SMARCA4/2 loss inhibits chemotherapy-induced apoptosis by restricting IP3R3-mediated Ca^{2+} flux to mitochondria

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Abstract

Inactivating mutations in *SMARCA4* and concurrent epigenetic silencing of *SMARCA2* characterize subsets of ovarian and lung cancers. Concomitant loss of these key subunits of SWI/SNF chromatin remodeling complexes in both cancers is associated with chemotherapy resistance and poor prognosis. Here, we discover that SMARCA4/2 loss inhibits chemotherapy-induced apoptosis through disrupting intracellular organelle calcium ion (Ca^{2+}) release in these cancers. By restricting chromatin accessibility to *ITPR3*, encoding Ca^{2+} channel IP3R3, SMARCA4/2 deficiency causes reduced IP3R3 expression leading to impaired Ca^{2+} transfer from the endoplasmic reticulum to mitochondria required for apoptosis induction. Reactivation of SMARCA2 by a histone deacetylase inhibitor rescues IP3R3 expression and enhances cisplatin response in SMARCA4/2-deficient cancer cells both *in vitro* and *in vivo*. Our findings elucidate the contribution of SMARCA4/2 to Ca^{2+}-dependent apoptosis induction, which may be exploited to enhance chemotherapy response in SMARCA4/2-deficient cancers.

64   The SWI/SNF family of ATP-dependent chromatin remodeling complexes control gene

65   expression by regulating chromatin organization [1, 2]. They also directly participate in DNA

66   replication, repair and recombination through modifying chromatin or recruiting relevant proteins

67   [3]. Cancer genome-sequencing efforts have revealed mutations in SWI/SNF subunits in more than

68   20% of all human cancers, highlighting their critical roles in tumorigenesis [4]. However, identifying

69   the diver mechanisms of SWI/SNF loss in promoting cancer remains a challenge.

70   SMARCA4 (BRG1) and SMARCA2 (BRM) are the two mutually exclusive ATPase

71   subunits of SWI/SNF. SMARCA4 is inactivated by mutations or other mechanisms in ~ 10% of

72   non-small cell lung cancer (NSCLC) [5, 6, 7, 8, 9]. Furthermore, concomitant loss of SMARCA4/2

73   protein expression occurs in a subset of NSCLC associated with a poor prognosis [6, 10]. In addition

74   to NSCLC, deleterious *SMARCA4* mutations have been found to be the sole genetic driver in ~100%

75   of small cell carcinoma of the ovary, hypercalcemic type (SCCOHT), a rare and aggressive ovarian

76   cancer affecting young women [11, 12, 13, 14, 15]. SCCOHT is also characterized by concurrent loss of

77   SMARCA4/2 protein expression, where *SMARCA2* is epigenetically silenced and its reactivation

78   strongly suppressed SCCOHT growth [16, 17]. In contrast to other cancer types where experimental

79   SMARCA2 inhibition is synthetic lethal with SMARCA4 loss [18, 19, 20], *SMARCA2* silencing may

80   cooperate with SMARCA4 loss in SMARCA4/2-deificient SCCOHT and NSCLC for cancer

81   development [10, 21]. However, the underlying mechanisms are not understood.

82   In addition to regulating gene expression, SWI/SNF components, including SMARCA4*,*

83   have also been implicated in DNA-damage repair (DDR) [22, 23, 24]. Thus, their inactivation may also

84   lead to compromised DDR and genome instability which are widely recognized as driving events

85   in cancer development [25]. However, SCCOHT has a simple genome and harbors few mutations or

86　chromosomal alterations other than inactivating mutations in *SMARCA4* [15, 26, 27], suggesting that

87　altered transcriptional regulation may be the predominant driver of tumorigenesis in this cancer [28].

88　　　Platinum-based chemotherapies, such as cisplatin, induce DNA damage leading to cancer

89　cell apoptosis and have been widely used in clinical practice for treating lung and ovarian cancers

90　[29, 30]. The involvement of SWI/SNF in DDR support the use of these genotoxic agents for treating

91　cancers with SMARCA4/2 deficiency, which does not often cooccur with other druggable

92　oncogenic mutations. Indeed, previous studies have shown that experimental inhibition of

93　SMARCA4 in SMARCA4-proficient cancer cells enhanced response to DNA damaging agents [31,

94　[32, 33]. However, conventional chemotherapies are rarely effective for SCCOHT patients [15, 34] and

95　compared to other ovarian cancer types, SCCOHT cell lines show substantial resistance to these

96　drugs [26, 35]. In line with this, NSCLC patients with concomitant loss of SMARCA4/2 have a poorer

97　prognosis than others [6, 10] while adjuvant chemotherapy remains among primary treatment options

98　for this cancer [29]. Thus, while SWI/SNF deficiencies have been widely associated to cancer

99　progression, the mechanism by which SMARCA4/2-deficient cancer cells have adapted to resist

100　chemotherapy is unknown.

101　　　In this study, we sought to examine the role of SMARCA4/2 in modulating chemotherapy

102　responses in SCCOHT and NSCLC where SMARCA4/2 deficiency are frequently observed. Our

103　results reveal a mechanism linking SMARCA4/2 loss to chemoresistance by inhibiting apoptosis

104　induction and suggest a potential therapeutic strategy for improving treatment for SMARCA4/2-

105　deficient cancers.

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Results

SMARCA4/2 loss confers resistance to chemo-induced apoptosis in cancer cells

SCCOHT harbors few mutations or chromosomal alterations other than inactivating mutations in *SMARCA4* but is typically resistant to conventional chemotherapy in patients [15, 34], suggesting a potential connection between SMARCA4 deficiency and chemotherapy resistance. Since SMARCA4 is also frequently inactivated in NSCLC, we investigated the association of *SMARCA4* expression with chemotherapy response in this cancer type. We first analyzed the most comprehensive NSCLC microarray gene expression data set with clinical outcome from the Director's Challenge data set of lung adenocarcinoma (LUAD, the most common NSCLC subtype) of diverse tumor staging [36]. For our analysis, we chose *SMARCA4* "Jetset probe" unbiasedly identified by Kaplan–Meier (KM) Plotter [37, 38], which is the optimal probe set for specificity, coverage, and degradation resistance without pre-association with patient outcome. We stratified the patients within each treatment group based on median of *SMARCA4* expression and found that low *SMARCA4* expression was significantly associated with worse survival with adjuvant therapies (chemotherapy and radiation) when compared to high *SMARCA4* expression (Figure S1A). This was supported by similar results obtained from KM Plotter analyzing multiple available LUAD data sets of diverse tumor staging using the same probe (Figure S1B). A similar trend in UT lung SPORE data set [39] was also observed although not statistically significant (Figure S1C). Together, these patient outcome results suggest that SMARCA4 deficiency is associated with chemotherapy resistance in NSCLC, similar to that seen in SCCOHT.

Because patient outcomes from the data sets described above may be influenced by other variable factors such as treatment history, we next examined the role of SWI/SNF loss in mediating chemoresistance in more controlled experimental settings using cancer cell lines. First, we

investigated the correlation between chemotherapy responses and mRNA expression levels of *SMARCA4/2* in a large cohort of cell lines (n=436) across different cancer types (Figure S2A), by integrating publicly available drug sensitivity data from Genomics of Drug Sensitivity in Cancer (GDSC) [40] and RNA sequencing (RNA-seq) data from Cancer Cell Line Encyclopedia (CCLE) [41, 42]. We stratified these pan cancer cell lines (n=436) based on their *SMARCA4/2* expression in tertiles (Figure S2B) and found that *SMARCA4*[Low]/ *SMARCA2*[Low] (*A4*[L]/*A2*[L], bottom tertile for both genes) group (n=53) has the highest half maximal inhibitory concentration (IC$_{50}$) among all 4 groups, for common chemotherapy drugs with different mechanisms of action, including cisplatin, cyclophosphamide, topotecan, paclitaxel, etoposide and 5FU (Figure 1A, Figure S2C). Notably, IC$_{50}$ difference between *A4*[L]/*A2*[L] and the *SMARCA4*[High]/ *SMARCA2*[High] (*A4*[H]/*A2*[H] top tertile for both genes) group (n=50) was statistically significant for all of these drugs. The *SMARCA4*[Low]/ *SMARCA2*[High] (*A4*[L]/*A2*[H]) group (n=24) had the second highest IC$_{50}$ which was significantly higher than that of the *A4*[H]/*A2*[H] group in 3 of the 6 drugs including cisplatin. We also observed a consistent trend of higher IC$_{50}$ in the *SMARCA4*[High]/ *SMARCA2*[Low] (*A4*[H]/*A2*[L]) group (n=34) compared to *A4*[H]/*A2*[H] although it was not statistically significant. Similar results were also obtained when analyzing lung cancer cell lines only (Figure 1B, Figure S2D), which represented the largest cancer type (n=103) among the CCLE panel (Figure S2A). Together, these observations show that reduced *SMARCA4/2* expression correlates with resistance to different chemotherapies, including cisplatin, and suggest that SMARCA4 may play a dominant role in regulating drug responses in cancer cells.

To help unbiasedly assess the potential roles of SWI/SNF genes in modulating cisplatin responses, we performed a pooled CRISPR knockout screen targeting 496 epigenetic modifiers in OVCAR4, a SMARCA4/2-proficient high grade serous ovarian carcinoma (HGSC) cell line

(Figure 1C). Upon screen completion, we analyzed the data using the MAGeCK statistical software package [43, 44] to search for candidate genes whose knockout may confer cisplatin resistance. Validating the screen, we identified *EP300* and *CARM1* among the top candidates (ranked #1 and #5, respectively; Table S1 and Figure 1D), whose suppression is known to confer cisplatin resistance [45, 46, 47]. In keeping with our above findings in patient outcome and CCLE cell lines responses to chemotherapies, *SMARCA4* was also highly ranked (#11) in our screen suggesting that SMARCA4 loss confers cisplatin resistance (Figures 1C, 1D and Table S1). *SMARCA2* was not significantly enriched (ranked #162), suggesting that SMARCA4 plays a dominant role in controlling cisplatin response, with SMARCA2 only compensating when SMARCA4 is lost.

To validate the above screen results, we knocked out *SMARCA4* in OVCAR4 cells using CRISPR/Cas9 genome editing system and investigated their apoptotic responses known to be induced by cisplatin treatment. Compared to the parental control, *SMARCA4* knockout ($A4^{KO}$) cells were more resistant to cisplatin-induced elevation of annexin V (cell death marker; Figure S3A), cleaved PARP and cleaved caspase 3 (apoptosis markers; Figure 1E). They also exhibited reduced annexin V^+/ propidium iodide (PI)$^-$ apoptotic cell population (Figure 1F), and had fewer morphological defects, a characteristic of the apoptotic cell (Figure 1G) in response to cisplatin treatment. Similarly, *SMARCA4* knockout also protected OVCAR4 cells against paclitaxel-induced apoptosis (Figure S3B). Furthermore, knockdown of *SMARCA2* using two independent shRNAs in these $A4^{KO}$ cells led to increased resistance to above-described apoptotic responses induced by cisplatin (Figures 1E-G, Figure S3A). Similar results were obtained in HEC116 ovarian endometrial cancer cell line (Figures S3C, S3D), further validating above results in OVCAR4 cells. We also noted that high dose cisplatin treatment in OVCAR4 control cells led to reduced

178   SMARCA4/2 protein expression (Figure 1E), suggesting a potential negative feedback regulation

179   or a selection for cells expressing low SMARAC4/2. To corroborate our results, we sought to

180   perform the reverse experiments by restoring SMARCA4 or SMARCA2 in SMARCA4/2-

181   deficient cancer cells. SMARCA4/2 restoration in SCCOHT cells both strongly suppressed their

182   growth [16, 17], which limited the experimental window to study apoptosis regulation upon

183   subsequent cisplatin treatment. In contrast, SMARCA4/2-deficient NSCLC cells including H1703

184   can tolerate restoration of SMARCA4/2 [48] and thus are better suited for this analysis. Ectopic

185   expression of SMARCA4 or SMARCA2 sensitized H1703 cells to cisplatin treatment and led to

186   strong induction of apoptosis, indicated by elevation of annexin V, cleaved PARP and cleaved

187   caspase 3, a marked increase of the annexin V^+/ PI^- apoptotic cell population, acquisition of

188   apoptotic cell morphology, and impaired growth (Figures 1H-J, Figures S3E, F). Further

189   supporting this, CRISPR/Cas9-mediated *SMARCA4* knockout in SMARCA4/2-proficient H1437

190   NSCLC cancer cells conferred resistant to apoptosis induced by cisplatin treatment; knockdown

191   of *SMARCA2* in these *A4*^{KO} cells led to further increased resistance to cisplatin, indicated by

192   reduction of cleaved PARP and cleaved caspase 3(Figures S3G, H).

193   We further examined the effect of SMARCA4 loss in response to other common

194   chemotherapeutics using above-described isogenic cell pairs of HEC116 and H1703 that differ

195   only in SMARCA4 status. Consistent with cisplatin results, *SMARCA4* knockout in HEC116 cells

196   suppressed elevation of cleaved PARP and cleaved caspase 3 induced by cyclophosphamide,

197   topotecan and paclitaxel (Figure S4A) and led to increased cell viability in the presence of these

198   agents (Figure S4B). Conversely, SMARCA4 restoration sensitized H1703 cells to the treatment

199   with these drugs, as indicated by elevation of apoptosis and growth suppression (Figures S4C, D).

200 <mark>Together, our data indicate that SMARCA4/2 loss inhibits chemotherapy-induced apoptotic</mark>

201 <mark>responses in ovarian and lung cancer cells.</mark>

SMARCA4/2 loss results in altered intracellular Ca^{2+} homeostasis in cancer cells

203   To understand how SMARCA4/2 regulate chemotherapy sensitivity and apoptosis

204 induction, we analyzed the transcriptome regulated by SMARCA4 using SCCOHT cells, taking

205 advantage of their simple genetic background. Gene Set Enrichment Analysis (GSEA) of RNA-

206 seq data generated in SCCOHT-1 and BIN-67 cells ± SMARCA4 restoration [49] reveals top 10

207 Gene Ontology (GO) terms regulated by SMARCA4 consistently shared by these two SCCOHT

208 cell lines (Figures S5A, B). Multiple terms associated with ion/calcium homeostasis were

209 identified including "ion transmembrane transporter" and "calcium ion binding" (Figures 2A, B).

210 The established crucial role of calcium ion (Ca^{2+}) homeostasis in apoptosis induction [50] makes

211 these GO terms particularly interesting. Transient Ca^{2+} release from the endoplasmic reticulum

212 (ER), the major intracellular Ca^{2+} store, to the cytosol and subsequent transfer to mitochondria is

213 important for cellular signal transductions as well as ATP production [51]. However, excessive ER-

214 Ca^{2+} release leads to mitochondrial Ca^{2+} overload and cell death, which has recently been

215 associated to the selective vulnerability of cancer cells [52, 53, 54]. Together, these transcriptome

216 analyses in SCCOHT cell lines indicate that Ca^{2+} homeostasis may be a commonly altered cellular

217 process by SMARCA4, contributing to their roles in apoptosis regulation and cancer cell survival.

218   Given the crucial role of intracellular Ca^{2+} signaling in apoptosis induction, we reasoned

219 that SMARCA4/2 may affect apoptosis by regulating intracellular Ca^{2+} flux. To validate the role

220 of SMARCA4/2 in Ca^{2+} homeostasis and transfer to mitochondria, we measured the changes in

221 cytosolic and mitochondrial Ca^{2+} content of SCCOHT-1 cells, ± SMARCA4 restoration, in

222 response to histamine, an inositol trisphosphate (IP3) agonist activating ER Ca^{2+} release via

223   inositol trisphosphate receptor (IP3R) [55]. In order to monitor intracellular Ca^{2+} dynamics, we

224   expressed genetically-encoded Ca^{2+} indicators (GECI) targeted to the cytosol (R-GECO) [56] or

225   mitochondria (CEPIA-2mt) [57] and monitored GECI fluorescence upon ER-Ca^{2+} release stimulation

226   by spinning disk confocal microscopy (Figure S6). While histamine stimulation induced little

227   changes in cytosolic or mitochondrial Ca^{2+} in SCCOHT-1 control cells, it strongly elevated Ca^{2+}

228   content in both compartments in SMARCA4-restored cells (Figures 2C-E). Consistent with this,

229   restoration of SMARCA4 in H1703 cells also significantly increased ER-Ca^{2+} release to the

230   cytosol and Ca^{2+} transfer to the mitochondria upon histamine stimulation, compared to control

231   cells (Figures 2F-H). These data indicate that SMARCA4 plays a causal role in regulating

232   intracellular Ca^{2+} homeostasis by enabling ER-Ca^{2+} release to the cytosol and mitochondria.

233   The increased cytosolic and mitochondrial Ca^{2+} content observed upon SMARCA4

234   restoration could be due to either direct enhanced Ca^{2+} release from the ER or elevated capacity of

235   the ER-Ca^{2+} content. To distinguish these possibilities, we measured the cytosolic Ca^{2+} changes in

236   above isogenic cell pairs of SCCOHT-1 and H1703 in response to thapsigargin, an inhibitor of

237   sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA), which can entirely deplete ER Ca^{2+}

238   stores [55]. Interestingly, restoration of SMARCA4 does not increase maximal cytosolic ER-Ca^{2+}

239   release induced by thapsigargin treatment in SCCOHT-1 or H1703 cells (Figures S7A, B),

240   suggesting that SMARCA4 promotes Ca^{2+} release from the ER rather than an increase in ER-Ca^{2+}

241   storage capacity. Further supporting this, SMARCA4 knockout in OVCAR4 and H1437 cells

242   significantly decreased the induction of cytosolic and mitochondrial Ca^{2+} upon histamine treatment

243   (Figures 2I-N), even though *SMARCA4* knockout had increased ER Ca^{2+} stores as indicated by an

244   increase in cytosolic Ca^{2+} in OVCAR4 cells, but not in H1437 cells, following thapsigargin

245   stimulation (Figures S7C, D). Finally, to rule out the potential contribution of the mitochondrial

246   Ca^{2+} uptake machinery in this phenotype, we showed that protein levels of the mitochondrial

247   calcium uniporter (MCU) and its regulators [58, 59] were unchanged in these cell lines, indicating that

248   Ca^{2+} transfer defects were not due to defective mitochondrial Ca^{2+} import machinery (Figure S7E).

249   Together, these results suggest that SMARCA4/2 regulate intracellular Ca^{2+} homeostasis and

250   mitochondrial Ca^{2+} content likely by controlling Ca^{2+} release from the ER.

251

252   SMARCA4/2 directly regulates *ITPR3* expression

253   To dissect the detailed mechanism by which SMARCA4/2 regulate Ca^{2+} homeostasis, we

254   further investigated Ca^{2+}-related genes in ion/calcium associated GO terms identified from the

255   above transcriptome analysis in SCCOHT cells (Figures 2A-B). Overlapping the two datasets

256   yielded 198 common genes affected by SMARCA4 restoration in both SCCOHT-1 and BIN-67

257   cells (Figure 3A; Table S2). To help identify direct targets of SMARCA4, we examined these 198

258   commonly regulated genes in a Chromatin Immunoprecipitation Sequencing (ChIP-Seq) data set

259   profiling SMARCA4 occupancy in BIN-67 cells ± SMARCA4 restoration [60]. This analysis

260   revealed 69 of the 198 genes showing SMARCA4 occupancy in their loci (Figure 3A; Table S2).

261   Considering that SMARCA4 and SMARCA2 may regulate the same target genes and that

262   SMARCA4 also modulates Ca^{2+} homeostasis in NSCLC cells (Figures 2F-H), we then examined

263   the regulation of these 69 genes in an independent RNA-seq dataset of BIN-67 cells ± SMARCA2

264   restoration (Pan et al., 2019) and a microarray dataset of NSCLC cell line H1299 ± SMARCA4

265   restoration [61]. Notably, all of the 69 SMARCA4-affected genes were also regulated by SMARCA2

266   in BIN-67 cells, indicating that SMARCA4/2 may have redundant function in controlling Ca^{2+}

267   homeostasis (Figure 3B). In keeping with the fact that lung cancer cells have more complex genetic

268   landscapes than SCCOHT [15, 62], only 4 genes, namely *ITPR3*, *MATN2, EHD4* and *ATP2B4*, were

269   consistently upregulated by SMARCA4 in both cancer types (Figure 3B).

270   Among these 4 common genes, *ITPR3* encodes inositol 1,4,5-trisphosphate receptor type 3

271   (IP3R3), one of the IP3R family members that form Ca^{2+} channels on the ER and play critical roles

272   in intracellular Ca^{2+} homeostasis and cell apoptosis [52, 63]. IP3R3 localizes at the mitochondria-

273   associated membranes (MAMs), a signaling platform allowing the generation of high-

274   microdomains of Ca^{2+} concentration required for efficient mitochondrial Ca^{2+} uptake [64], and

275   preferentially transmits apoptotic Ca^{2+} signals into mitochondria over other IP3Rs [65]. Tumor

276   suppressors such as PTEN, BAP1 and PML have been shown to inhibit apoptosis induction in

277   cancer cell by promoting IP3R3-mediated Ca^{2+} flux from the ER to mitochondria [66, 67, 68]. Thus,

278   we hypothesized that SMARCA4/2 may promote Ca^{2+} flux to the mitochondria and apoptosis

279   induction by directly regulating *ITPR3* gene expression. Corroborating our transcriptome data

280   above (Figure 3B), ectopic expression of SMARCA4 or SMARCA2 in both SCCOHT (BIN-67,

281   SCCOHT-1) and NSCLC (H1299, H1703) cells resulted in elevated mRNA and protein expression

282   of IP3R3 (Figures 3C, 3D). Conversely, *SMARCA4* knockout in OVCAR4, HEC116 and H1437

283   cells suppressed IP3R3 expression which was further downregulated upon subsequent *SMARCA2*

284   knockdown (Figure S8). These data established that SMARCA4/2 promotes IP3R3 expression in

285   both ovarian and lung cancer cells, likely through direct regulation of transcription.

286   Given the chromatin remodeling role of SWI/SNF, we then focused on the chromatin

287   architecture of the *ITPR3* locus and its potential regulation by SMARCA4/2. Indeed, SMARCA4

288   occupancy was observed at the *ITPR3* promoter in ChIP-seq data of the BIN-67 cells upon

289   SMARCA4 restoration (Figure 3E) [60]. We also detected this SMARCA4 occupancy in H1703 cells

290   with SMARCA4 restoration [48] and in H1299 cells expressing inducible SMARCA4 [61] (Figure 3E).

291   These data suggest that SMARCA4/2 may directly regulate *ITPR3* expression. Consistent with

292   this, we found that ChIP-seq signals of H3K27Ac, a chromatin mark associated with active

293   promoter and enhancer, were elevated at the up-stream and gene body regions of *ITPR3* in BIN-

294   67 cells after SMARCA4 restoration [60] and in H1703 cells after restoration of SMARCA4 or

295   SMARCA2 [48] (Figure 3F, upper panel). Furthermore, the Assay for Transposase-Accessible

296   Chromatin using sequencing (ATAC-seq) peaks at these *ITPR3* genomic regions were also

297   elevated upon SMARCA4/2 restoration in H1703 cells (Figure 3F, lower panel), indicating an

298   enhanced chromatin accessibility at the *ITPR3* locus when SMARCA4/2 were present. Together,

299   these data suggest that SMARCA4/2 promotes *ITPR3* transcription by directly remodeling

300   chromatin structure at its gene locus.

301

302   **SMARCA4/2 loss inhibits apoptosis by restricting IP3R3-mediated Ca^{2+} flux to**

303   **mitochondria**

304   Next, we investigated whether reduced IP3R3 expression accounts for compromised Ca^{2+}

305   flux in SMARCA4/2-deficient SCCOHT and NSCLC cells (Figures 2C-N). To this end, we

306   performed rescue experiments by suppressing SMARCA4-mediated IP3R3 induction in

307   SCCOHT-1 and H1703 cells. Accompanied by an increase of IP3R3 levels (Figure 4A), ectopic

308   SMARCA4 expression in SCCOHT-1 cells strongly elevated cytosolic (Figure 4B) and

309   mitochondrial (Figure 4C) Ca^{2+} contents in response to histamine stimulation. Notably, in these

310   SMARCA4-restored cells, shRNA-mediated knockdown of IP3R3 to levels similar to control cells

311   prevented ER-Ca^{2+} release, characterized by a significant decrease of cytosolic and mitochondrial

312   Ca^{2+} contents (Figures 4A-C). These results were confirmed in H1703 cells where suppression of

313   IP3R3 was achieved by siRNA (Figures 4D-F). Furthermore, cytosolic Ca^{2+} measurement upon

314 thapsigargin stimulation in the above SCCOHT-1 and H1703 cells indicated that ER Ca^{2+} storage

315 capacity was not significantly altered upon *ITPR3* knockdown (Figure S9). Together, these data

316 indicate that reduced IP3R3 expression is the critical contributor to the compromised Ca^{2+} flux in

317 SMARCA4/2-deficient cells.

318   In line with the established role of IP3R3 in Ca^{2+}-mediated apoptosis, suppression of IP3R3

319 in OVCAR4 cells prevented cisplatin-induced apoptosis as indicated by reduced levels of cleaved

320 PARP and cleaved caspase 3 (Figure 4G) and the annexin V^+/PI^- apoptotic cell population (Figure

321 4H). Conversely, ectopic expression of IP3R3 in H1703 cells enhanced apoptotic induction and

322 growth suppression after cisplatin treatment (Figure 4I, Figures S10A, B). Similarly, ectopic

323 IP3R3 expression also sensitized BIN-67 cells to cisplatin treatment (Figures S10C, D). Thus,

324 IP3R3 seems to be necessary and sufficient to mediate cisplatin-induced apoptosis in these models.

325 Given that SMARCA4/2 directly activates *ITPR3* expression (Figure 3), we then investigated

326 whether reduced IP3R3 expression in SMARCA4/2-deficient cells drives resistance to

327 chemotherapy-induced apoptosis. As shown in Figures 4J, K, while SMARCA4 restoration in

328 H1703 led to increased IP3R3 expression with concomitant elevation of cleaved PARP and

329 cleaved caspase 3 as well as the annexin V^+/PI^- apoptotic cell population after cisplatin treatment,

330 knockdown of IP3R3 markedly suppressed the induction of these apoptosis markers in these

331 SMARCA4-expressing cells, corroborating Ca^2 signaling defects in these cells (Figures 4D-F).

332 Together, these data suggest that SMARCA4/2 loss inhibits chemotherapy-induced apoptosis by

333 constricting IP3R3-mediated Ca^{2+} flux to mitochondria.

334

335 **IP3R3 expression is reduced in SMARCA4/2-deficient cancers**

14

336   To further validate our findings of *ITPR3* regulation by SMARCA4/2 in cell models with

337   genetic perturbation, we analyzed mRNA expression of *ITPR3* and *SMARCA4/2* in RNA-seq data

338   sets of ovarian (n=47) and lung cancer (n=192) cell lines available from CCLE [41, 42]. For both

339   cancer types, cell lines with low *SMARCA4* expression (bottom quartile) also expressed lower

340   levels of *ITPR3* compared to the rest of cell lines with high *SMARCA4* expression (Figure S11A).

341   Furthermore, we observed a significant positive correlation between *ITPR3* and *SMARCA2* in these

342   ovarian (n=11, r = 0.825) and lung (n=48, r = 0.584) cancer cell lines with low *SMARCA4*

343   expression (Figure 5A). Moreover, in a panel of 20 NSCLC cell lines, reduced IP3R3 protein was

344   observed in SMARCA4-deficient cells compared to SMARCA4-proficient cells; overall

345   SMARCA4/2 dual deficient cell lines expressed the lowest levels of IP3R3 (Figure 5B). These

346   results are in line with our above functional data, supporting that IP3R3 expression is reduced in

347   SMARCA4/2-deficient ovarian and lung cancer cells.

348   Next, we investigated the relationship between IP3R3 and SMARCA4/2 expression in

349   patient tumors. We analyzed the available TCGA RNA-seq data sets of ovarian serous

350   cystadenocarcinoma (OV) [69], lung adenocarcinoma (LUAD) and lung squamous cell carcinoma

351   (LUSC) tumors [7, 70]. Similar to the above observations in cell lines, *ITPR3* mRNA in patient tumors

352   with the bottom quartile of *SMARCA4* expression is significantly reduced compared the other

353   tumors in all three data sets (Figure S11B). Confirming the cell line results (Figure 5A), *ITPR3*

354   was also significantly correlated with *SMARCA2* mRNA in these tumors with low *SMARCA4*

355   expression (Figure 5C, Figure S11C). Furthermore, we analyzed *ITPR3* mRNA expression in

356   SCCOHT patient tumors (n=13) characterized by concomitant loss of SMARCA4/2 protein

357   expression. In keeping with above analysis, *ITPR3* mRNA in SCCOHT tumors is similar to OV

358   tumors with low expression of *SMARCA4/2* (n=42) while significantly lower than OV tumors with

15

359   high expression of *SMARCA4/2* (n=50) [69] (Figure 5D). Using immunohistochemistry (IHC), we

360   also examined IP3R3 protein expression in patient tumors of SCCOHT and HGSC with an IP3R3

361   antibody whose IHC specificity was verified by RNAi (Figure S12). As shown in Figures 5E and

362   F, SCCOHT tumors (n=45) expressed significantly lower levels of IP3R3 than HGSCs (n=45).

363   Consistently, NSCLC tumors with low SMARCA4 expression (n=9, H-score $\leq$ 100) expressed

364   significantly lower IP3R3 protein than those with higher SMARCA4 expression (n=50, H-

365   score >200) (Figures 5G, H). Together, these results from multiple cohorts of cell lines and patient

366   tumor samples support the cooperative roles of SMARCA4/2 in regulating *ITPR3* and confirm

367   reduced IP3R3 expression in SMARCA4/2-deficient cancers.

368   Given that suppressed IP3R3-mediated Ca^{2+} flux and apoptosis has been linked to other

369   major tumor suppressors PTEN, BAP1 and PML, in driving tumorigenesis [66, 67, 68], our above

370   analyses suggest that this may also play a role in SMARCA4/2-deficient cancers. We examined

371   this possibility *in vivo* using a xenograft model of H1703 cells with exogenous SMARCA4

372   expression, using a validated doxycycline-controlled expression system [49]. Upon tumor

373   establishment, we induced SMARCA4 expression with doxycycline treatment, which indeed

374   resulted in suppression of tumor growth (Figure 5I). Furthermore, IHC analysis of endpoint

375   tumors showed that induced-SMARCA4 expression led to elevated expression of IP3R3 and

376   cleaved caspase 3 (Figures 5J, K). While this requires further studies, these data support that

377   reduced IP3R3 expression in SMARCA4/2-deficient cancers may directly contribute to the

378   tumorigenesis through suppression of apoptosis.

379

380   **Histone deacetylase inhibitor rescues IP3R3 expression and enhances cisplatin response in**

381   **SMARCA4/2-deficient cancer cells**

382   Our data show that SMARCA4/2-deficient cancer cells are resistant to cisplatin in part

383   through suppression of IP3R3 and that ectopic IP3R3 expression can sensitize these cancer cells

384   to cisplatin-induced apoptosis (Figures 4G-K, Figure S10). Although IP3R3 is not targetable, its

385   expression is directly activated by SMARCA4/2 (Figure 3). In contrast to deleterious mutations in

386   *SMARCA4*, SMARCA2 loss is caused by epigenetic silencing in SCCOHT and NSCLC [17, 71, 72, 73].

387   Furthermore, histone deacetylase inhibitor (HDACi), a class of anti-cancer drugs that block the

388   deacetylation of chromatin and other cellular substrates involved in cancer initiation and

389   progression [74, 75], has also been shown to reactivate SMARCA2 expression in SCCOHT and lung

390   cancer cells [17, 76, 77]. Indeed, treatments with a second generation HDACi quisinostat [78] resulted in

391   strong activation of SMARCA2 with concomitant elevation of IP3R3 at both mRNA and protein

392   levels in SCCOHT (Figure S13) and SMARCA4/2-deficient NSCLC cancer cells (Figures 6A, B).

393   Consistent with this, quisinostat treatment strongly elevated cytosolic (Figure S14A) and

394   mitochondrial (Figure S14B) Ca^{2+} contents in response to histamine stimulation, similar to the

395   levels induced by ectopic SMARCA4 expression in H1703 cells (Figures S14A-C). Notably,

396   siRNA-mediated knockdown of IP3R3 in these quisinostat-treated cells prevented ER-Ca^{2+} release,

397   characterized by a significant decrease of cytosolic and mitochondrial Ca^{2+} contents (Figures

398   S14A-C). Together, these data indicate that quisinostat treatment can indirectly restore IP3R3

399   expression and rescue Ca^{2+} flux in SMARCA4/2-deficient cancer cells.

400   Next, we explored the possibility of using HDACi to restore chemotherapy sensitivity in

401   SMARCA4/2-deficient cancer cells. Remarkably, the combination treatment of cisplatin and

402   quisinostat in H1703 cells resulted in strong elevation of cleaved PARP and cleaved caspase 3

403   (Figure 6B), the annexin V$^+$/PI$^-$ apoptotic cell population (Figure 6C), and growth suppression

404   (Figure 6D). Given that HDACi is expected to activate expression of genes other than *SMARCA2*,

it was important to verify the essential contribution of SMARCA2 reactivation to apoptosis induction by this drug combination. Supporting this, CRISPR/Cas9-mediated *SMARCA2* knockout in H1703 cells blunted the elevation of IP3R3 and cell apoptosis markers induced by combination treatment of quisinostat and cisplatin (Figure 6E). Furthermore, siRNA-mediated knockdown of IP3R3 also prevented the elevation of apoptosis markers in H1703 cells induced by this treatment combination (Figure 6F). Finally, confocal live cell imaging demonstrated that the combination of cisplatin and quisinostat strongly induced an increase of basal mitochondrial Ca^{2+} levels in these cells (Figures 6G, H). These results demonstrate that quisinostat can stimulate SMARCA2-dependent IP3R3 expression to restore ER-Ca^{2+} release-induced mitochondrial Ca^{2+} flux and chemotherapy sensitivity in SMARCA4/2-deficient cancer cells.

Finally, we validated the antitumor effect of this cisplatin and quisinostat drug combination *in vivo* using a xenograft model of H1703 cells. After tumor establishment, animals were treated with cisplatin (4 mg kg^{-1}), quisinostat (10 mg kg^{-1}) or their combination. Consistent with our *in vitro* results, the combination more effectively suppressed tumor growth than each single drug alone, as indicated by a significant reduction of both tumor volume and weight (Figure 6I). We noted that some animals treated with cisplatin or the combination, but not quisinostat alone, showed body weight loss (Figure S15A), likely associated with chemotherapy-induced side effects. Nevertheless, when normalized to the animal body weight, the drug combination still showed significant reduction of both tumor volume and weight compared to single treatments (Figures S15B, C). Furthermore, IHC analysis of endpoint tumors revealed that quisinostat treatment was able to induce protein expression of SMARCA2 and IP3R3 (Figures 6J, K) and, when combined with cisplatin, synergistically elicited a strong apoptotic response as indicated by a marked increase of cleaved caspase 3 levels (Figures 6J, K). Taken together, our data provide a proof-of-

428 concept treatment strategy for enhancing chemotherapy response in patients affected by

429 SMARCA4/2-deficient cancers.

430

Discussion

We show that SMARCA4/2 deficiency impairs chemotherapy-induced apoptotic responses in ovarian and lung cancers at least in part by altering ER to mitochondria Ca^{2+} flux. By directly restricting *ITPR3* expression, SMARCA4/2 loss inhibits Ca^{2+} transfer from the ER to mitochondria required for apoptosis induction. Consequently, stimulation of *ITPR3* expression through SMARCA2 reactivation by HDACi enhanced chemotherapy response in SMARCA4/2-deficient cancer cells.

SWI/SNF subunits are frequently mutated in human cancers [4], which has been connected to hallmarks of cancers including aberrant cell proliferation, linage differentiation, and altered metabolism [1, 28]. Our findings establish a new functional link between SMARCA4/2 loss and dampened IP3R3-mediated Ca^{2+} flux in resisting programmed cell death. While our current study mostly focuses on chemoresistance, we also found that SMARCA4 restoration alone suppressed tumor growth of H1703 xenografts associated with increased expression of IP3R3 and cleaved caspase 3. This suggests that altered Ca^{2+} homeostasis may also directly contribute to the tumorigenesis of SMARCA4/2 loss through suppression of apoptosis, as previously shown for other major tumor suppressors PTEN, BAP1 and PML [66, 67, 68]. Additional investigations are warranted to further confirm these results. Given the cooperative roles of SMARCA4/2 in regulating ER to mitochondria Ca^{2+} flux and apoptosis, it is likely that they exert these functions in a SWI/SNF-dependent manner. Therefore, exploring the potential role of other SWI/SNF subunits frequently altered in cancers, such as ARID1A [4], in Ca^{2+} homeostasis and apoptosis may help understand the oncogenic mechanisms underlying other SWI/SNF-deficient cancers.

Our study examined the roles of SMARCA4/2 in regulating chemotherapy response and apoptosis induction using cancer cell lines that naturally harbor SMARAC4/2 alterations. This is

454   different from previous studies employing RNAi-mediated *SMARCA4* knockdown in SMARCA4-

455   proficient cancer cells which led to enhanced response to DNA damaging agents [31, 32, 33]. We found

456   that naturally occurred SMARCA4/2-deficient cancer cells are more resistance to chemotherapy,

457   which is in line with previous reports showing that SCCOHT is typically more resistant to

458   conventional chemotherapy in both cell models and patients [15, 34]. Similarly, experimental

459   suppression of SMARCA2 has been shown to be selective lethal to SMARCA4

460   deficient/SMARCA2 proficient cancer cells [18, 19, 20]. However, concomitant loss of SMARCA4/2

461   occurs in almost all SCCOHTs and a subset of NSCLCs associated with poorer prognosis in

462   patients[6, 10, 16, 17]. Therefore, naturally occurred SMARC4A/2-deficient cancers may represent a

463   unique group with distinct properties such as altered Ca^{2+} homeostasis leading to chemotherapy

464   resistance.

465   Similar to SCCOHT, our analysis in multiple NSCLC datasets of diverse tumor staging

466   including the most comprehensive Director's Challenge dataset suggests that reduced *SMARCA4*

467   expression is associated with chemoresistance in NSCLC. A previous report [79] analysing the

468   JBR.10 data set of NSCLCs from early stages [80] showed that patients whose tumors expressed low

469   *SMARCA4*, but not high *SMARCA4,* benefited from the adjuvant therapy of cisplatin and

470   vinorelbine (a microtubule inhibitor). This discrepancy is likely due to differences in *SMARCA4*

471   microarray probe sets chosen, patient cohort compositions, and data analysis methods. While we

472   used the optimal "Jetset probe" unbiasedly identified by the KM Plotter without pre-association

473   with patient outcome, microarray technology has limited sensitivity and specificity in quantifying

474   gene expression. Thus, these results require further confirmation using better tools such RNA-seq.

475   In addition, we recognize that patient outcome is often influenced by multiple factors such as

476   treatment history, which was not uniform among all patients analysed. Therefore, additional

477 clinical studies are needed to better control these variants and evaluate roles of SMARCA4/2

478 expression in predicting chemotherapy responses in NSCLC patients.

479 HDACi have been clinically approved for the treatment of several hematological

480 malignancies but their activity in solid tumors has been limited as single agents [74, 75]. Thus,

481 identifying genetic vulnerability of HDACi and effective drug combinations may enhance their

482 clinical utility. SCCOHT cells have been shown to be more sensitive to HDACi than

483 SMARCA4/2-deficient NSCLC cells [76]. This may be because NSCLC have a more complex

484 genetic make-up than SCCOHT [15, 62]. Our study provided proof-of-principle data supporting that

485 HDACi may be a potential therapeutic strategy to stimulate *ITPR3* transcription through

486 SMARCA2 reactivation and sensitize SMARCA4/2-deficient cancers to chemotherapy. Other

487 strategies may also be explored. For example, GGTi-2418, a geranylgeranyl transferase inhibitor,

488 sensitizes A549 cells to apoptosis induction by photodynamic therapy both *in vitro* and in

489 xenograft models *via* stabilizing the IP3R3 protein [66]. Of note, A549 is also a SMARCA4-deficient

490 NSCLC cell line and this independent study does further support the notion of elevating IP3R3

491 expression to enhance chemotherapy response in SMARCA4/2-deficient cancers. However, both

492 HDACi and GGTi-2418 intervene IP3R3 expression indirectly and may cause unexpected toxicity.

493 Therefore, other agents that directly facilitate Ca^{2+} flux from the ER to mitochondria need to be

494 investigated in the future. In addition to IP3R3, other common targets of SMARCA4/2 may also

495 play a role in altered Ca^{2+} homeostasis impacting apoptosis, which could serve as potential drug

496 targets in SMARCA4/2-deficient cancers and will require further studies.

497

498

499

500   In summary, we have uncovered that SMARCA4/2 loss restricts IP3R3-mediated Ca^{2+} flux

501   from the ER to mitochondria, leading to resistance to chemotherapy-induced apoptosis in ovarian

502   and lung cancers. Our study provides novel insights into the molecular mechanisms of SWI/SNF

503   loss in promoting drug resistance and suggests a potential therapeutic strategy to enhance

504   chemotherapy response in patients affected by SMARCA4/2-deficient cancers.

Author contributions

Y.X., J.L.M., K.Y., Z.F., X.Z., B.M., L.W., G.M., A.M. and V.P. carried out experiments. Y.X. and J.L.M. performed statistical analyses. Y.X., F.J. and W.L. conducted bioinformatic analysis. A.Y., T.G., M.C., S. J., A.V.G., L.M.P., J.S. and W.H.G. contributed samples and provided advice. P. F., S.C.B., L.F., and M.C.G. provided pathology expertise. Y.X., J.P. and S.H. wrote the manuscript with inputs from all authors. J.R., M.P., W.D.F., J.P. and S.H. supervised the experiments. J. P. and S.H. conceived and oversaw the study. All authors read and approved the final manuscript.

Ethics declarations

Competing interests

The authors declare no competing interests.

529   **Figure legends**

530

531   **Figure 1 SMARCA4/2 loss causes resistance to chemotherapeutics in ovary and lung cancers**

532   (A, B) The half maximal inhibitory concentration (IC_{50}) of cisplatin in pan cancer (A) and lung

533   cancer (B) cell lines with differential mRNA expression for *SMARCA4* and *SMARCA2* (see Figure

534   S2B for stratification). *A4*^{H}: *SMARCA4*^{High}; *A4*^{L}: *SMARCA4*^{Low}; *A2*^{H}: *SMARCA2*^{High}; *A2*^{L}:

535   *SMARCA2*^{Low}. Cell line numbers are indicated in grey below each group. Kruskal–Wallis test

536   followed by Dunn's test for multiple comparisons to *A4*^{H}*A2*^{H} group, **$p < 0.01$, ****$p < 0.0001$.

537

538   (C) Schematic outline of a pooled CRISPR screen with a sgRNA knockout library against

539   epigenetic regulators to identify genes required for cisplatin response in OVCAR4 cells.

540

541   (D) MAGeCK analysis [43, 44, 81] for screen in (C). Genes were ranked by robust rank aggregation

542   (RRA).

543

544   (E-G) Immunoblots (E), annexin V^{+}/PI^{-} apoptotic cell population determined by flow cytometry

545   (F), and representative phase-contrast images (G) of OVCAR4 cells with indicated *SMARCA4/2*

546   perturbations and cisplatin treatments (E, F, 48 hours).

547

548   (H-J) Immunoblots (H), annexin V^{+}/PI^{-} apoptotic cell population (I), and representative phase-

549   contrast images (J) of H1703 cells with indicated *SMARCA4/2* perturbations and cisplatin

550   treatments (H, I, 72 hours).

551

552 (E- J) Ctrl: Control; $A4^{KO}$: *SMARCA4* knockout; sh*A2*: shRNA targeting *SMARCA2*; cl. PARP:

553 cleaved PARP; cl. caspase 3: cleaved caspase 3; *A4*: *SMARCA4*; *A2*: *SMARCA2*. Scale bar, 150

554 µm. Mean ± SD, n = 3 independent experiments, one-way ANOVA followed by Dunnett's test for

555 multiple comparisons, *p< 0.05, **p < 0.01, ****p < 0.0001.

556

557 **Figure 2 SMARCA4 modulates Ca^{2+} flux from the ER to mitochondria**

558 (A, B) Gene Set Enrichment Analysis (GSEA) plots of indicated gene ontology terms in SCCOHT-

559 1 (A) and BIN-67 (B) cells ± SMARCA4 restoration [49]. FDR: False Discovery Rate.

560

561 (C) Immunoblots of indicated proteins in SCCOHT-1 cells ± SMARCA4 restoration.

562

563 (D, E) Changes of cytosolic (D) and mitochondrial (E) Ca^{2+} contents in SCCOHT-1 cells ±

564 SMARCA4 restoration upon histamine stimulation. For cytosolic Ca^{2+}, 44 control (Ctrl) and 21

565 ectopic *SMARCA4* (*A4*) expressing cells from four independent experiments were analyzed. For

566 mitochondrial Ca^{2+}, 44 Ctrl cells and 20 *A4* cells from four independent experiments were analyzed.

567

568 (F) Immunoblots of indicated proteins in H1703 cells ± SMARCA4 restoration.

569

570 (G, H) Changes of cytosolic (G) and mitochondrial (H) Ca^{2+} contents in H1703 cells ± SMARCA4

571 restoration upon histamine stimulation. For cytosolic Ca^{2+}, 41 Ctrl cells and 74 *A4* cells from three

572 independent experiments were analyzed. For mitochondrial Ca^{2+}, 45 Ctrl cells and 63 *A4* cells

573 from three independent experiments were analyzed.

574

(I) Immunoblots of indicated proteins in OVCAR4 cells ± SMARCA4 knockout.

(J, K) Changes of cytosolic (J) and mitochondrial (K) Ca^{2+} contents in OVCAR4 cells with *SMARCA4* knockout ($A4^{KO}$) upon histamine stimulation. For cytosolic Ca^{2+}, 60 Ctrl cells and 53 $A4^{KO}$ cells from three independent experiments were analyzed, and for mitochondrial Ca^{2+}, 41 Ctrl cells and 40 $A4^{KO}$ cells from three independent experiments were analyzed.

(L) Immunoblots of indicated proteins in H1437 cells ± SMARCA4 knockout.

(M, N) Changes of cytosolic (M) and mitochondrial (N) Ca^{2+} contents in H1437 cells with *SMARCA4* knockout ($A4^{KO}$) upon histamine stimulation. For cytosolic Ca^{2+}, 39 Ctrl cells and 37 $A4^{KO}$ cells from three independent experiments were analyzed, and for mitochondrial Ca^{2+}, 38 Ctrl cells and 42 $A4^{KO}$ cells from three independent experiments were analyzed.

(D, E, G, H, J, K, M, N) Left: traces of cytosolic and mitochondrial Ca^{2+} contents in indicated cell lines upon 100 μM histamine stimulation (mean ± SEM). Right: Quantification of the maximal Ca^{2+} signal peaks induced by histamine stimulation (mean ± SD). The Ca^{2+} probes R-GECO (R-GECO F/F0, A.U) and CEPIA-2mt (CEPIA-2mt F/F0, A.U) were used to monitor cytosolic and mitochondrial Ca^{2+}, respectively. Two-tailed *t*-test, *p < 0.05, **p < 0.01, ***p < 0.001.

Figure 3 SMARCA4/2 regulates *ITPR3* transcription through remodeling chromatin accessibility at its gene locus

597 (A) Venn diagram of Ca^{2+} related genes from Figures 2A-B that are enriched in SCCOHT-1 and

598 BIN-67 cells with SMARCA4 restoration.

599

600 (B) Heatmap of Ca^{2+} related genes bound by SMARCA4 (n = 69) in indicated SCCOHT

601 (SCCOHT-1 and BIN-67) and NSCLC (H1299) cell lines with SMARCA4/2 restoration. Left:

602 normalized reads from RNA-seq data of BIN-67 and SCCOHT-1 cells with SMARCA4 restoration

603 [49]. Middle: normalized reads from RNA-seq data of BIN-67 cells with SMARCA4/2 restoration

604 [60]. Right: normalized signal from microarray data of H1299 cells with SMARCA4 restoration [61].

605 Row scaling was used to generate the heatmap. The last column represents changes of genes in

606 H1299 cells ± SMARCA4 restoration: ns: not significant; Up: up-regulated; Down: down-

607 regulated.

608

609 (C) RT-qPCR measurements of *ITPR3* mRNA expression in indicated SCCOHT and NSCLC cell

610 lines with SMARCA4/2 restoration. GAPDH was used for normalization. Mean ± SD, n = 3 or 4

611 independent experiments, one-way ANOVA followed by Dunnett's test for multiple comparisons

612 to the control group (BIN-67, SCCOHT-1, H1703) or two-tailed *t*-test (H1299), *p < 0.05, **p <

613 0.01, ***p < 0.001, **** p < 0.0001.

614

615 (D) Immunoblots of indicated proteins in indicated SCCOHT and NSCLC cell lines ±

616 SMARCA4/2 restoration.

617

618 (E) SMARCA4 occupancy in vicinity of the *ITPR3* locus assessed by chromatin

619 immunoprecipitation sequencing (ChIP-seq) in indicated SCCOHT and lung cancer cell lines ±

620   SMARCA4 restoration. SMARCA4 in H1299 cells was induced by doxycycline (Dox) [61]. Track

621   height is normalized to relative number of mapped reads.

622

623   (F) Chromatin structure changes in vicinity of the ITPR3 locus assessed by H3K27Ac ChIP-seq

624   and assay for transposase-accessible chromatin sequencing (ATAC-seq) in indicated SCCOHT

625   and lung cancer cell lines ± SMARCA4/2 restoration. Track height is normalized to relative

626   number of mapped reads.

627

628   (A-F) Ctrl: Control; *A4*: *SMARCA4*; *A2*: *SMARCA2*.

629

630   **Figure 4 SMARCA4/2 loss inhibits apoptosis by constricting IP3R3-mediated Ca^{2+} flux**

631   (A) Immunoblots of indicated proteins in SCCOHT-1 cells with indicated *SMARCA4* and *ITPR3*

632   perturbations. *A4*: *SMARCA4*; sh*R3*: shRNA targeting *ITPR3*.

633

634   (B, C) Changes of cytosolic (B) and mitochondrial (C) Ca^{2+} contents in SCCOHT-1 cells with

635   indicated *SMARCA4* and *ITPR3* perturbations upon histamine stimulation. For cytosolic Ca^{2+}, 43

636   control (Ctrl) cells, 30 *A4* cells, 51 *A4* Sh*R3*#1 cells and 50 *A4* Sh*R3*#2 cells from four independent

637   experiments were analyzed. For mitochondrial Ca^{2+}, 31 Ctrl cells, 30 *A4* cells, 50 *A4* Sh*R3*#1 cells

638   and 50 *A4* Sh*R3*#2 cells from four independent experiments were analyzed.

639

640   (D) Immunoblots of the indicated proteins in H1703 cells with indicated *SMARCA4* and *ITPR3*

641   perturbations. si*R3*: siRNA targeting *ITPR3*.

642

643 (E, F) Changes of cytosolic (E) and mitochondrial (F) Ca^{2+} contents in H1703 cells with indicated

644 *SMARCA4* and *ITPR3* perturbations upon histamine stimulation. For cytosolic Ca^{2+}, 64 Ctrl cells,

645 70 *A4* cells and 64 *A4* si*R3*cells from three independent experiments were analyzed. For

646 mitochondrial Ca^{2+}, 53 Ctrl cells, 53 *A4* cells and 50 *A4* si*R3* cells from three independent

647 experiments were analyzed.

648

649 (G) Immunoblots of the indicated proteins in OVCAR4 cells with *ITPR3* knockdown. Cells were

650 collected 48 hours after the cisplatin treatment. cl. PARP: cleaved PARP; cl. caspase 3: cleaved

651 caspase 3.

652

653 (H) Annexin V^{+}/PI^{-} apoptotic cell population determined by flow cytometry in OVCAR4 cells

654 described in (G).

655

656 (I) Immunoblots of the indicated proteins in H1703 cells with *ITPR3* overexpression. Cells were

657 collected 72 hours after the treatment.

658

659 (J) Immunoblots of the indicated proteins in H1703 cells indicated *SMARCA4* and *ITPR3*

660 perturbations. Cells were collected 72 hours after the treatment.

661

662 (K) Annexin V^{+}/PI^{-} apoptotic cell population determined by flow cytometer in H1703 cells

663 described in (J).

664

665 (B, C, E, F) Left: traces of cytosolic and mitochondrial Ca^{2+} contents in the indicated cell lines

666 upon 100 μM histamine stimulation (mean ± SEM). Right: Quantification of the maximal Ca^{2+}

667 signal peaks induced by histamine stimulation (mean ± SD). The Ca^{2+} probes R-GECO (R-GECO

668 F/F0, A.U) and CEPIA-2mt (CEPIA-2mt F/F0, A.U) were used to monitor cytosolic and

669 mitochondrial Ca^{2+}, respectively. One-way ANOVA followed by Dunnett's test for multiple

670 comparisons to the control group, **p < 0.01, ***p < 0.001; ns, not significant.

671

672 (H, K) Mean ± SD, n = 3 independent experiments, one-way ANOVA followed by Dunnett's test

673 for multiple comparisons, ****p < 0.0001.

674

675

676 **Figure 5 IP3R3 expression is reduced in SMARCA4/2-deficient cancers**

677 (A) Correlation of *ITPR3* and *SMARCA2* mRNA in ovarian (left, n=11) and lung (right, n=48)

678 cancer cell lines with low expression of *SMARCA4*. Gene expression data were obtained from

679 Cancer Cell Line Encyclopedia (CCLE) and in Reads Per Kilobase Million (RPKM) [42]. *A4*^{Low}:

680 *SMARCA4*^{Low}, cell lines with the bottom quartile of *SMARCA4* expression. Number of cell lines

681 is indicated in grey. r, Pearson correlation.

682

683 (B) Immunoblots of indicated proteins in a panel of lung cancer cell lines with indicated

684 SMARCA4/2 status. A4: SMARCA4; A4/2: SMARCA4/2,; Pro: proficient; Def: deficient; *

685 *KRAS* mutant.

686

687   (C) Correlation of *ITPR3* and *SMARCA2* mRNA in ovarian cancer (left, n=89) and lung

688   adenocarcinoma (LUAD, right, n=128) patient tumors with low expression of *SMARCA4*. Gene

689   expression data were obtained from UCSC Xena and in Fragments Per Kilobase Million (FPKM).

690    $A4^{Low}$: $SMARCA4^{Low}$, patient tumors with the bottom quartile of SMARCA4 expression. Number

691   of tumor samples is indicated in grey. r, Pearson correlation.

692

693   (D) *ITPR3* mRNA expression in SCCOHT and ovarian cancer patient tumors with different

694   expression of *SMARCA4/2*. TCGA ovarian cancer tumors (n=379) were stratified based on the

695   expression of *SMARCA4/2* as indicated in Figure S2B. Number of patient tumors in each category

696   is indicated in grey. Expression of *ITPR3* mRNA was measured by FPKM and then normalized

697   by housekeeping gene *ACTB*. $A4^{H}$: $SMARCA4^{High}$; $A4^{L}$: $SMARCA4^{Low}$; $A2^{H}$: $SMARCA2^{High}$; $A2^{L}$:

698    $SMARCA2^{Low}$. Number of tumor samples is indicated in grey. Brown-Forsythe and Welch

699   ANOVA followed by Dunnett's test for multiple comparisons to $A4^{H}A2^{H}$ group or *t*-test between

700    $A4^{L}A2^{L}$ group and SCCOHT group, $*p < 0.05$, $***p < 0.001$, $****p < 0.0001$; ns, not significant.

701

702   (E) Representative images of immunohistochemistry (IHC) analysis for IP3R3 in SCCOHT and

703   HGSC patient tumors. H-score of each image is indicated in grey. Scale bar, 100 μm.

704

705   (F) H-score of IHC analysis for IP3R3 in SCCOHT and HGSC patient tumors. Number of tumor

706   samples is indicated in grey. Mann-Whitney test, $**p < 0.01$.

707

708   (G) Representative images of IHC analysis for IP3R3 and SMARCA4 in NSCLC patient tumors.

709   H-score of each image is indicated in grey. Scale bar, 100 μm.

710

(H) H-score of IHC analysis for IP3R3 and SMARCA4 in NSCLC patient tumors. Number of

tumor samples is indicated in grey. Mann-Whitney test, **p < 0.01.

713

(I) Tumor growth in xenograft models of H1703 cells with exogenous SMARCA4 expression

under Tet-on inducible system. Doxycycline (Dox) was given daily starting on day 21 (denoted by

arrow). Upper, tumor size; lower, endpoint tumor weight measured after surgery. Mean ± SEM,

two-way ANOVA (upper), Two-tailed *t*-test (lower), **p < 0.01, ***p < 0.001, ****p < 0.0001.

718

(J, K) Representative images (J) and digital quantification (K) of IHC analysis for SMARCA4,

IP3R3 and cleaved caspase 3 in endpoint tumors described in (I). Scale bar, 100 μm. Mean ± SD,

two-tailed *t*-test (lower), **p < 0.01, ****p < 0.0001.

722

723

Figure 6 The histone deacetylase inhibitor quisinostat rescues IP3R3 expression and
enhances cisplatin response in SMARCA4/2-deficient cancer cells

726

(A) RT-qPCR measurements of *SMARCA2* (left) and *ITPR3* (right) mRNA expression in H1703

cells treated with quisinostat. Cells were collected 48 hours after the treatment. n = 3 independent

experiments. Mean ± SD, one-way ANOVA followed by Dunnett's tests for multiple comparisons

to the control group, **p<0.01, ***p < 0.001, ****p < 0.0001.

731

732   (B, C) Immunoblots of indicated proteins (B) and annexin V^+/PI^- apoptotic cell population (C) in

733   H1703 cells treated with cisplatin and quisinostat. Cells were collected 72 hours after the treatment.

734   Cisplatin: 3 µM; quisinostat: 10 nM; cl. PARP: cleaved PARP; cl. caspase 3: cleaved caspase 3.

735   Mean ± SD, n = 3 independent experiments, one-way ANOVA followed by Dunnett's test for

736   multiple comparisons, $**p<0.01$, $***p < 0.001$, $****p < 0.0001$.

737

738   (D) Colony formation assay for H1703 cells treated with cisplatin and quisinostat. Cells were fixed

739   and stained 12 days after plating. Drugs were refreshed every 3 days.

740

741   (E, F) Immunoblots of indicated proteins in H1703 cells ± SMARCA2 knockout (E) or ± IP3R3

742   knockdown (F) treated with cisplatin and quisinostat for 72 hours. Cisplatin: 3 µM; quisinostat: 10

743   nM.

744

745   (G) Representative images from confocal live cell imaging of the mitochondrial Ca^{2+} probe

746   CEPIA-2mt overexpressing H1703 cells treated with quisinostat or/and cisplatin and stained with

747   Mitotracker deep red. Cisplatin: 2 µM for 24 hours; quisinostat: 40 nM for 72 hours. Scale bar, 25

748   µm.

749

750   (H) Quantification of basal mitochondrial Ca^{2+} levels from (G), showing the ratio of the

751   mitochondrial Ca^{2+} probe CEPIA 2mt / the mitochondrial marker Mitotracker deep red

752   fluorescence intensities compared to control (U.A.). Control (Ctrl) 43 cells, cisplatin 42 cells,

753   quisinostat 38 cells and quisinostat/cisplatin 46 cells from three independent experiments were

754 analyzed. One-way ANOVA followed by Dunnett's test for multiple comparisons to the control

755 group, **$p < 0.01$; ns, not significant.

756

757 (I) Tumor growth in xenograft models of H1703 cells treated with cisplatin and quisinostat.

758 Cisplatin, once per week at $4\,\text{mg}\,\text{kg}^{-1}$; quisinostat, three times per week at $10\,\text{mg}\,\text{kg}^{-1}$. Upper,

759 tumor size, mean $\pm$ SEM, two-way ANOVA; lower, final tumor weight measured after surgery,

760 one-way ANOVA followed by Dunnett's tests for multiple comparisons to the combination group.

761 *$p<0.05$, ***$p < 0.001$, ****$p < 0.0001$.

762

763 (J, K) Representative images (J) and digital quantification (K) of IHC analysis for SMARCA2,

764 IP3R3 and cleaved caspase 3 in endpoint tumors described in (I). Scale bar, 100 μm. Mean $\pm$ SD,

765 one-way ANOVA followed by Dunnett's test for multiple comparisons, *$p < 0.05$, **$p < 0.01$,

766 ***$p < 0.001$, ****$p < 0.0001$.

767

768

769

Supplemental figure legends

Figure S1 Reduced *SMARCA4* expression is associated with chemoresistance in NSCLC, related to Figure 1

(A) Kaplan–Meier (KM) curves of overall survival in lung adenocarcinoma patients ± adjuvant chemotherapy (ACT). Director's Challenge Consortium for the Molecular Classification of Lung Adenocarcinoma [36] was analyzed and patients were stratified based on median of *SMARCA4* mRNA expression (jetset probe, Affy ID 213720_s_at). One-tailed Mantel-Cox test.

(B) KM curves of overall survival in lung adenocarcinoma patients ± chemotherapy. Kaplan–Meier Plotter [37] was used and patients were stratified based on auto select best cut-off of *SMARCA4* mRNA expression (jetset probe, Affy ID 213720_s_at). One-tailed Mantel-Cox test.

(C) KM curves of overall survival in lung cancer patients ± adjuvant chemotherapy. The UT Lung SPORE dataset [39] was analyzed and patients were stratified based on median of *SMARCA4* mRNA expression (jetset probe, Affy ID 213720_s_at). One-tailed Mantel-Cox test.

Figure S2 Reduced SMARCA4/2 expression is associated with resistance to chemotherapeutics in cancer cell lines, related to Figure 1

(A) Pie chart depicting the tissue of origins of cell lines with both mRNA expression and IC_{50} data available. Numbers of cell lines are indicated in parentheses for each tissue type.

792   (B) Stratification of cell lines according to the mRNA expression of *SMARCA4/2*. *A4*[H]:

793   *SMARCA4*[High]; *A4*[L]: *SMARCA4*[Low]; *A2*[H]: *SMARCA2*[High]; *A2*[L]: *SMARCA2*[Low].

794

795   (C, D) IC_{50} of indicated chemotherapy drugs in pan cancer cell lines (C) and lung cancer cell lines

796   (D) with different mRNA expression levels for *SMARCA4* and *SMARCA2*. Cell line numbers are

797   indicated in grey below each group. Kruskal–Wallis test followed by Dunn's tests for multiple

798   comparisons to *A4*[H]*A2*[H] group, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

799

800   **Figure S3 SMARCA4/2 loss causes resistance to chemotherapy drug in ovary and lung**

801   **cancers, related to Figure 1**

802

803   (A) Annexin V staining of OVCAR4 cells with the indicated *SMARCA4* perturbation and cisplatin

804   treatment.

805

806   (B) Immunoblot analysis of OVCAR4 cells with the indicated *SMARCA4* perturbation and

807   paclitaxel treatments. Cells were collected 48 hours after the treatment.

808

809   (C, D) Immunoblot analysis (C) and colony formation (D) of HEC116 cells with indicated

810   *SMARCA4/2* perturbations and cisplatin treatments. Cells were fixed and stained 12 days after

811   plating. Drugs were refreshed every 3 days (C). Cells were collected 48 hours after the treatment.

812

813   (E) Annexin V staining of H1703 cells with the indicated *SMARCA4* perturbation and cisplatin

814   treatments.

815

(F) Colony formation of H1703 cells with the indicated *SMARCA4/2* perturbations and cisplatin

treatments. Cells were fixed and stained 12 days after plating. Drugs were refreshed every 3 days

(B).

(G, H) Immunoblot analysis (G) and cell viability assay (H) of H1437 cells with indicated

SMARCA4/2 perturbations and cisplatin treatments. Cells were collected 48 hours after the

treatment (G).

Ctrl: Control; $A4^{KO}$: *SMARCA4* knockout; sh*A2*: shRNA targeting *SMARCA2*; cl. PARP: cleaved

PARP; cl. caspase 3: cleaved caspase 3; *A4*: *SMARCA4*; *A2*: *SMARCA2*. A, E, mean $\pm$ SD, n = 3

independent experiments, two-way ANOVA, **p < 0.01, ****p < 0.0001.

Figure S4: SMARCA4/2 loss causes resistance to cyclophosphamide, topotecan and

paclitaxel in ovary and lung cancer cells, related to Figure 1

(A-D) Immunoblot analysis (A, C) and cell viability assay (B, D) of HEC116 (A, B) and H1703

(C, D) cells with indicated *SMARCA4/2* perturbations and treatments of cyclophosphamide,

topotecan and paclitaxel. For A, C, cells were collected 48 hours after the treatment of 2mM

cyclophosphamide, 4nM topotecan and 2nM paclitaxel. $A4^{KO}$: *SMARCA4* knockout; *A4*:

SMARCA4.

Figure S5 GSEA of published gene expression datasets in SCCOHT with SMARCA4 restoration, related to Figure 2

(A, B) Top 10 enriched gene ontology terms in SCCOHT-1(A) and BIN-67 (B) cells with SMARCA4 restoration [49]. MF: gene sets derived from the GO Molecular Function Ontology. FDR: False Discovery Rate. Calcium/ion transportation terms are highlighted with red.

Figure S6 SMARCA4 modulates Ca^{2+} flux from ER to mitochondria, related to Figure 2

(A) Representative confocal time-lapse images of SMARCA4 expressing H1703 cells transfected with the cytosolic Ca^{2+} probe R-GECO. 100 μM histamine final was added at t = 10s. Scale bar, 25 μm. Corresponding to Figure 2H.

(B) Representative confocal time-lapse images of SMARCA4 expressing H1703 cells transfected with the mitochondrial Ca^{2+} probe CEPIA-2mt. 100 μM histamine final was added at t = 10s. Scale bar, 25 μm. Corresponding to Figure 2I.

(C) Representative confocal time-lapse images of SMARCA4 expressing H1703 cells transfected with the cytosolic Ca^{2+} probe R-GECO. 10 μM thapsigargin final was added at t = 10s. Scale bar, 25 μm. Corresponding to Figure S6B.

Figure S7 SMARCA4 loss does not reduce Ca^{2+} storage in the ER, related to Figure 2

(A) Changes of cytosolic Ca^{2+} content in SCCOHT-1 cells ± SMARCA4 restoration upon thapsigargin stimulation. 21 control (Ctrl) cells and 29 *SMARCA4* (*A4*)- expressing cells from four independent experiments were analyzed.

860

(B) Changes of cytosolic Ca^{2+} content in H1703 cells ± SMARCA4 restoration upon thapsigargin stimulation. 31 Ctrl cells and 39 *A4* restored cells from three independent experiments were analyzed.

(C) Changes of cytosolic Ca^{2+} content in OVCAR4 cells ± *SMARCA4* knockout ($A4^{KO}$) upon thapsigargin stimulation. 57 Ctrl cells and 59 $A4^{KO}$ cells from three independent experiments were analyzed.

(D) Changes of cytosolic Ca^{2+} content in H1437 cells ± *SMARCA4* knockout ($A4^{KO}$) upon thapsigargin stimulation. 46 Ctrl cells and 48 $A4^{KO}$ cells from three independent experiments were analyzed.

(E) Immunoblots of the indicated proteins in OVCAR4, H1703 and SCCOHT-1 cells with the indicated *SMARCA4* perturbations.

(A-D) Left: traces of cytosolic Ca^{2+} content in indicated cell lines upon 10 μM thapsigargin stimulation (mean ± SEM). Middle: Quantification of the maximal Ca^{2+} signal peaks induced by thapsigargin stimulation (mean ± SD). Right: quantification of the area under the curve (AUC) from (A). The Ca^{2+} probe R-GECO (R-GECO F/F0, A.U) was used to monitor cytosolic Ca^{2+}. Two-tailed unpaired t-test, $**p < 0.01$; ns, not significant.

(A-E) Ctrl: Control; *A4*: *SMARCA4*; $A4^{KO}$: *SMARCA4* knockout.

883

Figure S8 SMARCA4/2 regulates IP3R3 expression in ovarian and lung cancer cells, related to Figure 3

(A, B, C) Immunoblots of indicated proteins in OVCAR4 (A), HEC116 (B) and H1437 (C) cancer cell lines with indicated *SMARCA4/2* perturbations. *A4*[KO]: *SMARCA4* knockout; sh*A2*: shRNA targeting *SMARCA2*.

Figure S9 Perturbations of *ITPR3* do not affect Ca^{2+} storage in the ER, related to Figure 4

(A) Changes of cytosolic Ca^{2+} content in SCCOHT-1 cells with indicated *SMARCA4* and *ITPR3* perturbations upon thapsigargin stimulation. 25 Ctrl cells, 29 *A4* cells, 52 *A4* Sh*R3*#1 cells and 58 *A4* Sh*R3*#2 cells from four independent experiments were analyzed. Ctrl: control; *A4*: *SMARCA4*; sh*R3*: shRNA targeting *ITPR3*. Corresponding to Figures 4A-C.

(B) Changes of cytosolic Ca^{2+} content in H1703 cells with indicated *SMARCA4* and *ITPR3* perturbations upon thapsigargin stimulation. 50 Ctrl cells, 50 *A4* cells and 50 *A4* si*R3*#1 cells from three independent experiments were analyzed. Ctrl: control; *A4*: *SMARCA4*; si*R3*: siRNA targeting *ITPR3*. Corresponding to Figures 4D-F.

(A, B) Left: traces of cytosolic Ca^{2+} content in the indicated cell lines upon 10 μM thapsigargin stimulation (mean ± SEM). Middle: Quantification of the maximal Ca^{2+} signal peaks induced by thapsigargin stimulation (mean ± SD). Right: quantification of the area under the curve (AUC) from (A). The Ca^{2+} probe R-GECO (R-GECO F/F0, A.U) was used to monitor cytosolic Ca^{2+}.

906

907

908

909

910 **Figure S10 Ectopic expression of *ITPR3* sensitizes SMARCA4/2 deficient cancer cells to**

911 **cisplatin, related to Figure 4**

912 (A) Immunoblots of BIN-67 cells with ectopic *ITPR3* expression.

913

914 (B) Colony formation of BIN-67 cells with ectopic *ITPR3* expression cultured with indicated

915 cisplatin treatments. Cells were fixed and stained 18 days after plating. Drugs were refreshed every

916 3 days.

917

918 (C) Immunoblots of H1703 cells with ectopic *ITPR3* restoration.

919

920 (D) Colony formation of H1703 cells with ectopic *ITPR3* expression cultured with indicated

921 cisplatin treatments. Cells were fixed and stained 12 days after plating. Drugs were refreshed every

922 3 days.

923

924 **Figure S11 *ITPR3* mRNA expression is reduced in ovarian and lung cancers expressing lower**

925 **levels of *SMARCA4/2*, related to Figure 5**

926 (A) *ITPR3* mRNA expression in ovarian (left, n=47) and lung (right, n=193) cancer cell lines with

927 differential *SMARCA4* expression. Gene expression data were obtained from Cancer Cell Line

928 Encyclopedia (CCLE) and in Reads Per Kilobase Million (RPKM) [42]. $A4^L$: $SMARCA4^{Low}$, cell

929 lines with the bottom quartile of *SMARCA4* expression; $A4^H$: $SMARCA4^{High}$, the other cell lines.

930 Number of cell lines is indicated in grey. Two-tailed *t*-test, *p < 0.05. Corresponding to Figure 5A.

931

932 (B) *ITPR3* mRNA expression in ovarian cancer (Left, n=454), lung adenocarcinoma (LUAD,

933 middle, n=510) and Lung Squamous Cell Carcinoma (LUSC, right, n =496) patient tumors with

934 different expression of *SMARCA4*. Gene expression data were obtained from UCSC Xena and in

935 Fragments Per Kilobase Million (FPKM). $A4^L$: $SMARCA4^{Low}$, tumors with the bottom quartile of

936 *SMARCA4* expression; $A4^H$: $SMARCA4^{High}$, the other cell lines. Number of tumor samples is

937 indicated in grey. Two-tailed *t*-test, ****p < 0.0001. Corresponding to Figure 5C.

938

939 (C) Correlation of *ITPR3* and *SMARCA2* mRNA in LUSC (n=124) patient tumors with low

940 expression of *SMARCA4*. Gene expression data were obtained from UCSC Xena and in FPKM.

941 $A4^{Low}$: $SMARCA4^{Low}$, patient tumors with the bottom quartile of SMARCA4 expression. Number

942 of tumor samples is indicated in grey. r, Pearson correlation.

943

944 **Figure S12 Specificity validation of IP3R3 antibody by shRNA knockdown, related to Figure**

945 **5**

946 (A) Immunoblot analysis of IP3R3 protein expression in OVCAR4 cells expressing control vector

947 or shRNAs targeting *ITPR3* (*R3*).

948

949 (B) Representative images of immunohistochemistry analysis for IP3R3 in OVCAR4 cells

950 described in (A). Scale bar, 100 μm.

951

952 **Figure S13 HDAC inhibitor activates *SMARCA2* and *ITPR3* expression in SCCOHT cells,**

953 **related to Figure 6**

954 (A, B) RT-qPCR measurements of *SMARCA2* (upper) and *ITPR3* (lower) mRNA expression in

955 BIN-67 (A) and SCCOHT-1 (B) cells treated with quisinostat. Cells were collected 48 hours after

956 the treatment. n = 2 or 3 independent experiments. Mean ± SD, two-tailed *t*-test (H1299), *p <

957 0.05, ***p < 0.001, **** p < 0.0001.

958

959 (C, D) Immunoblot analysis of SMARCA2 and IP3R3 protein expression in BIN-67 (C) and

960 SCCOHT-1 (D) cells treated with quisinostat. Cells were collected 48 hours after the treatment.

961

962 **Figure S14 HDAC inhibitor restores Ca^{2+} flux in H1703 cells, related to Figure 6**

963 (A, B) Changes of cytosolic (A) and mitochondrial (B) Ca^{2+} contents in H1703 cells with indicated

964 *SMARCA4*, *ITPR3* perturbations and quisinostat treatment, upon histamine stimulation. For

965 cytosolic Ca^{2+}, 63 control (Ctrl) cells, 61 Ctrl, quisinostat cells, 51 Ctrl, quisinostat, si*ITPR3* cells,

966 50 *A4* restored cells and 53 *A4* restored, si*ITP3R3* cells, from three independent experiments were

967 analyzed. For mitochondrial Ca^{2+}, 50 control (Ctrl) cells, 51 Ctrl, 58 Ctrl, quisinostat, si*ITPR3*

968 cells, 52 *A4* restored cells and 50 *A4* restored, si*ITPR3* cells from three independent experiments

969 were analyzed. Quisinostat: 40 nM for 72 hours.

970 (C) Immunoblot analysis of the indicated proteins in H1703 cells with indicated *SMARCA4* and

971 *ITP3R3* perturbations and quisinostat treatment. Quisinostat: 40 nM for 72 hours.

972

973 Left: traces of cytosolic and mitochondrial Ca^{2+} contents in indicated cell lines upon 100 μM

974 histamine stimulation (mean ± SEM). Right: Quantification of the maximal Ca^{2+} signal peaks

975 induced by histamine stimulation (mean ± SD). The Ca^{2+} probes R-GECO (R-GECO F/F0, A.U)

976 and CEPIA-2mt (CEPIA-2mt F/F0, A.U) were used to monitor cytosolic and mitochondrial Ca^{2+},

977 respectively. Two-tailed *t*-test, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

978

979 **Figure S15 Effects of cisplatin, quisinostat or their combination on tumor growth normalized**

980 **to body weight of mice, related to Figure 6**

981 (A) Body weight of mice in xenograft models of H1703 cells treated with cisplatin, quisinostat or

982 their combination. Corresponding to Figures 5J, K.

983

984 (B) Tumor growth normalized to body weight in xenograft models of H1703 cells treated with

985 cisplatin, quisinostat or their combination. Mean ± SEM, two-way ANOVA, **$p < 0.01$, **** $p <$

986 0.0001. Corresponding to Figures 5J, K.

987

988 (C) Final tumor weight normalized to body weight at the end point of the experiment. One-way

989 ANOVA followed by Dunnett's tests for multiple comparisons to the combination group, *$p <$

990 0.05, ***$p < 0.001$. Corresponding to Figures 5J, K.

991

992 **Figure S16 Quantification of key immunoblots**

993 The histograms show the quantification of cleaved PARP, cleaved caspase 3 or IP3R3

994 corresponding to indicated figures. The quantification was performed by ImageJ from two

995 independent experiments and normalized to the loading control Actin.

996

997 **Figure S17 Uncropped scans for the immunoblots presented in main figures**

998

Figure S18 Gating strategy using Guava flow cytometer

(A-D) The forward and side scatter gating (left column with the circle) and the fluorescence gating (right column with the 4 quadrants) of H1703 cells from the following conditions: without the addition of annexin V and propidium iodide (PI) fluorescent probes (A); with the addition of PI fluorescent probe only (B); with the addition of annexin V fluorescent probe only (C); with the addition of both annexin V and PI fluorescent probes (D).

1005

1006

Table S1

Ranking of genes by RRA (robust rank aggregation) scores in a CRISPR screen with OVCAR4 cells treated $\pm$ cisplatin (100nM). Cisplatin was refreshed every 3 days for 11 days before harvesting. Data was analysed by the MAGeCK statistical software package.

1011

Table S2

Lists of SMARCA4 regulated genes related to Ca^{2+} and SMARCA4 bound genes in SCCOHT cells. SMARCA4 regulated genes were from ontology terms of ion transmembrane transporter and calcium ion binding in Figures 2A, B. SMARCA4 bound genes whose loci showed SMARCA4 occupancy within 3 kbp from their transcription start sites were identified from ChIP-seq data in BIN-67 (GSE117734).

1018

Methods

Cell culture

All cell lines were cultured in Roswell Park Memorial Institute 1640 Medium (Thermo Fisher Scientific, Cat# 11875-093) with 7% fetal bovine serum (Sigma, Cat# F1051), 1% penicillin/ streptomycin (Thermo Fisher Scientific, Cat# 15140-122) and 2mM L-glutamine (Thermo Fisher Scientific, Cat# 25030-081), except for 293T with Dulbecco's Modified Eagle Medium (Thermo Fisher Scientific, Cat# 11995-065). Cells were maintained at 37°C and 5% CO_2 and regularly tested for Mycoplasma using Mycoalert Detection Kit (Lonza, Cat # LT07-318). All cell lines came directly from ATCC or have been validated by Short Tandem Repeat analysis.

Lentivirus Production and Infection

All experiments with ectopic expression, short hairpin RNA (shRNA) knockdown and CRISPR single guide RNA (sgRNA) knockout were performed using lentiviral constructs. For lentivirus production, 2.5×10^6 293T cells were plated in 2 ml of DMEM medium per well in a 6-well plate and transfected after ~8 hours with lentiviral constructs, the packaging (psPAX2) and envelope plasmid (pMD2.G) by $CaCl_2$. Virus containing medium were harvested at 24 and 36 hours after transfection before use or stored at −80 °C. For infection, ~ 5×10^5 target cells were plated the day before and infected with virus for ~ 8 hours. After ~20 hours recovery, cells were selected in medium containing 2 μg/ml puromycin or 5 μg/ml blasticidin for 2–3 days and harvested for the experiments.

Compounds and antibodies

1041 Cisplatin (S1166), Quisinostat (S1096), Paclitaxel (S1150), and Topotecan (S9321) were

1042 purchased from Selleck Chemicals. Cyclophosphamide (CA80500-080), Histamine (H7125), and

1043 thapsigargin (T9033) were from Sigma-Aldrich. Mitotracker deep red FM was from Themo Fisher

1044 Scientific (M22426). Antibodies against calregulin (Cat# sc-166837), HSP90 (Cat# sc-13119) and

1045 β-Actin (Cat# sc-47778) were from Santa Cruz Biotechnology; antibodies against SMARCA2

1046 (Cat# 11996), cleaved PARP(Cat# 5625) and cleaved caspase-3 (Cat# 9664) were from Cell

1047 Signaling; Antibody against MICU2 (Cat# ab-101465), VDAC1 (Cat# ab-14734) and GRP75

1048 (Cat# ab-2799) were from Abcam; Antibody against SMARCA4 (Cat# A300-813A) were from

1049 Bethyl Laboratories (A300-813A); Antibody against IP3R3 (Cat# 610312) was from BD

1050 Pharmingen); Antibody against vinculin (Cat# V4505) was from Sigma-Aldrich; Antibody against

1051 MCU (Cat# HPA0168480) was from Atlas; Antibody against MICU1 (Cat# orb-323178) was from

1052 Biorbyt. Antibody against SMARCA4 was used with 1:5000 dilution and all others with 1:1000

1053 dilution. Antibodies for IHC are listed in the corresponding method section below.

1054

1055 *Plasmids*

1056 Individual shRNA vectors used were from the Mission TRC library (Sigma) provided by McGill

1057 Platform for Cellular Perturbation (MPCP) of Rosalind and Morris Goodman Cancer Research

1058 Centre and Biochemistry at McGill University: sh*SMARCA2*#1 (TRCN0000358828);

1059 sh*SMARCA2*#2 (TRCN0000020333); sh*ITPR3*#1 (TRCN0000061324), sh*ITPR3*#2

1060 (TRCN0000061326). For shRNA experiments, pLKO vector control was used. Scramble control

1061 sgRNA (SCR_6); dual-sgRNAs targeting *SMARCA4* (TEDH-1074701) or *SMARCA2* (TEDH-

1062 1074696) were from the transEDIT-dual CRISPR Library (Transomic) provided by MPCP.

1063 Additional sgRNA (GCTGGCCGAGGAGTTCCGCCC) targeting *SMARCA4* was cloned into

1064 pLentiCRISPRv2. pReceiver control vector, pReceiver-*SMARCA4* and pReceiver-*SMARCA2*

1065 were purchased from GeneCopoeia. pLX304-*ITPR3* were generated by gateway cloning with

1066 pENTR223.1-*ITPR3* (BC172406) and pLX304-*GFP* control (ccsbBroad304_07515) were from

1067 Transomic provided by MPCP. transEDIT[TM] pCLIP-All-EFS-Puro Epigenetics CRISPR

1068 Screening library was from Transomic (Cat# CAHD9001). pLentiCas9-Blast (Cat# 52962),

1069 pLentiCRISPRv2 (Cat# 52961), pCMV-R-GECO1 (Cat# 32444) and pCMV-CEPIA2mt (Cat#

1070 58218) were from Addgene. pIN20 and pIN20-SMARCA4[82] were provided by Dr. Jannik N.

1071 Andersen (The University of Texas, MD Anderson Cancer Center).

1072

1073 ***CRISPR/Cas9 editing***

1074 Plasmid-based CRISPR/Cas9 editing was used to generate SMARCA4 knockout in OVCAR4 and

1075 H1437 cells using standard lentiviral delivery followed by single cell cloning. For HEC116 cells,

1076 Ribonucleoprotein (RNP) delivery was used. cRNA targeting SMARCA4 (sequence =

1077 GCGGTGGCATCACGGGCG) and tracrRNA duplexes (1 µM) were formed by heating at 95°C

1078 and gradual cool down to room temperature (RT). RNP complexes were formed by combining the

1079 1 µM guide RNA oligos with 1 µM Alt-R *S.pyogenes* Cas9 endonucleases (IDT) in Gibco Opti-

1080 MEM media (ThermoFisher Scientific) for 5 minutes at RT. Transfection complexes containing

1081 the RNP complex and Liptofectamine RNAiMAX transfection reagent (ThermoFisher Scientific)

1082 were diluted in Opti-MEM media and incubated at RT for 20 minutes. HEC116 endometrial

1083 cancer cells were added to transfection complexes in the wells of a 24-well tissue culture plate to

1084 achieve a final concentration of 40000 cells/well and final concentration of RNP of 10 nM. Flow

1085 cytometry (University of Alberta, Faculty of Medicine and Dentistry, Flow Cytometry Facility)

1086 was utilized to enrich for CRISPR transfected cells positive for tracrRNA-ATTO[TM]550

1087   fluorescence. Single clones were either generated by flow cytometry plating a single cell per well

1088   into a 96-well plate or manually plating of 0.5 cells/well into a 96-well plate upon filtration through

1089   a cell strainer. Single cell-derived clones were validated by Sanger sequencing over the guided

1090   nuclease target region.

1091

1092   ***CRISPR sgRNA screen***

1093   The transEDIT[TM] pCLIP-All-EFS-Puro Epigenetics CRISPR Screening library (Transomic)

1094   containing 5080 sgRNAs targeting 496 epigenetic genes was used in this study. Library virus was

1095   generated in 293T cells as described above. OVCAR4 cells were infected with library virus at low

1096   multiplicity of infection (MOI) achieving single sgRNA integration. After selection, ~5x10^6 cells

1097   (1000-time coverage) were plated in 15 cm dishes and treated $\pm$ 100 nM cisplatin for 14 days

1098   before harvesting. Genomic DNA was isolated with High Pure PCR Template Preparation Kit

1099   (Roche) by following the manufacturer's instruction. Library preparation for Next Generation

1100   Sequencing was done as described before [83]. Briefly, the gRNA sequences were amplified from

1101   20 µg genomic DNA by PCR using Phusion HF DNA polymerase (ThermoFisher Scientific) using

1102   a 2-step amplification adding a unique 6-bp index per sample and sequencing adapter sequences.

1103   PCR products were purified using the High Pure PCR Product Purification Kit (Roche) and

1104   quantified using the Quant-iT™ PicoGreen™ dsDNA Assay Kit (ThermoFisher Scientific) before

1105   sequencing on a HiSeq2500 System (Illumina). Sequencing reads were mapped to the library using

1106   xcalibr and counts were then analyzed with MAGeCK (version 0.5.8) using the Robust Rank

1107   Aggregation (RRA) algorithm.

1108   Primers used are as follows: PCR1: PTRC_index (forward, NNNNNN=index sequence): 5'

1109   ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNGGCTTTATATATCTTGTGG

1110   AAAGGACG   3',   IllSeqR_CR_r   (reverse):   5'

1111   GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTGACGGGCACCGGAGCCAATT

1112   CC   3'.   PCR2:   P5_Illuseq   (forward):   5'

1113   AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATC

1114   T   3',   P7_index_IR_r   (reverse,   NNNNNN=index   sequence):   5'

1115   CAAGCAGAAGACGGCATACGAGATNNNNNNGTGACTGGAGTTCAGACGTGTGCTCT

1116   TCCGATCT 3'. Index sequences: Control: ACATCG, cisplatin: GCCTAA.

1117

1118   *Colony formation assays*

1119   Since different cell lines have variable proliferation rates and sizes, plating densities for each line

1120   were first optimized to allow about two weeks of drug treatment, before cells reach 90%

1121   confluency in 6-well plates. Single cell suspensions of all cell lines were then counted and seeded

1122   into 6-well plates with the densities predetermined (2-8x10^4 cells/well). Cells were treated with

1123   vehicle control or drugs on the next day and culture medium was refreshed every 3 days for 10-14

1124   days in total. At the endpoints of colony formation assays, cells were fixed with 3.75%

1125   formaldehyde, stained with crystal violet (0.1%w/v) and photographed. All relevant assays were

1126   performed independently at least three times.

1127

1128   *Cell viability assays*

1129   Cultured cells were seeded into 96-well plates (1,000–6,000 cells per well). Twenty-four hours

1130   after seeding, different dilutions of compounds were added to cells. Cells were then incubated for

1131   4 days and cell viability was measured using the CellTiter-Blue viability assay (Promega) by

1132   measuring the fluorescence (560/590 nm) in a microplate reader. Relative survival in the presence

1133   of drugs was normalized to the untreated controls after background subtraction.

1134

1135   ***Protein lysate preparation and immunoblots***

1136   Cells were first seeded in normal medium without inhibitors. After 24 hours, the medium was

1137   replaced with fresh medium containing the inhibitors as indicated in the text. After the drug

1138   stimulation, cells were washed with cold PBS, lysed with protein sample buffer and processed

1139   with Novex® NuPAGE® Gel Electrophoresis Systems (ThermoFisher Scientific). HSP90, actin,

1140   vinculin or calreticulin were used as loading controls. The quantification of immunoblots was

1141   performed on two independent experiments using Image J and normalized to loading controls are

1142   displayed in Figure S16. Uncropped immunoblots presented in main figures are displayed in

1143   Figure S17.

1144

1145   ***Annexin-V staining and IncuCyte imaging***

1146   Cells in 96-well plates were treated with cisplatin at different concentrations in medium containing

1147   IncuCyte® Annexin V for Apoptosis (Essen Bioscience, Cat# 4641). IncuCyte® live-cell analysis

1148   imaging system was used to record 4 images every 2-4 hours. Images were analyzed by IncuCyte®

1149   Zoom (2016B) software and annexin V positive cells were normalized to phase contrast

1150   confluency for each well.

1151

1152   ***Annexin-V and Propidium Iodide Flow Cytometry***

1153   For apoptosis assays, negative controls were prepared by incubating cells in the absence of

1154   inducing agent and positive controls for apoptosis were prepared using 10 μm H_2O_2. Cells were

1155   harvested after treatment and washed in cold phosphate buffered saline and resuspended in 1X

1156 Annexin Binding Buffer (BMS500BB) to 10^6 cells/mL. 100μL of cell suspension was used per

1157 assay and 5 μL of FITC annexin V (A13199) and 1μL of propidium Iodide (PI; P1304MP) diluted

1158 to 100 μg/mL in annexin V binding buffer was added to each suspension. Cells were incubated

1159 following addition of fluorescent reagents for 15 minutes at RT. 400 μL of 1X Annexin V Binding

1160 Buffer was added to each suspension following incubation and the samples were mixed gently and

1161 kept in the dark and on ice prior to analysis.

1162 Flow cytometry was performed using Guava easyCyte HT (Sigma) with the guavaSoft 2.5

1163 software (Sigma) based on the manufacturer's instructions. Fluorescence emission was measured

1164 at 530 nm and >575 nm to separate between the annexin V^+ and PI^+ populations in green and red.

1165 Technical controls for gating were prepared with uninduced cells with both PI and annexin V stains,

1166 with either PI or annexin V only, or in the absence of both. Apoptotic cell population (annexin

1167 V^+/PI^-) showed green fluorescence only. Gating strategy is exemplified in Figure S18.

1168

1169 ***RNA isolation and qRT-PCR***

1170 After indicated drug treatment or genetic modifications, cells were harvested for RNA isolation

1171 using Trizol (ThermoFisher Scientific, Cat # 15596018) the next day. Synthesis of complementary

1172 DNAs (cDNAs) using Maxima First Strand cDNA Synthesis Kit (ThermoFisher Scientific, Cat#

1173 K1642) and real-time quantitative reverse transcription PCR (qRT-PCR) assays using PowerUp™

1174 SYBR™ Green Master Mix (ThermoFisher Scientific, Cat# A25742) were carried-out according

1175 to manufacturer protocols. Relative mRNA levels of each gene shown were normalized to the

1176 expression of the housekeeping gene *GAPDH*. The sequences of the primers are as follows:

1177 GAPDH_qPCR_For: AAGGTGAAGGTCGGAGTCAA

1178 GAPDH_qPCR _Rev: AATGAAGGGGTCATTGATGG

1179   ITPR3_ qPCR_For: TATGCAGTTTCGGGACCACC

1180   ITPR3_qPCR_Rev: TGCCCTTGTACTCGTCACAC

1181   SMARCA2_ qPCR_For: AGGGGATTGTAGAAGACATCCA

1182   SMARCA2_ qPCR_Rev: TTGGCTGTGTTGATCCATTGG

1183

1184   *Survival Analysis*

1185   Survival analyses were performed on lung adenocarcinoma patients from the following datasets

1186   with available information on adjuvant chemotherapy status: Director's Challenge Consortium for

1187   the Molecular Classification of Lung Adenocarcinoma [36], KMPlotter [37] and The UT Lung SPORE

1188   (GSE42127) [39]. For all Affymetrix microarray datasets, 213720_s_at was the probe used to assess

1189   *SMARCA4* expression based on criteria for probe selection previously described [38]. Director's

1190   challenge and The UT Lung SPORE (GSE42127) datasets were analyzed by stratifying patients

1191   into *SMARCA4* high and low groups, separated by median *SMARCA4* level. The survival data were

1192   analyzed by one-tailed Mantel-Cox analysis in GraphPad Prism. Parameters for kmplot.com query

1193   were: gene symbol - *SMARCA4*; probe set options – use JetSet best probe set; split patients by –

1194   auto select best cutoff; survival – censor at threshold; histology – adenocarcinoma; and all other

1195   default settings. Patients with and without adjuvant chemotherapy were analyzed separately in all

1196   datasets.

1197

1198   *Transcriptome analysis*

1199   Cell lines

1200 There were three sets of transcriptome data used in this study, namely SMARCA4 restoration in

1201 BIN-67 and SCCOHT-1 cells (GSE120297, RNAseq), SMARCA4/2 restoration in BIN-67 cells

1202 (GSE117735, RNAseq) and SMARCA4 restoration in H1299 cells (GSE109010, microarray).

1203 Processed gene expression data were retrieved from original study for GSE120297 and by

1204 GEOquery (2.56.0) [84] for GSE109010, respectively. For GSE117735, sequencing files were

1205 downloaded from Sequence Read Archive (SRA) and mapped to reference human genome

1206 sequence (hg19) with STAR (2.6.1c) [85]. Gene expression counts were calculated by featureCounts

1207 (v1.6.4) [86] with UCSC hg19 gene annotation GTF file. Heatmaps for gene expression were

1208 generated with pheatmap (1.0.12) after normalization. Differential expression genes were

1209 identified with DESeq2 (version 1.19.38) for GSE120297, with GEO2R analysis for GSE109010

1210 and from original study [60] for GSE117735.

1211 Patient tumors

1212 Total RNA from 3 SCCOHT patient tumors was extracted with the RNeasy Mini Kit (Qiagen, Cat

1213 # 74104) and subjected for RNA-Seq at Genome Quebec. Briefly, quantification was performed

1214 using a NanoDrop Spectrophotometer ND-1000 (NanoDrop Technologies, Inc.) and its integrity

1215 was assessed using a 2100 Bioanalyzer at Genome Quebec. Libraries were generated from 250 ng

1216 of total RNA using the TruSeq stranded mRNA Sample Preparation Kit (Illumina, Cat# RS-122-

1217 2101), as per the manufacturer's recommendations. Libraries were quantified using the Quant-

1218 iT™ PicoGreen® dsDNA Assay Kit (ThermoFisher Scientific, Cat# P7589) and the Kapa Illumina

1219 GA with Revised Primers-SYBR Fast Universal kit (Kapa Biosystems). Average size fragment

1220 was determined using a LabChip GX (PerkinElmer) instrument. RNA-Seq data of another ten

1221 SCCOHT patient tumors were obtained from a previous study [87]. Sequencing results were

1222 processed by following mRNA quantification analysis pipeline of Genomic Data Commons.

1223   https://docs.gdc.cancer.gov/Data/Bioinformatics_Pipelines/Expression_mRNA_Pipeline/

1224   RNA-seq read counts of 379 ovarian cancer tumors were obtained from UCSC xena

1225   (http://xena.ucsc.edu/) which followed the exact same pipeline. The Fragments per Kilobase of

1226   transcript per Million mapped reads (FPKM) for each gene was calculated as below:

1227

$$FPKM = \frac{\text{RCg} * 109}{\text{RCpc} * \text{L}}$$

1228

1229   RCg: Number of reads mapped to the gene; RCpc: Number of reads mapped to all protein-coding

1230   genes; L: mean of lengths of isoforms of a gene.

1231

1232   ***Gene set enrichment analysis***

1233   Pre-ranked gene listed were generated on the $\log_2$ transformed fold change for significantly

1234   changed genes (adjusted *p*-value smaller than 0.05). The R package clusterProfiler (v3.12.0) [88] was

1235   used to perform gene set enrichment analysis (GSEA) with the following parameters: ont ="MF",

1236   nPerm = 10000, minGSSize = 3, maxGSSize = 800, pvalueCutoff = 0.05.

1237

1238   ***siRNA and plasmids transfection***

1239   For small-interfering RNA (siRNA) experiments, cells were transfected using Lipofectamine

1240   RNAimax (ThermoFisher Scientific, Cat# 13778150) with 20 nM SMARTPool ON-TARGETplus

1241   HUMAN *ITPR3* siRNA (Horizon Discovery, cat# L-006209-00-0005) for 3 days according to

1242   manufacturer's recommendations. Plasmids were transfected for 24 hours using FuGENE HD

1243   (Promega, Cat# E2311) following manufacturer's recommendations.

1244

1245 *Intracellular Ca^{2+} measurements*

1246 To measure cytosolic or mitochondrial Ca^{2+}, OVCAR4, H1703, SCCOHT1 and H1437 cells were

1247 cultured on Nunc Lab-Tek chambered 8-well cover glass (ThermoFisher Scientific) and transiently

1248 transfected with the cytosolic R-GECO1 (Addgene, cat# 32444) [56] or mitochondrial CEPIA-2mt

1249 (Addgene, cat# 58218) [57] Ca^{2+} reporter probes. Cells were washed three times in a balanced salt

1250 solution buffer + Ca^{2+} (BSS) (120 mM NaCl, 5.4 mM KCl, 0,8 mM MgCl$_2$, 6 mM NaHCO3, 5.6

1251 mM D-glucose, 2 mM CaCl$_2$, and 25 mM HEPES [pH 7.3]). Fluorescence values were then

1252 collected every 2 seconds for 3 minutes (min). ER-Ca^{2+} release was stimulated by injection of 100

1253 μM histamine final in BSS+Ca^{2+} at 10 seconds. Images were acquired using a 40x objective of the

1254 Nikon Eclipse Ti-E microscope, coupled to an Andor Dragonfly spinning disk confocal system

1255 equipped with an Andor Ixon camera, exciting with 488 nm or 561 nm laser for CEPIA-2mt or R-

1256 GECO1, respectively.

1257 To measure total ER Ca^{2+} content, OVCAR4, H1703, SCCOHT1 and H1437 cells were cultured

1258 on Nunc Lab-Tek chambered 8-well cover glass (ThermoFisher Scientific) and transiently

1259 transfected with the cytosolic R-GECO Ca^{2+} reporter probe. Cells were washed three times in a

1260 BSS-Ca^{2+} (120 mM NaCl, 5.4 mM KCl, 0.8 mM MgCl$_2$, 6 mM NaHCO3, 5.6 mM D-glucose and

1261 25 mM HEPES [pH 7.3]). Fluorescence values were then collected every 2 seconds for 5 minutes.

1262 ER-Ca^{2+} release was stimulated by injection of 10 μM thapsigargin final in BSS-Ca^{2+} at 10 seconds.

1263 Images were acquired using microscope and laser described above.

1264 To measure basal mitochondrial Ca^{2+} pools, H1703 cells were cultured on Nunc Lab-Tek

1265 chambered 8-well over glass (ThermoFisher Scientific), treated with appropriate drugs and

1266 transiently transfected with the mitochondrial CEPIA-2mt Ca^{2+} reporter probe. Cells were stained

1267 with 100 nM Mitotracker deep red (ThermoFisher Scientific) prior to imaging for 20 minutes

1268   followed by 3 washes in complete culture media. Fluorescence values were then collected every 2

1269   seconds for 30 seconds. Images were acquired using a 40x objective of the Nikon Eclipse Ti-E

1270   microscope, coupled to an Andor Dragonfly spinning disk confocal system equipped with an

1271   Andor Ixon camera, exciting with 488 nm and 647 nm lasers for CEPIA-2mt and Mitotracker deep

1272   red, respectively.

1273

1274   *Immunohistochemistry*

1275   Tissue microarrays (TMAs) of tumor samples of HGSC and NSCLC patients used in this study

1276   were previously described [48, 49]. A new TMA of 52 SCCOHT patient tumors was constructed for

1277   this study. Studies on SCCOHT patient tumors were approved by the Institutional Review Board

1278   (IRB) at McGill University, McGill IRB # A08-M61-09B. Studies on 59 pathologist-confirmed

1279   (B.A.C.) ovarian HGSC samples were approved by the ethics boards at the University Hospitals

1280   Network and the Jewish General Hospital respectively. Informed consent was obtained from all

1281   participants in accordance with the relevant IRB approvals. Hematoxylin and eosin (H&E)-stained

1282   sections of the 50 SCCOHTs (confirmed by DNA mutation analysis or/and SMARCA4 IHC) and

1283   52 HGSC cases were reviewed and areas containing tumor only were demarcated and cored to

1284   construct tissue microarrays (TMAs) using duplicate 0.6-mm cores from the demarcated areas. A

1285   panel of 100 resected lung adenocarcinoma patient tumors were analyzed. This study was

1286   approved by the ethics boards at the McGill University Health Centre (F11HRR, 17212). The

1287   NSCLC TMA was comprised of 4 mm cores from the selected paraffin-embedded tissue blocks.

1288   For all IHC analysis, cores with low tumor cellularity and artifacts were not included in the analysis.

1289   The 4 µm thick sections from these TMAs were cut, deparaffinized and stained using the

1290   BenchMark Ultra system (Ventana Medical Systems Inc). Heat-induced epitope retrieval (HIER)

1291   was performed with Ultra Cell Conditioning Solution (CC1) for 64 minutes at 95 °C, followed by

1292   16 min of incubation at 36 °C with the rabbit monoclonal antibody against SMARCA4 (clone

1293   EPNCIR111A; dilution, 1:100; Abcam). For IP3R3, HIER was performed in CC1 for 48 minutes

1294   at 100 °C followed by a 48 minutes of incubation at 36 °C with the Mouse Anti-IP3R3 (BD

1295   Transduction Laboratories). After primary antibody incubation, detection was performed using the

1296   default OptiView DAB protocol as per the manufacturer's directions (Ventana). The slides were

1297   digitalized using an Aperio scanner and the Lumenera INFINITY X CMOS Camera.

1298   For patient tumors: assessment of SMARCA4, unequivocally absent staining in the nuclei of viable

1299   tumor cells as opposed to strong staining in background stromal cells was considered IHC negative.

1300   Expression in the tumor cells that is equivalent to the staining of non-neoplastic cells in the

1301   background was considered IHC positive. Positive cells were analyzed according to the staining

1302   intensity on a scale of 0–3 (0 = negative, 1 = weak, 2 = moderate, 3 = strong). H-scores were

1303   calculated as the sum of the percent of cells at each intensity (Pi) multiplied by the intensity score

1304   (i). H-score = Σ (Pi(i)) × 100. Score values range between 0 and 300. Cores with low tumor

1305   cellularity and artifacts were not included in the analysis.

1306   For xenograft tumor sections: quantification of percentage positive staining for SMARCA4,

1307   SMARCA2, and cleaved caspase 3 were performed unbiasedly using the Aperio Nuclear algorithm

1308   on Aperio ImageScope. Quantification of percentage positive staining for IP3R3 was performed

1309   unbiasedly using the Aperio Cytoplasm Algorithm on Aperio ImageScope. Weak IP3R3 (+)

1310   staining resulting from the background was not considered in the analysis.

1311

1312   ***Mouse xenografts and in vivo drug studies***

1313 Animal experiments were carried-out according to standards outlined in the Canadian Council on

1314 Animal Care Standards (CCAC) and the Animals for Research Act, R.S.O. 1990, Chapter c. A.22,

1315 and by following internationally recognized guidelines on animal welfare. All animal procedures

1316 (Animal Use Protocol) were approved by the Institutional Animal Care Committee according to

1317 guidelines of the Canadian Council of Animal Care. All animal experiments were carried-out at

1318 the Goodman Cancer Research Center of McGill University, using 8–12-week-old in house bred

1319 male NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ (NSG) mice.

1320 For in vivo drug studies, Quisinostat (SelleckChem) was resuspended in 20% hydroxypropyl-β-

1321 cyclodextrin (Sigma Aldrich) buffer (pH = 8.70) at a concentration of 50 mg ml^{-1} (administrated

1322 intraperitoneally at 10 mg kg^{-1} dose for a 25-28 g mouse in a volume of 100 µl). Cisplatin

1323 (SelleckChem) was resuspended in 0.9% Sodium Chloride Solution (administrated

1324 intraperitoneally at 4 mg kg^{-1} dose for a 25-28 g mouse in a volume of 200 µl). These two reagents

1325 are stored at −80 °C. Tubes were thawed overnight at 4 °C.

1326 For the tumor model, single-cell suspension was created by dissociating a sufficient number of

1327 sub-confluent flasks of cells to produce 4x10^6 cells (H1703 or H1703 expressing pIN20-*SMARCA4*)

1328 in 200 µl of Matrigel HC in a 50:50 ratio (Corning Matrigel HC, VWR). The tumor cell suspension

1329 was subcutaneously injected into the left flank of each NSG mouse. For the doxycycline inducible

1330 model using H1703 cells expressing pIN20-*SMARCA4*, as tumor volumes (V = (H × W2)/2)

1331 reached ~150 mm^3 (21 days post inoculation), experimental mice (n=6) were injected with 2.5

1332 mg/ml doxycycline (Millipore Sigma) intraperitoneally followed by 2 mg/ml in sucralose

1333 (MediDropR, ClearH20) solution ad libitum. Experimental mice were again injected

1334 intraperitoneally with doxycycline at day 32 to ensure they were acquiring adequate drug. Control

1335 mice (n=4) received intraperitoneal injections of saline (diluent) and received sucralose ad libitum.

1336   All mice were placed on sucralose a week prior to the experiment to acclimatize mice to the taste.

1337   For the chemo drug treatment experiment, when tumor volumes reached ~150 mm^3 (20 days post

1338   inoculation), which was assigned as day 0, the mice were entered into the treatment regimen (21

1339   days). Mice were randomly allocated to control (vehicle, n=6), quisinostat (10 mg kg^{-1} quisinostat,

1340   [76], three times per week, n=5), cisplatin (4 mg kg^{-1} cisplatin, [89], once per week, n=5) or

1341   combination (10 mg kg^{-1} quisinostat and 4 mg kg^{-1} cisplatin, n=5) group. Mice were housed in

1342   groups of 4–5, with each group consisting of both vehicle control and treatment animals matched

1343   for tumor size on day 0 of treatment. Tumor progression was monitored and measurements using

1344   digital calipers (VWR) were recorded twice weekly. The persons who performed all the tumor

1345   measurements and the IHC analysis for the endpoint tumor samples were blinded to the treatment

1346   information.

1347

1348   *Statistical analysis*

1349   GraphPad Prism 8 software was used to generate graphs and statistical analyses. Methods for

1350   statistical tests, exact value of n and definition of error bars are indicated in figure legends.

1351   *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

1352

1353   **Data Availability**

1354   Original data for IC$_{50}$ of chemotherapy drugs are from Genomics of Drug Sensitivity in Cancer

1355   (https://www.cancerrxgene.org/). mRNA expression data of SMARCA4/2 and ITPR3 are obtained

1356   from the Cancer Cell Line Encyclopedia (https://portals.broadinstitute.org/ccle) for cell lines and

1357   downloaded from UCSC Xena (https://xenabrowser.net/datapages/) for TCGA tumors of lung and

1358   ovarian cancer patients. Out of 13 SCCOHT patient tumors, RNA-seq data of 10 cases were

1359 obtained from a previous study [87] and that of the other three cases will be deposit before publication.

1360 Source data for RNA-seq, microarray, ChIP-seq and ATAC-seq can be found using the accession

1361 number GSE120297 [49], GSE117735 [60], GSE121755 [48], GSE109010 and GSE109020 [61]. All other

1362 data supporting the findings of this study are available from the corresponding author upon

1363 reasonable request.

1364

1365

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1661

Figure 1

A Pan cancer cell lines

B Lung cancer cell lines

C Epigenetic CRISPR library

D Cisplatin resistance ranking

E

Cisplatin (μM)

OVCAR4

F

OVCAR4

G

$A4^{KO}$

OVCAR4

H

Cisplatin (μM)

H1703

I

H1703

J

H1703

Figure 2

A *A4* vs Ctrl_SCCOHT-1

Ion transmembrane transporter

Calcium ion binding

B *A4* vs Ctrl_BIN-67

Ion transmembrane transporter

Calcium ion binding

C

SCCOHT-1

D Cytosolic Ca^{2+}_SCCOHT-1

E Mitochondrial Ca^{2+}_SCCOHT-1

F

H1703

G Cytosolic Ca^{2+}_H1703

H Mitochondrial Ca^{2+}_H1703

I

OVCAR4

J Cytosolic Ca^{2+}_OVCAR4

K Mitochondrial Ca^{2+}_OVCAR4

L

H1437

M Cytosolic Ca^{2+}_H1437

N Mitochondrial Ca^{2+}_H1437

Figure 3

A

A4 vs Ctrl
SCCOHT-1

A4 vs Ctrl
BIN-67

RNAseq

RNAseq

120   129   116

69

42   30

4927

ChIPseq

SMARCA4 bound
BIN-67 + *A4*

C

BIN-67   SCCOHT-1   H1299   H1703

ITPR3 mRNA

Ctrl A2 A4   Ctrl A2 A4   Ctrl A4   Ctrl A2 A4

D

A4: - - +   - - +   - +   - - +
A2: - + -   - + -   - -   - + -

SMARCA4   250
SMARCA2   250
IP3R3   250
Actin   37
kDa

BIN-67   SCCOHT-1   H1299   H1703

B

Z-score

ITPR3
MATN2
FBLN2
EHD4
ATP2B4

E

Chr 6   33,580,000   33,620,000   33,660,000
ITPR3   *UQCC2*

SMARCA4

Ctrl-1   [0 – 1.7]
Ctrl-2   [0 – 1.7]   BIN67
A4   [0 – 1.7]
input   [0.5 – 2.7]   H1703
A4   [0.5 – 5]
- Dox   [0 – 51]   H1299
+Dox   [0 – 51]
(*A4*)

F

Chr 6   33,580,000   33,620,000   33,660,000
ITPR3   *UQCC2*

H3K27Ac

Ctrl   [0 – 6]   BIN67
A4   [0 – 6]
Ctrl   [0 – 60]
A4   [0 – 60]   H1703
A2   [0 – 60]

ATAC

Ctrl   [0 – 140]
A4   [0 – 300]
A2   [0 – 180]

Figure 4

A

A4: − + + +
sh*R3*#1: − − + −
sh*R3*#2: − − − +

SMARCA4
IP3R3
Calreticulin
Vinculin

185
185
50
115 kDa

SCCOHT-1

B

Cytosolic Ca²⁺_SCCOHT-1

Histamine

$F/F0$ (R-GECO, A.U)

Time (s)

Maximum $F/F0$

***

A4: − + + +
sh*R3*#1: − − + −
sh*R3*#2: − − − +

● Ctrl ▼ *A4*, sh*R3*#1
■ *A4* ▼ *A4*, sh*R3*#2

C

Mitochondrial Ca²⁺_SCCOHT-1

Histamine

$F/F0$ (CEPIA-2mt, A.U)

Time (s)

Maximum $F/F0$

**** ns ns

A4: − + + +
sh*R3*#1: − − + −
sh*R3*#2: − − − +

● Ctrl ▼ *A4*, sh*R3*#1
■ *A4* ▼ *A4*, sh*R3*#2

D

A4: − + +
si*R3*: − − +

SMARCA4
IP3R3
Calreticulin
Vinculin

185
185
50
115 kDa

H1703

E

Cytosolic Ca²⁺_H1703

Histamine

$F/F0$ (R-GECO, A.U)

Time (s)

Maximum $F/F0$

** ns

A4: − + +
si*R3*: − − +

● Ctrl ■ *A4* ▼ *A4*, si*R3*

F

Mitochondrial Ca²⁺_H1703

Histamine

$F/F0$ (CEPIA-2mt, A.U)

Time (s)

Maximum $F/F0$

*** ns

A4: − + +
si*R3*: − − +

● Ctrl ■ *A4* ▼ *A4*, si*R3*

G

Cisplatin (µM) 0 3
sh*R3*#1: − + − − + −
sh*R3*#2: − − + − − +

IP3R3
cl. PARP
cl. caspase3
Actin

250
100
15
37 kDa

OVCAR4

H

OVCAR4

% Annexin V⁺/ PI⁻ cells

**** ****

sh*R3*#1: − + − − + −
sh*R3*#2: − − + − − +
Cisplatin (µM) 0 3

I

Cisplatin (µM) 0 2
R3: − + − +

IP3R3
cl. PARP
cl. caspase3
Actin

250
100
15
37 kDa

H1703

J

Cisplatin (µM) 0 2
A4: − + + + − + + +
sh*R3*#1: − − + − − − + −
sh*R3*#2: − − − + − − − +

SMARCA4
IP3R3
cl. PARP
cl. caspase3
Actin

250
250
100
15
37 kDa

H1703

K

H1703

% Annexin V⁺/ PI⁻ cells

**** ****

A4: − + + + − + + +
sh*R3*#1: − − + − − − + −
sh*R3*#2: − − − + − − − +
Cisplatin (µM) 0 2

Figure 5

Figure 6

A SMARCA2 mRNA, ITPR3 mRNA — Quisinostat (nM) 0, 10, 40. H1703

B Quisinostat/Cisplatin blots: SMARCA2 (250), IP3R3 (250), cl. PARP (100), cl. caspase3 (15), Actin (37 kDa). H1703

C Apoptosis — % Annexin V+/PI- cells; Ctrl, Cisplatin, Quisinostat, Combination

D Quisinostat (nM) 0, 2.5, 5; Cisplatin (µM) 0, 1.5. H1703

E sgA2 / Quisinostat / Cisplatin blots: SMARCA2 (250), IP3R3 (250), cl. PARP (100), cl. caspase3 (15), Actin (37 kDa). H1703

F siR3 / Quisinostat / Cisplatin blots: SMARCA2 (250), IP3R3 (250), cl. PARP (100), cl. caspase3 (15), Actin (37 kDa). H1703

G Ctrl, Cisplatin, Quisinostat, Combination — CEPIA-2mt, Mitotracker, Overlay. H1703

H Basal Mito-Ca^{2+} — CEPIA-2mt / mitotracker; Ctrl, Cisplatin, Quisinostat, Combination

I H1703 xenografts — Tumor volume (mm^3) vs Days of treatment; Vehicle, Cisplatin, Quisinostat, Combination. Endpoint tumor weight (g).

J Vehicle, Cisplatin, Quisinostat, Combination — SMARCA2, IP3R3, cl. caspase 3. H1703 xenografts

K SMARCA2+ (%), IP3R3++ (%), Cl. caspase 3+ (%); Vehicle, Cisplatin, Quisinostat, Combination