

SUPPLEMENTARY FIGURES

**for**

**Biodistribution of Therapeutic Small Interfering RNAs Delivered with  
Lipid-Substituted Polyethylenimine-Based Delivery Systems**

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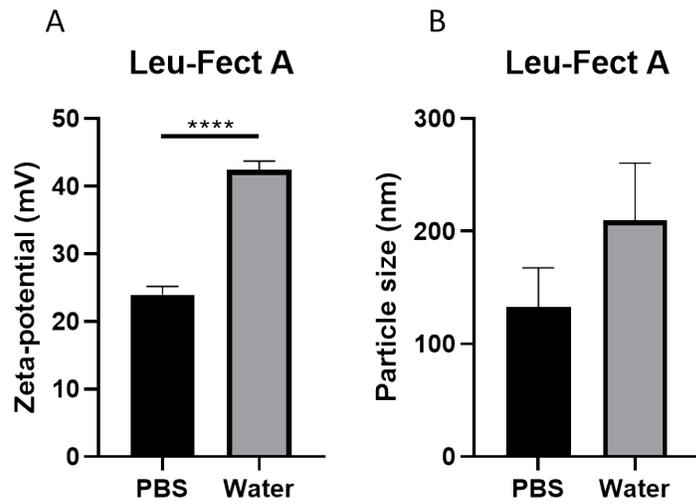
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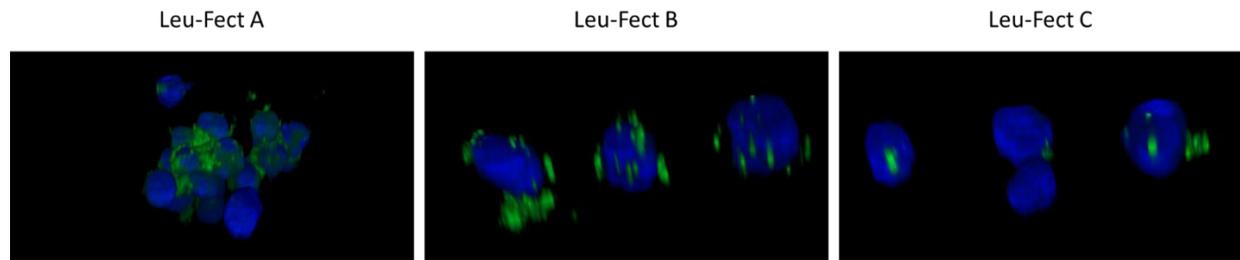
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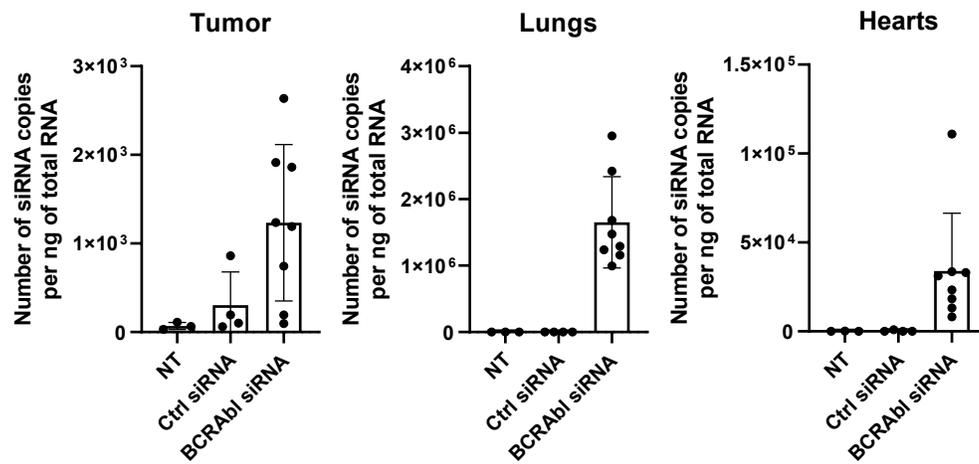
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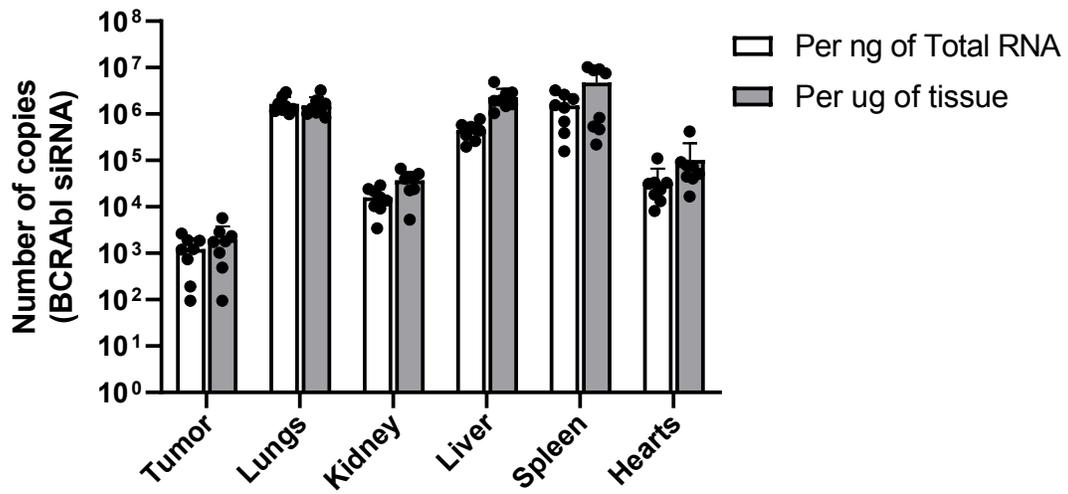
Supplementary Figure 1. Effects of solvent on  $\zeta$ -potential and particle size: (A) Preparation of siRNA complexes in water results in a significantly higher  $\zeta$ -potential when compared to PBS. (B) Particle size shows a trend towards larger sizes when the complexes were prepared in water, but does not reach statistical significance.



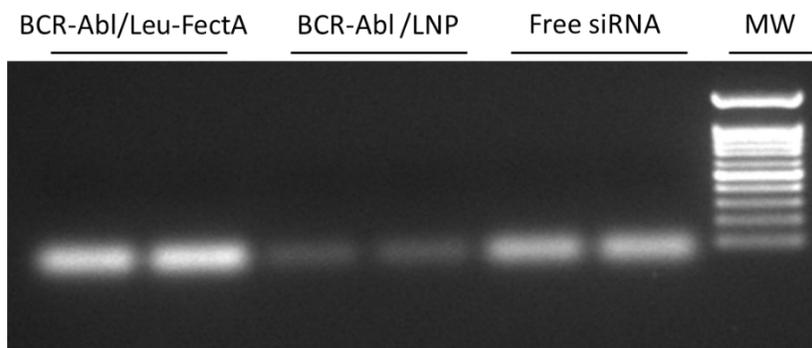
**Supplementary Figure 2:** 3D reconstruction of images obtained by confocal microscopy of MV4:11 cells treated with 60 nM of FAM-siRNA at a 1:7.5 siRNA/carrier ratio.



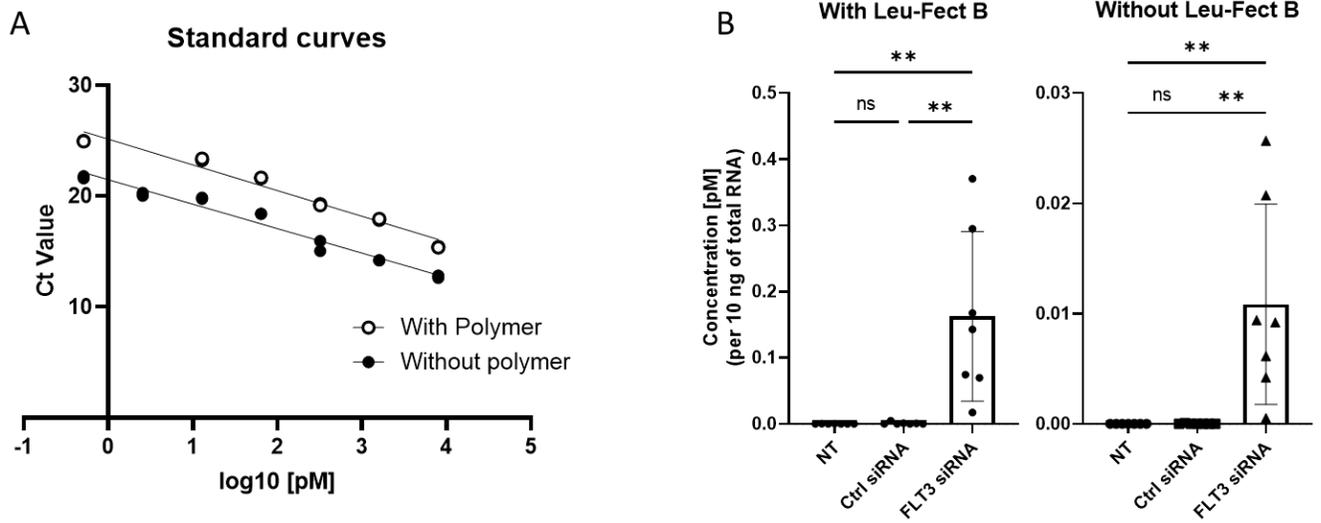
Supplementary Figure 3. Biodistribution of siRNA in different organs in a xenograft CML model: Amount of BCR-Abl siRNA in the tumor and other organs of mice injected with either vehicle (NT), control siRNA or BCR-Abl siRNA, expressed as number of copies per nanogram of total RNA. Organ collection was performed 48 hours after the last siRNA injection.



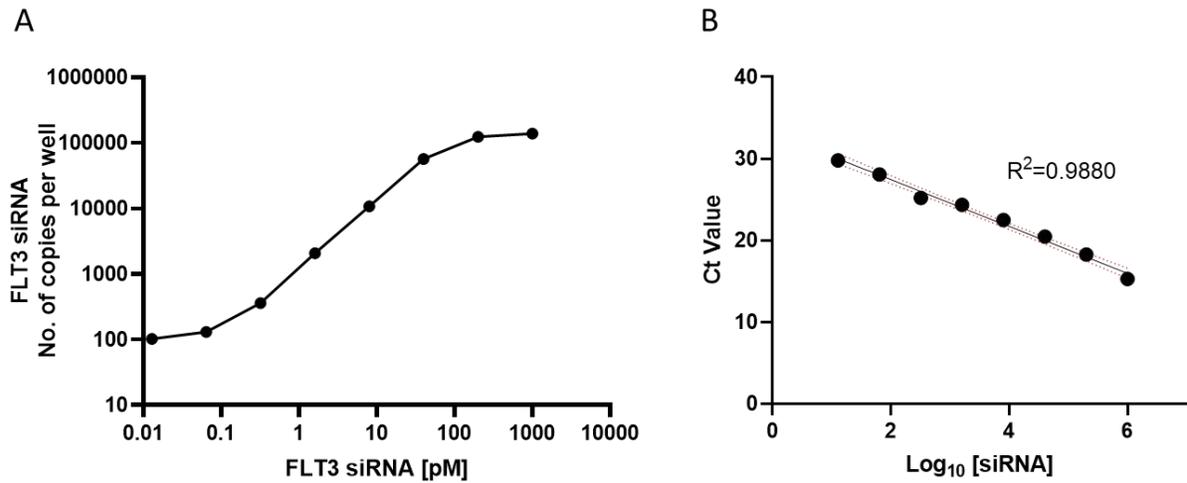
Supplementary Figure 4. Summary of number of BCR-Abl siRNA copies in each organ after 2 days of last BCR-Abl siRNA injection. The siRNA copies were normalized over amount (ng) of total RNA as well as the mass ( $\mu\text{g}$ ) of tissue. Both methods of normalization show similar results.



**Supplementary Figure 5.** Equal proportions of the final volume of the complexes injected into mice were analyzed by electrophoresis to extrapolate the total amount of BCR-Abl siRNA injected in different groups (duplicate lanes). The amount of siRNA injected in animals in the LNP group was 7  $\mu\text{g}$  siRNA per animal (maximum dose that could be injected based on LNP loading), while animals in the Leu-Fect A group were injected with 25  $\mu\text{g}$  siRNA. Last two lanes contain free BCR-Abl siRNA for reference.

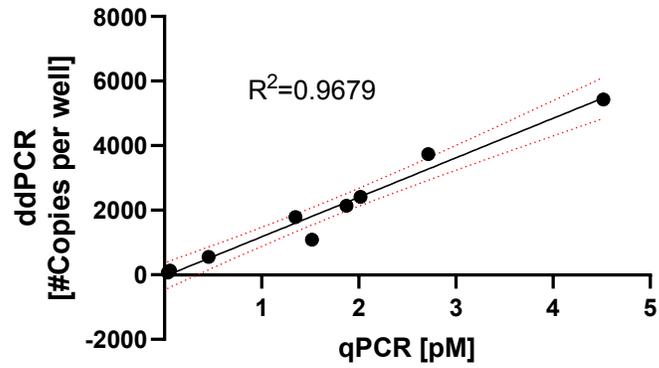


Supplementary Figure 6: (A) A standard curve of FLT3 siRNA was prepared, including an initial concentration of 8 nM followed by 1:5 serial dilutions to assess the effect of the presence of the polymer (Leu-Fect B) in the qPCR reaction. (B) Tumor samples of vehicle (NT: no treatment), control siRNA, and FLT3 siRNA treated animals were analyzed and concentration of FLT3 siRNA was calculated by extrapolating to a standard curve prepared including the carrier (left) or without it (right).



Supplementary Figure 7: A standard curve of FLT3 siRNA was prepared, including an initial concentration of 1 nM followed by 1:5 serial dilutions to assess the dynamic range of both methodologies. **(A)** Samples were run by ddPCR and the number of FLT3 siRNA copies detected vs. concentration was plotted. **(B)** Samples run by qPCR and the Ct Values vs. Log<sub>10</sub> of siRNA concentration was plotted. Analysis was done by simple linear regression. Red dotted lines represent CI95.

### Correlation of FLT3 levels Heart samples



**Supplementary Figure 8:** Amount of FLT3 siRNA in the hearts of non-treated mice or injected with FLT3 siRNA, expressed as concentration per 10 ng of total RNA (qPCR) or number of copies per well (ddPCR). Organ collection was after 72 hours of last injection. Analysis was done by simple linear regression. Red dotted lines represent CI95. ( $R^2 = 0.9679$ ;  $N=9$ ).