siRNA mediated gene knockdown

For *SPTLC* triple knock down (TKD) and *UGCG* single knock down (KD) experiments, freshly-isolated $CD4^+$ cells were suspended in Optimem I (Invitrogen) and transfected with siGenome SMARTpool small interference RNA (siRNA) oligonucleotides (Dharmacon) using the nucleofection technique by Lonza. Scrambled non-targeting siRNA (5'-AAUUCUCCGAACGUGUCACGU-3') was used as control (Sigma). Briefly, four million cells were transfected with 12 µg of *SPTLC*-targeting siRNAs (4 µg of SMARTpool *SPTLC1* siRNA M-006673-02; 4 µg of SMARTpool *SPTLC2* siRNA M-006674-01; and 4 µg of SMARTpool *SPTLC3* siRNA M-010285-02) or 12 µg of Scramble siRNA. For UGCG single knockdown experiments 12 µg of UGCG-targeting siRNA (siGenome SMARTpool, M-006441-02) were used. Cells were rested for 24h in RPMI 1640 medium (Sigma-Aldrich) supplemented with penicillin/streptomycin, 2 mM L-glutamine and 10% FCS and subsequently activated and cultured under Th17 conditions. *SPTLC1* and *SPTLC2* knockdown was validated by western blot at 24 hours, *UGCG* and *SPTLC3* knockdown was determined using quantitative real-time PCR (at 12 and 72 hours, respectively).