Small Molecule End Group of Linear Polymer Determines Cell-type Gene Delivery Efficacy

By Joel Sunshine, Jordan J. Green, Kerry Mahon, Fan Yang, Ahmed A. Eltoukhy, David N. Nguyen, Robert Langer, and Daniel G. Anderson

Supporting Information

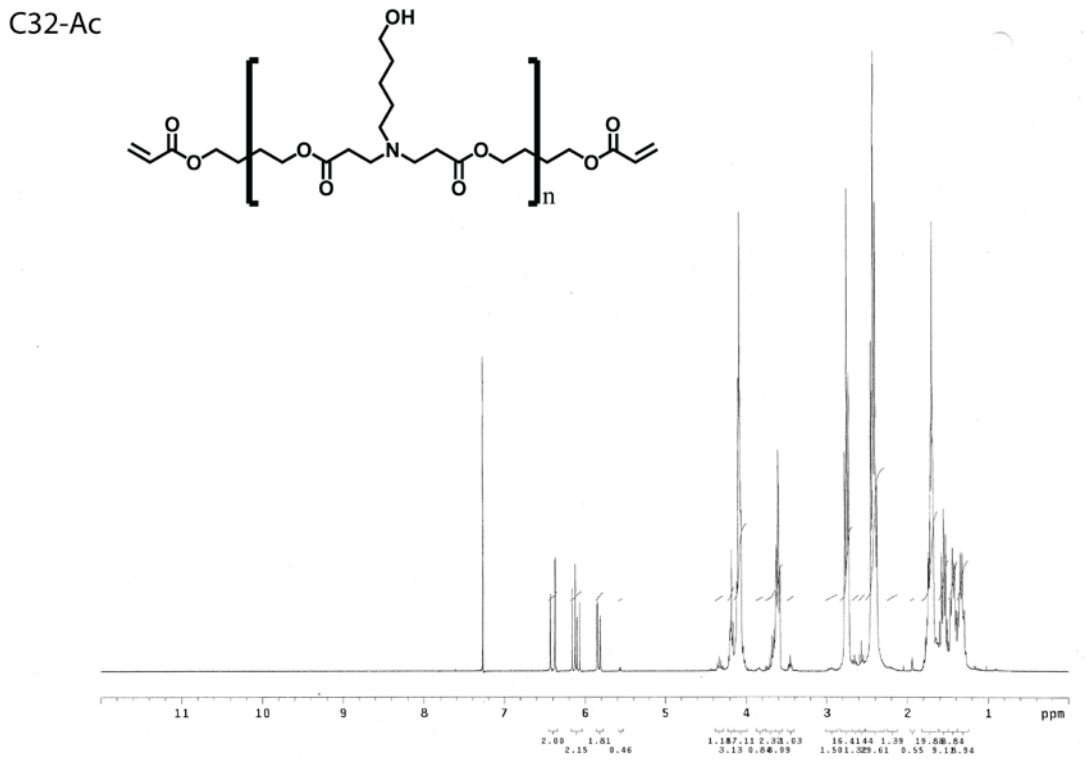


Figure S1a: 1H-NMR of acrylate-terminated (1.2:1 acrylate: amine) C32

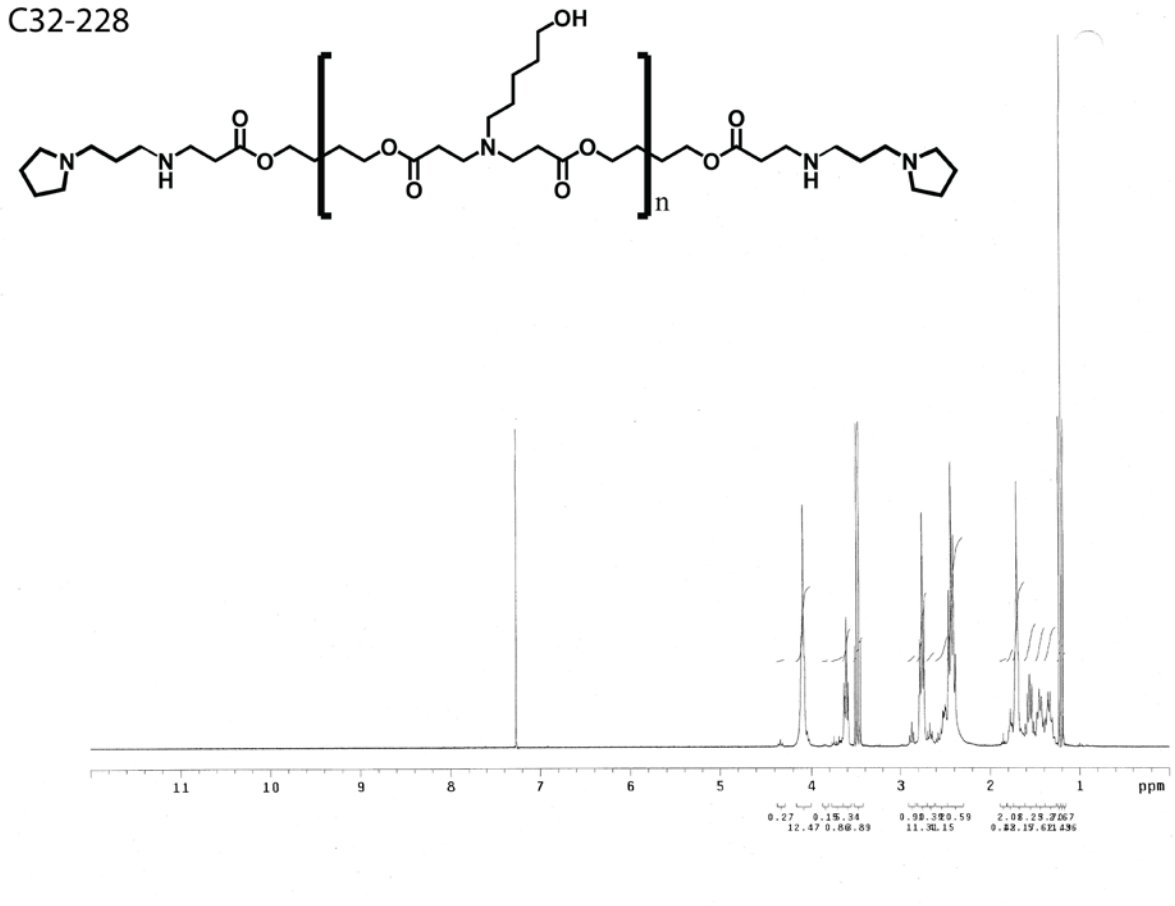


Figure S1b: 1H-NMR of C32-228. Note the complete disappearance of the peaks between 5 and 7 ppm.

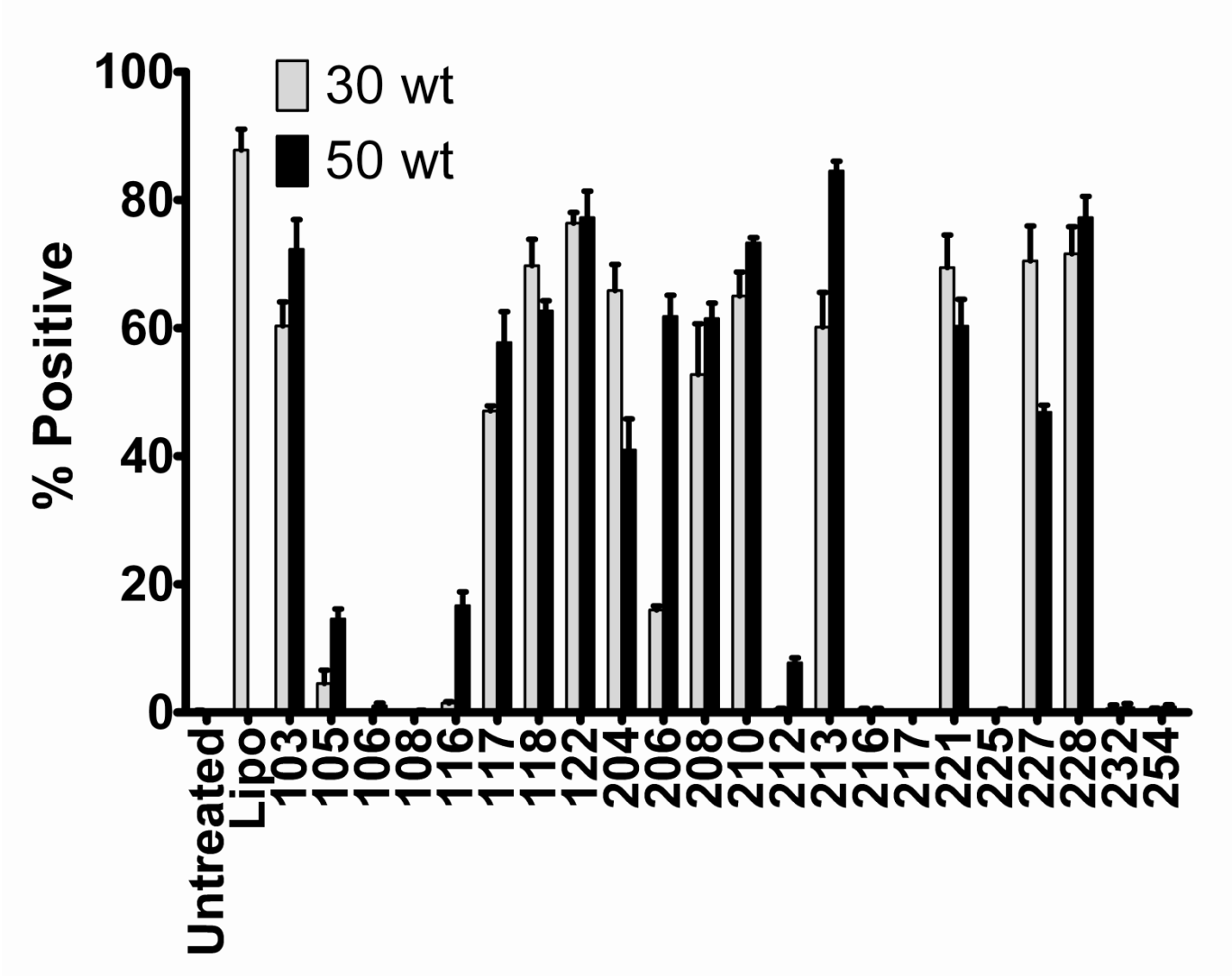


Figure S2: Transfection of HeLa cells using end-modified polymers. Percent positive cells determined by GFP expression and FACS. Mean + SD shown. Polymers are used at 30 w/w and 50 w/w and Lipofectamine 2000 is used following manufacturer instructions.

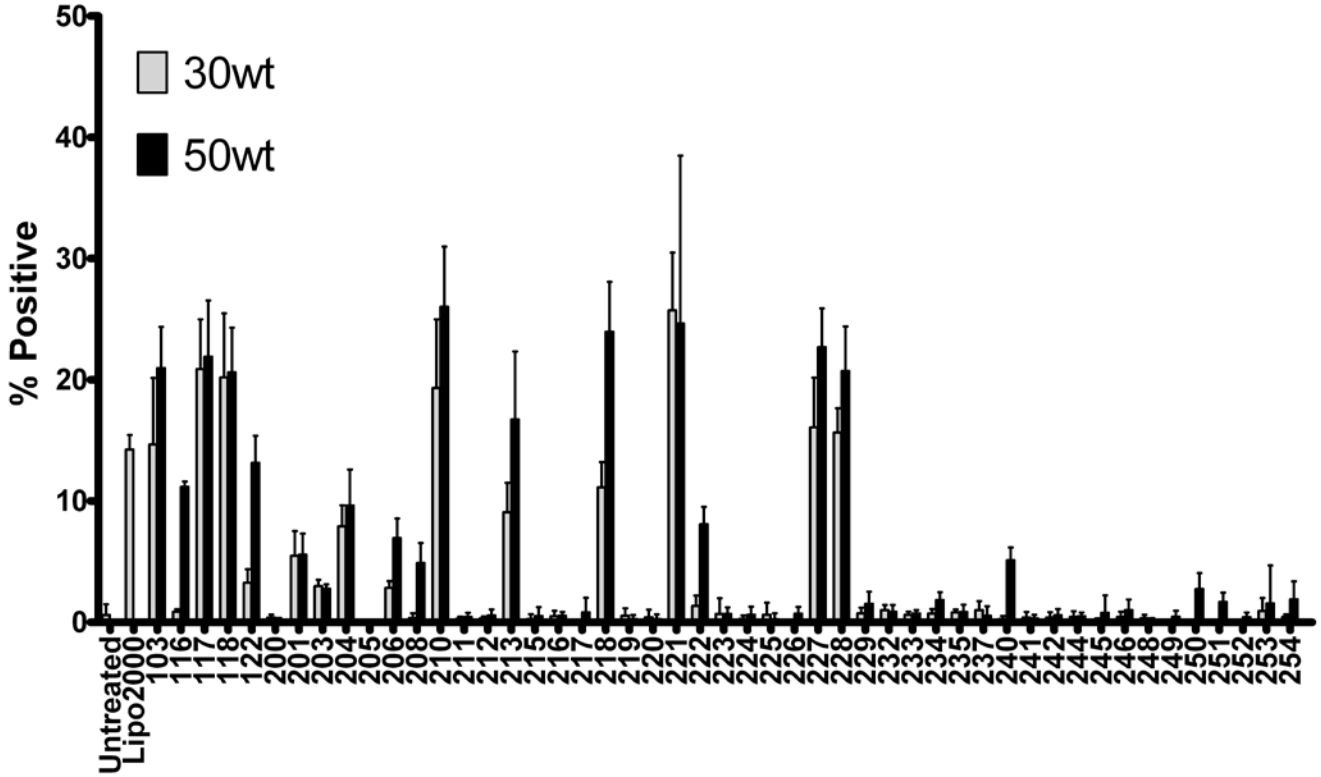


Figure S3: Transfection of HEPG2 cells using end-modified polymers. Percent positive cells determined by GFP expression and FACS. Mean + SD shown. Polymers are used at 30 w/w and 50 w/w and Lipofectamine 2000 is used following manufacturer instructions.

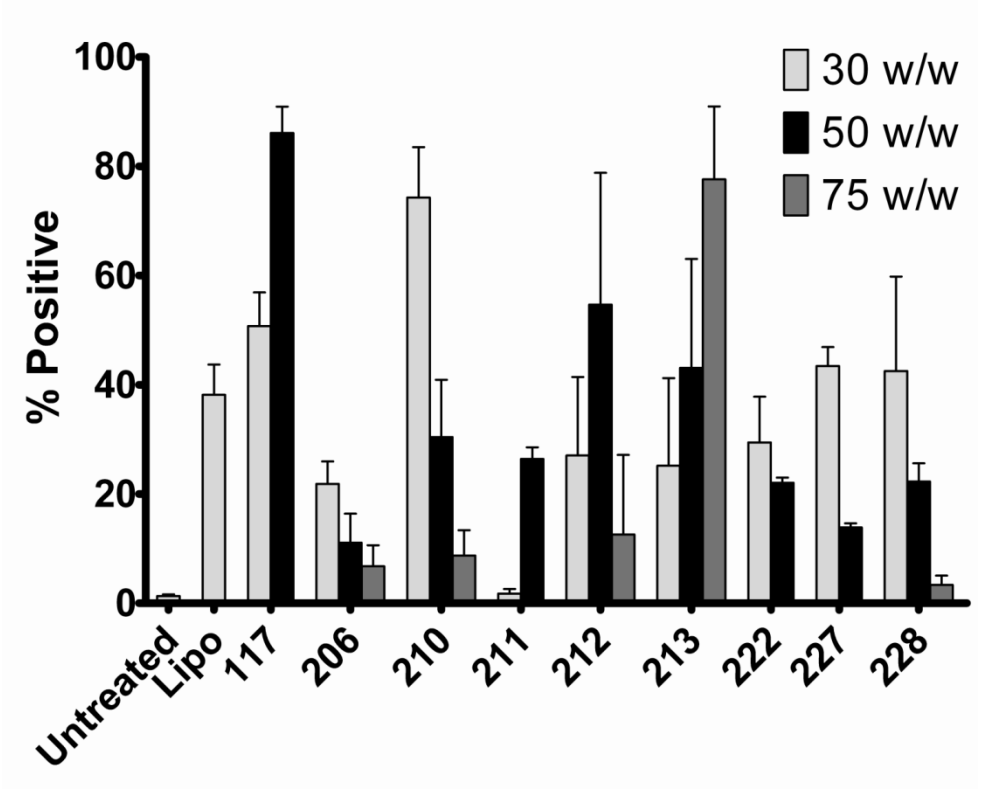


Figure S4: Transfection of HUVECs using end-modified polymers. Percent positive cells determined by GFP expression and FACS. Mean + SD shown. Polymers are used at 30 w/w, 50 w/w, and 75 w/w and Lipofectamine 2000 is used following manufacturer instructions.

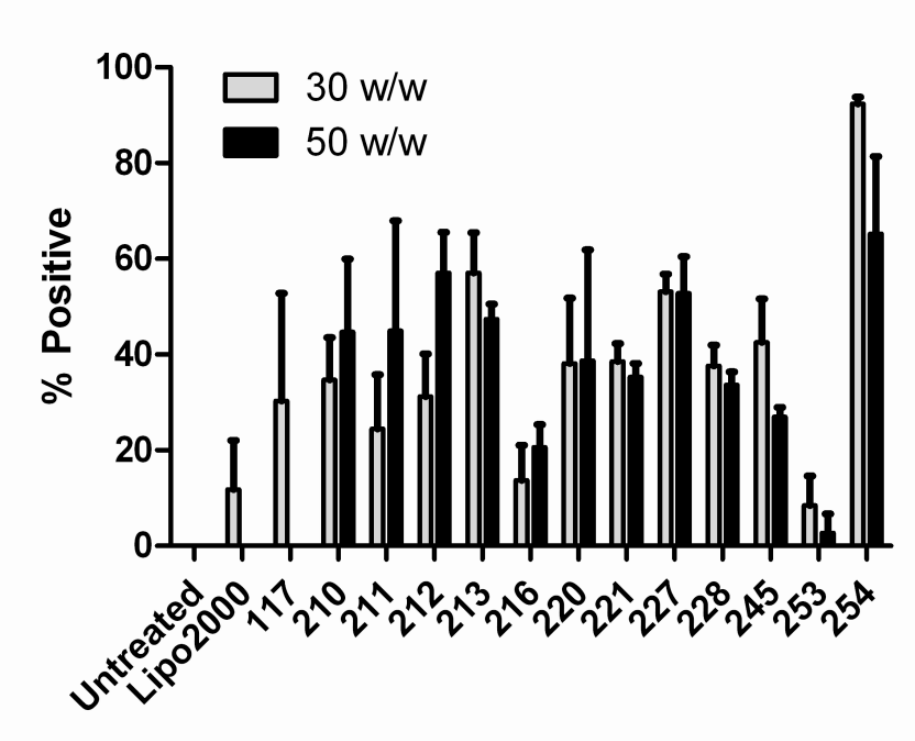


Figure S5: Transfection of DC2.4s using end-modified polymers. Percent positive cells determined by GFP expression and FACS. Mean + SD shown. Polymers are used at 30 w/w and 50 w/w and Lipofectamine 2000 is used following manufacturer instructions.

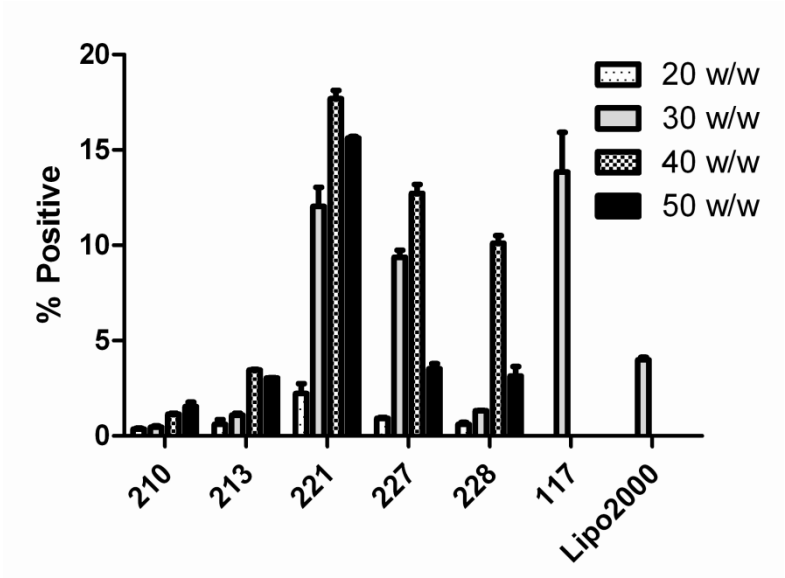


Figure S6a: Transfection of Human Mesenchymal Stem Cells using end-modified polymers at regular DNA dose. Percent positive cells determined by GFP expression and FACS. Mean + SD shown. Polymers are used at 20, 30, 40 and 50 w/w and Lipofectamine 2000 is used following manufacturer instructions.

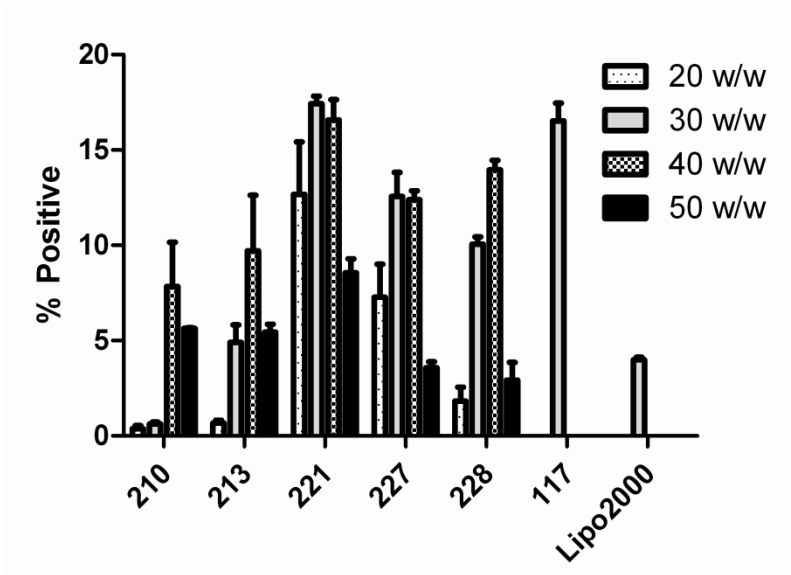


Figure S6b: Transfection of Human Mesenchymal Stem Cells using end-modified polymers and a double DNA dose. Percent positive cells determined by GFP expression and FACS. Mean + SD shown. Polymers are used at 20, 30, 40 and 50 w/w and Lipofectamine 2000 is used following manufacturer instructions.

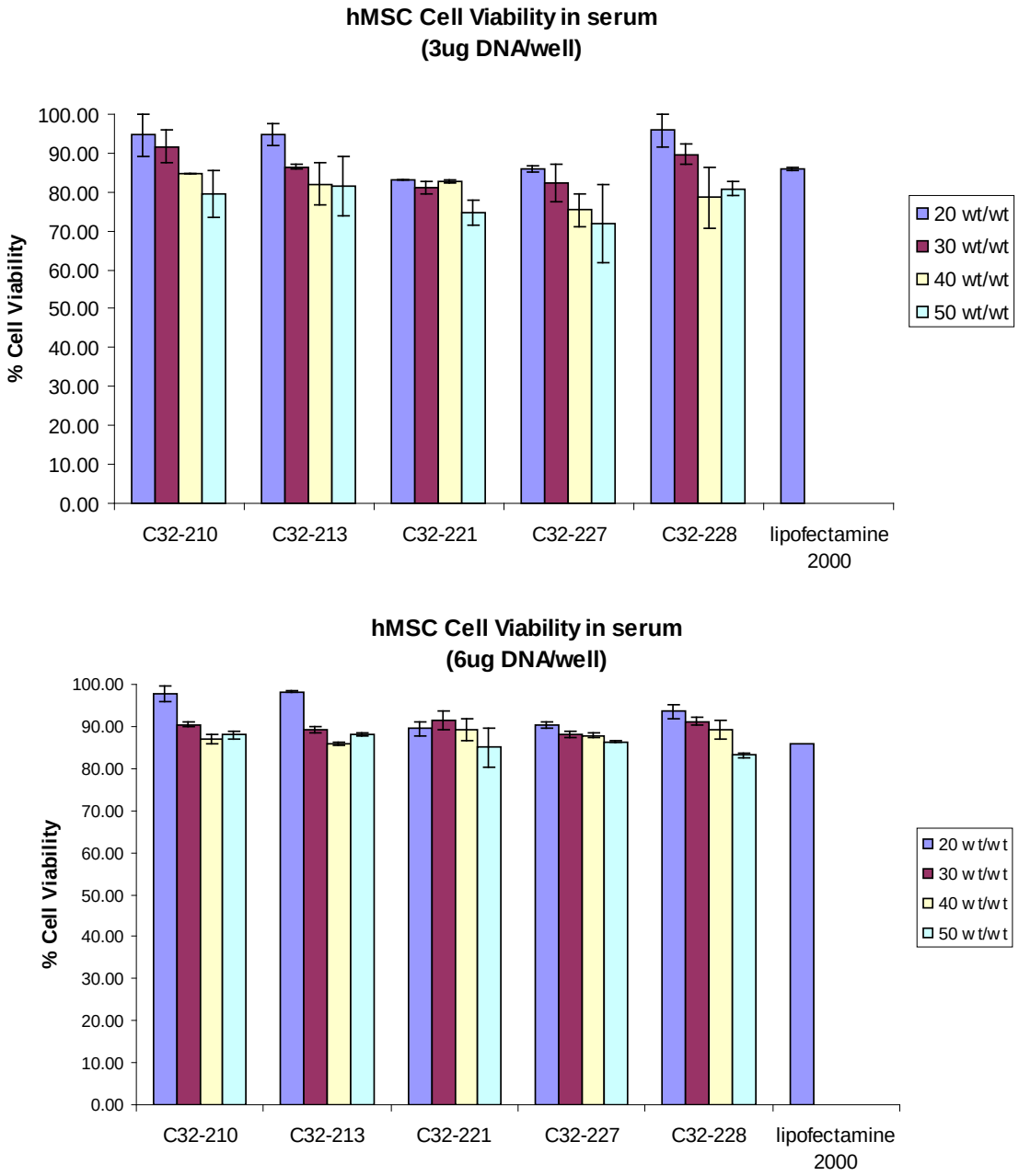
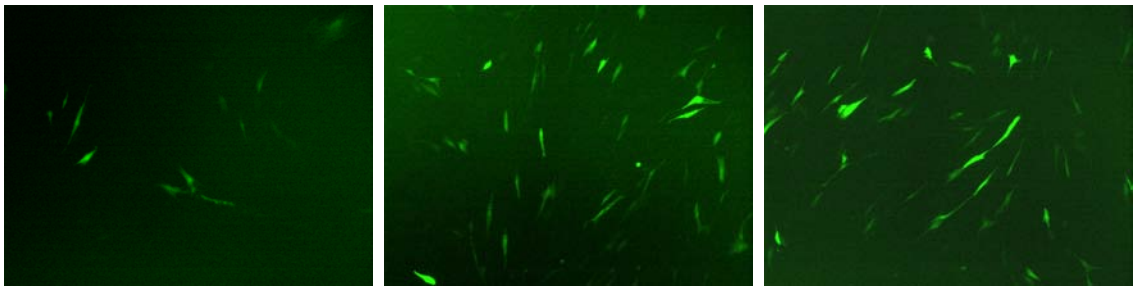


Figure S6c and S6d: Transfection of Human Mesenchymal Stem Cells using end-modified polymers and regular or double DNA doses. Percent viable cells as compared to untreated controls. Mean + SD shown. Polymers are used at 20, 30, 40 and 50 w/w and Lipofectamine 2000 is used following manufacturer instructions.



Lipo 3 μg 40w/w C32-221 3 μg 40w/w C32-227

Figure S6e: GFP Micrographs showing transfection of Human Mesenchymal Stem Cells using end-modified polymers.

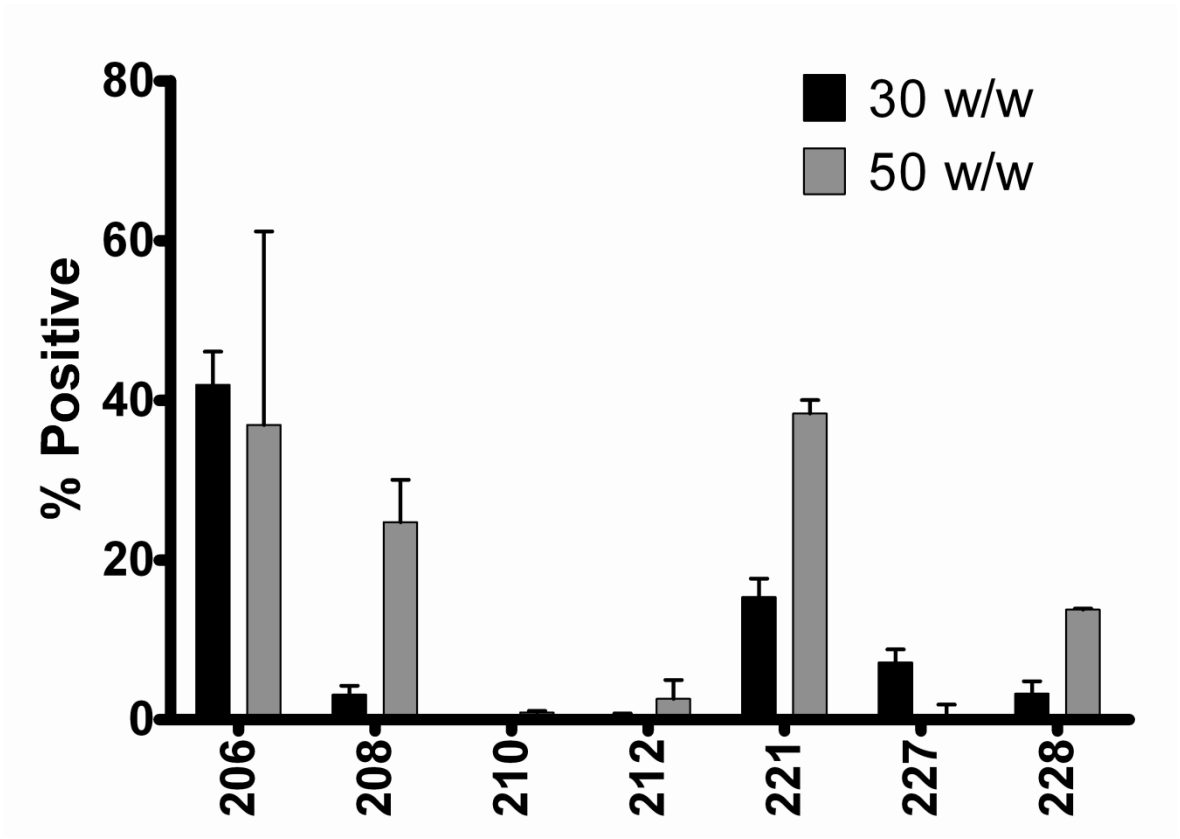


Figure S7a. Transfection of COS-7 cells using end-modified polymers in the presence of 100% serum. Percent positive cells determined by GFP expression and FACS. Mean + SD shown. Polymers are used at 30 w/w and 50 w/w and Lipofectamine 2000 is used following manufacturer instructions.

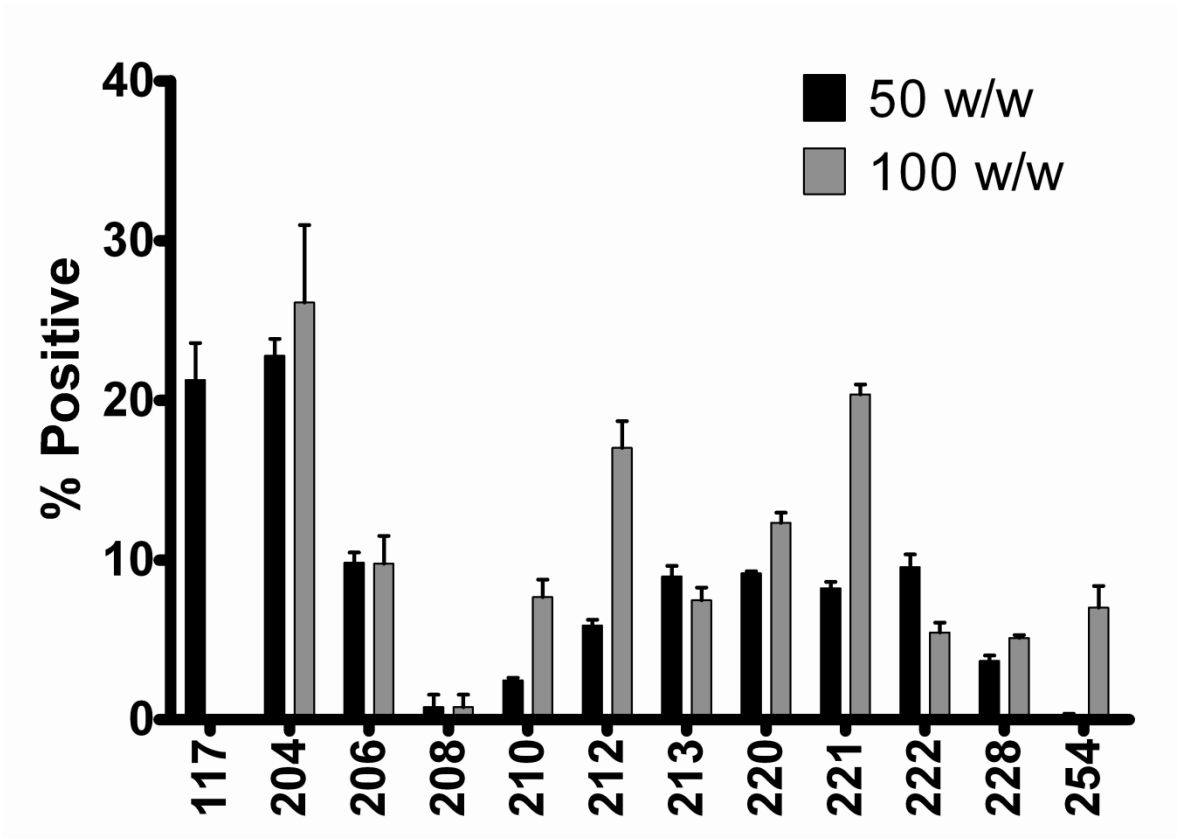


Figure S7b. Transfection of HUVECs using end-modified polymers in the presence of 100% serum. Percent positive cells determined by GFP expression and FACS. Mean + SD shown. Polymers are used at 50 w/w and 100 w/w and Lipofectamine 2000 is used following manufacturer instructions.

Polymer	Mn	Mw	PDI
117	4500	8300	1.84
200	5200	9100	1.75
206	3400	8600	2.53
210	5200	12400	2.38
212	3500	7900	2.26
213	3100	8000	2.58
218	3100	7300	2.35
221	9100	19700	2.16
227	8100	16700	2.06
228	7700	15700	2.04
240	3900	8800	2.26
241	4400	10800	2.45
242	4900	12300	2.51
243	3900	8600	2.21
246	3400	7100	2.09
248	2800	6300	2.25
249	3400	8300	2.44
250	4700	9100	1.94
251	3500	8300	2.37
252	4300	8700	2.02
253	4200	8500	2.02
254	9300	14200	1.53
255	4300	8700	2.02
256	4200	8500	2.02
257	4300	8600	2.00
258	4000	8400	2.10
259	4900	8700	1.78

Figure S8: Gel permeation chromatography characterization of polymers

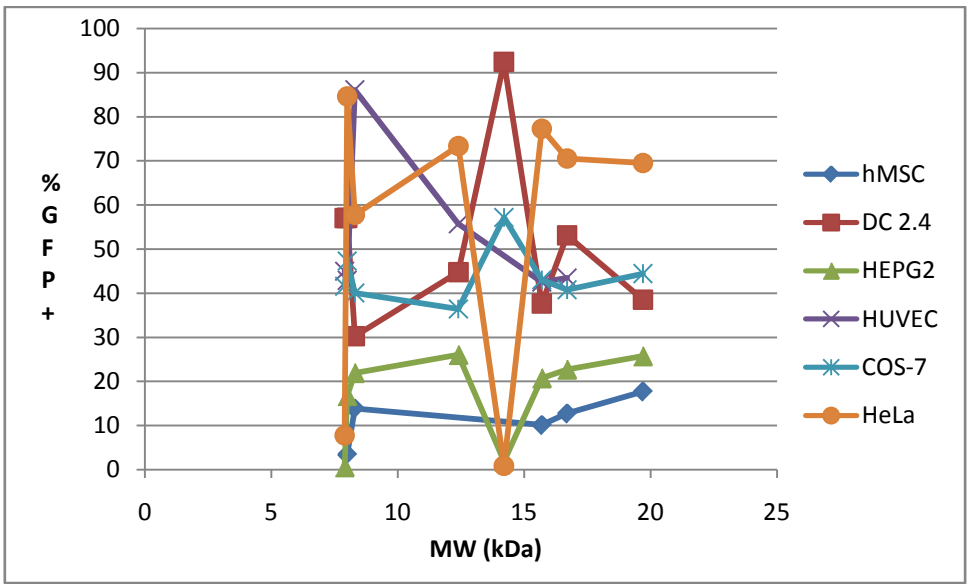


Figure S9: Molecular weight (MW) of end-modified polymers vs. transfection efficacy across all cell types. Molecular weight does not correlate to transfection efficacy. Polymers shown are the lead polymers from Figure 3.

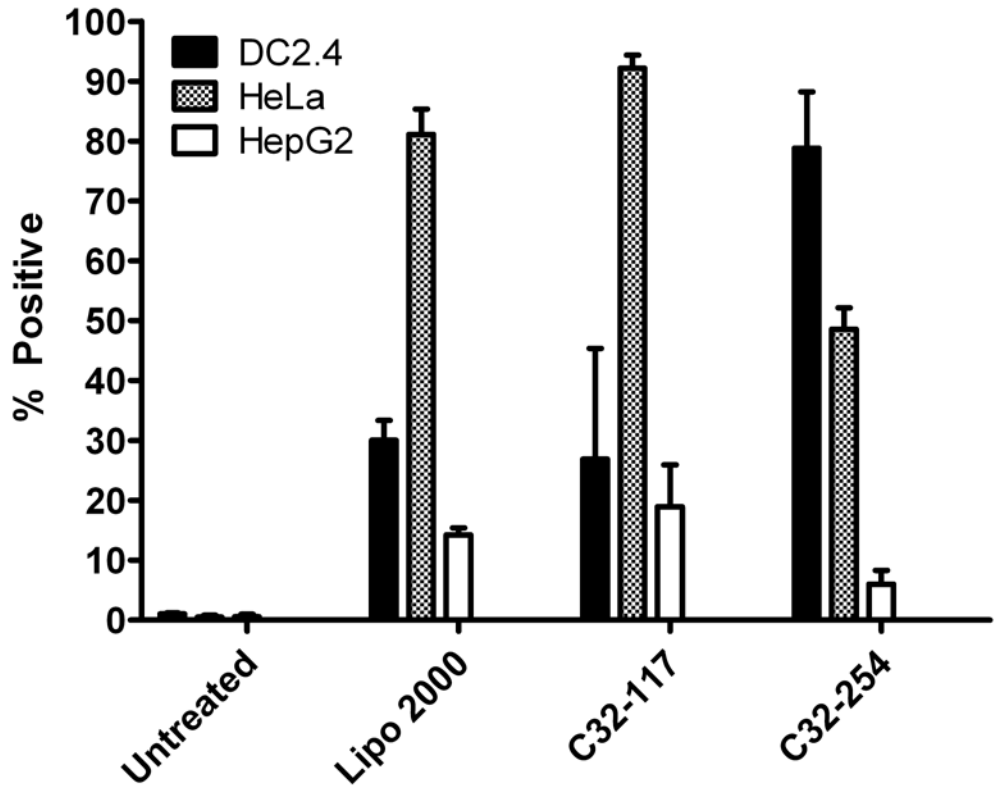


Figure S10: Transfection of of multiple cells types side-by-side using end-modified polymers and a double DNA dose. Percent positive cells determined by GFP expression and FACS. Mean + SD shown. Polymers are used at 30 w/w and Lipofectamine 2000 is used following manufacturer instructions. There is no observable change in cytotoxicity at this higher DNA dose.