Supplemental Information

PRMT5 promotes resistance to immunotherapy in triple negative breast cancer by methylating KEAP1 and inhibiting ferroptosis

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Supplementary Table S1

Oligonucleotides
siRNA: scrambled control RNA
siRNA: PRMT5#1: GGACCTGAGAGATGATATA
siRNA: PRMT5#2: CCAGAAGAGGAGAAGGATA
siRNA: TRIM25#1: AACAAGAATACACGGAAATGAAG
siRNA: TRIM25#2: CAGCAAGTTTGACACCATTTATC
Primer: PRMT5 (F):5'-CCTGTGGAGGTGAACACAGT-3'
Primer: PRMT5 (R):5'-AGAGGATGGGAAACCATGAG-3
Primer: KEAP1 (F):5'- CTGGAGGATCATACCAAGCAGG-3'
Primer: KEAP1 (R):5'- GGATACCCTCAATGGACACCAC -3'
Primer: NRF2 (F):5'- TCAGCGACGGAAAGAGTATGA-3'
Primer: NRF2 (R):5'- CCACTGGTTTCTGACTGGATGT-3'
Primer: HMOX1 (F):5'- AAGACTGCGTTCCTGCTCAAC-3'
Primer: HMOX1 (R):5'- AAAGCCCTACAGCAACTGTCG-3'
Primer: SLC7A11 (F):5'- TCTCCAAAGGAGGTTACCTGC-3'
Primer: SLC7A11 (R):5'- AGACTCCCCTCAGTAAAGTGAC-3'
Primer: GPX4 (F):5'- GAGGCAAGACCGAAGTAAACTAC-3'
Primer: GPX4 (R):5'- CCGAACTGGTTACACGGGAA-3'
Primer: GAPDH(F):5'- GAAAGCCTGCCGGTGACTAA-3'
Primer: GAPDH(R):5'- GCCCAATACGACCAAATCAGAGA-3'

Supplementary Table S2

PRmePred

Position	Peptide	Score
6	MQPDPRPSGAGACCR	0.903601
15	RPSGAGACCRFLPLQSQCP	0.82419
362	LRLADLQVPRSGLAGCVVG	0.832482
415	CAPMSVPRNRIGVGVIDGH	0.903907
596	DTDTWSEVTRMTSGRSGVG	0.835933
601	SEVTRMTSGRSGVGVAVTM	0.955328

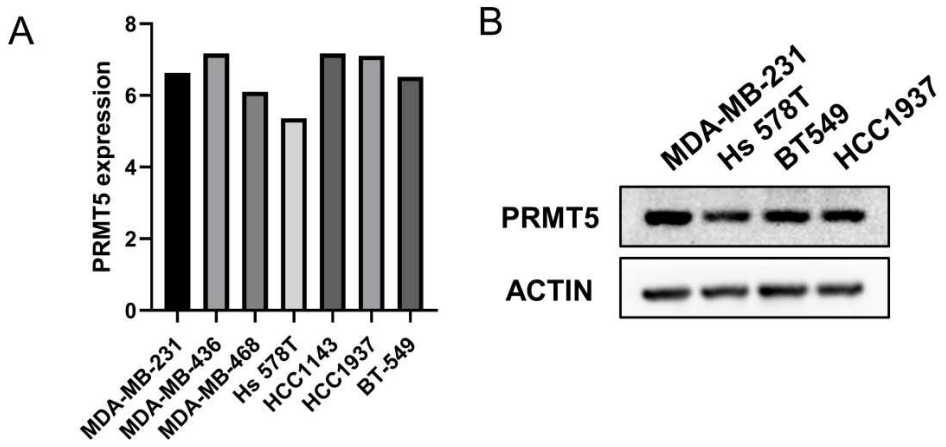
GPS-MSP

Position	Peptide	Score
71	AFGIMNELRLSQQLCDV	3.50
116	KAMFTNGLREQGMEVVS	3.63
234	CQLVTLISRDDLNVRCE	3.71
596	DTDTWSEVTRMTSGRSGVG	4.17

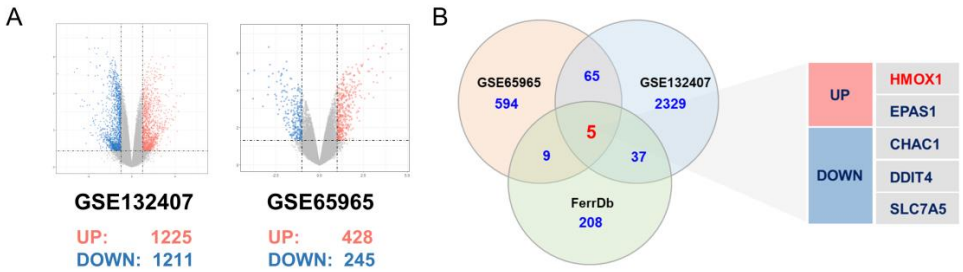
Supplementary Table S3

The PRMT5 and KEAP1 protein expression levels are positively correlated in human breast cancer specimens.

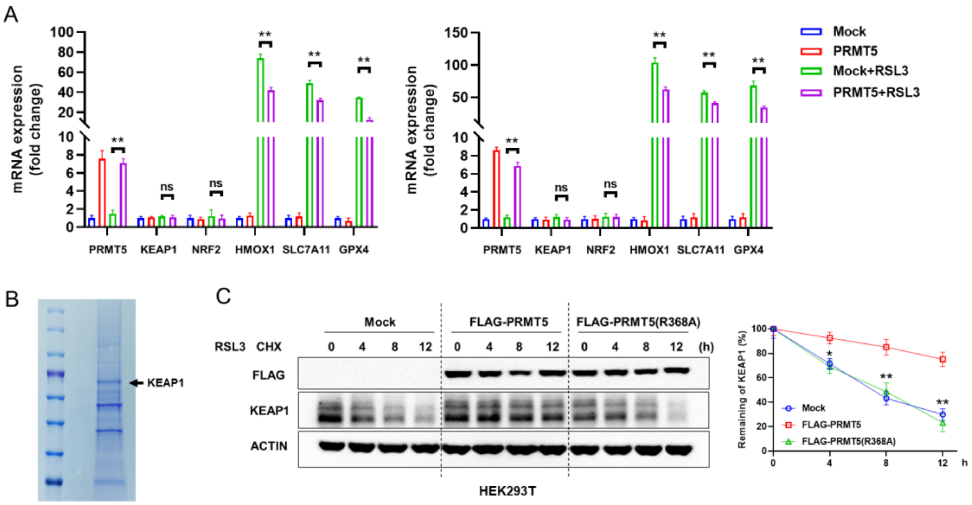
R=0.443		PRMT5		
P=0.002		Positive	Negative	Total
KEAP1	Positive	23	7	30
	Negative	5	11	16
	Total	28	18	46



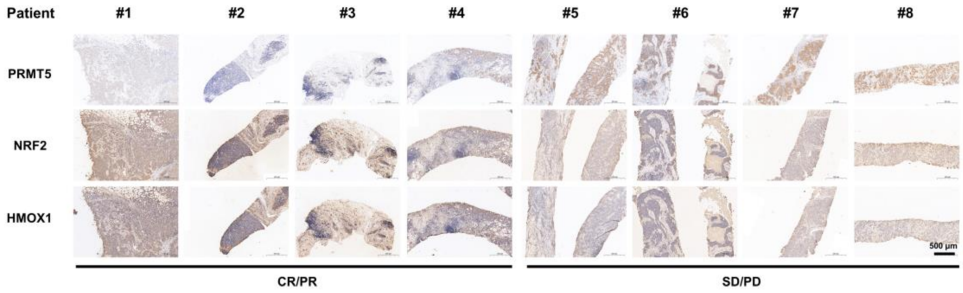
Supplementary Fig. S1 The levels of PRMT5 mRNA and protein expression were comparable among triple negative breast cancer cell lines A. The mRNA levels of triple negative breast cancer cell lines gained from Cancer Cell Line Encyclopedia (Cancer Cell Line Encyclopedia (CCLE) (broadinstitute.org)) dataset. **B**. The PRMT5 protein expression of four triple negative breast cancer cells were detected by immunoblotting.



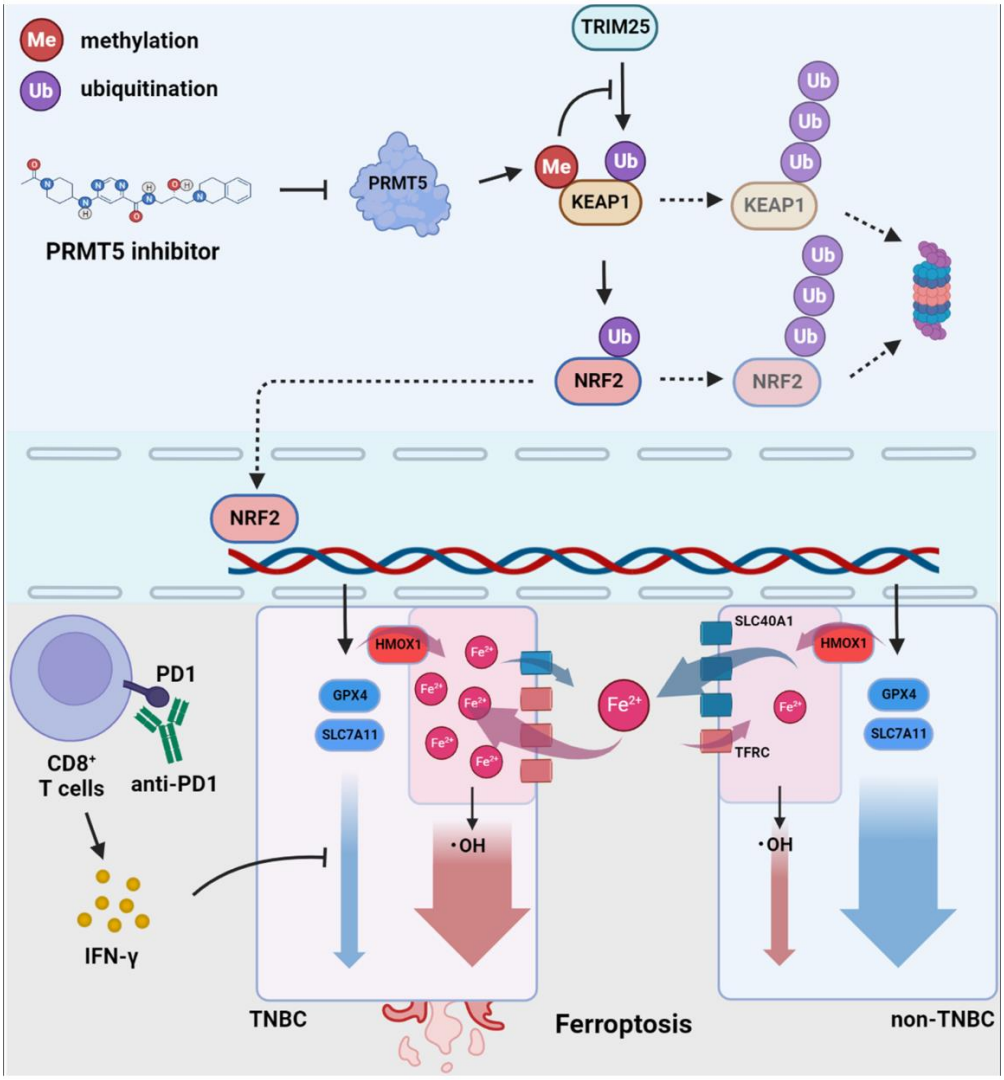
Supplementary Fig. S2 The ferroptosis associated genes regulated by PRMT5. A. Volcano plot of differentially expressed genes reveals putative genes regulated by PRMT5. **B**. Venn diagram of differentially expressed genes and ferroptosis associated genes highlights 5 candidates for ferroptosis associated genes regulated by PRMT5.



Supplementary Fig. S3 PRMT5 overexpression had no effect on the mRNA expression of KEAP1 and NRF2 but significantly extended the half-life of KEAP1 with pre-treated RSL3. A. PRMT5 overexpression had no effect on KEAP1 and NRF2 mRNA levels in MDA-MB-231 and HCC1937 cells in the presence of RSL3 but significantly decreased the mRNA levels of HMOX1, SLC7A11 and GPX4. **B**. Liquid chromatography-tandem mass spectrometry identified potential substrates of PRMT5. **C**. PRMT5 overexpression extended the half-life of KEAP1 with pre-treated RSL3. HEK293T cells were transfected with Flag-PRMT5 or Flag-PRMT5-R368A for 48h and then treated with RSL3 (3μM) for 6h, following CHX for the indicated times. The graph shows the quantitative results from the left panel. Average of three experiments. *P＜0.05. ** P＜0.01.



Supplementary Fig. S4 Expression of PRMT5, NRF2 and HMOX1 protein in human specimen derived from eight TNBC patients that received immunotherapy.



Supplementary Fig. S5 Schematic of the proposed model showing that PRMT5 inhibits triple negative breast cancer ferroptosis by suppressing NRF2/HMOX1 pathway and PRMT5-targeted drugs combined with immunotherapy may be a potential treatment strategy for triple negative breast cancer.