

Figure S1. Methods of gene delivery to liver. (A) Outline of tail vein injection of lentivirus mediated GFP-Luc reporter. (B) Representative figures show luciferase signal during the following week after lentivirus injection. (C) Paraffin sections of liver are stained with antibody against GFP. (D) Outline of hydrodynamic tail vein injection of GFP-Luc plasmid. (E) The consecutive figures show dynamic luciferase signals during the following week after injection of GFP-Luc plasmid. (F) Representative images show strong and uniform GFP signal in liver 4 days after injection.

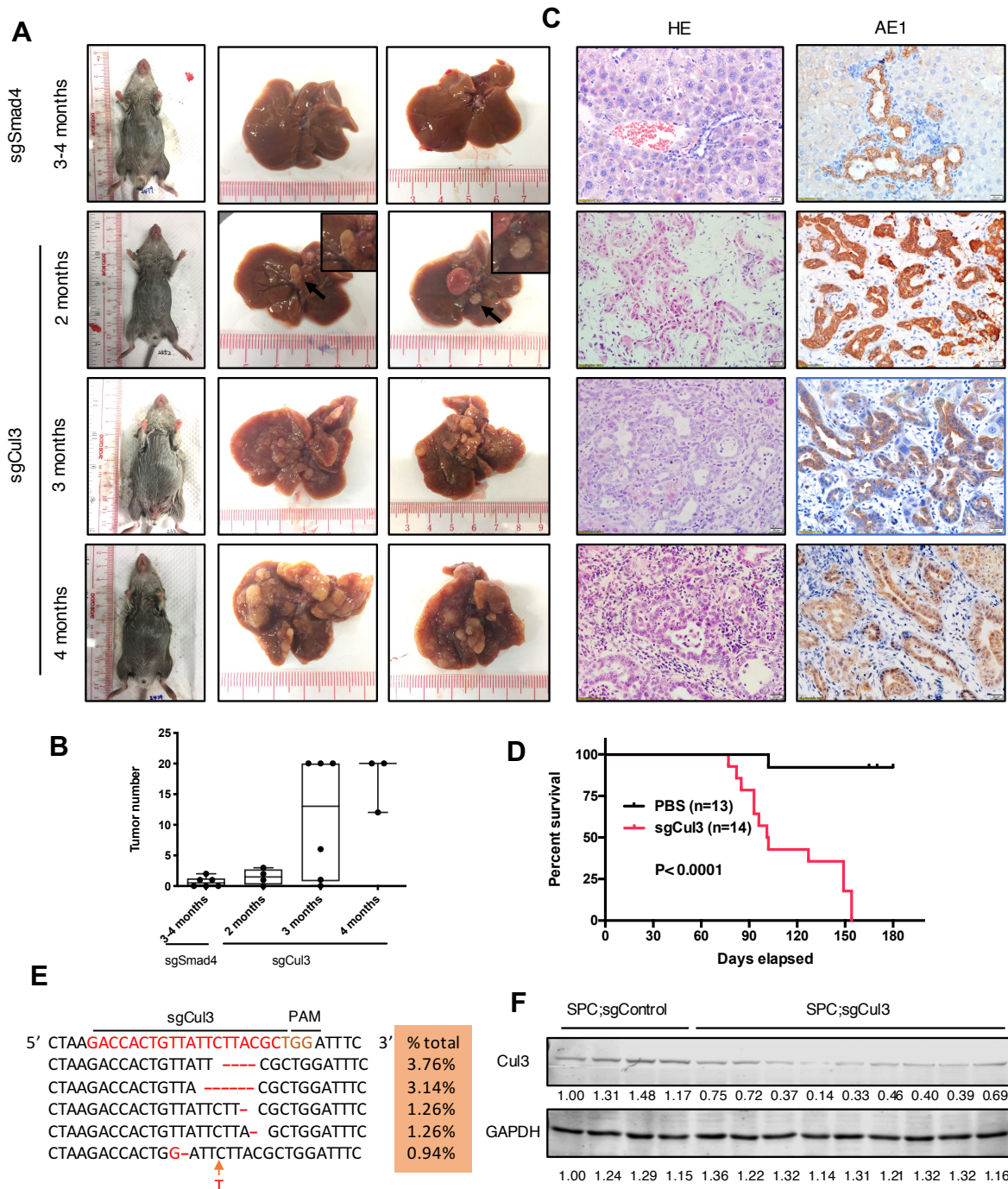


Figure S2. Inactivation of Cul3 induces CC formation in SPC mice. (A) Representative images of the liver at 2, 3 and 4 months of age, which are hydrodynamically injected with sgRNA targeting Cul3 at the age of 1 month. (B) Quantification of tumor numbers at 2, 3 and 4 months of age. (C) Histological analysis of tumors. (D) Kaplan-Meier survival curve for SPC and SPC;sgCul3 mice ($P < .0001$). (E) High-throughput sequencing shows indel mutations of the Cul3 targeting site and the percentage of each read. (F) Western blot analysis shows lower Cul3 protein levels in tumors from SPC;sgCul3 mice at 3-4 months compared with tumors from SPC;sgControl mice at 6-7 months.

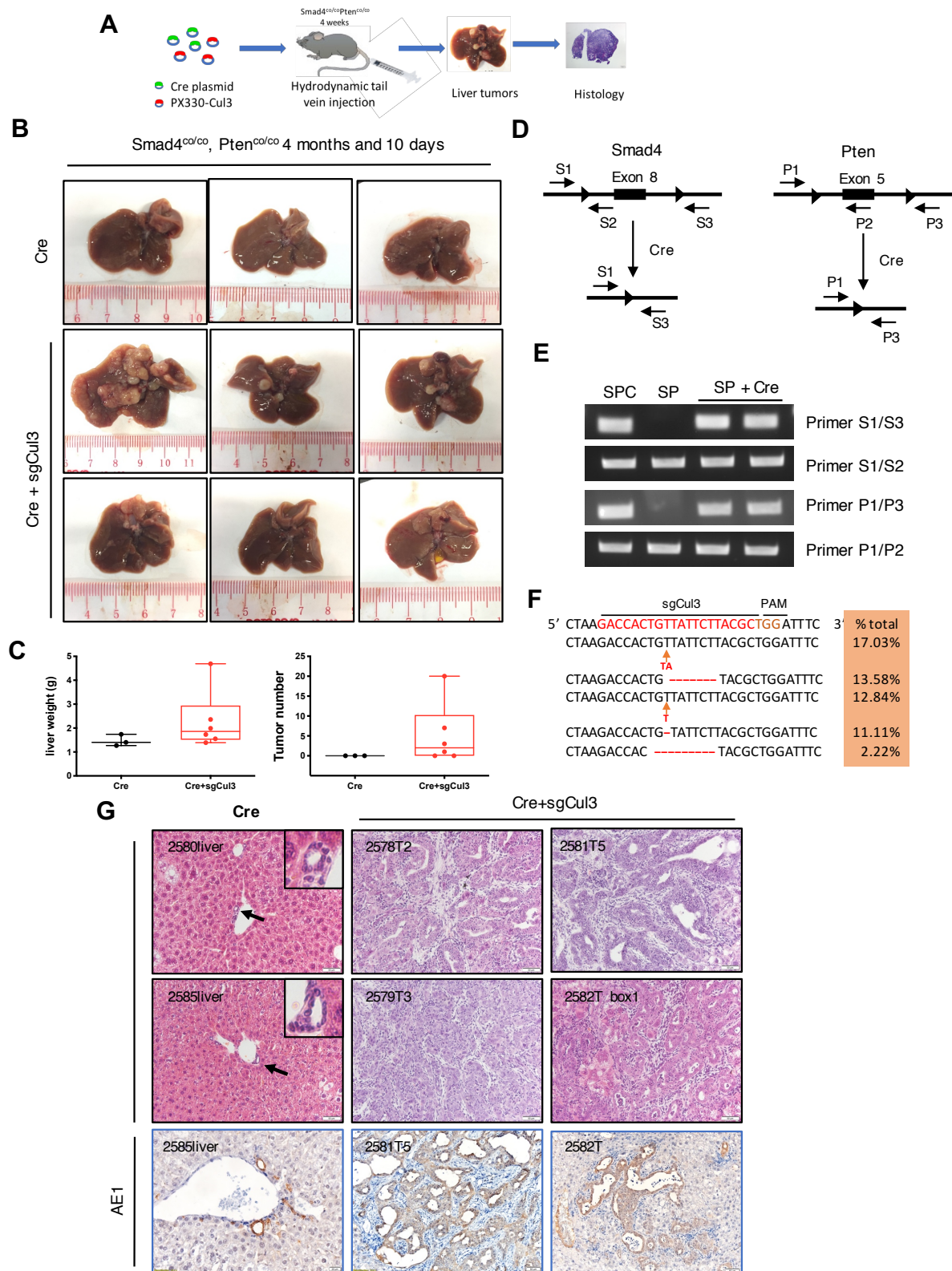


Figure S3. Triple knockout of Smad4, Pten and Cul3 induces liver tumors in wildtype mice. (A) Outline of hydrodynamic tail vein injection of Cre plasmid and sgRNA targeting Cul3. **(B)** Macroscopic images of livers injected with Cre or Cre and sgCul3. **(C)** Statistics of tumor number and liver weight. **(D)** Schematic diagram shows the exon deletion by Cre. **(E)**

PCR products amplified by indicated primers demonstrate the exon deletion. The Alb-Cre in first column is set as a positive control, while the second column without Cre plasmid injection is set as a negative control. **(F)** NGS sequence shows indel mutations of Cul3 targeting site and the percentage of each read. **(G)** Histology analysis of tumors. AE1 indicates cholangiocyte.

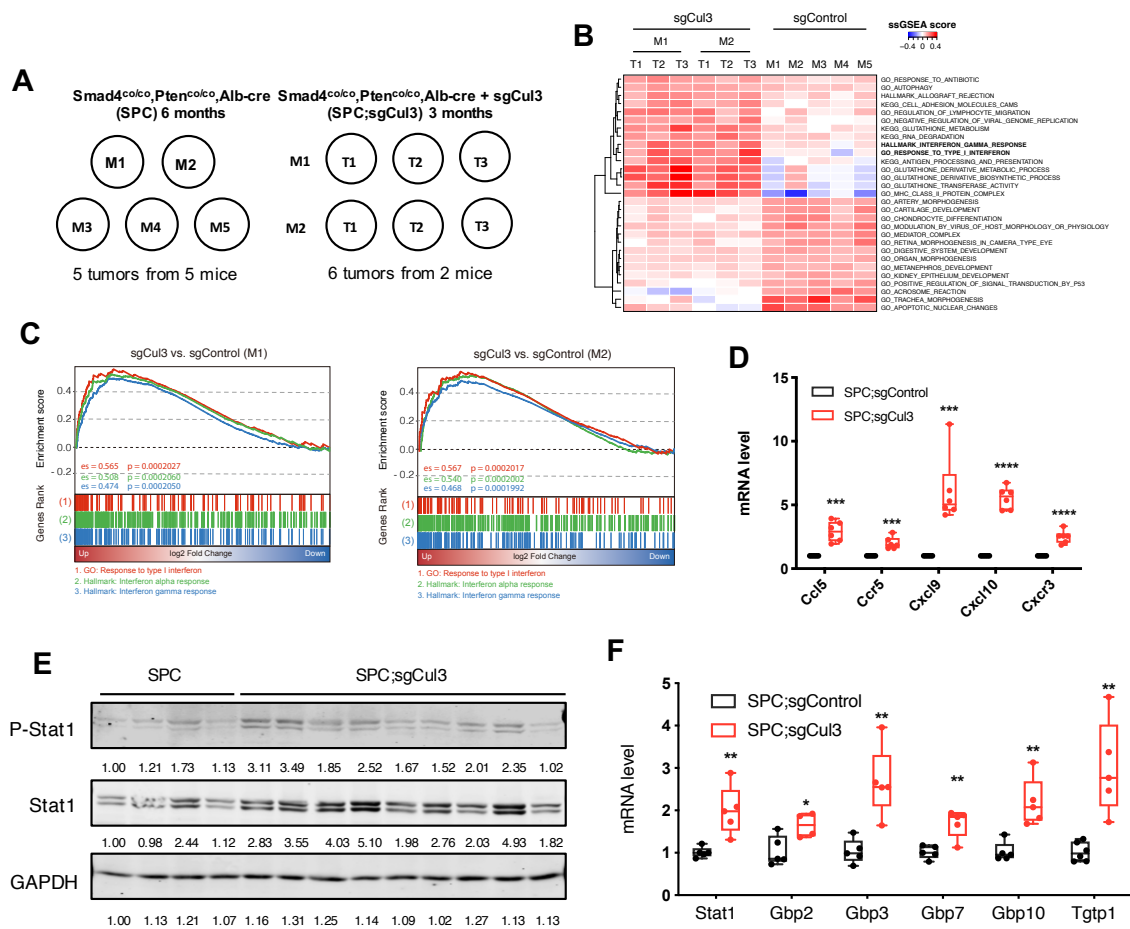


Figure S4. Cul3 deficiency induces inflammation in the tumor. (A) Diagram showing the scheme used for transcriptome analyses. **(B)** The heat-map shows the up- or down-regulated pathways analysed by Gene set enrichment analysis (GSEA). M1-M5 in SPC group indicate 5 mice, and T1-T3 in SPC;sgCul3 group indicate 3 tumors from each mouse. **(C)** GSEA profiles show enrichment of gene sets associated with inflammation. **(D)** RNA-seq data shows ligand-receptor expression changes between tumors from SPC;sgCul3 and SPC mice. **(E)** Western blot and **(F)** qRT-PCR results reveal the activation of Stat1 pathway. * $P < .05$, ** $P < .01$, *** $P < .001$, **** $P < .0001$, # $P > .05$.

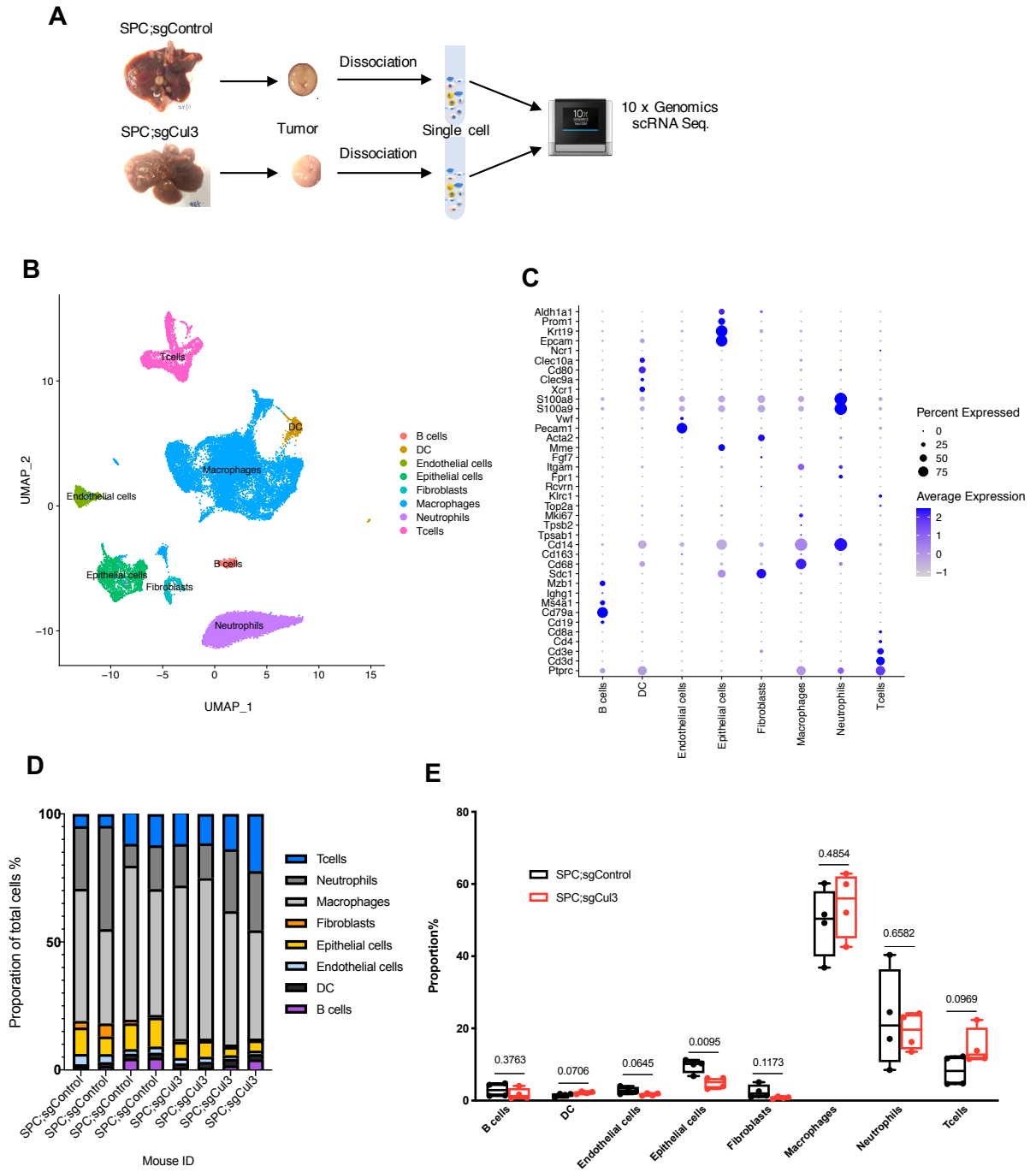


Figure S5. scRNA-Seq identifies major cell clusters in tumors from SPC and SPC;sgCul3 mice. (A) Schematic diagram shows sample collection and single cell preparation for 10× Genomics. The tumors with approximately equal size are isolated from 3-month old SPC;sgCul3 mice and 6-month-old SPC mice. (B) t-SNE plots show the identified cell clusters. (C) Heat-map shows the expression levels of marker genes for each cell population. (D, E) Quantification of each lineages.

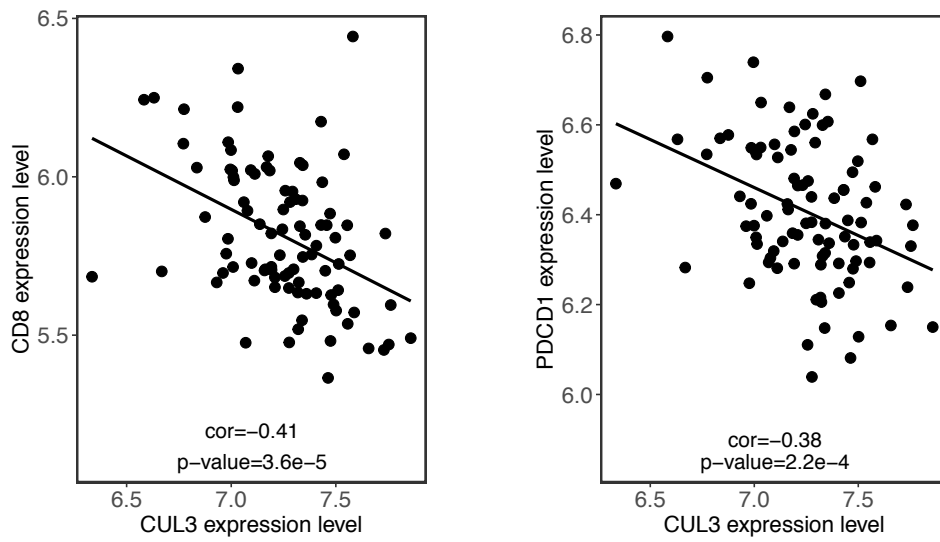


Figure S6. Correlation between CUL3 and CD8 or PD1 in human CC cohort (GSE76297).

Supplementary Table 1 The drug library containing 132 drugs.

No.	Drug	Indication	Target	Approved status
1	10-DAB	Cancer chemotherapy	Microtubule	Not approved (precursor of the approved taxane)
2	2-Methoxyestradiol	Cancer targeted therapy	HIF	Not Approved (Phase 2 Clinical trials)
3	Abiraterone acetate	Cancer hormone therapy	Androgen synthesis	FDA
4	Abitrexate	Cancer chemotherapy	DHFR	FDA
5	Adrucil	Cancer chemotherapy	DNA/RNA Synthesis	FDA
6	Altretamine	Cancer chemotherapy	DNA/RNA Synthesis	FDA (Olaparib approved)
7	Amuvatinib	Cancer targeted therapy	c-Kit, PDGFR, FLT3	INN, USAN (Phase 2 clinical trials)
8	Anastrozole	Cancer hormone therapy	Aromatase	FDA
9	Ancitabine hydrochloride	Cancer chemotherapy	DNA/RNA Synthesis	INN, MI
10	Artemether			
11	Aspirin			
12	Atazanavir sulfate			
13	Axitinib	Cancer targeted therapy	VEGFR, PDGFR, c-Kit	FDA
14	Azacitidine	Cancer chemotherapy	DNA/RNA Synthesis	FDA
15	Azaguanine-8	Cancer chemotherapy	DNA/RNA Synthesis	Not Approved (Clinical trials)
16	Azithromycin			
17	Bendamustine hydrochloride	Cancer chemotherapy	DNA/RNA Synthesis	FDA
18	Bepotastine Besilate			
19	Bergapten			
20	Bindarit	Anti-inflammatory	NF-kB	
21	Bleomycin sulfate	Cancer chemotherapy	DNA/RNA Synthesis	FDA
22	Bosutinib	Cancer targeted therapy	Src,Abl	FDA
23	Busulfan	Cancer chemotherapy	DNA/RNA Synthesis	FDA
24	Cantharidin			
25	Capecitabine	Cancer chemotherapy	DNA/RNA Synthesis	FDA
26	Carbazochrome sodium sulfonate			
27	Carboplatin	Cancer chemotherapy	DNA/RNA Synthesis	FDA
28	Carmofur	Cancer chemotherapy	DNA/RNA Synthesis	FDA
29	Cephalomannine			
30	Chlorambucil	Cancer chemotherapy	DNA/RNA Synthesis	FDA
31	Cinacalcet hydrochloride			
32	Cisplatin	Cancer chemotherapy	DNA/RNA Synthesis	FDA
33	Cladribine	Cancer chemotherapy	DNA/RNA Synthesis	FDA
34	Clofarabine	Cancer chemotherapy	DNA/RNA Synthesis	FDA
35	Clomifene citrate			
36	Clorsulon			
37	Crizotinib	Cancer targeted therapy	c-Met	FDA
38	Cyclophosphamide monohydrate	Cancer chemotherapy	DNA/RNA Synthesis	FDA
39	Cytarabine	Cancer chemotherapy	DNA/RNA Synthesis	FDA
40	Dacomitinib	Cancer targeted therapy	EGFR	INN, USAN (Phase 2 clinical trials)

41	Danuserib	Cancer targeted therapy	Aurora kinase	INN (Phase 2 clinical trials)
42	DAPT (GSI-IX)			
43	Dasatinib	Cancer targeted therapy	Src, Bcr-Abl, c-Kit	FDA
44	Diethylstilbestrol			
45	Dovitinib	Cancer targeted therapy	FGFR	INN, USAN (Phase 2 clinical trials)
46	Doxifluridine	Cancer chemotherapy	DNA/RNA Synthesis	INN, JAN
47	Doxorubicin	Cancer chemotherapy	Topoisomerase	FDA
48	Epirubicin hydrochloride	Cancer chemotherapy	Topoisomerase	FDA
49	Erlotinib hydrochloride	Cancer targeted therapy	EGFR	FDA
50	Esomeprazole sodium			
51	Estramustine	Cancer chemotherapy	Microtubule	FDA
52	Everolimus	Cancer targeted therapy	mTOR	FDA
53	Fludarabine	Cancer chemotherapy	DNA/RNA Synthesis	FDA
54	Flunarizine dihydrochloride			
55	Flutamide	Cancer hormone therapy	Androgen receptor	FDA
56	Formestane	Cancer hormone therapy	Aromatase	INN, BAN
57	Ftorafur	Cancer chemotherapy	DNA/RNA Synthesis	FDA
58	Fulvestrant	Cancer hormone therapy	Estrogen/progestogen Receptor	FDA
59	Gefitinib	Cancer targeted therapy	EGFR	FDA
60	Gemcitabine Hydrochloride	Cancer chemotherapy	DNA/RNA Synthesis	FDA
61	Geniposidic acid			
62	Genistein	Cancer chemotherapy	Topoisomerase	Not Approved (Phase 2 Clinical trials)
63	Histamine dihydrochloride	Cancer immunotherapy	Enhancing IL-2	FDA
64	Ifosfamide	Cancer chemotherapy	DNA/RNA Synthesis	FDA
65	Imatinib mesylate	Cancer targeted therapy	PDGFR, c-Kit, Bcr-Abl	FDA
66	Irinotecan	Cancer chemotherapy	Topoisomerase	FDA
67	Itraconazole			
68	Lamotrigine			
69	Lapatinib Ditosylate	Cancer targeted therapy	EGFR, HER2	FDA
70	Lenalidomide	Cancer immunotherapy	Immunomodulator	FDA
71	Letrozole	Cancer hormone therapy	Aromatase	FDA
72	Linagliptin			
73	Lomustine	Cancer chemotherapy	DNA/RNA Synthesis	FDA
74	Lonidamine	Cancer targeted therapy	Hexokinase	INN, BAN, (Phase 3 clinical trials)
75	Masitinib	Cancer targeted therapy	c-Kit, PDGFR, FGFR	INN (Phase 2/3 clinical trials)
76	MDV3100	Cancer hormone therapy	Androgen Receptor	FDA
77	Mechlorethamine HCL			
78	Megestrol acetate	Cancer hormone therapy	Progesterone analog	INN, BAN
79	Mercaptopurine	Cancer chemotherapy	DNA/RNA Synthesis	FDA
80	Mesna (Uromitexan, Mesnex)			
81	Methacycline hydrochloride			
82	Methazolastone	Cancer chemotherapy	DNA/RNA Synthesis	FDA
83	Miltefosine			

84	Mitotane	Cancer chemotherapy	Adrenal cortex	FDA
85	Moroxydine			
86	Naloxone HCl			
87	Nelarabine	Cancer chemotherapy	DNA/RNA Synthesis	FDA
88	Nilotinib	Cancer targeted therapy	Bcr-Abl	FDA
89	Nilvadipine	Cancer targeted therapy	PARP	FDA
90	Oxaliplatin	Cancer chemotherapy	DNA/RNA Synthesis	FDA
91	Paclitaxel	Cancer chemotherapy	Microtubule	FDA
92	Paeoniflorin	Anti-inflammatory	NF-kB	
93	Pazopanib hydrochloride	Cancer targeted therapy	VEGFR, PDGFR, c-Kit	FDA
94	Pemetrexed disodium	Cancer chemotherapy	DHFR	FDA
95	Phenylbutazone			
96	Phenylbutyric acid			
97	Pioglitazone			
98	Pomalidomide	Cancer immunotherapy	Immunomodulator	FDA
99	Ponatinib	Cancer targeted therapy	Abl	FDA
100	Procarbazine hydrochloride	Cancer chemotherapy	DNA/RNA Synthesis	FDA
101	Regorafenib	Cancer targeted therapy	VEGFR	FDA
102	Rosiglitazone			
103	Ruxolitinib	Cancer targeted therapy	JAK	FDA
104	Saracatinib	Cancer targeted therapy	Src, Bcr-Abl	INN, USAN (Phase 2 clinical trials)
105	Sorafenib	Cancer targeted therapy	VEGFR, PDGFR, Raf	FDA
106	Streptozotocin	Cancer chemotherapy	DNA/RNA Synthesis	FDA
107	Sulindac			
108	Sunitinib malate	Cancer targeted therapy	VEGFR, PDGFR, c-Kit, Flt	FDA
109	Temocapril hydrochloride			
110	Temsirolimus	Cancer targeted therapy	mTOR	FDA
111	Teniposide	Cancer chemotherapy	Topoisomerase	FDA
112	Tofacitinib citrate	Cancer targeted therapy	JAK	FDA
113	Tolbutamide			
114	Tolnaftate			
115	Topotecan hydrochloride			
116	Tretinoin	Cancer targeted therapy	PML-RAR fusion protein	FDA
117	Vandetanib	Cancer targeted therapy	VEGFR	FDA
118	Vatalanib	Cancer targeted therapy	VEGFR, PDGFR, c-Kit	INN (Phase 2 clinical trials)
119	Vemurafenib	Cancer targeted therapy	B-Raf	FDA
120	Vinorelbine	Cancer chemotherapy	Microtubule	FDA
121	Vismodegib	Cancer targeted therapy	Hedgehog	FDA
122	Vorinostat	Cancer targeted therapy	HDAC	FDA
123	XL-184 (Cabozantinib)	Cancer targeted therapy	c-Met	
124	Zoledronic acid			
125	Toremifene	Cancer hormone therapy	Estrogen receptor	
126	Cepharanthine	Anti-inflammatory		
127	AZD2461	Cancer targeted therapy	PARP	Not Approved (Phase 1 clinical trials)

128	Olaparib	Cancer targeted therapy	PARP	FDA
129	Tetrabromo azabenzimidazole	Cancer targeted therapy	Casein kinase-2 (CK2)	
130	Ammonium tetramolybdate	Cancer chemotherapy	Chelating copper	Not Approved (Phase 2 clinical trials)
131	Apatinib	Cancer targeted therapy	VEGFR, PDGFR, c-Kit	FDA
132	Lenvatinib	Cancer targeted therapy	VEGFR, PDGFR, Raf	FDA