Supporting Information

Christianson et al. 10.1073/pnas.1424994112

SI Materials and Methods

Cell Lines. Primary LECs isolated from afferent LyVs were provided by J.E.G. The primary LECs were maintained in endothelial cell growth medium microvascular (EGM MV) media (Lonza) supplemented with 10% (vol/vol) FBS, penicillin, and streptomycin. C8161, WM2664, and MeWo (ATCC) were maintained in MEM (Invitrogen) supplemented with 10% (vol/vol) heat-inactivated FBS, 1% sodium pyruvate, 1% nonessential amino acids, 1% vitamins, and 1 mM penicillin, streptomycin, and glutamine (GIBCO). The NCI60 cell lines LOX IMVI, MALME-3M, MDA-MB-435, M14, SK Mel 5, SK Mel 2, SK Mel 28, UACC 62, and UACC 257 were maintained in RPMI (Sigma) supplemented with 10% (vol/vol) FBS, penicillin, streptomycin, and glutamine. A357 and A2058 (ATCC) were maintained in DMEM supplemented with 10% (vol/vol) FBS, penicillin, and streptomycin. Human umbilical vein endothelial cells, HDLECs, and normal human melanocytes were obtained from PromoCell and maintained according to the company's instructions.

Lymphatic Tissue. Human afferent LyV tissue segments were obtained from residual tissue obtained during sentinel LN biopsy procedures performed by J.E.G. These tissue samples were selected from melanoma patients with antecedents of malignant melanoma undergoing sentinel LN dissection as a diagnostic/prognostic procedure. Immediately after surgical resection, the LyV was microscopically separated from surrounding tissue.

Ex Vivo Lymphatic Endothelial Vessel Screening. After isolated, afferent LyV tissue segments were cleaned of surrounding connective tissue, and each end of the vessel was cannulated centripetally with a sterile micropipette tip to ensure that only the lumen of the vessel would be exposed for screening with the phage library. Warm saline was used to keep the vessels from drying out during the procedure. The phage CX_7C peptide library [10^9 transducing units (T.U.)] was diluted in DMEM, gently introduced into the lumen of the LyV, and allowed to incubate for 15 min. After incubation, the lumen of the vessel was gently flushed with DMEM to remove unbound phage particles. LyVs were frozen at –80 °C until processed for phage recovery. Frozen vessels were thawed on ice and then weighed. Bound phages were recovered separately for each of the vessels by homogenizing the vessel in DMEM containing 1% BSA and protease inhibitors in a glass homogenizer (VWR). The homogenate was centrifuged to pellet the phage-bound tissue, and phages were recovered using bacterial infection. Bacterial colonies were counted the following day to determine the number of phages recovered per gram of tissue. Colonies from the second (271) and third (359) rounds were picked, PCR-amplified, and sequenced. The phage cultures grown overnight were each purified using the procedure described above. This pool was used for the subsequent round of screening.

Abs. Anti-PPP2R1A (A300-963A) was purchased from Bethyl Laboratories. Phycoerythrin (PE) anti-human podoplanin Ab (337004) was acquired from BioLegend, and allophycocyanin (APC) anti-human melanoma Ab (130–091-252) was purchased from Miltenyi Biotec Inc. HRP anti-Actin (sc-1616 HRP; Santa Cruz Biotechnology) was used for loading control detection.

Peptides. Synthetic peptides were chemically synthesized, cyclized by cysteines, and HPLC-purified to >99% purity by PolyPeptide Laboratories.

Phage Particle Production. A filamentous bacteriophage (phage) randomized 7-aa library flanked by cysteine residues for cyclization (CX_7C; C indicates cysteine, and X indicates any amino acid residue) was used for the library screenings. Phage particles were propagated, and phage titers were calculated by bacterial infectivity as described previously (1).

PCR and Sequencing. Colonies from all screenings were transferred into 30 µL 10% (vol/vol) glycerol and stored at −20 °C. The PCR mixture contained 2 µL bacteria/glycerol mixture, 8 pmol forward and reverse primers for fUSE5 (5′-TAATACGACTCACTATAGGGCAAGCTGATAAACCGATACAATT-3′ and 5-CCCTCATAGTTAGCGTAACGATCT-3, respectively), 1 µL 2.5 mM deoxynucleotide triphosphate mix (Promega), 2 µL 10× Taq polymerase buffer (Promega), and 2.5 units Taq DNA polymerase. The PCR conditions were as follows: 94 °C for 3 min and 30 cycles of 94 °C for 10 s, 60 °C for 30 s, and 72 °C for 30 s followed by 72 °C for 5 min. DNA was sequenced by The University of Texas MD Anderson Cancer Center Sequencing Core Facility.

LEC Screening. LECs were isolated as primary cultures from similar afferent LyV tissue. Collagenase II was introduced into the lumen to detach and remove the LECs. Cultures at passages III–VI were provided for the phage library screenings; 0.25% trypsin and 1 mM EDTA were used to detach the strongly adherent cultured LECs. The cells were then placed in a new flask containing fresh EGM MV medium, which was placed on a rocker for 6.5 h under normal growth conditions to allow for receptor regeneration. The cells were pelleted, washed with DMEM, and then, resuspended in DMEM containing 1% BSA at a concentration of $3–10 \times 10^5$ cells/200 µL. The Biopanning and Rapid Analysis of Selective Interactive Ligands approach was used (2) for the phage library screenings. The recovered phage library was added (10^9 T.U.) to the cells, incubated on ice for 4 h, and processed as described (1).

Phage Binding Assay. The Biopanning and Rapid Analysis of Selective Interactive Ligands method (2) was used to validate single-phage clones. The number of colonies formed relative to controls determines specific binding.

Peptide Sequence Alignment and Bioinformatics. Sequence alignment of peptides was performed with ClustalW software tools (www.ebi.ac.uk/clustalw/). Peptide sequence analysis and character pattern recognition were performed with an automated analysis program using SAS (version 8.1.2; SAS Institute) and Perl (version 5.8.1).

Polyclonal Antipeptide Serum. The GLTFKSL peptide was synthesized, conjugated to Keyhole limpet hemocyanin, and used to immunize New Zealand White rabbits for the generation of polyclonal Abs (Genemed Synthesis). In brief, the rabbits were immunized with 200 µg Keyhole limpet hemocyanin-conjugated peptide in Freund's complete adjuvant and boosted every 15 d with 100 µg. Preimmunized serum was collected before the first immunization and at 2-wk intervals after immunization.

Affinity Purification. GLTFKSL peptide was coupled to a column using the Carboxyl-Link Kit (Pierce) following the manufacturer's instructions. The peptide column was first equilibrated with 20 mL of Tris-buffered saline (TBS; 0.3 M NaCl, 20 mM Tris/HCl) containing 0.01% Tween-20 and 0.01% NaN_3 (T-TBS sample

buffer). The serum was diluted with sample buffer by adding 1 mL T-TBS and 0.55 mL 4 M NaCl to every 10 mL serum. The serum was then filtered through a 0.45-μm filter and batch-cycled by gravity through the column using silicone tubing connected to a Pharmacia Biotech Pump P-1 for 4 h. The column was then washed with 20 mL T-TBS containing 0.5 M NaCl until the absorbance of the flow-through was <0.02 (Aset). Bound Ab was then eluted from the column with 0.1 M glycine (pH 2.8) and collected as 1-mL fractions into 50 μL neutralization buffer (1 M Tris, pH 8.0). Each fraction was dialyzed using Slide-A-Lyzer (10,000 molecular weight cutoff; Thermo Scientific). Similarly, as a negative control, the preimmunized serum was run on the column to collect nonspecific binding Abs. IgG concentration was determined by the absorbance at 280 nm divided by the extinction coefficient factor of 1.4.

Immunofluorescence. Cells were seeded at 5×10^4 cells/well in an eight-chamber slide (Lab-Tek II; Nalge Nunc International) and allowed to adhere overnight at 37 °C. The cells were washed three times with phosphate-buffered saline (PBS) and fixed in 4% (vol/vol) paraformaldehyde (PFA, Electron Microscopy Science) for 15 min at room temperature (RT) followed by three PBS washes. For intracellular staining, the cells were permeabilized with 0.2% Triton X-100 for 10 min, washed three times with PBS, and then blocked with PBS containing 5% (vol/vol) normal goat serum (or normal serum corresponding to the species of the secondary Ab used) and 1% BSA for 30 min. Primary Abs were diluted in 1% BSA, incubated with cells for 2 h at RT, washed five times with PBS, and then, incubated with secondary Abs for 1 h at RT. For nuclear identification, VECTASHIELD mounting medium containing 4′,6-diamidino-2-phenylindole (DAPI, Vector Laboratories) was used. Fluorescence images were acquired on an Olympus fluorescence microscope.

Transmission EM. For EM, cells were fixed in 2% PFA and 0.1% glutaraldehyde in PBS (pH 7.3) for 1 h at RT followed by three 5-min washes with PBS and a 15-min incubation with 0.1 M sodium borohydride to quench the aldehydes. The samples were again washed three times for 5 min with PBS, and for intracellular staining samples, PBS containing 0.2% Triton X-100 was added for 15 min and then followed by three PBS washes. Each sample was then blocked with 2% (vol/vol) normal goat serum for 15 min. Primary Abs (anti-PPP2R1A and anti-GLTFKSL; 5 μg/mL) were added and incubated overnight at 4 °C. The next day, samples were washed three times for 10 min each with PBS, and an 18-nm gold-labeled anti-rabbit IgG (1:20; Jackson ImmunoResearch Laboratories) was added to the samples and incubated overnight at 4 °C. Each well was then washed three times for 10 min with PBS, fixed, and stored in 2% (vol/vol) gluteraldehyde. Samples were subsequently processed by the High-Resolution Electron Microscopy Facility at The University of Texas MD Anderson Cancer Center for EM. Samples were postfixed in 1% buffered osmium tetroxide for 1 h and stained with 1% Millipore-filtered uranyl acetate. The samples were dehydrated with increasing concentrations of ethanol, infiltrated, and embedded in Spurr's low-viscosity medium. Polymerization followed in a 70 °C oven for 2 d. The polymerized blocks were removed from the glass by dipping them in liquid nitrogen, and then, ultrathin sections were cut in a Leica Ultracut Microtome (Leica). The sections were stained with uranyl acetate and lead acetate in a Leica EM Stainer, and images were acquired with a JEN 1010 transmission electron microscope (JEOL).

Protein Microarray. The protein array used to identify the GLTFKSL mimic protein was designed and performed as previously described (3). In brief, cDNA was reverse-transcribed from RNA prepared from fetal brain tissue and cloned into the expression vector pQE30NST (Strategene). These arrays were provided by the German Resource Center for Genome Research. Protein expression and purification yielded 37,200 individual protein-expressing clones. These clones were spotted in duplicate onto PVDF membranes and processed in situ to express the proteins. The proteins were then directly immobilized onto PVDF sheets after bacteria lysis. Before screening, the desiccated bacteria were washed from the arrays. Each sheet contained up to 27,000 proteins. The sheets were blocked for 2 h in TBS containing 0.1% Tween-20 and 3% (wt/vol) nonfat dry milk and then incubated with the serum samples diluted (1:1,000) in TBS Tween-20 (0.1%) (TBST) containing 2% (wt/vol) nonfat milk for 16 h. The membranes were then washed with TBST three times for 10 min each and incubated with alkaline phosphatase-labeled anti-rabbit IgG (1:5,000; Sigma-Aldrich) diluted in 2% (wt/vol) nonfat milk and TBST for 1 h. After three 10-min washes in TBST, the membranes were washed for an additional 10 min in alkaline phosphatase (AP) buffer (1 mM $MgCl_2$, 0.1 M Tris, pH 9.5) followed by a 5-min incubation with 25 mM Attophos (Promega) in AP buffer. Images were obtained with a high-resolution Fuji Las-3000 CCD Imager and analyzed with VisualGrid (GPC-Biotech).

Protein ELISA. Recombinant human PPP2R1A protein (Novus Biologicals) was coated (500 ng/well) in triplicate in a 96-well plate (Maxi Sorp; Nalge Nunc) overnight at 4 °C. Negative coating controls were 3% (wt/vol) BSA and glutatione *S*-transferase. Wells were blocked at RT for 1 h with PBS containing 3% (wt/vol) BSA and then washed three times with PBS containing 0.05% Tween-20 (PBST; Sigma) and 1% BSA. Primary Abs were diluted in PBS containing 0.1% BSA and incubated for 2 h at RT followed by six washes with PBST. HRP-conjugated secondary anti-rabbit (1:4,000 dilution; Zymed) was incubated for 30 min followed by three PBST washes and detection with 3,3′,5,5′-tetramethylbenzidine. The inhibition ELISA was performed by preincubating the anti-GLTFKSL Ab with 100 mM excess of the synthetic GLTFKSL peptide.

Western Blot. Cells were grown to 80% confluence and washed three times with cold PBS, and cell extracts were obtained with Nonidet P-40 Lysis Buffer [50 mM Tris·HCl, pH 7.4, 1% Nonidet P-40, 0.25% Deoxycholate, 135 mM NaCl, 1 mM EDTA, 1 mM NaF, 1 mM Sodium Orthovanadate, 10% (vol/vol) Glycerol, antiphosphatase and antiprotease mixture]. Extracts were centrifuged at $18,210 \times g$ for 20 min at 4 °C. The lysate supernatant was transferred to a new tube, and concentration was determined using the BCA Protein Assay (Pierce). The cell extracts were separated by SDS/PAGE under reducing conditions and transferred to a 0.45 μM nitrocellulose membrane (Biorad). Membranes were blocked for 30 min in 5% (wt/vol) nonfat milk, and primary Abs were diluted in TBS Tween-20 (0.05%) containing 5% (wt/vol) BSA and then incubated overnight at 4 °C. Membranes were washed three times with TBST for 10 min; then, HRP-labeled secondary Abs were incubated for 30 min to 1 h at RT followed by three washes with TBST. The substrate ECL Plus was used for development (GE Healthcare).

Immunoprecipitation. Eighty percent confluent C8161 cells were washed three times with cold PBS; then, total cell lysate was obtained with the Nonidet P-40 Lysis Buffer supplemented with protease inhibitors. Magnetic Protein G Beads (Millipore) were precleared with 0.1% BSA in Nonidet P-40 Buffer and rocked for 30 min at 4 °C. Abs were added (anti-PPP2R1A, anti-GLTFKSL, and Rb-IgG control) and incubated overnight with rocking at 4 °C. C8161 cell extract was added for 2 h at 4 °C. Western detection was performed with anti-PPP2R1A Ab.

Flow Cytometry. Adherent cell lines were detached with 0.5 M EDTA. Human melanoma samples were washed three times with

FACS buffer [4% (vol/vol) FBS in PBS]. Cells were incubated with anti-PPP2R1A Ab (1:200) at 4 °C for 20 min and washed three times with FACS buffer. Cells were then incubated with diluted Alexa-488 (1:100) at 4 °C for 20 min. Cells were washed three times with FACS buffer. Cells were incubated with anti-podoplanin-PE Ab (1:100) and antimelanoma–APC Ab (1:11) at 4 °C for 20 min. Cells were washed three times with FACS buffer. Cells were fixed at 4 °C with either alcohol for 2 h or 2% (vol/vol) PFA for 10 min. Cells were washed three times with FACS buffer before analysis with a flow cytometer.

Cell–Cell Interaction Assay. HDLECs were seeded in 16-well chamber slides at a concentration of 8,000 cells/well and allowed to form a monolayer. Next, C8161 cells (20×10^6 cells/mL) were labeled with equal volumes of carboxyfluorescein succinimidyl ester (CFSE) (Invitrogen) at a final concentration of 15 μM. Labeled melanoma cells (7,500 cells/well) were mixed with the highest noncytotoxic concentrations of ligand peptide or specific Abs previously determined and incubated for 4 h in normal growth conditions. PPP2R1A knockdown and NT C8161 cells were similarly prepared and evaluated. Experimental slides were extensively washed in PBS and mounted in the presence of DAPI. Double-positive CFSE (green) and DAPI (blue) cells were counted in at least 10 nonoverlapping fields.

Invasion Assay. BioCoat Matrigel invasion chambers (BD Biosciences) were primed according to the manufacturer's directions. A solution of 20% (vol/vol) FBS in cell culture medium was placed in the lower well to act as a chemoattractant. Parental C8161 melanoma cells were placed in the upper chamber with or without peptides (10 μM) or Abs (10 μg/mL) at 2.5×10^3 cells in serum-free medium. PPP2R1A knockdown and NT C8161 cells were similarly prepared without addition of GLTFKSL peptide or anti-PPP2R1A Ab. Plates were incubated at 37 °C for 24, 48, and 72 h. Melanoma cells on the lower surface of the filter were stained with the Protocol Hema3 Stain Set (Fisher Scientific). Each sample was analyzed with a Nikon Microphot-FXA Microscope at 10× magnification. Images were acquired with a Leica DFC 320 R2 Digital Camera and software.

Immunocapture Assay. Immunocapture experiments were performed with anti-PPP2R1A or IgG control Abs as described (4). After blocking with PBS containing 3% BSA, 30 μg protein from cell membrane extracts were added into the wells for overnight incubation. After washes, phage (2×10^9 T.U.) was added to each well. Bound phage was recovered by bacterial infection as described above.

shRNA. Silencing experiments were performed with the retroviral vector pLKO.1 from the TRC Lentiviral shRNA Library (Open Biosystems) expressing specific shRNAs for human PPP2R1A (oligonucleotide TRCN0000002566 referred to as shRNA#1, and TRCN0000002567 referred to as shRNA#2). The lentiviral vectors (pCCLsin.PTT.PGK.EGFP.Wpre, pMDLg/pRRE, pRSV-Rev, and pMD2.VSVG) were a gift from Luigi Naldini (San Raffaele Telethon Institute for Gene Therapy, Milan, Italy), and the recombinant lentiviruses were produced. Briefly, 293FT cells were transiently transfected using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions, after which the lentiviruses were harvested 24 and 48 h later and filtered through 0.22-μm-pore cellulose acetate filters. Recombinant lentiviruses were used either as supernatant or concentrated by ultracentrifugation for 2 h at $50{,}000 \times g$. Vector infectivity was evaluated by infecting cells with a GFP vector or puromycin drug selection. Equal amounts of protein were loaded and resolved by SDS/PAGE followed by Western blot analysis with the appropriate Abs to verify PPP2R1A silencing. In addition, tumor-homing phage binding activity was examined in PPP2R1A-silenced cells.

Phage Homing in Vivo. In vivo phage homing was performed as described (5). For homing of selected phage in vivo, animals ($n = 3$ in each group) were deeply anesthetized and received 10^{10} T.U. targeted phage or insertless control phage i.v. After 6 h, animals were perfused through the heart with 10 mL DMEM.

Immunofluorescence. Immunofluorescence was performed on 4% (vol/vol) PFA-fixed cryostat sections (10- to 60-μm thickness). Tumors were washed three times with PBS and one time with PBS containing 0.3% Triton X-100 followed by blocking with 5% (vol/vol) normal goat serum diluted in PBS containing 0.3% Triton X-100. Tissue sections were incubated with specific Abs diluted in PBS containing 1% appropriate goat serum and 0.3% Triton X-100 for 1 h at RT. Sections were stained for 1 h with Cy3-, Cy5-, and FITC-conjugated secondary Abs (Jackson ImmunoResearch Laboratories). Confocal images were acquired on a laser-scanning confocal microscope (Zeiss LSM510) equipped with krypton–argon and helium–neon lasers. Image analysis was performed with the Zeiss LSM 3.2 software package.

Immunohistochemistry. Immunohistochemical staining of sections of fixed, paraffin-embedded tissue was performed on an automated autostainer (Lab Vision Corp.) according the manufacturer's instructions.

1. Christianson DR, Ozawa MG, Pasqualni R, Arap W (2007) Techniques to decipher molecular diversity by phage display. *Methods Mol Biol* 357:385–406.
2. Giordano RJ, Cardó-Vila M, Lahdenranta J, Pasqualini R, Arap W (2001) Biopanning and rapid analysis of selective interactive ligands. *Nat Med* 7(11):1249–1253.
3. O'Connell DJ, et al. (2010) Integrated protein array screening and high throughput validation of 70 novel neural calmodulin-binding proteins. *Mol Cell Proteomics* 9(6): 1118–1132.
4. Marchiò S, et al. (2004) Aminopeptidase A is a functional target in angiogenic blood vessels. *Cancer Cell* 5(2):151–162.
5. Kolonin MG, et al. (2006) Synchronous selection of homing peptides for multiple tissues by in vivo phage display. *FASEB J* 20(7):979–981.

Sequence Alignment with PPP2R1A

(Human Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform)

```
1   MAAADGDDSLYPIAVLIDELRNEDVQLRLNSIKKLSTIALALGVERTRSE    50
51  LLPFLTDTIYDEDEVLLALAEQLGTFTTLVGGPEYVHCLLPPLESLATVE   100
101 ETVVRDKAVESLRAISHEHSPSDLEAHFVPLVKRLAGGDWFTSRTSACGL   150
151 FSVCYPRVSSAVKAELRQYFRNLCSDDTPMVRRAAASKLGEFAKVLELDN   200
201 VKSEIIPMFSNLASDEQDSVRLLAVEACVNIAQLLPQEDLEALVMPTLRQ   250
251 AAEDKSWRVRYMVADKFTELQKAVGPEITKTDLVPAFQNLMKDCEAEVRA   300
301 AASHKVKEFCENLSADCRENVIMSQILPCIKELVSDANQHVKSALASVIM   350
351 GLSPILGKDNTIEHLLPLFLAQLKDECPEVRLNIISNLDCVNEVIGIRQL   400
401 SQSLLPAIVELAEDAKWRVRLAIIEYMPLLAGQLGVEFFDEKLNSLCMAW   450

451 LVDHVYAIREAATSNLKKLVEKFGKEWAHATIIPKVLAMSGDPNYLHRMT   500
Protein 1:AIREAATSNLKKLVEKFGKEWAHATIIPKVLAMSGDPNYLHRMT
Protein 2:                    KEWAHATIIPKVLAMSGDPNYLHRMT

501 TLFCINVLSEVCGQDITTKHMLPTVLRMAGDPVANVRFNVAKSLQKIGPI   550
    TLFCINVLSEVCGQDITTKHMLPTVLRMAGDPVANVRFNVAKSLQKIGPI
    TLFCINVLSEVCGQDITTKHMLPTVLRMAGDPVANVRFNVAKSLQKIGPI

551 LDNSTLQSEVKPILEKLTQDQDVDVKYFAQEALTVLSLA              589
    LDNSTLQSEVKPILEKLTQDQDVDVKYFAQEALTVLSLA
    LDNSTLQSEVKPILEKLTQDQDVDVKYFAQEALTVLSLA
```

Fig. S1. Alignment of proteins identified by microarray. Proteins were sequenced and then matched to the listed proteins with BLAST based on sequence alignment for the two identified proteins at the C-terminal identified region of the PPP2R1A protein. The two proteins had 100% identity with the C terminus of PPP2R1A, with protein 1 matched for residues 457–589 (red letters) and protein 2 matched for amino acids 475–589 (blue letters).

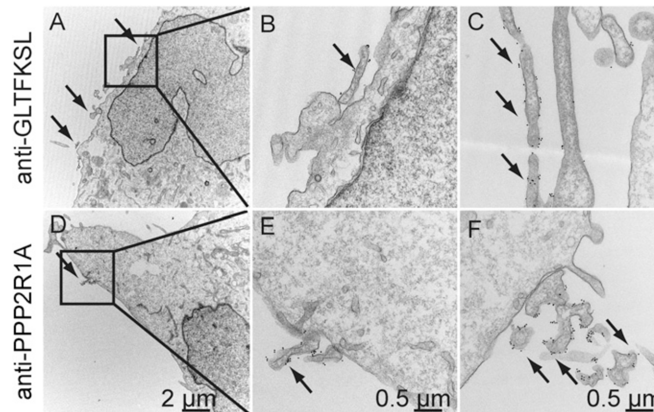

Fig. S2. Transmission EM of anti-GLTFKSL and anti-PPP2R1A binding to C8161 cells. Secondary Abs labeled with 18-nm gold particles were used for detection of the (*A*–*C*) anti-GLTFKSL and (*D*–*F*) anti-PPP2R1A, which are seen binding to the surface (arrows) of membrane-intact C8161 cells. (Scale bars: *A* and *D*, 2 µm; *B*, *C*, *E*, and *F*, 500 nm.)

```
  1 MAAADGDDSLYPIAVLIDELRNEDVQLRLNSIKKLSTIALALGVERTRSELLPFLTDTIYDEDEVLLALAEQLGTFTTLVGGPEYVHCLLPPLESLATVE
      AADGRRH  PIAVDFV                     VALGVPW                      SGEQLGP              PGGLESL
                                               VGRTRAQ              SPLARAE                   SMLESPA
                                                LRTRSNL
                                          VLATGVA

101 ETVVRDKAVESLRAISHEHSPSDLEAHFVPLVKRLAGGDWFTSRTSACGLFSVCYPRVSSAVKAELRQYFRNLCSDDTPMVRRAAASKLGEFAKVLELDN
           ASESLFV    HSPSLPH       GGLTGGD       AWGLSSV  RRVSSEF
        RDKAVTA                      VSARGDW      ACGHFWC
                                          SVFPSRT    LFSVDSR
                                            FGSRTRV

201 VKSEIIPMFSNLASDEQDSVRLLAVEACVNIAQLLPQEDLEALVMPTLRQAAEDKSWRVRYMVADKFTELQKAVGPEITKTDLVPAFQNLMKDCEAEVRA
           MFSNLRV  GDSVALA                                                LAVGPWDRLTDLPP
                       TRLLSVD                                              AVGPEPK
                           MVEANVT

301 AASHKVKEFCENLSADCRENVIMSQILPCIKELVSDANQHVKSALASVIMGLSPILGKDNTIEHLLPLFLAQLKDECPEVRLNIISNLDCBNEVIGIRQL
    DT                              LVSTAWE   SALGSEL
                                                LASVVRD
                                                LASRIGF
                                           DSKSALG   KGLSPRS
                                                YRSVGMG
                                             RFAKASV

401 SQSLLPAIVELAEDAKWRVRLAIIEYMPLLAGQLGVEFFDEKLNSLCMAWLVDHVYAIREAATSNLKKLVEKFGKEWAHATIIPKVLAMSGDPNYLHRMT
     PSLLGAV                      HGRLGVD                 EFAISEA                             GDWNYSG
                                   GRLGVLT
                            SSGPLLA VMGVEVF
501 TLFCINVLSEVCGQDITTKHMLPTVLRMAGDPVANVRFNVAKSLQKIGPILDNSTLQSEVKPILEKLTQDQDVDVKYFAQEALTVLSLA
                               PAGDPGW                                              RALTGLV
                                GGDNVAR                                                GRLSLAP
                            RGRMAGF  GAVRFAV                                         GLTFKSL
                               RAGDAVL
```

Fig. S3. Sequence alignment of peptides from the third round of screening on the afferent LyVs and LECs to PPP2R1A. The GLTFKSL peptide mimics the C-terminal region of PPP2R1A.

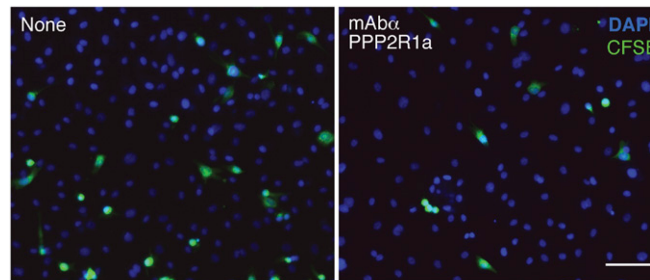

Fig. S4. Representative view of C8161 cells labeled with CFSE. Lymphatic cells (monolayer) were stained with DAPI (blue). Attached melanoma cells (green) were counted over at least 10 random fields.