Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary Information

Anti-DLL4 VNAR targeted nanoparticles for targeting of both tumour and tumour associated vasculature

Adam Leach,[‡a] Peter Smyth,[‡a] Laura Ferguson,[b] John Steven,[b] Michelle K. Greene,[a] Cristina M. Branco,[a] Aidan P. McCann,[a] Andrew Porter,[b] Caroline Barelle,*[b] Christopher J. Scott*[a]

a. The Patrick G. Johnston Centre for Cancer Research, School of Medicine, Queen's University Belfast, Belfast, UK. Email: c.scott@qub.ac.uk

b. Elasmogen Ltd, Aberdeen, UK. E-mail: caroline.barelle@elasmogen.com

‡These authors contributed equally.

* Corresponding Author.

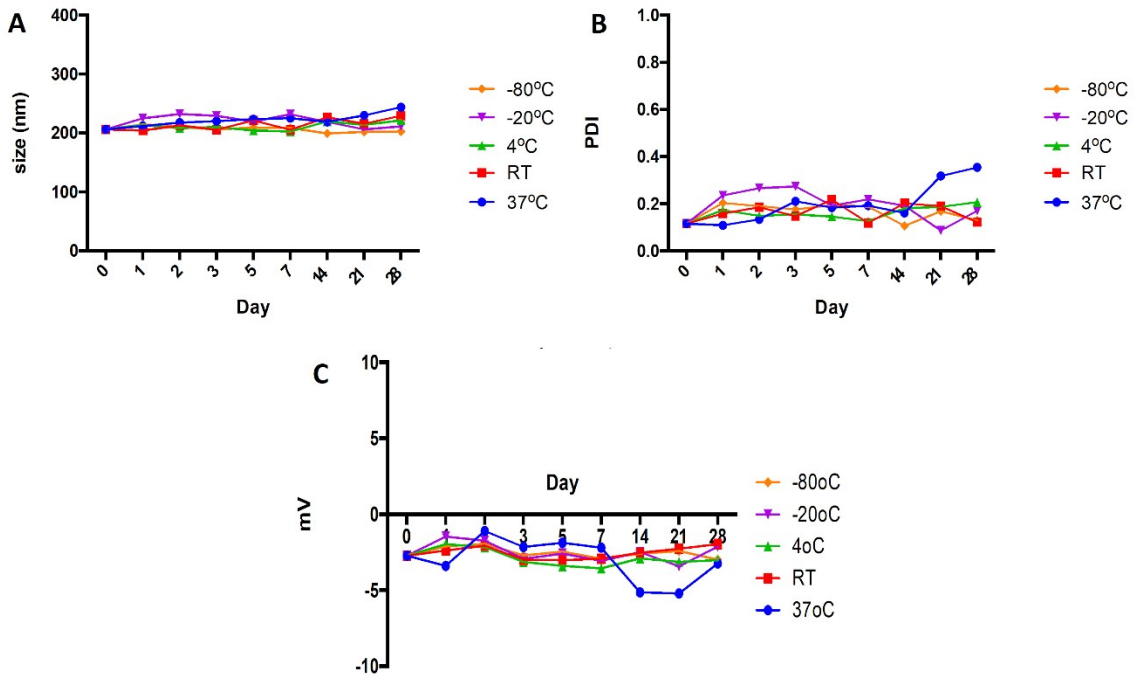

Figure S1. PLGA-PEG-Mal polymeric NPs are stable for at least 28 days under a variety of storage conditions. PLGA-PEG-Mal polymeric NPs were pelleted and stored at a range of temperatures (-80, -20, 4, ambient room temperature and 37°C). At predetermined intervals the NPs were resuspended in PBS (1 mg/ml) and reanalysed by DLS in terms of **(A)** size, **(B)** PDI and **(C)** zeta potential.

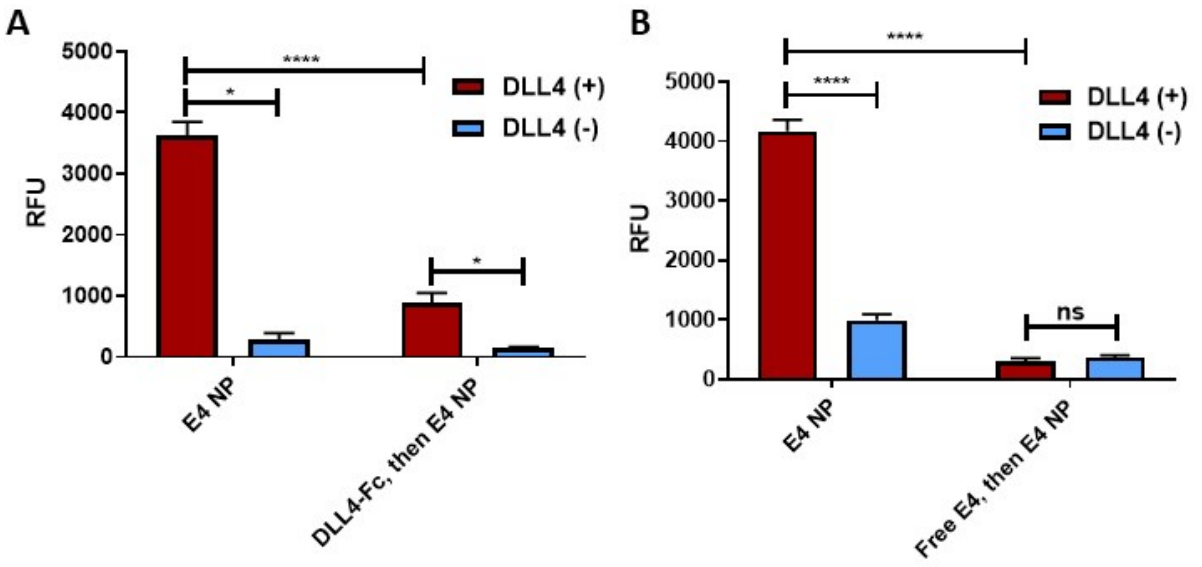

Figure S2. Blocking of the VNAR paratope or DLL4 epitope abrogates binding of E4-conjugated NPs. (A) E4-conjugated NP binding ± incubation with DLL4 Fc prior to plate incubation, (**B)** E4-conjugated NP binding to plate previously incubated with free E4 at 30 µg mL^{-1} for 30 minutes. N=2. Measurements performed in triplicate and data presented as mean of two independent experiments. Statistical significance was established by two-way ANOVA and Tukey's post-hoc test (****$p \leq 0.0001$, ** $p \leq 0.01$, * $p \leq 0.05$, ns $p > 0.05$).

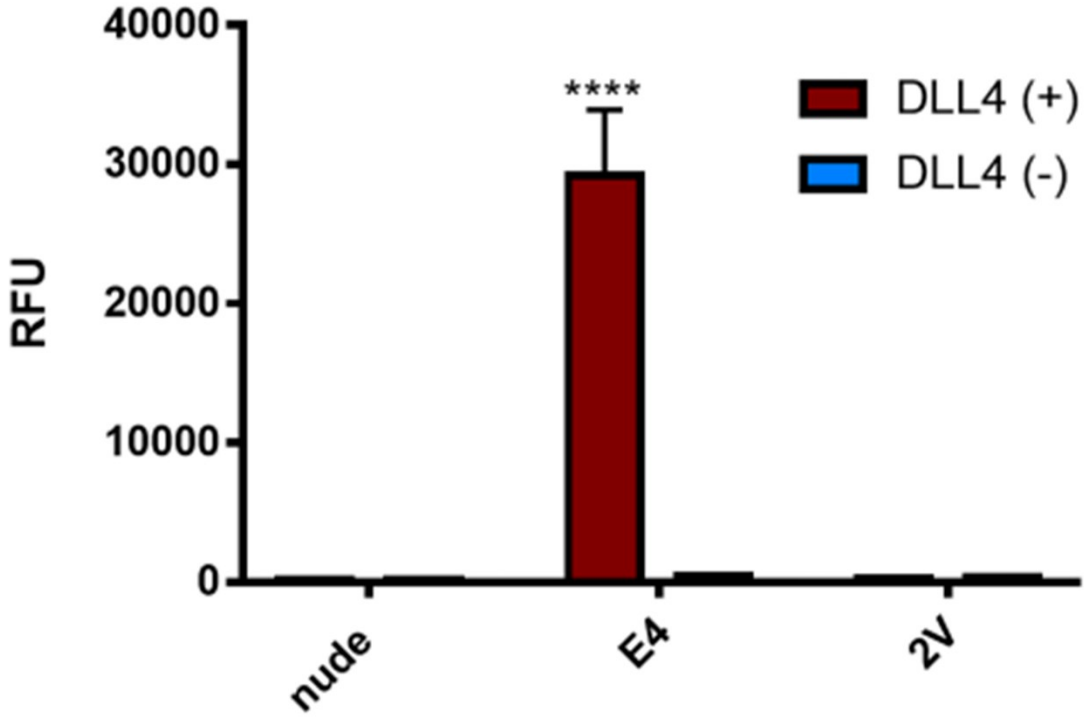

Figure S3. E4-conjugated NPs specifically bind to immobilized DLL4-Fc in bio-relevant media. Binding was assessed in media for E4-conjugated, nude and isotype 2V-conjugated NPs. Assay performed in triplicate. Data presented as mean of three independent experiments. Statistical significance was established by one-way ANOVA and Tukey's post-hoc test (****$p \leq 0.0001$, significance of E4 +DLL4 against all other treatment groups displayed).

Formulation	CPT added (ug/mg)	Size (nm)	PDI	Zeta Potential (mV)	CPT entrapped (ug/mg)	CPT entrapped (%)
PLGA-PEG-Mal (CPT)	5	199.0	0.122	-2.50	2.22	44.32

Figure S4. PLGA-PEG-Mal NPs efficiently entrap camptothecin (CPT). CPT loaded NPs composed of 75% PLGA 502H and 25% PLGA-PEG-Maleimide were produced and assessed in terms of size, PDI, zeta potential and CPT entrapment via comparison of CPT fluorescent signal to a standard calibration curve.