

## Supporting Information *Journal of Materials Chemistry B*

Optimized effective charge density and size of polyglycerol amines leads to strong knockdown efficacy *in vivo*

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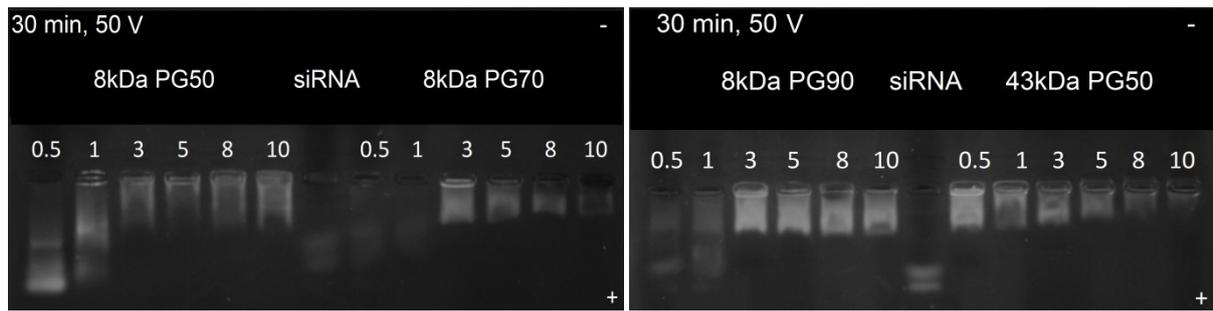
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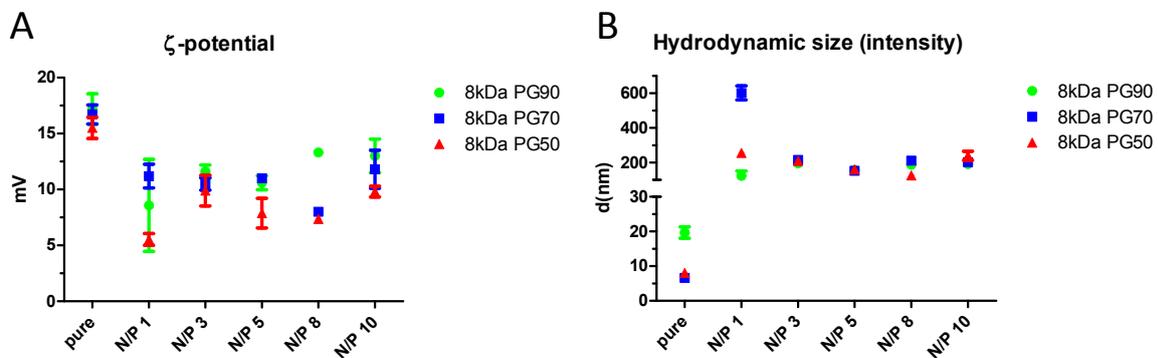
1. Gel electrophoreses data
2. Zeta-potential and hydrodynamic size data of 8kDa hPG amines
3. Zeta-potential and hydrodynamic size data of 43kDa PG50 vs. 8kDa PG90
4. Calculation of the amine density on the surface
5. *In vitro* evaluation of all 8kDa hPG amines
6. Additional information on the pro-inflammatory cytokines (8kDa hPG)
7. *In vitro* evaluation of hPG amines 43kDa PG50 vs. 8kDa PG90
8. Additional information on the pro-inflammatory cytokines (43kDa PG50 vs. 8kDa PG90)
9. Evaluation of tumor surface area and body weight during luciferase knockdown study *in vivo* with hPG amines 43kDa PG50 vs. 8kDa PG90
10. Histological analysis of tumor *sections* treated with 43kDa PG50 vs. 8kDa PG90
11. *In vivo* silencing of the PLK-1 gene using 43kDa PG50

## 1. Gel electrophoreses data



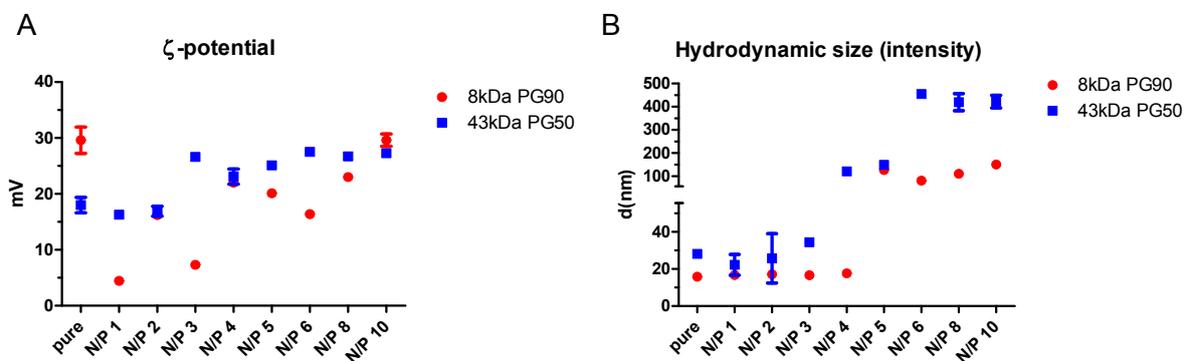
**Figure S 1.** Gel electrophoreses study performed for 8kDa PG50, 8kDa PG70, 8kDa PG90 and 43kDa PG50 at N/P ratios 1, 3, 5, 8 and 10. The resulting pictures were obtained using a digital camera showing a stable polyplex formation for all complexes starting at an N/P ratio of three.

## 2. Zeta-potential and hydrodynamic size data of 8kDa hPG amines



**Figure S 2.** Zeta-potential (A) and hydrodynamic size (B) measurements of pure 8kDa PG90, 8kDa PG70 and 8kDa PG50 and in complexation with siRNA at different N/P ratios in PBS buffer (10 mM, pH 7.4), respectively. The results are shown in mean  $\pm$  SD of at least three independent experiments. The PDI of all performed hydrodynamic size measurements was between 0.03 and 0.13.

## 3. Zeta-potential and hydrodynamic size data of 43kDa PG50 vs. 8kDa PG90



**Figure S 3.** Zeta-potential (A) and hydrodynamic size (B) of pure 8kDa PG90 and 43kDa PG50 and in complexation with siRNA at different N/P ratios in HyClone™ HyPure water, respectively. The results are shown in mean  $\pm$  SD of at least three independent experiments. Aggregations were detected at N/P ratio of 6-10 for 43kDa PG50 led to the higher hydrodynamic sizes. The PDI of all hydrodynamic size measurements was 0.03-1.3 for 8kDa PG90 and 0.2-0.6 for 43kDa PG50.

#### 4. Calculation of the amine density on the surface

For the calculation of the amine groups per surface area per particle, the hydrodynamic size of the cores measured by DLS in millipore water were used. Having assumed that the particle was spherical, the surface area could be calculated with Equation (1).

$$A = 4\pi r^2 \quad (1)$$

The total amount of functional groups per particle (FG) was obtained using the following Equation (2).

$$FG = \frac{\text{molecular mass (polymer) g/mol}}{\text{molecular mass (monomer)g/mol}} \quad (2)$$

Consequently the total amount of amine group per particle (AG) could be consequently calculated using Equation (3).

$$AG = FG \times \text{Percentage of amine loading} \quad (3)$$

Finally the amines per surface area per particle were received by Equation (4) based on the assumption that only 60% of the functional groups are on the surface<sup>1</sup>

$$\text{Number of amines} \frac{\text{groups}}{\text{nm}^2} = \frac{AG}{A} \times 60\% \quad (4)$$

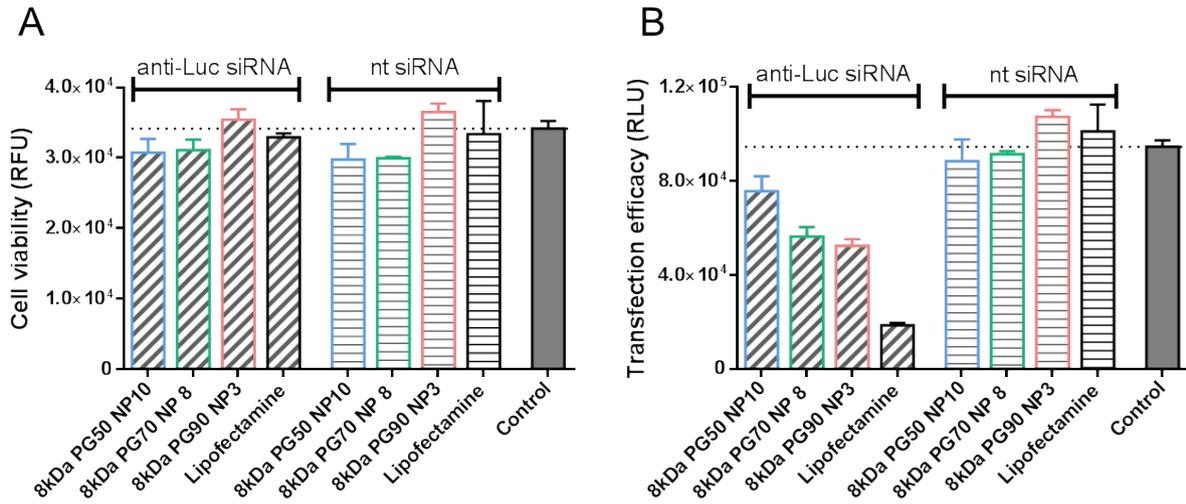
The results are shown in Table S 1.

**Table S 1.** Calculation parameters to receive the total amount of amine groups per nm<sup>2</sup> per particle.

	8kDa PG 90	43kDa PG50
<b>Molecular mass / Da (M<sub>n</sub>)</b>	8100	43602
<b>Hydrodynamic size /d(nm)</b>	6.2	8.2
<b>Surface area (A)/ nm<sup>2</sup></b>	120.7	211.2
<b>Functional groups (FG)</b>	109	589
<b>Amine groups (AG)</b>	99	295
<b>Number of amines/ nm<sup>2</sup></b>	0.5	0.8

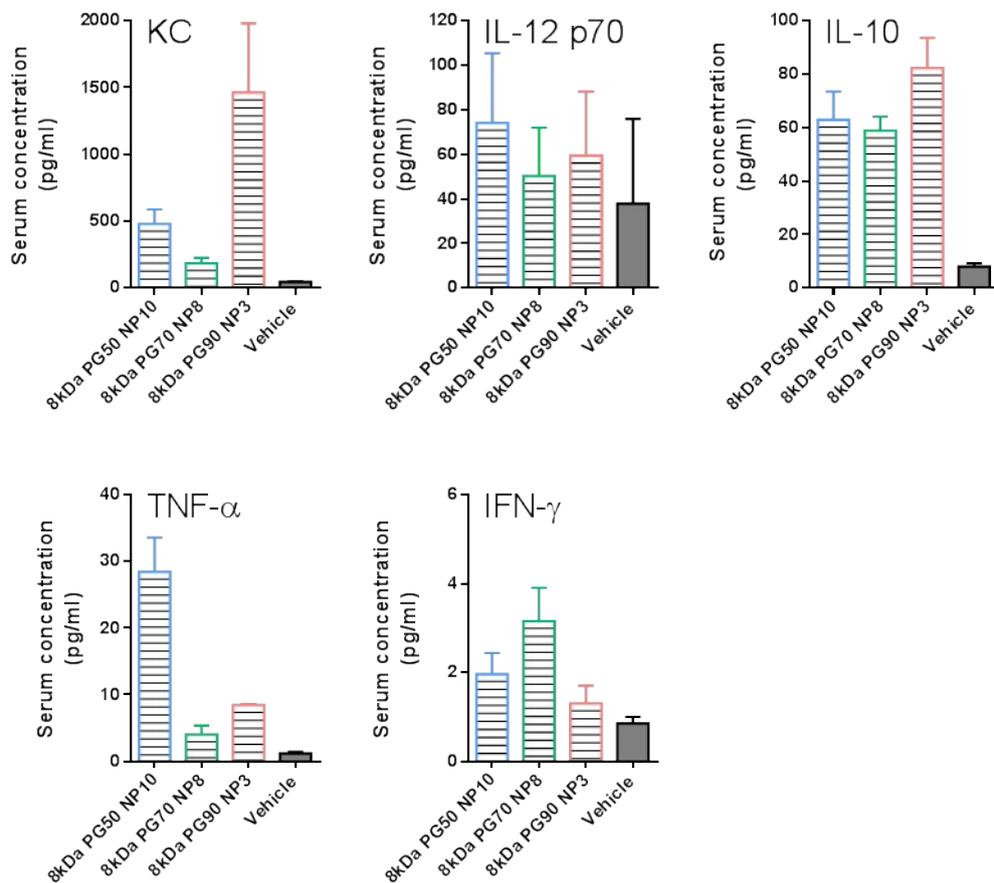
<sup>1</sup> A. Sunder, H. Frey and R. Mülhaupt, *Macromol. Symp.*, 2000, **153**, 187–196.

## 5. In vitro evaluation of the 8kDa hPG amines



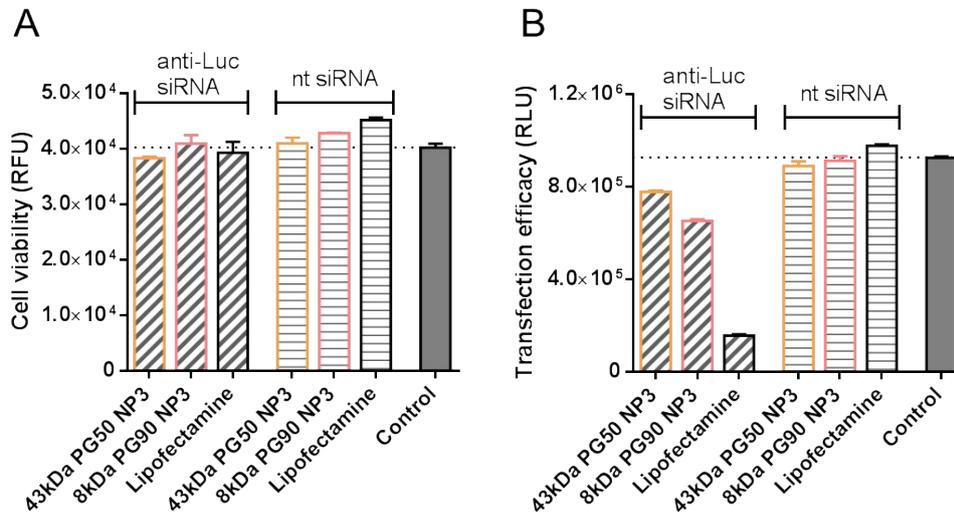
**Figure S 4.** *In vitro* transfection efficacy and tolerability of 8kDa hPG amines: 786-O Luc transgenic cells were transfected with luciferase-specific (anti-Luc siRNA) and non-targeting (nt) siRNA (ON-TARGETplus Non-targeting siRNA, Dharmacon) complexed with 8kDa PG50, PG70 and PG90 at N/P ratio of 10, 8, and 3, respectively, for 48 h. Lipofectamine was used as positive control and untreated cells as negative control. **A)** Cell viability and **B)** transfection efficacy were determined by using the commercial ONE-Glo™ + Tox Luciferase Reporter and Cell Viability Assay (Promega). Results are shown as mean±SD of triplicates.

## 6. Evaluation of pro-inflammatory cytokine secretion after systemic administration of 8kDa hPG amines (additional information)



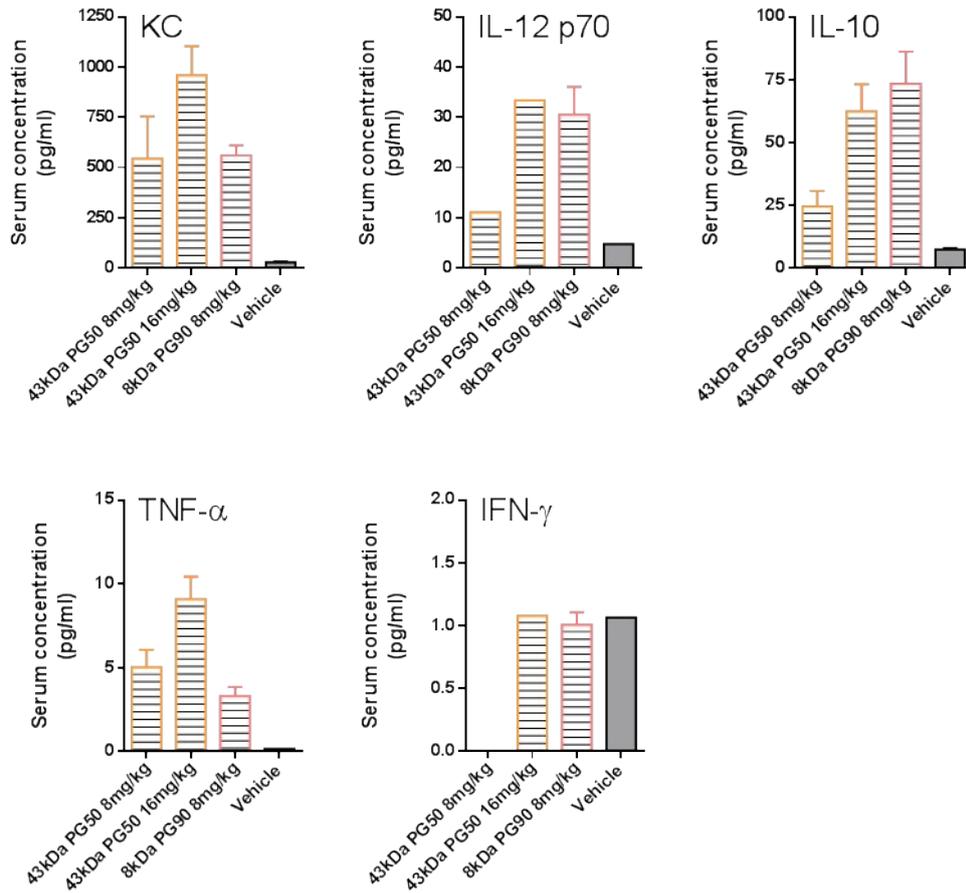
**Figure S5.** Determination of pro-inflammatory cytokine levels after systemic administration of 8kDa hPG amines: three BALB/c mice per group were treated intravenously with 8 mg/kg 8kDa PG50, 8kDa PG70 or 8kDa PG90, complexed with non-targeting siRNA (ON-TARGETplus Non-targeting siRNA, Dharmacon) at N/P ratios of 10, 8, and 3, respectively. HyClone™ HyPure water was used as negative control (vehicle). Retrobulbar blood was taken 1h after injection and the serum concentrations (pg/ml) of the 7 pro-inflammatory cytokines IL-6, IL-1 $\beta$ , KC (keratinocyte chemoattractant), IL-12p70, IL-10, TNF- $\alpha$  and IFN- $\gamma$  were examined via Meso Scale Discovery Multi-Spot Assay System, Mouse ProInflammatory 7-Plex Assay Ultra-Sensitive Kit. Results are shown as mean $\pm$ SD of triplicates.

## 7. *In vitro* evaluation of hPG amines 43kDa PG50 vs. 8kDa PG90



**Figure S 6.** *In vitro* transfection efficacy and tolerability of 43kDa PG50 vs. 8kDa PG90: 786-O Luc transgenic cells were transfected with luciferase specific and non-targeting siRNA (ON-TARGETplus Non-targeting siRNA, Dharmacon) complexed with nanocarriers 8kDa PG90 and 43kDa PG50 at NP ratio of 3 for 48 h. Lipofectamine was used as positive control and untreated cells as negative control. **A**) Cell viability and **B**) silencing efficacy was measured by using the commercial ONE-Glo™ + Tox Luciferase Reporter and Cell Viability Assay (Promega). Results are shown as mean±SD of triplicates.

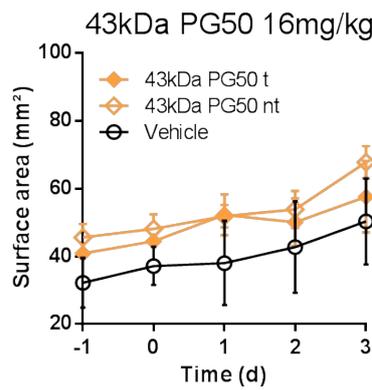
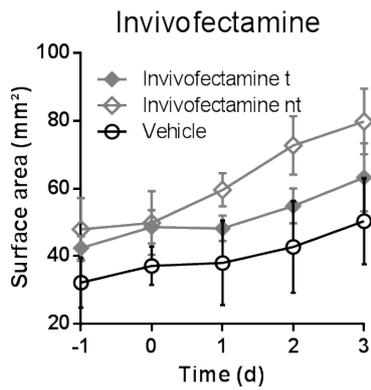
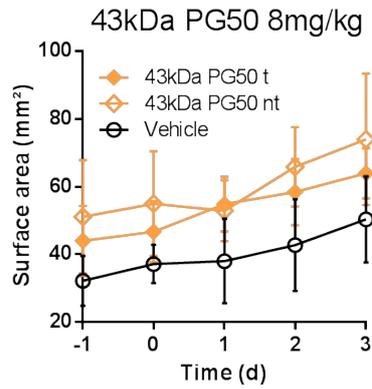
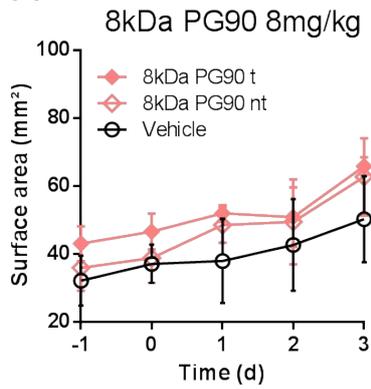
## 8. Evaluation of pro-inflammatory cytokine secretion after systemic administration of 43kDa PG50 vs. 8kDa PG90 (additional information)

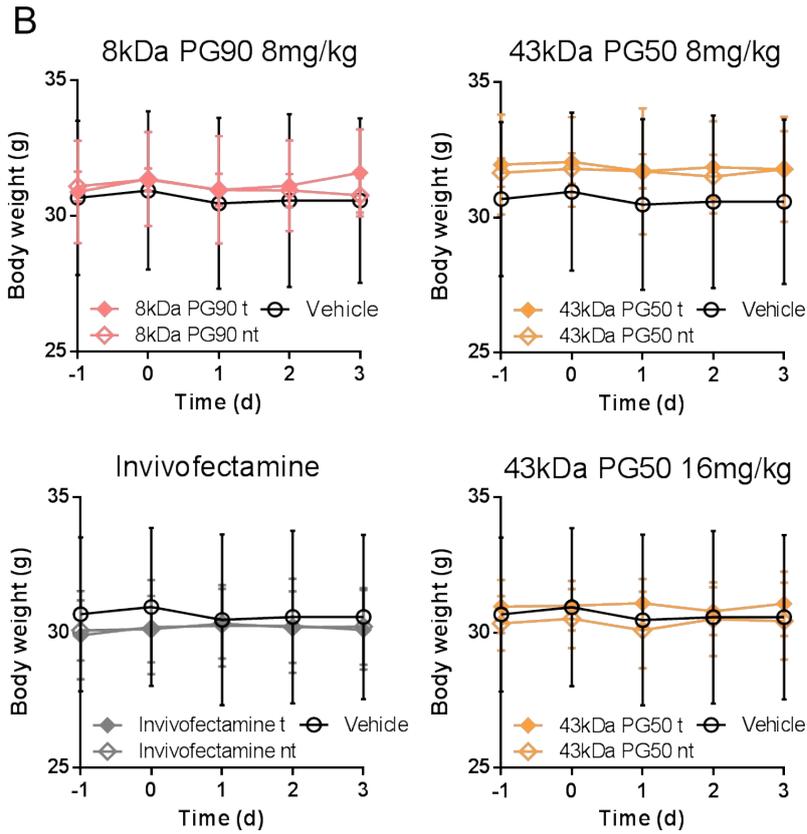


**Figure S7.** Determination of pro-inflammatory cytokine levels after systemic administration of 8kDa PG90 vs. 43kDa PG50: three BALB/c mice per group were treated intravenously with 8 mg/kg or 16 mg/kg 43kDa PG50 or 8 mg/kg 8kDa PG90 complexed with non-targeting siRNA at a N/P ratio of 3. HyClone™ HyPure water was used as negative control (vehicle). Retrobulbar blood was taken 1h after injection and the serum concentrations (pg/ml) of the 7 pro-inflammatory cytokines IL-6, IL-1 $\beta$ , KC (keratinocyte chemoattractant), IL-12p70, IL-10, TNF- $\alpha$  and IFN- $\gamma$  were examined via Meso Scale Discovery Multi-Spot Assay System, Mouse Proinflammatory 7-Plex Assay Ultra-Sensitive Kit. Results are shown as mean $\pm$ SD of triplicates.

**9. Evaluation of tumor surface area and body weight during luciferase knockdown study *in vivo* with hPG amines 43kDa PG50 vs. 8kDa PG90**

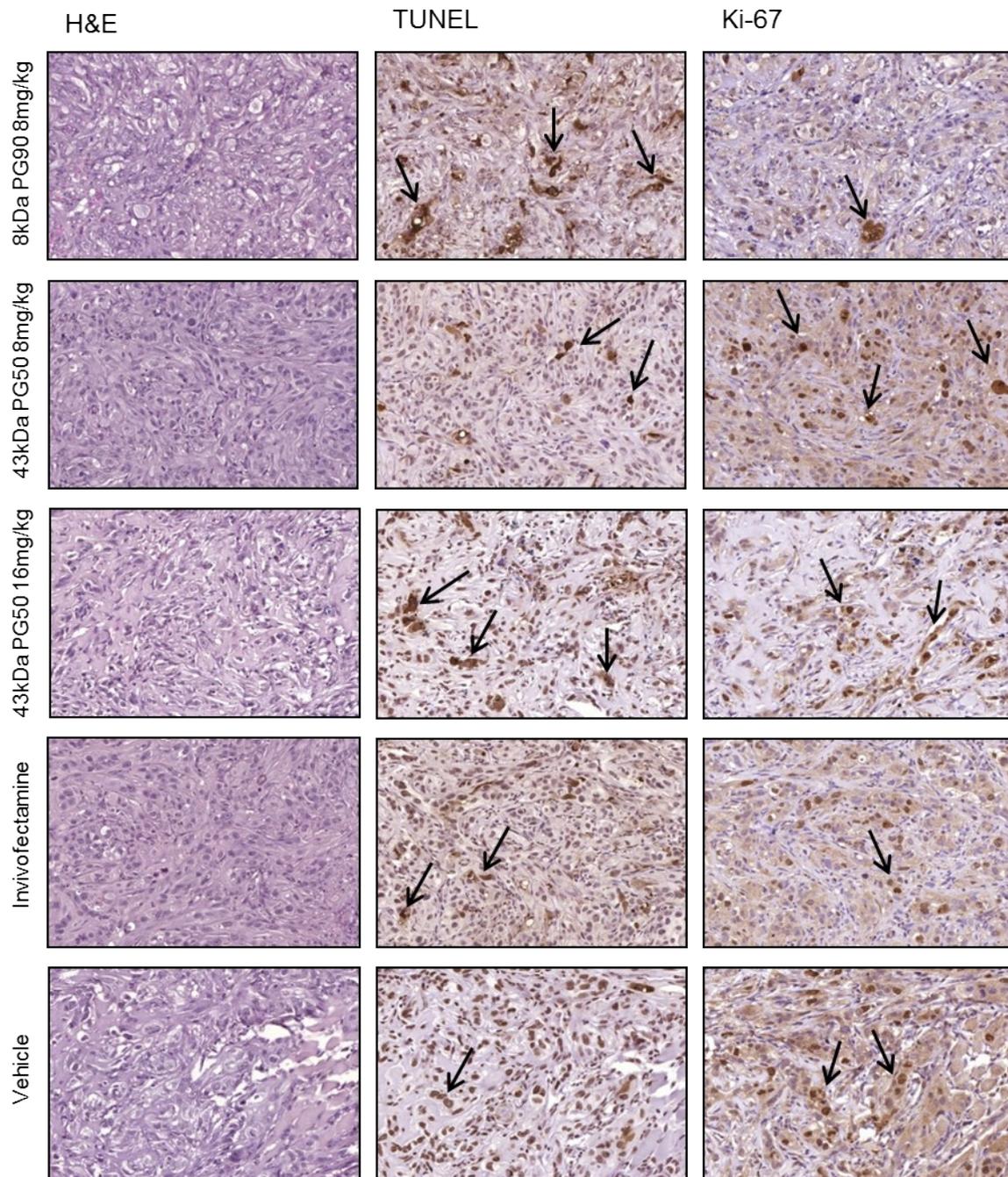
**A**





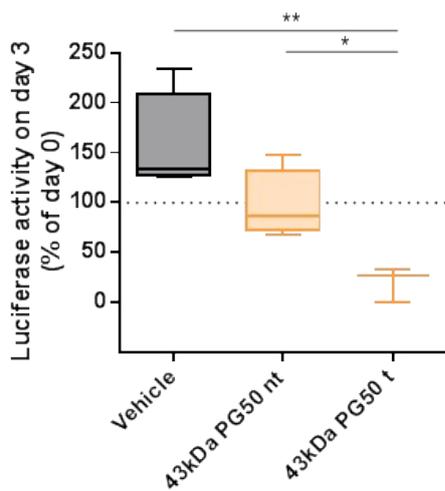
**Figure S 8.** *In vivo* knockdown study with 43kDa PG50 vs. 8kDa PG90: 786-O cells were implanted subcutaneously into NMRI nu/nu mice. **A)** Tumor growth was monitored by caliper measurement and **B)** body weight was detected by automatic weighing-machine. The treatment was started when tumors reached a size of 50mm<sup>2</sup>. Intratumoral injections of 8 mg/kg 8kDa PG90 and 8 mg/kg or 16 mg/kg 43kDa PG50, respectively, complexed with luciferase specific siRNA (t) or non-targeting siRNA (nt) at N/P ratios of 3 were given. Invivofectamine 2.0 Reagent (IF) complexed with anti-Luc siRNA was used as technical control and, HyClone™ HyPure water as negative control (vehicle). The results are shown in mean ± SD of quadruplicates. The tumor growth and body weight were not affected by the performed treatment.

## 10. Histological analysis of tumor sections treated with 43kDa PG50 vs. 8kDa PG90



**Figure S 9.** Histological analysis of tumor sections treated with 43kDa PG50 vs. 8kDa PG90: tumors were stained for H&E, DNA strand breaks (TUNEL) or Ki-67 using DAB chromagen and representative photographs were taken applying brightfield microscopy (x50). There was no crucial change in cell death (TUNEL) or proliferation (Ki-67) in tumors treated with 8mg/kg 8kDa PG90, 8mg/kg 43kDa PG50, 16mg/kg 43kDa PG50 or Invivofectamine compared to those treated with vehicle.

## 11. *In vivo* silencing of the PLK-1 gene using 43kDa PG50



**Figure S 10.** *In vivo* knockdown study with 43kDa PG50: silencing of the PLK-1 gene was investigated on day 3 after intratumoral injection of 16 mg/kg 43kDa PG50, complexed with PLK-1 specific siRNA (t) or non-targeting siRNA (nt) at N/P ratio 3 in NMRI nu/nu mice bearing 786-O Luc tumors on three consecutive days starting at day 0. HyClone™ HyPure water was used as negative control (vehicle). The results are shown in mean  $\pm$  SD of quadruplicates. Statistical analysis was performed to compare the luciferase activity of tumors treated with anti-Luc siRNA and those with non-targeting siRNA by using the unpaired one-sided t-test with logarithmized data (\*  $p < 0,05$ , \*\* $p < 0,01$ ).