Supplementary Information

Beta 2 adrenergic receptor antagonist propranolol and opioidergic receptor antagonist naltrexone produce synergistic effects on breast cancer growth prevention by acting on cancer cells and immune environment in a preclinical model of breast cancer.

Sengottuvelan Murugan, Bénédicte Rousseau, and Dipak K. Sarkar

Supplementary Figures

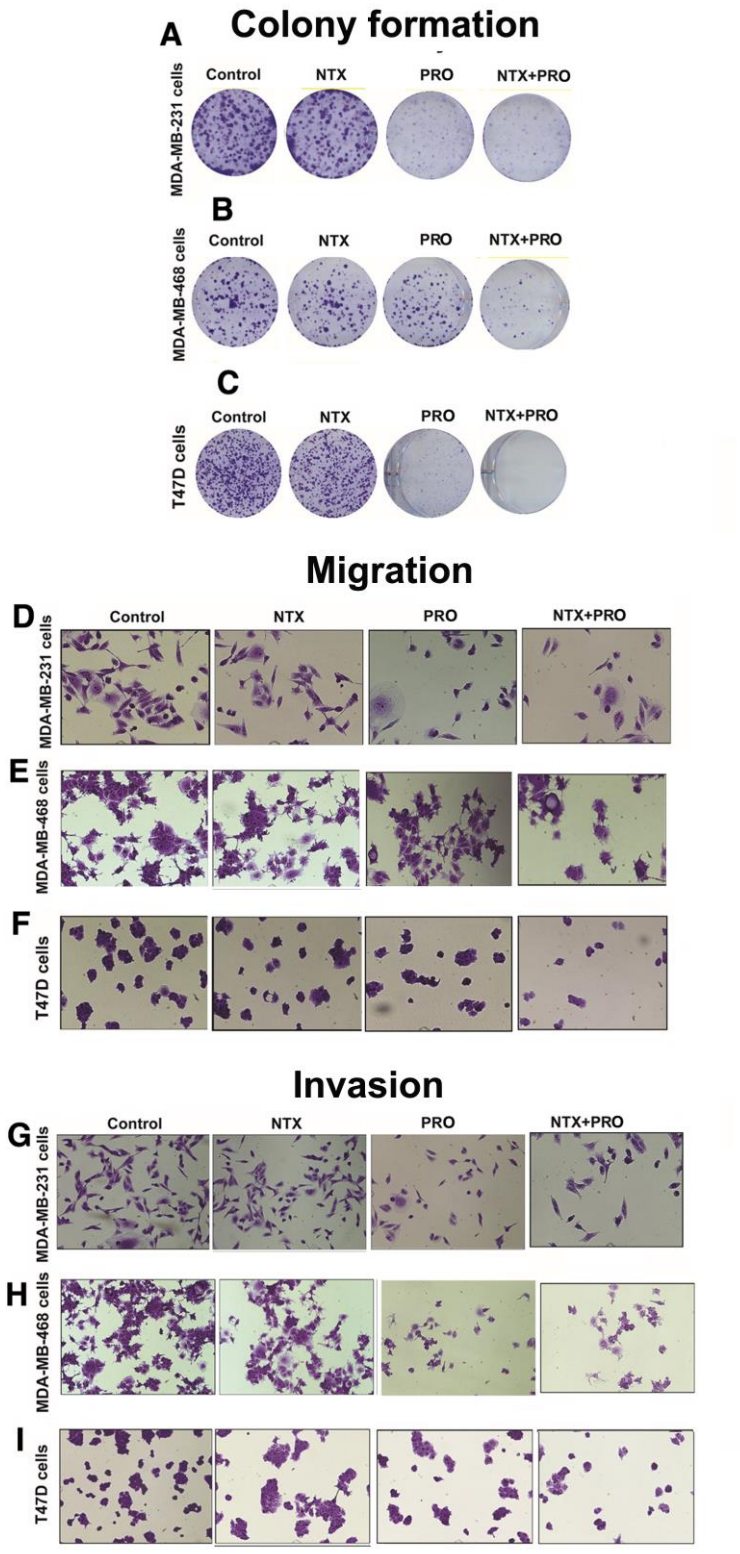


Figure S1. Beta-adrenergic and opioidergic agents suppress breast tumor cells' clonogenic behavior, cell migration, and invasion. Representative images of MDA-MB-231 (**A**), MDA-MB-468 (**B**), and T47D (**C**) crystal violet stained cell colonies after 14 days of treatment with a 100 μM dose of PRO and NTX alone or in combination. The crystal violet stained cell colonies were extracted with 10% acetic acid and used for optical density (OD) measurements for determination of colony growth. These data are presented in Fig. 1B-D. Cell migration was determined using transwell migration assay. Cells were stained with 0.5% crystal violet from control or after NTX, PRO, or NTX+PRO treatments as described in materials and methods. Representative images of migrated MDA-MB-231 cells (**D**), MDA-MB-468 cells (**E**), and T47D cells (**F**) are shown. The mean ± SEM values of cell migration data are presented in Figure 1E-G. Cell invasion was tested on a Matrigel™ coated membrane. Cells were treated with the drugs and stained

with 0.5% crystal violet after completion of the test. Representative images are shown here for MDA-MB-231 cells (**G**), MDA-MB-468 cells (**H**), and T47D cells (**I**), and mean ± SEM values of cell invasion data are presented in Figure 1H–J.

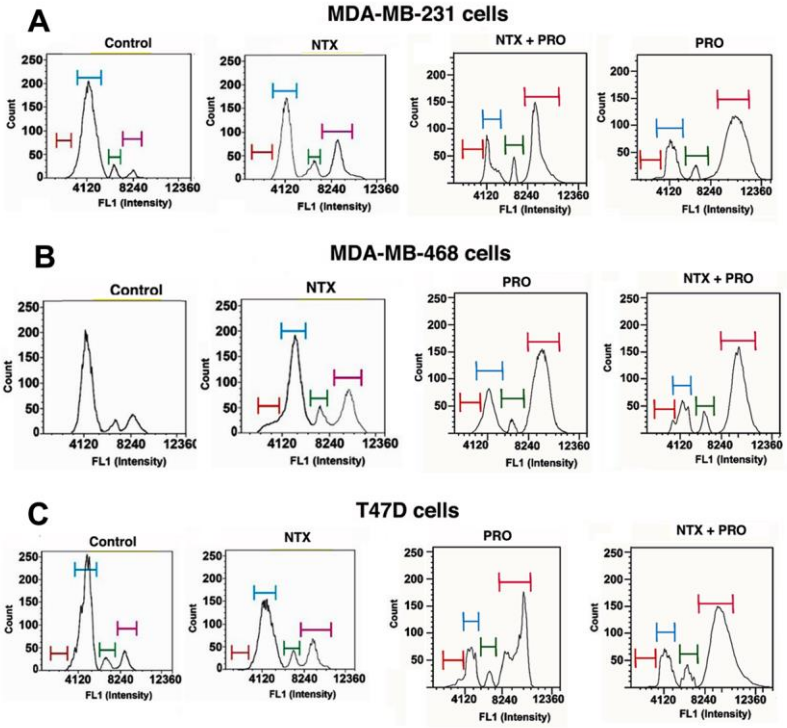


Figure S2. Beta-adrenergic and opioidergic agents increase the cell growth arrest in breast cancer cell lines. Cell cycle analysis of MDA-MB-231 (**A**), MDA-MB-468 (**B**), and T47D (**C**) cells following treatments with NTX, PRO, or NTX+PRO were conducted as described in materials and methods. Cells were treated with a 100 μM concentration of the beta-adrenergic and opioidergic drugs alone or in combination or vehicle alone for 24 hours. After the treatment period, cells were stained with PI, analyzed for cell cycle distribution using flow cytometry, and shown as grafts. Proportions of cells in each phase were quantified and shown as histograms in Figure 2A–C.

Figure S3 Original gel blots (Refer to Figure4,5,7,8):

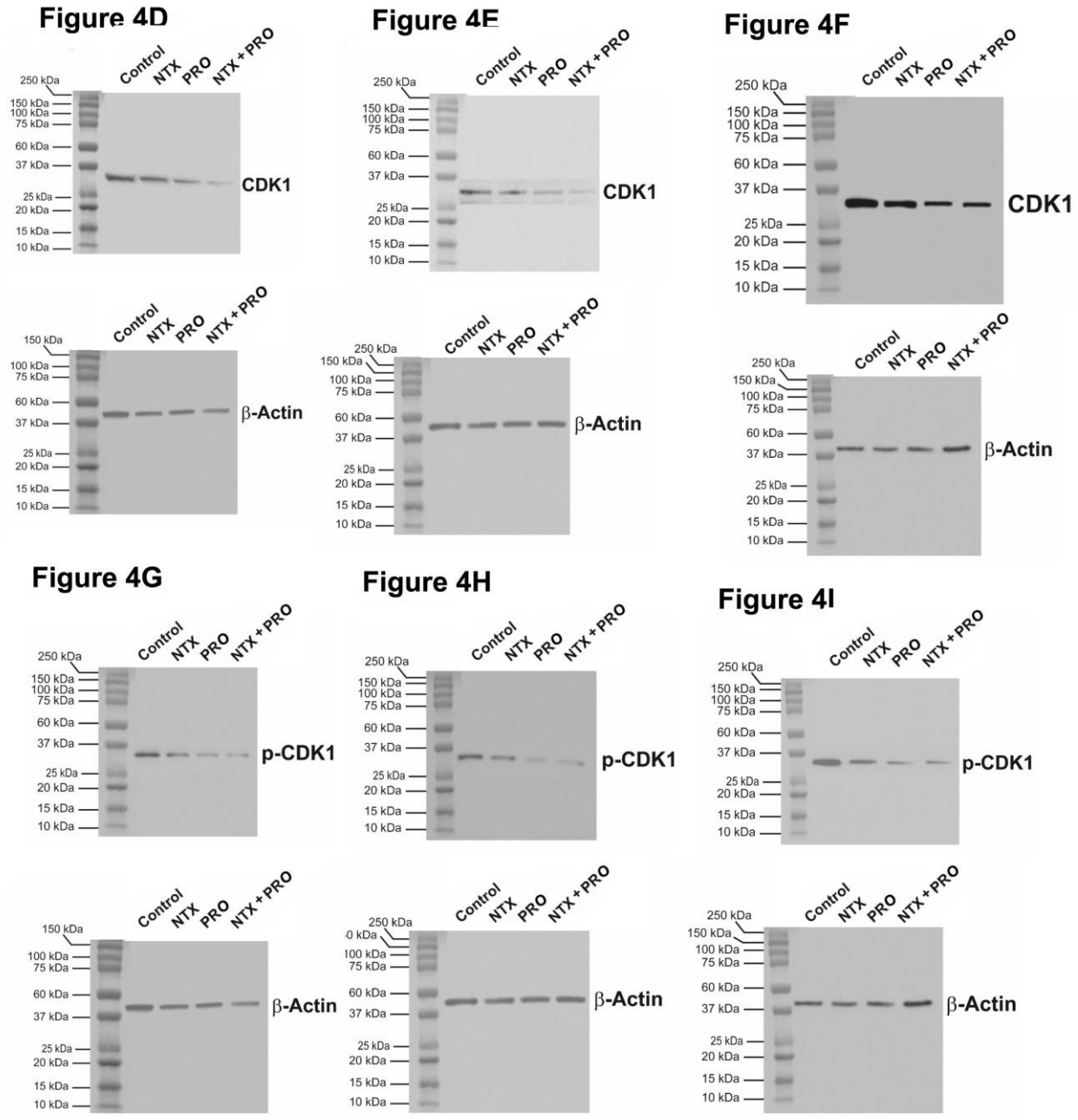
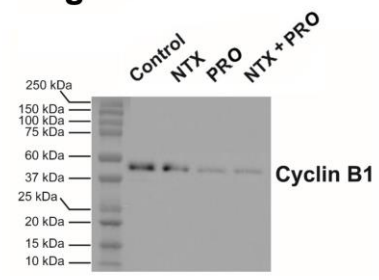


Figure 4J



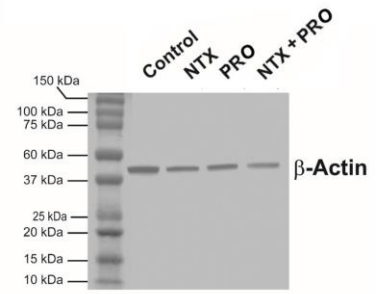
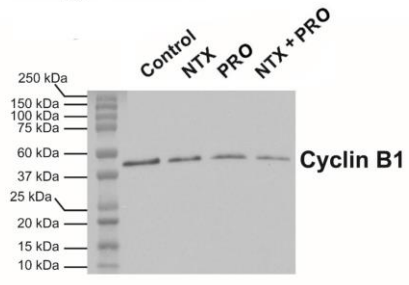
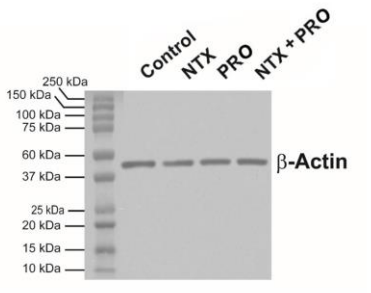


Figure 4K





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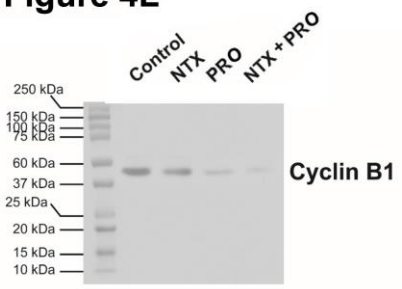

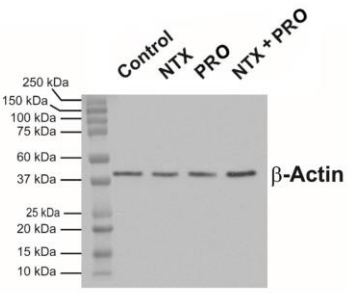

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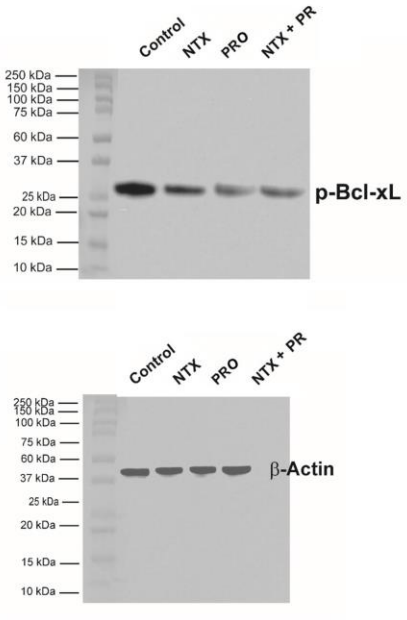

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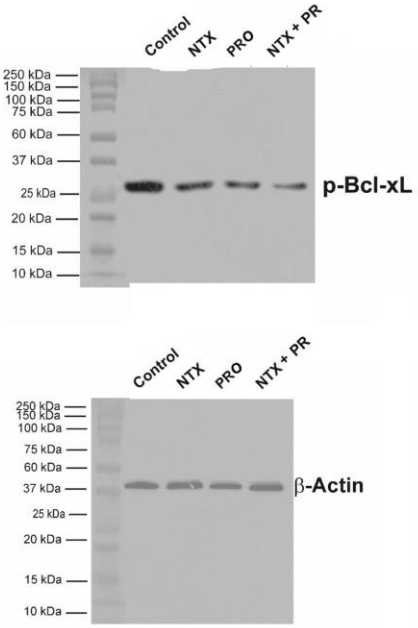

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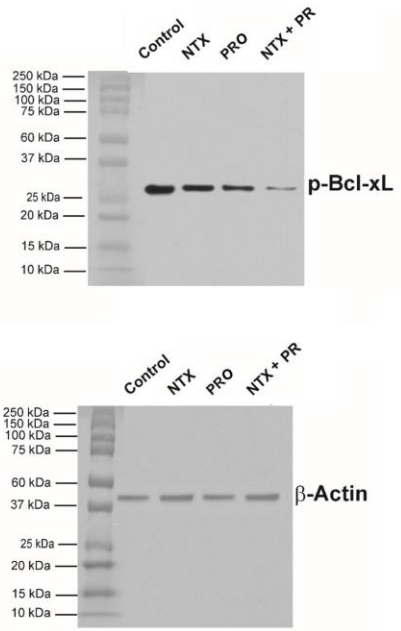

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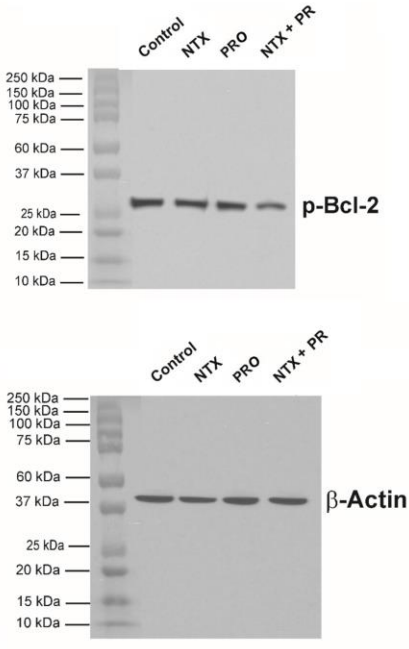

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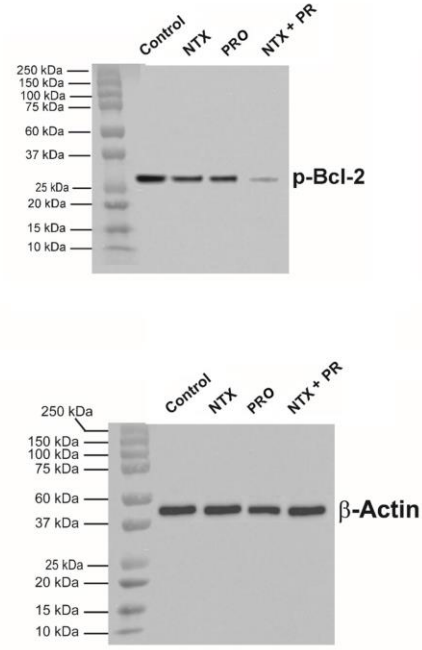

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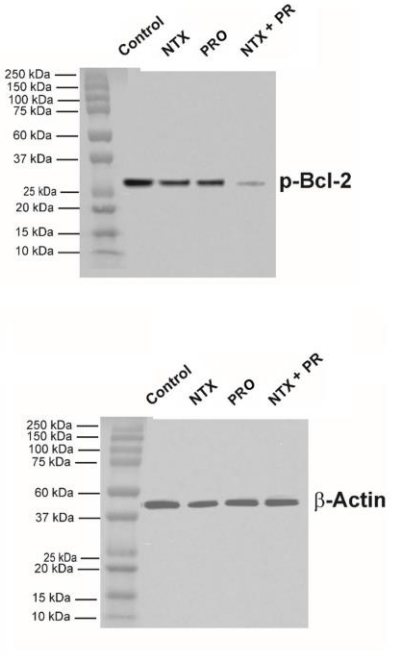

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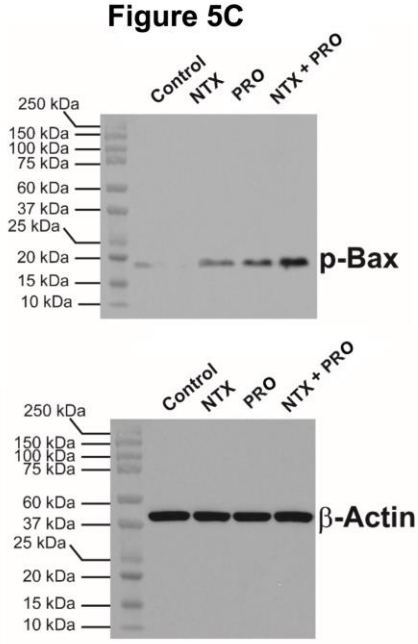

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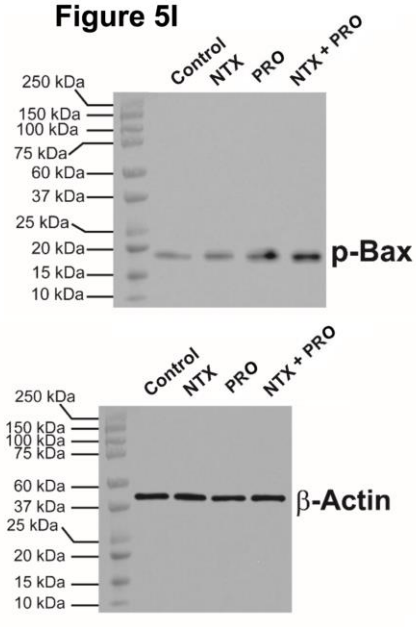

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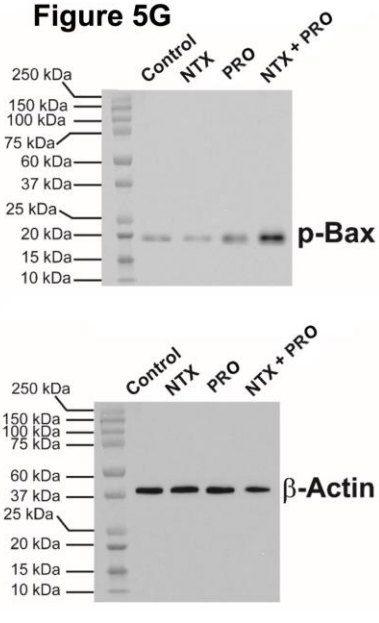

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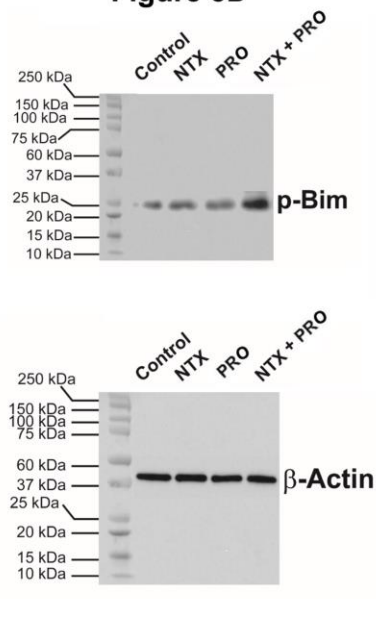

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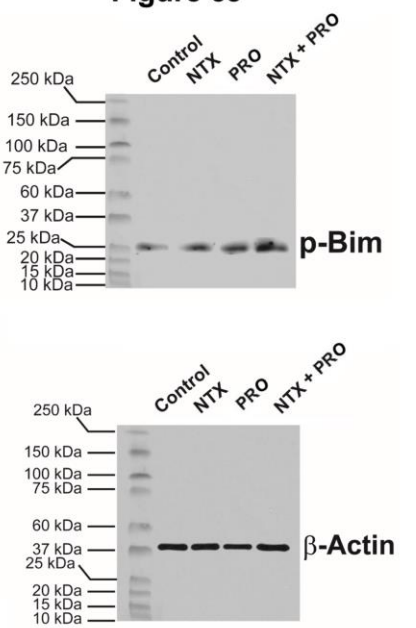

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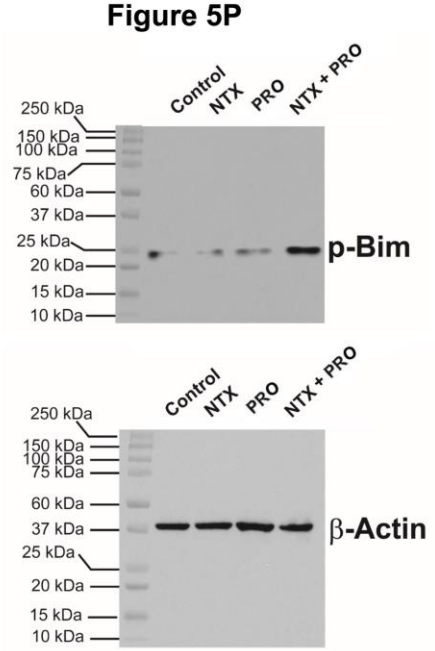

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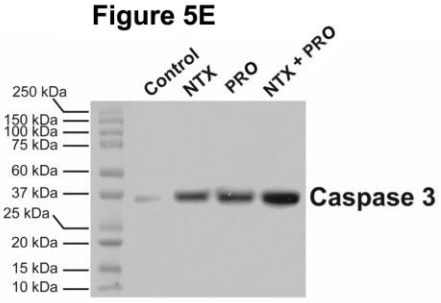

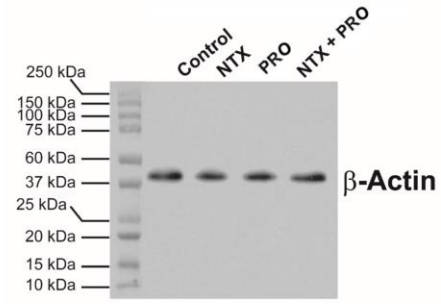

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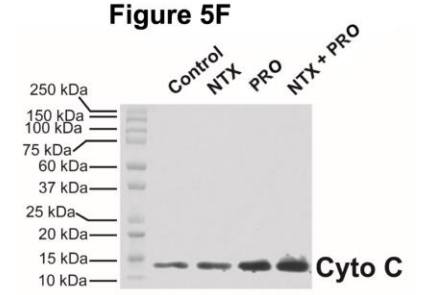

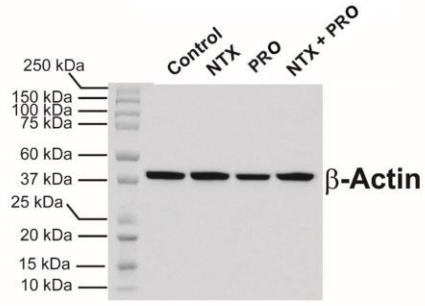

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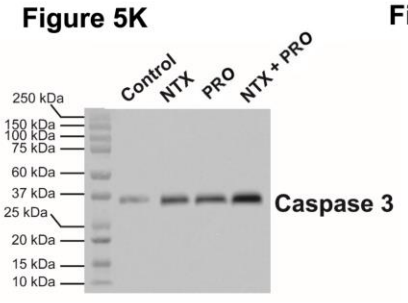

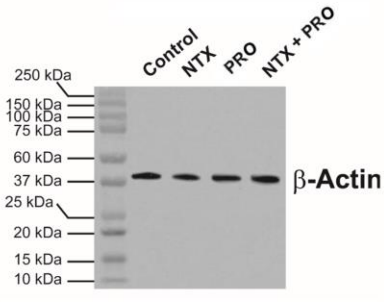

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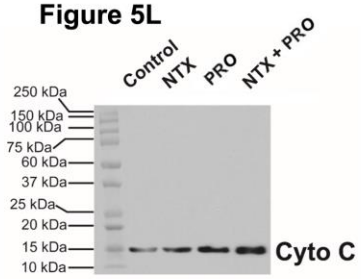

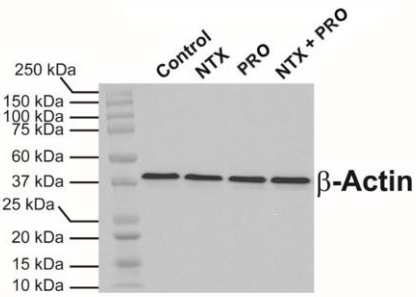

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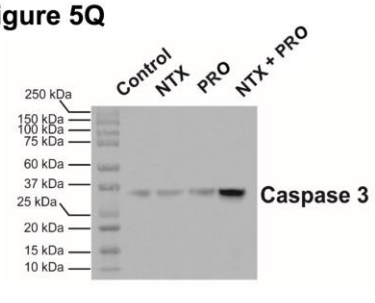

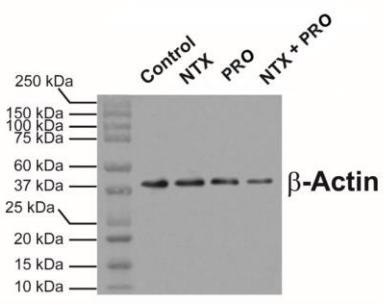

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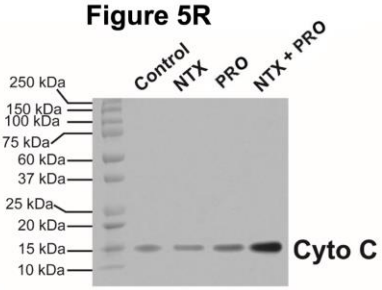

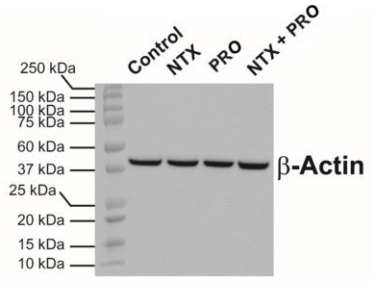

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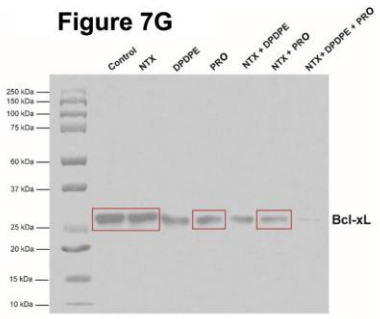

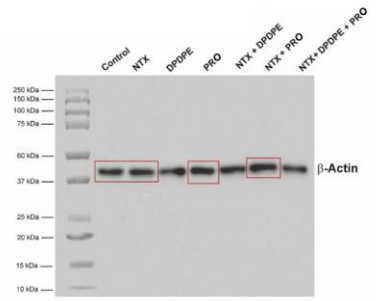

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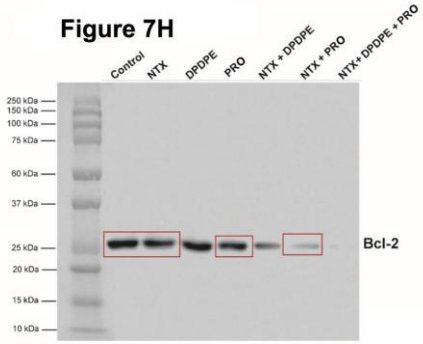

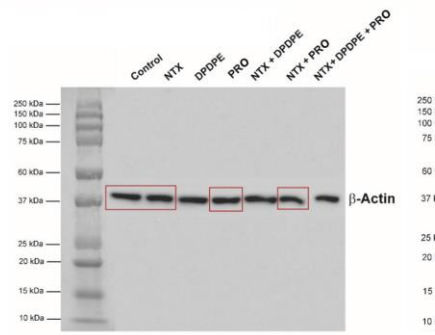

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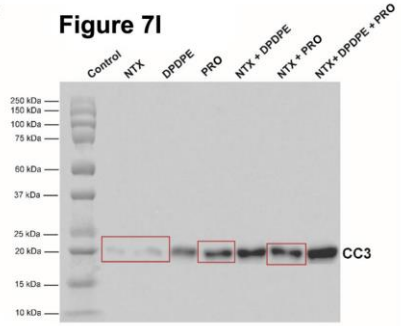

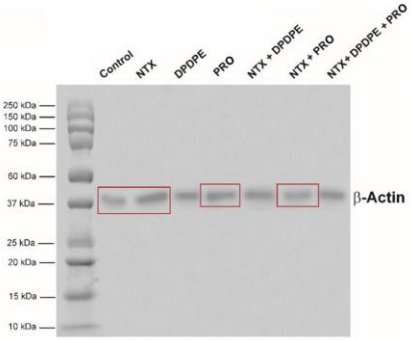

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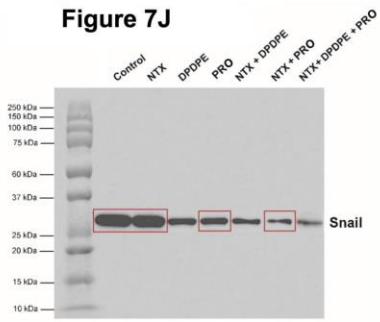

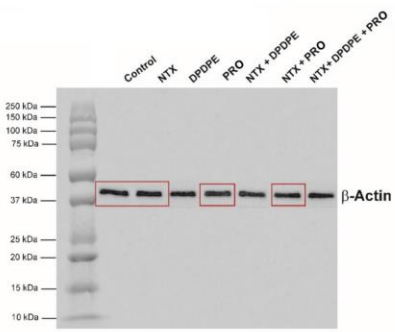

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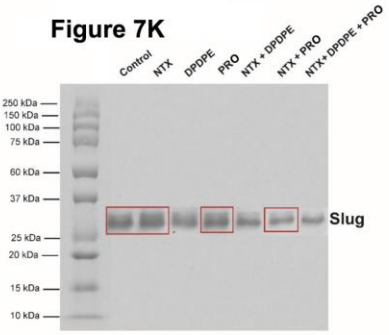

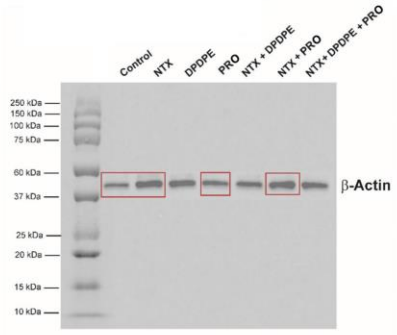

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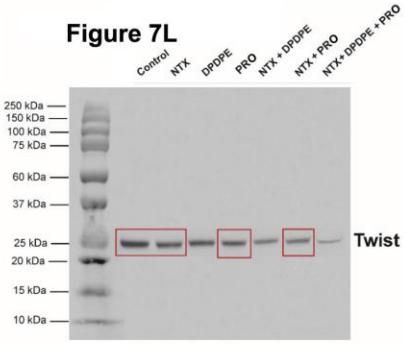

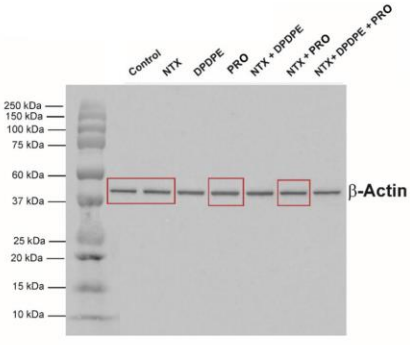

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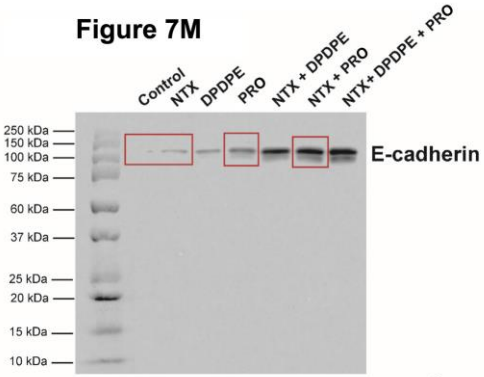

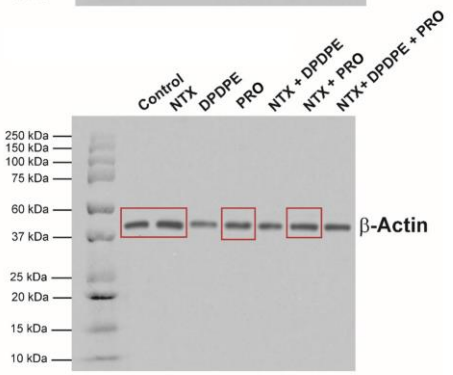

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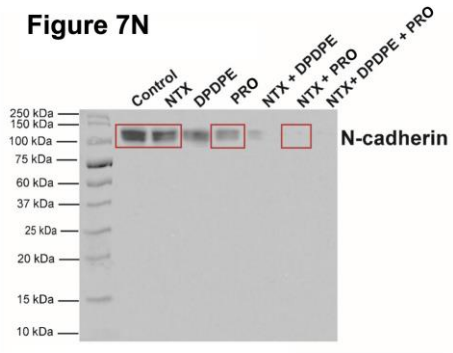

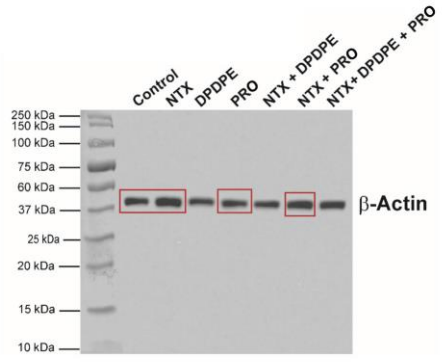

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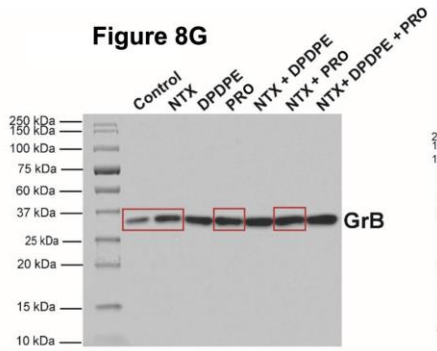

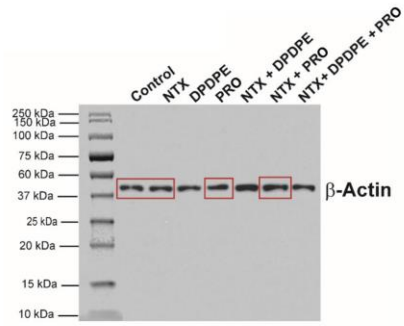

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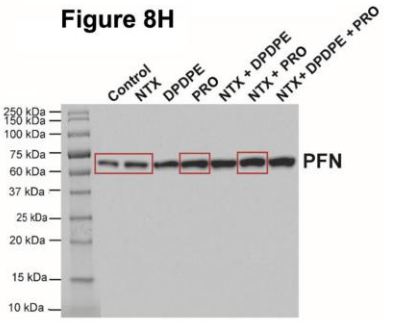


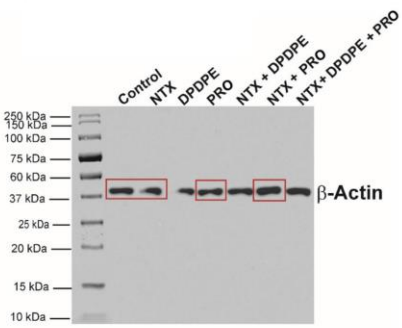
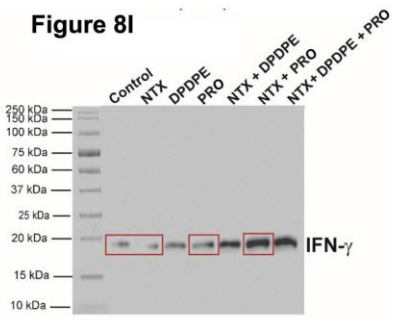
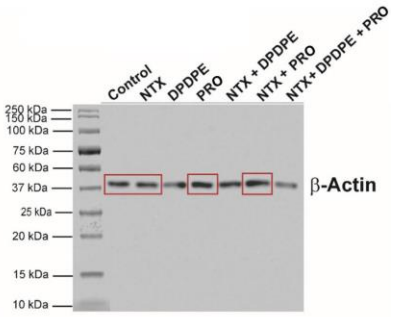


Figure 8I





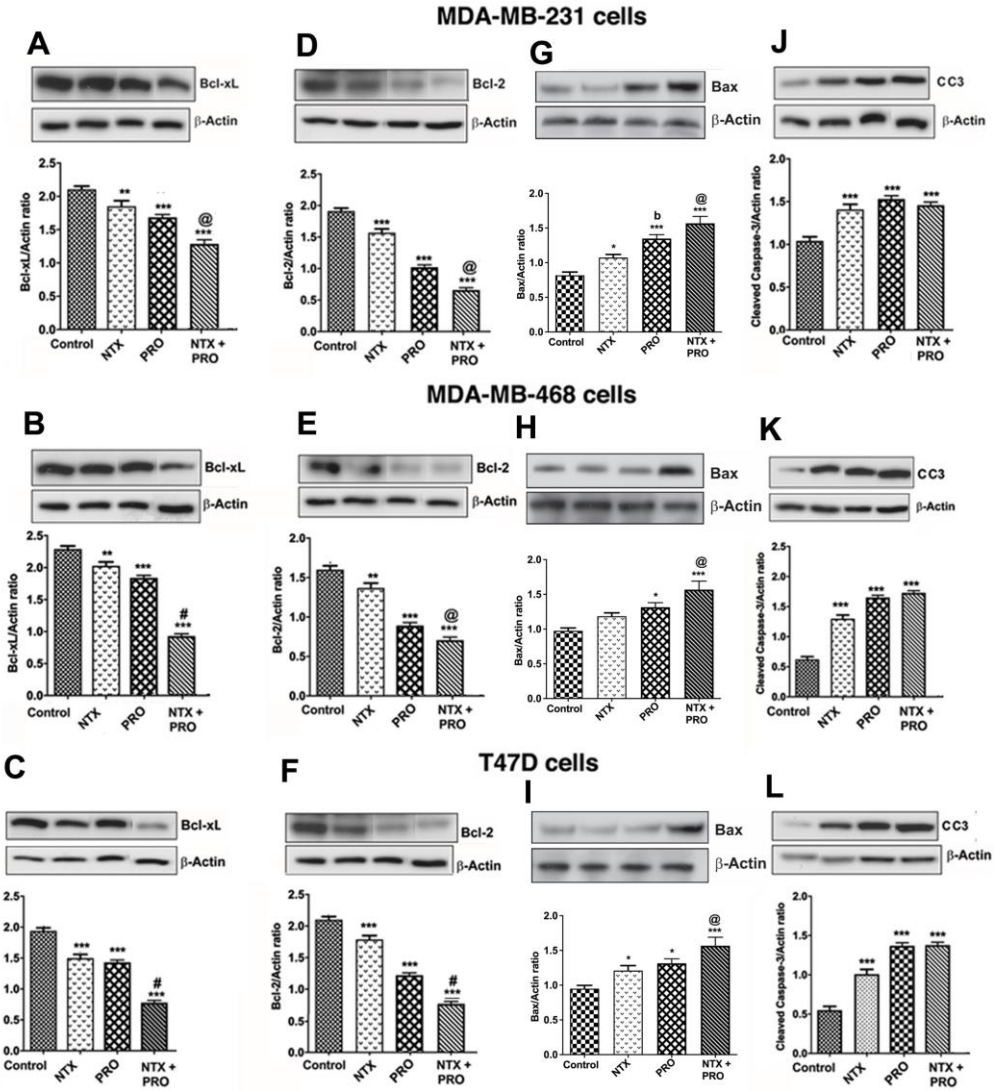


Figure S4. Beta-adrenergic and opioidergic agents increase apoptotic protein levels in breast cancer cell lines. Apoptotic regulatory protein levels of MDA-MB-231 (**A,D,G,J**), MDA-MB-468 (**B,E,H,K**), and T47D (**C,F,I,L**) cells following treatments with NTX, PRO, or NTX+PRO were conducted as described in materials and methods. Cells were treated with a 100 μM concentration of the beta-adrenergic and opioidergic drugs alone or in combination or vehicle alone for 48 hours. After the treatment period, some cells were extracted and used for Western blot analysis of Bcl-xL, Bcl-2, Bax, and CC3. Representative blots were presented on the top and mean densitometric values are presented as ratio of β-actin in the histograms. Data presented are mean ± SEM (n = 5–6 samples/group) and were analyzed using one-way ANOVA with the Newman-Keuls multiple

Supplementary Tables

Table S1: ***List of antibodies used for immunocytochemistry and Western blot analysis & cell lines used in this study***

REAGENT or RESOURCE	Dilution	SOURCE	IDENTIFIER
Snail (C15D3) Rabbit mAb antibody	1:250	Cell Signaling Technology, Danvers, MA	Cat# 3879, RRID:AB_2255011
Mouse Anti-SLUG Monoclonal Antibody, Unconjugated, Clone A-7	1:250	Santa Cruz Biotechnology, Dallas, Texas	Cat# sc-166476, RRID:AB_2191897
Twist (Twist2C1a) antibody	1:250	Santa Cruz Biotechnology, Dallas, Texas	Cat# sc-81417, RRID:AB_1130910
Rabbit Anti-E Cadherin Polyclonal Antibody, Unconjugated	1:1000	Abcam, Branford, CT	Cat# ab53033, RRID:AB_868611
Rabbit Anti-N Cadherin Polyclonal Antibody, Unconjugated	1:1000	Abcam, Branford, CT	Cat# ab18203, RRID:AB_444317
Anti-Bcl-XL antibody	1:500	Abcam, Branford, CT	Cat# ab32370, RRID:AB_725655
Anti-Bcl-2 antibody	1:500	Abcam, Branford, CT	Cat# ab194583, RRID:AB_2783814
Cleaved Caspase-3 (Asp175) (5A1E) Rabbit mAb antibody	1:5000	Cell Signaling Technology, Danvers, MA	Cat# 9664, RRID:AB_2070042
IFN-gamma (G-30) antibody	1:500	Santa Cruz Biotechnology, Dallas, Texas	Cat# sc-57208, RRID:AB_783952
Granzyme B (2C5) antibody	1:500	Santa Cruz Biotechnology, Dallas, Texas	Cat# sc-8022, RRID:AB_2232723
Perforin 1 (E-5) antibody	1:500	Santa Cruz Biotechnology, Dallas, Texas	Cat# sc-374346, RRID:AB_10988266
Ki67 antibody - Proliferation Marker	1:500	Abcam, Branford, CT	Cat# ab15580, RRID:AB_443209
β-Actin (8H10D10) Mouse mAb antibody	1:5000	Cell Signaling Technology, Danvers, MA	Cat# 3700, RRID:AB_2242334
MOUSE ANTI RAT CD161 antibody	1:500	Bio-Rad, Philadelphia, PA	Cat# MCA1427, RRID:AB_2234351
MOUSE ANTI RAT CD163 antibody	1:500	Bio-Rad, Philadelphia, PA	Cat# MCA342R, RRID:AB_321966
Anti-μ-opioid receptor antibody	1:1000	Millipore, Danvers, MA	Cat# AB 5511 RRID:AB_177512
Anti-μ-opioid receptor antibody	1:1000	Millipore, Danvers, MA	Cat# AB 1560 RRID:AB_90778
Anti-β-adrenergic receptor antibody	1:1000	Abcam, Branford, CT	Cat# Ab61778 RRID:AB_940556
Phospho-Bcl-xL (Thr47) Polyclonal Antibody	1:500	Thermo Fisher Scientific, Waltham, MA	Cat# PA5104974 RRID: AB_2816447
Phospho-BCL-2 (Thr69) Polyclonal Antibody	1:500	Thermo Fisher Scientific, Waltham, MA	PA5104784 RRID: AB_2816257
Caspase 3 Polyclonal Antibody	1:500	Thermo Fisher Scientific, Waltham, MA	Cat# PA577887 RRID: AB_2735574
Bax Monoclonal Antibody (6A7)	1:500	Thermo Fisher Scientific, Waltham, MA	Cat# MA5-14003 RRID: AB_10979735
Phospho-Bax (Ser184) Polyclonal Antibody	1:500	Thermo Fisher Scientific, Waltham, MA	Cat# PA5-39778 RRID: AB_2556329
Phospho-Bim (Ser44, Ser104) Polyclonal Antibody	1:500	Thermo Fisher Scientific, Waltham, MA	Cat# PA5-105973 RRID: AB_2817372
Cytochrome C Monoclonal Antibody (7H8.2C12)	1:1000	Thermo Fisher Scientific, Waltham, MA	Cat# 33-8500 RRID: AB_2533142
CDK1 Polyclonal Antibody	1:1000	Thermo Fisher Scientific, Waltham, MA	Cat# PA5-82086 RRID: AB_2789247
Phospho-CDK1 (Thr161) Polyclonal Antibody	1:1000	Thermo Fisher Scientific, Waltham, MA	Cat# PA5-105745 RRID: AB_2817144

Table S2: List of antibodies & dyes used in flow cytometry

Cell type	*Reactivity*	*Conjugate*	*Clone*	*Company*	Catalogue #	*Dilution*

Monocytes	Mouse anti rat CD11b	FITC	ED8	Bio-Rad, Philadelphia, PA	MCA619F	1:100
B-cells	Mouse anti-rat CD45RA	APC-Cy™7	OX-33	BD Biosciences, San Jose, CA	561624	1:100
NK cells	Mouse anti-rat CD161a	PE	10/78	BD Biosciences, San Jose, CA	555009	1:100
Macrophage	Mouse anti-rat RT1B	PerCP	OX-6	BD Biosciences, San Jose, CA	557016	1:100
Live/Dead cells		DAPI		Sigma, St. Louis, MO	D-9542	1µg/mL

Table S3: List of isotype control & beads used in flow cytometry

Reactivity	*Conjugate*	*Clone*	*Company*	**Catalogue #**	*Dilution*
Mouse IgG1 Negative Control	FITC		Bio-Rad, Philadelphia, PA	MCA1209F	1:100
Mouse IgG1, κ Isotype Control	APC-Cy™7	MOPC-21	BD Biosciences, San Jose, CA	557873	1:100
Mouse IgG1, κ Isotype Control	PE	MOPC-31C	BD Biosciences, San Jose, CA	550617	1:100
Mouse IgG1, κ Isotype Control	PerCP	MOPC-31C	BD Biosciences, San Jose, CA	550672	1:100
Anti-Rat Ig, κ/Negative Control Compensation Particles Set			BD Biosciences, San Jose, CA	552844	

Table S4: CI values for Individual Data Point (Concentration Values) for the combination of NTX+PRO on MDA-MB-231 cells (24 hrs)

Drug Concentration (uM)	Log (CI) Values	CI Values	Fraction Affected (Fa)	Effects
0.001	**-1.362710452**	**0.04338**	**93.68**	**Synergistic**
0.002	**-1.377475138**	**0.04193**	**92.26**	**Synergistic**
0.02	**-0.658921763**	**0.21932**	**90.75**	**Synergistic**
0.1	**-0.065082606**	**0.86083**	**90.12**	**Synergistic**
0.2	**-0.312185764**	**0.48732**	**86.17**	**Synergistic**
1	**-0.050171041**	**0.8909**	**82.11**	**Synergistic**
2	0.293976542	1.96778	82.55	Antagonistic
5	0.500656291	3.16706	80.53	Antagonistic
10	0.653350501	4.50143	78.84	Antagonistic
20	0.689543262	4.89264	75.55	Antagonistic
30	0.570937362	3.72338	71.48	Antagonistic
40	0.479627129	3.01736	68.23	Antagonistic
50	0.409635606	2.56824	65.58	Antagonistic
60	0.330943128	2.14261	62.97	Antagonistic
70	0.168939214	1.4755	59.03	Antagonistic
80	**-0.200094546**	**0.63082**	**51.33**	**Synergistic**
100	**-0.254082433**	**0.55708**	**48.55**	**Synergistic**
120	**-0.619228157**	**0.24031**	**40.40**	**Synergistic**
140	**-0.644088949**	**0.22694**	**38.75**	**Synergistic**
160	**-1.079302866**	**0.08331**	**30.31**	**Synergistic**

180	**-1.10419085**	**0.07867**	**29.09**	**Synergistic**
200	**-1.300248968**	**0.05009**	**25.39**	**Synergistic**

Note: Drug concentration that have synergistic effects are highlighted in bold letters

CI values for Individual Data Point (Concentration Values) for the combination of NTX+PRO on MDA-MB-231 cells (48 hrs)

Drug Concentration (uM)	Log (CI) Values	CI Values	Fraction Affected (Fa)	Effects
0.001	**-1.339798799**	**0.04573**	**93.6**	**Synergistic**
0.002	**-1.331055266**	**0.04666**	**92.28**	**Synergistic**
0.02	**-0.611561303**	**0.24459**	**90.78**	**Synergistic**
0.1	**-0.031073644**	**0.93095**	**90.07**	**Synergistic**
0.2	**-0.260056014**	**0.54947**	**86.26**	**Synergistic**
1	**-0.001452971**	**0.99666**	**82.2**	**Synergistic**
2	0.347031274	2.22347	82.68	Antagonistic
5	0.598108446	3.96377	81.16	Antagonistic
10	0.614568208	4.11688	77.92	Antagonistic
20	0.65443935	4.51273	74.6	Antagonistic
30	0.54856261	3.53641	70.65	Antagonistic
40	0.595957828	3.94419	69.5	Antagonistic
50	0.372983766	2.36039	64.49	Antagonistic
60	0.284405916	1.92489	61.71	Antagonistic
70	0.025043571	1.05936	56.07	Antagonistic
80	**-0.169180453**	**0.67736**	**51.57**	**Synergistic**
100	**-0.237629077**	**0.57859**	**48.59**	**Synergistic**
120	**-0.558210633**	**0.27656**	**41.43**	**Synergistic**
140	**-0.521318093**	**0.30108**	**40.9**	**Synergistic**
160	**-1.092534893**	**0.08081**	**30.38**	**Synergistic**
180	**-1.120388093**	**0.07579**	**29.16**	**Synergistic**
200	**-1.253599356**	**0.05577**	**26.5**	**Synergistic**

Note: Drug concentration that have synergistic effects are highlighted in bold letters

CI values for Individual Data Point (Concentration Values) for the combination of NTX+PRO on MDA-MB-231 cells (72 hrs)

Drug Concentration (uM)	Log (CI) Values	CI Values	Fraction Affected (Fa)	Effects
0.001	**-1.306888885**	**0.04933**	**92.67**	**Synergistic**
0.002	**-1.245881106**	**0.05677**	**91.53**	**Synergistic**
0.02	**-0.64535383**	**0.22628**	**89.27**	**Synergistic**
0.1	**-0.233364114**	**0.5843**	**87.33**	**Synergistic**
0.2	**-0.318369881**	**0.48043**	**84.25**	**Synergistic**
1	**-0.028603531**	**0.93626**	**80.34**	**Synergistic**
2	0.431289767	2.69954	81.94	Antagonistic
5	0.577918991	3.78372	79.36	Antagonistic
10	0.669018259	4.66679	77	Antagonistic
20	0.689042339	4.887	73.55	Antagonistic
30	0.573506323	3.74547	69.63	Antagonistic
40	0.548584715	3.53659	67.49	Antagonistic
50	0.383129285	2.41618	63.56	Antagonistic
60	0.261755187	1.82707	60.42	Antagonistic
70	0.011308181	1.02638	55.27	Antagonistic
80	**-0.217634888**	**0.60585**	**50.5**	**Synergistic**
100	**-0.234980019**	**0.58213**	**48.59**	**Synergistic**
120	**-0.589290235**	**0.25746**	**41.41**	**Synergistic**
140	**-0.940853281**	**0.11459**	**34.78**	**Synergistic**
160	**-1.179798541**	**0.0661**	**30.38**	**Synergistic**
180	**-1.221342368**	**0.06007**	**29.07**	**Synergistic**
200	**-1.364717362**	**0.04318**	**26.5**	**Synergistic**

Note: Drug concentration that have synergistic effects are highlighted in bold letters

CI values for Individual Data Point (Concentration Values) for the combination of NTX+PRO on MDA-MB-468 cells (24 hrs)

Drug Concentration (uM)	Log (CI) Values	CI Values	Fraction Affected (Fa)	Effects
0.001	**-1.046675304**	**0.08981**	**93.60**	**Synergistic**
0.002	**-1.034469056**	**0.09237**	**92.28**	**Synergistic**
0.02	**-0.311740398**	**0.48782**	**90.78**	**Synergistic**
0.1	0.270089286	1.86247	90.07	Antagonistic
0.2	0.046791616	1.11376	86.26	Antagonistic
1	0.309677001	2.04022	82.20	Antagonistic
2	0.657722089	4.54697	82.68	Antagonistic
5	0.859759561	7.24035	80.61	Antagonistic
10	1.015967175	10.3745	78.96	Antagonistic
20	1.053205254	11.3033	75.69	Antagonistic
30	0.929193545	8.49559	71.56	Antagonistic
40	0.842603	6.9599	68.40	Antagonistic
50	0.759339923	5.74566	65.56	Antagonistic
60	0.690190762	4.89994	63.13	Antagonistic
70	0.518333604	3.29863	59.07	Antagonistic

80	0.1532049	1.423	51.57	Antagonistic
100	**-0.002176919**	**0.995**	**48.59**	**Synergistic**
120	**-0.293435826**	**0.50882**	**40.43**	**Synergistic**
140	**-0.199661032**	**0.63145**	**40.90**	**Synergistic**
160	**-0.774148189**	**0.16821**	**30.38**	**Synergistic**
180	**-0.802609159**	**0.15754**	**29.16**	**Synergistic**
200	**-1.008021209**	**0.09817**	**25.50**	**Synergistic**

Note: Drug concentration that have synergistic effects are highlighted in bold letters

CI values for Individual Data Point (Concentration Values) for the combination of NTX+PRO on MDA-MB-468 cells (48 hrs)

Drug Concentration (uM)	Log (CI) Values	CI Values	Fraction Affected (Fa)	Effects
0.001	**-1.054826954**	**0.08814**	**93.6**	**Synergistic**
0.002	**-1.041914151**	**0.0908**	**92.28**	**Synergistic**
0.02	**-0.318514541**	**0.48027**	**90.78**	**Synergistic**
0.1	0.263586364	1.83479	90.07	Antagonistic
0.2	0.041444008	1.10013	86.26	Antagonistic
1	0.305198695	2.01929	82.2	Antagonistic
2	0.653154604	4.4994	82.68	Antagonistic
5	0.855554891	7.17059	80.61	Antagonistic
10	1.012014237	10.2805	78.96	Antagonistic
20	1.049683089	11.212	75.69	Antagonistic
30	0.926104137	8.43537	71.56	Antagonistic
40	0.839777111	6.91476	68.4	Antagonistic
50	0.756709879	5.71097	65.56	Antagonistic
60	0.68770104	4.87193	63.13	Antagonistic
70	0.516027409	3.28116	59.07	Antagonistic
80	0.151087789	1.41608	51.57	Antagonistic
100	**-0.001304842**	**0.997**	**48.59**	**Synergistic**
120	**-0.295626385**	**0.50626**	**40.43**	**Synergistic**
140	**-0.201839853**	**0.62829**	**40.9**	**Synergistic**
160	**-0.776841658**	**0.16717**	**30.38**	**Synergistic**
180	**-0.805430161**	**0.15652**	**29.16**	**Synergistic**
200	**-1.011218157**	**0.09745**	**25.5**	**Synergistic**

Note: Drug concentration that have synergistic effects are highlighted in bold letters

CI values for Individual Data Point (Concentration Values) for the combination of NTX+PRO on MDA-MB-468 cells (72 hrs)

Drug Concentration (uM)	Log (CI) Values	CI Values	Fraction Affected (Fa)	Effects
0.001	**-1.051000546**	**0.08892**	**93.67**	**Synergistic**
0.002	**-1.048662481**	**0.0894**	**92.33**	**Synergistic**
0.02	**-0.331959046**	**0.46563**	**90.82**	**Synergistic**
0.1	0.251516552	1.7845	90.13	Antagonistic
0.2	0.012449138	1.02908	86.25	Antagonistic
1	0.276758126	1.89129	82.24	Antagonistic

2	0.617284533	4.14271	82.64	Antagonistic
5	0.82174928	6.6336	80.61	Antagonistic
10	0.97684865	9.48088	78.96	Antagonistic
20	1.008331848	10.1937	75.64	Antagonistic
30	0.884814049	7.67033	71.54	Antagonistic
40	0.797700234	6.27625	68.39	Antagonistic
50	0.715353086	5.19222	65.58	Antagonistic
60	0.644811666	4.41379	63.14	Antagonistic
70	0.471269702	2.95985	59.07	Antagonistic
80	**0.106289512**	**0.67736**	**51.6**	**Synergistic**
100	**-0.005243055**	**0.52736**	**48.59**	**Synergistic**
120	**-0.344467804**	**0.45241**	**40.41**	**Synergistic**
140	**-0.371406744**	**0.4252**	**38.78**	**Synergistic**
160	**-0.824169396**	**0.14991**	**30.38**	**Synergistic**
180	**-0.858550227**	**0.1385**	**29.07**	**Synergistic**
200	**-1.057694247**	**0.08756**	**25.5**	**Synergistic**

Note: Drug concentration that have synergistic effects are highlighted in bold letters

CI values for Individual Data Point (Concentration Values) for the combination of NTX+PRO on T47D cells (24 hrs)

Drug Concentration (uM)	Log (CI) Values	CI Values	Fraction Affected (Fa)	Effects
0.001	**-1.362710452**	**0.04338**	**93.68**	**Synergistic**
0.002	**-1.377475138**	**0.04193**	**92.26**	**Synergistic**
0.02	**-0.658921763**	**0.21932**	**90.75**	**Synergistic**
0.1	**-0.065082606**	**0.86083**	**90.12**	**Synergistic**
0.2	**-0.312185764**	**0.48732**	**86.17**	**Synergistic**
1	**-0.050171041**	**0.8909**	**82.11**	**Synergistic**
2	0.293976542	1.96778	82.55	Antagonistic
5	0.500656291	3.16706	80.53	Antagonistic
10	0.653350501	4.50143	78.84	Antagonistic
20	0.689543262	4.89264	75.55	Antagonistic
30	0.570937362	3.72338	71.48	Antagonistic
40	0.479627129	3.01736	68.23	Antagonistic
50	0.409635606	2.56824	65.58	Antagonistic
60	0.330943128	2.14261	62.97	Antagonistic
70	0.168939214	1.4755	59.03	Antagonistic
80	**-0.200094546**	**0.63082**	**51.33**	**Synergistic**
100	**-0.254082433**	**0.55708**	**48.55**	**Synergistic**
120	**-0.619228157**	**0.24031**	**40.40**	**Synergistic**
140	**-0.644088949**	**0.22694**	**38.75**	**Synergistic**
160	**-1.079302866**	**0.08331**	**30.31**	**Synergistic**
180	**-1.10419085**	**0.07867**	**29.09**	**Synergistic**
200	**-1.300248968**	**0.05009**	**25.39**	**Synergistic**

Note: Drug concentration that have synergistic effects are highlighted in bold letters

CI values for Individual Data Point (Concentration Values) for the combination of NTX+PRO on T47D cells (48 hrs)

Drug Concentration (uM)	Log (CI) Values	CI Values	Fraction Affected (Fa)	Effects
0.001	**-1.339798799**	**0.04573**	**93.6**	**Synergistic**
0.002	**-1.331055266**	**0.04666**	**92.28**	**Synergistic**
0.02	**-0.611561303**	**0.24459**	**90.78**	**Synergistic**
0.1	**-0.031073644**	**0.93095**	**90.07**	**Synergistic**
0.2	**-0.260056014**	**0.54947**	**86.26**	**Synergistic**
1	**-0.001452971**	**0.99666**	**82.2**	**Synergistic**
2	0.347031274	2.22347	82.68	Antagonistic
5	0.598108446	3.96377	81.16	Antagonistic
10	0.614568208	4.11688	77.92	Antagonistic
20	0.65443935	4.51273	74.6	Antagonistic
30	0.54856261	3.53641	70.65	Antagonistic
40	0.595957828	3.94419	69.5	Antagonistic
50	0.372983766	2.36039	64.49	Antagonistic
60	0.284405916	1.92489	61.71	Antagonistic
70	0.025043571	1.05936	56.07	Antagonistic

80	**-0.169180453**	**0.67736**	**51.57**	**Synergistic**
100	**-0.237629077**	**0.57859**	**48.59**	**Synergistic**
120	**-0.558210633**	**0.27656**	**41.43**	**Synergistic**
140	**-0.521318093**	**0.30108**	**40.9**	**Synergistic**
160	**-1.092534893**	**0.08081**	**30.38**	**Synergistic**
180	**-1.120388093**	**0.07579**	**29.16**	**Synergistic**
200	**-1.253599356**	**0.05577**	**26.5**	**Synergistic**

Note: Drug concentration that have synergistic effects are highlighted in bold letters

CI values for Individual Data Point (Concentration Values) for the combination of NTX+PRO on T47D cells (72 hrs)

Drug Concentration (uM)	Log (CI) Values	CI Values	Fraction Affected (Fa)	Effects
0.001	**-1.308918508**	**0.0491**	**92.67**	**Synergistic**
0.002	**-1.248259126**	**0.05646**	**91.53**	**Synergistic**
0.02	**-0.648474245**	**0.22466**	**89.27**	**Synergistic**
0.1	**-0.23712646**	**0.57926**	**87.33**	**Synergistic**
0.2	**-0.323096151**	**0.47523**	**84.25**	**Synergistic**
1	**-0.034520778**	**0.92359**	**80.34**	**Synergistic**
2	0.425856887	2.66598	81.94	Antagonistic
5	0.571706503	3.72998	79.36	Antagonistic
10	1.23661031	17.2429	83	Antagonistic
20	0.681083777	4.79826	73.55	Antagonistic
30	0.564359465	3.66741	69.63	Antagonistic
40	0.53878103	3.45765	67.49	Antagonistic
50	0.372094171	2.35556	63.56	Antagonistic
60	0.249704535	1.77707	60.42	Antagonistic
70	**-0.002486928**	**0.99429**	**55.27**	**Synergistic**
80	**-0.233156047**	**0.58458**	**50.5**	**Synergistic**
100	**-0.251222973**	**0.56076**	**48.59**	**Synergistic**
120	**-0.608535588**	**0.2463**	**41.41**	**Synergistic**
140	**-0.96333119**	**0.10881**	**34.78**	**Synergistic**
160	**-1.204745817**	**0.06241**	**30.38**	**Synergistic**
180	**-1.247106845**	**0.05661**	**29.07**	**Synergistic**
200	**-1.392223396**	**0.04053**	**26.5**	**Synergistic**

Note: Drug concentration that have synergistic effects are highlighted in bold letters