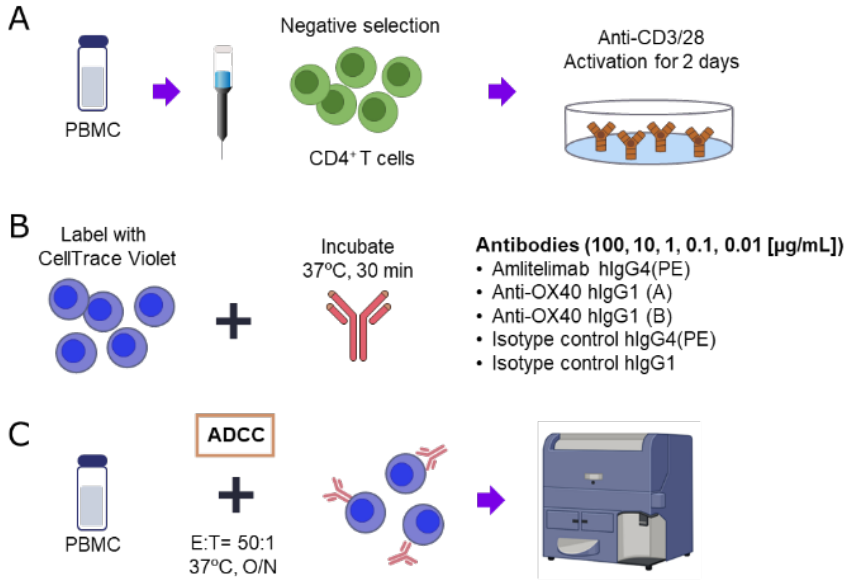

Fig S1. In vitro assay to evaluate ADCC activity against activated CD4$^+$ T cells and Foxp3$^+$ Tregs by amlitelimab and anti-OX40 antibodies

(A) CD4$^+$ T cells were enriched from PBMCs by negative selection and activated by anti-CD3 (10µg/mL)/anti-CD28 (2µg/mL) to induce OX40 expression. (B) Activated CD4$^+$ T cells (target cells) were labeled and co-cultured with unlabeled autologous NK-containing PBMCs (effector cells) in the presence of amlitelimab, two anti-OX40 IgG1 antibodies [anti-OX40 hIgG1 (A) and (B)], and respective isotype controls. (C) ADCC activity was assessed by flow cytometry evaluating the viability of activated CD4$^+$ T cells (CD4$^+$CD25$^+$Foxp3$^-$) and Foxp3$^+$ Tregs (CD4$^+$CD25$^+$Foxp3$^-$CD127$^-$).

ADCC, antibody-dependent cellular cytotoxicity; CD, cluster of differentiation; h, human; Ig, immunoglobulin; NK, natural killer; O/N, overnight; PBMC, peripheral blood mononuclear cell; PE, S228P L235E; Treg, regulatory T cell.

Fig S2. In vitro assay to evaluate ADCC activity of OX40L IgG1 and IgG4 antibodies

OX40L expression on HEK cells was confirmed. Activity of amlitelimab was compared to two different depleting anti-OX40L IgG1 antibodies [anti-OX40L hIgG1 (C) and (D)] and isotype controls using the DELFIA™ cell cytotoxicity kit. Fluorescence proportional to target-cell lysis was then measured to assess ADCC activity.

Ab, antibody; ADCC, antibody-dependent cellular cytotoxicity; EuTDA, non-radioactive Europium 2,2':6'2"-terpyridine-6,6"-dicarboxylic acid; HEK, human embryonic kidney; h, human; Ig, immunoglobulin; NK, natural killer; PE, S228P L235E; TRF, time-resolved fluorescence.