

SUPPLEMENTARY FIGURE LEGENDS

Figure S1 – Establishment of a neuronal cell line with stably silenced ATXN3. (A, B)

ATXN3^{shRNA} cells showed a high decrease in ATXN3 expression, as compared to SCR^{shRNA} and empty vector (pLKO.1) control cells. The ATXN3 level in the pLKO.1 cells expressing the empty vector was considered as 100% and α -tubulin was used as a loading control. (C) qRT-PCR analysis showed that transcript levels of genes encoding ATXN3L and JOSD1 and JOSD2 proteins were similar between ATXN3^{shRNA} and SCR^{shRNA} cells. (D, E) PC12_ATXN3^{shRNA} cells showed a high decrease in ATXN3 levels, as compared to PC12_SCR^{shRNA} cells. The results were normalized for MCT4 levels. (F) Increased expression of *ITGA5* in both SCR^{shRNA} and ATXN3^{shRNA} cells overexpressing *ITGA5*. mRNA levels were normalized to *HBMS* gene. * $p < 0.05$.

Figure S2 – Survival and cell cycle progression are not altered in non-differentiated neuronal cells lacking ATXN3. (A) ATXN3^{shRNA} cells were flattened as compared with the SCR^{shRNA} cells. (B) ATXN3 knockdown increased SH-SY5Y cell migration in a wound scratch assay. Bars represent migration rate expressed as a percentage of control and calculated as the proportion of the mean distance that remained cell-free after 24 hours. (C, D) Flow cytometry analysis using PI showed no alterations of the cell cycle progression in ATXN3^{shRNA} cells. (E) No effect on cell survival measured by propidium iodide (PI) incorporation caused by silencing of ATXN3. (F) Phalloidin staining (green) showed no major defects on the actin cytoskeleton in ATXN3^{shRNA} cells as compared with the controls. Nuclei were counterstained with DAPI (purple). Scale bar: 20 μ m. *** $p < 0.001$.

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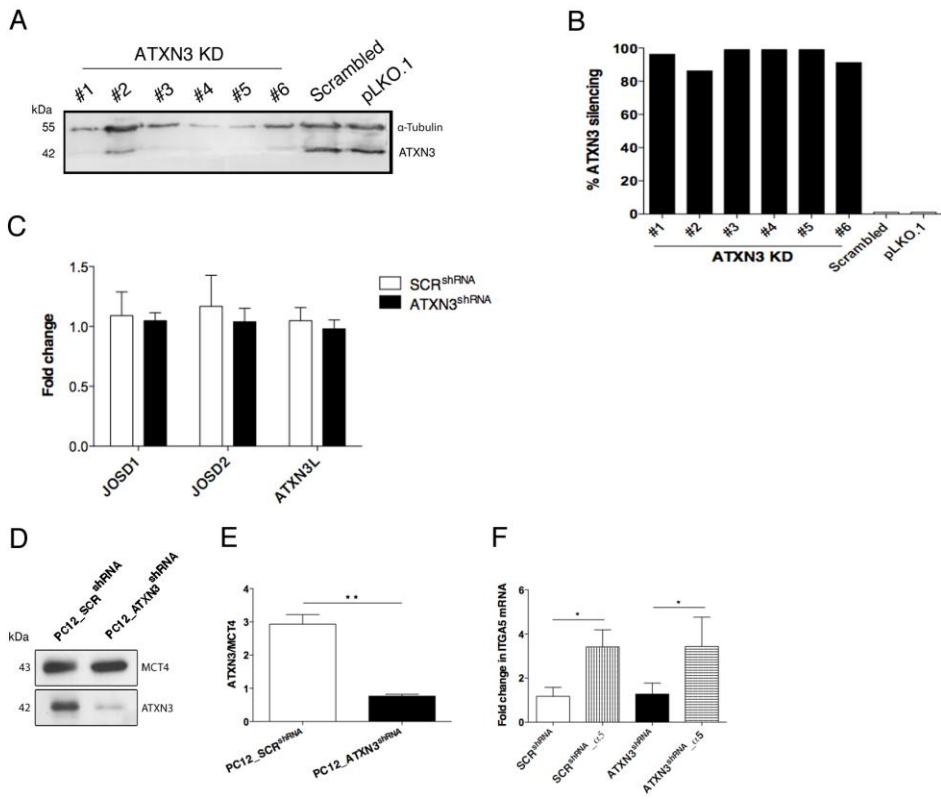
Figure S3 – ATXN3 silencing does not affect expression of RA-responsive genes and does not alter the mRNA levels of several integrin subunits (A) Transcript levels of *tTG*, a RA-responsive gene, were not altered in ATXN3^{shRNA} cells suggesting that these cells respond to RA treatment. (B) ATXN3 did not regulate the amounts of *ITGA5* in SH-SY5Y cells as measured by qRT-PCR. mRNA levels are normalized to *HBMS* gene.

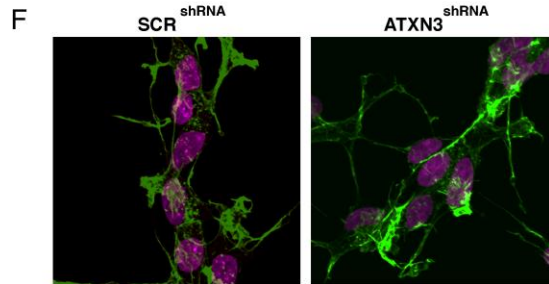
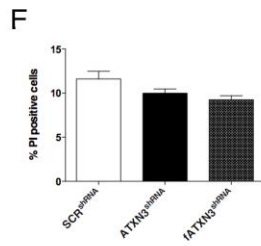
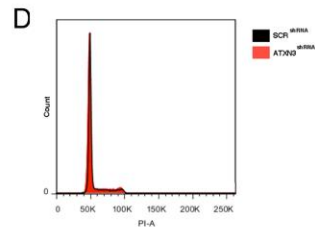
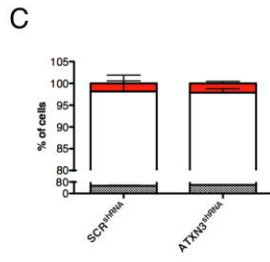
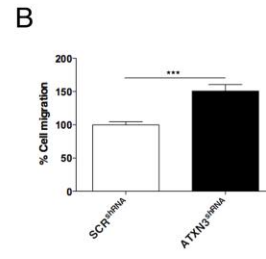
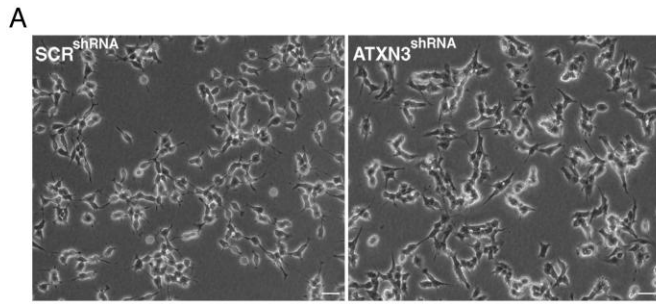
mRNA levels are normalized to *HBMS* gene.

Figure S4 – The phenotype of ATXN3^{shRNA} cells is ITGA5-related. (A) Western blot analysis showed no differences in alpha-1 integrin levels comparing ATXN3^{shRNA} and the SCR^{shRNA} control cells. (B) Wound scratch assay showed no differences on migration comparing ATXN3^{shRNA} and the SCR^{shRNA} control cells, when using Laminin or Poly-D-Lysine coatings, 24 h after scratching.

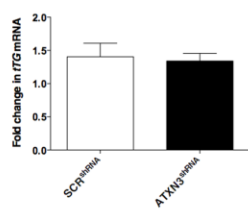
Figure S5 – Overexpression of both catalytic and expanded mutant versions of ATXN3 results in an abnormal cell morphology. (A) Overexpression of wild type (ATXN3_28Q), catalytic mutant (ATXN3_C14A) or expanded ATXN3 (ATXN3_83Q) in SH-SY5Y cells. mRNA levels were normalized to *HMBS* gene. (B) Cells overexpressing catalytic mutant (ATXN3_C14A) or expanded ATXN3 (ATXN3_83Q) cells failed to acquire a neuron-like morphology as compared with their counterpart controls, after RA treatment. Scale bar: 100 μ m. * $p < 0.05$, *** $p < 0.001$.

Figure S6 – Characterization of a PC12 neuronal cell line with stably silenced ATXN3. (A) PC12_ATXN3^{shRNA} cells were less elongated and showed shorter extensions as compared with the PC12_SCR^{shRNA} control cells after NGF treatment. Scale bar: 100 μ m. NGF treatment did not inhibit proliferation of PC12_ATXN3^{shRNA} cells as seen by optical microscopy (B) and (C, D) Ki-67 staining (red). Nuclei were counterstained with DAPI (blue). Scale bar: 200 μ m. (E) Flow cytometry analysis using propidium iodide (PI) showed increased cell death in PC12_ATXN3^{shRNA} cultures. (F) qRT-PCR analysis of the variation of neuronal markers after NGF treatment showed that mRNA levels were decreased for β III-tubulin and Tau in PC12_ATXN3^{shRNA} cultures. Transcript levels were normalized to levels in undifferentiated cells and to *HMBS* gene expression. (G) Phalloidin staining (green) showed that the actin cytoskeleton was less spread than in control cells, with less extensions, and that actin filaments were disorganized and not parallel in PC12_ATXN3^{shRNA} cells. Nuclei were counterstained with DAPI (purple). Arrows mark evidences for higher adhesion in control cells and asterisks mark microspikes in PC12_ATXN3^{shRNA} cells. Scale bar: 20 μ m. (H, I) Western blot analysis showed a decrease of ITGA5 levels in PC12_ATXN3^{shRNA} cells. The results were normalized for MCT4 expression. (J) ATXN3 did not regulate the amounts of ITGA5 mRNA expression in PC12 cells, measured by qRT-PCR. mRNA levels were normalized to *HMBS* gene expression.

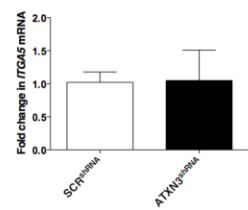


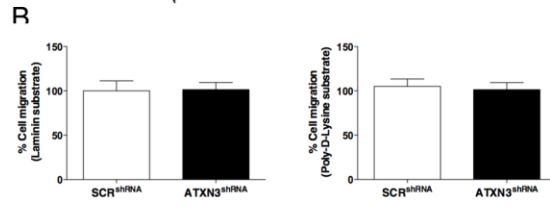
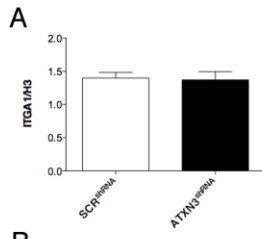


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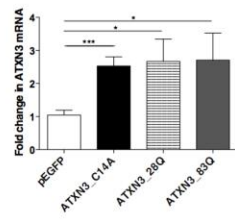


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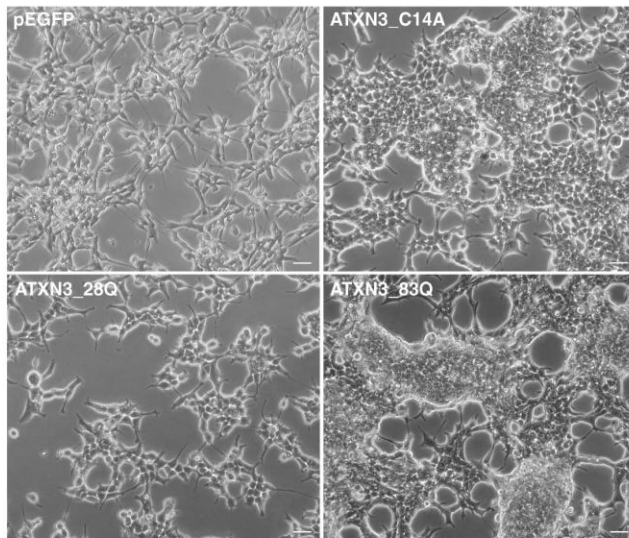




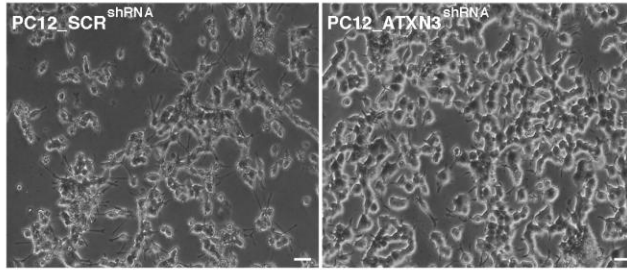
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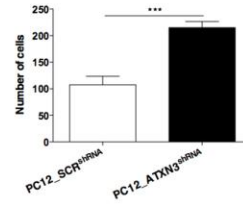
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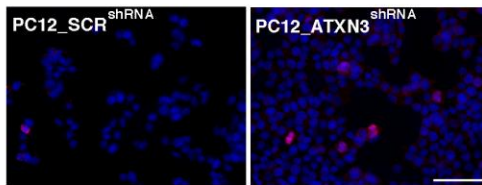
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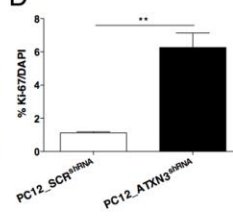
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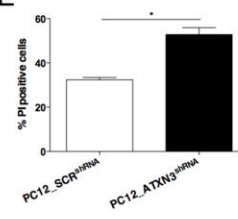
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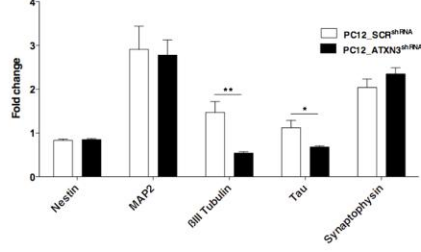
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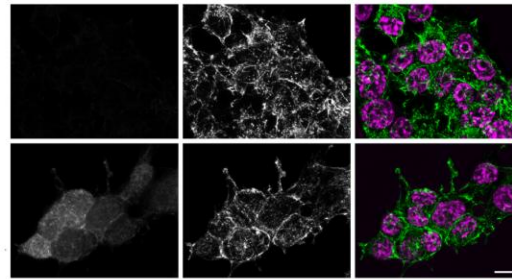
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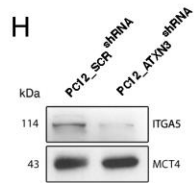
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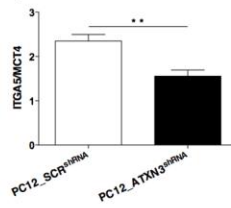
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