

# Supporting Information

## Blood–Brain Barrier- and Blood–Brain Tumor Barrier-Penetrating Peptide-Derived Targeted Therapeutics for Glioma and Malignant Tumor Brain Metastases

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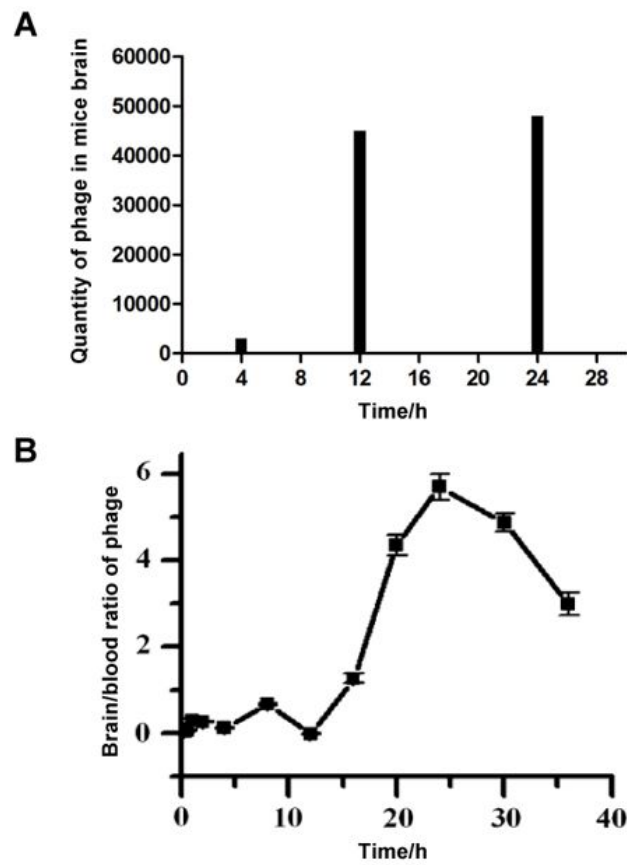
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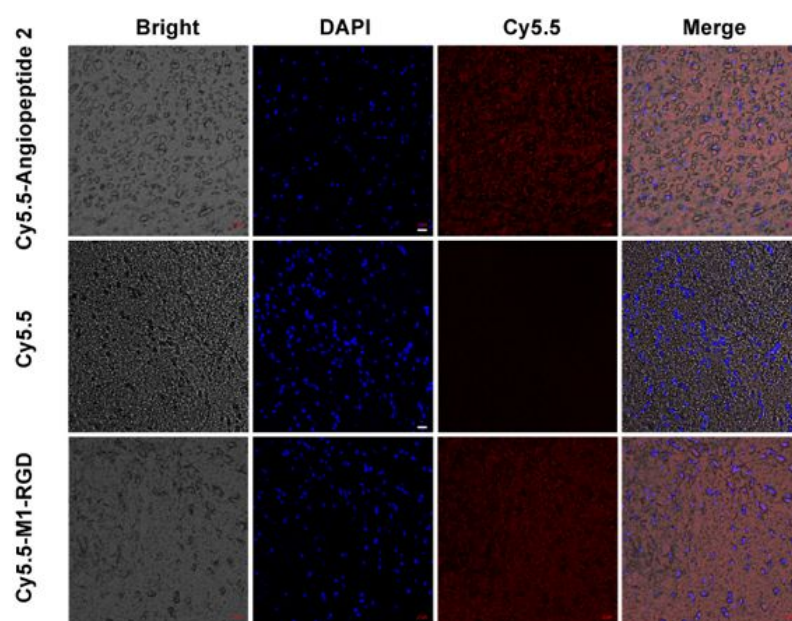
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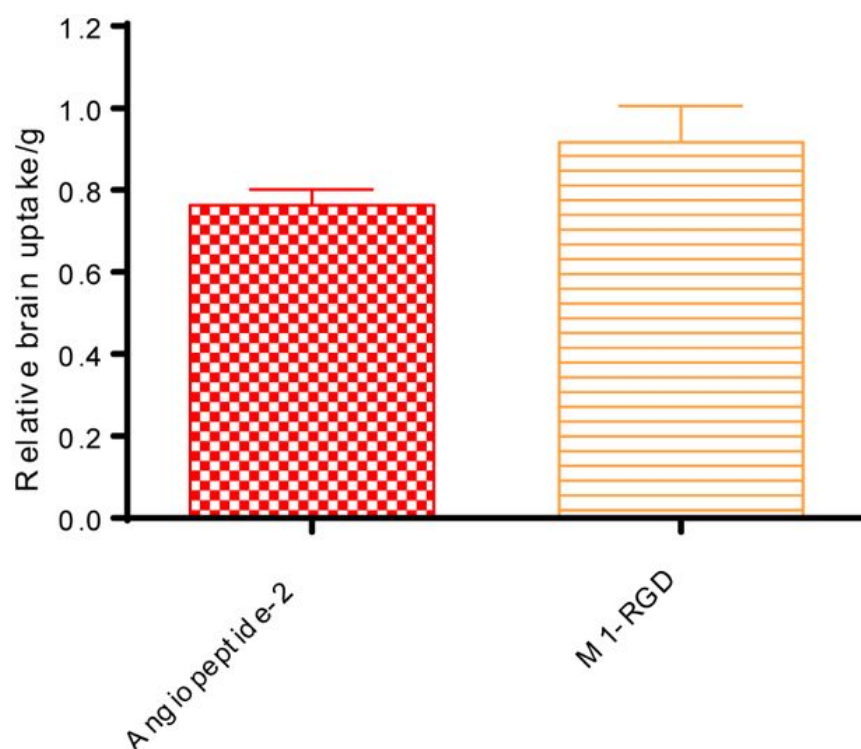
**Figure S1.** Sequences alignment of randomly picked colonies.



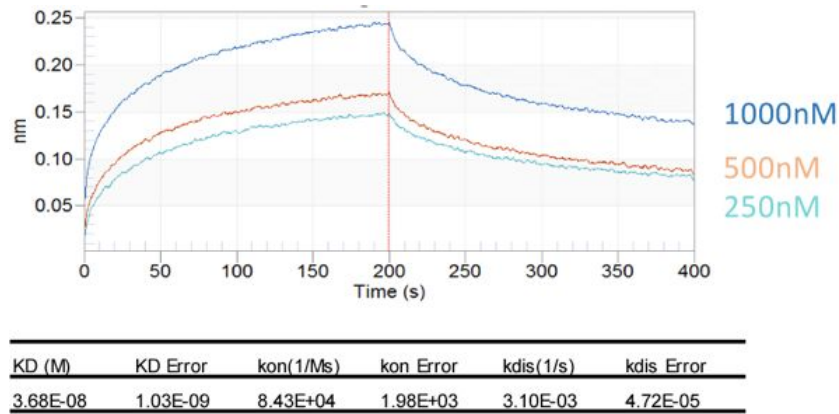
**Figure S2.** (A) Amount of phage in mouse brain at different times; (B) Brain blood ratio of phage at different times.



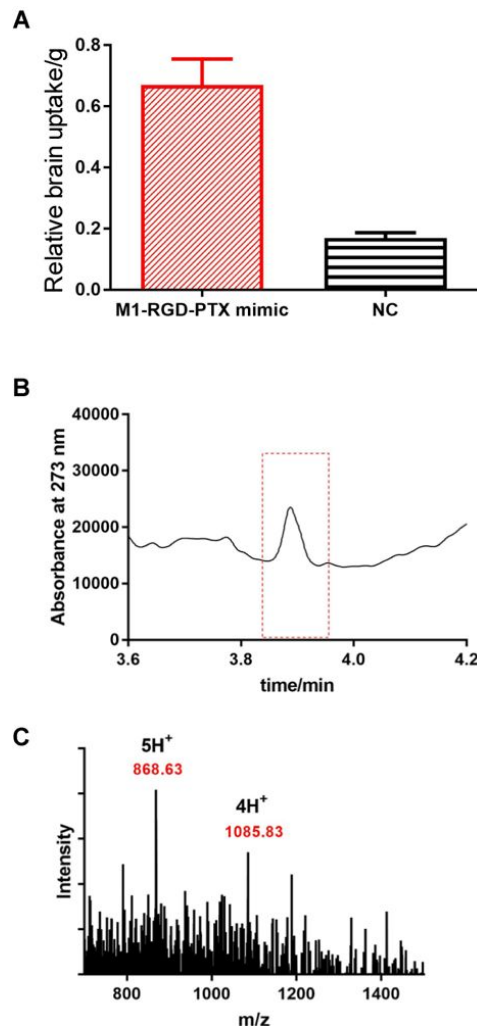
**Figure S3.** Confocal microscopy imaging of brain slices showing the penetration of peptides to blood-brain barrier. Scale bars represent 10  $\mu$ m.



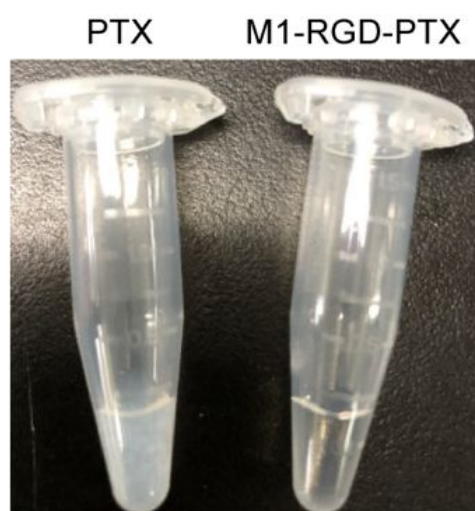
**Figure S4.** Quantitatively analysis and comparison of the ability for the two peptides to cross blood-brain barrier.



**Figure S5.** Binding curves of Angiopeptide-2 fusion to LPR-1.

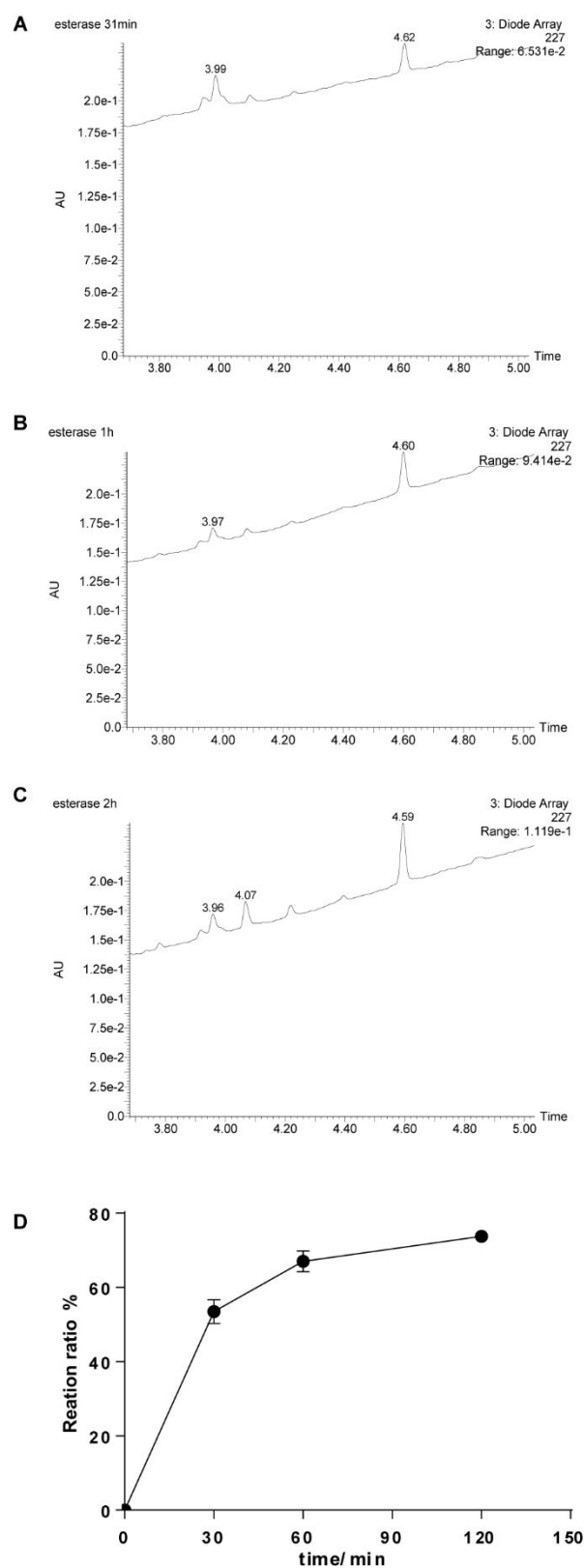


**Figure S6.** (A) Quantitatively analysis of the ability of M1-RGD-PTX to cross BBB using a M1-RGD-PTX mimic; (B) Liquid chromatography analysis of the brain extracts administered with M1-RGD-PTX; (C) Mass detected for the peak shown in B labeled with red box.



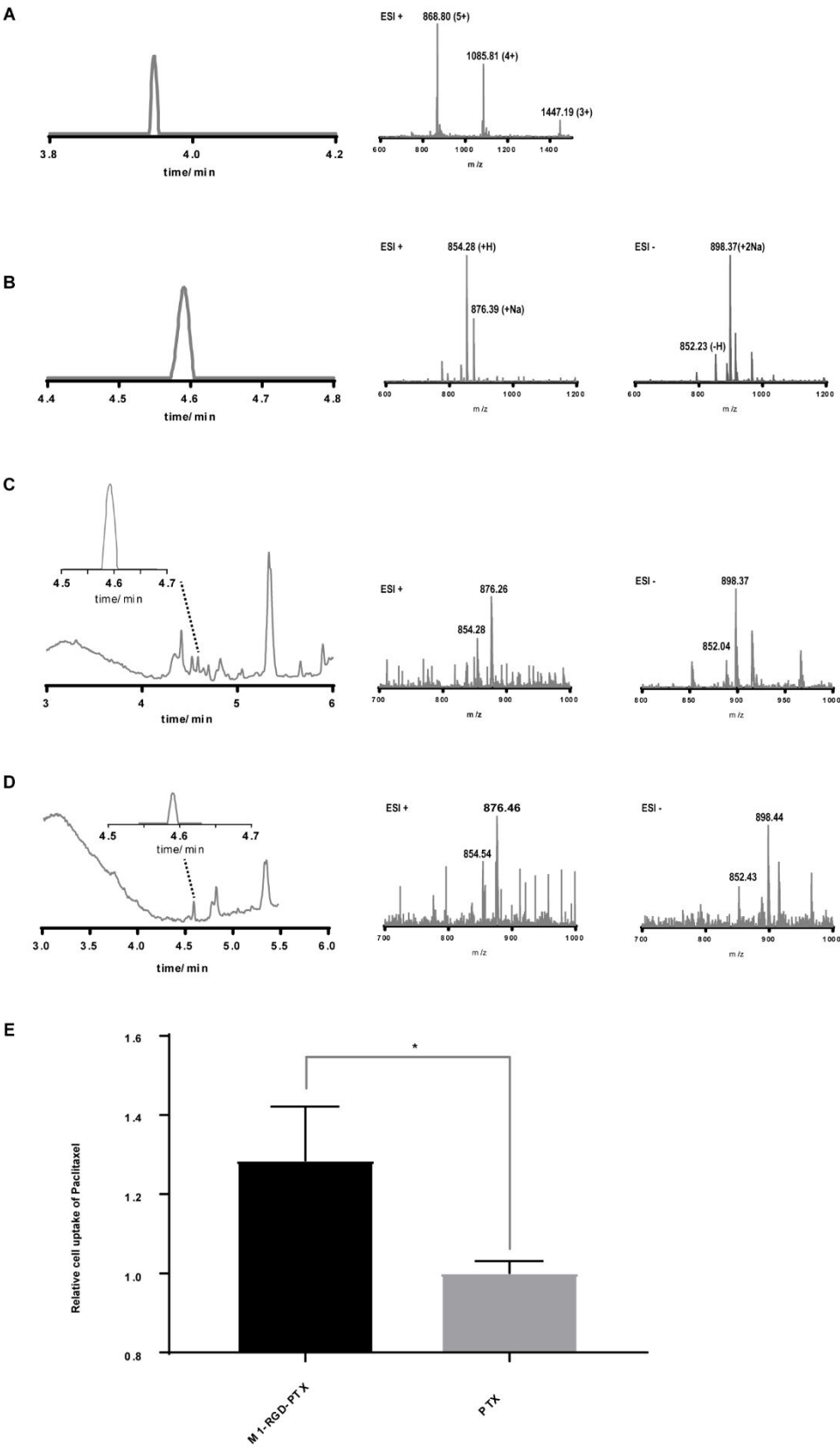
> 665 ug/ml in  
pH6.5 Glycine-HCl buffer

**Figure S7.** Image showing the difference in solubility for paclitaxel and M1-RGD-PTX. More than 665  $\mu\text{g/ml}$  for M1-RGD-PTX was detected.



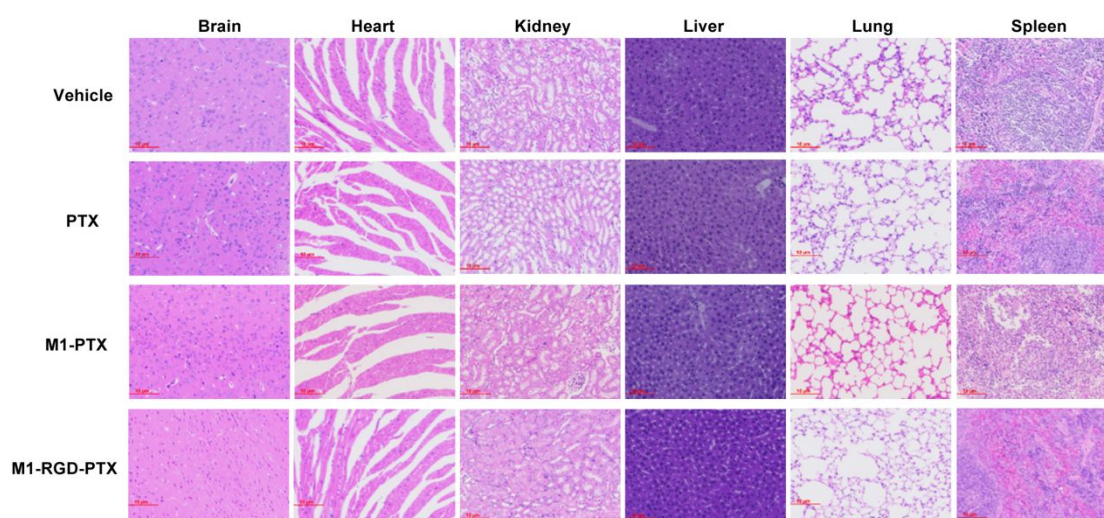
**Figure S8.** Hydrolysis of M1-RGD-PTX by esterase. LC trace of M1-RGD-PTX treated with esterase for (A) 30 min, (B) 60 min, and (C) 120 min. Peaks with retention times of 3.96-3.99 represented intact M1-RGD-PTX. Peaks with retention times of 4.59-4.62

represented released paclitaxel. (D) Paclitaxel release curve. Error bars indicate  $\pm$ S.D.; n=3.

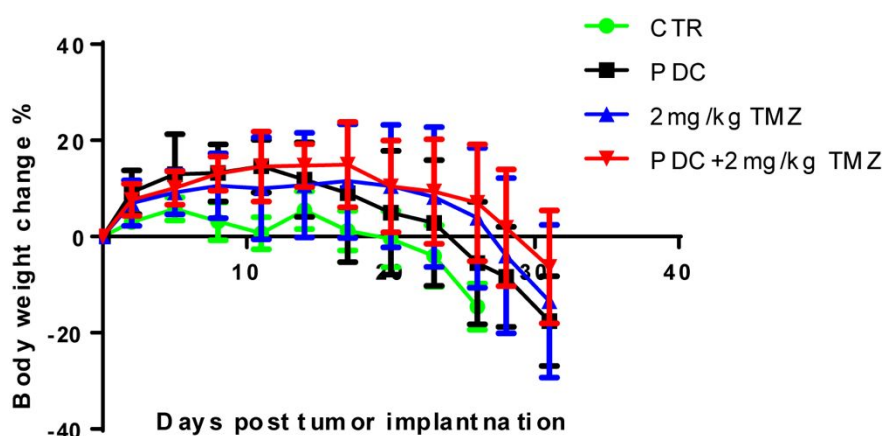




**Figure S9.** (A) LC trace and MS (ESI+) for M1-RGD-PTX standard. (B) LC trace and MS (ESI+/ESI-) for paclitaxel standard. (C) LC trace of cell lysate treated with M1-RGD-PTX (left) and detected MS ion peaks of released paclitaxel (right). (D) LC trace of cell lysate treated with free paclitaxel (left) and detected MS ion peaks of paclitaxel (right). (E) Relative cellular uptake of paclitaxel treated with M1-RGD-PTX or PTX. Intact M1-RGD-PTX has a retention time of ~3.96 min. Free paclitaxel has a retention time of ~4.59 min. Error bars indicate  $\pm$ S.D.; n=3.

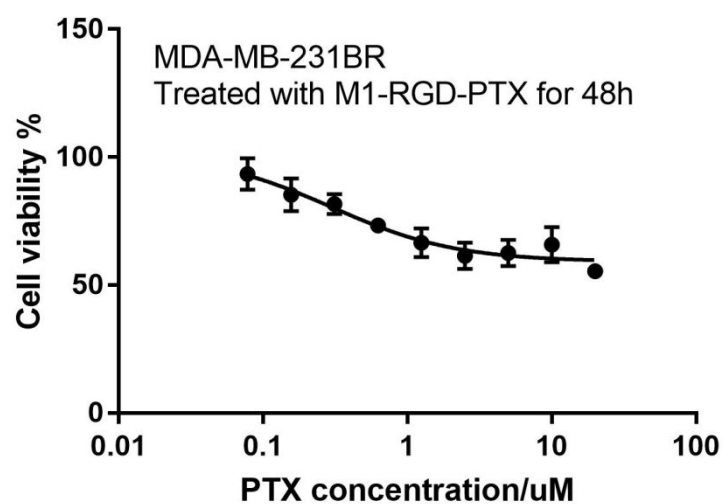


**Figure S10.** H&E stained tissue slices for animals shown in Figure 5C. Scale bars represent 10  $\mu$ m.



**Figure S11.** Body weights changes for animals used in Figure 6.





**Figure S12.** *In vitro* MDA-MB-231BR cytotoxicity of M1-RGD-PTX treated for 72 hours. Scale bars represent  $\pm$ SD, n=4.

**Table S1.** Survival states for animals used for testing LD50

Test Seq.	Animal ID	Dose (mg/kg)	Short-term Result	Long-term Result
1	1	125	X	X
2	2	100	X	X
3	3	75	X	X
4	4	125	X	X
5	5	100	X	X
6	6	75	X	X
7	7	58	O	O
8	8	39	O	O
9	9	22	O	O
10	10	12	O	O
11	11	54	O	O
12	16	34	O	O
13	13	19	O	O
14	14	10	O	O

(X = Died, O = Survived)

The dosage above was converted to PTX dosage

Estimated LD50: 152 mg/kg for M1-RGD-PTX, equivalent to 60 mg/kg PTX, iv.  
95% confidence interval: 58-75 mg/kg PTX

**Table S2** Comparison of M1-RGD-PTX with lead compound ANG1005

	Dosage		MST (Days)	Increased life span	P-values
ANG1005	0	Q3XD 5	16.5		
	50mg/kg (equal 25 mg/kg PTX)		19	15%	0.02
M1-RGD- PTX	0	Q3XD 5	20		
	44 mg/kg (equal 17.3 mg/kg PTX)		25	25%	0.0044
	0		24		
	44 mg/kg (equal 17.3 mg/kg PTX)		29	21%	0.014
	0		28		
	44 mg/kg (equal 17.3 mg/kg PTX)		33	18%	0.0017