

Figure S1, related to Figure 1. *STC1* correlates to cancer resistance to immunotherapy

(A) qPCR showing the top 8 gene transcripts in murine tumor cell lines. Data are presented as mean \pm SEM, 2 tail T-test was used for two-way comparisons ($n = 3-5$, $*p < 0.05$, $**p < 0.01$).

(B) Murine tumor *STC1* released in culture medium measured with ELISA. One of 3 experiments is shown.

(C) Relationship of *STC1* expression with T cell activation in cohort 1. ($n = 14$, $p = 0.031$). The dash line represents the median value, the bottom and top of the boxes are the 25th and 75th percentiles (interquartile range). Whiskers encompass 1.5 times the inter-quartile range.

(D) Relationship of *STC1* expression with cancer patient survival. Two combined cohorts treated with anti-CTLA4 (Van-Allen et al., 2015, Nathanson et al., 2017). High ($n = 15$) and low ($n = 50$) levels of *STC1* expression, $p = 0.0167$.

(E) Forest plot representing the adjust value of Cox proportional hazard ratio (HR) in patients treated with anti-PD-1, anti-CTLA4, and the combination as 95% confidential interval (CI) of overall survival.

(F) Relationship of *STC1* expression with cancer patient survival analyzed on primary SKCM. High ($n = 38$) and low ($n = 37$) levels of *STC1* expression, $p = 0.0387$.

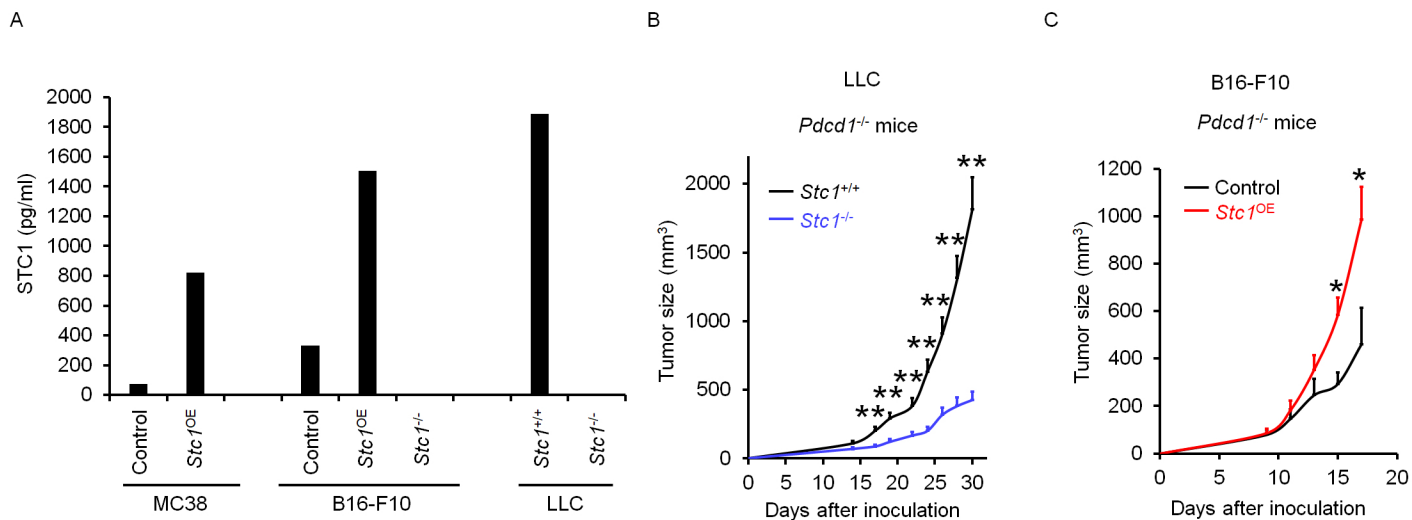


Figure S2, related to Figure 2. Tumor STC1 is critical for intrinsic resistance to tumor immunity

(A) Tumor STC1 released in culture measured with ELISA. One of 3 experiments is shown.

(B-C) Tumor growth curves of *Stc1*^{+/+} and *Stc1*^{-/-} LLC (B) or control and *Stc1*^{OE} B16-F10 (C) in *Pdcd1*^{-/-} C57BL/6J mice. Tumor volume was monitored. Data are shown as mean ± SEM, 2 tail T-test was used for two-way comparisons (n = 5-8, *p < 0.05, **p < 0.01).

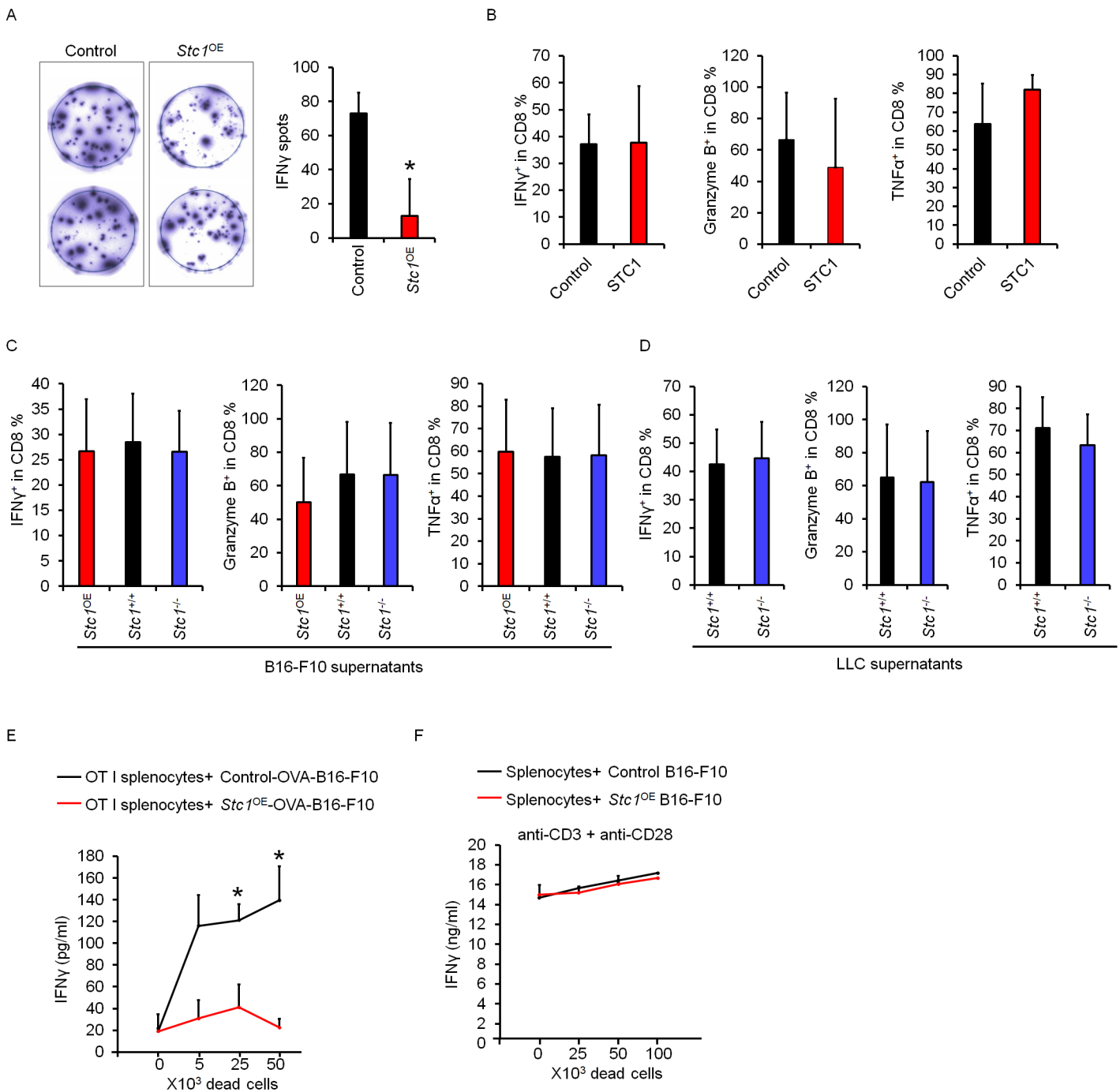


Figure S3, related to Figure 3. Tumor STC1 impairs anti-tumor CD8⁺ T cell responses

(A) IFN γ ELISPOT in tumor drained lymph node T cells from mice bearing control or *Stc1^{OE}* MC38 tumors. Data are shown as mean \pm SEM, 2 tail T-test was used for two-way comparisons (n = 5, *p < 0.05).

(B-D) Effect of STC1 on T cell activation. Splenocytes were activated for 72 hours with anti-CD3 and anti-CD28 in the presence of recombinant STC1 (A), or B16-F10 cell culture supernatants (B), or LLC cells culture supernatants (C). FACS showed the percentages of IFN γ^+ , granzyme B $^+$, and TNF α^+ CD8 $^+$ T cells. Data are shown as mean \pm SEM, 2 tail T-test was used for two-way comparisons (n = 3, *p < 0.05).

(E) Effect of STC1 on OT-I cell activation. OT-I splenocytes were cultured with dead tumor cells from OVA loaded-B16-F10 cells and OVA loaded- *Stc1^{OE}*-B16-F10 cells for 3 days. ELISA showed IFN γ production. Data are shown as mean \pm SEM, 2 tail T-test was used for two-way comparisons (n = 3-4, *p < 0.05).

(F) Effect of STC1 on T cell activation. Splenocytes were activated with anti-CD3 and anti-CD28 for 72 hours in the presence or absence of dead tumor cells from B16-F10 control and *Stc1^{OE}* cells. ELISA showed IFN γ production. Data are shown as mean \pm SEM (n = 3-4, *p < 0.05).

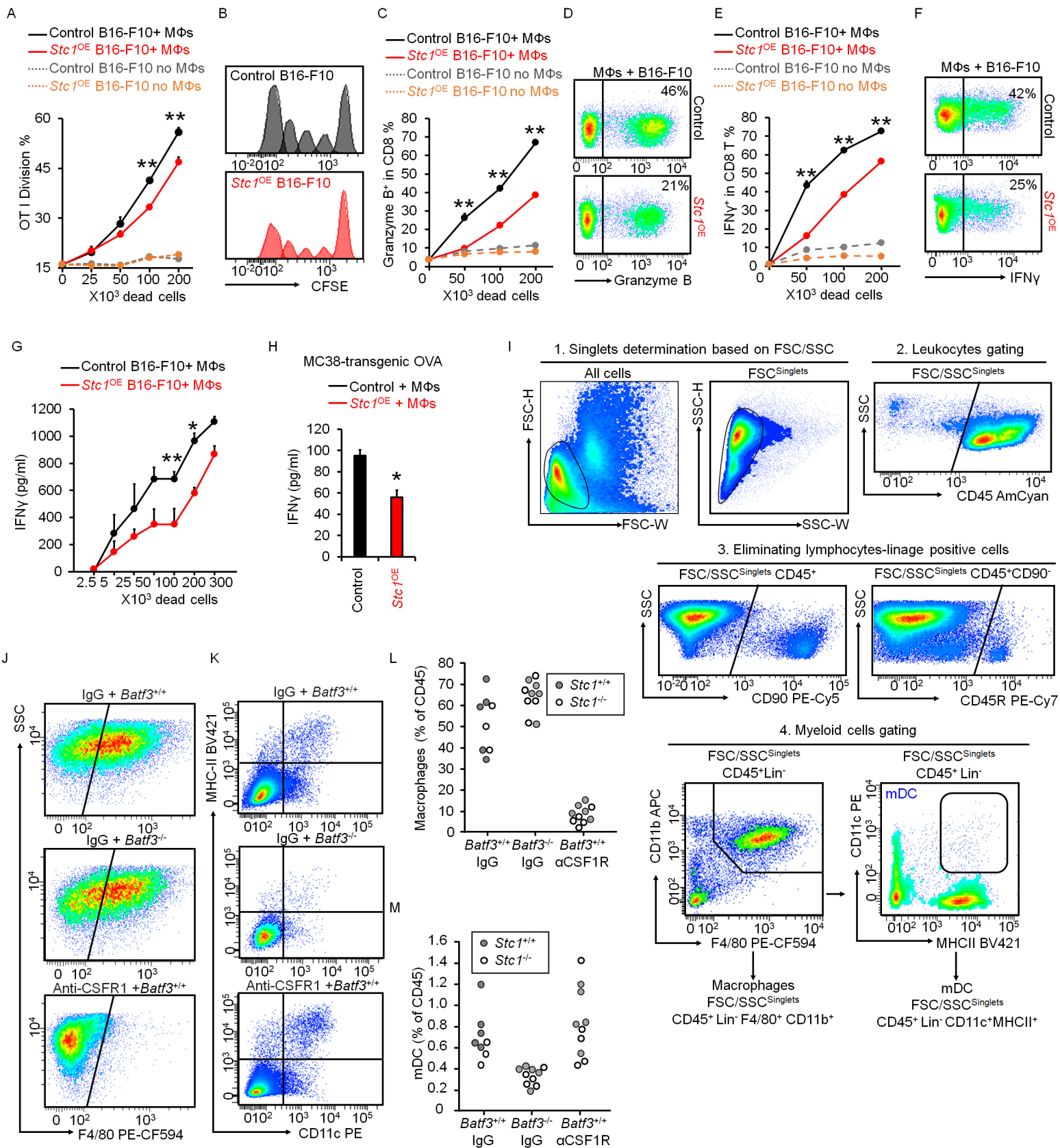


Figure S4, related to Figure 4. STC1 abrogates tumor immunogenicity via targeting APCs

(A-F) Effect of STC1 on OT-I cell activation. B16-F10 cells and *Stc1^{OE}* B16-F10 cells were loaded with OVA and killed with UV-irradiation. CFSE-labeled OT-I cells were cultured for 3 days with different numbers of dead B16-F10 cells and *Stc1^{OE}* B16-F10 cells in the presence of macrophages. FACS showed CFSE dilution (A-B), granzyme B⁺ (C-D), and IFN γ ⁺ (E-F) OT-I cells. Data are presented as mean \pm SEM, 2 tail T-test was used for two-way comparisons (n = 4-5, * p < 0.05, ** p < 0.01).

(G) ELISA detected OT-I cell released IFN γ in the setting as (A-F). Data are presented as mean \pm SEM, 2 tail T-test was used for two-way comparisons (n = 4-5, * p < 0.05, ** p < 0.01).

(H) ELISA detected OT-I cell released IFN γ . The experimental setting is similar to (A-F), but OVA-loaded B16-F10 cells were replaced with OVA transgenic control or *Stc1^{OE}* MC38 cells. Data are presented as mean \pm SEM, 2 tail T-test was used for two-way comparisons (n = 5, * p < 0.05).

(I) Representative FACS gating strategy to define tumor-infiltrating myeloid subsets from LLC tumors.

(J-M) FACS showing the changes of macrophages and DCs in LLC tumors under the treatment of IgG or anti-CSF1R in WT or *Batf3^{-/-}* mice.

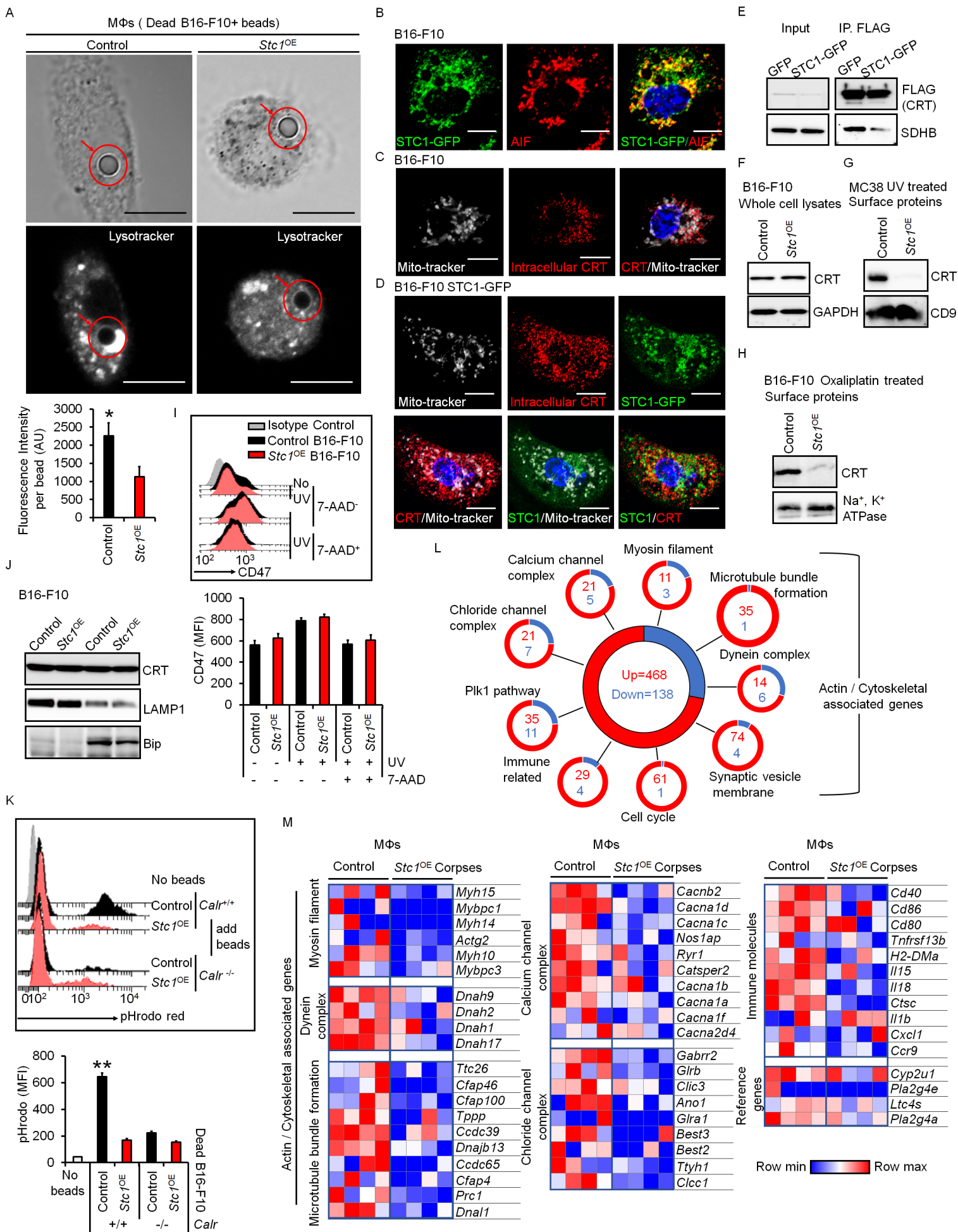


Figure S5, related to Figure 5. Tumor STC1 traps CRT to inhibit macrophage function

(A) Effect of STC1 on macrophage-mediated bead up-take. Macrophages were incubated with dead cells from B16-F10 cells and *Stc1*^{OE} B16-F10 cells. Latex beads were added (arrow). Macrophages were stained with lysotracker dye (white). Scale bars: 10 μ m. Results are shown as the fluorescence intensity of lysotracker dye at the bead areas in macrophage phagosomes, mean \pm SEM (n = 6, **p < 0.05).

(B) Localization of STC1. UV-treated STC1-GFP expressing B16-F10 cells were stained for GFP (STC1-GFP) (Green), AIF (red), and DAPI (blue). Confocal images showed the co-localization of STC1 and AIF. Scale bars: 10 μ m. One of 3 experiments is shown.

(C) Localization of CRT. UV-treated B16-F10 cells were stained for mito-tracker (white), CRT (Red) and DAPI (blue). Confocal images showed the co-localization of mito-tracker and CRT. Scale bars: 10 μ m. One of 3 is shown.

(D) Co-localization of STC1 and CRT. UV-treated STC1-GFP expressing B16-F10 cells were stained for mito-tracker (white), CRT (Red), GFP (STC1-GFP) (Green), and DAPI (blue). Confocal images showed the co-localization of mito-tracker, CRT, and STC1 (STC1-GFP) (Green). Scale bars: 10 μ m. One of 3 experiments is shown.

(E) Co-IPs of CRT-FLAG with SDHB were performed in cell lysates from UV-treated B16-F10 cells. One of 2 experiments is shown.

(F) Western blots showing expression of CRT in whole cell lysates from UV-treated B16-F10 cells and *Stc1*^{OE} B16-F10 cells. One of 2 experiments is shown.

(G) MC38 cell membrane CRT. Surface proteins from UV-treated or un-treated MC38 vehicle control cells and *Stc1*^{OE} cells were labeled with biotin. Western blots showed cell membrane CRT and CD9 in biotin-labeled proteins. CD9 serves as membrane protein control. One of 2 experiments is shown.

(H) B16-F10 cell membrane CRT. Surface proteins from oxaliplatin treated B16-F10 control cells and *Stc1*^{OE} cells were labeled with biotin. Western blots showed cell membrane CRT and Na⁺, K⁺ ATPase α 1 in biotin-labeled proteins. Na⁺, K⁺ ATPase α 1 serves as membrane protein control. One of 2 experiments is shown.

(I) FACS showing mean fluorescence intensity (MFI) of CD47 on UV-treated or un-treated B16-F10 control cells and *Stc1*^{OE} cells. Gated on either 7-AAD⁻ or 7-AAD⁺. Data are shown as mean \pm SEM (n = 4).

(J) Western blots showing distribution of CRT in lysosome (LAMP1) and ER (Bip) from UV-treated B16-F10 cells and *Stc1*^{OE} B16-F10 cells. One of 2 experiments is shown.

(K) Macrophage-mediated bead up-take. Macrophages were incubated with dead cells from *Calr*^{+/+} or *Calr*^{-/-} B16-F10 and *Stc1*^{OE} B16-F10 cells for 20 hours. pHrodo™-SE labeled 3 μ m latex beads were added for an additional 1 hour. FACS showed mean red fluorescence intensity (MFI) in macrophages. Gated on CD11b⁺CD45⁺. Data are shown as mean \pm SEM (n = 4, **p < 0.01).

(L-M) RNA-seq of macrophages engulfed control B16-F10 tumors and *Stc1*^{OE} B16-F10 tumors. Gene signatures were normalized to macrophages without engulfment. The heatmap depicts representative gene clusters differentially expressed between macrophages engulfed control B16-F10 tumors and *Stc1*^{OE} B16-F10 tumors.

Table S1, related to Figure 5. Mass spectrometry analysis of proteins interacting with STC1

Identified Proteins	Accession Number	Total Spectrum Count		Ratio (STC1 ^{FLAG} /Control)
		Control	STC1 ^{FLAG}	
Stanniocalcin-1	sp O55183 STC1_MOUSE	0	71	N/A
Protein disulfide-isomerase A3	sp P27773 PDIA3_MOUSE	0	31	N/A
Metastasis-associated protein MTA2	sp Q9R190 MTA2_MOUSE	0	26	N/A
Calreticulin	sp P14211 CALR (CRT)_MOUSE	0	19	N/A
Transforming acidic coiled-coil-containing protein 1	sp Q6Y685 TACC1_MOUSE	0	17	N/A
Heat shock protein HSP 90-alpha	sp P07901 HS90A_MOUSE	0	10	N/A
Elongation factor 1-alpha 1	sp P10126 EF1A1_MOUSE	0	9	N/A
Intracellular hyaluronan-binding protein 4	tr E9QKB2 E9QKB2_MOUSE	0	9	N/A
Isoform 2 of 14-3-3 protein theta	sp P68254-2 1433T_MOUSE	0	8	N/A
U5 small nuclear ribonucleoprotein 200 kDa helicase	sp Q6P4T2 U520_MOUSE	0	7	N/A
14-3-3 protein eta	sp P68510 1433F_MOUSE	0	7	N/A
Tight junction protein ZO-1	sp P39447 ZO1_MOUSE	0	6	N/A
Methyl-CpG-binding domain protein 2	sp Q9Z2E1 MBD2_MOUSE	0	6	N/A
Protein disulfide-isomerase A6	tr Q3TML0 Q3TML0_MOUSE	0	6	N/A
Pre-mRNA-processing-splicing factor 8	sp Q99PV0 PRP8_MOUSE	0	5	N/A
Polyadenylate-binding protein 1	sp P29341 PABP1_MOUSE	0	5	N/A
26S protease regulatory subunit 7	tr Q8BVQ9 Q8BVQ9_MOUSE	0	5	N/A
Nucleophosmin	sp Q61937 NPM_MOUSE	0	5	N/A
Protein RCC2	sp Q8BK67 RCC2_MOUSE	0	5	N/A
Tubulin beta-5 chain	sp P99024 TBB5_MOUSE	0	5	N/A
Eukaryotic translation initiation factor 3 subunit C	sp Q8R1B4 EIF3C_MOUSE	0	5	N/A
Isoform 2 of F-actin-capping protein subunit beta	sp P47757-2 CAPZB_MOUSE	0	5	N/A
Dynactin subunit 2	sp Q99KJ8 DCTN2_MOUSE	0	5	N/A
Chromobox protein homolog 3	sp P23198 CBX3_MOUSE	0	5	N/A
Endoplasmic	sp P08113 ENPL_MOUSE	0	5	N/A
Chromodomain-helicase-DNA-binding protein 4	sp Q6PDQ2 CHD4_MOUSE	0	4	N/A
Aminoacyl tRNA synthase complex-interacting multifunctional protein 1	sp P31230 AIMP1_MOUSE	0	4	N/A
Cyclin-dependent kinase 2-associated protein 1	sp O35207 CDKA1_MOUSE	0	4	N/A
Single-stranded DNA-binding protein	tr Q8R2K3 Q8R2K3_MOUSE	0	4	N/A
TBC1 domain family member 5	sp Q80XQ2 TBCD5_MOUSE	0	4	N/A
Pre-mRNA-splicing factor RBM22	sp Q8BHS3 RBM22_MOUSE	0	4	N/A
10 kDa heat shock protein, mitochondrial	sp Q64433 CH10_MOUSE	0	4	N/A
Src substrate cortactin	sp Q60598 SRC8_MOUSE	0	4	N/A
Peroxisome oxidoreductin-2	sp Q61171 PRDX2_MOUSE	0	4	N/A
40S ribosomal protein S19	sp Q9CZX8 RS19_MOUSE	0	4	N/A
60S acidic ribosomal protein P0	sp P14869 RLA0_MOUSE	0	4	N/A
Elongation factor 2	sp P58252 EF2_MOUSE	0	4	N/A
Protein TSSC4	sp Q9JHE7 TSSC4_MOUSE	0	4	N/A
Tubulin beta-4B chain	sp P68372 TBB4B_MOUSE	0	4	N/A
Isoleucine--tRNA ligase, cytoplasmic	sp Q8BU30 SYIC_MOUSE	0	3	N/A
Protein Qars	tr D3Z158 D3Z158_MOUSE	0	3	N/A
KN motif and ankyrin repeat domain-containing protein 4	sp Q6P9J5 KANK4_MOUSE	0	3	N/A
Stromal cell-derived factor 2	tr A0A0R4IZW9 A0A0R4IZW9_MOUSE	0	3	N/A
Alpha-centractin	sp P61164 ACTZ_MOUSE	0	3	N/A
Elongation factor 1-gamma	sp Q9D8N0 EF1G_MOUSE	0	3	N/A
oxoglutarate dehydrogenase complex, mitochondrial	sp Q9D2G2 ODO2_MOUSE	0	3	N/A
U2 small nuclear ribonucleoprotein A	sp P57784 RU2A_MOUSE	0	3	N/A
Protein disulfide-isomerase	sp P09103 PDIA1_MOUSE	0	3	N/A
Eukaryotic translation initiation factor 3 subunit G	sp Q9Z1D1 EIF3G_MOUSE	0	3	N/A
PERQ amino acid-rich with GYF domain-containing protein 2	sp Q6Y7W8 PERQ2_MOUSE	0	3	N/A
Eukaryotic translation initiation factor 3 subunit K	sp Q9DBZ5 EIF3K_MOUSE	0	3	N/A
60S ribosomal protein L12	sp P35979 RL12_MOUSE	0	3	N/A
Isoform 2 of Polycomb protein EED	sp Q921E6-2 EED_MOUSE	0	3	N/A

40S ribosomal protein SA	sp P14206 RSSA_MOUSE	0	3	N/A
Reticulocalbin-2	sp Q8BP92 RCN2_MOUSE	0	3	N/A
Nucleolar RNA helicase 2	sp Q9JIK5 DDX21_MOUSE	0	3	N/A
Complement component 1 Q subcomponent-binding protein	tr Q8R5L1 Q8R5L1_MOUSE	0	3	N/A
Small nuclear ribonucleoprotein Sm D1	sp P62315 SMD1_MOUSE	0	3	N/A
26S protease regulatory subunit 6B	sp P54775 PRS6B_MOUSE	0	3	N/A
40S ribosomal protein S20	sp P60867 RS20_MOUSE	0	3	N/A
Eukaryotic translation initiation factor 3 subunit M	sp Q99JX4 EIF3M_MOUSE	0	3	N/A
Endoplasmic reticulum resident protein 29	sp P57759 ERP29_MOUSE	0	3	N/A
Vesicle-associated membrane protein-associated protein B	sp Q9QY76 VAPB_MOUSE	0	3	N/A
26S proteasome non-ATPase regulatory subunit 11	sp Q8BG32 PSD11_MOUSE	0	3	N/A
Isoform 2 of Poly(U)-binding-splicing factor PUF60	sp Q3UEB3-2 PUF60_MOUSE	0	3	N/A
Aspartate--tRNA ligase, cytoplasmic	sp Q922B2 SYDC_MOUSE	0	3	N/A
Isoform 2 of Peptidyl-prolyl cis-trans isomerase H	sp Q9D868-2 PPIIH_MOUSE	0	3	N/A
Polyadenylate-binding protein 2	sp Q8CCS6 PABP2_MOUSE	0	2	N/A
LanC-like protein 1	sp O89112 LANC1_MOUSE	0	2	N/A
Collagen alpha-5(VI) chain	sp A6H584 CO6A5_MOUSE	0	2	N/A
Probable cytosolic iron-sulfur protein assembly protein CIAO1	sp Q99KN2 CIAO1_MOUSE	0	2	N/A
Eukaryotic translation initiation factor 4B	sp Q8BGD9 IF4B_MOUSE	0	2	N/A
U1 small nuclear ribonucleoprotein A (Fragment)	tr D3Z0S6 D3Z0S6_MOUSE	0	2	N/A
Dehydrogenase/reductase SDR family member 4	sp Q99LB2 DHRS4_MOUSE	0	2	N/A
Ferritin light chain 1	sp P29391 FRIL1_MOUSE	0	2	N/A
Coatomer subunit beta	sp O55029 COPB2_MOUSE	0	2	N/A
CD2 antigen cytoplasmic tail-binding protein 2	sp Q9CWX3 CD2B2_MOUSE	0	2	N/A
Leucine--tRNA ligase, cytoplasmic	sp Q8BMJ2 SYLC_MOUSE	0	2	N/A
mitochondrial	sp Q9CQA3 SDHB_MOUSE	2	17	8.5
Rho guanine nucleotide exchange factor (GEF7), isoform CRA_a	tr A0A0R4J0X8 A0A0R4J0X8_MOUSE	4	23	5.8
(Bos taurus) similar to alpha-tubulin I isoform 1	ENSEMBL:ENSBTAP00000016242	2	9	4.5
Isoform 2 of Cellular nucleic acid-binding protein	sp P53996-2 CNBP_MOUSE	3	12	4.0
Stromal cell-derived factor 2-like protein 1	sp Q9ESP1 SDF2L_MOUSE	3	11	3.7
Transcriptional repressor p66-beta	sp Q8VHR5 P66B_MOUSE	14	46	3.3
Isoform 2 of Methyl-CpG-binding domain protein 3	sp Q9Z2D8-2 MBD3_MOUSE	5	16	3.2
Metastasis-associated protein MTA1	sp Q8K4B0 MTA1_MOUSE	15	44	2.9
Transcriptional repressor p66 alpha	sp Q8CHY6 P66A_MOUSE	13	38	2.9
Splicing factor 3B subunit 3	sp Q921M3 SF3B3_MOUSE	5	14	2.8
Bifunctional glutamate/proline--tRNA ligase	sp Q8CGC7 SYEP_MOUSE	6	16	2.7
Lysine--tRNA ligase	sp Q99MN1 SYK_MOUSE	9	24	2.7
Elongation factor 1-delta	sp P57776 EF1D_MOUSE	2	5	2.5
Elongation factor 1-beta	sp O70251 EF1B_MOUSE	4	10	2.5
116 kDa U5 small nuclear ribonucleoprotein component	sp O08810 U5S1_MOUSE	4	10	2.5
Transmembrane glycoprotein NMB	sp Q99P91 GPNMB_MOUSE	6	15	2.5
Isoform 3 of Transforming acidic coiled-coil-containing protein 2	sp Q9JJG0-3 TACC2_MOUSE	43	105	2.4
cAMP-dependent protein kinase type I-alpha regulatory subunit	sp Q9DBC7 KAP0_MOUSE	5	12	2.4
Peroxiredoxin-1	sp P35700 PRDX1_MOUSE	3	7	2.3
Isoform HSP105-beta of Heat shock protein 105 kDa	sp Q61699-2 HS105_MOUSE	4	9	2.3