

## **Immunophenotyping Details**

For analysis of co-stimulatory molecules in CD4<sup>+</sup> or CD8<sup>+</sup> T cells and CD4<sup>+</sup>CD25<sup>+</sup>CD127<sup>-</sup> Treg cells, after lysis of red blood cells (Immunoprep reagent systems, Beckman Coulter), cells (1 x 10<sup>6</sup> cells) were stained with the following combinations of monoclonal antibodies at 4°C for 20 min: 1) anti-ICOS-FITC (clone ISA-3, eBioscience), anti-PD1-PE (clone MIH4, BD Biosciences), anti-CD3-ECD (clone UCHT1, Beckman Coulter), anti-CD8-PE/Cy5 (clone B9.11, Beckman Coulter), and anti-CD4-PE/Cy7 (SFC112T4D11, Beckman Coulter); and 2) anti-CD3-FITC (clone UCHT1, Beckman Coulter), anti-CD137-PE (clone 4B4-1, BD Biosciences), anti-CD8-ECD (clone SFC121ThyD3, Beckman Coulter), anti-OX40-PE/Cy5 (clone ACT35, BD Biosciences), and anti-CD4-PE/Cy7 (SFC112T4D11, Beckman Coulter) and 3) anti-CD3-FITC (clone UCHT1, Beckman Coulter), anti-CD127-PE (clone R34.34, Beckman Coulter), anti-CD25-ECD (clone B1.49.9, Beckman Coulter), anti-CD27-PE/Cy5 (clone 1A4CD27, Beckman Coulter), and anti-CD4-PE/Cy7 (SFC112T4D11, Beckman Coulter). For analysis of Foxp3<sup>+</sup> Treg cells, whole blood cells (200 µl) were lysed (BD Pharm Lyse, BD Biosciences) and stained with the cocktail of anti-CD3-ECD (clone UCHT1, Beckman Coulter), anti-CD25-PE/Cy5 (clone B1.49.9, Beckman Coulter), and anti-CD4-PE/Cy7 (SFC112T4D11, Beckman Coulter) at 4°C for 20 min. Subsequently, after fixation and permeabilization according to the manufacturer's instructions (eBioscience), cells were stained with anti-Foxp3-FITC (clone PCH101, eBioscience) and anti-CTLA-4-PE (clone BNI3, Beckman Coulter) at 4°C for 30 min.