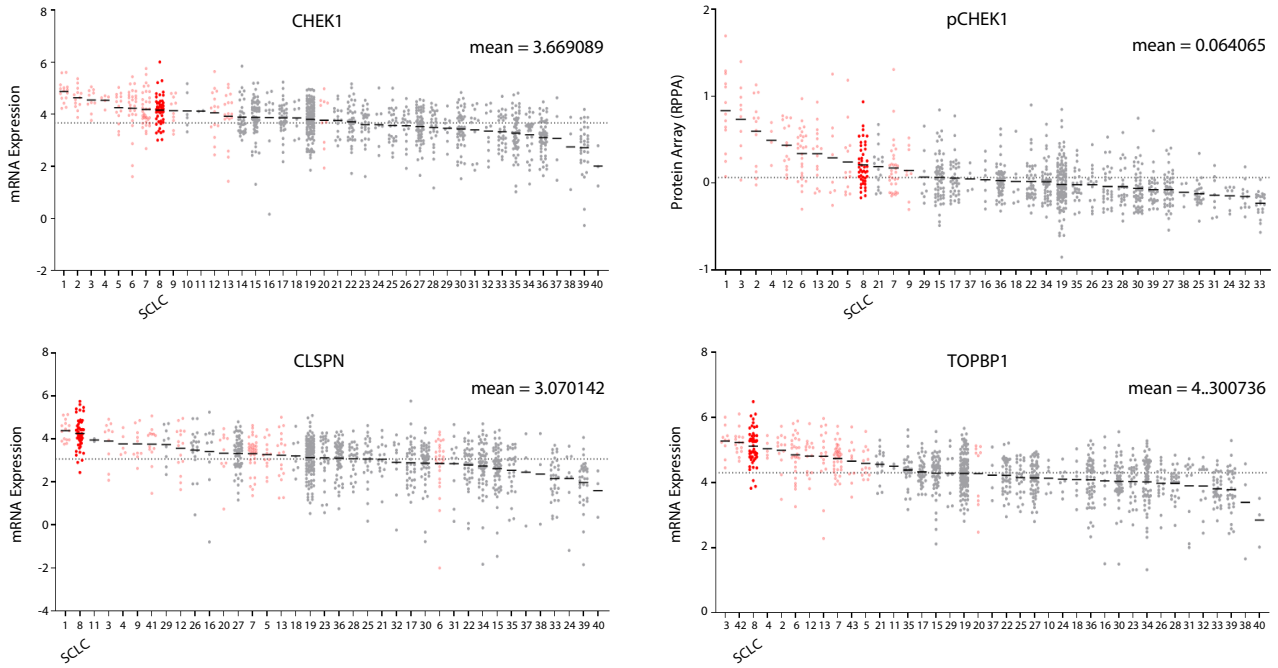
Figure S1

A



1	T-cell ALL	6	Mult. Myeloma	11	Meningioma	16	Ewing Sarc.	21	Neuroblastoma	26	Soft tissue	32	Thyroid	38	Chondrosarcoma
2	B-cell ALL	7	AML	12	B-cell Lymph. (other)	17	Stomach	22	Pancreas	27	Glioma	33	Kidney	39	Upper aerodigestive
3	Burkitt Lymph.	8	SCLC	13	DLBCL	18	Prostate	23	Endometrium	28	Liver	34	Breast	40	Giant cell tumor
4	Leukemia (other)	9	T-cell Lymph. (other)	14	NA	19	NSCLC	24	Bile duct	29	Osteosarcoma	35	Esophogus	41	B-cell Lymph.
5	CML	10	other	15	Colorectal	20	Lymph. (Hodgkin)	25	Urinary tract	30	Ovary	36	Melanoma	42	T-cell Leuk.
										31	Mesothelioma	37	Medullobastoma	43	T-cell Lymph.

B

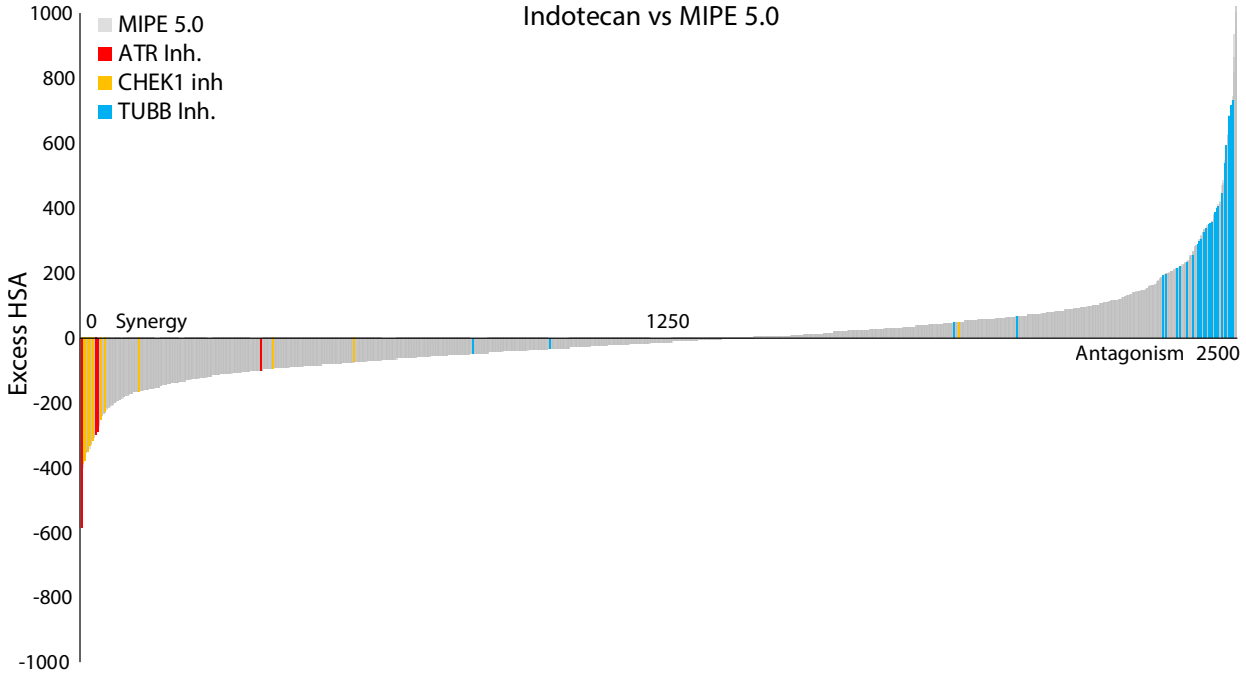
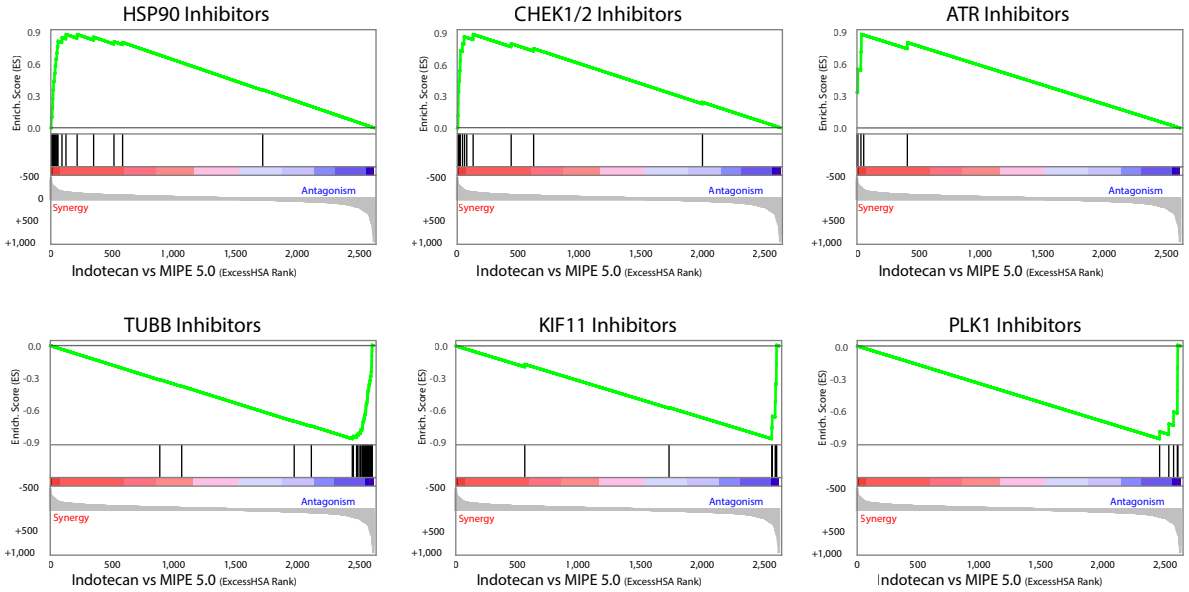


Figure S1 (cont.)

C



D

Notable Synergies	ExcessHSA value
Rank # 1: Indotecan + BAY-1895344 (ATR i)	-585.8
Rank # 5: Indotecan + Ganetesipib (HSP90 i)	-408.7
Rank # 7: Indotecan + CHIR-124 (CHK1 i)	-390.7
Rank # 8: Indotecan + AZD-6738 (ATR i)	-378.7
Rank # 9: Indotecan + Prexasertib (CHK1 i)	-376.7
Rank # 10: Indotecan + AZD-7762 (CHK1/2 i)	-372

Notable Synergies	ExcessHSA value
Rank # 15: Indotecan + SAR-020106 (CHK1 i)	-352.3
Rank # 19: Indotecan + Alvespimycin (HSP90 i)	-340.8
Rank # 20: Indotecan + Rabusertib (CHK1 i)	-332.4
Rank # 32: Indotecan + M6620 (ATR i)	-300.5
Rank # 36: Indotecan + VE-821 (ATR i)	-286.9
Rank # 54: Indotecan + MK-8776 (CHK1 i)	-229.8

E

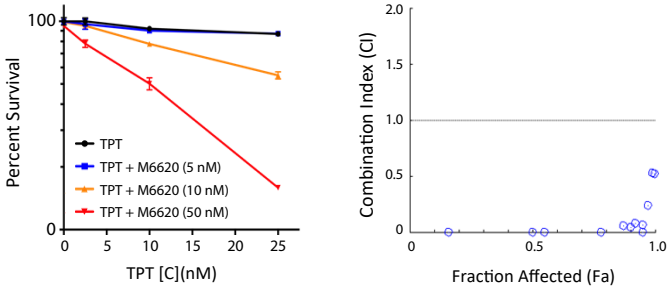


Figure S1: Combinatorial activity of agents targeting replication stress in SCLC cell lines, related to Figure 1. **A)** CHK1, CLSPN, and TOPBP1 mRNA expression and phosphoCHK1 in the CCLE dataset. Red dots and light pink dots indicate small cell lung cancer and hematopoietic malignancy cell lines, respectively. **B)** Ranking of synergy and antagonism of 2480 agents in the MIPE 5.0 library of approved and investigational drugs based on the Excess HSA metric. Prominent mechanistic classes including ATR, CHK1 and tubulin inhibitors are highlighted. **C)** Drug target enrichment analysis (DTEA) plots of the indotecan drug-combination screen highlighting synergy or antagonism enrichments for key drug classes including HSP90, CHK1, ATR, tubulin, KIF11 and PLK inhibitors. **D)** Examples and rankings of notable synergistic indotecan + drug combinations. **E)** Evaluation of H446 cell survival (mean +/- standard deviation, n = 3) following exposure to topotecan and M6620 using fixed ratio dosing and assessment of the combination index (CI) and fraction affected (Fa) for the combination

Figure S2

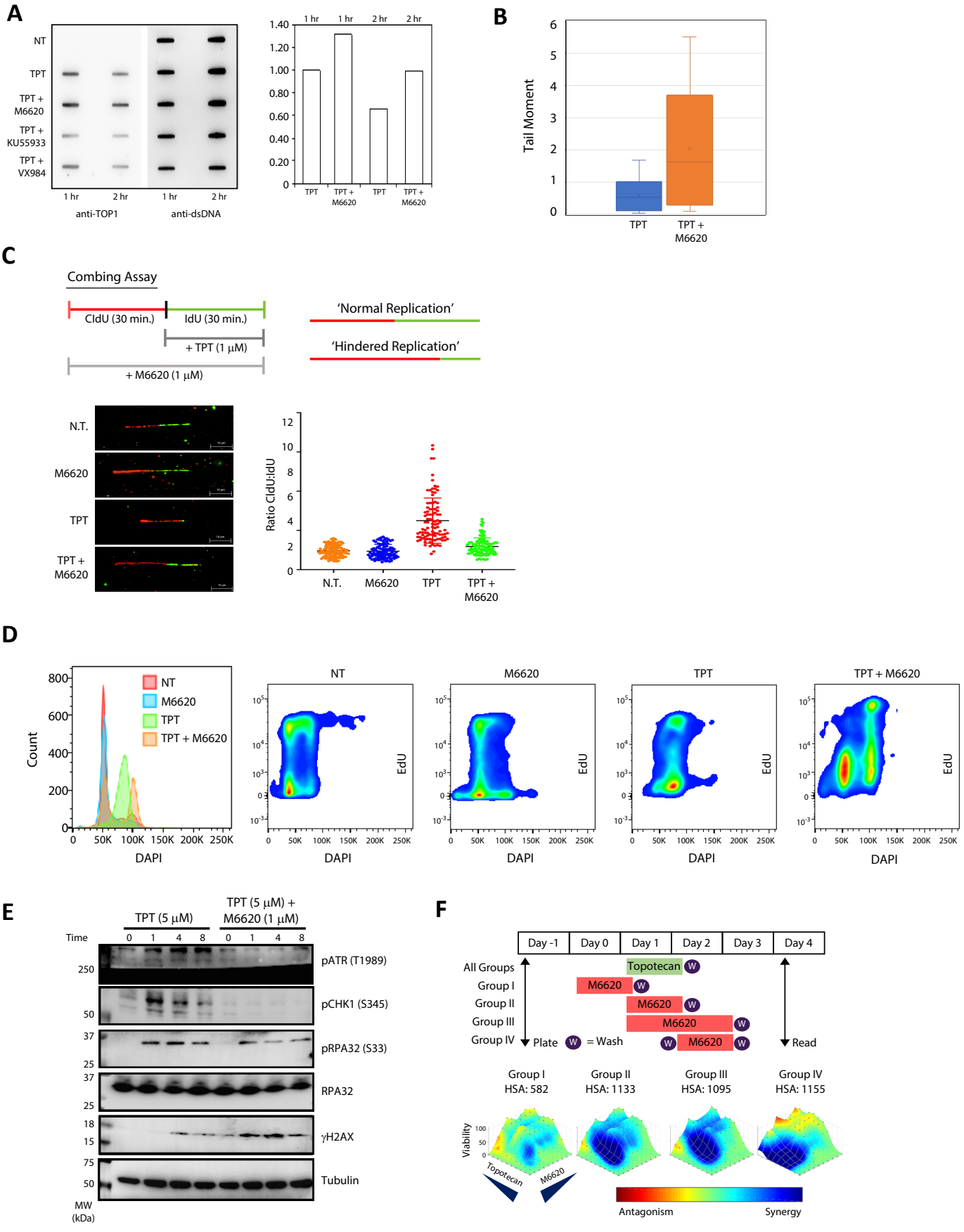

A
NT
TPT
TPT + M6620
TPT + KU55933
TPT + VX984
1 hr
2 hr
1 hr
2 hr
anti-TOP1
anti-dsDNA
1 hr
1 hr
2 hr
2 hr
TPT
TPT + M6620
TPT
TPT + M6620
B
Tail Moment
TPT
TPT + M6620
C
Combing Assay
CldU (30 min.)
IdU (30 min.)
+ TPT (1 μM)
+ M6620 (1 μM)
'Normal Replication'
'Hindered Replication'
N.T.
M6620
TPT
TPT + M6620
Ratio CldU:IdU
N.T.
M6620
TPT
TPT + M6620
D
Count
NT
M6620
TPT
TPT + M6620
DAPI
NT
M6620
TPT
TPT + M6620
EdU
DAPI
E
TPT (5 μM)
TPT (5 μM) + M6620 (1 μM)
Time
0 1 4 8 0 1 4 8
pATR (T1989)
pCHK1 (S345)
pRPA32 (S33)
RPA32
γH2AX
Tubulin
MW (kDa)
F
Day -1
Day 0
Day 1
Day 2
Day 3
Day 4
All Groups
Group I
Group II
Group III
Group IV
Topotecan
M6620
Plate
= Wash
Read
Group I HSA: 582
Group II HSA: 1133
Group III HSA: 1095
Group IV HSA: 1155
Viability
Topotecan
M6620
Antagonism
Synergy

Figure S2 (cont.)

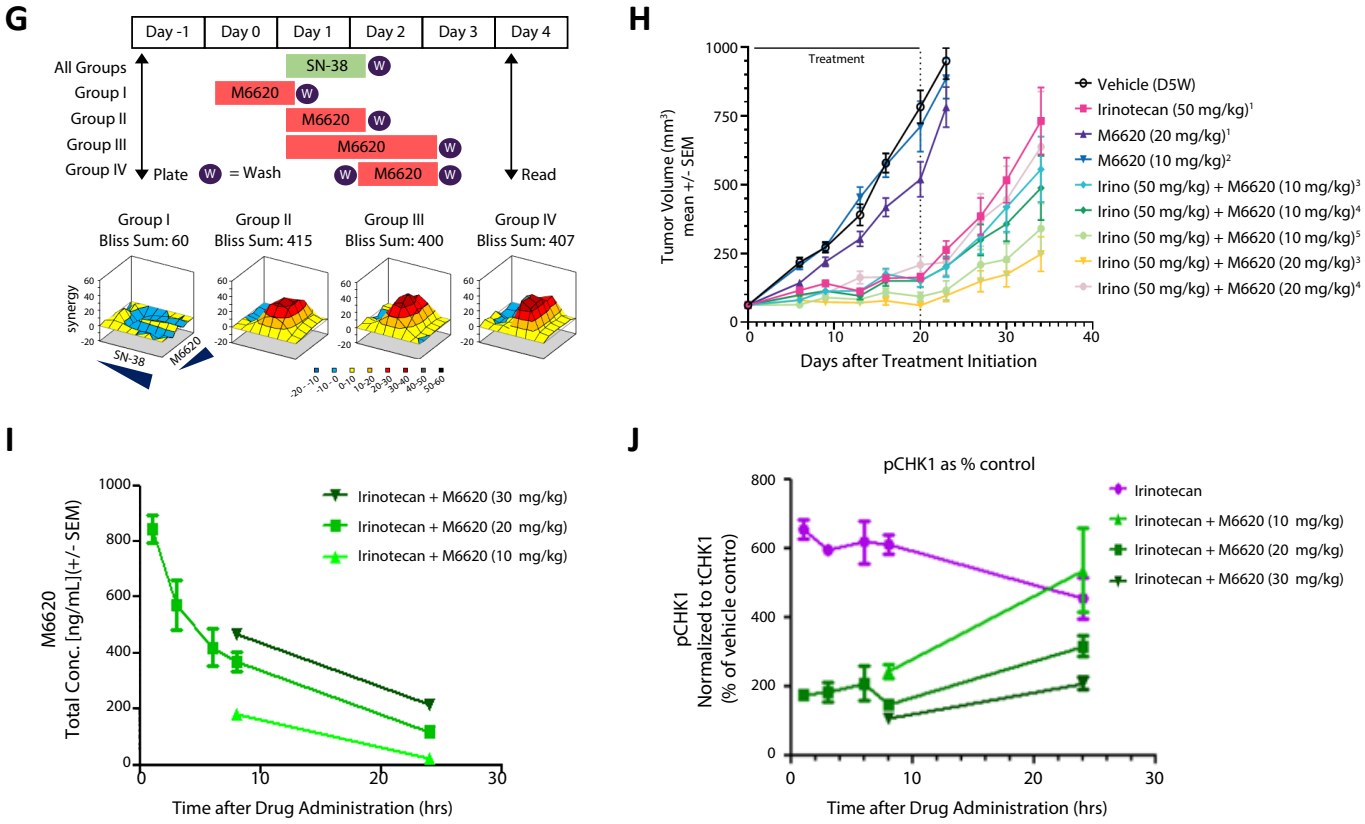
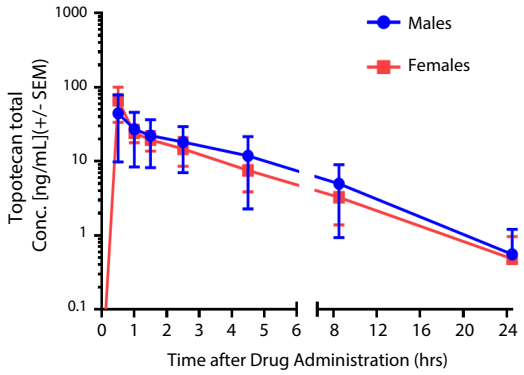


Figure S2: In vitro and in vivo synergy, mechanistic basis, pharmacokinetic and pharmacodynamic evaluations of the combination of ATR and TOP1 inhibition, related to Figure 2. A) Evaluation of TOP1 DNA-protein crosslinks (TOP1-DPCs) following exposure to topotecan and M6620. **B)** Quantitation of Comet Assay (single cell gel electrophoresis) demonstrating the increased DNA strand breaks caused by the co-administration of topotecan and M6620. Top and bottom of the boxes are maximum and minimum values, middle line is median and asterisk is mean. Whiskers are upper and lower quartiles. **C)** Schematic illustrating the combing assay (spatiotemporal analysis of DNA replication) for the detection of replication stress and specified imaging outcomes, highlighting the re-establishment of normal replication when M6620 is added to topotecan in the SCLC cell model H209. **D)** Cell cycle evaluation following exposure to topotecan, M6620 or the combination of these agents as flow cytometry and FACS analysis. **E)** Western blot analysis of pCHK1, pRPA and gH2AX with topotecan and the combination of topotecan + M6642 across three time points (1, 4 and 8 hours). **F)** Cell viability with topotecan and M6620 across different treatment schedules in the SCLC cell line H446. Pre-administration of M6620 followed by topotecan does not yield a synergistic effect on cell viability, while pre-administration of topotecan followed by M6620 and co-administration of these drugs creates a synergistic effect. Group I: M6620 for 24 hours followed by 24 hours of topotecan; Group II: concurrent M6620 and topotecan (24 hours); Group III: concurrent M6620 and topotecan (24 hours) followed by an additional 24 hours of M6620; Group IV: topotecan for 24 hours followed by 24 hours of M6620.

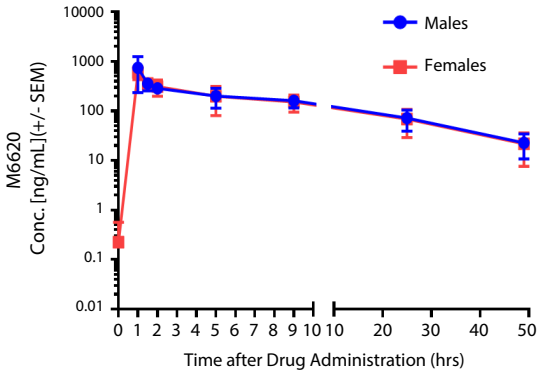
G) Cell viability with SN-38 and M6620 across different treatment schedules in the colorectal cancer cell line HT-29. Pre-administration of M6620 followed by SN-38 does not yield a synergistic effect on cell viability, while pre-administration of SN-38 followed by M6620 and co-administration of these drugs creates a synergistic effect. Group I: M6620 for 24 hours followed by 24 hours of SN-38; Group II: concurrent M6620 and SN-38 (24 hours); Group III: concurrent M6620 and SN-38 (24 hours) followed by an additional 24 hours of M6620; Group IV: SN-38 for 24 hours followed by 24 hours of M6620. **H)** Xenograft from human colorectal adenocarcinoma model (Colo205) showing the schedule-dependent effect of irinotecan and M6620. Dosing schedule #1: single agent treatment on day 0; #2: single agent treatment on days 0 and 1; #3: irinotecan day 0 + M6620 day 0; #4: irinotecan day 0 + M6620 day 1; #5: irinotecan day 0 + M6620 days 0 and 1. **I)** Pharmacokinetics of M6620 in tumor bearing mice (HSD athymic) following administration of irinotecan (50 mg/kg) with three different doses of M6620 (10, 20 and 30 mg/kg). **J)** Pharmacodynamic evaluation of phosphoCHK1 (normalized to total CHK1) in tumor bearing mice (HSD athymic) following administration of irinotecan (50 mg/kg) and the combination of irinotecan with three different doses of M6620 (10, 20 and 30 mg/kg).

Figure S3

A



B

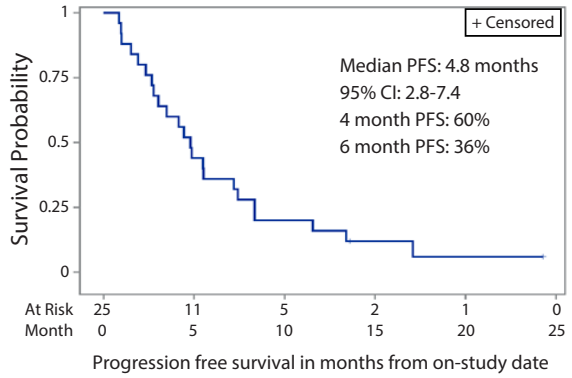


C

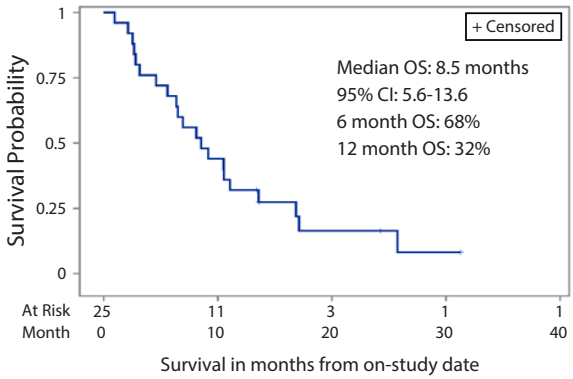
Topotecan and M6620 pharmacokinetic parameters

Parameter	Mean	%CV
Topotecan		
Cmax (ng/mL)	55.2	64.10%
AUCinf (hr*ng/mL)	143.3	59.70%
T1/2 (hr)	4.14	12.90%
Vd (L)	102.8	41.20%
CL (L/hr)	20.7	38.60%
M6620		
Cmax (ng/mL)	663.7	57.20%
AUCinf (hr*ng/mL)	5320	33.30%
T1/2 (hr)	13.4	20.10%
Vd (L)	1509	42.60%
CL (L/hr)	84	33.60%

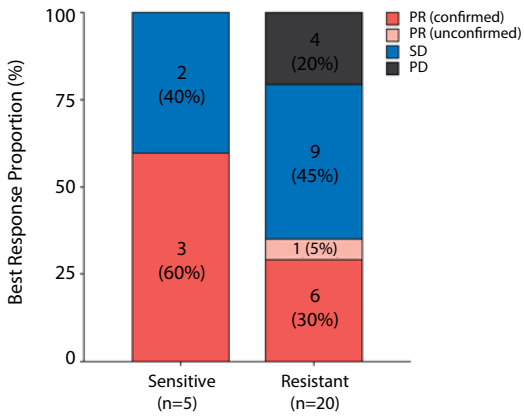
D



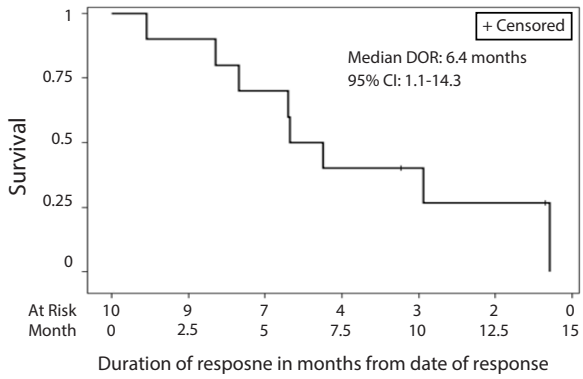
E



F



G



H

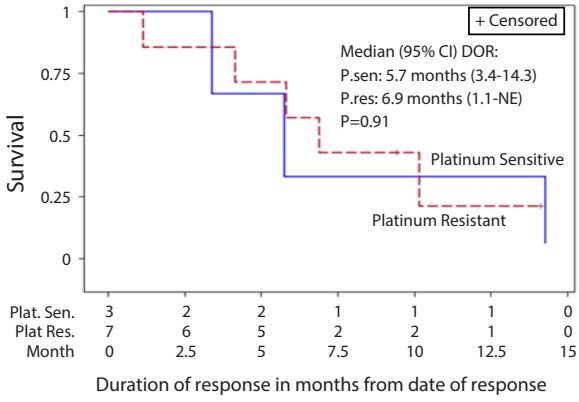
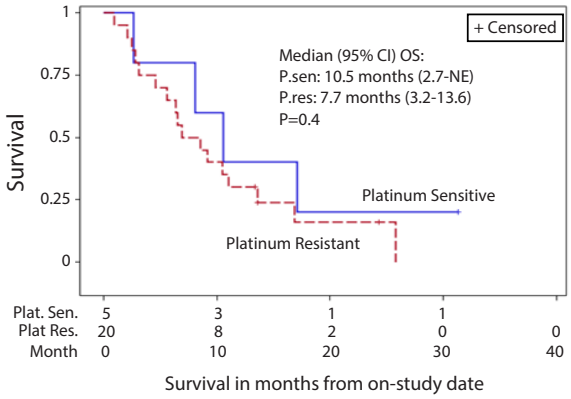
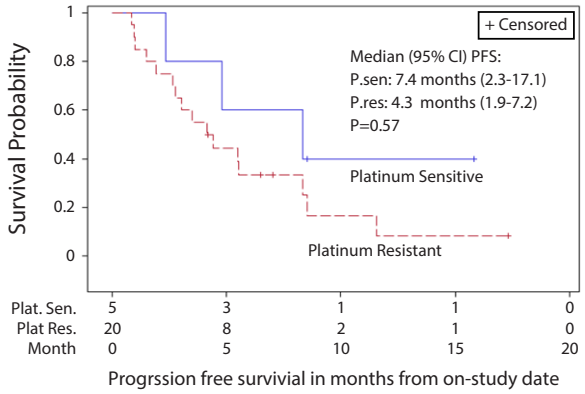


Figure S3 (cont.)

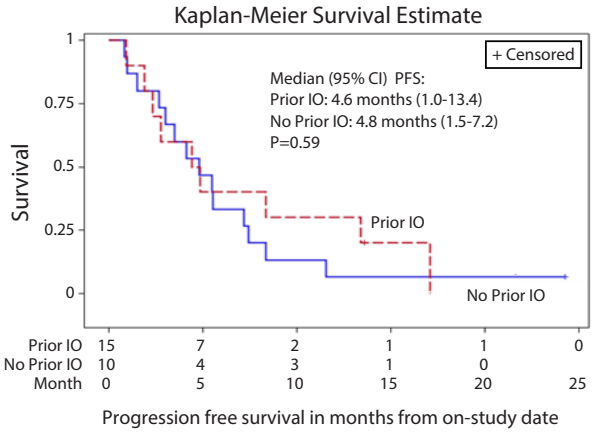
I



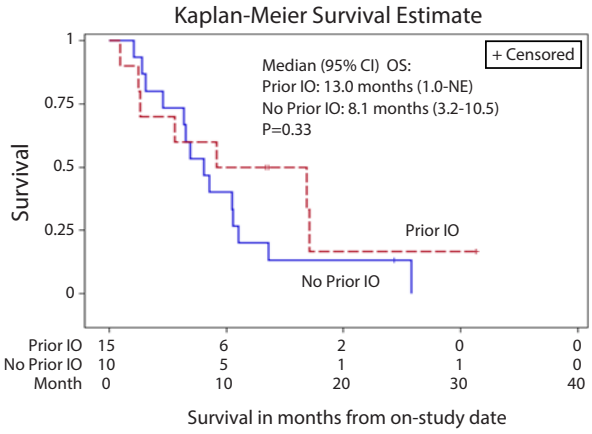
J



K



L



M

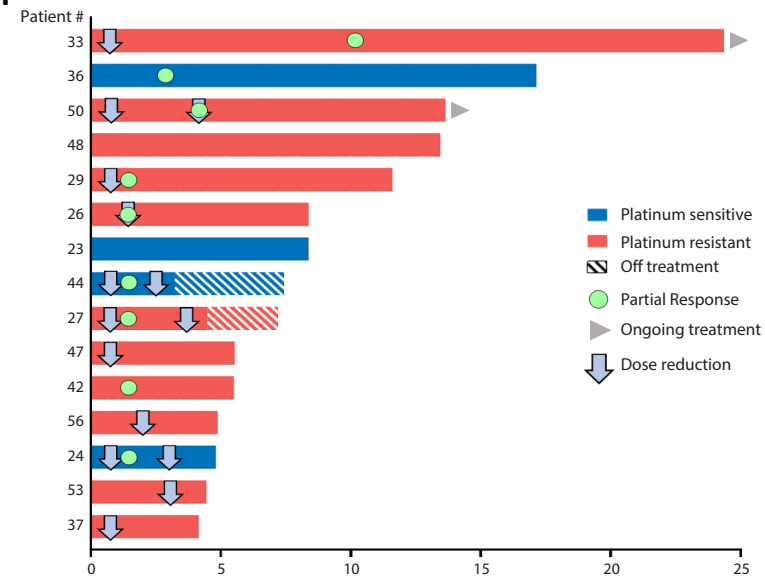


Figure S3: Pharmacokinetics and efficacy of the combination of topotecan and M6620 in patients with SCLC, related to Figure 3. A) Pharmacokinetics of topotecan in humans following intravenous administration (1.25 mg/m2) co-administered with M6620 with data separated by sex (6 males and 7 females). **B)** Pharmacokinetics of M6620 in humans following intravenous administration (210 mg/m2) co-administered with topotecan with data separated by sex (6 males and 6 females). **C)** Key PK parameters. Kaplan-Meier curves showing **D)** progression-free survival and **E)** overall survival in the overall trial population. **F)** Best response according to platinum sensitivity. **G)** Kaplan-Meier curve showing duration of response. Kaplan-Meier curves showing **H)** duration of response **I)** overall survival and **J)** progression-free survival in platinum-sensitive and resistant patients. **K)** progression-free survival and **L)** overall survival in patients who were previously treated with immunotherapy versus those who were not. M) Dose reductions, tumor response and duration of treatment.

Figure S4

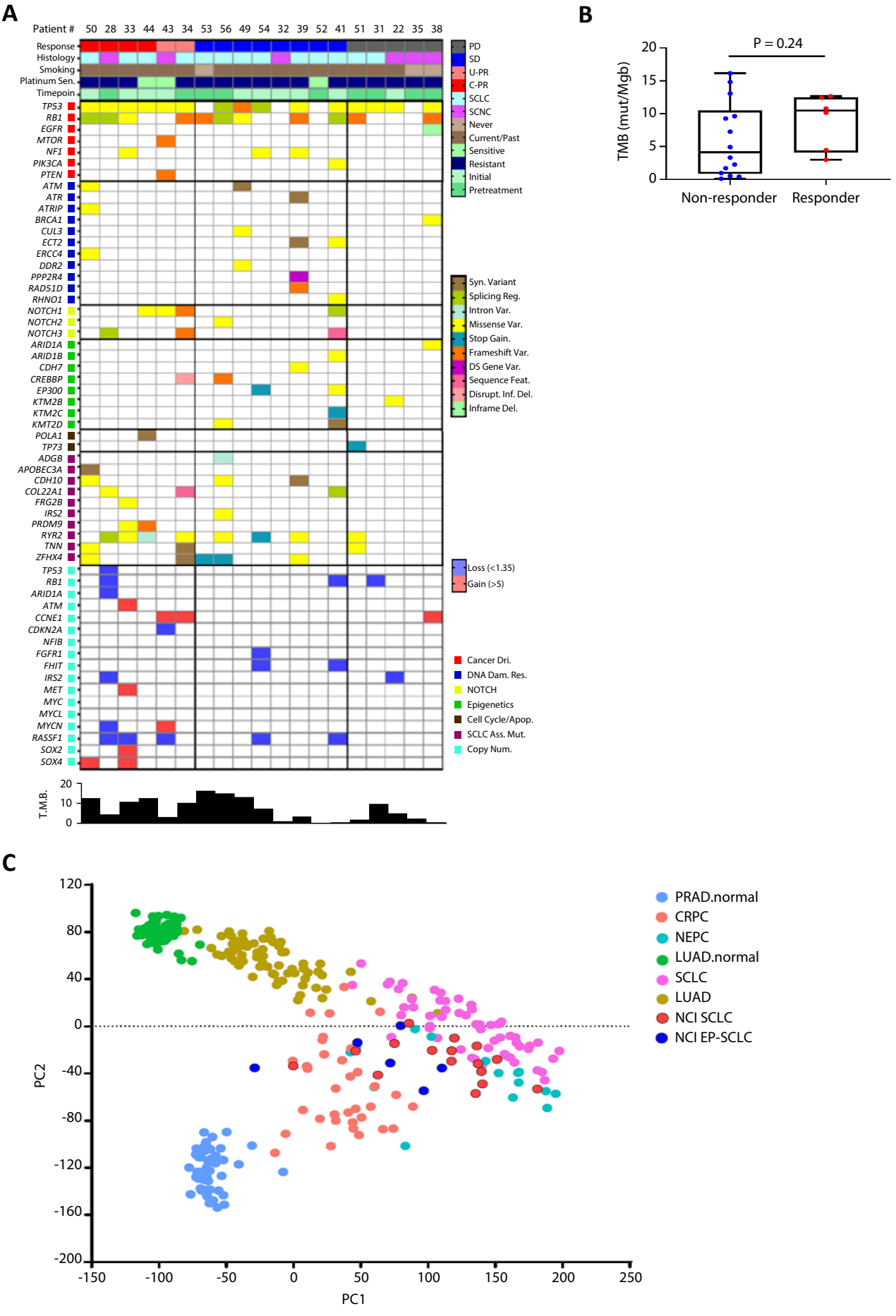

A
Patient # 50 28 33 44 43 34 53 56 49 54 32 39 52 41 51 31 22 35 38
Response
Histology
Smoking
Platinum Sen.
Timepoin
TP53
RB1
EGFR
MTOR
NF1
PIK3CA
PTEN
ATM
ATR
ATRIP
BRCA1
CUL3
ECT2
ERCC4
DDR2
PPP2R4
RAD51D
RHNO1
NOTCH1
NOTCH2
NOTCH3
ARID1A
ARID1B
CDH7
CREBBP
EP300
KTM2B
KTM2C
KMT2D
POLA1
TP73
ADGB
APOBEC3A
CDH10
COL22A1
FRG2B
IRS2
PRDM9
RYR2
TNN
ZFHX4
TP53
RB1
ARID1A
ATM
CCNE1
CDKN2A
NFIB
FGFR1
FHIT
IRS2
MET
MYC
MYCL
MYCN
RASSF1
SOX2
SOX4
T.M.B.
20
10
0
PD
SD
U-PR
C-PR
SCLC
SCNC
Never
Current/Past
Sensitive
Resistant
Initial
Pretreatment
Syn. Variant
Splicing Reg.
Intron Var.
Missense Var.
Stop Gain.
Frameshift Var.
DS Gene Var.
Sequence Feat.
Disrupt. Inf. Del.
Inframe Del.
Loss (<1.35)
Gain (>5)
Cancer Dri.
DNA Dam. Res.
NOTCH
Epigenetics
Cell Cycle/Apop.
SCLC Ass. Mut.
Copy Num.
B
P = 0.24
TMB (mut/Mgb)
20
15
10
5
0
Non-responder
Responder
C
PC2
PC1
120
80
40
0
-40
-80
-120
-160
-200
-150
-100
-50
0
50
100
150
200
250
PRAD.normal
CRPC
NEPC
LUAD.normal
SCLC
LUAD
NCI SCLC
NCI EP-SCLC

Figure S4 (cont.)

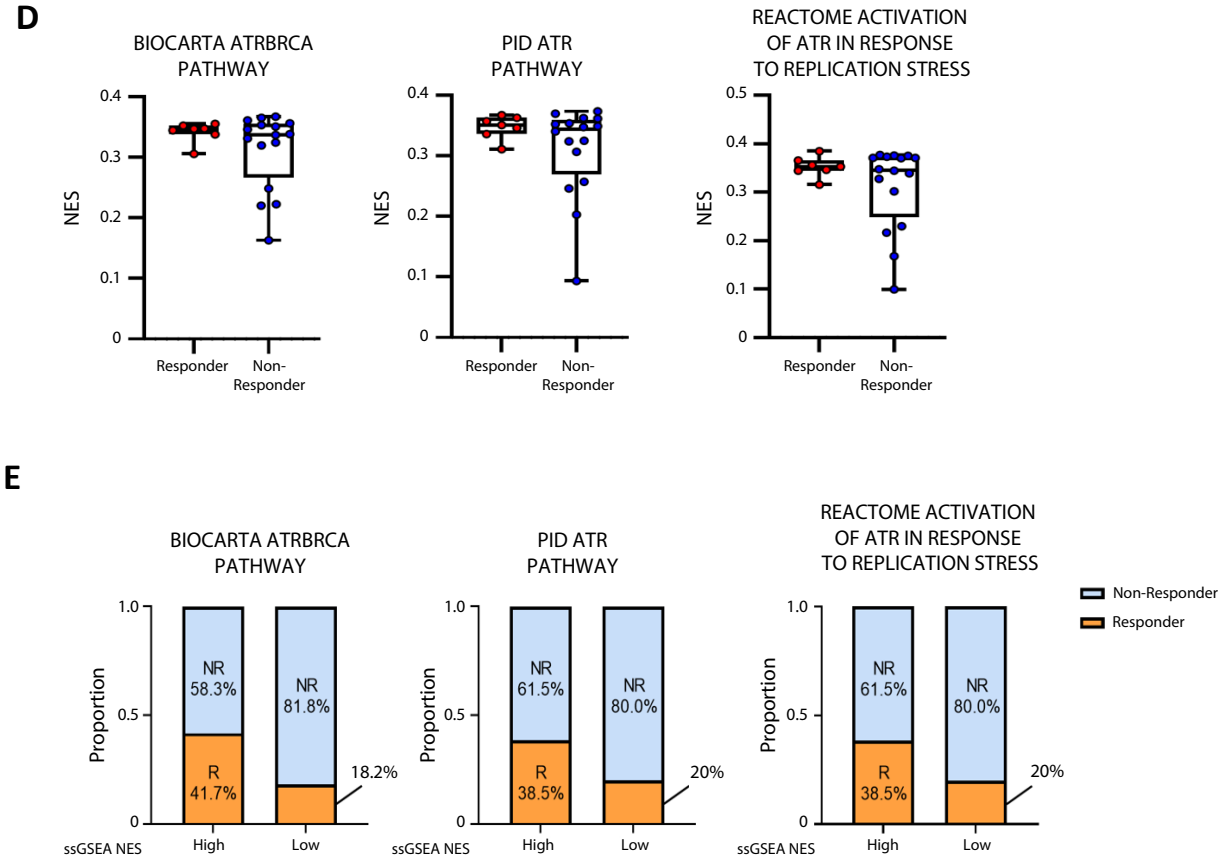


Figure S4: Tumor exome, transcriptome, and replication stress signatures in responders and non-responders, related to Figure 5. A) Mutations classified by type (e.g. intron variant, missense variant) and pathway (e.g. NOTCH signaling, epigenetics), and copy number variations among responders and non-responders. **B)** Boxplot showing the mutational burden of responders and non-responders (mut/Mb: mutations per megabase). **C)** Transcriptomic relationship of current cohort and previously published SCLC, lung adenocarcinoma, and prostate cancer cohorts. PRAD, prostate adenocarcinoma; CRPC, castration-resistant prostate cancer; NEPC, neuroendocrine prostate cancer; SCLC, small cell lung cancer; LUAD, lung adenocarcinoma; NCI SCLC and NCI EP-SCNC are samples from the current study. **D)** Box plot analysis of enrichment (NES) for selected ATR and replication stress-associated transcriptional signatures among responders and non-responders. **E)** Proportional analysis of responders and non-responders based on selected ATR and replication stress-associated transcriptional signatures.

Figure S5

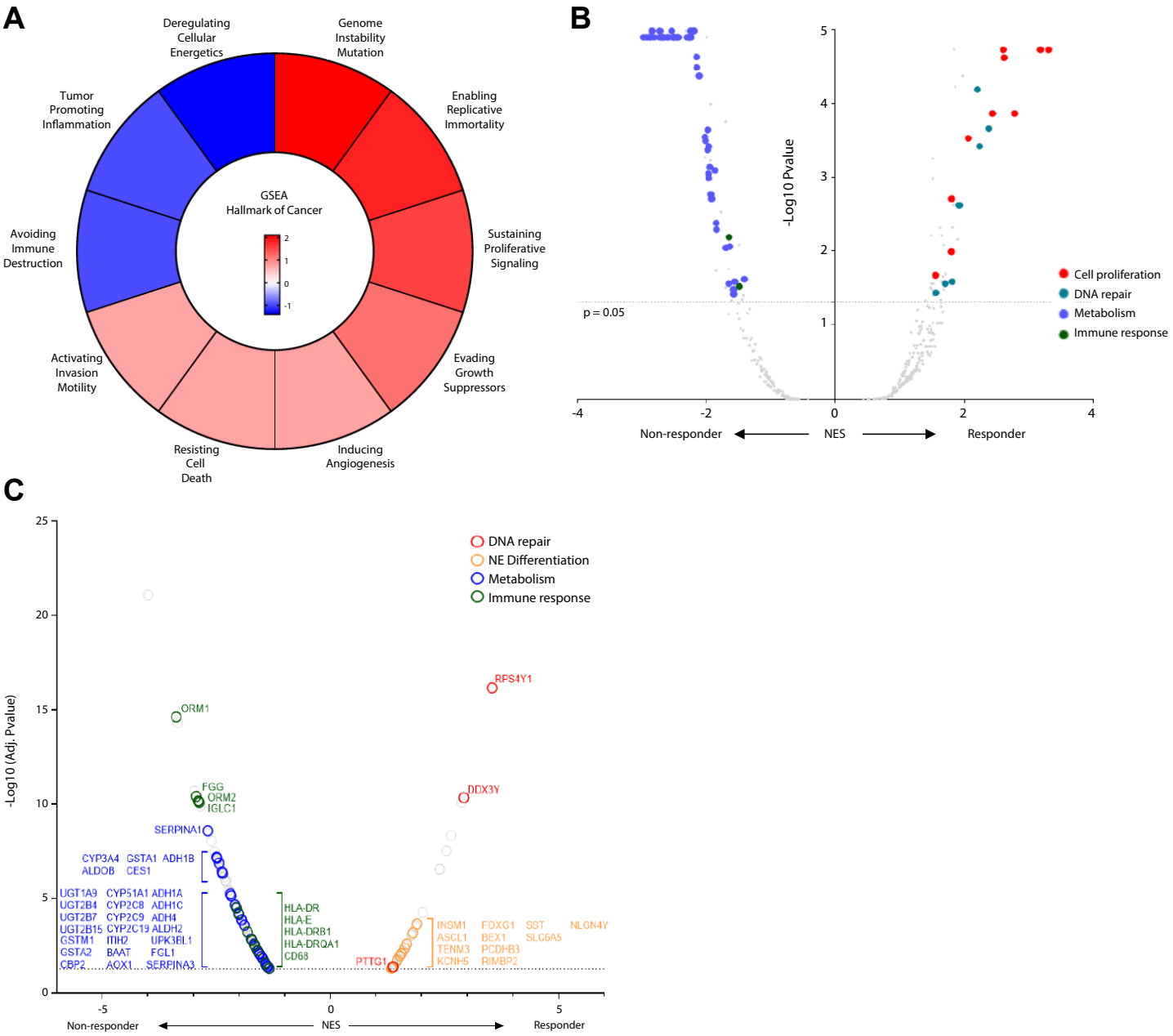
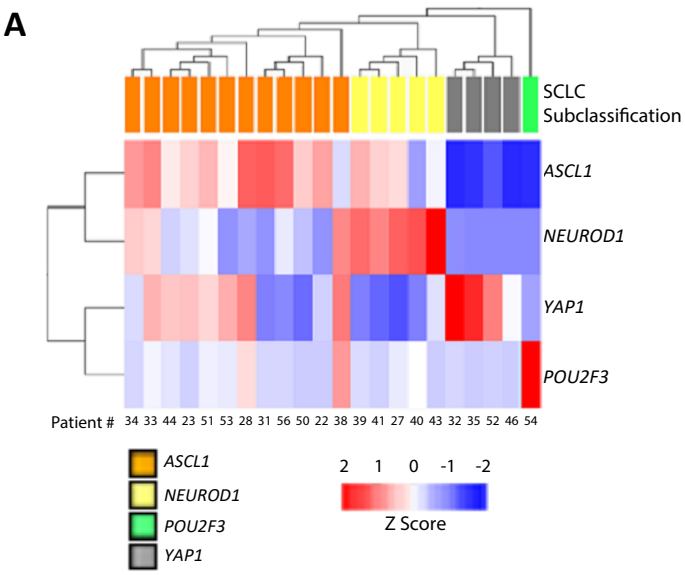
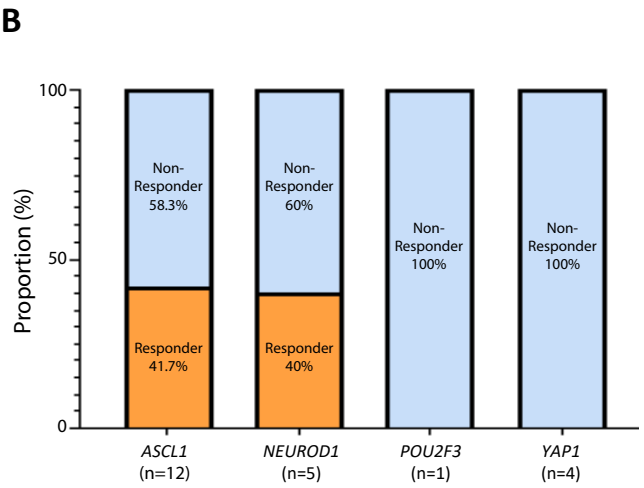
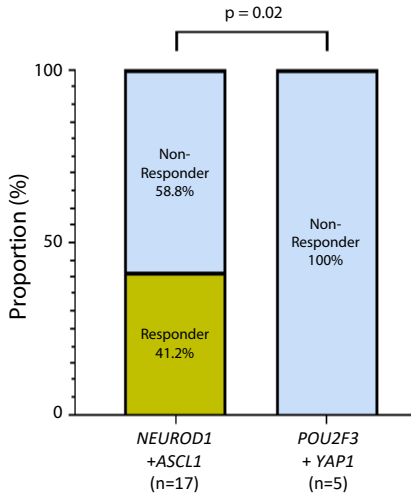


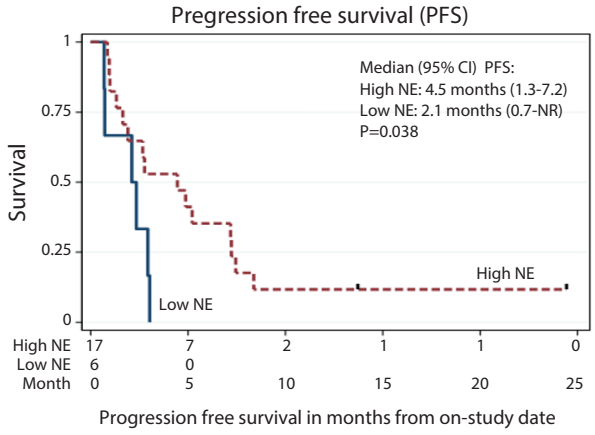
Figure S5: Cancer hallmarks, genes, and gene sets differentially enriched among responders, related to Figure 5. A) GSEA of cancer hallmarks based on differentially expressing genes between non-responders to M6620 and topotecan. **B)** Volcano plot showing gene set enrichments among SCLC responders and non-responders to M6620 and topotecan. Selected gene sets related to cell proliferation, DNA damage and replication, metabolism, and immune response are highlighted. **C)** Volcano plot showing differentially expressed genes between SCNC responders and non-responders to M6620 and topotecan. Selected genes related to DNA/RNA synthesis, metabolism, immune response and NE differentiation are highlighted.

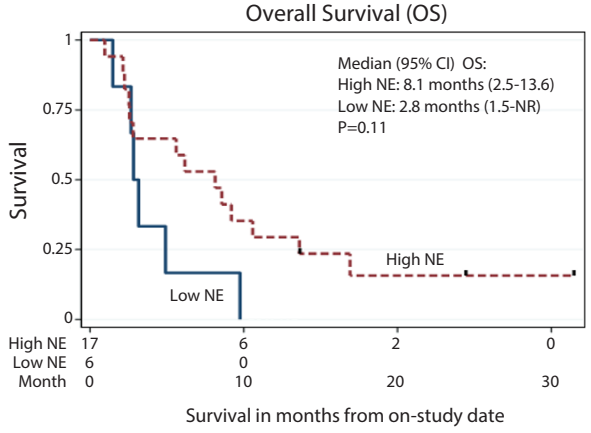
Figure S6


A
SCLC Subclassification
ASCL1
NEUROD1
YAP1
POU2F3
Patient # 34 33 44 23 51 53 28 31 56 50 22 38 39 41 27 40 43 32 35 52 46 54
ASCL1
NEUROD1
POU2F3
YAP1
2 1 0 -1 -2
Z Score


B
100
50
0
Proportion (%)
Non-Responder 58.3%
Responder 41.7%
Non-Responder 60%
Responder 40%
Non-Responder 100%
Non-Responder 100%
ASCL1 (n=12)
NEUROD1 (n=5)
POU2F3 (n=1)
YAP1 (n=4)


C
p = 0.02
100
50
0
Proportion (%)
Non-Responder 58.8%
Responder 41.2%
Non-Responder 100%
NEUROD1 +ASCL1 (n=17)
POU2F3 + YAP1 (n=5)


D
Pregression free survival (PFS)
Median (95% CI) PFS:
High NE: 4.5 months (1.3-7.2)
Low NE: 2.1 months (0.7-NR)
P=0.038
Survival
1
0.75
0.5
0.25
0
High NE
Low NE
High NE 17 7 2 1 1 0
Low NE 6 0
Month 0 5 10 15 20 25
Progression free survival in months from on-study date


E
Overall Survival (OS)
Median (95% CI) OS:
High NE: 8.1 months (2.5-13.6)
Low NE: 2.8 months (1.5-NR)
P=0.11
Survival
1
0.75
0.5
0.25
0
High NE
Low NE
High NE 17 6 2 0
Low NE 6 0
Month 0 10 20 30
Survival in months from on-study date

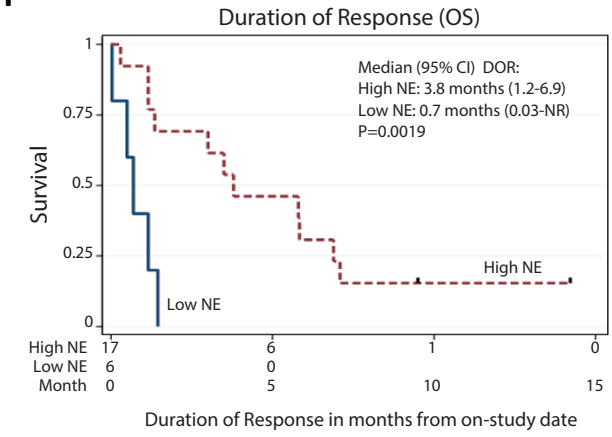

F
Duration of Response (OS)
Median (95% CI) DOR:
High NE: 3.8 months (1.2-6.9)
Low NE: 0.7 months (0.03-NR)
P=0.0019
Survival
1
0.75
0.5
0.25
0
High NE
Low NE
High NE 17 6 1 0
Low NE 6 0
Month 0 5 10 15
Duration of Response in months from on-study date

Figure S6: SCNC transcriptional subtypes and response to M6620 and topotecan, related to Figure 5. A) Hierarchical clustering of pre-treatment tumor transcriptomes based on expression of ASCL1, NEUROD1, YAP1 and POU2F3. **B and C)** Proportion of responses among SCNCs classified as ASCL1, NEUROD1, YAP1 and POU2F3. Kaplan-Meier curves showing **D)** progression-free survival and **E)** overall survival and **F)** duration of response based on high and low neuroendocrine signatures.