

SUPPLEMENTARY MATERIALS

Supplementary materials and methods

CM-specific CD4⁺ T cell stimulation and characterization of CM-reactive CD4⁺ T cells

To isolate CM-reactive T cells, frozen PBMCs were revived, rested and stimulated ($0.5 \times 10^6/\text{ml}$) using 100 $\mu\text{g}/\text{ml}$ cow milk protein (CM) (*Bos taurus*, Greer Laboratories, #XPF395D3A25), 1 $\mu\text{g}/\text{ml}$ CD40 (clone: HB14, Miltenyi Biotec., #130-094-133) and 1 $\mu\text{g}/\text{ml}$ CD28 (clone:15E8, Miltenyi Biotec.,#130-093-375) functional grade antibodies for 6 hours in RPMI media containing 10% human serum (5% CO₂, 37°C). After 6 hours, PBMCs were washed and stained with fluorescent-labeled antibodies and surface labeled for 30 mins at 4°C prior to fluorescent activated cell sorting (FACS) of CD154⁺ (Tcon^{CM+}) and CD137⁺ (Treg^{CM+}) on BD FACS Aria III. For intracellular staining of Foxp3 and Helios, eBioscience Foxp3/transcription factor staining buffer set (ThermoFisher, #00-5523) was used and acquired on Cytex Aurora. The following are the entire list of antibodies used:

<i>Antibody</i>	<i>Source</i>	<i>Identifier</i>
CD3 - BV785 (Clone: SK7)	BioLegend	344842
CD4 - BUV496 (Clone: SK3)	BD Horizon™	564651
CD8 - Pacific blue (Clone: SK1)	Biolegend	344718
CD25 - BV711 (Clone: 2A3)	BD Horizon™	563159
CD127 (IL-7R α) - AF700 (Clone: A019D5)	Biolegend	351344
CD154 (CD40L) - APC (Clone: 89-76)	BD Biosciences	648887
CD137 MicroBead Kit, human	Miltenyi Biotec	130-093-476
CD137 PE (Clone: 4B4-1)	BD Pharmingen™	555956
CD183 (CXCR3) - PE-Cy7 (Clone: G025H7)	Biolegend	353720
CD194 (CCR4) - BV510 (Clone: L291H4)	Biolegend	359416
CD196 (CCR6) - BV605 (Clone: G034E3)	Biolegend	353420
CCR10 - PerCP-Cy5.5 (Clone : 1B5)	BD Pharmingen	564772
FOXP3 - PE-Cy5 (Clone: PCH101)	Thermo Fisher Scientific	15-4776-42
HELIOS - FITC (Clone: REA829 22F6)	Miltenyi Biotec	130-112-629
Zombie NIR™ Fixable Viability Kit	BioLegend	423106