

Guide Cells Support Muscle Regeneration and Affect Neuro-Muscular Junction Organization

Flavio L. Ronzoni^{1,2,†}, Nefele Giarratana^{3,4,†}, Stefania Crippa⁵, Mattia Quattrocelli⁶, Marco Cassano⁷, Gabriele Ceccarelli¹, Laura Benedetti¹, Jens Van Herck⁸, Maria Gabriella Cusella De Angelis¹, Marco Vitale⁹, Daniela Galli^{9,*} and Maurilio Sampaolesi^{1,3,*}

¹ Department of Public Health, Experimental and Forensic Medicine, Human Anatomy Unit, University of Pavia, 27100, Pavia, Italy

² Department of Biomedical Sciences, Humanitas University, 20090, Milan, Italy.

³ Translational Cardiomyology laboratory, Department of Development and Regeneration, Stem Cell Institute, KULeuven, 3000, Leuven, Belgium.

⁴ Stem Cell Laboratory, Department of Pathophysiology and Transplantation, University of Milan, Unit of Neurology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Centro Dino Ferrari, 20122, Milan, Italy.

⁵ San Raffaele Telethon Institute for Gene Therapy, Unit of Pathogenesis and therapy of primary immunodeficiencies - Vita Salute San Raffaele University, 20133, Milan, Italy.

⁶ Department of Pediatrics, University of Cincinnati College of Medicine and Molecular Cardiovascular Biology Division, Heart Institute, Cincinnati Children's Hospital Medical Center, Cincinnati, 45229, OH, USA.

⁷ Department of Biosciences, University of Milan, 20133, Milan, Italy.

⁸ Department of Human Genetics, Laboratory of Reproductive Genomics, KU Leuven, 3000, Leuven, Belgium.

⁹ Department of Medicine and Surgery, Division of Biomedical, Biotechnological and Translational Sciences, University of Parma, 43121, Parma, Italy

[†] These authors contributed equally to this work

^{*} Corresponding author daniela.galli@unipr.it (D.G.); maurilio.sampaolesi@kuleuven.be (M.S.)

Supplementary Figure Legends

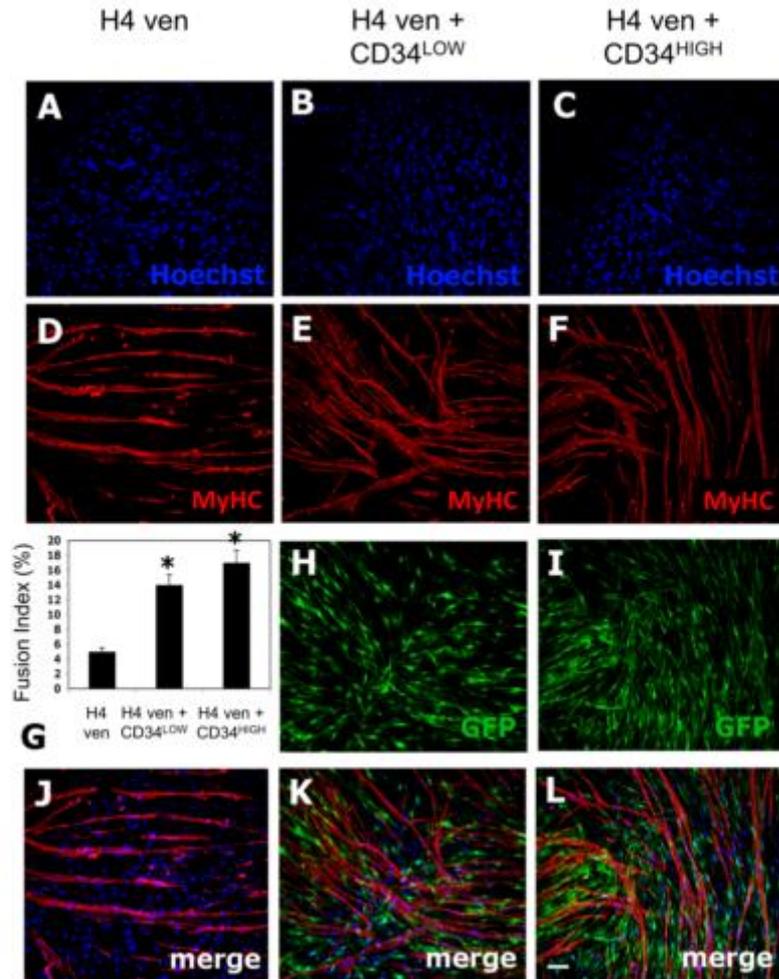


Figure S1: Myogenic differentiation (5 days) of H4ven mesoangioblasts co-cultured with guide cells. (A-C), Hoechst nuclei staining of H4ven (A), H4ven co-cultured with CD34^{LOW} (B), or with and CD34^{HIGH} cells (C). (D-F), Myosin Heavy Chain (MyHC) immunofluorescence analysis on H4ven (D), H4ven co-cultured with CD34^{LOW} (E) or CD34^{HIGH} cells (F). (G), fusion index (see results) of H4ven, H4ven co-cultured with CD34^{LOW} or CD34^{HIGH} cells; *p<0.03. (H, I), GFP immunofluorescence analysis on H4ven co-cultured with CD34^{LOW} (H) or CD34^{HIGH} cells (I). (J) merge of A and D panels. (K) merge of B, E and H panels. (L) merge of panels C, F, I. Scale bar = 40 μ m.

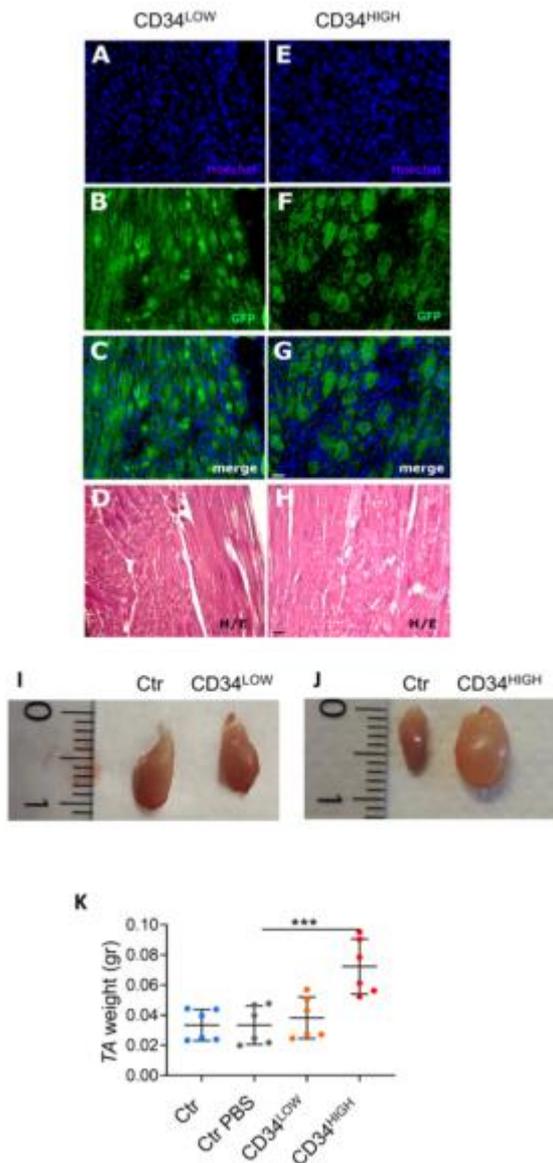


Figure S2: Cardiotoxin-injured *tibialis anterior* muscles injected with guide cells. (A, E), Hoechst staining of muscles injected with CD34^{LOW} (A) and CD34^{HIGH} cells (E). (B, F), GFP immunofluorescence in muscles injected with CD34^{LOW} (B) and CD34^{HIGH} cells (F). (C), merge of A, B panels; (G) merge of E, F panels. Scale bar in G for panels A-G, 50 μm. (D, H), hematoxylin and eosin (H/E) staining of muscles injected with CD34^{LOW} (D) and CD34^{HIGH} cells (H). Scale bar for panels D-H, 100 μm. (I, J) The images show CTX injured *tibialis anterior* muscles, 4-weeks after the injection of PBS (Ctr) or CD34^{LOW} cells (CD34^{LOW}) and CD34^{HIGH} cells (CD34^{HIGH}), respectively. (K) Weight (gr) of TA in un-injected mice (Ctr, blue dots), PBS injected mice (Ctr PBS, grey dots), CD34^{LOW} cells injected mice (CD34^{LOW}, orange dots) and CD34^{HIGH} cells injected mice (CD34^{HIGH}, red dots); N=6 per group, ***p<0.001.

Supplementary Video Legends

Video S1: Time-lapse confocal microscopy of CD44, Sox2, and Ncam2 positive cells.

Video S2: Time-lapse confocal microscopy of CD44, Sox2, Ncam2 and CD34^{LOW} positive cells.

Video S3: Time-lapse confocal microscopy of CD44, Sox2, Ncam2 and CD34^{HIGH} positive cells.