Identification and validation of a non-genetically encoded vulnerability to XPO1 inhibition in malignant rhabdoid tumors – expanding patient-driven discovery beyond the N-of-1.

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Abstract

Malignant rhabdoid tumors (MRTs) are rare, aggressive pediatric solid tumors, characterized by a 22q11 deletion that inactivates the SMARCB1 gene. Outcomes remain poor despite multimodality treatment. MRTs are among the most genomically stable cancers and lack therapeutically targetable genetic mutations. We utilized metaVIPER, an extension of the Virtual Inference of Protein-activity by Enriched Regulon (VIPER) algorithm, to computationally infer activated druggable proteins in the tumor of an eight month old patient and then expanded the analysis to TCGA and TARGET cohorts. *In vitro* studies were performed on a panel of MRT and atypical teratoid/rhabdoid tumor cell lines. Two patient-derived xenograft (PDX) mouse models of MRT were used for *in vivo* efficacy studies. MetaVIPER analysis from the patient's tumor identified significantly high inferred activity of nuclear export protein Exportin-1 (XPO1). Expanded metaVIPER analysis of TCGA and TARGET cohorts revealed consistent elevations in XPO1 inferred activity in MRTs compared to other cancer types. All MRT cell lines demonstrated baseline activation of XPO1. MRT cell lines demonstrated *in vitro* sensitivity to the XPO1 inhibitor, selinexor which led to cell cycle arrest and induction of apoptosis. Targeted inhibition of XPO1 in patient-derived xenograft models of MRT using selinexor resulted in abrogation of tumor growth. Selinexor demonstrates efficacy in preclinical models of MRT. These results support investigation of selinexor in a phase II study in children with MRT and illustrate the importance of an N-of-1 approach in driving discovery beyond the single patient.

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Statement of Translational Relevance:

We describe the patient-driven discovery of XPO1 activation as a non-genetically encoded vulnerability in MRTs. The application of metaVIPER analysis to tumors lacking actionable oncogenic alterations represents a novel approach for identifying potential therapeutic targets and biomarkers of response. Our preclinical validation of selinexor confirms XPO1 inhibition as a promising therapeutic strategy for the treatment of MRT.

Introduction

Comprehensive genomic and transcriptomic profiling of tumors has the potential to impact patient care and guide treatment decisions for individual patients, especially those with rare cancers lacking efficacious conventional therapeutic options (1). Malignant rhabdoid tumors (MRTs) are rare, aggressive pediatric tumors associated with extremely poor prognosis. MRTs are characterized by the biallelic inactivation of the *SMARCB1* gene (also known as *SNF5*, *INI1*, or *BAF47*) through chromosomal deletions and nonsense, missense, or frameshift mutations, with as many as 35% of cases associated with germline variants in *SMARCB1* (2,3). MRTs occur throughout the body but are most frequently localized to the kidney and the brain, where they are referred to as atypical teratoid/rhabdoid tumors (ATRTs). The peak incidence of MRT is in infancy, with a median age of 11 months at diagnosis (4). Despite multimodal therapy,

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86 outcomes remain poor with an overall 4-year survival of 23.2%, and only 8.8% in

87 patients younger than six months (5).

88

89 Compared to adult tumors, genomic characterization of pediatric tumors

90 generally demonstrates few single nucleotide variants (SNV) and low overall

91 tumor mutational burden (6).   Furthermore, MRTs are one of the most

92 genomically stable of all pediatric cancers with a mutation rate of only 0.084 per

93 megabase, which is 10 to 50-fold lower than the majority of adult tumors (7).

94 SMARCB1, characteristically inactivated in MRT, is a core subunit of the highly

95 conserved   nucleosome-remodeling   SWItch/Sucrose   Non-Fermentable

96 (SWI/SNF) complex which regulates nucleosome positioning and occupancy in

97 an ATP-dependent manner, thereby controlling DNA accessibility (8). Within the

98 SWI/SNF complex, SMARCB1 is required for efficient binding to DNA,

99 transcriptional activation of enhancers of lineage-specific genes, and resolution

100 of bivalency at promoters of lineage-specific genes (9). Loss of SMARCB1

101 results in gene expression changes that drive tumorigenesis by multiple

102 mechanisms, including epigenetic perturbations of differentiation and the cell

103 cycle (9). Due to the lack of targetable genomic alterations in MRT, new methods

104 for discovering candidate, non-genetically-encoded tumor vulnerabilities are

105 needed.

106

107 Both genetic and epigenetic alterations, in combination with complex networks

108 that regulate gene expression and post-translational modifications, contribute to

4

109 dysregulated protein activity (10). The Virtual Inference of Protein-activity by

110 Enriched Regulon analysis (VIPER) algorithm is a computational tool for the

111 inference of protein activity in cancer at the individual tumor level (11,12). The

112 ability of VIPER to accurately and reproducibly infer differential protein activity

113 has been extensively validated in several contexts: 1) to identify candidate

114 'Master Regulators' (MRs) whose aberrant activity is critical to tumor viability and

115 growth *in vivo*; 2) to identify regulatory proteins whose differential activity

116 associates with tumor subtypes, metastatic state, treatment sensitivity,

117 mechanisms of resistance, and prognosis; and 3) to identify drugs/combinations

118 that disrupt the regulatory proteins responsible for a tumor's homeostatic state as

119 a predictor of drug sensitivity (13-19).

120

121 We report the case of an infant diagnosed with MRT, whose tumor was assessed

122 using whole exome and RNA sequencing. Using the VIPER algorithm in a N-of-1

123 application, we identified aberrant XPO1 activity by comparison to other cancer

124 types. Leveraging this patient-driven observation, we demonstrate XPO1

125 inhibition as a class-specific vulnerability for MRTs, suggesting a new therapeutic

126 approach for this disease.

127

128 **Results:**

129 *Case Report*

130 A previously healthy eight month old girl presented with a firm abdomen and was

131 found to have a 10 cm right hepatic mass on ultrasound. An abdominal computed

132   tomography (CT) scan showed a 10.1 x 7.2 x 11.5 cm lobulated heterogeneous

133   mass arising from the right hepatic lobe (Figure 1A). She was also noted to have

134   multiple small pulmonary nodules consistent with metastatic disease on chest

135   CT. Laboratory workup demonstrated an alpha feto-protein (AFP) of 71.5 ng/ml

136   (mean AFP for age 8ng/ml, normal range 0.8-87ng/ml)(20). Histopathological

137   evaluation of an incisional biopsy of the liver mass showed small cell morphology

138   (Fig. 1B) with immunohistochemistry (IHC) showing loss of INI1 protein

139   expression (Fig. 1C) (21). In the absence of rhabdoid morphology the tumor was

140   classified as small cell undifferentiated (SCUD) hepatoblastoma. Under the

141   current pediatric liver tumor classification, in the absence of rhabdoid morphology

142   INI1 negative IHC is insufficient to diagnose MRT, however, recent studies have

143   shown that presence of INI1 negativity with IHC in a pediatric liver tumor is

144   diagnostic of MRT even in the absence of rhabdoid morphology (22-24). Given

145   these results, a presumptive diagnosis of high risk hepatoblastoma was rendered

146   due to non-elevated AFP and metastatic disease status. The patient was enrolled

147   on Children's Oncology Group (COG) study AHEP0731, high-risk stratum, and

148   received 2 cycles of vincristine, irinotecan, and temsirolimus (VIT) and 4 cycles of

149   cisplatin, 5-fluorouracil, and vincristine (C5VD) prior to a right partial

150   hepatectomy.

151

152   Review of the surgical resection specimen demonstrated focal areas of large

153   polygonal tumor cells with eccentric nuclei and dense eosinophilic cytoplasm

154   consistent with a rhabdoid morphology. For further evaluation, whole exome

6

155 sequencing (WES) and RNA sequencing were performed and copy number

156 analysis confirmed homozygous deletion of *SMARCB1* on chromosome

157 22q11.23 with consequent complete loss of mRNA expression (25). Based on

158 this information the tumor was reclassified as an MRT (21,23-25). WES did not

159 identify any additional known oncogenic variants.

160

161 Following 6 total cycles of chemotherapy, the patient experienced on therapy

162 progression in the lungs prompting removal from trial therapy. She subsequently

163 received empiric ifosfamide and etoposide alternating with vincristine,

164 doxorubicin, and cyclophosphamide for five cycles with minimal benefit, followed

165 by metastatecomy of four lung lesions. This was followed by high dose

166 chemotherapy with melphalan, carboplatin, etoposide, and autologous stem cell

167 rescue, whole lung (1050 cGy) and abdomen (1200 cGy) radiation, and repeat

168 gross total resection, but the patient ultimately succumbed to inexorable disease

169 progression.

170

171 *Increased XPO1 Activity in Patient's Tumor*

172 Due to the lack of targetable genomic alterations from tumor WES profiling, we

173 utilized the VIPER algorithm to infer protein activity from the transcriptome

174 profile. VIPER leverages cancer type-specific gene regulatory networks, such as

175 those constructed by ARACNe (Algorithm for the Reconstruction of Accurate

176 Cellular Networks), with sample-specific gene expression signatures. It infers

177 differential protein activity by using the expression of tens to hundreds of target

178 genes of each regulatory protein as a robust and sensitive reporter for its activity

179 (11,12,26). Because there was an insufficient number of MRTs profiled in the

180 TARGET database to generate an MRT-specific network, we utilized metaVIPER

181 (27), a recently published extension of VIPER that can effectively infer protein

182 activity by integrating the results of VIPER using all available cancer networks at

183 the protein level. metaVIPER analysis was used to computationally infer the

184 differential activity of 412 proteins currently classified as 'druggable' using the

185 patient's gene expression profile. This approach identified several proteins with

186 elevated inferred activity, including key regulators of actin cytoskeleton and cell

187 polarity (ROCK1/2), DNA damage response (ATM and ATR), cell signaling

188 (PIK3CA and BRAF), and protein trafficking (XPO1) (Fig. 1D and Supplementary

189 Table 1). XPO1 is a nuclear export protein that selectively transports tumor

190 suppressor and growth regulatory proteins, including protein 53 (P53),

191 retinoblastoma 1 (RB1), and cyclin-dependent kinase inhibitor 1B (CDKN1B,

192 p27^{Kip1}), out of the nucleus and inhibits their function (24,25). An emerging class

193 of small-molecule orally bioavailable XPO1 inhibitors, the selective inhibitors of

194 nuclear export (SINE), have already shown preclinical efficacy in several

195 hematologic and solid malignancies and recently gained FDA approval for the

196 treatment of multiple myeloma (28). Selinexor is currently in early phase trials in

197 pediatrics (29,30).

198

199 *Effects of Selinexor Treatment in Patient-Specific PDX Model*

200   A patient-derived xenograft (PDX) model (CUHEP-14531) was generated using

201   tumor material obtained from the patient's biopsy specimen and confirmed to

202   harbor the *SMARCB1* deletion (Supplementary Table 2). Cells harvested from

203   the PDX model were expanded and treated with selinexor *ex vivo* under short-

204   term culture conditions. Results from a dose-response assay revealed that cells

205   were highly sensitive to selinexor (IC_{50} of 5.2 nM 95% CI 4.2-6.3nM) (Fig. 1E) in

206   comparison to multiple myeloma cells (median IC_{50} 165 nM in 12 cell lines) for

207   which selinexor has been granted FDA approval (31). Additionally,

208   pharmacokinetic studies of selinexor have shown that the FDA-approved dose of

209   80 mg twice a week achieves a peak plasma concentration of 1-1.5 uM

210   suggesting *in vitro* sensitivity at clinically achievable levels (29,30).

211

212   We next assessed the *in vivo* efficacy of selinexor in a PDX model derived from

213   the case patient's tumor (CUHEP-14531). Treatment of mice with established

214   MRT tumors demonstrated tumor regression over the 25 days of treatment with

215   selinexor, however, the abrogation of tumor growth was dependent upon

216   sustained treatment (Fig. 1F). The magnitude of response to selinexor was

217   comparable to standard of care therapy with ifosfamide and etoposide (Fig. S1).

218   Unfortunately, the patient was unable to enroll in the selinexor pediatric trial

219   ADVL1414 (NCT02323880) due to body surface area requirements and lack of

220   oral suspension.

221

222   *Increased XPO1 Activity in MRT*

223   To evaluate the relevance of XPO1 as a target across MRTs, we performed

224   metaVIPER analysis on TCGA and selected TARGET tumor types (Fig 2A).

225   Notably, XPO1 was a significantly activated protein in 94% of MRTs (64 of 68

226   samples with FDR p-value < 0.01). Based on both within sample protein

227   normalized enrichment score (NES) (Fig 2A) and rank (Fig. S2A), the overall

228   XPO1 inferred activity in MRTs was higher compared to most other cancers.

229   Other tumors with high XPO1 inferred activity included Wilms tumor, testicular

230   germ cell tumors, esophageal carcinoma, and acute myeloid leukemia (AML)

231   (Figs. 2A, S2A). The median rank of XPO1 activity in MRTs was 6.5, making it

232   the third most frequently activated druggable protein across MRTs, behind DNA

233   topoisomerase II alpha (TOP2A) and dual specificity protein kinase TTK, a

234   protein associated with cell proliferation and chromosome alignment (Fig. S2B).

235   Additional recurrently activated therapeutic targets identified in MRTs include the

236   purine biosynthesis enzyme GART, the kinesin-like spindle protein KIF11, Haspin

237   (GSG2), and DNMT3B (Fig. S2B). Unlike most other tumor types, in which XPO1

238   activity was distributed over a wide range of values, MRTs have a relatively high

239   and uniform XPO1 inferred activity (Fig. 2A: IQR for NES 4.59 to 6.08).

240

241   To gain insight into whether genetic disruption of the SWI/SNF complex is

242   associated with aberrant XPO1 activity, we performed co-segregation analysis

243   between genomic events, i.e., somatic single nucleotide variants (SNVs) and

244   copy number variants (CNVs), and XPO1 activity using 33 cancer cohorts in

245   TCGA (N=8,348 tumors). Across this pan-cancer atlas, XPO1 activity was found

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246 to co-segregate with SNVs (p=0.00016) and deletions (p=0.00052) in genes

247 encoding the SWI/SNF complex subunits (Fig. 2B). Notably, XPO1 activation

248 occurs in association with mutations in SWI/SNF complex "core" subunits,

249 including SMARCB1, plus the AT-rich DNA binding subunits ARID1A and B, but

250 not with accessory subunits (Fig. 2B) (8), suggesting that a broader correlation

251 exists between loss of SWI/SNF function and increased XPO1 activity.

252

253 *MRT/ATRT cell lines demonstrate XPO1 activation and differential sensitivity to*

254 *selinexor*

255

256 To experimentally validate the *in silico* results above, we assembled a panel of

257 MRT and AT/RT cell lines and utilized metaVIPER analysis to compare their

258 XPO1 inferred activity to reference cell lines profiled in the Cancer Cell Line

259 Encyclopedia (CCLE). Our analysis confirmed high XPO1 activity (Fig. S2C) in

260 the selected cell lines, indicating that MRT/ATRT cell lines recapitulated the

261 XPO1 activation observed in patient tumors. Protein expression of XPO1 was

262 variable across a range of MRT/ATRT and other cancer cell lines and had overall

263 higher expression compared to selected non-cancer cell lines (Fig. S2D). As

264 expected, all MRT/ATRT cell lines were SMARCB1 negative, while the non-

265 MRTs expressed SMARCB1 (Fig. S2D).

266

267 *In vitro* sensitivity to selinexor was then evaluated across the same panel of

268 MRT/ATRT cell lines. The median IC_{50} for MRT cell lines following 72 hour

11

269   treatment with selinexor was 200 nM (IQR 170-440 nM) for MRTs, 460 nM (IQR

270   400 nM-1.4 uM) for ATRTs, and 1.1 uM (IQR 580 nM-1.4 uM) for 5 non-MRT cell

271   lines (Fig. 2C), demonstrating differential sensitivity of MRT cell lines to XPO1

272   inhibition compared to non-MRT cell lines. Next, we generated a tumor xenograft

273   MRT model utilizing the G401 established MRT cell line. Treatment with

274   selinexor over a period of 21 days significantly inhibited tumor growth (TGI=

275   71%) (p=0.0002) (Fig. 2D), confirming the sensitivity of MRT cells to selinexor *in*

276   *vivo.*

277

278   *Selinexor inhibits XPO1 activity and induces cell cycle arrest and apoptosis*

279   To gain insight on the mechanism of action of selinexor we treated selected MRT

280   cell lines with vehicle (DMSO) or with a sublethal concentration of selinexor (30

281   nM) and performed post-perturbation RNAseq profiling and metaVIPER analysis

282   to evaluate differential protein activity induced by the drug. This analysis

283   demonstrated a predictable and significant decrease in XPO1 activity that

284   became more profound between 6 and 24 hours in all four cell lines, and, in turn,

285   a compensatory increase in XPO1 mRNA abundance (Fig 3A). In addition to

286   confirming that the analysis is sufficiently sensitive to infer changes in XPO1

287   activity as a consequence of pharmacologic inhibition, it also highlights other

288   potential mechanistic targets of selinexor (Fig. 3A, Fig. S3A). Next, we used the

289   differential protein activity signature computed by metaVIPER and ran pathway

290   enrichment analysis to determine which cancer hallmark pathways were most

291   significantly impacted at this sublethal concentration of selinexor. We observed a

12

292 marked downregulation in pathways involving cell cycle progression (E2F

293 targets, G2M checkpoint), downregulation in the MYC pathway, and an increase

294 in epithelial-mesenchymal transition, TNF-alpha signaling, and KRAS signaling

295 (Fig. 3B).

296

297 In addition, we examined changes in the inferred activity of other druggable

298 proteins whose activity strongly co-segregated with XPO1 in MRTs, including

299 several epigenetic regulators such as DNMT1, EZH2, KDM1A, EP300, and

300 BRD4, as well as several proteins involved in DNA repair (Fig. S3B).

301 Interestingly, inhibiting XPO1 led to a loss of activity in the majority of these

302 proteins suggesting a functional interdependency. Alternatively, there was

303 increased activity of BRAF, the Rho-associated kinases ROCK1/ROCK2, and

304 cereblon (CRBN), the target of lenalidomide in the E3-ubiquitin ligase pathway

305 (Fig. S3B).

306

307 Consistent with the observed gene expression/protein activity changes, selinexor

308 treatment of MRT/ATRT cell lines *in vitro* demonstrated induction of apoptosis at

309 various levels in most cells tested (Fig. 3C) and cell cycle arrest with a decrease

310 in the percentage of cells in S phase and reciprocal increase in the percentage of

311 cells in G1 and G2/M phase (Fig. 3D). We then evaluated the protein levels and

312 the cellular localization of XPO1 and XPO1 target proteins mediating cell cycle

313 and apoptosis. Analysis of protein expression following selinexor treatment in 4

314 MRT cell lines showed decreased XPO1 expression (Fig. 3E). We also observed

13

315   decreases in phosphorylation of RB (Fig. 3E), confirming that selinexor alters the

316   expression of major cell cycle regulators. In parallel, we observed increased

317   levels of cleaved PARP, consistent with an induction of apoptosis in MRT cells

318   upon treatment with selinexor (Fig. 3E). Subcellular localization of XPO1 targets

319   following selinexor treatment showed increased nuclear sequestration of

320   mediators of cell cycle arrest p53, p27, and p21 (Fig. S3C), confirming that

321   selinexor blocked the ability of XPO1 to shuttle its targets across the nuclear

322   membrane.

323

324   *Effects of Selinexor Treatment in PDX MRT Model*

325   Although cell lines were used to evaluate selinexor sensitivity of MRT *in vitro*, the

326   mutational analysis performed using MSK-IMPACT (32), our institutional next-

327   generation sequencing targeted panel, showed that most cell lines, including

328   G401, harbored mutations in *TP53*, which are not common in rhabdoid patient

329   tumors (Supplemental Table 2). Therefore, we assessed the *in vivo* effects of

330   selinexor in a second MRT PDX model. Model MSKMRT-31222 was generated

331   from primary tumor tissue obtained from the resection of a pelvic soft tissue mass

332   in a 16 year old girl with treatment-refractory MRT. The model had absence of

333   INI1 staining by IHC, and genomic characterization confirmed homozygous

334   deletion of 22q11.23 involving *SMARCB1* with resultant loss of mRNA

335   expression, but no mutations in *TP53* (Supplementary Table 2).

336

337   Treatment of MSKMRT-31222 PDX tumors with selinexor demonstrated an anti-

338   tumor effect with stabilization of tumor volume (Fig. 4A). Similar to *in vitro*

339   treatment of MRT cell lines, analysis of CUHEP-14531 PDX tumors collected

340   after one week of selinexor treatment showed decreased XPO1, RB1-pSer780,

341   and c-MYC (Fig. 4B) consistent with the anti-proliferative response observed *in*

342   *vitro*. MSKMRT-31222 PDX tumors showed only a significant decrease in c-MYC

343   (Fig. 4C). Interestingly, while the CUHEP-14531 tumors also demonstrated

344   increased p53, p27, and p21 expression, we could not detect the expression of

345   p53 by immunoblotting in MSKMRT-31222 tumors (data not shown), although

346   *TP53* was not deleted or mutated based on MSK-IMPACT analysis.

347   Immunohistochemical analysis of MSKMRT-31222 treated mice showed

348   significantly decreased Ki67 staining (Fig. 4D) confirming selinexor's primary

349   effect on cell proliferation. Notably, the durability of anti-tumor response was

350   dependent upon continued treatment with selinexor since tumor regrowth was

351   observed upon discontinuation of treatment in both models as confirmed by the

352   increase in the percentage of Ki67 positive cells following treatment interruption

353   in the MSKMRT-31222 model (Fig. 1F and 4A,D). Nonetheless, we observe

354   consistent abrogation of tumor growth in MRT models *in vivo* through XPO1

355   inhibition.

356

357   **Discussion:**

358   The lack of a standard approach for the treatment of recurrent or progressive

359   disease in MRT and other childhood tumors represents a significant challenge to

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360 oncologists. In pediatrics, this challenge is made even more difficult by the

361 paucity of targetable mutations despite increasing access to various next-

362 generation genomic profiling technologies. Hence, novel methodologies are

363 required to interrogate genomic data for non-genetically encoded therapeutic

364 targets. We report on the application of a systems biology approach for

365 identifying non-genetically encoded vulnerabilities in a genomically quiescent

366 cancer within the context of a pediatric precision medicine program. Furthermore,

367 we describe the patient-driven discovery of XPO1 as a novel MRT vulnerability

368 and demonstrate the potential utility of XPO1 inferred activity as a biomarker of

369 response to XPO1 inhibition to identify additional disease indications that would

370 not otherwise be considered for treatment with an XPO1 inhibitor. This

371 experience highlights that although a patient-centric N-of-1 approach begins by

372 focusing on a single patient, the knowledge gained can drive novel discoveries

373 with impact beyond the individual patient.

374

375 Partnering an N-of-1 approach with the use of metaVIPER to identify aberrantly

376 activated, druggable proteins offers a novel strategy for precision oncology

377 therapeutics in pediatrics (14). The current approach of identifying and matching

378 targetable mutations with molecularly-targeted drugs (e.g. NCI-MATCH) have

379 resulted in tepid preliminary results (33). Furthermore, while the FDA has

380 approved over 115 targeted antineoplastic drugs over the past two decades,

381 there are only 12 unique genomic alterations linked as companion diagnostics to

382 these drugs (28). MetaVIPER infers the relative activity of over 6,000 proteins,

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383 including ~400 currently druggable proteins. This allows for the identification not

384 only of proteins that are directly activated by a genomic alteration, but also of

385 proteins that become highly activated as the result of a complex interplay of

386 expression, protein-protein interactions, and post-translational modifications.

387

388 Using the metaVIPER algorithm, we have identified XPO1 as a highly active

389 protein in MRT and demonstrated that targeting XPO1 through the use of

390 selinexor is efficacious in preclinical models of MRT. We found sub-micromolar

391 IC_{50} values for selinexor across a panel of MRT human cell lines and evidence of

392 cell cycle arrest and apoptosis. *In vivo* treatment of MRT PDX models with

393 selinexor further confirmed a therapeutic response to selinexor, although the

394 extent of the response varied across models.

395

396 Selinexor has demonstrated efficacy in patients with refractory hematologic

397 malignancies, as well as those with advanced or metastatic solid tumors

398 (29,30,34). It is currently being evaluated in adult trials for the treatment of a

399 range of cancers including AML, sarcoma, and other solid tumors, and recently

400 garnered FDA accelerated approval for the treatment of multiple myeloma and

401 diffuse large B-cell lymphoma (DLBCL) (28). Notably, our analysis showed that

402 AML, DLBCL, and multiple myeloma have high XPO1 inferred activity, though not

403 as uniformly high as in MRT, supporting the idea that high XPO1 activity may

404 predict response to selinexor. In a multiple myeloma trial of selinexor and

405 dexamethasone, metaVIPER was used with a linear discriminant analysis

406   classifier that identified a four-protein classifier with high predictive performance

407   of patient response to selinexor while using differential gene-expression data did

408   not produce an effective classifier suggesting another potential use of

409   metaVIPER (35).   Selinexor is also being studied in a phase 1 pediatric trial in

410   refractory solid tumors and brain tumors (NCT02323880) and is tolerable in

411   combination with fludarabine and cytarabine in a pediatric phase 1 trial of

412   relapsed or refractory acute leukemia (36). This data on the safety and efficacy in

413   children will be helpful for informing future trials in patients with MRT.

414

415   Our case illustrates that MRTs are aggressive pediatric malignancies with poor

416   survival rates despite the use of multimodal chemotherapeutic, radiotherapeutic

417   and surgical interventions. Furthermore, intensification of standard therapy

418   approaches has not improved prognosis highlighting the need for more effective

419   therapeutic approaches. Agents targeting downstream effectors of *SMARCB1*

420   loss are currently under clinical investigation for MRTs. The CDK4/6 inhibitor

421   ribociclib previously demonstrated stable disease in several ATRT patients (37)

422   and is currently under investigation in a phase 1 trial in combination with

423   everolimus in pediatric patients with recurrent or refractory ATRT or other

424   malignant brain tumors (NCT03387020). The Aurora-A kinase (AURKA) inhibitor

425   alisertib is currently being studied in a phase 2 trial in pediatric patients with

426   ATRT or MRT (NCT02114229). The EZH2 inhibitor tazemetostat is currently in

427   early phase trials (NCT02601937 and NCT03213665) including the Pediatric

428   MATCH treatment arm for patients with relapsed or refractory *SMARCB1*-deleted

18

429 tumors, and recently gained FDA approval to treat epithelioid sarcoma (38). It

430 has been previously established that *SMARCB1* mutant MRTs are dependent on

431 the enzymatic activity of EZH2 and that EZH2 inhibition induced tumor regression

432 in pre-clinical models (39). We show that EZH2 inferred activity positively co-

433 segregates with XPO1 activity in MRTs. However, while EZH2 activity was

434 generally increased in MRT, it was not increased to the same extent, and not as

435 universally as XPO1, ranking as the 17th most dysregulated druggable protein

436 across MRTs compared to 3rd for XPO1 (Fig. S2B and Supplementary Table 3).

437 Interestingly, we found that selinexor treatment not only inhibited XPO1 activity,

438 but also significantly decreased the inferred activity of EZH2 (Fig. S3B),

439 suggesting that part of selinexor's effects may be due to reduced EZH2 activity,

440 in addition to its effects on cell cycle regulators.

441

442 Although selinexor can induce significant anti-tumor responses *in vivo*,

443 therapeutic responses were dependent upon sustained drug exposure,

444 suggesting that combination therapies incorporating selinexor with additional

445 agents may be needed to obtain long-term durable responses. Pre-clinical

446 studies of AML show a potential benefit of selinexor in combination with

447 topoisomerase II inhibitors due to increased nuclear retention of topoisomerase II

448 following XPO1 inhibition (40). Furthermore, selinexor treatment was able to

449 restore TOP2A nuclear localization even in cells that were resistant to

450 topoisomerase inhibitor due to aberrant nuclear export and cytoplasmic

451 localization of TOP2A (41). The topoisomerase II inhibitor, etoposide, may

19

452 therefore be a viable agent for combination with selinexor since it is a principal

453 component of MRT therapy and TOP2A is identified by metaVIPER analysis to

454 be the most activated protein across MRTs in the TARGET cohort. If selinexor

455 demonstrates clinical activity in MRT, a precision medicine approach that couples

456 selinexor with drugs that target proteins uniquely activated in subsets of MRT

457 may also be a viable next step in developing rational combinations that may exert

458 a more durable therapeutic response. In addition, the finding that selinexor

459 treatment increased the activity of particular druggable proteins, including BRAF,

460 PIK3CA, and cereblon, suggests potential targets that can be tested in

461 combination with selinexor to improve response.

462

463 In conclusion, we identify XPO1 as a high aberrantly activated protein in MRT

464 and a promising non-genetically encoded therapeutic target. These findings

465 demonstrate the value of metaVIPER analysis in tumors lacking actionable

466 oncogenic alterations to identify potential therapeutic targets and biomarkers of

467 response and illustrate the impact of an N-of-1 approach in identifying viable

468 therapeutic strategies for genomically quiescent tumors beyond the single

469 patient.

470 XPO1 inhibition as a promising therapeutic strategy for the treatment of MRT will

471 be explored further in the context of a clinical trial of selinexor in pediatric

472 patients with MRT or Wilms tumor, another pediatric tumor demonstrated to have

473 high inferred XPO1 activity.

474

Methods:

Cell Culture

1. Rhabdoid cell lines G401 (ECACC Cat# 87042204, RRID:CVCL_0270) and A204 (ATCC Cat# HTB-82, RRID:CVCL_1058) were obtained from the American Type Culture Collection (ATCC). Rhabdoid cell lines MP-MRT-AN (RRID:CVCL_7049), KP-MRT-NS (RRID:CVCL_7050), KP-MRT-RY (RRID:CVCL_7051), and KP-MRT-YM (RRID:CVCL_7052) were obtained courtesy of Dr. H. Hosoi (42-45). The ATRT cell line CHLA-266 (RRID:CVCL_M149) was obtained from the COG cell line repository. Cell lines were authenticated using STR profiling. Cells were cultured in DMEM with 10% FBS and 1% Anti-Anti (Thermo Fisher Scientific). All cells were tested for *Mycoplasma* approximately once every 4 months (last performed May 2018) (46).

Cell viability

Cells (2,500 cells/well) were seeded in 96-well plates and after 24 hours were incubated with selinexor (Selleckchem) for 72 hours. The viability of the cells was determined using CellTiter-Glo Luminescent Cell Viability Assay (Promega) according to the manufacturer's recommendations. IC_{50} concentrations were determined using GraphPad Prism Software Version 7.0.

Cell cycle assay

Cells were treated with 400 nM selinexor for 48 hours, fixed in 70% ethanol and stored at 4° C for 24 hours. Cells were then washed and stained in 1 x PBS

498   containing 50 µg/ml RNase H (Sigma Aldrich) and 50 µg/ml propidium iodide (PI)

499   (Sigma Aldrich), followed by flow cytometric analysis.

500

501   *Apoptosis assay*

502   Cells were treated with 200 nM selinexor for 48 hours. Apoptosis of cells was

503   determined using Caspase-Glo 3/7 Assay (Promega) according to the

504   manufacturer's recommendations.

505

506   *Immunoblotting*

507   Cells and PDX tumor samples were resuspended in 1% SDS-RIPA Buffer

508   (Sigma Aldrich). Tissues were disrupted and homogenized with a Qiagen

509   Bioruptor for 2 minutes at 50Hz (2 rounds). Protein concentrations were

510   determined using the BCA Protein Assay Kit (Pierce) according to manufacturer's

511   recommendations. The isolated protein samples were separated on 4-12% SDS-

512   PAGE gels and were subsequently transferred to a nitrocellulose membrane

513   using the iBlot Gel Transfer Device and iBlot Gel Transfer Stacks (Thermo

514   Scientific). The blots were developed using LI-COR secondary antibodies

515   (680RD and 800CW). Antibodies are listed in supplementary materials and

516   methods

517

518   *Ethics approval and consent to participate*

519   Informed and signed consent was obtained and archived for the research

520   performed and publication of the results. Memorial Sloan Kettering Cancer

22

521 Center (MSKCC) patients were enrolled onto the MSKCC targeted gene

522 sequencing research study [Genomic profiling in cancer patients; NCT01775072]

523 with approval from the MSKCC Institutional Review Board under protocol IRB#

524 12-245, #06-107, and #17-387. For xenografts generated at Columbia University

525 Medical Center, the patient was consented for clinical sequencing at Columbia

526 University Medical Center (CUMC) through the Precision in Pediatric Sequencing

527 Program (PIPseq) and PDX tumor mouse model generation under the CUMC

528 Institutional Review Board (IRB)-approved protocol AAAN8404.

529

530 *Animal Studies*

531 All experiments were performed in accordance with institutional guidelines and

532 under an approved protocol from the CUMC Institutional Animal Care and Use

533 Committee (Protocol AAAF5850) or the MSKCC Institutional Animal Care and

534 Use Committee (#16-08-011).

535

536 *Patient-derived xenograft and xenograft generation*

537 The patient-derived xenograft (PDX) models (CUHEP-14531 and MSKMRT-

538 31222) were developed by transplanting a 2 mm fragment from a patient's biopsy

539 specimen into the flank of NOD (NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ) SCID gamma

540 (NSG) mice to generate the passage 0 (P0) generation. Tumors were serially

541 propagated and expanded from the PDX P0 generation for therapeutic studies.

542 The G401 cell line xenograft models were developed by injecting 2 million G401

543 cells into the flank of NSG mice. Tumor growth was measured biweekly using

23

544   calipers. Treatment started when flank tumors achieved a tumor volume (TV) of ~

545   150 mm^3 (TV = width^2 * length/2). PDX models were dosed with selinexor by oral

546   gavage three times a week at a starting dose of 15 mg/kg which escalated to 20

547   mg/kg in the second week. Tumors were collected and fragments were either

548   fixed in 10% formalin for histologic analysis or snap frozen in liquid nitrogen for

549   subsequent DNA, RNA, and protein isolation and analyses.

550

551   *Immunofluorescence*

552   Immunofluorescence staining was performed in the Molecular Cytology Core

553   Facility of MSKCC using the Discovery XT processor (Ventana Medical

554   Systems). The tissue sections were deparaffinized with EZPrep buffer (Ventana

555   Medical Systems), and antigen retrieval was performed with CC1 buffer (Ventana

556   Medical Systems). Sections were blocked for 30 minutes with Background Buster

557   solution (Innovex), followed by avidin-biotin blocking for 8 minutes (Ventana

558   Medical Systems). Sections were incubated with human-specific anti-Ki67

559   (DAKO, cat# M7240) or human specific anti-ClCaspase3 (Cell Signaling, cat#

560   9661), followed by a 60 minute incubation with biotinylated horse anti-mouse IgG

561   (Vector labs, cat# MKB-22258) or biotinylated goat anti-rabbit (Vector Labs, cat#

562   PK6101) respectively. The detection was performed with Streptavidin-HRP D

563   (part of DABMap kit, Ventana Medical Systems), followed by incubation with

564   Tyramide Alexa Fluor 568 (Invitrogen, cat# T20949) prepared according to

565   manufacturer's instructions with predetermined dilutions. After staining, all slides

24

566   were counterstained with DAPI (Sigma Aldrich, cat# D9542) for 10 min and

567   cover-slipped with Mowiol (Sigma Aldrich, cat# 81381).

568

569   *Nucleic acid extraction, clinical sequencing, and analysis*

570   DNA from frozen tissue or paraffin embedded tissue was extracted using the

571   QIAGEN QIAamp Tissue Kit (for tissue samples) on the QIAcube system. RNA

572   was extracted using the QIAGEN RNeasy Kit (fresh frozen tissue). All slides

573   were evaluated by a pathologist to ensure that a minimum of 50 % viable tumor

574   was present for subsequent extraction and analyses. Whole exome sequencing

575   (WES) was performed using the Agilent SureSelectXT All Exon V5 + UTRs

576   capture kit for library generation and sequenced on the HiSeq 2500 System

577   (Illumina), using paired-end 100 cycle × 2 sequencing. RNA was sequenced

578   using the TruSeq Stranded Total RNA LT Sample Prep Kit (Illumina), with 100

579   cycles × 2 paired-end sequencing on the HiSeq 2500. DNA sequencing reads

580   were de-multiplexed and converted to FASTQ files using CASAVA from Illumina.

581   Following mapping and variant calling of both tumor and normal samples by

582   NextGENe, resulting variants were subject to filtering. Variants in normal DNA

583   were passed through a "reference range filter" of cancer predisposition genes,

584   genes relevant to pharmacogenomics, and variants relevant to patient care; a

585   "reportable range filter" which includes COSMIC variants in the patient's mutation

586   report file and variants in genes on the list of ACMG (American College of

587   Medical Genetics and Genomics) recommendations for reporting of secondary

588   findings; as well as a frequency filter. Memorial Sloan Kettering-Integrated

25

589 Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) testing was

590 performed as described previously (32).

591

592 *MetaVIPER Analysis*

593 All regulatory networks used for metaVIPER analysis were reverse engineered

594 by ARACNe (26). Twenty four core TCGA RNA-Seq derived networks are

595 publicly available in the R Bioconductor package ARACNe networks. In the case

596 of signaling proteins, ARACNe detects maximum information path targets to

597 define its set of "indirectly regulated" target genes.

598

599 After standard read alignment of RNA-Seq data by STAR (47) to the GRCh38

600 reference genome build and summarization of expression quantities at the gene

601 count level, gene expression was normalized by variance stabilization. The

602 stabilization parameters are stored from a single run of the Variance Stabilizing

603 Transformation (VST), as implemented in the DESeq2 package (48), across all

604 TCGA and TARGET samples and these parameters are then applied to

605 individual samples. In the case of novel samples, post-normalization gene

606 expression profiles are compared between the novel samples and tissue-relevant

607 samples from TCGA/TARGET through methods such as principal component

608 analysis and t-SNE to confirm the absence of any significant batch effects that

609 may be attributed to instrument/library-specific biases. In the case of cell line

610 samples, an analogous procedure is performed through the initial application of

611 VST to all CCLE samples.

612

613   For the purposes of computing differential protein activity of TARGET MRTs, a

614   gene expression signature (GES) was computed between each MRT versus a

615   pan-cancer reference group that includes gene expression profiles of ~12,000

616   tumors belonging to 33 cancer types, followed by application of the analytic

617   Rank-based enrichment analysis (aREA) using each of the available networks

618   (11,12,27). As VIPER is a rank based enrichment method, GES's are generated

619   through a double-rank transformation process. First, the normalized expression

620   of each gene in the sample is ranked relative to the distribution of the expression

621   of this gene across all TCGA and TARGET samples. Expression distributions for

622   all genes have been fitted to a spline-model to ensure comparable ranks. Next,

623   genes within a sample are ranked based on their relative expressions. Following

624   generation of a GES, aREA tests for a global shift in the positions of a regulatory

625   protein's target genes in the rank-sorted GES and is an extension of other efforts

626   at computationally efficient method to approximate GSEA (11,49). aREA uses the

627   mean of the rank-transformed positions as the test statistic (enrichment score).

628   The enrichment score is computed first by a 1-tail approach, rank-transforming

629   the absolute values of the GES and secondly by a 2-tail approach, where the

630   positions of the repressed target genes (as inferred in the network) are inverted

631   in the gene expression signature before computing the enrichment score. The

632   contribution of each target gene to the enrichment score is also weighted based

633   on the regulator-target gene mutual information computed by ARACNe. In the

634   final step of aREA, in the case of single sample analysis, statistical significance

635   for the enrichment score is computed by an analytic approach that approximates

636   shuffling the genes in the signature at random, outputting a normalized

637   enrichment score (NES). Gene shuffling is approximated as follows: according to

638   the central limit theorem, the mean of a sufficiently large number of independent

639   random variables is ~normally distributed. We ensure a mean of zero and

640   variance equal to one for the enrichment score under the null hypothesis by

641   applying a quantile transformation based on the normal distribution to the rank-

642   transformed GES before computing the enrichment score. Then, under the null

643   hypothesis, the enrichment score will be normally distributed with mean of zero

644   and variance n^{-1}, where n is the regulon size, which is further scaled to the

645   standard variable. In the single sample analysis, aREA cannot directly account

646   for biological correlation between the expression of various genes in the given

647   biological context, and so conservative NES/p-value thresholds are empirically

648   used with the assumption that this approach underestimates the p-value. In the

649   final step of metaVIPER analysis, NES's generated by the application of VIPER

650   using each of the available networks are integrated at the individual regulatory

651   protein level by a weighted average, with greater absolute NES's contributing a

652   greater weight to the final integrated score to maximize the contribution of signal

653   over noise, as bench marked in (27). As noted, alternative methods including a

654   simple average of NES scores appear to provide an almost equivalent result.

655

656   Similarly, metaVIPER was used to infer differential protein activity between

657   samples from each of four selinexor treated MRT/ATRT cell lines and

658 corresponding DMSO treated controls. As these experiments were done in

659 replicate, the GES is computed through gene-wise modified student t-test

660 between condition and control. Pathway analysis on an integrated differential

661 protein activity signature in MRT/ATRT cell lines was performed with 'Cancer

662 Hallmark', 'Gene Ontology', and 'Oncogenic Signature' gene sets provided in the

663 Broad MSigDB collections (50). Pathway enrichment analysis was performed by

664 using the single-tail aREA method described above as a rapid approximation to

665 the KS-test used in GSEA, inputting the pathway genes and the sorted

666 differential protein activity signature. Unlike other statistical tests used for

667 pathway enrichment analysis, such as the Fisher's exact test on a thresholded

668 list of differentially expressed genes or the repeated application of the

669 Kolmogorov-Smirnov test in classical GSEA, aREA does not require the arbitrary

670 binarization into significant/non-significant hits for target genes in the signature

671 but takes into account their relative position in the signature.

672

673 *Co-Segregation Analysis of XPO1 Activity*

674 In order to identify potential genomic events that causally or incidentally

675 associate with increased XPO1 activity, a co-segregation analysis was performed

676 to determine somatic events that are enriched in tumors that are inferred to have

677 high XPO1 activity by metaVIPER across 33 TCGA cancer type cohorts. Publicly

678 available somatic SNV and CNV (GISTIC) calls from the Broad TCGA Genome

679 Data Analysis Center (GDAC) Firehose were downloaded in order to perform this

680 analysis. Enrichment analysis by aREA was performed by arranging tumors in

29

681 order of lowest to highest XPO1 activity; a null model derived from enrichment

682 analysis of each genomic event with all proteins that metaVIPER infers activity

683 on was developed to estimate the statistical significance of the co-segregation.

684 Analogously, the co-segregation analysis was performed between increased

685 XPO1 activity and the activity of about 411 other 'druggable' proteins in both the

686 TARGET malignant rhabdoid tumor dataset and the TCGA datasets to develop

687 hypotheses on biologically relevant protein interactions and potential synergistic

688 drug combinations to treat tumors with elevated XPO1 activity.

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Legend

Figure 1: Clinical presentation, MetaVIPER analysis, and sensitivity to small molecule inhibitor selinexor in patient-derived pre-clinical model. **A)** Diagnostic imaging demonstrating the presence of a large hepatic tumor. **B)** Hepatic tumor biopsy with H&E stain demonstrates small cell morphology. 600x **C)** INI1 immunohistochemistry stain highlights an entrapped bile duct (positive internal control), tumor cells are negative. 600x **D)** MetaVIPER inferred activity of all 'druggable' proteins in the patient tumor biopsy from which the CUHEP-14531 PDX model was developed. The inset to the right highlights the top 8 most aberrantly activated targets. **E)** IC_{50} of MRT cells cultured from CUHEP-14531 PDX and treated with selinexor for 72 hours. **F)** Tumor response in CUHEP-14531 PDX (n=8 per arm) treated with selinexor.

Figure 2: MetaVIPER inference of XPO1 activity and sensitivity of rhabdoid cell lines to the XPO1 small molecule inhibitor selinexor. **A)** Boxplots representing the distribution of metaVIPER inferred XPO1 activity for 33 TCGA tumor cohorts, 3 TARGET pediatric tumor cohorts (MRT, Wilms, osteosarcoma), and a neuroendocrine tumor cohort. The median and interquartile range for NES values is represented by each box for the respective tumor cohort. NES values from enrichment analysis are comparable to Z-scores, with higher scores representing increased activity. **B)** Co-segregation of SNV and CNV events in SWI/SNF complex genes with XPO1 activity. Top: Barplot of XPO1 activity in each TCGA tumor. Middle: SNVs in SWI/SNF complex genes. SNVs in core subunits, such as SMARCA4 and SMARCB1, and ARID1B most strongly co-segregate with XPO1 activation. Estimated p-values from enrichment analysis are to the right. Bottom: CNVs are far more common than SNVs in SWI/SNF complex genes. Heterozygous (light blue) and homozygous (dark blue) deletions in several SWI/SNF complex genes co-segregate with increased XPO1 activity, as do amplifications (red) in ACTL6A. However, SWI/SNF deletions do not universally correspond with high XPO1 activity, as can be seen in several tumors towards the left of this plot. **C)** Dot plot showing IC_{50} of selinexor in MRT and ATRT vs non-MRT cell lines following 72 hours of treatment. **D)** Tumor response in G401 mouse xenograft (n=8 per arm) treated with selinexor. TCGA – the Cancer Genome Atlas; TARGET – Therapeutically Applicable Research to Generate Effective Treatments; NES – normalized enrichment score. MRT – malignant rhabdoid tumor; ATRT – atypical teratoid/rhabdoid tumor.

Figure 3: Pharmacodynamic analysis of selinexor in rhabdoid tumor cell lines using next generation sequencing, flow cytometry, and western blot. **A)** RNA-Seq was performed on four rhabdoid cell lines at baseline and after treatment with selinexor 30 nM at 6 and 24 hours. Changes in mRNA abundance positively correlate with MetaVIPER inferred activity at 24 hours, but XPO1 is amongst a small group of proteins (inset) whose activity is markedly decreased by selinexor in spite of significant compensatory increase in mRNA. **B)** The differential protein

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935 activity signature induced by selinexor—i.e., the inferred change in protein
936 activity of about 6000 proteins including XPO1, was further analyzed using
937 pathway enrichment analysis on the cancer hallmarks set from MSigDB. The
938 pathways that are most significantly deactivated are in blue, while pathways with
939 significantly increased activity after selinexor perturbation are in red. **C)**
940 Apoptosis assay of cell lines treated with DMSO or 200 nM selinexor for 48
941 hours. **D)** Cell cycle analysis across MRT/ATRT cell lines treated with DMSO or
942 400 nM selinexor for 48 hours showing cell cycle arrest with a decreased
943 percentage of cells in S phase and increased in G1 and G2/M. **E)** Immunoblot
944 showing expression levels of the indicated proteins in G401, A204, KPMRT-NS
945 and KPMRT-RY cell lines treated with selinexor at varying concentrations for 48
946 hours. Mean and standard deviation of three independent experiments is shown
947 in (C) and (D). *=p<0.05, **=p<0.01, ***=p<0.001

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949 **Figure 4**: MRT PDX models are sensitive to selinexor *in vivo*. **A)** Tumor
950 response in MSKMRT-31222 PDX (n=8 per arm) treated with selinexor. **B)**
951 CUHEP-14531 and **C)** MSKMRT-31222 immunoblot showing the expression
952 levels of indicated proteins in PDX tumor harvested 3 days following initiation of
953 selinexor or vehicle. Each lane represents tumor from one mouse. Protein
954 expression quantitated by densitometry expressed relative to alpha-tubulin and
955 normalized to vehicle control. (**D)** Immunohistochemistry of PDX tumor following
956 treatment with selinexor evaluating Ki67 staining.

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958 **Supplementary Figure 1**: Tumor response of a malignant rhabdoid tumor (MRT)
959 PDX (CUHEP-14531) treated with ifosfamide/etoposide for 15 days.

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961 **Supplementary Figure 2:**
962 **A)** Boxplots representing the distribution of rank of XPO1 activity relative to 412
963 'druggable' proteins for which metaVIPER infers activity for 33 TCGA tumor
964 cohorts, 3 TARGET pediatric tumor cohorts (MRT, Wilms, osteosarcoma), and a
965 neuroendocrine tumor cohort. **B)** Boxplots representing inferred activity of the
966 top 30 'druggable' proteins in 68 MRTs in TARGET. **C)** Boxplots representing the
967 distribution of metaVIPER inferred XPO1 activity in a cohort of nine rhabdoid
968 (MRT and ATRT) cell lines compared to 27 cohorts of cancer cell lines profiled in
969 the publicly available CCLE. The median and interquartile range for NES values
970 is represented by each box for the respective cell line cohort. NES values from
971 enrichment analysis are comparable to Z-scores, with higher scores representing
972 increased activity. **D)** Protein expression of XPO1 and SMARCB1 across MRT,
973 ATRT, and non-MRT cell lines.

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975 **Supplementary Figure 3:**
976 **A)** RNA-Seq was performed on four rhabdoid cell lines at baseline and after
977 treatment with selinexor 30 nM at 6 and 24 hours. Changes in mRNA abundance
978 positively correlate with MetaVIPER inferred activity at 24 hours in each cell line,
979 but XPO1 is amongst a small group of proteins (labeled) whose activity is
980 markedly decreased by selinexor in spite of significant compensatory increase in

mRNA. The data represented in Figure 3A is summarized across all four cell lines shown independently here. **B)** Co-segregation analysis on protein activity in 68 rhabdoid tumors from TARGET. Rhabdoid tumors with low to high XPO1 activity are organized from left to right. Dark red boxes represent significant (FDR p <0.01) activation of the respective protein. Selinexor treatment decreases the activity of the majority of proteins whose activity co-segregates with elevated XPO1 activity. **C)** Immunoblot showing expression levels and subcellular localization of the indicated proteins in G401, A204, KPMRT-NS, and KPMRT-RY cells following selinexor treatment.

Supplementary Table 1: MetaVIPER inferred activity of 412 'druggable' proteins in the patient tumor biopsy from which the CUHEP-14531 PDX model was developed.

Supplementary Table 2: MSK-IMPACT results of MRT cell lines and PDX samples.

Supplementary Table 3: MetaVIPER inferred activity of 412 'druggable' proteins in MRT. The mean rank position of XPO1 activity compared to all druggable proteins amongst the 68 rhabdoid tumors in TARGET is provided in the table.
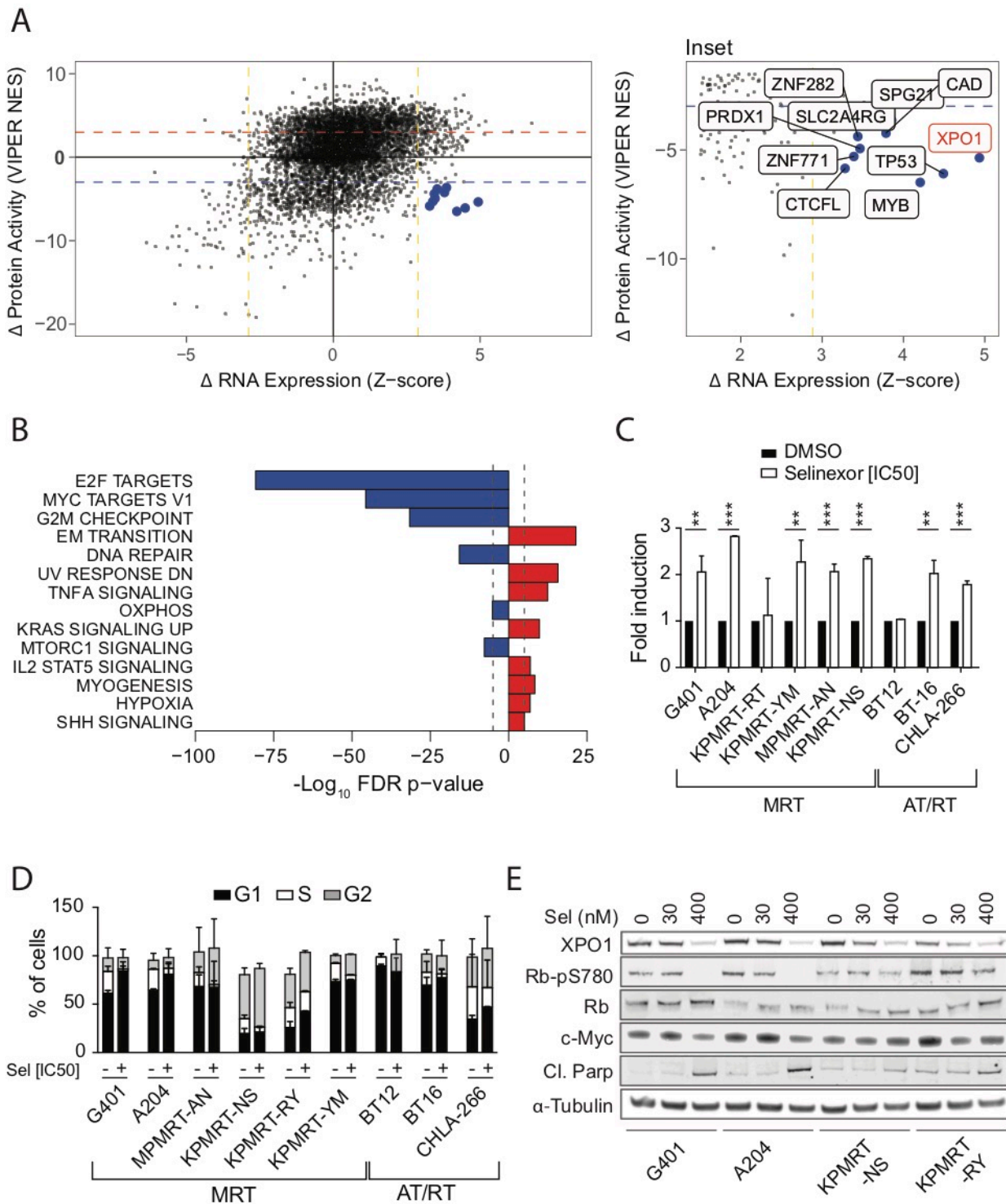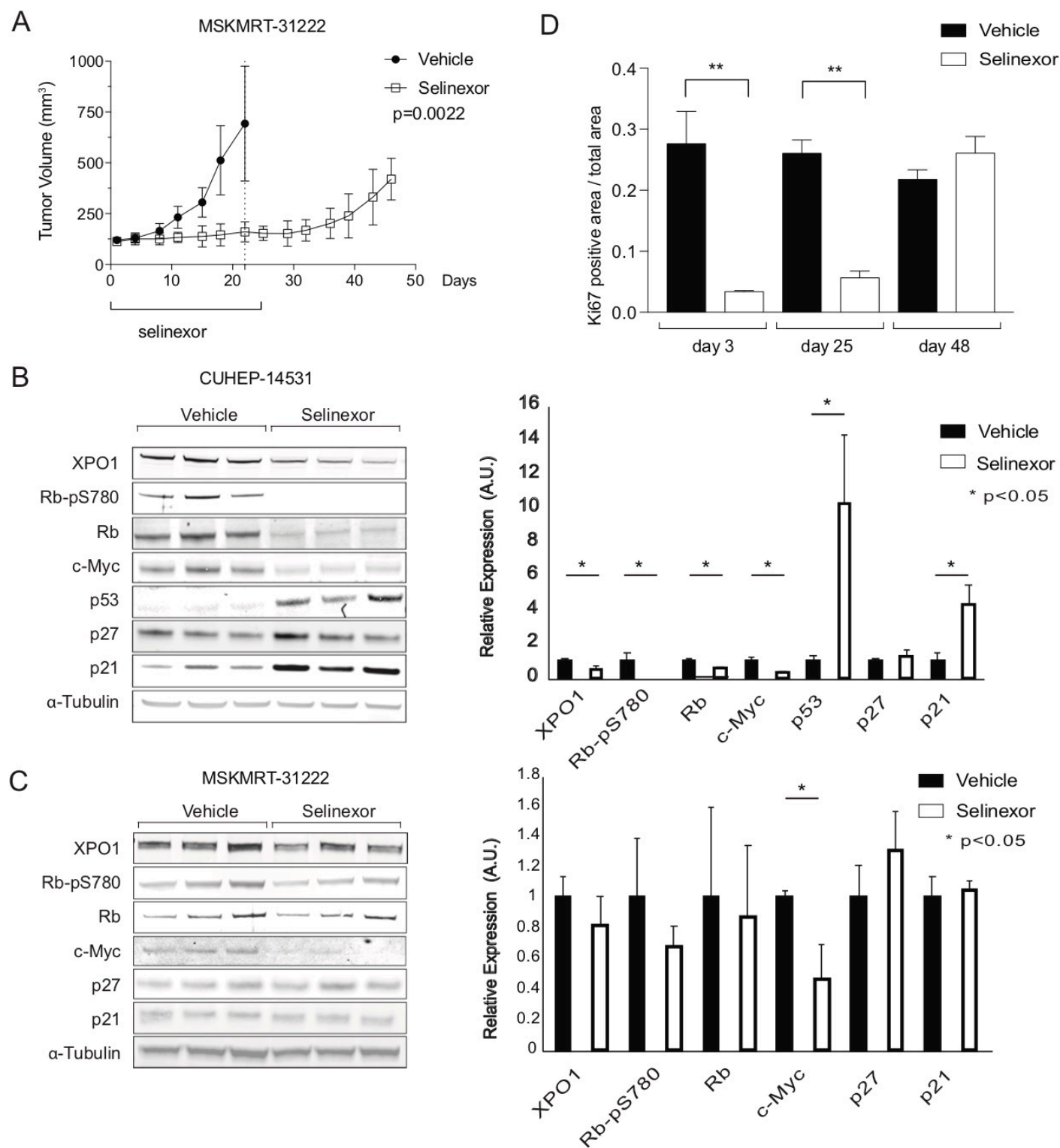
FIGURE 1
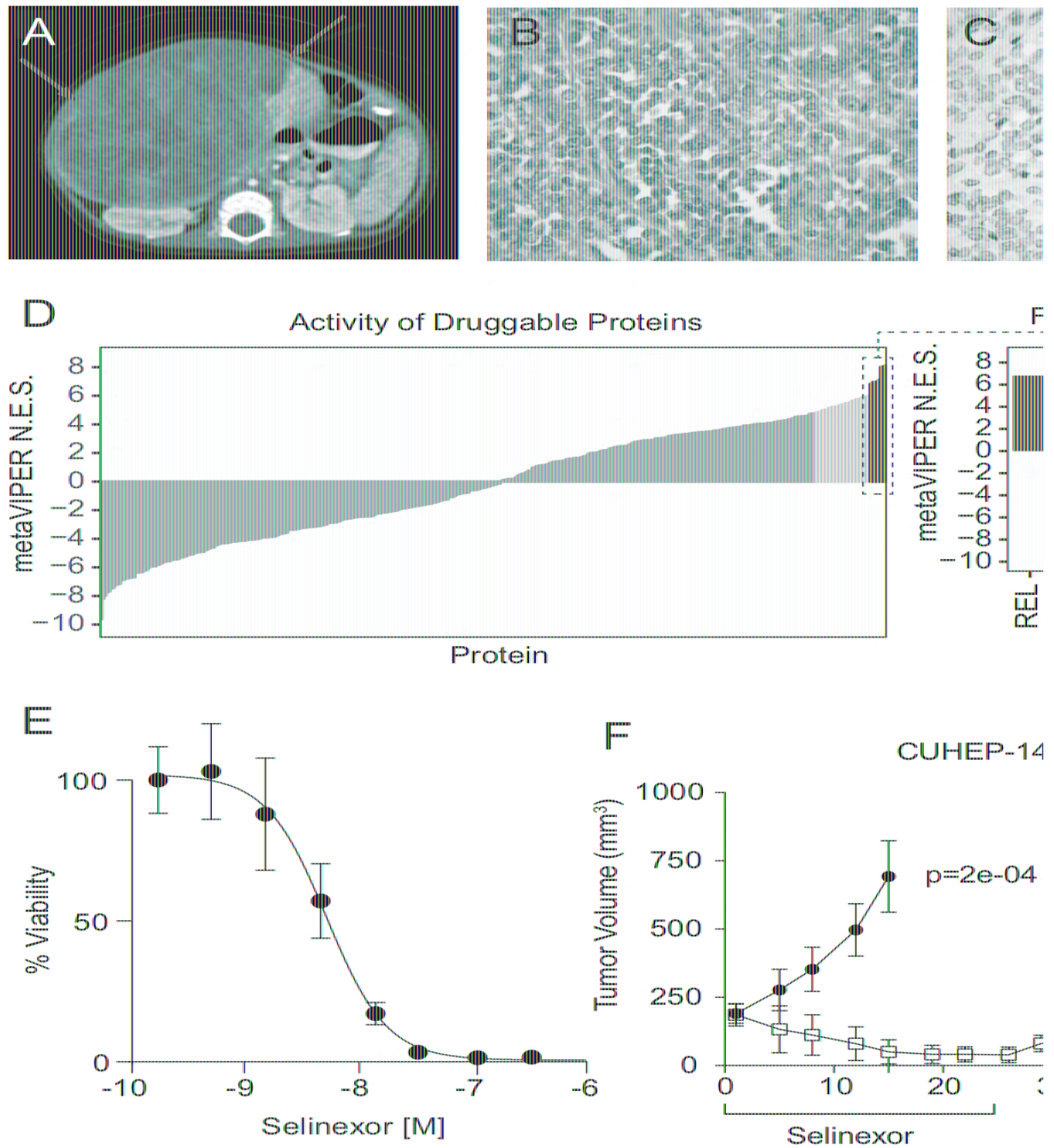
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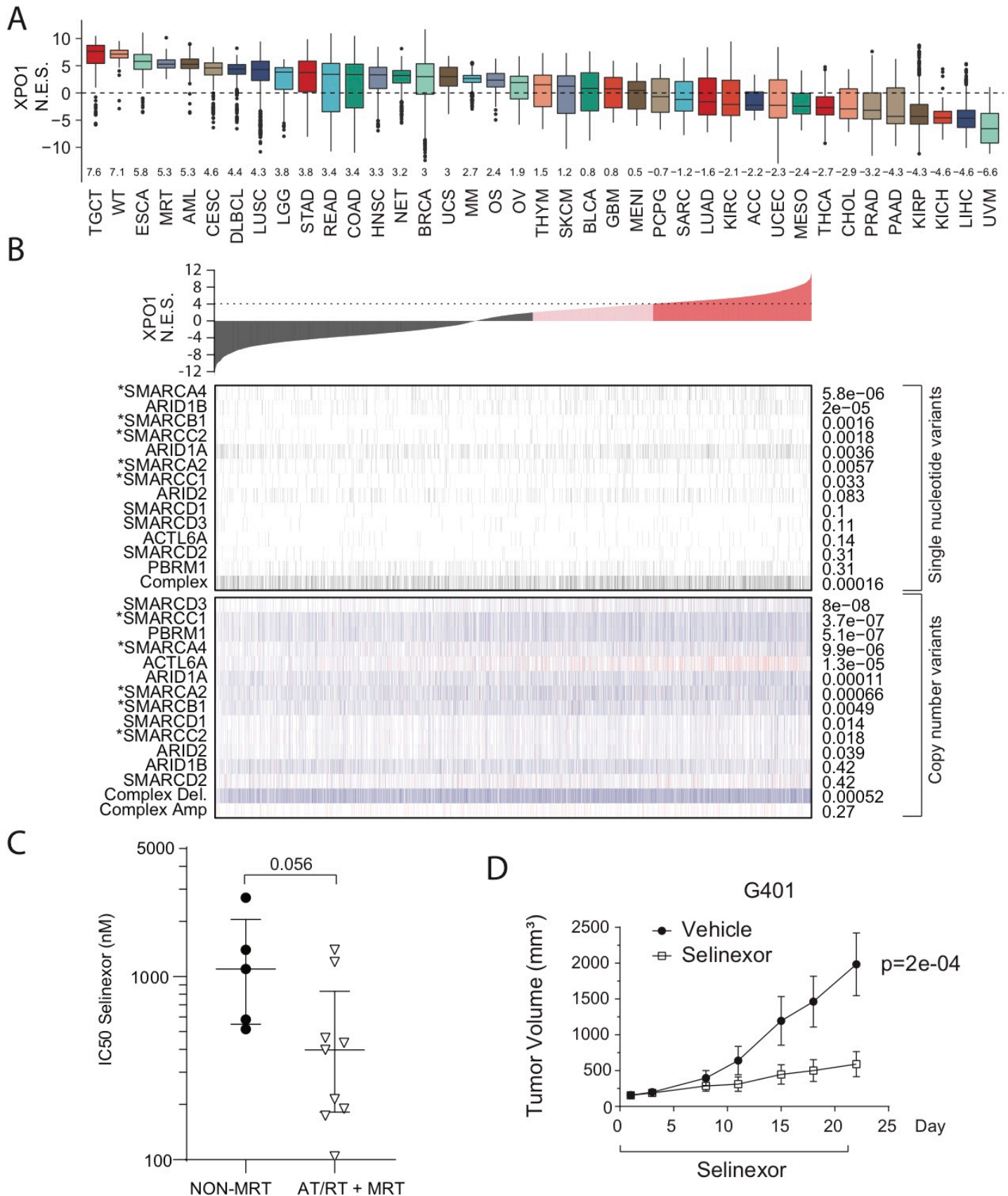
FIGURE 3

FIGURE 4

FIGURE S1

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FIGURE S2

FIGURE S3