Supporting Information

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SI Materials and Methods

Histopathological Procedures and Analysis. Normal C57BL/6J or melanoma-bearing mice were euthanized, photographed, and dissected. Brain, lungs, liver, spleen, pinnae, tail, melanomas, and lymph nodes were isolated, cut into small cubes, and immediately frozen on dry ice. A collection of fresh tissue samples from each mouse was embedded in optimum cutting temperature (O.C.T.) compound (Tissue-Tek, VWR) and stored at −80 °C before sectioning. The composite tissue blocks were sectioned using a cryostat (Leica Microsystems Inc.) at 6- to 10-μm thickness and were thaw-mounted on glass slides (Fisher Scientific). The sections were fixed in 100% acetone for 10 min, air-dried, rehydrated, and hematoxylin and eosin (H&E)-stained, followed by dehydration in sequential ethanols and xylene. The slides were mounted in Permount (Fisher Scientific). Histologic slides were examined and photographed using Zeiss microscopes equipped with a CCD or digital camera. For melanin-bleached tissue sections, paraffin sections were rehydrated and treated with 1% potassium permanganate for 30–60 min, followed by discolorization with 1% oxalic acid for 5 min. The bleached slides were then H&E-stained.

RT-PCR for Transgenic Mice. Fresh-frozen tissues were pulverized, and the tissue powders were either stored at −80 °C for later use or immediately lysed in RNA lysis buffer. Total RNA was isolated following the protocol of the RNeasy spin kit with two additional steps: (*i*) the use of the Qia shredder spin column to eliminate insoluble pieces of tissues and (*ii*) DNase I treatment of the RNA purification columns to remove any residual amounts of genomic DNA. Approximately 1 μg of total RNA was used for PCR and one-step RT-PCR for both rat mGluR5 and mouse β-actin control transcripts, according to the protocol of QIAGEN's OneStep RT-PCR kit. The primers for mGluR5 were 5′-CAG TCA GCC TCA GTG CCA CAG TGG CCC TGG-3′ and 5′-GCG CGG CCT GGC AGC CGC GGG GAA GCT CTC-3′. β-Actin–positive control primers flanked intron/exon junction areas to prevent them from annealing to genomic β-actin sequence in case there was any DNA contamination in the total RNA preparation. The primers were 5′-CCT TCT TGG GTA TGG AAT-3′ and 5′-AGG AGC AAT GAT CTT GAT-3′. Reverse transcriptase reaction was set at 50 °C for 30 min, followed by heating at 95 °C for 15 min for inactivation of reverse transcriptase and activation of TaqGold. PCR cycling for mGluR5 consisted of 30 cycles of 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min; and PCR cycling for β-actin was the same except the annealing temperature was 50 °C. The PCR products were detected in 1.5% agarose gel and verified by DNA sequencing after being subcloned to a TA cloning vector (pCRII, Invitrogen).

Western Blotting. The Bullet Blender (Next Advance) was used for lysing ear, tail, and brain tissues at 4 °C in lysis buffer (50 mM Tris·HCl, 150 mM NaCl, 2% Triton X-100, 1% SDS, 10 mM EDTA, and 10% glycerol) with complete protease inhibitor mixture tablets (Roche Applied Science). After quantification of the protein concentration of each sample with Pierce BCA protein assay reagent (Thermo Scientific), the lysates were subject to SDS/PAGE and Western blotting with the indicated antibodies. The bands were visualized with the SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific). Myc, phospho ERK1/2, ERK1/2, and α-tubulin antibodies were purchased from Cell Signaling Technology. EGFR, PCNA, and β-actin antibodies were purchased from Abcam, Santa Cruz Biotechnology, and Applied Biological Materials, respectively.

Fluorescence-Based Immunohistochemistry. The tumor tissue samples were embedded in O.C.T. (Tissue-Tek, VWR) and stored at −80 °C before sectioning, and the tissue blocks were sectioned using a cryostat (Leica Microsystems Inc.) at 6- to 10-μm thickness and were thaw-mounted on glass slides (Fisher Scientific, Inc.). The sections were fixed in 100% acetone for 10 min, air-dried, and rehydrated. Then the sections were permeabilized and blocked with 10% goat serum in PBS and then incubated with rabbit anti-Myc antibody (Sigma). Alexa568-conjugated goat anti-rabbit secondary antibody (Invitrogen) and Hoechst were incubated for detection of signals.

Quantitative RT-PCR. Human melanoma cell lines were kindly provided by Dr. Nicholas P. Restifo at the National Cancer Institute/National Institutes of Health (Bethesda, MD), and human metastatic melanoma tissue samples were purchased from OriGene, Inc. The human melanoma samples were collected from patients with metastatic melanoma, and they are all histopathologically diagnosed and verified. O.C.T. blocks of fresh-frozen melanoma tissue were made by snap-freezing in liquid nitrogen and stored at −80 °C before use.

Total RNA was purified using Invitrogen's RNeasy mini kit following the manufacturer's manual with modifications. In brief, ~5×10^6 human melanoma cells and 30 mg of pulverized melanoma tissue were lysed in RNA lysis buffer, and the insolubles were eliminated through a Qia Shredder spin column. Total RNA was eluted in 50 μL of RNase-free water via a RNeasy spin column pretreated with DNase I. Total RNA concentration was measured in a range of 0.5 μg/μL. Reverse transcription (RT) was then performed by mixing 10 μL of 2× RT master mix with 1–2 μg/10 μL of total RNA and by incubating at 37 °C for 2 h using ABI's High-Capacity cDNA RT Kit and protocol. The cDNAs were stored at −80 °C before use.

To quantify mRNA expression level in human melanomas, real-time RT-PCR was carried out using ABI's StepOne PCR machine, Power SYBR-GREEN kit and protocol, and intron-flanking primers designed to eliminate any chance of genomic DNA use in the reaction. Primer sequences used are the following: human mGluR1 (5′-gtggtttgatgagaaaggag-3′ and 5′-gttgctccactcaagatagc-3′), human mGluR5 (5′-tggagatacgatcctattcg-3′ and 5′-ccaaggcaggcaaacaccac-3′), and human β-actin (5′-ccttcctgggcatggagt-3′ and 5′-aggagcaatgatcttgat-3′). Approximately 1 μg of cDNA, 20 pmol of each primer, and 15 μL of SYBR-GREEN were subjected to a 30-μL real-time PCR with 40 cycles of 1 min at each step of 94 °C, 55 °C, and 72 °C. Human brain and normal human melanocyte cDNAs were also tested as controls side by side with cDNAs from human melanoma cell lines and tissue samples. The PCR products were all confirmed to be a single band in agarose gels, and each reaction was done in duplicate. Relative Ct values were calculated for each sample when the cycle threshold of mGluR1 and mGluR5 were normalized or adjusted with that of β-actin in the same sample.

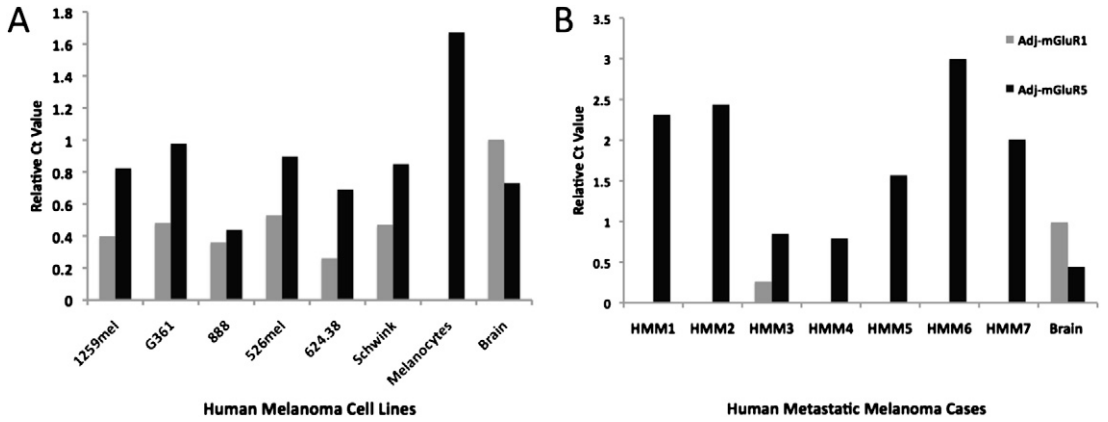

Fig. S1. Real-time RT-PCR of mGluR1 and mGluR5 in human melanoma cell lines and metastatic melanoma tissue. Human mGluR5 was detected in both human melanoma cell lines and metastatic melanoma tissue by quantitative RT-PCR. Although the amount of mGluR1 was higher than that of mGluR5 in human brain tissue ("Brain," *A* and *B*), mGluR5 was generally higher than mGluR1 in melanoma cell lines (*A*) as well as in normal human melanocytes ("Melanocytes," *A*), in which mGluR1 was not detected. In addition, mGluR5 was highly expressed in all seven human metastatic melanomas (*B*, HMM1–7), whereas mGluR1 was positive in only one of seven samples (*B*, HMM3). The expression level of mGluR1 and mGluR5 was normalized to the level of β-actin for each sample tested.