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(54) Title: HUMAN EIF3L BINDING MOLECULES AND CANCER EXOSOME DIAGNOSTICS AND ANTI-THROMBOTIC TREATMENT

(57) Abstract: Provided herein are human EIF3L binding molecules and nucleic acid sequences encoding such molecules. In particular embodiments, provided herein are human EIF3L binding molecules (e.g., monoclonal antibodies or antigen binding fragments thereof) having particular light and/or heavy chains variable regions, or light chain and/or heavy chain CDRs, and methods for using such molecules, and/or other EIF3L binding molecules, and/or CD63 binding molecules, to treat a prothrombotic condition that is accompanied by pathological exosome production in subjects (e.g., human subjects with tumor-mediated thrombosis). In some embodiments, the presence or level of cancer is detected by detecting CD63 positive, and/or PCA3 positive, and/or EIF3L-positive, exosomes in a sample.



HUMAN EIF3L BINDING MOLECULES AND CANCER EXOSOME DIAGNOSTICS AND ANTI-THROMBOTIC TREATMENT

The present application claims priority to U.S. provisional application serial number
5 63/503,332, filed May 19, 2023, which is herein incorporated by reference in its entirety.

This invention was made with government support under HL142772 awarded by the
National Institutes of Health. The government has certain rights in the invention.

10 FIELD OF THE INVENTION

Provided herein are human EIF3L binding molecules and nucleic acid sequences
encoding such molecules. In particular embodiments, provided herein are human EIF3L
binding molecules (e.g., monoclonal antibodies or antigen binding fragments thereof) having
particular light and/or heavy chains variable regions, or light chain and/or heavy chain CDRs,
15 and methods for using such molecules, and/or other EIF3L binding molecules, and/or CD63
binding molecules, to treat a prothrombotic condition that is accompanied by pathological
exosome production in subjects (e.g., human subjects with tumor-mediated thrombosis). In
some embodiments, the presence or level of cancer is detected by detecting CD63 positive,
and/or PCA3 positive, and/or EIF3L positive, exosomes in a sample.

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BACKGROUND OF THE INVENTION

Thrombosis is one of the main complications and a major cause of mortality in cancer
patient⁸⁻³. It is estimated that up to 30% of all venous thromboembolic cases are associated
with cancer and metastatic cancers pose an even higher risk⁴⁻⁷. The reciprocal relationship
25 between platelets and tumor progression has been established for over 170 years, yet, the
mechanism underlying this relationship remains obscure. Since thrombosis affects a large
number of patients each year and determines patient morbidity and disease-related mortality,
discerning all the mechanisms of cancer-associated thrombosis is of critical importance for
the development of better-targeted therapies.

30 Cancer promotes a prothrombotic state by producing pro-coagulant microvesicles
(MVs), adhesion molecules, and cytokine¹⁵. Tumor-MVs are small heterogeneous membrane-
bound vesicles possessing unique morphological traits and function¹⁶. One of the best-
characterized MVs are exosomes, which are 30-150 nm vesicles generated in multivesicular
endosomes (MVEs)⁹. High levels of circulating exosomes have been detected in the plasma

of patients with various cancer⁸⁰⁻¹³. Tumor exosomes might carry cancer-specific lipids, proteins, and nucleic acids that aid metastasi⁸⁴. Most methods for exosome purification co-isolate heterogeneous populations of EVs of diverse biogenic origin, including ectosomes that bud directly from the plasma membrane¹⁵.

5 The current treatment for cancer thrombosis involves the administration of anticoagulants that may result in bleeding complications and delay or discontinuation of chemotherapy, diminishing patient quality of life^{16, 17}. Therefore, timely identification of cancer patients with a high risk of thrombosis is vital for successful treatment. Unfortunately, however, thrombosis frequently goes undetected until post-mortem examination^{2, 18}.

10 SUMMARY OF THE INVENTION

Provided herein are human EIF3L binding molecules and nucleic acid sequences encoding such molecules. In particular embodiments, provided herein are human EIF3L binding molecules (e.g., monoclonal antibodies or antigen binding fragments thereof) having
15 particular light and/or heavy chains variable regions, or light chain and/or heavy chain CDRs, and methods for using such molecules, and/or other EIF3L binding molecules, and/or CD63 binding molecules, to treat a prothrombotic condition that is accompanied by pathological exosome production in subjects (e.g., human subjects with tumor-mediated thrombosis). In some embodiments, the presence or level of cancer is detected by detecting CD63 positive,
20 and/or PCA3 positive, and/or EIF3L positive, exosomes in a sample.

In some embodiments, provided here are compositions comprising a human EIF3L binding molecule, and/or one or more nucleic acid molecules encoding the human EIF3L binding molecule, wherein the human EIF3L binding molecule comprises: a) a heavy chain variable region, wherein the heavy chain variable region comprises: i) a CDRH1 amino acid
25 sequence comprising SEQ ID NO: 6, or SEQ ID NO:6 with one with one or two conservative amino acid changes, ii) a CDRH2 amino acid sequence comprising SEQ ID NO: 7, or SEQ ID NO:7 with one or two conservative amino acid changes, and iii) a CDRH3 amino acid sequence comprising SEQ ID NO: 8, or SEQ ID NO:8 with one with one or two conservative amino acid changes, and/or; b) a light chain variable region, wherein the light chain variable
30 region comprises: i) a CDRL1 amino acid sequence comprising SEQ ID NO: 10, or SEQ ID NO:10 with one with one or two conservative amino acid changes, ii) a CDRL2 amino acid sequence comprising SEQ ID NO: 11, or SEQ ID NO:11 with one with one or two conservative amino acid changes, and iii) a CDRL3 amino acid sequence comprising SEQ ID NO: 12, or SEQ ID NO:12 with one with one or two conservative amino acid changes.

In some embodiments, provided herein are method of treating or preventing a prothrombotic condition that is accompanied or caused by pathological exosome production, in a subject with said pro-thrombotic condition comprising: treating a subject with: i) a human EIF3L binding molecule, or one or more mRNAs encoding said EIF3L binding
5 molecules, or an expression vector comprising one or more nucleic acid molecules encoding said human EIF3L binding molecule, optionally wherein said human EIF3L binding molecule is as recited herein, ii) a human CD63 binding molecule, or one or more mRNAs encoding said human CD63 binding molecule, or an expression vector comprising one or more nucleic acid molecules encoding said CD63 binding molecule, and wherein said subject has, or is
10 suspected to develop, said prothrombotic condition.

In certain embodiments, provided herein are methods of treating or preventing a prothrombotic condition that is accompanied or caused by pathological exosome production, in a subject with said pro-thrombotic condition comprising: treating a subject with a human EIF3L binding molecule, or an expression vector comprising said one or more nucleic acid
15 molecules encoding said human EIF3L binding molecule, as recited above and herein (e.g., using a commercial antibody as described in Example 1), and wherein said subject has, or is suspected to develop, said prothrombotic condition. In some embodiments, wherein: i) said subject has cancer and has, or is suspected to develop, tumor-mediated thrombosis and/or a tumor-mediated thrombotic disorder and/or thrombosis associated with anti-tumor therapy,
20 and optionally wherein said tumor-mediated thrombotic disorder is selected from the group consisting of: heart attack, acute ischemic stroke, transient ischemic attack, deep vein thrombosis, a pulmonary embolism, and phlebitis; and/or iii) said prothrombotic condition is selected from: Sepsis, septic shock, viral infection, Sars-Cov2 infection, sickle cell disease, cardiovascular disease, acute coronary syndrome (ACS), stroke, acute inflammations,
25 infections-sepsis, and, acute coronary syndrome. In further embodiments, the human CD63 binding molecule binds a glycosylated version of human CD63.

In some embodiments, i) the CDRH1 amino acid sequence comprises SEQ ID NO: 6; ii) the CDRH2 amino acid sequence comprises SEQ ID NO: 7; iii) the CDRH3 amino acid sequence comprises SEQ ID NO: 9; iv) the CDRL1 amino acid sequence comprises SEQ ID
30 NO: 10; v) the CDRL2 amino acid sequence comprises SEQ ID NO: 11; and vi) the CDRL3 amino acid sequence comprises SEQ ID NO: 12. In certain embodiments, the human EIF3L binding molecule is an antibody, minibody, diabody, scFv, or antibody fragment capable of binding human EIF3L. In additional embodiments, the antibody fragment is a Fab, F(ab')₂ or

Fv antibody fragment. In some embodiments, the antibody or antibody fragment comprises at least an antigen binding portion of the A1806–3A1-3 antibody

In particular embodiments, the heavy chain and/or light chain variable region comprises a human framework region. In additional embodiments, the human EIF3L binding molecule further comprises a light chain constant region and a CH1 heavy chain constant region. In certain embodiments, the EIF3L binding molecule further comprises a CH2 heavy chain constant region and/or a CH3 heavy chain constant region. In other embodiments, the light chain constant region is human or a humanized murine, and/or wherein the CH1, CH2, and CH3 heavy chain constant regions are human or are humanized murine. In other embodiments, the human EIF3L binding molecule comprises an antibody, wherein the light chain constant region of the antibody is selected from: IgG Kappa and IgG Lambda, and wherein the heavy chain constant region of the antibody is selected from: IgG1, IgG2, IgG3, and IgG4. In particular embodiments, the human EIF3L binding molecule comprises an antibody, or antigen binding portion thereof, which is glycosylated or non-glycosylated.

In certain embodiments, the compositions herein further comprise a physiologically tolerable buffer. In additional embodiments, the heavy chain variable regions comprises SEQ ID NO: 5, or SEQ ID NO:5 with one or more conservative amino acid changes. In further embodiments, the light chain variable region comprises SEQ ID NO: 9, or SEQ ID NO:9 with one or more conservative amino acid changes. In additional embodiments, the composition comprises the one or more nucleic acid molecules. In other embodiments, the one or more nucleic acid molecules comprise: i) a first nucleic acid sequence encoding the heavy chain variable region, and ii) a second nucleic acid sequence encoding the light chain variable region. In additional embodiments, the compositions further comprise an expression vector, and wherein the first and/or second nucleic acid sequences are present in the expression vector.

In some embodiments, provided herein are methods of detecting CD63-positive, or PCA3-positive, or EIF3L-positive, small extracellular vesicles (sEVs) in a sample comprising: a) contacting a sample with: i) anti-CD63 antibodies, or antigen binding portions thereof, and/or ii) anti-PCA3 antibodies, or antigen binding portions thereof, and/or anti-EIF3L antibodies, or antigen binding portions thereof, wherein the sample comprises purified sEVs derived from a blood, plasma, or serum sample from a subject with cancer, and wherein the sample is suspected of containing CD63-positive, or PCA3-positive, or EIF3L-positive, sEVs, wherein the anti-CD63 antibodies, or antigen binding portions thereof, form first complexes with the CD63-positive sEVs if present in the sample, and wherein the anti-PCA

antibodies, or antigen binding portions thereof, form second complexes with the PCA3-positive sEVs if present in the sample, wherein the anti-EIF3L antibodies, or antigen binding portions thereof, form third complexes with the EIF3L-positive sEVs, if present in the sample; and b) detecting the presence or absence of the first complexes and/or the second complexes and/or third complexes, in the sample.

Anti-CD63 antibodies are described herein and available commercially (see, e.g., ab231975 and ab68418 from Abcam; EXOAB-CD63A-1 from System Biosciences; EWI018 from Kerfast; Clone H5C6 from BD Biosciences; and ExoBrite™ CD63 Flow Antibody from Biotium). Anti-PCA-3 antibodies are available commercially (see, e.g., HUF103418 from AssayGenie, and DEIA-LL272 from Creative Diagnostics). Anti-EIF3L are described herein and are available commercially (see, e.g., from MyBioSource, Solarbio Life Sciences, Bethyl laboratories, Aviva Systems Biology, GeneTex, and Biorbyt).

In certain embodiments, the sample is from a subject that has, or is suspected to develop, a prothrombotic condition that is accompanied by pathological exosome production, such as those described above, and/or thrombosis, and/or a thrombotic disorder. In other embodiments, the human anti-CD63, or anti-EIF3L, antibodies, or antigen binding portions thereof, comprise a detectable label, and/or wherein the anti-CD63, or anti-EIF3L, antibodies, or antigen binding portions thereof, are as described above and herein. In some embodiments, the methods further comprise contacting the sample with a conjugate molecule capable of binding to the: i) anti-CD63, or anti-EIF3L, antibodies, or antigen binding portions thereof, or ii) anti-PCA3 antibodies, or antigen binding portions thereof, wherein the conjugate molecule comprises a detectable label.

DESCRIPTION OF THE FIGURES

Figure 1. Platelets take up cancer cell-derived sEVs (small extracellular vesicles or exosomes). A. FACS analysis of isolated murine platelets alone (Control) or incubated for 60 minutes with Alexa Fluor 488 labeled synthetic, fibroblast-derived, or LNCaP-C4-2/cancer-derived sEVs (10 µg/ml) (n=3). B. Bar graph representing the mean percentage of sEV-positive (Alexa Fluor 488-positive) platelets from FACS analysis in panel A (n=3). C. Representative confocal images of isolated murine platelets labeled with WGA-Alexa Fluor 594 (red) after incubation for 60 min with WGA-Alexa Fluor 488 labeled LNCaP-C4-2/cancer-sEVs (green) (n=4). D. Bar graph showing quantification of the levels of synthetic (Control) and LNCaP-C4-2/cancer-sEVs (Alexa Fluor 488-mean fluorescence intensity (MFI)) detected in platelets by confocal microscopy in panel C (n=3). E. TEM of isolated

murine platelets incubated with LNCaP-C4-2-sEVs. The red arrows indicate LNCaP-C4-2-sEVs inside the platelets (n=4). F. A representative time course (out of three) plot of LNCaP-C4-2-sEV uptake by platelets in relation to 0 min time-point at a concentration of 10µg/ml as detected by FACS (n=3). G. PCR detection of hRPL28 RNA in platelets isolated from NSG mice carrying LNCaP-C4-2 tumor xenografts. Lanes represent C57BL/6J- WT (Platelets), NSG mice treated with IgM isotype control (Control + IgM), and NSG mice carrying the tumor xenografts treated with IgM (Tumor + IgM) or blocking antibody (Tumor + anti-sEV ab) (n=3). H. The panel on the left shows the detection of lncRNAs PCA3 and EST00000501280 by nested PCR in LNCaP-sEVs and cells, but not in murine platelets. The right panel shows the detection of PCA3 RNA in murine platelets pre-incubated with LNCaP-sEVs or vehicle (Control) and LNCaP cells. Mouse-GAPDH was used as a loading control for equal platelets. I. PCR detection of PCA3 in platelets isolated from healthy donors and cancer patients prior to prostatectomy (Pre-Op) and post-prostatectomy (Post-Op). LNCaP and VCaP cells were used as positive controls. Human-GAPDH serves as the loading control. (n=11 healthy donors (controls), and 32 Pre-Op and Post-Op patients). J. Bar graph representing the number of Pre-Op and Post-Op patient samples positive for PCA3 detection (n=30 Pre-Op and Post-Op patients). Graphs represent mean±SEM (B, D) and n is biological replicates or donors (I, J). P value was determined by non-parametric Kruskal-Wallis test followed by Dunn's post hoc multiple comparison test (B) and non-parametric Mann-Whitney test (D).

Figure 2. Platelet activation by cancer-sEVs. A. Mean percentage of activated platelets as measured by FACS. 2×10^8 /ml gel-filtered murine platelets were incubated for 60 minutes with vehicle alone (resting), thrombin (0.05 U/ml for 5 mins) and LNCaP-C4-2/cancer-sEVs at shown concentrations. Following the incubations, integrin α IIb β 3 activation was accessed with JON/A (PE) antibody using FACS (n=3). B. FACS analysis of depicting the time course of platelet activation by 20 µg/ml of LNCaP-C4-2/cancer-sEVs and thrombin (0.05 U/ml for 5 mins) (n=4). C. FACS analysis of platelet activation with JON/A (PE) antibody upon treatment with synthetic sEVs, LNCaP-C4-2/cancer-sEVs, for 60 mins and ADP (10 µM for 5 mins). D. Bar graph representing the mean percentage of activated platelets from panel C (n=4). E. Vascular occlusion times of mice as accessed by 12% FeCl₃-induced injury of the carotid artery 60 minutes after injection with 100µl PBS, synthetic, and LNCaP-C4-2 cancer-sEVs (10 µg/ml) (n=5, 8 and 12 mice respectively, per group). F-G. WT and APOE^{-/-} mice on a Western diet for 15 weeks carrying prostate cancer cell (RM1) xenografts or PBS (Control) were subjected to 12% FeCl₃-induced injury. Vascular

occlusion times in WT mice (n=6 and 4 per group) (F) and APOE^{-/-} mice (n=4) (G) are shown. A-G. Graphs represent mean±SEM and n is biological replicates. P value was determined by Kruskal-Wallis test followed by Dunn's post hoc multiple comparison test (A, B, D, E) and Mann-Whitney test (F, G).

5 Figure 3. N-linked glycosylated CD63-mediated cancer sEV uptake by platelets. A. Representative confocal images of platelets labeled with WGA-Alexa Fluor 594 (red) and sEVs labeled with BODIPY-488 (green). LNCaP-C4-2/cancer-sEVs were treated with PNGaseF or buffer alone overnight, followed by staining with BODIPY-488. SEVs were washed and incubated with gel-filtered murine platelets for 60 minutes. Platelets were then
10 washed and imaged by confocal microscopy (n=4). B. Bar graph representing the MFI levels of BODIPY-488 labeled PNGaseF-treated sEVs taken up by platelets in relative comparison to non-treated (control) sEVs as observed with confocal microscopy (n=4 experiments; cells from 3-4 fields analyzed/experiment). C. Representative confocal images of platelets incubated with anti-CD63 treated sEVs. LNCaP-C4-2-sEVs were incubated with anti-CD63
15 blocking antibody, isotype-IgG control, or vehicle (control) overnight, washed by 100,000 g centrifugation and then labeled with WGA-Alexa Fluor 488 for 4hrs. The treated sEVs were then incubated with isolated murine platelets pre-stained with WGA-Alexa Fluor 594 (red) for 60 minutes (n=5). D. Bar graph representation of quantification of the sEV levels (Alexa Fluor 488-MFI) inside platelets (red) observed by confocal microscopy in panel C. Anti-CD9
20 or anti-TSP1 treated sEVs are additional controls (n=5 for control and Anti-CD63 groups, n=3 for IgG and n=4 for CD9/TSP1; cells from 3 fields analyzed/experiment)). E. Quantification of MFI levels of PNGaseF, anti-CD63, a combination of PNGaseF and Anti-CD63, and O-glycosidase (O-glyco) treated sEVs in relative comparison with untreated sEVs (control) inside platelets as accessed by confocal microscopy (n=7 for Control and anti-CD63
25 and n=3 for others). F. MFI levels of sEVs inside platelets that were pre-treated with cytoskeleton inhibitors cytochalasin D (50 μM), ML141 (2 μM), wiskostatin (10 μM), compared relative to vehicle alone (control) as quantitated by confocal microscopy (n=3). A-F. Graphs represent mean±SEM and n is biological replicates. P value was determined by Kruskal-Wallis test followed by Dunn's post hoc multiple comparison test (D, E, F) and
30 Mann-Whitney test (B).

Figure 4. Inhibition of cancer sEV-induced platelet activation. A. Representative FACS histogram of platelet activation by LNCaP-C4-2-sEVs treated with fab fragments of the anti-CD63 or vehicle alone. ADP (10μM) was used as a platelet agonist (n=4). B. MFI of platelet activation with the various treatments represented in relation to sEVs treatment as

measured by FACS from panel A (n=4). C. Percentage of activated platelets as measured by FACS for integrin $\alpha\text{IIb}\beta 3$ activation with JON/A (PE) antibody from panel A (n=5 for all groups except ADP (n=4)). D. Representative bar graph demonstrating platelet P-selectin expression under basal (resting) conditions or in presence of LNCaP-C4-2-sEVs, sEVs treated with IgG isotype control, and sEVs treated with anti-CD63 antibody (n=3). E. FACS analysis of platelet activation by 20 $\mu\text{g}/\text{ml}$ PC3 cell line derived sEVs that were pre-treated with Fab fragments of anti-CD63 or IgG isotype control (n=4). F. Percentage of activated platelets as measured by FACS for integrin $\alpha\text{IIb}\beta 3$ activation with JON/A (PE) antibody from panel E (n=4). G. PCR analysis of hRPL28 in murine platelets incubated with LNCaP-C4-2-sEVs treated with Fab fragments of anti-CD63 or IgG control. mRNA from LNCaP-C4-2 cells serves as a positive control for hRPL28. Murine-GAPDH serves as a loading control for platelets. Graphs represent mean $\pm$ SEM and n is biological replicates. P value was determined by Kruskal-Wallis test followed by Dunn's post hoc test.

Figure 5. Platelet-RPTP α and sEV-CD63 mediated platelet activation signaling cascade. A. Isolated murine platelets stained with WGA-594 (red) were incubated for 60 minutes with WGA-Alexa fluor 488 (green)-labeled LNCaP-C4-2-sEVs (10 $\mu\text{g}/\text{ml}$) or sEVs pretreated with IgG isotype (control) and anti-CD63 antibody. In parallel, platelets were also pretreated with anti-RPTP α antibody for 30 mins followed by incubation with labeled sEVs. Unbound sEVs were removed by centrifugation, platelets were fixed in 4% PFA and visualized using confocal microscopy. Representative confocal images of platelets with sEVs are shown (n=4). B. Bar graph showing the mean levels (MFI of Alexa fluor-488 per platelet in relative comparison to sEVs+ group (black bar)) of LNCaP-C4-2-sEVs in platelets detected by confocal microscopy in A (n=4 for column 2 and 5, n=5 for RPTP, and n=6 for others). C-H. Isolated murine platelets ($2 \times 10^8/\text{ml}$) were incubated with LNCaP-C4-2-sEVs (20 $\mu\text{g}/\text{ml}$) or vehicle alone (Cont) for the indicated time. Platelets were then washed, lysed, and analyzed for the indicated proteins by western blotting. Representative Immunoblots and their respective quantitative densitometry analysis are shown on the right (D. n=6 for RP and 5min and n=3 for others; F. n=3; H. n=4 for groups RP and 90min on the left panel and n=3 for others). Graphs represent mean $\pm$ SEM and n is biological replicates. P value was determined by Kruskal-Wallis test followed by Dunn's post hoc test.

Figure 6. RPTP α is required for sEVs-induced signaling pathway in platelets. Isolated murine platelets ($2 \times 10^8/\text{ml}$) were pretreated with RPTP α antibody or IgG (0.01 $\mu\text{g}/\text{ml}$) for 30 minutes and washed to remove the unbound antibody. These platelets were then incubated with LNCaP-C4-2-sEVs (20 $\mu\text{g}/\text{ml}$) or with vehicle alone (Cont) for 5 min (A, B) and 90

minutes (C-G) and, thrombin (0.05 U/ml) for 15 minutes. Platelets were then washed and subjected to western blot analysis to detect the indicated proteins. Western blots (C, D, and F) were reprobbed with PLC γ 2, Akt, and P38 antibodies and have the same GAPDH as shown. Their respective quantitative densitometry analysis is shown in the bottom panels (A-B, D: n=6 for cont and -ve, n=3 for +ve, C, E: n=3, F: n=4, for cont and -ve, n=3 for +ve). Graphs represent mean $\pm$ SEM and n is biological replicates. P value was determined by Kruskal-Wallis test followed by Dunn's post hoc multiple comparison test.

Figure 7. CD63 blocking antibody inhibits cancer sEV-mediated platelet activation cascade and thrombosis *in vivo*. A. Representative Immunoblots of murine platelets incubated with LNCaP-C4-2-sEVs that were pre-treated with the Fab fragments of CD63 or CD9 blocking antibodies (0.01 μ g/ml) or IgG. B. Quantitative densitometry analysis of immunoblots in A, showing the levels of phosphoproteins with anti-CD63 and anti-CD9 treatments in relative comparison with IgG treatment after normalization to their respective total protein (n=3). C. Occlusion times of WT mice injected with LNCaP-C4-2-sEVs pre-treated with Fab fragments of anti-CD63 (0.01 μ g/ml) or IgG followed by 10% FeCl₃-induced carotid artery thrombosis injury (n=5, 7, and 4 for each group). D. Schematic depicting the mechanism of cancer-sEVs-mediated thrombus formation in circulation. Graphs represent mean $\pm$ SEM and n is biological replicates. P value was determined by Kruskal-Wallis test followed by Dunn's post hoc multiple comparison test.

Figure 8. Characterization of sEVs. A. Representative western blot of LNCaP-C4-2 cell and -sEV lysates. sEV-specific markers CD63 and Caveolin-1 were mainly detected in sEV lysates, with only minimal amounts observed in cell lysates. Annexin II, Ezrin, α -actinin 4, and β -actin were undetectable in the sEV fraction. B. Transmission electron microscopy (TEM) images of sEVs of different sizes showing sEV purity and structural integrity. n=3 biological replicates. C. Histogram representing the percentage frequency distribution of LNCaP-C4-2 sEVs size analyzed from the TEM images. n=3 biological replicates. D. NanoFACS analysis of the LNCaP-C4-2 sEV fraction, demonstrating the purity of isolated sEVs. More than 95% of the purified particles fall below the size of 180 nm, corresponding to the characteristic 30-150 nm size of sEVs, especially exosomes. Beads of different sizes and the sEVs-containing buffer (filtered PBS) were used as controls. E. Representative NTA of LNCaP-C4-2 sEVs purified by differential ultra-centrifugation (UC-sEVs) and control is 0.1 μ m filtered PBS that was used to resuspend sEVs. n=6 biological replicates. Particle size (diameter in μ M) is shown on the x-axis.

Figure 9. Human platelets uptake LNCaP-C4-2 sEVs. A. WGA-Alexa fluor-488 labeled LNCaP-C4-2 sEVs were treated with anti-CD63 antibody or IgG and incubated with purified human platelets (2×10^8 /ml) for 60 min. The platelets were washed and analyzed by confocal microscopy. Representative 3D reconstituted images are shown with sEVs in green and platelets in red. B. Representative TEM images of human platelets co-incubated with LNCaP-C4-2 sEVs or vehicle alone. Panels on the left show an entire platelet. The areas outlined in yellow are enlarged and shown in the panels on the right. The blue arrows indicate LNCaP-C4-2 sEVs within the platelets. C. Bar graphs (quantified from TEM images of B) represent the mean ($\pm$ SEM) number of sEVs that are membrane-bound and internalized observed per cross-section of mouse and human platelets. $n=4$ experiments each for mouse and human platelets with 20 and 30 cross-sections analyzed total for mouse and human, respectively. P value was determined by non-parametric Mann-Whitney test. D. Histogram representing FACS analysis of isolated murine platelets incubated for 60 minutes with Alexa Fluor 488 labeled fibroblast-derived, mouse prostate cancer cell line (RM1)-derived sEVs ($10 \mu\text{g/ml}$) and vehicle (Control).

Figure 10. Murine platelets interact with (bind/uptake) sEVs of various cancer origins. A-C. sEVs from LNCaP-C4-2, LNCaP, PC3, and SK-RC-26b were labeled with WGA-Alexa Fluor-488 and washed by ultracentrifugation. These labeled cancer sEVs ($20 \mu\text{g/ml}$) were then incubated with mouse platelets (2×10^8 /ml) for 60 min. The platelets were washed and analyzed for sEV uptake with FACS. Resting platelets were control-treated with the vehicle alone.

Figure 11. Detection of sEV-specific markers. A. Reverse transcriptase-PCR analysis of various mRNAs known to be enriched in cancers. Total RNA was isolated from cells and sEVs of breast cancer (MDA-MB-231), prostate cancer (LNCaP-C4-2), and renal cancer (SK-RC-26b), and then analyzed by PCR. GAPDH was used as a loading control. B. PCR analysis for prostate-specific membrane antigen (PSMA) in sEVs and cells of LNCaP, PC3 (prostate cancer), MDA-MB-231 (breast cancer), SK-RC-26b (renal cancer), and murine platelets. C. Gel-filtered murine platelets were incubated with LNCaP-C4-2 sEVs or vehicle alone for 60 minutes in Tyrode's buffer. Platelets were washed, total RNA was isolated and analyzed by PCR for RPL28 transcript. Total RNA from LNCaP-C4-2 cells was used as a positive control. D. Blood was collected from the inferior vena cava of mice into ACD anticoagulant with PGE1 and $100 \mu\text{l}$ of blood was incubated with LNCaP-sEVs ($10 \mu\text{g/ml}$) for 60 min at 37°C . Platelets were then isolated by gel filtration and analyzed for the presence of RPL28 transcript by PCR. E. $150 \mu\text{l}$ of LNCaP-C4-2-sEVs in PBS ($20 \mu\text{g/ml}$) were

injected into the mouse bloodstream via the tail vein. Control mice were injected with 150 μ l of PBS alone. Mice were allowed to rest for 60 min, and then blood was collected from the inferior vena cava. Leukocytes were isolated, platelets were purified by gel filtration, and total RNA was isolated from these cells. The RNA samples were analyzed by PCR for the presence of RPL28. GAPDH was used as a loading control.

Figure 12. Detection of PCA3 in platelets of prostate cancer patients. A. Platelets were isolated from the blood of healthy donors or prostate cancer patients as described in the Materials and Methods section. Total platelet RNA was isolated and analyzed for the presence of prostate cancer markers PCA3 and EST00000501280 by nested PCR. GAPDH served as a loading control. B. PCA3 was amplified by PCR from LNCaP cells and platelets of prostate cancer patients and subjected to restriction digestion with BsgI and ScaI. C. Purified platelets from healthy controls, and cancer patients prior to prostatectomy (Pre-Op) and post-prostatectomy (Post-Op), were analyzed for the presence of PCA3 with nested PCR. (n=11 healthy donors (controls), and 32 Pre-Op and Post-Op patients). LNCaP and VCaP cDNA were used as positive controls for PCA3, and GAPDH served as the loading control. D-E. NTA analysis of sEVs isolated from plasma of healthy volunteers and cancer patients. D. Representative histograms of sEV size distribution and concentration of (sEVs/ml of plasma) in healthy and, prostatectomy Pre-Op and Post-Op patients. E. Bar graph representing the mean sEV concentration per ml of plasma. n=7 donors for control, and 10 each for Pre-Op and Post-Op patients. Graphs represent mean $\pm$ SEM and n is biological replicates. P value was determined by One-way ANOVA followed by Tukey's post hoc multiple comparison test.

Figure 13. Detection of additional markers of cancer-sEVs in platelets of prostate cancer patients. A. PCR analysis of lncRNA pCAT1 in LNCaP sEVs, platelets of patient samples, and healthy donors. B. PCR detection of pCAT1 in platelets of prostate cancer patients before and after prostatectomy. Human GAPDH (hGAPDH) serves as a loading control. C. Bar graph representing the number of Pre-Op patient samples positive for PCAT1 detection by PCR (n=30 donors). D, E. PCR analysis and quantification of patient samples positive for the lncRNA TMPRSS2-ERG (n=14 donors). F. Synergistic effect of sEVs on ADP-mediated platelet aggregation. Platelets in 1x Tyrode's buffer containing calcium and fibrinogen were incubated with the indicated concentrations of LNCaP-C4-2 sEVs for 45 minutes and then accessed for aggregation in presence of 10 μ M ADP. The graph represents the percentage of aggregation at 10 minutes post ADP treatment. Graphs represent

mean $\pm$ SEM and n=3 biological replicates. P value was determined by Kruskal-Wallis test with the Dunn's post hoc test.

Figure 14. Platelet activation by sEVs from PC3 and SK-RC-26b cancer cell lines. A-B. PC3, LNCaP-C4-2, and SK-RC-26b cell-derived sEVs (20 μ g/ml) pre-treated with fab anti-CD63 or fab IgG or without treatment were incubated with murine platelets (2x10⁸/ml) for 60 min. The platelets were washed and analyzed for platelet integrin α IIb β 3 activation with JON/A (PE) antibody using FACS. Resting platelets were control-treated with the vehicle alone. n=3 biological replicates. C. Representative flow cytometry histogram demonstrating platelet P-selectin expression under basal (resting) conditions or in presence of LNCaP-C4-2 sEVs, sEVs treated with IgG isotype control, and sEVs treated with anti-CD63 antibody. n=3 biological replicates. D. Flow cytometry histogram demonstrating platelet Annexin-V expression under basal (resting) conditions or in presence of LNCaP-C4-2 sEVs, and sEVs treated with anti-CD63 antibody.

Figure 15. CD63-mediated cancer sEVs uptake by platelets. A-D. Labeled LNCaP-C4-2 sEVs were incubated with platelets isolated from WT, Kindlin3 deficient (K3hypo, n=3 mice), CD36 knockout (CD36^{-/-}, n=4 mice), AKT3 knockout (AKT3^{-/-}, n=5 mice), and TLR2 knockout (TLR2^{-/-}, n=3 mice). The MFI levels of LNCaP-C4-2 sEVs were taken up by platelets as quantified from confocal images and represented in relative comparison with WT. Graphs represent mean $\pm$ SEM and n is biological replicates. P value was determined by two-tailed unpaired t-test.

Figure 16. Cancer-sEVs induced platelet activation signaling mechanism. A. WT mouse platelets (2x10⁸/ml) were incubated with LNCaP-C4-2 sEVs (20 μ g/ml) or ADP (10 μ M) or KODA-PC (which can activate PLC γ 2 through TLR2, 20 μ M) or vehicle alone (Cont) for the indicated time. Platelets were then washed, lysed, and analyzed by western blot analysis to detect the indicated proteins. n=3 biological replicates. B-D. Platelets (2x10⁸/ml) were pretreated with different inhibitors - PI3K inhibitor (LY294002, 20 μ M), Src inhibitor (Dasatinib, 10 μ M), Akt inhibitor (MK2206, 10 μ M), ERK inhibitor (PD98059, 10 μ M), phospholipase C inhibitor (U73122) for 15 min and then incubated with LNCaP-C4-2 sEVs (20 μ g/ml) for 90 min. To detect the indicated proteins, platelets were then washed, lysed, and analyzed by western blot analysis. n=3 biological replicates. E. Quantitative densitometry analysis of immunoblots from panels B-D, showing the levels of phosphoproteins in platelets treated with Src inhibitor (Dasatinib, 10 μ M), PI3K inhibitor (LY294002, 20 μ M), and Akt inhibitor (MK2206, 10 μ M) followed by incubation with 20 μ g/ml of LNCaP-C4-2 sEVs for 90 minutes in relative comparison to untreated platelets incubated with LNCaP-C4-2 sEVs.

Graphs represent mean $\pm$ SEM and n=3 biological replicates. P value was determined by Kruskal-Wallis test with the Dunn's post hoc test.

Figure 17. A. Inhibition of sEVs-mediated platelet activation with pharmacological inhibitors in vitro. Purified mouse platelets (2×10^8 /ml) were pretreated with inhibitors LY294002, MK2206, PD98059, or U73122 for 15 min, then incubated with LNCaP-C4-2 sEVs (20 μ g/ml) for 90 min, washed, and analyzed for integrin α IIb β 3 activation using FACS analysis. Thrombin (0.05U/ml) for 15 min was used as a positive control. The actual MFI levels of a representative experiment are shown as a bar graph (representative of two biological replicates with platelets isolated from two animals for each replicate). B. Isolated murine platelets (2×10^8 /ml) were incubated with PC3 sEVs (20 μ g/ml) or vehicle alone (Cont) for 90 min. Platelets were then washed, lysed, and analyzed by western blot analysis (representative of two biological replicates). C. Elimination of sEVs independent signaling pathways in platelet activation. Quantitative densitometry analysis of immunoblots showing the levels of phosphoproteins in sEV-treated platelets in the presence of indicated inhibitors compared relative to sEV-treated platelets alone. Platelets were treated with apyrase (1 U/ml) and RGD cyclic peptide (RGD, 100 μ M) simultaneously with LNCaP-C4-2 sEVs (20 μ g/ml), followed by a 90 min incubation. Daltroban (100 μ M) was used to pretreat the platelets for 15 min, before the subsequent 90-min incubation with LNCaP-C4-2 sEVs (20 μ g/ml) (n=3 biological replicates for sEVs and sEVs + Apyrase; n=2 biological replicates for sEV+RGD and +Daltroban). Graphs represent mean $\pm$ SEM.

Figure 18 shows a schematic representation of an exemplary IgG molecule with the various regions and sections labeled. The CDRs and framework regions (FR) of one of the two variable region light chains, and one of the two variable region heavy chains, are also labeled.

Figure 19A shows the A1806–3A1-3 heavy chain amino acid sequence (SEQ ID NO:1), including a leader sequence (in italics). Figure 19B shows the A1806–3A1-3 heavy chain nucleic acid sequence (SEQ ID NO:2). Figure 19C shows the A1806–3A1-3 light chain amino acid sequence (SEQ ID NO:3), including a leader sequence (in italics). Figure 19D shows the A1806–3A1-3 light chain nucleic acid sequence (SEQ ID NO:4).

Figure 20A shows the A1806–3A1-3 variable heavy chain amino acid sequence (SEQ ID NO:5), including CDRH1 (SEQ ID NO:6), CDRH2, (SEQ ID NO:7), and CDRH3 (SEQ ID NO:8). Figure 20B shows the A1806–3A1-3 variable light chain amino acid sequence (SEQ ID NO:9), including CDRL1 (SEQ ID NO:10), CDRL2, (SEQ ID NO:11), and CDRL3 (SEQ ID NO:12).

Figure 21. Occlusion times of NSG (immunodeficient mice used to grow human LNCaP-C4-2 tumors) mice injected bilaterally with LNCaP-C4 tumors. Mice were treated intravenously with control IgM or with 3A1-3 antibody. Then occlusion time was measured in FeCl₃-induced carotid artery thrombosis injury model. Figure 21A shows the graphed time to occlusion. Figure 21B shows photographs of skin without tumor, next row- tumors in mice treated with control abs and the bottom two tumors in mice treated with 3A1-3 abs. Note diminished intratumoral thrombosis/hemorrhage as a result of 3A1-3 treatment.

Figure 22. 3A13 immunoprecipitates EIF3L from both LNCaP-C4 exosomes and cells. Confirmation of EIF3L (67kd) binding by Reciprocal IP with 3A13 (Figures 22 A and C) and commercial Anti-EIF3L polyclonal Abs (Figures 22 B and D). Figure 22A shows a gel probed with a commercial anti-EIF3L antibody, Figure 22B shows a gel probed with 3A13 antibody (showing it binds to EIF3L), Figure 22C shows a gel probed with 3A13 antibody, and Figure 22D shows a gel probed with commercial anti-EIF3L antibodies. These are two independent validation methods (in addition to mass spectrometry) to show that 3A13 recognizes EIF3L.

Figure 23. Direct binding of 3A13 to fluorescently labeled exosomes using MST assay. Figure 23A shows capillary scans of Microscale Thermophoresis Assays. Figure 23B shows that mAb-3A13 binds to LNCaP-C42 exosomes (target complex, lowest green line) and commercial recombinant EIF3L can diminish this complex by interfering with the interaction (blue line), but not change the strength of interaction (indicated by the vertical shift).

Figure 24 shows that mAb 3A13 likely blocks LNCaP-C42 exosome uptake in vivo and in vitro. Figure 24A shows that short treatment with 3A13 does not diminish tumor size (at least when injected late), but the uptake of exosomes by platelets is blocked. Figure 24B shows 3A13 blocks exosomes uptake by platelets measured by the presence in murine platelets cancer-specific human marker hRPL28. Note the lack of the band after 3A13 treatment. Figure 24C shows similar (but less efficient) inhibition can be achieved by anti-EIF4L antibodies (bottom left). Figure 24D shows 3A13, and another of our antibodies 3H9B, both inhibit uptake of cancer exosomes.

Figure 25 shows mAb-3A13 prevents platelet activation induced by prostate cancer LNCaP-C4-2 exosomes as measured by FACS (Figure 25A and summary graph in Figure 25C). Platelet activation was accessed with JON/A (PE) antibody using FACS. Figures 25 B and D: anti-EIF3L abs are also capable of inhibiting platelet activation, showing an

importance of EIF3L in this process. Figures 25 C and D are summaries from the FACs curves shown on top.

Figure 26 shows EIF3L is detected in renal cancer cell line (SK-rc26b) exosomes.

Figure 27 shows mAb-3A13 prevents platelet activation induced by renal cancer SK-
5 Rc-26b exosomes as measured by FACS (Figure 27A and in summary in Figure 27C). Platelet activation was assessed with JON/A (PE) antibody using FACS. Figure 27B and D show 3A13 prevents platelet activation induced by prostate cancer LNCaP-C4-2 exosomes as measured by FACS. Platelet activation was assessed with JON/A (PE) antibody using FACS.

Figure 28 shows EIF3L is detected in exos derived from colorectal, renal and prostate
10 cancer cell lines. Both anti-EIF3L antibodies and 3A13 abs (but not 3H9B abs) react with three different lines of colorectal cancer exosomes (CaCo2, HT29 and HCT) evidenced by dot blot analysis.

Figure 29 shows that levels of cancer exosomes in blood of control cancer-free
subjects (control) and prostate cancer patients prior (pre-op) and after radical prostatectomy
15 (post-op). Figure 29A shows exosomes profile in control blood, Figure 29B and C show exosomes from blood of prostate cancer patients prior (pre-op) and after radical prostatectomy (post-op). Figure 29D shows bar graph summarizing the results shown in Figures 29A-C.

Figure 30 shows results of screening of prostate cancer patient plasma with mAb-
20 3A13 and anti-EIF3L. Figure 30A. Purified platelets from healthy controls, and cancer patients prior to prostatectomy (Pre-OP) and post-prostatectomy (Post-Op), were analyzed for the presence of PCA3 with nested PCR. (n=11 healthy donors (controls), and 32 Pre-Op and Post-Op patients). LNCaP and VCaP cDNA were used as positive controls for PCA3, and GAPDH served as the loading control. Figure 30B shows that 3A13 antibody binds to
25 exosomes from prostate cancer patients prior prostatectomy but not after cancer removal, thus, 3A13 reacts to patients' circulating exosomes (might be used for diagnostics). Figure 30C and D show that anti-EIF3L antibody also recognize the same samples from prostate cancer patients that are recognized by 3A13 (in Figure 30B). Dots of plasma from patients were placed on the immunoblotting paper and developed using 3A13 antibodies (Figure 30B) or antibodies against EIF3L (Figures 30C and D). This simple dot-blot assay required a drop
30 of plasma or serum and can be used for cancer screening, detection and staging.

Figure 31 shows a second screening of prostate cancer patients' plasma. 3A13 antibody does not react with plasma from healthy donors (Figure 32A and B), but binds to plasma from prostate cancer patients prior to prostatectomy but not after cancer removal. In

Figure 31A, the dots of plasma from patients were placed on the immunoblotting paper and developed using 3A13 antibodies and in Figure 31B the dots of plasma from patients were placed on the immunoblotting paper and developed using anti-EIF3L antibodies. Figures 31C and D show optimization and increase of dot-blot sensitivity using different preparation of plasma samples.

Figure 32 shows mAb-3A13 binds to EIF3L LNCAP-C4 cells. Figure 32A shows mAb-3A13 pulls down EIF3L in LnCap-C42 cell lysates (indicated by an arrow). Figure 32B shows an opposite experiment: antibodies against EIF3L immunoprecipitated antigen that is recognized by 3A13 antibodies. These are two independent validation methods (in addition to mass spectrometry) to show that 3A13 recognize EIF3L.

Figure 33 shows 3A1-3 mAb reduces colorectal cancer-exosome induced platelet activation. Figure 33A shows profiles of FACS analysis (gray- control unstained platelets, blue- resting untreated platelets, red- platelets activated with CaCo-2 and treated with control mouse IgM Kappa isotype abs, green- platelets activated by exosomes pre-treated with 3A1-3 MAB). Figure 33B shows the summary bar graph from 5 independent experiments. Details: Caco-2 cells (ATCC #HTB-37™) are epithelial cells isolated from colon tissue derived from a 72-year-old, White, male with colorectal adenocarcinoma. Exosome isolation was done by differential centrifugation of cell supernatant. Exosomes were pre-treated overnight with 3A1-3 mAb or mouse IgM Kappa isotype Control. Mouse platelets were purified by gel filtration (Sephadex CL-2B column) and incubated with pre-treated exosomes for 45 min. Platelet integrin α IIb β 3 activation was assessed using JON/A (PE) antibody and FACS analysis using CytoFLEX flow cytometer and analyzed with FlowJo 10 software.

DEFINITIONS

To facilitate an understanding of the invention, a number of terms are defined below.

As used herein, the terms “exosomes” and “small extracellular vesicles” (aka “sEVs”) refer to vesicles are less than 200 nm in diameter as described in the MISEV2018 guidelines, published in the Journal of Extracellular Vesicles.

The term "antibody," as used herein, is intended to refer to immunoglobulin molecules comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or VL) and a

light chain constant region. The light chain constant region is comprised of one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each variable region (VH or VL) contains 3 CDRs, designated CDR1, CDR2 and CDR3 (see, Figure 18). Each variable region also contains 4 framework sub-regions, designated FR1, FR2, FR3 and FR4 (see, Figure 18), which may be human framework sub-regions.

As used herein, the term "antibody fragment or portion" refers to a portion of an intact antibody. Examples of antibody fragments or portions include, but are not limited to, linear antibodies, single-chain antibody molecules, Fv, Fab and F(ab')₂ fragments, and multi-specific antibodies formed from antibody fragments. The antibody fragments preferably retain at least part of the heavy and/or light chain variable region.

As used herein, the terms "complementarity determining region" and "CDR" refer to the regions that are primarily responsible for antigen-binding. There are three CDRs in a light chain variable region (CDRL1, CDRL2, and CDRL3), and three CDRs in a heavy chain variable region (CDRH1, CDRH2, and CDRH3).

As used herein, the term "framework" refers to the residues of the variable region other than the CDR residues. There are four separate framework sub-regions that make up the framework: FR1, FR2, FR3, and FR4 (see, Figure 18). In order to indicate if the framework sub-region is in the light or heavy chain variable region, an "L" or "H" may be added to the sub-region abbreviation (e.g., "FRL1" indicates framework sub-region 1 of the light chain variable region). It is noted that, in certain embodiments, the human EIF3L binding molecules of the present invention may have less than a complete framework (e.g. the human CD63 binding molecule may have a portion of a framework that only contains one or more of the four sub-regions).

As used herein, the term "fully human framework" means a framework with an amino acid sequence found naturally in humans. Examples of fully human frameworks, include, but are not limited to, KOL, NEWM, REI, EU, TUR, TEI, LAY and POM (See, e.g., Kabat et al., (1991) Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, NIH, USA; and Wu et al., (1970) J. Exp. Med. 132, 211-250, both of which are herein incorporated by reference). In certain embodiments, the human CD63 binding molecules herein have a fully human framework.

As used herein, the terms "subject" and "patient" refer to any animal, such as a mammal like a dog, cat, bird, livestock, and preferably a human.

As used herein, the term "codon" or "triplet" refers to a group of three adjacent nucleotides which specify one of the naturally occurring amino acids found in polypeptides. The term also includes codons which do not specify any amino acid. It is also noted that, due to the degeneracy of the genetic code, there are many codons that code for the same amino acid. As such, many of the bases of the nucleic acid sequences of the present invention can be changed without changing the actual amino acid sequence that is encoded. The present disclosure is intended to encompass all such nucleic acid sequences.

As used herein, the terms "an oligonucleotide having a nucleotide sequence encoding a polypeptide," "polynucleotide having a nucleotide sequence encoding a polypeptide," and "nucleic acid sequence encoding a peptide" means a nucleic acid sequence comprising the coding region of a particular polypeptide. The coding region may be, for example, present in a cDNA, genomic DNA, or RNA form. When present in a DNA form, the oligonucleotide or polynucleotide may be single-stranded (i.e., the sense strand) or double-stranded. Suitable control elements such as enhancers/promoters, splice junctions, polyadenylation signals, etc. may be placed in close proximity to the coding region of the gene if needed to permit proper initiation of transcription and/or correct processing of the primary RNA transcript. Alternatively, the coding region utilized in the expression vectors of the present invention may contain endogenous enhancers/promoters, splice junctions, intervening sequences, polyadenylation signals, etc., or a combination of both endogenous and exogenous control elements.

Also, as used herein, there is no size limit or size distinction between the terms "oligonucleotide" and "polynucleotide." Both terms simply refer to molecules composed of nucleotides. Likewise, there is no size distinction between the terms "peptide" and "polypeptide." Both terms simply refer to molecules composed of amino acid residues.

As used herein, the term "the complement of" a given sequence is used in reference to the sequence that is completely complementary to the sequence over its entire length. For example, the sequence 5'-A-G-T-A-3' is "the complement" of the sequence 3'-T-C-A-T-5'.

The term "isolated" when used in relation to a nucleic acid, as in "an isolated oligonucleotide" or "isolated polynucleotide" or "isolated nucleic acid sequence encoding a CD63 binding molecule" refers to a nucleic acid sequence that is identified and separated from at least one contaminant nucleic acid with which it is ordinarily associated (e.g. host cell proteins).

As used herein, the term "purified" or "to purify" refers to the removal of contaminants from a sample. For example, EIF3L binding molecules (e.g., antibodies or

antibody fragments) may be purified by removal of contaminating non-immunoglobulin proteins; they are also purified by the removal of immunoglobulins that do not bind to the same antigen. The removal of non-immunoglobulin proteins and/or the removal of immunoglobulins that do not bind the particular antigen results in an increase in the percentage of antigen specific immunoglobulins in the sample. In another example, recombinant antigen-specific polypeptides are expressed in bacterial host cells and the polypeptides are purified by the removal of host cell proteins; the percentage of recombinant antigen-specific polypeptides is thereby increased in the sample.

As used herein, the term "Fc region" refers to a C-terminal region of an immunoglobulin heavy chain. The "Fc region" may be a native sequence Fc region or a variant Fc region (e.g., with increased or decreased effector functions).

DESCRIPTION OF THE INVENTION

Provided herein are human EIF3L binding molecules and nucleic acid sequences encoding such molecules. In particular embodiments, provided herein are human EIF3L binding molecules (e.g., monoclonal antibodies or antigen binding fragments thereof) having particular light and/or heavy chains variable regions, or light chain and/or heavy chain CDRs, and methods for using such molecules, and/or other EIF3L binding molecules, and/or CD63 binding molecules, to treat a prothrombotic condition that is accompanied by pathological exosome production in subjects (e.g., human subjects with tumor-mediated thrombosis). In some embodiments, the presence or level of cancer is detected by detecting CD63 positive, and/or PCA3 positive, exosomes in a sample.

In work conducted during development of embodiments herein, we show that platelet uptake of cancer cell-derived sEVs in vitro and in vivo via sEV-tetraspanin CD63 or EIF3L and delineated the pathway leading to platelet activation. Platelets accumulate cancer cell-derived mRNAs, which could serve as predictive markers of the prothrombotic state in cancer patients.

Platelet hyperreactivity and thrombosis are major life-threatening complications in cancer patients. Tumor-induced platelet activation is a complex process that may involve direct interactions. Understanding the mechanisms of tumor-platelet communication and platelet activation can help develop targeted therapies for preventing thrombosis in cancer patients. In work conducted during the development of embodiments herein, we determined a novel mechanism of sEV-mediated cancer-platelet communications involving CD63 and EIF3L on cancer-sEVs and RPTP α on platelets and leading to the accumulation of cancer

markers in platelets, their hyperreactivity, and thrombosis. Platelets selectively uptake sEV from malignant cells, thereby accumulating tumor- markers. The prostate cancer marker PCA3 is present in platelets of prostate cancer patients before but not after prostatectomy suggesting the value of cancer-sEVs markers in platelets for both cancer detection and

5 prediction of thrombotic events. Cancer sEV uptake occurs via a novel mechanism involving CD63 and EIF3L on sEV and platelet RPTP α , leading to platelet activation and subsequent thrombosis. Interference with this mechanism using anti-CD63 antibodies, or fragments thereof, or anti-EIF3L antibodies or fragments thereof, prevents both sEVs uptake and thrombosis in vivo. As such, sEV-CD63 and EIF3L are a therapeutic targets (e.g., using anti-

10 CD63 antibodies or anti-EIF3L antibodies) for treating and preventing thrombosis in cancer patients.

In certain embodiments, the human EIF3L binding molecules comprise one or more of the antibodies, variable regions, or CDRs shown in SEQ ID NOS:1, 3, 5-12 and/or variable regions or CDRs with one or more conservative or non-conservative amino acid changes in

15 these SEQ ID NOS: 1, 3, 5-12, and nucleic acid sequences encoding SEQ ID NOS: 1, 3, 5-12 (e.g., using at least a portion of the nucleic acid sequences in SEQ ID NOS: 2 or 4). Changes to the amino acid sequences of the CDRs or variable regions (see Figures 19-20) may be generated by changing the nucleic acid sequence encoding the amino acid sequence. A nucleic acid sequence encoding a variant of a given CDR or variable region may be prepared

20 by methods known in the art using the guidance of the present specification for particular sequences. These methods include, but are not limited to, preparation by site-directed (or oligonucleotide-mediated) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared nucleic acid encoding the CDR or variable region.

Briefly, in carrying out site-directed mutagenesis of DNA, the starting DNA is altered

25 by first hybridizing an oligonucleotide encoding the desired mutation to a single strand of such starting DNA. After hybridization, a DNA polymerase is used to synthesize an entire second strand, using the hybridized oligonucleotide as a primer, and using the single strand of the starting DNA as a template. Thus, the oligonucleotide encoding the desired mutation is incorporated in the resulting double-stranded DNA.

30 PCR mutagenesis is also suitable for making amino acid sequence variants of the starting CDR (see, e.g., Vallette et. al., (1989) Nucleic Acids Res. 17: 723-733, hereby incorporated by reference). Briefly, when small amounts of template DNA are used as starting material in a PCR, primers that differ slightly in sequence from the corresponding region in a template DNA can be used to generate relatively large quantities of a specific

DNA fragment that differs from the template sequence only at the positions where the primers differ from the template.

Another method for preparing variants, cassette mutagenesis, is based on the technique described by Wells et al., (1985) Gene 34: 315-323, hereby incorporated by
5 reference. The starting material is the plasmid (or other vector) comprising the starting CDR or variant region DNA to be mutated. The codon(s) in the starting DNA to be mutated are identified. There should be a unique restriction endonuclease site on each side of the identified mutation site(s). If no such restriction sites exist, they may be generated using the above-described oligonucleotide-mediated mutagenesis method to introduce them at
10 appropriate locations in the starting polypeptide DNA. The plasmid DNA is cut at these sites to linearize it. A double-stranded oligonucleotide encoding the sequence of the DNA between the restriction sites but containing the desired mutation(s) is synthesized using standard procedures, wherein the two strands of the oligonucleotide are synthesized separately and then hybridized together using standard techniques. This double-stranded
15 oligonucleotide is referred to as the cassette. This cassette is designed to have 5' and 3' ends that are compatible with the ends of the linearized plasmid, such that it can be directly ligated to the plasmid. This plasmid now contains the mutated DNA sequence.

Alternatively, or additionally, the desired amino acid sequence encoding a CDR variant, or variable region variant, can be determined, and a nucleic acid sequence encoding
20 such amino acid sequence variant can be generated synthetically. Conservative modifications in the amino acid sequences of the CDRs or variable region may also be made. Naturally occurring residues are divided into classes based on common side-chain properties:

- (1) hydrophobic: norleucine, met, ala, val, leu, ile;
- (2) neutral hydrophilic: cys, ser, thr;
- 25 (3) acidic: asp, glu;
- (4) basic: asn, gln, his, lys, arg;
- (5) residues that influence chain orientation: gly, pro; and
- (6) aromatic: trp, tyr, phe.

Conservative substitutions will entail exchanging a member of one of these classes for
30 another member of the same class in a particular antibody, variable region, or CDR, such as in SEQ ID NOS: 1, 3, 5-12.

The CDRs of the present invention may be employed with any type of suitable framework. In some embodiments, the CDRs are used with fully human frameworks, or framework sub-regions. For example, the NCBI web site contains the sequences for known

human framework regions. Examples of human VH sequences include, but are not limited to, VH1-18, VH1-2, VH1-24, VH1-3, VH1-45, VH1-46, VH1-58, VH1-69, VH1-8, VH2-26, VH2-5, VH2-70, VH3-11, VH3-13, VH3-15, VH3-16, VH3-20, VH3-21, VH3-23, VH3-30, VH3-33, VH3-35, VH3-38, VH3-43, VH3-48, VH3-49, VH3-53, VH3-64, VH3-66, VH3-7, 5 VH3-72, VH3-73, VH3-74, VH3-9, VH4-28, VH4-31, VH4-34, VH4-39, VH4-4, VH4-59, VH4-61, VH5-51, VH6-1, and VH7-81, which are provided in Matsuda et al., (1998) J. Exp. Med. 188:1973-1975, that includes the complete nucleotide sequence of the human immunoglobulin chain variable region locus, herein incorporated by reference. Examples of human VK sequences include, but are not limited to, A1, A10, A11, A14, A17, A18, A19, 10 A2, A20, A23, A26, A27, A3, A30, A5, A7, B2, B3, L1, L10, L11, L12, L14, L15, L16, L18, L19, L2, L20, L22, L23, L24, L25, L4/18a, L5, L6, L8, L9, O1, O11, O12, O14, O18, O2, O4, and O8, which are provided in Kawasaki et al., (2001) Eur. J. Immunol. 31:1017-1028; Schable and Zachau, (1993) Biol. Chem. Hoppe Seyler 374:1001-1022; and Brensing-Kuppers et al., (1997) Gene 191:173-181, all of which are herein incorporated by reference. 15 Examples of human VL sequences include, but are not limited to, V1-11, V1-13, V1-16, V1-17, V1-18, V1-19, V1-2, V1-20, V1-22, V1-3, V1-4, V1-5, V1-7, V1-9, V2-1, V2-11, V2-13, V2-14, V2-15, V2-17, V2-19, V2-6, V2-7, V2-8, V3-2, V3-3, V3-4, V4-1, V4-2, V4-3, V4-4, V4-6, V5-1, V5-2, V5-4, and V5-6, which are provided in Kawasaki et al., (1997) Genome Res. 7:250-261, herein incorporated by reference. Fully human frameworks can be selected 20 from any of these functional germline genes. Generally, these frameworks differ from each other by a limited number of amino acid changes. These frameworks may be used with the CDRs described herein. Additional examples of human frameworks which may be used with the CDRs of the present invention include, but are not limited to, KOL, NEWM, REI, EU, TUR, TEI, LAY and POM (See, e.g., Kabat et al., (1991) Sequences of Proteins of 25 Immunological Interest, US Department of Health and Human Services, NIH, USA; and Wu et al., (1970), J. Exp. Med. 132:211-250, both of which are herein incorporated by reference).

In certain embodiments, the human EIF3L binding molecules of the present invention comprise antibodies or antibody fragments (e.g., comprising one or more of the CDRs described herein, such as in Figure 20). An antibody, or antibody fragment, of the present 30 invention can be prepared, for example, by recombinant expression of immunoglobulin light and heavy chain genes in a host cell. For example, to express an antibody recombinantly, a host cell may be transfected with one or more recombinant expression vectors carrying DNA fragments encoding the immunoglobulin light and heavy chains of the antibody such that the light and heavy chains are expressed in the host cell and, preferably, secreted into the

medium in which the host cell is cultured, from which medium the antibody can be recovered. Standard recombinant DNA methodologies may be used to obtain antibody heavy and light chain genes, incorporate these genes into recombinant expression vectors and introduce the vectors into host cells, such as those described in Sambrook, Fritsch and
5 Maniatis (eds), Molecular Cloning; A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), Ausubel, F. M. et al. (eds.) Current Protocols in Molecular Biology, Greene Publishing Associates, (1989) and in U.S. Pat. No. 4,816,397 by Boss et al., all of which are herein incorporated by reference.

10 In certain antibodies, the anti-EIF3L antibodies, or fragments, thereof prepared herein have an IgG isotype constant regions as shown in Table 1 below.

TABLE 1

IgG Isotype Constant region	Accession #
IgG Light Kappa	P0DOX7.1
IgG Light Lambda	P0DOX8.1
IgG1 Heavy	P01857
IgG2 Heavy	P01859
IgG3 Heavy	P01860
IgG4 Heavy	P01861

15 To express an antibody with one or more of the CDRs herein, DNA fragments encoding the light and heavy chain variable regions are first obtained. These DNAs can be obtained by amplification and modification of germline light and heavy chain variable sequences using the polymerase chain reaction (PCR).

20 Once the germline VH and VL fragments are obtained, these sequences can be mutated to encode one or more of the CDR amino acid sequences disclosed herein (see, Figure 20). The amino acid sequences encoded by the germline VH and VL DNA sequences may be compared to the CDRs sequence(s) desired to identify amino acid residues that differ from the germline sequences. Then the appropriate nucleotides of the germline DNA

sequences are mutated such that the mutated germline sequence encodes the selected CDRs, using the genetic code to determine which nucleotide changes should be made. Mutagenesis of the germline sequences may be carried out by standard methods, such as PCR-mediated mutagenesis (in which the mutated nucleotides are incorporated into the PCR primers such
5 that the PCR product contains the mutations) or site-directed mutagenesis. In other embodiments, the variable region is synthesized de novo (e.g., using a nucleic acid synthesizer).

Once DNA fragments encoding the desired VH and VL segments are obtained (e.g., by amplification and mutagenesis of germline VH and VL genes, or synthetic synthesis, as
10 described above), these DNA fragments can be further manipulated by standard recombinant DNA techniques, for example to convert the variable region genes to full-length antibody chain genes, to Fab fragment genes or to a scFv gene. In these manipulations, a VL- or VH-encoding DNA fragment is operably linked to another DNA fragment encoding another polypeptide, such as an antibody constant region or a flexible linker. The isolated DNA
15 encoding the VH region can be converted to a full-length heavy chain gene by operably linking the VH-encoding DNA to another DNA molecule encoding heavy chain constant regions (CH1, CH2 and CH3). The sequences of mouse and human heavy chain constant region genes are known in the art and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The heavy chain constant region can be, for
20 example, an IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM or IgD constant region. For a Fab fragment heavy chain gene, the VH-encoding DNA can be operably linked to another DNA molecule encoding only the heavy chain CH1 constant region.

The isolated DNA encoding the VL region can be converted to a full-length light chain gene (as well as a Fab light chain gene) by operably linking the VL-encoding DNA to
25 another DNA molecule encoding the light chain constant region, CL. The sequences of mouse and human light chain constant region genes are known in the art (see e.g., Kabat, E. A., et al., (1991) Sequences of Proteins of immunological Interest, Fifth Edition, U.S. Department of Health and Human Services. NIH Publication No. 91-3242) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The
30 light chain constant region can be a kappa or lambda constant region.

To create a scFv gene, the VH- and VL-encoding DNA fragments may be operably linked to another fragment encoding a flexible linker, e.g., encoding the amino acid sequence (Gly₄-Ser)₃, such that the VH and VL sequences can be expressed as a contiguous single-chain protein, with the VL and VH regions joined by the flexible linker (see e.g., Huston et

al., (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883; and McCafferty et al., (1990) Nature 348:552-554), all of which are herein incorporated by reference).

To express the antibodies, or antibody fragments of the invention, DNAs encoding partial or full-length light and heavy chains, (e.g. obtained as described above), may be
5 inserted into expression vectors such that the genes are operably linked to transcriptional and translational control sequences. In this context, the term "operably linked" is intended to mean that an antibody gene is ligated into a vector such that transcriptional and translational control sequences within the vector serve their intended function of regulating the transcription and translation of the antibody gene. The expression vector and expression
10 control sequences are generally chosen to be compatible with the expression host cell used. The antibody light chain gene and the antibody heavy chain gene can be inserted into separate vectors or, more typically, both genes are inserted into the same expression vector. The antibody genes may be inserted into the expression vector by standard methods (e.g., ligation of complementary restriction sites on the antibody gene fragment and vector, or blunt
15 end ligation if no restriction sites are present). Prior to insertion of the light or heavy chain sequences, the expression vector may already carry antibody constant region sequences. For example, one approach to converting the VH and VL sequences to full-length antibody genes is to insert them into expression vectors already encoding heavy chain constant and light chain constant regions, respectively, such that the VH segment is operably linked to the CH segment(s) within the vector and the VL segment is operably linked to the CL segment within
20 the vector. Additionally, or alternatively, the recombinant expression vector can encode a signal peptide that facilitates secretion of the antibody chain from a host cell. The antibody chain gene can be cloned into the vector such that the signal peptide is linked in-frame to the amino terminus of the antibody chain gene. The signal peptide can be an immunoglobulin
25 signal peptide or a heterologous signal peptide (i.e., a signal peptide from a non-immunoglobulin protein).

In addition to the antibody chain genes, the recombinant expression vectors of the disclosure may carry regulatory sequences that control the expression of the antibody chain genes in a host cell. The term "regulatory sequence" is intended to include promoters,
30 enhancers and other expression control elements (e.g., polyadenylation signals) that control the transcription or translation of the antibody chain genes. Such regulatory sequences are described, for example, in Goeddel; Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, Calif. (1990), herein incorporated by reference. It will be appreciated by those skilled in the art that the design of the expression vector, including the

selection of regulatory sequences may depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. In certain embodiments, regulatory sequences for mammalian host cell expression include viral elements that direct high levels of protein expression in mammalian cells, such as promoters and/or enhancers
5 derived from cytomegalovirus (CMV) (such as the CMV promoter/enhancer), Simian Virus 40 (SV40) (such as the SV40 promoter/enhancer), adenovirus, (e.g., the adenovirus major late promoter (AdMLP)) and polyoma virus. For further description of viral regulatory elements, and sequences thereof, see e.g., U.S. Pat. No. 5,168,062 by Stinski, U.S. Pat. No. 4,510,245 by Bell et al. and U.S. Pat. No. 4,968,615 by Schaffner et al., all of which are
10 herein incorporated by reference.

In addition to the antibody chain genes and regulatory sequences, the recombinant expression vectors of the invention may carry additional sequences, such as sequences that regulate replication of the vector in host cells (e.g., origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the
15 vector has been introduced (see e.g., U.S. Pat. Nos. 4,399,216, 4,634,665 and 5,179,017, all by Axel et al.). For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in dhfr- host cells with methotrexate selection/amplification) and the neomycin gene (for
20 G418 selection).

For expression of the light and heavy chains, the expression vector(s) encoding the heavy and light chains may be transfected into a host cell by standard techniques. The various forms of the term "transfection" are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or
25 eukaryotic host cell, e.g., electroporation, calcium-phosphate precipitation, DEAE-dextran transfection and the like.

In certain embodiments, the expression vector used to express the human EIF3L binding molecules of the present invention are viral vectors, such as retro-viral vectors. Such viral vectors may be employed to generate stably transduced cell lines (e.g. for a continuous
30 source of the EIF3L binding molecules). In some embodiments, the GPEX gene product expression technology (from Catalent, Somerset, NJ) is employed to generate CD63 binding molecules (and stable cell lines expressing the EIF3L binding molecules). In particular embodiments, the expression technology described in WO0202783 and WO0202738 to Bleck et al. (both of which are herein incorporated by reference in their entireties) is employed.

Mammalian host cells for expressing the recombinant antibodies of the invention include, for example, Chinese Hamster Ovary (CHO cells) (including dhfr- CHO cells, described in Urlaub and Chasin, (1980) Proc. Natl. Acad. Sci. USA 77:4216-4220, used with a DHFR selectable marker, e.g., as described in R. J. Kaufman and P. A. Sharp (1982) Mol. Biol. 159:601-621), NSO myeloma cells, COS cells and SP2 cells. In other embodiments, the host cells express GnT III as described in WO9954342 and U.S. Pat. Pub. 20030003097, both herein incorporated by reference, such that expressed EIF3L binding molecules have increased ADCC activity. When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are generally produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more preferably, secretion of the antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods.

Host cells can also be used to produce portions of intact antibodies, such as Fab fragments or scFv molecules. It will be understood that variations on the above procedure are within the scope of the present disclosure. For example, it may be desirable to transfect a host cell with DNA encoding either the light chain or the heavy chain of an antibody of this disclosure. Recombinant DNA technology may also be used to remove some or all of the DNA encoding either or both of the light and heavy chains that is not necessary for binding to EIF3L. The molecules expressed from such truncated DNA molecules are also encompassed by the antibodies of the invention. In addition, bi-functional antibodies may be produced in which one heavy and one light chain are an antibody of the invention and the other heavy and light chain are specific for an antigen other than EIF3L (e.g., by crosslinking an antibody of the invention to a second antibody by standard chemical crosslinking methods).

In certain embodiments, the antibodies and antibody fragments of the present invention are produced in transgenic animals. For example, transgenic sheep and cows may be engineered to produce the antibodies or antibody fragments in their milk (see, e.g., Pollock DP, et al., (1999) Transgenic milk as a method for the production of recombinant antibodies. J. Immunol. Methods 231:147-157, herein incorporated by reference). The antibodies and antibody fragments of the present invention may also be produced in plants (see, e.g., Larrick et al., (2001) Production of secretory IgA antibodies in plants. Biomol. Eng. 18:87-94, herein incorporated by reference). Additional methodologies and purification protocols are provided in Humphreys et al., (2001) Therapeutic antibody production technologies: molecules applications, expression and purification, Curr. Opin. Drug Discov. Devel. 4:172-

185, herein incorporated by reference. In certain embodiments, the antibodies or antibody fragments of the present invention are produced by transgenic chickens (see, e.g., US Pat. Pub. Nos. 20020108132 and 20020028488, both of which are herein incorporated by reference).

5 In certain embodiments, the human EIF3L binding molecules of the present invention (e.g., as antibodies or antibody fragments), or other EIF3L binding molecules, and/or CD63 binding molecules, are useful for immunoassays which detect or quantify human EIF3L and/or CD63 containing exosomes in a sample (e.g., a purified blood sample from a subject). In some embodiments, an immunoassay for CD63 or EIF3L typically comprises incubating a
10 biological sample in the presence of a detectably labeled antibody or antibody fragment of the present invention capable of selectively binding to CD63 and/or EIF3L present on exosomes (e.g., where the CD63 may be methylated), and detecting the labeled peptide or antibody which is bound in a sample. Various clinical assay procedures are well known in the art.

 The present disclosure provides immunoassay methods for determining the presence,
15 amount or concentration of human exosomes with CD63 and/or EIF3L on their surface in a test sample. Any suitable assay known in the art can be used in such a method. Examples of such assays include, but are not limited to, immunoassay, such as sandwich immunoassay (e.g., monoclonal-polyclonal sandwich immunoassays, including radioisotope detection (radioimmunoassay (RIA)) and enzyme detection (enzyme immunoassay (EIA) or enzyme-
20 linked immunosorbent assay (ELISA) (e.g., Quantikine ELISA assays, R&D Systems, Minneapolis, Minn.)), competitive inhibition immunoassay (e.g., forward and reverse), fluorescence polarization immunoassay (FPIA), enzyme multiplied immunoassay technique (EMIT), an ARCHITECT assay (ABBOTT), a bioluminescence resonance energy transfer (BRET), and homogeneous chemiluminescent assay, etc.

25 A human CD63 and/or EIF3L binding molecule can be captured on beads or nitrocellulose, or on any other solid support which is capable of immobilizing soluble proteins (e.g., magnetic beads). A human CD63 and/or EIF3L containing sample (e.g., exosomes with CD63 and/or EIF3L on their surface) is then added to the support which is subsequently washed with suitable buffers to remove unbound proteins. A second, detectably
30 labeled, molecule (e.g., antibody or peptide) that can bind to the human CD63 and/or EIF3L binding molecule is added to the solid phase support that can then be washed with the buffer a second time to remove unbound molecules. The amount of bound label on the solid support can then be detected by known methods.

Detectably labeling the human CD63 and/or EIF3L binding molecule can be accomplished by coupling to an enzyme for use in an enzyme immunoassay (EIA), or enzyme-linked immunosorbent assay (ELISA). The linked enzyme reacts with the exposed substrate to generate a chemical moiety which can be detected, for example, by

5 spectrophotometric, fluorometric or by visual means. Enzymes which can be used to detectably label the human CD63 and/or EIF3L binding molecules of the present invention include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase,

10 glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase and acetylcholinesterase.

In some embodiments of the present invention, human CD63 and/or EIF3L which is detected by the above assays can be present in a biological sample (e.g., containing cancer cell generated exosomes). Any sample containing human CD63 and/or EIF3L can be used.

15 In certain embodiments, the sample is a biological fluid such as, for example, blood, brain tissue, serum, lymph, urine, cerebrospinal fluid, amniotic fluid, synovial fluid, a tissue extract or homogenate, and the like. However, the invention is not limited to assays using only these samples, as it is possible for one of ordinary skill in the art to determine suitable conditions which allow the use of other samples.

20 In certain embodiments, provided here are kits for the detection of CD63 and/or EIF3L that include a human CD63 and/or EIF3L detection molecule. Such kits may include any of the immunodiagnostic reagents described herein and may further include instructions for the use of the immunodiagnostic reagents in immunoassays for determining the presence of human CD63 and/or EIF3L in a test sample (e.g., a test sample containing CD63 and/or

25 EIF3L expressing exosomes from a cancer cells). The kits may also include other reagents required to conduct a diagnostic assay or facilitate quality control evaluations, such as buffers, salts, enzymes, enzyme co-factors, substrates, detection reagents, and the like. Other components, such as buffers and solutions for the isolation and/or treatment of a test sample (e.g., pretreatment reagents), also can be included in the kit. The kit can additionally include

30 one or more other controls. One or more of the components of the kit can be lyophilized, in which case the kit can further comprise reagents suitable for the reconstitution of the lyophilized components.

The various components of the kit may be provided in suitable containers as necessary, e.g., a microtiter plate. The kit can further include containers for holding or storing

a sample (e.g., a container or cartridge for a sample). Where appropriate, the kit optionally also can contain reaction vessels, mixing vessels, and other components that facilitate the preparation of reagents or the test sample. The kit can also include one or more instrument for assisting with obtaining a test sample, such as a syringe, pipette, forceps, measured spoon, or
5 the like.

EXAMPLES

The following examples are provided in order to demonstrate and further illustrate certain preferred embodiments and aspects of the present invention and are not to be
10 construed as limiting the scope thereof.

EXAMPLE 1

Mechanism of tumor-platelet communications in cancer

Thrombosis is one of the main complications in cancer patients often leading to
15 mortality. However, the mechanisms underlying platelet hyper-activation are poorly understood. Murine and human platelets were isolated and treated with small extracellular vesicles (sEVs) from various cancer cell lines. We demonstrate that platelets very effectively take up sEVs from aggressive prostate cancer cells. The process of uptake is fast, proceeds effectively in circulation in mice, and is mediated by the abundant sEV membrane protein-
20 CD63. The uptake of cancer-sEVs leads to the accumulation of cancer cell-specific RNA in platelets *in vitro* and *in vivo*. The human prostate cancer-sEV-specific RNA marker PCA3 is detected in platelets of ~70% of prostate cancer patients. This was markedly reduced following prostatectomy. *In vitro* studies showed that platelet uptake of cancer-sEVs induces strong platelet activation in a CD63- RPTP α -dependent manner. In contrast to physiological
25 agonists ADP and thrombin, sEVs activate platelets via a non-canonical mechanism dependent upon active translation. Intravital studies demonstrated accelerated thrombosis both in murine tumor models and in mice that received intravenous injections of cancer-sEVs. The prothrombotic effects of sEVs were rescued by blocking CD63.

Tumors communicate with platelets by the means of sEVs, which deliver cancer
30 markers and activate platelets in a CD63-dependent manner leading to thrombosis. This emphasizes the diagnostic and prognostic value of platelet-associated cancer markers and identifies new pathways for intervention.

MATERIALS AND METHODS

Patient samples

Blood samples (2-5 ml) from healthy volunteers and prostate cancer patients were obtained from the Cleveland Clinic Glickman Urological and Kidney Institute with patient consent using protocols approved by the Institutional Review Board of the Cleveland Clinic Foundation. The healthy volunteers were picked at random and, included six males and five females. Further details, such as PSA levels and tumor histology from patients that had undergone prostatectomy, are shown in Table 2.

Table 2. Patient Tumor metrics. Prostate-specific antigen (PSA), tumor size, Gleason grade, and androgen deprivation therapy (ADT) parameters from prostate cancer patients shown in this Example.

Pt number	PSA mg/mL	Nodule dimension (cm)	RP Gleason Grade	Pre-op ADT
1402	14.25	1.5	3+4	No
1403	7.21	1.6	3+4	No
1417	6.07	1.9	3+4	No
1418	4.5	2.1	3+4	No
1419	4.55	3.4	3+4	No
1422	5.45	2.1	3+4	No
1423	5.51	1.9	3+4	No
1424	5	2.0	4+3	No
1426	4.59	2.1	3+4	No
1427	9.39	1.3	4+3	Yes
1429	5.4	2.0	3+4	No
1430	4.23	2.0	3+4	No
1432	3.09	2.1	4+3	No
1434	3.5	1.8	3+4	No
1435	25.86	3.0	small cell cancer	ADT + chemo
1436	62.2	4.1	4+4	Yes
1437	10.9	1.9	5+4	No

Reagents

Synthetic sEVs (plain liposomes) were purchased from Encapsula Nano Sciences (catalog#: CEP-500). CD63 blocking antibody (catalog#: H-193) was purchased from Santa Cruz Biotechnology (catalog#: sc-15363). The Fab fragment of CD63 was generated by papain digestion. PNGaseF (catalog#: P0704S) and O-glycosidase (catalog#: P0733S) were purchased from New England Bio Labs. Cytochalasin D (catalog#: C8273), wiskostatin (catalog#: W2270), and ML141 (catalog#: SML0407) were purchased from Sigma-Aldrich. We purchased dasatinib (catalog#: 11498) and RGD (catalog#: 14501) from Caymanchem; LY294002 (catalog#: S1105) from Selleckchem; apyrase (catalog#: A6410-200UN), and daltraban (catalog#: D7441) from Sigma Aldrich. Conjugated anti-murine activated

$\alpha_{11b}\beta_3$ (clone JON/A) (catalog#: M023-2) monoclonal antibodies were purchased from Emfret Analytics.

Cell lines

BJ normal human fibroblast cell line (cat.# CRL-2522), LNCaP-C4-2 human bone metastatic prostate cancer cell line (cat.# CRL-3314), LNCaP human prostate cancer cell line (cat.# CRL-1740), PC3 human prostate cancer cell line (cat.# CRL-1435), MDA-MB-231 human breast cancer cell line (cat.# HTB-26) were all obtained from ATCC. RM1 mouse prostate cancer cell line and SK-RC-26b renal cancer cell line were obtained from the labs of W.D. Heston and James Finke. LNCaP-C4-2 cell cultures under 15 passages and others between 4-12 passages were used in this study. The cells were examined for the presence of mycoplasma using the MycoAlert™ PLUS Assay.

Animals

Male mice between the ages of 8-12 weeks with weights averaging between 25-30g were used. C57BL/6J and NSG (strain#:005557) mice were obtained from JAX labs. Kindlin3 hypomorphic mice ($K3^{hypo}$) that express very low levels of mutant Kindlin3 protein were generated as previously described^{43, 62}. CD63 KO mice were obtained from Roy L. Silverstein's lab⁴², AKT3 KO mice were obtained from the Hay lab⁶³, and TLR2 KO mice were obtained from Dr. S. Akira (Osaka University)⁶⁴. APOE KO mice (Strain #:002052) and their controls injected with RMI tumors were 6 months old. The APOE KO mice were fed high cholesterol Western diet (Envigo Teklad, TD.96121) for 15 weeks. The mice were housed in microisolator cages with individual ventilation in a pathogen-free facility and subjected to a light-dark cycle consisting of 12 hours of light and 12 hours of darkness. No non-inclusion or exclusion parameters were used in our studies. Considering that the primary in vivo experiments were conducted using prostate cancer cell lines -sEVs and -tumor xenografts derived from male-specific prostate tissue, and taking into account that male gender has been reported to influence prostate tumor growth and outcomes, we restricted our study to male subjects. Randomization and Blinding: Male litter mates were assigned to different experimental groups for the tumor xenograft model and sEVs injections, using a randomization process that alternated animals to each group. The intravital thrombosis study was performed and evaluated in a blinded manner, with the personnel conducting the assay being unaware of the animal's group allocation.

Platelet isolation

Platelets were purified by gel filtration (Sephacose CL-2B column) as described previously⁶⁵ and diluted to a concentration of 2×10^8 platelets/ml in HEPES-buffered Tyrode's solution for functional studies.

5 Small EVs isolation and characterization

LnCaP-C4-2 cell cultures under 15 passages and others between 4-12 passages were used in this study. Cells were cultured in RPMI (for LNCaP-C4-2) or DMEM/F12 media supplemented with 15% FBS, 100 U/ml penicillin and streptomycin, 0.25 μ g/ml amphotericin B, and supplementation of non-essential amino acids (NEAA). After the cells have attained
10 ~75% confluency, the culture flasks were washed with filtered PBS five times with gentle shaking on a shaker at 45 RPM. Serum-free media supplemented with 0.1% ultra-centrifuged (110,000g) bovine serum albumin (BSA) that is sEV-free was added to the cells. After 24hrs, the harvested conditioned media from four T175 flasks were pooled (~120ml conditioned
15 with $\sim 5 \times 10^7$ cells in total) was subjected to differential centrifugation at 4°C, 300 g for 10min, and 2000 g for 10min to remove cell debris and apoptotic cell bodies. The supernatant was then centrifuged at 10,000 g for 20min at 4°C in a Sorvall-Bios 16 centrifuge to remove large EVs. Next, the supernatant was filtered through 0.22 μ m filters to remove EVs larger than 200nm (medium and large EVs) and then centrifuged at 110,000 g for 90min at 4°C using a Beckman 75Ti rotor to pellet sEVs. The sEV pellet was resuspended and washed with
20 ice-cold 0.1 μ m filtered PBS by 110,000g ultracentrifugation and the resulting sEV pellet was resuspended in 0.1 μ m filtered PBS.

Small EVs quantification

The sEVs were characterized by transmission electron microscopy (TEM), apogee FACS, nanoparticle tracking analysis (NTA), BCA protein assay (Thermo Fisher Scientific),
25 and western blotting. We have analyzed several batches of sEVs by BCA protein assays and NTA for reproducibility. Based upon BCA protein estimation and Nano-FACS or NTA analysis, 10 μ g/ml sEVs on average equals 1×10^{11} particles/ml. The serum-free media supplemented with 0.1% ultra-centrifuged (110,000g) bovine serum albumin (BSA) that is sEV-free was subjected to sEV isolation protocol and analyzed by protein estimation and
30 NTA to confirm EV depletion in culture media.

Small EVs isolation from plasma

Plasma was collected from blood samples and stored at -80°C before use. Briefly, 20 μ l of plasma was diluted with 0.1 μ m filtered PBS and centrifuged at 2000 g for 10min to remove cell debris and apoptotic cell bodies. The supernatant was then centrifuged at 10,000

g for 20min at 4°C in a Sorvall-Bios 16 centrifuge to remove large EVs. Next, the supernatant was filtered through 0.22 µm filters to remove EVs larger than 200nm (medium and large EVs) and then centrifuged at 110,000 g for 90min at 4°C using a Beckman 75Ti rotor to pellet sEVs. The sEV pellet was resuspended and washed with ice-cold 0.1 µm filtered PBS by 110,000g ultracentrifugation and the resulting sEV pellet was resuspended in 0.1 µm filtered PBS.

Apogee FACS

The Apogee A50 (Apogee Flow Systems) can resolve extracellular vesicle (EV)-sized nanoparticles and is routinely used for characterizing EVs. Size-calibrated non-fluorescent silica beads (0.18, 0.24, 0.30, 0.59, 0.88, and 1.30 µm) were used to calibrate and gate with small angle light scattering (SALS) and large angle light scatter (LALS). The system was cleaned with 10% bleach to remove any adhered particles and then washed with 0.22µm filtered PBS. sEVs resuspended in 0.22µm filtered in PBS were analyzed at a flow rate of 1.5 µL /min and an accumulation time of 180 seconds.

Nanoparticle tracking analysis (NTA)

ZetaView (Particle Metrix, Germany) instrument was calibrated with 100 nm polystyrene nanoparticles and operated according to the manufacturer's instructions with default software settings for EVs. For each run, 1 ml of the diluted samples in 0.1µm filtered PBS was injected into the sample chamber with an ideal concentration of 50-200 particles/frame. For each measurement, three cycles were performed by scanning 11 cell positions each and capturing 60 frames per position. The captured videos were analyzed with the built-in ZetaView Software 8.02.31. The following parameters were applied: Maximum particle size of 1000, the minimum particle size of 5, and minimum particle brightness of 20.

RNA extraction and PCR

Total RNA from sEVs or platelets was isolated using miRCURY™ RNA Isolation Kit (Exiqon) according to the manufacturer's protocol. Total RNA from platelets and sEVs was reverse transcribed to cDNA with random hexamers using the QuantiTect Reverse Transcription Kit (Qiagen) according to the manufacturer's recommendations. The following primers were used:

FTH1 134bp

For 5'AGGTGGCCGAATCTTCCT (SEQ ID NO:13)

Rev 5'CCAGTTTGTGCAGTTCCAGT (SEQ ID NO:14)

Rab13 107bp

For 5'CGGGACATCTTGCTCAAGT (SEQ ID NO:15)

- Rev 5'AGGGAGCACTTGTGGGTGTT (SEQ ID NO:16)
RPPH1 89bp
For 5'CGGAGGGAAGCTCATCAGTG (SEQ ID NO:17)
Rev TGGCCCTAGTCTCAGACCTT (SEQ ID NO:18)
- 5 RPL28 215bp
For 5'CTTCCGCTACAACGGACTGA (SEQ ID NO:19)
Rev 5'CCATGCGCAGGTCGG (SEQ ID NO:20)
PRC1 340bp
For 5'CTATGATATTGACAGTGCCTCAGTGC (SEQ ID NO:21)
- 10 Rev 5'TATTTGCAACCTGTCCCAGAGCTCTCG (SEQ ID NO:22)
hGAPDH 229bp
For 5'GAAGGTGAAGGTCGGAGTC (SEQ ID NO:23)
Rev 3'TCAGAAGATGGTGATGGGATTTC (SEQ ID NO:24)
mGAPDH 154bp
- 15 For 5'ACTCCCCTCTTCCACCTTC (SEQ ID NO:25)
Rev 5'TCCAGGGTTTCTTACTCCTTG (SEQ ID NO:26)
PSMA 177bp
For 5'CATAGTGCTCCCTTTTGATTGTC (SEQ ID NO:27)
Rev 5'CTCTCACTGAACTTGGAAGCAAT (SEQ ID NO:28)
- 20 PCAT1 322bp
Step1 For 5'GTGGAGAAGAGGCAGAAACA (SEQ ID NO:29)
Step1 Rev 5'TGACTGCACTGTACCTTCATTAG (SEQ ID NO:30)
Step2 For 5'ATGACGCAAAGGAACCTAACT (SEQ ID NO:31)
Step2 Rev 5'CTTCCAATGGCTGGTCACTAT (SEQ ID NO:32)
- 25 TMPRSS2ERG 600bp
Step1 For 5'CAGGAGGCGGAGGCGGA (SEQ ID NO:33)
Step1 Rev 5'GGCGTTGTAGCTGGGGGTGAG (SEQ ID NO:34)
Step2 For 5'GGAGCGCCGCTGGAG (SEQ ID NO:35)
Step2 Rev 5'CCATATTCTTTACCGCCCACTCC (SEQ ID NO:36)
- 30 LncRNA 273bp
For 5'TGAGCACTTTCCCACCATAC (SEQ ID NO:37)
Rev 5'CCTCATTCAACCCTTCCAATCT (SEQ ID NO:38)
FLT 130bp
For 5'GCTACGAGCGTCTCCTGAAG (SEQ ID NO:39)

Rev 5'GGCCTGGTTCAGCTTTTTCT (SEQ ID NO:40)

PCA3 (120bp) primers nested PCR as described previously: Step1: For-

5' AGTCCGCTGTGAGTCT3' (SEQ ID NO:41), Rev-5'CCATTTTCAGCAGATGTGTGG3'

(SEQ ID NO:42). Step2: For-5'ATCGACGGCACTTTCTGAGT3' (SEQ ID NO:43), Rev-

5 5'TGTGTGGCCTCAGATGGTAA3' (SEQ ID NO:44)^{59, 66}. The following PCR cycling conditions were used generally: 94 °C for 3 min, 35 cycles of 94 °C for 30 s, 60 °C for 45 s, and 72 °C for 1 min, with the final elongation step at 72 °C for 10 min. Primers were used at a concentration of 500 nM in Choice Taq Blue Master Mix (Cat.# CB4065-8, Denville). The PCR products were subjected to electrophoresis in a 2% agarose gel at 110 V for 30-40 min and visualized using SYBR™ Safe DNA Gel Stain (Cat.# S33102, ThermoFisher) under UV light.

Immunostaining and Confocal Microscopy

The dyes WGA-Alexa Fluor-488 (cat.# W11261) and WGA-Alexa Fluor-594 (cat.# W11262), both from ThermoFisher Scientific were dissolved in filtered PBS according to the manufacturer's recommendation (but without sodium azide) and centrifuged at 110,000 g for 90min at 4⁰C using a Beckman 75Ti rotor to pellet and remove any undissolved particles or aggregates formed in the solution. The supernatant of the dye was used for staining sEVs and platelets at a final concentration of 5µg/ml. The WGA-Alexa Fluor-488 was added to sEVs or PBS alone (-ve control) overnight on a rotator at 4⁰C followed by washing with ice-cold filtered PBS at 110,000 g for 90min at 4⁰C using a Beckman 75Ti rotor. The pellets of stained sVs and -ve control were then resuspended in either filtered Tyrode's buffer or PBS. Platelets were stained with WGA-Alexa Fluor-594 for 20mins followed by washing with Tyrode's buffer by 300 g centrifugation at room temperature. The stained platelets were co-incubated with stained sEVs or -ve control. Following the co-incubation experiments, the platelets were washed, fixed with 4% PFA for 20 min, washed with PBS, and mounted onto slides with ProLong™ Diamond Antifade Mountant (cat.# P36961; ThermoFisher Scientific). Confocal images were obtained using a Leica SP5 confocal/multi-photon microscope in the Imaging Core of the Lerner Research Institute and a Leica TCS SPE confocal microscope. Platelets were imaged with 63x objectives and a step size of 0.5µm spanning the height of the platelets. The images were processed and staining intensity was quantified using Volocity software and Image J. The 3-D images were prepared by merging the confocal stacks using Volocity.

Transmission Electron Microscopy

Negative staining TEM of sEVs: The sEVs were isolated and then suspended in 2.5% glutaraldehyde-0.1 M phosphate buffer for overnight fixation at 4°C. Subsequently, the samples were placed on Formvarcarbon-coated grids and air-dried for 1 hour. The grids were
 5 rinsed five times with 0.1 M phosphate buffer and distilled water, and then treated with a contrast agent and embedded in a mixture of 4% uranyl acetate and 2% methylcellulose at a ratio of 1:9. The grids were air dried and observed with an FEI Tecnai G2 Spirit BioTWIN (Transmission Electron Microscope; FEI, Hillsboroboro, OR) equipped with Orius 832 CCD Camera, 11 megapixels (Gatan, Inc., Pleasanton, CA) and DigitalMicrograph software
 10 (Gatan, Pleasanton, CA).

Platelets were fixed in 2.5% glutaraldehyde- 0.1 M phosphate buffer for 90 min. The fixed cells were centrifuged at 4 °C and the platelet pellets were washed three times in 0.1 M phosphate buffer, post-fixed with 1% osmium tetroxide for 1 hour at 4 °C. They were then dehydrated in a graded series of ethanol (50, 70, 90, 96, and 100%), and embedded in Epon.
 15 Thin sections were cut with EM UC7 Ultramicrotome (Leica Microsystems GmbH, Vienna, Austria) and stained with uranyl acetate and lead citrate, then examined using an FEI Tecnai G2 Spirit BioTWIN (Transmission Electron Microscope; FEI, Hillsboroboro, OR) equipped with Orius 832 CCD Camera, 11 megapixels (Gatan, Inc., Pleasanton, CA) and DigitalMicrograph software (Gatan, Pleasanton, CA).

20 Western blot analysis

Platelets were pelleted down after treatment and lysed with RIPA buffer on ice. Platelet lysates or cell lysates or sEV extracts were separated on 12% polyacrylamide slab gels in a Mini-Protean II system (cat.# 4561044 Bio-Rad Laboratories). Then proteins were electrophoretically transferred to Immobilon-P, PVDF membrane (catalog#: IPVH00010
 25 Millipore) and membranes were blocked with 5% nonfat dry milk or 5% BSA in TTBS (TTBS; 0.2 M Tris [pH 7.4], 1.5 M NaCl, 0.1% thimerosal and 0.5 % Tween 20). Membranes were washed with TTBS and incubated with primary antibody at 4°C overnight. The following commercially-available primary antibodies were used: Anti-CD63 (E-12) (cat.# sc-365604), CD9 (ALB6) (cat.# sc-59140), caveolin-1 (7C8) (cat.# sc-53564), and Annexin-
 30 2/ANXA2 Antibody (H-5) (cat.# sc-48397) (all from Santa Cruz Biotechnology). Phospho-SAPK/JNK (Thr183/Tyr185) (81E11) (cat.# 4668S), Phospho-p38 MAPK (Thr180/Tyr182) (D3F9) (cat.# 4511T), p44/42 MAPK (Erk1/2) (L34F12) (cat.# 4696), Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (cat.# 9101S), Akt (pan) (11E7) (cat.# 4685), Phospho-Akt (Ser473) (193H12) (cat.# 4058), PLCγ2 (cat.# 3872S), Phospho-PLCγ2 (Tyr1217) (cat.#

3871S), Src (36D10) (cat.# 2109S), Phospho-Src Family (Tyr416) (cat.# 2101S), Ezrin (cat.# 3145), GAPDH (14C10) (cat.# 2118S), α -Actinin (D7U5A) (cat.# 15145S), and β -Actin (cat.# 4967S) (all from Cell signaling). Phospho RPTP α (Ser180) from Biossusa (cat.# BS-5175R) and RPTP α from Novus Biologicals (cat.# NBP2-57255). Then membranes were
 5 washed in TTBS and incubated with the appropriate secondary antibody (cat.# A0545 Amersham) (cat.# A3682 Amersham) conjugated to horseradish peroxidase at a 1:5,000 dilution for 1 hr at room temperature. They were then washed with TTBS twice for 15 min each, then four times for 5 min each, and developed with an enhanced chemiluminescence (ECL) kit (cat.# 32132 Amersham).

10 **Tail vein injection**

sEVs were isolated from cultured cancer cells by differential centrifugation as described above and 100 μ l of sEVs in 0.22 μ m filtered PBS at a final concentration of 20mg/ml were injected into the mouse via the tail vein. Mice were anesthetized and blood samples were taken 60 min later from the inferior vena cava.

15 **Intravital thrombosis assay**

The intravital thrombosis study was performed using FeCl₃-induced carotid artery thrombosis in C57BL/6J male mice as previously described³². Mice were injected either with 100 μ l of cancer cell-derived sEVs in 0.22 μ m filtered PBS or 0.22 μ m filtered PBS (control) via the tail vein. Sixty min after injection, mice were anesthetized with an intraperitoneal
 20 injection of ketamine/xylazine mixture (100 mg ketamine/kg body weight, 10 mg xylazine/kg body weight). The carotid artery was exposed and injury was induced with 12% FeCl₃ (10% FeCl₃ for the anti-CD63 experiment). The area of injury on the artery was observed under a Leica DM LFS microscope (Leica, Germany) with $\times$ 10/0.30 objectives. Images were acquired using a cooled high-speed digital camera (QImaging Retiga EXi Fast 1394) and
 25 with Streampix acquisition software³². All mice were included in the study, no exclusions were made.

Xenograft model

4-6 week-old NSG. Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ (purchased from Jackson Laboratory) male mice were used for the xenograft model. Mice were anesthetized with a
 30 ketamine/xylazine mixture (100 mg ketamine/kg body weight, 10 mg xylazine/kg body weight). LNCaP-C4-2 cells (4 $\times$ 10⁵ cells resuspended in 100 μ l of 50% matrigel diluted with PBS) were injected subcutaneously into either flank and the tumors were allowed to grow at around 1 cm³ before isolating platelets¹⁹. The mice carrying the human tumor xenografts

were injected with five doses of sEV blocking antibody (anti-sEV ab) or IgM as control during this time. All mice were included in the study, no exclusions were made.

FACS analysis

Platelets isolated by gel filtration were incubated with sEVs isolated from cancer or control cells for 1hr and platelet integrin $\alpha\text{IIb}\beta_3$ activation was assessed using JON/A (PE) antibody (Emfret, cat.# M023-2) and FACS analysis as previously described⁶⁷. Platelets AnnexinV and P-selectin expression were assessed with AnnexinV FITC conjugated antibody (BD Pharmingen, cat.# 51-6587) and CD62P/P-Selectin Antibody (Psel.KO2.3), PE conjugate (ThermoFisher Scientific, cat.# A16339). Data were acquired using a FACS Calibur II instrument (Becton Dickinson, San Jose, CA) and analyzed using FlowJo 10 software (Tree Star, Ashland, OR). Weak physiological agonist ADP (10 μ M) and strong physiological agonist thrombin (0.05U/ml) were used as positive controls.

Statistics

All data are represented as mean $\pm$ standard error of the mean (SEM). Prism 9 software (GraphPad) was used for statistical testing and graph creation. Shapiro-Wilk normality and longnormality test was used to determine data distribution if $n \geq 6$. For a small sample size with $n < 6$ and for not normally distributed data with $n \geq 6$, we used a non-parametric Mann-Whitney test to compare two groups, and Kruskal-Wallis test with the Dunn's post hoc test for 3 and more groups. P-values less than 0.05 were considered significant. For the experiments with $n=3$, the minimum achievable p-value for the non-parametric tests is 0.1000.

RESULTS

Isolated murine platelets take up sEVs. sEVs generated by the highly-metastatic human prostate carcinoma cell line (LNCaP-C4-2) and other cancer cell lines were isolated and characterized according to MISEV 2018 (detailed in the supplementary methods). Western blotting analysis demonstrated the presence of sEV-membrane marker CD63 and sEV-cytosolic marker caveolin-1, and the absence of α -actinin 4 (an sEV negative marker) in sEVs compared to LNCaP-C4-2 cell lysates (Figure 8A). At the same time, annexin II and β -actin the markers for prostasomes and cytoplasmic contaminants, respectively^{20, 21} were absent from the sEV lysates (Figure 8A). The absence of the ectosome marker, ezrin¹⁵, indicates a predominant exosome population (Figure 8A). The size and structural integrity of the purified sEVs were assessed by transmission electron microscopy (TEM; Figure 8B, C).

The negatively-stained sEVs were observed as cup-shaped bilayered membrane vesicles, and 98% were within the range of 30 to 150 nm and had a mean diameter of 83.5 nm (Figure 8B, C). Apogee FACS analysis confirmed that 95% of the sEV fraction was within the 30 to 150 nm range (Figure 8D). Additionally, NTA analysis was used to show the particle numbers, concentration, and size (Figure 8E).

Next, using FACS we show that platelets readily uptake LNCaP-C4-2/cancer sEVs, while the interaction of synthetic-sEVs with platelets was negligible and did not significantly differ from fibroblast-derived sEVs (Figure 1A, B), demonstrating the selectivity and cancer specificity of the process. The uptake of cancer-sEVs by murine and human platelets was confirmed by confocal microscopy (Figure 1C, D and Figure 9A) and TEM analysis demonstrating the presence of LNCaP-C4-2 derived cancer-sEVs inside the platelets, while only a small fraction of the sEVs were associated with the platelet surface (Figure 1E and Figure 9B, C). Murine platelets also preferentially uptake sEVs derived from the mouse prostate cancer cell line (RM1) as compared to human fibroblast-sEVs (Figure 9D), confirming that the uptake of human cancer-sEVs is not species but cancer-specific. The uptake of cancer sEVs by platelets was rapid, with a significant amount of sEVs detected inside platelets within 15-20min of incubation, and reaching a plateau after 45-60min (Figure 1F).

Similar results were observed using alternative prostate cancer lines PC3 and LNCaP and other cancer cells, i.e. metastatic renal cell carcinoma cell line (SK-RC-26b) (Figure 17A-C). Thus, this appears to be a rather general cancer-specific phenomenon.

Cancer-sEVs transfer cancer cell mRNA to platelets. In search for cancer markers transferred by sEVs to platelets, we analyzed several previously reported candidates. GTPase RAB13, ferritin heavy chain1 (FTH1), ferritin light chain (FTL), and lncRNA RPPH1²²⁻²⁵ were detected in both murine and human platelets in the absence of sEVs (Figure 11A). Other markers, polycomb repressive complex 1 (PRC1) and prostate cancer marker PSMA²⁶ were not detected in cancer-sEVs (Figure 11A, B). Only ribosomal protein L28 (RPL28)²⁷ was highly enriched in LNCaP-C4-2 cancer-sEVs, but absent in both murine and human platelets (Figure 11A), making it the most appropriate cancer-sEV marker in platelets.

Isolated murine platelets were incubated with LNCaP-C4-2 derived cancer-sEVs in Tyrode's buffer, washed to remove unbound sEVs, and then analyzed by PCR. RPL28 mRNA was readily detected in platelets (Figure 11C). Experiments performed in whole murine blood also revealed the transfer of RPL28 mRNA to platelets (Figure 11D). Next, we intravenously injected LNCaP-C4-2 derived cancer-sEVs or the vehicle and collected blood 60min later.

RPL28 was successfully detected in platelets from mice injected with sEVs, but not controls (Figure 11E). In contrast to platelets, the levels of RPL28 in leukocytes were not significantly altered by sEVs injection. To show the importance of this mechanism *in vivo*, we implanted LNCaP-C4-2 tumors into the immunodeficient NSG mice followed by treatments with sEV-
5 blocking antibodies or IgM isotype controls. Only platelets isolated from the mice with xenografts were positive for RPL28, whereas platelets from controls and tumor-bearing mice treated with sEV-blocking antibodies were negative for RPL28 (Figure 1G). Thus, the direct transfer of cancer markers to platelets occurs *in vivo* and can be prevented using antibodies to sEVs.

10 Detection of the human prostate cancer marker PCA3 in cancer-sEVs and in the patient platelets. Since RPL28 was detected at a low level in leukocytes (Figure 11E), we continued our search for more specific markers for human studies. LncRNAs PCA3 and ENST00000501280 are enriched in the sEVs of prostate cancer cell line^{9,29}. Using nested PCR, we show that PCA3 was highly enriched in sEVs, while ENST00000501280 was
15 detected in both cells and sEVs and both markers were absent in platelets (Figure 1H, left panel), thereby making PCA3 the preferred marker. Similar to RPL28, PCA3, and ENST00000501280 were detected in murine platelets treated with LNCaP-sEVs *in vitro* by nested PCR (Figure 1H, right panel). We next tested blood samples from prostate cancer patients and healthy volunteers for the presence of PCA3 and ENST00000501280. Both
20 markers were detected in the patient's platelets, but not in the control (healthy volunteers) platelets (Figure 12A). The amplification of PCA3 by PCR in sEVs and patient samples was confirmed by restriction digestion mapping of the amplification product (Figure 12B).

A larger-scale analysis of PCA3 in platelets from 32 prostate cancer patients revealed the presence of this marker in 69% of patients' samples while all controls were negative
25 (Figure 1I, J, and Figure 12C). Importantly, PCA3 was mostly undetectable in the same patients two months after prostatectomy (Post-Op) (Figure 1I, J and Figure 12C). NTA of sEVs purified from plasma revealed increased levels of sEVs in cancer patients and a tendency to its reduction post-prostatectomy (Figure 12D-E). However, a correlation between sEV levels and the presence of PCA3 was not identified. In contrast to PCA3, lncRNA's
30 PCAT1 and TMPR16-ERG, also known to be enriched in prostate tumor-sEV^{10,31}, were less suitable as biomarkers in platelets since they were detected only in ~25% of platelet samples from prostate cancer patients (Figure 13A-E). These results demonstrate that PCA3 in platelets is of prostate cancer-sEV origin. Thus, measurements of PCA3 in platelets from prostate cancer patients might be a promising approach for cancer detection and staging.

Cancer-sEVs induce platelet activation and promote thrombosis. Incubation of murine platelets with LNCaP-C4-2/cancer-sEVs induced activation of platelet integrin $\alpha_{IIb}\beta_3$, in a concentration- and time- dependent manner (Figure 2A, B). The activation of platelets by cancer-sEVs was comparable to activation induced by the physiological agonists ADP and thrombin (Figure 2A-D). The platelet activation was specific for cancer-sEVs, as synthetic and fibroblast sEVs were not effective (Figure 2C, D). Interestingly, while platelet activation by ADP and thrombin occurred in seconds, the activation by sEVs was substantially slower, reaching a maximum at 60 min (Figure 2B). Platelet aggregation assay with ADP in the presence of increasing concentrations of cancer-sEVs demonstrated a synergistic effect of cancer-sEVs on ADP-induced platelet aggregation (Figure 13F), indicating that cancer-sEVs might prime platelets for subsequent activation with conventional agonists. A similar platelet activation profile was observed using other metastatic cancer lines of prostate and renal cancers PC3 and SK-RC-26b, respectively (Figure 14A, B).

In the in vivo arterial thrombosis model, the LNCaP-C4-2 cancer-sEVs significantly shortened the occlusion time in WT mice, while synthetic-sEVs had no effect (Figure 2E). In mice bearing mouse prostate cancer tumors (RM1 tumor cells), an arterial occlusion was also accelerated (Figure 2F), mirroring the results observed using cancer-sEVs injection. A significant number of prostate cancer patients suffer from cardiovascular diseases and a combination of these pathologies might exacerbate thrombosis. To show this in mice, we implanted RM1 tumors into hyperlipidemic APOE-deficient (APOE^{-/-}) mice fed a Western diet. The presence of tumor significantly accelerated thrombosis in the atherosclerosis-prone APOE^{-/-} mice, even though the effect was smaller than that in WT mice (Figure 2G), likely due to the already-elevated platelet reactivity in these mice³². Taken together, these results strongly suggest that platelet uptake of cancer cell-derived sEVs, and the subsequent activation of platelets, promotes thrombosis.

Tetraspanin CD63 and N-linked glycosylation on cancer-sEVs mediate their uptake by platelets. sEV membrane proteins are highly N-linked glycosylated³³. Changes in glycosylation might define the interaction with receptor¹⁷⁴ and, thereby, influence platelet uptake. We hence removed N-linked glycans from sEV glycoproteins by PNGase-F which resulted in a significant decrease of the EVs uptake by platelets (Figure 3A, B). One of the markers of cancer-sEVs is the tetraspanin CD63 which is crucial for sEVs secretion and protein packaging^{35, 36}. CD63 is also highly expressed in sEVs across several cancers in vitro and in vivo³⁷. A well-characterized blocking antibody against CD63 (anti-CD63)^{38, 39} significantly decreased the uptake of sEVs by platelets compared to sEVs alone, while IgG-

isotype matched abs were not effective ne(Figure 3C, D). At the same time, blocking of another sEVs' tetraspanin -CD9 with an anti-CD9 antibody had no effect on sEV uptake (Figure 3D).

The combination of N-deglycosylation and anti-CD63 blocking antibody did not show an additive effect on sEV uptake by platelets (Figure 3E), suggesting a possible role for N-glycosylated CD63 in the interaction between cancer-sEVs and platelets. Treatment with O-glycosidase had no significant effect on sEV uptake by platelets (Figure 3E). CD63-mediated sEV platelet uptake seems to be an active process, since inhibitors of cytoskeleton rearrangement, i.e. wiskostatin, significantly suppressed platelet uptake of cancer-sEVs (Figure 3F).

CD63 blocking antibody inhibits cancer-sEV mediated platelet activation. Integrin $\alpha_{IIb}\beta_3$ activation induced by LNCaP-C4-2 derived-sEVs was similar to ADP treatment, while activation induced by sEVs in the presence of anti-CD63 but not isotype-matched antibodies was not significantly different from resting platelets (Figure 4A-C), directly connecting CD63-mediated uptake of sEVs to platelet activation. Likewise, cancer-sEVs induce other platelet responses such as granule secretion and P-selectin surface exposure, whereas the activation in the presence of anti-CD63 but not isotype control antibodies was not statistically different from non-stimulated platelets (Figure 4C and Figure 4D). Similar effects were observed on PS exposure measured by Annexin-V binding (Figure 4D). Likewise, while PC3-derived sEVs induced platelet $\alpha_{IIb}\beta_3$ activation, anti-CD63 antibody decrease activation to the level of resting platelets (Figure 4E, F). Preincubation of cancer-sEVs with the anti-CD63 also reduced platelet hRPL28 mRNA level as compared to vehicle or isotype IgG control (Figure 4G). Thus, the anti-CD63 antibody inhibits cancer-sEVs uptake by platelets and reduces subsequent platelet activation.

Platelet activation by cancer-sEVs is mediated by RPTP α , Akt, and MAPKs. We next assessed the contributions of platelet surface receptors, including integrins and scavenger receptors, as possible regulators of sEVs uptake⁴⁰. However, platelets obtained from mice deficient in integrin function (Kindlin3-hypomorph), scavenger receptor (CD36), micropinocytosis regulator (AKT3), and innate immune receptor (TLR2)^{32, 41-43} did not affect sEV uptake (Figure 15A-D).

It was reported that CD63 interacts with transmembrane receptor-like protein tyrosine phosphatase alpha (RPTP α) expressed on platelet¹¹⁴, leading to Src activation shown in kidney cell¹¹⁵. Accordingly, we examined the effect of RPTP α inhibition alone or in combination with anti-CD63 on cancer-sEVs uptake by platelets. LNCaP-C4-2 cancer-sEVs

treated with anti-CD63 antibody but not IgG isotype control showed a 75% reduction in sEVs uptake by platelets (Figure 5A, B). Platelets treated with anti-RPTP α antibody also showed a substantial reduction in sEVs uptake, however, a combination of sEVs treated with anti-CD63 and platelets treated with anti-RPTP α did not exhibit any synergistic effect (Figure 5A, B). This indicates similar roles for CD63 on sEVs and RPTP α on platelets in the uptake of cancer-sEVs by platelets.

At the molecular level, a significant increase in phosphorylation of RPTP α and its downstream target Src was observed in platelets within 5 mins of incubation with LNCaP-C4-2 cancer-sEVs, followed by a gradual decrease over 90min (Figure 5C, D). Following these early events, significant phosphorylation of Akt and PLC γ 2 was detected at the later time point of 90min after adding sEVs as compared to resting platelets at the same time point (Figure 5E, F). In comparison, ADP induced the phosphorylation of Akt but not PLC γ 2 within 15 mins (Figure 16A). Akt and PLC γ 2 are known to regulate MAPKs, which, in turn, contribute to α IIb β 3 integrin activation⁴⁶. We observed that sEVs induced phosphorylation of ERK, p38, and JNK in platelets, reaching a peak at 90min (Figure 5G, H). In contrast, ADP-induced platelet activation led to phosphorylation of Erk alone, and at a much shorter time of 15 mins (Figure 16A).

We next used various pharmacological inhibitors to probe this signaling pathway (Figure 16B-E) leading to α IIb β 3 activation (Figure 80A). Murine platelets were incubated with the inhibitors for 15 mins prior to the treatment with LNCaP-C4-2 derived cancer-sEVs. Src inhibitor (dasatinib) and PI3K inhibitor (LY294002) reduced the phosphorylation of Akt, Erk, and PLC γ 2 (Figure 16B-E) suggesting the upstream nature of Src and PI3K. Akt inhibitor (MK2206) successfully inhibited the phosphorylation of Erk and Akt but not PLC γ 2 (Figure 16B-D) suggesting that Erk is downstream of Akt, while PLC γ 2 might be independent. This was confirmed by the observation that Erk inhibitor (PD98059) had no effect on either Akt or PLC γ 2, while PLC γ 2 inhibitor (U73122) inhibited PLC γ 2 alone but no other molecule in the pathway (Figure 16B-D). Most of these inhibitors are known to affect integrin α IIb β 3 activation in platelets, including activation by sEVs (Figure 80A). A similar sequence of signaling events was also observed using PC3 cancer-sEVs (Figure 80B), suggesting these pathways are common to cancer-derived sEVs during platelet activation. We observed that the integrin antagonist, cilengitide (cyclic RGD pentapeptide) known to inhibit α IIb β 3 at high concentration of 17-170 μ M⁴⁷, does not affect sEV-mediated phosphorylation events (Figure 80C) that together with the results using Kindlin3 deficient mouse platelets, indicates that this process is independent of integrin function. Similarly,

apyrase (which hydrolyses ADP released from platelets) and TXA receptor antagonist, daltroban also had no effect (Figure 80C). Thus, cancer sEVs-mediated platelet activation is not secondary to integrin signaling and ADP or TXA₂ release from platelets.

The blockade of CD63-RPTP α interaction inhibits cancer-sEVs-induced platelet
 5 activation and accelerated thrombosis. Treatment of platelets with RPTP α blocking antibody for 30 mins followed by 5 mins incubation with LNCaP-C4-2 derived cancer-sEVs resulted in decreased phosphorylation of Src, PLC γ 2, Akt, Erk and downstream targets emphasizing the key role for RPTP α in initiation of this signaling cascade (Figure 6A-G). KODA-PC,
 10 known to induce platelet activation by a different TLR2-dependent mechanism was used as a negative control for anti-RPTP α antibody (Figure 16A). Similar to RPTP α , the blocking CD63 abs inhibited the components of the signaling cascade, Akt, PLC γ 2, Erk, JNK and p38, induced by cancer-sEVs and leading to α IIb β 3 activation (Figure 7A, B). Finally, while sEVs shorted occlusion time in the FeCl₃-induced carotid artery thrombosis assay, anti-CD63 blocking antibodies but not control IgG had an inhibitory anti-thrombotic effect (Figure 7C).
 15 These observations again demonstrate the key role for CD63 in cancer-sEV uptake, activation of platelets, and acceleration of thrombosis.

Tumors might induce platelet activation by direct interaction with platelets or by the release of procoagulant factors such as tissue factor (TF), podoplanin, ADP, and tumor-
 20 EV11⁸⁻⁵⁰. TF on the surface of tumor-EVs has been implicated in platelet aggregation and thrombosis^{119, 51}. However, more recent studies have shown that platelet aggregation can be driven by TF-independent pathways as well⁵¹, or more importantly, by as yet unknown mechanisms as seen in breast cancer⁵². This shows that the mechanism of tumor-EV-induced platelet activation is complex and that several alternative mechanisms may exist. Tumor cells,
 25 especially aggressive metastatic tumors, produce and shed high levels of sEVs (35-117 μ g sEVs/ml) that are detected in the plasma of patients with prostate, breast, colorectal, and other cancer^{80, 12, 13, 53}. Thus, in this Example, we focused on cancer cell-derived sEVs containing tumor-specific markers as a potential mechanism of tumor-platelet communication. Cancer cell line-derived sEVs rather than patient-derived sEVs were
 30 employed to overcome the limitations on sEV yield, purity, and high variability in sEVs concentration and composition between cancer patients. Our findings show that platelet activation is a direct consequence of sEV uptake via CD63.

Based upon the characteristic size of <200nm, shape, and expression of the sEV markers, the EVs used in this study were classified and referred to as sEVs in accordance

with MISEV2018. Our findings demonstrate that platelets specifically take up cancer-sEVs *in vitro* and *in vivo* in circulation at a higher rate compared to other circulating sEVs. These findings suggest that the mechanism of “information” transfer from tumor to platelet, and its consequences on platelet biology and reactivity, is likely sEV-dependent. *In vivo* experiments using either injection of cancer cell-derived sEVs into the circulation or murine tumor xenograft models demonstrated accelerated thrombosis, suggesting that this process may contribute to thrombosis in cancer patients. Significant numbers of prostate cancer patients suffer from cardiovascular diseases that exacerbate thrombosis^{12,4}. Interestingly, the presence of tumor significantly accelerated thrombosis in atherosclerosis-prone APOE^{-/-} mice, indicating that cancer-sEVs can further increase platelet reactivity and promote thrombosis even in conditions of extreme hyperlipidemia.

High levels of CD63-positive sEVs are associated with cancer progression and represent a predictive biomarker, especially for malignant cancer^{12,5,56}. Our studies reveal that CD63, possibly in its glycosylated form, on the tumor-sEV surface is crucial for recognition and uptake by platelets. We further showed that sEV uptake is an active process, which involves platelet cytoskeleton rearrangements, i.e. CD63-dependent endocytosis leading to platelet activation. This role is specific, since blocking CD63 had no effect on platelet activation by physiological agonists such as ADP. This is in agreement with previous reports that the lack of CD63 does not diminish platelet aggregation⁵⁷ and does not lead to bleeding in mice^{55,57}. Importantly, the CD63 blocking antibody was able to attenuate thrombosis in mice carrying tumor-sEVs.

Recent work demonstrated that so-called “tumor-educated platelets” are characterized by changes in their RNA profile⁵⁸. This was suggested to be mediated by the transfer of tumor-EVs carrying the tumor marker lncRNA PCA3 to platelet^{12,9}. We now provide evidence that this process depends upon cancer cell-sEV uptake. PCA3, a prostate cancer-specific biomarker, was recently detected in sEVs in the urine of prostate cancer patient⁹. In agreement with these studies, we detected PCA3 in LNCaP-sEVs and platelets of 69% of prostate cancer patients, but not healthy donors. In support of the tumor origin of this marker in platelets, we found that two months after radical prostatectomy, almost all patients from the same cohort became PCA3-negative. These findings open additional opportunities for the early detection of cancer and a novel approach for assessing the risk of platelet hyper-reactivity and thrombosis in cancer patients.

Platelet activation by sEVs was distinct from the physiological platelet agonists ADP or thrombin. While it was strong, it required longer incubation with platelets and did not

involve the platelet receptors of similar ligands, CD36, TLR2, and integrins indicating the involvement of a non-canonical pathway. In kidney cells, CD63 interacts with RPTP α ⁴⁵, a receptor that is also expressed on platelet^{11,4}. Blocking sEVs-CD63 or platelet-RPTP α inhibited platelet activation induced by cancer-sEVs but not with ADP or thrombin suggesting a novel pathway. Interestingly, RPTP α mediated activation of Src is followed by a delay in activation of PI3K, PLC γ 2, Akt, and MAPKs in platelet activation (Figure 7D).

Platelet activation and surface receptor engagement can initiate the translation of multiple proteins including Bcl-3, IL-1 β , and other^{13,61}. The involvement of such a translation mechanism could account for the prolonged signaling cascade specific to cancer-sEVs. The activation of PLC γ 2 is independent of PI3K, Akt, or MAPKs but depends on Src indicating two parallel pathways in sEVs-induced platelet activation (Figure 7D). Platelet MAPKs can trigger granule secretion and release of ADP or thromboxane (TXA₂), which can in turn platelet^{11,6}. Our observations of sEVs-induced platelet activation with sEVs derived from various cancer cell lines including renal cancer indicate this to be a general cancer-related phenomenon.

Taken together, our data reveal a novel mechanism of communication between cancer cells and platelets linked to non-canonical platelet activation and accelerated thrombosis. An understanding of this process opens new opportunities for cancer detection and for the prevention and treatment of thrombosis, which is a major cause of death in patients with metastatic cancers.

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- 20

All publications and patents mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the
25 scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in chemistry, medicine, and molecular biology or related fields are intended to be within the
30 scope of the following claims.

CLAIMS:

We claim:

1. A composition comprising a human EIF3L binding molecule, and/or one or more nucleic acid molecules encoding said human EIF3L binding molecule, wherein said human EIF3L binding molecule comprises:
 - a) a heavy chain variable region, wherein said heavy chain variable region comprises:
 - i) a CDRH1 amino acid sequence comprising SEQ ID NO: 6, or SEQ ID NO:6 with one with one or two conservative amino acid changes,
 - ii) a CDRH2 amino acid sequence comprising SEQ ID NO: 7, or SEQ ID NO:7 with one or two conservative amino acid changes, and
 - iii) a CDRH3 amino acid sequence comprising SEQ ID NO: 8, or SEQ ID NO:8 with one with one or two conservative amino acid changes, and/or;
 - b) a light chain variable region, wherein said light chain variable region comprises;
 - i) a CDRL1 amino acid sequence comprising SEQ ID NO: 10, or SEQ ID NO:10 with one with one or two conservative amino acid changes,
 - ii) a CDRL2 amino acid sequence comprising SEQ ID NO: 11, or SEQ ID NO:11 with one with one or two conservative amino acid changes, and
 - iii) a CDRL3 amino acid sequence comprising SEQ ID NO: 12, or SEQ ID NO:12 with one with one or two conservative amino acid changes.
2. The composition of claim 1, wherein:
 - i) said CDRH1 amino acid sequence comprises SEQ ID NO: 6;
 - ii) said CDRH2 amino acid sequence comprises SEQ ID NO: 7;
 - iii) said CDRH3 amino acid sequence comprises SEQ ID NO: 9;
 - iv) said CDRL1 amino acid sequence comprises SEQ ID NO: 10;
 - v) said CDRL2 amino acid sequence comprises SEQ ID NO: 11; and
 - vi) said CDRL3 amino acid sequence comprises SEQ ID NO: 12.
3. The composition of Claim 1, wherein said human EIF3L binding molecule is an antibody, minibody, diabody, scFv, or antibody fragment capable of binding human EIF3L.

4. The composition of Claim 3, wherein said antibody fragment is a Fab, F(ab')₂ or Fv antibody fragment.
5. The composition of Claim 2, wherein said antibody or antibody fragment comprises at least an antigen binding portion of the A1806-3A1-3 antibody
6. The composition of Claim 1, wherein said heavy chain and/or light chain variable region comprises a human framework region.
7. The composition of Claim 1, wherein said human EIF3L binding molecule further comprises a light chain constant region and a CH1 heavy chain constant region.
8. The composition of Claim 7, wherein said EIF3L binding molecule further comprises a CH2 heavy chain constant region and/or a CH3 heavy chain constant region.
9. The composition of Claim 8, wherein said light chain constant region is human or a humanized murine, and/or wherein said CH1, CH2, and CH3 heavy chain constant regions are human or are humanized murine.
10. The composition of Claim 1, wherein said human EIF3L binding molecule comprises an antibody, wherein the light chain constant region of said antibody is selected from: IgG Kappa and IgG Lambda, and wherein the heavy chain constant region of said antibody is selected from: IgG1, IgG2, IgG3, and IgG4.
11. The composition of Claim 1, wherein said human EIF3L binding molecule comprises an antibody, or antigen binding portion thereof, which is glycosylated or non-glycosylated.
12. The composition of Claim 1, further comprising a physiologically tolerable buffer.
13. The composition of Claim 1, wherein said heavy chain variable regions comprises SEQ ID NO: 5, or SEQ ID NO:5 with one or more conservative amino acid changes.

14. The composition of Claim 1, wherein said light chain variable region comprises SEQ ID NO: 9, or SEQ ID NO:9 with one or more conservative amino acid changes.

15. The composition of Claim 1, wherein said composition comprises said one or more nucleic acid molecules.

16. The composition of Claim 1, wherein said one or more nucleic acid molecules comprise: i) a first nucleic acid sequence encoding said heavy chain variable region, and ii) a second nucleic acid sequence encoding said light chain variable region.

17. The composition of Claim 16, further comprising an expression vector, and wherein said first and/or second nucleic acid sequences are present in said expression vector.

18. A method of treating or preventing a prothrombotic condition that is accompanied or caused by pathological exosome production, in a subject with said pro-thrombotic condition comprising:

treating a subject with:

i) a human EIF3L binding molecule, or one or more mRNAs encoding said EIF3L binding molecules, or an expression vector comprising one or more nucleic acid molecules encoding said human EIF3L binding molecule, optionally wherein said human EIF3L binding molecule is as recited in any of Claims 1-17,

ii) a human CD63 binding molecule, or one or more mRNAs encoding said human CD63 binding molecule, or an expression vector comprising one or more nucleic acid molecules encoding said CD63 binding molecule, and

wherein said subject has, or is suspected to develop, said prothrombotic condition.

19. The method of Claim 18, wherein:

i) said subject has cancer and has, or is suspected to develop, tumor-mediated thrombosis and/or a tumor-mediated thrombotic disorder and/or thrombosis associated with anti-tumor therapy, and optionally wherein said tumor-mediated thrombotic disorder is selected from the group consisting of: heart attack, acute ischemic stroke, transient ischemic attack, deep vein thrombosis, a pulmonary embolism, and phlebitis; and/or

iii) said prothrombotic condition is selected from: Sepsis, septic shock, viral infection, Sars-Cov2 infection, sickle cell disease, cardiovascular disease, acute coronary

syndrome (ACS), stroke, acute inflammations, infections-sepsis, and acute coronary syndrome.

20. The method of Claim 18, wherein said human CD63 binding molecule, and/or said human EIF3L binding molecule, is an antibody or antigen binding portion thereof.

21. The method of Claim 18, wherein said antibody or antigen binding portion thereof is a human antibody or antigen binding portion thereof.

22. The method of Claim 18, wherein said antibody or antigen binding portion thereof is a humanized antibody or antigen binding portion thereof.

23. The method of Claim 18, wherein said heavy chain variable region of said human EIF3L binding molecule comprises SEQ ID NO: 5, or SEQ ID NO:5 with one or more conservative amino acid changes.

24. The composition of Claim 18, wherein said light chain variable region of said human EIF3L binding molecule comprises SEQ ID NO: 9, or SEQ ID NO: 9 with one or more conservative amino acid changes.

25. The composition of Claim 18, wherein said human CD63 binding molecule binds a glycosylated version of human CD63.

26. A method of detecting CD63-positive, PCA3-positive, and/or EIF3L-positive small extracellular vesicles (sEVs) in a sample comprising:

a) contacting a sample with: i) anti-CD63 antibodies, or antigen binding portions thereof, and/or ii) anti-PCA3 antibodies, or antigen binding portions thereof, and/or iii) anti-EIF3L antibodies, or antigen binding portions thereof,

wherein said sample comprises purified sEVs derived from a blood, plasma, or serum sample from a subject with cancer, and wherein said sample is suspected of containing CD63-positive, or PCA3-positive, or EIF3L-positive, sEVs,

wherein said anti-CD63 antibodies, or antigen binding portions thereof, form first complexes with said CD63-positive sEVs if present in said sample, and wherein said anti-PCA antibodies, or antigen binding portions thereof, form second complexes with said

PCA3-positive sEVs if present in said sample, and wherein said anti-EIF3L antibodies, or antigen binding portions thereof, form third complexes with said EIF3L-positive sEVs if present in said sample; and

b) detecting the presence or absence of said first complexes and/or said second complexes and/or said third complexes in said sample.

27. The method of Claim 26, wherein said sample is from a subject that has, or is suspected to develop, a prothrombotic condition, including those recited in Claim 19.

28. The method of Claim 26, wherein said human anti-EIF3L antibodies, or antigen binding portions thereof, comprise a detectable label, and/or wherein said anti-EIF3L antibodies, or antigen binding portions thereof, are as recited in Claim 1-17.

29. The method of Claim 26, further comprising contacting said sample with a conjugate molecule capable of binding to said: i) anti-CD63 antibodies, or antigen binding portions thereof, and/or ii) anti-PCA3 antibodies, or antigen binding portions thereof, and/or iii) anti-EIF3L antibodies, or antigen binding portions thereof, wherein said conjugate molecule comprises a detectable label.

FIGURE 1A-J

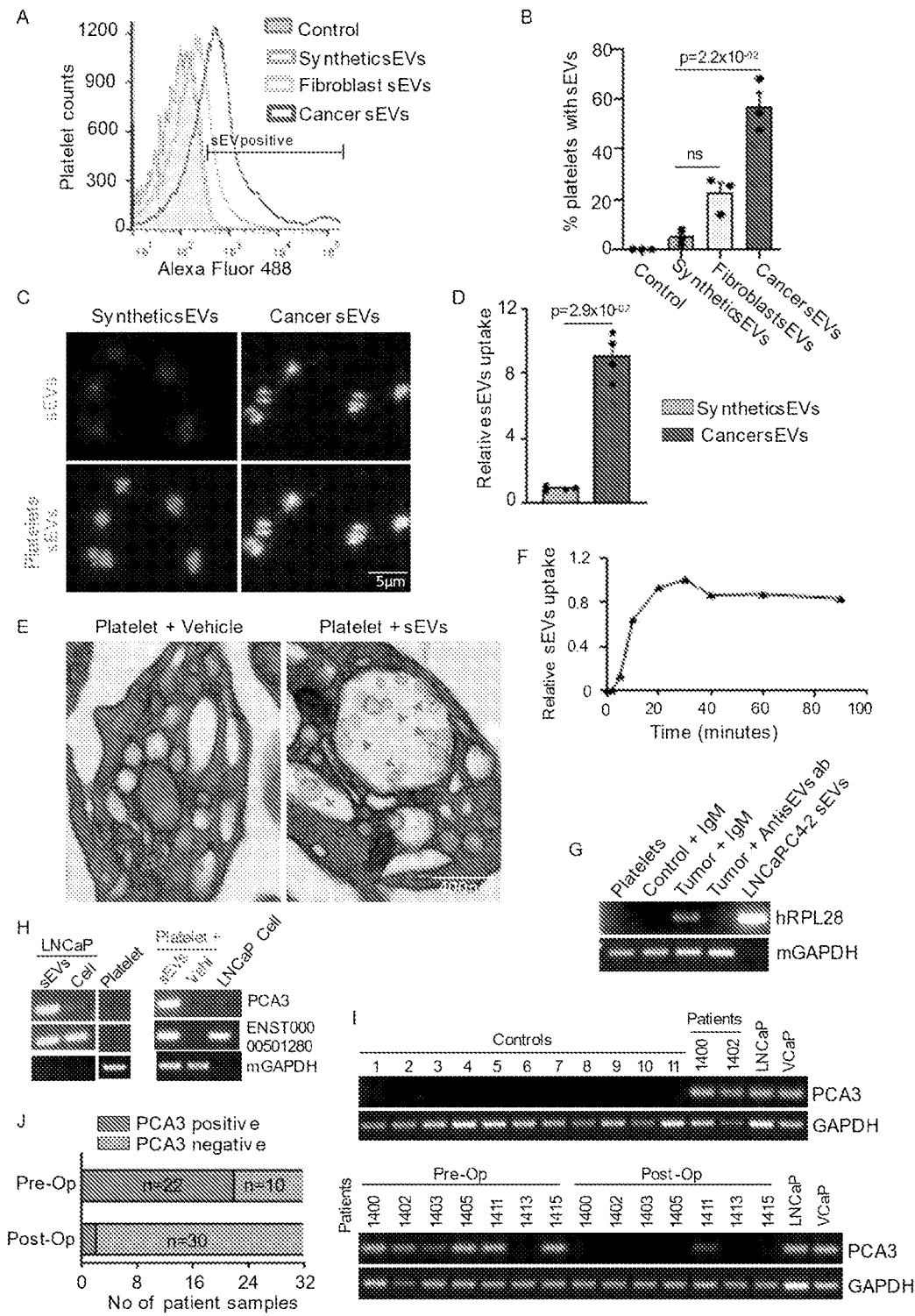


FIGURE 2A-G

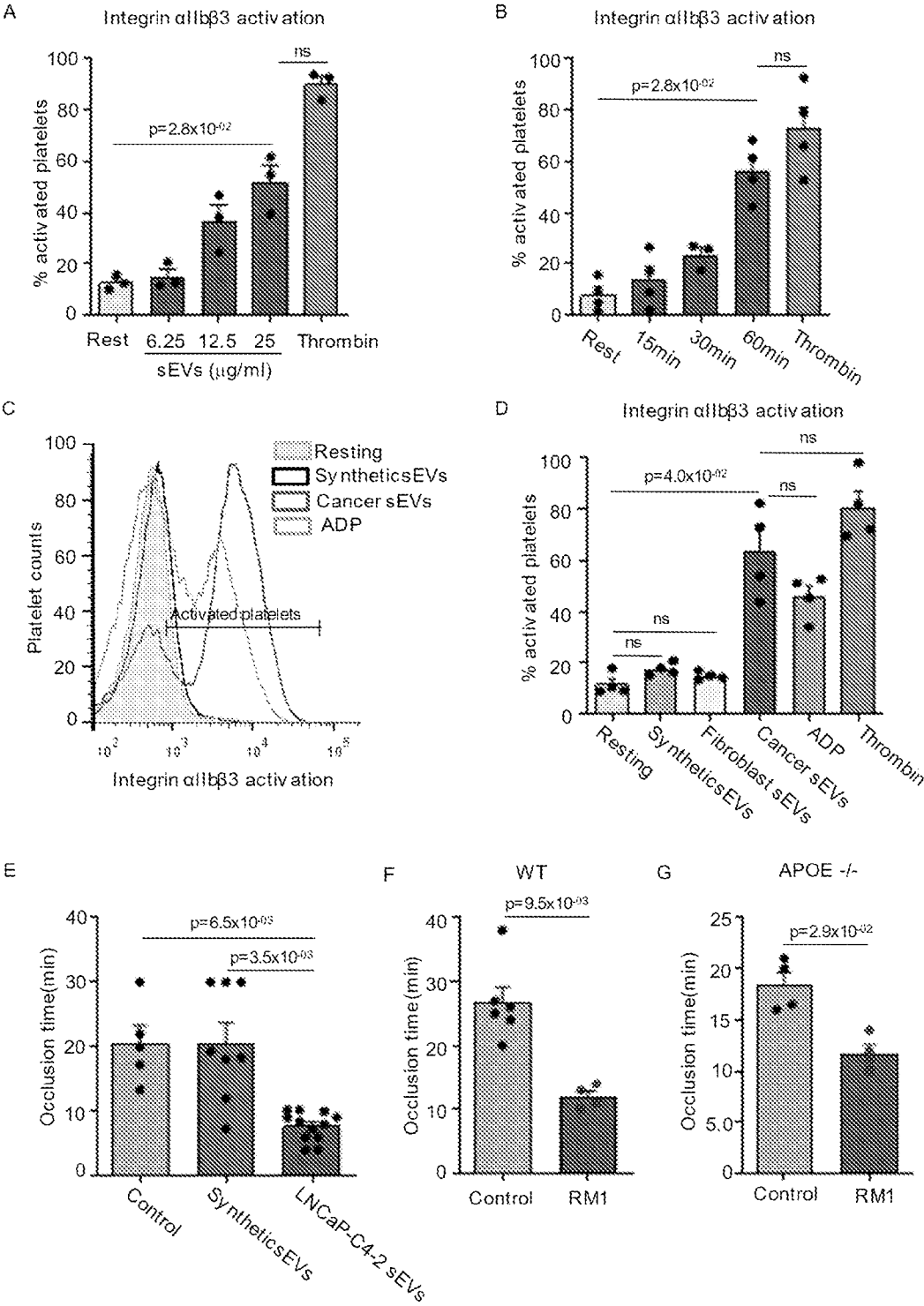


FIGURE 3A-F

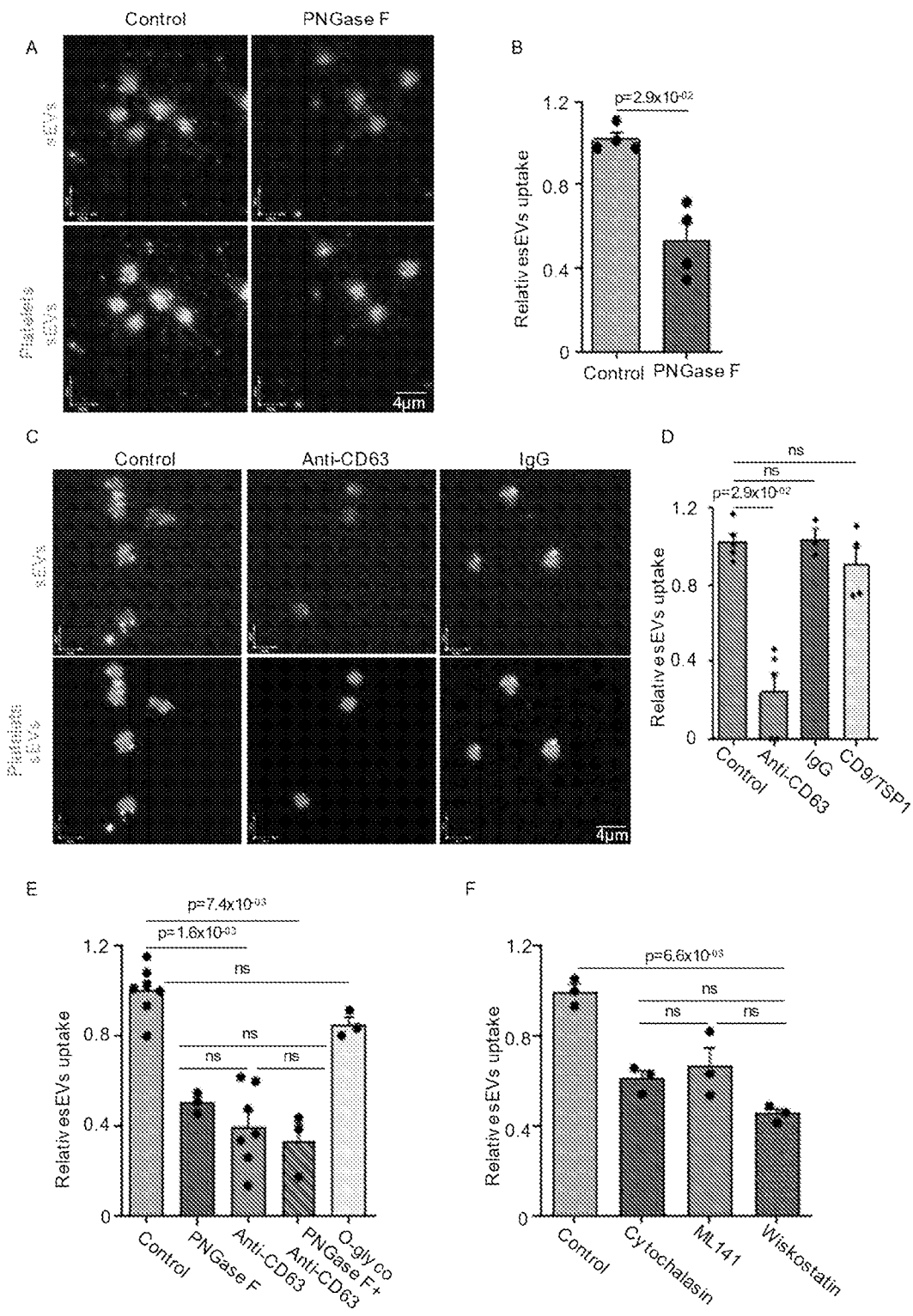


FIGURE 4A-G

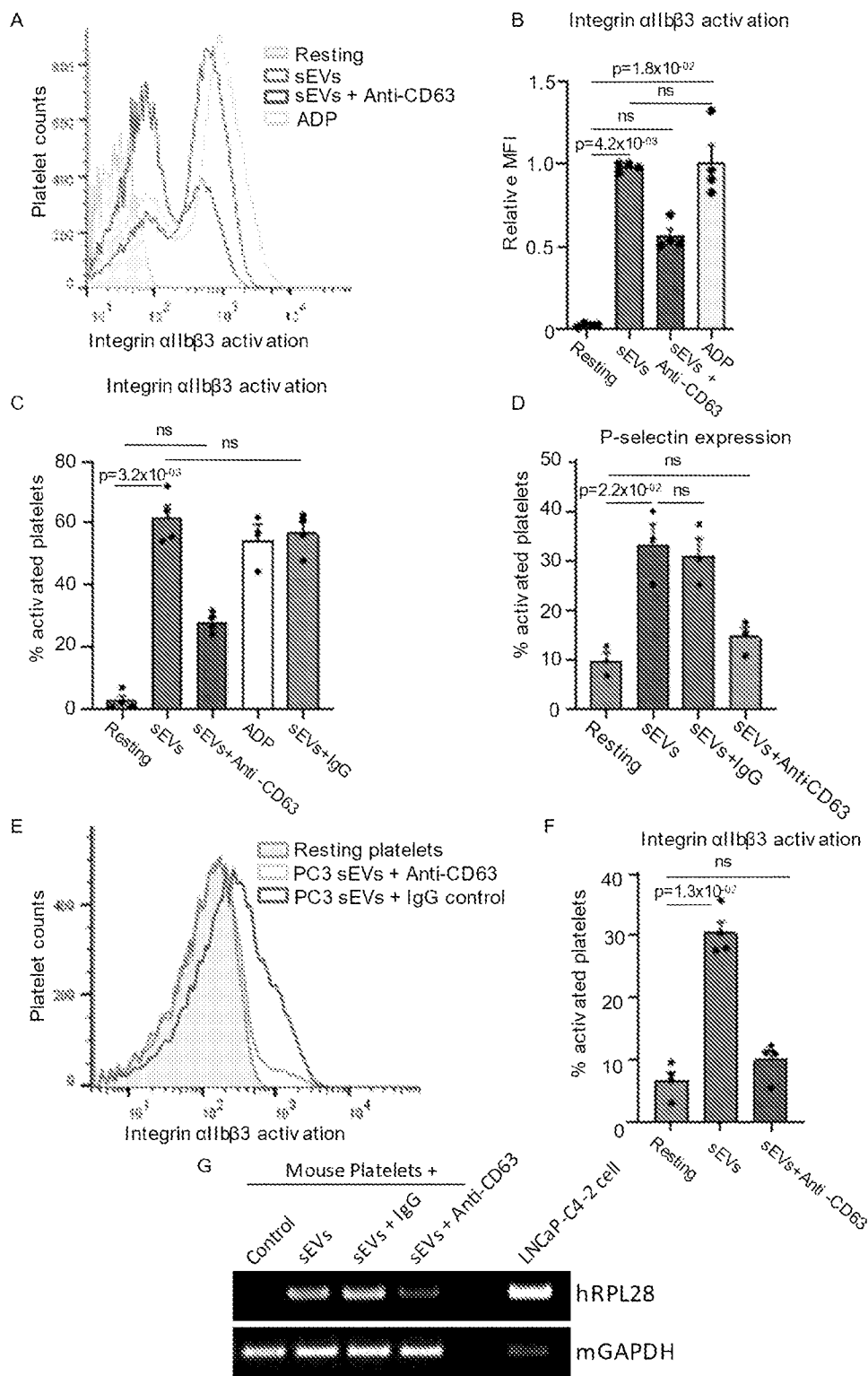


FIGURE 5A-H

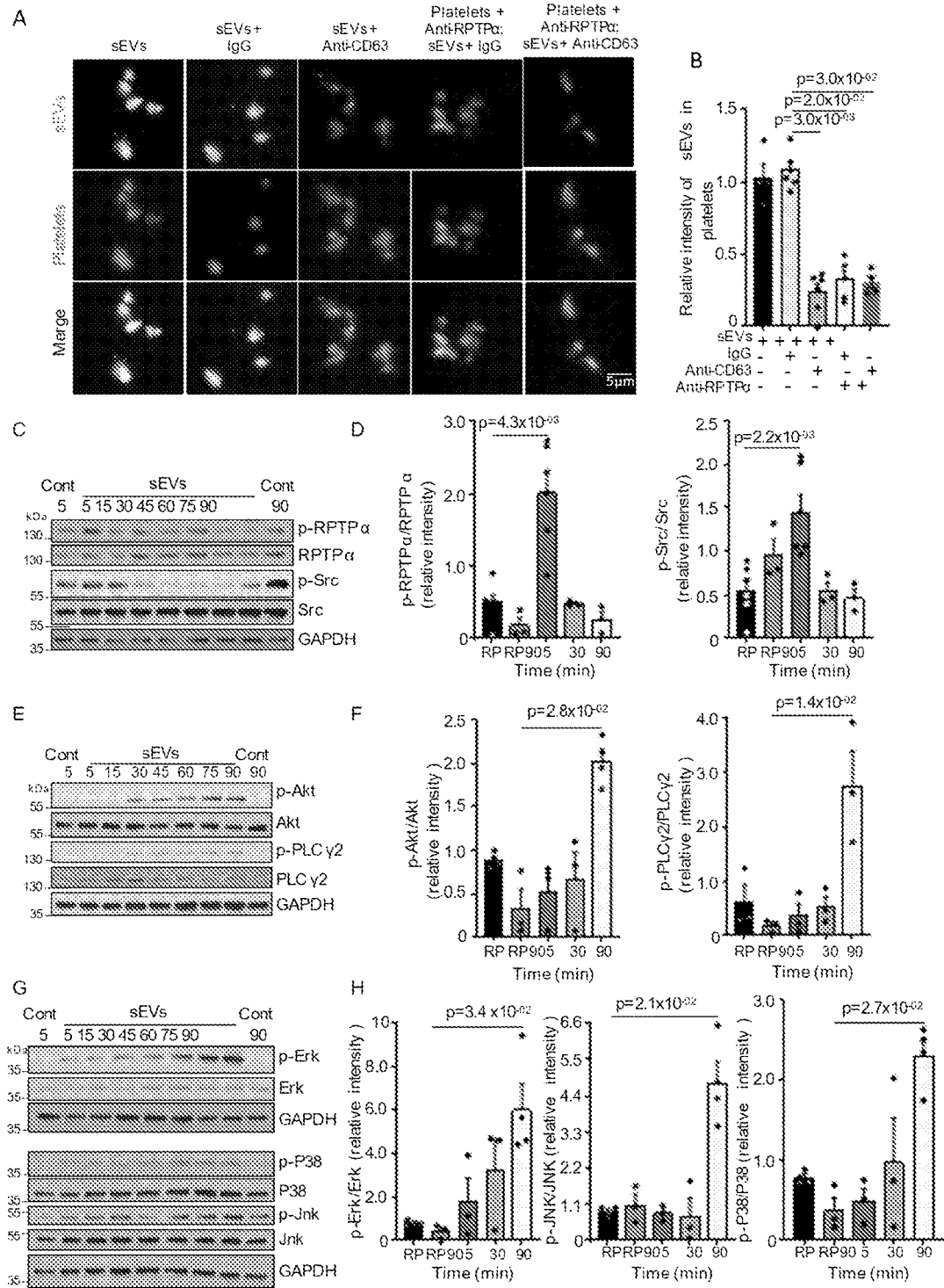


FIGURE 6A-G

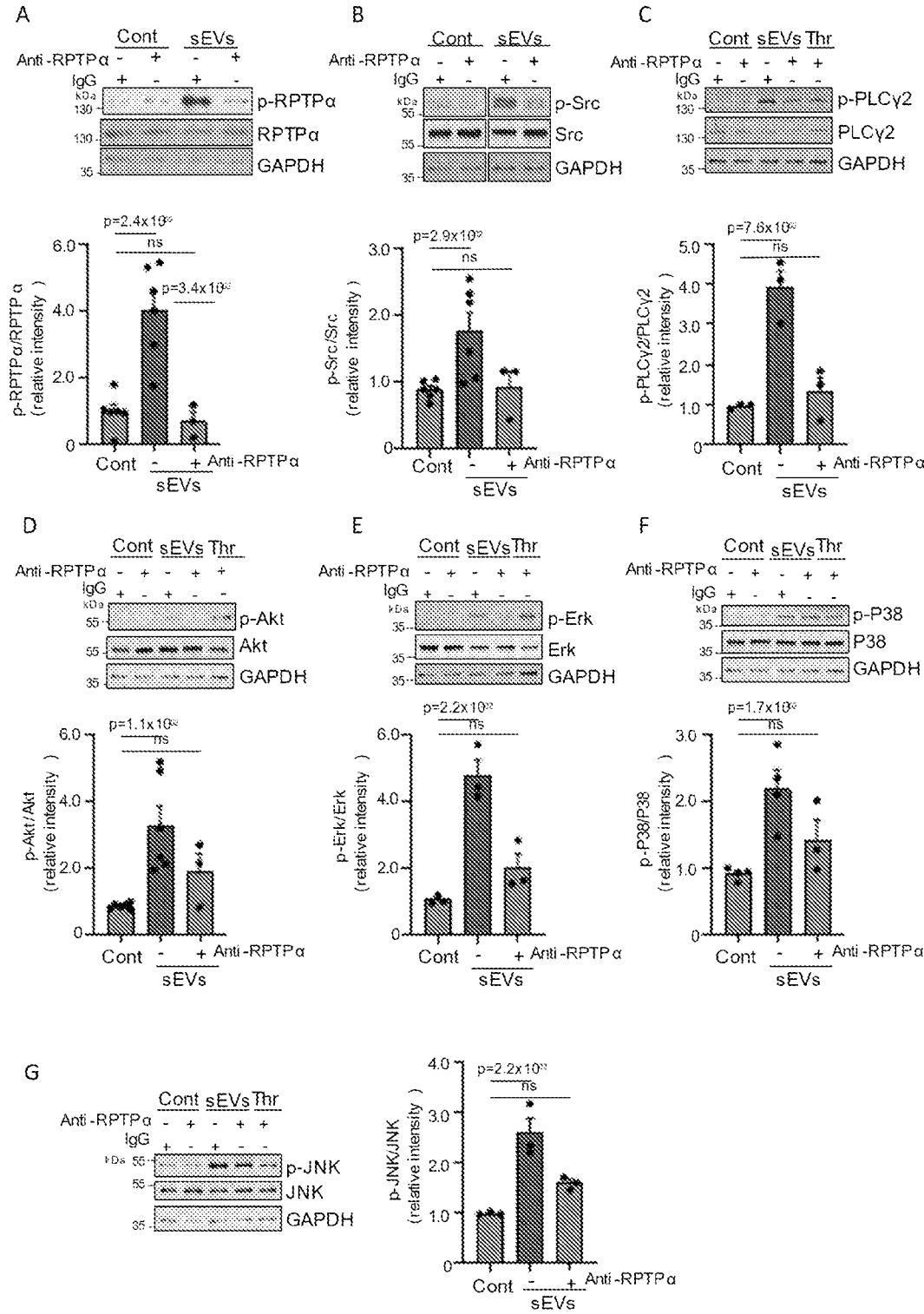


FIGURE 7A-D

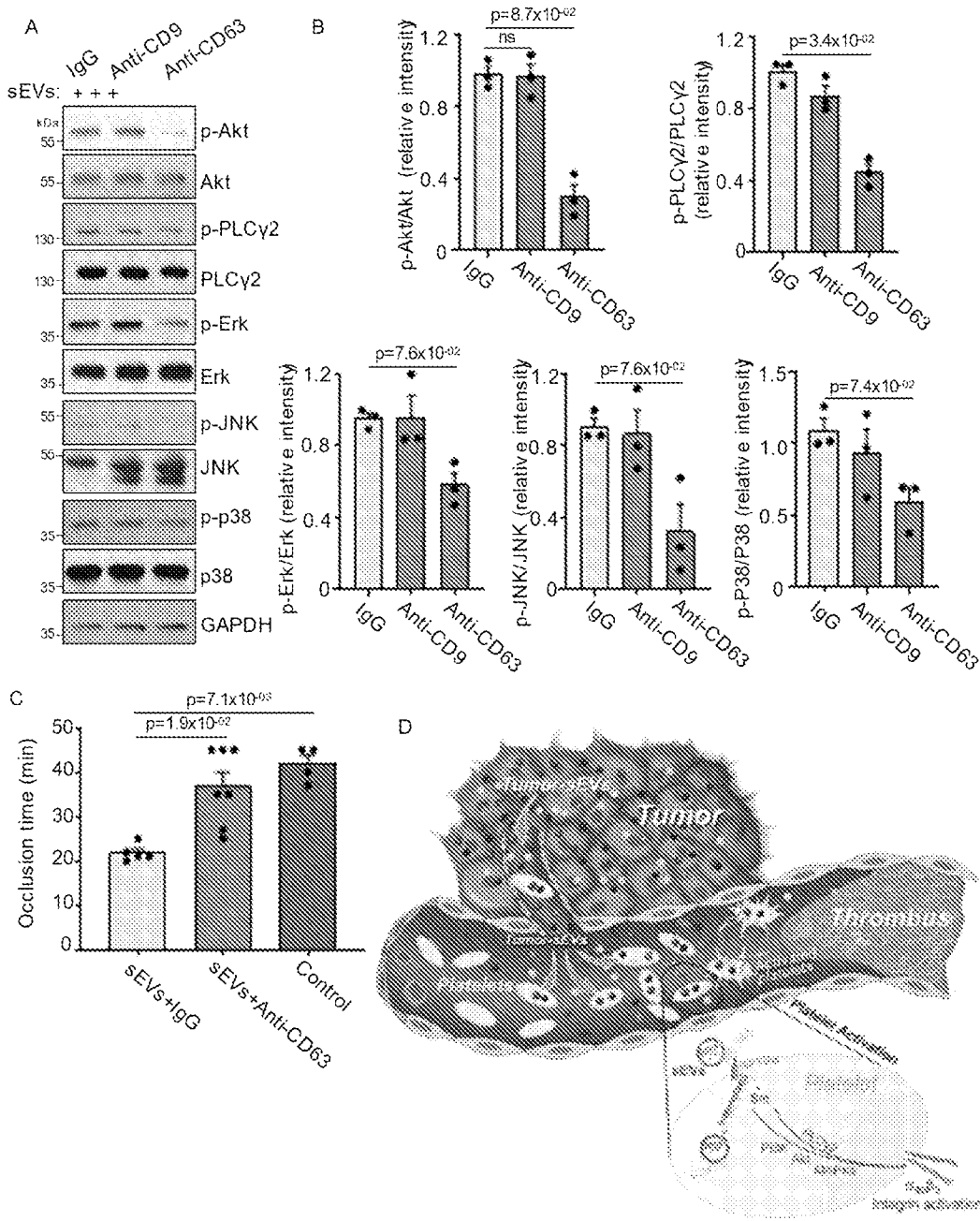


FIGURE 8A-E

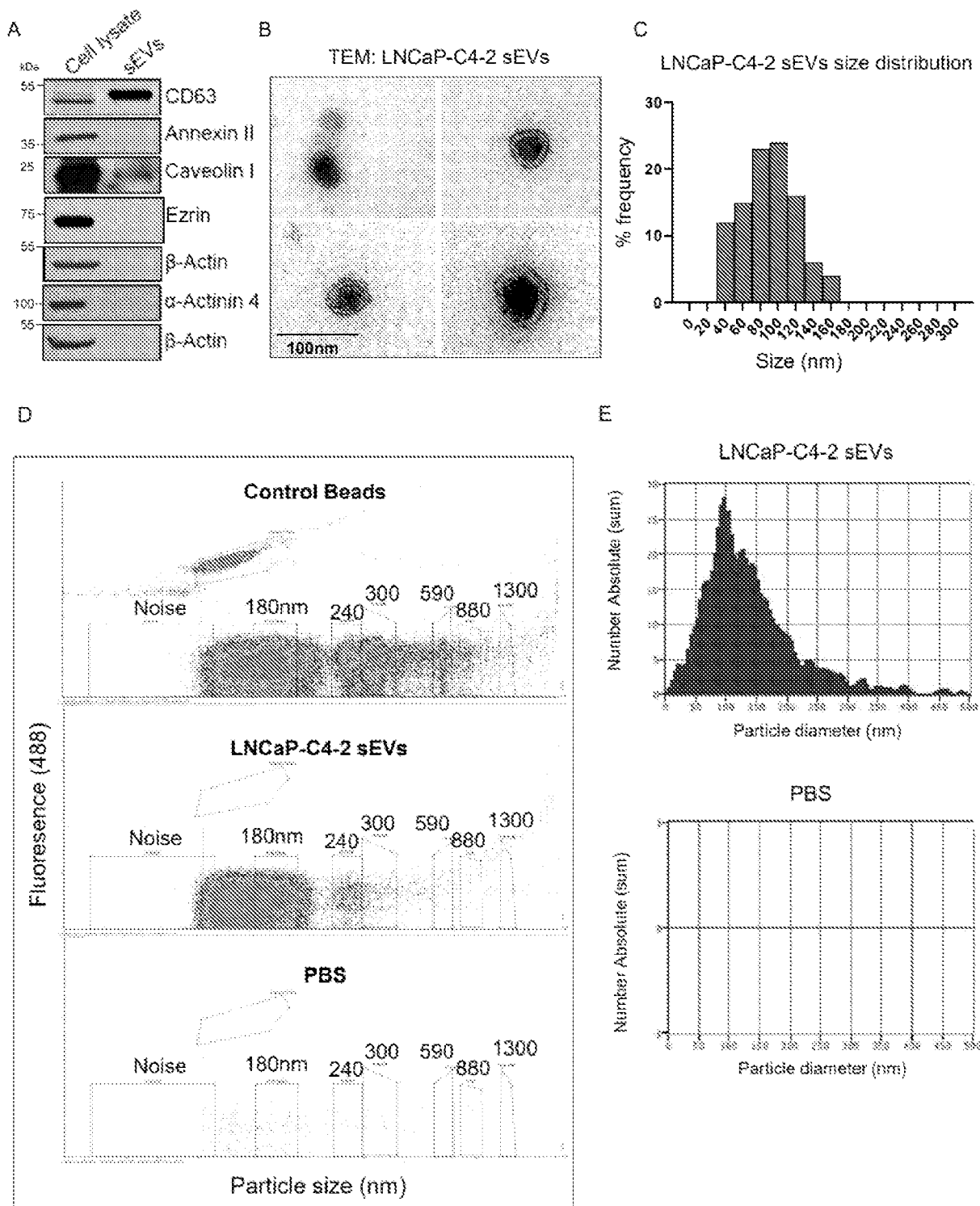


FIGURE 9A-D

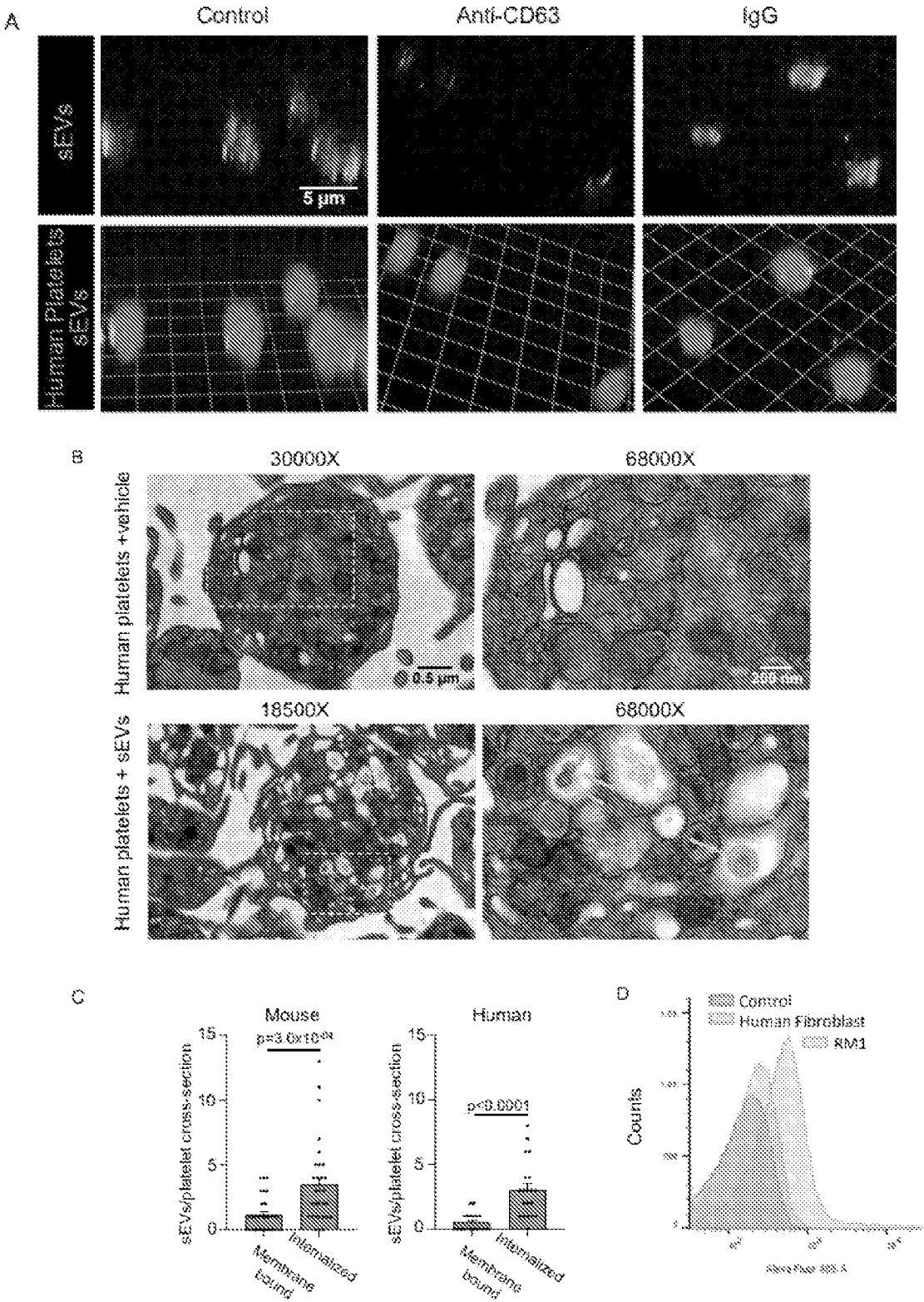


FIGURE 10A-C

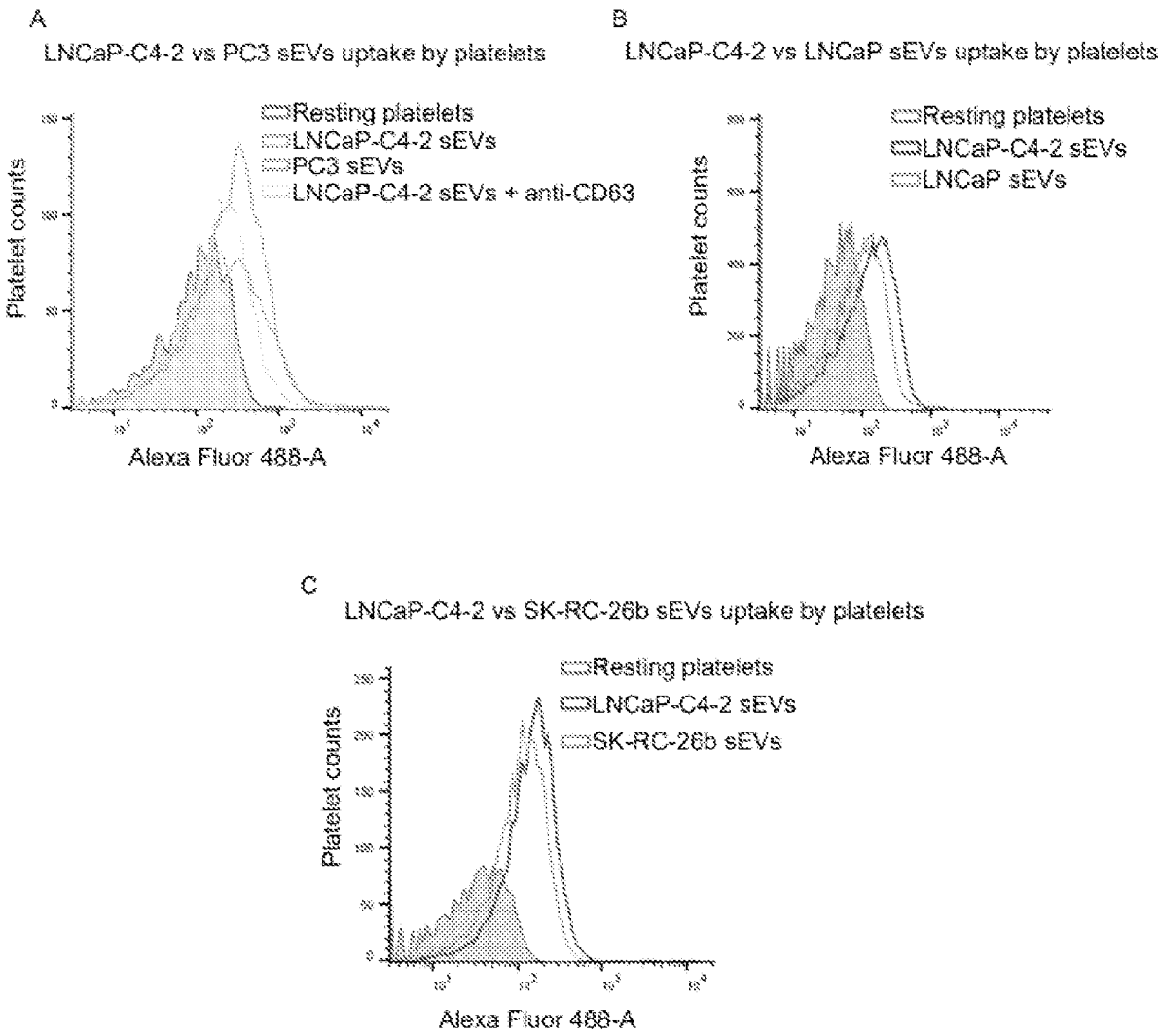


FIGURE 11A-E

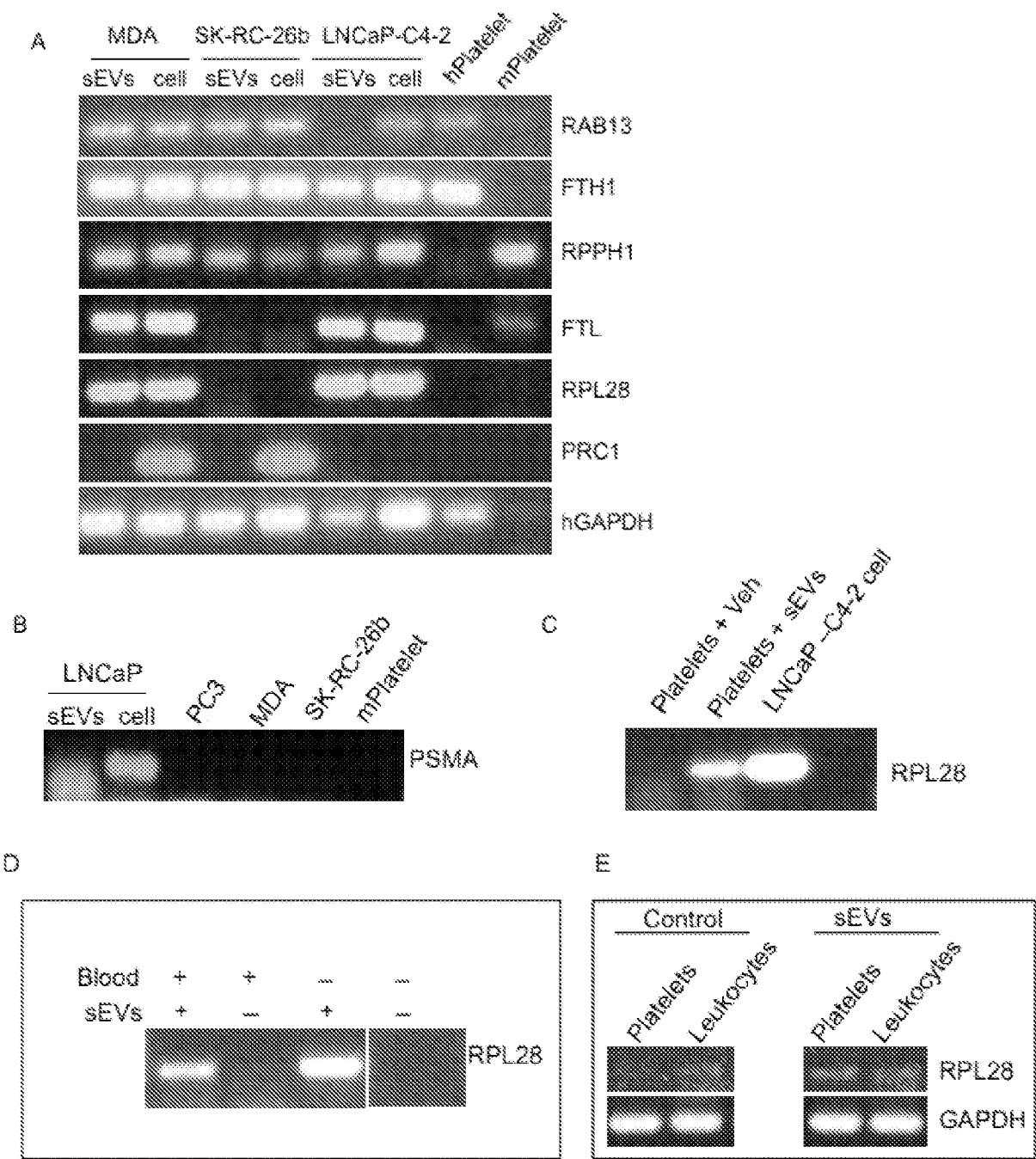


FIGURE 12A-E

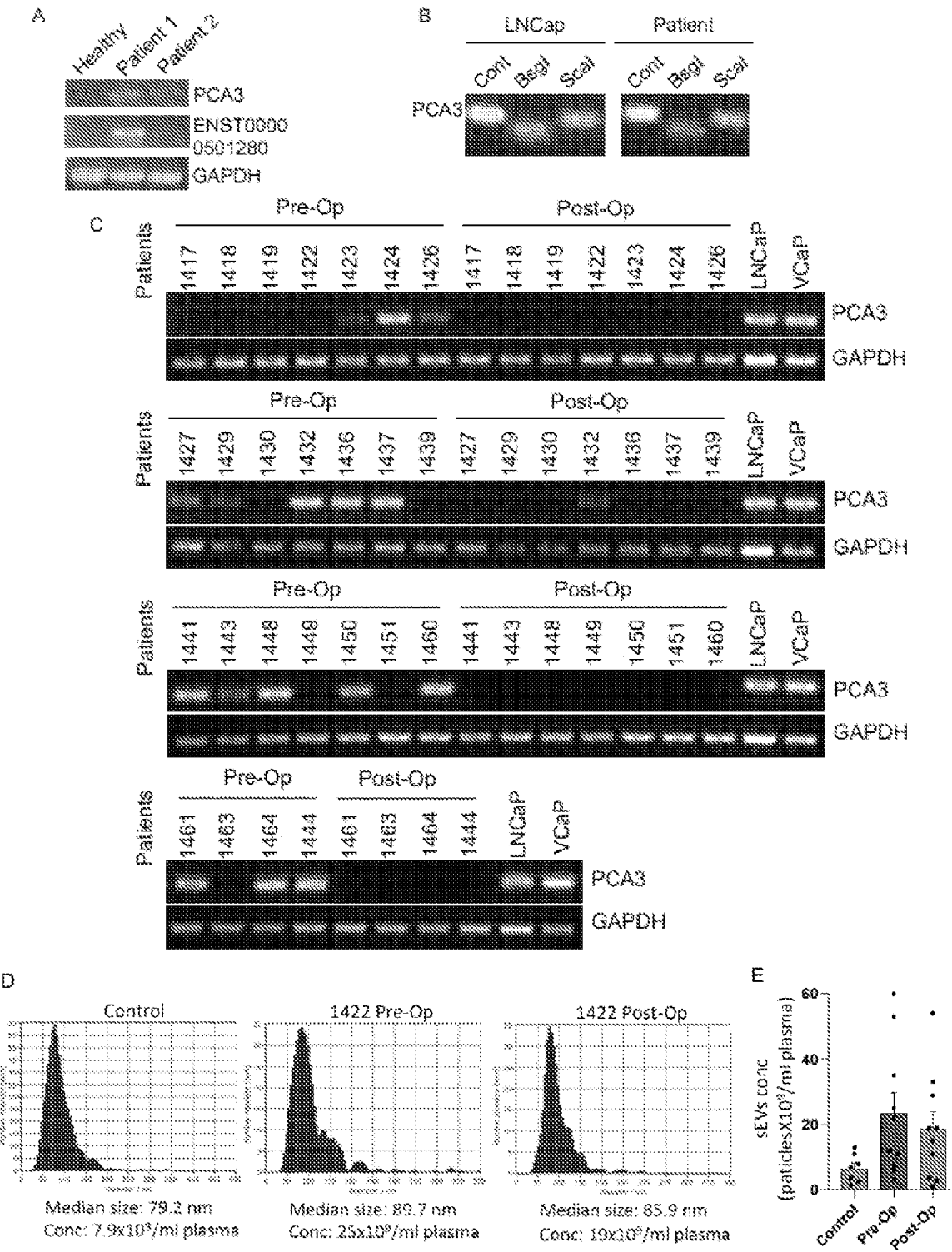


FIGURE 13A-F

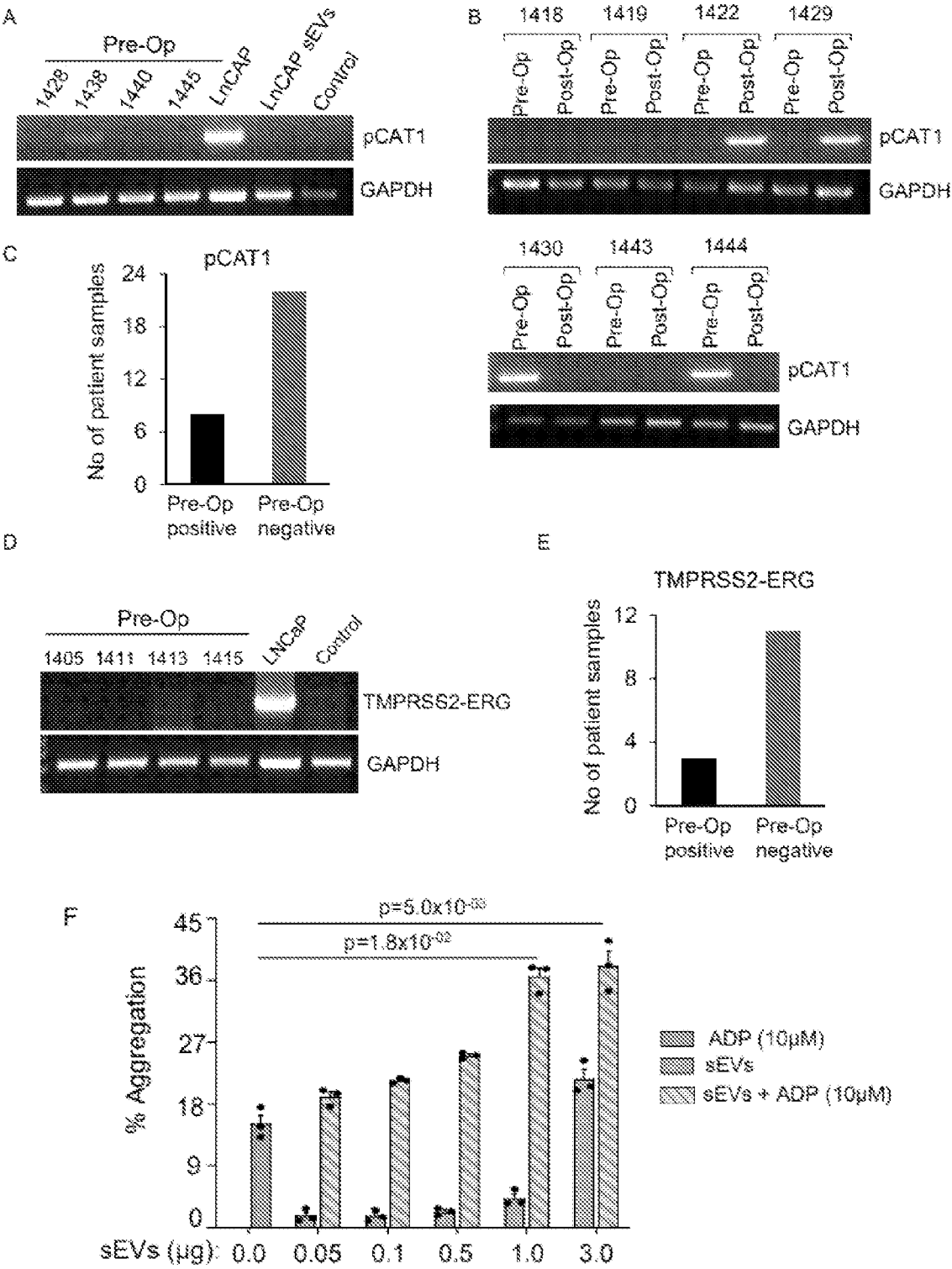


FIGURE 14A-D

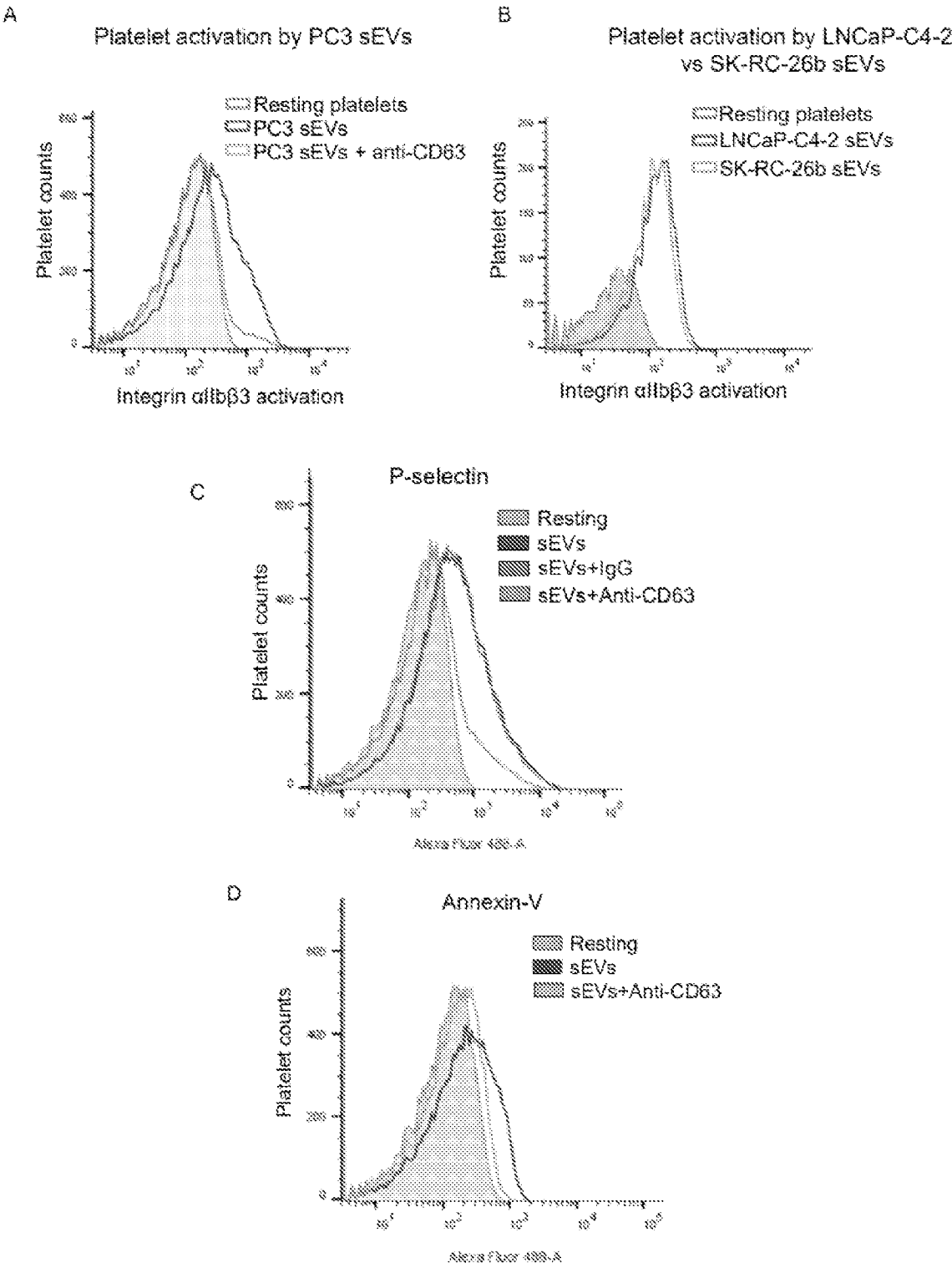


FIGURE 15A-D

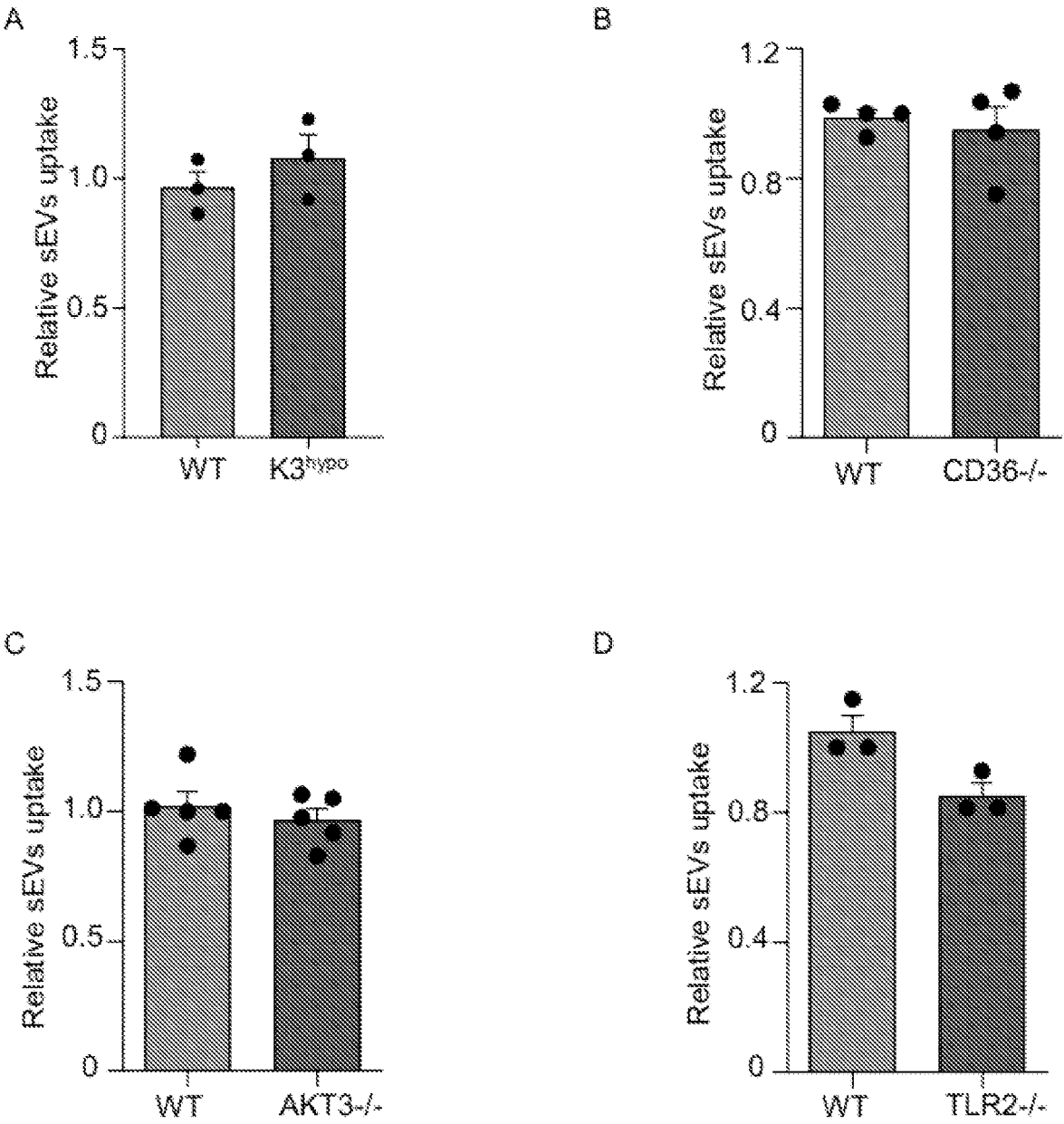


FIGURE 16A-G

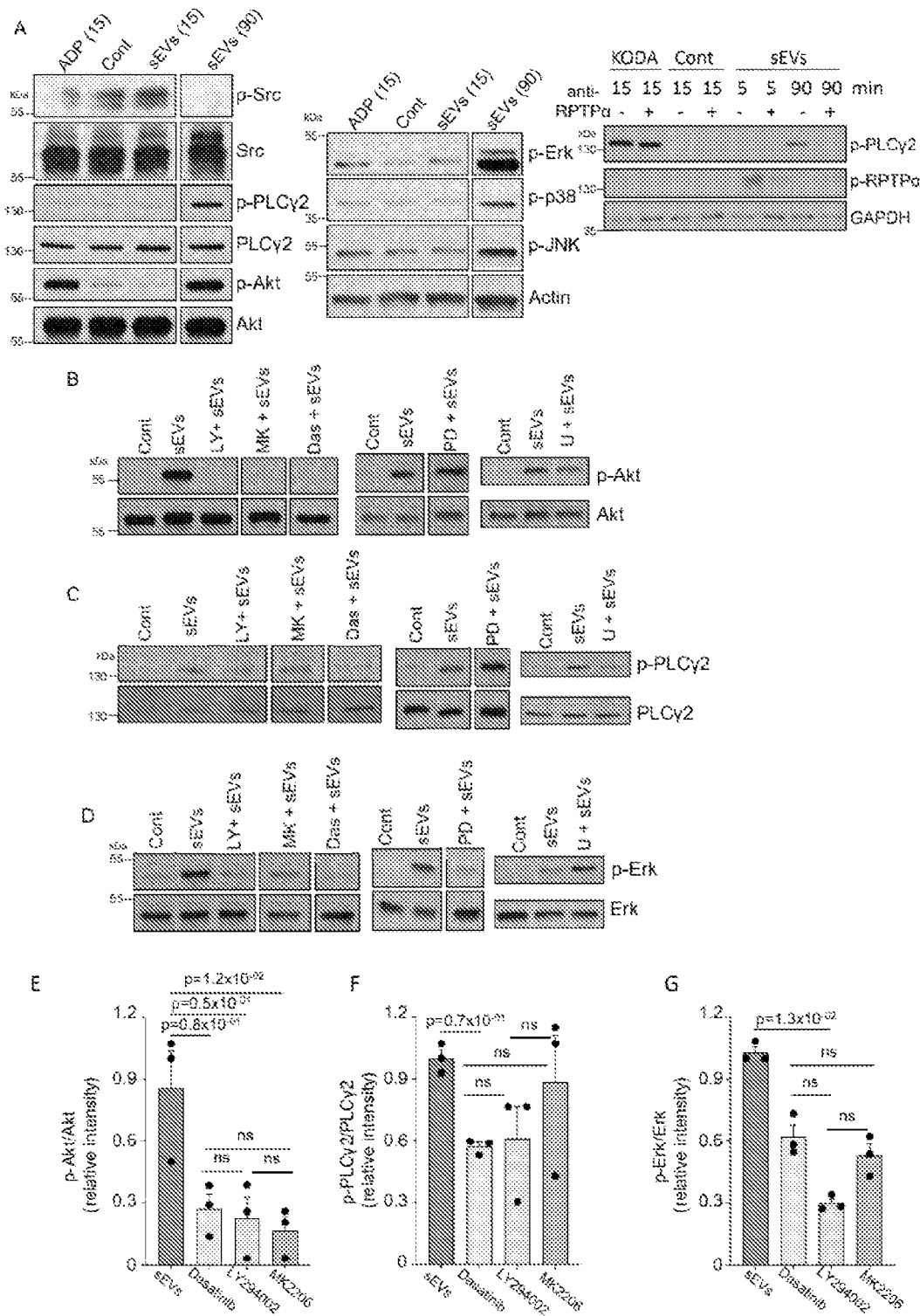


FIGURE 17A-C

Figure S10

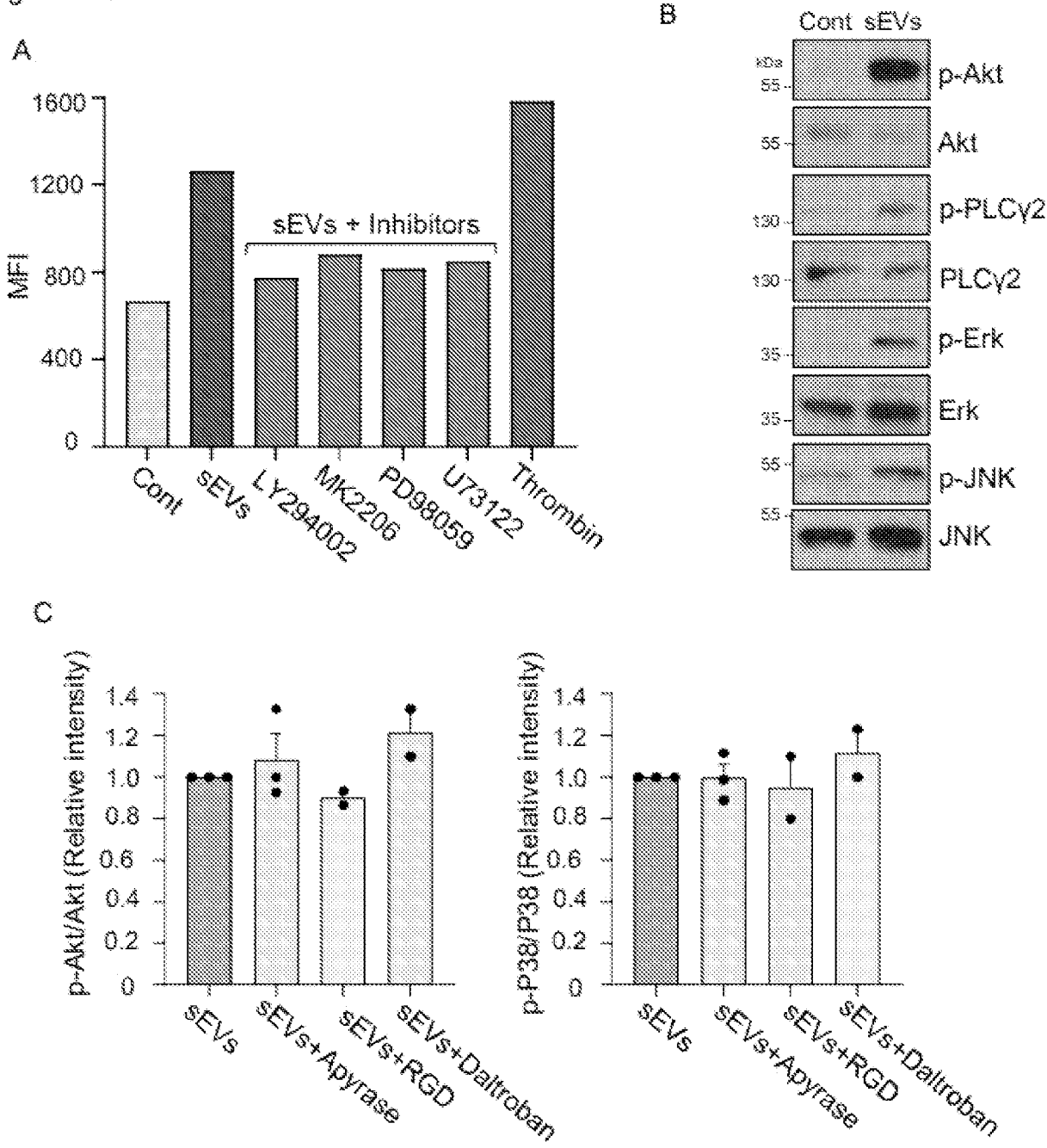


FIGURE 19A-D

A. A1806-3A1-3 Heavy Chain Amino Acid Sequence (SEQ ID NO:1)

*MRVLILLWLFTA****FP****GILSDVQLQESGPGLVKPSQSL****SLTCTVT****GYSITSDY****AWN****WIRQFPGNKLEWMGYIS*
*YSGTSYNPSL****KSRISITR****DTSKNQFFLQLNSVT****TEDTATYYC****ASMITAWFAY****WGQ****GLVTVSAESQ****SFP***
*NVFPLVSCESPLSDKNLVAMGCLARDFLPSTISFTWNYQN****TE****VIQGI****RT****FTLRTGGKYLATSQVLLSPKSI*

Leader sequence in italics, VH in bold, constant region has no italics and not bold, and CDRs underlined

B. A1806-3A1-3 Heavy Chain Nucleic Acid Sequence (SEQ ID NO:2)

ATGAGAGTGCIGATTCTTTTGTGGCTGTTACAGCCTTTCTCGGATCCTGTCTGATGTGCAGCTTCAGGAGTCGGGACCTGGCCT
CGTGAACCTTCTCACTCTCTGTCCCTCACCTCCACTGTCAGTCTCAATCACCAGTGATTATGCCTCGAAGTCGATCCGGC
AGTTTCCAGGAAACAACTGGAGTGGATGGGCTACATAAGCTACACTGGTAGCTAGCTACAACCCATCTCTCAAAAGTCGAATC
TCTATCACTCGAGACACATCCAAGAACCAGTTCTTCTGTCAGTTGAATTCTGTGACTACTGAGGACACAGCCACATATTACTGTGC
AAGTATGATTACGGCCTGGTTTGCTTACTGGGGCCAAGGGACTCTGGTCACTGCTCTGTCAGAGAGTCAGTCCTTCCCAAATGTCT
TCCCCCTCGTCTCTGCGAGAGCCCCCTGTCTGATAAGAATCTGGTGGCCATGGGCTGCCTGGCCCCGGGACTTCTGCCCAGCACC
ATTTCTTCACTGGAACACCAGAACAACTGAAGTCATCCAGGGTATCAGAACCTTCCCAACACTGAGGACAGGGGCAAGTA
CCTAGCCACCTCGCAGGTCTTGCTGTCTCCCAAGAGCATC

C. A1806-3A1-3 Light Chain Amino Acid Sequence (SEQ ID NO:3)

*MEKDTLLLWVLLLWVPGSTG****DIVLTQSPASLAVSLGQRATIS****CRASESV****DNYGIS****FMNWFQ****QKPGQPPKL***
LIYAAS*NQGS****GV****PARFSGSG****SGTDFSLNIHPMEED******TAM****YFCQOSKEVP****YTF****GGG****TKLEIKRADAAPT***
*VSIFPPSSEQLTSGGASVVCFLNNFY****PKDINVKWKIDG****SERQNGVLNSWTDQDSKDSTYSMSSTLT****LT******TK***

Leader sequence in italics, VL in bold, constant region has no italics and not bold, and CDRs underlined

D. A1806-3A1-3 Light Chain Nucleic Acid Sequence (SEQ ID NO:4)

ATGGAGAAAGACACACTCCTGCTATGGGTCTCTCTCTGGGTCCAGGTTCCACAGGTGACATTCTGCTGACCCAATCTCCAGC
TTCTTTGGCTGTGTCTCTAGGGCAGAGGGCCACCATCTCCTGCAGAGCCAGCGAAAGTGTGATAATTATGGCATTAGTTTATGA
ACTGGTTCCAACAGAAACCAGGACAGCCACCCAACTCCTCATCTATGCTGCATCCAACCAAGGATCCGGGGTCCCTGCCAGGTTT
AGTGGCAGTGGGTCTGGGACAGACTTCAGCCTCAACATCCATCCATGGAGGAGGATGATACTGCAATGTATTTCTGTCAGCAAAG
TAAGGAGGTTCCGTACAGTTCCGAGGGGGGACCAAGCTGGAATAAAACGGGCTGATGCTGCACCAACTGTATCCATCTTCCCAC
CATCCAGTGAGCAGTTAACATCTGGAGGTGCCTCAGTCGTGTGCTTCTTGAACAACCTTACCCCAAAGACATCAATGTCAAGTGG
AAGATTGATGGCAGTGAACGACAAAATGGCGTCTGAACAGTTGGACTGATCAGGACAGCAAAGACAGCACCTACAGCATGAGCAG
CACCTCACGTTGACCAAG

FIGURE 20A-B

A. A1806-3A1-3 Variable Heavy Chain Amino Acid Sequence (SEQ ID NO:5)

DVQLQESGPGLVKPSQSLSLTCTVTGYSITSDYAWN WIRQFPGNKLEWMGYISYSGSTSY

(CDRH1, SEQ ID NO:6)

(CDRH2, SEQ ID NO:7)

NPSLKSRISITRDTSKNQFFLQLNSVTTEDTATYYCASMITAWFAYWGQGLVTVSA

(CDRH3, SEQ ID NO:8)

B. A1806-3A1-3 Variable Light Chain Amino Acid Sequence (SEQ ID NO:9)

DIVLTQSPASLAVSLGQRATISCRASESVDNYGISFMNWFQKPGQPPKLLIYAASNQGS

(CDRL1, SEQ ID NO:10)

(CDRL2, SEQ ID NO:11)

VPARFSGSGSGTDFSLNIHPMEEDDTAMYFCQQSKEVPYTFGGGTKLEIK

(CDRL3, SEQ ID NO:12)

FIGURE 21A-B

3A1-3 mAb rescues exosome induced occlusion time (decreases thrombosis)

➤ In vivo model 2: LNCaP-C4-2 tumor xenograft

- 9 NSG mice each for Tumor+IgM, Tumor+mAb 3A1-3 and Control+IgM
- LNCaP-C4-2 Xenograft:
 - Bilateral tumors
 - Inject IgM or 3A1-3 four times (5th day, 10th day, 24 hrs, 14 hrs, and 3hrs before thrombosis).

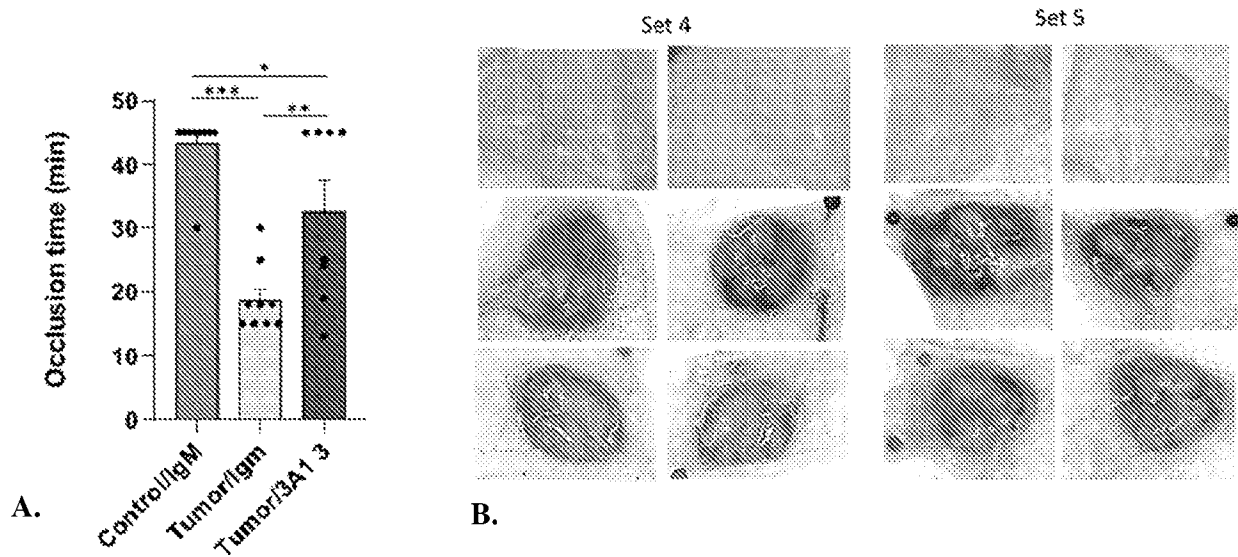


FIGURE 22A-D

LNCaP-C42 cell lysates: Confirmation of EIF3L (67kd) by Reciprocal IP with 3A13 and commercial Anti-EIF3L polyclonal Abs

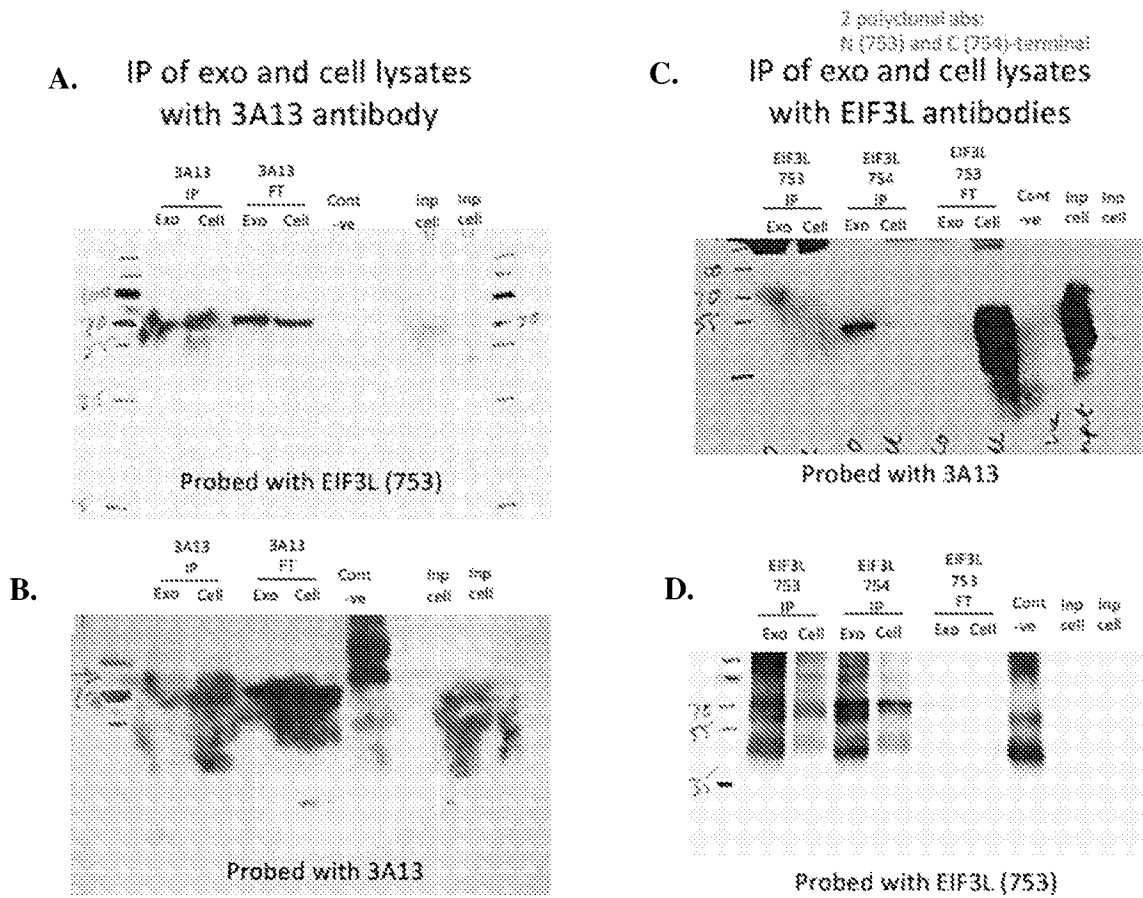


FIGURE 23A-B

mAb-3A13 binds EIF3L on LNCaP-C42 exosome surface

Microscale Thermophoresis assays (MST assays) measure the motion of molecules along microscopic temperature gradients that changes upon ligand binding.

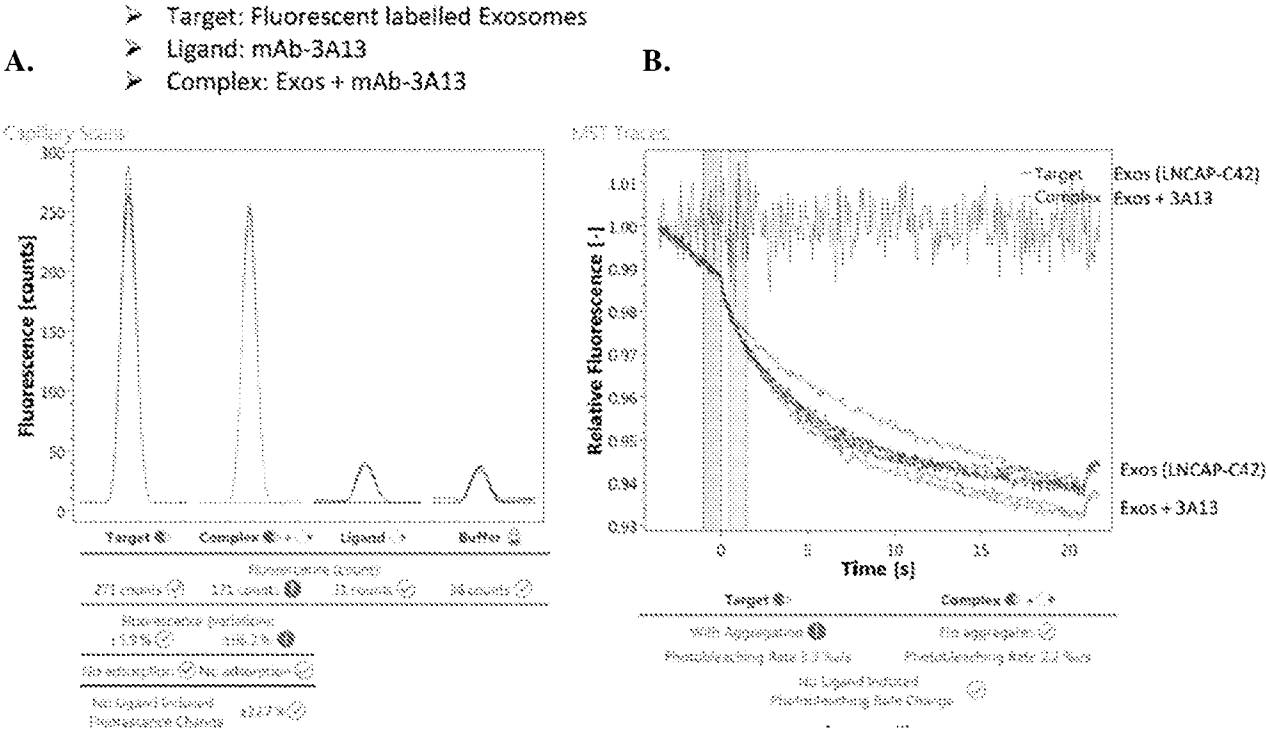
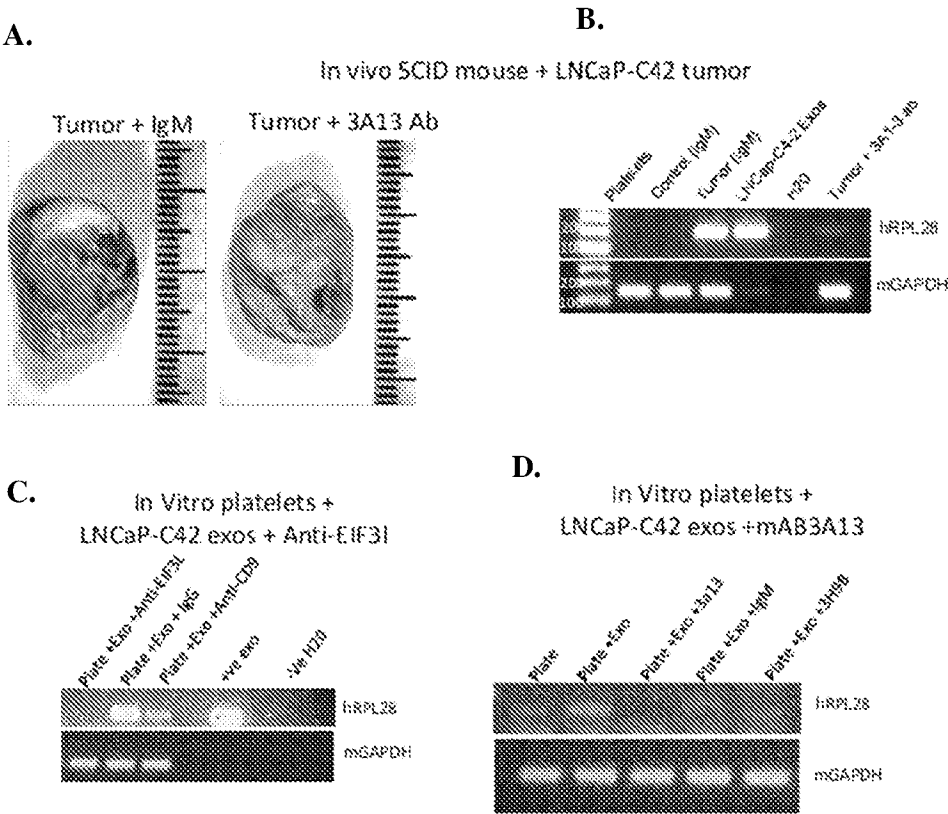


FIGURE 24A-D



Result: 3A13 and Anti-EIF3I block uptake of tumor exosomes by platelets as evidenced by low levels of tumor exosome marker hRPL28 in platelets of mice treated with the Abs.

FIGURE 25A-D

mAb-3A13 blocks prostate cancer exosome mediated platelet activation

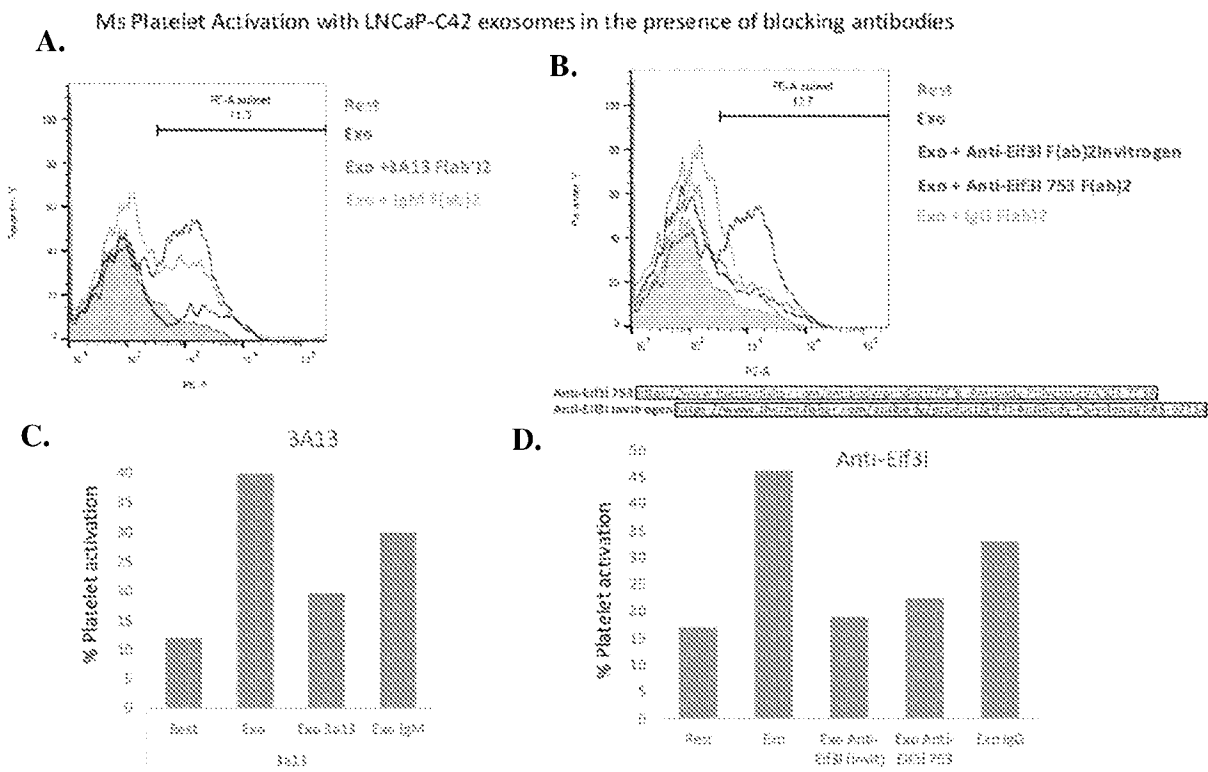
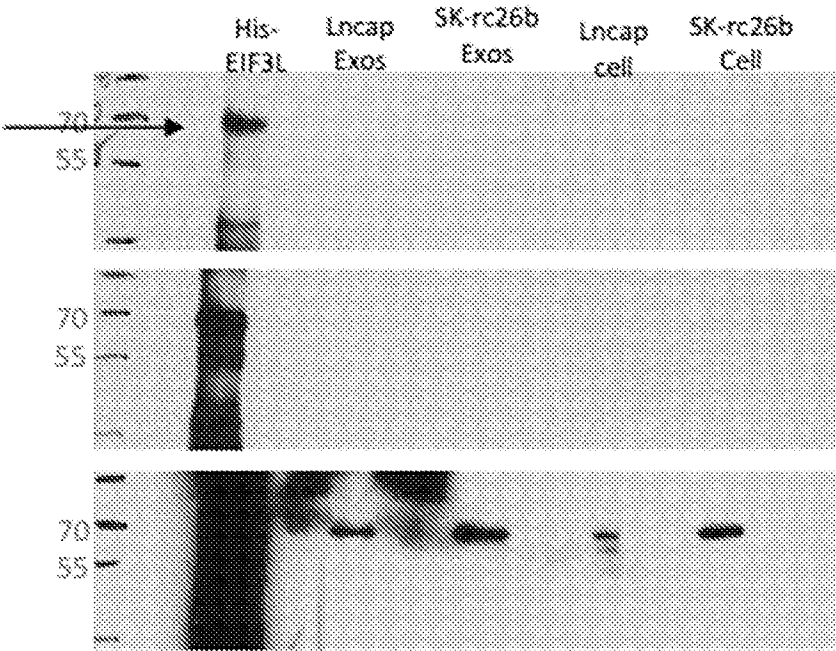


FIGURE 26

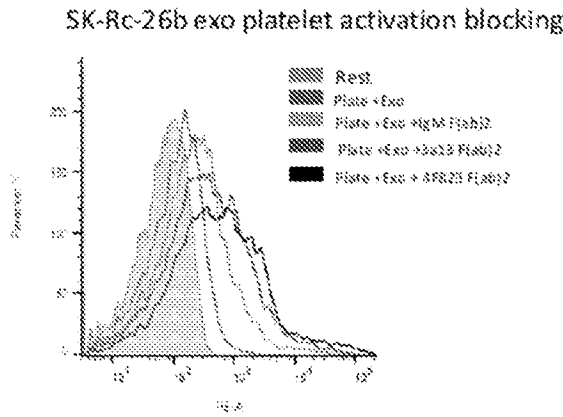
EIF3L is detected in renal cancer cell line (SK-rc26b) exosomes



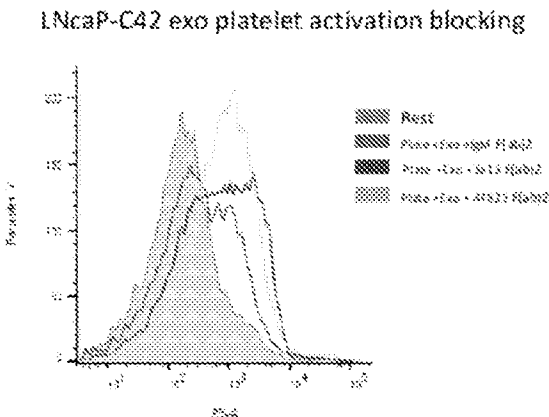
Probed with anti-EIF3L (753)

FIGURE 27A-D

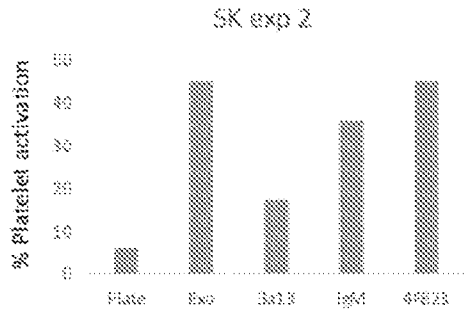
A.



B.



C.



D.

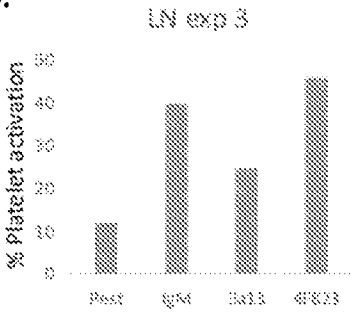


FIGURE 28

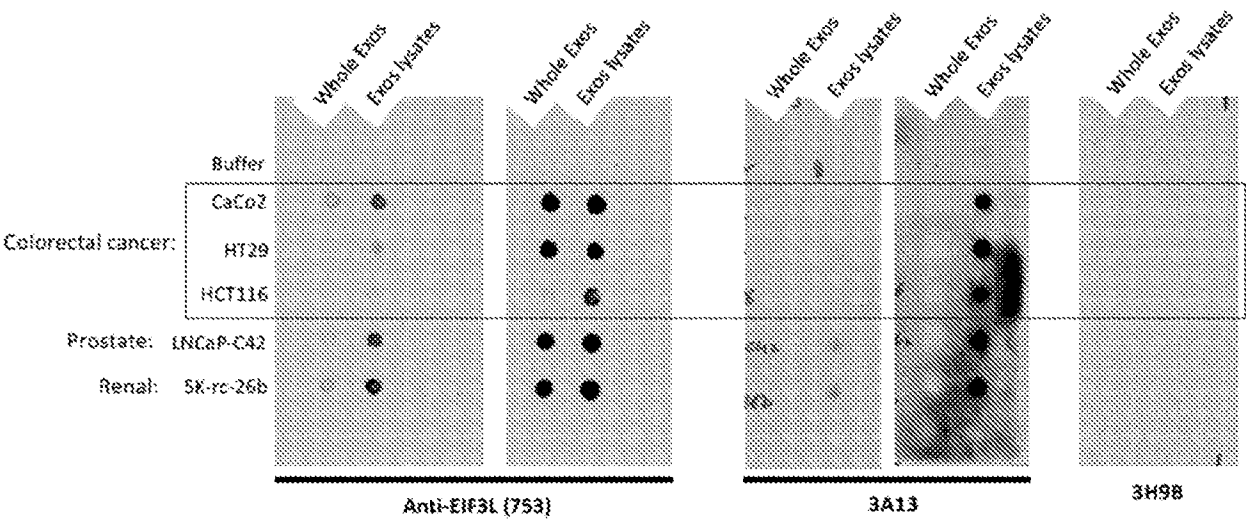


FIGURE 29A-D

Screening of prostate cancer patient plasma with mAb-3A13 and anti-EIF3L

Higher levels of circulating exosomes in prostate cancer patients

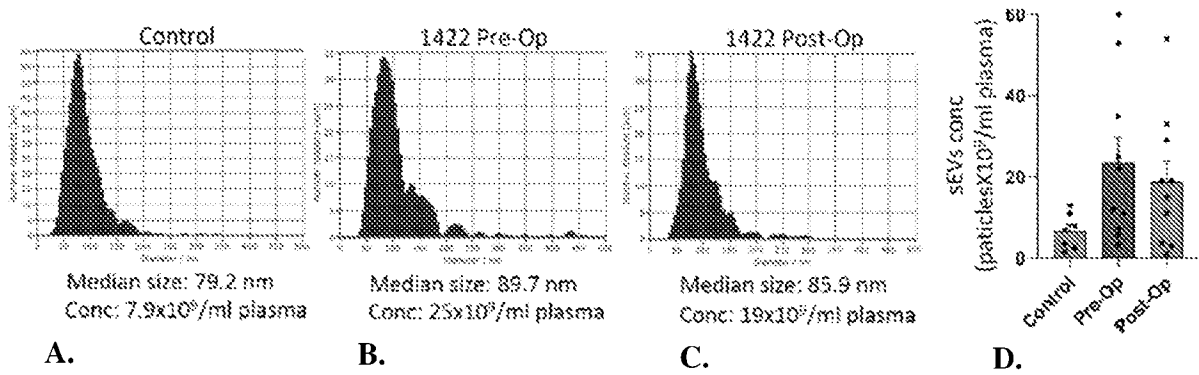


FIGURE 30A-D

Control: 5 healthy donors

Pre-prostatectomy: Pre-Op {1400,1402, 1403, 1411, 1413, 1415}

Post-prostatectomy: Post-Op {1400,1402, 1403, 1411, 1413, 1415}

Dot blot with Equal volume (25ul PPP + 75ul PBS)

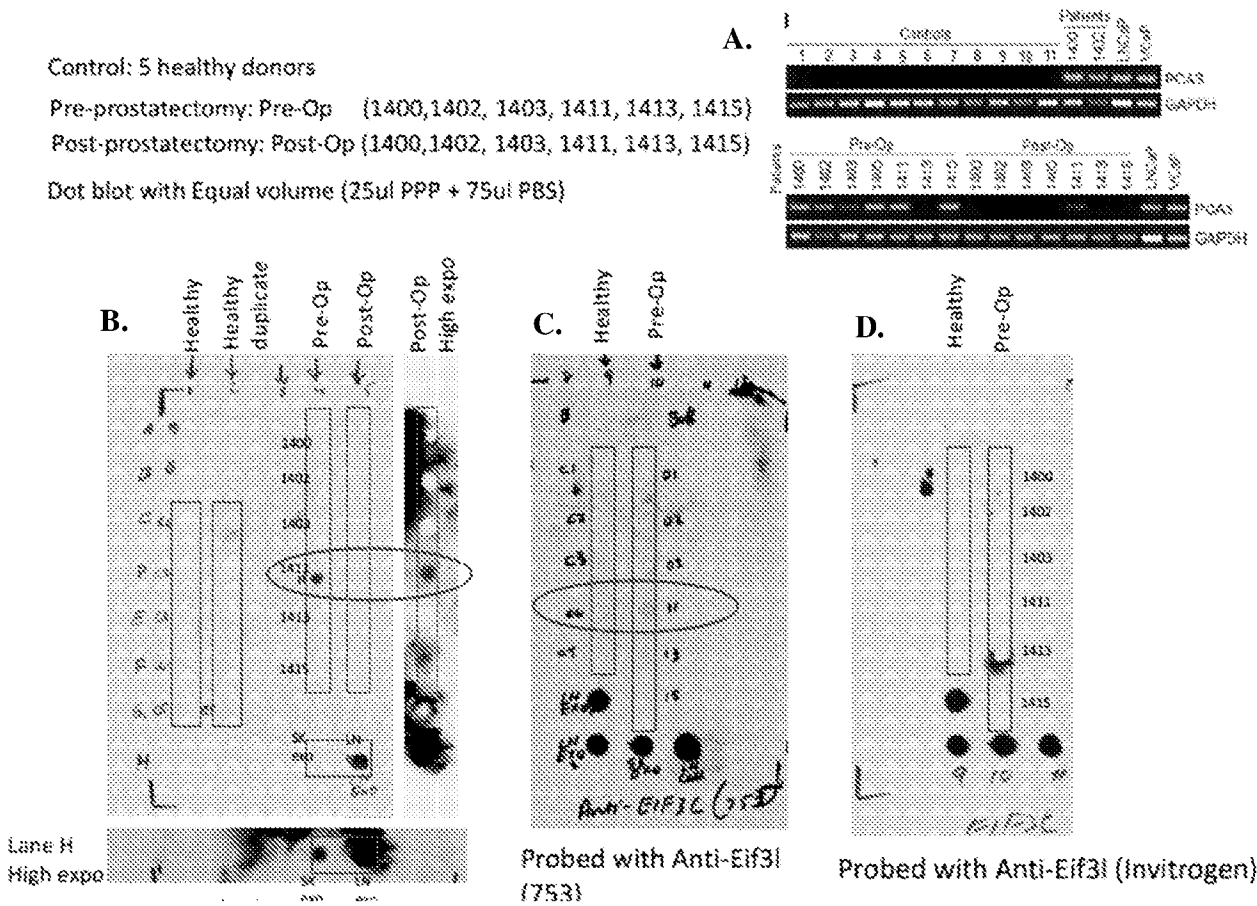


FIGURE 31A-D

Dot blot with Prostate cancer patient plasma samples

Screening of prostate cancer patient plasma with mAb-3A13 and anti-EIF3L

Control: 5 healthy donors

Pre-prostatectomy: Pre-Op {1400,1402,1403,1411,1413,1415}

Post-prostatectomy: Post-Op {1400,1402,1403,1411,1413,1415}

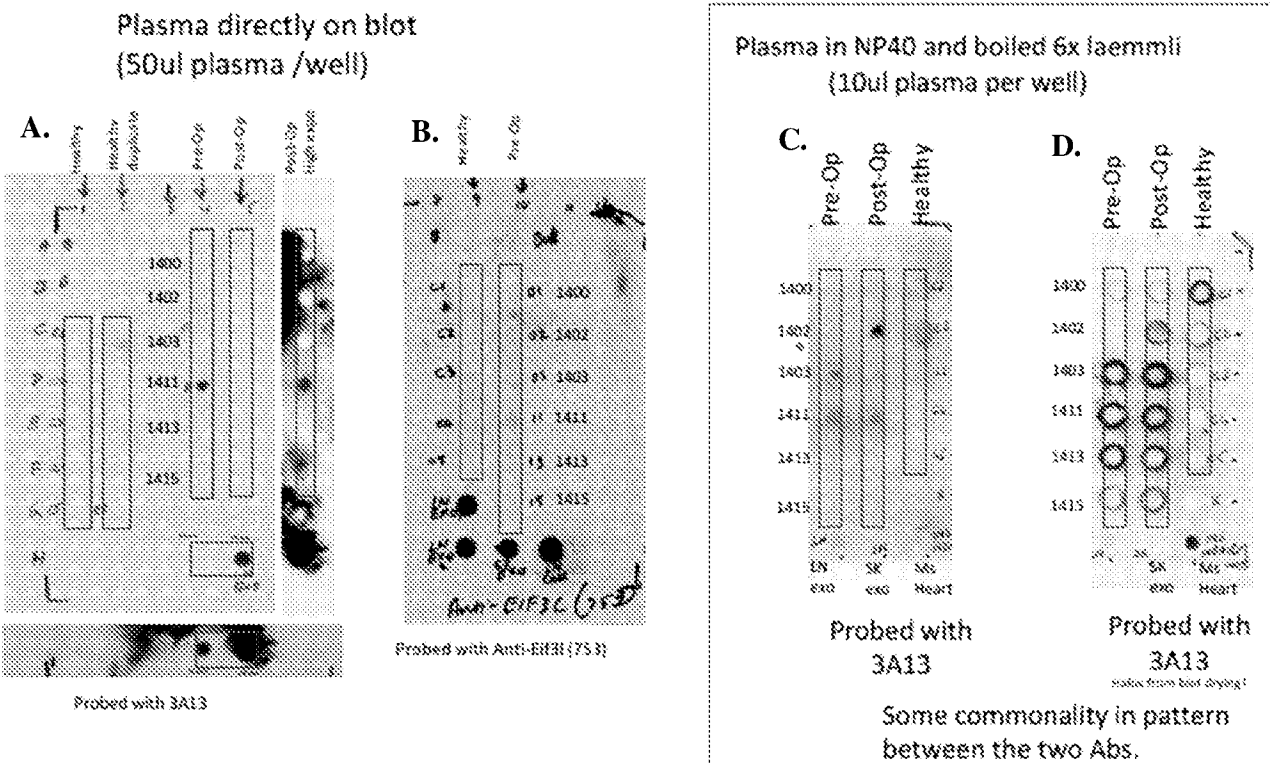


FIGURE 32A-B

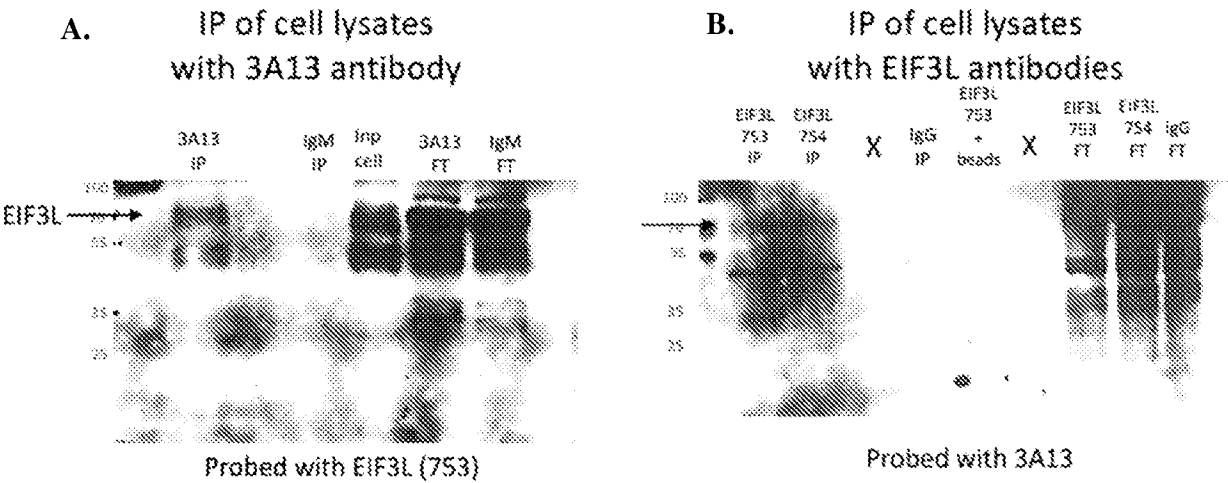


FIGURE 33A-B

