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Supplemental Information

Improving the Repeatability and Efficacy of Intradermal Electroporated Self-Replicating mRNA

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Supplemental data

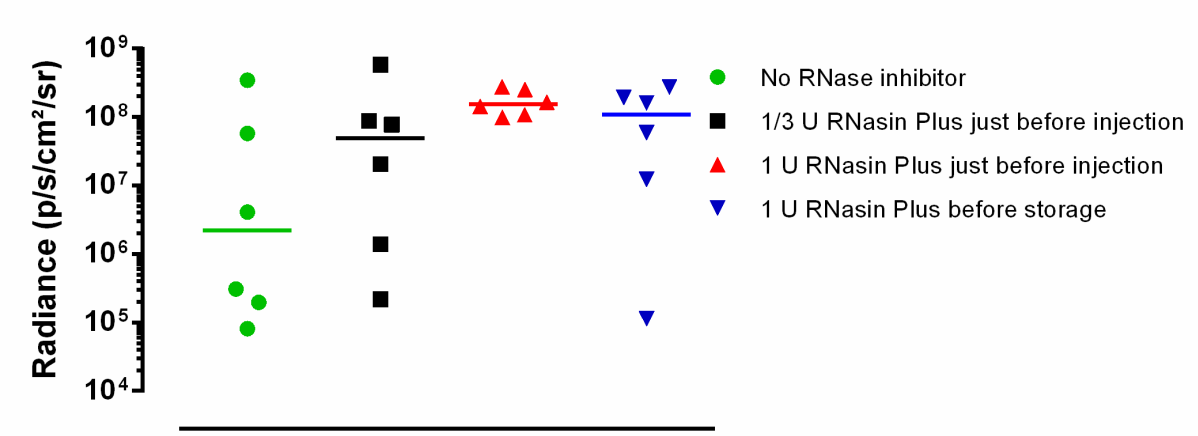

Fig. S1. Quantification of the effect of different doses of RNase inhibitor on the expression of sr-mRNA after intradermal electroporation in the flank of mice. The luciferase expression was quantified by calculating the AUCs of the curves in Fig. 2 (n=6). The median of these AUC-values as well as the individual values are shown. The median AUC in the absence of RNase inhibitor (green line) increased 70-fold when the RNase inhibitor (1 units/µl, red line) was added to the sr-mRNA (5 µg/50 µl) just before administration. The data in red and black were obtained when the RNase inhibitor was added just before intradermal electroporation of 5 µg luciferase encoding sr-mRNA, while the data in blue were obtained with sr-mRNA that had been stored with the RNase inhibitor at -80°C.

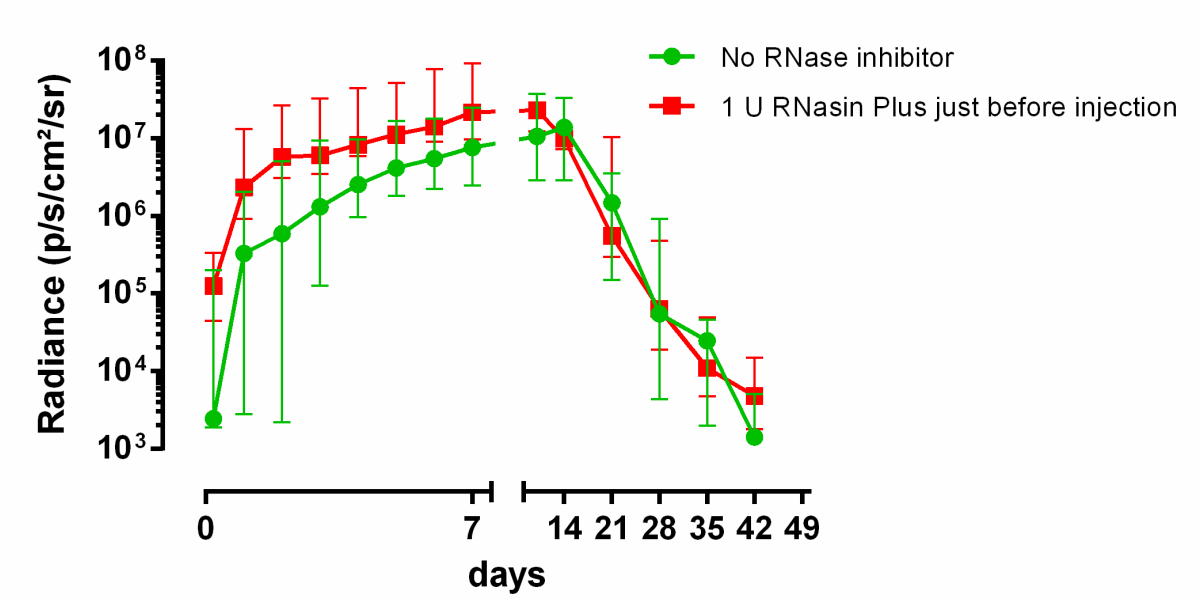

Fig. S2. Effect of RNase inhibitor on the expression of naked sr-mRNA after intradermal administration without electroporation. The median bioluminescence signals with interquartile range obtained after intradermal injection of naked sr-mRNA with or without RNase inhibitor supplementation. Five microgram of luciferase encoding sr-mRNA dissolved in 50 µl PBS (without Ca^{2+} and Mg^{2+}) was injected at the tail base of mice (n=6). The green curve shows the luciferase expression without supplementation with RNase inhibitor, while the red curve shows the expression after adding 1.0 units/µl of RNase inhibitor to the sr-mRNA just before injection. No significant differences were observed. Only the variation of the expression was clearly higher in the first 4 days when no RNase inhibitor was used (green curve).

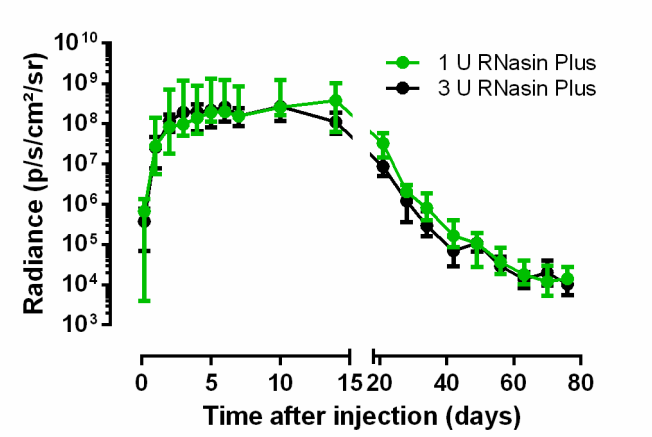

Fig. S3. Increasing the RNase inhibitor dose above 1 unit per microliter sr-mRNA solution did not further improve the expression or repeatability. The median bioluminescence signals over time after intradermal electroporation of a luciferase encoding sr-mRNA (5 µg/50 µl PBS) to which 1.0 (green curve) or 3.0 units/µl (black curve) of RNase inhibitor was added just before injection. No significant differences were observed between the groups. Error bars represent interquartile range (n=6).

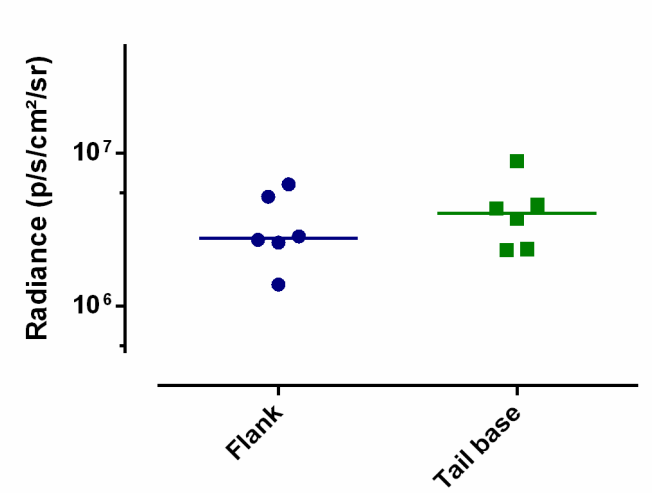

Fig. S4. Comparison of the penetration of bioluminescent light through the skin of the flank and tail base. Mice were intradermally injected at the flank and at the tail base with an equal amount of 4T1 cells (10^6) that stably express luciferase. Directly after administration of the bioluminescent cells, luciferin was intraperitoneal injected and 15 minutes later the bioluminescent signals were measured. The graph represents the median bioluminescence signals and the individual signals after flank (blue) or tail base (green) administration. No significant difference was observed.

	Buffer	n-value	Mean	SD	Significant difference
PARTICLE SIZE (diameter nm)	**PBS**	4	920	174	ns
	Ca^{2+} PBS	6	1155	309	
ZETA POTENTIAL (mV)	**PBS**	4	-7	4	**
	Ca^{2+} PBS	9	-29	1	p = 0.0028

Table S1. Effect of calcium ions on the hydrodynamic diameter and zeta potential of sr-mRNA. Ten µg of sr-mRNA was dissolved in 100 µl PBS without or with $CaCl_2$ (3mEq/l Ca^{2+}). After 30 minutes of incubation at room temperature, the sr-mRNA solutions were diluted with either PBS or $CaCl_2$-containing PBS to 1 ml, keeping a constant Ca^{2+} concentration. Subsequently, the size and zeta potential were measured.