**Supplementary Material and Methods**

**Antibodies and flow cytometry**

The following anti-mouse mAbs were purchased from eBioscience: APC conjugated anti-CD62L (clone: MEL-14, Cat.#: 17-0621-83), PE conjugated anti-Ki-67 (clone: SolA15, Cat.#: 12-5698-80), APC conjugated anti-CD27 (clone: LG.7F9, Cat.#: 17-0271-81), APC conjugated anti-CD8α (clone: 53-6.7 Cat.#: 17-0081-83), eFluor450 conjugated anti-Vα2 TCR(clone: B20.1, Cat.#: 48-5812-80). The following mAbs were purchased from BioLegend: PE conjugated anti-CD44 (clone: IM7, Cat.#: 103008), PE/Cy7 conjugated anti-CD25 (clone: OC61, Cat.# 102016), APC/Cy7 conjugated anti-CD4 (clone: RM4-5, Cat.#: 100526), PE/Cy7 conjugated anti-CD3 (clone: 145-2C11, Cat.#: 100320), Pacific Blue conjugated anti-CD8α (clone: 53-6.7, Cat.#: 100725), FITC conjugated anti-TCRβ (clone: H57-597, Cat.# 109215), APC conjugated anti-CD8α (clone: 53-6.7, Cat.# 17-0081-83), PE/Cy7 conjugated anti-CD45.1 (clone: A20, Cat.#: 2020-01-31), APC/Cy7 conjugated anti-mCD90.2 (clone: 30-H12, Cat#: 105328), PE conjugated anti-CTLA-4 (clone: UC10-4B9, Cat.#: 106305), APC conjugated anti-Tim3 (clone: B8.2C12, Cat.#: 134007), PercP/Cy5.5 conjugated anti-PD1 (clone: RMPI-30, Cat.#: 109120). The following mAb was purchased from BD Bioscience: PE conjugated anti-Vβ 5.1, 5.2 TCR (clone:F23.1 Cat#: BDB553862). Intracellular staining was performed using the BD Cytofix/Cytoperm and Perm/Wash buffers or, for intracellular FoxP3 (APC, clone: FJK-16s; eBioscience Cat.#: 17-5773-82) staining, the eBioscience FoxP3 staining buffer set. For intracellular staining of IFN-γ (PE-labeled, clone: XMG1.2; Biolegend Cat.#: 505808) TNF-α (APC-labeled, clone: MP6-XT22; eBioscience Cat.#: 506308), IL-17 (PercP/Cy5 labeled, clone: eBio17B7 cat.#: 45-7177-82) T cells were incubated for 4 h at 37 °C in ionomycin (750 ng/ml) and PMA (20ng/ml). For the last 3 h, Brefeldin (eBioscience, 1000X Solution) was added to the cultures. For tetramer staining cells were labelled for 2 h at 37 °C with H-2Kb OVA Tetramer SIINFEKL-PE (MBL, iTAg MHC tetramer, Cat. #: T0300). Activated caspases were quantified using Vybrant® FAM Poly Caspases Assay Kit (Invitrogen, Cat.#: V35117), according to the manufacturer’s protocol.

The following anti-human proteins mAbs were purchased from Life Technologies: PE-Texas Red conjugated anti-CD4 (clone: S3.5, Cat.# MHCD0417), QD655 conjugated anti-CD45RA (clone: MEM-56, Cat#. Q10069). The following mAbs were purchased from BioLegend: PE conjugated anti-CCR7 (clone: G043H7, Cat#. 353204), PE/Cy5 conjugated anti-CD25 (clone: BC96, Cat.#: 302608). FITC conjugated anti-CD8 (clone: B9.11, Cat.#: A07756). Samples were acquired on a LSRFortessa (BD Bioscience) flow cytometer. Data were analyzed using FlowJo software (TreeStar) or FACS Diva software (BD Bioscience).

**Real-time quantitative reverse transcription PCR (qRT-PCR)**

Total RNA from FACS sorted cells was precipitated in Trizol (Invitrogen) and reverse transcribed to cDNA using Random hexamers (Roche, Cat.*#*: R 15504) and M-MLV reverse-transcriptase (Invitrogen, Cat.*#*: 28025-013) following manufacturer’s protocol. mRNA samples were treated with 2 U/sample of DNase (Applied Biosystems). Transcripts were quantified by real-time PCR on an ABI PRISM 7700 Sequence Detector with predesigned TaqMan Gene Expression Assays and reagents according to the manufacturer’s instructions (<https://www.lifetechnologies.com>). The following probes were used for mouse cells: *Cdkn1a* (Mm00432448\_m1), *Cdkn1b* (Mm00432448\_m1), *P2rx1* (Mm00435460\_m1), *P2rx2* (Mm01202368\_g1), *P2rx3* (Mm00523699\_m1), *P2rx4* (Mm00501787\_m1), *P2rx5* (Mm00473677\_m1), *P2rx6* (Mm00440591\_m1), *P2rx7* (Mm01199500\_m1), *Trp53* (Mm01731290\_g1), *Gadd45b* (Mm00435123\_m1). The following probes were used for human cells: *CDKN1A* (Hs00355782\_m1), *P2RX7* (Hs00175721\_m1). All reactions were performed in triplicate. The relative amounts of mRNAs were calculated by the ΔCT method. *Hprt* for mouse cells and *TBP* for human cells were used as internal housekeeping genes.

**Collections of cancer transcriptomes**

Bulk RNA-seq data of purified populations of CD8+ T cells isolated from primary lung tumors were derived from ref. 26. Transcriptional data were downloaded from the series matrix file of GSE90728 and used as are. The status of CD103 (ITGAE) gene in NSCLC CD8+ TILs was derived from table S1 of ref. 26. The expression level of the *P2rx7-/-* up-regulation signature (FDR≤5% and fold change≥1.5; see table S2) has been calculated as the standardized average expression of all *P2rx7-/-* up-regulated genes in CD103lowCD39-, CD103intermediate, and CD103highCD39+ TIL subgroups.

To identify groups of tumors with either high or low *P2rx7-/-* up-regulation signature, we used the classifier described in Adorno et al. (1), that is a classification rule based on a signature score obtained summarizing the standardized expression levels of signature genes into a combined score with zero mean. Tumors were classified as *P2rx7-/-* up-regulation signature “low” if the combined score was negative and as *P2rx7-/-* up-regulation signature “high” if the combined score was positive. This classification was applied to expression values of a non-small-cell lung cancer (NSCLC) compendium and of the TCGA skin cutaneous melanoma (SKCM) dataset. The NSCLC compendium has been created from 7 major datasets comprising microarray data of lung cancer samples annotated with clinical outcome. All data were measured on Affymetrix arrays and have been downloaded from NCBI Gene Expression Omnibus GSE3141, GSE10245, GSE14814, GSE19188, GSE31210, and GSE68465 and from the Ladanyi and Gerald Laboratories Lung Adenocarcinoma microarray repository (<http://cbio.mskcc.org/public/lung_array_data/>). Prior to analysis, we eliminated duplicate samples and renamed all original sets after the medical center where patients were recruited. This re-organization returned 1,136 unique samples from 10 independent cohorts comprising 989 adenocarcinomas (778 of which with complete clinical outcome information) and 147 squamous cell carcinomas. The type and content of clinical and pathological annotations of the compendium samples have been derived from the original cohorts. Since raw data (.CEL files) were available for all samples, the integration, normalization and summarization of gene expression signals has been obtained applying the procedure described in Rustighi et al (2). Briefly, expression values were generated from intensity signals using a custom CDF obtained merging HG-U133A, HG-U133A2 and HG-U133 Plus2 original CDFs and transforming the original CEL files accordingly. Intensity values for a total of 21,995 probe sets have been background-adjusted, normalized using quantile normalization, and gene expression levels calculated using median polish summarization (multi-array average procedure, RMA). Clinical information among the various datasets has been standardized as described in Cordenonsi et al (3). Gene expression data (raw counts) and clinical information of the TCGA skin cutaneous melanoma (SKCM) dataset were downloaded from the Genomic Data Commons Portal using functions of the *TCGAbiolinks* R package. Raw counts were normalized and gene expression levels quantified as counts per million (cpm) using functions of the *edgeR* R package. To evaluate the prognostic value of the *P2rx7-/-* up-regulation signature, we estimated, using the Kaplan– Meier method, the probability that patients would remain metastasis-free or alive. To confirm these findings, the Kaplan–Meier curves were compared using the log-rank (Mantel–Cox) test. P-values were calculated according to the standard normal asymptotic distribution. Survival analysis was performed in GraphPad Prism.

***In vitro* monitoring of cell survival and proliferation**

T naïve or TEM cells were grown in a 96 flat-bottom well plate in RPM-1640 supplemented with 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin/streptomycin and 100 U/mL kanamycin and maintained in 5% CO2 at 37°C. Cells were labeled with CellTrace violet (Thermo Fisher, Cat.*#*: C34557) and stimulated by plate-bound anti-CD3ε mAb (2µg/mL) with co-immobilized anti-CD28 mAb (2µg/mL) (eBioscience); for stimulation with cytokines, cells were cultured with IL-2 at 50 U/mL or IL-7 at 10 ng/ml. CellTrace dilution was measured in viable cells through the exclusion of dead and apoptotic cells by electronically gating PI and Annexin V negative cells. FACS acquisitions were standardized by fixed numbers of calibration beads (BD Biosciences). Half-life and cell cycling activity was calculated as previously described (4). In brief, purified TEM cells were stained with CFSE, stimulated with CD3/CD28 antibodies and monitored for proliferation in flow cytometry every 8 hours. Calibrating beads were added before cell harvest to determine cell number. Dead cells were excluded from the analysis by PI and Annexin V staining. The following formula was applied to calculate the number of live cells in each division class: total number of cells (within division peak) = total number of cells × fraction of total cells in division. A table of precursor cohort numbers was then created by dividing the total number of cells in the *i*th division by 2*i*, where *i* is the division number. For each time point a normal distribution was fitted to the precursor cohort plots to determine the mean division number at each harvest time. Division time was calculated by plotting the means of precursor cohorts against harvest time and a linear regression analysis was performed. The extrapolation to the intercept gives the mean time taken for lymphocytes to enter each division. To estimate the half-life of cells, total live cell number was plotted against the time of harvest and an exponential function fitted. The format of the equation used is *y* = e−*kx*, where *k* is the exponential decay constant. The half-life of cells that die before first division was then calculated by using the following formula: half-life = (ln(2))/*k* (4).

**Quantification of β-galactosidase and mitochondria-associated ROS**

To measure cellular senescence, purified TEM cells were stimulated for 3 days with anti-CD3 and CD28 mAbs in the presence or absence of 70 µM BzATP. The following protocol was used also for OT-I cells were collected from tumors. Cells were incubated for 2 h with 100 nM bafilomycin A1 to inhibit lysosomal β-galactosidase before adding the β-galactosidase substrate C12FDG (Thermo Fisher). Relative β-galactosidase activity was assessed as fluorescence emission at FACS (with 488 nm laser). Mitochondria-associated ROS levels were measured by staining cells with MitoSOX red (Molecular Probes/Invitrogen) at 5 µM for 40 min at 37ºC. Cells were then washed and resuspended in PBS for FACS analysis (with laser for PE).

**Western blotting**

For western blot analysis TEM cells were washed with ice cold PBS and lysed with RIPA buffer 1x (Sigma) or with Urea 9 M to detect nuclear protein, both supplemented with phosphatase inhibitor cocktail (Sigma-Aldrich) and protease inhibitor cocktail (Roche). Samples were centrifuged at 14.000 rpm for 10 min at 4°C and snap frozen. Cleared protein lysate was denatured with loading buffer supplemented with DTT 0.1 μM for 10 min at 65°C. Samples were run on precast 4-12% bis-tris protein gels (BioRad) and then transferred onto PVDF membranes using Trans-Blot Turbo Transfer System (BioRad). Membranes were blocked with 10% (wt/vol) nonfat dry milk (Bio-Rad) and 0.1% Tween-20 in TBS and incubated with appropriate antibodies in TBS with 0.1% Tween-20 for 16 h at 4 °C. The following antibodies were purchased from Cell Signaling Technology: anti-p38 MAPK (rabbit, Cat. #9212), anti-phospho p38MAPK (T180/Y182) (rabbit, clone: D3F9, Cat. #4511), anti-phospho H2A.X (S139) (rabbit, clone: 20E3, Cat. #9718), anti-p21 (rabbit, clone 12D1 Cat.#2947) and anti-Vinculin (rabbit, clone:E1E9V Cat.#:13901). Anti-GAPDH antibody (mouse, clone: 6C5 Cat. #MAB374) was purchased from Millipore. All incubations with primary antibody were followed by incubation with secondary HRP-conjugated anti-rabbit (Cat. #7074, Cell Signaling Technology), or anti-mouse (Cat. #7076, Cell Signaling Technology) IgG antibodies in TBS with 0.1% Tween-20. Membranes were developed using the Pierce ECL Western blotting substrate (Thermo Scientific, Cat.# 32209), signals were detected with the ImageQuant LAS 4000 system in the standard acquisition mode (GE Healthcare Life Sciences), and bands were quantified using the Multi Gauge Analysis tool (Fujifilm).

**Identification of human SNP variants, TEM cells isolation and transfection**

The Progressione della Lesione Intimale Carotidea (PLIC) Study (a sub-study of the CHECK study) is a large survey of the general population of the northern area of Milan (n= 2.606) (5,6), followed at the Center for the Study of Atherosclerosis, Bassini Hospital (Cinisello Balsamo, Milan, Italy). The Study was approved by the Scientific Committee of the Università degli Studi di Milano (“Cholesterol and Health: Education, Control and Knowledge – Studio CHECK (SEFAP/Pr.0003) – reference number Fa-04-Feb-01) in February 4th 2001. An informed consent was obtained by subjects in accordance with the Declaration of Helsinki. Within the PLIC Cohort, 2,274 subjects were genotyped for two variants in the *P2RX7* locus: a) the rs11065464, g.36458C>A intron variant, associated with loss-of-function P2X7 pore forming (7) and the rs1718119 Ala228Thr missense variant, determining increase in receptor activity (7). Both variants were not in linkage disequilibrium and their respective minor allele frequencies were in accordance to the Hardy-Weinberg equilibrium. For the first variant, out of 2,274, we found 1,183 homozygous wild-type (CC), 907 heterozygous (CA) and 184 mutated form (AA) homozygous carriers. For the missense variant, out of 2,274, we found 1,008 homozygous wild-type (CC), 1,011 heterozygous (CA) and 255 mutated form (AA) homozygous carriers. Genomic DNA was extracted using Flexigene DNA kit (Qiagen, Milan ,Italy) and the genotyping was performed by quantitative real time PCR as previously described (8).

The permission to perform the experiments is authorized according to the Swiss law by the Federal Office for the Environment (FOEN) under the notification number A080059. Peripheral blood mononuclear cells (PBMCs) were isolated from blood by density-gradient centrifugation using Ficoll-Paque™ Plus gradient (GE-Healthcare). CD4+ and CD8+ T cells were enriched using human anti-CD4 or anti-FITC MicroBeads (Miltenyi Biotec) and then CD4+ and CD8+ TEM cells were sorted with a FACSAria (BD) as CD4+/-, CD8+/-, CCR7-, CD45RA- and CD25-. To knock-down *P2RX7* gene, TEM cells were electroporated with Neon transfection kit and device (Invitrogen). A total of 5x105 TEM cells were washed three times with PBS and resuspended in 10 μL of Buffer T (Neon kit, Invitrogen). 40 pmol of *P2RX7* Silencer Select Pre-designed siRNA (Ambion) or, as a control, siGLO Green Transfection Indicator (Dharmacon) were added to the cells to a final volume of 11 μL and mixed. 10 μL of the suspension was electroporated with Neon electroporation Device (Invitrogen; 2150 V, 30 ms, 1 pulse). Electroporated cells were then transferred in RPMI-1640 supplemented with 10% FBS and without antibiotics for 24 hours. For analysis of cell proliferation, electroporated TEM cells were labelled with CellTrace violet (Thermo Fisher, Cat.*#*: C34557) and plated on 96 well NUNC immunoplate coated with anti-CD3 (5 μg) and anti-CD28 (1 μg) antibodies for 5 days.

**References**

1. Adorno M, Cordenonsi M, Montagner M, Dupont S, Wong C, Hann B*, et al.* A Mutant-p53/Smad complex opposes p63 to empower TGFbeta-induced metastasis. Cell **2009**;137:87-98

2. Rustighi A, Zannini A, Tiberi L, Sommaggio R, Piazza S, Sorrentino G*, et al.* Prolyl-isomerase Pin1 controls normal and cancer stem cells of the breast. EMBO Mol Med **2014**;6:99-119

3. Cordenonsi M, Zanconato F, Azzolin L, Forcato M, Rosato A, Frasson C*, et al.* The Hippo transducer TAZ confers cancer stem cell-related traits on breast cancer cells. Cell **2011**;147:759-72

4. Hawkins ED, Hommel M, Turner ML, Battye FL, Markham JF, Hodgkin PD. Measuring lymphocyte proliferation, survival and differentiation using CFSE time-series data. Nat Protoc **2007**;2:2057-67

5. Baragetti A, Norata GD, Sarcina C, Rastelli F, Grigore L, Garlaschelli K*, et al.* High density lipoprotein cholesterol levels are an independent predictor of the progression of chronic kidney disease. J Intern Med **2013**;274:252-62

6. Norata GD, Garlaschelli K, Ongari M, Raselli S, Grigore L, Catapano AL. Effects of fractalkine receptor variants on common carotid artery intima-media thickness. Stroke **2006**;37:1558-61

7. Todd JN, Poon W, Lyssenko V, Groop L, Nichols B, Wilmot M*, et al.* Variation in glucose homeostasis traits associated with P2RX7 polymorphisms in mice and humans. J Clin Endocrinol Metab **2015**;100:E688-96

8. Baragetti A, Palmen J, Garlaschelli K, Grigore L, Pellegatta F, Tragni E*, et al.* Telomere shortening over 6 years is associated with increased subclinical carotid vascular damage and worse cardiovascular prognosis in the general population. J Intern Med **2015**;277:478-87