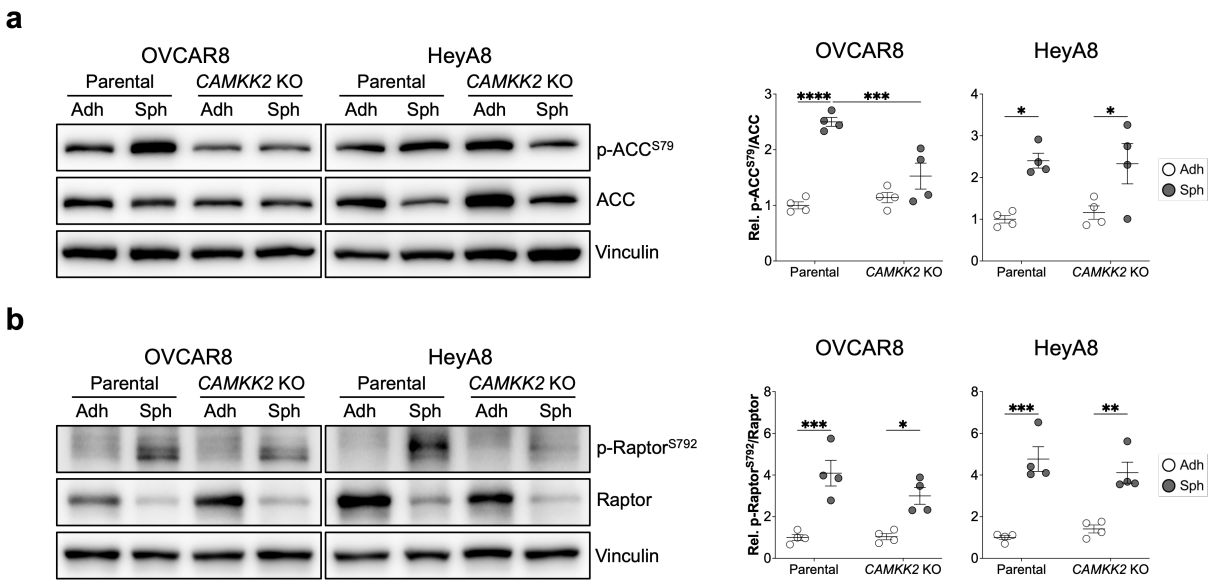
CAMKKβ supports growth and viability of epithelial ovarian cancer in vitro and in vivo

Matthew J. Borrelli, Adrian Buensuceso, Yudith Ramos-Valdes, Tiffany P.A. Johnston, Jacob Haagsma, and Trevor G. Shepherd

Supplementary information

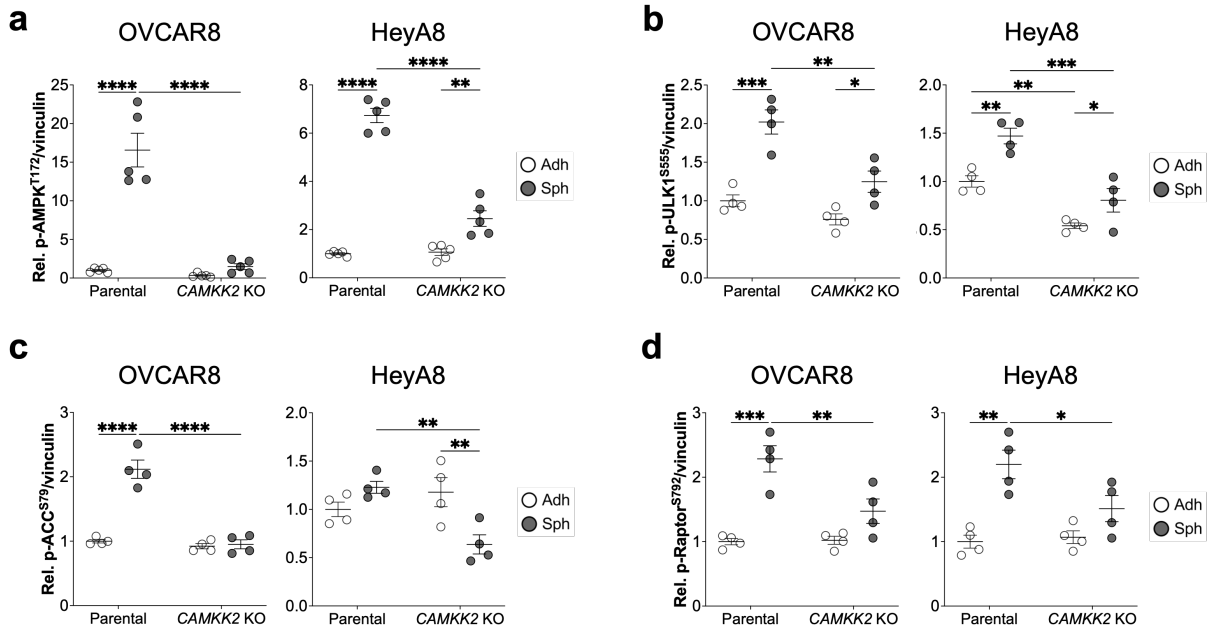
Supplementary Figures S1–S5 and Supplementary Tables S1–S2.



Supplementary Figure S1. Effects of *CAMKK2* ablation on phosphorylation of AMPK targets in EOC spheroids.

(a, b) Western blot analysis and densitometric quantification of **(a)** ACC^{S79} and **(b)** Raptor^{S792} phosphorylation in OVCAR8 parental, OVCAR8 *CAMKK2* KO, HeyA8 parental, and HeyA8 *CAMKK2* KO adherent cells (Adh) and spheroids (Sph).

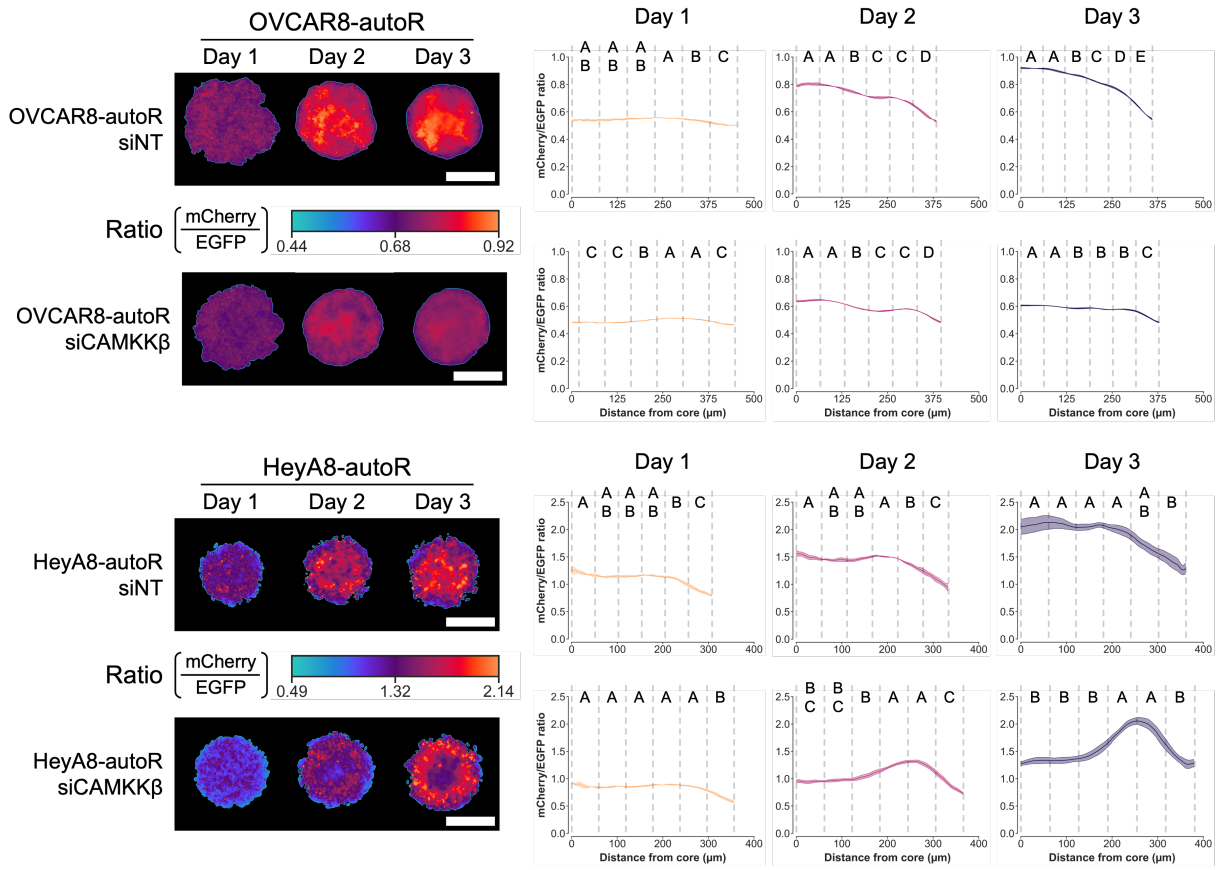
Quantified western blot data represent mean normalized phospho-protein abundance ± SEM for n=4 independent experiments. Signal for each phospho-target was normalized against the respective total target signal. Data are expressed relative to the parental adherent sample, for which the mean was set to 1. Data were analyzed via two-way ANOVA and groups with shared lineage (i.e., parental Adh vs. parental Sph) or shared culture condition (i.e., parental Adh vs. *CAMKK2* KO Adh) were compared using the Holm-Šídák method (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).



Supplementary Figure S2. Effects of CAMKKβ ablation on levels of phosphorylated AMPK and its downstream targets in EOC spheroids.

(a–d) Densitometric quantification of overall **(a)** p-AMPK^{T172}, **(b)** p-ULK1^{S555}, **(c)** p-ACC^{S79}, and **(d)** p-Raptor^{S792} abundance in OVCAR8 parental, OVCAR8 *CAMKK2* KO, HeyA8 parental, and HeyA8 *CAMKK2* KO cells under adherent and spheroid culture conditions. Extended analysis of western blots in Figures 1 and S1.

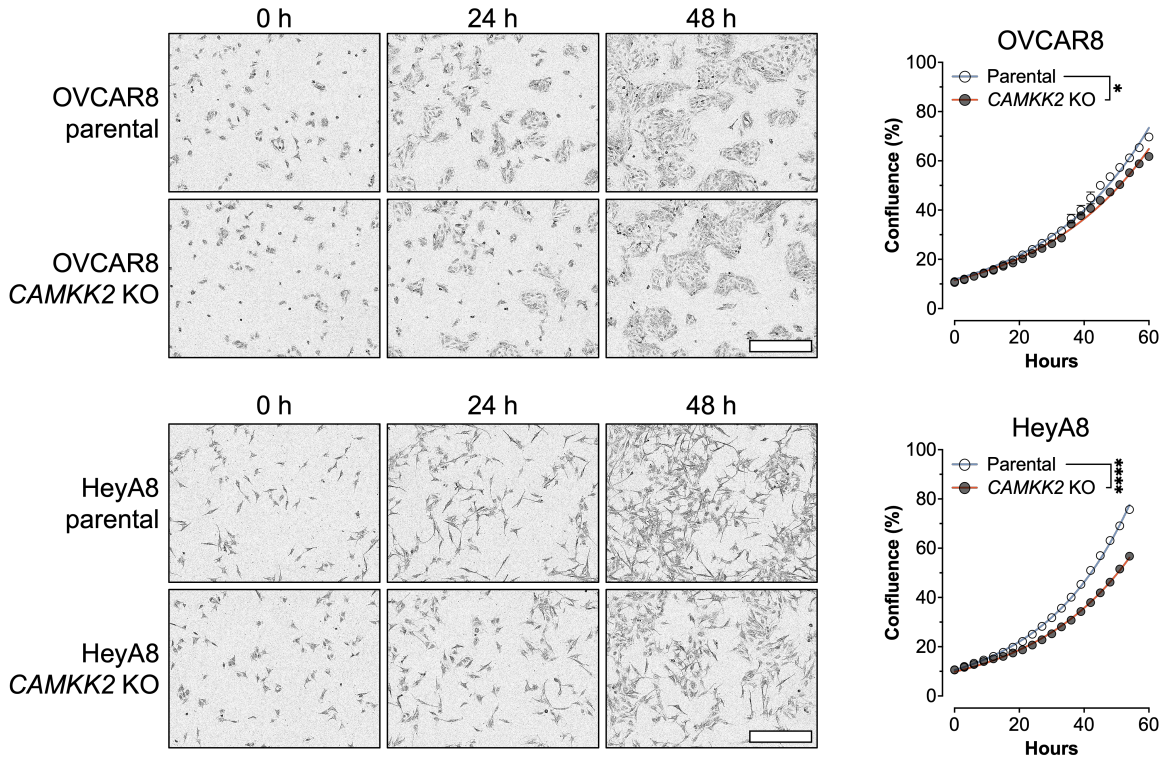
Quantified western blot data represent mean normalized phospho-protein abundance ± SEM for n=5 (p-AMPK^{T172}) or n=4 (other targets) independent experiments. Phospho-protein signals were normalized against vinculin. Data are expressed relative to the parental adherent sample, for which the mean was set to 1. Data were analyzed via two-way ANOVA and groups with shared lineage (i.e., parental Adh vs. parental Sph) or shared culture condition (i.e., parental Adh vs. *CAMKK2* KO Adh) were compared using the Holm-Šídák method (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).



Supplementary Figure S3. Loss of CAMKKβ attenuates autophagy induction and alters its spatial regulation in EOC spheroids.

Representative ratio-mapped images and Spatial Profiling of Ratiometric Trends in Spheroids (SPoRTS) analysis of mCherry/EGFP ratio in mCherry-EGFP-LC3B (autoR) expressing OVCAR8 (OVCAR8-autoR) and HeyA8 (HeyA8-autoR) spheroids under control (siNT) and CAMKKβ knockdown (siCAMKKβ) conditions.

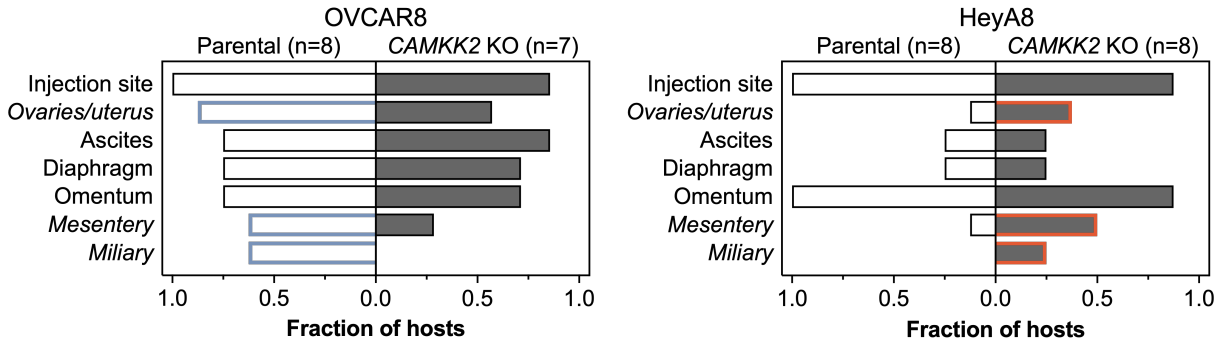
Ratio-mapped images are pseudocoloured according to mCherry/EGFP ratio using a common colour scale for both conditions for a given cell line as indicated by the respective colour bars. SPoRTS ratio profiles represent mean mCherry/EGFP ratio ± SEM for n=3 independent experiments, each of which comprised 5 individual spheroids (15 spheroids in total). For statistical analysis, ratio profile data were segmented into 6 equally spaced regions and the mean ratio value for each region was calculated for each experiment. The resulting data were analyzed via ordinary one-way ANOVA with Tukey's multiple comparisons test. Common letter labels identify groups with means that did not differ significantly from one another ($p \geq 0.05$). Scale bars: 500 μm.



Supplementary Figure S4. CAMKKβ drives proliferation of EOC cells grown under adherent conditions.

Representative phase contrast images of OVCAR8 parental, OVCAR8 *CAMKK2* KO, HeyA8 parental, and HeyA8 *CAMKK2* KO adherent cells and quantification of confluence over time.

Confluence over time data represent mean confluence (%) ± SEM for n=4 independent experiments, each of which comprised 4 fields of view in each of 4 wells (64 fields of view in total). Confluence data were collected using an IncuCyte ZOOM system. Data were analyzed by using the extra sum-of-squares F Test to compare growth constants of exponential curves fitted to confluence-over-time data using least squares regression (*p<0.05, ****p<0.0001). Scale bars: 500 μm.



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64 **Supplementary Figure S5. Comparison of parental and *CAMKK2* KO xenograft tumour**
65 **distributions.**

66 Sites where loss of CAMKKβ was associated with a ≥25% difference in tumour prevalence are
67 marked with italicized labels and coloured outlines. Data represent the fraction of xenograft
68 hosts in which tumours were found in the indicated tissue. Data were not analyzed statistically.

Supplementary Table S1. Cell seeding parameters.

Vessel type	Vessel format	Usage	Cell number		Total volume
			OVCAR8	HeyA8	
Tissue culture (adherent)	100 mm (Sarstedt, 83.3902)	Lysate preparation	7.5×10^5	6.0×10^5	15.0 mL
	6-well (Sarstedt, 83.3920)	Transfection (plasmid) Transfection (siRNA)	4.0×10^5 1.75×10^5	3.2×10^5 1.5×10^5	2.0 mL
	24-well (Sarstedt, 83.3922)	Scratch closure	2.5×10^4		1.0 mL
	96-well (Sarstedt, 83.3924)	Doubling time	1.2×10^3		200 µL
	96-well (Falcon, 353072)	Mesothelial clearance	1.0×10^4		200 µL
Ultra-low attachment (ULA) (spheroid)	6-well (Corning, 3471)	Lysate preparation	5.0×10^5		5.0 mL
	24-well (Corning, 3743)	Trypan blue exclusion cell counting	5.0×10^4		1.0 mL
	96-well round-bottom (Corning, 7007)	SPoRTS analyses Mesothelial clearance	1.0×10^4 2.0×10^3		200 µL

71 **Supplementary Table S2. Details of primary antibody usage.**

Antibody ID	Blocking agent (TBS-T)	Diluent (TBS-T)	Dilution factor
Rabbit monoclonal anti-CAMKK2 Cell Signaling Technology, 16810 RRID: AB_2798771	5% non-fat milk	5% BSA	1:1,000
Rabbit monoclonal anti-phospho-AMPKα (Thr172) Cell Signaling Technology, 2535 RRID: AB_331250	5% BSA	5% BSA	1:1,000
Rabbit monoclonal anti-AMPKα Cell Signaling Technology, 5832 RRID: AB_10624867	5% BSA	5% BSA	1:1,000
Rabbit polyclonal anti-phospho-acetyl-CoA carboxylase (ACC) (Ser79) Cell Signaling Technology, 3661 RRID: AB_330337	5% BSA	5% BSA	1:1,000
Rabbit anti-acetyl-CoA carboxylase (ACC) Cell Signaling Technology, 3676 RRID: AB_2219397	5% BSA	5% BSA	1:1,000
Rabbit monoclonal anti-phospho-ULK1 (Ser555) Cell Signaling Technology, 5869 RRID: AB_10707365	5% non-fat milk	5% BSA	1:1,000
Rabbit monoclonal anti-ULK1 Cell Signaling Technology, 8054 RRID: AB_11178668	5% BSA	5% BSA	1:1,000
Mouse monoclonal anti-Vinculin MilliporeSigma, V9264 RRID: AB_10603627	5% BSA	5% BSA	1:10,000

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