

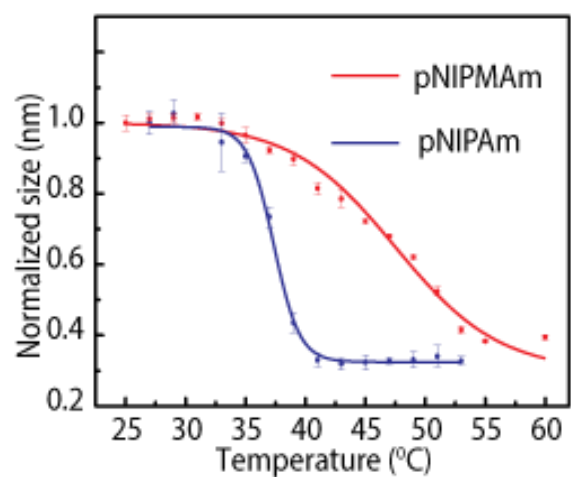
# Supporting Information

## Enhancing Gene Knockdown Efficiency of Poly(*N*-isopropylacrylamide) Nanogels

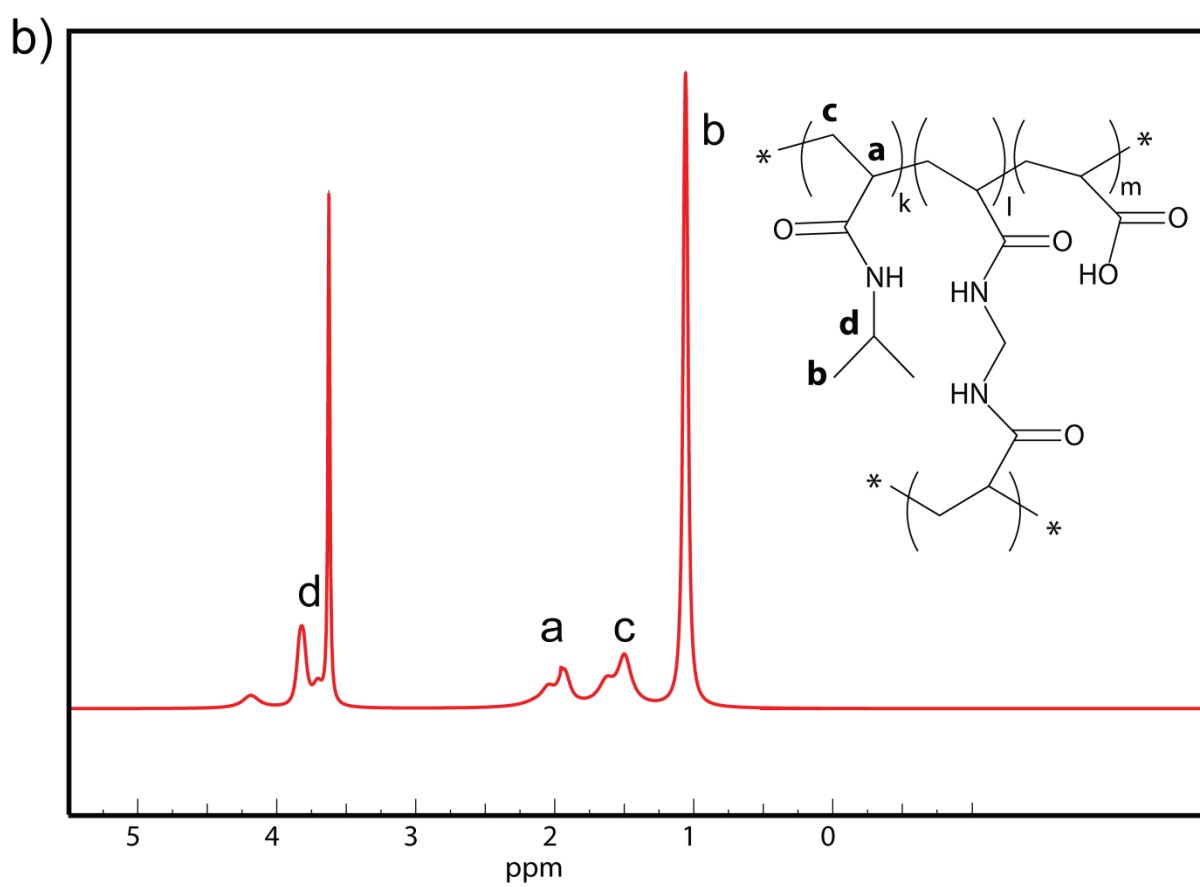
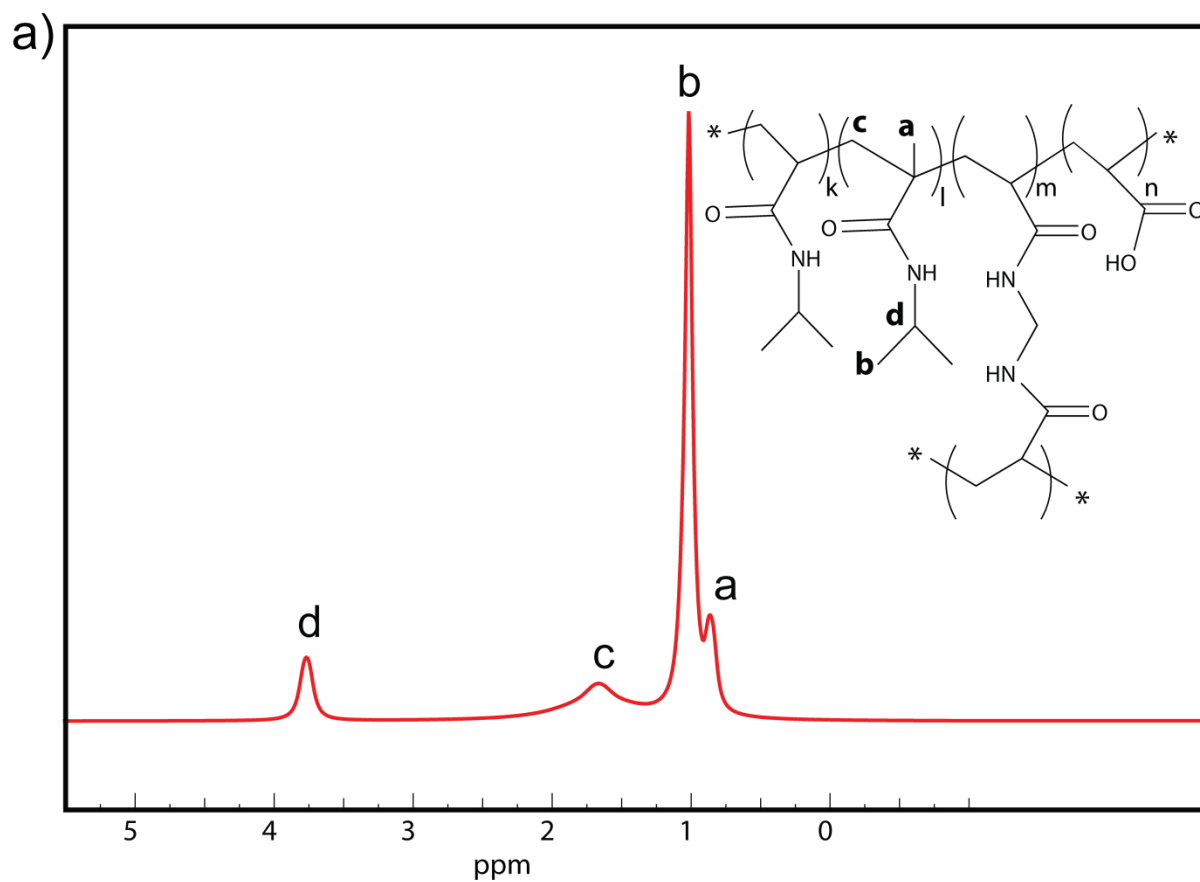
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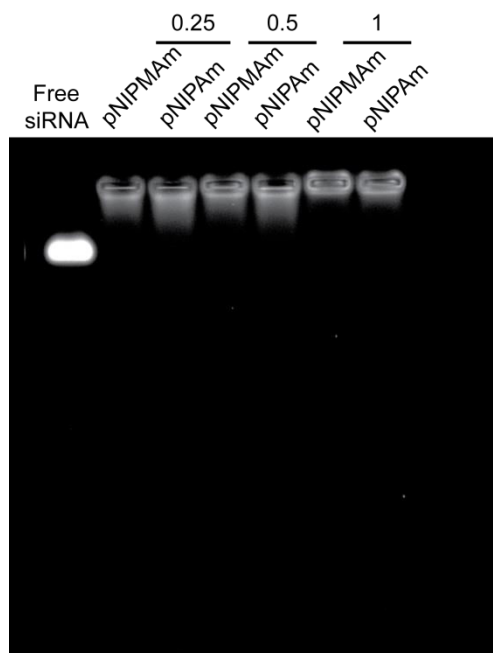
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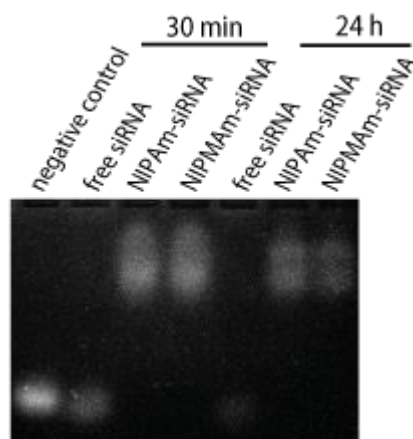
**Figure S1.** Temperature dependent size of pNIPAm and p(NIPMAm-*co*-NIPAm) nanogels by DLS. Note: p(NIPMAm-*co*-NIPAm) is denoted as pNIPMAm.



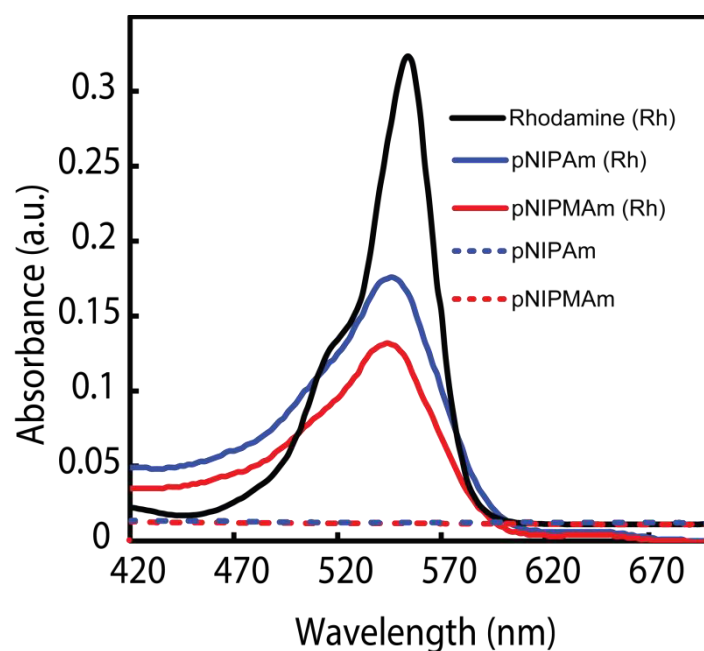
**Figure S2.** NMR of a) p(NIPMAm-co-NIPAm) and b) pNIPAm nanogels.



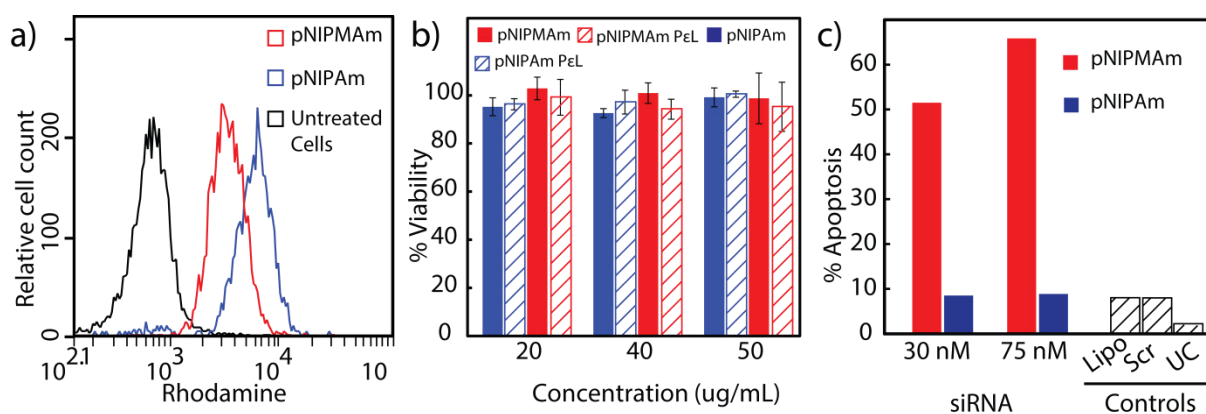
**Figure S3:** Optimization of siRNA loading. The numbers indicate nanogel:siRNA ratio. (Image contrast adjusted using ImageJ). Note: p(NIPMAM-co-NIPAm) is denoted as pNIPMAM.



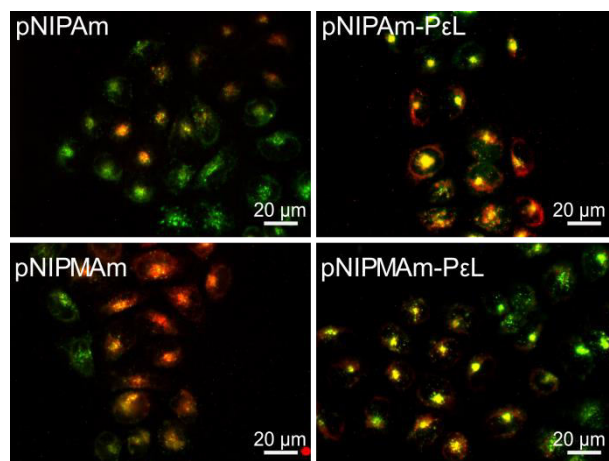
**Figure S4.** Serum stability of siRNA loaded into nanogels. (Image contrast adjusted using ImageJ). Negative control: free siRNA without serum treatment. Note: p(NIPMAM-co-NIPAm) is denoted as pNIPMAM.



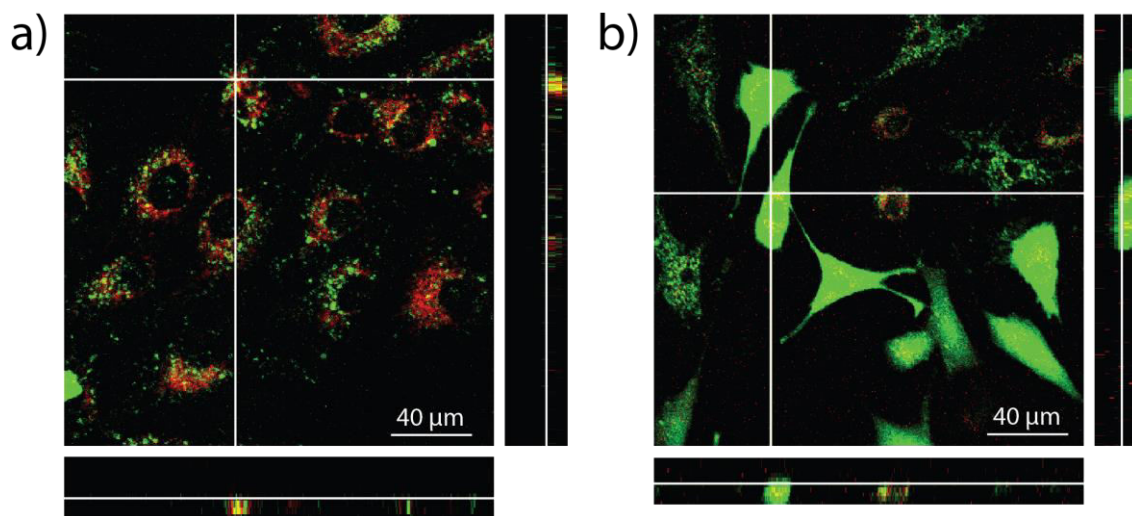
**Figure S5.** Absorbance spectra of rhodamine loaded nanogels. Note: p(NIPMAm-co-NIPAm) is denoted as pNIPMAm.



**Figure S6.** a) Uptake of rhodamine tagged nanogels by MDA-MB-231 cell line. b) Cytocompatibility of nanogels by MTT assay in MDA-MB-231 cell line. The error bars indicate standard deviation between triplicates. c) Flow cytometry analysis of apoptosis induced by knockdown of PKL1 gene, by siRNA loaded nanogels. Lipo: Lipofectamine loaded with 75 nM PKL1 siRNA, Scr: nanogel with 75 nM scrambled siRNA, UC: untreated cells. Note: p(NIPMAm-co-NIPAm) is denoted as pNIPMAm.



**Figure S7:** Co-localization of nanogels and endosomes, studied using rhodamine labelled nanogels and LysoTracker blue DND 22, by fluorescence microscopy. Red=nanogels and Green=endosomes (pseudo colored using ImageJ). Magnification: 40 $\times$ . Note: p(NIPMAm-*co*-NIPAm) is denoted as pNIPMAm.



**Figure S8.** Calcein release into cell cytoplasm analyzed by orthogonal confocal microscopy for MG-63 cells co-incubated with calcein and rhodamine labelled a) p(NIPMAm-*co*-NIPAm) and b) p(NIPMAm-*co*-NIPAm)-P $\epsilon$ L.