

Supplementary Data for:

¹⁸F-FLT Positron Emission Tomography (PET) is a Pharmacodynamic Marker for EWS-FLI1 Activity and Ewing Sarcoma

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Supplementary Table 1

Gene	Cell	P	FDR q	95% FCI Lower	95% FCI Upper
DRD2	TC32	<0.0001	<0.0001	3.618	4.212
	TC71	<0.0001	<0.0001	4.851	5.168
	EW8	<0.0001	<0.0001	4.781	5.094
FOLR1	TC32	<0.0001	<0.0001	2.539	3.051
	TC71	<0.0001	<0.0001	2.393	2.902
	EW8	<0.0001	<0.0001	2.79	3.091
ESR2	TC32	<0.0001	<0.0001	1.659	1.993
	TC71	0.0003	0.0004	1.75	2.487
	EW8	<0.0001	<0.0001	2.501	2.884
DRD3	TC32	<0.0001	<0.0001	1.643	1.902
	TC71	<0.0001	0.0001	1.768	2.34
	EW8	0.0018	0.0024	1.33	1.847
GRM5	TC32	0.0002	0.0003	1.55	2.039
	TC71	<0.0001	<0.0001	2.145	2.503
	EW8	0.0002	0.0003	1.465	1.899
SLC7A11	TC32	0.0016	0.0023	1.216	1.547
	TC71	0.0017	0.0024	1.207	1.531
	EW8	0.0098	0.0127	1.109	1.436
AR	TC32	0.0261	0.0313	1.068	1.453
	TC71	<0.0001	<0.0001	1.306	1.467
	EW8	0.0176	0.0223	1.092	1.478
HK1	TC32	0.0254	0.0313	1.055	1.36
	TC71	0.1697	0.1852	0.968	1.222
	EW8	0.2179	0.2324	0.957	1.212
TSPO	TC32	0.3524	0.3678	0.89	1.322
	TC71	0.6615	0.6615	0.885	1.181
	EW8	0.6615	0.6615	0.885	1.181
ESR1	TC32	0.0358	0.042	1.029	1.263
	TC71	0.037	0.0423	1.028	1.261
	EW8	0.1045	0.1166	0.988	1.258
ENT1	TC32	<0.0001	<0.0001	0.54	0.683
	TC71	<0.0001	<0.0001	0.536	0.62
	EW8	0.0008	0.0012	0.711	0.872
SLC1A5	TC32	<0.0001	<0.0001	0.426	0.624
	TC71	<0.0001	<0.0001	0.581	0.707
	EW8	<0.0001	<0.0001	0.645	0.759
ENT2	TC32	<0.0001	<0.0001	0.395	0.525
	TC71	<0.0001	<0.0001	0.449	0.574
	EW8	0.0001	0.0002	0.783	0.882
ALK	TC32	<0.0001	<0.0001	0.328	0.499
	TC71	<0.0001	<0.0001	0.459	0.551
	EW8	<0.0001	<0.0001	0.312	0.447
TK1	TC32	<0.0001	<0.0001	0.257	0.398
	TC71	<0.0001	<0.0001	0.35	0.502
	EW8	<0.0001	<0.0001	0.313	0.49
EWS-FLI1	TC32	<0.0001	<0.0001	0.085	0.148
	TC71	<0.0001	<0.0001	0.223	0.323
	EW8	<0.0001	<0.0001	0.098	0.112

Supplementary Table 1: Results table for initial screening of genes simultaneously suppressed with ESW-FLI1 (Fig. 1b). FDR q: Benjamini-Hochberg false-discovery rate adjusted p-values. FCI: false coverage interval.

Supplementary Table 2

Gene	Cell	P	FDR q	FCI Lower	FCI Upper
ENT1	TC32	<0.0001	<0.0001	0.5377	0.6223
	TC71	<0.0001	<0.0001	0.6629	0.7571
	EW8	<0.0001	<0.0001	0.5429	0.6371
	A673	<0.0001	<0.0001	0.5338	0.5862
ENT2	TC32	<0.0001	<0.0001	0.47	0.57
	TC71	<0.0001	<0.0001	0.4733	0.5467
	EW8	<0.0001	<0.0001	0.4314	0.5466
	A673	<0.0001	<0.0001	0.499	0.541
TK1	TC32	<0.0001	<0.0001	0.3108	0.3492
	TC71	<0.0001	<0.0001	0.2986	0.3614
	EW8	<0.0001	<0.0001	0.3481	0.4319
	A673	<0.0001	<0.0001	0.309	0.351

Supplementary Table 2: Results table for confirmatory PCR on ENT1, ENT2, and TK1 in four cell-lines (Fig. 1c). FDR q: Benjamini-Hochberg false-discovery rate adjusted p-values. FCI: false coverage interval.

Supplementary Table 3

A

Cell	Treatment	P	FDR q	FCI Lower	FCI Upper
ENT1	50 nM Mithramycin	<0.0001	<0.0001	0.3949	0.6292
	50 nM EC8042	<0.0001	<0.0001	0.47	0.5476
	15 nM EC8105	<0.0001	<0.0001	0.0801	0.11
	24 uM 5-FU	<0.0001	<0.0001	2.1432	2.386
ENT2	50 nM Mithramycin	<0.0001	<0.0001	0.0873	0.3311
	50 nM EC8042	<0.0001	<0.0001	0.2569	0.3221
	15 nM EC8105	<0.0001	<0.0001	0.024	0.0345
	24 uM 5-FU	<0.0001	<0.0001	1.3582	1.5416
TK1	50 nM Mithramycin	<0.0001	<0.0001	0.4401	0.6789
	50 nM EC8042	<0.0001	<0.0001	0.646	0.7338
	15 nM EC8105	<0.0001	<0.0001	0.4036	0.5408
	24 uM 5-FU	<0.0001	<0.0001	1.7067	1.9526

B

Cell	Treatment	Gene	P	FDR q	FCI Lower	FCI Upper
MCF7	Mithramycin	TK1	0.0372	0.0577	0.8450	0.9874
		ENT2	0.0385	0.0577	0.8062	0.9852
		ENT1	0.5341	0.5898	0.9135	1.1534
	5 FU	TK1	<0.0001	<0.0001	1.6289	1.7471
		ENT2	0.5898	0.5898	0.8940	1.0649
		ENT1	0.0003	0.0010	1.4130	1.7249
A2058	Mithramycin	TK1	0.8956	0.8956	0.8099	1.2164
		ENT2	0.0028	0.0113	1.3652	1.8328
		ENT1	0.0279	0.0418	1.0619	1.3426
	5 FU	TK1	0.0180	0.0361	1.1847	1.7749
		ENT2	0.5939	0.7127	0.6613	1.1958
		ENT1	0.0038	0.0113	1.4773	2.1597
RD	Mithramycin	TK1	0.6664	0.6881	0.8274	1.1172
		ENT2	0.6881	0.6881	0.8035	1.2814
		ENT1	0.6208	0.6881	0.7766	1.1428
	5 FU	TK1	<0.0001	0.0005	1.8409	2.2739
		ENT2	0.4979	0.6881	0.8184	1.3411
		ENT1	0.0016	0.0047	1.3135	1.7052
RH30	Mithramycin	TK1	0.6879	0.6879	0.8544	1.0991
		ENT2	0.5188	0.6225	0.8683	1.2498
		ENT1	0.0986	0.1480	0.9812	1.3722
	5 FU	TK1	0.0038	0.0077	1.3742	1.9660
		ENT2	0.0022	0.0065	0.8938	0.9530
		ENT1	0.0001	0.0008	1.6301	1.9653

Supplementary Table 3: (a) Results table for the treatment of TC32 Ewing sarcoma cells with the mithramycin analogs EC-8042 and EC-8105 (Fig. 2b). **(b)** Results table testing the Expression of ENT1, ENT2, and TK1 in 4 control cell lines that do not express EWS-FLI1 (Fig. 2c). FDR q: Benjamini-Hochberg false-discovery rate adjusted p-values. FCI: false coverage interval.

Supplementary Table 4

A

Comparison	Estimated Difference	P	95% FCI Lower	95% FCI Upper
siEWS - siNeg	0.7775	< 0.0001	0.6929	0.8621
MMA - siNeg	0.8475	< 0.0001	0.7629	0.9321
8105 - siNeg	0.7960	< 0.0001	0.7114	0.8806
8042 - siNeg	0.8431	< 0.0001	0.7584	0.9277
5FU - siNeg	-1.1000	< 0.0001	-1.1846	-1.0154

B

Comparison	Estimated Difference	P	95% FCI Lower	95% FCI Upper
siEWS - siNeg	0.0175	0.7909	-0.1100	0.1450
MMA - siNeg	0.1225	0.1897	-0.0050	0.2500
8105 - siNeg	-0.02	0.7909	-0.1475	0.1075
8042 - siNeg	0.075	0.4398	-0.0525	0.2025
5FU - siNeg	0.57	0.0000	0.4425	0.6975

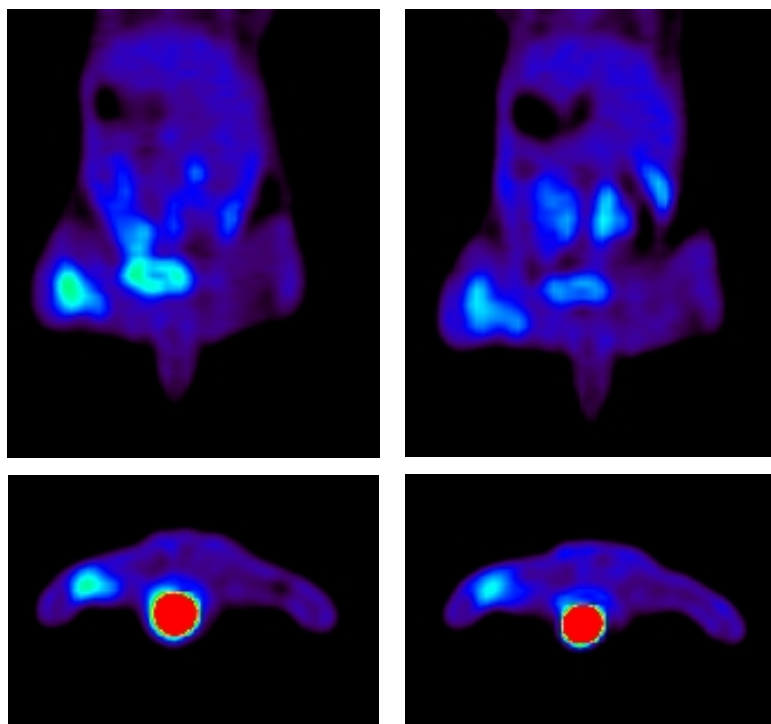
Supplementary Table 4: (a) Results table for changes in ^{18}F -FLT PET in TC32 Ewing Sarcoma cells treated with Mithramycin and its analogs EC-8042 and EC-8105 (b) Results table for changes in ^{18}F -FDG PET in TC32 Ewing Sarcoma cells treated with Mithramycin and its analogs EC-8042 and EC-8105. (Fig. 3a). FDR q : Benjamini-Hochberg false-discovery rate adjusted p -values. FCI: false coverage interval.

Supplementary Figure 1

Mithramycin

T = 0 h

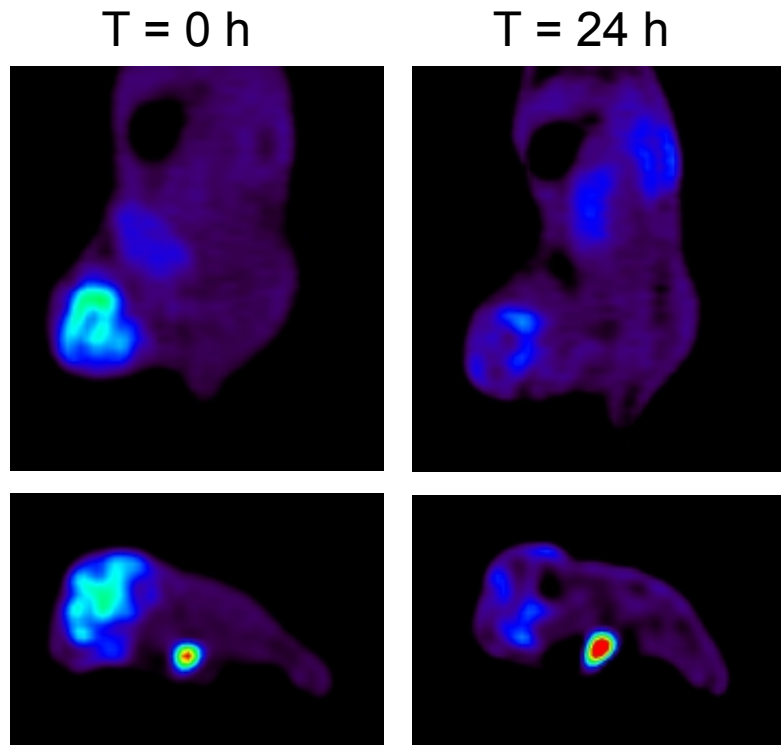
T = 24 h



Supplementary Figure 1: Treatment of mice bearing a Ewing sarcoma xenograft with mithramycin. Mithramycin (1 mg/kg) suppressed ^{18}F -FLT activity at 24 h. Bladder cross section (lower panel) confirms similar amounts of tracer administered.

Supplementary Figure 2

EC-8105



Supplementary Figure 2: Treatment of mice bearing a Ewing sarcoma xenograft with EC-8105. EC-8105 (1.5 mg/kg) suppressed ^{18}F -FLT activity at 24 h. Bladder cross section (lower panel) confirms similar amounts of tracer administered.

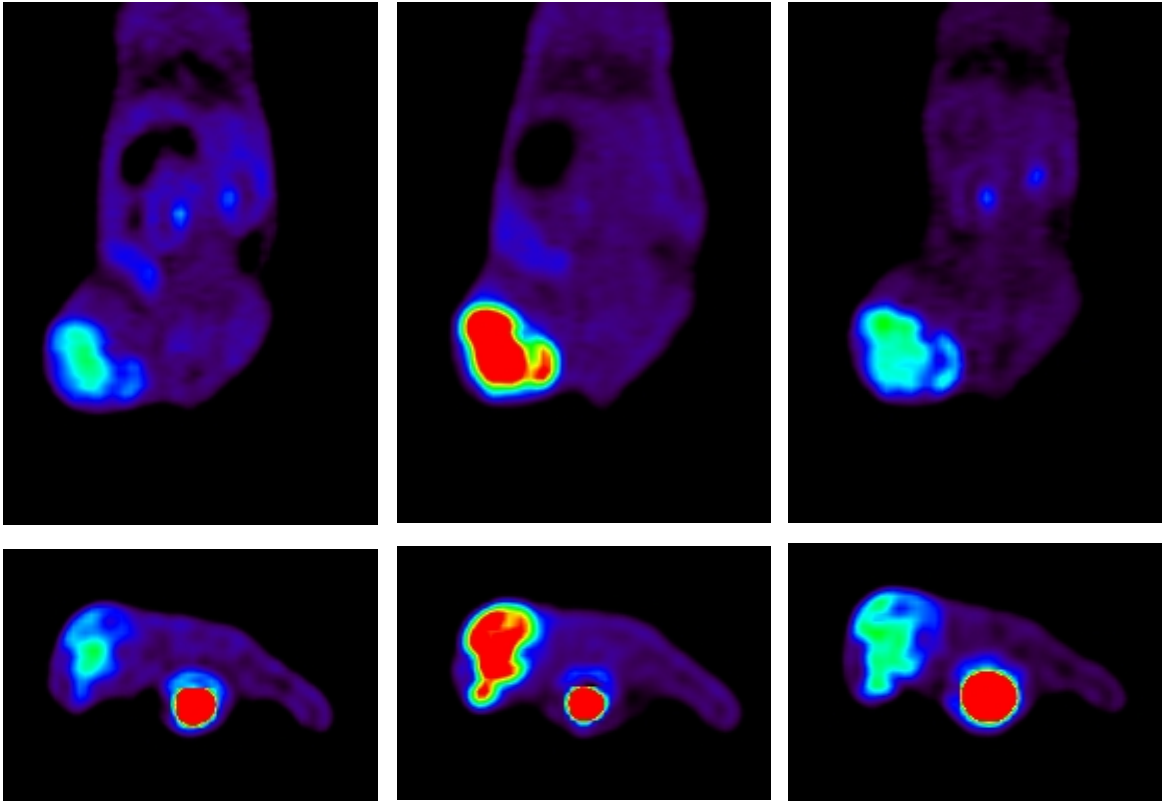
Supplementary Figure 3

5-FU

T = 0 h

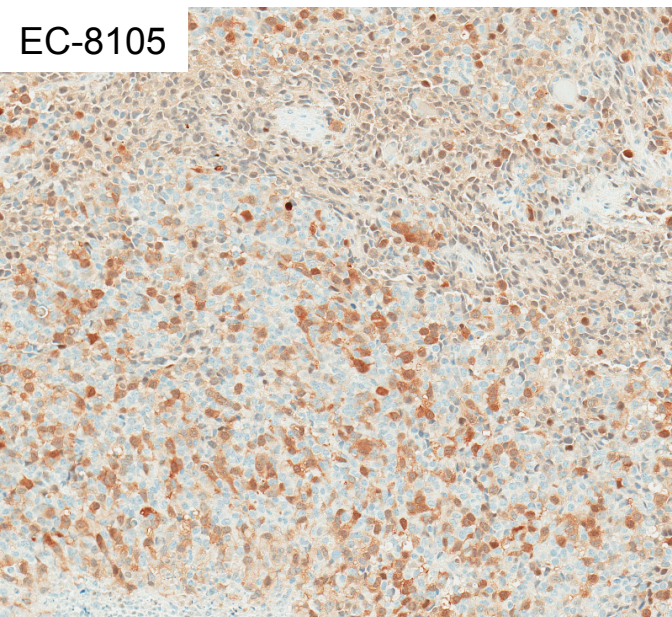
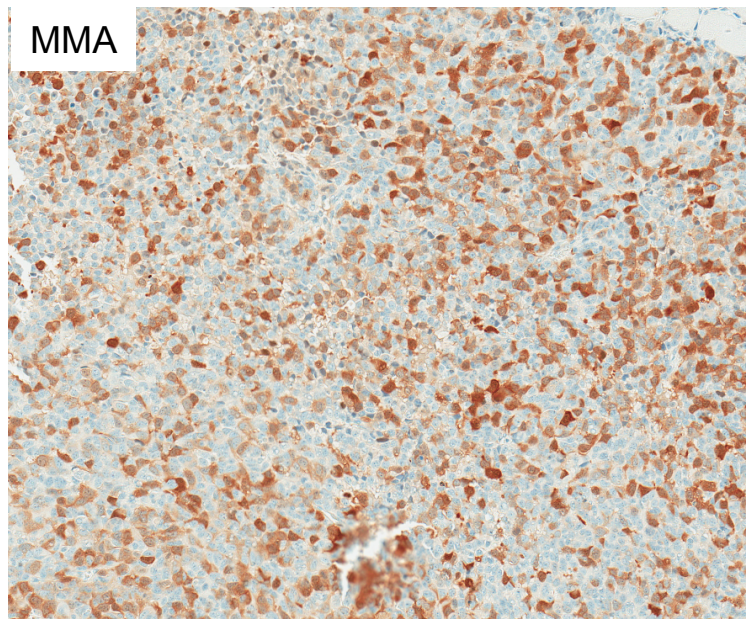
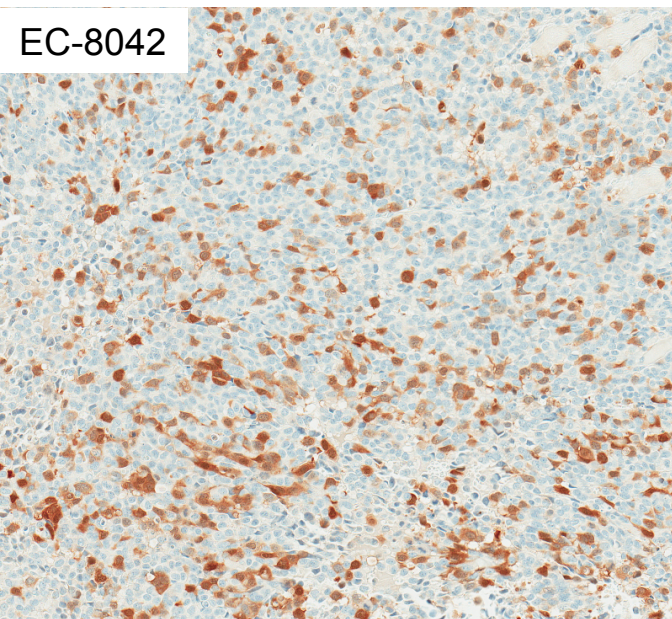
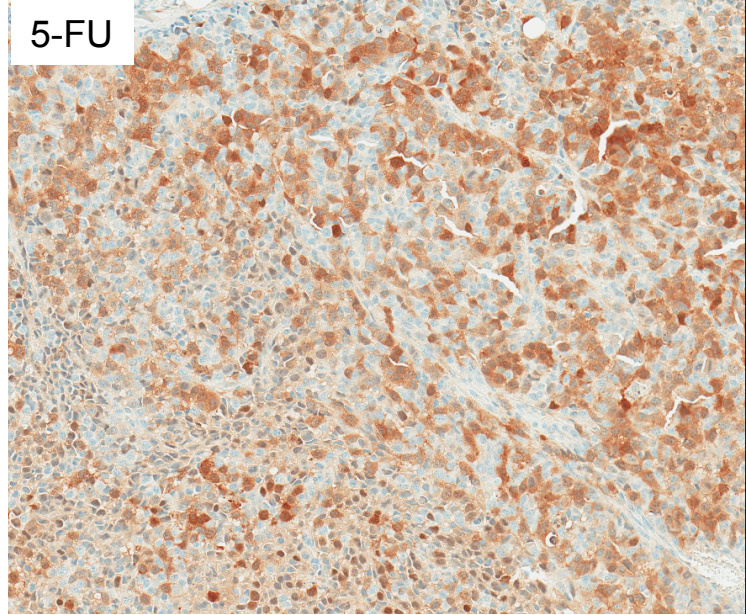
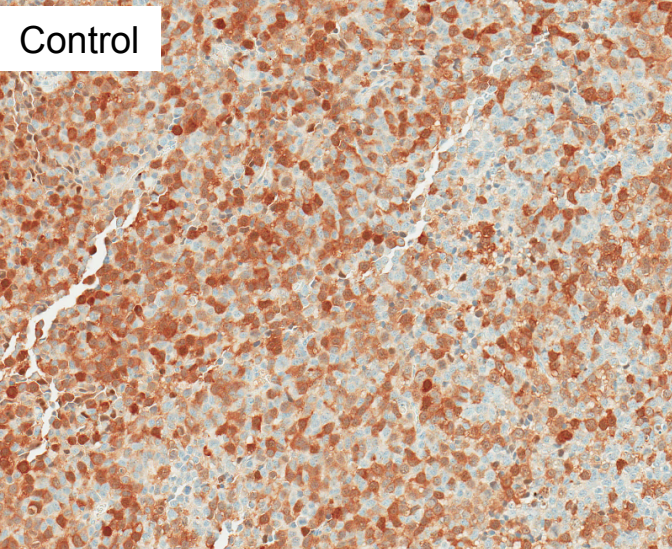
T = 6 h

T = 24 h



Supplementary Figure 3: Treatment of mice bearing a Ewing sarcoma xenograft with 5-FU. 5-FU (50 mg/kg) induced ^{18}F -FLT activity at 6 h. Bladder cross section (lower panel) confirms similar amounts of tracer administered.

Supplementary Figure 4



Supplementary Figure 4: Expanded 10X view of tissue collected at 24 hours stained for TK1 following treatment with Vehicle (control), 5-FU, EC-8042, Mithramycin (MMA), or EC-8105