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*Original Article*

# Growth inhibition by bacterial Cas2Em proteins expressed in mammalian cells

Xin Yu[1,2*], Lei Wang[1*], Lin Zou[1], Mengjie Li[1], Tiansheng Li[1], Linlin Hou[1], Yameng Guo[1], Danfeng Shen[1], Guiqin Sun[3], Di Qu[1], Xunjia Cheng[1], Li Chen[1]

*[1]Department of Medical Microbiology, Key Laboratory of Medical Molecular Virology of Ministries of Education and Health, School of Basic Medical Sciences, Fudan University, Shanghai 200032, China; [2]Roche Innovation Center Shanghai, Shanghai 201203, China; [3]College of Medical Technology, Zhejiang Chinese Medical University, Hangzhou 310053, Zhejiang, China. *Equal contributors.*

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**Abstract:** Background: Clustered regularly interspaced short palindromic repeats (CRISPRs) and the CRISPR-associated (Cas) proteins are bacterial adaptive immune system for survival. In our previous study, we demonstrate that polyploid giant bacterial cells (PGBC) induced by Cas2 protein is a step required by new spacer acquisition reaction catalyzed by Cas1/Cas2 complex. We also demonstrated that a carboxyl terminal domain on Cas2Em (the protein Cas2 cloned from *Elizabethkingia meningoseptica*) is sufficient and enough for PGBC. Thus, the potential role of Cas2Em in microbial-host interaction was explored in this study. Methods: The impacts of Cas2Em on growth of both CHO-K1 and Hela cells were investigated. The subcellular localization and potential molecular target of Ca2Em were studied. Results: The growth of mammalian cells were inhibited by Cas2Em protein via G1 arresting and apoptosis. In addition, we also demonstrated that Cas2Em was tightly associated with nuclear outer membrane and could be immunoprecipitated with 14-3-3γ through a 30 amino acid domain (homology of CLK2). Conclusion: Cas2Em significantly suppressed the growth of mammalian cells indicating Cas2 proteins play an important role in mammalian cells.

**Keywords:** Cas2, microbial host interaction, 14-3-3, CLK2

## Introduction

CRISPR genomic loci consists of arrays of direct repeats separated by variable sequences, called spacers, which are commonly based on invader genetic elements [1, 2]. In CRISPR-Cas system, Cas are composed of Cas9, Cas1 and Cas2. Recent reports have indicated that Cas proteins are encoded by genes which were situated in the vicinity of the CRISPR loci [3, 4]. These proteins are participated in the three major steps of the CRISPR-Cas system action: adaptation, expression and interference [5]. Cas1 and Cas2 proteins sustain new spacer acquisition [6]. These two proteins form a stable complex in which a Cas2 dimer links two Cas1 dimers [7]. Cas2 recognizes the double-stranded region while Cas1 binds the 3' single-stranded flanks. At this stage, Cas1 may cleave the protospacer at the correct position with respect to its PAM [8], and catalyze its integration as a new spacer at a CRISPR locus [9].

Our recent investigation also revealed that Cas2Em (Em means the abbreviation of *Elizabethkingia meningoseptica*. Cas2EM indicates the protein Cas2 cloned from *Elizabethkingia meningoseptica*) could induce polyploid giant bacterial cells (PGBC) [10]. Moreover, historical studies found that molecules that cause the filamentous bacteria also have anti-tumor effects [11-13]. All these data indicated that Cas2Em may have anti-cancer ability. However, the inhibitory effect of Cas2Em on mammalian cell growth remains unclear. Therefore, we aimed to investigate the inhibitory effect of Cas2Em on mammalian cell growth and explore the underlying mechanisms.

## Material and methods

### Cell culture

CHO-K1 and Hela cell lines were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). CHO-K1 cells were cultured in Ham's F12 medium (Thermo Fisher Scientific, Waltham, MA, USA) with 10% fetal bovine serum (FBS, Thermo Fisher Scientific, Waltham, MA, USA), 1% penicillin-streptomycin (Thermo Fisher Scientific, Waltham, MA, USA) at 37°C, 5% $CO_2$. Hela cells were cultured in DMEM medium (Thermo Fisher Scientific, Waltham, MA, USA) with 10% fetal bovine serum (FBS, Thermo Fisher Scientific, Waltham, MA, USA), 1% penicillin-streptomycin (Thermo Fisher Scientific, Waltham, MA, USA) at 37°C, 5% $CO_2$.

### *Cell transfection*

Transient expression experiments were conducted to verify the expression of Cas2Em *in vitro*. CHO-K1 or Hela cells were cultured in 96-well plates and transfected with either GFP vector only or Cas2-GFP using Lipofectamine 3000 reagent kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. After transfection, cells were stained with Hoechst 33342 and the cell counting was measured by using Opera high content screening platform (Perkin Elmer Inc, USA). High content screening is a further development of cell-based screening in which multiple fluorescence readouts are measured simultaneously in intact cells by means of imaging techniques. The outcome of cell proliferation was based on counting cell number in a time course manner.

### *Cell cycle detection*

Cell cycle was determined by flow cytometry using Cycle Detection Kit I (BD Biosciences, Franklin Lake, NJ, USA). CHO-K1 or Hela cells were seeded in 6-well plate one day before transfection with Cas2-GFP. After 24 h of transfection, the cells were lifted and fixed in pre-cold 70% ethanol at 4°C overnight. Then, cells were treated with 100 μl PI/RNase Staining Buffer (Thermo Fisher Scientific, Waltham, MA, USA) at room temperature in the dark for 30 min. Finally, Flow cytometry (BD Biosciences, Franklin Lake, NJ, USA) was used to detect the cell cycle distribution and the data was analyzed using the Flowjo software (BD, Franklin Lake, NJ, USA).

### *Cell apoptosis analysis*

Hela were seeded in 6-well plate and transfected with GFP, Cas2-GFP or Cas2C30-GFP, respectively. Cells were lifted and re-suspended with 100 μl binding buffer after centrifuged at 1200 rpm/min for 5 min at different time points. Then, 5 μl Annexin V-PE was added in the cell suspension for 15 min. The apoptosis rate in cells was measured by flow-cytometer (BD, Franklin Lake, NJ, USA) and the data was analyzed using the Flowjo software (BD, Franklin Lake, NJ, USA).

### *Mass spectrum (MS) analysis*

In this study, we have provided details of the novel technique of immunoprecipitation MS analysis combined with on-membrane digestion for the analysis of protein-protein interactions. MS analysis was done as described previously [14].

### *Nuclei extraction*

Hela cells were seeded in 6 cm dish overnight and transfected with GFP or Cas2-GFP for 24 h. After that, cells were washed with ice-cold PBS containing phosphatase inhibitor and harvested using cell lifter. Then, nuclei were released using nuclear extraction kit (Active Motif, Carlsbad, CA, USA) following the manufacturer's instruction; nuclei were examined under inverted fluorescent microscope.

### *Immunofluorescence*

Hela cells were seeded in 24-well plates overnight and transfected with GFP or Cas2-GFP for 24 h. Then, the cells were prefixed in 4% paraformaldyhyde for 10 min, and fixed in pre-cold methanol for another 10 min. Next, cells were permeabilized and blocked in PBS containing 0.1% Triton X-100 and 10% goat serum for 1 h, then cells were incubated with anti-14-3-3γ (Abcam; 1:500) overnight at 4°C; alexafluor 594 goat anti-rabbit IgG antibody (Invitrogen; 1:1000) was used as the secondary antibody and DAPI was used for nuclear staining. The samples were visualized by confocal microscope (Zeiss LSM710, German) immediately.

### *Western blot*

Whole-cell lysates were collected using RIPA buffer. Proteins were separated by using 4-12% SDS polyacrylamide gel and proteins were transported to PVDF membranes (Thermo Fisher Scientific, CA, USA). The PVDF membranes were blocked with 5% skim milk in TBST at room temperature for 1 h. Later on, PVDF membranes were incubated with primary antibodies: HRP conjugated anti-Flag (Sigma; 1:1000), rabbit anti-GFP (Invitrogen; 1:1000), HRP conjugated anti-His (Sigma; 1:1000) and HRP conjugated anti-GAPDH (Sigma; 1:1000) overnight at 4°C. After that, the PVDF membrane was incubated with secondary anti-rabbit IgG, HRP-linked antibody (CellSignal; 1:3000) at room temperature for 1 h. Finally, the PVDF membranes were visualized with the enhanced chemiluminescent detection system (Thermo Fisher Scientific, CA, USA). GAPDH was used as an internal control.

### *Co-immunoprecipitation*

Co-immunoprecipitation of proteins was executed as previously described [15] with modifications. In brief, Hela cell protein supernatants were pre-treated with 50 μL A/G beads (Selleck Chemicals, Houston, USA) before immunoprecipitation. Then, cells were treated with 5 μg control IgG (Santa Cruz Biotechnology), His-NTA, or anti-GFP magnetic beads overnight at 4°C. After incubation with 50 μL A/G beads at 4°C for 6 h, the immune-precipitates were eluted with ice-cold PBS containing 0.2% NP-40 for 5 times. Subsequently, these immunoprecipitated proteins were electrophoresed on SDS-PAGE, and target proteins were visualized by western blot.

### *Sequence alignment*

Amino acid sequence alignment and annotation were carried out using ESPript 3.0 (http://espript.ibcp.fr/ESPript/ESPript/index.php). Homologous structure template was identified by BLASTp against the PDB database using the amino acid sequences of rice F3'Hs as queries. The protein structure of Cas2 with the highest amino acid identity was used for the model development.

### *Statistical analysis*

Each group were performed at least three independent experiments and all data were expressed as the mean ± standard deviation (SD). The comparisons among multiple groups were made with one-way analysis of variance (ANOVA) followed by Tukey's test (Graphpad Prism7). $P<0.05$ was considered to indicate a statistically significant difference.

## Result

### *Cas2Em significantly induced cell growth inhibition*

In order to investigate the effect of Cas2Em on proliferation of CHO-K1 and Hela cells, High content screening was used. As showed in **Figure 1A**, Cas2Em notably decreased the growth of CHO-K1 cells. Consistently, Cas2Em time-dependently inhibited the proliferation of Hela cells (**Figure 1B**). All these data indicated that Cas2Em exhibited inhibitory effect on growth of CHO-K1 and Hela cells.

### *Cas2Em induced G1-arrest in CHO-K1 and Hela cells*

To explore the role of Cas2Em during the proliferation of mammalian cells, cell cycle test was performed by using flow-cytometry. As indicated in **Figure 2A** and **2B**, G1-G0 arrest of CHO-K1 cells was markedly induced by Cas2Em. Additionally, Cas2Em significantly induced G1-G0 arrest of Hela cells (**Figure 2C** and **2D**). All these results revealed that Cas2Em could significantly inhibit the proliferation of mammalian cells.

### *Cas2Em induced apoptosis of Hela cells*

Next, the apoptosis of Hela cells was measured with flow-cytometer. As illustrated in **Figure 3A** and **3B**, the apoptosis rate of Hela cells transfected with Cas2Em was significantly increased time-dependently. This data showed that Cas2Em significantly promoted the apoptosis of cancer cells.

### *Cas2Em associated with nuclear outer membrane*

To explore the mechanism underneath above observations, fractionation of cytosol and nuclei in Hela cells was extracted. When the nuclei were released, Cas2-GFP was visualized significantly associated on the surface of nuclei. However, GFP was barely visualized in the same condition. This data indicated that Cas2Em might associate with perinuclear subcellular structure (**Figure 4A**).

### *Cas2Em interacted with 14-3-3γ*

To further explore the subcellular localization by which Cas2Em might interact to inhibit the cell growth, MS was performed. As illustrated in **Table 1**, 14-3-3γ interacting proteins was

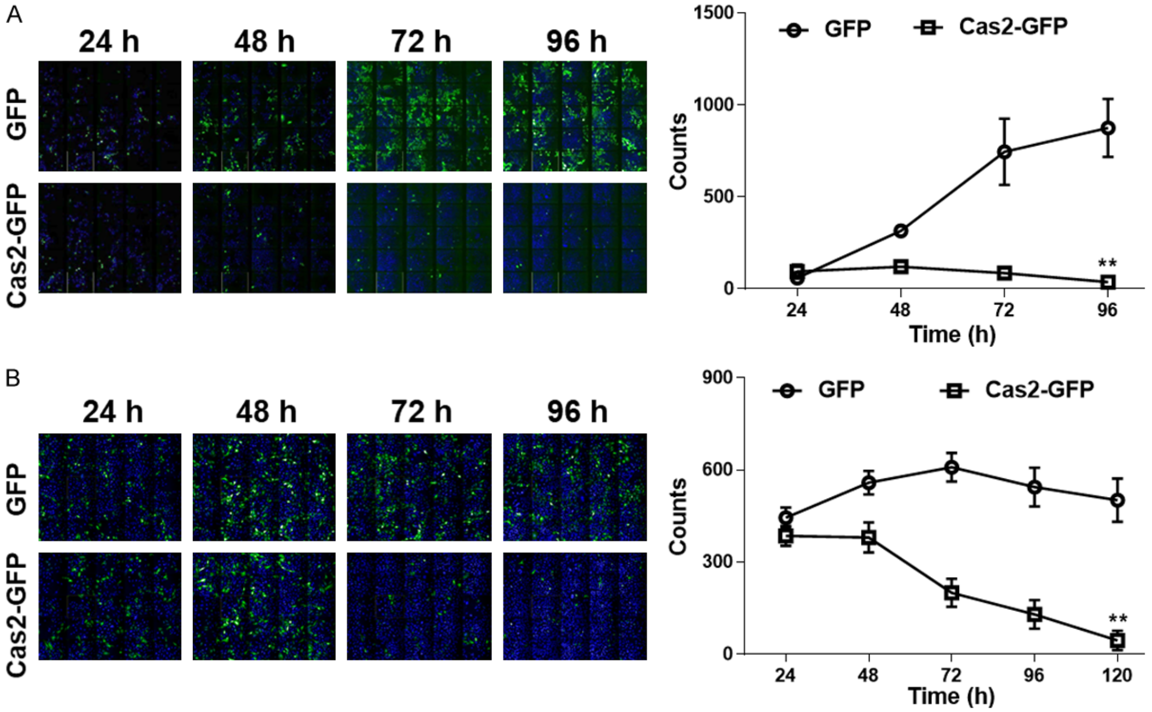

**Figure 1.** Cas2Em significantly induced cell growth inhibition. A, B. After Cas2Em transfection, representative images of CHO-K1 and Hela cells were captured and counted using Opera High content screening platform every 24 h. Each group were performed at least three independent experiments and all data were expressed as the mean ± standard deviation (SD). $^{**}P<0.01$ compared to GFP group.

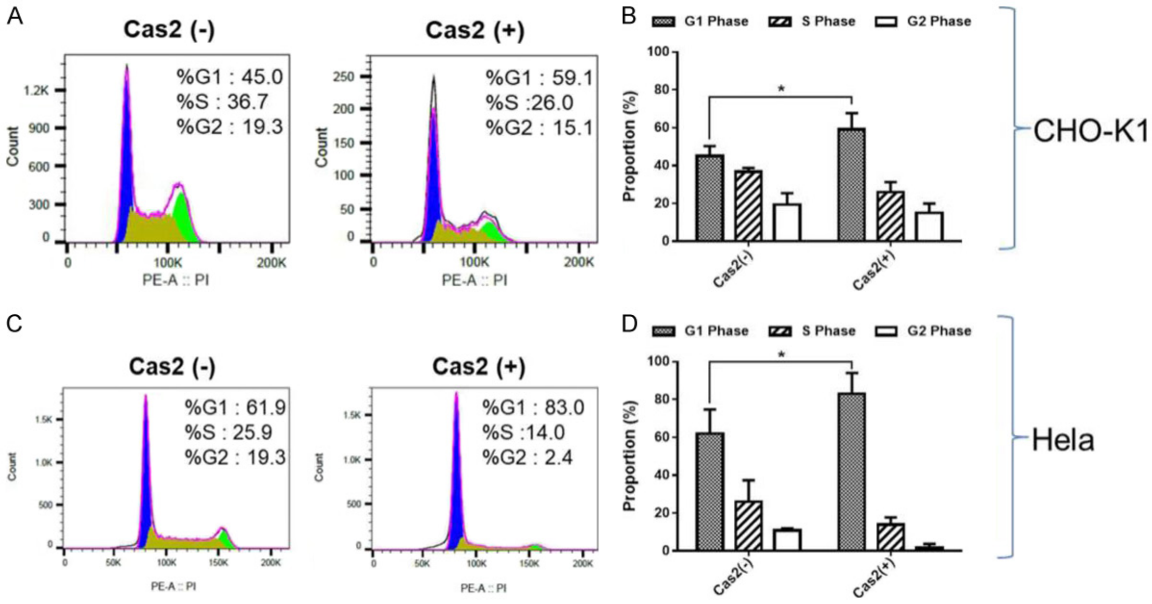

**Figure 2.** Cas2Em induced G1-arrest in CHO-K1 and Hela cells. A, B. The cell cycle distribution (G0/G1, S, and G2 phase) in CHO-K1 cells transfected with Cas2-GFP for 24 h were determined by FACS. C, D. The cell cycle distribution (G0/G1, S, and G2 phase) in Hela cells transfected with Cas2-GFP for 24 h were determined by FACS. Each group were performed at least three independent experiments and all data were expressed as the mean ± standard deviation (SD). $^{*}P<0.05$

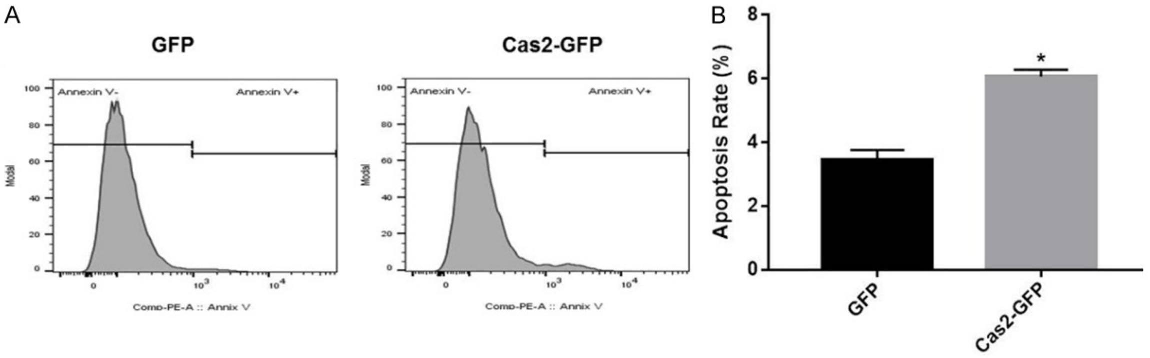

**Figure 3.** Cas2Em induced apoptosis of Hela cells. A, B. The apoptosis rate in Hela cells was measured by FACS after staining with Annexin V. X axis: the level of Annexin-V PE fluorescence; Y axis: count (%). Each group were performed at least three independent experiments and all data were expressed as the mean ± standard deviation (SD). $^{**}P<0.01$.

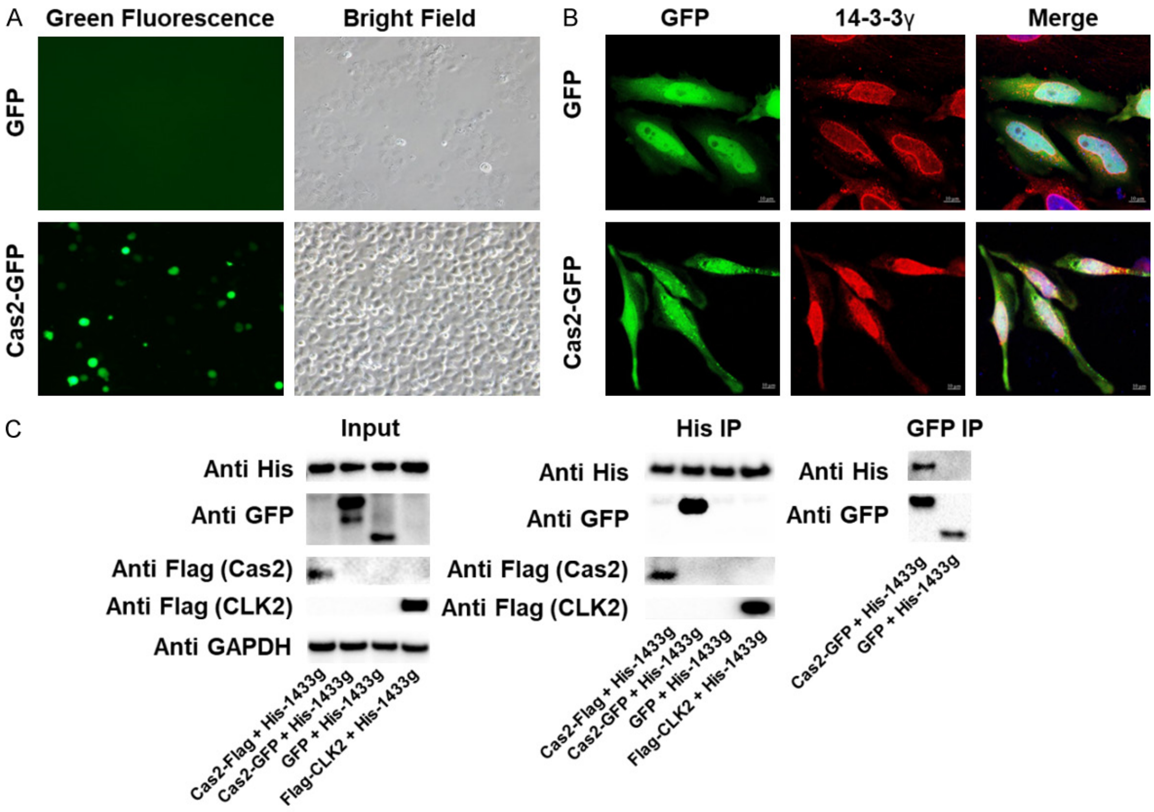

**Figure 4.** Cas2Em interacted with 14-3-3γ. A. Hela cells were seeded in 6 cm dish overnight and transfected with GFP or Cas2-GFP for 24 h. cells were washed with ice-cold PBS containing phosphatase inhibitor and harvested using cell lifter, and then the nuclei were extracted and examined under microscope. B. Expression of 14-3-3γ in Hela cells were detected using immunofluorescence staining. The green fluorescence indicated Hela cells expressing GFP or Cas2-GFP. The red fluorescence showed 14-3-3γ-positive Hela cells. Merge indicated the co-localization of 14-3-3γ and Cas2-GFP. The blue fluorescence indicated the nucleus. C. Equal amounts of total lysates were applied to co-immunoprecipitation with His-NTA or anti-GFP magnetic beads. IP analysis of Hela cells treated with His-14-3-3γ and Cas2 or GFP or Flag-CLK2. Then, expressions of His-14-3-3γ, Flag CLK2, GFP and Cas2 in Hela cells were detected by western-blot.

largely found (19.5%) in immunoprecipitation purified products of Cas2Em expressed Hela cells. This data indicated that Cas2Em might interact with 14-3-3γ. Moreover, immunofluo-

**Table 1.** The data of MS

| Accession | Description | Score | Coverage | # Proteins | # Unique Peptides | # Peptides | # PSMs | # AAs | MW [kDa] | calc. pI |
|---|---|---|---|---|---|---|---|---|---|---|
| P31946 | 14-3-3 protein beta/alpha OS=Homo sapiens GN=YWHAB PE=1 SV=3 - [1433B_HUMAN] | 67.10 | 15.85 | 3 | 1 | 4 | 4 | 246 | 28.1 | 4.83 |
| P62258 | 14-3-3 protein epsilon OS=Homo sapiens GN=YWHAE PE=1 SV=1 - [1433E_HUMAN] | 111.20 | 14.51 | 3 | 2 | 4 | 4 | 255 | 29.2 | 4.74 |
| P61981 | 14-3-3 protein gamma OS=Homo sapiens GN=YWHAG PE=1 SV=2 - [1433G_HUMAN] | 102.12 | 21.05 | 3 | 3 | 5 | 5 | 247 | 28.3 | 4.89 |
| P27348 | 14-3-3 protein theta OS=Homo sapiens GN=YWHAQ PE=1 SV=1 - [1433T_HUMAN] | 103.84 | 16.73 | 3 | 1 | 4 | 4 | 245 | 27.7 | 4.78 |
| P63104 | 14-3-3 protein zeta/delta OS=Homo sapiens GN=YWHAZ PE=1 SV=1 - [1433Z_HUMAN] | 144.58 | 26.12 | 3 | 3 | 6 | 6 | 245 | 27.7 | 4.79 |
| P62333 | 26S protease regulatory subunit 10B OS=Homo sapiens GN=PSMC6 PE=1 SV=1 - [PRS10_HUMAN] | 148.40 | 14.40 | 1 | 5 | 5 | 5 | 389 | 44.1 | 7.49 |
| P62191 | 26S protease regulatory subunit 4 OS=Homo sapiens GN=PSMC1 PE=1 SV=1 - [PRS4_HUMAN] | 97.94 | 12.95 | 2 | 4 | 5 | 5 | 440 | 49.2 | 6.21 |
| P17980 | 26S protease regulatory subunit 6A OS=Homo sapiens GN=PSMC3 PE=1 SV=3 - [PRS6A_HUMAN] | 53.06 | 7.52 | 1 | 3 | 3 | 3 | 439 | 49.2 | 5.24 |
| P43686 | 26S protease regulatory subunit 6B OS=Homo sapiens GN=PSMC4 PE=1 SV=2 - [PRS6B_HUMAN] | 91.37 | 19.14 | 1 | 6 | 6 | 6 | 418 | 47.3 | 5.21 |
| P35998 | 26S protease regulatory subunit 7 OS=Homo sapiens GN=PSMC2 PE=1 SV=3 - [PRS7_HUMAN] | 137.72 | 19.86 | 1 | 7 | 7 | 8 | 433 | 48.6 | 5.95 |
| P62195 | 26S protease regulatory subunit 8 OS=Homo sapiens GN=PSMC5 PE=1 SV=1 - [PRS8_HUMAN] | 181.61 | 16.01 | 2 | 5 | 6 | 6 | 406 | 45.6 | 7.55 |
| Q99460 | 26S proteasome non-ATPase regulatory subunit 1 OS=Homo sapiens GN=PSMD1 PE=1 SV=2 - [PSMD1_HUMAN] | 84.30 | 4.20 | 1 | 4 | 4 | 4 | 953 | 105.8 | 5.39 |
| O00231 | 26S proteasome non-ATPase regulatory subunit 11 OS=Homo sapiens GN=PSMD11 PE=1 SV=3 - [PSD11_HUMAN] | 110.94 | 14.22 | 1 | 6 | 6 | 6 | 422 | 47.4 | 6.48 |
| O00232 | 26S proteasome non-ATPase regulatory subunit 12 OS=Homo sapiens GN=PSMD12 PE=1 SV=3 - [PSD12_HUMAN] | 107.51 | 11.18 | 1 | 4 | 4 | 4 | 456 | 52.9 | 7.65 |
| Q9UNM6 | 26S proteasome non-ATPase regulatory subunit 13 OS=Homo sapiens GN=PSMD13 PE=1 SV=2 - [PSD13_HUMAN] | 119.88 | 9.57 | 1 | 3 | 3 | 4 | 376 | 42.9 | 5.81 |
| O00487 | 26S proteasome non-ATPase regulatory subunit 14 OS=Homo sapiens GN=PSMD14 PE=1 SV=1 - [PSDE_HUMAN] | 62.44 | 4.19 | 1 | 1 | 1 | 1 | 310 | 34.6 | 6.52 |
| Q13200 | 26S proteasome non-ATPase regulatory subunit 2 OS=Homo sapiens GN=PSMD2 PE=1 SV=3 - [PSMD2_HUMAN] | 264.45 | 16.08 | 1 | 11 | 11 | 12 | 908 | 100.1 | 5.20 |
| O43242 | 26S proteasome non-ATPase regulatory subunit 3 OS=Homo sapiens GN=PSMD3 PE=1 SV=2 - [PSMD3_HUMAN] | 100.40 | 13.11 | 1 | 6 | 6 | 6 | 534 | 60.9 | 8.44 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| P55036 | 26S proteasome non-ATPase regulatory subunit 4 OS=Homo sapiens GN=PSMD4 PE=1 SV=1 - [PSMD4_HUMAN] | 70.00 | 2.65 | 1 | 1 | 1 | 1 | 377 | 40.7 | 4.79 |
| Q15008 | 26S proteasome non-ATPase regulatory subunit 6 OS=Homo sapiens GN=PSMD6 PE=1 SV=1 - [PSMD6_HUMAN] | 74.01 | 9.77 | 1 | 4 | 4 | 4 | 389 | 45.5 | 5.62 |
| P51665 | 26S proteasome non-ATPase regulatory subunit 7 OS=Homo sapiens GN=PSMD7 PE=1 SV=2 - [PSMD7_HUMAN] | 49.28 | 11.11 | 1 | 3 | 3 | 4 | 324 | 37.0 | 6.77 |
| P48556 | 26S proteasome non-ATPase regulatory subunit 8 OS=Homo sapiens GN=PSMD8 PE=1 SV=2 - [PSMD8_HUMAN] | 96.71 | 7.71 | 1 | 3 | 3 | 4 | 350 | 39.6 | 9.70 |
| Q99714 | 3-hydroxyacyl-CoA dehydrogenase type-2 OS=Homo sapiens GN=HSD17B10 PE=1 SV=3 - [HCD2_HUMAN] | 50.34 | 7.28 | 1 | 2 | 2 | 2 | 261 | 26.9 | 7.78 |
| P62280 | 40S ribosomal protein S11 OS=Homo sapiens GN=RPS11 PE=1 SV=3 - [RS11_HUMAN] | 85.65 | 16.46 | 1 | 3 | 3 | 3 | 158 | 18.4 | 10.30 |
| P25398 | 40S ribosomal protein S12 OS=Homo sapiens GN=RPS12 PE=1 SV=3 - [RS12_HUMAN] | 37.94 | 13.64 | 1 | 2 | 2 | 2 | 132 | 14.5 | 7.21 |
| P62277 | 40S ribosomal protein S13 OS=Homo sapiens GN=RPS13 PE=1 SV=2 - [RS13_HUMAN] | 47.28 | 16.56 | 1 | 2 | 2 | 2 | 151 | 17.2 | 10.54 |
| P62263 | 40S ribosomal protein S14 OS=Homo sapiens GN=RPS14 PE=1 SV=3 - [RS14_HUMAN] | 64.32 | 15.89 | 1 | 2 | 2 | 2 | 151 | 16.3 | 10.05 |
| P62249 | 40S ribosomal protein S16 OS=Homo sapiens GN=RPS16 PE=1 SV=2 - [RS16_HUMAN] | 87.86 | 14.38 | 1 | 2 | 2 | 2 | 146 | 16.4 | 10.21 |
| P0CW22 | 40S ribosomal protein S17-like OS=Homo sapiens GN=RPS17L PE=1 SV=1 - [RS17L_HUMAN] | 34.00 | 30.37 | 1 | 3 | 3 | 4 | 135 | 15.5 | 9.85 |
| P62269 | 40S ribosomal protein S18 OS=Homo sapiens GN=RPS18 PE=1 SV=3 - [RS18_HUMAN] | 188.62 | 43.42 | 1 | 7 | 7 | 7 | 152 | 17.7 | 10.99 |
| P39019 | 40S ribosomal protein S19 OS=Homo sapiens GN=RPS19 PE=1 SV=2 - [RS19_HUMAN] | 25.71 | 6.90 | 1 | 1 | 1 | 1 | 145 | 16.1 | 10.32 |
| P62266 | 40S ribosomal protein S23 OS=Homo sapiens GN=RPS23 PE=1 SV=3 - [RS23_HUMAN] | 68.63 | 15.38 | 1 | 2 | 2 | 3 | 143 | 15.8 | 10.49 |
| P62847 | 40S ribosomal protein S24 OS=Homo sapiens GN=RPS24 PE=1 SV=1 - [RS24_HUMAN] | 24.89 | 8.27 | 1 | 1 | 1 | 1 | 133 | 15.4 | 10.78 |
| P62851 | 40S ribosomal protein S25 OS=Homo sapiens GN=RPS25 PE=1 SV=1 - [RS25_HUMAN] | 109.52 | 20.80 | 1 | 3 | 3 | 4 | 125 | 13.7 | 10.11 |
| P62854 | 40S ribosomal protein S26 OS=Homo sapiens GN=RPS26 PE=1 SV=3 - [RS26_HUMAN] | 51.54 | 7.83 | 2 | 1 | 1 | 1 | 115 | 13.0 | 11.00 |
| P62273 | 40S ribosomal protein S29 OS=Homo sapiens GN=RPS29 PE=1 SV=2 - [RS29_HUMAN] | 57.63 | 19.64 | 1 | 1 | 1 | 1 | 56 | 6.7 | 10.13 |
| P23396 | 40S ribosomal protein S3 OS=Homo sapiens GN=RPS3 PE=1 SV=2 - [RS3_HUMAN] | 62.90 | 11.93 | 1 | 3 | 3 | 3 | 243 | 26.7 | 9.66 |
| P62861 | 40S ribosomal protein S30 OS=Homo sapiens GN=FAU PE=1 SV=1 - [RS30_HUMAN] | 91.38 | 32.20 | 1 | 3 | 3 | 3 | 59 | 6.6 | 12.15 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| P61247 | 40S ribosomal protein S3a OS=Homo sapiens GN=RPS3A PE=1 SV=2 - [RS3A_HUMAN] | 28.44 | 3.79 | 1 | 1 | 1 | 1 | 264 | 29.9 | 9.73 |
| P62701 | 40S ribosomal protein S4, X isoform OS=Homo sapiens GN=RPS4X PE=1 SV=2 - [RS4X_HUMAN] | 200.45 | 25.48 | 3 | 7 | 7 | 8 | 263 | 29.6 | 10.15 |
| P46782 | 40S ribosomal protein S5 OS=Homo sapiens GN=RPS5 PE=1 SV=4 - [RS5_HUMAN] | 54.38 | 4.41 | 1 | 1 | 1 | 1 | 204 | 22.9 | 9.72 |
| P62081 | 40S ribosomal protein S7 OS=Homo sapiens GN=RPS7 PE=1 SV=1 - [RS7_HUMAN] | 59.17 | 15.98 | 1 | 2 | 2 | 2 | 194 | 22.1 | 10.10 |
| P08865 | 40S ribosomal protein SA OS=Homo sapiens GN=RPSA PE=1 SV=4 - [RSSA_HUMAN] | 56.50 | 10.85 | 1 | 3 | 3 | 3 | 295 | 32.8 | 4.87 |
| P08195 | 4F2 cell-surface antigen heavy chain OS=Homo sapiens GN=SLC3A2 PE=1 SV=3 - [4F2_HUMAN] | 72.61 | 3.33 | 1 | 2 | 2 | 2 | 630 | 68.0 | 5.01 |
| P10809 | 60 kDa heat shock protein, mitochondrial OS=Homo sapiens GN=HSPD1 PE=1 SV=2 - [CH60_HUMAN] | 320.76 | 25.83 | 1 | 10 | 10 | 11 | 573 | 61.0 | 5.87 |
| P05388 | 60S acidic ribosomal protein P0 OS=Homo sapiens GN=RPLP0 PE=1 SV=1 - [RLA0_HUMAN] | 26.44 | 3.47 | 2 | 1 | 1 | 2 | 317 | 34.3 | 5.97 |
| P05387 | 60S acidic ribosomal protein P2 OS=Homo sapiens GN=RPLP2 PE=1 SV=1 - [RLA2_HUMAN] | 69.91 | 26.96 | 1 | 2 | 2 | 2 | 115 | 11.7 | 4.54 |
| P62906 | 60S ribosomal protein L10a OS=Homo sapiens GN=RPL10A PE=1 SV=2 - [RL10A_HUMAN] | 21.88 | 3.69 | 1 | 1 | 1 | 1 | 217 | 24.8 | 9.94 |
| P62913 | 60S ribosomal protein L11 OS=Homo sapiens GN=RPL11 PE=1 SV=2 - [RL11_HUMAN] | 0.00 | 5.06 | 1 | 1 | 1 | 1 | 178 | 20.2 | 9.60 |
| P30050 | 60S ribosomal protein L12 OS=Homo sapiens GN=RPL12 PE=1 SV=1 - [RL12_HUMAN] | 56.12 | 5.45 | 1 | 1 | 1 | 1 | 165 | 17.8 | 9.42 |
| P26373 | 60S ribosomal protein L13 OS=Homo sapiens GN=RPL13 PE=1 SV=4 - [3_HUMAN] | 95.79 | 22.27 | 1 | 5 | 5 | 5 | 211 | 24.2 | 11.65 |
| P35268 | 60S ribosomal protein L22 OS=Homo sapiens GN=RPL22 PE=1 SV=2 - [RL22_HUMAN] | 97.10 | 18.75 | 1 | 2 | 2 | 2 | 128 | 14.8 | 9.19 |
| P62829 | 60S ribosomal protein L23 OS=Homo sapiens GN=RPL23 PE=1 SV=1 - [RL23_HUMAN] | 97.56 | 23.57 | 1 | 3 | 3 | 5 | 140 | 14.9 | 10.51 |
| P62750 | 60S ribosomal protein L23a OS=Homo sapiens GN=RPL23A PE=1 SV=1 - [RL23A_HUMAN] | 73.29 | 13.46 | 1 | 2 | 2 | 3 | 156 | 17.7 | 10.45 |
| P83731 | 60S ribosomal protein L24 OS=Homo sapiens GN=RPL24 PE=1 SV=1 - [RL24_HUMAN] | 45.04 | 5.73 | 1 | 1 | 1 | 1 | 157 | 17.8 | 11.25 |
| P61254 | 60S ribosomal protein L26 OS=Homo sapiens GN=RPL26 PE=1 SV=1 - [RL26_HUMAN] | 22.98 | 6.21 | 1 | 1 | 1 | 1 | 145 | 17.2 | 10.55 |
| P46776 | 60S ribosomal protein L27a OS=Homo sapiens GN=RPL27A PE=1 SV=2 - [RL27A_HUMAN] | 65.39 | 14.19 | 1 | 2 | 2 | 2 | 148 | 16.6 | 11.00 |
| P46779 | 60S ribosomal protein L28 OS=Homo sapiens GN=RPL28 PE=1 SV=3 - [RL28_HUMAN] | 55.39 | 16.79 | 1 | 2 | 2 | 2 | 137 | 15.7 | 12.02 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| P47914 | 60S ribosomal protein L29 OS=Homo sapiens GN=RPL29 PE=1 SV=2 - [RL29_HUMAN] | 47.85 | 14.47 | 1 | 2 | 2 | 2 | 159 | 17.7 | 11.66 |
| P62899 | 60S ribosomal protein L31 OS=Homo sapiens GN=RPL31 PE=1 SV=1 - [RL31_HUMAN] | 105.35 | 44.80 | 1 | 6 | 6 | 6 | 125 | 14.5 | 10.54 |
| P42766 | 60S ribosomal protein L35 OS=Homo sapiens GN=RPL35 PE=1 SV=2 - [RL35_HUMAN] | 61.36 | 15.45 | 1 | 2 | 2 | 3 | 123 | 14.5 | 11.05 |
| Q9Y3U8 | 60S ribosomal protein L36 OS=Homo sapiens GN=RPL36 PE=1 SV=3 - [RL36_HUMAN] | 33.55 | 8.57 | 1 | 1 | 1 | 1 | 105 | 12.2 | 11.59 |
| P83881 | 60S ribosomal protein L36a OS=Homo sapiens GN=RPL36A PE=1 SV=2 - [RL36A_HUMAN] | 31.00 | 7.55 | 2 | 1 | 1 | 1 | 106 | 12.4 | 10.58 |
| P61513 | 60S ribosomal protein L37a OS=Homo sapiens GN=RPL37A PE=1 SV=2 - [RL37A_HUMAN] | 24.72 | 9.78 | 1 | 1 | 1 | 1 | 92 | 10.3 | 10.43 |
| P63173 | 60S ribosomal protein L38 OS=Homo sapiens GN=RPL38 PE=1 SV=2 - [RL38_HUMAN] | 168.60 | 47.14 | 7 | 4 | 4 | 7 | 70 | 8.2 | 10.10 |
| P46777 | 60S ribosomal protein L5 OS=Homo sapiens GN=RPL5 PE=1 SV=3 - [RL5_HUMAN] | 52.53 | 15.15 | 1 | 4 | 4 | 4 | 297 | 34.3 | 9.72 |
| P11021 | 78 kDa glucose-regulated protein OS=Homo sapiens GN=HSPA5 PE=1 SV=2 - [GRP78_HUMAN] | 528.88 | 28.29 | 2 | 12 | 14 | 17 | 654 | 72.3 | 5.16 |
| P68032 | Actin, alpha cardiac muscle 1 OS=Homo sapiens GN=ACTC1 PE=1 SV=1 - [ACTC_HUMAN] | 174.17 | 24.93 | 5 | 1 | 9 | 14 | 377 | 42.0 | 5.39 |
| P60709 | Actin, cytoplasmic 1 OS=Homo sapiens GN=ACTB PE=1 SV=1 - [ACTB_HUMAN] | 269.16 | 29.07 | 8 | 2 | 10 | 18 | 375 | 41.7 | 5.48 |
| P23526 | Adenosylhomocysteinase OS=Homo sapiens GN=AHCY PE=1 SV=4 - [SAHH_HUMAN] | 91.88 | 9.03 | 1 | 3 | 3 | 3 | 432 | 47.7 | 6.34 |
| P30566 | Adenylosuccinate lyase OS=Homo sapiens GN=ADSL PE=1 SV=2 - [PUR8_HUMAN] | 36.57 | 2.27 | 1 | 1 | 1 | 1 | 484 | 54.9 | 7.11 |
| P05141 | ADP/ATP translocase 2 OS=Homo sapiens GN=SLC25A5 PE=1 SV=7 - [ADT2_HUMAN] | 96.62 | 16.11 | 4 | 5 | 5 | 6 | 298 | 32.8 | 9.69 |
| Q9ULA0 | Aspartyl aminopeptidase OS=Homo sapiens GN=DNPEP PE=1 SV=1 - [DNPEP_HUMAN] | 59.26 | 5.05 | 1 | 2 | 2 | 2 | 475 | 52.4 | 7.42 |
| P25705 | ATP synthase subunit alpha, mitochondrial OS=Homo sapiens GN=ATP5A1 PE=1 SV=1 - [ATPA_HUMAN] | 42.94 | 1.81 | 1 | 1 | 1 | 1 | 553 | 59.7 | 9.13 |
| P53396 | ATP-citrate synthase OS=Homo sapiens GN=ACLY PE=1 SV=3 - [ACLY_HUMAN] | 109.76 | 5.09 | 1 | 7 | 7 | 8 | 1101 | 120.8 | 7.33 |
| O95816 | BAG family molecular chaperone regulator 2 OS=Homo sapiens GN=BAG2 PE=1 SV=1 - [BAG2_HUMAN] | 132.00 | 13.74 | 1 | 3 | 3 | 3 | 211 | 23.8 | 6.70 |
| P61769 | Beta-2-microglobulin OS=Homo sapiens GN=B2M PE=1 SV=1 - [B2MG_HUMAN] | 27.71 | 16.81 | 1 | 2 | 2 | 3 | 119 | 13.7 | 6.52 |
| P62158 | Calmodulin OS=Homo sapiens GN=CALM1 PE=1 SV=2 - [CALM_HUMAN] | 100.89 | 12.75 | 2 | 3 | 3 | 4 | 149 | 16.8 | 4.22 |

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| P31944 | Caspase-14 OS=Homo sapiens GN=CASP14 PE=1 SV=2 - [CASPE_HUMAN] | 40.62 | 3.31 | 1 | 1 | 1 | 1 | 242 | 27.7 | 5.58 |
| Q9Y6A4 | Cilia- and flagella-associated protein 20 OS=Homo sapiens GN=CFAP20 PE=1 SV=1 - [CFA20_HUMAN] | 46.79 | 5.70 | 1 | 1 | 1 | 1 | 193 | 22.8 | 9.76 |
| Q00610 | Clathrin heavy chain 1 OS=Homo sapiens GN=CLTC PE=1 SV=5 - [CLH1_HUMAN] | 57.64 | 1.13 | 1 | 2 | 2 | 2 | 1675 | 191.5 | 5.69 |
| P09496 | Clathrin light chain A OS=Homo sapiens GN=CLTA PE=1 SV=1 - [CLCA_HUMAN] | 58.47 | 7.26 | 1 | 2 | 2 | 2 | 248 | 27.1 | 4.51 |
| O43809 | Cleavage and polyadenylation specificity factor subunit 5 OS=Homo sapiens GN=NUDT21 PE=1 SV=1 - [CPSF5_HUMAN] | 397.35 | 55.51 | 1 | 10 | 10 | 15 | 227 | 26.2 | 8.82 |
| Q07021 | Complement component 1 Q subcomponent-binding protein, mitochondrial OS=Homo sapiens GN=C1QBP PE=1 SV=1 - [C1QBP_HUMAN] | 57.32 | 13.12 | 1 | 2 | 2 | 3 | 282 | 31.3 | 4.84 |
| P17812 | CTP synthase 1 OS=Homo sapiens GN=CTPS1 PE=1 SV=2 - [PYRG1_HUMAN] | 31.38 | 1.69 | 1 | 1 | 1 | 1 | 591 | 66.6 | 6.46 |
| P42771 | Cyclin-dependent kinase inhibitor 2A, isoforms 1/2/3 OS=Homo sapiens GN=CDKN2A PE=1 SV=2 - [CD2A1_HUMAN] | 40.51 | 7.69 | 1 | 1 | 1 | 1 | 156 | 16.5 | 5.81 |
| P35520 | Cystathionine beta-synthase OS=Homo sapiens GN=CBS PE=1 SV=2 - [CBS_HUMAN] | 64.05 | 6.17 | 1 | 3 | 3 | 3 | 551 | 60.5 | 6.65 |
| P01040 | Cystatin-A OS=Homo sapiens GN=CSTA PE=1 SV=1 - [CYTA_HUMAN] | 27.82 | 12.24 | 1 | 1 | 1 | 1 | 98 | 11.0 | 5.50 |
| P49589 | Cysteine--tRNA ligase, cytoplasmic OS=Homo sapiens GN=CARS PE=1 SV=3 - [SYCC_HUMAN] | 32.16 | 1.34 | 1 | 1 | 1 | 1 | 748 | 85.4 | 6.76 |
| P08574 | Cytochrome c1, heme protein, mitochondrial OS=Homo sapiens GN=CYC1 PE=1 SV=3 - [CY1_HUMAN] | 54.03 | 3.69 | 1 | 1 | 1 | 1 | 325 | 35.4 | 9.00 |
| O43175 | D-3-phosphoglycerate dehydrogenase OS=Homo sapiens GN=PHGDH PE=1 SV=4 - [SERA_HUMAN] | 86.97 | 8.63 | 1 | 4 | 4 | 4 | 533 | 56.6 | 6.71 |
| Q9H773 | dCTP pyrophosphatase 1 OS=Homo sapiens GN=DCTPP1 PE=1 SV=1 - [DCTP1_HUMAN] | 51.37 | 7.65 | 1 | 1 | 1 | 1 | 170 | 18.7 | 5.03 |
| P33316 | Deoxyuridine 5'-triphosphate nucleotidohydrolase, mitochondrial OS=Homo sapiens GN=DUT PE=1 SV=4 - [DUT_HUMAN] | 109.53 | 14.68 | 1 | 3 | 3 | 4 | 252 | 26.5 | 9.36 |
| P81605 | Dermcidin OS=Homo sapiens GN=DCD PE=1 SV=2 - [DCD_HUMAN] | 105.88 | 20.00 | 1 | 2 | 2 | 4 | 110 | 11.3 | 6.54 |
| Q02413 | Desmoglein-1 OS=Homo sapiens GN=DSG1 PE=1 SV=2 - [DSG1_HUMAN] | 26.75 | 1.72 | 1 | 1 | 1 | 1 | 1049 | 113.7 | 5.03 |
| P15924 | Desmoplakin OS=Homo sapiens GN=DSP PE=1 SV=3 - [DESP_HUMAN] | 78.84 | 1.71 | 1 | 5 | 5 | 5 | 2871 | 331.6 | 6.81 |
| Q9Y295 | Developmentally-regulated GTP-binding protein 1 OS=Homo sapiens GN=DRG1 PE=1 SV=1 - [DRG1_HUMAN] | 65.01 | 7.90 | 1 | 2 | 2 | 2 | 367 | 40.5 | 8.90 |
| Q9NR28 | Diablo homolog, mitochondrial OS=Homo sapiens GN=DIABLO PE=1 SV=1 - [DBLOH_HUMAN] | 112.36 | 12.97 | 1 | 3 | 3 | 3 | 239 | 27.1 | 5.90 |

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|---|---|---|---|---|---|---|---|---|---|---|
| P33992 | DNA replication licensing factor MCM5 OS=Homo sapiens GN=MCM5 PE=1 SV=5 - [MCM5_HUMAN] | 0.00 | 1.77 | 1 | 1 | 1 | 1 | 734 | 82.2 | 8.37 |
| P31689 | DnaJ homolog subfamily A member 1 OS=Homo sapiens GN=DNAJA1 PE=1 SV=2 - [DNJA1_HUMAN] | 30.33 | 5.04 | 1 | 2 | 2 | 2 | 397 | 44.8 | 7.08 |
| Q7Z6Z7 | E3 ubiquitin-protein ligase HUWE1 OS=Homo sapiens GN=HUWE1 PE=1 SV=3 - [HUWE1_HUMAN] | #### | 13.26 | 2 | 45 | 45 | 53 | 4374 | 481.6 | 5.22 |
| P68104 | Elongation factor 1-alpha 1 OS=Homo sapiens GN=EEF1A1 PE=1 SV=1 - [21A1_HUMAN] | 63.28 | 2.60 | 2 | 1 | 1 | 2 | 462 | 50.1 | 9.01 |
| P24534 | Elongation factor 1-beta OS=Homo sapiens GN=EEF1B2 PE=1 SV=3 - [21B_HUMAN] | 20.78 | 4.00 | 1 | 1 | 1 | 1 | 225 | 24.7 | 4.67 |
| Q13868 | Exosome complex component RRP4 OS=Homo sapiens GN=EXOSC2 PE=1 SV=2 - [EXOS2_HUMAN] | 25.04 | 3.75 | 1 | 1 | 1 | 1 | 293 | 32.8 | 7.50 |
| Q9NPD3 | Exosome complex component RRP41 OS=Homo sapiens GN=EXOSC4 PE=1 SV=3 - [EXOS4_HUMAN] | 37.68 | 4.08 | 1 | 1 | 1 | 1 | 245 | 26.4 | 6.52 |
| P52907 | F-actin-capping protein subunit alpha-1 OS=Homo sapiens GN=CAPZA1 PE=1 SV=3 - [CAZA1_HUMAN] | 155.96 | 40.56 | 1 | 6 | 8 | 8 | 286 | 32.9 | 5.69 |
| P47755 | F-actin-capping protein subunit alpha-2 OS=Homo sapiens GN=CAPZA2 PE=1 SV=3 - [CAZA2_HUMAN] | 80.76 | 12.24 | 1 | 1 | 3 | 3 | 286 | 32.9 | 5.85 |
| P49327 | Fatty acid synthase OS=Homo sapiens GN=FASN PE=1 SV=3 - [FAS_HUMAN] | 150.95 | 4.50 | 1 | 8 | 8 | 8 | 2511 | 273.3 | 6.44 |
| Q01469 | Fatty acid-binding protein, epidermal OS=Homo sapiens GN=FABP5 PE=1 SV=3 - [FABP5_HUMAN] | 37.12 | 6.67 | 1 | 1 | 1 | 1 | 135 | 15.2 | 7.01 |
| P20930 | Filaggrin OS=Homo sapiens GN=FLG PE=1 SV=3 - [FILA_HUMAN] | 41.49 | 1.97 | 1 | 2 | 2 | 2 | 4061 | 434.9 | 9.25 |
| Q5D862 | Filaggrin-2 OS=Homo sapiens GN=FLG2 PE=1 SV=1 - [FILA2_HUMAN] | 80.63 | 4.35 | 1 | 4 | 4 | 5 | 2391 | 247.9 | 8.31 |
| P21333 | Filamin-A OS=Homo sapiens GN=FLNA PE=1 SV=4 - [FLNA_HUMAN] | 294.64 | 9.71 | 3 | 21 | 21 | 23 | 2647 | 280.6 | 6.06 |
| Q08380 | Galectin-3-binding protein OS=Homo sapiens GN=LGALS3BP PE=1 SV=1 - [LG3BP_HUMAN] | 42.26 | 5.81 | 1 | 3 | 3 | 3 | 585 | 65.3 | 5.27 |
| P47929 | Galectin-7 OS=Homo sapiens GN=LGALS7 PE=1 SV=2 - [LEG7_HUMAN] | 28.26 | 8.09 | 1 | 1 | 1 | 1 | 136 | 15.1 | 7.62 |
| P78347 | General transcription factor II-I OS=Homo sapiens GN=GTF2I PE=1 SV=2 - [GTF2I_HUMAN] | 408.12 | 23.75 | 1 | 19 | 19 | 27 | 998 | 112.3 | 6.39 |
| P15104 | Glutamine synthetase OS=Homo sapiens GN=GLUL PE=1 SV=4 - [GLNA_HUMAN] | 31.71 | 2.14 | 1 | 1 | 1 | 1 | 373 | 42.0 | 6.89 |
| P00390 | Glutathione reductase, mitochondrial OS=Homo sapiens GN=GSR PE=1 SV=2 - [GSHR_HUMAN] | 76.57 | 15.13 | 1 | 5 | 5 | 5 | 522 | 56.2 | 8.50 |
| P28161 | Glutathione S-transferase Mu 2 OS=Homo sapiens GN=GSTM2 PE=1 SV=2 - [GSTM2_HUMAN] | 65.81 | 10.09 | 4 | 1 | 2 | 2 | 218 | 25.7 | 6.37 |

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|---|---|---|---|---|---|---|---|---|---|---|
| P21266 | Glutathione S-transferase Mu 3 OS=Homo sapiens GN=GSTM3 PE=1 SV=3 - [GSTM3_HUMAN] | 79.77 | 14.67 | 2 | 2 | 3 | 4 | 225 | 26.5 | 5.54 |
| P04406 | Glyceraldehyde-3-phosphate dehydrogenase OS=Homo sapiens GN=GAPDH PE=1 SV=3 - [G3P_HUMAN] | 46.18 | 2.39 | 1 | 1 | 1 | 1 | 335 | 36.0 | 8.46 |
| Q0VDF9 | Heat shock 70 kDa protein 14 OS=Homo sapiens GN=HSPA14 PE=1 SV=1 - [HSP7E_HUMAN] | 22.65 | 1.96 | 1 | 1 | 1 | 1 | 509 | 54.8 | 5.59 |
| P08107 | Heat shock 70 kDa protein 1A/1B OS=Homo sapiens GN=HSPA1A PE=1 SV=5 - [HSP71_HUMAN] | 753.39 | 41.03 | 2 | 16 | 19 | 28 | 641 | 70.0 | 5.66 |
| P11142 | Heat shock cognate 71 kDa protein OS=Homo sapiens GN=HSPA8 PE=1 SV=1 - [HSP7C_HUMAN] | 842.86 | 43.65 | 2 | 17 | 21 | 35 | 646 | 70.9 | 5.52 |
| P04792 | Heat shock protein beta-1 OS=Homo sapiens GN=HSPB1 PE=1 SV=2 - [HSPB1_HUMAN] | 29.79 | 3.90 | 1 | 1 | 1 | 1 | 205 | 22.8 | 6.40 |
| P07900 | Heat shock protein HSP 90-alpha OS=Homo sapiens GN=HSP90AA1 PE=1 SV=5 - [HS90A_HUMAN] | 86.58 | 6.42 | 3 | 1 | 4 | 4 | 732 | 84.6 | 5.02 |
| P08238 | Heat shock protein HSP 90-beta OS=Homo sapiens GN=HSP90AB1 PE=1 SV=4 - [HS90B_HUMAN] | 100.32 | 11.19 | 4 | 4 | 7 | 8 | 724 | 83.2 | 5.03 |
| P52597 | Heterogeneous nuclear ribonucleoprotein F OS=Homo sapiens GN=HNRNPF PE=1 SV=3 - [HNRPF_HUMAN] | 28.75 | 2.41 | 3 | 1 | 1 | 1 | 415 | 45.6 | 5.58 |
| Q00839 | Heterogeneous nuclear ribonucleoprotein U OS=Homo sapiens GN=HNRNPU PE=1 SV=6 - [HNRPU_HUMAN] | 61.90 | 1.82 | 1 | 1 | 1 | 1 | 825 | 90.5 | 6.00 |
| Q9UBN7 | Histone deacetylase 6 OS=Homo sapiens GN=HDAC6 PE=1 SV=2 - [HDAC6_HUMAN] | 48.74 | 1.48 | 1 | 2 | 2 | 2 | 1215 | 131.3 | 5.30 |
| P16403 | Histone H1.2 OS=Homo sapiens GN=HIST1H1C PE=1 SV=2 - [H12_HUMAN] | 74.55 | 10.80 | 5 | 2 | 2 | 2 | 213 | 21.4 | 10.93 |
| P16401 | Histone H1.5 OS=Homo sapiens GN=HIST1H1B PE=1 SV=3 - | 74.50 | 10.18 | 1 | 2 | 2 | 2 | 226 | 22.6 | 10.92 |
| Q96KK5 | Histone H2A type 1-H OS=Homo sapiens GN=HIST1H2AH PE=1 SV=3 - [H2A1H_HUMAN] | 91.04 | 35.16 | 15 | 3 | 3 | 3 | 128 | 13.9 | 10.89 |
| O60814 | Histone H2B type 1-K OS=Homo sapiens GN=HIST1H2BK PE=1 SV=3 - [H2B1K_HUMAN] | 61.19 | 15.87 | 15 | 2 | 2 | 2 | 126 | 13.9 | 10.32 |
| P62805 | Histone H4 OS=Homo sapiens GN=HIST1H4A PE=1 SV=2 - [H4_HUMAN] | 68.23 | 17.48 | 1 | 2 | 2 | 2 | 103 | 11.4 | 11.36 |
| Q86YZ3 | Hornerin OS=Homo sapiens GN=HRNR PE=1 SV=2 - [HORN_HUMAN] | 239.82 | 12.91 | 1 | 9 | 9 | 11 | 2850 | 282.2 | 10.04 |
| P00492 | Hypoxanthine-guanine phosphoribosyltransferase OS=Homo sapiens GN=HPRT1 PE=1 SV=2 - [HPRT_HUMAN] | 128.07 | 24.77 | 1 | 5 | 5 | 5 | 218 | 24.6 | 6.68 |
| Q9H2U2 | Inorganic pyrophosphatase 2, mitochondrial OS=Homo sapiens GN=PPA2 PE=1 SV=2 - [IPYR2_HUMAN] | 50.28 | 3.59 | 1 | 1 | 1 | 1 | 334 | 37.9 | 7.39 |
| P12268 | Inosine-5'-monophosphate dehydrogenase 2 OS=Homo sapiens GN=IMPDH2 PE=1 SV=2 - [IMDH2_HUMAN] | 120.97 | 9.53 | 2 | 4 | 4 | 4 | 514 | 55.8 | 6.90 |

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| P14923 | Junction plakoglobin OS=Homo sapiens GN=JUP PE=1 SV=3 - [PLAK_HUMAN] | 42.61 | 2.82 | 1 | 2 | 2 | 2 | 745 | 81.7 | 6.14 |
| P13645 | Keratin, type I cytoskeletal 10 OS=Homo sapiens GN=KRT10 PE=1 SV=6 - [K1C10_HUMAN] | #### | 46.40 | 13 | 23 | 26 | 61 | 584 | 58.8 | 5.21 |
| P02533 | Keratin, type I cytoskeletal 14 OS=Homo sapiens GN=KRT14 PE=1 SV=4 - [K1C14_HUMAN] | 513.42 | 26.27 | 11 | 4 | 11 | 17 | 472 | 51.5 | 5.16 |
| P08779 | Keratin, type I cytoskeletal 16 OS=Homo sapiens GN=KRT16 PE=1 SV=4 - [K1C16_HUMAN] | 566.68 | 28.12 | 11 | 6 | 12 | 18 | 473 | 51.2 | 5.05 |
| Q04695 | Keratin, type I cytoskeletal 17 OS=Homo sapiens GN=KRT17 PE=1 SV=2 - [K1C17_HUMAN] | 188.44 | 16.20 | 10 | 3 | 7 | 8 | 432 | 48.1 | 5.02 |
| P05783 | Keratin, type I cytoskeletal 18 OS=Homo sapiens GN=KRT18 PE=1 SV=2 - [K1C18_HUMAN] | 103.03 | 8.60 | 8 | 3 | 4 | 4 | 430 | 48.0 | 5.45 |
| P35527 | Keratin, type I cytoskeletal 9 OS=Homo sapiens GN=KRT9 PE=1 SV=3 - [K1C9_HUMAN] | 978.70 | 36.44 | 2 | 17 | 18 | 29 | 623 | 62.0 | 5.24 |
| P04264 | Keratin, type II cytoskeletal 1 OS=Homo sapiens GN=KRT1 PE=1 SV=6 - [K2C1_HUMAN] | #### | 43.01 | 3 | 23 | 31 | 60 | 644 | 66.0 | 8.12 |
| P35908 | Keratin, type II cytoskeletal 2 epidermal OS=Homo sapiens GN=KRT2 PE=1 SV=2 - [K22E_HUMAN] | #### | 64.16 | 3 | 27 | 35 | 46 | 639 | 65.4 | 8.00 |
| P13647 | Keratin, type II cytoskeletal 5 OS=Homo sapiens GN=KRT5 PE=1 SV=3 - [K2C5_HUMAN] | 534.03 | 29.15 | 5 | 9 | 21 | 27 | 590 | 62.3 | 7.74 |
| P02538 | Keratin, type II cytoskeletal 6A OS=Homo sapiens GN=KRT6A PE=1 SV=3 - [K2C6A_HUMAN] | 529.78 | 32.09 | 6 | 2 | 22 | 29 | 564 | 60.0 | 8.00 |
| P04259 | Keratin, type II cytoskeletal 6B OS=Homo sapiens GN=KRT6B PE=1 SV=5 - [K2C6B_HUMAN] | 510.46 | 31.74 | 5 | 1 | 21 | 28 | 564 | 60.0 | 8.00 |
| Q8N1N4 | Keratin, type II cytoskeletal 78 OS=Homo sapiens GN=KRT78 PE=2 SV=2 - [K2C78_HUMAN] | 57.59 | 1.73 | 1 | 1 | 1 | 1 | 520 | 56.8 | 6.02 |
| P05787 | Keratin, type II cytoskeletal 8 OS=Homo sapiens GN=KRT8 PE=1 SV=7 - [K2C8_HUMAN] | 484.20 | 19.46 | 8 | 6 | 13 | 23 | 483 | 53.7 | 5.59 |
| O60282 | Kinesin heavy chain isoform 5C OS=Homo sapiens GN=KIF5C PE=1 SV=1 - [KIF5C_HUMAN] | 30.09 | 1.25 | 3 | 1 | 1 | 1 | 957 | 109.4 | 6.19 |
| P00338 | L-lactate dehydrogenase A chain OS=Homo sapiens GN=LDHA PE=1 SV=2 - [LDHA_HUMAN] | 39.44 | 3.01 | 1 | 1 | 1 | 1 | 332 | 36.7 | 8.27 |
| P14174 | Macrophage migration inhibitory factor OS=Homo sapiens GN=MIF PE=1 SV=4 - [MIF_HUMAN] | 201.58 | 26.96 | 1 | 4 | 4 | 6 | 115 | 12.5 | 7.88 |
| Q9NZL9 | Methionine adenosyltransferase 2 subunit beta OS=Homo sapiens GN=MAT2B PE=1 SV=1 - [MAT2B_HUMAN] | 43.82 | 10.48 | 1 | 2 | 2 | 2 | 334 | 37.5 | 7.36 |
| Q9BQA1 | Methylosome protein 50 OS=Homo sapiens GN=WDR77 PE=1 SV=1 - [MEP50_HUMAN] | 65.26 | 5.56 | 1 | 2 | 2 | 2 | 342 | 36.7 | 5.17 |

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| O43684 | Mitotic checkpoint protein BUB3 OS=Homo sapiens GN=BUB3 PE=1 SV=1 - [BUB3_HUMAN] | 59.66 | 18.90 | 1 | 5 | 5 | 5 | 328 | 37.1 | 6.84 |
| Q9UI30 | Multifunctional methyltransferase subunit TRM112-like protein OS=Homo sapiens GN=TRMT112 PE=1 SV=1 - [TR112_HUMAN] | 20.43 | 11.20 | 1 | 1 | 1 | 1 | 125 | 14.2 | 5.26 |
| P22234 | Multifunctional protein ADE2 OS=Homo sapiens GN=PAICS PE=1 SV=3 - [PUR6_HUMAN] | 30.23 | 2.35 | 1 | 1 | 1 | 1 | 425 | 47.0 | 7.23 |
| P60660 | Myosin light polypeptide 6 OS=Homo sapiens GN=MYL6 PE=1 SV=2 - [MYL6_HUMAN] | 69.94 | 28.48 | 1 | 4 | 4 | 4 | 151 | 16.9 | 4.65 |
| P19105 | Myosin regulatory light chain 12A OS=Homo sapiens GN=MYL12A PE=1 SV=2 - [ML12A_HUMAN] | 180.34 | 31.58 | 2 | 2 | 5 | 5 | 171 | 19.8 | 4.81 |
| P24844 | Myosin regulatory light polypeptide 9 OS=Homo sapiens GN=MYL9 PE=1 SV=4 - [MYL9_HUMAN] | 168.36 | 25.58 | 1 | 1 | 4 | 4 | 172 | 19.8 | 4.92 |
| P35579 | Myosin-9 OS=Homo sapiens GN=MYH9 PE=1 SV=4 - [MYH9_HUMAN] | 186.10 | 6.48 | 2 | 12 | 12 | 12 | 1960 | 226.4 | 5.60 |
| Q9Y266 | Nuclear migration protein nudC OS=Homo sapiens GN=NUDC PE=1 SV=1 - [NUDC_HUMAN] | 64.52 | 5.74 | 1 | 2 | 2 | 2 | 331 | 38.2 | 5.38 |
| P67809 | Nuclease-sensitive element-binding protein 1 OS=Homo sapiens GN=YBX1 PE=1 SV=3 - [YBOX1_HUMAN] | 65.97 | 19.75 | 2 | 3 | 4 | 4 | 324 | 35.9 | 9.88 |
| P19338 | Nucleolin OS=Homo sapiens GN=NCL PE=1 SV=3 - [NUCL_HUMAN] | 141.58 | 11.69 | 1 | 9 | 9 | 9 | 710 | 76.6 | 4.70 |
| P06748 | Nucleophosmin OS=Homo sapiens GN=NPM1 PE=1 SV=2 - [NPM_HUMAN] | 65.31 | 7.48 | 1 | 2 | 2 | 2 | 294 | 32.6 | 4.78 |
| P15531 | Nucleoside diphosphate kinase A OS=Homo sapiens GN=NME1 PE=1 SV=1 - [NDKA_HUMAN] | 68.18 | 21.05 | 3 | 3 | 3 | 3 | 152 | 17.1 | 6.19 |
| Q99733 | Nucleosome assembly protein 1-like 4 OS=Homo sapiens GN=NAP1L4 PE=1 SV=1 - [NP1L4_HUMAN] | 77.30 | 5.33 | 2 | 2 | 2 | 2 | 375 | 42.8 | 4.69 |
| P23284 | Peptidyl-prolyl cis-trans isomerase B OS=Homo sapiens GN=PPIB PE=1 SV=2 - [PPIB_HUMAN] | 153.57 | 23.61 | 1 | 5 | 5 | 6 | 216 | 23.7 | 9.41 |
| Q06830 | Peroxiredoxin-1 OS=Homo sapiens GN=PRDX1 PE=1 SV=1 - [PRDX1_HUMAN] | 33.78 | 10.55 | 1 | 2 | 2 | 2 | 199 | 22.1 | 8.13 |
| Q9Y285 | Phenylalanine--tRNA ligase alpha subunit OS=Homo sapiens GN=FARSA PE=1 SV=3 - [SYFA_HUMAN] | 84.00 | 8.07 | 1 | 4 | 4 | 4 | 508 | 57.5 | 7.80 |
| Q9NSD9 | Phenylalanine--tRNA ligase beta subunit OS=Homo sapiens GN=FARSB PE=1 SV=3 - [SYFB_HUMAN] | 36.37 | 3.40 | 1 | 2 | 2 | 2 | 589 | 66.1 | 6.84 |
| Q00325 | Phosphate carrier protein, mitochondrial OS=Homo sapiens GN=SLC25A3 PE=1 SV=2 - [MPCP_HUMAN] | 33.70 | 3.31 | 1 | 1 | 1 | 1 | 362 | 40.1 | 9.38 |
| Q8NC51 | Plasminogen activator inhibitor 1 RNA-binding protein OS=Homo sapiens GN=SERBP1 PE=1 SV=2 - [PAIRB_HUMAN] | 94.64 | 6.62 | 1 | 2 | 2 | 2 | 408 | 44.9 | 8.65 |
| Q15365 | Poly(rC)-binding protein 1 OS=Homo sapiens GN=PCBP1 PE=1 SV=2 - [PCBP1_HUMAN] | 91.47 | 7.87 | 2 | 3 | 3 | 3 | 356 | 37.5 | 7.09 |

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|---|---|---|---|---|---|---|---|---|---|---|
| Q9UHX1 | Poly(U)-binding-splicing factor PUF60 OS=Homo sapiens GN=PUF60 PE=1 SV=1 - [PUF60_HUMAN] | 259.06 | 22.72 | 1 | 11 | 11 | 14 | 559 | 59.8 | 5.29 |
| P0CG48 | Polyubiquitin-C OS=Homo sapiens GN=UBC PE=1 SV=3 - [UBC_HUMAN] | 71.41 | 32.85 | 4 | 2 | 2 | 2 | 685 | 77.0 | 7.66 |
| Q9UHV9 | Prefoldin subunit 2 OS=Homo sapiens GN=PFDN2 PE=1 SV=1 - [PFD2_HUMAN] | 29.75 | 5.84 | 1 | 1 | 1 | 1 | 154 | 16.6 | 6.58 |
| Q9H5Z1 | Probable ATP-dependent RNA helicase DHX35 OS=Homo sapiens GN=DHX35 PE=1 SV=2 - [DHX35_HUMAN] | 45.24 | 3.70 | 1 | 2 | 2 | 2 | 703 | 78.9 | 8.59 |
| O75340 | Programmed cell death protein 6 OS=Homo sapiens GN=PDCD6 PE=1 SV=1 - [PDCD6_HUMAN] | 131.22 | 24.61 | 1 | 5 | 5 | 6 | 191 | 21.9 | 5.40 |
| P12273 | Prolactin-inducible protein OS=Homo sapiens GN=PIP PE=1 SV=1 - [PIP_HUMAN] | 66.98 | 13.70 | 1 | 2 | 2 | 2 | 146 | 16.6 | 8.05 |
| Q06323 | Proteasome activator complex subunit 1 OS=Homo sapiens GN=PSME1 PE=1 SV=1 - [PSME1_HUMAN] | 220.28 | 34.94 | 1 | 7 | 7 | 7 | 249 | 28.7 | 6.02 |
| Q9UL46 | Proteasome activator complex subunit 2 OS=Homo sapiens GN=PSME2 PE=1 SV=4 - [PSME2_HUMAN] | 25.21 | 10.04 | 1 | 2 | 2 | 2 | 239 | 27.4 | 5.73 |
| P25786 | Proteasome subunit alpha type-1 OS=Homo sapiens GN=PSMA1 PE=1 SV=1 - [PSA1_HUMAN] | 110.45 | 15.97 | 1 | 4 | 4 | 5 | 263 | 29.5 | 6.61 |
| P25788 | Proteasome subunit alpha type-3 OS=Homo sapiens GN=PSMA3 PE=1 SV=2 - [PSA3_HUMAN] | 69.31 | 15.69 | 1 | 4 | 4 | 4 | 255 | 28.4 | 5.33 |
| P25789 | Proteasome subunit alpha type-4 OS=Homo sapiens GN=PSMA4 PE=1 SV=1 - [PSA4_HUMAN] | 57.66 | 7.28 | 1 | 2 | 2 | 2 | 261 | 29.5 | 7.72 |
| P28066 | Proteasome subunit alpha type-5 OS=Homo sapiens GN=PSMA5 PE=1 SV=3 - [PSA5_HUMAN] | 99.34 | 15.35 | 1 | 3 | 3 | 3 | 241 | 26.4 | 4.79 |
| P60900 | Proteasome subunit alpha type-6 OS=Homo sapiens GN=PSMA6 PE=1 SV=1 - [PSA6_HUMAN] | 145.10 | 22.76 | 1 | 5 | 5 | 6 | 246 | 27.4 | 6.76 |
| O14818 | Proteasome subunit alpha type-7 OS=Homo sapiens GN=PSMA7 PE=1 SV=1 - [PSA7_HUMAN] | 84.38 | 14.92 | 2 | 4 | 4 | 4 | 248 | 27.9 | 8.46 |
| P49721 | Proteasome subunit beta type-2 OS=Homo sapiens GN=PSMB2 PE=1 SV=1 - [PSB2_HUMAN] | 40.73 | 17.91 | 1 | 3 | 3 | 3 | 201 | 22.8 | 7.02 |
| P49720 | Proteasome subunit beta type-3 OS=Homo sapiens GN=PSMB3 PE=1 SV=2 - [PSB3_HUMAN] | 47.95 | 8.78 | 1 | 1 | 1 | 1 | 205 | 22.9 | 6.55 |
| P28070 | Proteasome subunit beta type-4 OS=Homo sapiens GN=PSMB4 PE=1 SV=4 - [PSB4_HUMAN] | 76.97 | 12.12 | 1 | 2 | 2 | 2 | 264 | 29.2 | 5.97 |
| P28074 | Proteasome subunit beta type-5 OS=Homo sapiens GN=PSMB5 PE=1 SV=3 - [PSB5_HUMAN] | 130.49 | 17.11 | 1 | 4 | 4 | 4 | 263 | 28.5 | 6.92 |
| P28072 | Proteasome subunit beta type-6 OS=Homo sapiens GN=PSMB6 PE=1 SV=4 - [PSB6_HUMAN] | 53.08 | 4.60 | 1 | 1 | 1 | 1 | 239 | 25.3 | 4.92 |
| Q99436 | Proteasome subunit beta type-7 OS=Homo sapiens GN=PSMB7 PE=1 SV=1 - [PSB7_HUMAN] | 80.00 | 11.19 | 1 | 3 | 3 | 3 | 277 | 29.9 | 7.68 |

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|---|---|---|---|---|---|---|---|---|---|---|
| O14744 | Protein arginine N-methyltransferase 5 OS=Homo sapiens GN=PRMT5 PE=1 SV=4 - [ANM5_HUMAN] | 35.77 | 2.67 | 1 | 2 | 2 | 2 | 637 | 72.6 | 6.29 |
| Q15084 | Protein disulfide-isomerase A6 OS=Homo sapiens GN=PDIA6 PE=1 SV=1 - [PDIA6_HUMAN] | 59.04 | 6.36 | 1 | 2 | 2 | 2 | 440 | 48.1 | 5.08 |
| Q99497 | Protein DJ-1 OS=Homo sapiens GN=PARK7 PE=1 SV=2 - [PARK7_HUMAN] | 312.71 | 45.50 | 1 | 8 | 8 | 15 | 189 | 19.9 | 6.79 |
| P31949 | Protein S100-A11 OS=Homo sapiens GN=S100A11 PE=1 SV=2 - [S10AB_HUMAN] | 41.55 | 8.57 | 1 | 1 | 1 | 1 | 105 | 11.7 | 7.12 |
| P06703 | Protein S100-A6 OS=Homo sapiens GN=S100A6 PE=1 SV=1 - [S10A6_HUMAN] | 37.10 | 8.89 | 1 | 1 | 1 | 1 | 90 | 10.2 | 5.48 |
| P31151 | Protein S100-A7 OS=Homo sapiens GN=S100A7 PE=1 SV=4 - [S10A7_HUMAN] | 68.20 | 18.81 | 1 | 1 | 1 | 1 | 101 | 11.5 | 6.77 |
| P05109 | Protein S100-A8 OS=Homo sapiens GN=S100A8 PE=1 SV=1 - [S10A8_HUMAN] | 58.96 | 20.43 | 1 | 2 | 2 | 3 | 93 | 10.8 | 7.03 |
| P06702 | Protein S100-A9 OS=Homo sapiens GN=S100A9 PE=1 SV=1 - [S10A9_HUMAN] | 48.61 | 20.18 | 1 | 2 | 2 | 2 | 114 | 13.2 | 6.13 |
| Q9BRJ7 | Protein syndesmos OS=Homo sapiens GN=NUDT16L1 PE=1 SV=1 - [SDOS_HUMAN] | 204.48 | 35.07 | 2 | 6 | 6 | 7 | 211 | 23.3 | 8.91 |
| Q15436 | Protein transport protein Sec23A OS=Homo sapiens GN=SEC23A PE=1 SV=2 - [SC23A_HUMAN] | 65.47 | 4.18 | 1 | 3 | 3 | 3 | 765 | 86.1 | 7.08 |
| Q15437 | Protein transport protein Sec23B OS=Homo sapiens GN=SEC23B PE=1 SV=2 - [SC23B_HUMAN] | 71.65 | 3.00 | 1 | 1 | 2 | 2 | 767 | 86.4 | 6.89 |
| P61619 | Protein transport protein Sec61 subunit alpha isoform 1 OS=Homo sapiens GN=SEC61A1 PE=1 SV=2 - [S61A1_HUMAN] | 37.10 | 4.20 | 2 | 2 | 2 | 2 | 476 | 52.2 | 8.06 |
| Q5VSP4 | Putative lipocalin 1-like protein 1 OS=Homo sapiens GN=LCN1P1 PE=5 SV=1 - [LC1L1_HUMAN] | 51.03 | 6.79 | 2 | 1 | 1 | 1 | 162 | 17.9 | 5.00 |
| P14618 | Pyruvate kinase PKM OS=Homo sapiens GN=PKM PE=1 SV=4 - [KPYM_HUMAN] | 151.89 | 10.92 | 1 | 6 | 6 | 6 | 531 | 57.9 | 7.84 |
| P31153 | S-adenosylmethionine synthase isoform type-2 OS=Homo sapiens GN=MAT2A PE=1 SV=1 - [METK2_HUMAN] | 59.58 | 6.58 | 1 | 2 | 2 | 2 | 395 | 43.6 | 6.48 |
| P02810 | Salivary acidic proline-rich phosphoprotein 1/2 OS=Homo sapiens GN=PRH1 PE=1 SV=2 - [PRPC_HUMAN] | 27.74 | 10.24 | 1 | 1 | 1 | 1 | 166 | 17.0 | 4.84 |
| Q9UHR5 | SAP30-binding protein OS=Homo sapiens GN=SAP30BP PE=1 SV=1 - [S30BP_HUMAN] | 119.60 | 11.36 | 1 | 3 | 3 | 3 | 308 | 33.8 | 4.84 |
| P10398 | Serine/threonine-protein kinase A-Raf OS=Homo sapiens GN=ARAF PE=1 SV=2 - [ARAF_HUMAN] | 26.62 | 1.49 | 2 | 1 | 1 | 1 | 606 | 67.5 | 9.01 |
| P30153 | Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform OS=Homo sapiens GN=PPP2R1A PE=1 SV=4 - [2AAA_HUMAN] | 92.04 | 6.96 | 1 | 4 | 4 | 5 | 589 | 65.3 | 5.11 |

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|---|---|---|---|---|---|---|---|---|---|---|
| P62714 | Serine/threonine-protein phosphatase 2A catalytic subunit beta isoform OS=Homo sapiens GN=PPP2CB PE=1 SV=1 - [PP2AB_HUMAN] | 42.66 | 3.56 | 2 | 1 | 1 | 1 | 309 | 35.6 | 5.43 |
| P02768 | Serum albumin OS=Homo sapiens GN=ALB PE=1 SV=2 - [ALBU_HUMAN] | 54.07 | 4.11 | 1 | 2 | 2 | 2 | 609 | 69.3 | 6.28 |
| P37108 | Signal recognition particle 14 kDa protein OS=Homo sapiens GN=SRP14 PE=1 SV=2 - [SRP14_HUMAN] | 38.45 | 36.03 | 1 | 3 | 3 | 4 | 136 | 14.6 | 10.04 |
| O60232 | Sjoegren syndrome/scleroderma autoantigen 1 OS=Homo sapiens GN=SSSCA1 PE=1 SV=1 - [SSA27_HUMAN] | 55.51 | 11.56 | 1 | 2 | 2 | 2 | 199 | 21.5 | 5.24 |
| P62306 | Small nuclear ribonucleoprotein F OS=Homo sapiens GN=SNRPF PE=1 SV=1 - [RUXF_HUMAN] | 50.92 | 13.95 | 1 | 1 | 1 | 1 | 86 | 9.7 | 4.67 |
| P62316 | Small nuclear ribonucleoprotein Sm D2 OS=Homo sapiens GN=SNRPD2 PE=1 SV=1 - [SMD2_HUMAN] | 28.31 | 16.10 | 1 | 2 | 2 | 2 | 118 | 13.5 | 9.91 |
| P14678 | Small nuclear ribonucleoprotein-associated proteins B and B' OS=Homo sapiens GN=SNRPB PE=1 SV=2 - [RSMB_HUMAN] | 55.74 | 6.67 | 2 | 2 | 2 | 2 | 240 | 24.6 | 11.19 |
| Q15459 | Splicing factor 3A subunit 1 OS=Homo sapiens GN=SF3A1 PE=1 SV=1 - [SF3A1_HUMAN] | 27.16 | 3.15 | 1 | 2 | 2 | 2 | 793 | 88.8 | 5.22 |
| O75533 | Splicing factor 3B subunit 1 OS=Homo sapiens GN=SF3B1 PE=1 SV=3 - [SF3B1_HUMAN] | 37.73 | 2.76 | 1 | 2 | 2 | 2 | 1304 | 145.7 | 7.09 |
| P38646 | Stress-70 protein, mitochondrial OS=Homo sapiens GN=HSPA9 PE=1 SV=2 - [GRP75_HUMAN] | 180.97 | 7.95 | 1 | 6 | 6 | 6 | 679 | 73.6 | 6.16 |
| P31040 | Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial OS=Homo sapiens GN=SDHA PE=1 SV=2 - [SDHA_HUMAN] | 44.44 | 1.66 | 1 | 1 | 1 | 1 | 664 | 72.6 | 7.39 |
| Q6UWP8 | Suprabasin OS=Homo sapiens GN=SBSN PE=2 SV=2 - [SBSN_HUMAN] | 46.36 | 9.15 | 1 | 1 | 1 | 1 | 590 | 60.5 | 7.01 |
| P78371 | T-complex protein 1 subunit beta OS=Homo sapiens GN=CCT2 PE=1 SV=4 - [TCPB_HUMAN] | 54.99 | 4.11 | 1 | 2 | 2 | 2 | 535 | 57.5 | 6.46 |
| P50991 | T-complex protein 1 subunit delta OS=Homo sapiens GN=CCT4 PE=1 SV=4 - [TCPD_HUMAN] | 42.73 | 1.48 | 1 | 1 | 1 | 1 | 539 | 57.9 | 7.83 |
| P48643 | T-complex protein 1 subunit epsilon OS=Homo sapiens GN=CCT5 PE=1 SV=1 - [TCPE_HUMAN] | 80.74 | 3.70 | 1 | 2 | 2 | 2 | 541 | 59.6 | 5.66 |
| Q99832 | T-complex protein 1 subunit eta OS=Homo sapiens GN=CCT7 PE=1 SV=2 - [TCPH_HUMAN] | 45.65 | 2.95 | 1 | 2 | 2 | 2 | 543 | 59.3 | 7.65 |
| P49368 | T-complex protein 1 subunit gamma OS=Homo sapiens GN=CCT3 PE=1 SV=4 - [TCPG_HUMAN] | 71.12 | 5.87 | 1 | 3 | 3 | 3 | 545 | 60.5 | 6.49 |
| P50990 | T-complex protein 1 subunit theta OS=Homo sapiens GN=CCT8 PE=1 SV=4 - [TCPQ_HUMAN] | 82.04 | 10.77 | 1 | 6 | 6 | 6 | 548 | 59.6 | 5.60 |

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|---|---|---|---|---|---|---|---|---|---|---|
| P40227 | T-complex protein 1 subunit zeta OS=Homo sapiens GN=CCT6A PE=1 SV=3 - [TCPZ_HUMAN] | 70.60 | 3.95 | 1 | 1 | 1 | 1 | 531 | 58.0 | 6.68 |
| Q86V81 | THO complex subunit 4 OS=Homo sapiens GN=ALYREF PE=1 SV=3 - [THOC4_HUMAN] | 57.10 | 15.95 | 1 | 2 | 2 | 2 | 257 | 26.9 | 11.15 |
| Q15369 | Transcription elongation factor B polypeptide 1 OS=Homo sapiens GN=TCEB1 PE=1 SV=1 - [ELOC_HUMAN] | 33.41 | 8.93 | 1 | 1 | 1 | 1 | 112 | 12.5 | 4.78 |
| Q15370 | Transcription elongation factor B polypeptide 2 OS=Homo sapiens GN=TCEB2 PE=1 SV=1 - [ELOB_HUMAN] | 24.48 | 6.78 | 1 | 1 | 1 | 1 | 118 | 13.1 | 4.88 |
| P55072 | Transitional endoplasmic reticulum ATPase OS=Homo sapiens GN=VCP PE=1 SV=4 - [TERA_HUMAN] | 46.08 | 2.48 | 2 | 2 | 2 | 2 | 806 | 89.3 | 5.26 |
| P29401 | Transketolase OS=Homo sapiens GN=TKT PE=1 SV=3 - [TKT_HUMAN] | 35.40 | 4.98 | 1 | 2 | 2 | 3 | 623 | 67.8 | 7.66 |
| Q9Y3I0 | tRNA-splicing ligase RtcB homolog OS=Homo sapiens GN=RTCB PE=1 SV=1 - [RTCB_HUMAN] | 37.29 | 2.18 | 1 | 1 | 1 | 1 | 505 | 55.2 | 7.23 |
| P07951 | Tropomyosin beta chain OS=Homo sapiens GN=TPM2 PE=1 SV=1 - [TPM2_HUMAN] | 72.85 | 4.58 | 2 | 1 | 1 | 1 | 284 | 32.8 | 4.70 |
| P07477 | Trypsin-1 OS=Homo sapiens GN=PRSS1 PE=1 SV=1 - [TRY1_HUMAN] | 368.66 | 12.15 | 3 | 2 | 2 | 15 | 247 | 26.5 | 6.51 |
| P35030 | Trypsin-3 OS=Homo sapiens GN=PRSS3 PE=1 SV=2 - [TRY3_HUMAN] | 88.09 | 4.28 | 1 | 1 | 1 | 3 | 304 | 32.5 | 7.49 |
| Q9BQE3 | Tubulin alpha-1C chain OS=Homo sapiens GN=TUBA1C PE=1 SV=1 - [TBA1C_HUMAN] | 185.60 | 28.95 | 9 | 10 | 10 | 10 | 449 | 49.9 | 5.10 |
| P07437 | Tubulin beta chain OS=Homo sapiens GN=TUBB PE=1 SV=2 - [TBB5_HUMAN] | 239.15 | 28.38 | 7 | 2 | 9 | 10 | 444 | 49.6 | 4.89 |
| P68371 | Tubulin beta-4B chain OS=Homo sapiens GN=TUBB4B PE=1 SV=1 - [TBB4B_HUMAN] | 209.93 | 28.31 | 6 | 2 | 9 | 10 | 445 | 49.8 | 4.89 |
| P09661 | U2 small nuclear ribonucleoprotein A' OS=Homo sapiens GN=SNRPA1 PE=1 SV=2 - [RU2A_HUMAN] | 66.64 | 5.49 | 1 | 1 | 1 | 1 | 255 | 28.4 | 8.62 |
| Q9Y224 | UPF0568 protein C14orf166 OS=Homo sapiens GN=C14orf166 PE=1 SV=1 - [CN166_HUMAN] | 31.58 | 3.69 | 1 | 1 | 1 | 1 | 244 | 28.1 | 6.65 |
| Q9BRX9 | WD repeat domain-containing protein 83 OS=Homo sapiens GN=WDR83 PE=1 SV=1 - [WDR83_HUMAN] | 42.91 | 3.81 | 1 | 1 | 1 | 3 | 315 | 34.3 | 5.58 |
| P16989 | Y-box-binding protein 3 OS=Homo sapiens GN=YBX3 PE=1 SV=4 - [YBOX3_HUMAN] | 49.66 | 7.26 | 2 | 1 | 2 | 2 | 372 | 40.1 | 9.77 |

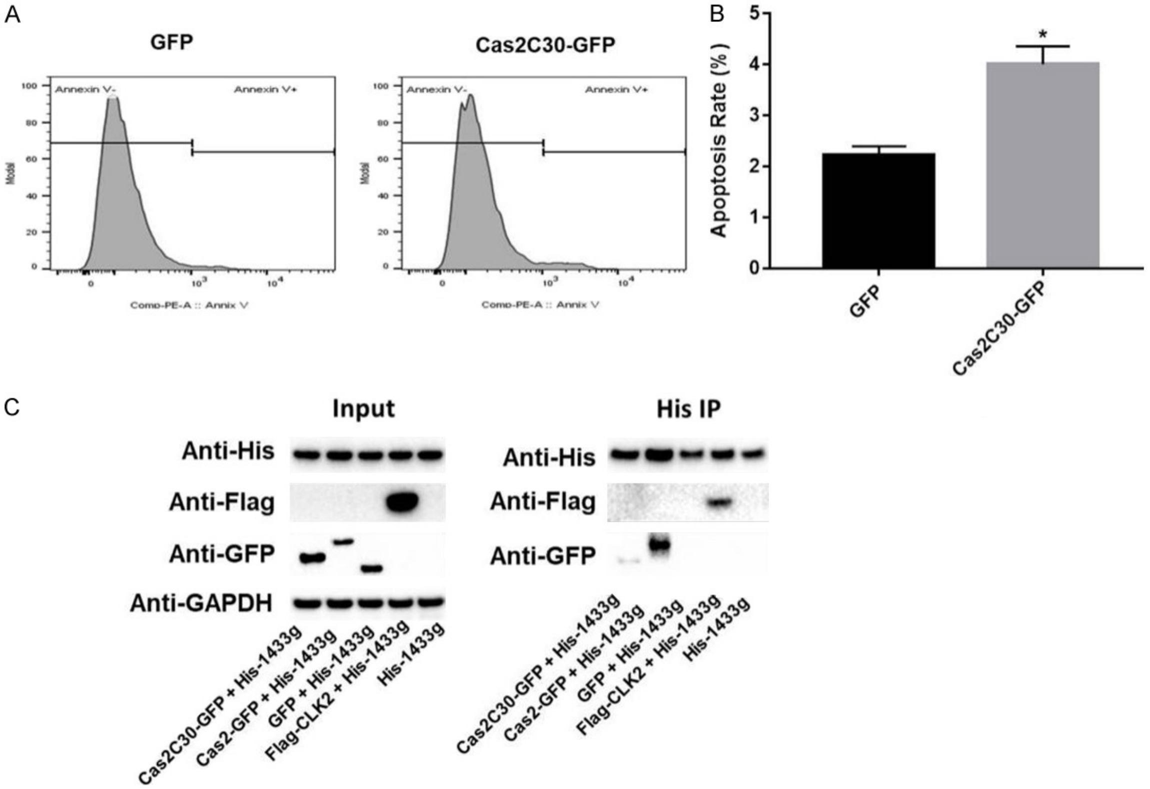

**Figure 5.** C-terminal of Cas2Em induced growth inhibition of Hela cells through interacting with 14-3-3γ. A, B. The apoptosis rate in Hela cells was detected by FACS after staining with Annexin V. X axis: the level of Annexin-V PE fluorescence; Y axis: count (%). C. Hela cells were co-transfected with His-14-3-3γ and Cas2C30-GFP or Cas2-GFP or GFP or Flag-CLK2 for 24 h, and the cell lysates were applied to immunoprecipitation with His-NTA. Then, expressions of Flag CLK2, His and GFP (Cas2-GFP, Cas2C30-GFP and GFP) in Hela cells were detected by western-blot. Each group were performed at least three independent experiments and all data were expressed as the mean ± standard deviation (SD). $^{**}P<0.01$.

rescent staining revealed co-localization of 14-3-3γ and Cas2Em proteins in Hela cells was observed (**Figure 4B**). To verify this result, His-tagged 14-3-3γ was co-expressed with Cas2Em in Hela cells and co-immunoprecipitation assay was used. The result indicated that Cas2Em and 14-3-3γ shared the same protein complex in Hela cells (**Figure 4C**). All these results demonstrated that Cas2Em physically interacted with 14-3-3γ in mammalian cells.

### *C-terminal of Cas2Em (Cas2C30) was involved in growth inhibition of Hela cells through interacting with 14-3-3γ*

The amino acid alignment was performed to explore the mechanism by which Cas2Em exhibited its inhibitory effect on cell growth. As revealed in Figure S1, a homologous domain of human CLK2 was found in C-terminal of Cas2Em (Cas2C30). This data suggested that C-terminal of Cas2Em was involved in the inhibition of mammalian cell growth. As expected, the apoptosis of Hela cells was significantly enhanced by C-terminal of Cas2Em (**Figure 5A**, **5B**). Moreover, 14-3-3γ interacted with the full-length and the C-terminal part of Cas2Em (CLK2) (**Figure 5C**). All these data revealed that C-terminal of Cas2Em was involved in growth inhibition of Hela cells through interacting with 14-3-3γ.

### *Cas2C30 induced G1 arrest in CHO-K1 and Hela cells*

Finally, flow cytometry was used to detect the effect of Cas2C30 on cell cycle. As showed in **Figure 6A-D**, Cas2C30 significantly induced the G1 arrest in CHO-K1 and Hela cells. These results further confirmed that C-terminal of Cas2Em was involved in growth inhibition of CHO-K1 and Hela cells.

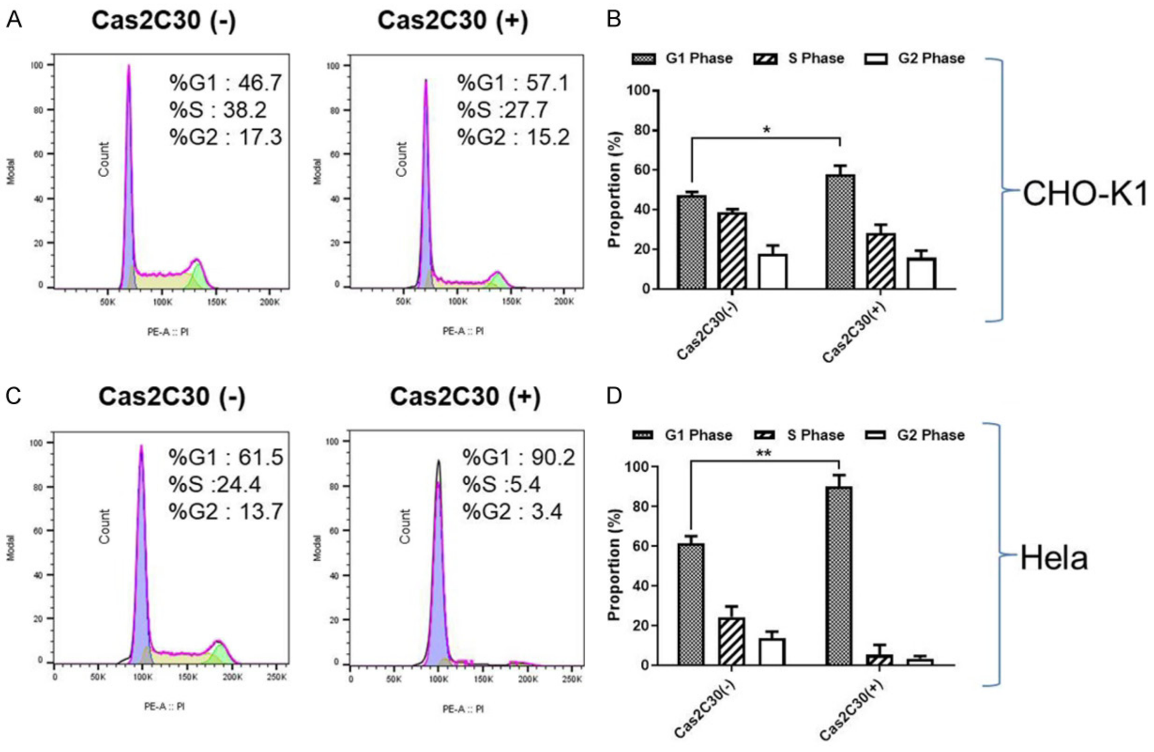

**Figure 6.** C-terminal of Cas2Em induced G1 arrest in CHO-K1 and Hela cells. A, B. The cell cycle distribution (G0/G1, S, and G2 phase) in CHO-K1 cells transfected with Cas2-GFP for 24 h were determined by FACS. C, D. The cell cycle distribution (G0/G1, S, and G2 phase) in Hela cells transfected with Cas2-GFP for 24 h were determined by FACS. Each group were performed at least three independent experiments and all data were expressed as the mean ± standard deviation (SD). $^{*}P<0.05$, $^{**}P<0.01$.

## Discussion

In this research, we firstly found that Cas2Em could inhibit the growth of mammalian cells. In addition, it could induce G1-arrest and apoptosis in Hela cells. Moreover, we found Cas2Em significantly suppressed the proliferation of Hela cells. Wang L et al found that Cas2 could inhibit the proliferation of oral squamous cells [16]. Our finding was similar to this result, suggesting that Cas2Em could be an anti-tumor agent which may be used as a potential novel target for the treatment of cancer.

In immunoprecipitation and immunofluorescence staining, we found that Cas2Em physically interacted with 14-3-3γ. 14-3-3γ was a member of 14-3-3 protein family. It has been reported that the 14-3-3 protein family comprises a group of small, dimeric, acidic proteins that regulate multiple cellular pathways [17, 18]. Additionally, 14-3-3 proteins have been regarded to bind to ligands (cdc25C, Raf1 or IRS-1) via two consensus motifs (modes I and II) interacting with 14-3-3 [19-21]. Hiraoka E et al indicated that the role of 14-3-3γ in breast cancer invasiveness might be to promote cell motility [22]. Our data were consistent to these studies indicating that Cas2Em could inhibit cancer cells growth via interacting 14-3-3γ. Besides, Hosing AS et al found that the override of checkpoint observed in 14-3-3γ knockdown cells was due to failure to inhibit cdc25C function [23]. This data supported our findings that Cas2Em could significantly induce G1 arrest in cancer cells [10]. Altogether, our findings indicated that Cas2Em suppressed mammalian cell growth via interacting 14-3-3γ.

In addition, Jin J et al has reported that CLK2 is an interacting protein of 14-3-3γ in an earlier human proteomic study [24]. Our study firstly demonstrated that CLK2 was co-immunoprecipitaed by 14-3-3γ. Our study also found that C-terminal part of Cas2Em (a homologous domain of human CLK2) could induce the apoptosis of Hela cells. These data suggested that Cas2Em might inhibit the tumorigenesis via interaction with 14-3-3γ and CLK2. Based on these results, we will further confirm the role of CLK2 during the tumorigenesis. Meanwhile, it has been reported that Cdc2-like kinase 2 (CLK2) could act as a key regulator of the cell cycle via FOXO3a/p27 in glioblastoma [25]. Yoshida T et al found that CLK2 was an oncogenic kinase and splicing regulator in breast cancer [26]. Moreover, Salvador F et al found that CLK2 blockade could compromise MYC-driven breast tumors via modulating alternative splicing [27]. The CoIP result that C-terminal of Cas2Em interacted 14-3-3γ also demonstrated that C-terminal of Cas2Em was a necessary domain for its function. These results were consistent with our study that C-terminal of Cas2Em was involved in growth inhibition of Hela cells through interacting with 14-3-3γ. Since Shen Q found that 14-3-3γ overexpression could modify AKT-Foxo signaling [28], we will further investigate the effect of Cas2Em on AKT-Foxo pathway in future.

In our previous study, we found that Cas2 might be a key factor for polyploid giant bactetial cells (PGBC) to control the risks of genomic reprogramming [10]. Giant cells with similar function including eggs for reproduction and polyploid giant cancer cells (PGCC) in tumor, which were observed in various biological systems [29, 30]. Moreover, we indicated that Cas2 induced a G1/S arresting in mammalian cells in this research. The impact of Cas2Em protein on polyploidy formation and genomic reprogramming of host cells is an interesting open field.

In summary, Cas2Em could suppress mammalian cell growth via interacting 14-3-3γ, which may serve as a novel potential target for the treatment of cancer.

## Disclosure of conflict of interest

None.

**Address correspondence to:** Li Chen, Department of Medical Microbiology, Key Laboratory of Medical Molecular Virology of Ministries of Education and Health, School of Basic Medical Sciences, Fudan University, 131 Dongan Road, Shanghai 200032, China. E-mail: lichen_bk@fudan.edu.cn

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Alignment

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P49760 CLK2_HUMAN      1    MPHPRRYHSSERGSRGSYREHYRSRKHKRRRSRSWSSSSDRTRRRRREDSYHVRSRSSYD      60
Cas2Em                 1    ------------------------------------------------------------       0

P49760 CLK2_HUMAN     61    DRSSDRRVYDRRYCGSYRRNDYSRDRGDAYYDTDYRHSYEYQRENSSYRSQRSSRRKHRR     120
Cas2Em                 1    ------------------------------------------------------------       0

P49760 CLK2_HUMAN    121    RRRRSRTFSRSSSQHSSRRAKSVEDDAEGHLIYHVGDWLQERYEIVSTLGEGTFGRVVQC     180
Cas2Em                 1    ------------------------------------MWVLVLYDLPTETKENMRDANLFR      24
                                                                 *:   *:: :   *.  .  :

P49760 CLK2_HUMAN    181    VDHRRGGA-RVALKIIKNVEKYKEAARLEINVLEKINEKDPDNKNLCVQMFDWFDYHGHM     239
Cas2Em                25    KRLLDDGFSLFQFSMYIRHCPSRENAEVHIKRVKV--------------MLPKAGKVAIM      70
                                 .*   . :.:  .    :* *.:.*: ::               *:   .  . *

P49760 CLK2_HUMAN    240    CISFELLGLSTFDFLKDNNYLPYPIHQVRHMAFQLCQAVKFLHDNKLTHTDLKPENILFV     299
Cas2Em                71    CITDKQFGDIE-------------------------------------------------      81
                            **: : :*

P49760 CLK2_HUMAN    300    NSDYELTYNLEKKRDERSVKSTAVRVVDFGSATFDHEHHSTIVSTRHYRAPEVILELGWS     359
Cas2Em                82    ------------------------------------------------------------      81

P49760 CLK2_HUMAN    360    QPCDVWSIGCIIFEYYVGFTLFQTHDNREHLAMMERILGPIPSRMIRKTRKQKYFYRGRL     419
Cas2Em                82    ------------------------------------------------------------      81

P49760 CLK2_HUMAN    420    DWDENTSAGRYVRENCKPLRRYLTSEAEEHHQLFDLIESMLEYEPAKRLTLGEALQHPFF     479
Cas2Em                82    ---------------------------------------------------------IFF      84
                                                                                      **

P49760 CLK2_HUMAN    480    ARLRAEPPNKLWDSSRDISR                                             499
Cas2Em                85    ARNKEEPPPTFQQLELF---                                             101
                            ** : *** .: : .
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**Figure S1.** A homologous domain of human CLK2 was found in C-terminal of Cas2Em through the amino acid alignment.