Table S1. Sensitivity of parental and BR melanoma cell lines to ADI-PEG20

Cell line	BRAF (V600E)	ASS1 level [a]	IC50 of ADI-PEG20[b]	Caspases activity (%)[c]	IC50 of BRAFi[b]	IC50 of MEKi
A375	Mut	(-) → (-)	181±10.5 ng/ml	27.2±3.1%	0.5±0.2 μM	9.8±1.1 nM
A375BR	Mut	(-) → (-)	68±8.2 ng/ml	50.3±7.8%	10.2±0.5 μM	5.6±0.8 nM
A375BMR	Mut	(-) → (-)	59± 6.6 ng/ml	55.1±6.3%	> 10 μM	17.1±2.1 nM
A2058	Mut	(-) → (+)	425±18.5 ng/ml	3.2±0.5%	5.1±0.4 μM	19±2.1 nM
A2058BR	Mut	(-) → (-)	125±7.8 ng/ml	27.9±4.8%	25±1.3 μM	15.3± 1.4 nM
A2058BMR	Mut	(-) → (-)	105±4.5 ng/ml	33.4±8.2%	> 25 μM	33.1±1.8 nM
MEL-1220	Mut	(-) → (-)	215±12 ng/ml	2.6±1.1%	4.2±0.5 μM	7.8±1.2 nM
MEL-1220BR	Mut	(-) → (-)	62.5±3.3 ng/ml	26.5±3.3%	15.2±0.8 μM	5.5±0.6 nM
SK-MEL-28	Mut	(-) → (+)(+)	222±4.3 ng/ml	9.8±2.3%	2.5±0.8 μM	1.2±0.3 nM
SK-MEL-28BR	Mut	(-) → (+)	103±8.4 ng/ml	20.5±3.7%	10±1.3 μM	2.0±0.5 nM
SK-MEL-28BMR	Mut	(-) → (-)	78.9±6.3 ng/ml	31.4±2.2%	> 10 μM	5.2±1.1nM
UACC-62	Mut	(-) → (+)	168±7.3 ng/ml	2.9±1.5%	7.8±0.6 μM	2.5±0.3 nM
UACC-62BR	Mut	(-) → (-)	92±5.5 ng/ml	14.9±5.5%	17.2±2.1 μM	1.9±0.5 nM
BJ	Skin fibroblast	(+)(+)→(+)(+)	ND	ND	ND	ND

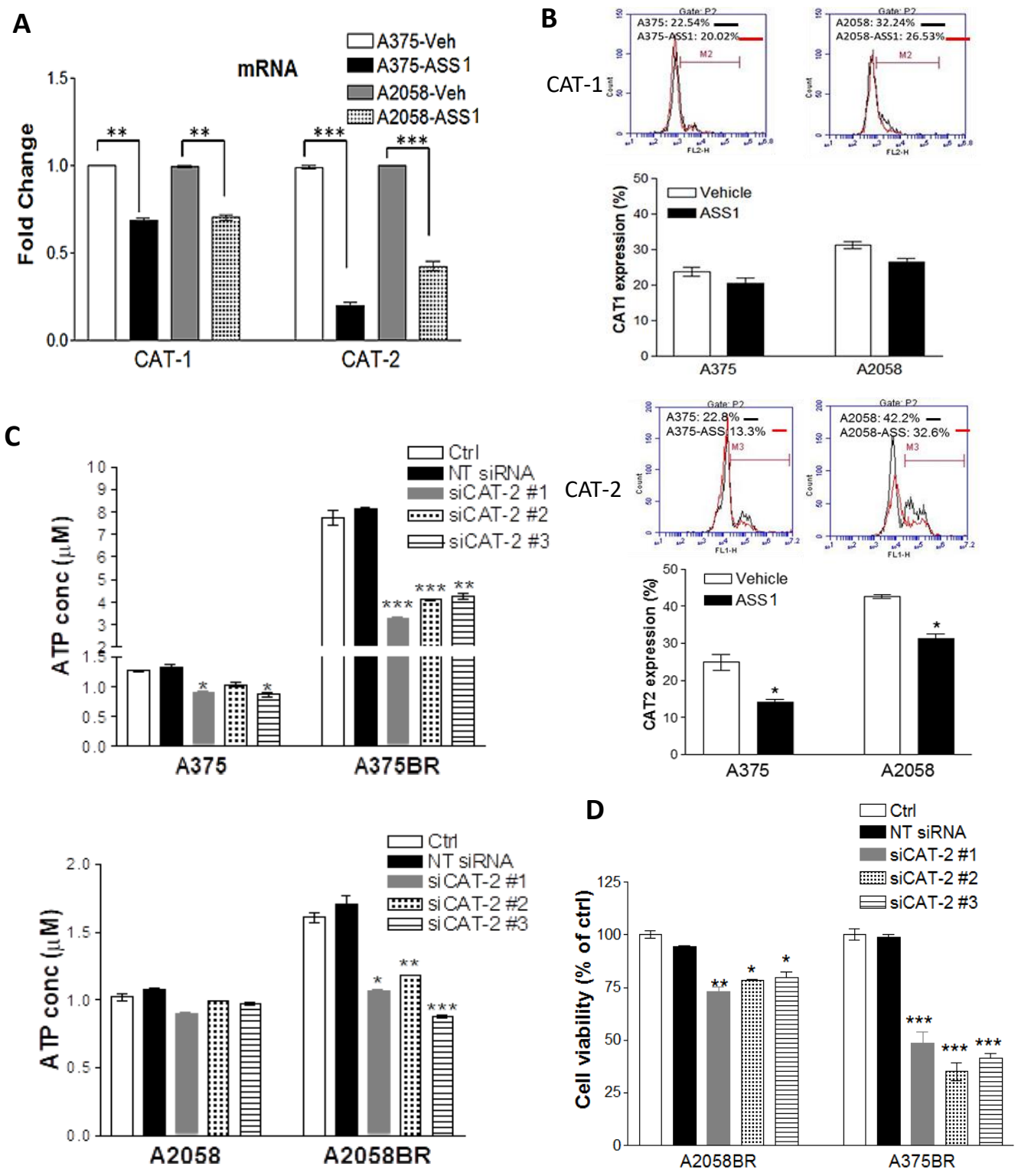
[a] The inducible ASS expression has been identified by qRT-PCR and immunoblotting following ADI-PEG20 treatment (100 ng/ml) for 48 hr.

[b] IC50 values are based on cell viability curves, corresponding to previous study (Li, et al., 2016).

[c] The percentages of caspase activity were quantified by FACS following ADI-PEG20 treatment (100 ng/ml) for 72 hr. The proportions of treatment groups are normalized by those of untreated groups.

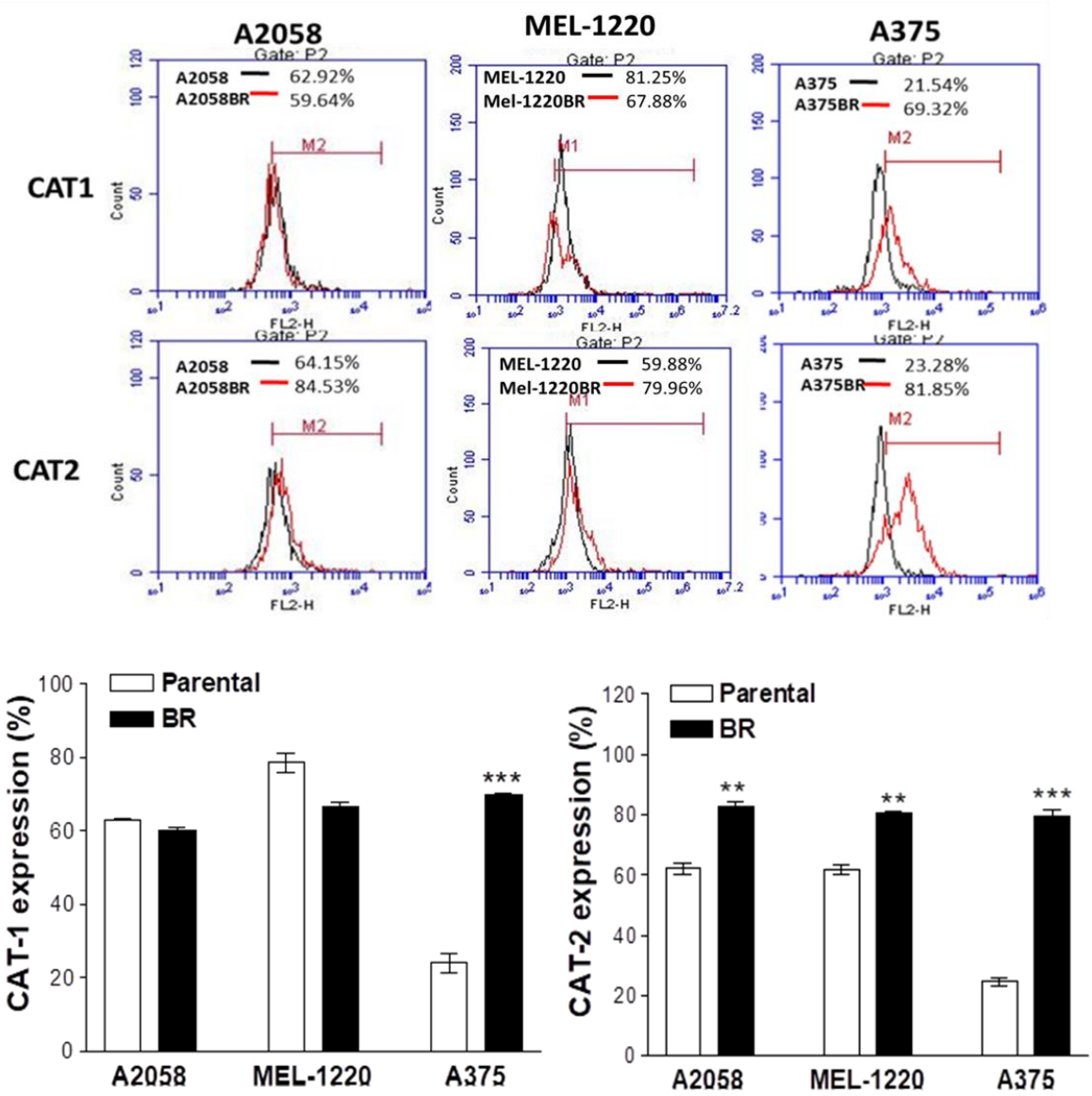
ND = Not detected, Mut = Mutant, BRAFi = vemurafenib, MEKi = trametinib

Suppl. Fig. 1



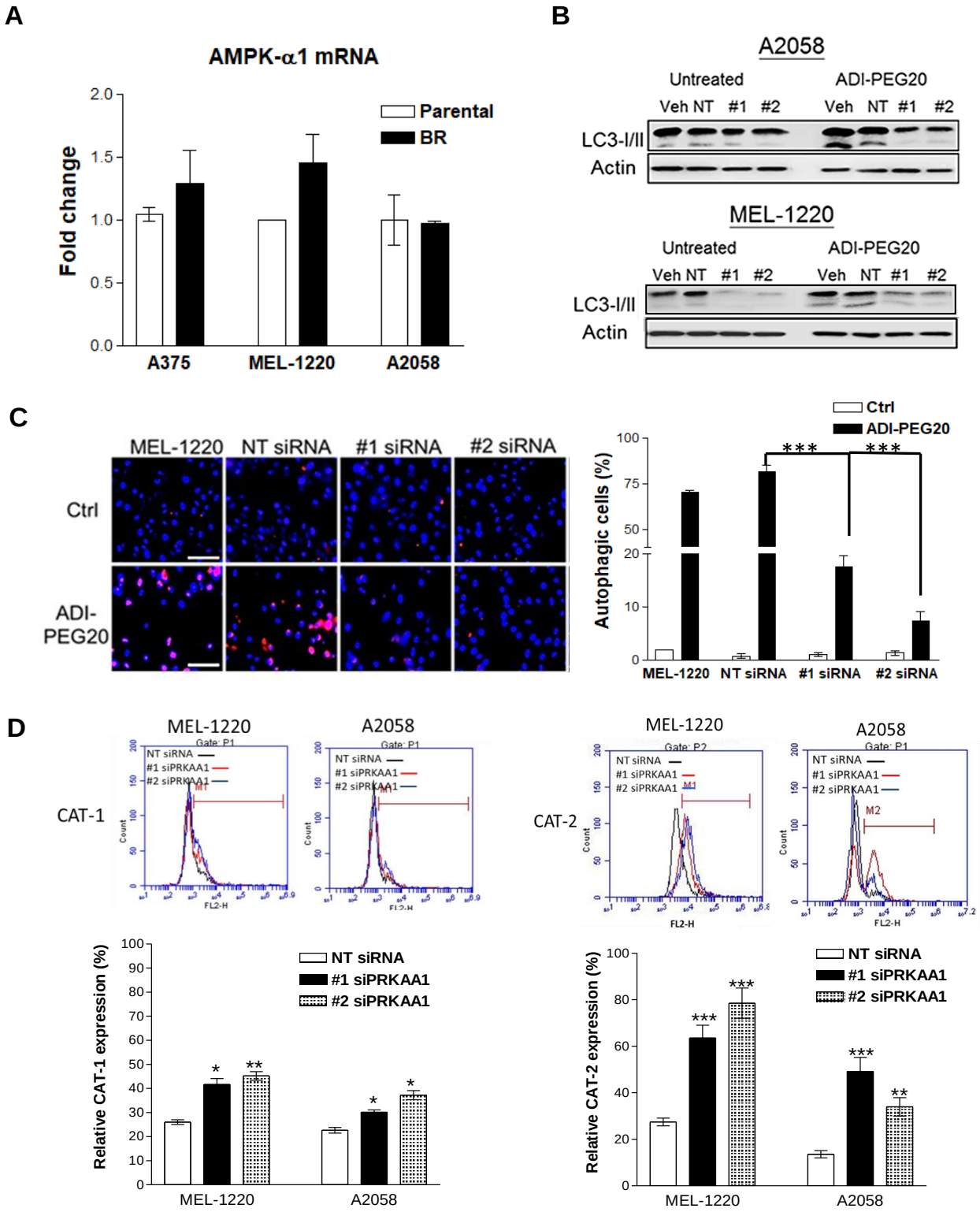
Suppl. Fig. 1. Arginine transporter CAT-2 is needed for ASS1-negative melanoma cells to obtain exogenous arginine for energy source. A375 and A2058 cells were transfected with the plasmid encoding ASS1 and vehicle plasmid, respectively. (A) CAT-1 and CAT-2 RNA levels were analyzed by qRT-PCR. (B) CAT-2 expression was detected by FACS and quantitated upon the percentage of right shift (M2 or M3 gate). (C) Knockdown of CAT-2 using 50 nM of siRNA (siCAT-2 #1, siCAT-2 #2, or siCAT-2 #3) was carried out in parental and BR cells for 48 hr and then their intracellular ATP concentration was detected. Intracellular ATP concentration was normalized by protein content. Non-targeting (NT) siRNA was used as a negative control. (D) The viability of BR cells was determined by MTT assay (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.005$).

Suppl. Fig. 2



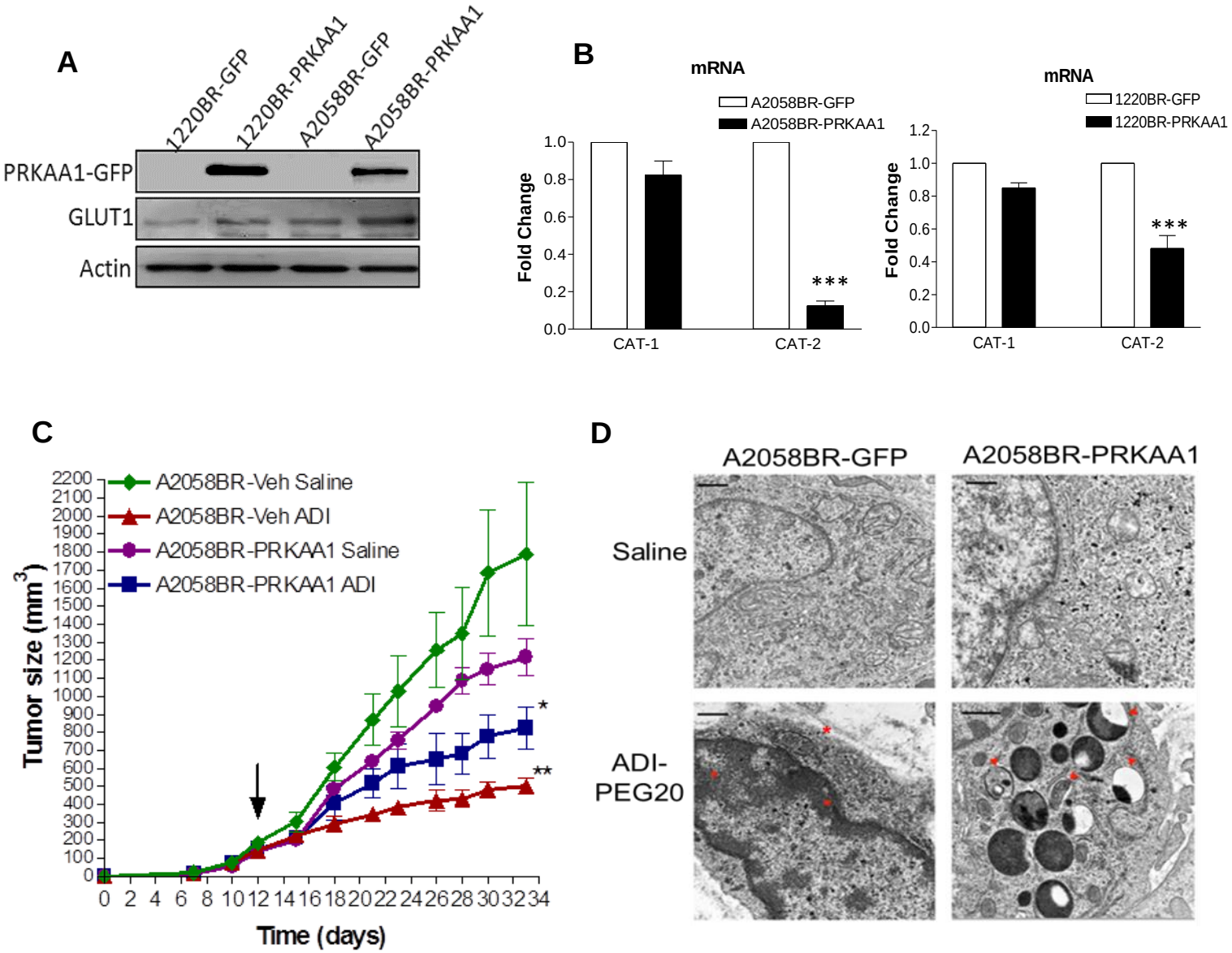
Suppl. Fig. 2. BRAFi resistant (BR) melanoma cells acquire more exogenous arginine by expressing higher levels of arginine transporter CAT-2 compared to their parental counterparts. Melanoma cells were incubated with anti-CAT-1 or anti-CAT-2 antibody and second antibody conjugated with Alexa Fluor® 555 and then analyzed by flow cytometry (FACS). The black and red curves in histograms respectively indicated parental and BR cells. The relative expression of CAT-1 or CAT-2 shown in bar graphs was based on the percentage of M1 or M2 gate. (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.005$).

Suppl. Fig. 3



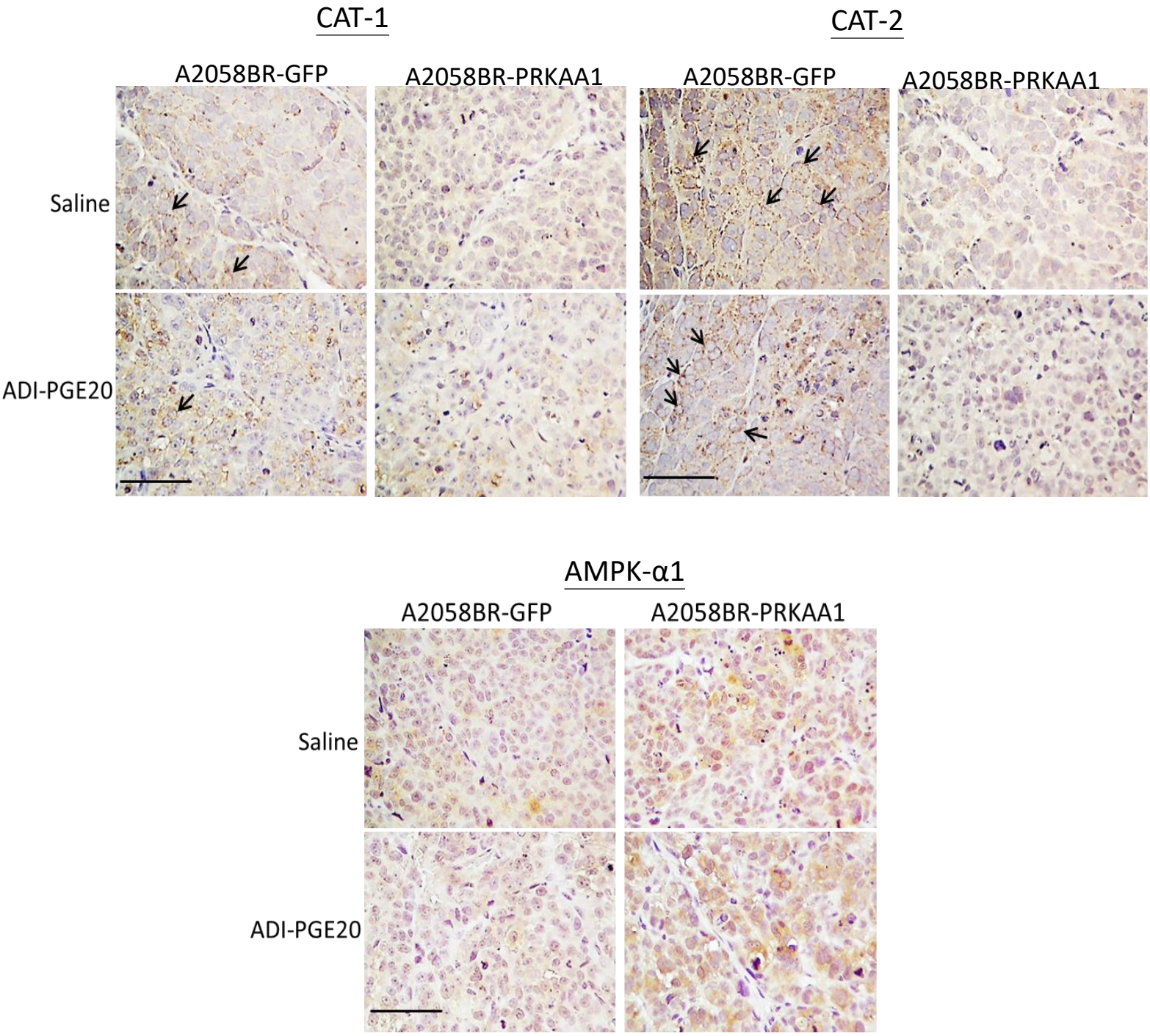
Suppl. Fig. 3. Silencing AMPK-α1 expression results in abrogation of autophagy and significant upregulation CAT-2 expression and slightly enhances CAT-1 expression in parental cells. (A) There was no significant difference of AMPK-α1 RNA levels between parental and BR cells. (B) A2058 and MEL-1220 cells were transfected with individual siRNAs against AMPK-α1 or a non-targeting (NT) siRNA (50 nM). After cultured in arginine free or completed medium (ctrl), the cell lysates of these transfectants were subjected for to immunoblot for detection of LC3-I/II expression. (C) The autophagosomes and nuclei in MEL-1220 transfectants were stained with lysotracker (red) and DAPI (blue), respectively (scale bar = 50 μm). Autophagy positive cells were quantitated and shown in a bar graph. (D) The levels of CAT-1 or CAT-2 of these transfectants were detected by anti-CAT-1 or anti-CAT-2 antibody and second antibody conjugated with Alexa Fluor® 555, and quantitated using FACS. Their relative expressions in bar graphs were based on the percentages of M1 or M2 gates in histograms (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.005$).

Suppl. Fig. 4



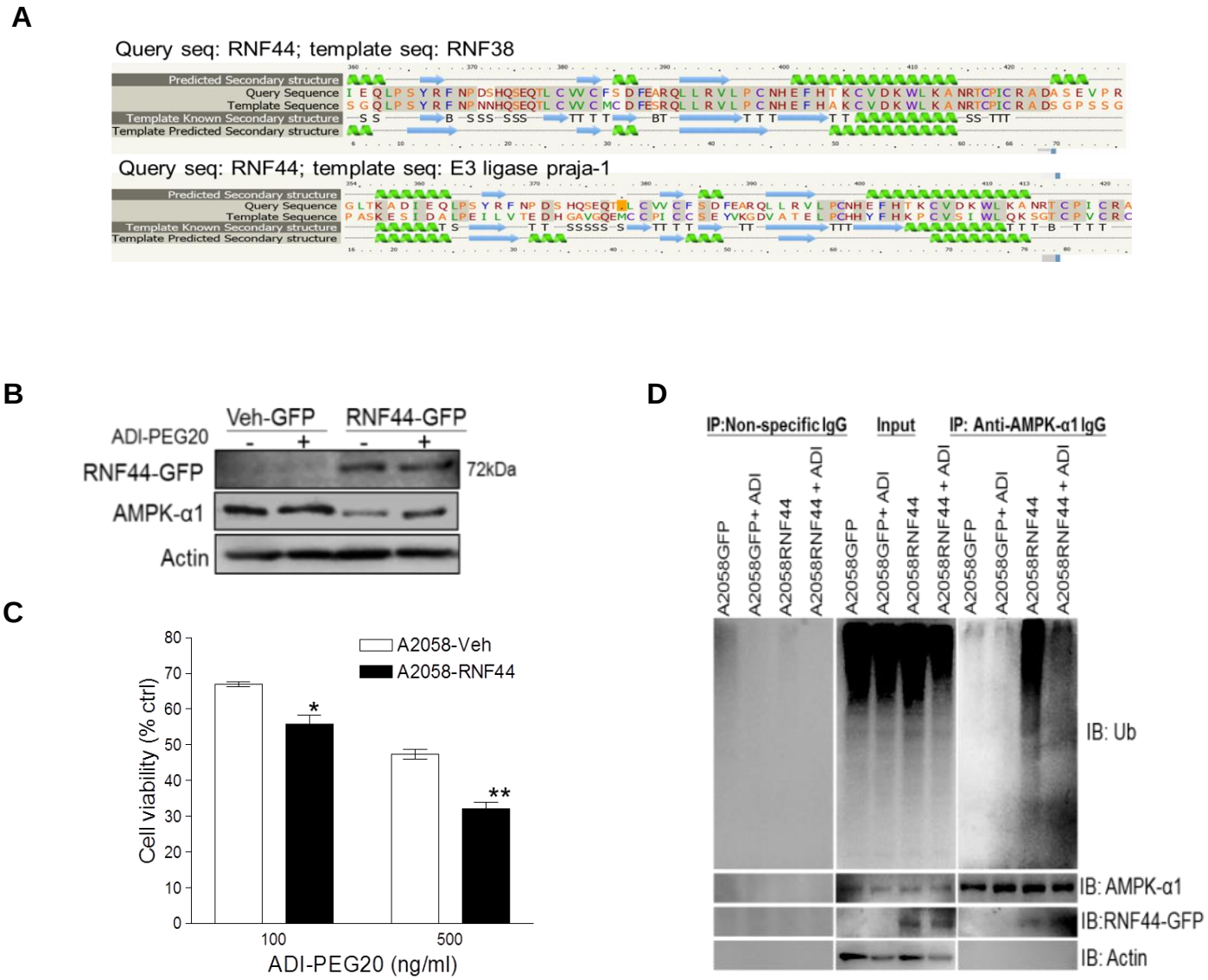
Suppl. Fig. 4. Overexpressing AMPK-α1 (PRKAA1)-GFP in BR cells restores the ability to undergo autophagy and switches acquisition of arginine to glucose. A2058BR and MEL-1220BR cells were transfected with plasmids containing AMPK-α1-GFP or GFP alone (vehicle). (A) The levels of AMPK-α1 and GLUT1 in transfectants were determined by immunoblotting. (B) CAT-1 and CAT-2 expressions were confirmed by qRT-PCR (C) 1 x 10^6 transfectants were injected into mice. Mice bearing A2058BR-GFP or A2058BR-PRKAA1 melanoma xenografts were treated with 100 ng/ml ADI-PEG20 or saline when the tumor volume reached around 100 nm^3. Tumor volumes were represented as mean ± SEM (n = 5); *p < 0.05, **p < 0.01, and ***p < 0.005. (D) *In vivo* cell death (red asterisks) and autophagy (red arrows) were visualized by TEM, 25000X. Scale bar = 500 nm.

Suppl. Fig. 5



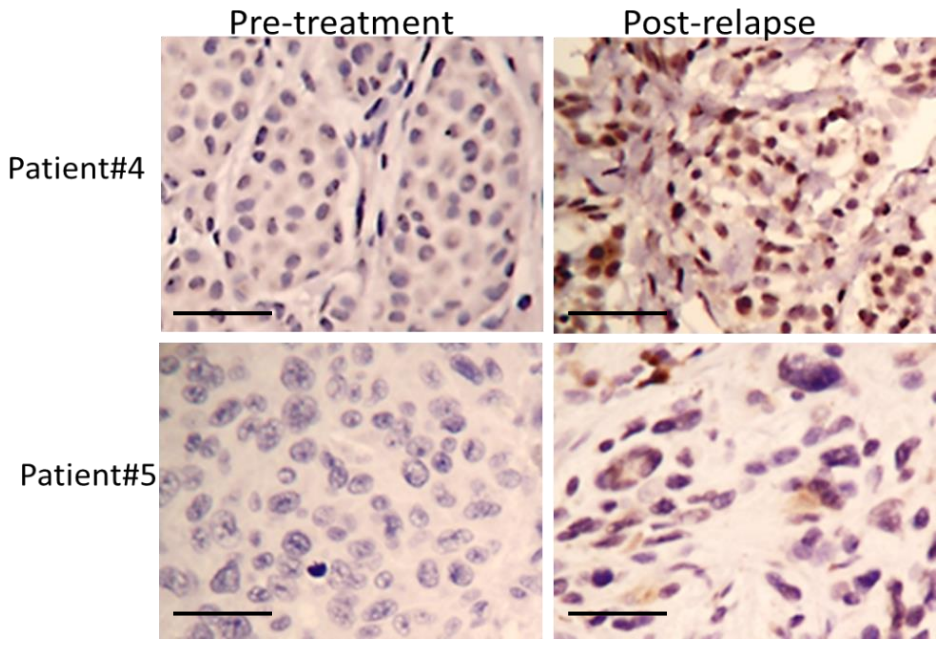
Suppl. Fig. 5. Mouse xenograft models demonstrate that overexpression of AMPK-α1 (PRKAA1) in A2058BR cells drastically reduces CAT-2 expression, corresponding to Suppl. Fig. 4. CAT-1 and CAT-2 expressions (black arrows) were detected by IHC staining (scale bar = 100 µm). A2058BR-GFP cells expressed high levels of CAT-2 and low levels of CAT-1. Decreased CAT-1 and CAT-2 expressions can be seen in A2058BR-PRKAA1 cells. AMPK-α1 staining was used to confirm PRKAA1 overexpression.

Suppl. Fig. 6



Suppl. Fig. 6. Overexpressing RNF44 in A2058 cells renders A2058 cells more sensitive to arginine deprivation due to enhanced ubiquitin-dependent AMPK-α1 degradation. (A) The partial protein sequence of RNF44 analyzed by a server Phyre2 and PDB database is similar to RNF38 and E3 ligase praja-1. (B) Overexpression of RNF44 was confirmed by immunoblotting. (C) Cell viability of A2058 transfectants which overexpress RNF44-GFP or GFP (vehicle, veh) were assayed by MTT following ADI-PEG20 (100 or 500 ng/ml) treatment for 72 hr (n = 3, *p < 0.05, **p < 0.01, and ***p < 0.005). (D) RNF44-GFP and ubiquitin (Ub) were co-immunoprecipitated with AMPK-α1, and subsequently analyzed by immunoblotting.

Suppl. Fig. 7



Suppl. Fig. 7. RNF44 expression appears in tumor tissues from patients who failed BRAFi (patient #4) or BRAFi/MEKi (patient #5) treatment, corresponding to Fig. 7C.RNF44 expression was detected by IHC staining (400 X) (scale bar = 50 μm).