

Decentralized Immunity: intercellular telomere transfer extends T cell lifespan

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The common view is that T-lymphocytes activate telomerase, a DNA polymerase that extends telomeres at chromosome ends, to delay

senescence. We show that independently of telomerase, T cells elongate telomeres by acquiring telomere vesicles from antigen-presenting cells (APCs). Upon contact with T cells, APCs degraded shelterin to donate telomeres, which were cleaved by the telomere trimming factor TZAP, and then transferred in extracellular vesicles at the immunological synapse. Telomere vesicles retained the Rad51 recombination factor that enabled telomere fusion with T cell chromosome ends lengthening them by an average of ~3000 base pairs. This previously unknown telomere transfer program supports T cell lifespan, representing a new form of ‘decentralized immunity’ whereby APCs distribute ‘telomere tokens’ needed for generation of stem-like memory T cells that provide long lasting protection against infections, and possibly, cancer.

Telomeres are TTAGGG repeats that protect chromosome ends and promote cellular lifespan¹. In cells with short telomeres (<4kb), proliferative activity ceases and replicative senescence occurs rather rapidly². Telomere shortening is observed in age-related pathologies, providing a common mechanism linking senescence, cancer and ageing³. Although cells prevent telomere shortening by telomerase-dependent and independent pathways^{4,5}, it is unknown whether telomeres can be transferred between cells as part of a telomere maintenance program.

We found that naïve T cells elongate their telomeres by acquiring telomeres in extracellular vesicles (EVs) from APCs. Telomere acquiring T cells become stem-like long lived memory cells while other T cells progress towards senescence.

This is a new form of decentralized immunity whereby APCs distribute telomeres to T cells as 'longevity tokens', that enable long-lasting immunity.

Discovery of telomere transfer.

While studying conjugates of primary human CD27⁺ CD28⁺ CD3⁺ CD4⁺ T cells and autologous APCs, we noticed T cell telomere elongation by up to 3 kb and a concurrent telomere shortening in APCs after synapse formation (**Fig. 1a**). Fluorescence *in-situ* hybridization (FISH) and quantitative polymerase chain reaction (qPCR) generated similar results (**Extended Data Fig. 1a-b**). Telomere lengthening was antigen dependent.

T cells deficient in telomerase catalytic subunit (TERT-KO T cells; **Extended Data Fig. 1c-d**) did not require telomerase to elongate telomeres after exposure to APCs (**Extended Data Fig. 1e**). Telomere extension was also not a result of alternative-lengthening of telomeres (ALT)⁵, a DNA break-induced recombination pathway that extends telomeres independently of telomerase, because it occurred in the absence of telomeric DNA synthesis (**Extended Data Fig. 1f-g**).

We postulated that APCs donate telomeres to T cells. Indeed, APCs presented their clustered telomeres in ~70% antigen-specific conjugates (**Fig. 1b**). No telomere clusters were detected if APCs were not coupled with T cells. Clusters of molecules at the synapse often precede their transfer into the adjacent cell⁶. In fact, using APCs with live telomere probe incorporation⁷ (**Extended Data Fig. 2**), we visualized telomere clusters exiting APC nuclei and accumulating at the

synapse with T cells (**Extended Data Fig. 3a**). These data show that APC telomeres are poised for cross-synaptic transfer into T cells.

We confirmed that APCs release telomeric DNA upon antigen-specific contacts with T cells (**Fig. 1c**), protected by a vesicle membrane (**Fig. 1d**). We also excluded that isolated T cells and APCs could spontaneously release telomeres (**Extended Data Fig. 3b**). Telomeric DNA in vesicles was insensitive to non-telomeric enzymatic digestion (**Fig. 1e**). Only trace levels of other forms of DNA, such as Alu repeats, that did not increase upon antigen challenge were detected (**Extended Data Fig. 3b**).

Using the calcium ionophore ionomycin which stimulates vesicle release⁸, and telomere lipid PKH67 dye labelling, we obviated the need of T cells for APC stimulation, and successfully visualised telomere vesicles within ~10% of the total APC single particle vesicle fraction by fluorescence activated vesicle sorting (FAVS)⁹ (**Extended Data Fig. 3c-d**). qPCR confirmed that purified telomere vesicles contained telomeric DNA (**Extended Data Fig. 3e**). Vesicle free telomeres were also found but in minor amounts (~1%; **Extended Data Fig. 3f**). Dot blot analysis further showed that telomeric DNA is mainly present in the APC vesicle fraction as double-stranded DNA (**Extended Data Fig. 3g**).

We performed super-resolution microscopy and observed that membrane lipids and telomeric DNA were co-localized in purified telomere vesicles released by APCs (**Extended Data Fig. 3h**). We also detected telomeric DNA within vesicles using transmission electron microscopy (**Fig. 1f**) and confirmed that most

telomere vesicles were 30-100nm, with a minor vesicle subset (~5%) between 100-300 nm (**Extended Data Fig. 3i**). Similar observations were made by field emission scanning electron microscopy (**Fig. 1g**).

Using EdU⁺ telomere vesicles derived from APCs, we discovered that ~8% of T cell chromosome ends contained EdU labelled telomeric DNA (**Fig. 1h and Extended Data Fig. 3j**). Similar results were obtained using fluorescently labelled telomere vesicles such that only APC, but not endogenous T cell, telomeres could be detected (**Extended Data Fig. 4a**). Additionally, telomere vesicles on T cell chromosomes were destroyed upon application of T7 endonuclease that cleaves sites of DNA integration¹⁰ (**Extended Data Fig. 4b**), were not immobilized on the T cell plasmalemma (**Extended Data Fig. 4c**), and co-immunoprecipitated with the telomere protein POT1 in the T cells (**Extended Data Fig. 4d**).

Mechanism of telomere vesicle release and recombination.

Notably, APCs, activated on planar bilayers constructed with antibodies to CD3 and the integrin-ligand ICAM-1 onto which T cells secrete the antigen receptor⁶ (**Fig. 2a**), spontaneously released telomeres (**Fig. 2b**). The released telomeres colocalized with APC CD63 (**Fig. 2b**), suggesting vesicular nature, and were released directly on the bilayer (**Extended Data Fig. 5a**). This was confirmed using telomere probes and PKH67 lipid dye (**Fig. 2c and Extended Data Fig. 5b**), and isotype antibodies (**Extended Data Fig. 5c**). Telomere vesicles were

not released on bilayers prepared without anti-CD3 or with antigen-free APCs (**Fig. 2d**). These data highlight the role of antigen-specific contacts via the TCR-MHC-II complex to trigger telomere transfer, likely promoting calcium flux in APCs, such that pre-treating APCs with a Syk inhibitor that blocks calcium signalling⁶ is sufficient to blunt telomere release on bilayer (**Fig. 2d**). However, MHC signalling was not required for telomere fusion after vesicle transfer (**Extended Data Fig. 5d**).

When using FAVS, we found that while resting APCs spontaneously release little or no telomere vesicles, calcium (ionomycin)-activated APCs release ~10,000 telomere vesicles per 10⁶ cells (**Fig. 3a, 3e**) with no evidence of cell death (**Extended Data Fig. 6a**), blebbing (**Extended Data Fig. 6b**), nor need for T cells or their receptor on the bilayers. APCs also did not die upon antigen-specific contact with T cells (**Extended Data Fig. 6c**). An APC subset sorting strategy revealed that myeloid APCs (DCs and monocytes) released the highest levels of telomere vesicles while B cells released a lower amount (**Extended Data Fig. 6d-e**).

Telomere vesicles contained exosome (CD63, TSG101), microvesicle (C1q), histocompatibility antigen (MHC II) and telomere proteins (TZAP) (**Extended Data Fig. 7a-b**). Ionomycin-induced calcium activation increased both TZAP expression and telomere-trimming activity in APCs (**Extended Data Fig. 7c, d**). That TZAP is required for telomere vesicle release was confirmed by siRNA depletion (**Extended Data Fig. 7e-f**). TZAP was also the molecule transferred at

the synapse between T cells and TZAP-over-expressing APCs (**Extended Data Fig. 7g**).

Shelterin is a telomere-binding complex that protects telomeres from the DNA damage response, ensuring telomere stability. We found that APCs, ionomycin activated or in synapse with T cells, down-regulated expression of both telomere-binding and shelterin-assembly factors, POT1 and TRF2^{11,12} consistent with TZAP cleavage at telomeres with reduced shelterin amounts¹³ (**Fig. 3b,c; Extended Data Fig. 8a**). Shelterin down-regulation was prevented by treating APCs with the proteasome inhibitor MG-132 prior to synapse formation (**Extended Data Fig. 8a**), suggesting that shelterin undergoes calcium-dependent proteasomal degradation¹⁴. Telomere-shelterin co-localization was not absolute (**Fig. 3b**), suggesting some telomeres in primary human APCs spontaneously lose shelterin due to prior episodes of telomere transfer *in vivo* and/or baseline activation (e.g. without ionomycin) by the culture conditions *per se*. In fact, we found that telomere vesicles did not contain TRF2 or POT1 (**Fig. 3d**). Shelterin expression by POT1/TRF2 transduction in ionomycin-activated APCs (**Extended Data Fig. 8b**) suppressed telomere vesicle release, as shown by both FAVS (**Fig. 3a, e**) and dot blot analysis (**Fig. 3f**). Global demolition of shelterin by combined siRNA to POT1 and TRF2¹² (**Extended Data Fig. 8c**) resulted in opposite results (**Fig. 3a, e and Extended Data Fig. 8d**) with no need for ionomycin activation. Thus, shelterin proteins prevent telomere transfer and once these proteins are degraded, TZAP can lead to telomere excision and release in EVs.

1 Telomere vesicles retained Rad51, the homologous recombination factor
2 involved in telomere elongation¹⁵ (**Fig. 3g**), as well other DNA damage factors
3 (γ H2Ax and BRCA2; **Extended Data Fig. 9a**). We silenced Rad51 with siRNA in
4 APCs and found that telomere vesicles depleted of Rad51 (**Extended Data Fig.**
5 **9b**) retained vesicle size (**Extended Data Fig. 9c**), key telomere vesicle
6 molecules (CD63, MHC-II, TZAP; **Extended Data Fig. 9d**) and vesicle number
7 (**Fig. 3a**) but had defective recombinogenic potential (lack of BRCA2 and Rad51
8 and shorter single-strand DNA, likely corresponding to telomere overhangs
9 needed for recombination-based telomere extension¹⁵; **Extended Data Fig. 9d-**
10 **e**). Transferring red APC telomeres into green T cell telomere nuclei, we found
11 that siRad51 telomere vesicles were no longer co-localised with T cell telomeres,
12 suggesting defective recombination in the absence of Rad51 (**Fig. 3h**). The
13 siRad51 vesicle generated T cell chromosomes were indeed ~1.2kb shorter than
14 siCtrl generated (**Extended Data Fig. 9f**), despite comparable telomere length
15 between siRad51 and siCtrl telomere vesicles (**Extended Data Fig. 9g**). qPCR
16 confirmed that the fusion between APC and T cell telomeres requires Rad51
17 present in the telomere vesicles (**Extended Data Fig. 9h**).

18 **Generation of long-lasting immunity by telomere transfer.**

19 Human T cells activated in the presence of autologous, heterologous, or even
20 mouse telomere vesicles expanded ~3 fold, over 30-40 days, compared to cells
21 left without any vesicles or stimulated with telomere depleted vesicles (**Fig. 4a**
22 **and Extended Data Fig.10a**). The telomere vesicle enhanced expansion was
23 greater than that produced by CRISPR-mediated telomerase activation
24 (**Extended Data Fig.10b-c**). That was not observed in T cells exposed to
25 telomere vesicles depleted of Rad51 (**Fig. 4a**). Telomere vesicles also supported

proliferative expansion of TERT-KO T cells (**Extended Data Fig.10d**), and reduced the burden of ultra-short telomeres (**Extended Data Fig. 10e**). Vesicle-free telomeres were ineffective. Inhibiting TZAP in APCs with siRNA resulted in poor T cell expansion upon telomere transfer (**Extended Data Fig. 10f**), likely due to a lower absolute count of siTZAP telomere vesicles (**Fig. 3a, e**) rather than a functional defect of the vesicle per se, in notable contrast to depletion of Rad51, while transfer of containing TZAP⁺ vesicles produced the opposite effect (**Extended Data Fig. 10g**). Telomere vesicles also abrogated expression of senescence markers, including beta-galactosidase^{16,17} and sestrin¹⁸, compared to T cells grown with telomere-depleted vesicles or siRad51 telomere vesicles (**Fig. 4b and Extended Data Fig. 11**). Conversion of naïve to senescent T cells was also strongly reduced by telomere vesicles (**Fig. 4c**).

Extensive phenotypic analysis revealed that naïve T cells treated with telomere vesicles switched to CD28⁺ CD62⁺ CD45RA⁺ CD95⁺ stem-like memory T cells with intermediate characteristics between effector and memory cells, a well-recognized phenotype whose mechanistic basis is poorly understood^{19,20} (**Fig. 4d**). The percentage of CD28⁺ CD45RA⁻ central memory cells also increased upon transfer of telomere vesicles. Further phenotypic modulation can be found, with similar human phenotype modulation by both human and mouse vesicles (**Extended Data Fig. 12**). When testing antigen-specific telomere transfer, we found that naïve and central memory cells, which are associated with elevated long-term potential, but not poorly proliferative senescent T cells, acquired telomeres from APCs (**Extended Data Fig. 13**).

When studying telomere transfer *in vivo*, we found that ~50% OT-II T cells had acquired telomeres from OVA-pulsed APCs in their popliteal lymph nodes (**Fig. 4e**). Metaphase spread imaging confirmed that APC telomeres reside in T cell nuclei upon telomere transfer *in vivo* (**Fig. 4f**). We are yet to find T cells that do not contain telomeres in their nuclei upon telomere vesicle uptake from APCs. We also confirmed that ~8% mouse T cell chromosomes contained telomeres of APC origin when Edu-free T cells acquired telomeres from APCs that had been previously labelled with Edu *in vivo* (**Extended Data Fig. 14**).

When assessing the *in vivo* behaviour of OT-II T cells that had acquired telomeres from OVA-pulsed APCs (Tel⁺; **Extended Data Fig. 15a**) by adoptive transfer, there was both short-term (5 days; **Fig. 4g** and **Extended Data Fig. 15b**) expansion and long-term (90 days) maintenance in all animals tested (**Fig. 4h** and **Extended Data Fig. 15c**). Similarly, to human T cells with APC telomeres, mouse Tel⁺ T cells also switched to a stem-like memory phenotype (**Fig. 4i**). OT-II cells without APC telomeres (Tel⁻) differentiated as short-lived, likely senescent, effectors *in vivo* (**Extended Data Fig. 15c**). Similar results were found in lymph nodes (**Extended Data Fig. 15d**), but not in the blood of vaccinated mice (**Extended Data Fig. 15e**), suggesting that T cells with APC telomeres migrate rapidly to long-term reservoirs to maintain long-term immunological memory. Similar results with purified telomere vesicles directly applied to T cells prior to transfer in recipients but only if the vesicles contained Rad51 (**Fig. 4j** and **Extended Data Fig. 15f-g**). Rad51 is required for the long-term boosting potential of the telomere vesicles.

When injecting T cells with or without APC telomeres from mice that had been previously immunized with influenza vaccines into naive recipients, followed by H1N1 flu infection (**Fig. 4k**), we found that immediate infection was resolved by mice receiving both types of T cells (**Fig. 4l**), but only mice that received T cells with APC telomeres survived upon delayed infection (**Fig. 4m**).

In conclusion, APCs donate telomere vesicles to extend T cell lifespan (**Extended Data Fig. 16**). Thus, while telomerase maintains telomeres throughout T cell divisions²¹, telomere transfer sets the initial chromosome length during APC-T cell interactions. We propose a binary model whereby naive T cells either acquire telomeres from APCs shifting towards a stem-like long-lived memory state or differentiate into short-lived senescent T cell progenitors in the absence of telomere transfer. This is different from both previously described linear and asymmetric models for T cell differentiation^{22,23} because a single T cell can undergo opposite fates pending on telomere transfer. Because each round of telomere transfer extends T cell telomeres by 3,000 bp, and because the average difference in telomere length between naïve and senescent T cells is less than 3,000bp²⁴, it may only require one failed round of telomere transfer to initiate T cell senescence. The idea that senescent T cells accumulate linearly due to repeated proliferation^{25,26} should be revised. The breaking apart of telomeres and their distribution in vesicles represent a new form of decentralized immunity, whereby T cells hold ‘telomere tokens’ as currency needed to achieve Immune Optical Scalable (ImmunOS) effects. The ImmunOS is optical (O) because it is fast and spreads on like light on inverse square law (such that the adjacent T cell closer to the APC is more likely to benefit from telomere transfer); it is scalable

(S) as it mounts large pools of antigen-specific memory cells that confer immune protection over a lifetime.

Supplementary discussion.

We showed that T cells with APC telomeres shift towards a stem-like memory state with intermediate characteristics between naïve and memory cells. These findings do not support the linear model of T cell differentiation and memory cell generation whereby a single naïve T cell exclusively originate a large pool of well-differentiated effector cells which are rapidly cleared when the immune-challenge subsides leaving a small pool of antigen-specific memory cells that progressively differentiate towards senescence²⁷. Rather, ageing fate decisions of T cells are made immediately, during initial synaptic contacts with APCs pending on telomere transfer. It has been known for sometime that a naïve T cell can divide asymmetrically and generate both effector and memory cells at the initiation of the adaptive immune response²³. Our data support further the generation of memory cells directly from naïve ones, precisely stem-like memory cells that can then subsequently originate all other types of T cells, including central memory cells. It was suggested previously that as yet undefined signals must be responsible for terminal differentiation and senescence of T cells²⁸. We now propose telomere transfer to be such signal. This is also different from senescent T cells being thought to accumulate during chronic viral infections, natural ageing and cancer because these cells, unlike T cells with APC telomeres, do not possess the ability to undergo antigen-specific expansion²⁹. An

important but as yet undefined function of the synapse is therefore the control of T cell senescence. The fact that 24 hours are needed to complete telomere transfer fits well with prolonged immune-synaptic interactions being required to generate durative memory T cells responses³⁰ due to previously unknown reasons. This model does not exclude those other types of self-renewing/highly proliferative T cells such as central memory cells that also acquire telomeres to maintain their long-term potential. However, the fact that central memory cells retroactively switch to a stem-like (naïve) memory state, and that telomere transfer firstly generates stem-like memory cells from naïve cells followed by central memory cell generation (on time course experiments; data not shown) argues against the redefinition of these cells as the truly stem cell memory subset^{31,32}. Instead, a key role for the naïve-like T cell being the truly stem memory cell both upstream and downstream central memory T cell generation. Ultimately, this fate decision may be based on the type of metabolism displayed by the naive T cell encountering APCs, such that only fit T cells would receive telomere donations. This remains to be tested.

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12 **Figure 1. APCs donate telomeres to T cells.** (a) Telomere restriction fragment
13 (TRF) analysis (n = 7) of APCs and T cells left either unconjugated or conjugated
14 in the presence of an antigen mix for 24h. (b) Telomere clustering in APC-T cell
15 conjugates (n = 37). APCs were conjugated with Cell Trace Violet (CTV) T cells
16 for 2h, fixed and analysed by IF-FISH with TelC probe. Scale bar, 5 μ m. (c)
17 Telomere donation by APCs (n = 3). Telomeric DNA donated by BrdU-labelled
18 APCs was immunoprecipitated with anti-BrdU following Triton-X 100
19 permeabilization from cell-free supernatants and telomeres detected with TelC
20 probes. Input, APC genomic DNA (200 ng). (d) Telomere vesicle protection
21 assays (n = 3) by qPCR in the presence or in the absence of Triton-X 100 to
22 disrupt vesicle membranes. (e) TRF analysis (n = 2) of APC telomeric DNA
23 present in the EVs isolated by ultracentrifugation. APC gDNA, positive control.
24 Dot blots with anti-dsDNA, loading controls. (f) Transmission electron microscopy
25 (TEM) of APC telomere vesicles with biotin-labelled TelC probes. APCs were

1 stimulated with ionomycin for 18h prior to supernatant ultracentrifugation. Scale
 2 bar, 100nm. **(g)** Ultrastructural analysis of telomere vesicles (n = 204) by field
 3 emission scanning electron microscopy (FESEM). **Experimental details,**
 4 **Extended Data Fig.2,3.** Magnification, 100,000X; scale bar, 100nm. **(h)**
 5 Detection of T cell chromosomes with APC telomere by IF-FISH (n = 2,425).
 6 APCs were labelled with Cy-5 TelC probes and EdU, treated with ionomycin, and
 7 FAVS-purified telomere vesicles transferred to separate T cell cultures that had
 8 not labelled with EdU prior to vesicle transfer. Metaphase spreads were analysed
 9 by IF-FISH with anti-EdU and FITC-TelC probes. Co-localization EdU-TelC, T cell
 10 chromosome ends with APC telomeres. No vesicle control of T cell
 11 chromosomes is shown on the right. Scale bar, 2 μ m. In **(a, c)**, paired Student's t
 12 test. $**P < 0.01$. In **(b)**, Mann-Whitney test. Error bars indicate S.E.M. throughout.

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 14 **Figure 2. Synaptic TCRs are sufficient to extract telomere vesicles from**
 15 **APCs.** **(a)** Schematic representation of telomere transfer on artificial synapse
 16 bilayers. **(b)** Z-stack 3D reconstruction showing release of CD63⁺ telomere
 17 vesicles from APCs activated on bilayers. APCs were live-labelled with TelC
 18 telomere probes and anti-CD63 prior to transfer on bilayers. Scale bar, 10, 5 or 1
 19 μ m as shown. **(c)** 3D reconstruction of telomere vesicle release from APCs on
 20 planar bilayers constricted as in **(a)**. APCs were live labelled with TelC probes
 21 and PKH67 lipid dye then incubated onto bilayers containing synaptic TCRs for
 22 24h. **(d)** Quantification of telomere release by APCs on bilayers in the absence
 23 of any CD63 or other staining antibody. Data pooled from 4 donors (Ag) or 2
 24 donors (No Ag, No TCR, SYK In). Where indicated, APCs were pre-treated with
 25 SYK inhibitor for 30 min prior to transfer on bilayers. The percentage of telomere

releasing APCs was normalized to the total number of APCs on the bilayer. In (d), Kruskal-Wallis with Dunn's post-correction test. $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$. Error bars indicate S.E.M.

Figure 3. APCs dismantle shelterin to donate telomeres. (a) FAVS of telomere vesicles (n = 4, 5 or 7) released by APCs in culture media following the indicated treatments, with or without ionomycin for 18h. (b) Shelterin levels in APCs without (top two) or following activation (bottom two) with ionomycin as in (a). In quantification analysis (right), each dot is an individual cell. Dashed lines demarcate panel (b). Scale bar, 2 μ m. (c) Shelterin downregulation (n = 3) was confirmed by immunoblotting. (d) APCs donate shelterin-devoid telomeres. TelC-labelled APCs were activated with ionomycin, then analysed by IF to POT1 (n = 30). Arrows indicate 'shelterin-devoid' telomeres released by APCs. Scale bar, 10 μ m. (e) FAVS profiles as in (a). (f) Telomere blot (n = 3) on BrDU immunoprecipitates from APCs transduced with mock or shelterin over-expressing (Shelterin Ov.) vectors to TRF2 + POT1. (g) Telomere vesicles were analysed by IF to Rad51 (n = 42 microscopy fields). Scale bars, 2 μ m. (h) Role of vesicular Rad51 in APC-T cell telomere colocalization (n = 6). APCs were transfected with siCtrl or siRad51 for 36h, live-labelled with red (Cy3) telomere probes then activated by ionomycin for 18h. The cell-free supernatants containing fluorescent APC-telomere vesicles were then transferred into T cells with green (FITC) telomere labelling. Analysis carried 24h later. Arrowheads, APC-T cell telomere co-localization. Note that only green signals are endogenous T cell telomeres. Scale bar, 5 μ m. In (a, b, c, d, f, h), Mann-Whitney test. $**p < 0.01$ $***P < 0.001$. Error bars indicate S.E.M. throughout.

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2 **Figure 4. Generation of long-lasting immunity by telomere transfer. (a)**

3 Expansion of T cells activated with anti-CD3 plus anti-CD28 every 10 days in the
4 absence or presence of 1,000 siCtrl or siRad51 telomere vesicles (Tel⁺) purified
5 from APCs. Control T cells were cultured with 1,000 telomere depleted vesicles
6 (Tel⁻) or left without any vesicles (no vesicles) throughout. **(b)** Senescence-
7 associated beta-galactosidase activity (n = 4) in T cells as in **(a)** for 10 days. **(c)**
8 Prevention of T_{sen} generation by telomere transfer. Naïve T cells were activated
9 as in **(a)** then treated with 250 telomere vesicles for 15 days. **(d)** Induction of T
10 stem-like memory state by telomere transfer of cells as in **(c)**. **(e)** Demonstration
11 of *in vivo* telomere transfer by flow cytometry (n = 3). OVA-pulsed APCs with
12 TelC labelled-telomeres were injected into the right footpad of recipients followed
13 by adoptive-transfers of OT-II cells identified by Cell Trace Violet dye 18h later.
14 Animals were culled 18h after the addition of T cells and popliteal lymph nodes
15 collected for analysis of telomere transfer. **(f)** Presence of APC-derived telomeres
16 into the nuclei of mouse T cells (metaphase spreads) following *in vivo* telomere
17 transfer as in **(d)**. Each red dot is an APC telomere in the nucleus of T cells.
18 Scale bar, 5 µm. **(g-i)** Tel⁺ versus Tel⁻ (naïve) CD45.2 OT-II cells were derived
19 upon conjugation with TelC labelled APCs for 24 hours in the presence of OVA
20 prior to FACS sorting. Tel⁺ versus Tel⁻ (1,000 cells each) were then adoptively
21 transferred into CD45.1 congenic recipients, and animals immunized with OVA
22 18 hours later throughout. In **(g)**, the effector T cell expansion, in **(h)** the long
23 term maintenance, and in **(i)** the stem cell memory switch characteristics of
24 telomere transfer. In **(g)**, analysis after 5 days after transfer, **(h, i)** a second of
25 OVA stimulation 40 days after transfer and analysis after an additional 50 days

(90 days in total). (j) Vesicular Rad51 is required for telomere vesicles to extend T cell lifespan. CD45.2 T cells (0.5×10^6) were treated with 500 telomere vesicles, Rad51 depleted vesicles or telomere depleted vesicles from siRNA transfected APCs for 24 hours then transferred into recipient CD45.1 mice. Mice were challenged with OVA 18 hours later, rested for 40 days, followed by OVA restimulation for another 30 days. Analysis were carried out 70 days post transfer. (k) Experimental design. (l) Survival of mice receiving either Tel⁺ or Tel⁻ T cells as in (a) and infected immediately. Ctrl, animals injected with T cells that had not been exposed to the vaccine (yellow line). (m) Survival of mice receiving either Tel⁺ or Tel⁻ T cells as in (a) and infected 15 days later. In (a, b, c), in ANOVA with Bonferroni post-test correction; in (d, e, f, g, h, i, j, l, m), paired student T test. * $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$. Error bars indicate S.E.M. throughout.

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Disclosure. A.L. is Founder of SenTcell and ElecTra Life Sciences ltd.

Author contribution. A.L. imagined and further conceived of, performed, and directed the study, analysed and interpreted the data, provided funding and laboratory infrastructures, formed collaborations, and wrote the paper; B.V., C.D. and C.V. designed and performed experiments and analysed individual data sets; S.V. performed lipid bilayer experiments; V.C. performed immune-phenotyping, cell-death analysis and provided key samples; O.D. performed initial FISH and collected lymph-nodes; M.S. performed fluorescence activated vesicle sorting;; M. D. B. performed APC subset isolation; A.N.A. provided initial infrastructures; G.B. performed APC subset reanalysis of telomere transfer designed and guided by A.L.; D.W.G. performed *in vivo* manipulations; M.L.D. provided lipid bilayer infrastructure, conceptual framework for synaptic vesicles, feedback and advice; B.B: helped developing decentralized immunity concept; and M.K. inspired A.L., provided feedback, advice, and supported experimental revisions of the final version of the manuscript, which was read, commented and approved by all authors. B.V., C.D., C.V., and S.V. contributed equally to this work.

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5 **Materials and Methods.**

6 Human studies.

7 We collected heparinized peripheral blood samples from 140 healthy donors
8 (aged 20-65, male 55% and female 45%). All human specimens were obtained
9 with the approval of the Ethical Committee of Royal Free and University College
10 Medical School, the University of Oxford or the Fondazione Santa Lucia Ethical
11 Committee (CE/prog. 754) with voluntary informed consent, in accordance with
12 the Declaration of Helsinki.

13

14 Cell isolation.

15 Primary human conventional CD27⁺ CD28⁺ CD4⁺ T cells were purified using
16 commercially available kits from Miltenyi. Briefly, peripheral blood mononuclear
17 cells (PBMCs) were isolated by Ficoll followed by negative selection for CD4
18 (130-096-533). The untouched population was then purified by CD27 microbeads
19 first (130-051-601) yielding CD27⁺ CD28⁺ CD4⁺ T cells. For isolation of mouse T
20 cells from splenocytes, CD4 MicroBeads (L3T4; 130-117-043) were used. For
21 APC purification (CD3⁺-depleted autologous cells), human CD3 MicroBeads
22 (130-050-101) and mouse CD3e Microbead kit (130-094-973) were used to
23 deplete T cells. In BrdU labelling experiments, human APCs were expanded with
24 IL-4 and GM-CSF for 48h prior to downstream application to allow BrdU
25 incorporation in APC DNA. In all other cases, APCs were directly used as CD3

negative cells.

Signalling studies.

Telomere vesicles were stained with antibodies to Rad51 (1:100; ab63801, Abcam) and TRF2 (1:300; ab13579, Abcam) and POT1 (1:300; PA-66996, ThermoFisher). Activated T cells were stained with antibodies to sestrin 1 (1:300, ab134091, Abcam). Shelterin expression in APCs was evaluated with antibodies to TRF2 and POT1 as per telomere vesicles.

Telomerase modulation by CRISPR.

Human T cells ($2-5 \times 10^6$) were transfected with 3 mg of TERT KO CRISPR/Cas9 plasmid (h) (sc-400316, Santa Cruz) according to the manufacturer instructions. TERT-KO (GFP⁺) T cells were activated by anti-CD3 plus anti-CD28 and then purified by FACS 96h post-transfection. Control T cells were transfected with 3 mg of control CRISPR/Cas9 Plasmid (sc-418922, Santa Cruz). Knock-out efficiency was confirmed by qPCR (Hs00972656_m1; TaqMan). Transcript expression was normalized to that of the housekeeping gene *GAPDH* by the change-in-cycling-threshold method ($\Delta\Delta C_t$) for the calculation of values supplied by Applied Biosystems. In experiments with telomerase enhancement, 10^7 T cells were transduced with 3 μ g of CRISPR telomerase activation particles (sc-400316-ACT) and then used 96h post transduction. For immunoblots to TERT (1:1000; sc-3093013, Santa Cruz) on purified GFP⁺ T cells as previously described³³.

Telomere Immunofluorescence *In situ* hybridization FISH (IF-FISH).

Sections were fixed in ice-cold 3.7% formaldehyde for 15 min, washed in PBS

and treated 20 min with -20 °C ethanol. Blocking was performed for 1 h at room temperature in PBS-TT (8% BSA, 0.5% Tween-20 and 0.1% Triton X-100 in PBS). Primary staining's were performed overnight at 4 °C with antibodies to TRF2 and POT-1 (1:300; Abcam and ThermoFisher,); CD3 (1:1000; BIO-RAD MCA463G). Secondary staining's were performed for 1 h at room temperature in the dark with biotinylated goat anti-rabbit IgG AlexaFluor 405 (1:400; A-31556, Life Technologies) and goat anti-mouse IgG1 AlexaFluor 647 (1:800; Life Technologies A-21240, Life Technologies). Cross-linking was performed 20 min in 4% paraformaldehyde. Sections were dehydrated in graded ethanol, treated with DNase-free RNase (100 ng/mL; Thermofisher) for 60' then hybridized with 40 pM PNA probe solution (Panagene, TelC Cy3, 14 1224PL-01) in hybridization buffer (1M Tris pH 7.2; magnesium chloride buffer; deionised formamide; blocking buffer, Roche; deionised water). DNA denaturation was performed 10 min at 82 °C. Hybridisation was allowed 2 h at room temperature, followed by subsequent washing in 70% formamide, saline sodium citrate (SSC) buffer and PBS, respectively. DNA was stained for 5 minutes in PBS with 1 ug/ml of DAPI and coverslips mounted with Pro-Long Gold (Invitrogen). Imaging was performed using a Leica SPE2 confocal microscope using LAS X version 3.3.0 software (Leica Microsystems, Wetzlar, Germany) or super-resolution ZEISS LSM 800 Airyscan (Zeiss). Sections were z-stacked and the average telomere integrated fluorescence per cell nucleus was quantified by ImageJ software.

Antigen-specific conjugates.

Human APCs were pre-pulsed for 4h at 37 °C with 1:50 antigen mix (HBV, influenza and CMV lysates; bulk telomere measurements) or 1:50

cytomegalovirus (CMV)-lysates (Zeptomatrix Corporation; IF-FISH studies). APC-T cell conjugates were then formed by adding autologous CD27⁺ CD28⁺ CD3⁺ CD4⁺ T cells in a 3:1 ratio, spun at 300 rpm for 5', then incubated at 37 °C as indicated.

Isolation of extracellular vesicles (EVs) by ultracentrifugation.

Vesicles were isolated from APC supernatants following sequential centrifugation (300g 10 min; 2,000g 10 min; 10,000g for 30 min). The 300g centrifugation is used to remove cells; the 2,000g centrifugation removes cell debris; and the 10,000g removes apoptotic bodies and other non-EV structures. Where indicated, the 10,000g centrifuged APC supernatants were further passed through 0.3 µM filters to remove vesicle clusters, and EVs pelleted at 100,000g for 90min to derive exosome-enriched preparations.

Vesicle Immunoblotting.

APCs were activated with ionomycin for 18h prior to being removed by centrifugation at 300g 10min. The APC supernatants were then subjected to sequential centrifugation at 2,000g 10 min; 10,000g for 30 min and, following 0.3 µM filtration, at final 100,000g for 90min. The pellets obtained after each sequential centrifugation step were resuspended in PBS and used for vesicle immunoblotting with the primary antibodies used in ELISA applications (see below) plus p-H2AX S139 (Abcam, ab26350) and BRCA1 (GeneTex, GTX70113). Input was 20% APC whole cell lysates. Fractionation purity was confirmed with antibodies to cytochrome C (BD pharmigen, 556432). Antibodies were used at 1:1000 dilution.

Generation of APCs with fluorescent telomeres.

APCs were adhered overnight on glass coverslips in complete RPMI-1640 medium (supplemented with 10% heat-inactivated FCS, 100 U/ml penicillin, 100 mg/ml streptomycin, 50 µg/ml gentamicin, and 2 mM L-glutamine). Cy-3 TelC-PNA probes were dissolved 1:50 in telomere loading buffer (80 mM KCl, 10 mM K₂PO₄, 4 mM NaCl, pH 7.2) and gently introduced on adherent live-APCs by rolling pH 7.2. alkali-washed glass-beads (G8772; Sigma Aldrich) for 5 min. Alkali bead washing was performed overnight in 1M NaOH. TelC-Cy3-probe labelled APCs were washed in PBS twice after rolling, and cultured in complete RPMI.

Fluorescence activated vesicle sorting.

Telomere vesicles were extracted from APCs live-labelled with Cy3 TelC telomere probes and the membrane lipid dye PKH67 (Sigma Aldrich). In experiments with modulation of telomere transfer, APC (10⁷) were transfected for 48h with siCtrl (10 nM; sc-37007, Santa Cruz) or siRad51 (10 nM; sc-36361, Santa Cruz), siTZAP (10nm; sc-78823, Santa Cruz), siPOT1 (10nm; sc-44032, Santa Cruz), siTRF2 (10nm; sc-38505, Santa Cruz) as described in **Fig. 3a**. After transfection, 10⁶/condition were stimulated with ionomycin for an additional 18h and the culture media collected. After sequential centrifugation (300g, 2000g and 10000g), the APC supernatants were analysed by fluorescence activated vesicle sorting. Fluorescent telomere vesicles (PKH67+ TTAGGG+ single particles) were depleted by MoFlo Astrios-EQ flow cytometer (Beckman Coulter) equipped with 5 lasers (355, 405, 488, 561 and 640 nm wavelengths). To reduce instrument background noise, the system is also equipped with inline sheath filter with 40 nm pore size. The applied pressure to

sheath fluid was 60 psi. Routinely alignments were performed with Ultra Rainbow Fluorescent Particles 3.0 μm (Spherotech, URFP-30-2). The triggering threshold was applied to the side scatter on the 488-SSC channel and the relative applied voltage was determined by using size reference beads (Apogee flow systems, 1493) to exclude vesicle aggregates >300 nm. The Instrument setup to identify microvesicles was obtained by balancing triggering threshold and SSC voltage in order to reduce and maintain over time the background noise at 150-300 events/sec.

DNA content of telomere vesicles.

To confirm presence of telomeric DNA in telomere vesicle preparations derived from APCs, 1,000 telomere vesicles were purified by fluorescence activated vesicle sorting and analysed by qPCR using telomere specific primers. Absence of non-telomeric DNA in telomere vesicle preparations was confirmed using random primers (Thermo Scientific). T cells nuclear extracts served as positive control for non-telomeric amplification. Absence of substantial non-telomeric DNA was confirmed by DIG-Alu probe followed by slot blot. APC genomic DNA served as control. In protection assays, telomere vesicles derived from APCs were treated with the endonuclease DNase I to digest DNA, in the presence or in the absence of Triton X-100 followed by performed qPCR with telomere specific primers. DNAase I treatment destroyed telomeric DNA only in the presence of Triton X-100, confirming that the released telomeres were protected by a vesicle membrane.

Protein cargo of telomere vesicles.

1 Telomere positive and negative vesicles were purified as above described.
2 Protein cargo was analysed by indirect ELISA after overnight incubation of
3 vesicle extracts (obtained by 1% Triton x-100) onto binding plates with a final
4 volume of 50 μ l. After overnight incubation, plates were washed 3 times in 0.1%
5 Tween-20 in TBS and incubated overnight at 4C with antibodies to: MHC II
6 (Abcam ab55152), TZAP (Abnova H00003104-B01P), CD63 (GeneTex
7 GTX28219), BRCA2 (Abcam ab123491), TSG101 (GeneTex GTX70255),
8 Rad51(Abcam ab63801) (all at 1:100 dilution) Background control plates were
9 incubated with PBS. Antibody specificity was confirmed by staining parallel
10 vesicle extracts with IgG (isotype control Cell Signaling, 3900S). Primary
11 antibodies were then detected using horseradish peroxidase (HRP) conjugated
12 antibodies (anti-mouse or anti-rabbit IgG, Invitrogen) at 1:1,000 dilution in 5%
13 non-fat dry milk 0.1% Tween-20 in TBS, at room temperature for 2h. After
14 washing secondary antibodies 3 times in 0.1% Tween-20 in TBS, 50 μ l TMB
15 substrate (ThermoScientific) was added in each well to detect signals for 15
16 minutes followed by 50 μ l stop solution (ThermoScientific). Absorbance of triplicate
17 wells was detected at 450nm with an Elisa reader.

18 Telomere restriction fragment (TRF) analysis.

20 APCs and T cells were purified as described above. Primary human T cells
21 (5×10^6) were conjugated 1:1 with autologous APCs for 24h in the presence of
22 1:50 antigen mix. The day after, conjugates were broken using a syringe with
23 cold PBS plus 5mM EDTA and T cell and APC fractions purified by anti-CD3. In
24 parallel, additional 5×10^6 T cells and APCs were left unconjugated for comparison
25 of telomere length before and after forming the synapse. Pre and post synapse T

cells and APC fractions were then pelleted and genomic DNA extracted using the PureLink™ Genomic DNA Mini Kit (K182001, ThermoScientific). Telomere length was analysed using the TeloTAGGG™ Telomere Length Assay (12209136001, Sigma-Aldrich). Briefly, genomic DNA (1.5 µg/sample) was digested with HinF I/Rsa I restriction enzymes for 2h at 37 °C. Digested DNA was then subjected to gel electrophoresis (10h 4V/cm) on a 1% Agarose gel. The gel was denatured in 0.5 M NaOH, 1.5 M NaCl, neutralized in 0.5 M Tris-HCl, 3 M NaCl, pH 7.5 then over-night transferred by capillarity to an Amersham Hybond-N+ nylon membrane (GE). Transferred DNA on membrane was UV cross-linked, finally hybridized for 3h at 42 °C with DIG-labelled telomere probes and bulk telomere length analysed as recommended by the manufacturer (Telo-TAGGG Telomere Length Kit Assay; Roche). Briefly, a grid was composed on the telomere shift that was divided in at least 30 squares per lane, signal intensity (OD) of each square calculated on ImageJ and where the corresponding length (L) was the length of the midpoint of each square. Mean Telomere length was derived according to the formula :

$$TRF = \Sigma (OD_x) / \Sigma (OD_x / L_x)$$

For TRF on APC vesicle preparations, 1.5 µg of DNA (purified from pellets recovered after final 100,000g ultra-centrifugation of APC supernatants) were analysed in the presence or in the absence of HinF I/Rsa I restriction enzymes to digest non-telomeric DNA.

Flow-FISH.

T cells were conjugated with autologous APCs and spun 2 min at 300 rpm. Telomere length was then measured by a modified version of a published

fluorescence *in situ* hybridization–flow cytometer method over a 48h time course. For determination of absolute telomere length (kb) by Flow-FISH, we used a previously generated standard curve formed by cryopreserved samples with known telomere length as examined by Southern blot of telomeric restriction factors (TRFs). This standard curve allows conversions of Flow-FISH MFI values into TRF kb by calculating Molecules of Equivalent Soluble Fluorochrome (MESF) units^{36,37}.

Generation of T cell chromosomes with APC telomeres.

Primary human T cells were activated with anti-CD3 plus anti-CD28 antibodies (0.5 µg/mL) and treated with 3,000 TelC-Cy5⁺ PKh67⁺ telomere vesicles derived from 10 µM EdU labelled, ionomycin-conditioned APC medium and separated by FACS sorting. Control metaphases were generated from T cell cultures not activated with vesicles or with telomere depleted vesicles. For mouse experiments, APC telomere vesicles mice were *in vivo* labelled by IP injection with 100 µl of 1 mg/ml EdU in PBS. Mice were then administered 0.3 mg/ml EdU in drinking water ad libitum for 14 days. Water was replaced with fresh Edu every 2 days. The TelC-Cy5⁺ PKh67⁺ telomere vesicles separated by Edu *in vivo* labelled APCs.

T cells were treated 4h with colcemid (0.2 µg/mL; Life technologies) after 24h activation. T cell metaphase spreads were swollen 5 min in KCl buffer (12.3 mM HEPES, 0.53 mM EGTA, 64.4 mM KCl), fixed 5 min in methanol: glacial acetic (3:1 solution) then dried 60 min onto pre-warmed glass-slides. For Edu telomere vesicle localization at T cell chromosomes ends, endogenous T cell telomeres

1 were detected by standard telomere FISH with Cy-3 TelC probes, and
2 colocalization events enumerated. For metaphase Q-FISH, T cell chromosomes
3 with APC-telomeres were generated as above described in the absence of any
4 standard telomere FISH such that only APC telomeres but not endogenous T cell
5 telomeres could be detected, and directly quantified by Q-FISH coupled to TFL-
6 software analysis in parallel with internal standards with known telomere length.
7 For endonuclease analysis, enzymatic digestion was performed 30 min with 1
8 unit T7 endonuclease (New England Biolabs) at 37 °C. Chromosomes were
9 stained with DAPI. Sections were z-stacked and the %T cell chromosomes
10 bearing APC-telomeres before and after the T7 endonuclease treatment was
11 calculated within a single stack. The total number of APC-telomeres remained
12 unaffected.

14 APC-T cell telomere fusion.

15 T cells were cultured for 48h with 5,000 telomere vesicles or 5,000 telomere
16 depleted vesicles (both obtained by fluorescence activated vesicle sorting as
17 described below). In some experiments, blocking antibodies to MHC class II
18 (1µg/mL) were added to the vesicle preparations prior to transfer into T cells, as
19 indicated. T cell nuclear extracts were then analysed by qPCR using telomere
20 specific and standard primers (8918, ScienceCell research Laboratories) using
21 standard 6800 Roche Analyzer.

23 In vivo Telomere Transfer.

24 Six week-old (wild type) C57BL/6J mice and OT II mice were purchased from
25 Charles River. TelC-Cy3-probe labelled mouse APCs were loaded with 3 µM

OVA peptide (323-339; GeneScript) and injected into the right footpad of wild type C57BL/6J recipients. The day after, OVA-specific OT II cells (identified by Cell Trace Violet dye staining) were adoptively transferred, popliteal lymph nodes harvested after additional 18 h and APC->T cell telomere transfer examined by flow cytometry. In control experiments, APCs were not loaded with OVA antigens.

Memory/Effector responses.

Naïve CD4⁺ OTII TelC-Cy3⁺/⁻ cells were sorted from OVA stimulated (1:40) APC-T conjugates. 1×10^3 cells³⁴ were injected per mouse and adoptively transferred after OVA vaccination eighteen hours later. CD4⁺ OTII cells were enriched from blood, lymph nodes and spleen by using 2ug/ml CD45.2-biotin antibody (130-124-089, Miltenyi) and pulled down with 50 ul of Streptavidin Microbeads (130-048-101, Miltenyi). Mouse T cells were stained with anti-CD4 (Miltenyi 130118955) anti-CD44 (Miltenyi, Cat. 130119121), anti -CD62L (Miltenyi 130112649), anti-CD45.1 (Miltenyi 130121221), anti-CD45.2 (Miltenyi 130124080), anti-CD95 (Miltenyi 130122950). For effector responses, T cells were analysed 5 days after OVA vaccination in vivo; for memory responses mice were stimulated with a second round of OVA vaccination 40 days post transfer, rested for 50 days and memory responses assessed 90 days after adoptive transfer.

Recombinogenic potential of Rad51 telomere vesicles *in vivo*.

Six week-old (wild type) C57BL/6J (CD45.1/CD45.2) mice and OT II mice were purchased from Charles River. TelC-Cy3 ^{-/+} vesicles were sorted from CTRL mouse and siRad51 APC supernatants. For generation of mouse siRad51

vesicles, APCs were transfected with mouse siRad51 (10nm; sc-36360, Santa Cruz). 5×10^5 total CD4 OTII /CD45.2 were then incubated 18h with 500 vesicles and then I.V injected into wild type C57BL/6J (CD45.1) recipients recipient (adoptive transfer). Eighteen hours later, mice were vaccinated with 3 μ M OVA peptide (323-339; GeneScript). Forty days later, mice were revaccinated with OVA (second round *in vivo*) then culled after an additional 30-day observation (a 70 day experiment), OTII cells from lymph nodes and spleen were examined by flow cytometry.

Survival studies.

Eight wild type mice were primed with FLUAD vaccine (1:20) and killed 5 days later. Total CD4+ were incubated with EVs TelC-Cy3-/+ for 18h and then Intravenous injected in wild type recipients (5×10^6 cells per mouse). Mice were early and delayed infected with H1N1 flu virus (10^5 PFU dose per mouse) and monitored for 15 days for weight loss; fur aspect; mobility; hunched back; and breathing, When severe dyspnoea occurred, mice were killed. In all other cases, mice were killed when clinical scores were equal or above 10.

Measurement of Telomerase activity.

Telomerase activity was assessed with a TeloTAGGG telomerase ELISA kit according to the manufacturer's instructions (Roche) and extracts of 2×10^3 viable T cells as described previously^{18,33}.

Long-term cultures.

Primary human T cells were activated by anti-CD3/anti-CD28 antibodies (0.5

1 $\mu\text{g/mL}$ each) every 10 days in the presence or absence of telomere vesicles or
2 telomere depleted vesicles as control and their population doublings calculated
3 as described previously¹².

4
5 Immunological analysis.

6 Primary human T cells were activated as above described for 10 days in the
7 presence of telomere vesicles (purified from either human or mouse APCs),
8 telomere depleted vesicles or left without any vesicle. Ten days later, T cells were
9 analysed on a Beckman Coulter flow cytometry with multiparametric settings (10
10 colors). For the immunophenotype characterization, T cells were stained with
11 anti-CD4 Alexa 700 (clone OKT4, Biolegend, Cat. 317626, 1:100), anti-CD27
12 PerCP5.5 (clone M-T271, Biolegend, Cat. 356408, 1:100), anti-CD28 APC-
13 Vio770 (clone REA612, Miltenyi Biotec, Cat. 130-116-506, 1:100), anti-CD45RA
14 BV421 (clone HI100, Biolegend, Cat. 3104130, 1:100), anti-CD45RO ECD (clone
15 UCHL1, Beckman Coulter, Cat. M2712U, 1:100), anti-CD75 (Miltenyi Cat.
16 130113068), anti-PD1 APC (clone NAT105, Biolegend, Cat. 367406, 1:100), anti-
17 FasL PE-Cy7 (clone NOK-1, Biolegend, Cat. 306418, 1:100), anti-CTLA4 PE
18 (clone L3D10, Biolegend, Cat. 349906, 1:100) and anti-TIM-3 VioBright FITC
19 (clone REA602, Miltenyi Biotec, Cat. 130-109-711, 1:50). Cells were washed in
20 PBS and analysed on a Cytoflex flow cytometer (Beckman Coulter).

21
22 Cell death analysis.

23 APCs were activated with ionomycin as above or conjugated with autologous T
24 cells (3:1) in the presence or absence of antigen mix. Cell death was analysed

18h later by FITC Annexin V/PI Apoptosis Detection Kit with PI (640914; Biolegend).

Measurement of senescence-associated-β-galactosidase.

Human T cells were activated by anti-CD3 plus anti-CD28 either in the presence or in the absence of telomere vesicles from APCs for 10 days. After overnight resting at 37 degrees °C, cells were cytopun 5' at 500 rpm and senescence-associated beta-galactosidase activity measured as recommended by the manufacturer (Cell Signaling Technology) on a Zeiss-Axio inverted phase-contrast microscope. Control APCs were activated either in the absence of vesicles or with telomere depleted vesicles.

APC-T cell Telomere co-localization.

APCs were nucleofected with siCtrl (10 nM; sc-37007, Santa Cruz) or siRad51 (10 nM; sc-36361, Santa Cruz), labelled with Cy3-TelC PNA probes and extracted as above described. APC-derived telomeres were then added drop by drop into autologous T cells that had been previously labelled with FITC-TelC PNA probes and cultured onto poly-lysine-coated coverslips. APC-T cell telomere co-localization (Pearson's co-localization test) into the nuclei of recipient T cells was assessed by ImageJ software. Costes method was used to verify co-localization thresholds against randomly generated images¹⁹. Co-localization frequencies shown in **Figure 3f** were calculated within a single stack with the following formula:

$$Z/(X+Y-Z)*100\%$$

where Z is number of co-localization events; X is the number of T cell telomeres;

and Y is the number of transferred telomeres of APC-origin.

Chromatin Immunoprecipitation.

Primary human T cells (10^7) were activated overnight by anti-CD3 plus anti-CD28 in the presence or absence of Cy3-TelC-labelled APC-derived telomeres (extracted and purified as above described). Cells were cross-linked 10 min with 1% formaldehyde, lysed and their nuclei digested with MNase-based Thermofisher Pierce Agarose Chip kit. Digested chromatin was immunoprecipitated with polyclonal anti-POT1 or control rabbit IgG antibodies at 4C on a rotary shaker overnight followed by incubation with Chip-grade A/G agarose beads at 4C for 3 hours. Presence of APC-derived telomeres in control and POT-1 immunoprecipitated T-cell reactions was quantified as Cy3-absorbance emission of triplicate wells using a micro-plate reader. Presence of APC-derived telomeres was further confirmed by adding DNase directly to the POT1 IPs for 10 min prior to reading. Background fluorescence was calculated in parallel POT1 immunoprecipitation reactions from activated T cells not treated with APC-derived telomeres.

Supported planar bilayers.

Glass coverslips were cleaned 20 min with Piranha solution, rinsed extensively and dried. Coverslips were plasma cleaned 4 min then assembled into ibidi chambers for bilayer formation. Lipid bilayers containing 12.5% mol NTA (1,2-dioleoyl-*sn*-glycero-3-*N*-5-amino-1-carboxypentyl iminodiacetic acid succinyl) and 0.004% CapBio (1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-*N*-cap biotiny) in DOPC (1,2-dioleoyl-*sn*-glycero-3-phosphocholine) were incubated 20

1 min at a total phospholipid concentration of 0.4 mM. After washing in 0.1%
2 HBS/BSA, bilayers were blocked 20 min with 2% BSA/HBS containing 100 μ M
3 NiSO₄, washed extensively, and loaded 20 min with 4 μ g/mL streptavidin.
4 Streptavidin-coated bilayers were subsequently coated 20 min with 1.1 μ g/ml
5 UCHT1 568 (final density: 30 molecules/ μ m²) and 0.39 μ g/ml ICAM-1 405 (final
6 density: 200 molecules/ mm²). Bilayers were washed and immediately used for
7 synapse formation. T cells were activated on bilayers for 20 min, fixed 10 min
8 with 2% PFA then imaged in TIRF mode with Olympus 150x 1.45 NA objective.
9 Primary human T cells were loaded onto bilayers directly *ex vivo* or after over-
10 night resting in complete RPMI at 37°C.

11

12 Extraction of APC-telomeres on planar bilayers.

13 Planar bilayers were constructed as described earlier and incubated with primary
14 human T cells. After 20 min activation on bilayers, T cells were removed with cold
15 PBS. The released synaptic TCRs were retained on the bilayer. Next, telomere-
16 labelled APCs were loaded with antigen mix and transferred on the TCR coated
17 planar bilayers. The % of APCs releasing telomeres on bilayers was quantified
18 24h later. In control experiments, APCs were not loaded with antigen mix; or they
19 were transferred onto bilayers constructed with ICAM-1 molecules alone. The
20 vesicular nature of the released telomeres was further tested by pre-incubating
21 TelC labelled APCs with antibodies to the exosome marker CD63 (clone H5c6;
22 Biolegend Cat. 353015) at 1:50 dilution prior to transfer on bilayers.

23

24 Dot blot analysis of telomere vesicles.

1 Human APCs were pulsed with BrdU (10 μ g/mL) and cultured in the presence of
2 IL-4 (50 ng/mL) plus GM-CSF (10 ng/mL) for 48 hours, at which point BrdU had
3 been uniformly up-taken by APCs (data not shown). APCs were then washed
4 with PBS, loaded for 4h with CMV antigens, and allowed to form synapses with
5 BrdU-unlabelled (autologous) T cells. Twenty four hours later, supernatants were
6 collected, purified by sequential centrifugation (as above described), and DNA
7 secreted by APCs was firstly concentrated 60 min at -80 °C by adding
8 Ethanol/sodium acetate, then immunoprecipitated with anti-BrdU in the presence
9 of 1% Triton-X100 at 4°C. The next day, BrdU immunoprecipitates were
10 incubated with Agarose A/G beads (1:100; Santa Cruz Biotechnology) and kept
11 rotating at 4°C for 3h. Beads were subsequently washed twice with ice-cold
12 HNGT buffer (50 mM HEPES, pH 7.5, 150 mM EDTA, 10 mM sodium
13 pyrophosphate, 100 mM sodium orthovanadate, 100 mM sodium fluoride, 10
14 mg/ml aprotinin, 10 mg/ml leupeptin, and 1 mM phenylmethylsulfonyl fluoride)
15 and once with TE buffer. Beads were then incubated in elution buffer (1% SDS in
16 TE buffer) for 15 min at 65 °C. Telomere vesicles were then eluted by
17 centrifuging at 13,000 rpm for 30 min and dot blotted on Amersham Hybond-N+
18 nylon membrane (GE). The membrane was denaturated in 0.5 M NaOH, 1.5 M
19 NaCl, neutralized and UV-cross linked for 2 min. Telomere dot blot was
20 performed by 3h hybridization at 42°C with DIG-labelled telomere probe as
21 recommended by the manufacturer (Telo-TAGGG Telomere Length Kit Assay;
22 Roche). Control Input was 200 ng genomic DNA from APCs. Alternatively,
23 telomeric DNA was pelleted from APC vesicles isolated by sequential
24 centrifugation. DNA was sonicated (5 cycles 20 seconds/each) and spotted onto
25 Nylon membranes. Presence of single strand versus double strand telomeric

DNA was confirmed by 10mM EDTA plus 100mM NaOH denaturation followed by 10min at 95°C prior to transfer onto Nylon membranes. Pellets were also recovered from all previous centrifugation steps as a control, largely showing absence of telomeric DNA in earlier purification steps. For detection of non-telomeric DNA, vesicles were analysed by slot blot with DIG-Alu DNA ctrl probe (Bioptica; Cat. T-1053-400). In experiments with ionomycin activation, APCs were treated as above but stimulated with ionomycin rather than T cells, followed by supernatant collection, lysis in Triton X-100, and TelC detection throughout.

Shelterin modulation.

Human APCs were cultured with IL-4 (50 ng/mL) plus GM-CSF (10 ng/mL) for 48h, then transduced with POT1 (sc-403275-LAC) and TRF2 (sc-401289-LAC) lentiviral activation particles (both from Santa-Cruz Biotechnology). Control APCs were transduced with control lentiviral activation particles (sc-437282-lac). Four days post-transduction, APCs were activated with ionomycin and analyzed by IF-FISH with anti-POT1 and anti-TRF2 antibodies as well telomere dot blot as above described. For IF-FISH to POT1 we used either NB500-176 (Novusbio) or PA-66996, (ThermoFisher) (both at 1;300 dilution), with similar results. The TRF2 antibody as above described (signaling studies section). Conversely, shelterin-deficient APCs were generated by siTRF2 plus siPOT1 nucleofection from 10⁷ human APCs and their telomere vesicle release analysed 72h later as above described.

Universal-STELA.

1 Briefly, 10 ng of MseI and NdeI digested DNA was incubated with 12-mer and 42-
2 mer panhandles at 65 °C then cooled to 16°C in 49 minutes. T4 DNA ligase was
3 then added to the mixture overnight and kept at 16°C (final volume, 15 µl). The
4 digested DNA was then incubated with 10⁻³ µM terminal adapters (telorettes) in a
5 final volume of 25 µl and incubated overnight at 35°C. Next, a PCR reaction was
6 set using 40 pg (i.e. 5 genome equivalent) of ligated DNA, in the presence of 0.1
7 µM adapter and teltail primers and using failsafe master mix and failsafe enzyme.
8 The PCR conditions were as follows: 1 cycle at 68°C for 5 min; 1 cycle at 95 °C
9 for 2 min; 26 cycles at 95 °C for 15 sec; 26 cycles at 58 °C for 30 sec; 26 cycles
10 at 72°C for 12 min; 1 cycle at 72 °C for 15 min. The PCR product was then
11 resolved as per TRF analysis on a 0.8% Agarose gel and the load of ultra-short
12 telomeres was calculated as number of telomere bands <3kb/ genome equivalent
13 as described(19). U-STELA PCR primers (5'—3') were as follows: 11+2-mer-
14 panhandle TAC CCG CGT CCG C
15 42-mer-panhandle TGT AGC GTG AAG ACG ACA GAA AGG GCG TGG TGC
16 GGA CGC GGG
17 Telorette 1 TGC TCC GTG CAT CTG GCA TCC CCT AAC
18 Telorette 2 TGC TCC GTG CAT CTG GCA TCT AAC CCT
19 Telorette 3 TGC TCC GTG CAT CTG GCA TCC CTA ACC
20 Telorette 4 TGC TCC GTG CAT CTG GCA TCC TAA CCC
21 Telorette 5 TGC TCC GTG CAT CTG GCA TCA ACC CTA
22 Telorette 6 TGC TCC GTG CAT CTG GCA TCA CCC TAA
23 Adapter primer TGT AGC GTG AAG ACG ACA GAA
24 Teltail primer TGC TCC GTG CAT CTG GCA TC-DIG

Analysis of telomere single-stranded DNA (ssDNA) by qPCR. Vesicle DNA was purified and analysed by quantitative amplification of ssDNA ³⁸. Briefly, DNA was amplified by two PCR rounds (2xPCR). In the first round, 4 ng of DNA was added to a 25 µl of a master mix comprising (final concentration in parentheses): water, 5X Colorless GoTaq® Reaction Buffer (1x), dNTPs (250 µM), telomere primer set (0,25 µl) and GoTaq® G2 DNA Polymerase (0.025 U/µl) and the samples subjected to: 2 cycles; 40°C for 5 min ramp to 72°C, at 2°C per min, and 10 min 72°C. PCR products were purified (QIAquick PCR Purification Kit, 28104, Qiagen) and subjected to a second qPCR round using the Absolute Human Telomere Length Quantification qPCR Assay Kit (8918, Sciencell). Vesicle DNA (4 ng) was also analysed by single round qPCR (1xPCR) using the same kit. Percentage of ssDNA was calculated as:

$$ssDNA(\%) = \frac{kb(2xPCR) - kb(1xPCR)}{kb(1xPCR)} * 100\%$$

Field Emission Scanning Electron Microscopy.

Samples (APCs or telomere vesicles purified by fluorescence-activated vesicle sorting), were adhered on Ply-L-Lysine-coated round glass coverslips of 1 cm diameter, then fixed with 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer (pH 7.4) at room temperature for 1h. Fixed samples were rinsed 10 minutes with 0.1M sodium cacodylate buffer (3 times), then dehydrated through ethanol gradients (30%, 50%, 70%, 85%, 95%, 100% - 10 mins at room temperature for each ethanol concentration). A 1:1 ethanol: hexamethyldisilazane (HMDS) mix was then added to the samples for 5 min at room temperature followed by a final 5 min step incubation in HMDS. All samples were then left drying overnight. The upper base of each aluminium stub was coated with colloidal silver paint and the

coverslips were pasted onto them. Samples mounted on stubs were coated with a gold layer by Q150R S Rotary-Pumped Sputter Coater (Quorum Technologies) and examined by FESEM (Sigma-Zeiss) at an accelerating voltage of 2 kV using secondary electron (SE) detection. A number of regions of interest (ROIs) containing single or multiple APCs for each sample were recorded to confirm absence of glass beads (fig. S2B) or to depict structural alterations (fig. S8C). Micrographs of telomere vesicles were used to perform particle size distribution analysis by measuring the diameter of each adhered telomere vesicle.

Immunogold analysis of telomere vesicles.

Human APCs (10^8) were live-labelled with TelC-biotinylated PNA probes as above described, then stimulated with ionomycin for 18h. The 10,000g ultra-centrifuged supernatants (1mL) were then loaded over 100 ul of 30% sucrose solution in PBS without mixing the two layers, and ultracentrifuged at 100,000g for 90 minutes at 4 °C using Sorvall™ WX 90+ ultracentrifuge (Thermo Fisher Scientific). The supernatants were then discarded and the sucrose layer (~200 ul) washed with 1 ml ice-cold PBS followed by further ultracentrifugation at 100,000g for 90 minutes. Vesicle pellets were resuspended in 40 µL fixing solution (2% paraformaldehyde, 0.125% glutaraldehyde in 0.1 M sodium phosphate buffer), then fixed for 1h at 4°C on a rocker. 5-µl of vesicle preparations were mounted on Formvar/Carbon 200 Mesh Cu grids (Agar Scientific Ltd), and the grids left to air dry for 10 min. Vesicles were permeabilized with 0.1 % saponin diluted in PBS for 30 min, and washed twice in

1 PBS. Samples were incubated in blocking buffer (1% Bovine Serum Albumin
2 (BSA), 20 mM glycine in PBS) for 45 min, then labelled with Streptavidin-collodial
3 10 nm gold conjugate (S9059, Sigma Aldrich; 1:10 dilution) in
4 blocking/permeabilizing buffer at 4 °C for 1h on a rocker. The vesicles were
5 washed 6 times in blocking buffer (1:10 in PBS) followed by incubation in 1%
6 (v/v) glutaraldehyde for 5 min and washed in ddH₂O. Grids were negatively
7 stained using 1% phosphotungstic acid (Electron Microscopy Sciences) for 1 min
8 and the excess removed using a filter paper. Finally, the grids were air dried for
9 10 min and examined on a Philips EM 208S (FEI) transmission electron
10 microscope with an accelerating voltage of 80 kV. Images were captured on a
11 MegaView III (Olympus Soft Imaging Solutions). In control experiments, APCs
12 were not labeled with TelC-biotin probes prior to ionomycin-based activation and
13 EV purification that confirmed absence of telomere vesicles with immunogold-
14 labelling (data not shown)

15

16 Exclusion of APC-telomeres in T cell plasma membranes.

17 APC-telomeres were live-labelled with TelC telomere probes, extracted by
18 ionomycin, and transferred to autologous T cells activated by anti-CD3 plus anti-
19 CD28. Twenty-four hours later, T cell plasma membranes were purified with
20 Plasma Membrane Protein Extraction Kit (ab65400; Abcam) and absence APC
21 telomeres assessed by confocal imaging on Leica SP2. Plasma membrane
22 purification was confirmed by CellMask dye (Invitrogen).

23

24 Statistical analysis.

25 GraphPad Prism was used to perform statistical analysis. For pairwise

comparisons, a paired Student's *t*-test was used. For three matched groups, a one-way analysis of variance (ANOVA) for repeated measures with a Bonferroni post-test correction was used. For synapse studies, Mann-Whitney test was used. **P* < 0.05, ***P* < 0.01 and ****P* < 0.001 and error bars indicate S.E.M. throughout.

Data availability.

The data generated in this study are included in the main paper and its supplementary information files.

Extended Data Figure 1. Telomere elongation without DNA synthesis. (a)

Representative IF-FISH and **(b, top)** pooled data showing telomere elongation in CD27⁺ CD28⁺ CD4⁺ T cells (purity 98.8%) and concomitant telomere shortening in APCs after forming the synapse. T cells were allowed to interact with autologous CMV-loaded APCs for the indicated time points. Conjugates were fixed, and analysed by IF-FISH with TelC telomere probe, DAPI (nuclei) and anti-CD3 (T cells). Three donors. Scale bar, 10 μm. T=0, initial time at which conjugates are observed (20 min). Images were z-stacked and the raw telomere integrated fluorescence signals (AU, arbitrary units) are shown. 102 conjugates were analysed. **(b, bottom)** Analysis of telomere length by qPCR in APCs and T cells after forming conjugates. **(c-d)** T cells were transfected with sgCtrl or sgTERT CRISPR constructs, activated with anti-CD3 plus anti-CD28, and elimination of telomerase was confirmed by **(c)** qPCR and **(d)** TRAP assay. **(e)** Telomerase positive and negative CD27⁺ CD28⁺ CD4⁺ T cells (purity 98.8%) were exposed to APCs and telomere content was quantified by flow-FISH using TelC telomere probe. Absolute telomere length by Flow-FISH was determined

from Mean Fluorescence Intensity (MFI) values using a standard curve formed by cryopreserved samples with known telomere length as determined by TRF. **(f)** Telomerase positive and negative T cells (identified by GFP expression) were pre-incubated with BrdU (a thymidine analogue incorporated into nascent DNA) for 4h, washed to remove any BrdU excess, then stimulated with APCs for 48h. Telomere content was then measured by flow-FISH using either TelC or TelG telomere specific probes coupled to anti-BrdU detection (to monitor telomere elongation vs DNA synthesis in T cells). Flow FISH demonstrated that T cells stimulated with APCs elongated telomeres without synthesizing DNA. The T cells remained indeed BrdU⁻ while increasing their telomere content. This suggested that the source of telomeres may be provided directly by the APCs. **(g)** Telomere length by flow-FISH demonstrating telomere elongation in T cells treated with DNA polymerase inhibitors aphidicolin and thymidine (both at 0.5 μ g/mL) prior to exposure to APCs for 48h. Note telomere extension occurs even when DNA synthesis is halted. In **(b, top)** ANOVA with Bonferroni-post test correction, in **(b, bottom, c, d)** student's t test. * $P < 0.05$ and *** $P < 0.001$. Error bars indicate S.E.M. throughout.

Extended Data Figure 2. Generation of live APCs with fluorescent telomeres. **(a)** PNA-telomere probes were dissolved 1:50 in telomere loading buffer (80 mM KCl, 10 mM K₂PO₄, 4 mM NaCl, pH 7.2) and gently introduced on adherent APCs by rolling large glass beads (size: 400-600 nm). The beads are used to produce small holes in the APC plasma membranes that allow telomere labeling while preserving viable APCs (no cell fixative) needed for subsequent synapse studies with T cells. Arrow indicates beads. The beads are completely

1 removed by washing with PBS prior to assays. **(b)** Absence of any residual glass
 2 beads from APCs was confirmed by field emission scanning electron microscopy
 3 (FESEM). Scale bar, 2 μ m. **(c)** Identical telomere detection by IF-FISH (fixed
 4 cells) or glass bead-mediated telomere PNA probe delivery (live cells) in APCs.
 5 Four experiments. Scale bar, 20 μ m. **(d)** Manders co-localization scores of
 6 experiments as in **(c)**. Negative control, APCs with unlabelled telomeres. **(e)**
 7 POT1 recruitment to telomeres detected by microbead-mediated telomere PNA
 8 probe delivery in human APCs, directly *ex vivo*. Scale bar, 10 μ m. **(f)** Manders
 9 co-localization scores of experiments as in **(e)**. Two experiments. Error bars
 10 indicate S.E.M. throughout.

11

12 **Extended Data Figure 3. APCs donate telomere in vesicles.** **(a)** Z-stack 3D
 13 reconstruction showing telomere transfer through the immune synapse. APCs
 14 were live labelled with TelC probes then conjugated with telomere-unlabelled
 15 CD27⁺ CD28⁺ CD4⁺ T cells (purity 97%) for 12 hours. Scale bar, 5 μ m. **(b, top**
 16 **left)** Telomere vesicle release triggered by antigen-specific contacts. Vesicles
 17 were ultracentrifugated from culture medium, treated with DNAase (10 U, 45') to
 18 remove extra-vesicular DNA then lysed with addition of 1% non-ionic detergent
 19 (Triton X-100). **(b, top left)** Alu release in cells as in **(b, bottom left)**. **(b, right)**.
 20 Note that, unlike telomeric DNA, Alu release is minimal and does not increase
 21 upon antigen challenge. The results from six independent experiments are
 22 shown. **(c, left and middle)** Side scatter (SSC) threshold and calibration beads
 23 used in FAVS-based vesicle purifications. Mixed silica/polystyrene nanobeads
 24 were used to determine particle size and calibrated on the forward scatter (FSC)
 25 axis, gated on singlets, to accommodate the very small size of the telomere

1 vesicles. **(c, right)** All vesicles produced by APCs were identified by staining the
 2 cells with PKH67 lipid dye (488 channel) prior to ionomycin activation. An
 3 unstained PKH67 control is also shown to identify the threshold for the positive
 4 population in the stained sample. A gating strategy was applied to purify
 5 individual vesicles <100 nm up to 300 nm. Larger particles (>300 nm) were
 6 instead due to the formation of vesicle aggregates and were therefore excluded
 7 from sorting. The size of the vesicles determined by flow cytometry was bigger
 8 than that calculated by electron microscopy, possibly due to heterogeneity in the
 9 refractive index of vesicles compared to that of the calibration beads. **(d, left)**
 10 Single staining control (PKH67 staining) and **(d, middle and right)** Presence of
 11 telomere vesicles (TTAGGG⁺ PKH67⁺ single particles) in ~10% of the total APC
 12 single particle vesicle fraction. APCs were live labelled with the membrane lipid
 13 dye PKH67 and the TelC telomere probe using glass beads and activated with
 14 ionomycin for 18h. Cell free supernatants were subjected to fluorescence
 15 activated vesicle sorting. **(d, far right)** The pooled data from 9 depletion
 16 experiments are shown. **(e)** The telomere content in purified vesicles was
 17 confirmed by qPCR as shown. **(f)** Presence of a small population (~1%) of
 18 vesicle free telomeres released by APCs treated as in **(d)**. **(g)** Dot-blot analysis of
 19 telomeric DNA isolated in EV pellets by sequential centrifugation of APC
 20 supernatants under native or denaturing conditions. APC genomic DNA (gDNA)
 21 served as loading control. **(h)** Super-resolution microscopy of FAVS-purified
 22 TTAGGG⁺ PKH67⁺ vesicles (Tel⁺ vesicles). Dashed line indicates vesicle
 23 magnification. Scale bar, 200 nm. **(i)** TEM analysis of Tel⁻ vs Tel⁺ vesicles
 24 performed as in **Fig. 1f**. Examples for exosome-sized vesicles **(top)** and
 25 microvesicles **(bottom)** are shown for both Tel⁻ and Tel⁺. Quantifications of

1 telomeric DNA in exosome-sized vesicles vs microvesicles are shown (**right**). (**j**)
 2 Further examples of T cell chromosomes with APC telomeres upon telomere
 3 transfer as per **Fig. 1h**. In (**b, d, e**), student's t test, in (**i**) Mann-Whitney test
 4 *** $P < 0.001$.

5 **Extended Data Figure 4. APC telomeres at T cell chromosome ends. (a)**

6 Representative metaphase spreads showing T cell chromosomes with APC-
 7 derived telomeres. CD4⁺ T cells (purity 97%) T cells were incubated with cell-free
 8 supernatants from ionomycin-activated APCs that were live-labelled with TelC
 9 probes using glass beads. Scale bar, 0.85 μ m. TelC PNA probe control assay is
 10 shown below. No PNA signal could be detected in APC supernatants upon DNA
 11 digestion with DNAase, demonstrating that the fluorescent signals originates from
 12 real telomeric DNA. Note that DNA digestion only takes place in the presence of
 13 Triton-X 100 demonstrating that the fluorescent signals are real telomeric DNA
 14 not just an excess of PNA probes in the fluorescent supernatants that are
 15 themselves resistant to the enzymatic digestion. (**b**) Metaphase spreads
 16 generated as in (**a**) were treated with 1unit T7 endonuclease for 30 min at 37 °C.
 17 The number of T cell chromosomes with APC telomeres before (black values)
 18 and after (red values) T7 endonuclease treatment is shown. Endonuclease
 19 treatment destroyed T cell chromosomes with APC telomeres while the total
 20 number of APC telomeres remained unaffected. (**c**) Presence of APC-derived
 21 telomeres in purified T cell plasma membranes vs T cell nuclei 24h after transfer
 22 of APC-free supernatants derived as in (**a**). (**d**) Quantification of APC telomere
 23 signal after T cell chromatin immunoprecipitation. Telomeres were extracted from
 24 Cy-3-PNA labelled APCs with ionomycin then transferred to T cells. The
 25 endogenous T cell telomere complex was immunoprecipitated (IP) 24h later with

anti-POT1 and the Cy3-fluorescence of APC-derived telomeres quantified with a microplate reader. Control IP, T cell extracts precipitated with irrelevant IgG. Presence of APC-derived telomeres was confirmed by adding DNase directly to the POT1 IP for 10 min at RT prior to fluorescence reading. Two donors. Error bars indicate S.E.M. throughout.

Extended Data Figure 5. APC-T cell telomere fusion does not require MHC

signalling. (a) Release of CD63⁺ telomere vesicles on planar bilayers documented by brightfield illumination. Scale bar, 10 μ m. (b) Further examples of telomere vesicles release on bilayers from APCs treated as in **Fig. 2c**. (c) IgG isotype control for experiment in **Fig.1b** and **Extended Data fig.5a**. (d) CD27⁺ CD28⁺ CD4⁺ T cells (purity 97.5%) were cultured for 48h with 5,000 telomere vesicles (Tel⁺) purified from APCs by fluorescence activated vesicles sorting, in the presence or absence of blocking MHC II antibodies (1 μ g/mL). T cell nuclear extracts were then analysed by qPCR with telomere and single copy reference primers. 5,000 telomere depleted vesicles (Tel⁻) served as control. In (d) Anova with Bonferroni post correction test, ****P** < 0.01. ns, not significant. Error bars indicate S.E.M. throughout.

Extended Data Figure 6. Telomere transfer does not cause APC death. (a)

Analysis of cell death of APCs after stimulation with ionomycin for 18h. These experiments were performed 7 times. Representative plots (**left**) and percentages (**right**) of total dead cells (Annexin V+/PI-cells, Annexin V+/PI+ and Annexin V-/PI+ cells are shown. (b) FESEM micrographs (10,000x) of resting

APCs or activated APCs upon treatment with ionomycin or H₂O₂ for 18h. Scale bar 1µm. Pooled data from 12 micrographs (3-5 APCs per micrograph at 10,000x magnification) depicting %APCs with structural alterations (blebbing or membrane damage) are shown. Note that ionomycin treatment does not induce membrane blebbing. APCs treated with H₂O₂ (500µM) served as positive control throughout experiments. **(c, left)** APCs were coupled to T cells in the presence or in the absence of antigens for 18h then analysed as in **(a)**. **(c, right)** These experiments were performed 3 times. **(d)** APCs were separated into their main subsets of DCs, Monocytes and B cells by FACS sorting then 10⁶ cells/subset were live labelled with TelC probes and PKH67 lipid dye, stimulated with ionomycin for 18h, followed by FAVS analysis. **(d, right)** Cumulative data from 3-4 experiments are shown. **(e)** The sorting strategies and related purities; to derive DCs (99.1%), Monocytes (99.3) and B cells (98.8%) for experiments in **(d)**. In **(d)** Anova with Bonferroni post correction test, ****P** < 0.01. Error bars indicate S.E.M. throughout.

Extended Data Figure 7. TZAP is required for telomere transfer. **(a)** Immunoblot analysis of EV pellets derived by sequential centrifugation of APC supernatants after activation with ionomycin for 18h. APC cell lysates (20%) were used as input control. Representative of 3 experiments. **(b)** Protein cargo analysis in FAVS purified telomere vesicles (Tel⁺) or telomere depleted vesicles (Tel⁻) by indirect ELISA. The results from nine experiments are shown. **(c)** APCs treated as indicated for 18h were analysed by immunoblot against cleaved-caspase 3 as apoptosis marker, and the telomere trimming factor TZAP. Representative of 3 experiments. Tubulin served as loading control. **(d)**

1 Representative TRF analysis (**left**) and pooled data (n=3; **right**) of TZAP
 2 dependent telomere trimming *in vitro*. TZAP was immunoprecipitated from APCs
 3 cultured with or without ionomycin for 18h and the immunoprecipitates were
 4 incubated for additional 18h at 30°C with genomic DNA extracted from resting
 5 APCs. TRF analysis determined telomere shortening upon incubation with TZAP
 6 but not control IgG immunoprecipitates that was enhanced by ionomycin. (**e**)
 7 Validation of TZAP depletion. Human APCs were transfected with siCtrl or
 8 siTZAP for 72h followed by immunoblotting to TZAP. GAPDH served as loading
 9 control. The numbers indicate quantification of knock-down efficiency. (**f**)
 10 Analysis of telomeric DNA in EV pellets isolated by ultra-centrifugation from
 11 APCs transfected as in (**e**) and activated with ionomycin for 18h. Representative
 12 results and pooled data from 5 experiments are shown (**right**). (**g**) TZAP transfer
 13 at the immune synapse. APCs were transduced with lentiviral vectors encoding
 14 TZAP tagged to cyan-fluorescent-protein (CFP-TZAP), conjugated 24h with T
 15 cells in the presence of antigen mix then analysed by IF-FISH with TelC probe. In
 16 APCs transduced with mock vector, no transfer was observed (data not shown).
 17 In (**b**, **f**), student's t test In (**d**), Anova with Bonferroni post correction test, $*P <$
 18 0.05 , $**P < 0.01$. $***P < 0.001$. Error bars indicate S.E.M. throughout.

21 **Extended Data Figure 8. Additional shelterin data.** (**a**) Representative images
 22 (**left**) and quantification (**right**) of TRF2 levels by immunofluorescence in APCs
 23 left either untreated, or pre-incubated with the proteasome inhibitor MG-132
 24 ($1\mu\text{M}$) then conjugated with CD27⁺ CD28⁺ CD4⁺ T cells (purity 97.5%) for 24h.
 25 T=0, initial time at which conjugates are observed (20 min). Images were z-

stacked and the raw telomere integrated fluorescence signals (AU, arbitrary units) are shown. 48 conjugates were analysed. Scale bar, 10 μ m. **(b, top)** Validation of shelterin overexpression (TRF2 and POT1) by immunoblotting in APCs. Numbers indicate shelterin overexpression efficiency. H2B, loading control. **(b, bottom)** IF-FISH demonstrating recruitment of artificial shelterin factors (POT1 and TRF2) to telomeres in APCs transduced with lentiviral vectors and activated with ionomycin for 18h. The Pearson's co-localization score for artificial POT1 and TRF2 with APC telomeres are shown. Scale bar, 5 μ m. **(c)** Immunoblot analysis of TRF2 and POT1 following indicated siRNA treatment in human APCs. H2B served as loading control. The numbers indicate knock-down efficiency. Representative of 3 separate experiments. **(d)** Dot-blot analysis of telomeric DNA from the EVs released by APCs transfected as in **(c)** and activated with ionomycin for 18h. Quantification of the arbitrary units (AU) from 4 independent experiments **(top)** and representative dot-blot using TelC probe **(bottom)**. In **(a)**, Kruskal-Wallis with Dunn's post-correction test; in **(d)**, Mann-Whitney test. $**P < 0.01$. Error bars indicate S.E.M. throughout.

Extended Data Figure 9. Defective recombinogenic potential in Rad51-deficient telomere vesicles **(a)** Immunoblot analysis of the indicated DNA damage factors in EV pellets derived by sequential centrifugation of APC supernatants following activation with ionomycin for 18h. APCs served as whole cell lysate control. Representative of 3 separate experiments. **(b)** Immunoblots of Rad51 following indicated siRNA treatment in human APCs. H2B served as loading control. The numbers indicate knockdown efficiency. Representative of 3 separate experiments. **(c)** FESEM analysis of 200 siRad51 telomere vesicles

purified by fluorescence activated vesicle sorting among cell-free supernatants of APCs transfected as in (b) then activated with ionomycin for 18h. (d) Protein cargo in siRad51 or siCtrl telomere vesicles by indirect ELISA. Telomere vesicles (250 per sample, in triplicate wells) were purified by FAVS from cell-free supernatants of APCs transfected with either siCtrl or siRad51 short-interference RNA, then stimulated with ionomycin for 18h. Pooled data from 4 experiments are shown. (e) Pooled data (n=3) showing reduced single-strand telomeric DNA in siRad51 vs siCtrl telomere vesicles by quantitative amplification of single-stranded DNA (QAOS). (f) Metaphase Q-FISH coupled to TFL software analysis showing elongation of individual T cell chromosome ends upon transfer of telomere vesicles derived from APCs. Each dot represents an individual T cell chromosome with APC-derived telomeres; 370 (siCtrl) and 378 (siRad51). (g) TRF analysis comparison of siCtrl vs siRad51 EVs isolated by 100,000g ultracentrifugation following activation with ionomycin for 18h. Pooled data from 3 experiments are shown (right). (h) Reduced fusion (elongation) between siRad51 telomere vesicles and T cell telomeres. Recipient CD27⁺ CD28⁺ CD4⁺ T cells (purity 98.7%) T cells were treated with 5,000 FACS-purified telomere vesicles (Tel⁺) from siCtrl or siRad51 APCs and total nuclear T cell extracts were analysed by qPCR 48h later. 5,000 telomere depleted vesicles (Tel⁻) were also transferred as control. In (d, e) student's t test, in (f) Mann-Whitney test, in (h) Anova with Bonferroni post-correction test. *p<0.05, **p<0.001 ***P < 0.001. Error bars indicate S.E.M. throughout.

Extended Data Figure 10. Telomere vesicles, but not telomerase reactivation, eliminate ultra-short telomeres. (a) Expansion of human T cells

1 by heterologous telomere vesicles. Control T cells were cultured 10 days in the
 2 presence of 1,000 telomere vesicles (Tel⁺) or telomere depleted vesicles (Tel⁻)
 3 purified by FAVS and derived from either mismatched human (h) or mouse (h)
 4 APCs, as indicated. T cells cultured without any vesicles are also shown. Each
 5 dot is an independent T cell culture. **(b)** Confirmation of CRISPR-based
 6 telomerase enhancement in CD27⁺ CD28⁺ CD4⁺ T cells (purity 97.5%) T cells 96
 7 hours after transduction by TRAP assay (**top**) and immunoblots (**bottom**). The
 8 numbers indicate efficiency of CRISPR-based TERT overexpression (TERT-OE).
 9 Controls were transduced with mock vectors throughout. **(c)** Population doublings
 10 of T cells treated as in **(b)**, then cultured for up to 30 days. **(d)** Telomere transfer
 11 effects are telomerase independent. Telomerase positive and negative T cells
 12 were activated with anti-CD3 and anti-CD28 for 10 days in the presence of 250
 13 telomere vesicles derived from APCs and their numbers enumerated. **(e)** PCR
 14 based assays (U-STELA) showing reduced load of ultra-short telomeres (<3kb)
 15 in T cells activated by anti-CD3 plus anti-CD28 for 48h followed by transfer of
 16 1,000 FAVS-purified telomere vesicles (Tel⁺) or vector-based telomerase
 17 enhancement as in **(b)**. Controls were transduced with mock vector or 1,000
 18 telomere depleted vesicles (Tel⁻). Results from 5-8 experiments were normalized
 19 to the number of genome equivalent used in PCR amplification reactions (5
 20 genome equivalent, 1 genome equivalent = 8pg DNA). **(f)** Defective proliferation
 21 in T cells supplemented with siTZAP telomere vesicles compared to those
 22 expressing TZAP. **(g)** APCs were transduced with a lentiviral vector encoding for
 23 a cyan fluorescent variant of TZAP (artificial TZAP), labelled with PKH67 lipid
 24 dye, and stimulated with ionomycin for 18 hours. The vesicle enriched
 25 supernatants were analysed by FAVS. Note that only telomere vesicles contain

TZAP (**left**). Every dot is a PKH67⁺ vesicle. TZAP⁺ vesicles are indeed capable to boost T cell expansion as much as telomere vesicles without fluorescent tagging of TZAP over a 10 day experiment (**right**). In (**a, c-g**), Anova with Bonferroni post-correction test, in (**b**) student's t test, and. * $p < 0.05$, ** $p < 0.001$ *** $P < 0.001$. Error bars indicate S.E.M. throughout.

Extended Data Figure 11. T cells with APC telomeres do not upregulate sestrins. (**a**) Telomere vesicles were extracted with ionomycin from APCs that had been previously transfected with either siCtrl or siRad51 RNAs then transferred to T cells. CD27⁺ CD28⁺ CD4⁺ T cells (purity 98.5%) were then activated by anti-CD3 plus anti-CD28 for 10 days. IF demonstrated that activated T cells do not up-regulate senescence-associated sestrin proteins (sestrin 1) upon transfer of Tel⁺ vesicles. By contrast, T cells up-regulated sestrins (albeit to a lesser extent) upon transfer of siRad51 Tel⁺ vesicles. Two donors. In (**b**), Kruskal-Wallis with Dunn's post-correction test. * $P < 0.05$; *** $P < 0.001$.

Extended Data Figure 12. Phenotypic analysis of T cells with APC telomeres. Human CD27⁺ CD28⁺ CD4⁺ T cells (purity 97.5%) were activated with anti-CD3 and IL-2 for 10 days in the presence of 250 FACS-purified telomere vesicles (Tel⁺) derived from either human or mouse APCs prior to multiparametric flow cytometry. Control T cells were activated without any vesicle or with 250 telomere depleted vesicles (Tel⁻) obtained by fluorescence activated vesicle sorting. (**a**) Representative plots and (**b**) pooled data from 5 experiments are shown. In (**b**) Anova with Bonferroni post correction test. * $P < 0.05$; ** $P < 0.001$ and *** $P < 0.001$.

1

2 **Extended Data Figure 13. Naïve and central memory, but not senescent,**
3 **cells acquire telomeres from APCs.** (a) Analysis of telomere transfer by flow-
4 fish flow cytometry upon conjugation of Tel-live labelled APCs and total CD3⁺ T
5 cells for 24 hours. Each dot is an individual experiment (n = 3-4). Control, APC
6 loaded with antigens and stimulated with T cells but without telomere labelling
7 throughout experiments (no APC telomere). No antigen control is also shown
8 confirming antigen dependency. (b) Naïve and central memory, but not
9 senescent, cells acquire telomeres from APCs. Purified T cell populations (naïve
10 98.7%; central memory 95%; effector memory 97.5%; emra 94%) were treated
11 as in (a) and telomere transferred was measured by flow FISH (n = 3-4).

12

13 **Extended data fig. 14. Existence of T cell chromosomes with telomeres of**
14 **APC origin in mice.** Quantification of APC telomeres at mouse T cell
15 chromosomes upon *in vivo* APC labelling. Mice were administered Edu in
16 drinking water for 14 days, culled, then Edu-labelled APCs were coupled with
17 unlabelled CD4⁺ T cells (purity 99%) from congenic mice not exposed to Edu. IF-
18 FISH on metaphase spreads obtained by colcemid demonstrated presence of
19 APC telomeres at T cell chromosome ends, similarly to their human counterparts.
20 Two representative examples and relative ZOOMs are shown. As control, mouse
21 T cells were analysed by IF-FISH in the absence of telomere vesicles (no vesicle
22 control) or in the presence of telomere depleted vesicles (**not shown**) with
23 identical results (no Edu labelling). Scale bar, 2µm. Edu incorporation control in
24 donor APC prior to telomere transfer is also shown in the FACS plot.

25

Extended data fig. 15. Enhanced expansion and longevity of T cells with APC telomeres in the spleen, LN, but not in the blood, of recipient mice *in vivo*. (a) Naïve CD45.2 OTII T cells were incubated with TelC labelled APCs in the presence of OVA for 18 hours then sorted into Tel⁺ (T cells with APC telomeres) versus Tel⁻ (T cells without APC telomeres) subpopulations based on telomere transfer prior to transfer into CD45.1 recipients and vaccination with OVA. Effector responses were assessed 5 days post-transfer; for memory responses mice were re-vaccinated with OVA 40 days after the first vaccination and observed for additional 50 days (90 days). Note that the *in vitro* efficacy of telomere transfer is much lower than that observed *in vivo*, possibly due to the well-recognized lower efficiency of *in vitro* APC-T cell conjugates versus their physiological counterparts *in vivo*³⁵. (b, left) Absolute numbers of Tel⁺ versus Tel⁻ for experiments as in **Fig. 4g** (5 days post-transfer; effector response). (b, right) Phenotypic analysis of Tel⁺ versus Tel⁻ during effector responses as in **Fig. 4g**. (c, left) Absolute numbers of Tel⁺ versus Tel⁻ for experiments as in **Fig. 4h** (90 days post-transfer; memory response). (c, right) Phenotypic analysis of Tel⁺ versus Tel⁻ during memory responses for experiments as in **Fig. 4h**. (d) Percentage of Tel⁺ versus Tel⁻ in the lymph-nodes and (e) blood of recipient mice vaccinated with OVA during effector and memory responses. (f) Phenotypic analysis of CD45.2 OT II T cells cultured in the presence of telomere vesicles, (siCtrl tel) telomere depleted vesicles or telomere vesicles depleted of Rad51 (siRad51 tel) from APCs, adoptively transferred into CD45.1 recipients, then vaccinated with OVA two times (immediately and 40 days post-transfer). The mice were observed for 70 days as per experiments in **Fig. 4j** (g) Percentage of cells as in (f) in the LNs of recipient mice. In (b, c, d) student's t test; in (f, g) Anova with Bonferroni

1 post-correction test. * $p < 0.05$, ** $p < 0.001$ *** $P < 0.001$. Error bars indicate S.E.M.
2 throughout.

3

4 **Extended data fig. 16. APCs donate telomere vesicles to promote T cell**
5 **lifespan.** Upon antigen-specific contacts with T cells (1-2), APCs degrade
6 shelterin (3) to donate telomeres (4), which are cleaved in TZAP-dependent
7 manner and then transfer in vesicles at the synapse with T cells (5). Telomere
8 vesicles are devoid of shelterin but retain the Rad51 recombination factor that
9 mediates APC-T cell telomere fusion causing an average lengthening of 3000
10 base pairs (6). The result is a T cell that escapes senescence within 24 hours of
11 synaptic interactions (7). That T cell will become an antigen-specific stem like
12 memory cell.

13

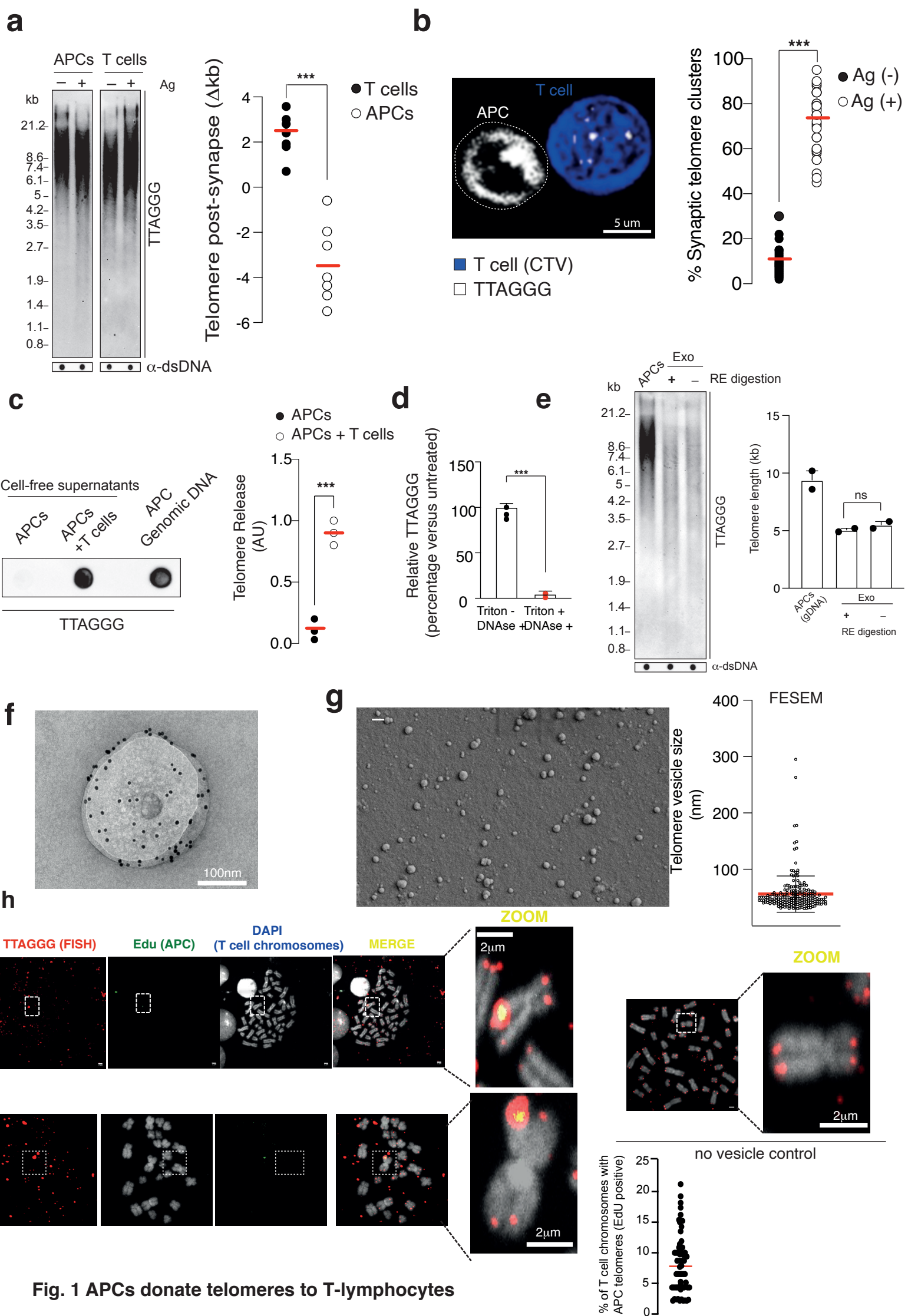


Fig. 1 APCs donate telomeres to T-lymphocytes

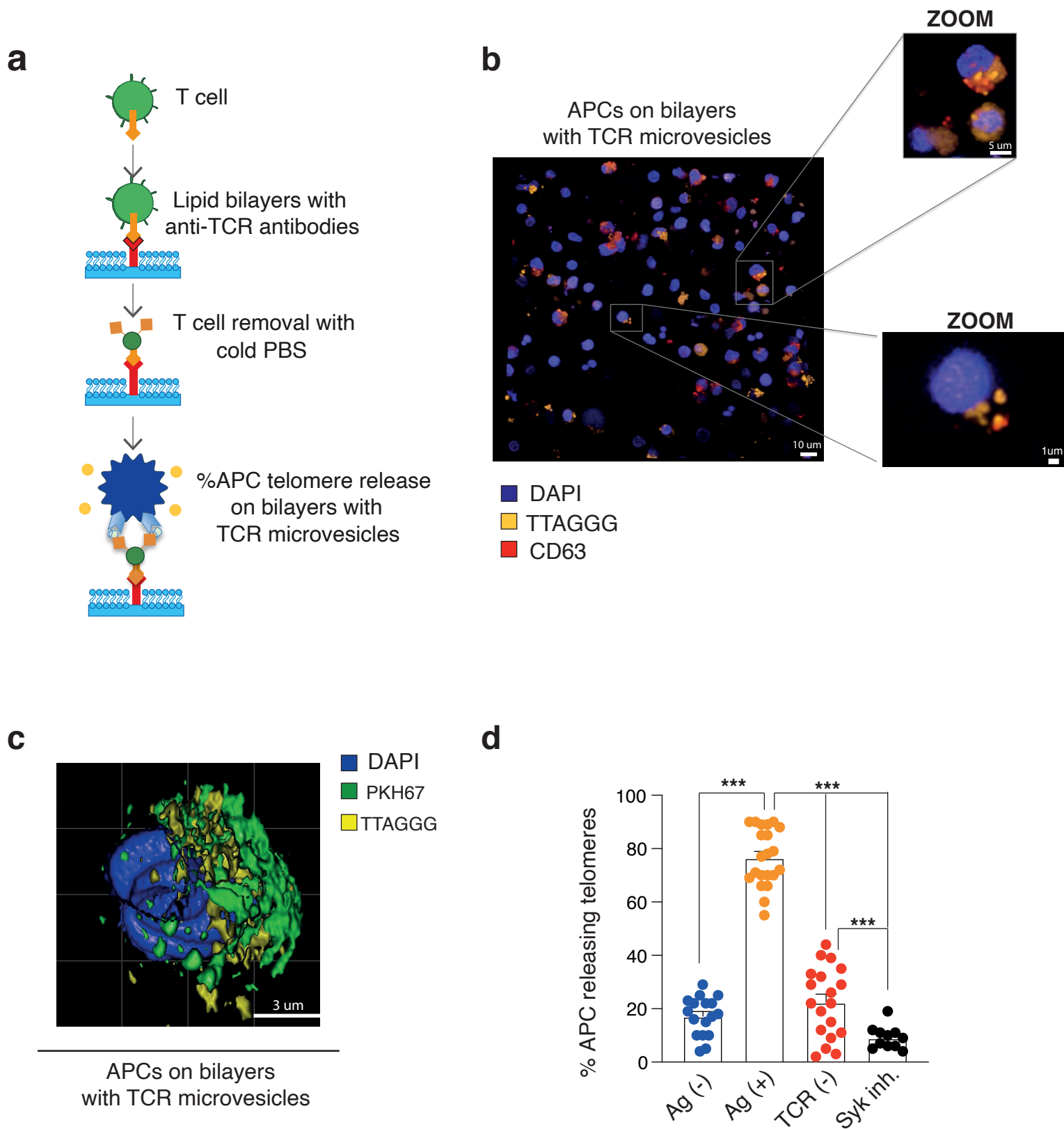
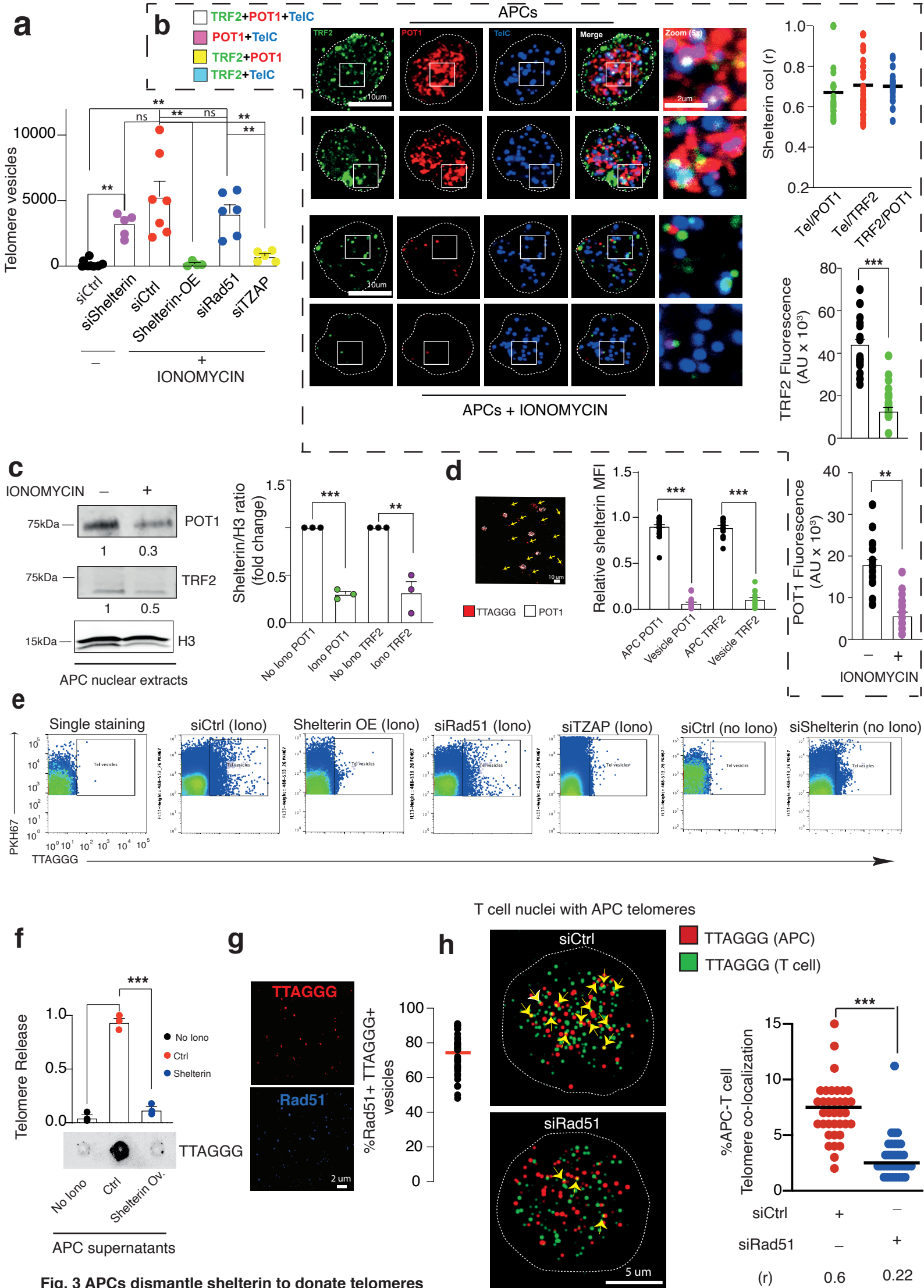


Fig . 2 Synaptic TCRs are sufficient to extract telomere vesicles from APCs



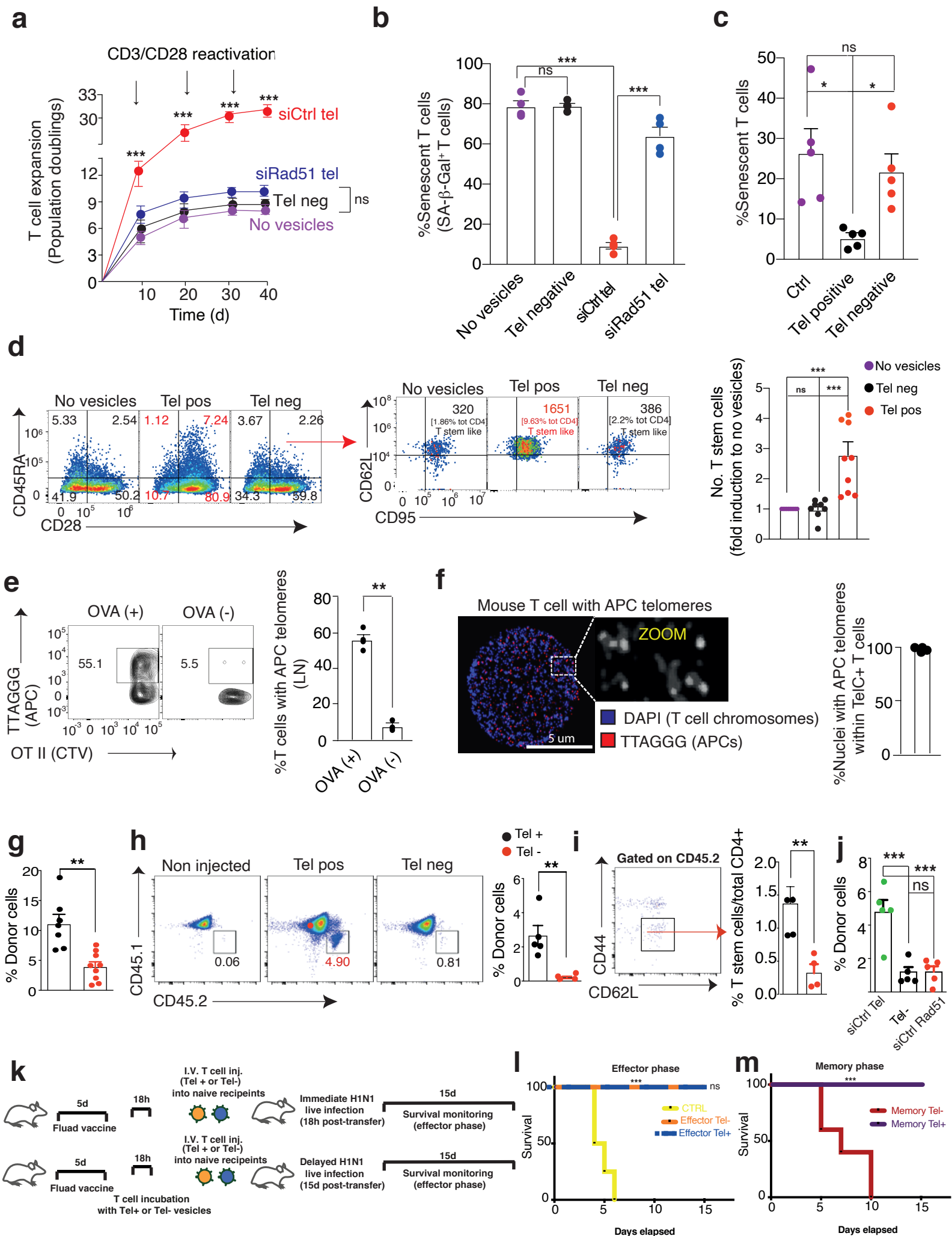


Fig. 4 Generation of long lasting immunity by telomere transfer