An ultraspecific monoclonal antibody recognises a novel marker on stem memory T cells and induce cell proliferation and differentiation *in vitro* and *in vivo*

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INTRODUCTION

Stem memory T cells (TSCMs)

- TSCMs can be found in humans, mice and non-human primates at low frequency (2-4%).
- TSCMs possess higher proliferative, greater survival responses and superior anti-tumour capacity compared to central memory (TCM) and effector memory (TEM) T cells.
- TSCMs have self-renewal capacity and multipotency, capable of reconstituting the full diversity of the effector and memory T cell compartments on serial transplantation.
- Clinical exploitation of TSCMs is currently not feasible due to their relative low percentage in the periphery and lack of robust clinical grade protocols for isolating and expanding these cells.

2811 monoclonal antibody (mAb)

- Highly specific mAb against SSEA-4.
- Recognises human and mouse TSCMs.
- Induces TSCM proliferation and differentiation.

2811 mAb- ultraspecific against SSEA-4

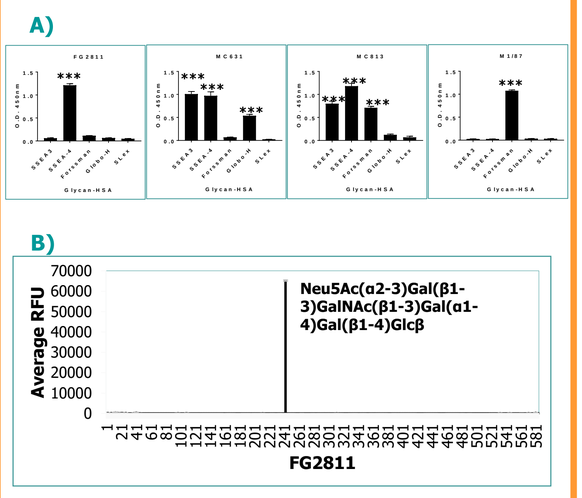


Figure 1. Assessment of 2811 mAb specificity towards SSEA-4.
A, Binding of 2811 mAb to HSA-coupled glycan antigens was assessed by ELISA. Commercial mAb MC631 (anti-SSEA-3), MC813 (anti-SSEA-4) and M1/87 (anti-Forssman) were included as comparisons. Biotinylated anti-mouse IgG secondary antibody (no primary mAb) was used as negative control. Antibody activity was measured by absorbance at 450nm.

B, Binding of 2811 mAb to 600 glycans on Consortium for Functional Glycomics glycan array.

2811 mAb recognises human TSCMs

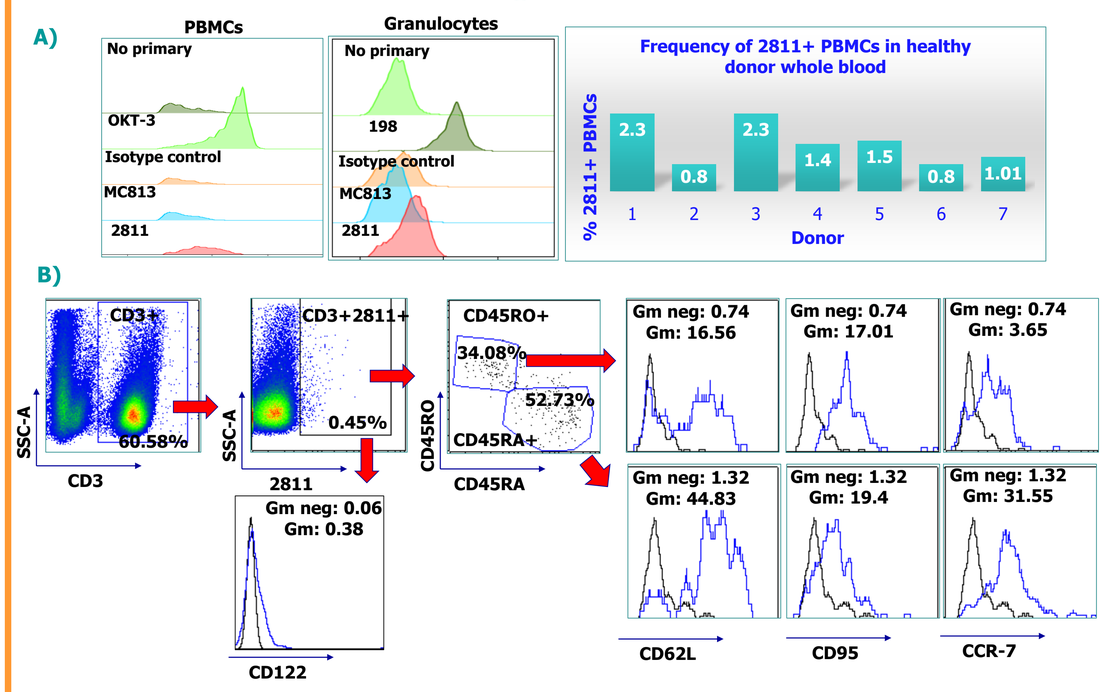


Figure 2. The 2811 mAb bound to healthy normal donor blood cells.
A, Binding of 7 healthy donor whole blood with 2811, MC813, mouse IgG1 isotype control antibody, OKT3 (anti-CD3), 198 (anti-CEACAM6) and secondary antibody alone (no primary mAb) was assessed by indirect immunofluorescence staining and flow cytometric analysis (result is representative of 7 donors)

B, Successive panels depicting the flow cytometric gating strategy used to phenotype $CD3^+2811^+$ T cells. The $CD3^+2811^+$ T cells were checked for the expression of CD122, CD45RA and CD45RO markers. The $CD45RA^+$ and $CD45RO^+$ populations were further checked for the expression of CD62L, CD95 and CCR-7 markers (result is representative of 3 donors).

2811 mAb induces human T cell proliferation *in vitro*

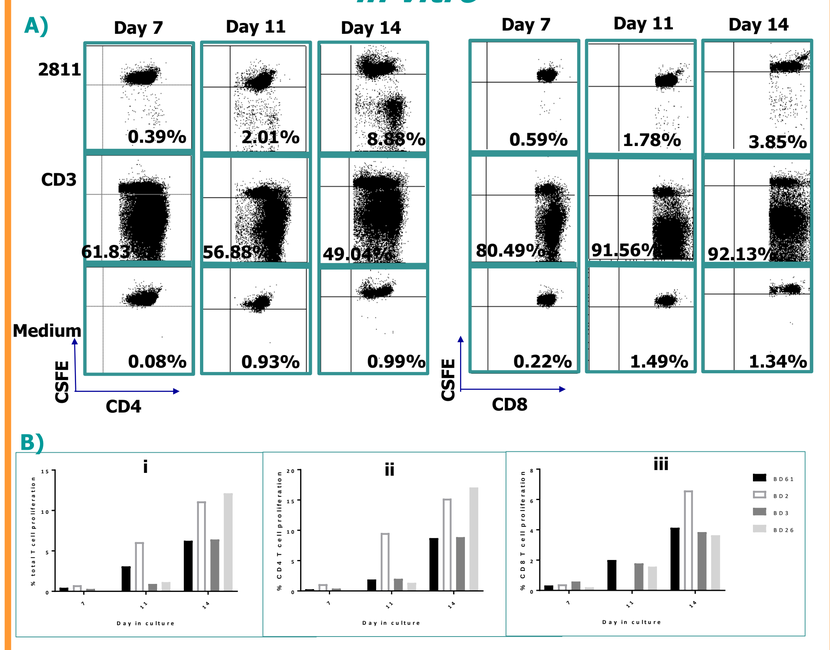


Figure 3. Plate bound 2811 mAb induced T cell proliferation.
A, CSFE labelled T cells from healthy donors were stimulated with plate bound 2811 mAb, anti-CD3 mAb and medium; cells were collected at day 7, 11 and 14 to check for CD4 and CD8 T cell proliferation. Percentages of specific T cell population proliferation were assessed via CSFE dye dilution analysis (result is representative of 4 donors).

B, Summary of i) total T cell proliferation, ii) CD4 T cell proliferation and iii) CD8 T cell proliferation from 4 healthy donors, as assessed by CSFE dye dilution analysis.

2811 mAb stimulated T cells remains viable *in vitro* for more than 2 months

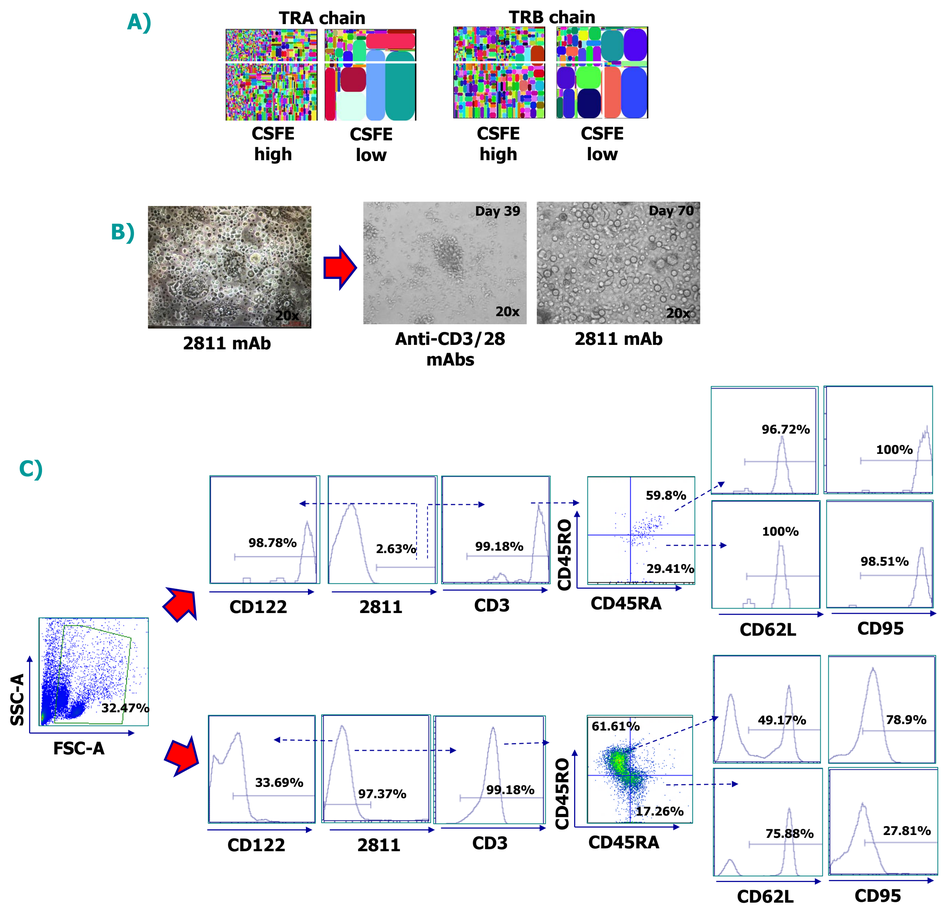


Figure 4. 2811 stimulated T cells remained viable *in vitro* for more than 2 months without any exogeneous cytokines
A, Assessment of TCR repertoire clonotype in 2811 stimulated T cells. T cell repertoire is detected from the extracted RNA of CSFE high and low 2811 stimulated T cells from 2 healthy donors. TCR repertoire diversity is illustrated in tree maps where each rounded rectangular represents a unique entry: V-J-uCDR3 and the size of the spot denotes the relative frequency.

B, At day 35, under light microscope, cells stimulated with anti-CD3 mAb and unstimulated cells were all dead except cells stimulated with 2811 mAb.

C, Successive panels depicting the flow cytometric gating strategy used to phenotype viable cells at day 35. Gates were drawn for analysis on 2811^+ and 2811^- cells; they were checked for CD3 and CD122 expressions. The $CD3^+$ cells were further checked for the expression of CD45RA, CD45RO, CD62L and CD95 markers.

Self-sustaining cytokines for 2811+ T cells

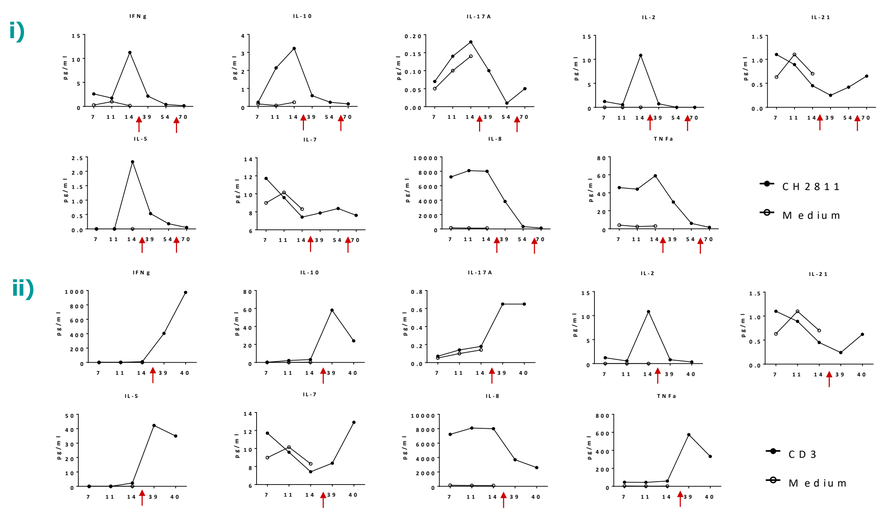
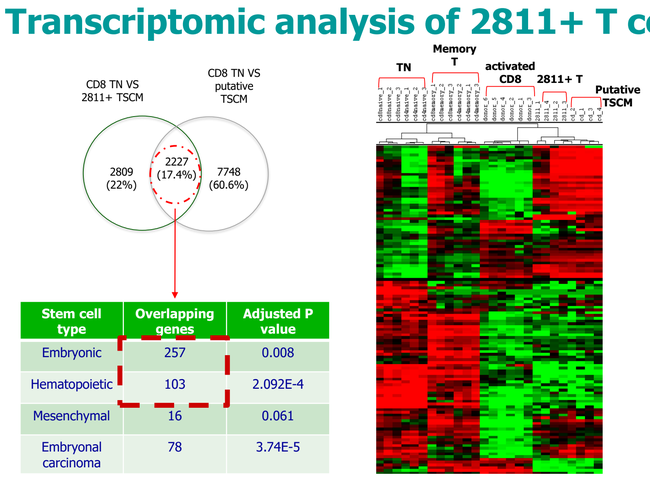


Figure 5. IL-7 and IL-21 could be crucial self-sustaining cytokines for the *in vitro* long-term survival of 2811 stimulated T cells. Representative cytokine/ chemokine expression levels (pg/ml) in i) 2811 and ii) anti-CD3/28 restimulated T cells. T cells were stimulated with CH2811 at day 0 followed by restimulation with either 2811 at day 33 and day 64 or with anti-CD3/28 antibodies at day 33. Supernatants were collected at day 7, 11, 14, 39, 54 and 70 and assessed for the concentration of cytokines/chemokines(pg/ml). Arrows depicted antibody restimulation day.

Transcriptomic analysis of 2811+ T cells



Stem cell type	Overlapping genes	Adjusted P value
Embryonic	257	0.008
Hematopoietic	103	2.092E-4
Mesenchymal	16	0.061
Embryonal carcinoma	78	3.74E-5

Figure 6. Transcriptomic analysis of 2811-enriched TNs and CD95/CD122-enriched TNs from 4 healthy donors using bulk RNA sequencing.
Venn diagram showing the common genes between the two sets of differentially expressed (DE) genes obtained through comparing CD8 TNs (GSE83808) with 2811-enriched TNs and CD95/CD122 TNs, respectively. The 2227 common genes displayed significant overlap with stemness signatures from embryonic and hematopoietic stem cells (StemChecker).
Heatmaps and hierarchical clustering of selected genes from 2811-enriched and CD95/CD122-enriched transcriptomic profiles, based on the DE genes between TN and TSCM (GSE23321).

T cell agonistic effect in C57/B6 mice

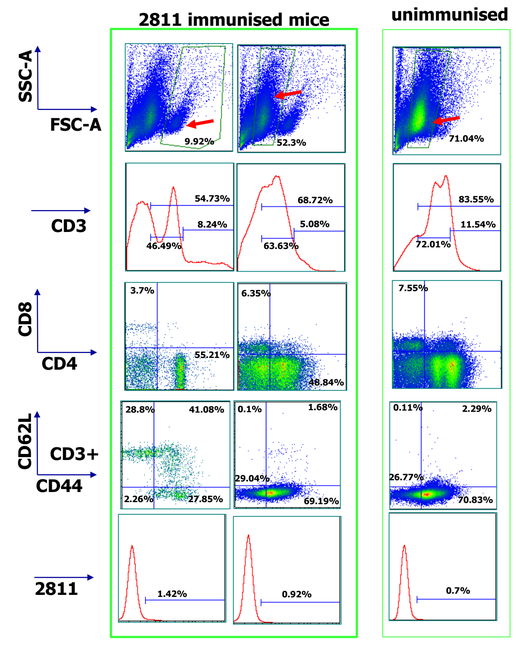


Figure 7. 2811 mAB induced phenotypic TSCM cells in C57/B6 mice.
C57/B6 mice were immunised with 2811 mAb at day 0; unimmunised mice were included as control. At day 16, mice were euthanised and splenocytes were cultured *in vitro* for 7 days without any exogeneous cytokines. 7 days later, splenocytes were harvested and stained with CD3, CD4, CD8, CD44, CD62L and 2811 mAbs and assessed using flow cytometric analysis.

Conclusions

- **SSEA-4 is a novel marker for human and mouse TSCMs.**
- **2811 mAb able to identify, isolate and expand putative TSCM *in vitro* and *in vivo*.**
- **2811+ T cells are potential candidates for genetic manipulation to express antigen specific TCRs or CARs.**
- **2811 is a potential T cell agonistic mAb**