

Delivery and immunogenic performance of naked DNA vaccines visualized by *in vivo* imaging of a novel near-infrared fluorescent reporter.

Juris Jansons

DNA electroporation

The procedure improves plasmid delivery by a factor of 10–1,000 fold over naked DNA delivery alone

Inflammatory response stimulated by electroporation may also be essential for enhancing immune responses to DNA vaccines.

Increased gene expression and inflammatory cell infiltration caused by electroporation are both important for improving the efficacy of DNA vaccines

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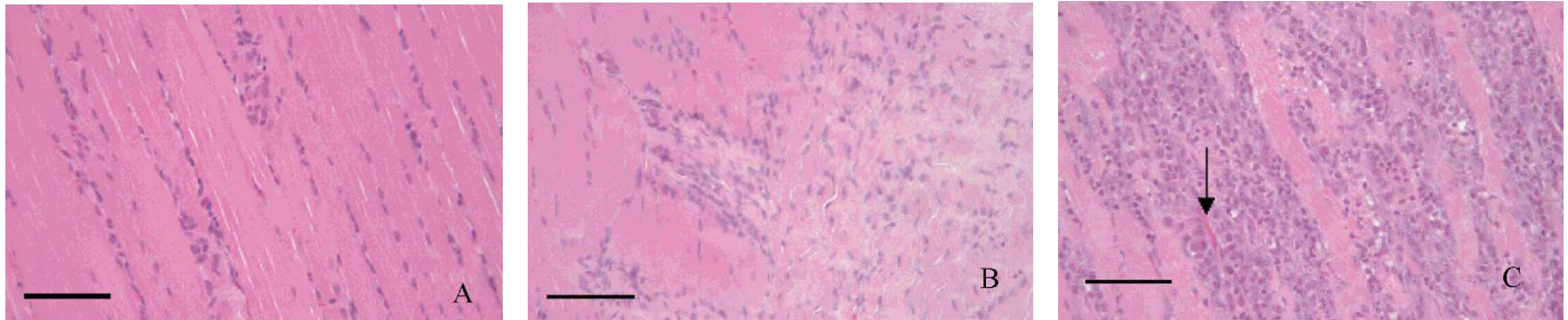
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Histological examination of muscle (HE stain) 48 h following plasmid administration. (A) Muscle injected with plasmid; (B) muscle injected with plasmid followed by electroporation (two pulses 100 V); (C) muscle injected with plasmid followed by electroporation (six pulses 200 V) representative of (two pulses 200 V).

Mild infiltration of macrophages and neutrophils was observed in (A) and severe infiltration of macrophages and neutrophils was observed in (B) and (C) with scattered necrotic myofibers (arrow) were also noted. Bar is 100 μ m.

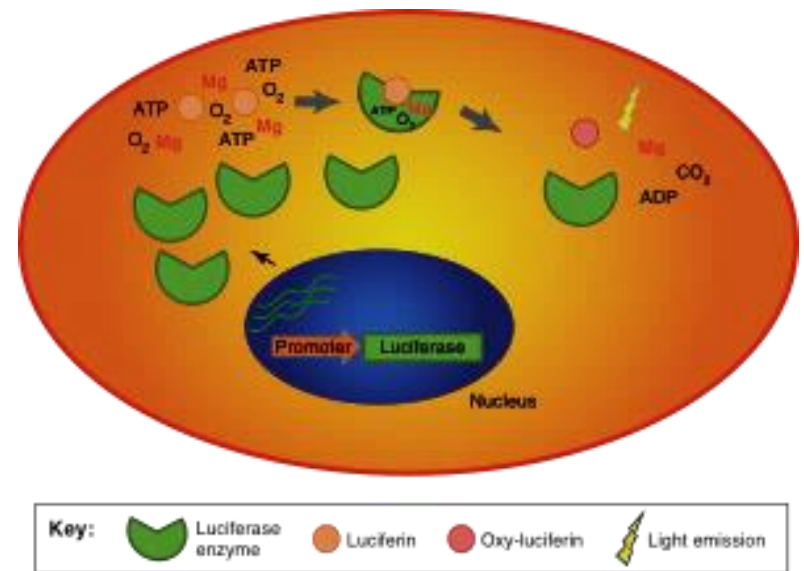
The luciferase reporter gene

- Luciferases

Firefly, Renilla/Gaussia, Bacterial

Generate luminescent light (490 – 560 nm)

Low immunogenicity



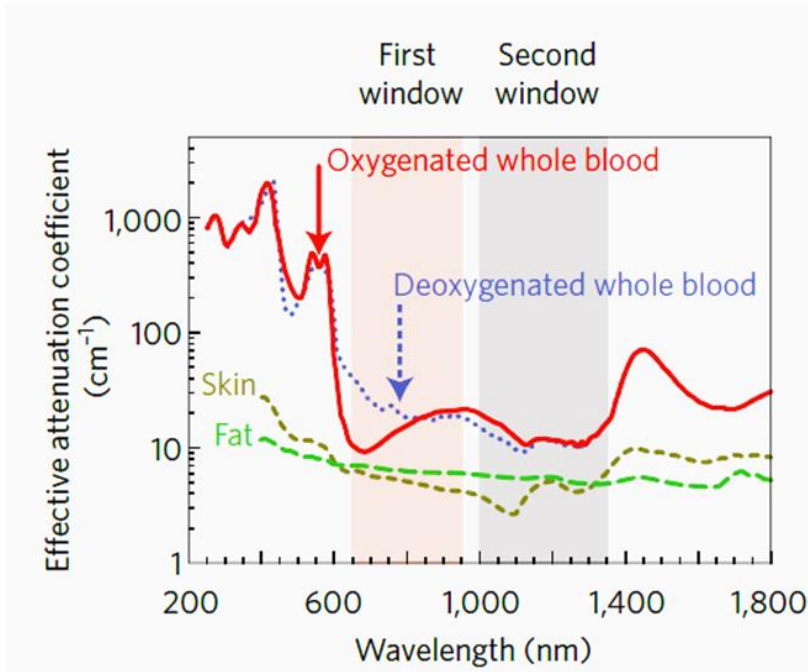
Near-infrared fluorescent proteins

Derived from bacterial phytochrome photoreceptors (BphP)

Suitable for deep optical imaging in mammalian tissues

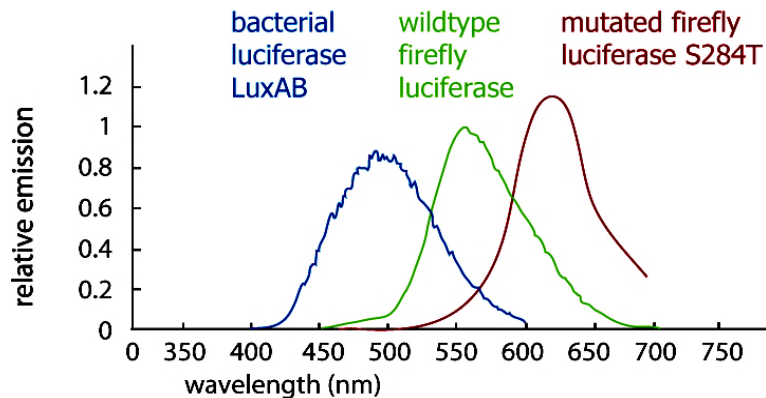
Shcherbakova, D. M., & Verkhusha, V. V. (2013). *Nature Methods*.

Luminescence vs fluorescence in tissue

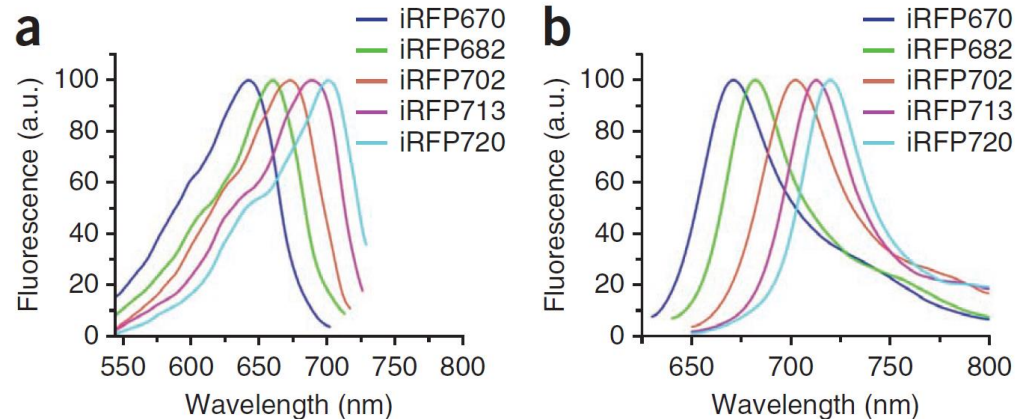


Near-infrared optical window

- Well-defined window for imaging 650-950 nm
- High tissue penetration due to low scatter and absorbance
- Reduced autofluorescence

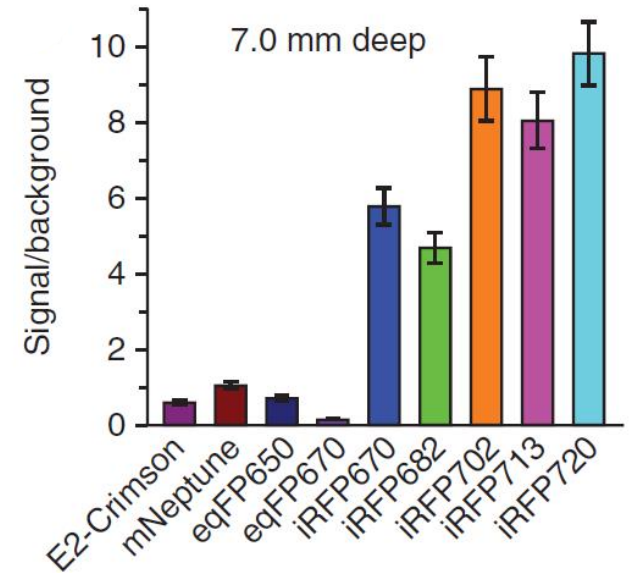
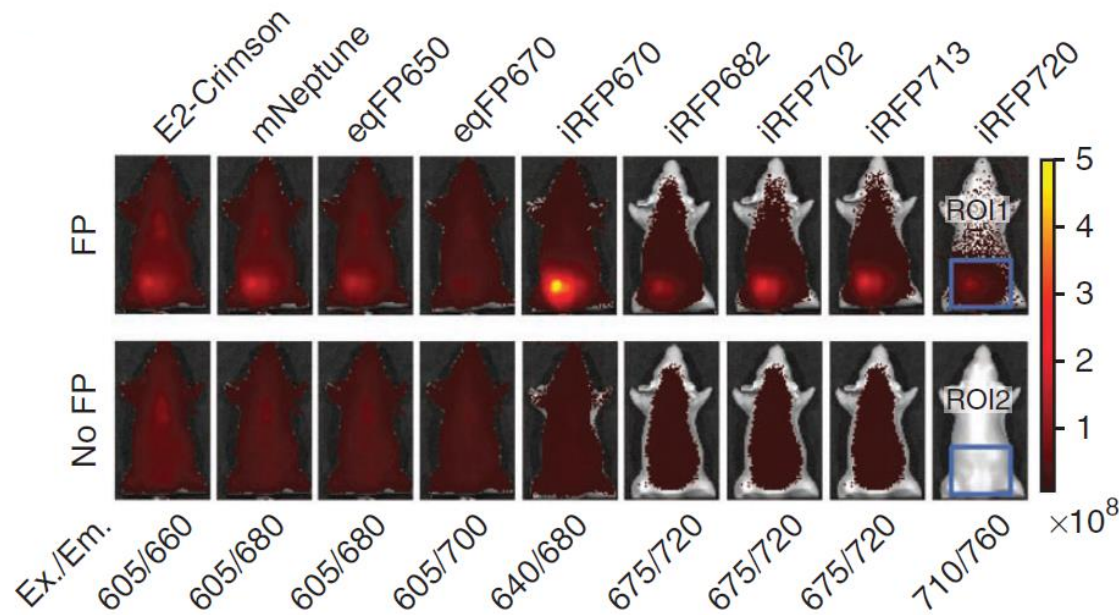


Light emission of luciferase

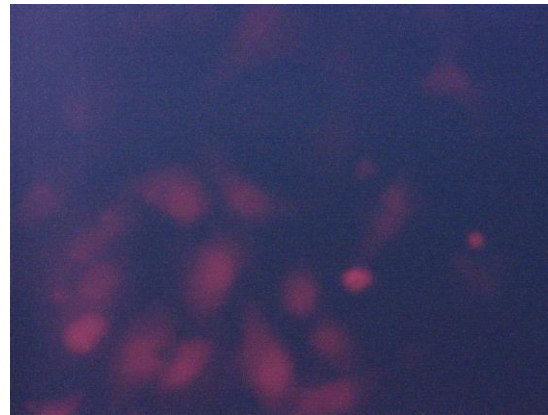
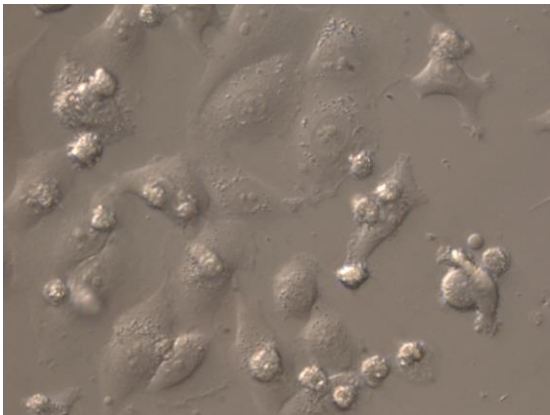


Excitation (a) and emission (b) of BphP-based fluorescent proteins.

Comparison of iRFPs with GFP-like far-red FPs as fluorescent probes in deep-tissue imaging.

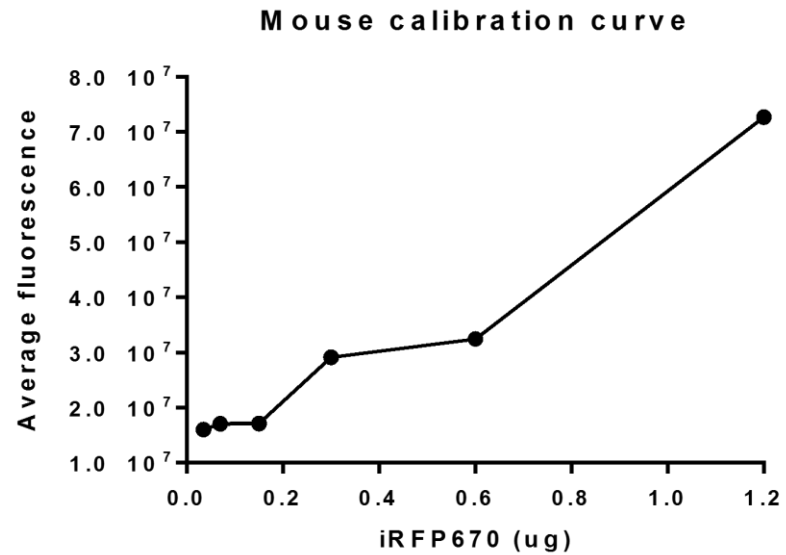
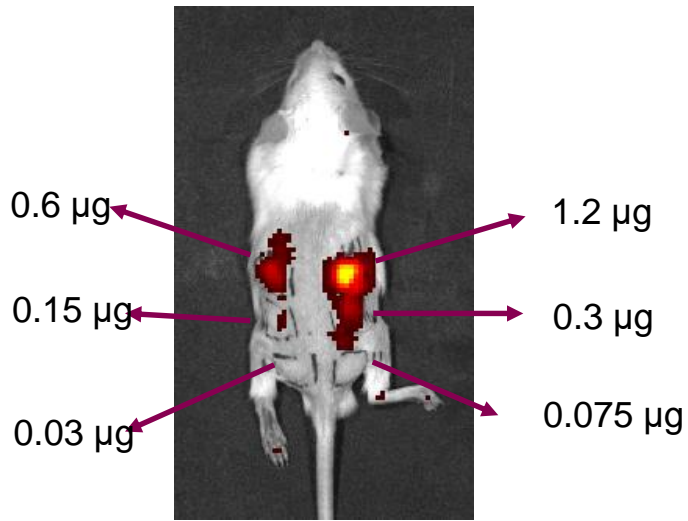


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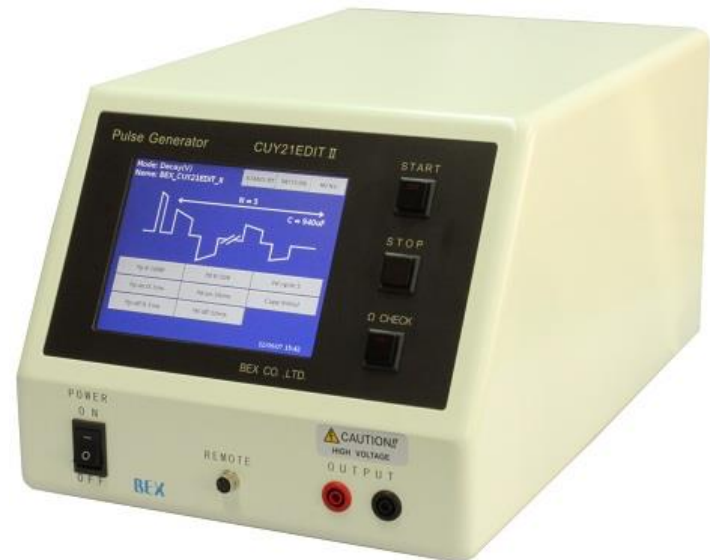
Expression of iRFP670 in HeLa cells detected by fluorescent microscopy

iRFP670 protein detection by IVIS Spectrum CT



CUY21EDIT II pulse generator

- In vivo and in vitro electroporation
- Patterns of electroporation pulses
 - Square
 - Decaying
 - Change of polarity
- First constant current electroporator
 - The user can set desired current



Electrodes for skin electroporation



□ 2-needle electrode array, BTX



□ Multineedle array electrodes, BTX



□ Platinum-coated tweezers with plate electrodes, BEX



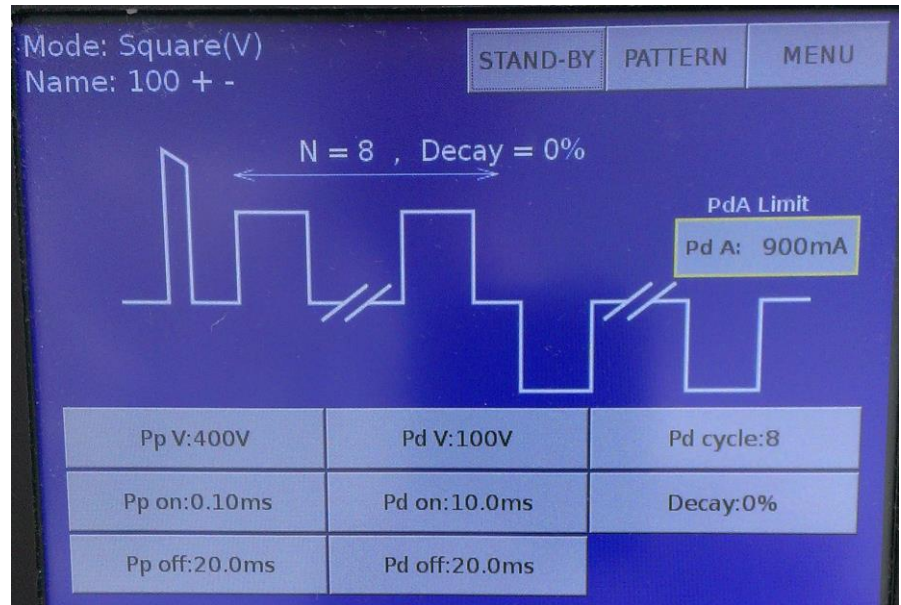
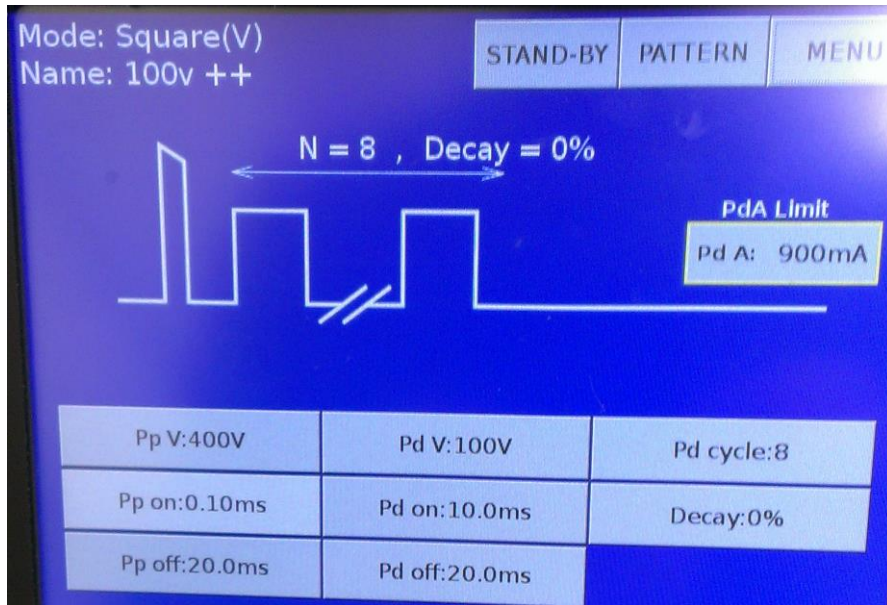
□ Tweezers with fork and a plate electrode, BEX

Optimization of iRFP670 delivery

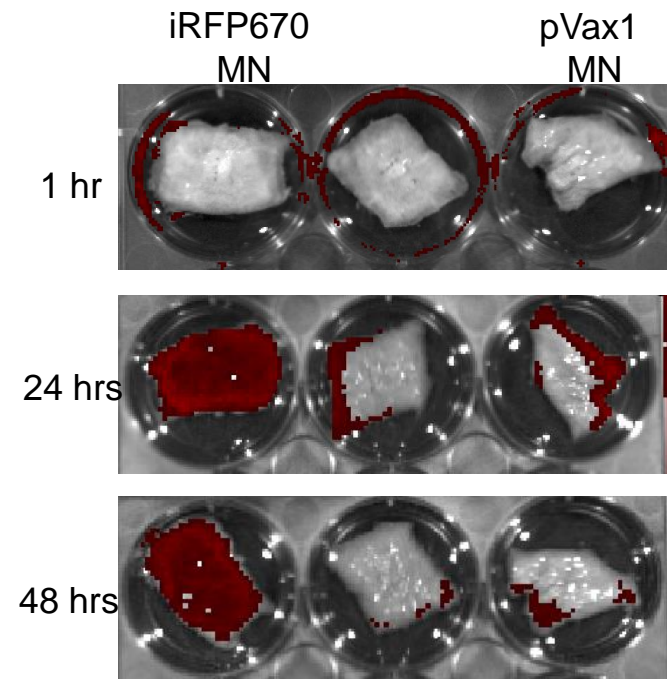
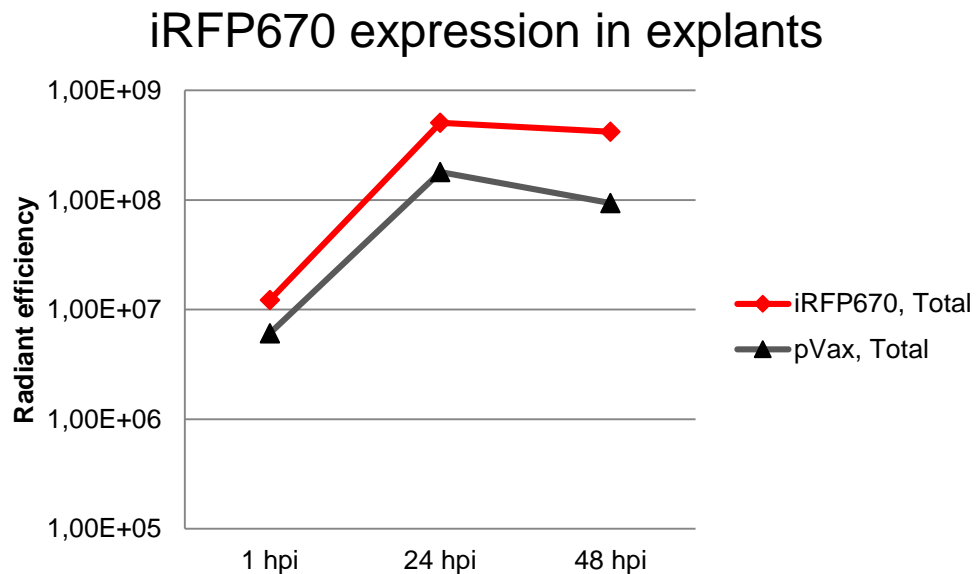
- 4 important parameters:
 - Electrodes
 - Multineedle
 - 2-needle
 - Plate
 - Plate-fork
 - Voltage
 - Polarity
 - Dose

Immunization parameters

- Intradermal injection of plasmid encoding reporter gene dissolved in 20 μ l PBS
- Electroporation immediately after injection

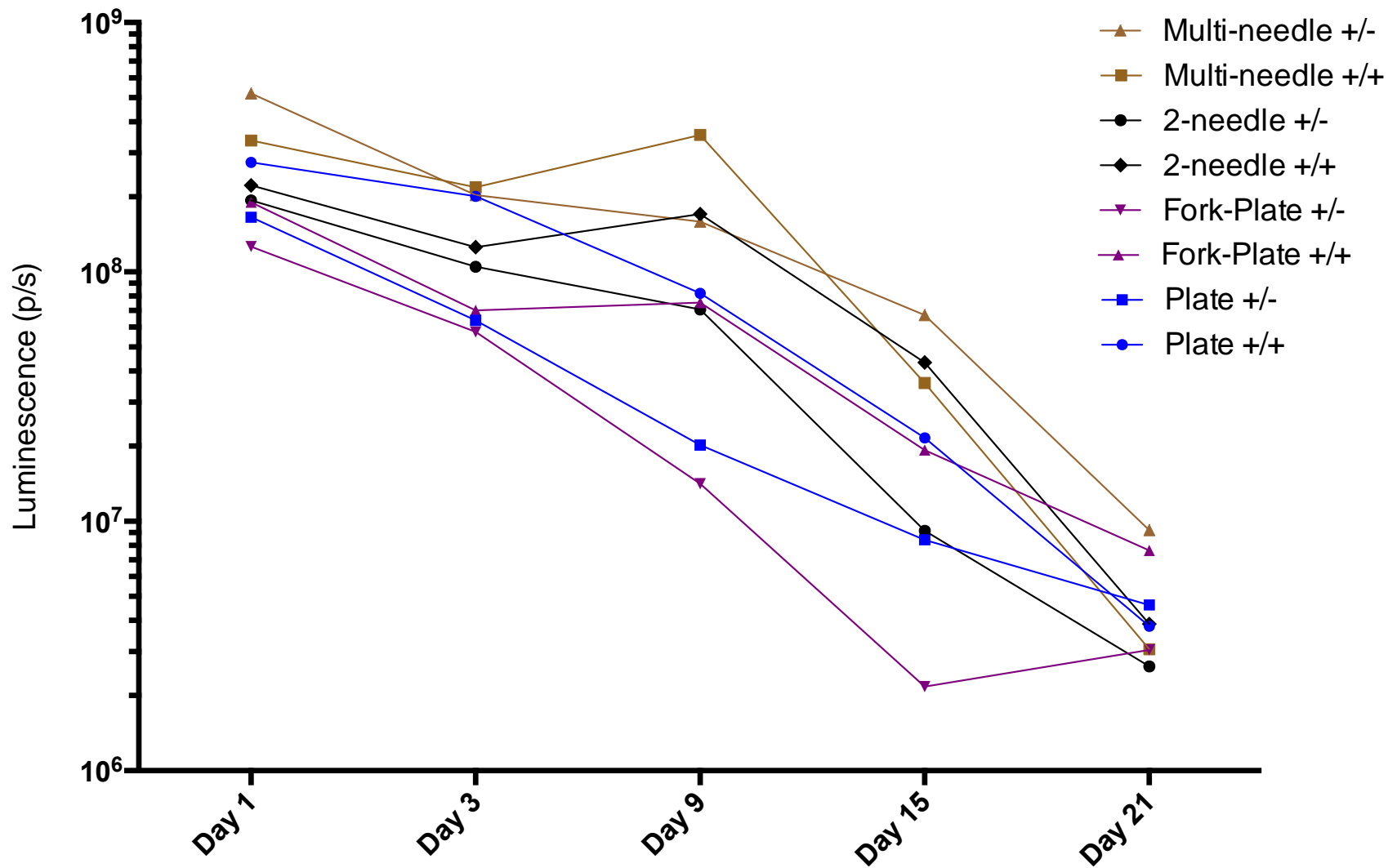


Optimization in human skin explants (50 μ g iRFP670)

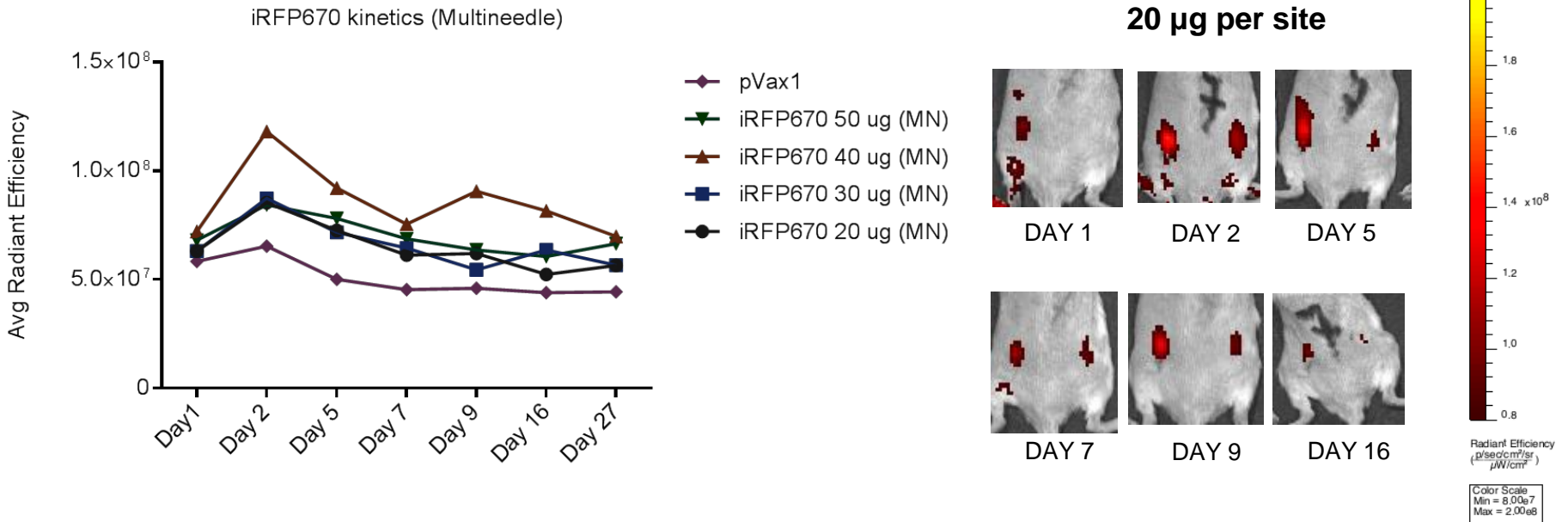


Detectable difference in fluorescence between vector and iRFP670 inoculated explants

Longitudinal monitoring of luciferase activity after *in vivo* transfection

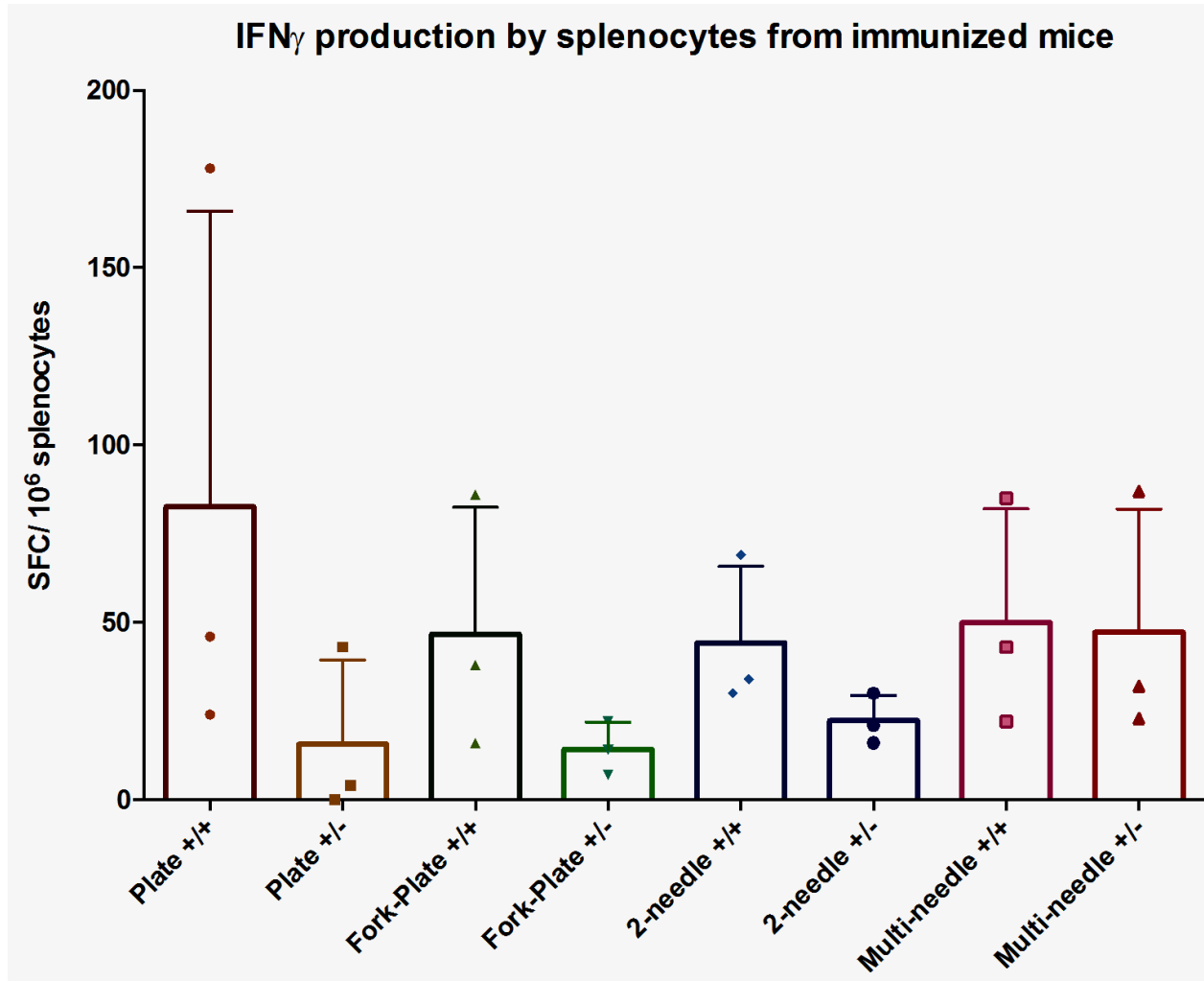


Longitudinal monitoring of fluorescence after *in vivo* transfection



The data confirms saturation at not more than 40 μ g. Expression persists longer than 27 days.

Anti-luciferase immunological response



Issues with *in vivo* imaging

Luciferase

vs

iRFP

1. Poor deep-tissue detection due to spectral properties
 2. Luciferase detection requires substrate (D-luciferin) and ATP
1. Background signal due to post-electroporational inflammation
 2. Requires excitation light and emission filter
3. Immunological response to the both proteins is equally low

Conclusions

1. Expression of iRFP670 reporter was evaluated in short- and long term experiments
2. Immunogenicity and toxicity of iRFP670 *in vivo* are low
3. iRFP670 is a promising candidate for both *in vivo* and *ex vivo* imaging of transfected tissue

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