Supporting Information

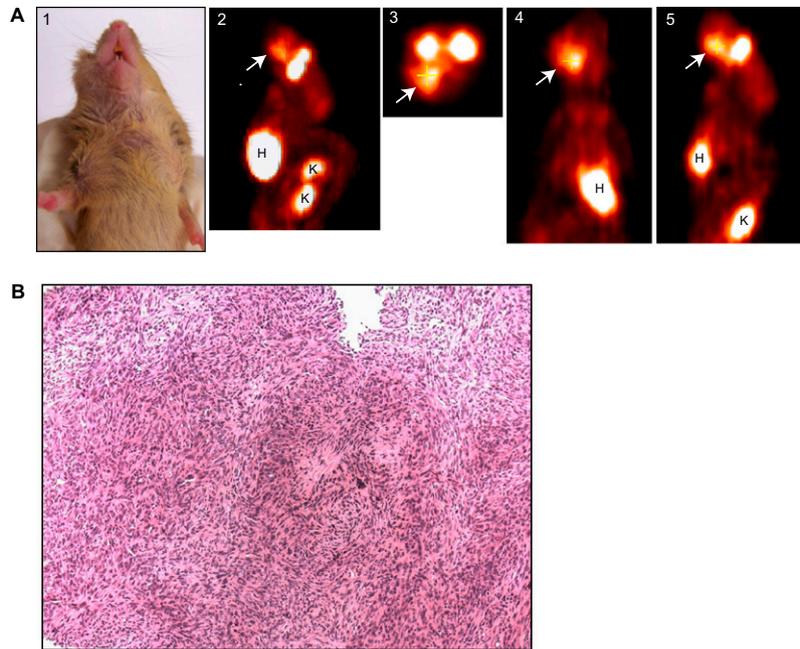## Smith et al. 10.1073/pnas.0913297108

Fig. S1. PET analysis of *Ini1*^{+/−} mouse with a primary rhabdoid tumor resistant to flavopiridol. (*A1*) Digital image of mouse E66, showing a visible mass at the face; (*A2*) PET projection image revealing increased ^{18}F-FDG uptake in the face; (*A3–A5*) Transaxial, coronal, and sagittal sections show various cross-sections of this particular PET scan. White arrows indicate the location of tumor. H, heart; K, kidney. (*B*) H&E-stained section of the primary tumor from mouse E66.
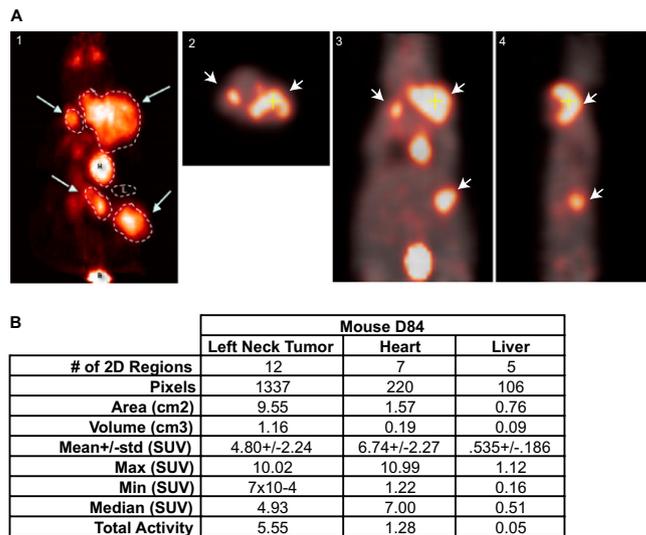

	Mouse D84		
	Left Neck Tumor	**Heart**	**Liver**
# of 2D Regions	12	7	5
Pixels	1337	220	106
Area (cm2)	9.55	1.57	0.76
Volume (cm3)	1.16	0.19	0.09
Mean+/-std (SUV)	4.80+/-2.24	6.74+/-2.27	.535+/-.186
Max (SUV)	10.02	10.99	1.12
Min (SUV)	7x10-4	1.22	0.16
Median (SUV)	4.93	7.00	0.51
Total Activity	5.55	1.28	0.05

Fig. S2. Quantification of rhabdoid tumors using PET. (*A*) Quantitation of tumor masses by PET imaging. (*A1*) Projection image of mouse D84, with dotted white lines drawn around areas of intense 2-[(18)F]fluoro-2-deoxyglucose (^{18}F-FDG) uptake to create a region of interest (ROI) surrounding each tumor mass. Transaxial (*A2*), coronal (*A3*), and sagittal (*A4*) sections show various cross-sections of the same PET scan. White arrows indicate the location of the masses. (*B*) Table with an example of the values obtained after defining ROIs in three dimensions and calculating the standardized uptake values (SUVs) within these regions.
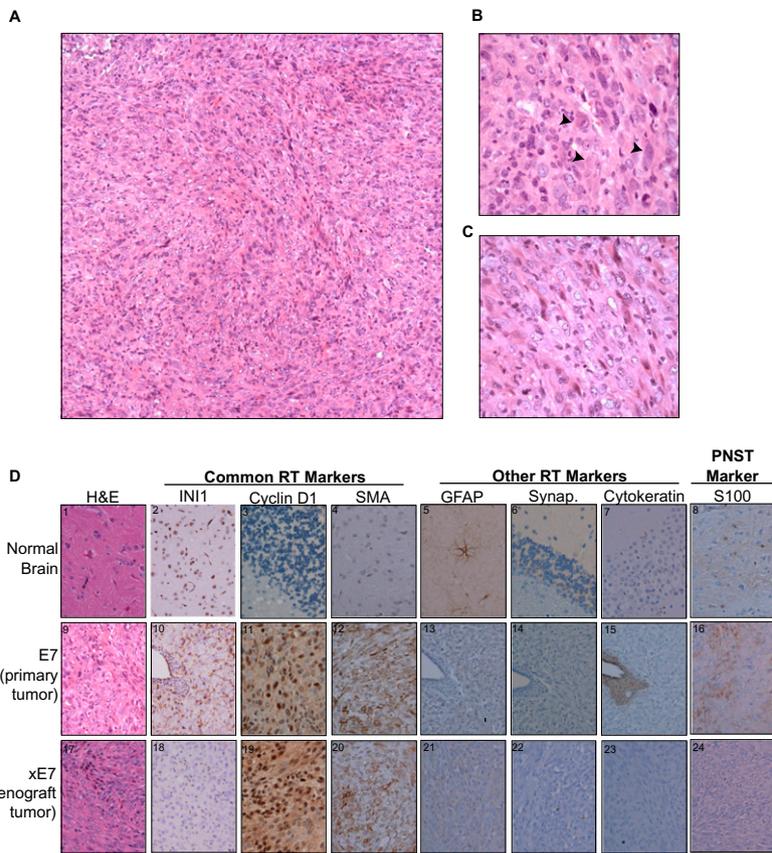
Fig. S3. Flavopiridol is effective in inhibiting primary rhabdoid tumors arising in *Ini1*^{+/−} mice. (A) Digital and PET projection images of *Ini1*^{+/−} mouse D84 treated with flavopiridol. *Upper:* Digital images; *Lower:* PET images that correspond to the digital images above. (A1 and A4) Images taken after 2-wk treatment break from flavopiridol (day 29); (A2 and A5) after the second round of treatment (day 49); (A3 and A6) 10 wk after the last treatment was given (day 120). White arrows point to the locations of tumor masses. H, heart; B, urinary bladder. (B) Digital and PET images of *Ini1*^{+/−} mouse M3 treated with flavopiridol. (B1) Digital image showing the hind leg clasping behavioral abnormality; (B2) PET image before treatment, showing increased uptake in the cerebellum; (B3) PET image after first round of flavopiridol treatment, showing that uptake in the hindbrain had returned to normal levels; (B4) PET image after 2 wk treatment interruption; (B5) PET image after second round of treatment.

Fig. S4. Histological and immunohistochemical characterization of the flavopiridol-resistant rhabdoid tumor. (*A–C*) H&E-stained sections of the primary tumor. (*A*) Representative section of the primary tumor. (Magnification: 10×.) (*B*) Area of the tumor with characteristic rhabdoid cells, indicated by black arrowheads. (Magnification: 40×.) (*C*) Area of the tumor highly characteristic of a spindle cell sarcoma. (Magnification: 40×.) (*D*) Immunohistochemical analysis of: normal brain tissue adjacent to the tumor, used as a control (*D1–D8*); primary resistant tumor (*D9–D16*); and an orthograft-passaged portion of the tumor (*D17–D24*). Antibodies used for staining are indicated above each column. These include common rhabdoid tumor (RT) markers INI1 (*D2*, *D10*, and *D18*), cyclin D1 (*D3*, *D11*, and *D19*), and smooth muscle actin (SMA) (*D10* and *D11*); other RT markers, including glial fibrillary acidic protein (GFAP) (*D5*, *D13*, and *D21*), synaptophysin (Synap.) (*D6*, *D14*, and *D22*), and cytokeratin (*D7*, *D15*, and *D23*); and S100, a marker of peripheral nerve sheath tumor or schwannomatosis (*D8*, *D16* and *D24*). Note that any positive staining for INI1 in *D10* and *D11* is in nontumor stromal cells, endothelial cells, and infiltrating immunocytes. Also note overexpression of cyclin D1 (*D11* and *D19*) and SMA (*D12* and *D20*) within the tumor.
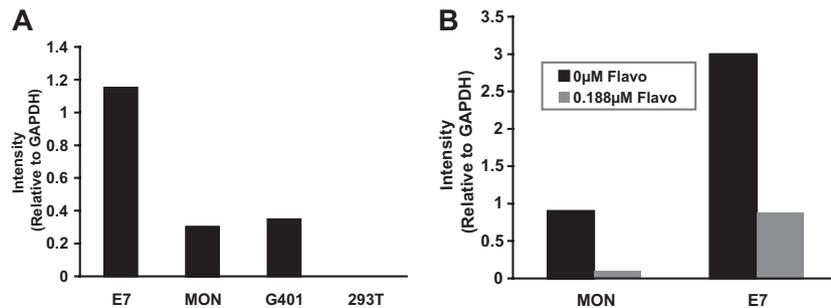

Fig. S5. Densitometry analysis of immunoblots shown in Fig. 4 (main text). (*A*) Densitometric analysis of immunoblot (shown in Fig. 4*C*, main text) quantifying expression of cyclin D1 in E7 cells compared with MON, G401, and 293T cells. (*B*) Densitometric analysis of immunoblot (shown in Fig. 4*D*, main text) quantifying expression of cyclin D1 in MON and E7 cells untreated or treated with 0.188 μM flavopiridol.
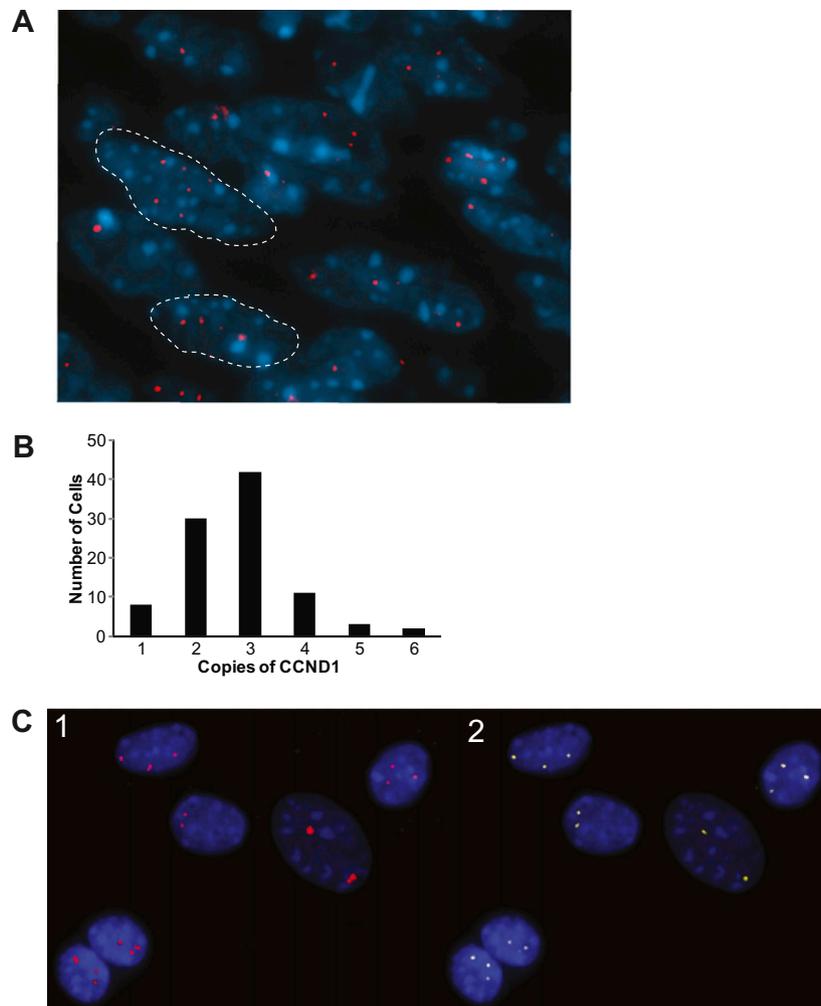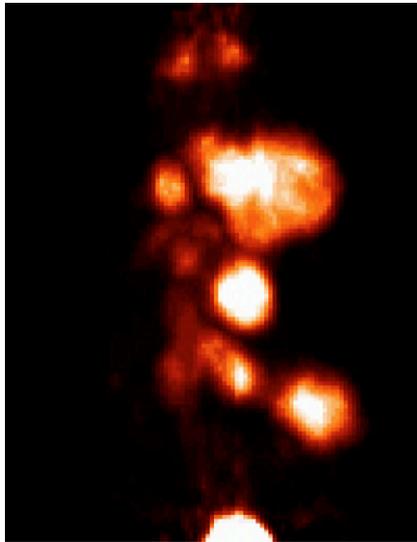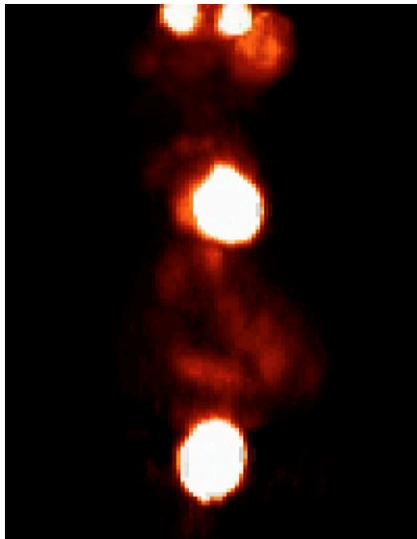
Fig. S6. FISH analysis showing CCND1 amplification in resistant tumors and cells. (*A*) FISH using probe RP24-186B2 on a paraffin section of the E7 tumor tissue. (*B*) Quantitation of *CCND1* signal counts in tumor cells based on FISH analysis of paraffin section of E7 resistant tumor (example of an image is shown in *A*). (*C*) Interphase E7 cells hybridized with RP24-186B2 (*C1*) and RP24-316N16 (*C2*). Note the gain of signals for both *CCND1* and the 3′ flanking region.

Fig. S7. Karyotype and *CCND1* copy number analysis in metaphase spreads of tumor cells. (*A*) Metaphase preparations of a control spleen cell (control) and resistant tumor cells (cells #1–7) stained with DAPI (blue) and probed with RP24-186B2 (C*CND1*, in red). *CCND1* signals are indicated by white arrows. The control hybridization panel shows metaphase spleen preps from C57BL/6J mice hybridized with labeled probes to provide a control for probe specificity. (*B*) Full karyotype from cell 3 from *A*, indicating additional copies of *CCND1* on chromosome 19. (*C*) Karyotpye data showing CCND1 signals on both chromosome 7 and chromosome 19 in a selection of cells from the resistant E7 tumor.

Movie S1. Image from mouse D84 taken on day 1, before treatment.

Movie S1

Movie S2. Image from mouse E7 taken on day 1, before treatment.

Movie S2

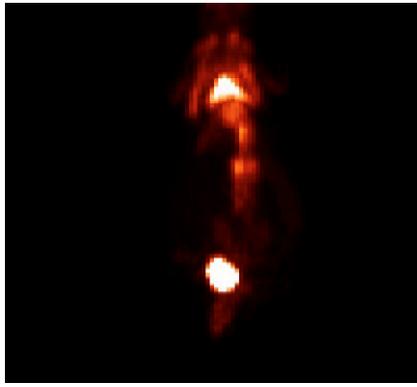Smith et al. www.pnas.org/cgi/content/short/0913297108
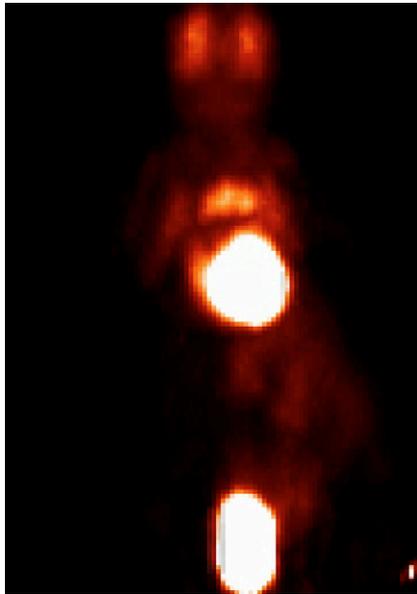
Movie S3. Image from mouse D84 taken on day 18, after completion of round 1 of flavopiridol treatment.

Movie S3
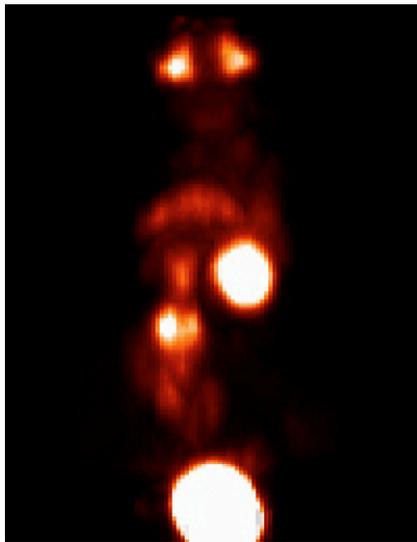
Movie S4. Image from mouse D84 taken on Day 29, after 2-wk treatment interruption.
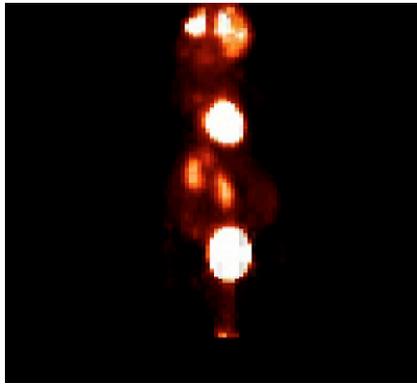
Movie S4

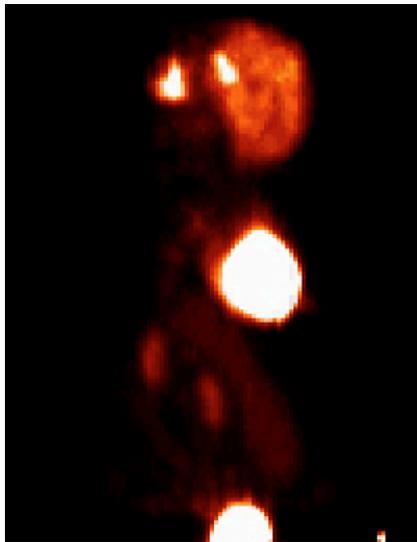**Movie S5.**   Image from mouse D84 taken on day 49, after round 2 of treatment.

[Movie S5](#)
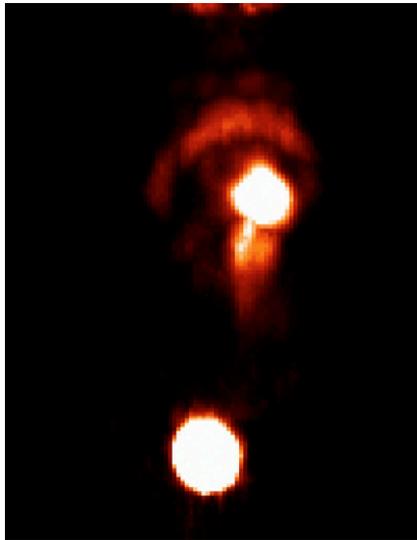
Movie S6.   Image from mouse D84 taken on day 120, 10 wk after the last treatment was given.

[Movie S6](#)

Movie S7. Image from mouse E7 taken on day 12 after 2 wk of treatment.

Movie S7

Movie S8. Image from mouse E7 taken on day 56, after 7 wk of continuous treatment.

Movie S8