

Electronic Supplementary Material

Tumor Necrosis Factor α Reduces SNAP29 Dependent Autolysosome Formation to Increase Prion Protein Level and Promote Tumor Cell Migration

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Table S1 Primers used to construct plasmids.**Gene name and sequence**

FOXP3

Sense: 5'-CCCAAGCTTGCCACCATGCCCAACCCCAGGCCT-3'

Antisense: 5'-CGCGGATCCTCAGGGGCCAGGTGTAGGGTTGGA-3'

PGL-1-2000-promotor

Sense: 5'-CGACGCGTCGAATGTGCTGACTAGCTAGCATGAGTTTGATTCA-3'

Antisense: 5'-CCCAAGCTTTCCTTCCGCGCGTTCGCGCGCAGGTGGCCCCGCCCT-3'

PGL-1-1000-promotor

Sense: 5'-CGACGCGTCGCCCCCAGCCTCGTAGAATAACACTGCGCAGTTG-3'

Antisense: 5'-CCCAAGCTTTCCTTCCGCGCGTTCGCGCGCAGGTGGCCCCGCCCT-3'

PGL-1-500-promotor

Sense: 5'-CGACGCGTCTCCACGCTTCGTCACCCCAG-3'

Antisense: 5'-CCCAAGCTTTCCTTCCGCGCGTTCGCGCGCAGGTGGCCCCGCCCT-3'

PGL-Δ1-500-promotor

Sense: 5'-CGACGCGTCGAATGTGCTGACTAGCTAGCATGAGTTTGATTCA-3'

Antisense: 5'-CCCAAGCTTGCGGGACGAGGGTCTGTGGG-3'

PGL-Δ(161-168)-promotor

Sense: 5'-CGCCACGTGACCTCTCAGTACTAGATAGGGC-3'

Antisense: 5'-GCCCTATCTAGTACTGAGAGGTCACGTGGCG-3'

Phage-flag-PRNP

Sense: 5'-TGCTCTAGAGCCACCATGGCGAACCTTTGGCTGCT-3'

Antisense: 5'-CGCGGATCCTCATCCCACTATCAGGA-3'

Table S2 RNA oligos used to knockdown *PRNP*, *SNAP29*, *FOXP3* in 549 cells or knockout *FOXP3*, *SNAP29* in M2 cells.

Name	sequence
(A549)shSNAP29-1#:	5'-GGTGGACAAGATGGACCAAGA-3'
(A549)shSNAP29-2#:	5'-GCCCAACAACAGATTGAAAGA-3'
(A549)shFOXP3-6#:	5'-GCAAATGGTGTCTGCAAGTGG-3'
(A549)shFOXP3-8#:	5'-GGAAACAGCACATTCCCAGAG-3'
(M2)SNAP29-sgrna:	5'-CAATCCGTTTCGACGACGACG-3'
(M2)FoxP3-sgrna-1#:	5'-AGGAGCCTCGCCCAGCTGGA-3'
(M2)FoxP3-sgrna-2#:	5'-ACCCAAAGCCTCAGACCTGC-3'
(A549)shRNAi-PrP-2#:	5'-ATGAGCAGGCCCATCATACAT -3'
(A549)shRNAi-PrP-3#:	5'-GAGGGAATCTCAGGCCTATTA -3'

Table S3 Primers were used in RT-PCR assay.

Gene name	and sequence
<i>PRNP</i>	
	Sense : 5'-GTGACTATGAGGACCGTTACTATC-3'
	Antisense: 5'-TGACCGTGTGCTGCTTGA-3'
<i>SNAP29</i>	
	Sense : 5'-ACTGATGCTTACCCAAAGAACC-3'
	Antisense: 5'-GTCCTTCAGACGACCCAGTC-3'
<i>LAMP2</i>	
	Sense: 5'-GAAAATGCCACTTGCCTTTATGC-3'
	Antisense: 5'-AGGAAAAGCCAGGTCCGAAC-3'
<i>FOXP3</i>	
	Sense: 5'-GTGGCCCGGATGTGAGAAG-3'
	Antisense: 5'-GGAGCCCTTGTCGGATGATG-3'
<i>ACTB</i>	
	Sense: 5'-ATCGTGCGTGACATTAAGGAG-3'
	Antisense: 5'-GGAAGGAAGGCTGGAAGAGT-3'
<i>TNFA</i>	
	Sense: 5'-GCCGCATCGCCGTCTCCTAC-3'
	Antisense: 5'-CCTCAGCCCCCTCTGGGGTC-3'

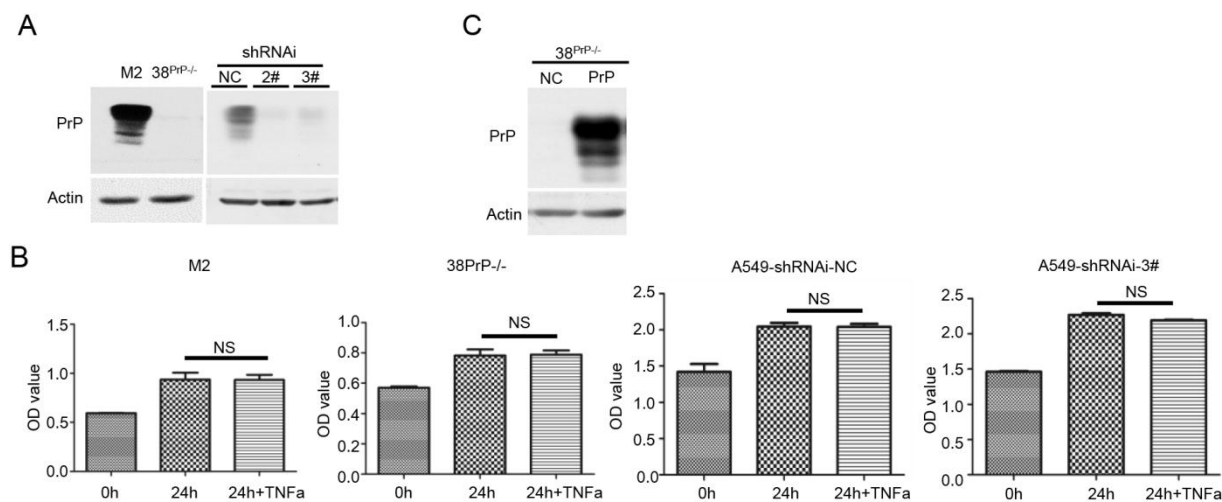


Fig. S1 Expressing PrP in M2 and A549 cells does not affect cell proliferation under TNF α treatment. **A** Immunoblotting showed that *PRNP* was silenced in M2 and A549 cells. Cell lysates from M2 or *PRNP* null M2, A549 or shRNA-*PRNP* A549 cells were probed with PrP specific monoclonal antibody 4H2. Actin was blotted as a loading control. **B** 24 h TNF α treatment does not alter cancer cells proliferation with or without PrP expression. 38PrP^{-/-} was the *PRNP* null M2 cells; 2# & 3# were two different shRNAi-*PRNP* A549 cells. These experiments were repeated three times with similar results. **C** Rescuing PrP in *PRNP* null M2 cells. Immunoblotting of cell lysates from control and PrP expressing *PRNP* null M2 cells with 4H2 revealed that expression of PrP was successfully rescued.

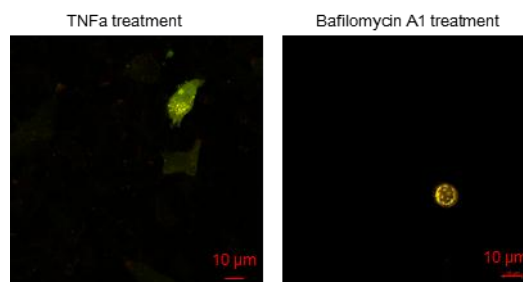


Fig. S2 TNF α treatment significantly reduces the fusion between autophagosome and lysosome in A549 cells. When treated only with TNF α , we detected reduction of fusion between autophagosome and lysosome (left panel). However, when bafilomycin A1 was added into the A549 cell culture medium for additional two h, we detected all yellow dots, indicating a total loss of fusion between autophagosome and lysosome (right panel).

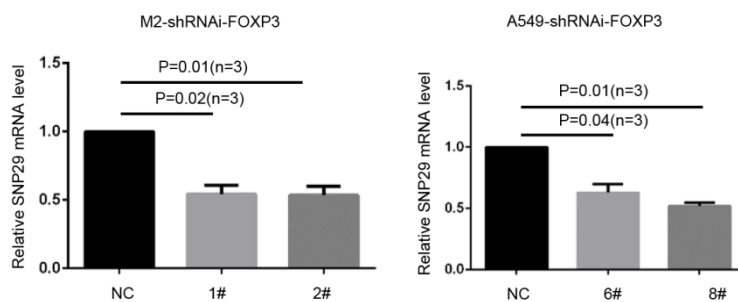


Fig. S3 Silencing *FOXP3* significantly decreases *SNAP29* transactivation. mRNA level of *SNAP29* was quantified with primers specific for *SNAP29* by Q-PCR. *P*-values were indicated. Error bar represented SEM of indicated experiments. Other than indicated, all the experiments were repeated at least three times.