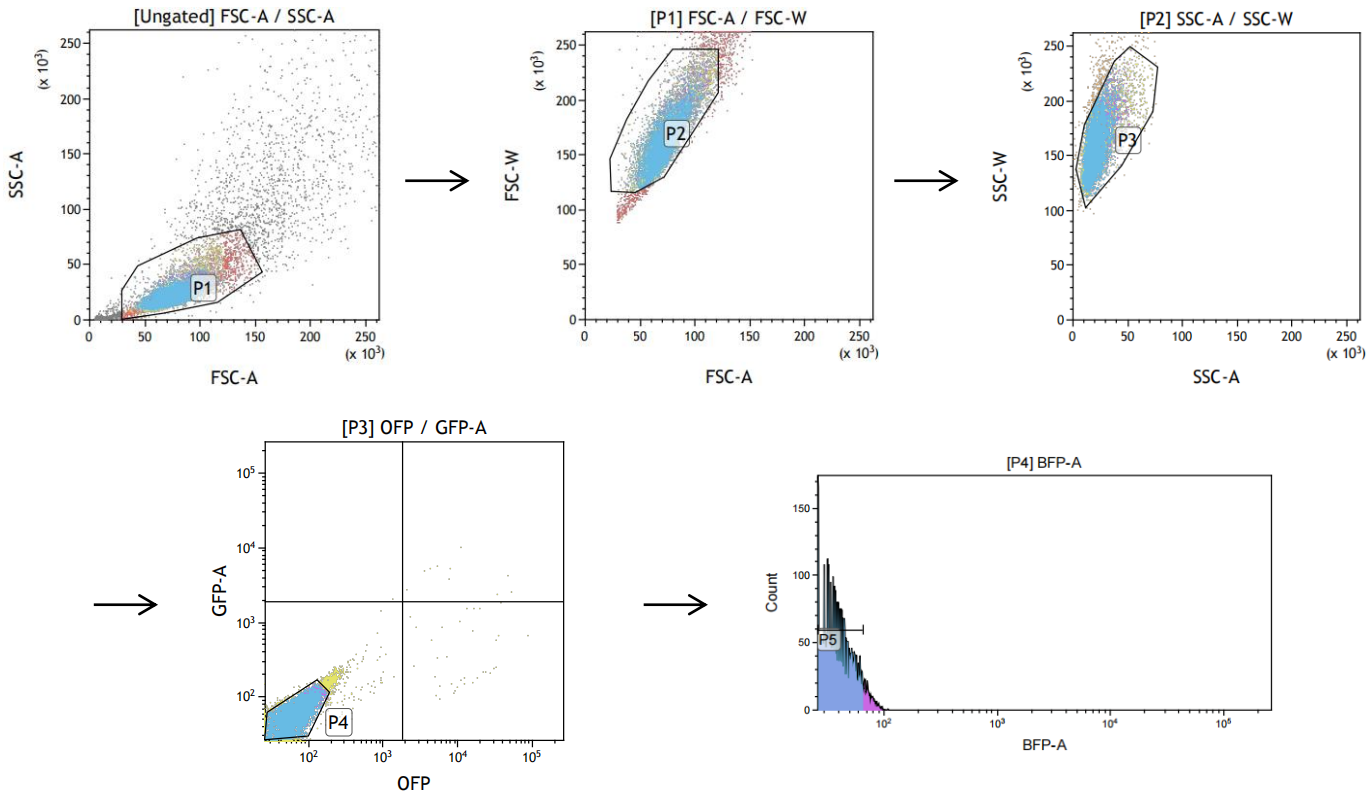
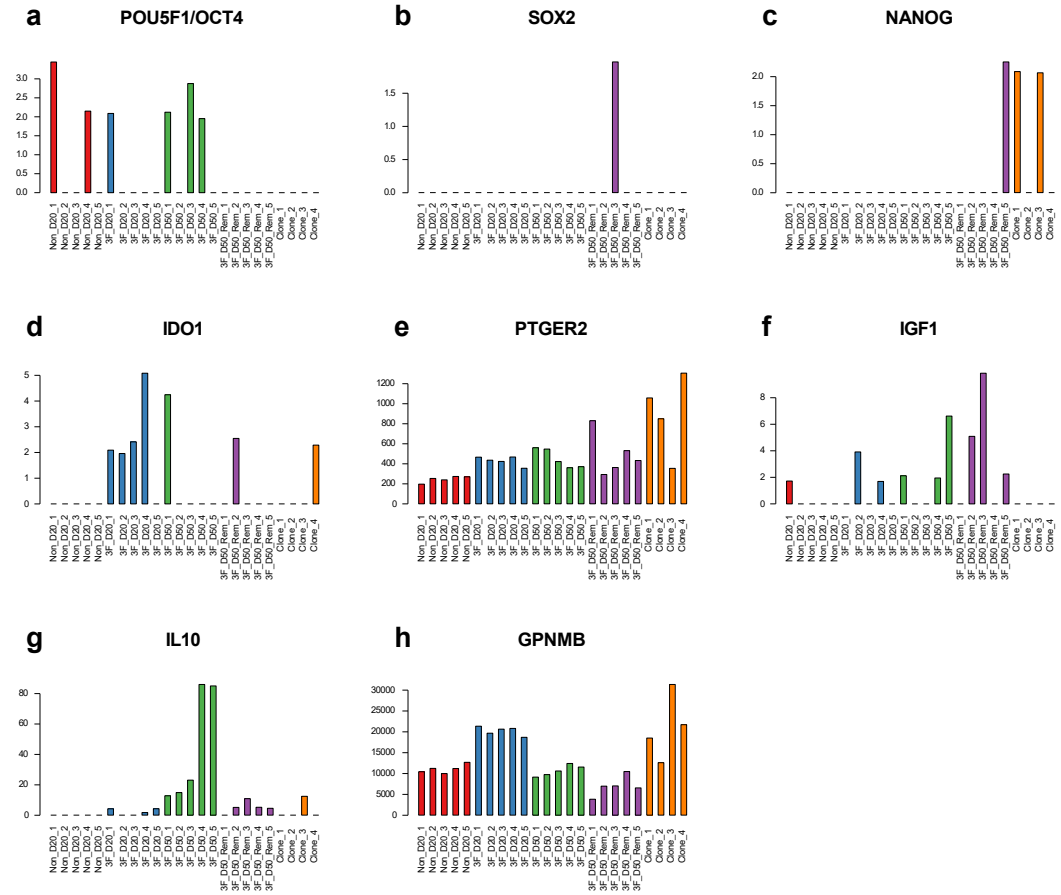
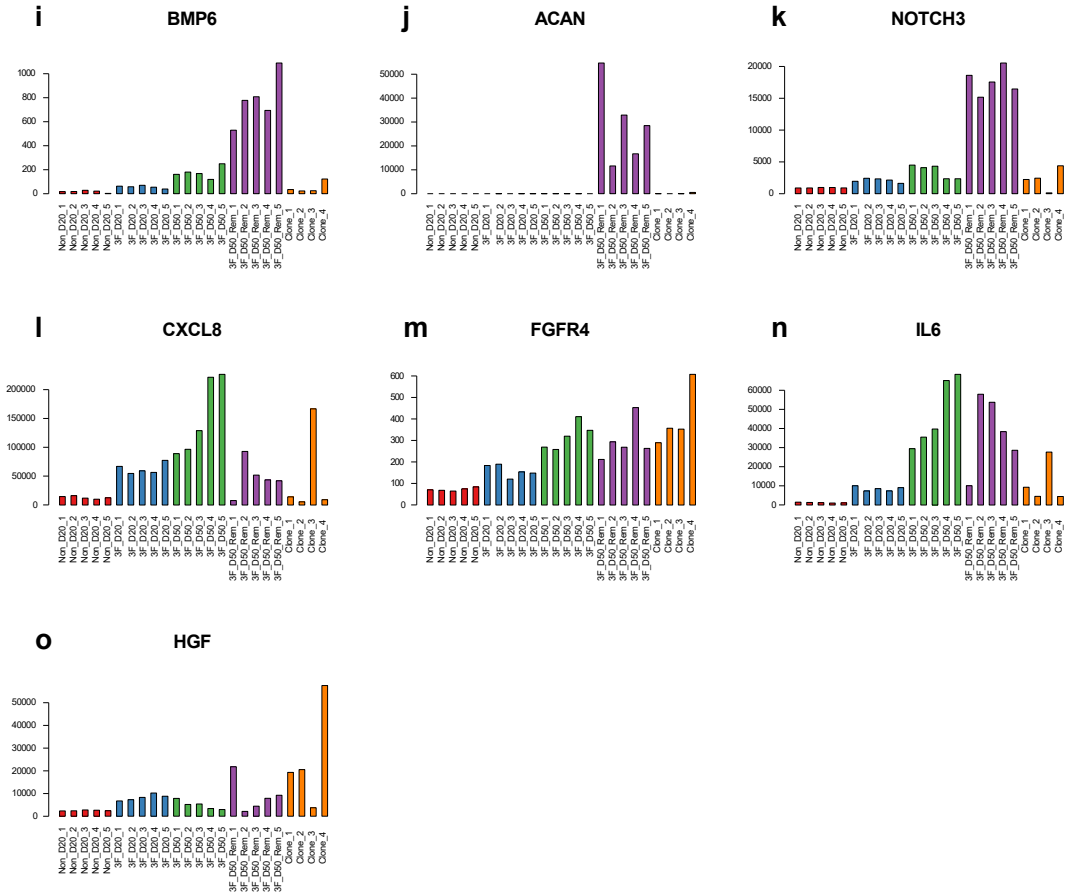


Supplementary Figure S1. Gating strategy for sorting fluorescence-negative cells.
Sequential gating steps are shown: P1, FSC/SSC; P2, FSC/FSC-width (FSC singlets); P3, SSC/SSC-width (SSC singlets); P4, GFP/OFP double-negative population (two-parameter plot); P5, BFP-negative population (histogram). The final gate (P5) was used to isolate fluorescence-negative cells.

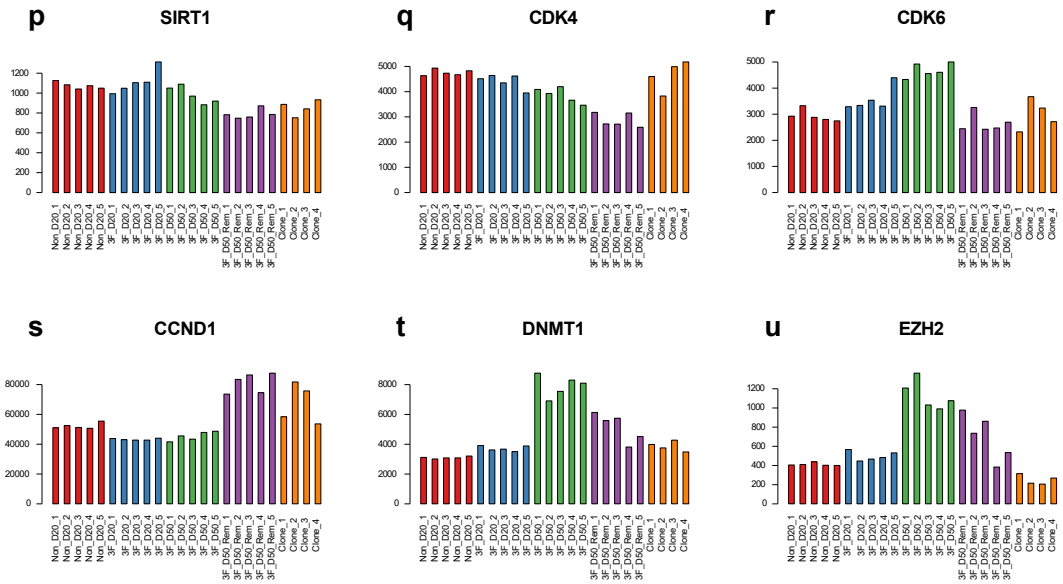
Immunomodulation related gene



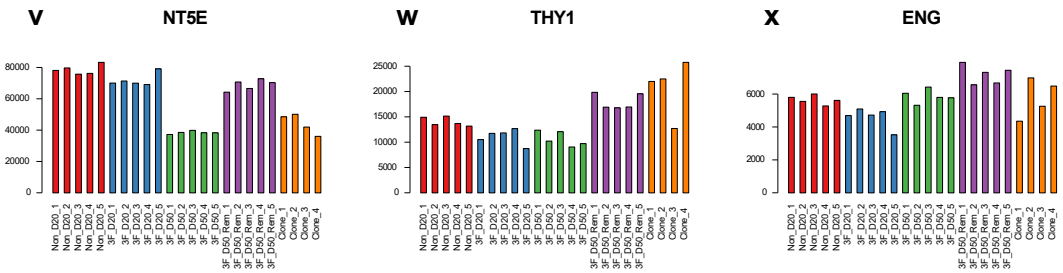
Regenerative/mesenchymal gene



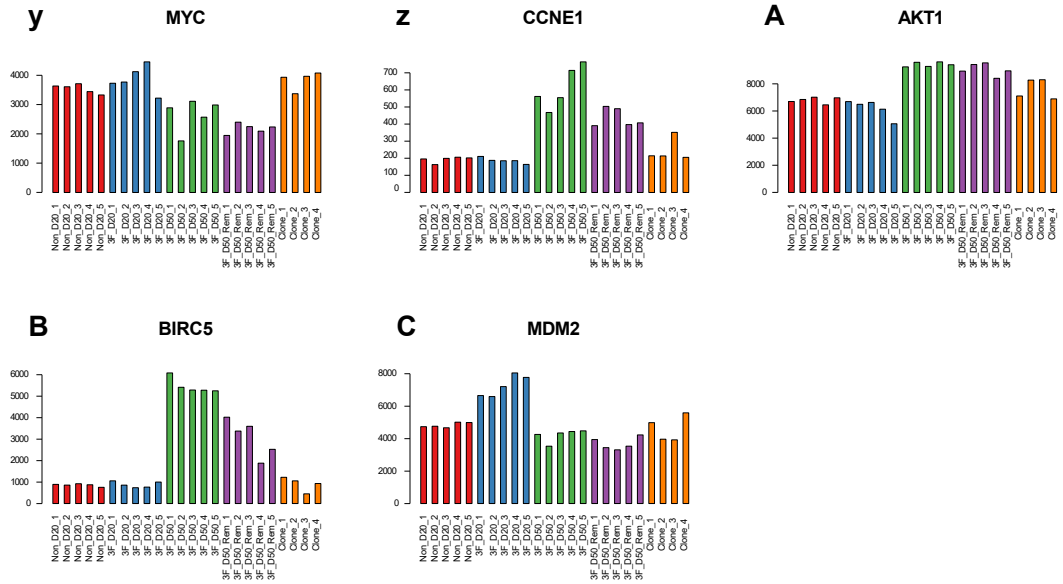
Cell cycle check point related gene

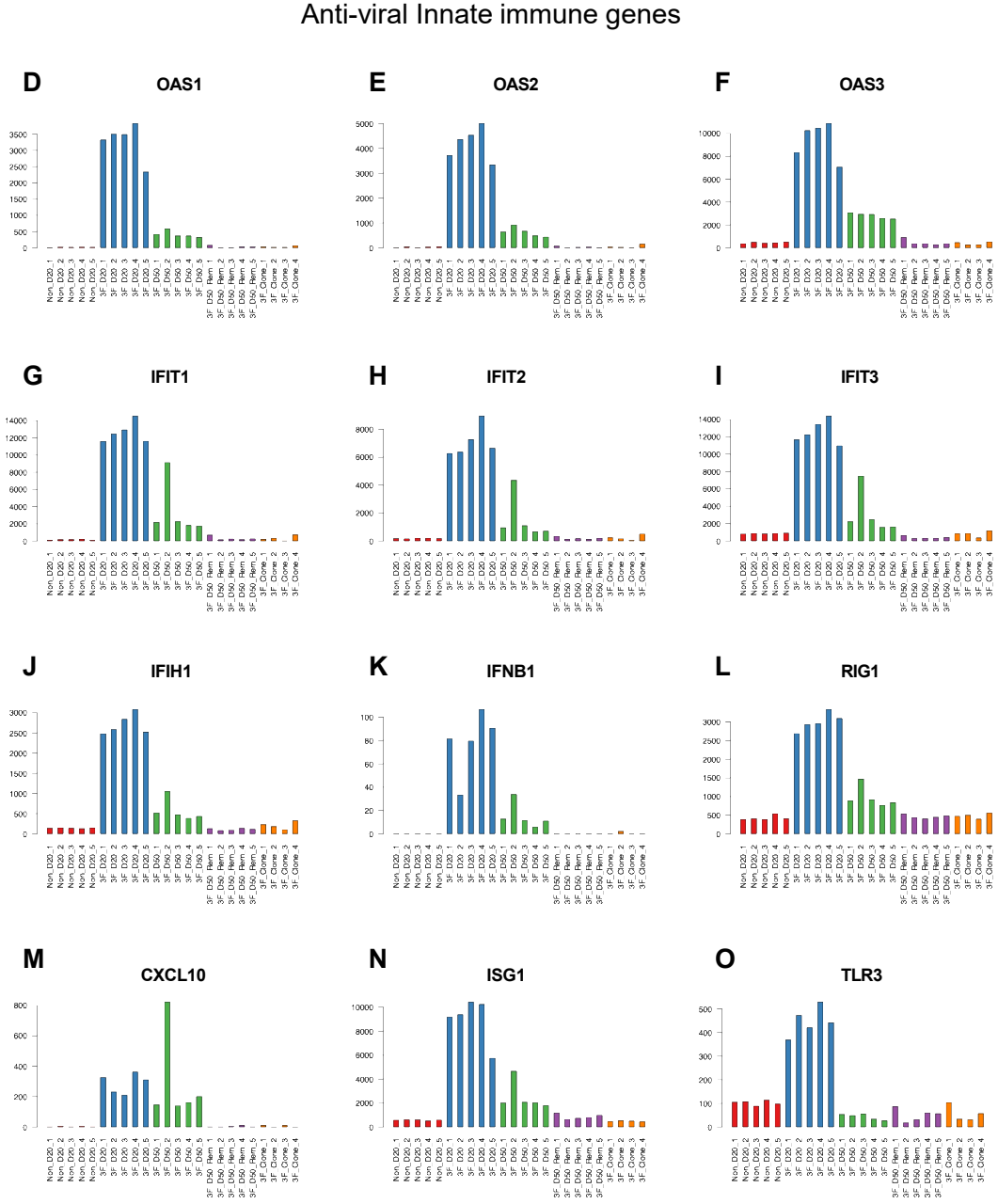


Mesenchymal surface markers

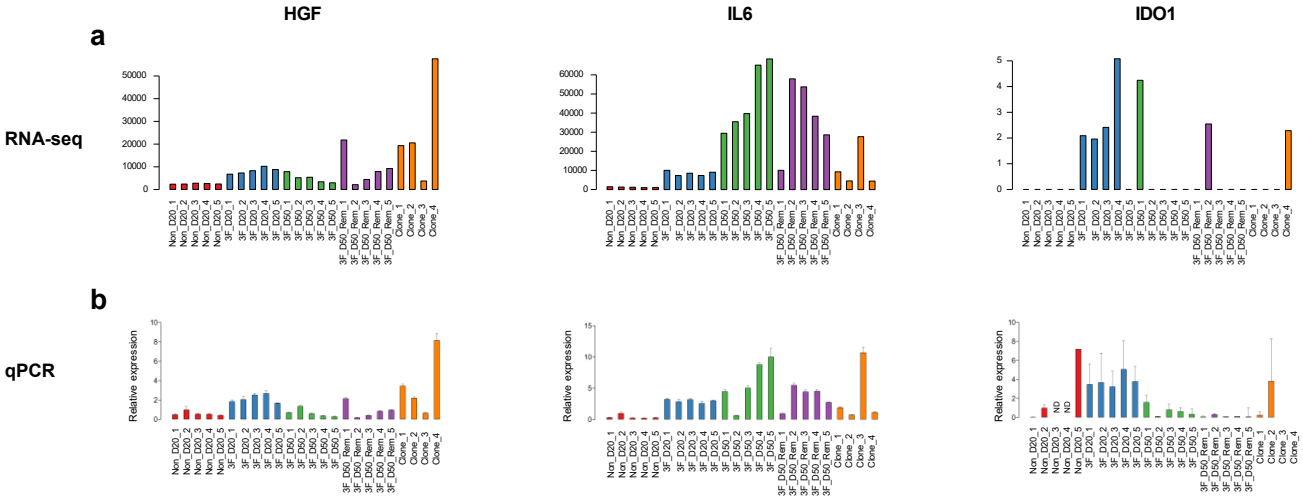


Oncogenesis related gene



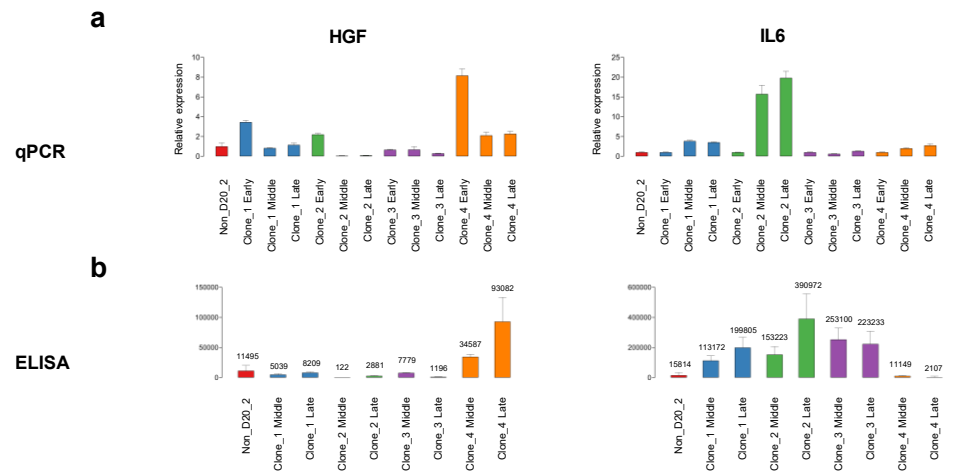


Supplementary Figure S2. Time-dependent changes in key gene expression during rejuvenation. Expression patterns of immunomodulation-related, regenerative/mesenchymal, cell cycle check point-related, mesenchymal surface marker, oncogenesis-related, and anti-viral innate immunity-related genes highlighting molecular transitions form SeV infection to removal. The y-axes indicate TMM-normalized expression levels.



Supplementary Figure S3. Comparison of RNA-seq and qPCR analyses for HGF, IL6, and IDO1 expression across rejuvenated MSC populations and clones.

Gene expression levels of HGF, IL6, and IDO1 were assessed by RNA sequencing (upper panels) and quantitative PCR (qPCR; lower panels) across parental MSCs, SeV-infected cells, SeV-removed populations, and clonally derived MSC lines. The y-axes of RNA-seq data indicate TMM-normalized expression levels. qPCR data are presented as relative expression levels normalized to internal reference gene.



Supplementary Figure S4. Comparison of qPCR and ELISA analyses of HGF and IL6 expression in rejuvenated MSC clones.

The expression of HGF and IL6 was analyzed at the mRNA level by quantitative PCR (qPCR; upper panels) and at the protein level by enzyme-linked immunosorbent assay (ELISA; lower panels) in parental MSCs and clonally derived rejuvenated MSCs. qPCR data are presented as relative expression levels normalized to internal reference gene, while ELISA results are shown as protein concentrations measured in conditioned media. Early, Middle, and Late indicate the different stages of the culture period.

Supplementary Table S1. Spearman's rank correlations between rej-MSC differentiation capacity and gene expression.

Neuron	ENO2	NES	TUBB3	GFAP	MAP2	PAX6	NEUROD1	SOX2		
	-0.4599	-0.1969	0.46773	-0.2716	-0.2445	0.5831	-0.1907	0.5303		
Adipocyte	PPARG	CEBPA	CEBPB	FABP4	LPL					
	-0.0851	-0.3818	-0.4061	-0.0182	-					
Osteoblast	RUNX2	TGFB1	ALPL	BMP1	BMP2	BMP3	BMP4	BMP6	BMP7	BGLAP
	-0.5394	0.1636	0.7091	0.0061	0.0303	-0.4479	-0.0424	0.2121	-0.4062	0.2076
Chondroncyte	COL2A1	SOX9	ACAN	RUNX2						
	0.0651	0.2492	-0.0061	-0.4134						