	IC_{50} (μM)
Gos3	1.5
U87	1.6
A172	2.0
SW1783	2.2
U118	2.5
U373	2.9
T98	3.0
SW1088	3.5
CCF	3.5
LN405	4.0

Cell line	BCNU IC_{50} (μM)	TMZ IC_{50}(μM)
Gos3	100	250
U87MG	400	400
A172	>400	>400
SW1783	300	200
U118	350	>400
U373	375	>400
T98	>>400	>400
SW1088	>400	350
CCF	>400	>400
LN405	>400	>400

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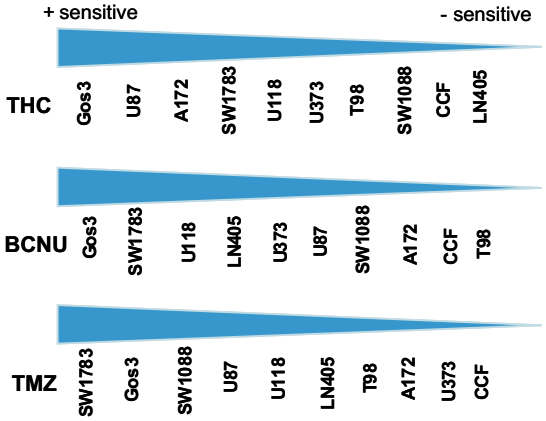

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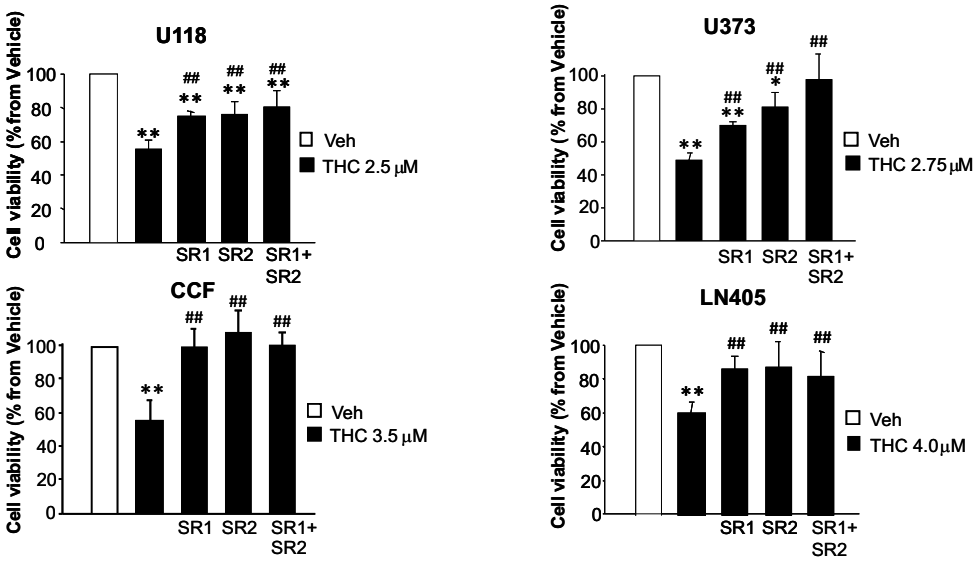
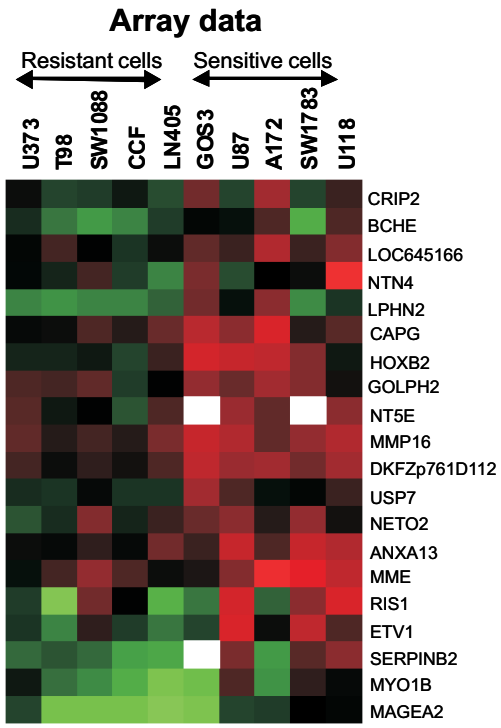


Supplementary Figure 1. Differences in the sensitivity of human glioma cells to different treatments

(a) THC IC_{50} values (cell viability; 72 h) for the human glioma cell lines used in this study. (b) Temozolomide (TMZ) and carmustine (BCNU) IC_{50} values (cell viability; 72 h) for the human glioma cell lines used in this study. (c) The graphic represents the ten human glioma cell lines according to their sensitivity to THC, TMZ and BCNU. (d) Effect of THC, SR1 (1? M) and SR2 (1? M) on the viability (72h) of U118, U373, CCF and LN405 cells lines (mean ± s.d; n = 3; **p < 0.01 from vehicle-treated cells, $^{\#\#}$p < 0.01 from THC-treated cells; Veh: Vehicle, THC: Δ^9-tetrahidrocannabinol).

a



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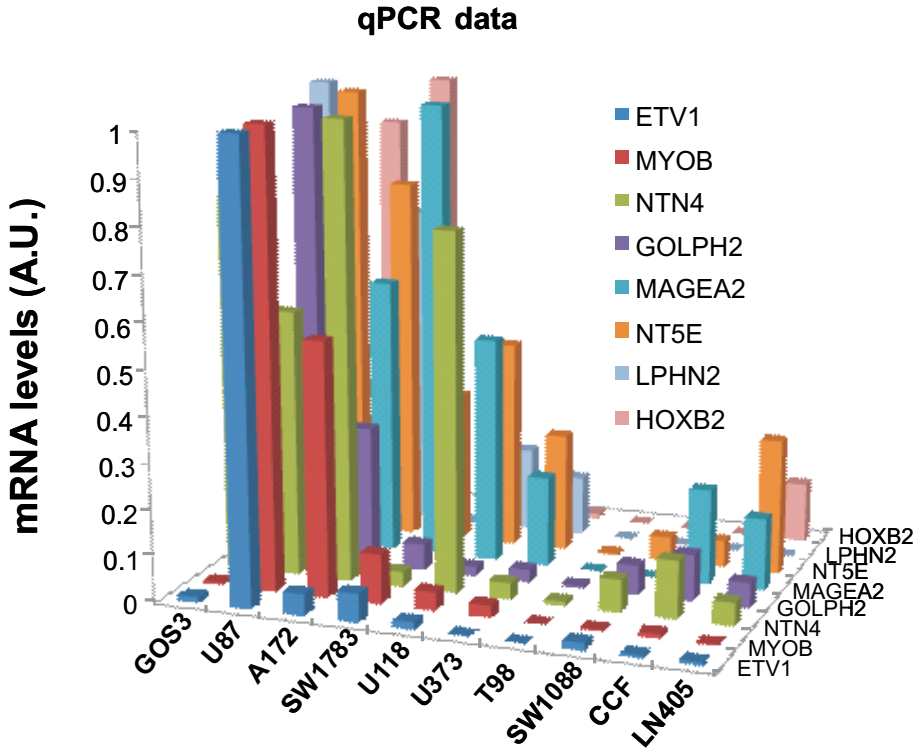
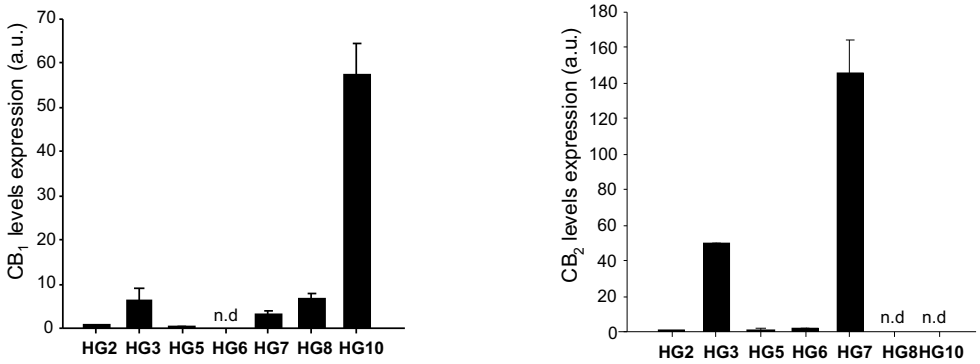


Supplementary Figure 2. Identification of the gene expression profile characteristic of THC-sensitive glioma cells

(a) Array data: selection of genes that exhibit different basal expression levels between THC-sensitive and THC-resistant human glioma cell lines. Data correspond to genes whose expression was significantly increased (FDR < 0.2) at least 2.5 fold in 3 or more sensitive glioma cell lines with respect to 3 or more resistant glioma cell lines. Each square represents the fluorescence ratio (log_2 scale) between sample RNA (cell lines) and reference RNA for one gene; red squares represent positive values, green squares represent negative values and white squares represent non-determined values. (b) mRNA levels, as determined by quantitative real-time PCR, of 8 genes associated with sensitivity to THC-induced cell death in human glioma cell lines. Data correspond to mRNA levels for each gene relative to the highest mRNA expression level for that gene detected in any of the ten human glioma cell lines and are expressed in arbitrary units (a.u.) in a 0 to 1 scale.

	IC_{50} (μM)
HG 3	5
HG 8	5
HG 1	5.5
HG 5	5.5
HG 9	5.7
HG 10	6
HG 6	6
HG 2	8
HG 7	>8

b



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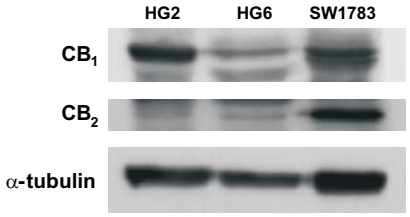

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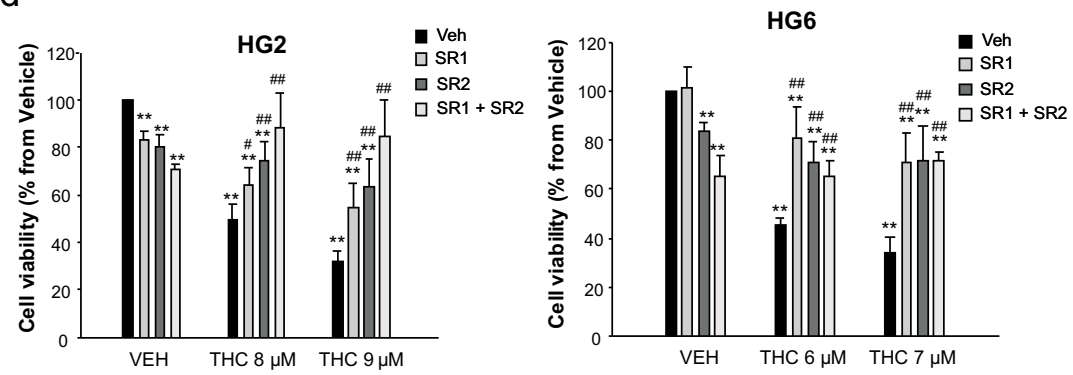
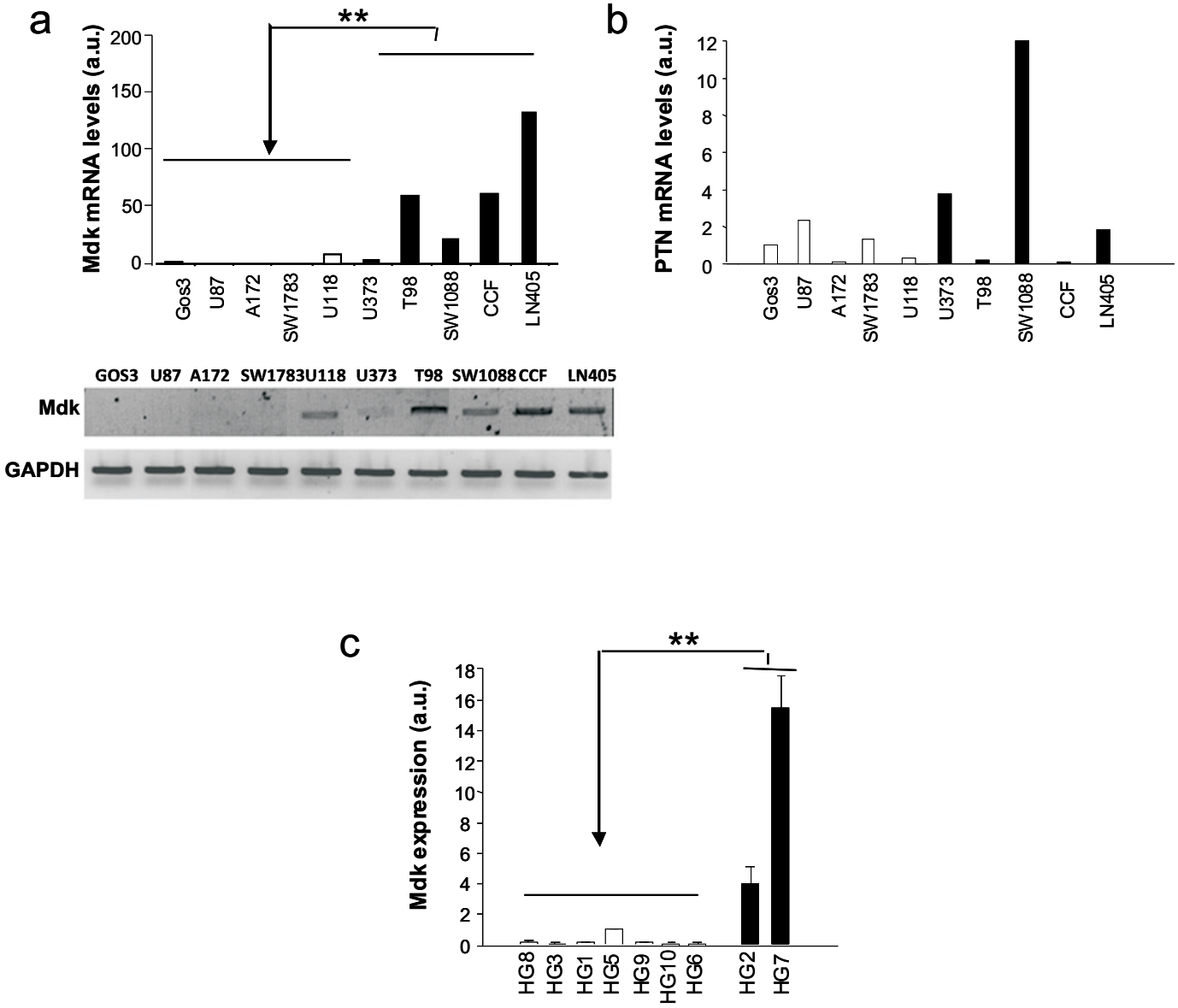


Supplementary Figure 3. THC promotes cell death of primary cultures of human glioma cells via cannabinoid receptors.

(a) THC IC_{50} values (cell viability; 72 h) for primary cultures of human glioma cells used in this study. (b) CB_1 (left panel) and CB_2 (right panel) mRNA levels (as determined by real-time quantitative PCR) of the different primary cultures of human glioma cells used in this study. Data correspond to CB_1 or CB_2 mRNA levels relative to CB_1 or CB_2 mRNA levels of HG2 cells and are expressed in arbitrary units (a.u.) (mean ± s.d; n = 3). N.d: not detected. (c) CB1 and CB2 protein levels of HG2 and HG6 cells (n = 2). (d) Effect of THC, SR1 (1 μM) and SR2 (1 μM) on the viability (72h) of HG2 and HG6 cells (mean ± s.d; n = 3; **p < 0.01 from vehicle-treated cells, ##p < 0.01 from THC-treated cells; Veh: Vehicle, THC: Δ^9-tetrahidrocannabinol).



Suplementary Figure 4. ALK and Mdk expression in human glioma cell lines
(a) Mdk mRNA levels (as determined by real-time quantitative PCR, upper panel) or RT-PCR (bottom panel) of ten human glioma cell lines. Data correspond to Mdk mRNA levels of each cell line relative to Mdk mRNA levels of GOS-3 cells are expressed in arbitrary units (a.u) (mean ± s.d; n = 3; **p < 0.01 from each THC-sensitive human glioma cell line). Bottom panel: a representative RT-PCR experiment is shown. (b) Pleiotrophin (PTN) mRNA levels (as determined by quantitative real-time PCR) of the human glioma cell lines. Data correspond to PTN mRNA levels relative to PTN mRNA levels of GOS3 cells and are expressed in a.u. (c) Mdk mRNA levels (as determined by real-time quantitative PCR) of nine primary cultures of human glioma cells. Data correspond to Mdk mRNA levels of each primary culture relative to Mdk mRNA levels of HG5 cells are expressed in arbitrary units (a.u) (mean ± s.d; n = 3; **p < 0.01 from each THC-sensitive primary culture of human glioma cells).

Lorente et al., Supplementary Figure 5

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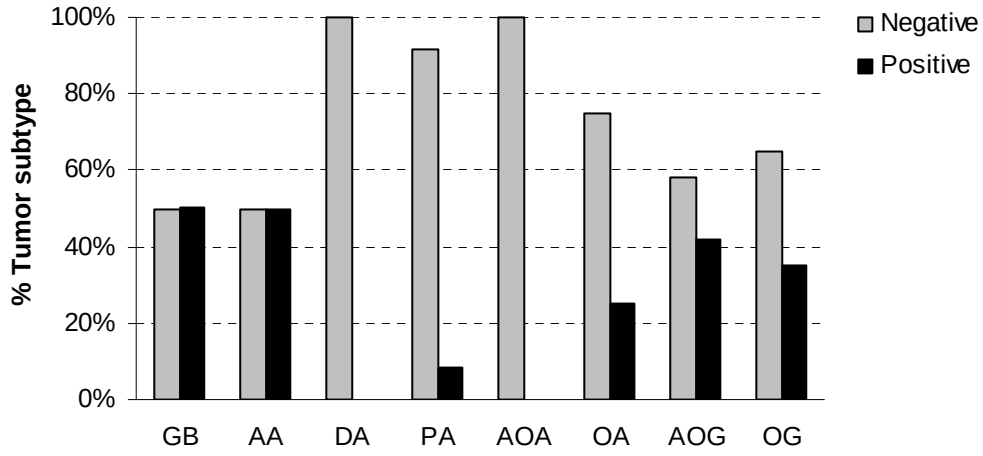

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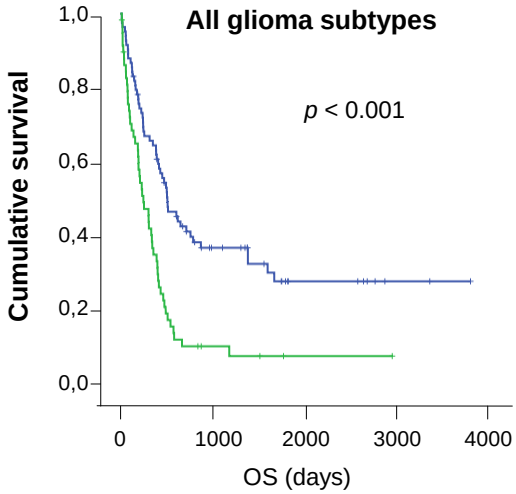

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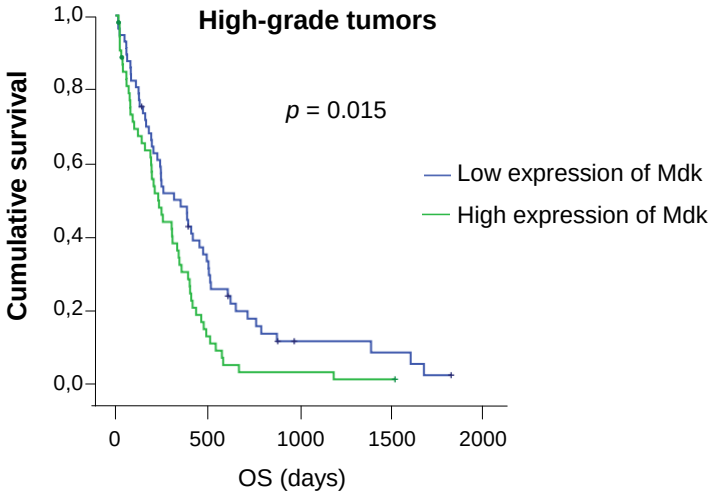

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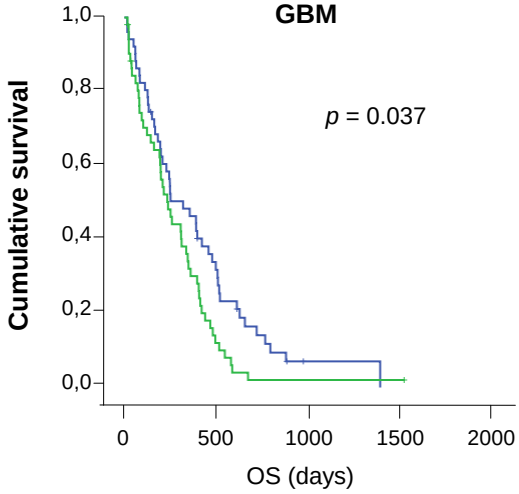

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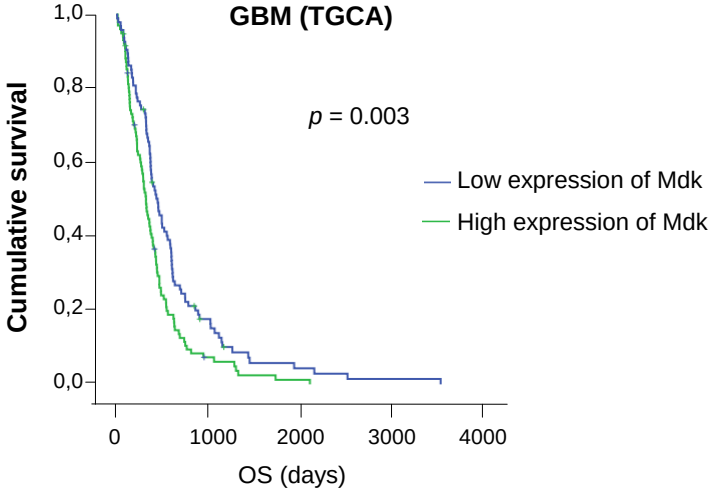

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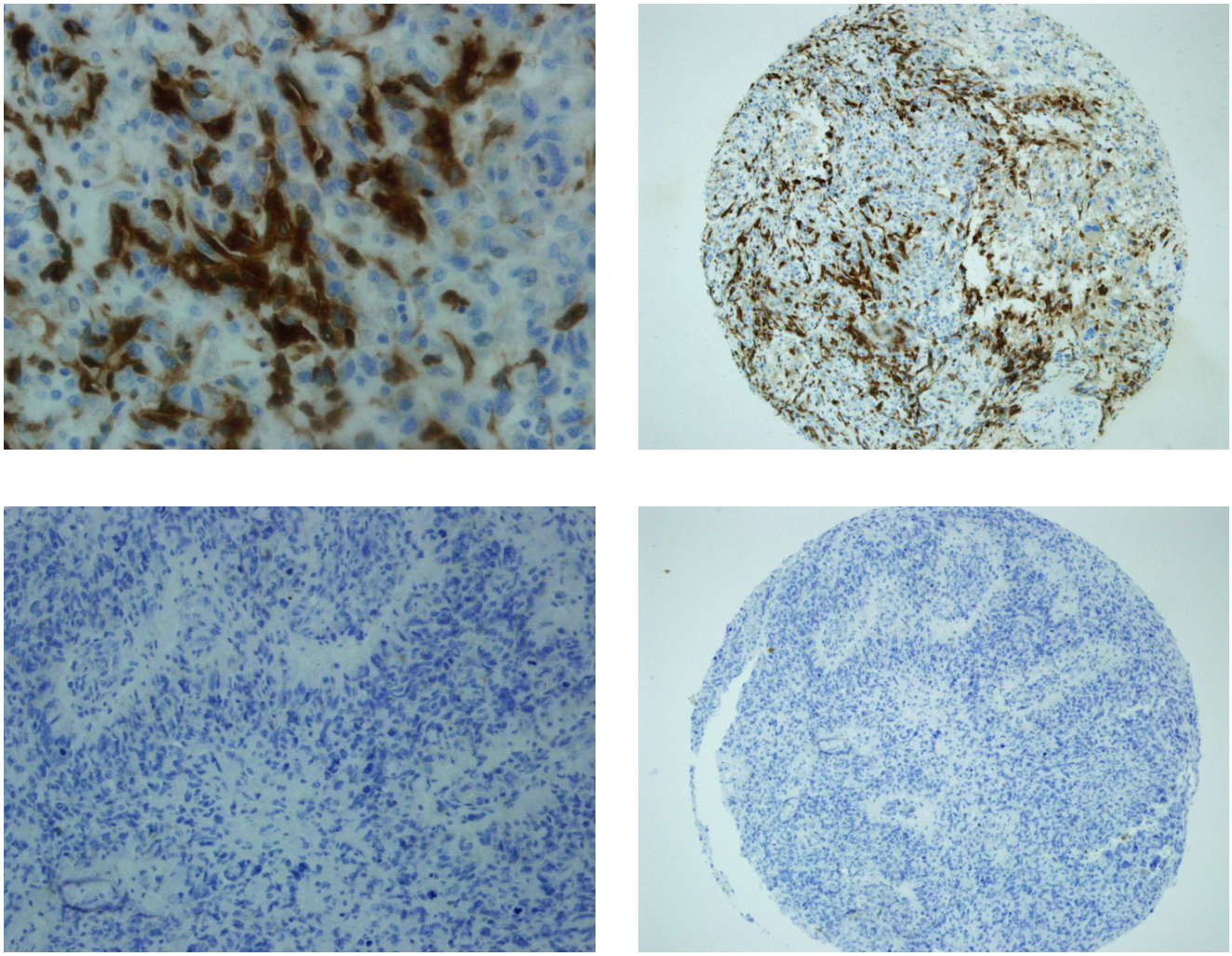
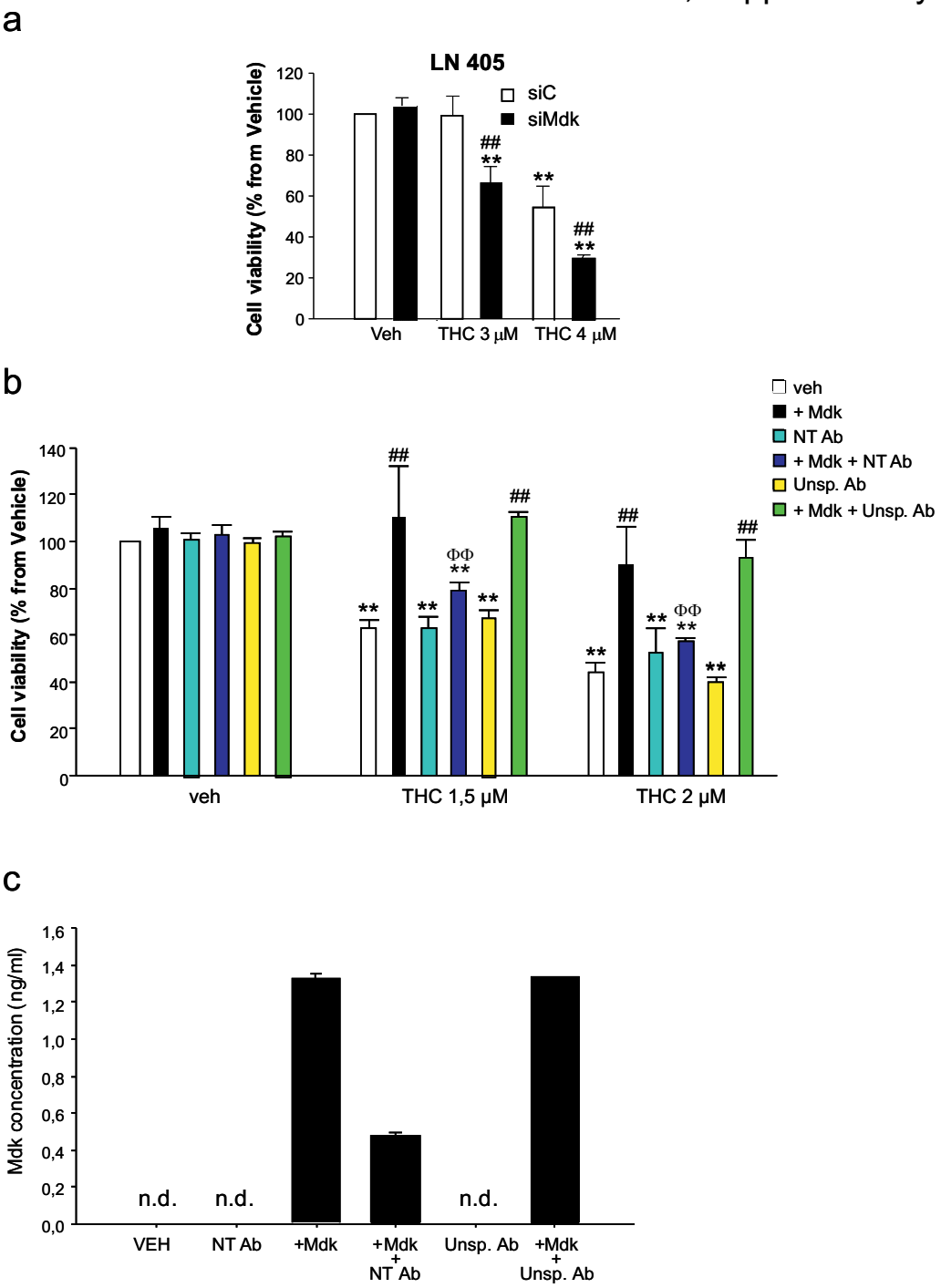
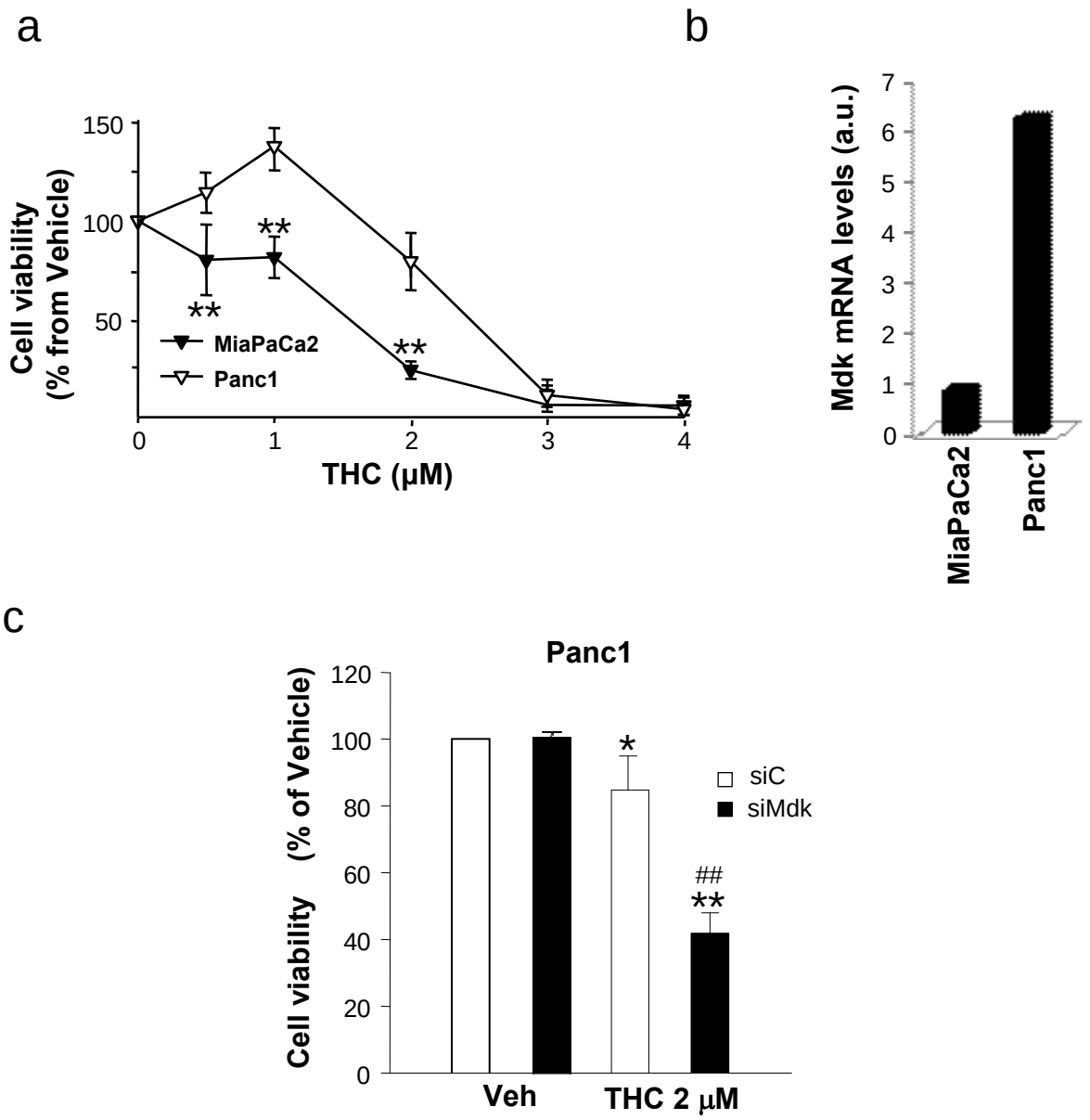


Supplementary Figure 5. Survival analyses of patients with primary gliomas
(a) Percentage of each subtype of brain tumor collected at the Virgen de la Salud Hospital (Toledo) showing positive or negative Mdk expression. (b-e) Kaplan–Meier survival curves are shown for all glioma subtypes (b); high-grade gliomas (glioblastomas, anaplastic astrocytomas and anaplastic oligoastrocytomas) (c); and glioblastoma tumors from patients collected at the Virgen de la Salud Hospital (Toledo) (d); 202 glioblastomas of the TCGA series reported by Verhaak et al (e). MDK high or low expression is shown by green or blue lines, respectively. OS: overall survival. (f) Representative images of two GBM tumors collected at the Virgen de la Salud Hospital (Toledo) showing positive (upper panels) and negative (lower panels) Mdk expression.



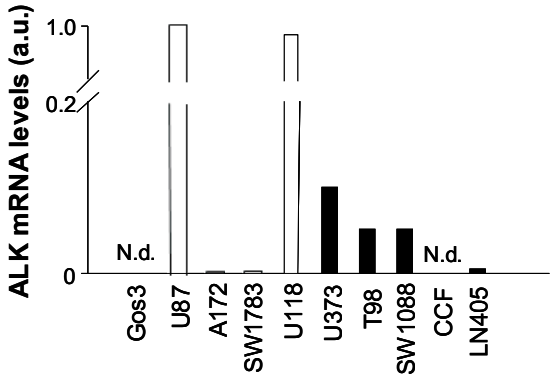
Supplementary Figure 6. Mdk confers human glioma cells resistance to THC
(a) Effect of THC on the viability (72 h) of LN-405 cells transfected with control (siC) or midkine-selective (siMdk) siRNA (mean ± s.d; n = 5, **p < 0.01 from vehicle-treated, siC-transfected cells and from vehicle-treated, siMdk-transfected cells and $^{\#\#}$p < 0.01 from THC-treated, siC-transfected cells). Mdk mRNA levels (as determined by real-time quantitative PCR) were reduced in siMdk-transfected cells relative to their corresponding siC-transfected cells by 84±12 % (n = 5). (b) Effect of THC on the viability of U87 cells incubated with a medium to which exogenous Mdk (5 ng/ml) or vehicle had been added. Before addition to the cells, media were pre-incubated with anti-Mdk (NT-Ab) or isotype unspecific (Unsp.-Ab) antibodies coupled to sepharose beads (mean ± s.d; n = 4; **p < 0.01 from vehicle-treated cells, $^{\#\#}$p < 0.01 from THC-treated, U87 cells incubated on a medium without exogenous Mdk and $^{\phi\phi}$p < 0.01 from THC-treated, U87 cells incubated on a medium containing exogenous Mdk). (c) Mdk concentration, as determined by ELISA, in the supernatant of U87 cells incubated with a exogenous Mdk-enriched medium and pre-incubated with an anti-Mdk (NT-Ab) or isotype unspecific (Unesp.-Ab) antibody coupled to sepharose beads (mean ± s.d; n = 2). N.d: not detected.



Supplementary Figure 7. Increased Mdk expression confers resistance to THC-treatment in pancreatic cancer cell lines

(a) Effect of THC on the viability (72 h) of the human pancreatic cancer cell lines MiaPaCa2 and Panc1 (mean ± s.d, n = 5). (b) Mdk mRNA levels (as determined by quantitative real-time PCR) of MiaPaCa2 and Panc1 cells. Data correspond to Mdk mRNA levels relative to Mdk mRNA levels in MiaPaCa2 cells and are expressed in arbitrary units (a.u). (c) Effect of THC on the viability (72 h) of Panc1 cells transfected with siC or siMdk (mean ± s.d; n = 5; *$p < 0.05$ or **$p < 0.01$ from vehicle-treated, siC-transfected cells and from vehicle-treated, siMdk-transfected cells and $^{\#\#}p < 0.01$ from THC-treated, siC-transfected cells). Mdk mRNA levels (as determined by real-time quantitative PCR) were reduced in siMdk-transfected cells relative to their corresponding siC-transfected cells by 87±4% (n = 5).

a



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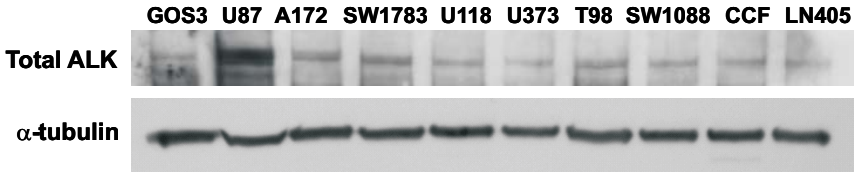

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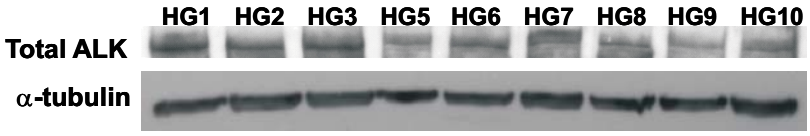

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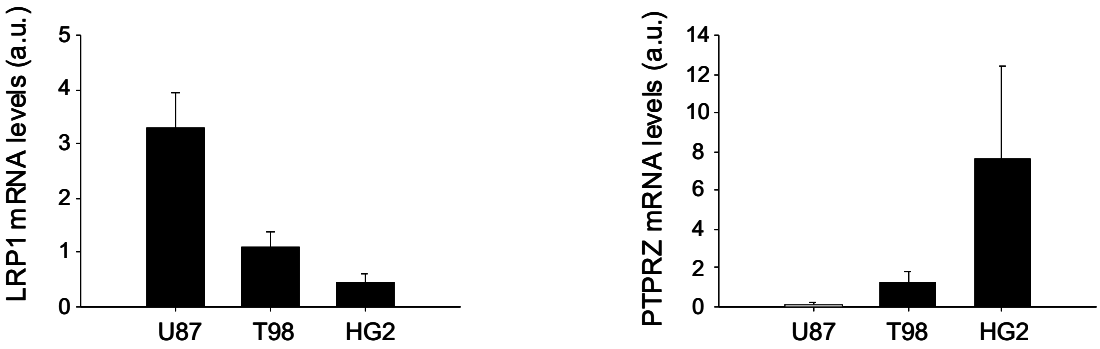
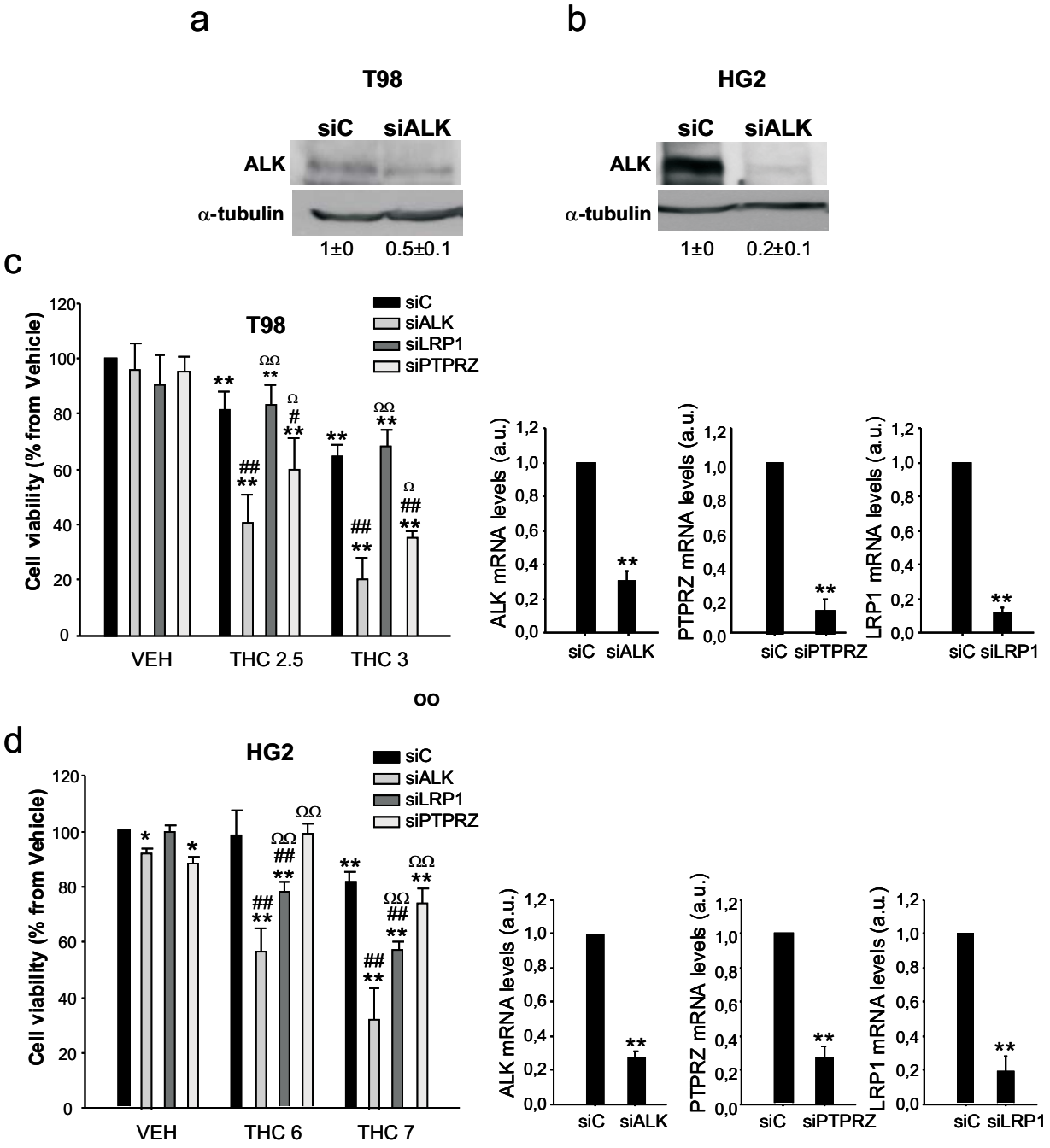
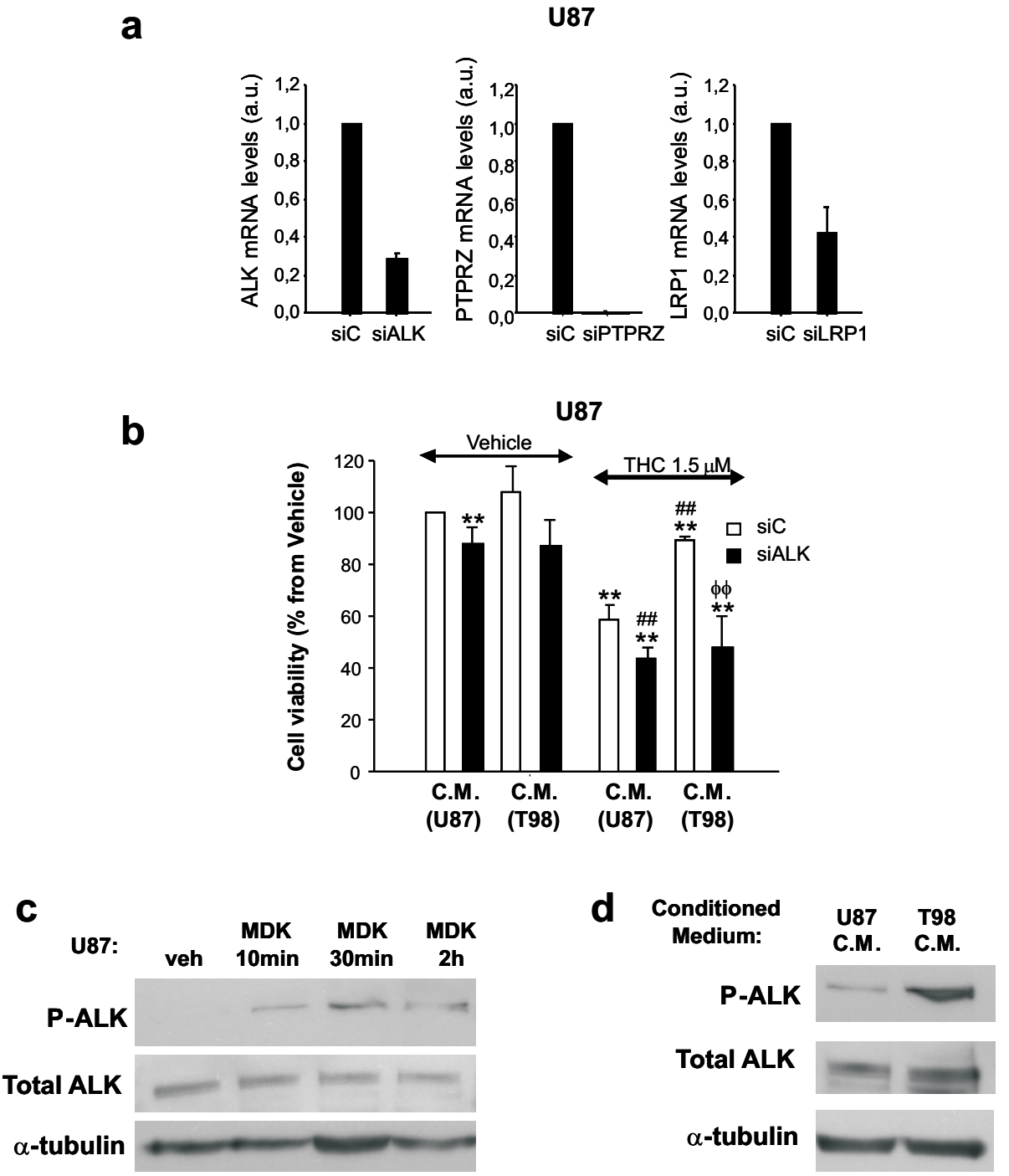


Supplementary Figure 8. ALK is expressed in human glioma cells
(a) ALK mRNA levels (as determined by real-time quantitative PCR), of ten human glioma cell lines. Data correspond to ALK mRNA levels of each cell line relative to ALK mRNA levels of U87 cells and are expressed in arbitrary units (a.u) (mean ± s.d; n = 3). N.d: not detected. (b-c) ALK expression as determined by Western blot of ten human glioma cell lines (b) and nine primary cultures of human glioma cells (c). A representative Western blot of 3 (b) and 2 (c) experiment is shown. (d) LRP1 (left panel) and PTPRz (right panel) mRNA levels (as determined by real-time quantitative PCR) of U87, T98 and HG2 cells. Data correspond to LRP1 (left panel) or PTPRz (right panel) mRNA levels relative to LRP1 and PTPRz mRNA levels of T98 cells and are expressed in a.u. (mean ± s.d; n = 3).



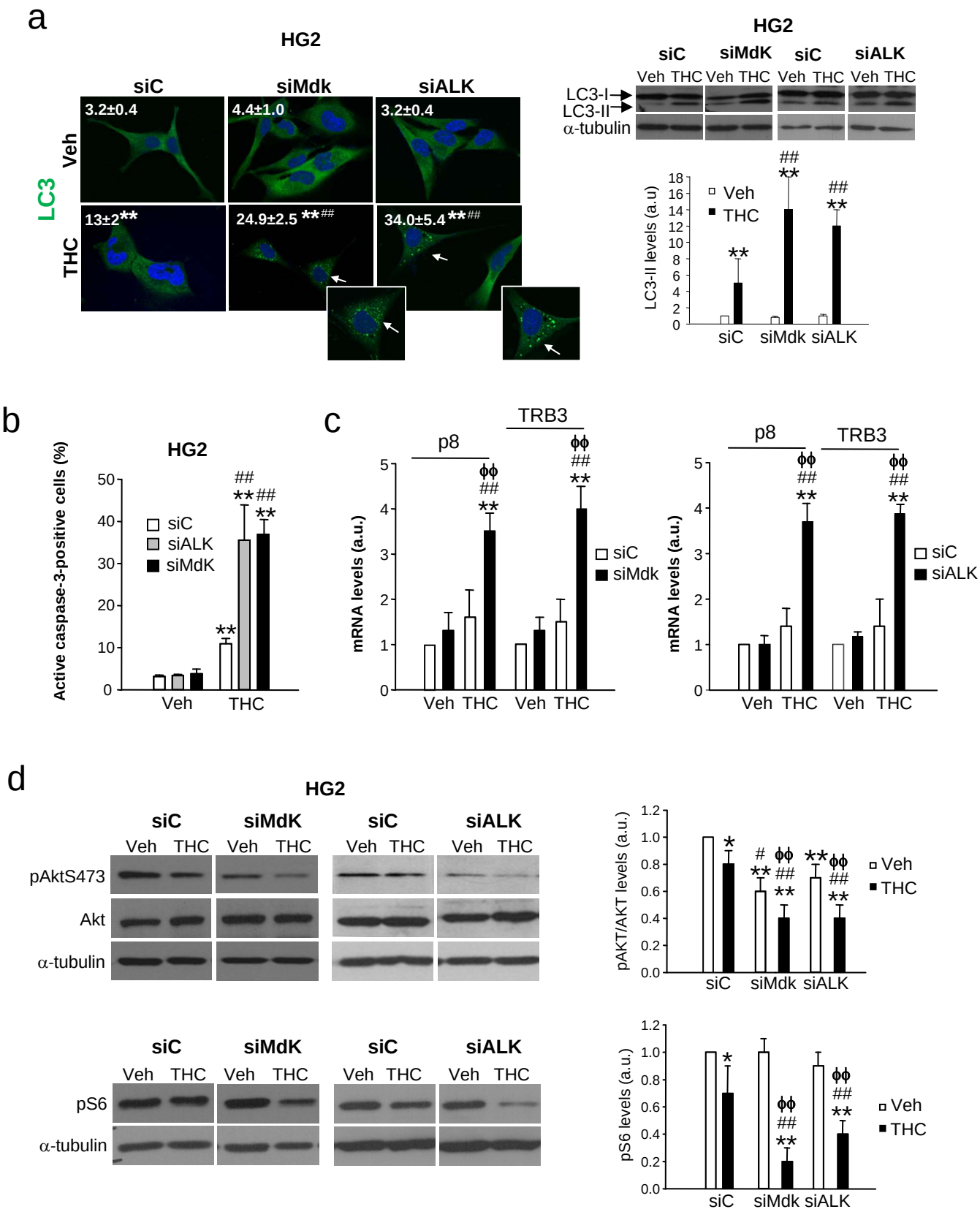
Supplementary Figure 9. ALK silencing sensitizes T98 and HG2 cells to THC treatment

(a-b) Transfection with siALK reduces ALK protein levels of T98 (a) and HG2 (b) cells. Values correspond to the densitometric analysis of the total ALK/tubulin ratio for each condition relative to siC-transfected cells. (c-d) Effect of THC on the viability of T98 (c) or HG2 (b) cells transfected with siC, siALK, siLRP1 ot siPTPRZ (mean ± s.d; n = 4 for T98 and HG2 cells; $^{**}p < 0.01$ from vehicle-treated, siC-transfected cells and from vehicle-treated, siALK-transfected cells, $^{\#\#}p < 0.01$ or $^{\#}p < 0.05$ from THC-treated, siC-transfected cells and $^{\Omega\Omega}p < 0.01$ from siALK-transfected, THC treated cells). Right panels: ALK, LRP1 and PTPRz mRNA levels (as determined by real-time quantitative PCR) of T98 (c) and HG2 (d) cells transfected with siALK, siLRP1 or siPTPRZ. Data correspond to ALK, LRP1 or PTPRZ mRNA levels of each cell line relative to siC-transfected T98 (c) or HG2 (d) cells and are expressed in arbitrary units (a.u) (mean ± s.d; n = 4; $^{**}p < 0.01$ from the corresponding siC-transfected cells).



Supplementary Figure 10. Mdk promotes resistance of U87 cells to THC treatment via ALK

(a) ALK, LRP1 and PTPRz mRNA levels (as determined by real-time quantitative PCR) of U87 cells transfected with siALK, siLRP1 or siPTPRZ. Data correspond to ALK, LRP1 or PTPRZ mRNA levels relative to siC-transfected U87 cells and are expressed in arbitrary units (a.u) (mean ± s.d; n = 3). (b) Effect of THC on the viability of siC or siALK-transfected U87 cells incubated with U87 C.M. or T98 C.M. (mean ± s.d; n = 5; $^{**}p < 0.01$ from vehicle-treated, U87 C.M.-incubated, siC-transfected cells, $^{\#\#}p < 0.01$ from THC-treated, U87 C.M.-incubated, siC-transfected cells and $^{\phi\phi}p < 0.01$ from THC-treated, U87 C.M.-incubated, siALK-transfected cells). (c-d) Effect of exogenous Mdk (c) or T98 C.M. (2 h; e) on ALK phosphorylation of U87 cells. Representative Western blots of one of 3 different experiments are shown.


a
HG2
siC
siMdk
siALK
Veh
THC
LC3
3.2±0.4
4.4±1.0
3.2±0.4
13±2**
24.9±2.5 **##
34.0±5.4 **##
HG2
siC
siMdK
siC
siALK
Veh THC Veh THC Veh THC Veh THC
LC3-I
LC3-II
α-tubulin
LC3-II levels (a.u)
Veh
THC
siC siMdk siALK
b
HG2
Active caspase-3-positive cells (%)
siC
siALK
siMdK
Veh
THC
c
p8
TRB3
mRNA levels (a.u.)
siC
siMdk
siALK
Veh THC Veh THC
d
HG2
siC
siMdK
siC
siALK
Veh THC Veh THC Veh THC Veh THC
pAktS473
Akt
α-tubulin
pAKT/AKT levels (a.u.)
Veh
THC
siC siMdk siALK
pS6
α-tubulin
pS6 levels (a.u.)
Veh
THC
siC siMdk siALK

Suplementary Figure 11. Mdk promotes resistance to THC-induced autophagy and apoptosis in HG2 cells

(a) Left panel: Effect of THC (7 μM, 24 h) on LC3 immunostaining of siC-, siMdk- or siALK-transfected HG2 cells. Values in the upper left corner at each photomicrograph correspond to the percentage of cells with LC3 dots relative to the total number of cells (mean ± s.d; n = 3; representative photomicrographs of each condition are shown; **p < 0.01 from vehicle-treated, siC-transfected cells; $^{\#\#}$p < 0.01 from THC-treated, siC-transfected cells). Right panel: Effect of THC (7 μM, 24 h) on LC3 lipidation of siC-, siMdk- or siALK-transfected HG2 cells. A representative experiment of 3 is shown. Lower panel: densitometric analysis of LC3 lipidation. Data correspond to LC3-II optical density values (O.D.) for each condition relative to vehicle-treated, siC-transfected cells (mean ± s.d; n = 3; **p < 0.01 from the corresponding vehicle-treated cells, $^{\#\#}$p < 0.01 from THC-treated, siC-transfected cells). (b) Effect of THC (7 μM, 24 h) on apoptosis (as determined by active caspase-3 immunostaining) of siC-, siALK- and siMdk-transfected HG2 cells. Data correspond to the percentage of active caspase-3-positive cells relative to the total number of cells (mean ± s.d; n = 3; **p < 0.01 from vehicle-treated, siC-, siALK- and siMdk-transfected cells; $^{\#\#}$p < 0.01 from THC-treated, siC-transfected cells). (c) Effect of THC (7 μM, 6 h) on p8 and TRB3 mRNA levels as determined by quantitative real-time PCR of siC- and siMdk- (left panel) or siC- and siALK- (right panel) -transfected cells (mean ± s.d; n = 4; **p < 0.01 from vehicle-treated, siC-transfected cells; $^{\#\#}$p < 0.01 from vehicle- treated, siMdk-transfected (left panel) or from vehicle-treated siALK -transfected (right panel) cells; $^{\phi\phi}$p < 0.01 from THC-treated, siC-transfected cells). (d) Effect of THC (7 μM, 24 h) on AKT and S6 phosphorylation of siC-, siMdk- or siALK-transfected HG2 cells. A representative experiment of 3 is shown. Right panel: densitometric analysis of Akt and S6 phosphorylation. Data correspond to phospho-Akt, Akt, phospho-S6 and tubulin O.D. values and are expressed as the ratio phosphoAKT/total Akt or phospho S6/tubulin for each condition relative to vehicle-treated, siC-transfected cells (mean ± s.d; n = 3; *p < 0.05 or **p < 0.01 from vehicle-treated, siC- transfected cells; $^{\#\#}$p < 0.01 from THC-treated, siC- transfected cells; $^{\phi\phi}$p < 0.01 from vehicle-treated, siMdk- and from vehicle-treated, siALK- transfected cells).

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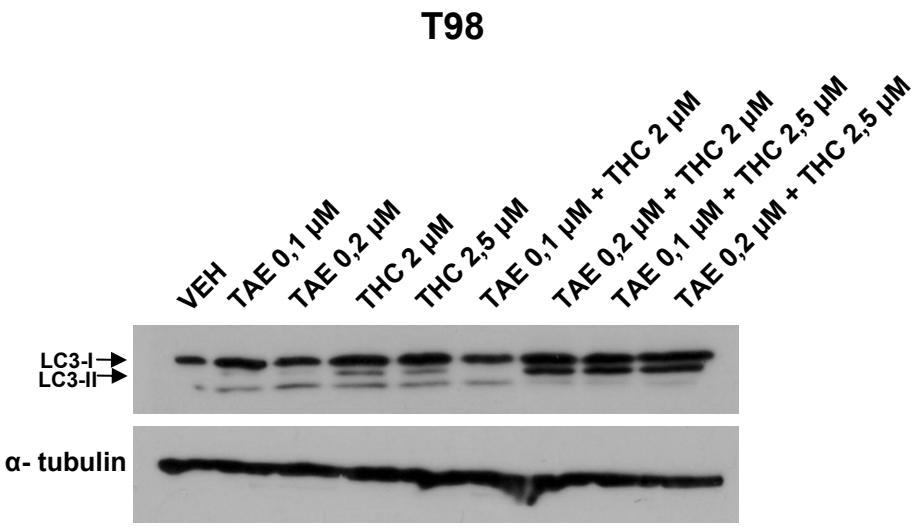

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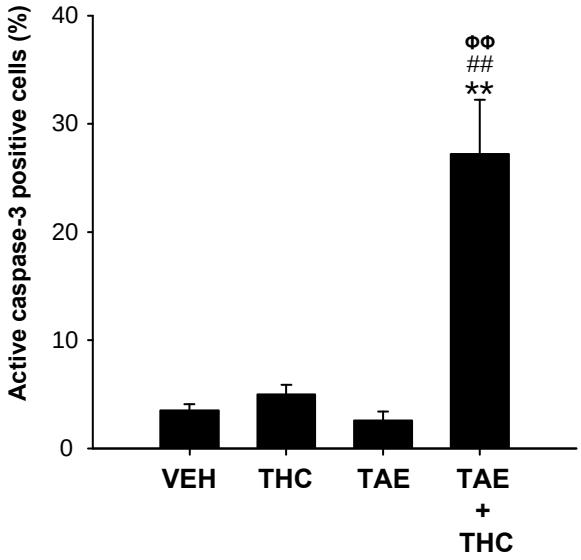
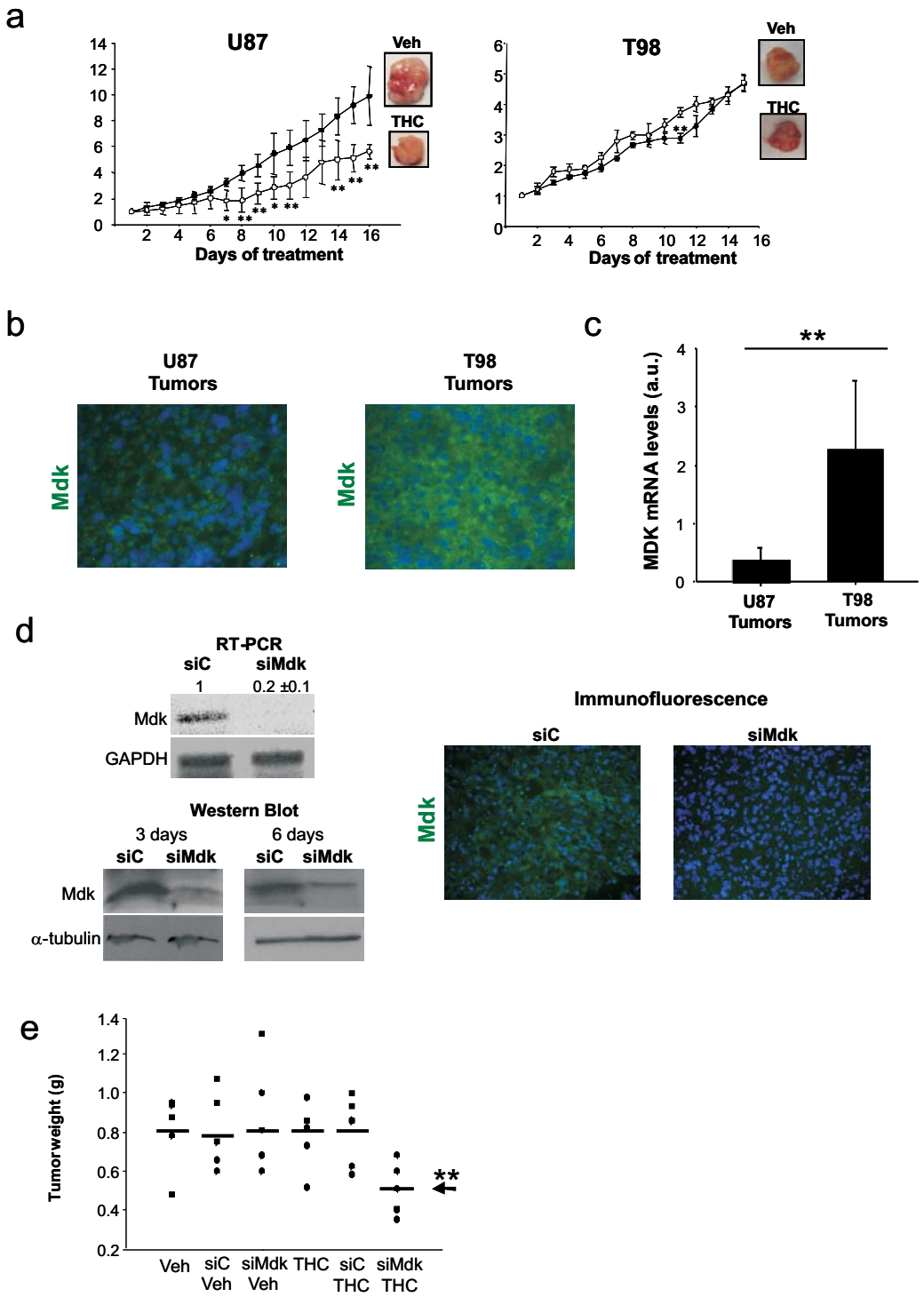


Supplementary Figure 12. Pharmacological inhibition of ALK enhances THC-induced autophagy and apoptosis.
(a) Effect of TAE and THC (24 h) on LC3 lipidation of T98 cells. A representative experiment of 2 is shown. (b) Effect of TAE (0.1 μM) and THC (2 μM) (24 h) on apoptosis (as determined by active-caspase 3 immunostaining) of T98 cells. Data correspond to the percentage of active caspase 3-positive cells relative to the total number of cells (mean ± s.d; n = 3; **$p < 0.01$ from vehicle-treated cells; $^{\#\#}p < 0.01$ from THC-treated cells and $^{\phi\phi}p < 0.01$ f from TAE treated cells).



Suplementary Figure 13. In vivo silencing of Mdk sensitizes T98 cell-derived tumours to THC anti-tumoral action

(a) Effect of THC treatment on the growth of tumor xenografts derived from U87 (upper left panel) or T98 (upper right panel) cells (mean ± s.d; n = 6 for each condition; *p < 0.05 and **p < 0.01 from vehicle-treated tumors). (b) Mdk expression as determined by immunostaining of U87 (left panel) or T98 (right panel) tumors. Representative microphotographs of one U87 and one T87 cell-derived tumor are shown. (c) Mdk mRNA levels as determined by or by real-time quantitative PCR of U87 and T98 tumors. Data correspond to MDK mRNA levels relative to one of the U87 tumors and are expressed in a.u. (mean ± s.d; n = 3 for each condition; **p < 0.01 from U87 tumors). (d) Effect of Mdk silencing on Mdk expression of T98 cell-derived tumors. Left panel: Mdk mRNA levels of siC- and siMDK-transfected T98 tumors (n = 7; a representative RT-PCR experiment is shown), values correspond to Mdk mRNA levels as determined by quantitive real-time PCR]. Right panel: Mdk protein levels as determined by Western blot [(upper panel) n = 3 for each condition. A representative Western blot is shown]; or immunofluorescence [(lower panel); n=5; representative photomicrographs are shown]. (e) Effect of Mdk silencing and THC treatment on the weight of T98 cell-derived tumor xenografts (n=5 for each treatment; **p < 0.01 from the rest of treatments; each closed-circle represents a single tumor).