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Supplemental Information

Cathelicidin Suppresses Colon Cancer Metastasis via a P2RX7-Dependent Mechanism

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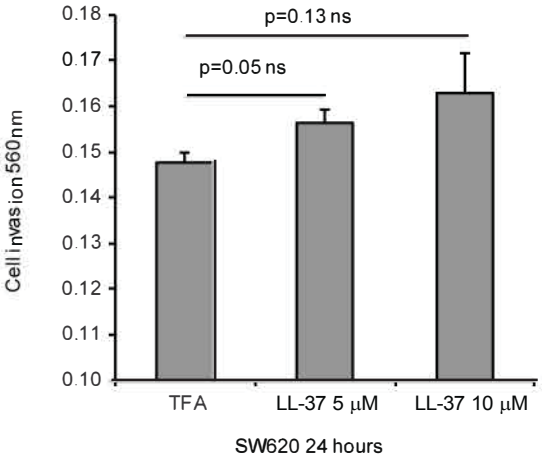
S1 Figure

A



B

HT29

(pg/ml)	TFA	LL37		
		1μM	5μM	10μM
MMP1	77.96±5.19	96.46±22.45	64.04±5.59	75.86±7.38
TIMP1	2168.46±190.77	2248.87±233.29	2517.51±375.07	2729.54±426.82
TIMP2	793.47±16.91	1153.31±112.30*	1330.89±151.72*	1558.96±168.39*
TGF-β1	10.44±0.84	15.20±2.83	9.93±1.35	11.63±1.72
VEGF	353.66±4.36	534.60±60.72*	512.36±46.78*	511.33±51.16*

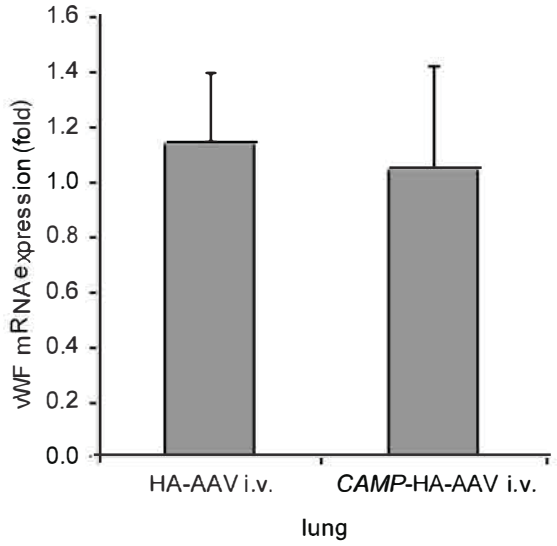
SW620

(pg/ml)	TFA	LL37		
		1μM	5μM	10μM
MMP1	31.51±0.38	35.49±1.93	34.69±1.32*	37.00±2.37*
TIMP1	1319.95±35.87	1571.43±84.89*	1555.83±106.05*	1579.85±62.79*
TIMP2	7266.35±338.18	7389.73±518.3	7958.51±640.92	9695.52±1372.38
TGF-β1	268.77±12.22	246.11±26.70	263.59±23.66	219.35±25.80
VEGF	279.95±6.55	304.43±23.14	320.53±30.75	331.56±38.38

SW480

(pg/ml)	TFA	LL37		
		1μM	5μM	10μM
MMP1	15.94±0.68	19.22±1.62	16.30±0.30	23.82±3.55
TIMP1	1791.33±38.95	2708.53±132.83*	3149.63±278.65*	2702.13±90.64*
TIMP2	Not determined	Not determined	Not determined	Not determined
TGF-β1	267.77±3.17	325.34±4.93*	293.43±23.82	232.49±11.34
VEGF	418.70±8.67	458.25±61.95	399.00±14.82	475.07±14.18*

C



D

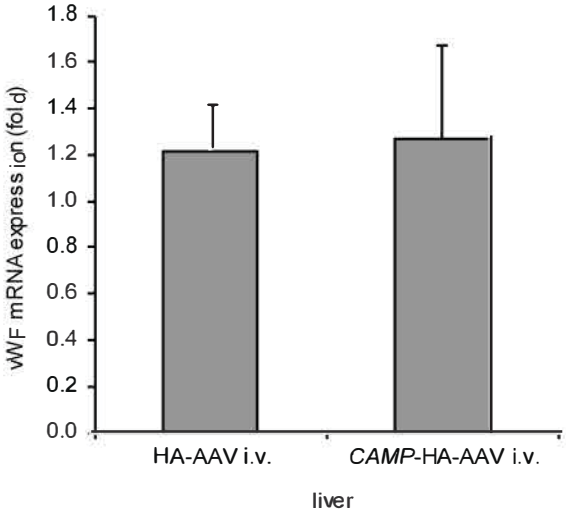
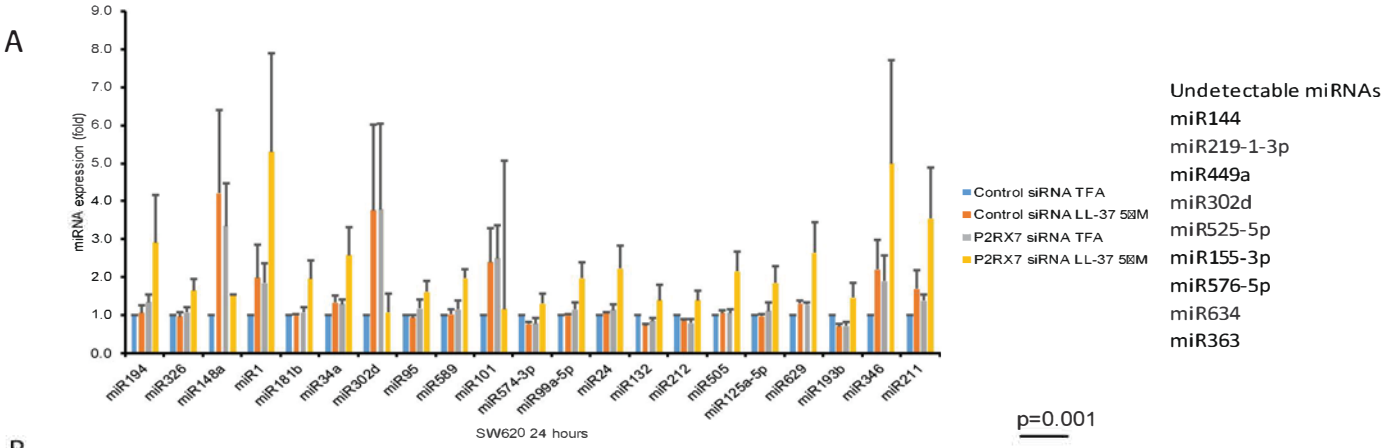
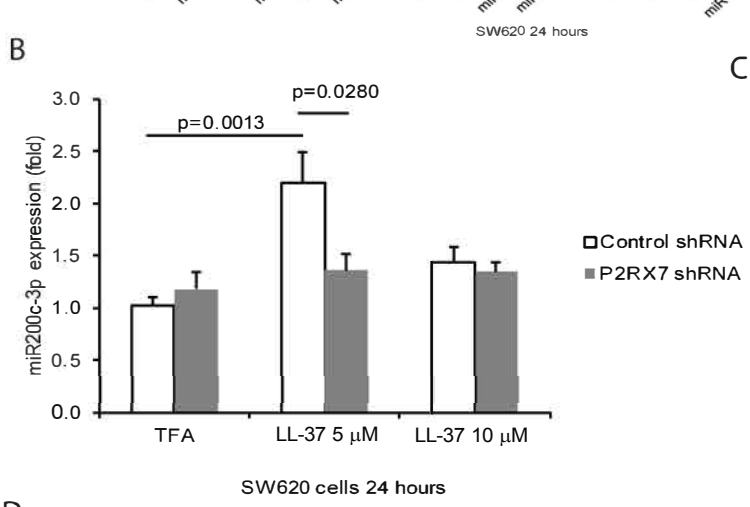


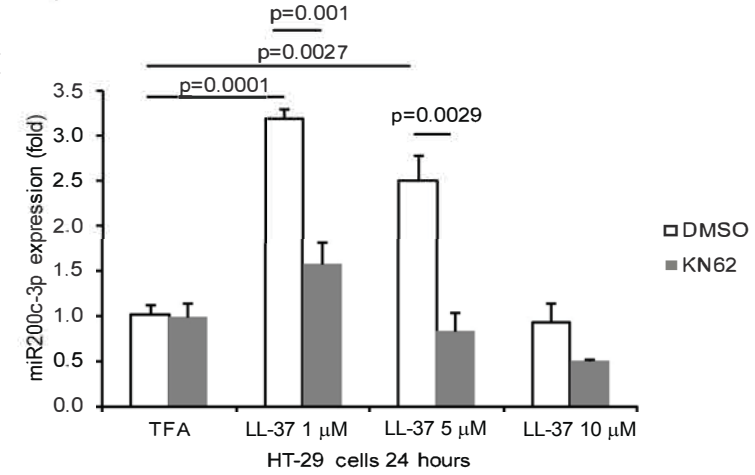
Figure S1 Cathelicidin did not affect colon cancer cell invasion.

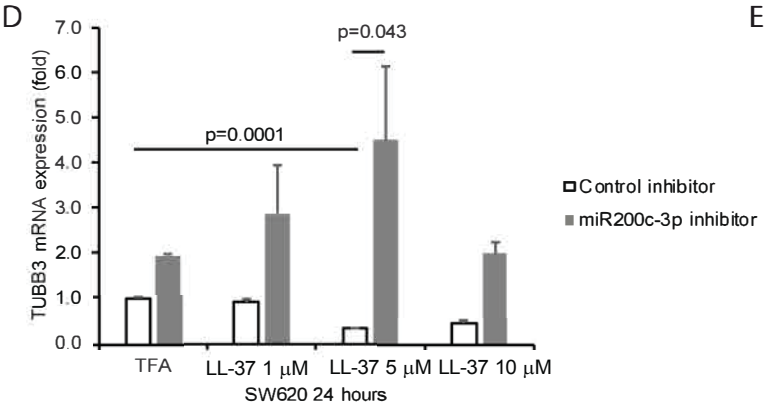
(A) Cell invasion assay of SW620 cells. Results were pooled from three independent experiments. (B) HT-29, SW480, and SW620 cells were incubated with TFA or LL-37. The soluble mediators in the conditioned media were measured by ELISA. Changes in mediator levels with statistically significant differences were marked with *. Results were pooled from six independent experiments. (C and D) vWF mRNA expression in the lungs and liver of HT-29-loaded nude mice.

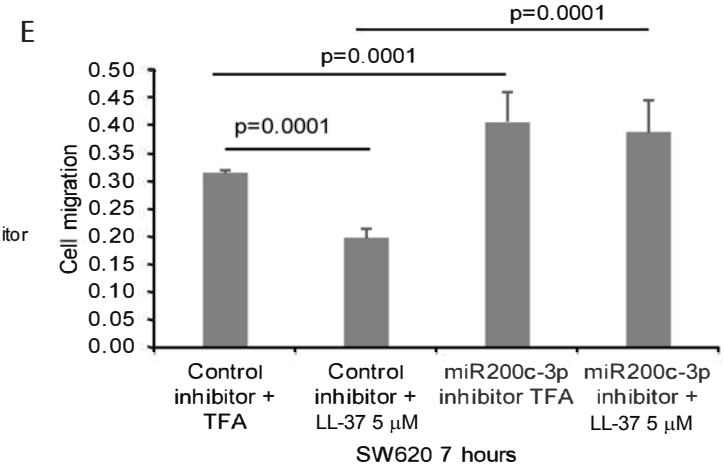
S2 Figure


A
miRNA expression (fold)
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
miR194
miR326
miR148a
miR1
miR181b
miR34a
miR302d
miR95
miR589
miR101
miR574-3p
miR99a-5p
miR24
miR132
miR212
miR505
miR125a-5p
miR629
miR193b
miR346
miR211
SW620 24 hours
Control siRNA TFA
Control siRNA LL-37 5ΜM
P2RX7 siRNA TFA
P2RX7 siRNA LL-37 5ΜM
Undetectable miRNAs
miR144
miR219-1-3p
miR449a
miR302d
miR525-5p
miR155-3p
miR576-5p
miR634
miR363


B
miR200c-3p expression (fold)
p=0.0280
p=0.0013
Control shRNA
P2RX7 shRNA
TFA
LL-37 5 μM
LL-37 10 μM
SW620 cells 24 hours


C
p=0.001
p=0.0027
p=0.0001
p=0.0029
miR200c-3p expression (fold)
DMSO
KN62
TFA
LL-37 1 μM
LL-37 5 μM
LL-37 10 μM
HT-29 cells 24 hours


D
TUBB3 mRNA expression (fold)
p=0.043
p=0.0001
Control inhibitor
miR200c-3p inhibitor
TFA
LL-37 1 μM
LL-37 5 μM
LL-37 10 μM
SW620 24 hours


E
p=0.0001
p=0.0001
p=0.0001
Cell migration
Control inhibitor + TFA
Control inhibitor + LL-37 5 μM
miR200c-3p inhibitor TFA
miR200c-3p inhibitor + LL-37 5 μM
SW620 7 hours

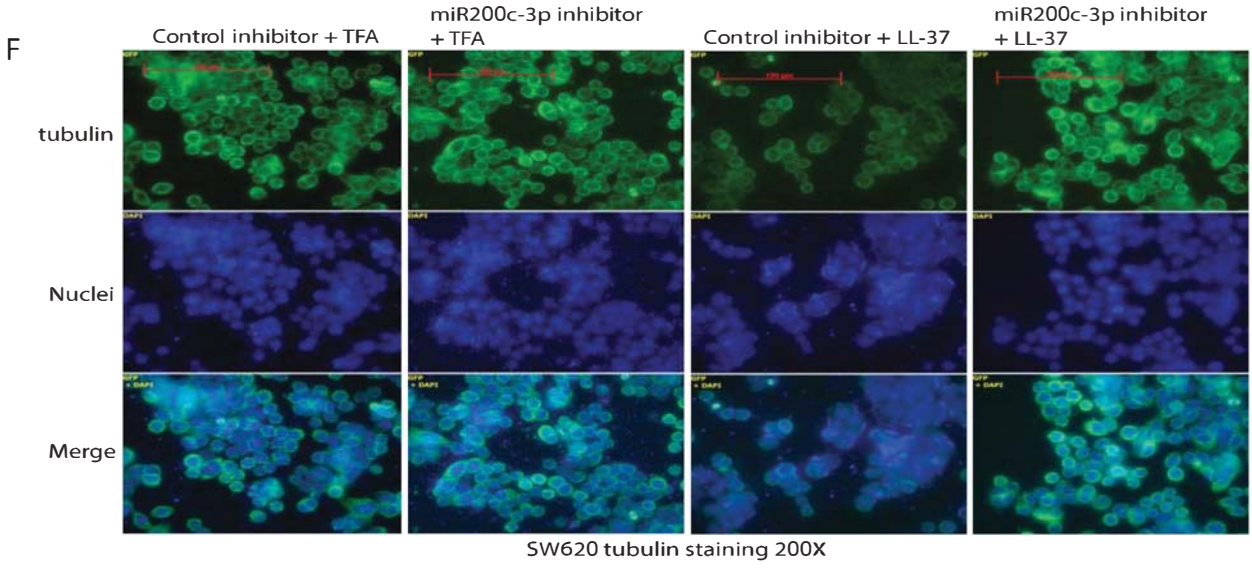

F
Control inhibitor + TFA
miR200c-3p inhibitor + TFA
Control inhibitor + LL-37
miR200c-3p inhibitor + LL-37
tubulin
Nuclei
Merge
SW620 tubulin staining 200X

Figure S2 miR200c is associated with cathelicidin-mediated TUBB3 inhibition.

(A) miRNA expression was profiled by a PCR array. (B) miR200c-3p expression in SW620 cells. (C) HT-29 cells were treated with DMSO or KN62 (10μM) for 30 minutes, followed by exposure to LL-37. (D) SW620 cells were pretreated with control inhibitor and miR200c-3p inhibitor (50nM) overnight, followed by exposure to LL-37 (5μM) for 24 hours. TUBB3 mRNA expression. (E) SW620 cells were pretreated with control inhibitor or miR200c-3p inhibitor (50nM) overnight, followed by LL-37 exposure for 7 hours. Cell migration of SW620 cells. (F) Green tubulin tracker staining with blue nuclear staining in human cancer SW620 cells. SW620 cells were pretreated with control inhibitor and miR200c-3p inhibitor (50nM) overnight, followed by exposure to LL-37 (5μM) for 24 hours. LL-37 reduced tubulin expression in SW620 cells that was reversed by miR200-3p inhibitor.