

**Secretome of *in vitro* cultured human embryos contains extracellular vesicles that are uptaken
by the maternal side**

**Elisa Giacomini¹, Riccardo Vago^{2,3}, Ana Maria Sanchez¹, Paola Podini⁴, Natasa Zarovni⁵,
Valentina Murdica², Roberta Rizzo⁶, Daria Bortolotti⁶,
Massimo Candiani^{3,7} and Paola Viganò^{1,*}**

¹Reproductive Sciences Laboratory, Division of Genetics and Cell Biology, IRCCS San Raffaele Scientific Institute, Milano, 20132, Italy.

²Urological Research Institute, IRCCS Ospedale San Raffaele, Milan, 20132, Italy

³Università Vita-Salute San Raffaele, Milan, 20132, Italy

⁴Department of Neuroscience, Institute of Experimental Neurology, IRCCS San Raffaele Scientific Institute, 20132 Milan, Italy

⁵Exosomics Siena S.p.A, Siena, 53100, Italy

⁶Department of Medical Sciences, Section of Microbiology and Medical Genetics, University of Ferrara, 44121, Ferrara, Italy.

⁷Obstetrics and Gynecology Unit, IRCCS San Raffaele Scientific Institute, Milano, 20132, Italy.

Corresponding author*:

Viganò Paola, PhD

Reproductive Sciences Laboratory, Division of Genetics and Cell Biology, IRCCS San Raffaele

Scientific Institute,

Via Olgettina, 60, 20132, Milano, Italy

Tel: +39 02 2346 6228

Fax: + 39 02 26434311

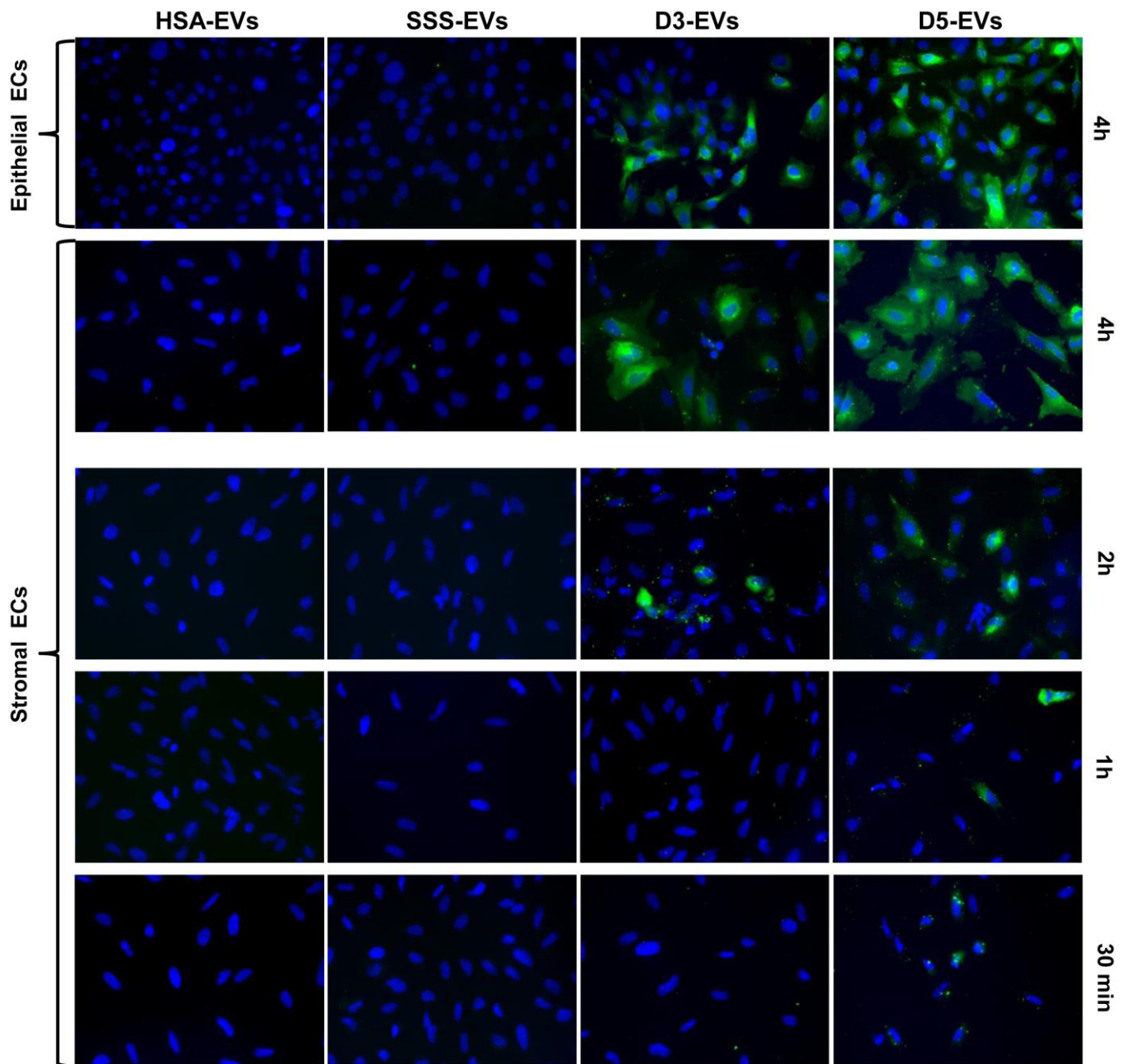
E-mail: vigano.paola@hsr.it

SUPPLEMENTARY INFORMATION

Supplementary Table S1. Sequences of primers used in this study.

Gene	Sequence (5'-3')	Product size (bp)
<i>ACTB Forward</i>	GGCACCCAGCACAATGAAG	66
<i>ACTB Reverse</i>	CCGATCCACACGGAGTACTTG	
<i>NANOG Forward</i>	ACCTTGGCTGCCGTCTCTGG	151
<i>NANOG Reverse</i>	TGTTTGGGATTGGGAGGCTTTGCT	
<i>POU5F1 Forward</i>	TTTTGGTACCCCAGGCTATG	136
<i>POU5F1 Reverse</i>	ATTCAAAGTGAAGTGCCTGC	
<i>SOX2 Forward</i>	GAGCTTTGCAGGAAGTTTGC	190
<i>SOX2 Reverse</i>	GCAAGAAGCCTCTCCTTGAA	
<i>KLF4 Forward</i>	GCAGCCACCTGGCGAGTCTG	130
<i>KLF4 Reverse</i>	CCCCGAATAACCGCTGGCGG	

Supplementary Figure S1



Supplementary Figure S1. Epithelial and stromal ECs can incorporate embryo-derived EVs. Representative images of primary epithelial and stromal ECs treated for 4 hours with 10 $\mu\text{g}/\text{ml}$ of Vybrant DiO-labelled EVs derived from 5% HSA-supplemented fresh medium (HSA-EVs), from 10% SSS-supplemented fresh media (SSS-EVs) and from conditioned media of day 3 embryos (D3-EVs) and of day 5 embryos (D5-EVs). Endometrial stromal cells were also treated for different time (30 minutes, 1, 2 or 4 hours) with 10 $\mu\text{g}/\text{ml}$ of Vybrant DiO-labelled EVs derived from 5% HSA-supplemented fresh medium (HSA-EVs), from 10% SSS-supplemented fresh media (SSS-EVs) and from conditioned media of day 3 embryos (D3-EVs) and of day 5 embryos (D5-EVs) with D3-EVs and D5-EVs. Blue = nuclei.