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Original Article

In vivo antibody library screening identifies PKM2-targeting M1 antibody with antitumor activity in melanoma

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Abstract: In this study, we introduce an innovative in vivo antibody screening method to identify antibodies that can inhibit melanoma cell growth and induce apoptosis. By using a lentiviral ScFv library, we developed a platform that allows for the direct suppression of melanoma cell proliferation within a living mouse model. Through this approach, we identified the M1 antibody, which targets PKM2, a key protein involved in tumor progression. The M1 antibody was found to significantly inhibit melanoma cell growth by disrupting the function of PKM2. Although PKM2 is widely recognized as an important factor in various cancers, no commercial therapeutic agents currently target this protein. Our findings indicate that the in vivo antibody screening method is a reliable and effective approach for isolating antibodies for melanoma therapy. Moreover, the M1 antibody shows great potential as a promising candidate for developing novel treatments for human melanoma.

Keywords: Antibody selection, antibody library, melanoma, PKM2, cancer

Introduction

Melanoma is a serious type of skin cancer that begins in the melanocytes, the cells responsible for producing melanin, the pigment that gives our skin its color. Although it is less common that other types of skin cancer, melanoma is a highly aggressive skin cancer with a strong tendency to metastasize to other parts of the body if not detected and treated early. It often appears on sun-exposed areas of the body, such as the back, legs, arms, and face, but can also develop in less expected places like the soles of the feet, palms, and under the nails. Exposure to UV radiation and genetic predisposition significantly increase the risk of developing melanoma, making the critical need for prevention, early detection, and successful treatments [1, 2].

The treatment approach for melanoma depends on the stage of the cancer at the time of diagnosis and the overall health condition of the patient. The primary treatment is surgical removal for early-stage melanoma, while more advanced cases may require targeted therapies that inhibit specific pathways involved in tumor growth, especially in patients with BRAF mutations. Recently immunotherapy that boosts the body's immune system to fight cancer has also shown significant promise in treating melanoma. Other options include radiation therapy, chemotherapy, and newer methods like oncolytic virus therapy. Despite these advances, the treatment of extensively metastatic melanoma remains a major challenge, underlining the need for ongoing research and participation in clinical trials to develop more effective treatments [3-7].

For many years, high-throughput target-based screening like phage display and hybridoma technology was the standard method for isolating antibodies, but it has become increasingly difficult due to the limited number of validated targets in this field [8-10]. However, recent advancements in combinatorial antibody libraries and innovative delivery methods have opened up new possibilities for isolating functional antibodies directly within cellular environ-

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ments. These antibodies can alter cell fate by being secreted, anchored on the plasma membrane, expressed in the cytoplasm, or implanted in the endoplasmic reticulum. In stem cell research, these functional antibodies have shown the potential to regulate pluripotency and lineage specification, providing new insights into cell surface signaling and the intricacies of signal transduction [11-13].

In vivo screening provides researchers with a more accurate and physiologically relevant understanding of how treatments function within the complex biological environment of a living organism. Unlike in vitro assays, which are limited to isolated cells under artificial conditions, in vivo studies capture critical systemic factors such as tissue architecture, immune responses, pharmacokinetics, and cell-cell interactions. Advanced technologies such as CRISPR/Cas9 and RNA interference (RNAi) have further expanded the capabilities of in vivo screening by enabling direct gene modification or silencing. These tools support high-throughput, genome-wide analyses of gene function and disease mechanisms, although their application can be limited by technical challenges such as delivery efficiency, off-target effects, and complexity [14, 15].

Building on this foundation, in vivo antibody screening has emerged as a powerful and versatile platform for discovering therapeutically relevant antibodies. Unlike traditional cell-based assays or phage display, in vivo antibody selection enables researchers to identify antibodies that not only bind to disease-associated antigens but also demonstrate functional activity within physiological environments such as the tumor microenvironment (TME) [16]. By introducing lentiviral antibody libraries into tumor cells prior to implantation in animals, antibodies can be selected under natural conditions where immune interactions, vasculature, and extracellular matrix are preserved. In our study, we employed this approach to identify functional antibodies against melanoma. The resulting scFv library exhibited measurable antitumor activity, and among the selected antibodies, M1 showed robust inhibitory effects across multiple melanoma cell lines, underscoring the translational value of in vivo antibody discovery [17].

Beyond oncology, in vivo antibody screening plays a pivotal role in regenerative medicine, particularly for studying cell migration and differentiation. Similar to autocrine signaling-based selection, this approach allows identification of antibodies from combinatorial libraries that influence stem cell fate and movement within the body. Our group and others have demonstrated that antibodies can induce hematopoietic stem cells (HSCs) to differentiate into specific lineages such as microglia, macrophages, brown adipocytes, and beta-like cells, and subsequently migrate to their target tissues. For example, antibody agonists have been shown to drive microglial differentiation from bone marrow cells, leading to functional integration in the brain. Similarly, antibodies targeting proteins such as IYD and periostin (POSTN) facilitated the differentiation and migration of cells to the heart and pancreatic islets, respectively. These findings highlight the potential of in vivo antibody screening not only to regulate cell fate but also to reconstitute damaged organ systems by mimicking developmental processes. Despite technical limitations such as the difficulty in precisely characterizing induced cell types and understanding antibody mechanisms this strategy provides a powerful framework for developing targeted therapies for diseases including cancer, autoimmune disorders, metabolic diseases, and heart conditions [11-13, 17-20].

In cancer research, functional antibodies have shown the ability to regulate cell proliferation and differentiation, sometimes by activating pathways different from those induced by natural ligands. This receptor pleiotropism highlights the complexity of cellular signaling and offers opportunities for discovering new therapeutic targets. For example, antibodies derived from combinatorial antibody libraries have been used to either promote or inhibit cancer growth depending on their interaction with specific binding receptors. This approach highlights the potential of antibody libraries in identifying targets and developing treatments that can effectively regulate cancer progression, making it a valuable strategy in advancing cancer therapy [11, 21].

In this study, our goal was to develop an in vivo antibody selection method for melanoma therapy. We identified that selected M1 antibody plays a key role in regulating human melanoma cell growth through our novel method. By screening M1 from a human antibody library, we found that it successfully induces cell prolif-

eration arrest by blocking PKM2. These results indicate that our method is highly robust and efficient for the novel antibody selection in vivo melanoma. Moreover, PKM2 has the potential to act as a target for melanoma immune therapy.

Materials and methods

Mice and cell lines

All experiments were performed using C57-BL/6J mice (The Jackson Laboratory, USA). The mice were housed and maintained at temperatures between 20 and 26°C, adhering to protocols approved by the Institutional Animal Care and Use Committee at Hannam University (12-0029/HNU2021-6). Cell lines used in the study included HEK 293T human embryonic kidney cells, B16F10 mouse melanoma cells, the normal fibroblast cell line CCD-986Sk, and the A375P and A375SM human melanoma cell lines, which were cultured in DMEM medium (Corning, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, USA) and 1% penicillin/streptomycin (Gibco, USA). The FreeStyle™ 293-F cell line (Invitrogen, USA) was cultured in FreeStyle 293 Expression Medium (Invitrogen, USA). All cell cultures were maintained at 37°C in a humidified incubator with 5% CO_2.

Lentivirus transfection

HEK293T cells were seeded at a density of 8 × 10^5 cells per well in a 6-well plate. After overnight incubation, the cells were transfected with 1.3 μg each of pLV plasmid, pCMVD packaging plasmid, and pVSVG envelope plasmid using Lipidofect-P (Lipidomia, Korea) as the transfection reagent. Following a 4-hour incubation, the culture medium was replaced with fresh growth medium. After 72 hours of incubation, the culture supernatant was harvested, filtered through CA filters, and analyzed by Western blotting using an anti-HIV-1 p24 antibody (Santa Cruz, USA), with recombinant HIV-1 p24 (Abcam, USA) serving as an assay control. Lentiviral supernatants with confirmed production were stored at -80°C.

MTS assay

Cells were seeded into 96-well plates at a density of 5 × 10^3 cells per well and infected with M1 lentivirus in the presence of 10 μg/ml polybrene (Millipore, USA). After 12 hours, the medium was replaced with 100 μl of fresh growth medium. The cells were then cultured for an additional 3-4 days, followed by the addition of 20 μl of CellTiter 96® AQueous One Solution (Promega, USA) to each well. The plates were incubated at 37°C for 4 hours, and the optical density (OD) was measured at 490 nm using a SpectraMax 190 Microplate Reader (Molecular Devices, USA).

Colony formation assay

Cells were seeded at a clonogenic density of 1 × 10^3 cells per well in 12-well plates. After 24 hours, cells were infected with M1 lentivirus in the presence of 10 μg/ml polybrene (Millipore, USA) for 12 hours, after which the culture medium was replaced. Forty-eight hours later, the infection procedure was repeated. Cells were incubated for 10-12 days, with the culture medium refreshed every 3 days. Following incubation, colonies were fixed with 4% paraformaldehyde in PBS for 20 minutes, rinsed with PBS, and stained with 0.03% crystal violet in water for 20 minutes. Stained colonies were quantified using ImageJ software (version 1.8.0). All experiments were performed in triplicate.

Wound healing assay

Cells were seeded at a density of 1 × 10^5 cells per well in 12-well plates and incubated for 24 hours. A linear scratch was made across the cell monolayer using a pipette tip to simulate a wound. Fresh culture medium supplemented with M1 lentivirus and 10 μg/ml polybrene (Millipore, USA) was then added to each well. Images of the wound area were captured at 0, 24, and 48 hours. The rate of cell migration was assessed by measuring the remaining wound area at each time point relative to the initial wound area at 0 hours, using ImageJ software for quantitative analysis.

Immunoblotting

Cells were washed twice with ice-cold PBS and lysed in IP or RIPA buffer (Thermo Scientific, USA). Lysates were centrifuged at 15,000 rpm for 25 minutes at 4°C, and protein concentrations were determined using the BCA Protein Assay Kit (Thermo Scientific, USA). Equal amounts of protein were denatured by boiling in SDS-PAGE loading buffer at 70°C for 5 min-

utes, separated by SDS-PAGE, and transferred onto nitrocellulose (NC) membranes using the Trans-Blot Turbo Transfer System (Bio-Rad). Membranes were blocked in 5% blocking buffer (0.2% Tween-20 and 5% skim milk powder in TBS) for 1 hour at room temperature, then incubated overnight at 4°C with primary antibodies against M1, PARP, Caspase-3, cleaved Caspase-3, $p21^{Waf1/Cip1}$, BAX, β-actin (Cell Signaling Technology, USA), and p53 (Millipore, USA). After three washes with TBST (TBS containing 0.1% Tween-20) for 15 minutes each, membranes were incubated with HRP-conjugated secondary antibodies (anti-IgG1, anti-rabbit, or anti-mouse; Cell Signaling Technology, USA) for 1 hour at room temperature. Following three additional 10-minute washes in TBST, protein bands were visualized using Clarity Western ECL Substrate (Bio-Rad) or SuperSignal™ West Dura Substrate (Thermo Scientific, USA).

Immunoprecipitation

Immunoprecipitation was performed using the Pierce™ Crosslink IP Kit (Thermo Scientific, USA). The M1-Fc antibody (100 μg/ml) was conjugated to Protein A/G Agarose and subsequently cross-linked using Disuccinimidyl Suberate (DSS). Cell lysates from HEK-293T, A375P, and A375SM cells (1×10^6 cells per sample) were prepared using the IP Lysis/Wash Buffer and pre-cleared with Pierce Control Agarose Resin to minimize non-specific binding. The cross-linked M1-Fc antibody was then incubated with the lysates to immunoprecipitate the target antigen.

Silver stain and LC-MS

The immunoprecipitated eluates were separated by SDS-PAGE using Mini-PROTEAN® TGX™ 10% precast gels (BIO-RAD, USA). After electrophoresis, the gels were silver-stained with the SilverQuest™ Staining Kit (Invitrogen, USA). The silver-stained bands that were specifically identified by the M1 antibody were subsequently analyzed by liquid chromatography-mass spectrometry at the National Institute for Basic Science Research (Korea).

PKM2 small interfering RNA (siRNA) transfection

PKM2-specific siRNA oligonucleotides and non-specific siRNA (siNC) were synthesized (Jinpama, China). The siRNAs were transfected into A375P and A375SM cells using Lipidofect-P (Lipidomia, Korea) following the manufacturer's instructions. After siRNA transfection, the cells were incubated for 24-48 hours. Subsequently, cell lysates were collected and analyzed by Western blot to assess the impact of siRNA. The transfected cells were then subjected to colony-forming assays. The specific sequences of the siRNAs were as follows:

Name	Sequence
siPKM2 #1	5'-GCCAUAAUCGUCCUCACCA-3'
siPKM2 #2	5'-CCAUAAUCGUCCUCACCAA-3'
siNC	5'-CUUACGCUGAGUACUUCGA-3'

Statistical analyses

All data are presented as mean ± standard error of the mean (s.e.m.), based on experiments independently repeated at least three times. Statistical analyses were performed using multiple unpaired t-tests or one-way ANOVA, as appropriate. Significance levels were indicated as follows: NS, not significant; *P < 0.05; *P < 0.01; *P < 0.001. Statistical analysis was conducted using GraphPad Prism version 9 (Graphpad software, USA).

Results

In vivo antibody selection system for inhibiting melanoma cell growth

We established a novel in vivo antibody screening method to select antibodies that can suppress melanoma growth (**Figure 1**). This approach uses a human single-chain fragment variable (ScFv) phage library to generate a human ScFv lentiviral combinatorial antibody library including 10^8 specific individual clones. The antibodies were presented on the cell surface by using the methods we previously published [11-13, 17, 19, 20]. B16F10 mouse melanoma cells are transduced with an ScFv lentiviral library and then transplanted subcutaneously into the hind flank of a mouse. After about three weeks, candidate antibodies are identified from cells whose growth has been suppressed by the antibodies.

The results indicated that our antibody selection method is effective on melanoma cells, showing a significant reduction in cell growth following antibody library treatment compared

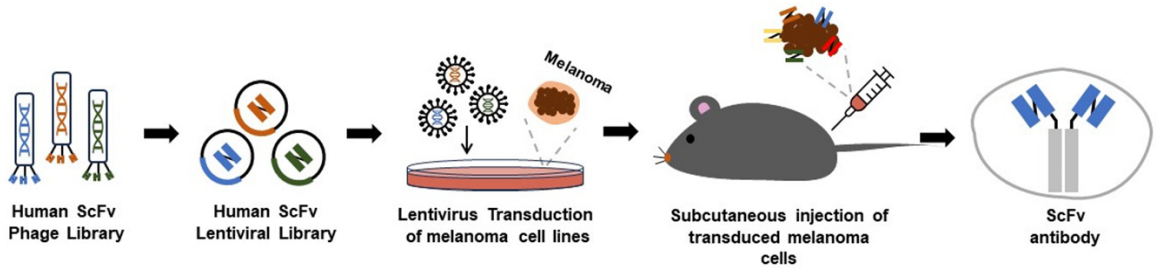


Figure 1. Antibody selection scheme for suppressing melanoma growth in vivo. A scheme was developed to select antibodies that inhibit cell growth. To achieve this, genes from a human ScFv phage library, comprising 108 members, were cloned into a lentiviral vector to make a lentiviral antibody library. In this library, antibodies are anchored to the plasma membrane and displayed on the cell surface. B16F10 melanoma cells were then infected with this antibody library in vitro and cultured for two days. Because of an autocrine basis, each cell expressed distinct antibodies. Subsequently, the cells were subcutaneously transplanted into C57BL/6 mice and allowed to grow for three weeks. Afterward, the melanoma cells were harvested and analyzed using PCR to identify the antibody genes that effectively inhibited tumor cell growth.

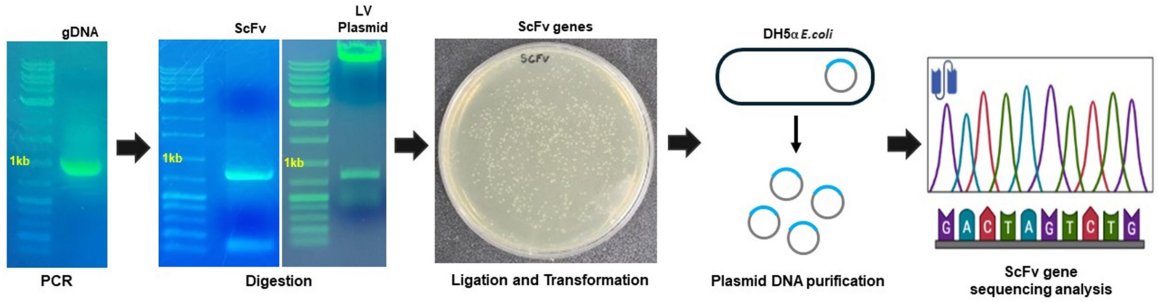


Figure 2. Candidate antibody identification. ScFv DNA was amplified from genomic DNA (gDNA). The amplified ScFv DNA and the lentiviral vectors (LV) were digested using the SfiI restriction enzyme and subsequently ligated. The resulting constructs were transformed into DH5α bacterial cells. Following colony selection, Plasmid prep and sequencing analysis were performed to confirm successful cloning.

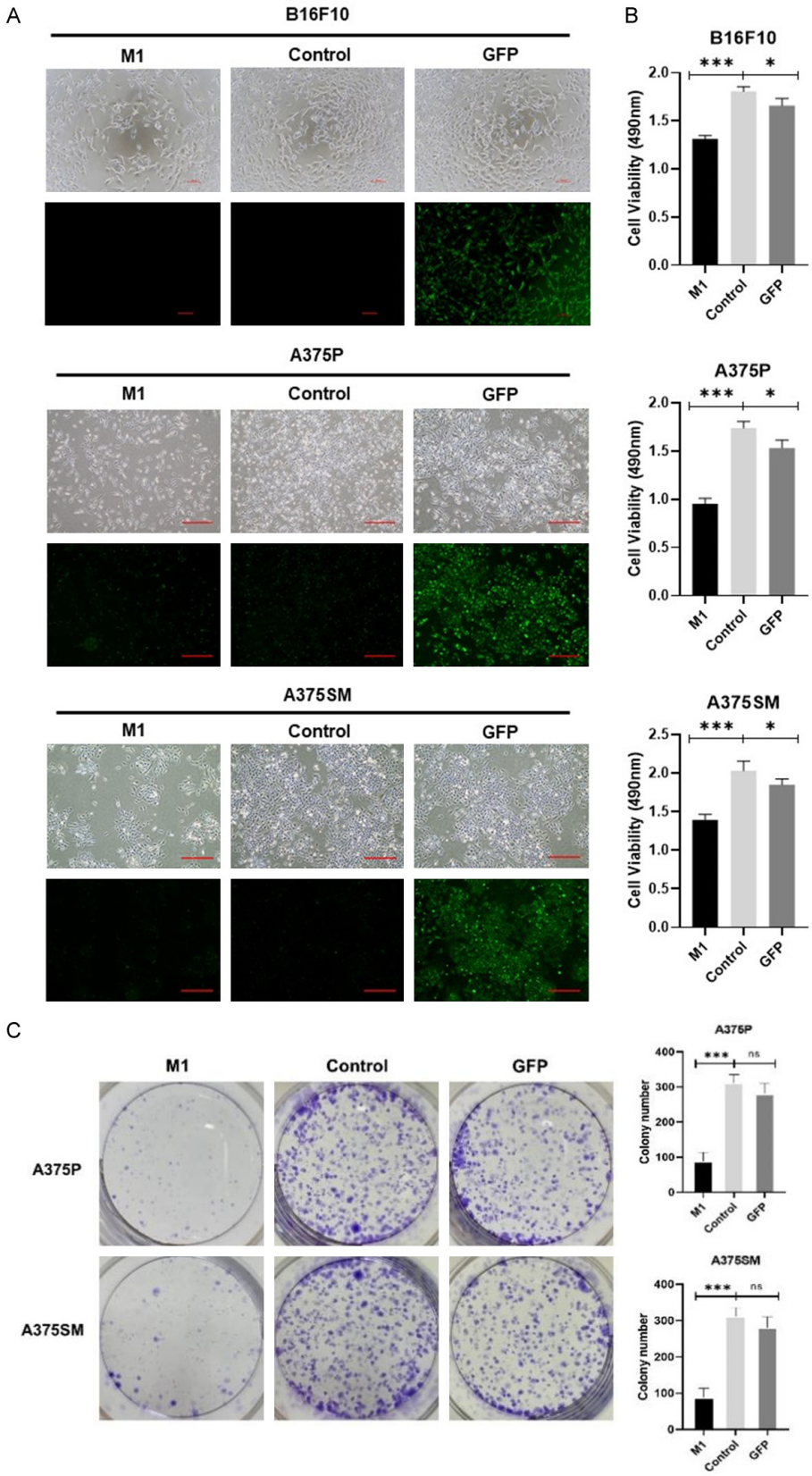
to the control group (Figure S1). Candidate antibodies were identified through genomic DNA PCR, cloning, and sequencing of the antibody library-treated cells isolated from the in vivo selection process (**Figure 2**). To determine whether a single antibody from the selected antibodies could inhibit melanoma growth, we focused on the M1 gene, which consistently appeared with significant redundancy in the sequencing results from the harvested melanoma cells.

Selected M1 antibody inhibits proliferation and migration of melanoma cell lines

The results showed a substantial reduction in cell viability in multiple cell lines treated with M1 compared to the control. A clear reduction in cell growth was observed following treatment, particularly in melanoma cell lines. B16F10 cells showed a 27.3% decrease, A375P cells dropped by 45.1%, and A375SM cells by 31.3%. Liver (HepG2) and lung (A549) cancer cells also showed notable declines in viability, with reductions of 21.0% and 18.8%, respectively. In contrast, the colon cancer cell line HCT116 showed only a slight decrease of 1.6%, indicating no meaningful difference from the untreated control (**Figures 3A**, **3B**, S3). In addition, the colony formation assay showed a significant decrease in the colony count after treatment with M1 Ab compared to the control (**Figure 3C**). This finding was corroborated by the wound healing assay, which demonstrated a considerable reduction in cell migration following M1 Ab treatment (**Figure 3D**). Collectively, these results indicated that M1 Ab markedly inhibit human melanoma cell proliferation, colony formation, and migration in vitro.

Selected M1 antibody induces apoptosis in melanoma

To determine whether M1 antibody triggers apoptosis in human melanoma cells, we observed the levels of key cell death markers, including PARP, cleaved PARP, p53, $p21^{Waf1/Cip1}$, and BAX using Western blot analysis after treat-


A
B16F10
M1
Control
GFP
A375P
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B
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Cell Viability (490nm)
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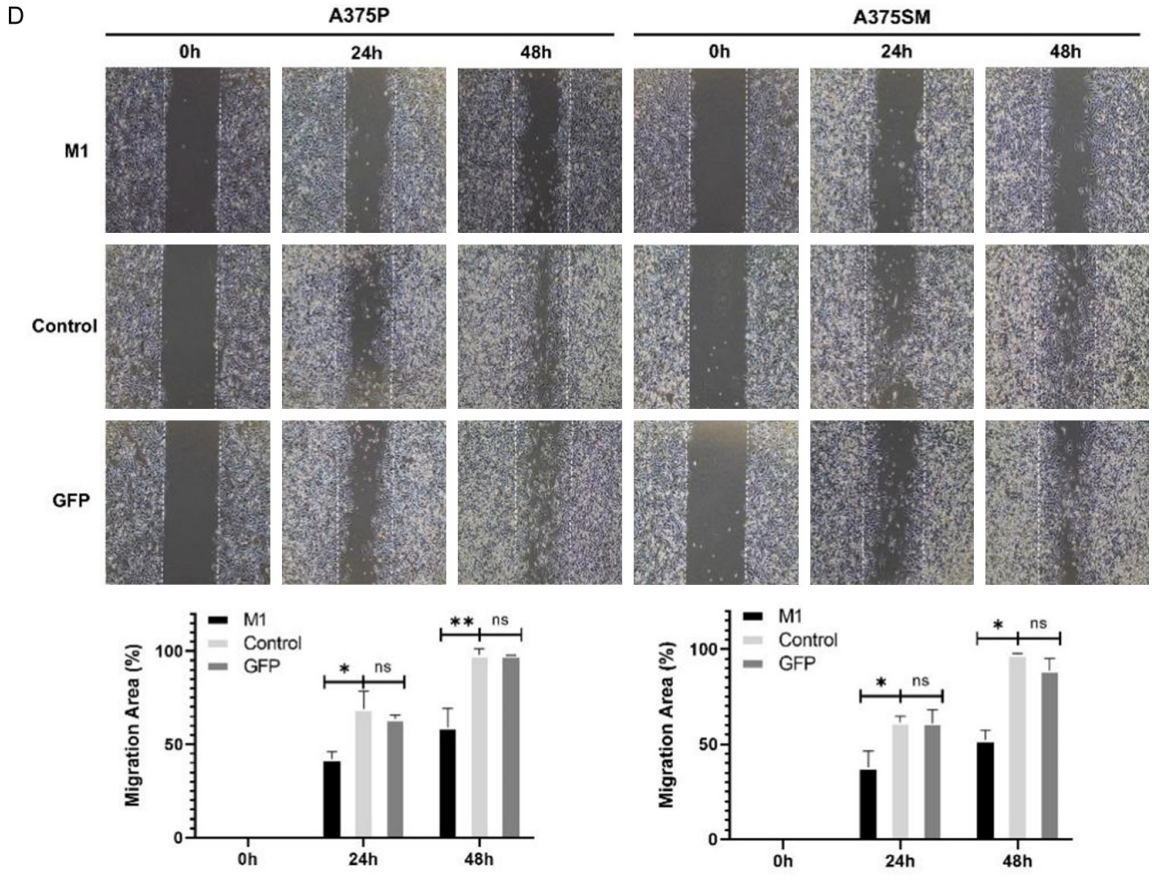


Figure 3. M1 antibody suppresses human melanoma cells growth. A. B16F10, A375P, and A375SM cells were observed in culture following M1 treatment. Scale bar: 100 μm. B. The MTS assay was used to assess the proliferation of M1 treated B16F10, A375P, and A375SM cells. C. Colony formation was analyzed in both cell lines, with the number of colonies quantified through ImageJ. D. The impact of M1 on cell migration was evaluated using wound healing assays. Representative images show the scratched and healing areas (indicated by white dotted lines) on confluent layers of melanoma cells transduced with M1. Results are expressed as mean ± s.e.m., based on an experiment repeated independently at least three times. Statistical significance was determined using multiple t-tests and one-way ANOVA: NS, not significant; *P < 0.05, **P < 0.01, ***P < 0.001.

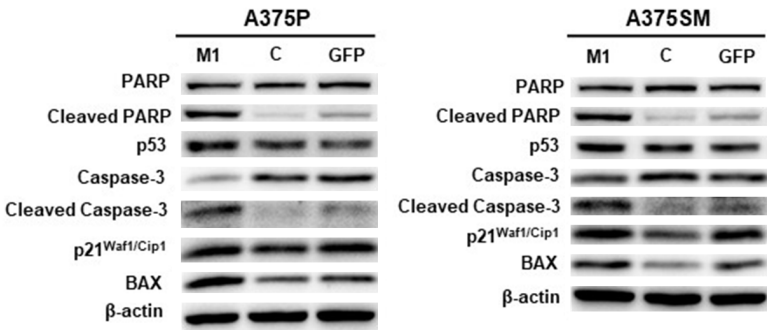


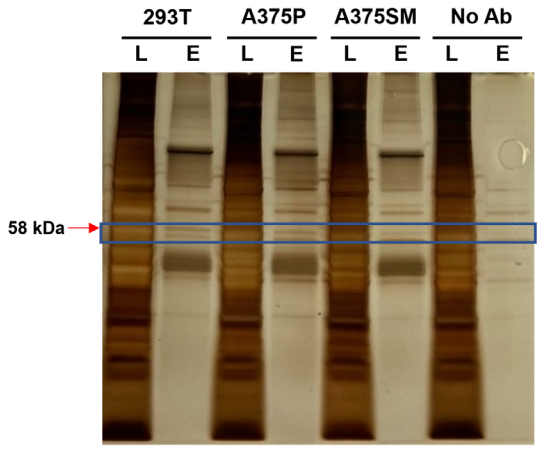
Figure 4. M1 Ab regulates apoptosis-related protein expression in human melanoma. A375P and A375SM melanoma cells were lysed after treatment with M1 Ab and control. The protein level of PARP, cleaved PARP, p53, $p21^{Waf1/Cip1}$, Caspase-3, cleaved Caspase-3, and BAX was measured using Western blotting.

ing the cells with M1 antibody delivered through lentiviral transduction. Cleaved PARP, p53, and $p21^{Waf1/Cip1}$ and BAX proteins associated with are apoptotic cell death were significantly increased in A375P and A375SM cells treated with M1 antibody (**Figure 4**). These results demonstrated that M1 antibody plays a role in activating apoptosis pathway in human melanoma cell lines.

Identification of a target protein

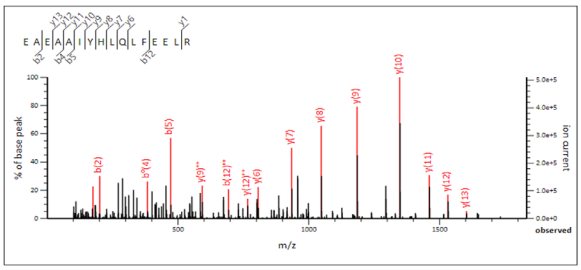
To identify the antigenic protein recognized by M1 Ab, M1 antibody was first produced and purified, after which M1 Ab was incubated with A375P and A375SM cell lysates. Immune

A



B

Description	Coverage	Matches
Pyruvate kinase PKM	62.7	63
T-complex protein 1 subunit alpha	30.2	16
Phosphoglucomutase-1 subunit alpha	18.1	8
Dihydropyrimidinase-related protein 2	10.1	4



>P14618, Pyruvate kinase PKM OS=Homo sapiens OX=9606 GN=PKM

Figure 5. Identification of a target protein recognized by M1 antibody. A. HEK 293T, A375P, and A375SM cell lysates were incubated with M1 antibody for immunoprecipitation. Immunoprecipitated elutes were analyzed by silver staining and LC-MS (L: cell lysate, E: elution). B. The candidate antigens were selected from the LC-MS analysis results.

complexes formed were then captured using a protein A column. The proteins interacting with M1 antibody, were subsequently analyzed by silver stain and identified through mass spectrometry (**Figure 5A**, **5B**). A specific band with an apparent molecular weight of approximately 58 kDa was excised from the gel and subjected to LC-MS analysis. Among the detected proteins, pyruvate kinase M2 (PKM2) showed the highest coverage and matches (**Figure 5B**). PKM2 was identified as the most likely target antigen of M1 Ab.

The interaction between the PKM2 antigen and M1 antibody

To confirm PKM2 as the target antigen, a western blot analysis was performed. This analysis demonstrated that M1 antibody bound to lysates from A375P and A375SM cells specifically at the known molecular size of PKM2, but not to lysates from the normal fibroblast cell line CCD-986Sk, (**Figure 6A**). To further investigate whether the interaction between M1 antibody and PKM2 regulate melanoma cell growth, we used two siRNAs (siPKM2#1-2), silence control, and control to knock down PKM2 expression in human melanoma A375SM cells (**Figure 6B**). Following the knockdown, cells were treated with the M1 antibody and a colony forming assay was performed. Without PKM2, M1 antibody treatment did not inhibit melanoma cell growth. However, when PKM2 was not silenced in the control group, the M1 antibody showed consistent inhibition on melanoma cells (**Figure 6C**, **6D**). These results demonstrated that the M1 antibody suppresses melanoma cell growth by interacting with PKM2.

Discussion

In this study, we investigated to determine if specific antibodies from human Scfv antibody library could inhibit cells growth or induce cells death in melanoma cells implanted under the skin of mice in vivo. The newly developed in vivo antibody selection method performed as expected, allowing us to identify promising antibody candidates. Notably, among the selected antibodies, the M1 antibody showed significant effectiveness in regulating the growth of human melanoma cells through PKM2.

In vivo antibody selection offers a significant advantage over traditional in vitro cell culture or phage display. By directly injecting cells treated with lentiviral antibody libraries into animals, this approach allows for the isolation of antibodies and identification of tissue specific antigens [12]. This method enables the isolation of more precise and capable antibody candidates showing promise for developing targeted therapies. We applied this method to find new antibody candidates for treating melanoma. It showed further results that the in vivo antibody screening method is consistently robust and effective.

Our results indicated pyruvate kinase M2 (PKM2) is the target antigen by M1 antibody.

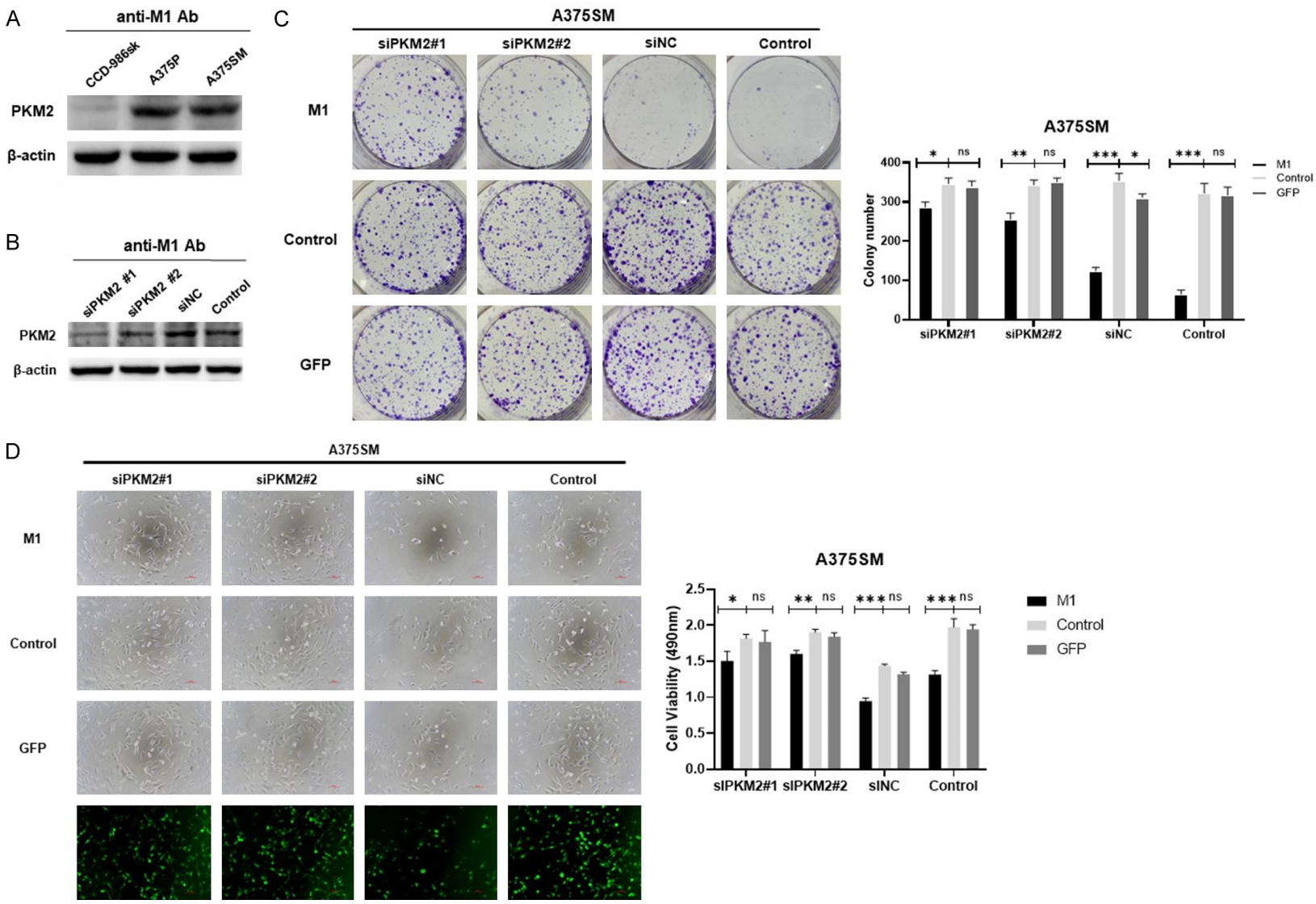


Figure 6. Identification of a target protein recognized by M1 antibody. A. Western blot analysis indicated that the M1 antibody recognized the protein in not CCD-986, but A375P and A375SM cells. B. Western blot analysis confirmed the binding of the M1 antibody to PKM2 in A375SM cell lysates from untreated cells, siNC-treated cells, and cells treated with siRNA targeting PKM2 (si PKM2#1-2). C. Given that si PKM2#1,2 successfully knocked down PKM2 expression in melanoma cells, these siRNA-treated cells, along with those treated with the M1 antibody, were further assessed through a colony forming assay. D. Cells treated with siPKM2#1, siPKM2#2, and the M1 antibody were subsequently analyzed using the MTS assay. Results are expressed as mean ± s.e.m., based on an experiment repeated independently at least three times. Statistical significance was determined using multiple t-tests and one-way ANOVA: NS, not significant; *P < 0.05, **P < 0.01, ***P < 0.001.

PKM2 is an isoenzyme of pyruvate kinase that plays a crucial role in the glycolysis pathway. PKM2 also contributes to regulate multiple mechanisms in various cancers, including gastrointestinal, pancreatic, lung, ovarian, and renal cancers [22, 23]. It playing a significant role in the Warburg effect, where cancer cells preferentially convert glucose to lactate even in the presence of oxygen [24]. This metabolic shift supports rapid cell growth and proliferation by allowing the diversion of glycolytic intermediates into biosynthetic pathways. It also contributes to cancer metastasis by facilitating the epithelial-mesenchymal transition (EMT), which grants cancer cells the ability to migrate and invade distant organs. It interacts with various proteins involved in cell migration and invasion, further enhancing metastatic potential [25-29]. PKM2 is primarily known as a cytoplasmic enzyme involved in glycolysis. However, several recent researches suggest that PKM2 can also be associated with the cell membrane, particularly in aggressive cancer cells. In these cases, PKM2 interacts with membrane proteins, such as integrins, which are involved in cell adhesion and signaling, thereby promoting cancer cell migration and invasion (Figure S2) [29-32]. Additionally, PKM2 can be expressed on the surface of cancer cells, where it may play a role in immune evasion and extracellular interactions that contribute to tumor progression. Due to its central role in cancer cell metabolism and gene regulation, PKM2 is considered a promising therapeutic target. Inhibiting PKM2 could disrupt cancer cell metabolism, inhibit tumor growth, and prevent metastasis, making it a focus of ongoing cancer research.

We have yet to fully understand how M1 antibody interacts with PKM2 to inhibit melanoma growth. To address this, we are investigating studies on the cellular mechanisms involved and testing the effectiveness of M1 antibody treatment in immunodeficiency mouse model.

In summary, our innovative antibody screening method showed great promise, not only because it can be applied to melanoma, but also for its potential to all types of solid cancers. Additionally, our studies suggest that PKM2 could serve as an important target for the treatment of human melanoma. These findings point to the possibility of developing M1 antibody into an effective therapeutic agent, offering new possibilities for future treatment strategies.

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Disclosure of conflict of interest

None.

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References

[1] Schadendorf D, van Akkooi ACJ, Berking C, Griewank KG, Gutzmer R, Hauschild A, Stang A, Roesch A and Ugurel S. Melanoma. Lancet 2018; 392: 971-984.

[2] Franceschi S, Levi F, Randimbison L and La Vecchia C. Site distribution of different types of skin cancer: new aetiological clues. Int J Cancer 1996; 67: 24-28.

[3] Ahmed B, Qadir MI and Ghafoor S. Malignant melanoma: skin cancer-diagnosis, prevention, and treatment. Crit Rev Eukaryot Gene Expr 2020; 30: 291-297.

[4] Long GV, Swetter SM, Menzies AM, Gershenwald JE and Scolyer RA. Cutaneous melanoma. Lancet 2023; 402: 485-502.

[5] Xu Z, Lee CC, Ramesh A, Mueller AC, Schlesinger D, Cohen-Inbar O, Shih HH and Sheehan JP. BRAF V600E mutation and BRAF kinase inhibitors in conjunction with stereotactic radiosurgery for intracranial melanoma metastases. J Neurosurg 2017; 126: 726-734.

[6] Pasquali S, Chiarion-Sileni V, Rossi CR and Mocellin S. Immune checkpoint inhibitors and targeted therapies for metastatic melanoma: a network meta-analysis. Cancer Treat Rev 2017; 54: 34-42.

[7] Gaudy-Marqueste C, Dussouil AS, Carron R, Troin L, Malissen N, Loundou A, Monestier S, Mallet S, Richard MA, Regis JM and Grob JJ. Survival of melanoma patients treated with targeted therapy and immunotherapy after systematic upfront control of brain metastases by radiosurgery. Eur J Cancer 2017; 84: 44-54.

[8] Kumar R, Parray HA, Shrivastava T, Sinha S and Luthra K. Phage display antibody libraries: a robust approach for generation of recombinant human monoclonal antibodies. Int J Biol Macromol 2019; 135: 907-918.
[9] Posner J, Barrington P, Brier T and Datta-Mannan A. Monoclonal antibodies: past, present and future. Handb Exp Pharmacol 2019; 260: 81-141.
[10] Hanack K, Messerschmidt K and Listek M. Antibodies and selection of monoclonal antibodies. Adv Exp Med Biol 2016; 917: 11-22.
[11] Lin CW and Lerner RA. Antibody libraries as tools to discover functional antibodies and receptor pleiotropism. Int J Mol Sci 2021; 22: 4123.
[12] Han KH, Arlian BM, Macauley MS, Paulson JC and Lerner RA. Migration-based selections of antibodies that convert bone marrow into trafficking microglia-like cells that reduce brain amyloid beta. Proc Natl Acad Sci U S A 2018; 115: E372-E381.
[13] Han KH, Gonzalez-Quintial R, Peng Y, Baccala R, Theofilopoulos AN and Lerner RA. An agonist antibody that blocks autoimmunity by inducing anti-inflammatory macrophages. FASEB J 2016; 30: 738-747.
[14] Weber J, Braun CJ, Saur D and Rad R. In vivo functional screening for systems-level integrative cancer genomics. Nat Rev Cancer 2020; 20: 573-593.
[15] Chow RD and Chen S. Cancer CRISPR screens in vivo. Trends Cancer 2018; 4: 349-358.
[16] Andre AS, Moutinho I, Dias JNR and Aires-da-Silva F. In vivo phage display: a promising selection strategy for the improvement of antibody targeting and drug delivery properties. Front Microbiol 2022; 13: 962124.
[17] Lin CW and Han KH. Antibody libraries as platforms to exploring target and receptor pleiotropy. Israel Journal of Chemistry 2023; 63.
[18] Lee EJ, Baek SH, Song CH, Choi YH and Han KH. Agonist (P1) antibody converts stem cells into migrating beta-like cells in pancreatic islets. J Microbiol Biotechnol 2022; 32: 1615-1621.
[19] Kang S, Park HW and Han KH. Antibodies regulate dual-function enzyme IYD to induce functional synergy between metabolism and thermogenesis. Int J Mol Sci 2022; 23: 7834.
[20] Han KH, Arlian BM, Lin CW, Jin HY, Kang GH, Lee S, Lee PC and Lerner RA. Agonist antibody converts stem cells into migrating brown adipocyte-like cells in heart. Cells 2020; 9: 256.
[21] Lin CW, Xie J, Zhang D, Han KH, Grande G, Wu NC, Yang Z, Yea K and Lerner RA. Immunity against cancer cells may promote their proliferation and metastasis. Proc Natl Acad Sci U S A 2020; 117: 426-431.
[22] Zhu S, Guo Y, Zhang X, Liu H, Yin M, Chen X and Peng C. Pyruvate kinase M2 (PKM2) in cancer and cancer therapeutics. Cancer Lett 2021; 503: 240-248.
[23] Zahra K, Dey T, Ashish, Mishra SP and Pandey U. Pyruvate kinase M2 and cancer: the role of PKM2 in promoting tumorigenesis. Front Oncol 2020; 10: 159.
[24] Zhong X, He X, Wang Y, Hu Z, Huang H, Zhao S, Wei P and Li D. Warburg effect in colorectal cancer: the emerging roles in tumor microenvironment and therapeutic implications. J Hematol Oncol 2022; 15: 160.
[25] Zhou S, Li D, Xiao D, Wu T, Hu X, Zhang Y, Deng J, Long J, Xu S, Wu J, Li G, Peng M and Yang X. Inhibition of PKM2 enhances sensitivity of olaparib to ovarian cancer cells and induces DNA damage. Int J Biol Sci 2022; 18: 1555-1568.
[26] Yin C, Lu W, Ma M, Yang Q, He W, Hu Y and Xia L. Efficacy and mechanism of combination of oxaliplatin with PKM2 knockdown in colorectal cancer. Oncol Lett 2020; 20: 312.
[27] Zhang Y, Li L, Liu Y and Liu ZR. PKM2 released by neutrophils at wound site facilitates early wound healing by promoting angiogenesis. Wound Repair Regen 2016; 24: 328-336.
[28] Jiang Y, Wang Y, Wang T, Hawke DH, Zheng Y, Li X, Zhou Q, Majumder S, Bi E, Liu DX, Huang S and Lu Z. PKM2 phosphorylates MLC2 and regulates cytokinesis of tumour cells. Nat Commun 2014; 5: 5566.
[29] Wang LY, Liu YP, Chen LG, Chen YL, Tan L, Liu JJ, Jazag A, Ren JL and Guleng B. Pyruvate kinase M2 plays a dual role on regulation of the EGF/EGFR signaling via E-cadherin-dependent manner in gastric cancer cells. PLoS One 2013; 8: e67542.
[30] Han H, Zhang Y, Peng G, Li L, Yang J, Yuan Y, Xu Y and Liu ZR. Extracellular PKM2 facilitates organ-tissue fibrosis progression. iScience 2021; 24: 103165.
[31] Kim H, Kim SH, Hwang D, An J, Chung HS, Yang EG and Kim SY. Extracellular pyruvate kinase M2 facilitates cell migration by upregulating claudin-1 expression in colon cancer cells. Biochem Cell Biol 2020; 98: 219-226.
[32] Yang P, Li Z, Wang Y, Zhang L, Wu H and Li Z. Secreted pyruvate kinase M2 facilitates cell migration via PI3K/Akt and Wnt/beta-catenin pathway in colon cancer cells. Biochem Biophys Res Commun 2015; 459: 327-332.

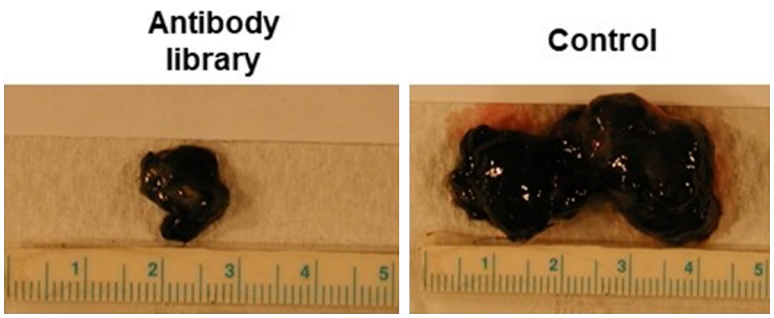

Figure S1. Antibody Selection on melanoma cells in vivo. B16F10 melanoma cells were then infected with this antibody library in vitro and cultured for two days. Subsequently, the cells were subcutaneously transplanted into C57BL/6 mice and allowed to grow for three weeks. Afterward, the melanoma cells were harvested and measured tissue size.

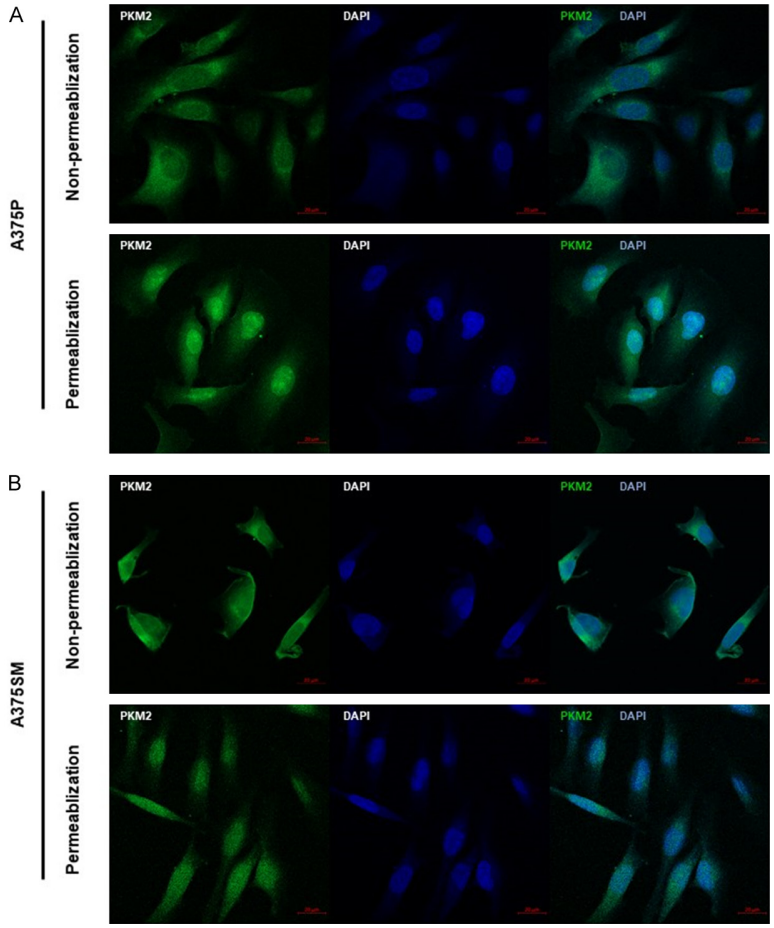

Figure S2. Immunocytochemical detection of M1 antibody binding to PKM2 in A375P and A375SM cells. Immunocytochemistry was used to visualize the binding of the M1 antibody to PKM2 (labeled with FITC, green) and to stain cell nuclei (DAPI, blue) in A375P and A375SM human melanoma cells, with and without permeabilization. The M1 purified antibody was used as the primary antibody, followed by a FITC-conjugated Mouse Anti-Human IgG1 (Fc-region) secondary antibody (Alpha Diagnostics, USA). DAPI-containing mounting medium (Thermo, USA) was used for nuclear staining. In cells that were not permeabilized, PKM2 staining was primarily seen at the cell surface or membrane. However, in permeabilized cells, a stronger signal was observed within the cells, clearly showing PKM2 localization in both the cytoplasm and the nucleus. All images were acquired using a Zeiss LSM 900 confocal microscope. Scale bar = 20 μm.

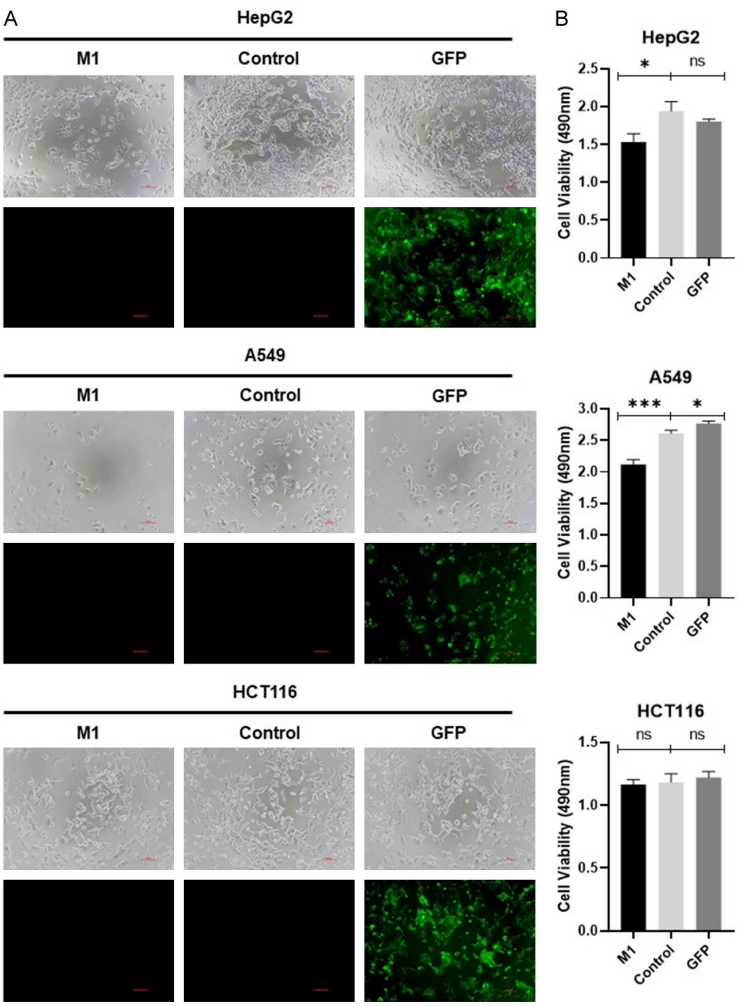

Figure S3. M1 antibody suppresses cell proliferation in multiple cancer cell lines. A, B. Cell proliferation assays (MTS) were performed on HepG2 (liver), A549 (lung), and HCT116 (colon) cancer cell lines following treatment with the M1 antibody. Significant inhibition of cell growth was observed in HepG2 and A549 cells, while HCT116 cells exhibited no significant difference compared to the control. Data are presented as mean ± s.e.m. from at least three independent experiments. Statistical significance was assessed using multiple t-tests and one-way ANOVA. NS, not significant; *P < 0.05; **P < 0.01; ***P < 0.001.