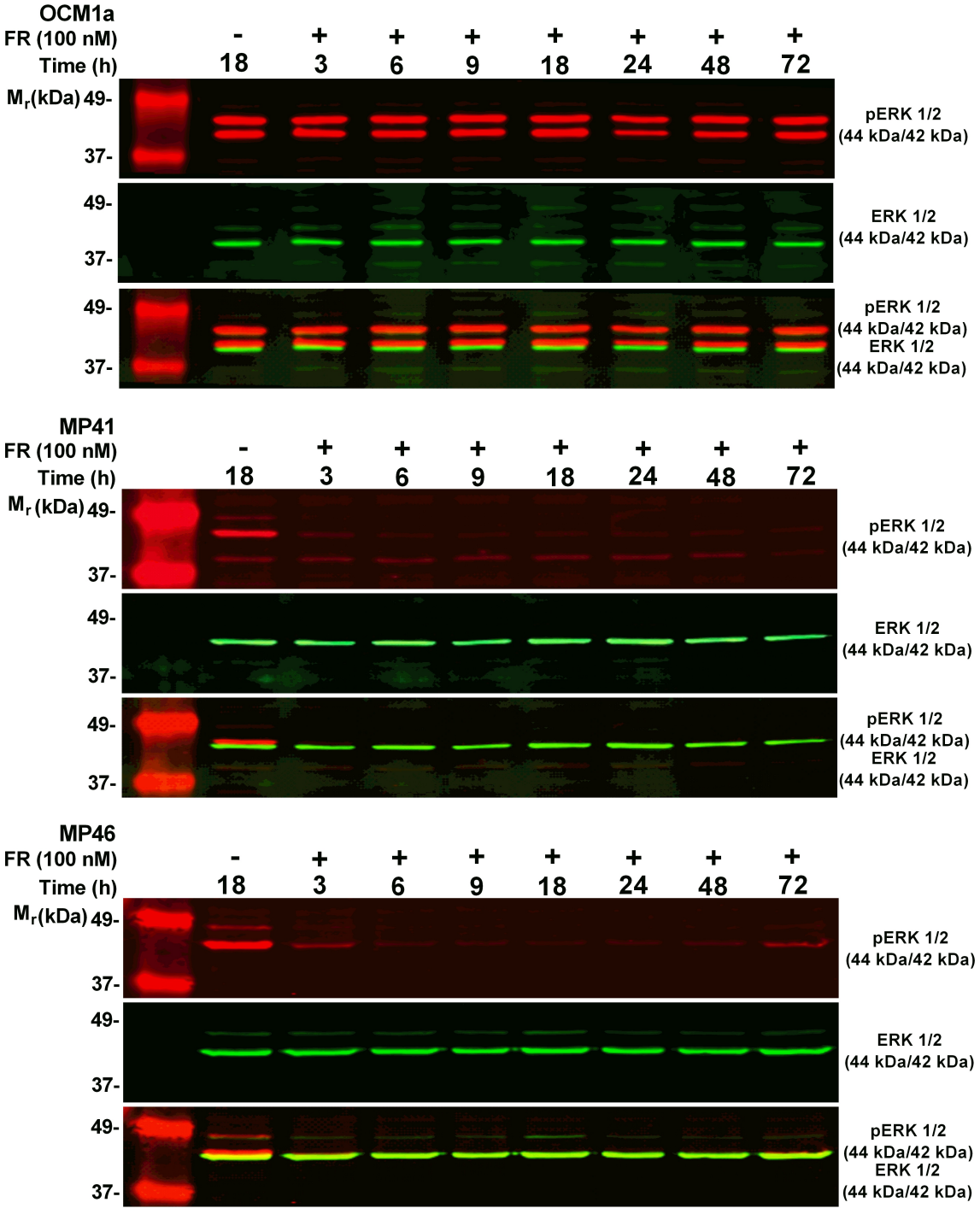
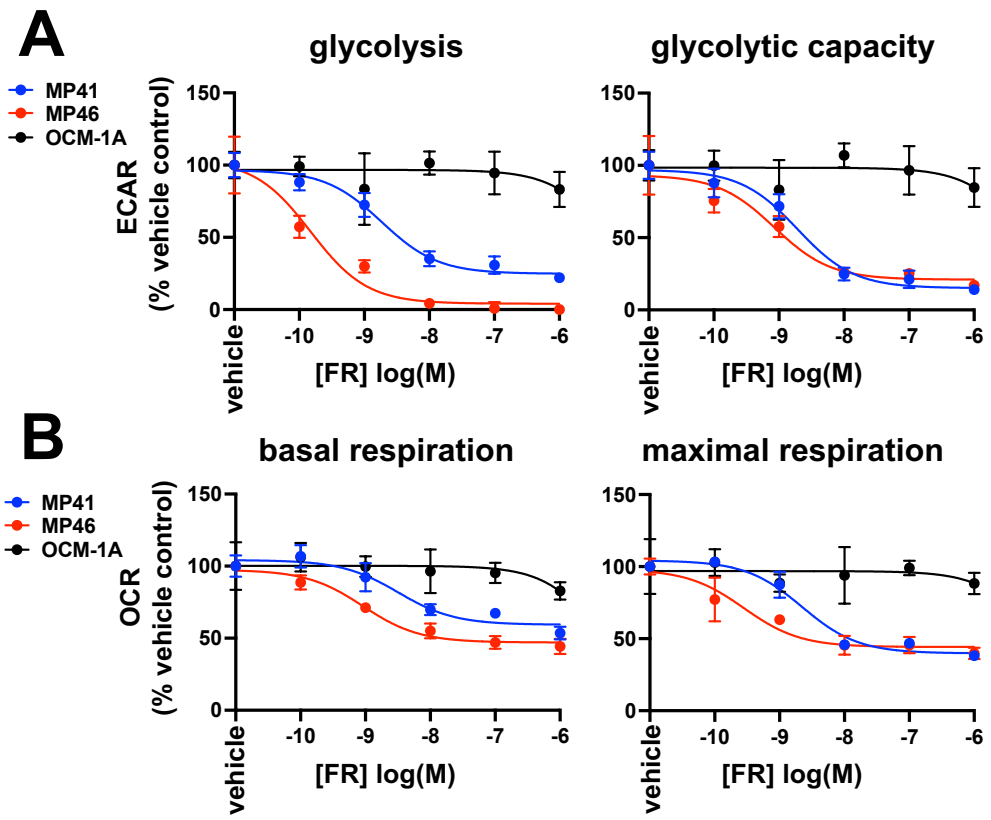
Supplemental Figure S1



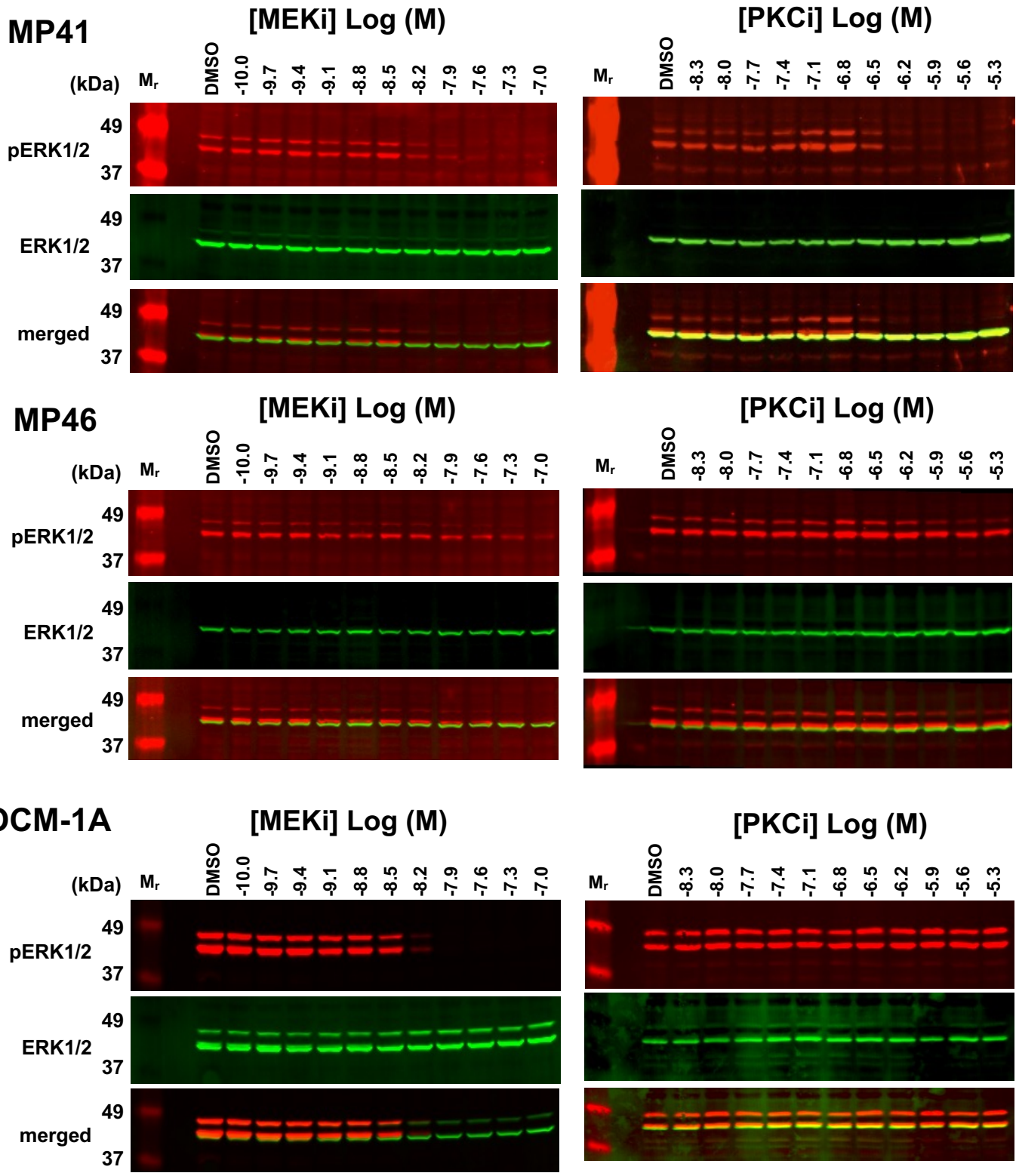
Supplemental Figure S1. ERK immunoblots for FR time course. Representative blots from three independent experiments. MP41, MP46, and OCM-1A cells were treated with 100 nM FR for the indicated time, and then lysed to collect protein. Lysates were subjected to PAGE and immunoblotted for pERK1/2 (44 kDa / 42 kDa; red) or total ERK1/2 (44 kDa / 42 kDa; green). Imaging and fluorescence intensity measurements were performed on a LI-COR Odyssey system.

Supplemental Figure S2



Supplemental Figure S2. FR dose response curves for UM cell metabolism. Representative graphs from three independent experiments. MP41, MP46, and OCM-1A cells were treated with the indicated concentrations of FR or vehicle (DMSO) for 18 h prior to Seahorse analysis. ECAR and OCR values were normalized to cell number per well based on DAPI staining, and then values for each cell line were normalized to vehicle control for that line. A. Graphs show glycolysis and glycolytic capacity as measured using the glycolytic stress test on the Seahorse analyzer. B. Graphs show basal and maximal respiration as measured using the mitochondrial stress test on the Seahorse analyzer. Gq/11-driven MP41 and MP46 cells show dose-dependent response to FR within a nanomolar dose range. No dose-response is seen in Gq/11-wildtype OCM-1A cells.

Supplemental Figure S3



Supplemental Figure S3. ERK immunoblots for MEKi and PKCi dose curve. Representative blots from four independent experiments. MP41, MP46, and OCM-1A cells were treated with MEKi or PKCi at the indicated doses for 18 hours, and then lysed to collect protein. Lysates were subjected to PAGE and immunoblotted for pERK1/2 (44 kDa / 42 kDa; red) or total ERK1/2 (44 kDa / 42 kDa; green). Imaging and fluorescence intensity measurements were performed on a LI-COR Odyssey system.

Supplemental Table S1

Cell line name	Driver oncogene	Molecular class	Source	Research Resource Identifier (RRID)
MP41	*GNA11*(Q209L)	1	UM PDX	CVCL_4D12
MP46	*GNAQ*(Q209L)	2 (BAP1-null)	UM PDX	CVCL_4D13
92.1	*GNAQ*(Q209L)	1	UM	CVCL_8607
Mel202	*GNAQ*(Q209L)	1	UM	CVCL_C301
Mel270	*GNAQ*(Q209P)	1	UM	CVCL_C302
OCM-1A	*BRAF*(V600E)	1	UM	CVCL_6934
A375	*BRAF*(V600E)	n/a	CM	CVCL_0132
MeWo	*NF1*-null	n/a	CM	CVCL_0445
SK-mel-2	*NRAS*(Q61R)	n/a	CM	CVCL_0069

Supplemental Table S4. Cell lines used for this project. The driver oncogenic mutation is given for each cell line. Gene expression class (tumor grade) is given for cell lines derived from UM tumors. MP41 and MP46 were initially derived as patient-derived xenografts in mice and then moved to cell culture. All other cells lines were derived initially in culture.

Supplemental Table S2

Materials and Reagents		
Description	Company	Catalog Number
Internal Standards		
13C3 Sodium Pyruvate	Cambridge Isotope Laboratories, Inc.	CLM-2440-0.5
13C3 Sodium Lactate	Cambridge Isotope Laboratories, Inc.	CLM-1579-0.5
13C6 Citric Acid	Cambridge Isotope Laboratories, Inc.	CLM-9021-PK
13C4 alpha-ketoglutaric acid sodium salt	Cambridge Isotope Laboratories, Inc.	CLM-4442-0.1MG
13C4 Succinic acid	Cambridge Isotope Laboratories, Inc.	CLM-1571-0.1MG
13C4 Fumaric acid	Cambridge Isotope Laboratories, Inc.	CLM-1529-0.1MG
13C4 L-Malic acid	Cambridge Isotope Laboratories, Inc.	CLM-8065-0.1MG
Stable isotope labeled canonical amino acid mix	Cambridge Isotope Laboratories, Inc.	MSK-CAA-1
U-13C16 Sodium Palmitate	Cambridge Isotope Laboratories, Inc.	CLM-6059-1
Derivatization materials		
Methoxyamine hydrochloride	Sigma-Aldrich	226904-1G
Pyridine anhydrous 99.8%	Sigma-Aldrich	270970-25ML
MTBSTFA (with 1% t-BDMCS)	Sigma-Aldrich	M-108-5x1ML
GC vials and inserts		
Vial inserts	National	C4011-631
Clear glass autosampler vials	National	C4000-1
Vial caps	National	C5000-51B
GC-MS instrumentation		
Agilent 7890A gas chromatograph	Agilent	
Agilent 5975C mass spectrometer	Agilent	
ChemStation E.02.02.1431	Agilent	
BCA Assay		
Pierce BCA Protein Assay Kit	Thermo Fisher	23227
RIPA Lysis Buffer system	Santa Cruz Biotechnology	Sc-24948A

Supplemental Table S5. Materials and reagents used for GC-MS experiments. Sources are listed for reagents and equipment used in this study. Catalog numbers are included for reagents.

Supplemental Table S3

Metabolite ions monitored for FR900359 studies	
Metabolites (IS in bold)	**Ions**
Pyruvate, **$^{13}C_3$ Pyruvate**	174.0, **177.0**
Lactate, **$^{13}C_3$ Lactate**	261.1, **264.1**
Citrate, **$^{13}C_6$ Citrate**	459.3, **465.3**
alpha-ketoglutaric acid, **$^{13}C_4$ alpha-ketoglutaric acid**	346.1, **350.1**
Succinic acid, **$^{13}C_4$ Succinic acid**	289.1, **293.1**
Fumaric acid, **$^{13}C_4$ Fumaric acid**	287.1, **291.1**
Malic acid, **$^{13}C_4$ Malic acid**	419.3, **423.3**

Supplemental Table S6. Metabolite ions monitored for FR responses.
Ion values are listed for each metabolite and its labeled controls (bold).