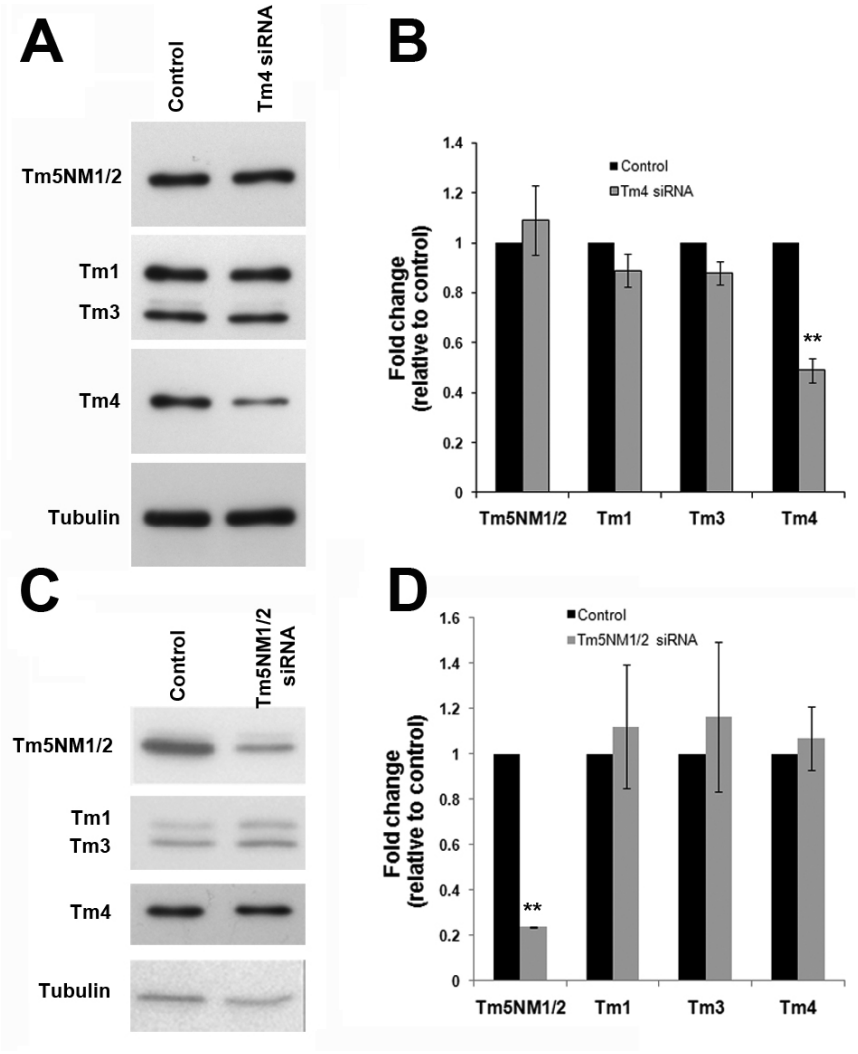
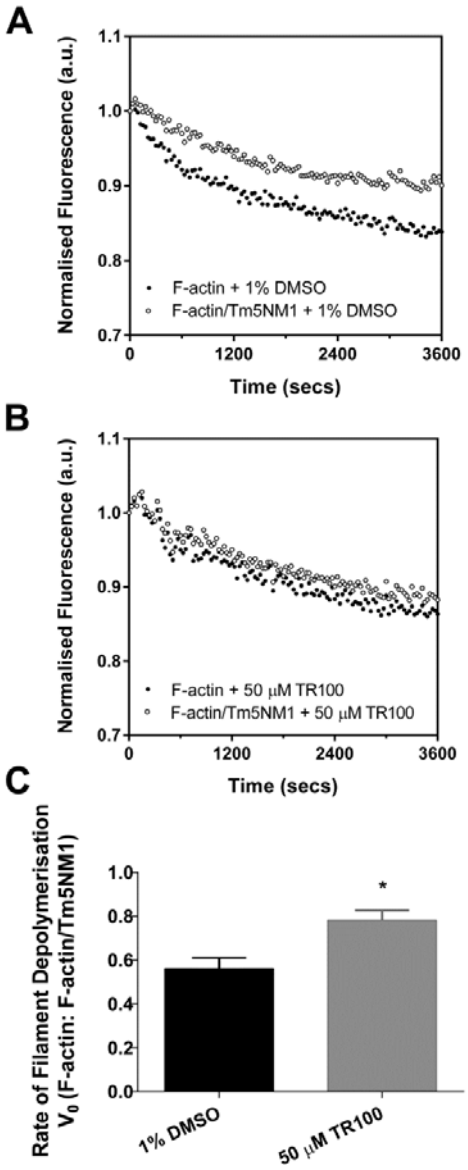
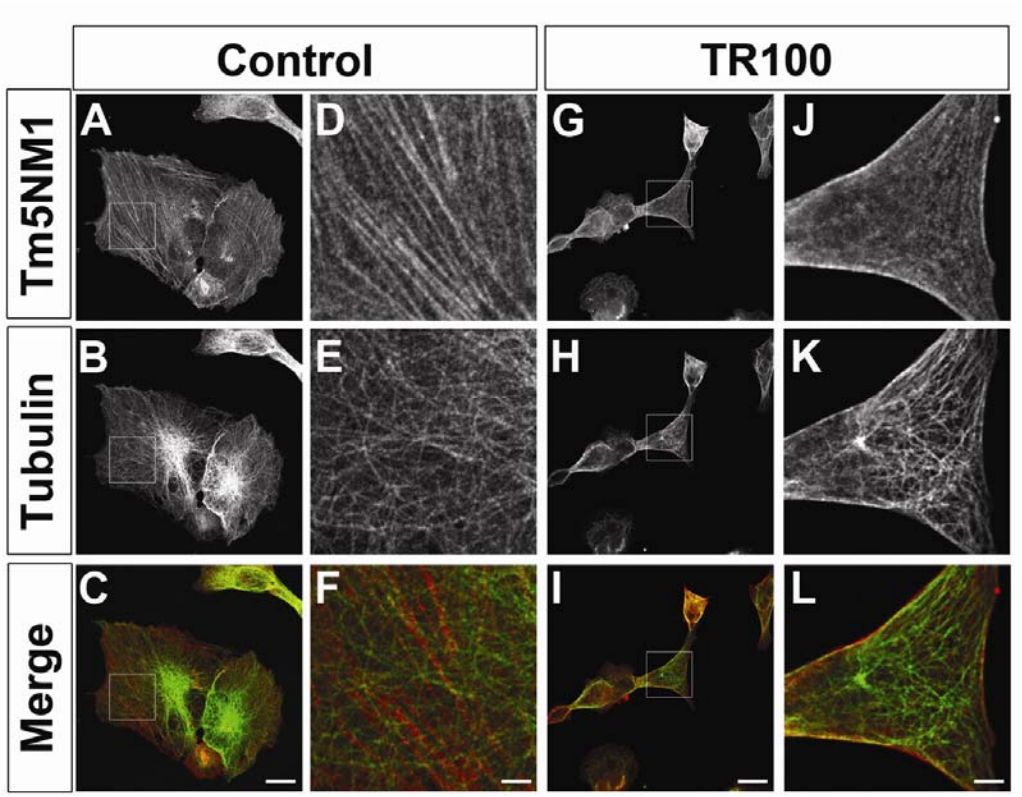
Supplementary Data



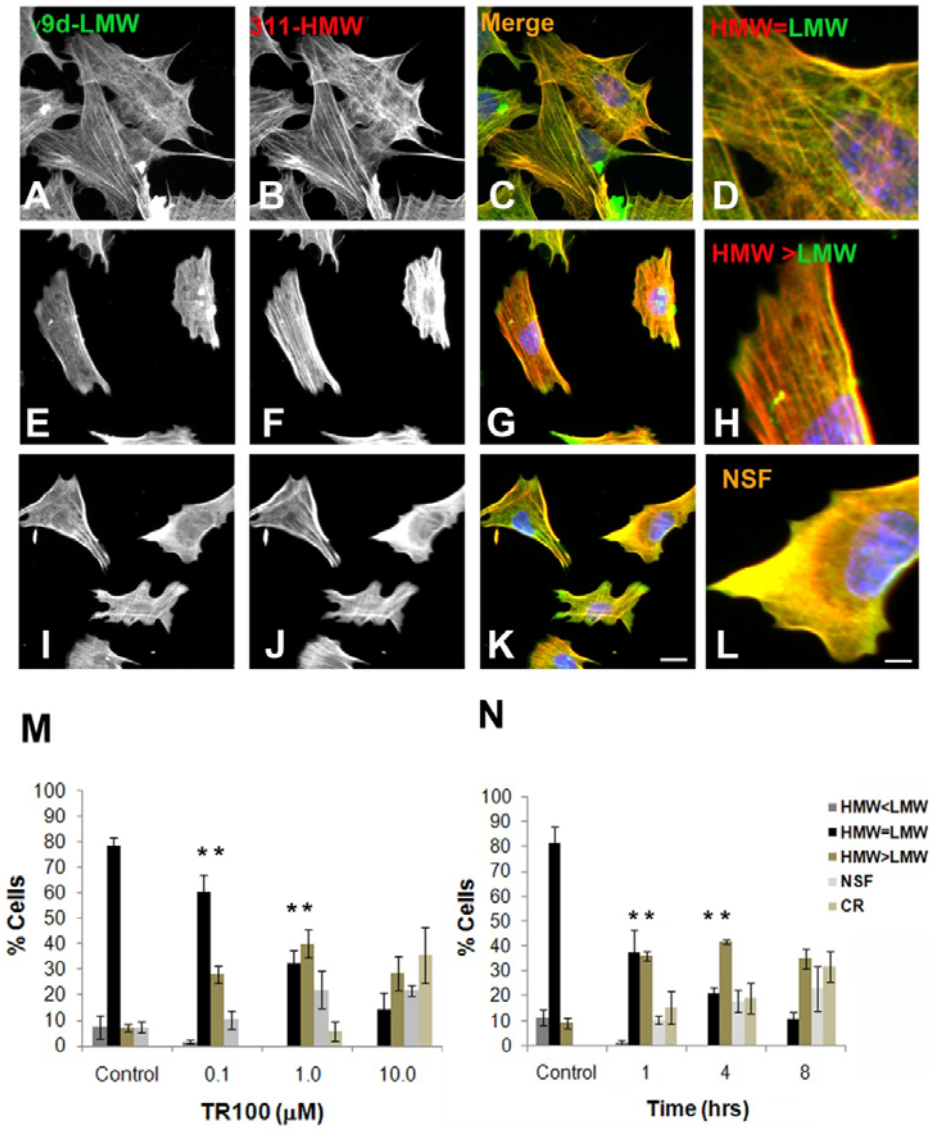
Supplementary Figure 1: Tropomyosin siRNA knockdown is isoform specific and does not result in any compensation. Western blot analysis showing levels of endogenous Tm5NM1/2, Tm1, Tm3 and Tm4 expression in SH-EP cells transfected with Tm4 (**A**) or Tm5NM1/2 (**C**) siRNA. Quantitation of tropomyosin expression in SH-EP cells treated with non-silencing (black bar) or silencing (grey bar) Tm4 (**B**) or Tm5NM1/2 (**D**) siRNA. Data points represent the average of n=3 ±SEM. **P<0.01



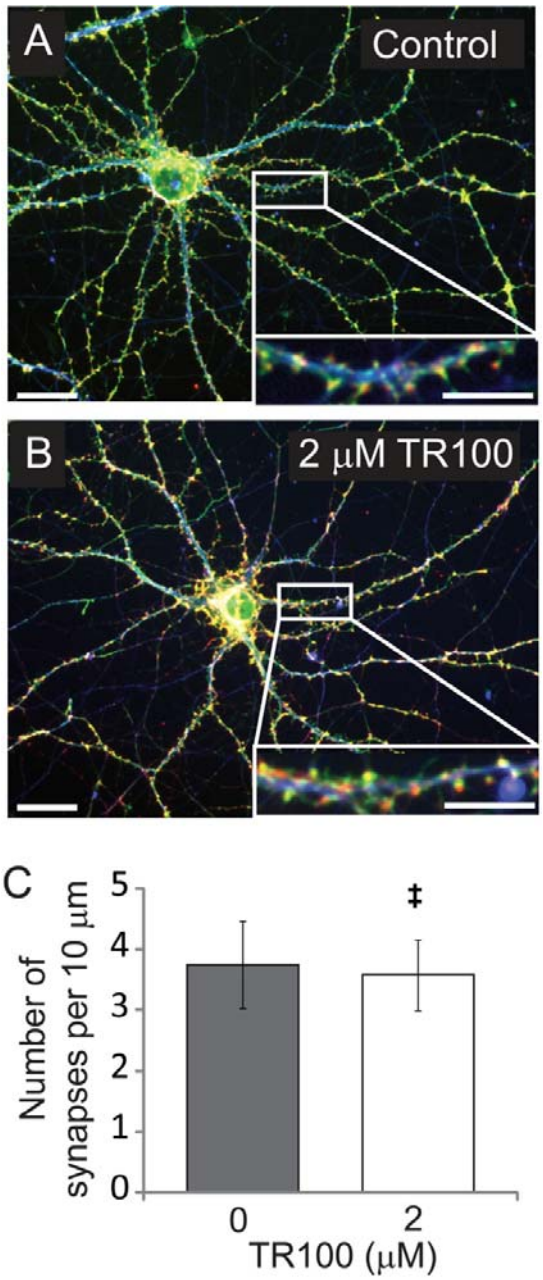
Supplementary Figure 2: **TR100 nullifies the impact of Tm5NM1 on actin filament depolymerization kinetics.** Impact of 1 % (v/v) DMSO **(A)** or 50 μM TR100 **(B)** on the depolymerization of 6 μM actin filaments (35 % pyrene labeled) in the presence or absence of saturating amounts (10 μM) of Tm5NM1. Filaments were diluted 12-fold into F-actin buffer and depolymerization was measured as a decrease in fluorescence over time. Depolymerization data is normalized to the initial fluorescence value. **(C)** Initial rates of depolymerization (V_0) calculated from **A** and **B**, represented as a ratio between F-actin alone and F-actin/Tm5NM1. Data represents the average of n=6 replicates ± SEM. * $P<0.05$.



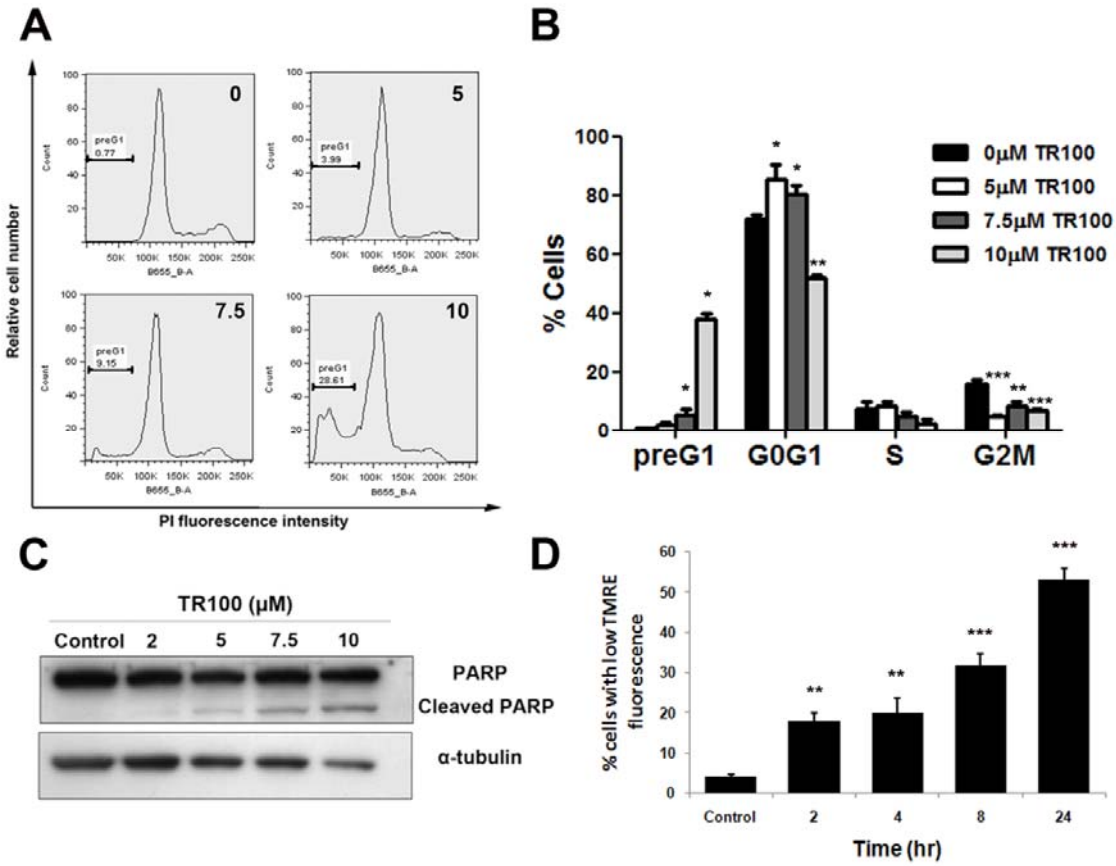
Supplementary Figure 3: **Anti-Tropomyosin compound impact on the actin cytoskeleton assessed by the microfilament disruption assay.** Representative IF images of SH-EP cells treated with DMSO alone (**A-F**) or 5 μM TR100 (**G-L**) for 24 hr and stained with γ9d antibody (**A,D,G and J**) to visualize Tm5NM1/2 or α-tubulin (**B,E,H and K**) to visualize the microtubules. Merged images shown in C,F,I and L. Scale bar = 10 μm (5 μm inset).



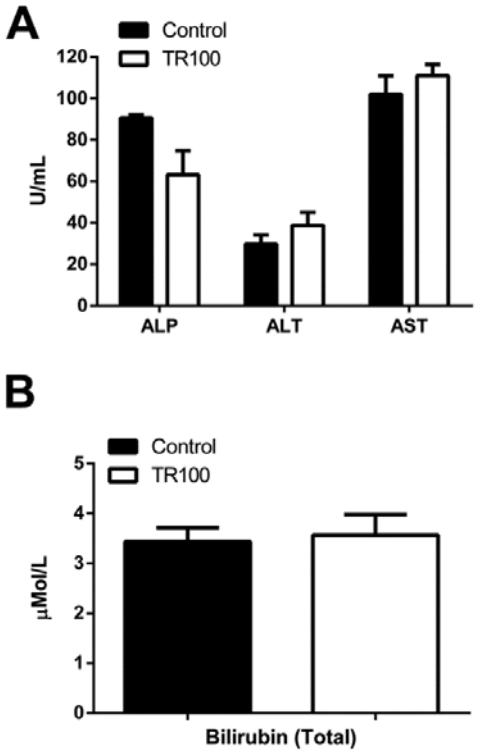
Supplementary Figure 4: TR100 targets the actin cytoskeleton and preferentially disrupts LMW-cytoskeletal tropomyosin containing filaments. For cell scoring endogenous LMW tropomyosin containing filaments were visualized in MEFs untreated (**A-D**) or treated with 10 μM TR100 for 4 hr (**E-H**) or 8hr (**I-J**) using the γ9d antibody (**A,E** and **I**) and HMW containing filaments using the 311 antibody (**B, F** and **J**) with merged images shown in **C**, **G** and **K** and enlarged insets **D, H** and **L**. MEFs were independently scored (blinded) and categorized based on the proportion of HMW to LMW tropomyosin containing filaments within individual cells for both the dose response (**M**) and time course (**N**) experiments. Statistical analysis compared drug treated cells in each condition to the DMSO control for n=3 independent experiments (total of >100 cells per condition analyzed). Scale bar = 10 μm (2 μm inset). * $P<0.05$.



Supplementary Figure 5: TR100 shows no overt impact on synaptic integrity of hippocampal neurons. Long term cultures (19 days in vitro (DIV)) of hippocampal neurons were treated with 0 (**A**) or 2 μM (**B**) TR100 and fixed 48 hr post treatment. The cells were co-stained for synaptophysin as presynaptic marker and fluorophore-tagged phalloidin (postsynaptic marker) to visualize synaptic connections. The number of synaptic connections/ length of dendrite sections were counted from control and 2 μM TR100 treated neurons (**C**). Data points represent the average of 72 dendrite sections from 18 neurons per condition ± Std.Dev. ‡Not significant NS. Scale bar = 25 μm (10 μm insets).



Supplementary Figure 6: TR100 induces apoptosis in tumor cells via a mitochondrial pathway. (**A**) Representative cell cycle plots for SKMEL-28 cells treated with 0, 5, 7.5 or 10 μM of TR100 (24 hr). (**B**) Quantitation of % cells in sub G1 (apoptotic), G_0G_1, S or G_2M of the cell cycle. (**C**) Western blot analysis of cleaved PARP levels in SK-MEL-28 cells after treatment with 0, 2, 5, 7.5 and 10 μM TR100 for 24 hr. (**D**) TMRE fluorescence of SK-MEL-28 cells treated with 2 μM TR100 for 0-24 hr. * P<0.05 **P<0.01 ***P<0.001.



Supplementary Figure 7: TR100 does not impact liver function *in vivo.* (A) Levels of alkaline phosphatase (ALP), liver transaminases (alanine transaminase (ALT) and aspartate transaminase (AST)) were measured to evaluate the impact of TR100 on liver cell integrity. **(B)** Levels of total bilirubin were also measured to evaluate the impact of TR100 on overall liver function. Graph bars represent an average of $n \geq 8$ animals per group ± SEM.

Tumor Type	Cell line	TR100 EC_{50} (μM)
Neuroblastoma	SH-EP	1.9 ± .08
	SKNBe2C	3.24 ± 0.46
	IMR-32	1.24 ± 0.85
	SKN-SH	2.84 ± 0.29
	SKN-AS	3.65 ± 0.19
	CHP134	4.46 ± 0.52
	CHLA20	3.01 ± 0.27
Melanoma	B16/F10	2.61 ± 0.81
	SKMEL-28	2.83 ± 0.74
	WM793	1.85 ± 0.40
	1205Lu	2.99 ± 0.7
	WM35	3.23 ± 1.01
	451Lu	2.07 ± 0.32
	WM164	4.34 ± 1.96
	C8161	4.73 ± 1.19
MPNST	S462-TY	3.45 ± 1.29
	STS26T	4.99 ± 0.24
	ST88-14	3.40 ± 1.06
	T265p21	4.27 ± 0.74
	S462	3.42 ± 1.63
	CMTRL-100	4.50 ± 0.64
Ewings	A673	~ 4.02
	TC71	4.48 ± 1.78
Leukemia	K562	3.57 ± 0.15
	HL 60	4.59 ± 0.69
	Ocl/AML3	4.17 ± 0.9
T-cell Lymphoma	H9	2.98 ± 1.06

Supplemental Table 1: Summary of impact of TR100 on tumor cell viability. Effect of the anti-Tm compounds on tumor cell viability was assessed in a panel of tumor cell lines using MTT cytotoxicity assays. Effective concentration (EC_{50}) was determined using Graph Pad Prism 5.