**SUPPLEMENTARY MATERIALS AND METHODS**

**Flow cytometry.** Single cell suspensions were obtained from cell cultures or by digestion of prostates and LN tissues with collagenase IV (1600 units/ml) for 1 hour at 37°C, incubated 10 minutes with FcR blocker (BD-Biosciences), labeled for 15 minutes at 4°C with fluorochrome-conjugated monoclonal antibodies or isotype controls (all from BD-Biosciences or BioLegend) and acquired by BD FACSCanto™. Dead cells were excluded by 7AAD staining. Data were analyzed using FlowJo software. For intracellular staining, cells were stained for surface markers, fixed with 2% PFA, and permeabilized with saponin (0.5% in PBS), before incubation with the desired antibody. For intracellular detection of IFNγ, blasts were stimulated 4h with PMA (120 ng/ml) and ionomicin (1μg/ml), or, when indicated, with OVA323-339 or OVA257-264 peptides (1μg/ml), adding brefeldin A (10μg/ml) in the last 3h, as previously described ([1](#_ENREF_1)). For intracellular detection of phosphorylated Zap70, ERK2 and STAT5, cells were stimulated with 1μl of anti CD3 beads or 50IU/ml of IL-2, respectively, and stained according to the BD-Phosflow kit protocol (BD-Biosciences). F-actin was quantitated by staining with FITC-conjugated phalloidin. Briefly, splenocytes from C57BL/6 mice were pre-warmed for 10 min at 37°C in TCM, treated with 5μg/ml of CCD, or incubated with prostate CSCs (10:1 ratio) for 25 min if indicated, stimulated 5 min with PMA (100nM), fixed with 3.7% PFA and permeabilized on ice for 2 min with Triton (0,1% in PBS). Thereafter, cells were stained with FITC-Phalloidin (2 μg/ml, Sigma) at 37°C for 30 min and analyzed by BD FACSCanto™.

***In vitro* migration.** TPIN-SCs were seeded in the upper chamber of an 8μm transwell system, and cultured overnight in absence or presence of recombinant murine CXCL12 (2.5nM; Peprotech, Rocky Hill, NJ), and/or the CXCR4 inhibitor AMD3100 (10 M) or the CXCR4 antagonist Peptide R [10M; ([2](#_ENREF_2))]. Cells migrated in the lower chamber were quantified by FACS.

**Immunization procedure.** Dendritic cells pulsed with the Tag 404-411 peptide were prepared as described ([1](#_ENREF_1)). Mice were sacrificed one week after vaccination, and their splenocytes re-stimulated *in vitro* for 5 days with Tagpeptidein the presence of irradiated prostate CSCs (10:1 ratio), and tested for IFN production and cytotoxic activity in a standard 4h 51Cr release assay ([1](#_ENREF_1)). Lytic units (LU) were determined as the number of effector cells capable to kill 30% of target cells, and were expressed as 106.

**Tumor challenge.** 2x106 TPIN-SCs shRNA-TNC or shRNA-ctr were diluted 1:1 in Matrigel™ High Concentration (BD-Biosciences, Milan, Italy; 354248) and injected subcutaneously in male C57BL/6 or RAG-1-/- OTI recipients. Mice were monitored twice/week and sacrificed when tumor size reached an area of 100mm2 or 100 days after challenge.

***In vivo* proliferation.** C57BL6J mice were challenged subcutaneously with 2x105 B16-OVA cells. When tumors reached a mean diameter of 4x4 mm, mice were inoculated intra-spleen with 30x106 CFSE labeled splenocytes from RAG-1-/- OTI mice, together or not with 3x106 prostate CSCs. Animals were killed 5 days later, spleens were processed on a 70m cell stranier, while tumors were digested in collagenase D (Roche, Monza, Italy) for 1 hour at 37°C. Single cell suspensions were analyzed by FACS.

**Multichannel time-lapse fluorescent live cell imaging.** CFSE-labeled prostate CSCs were cultured with CMTMR-labeled CD8 T cells purified from RAG-1-/-OT1 transgenic mice (ratio 1:10) in the presence of anti-CD3/CD28 beads and IL-2. Cells contacts were recorded during the first 4 hours of co-culture; ΔT=3 min. Time-lapse experiments were performed on TCS SP2 confocal microscope (Leica, Milan, Italy).

**SILAC, mass spectrometry and data Analysis.** OTI cells were activated as described above and cultured in SILAC medium containing light (12C and 14N) or heavy (13C 15N) labeled L-lysine and L-arginine, in the presence of irradiated prostate CSCs. After 7 days of co-culture, CD8+ T cells from either condition were sorted for activated (CD8+CD62Llow) or inhibited (CD8+CD62Lhigh) phenotype and lysed in RIPA buffer (Cell Signaling Technologies, Boston, MA, USA). Heavy and light lysates were mixed 1:1 for the protein content; proteins were then resolved onto a 4-12% NuPAGE® pre-cast gel (Invitrogen) and stained by Coomassie colloidal blue. The gel lane was cut into 12 slices each of which has been reduced, alkylated and digested with trypsin as reported ([3](#_ENREF_3)). Peptides mixtures were desalted and concentrated on a home-made C18 desalting tip, then peptides were injected in a nanoHPLC (EesyLC Proxeon, Denmark). Peptides separation occurred onto a 25 cm long column, reverse phase spraying fused silica capillary column (75 μm i.d.) home-made packed with 3μm ReproSil AQ C18 (Dr. Maisch GmbH, Germany). A gradient of eluents A (pure water with 2% v/v ACN, 0.5% v/v acetic acid) and B (ACN with 20% v/v pure water with 0.5% v/v acetic acid) was used to achieve separation, from 20% to 40% of B in 70 minutes, at a constant flow rate of 150 nl/min. The LC system was connected to an LTQ Orbitrap mass spectrometer (ThermoScientific, Bremen, Germany) equipped with a nanoelectrospray ion source (Proxeon Biosystems, Odense, Denmark). Full scan mass spectra were acquired in the LTQ Orbitrap mass spectrometer with the resolution set to 30,000. For accurate mass measurements the lock-mass option was used ([4](#_ENREF_4)). The acquisition mass range for each sample was from m/z 300 to 1750 Da and the analyses were made in duplicates. The ten most intense doubly and triply charged ions were automatically selected and fragmented in the ion trap after accumulation to a ‘target value’ of 10,000. Target ions already selected for the MS/MS were dynamically excluded for 300 s. Identification and quantification of peptides and proteins were performed with MaxQuant 1.3.0.2 against the mouse Uniprot complete proteome set, having identified a protein with at least 2 peptides (1 unique), 6 aminoacids of minimal length, FDR<1% and quantified with at least 2 ratio counts. Significant outliers scores were calculated using Perseus 1.2.0.16 ([5](#_ENREF_5)) and those with a p-value <0.05 have been selected for further analysis. Pathway analysis was performed using the String software, available online (http://string-db.org/). Pathways with the highest probability score and the highest number of hits have been considered further.

**Microarray-based gene expression profiling.** Total RNA extracted using the RNeasy Micro and Mini kit (Qiagen, Chatsworth, CA, USA) was analyzed with Affimetrix Mouse Gene 1.0 ST Array. Quality control of hybridization was performed by Image Quality, MAplots, Boxplot and Density Plot. Array normalization was executed by the RMA algorithm. Divisive clustering algorithms were used to obtain dendrograms, where the biological samples were clustered based on the differentially expressed genes. The hierarchical clustering algorithms employed were: a) distances (euclidian), b) linkage (complete, single, ward, centroid). The differentially expressed genes (DEGs) were obtained based on: a) t-test moderated empirical Bayes, b) p-value (FDR adjusted 0.05), c) cut-off (1 log2 Fold Change, FC).

**Immunohistochemistry and immunofluorescence.** TRAMP and human prostate or LN specimens were embedded in paraffin. 5μM sections were stained with Mayer-Hematoxylin and Eosin (BioOptica, Milan, Italy) and evaluated by an expert pathologist ([1](#_ENREF_1)). Alternatively, after re-hydratation, antigen retrieval in 10mM citric acid and blocking with 5% NGS, slides were incubated with the anti mouse/human TNC or pan-cytokeratin primary antibody (1:200; Abcam, Cambridge, UK) overnight at 4°C. A biotinylated secondary antibody was used 1:200 for 1h at room temperature. Colorimetric revelation was made with Novared chromogen (Vector Labs, Burlingame, CA, USA). Slides were finally contrasted with Mayer-Hematoxylin (BioOptica), mounted with cover glass and examined under microscope (Carl Zeiss, Axioscope 40FL, Varese, Italy). Prostate CSCs were prepared as single cell suspension of 105cells/100μl, spotted on a poly-lysine coated glass slide by cytocentrifuge, fixed with 4% PFA, and permeabilized with PBS containing 0,5% saponin. After blocking with Triton X-100 0.1% and 5% NGS for 1 h at room temperature, cells were incubated with rat anti-mouse/human TNC (Abcam; 1:200), rat anti-mouse STEAP and rabbit anti-mouse PSCA antibodies (Santa Cruz Biotechnology, Heidelberg, Germany: 1:200) for 2h at room temperature, and then with anti rabbit Alexa 488 or anti rat Alexa 546 conjugated secondary antibodies (Invitrogen; 1:200). Nuclei were stained with 0.1 μg/mL DAPI and sections were examined under microscope (Axioscope 40FL).

**Silencing of TNC.** TPIN-SCs were stably infected with TNC shRNA Lentiviral Particles or with control shRNA Lentiviral Particles (Santa Cruz Biotechnology, Inc) at 0,4/4/40 MOI (molteplicity of infection), according to the manifacturer’s protocol. Briefly, 5000 cells/well were plated in a mixture of medium and Polybrene (Santa Cruz Biotechnology, Inc). At day 2 lentiviral particles were added. At day 5 after infection, 2μg/ml of puromycin dihydrochloride (Santa Cruz Biotechnology, Inc) were added to select cells that had integrated the lentiviral particles. Cells were then propagated extensively and cloned.

**Western blot.** Cells were lysed in RIPA buffer (Cell Signaling Technologies). Lysates were run on SDS page and transferred on a PVDC membrane. Membrane was incubated 1h at room temperature with 10% milk diluted in PBS 0.5% Tween, and then with primary anti mouse TNC antibody (1:2000) or anti β-actin antibody (1:5000) overnight at 4°C. After washing, membrane was incubated with HRP conjugated secondary antibody (1:5000) 1h at room temperature. Staining was revealed with ECL solution (GE Healthcare Europe, Milan, Italy) and developed of an X-ray film. Protein expression and TNC/β-actin ratio were measured with ImageJ software.

**Real Time PCR.** Total RNA from prostate or LN was extracted using the RNeasy Micro and Mini kit (Qiagen, Chatsworth, CA, USA). cDNA was obtained from 500ng of RNA using the M-MLV- Reverse Transcriptase kit (Promega, Madison, WI, USA). Real-Time PCR was performed in a total volume of 25μL using the Taqman® Universal PCR Master Mix (Applied Biosystems, Monza, Italy), 5μL of cDNA (prediluted 1:8) and specific probes for CXCL12, TNC or TBP (Applied Biosystems, Italy). Values were normalized to internal control (TBP) and to positive control (a prostate of a TRAMP mouse) using the ΔΔCT method.

**SUPPLEMENTARY REFERENCES**

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