

***Toxoplasma* GRA16 attenuates tau hyperphosphorylation and enhances autophagy in thrombin-treated HT-22 hippocampal neuronal cells**

Seung-Hwan Seo^{1, 2}, Do-Won Ham², Ji-Eun Lee², Eun-Hee Shin^{1, 2, 3, *}

¹Institute of Endemic Diseases, Medical Research Center, Seoul National University, Seoul, Korea.

²Department of Tropical Medicine and Parasitology, Seoul National University College of Medicine, Seoul, Korea.

³Seoul National University Bundang Hospital, Seongnam, Korea.

*Corresponding author

Eun-Hee Shin, Ph. D.

Tel: +82-2-740-8344

E-mail: ehshin@snu.ac.kr

Raw data

Figure 1.

Thrombin induces tau hyperphosphorylation and APOE expression, which are pathologically relevant targets of AD, and partially attenuates the expression of autophagy-related factors regulated by FOXO3A

Figure 1A. Fluorescence expression of p-Tau (AT8) in Thrombin-treated HT-22 control cells

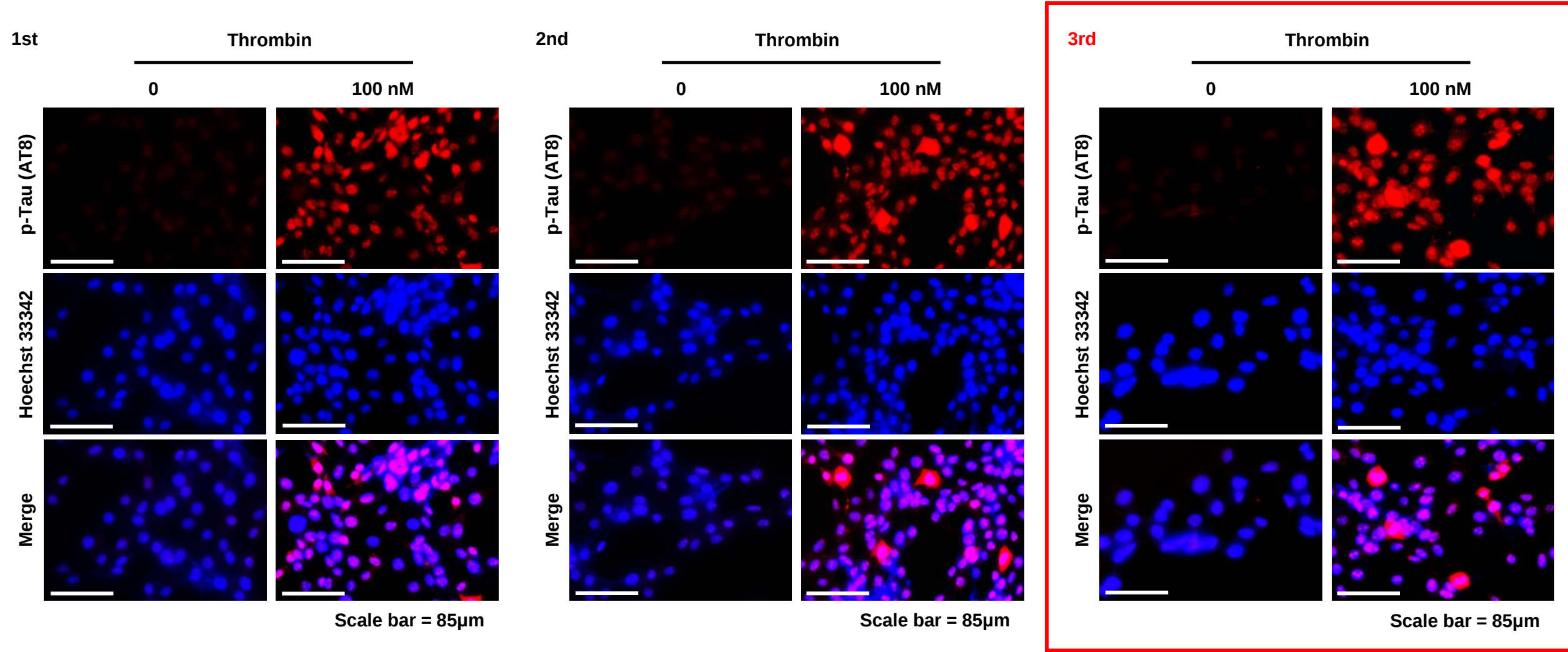
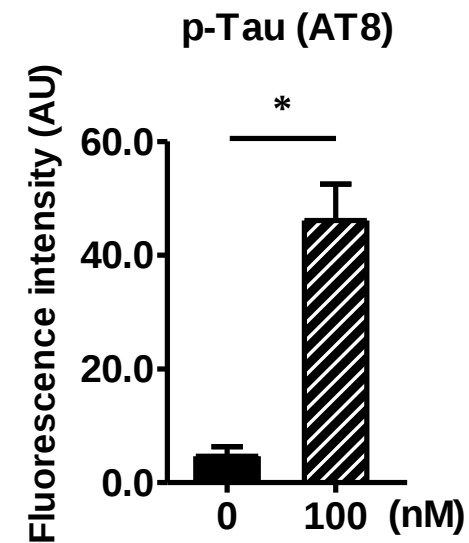
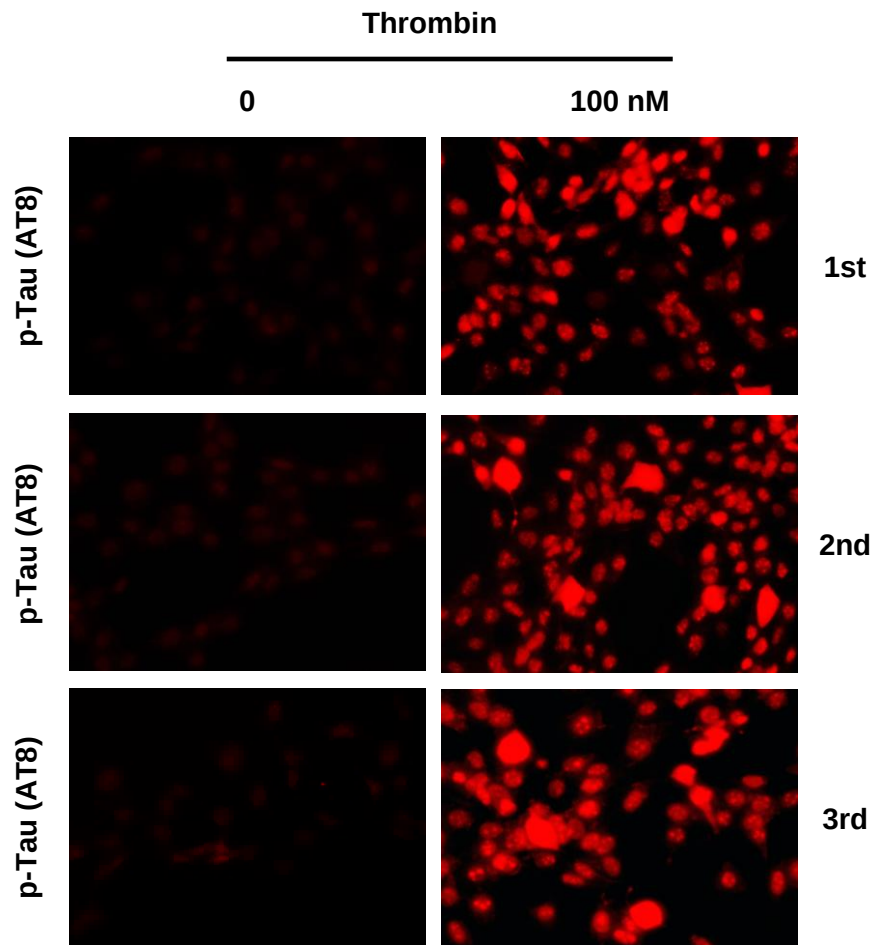


Figure 1B. Fluorescence intensity of p-Tau (AT8) in Thrombin-treated HT-22 control cells



	HT-22 cells	
Thrombin	0	100nM
1	4.844	38.815
2	6.225	50.785
3	2.833	48.692
Ave.	4.634	46.097

Fluorescence intensity using ImageJ

Figure 1C. Tau and p-Tau (S202/T205) expression in Thrombin-treated HT-22 control cells

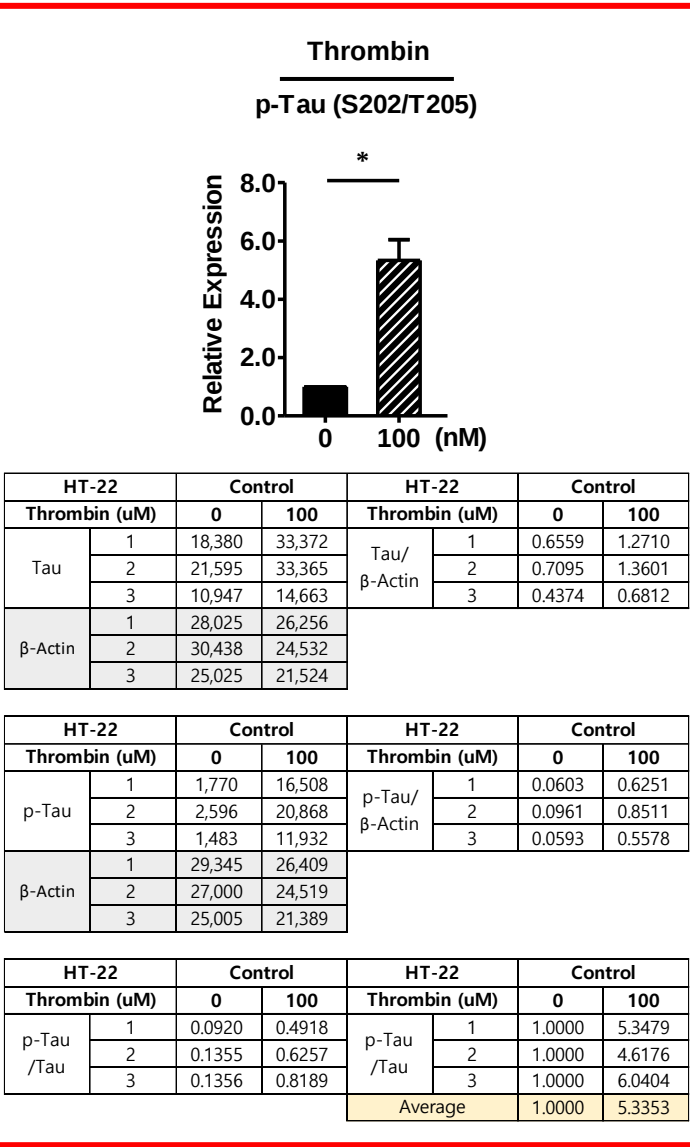
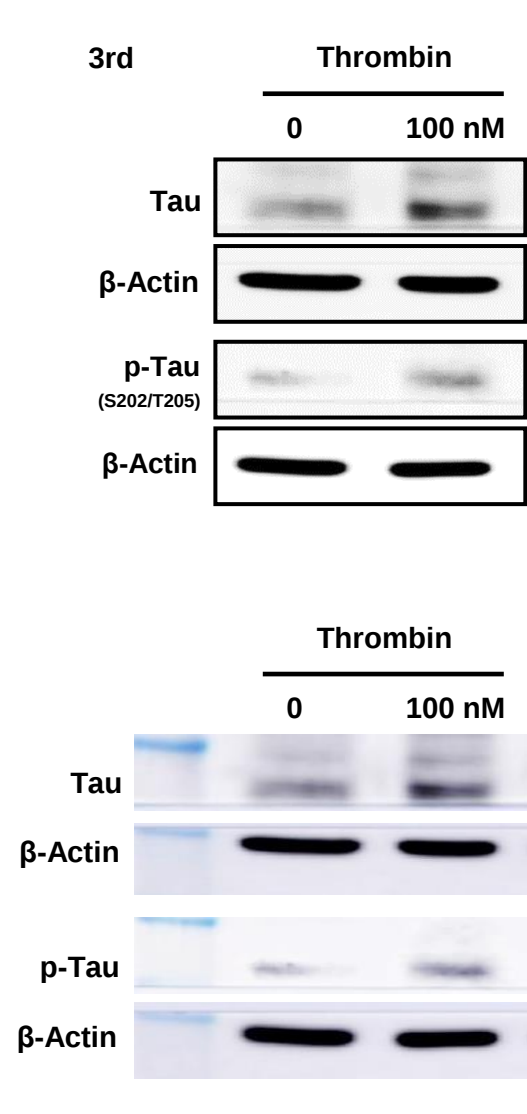
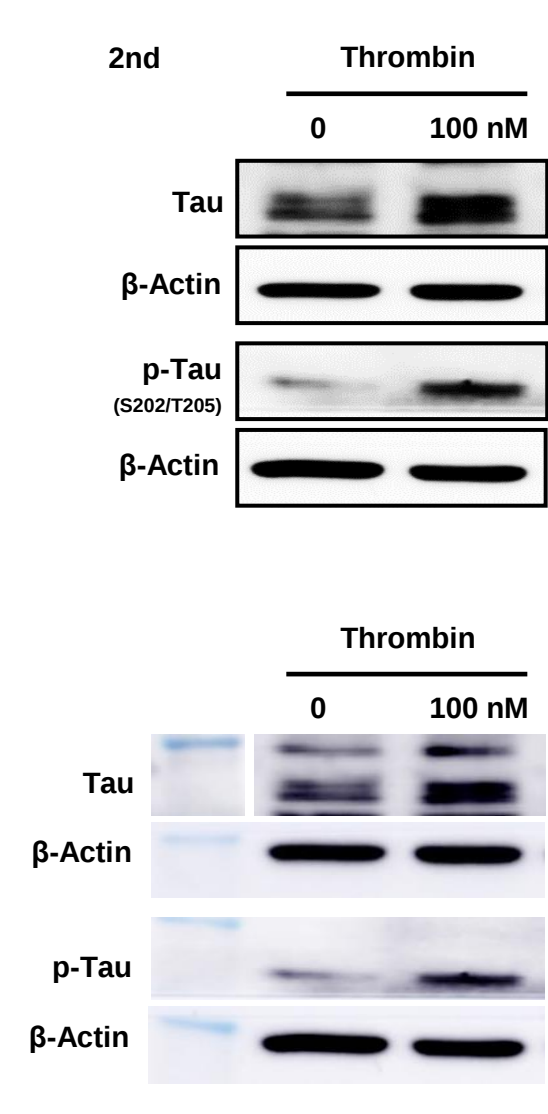
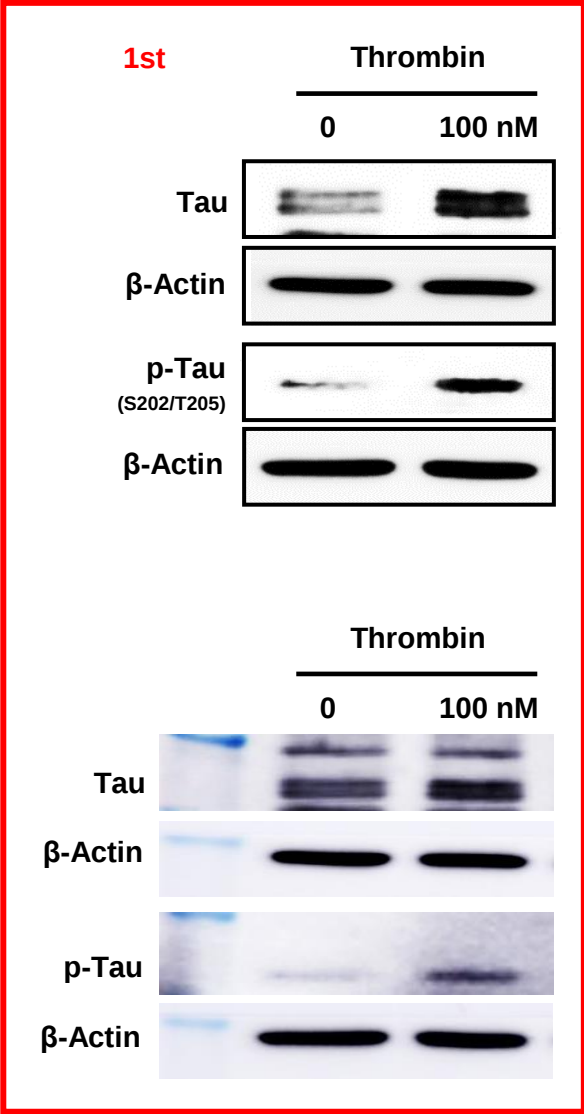


Figure 1D. Tau and p-Tau (S422) expression in Thrombin-treated HT-22 control cells

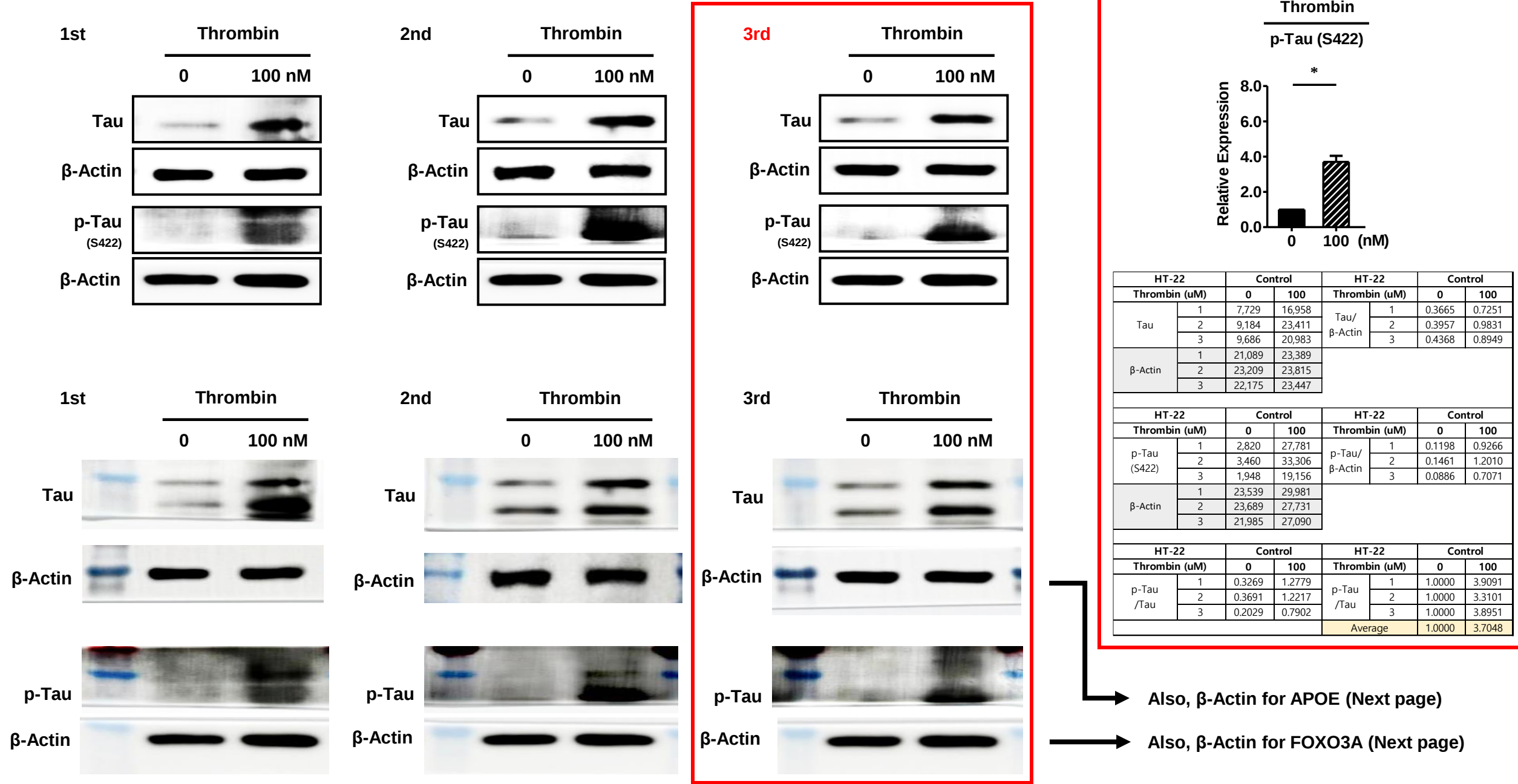
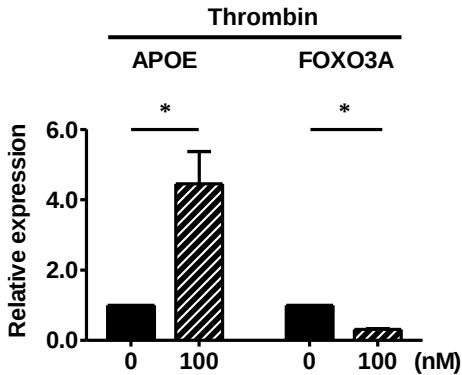
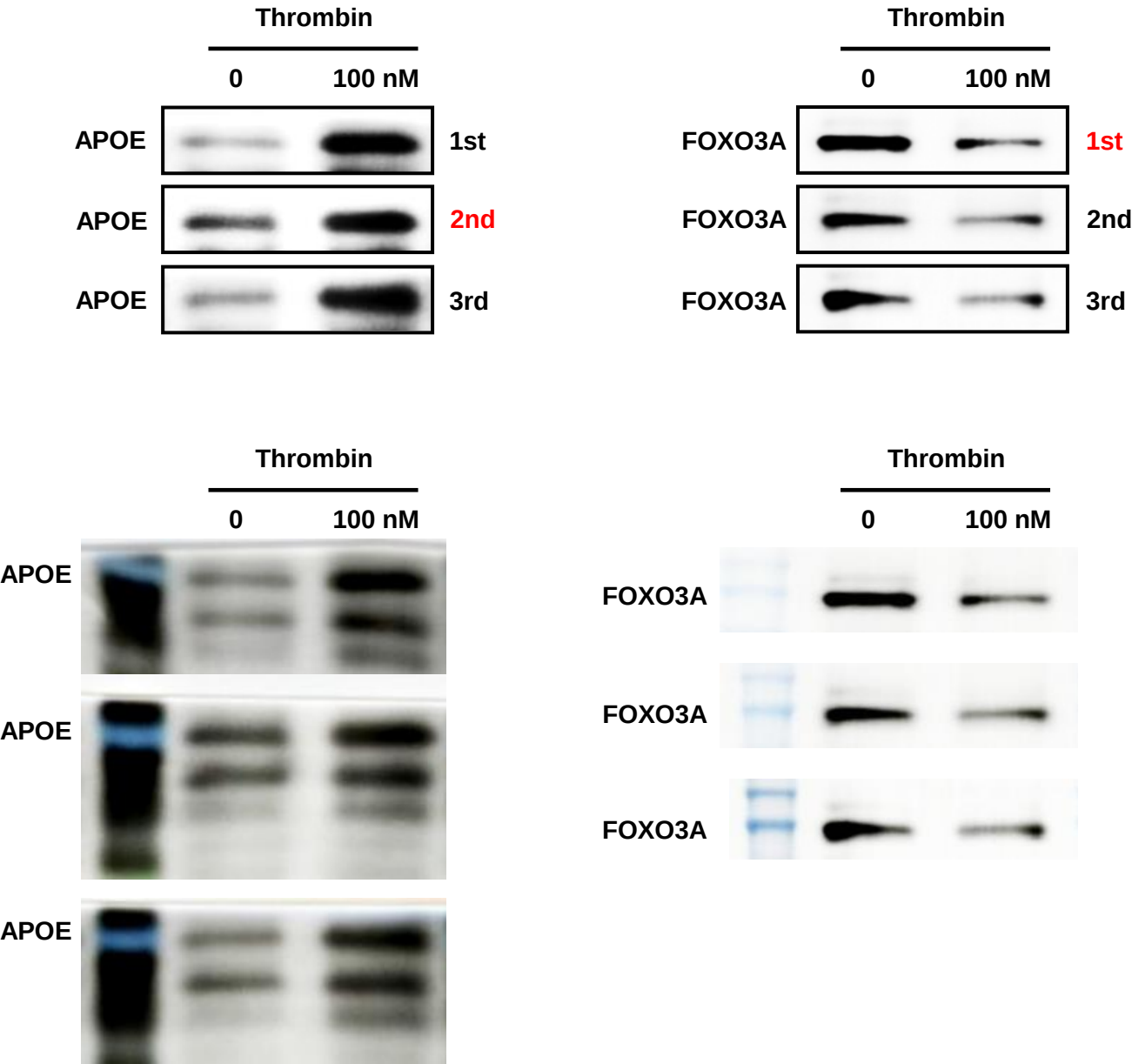


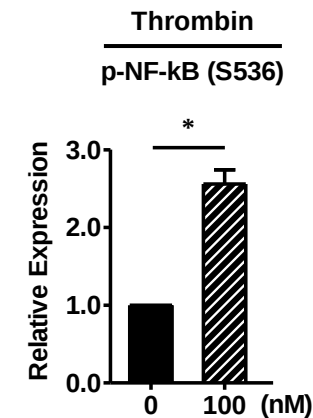
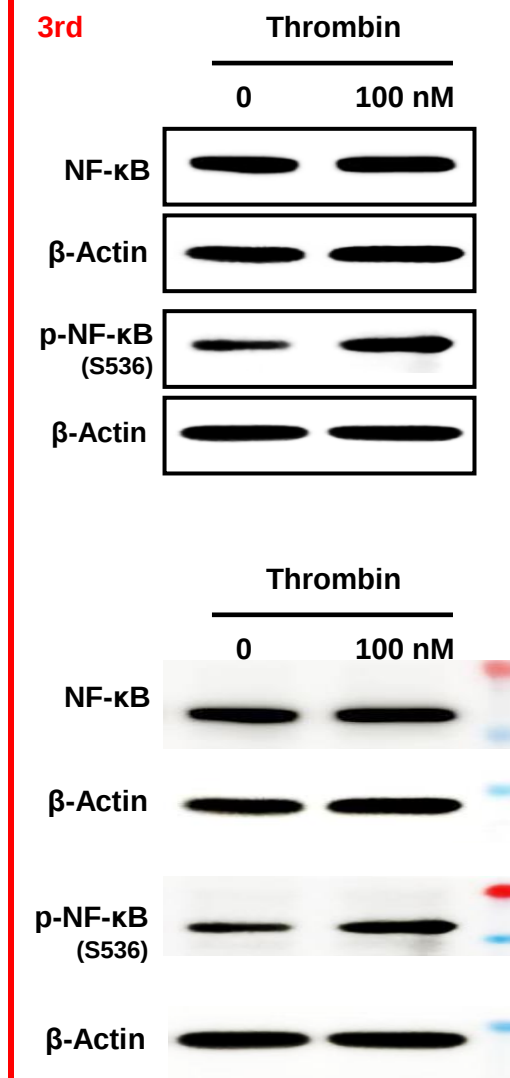
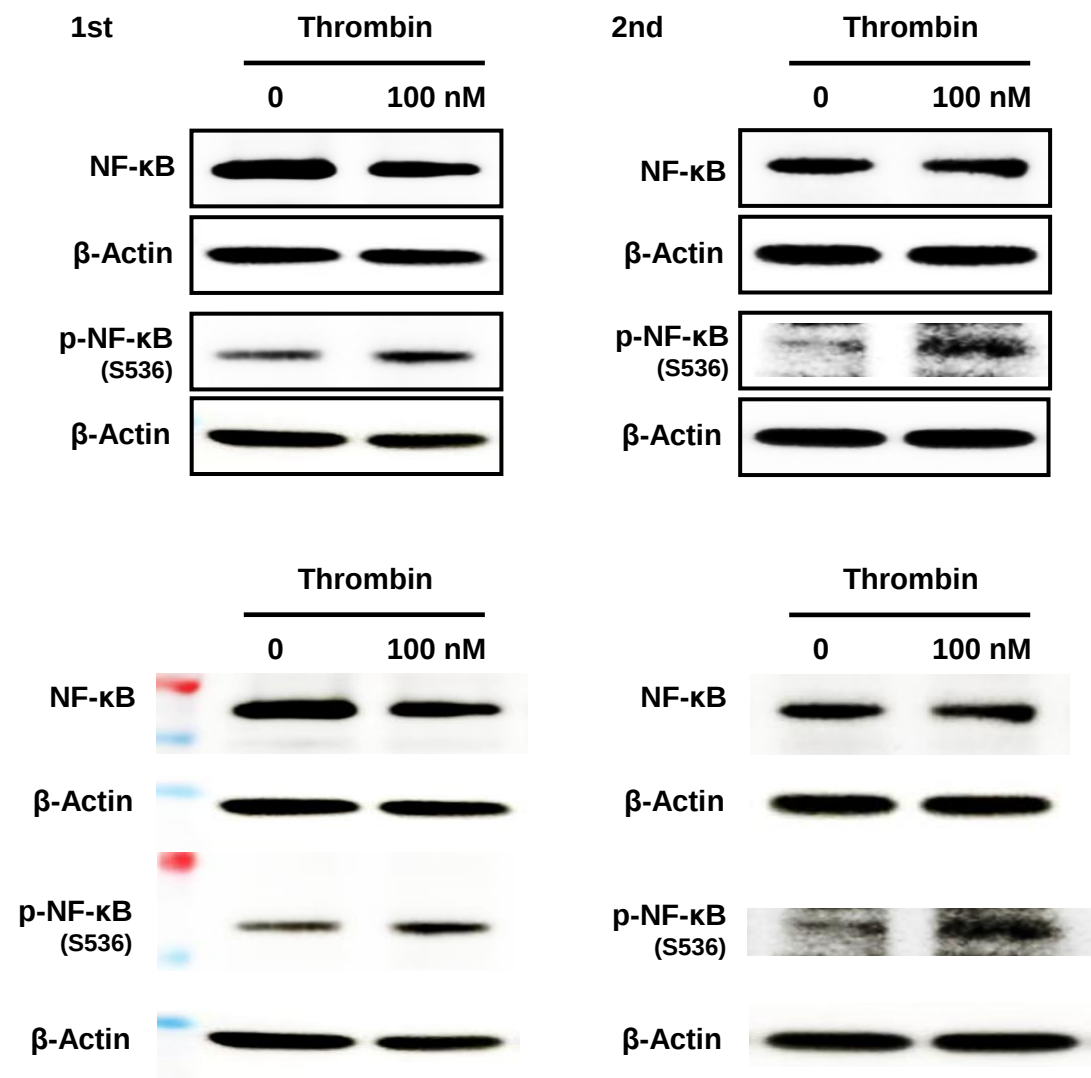
Figure 1F. APOE, FOXO3A expression in Thrombin-treated HT-22 control cells



HT-22		Control		HT-22		Control	
Thrombin (uM)		0	100	Thrombin (uM)		0	100
APOE	1	4,187	23,849	APOE/ β-Actin	1	0.1985	1.0197
	2	6,392	22,329		2	0.2754	0.9376
	3	5,223	26,595		3	0.2355	1.1343
β-Actin	1	21,089	23,389	HT-22		Control	
	2	23,209	23,815	Thrombin (uM)		0	100
	3	22,175	23,447	APOE/ β-Actin	1	1.0000	5.1363
					2	1.0000	3.4044
					3	1.0000	4.8159
				Average		1.0000	4.4522

HT-22		Control		HT-22		Control	
Thrombin (uM)		0	100	Thrombin (uM)		0	100
FOXO3A	1	20,398	7,869	FOXO3A/ β-Actin	1	0.8666	0.2625
	2	16,583	6,480		2	0.7000	0.2337
	3	16,989	6,228		3	0.7728	0.2299
β-Actin	1	23,539	29,981	HT-22		Control	
	2	23,689	27,731	Thrombin (uM)		0	100
	3	21,985	27,090	FOXO3A/ β-Actin	1	1.0000	0.3029
					2	1.0000	0.3338
					3	1.0000	0.2975
				Average		1.0000	0.3114

Figure 1E. NF- κ B and p-NF- κ B (S536) expression in Thrombin-treated HT-22 control cells



HT-22		Control		HT-22		Control	
Thrombin (uM)		0	100	Thrombin (uM)		0	100
NF-kB	1	25,351	16,601	NF-kB/ β-Actin	1	0.8990	0.6933
	2	15,112	14,684		2	0.6048	0.5832
	3	15,796	17,493		3	0.7662	0.7032
β-Actin	1	28,199	23,946				
	2	24,989	25,178				
	3	20,617	24,876				

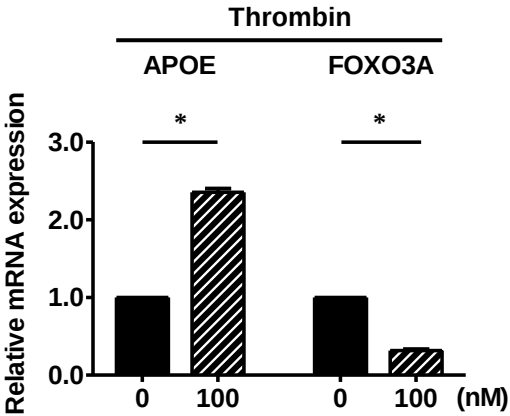
HT-22		Control		HT-22		Control	
Thrombin (uM)		0	100	Thrombin (uM)		0	100
p-NF-kB	1	10,106	13,676	p-NF-kB /β-Actin	1	0.3774	0.7222
	2	7,388	21,786		2	0.2908	0.8159
	3	6,647	14,280		3	0.2922	0.6134
β-Actin	1	26,780	18,937				
	2	25,402	26,701				
	3	22,751	23,281				

HT-22		Control		HT-22		Control	
Thrombin (uM)		0	100	Thrombin (uM)		0	100
p-NF-kB /NF-kB	1	0.4198	1.0417	p-NF-kB /NF-kB	1	1.0000	2.4816
	2	0.4809	1.3990		2	1.0000	2.9091
	3	0.3813	0.8722		3	1.0000	2.2874
				Average		1.0000	2.5594

Figure 1G. Relative mRNA expressions in Thrombin-treated HT-22 control cells

HT-22			APOE			HT-22			APOE			HT-22			APOE		
Δ CT			0	100 nM		$\Delta\Delta$ CT			0	100 nM		$2^{-\Delta\Delta$ CT}			0	100 nM	
1	8.6521	7.4621	1	0.0000	-1.1901	1	1.0000	2.2816				1	1.0000	2.2816			
2	8.7023	7.4852	2	0.0000	-1.2171	2	1.0000	2.3248				2	1.0000	2.3248			
3	8.7286	7.4823	3	0.0000	-1.2463	3	1.0000	2.3723				3	1.0000	2.3723			
4	8.7343	7.4763	4	0.0000	-1.2580	4	1.0000	2.3917				4	1.0000	2.3917			
5	8.7329	7.4723	5	0.0000	-1.2606	5	1.0000	2.3959				5	1.0000	2.3959			
Average	8.7101	7.4756	Average	0.0000	-1.2344	Average	1.0000	2.3533				Average	1.0000	2.3533			

HT-22			FOXO3A			HT-22			FOXO3A			HT-22			FOXO3A		
Δ CT			0	100 nM		$\Delta\Delta$ CT			0	100 nM		$2^{-\Delta\Delta$ CT}			0	100 nM	
1	8.8390	10.4690	1	0.0000	1.6300	1	1.0000	0.3231				1	1.0000	0.3231			
2	8.8558	10.4857	2	0.0000	1.6299	2	1.0000	0.3231				2	1.0000	0.3231			
3	8.8811	10.4761	3	0.0000	1.5951	3	1.0000	0.3310				3	1.0000	0.3310			
4	8.8426	10.4804	4	0.0000	1.6378	4	1.0000	0.3213				4	1.0000	0.3213			
5	8.8496	10.6510	5	0.0000	1.8014	5	1.0000	0.2869				5	1.0000	0.2869			
Average	8.8536	10.5124	Average	0.0000	1.6588	Average	1.0000	0.3171				Average	1.0000	0.3171			



HT-22			CDK5			HT-22			CDK5			HT-22			CDK5		
Δ CT			0	100 nM		$\Delta\Delta$ CT			0	100 nM		$2^{-\Delta\Delta$ CT}			0	100 nM	
1	6.9528	6.8871	1	0.0000	-0.0657	1	1.0000	1.0466				1	1.0000	1.0466			
2	6.9625	6.8712	2	0.0000	-0.0913	2	1.0000	1.0653				2	1.0000	1.0653			
3	6.9514	6.8536	3	0.0000	-0.0978	3	1.0000	1.0701				3	1.0000	1.0701			
4	7.0246	6.9451	4	0.0000	-0.0795	4	1.0000	1.0567				4	1.0000	1.0567			
5	7.0278	6.9434	5	0.0000	-0.0843	5	1.0000	1.0602				5	1.0000	1.0602			
Average	6.9838	6.9001	Average	0.0000	-0.0837	Average	1.0000	1.0598				Average	1.0000	1.0598			

HT-22			PINK1			HT-22			PINK1			HT-22			PINK1		
Δ CT			0	100 nM		$\Delta\Delta$ CT			0	100 nM		$2^{-\Delta\Delta$ CT}			0	100 nM	
1	6.8915	3.9677	1	0.0000	-2.9238	1	1.0000	7.5886				1	1.0000	7.5886			
2	6.8904	4.0244	2	0.0000	-2.8660	2	1.0000	7.2906				2	1.0000	7.2906			
3	7.0105	4.0099	3	0.0000	-3.0006	3	1.0000	8.0033				3	1.0000	8.0033			
4	7.0086	4.1312	4	0.0000	-2.8774	4	1.0000	7.3483				4	1.0000	7.3483			
5	6.9419	4.1130	5	0.0000	-2.8289	5	1.0000	7.1054				5	1.0000	7.1054			
Average	6.9486	4.0492	Average	0.0000	-2.8994	Average	1.0000	7.4672				Average	1.0000	7.4672			

HT-22			OPTN			HT-22			OPTN			HT-22			OPTN		
Δ CT			0	100 nM		$\Delta\Delta$ CT			0	100 nM		$2^{-\Delta\Delta$ CT}			0	100 nM	
1	6.2082	3.3937	1	0.0000	-2.8145	1	1.0000	7.0347				1	1.0000	7.0347			
2	6.1633	3.3996	2	0.0000	-2.7637	2	1.0000	6.7912				2	1.0000	6.7912			
3	6.1656	3.4117	3	0.0000	-2.7539	3	1.0000	6.7456				3	1.0000	6.7456			
4	6.2642	3.4384	4	0.0000	-2.8257	4	1.0000	7.0898				4	1.0000	7.0898			
5	6.2255	3.4705	5	0.0000	-2.7550	5	1.0000	6.7505				5	1.0000	6.7505			
Average	6.2054	3.4228	Average	0.0000	-2.7826	Average	1.0000	6.8824				Average	1.0000	6.8824			

HT-22			ATG12			HT-22			ATG12			HT-22			ATG12		
Δ CT			0	100 nM		$\Delta\Delta$ CT			0	100 nM		$2^{-\Delta\Delta$ CT}			0	100 nM	
1	7.4204	7.7278	1	0.0000	0.3074	1	1.0000	0.8081				1	1.0000	0.8081			
2	7.3978	7.7710	2	0.0000	0.3732	2	1.0000	0.7721				2	1.0000	0.7721			
3	7.5058	7.9165	3	0.0000	0.4106	3	1.0000	0.7523				3	1.0000	0.7523			
4	7.5443	7.9276	4	0.0000	0.3833	4	1.0000	0.7667				4	1.0000	0.7667			
5	7.4907	7.9102	5	0.0000	0.4194	5	1.0000	0.7477				5	1.0000	0.7477			
Average	7.4718	7.8506	Average	0.0000	0.3788	Average	1.0000	0.7694				Average	1.0000	0.7694			

HT-22			BECN1			HT-22			BECN1			HT-22			BECN1		
Δ CT			0	100 nM		$\Delta\Delta$ CT			0	100 nM		$2^{-\Delta\Delta$ CT}			0	100 nM	
1	7.7672	5.3836	1	0.0000	-2.3836	1	1.0000	5.2184				1	1.0000	5.2184			
2	7.7002	5.3940	2	0.0000	-2.3062	2	1.0000	4.9458				2	1.0000	4.9458			
3	7.6906	5.3804	3	0.0000	-2.3102	3	1.0000	4.9594				3	1.0000	4.9594			
4	7.6839	5.4533	4	0.0000	-2.2306	4	1.0000	4.6934				4	1.0000	4.6934			
5	7.7777	5.4743	5	0.0000	-2.3034	5	1.0000	4.9361				5	1.0000	4.9361			
Average	7.7239	5.4171	Average	0.0000	-2.3068	Average	1.0000	4.9506				Average	1.0000	4.9506			

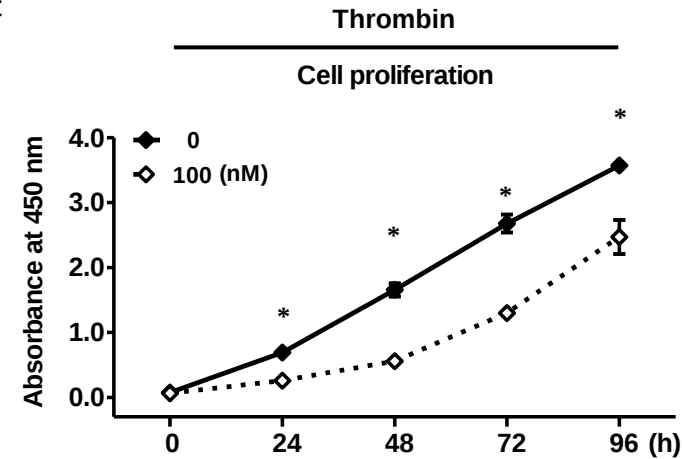
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Δ CT			0	100 nM		$\Delta\Delta$ CT			0	100 nM		$2^{-\Delta\Delta$ CT}			0	100 nM	
1	2.5679	-0.2589	1	0.0000	-2.8268	1	1.0000	7.0952				1	1.0000	7.0952			
2	2.5576	-0.2606	2	0.0000	-2.8182	2	1.0000	7.0529				2	1.0000	7.0529			
3	2.5665	-0.2519	3	0.0000	-2.8184	3	1.0000	7.0538				3	1.0000	7.0538			
4	2.5602	-0.2529	4	0.0000	-2.8131	4	1.0000	7.0278				4	1.0000	7.0278			
5	2.4980	-0.1719	5	0.0000	-2.6700	5	1.0000	6.3641				5	1.0000	6.3641			
Average	2.5501	-0.2392	Average	0.0000	-2.7893	Average	1.0000	6.9188				Average	1.0000	6.9188			

HT-22			BNIP3			HT-22			BNIP3			HT-22			BNIP3		
Δ CT			0	100 nM		$\Delta\Delta$ CT			0	100 nM		$2^{-\Delta\Delta$ CT}			0	100 nM	
1	6.4695	7.7141	1	0.0000	1.2446	1	1.0000	0.4220				1	1.0000	0.4220			
2	6.4767	7.7300	2	0.0000	1.2532	2	1.0000	0.4195				2	1.0000	0.4195			
3	6.4954	7.7372	3	0.0000	1.2418	3	1.0000	0.4229				3	1.0000	0.4229			
4	6.4812	7.8238	4	0.0000	1.3426	4	1.0000	0.3943				4	1.0000	0.3943			
5	6.4813	7.8608	5	0.0000	1.3795	5	1.0000	0.3843				5	1.0000	0.3843			
Average	6.4808	7.7731	Average	0.0000	1.2923	Average	1.0000	0.4086				Average	1.0000	0.4086			

HT-22			LC3			HT-22			LC3			HT-22			LC3		
Δ CT			0	100 nM	$\Delta\Delta$ CT			0	100 nM	$2^{-\Delta\Delta CT}$			0	100 nM	$2^{-\Delta\Delta CT}$		
1			10.2901	10.8561	1			0.0000	0.5660	1			1.0000	0.6755	1		
2			10.3365	10.8338	2			0.0000	0.4973	2			1.0000	0.7084	2		
3			10.3347	10.8105	3			0.0000	0.4757	3			1.0000	0.7191	3		
4			10.2425	10.7600	4			0.0000	0.5176	4			1.0000	0.6986	4		
5			10.2202	10.8272	5			0.0000	0.6070	5			1.0000	0.6565	5		
Average			10.2848	10.8175	Average			0.0000	0.5327	Average			1.0000	0.6916	Average		

Figure 1H. Cell proliferation of Thrombin-treated HT-22 control cells

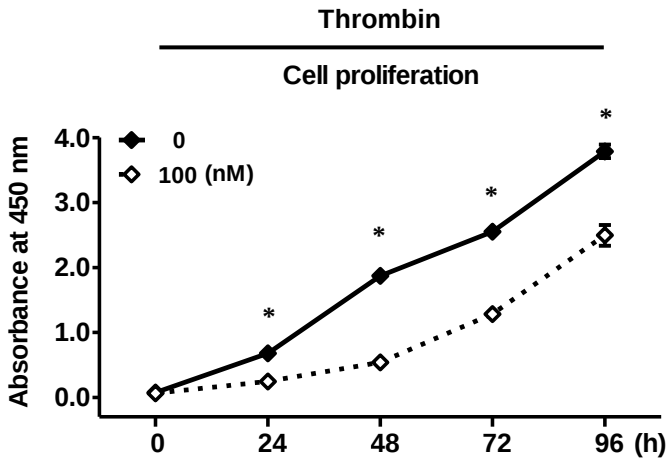
1st



HT-22	HT-22 Control cells + None (0 nM)					Average
	1	2	3	4	5	
0h	0.0760	0.0654	0.0713	0.0777	0.0799	0.0741
24h	0.6497	0.6248	0.7027	0.8116	0.6690	0.6916
48h	1.7551	1.4880	1.6457	1.7181	1.6652	1.6544
72h	2.6846	2.4453	2.6816	2.7802	2.7904	2.6764
96h	3.6798	3.5847	3.5577	3.5868	3.4411	3.5700

HT-22	HT-22 Control cells + Thrombin (100 nM)					Average
	1	2	3	4	5	
0h	0.0642	0.0633	0.0613	0.0651	0.0626	0.0633
24h	0.2549	0.2465	0.2629	0.2651	0.2489	0.2557
48h	0.5355	0.5735	0.5582	0.5381	0.5783	0.5567
72h	1.3385	1.3158	1.2123	1.3539	1.2644	1.2970
96h	2.1948	2.6852	2.1703	2.6591	2.6405	2.4700

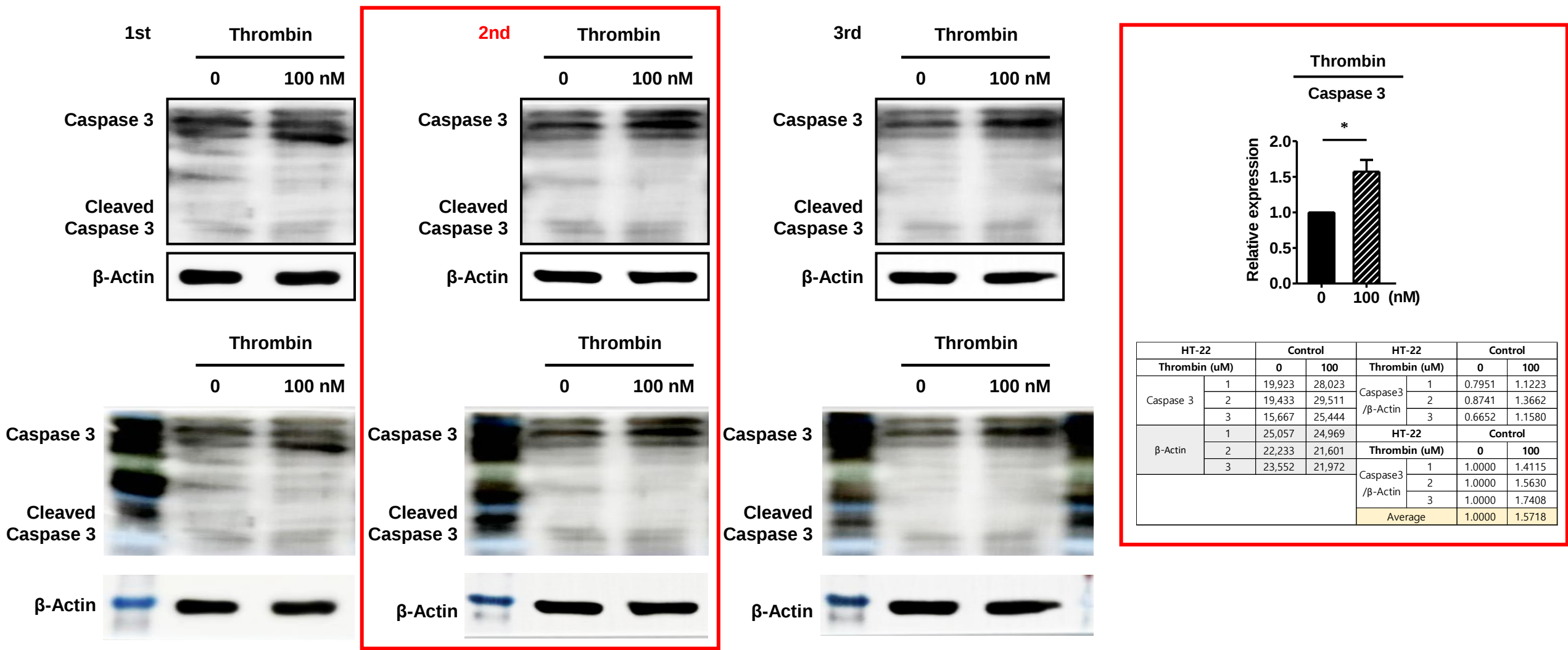
2nd



HT-22	HT-22 Control cells + None (0 nM)					Average
	1	2	3	4	5	
0h	0.0761	0.0745	0.0714	0.0742	0.0791	0.0751
24h	0.6645	0.6760	0.7949	0.5704	0.6922	0.6796
48h	1.7821	1.9177	1.9626	1.8412	1.8482	1.8704
72h	2.5613	2.4932	2.5160	2.5166	2.6739	2.5522
96h	3.7739	3.7399	3.9641	3.7699	3.6889	3.7873

HT-22	HT-22 Control cells + Thrombin (100 nM)					Average
	1	2	3	4	5	
0h	0.0602	0.0619	0.0635	0.0619	0.0619	0.0619
24h	0.2419	0.2396	0.2547	0.2483	0.2440	0.2457
48h	0.5428	0.5182	0.5503	0.5466	0.5324	0.5381
72h	1.2320	1.2475	1.3606	1.2963	1.2719	1.2817
96h	2.6304	2.6256	2.2483	2.4394	2.5325	2.4952

Figure 1. Caspase 3 expression in Thrombin-treated HT-22 control cells



Thrombin

Caspase 3

Relative expression

0.02.0

0100 (nM)

*

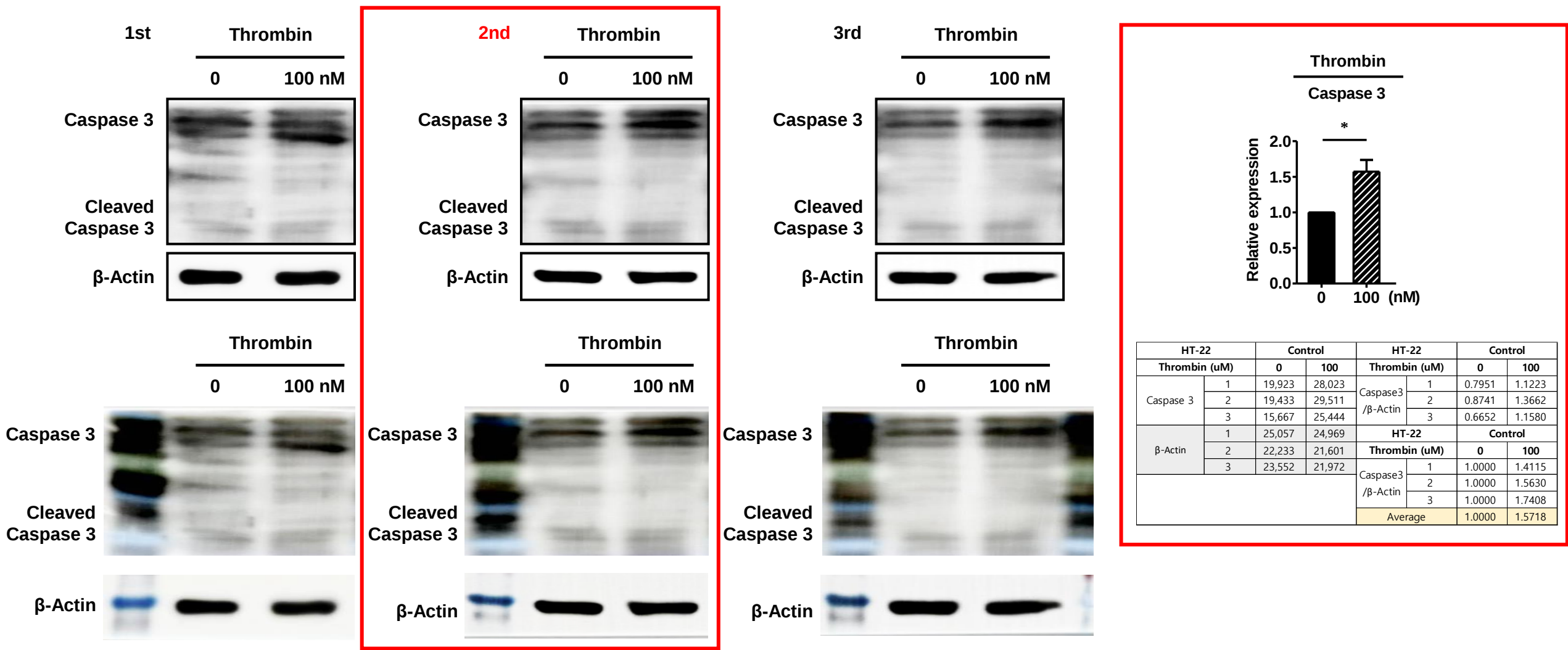


Figure 2.

GRA16 affects upstream signaling pathways that modulate both the inhibition and activation of tau hyperphosphorylation

Figure 2A. GRA16 expression in HT-22 stable cells

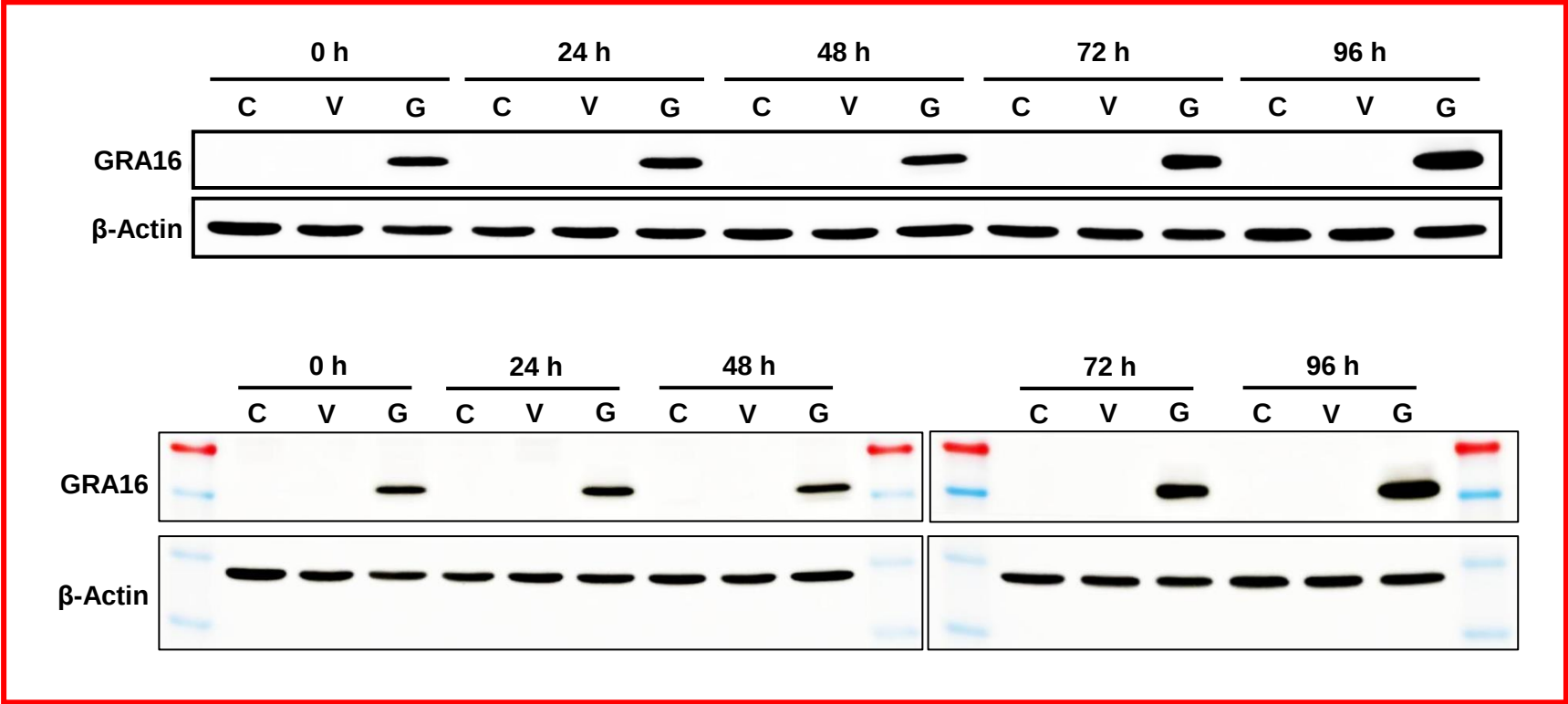


Figure 2B. PP2A-B55 expression in Thrombin-treated HT-22 stable cells

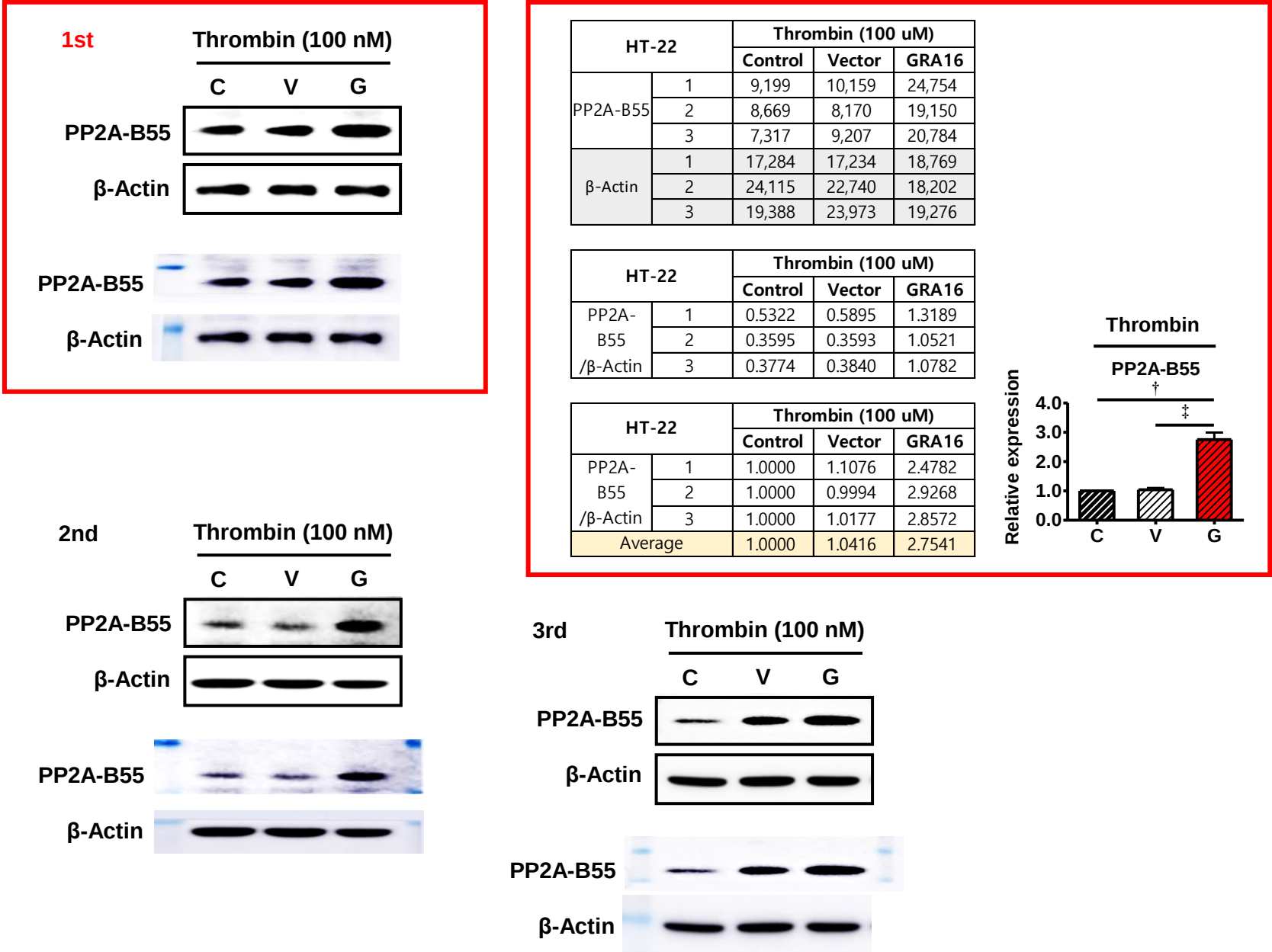
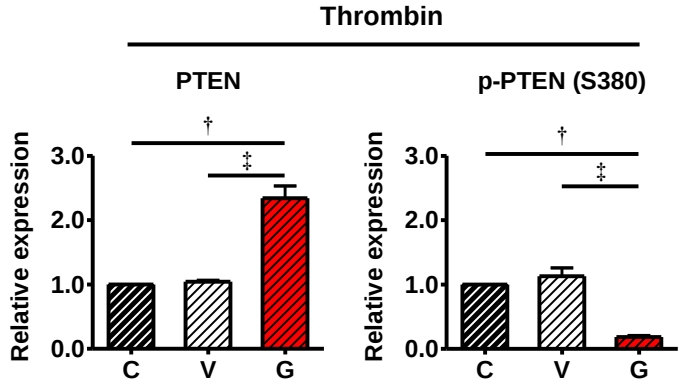
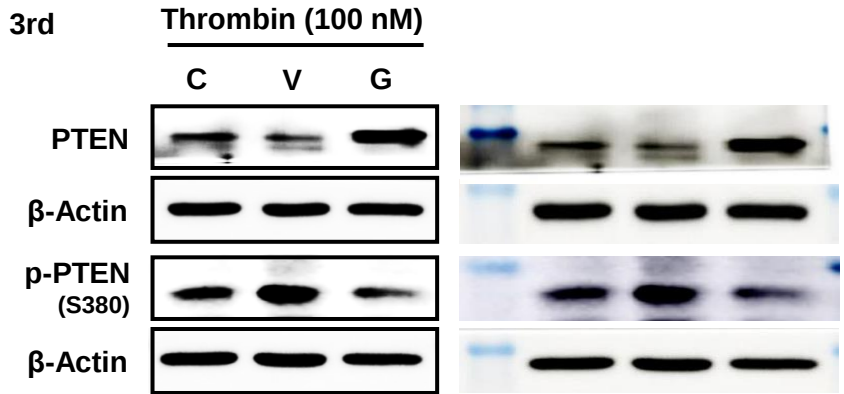
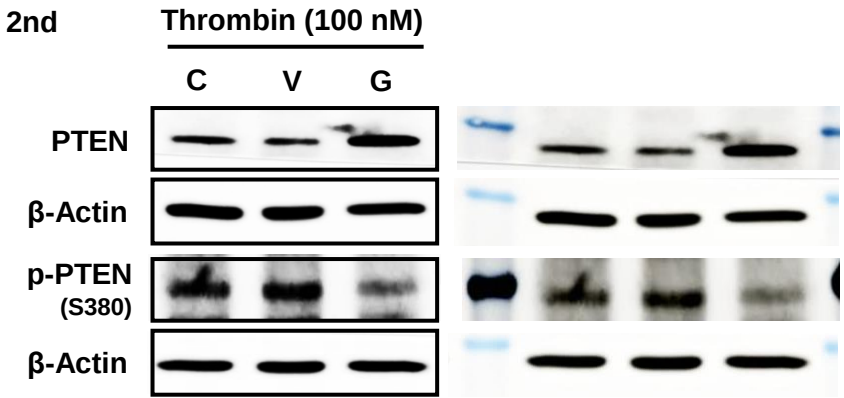
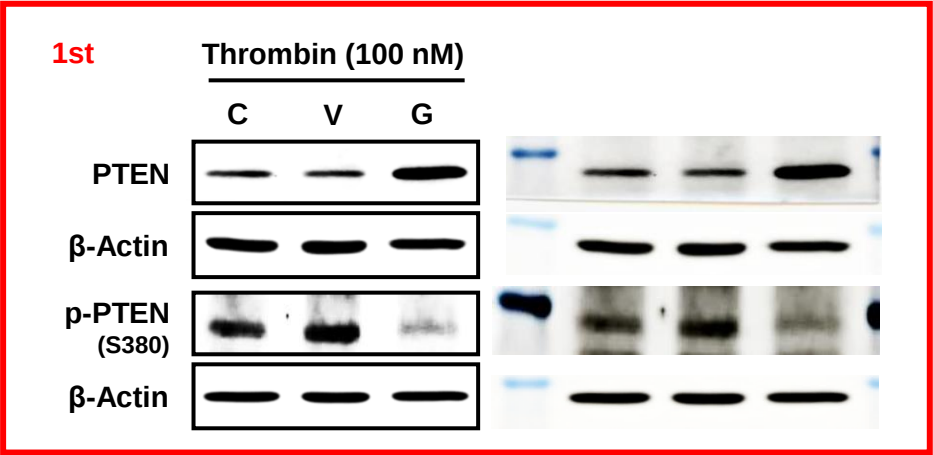


Figure 2C. PTEN and p-PTEN (S380) expression in Thrombin-treated HT-22 stable cells

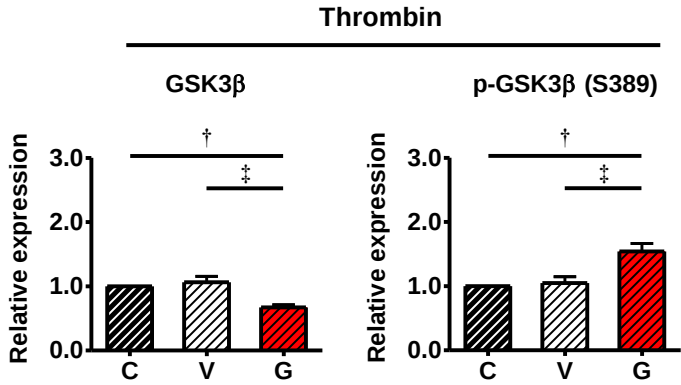
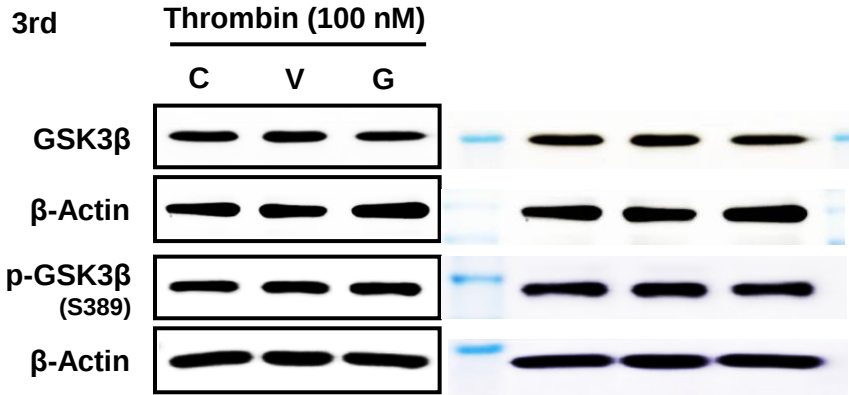
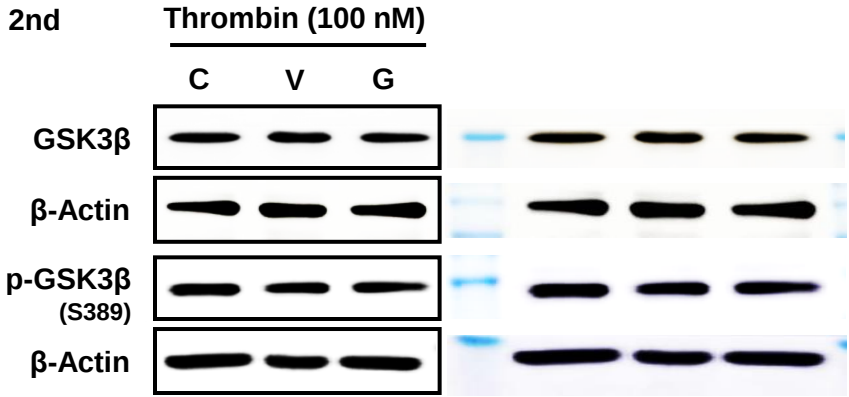
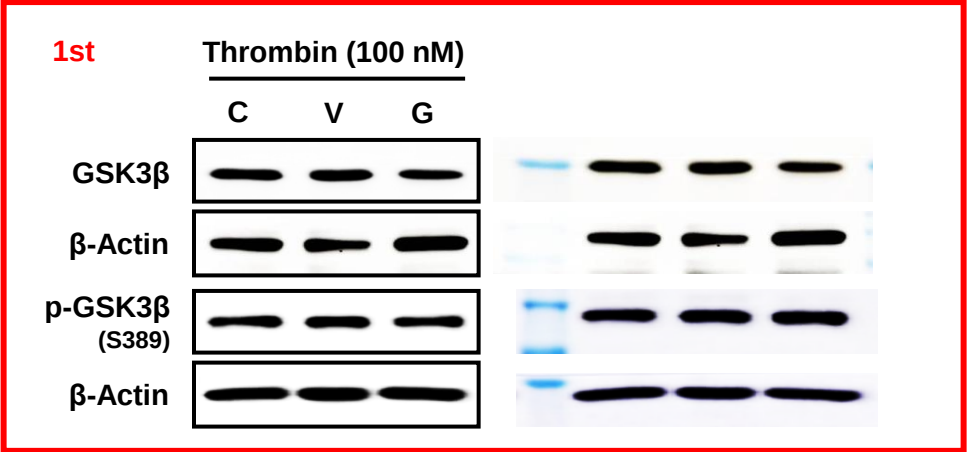


HT-22		Thrombin (100 uM)			HT-22		Thrombin (100 uM)		
		Control	Vector	GRA16			Control	Vector	GRA16
PTEN	1	7,753	7,481	19,247	p-PTEN	1	14,175	16,549	5,839
	2	10,130	8,613	19,992		2	15,169	16,006	6,042
	3	14,074	12,114	28,436		3	11,464	14,600	4,884
β -Actin	1	20,827	19,750	20,327	β -Actin	1	21,995	21,286	20,456
	2	24,010	19,324	20,489		2	19,338	19,702	20,595
	3	22,720	18,534	21,227		3	19,781	19,531	18,295

HT-22		Thrombin (100 uM)			HT-22		Thrombin (100 uM)		
		Control	Vector	GRA16			Control	Vector	GRA16
PTEN/ β -Actin	1	0.3723	0.3788	0.9469	p-PTEN / β -Actin	1	0.6444	0.7775	0.2854
	2	0.4219	0.4457	0.9758		2	0.7844	0.8124	0.2934
	3	0.6194	0.6536	1.3396		3	0.5796	0.7475	0.2670

HT-22		Thrombin (100 uM)			HT-22		Thrombin (100 uM)		
		Control	Vector	GRA16			Control	Vector	GRA16
PTEN/ β -Actin	1	1.0000	1.0175	2.5434	p-PTEN /PTEN	1	1.0000	1.1857	0.1741
	2	1.0000	1.0564	2.3127		2	1.0000	0.9804	0.1617
	3	1.0000	1.0552	2.1626		3	1.0000	1.2224	0.2130
Average		1.0000	1.0430	2.3396	Average		1.0000	1.1295	0.1829

Figure 2D. GSK3β and p-GSK3β (S389) expression in Thrombin-treated HT-22 stable cells

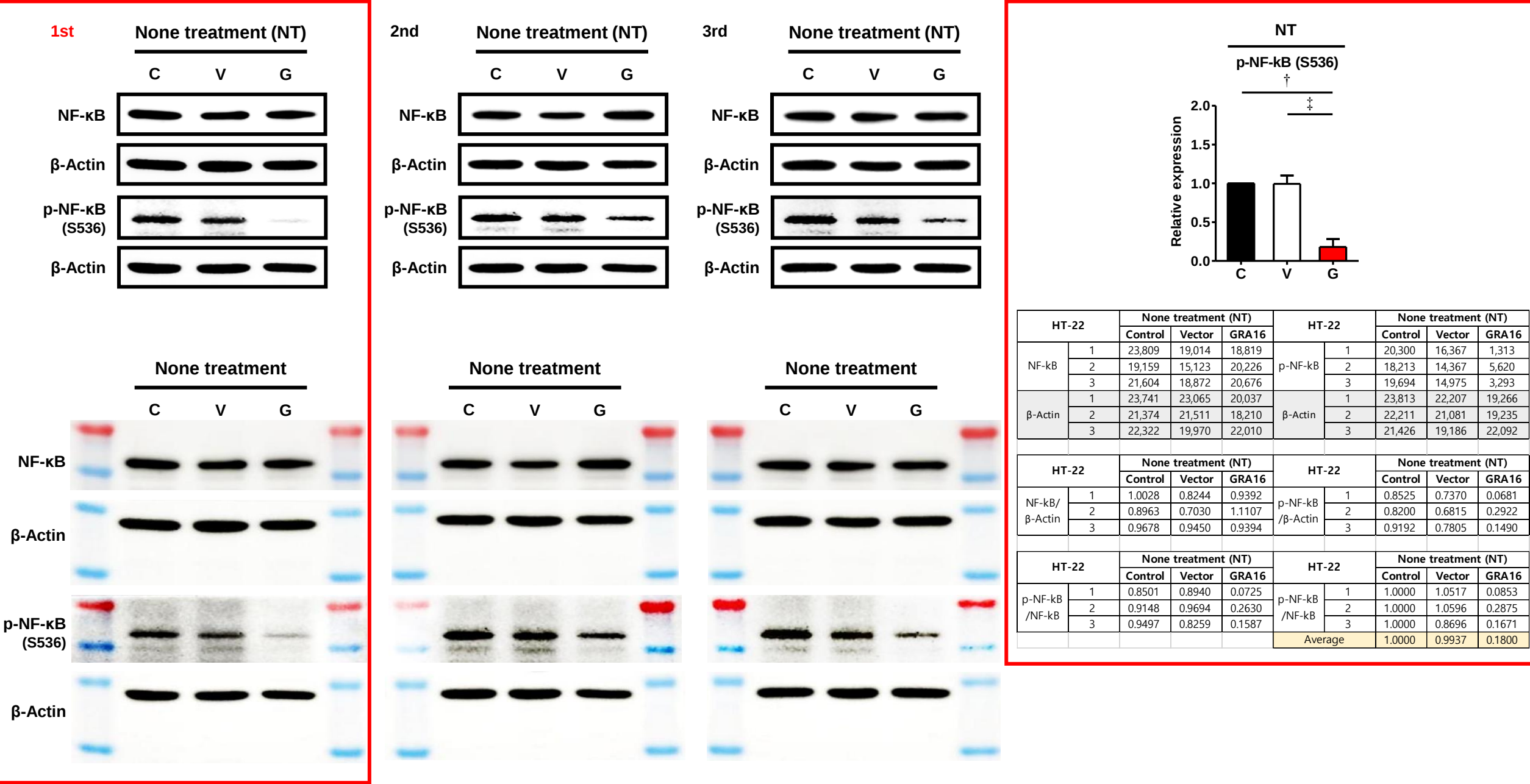


HT-22		Thrombin (100 uM)			HT-22		Thrombin (100 uM)		
		Control	Vector	GRA16			Control	Vector	GRA16
GSK3β	1	21,328	19,695	15,895	p-GSK3β	1	16,144	16,740	16,050
	2	18,397	18,134	15,505		2	22,609	16,299	18,487
	3	19,178	19,616	17,236		3	16,283	17,194	18,551
β-Actin	1	19,149	15,468	22,901	β-Actin	1	20,257	19,615	21,068
	2	17,464	17,900	21,231		2	27,105	18,034	22,507
	3	18,101	17,123	23,341		3	22,062	19,923	21,606
HT-22		Thrombin (100 uM)			HT-22		Thrombin (100 uM)		
		Control	Vector	GRA16			Control	Vector	GRA16
GSK3β/ β-Actin	1	1.1138	1.2733	0.6941	p-GSK3β /β-Actin	1	0.7970	0.8534	0.7618
	2	1.0534	1.0131	0.7303		2	0.8341	0.9038	0.8214
	3	1.0595	1.1456	0.7384		3	0.7380	0.8630	0.8586
HT-22		Thrombin (100 uM)			HT-22		Thrombin (100 uM)		
		Control	Vector	GRA16			Control	Vector	GRA16
p-GSK3β /GSK3β	1	1.0000	1.1432	0.6232	p-GSK3β /GSK3β	1	1.0000	0.9367	1.5339
	2	1.0000	0.9617	0.6932		2	1.0000	1.1267	1.4205
	3	1.0000	1.0812	0.6969		3	1.0000	1.0815	1.6693
Average		1.0000	1.0620	0.6711	Average		1.0000	1.0483	1.5412

Figure 3.

NF- κ B inhibition by GRA16 suppresses APOE and upregulates autophagy-related factors via FOXO3A increase in thrombin-induced HT-22 cells

Figure 3A. NF-κB and p-NF-κB (S536) expression in Non-treated HT-22 stable cells



NT

p-NF-κB (S536)

†

†

Relative expression

2.0

1.5

1.0

0.5

0.0

C

V

G

Figure 3C. Relative mRNA expressions in Non-treated HT-22 stable cells

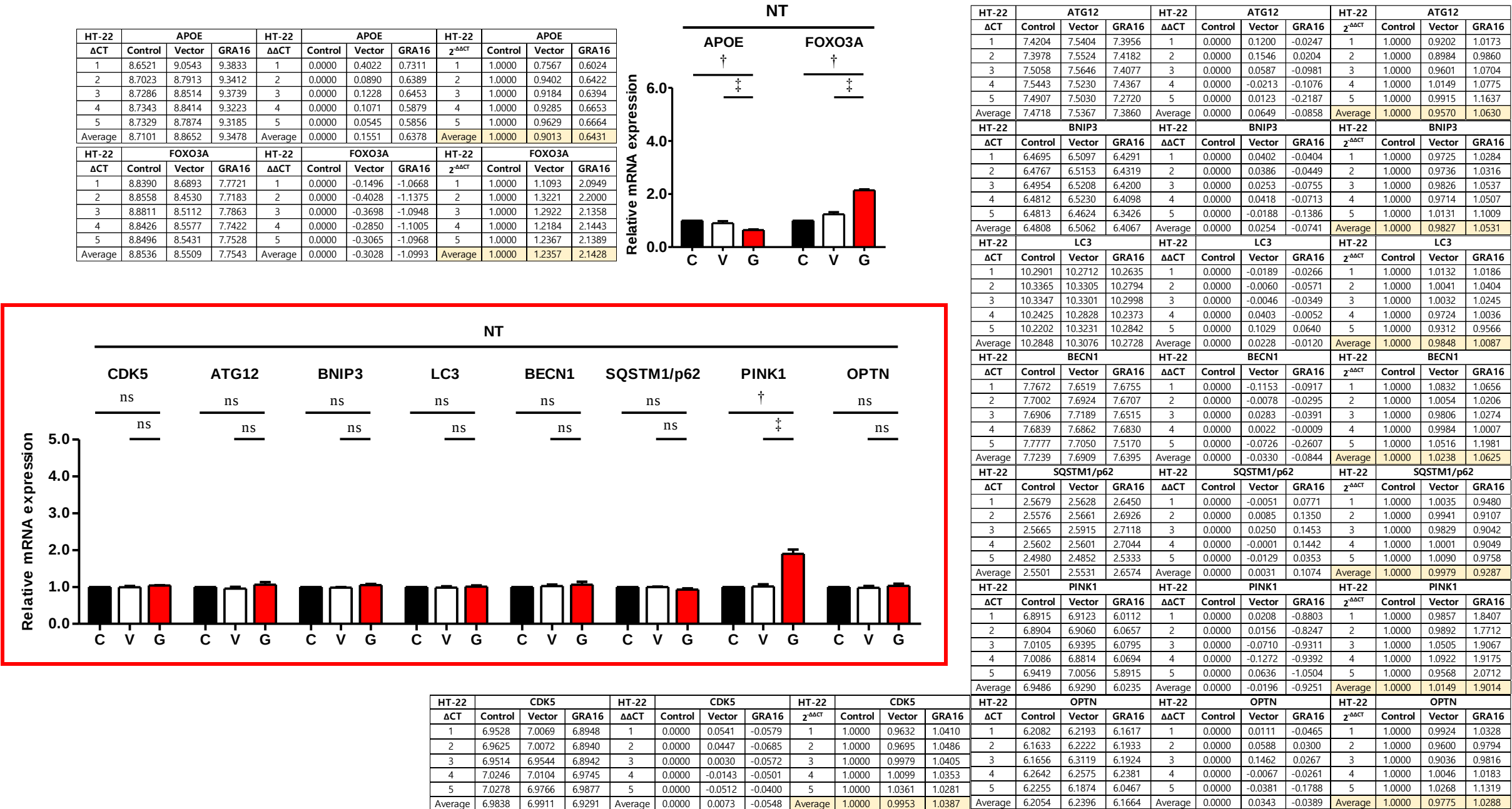
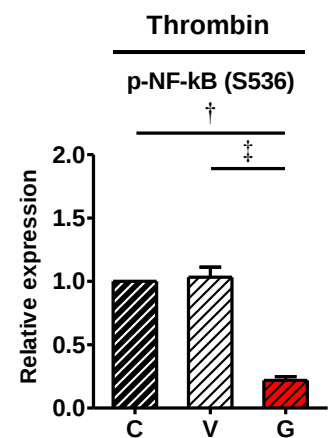
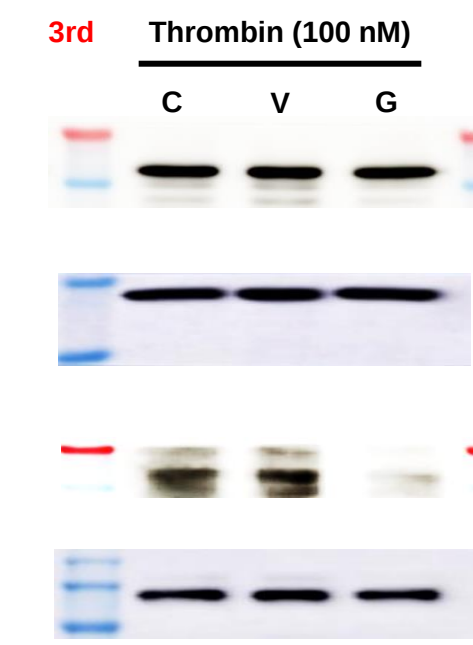
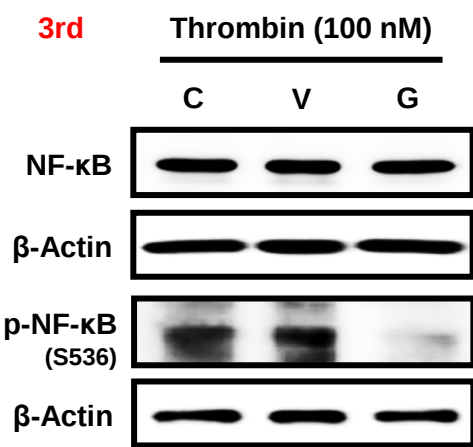
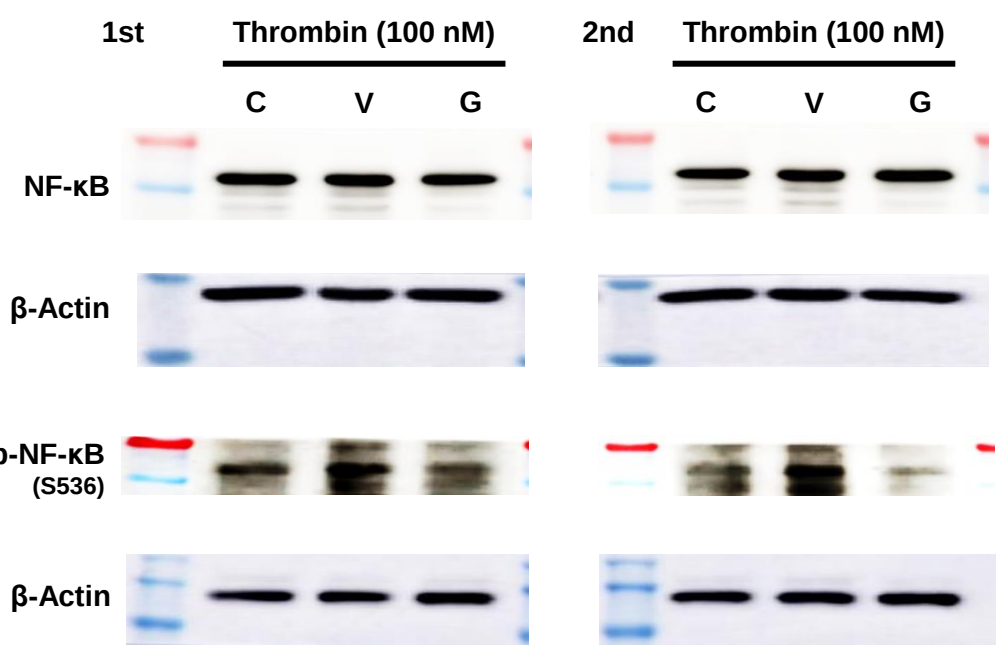
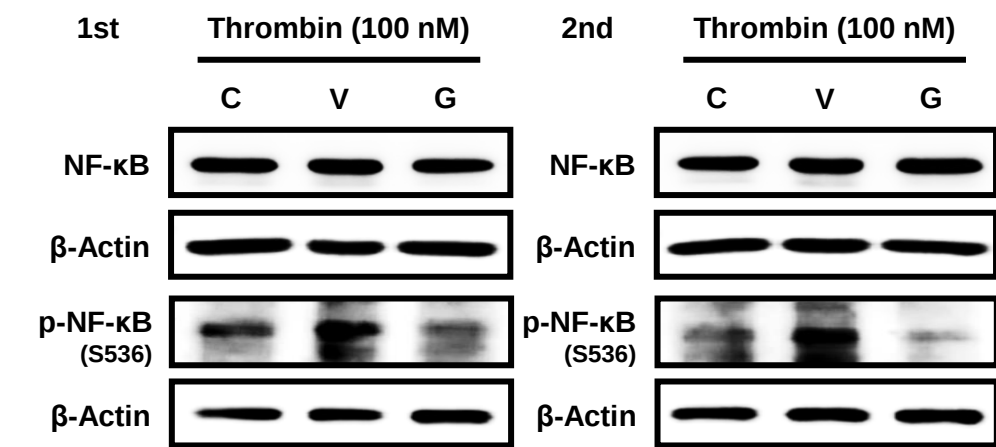


Figure 3D. NF-κB and p-NF-κB (S536) expression in Thrombin-treated HT-22 stable cells



HT-22		Thrombin (100 uM)			HT-22		Thrombin (100 uM)		
		Control	Vector	GRA16			Control	Vector	GRA16
NF-κB	1	22,125	19,704	18,714	p-NF-κB	1	10,197	12,699	3,231
	2	19,376	20,024	22,266		2	13,219	16,996	4,070
	3	19,734	19,209	19,602		3	17,022	16,140	2,930
β-Actin	1	28,983	21,666	24,334	β-Actin	1	17,397	16,836	22,127
	2	25,766	23,849	25,139		2	19,985	21,358	23,706
	3	23,318	22,587	24,224		3	18,706	18,746	17,791

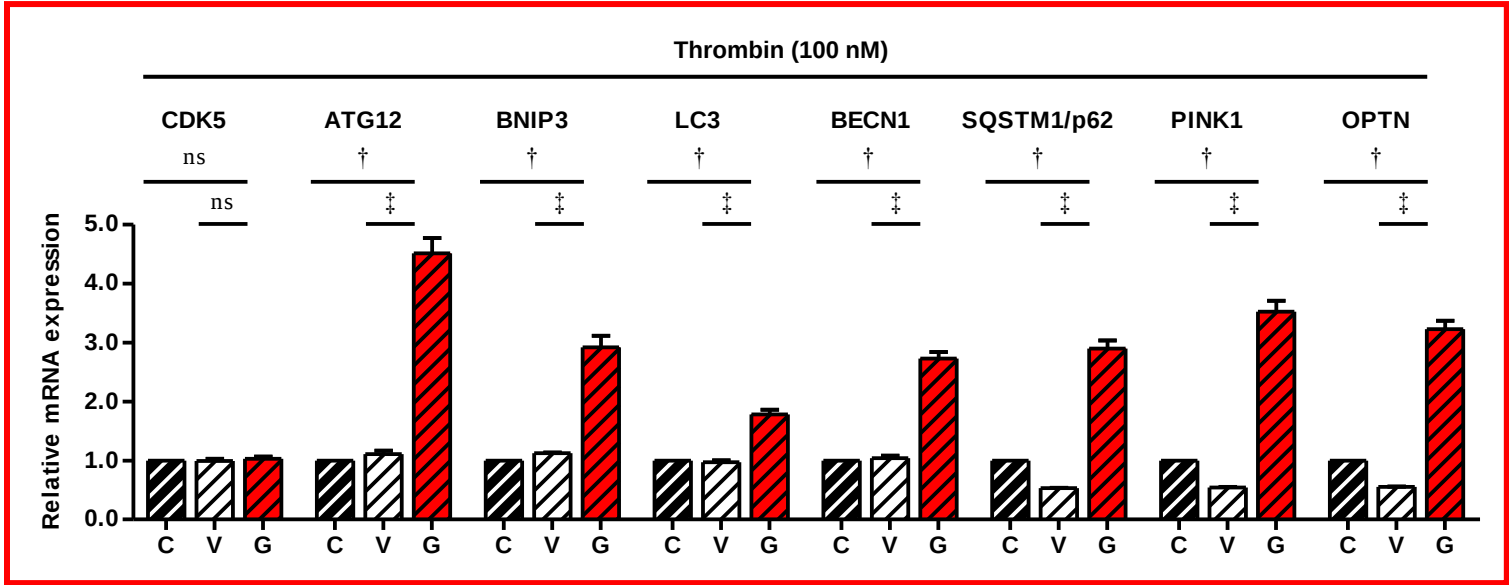
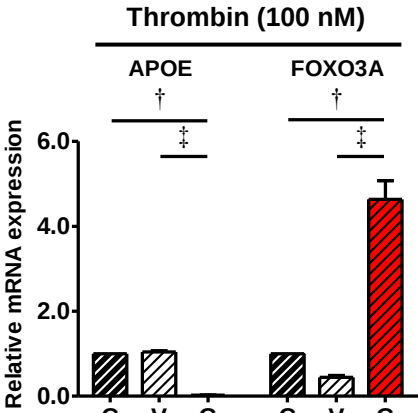
HT-22		Thrombin (100 uM)			HT-22		Thrombin (100 uM)		
		Control	Vector	GRA16			Control	Vector	GRA16
NF-κB/ β-Actin	1	0.7634	0.9095	0.7691	p-NF-κB /β-Actin	1	0.5862	0.7543	0.1460
	2	0.7520	0.8396	0.8857		2	0.6615	0.7958	0.1717
	3	0.8463	0.8505	0.8092		3	0.9100	0.8610	0.1647

HT-22		Thrombin (100 uM)			HT-22		Thrombin (100 uM)		
		Control	Vector	GRA16			Control	Vector	GRA16
p-NF-κB /NF-κB	1	0.7679	0.8294	0.1899	p-NF-κB /NF-κB	1	1.0000	1.0801	0.2473
	2	0.8797	0.9478	0.1938		2	1.0000	1.0774	0.2204
	3	1.0752	1.0124	0.2035		3	1.0000	0.9415	0.1893
		Average					1.0000	1.0330	0.2190

Figure 3F. Relative mRNA expressions in Thrombin-treated HT-22 stable cells

HT-22				APOE				HT-22				APOE			
ΔCT	Control	Vector	GRA16	ΔΔCT	Control	Vector	GRA16	2 ^{-ΔΔCT}	Control	Vector	GRA16	ΔCT	Control	Vector	GRA16
1	7.4621	7.4564	13.0162	1	0.0000	-0.0057	5.5541	1	1.0000	1.0040	0.0213	2	7.4852	7.5012	13.0567
2	7.4823	7.4918	13.1193	3	0.0000	0.0095	5.6370	3	1.0000	0.9934	0.0201	3	7.4763	7.4940	13.1489
4	7.4763	7.4940	13.1489	4	0.0000	0.0177	5.6726	4	1.0000	0.9878	0.0196	5	7.4723	7.5219	13.5371
5	7.4723	7.5219	13.5371	5	0.0000	0.0496	6.0648	5	1.0000	0.9662	0.0149	Average	7.4756	7.4931	13.1757
Average	7.4756	7.4931	13.1757	Average	0.0000	0.0174	5.7000	Average	1.0000	0.9881	0.0194				

HT-22				FOXO3A				HT-22				FOXO3A			
ΔCT	Control	Vector	GRA16	ΔΔCT	Control	Vector	GRA16	2 ^{-ΔΔCT}	Control	Vector	GRA16	ΔCT	Control	Vector	GRA16
1	10.4690	11.8888	8.3214	1	0.0000	1.4198	-2.1476	1	1.0000	0.3738	4.4308	2	10.4857	11.6193	8.3535
2	10.4857	11.6193	8.3535	2	0.0000	1.1336	-2.1322	2	1.0000	0.4558	4.3838	3	10.4761	11.6172	8.3468
3	10.4761	11.6172	8.3468	3	0.0000	1.1411	-2.1293	3	1.0000	0.4534	4.3751	4	10.4804	11.6050	8.2864
4	10.4804	11.6050	8.2864	4	0.0000	1.1246	-2.1940	4	1.0000	0.4586	4.5757	5	10.6510	11.6930	8.2156
5	10.6510	11.6930	8.2156	5	0.0000	1.0420	-2.4354	5	1.0000	0.4857	5.4092	Average	10.5124	11.6847	8.3048
Average	10.5124	11.6847	8.3048	Average	0.0000	1.1722	-2.2077	Average	1.0000	0.4454	4.6349				



HT-22		CDK5		HT-22		CDK5		HT-22		CDK5	
ΔCT	Control	Vector	GRA16	ΔΔCT	Control	Vector	GRA16	2 ^{-ΔΔCT}	Control	Vector	GRA16
1	6.8871	6.8876	6.8869	1	0.0000	0.0005	-0.0001	1	1.0000	0.9996	1.0001
2	6.8712	6.9241	6.8273	2	0.0000	0.0528	-0.0439	2	1.0000	0.9640	1.0309
3	6.8536	6.9434	6.8673	3	0.0000	0.0898	0.0137	3	1.0000	0.9397	0.9906
4	6.9451	6.9244	6.8569	4	0.0000	-0.0207	-0.0882	4	1.0000	1.0145	1.0630
5	6.9434	6.8888	6.8516	5	0.0000	-0.0546	-0.0918	5	1.0000	1.0386	1.0657
Average	6.9001	6.9136	6.8580	Average	0.0000	0.0136	-0.0421	Average	1.0000	0.9913	1.0301

HT-22				ATG12				HT-22				ATG12			
ΔCT	Control	Vector	GRA16	ΔΔCT	Control	Vector	GRA16	2 ^{ΔΔCT}	Control	Vector	GRA16	ΔCT	Control	Vector	GRA16
1	7.7278	7.6497	5.6962	1	0.0000	-0.0780	-2.0316	1	1.0000	1.0556	4.0886	2	7.7710	7.6517	5.6218
2	7.7710	7.6517	5.6218	2	0.0000	-0.1194	-2.1492	2	1.0000	1.0863	4.4358	3	7.9165	7.6579	5.6893
3	7.9165	7.6579	5.6893	3	0.0000	-0.2586	-2.2271	3	1.0000	1.1963	4.6821	4	7.9276	7.7454	5.6914
4	7.9276	7.7454	5.6914	4	0.0000	-0.1822	-2.2362	4	1.0000	1.1346	4.7114	5	7.9102	7.8124	5.6938
5	7.9102	7.8124	5.6938	5	0.0000	-0.0977	-2.2163	5	1.0000	1.0701	4.6470	Average	7.8506	7.7034	5.6785
Average	7.8506	7.7034	5.6785	Average	0.0000	-0.1472	-2.1721	Average	1.0000	1.1086	4.5130				

HT-22				BNIP3				HT-22				BNIP3			
ΔCT	Control	Vector	GRA16	ΔΔCT	Control	Vector	GRA16	2 ^{ΔΔCT}	Control	Vector	GRA16	ΔCT	Control	Vector	GRA16
1	7.7141	7.5385	6.2926	1	0.0000	-0.1755	-1.4215	1	1.0000	1.1294	2.6786	2	7.7300	7.5429	6.2335
2	7.7300	7.5429	6.2335	2	0.0000	-0.1871	-1.4964	2	1.0000	1.1384	2.8214	3	7.7372	7.5650	6.2172
3	7.7372	7.5650	6.2172	3	0.0000	-0.1722	-1.5200	3	1.0000	1.1267	2.8679	4	7.8238	7.6759	6.1957
4	7.8238	7.6759	6.1957	4	0.0000	-0.1479	-1.6281	4	1.0000	1.1079	3.0910	5	7.8608	7.7034	6.2035
5	7.8608	7.7034	6.2035	5	0.0000	-0.1573	-1.6572	5	1.0000	1.1152	3.1541	Average	7.7731	7.6051	6.2285
Average	7.7731	7.6051	6.2285	Average	0.0000	-0.1680	-1.5446	Average	1.0000	1.1236	2.9226				

HT-22				LC3				HT-22				LC3			
ΔCT	Control	Vector	GRA16	ΔΔCT	Control	Vector	GRA16	2 ^{ΔΔCT}	Control	Vector	GRA16	ΔCT	Control	Vector	GRA16
1	10.8561	10.8817	9.9418	1	0.0000	0.0256	-0.9143	1	1.0000	0.9824	1.8846	2	10.8338	10.8297	10.0269
2	10.8338	10.8297	10.0269	2	0.0000	-0.0041	-0.8069	2	1.0000	1.0029	1.7495	3	10.8105	10.8640	9.9989
3	10.8105	10.8640	9.9989	3	0.0000	0.0535	-0.8116	3	1.0000	0.9636	1.7551	4	10.7600	10.8784	10.0041
4	10.7600	10.8784	10.0041	4	0.0000	0.1183	-0.7560	4	1.0000	0.9213	1.6888	5	10.8272	10.8412	9.9454
5	10.8272	10.8412	9.9454	5	0.0000	0.0139	-0.8818	5	1.0000	0.9904	1.8427	Average	10.8175	10.8590	9.9834
Average	10.8175	10.8590	9.9834	Average	0.0000	0.0414	-0.8341	Average	1.0000	0.9721	1.7841				

HT-22				BECN1				HT-22				BECN1			
ΔCT	Control	Vector	GRA16	ΔΔCT	Control	Vector	GRA16	2 ^{ΔΔCT}	Control	Vector	GRA16	ΔCT	Control	Vector	GRA16
1	5.3836	5.2663	4.0267	1	0.0000	-0.1173	-1.3569	1	1.0000	1.0847	2.5613	2	5.3940	5.2907	3.9239
2	5.3940	5.2907	3.9239	2	0.0000	-0.1033	-1.4701	2	1.0000	1.0742	2.7705	3	5.3804	5.4138	3.9513
3	5.3804	5.4138	3.9513	3	0.0000	0.0334	-1.4291	3	1.0000	0.9771	2.6928	4	5.4533	5.4128	3.9305
4	5.4533	5.4128	3.9305	4	0.0000	-0.0405	-1.5228	4	1.0000	1.0285	2.8735	5	5.4743	5.4105	4.0159
5	5.4743	5.4105	4.0159	5	0.0000	-0.0638	-1.4584	5	1.0000	1.0452	2.7479	Average	5.4171	5.3588	3.9697
Average	5.4171	5.3588	3.9697	Average	0.0000	-0.0583	-1.4475	Average	1.0000	1.0419	2.7292				

HT-22				SQSTM1/p62				HT-22				SQSTM1/p62			
ΔCT	Control	Vector	GRA16	ΔΔCT	Control	Vector	GRA16	2 ^{ΔΔCT}	Control	Vector	GRA16	ΔCT	Control	Vector	GRA16
1	-0.2589	0.6634	-1.6898	1	0.0000	0.9223	-1.4309	1	1.0000	0.5277	2.6961	2	-0.2606	0.6595	-1.7886
2	-0.2606	0.6595	-1.7886	2	0.0000	0.9201	-1.5280	2	1.0000	0.5285	2.8838	3	-0.2519	0.6592	-1.7857
3	-0.2519	0.6592	-1.7857	3	0.0000	0.9110	-1.5338	3	1.0000	0.5318	2.8955	4	-0.2529	0.6589	-1.8037
4	-0.2529	0.6589	-1.8037	4	0.0000	0.9118	-1.5508	4	1.0000	0.5315	2.9298	5	-0.1719	0.7189	-1.7981
5	-0.1719	0.7189	-1.7981	5	0.0000	0.8908	-1.6262	5	1.0000	0.5393	3.0870	Average	-0.2392	0.6720	-1.7732
Average	-0.2392	0.6720	-1.7732	Average	0.0000	0.9112	-1.5339	Average	1.0000	0.5318	2.8984				

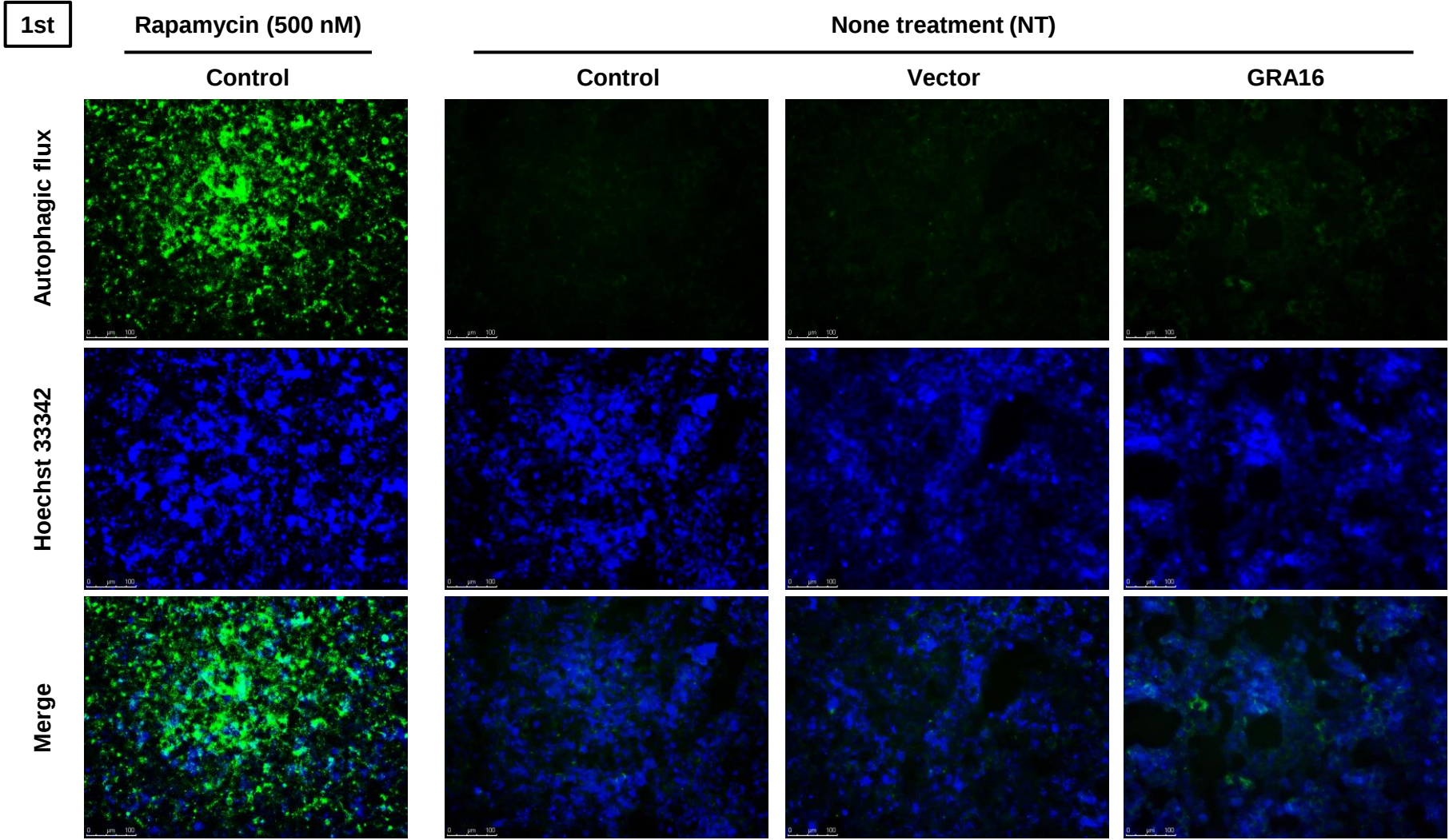
HT-22				PINK1				HT-22				PINK1			
ΔCT	Control	Vector	GRA16	ΔΔCT	Control	Vector	GRA16	2 ^{ΔΔCT}	Control	Vector	GRA16	ΔCT	Control	Vector	GRA16
1	3.9677	4.9001	2.2854	1	0.0000	0.9324	-1.6823	1	1.0000	0.5240	3.2093	2	4.0244	4.8960	2.1948
2	4.0244	4.8960	2.1948	2	0.0000	0.8716	-1.8296	2	1.0000	0.5465	3.5543	3	4.0099	4.9206	2.1892
3	4.0099	4.9206	2.1892	3	0.0000	0.9106	-1.8207	3	1.0000	0.5320	3.5325	4	4.1312	4.9623	2.2510
4	4.1312	4.9623	2.2510	4	0.0000	0.8312	-1.8802	4	1.0000	0.5621	3.6813	5	4.1130	5.0054	2.2460
5	4.1130	5.0054	2.2460	5	0.0000	0.8924	-1.8670	5	1.0000	0.5387	3.6478	Average	4.0492	4.9369	2.2333
Average	4.0492	4.9369	2.2333	Average	0.0000	0.8876	-1.8160	Average	1.0000	0.5407	3.5251				

HT-22				OPTN				HT-22				OPTN			
ΔCT	Control	Vector	GRA16	ΔΔCT	Control	Vector	GRA16	2 ^{ΔΔCT}	Control	Vector	GRA16	ΔCT	Control	Vector	GRA16
1	3.3937	4.2465	1.8128	1	0.0000	0.8528	-1.5810	1	1.0000	0.5537	2.9917	2	3.3996	4.2570	1.7231
2	3.3996	4.2570	1.7231	2	0.0000	0.8573	-1.6765	2	1.0000	0.5520	3.1966	3	3.4117	4.2817	1.7051
3	3.4117	4.2817	1.7051	3	0.0000	0.8700	-1.7066	3	1.0000	0.5471	3.2638	4	3.4384	4.2898	1.6975
4	3.4384	4.2898	1.6975	4	0.0000	0.8514	-1.7409	4	1.0000	0.5543	3.3425	5	3.4705	4.3082	1.7312
5	3.4705	4.3082	1.7312	5	0.0000	0.8376	-1.7393	5	1.0000	0.5596	3.3387	Average	3.4228	4.2766	1.7340
Average	3.4228	4.2766	1.7340	Average	0.0000	0.8538	-1.6889	Average	1.0000	0.5533	3.2267				

Figure 4.

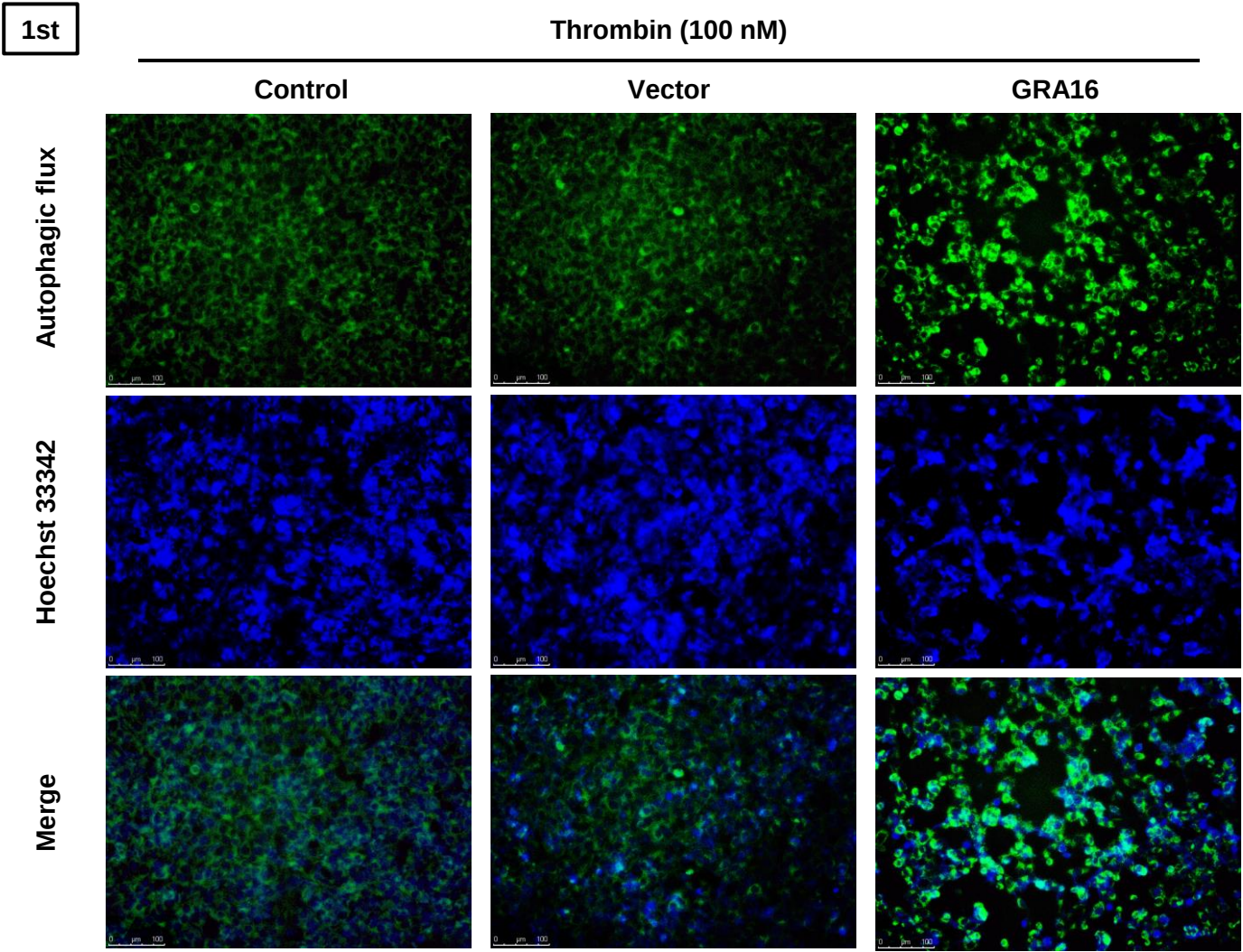
GRA16 blocks tau hyperphosphorylations by further enhancing the thrombin-induced autophagic flux

Figure 4A. Autophagic fluorescence in Rapamycin-treated HT-22 Control cells and Non-treated HT-22 stable cells (1st)



Scale bar = 100μm

Figure 4A. Autophagic fluorescence in Thrombin-treated HT-22 stable cells (1st)



Scale bar = 100μm

Figure 4A. Autophagic fluorescence in Rapamycin-treated HT-22 Control cells and Non-treated HT-22 stable cells (2nd)

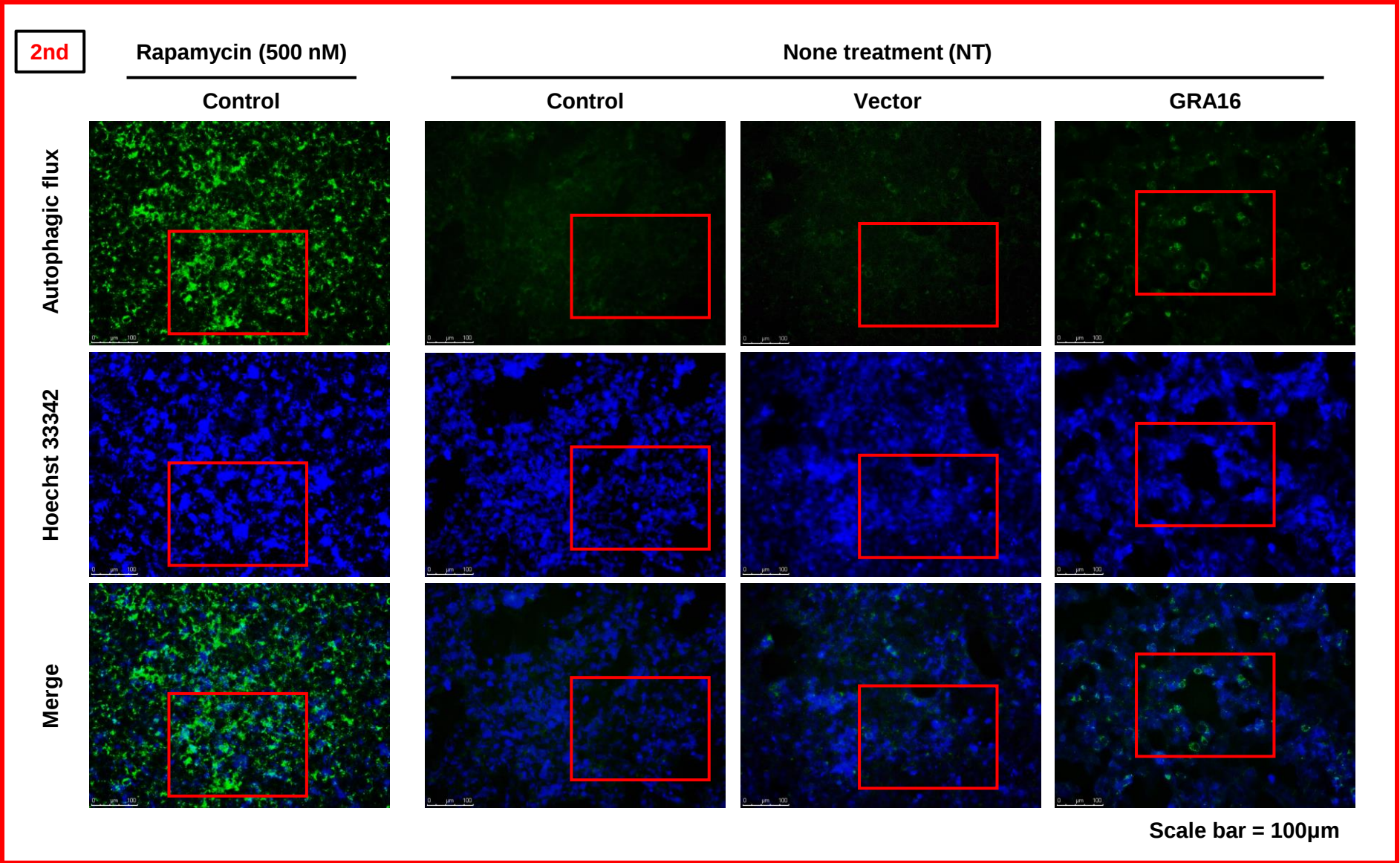


Figure 4A. Autophagic fluorescence in Thrombin-treated HT-22 stable cells (2nd)

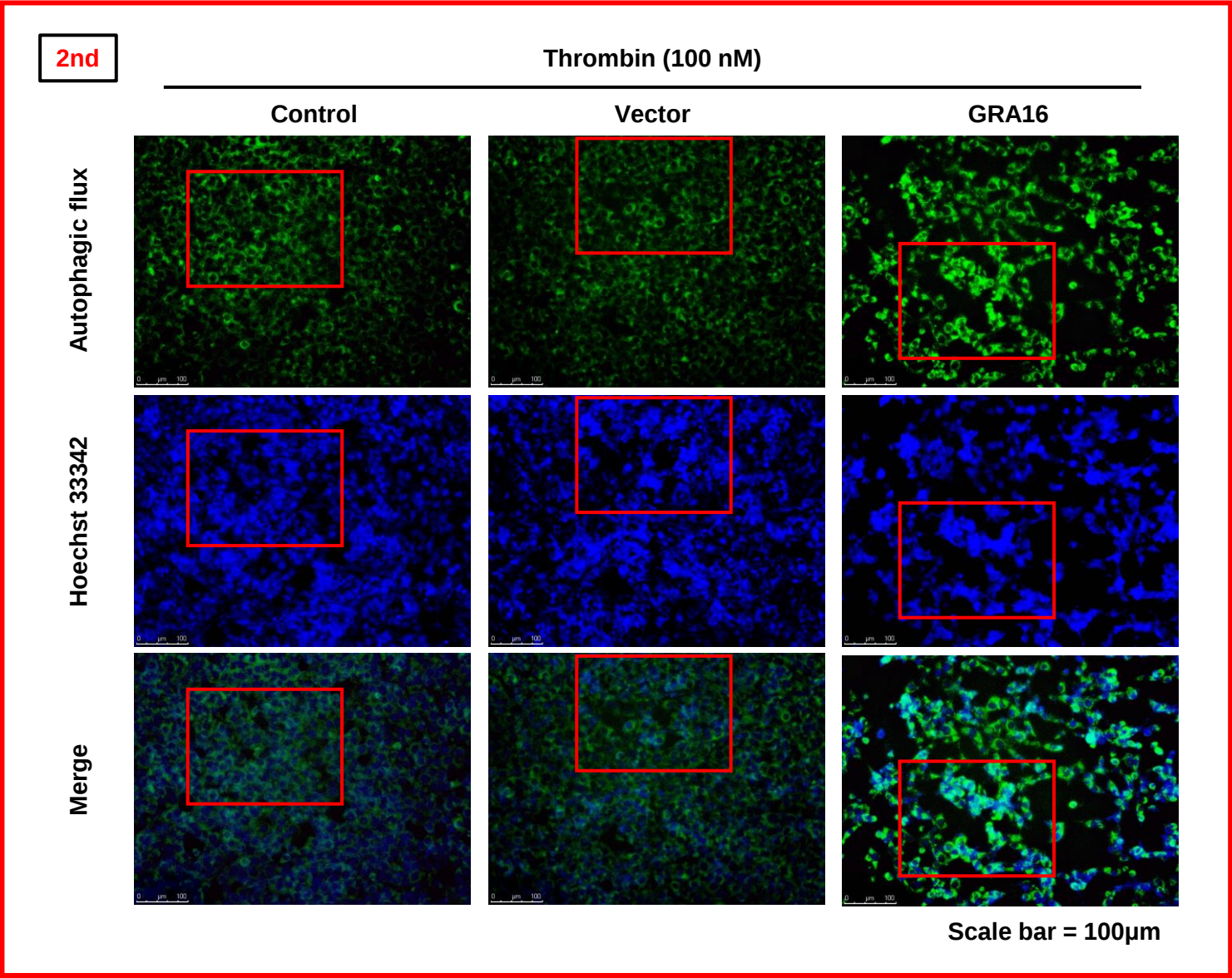
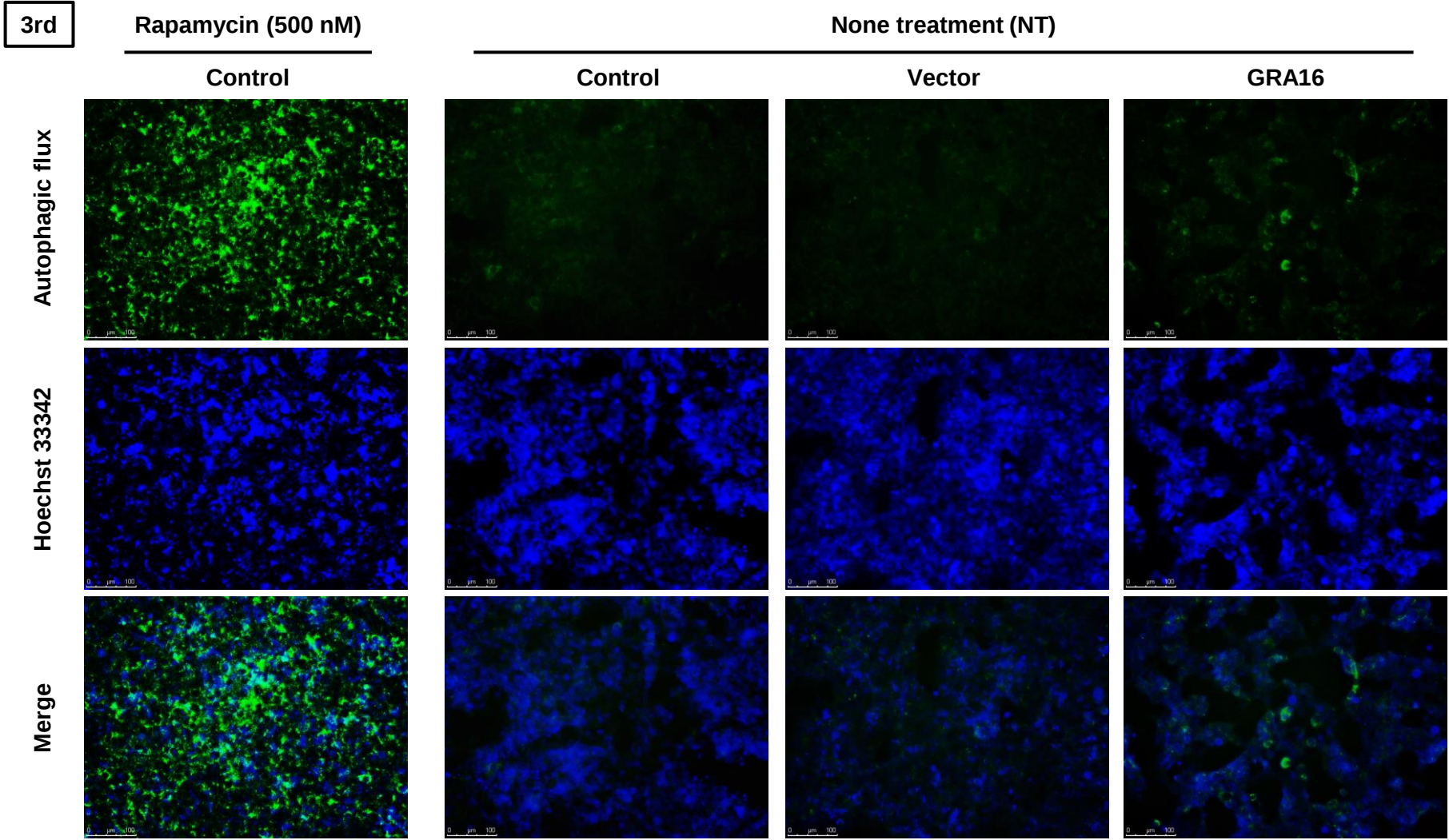


Figure 4A. Autophagic fluorescence in Rapamycin-treated HT-22 Control cells and Non-treated HT-22 stable cells (3rd)



Scale bar = 100μm

Figure 4A. Autophagic fluorescence in Thrombin-treated HT-22 stable cells (3rd)

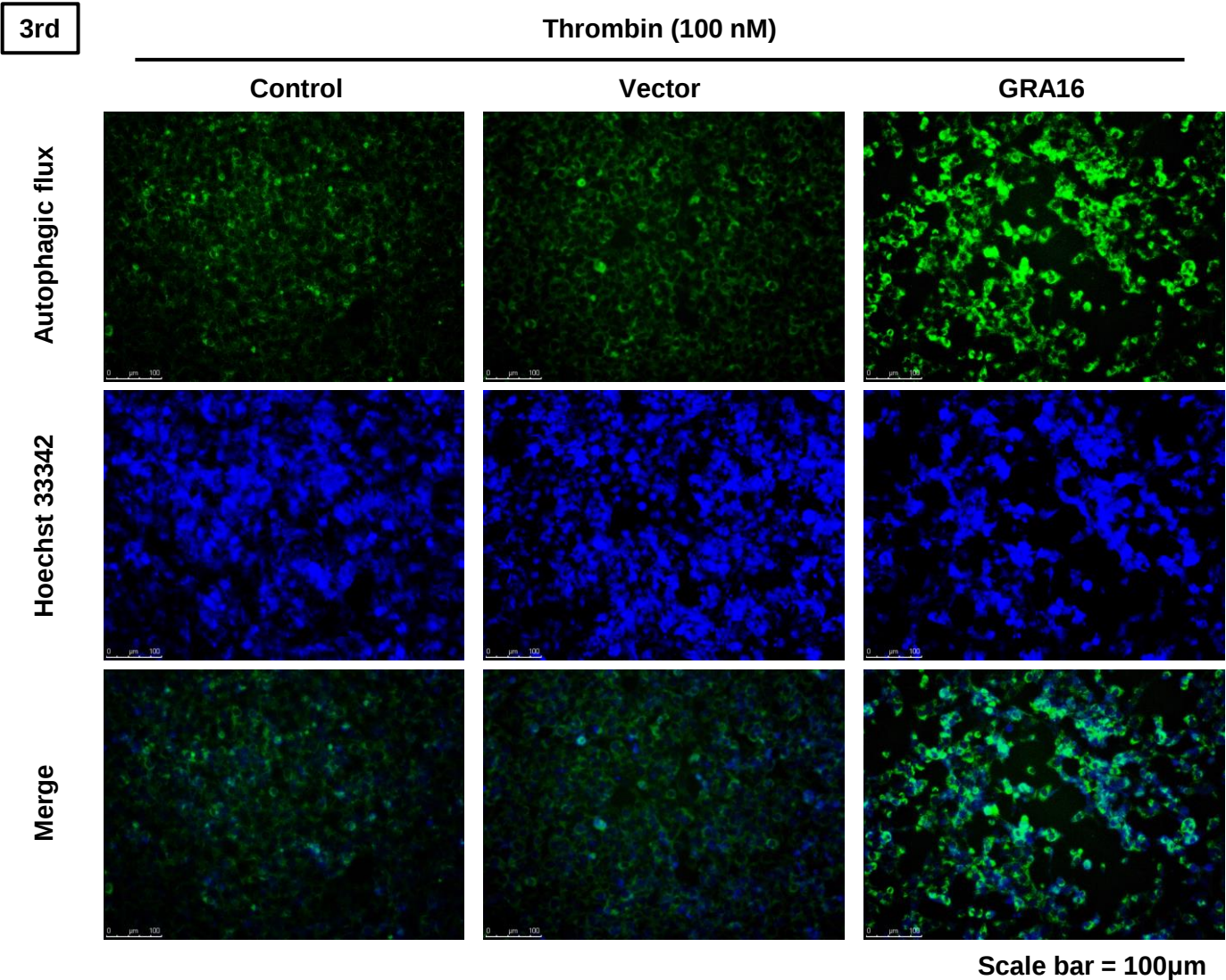
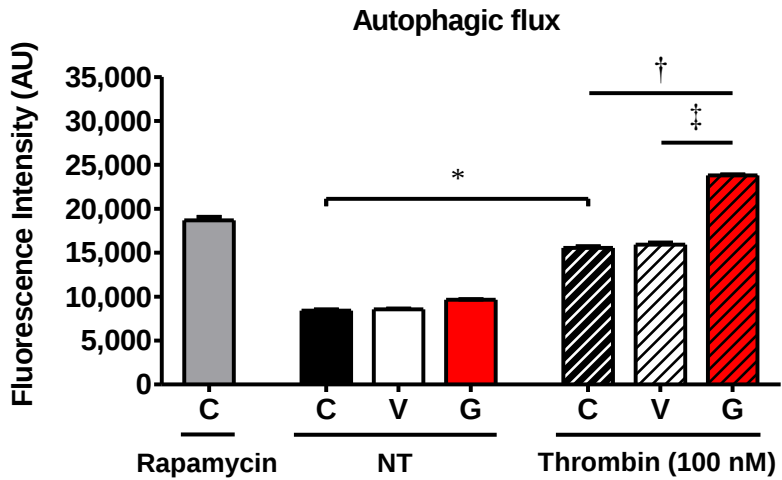


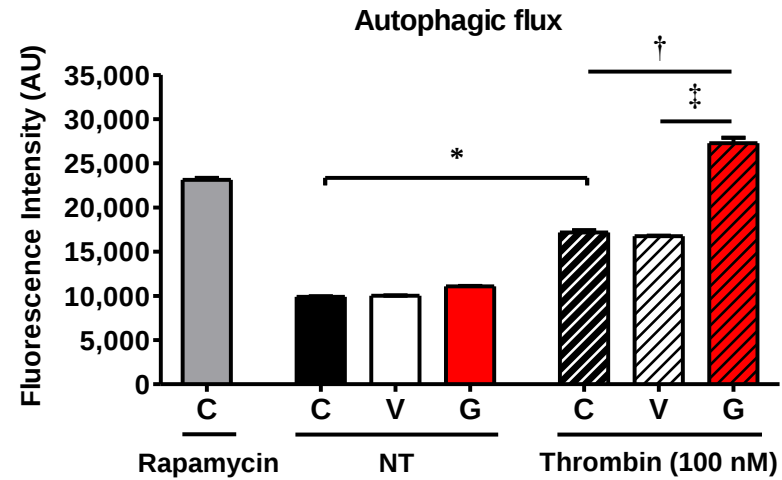
Figure 4B. Autophagic flux assay in Rapamycin-, None-, or Thrombin-treated HT-22 stable cells

1st



HT-22	Rapamycin	None treatment			Thrombin (100 nM)		
	Control	Control	Vector	GRA16	Control	Vector	GRA16
1	18,620	8,424	8,303	9,514	15,391	15,706	23,852
2	19,051	8,568	8,679	9,569	16,127	15,936	24,107
3	17,986	8,747	8,530	9,732	14,985	15,422	23,754
4	17,827	8,351	8,668	9,654	15,594	16,782	23,450
5	19,982	7,965	8,626	9,740	15,640	15,760	23,830
Average	18,693	8,411	8,561	9,642	15,547	15,921	23,799

2nd



HT-22	Rapamycin	None treatment			Thrombin (100 nM)		
	Control	Control	Vector	GRA16	Control	Vector	GRA16
1	22,865	9,917	10,076	11,052	16,906	16,719	26,367
2	22,416	9,670	9,876	11,036	17,801	16,885	25,373
3	23,302	9,962	10,005	11,165	17,789	16,720	28,160
4	23,368	9,907	10,078	11,166	16,768	16,737	28,959
5	23,738	9,961	9,990	11,029	16,610	16,785	27,505
Average	23,138	9,883	10,005	11,090	17,175	16,769	27,273

Figure 4C, 4E. Fluorescence expression of p-Tau (AT8) in None- and Thrombin-treated HT-22 stable cells)

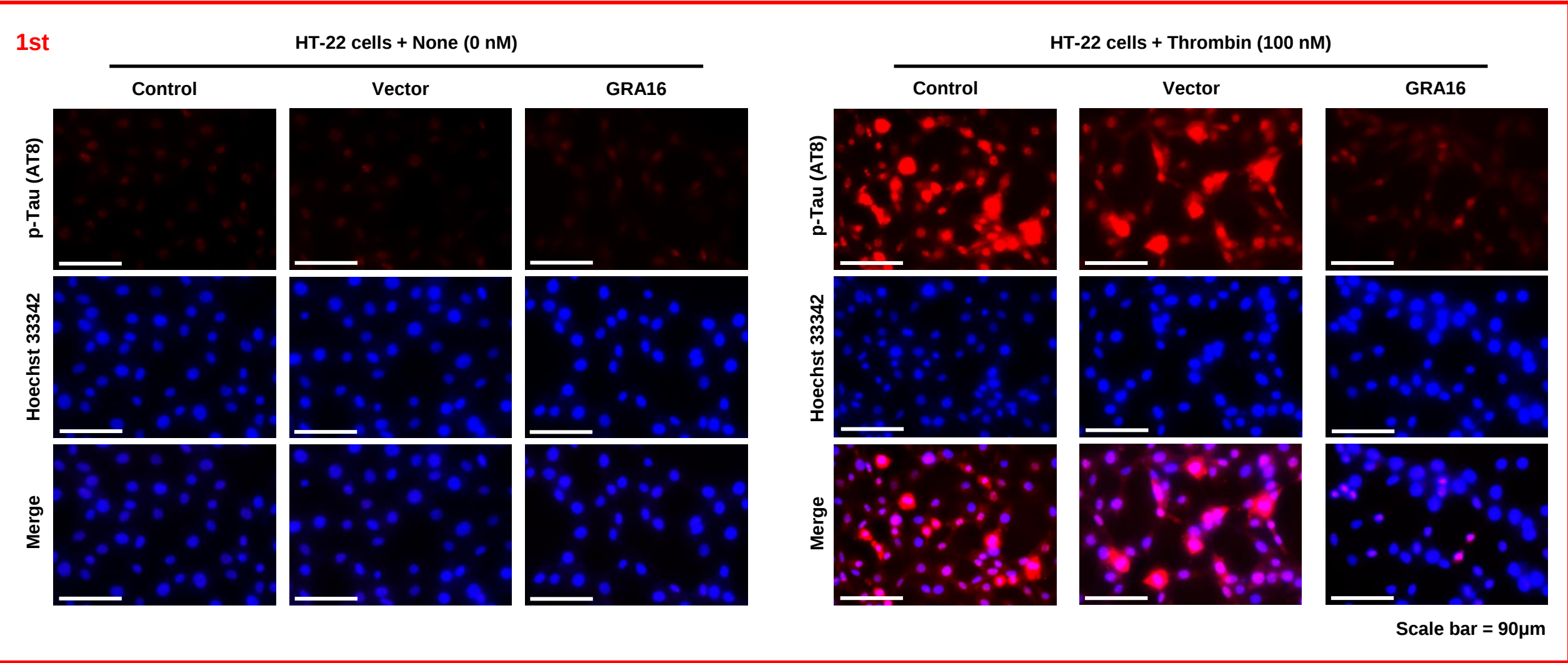


Figure 4C, 4E. Fluorescence expression of p-Tau (AT8) in None- and Thrombin-treated HT-22 stable cells

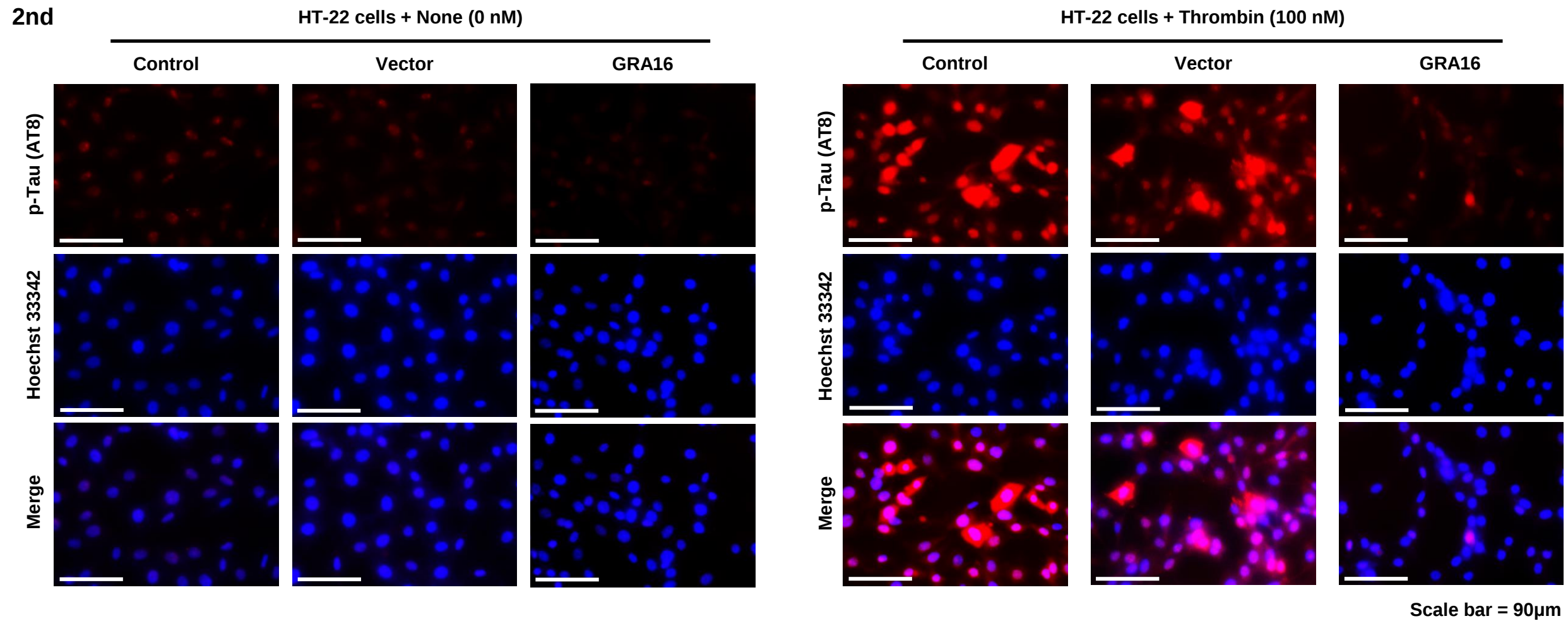


Figure 4C, 4E. Fluorescence expression of p-Tau (AT8) in None- and Thrombin-treated HT-22 stable cells

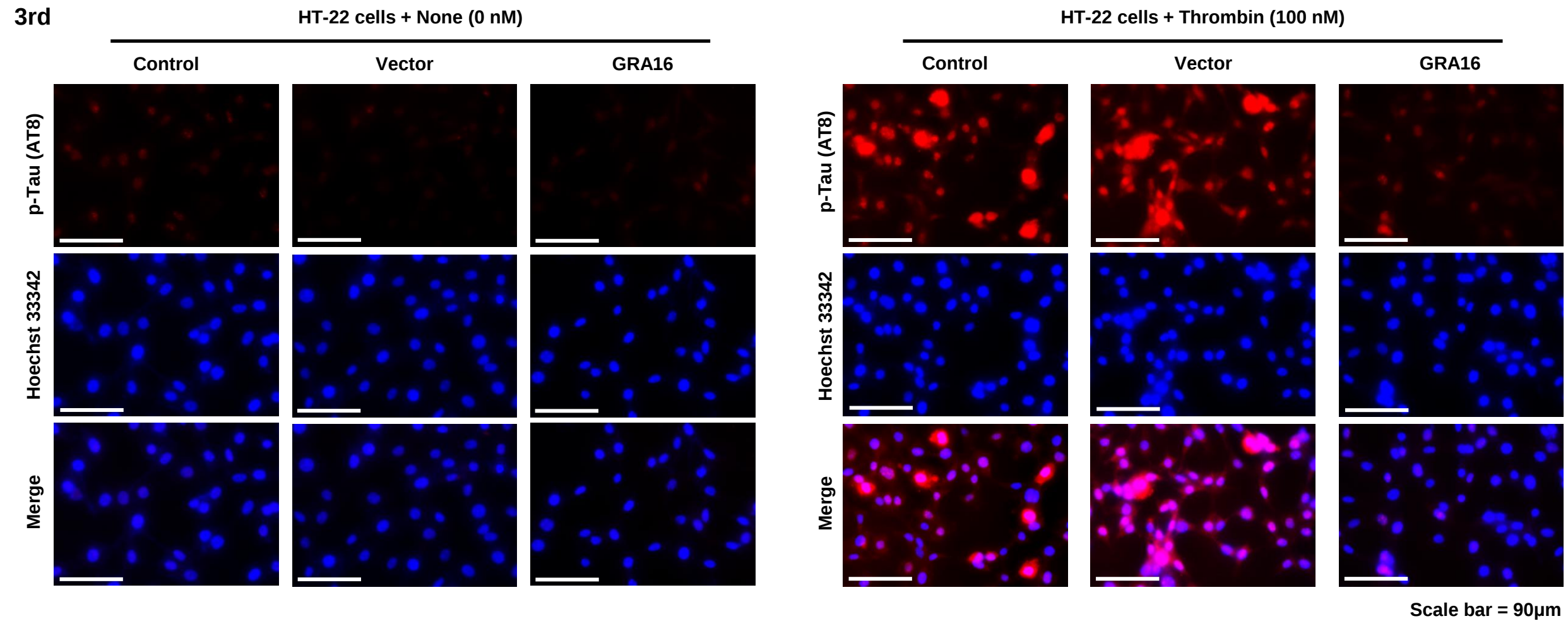


Figure 4D. Fluorescence intensity of p-Tau (AT8) in None-treated HT-22 stable cells

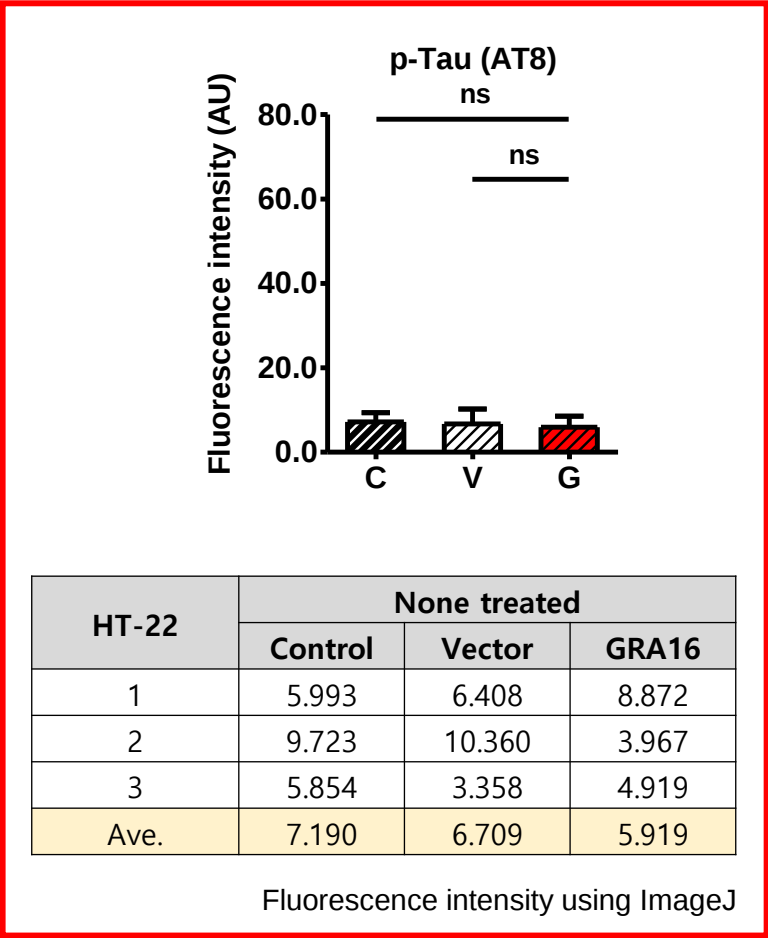
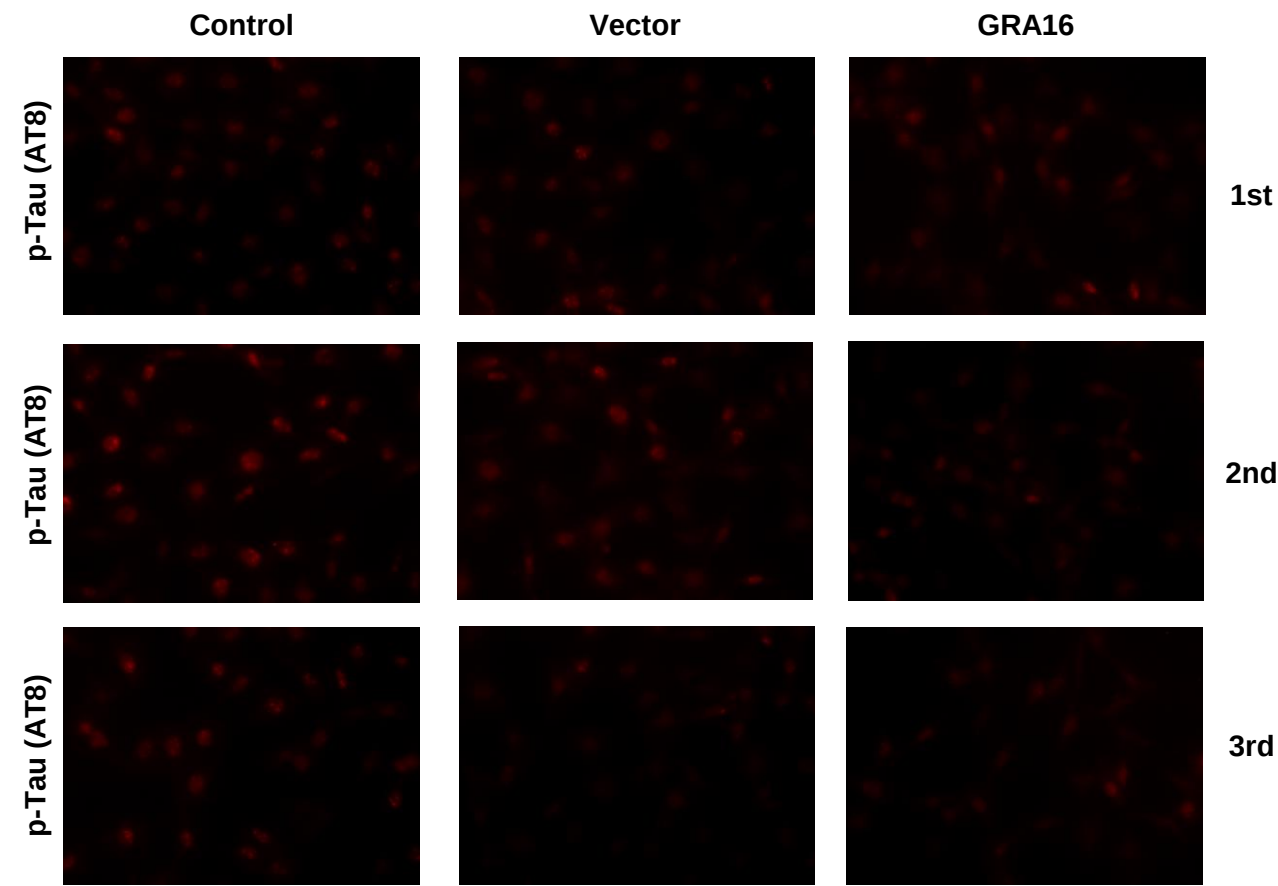


Figure 4F. Fluorescence intensity of p-Tau (AT8) in Thrombin-treated HT-22 stable cells

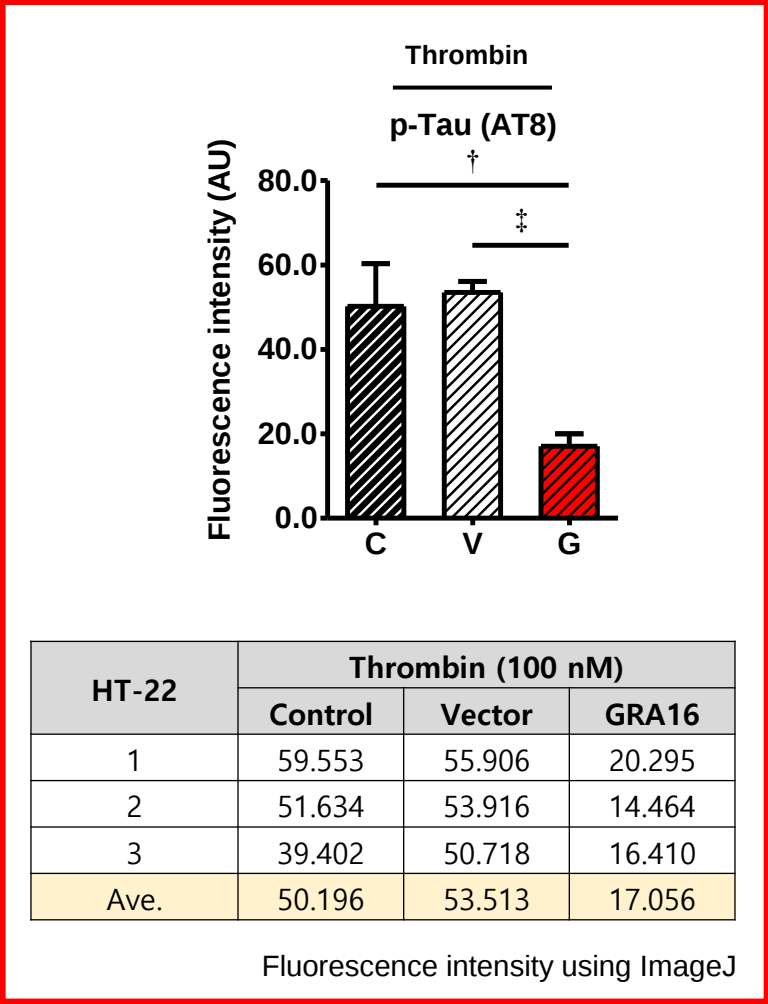
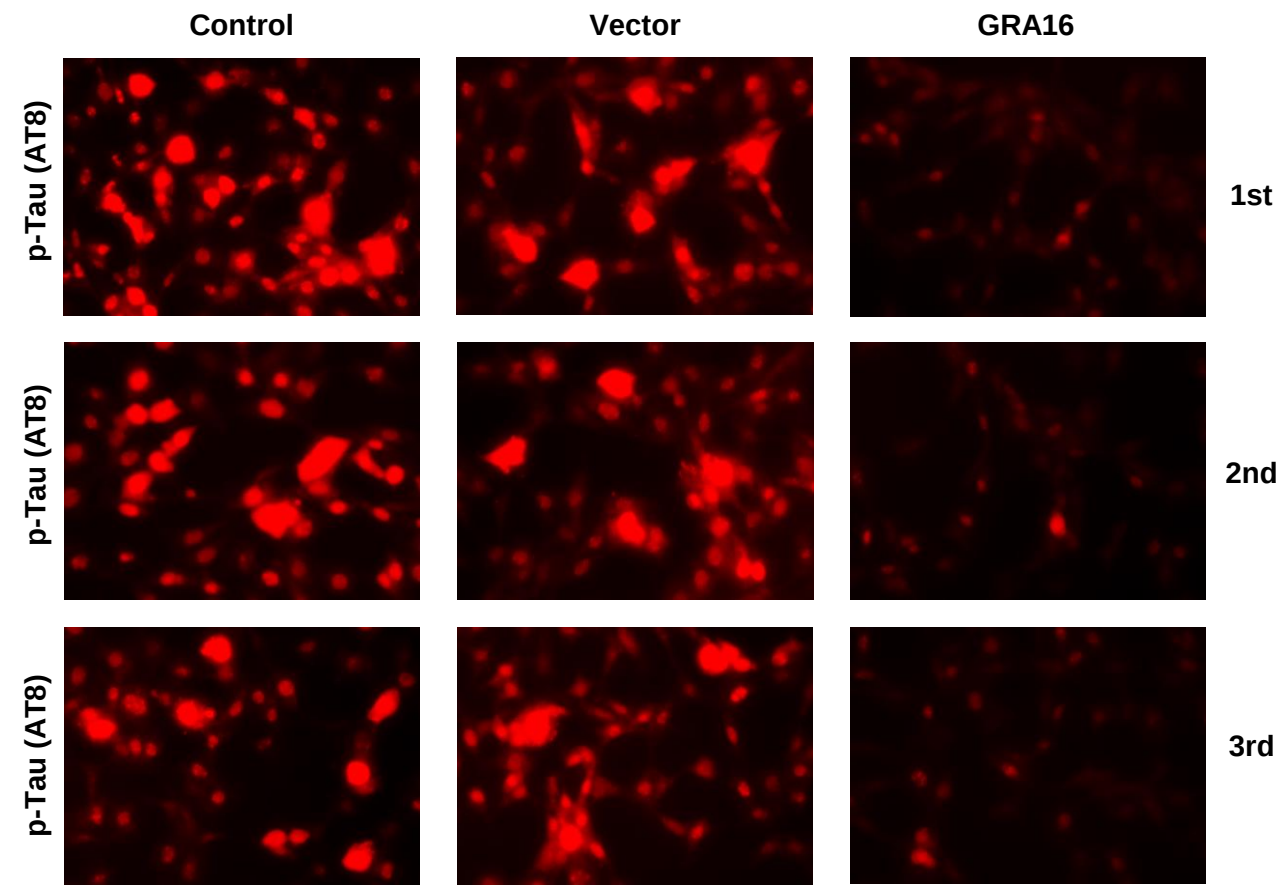
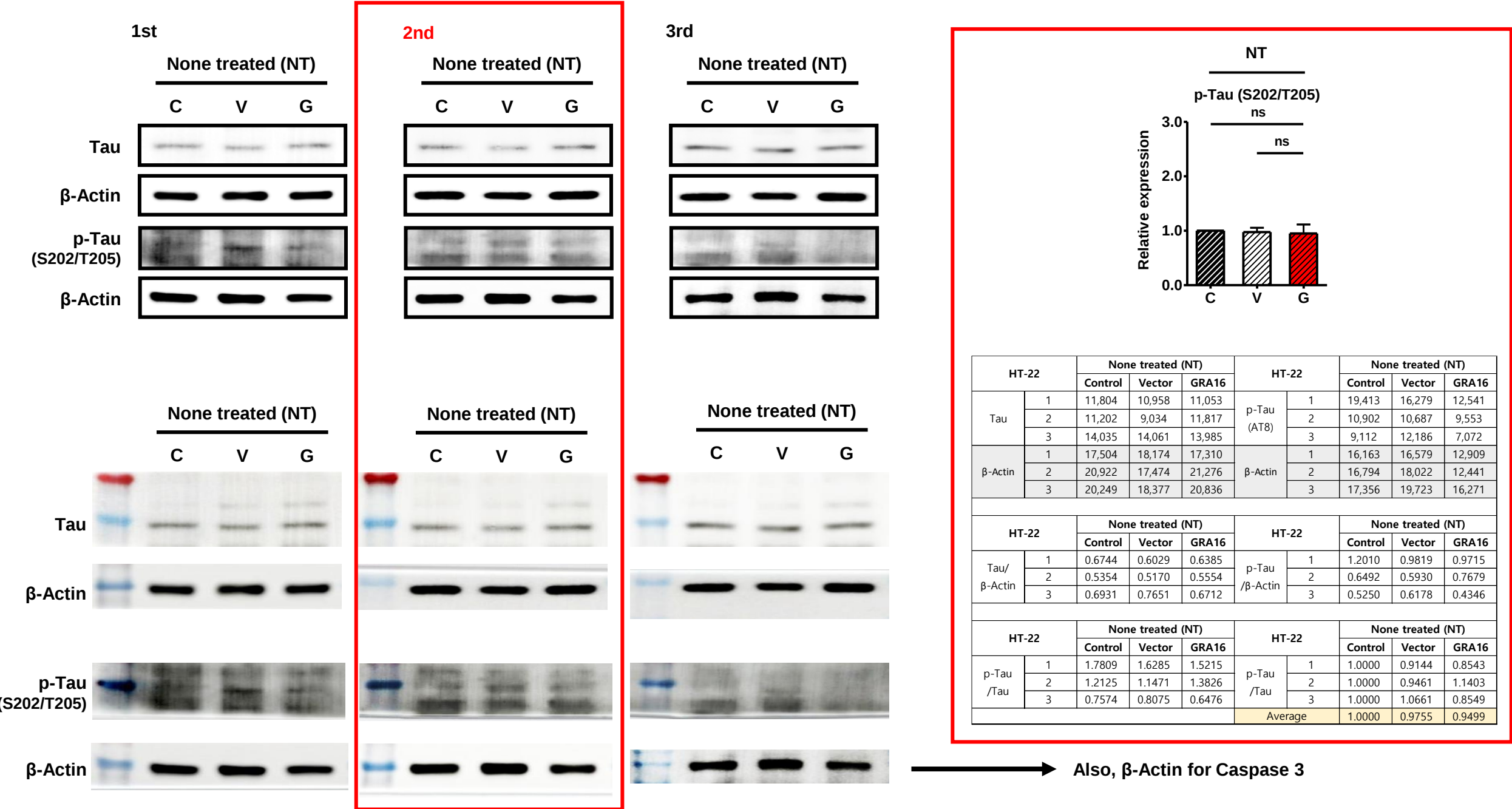


Figure 4G. Tau, p-Tau (S202/T205, AT8) expression of None-treated HT-22 stable cells



NT

p-Tau (S202/T205)

ns

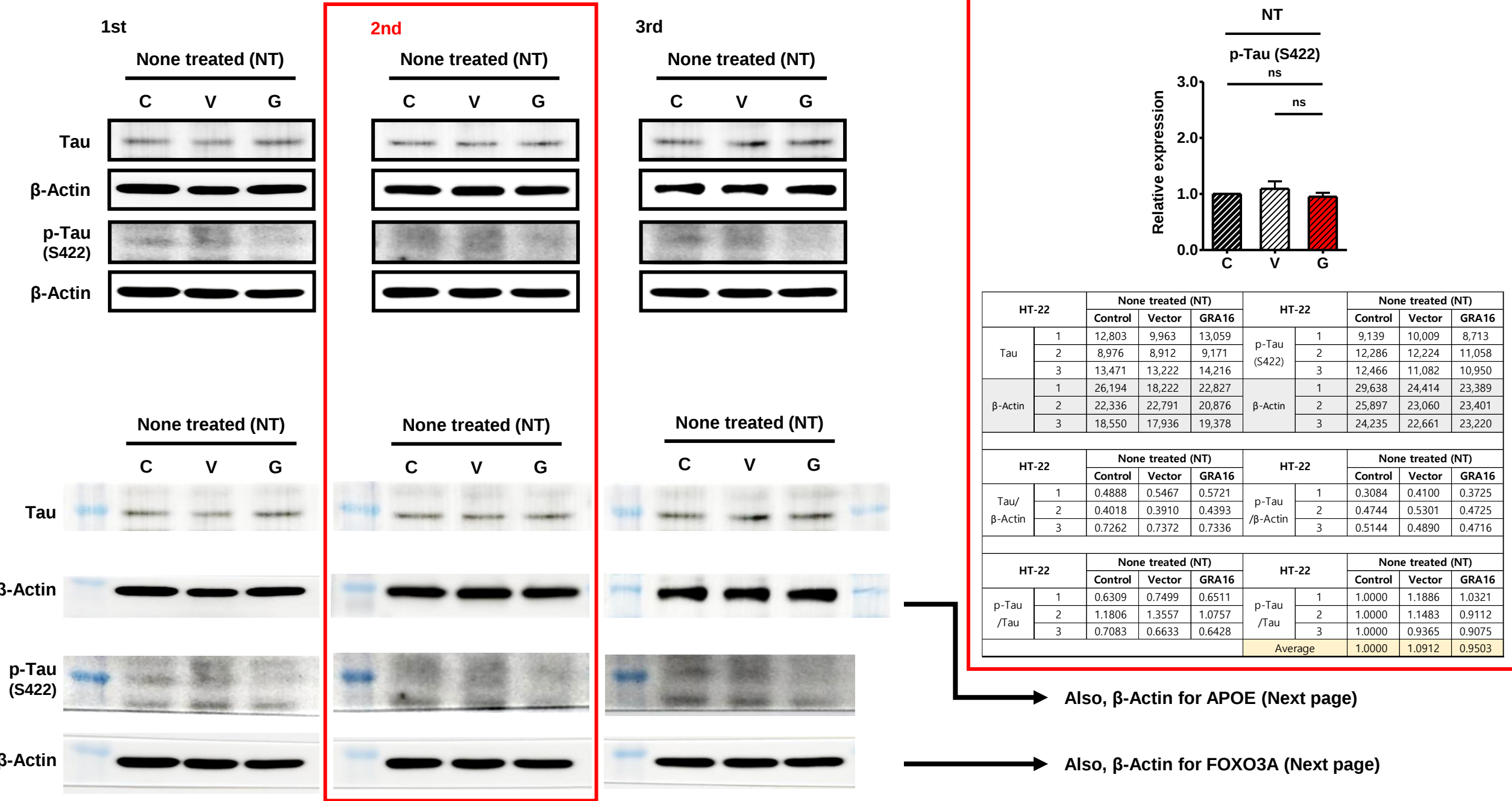
ns

Relative expression

C V G

Also, β-Actin for Caspase 3

Figure 4H. Tau, p-Tau (S422) expression of None-treated HT-22 stable cells



NT

p-Tau (S422)

ns

ns

Relative expression

3.0

2.0

1.0

0.0

C V G

HT-22

None treated (NT)

Control Vector GRA16

HT-22

None treated (NT)

Control Vector GRA16

HT-22

None treated (NT)

Control Vector GRA16

HT-22

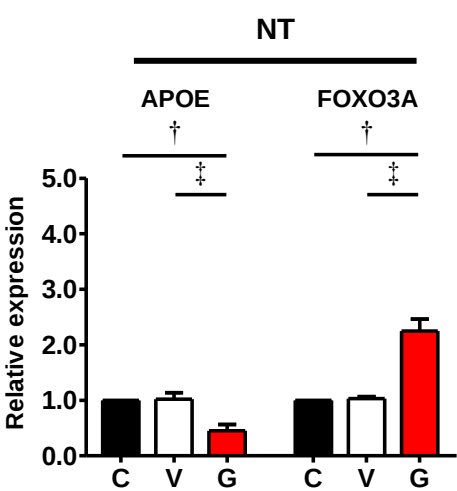
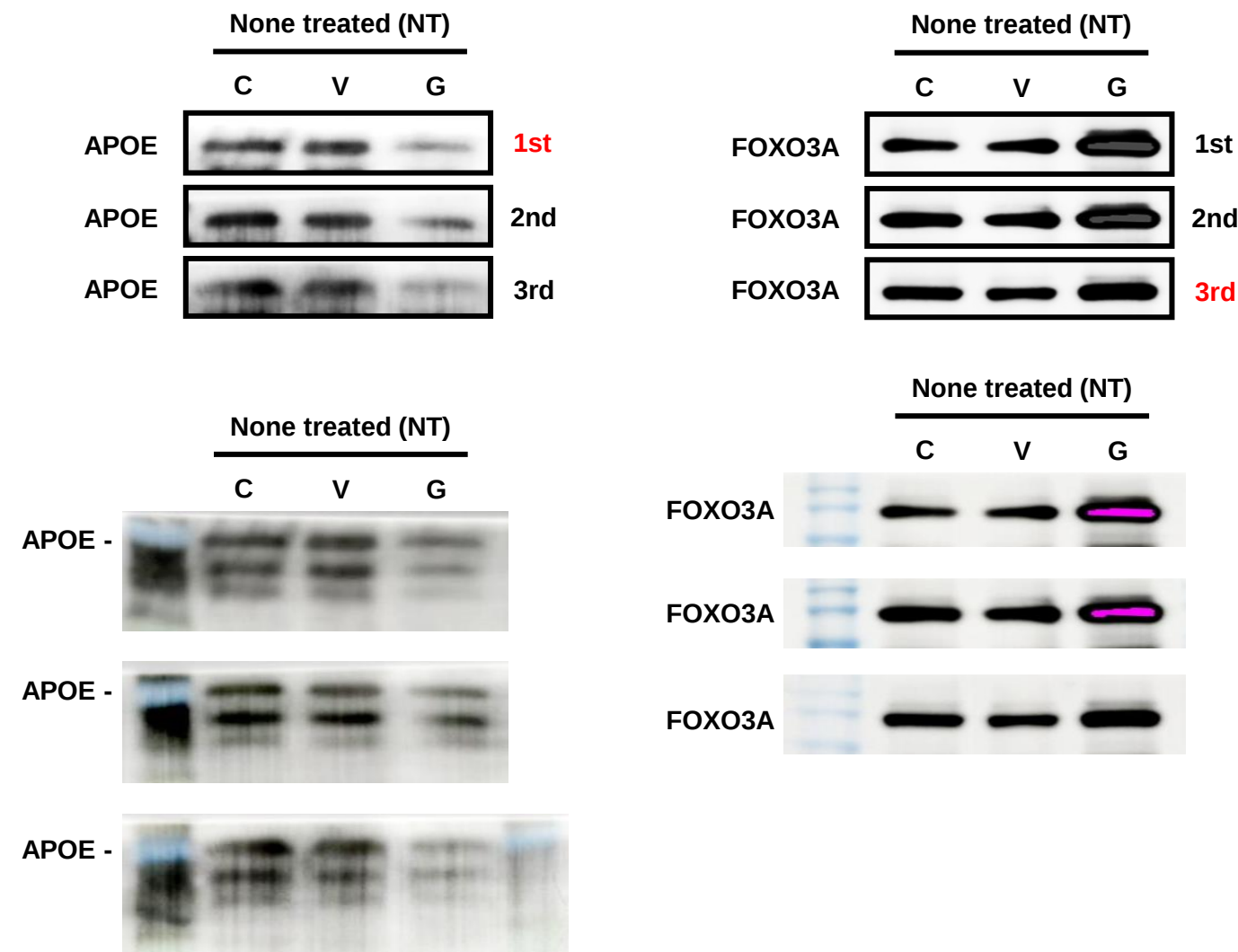
None treated (NT)

Control Vector GRA16

Also, β-Actin for APOE (Next page)

Also, β-Actin for FOXO3A (Next page)

Figure 3B. APOE, FOXO3A expression of None-treated HT-22 stable cells



HT-22		None treated (NT)			HT-22		None treated (NT)		
		Control	Vector	GRA16			Control	Vector	GRA16
APOE	1	13,328	10,642	5,853	APOE/ β-Actin	1	0.5088	0.5840	0.2564
	2	13,913	12,962	6,848		2	0.6229	0.5687	0.3280
	3	16,418	15,818	5,518		3	0.8851	0.8819	0.2847
β-Actin	1	26,194	18,222	22,827	APOE/ β-Actin	1	1	1.1478	0.5040
	2	22,336	22,791	20,876		2	1	0.9130	0.5266
	3	18,550	17,936	19,378		3	1	0.9964	0.3217
					Average		1	1.0191	0.4508

HT-22		None treated (NT)			HT-22		None treated (NT)		
		Control	Vector	GRA16			Control	Vector	GRA16
FOXO3A	1	11,641	9,772	22,879	FOXO3A /β-Actin	1	0.3928	0.4002	0.9782
	2	14,124	13,468	27,718		2	0.5454	0.5840	1.1845
	3	11,496	10,733	22,890		3	0.4744	0.4736	0.9858
β-Actin	1	29,638	24,414	23,389	FOXO3A /β-Actin	1	1	1.0191	2.4905
	2	25,897	23,060	23,401		2	1	1.0709	2.1719
	3	24,235	22,661	23,220		3	1	0.9984	2.0781
					Average		1	1.0295	2.2468

Figure 4I. Tau, p-Tau (S202/T205, AT8) expression of Thrombin-treated HT-22 stable cells

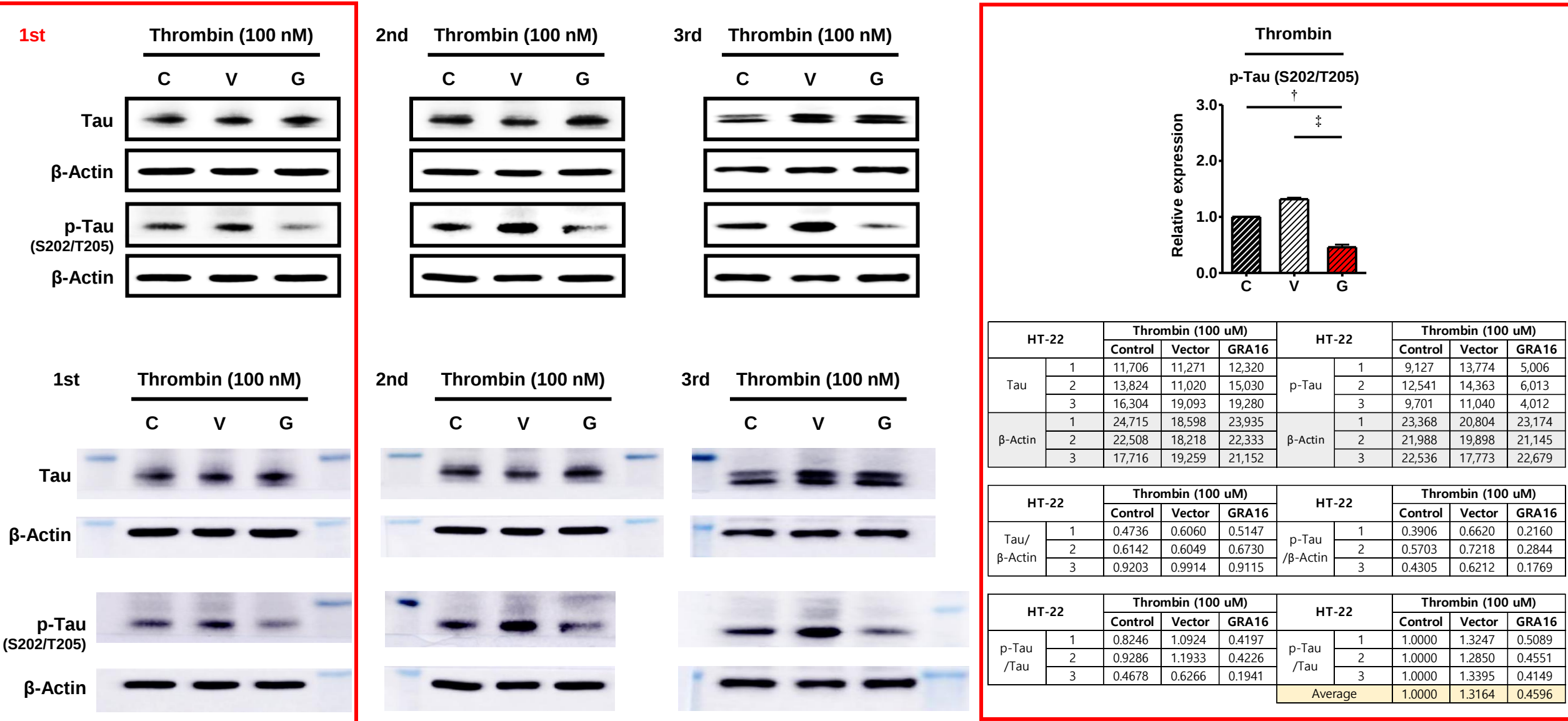


Figure 4J. Tau, p-Tau (S422) expression of Thrombin-treated HT-22 stable cells

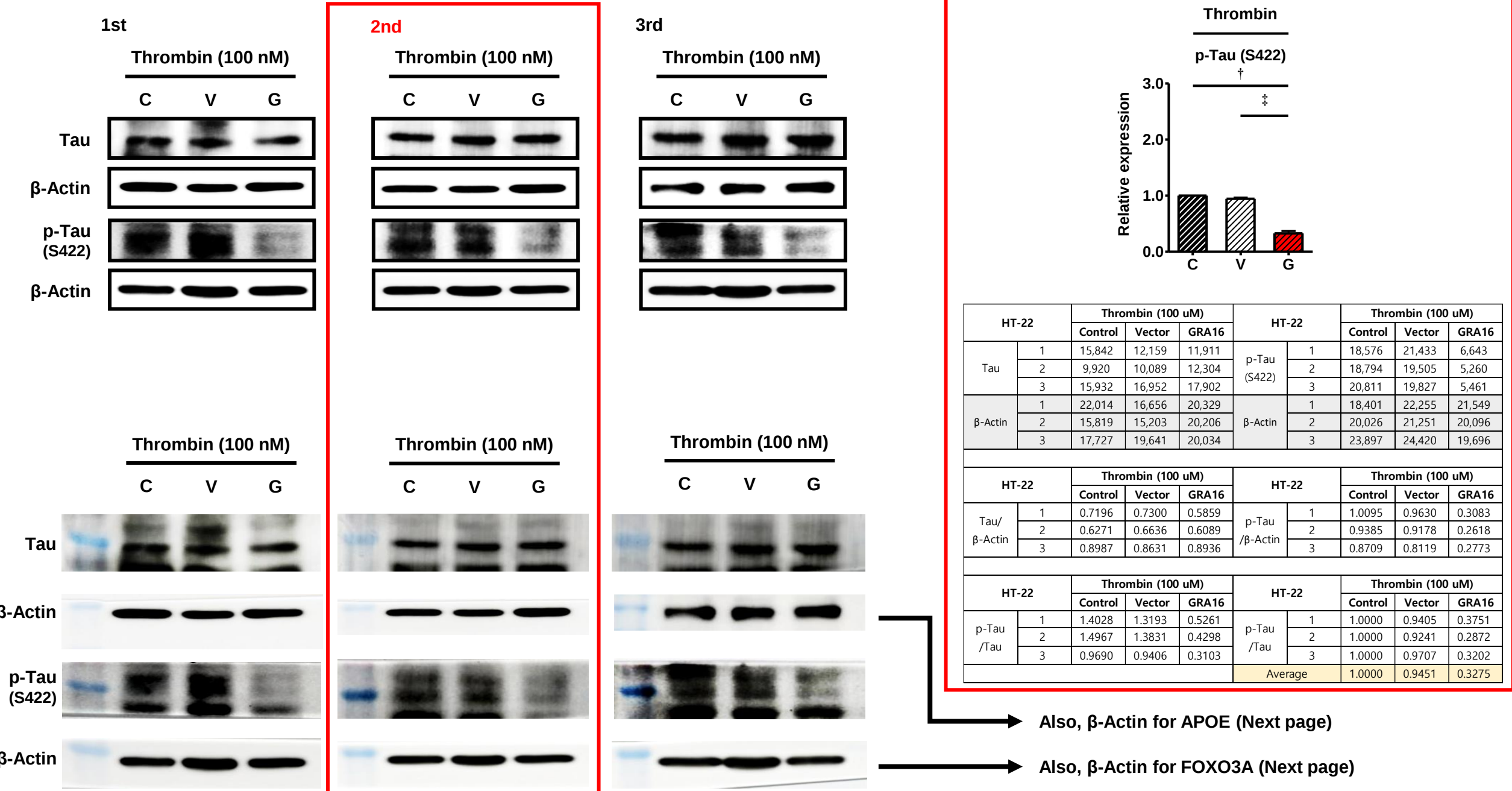
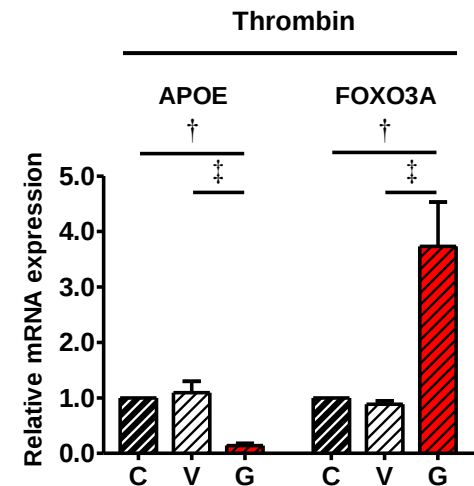
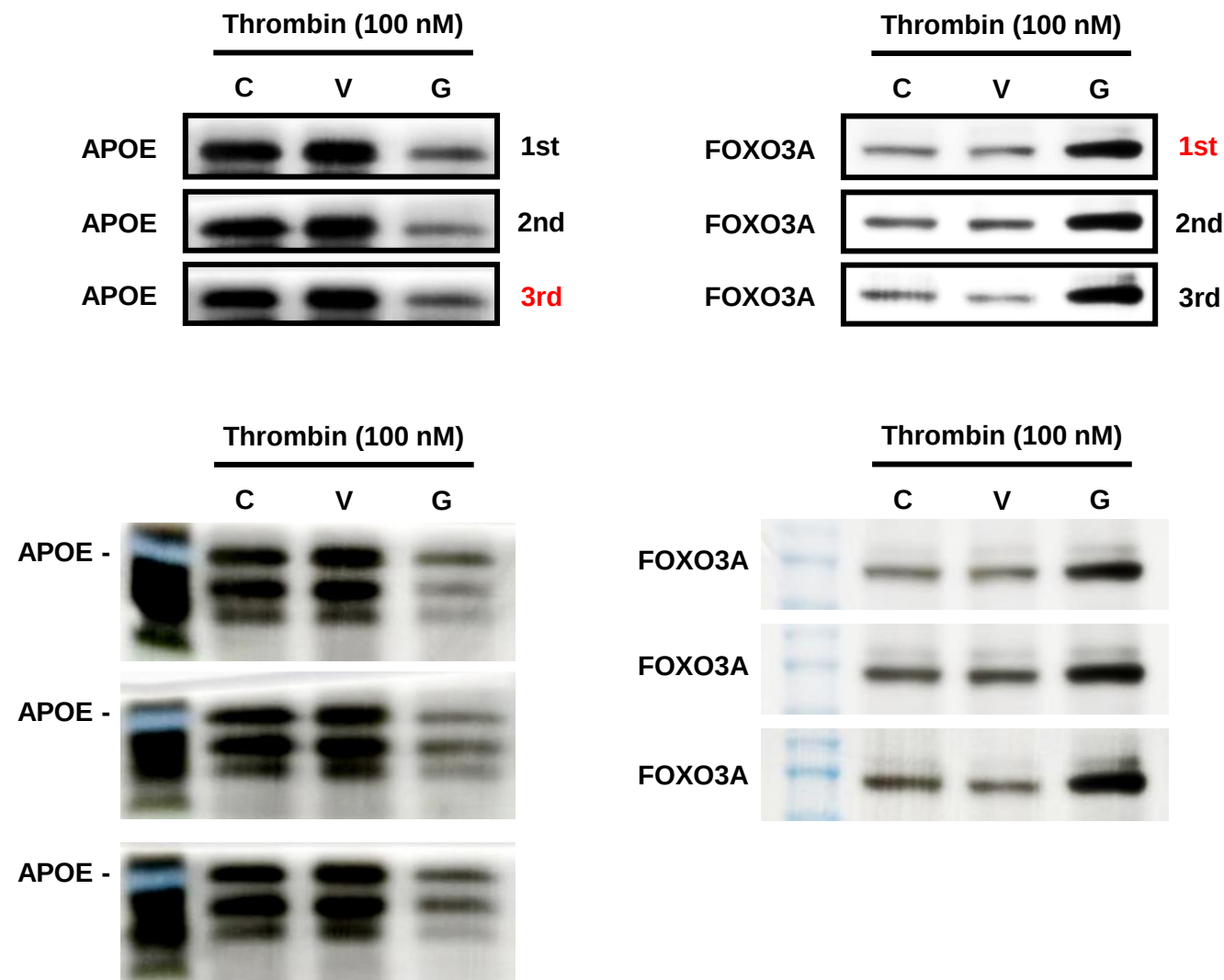


Figure 3E. APOE, FOXO3A expression of Thrombin-treated HT-22 stable cells

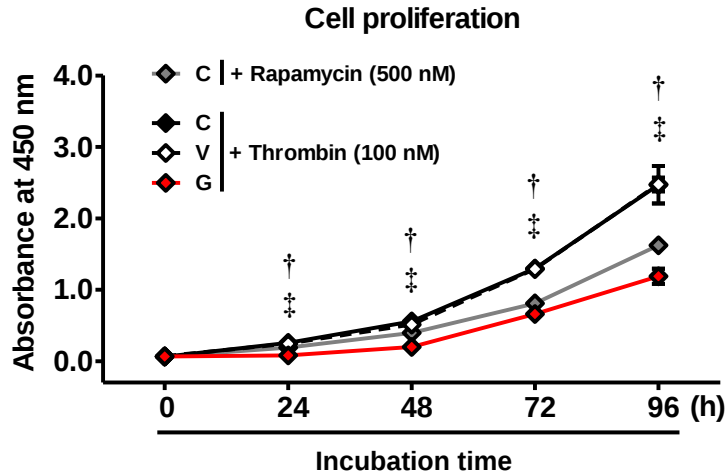


HT-22		Thrombin (100 uM)			HT-22		Thrombin (100 uM)		
		Control	Vector	GRA16			Control	Vector	GRA16
APOE	1	27,006	27,054	4,006	APOE/ β-Actin	1	1.2267	1.6242	0.1971
	2	26,893	26,586	3,333		2	1.7000	1.7487	0.1650
	3	27,220	27,954	5,021		3	1.5355	1.4233	0.2507
β-Actin	1	22,014	16,656	20,329	APOE/ β-Actin	1	1	1.3240	0.1606
	2	15,819	15,203	20,206		2	1	1.0287	0.0970
	3	17,727	19,641	20,034		3	1	0.9269	0.1632
					Average		1	1.0932	0.1403

HT-22		Thrombin (100 uM)			HT-22		Thrombin (100 uM)		
		Control	Vector	GRA16			Control	Vector	GRA16
FOXO3A	1	6,205	6,187	22,130	FOXO3A /β-Actin	1	0.3372	0.2780	1.0270
	2	6,156	6,188	21,897		2	0.3074	0.2912	1.0896
	3	5,892	5,332	22,385		3	0.2466	0.2184	1.1365
β-Actin	1	18,401	22,255	21,549	FOXO3A /β-Actin	1	1	0.8243	3.0452
	2	20,026	21,251	20,096		2	1	0.9473	3.5448
	3	23,897	24,420	19,696		3	1	0.8856	4.6097
					Average		1	0.8858	3.7332

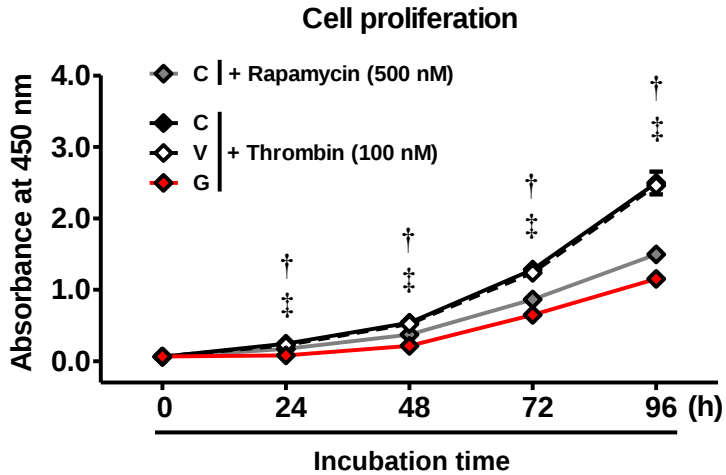
Figure 4K.
Cell proliferation in
Rapamycin- or Thrombin-
treated HT-22 stable cells

1st



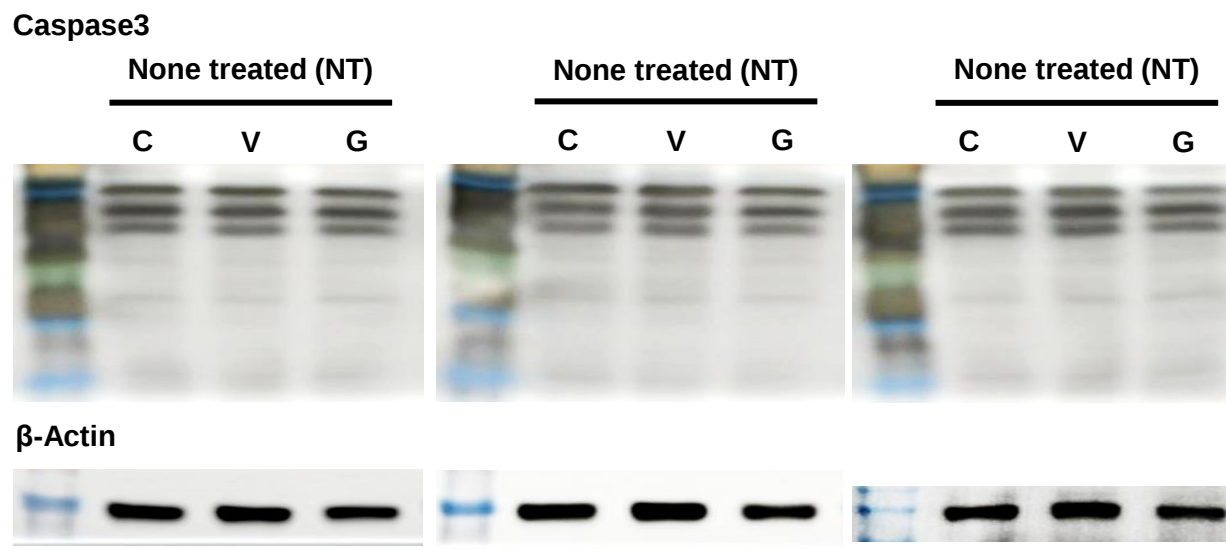
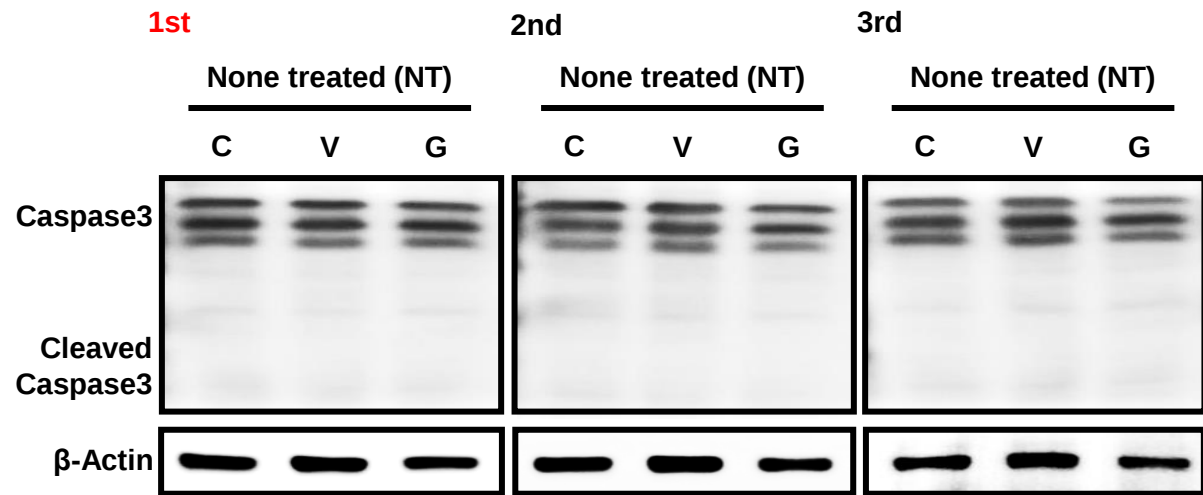
HT-22	Control cells + Rapamycin (500 nM)					
	1	2	3	4	5	Average
0h	0.0641	0.0628	0.0684	0.0645	0.0658	0.0651
24h	0.1918	0.1932	0.1870	0.1902	0.1847	0.1894
48h	0.4097	0.3871	0.4033	0.3916	0.3927	0.3969
72h	0.7704	0.8374	0.8186	0.8453	0.7737	0.8091
96h	1.6610	1.5314	1.5267	1.6733	1.7134	1.6212
HT-22	Control cells + Thrombin (100 nM)					
	1	2	3	4	5	Average
0h	0.0642	0.0633	0.0613	0.0651	0.0626	0.0633
24h	0.2549	0.2465	0.2629	0.2651	0.2489	0.2557
48h	0.5355	0.5735	0.5582	0.5381	0.5784	0.5567
72h	1.3385	1.3158	1.2123	1.3539	1.2644	1.2970
96h	2.1948	2.6852	2.1703	2.6591	2.6405	2.4700
HT-22	Vector cells + Thrombin (100 nM)					
	1	2	3	4	5	Average
0h	0.0666	0.0666	0.0618	0.0727	0.0691	0.0674
24h	0.2470	0.2481	0.2461	0.2241	0.2567	0.2444
48h	0.5474	0.5251	0.4959	0.5047	0.4783	0.5103
72h	1.3546	1.3544	1.2703	1.2608	1.2086	1.2897
96h	2.4005	2.5893	2.4386	2.5643	2.3796	2.4745
HT-22	GRA16 cells + Thrombin (100 nM)					
	1	2	3	4	5	Average
0h	0.0627	0.0624	0.0653	0.0673	0.0656	0.0647
24h	0.0864	0.0810	0.0895	0.0766	0.0735	0.0814
48h	0.2059	0.1995	0.1856	0.1958	0.2123	0.1998
72h	0.5616	0.6298	0.6456	0.7120	0.7467	0.6591
96h	1.2993	1.2333	1.1825	1.0155	1.2140	1.1889

2nd



HT-22	Control cells + Rapamycin (500 nM)					
	1	2	3	4	5	Average
0h	0.0636	0.0653	0.0643	0.0633	0.0633	0.0640
24h	0.1621	0.1779	0.1806	0.1700	0.1751	0.1731
48h	0.3737	0.3689	0.3733	0.3777	0.3735	0.3734
72h	0.8669	0.8610	0.8771	0.8640	0.8450	0.8628
96h	1.5512	1.4707	1.5521	1.5109	1.3893	1.4948
HT-22	Control cells + Thrombin (100 nM)					
	1	2	3	4	5	Average
0h	0.0602	0.0619	0.0635	0.0619	0.0619	0.0619
24h	0.2419	0.2396	0.2547	0.2483	0.2440	0.2457
48h	0.5429	0.5182	0.5503	0.5466	0.5324	0.5381
72h	1.2321	1.2475	1.3607	1.2964	1.2719	1.2817
96h	2.6304	2.6256	2.2483	2.4394	2.5325	2.4952
HT-22	Vector cells + Thrombin (100 nM)					
	1	2	3	4	5	Average
0h	0.0644	0.0607	0.0660	0.0652	0.0629	0.0638
24h	0.2460	0.2049	0.2204	0.2332	0.2191	0.2247
48h	0.5277	0.5420	0.4977	0.5127	0.5273	0.5215
72h	1.2379	1.2842	1.1997	1.2188	1.2515	1.2384
96h	2.5075	2.5097	2.3728	2.4402	2.4749	2.4610
HT-22	GRA16 cells + Thrombin (100 nM)					
	1	2	3	4	5	Average
0h	0.0651	0.0672	0.0661	0.0656	0.0664	0.0661
24h	0.0800	0.0769	0.0887	0.0844	0.0806	0.0821
48h	0.2196	0.2084	0.2143	0.2169	0.2126	0.2143
72h	0.6693	0.6812	0.5993	0.6343	0.6578	0.6484
96h	1.1037	1.2472	1.1176	1.1107	1.1789	1.1516

Figure 4L. Caspase 3 expression of None-treated HT-22 stable cells



Also, β -Actin for p-Tau (S202/T422)

Figure 4M. Caspase 3 expression of Thrombin-treated HT-22 stable cells

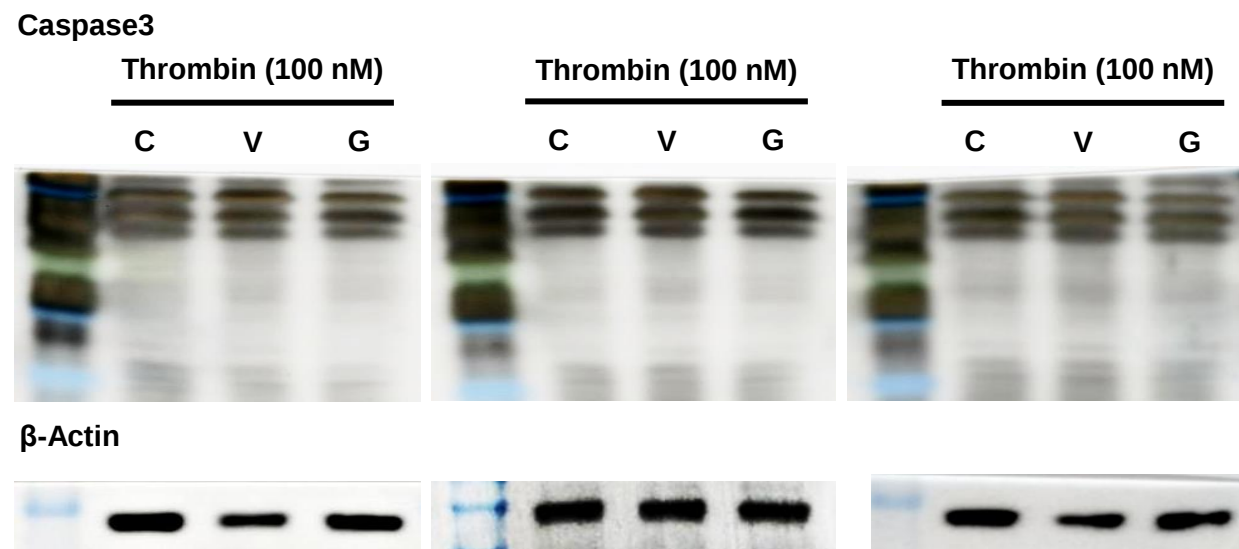
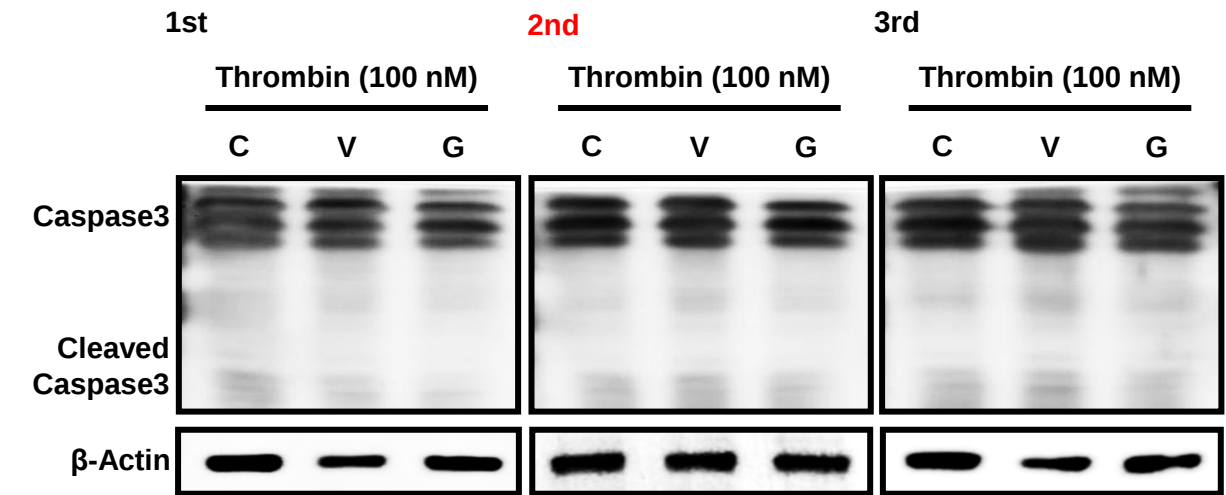


Figure 4L. Caspase 3 expression of None-treated HT-22 stable cells

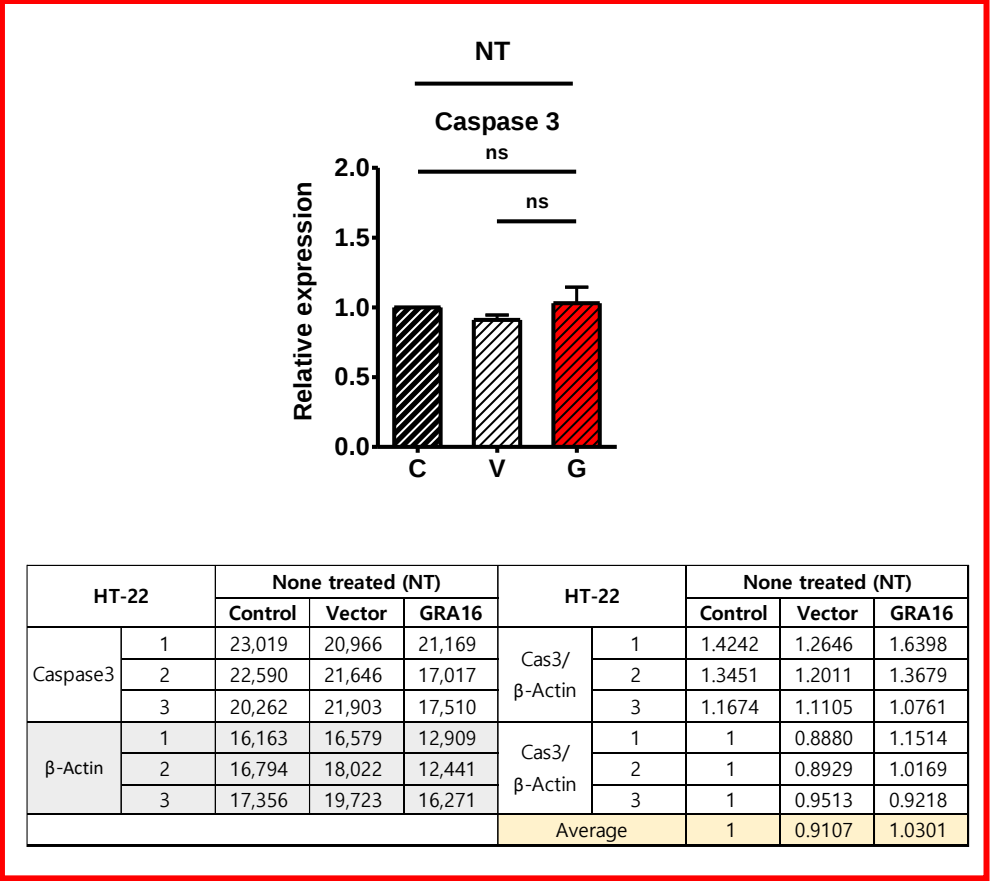


Figure 4M. Caspase 3 expression of Thrombin-treated HT-22 stable cells

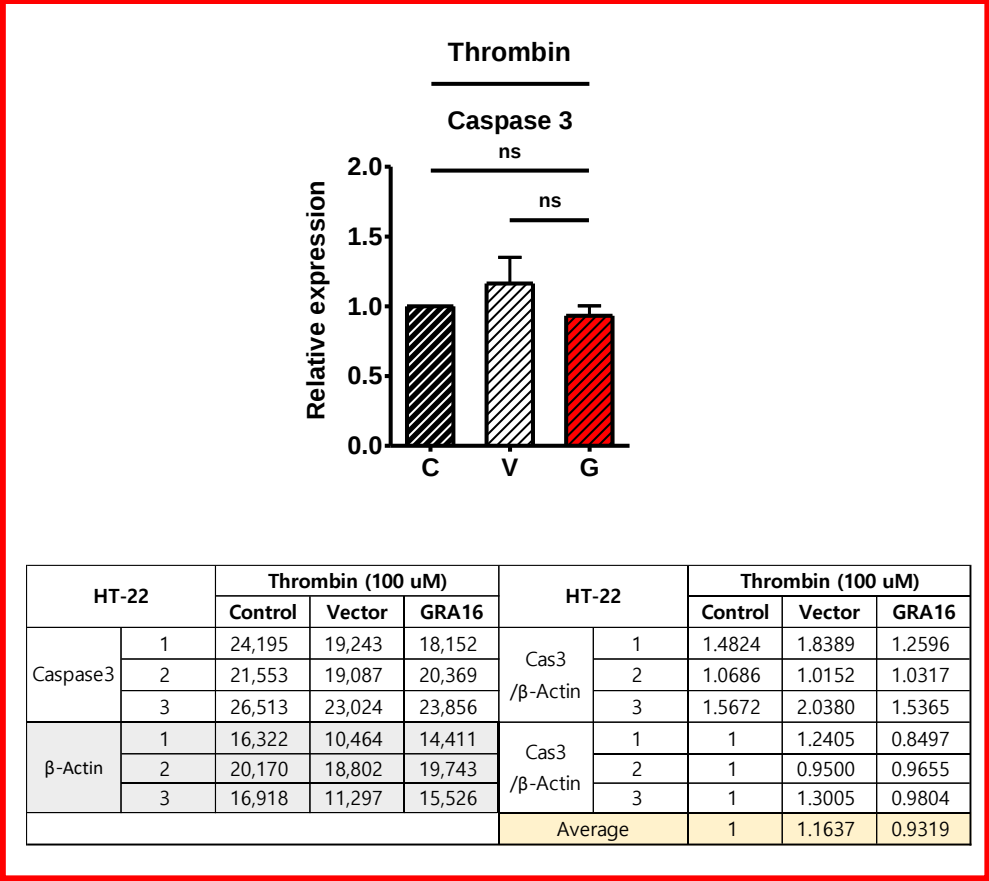
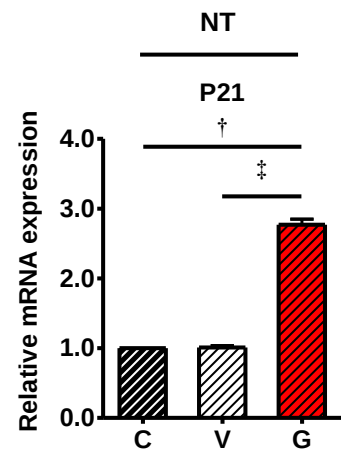
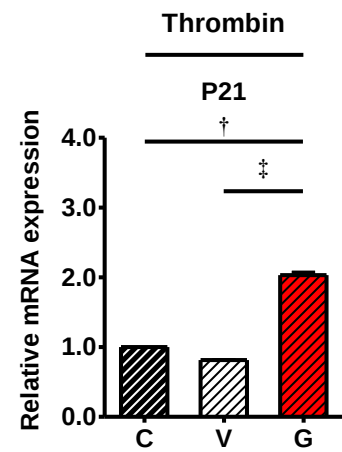


Figure 4O. P21 expression of None-treated HT-22 stable cells



HT-22	P21			HT-22	P21			HT-22	P21		
ΔCT	Control	Vector	GRA16	ΔΔCT	Control	Vector	GRA16	2 ^{-ΔΔCT}	Control	Vector	GRA16
1	7.7638	7.8077	6.3621	1	0.0000	0.0439	-1.4017	1	1.0000	0.9700	2.6421
2	7.8848	7.8812	6.3713	2	0.0000	-0.0036	-1.5135	2	1.0000	1.0025	2.8549
3	7.8780	7.8181	6.3891	3	0.0000	-0.0599	-1.4889	3	1.0000	1.0424	2.8068
4	7.8679	7.8514	6.4071	4	0.0000	-0.0165	-1.4608	4	1.0000	1.0115	2.7527
5	7.8748	7.8428	6.3948	5	0.0000	-0.0320	-1.4800	5	1.0000	1.0225	2.7896
Average	7.8539	7.8402	6.3849	Average	0.0000	-0.0136	-1.4690	Average	1.0000	1.0098	2.7692

Figure 4P. P21 expression of Thrombin-treated HT-22 stable cells



HT-22	P21			HT-22	P21			HT-22	P21		
ΔCT	Control	Vector	GRA16	ΔΔCT	Control	Vector	GRA16	2 ^{-ΔΔCT}	Control	Vector	GRA16
1	6.4342	6.7340	5.4298	1	0.0000	0.2998	-1.0044	1	1.0000	0.8124	2.0061
2	6.4327	6.7178	5.3701	2	0.0000	0.2850	-1.0627	2	1.0000	0.8207	2.0888
3	6.4177	6.7225	5.3815	3	0.0000	0.3048	-1.0362	3	1.0000	0.8096	2.0509
4	6.4187	6.7194	5.4307	4	0.0000	0.3007	-0.9880	4	1.0000	0.8119	1.9835
5	6.3785	6.6791	5.3594	5	0.0000	0.3006	-1.0191	5	1.0000	0.8119	2.0266
Average	6.4164	6.7146	5.3943	Average	0.0000	0.2982	-1.0221	Average	1.0000	0.8133	2.0312