14d post tumour induction in vivo, mice with TCS-conditioned, stromal-dense tumours had significantly fewer activated CD4+ and CD8+ CD25+ expressing T cells, both intratumourally and distally in the draining lymph nodes and spleen. They also had low levels of cytotoxic granzyme B-expressing CD8+ T cells. Interestingly, this suppression was sialylation-dependent. De-sialylation of TCS-conditioned stromal cells led to restoration of activated T cell levels in tumours and peripheral lymphoid tissues, as well as a marked increase in cytotoxic T cells (figure 3).

Conclusions These results demonstrate, for the first time, that tumour stromal cells suppress activated T cells through sialic acid-dependent interactions. We show that targeting stromal cell sialylation may represent a novel immune checkpoint to reactivate anti-tumour immunity.

Ethics Approval The animal study was approved by the Animals Care Research Ethics Committee of the National University of Ireland, Galway (NUIG) and conducted under individual and project authorisation licenses from the Health Products Regulatory Authority (HPRA) of Ireland (AE19125/077). The study using human samples was approved by University Hospital Galway Ethics committee under an ethically approved protocol (Clinical Research Ethics Committee, Ref: C.A. 2074).

Abstract 1444 Figure 1 Exposure of stromal cells to tumour cell secretome or microenvironment increases sialic acid and specific Siglec ligand expression. (A) Mouse bone marrow-derived mesenchymal stromal cells (MSCs) have elevated SNA I (α2,6 sialic acid) and Siglec E ligand expression following exposure to tumour cell secretome from CT26 murine colon cancer cells (MSCTCS). Isotype control is shown as green histogram. (B) Cancer-associated fibroblasts (CAFs) isolated from colorectal cancer patient biopsies express higher levels of SNA I and Siglec 9 ligand compared to fibroblasts isolated from non-cancerous (NAF), adjacent intestinal tissue. Data are mean ± SD; *p < 0.05, **p < 0.01, and ***p < 0.001 using (A) unpaired t-test and (B) ratio paired test; n = 4 biological replicates.

Abstract 1444 Figure 2 CAFs induce an exhausted phenotype in CD8+ T cells which is reversible by de-sialylation. (A) Schematic overview depicting the process of NAFs and CAFs isolation. (B) Frequency (%) of CD8+PD-1+ and CD8+PD-1+Tim-3+ T cells after co-culture with NAFs or CAFs. (C) Frequency (%) of CD8+Siglec-7+ T cells after co-culture with NAFs or CAFs. Frequency (%) of CD8+Siglec-7+ T cells after co-culture with NaFAs (Cii) or CAFs (Ciii) pre-treated or not with a sialytransferase inhibitor (SI). Data are mean ± SD; *p < 0.05, **p < 0.01, and ***p < 0.001 using (A) unpaired t-test and (B) ratio paired t-test; n = 4 biological replicates.
Ci and Di) one-way ANOVA with a Tukey post hoc test and (Cii, Ciii, Dii and Diii) a ratio paired t test. n = 3-4 biological replicates

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Figure 3

TCS-conditioned stromal cells inhibit T cell activation and cytotoxicity in vivo and these effects can be reversed by targeting stromal cell sialylation. 8-12 week old female Balb/c mice were injected subcutaneously with 5x10⁵ CT26 colon cancer cells with or without 1.5x10⁵ TCS MSCs or Sialyltransferase inhibited TCS MSCs (SI-TCS) where indicated. 13 days post-injection, tumours, draining lymph nodes and spleens were harvested and analysed for the presence of specific immune cell markers by flow cytometry. Tumour conditioned stromal cells suppress T cell activation in the tumour, spleen and draining lymph node, as determined by the stromal mediated immunosuppressive effects on cytotoxic CD8 T cells are restored in the draining lymph and spleen – and partially restored in the tumour. Data are mean ± SD; *p < 0.05, **p< 0.01, ***p< 0.001 and ****p, 0.0001 using one-way ANOVA with a Tukey post hoc test. n = 4-5 biological replicates.