

**Title:** Overexpression of *HSPA1A* enhances the osteogenic differentiation of bone marrow mesenchymal stem cells via activation of the Wnt/ $\beta$ -catenin signaling pathway

**Author names:** Wei Zhang, MD<sup>1\*</sup>, Deting Xue, MD<sup>1\*</sup>, Houfa Yin, MD<sup>2</sup>, Shengdong Wang, MD<sup>1</sup>, Chao Li, MD<sup>3</sup>, Erman Chen, MD<sup>1</sup>, Dongcai Hu, MD<sup>1</sup>, Yiqing Tao, MD<sup>1</sup>, Jiawei Yu, MD<sup>4</sup>, Qiang Zheng, MD<sup>1</sup>, Xiang Gao, MD<sup>1</sup>, Zhijun Pan, MD<sup>1#</sup>

**The affiliation and address of the authors:**

- 1- Department of Orthopedics, Second Affiliated Hospital, School of Medicine, Zhejiang University, 310009, Hangzhou, China.
- 2- Eye Center, Second Affiliated Hospital, School of Medicine, Zhejiang University, 310009, Hangzhou, China.
- 3- Department of Surgery, Second Affiliated Hospital of Zhejiang University School of Medicine, 310009, Hangzhou, Zhejiang Province, China.
- 4- Department of Orthopedics, Zhuji People's Hospital of Zhejiang Province, Zhuji, Zhejiang Province, China.

**#Corresponding author:**

Zhijun Pan, Tel: 0086-0571-87783567, E-mail: panzhijunzw@163.com

## Supplemental material legends

Supplemental material 1: Lentiviral overexpressing HSPA1A particles (Lenti-HSPA1A)

Supplemental material 2: Cell sheet preparation

Supplemental material 3: Animal model

## Supplemental material 1: lentiviral vector

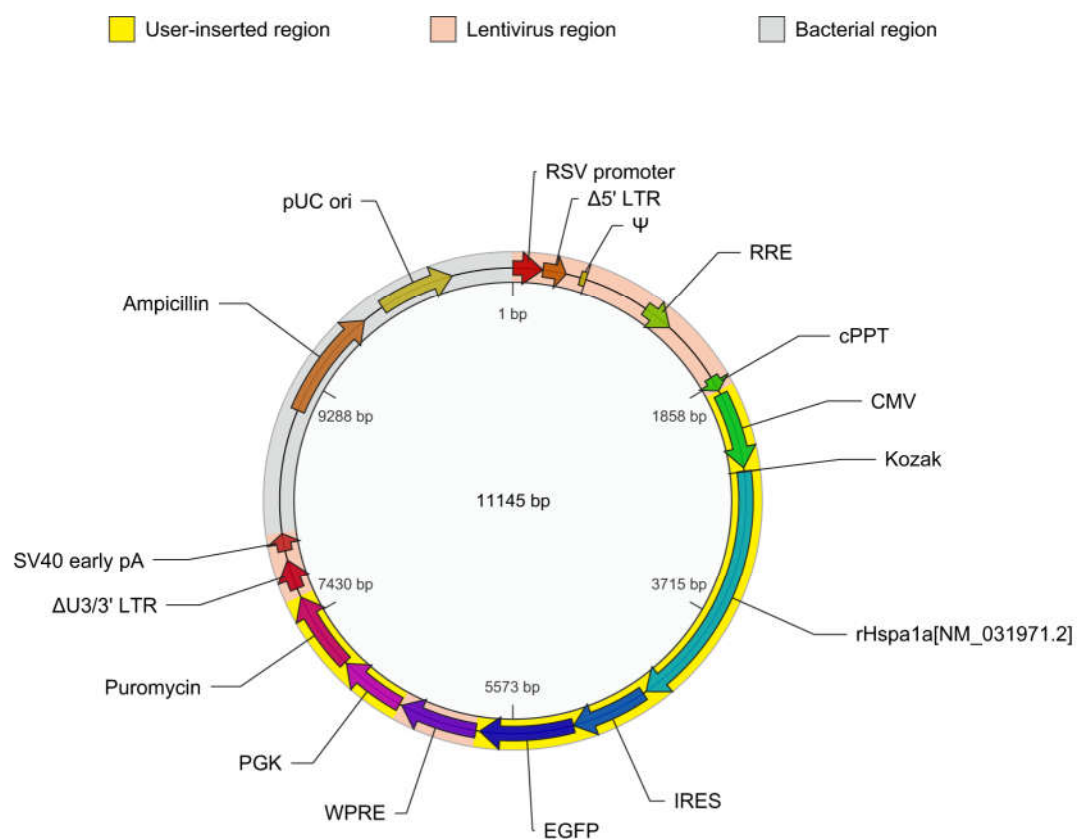
### Vector Summary

Vector ID	VB150330-10009
Vector Name (official)	pLV[Exp]-Puro-CMV>rHspa1a[NM_031971.2];IRES:EGFP
Date Created (Pacific Time)	2015-03-30
Size	11145 bp
Vector Type	Lentivirus gene expression vector (3rd generation)
Inserted Promoter	CMV
Inserted ORF	rHspa1a[NM_031971.2], EGFP
Inserted Linker	IRES
Inserted Marker	Puro
Copy Number	High
Bacterial Resistance	Ampicillin
Cloning Host	Stbl3

### User Annotation of Vector

Vector alias (from user)	None
Comment (from user)	None

### Vector Map



## Vector Components

Component Name	Nucleotide Position	Full Name	Description
RSV promoter	<b>1-229</b>	Rous sarcoma virus (RSV) enhancer/promoter	Allows Tat-independent production of viral mRNA.
$\Delta$ 5' LTR	<a href="#">230-410</a>	HIV-1 truncated 5' LTR	Permits viral packaging and reverse transcription of the viral mRNA.
$\Psi$	<b>521-565</b>	HIV-1 psi packaging signal	Allows viral packaging.
RRE	<a href="#">1075-1308</a>	HIV-1 Rev response element	Permits Rev-dependent nuclear export of unspliced viral mRNA.
cPPT	<b>1803-1920</b>	Central polypurine tract	Facilitates the nuclear import of HIV-1 cDNA through a central DNA flap.
CMV	<a href="#">1950-2538</a>	CMV	Component entered by user
Kozak	<a href="#">2563-2568</a>	Kozak	Component entered by user
rHspa1a[NM_031971.2]	<a href="#">2569-4494</a>	rHspa1a[NM_031971.2]	Component entered by user
IRES	<a href="#">4519-5106</a>	IRES	Component entered by user
EGFP	<a href="#">5107-5826</a>	EGFP	Component entered by user
WPRES	<b>5856-6453</b>	Woodchuck hepatitis virus posttranscriptional regulatory element	Facilitates effective transcription termination at the 3' LTR.
PGK	<a href="#">6472-6982</a>	Mouse phosphoglycerate kinase promoter	Allows high-level expression of the selection marker in mammalian cell lines.
Puromycin	<b>6995-7594</b>	Puromycin resistance gene	Permits selection of stably transduced mammalian cell lines.
$\Delta$ U3/3' LTR	<a href="#">7666-7900</a>	HIV-1 truncated 3' LTR	Allows viral packaging but self-inactivates the 5' LTR for biosafety purposes. The element also contains a polyadenylation signal for transcription termination and polyadenylation of mRNA in transduced cells.
SV40 early pA	<b>7972-8106</b>	SV40 early polyadenation signal	Allows transcription termination and polyadenylation of mRNA.
Ampicillin	<a href="#">9061-9921</a>	Ampicillin resistance gene	Allows selection of the plasmid in E.coli.
pUC ori	<b>10091-10679</b>	pUC origin of replication	Permits high-copy replication and maintenance in E.coli.

Note: (c) denotes complementary strand.

## User Annotation of Vector Components

Component Name	Comment by User
CMV	<i>None</i>
rHspa1a[NM_031971.2]	<i>None</i>
IRES	<i>None</i>
EGFP	<i>None</i>
Puro	<i>None</i>

## Supplemental material 2: Cell sheet preparation



## Supplemental material 3: Animal model

