Supporting Information

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SI Materials and Methods

Reagents and Antibodies. Human serum albumin (HSA), BSA, Accutase, Hepes, DMSO, NaCl, $MgCl_2$, $CaCO_3$ and chloroform (ACS grade with 0.5–1% ethanol added as stabilizer) were all obtained from Sigma-Aldrich. RPMI 1640 cell culture media, FBS, HBSS, penicillin-streptomycin (PenStrep), and Dulbecco's PBS (DPBS) were all obtained from Invitrogen. His-tagged recombinant human tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), his-tagged recombinant human E-selectin-IgG chimera (ES), and Annexin-V FITC Apoptosis Detection Kit were purchased from R&D Systems. 2′,7′-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein, acetoxymethyl ester solution and trypsin-EDTA solution were obtained from Invitrogen. PBS-based enzyme-free cell dissociation media was purchased from Millipore. L-α-lysophosphatidylcholine from egg (Egg PC), sphingomyelin from egg (Egg SM), ovine wool cholesterol (Chol), 1,2-dioleoyl-sn-glycero-3-[(*N*-(5-amino-1-carboxypentyl) iminodiacetic acid) succinyl] (nickel salt) (DOGS-Ni-NTA), and 23-(dipyrromethеneboron difluoride)-24-norcholesterol (Bdp-Chol, Ex/Em 490 nm/504 nm) either dissolved in chloroform (Egg PC, Egg SM, Chol, DOGS NTA-Ni) or in powder form (Bdp-Chol) were purchased from Avanti Polar Lipids. Rat anti-human CD62E (E-selectin) antibody was purchased from Abcam. Anti-human CD3, CD14, CD16, CD19, and CD56 conjugated with Pacific Blue, APC Cy7, PERCP-Cy5.5, APC, and PE, respectively, along with corresponding isotypes were all purchased from BD Biosciences.

Cell Lines and Cell Culture. Colon cancer cell line COLO 205 (ATCC number CCL-222) was obtained from ATCC and cultured in RPMI 1640 supplemented with 2 mM L-Glutamine, 25 mM Hepes, 10% (vol/vol) FBS and 100 U/mL PenStrep (complete media) under humidified conditions at 37 °C and 5% CO_2. Prostate cancer cell line PC-3 (ATCC number CRL-1435) was obtained from ATCC and cultured in F-12K medium supplemented with 10% (vol/vol) FBS and 100 U/mL PenStrep. Human umbilical vein endothelial cells (HUVECs) were purchased from Cascade Biologics and maintained in Medium 200 (Cascade Biologics) supplemented with low-serum growth supplement (Cascade Biologics) and 5% FBS (Invitrogen). HUVECs from passages 2–5 were used for experiments. For all experiments, >95% viability was assessed by trypan blue exclusion dye.

Preparation of Liposomes. Multilamellar liposomes, composed of egg L-α-lysophosphatidylcholine (Egg PC), egg sphingomyelin (Egg SM), ovine wool cholesterol (Chol), and 1,2-dioleoyl-sn-glycero-3-[(*N*-(5-amino-1-carboxypentyl) iminodiacetic acid) succinyl] (nickel salt) (DOGS NTA-Ni) at weight ratios 60–50%:30%:10%:0–10% (Egg PC/Egg SM/Chol/DOGS NTA-Ni), were prepared using a thin lipid film method. DOGS-NTA-Ni is a lipid conjugated to nickel-nitrilotriacetic acid (Ni-NTA) that allows for attachment to his-tagged proteins. Briefly, stock solutions of all lipids were prepared by dissolving powdered lipids in chloroform to produce a final concentration of 5 mg/mL Egg PC, 20 mg/mL Egg SM, 5 mg/mL Chol, and 20 mg/mL DOGS-NTA-Ni in glass containers and stored at –20 °C. Appropriate volumes of the lipids were taken from the stock solution to make lipids with varying concentrations of DOGS NTA-Ni in a glass tube and gently dried under nitrogen. To ensure complete removal of chloroform, the lipids were left under vacuum for an additional 12 h. With increasing amounts of DOGS NTA-Ni, the corresponding amount of Egg PC was decreased (Table S1). The lipid film was hydrated with a liposome buffer composed of 150 mM NaCl, 10 mM Hepes, and 1 mM $MgCl_2$ dissolved in nuclease-free water to create multilamellar liposomes. The resulting multilamellar liposomes were sized by repeated thawing and freezing, and then subjected to 15 extrusion cycles at 60 °C through two different pore size (200 and 100 nm) polycarbonate membranes (Nucleopore; Whatman) to produce unilamellar nanoscale liposomes. Recombinant human ES and TRAIL were dissolved in nuclease-free sterile water to a final concentration of 1 mg/mL, and 100 μg/mL aliquots of stock solutions were stored at –20 °C and used as needed within 60 d. Freshly prepared nanoscale liposomes were then incubated with ES (final concentration 71.43 nM) and TRAIL (250 nM final concentration) for 30 min at 37 °C and then overnight at 4 °C, to ensure maximum protein binding via the interaction between his-tag and Ni-NTA. Based on approximations for ligand density on liposomes suggested in previous work by Huang and Mason (1), there were ~65 TRAIL and 19 E-selectin proteins present on the surface of each liposome, assuming a unilamellar liposome diameter of 100 nm. To remove unbound TRAIL and ES, liposomes were diluted 1:6 with liposome buffer and subjected to ultracentrifugation at 100,000 × *g* for 3 h at 4 °C. The supernatant with unbound TRAIL and ES was carefully removed and collected for further evaluation, and the remaining liposomes were gently resuspended in buffer. A similar procedure was used to create fluorescent conjugated liposomes by replacing ovine wool cholesterol with 23-(dipyrromethеneboron difluoride)-24-norcholesterol. Freshly prepared nanoscale liposomes were diluted in buffer, and the mean particle diameter and surface charge (zeta potential) were measured by dynamic light scattering using a Malvern Zetasizer nano ZS (Malvern Instruments Ltd.), according to the manufacturer's protocols. Conjugated liposomes were measured to be 117.8 ± 10.3 nm in diameter, with a zeta potential of –5.7 ± 4.6 mV.

Static Experiments. COLO 205 cells were seeded in multiwell plates at a seeding density of 300,000 cells per mL, 1 d before experimentation to ensure that the cells were in the linear phase of the growth cycle. Media was changed before experimentation. Cells were incubated with either the supernatant from ultracentrifugation or 10 μL of conjugated and purified liposomes. The cells were maintained in culture conditions with the supernatant or nanoscale lipids for 24 h and later analyzed by an Annexin-V assay to quantify the proportion of viable cells.

Uniform Shear-Flow Experiments. To simulate the shear-stress conditions of blood flow, cancer cells were subjected to uniform shear in a cone-and-plate viscometer (2, 3). Cancer cells seeded 1 d before the experiment were gently detached from the surface using PBS-based enzyme-free cell-detachment solution or accutase. Cells were then washed twice in 1× DPBS and resuspended in buffer at a concentration of 1×10^6 cells per mL. Then, 10 μL of lipids was added to 490 μL of cell suspension (at 10^6 cells per mL) and immediately added to the cone-and-plate viscometer. Cell suspensions were exposed to shear flow at a shear rate of 188 s^{-1} for 2 h. All samples were exposed to shear flow at room temperature to prevent potential sample evaporation and/or drying in the cone-and-plate viscometer over prolonged periods of shear. After 2 h, the cells were removed and washed twice in resuspension buffer at 200 × *g* for 5 min. Cells were resuspended in complete media and cultured for 24 h. In the case of fluorescent lipids, an aliquot was taken for visual inspection on an inverted microscope (Olympus America Inc.) equipped with fluorescence and an intensified CCD digital camera (Cooke

Corporation) to record images. For spiking experiments, peripheral blood was collected into Vacutainer tubes containing heparin and allowed to equilibrate to room temperature before use. Then, 1×10^6 COLO 205 or PC-3 cells were tagged fluorescently with 3 μM BCECF AM solution for 15 min at 37 °C, washed twice, and collected via centrifugation at $200 \times g$ for 5 min. The supernatant was discarded, and the collected cells were resuspended in 1 mL of whole blood. Then, 10 μL of lipid was then added to 490 μL of spiked blood and immediately added to a cone-and-plate viscometer previously coated with 5% BSA. Spiked blood was subjected to a uniform shear rate of 188 s^{-1} for 2 h. As an additional comparison for spiking experiments, identical experiments were performed in buffer instead of whole blood. In leukocyte functionalization experiments, 10 μL of lipid was added to 490 μL of human blood, sheared in a cone-and-plate viscometer at a uniform shear rate of 188 s^{-1} for 2 h, and subsequently centrifuged to remove the plasma containing unbound liposomes. The removed plasma was replaced with freshly isolated plasma, and the blood samples were used for identical spiking experiments mentioned above. After shearing, the blood sample was collected from the device and carefully layered over 1.5 mL of Ficoll and centrifuged at $480 \times g$ for 50 min at room temperature. The buffy coat containing mononuclear cells (MNCs) and cancer cells was recovered and washed twice in resuspension buffer, collected, cultured for 24 h, and analyzed for viable fluorescent cancer cells using flow cytometry. To evaluate the effect of hematocrit, the number of RBCs was varied by removal via centrifugation. Volumes of RBCs were replaced with excess plasma from the same blood donor. Cancer cells were spiked into blood samples as mentioned earlier, at a concentration of 1×10^6 cells per mL. Then, 10 μL of liposome solution was added to 490 μL of blood and sheared for 2 h at 188 s^{-1}. Samples were collected, incubated, and analyzed for viability on a flow cytometer as described in *Flow Cytometry*. In some experiments, 490 μL of whole blood was sheared with 10 μL of liposome solution, and the plasma and any remaining unbound liposomes and/or liposome fragments were separated by centrifugation. To determine the effects of remaining unbound liposomes and/or liposome fragments on cancer-cell viability, the recovered plasma was incubated with 500,000 COLO 205 cells for 24 h at 37 °C and analyzed for cell viability using flow cytometry.

Polymorphonuclear and Mononuclear Cell Isolation. All human subject protocols were approved by the Institutional Review Board for Human Participants of Cornell University. Peripheral blood was collected from healthy donors after informed consent into Vacutainer tubes containing heparin and allowed to equilibrate at room temperature before use (2, 4). Then, 3 mL of blood diluted with resuspension buffer was carefully layered over 3 mL of 1-Step Polymorphs (Accurate Chemical & Scientific Corporation) and centrifuged at $480 \times g$ for 50 min at room temperature. Two separate layers of MNCs and polymorphonuclear cells (PMNs) were collected and washed twice with resuspension buffer.

Leukocyte Functionalization with ES/TRAIL Liposomes in Whole Blood. To assess the adhesion of ES/TRAIL liposomes to leukocytes in whole blood under flow, 490 μL of whole blood was sheared with 10 μL of fluorescent ES/TRAIL liposome solution for 30 min in a cone-and-plate viscometer at a shear rate of 188 s^{-1}. Leukocytes were then separated using 1-Step Polymorphs and assessed for adherent ES/TRAIL liposomes using confocal microscopy. To assess the fraction of leukocyte subpopulations that adhere to ES/TRAIL liposomes, leukocytes were labeled with anti-human CD3, CD14, CD16, CD19, and CD56, along with corresponding isotype controls, and analyzed for adherent ES/TRAIL liposomes using flow cytometry. To assess the specificity of the ES interaction with leukocytes, liposomes were incubated with a functional blocking anti-human E-selectin antibody before shearing in whole blood. To assess the fraction of leukocytes in blood adhered to ES/TRAIL liposomes before shear, fluorescent ES/TRAIL liposomes were added to whole blood and then separated using 1-Step Polymorphs.

Endothelial-Cell Viability Assay. Forty-millimeter-diameter circular glass coverslips (Thermo Scientific) were plasma-treated (Harrick Plasma Cleaner) for 2 min and subsequently incubated in 1% polyethylenemine (PEI) at room temperature for 10 min. Coverslips were then washed in water three times and treated with 0.1% glutaraldehyde (Sigma-Aldrich) in PBS at room temperature for 30 min. Coverslips were washed in water three times, dried, and treated with 0.1 mg/mL type I rat-tail collagen (Becton Dickinson) in Hepes (pH 8.0; Sigma-Aldrich) for 2 h at 4 °C. Coverslips were placed in Petri dishes (60 mm × 15 mm; Sigma-Aldrich), washed three times in PBS, and briefly sterilized via UV exposure for 15 min. HUVECs were plated on coverslips, at a density of 500,000 cells per coverslip, in Medium 200 supplemented with low-serum growth supplement (Cascade Biologics), 5% FBS (Invitrogen), and 100 U/mL PenStrep. HUVECs were cultured for 4 d on coverslips before experiments and then adhered to the plate of a cone-and-plate viscometer using vacuum grease. HUVECs were then treated with 2.94 mL of human blood and 60 μL of PBS, ES/TRAIL (TRAIL final concentration: 0.3 μg/mL), or soluble TRAIL in PBS (TRAIL final concentration: 0.3 μg/mL) for 4 h at 37 °C in a humidified cone-and-plate viscometer at a shear rate of 188 s^{-1}. As a positive control, HUVECs were treated with a high concentration of soluble TRAIL (15 μg/mL). Coverslips were removed from the viscometer, gently washed in PBS, and placed in Medium 200 supplemented with low-serum growth supplement, 5% FBS (Invitrogen), and 100 U/mL PenStrep. HUVEC morphology was assessed using brightfield and phase-contrast microscopy. HUVECs were immediately placed into culture for 8 h, maintained at 37 °C and 5% CO_2. HUVECs were then treated with 0.25% Trypsin-EDTA solution (Gibco) for 2 min at 37 °C, followed by treatment with an equal volume of trypsin neutralizer solution (Gibco). HUVECs were collected from coverslips, washed twice in PBS, and assessed for viability using an Annexin-V assay.

Liposome and COLO 205 Cell Injection in Mice. C57BL/6J mice aged 16–20 wk (both sexes), weighing 25–32 g, were obtained from The Jackson Laboratory. Mice were anesthetized using isoflurane (5%) for all procedures. Either 120 μL of saline, sTRAIL (15 μg/mL; TRAIL plasma concentration ~1.0 μg/mL), ES/TRAIL (TRAIL injection concentration 15 μg/mL; TRAIL plasma concentration ~1.0 μg/mL) liposomes, or ES liposomes suspended in saline were injected retro-orbitally using a 30-G needle, and animals were removed from anesthesia. Three mice were used in each group. Thirty minutes later, animals were reanesthetized, and $\sim 2 \times 10^6$ COLO 205 cells, labeled by 2 μg/mL Hoescht (H1399; Invitrogen) or 3 μM BCECF AM suspended in saline were injected into the tail vein. Animals were removed from anesthesia, and the cancer cells were allowed to circulate for 2 h. All animal procedures were approved by the Cornell University Institutional Animal Care and Use Committee.

Analysis of Circulating COLO 205 Cells. Animals were euthanized with a lethal dose of pentobarbital. Blood was removed from the heart via cardiac puncture and collected into sodium heparin-coated tubes. Leukocytes and circulating COLO 205 cells were separated using Ficoll-Paque PLUS. After centrifugation, the MNC buffy coat was collected, washed in buffer containing Ca^{2+} and cultured for 2–3 h in multiwell plates. Cell viability was assessed using flow cytometry.

Two-Photon Imaging of Lung Tissue. Animals were anesthetized and received injections of saline, sTRAIL, ES/TRAIL liposomes, or ES liposomes, followed by an injection of Hoescht-labeled cancer cells, as described in *Liposome and COLO 205 Cell Injection in Mice*. After 2 h of cancer-cell circulation, an Alexa Fluor 568-labeled Annexin-V probe (A13202; Invitrogen) was injected retro-orbitally and allowed to circulate for 2 h to ensure maximum detection of apoptotic cells. Animals were then given a lethal dose of pentobarbital. After euthanasia, intact lungs were resected and immediately imaged via two-photon excited fluorescence microscopy. Two-photon imaging was conducted on a locally designed microscope using a train of 800-nm, 87-MHz, 100-fs pulses from a Ti:sapphire laser oscillator (MIRA HP, pumped by a Verdi-V18; Coherent) for excitation. Laser scanning and data acquisition were controlled by ScanImage software. For high-resolution imaging of COLO 205 Hoechst-labeled nuclei and Annexin V labeling, a 20× (numerical aperture: 0.95) water-immersion objective (Olympus) was used. Fluorescence was detected using emission filters with 460-nm and 645-nm center wavelength with 65-nm bandwidth to image Hoescht and Alexa Fluor 568 (Invitrogen), respectively. Spectrally broad autofluorescence from the lung tissue was visible in both channels.

Counting and Viability Scoring of COLO 205 Cells in Lung. Hoechst-labeled COLO 205 cells were manually counted from ~10 representative two-photon image stacks taken from the lung in each mouse using Image J (NIH) cell counting software. The Hoechst signal was of similar magnitude to the background lung autofluorescence in the 460-nm channel. To aid in identifying nuclei, the Hoechst channel was compared with the 645-nm channel, where autofluorescence was also visible, but Hoescht was not and Alexa Fluor 568 signal was significantly brighter than autofluorescence. In addition, attributes such as size and shape to distinguish the labeled COLO 205 cells were used. Each imaged volume was about 0.022 mm^3. The total number of cells in the lung was estimated by scaling the imaged volumes to the total lung volume, which was measured via a volume-displacement method. Counts were recorded by two different observers, each blinded to the treatment received, and averaged. To determine which COLO 205 cells were apoptotic, we determined whether Alexa Fluor 568 Annexin V labeling was present at each of the COLO 205 cell nuclei we identified.

Flow Cytometry. Mode of cell death was analyzed using an Annexin-V apoptosis assay on an Accuri C6 flow cytometer. Samples were prepared per the manufacturer's instructions. Briefly, cells were classified into four categories based on dye uptake: viable cells [negative for Annexin-V and propidium iodide (PI)], early apoptotic cells (positive for Annexin-V only), late apoptotic cells (positive for Annexin-V and PI), and necrotic cells (positive for PI only). For blood spiking experiments, fluorescent untreated cancer cells and liposome-treated samples were assessed for viability using a flow cytometer. A gate was set based on a viable, untreated cancer-cell control. Equal volumes from all samples were used for analysis. Cell viability was determined by measuring the amount of cells positive for fluorescent BCECF staining. For in vivo animal experiments, 100 μL of each sample was processed. A gate denoting viable, BCECF AM-labeled cancer cells was established by processing a viable, fluorescent sample of COLO 205 cells in buffer. COLO 205 cells recovered from mouse blood were differentiated based on size and fluorescence using flow cytometry. The number of cancer cells per milliliter of mouse blood was determined based on the amount of mouse blood recovered from each animal.

Statistical Analysis. Where appropriate, Student *t* test and one-way ANOVA with Tukey posttest comparing all means were used at a significance level of $\alpha = 0.05$. All statistical analyses were performed using GraphPad Prism 5.0c for Mac OS X (GraphPad software) and Kaleidagraph (Synergy) software.

1. Huang C, Mason JT (1978) Geometric packing constraints in egg phosphatidylcholine vesicles. *Proc Natl Acad Sci USA* 75(1):308–310.
2. Mitchell MJ, King MR (2012) Shear-induced resistance to neutrophil activation via the formyl peptide receptor. *Biophys J* 102(8):1804–1814.
3. Mitchell MJ, King MR (2013) Fluid shear stress sensitizes cancer cells to receptor-mediated apoptosis via trimeric death receptors. *New J Phys* 15:015008.
4. Ball CJ, King MR (2011) Role of c-Abl in L-selectin shedding from the neutrophil surface. *Blood Cells Mol Dis* 46(3):246–251.

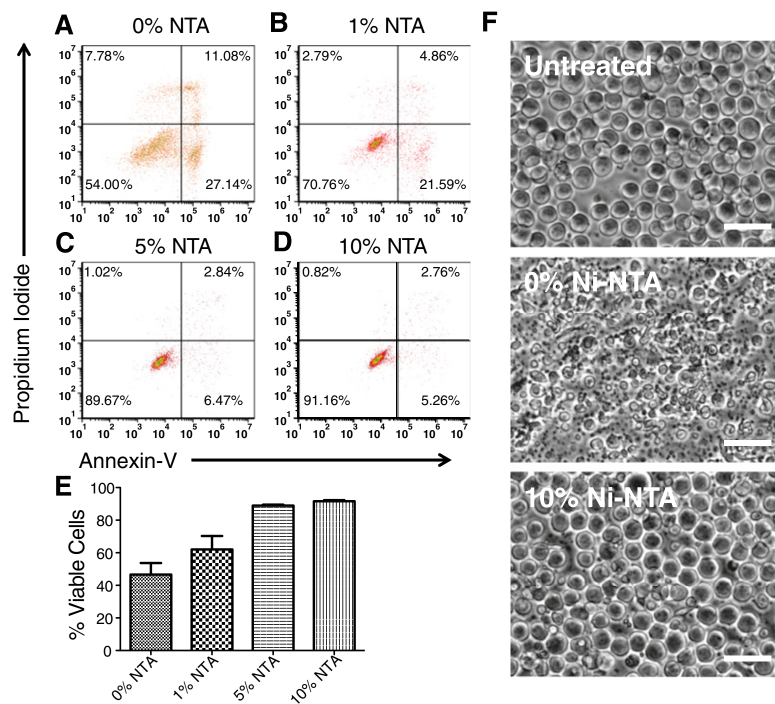

Fig. S1. Incorporation of Ni-NTA–conjugated lipids on liposomes maximizes protein conjugation to the liposome surface. To determine the optimal amount Ni-NTA-conjugated lipid required to bind TRAIL and ES to the liposome surface, COLO 205 cells were incubated with supernatant left after liposome preparation and assayed for cell viability. Increased cell death is indicative of more unbound TRAIL protein in solution. (*A*–*D*) Annexin-V apoptosis assay of COLO 205 cell viability after incubation with supernatant of liposomes conjugated to 0% (*A*), 1% (*B*), 5% (*C*), and 10% Ni-NTA (*D*) postultracentrifugation, with varying amounts of Ni-NTA conjugated to the liposome surface. Cells were classified into four categories based on dye uptake: viable cells (negative for Annexin-V and PI), early apoptotic cells (positive for Annexin-V only), late apoptotic cells (positive for Annexin-V and PI), and necrotic cells (positive for PI only). (*E*) COLO 205 cell viability after treatment with unbound TRAIL and ES in liposome supernatant. $n = 3$ for all samples. Bars represent the mean ± SD in each treatment group. (*F*) Representative micrographs of untreated COLO 205 cells (*Top*) and those treated with the supernatant of liposomes conjugated to 0% (*Middle*) and 10% Ni-NTA (*Bottom*). The 10% Ni-NTA–conjugated lipid on the liposome leads to nearly complete incorporation of TRAIL and ES onto the liposome surface. (Scale bar, 50 μm.)

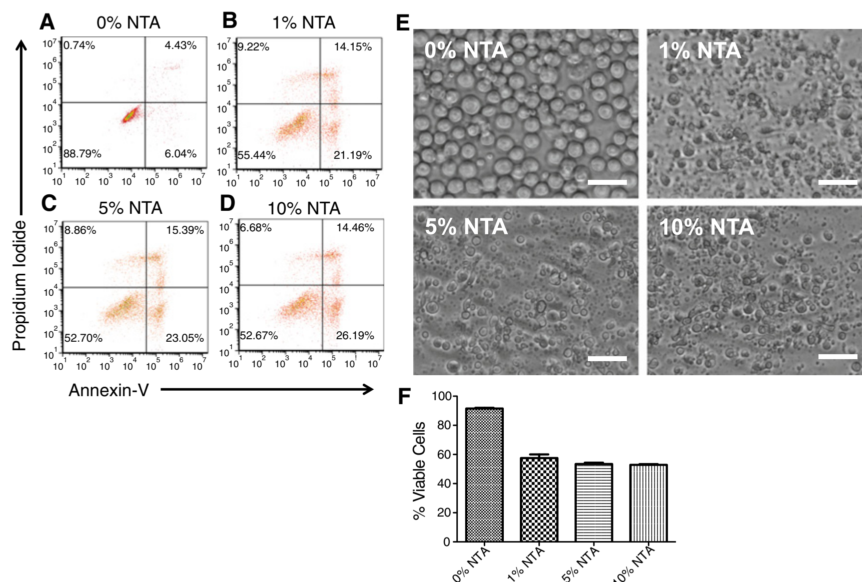

Fig. S2. ES/TRAIL liposomes are somewhat effective in targeting and killing COLO 205 cells under static conditions. (*A*–*D*) Annexin-V apoptosis plots of COLO 205 cells treated with ES/TRAIL liposomes consisting of 0% (*A*), 1% (*B*), 5% (*C*), and 10% Ni-NTA (*D*) for 24 h. (*E*) Representative micrographs showing COLO 205 cells after 24 h incubation with liposomes. (Scale bar, 50 μm.) (*F*) COLO 205 cell viability following incubation with liposomes with varying amounts of Ni-NTA on the liposome surface. $n = 3$ for all samples. Bars represent the mean ± SEM in each treatment group.

Fig. S3. ES/TRAIL liposomes adhesively interact with cancer cells. (*A*) Flow-cytometry plots of cancer cells positive for fluorescently tagged ES/TRAIL liposomes (green). Gray population represents fluorescence of COLO 205 cells in the absence of fluorescent ES/TRAIL liposomes. (*B*) Percent of COLO 205 cells adhered to liposomes following shear flow at a shear rate of $188 s^{-1}$. $n = 3$ for all samples. Bars represent the mean ± SEM in each treatment group. (*C* and *D*) Representative fluorescent (*C*) and brightfield (*D*) micrographs of COLO 205 cells adhered to fluorescently tagged liposomes following shear flow. (Scale bar, 20 μm.)

Fig. S4. Leukocyte functionalization with ES/TRAIL does not induce significant leukocyte death. (*A*–*C*) Representative propidium iodide/Annexin-V flow-cytometry plots of untreated mononuclear cells (*A*) and those treated with ES (*B*) and ES/TRAIL (*C*) liposomes under static conditions for 24 h. (*D*) Viability of mononuclear cells following incubation with liposomes for 24 h. $n = 3$ for all samples. Bars represent the mean ± SEM in each treatment group. NS, not significant (one-way ANOVA with Tukey posttest). (*E*–*G*) Representative propidium iodide/Annexin-V flow-cytometry plots of untreated mononuclear cells (*E*) and those treated with ES (*F*) and ES/TRAIL (*G*) liposomes under shear flow (shear rate: $188 s^{-1}$) for 2 h. (*H*) Viability of MNCs treated with liposomes under shear flow for 2 h. $n = 3$ for all samples. Bars represent the mean ± SD in each treatment group. NS, not significant (one-way ANOVA with Tukey posttest).

Fig. S5. Leukocyte functionalization with ES/TRAIL does not induce significant endothelial cell death. (*A*) Representative images of HUVECs immobilized on coverslips immediately after treatment in human blood, with various drug treatments, under shear flow (shear rate: 188 s^{-1}) for 4 h at 37 °C. (Scale bar, 100 μm.) (*B–D*) Representative propidium iodide/Annexin-V flow cytometry plots of untreated HUVECs (*B*) and those treated with ES/TRAIL liposomes (*C*) (TRAIL concentration 0.3 μg/mL) or soluble TRAIL (*D*) (TRAIL concentration 0.3 μg/mL) in human blood under shear flow for 4 h at 37 °C. (*E*) As a positive control, HUVECs were treated with a high dosage of TRAIL (15 μg/mL) in human blood under shear flow for 4 h at 37 °C. HUVECs were classified into four categories based on dye uptake: viable cells (negative for Annexin-V and PI), early apoptotic cells (positive for Annexin-V only), late apoptotic cells (positive for Annexin-V and PI), and necrotic cells (positive for PI only). (*F*) Percent viability of HUVECs after various drug treatments in human blood under shear flow for 4 h at 37 °C. $n = 3$ for all samples. Bars represent the mean ± SD in each treatment group. $^{**}P < 0.001$. NS, not significant (one-way ANOVA with Tukey posttest).

Fig. S6. Low toxicity of blood plasma after treatment with ES/TRAIL liposomes. (*A*) Viability of COLO 205 cells after treatment with plasma from normal healthy blood for 24 h. (*B*) Viability of COLO 205 cells after treatment with plasma extracted from blood that had been sheared with ES/TRAIL liposomes for 30 min. Cells were incubated with plasma, and any remaining unbound ES/TRAIL liposomes and/or liposome fragments in the plasma for 24 h, and analyzed for cell viability.

Table S1. Weight ratios of liposome formulations

DOGS NTA-Ni	Egg PC	Egg SM	Chol/Bdp-Chol
0% NTA	60	30	10
1% NTA	59	30	10
5% NTA	55	30	10
10% NTA	50	30	10

With increasing amounts of Ni-NTA–conjugated lipid, the corresponding amount of Egg PC lipid was decreased.