

Supplementary Information

Survivin and XIAP – two potential biological targets in follicular thyroid carcinoma

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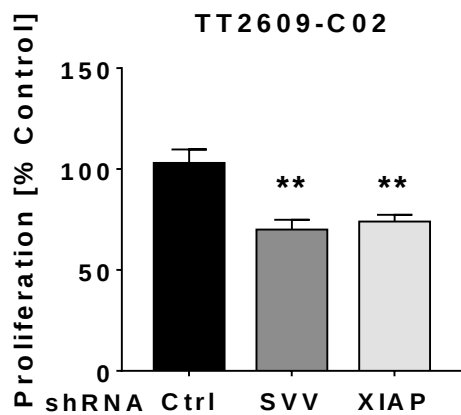
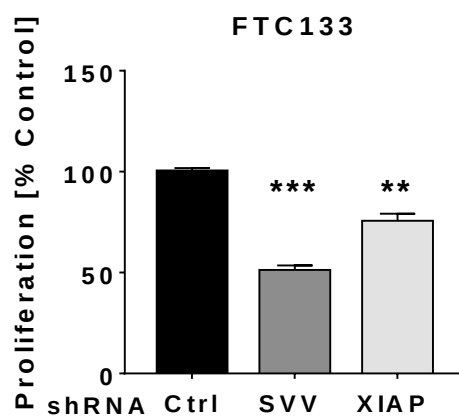
Correspondence to: Andreas Krieg, Department of Surgery (A), Heinrich-Heine-University and University Hospital Duesseldorf, Moorenstr. 5, Bldg. 12.46, 40225 Duesseldorf, Germany; Phone: + 49 211 81 19251; Fax: + 49 211 81 19205; Email: andreas.krieg@med.uni-duesseldorf.de

Supplementary Figure 1: Inhibition of survivin and XIAP impairs FTC cell proliferation in vitro. Gene-specific shRNA knockdown of survivin (SVV) and XIAP decreased cell proliferation in both FTC cell lines TT2609-C02 **(a)** and FTC133 **(b)**. Non-specific shRNA served as control (Ctrl). All values are expressed in means + SEM of at least three independent experiments. Statistical significance was calculated by two-tailed nonparametric Mann-Whitney test (** $p < 0.01$, *** $p < 0.001$).

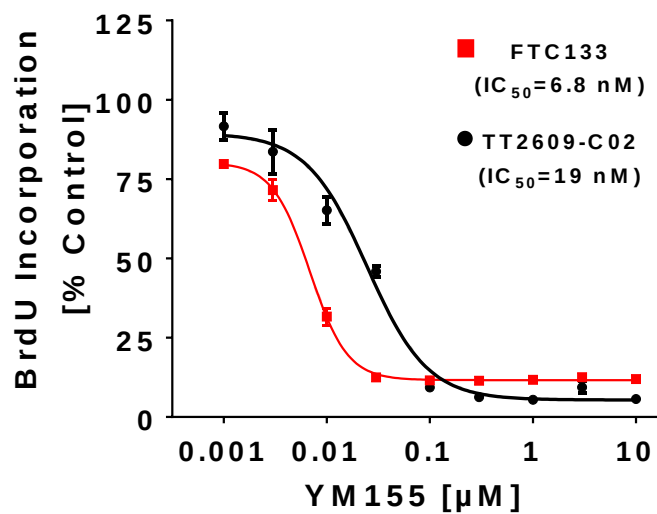
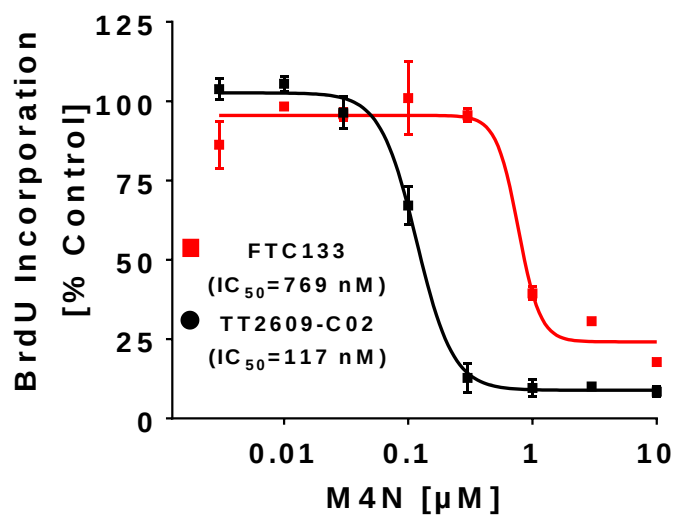
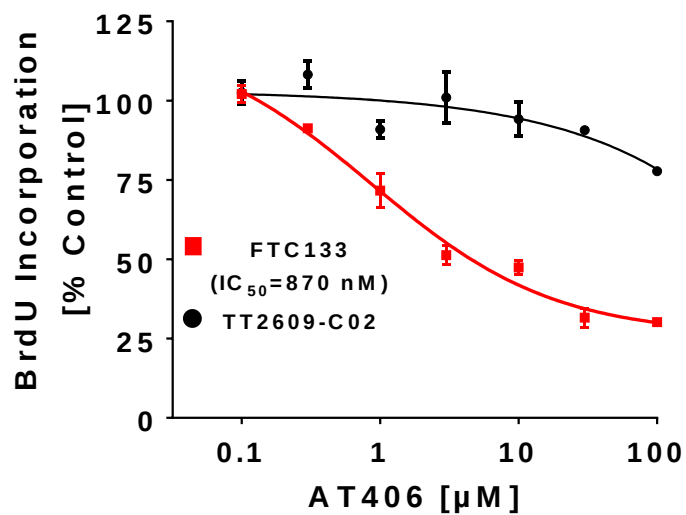
Supplementary Figure 2: Effect of small molecule survivin or XIAP inhibitors on proliferation of FTC cells. FTC cell lines TT2609-C02 and FTC133 were treated with increasing concentrations of **(a)** YM155, **(b)** M4N and **(c)** AT406. Changes in cell proliferation are illustrated in a logarithmic fashion to demonstrate the dose dependent effect of the respective compounds. IC₅₀ values represent the mean 50 % inhibitory concentration, where applicable.

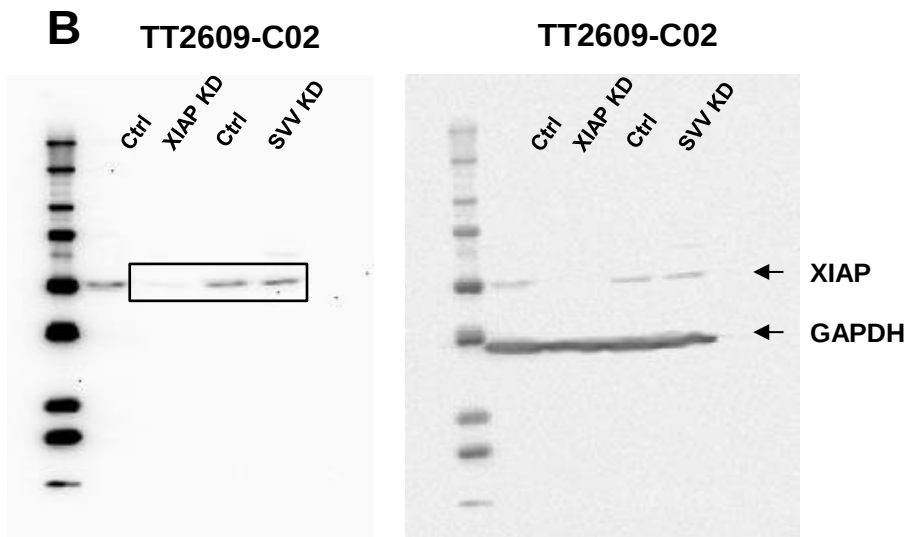
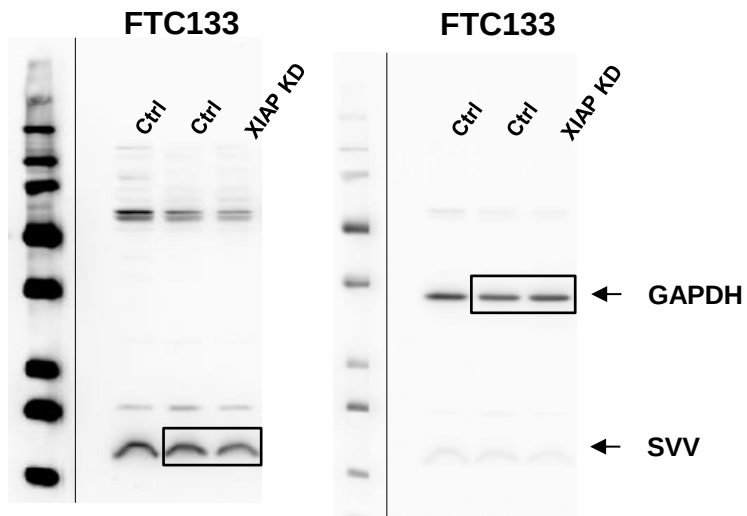
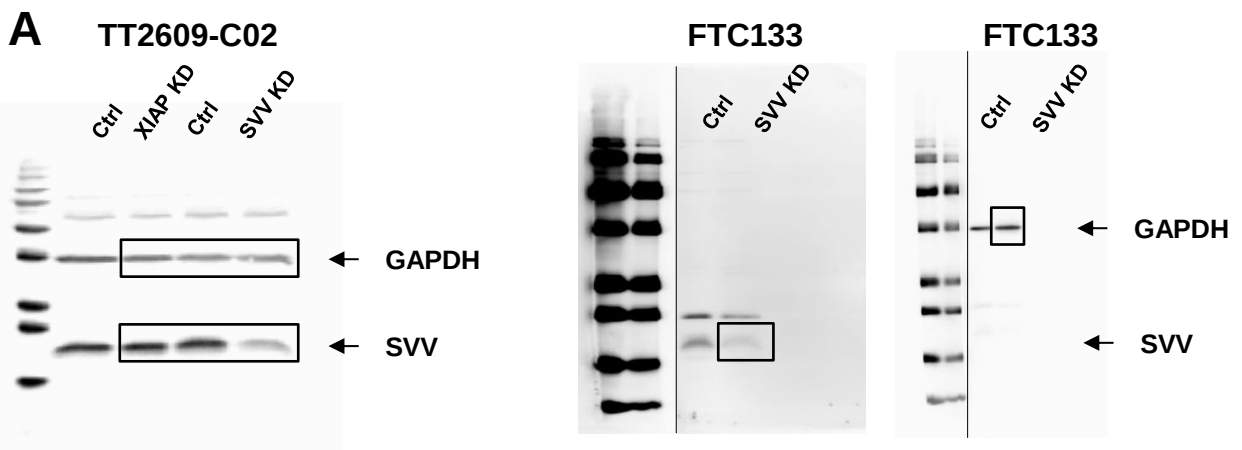
Supplementary Figure 3: Full-length western blots.

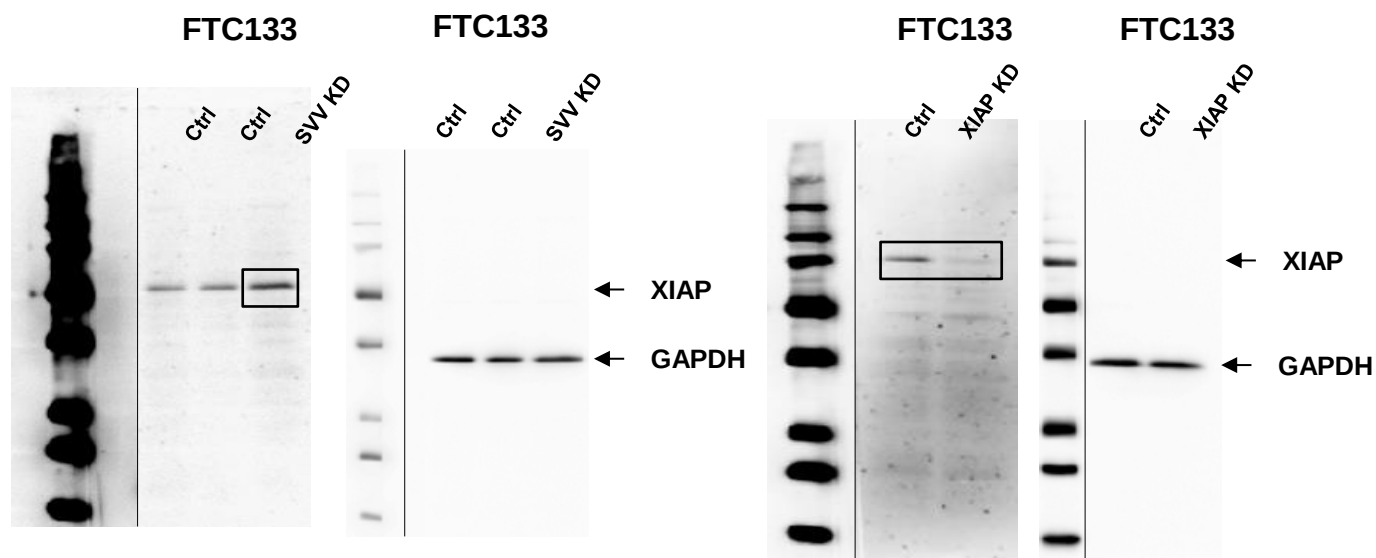
Blots display the protein expression of **(a)** survivin (SVV) and **(b)** XIAP in the gene-specific shRNA knockdown cell lines SVV KD (survivin knockdown) and XIAP KD (XIAP knockdown) as compared to the non-specific shRNA transfected control cell line (Ctrl). Both FTC cell lines TT2609-C02 and FTC133 were treated with increasing concentrations of YM155, M4N and AT406 and protein expression levels of IAP family members **(c)** survivin, **(d)** XIAP, **(e)** cIAP1 and **(f)** cIAP2 were investigated. Black lanes are inserted where lanes were not directly adjacent on the original blot. The used lanes are labelled by black rectangles. GAPDH served as loading control.

A**B**

Supplementary Figure 1

A**B****C****Supplementary Figure 2**



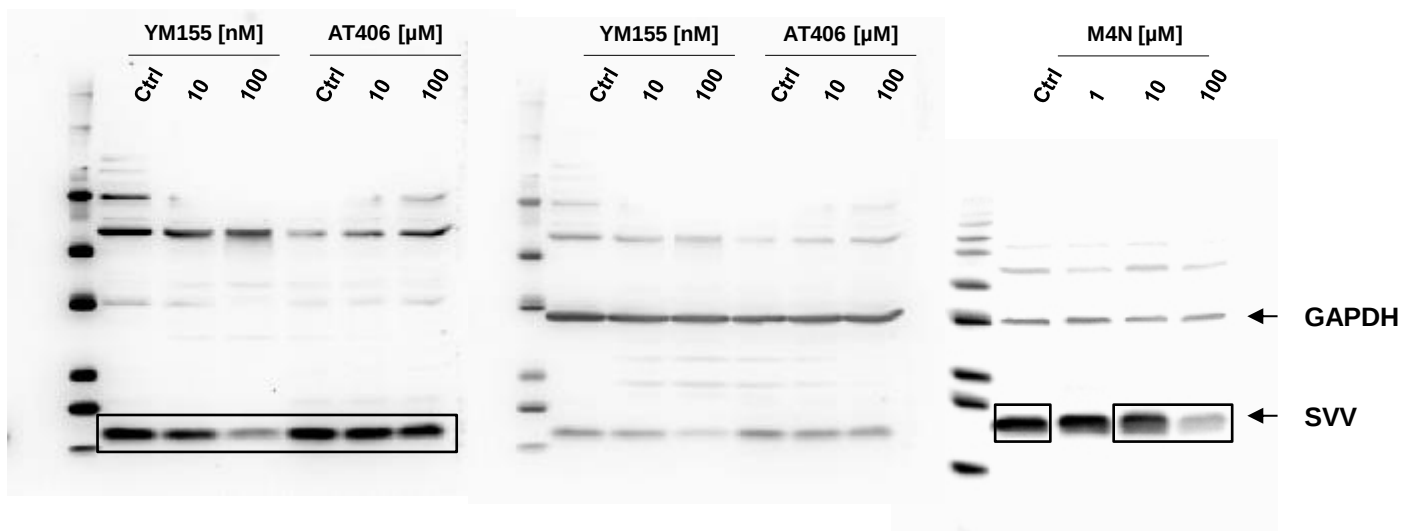


C

TT2609-C02

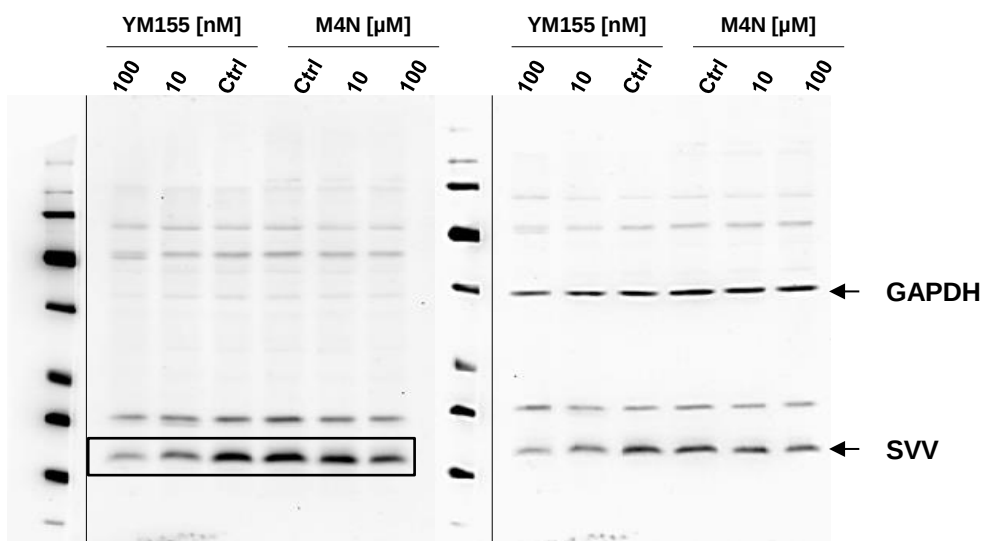
TT2609-C02

TT2609-C02



FTC133

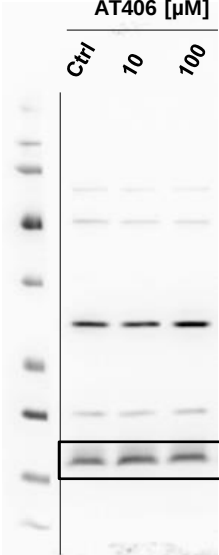
FTC133



FTC133

AT406 [μ M]

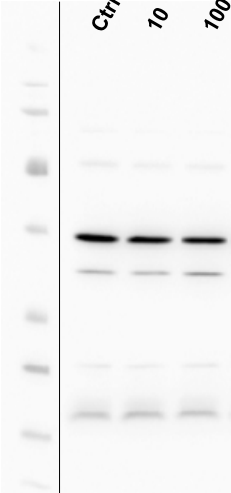
Ctrl 10 100



FTC133

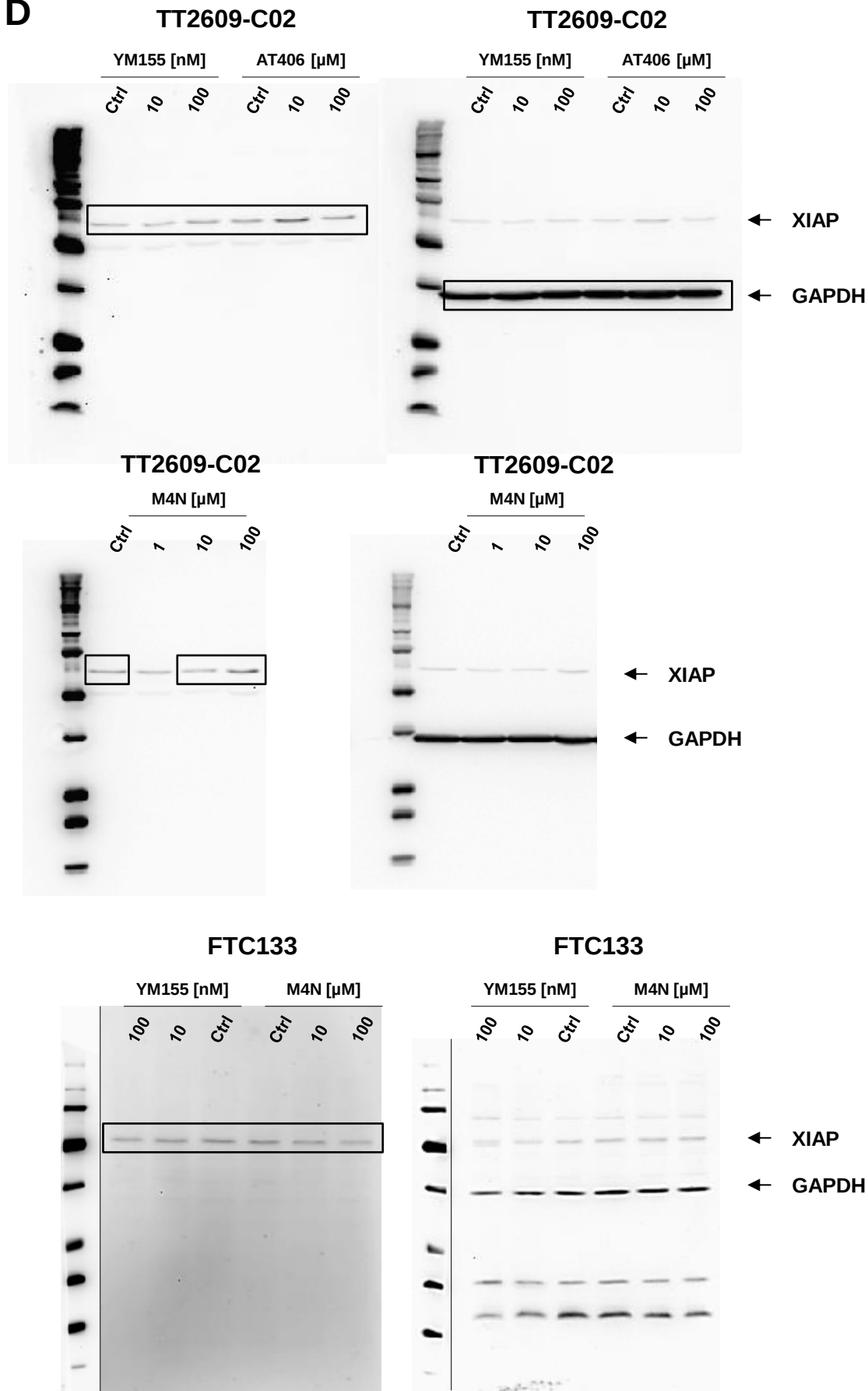
AT406 [μ M]

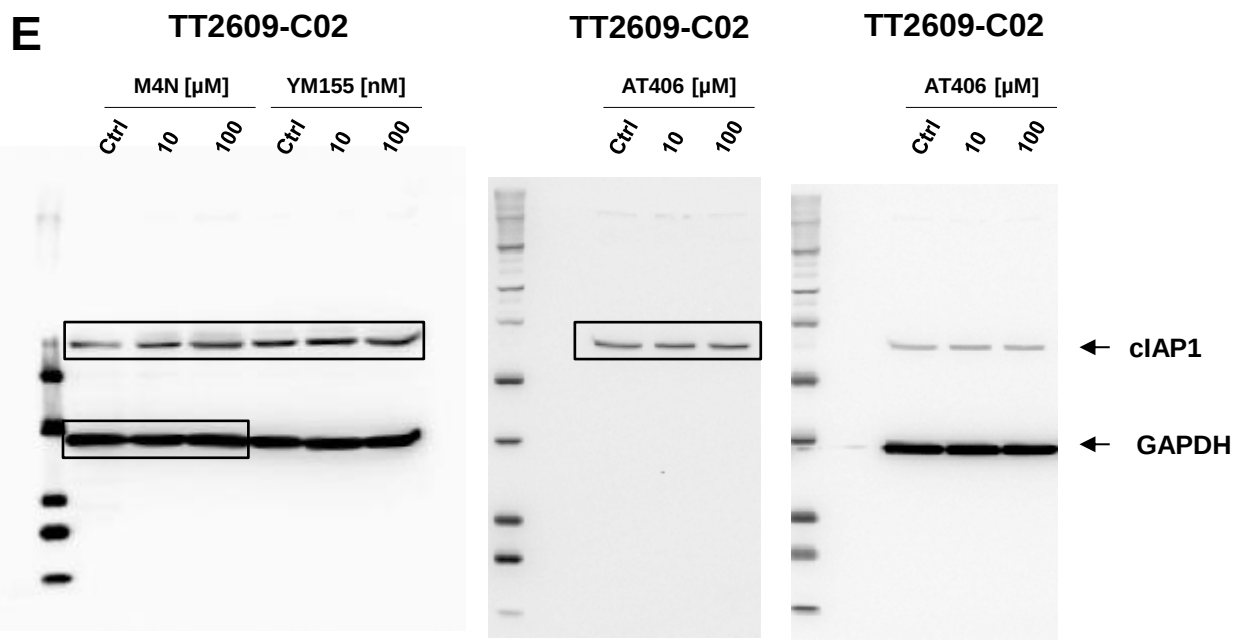
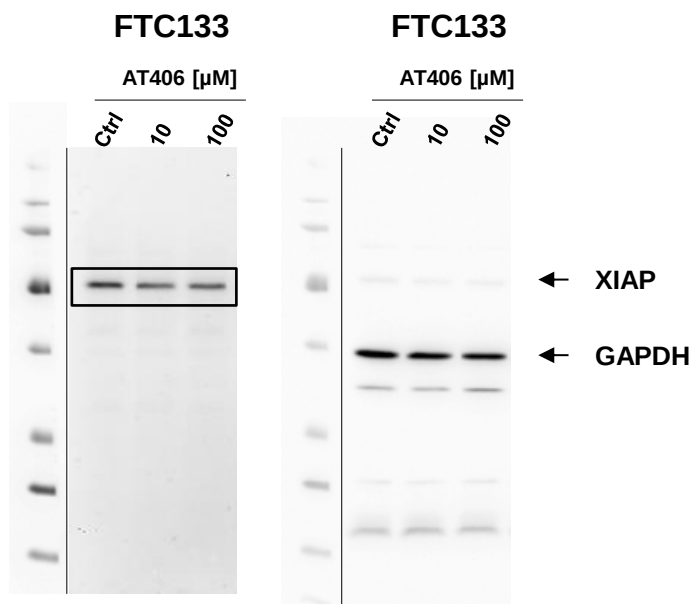
Ctrl 10 100



← GAPDH

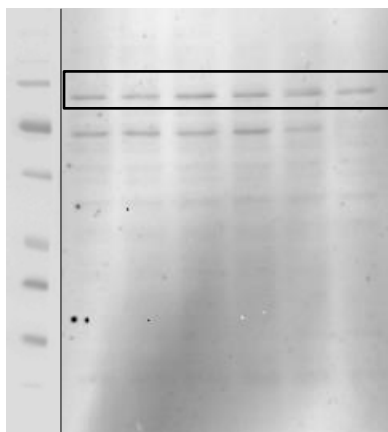
← SVV

D



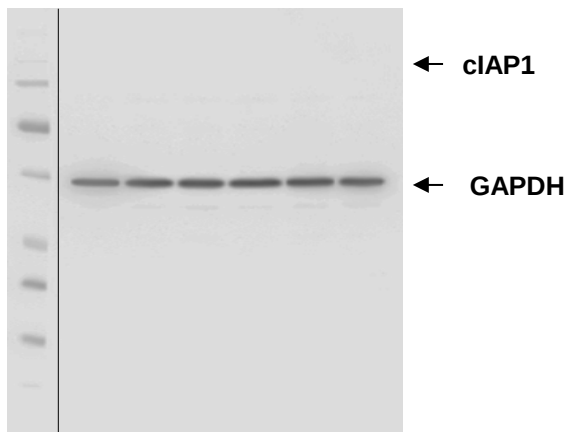
FTC133

M4N [μ M]			YM155 [nM]		
Ctrl	10	100	Ctrl	10	100



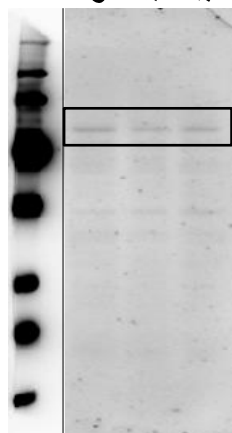
FTC133

M4N [μ M]			YM155 [nM]		
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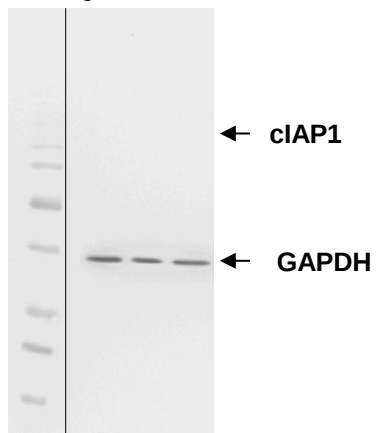
FTC133

AT406 [μ M]		
Ctrl	10	100



FTC133

AT406 [μ M]		
Ctrl	10	100



F

TT2609-C02

TT2609-C02

M4N [μ M]

YM155 [nM]

M4N [μ M]

YM155 [nM]

Ctrl

10

100

Ctrl

10

100

Ctrl

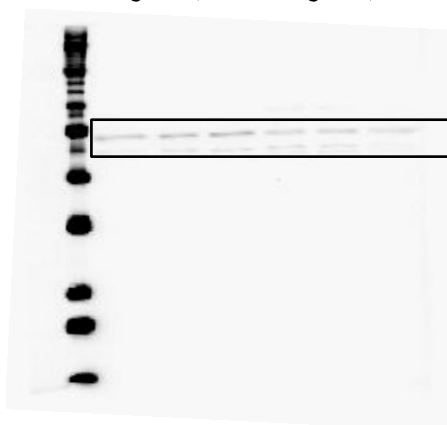
10

100

Ctrl

10

100



← cIAP2

← GAPDH

TT2609-C02

TT2609-C02

AT406 [μ M]

AT406 [μ M]

Ctrl

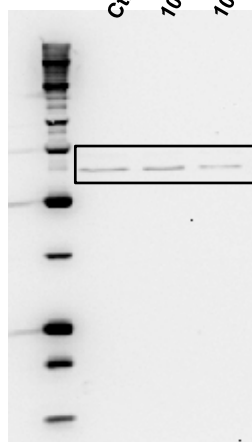
10

100

Ctrl

10

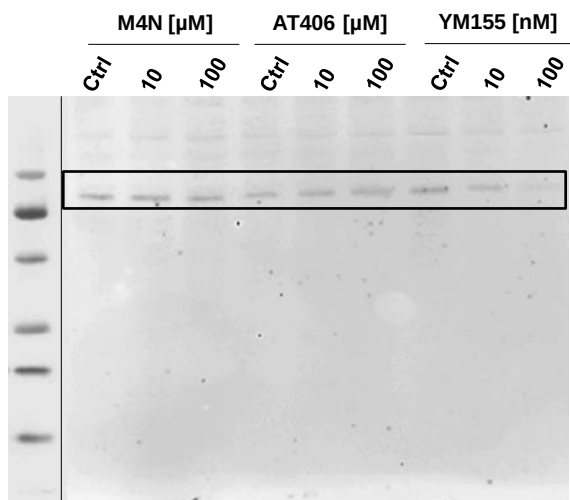
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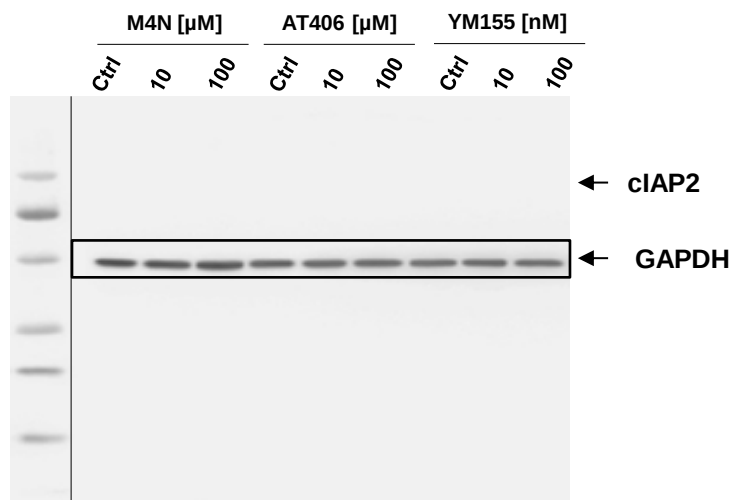
← cIAP2

← GAPDH

FTC133



FTC133



Supplementary Figure 3

Supplementary Table 1: Patient characteristics (n = 44)

Variables	No. of patients (%)
Total	44
Age	
Median (range); years	59 (17–85)
Gender	
Male	19 (43)
Female	25 (57)
Type of surgery	
Hemithyroidectomy	3 (7)
with unilateral ND	1 (2)
Subtotal thyroidectomy	11 (25)
with unilateral ND	4 (9)
with central LND	1 (2)
Total thyroidectomy	7 (16)
with central LND	4 (9)
with unilateral ND	2 (5)
with bilateral ND	11 (25)
Tumour stage	
T1/2	24 (55)
T3/4	20 (45)
Lymph node metastasis	
N0	41 (93)
N1a/b	3 (7)
Distant metastasis	
M0	38 (86)
M1	6 (14)
UICC stage	
UICC I/II	22 (50)
UICC III/IV	22 (50)

Abbreviations: ND = neck dissection; LND = lymph node dissection; UICC = Union internationale contre le cancer.

Supplementary Table 2: Correlation between survivin or XIAP and clinicopathological parameters in FTC

Variables	Survivin expression		<i>p</i> -value	XIAP expression		<i>p</i> -value
	Low, n = 14 (%)	High, n = 30 (%)		Low, n = 20 (%)	High, n = 24 (%)	
Age, mean $\pm$ s.d.	49 $\pm$ 14.7	60.5 $\pm$ 17.2	0.3319	60 $\pm$ 14	53 $\pm$ 19	0.7626
Gender						
Male	5 (36)	14 (47)	0.5340	9 (45)	10 (42)	1
Female	9 (64)	16 (53)		11 (55)	14 (58)	
Tumour stage						
T1/2	13(93)	11 (37)	0.0008***	10 (50)	14 (58)	0.7619
T3/4	1 (7)	19 (63)		10 (50)	10 (42)	
Lymph node metastasis						
N0	12 (86)	29 (97)	0.2336	19 (95)	22 (92)	1
N1a/b	2 (14)	1 (3)		1 (5)	2 (8)	
Distant metastasis						
M0	14 (100)	24 (80)	0.1547	18 (90)	20 (83)	0.6731
M1	0 (0)	6 (20)		2 (10)	4 (17)	
UICC stage						
UICC I/II	11 (79)	11 (37)	0.0217*	10 (50)	12 (50)	1
UICC III/IV	3 (21)	19 (63)		10 (50)	12 (50)	

Abbreviations: FTC = follicular thyroid carcinoma; XIAP = X-Linked inhibitor of apoptosis protein; UICC = Union internationale contre le cancer; * $p < 0.05$; *** $p < 0.001$.