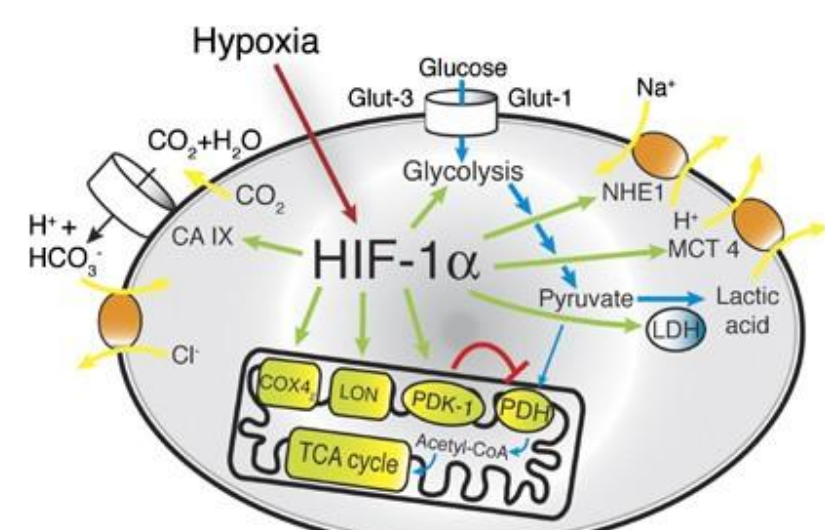




1. ABSTRACT

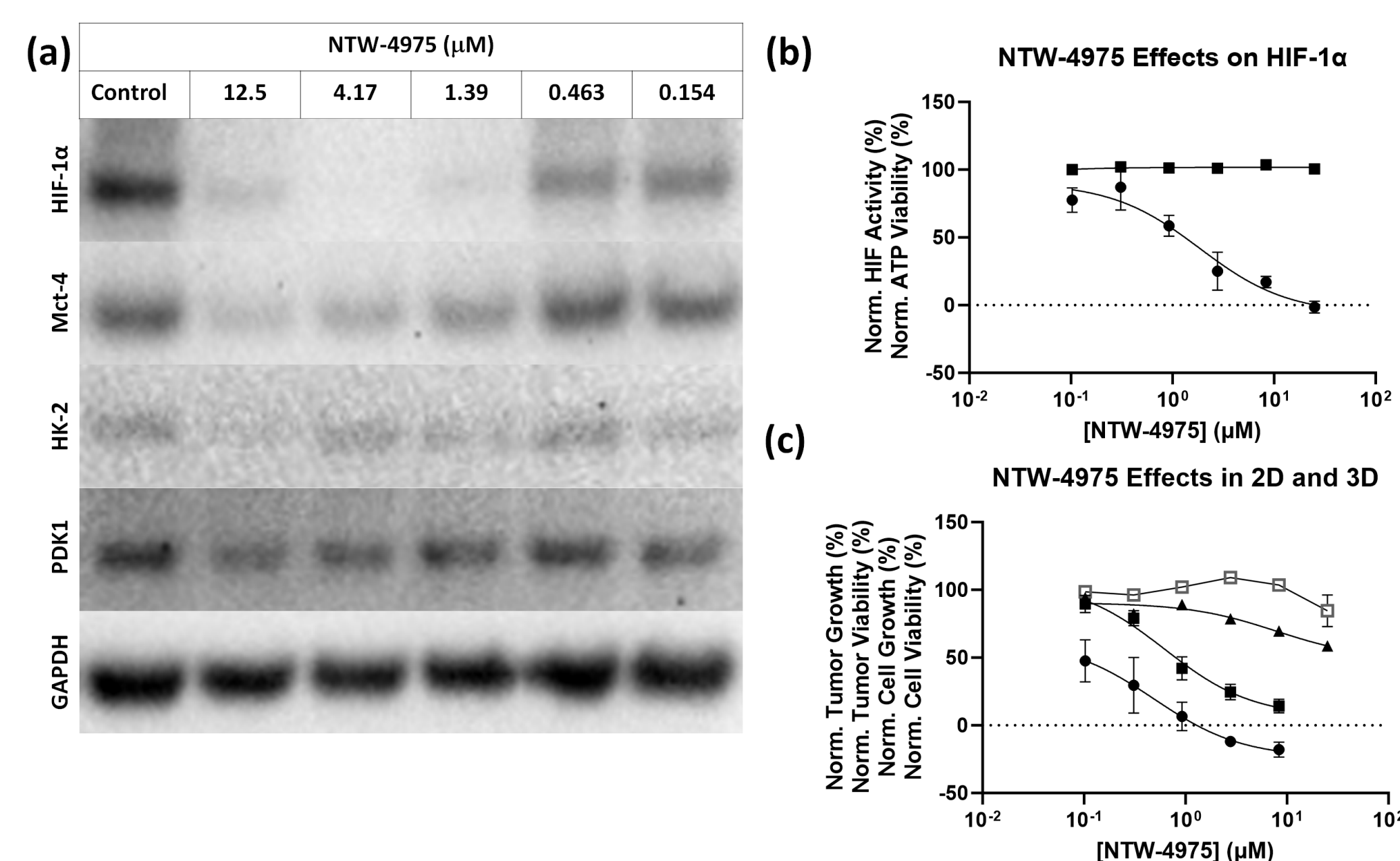
The monocarboxylic acid transporter 4 (Mct-4) is involved in the cellular response to hypoxia, as indicated by the hypoxic response element in its promoter region. Using a tumorsphere assay as an in vitro model for 3-dimensional cell proliferation, we identify a hypoxic response in the tumorsphere model which is distinct from that of cells grown under 2-dimensional normoxic conditions, as well as a key role for Mct-4 in enabling 3-dimensional growth. The tumorsphere model yields evidence of an essential role for Mct-4 in cell lines which were genetically modified to underexpress and overexpress Mct-4, evidence not apparent in a standard 2-D model of growth in the same cell lines. We show that the response to hypoxia may be circumvented by transfection with a CMV promoter driven Mct-4, which confers constitutive 3-D growth, wherein tumorsphere growth inhibition by small molecule HIF-1a inhibitors is mitigated. Finally, we identify a role for Mct-4 in cell migration using a chamber assay. Thus, the tumorsphere model may endeavor to provide a convenient, robust, and reproducible tool for elucidation of mechanisms of action underlying tumor growth and migration.



Weidemann, A., Johnson, R. Biology of HIF-1α. *Cell Death Differ* 15, 621–627 (2008).

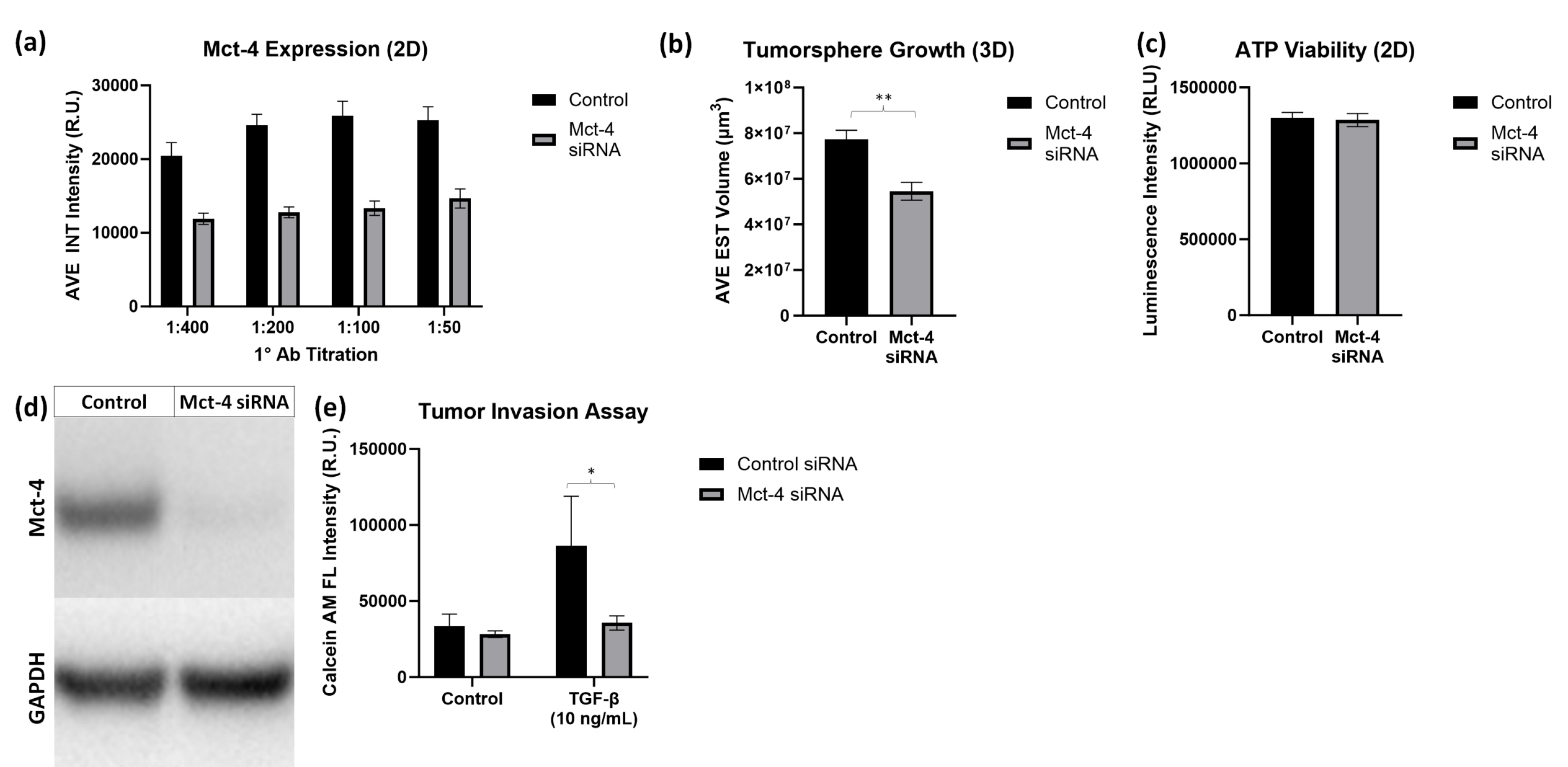


2. HYPOXIA INHIBITORY EFFECTS OF NTW-4975



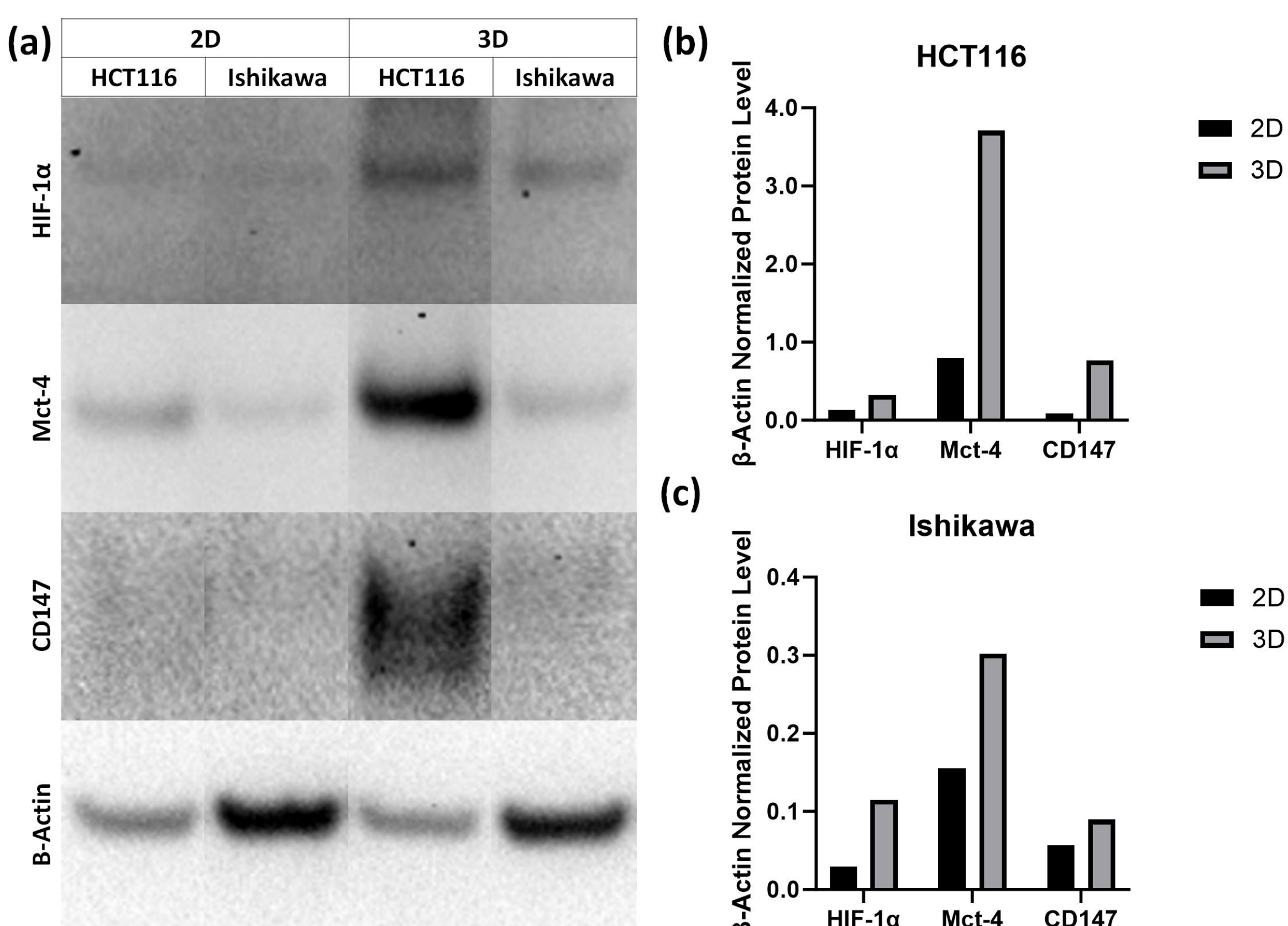
- (a) Inhibitory effects of hypoxia induced expression in Ishikawa cells with SDS-PAGE
- (b) HIF-1α activities and ATP viability (2D) in HepG2 exposed to hypoxia for 24 h
- (c) Tumor volume (3D - growth), tumor ATP (3D - viability), CellTiter Blue (2D – growth), and cell ATP (2D – viability) in Ishikawa cells grown under normoxic conditions for 72 h
- Standard deviations are calculated from quadruplicates

3. EFFECTS OF MCT-4 DEPLETION ON TUMOR GROWTH/VIABILITY



- Confirmation of Mct-4 depletion measured with Alex Fluor 488 fluorescence intensities using image cytometry
- Effects of Mct-4 depletion on (b) tumor volume (3D) and (c) ATP viability (2D) with HCT116
- (d) Confirmation of Mct-4 depletion in Panc1 cells via Western Blot
- (e) Effects of Mct-4 depletion on TGF-β stimulated tumor invasion using the Panc1 cells ($p > 0.05$). Standard deviations are calculated from quadruplicates.

4. EFFECTS OF MCT-4 OVEREXPRESSION ON TUMOR GROWTH/VIABILITY



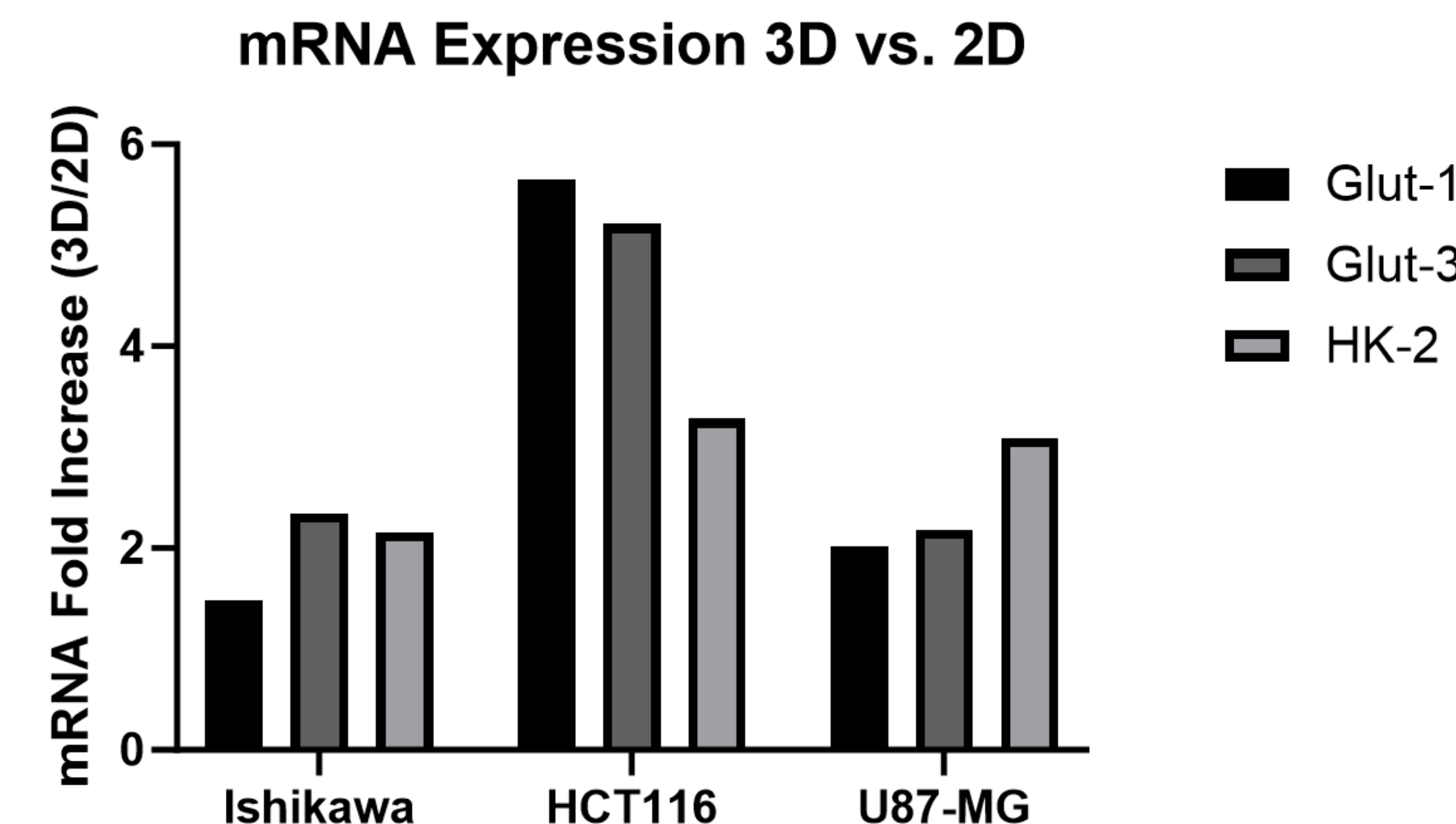
- (a) Confirmation of Mct-4 overexpression in Ishikawa cells shown with Western Blot
- Effects of Mct-4 overexpression on time-dependent (b) tumor volume (3D) and (c) ATP viability (2D) using the Ishikawa cells
- Reversing the inhibitory effects of Bay 87-2243 on Mct-4 overexpressing Ishikawa cells (Clone #11) in (d) 2D and (e) 3D models
- Standard deviations are calculated from quadruplicates

5. COMPARISON OF mRNA TRANSCRIPTION BETWEEN 2D AND 3D MODEL

Symbol	2D Normoxia	2D Hypoxia	3D Normoxia
	Compared to 2D Normoxia-DMSO		
	Fold Change	Fold Change	Fold Change
SLC2A3	-6.16	1.08	4.39
LGALS3	-1.14	1.37	3.94
NDRG1	1.72	53.90	3.31
ALDOC	-3.00	22.07	2.62
TFRC	1.10	1.71	2.36
NOS3	-1.48	4.88	2.28
B2M	1.07	1.28	2.23
VEGFA	1.72	17.81	2.13
HIF3A	-1.13	6.33	2.13
HPRT1	-1.05	1.37	2.11
HNF4A	1.11	1.47	2.07
PPC	-1.56	1.45	1.99
F10	-4.58	1.08	1.88
HK2	2.29	20.77	1.84

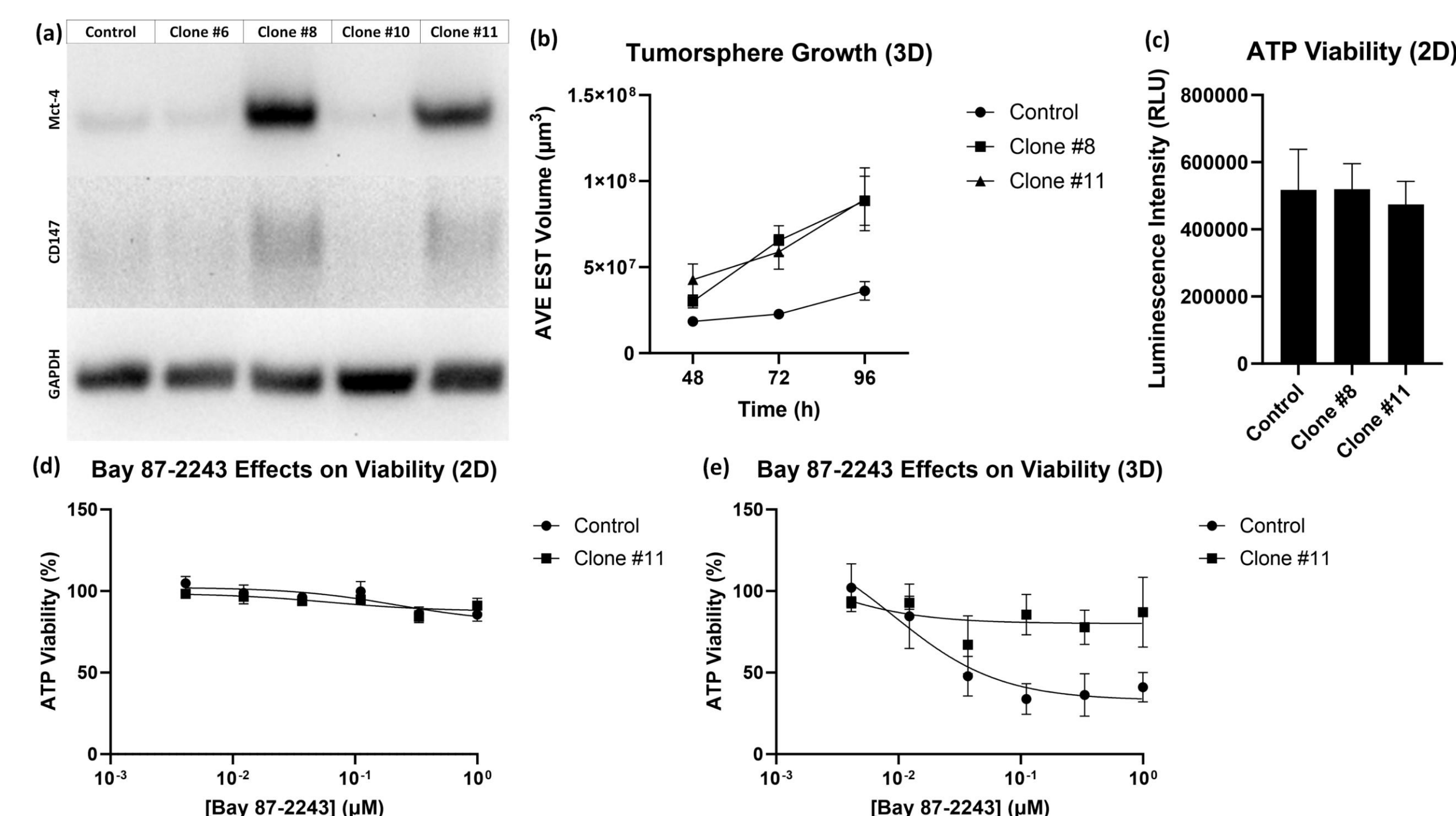
- The mRNA expression fold changes of different genes/proteins showing differences between 2D in normoxia, 2D in hypoxia, and 3D in normoxia
- Ishikawa cells were grown in 2D models under normoxia and hypoxia conditions, as well as 3D culture in normoxia conditions
- The various mRNA transcriptions increase from 2D to 3D indicated that 3D models allowed higher protein expression, potentially enabling increased growth and viability

6. COMPARISON BETWEEN mRNA EXPRESSION BETWEEN 3D AND 2D



- The mRNA expression fold changes of Glut-1, Glut-3, and HK-2 for Ishikawa, HCT116, and U87-MG cell lines between 3D and 2D models
- The results demonstrated that 3D models contain more mRNA expression than 2D, indicating the increase in protein expression within the 3D model
- Experiment was performed in duplicate, but data is shown from one experiment

7. HYPOXIA INDUCING PROTEIN EXPRESSION BETWEEN 2D AND 3D MODEL



- (a) Western Blot with increase in hypoxia induced expression grown in 2D and 3D conditions ($n = 2$)
- Quantified with the β-Actin normalized protein levels for (b) HCT116 and (c) Ishikawa cells
- Quantitation was performed using ImageLab™ (BioRad), where data is shown from one experiment

8. SUMMARY

- We have characterized and compared the effects of hypoxia response factors, specifically HIF-1α and Mct-4, on tumor growth, viability, and mobility, between 2D and 3D cell culture models
- Demonstration of differences between 2D and 3D models suggests the importance of the 3D model in recapitulating the tumor microenvironment
- With the employment of high-throughput image cytometers, both 2D and 3D models may be easily evaluated rapidly and reliably
- It may be useful to use 3D models to identify candidates for small molecules, antibodies, or cellular therapies on solid tumors