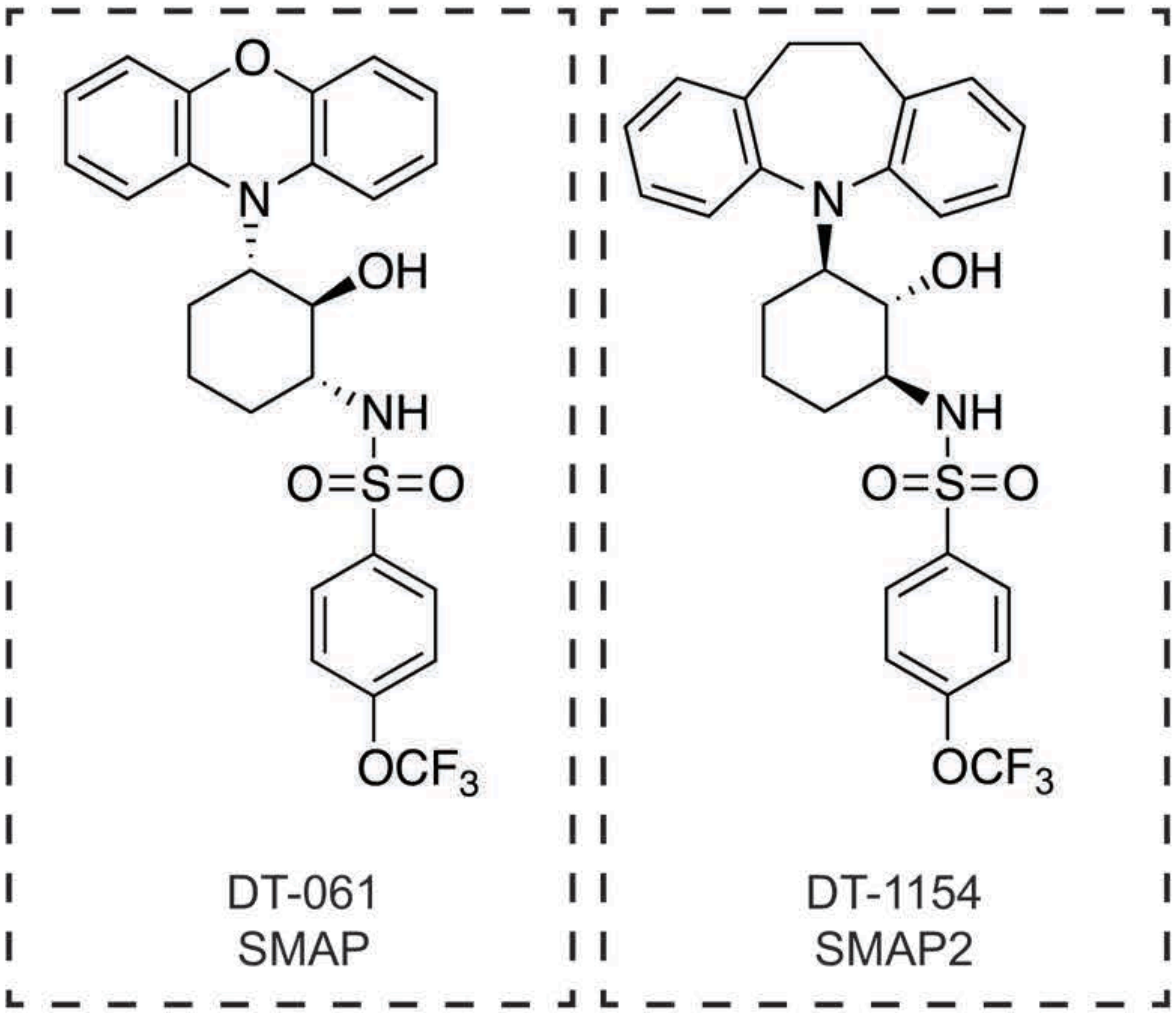
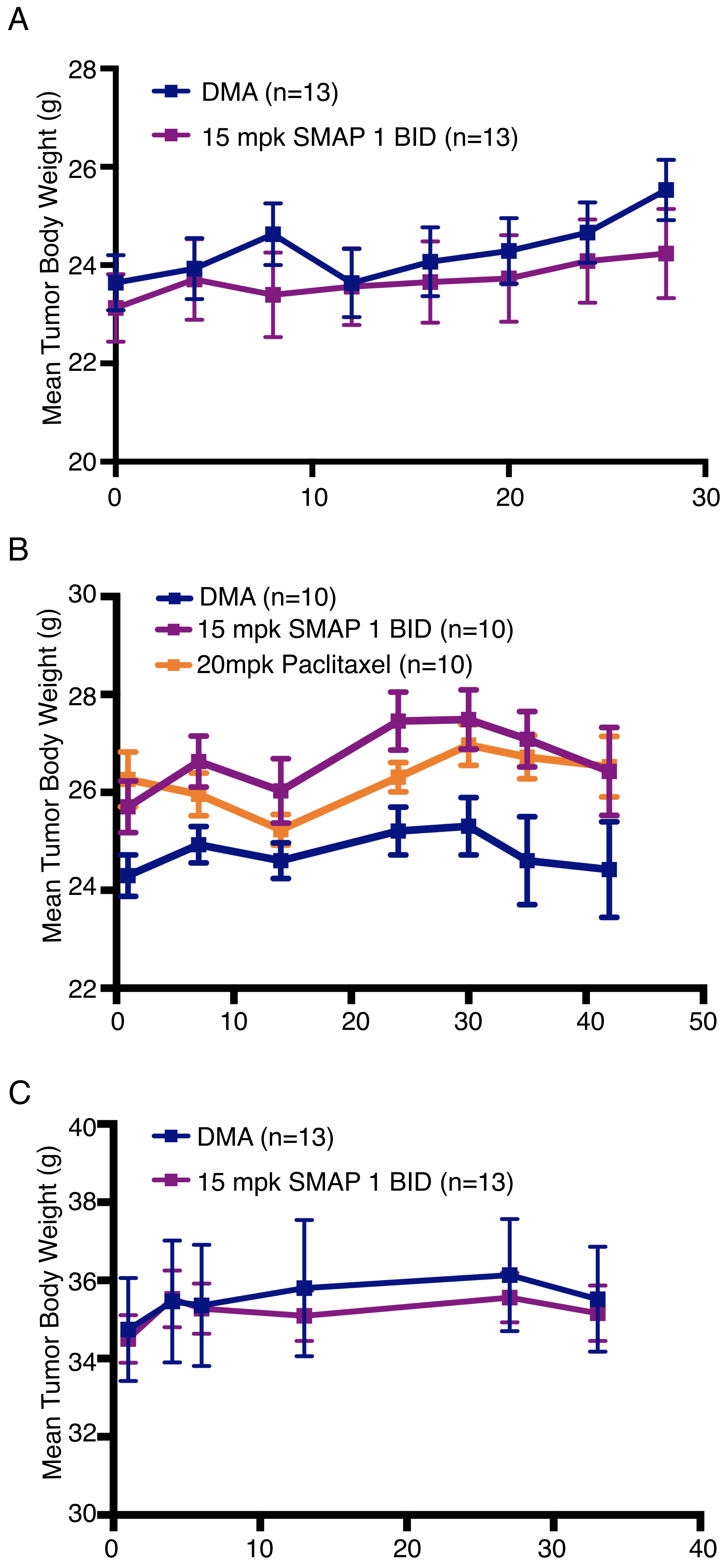


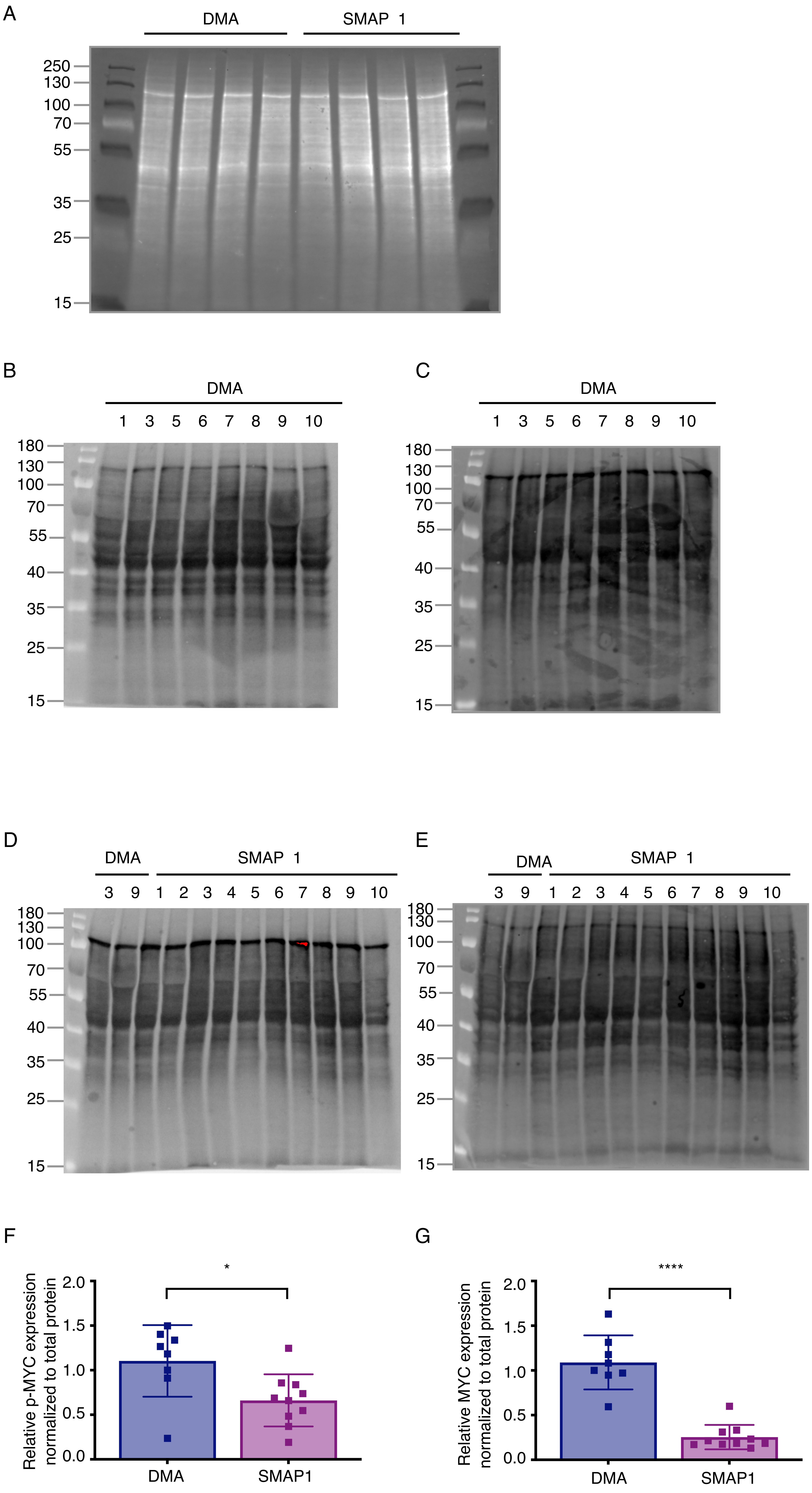
DT-061
SMAP

DT-1154
SMAP2

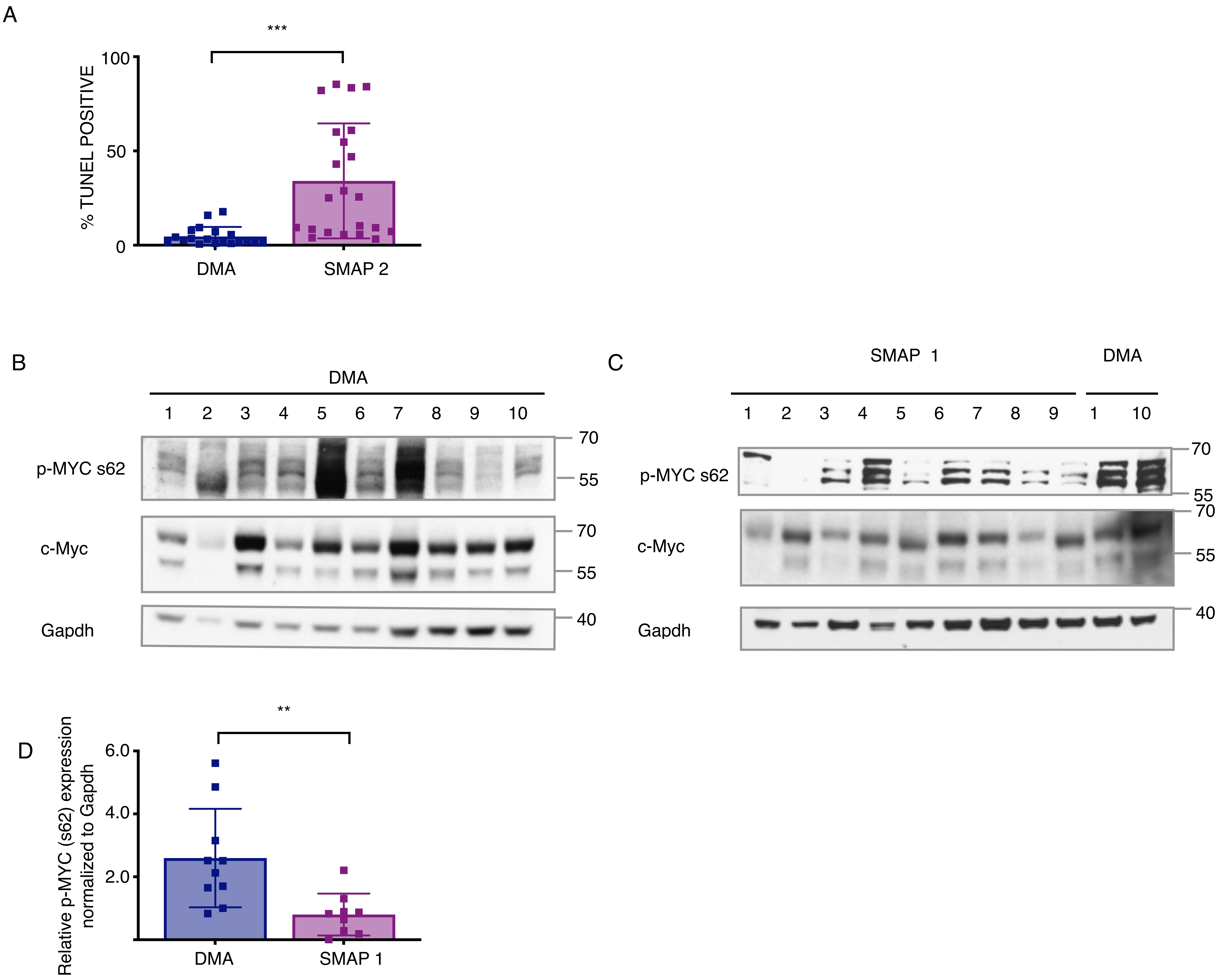
Supplemental Figure 1. Structure of SMAPs



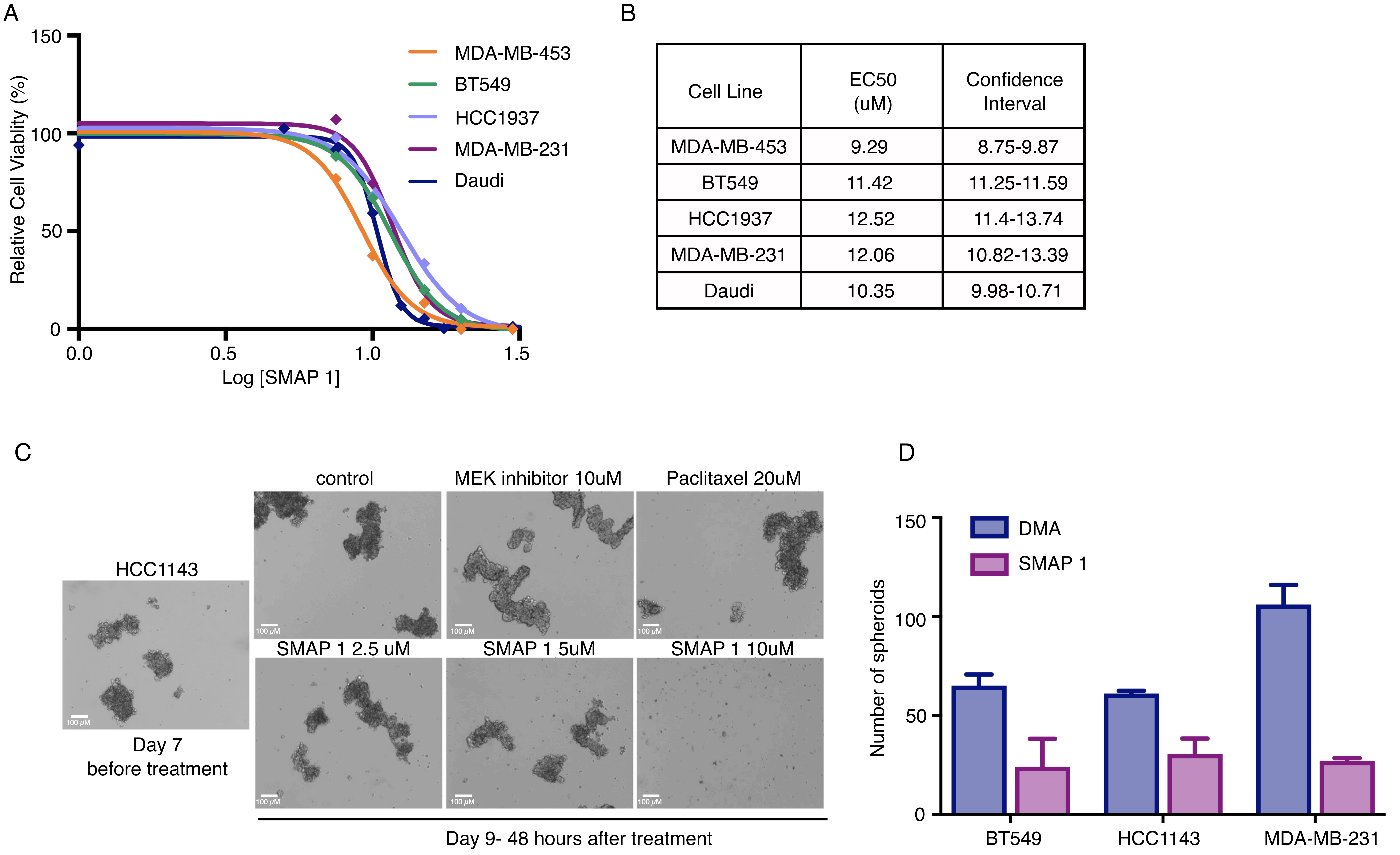
Supplemental Figure 2. SMAPs do not affect mouse body weight. (A) Changes in mouse weight over the course of the Daudi xenograft study (B) the MDA-MB-231 study and (C) the MDA-MB-453 study.



Supplemental Figure 3. (A) Image of membrane used for normalization of protein in Figure 1 C,D. (B) total protein loaded for figure 3C, phospho-MYC intensity in DMA tumors. (C) total protein loaded for Figure 3C western of total MYC in DMA tumors. (D) total protein loaded for figure 3C, phospho-MYC intensity in SMAP 1. treated vs DMA tumors. (E) total protein loaded for Figure 3C western of total MYC in SMAP 1 vs DMA tumors. (F) quantification of phospho MYC in figure 3C normalized to total protein and (G) quantification of total MYC in figure 3C normalized to total protein

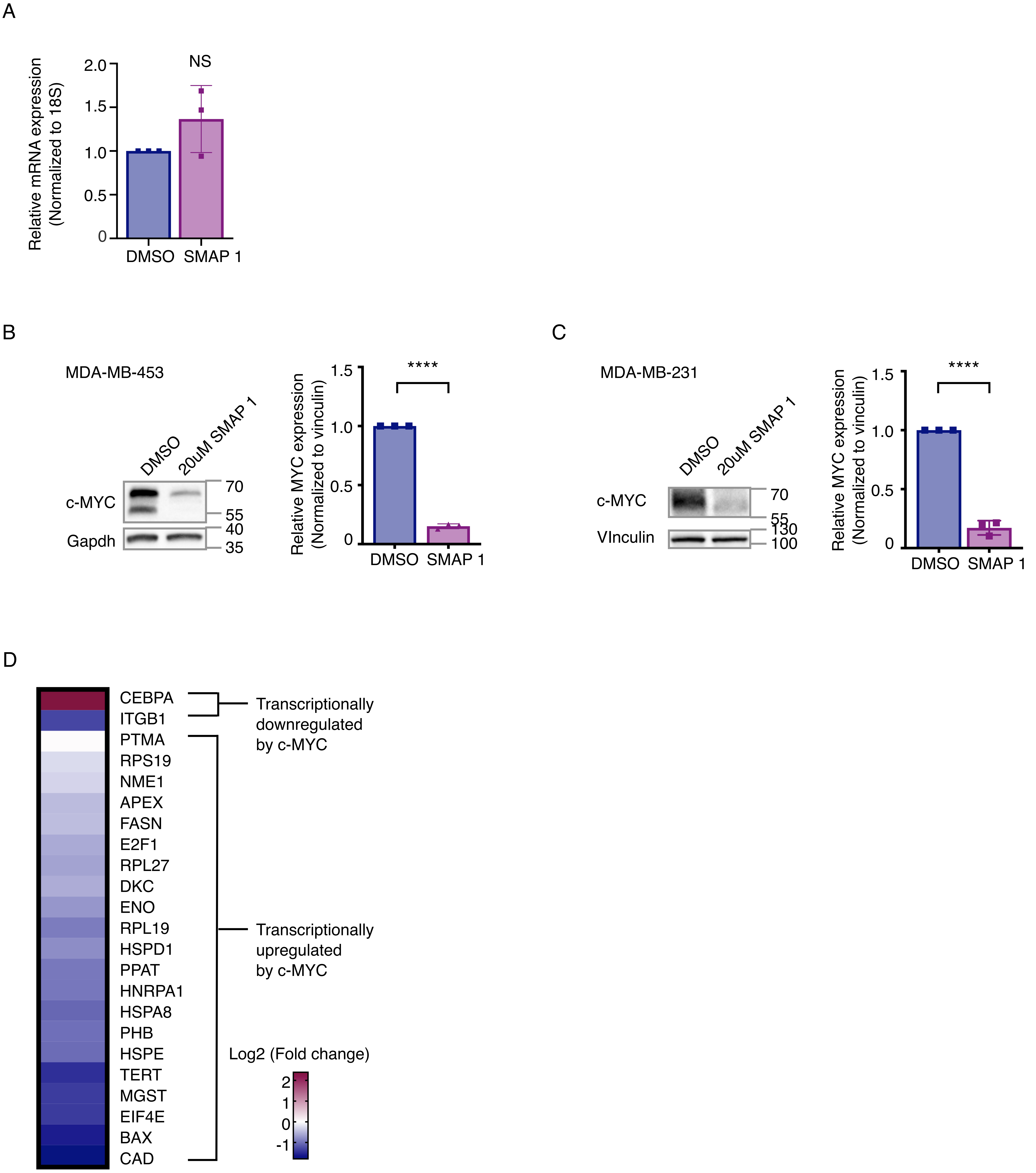


Supplemental Figure 4 SMAP treatment increases TUNEL staining and decrease c-MYC protein expression in NSCLC mouse models upon treatment with SMAPs (A) Quantificaiton of tunel staining from the KRAS LA2 mouse model of NSCLC in DMA versus SMAP 2 treatment (B)Tumor lysates from H441 xenograft treated with DMA and (C) SMAP 1 vs DMA treatment assessed by western blot for phospho c-MYC and total c-MYC protein expression. (D) Quantification of phospho-MYC expression Tumors normalized in quantification to DMA lysate 10 (quantification of total MYC shown in figure 2J). Respective quantifications are represented as mean ± SD. * $P < 0.05$ **$P < 0.01$; ***$P < 0.001$.



Cell Line	EC50 (uM)	Confidence Interval
MDA-MB-453	9.29	8.75-9.87
BT549	11.42	11.25-11.59
HCC1937	12.52	11.4-13.74
MDA-MB-231	12.06	10.82-13.39
Daudi	10.35	9.98-10.71

Supplemental Figure 5. SMAPs decrease cellular viability and spheroid growth in panel of cancer cell lines MTS and MTT viability assays in a panel of Burkitts and breast cancer cell lines upon treatment with varying concentrations of SMAP 1 (A) and calculation of EC50s in (B) Representative image of a mammosphere assay in HCC1143 treated with SMAP 1, MEK inhibitor or Paclitaxel (C) and quantification of n=2 of spheroids upon SMAP treatment in a panel of breast cancer cell lines (D).



Supplemental Figure 6. SMAPs do not induce changes to c-MYC mRNA in Burkitts or breast cancer cell lines and inhibit c-MYC protein in breast cancer cell lines **(A)** mRNA expression of c-MYC in Daudi cell line upon treatment with 20uM SMAP 1 at 6 hours **(B)** Western blot and n=3 quantification for MYC expression in MDA-MB-453 at 3 hours with SMAP 1 treatment and **(C)** Western blot and n=3 quantification for MYC expression in MDA-MB-231 at 3 hours with SMAP 1 treatmen. **(D)** Changes to mRNA expression of c-MYC target genes in MDA-MB-231 upon treatment with SMAP 1 for 6 hours

Target Gene	Primer Sequence
ITGB1 SET F1	CAGTCCAATCCAGAAAATTGG
ITGB1 SET R1	GAGTCGCGGAACAGCAG
HSPA8 SET F1	TGGAAAACACCCACACAAGA
HSPA8 SET R1	TCCTTCGTTATTGGAGCCAG
HSPE SET F1	TGCCTCCATATTCTGGGAGA
HSPE SET R1	AGTAGTCGCTGTTGGATCGG
HSPD1 SET F1	TTGACTGCCACAACCTGAAG
HSPD1 SET R1	CACCGTAAGCCTTTGGTCAT
PHB SET F1	TGGTGAAGATGCGAGGAAG
PHB SET R1	ACCACGTAATGTGCCAGTCA
EIF4E SET F1	CTGGGTTAGCAACCTCCTGA
EIF4E SET R1	CTAAGATGGCGACTGTCGAA
RPL19 SET F1	GCCCATCTTTGATGAGCTTC
RPL19 SET R1	GTGGCAAGAAGAAGGTCTGG
RPL27A SET F1	GCTTTCCCTTTCCCAGAACT
RPL27A SET R1	ACGGGTGAATGCTGCTAAAA
RPS19 SET F1	TCCCCCATAGATCTTGGTCA
RPS19 SET R1	AACTGGTTCTACACGCGAGC
FASN SET F1	TCTCCGACTCTGGCAGCTT
FASN SET R1	GCTCCAGCCTCGCTCTC
CAD SET F1	CAAAGCCCACACAGTTCTCA
CAD SET R1	ATTGGGAGCTGCATGAAGAG
PPAT SET F1	GATTTGAATGTTGGCACCG
PPAT SET R1	TGATCACTCTGGGACTCGTG
MGST1 SET F1	GCATAGGATGCAAAAGCCAT
MGST1 SET R1	TTGTCTGGTATCATGAATTTGGA
PSMB1 SET F1	GTAGCATGGCACTTGCTGAG
PSMB SET R1	TCGGTGGACTTGATGAAGAAG
DKC1 SET F1	GCAGGTAGAGATGACCGCTG
DKC1 SET R1	ATATGAGGACGGCATTGAGG
HNRPA1 SET F1	TCAACCCTCCAATGAAGAGC
HNRPA1 SET R1	GCCGAAGAAGCATCGTTAAA
NCL SET F1	TTGCCTTTCTTCTGAGGTATGA
NCL SET R1	CCTCCTCCAAAGGAGGTAGAA
CEBPA SET F1	TTCACATTGCACAAGGCACT
CEBPA SET R1	GAGGGACCGGAGTTATGACA
E2F1 SET F1	GGCCAGGTACTGATGGTCA
E2F1 SET R1	GACCCTGACCTGCTGCTCT
ENO1 SET F1	CCTGGCATGGATCTTGAGAA
ENO1 SET R1	TACGTTCACCTCGGTGTCTG
NME1 SET F1	CGGTCCTTCAGGTCAACGTA
NME1 SET R1	GCGTTTTGAGCAGAAAGGAT
APEX SET F1	CTGGGGCTTCTTCCTTTACC
APEX SET R1	TGCCACACTCAAGATCTGCT
PTMA SET F1	TTTCCTCATTAGCATTCCCG
PTMA SET R1	AGACACCAGCTCCGAAATCA
TERT SET F1	CAGGATCTCCTCACGCAGAC
TERT SET R1	GAGCTGACGTGGAAGATGAG
BAX1 SET F1	GGAGGAAGTCCAATGTCCAG
BAX1 SET R1	GGGTTGTCGCCCTTTTCTAC
CDKN1B SET F1	TTCATCAAGCAGTGATGTATCTGA
CDKN1B SET R1	AAGAAGCCTGGCCTCAGAAG
MYC SET F1	CACCGAGTCGTAGTCGAGGT
MYC SET R1	TTTCGGGTAGTGGAAAACCA

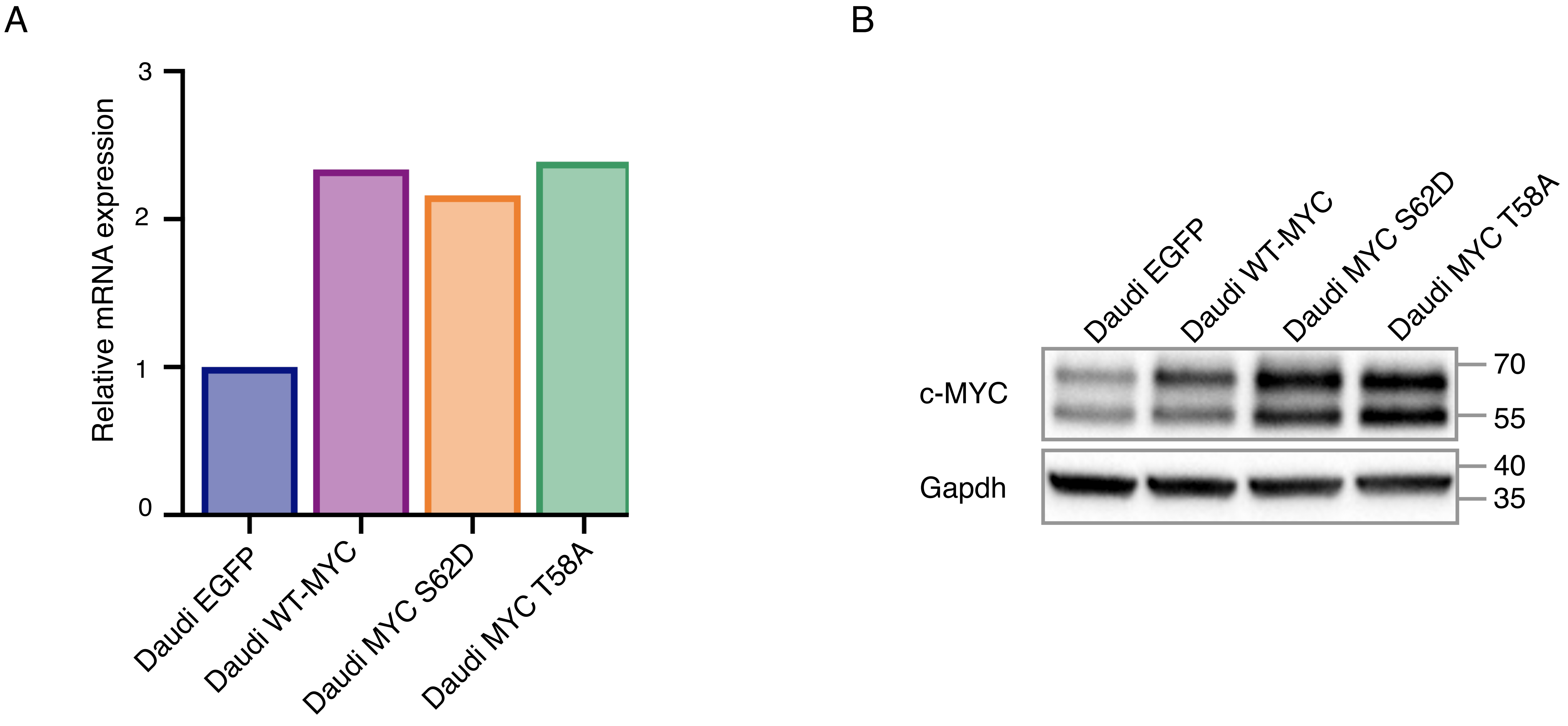
Supplemental Table 1: c-MYC target genes used to assess c-MYC transcriptional activity and corresponding primer sequences fo qRT-PCR.

Gene Target	Mean Log 2 (fold change)	Standard Deviation
CEBPA	6.28	0.27
ITGB1	-1.39	0.61
E2F1	0.32	0.27
RPS19	-0.21	0.25
RPL27	-0.83	0.06
APEX	-0.80	0.12
NCL-2	-0.65	0.29
BAX	-0.68	0.05
RPL19	-0.73	0.23
NME1	-0.73	0.22
FASN	-1.21	0.94
ENO	-1.21	0.61
DKC	-1.11	0.14
HSPA8	-1.17	0.47
PPAT	-1.15	0.15
PSMB	-1.18	0.33
PTMA	-1.30	0.34
HSPD1	-1.29	0.18
TERT	-1.39	0.42
HNRPA1	-1.44	0.49
PHB	-1.57	0.59
EIF4E	-1.68	0.54
HSPE	-1.91	0.24
CAD	-2.46	0.28

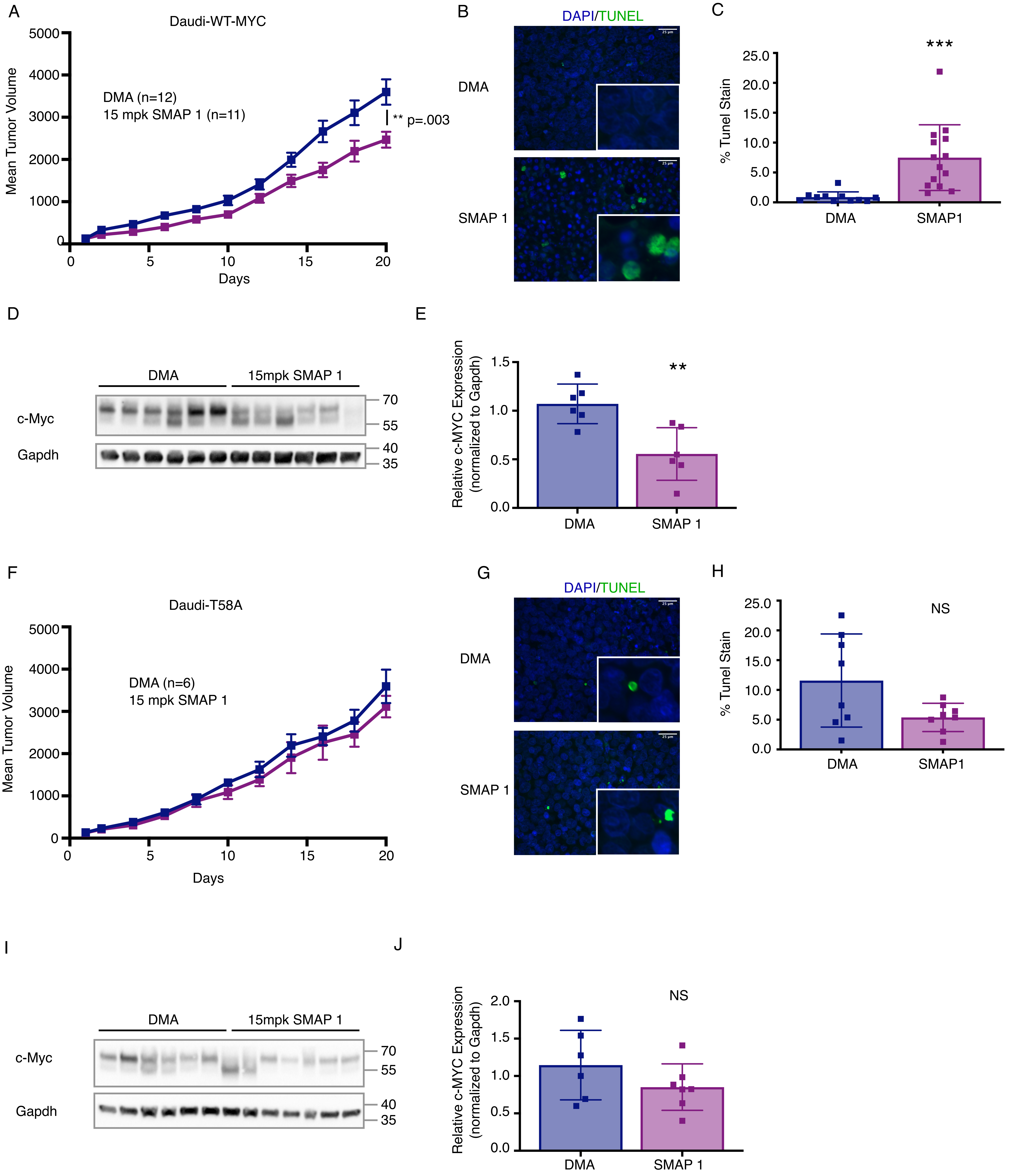
Supplemental Table 2. Changes to c-MYC target genes in the Daudi cell line. Mean (n=4) Log2(fold change) of c-MYC target genes after 6 hours of SMAP 1 exposure normalized to DMSO treated cells.

Gene Target	Mean Log 2 (fold change)	Standard Deviation
CEBPA	0.72	0.15
ITGB1	-0.39	0.26
PTMA	0.01	0.10
RPS19	-0.08	0.16
NME1	-0.09	0.12
APEX	-0.15	0.10
FASN	-0.14	0.04
E2F1	-0.18	0.13
RPL27	-0.20	0.17
DKC	-0.18	0.05
ENO	-0.22	0.18
RPL19	-0.28	0.30
HSPD1	-0.24	0.10
PPAT	-0.29	0.05
HNRPA1	-0.29	0.00
HSPA8	-0.32	0.20
PHB	-0.31	0.04
HSPE	-0.31	0.03
TERT	-0.44	0.26
MGST	-0.41	0.16
EIF4E	-0.42	0.02
BAX	-0.48	0.25
CAD	-0.54	0.26

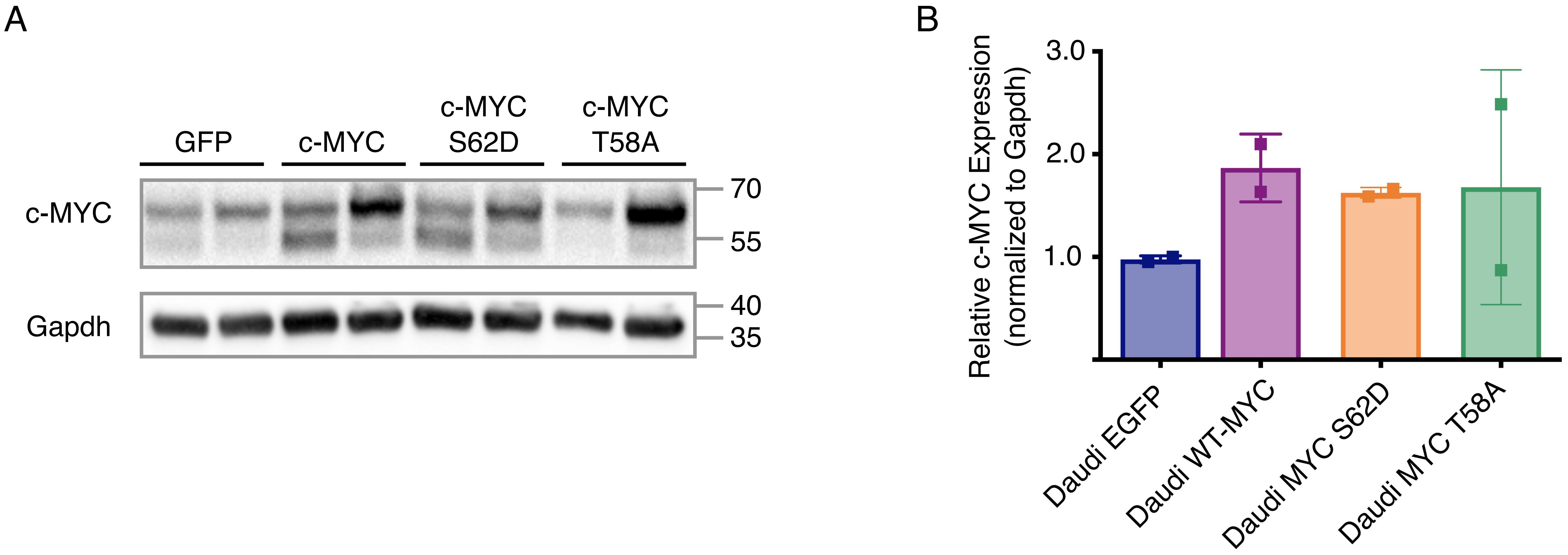
Supplemental Table 3. Changes to c-MYC target genes in the MDA-MB-231 cell line. Mean (n=3) Log2(fold change) of c-MYC target genes after 6 hours of SMAP 1 exposure normalized to DMSO treated cells.



Supplemental Figure 7. (A) Confirmation of MYC overexpression in Daudi cell line. **(B)** mRNA expression for MYC in EGFP, WT-MYC, MYC S62D and T58A overexpressing cell lines and corresponding expression of c-MYC protein.



Supplemental Figure 8. SMAP inhibition of tumor growth and changes to c-MYC expression is abrogated by mutation to c-MYC phosphodegron **(A)**Tumor growth in a xenograft model of Daudi cell line expressing WT c-MYC treated with DMA or SMAP 1 BID **(B)** Representative images of tunel staining of WT c-MYC tumors tumors treated with DMA or SMAP 1 **(C)** quantification of TUNEL in WT c-MYC tumors. **(D)**Western blotting for c-MYC in DMA and SMAP 1 treated WT c-MYC tumors (E) quantification of c-MYC protein in DMA and SMAP treated WT c-MYC tumors normalized to GAPDH **(F)**Tumor growth in a xenograft model of Daudi cell line expressing c-MYC with a T58A mutation treated with DMA or SMAP 1 BID **(G)**Representative images of tunel staining of MYC T58A tumors treated with DMA or SMAP 1 **(H)** quantificaiton of TUNEL in MYC T58 tumors. **(I)** Western blotting for c-MYC in DMA and SMAP 1 treated MYC T58A tumors **(J)** quantification of c-MYC protein in DMA and SMAP treated MYC T58A tumors normalized to GAPDH



Supplemental Figure 9. Confirmation of c-MYC band and overexpression in Daudi tumor lysates. (A) Lysates from two different tumors in each Daudi xenograft presetned in figure 5A-J and supplemental figure 8A-J, Daudi GFP, Daudi c-MYC, Daudi c-MYC s62d, Daudi c-MYC t58A for c-MYC. (B) quantification of MYC expression in A.