HUVEC: transfection of siRNA

VB-01LB-00, VB-01LB-01

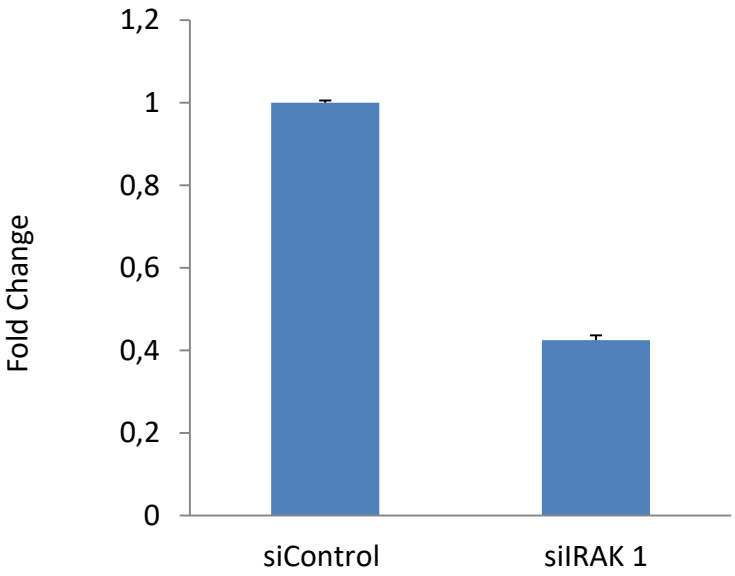


Fig. 1: Knock-down of IRAK 1 gene expression in HUVEC (human umbilical vein endothelial cells) after transfection with Viromer® BLUE

- 100nM siRNA (standard protocol for suspension cells)
- **60% knock-down** 48h post transfection

Dr. R. Teske, Cardiovascular Biology - University Hospital Giessen (Germany)

HUVEC: transfection of siRNA

VB-siBUNDLE-01

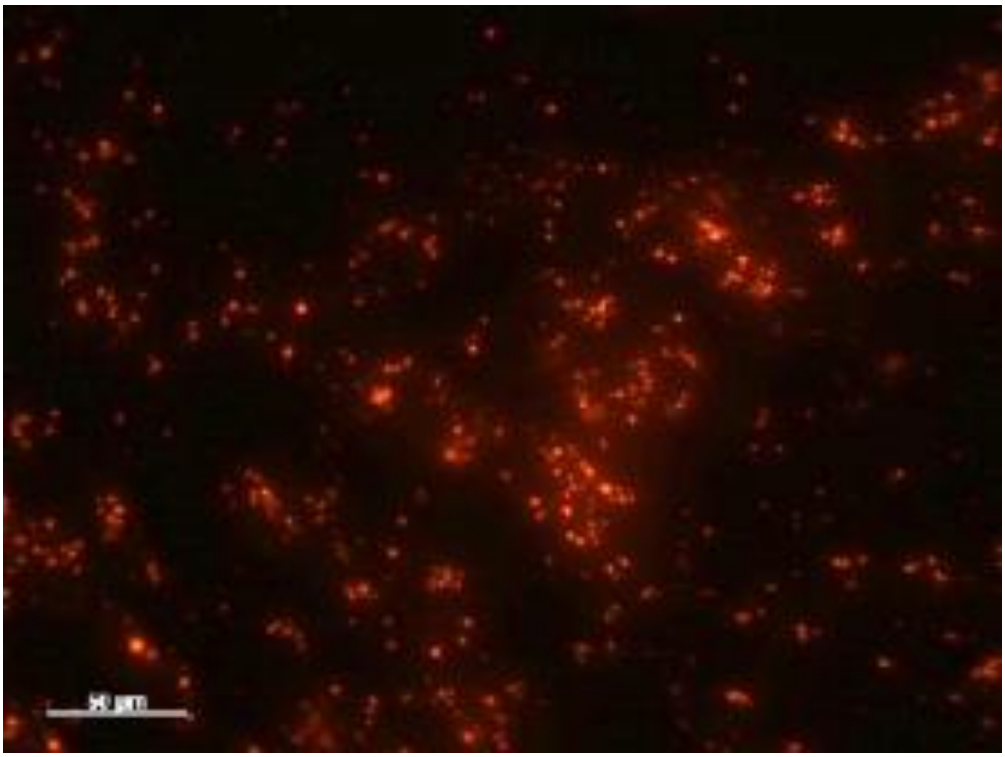
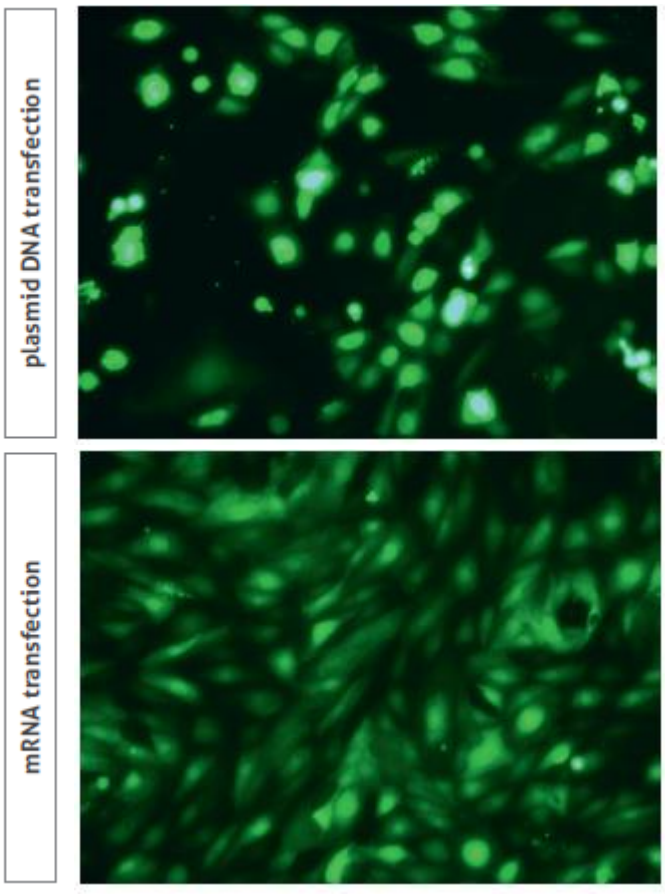
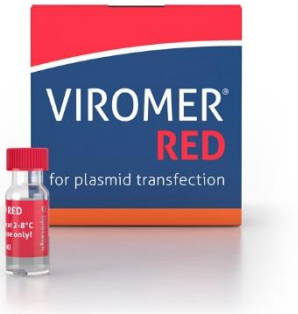


Fig. 2: Monitoring of transfection complex uptake by HUVEC cells transfected with a Cy3-labeled siRNA using Viromer® BLUE Start Positive Controls (fluorescence microscopy).

Internal data Lipocalyx GmbH

HUVEC: transfection of plasmid DNA and mRNA





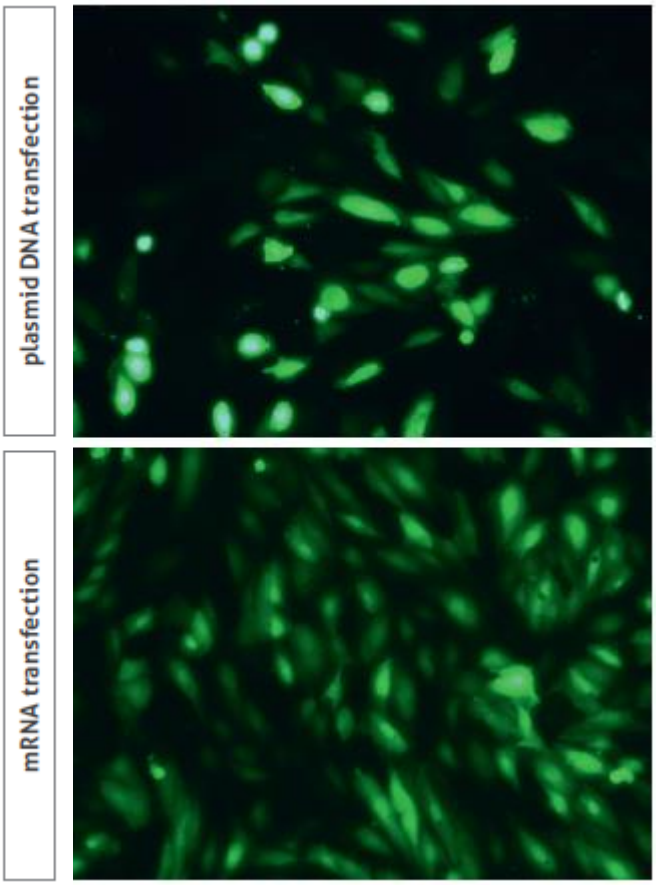
VR-01LB-00, VR-01LB-01

Fig. 3: Comparative GFP expression in HUVEC (human umbilical vein endothelial cells) after transfection of pDNA or mRNA with Viromer® RED

- transfection with pCMV-GFP plasmid and GFP-encoding mRNA
- monitored by fluorescence microscopy

Data from H. Cynis, Fraunhofer Institute for Cell Therapy and Immunology, Halle (Germany)

HUVEC: transfection of plasmid DNA and mRNA



VR-BUNDLE-01

Fig. 3: Comparative GFP expression in HUVEC (human umbilical vein endothelial cells) after transfection with Viromer® RED Start Positive® Controls

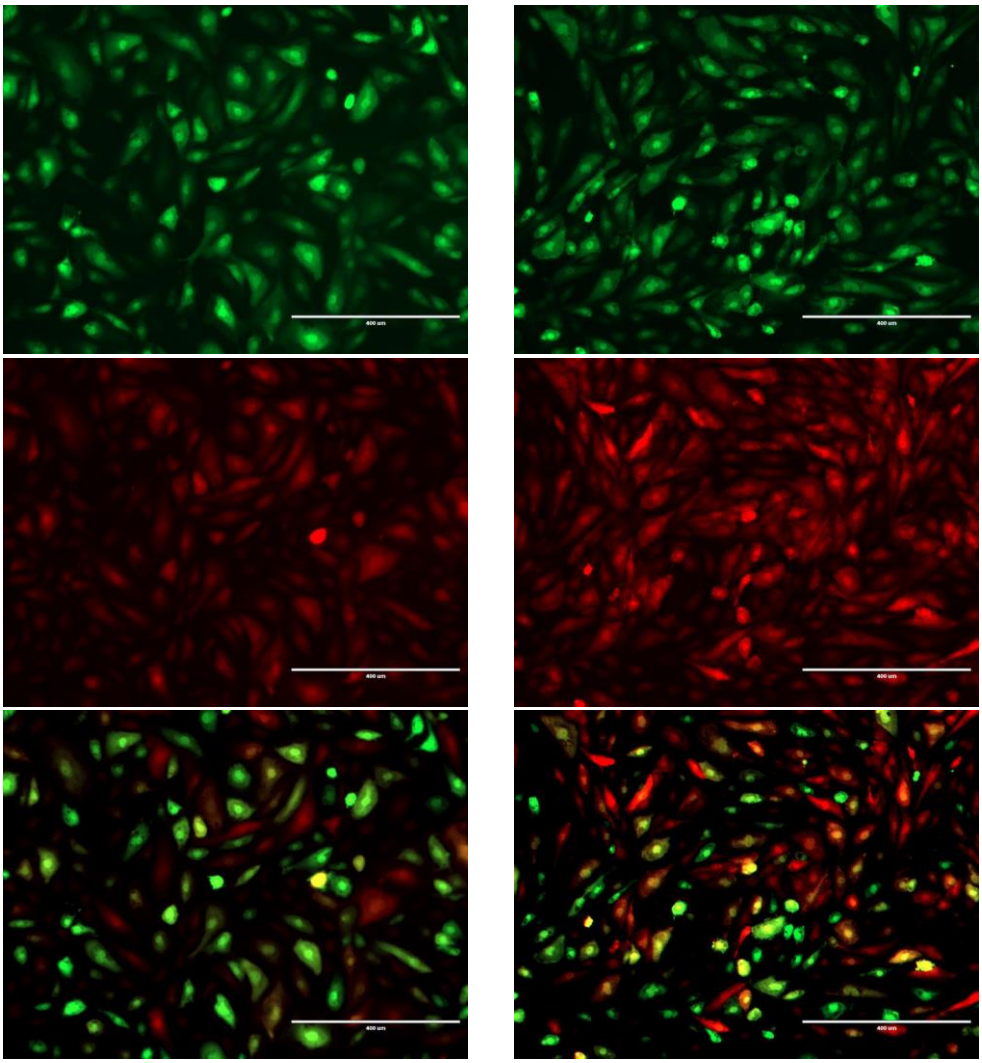
- transfection with preformed Viromer® RED complexed with pCMV-GFP plasmid and GFP-encoding mRNA
- monitored by fluorescence microscopy

Data from H. Cynis, Fraunhofer Institute for Cell Therapy and Immunology, Halle (Germany)

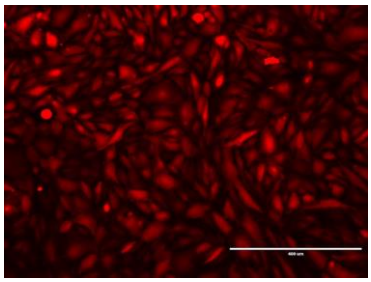
HUVEC: transfection of mRNA

Transfection scale x1

Transfection scale x1.5



VR-01LB-00, VR-01LB-01



control

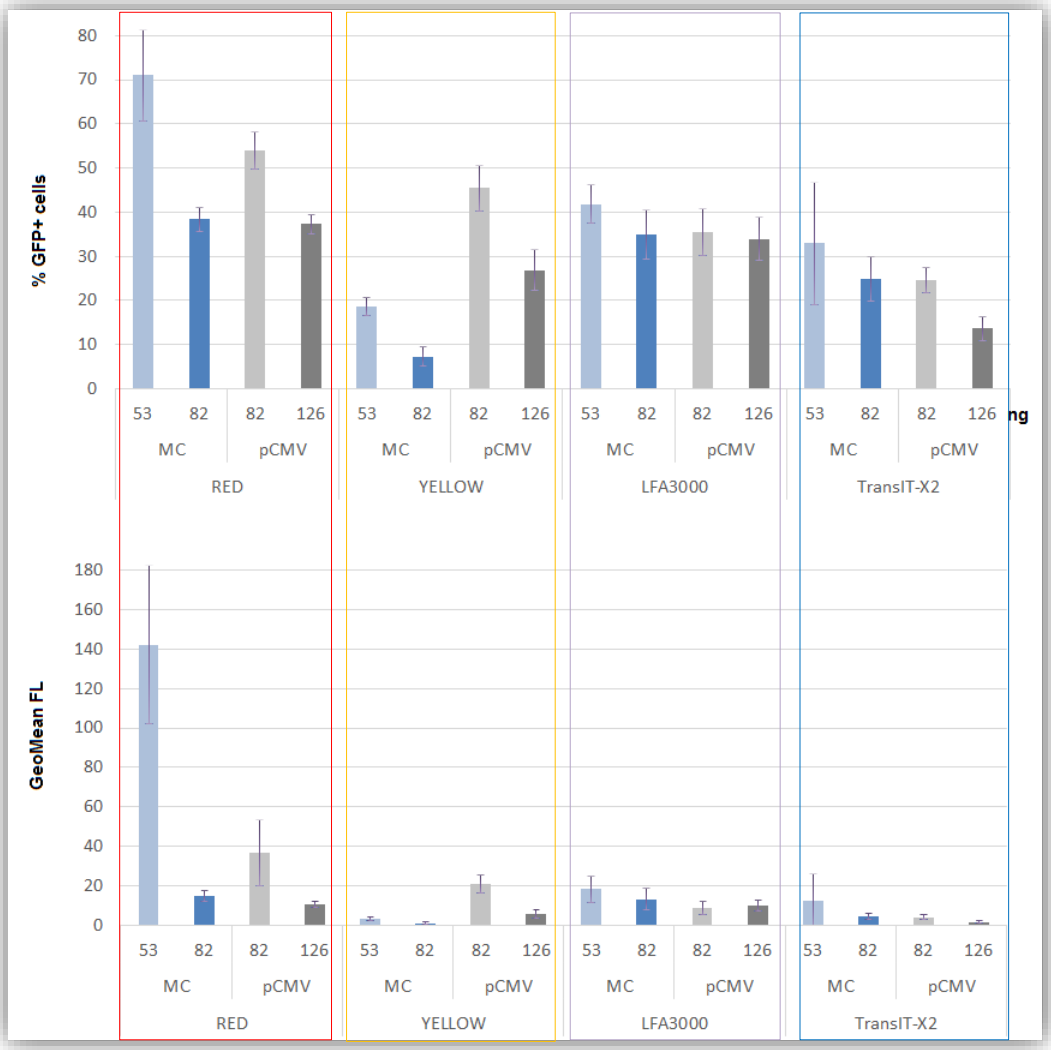
Fig. 3: Expression of GFP in HUVEC-RFP after mRNA transfection with Viromer® RED *(GFP/RFP/Overlay)*

- 24-well plate format, standard protocol with 2 transfection scales (x1 and x1.5)
- Transfection mix added on cells after rehydration of lyophilized mRNA:Viromer complex (mRNA from TriLink, US: CleanCap™ EGFP mRNA - 5moU)
- Pictures taken 24h post-transfection at 10x magnification using the EVOS FL Cell Imaging System (Thermo Fisher Scientific). Scale 400µm.

Data from Dr. Bohrane Guezguez,
University Medical Center of Johannes Gutenberg-University Mainz (Germany)

HUVEC: supplemental info
transfection of standard plasmids vs. minicircles

- Minicircles (MC) plasmids from Plasmid Factory and standard pCMV vectors encoding GFP used for complexation with **Viromer® RED**, **Viromer® YELLOW**, Lipofectamine® 3000 and Mirus TransIT®-X2
- Cells cultured in 96-well plates (5% CO_2, EGM-2, 10.000 cells/well)
- 80% confluency at the day of transfection
- 53 ng and 82 ng of MC are equimolar to 82 ng and 126 ng of pCMV, respectively
- FACS analysis 24h post-transfection



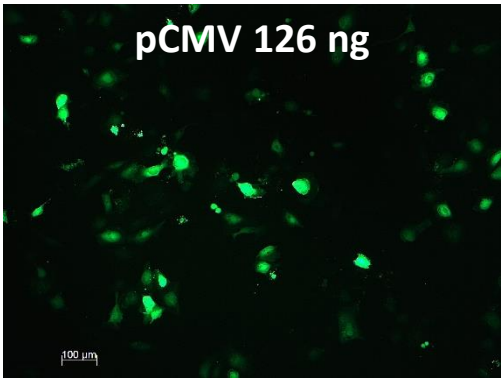
Transfection efficiency (% GFP+ cells)

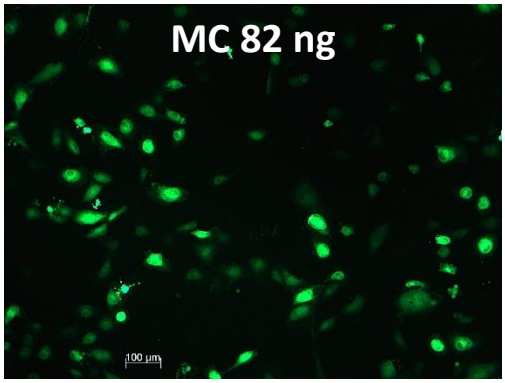
GFP expression level (mean fluo)

>> **Viromer® RED** outcompetes all other transfectants and shows a clear preference for minicircle pDNA

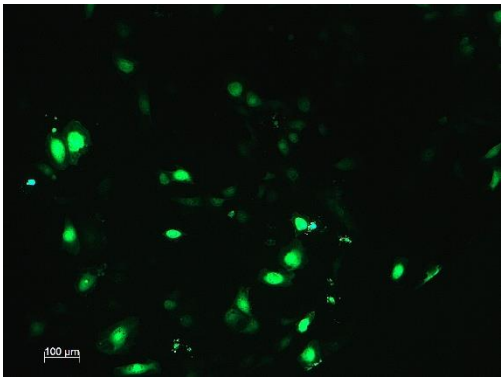
Corresponding microscopy pictures on next page

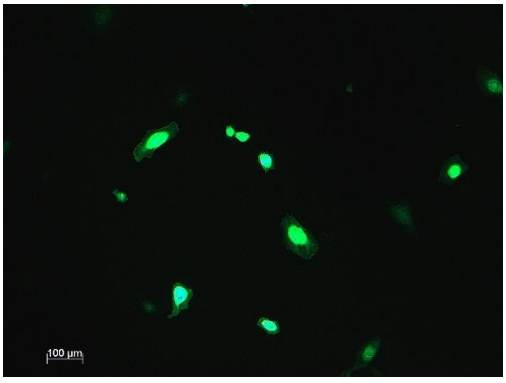
HUVEC: supplemental info
transfection of standard plasmids vs. minicircles



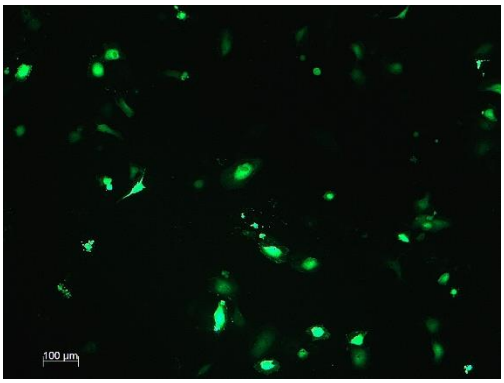


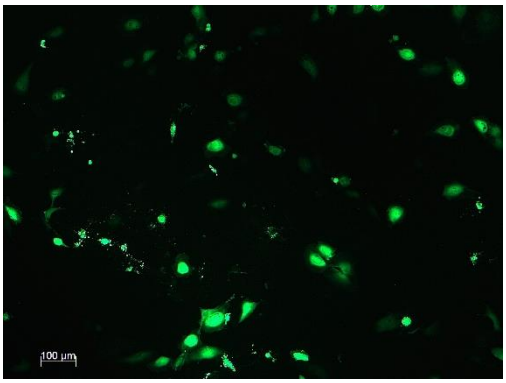
Viromer® RED



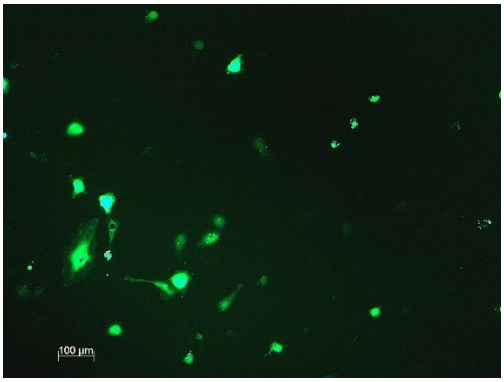


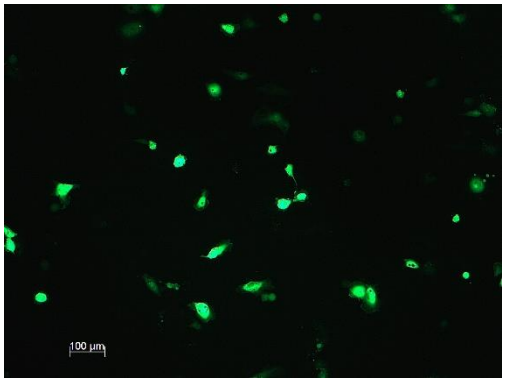
Viromer® YELLOW





Lipofectamine® 3000

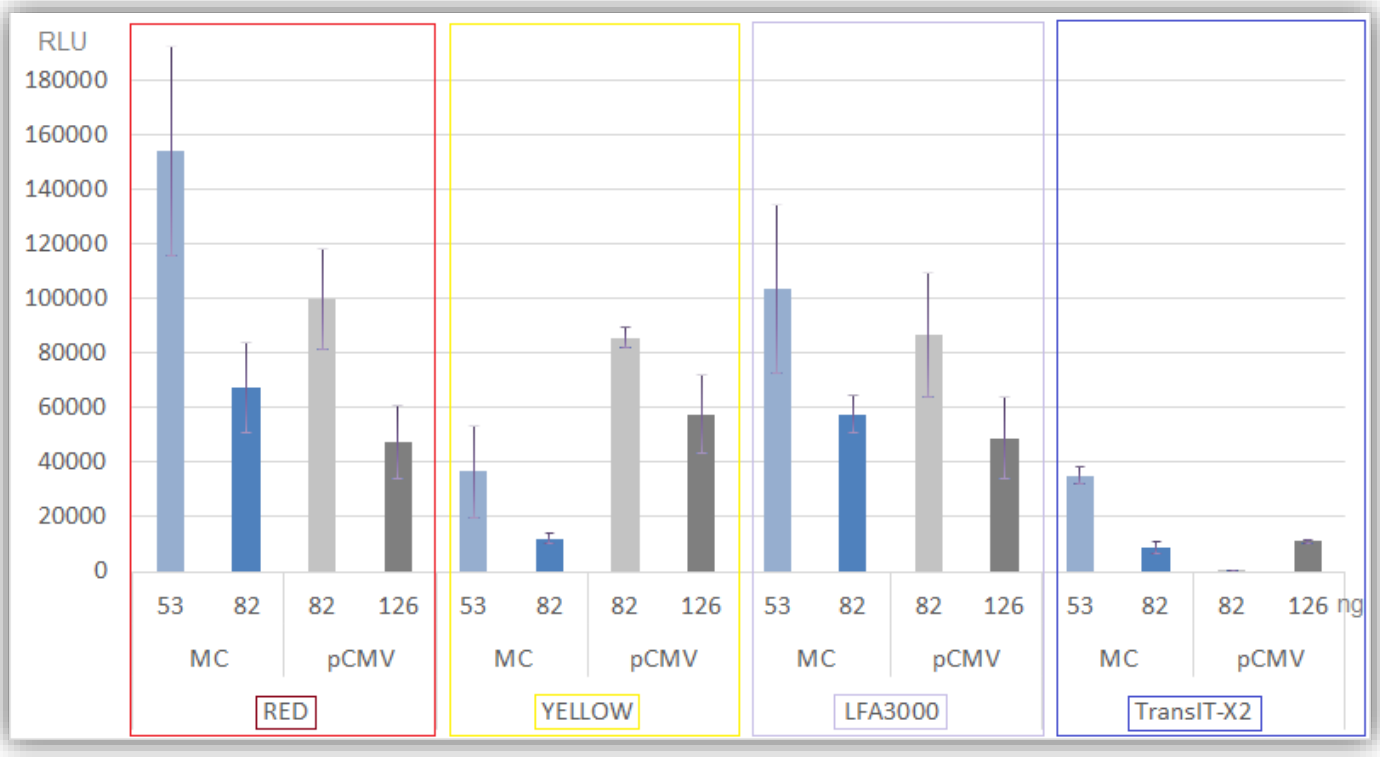




TransIT®-X2

HUVEC: supplemental info
transfection of standard plasmids vs. minicircles

- Same experimental design used with Minicircles (MC) plasmids and standard pCMV vectors encoding **Luciferase**



>> **Viromer® RED, Viromer® YELLOW** and Lipofectamine® 3000 give the highest efficiency with the standard pCMV
>> Use of minicircles is advantageous for all reagents except Viromer® YELLOW
>> Combination of **Viromer® RED** and minicircles outperforms all other complexation options

Data from H. Cynis, Fraunhofer Institute for Cell Therapy and Immunology, Halle (Germany)