TENT5C/FAM46C modulation *in vivo* reveals a trade-off between antibody secretion and tumor growth in multiple myeloma

Authors

Massimo Resnati,[1] Sara Pennacchio,[1] Lisa Viviani,[1] Tommaso Perini,[1,2] Maria Materozzi,[1] Ugo Orfanelli,[1] Jessica Bordini,[3] Raffaella Molteni,[1] Mario Nuvolone,[4,5] Matteo Da Vià,[6] Francesca Lazzaroni,[6] Niccolò Bolli,[6,7] Simone Cenci[1,8] and Enrico Milan[1,8]

[1]Age Related Diseases Unit, Division of Genetics and Cell Biology, IRCCS San Raffaele Scientific Institute, Milano; [2]Hematology and Bone Marrow Transplantation Unit, IRCCS San Raffaele Scientific Institute, Milano; [3]B-cell Neoplasia Unit, Division of Experimental Oncology, IRCCS San Raffaele Scientific Institute, Milano; [4]Department of Molecular Medicine, University of Pavia, Pavia; [5]Amyloidosis Research and Treatment Center, Fondazione IRCCS Policlinico San Matteo, Pavia; [6]Hematology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano; [7]Università degli Studi di Milano, Milano and [8]University Vita-Salute San Raffaele, Milano, Italy

Correspondence:
E. MILAN - milan.enrico@hsr.it

https://doi.org/10.3324/haematol.2023.284299

SUPPLEMENTARY TABLE

Antibody		
mouse anti-β-Actin	Sigma-Aldrich	A5441
rabbit anti-Calreticulin	GeneTex	GTX111627
rabbit anti Calnexin	Sigma-Aldrich	C4731
rabbit anti-ERGIC53	Sigma-Aldrich	E1031
rabbit anti-CD68	Cell Signaling	97778
rabbit anti-lambda light chains	Dako	A0194
rabbit anti-H3	Abcam	ab1791
rabbit anti-PDI	kindly provided by Dr. Ineke Braakman Lab	Utrecht, NL
rabbit anti-PRDX4	AbFrontier	PA0009
rabbit anti-PRDX4	Proteintech	10703-1-AP
rabbit anti-TENT5C	Proteintech	25038-1-AP
Alexa Fluor 546 goat anti-mouse IgG	Life Technologies	A11030
Alexa Fluor 546 goat anti-rabbit IgG	Life Technologies	A11010
Alexa Fluor 647 goat anti-rabbit IgG	Life Technologies	A21245
Alexa Fluor 647 goat anti-mouse IgG	Life Technologies	A21236
Anti-rabbit IgG, HRP-linked	Southern Biotech	4050-05
Anti-mouse IgG, HRP-linked	Southern Biotech	1031-05
PE mouse IgG1 k isotype control	Biolegend	400112
PE mouse IgG1 anti-human CD38	Biolegend	303506
PE mouse IgG1 anti-calreticulin clone FMC75	Abcam	Ab83220
Goat anti-Human IgG (H+L) Secondary Antibody, HRP	Thermo Scientific	A18805
Primers	**FW**	**REV**
hCNX/CANX	GTAGCCCTTCCTGTGTTCCT	TGACAGTGCCACCATCTTCT
hERGIC53/LMAN1	TCCGCAACAAACCCTATCCT	CGGCTCTTTTCCAGGTTCAG
hGAPDH	CCACATCGCTCAGACACCAT	GTGACCAGGCGCCCAATAC
hH3	GTGAAGAAACCTCATCGTTACAGGCCTGGT	CTGCAAAGCACCGATAGCTGCGCTCTGGAA
hHSPA5/BiP	TAGCGTATGGTGCTGCTGTC	TGACACCTCCCACAGTTTCA
hIGLV6-57	CTGGGCTCCACTACTTCTCACC	ATGGAGCCAGAGAACCGATCA

hMZB1	AAATCTGGCAAAGGCAGAGA	CTAAGTCCTGGGCCTGTGAG
hPDI/P4HB	TCACATCCTGCTGTTCTTGC	GTCGCTGTCGATGAAGATGA
hPRDX4	AACAGCTGTGATCGATGGAG	TCAAGTCTGTCGCCAAAAGC
hTENT5C/FAM46C	CAGGCATCAAAGTGCACGAC	AGCTTGTTCACACCCTCTGG
mCd206/Mrc1	ATGCCAAGTGGGAAAATCTG	TGTAGCAGTGGCCTGCATAG
mCd31/Pecam1	AGGGGACCAGCTGCACATTAGG	AGGCCGCTTCTCTTGACCACT
mCd45/Ptprc	GGAGACCAGGAAGTCTGTGC	GTTCTGGGCTCCTTCCTCTT
mCd68	CTGACAAGGGACACTTCGGG	AGGCCAATGATGAGAGGCAG
mH3	GTGAAGAAACCTCATCGTTACAGGCCTGGT	CTGCAAAGCACCAATAGCTGCACTCTGGAA
mTent5c	TCACCTCCTCTTCCAACGCC	AGGTTGGAAAGTTGCCTCGC

Table S1: List of antibodies and primers used.

SUPPLEMENTARY FIGURES:

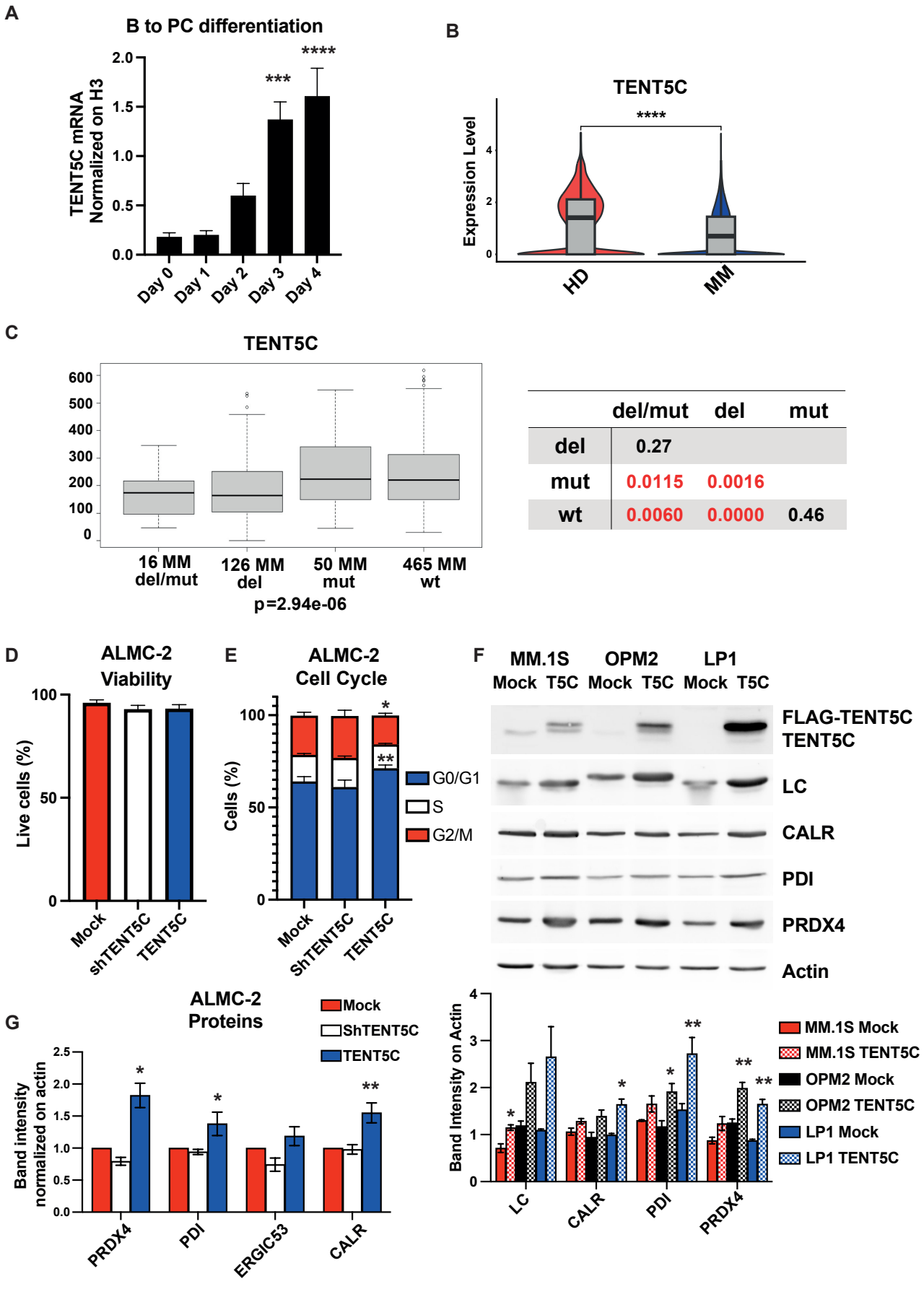

Figure S1 associated to Figure 1. (A) qRT-PCR analysis of TENT5C mRNA levels in LPS-activated murine splenic B cells, (mean ± SEM normalized on H3 mRNA; n=6; ***p < 0.001; ****p < 0.0001; Ordinary One-way ANOVA with Dunnett's multiple comparison versus Day 0). Primary murine PCs were obtained by LPS-stimulation of purified splenic B cells from C57BL/6 wild-type mice (Charles River), as previously described in Pengo et al., Nat Immunol, 2013. **(B)** TENT5C expression levels in 6 healthy donors (5 derived from the public dataset GSE230705, Duan et al., Cell Reports, 2023) and 18 newly diagnosed multiple myeloma (MM) patients were measured by single cell RNA sequencing and have been integrated in a single Seurat object. Primary bone marrow samples were collected at the Hematology Department of Fondazione IRCCS Cà Granda, Ospedale Maggiore Policlinico-Milano (Italy). This study was approved by the local Ethics Committee (949/21), and all patients signed written consent in accordance with the Declaration of Helsinki. Briefly, purified PCs were counted and loaded into the Chromium Single Cell Chip G (PN-1000120) to yield a recovery of 9,000 single-cell transcriptomes. Subsequently, the libraries were pooled and sequenced on a NovaSeq6000 platform (Illumina) with a 150bp Paired End protocol. Raw sequences were preprocessed using Cell Ranger (version 5.0.0) pipelines using GRCh38 human reference transcriptome. Batch effect was corrected by Harmony (github.com/immunogenomics/harmony, v1.0) using "place" state as the variable on which compute the correction. Vlnplot was generated using "vlnplot" fuction by Seurat. Wilcoxon test was applied throught "stat_compare_means" function: ****p < 0.0001. (**C**) Left, box plot of TENT5C expression level in 660 multiple myeloma cases freely accessible from MMRF CoMMpass Study (https://research.themmrf.org/) retrieved from the Interim Analysis 15a. Transcript per Million (TPM) reads values of the TENT5C transcript were retrieved using Salmon gene expression quantification data (MMRF_CoMMpass_IA15a_E74GTF_Salmon_V7.2_Filtered_Gene_TPM). Non-synonymous (NS) somatic mutation variants and counts data were obtained from whole exome sequencing (WES) analyses, and Copy Number Alteration (CNA) data were retrieved by means of NGS-based FISH (Miller et al., Blood, 2016). The presence of a specific CNA was considered when occurring in at least one of the investigated cytoband at a 20 percent cut-off for each considered chromosomal aberration (Todoerti et al., Haematologica. 2021). Patients were stratified based on the presence of both TENT5C deletions and mutations (16 MM del/mut); presence of TENT5C deletions (126 MM del); presence of TENT5C mutations (50 MM mut); absence of TENT5C deletions or mutations (465 MM wt). Right, the significant pairwise comparisons performed by the Dunn's test are marked red-bold in the table. (**D**) Equal numbers of mock, TENT5C-silenced or overexpressing ALMC-2 cells were seeded and counted with trypan blue staining after 2 days, (mean ± SEM; n=6). (**E**) Cytofluorimetric analysis of cell cycle phase in

control, TENT5C-silenced and overexpressing ALMC-2 cells. After fixation in 70% ethanol, ALMC-2 cells were stained with propidium iodide (50µg/mL PI, 10µg/mL RNase in PBS) and analyzed with a BD FACSCanto II (mean ± SEM; n=6; *p < 0.05; **p < 0.01; Repeated measure one-way ANOVA with Dunnett's multiple comparison versus Mock). (**F**) Immunoblots of selected endoplasmic reticulum (ER)-resident proteins and IgG λ in TENT5C control or overexpressing MM cells. Top, representative images; bottom, quantification of band intensities of 3 independent experiments normalized on actin (mean ± SEM; *p < 0.05; **p < 0.01; unpaired t test. (**G)** Quantification of band intensities of at least 5 independent experiments normalized on actin represented in **Figure 1E** (mean ± SEM; *p < 0.05; **p < 0.01; Kruskal-Wallis one-way test with Dunn's multiple comparison versus Mock).

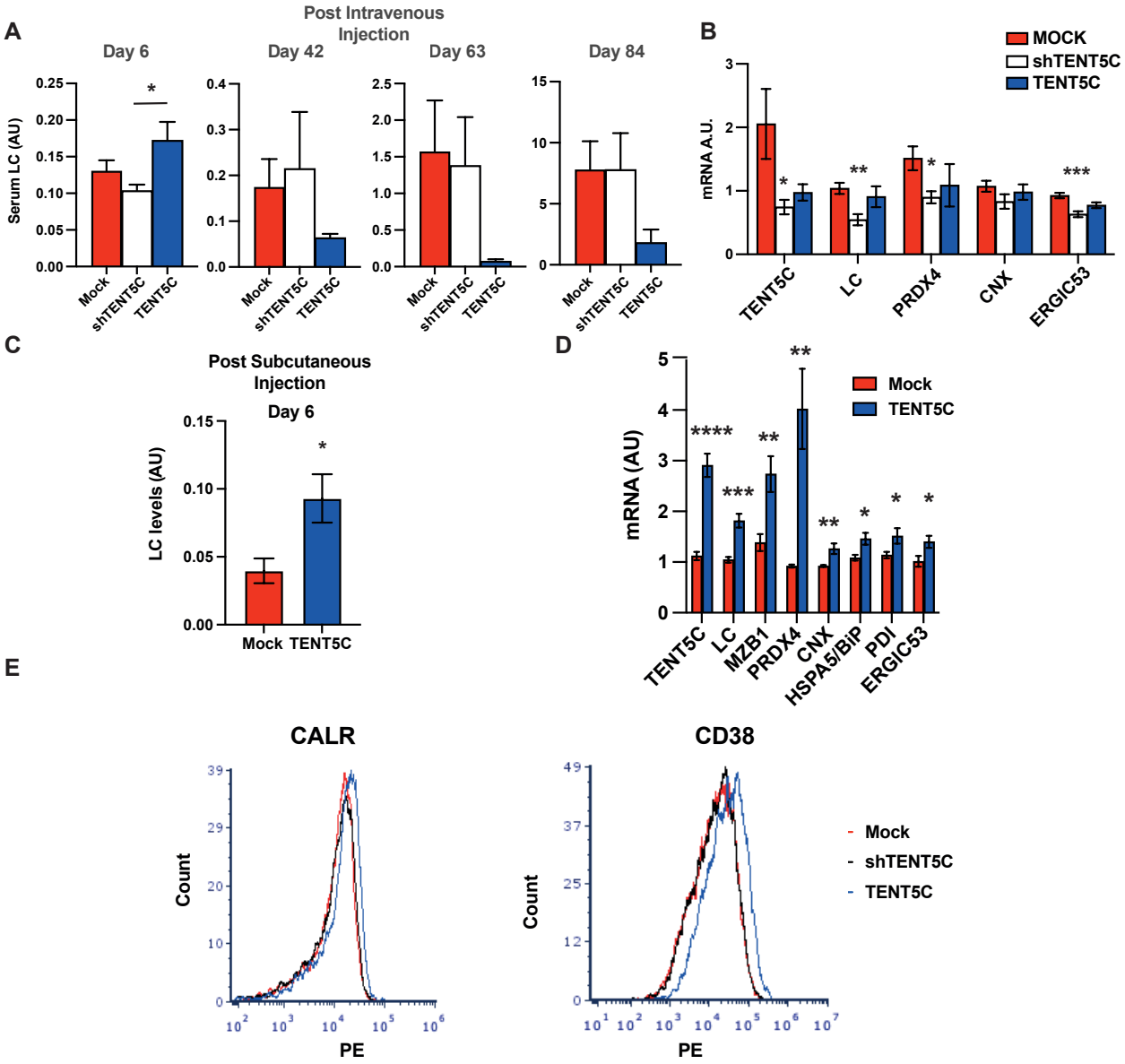

Figure S2 associated to Figure 2 and 3. (A) Quantifications of immunoblot analyses for the Ig λ LC in 1µl of serum collected at the indicated timepoints from mice intravenously injected with control, TENT5C-silenced or overexpressing ALMC-2 cells, (mean ± SEM; n=8 per group; *p < 0.05; Ordinary one-way ANOVA). **(B)** qRT-PCR analysis of TENT5C, IGLV6-57 and selected ER-targeted mRNAs in excised plasmacytomas (mean ± SEM normalized on GAPDH mRNA; n=6 Mock, 7 shTENT5C and 4 TENT5C; *p < 0.05; **p < 0.01; ***p < 0.001; Ordinary One-way ANOVA with Dunnett's multiple comparison versus Mock). Total RNA was extracted from tumor biopsies by homogenization in TriFAST (Euroclone, EMR507100). 1000 ng of RNA were retro-transcribed with PrimeScriptTM RT reagent Kit with gDNA Eraser (Takara, RR047A). qPCRs were performed using iTaq SYBR Green Supermix (Bio-Rad, 1725122) on Bio-Rad CFX96 PCR. **(C)** Quantifications of immunoblot analyses for the Ig λ LC in 1µl of serum collected from the retro-orbital vein at the indicated timepoint from mice subcutaneously injected with control or

TENT5C-overexpressing ALMC-2 cells (mean ± SEM; n=7 per group, *p < 0.05; unpaired t test). (**D**) qRT-PCR analysis of IGLV6-57 and selected ER-targeted mRNAs in excised subcutaneous tumors (mean ± SEM normalized on GAPDH mRNA; n=7 Mock, 6 TENT5C; *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; unpaired t test). (**E)** Representative cytofluorimetry histograms of CALR and CD38 surface expression in control, TENT5C-overexpressing and silenced ALMC-2 cells.