frontiers in Pharmacology

Homoharringtonine Exerts Anti-tumor Effects in Hepatocellular Carcinoma Through Activation of the Hippo Pathway

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H[illegible] (HCC) [illegible] 3–6/100,000 [illegible] A[illegible] HCC, [illegible] T[illegible] H[illegible] HCC, [illegible] -HCC [illegible] H[illegible] (HHT), [illegible] FDA- [illegible] [illegible] I[illegible] HHT, [illegible] fi[illegible] HCC, [illegible] [illegible] M[illegible] HHT [illegible] [illegible] [illegible] A[illegible] HHT [illegible] [illegible] S[illegible] [illegible] M[illegible] HHT- [illegible] [illegible] H[illegible] C[illegible] MST1/2, [illegible] M- MP-1, [illegible] HHT- [illegible] F[illegible] [illegible] [illegible] fi[illegible] HHT. T[illegible] I[illegible] fi[illegible] [illegible] HHT [illegible] HCC.

Keywords: homoharringtonine, hepatocellular carcinoma, apoptosis, hippo pathway, cell-cycle arrest, proliferation

INTRODUCTION

[illegible] ([illegible]). [illegible] ([illegible]), [illegible] 85– 0% [illegible] 3–6/100,000 ([illegible]). ([illegible]). [illegible] ( ) [illegible] ([illegible])

(., 2016 , 201 ., 2020). (., 2016). fi *Drosophila,* (., 2005 ., 2007). 20- 1/2 (, 1/2), 1 1 (, 1 1), 1 (, 1), 1/2 (, 1/2), ( ) /8 /8 1 (/8 /8 1/), (., 2011). (, 2010 ., 2013). (., 2006), fi (., 2015). fi (., 2014 ., 2015 , 201). ( ), *Cephalota, us harringtonia* fi ( ), ( ), ( ), ( ) (., 1 5 ., 1 6 ., 2011 ., 2013). 3) 1/2 3 (., 201 ., 201 ., 2020).

RESULTS

HHT Inhibits HCC Cell Proliferation and Colony Formation

in vitro, 2, 7, -7721

7 (25, 50, 100, 200, 400) 24, 48, 72 8 fi (**Figures 1A,B Supplementary Figures S1A,B**). 1 50 25, 50, 100 48 02 (**Supplementary Figure S1C**). 1 02 400 (50) 48 , 14 (**Figure 1C**). **Figure 1D,** fi

HHT Attenuates Cell Migration and Cell Invasion

(**Figure 2A**). fi (**Figures 2B,D**), 60, 80, 0% 2 80, 85, 0% 7 20, 50, 100 50, 80, 0% (**Figures 2C,E**).

HHT Induces HCC Cell Cycle Arrest and Apoptosis

fl 2 7 48 1 (**Figures 3A,B**).

100 .74 46. 4 .70 51.22 2 7 (**Figures 3C,D**). 3, **Figure 4A,**

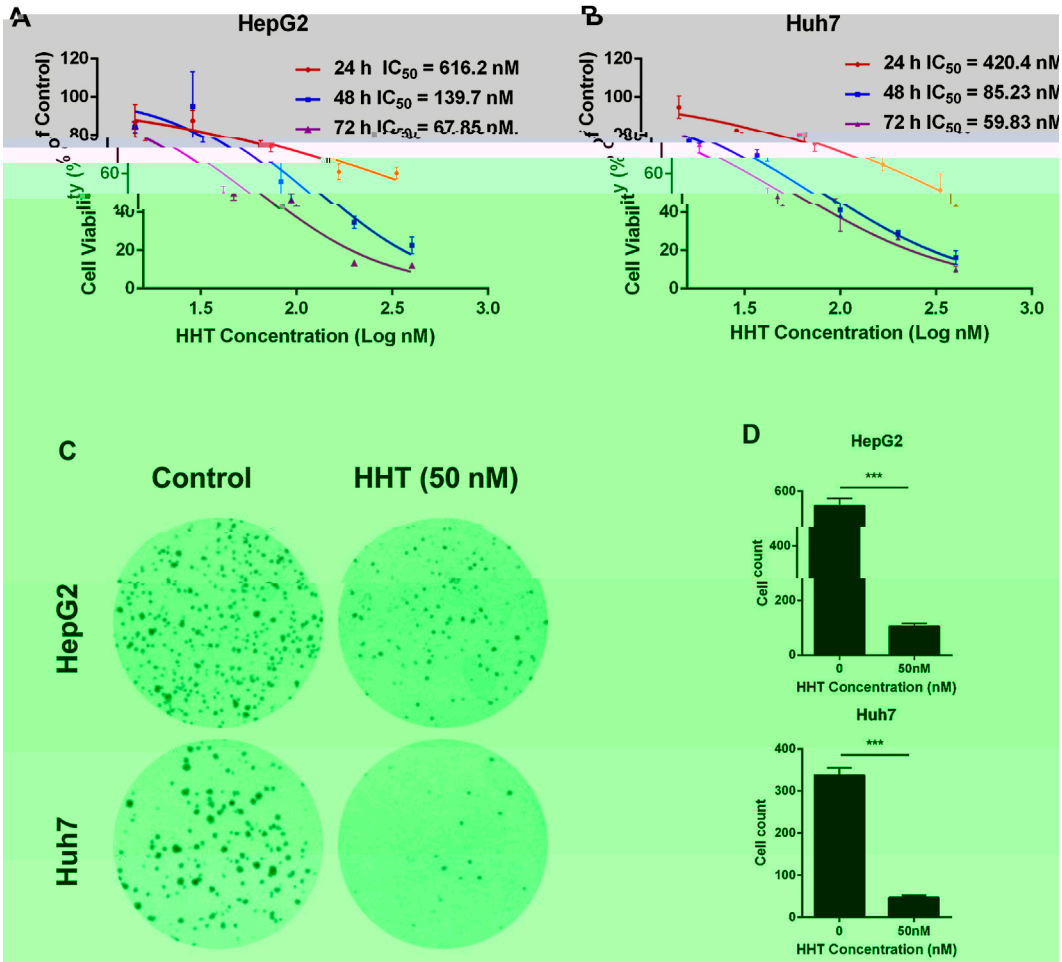

FIGURE 1 | HHT ... **(A, B)** H... G2... H...7... 96-... 3,000 ... HHT (0–400 M) ... 24, 48, ... 72 ... C... CCK-8 ... **(C)** H... G2... H...7, ... 6-... 1,000. ... HHT (0... 50 M) ... 14 ... T... L... DMI4000 ... **(D)** S... **(C)**. * < 0.05, ** < 0.01, *** < 0.001 ... ANO... A, ... D... T... N = 3. E... = S.D.

HHT Suppresses the Malignant Phenotype of HCC Cells via Activating the Hippo Pathway

... 2 ... 7 ... 48 ... 1 (183), 2 (180), 1 (35), 1 (107 0), (127) (**Figure 4B**). ... 2 ... 7 ... 1 μ ... -1, ... 1/2, ... 24 ... -1 ... 1, 1, (**Figures 4C,D Supplementary Figures S2A,B**). ... -1 ... 2 ... 7 ... -1 ...

HHT Inhibits HCC Cell Growth in Xenograft Models

... *in vivo*, ... 7 ... 10 ...

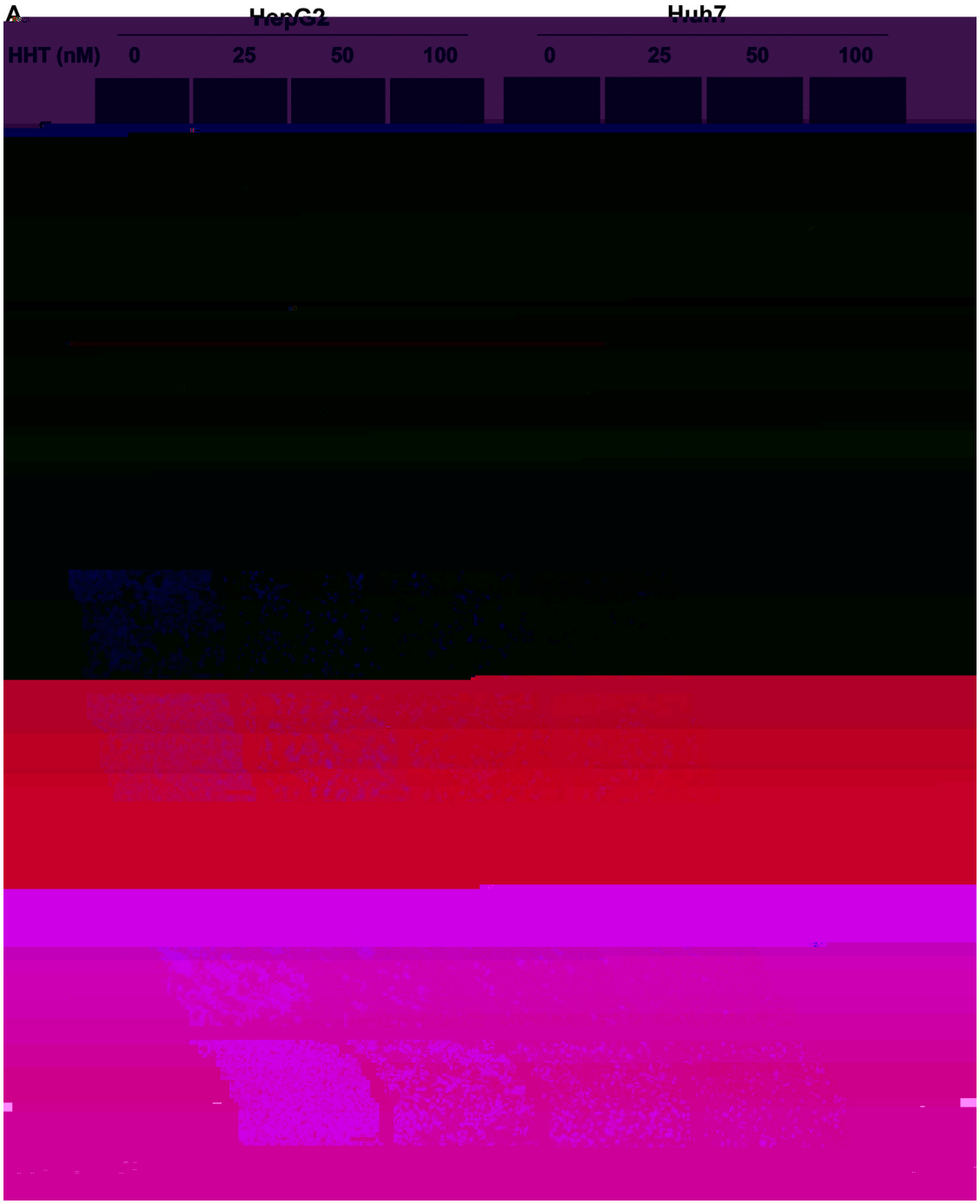

FIGURE 2 | HHT... **(A)** H... G2... H... 7... 6-... i... T... HHT... (0, 25, 50, 100 M)... 24, 48... 72... T... i... **(B)** T... HHT... R... fi... 24... **(C)** S... **(B)**. **(D)** R... M... 24... **(E)** S... **(D)**. S... : 200 μM * < 0.05, ** < 0.01, *** < 0.001... ANO... A, ... D... T... . N = 3. E... = S.D.

... 18... 0.... ... (... ., 201... ., 2020). ... *in vivo*

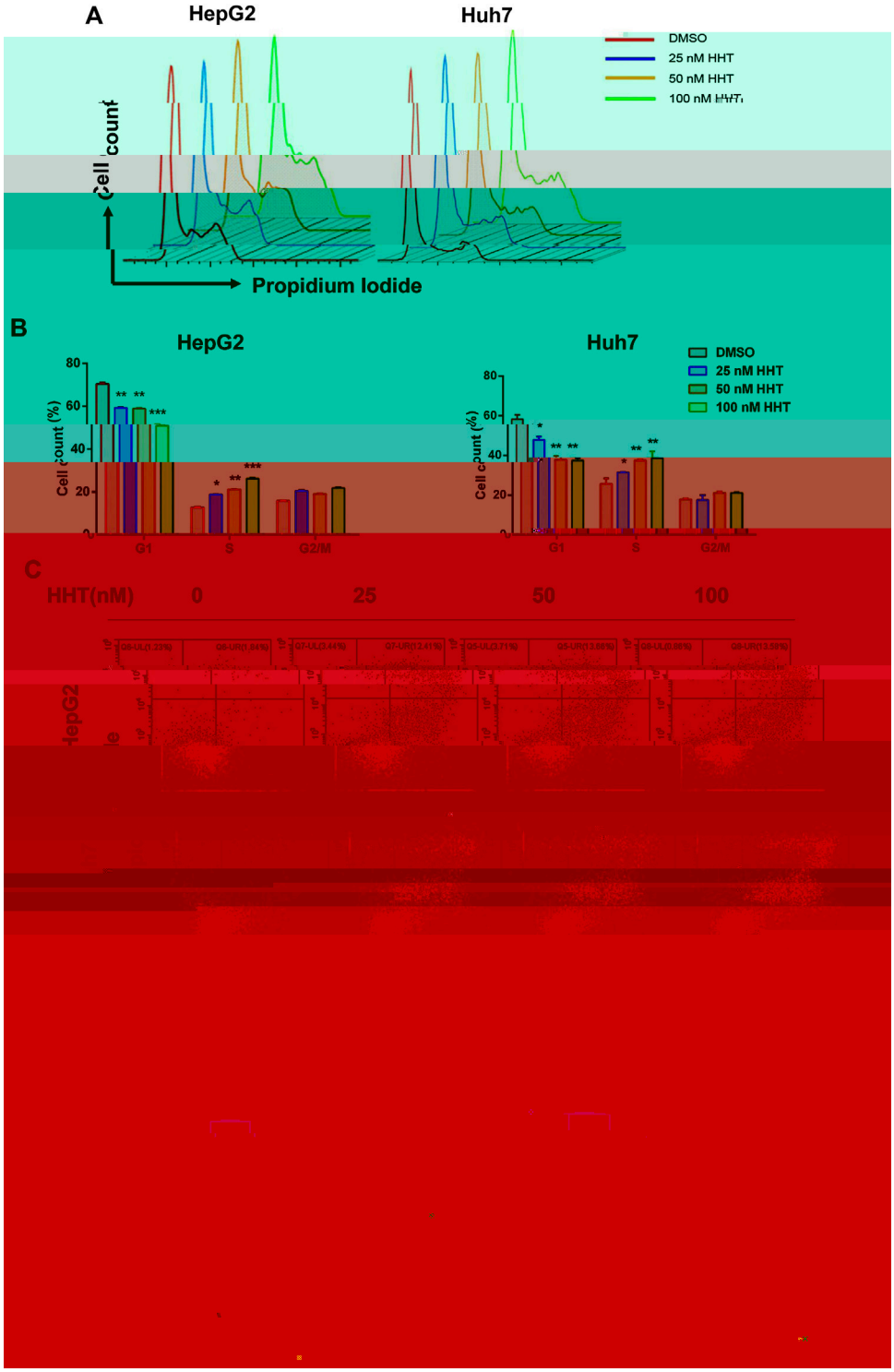

FIGURE 3 | HHT S (0, 25, 50, 100 M) 48 . C fl . **(A)**. **(C)** H G2 H 7, HHT (0, 25, 50, 100 M) 48 FITC A . C fl . **(B)** S **(A)**. **(C)** H G2 H 7, HHT (0, 25, 50, 100 M) 48 FITC A . C fl . **(D)** S **(C)**. * < 0.05, ** < 0.01, *** < 0.001 ANO A, D T . N = 3. E = S.D.

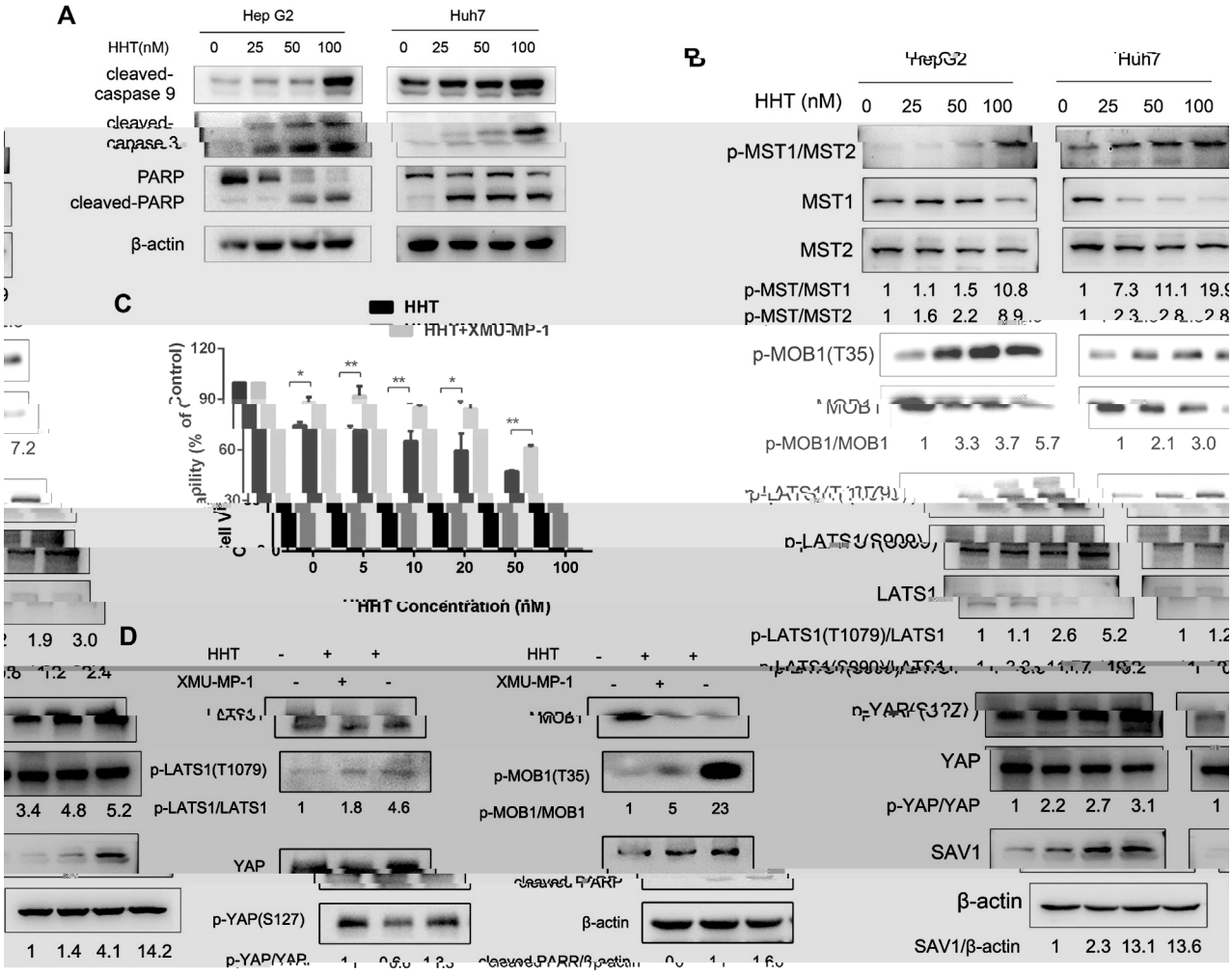

FIGURE 4 | HHT H **(A)** R 9 3 PARP H G2 H 7 HHT **(B)** R H MST1/2, MOB1, LATS1, SA 1 AP HHT **(C)** H 7 1 μM M -MP1 HHT (0, 5, 10, 20, 50, 100 M) 48 CCK-8. **(D)** H 7 1 μM M -MP1 HHT (0, 50 M) 48 H

(**Figures 5A,C**). 0. 30 50% (**Figure 5B**), I I - (**Figure 5D**).

3 3 (**Figure 5E**). fi *in vitro*, I I / *in vitro* *in vivo*.

DISCUSSION

(., 2017), 60% (., 201). ( ) fi (., 2018 ., 2020 ., 2020 ., 2020).

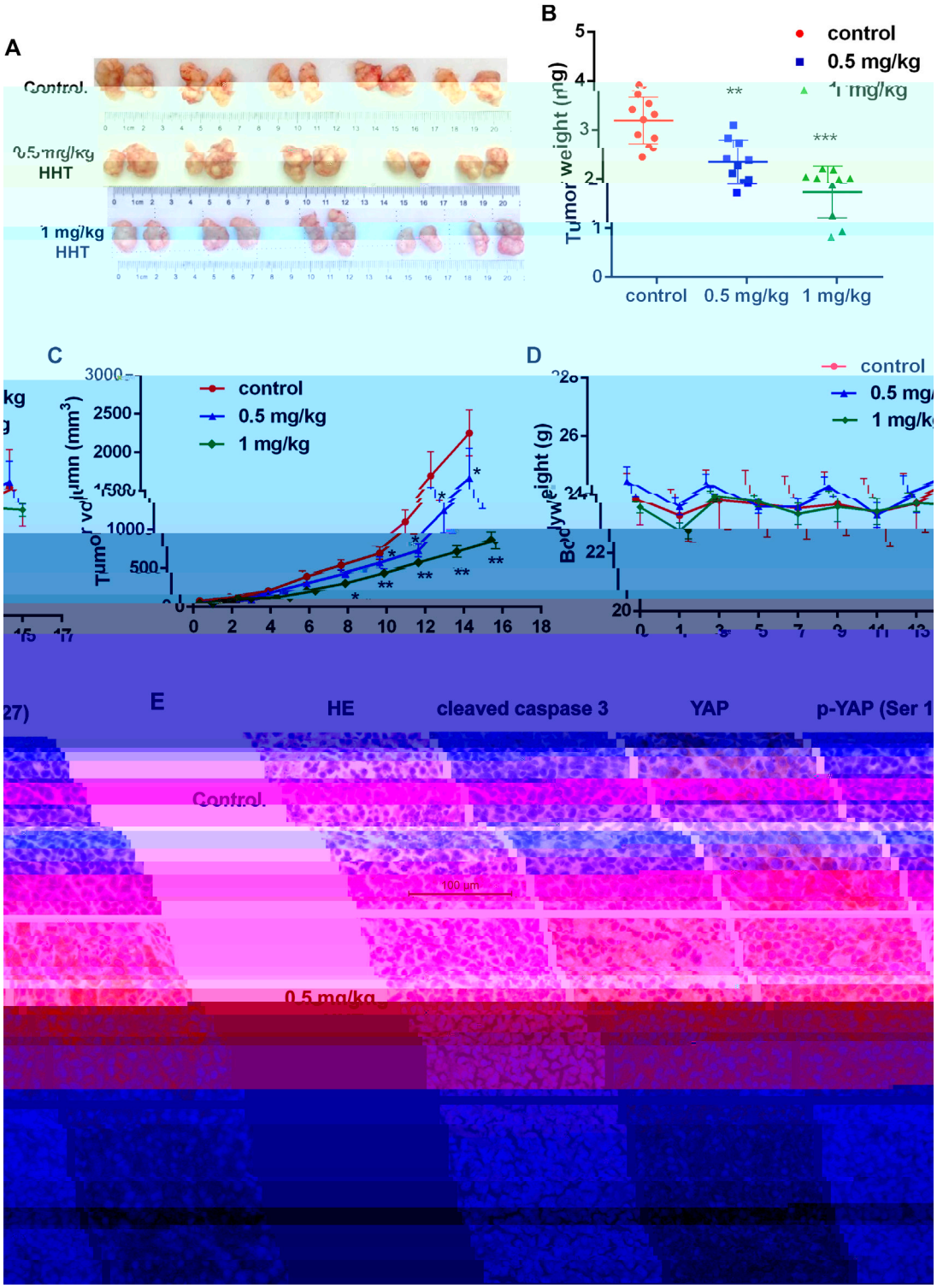

FIGURE 5 | HHT . **(A)** DMSO HHT- . **(B)** S **(A)**. **(C)** S HHT . **(D)** S HHT- . **(E)** HE 3, AP (S127) AP (×200 fi): 100 μM * < 0.05, ** < 0.01, *** < 0.001 ANO A, D Twi . N = 3. E = S.D.

MATERIALS AND METHODS

Cell Culture

2, 7, -7721 ( ), 7 02 -1640 , 10% (,), 100 100 37 5% 2.

Antibodies and Reagents

(. -56077) β- (. -8432) (# 542), 3 (# 661), 1 (#3682), 2 (#3 52), 1(183)/ 2(180) (#4 332), 1 (#3477), 1(0) (# 15), 1(107) (#8654), 1 (#13730), 1 (35) (#86), 1 (#13301), (#14074), (. 127) (#13008), (. 3 7) (#1361).

Cell Proliferation Assay

2, 7, 7, -7721, 02 6- 3,000 25, 50, 100, 200, 400 24, 48, 72 8 ( )/ 3 450 800).

Colony Formation Assay

50 48 1×10^3 6- 14 4% 0.5% ( ). 400

Wound Healing Assay

6- 70% 37 5% 24, 48, 72

Cell Migration and Invasion Assay

( ) ( ) ( ) 400 μ 20% 24 4% 15 0.5% 15

fi fi ×400.

Cell Cycle Arrest and Apoptosis Analysis

2 7 6- 70% 48 70% −20 4 (100 μ) 1 (50 μ) 3 fi ( )

Western Blot Analysis

(8 ., 2018). 10% (5%) 1 25 1 1,000 4 ( ) 1 5,000 1 25 4,600 1 (fi).

Tumor Xenograft Study

(6–8) 16 20 ( ). 7 (1×10^7) fl (0.5 /) (.) 18

Immunohistochemical Analysis

(-/ , 2005). fl fi 3% 15 5% ( ) 1 25 3, 1 400 5%

4 ( ) / / 1 ( ). 1 / 200 × ( ).

Statistical Analysis

fi fi / (20.0 1 6.0 ( ).

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/8 ( )

DATA AVAILABILITY STATEMENT

/Supplementary Material,

ETHICS STATEMENT

/ /

AUTHOR CONTRIBUTIONS

/8 I, /8 /8 /8 I

FUNDING

/ / 82000201, 81570124, 81800203, 81702683 (1 02036) (201 12 56) & (201 8/10300027) (2020003) (20180540088) (2018 13 0 5).

SUPPLEMENTARY MATERIAL

/// / / / /10.338 / .2021.5 2071/ #

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Conflict of Interest: