Supporting Information

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SI Materials and Methods

Mass Spectroscopy. Thapsigargin in culture medium was quantified by LC/MS/MS after sample clean-up by solid phase extraction (Supelco 52611-U, DSC-18T, 100 mg). Tg was isolated by reverse-phase C-18 HPLC using gradient elution with up to 95% methanol:5% 0.1% formic acid in water. Quantitation was based on peak area generated by scanning for cumulative product ions (*m/z* 513, 573, 612) from the protonated molecular ion parent (*m/z* 674). External standardization was used, with Excalibur Version 2.0 Software, interfaced with a Thermo-Finnigan LCQ DUO mass spectrometer and Thermo-Fisher Surveyor P5000 HPLC and Autosampler modules.

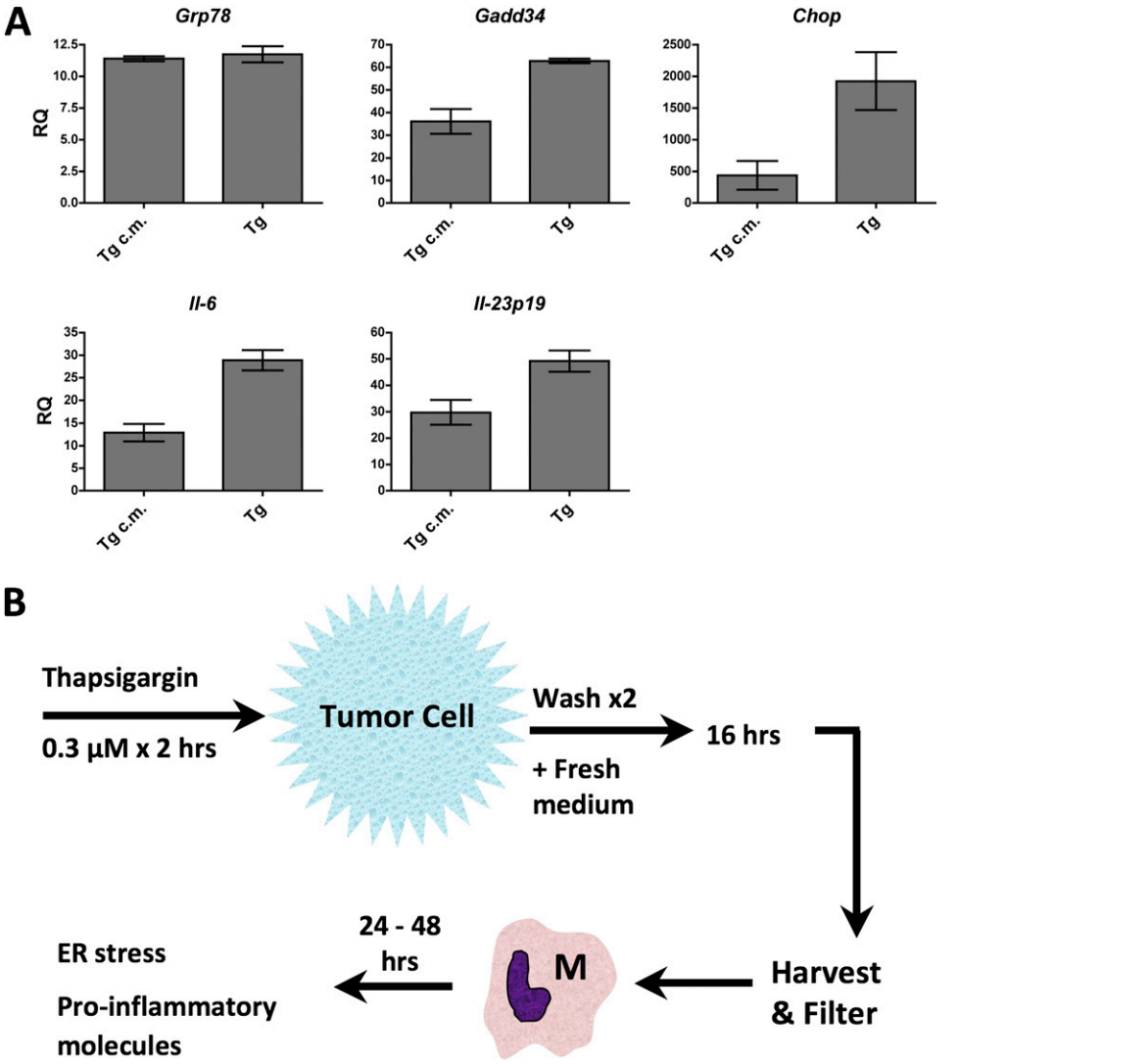

Fig. S1. Modeling the noncognate influence of tumor cell ER stress on macrophages. (*A*) TC1 cells were treated as in *B* (Tg c.m.) or continuously for 18 h with thapsigargin (Tg). RNA was isolated and analyzed by RT-qPCR for UPR activation and *Il-6 and Il-23p19* transcription. Data columns indicate fold difference in transcript level (RQ) between cells treated with Tg or an equal volume of vehicle control (100% ethanol) only. Error bars represent SEM of two biological replicates representative of three independent experiments. (*B*) Scheme of macrophage culture in ER-stressed tumor cell-conditioned medium. Briefly, tumor cells (TC1, B16.F10, LLC) were treated as indicated, conditioned medium harvested and transferred to macrophages for the indicated times, and macrophage ER stress response and cytokine production assayed by RT-qPCR and cytometric bead array.

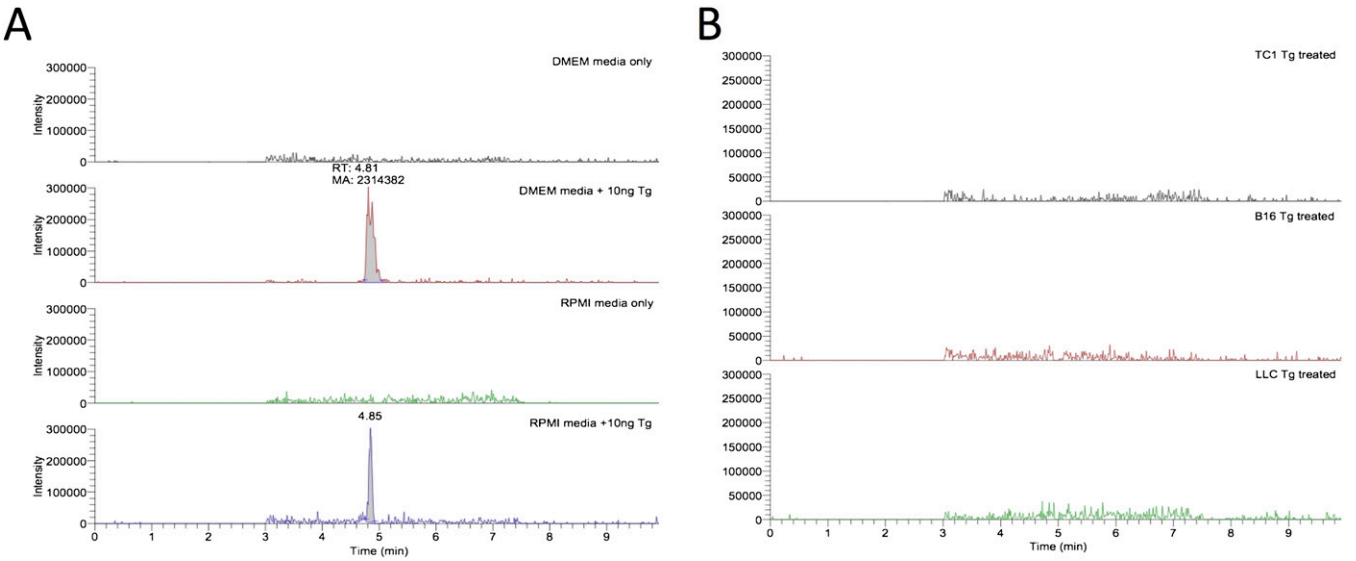

Fig. S2. ER stress tumor-conditioned media does not contain carry over thapsigargin. Samples of ER stress TC1, B16.F10, and LLC conditioned medium were analyzed for presence of Tg [identified by cumulative product ions (*m/z* 513, 573, 612) from the protonated molecular ion parent (*m/z* 674)] by mass spectroscopy and compared against complete culture medium (RPMI or DMEM) spiked or not spiked with 10 ng/mL Tg. Volumes analyzed were all 300 μL. (*A*) ion-chromatograms of blank media (complete RPMI and DMEM) as well as blank medium to which a known amount of Tg was added as positive control (equivalent to 10 ng/mL) (*B*) Ion-chromatograms of TC1, B16.F10, and LLC Tg c.m. as annotated.

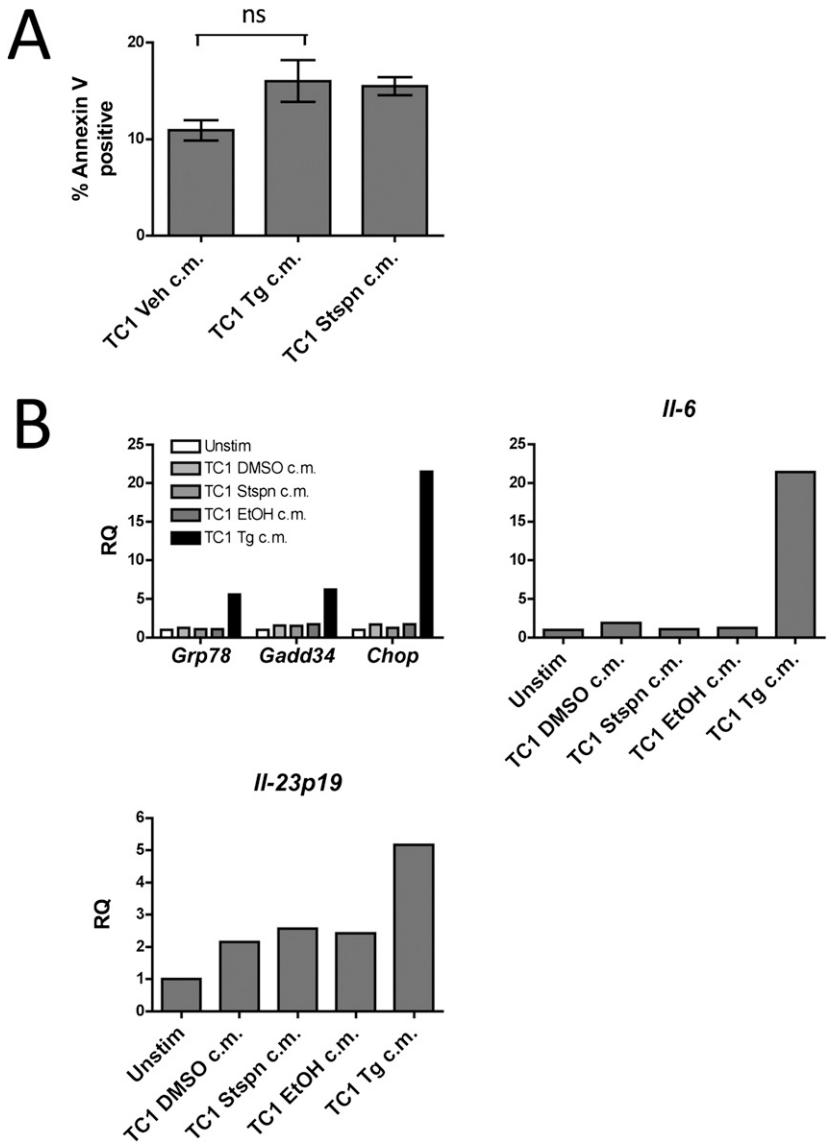

Fig. S3. Cell death in Tg-treated TC1 cells does not account for transmissible ER stress and associated proinflammation in macrophages. (*A*) TC1 cells were treated with 300 nM Tg, 100 nM staurosporine (Stspn), or equal volumes of vehicle (100% EtOH and DMSO, respectively) and treated as indicated in Fig. S1*B*, and analyzed for Annexin V positivity by flow cytometry. Error bars represent SEM of triplicate wells. ns, Not significant ($P > 0.05$, unpaired, two-tailed t test). (*B*) J774 macrophages were cultured in culture medium derived from TC1 treated with Tg (TC1 Tg c.m.), Stspn (TC1 Stspn c.m.), vehicles (100% EtOH or DMSO) only, or culture medium alone (Unstim) for 24 h. RNA was isolated and analyzed by RT-qPCR for UPR activation and proinflammatory cytokine transcription. Columns indicate fold increase in transcript level (RQ) of each treatment group. The value of unstimulated controls was set arbitrarily to 1. Data shown are representative of two independent experiments.

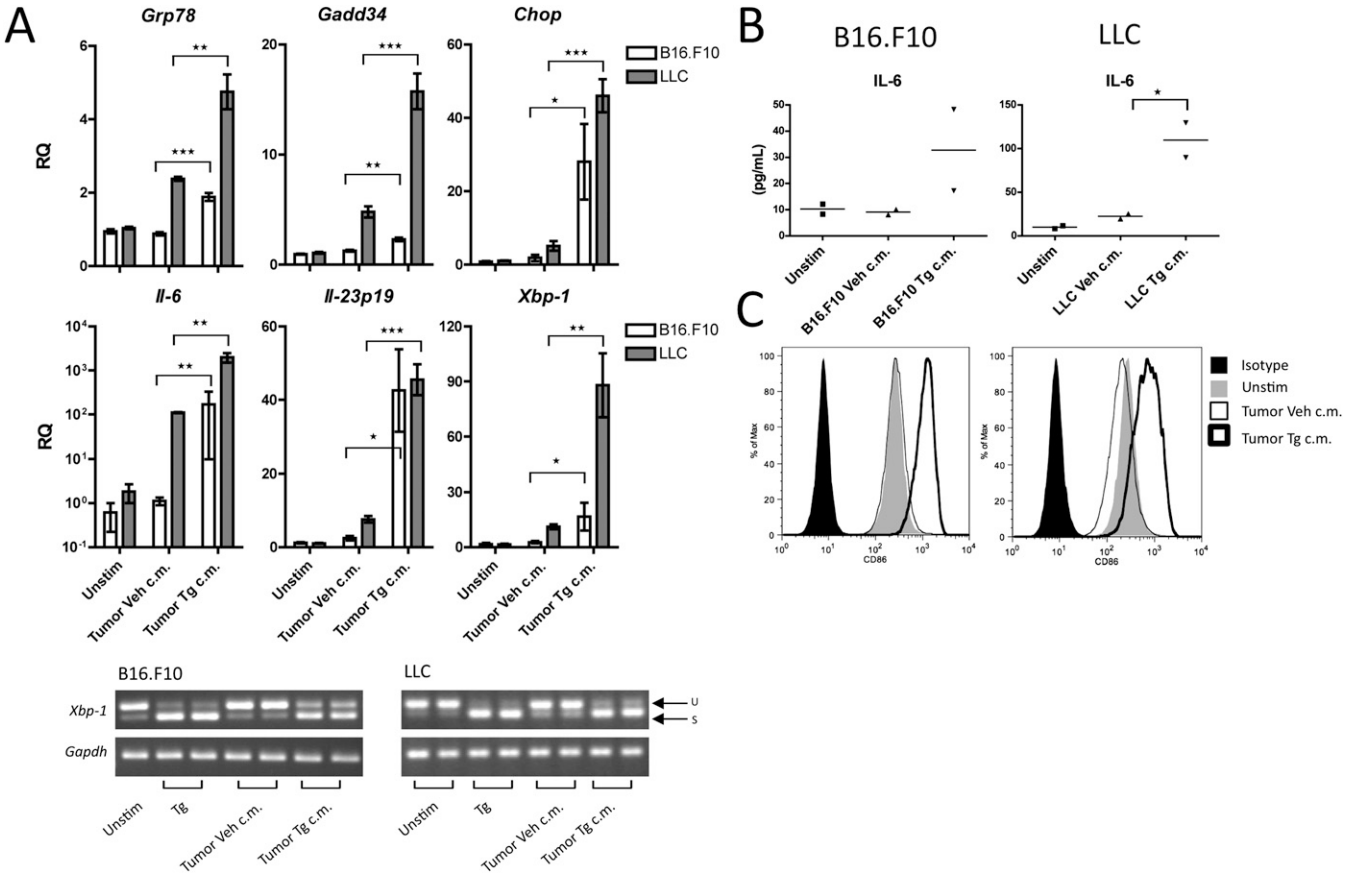

Fig. S4. ER-stressed conditioned medium from B16.F10 and LLC tumor cells also mediates transmissible ER stress in macrophages. J774 macrophages were cultured in conditioned medium of ER-stressed B16.F10 or LLC cells (B16.F10, LLC Tg c.m.), control cells (B16.F10, LLC Veh c.m.), or culture medium alone (Unstim) for 24 h. (*A*) RNA was isolated and analyzed by RT-qPCR for UPR activation and proinflammatory cytokine gene transcription. Columns indicate fold increase in transcript level (RQ) of each treatment group. The value of an unstimulated control was set arbitrarily to 1. Error bars represent SEM of two to four biological replicates. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, unpaired, two-tailed *t* test. u, Unspliced; s, spliced. (*B*) J774 supernatants were analyzed by the multiplex cytometric bead assay for presence of IL-6. $*P < 0.05$, unpaired, two-tailed *t* test. (*C*) Flow cytometry analysis of J774 macrophages treated for 24 h as indicated. Results are of representative of two independent experiments.

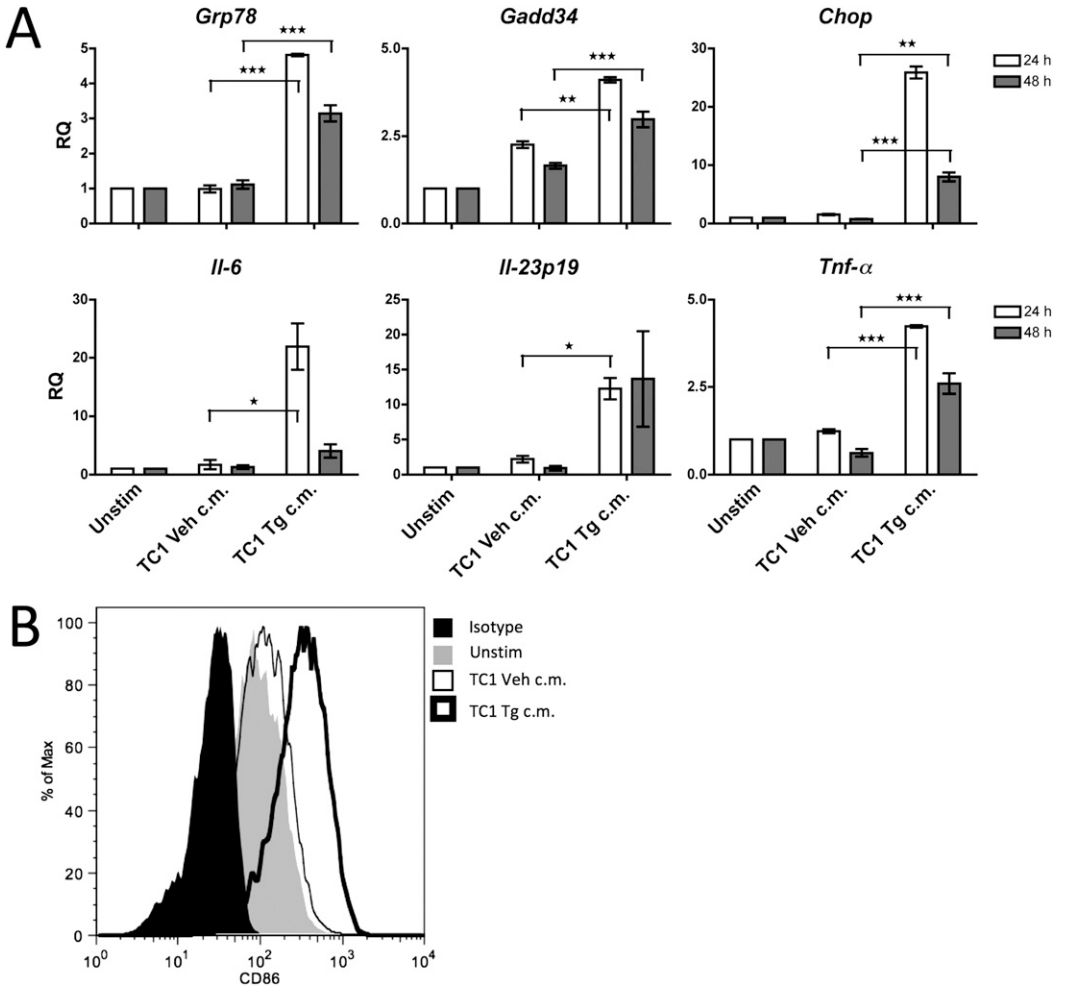

Fig. S5. BMDM experience transmissible ER stress and proinflammatory cytokine gene transcription. BMDM were cultured in conditioned medium of ER-stressed TC1 cells (TC1 Tg c.m.), control TC1 cells (TC1 Veh c.m.), or culture medium alone (Unstim) for 24 or 48 h. (*A*) RNA was isolated and analyzed by RT-qPCR for UPR activation and proinflammatory cytokine gene transcription. Columns indicate fold increase in transcript level (RQ) of each treatment group. The value of unstimulated controls was set arbitrarily to 1. Error bars represent SEM of two to five independent experiments. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, unpaired, two-tailed *t* test. (*B*) Flow cytometry analysis of BMDM treated as indicated for 48 h. Results are representative of three independent experiments.

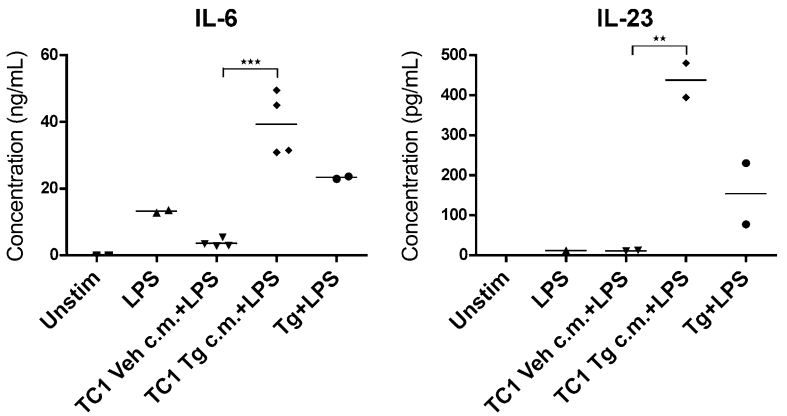

Fig. S6. TLR4 signaling potentiates the influence of ER-stressed tumor cell-conditioned medium on J774 macrophages. J774 macrophages were in conditioned medium of ER-stressed TC1 cells (TC1 Tg c.m.), control TC1 cells (TC1 Veh c.m.), or culture medium alone, with LPS (100 ng/mL) for 18 h. Macrophages cultured in medium containing Tg (300 nM) plus LPS serves as a control. Macrophage supernatants were analyzed by cytometric bead array assay for presence of IL-6. $**P < 0.01$, $***P < 0.001$, unpaired, two-tailed *t* test.

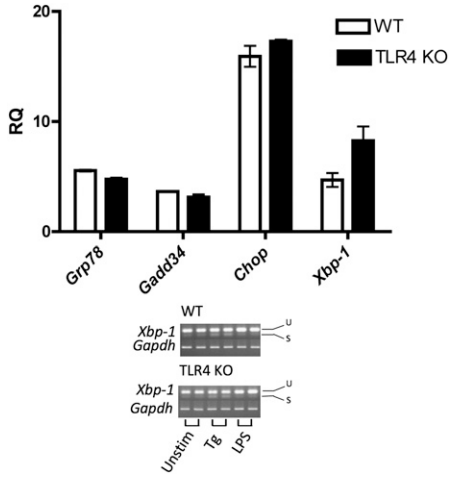

Fig. S7. TLR4 KO BMDM mount UPR comparable to WT BMDM. TLR4 KO and WT BMDM were treated with Tg (300 nM) for 24 h. RNA was isolated and analyzed by RT-qPCR for UPR activation and proinflammatory cytokine gene transcription. Columns indicate fold increase in transcript level (RQ) of each treatment group. The value of an unstimulated control was set arbitrarily to 1. Error bars represent SEM of two biological replicates. Values for WT and KO gene expression were not statistically different. $P \geq 0.05$, unpaired, two-tailed *t* test. u, Unspliced; s, spliced.

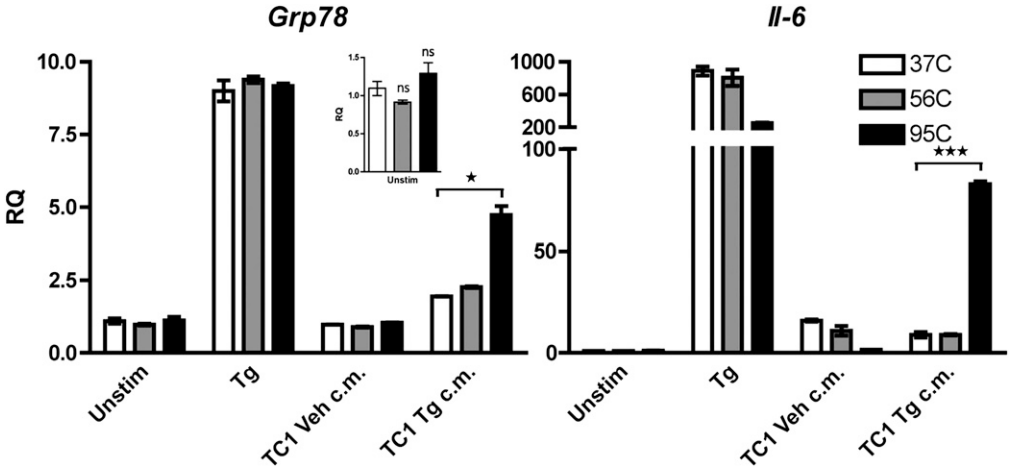

Fig. S8. Factor released by tumor cells under ER stress is not inactivated by heat. J774 macrophages were cultured for 24 h in conditioned medium of ER-stressed TC1 cells (TC1 Tg c.m.), control TC1 cells (TC1 Veh c.m.), or culture medium with (Tg) or without (Unstim) Tg (300 nM), each heated to 37 °C, 56 °C, or 95 °C for 30 min. Heated media were allowed to cool to room temperature on ice before use. RNA was isolated and analyzed by RT-qPCR for *Grp78* and *Il-6* transcription. Columns indicate fold increase in transcript level (RQ) of each treatment group relative to unstimulated controls at the same temperature, which was set arbitrarily to 1. (*Inset*) Gene expression of unstimulated macrophages at different temperatures relative to macrophages cultured in 37 °C medium, which was set arbitrarily to 1. Error bars represent SEM of two biological replicates. $*P < 0.05$, $***P < 0.001$; ns, not significant ($P \geq 0.05$, unpaired, two-tailed *t* test).

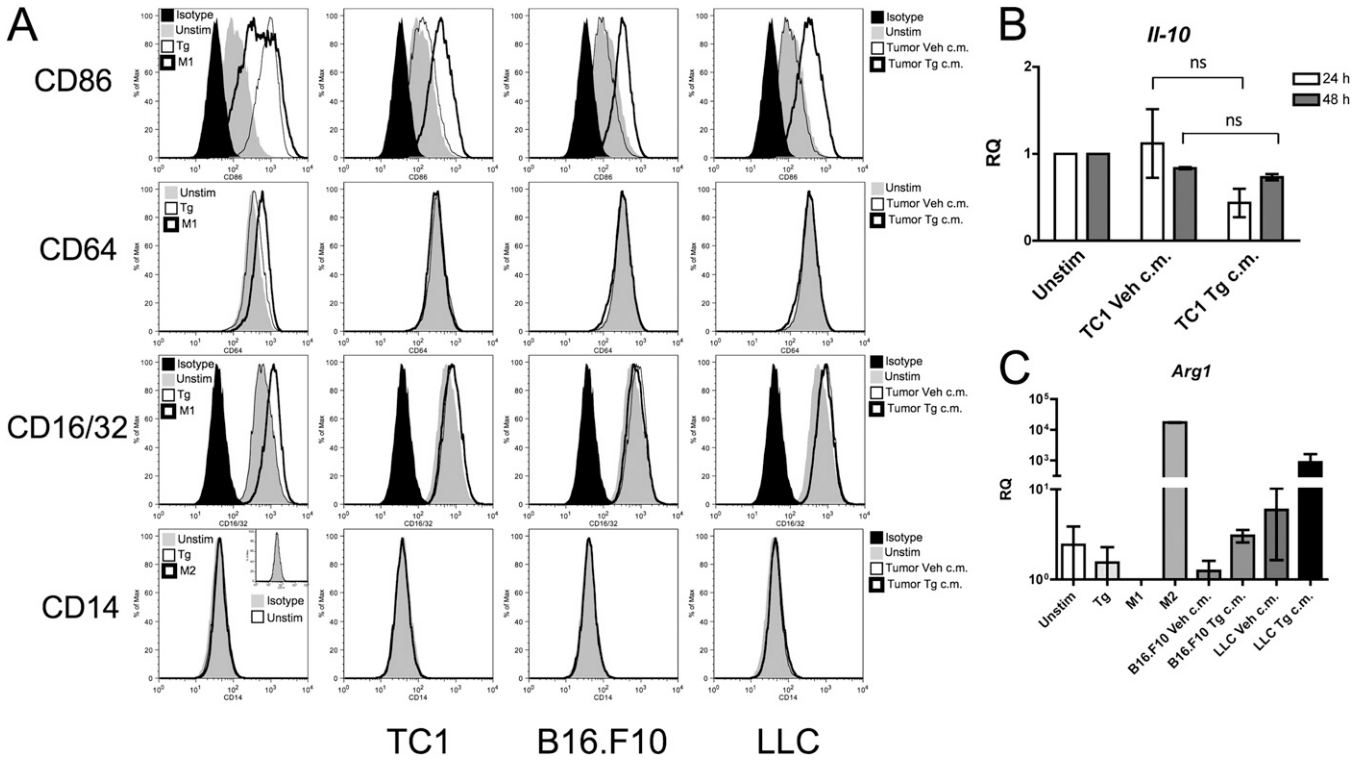

Fig. S9. Conditioned medium from ER-stressed tumor cells polarizes macrophages toward an inflammatory, tumor-associated macrophage phenotype. BMDM were cultured in conditioned medium of ER-stressed tumor cells (TC1, B16.F10, LLC Tg c.m.), control tumor cells (Veh c.m.), or culture medium with (Tg) or without (Unstim) Tg (300 nM) for 24 h. BMDM primed with IFN-γ (250 U/mL) and activated with LPS (100 ng/mL) (M1) or treated with IL-4 (50 ng/mL) (M2) served as controls. (*A*) Flow cytometry analysis of BMDM treated as indicated for macrophage cell surface phenotype markers. Results are of representative of three independent experiments. (*B*) Relative quantification of *Arg* transcription of BMDM by RT-qPCR. Columns indicate fold increase in transcript level (RQ) of each treatment group. The value of an unstimulated control was set arbitrarily to 1. Error bars represent SEM of two biological replicates. (*C*) *Il-10* transcription was quantified in BMDM treated for 24 or 48 h with conditioned medium of ER-stressed TC1 cells (TC1 Tg c.m.), control TC1 cells (TC1 Veh c.m.) by RT-qPCR. Columns indicate fold increase in transcript level (RQ) of each treatment group. The value of unstimulated controls was set arbitrarily to 1. Error bars represent SEM of two to three independent experiments. ns, Not significant ($P \geq 0.05$, unpaired, two-tailed *t* test).

Table S1. List of primers

mXbp-1	Forward	5′-ACACGCTTGGGAATGGACAC-3′
	Reverse	5′-CCATGGGAAGATGTTCTGGG-3′
mGapdh	Forward	5′-ACGGATTTGGTCGTATTGGGC-3′
	Reverse	5′-TTGACGGTGCCATGGAATTTG-3′
mXbp-1 (RT-qPCR)	1	5′-ACCAGGAGTTAAGAACACGC-3′
	2	5′-CAACAGTGTCAGAGTCCATGG-3′
	Probe	5′-AGGTGCAGGCCCAGTTGTCA-3′