Urine-derived Stem Cells (USCs) knock out for ATM effectively recapitulate ataxia-telangectasia cell pathology.

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Background: Ataxia-Telangiectasia (A-T) is a rare, multi-systemic and neurodegenerative disorder, caused by the functional loss of the Ataxia Telangiectasia Mutated (ATM) protein. ATM is a phosphatidylinositol-3-kinase -like protein that, in response to DNA double-strand breaks (DSBs), activates several targets (i.e. p53 and CHK2) that in turn regulate cell cycle and allow DNA repair. ATM mutations lead to dysfunction in many organs and cellular types including cerebellar and spinal neurons, glial cells and skeletal muscles resulting in cerebellar ataxia and muscle dystrophy. Nowadays, no treatment is available to slow down A-T symptoms. As a consequence, it is urgent to identify robust, versatile, yet personalized models for understanding A-T pathophysiology and developing novel therapies. Herein we describe an important step forward in the generation of novel ATM KO cellular models which may represent valuable cellular platforms for drug screening in A-T.

Methods: Stem cells were isolated from urine (USCs) of healthy donors and genetically modified cells by CRISPR/Cas9 technology to knock out ATM gene. ATM KO and wild type USCs were characterized for alterations in p53 pathway (by qPCR), cell cycle analysis (by FACS) and DNA damage response (by comet assay). Calcium homeostasis was also assessed by evaluating cytosolic and mitochondrial ATP-induced calcium transients using Fura-2 and MitFura-2, respectively. ATM KO USCs and wild type USCs were then differentiated into skeletal muscle cells (SkMCs) and extensively characterized as previously described for ATM KO USCs.

USCs ATM KO characterization maintain stem cells properties

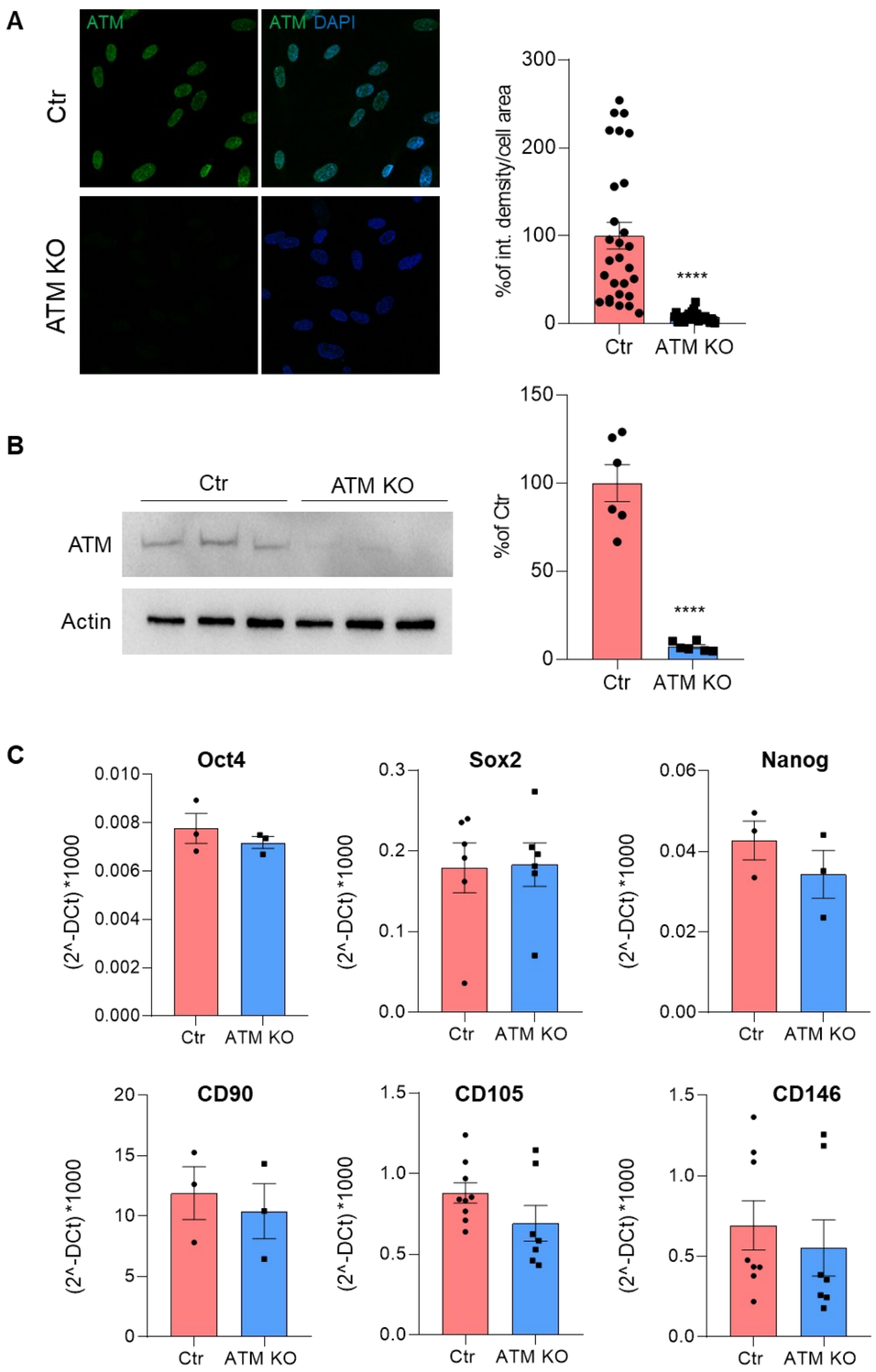

Figure 1. USCs ATM KO characteriztion. A) Representative IF analysis of ATM expression in non-transfected USCs (Ctr) and USCs ATM KO (Magnification 63X; Green: ATM; Blue: DAPI) and relative fluorescence quantification (% of integrated density/cell area). B) Representative Western blot and densitometric analysis of ATM expression in USCs Ctr and ATM KO (% of the Ctr). C) qPCR analysis of stem (Oct4, Sox2, Nanog) and mesenchymal (CD90, CD105, CD146) markers.

USCs ATM KO have impaired proliferation, cell cycle and DNA repair mechanism.

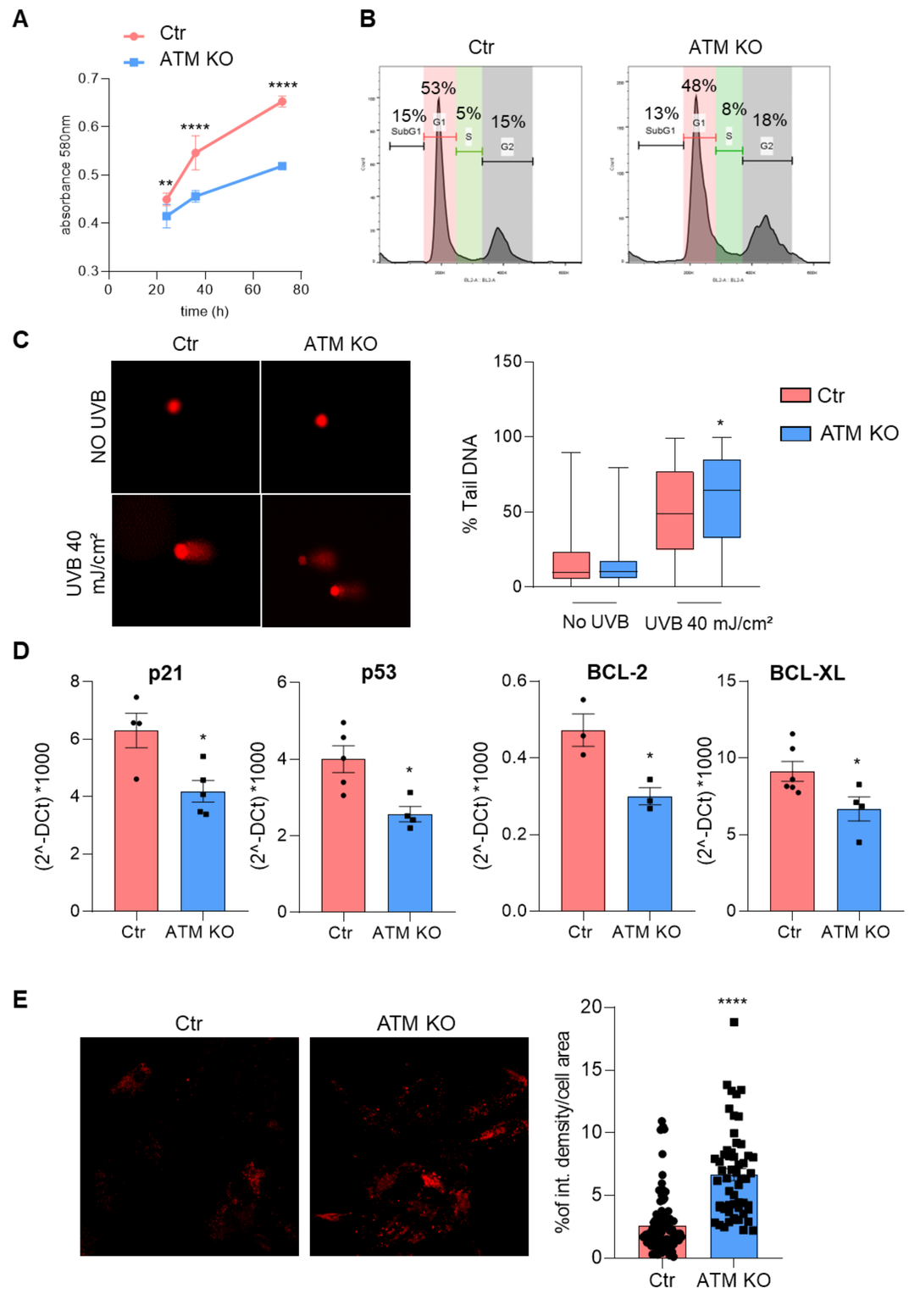

Figure 2. Figure 2. USCs ATM KO phenotype characterization. A) Crystal violet proliferation assay. B) Representative histograms of cell cycle analysis. C) Representative images and quantification of comet assay analysis. USCs and USC ATM KO were stimulated with UVB 40 mJ/cm 2 and, after 6 hours of recovery, stained with propidium iodide and separated by electrophoresis. D) Real-time analysis of apoptosis related genes. E) Representative confocal images and fluorescence quantification of mitoSOX staining of USCs Ctr and USCs ATM KO.

USCs ATM KO efficiently differentiate into skeletal muscle cells

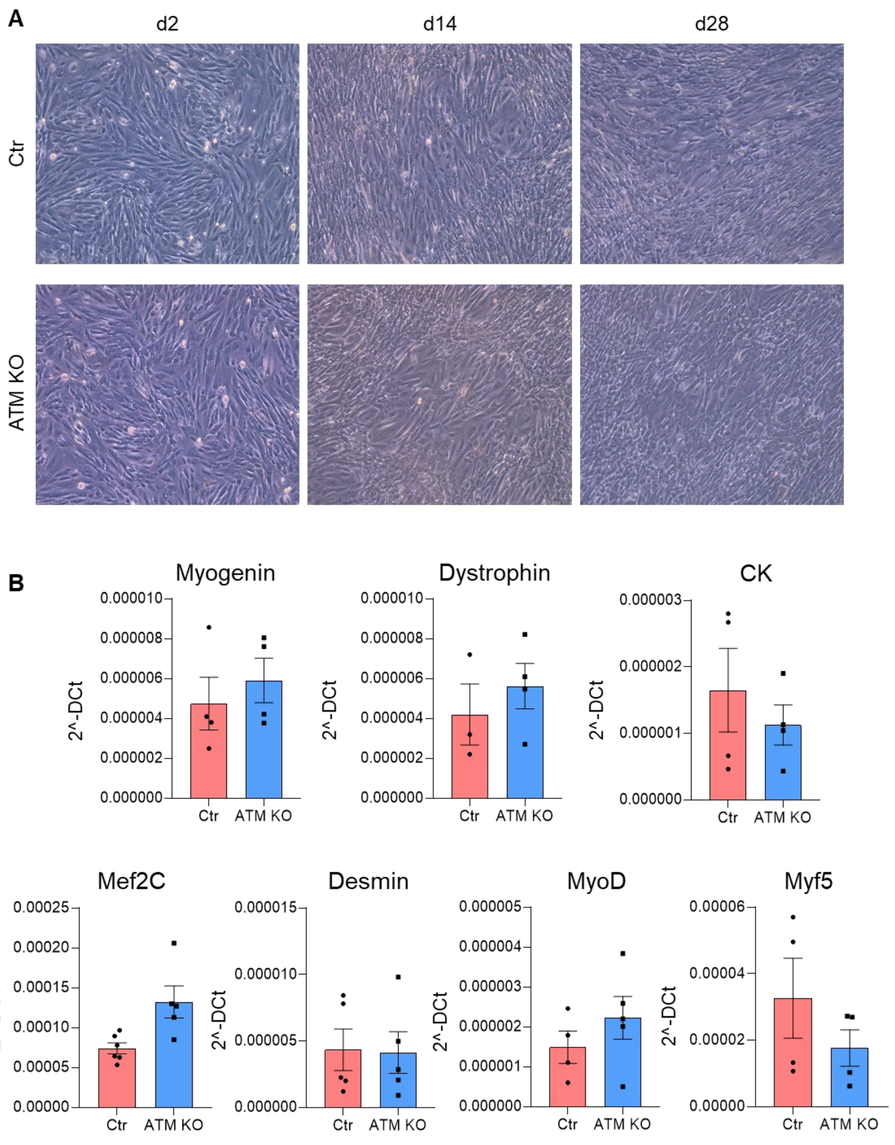

Figure 3. USC-SkMCs ATM KO characterization. A) Representative phase-contrast images of USCs Ctr and ATM KO at different stages of differentiation towards SkMCs (magnification 200x). B) qPCR analysis of skeletal muscle cells markers (myogenin, Myf5, Mef2C, desmin, MyoD, creatine kinase - CK) markers.

USCs ATM KO-derived skeletal muscle cells have different kinetic of contraction

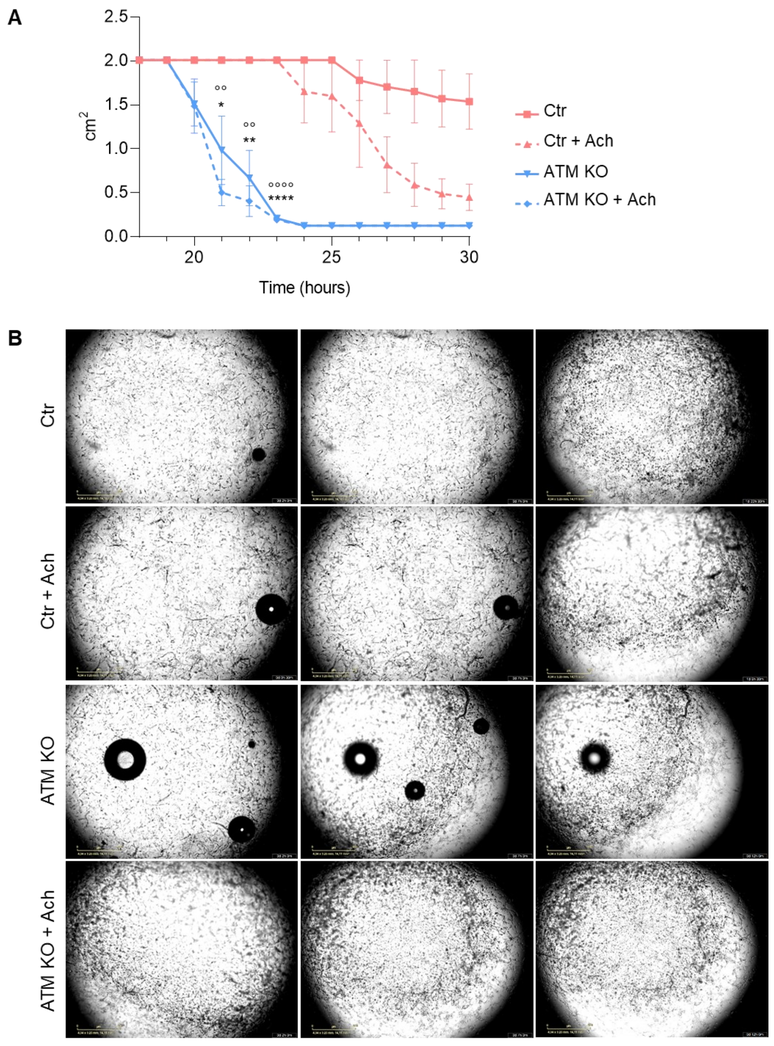

Figure 4. Collagen contraction assay. SkMCs were plated on collagen disc and treated with acetylcholine (100) µM. A) The collagen area was measured at the indicated time points. B) Representative phase-contrast images of collagen disc at progressive time points.

Conclusion: Both ATM KO-USCs and derived SkMCs faithfully recapitulate hallmarks of A-T pathology like cell cycle and DSB repair dysregulation, thus proving to be a reliable cellular model for A-T pathogenesis and for drug screening, starting from a biological sample easily recoverable from the donor in a non-painful, non-invasive and economic way. The results obtained by modeling the A-T pathology in USCs-ATM KO and SkMSc will be validated in USCs and SkMSc obtained from A-T patients, paving the way to the development of personalized A-T therapy.

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