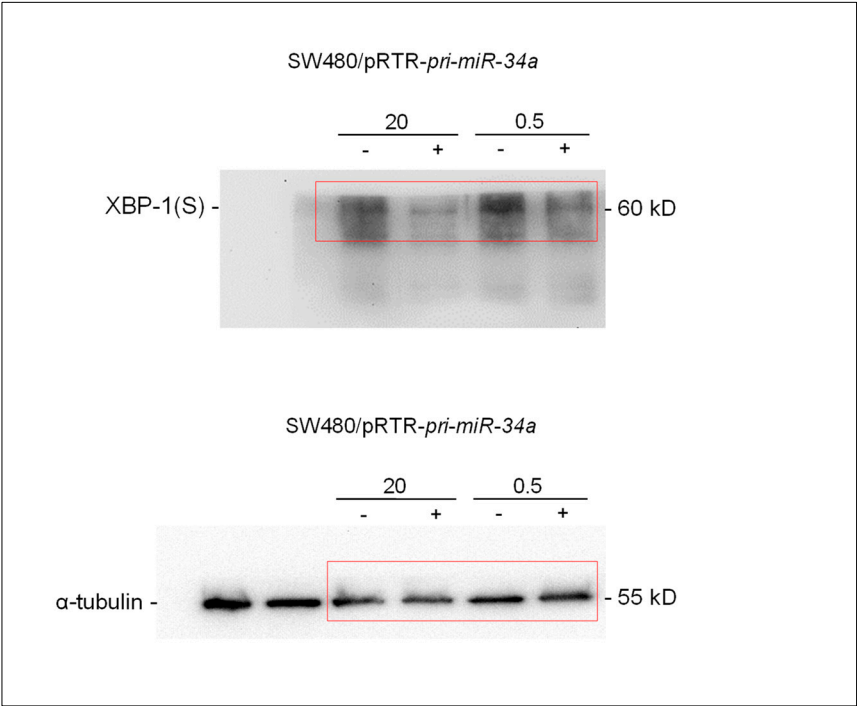
Related to Figure 1f



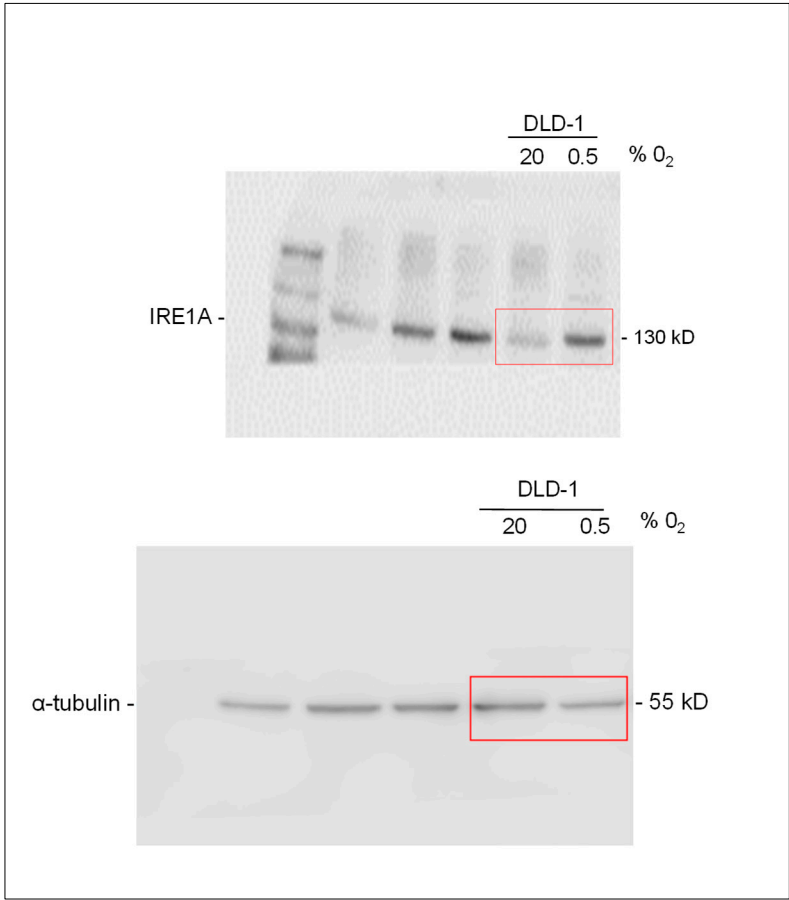
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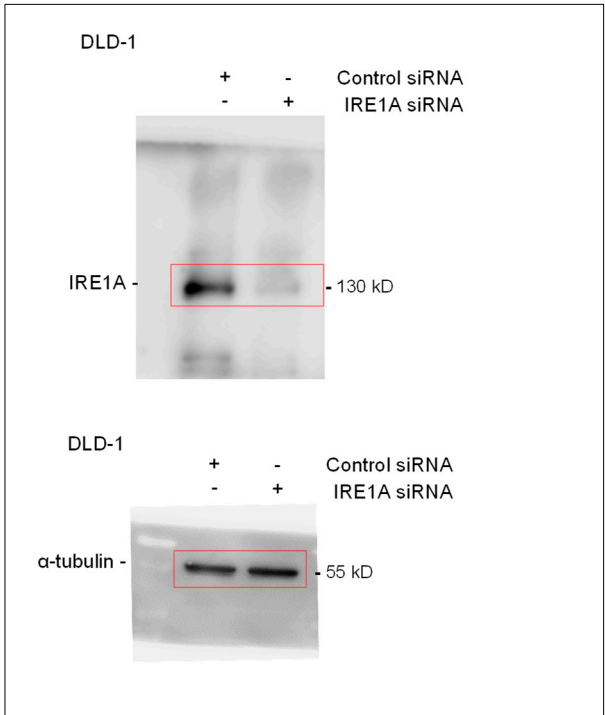
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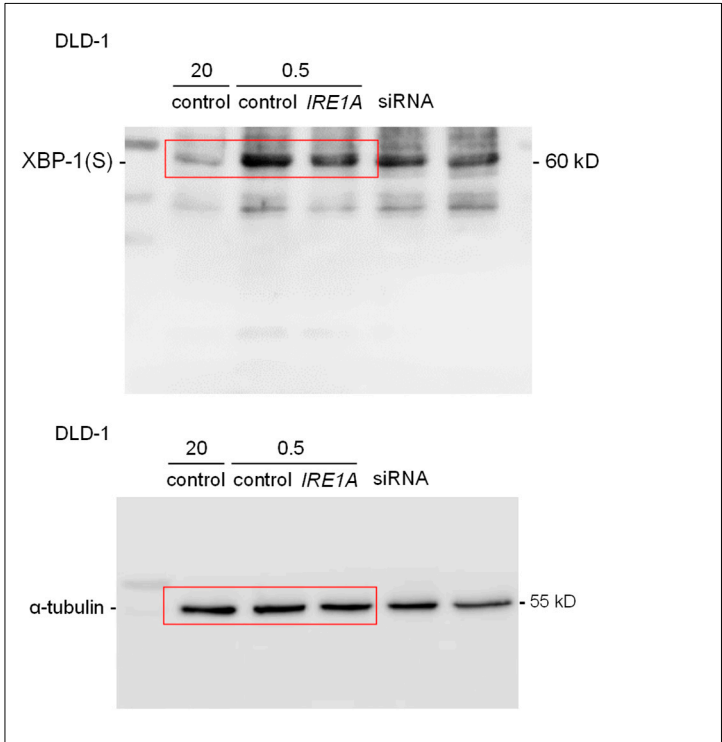
Related to Figure 2a



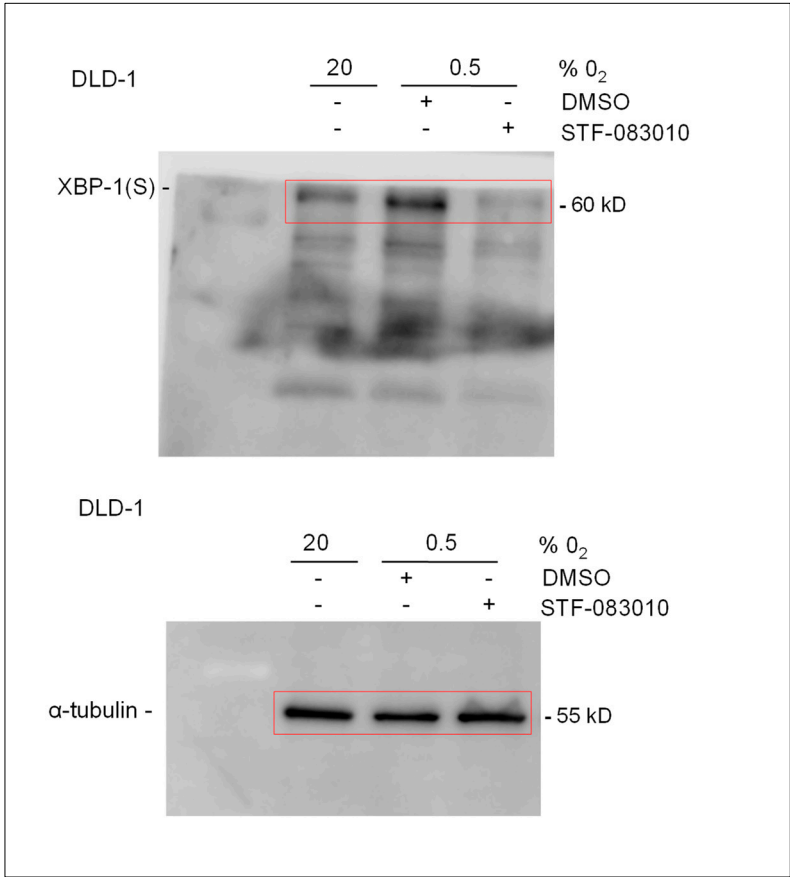
Related to Figure 2c



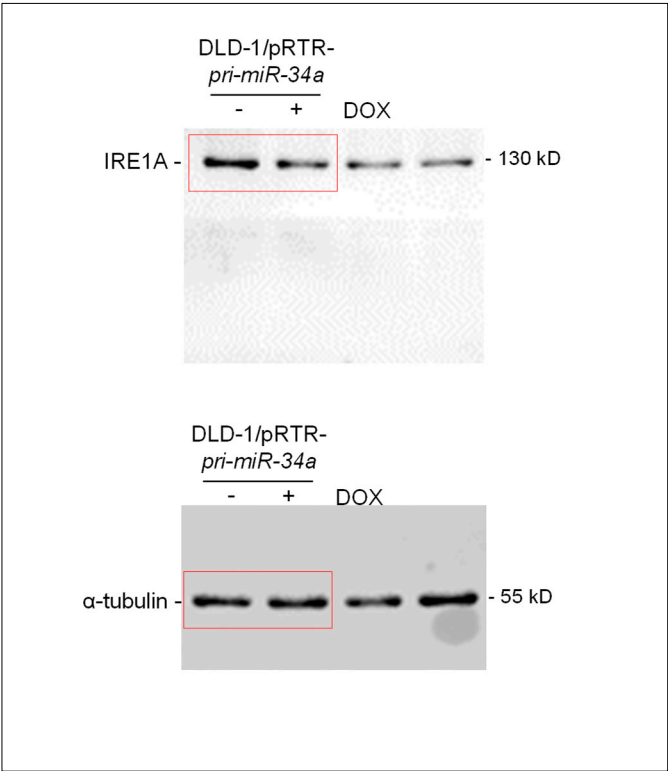
Related to Figure 2d



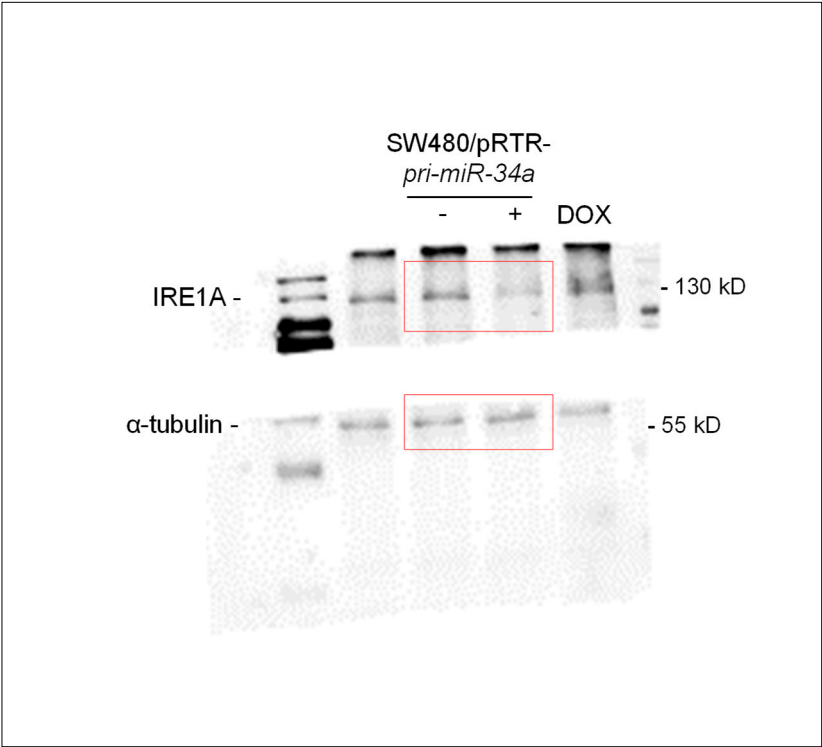
Related to Figure 2e



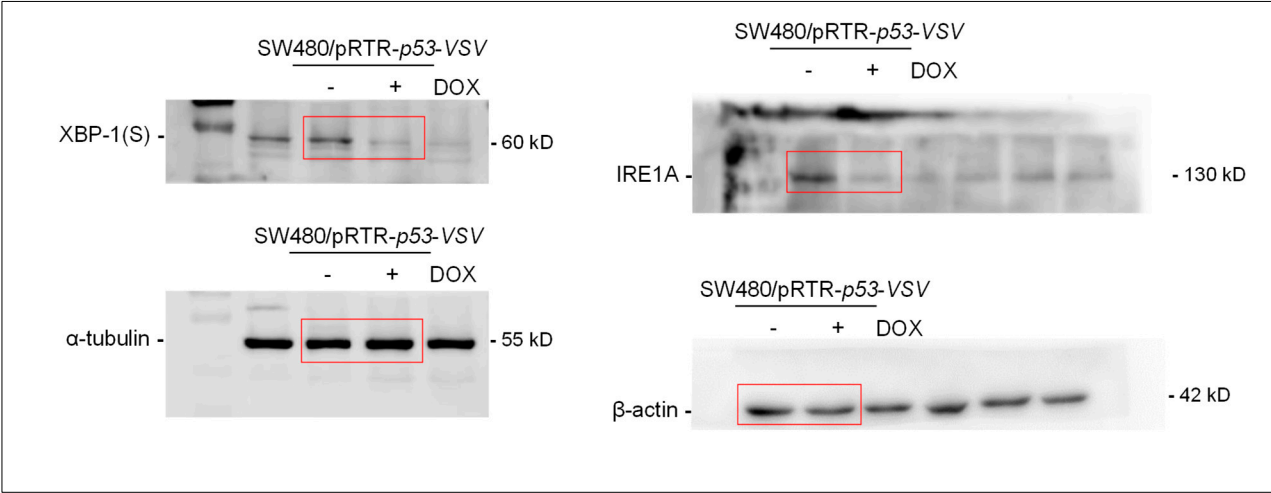
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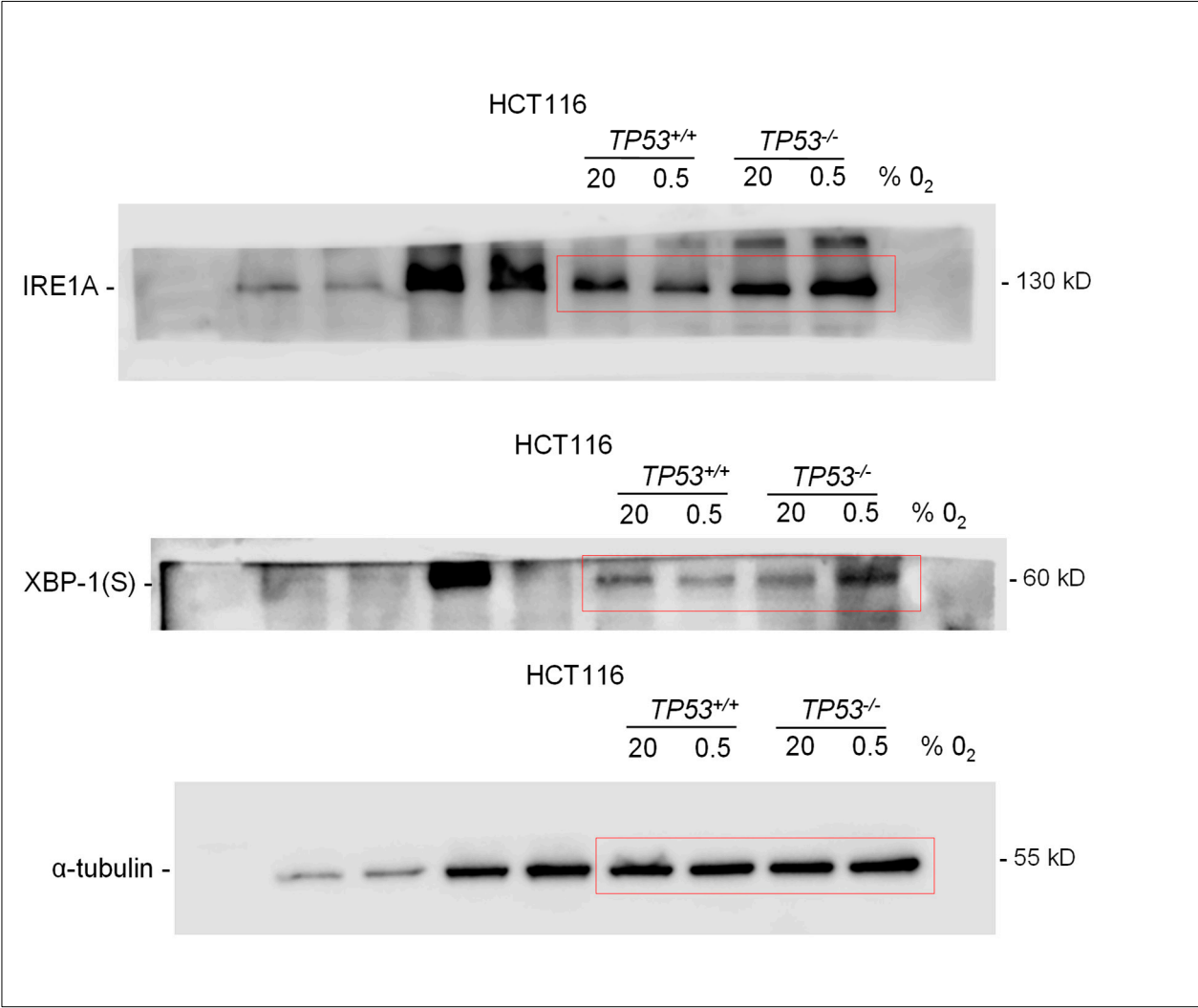
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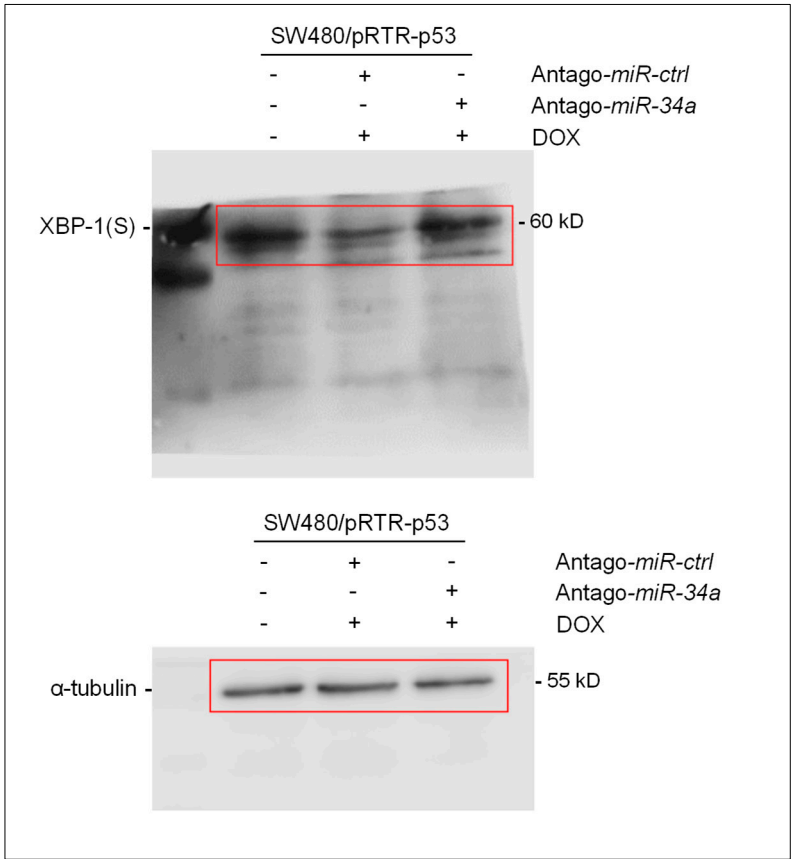
Related to Figure 3a



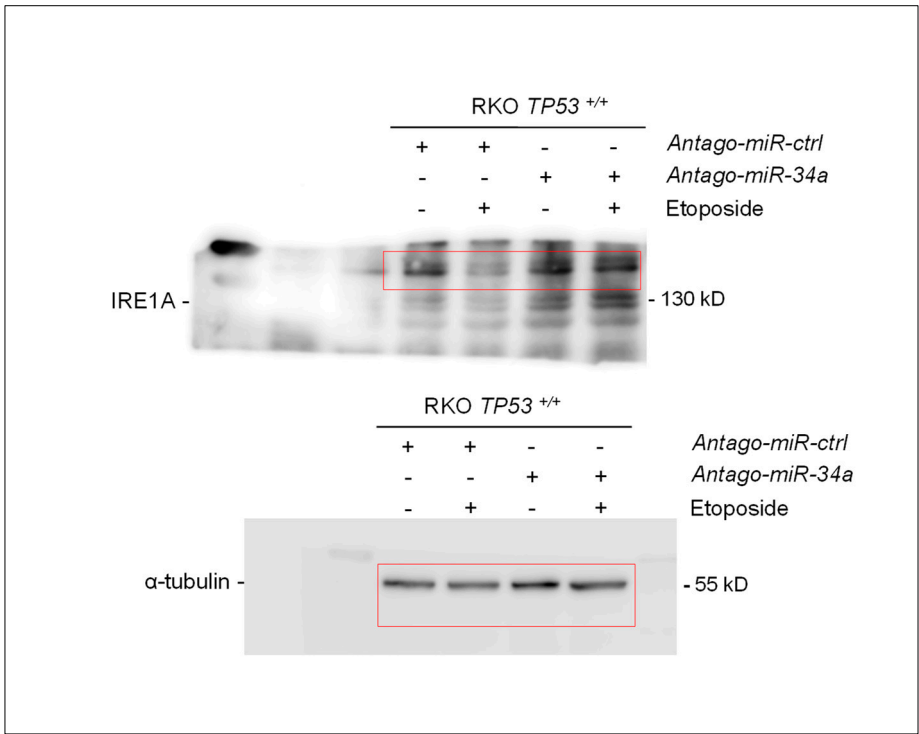
Related to Figure 3c



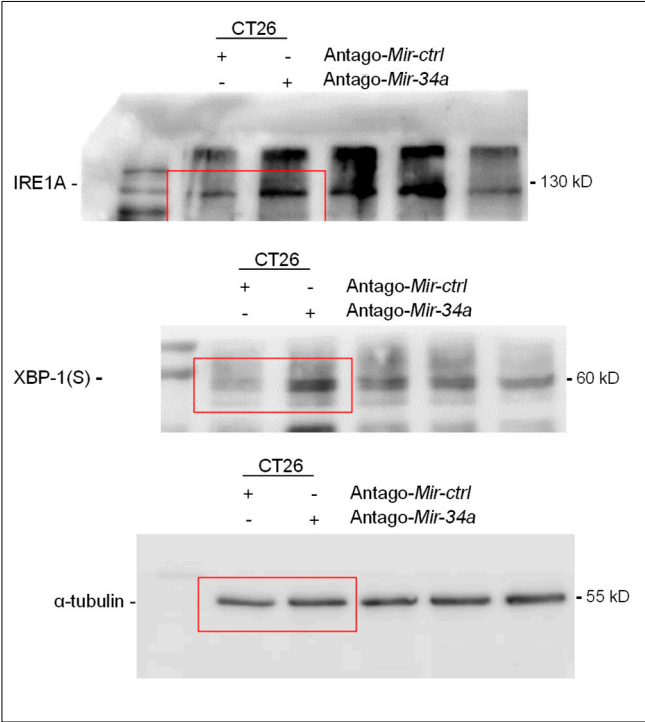
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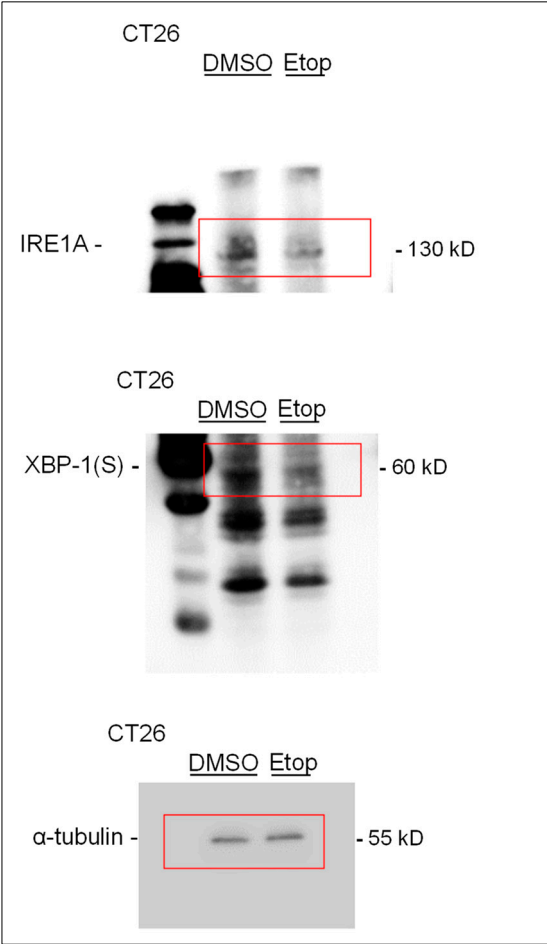
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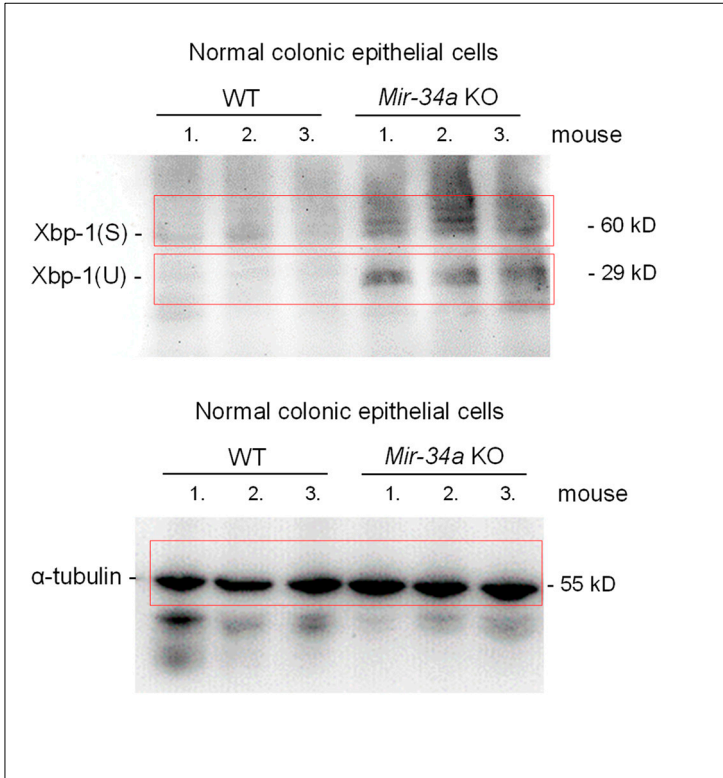
Related to Figure 4b



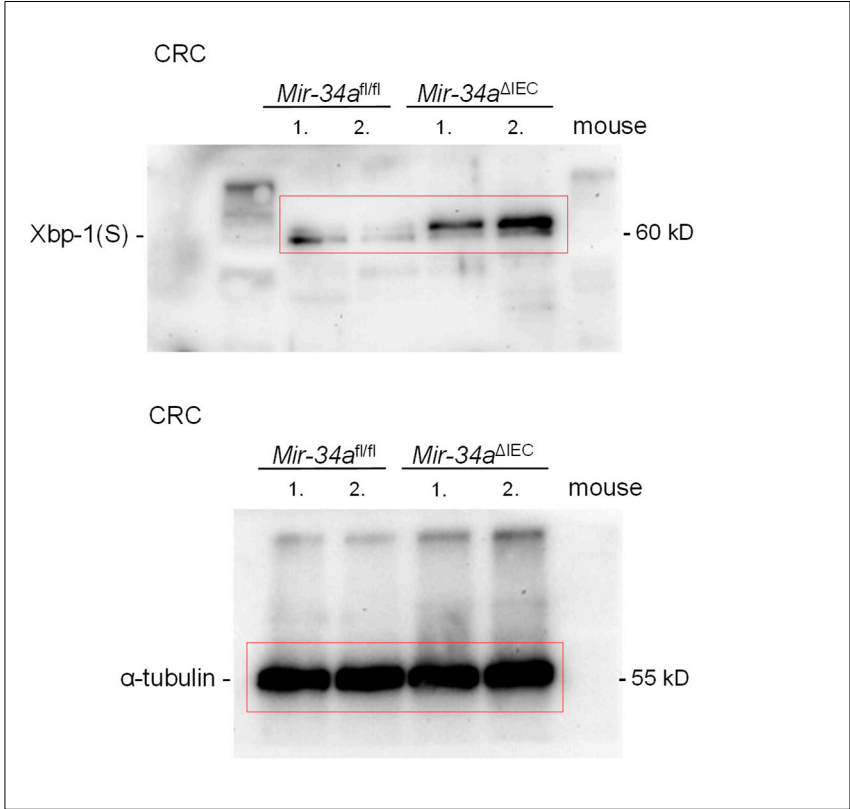
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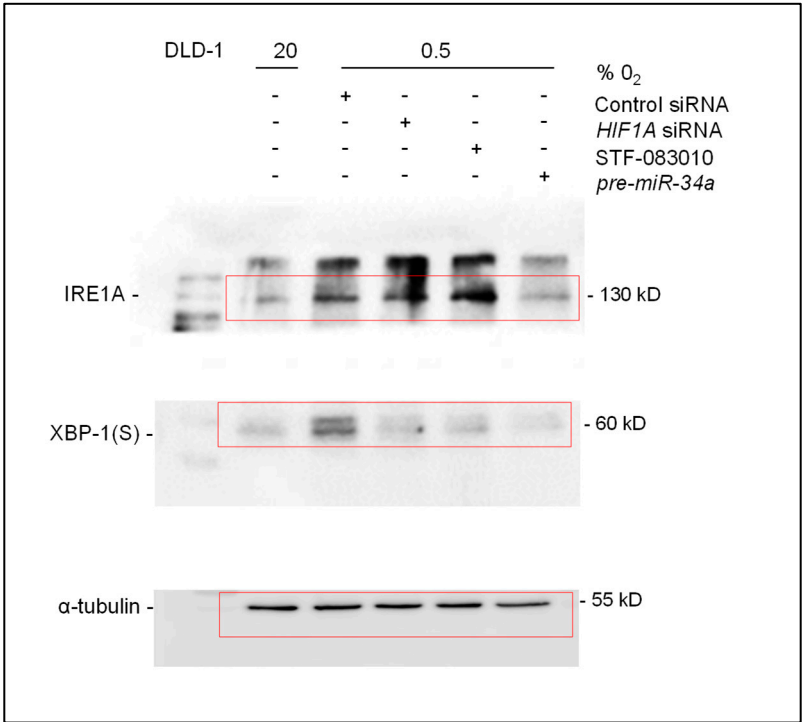
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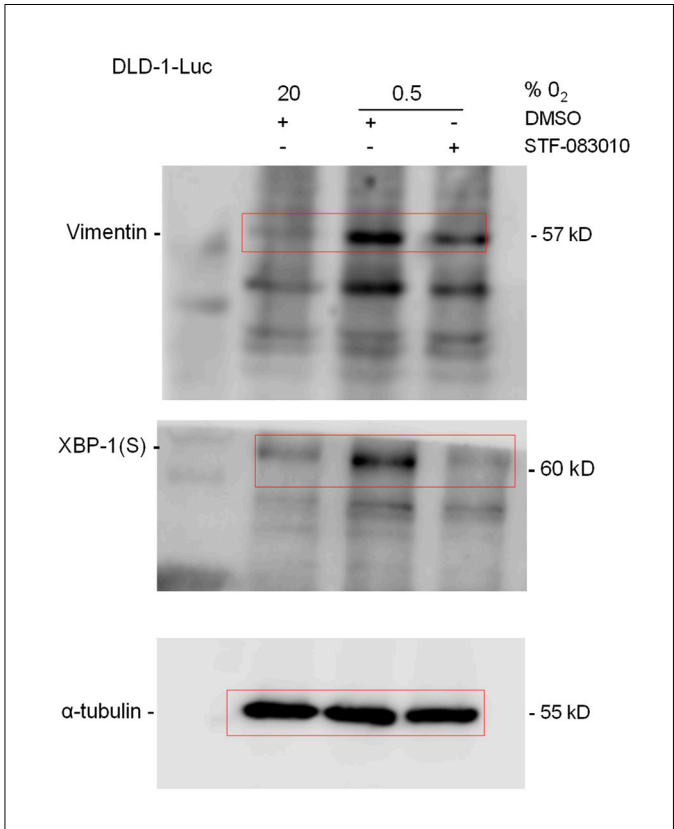
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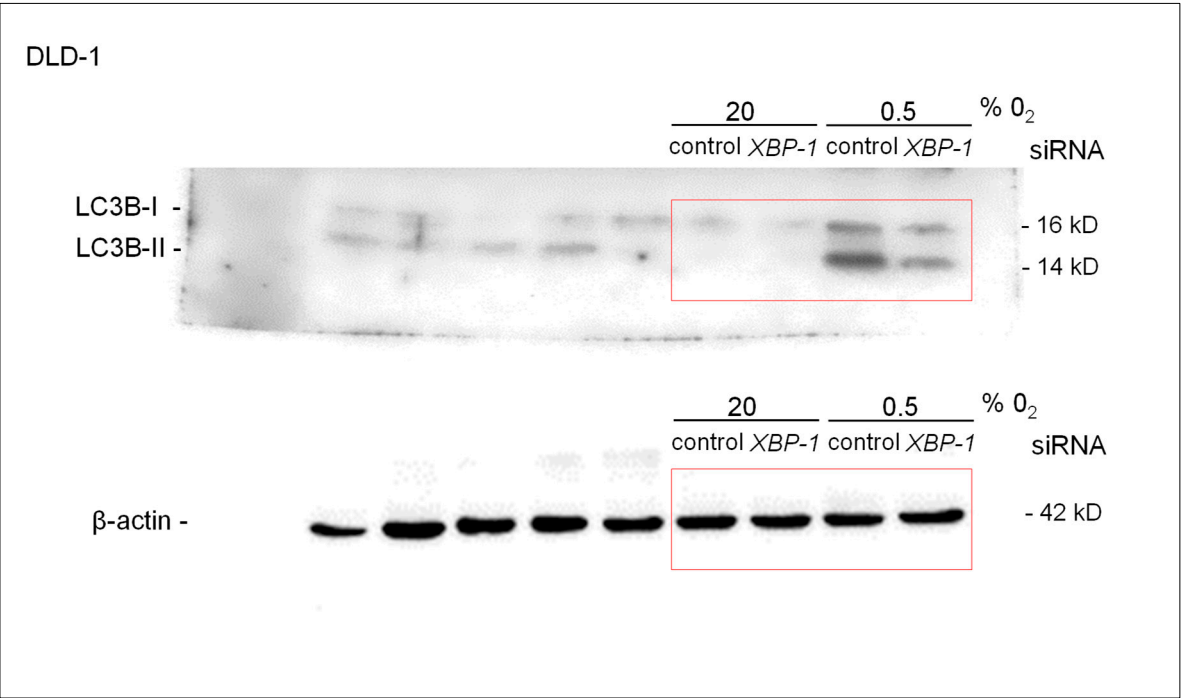
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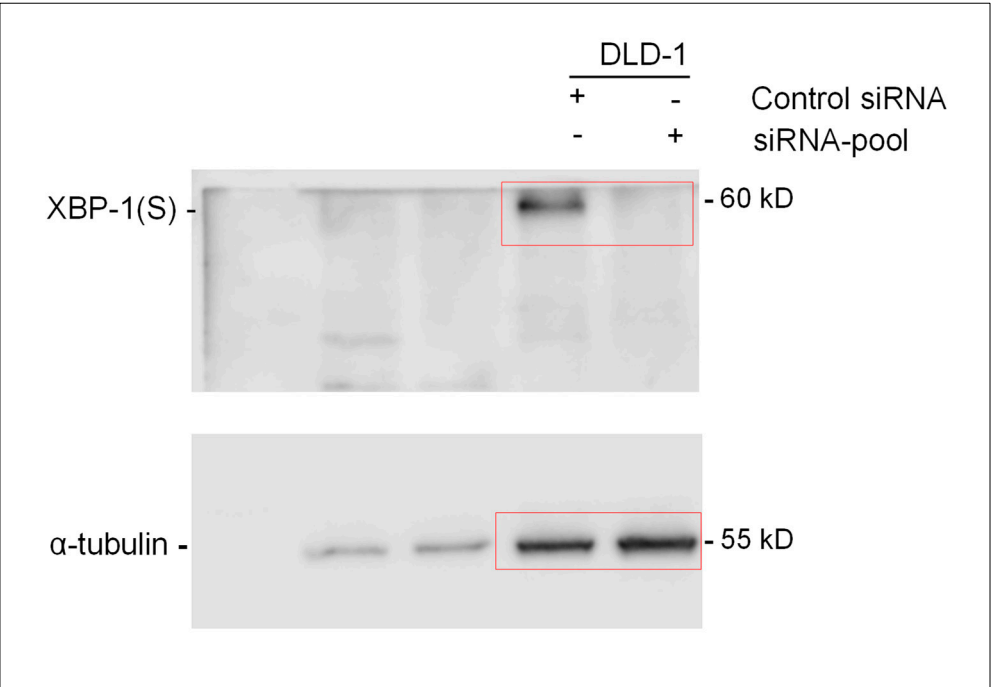
Related to Figure 7a



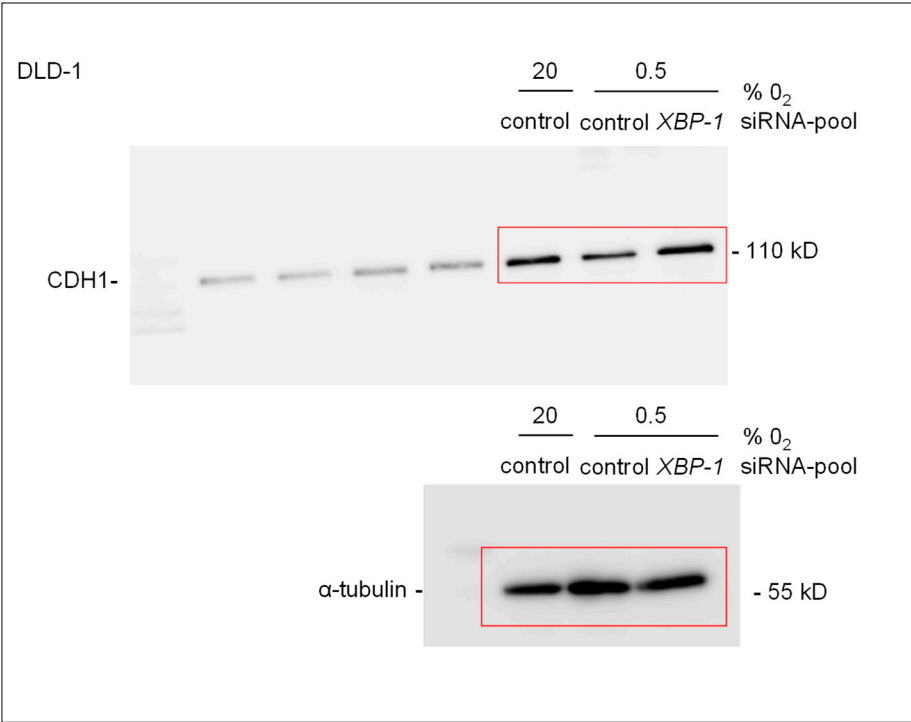
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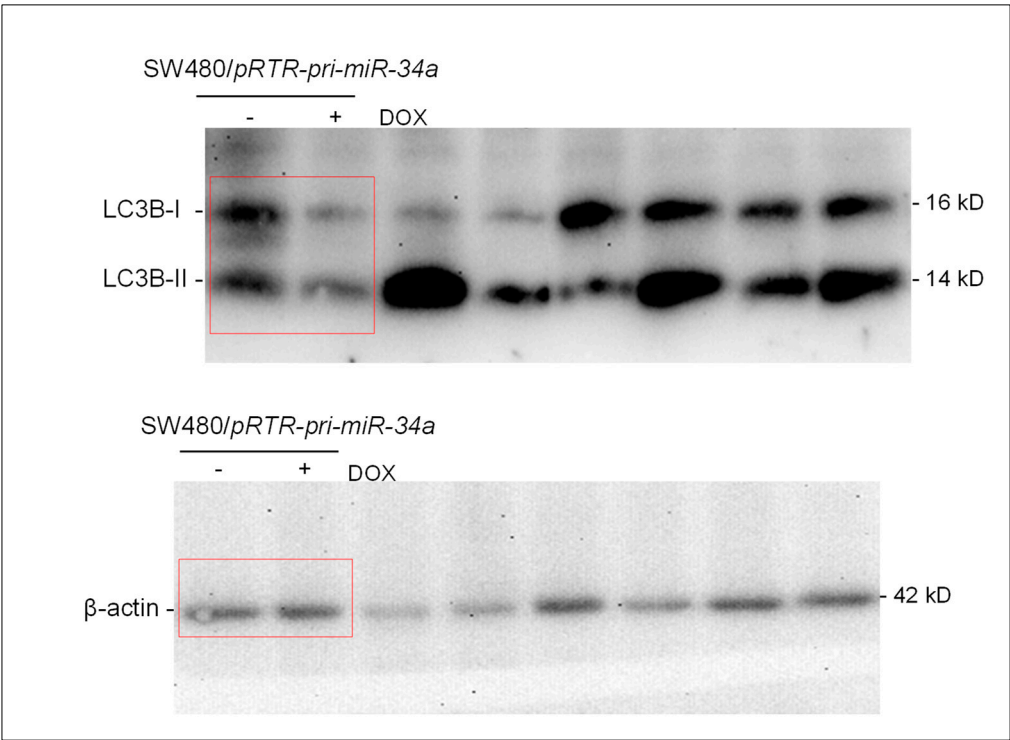
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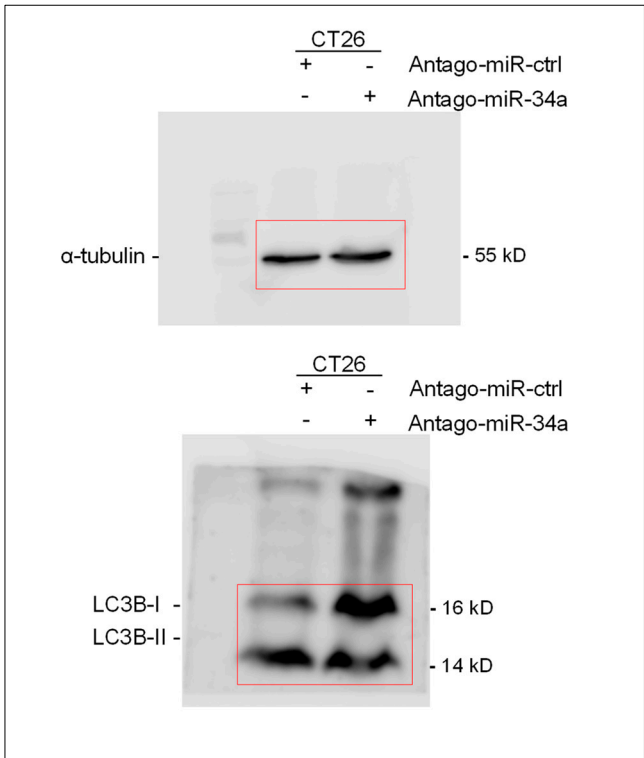
Related to Figure S1d



Related to Figure S2e



Related to Figure S2f



Supplementary Table S1: Oligonucleotides used for quantitative real-time PCR analyses

gene	forward (5' – 3')	reverse (5' – 3')
human <i>GAPDH</i>	GTTGCCATCAATGACCCCTT	CTCCACGACGTACTCAGCG
human <i>XPB-1</i>	AAGCCAAGGGGAATGAAGT	CCAGAATGCCCAACAGGATA
human <i>XPB1-(S)</i>	GCTGAGTCCGCAGCAGGTG	GCTGGCAGGCTCTGGGGAAG
human <i>IRE1A</i>	CGGGAGAACATCACTGTCCC	CCCGGTAGTGGTGCTTCTTA
human <i>pri-miR34a</i>	CGTCACCTCTTAGGCTTGGA	CATTGGTGTCTGTTGTGCT
human <i>VIMENTIN</i>	TACAGGAAGCTGCTGGAAGG	ACCAGAGGGAGTGAATCCAG
human <i>SNAIL</i>	GCACATCCGAAGCCACAC	GGAGAAGGTCCGAGCACAC
human <i>SLUG</i>	GGGGAGAAGCCTTTTTCTTG	TCCTCATGTTTGTGCAGGAG
mouse <i>Xbp-1(S)</i>	CTGAGTCCGCAGCAGGT	TGTCAGAGTCCATGGGAAGA
mouse <i>Ire1a</i>	CCGAGCCATGAGAAACAAGAA	GGGAAGCGGGAAGTGAAGTAG
mouse <i>TUBULIN</i>	AGTAAACCGTAGCCATGAGG	CCTCCCAGAACTTAGCACC

Supplemental Table S2: List of antibodies

Primary antibodies

epitope	source	company	catalog no.	use	dilution mouse	dilution human
α -Tubulin	mouse	Sigma-Aldrich	#T9026	WB	1:1000	1:1000
B-actin	rabbit	Sigma Aldrich	# A2066	WB	1:1000	1:1000
E-cadherin	mouse	Invitrogen	# 334000	IF		1:50
XPB-1U (29 kda)	rabbit	Cell signaling	#12782	WB	1:1000	1:1000
XPB-1S(56 kda)	rabbit	Cell signaling	#12782	WB, IHC	1:1000, 1:500	1:1000
IRE1A	rabbit	Cell signaling	#3294	WB	1:1000	1:1000
IRE1A (pS724)	rabbit	abcam	ab124945	WB		1:500
Vimentin	rabbit	Cell signaling	#5741	WB		1:1000
LC3B	rabbit	Cell signaling	#2775	WB, IF	1:1000, 1:500	1:1000

WB: Western Blotting; IHC: Immunohistochemistry; IF: Immunofluorescence

Secondary antibodies or conjugates

name	source	company	catalog no.	use	dilution
anti-mouse HRP	goat	Promega	#W4021	WB	1:1000
anti-rabbit HRP	goat	Sigma Aldrich	#A0545	WB, ICH	1:1000
Alexa Flour 555 conjugated anti-rabbit	goat	Invitrogen	#A11034	IF	1:500
Alexa Flour 555 conjugated anti-mouse	goat	Invitrogen	#A21422	IF	1:500

WB: Western Blotting; IHC: Immunohistochemistry; IF: Immunofluorescence

Supplemental Table S3: Oligonucleotides used for qChIP

gene	forward (5' – 3')	reverse (5' – 3')
<i>16q22</i>	CTACTCACTTATCCATCCAGGCTAC	ATTCACACACTCAGACATCACAG
<i>UPRE</i>	TCGCATCTTGTTGAATCCGG	AGGGCCTCTCGCCTGGA

Supplemental Table S4: Oligonucleotides used for cloning and mutagenesis of *XBP-1* and *IRE1A* 3'UTRs

gene	forward (5' – 3')	reverse (5' – 3')
Human <i>XBP-1</i> 3'UTR	GGACTAGTCCACATATATACCAAGCCCC	CGAGCTCCAGAAGCTACACTAGCAGG
Human <i>XBP-1</i> 3'UTR mutant	CTGCTTTTCATCCAGCTCGCGAGCAAAGC CATCTTCCTG	CAGGAAGATGGCTTTGCTCGC GAGCTGGATGAAAGCAG
Human <i>IRE1A</i> 3'UTR	GGACTAGTCTCCTGGCAGGAAGTCATCAG	CGAGCTCCCTTTATTGTGGTT GGCTCGAC
Human <i>IRE1A</i> 3'UTR mutant	CCCTTTTCCTTGACTATTACACGTGGTGGA GGATAGCAGAG	CTCTGCTATCCTCCACCACGT GTAATAGTCAAGGAAAAGGG

Supplemental Table S5: Vectors used in this study

Name	Insert	Reference
pRTR	/	[40]
pRTR/ <i>p53</i> -VSV	human <i>TP53</i>	[41]
pRTR/ <i>pri-miR34a</i>	human <i>pri-miR-34a</i>	[42]