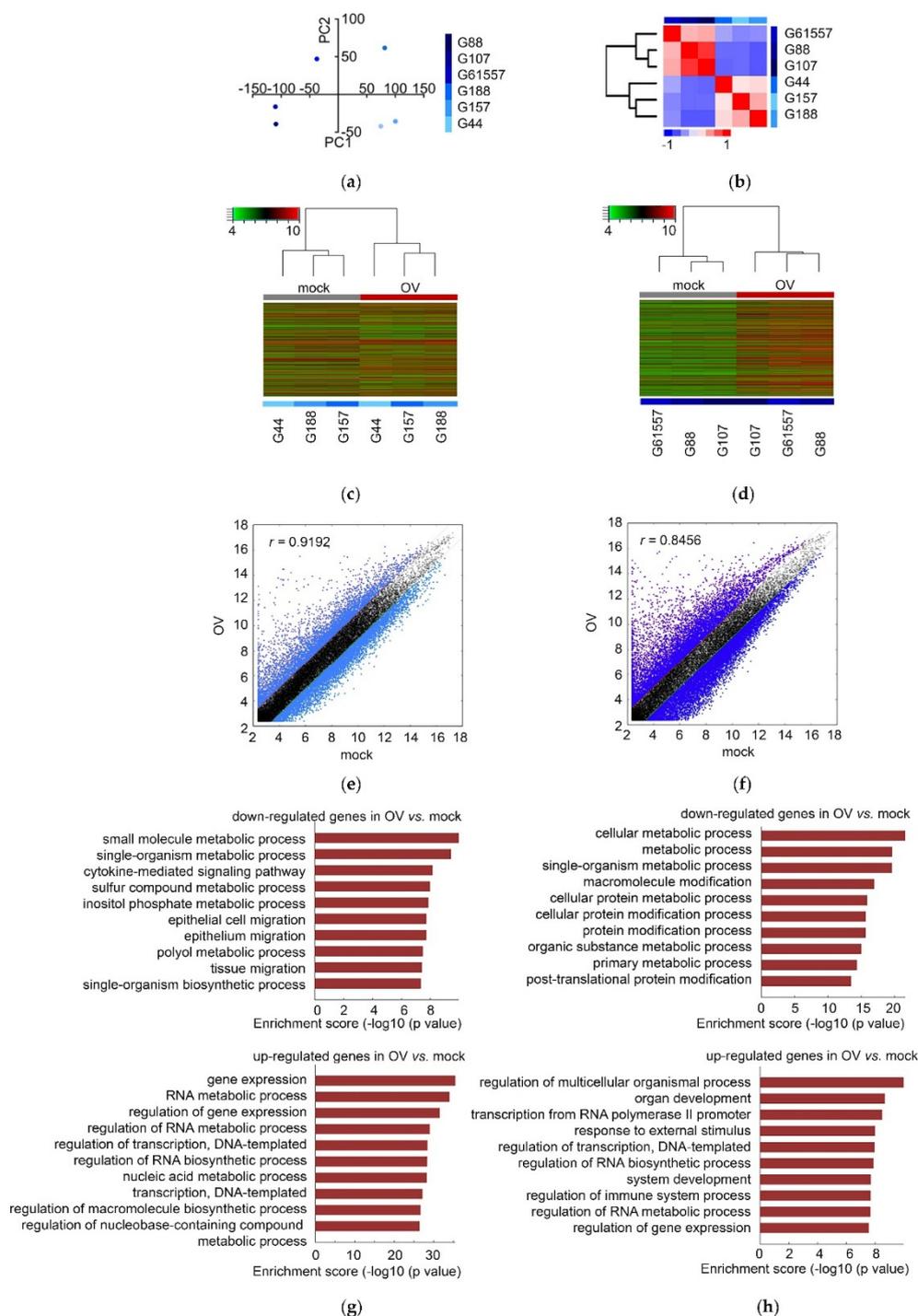


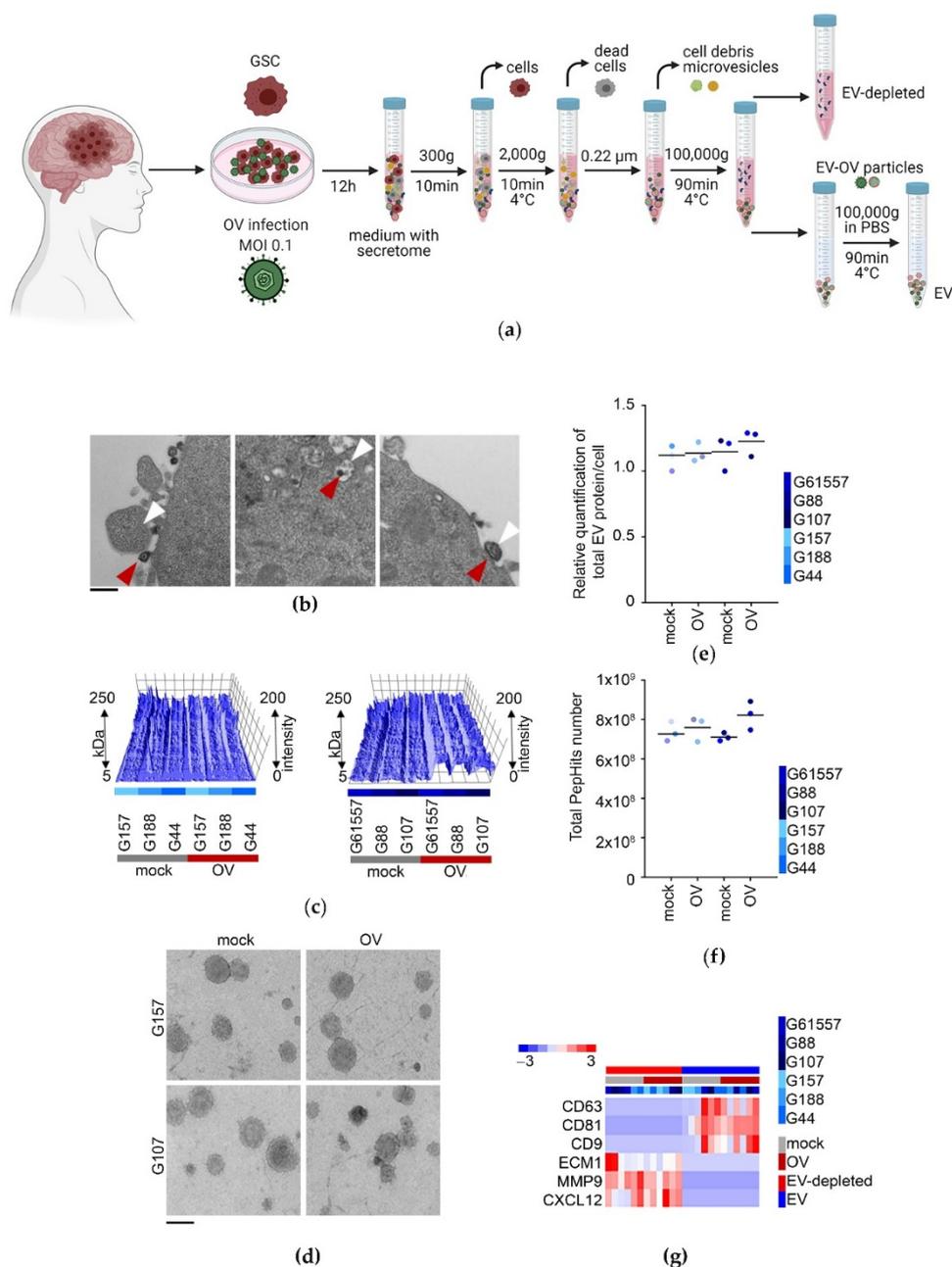
# Oncolytic Virus Therapy Alters the Secretome of Targeted Glioblastoma Cells

Jakub Godlewski, Mohamed Farhath, Franz L. Ricklefs, Carmela Passaro, Klaudia Kiel, Hiroshi Nakashima, E. Antonio Chiocca and Agnieszka Bronisz

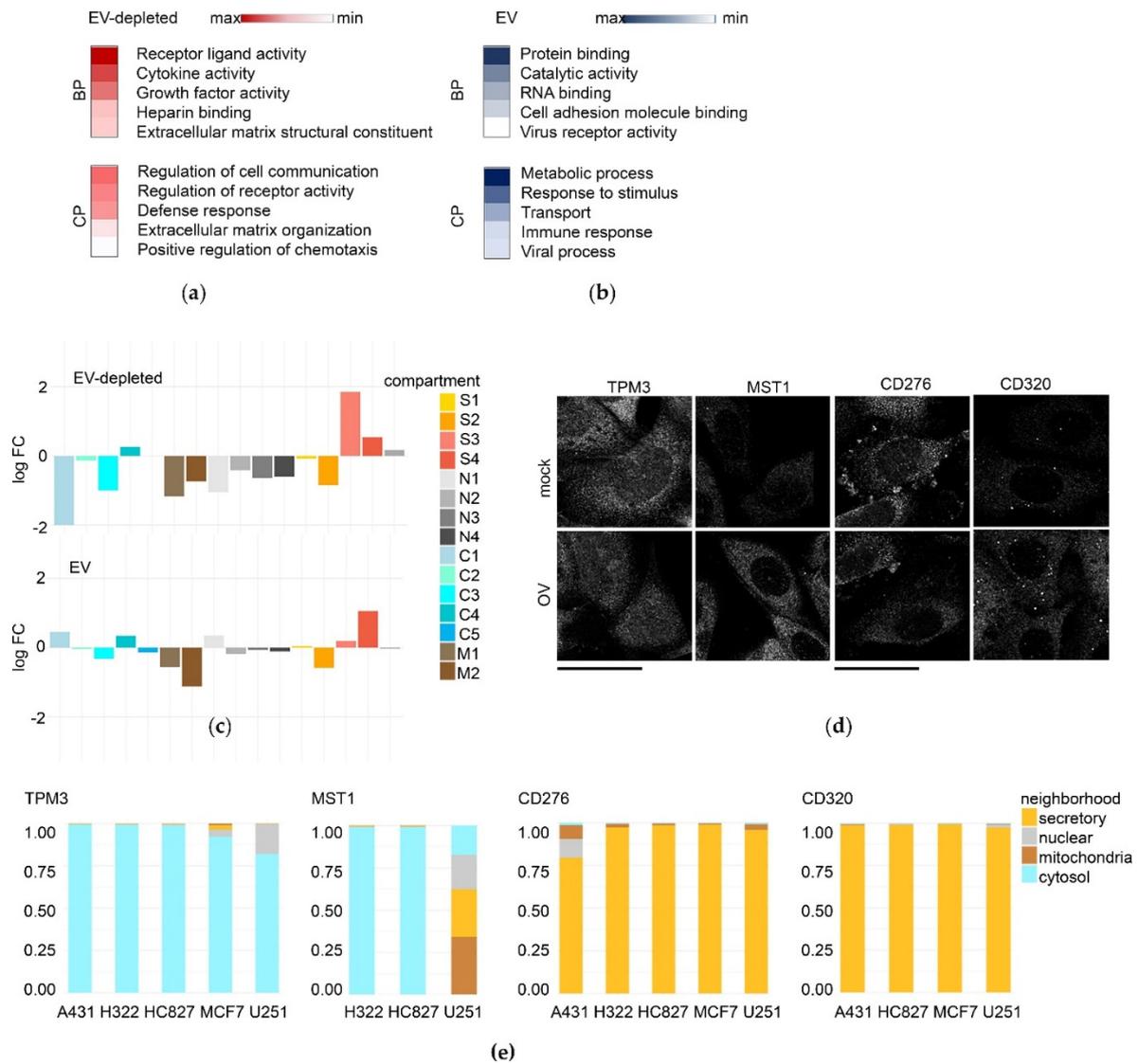


**Figure S1.** **a,b:** Principal component (PC) **(a)**, and matrix correlation analysis **(b)** based on all mRNA transcripts (Array-star™ human mRNA array,  $n = 6$ ). **c,d:** Heatmap with unsupervised hierarchical clustering for GSCs mock or OV infected are shown separately for OV slow-responding **(c)**,  $n = 3$  per variant – light blue) and OV fast-responding **(d)**,  $n = 3$  per variant

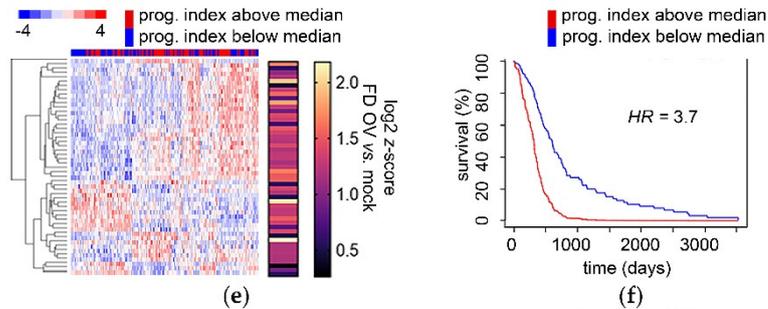
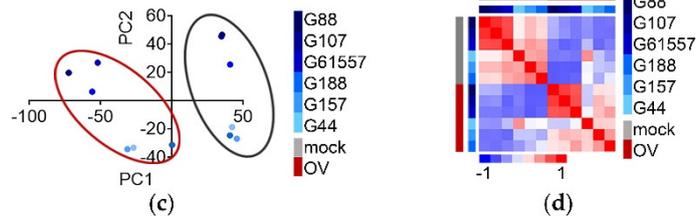
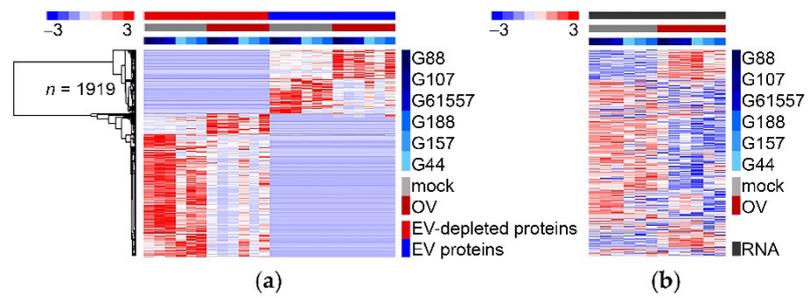
– dark blue) GSCs based on mRNA transcripts ( $n = 31555$  genes on Arraystar™ human mRNA array). **e,f**: Scatter plots of genes from GSCs mock, or OV infected are shown separately for OV slow-responding (**e**,  $n = 3$  per variant, affected genes – light blue) and OV fast-responding (**f**,  $n = 3$  per variant, affected genes – dark blue) GSCs. Pearson correlation values are disclosed. **g,h**: Gene ontology analysis of biological processes prevalent among genes deregulated upon OV infection in OV slow-responding (**g**, down-regulated – top, up-regulated – bottom), and OV fast-responding (**h**, down-regulated – top, up-regulated – bottom) GSCs.



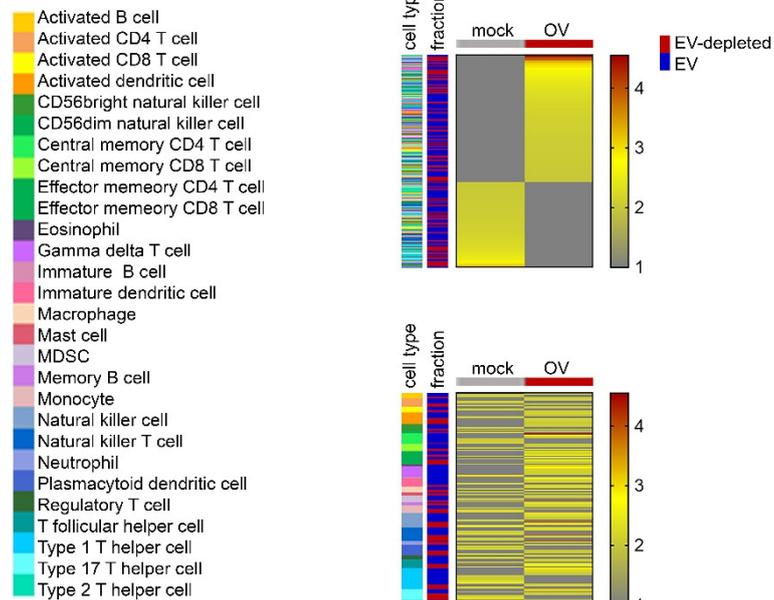
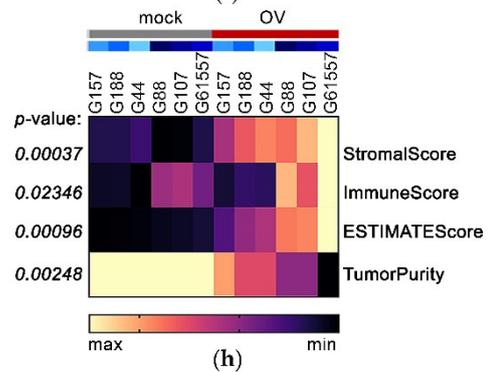
**Figure S2.** **a**: Flow chart of experimental procedures of secretome fractionation. BioRender (<https://biorender.com/>) software was used. **b**: Representative micrographs of OV/EV tandems by transmission electron microscopy. Red arrows - EV, white arrows – OV; scale bar: 500nm. **c**: Densitometric analysis of Coomassie Blue stained gels with EV-depleted proteins secreted by mock and OV infected GSCs. **d**: Representative micrographs of EVs secreted by mock or OV infected GSCs by transmission electron microscopy; scale bar: 500nm. **e**: Mass spectroscopy analysis of EV protein secreted by mock or OV infected GSCs by total peptide hits number. Data for human and viral proteins pooled ( $n = 3$  per variant). **f**: Mass spectroscopy analysis of EV protein secreted by mock and OV infected GSCs by relative quantification of total protein content per cell ( $n = 3$  per variant). **g**: Mass spectroscopy analysis of markers protein detected in EV and EV-depleted fraction respectively. Data represented by heatmap.



**Figure S3. a,b:** Gene ontology analysis of biological processes (BP) and cellular processes (CP) prevalent among proteins detected in EV-depleted (a), and EV (b) fractions of the secretome; the color bar for enrichment in each category GSCs. **c:** Bar analysis of protein localization from EV-depleted (top,  $n = 1203$ ) and EV fractions (bottom,  $n = 2088$ ) in Subcell Barcode dataset based on the mass-spectroscopy analysis of proteins in secretome (S), nucleus (N), cytoplasm (C), or mitochondrial (M) compartments. **d:** Representative micrographs of selected proteins in mock or OV infected GSCs by fluorescent confocal microscopy; with an antibody signal, scale bar:  $25\mu\text{m}$ . **e:** Bar analysis of cell-line-specific localization of TPM3, MST1, CD276, and CD320 in Subcell Barcode dataset based on the mass-spectroscopy analysis.



- $n = 50$  annotated genes
- 2.6e-0.6 Leukocyte activation
  - 4.3e-0.9 Immune system process
  - 7.1e-0.6 Leukocyte activation involved in immune response
  - 3.0e-0.9 Cell activation involved in immune response
  - 4.7e-0.6 Myeloid leukocyte activation



(i)

**Figure S4. a:** Heatmap with supervised hierarchical clustering for GSCs mock or OV infected ( $n = 6$  per variant) based on mass spectroscopy analysis in EV-depleted and EV fractions ( $n = 1919$  proteins, cutoffs between groups mock *vs.* OV in both fractions:  $p$ -value  $< 0.05$ ,  $FD > 2$ ). **b:** Heatmap with unsupervised clustering for GSCs mock or OV infected ( $n = 6$  per variant) based on mRNA transcripts ( $n = 1919$  genes encoding for proteins shown on a panel a, based on Arraystar™ human mRNA array). **c,d:** Principal component (PC) (**c**), and matrix correlation analysis (**d**) based on mRNA transcripts ( $n = 1919$  genes encoding for proteins shown on a panel a, based on Arraystar™ human mRNA array,  $n = 6$  per variant). **e,f:** The list of 1500 genes (see Fig. 4a) was filtered out to top-50 varied genes using TCGA data associated with glioblastoma. **e)** Heatmap of genes with color annotations according to prognostic index stratification cluster (horizontal cluster) and FD upon OV infection (vertical cluster). **f)** Kaplan-Meier curves show survival analysis stratified according to their prognostic index (see panel e); hazard ratio (HR). **g:** A hierarchical clustering tree summarizes the biological functions of top-50 survival-predictive genes. Bigger dots indicate more significant  $p$ -values. **h:** Heatmap classify GSCs mock, or OV infected ( $n = 6$  per variant) using four algorithms to estimate microenvironmental traits based on gene expression ( $n = 1500$ ) signatures. **i:** Heatmaps classify GSCs ( $n = 6$  per variant) to deconvolute secretome profiles into mock or OV infected cells (**top**) and immune cell type (**bottom**) of specific profiles based on immune response protein signatures ( $n = 220$ ); color bars indicate the type of immune cells and EV/EV-depleted proteins.

**Table S1.** Information about all reagents and resources used in the preparation of the manuscript.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
TPM3 Rabbit RRID AB_2633251	Thermo Fisher	720306
MST1 Rabbit RRID AB_11157025	Thermo Fisher	PA5-22015
CD276 Rabbit RRID AB_2789731	Thermo Fisher	PA5-82573
CD320 Rabbit RRID AB_2664869	Thermo Fisher	PA5-67296
CD63 Mouse (TS63)	Abcam	AB59479
Secondary Goat anti-Rabbit polyclonal	Thermo Fisher	32460
Bacterial and Virus Strains		
BL21 Star (DE3) Chemically Competent <i>E. coli</i>	Thermo Fisher	C601003
Oncolytic herpes simplex virus type 1 (oHSV) rQNestin34.5	48	NA
Biological Samples		
Patient-derived GSCs	49	N/A
Chemicals, Peptides, and Recombinant Proteins		
EGF	Peppo Tech	AF-100-15
FGF2	Peppo Tech	100-18C
B27	Gibco	21103049
Neurobasal	Gibco	21103049
TRIzol™ Reagent	ThermoFisher	15596018
Critical Commercial Assays		
Power Up SYBR Green Master Mix	Thermo Fisher	A25743
TaqMan Fast Advanced Master Mix	Thermo Fisher	4444963
Agilent whole human genome microarray	Arraystar	Agilent-026652 4x44K v2
iScript™ cDNA Synthesis Kit	Bio-Rad	1708890
Deposited Data		
Mass spectrometry	This paper	Supplemental spreadsheets Table 2
Genome microarray	This paper	Deposition: GSE155247
qPCR primers:		

TPM3: F: 5'-GA- GAGGTATGAAGGTTATTCA-3' R: 5'-ATCACCACCTTAC- GAGCCACC-3'		
MST1: F: 5'-GACAGCCCTCACGTAGTCAA - 3' R: 5'-AGGAGCCATCCAAAACGGG-3'		
CD276: F: 5'- GTGGGGCTGTCTGTCTGTCTCAT-3' R: 5'-CTGTCAGAG- TGTTTCAGAGGCT-3'	Invitrogen	N/A
CD320: F: 5'-CTGCGACAGGGACTTGGA-3' R: 5'-GGGTACATGGCTCAATCCTG- 3'		
18S rRNA: F: 5'-GGCCCTGTAATT- GGAATGAGTC-3' R: 5'-CCAAGATCCAACACTAC- GAGCTT-3'		
Recombinant DNA		
pCSCMV PalmtdTomato	50	N/A
Software and Algorithms		
dchip with R package version dChip 2010.01	51	<a href="https://sites.google.com/site/dchip-soft/home">https://sites.google.com/site/dchip-soft/home</a>
ShinyGO v0.61	52	<a href="http://bioinformatics.sdstate.edu/go/">http://bioinformatics.sdstate.edu/go/</a> accessed on: 01.03.2019
GBM biodiscovery portal	53	<a href="https://gbm-biodp.nci.nih.gov">https://gbm-biodp.nci.nih.gov</a> accessed on: 01.03.2019
GlioVis:	54	<a href="http://gliovis.bioinfo">http://gliovis.bioinfo</a> accessed on: 15.01.2020
TCGA GBM	28	<a href="https://www.cancer.gov/">https://www.cancer.gov/</a> accessed on: 01.03.2020
Subcellbarcode	55	<a href="http://www.subcellbarcode.org">http://www.subcellbarcode.org</a>
BioRender	NA	<a href="https://biorender.com/">https://biorender.com/</a>

**Table S2.** Mass spectroscopy analysis of two protein fractions EV-depleted and EV: please see the [link](#).