SUPPLEMENTARY INFORMATION

Supplementary Methods

- **H and E staining**
  1. Bake sections in oven at 65°C for 1 h
  2. Deparaffinize sections in xylene for 10 min
  3. Re-hydrate in absolute alcohol for 5 min, 95% alcohol and 70% alcohol for 2 min each
  4. Wash briefly in distilled water
  5. Stain in hematoxylin solution for 5 min
  6. Wash in running tap water for 5 min
  7. Differentiate in 1% acid alcohol for 10 s
  8. Wash running tap water for 1 min
  9. Bluing in 0.25% ammonia water for 2 min
  10. Wash in running tap water for 5 min
  11. Rinse in 75% alcohol for 10 s
  12. Counterstain in eosin-phloxine solution for 30 s
  13. Wash running tap water for 1 min
  14. Dehydrate through 95% alcohol and absolute alcohol for 2 min each, then natural drying
  15. Transparentizing in T0 transparent reagent I and II for 5 min each, then natural drying
  16. Mount with neutral glue.

- **IHC staining**
  1. Deparaffinization
     i. At 65°C for 1 h: Bake sections in oven
     ii. At room temperature (RT) 10 min each: wash slides in two changes of TO type transparent reagent.

  2. Rehydration (at RT)
     i. Three minutes: wash slides in 100% alcohol
     ii. Three minutes: wash slides in 95% alcohol
     iii. Three minutes: wash slides in 80% alcohol
     iv. Five minutes: gently rinse slides using distilled water.

  3. Antigen retrieval and blocking
     i. At 97°C for 40 min: steam slides in 0.01 M sodium citrate buffer, pH 6.0
     ii. At RT for 20 min: remove slides from heat and let cool in buffer
     iii. At RT for 30 min: apply 3% H_2O_2-methanol solution
     iv. At RT for 1 min: wash running tap water
     v. At 37°C in thermostatic incubator for 30 min: apply 5% BSA block, and then drain the 5% BSA block from the slides.

  4. Immunostaining
     i. At 4°C in fridge for overnight: apply primary antibody: TLR2 (1:400; Nonus: NB100-56720), TLR10 (1:500; Thermo Fisher Scientific: PA5-20054), and HMGB1 (1:2000; Abcam: ab18256)
     ii. At RT for 30 min: rewarm slides to RT
     iii. At RT for 1 min each: wash slides in three changes of distilled water
     iv. At 37°C in thermostatic incubator for 1 h: apply a biotinylated secondary antibody (AffiniPure Goat Anti-Rabbit IgG, Jackson)
     v. At RT for 1 min each: wash slides in three changes of distilled water
     vi. At RT for suitable time (controlled under microscope), apply DAB coloration kit
     vii. At RT for 1 min each: wash slides in three changes of distilled water
     viii. At RT for 5 min: Stain in hematoxylin
     ix. At RT for 1 min each: wash slides in three changes of distilled water.

  5. Dehydration (at RT)
     i. One minute each: Wash slides in two changes of 80% alcohol
     ii. One minute each: Wash slides in two changes of 95% alcohol
     iii. One minute each: Wash slides in two changes of 100% alcohol
     iv. One minute each: Wash slides in three changes of xylene
     v. Apply coverslip.

- **Frozen section immunofluorescence**

  A prostate chip stored in liquid nitrogen was made into frozen section (5 μm). Section was treated in the following:
  1. Fix in precooled 10% neutral buffered formalin for 5 min
  2. Wash in three changes of distilled water

3. At 37°C in thermostatic incubator for 30 min: apply 5% BSA block and then drain the 5% BSA block from the slides
4. At 4°C in fridge for overnight: apply primary antibody: TLR2 (1:400; Nonus: NB100-56720) and TLR10 (1:500; Thermo Fisher Scientific: PA5-20054)
5. At RT for 30 min: rewarm slides to RT
6. At RT for 1 min each: wash slides in three changes of distilled water
7. At 37°C in thermostatic incubator for 1 h: apply fluorescent secondary antibodies: Alexa Fluor 488 (Thermo Fisher Scientific) and Alexa Fluor 594 (Thermo Fisher Scientific)
8. At RT for 1 min each: wash slides in 3 changes of distilled water
9. At RT for 3 min: apply 0.1 ug ml^{-1} DAPI (Sigama)
10. At RT for 1 min each: wash slides in three changes of distilled water
11. Mount with antifade mounting medium and store at 4°C in the dark.

- **Cell immunofluorescence**

Cells were plant on 12 well plates with slides to proliferate reaching about 80%. Slides were treated in the following:

1. At RT for 3 min each: shake and wash slides in three changes of PBS
2. At RT for 15 min: fix with 200 ul 4% paraformaldehyde (cover slides)
3. At RT for 3 min each: shake and wash slides in three changes of PBS
4. At RT for 3 min: permeabilization with 200 ul 0.2% TritonTM X-100
5. At RT for 3 min each: shake and wash slides in three changes of PBS
6. At RT for 30 min: apply 1% BSA block
7. At RT for 3 min each: shake and wash slides in three changes of PBS
8. At 37°C in thermostatic incubator for 1 h: apply 200 ul primary antibodies (dissolved in the 1% BSA): TLR2 (1:400; Thermo Fisher Scientific: MA5-16200) and TLR10 (1:1000; Thermo Fisher Scientific: PA5-20054)
9. At RT for 3 min each: shake and wash slides in three changes of PBS
10. At 37°C in thermostatic incubator for 1 h: apply 200 ul fluorescent secondary antibodies (dissolved in the PBS): Alexa Fluor 488 (Thermo Fisher Scientific) and Alexa Fluor 594 (Thermo Fisher Scientific)
11. At RT for 3 min each (keep in dark): shake and wash slides in 3 changes of PBS
12. At RT for 3 min (keep in dark): apply 0.1 ug ml^{-1} DAPI (Sigama)
13. At RT for 3 min each (keep in dark): shake and wash slides in three changes of PBS
14. At RT for 1 min each (keep in dark): wash slides in two changes of distilled water
15. Mount with antifade mounting medium and store at 4°C in the dark.

Note: Skip permeabilization (4 and 5) when staining proteins on the plasma membrane.

- **RNA extraction, reverse transcription and qRT-PCR**

1. Total RNAs of collected prostate tissues or RWPE-1 cells were extracted with TRIzol
2. cDNAs were synthesis with iscript cDNA Sythesis Kit (Bio-rad)

One 20 μl reaction include:

5x Iscript reaction mix	4 ul
Iscript reverse transcriptase	1 ul
Nuclease-free water	x ul
RNA template	1 ug
Total volume	20 ul

3. Program Settings: 25°C 5 min; 42°C 30 min; 85°C 5 min; 12°C ∞. After reverse transcription, cDNA solutions were diluted 5 times with 80 ul RNase-free water.
4. Primers were follows:

Human TLR2 primer (100 bp):
Forward 5'-GATGCCTACTGGGTGGAGAA-3';
Reverse 5'-CGCAGCTCTCAGATTTACCC-3'.
Human TLR10 primer (115 bp):
Forward 5'-TCTCCACTTTGATCTGCCCT-3';
Reverse 5'-TGCGTGGAATCGGACATTTC-3'.
Human HMGB1 (163 bp):
Forward 5'- AGAGCGGAGAGAGTGAGGAG-3';
Reverse 5'- GATCTCCTTTGCCCATGTTT-3'.
Human IL-6 (342 bp):
Forward 5'- CAAATTCGGTACATCCTCGAC-3';
Reverse 5'-CTACGTTATTGGTGGGGACTG-3'.
Human IL-8 (302 bp):

Forward 5'- ATGACTTCCAAGCTGGCCGTG-3';
Reverse 5'-CTCTTCAAAAACTTCTCCCGACTCTTAAGTATT -3'.

Human PCNA (307 bp):
Forward 5'-GACACATACCGCTGCGATCG-3';
Reverse 5'-TCACCACAGCATCTCCAATAT-3'.

Human β-actin (342 bp):
Forward 5'- GCATGGAGTCCTGTGGCAT-3';
Reverse 5'-CTAGAAGCATTTGCGGTGG-3'.

5. Primers were diluted to 10 uM. PCR reactions used for three-step PCR were follows:

2x mix	5 ul
cDNA	2 ul
ddH2O	2 ul
P1 (10 uM)	0.5 ul
P2 (10 uM)	0.5 ul
Total volume	10 ul

- **Western blot**
  1. Cells and prostate tissues were ground into powder in liquid nitrogen. RIPA lyste solution was then used to prepare cell/tissue lysates
  2. Protein concentration was measured with BCA Protein Assay Kit (Beyotime Biotechnology)
  3. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), transfer membrane, blocking
  4. Primary antibodies incubation: TLR2 (1:1000; CST: 12276), TLR10 (1:250; R&D: MAB6619), phospho-NF-κB P65 (1:1000; CST: 3033T) and NF-κB P65 (1:1000; CST: 8242T)
  5. Correspondent secondary antibodies incubation;
  6. Chemiluminescence detection.

- **ELISA**

  Supernatants were collected for ELISA test. Manipulations were followed the instructions strictly;
  1. Adding samples: samples were added to accommodate a blank, a standard and test sample in the wells of the ELISA plates (100 μl well^{-1})
  2. Adding antibodies: biotinylated antibodies (IL-6, IL-8, or HMGB1) were placed into the wells (50 μl well^{-1}). Plates were put in thermostatic incubator for 1.5 h (at 37°C)
  3. Washing: wells were rinsed with 4 charges of washing buffer (300 μl well^{-1})
  4. Adding enzyme: streptavidin-HRP was added (100 μl well^{-1}). Plates were put in thermostatic incubator for 0.5 h (at 37°C)
  5. Washing: repeat step 3
  6. Coloration: TMB was added for coloration (100 μl well^{-1}). Plates were put in thermostatic incubator for 5–30 min (at 37°C, keep in dark). Termination of the reaction was according to the color depth of the holes
  7. Terminate reaction: stop solution was added to terminate reaction (100 μl well^{-1})
  8. Immediately (within 10 min) placed the plate into the socket of ELISA instrument and measured at λ = 450 nm.