

Sterile inflammation via TRPM8 RNA-dependent TLR3-NF-kB/IRF3 activation promotes antitumor immunity in prostate cancer

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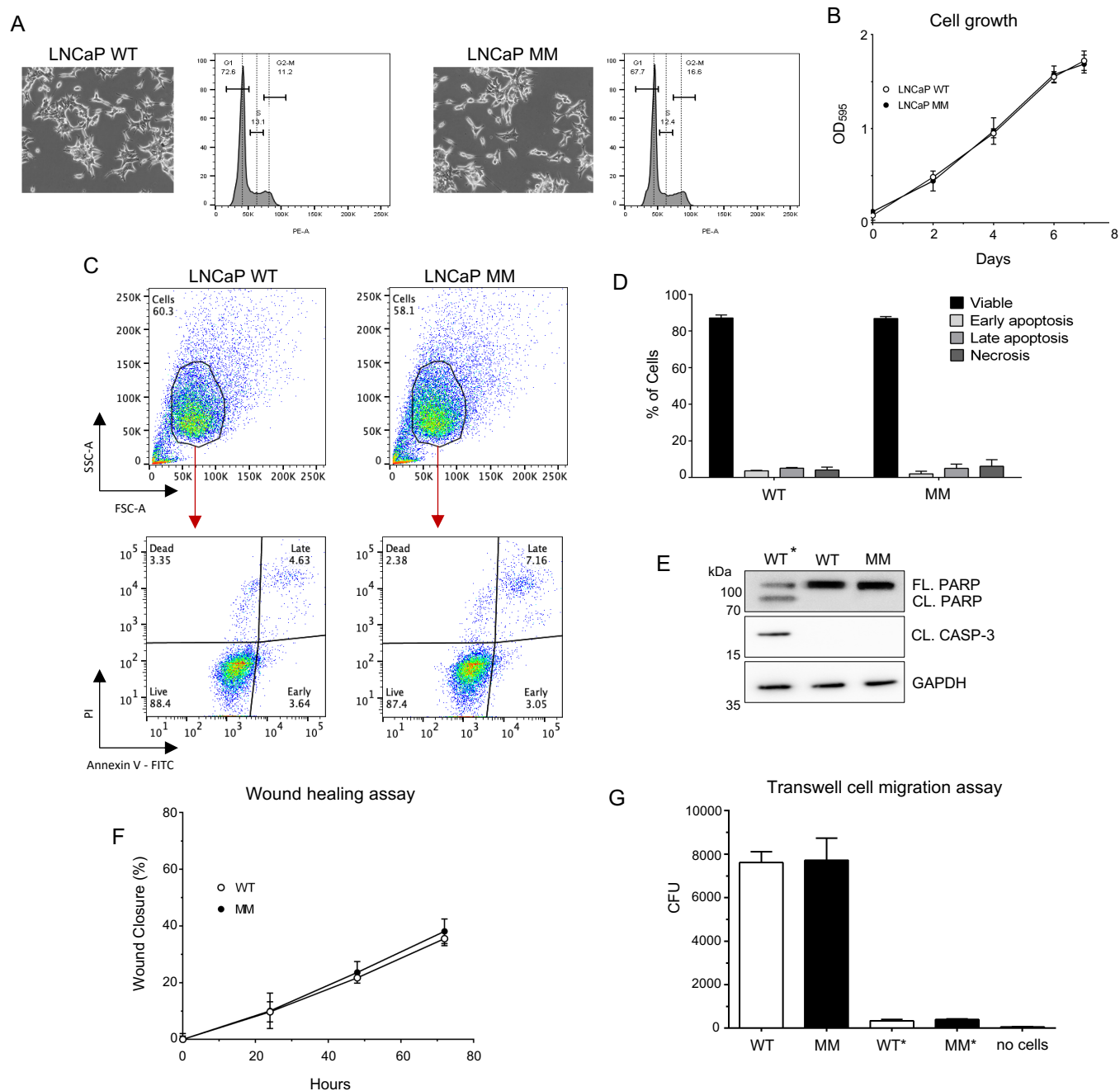
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Appendix Figure S1. Unaltered features of normal and inflamed prostate cancer cells

A, Morphology (left) and cell cycle analysis by flow cytometry (right) of LNCaP WT and LNCaP MM cell lines (LNCaP WT % of cells in G1 = 72,6; S = 13,1; G2/M = 11,2; LNCaP MM % of cells in G1 = 67,7; S = 12,4; G2/M = 16,6).

B, Cell growth analysis of LNCaP WT and LNCaP MM cell lines.

C and **D**, Cell viability analysis by flow cytometry with Annexin-V/PI staining (C) and relative quantification (D) of LNCaP WT and LNCaP MM cell lines.

E, Cell death study by Western blot analysis of full-length and cleaved PARP and cleaved Caspase 3 LNCaP WT and LNCaP MM cell lines. Staurosporin treatment (1 μ M, 6 hours) of WT LNCaP (WT*) was used as positive control of apoptotic cell death. GAPDH was used as loading control.

F and **G**, Wound healing (F) and Transwell migration assays (G, *migrated cells without chemoattractants) of LNCaP WT and LNCaP MM cell lines.

A

RNA	Native RIP			fCLIP		
	LNcAP			LNcAP		
	MM	WT	CAS	MM	WT	CAS
TRPM8 Ex 4-5	22.89	24.53	37.76	22.85	24.27	N/A
	23.02	24.28	N/A	23.56	24.87	N/A
	23.39	24.22	N/A	23.35	24.25	38.29
TRPM8 Ex 8-9	38.43	N/A	N/A	39.76	N/A	N/A
	N/A	39.42	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A
TRPM8 Ex 12-15	22,71	25.14	36.02	22.41	23.48	N/A
	23,11	24.33	37.95	24.76	24.22	39.21
	23.68	23.43	39.62	22.74	23.81	38.25
TRPM8 Ex 15-18	N/A	38.98	N/A	N/A	N/A	N/A
	39.75	N/A	N/A	37.87	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A
TRPM8 Ex 21-23	25.25	25.49	38.18	24.97	25.31	N/A
	25.59	25.81	N/A	25.40	25.69	38.98
	N/A	25.31	38.48	25.01	25.47	N/A
GAPDH	39.54	N/A	N/A	39.16	N/A	N/A
	N/A	N/A	N/A	N/A	38.09	N/A
	N/A	37.85	N/A	N/A	N/A	N/A
Y3	39.42	N/A	N/A	38.56	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A
	38.58	N/A	N/A	N/A	N/A	N/A

B

Ex 4-5 MM Native RIP
GCACCCAGATCAACCAAGTGAGAAATGGAAGTACAAGAAACACACCAAGGAATTTCTACCGA
CGCCTTTGGGGATATTCAAGTTTGAGACACTGGGGAAGAAAGGGAAGTATATACGCTGTCTGCTGC
GACACGGACGCGGAAATCCTTTACGAGCTGCTGACCCAGCACTGGCACCTGAAACACCCAAAC
CTGGTCAATTTCTGTGACCGGGGGCGCCAAAG

Ex 4-5 WT Native RIP
GAACTACAAGAAACACACCAAGGAATTTCTACCGACGCTTTGGGGATATTCAAGTTTGAGACA
CTGGGGAAGAAAGGGAAGTATATACGCTGTCTGCGACACGGACGCGGAAATCCTTTACGAG
CTGCTGACCCAGCACTGGCACCTGAAACACCCAACTGGTCAATTTCTGTGACCGGGGGCGCC
AAGAACTTGCCTTGAAGCCGCGCATGCGCAAGATCTTCAGCCGGCTCATCT

Ex 12-15 MM Native RIP
AGTCATGTTTACGGCTCTCATAAAGACAGACCCAAAGTTTGTCGCCCTCTTTCTGGAGAATGGC
TTGAACCTACGGAAGTTTCTCACCCATGATGTCCTCACTGAACTCTTCTCCAACCACTTCAGCAC
GCTTGTGTACCGGAATCTGCAGATCGCCAAGAAATCCTATAATGATGCCCTCCTCACTGTTGTCT
GGAACCTGGTTCGCAACTTCCGAAGAGGCTTCCGGAAGGAAGACAGAAATGGCCGGGACGAG
ATGGACATAGAAGTCCACGACGTGTCTCTATTACTCGGCACCCCTGCAAGCTCTCTTCATCT
GGGCCATTTCTCAGAATAAGAAGGA

Ex 12-15 WT Native RIP
CTGACCTTCAAGAAGTCATGTTTACGGCTCTCATAAAGACAGACCCAAAGTTTGTCCGCCCTTTT
CTGGAGAATGGCTTGAACCTACGGAAGTTTCTCACCCATGATGTCCTCACTGAACTCTTCTCCA
ACCACTTCAGCACGCTGTGTACCGGAATCTGCAGATCGCCAAGAAATCCTATAATGATGCCCTCTC
CCTCACGTTTGTCTGGAACCTGGTTCGCAACTTCCGAAGAGGCTTCCGGAAGGAAGACAGAAA
TGGCCGGGACGAGATGGACATAGAAGTCCACGACGTGTCTCTATTACTCGGCACCCCTGCA
AGCTCTCTCATCTGGGCCATCTTTCAGAATAAGAAGAACTTCCAAAGTCAATTTGGGAGCAG
ACCAAGGGGCTGCACCTTGGCAGCCCTGGGAGCCAGCAAGCTCTG

Ex 21-23 MM Native RIP
CAGGTGCCAGTGACGTGGATGGTACCAGTATGACTTTGCCCACTGCACCTTCACTGGGAAT
GAGTCCAAAGCACTGTGTGGAGCTGGATGAGCACAACCTGCCCGGTTCCCGAGTGGATC
ACCATCCCCCTGGTGTGCATCTACATGTTATCCACCAACATCTGCTGGTCAACCTGCTGGTGC
CCATGTTTGGCTACACGGTGGGACCGTCCAGGAGAACAATGACCAGGTCTGGAAGTTCCAGA
GGTACTTCTGGTGCAGGAGTACTGCAGCCGCCCTCAATATCCCCCTTCCCTTCATCGTCTTCGC
TTACTTCTACATGGTGGTGAAGAAGTCTCAAGTGTGCTGCAAGGAGAAAAACATGGAGTCT
TCTGTCTGCTGTTTCAAAATGAAGACAATGAGACTCTGGCATGG

Ex 21-23 WT Native RIP
TCGGCCAGGTGCCAGTGACGTGGATGGTACCAGTATGACTTTGCCCACTGCACCTTCACTG
GGAATGAGTCCAAAGCACTGTGTGGAGCTGGATGAGCACAACCTGCCCGGTTCCCGAGTGGATC
GGAATCACCCTCCCTGGTGTGCATCTACATGTTATCCACCAACATCTGCTGGTCAACCTGCTG
GGTGCATGTTTGGCTACACGGTGGGACCGTCCAGGAGAACAATGACCAGGTCTGGAAGTTCC
CCAGAGGTACTTCTGCTGCGAGGAGTACTGCAGCCGCCCTCAATATCCCCCTTCCCTTCATCGTCT
TTCGTTACTTCTACATGGTGGTGAAGAAGTCTCAAGTGTGCTGCAAGGAGAAAAACATGG
AGTCTTCTGTCTGCTGTTTCAAAATGAAGACAATGAGACTCTG

Ex 4-5 MM fCLIP
GCACCCAGATCAACCAAGTGAGAAATGGAAGTACAAGAAACACACCAAGGAATTTCTACCGA
CGCCTTTGGGGATATTCAAGTTTGAGACACTGGGGAAGAAAGGGAAGTATATACGCTGTCTGCTGC
GACACGGACGCGGAAATCCTTTACGAGCTGTGACCCAGCACTGGCACCTGAAACACCCAAAC
CTGGTCAATTTCTGTGACCGGGGGCGCCAAAGAACTTCGCCCTGAAGCCGCGCATGCGCAAGATC
TTACGCCGG

Ex 4-5 WT fCLIP
CAGATCAACCAAGTGAGAAATGGAAGTACAAGAAACACACCAAGGAATTTCTACCGACGCTT
TTGGGGATATTCAAGTTTGAGACACTGGGGAAGAAAGGGAAGTATATACGCTGTCTGCTGCGACAC
GGACCGGGAATCCTTTACGAGCTGTGACCCAGCACTGGCACCTGAAACACCCAACTGGT
CATTTCTGTGACCGGGGGCGCCAAAGAACTTCGCCCTGAAGCCGCG

Ex 12-15 MM fCLIP
CTTCAAGAAGTCAATGTTTACGGCTCTCATAAAGACAGACCCAAAGTTTGTCGCCCTCTTTCTGG
AGAATGGCTTGAACCTACGGAAGTTTCTCACCCATGATGTCCTCACTGAACTCTTCTCCAACCA
CTTACGACGCTTGTGTACCGGAATCTGCAGATCGCCAAGAAATCCTATAATGATGCCCTCTCTC
ACGTTTGTCTGGAACCTGGTTCGCAACTTCCGAAGAGGCTTCCGGAAGGAAGACAGAAATGGC
CGGGACGAGATGGACATAGAAGTCCACGACGTGTCTCTATTACTCGGCACCCCTGCAAGCT
CTCTTCATCTGGGCCATCTTTCAGAATAAGAAGAACTTCCAAAGTCAATTTGGGAGCAGACCA
GGGGCTGCACCTTGGCAGCCCTGGGAGCCAGCAAGCTTCTGAAG

Ex 12-15 WT fCLIP
AGTCATGTTTACGGCTCTCATAAAGACAGACCCAAAGTTTGTCCGCCCTTTTCTGGAGAATGGC
TTGAACCTACGGAAGTTTCTCACCCATGATGTCCTCACTGAACTCTTCTCCAACCACTTCAGCAC
GCTTGTGTACCGGAATCTGCAGATCGCCAAGAAATCCTATAATGATGCCCTCTCAGTGTGTCT
GGAACCTGGTTCGCAACTTCCGAAGAGGCTTCCGGAAGGAAGACAGAAATGGCCGGGACGAG
ATGGACATAGAAGTCCACGACGTGTCTCTATTACTCGGCACCCCTGCAAGCTCTTCTCATCT
GGGCCATCTTTCAGAATAAGAAGAACTTCCAAAGTCAATTTGGGAGCAGACCGGGCTGCA
CTCTGGCAGCCCTGGGAGCCAGCAAGCTTCTGAAGACTCTGGCCA

Ex 21-23 MM fCLIP
CAGGTGCCAGTGACGTGGATGGTACCAGTATGACTTTGCCCACTGCACCTTCACTGGGAAT
GAGTCCAAAGCACTGTGTGGAGCTGGATGAGCACAACCTGCCCGGTTCCCGAGTGGATC
ACCATCCCCCTGGTGTGCATCTACATGTTATCCACCAACATCTGCTGGTCAACCTGCTGGTGC
CCATGTTTGGCTACACGGTGGGACCGTCCAGGAGAACAATGACCAGGTCTGGAAGTTCCAGA
GGTACTTCTGGTGCAGGAGTACTGCAGCCGCCCTCAATATCCCCCTTCCCTTCATCGTCTTCGC
TTACTTCTACATGGTGGTGAAGAAGTCTTCAAGTGTGCTGCAAGGAGAAAAACATGGAGTCT
TCTGTCTGCTGTTTCAAAATGAAGACAATGAGACTCTGGCATGG

Ex 21-23 WT fCLIP
CAGTGACGTGGATGGTACCAGTATGACTTTGCCCACTGCACCTTCACTGGGAATGAGTCCAA
GCCACTGTGTGTGGAGCTGGATGAGCACAACCTGCCCGGTTCCCGAGTGGATCACCATCCCC
CCTGGTGTGCATCTACATGTTATCCACCAACATCTGCTGGTCAACCTGCTGGTGCCTATGTTT
GGCTACACGGTGGGACCGTCCAGGAGAACAATGACCAGGTCTGGAAGTTCCAGAGGTACTTC
CTGGTGACGAGTACTGCAGCCGCCCTCAATATCCCCCTTCCCTTCATCGTCTTCTGCTTCTCT
ACATGGTGGTGAAGAAGTCTTCAAGTGTGCTGCAAGGAGAAAAACATGGAGTCTTCTGTCTG
CTGTTTCAAAATGAAGACAATGAGACTCTGGCATGGAGGGTGT

Appendix Figure S2. Quantitation of TRPM8 RNA binding to TLR3

A, Table showing qPCR threshold cycle (CT) of TRPM8, GAPDH and Y3 transcripts RIP and fCLIP experiments performed with the TLR3 antibody NB100-5657 produced by Novus Biological.

B, Amplicons sequence.