Supplemental Methods

Mice

C57Bl/6 mice were from Charles River (Wilmington, MA, USA). CA>GFPstop>HA-*Uprt* and *Pf4*-Cre mice were from JAX (Bar Harbor, ME, USA). All transgenic animals were backcrossed >10 times onto the C57Bl/6 background and housed in a pathogen-free facility. All animal procedures were approved by the Temple University Institutional Animal Care and Use Committee.

Antibodies, oligonucleotides and reagents

Purified rat anti-mouse CD41 antibody was from BD Biosciences (San Jose, CA, USA). Mouse monoclonal anti-HA antibody was from Biolegend (San Diego, CA). Mouse monoclonal anti-human CD41 antibody was from Thermo (Grand Island, NY, USA). Rabbit anti-von Willebrand Factor antibody was from Abcam (Cambridge, MA, USA). Anti-Ago2, β-actin and cleaved caspase-3 antibodies were from Cell Signaling (Danvers, MA, USA). Fluorophore-conjugated secondary antibodies and pre-immune IgG were from Jackson (West Grove, PA, USA). Acridine orange was from Fisher (Pittsburgh, PA, USA). Molecular biology reagents were from New England Biolabs (Ipswich, MA, USA) except RNase T1 which was from Fisher (Pittsburgh, PA, USA). pCRIMZ plasmid harboring an E2-Crimson cassette was generated by introducing an Nhe1 restriction endonuclease site into pGIPZ (Dharmacon, Lafayette, CO, USA) and replacement of the Turbo GFP cassette with E2-Crimson derived from pEF.myc.ER-E2-Crimson (a gift from Benjamin Glick, Addgene plasmid #38770) using Nhe1/Not1 restriction sites. Oligonucleotides were from IDT (Coralville, IA, USA), except LNA/phosphorothioate oligonucleotides which were from Exiqon (Woburn, MA, USA). Control and FAM-conjugated RISC-free siRNA (siGLO) were from Dharmacon. Antagomir-24 oligonucleotide sequence is 5'-AGTAGGCA. DNA sequencing was provided by Genewiz (South Plainfield, NJ, USA). 4-thiouracil (4TU) was from Sigma Alrdich (St. Lous, MO, USA). Multi-cancer tissue microarray slides were from Protein Biotechnologies (Ramona, CA, USA).

Cell culture and transfections

Lewis lung carcinoma (LLC) and NIH3T3 cells were obtained from American Type Culture Collection (Rockville, MD, USA). MC-38 colon carcinoma cells were a generous gift of Ana Gamero (Lewis Katz School of Medicine at Temple University). Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) with 10% FBS in a humidified, 5% CO_2 atmosphere at 37°C. Cells were transfected using Lipofectamine reagent (Invitrogen) according to the manufacturer's instructions. LLC cells stably expressing E2-Crimson were generated by transfection with pCRIMZ, selected with 2 μg/ml puromycin (MP Biomedicals, Solon, OH, USA), and cloned by FACS-based sorting for E2-Crimson using a 633 nm laser at 1 cell/well. GFP-HRAS(G12V) NIH3T3 cells were generated by transfection and selection in media containing 2 mg/ml G418 sulfate followed by single cell sorting as described[1]. Colonies were sub-cloned after 2 weeks and transgene expression was confirmed by Western blot analysis. For propidium iodide (PI) labeling and flow cytometry, cell suspensions were labeled with PI at 50 μg/ml prior to scanning using a Cy5 emission on a Becton Dickinson LSR II flow cytometer.

Ago2 RISC complex immunoprecipitations and RNA extraction

PMP-treated (16 h) and untreated LLC cells were rinsed in PBS, trypsinized, pelleted and resuspended in PBS, then transferred to a petri dish. Cells in the dishes were subject to crosslinking by exposure to 312 nm light on a Foto/PrepII UV transilluminator (Fotodyne, Hartland, WI, USA) for 2 min, rotated 90°, and then exposed again for 2 min. Cells were collected, resuspended in lysis buffer (10 mm Tris-Cl, pH 7.5, 100 mm NaCl, 2 mm MgOAc, 0.5% Nonidet P-40, and a cocktail of protease inhibitors [Roche]) for 30 min on ice. Insoluble material and ribosomes were removed by ultracentrifugation at 30,000x*g* for 30 min at 4°C. Supernatants were pre-cleared by incubation with Protein G-coupled agarose beads (Roche) for 1 h at 4°C. Cleared lysates were incubated with 2 μg of Argonaute 2 (Ago2) antibodies for 16 h at 4°C, followed by antibody capture on Protein G-agarose beads for 1 h. Bead-bound complexes were pelleted by centrifugation, washed 5x in lysis buffer, resuspended in SDS buffer and separated by SDS–PAGE, followed by transfer to nitrocellulose membranes (see *Western blotting*). Membranes were stained with Ponceau S (Sigma) and the

prominent immunoglobulin bands appearing at 165 kDa were excised. RNA was isolated from the membrane slices via TRIzol extraction as described below.

Tumor cell isolation

Tumors were resected from euthanized mice and separated from fat, skin and connective tissues. Resected tumors were rinsed in PBS, diced with a razor blade, and suspended in PBS containing 1 mg/ml collagenase A for 45 min with gentle agitation at 37°C. The tissue/cell suspension was filtered through a 70-µm strainer. The filtered cell suspension was centrifuged for 5 minutes at 1,300 rpm, the cell pellet was washed once with 0.1% bovine serum albumin (BSA)/PBS, and then incubated with 25 µl anti-PECAM-1 (CD31) antibody-conjugated magnetic beads (Dynabeads) for 60 minutes at room temperature. Bead-bound cells were separated using a magnet (Invitrogen) and discarded to remove blood cells including platelets. Bead separation was repeated 2x. The remaining cell suspension was transferred to tissue culture plates or coverslips and incubated at 37°C, 5% CO_2 for 120 min. Non-adherent cells were removed by aspiration, plates were rinsed 3x in PBS and cells were collected by trypsinization. Cell suspensions were pelleted at 1000x*g*, resuspended in PBS and pelleted again 3x, assayed for platelets using a HEMAVET (samples were washed until platelet counts measured 0), and processed for immunocytochemistry, or cell pellets were stored at -80°C. In some cases, cell suspensions were separated by single cell sorting using a FACS Aria IIµ cell sorter (BD, Franklin Lakes, NJ, USA) equipped with a 633 nm laser, to select E2-Crimson$^+$ cells.

Platelets, platelet transfection, and microparticle generation and isolation

Murine platelets were isolated from blood by exsanguination under anesthesia, as described[2]. Briefly, mouse blood was collected from anesthetized mice into syringes containing 0.1 blood volume of 3.8% sodium citrate as anticoagulant, followed by platelet isolation by centrifugation. Human blood was obtained from a pool of healthy volunteers in a one-sixth volume of acid-citrate-dextrose. Red blood cells were removed by centrifugation at 100x*g* for 30 min at room temperature. Platelet-rich plasma was recovered, and platelets were pelleted at 400x*g* for 10 min at room temperature. The platelet pellet was suspended in Tyrode's buffer (pH 7.4)

containing 1 mg/ml BSA, to a density of 1.5 X 10^8 cells/ml. In some cases platelets were transfected with 2.4 nmol siRNA per 1x10^9 platelets according to the method of Hong et al.[3], using Lipofectamine reagent. Platelet and tumor cell mitochondria were labeled by incubation with 100 nM Mitotracker Red (Thermo Fisher Scientific) for 45 min at 37°C. Microparticle release was induced with 0.1 U/ml thrombin at 37°C for 2 hr with gentle agitation. Reactions were stopped with 10 mM EDTA, and platelets were pelleted by centrifugation twice. Microparticles were harvested from the supernatant by centrifugation at 15,000x*g*, 18°C for 90 min, suspended in cell culture media or PBS, and analyzed before applications by nanoparticle tracking analysis with a NanoSight NS300 fitted with a 488 nm laser (Malvern, Westborough, MA, USA). Filtered media or buffer (0.22 μm filter) was analyzed each time for background subtraction; typical counts were ~10^7 (i.e., ~1/100 of the particle counts in the samples). PMP counts were derived from averaging the final concentrations with background subtracted, for three scans per sample, 30 sec per scan. Addition of 0.1% Triton X-100 caused immediate disappearance of event counts, indicating that the particle counts represented membranous structures (results not shown). In some cases microparticle suspensions were added to cells in culture at a ratio of 2x10^5 microparticles/cell, or as otherwise indicated. A small aliquot of whole blood was used for Nanosight analysis to determine microparticle counts/ml of blood. Approval for this study was obtained from the Institutional Review Board of Temple University. Written informed consent was obtained after the nature and possible consequences of the study were explained.

Immunocytochemistry and immunohistochemistry

Cells were suspended with trypsin, which was inactivated with 2% serum. Cells were washed 3x in PBS and pelleted at 1000x*g* before being resuspended in complete medium. Cell suspensions were seeded on coverslips coated for 16 h with 5 μg/ml human fibronectin, 0.1 M $NaHCO_3$, and maintained for 1 hr. Non-adherent cells were washed away with PBS and coverslips were fixed in either ice-cold acetone for 10 min at -20°C, or 3.7% formaldehyde/PBS for 20 min followed by permeablization with 0.1% Triton X-100/PBS for 5 min, equilibrated in PBS, and processed for immunocytochemical staining. Tissues were fixed in 3.7% paraformaldehyde, embedded in paraffin, and 5 μm sections were collected on charged slides, immersed in

xylene for 5 minutes and rehydrated. Slides were incubated with 0.4% pepsin at 37°C for 20 minutes, washed 4x in TBS, followed in some cases (Figure 5D-E only) by 0.1% Triton X-100, washes, then incubated with primary antibodies at a dilution of 1:200 plus 1:100 dilution normal donkey serum, overnight at 4°C. The next day, sections were washed 4x, then 1 mg/ml solutions of fluorescein isothiocyanate (FITC)-, Cy3- or Cy5-conjugated antibodies were applied at a dilution of 1:100 (FITC) or 1:2000 (Cy3/5), for 1 h at room temperature, washed, and mounted with 30 nM DAPI under coverslips with 80% glycerol/TBS. Images were acquired on a Nikon E800 or a Leica DM IRE2 microscope with a TCS SL confocal system, using a 63x/1.40 NA oil-immersion objective at room temperature using Q-Capture or Leica software, respectively. Post-acquisition image processing was performed using ImageJ and Adobe Photoshop. Operations included brightness/contrast adjustment to all pixels in the images, and grouping of images.

Western blotting

Cell lysates were generated and normalized to total protein levels, and immunoblotting were performed as described[4].

RNA extraction, cDNA synthesis, and PCR

Cells were suspended with trypsin and harvested as above. RNA was extracted using TRIzol reagent according to the manufacturer's instructions, except that 95% EtOH was used for RNA washes to preserve small RNAs. cDNA libraries were constructed using the NCode miRNA First-Strand cDNA Synthesis Kit (Life Technologies, Grand Island, NY, USA) according to the manufacturer's instructions. Conventional PCR was carried out on an Eppendorf thermal cycler using 5' oligonucleotides corresponding to mature miRNA complete sequences as annotated in miRBase[5] and universal 3' primers from the NCode cDNA kit, or as indicated. Real-time PCR (qRT-PCR) analysis was performed using the LightCycler (Roche, Indianapolis, IN, USA) and the FastStart DNA Master SYBR Green I Kit, according to the manufacturer's instructions. RNA from mouse platelets was extracted using mirVana isolation kit (Life Technologies, Grand Island, NY, USA) and qRT-PCR performed using Taqman (Life Technologies) primer/probe sets in an iCycler (Bio-Rad, Hercules, CA, USA).

Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a housekeeping control for qRT-PCR experiments. Primers used for RT-PCR amplification included the following: murine mt-Nd2-F: 5'-ATCCTATCACCCTTGCCATC; murine mt-Nd2-R: 5'-TTGAGGCTGTTGCTTGTGTG; murine Snora75-F: 5'-AGTGATAGATTATGGATTCGCACG; murine Snora75-R: 5'-AATGTCTCACACAACAGCCA; GAPDH-F: 5'-CATGGCCTTCCGTGTTCCTA; GAPDH-R: 5′-CCTGCTTCACCACCTTCTTGAT; human mt-Nd2-F: 5'-CCTGGCCCAACCCGTCATCTACTCTACC; human mt-Nd2-R: 5`GGATGCGGTTGCTTGCGTGAGG; human Snora75-F: 5`-GCTCCTTTCTGTCTATCAGTGGC; human Snora75-R: 5'-GCTAAATATACCTCTGTAAAGACAGAAGG; Pf4-F: 5'-TTCTGCGCCTCACGCCC; Pf4-R: 5'-TGGGACGGACCTGGGAG . Gene expression levels relative to GAPDH were determined using the $2^{-\Delta Ct}$ method and expressed relative to controls.

Subcellular organelle fractionation

Mitochondria, nuclei and nucleoli were isolated from the same cell samples after hypotonic lysis using established protocols[6]. Briefly, cells were lysed in CHM buffer (10 mM Tris-Cl, pH 6.7, 150 mM $MgCl_2$, 10 mM KCl) by dounce homogenization, followed by addition of 1 M sucrose, and nuclei were pelleted at 1000x*g*, 5 min. Nuclei were further purified by resuspending nuclear pellets in Nu buffer 1 (0.25 M sucrose, 10 mM $MgCl_2$), layering the suspension over Nu buffer 2 (0.35 M sucrose, 0.5 mM $MgCl_2$), and pelleting the nuclei at 1430x*g*, 5 min. Nucleoli were extracted by sonicating a fraction of the nuclear pellets, and release of purified free nucleoli was confirmed by examination under a phase contract microscope. The nucleolar suspension was layered over Nu buffer 3 (0.88 M sucrose, 0.5 mM $MgCl_2$) buffer and nucleoli were collected by centrifugation at 3000x*g*, 10 min, resuspension of the nucleolar pellet in Nu buffer 2, and recollection at 1430x*g*, 5 min. Mitochondria were collected from post-nuclear supernatants by centrifugation at 5000x*g*, 10 min in a fixed angle rotor, resuspension of the pellets in 10 mM Tris-Cl, pH 6.7, 150 mM $MgCl_2$, 0.25 M sucrose, dounce homogenization, and repeat centrifugation at 5000x*g*, 10 min. All reagents and samples were maintained at 4°C throughout. Nuclear, nucleolar and mitochondrial purity and integrity in the final fractions were confirmed by examination under a phase contrast microscope.

$\Delta\psi_m$ and ATP production

Mitochondrial membrane potential measurement: Cells were loaded with mitochondrial membrane potential ($\Delta\Psi$m) indicator tetra-methyl rhodamine ethyl ester (TMRM; 50 nM) for 30 min and images were acquired by confocal microscopy using the same illumination and gain settings for all experiments. TMRM fluorescence was quantified using ZEN software.

Cellular ATP measurement: Total cellular ATP levels were assessed using the CellTiter-Glo® luminescent assay kit (Promega). Luminescence was measured using a VICTOR X5 2030 Multilabel reader (Perkin Elmer).

Statistical analysis

Data are presented as means ± s.e.m. Student's *t*-test was used for hypothesis testing in two-group comparisons. ANOVA and post-hoc student's *t*-tests were used for multiple comparisons. A p-value < 0.05 was considered significant. All data are representative of three or more independent experiments.

Table S1. Ambiguous miR-24:target RNA adduct clones.

Clone #	Alignment	Genetic region	miRNA segment	Target / adduct segment	poly(A) segment
1	multiple genes	5' and 3' UTRs	GTGCCTACTGAGCTGATATCAGT	TCCCAACACCCATATCATGCAGCTCATAACCACT	AAAAAAAAAAAAAAAAAACA
2	multiple genes	5' and 3' UTRs	GTGCCTACTGAGCTGATATCAGT	AGTCATGCCAATGTTTGATTNAGTAGAGTTCAGTGATGGCAGT	AAAAAAAAAAAA
5	multiple genes	5' and 3' UTRs	GTGCCTACTGAGCTGATATCAGT	TTGTCCTATTTTAC	AAAAAAAAAAAAAAAAAA
7	None	n.a.	GTGCCTACTGAGCTGATATCAGT	GTTGTGCTGTGCTGTCTGTGCTGTACTGTCTGTGCTGTCTGTGCT GTGCTGGCTGC	AAAAAAAAAAAAAAAAAA
9	None	n.a.	GTGCCTACTGAGCTGATATCAGT	NNNTGG	AAAAAAAAAAAAAAAAAAAA
10	None	n.a.	GTGCCTACTGAGCTGATATCAGT	NNNTGG	AAAAAAAAAAAAAAAAAAAA
11	multiple genes	CR and 3' UTRs	GTGCCTACTGAGCTGATATCAGT	CAGGGGCAGAGC	AAAAAAAAAAAAAAAA
13	None	n.a.	GTGCCTACTGAGCTGATATCAGT	CTGTGCTGTGCTGTGGTGTCTGTGCTGCGCTGTGGTGTCTGTGC TGTGCTGTGC	AAAAAAAAAAAAAAAAAAAA
16	Ch.8, 6267392 – 6267432	Unknown	GTGCCTACTGAGCTGATATCAGT	CGCCTTTGTTCTCCTGGTGTCCTCCCTTCTCTCTGTGTCCTTAG	AAAAAAAAAAAAAAAA
17	multiple genes	5' and 3' UTRs	GTGCCTACTGAGCTGATATCAGT	GATAGATTATGGATTCGCACGCAG	AAAAAAAAAAAAAAAAAAA
19	None	n.a.	GTGCCTACTGAGCTGATATCAGT	CTGTCTGTGCTGTGCTGTG	AAAAAAAAAAAAAAAAAAA
20	Ch. 14, 55837943 – 55838023	unknown	GTGCCTACTGAGCTGATATCAGT	GGTGTGTCCATCAAACAGGGTTTGGCTTCATACACTTTAATCCGC AGACACAACCCTTGCTCTTGTCTCTATCTTTTTCCC	AAAAAAAAAAAAAAAAAAA
22*	*Psmd14*¶	3' UTR	GTGCCTACTGAGCTGATATCAGT	AGCACAAATATATTTTGATTGTTGCTTACAAAATAAAATACATTTAC AATCC	AAAAAAAAAAGAAAAAAAAAAAA AAAAAAAA
24	multiple genes	5' and 3' UTRs	GTGCCTACTGAGCTGATATCAGT	TCATCTTTGATAGGTTTAACAT	AAAAAAAAAAAAAAAAAAAA
26	None		GTGCCTACTGAGCTGATATCAGT	TCTTCCCGC	AAAAAAAAAAAAAAAAAAAA
29	None	n.a.	GTGCCTACTGAGCTGATATCAGT	AGT	AAAAAAAAAAAAAAAAAAAACA
30	Ch. 17. 13552358 – 13552427	Unknown	GTGCCTACTGAGCTGATATCAGT	GCTGTCTGTGCTGTGCTGTGCTGTGCTGTGCTGTCTGTGCTGTG CTGTGCTGTGCTGTGCTGTGCTGTCTGG	AAAAAAAAAAGAAAAAAAAA
31	None	n.a.	GTGCCTACTGAGCTGATATCAGT	AAACTTTATTTC	AAACAAAAAAAAAAAAAAAAAA
35	Ch. 8, 68363370-68353386	unknown	GTGCCTACTGAGCTGATATCAGT	TGGGGGGATTTAGGTTT	AAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAA
38	multiple genes	5' and 3' UTRs	GTGCCTACTGAGCTGATATCAGT	TTAAGTATCAGGTTAATTT	AAAAAAAAAAAAAAAAAAA
39	Ch. 13, 92139951-92139975	unknown	GTGCCTACTGAGCTGATATCAGT	GTCAAGTTCCTCTGGTCTTGCCTTT	AAAAAAAAAAAAAAAA
40	None	n.a.	GTGCCTACTGAGCTGATATCAGT	AATTACT	AAAAAAAAAAAAAAAAAA
48	Ch. 8, 116742435-116741503	unknown	TTGCCTACTGAGCTGATATCAGT	TTCAGCCATCACATTTATTGCCATGTGTTATGGCAACCACAGGCA ACTAGCATACCATCACCTGGGAGGCC	AAAAAAAAAAAAAAAAAA
50	Ch. 8, 26090798-26090871	unknown	GTGCCTACTGAGCTGATATCAGT	ATTTCCTGGTGAGAAACCCTGGCTCAGATTCTCTCTACAGTCCTC TTTTCTCTCTTTGCTATCCTTTGGTTTTTTTG	AAAAAAAAAAAAAAAAAA
51	multiple genes	3' UTRs, CR, lncRNA	GTGCCTACTGAGCTGATATCAGT	TCTTCTGACTTG	AAAAAAAAAAAAAAAAAA
56	None	n.a.	GTGCCTACTGAGCTGATATCAGT	GCTGTGCTGTGCTGTCTGTGCTGTGC	AAAAGAAAAAAAAAAAAA

TA clones with inserts are shown for all clones not included in Figure 6B. The insert sequences are separated in the table into the apparent miRNA segment, the cognate target/adduct segment, and the poly(A) segment. Upon bacterial transformation, samples derived from untreated cells yielded no colonies, whereas those from PMP-treated cells yielded hundreds of colonies (not shown), indicating that the miR-24 PCR priming template in the TCs was from PMPs. We sequenced plasmid DNA from 56 colonies. 41 (73%) contained sequences corresponding to the mature miR-24-1-5p and a poly(dA) tail. Of those, 30 (73%) also

showed “insert” sequences between these segments, indicating *in vitro* chimerization of cognate target RNAs to miR-24. Inserts ranged from 12-96 nt, with an average length of 33.5 ± 4.9 nt, excluding one clone containing a 281-nt insert which corresponded to *mt-Nd2* (see Figure 6B). The unusually long *mt-Nd2* adduct sequence in the miRNA:target cloning scheme may have resulted from RNase protection afforded by the mitochondrial matrix, or coupling of an Ago2/P-body/miRNA:mRNA complex with mitochondrial membranes[80]. Interestingly, few inserts harbored sequences representing the exact complement of the miR-24 7-mer seed sequence (GTAGGCA), considered the primary determinant for miRNA hybridization to target mRNAs[7]. However, in most inserts, 1- or 2-nt variations could be construed. Similar results were observed with clones using miR-223 and miR-191 5’ primers, although in some cases exact seed complements were present (not shown). These observations suggest that there may be flexibility in seed complementarity for RNA targets of miRNAs in these cells, as has been observed in other systems[8].

[a] The adduct “insert” sequence in this clone (#22) is immediately 5’ to the poly(A) tail in the matching mRNA, and is preceded by a 6-mer identical to the 3’ end of miR-24. Hence, this clone was considered to have arisen from non-specific priming during the PCR reaction. qRT-PCR indicated no change in this mRNA in the presence of PMPs (not shown).

Supplementary Figures

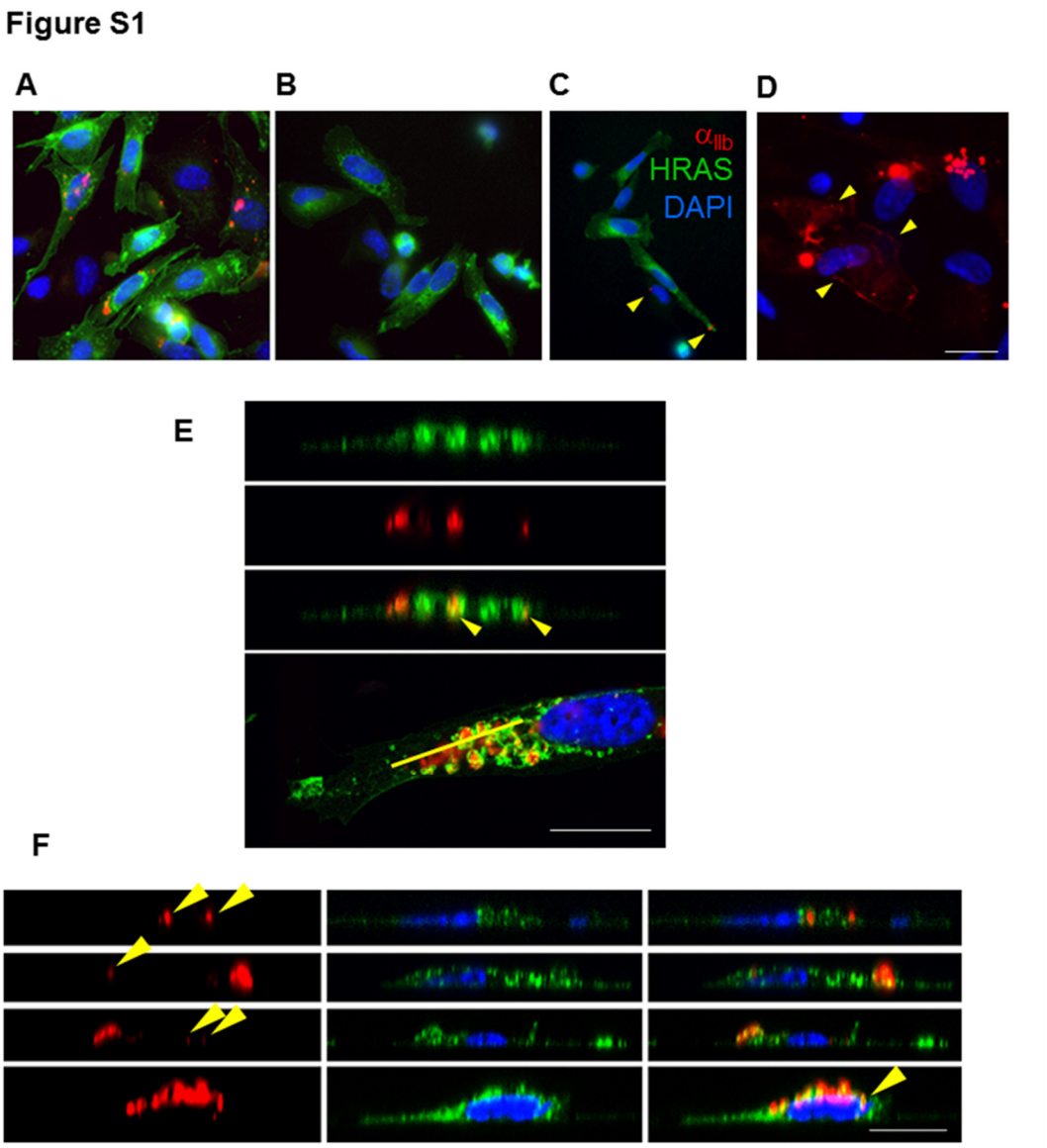

Figure S1. PMPs anchored to HRAS-transformed tumor cell surfaces and cargo internalization in solid tumor cells *in vivo*. (A) GFP-HRAS(G12V)-transformed tumor cells captured from allograft tumors resected and collagenase-digested at 21 d, stained with α_{IIb} integrin antibodies. (B) Cells from a 7 d tumor. (C) Tumor cells (21 d), 24 h after immune-induced thrombocytopenia (80% platelet depletion). PMPs indicated with arrowheads. (D) Cells from the same samples in (A), GFP omitted for clarity. Yellow arrowheads indicate α_{IIb} integrin at the plasma membrane. (E) confocal x-y and (F) z-plane scans of cells from (A). Arrowheads indicate examples of α_{IIb} integrin in recycling endosomes. Yellow line indicates plane of z-section. GFP-Ras, green; α_{IIb} integrin, red; DAPI, blue. Merged images are shown to the bottom in (A) and to the right in (B). Bars, 10 µm.

Figure S2

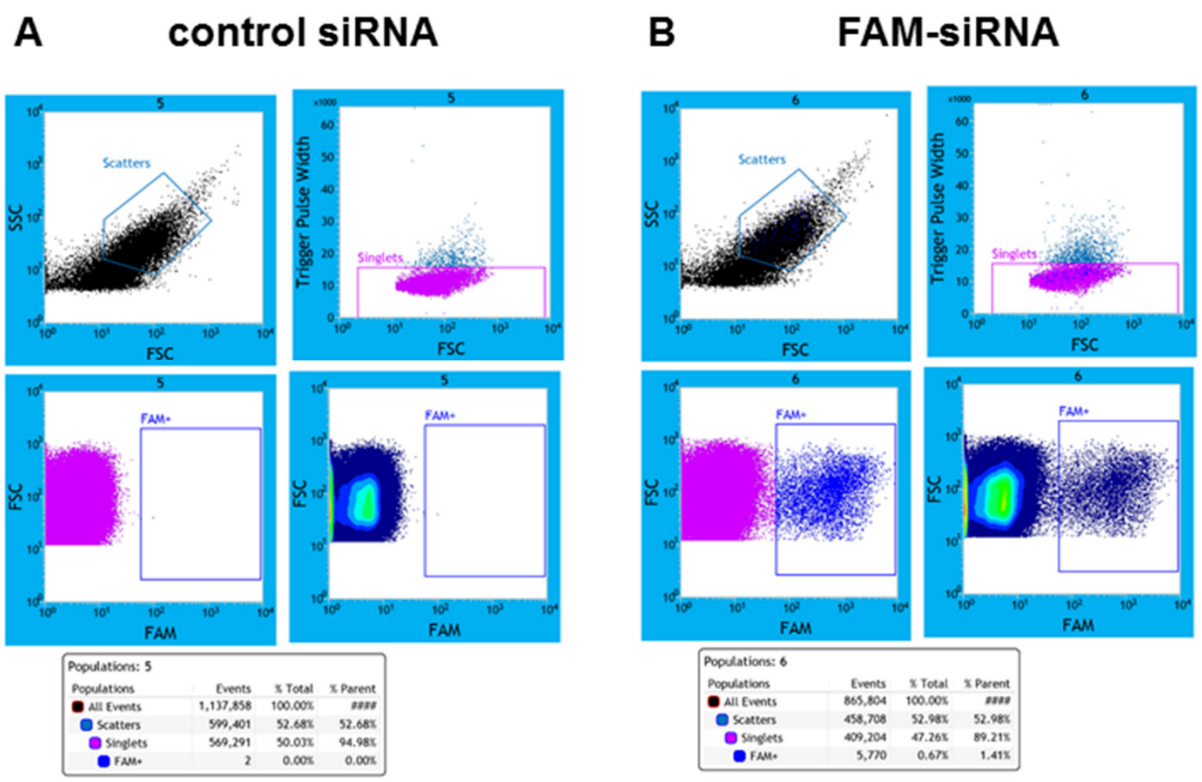

Figure S2. Transfection of human platelets with FAM-labeled siRNA. 1x10^9 freshly isolated, washed human platelets were transfected with 2 nmol unlabeled (A) or FAM-siGLO (B) RISC-free siRNA, as described in **Supplemental Methods**. Prior to transfusion, a sample of the transfected platelets was set aside for FACS analysis for FAM fluorescence. Representative intensity plots are shown, log-scale. SSC, side scatter; FSC, forward scatter. 2.2 ± 0.8% of platelets showed FAM-siRNA transfection by this method and the whole platelet sample was used for transfusion experiments.

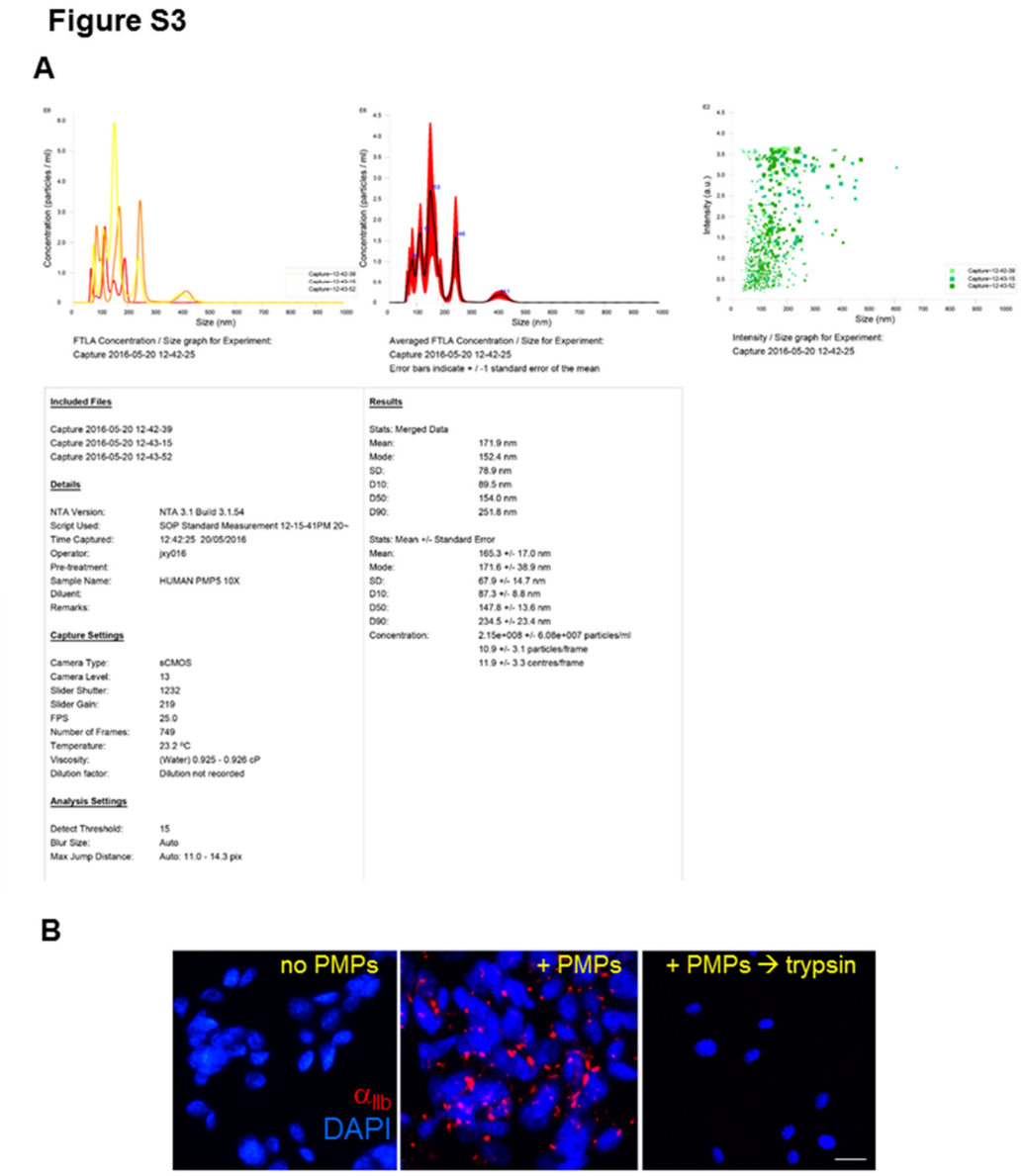

Figure S3. Characterization of collected PMPs and PMP anchorage to tumor cells and enzymatic removal of surface PMPs *in vitro*. (A) Representative nanoparticle tracking analyses from a 1:10 dilution of PMPs collected as described in **Supplemental Methods**. Shown are individual tracings for 3 replicates per sample (left panel), plots of average concentrations ± s.e.m. in red (center panel), and size and particle intensity distributions for all 3 replicates (right panel). (B) PMPs from induced platelets from freshly drawn human blood were collected by centrifugation and co-incubated with sub-confluent cultures of LLC cells seeded on coverslips, for 1 h (+ PMPs). Cells were trypsinized (+ PMPs → trypsin), pelleted at 1000x*g* and washed with PBS 3x, and re-seeded on coverslips, fixed with acetone with no permeablization, and stained. α_{IIb} integrin, red; DAPI, blue. Bar, 10 μm.

Figure S4

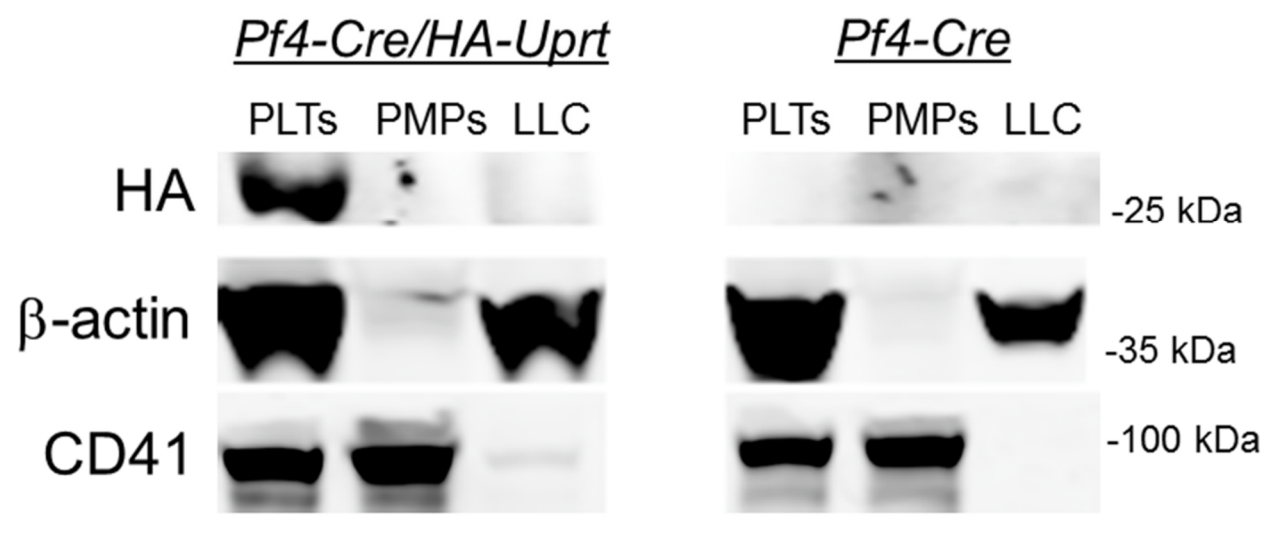

Figure S4. HA-UPRT protein does not transfer from platelets to tumor cells *in vivo*. E2-Crimson-LLC tumors were seeded in the flanks of *Pf4-Cre/HA-Uprt* mice or *Pf4-Cre* mice and allowed to grow for 21 days. Mice were exsanguinated and platelets (PLTs) were collected either for *ex vivo* generation of PMPs by thrombin/stirring, or for direct lysis for western blots. LLC tumor cells were isolated by FACS and lysed along with PMPs in RIPA buffer, and protein samples were subjected to non-reducing SDS-PAGE followed by western blotting with the indicated antibodies.

Figure S5

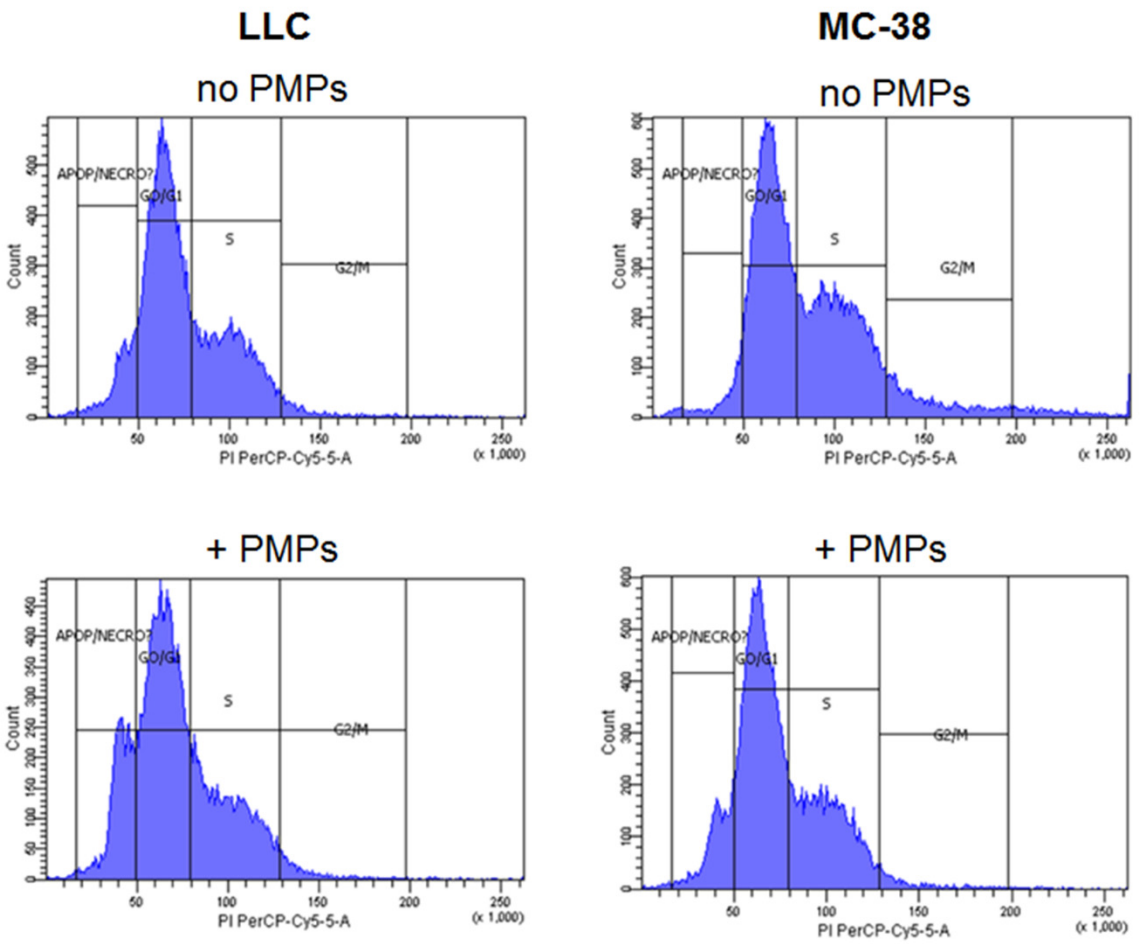

Figure S5. Cell cycle analysis of LLC and MC-38 cells exposed to PMPs *in vitro*. LLC or MC-38 cells grown in culture were treated with isolated PMPs as in Figure 4 (A-F). Cells were collected with trypsin and labeled with propidium iodide (PI), and singlet cells were analyzed by flow cytometry for PI staining. Cell cycle phases based on PI profiles are indicated with vertical lines. Representative of 3 independent experiments.

Figure S6

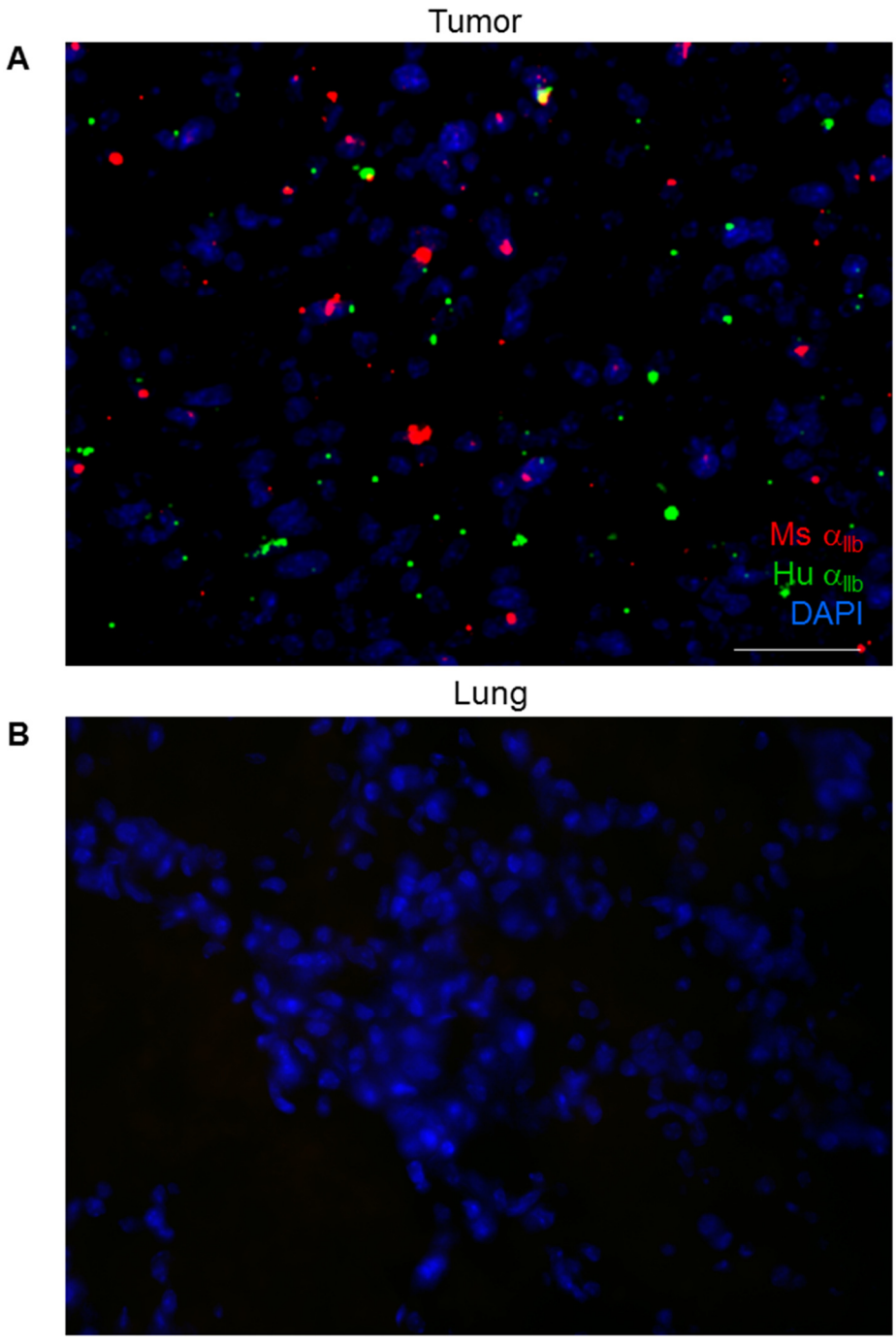

Figure S6. Tumor infiltration of endogenous and transfused PMPs. (A) LLC tumors resected from mice transfused daily with PMPs as in Figure 4, were processed for IHC with antibodies to human α_{IIb} integrin (green) and mouse α_{IIb} integrin (red). DAPI staining is shown in blue. (B) Lung tissue from the same mouse in (A), stained with the same antibodies. Bar for both images, 25 μm.

Figure S7

Figure S7. TA clone alignments and miR-24 seed sequence matches in *mt-Nd2* and *Snora75*. Match sequences were from PubMed. TA clone insert sequences are underlined, START codon in green, STOP codon in blue, and putative 1- or 2-nt variant miR-24 seed complementary sequences are shown in red. An exact 7-nt seed match to a putative isomiR of miR-24 is italicized.

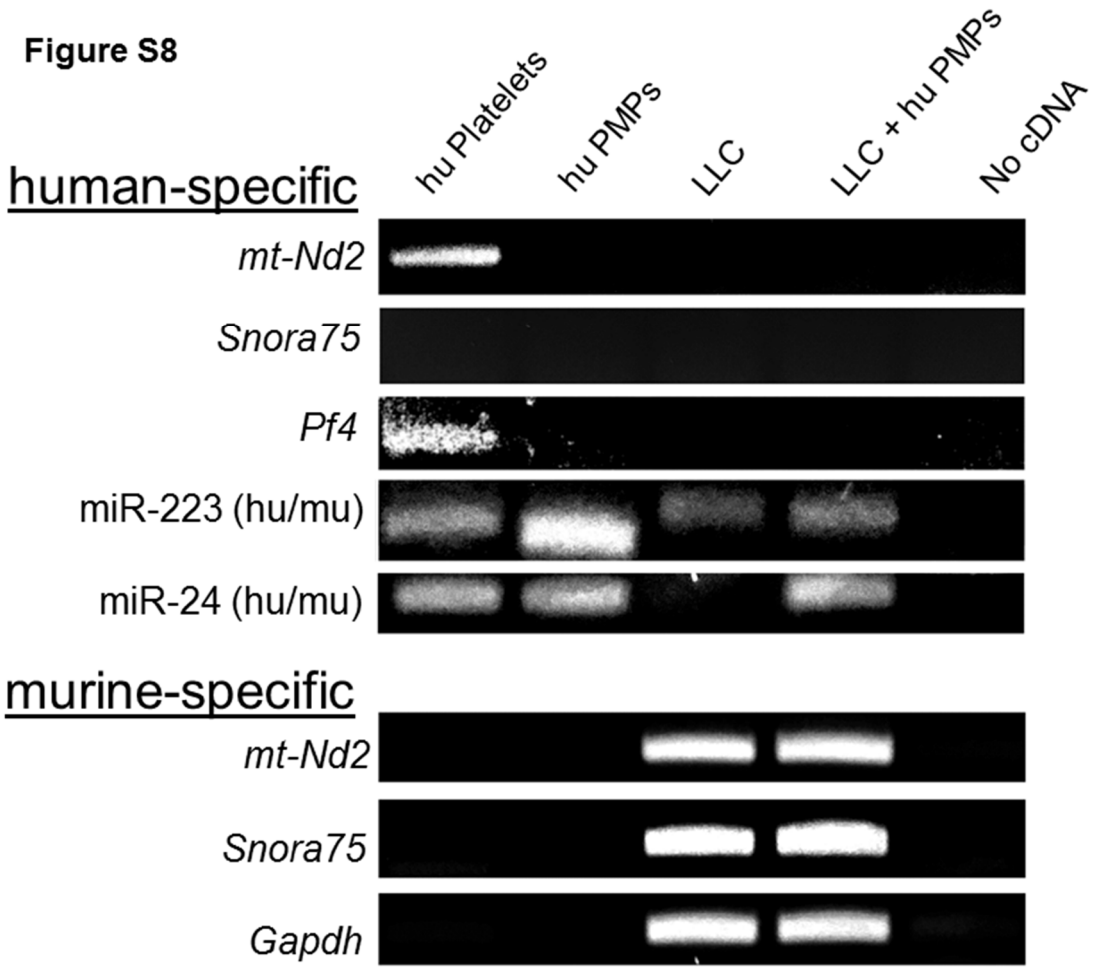

Figure S8. *Mt-Nd2* and *Snora75* RNA transcripts do not transfer from platelets to tumor cells via PMPs. LLC cells (murine, mu) grown in culture were exposed to isolated PMPs derived from human *ex vivo* (hu) platelets by thrombin stimulation under stirring conditions as described in the Methods. RNA was extracted from each fraction and converted to cDNA with poly(dT) primers after RNA poly(dA) tailing, then subject to PCR using species-specific primers for each transcript as indicated. miRNA PCRs used 5'-miR-specific forward primers as indicated and 3'-poly(dA) reverse primer. Platelet RNA was prepared from un-stimulated platelets. "No cDNA" PCR reactions included the indicated gene-specific primers in each case.

Figure S9

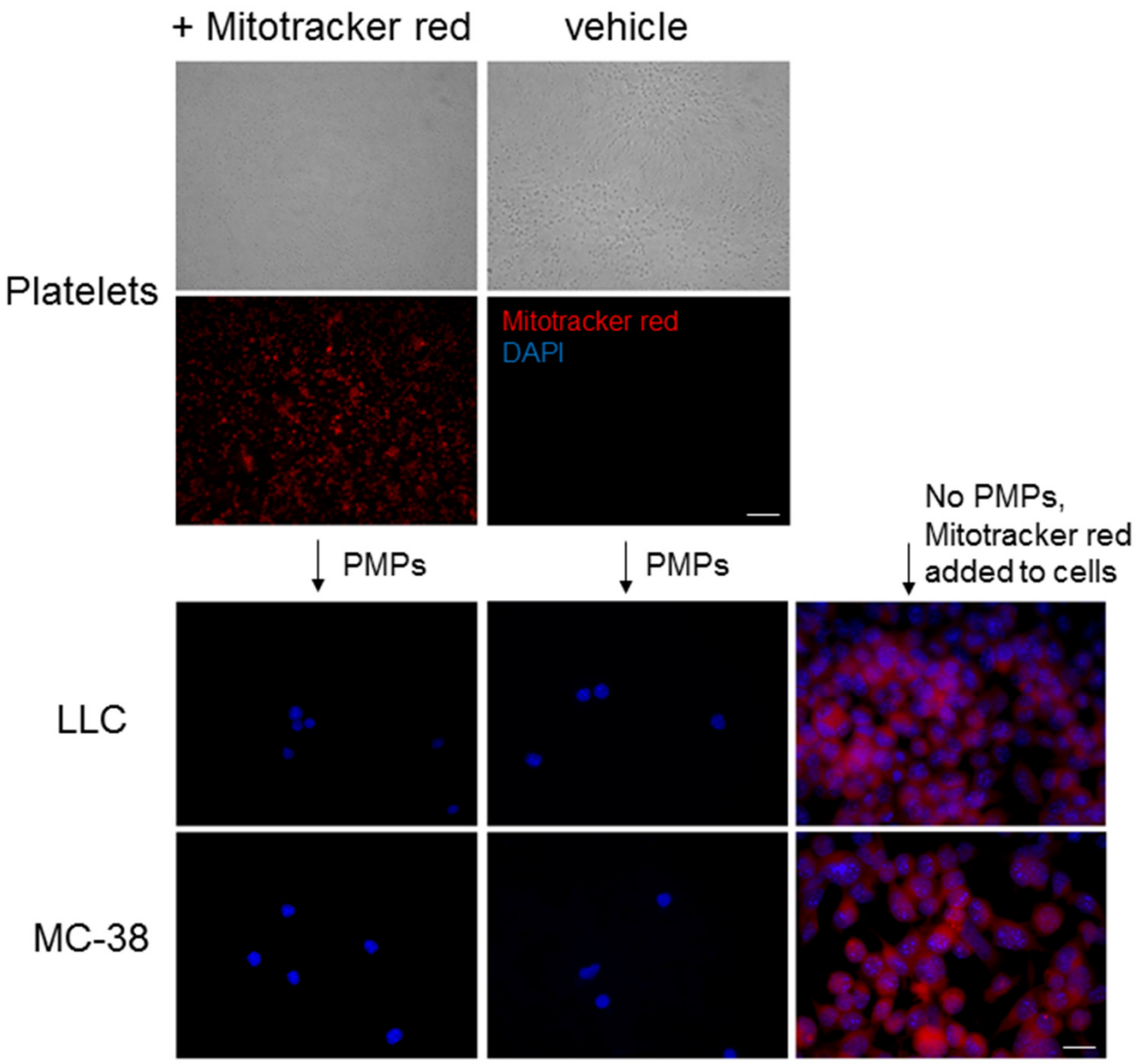

Figure S9. Platelet-derived mitochondria are not transferred to cancer cells from PMPs. Freshly isolated human washed platelets were treated with Mitotracker Red to label mitochondria, or vehicle, as described in Methods. The top panels show immobilized platelets after Mitotracker red treatment, captured on fibronectin-coated coverslips (top, brighfield, bottom, 647 nm, red). PMPs were generated from the treated platelets kept in suspension, and incubated for 16 hrs with LLC or MC-38 cells seeded on coverslips, and platelets and tumor cells were imaged for Mitotracker red (647 nm, red) and DAPI (blue). Bottom right panels show LLC and MC-38 cells treated directly with Mitotracker red without PMP exposure. Bars, 10 μm.

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