VIROMER® transfection – references in publications 2014-2019

Non-exhaustive list of citations selected among >200 references and ranked by cell types / applications

immune cells | neurons | hepatic cells | muscle cells | fibroblasts | keratinocytes | breast, ovaria, vagina cells | stem cells

CRISPR | Luciferase assays | siRNA and shRNA silencing | plasmid and mRNA transfection

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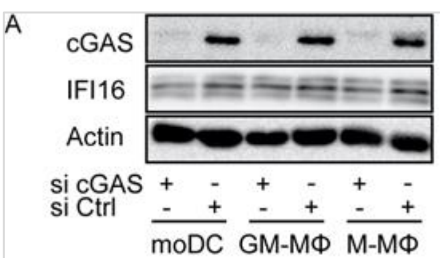
Full list and raw data: viromer-transfection.com

cells	Which Viromer®	Delivery of	info	citation	year	Open access
Immune cells: siRNA and miRNA transfection (1/2)						
PBMCs	GREEN	siRNA		Leber et al	2018	no
Primary CD34+ hematopoietic cells	GREEN	siRNA		Kew et al	2017	yes
Primary blood monocytes	GREEN	siRNA		Hadadi et al	2016	yes
Monocytes CD14+ from PBMCs	BLUE	siRNA		Machacek et al	2016	yes
Monocytes CD14+ from PBMCs	GREEN	siRNA		Takamura et al	2019	yes
Monocytes CD14+ from PBMCs	BLUE	siRNA		Ugolini et al	2018	yes
Blood monocytes and macrophages isolated from intestinal mucosa	GREEN	siRNA		Dennis et al	2018	no
Macrophages – monocyte-derived	BLUE	siRNA		Boliar et al	2019	yes
Macrophages – monocyte-derived	BLUE	siRNA		Fuchs et al	2018	yes
Macrophages – monocyte-derived	GREEN	siRNA		Unger et al	2018	yes
Macrophages – monocyte-derived	BLUE	siRNA	siRNA pool	Bercusson et al	2018	no
Macrophages – monocyte-derived	BLUE	siRNA		Chen et al	2017	yes
Macrophages – monocyte-derived	BLUE	siRNA	siRNA pool	Taylor et al	2018	no
Macrophages – monocyte-derived	BLUE	siRNA		Tasker et al	2016	yes
Macrophages – monocyte-derived	BLUE	siRNA		Chen et al	2016	yes
DCs and macrophages – mo-derived	BLUE	siRNA	siRNA pool	Paijo et al	2016	yes
Dendritic cells – monocyte-derived	GREEN	siRNA	siRNA pool	Gomez-Fernandez et al	2018	yes
Dendritic cells – monocyte-derived	GREEN	Pre and antimiR	protocol suspension cells	Pichulik et al	2016	yes
Dendritic cells – bone-marrow derived	GREEN	siRNA	see Fig S7	Liu et al	2016	yes



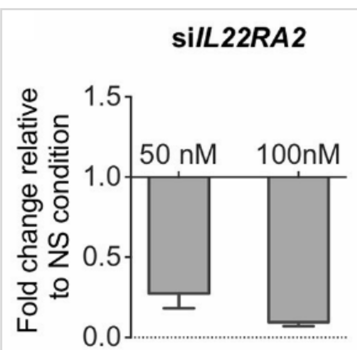
siRNA-mediated silencing of cGAS in monocyte-derived dendritic cells and macrophages using Viromer® BLUE

Fig 7A from Paijo J. et al. "cGAS senses human cytomegalovirus and induces type I interferon responses in human monocyte-derived cells." *PLoS pathogens* 12.4 (2016): e1005546.

cells	Which Viromer®	Delivery of	info	citation	year	Open access

Immune cells: siRNA and miRNA transfection (2/2)

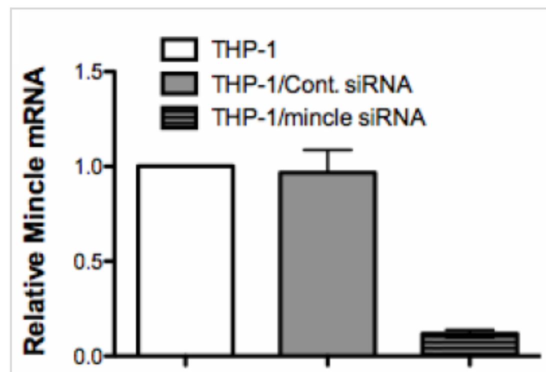
cells	Which Viromer®	Delivery of	info	citation	year	Open access
Macrophages – bone-marrow derived	GREEN	siRNA		Niu et al	2016	yes
Macrophages – bone-marrow derived	GREEN	siRNA	reverse transfection	Lin et al	2017	yes
Macrophages – bone-marrow derived	GREEN	siRNA		Van Opdenbosch et al	2017	yes
Macrophages – bone-marrow derived	BLUE	siRNA		Kim et al	2017	no
Macrophages – bone-marrow derived	BLUE	siRNA	siRNA pool	Yan et al	2016	yes
Macrophages – bone-marrow derived	GREEN	miRNA	miRNA mimics library HTS	Verjans et al	2019	yes
RAW and bone-marrow macrophages	BLUE	miRNA		Robertson et al	2016	yes
RAW 264.7 macrophages	BLUE	siRNA		Vilani et al	2019	yes
RAW 264.7 macrophages	BLUE	siRNA	siRNA pool	Nishiyama et al	2018	no
RAW 264.7 macrophages	BLUE	siRNA	see Fig S4	Egami et al	2017	yes
THP-1 and primary macrophages (MDM)	BLUE	siRNA	siRNA pool, see Fig 3	Goddard et al	2019	yes
THP-1 and primary macrophages	BLUE	siRNA		Haneklaus et al	2017	yes
THP-1	GREEN	siRNA	thesis	Lewis	2017	yes
THP-1 (differentiated)	GREEN	siRNA	siRNA pool, see Fig S2	Chinthamani et al	2017	yes
THP-1 (differentiated)	BLUE	siRNA		Curto et al	2019	yes
U937	BLUE	miRNA		Ciechomska et al	2017	no



RNA-mediated silencing using Viromer® GREEN

<< Dendritic cells – Fig 8D from Gómez-Fernández P. et al. "Long interleukin-22 binding protein isoform-1 is an intracellular activator of the unfolded protein response." *Frontiers in immunology* 9 (2018).

>> THP-1 monocytes – Fig S2 from Chinthamani S. et al. "Macrophage inducible C-type lectin (Mincle) recognizes glycosylated surface (S)-layer of the periodontal pathogen Tannerella forsythia." *PloS one* 12.3 (2017): e0173394



cells	Which Viromer®	Delivery of	info	citation	year	Open access

Immune cells: pDNA and mRNA transfection

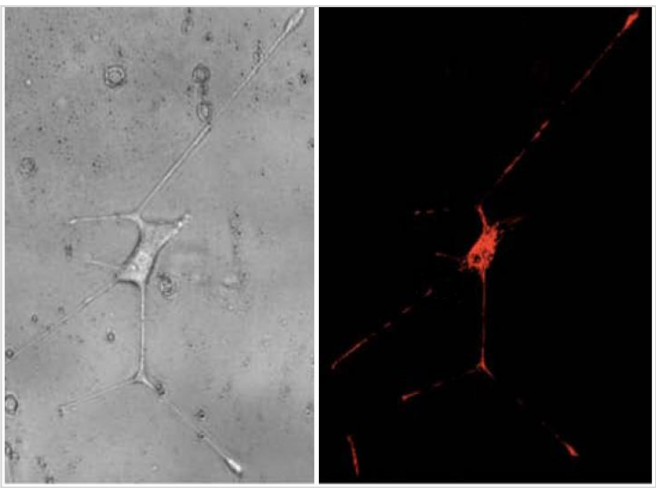
Dendritic cells	RED	mRNA	see Fig S1A	Kranz et al	2016	no
Monocytes - CD14+ from PBMC	RED	plasmid	Luciferase assay	Schwentner et al	2018	Yes
Macrophages – monocyte-derived	RED	plasmid	Luciferase assay	Nieto et al	2019	yes
Macrophages	RED	plasmid	co-transfection, Luc assay	Ringleb et al	2018	yes
Macrophages – monocytes-derived	RED	plasmid	CRISPR interference	Gupta et al	2018	no
Macrophages (RAW 264.7)	RED	plasmid	see protocol p.34	Bedair - thesis	2018	yes
Macrophages (RAW 264.7)	RED	plasmid	"significant changes in efficiency beyond 25 passages"	Farag et al	2017	yes
Macrophages (RAW 264.7)	RED	plasmid	Luciferase assay, see Fig 9B	Shen et al	2016	yes
Macrophages (J774 and BMDM)	RED	mRNA		Xu et al	2018	no
THP-1 and macrophages (BMDM)	RED	plasmid		Almeida - thesis	2016	yes
THP-1	RED	mRNA		Fiore et al	2018	yes
THP-1	RED	plasmid		Long et al	2017	yes
Leucocytes CD11b+ within muscle fiber	RED	mRNA	see Fig S9 (in vivo)	Kirschman et al	2017	yes

We generally advise against plasmid transfection in immune cells as it may be inactivated by endogenous DNase activity. However, there are here some positive feedbacks, in particular for use in luciferase assays and with model cell lines.

Microglia cells

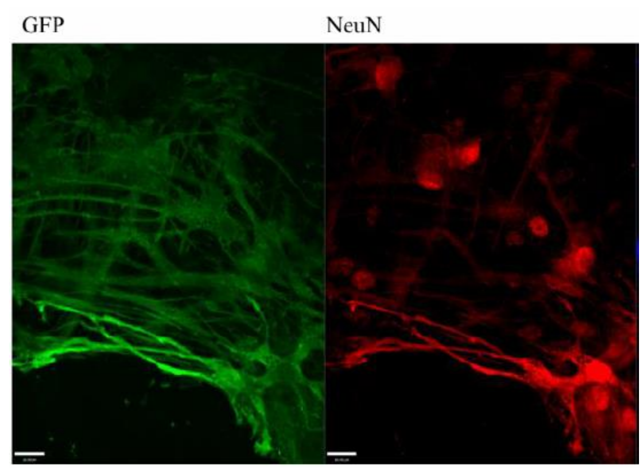
Microglia CD11b+ isolated from mice	BLUE	siRNA	see Fig 5E	Houtman et al	2019	no
BV-2 microglia-like cells	BLUE	siRNA	siRNA pool, see Fig 3C	Mathur et al	2017	yes
BV-2 microglia-like cells	BLUE	siRNA	see Fig 3Da	Kawasaki et al	2017	yes
BV-2 microglia-like cells	BLUE	siRNA	siRNA pool, see Fig 3H	Czirr et al	2017	yes
BV-2 microglia-like cells	BLUE	siRNA	siRNA pool , see Fig 3	Burai et al - patent	2017	yes
BV-2 microglia-like cells	RED	plasmid	See Fig 1(c)	Rao et al	2015	yes

cells	Which Viromer®	Delivery of	info	citation	year	Open access
Transfection of neurons						
Primary mesencephalic dopamine rat neurons	RED	plasmid		Pereira et al	2017	yes
Primary cerebellar neurons	RED	plasmid		Fujita et al	2017	yes
Primary rat cortical neurons	RED	mRNA	see Fig 4.3 (page 97)	Kirschman	2017	yes
PC12 neuron-like cells	RED	plasmid		Barillé et al	2018	yes
Dorsal Root Ganglion cells	RED	plasmid		Kolbe et al	2016	no



<< Visualization of travelling mitochondria in neurites of PC12 cells with DsRed2 fluorochrome reporter expressed after plasmid transfection using Viromer® RED

Modified from Fig 2 *in* Barillé R. et al. "Characterization of Cells Interactions with Patterned Azopolymer-Based Materials using SEM, AFM and Video Microscopy." *The Open Biomedical Engineering Journal* 12.1 (2018).



>> Colocalization of GFP expression with the neuron-specific nuclear marker NeuN in rat cortical neuronal cultures transfected with GFP-mRNA using Viromer® RED

Modified from Fig 4.3 *in* Kirschman, JL. *Characterization of in vitro transcribed MRNA for optimal expression in therapeutic applications*. Diss. Georgia Institute of Technology, 2017.

cells	Which Viromer®	Delivery of	info	citation	year	Open access

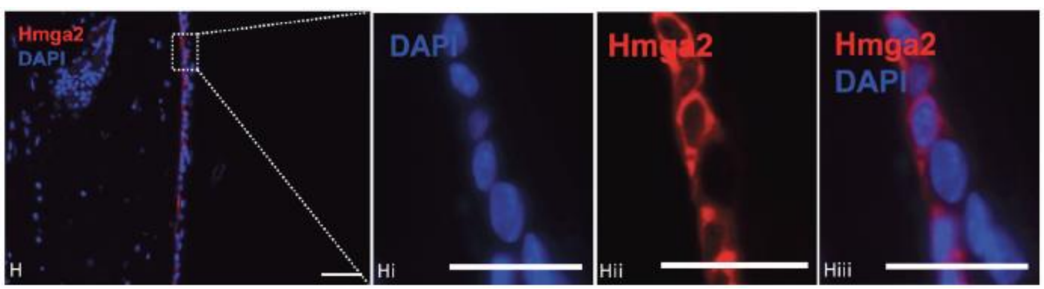
Transfection of glioma and neuroblastoma cells

C6 glioma	RED	plasmid	stable KO with shRNA	Kijewska et al	2017	yes
U-87 MG and LN18 glioblastoma	BLUE	siRNA	see Fig 3	Was et al	2019	yes
U251 glioblastoma	BLUE	siRNA	see Fig 2H	Hlavac et al	2019	no
U251 glioblastoma	GREEN	siRNA		Chavali et al	2017	yes
LN18 glioblastoma	BLUE	siRNA	siRNA pool, see Fig 2A-B	Ciechomska et al	2016	yes
LNT-229, LN-308, T98G, Tu-132 GBM	BLUE	siRNA	siRNA pool	Armento et al	2017	yes
SH-SY5Y neuroblastoma	GREEN	siRNA	see Fig 4	Ambrosio et al	2017	yes
SH-SY5Y neuroblastoma	GREEN	siRNA	see Fig 6D	Bompard et al	2018	yes
SH-SY5Y neuroblastoma	GREEN	siRNA	See Fig 8D-E	Prescott et al	2014	yes
SH-SY5Y neuroblastoma	RED	plasmid	DNA and shRNA	Hu et al	2018	no
Ntera2 and SH-EP neuroblastoma	RED	plasmid		Weber et al	2018	yes
Ntera2 neuroblastoma	RED	plasmid		Wright - thesis	2016	yes
1321N1 astrocytoma	BLUE	siRNA		Ruiz et al	2019	yes

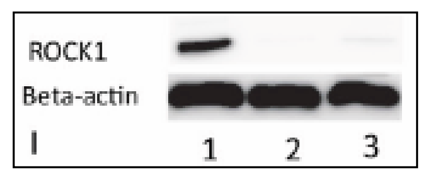
Transfection of fibroblasts (1/2)

L929 cells	GREEN	siRNA	HTS reverse transfection	V'kovski et al	2019	yes
Primary fibroblasts from fibrotic kidney	BLUE	siRNA	siRNA pool, see Fig 8b-c	Smith et al(1)	2017	yes
Primary fibroblasts from fibrotic kidney	YELLOW	plasmid	co-transfection Luc assay	Smith et al(1)	2017	yes
Primary fibroblasts from fibrotic kidney	BLUE	siRNA	siRNA pool	Smith et al(2)	2017	yes
Primary fibroblasts from fibrotic kidney	YELLOW	plasmid	co-transfection Luc assay	Smith et al(2)	2017	yes
Fibroblasts from healthy donor skin	BLUE	siRNA	siRNA pool	Sanchez-Garrido et al	2018	no
Primary foreskin fibroblasts	RED	mRNA	Comparative data plasmid versus mRNA	U.S. Patent App No. 15/747,136	2016	yes
Primary fibroblasts (breast cancer)	RED	plasmid	shRNA-mediated KD	Simkova et al	2016	yes
Primary MEFs	RED	plasmid	see Fig S2	Yuniati et al	2015	yes
BJ-hTERT fibroblasts	YELLOW	plasmid		Bianco et al	2019	yes

cells	Which Viromer®	Delivery of	info	citation	year	Open access
Transfection of fibroblasts (2/2)						
MDFs Mouse dermal fibroblasts	YELLOW	plasmid	CRISPR KO stable cells	Liccardi et al	2018	yes
TIG-1 fibroblasts	YELLOW	plasmid	High-throughput microscopy	Poletto et al	2016	yes
HAP1 fibroblast-like CML cells	RED	plasmid	CRISPR KO stable cells	Sarno et al	2019	yes
Transfection of keratinocytes						
Mouse primary KCs in co-culture with melanocytes	RED	plasmid	see Fig 6H	Mescher et al	2017	yes
Mouse primary KCs in skin cancer	RED	plasmid	see Fig 1C Luciferase assay	Li et al	2017	yes
Mouse primary KCs in skin cancer	RED	plasmid	see Fig 3H-I (shRNA silencing)	Li et al	2017	yes
Mouse primary KCs	RED	plasmid	see Fig 7H	Rübsam et al	2017	yes
Mouse primary KCs	RED	plasmid		Noethel et al	2018	yes
Human KCs from neonatal foreskin	RED	plasmid		McCormack et al	2017	yes
Human KCs from neonatal foreskin	RED	plasmid		Erasmus et al	2016	yes

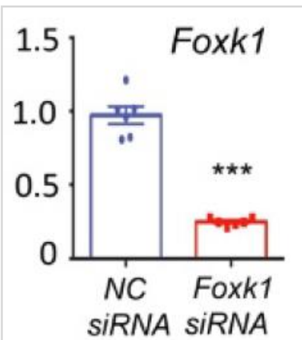


shRNA-mediated KD in primary KCs with Viromer® RED
<< Hmga2 expression with mock and ROCK1 KD



From Fig 3 Inhibition of Hmga translocation through ROCKS in: Li, Y et al. 2017"Hmga2 translocation induced in skin tumorigenesis." *Oncotarget* 8.18: 30019.

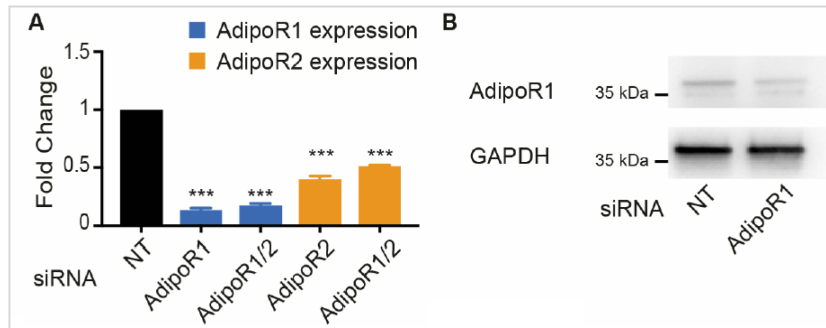
cells	Which Viromer®	Delivery of	info	citation	year	Open access
Transfection of hepatic cells						
Primary mouse WT hepatocytes	BLUE	siRNA	see Fig 5a	Wang et al	2019	no
Primary mouse hepatocytes	RED	plasmid	see Fig 5A-B	Ziros et al	2016	yes
HepG2	BLUE	siRNA	see Fig S4	Ruiz et al	2019	yes
McArdle hepatoma cells	RED	plasmid	Microscopy visualization of Golgi apparatus with mDsRed-Golgi-7	Krahmer et al	2019	no



siRNA-mediated silencing in cells transfected with Viromer® BLUE

<< Fig 5a from Wang, L. et al. "Identification of insulin-responsive transcription factors regulating hepatocyte glucose production." *Diabetes* (2019): db181236.

>> Fig S4 from Ruiz, M. et al. "AdipoR1 and AdipoR2 Maintain Membrane Fluidity in Most Human Cell Types and Independently of Adiponectin." *Journal of lipid research* (2019): jlr-M092494.



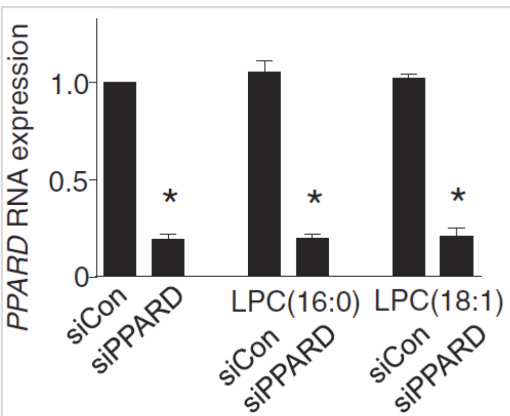
Transfection of muscle cells (1/2)

C2C12	RED	plasmid		Honda et al	2019	no
C2C12 (before differentiation)	RED	plasmid		Li et al	2018	no
C2C12 (before differentiation)	BLUE	siRNA		Li et al	2018	no
C2C12 (after differentiation)	RED	plasmid		Yang et al	2018	yes
C2C12	RED	plasmid	see Fig 4e	Lessard et al	2018	yes
C2C12 (before differentiation)	BLUE	siRNA	see Fig 2C-D	Wanatabe et al	2016	yes
Mouse tibialis muscle (in vivo)	RED	mRNA	see Fig S9 (leucocytes within muscle fibers actually transfected)	Kirschman et al	2017	yes

cells	Which Viromer®	Delivery of	info	citation	year	Open access

Transfection of muscle cells (2/2)

INS-1E sarcoma cells	RED	plasmid	see Fig S5 (localization study with a fused fluorescent protein)	Dwivedi et al	2018	yes
Synovial sarcoma (HS-SY-II, FUJI, 1273/99, CME-1, SYO-1)	RED	plasmid		Isfort et al	2019	no
Neonatal rat ventricular cardiomyocytes	YELLOW	plasmid	co-transfection, Luc assay	Falcon et al	2017	yes
Neonatal mouse atrial cardiomyocytes	YELLOW	plasmid	co-transfection, Luc assay	Furtado et al	2017	yes
Human myotubes differentiated from primary human myoblasts	BLUE	siRNA	siRNA pool, see Fig 2B	Ordelheide et al	2016	yes
Human myotubes differentiated from primary human myoblasts	BLUE	siRNA	siRNA pool, see Fig 3F-H	Klinger et al	2016	no
Human myotubes differentiated from primary human myoblasts	BLUE	siRNA	siRNA pool, see Fig 6D-F	Ingerslev et al	2017	yes
Muscle stem cells	BLUE	siRNA	see Fig 7	Tseng et al	2018	no



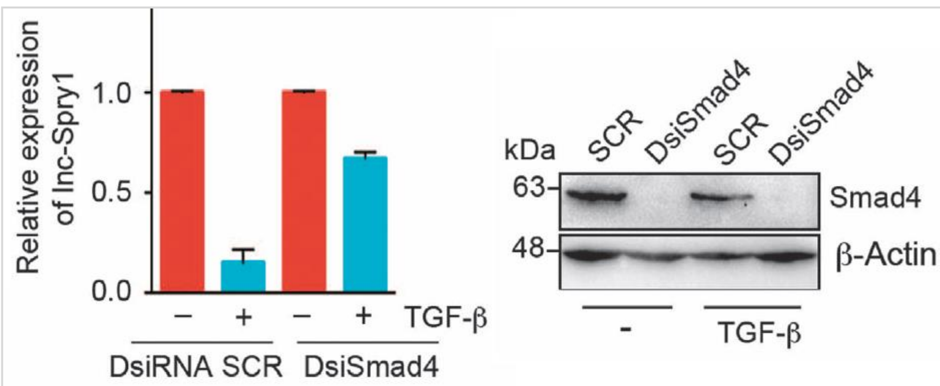
siRNA-mediated silencing in myotubes transfected with Viromer® BLUE

Fig 1F from Klingler, C. et al. "Lysophosphatidylcholines activate PPARδ and protect human skeletal muscle cells from lipotoxicity." *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids* 1861.12 (2016): 1980-1992.

cells	Which Viromer®	Delivery of	info	citation	year	Open access

Transfection of breast, ovaria and vagina cells

cells	Which Viromer®	Delivery of	info	citation	year	Open access
NMuMG	RED	plasmid	see Fig S5	Rodriguez-Mateo et al	2017	yes
NMuMG	BLUE	siRNA	see Fig 1f	Rodriguez-Mateo et al	2017	yes
MDA-MB-231	BLUE	siRNA	see Fig 6 and S1	Puchert et al	2018	no
SK-BR3 and BT474 breast cancer cells	RED	plasmid	shRNA silencing, see Fig 6	Dietel et al	2018	yes
MCF-10A	RED	plasmid		Miura – thesis	2019	yes
Kuramochi and OVCAR-3 ovarian cancer	RED	plasmid		Chen et al	2018	yes
SKOV-3 ovarian cancer cells	GREEN	siRNA		Schnitzer – thesis	2016	yes
VK2	GREEN	siRNA	see Fig 1G	Ali et al	2017	yes
VK2	BLUE	siRNA	see Fig S3	Mowbray et al	2018	yes



siRNA-mediated silencing in NMuMG mouse breast epithelial cells transfected with Viromer® BLUE

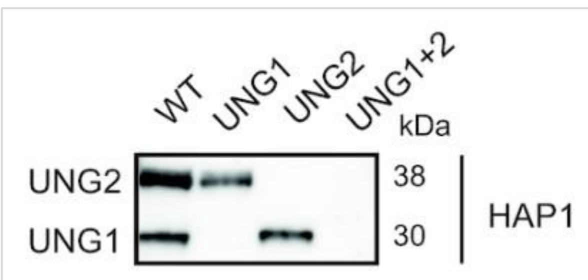
Fig 1f from Rodríguez-Mateo, C. et al. "Downregulation of Lnc-Spry1 mediates TGF-β-induced epithelial–mesenchymal transition by transcriptional and posttranscriptional regulatory mechanisms." *Cell death and differentiation* 24.5 (2017): 785.

cells	Which Viromer®	Delivery of	info	citation	year	Open access
Transfection of stem cells						
Primary CD34+ hematopoietic cells	GREEN	siRNA		Kew et al	2017	yes
Mesenchymal stem cells (BM-MSCs)	YELLOW	plasmid		Bigot et al	2015	yes
Muscle stem cells	BLUE	siRNA	see Fig 7	Tseng et al	2018	no
Pluripotent stem cells (hPS)	BLUE	siRNA	see Fig S17	Xue et al	2018	no
Corneal stroma stem cells (CSSC)	BLUE	siRNA	see protocol on p.6	Funderburgh	2016	yes
Corneal stroma stem cells (CSSC)	BLUE	siRNA	see Fig 4	Hertsenberg et al	2017	yes
Brain tumor stem-like cells (mBTSCs)	BLACK	miR and siRNA	see Fig 4m-q	Li et al	2018	yes
Neural stem cells	BLACK	DNA aptamers	see Fig 4-5	Shin et al	2018	yes

siRNA-mediated silencing in muscle stem cells with Viromer® BLUE

Modified from Fig 7 Tseng C. et al. "Markers of Accelerated Skeletal Muscle Regenerative Response in Murphy Roths Large Mice: Characteristics of Muscle Progenitor Cells and Circulating Factors." *STEM CELLS* 37.3 (2019): 357-367.

cells	Which Viromer®	Delivery of	info	citation	year	Open access
CRISPR						
Macrophages – monocytes-derived	RED	plasmid	CRISPRi	Gupta et al	2018	no
A549 and MDCK	RED	mRNA	encoding Cas13a	Bawage et al	2018	yes
THP-1	RED	gRNA	test immunogenicity	Cai et al	2018	yes
HGT-1	RED	plasmid	KO stable cells	Liszt et al	2017	yes
Hap1	RED	plasmid	KO stable cells	Sarno et al	2019	yes
HEp2	RED	plasmid	KO stable cells	Ko et al	2019	yes
MDFs Mouse dermal fibroblasts	YELLOW	plasmid	KO stable cells	Liccardi et al	2018	yes
HDFs Human dermal fibroblasts	RED	plasmid	low compared to RNP	Fuster-Garcia et al	2017	yes
A549, HEK, HeLa, C2C12, HUVECs, PDAC	CRISPR	RNP	poster launch reagent	Lagauzere et al	2019	yes



Generation of KO-HAP1 cells (UNG gene) through genome editing using plasmid transfection with Viromer® RED

Modified from Fig7B *in* Sarno A. et al. "Uracil–DNA glycosylase UNG1 isoform variant supports class switch recombination and repairs nuclear genomic uracil." *Nucleic Acids Research* (2019).

Recent advances around delivery methods have shown a great advantage of transfecting directly Cas active proteins as ribonucleoproteins already complexed with their gRNA. Compared to plasmid vectors, it enables a much faster action and avoid off-target effects.

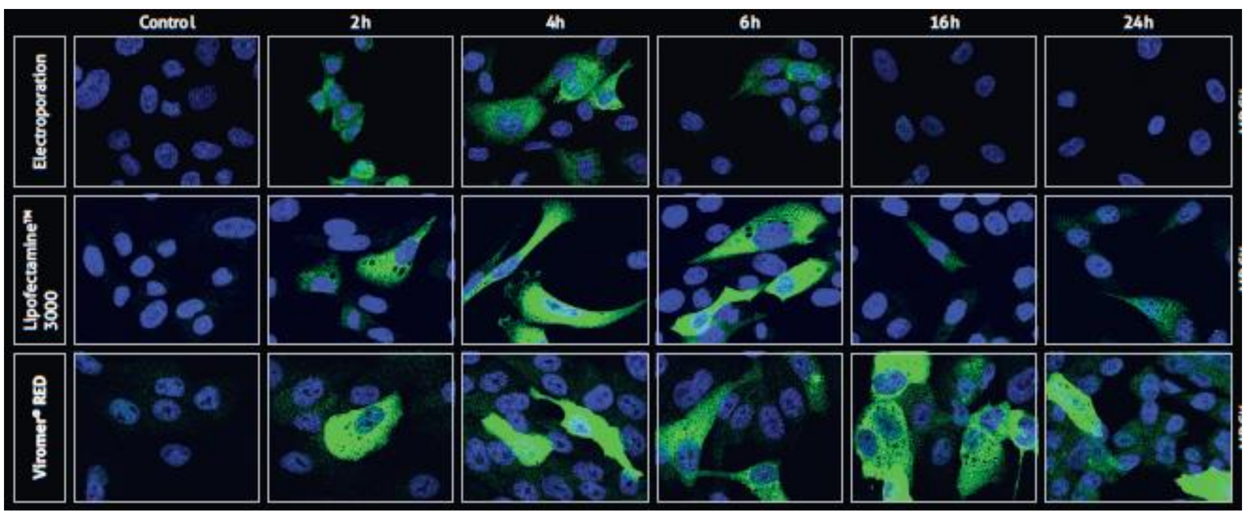
To learn more about RNP delivery, please watch this webinar hosted by OriGene on 03/15/2019!

cells	Which Viromer®	Delivery of	info	citation	year	Open access

mRNA transfection

Transfection of mRNA is THE alternative to achieve efficient transient protein overexpression in cells known as "resistant" to plasmid transfection.
It's also a powerful tool for cell reprogramming, CRISPR/Cas applications and immunotherapy assays.
While the following citations refer to **Viromer® RED**, we recommend use of the new premium reagents **Viromer® mRNA** and **Viromer® mRNA in vivo HQ**.

cells	Which Viromer®	Delivery of	info	citation	year	Open access
A549 and MDCK	RED	mRNA	encoding Cas13a	Bawage et al	2018	yes
A549, MDCK, neurons, cardiomyocytes	RED	mRNA	thesis	Kirschman	2017	yes
Primary foreskin fibroblasts	RED	mRNA	Comp. data plasmid vs mRNA	U.S. Pat. 15/747,136	2016	yes
Dendritic cells	RED	mRNA	see Fig S1A	Kranz et al	2016	no
Vero cells and Macrophages (J774 and BMDM)	RED	mRNA		Xu et al	2018	no
A549, Vero	RED	mRNA		Tiwari et al	2018	yes
A549, myoblasts	RED	mRNA		Bhosle et al	2018	no
THP-1	RED	mRNA		Fiore et al	2018	yes

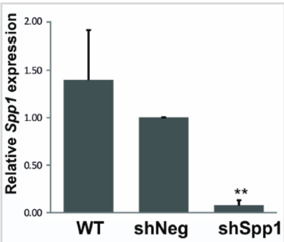


By using synthetic mRNA encoding the Cas13a CRISPR effector and guide RNAs targeting *Influenza* virus and human respiratory syncytial virus, this study shows a new way to reduce and prevent viral infections
(i.e. Cas13a induces a cleavage of viral RNA, activity here detected in MDCK cells by GFP fluorescence).

Viromer® RED was selected as transfection reagent given its outperformance versus electroporation and a lipid-based reagent.

Bawage et al. "Synthetic mRNA expressed Cas13a mitigates RNA virus infections." bioRxiv (2018) 370460

cells	Which Viromer®	Delivery of	info	citation	year	Open access
shRNA-mediated silencing						
C6 glioma	RED	plasmid	see Fig 6A	Kijewska et al	2017	yes
Breast cancer SK-BR3 and BT474	RED	plasmid		Dietel et al	2018	yes
SH-SY5Y	RED	plasmid		Hu et al	2018	no
Primary keratinocytes (skin cancer)	RED	plasmid		Li et al	2017	yes
Primary fibroblasts (breast cancer)	RED	plasmid		Simkova et al	2016	yes



shRNA-mediated silencing in C6 glioma cells transfected with Viromer® RED

Fig 6A from Kijewska, M. et al. "The embryonic type of SPP1 transcriptional regulation is re-activated in glioblastoma." *Oncotarget* 8.10 (2017): 16340.

Luciferase assays

HEK and HeLa	RED	plasmid	co-transfection	Plewka et al	2018	yes
HEK	YELLOW	plasmid	see Fig S5f	Anderson et al	2018	yes
C2C12	RED	plasmid		Honda et al	2019	no
Synovial sarcoma (HS-SY-II, FUJI, 1273/99, CME-1, SYO-1)	RED	plasmid		Isfort et al	2019	no
Macrophages – monocyte-derived	RED	plasmid		Nieto et al	2019	yes
Macrophages (RAW 264.7)	RED	plasmid	see Fig 9B	Shen et al	2016	yes
Macrophages	RED	plasmid	co-transfection	Ringleb et al	2018	yes
Monocytes - CD14+ from PBMC	RED	plasmid		Schwentner et al	2018	Yes
Neonatal rat ventricular cardiomyocytes	YELLOW	plasmid	co-transfection	Falcon et al	2017	yes
Neonatal mouse atrial cardiomyocytes	YELLOW	plasmid	co-transfection	Furtado et al	2017	yes
Primary fibroblasts from fibrotic kidney	YELLOW	plasmid	co-transfection	Smith et al	2017	yes
Mouse primary KCs in skin cancer	RED	plasmid	see Fig 1C Luciferase assay	Li et al	2017	yes