

**Targeted Downregulation of s36 Protein Unearths its Cardinal Role
in Chorion Biogenesis and Architecture during *Drosophila*
melanogaster Oogenesis**

**Athanassios D. Velentzas¹, Panagiotis D. Velentzas^{1,2}, Niki E. Sagioglou¹,
Eumorphia G. Konstantakou^{1,3}, Athanasios K. Anagnostopoulos⁴, Maria M.
Tsioka¹, Vassiliki E. Mpakou¹, Zoe Kollia⁵, Christos Consoulas⁶, Lukas H.
Margaritis¹, Issidora S. Papassideri¹, George Th. Tsangaris⁴, Evangelia
Sarantopoulou⁵, Alkiviadis-Constantinos Cefalas⁵ and Dimitrios J. Stravopodis^{1,*}**

¹Section of Cell Biology and Biophysics, Department of Biology, School of Science, National and Kapodistrian University of Athens (NKUA), Athens, Greece

²Present Address: Department of Cancer Biology, Medical School, University of Massachusetts, Worcester, Massachusetts (MA), USA

³Present Address: Department of Hematology and Medical Oncology, Winship Cancer Institute of Emory, Emory University School of Medicine, Atlanta, Georgia (GA), USA

⁴Proteomics Core Facility, Systems Biology Center, Biomedical Research Foundation of the Academy of Athens (BRFAA), Athens, Greece

⁵Theoretical and Physical Chemistry Institute, National Hellenic Research Foundation (NHRF), Athens, Greece

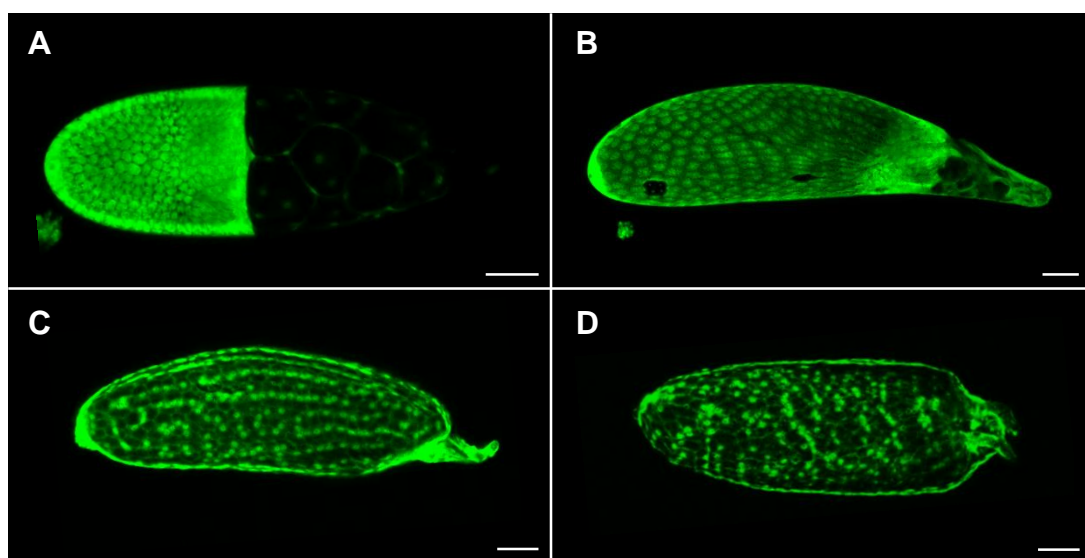
⁶Laboratory of Experimental Physiology, Medical School, National and Kapodistrian University of Athens (NKUA), Athens, Greece

***Corresponding Author:**

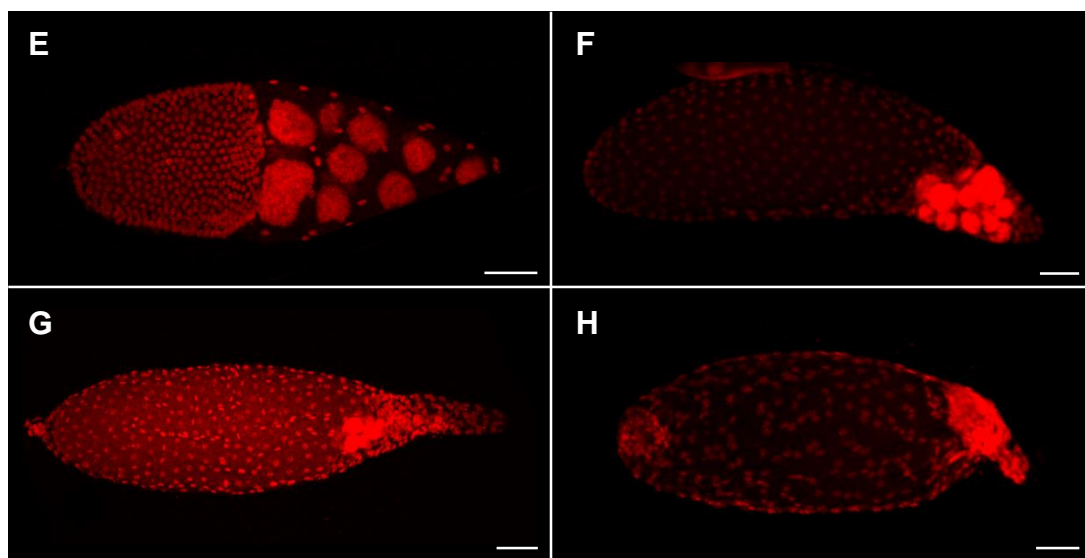
Dimitrios J. Stravopodis, *BSc, MSc, PhD*, Assistant Professor, Section of Cell Biology and Biophysics, Department of Biology, School of Science, National and Kapodistrian University of Athens (NKUA), Panepistimiopolis, Zografou 157 84, Athens, Greece (GR). Tel.: +30 210 727 4105, Fax: +30 210 727 4742, E-mail: dstravop@biol.uoa.gr, URL: <http://www.biol.uoa.gr>

Running Title: Indispensable Contribution of s36 Protein to Chorion Morphogenesis

c355>eGFP,s36_RNAi

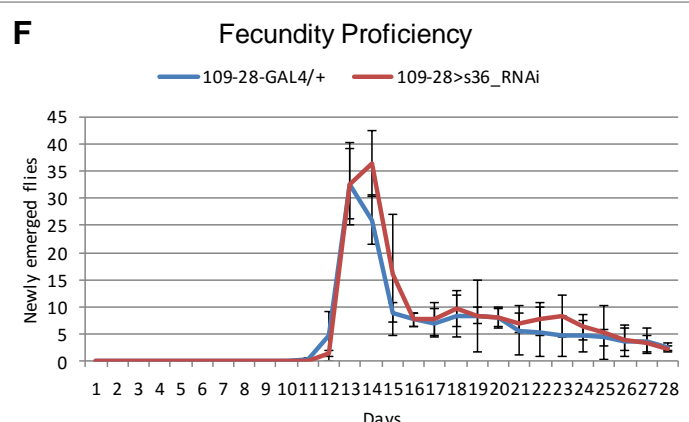
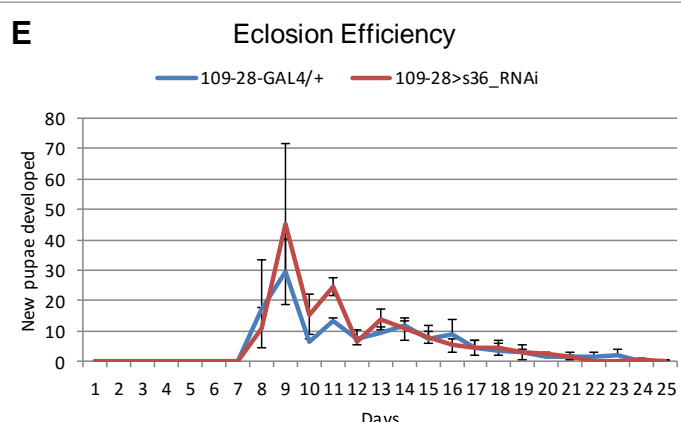
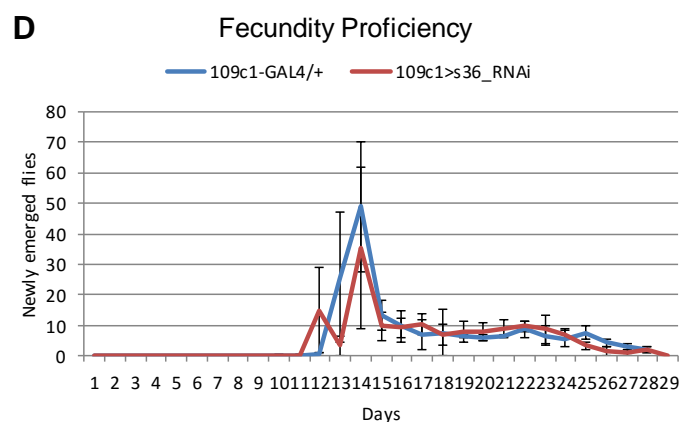
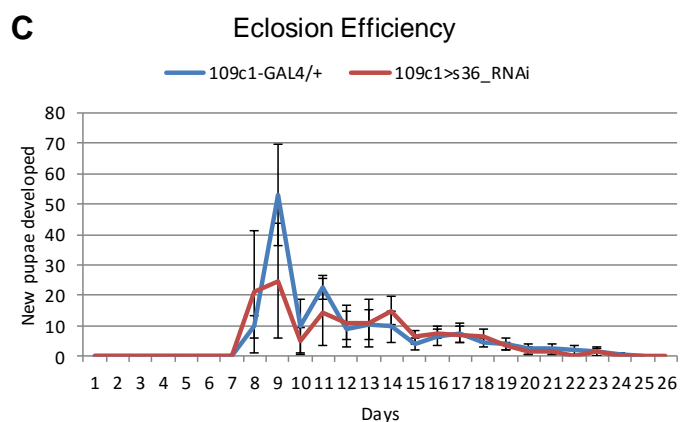
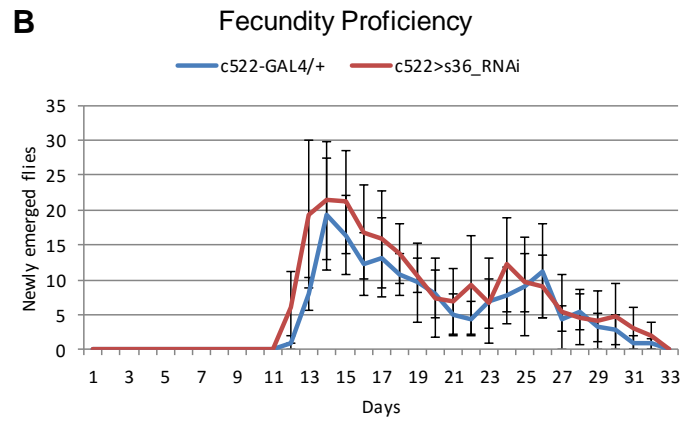
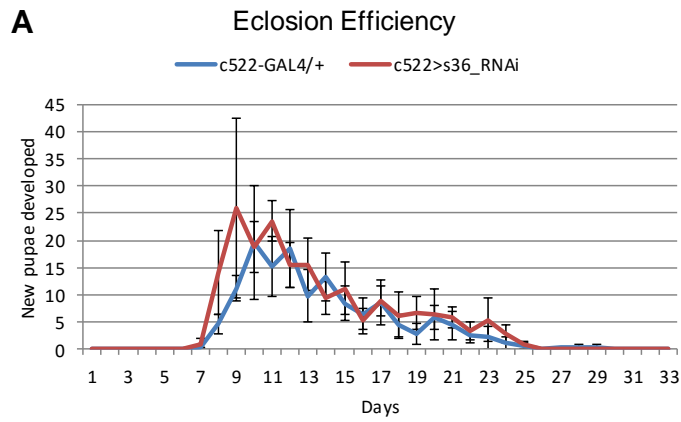


c355>s36_RNAi



Supplementary Figure S1

The developmental courses of follicle- and nurse-cell clusters remain unaffected in the absence of s36 chorionic protein. (A-D) Confocal laser scanning microscopy (CLSM) images of eGFP-protein expression profiling in the s36-depleted follicle-cell cluster of triple transgenic (*c355>eGFP,s36_RNAi*) ovarian follicles at developmental stage 10 (A), 13 (B) and 14 (C and D). (E-H) CLSM imaging of PI nuclear staining of follicle- and nurse-cell compartments in the s36-targeted follicles (*c355>s36_RNAi*) at developmental stage 10 (E), 12 (F), 13 (G) and 14 (H). Scale Bars: 50 μm .



Supplementary Figure S2

Genetic targeting of s36 chorionic protein in regionally specialized follicle-cell subpopulations does not harm typical eclosion capacity and fecundity integrity.

Eclosion-efficiency (A, C and E) and fecundity-proficiency (B, D and F) graphs of flies being targeted for the s36 chorionic protein specifically in follicular border cells (c522>s36_RNAi) (A and B), (anterior and posterior) polar cells (109c1>s36_RNAi) (C and D), or follicle cells of dorsal appendages (109-28>s36_RNAi) (E and F). Note the significant similarities with their respective control (c522-GAL4/+, 109c1-GAL4/+ and 109-28-GAL4/+) fly strains.

Gene Name	Primer Sequence	Molecular Size (bp) of PCR Product	Ta
Chorion protein 36 (CG1478)	F: 5'-CAA CTC GGT CTC TGG TTT GG-3'	195	57°C
	R: 5'-CTG TGA GGC GAT CTG ATT GG -3'		
Chorion protein 38 (CG11213)	F: 5'-CGA GAT CGA CCT ACA TTT G-3'	213	53°C
	R: 5'-ATT CGA GAG CAC CAG CAC C-3'		
Chorion protein 18 (CG6517)	F: 5'-GCC AAT GTG GGA TCT CAG TA-3'	195	55°C
	R: 5'-GCT GTG TCC GTA CTC CTG GT-3'		
Chorion protein 16 (CG6533)	F: 5'-AAG CTG GAC GGT GCT GAC T-3'	201	53°C
	R: 5'-GGT CTT CAG GTA CGC ATT C-3'		
Chorion protein 19 (CG6524)	F: 5'-GCT ACA ACG GTG GCA ACT A-3'	196	53°C
	R: 5'-CCA TAC TGG TTC TCT GGG A-3'		
Chorion protein 15 (CG6519)	F: 5'-AGG TTA TGG TGG TGG CTA CG-3'	190	55°C
	R: 5'-CTT GTA GGT TCC GGG ATT CA-3'		

Supplementary Table S1

Gene name, oligonucleotide primer sequence (F: forward; R: reverse), molecular size (bp) and annealing temperature (Ta) of the amplified PCR products for the herein examined genes

Supplementary Table S2 (a separate Microsoft Excel format file)

Protein library (N = 2,131 members) of s36-depleted fly ovaries (c355>s36_RNAi) indicating for each identified protein its: (a) UniProt accession number, (b) name and description together with fundamental features of LC-MS/MS proteomics analysis, such as (c) (Mascot) score, (d) sequence coverage, (e) number of unique peptides (n = 17,772 tryptic fragments), (f) number of amino-acid residues (AAs), (g) molecular weight (MW) in kDa and (h) (calculated) isoelectric point (pI)