

PREMATURE AGING

Inhibition of the acetyltransferase NAT10 normalizes progeric and aging cells by rebalancing the Transportin-1 nuclear import pathway

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Hutchinson-Gilford progeria syndrome (HGPS) is an incurable premature aging disease. Identifying deregulated biological processes in HGPS might thus help define novel therapeutic strategies. Fibroblasts from HGPS patients display defects in nucleocytoplasmic shuttling of the GTP-bound form of the small GTPase Ran (RanGTP), which leads to abnormal transport of proteins into the nucleus. We report that microtubule stabilization in HGPS cells sequestered the nonclassical nuclear import protein Transportin-1 (TNPO1) in the cytoplasm, thus affecting the nuclear localization of its cargo, including the nuclear pore protein NUP153. Consequently, nuclear Ran, nuclear anchorage of the nucleoporin TPR, and chromatin organization were disrupted, deregulating gene expression and inducing senescence. Inhibiting *N*-acetyltransferase 10 (NAT10) ameliorated HGPS phenotypes by rebalancing the nuclear to cytoplasmic ratio of TNPO1. This restored nuclear pore complex integrity and nuclear Ran localization, thereby correcting HGPS cellular phenotypes. We observed a similar mechanism in cells from healthy aged individuals. This study identifies a nuclear import pathway affected in aging and underscores the potential for NAT10 inhibition as a possible therapeutic strategy for HGPS and perhaps also for pathologies associated with normal aging.

INTRODUCTION

Hutchinson-Gilford progeria syndrome (HGPS) is a very rare, fatal premature aging syndrome caused by a de novo heterozygous point mutation within exon 11 of the *lamin A* (*LMNA*) gene (1, 2). *LMNA* encodes two type V intermediate filament proteins: lamin A and lamin C. Together with B-type lamins, these proteins form the nuclear lamina at the inner nuclear membrane, where they act as a scaffold to maintain nuclear architecture, nuclear pore complexes (NPCs), and chromatin organization and to regulate DNA replication and transcription. In HGPS, the mutation only affects lamin A and causes the production of a truncated, toxic form of the protein called progerin that acts as a dominant negative. Accumulation of progerin at the inner nuclear membrane alters nuclear lamina structure, causing abnormal nuclear morphology, aggregation of NPCs, chromatin disorganization due to loss of peripheral heterochromatin anchorage, and transcriptional deregulation, all of which ultimately lead to DNA damage accumulation and premature entry of HGPS cells into senescence (3–6). These cellular phenotypes are also observed in cells from healthy aged individuals. In HGPS patients, progerin accumulation triggers segmental premature aging, in which most tissues are affected but the patient displays accelerated aging only in some of them, usually starting within the first year

of life and leading to death at the average age of 14, mainly due to heart defects (7, 8). Although HGPS syndrome was first described over a century ago, the exact mechanisms by which progerin drives such striking cellular and organismal phenotypes are still being explored.

Various studies have reported disruption of nucleocytoplasmic transport in HGPS fibroblasts, due to an abnormal nucleocytoplasmic gradient of guanosine 5'-triphosphate (GTP)-bound Ran (RanGTP) (9–11). Ran is a small guanosine triphosphatase (GTPase) that shuttles between the cytoplasm and the nucleus and that cycles between guanosine diphosphate (GDP)-bound and GTP-bound states (12), with conversion between these states being catalyzed by cytoplasmic factors, such as Ran GTPase-activating protein (13), and by nuclear factors, such as the regulator of chromatin condensation 1 (RCC1) (14, 15). Therefore, Ran is predominantly in its GTP-bound form in the nucleus and in its GDP-bound form in the cytoplasm. This creates a RanGTP gradient across the nuclear envelope (16, 17), which allows active transport of protein cargos through NPCs by a family of carrier proteins called karyopherins. In HGPS, the presence of progerin induces defects in Ran nuclear localization. Consequently, nuclear import of proteins requiring Ran-dependent active transport through nuclear pores appeared more strongly affected than that of proteins passively diffusing through nuclear pores in these cells. One of the proteins displaying reduced nuclear import in HGPS cells is the nucleoporin translocated promoter region (TPR) (9, 10), a polypeptide of ~265 kDa that forms a homodimer and is thus one of the largest nucleoporins. TPR is the last protein recruited to the NPC, and its anchorage in the nuclear envelope requires the nuclear pore protein NUP153 (18, 19). We previously showed that disrupting NUP153 abundance or function affects Ran nuclear localization, thus impairing nuclear import of p53-binding protein 1 (53BP1), which is involved in DNA damage repair. This contributes to DNA damage accumulation in aging vascular smooth

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muscle cells (VSMCs) (20). Remodelin, a small-molecule inhibitor of N-acetyltransferase 10 (NAT10) that normalizes phenotypes of fibroblasts isolated from HGPS patients (21), restores NUP153 localization and nuclear Ran in VSMCs through an uncharacterized mechanism (20). This raises the question of why the abundance of nuclear Ran is altered in HGPS cells and which aspect of the nuclear transport machinery is disrupted because NUP153 can be imported by several import pathways (22–24).

Here, we show that the decrease of nuclear Ran abundance that occurs in cells from HGPS patients and in cells from aged individuals is linked to dysregulation of the nonclassic transport pathway mediated by Transportin-1 (TNPO1), also called karyopherin β 2 (25). Unlike classical importin proteins, which are general transporters, TNPO1 only mediates the nuclear import of a subset of proteins. In the nucleus, RanGTP binds to TNPO1 to stimulate release of the cargo (26). Among TNPO1 cargos, the most extensively characterized is the RNA binding protein heterogeneous nuclear ribonucleoprotein 1 (hnRNPA1) (27), which functions in several processes including mRNA biogenesis and promotion of transcription factor activity (28–30). NPC protein NUP153 is also a target for TNPO1-mediated nuclear import (24). Accordingly, we observed that hnRNPA1, NUP153, TPR, and nuclear Ran defects in HGPS cells were linked to deregulation of the TNPO1 nuclear-to-cytoplasmic ratio. NAT10 inhibition restored the proper subcellular distribution of TNPO1 and TNPO1-dependent nuclear import in HGPS cells, thereby promoting nuclear localization of its cargos such as hnRNPA1 and NUP153. This allowed proper NUP153-dependent recruitment of TPR to the NPC and restored chromatin reorganization and global gene transcription, thus preventing premature entry of HGPS cells into senescence. NUP153 depletion did not affect the TNPO1 pathway, supporting the notion that TNPO1 deregulation in HGPS is the cause and not the consequence of abnormal NUP153 nuclear localization. This study thus provides mechanistic insight into how NAT10 inhibition reverses HGPS cellular phenotypes and provides further evidence that it may also be relevant in normal aging.

RESULTS

Premature aging and normal aged cells display nucleocytoplasmic transport defects

We found by immunofluorescence staining that, as expected (31), most Ran was in the nuclei of interphase fibroblasts from a healthy 20-year-old individual (Fig. 1A). By contrast, and in line with previous reports (9, 10), we observed that Ran localization was abnormal in fibroblasts from an HGPS patient (HGPS 11513), with most Ran being cytoplasmic (Fig. 1A). Because evidence suggests that HGPS might recapitulate various aspects of normal aging (32), we also looked at the subcellular localization of Ran in fibroblasts from healthy individuals between 81 and 96 years of age (AG11240, AG04059, AG05248, and AG09602). Similarly to HGPS cells, nuclear Ran appeared to be depleted to various extents in cells from aged individuals (Fig. 1A). This reduction in nuclear Ran was not associated with an overall decrease in Ran abundance in fibroblasts from the HGPS patients or in fibroblasts from aged individuals (Fig. 1B), suggesting that changes in Ran subcellular localization reflected dysregulated RanGTP gradient formation across the nuclear envelope rather than changes in Ran abundance. As reported previously (10), decreased nuclear Ran in HGPS cells was also associated with

decreased nuclear staining intensity of the nucleoporin TPR (Fig. 1, C and D), as well as in cells from aged healthy individuals (fig. S1, A and B). These results confirmed previous findings in HGPS cells and also suggested that a similar nucleocytoplasmic transport defect may occur during normal aging.

Inhibition of NAT10 restores normal nucleocytoplasmic transport in HGPS cells

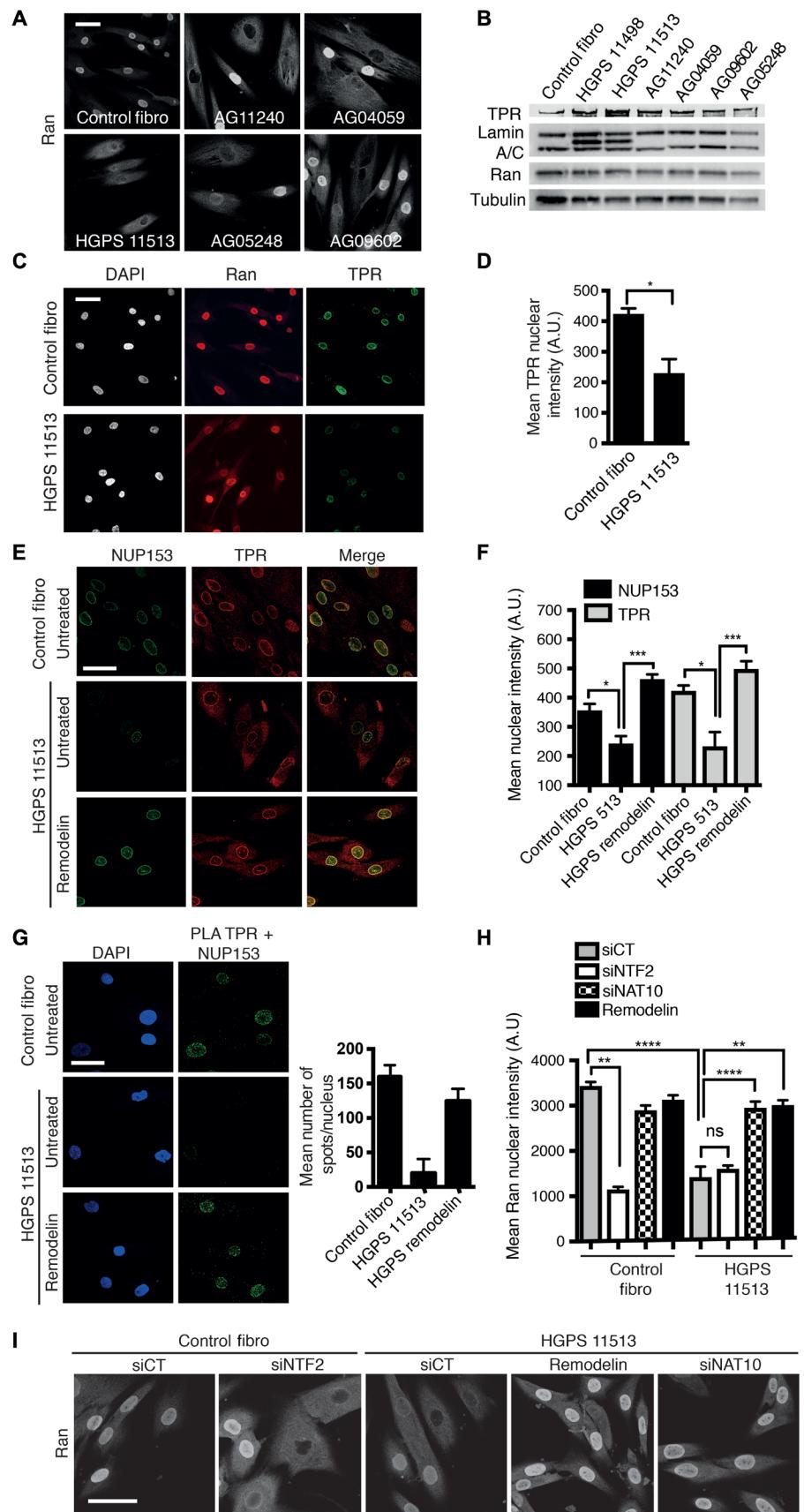
Because the NPC protein NUP153 is required to anchor TPR to the NPC (18), we assessed whether NUP153 was present at the nuclear periphery by immunofluorescence in fibroblasts from control individuals and from HGPS patients (hereafter referred to as control or unaffected and HGPS fibroblasts, respectively). NUP153 nuclear intensity was significantly reduced in HGPS cells compared to controls, in a manner that correlated with decreased nuclear TPR staining (Fig. 1, E and F, and fig. S2A). Because we had previously reported that inhibition of the N-acetyltransferase NAT10 reverses abnormal phenotypes of HGPS cells, as well as aged smooth muscle cells (20, 21), we investigated whether NAT10 inhibition affected NUP153 and TPR staining. When we treated HGPS cells with the NAT10 inhibitor remodelin (21), this increased the nuclear staining intensity of NUP153 and TPR (Fig. 1, E and F). To assess whether this reflected an enhanced interaction between the NUP153 and TPR proteins after remodelin treatment, we performed a proximity ligation assay (PLA) (33) using primary antibodies recognizing TPR and NUP153. Whereas NUP153 and TPR clearly interacted in control fibroblasts (Fig. 1G), the interaction was almost completely absent in untreated HGPS cells, in accord with these proteins being mislocalized. However, remodelin treatment greatly enhanced the interaction between NUP153 and TPR in HGPS cells (Fig. 1G). This suggested that NAT10 inhibition promoted normal nuclear localization and anchorage of both NUP153 and TPR at the nuclear periphery of HGPS cells.

We next looked at the effect of NAT10 inhibition on Ran subcellular localization in HGPS cells. Strikingly, small interfering RNA (siRNA)-mediated depletion of NAT10 or NAT10 inhibition by remodelin nearly completely abolished Ran nuclear localization defects in HGPS cells (Fig. 1, H and I, and fig. S2B). In agreement with nuclear transport factor 2 (NTF2) mediating Ran nuclear import (34, 35), siRNA depletion of NTF2 in control fibroblasts reduced Ran nuclear accumulation, similar to that observed in untreated HGPS cells (Fig. 1, H and I).

Reduction of nuclear Ran modifies chromatin compaction and triggers senescence

Because many cellular processes are disrupted in HGPS, we explored which might be associated with decreased nuclear Ran abundance. One of the phenotypes observed in HGPS cells is chromatin disorganization and loss of heterochromatin marks, including trimethylation of histone H3 at lysine 9 (H3K9me3) (36, 37). To assess whether disrupting Ran nuclear import could affect the state of chromatin at a global level, we transfected nonprogeric human bone osteosarcoma U2OS cells with siRNA targeting NTF2 (Fig. 2A) and looked at global chromatin compaction by treating nuclei harvested from these cells with micrococcal nuclease (MNase). The efficiency of MNase digestion varies with the degree of chromatin compaction. We used U2OS cells for this experiment instead of primary human cells because this assay requires a large number of cells. These assays established that disruption of nuclear Ran localization led to global chromatin

Fig. 1. NAT10 inhibition rescues the reduced nucleocytoplasmic transport in progeric and aged cells. (A) Representative immunofluorescence images showing subcellular localization of Ran in skin fibroblasts from a 20-year-old healthy male (control fibro), aged individuals (AG11240, an 81-year-old female; AG04059, a 96-year-old male; AG05248, an 87-year-old male; AG09602, a 92-year-old female), and an 8-year-old HGPS patient (HGPS 11513). $n = 3$ independent experiments. (B) Representative Western blot showing the abundance of the indicated proteins in extracts of fibroblasts from young, aged, and HGPS individuals. $n = 3$ independent experiments. (C) Representative immunofluorescence images showing nuclear Ran and TPR in HGPS cells as compared to control cells. DAPI, 4',6-diamidino-2-phenylindole. (D) Quantification of TPR nuclear intensity in the indicated cells. $n = 3$ independent experiments, $n > 100$ nuclei per experiment for each cell type. $*P = 0.016$ (Mann-Whitney test). Error bars indicate SEM. A.U., arbitrary units. (E) Representative immunofluorescence images showing localization of NUP153 and TPR in control and HGPS fibroblasts in the absence (untreated) or presence of the NAT10 inhibitor remodelin. (F) Quantification of NUP153 and TPR nuclear intensity from experiments in (E). $n = 3$ independent experiments, $n > 70$ nuclei per experiment for each cell type. P values from left to right: $*P = 0.0111$, $***P = 0.0002$, $*P = 0.0163$, $***P = 0.0007$ (Mann-Whitney test). Error bars indicate SEM. (G) PLA showing the interaction of TPR and NUP153 in control and HGPS fibroblasts in the absence (untreated) or presence of the NAT10 inhibitor remodelin. Results of the PLA were quantified as the mean number of dots per nucleus. $n = 3$ independent experiments, $n > 50$ nuclei per experiment for each cell type. (H and I) Ran nuclear localization in control and HGPS fibroblasts upon siRNA-mediated depletion of NTF2 (siNTF2) or NAT10 (siNAT10) or upon NAT10 inhibition (remodelin). siCT, control siRNA. $n = 3$ independent experiments, $n > 70$ nuclei per experiment for each cell type. P values from left to right: $***P = 0.0043$; $****P < 0.0001$; ns, not significant; $****P < 0.0001$; $**P = 0.0029$ (Mann-Whitney test). Error bars indicate SEM. Scale bars, 50 μ m.



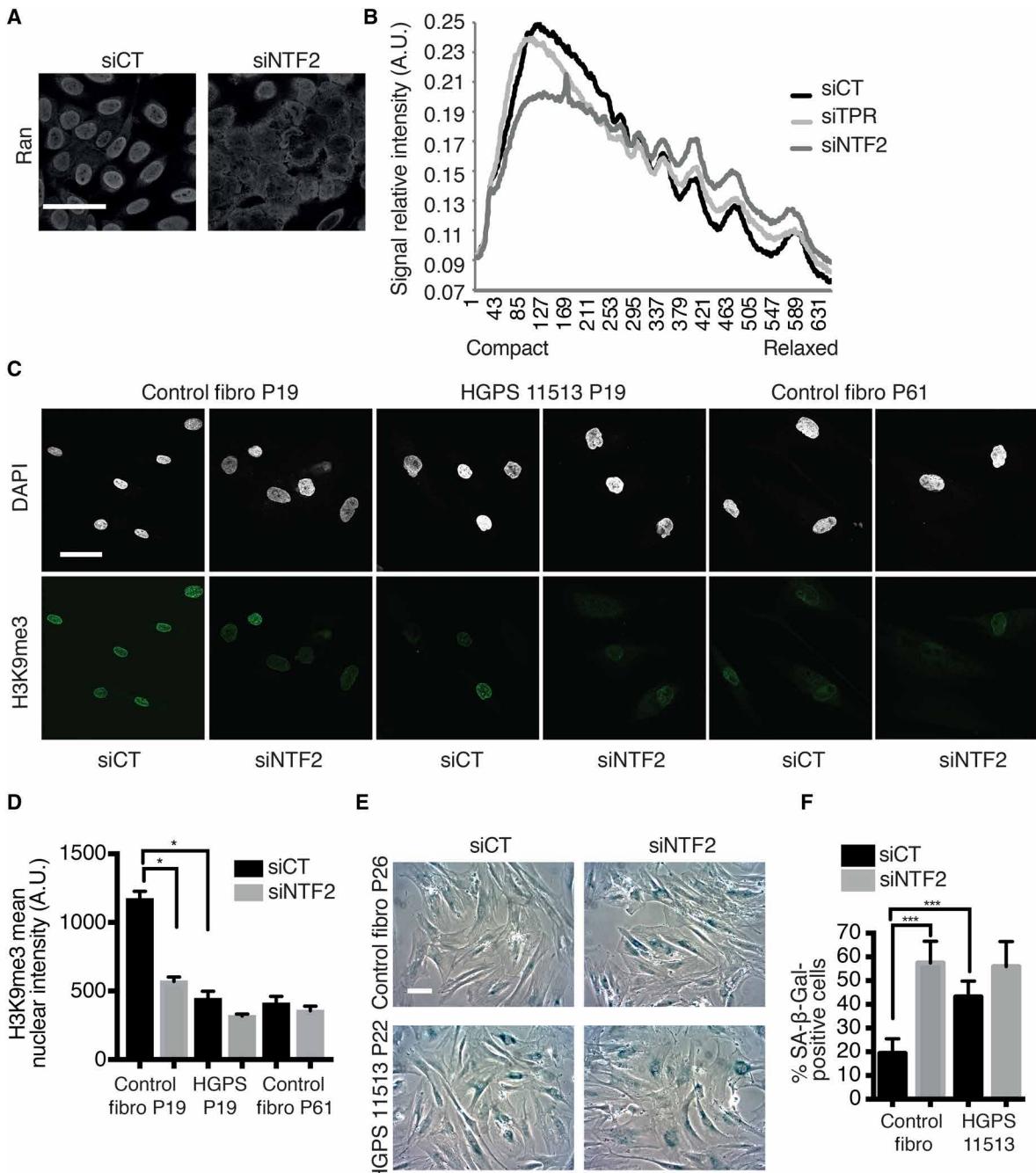


Fig. 2. Disruption of Ran induces chromatin relaxation and senescence. (A and B) Analysis of chromatin relaxation by MNase assay in U2OS cells upon siRNA-mediated TPR depletion (siTPR) or disruption of Ran nuclear import by NTF2 depletion (siNTF2). Scale bar, 30 μ m. Digested chromatin was analyzed on an agarose gel and quantified using ImageJ. The graph is a representative figure of three independent experiments and represents the relative band intensity (y axis) of the digested nucleosomes that migrate further from the well (x axis) because the chromatin is more relaxed and thus more accessible to the MNase enzyme. (C) Representative immunofluorescence images showing H3K9me3 staining in young fibroblasts at P19 (control fibro P19), old fibroblasts at P61 (control fibro P61), and HGPS cells at P19 (HPGS 11513 P19) upon transfection with siCT or siNTF2 to disrupt the RanGTP gradient. Scale bar, 50 μ m. (D) Quantification of H3K9me3 nuclear intensity from (C). $n = 2$ independent experiments, $n > 50$ nuclei per experiment for each cell type. P values from left to right: * $P = 0.0159$, ** $P = 0.014$ (Mann-Whitney test). Error bars indicate SEM. (E) Senescence-associated β -galactosidase (SA- β -Gal) staining (blue cells) upon transfection with siCT or siNTF2 siRNA in control (control fibro) and HGPS (HPGS 11513) fibroblasts at the indicated passage number (P). Scale bar, 50 μ m. (F) Quantification of blue cells from experiments in (C). $n = 3$ independent experiments, $n > 200$ nuclei per experiment for each cell type. P values from left to right: *** $P = 0.0003$, *** $P = 0.0002$ (Mann-Whitney test). Error bars indicate SEM.

decompaction (Fig. 2B) and suggested that, by affecting the bidirectional transport of many large cargo proteins, Ran deregulation might have broad effects on chromatin organization, as observed in HGPS (36, 37). Chromatin relaxation was also observed—albeit to a lesser extent—upon TPR depletion, reflecting the known role of TPR and NPCs in chromatin organization at the nuclear periphery (38, 39).

We next assessed the effect of NTF2 depletion on the heterochromatin mark H3K9me3 in control and HGPS fibroblasts. We observed a marked decrease in H3K9me3 staining intensity upon NTF2 depletion in control fibroblasts (Fig. 2, C and D), similar to the basal H3K9me3 staining observed in HGPS cells expressing a control siRNA. NTF2 depletion did not further reduce H3K9me3 in HGPS cells. Moreover, we observed a similar decrease in H3K9me3 in high passage number [passage number 61 (P61)] unaffected human fibroblasts, suggesting that the perturbation observed in HGPS cells resembles what happens when unaffected cells reach senescence. In addition to the effect on chromatin, disrupting nuclear Ran by depleting NTF2 also promoted the entry of control fibroblasts into senescence as measured by senescence-associated β -galactosidase staining (Fig. 2, E and F). However, depleting NTF2 in HGPS cells, which already had reduced nuclear Ran, did not significantly further increase the proportion of senescent cells. Collectively, these results suggested that the reduction in nuclear Ran observed in HGPS cells might, by globally affecting nucleocytoplasmic transport, contribute to downstream phenotypes of chromatin disorganization and premature entry into senescence.

NAT10 inhibition reverses TNPO1 nuclear import pathway defects in HGPS cells

Neither the abundance nor the subcellular localization of NTF2 appeared to be affected in HGPS cells (fig. S3A), implying that defects in the Ran nuclear import machinery were not the cause of reduced nuclear accumulation of Ran in HGPS cells. Because NUP153 disruption reduces the nuclear abundance of Ran (20), we hypothesized that Ran gradient deregulation occurring in HGPS might reflect the loss of NUP153 nuclear import. This led us to investigate which upstream pathway(s) could affect NUP153 nuclear import in HGPS cells. NUP153 can be imported by several proteins, but evidence suggests that NUP153 is imported into the nucleus of interphase cells by the nonclassic nuclear transport receptor TNPO1 (24), a karyopherin β protein that promotes nuclear import of only a specific subset of proteins, including NUP153 and hnRNPA1. We therefore assessed TNPO1 subcellular localization in control and HGPS fibroblasts by immunofluorescence staining. Whereas TNPO1 was nearly equally distributed between the cytoplasm and nucleus in unaffected fibroblasts, TNPO1 was depleted from the nucleus in HGPS cells (Fig. 3, A and B), similarly to what we had observed for Ran (Fig. 1). To determine whether changes in the nuclear-to-cytoplasmic ratio of TNPO1 in HGPS cells had an impact on its nuclear importer function, we assessed the subcellular localization of hnRNPA1, one of the best-described TNPO1 cargo proteins. In accord with TNPO1 pathway deficiency in HGPS cells, we observed a marked decrease of nuclear hnRNPA1 in these cells (Fig. 3A). Similar defects were observed for the Ewing sarcoma protein (EWS), another characterized TNPO1 cargo (fig. S3, B and C) (40). In contrast, the localization of NAT10, the subcellular distribution of which is not detectably affected in HGPS [fig. S4A and as described in (21)], was not altered upon interfering with TNPO1 function by expressing MBP-M9M, a peptide that binds to TNPO1 and competes with its natural sub-

strates (fig. S4B) (41). These results identify the TNPO1 nuclear import pathway as deregulated in HGPS.

We also observed loss of nuclear TNPO1 in cells from unaffected aged individuals (Fig. 3C), with no apparent change in the abundance of TNPO1 or hnRNPA1, similarly to HGPS cells (Fig. 3D). Because NAT10 inhibition normalized NUP153 and TPR nuclear accumulation and nuclear Ran abundance in HGPS cells (Fig. 1), we assessed the effects of NAT10 inhibition on the TNPO1 pathway. Both remodelin treatment and siRNA-mediated NAT10 depletion restored the nuclear-to-cytoplasmic ratio of TNPO1 in HGPS cells so that it was comparable to that of control fibroblasts (Fig. 3, A and B). Together, these results suggested that the TNPO1 pathway is affected both in HGPS cells and in cells from aged individuals, leading to defects in the nuclear import of TNPO1 and its cargos, including hnRNPA1 and NUP153, thereby contributing to downstream defects of the RanGTP gradient. We conclude that NAT10 inhibition results in global normalization of TNPO1 subcellular localization and function in HGPS cells, thereby alleviating downstream phenotypes. Although TNPO1 depletion itself reduced proliferation of normal retinal pigmented epithelial (RPE-1) cells (fig. S5A), the TNPO1 pathway defects observed in HGPS and in aging cells did not appear to be a mere consequence of slower cell growth. Mimicking reduced cell growth through serum starvation of RPE-1 cells and control fibroblasts did not discernibly affect the TNPO1 pathway nor the subcellular localization of Ran or H3K9me3 (fig. S5, B to D), all of which are strongly affected in HGPS cells.

Modulation of microtubule stability by NAT10 inhibition affects the TNPO1 pathway

We previously observed that HGPS fibroblasts have a more stable microtubule network than control cells (21) and that this contributes to HGPS cellular phenotypes. We also showed that NAT10 inhibition improved these phenotypes, at least in part by targeting and destabilizing microtubules, thus releasing cytoskeletal forces on the nucleus and normalizing nuclear shape (21). This is in accord with a pool of NAT10 being associated with the cytoskeleton, as observed by subcellular fractionation and immunofluorescence (fig. S6, A and B). To assess whether there was a link between accumulation of TNPO1 in the cytoplasm of HGPS cells and microtubule stability, we first performed a PLA between TNPO1 and α -tubulin. Despite similar TNPO1 and α -tubulin staining intensities observed by immunofluorescence (fig. S7A) and similar protein abundance observed by Western blotting (fig. S7B), the interaction between TNPO1 and α -tubulin was significantly greater in HGPS fibroblasts than in control fibroblasts, whether measured per cell or per defined area of cytoplasm (Fig. 4, A and B). The reason for measuring the number of PLA spots within a defined area was to account for the fact that HGPS cells are larger than unaffected cells, which could have biased the results. However, there was a significant increase in the number of PLA spots even within a defined area of cytoplasm (Fig. 4B, right panel), showing that the increased interaction was not a reflection of the increased size of HGPS cells. The interaction between TNPO1 and α -tubulin was confirmed by immunoprecipitation using extracts of control fibroblasts (fig. S7C).

To understand whether the effect of NAT10 inhibition on TNPO1 was associated with microtubule stabilization, we treated unaffected and HGPS fibroblasts with the microtubule stabilizing or destabilizing agents tubacin or nocodazole, respectively (Fig. 4C). Mimicking microtubule stabilization, such as occurs in HGPS cells, by treating

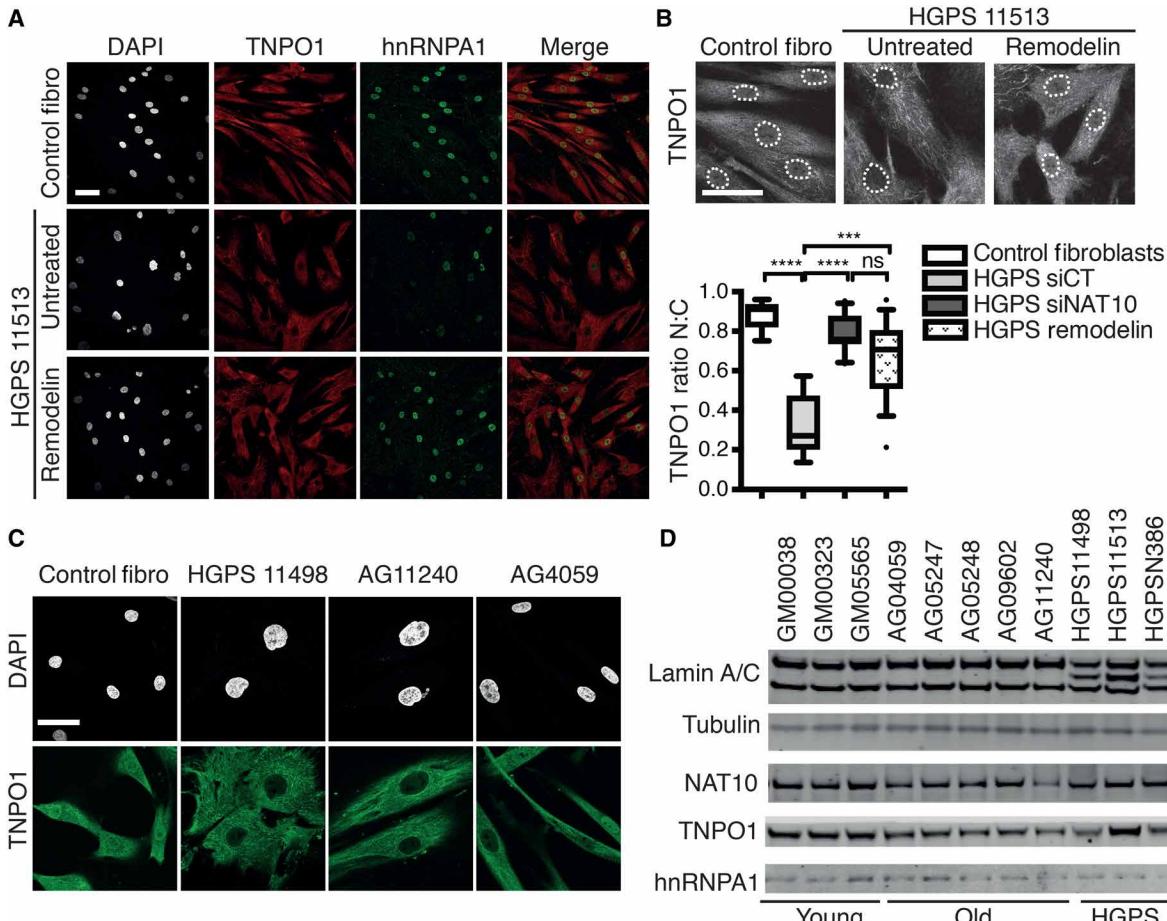


Fig. 3. The TNPO1 pathway is defective in HGPS cells and rescued by NAT10 inhibition. (A) Representative immunofluorescence images showing the subcellular localization of TNPO1 and its cargo hnRNPA1 in control and HGPS fibroblasts in the absence (untreated) or presence of the NAT10 inhibitor remodelin. (B) Higher-magnification images from (A) showing TNPO1 nuclear and cytoplasmic subcellular localization, quantified below as nuclear-to-cytoplasmic ratio (N:C). Nuclei are outlined with white dotted lines. Box plots represent median (line), 10th to 90th percentiles (whiskers), and outliers (dots). $n = 3$ independent experiments, $n > 50$ nuclei per experiment for each cell type. P values from left to right: **** $P < 0.0001$; *** $P = 0.0003$; *** $P < 0.0001$; ns, not significant (Mann-Whitney test). Error bars indicate SEM. (C) Representative immunofluorescence images showing TNPO1 subcellular localization in fibroblasts from other HGPS patients (HGPS 11498) and in cells from healthy aged individuals (AG11240 and AG059). $n = 3$ independent experiments. (D) Western blot showing total TNPO1 and hnRNPA1 abundance in the indicated cells. $n = 3$ independent experiments. Scale bars, 50 μ m.

control fibroblasts with tubacin led to significant defects in both TNPO1 and hnRNPA1 nuclear import (Fig. 4, C and D). By contrast, destabilizing microtubules in HGPS cells or in cells from unaffected aged individuals with nocodazole enhanced TNPO1 nuclear localization and, presumably by promoting TNPO1 transport function, increased the nuclear abundance of hnRNPA1 (Fig. 4, C and D, and fig. S8). These results suggested that the TNPO1 import pathway can be altered by microtubule stability and that, by modulating this, NAT10 inhibition rebalanced the TNPO1 nuclear-to-cytoplasm ratio and enhanced the nuclear import of TNPO1 cargo proteins.

Normalization of HGPS cells by NAT10 inhibition requires TNPO1

To determine whether the normalization of heterochromatin markers and decreased senescence that we previously observed in HGPS cells upon NAT10 inhibition (21) depended on TNPO1, we treated control and HGPS fibroblasts with siRNAs to deplete NAT10 alone or in combination with NUP153 or TNPO1 depletion. As we ob-

served previously (20), NUP153 depletion in control cells led to loss of nuclear Ran, probably because it affects the structure of NPCs that is important for RanGTP gradient formation. However, NUP153 depletion did not affect the TNPO1 pathway, as observed by the normal subcellular localization of TNPO1 and its downstream target hnRNPA1 (fig. S9). Cells impaired in nuclear Ran because of TNPO1 depletion (Fig. 5A and fig. S10) or as observed in HGPS cells (Fig. 5B) also displayed significantly reduced abundance of the heterochromatin marker H3K9me3 (Fig. 5C). This is in accordance with the chromatin decompaction that we observed upon Ran deregulation (Fig. 2, B and C) and suggested that TNPO1 depletion was sufficient to cause this phenotype. Furthermore, simultaneous depletion of NUP153 and NAT10 in control fibroblasts produced similar H3K9me3 phenotypes compared to NUP153 depletion alone (Fig. 5, A and C), identifying NUP153 as a critical mediator of NAT10-dependent cellular rebalancing. Whereas NAT10 depletion alone restored nuclear Ran and H3K9me3 staining in HGPS cells, this did not happen when NUP153 or TNPO1 was codepleted with NAT10

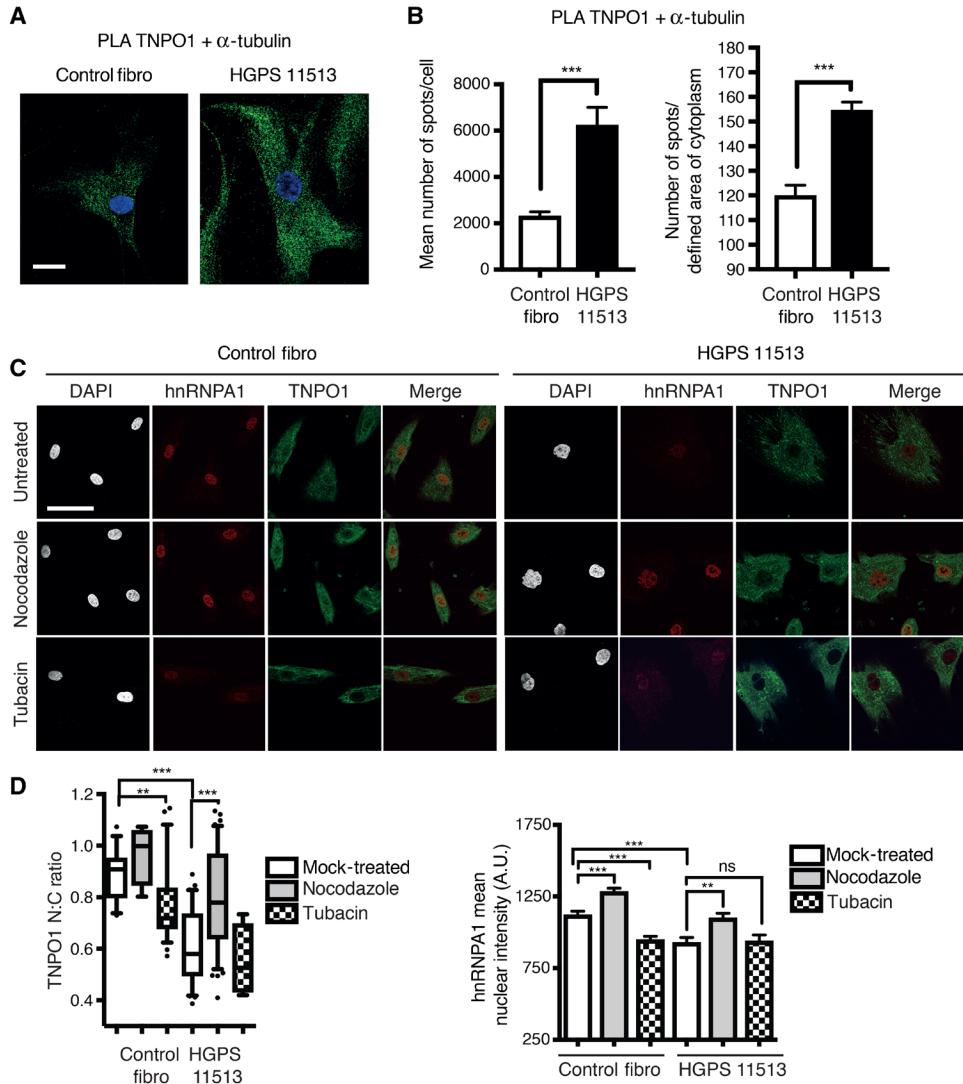


Fig. 4. Microtubule reorganization modulates TNPO1 localization and function. (A) Representative immunofluorescence images from PLAs in control and HGPS fibroblasts using antibodies recognizing TNPO1 and α -tubulin. Each dot represents an interaction between one molecule of TNPO1 and one molecule of α -tubulin. Scale bar, 20 μ m. (B) Quantification of the PLA shown in (A), representing both the total number of dots per cell (left panel) and the average number of spots in a defined area of cytoplasm (right panel). $n = 3$ independent experiments, $n > 46$ nuclei per experiment for each cell type. P values from left to right: *** $P = 0.0005$, *** $P = 0.0003$ (Mann-Whitney test). Error bars represent SEM. (C) Representative immunofluorescence images showing the effect of compounds that destabilize (nocodazole) or stabilize (tubacin) the microtubule network on the nuclear localization of TNPO1 and its cargo hnRNPA1. Scale bar, 50 μ m. (D) Quantification of the TNPO1 nuclear-to-cytoplasmic ratio and of hnRNPA1 nuclear intensity. Box plots represent median (line), 10th to 90th percentiles (whiskers), and outliers (dots). $n = 3$ independent experiments, $n > 50$ nuclei per experiment for each cell type. Left-graph P values from left to right: ** $P = 0.0011$; *** $P = 0.0002$; *** $P = 0.0002$; ns, not significant. Right-graph P values from left to right: *** $P = 0.0002$; *** $P = 0.0001$; *** $P = 0.0001$; ** $P = 0.0023$; ns, not significant (Mann-Whitney test). Error bars represent SEM.

(Fig. 5, B and C). Similarly, although NAT10 depletion strongly decreased the proportion of HGPS cells positive for senescence-associated β -galactosidase, this was not the case when NUP153 or TNPO1 was codepleted with NAT10 (Fig. 5, D and E). These results indicated that the normalization of phenotypes caused by NAT10 depletion in HGPS cells indeed occurred through restoration of TNPO1 pathway function. Thus, when TNPO1 or its cargo NUP153 is absent, NAT10-dependent phenotypic rescue does not occur.

TNPO1 or NUP153 depletion in control fibroblasts also promoted senescence, implying that the TNPO1 pathway is important for maintaining cellular homeostasis.

TNPO1 is required for NAT10 inhibition-mediated gene expression modulation

In light of the above observations, we hypothesized that enhancing TNPO1-mediated nuclear import in HGPS cells by inhibiting NAT10 might have an impact on gene expression. To test this idea, we performed global gene expression analysis on HGPS fibroblasts compared to control fibroblasts, both after 8 weeks of dimethyl sulfoxide (DMSO)-only or remodulin treatment (medium was supplemented with fresh remodulin every 3 days). Volcano plots of each individual differential expression analysis (Fig. 6A) show \log_{10} -adjusted P values plotted against \log_2 -fold gene expression changes. Significant genes [based on a fold change threshold of twofold (up or down) and an adjusted P value threshold of 0.05] are indicated by the red (increased expression) and blue (decreased expression) regions of the plots. As expected and as previously reported (32, 36, 42), we observed deregulation of gene expression in HGPS cells compared to control fibroblasts [Fig. 6, A (left panel) and B (row 1)], with 137 and 221 genes showing significantly increased or decreased expression, respectively. Notably, chronic long-term treatment with remodulin had very little effect on gene expression in control fibroblasts, with only 17 genes exhibiting significantly increased or decreased expression (Fig. 6A). In marked contrast, remodulin had a strong effect on HGPS cells (Fig. 6A), modifying the expression of 652 genes (table S1). Strikingly, these remodulin-induced gene expression changes in HGPS cells were largely reciprocal to the gene expression differences between HGPS cells and unaffected cells (Fig. 6B). Thus, long-term remodulin treatment had a strong rebalancing effect on the expression of most genes that are deregulated in HGPS cells.

Next, we asked whether the gene expression changes mediated by NAT10 inhibition would still occur in the absence of TNPO1. To address this question, we performed a separate microarray analysis after short-term depletion of NAT10, TNPO1, or both NAT10 and TNPO1 by siRNA. We used NAT10 siRNA instead of remodulin in this experiment to enable rigorous comparison to siRNA-mediated depletion of TNPO1. Because of the shorter time frame of siRNA experiments, we applied a lower threshold (1.5-fold up or down) in

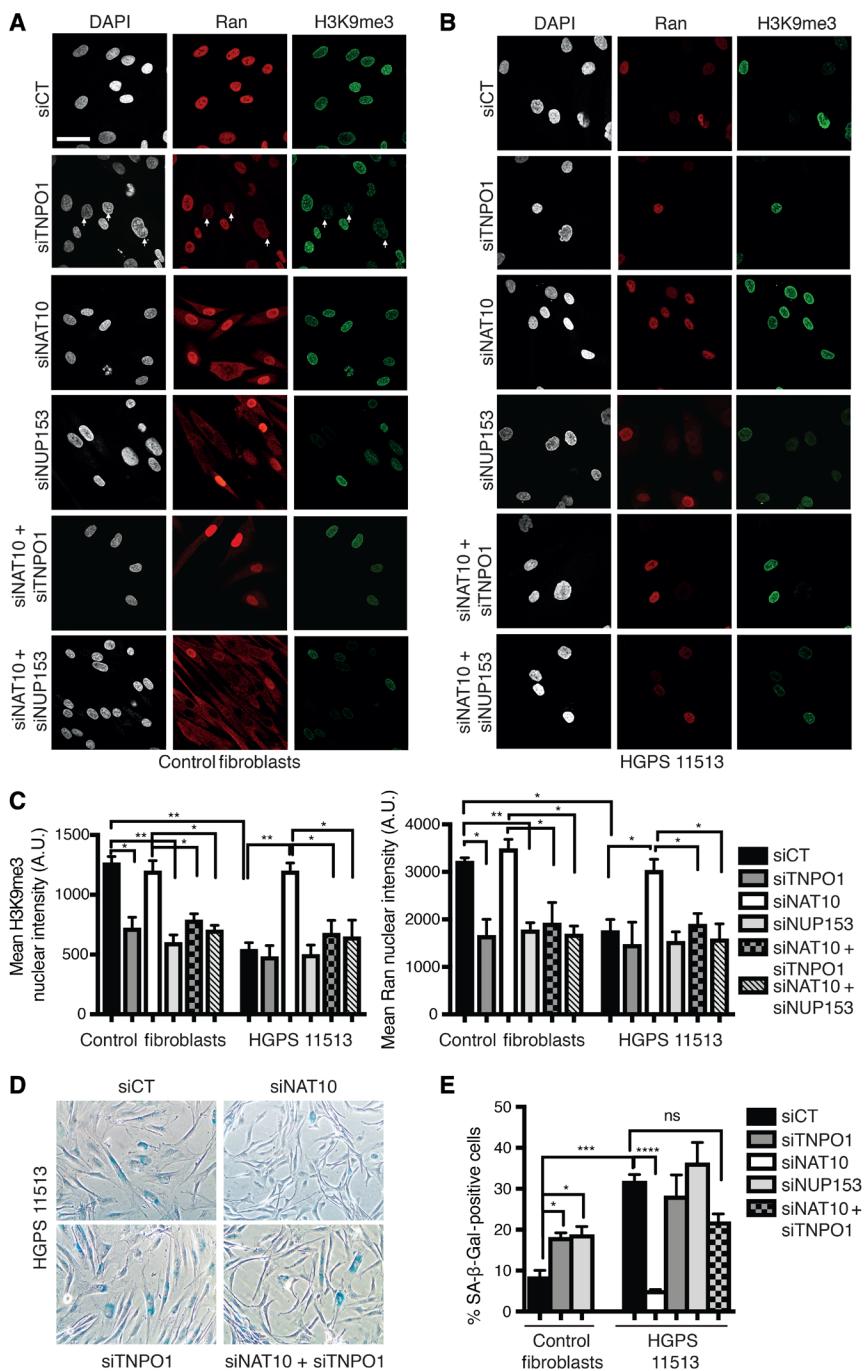


Fig. 5. TNPO1 is required for cellular normalization mediated by NAT10 inhibition. (A and B) Representative immunofluorescence images showing Ran and H3K9me3 staining in control (A) or HGPS (B) fibroblasts after transfection with the indicated siRNAs (see fig. S10 for protein knockdown efficiency). $n = 3$ independent experiments. Scale bar, 50 μ m. siTNPO1, siRNA-mediated depletion of TNPO1. (C) Quantification of H3K9me3 and Ran nuclear intensity from the cells in (A) and (B). $n = 3$ independent experiments, $n > 150$ cells per experiment for each cell type. P values from left to right: (H3K9me3 in control fibroblasts) * $P = 0.0195$, ** $P = 0.0087$, * $P = 0.0315$, * $P = 0.0197$, ** $P = 0.0064$; (H3K9me3 in HGPS fibroblasts) ** $P = 0.0097$, * $P = 0.03$, * $P = 0.0393$; (Ran in control fibroblasts) * $P = 0.025$, ** $P = 0.0075$, * $P = 0.0443$, * $P = 0.011$, * $P = 0.015$; (Ran in HGPS fibroblasts) * $P = 0.0342$, * $P = 0.0407$, * $P = 0.0368$ (unpaired t test). Error bars indicate SEM. (D) Senescence-associated β -galactosidase staining in HGPS fibroblasts that had been treated with the indicated siRNAs. (E) Quantification of senescence-associated β -galactosidase staining in (D). $n = 3$ independent experiments, $n > 200$ cells per experiment for each cell type. P values from left to right: * $P = 0.0107$, * $P = 0.049$, *** $P = 0.0001$; **** $P < 0.0001$; ns, not significant (unpaired t test). Error bars indicate SEM.

this analysis, which increased the number of deregulated genes in HGPS cells treated with a control siRNA (Fig. 6C) compared to earlier experiments with remodelin (Fig. 6A). Similar to remodelin treatment, NAT10 depletion modified the expression of more genes in HGPS cells than it did in control fibroblasts (Fig. 6C and table S2) and had a stronger effect than remodelin on control fibroblasts. siRNA-mediated NAT10 depletion abolished all functions of NAT10, such as its RNA helicase activity and its role in ribosome biogenesis (43). Although we did observe a rebalancing effect on some HGPS-deregulated genes (Fig. 6D), short-term NAT10 depletion by siRNA treatment did not have comparable effects to remodelin treatment on global gene expression rebalancing (Fig. 6D). This suggests that long-term NAT10 inhibition is required to observe global rebalancing of the HGPS gene expression profile.

To assess whether gene expression changes mediated by NAT10 depletion were occurring through TNPO1, we compared NAT10 depletion alone with simultaneous depletion of NAT10 and TNPO1 (Fig. 7A). We observed that 70% of the genes showing significantly increased or decreased expression upon NAT10 depletion alone in control fibroblasts were not modified anymore upon simultaneous depletion of TNPO1 (Fig. 7B and tables S3 and S4), despite similar NAT10 protein knockdown (Fig. 7A). In HGPS cells, this number was reduced to 40% (Fig. 7C), likely a result of the deregulation of additional pathways. However, because we observed that the TNPO1 pathway was required for the NAT10 inhibition-mediated phenotypic changes in HGPS cells, we hypothesized that this specific set of genes (Fig. 7C) would be mainly responsible for the HGPS cellular rescue. Gene ontology analysis of those genes significantly affected by NAT10 depletion only (table S5) and not by simultaneous depletion of NAT10 and TNPO1 in HGPS cells showed an enrichment for various cellular components (Fig. 7D), including cell-substrate junctions and focal adhesions, extracellular matrix, and endoplasmic reticulum for genes showing decreased expression or Golgi and small ribosomal subunits for genes showing increased expression. When we plotted this specific set of genes that we refer to as “siNAT10-only” genes on a heat map (Fig. 7E), we observed that the expression of about 40% of this specific set of genes was rebalanced toward the expression observed in control fibroblasts (Fig. 7E).

Finally, to assess whether the TNPO1 pathway was affected in HGPS, we compared the effect of TNPO1 depletion on gene expression profiles in control fibroblasts (Fig. 7F) with gene expression in HGPS fibroblasts treated with a control siRNA compared to control fibroblasts treated with a control siRNA (Fig. 7F). This showed that 58% of the genes

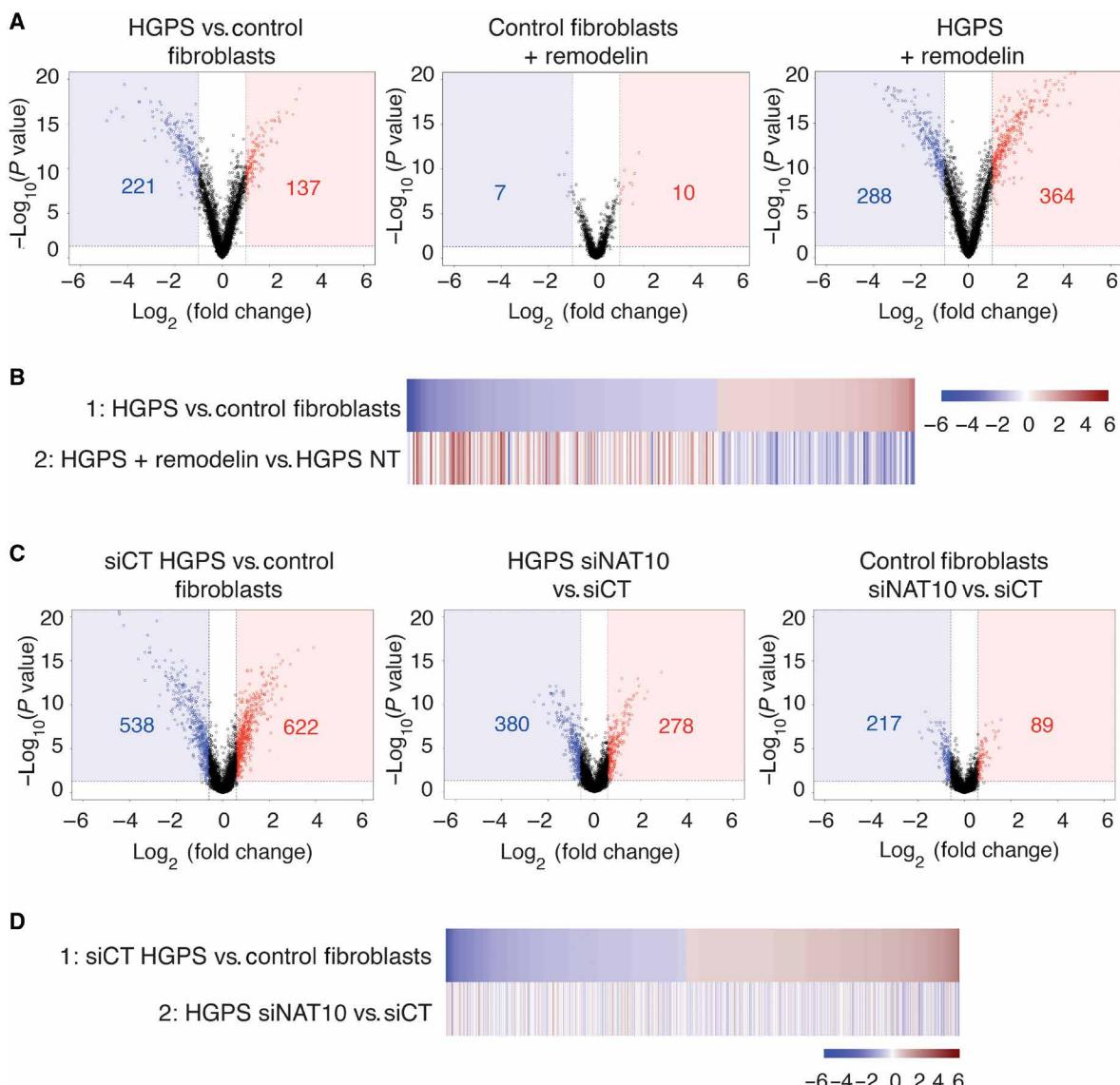


Fig. 6. Effects of NAT10 inhibition or depletion on gene expression. (A) Volcano plots from the microarray analysis showing the number of genes with decreased (blue) or increased (red) expression in HGPS fibroblasts compared to control fibroblasts, in control fibroblasts treated with remodelin, and in HGPS fibroblasts treated with remodelin. $n = 3$ independent experiments. The \log_{10} of the adjusted P value is used such that the more significant a gene's expression is, the higher it will appear on the y axis. The \log_2 of the fold change is used such that genes with increased (red) or decreased (blue) expression have equal weighting in the plot. Genes were classified as showing gene expression changes if they showed a change of twofold (up or down) or greater with an adjusted P value less than or equal to 0.05. For both control and HGPS fibroblasts, remodelin was used as a chronic treatment for 8 weeks (middle and right panels). (B) Heat maps of genes with significantly altered expression from (A) showing the effect of remodelin treatment on gene expression in HGPS cells. NT, nontreated. (C) Volcano plots from the microarray analysis showing the number of genes for which expression was decreased (blue) or increased (red) by 1.5-fold or greater under the indicated conditions. siNAT10 and siCT treatment were transient (5 days). $n = 3$ independent experiments. (D) Heat maps of genes presented with significantly altered expression from (C) showing the effect of NAT10 depletion on gene expression in HGPS cells.

exhibiting significantly increased or decreased expression upon TNPO1 depletion in control fibroblasts showed a similar trend in HGPS cells treated with control siRNA (Fig. 7F). TNPO1 depletion can be expected to have differential effects compared to a change of TNPO1 subcellular localization as observed in HGPS, where the protein is still expressed but is mislocalized (Fig. 7, A and F). Moreover, short-term TNPO1 depletion by siRNA might not recapitulate the effects of long-term decreased nuclear abundance of TNPO1 observed in HGPS cells. These results, however, support our hypothesis that the TNPO1 pathway is affected in HGPS and that NAT10 inhibition mediates gene expression changes, at least in part through

TNPO1. Collectively, our results suggest that NAT10 inhibition enhances global HGPS cell fitness by restoring TNPO1 function, thus rebalancing nucleocytoplasmic transport, which restores normal chromatin organization and control of gene expression and prevents premature entry into senescence.

DISCUSSION

TNPO1 dysfunction in HGPS drives nuclear Ran defects

Here, we established that the TNPO1 nuclear importer is mislocalized in cells derived from patients with HGPS. Furthermore, we found

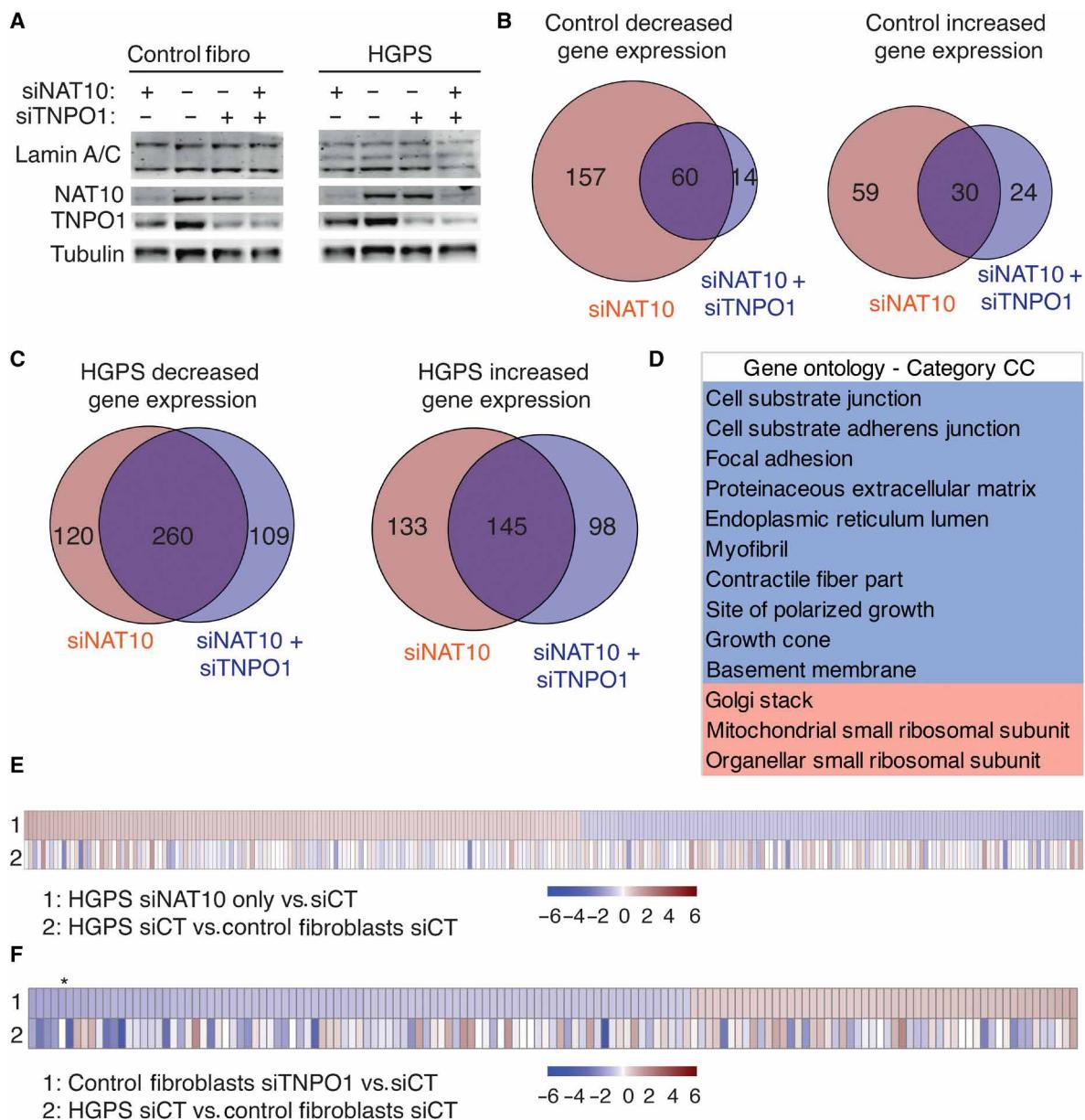


Fig. 7. NAT10 modulates gene expression through TNPO1. (A) Representative Western blot analysis showing the efficiency of siNAT10 and siTNPO1. $n = 2$ independent experiments. (B and C) Venn diagrams generated from the microarray analysis showing the overlap between genes with significantly increased or decreased expression upon NAT10 depletion alone or together with TNPO1 depletion (siNAT10 + siTNPO1) in control (B) and HGPS (C) fibroblasts. (D) Gene ontology analysis showing cellular component (CC) terms enrichment from genes with significantly decreased (blue) or increased (red) expression only upon NAT10 depletion in HGPS cells. (E) Heat maps of siNAT10-specific genes presented in (C) and (D), showing the gene expression changes for genes modified by siNAT10 only (compared to siCT) in HGPS cells (row 1) and their expression in HGPS cells compared to control fibroblasts (row 2). (F) Heat maps of genes with expression significantly modified by TNPO1 depletion in control fibroblasts (row 1) and their expression in HGPS cells compared to control fibroblasts (row 2). TNPO1 is indicated by an asterisk. All analyses were performed using three biological replicates.

that defective nuclear import of TNPO1 cargo proteins including NUP153 (24) and hnRNPA1 (27) appeared to contribute to downstream phenotypic defects in HGPS cells, including abnormal nuclear pore assembly, chromatin disorganization, gene expression changes, and premature entry into senescence. Although previous studies had reported RanGTP gradient defects in cells from HGPS patients (9, 10), the mechanism behind it was still unclear. Given the known link between oxidative stress and nucleocytoplasmic trans-

port [as reviewed in (44)], and because oxidative stress is higher in HGPS cells (11), it was thought to be the main cause of decreased nuclear Ran abundance in progeria. However, oxidative stress has been shown to affect classical importin-mediated nuclear import pathways (45), whereas we found here that the TNPO1 pathway appeared to be most affected in HGPS. Therefore, on the basis of our findings herein, we suggest that depletion of Ran from the nucleus in HGPS cells is a consequence of dysfunctional TNPO1 rather than

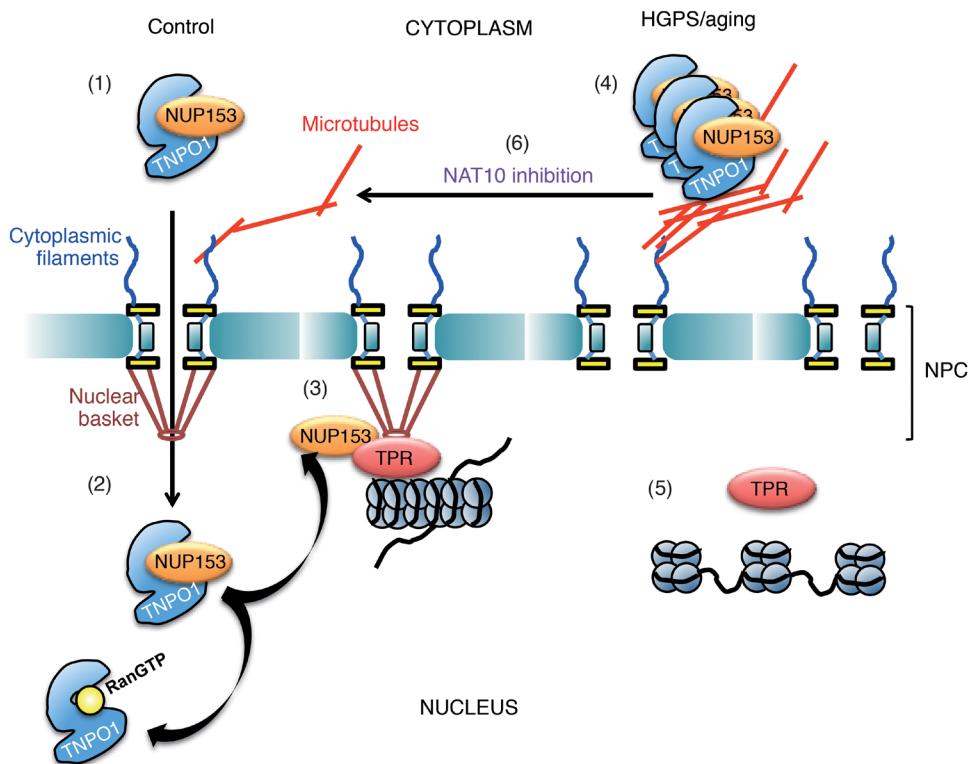


Fig. 8. Model for how NAT10 inhibition rescues HGPS phenotypes. (1) In control cells, TNPO1 binds to its cargo proteins, such as NUP153, in the cytoplasm. (2) The TNPO1-cargo complex translocates into the nucleus through the nuclear pore. (3) Once in the nucleus and upon RanGTP binding to TNPO1, the NUP153 cargo dissociates from TNPO1 and is incorporated into the NPC and recruits TPR to the NPC. Mature, fully functional NPCs mediate nucleocytoplasmaic transport and other roles, such as anchoring chromatin to the nuclear envelope through TPR. (4) In HGPS and aging, increased microtubule network stability sequesters TNPO1 in the cytoplasm. (5) TNPO1 cargos such as NUP153 are not properly imported into the nucleus. The absence of NUP153 results in decreased nuclear Ran abundance and defects in TPR anchorage at NPCs. This leads to chromatin disorganization, global gene expression changes, and premature entry into senescence. (6) By destabilizing the microtubule network, NAT10 inhibition releases TNPO1 from the cytoplasm, enhancing its nuclear translocation together with nuclear import of its cargos such as NUP153 and hnRNPA1. This then substantially normalizes chromatin organization, RanGTP-mediated nucleocytoplasmaic transport, and global transcription patterns.

a direct effect of the presence of progerin, oxidative stress, or NUP153 deregulation. In addition, by showing that NAT10 inhibition restores TNPO1-dependent nuclear import by releasing TNPO1 from the cytoplasm of HGPS cells, we have provided insights into how NAT10 inhibition, through its effects on microtubule stability, ameliorates cellular defects of HGPS cells. As described previously (21), NAT10 inhibition does not affect progerin abundance or localization, suggesting that its effects on HGPS cells occur through a progerin-independent pathway.

It is still unclear how NAT10 activity might increase during normal and premature aging. However, it has been suggested that NAT10 activity is modulated by autoacetylation and that sirtuin proteins can mediate NAT10 deacetylation (46). Sirtuin proteins have been linked to various age-related pathologies [reviewed in (47)], with their decreased activity being associated with senescence. In this context, reactivation of SIRT1 has been shown to extend health and life span in an HGPS mouse model (48). Therefore, we speculate that decreased sirtuin activity as occurs in aging might lead to increased NAT10 acetylation and activity, therefore contributing to increased microtubule stability and retention of TNPO1 in

the cytoplasm. Whether the acetylation of other reported NAT10 substrates such as p53 (49), the upstream binding factor (50), and ribosomal RNA (51) is also affected in HGPS warrants investigation.

Microtubule stability modulates nucleocytoplasmaic transport

On the basis of our findings, we propose a model for how TNPO1-mediated nuclear import affects the nucleocytoplasmaic RanGTP gradient and how sequestration of TNPO1 in the cytoplasm perturbs this process (Fig. 8). Step 1: Under normal circumstances, TNPO1 binds to specific cargo proteins, such as NUP153, in the cytoplasm. Step 2: The TNPO1-cargo complex is translocated into the nucleus through the nuclear pore, which is anchored to microtubules and microfilaments in the cytoplasm (52). Step 3: Once in the nucleus and upon RanGTP binding to TNPO1, the NUP153 cargo dissociates from TNPO1 and is incorporated as a component of the NPC basket structure, which is responsible for anchoring TPR (18, 19), the last nucleoporin to be assembled on the NPC (53). The resulting mature NPC is then fully functional for nucleocytoplasmaic transport and other NPC-dependent roles, such as anchoring chromatin. Step 4: During the progression of HGPS and during aging of unaffected cells, increased microtubule network stability appears to sequester TNPO1 and its cargo proteins in the cytoplasm. Step 5: As a consequence, TNPO1 cargos, such as NUP153, are not imported properly into the nucleus, which prevents

NUP153 from being incorporated efficiently into NPCs. This in turn affects the nuclear import of Ran, resulting in decreased nuclear Ran abundance, as well as defects of TPR anchorage at NPCs. Consequently, during the progression of HGPS and upon normal cell aging, there is progressive chromatin disorganization and decompaction, global gene expression changes, and premature entry into senescence. We speculate that the global dysregulation of gene expression observed in HGPS cells is probably a consequence of a direct effect both of abnormal nuclear architecture on chromatin organization and of nucleocytoplasmaic transport defects affecting the nuclear import of various proteins, including TPR, which directly binds chromatin (39) and hnRNPA1, which modulates the activity of transcription factors (28–30). Step 6: By destabilizing the microtubule network (21), NAT10 inhibition releases TNPO1 from the cytoplasm in HGPS or in cells from unaffected aged individuals, thus enhancing its nuclear translocation together with the ensuing nuclear import of its cargos such as NUP153 and hnRNPA1. This then substantially normalizes chromatin organization, Ran-mediated nucleocytoplasmaic transport, and global transcription patterns. In support of this model, wherein NAT10 inhibition ameliorates HGPS phenotypes through

the TNPO1 pathway, we found that NAT10 inhibition had little effect on HGPS phenotypes when the cells had been depleted of TNPO1.

In addition to helping to explain the mechanistic basis for HGPS cell dysfunction, our study highlights how specific components of the nucleocytoplasmic transport machinery are affected by a range of factors, in this case, cytoskeletal integrity. This correlates with the finding that mechanical forces can modulate nuclear pore opening and conformation, thereby modifying nucleocytoplasmic transport efficiency (54). Finally, our study underscores the potential for NAT10 inhibition as a possible therapeutic strategy for HGPS and perhaps also for certain pathologies associated with aging.

MATERIALS AND METHODS

Cell culture and transfections

Control skin primary fibroblasts from a young healthy individual (GM03440; 20 years old), from two HGPS patients (AG11498; 14 years old and AG11513; 8 years old), and from four aged individuals (AG11240; 81-year-old female, AG04059; 96-year-old male, AG05248; 87-year-old male, and AG09602; 92-year-old female) were purchased from Coriell Cell Repositories and used in passage-matched cells (unless indicated otherwise on the figure) between P9 and P25. Cells were grown in Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS; BioSera), 2 mM L-glutamine, penicillin (100 U/ml), and streptomycin (100 µg/ml). Normal RPE-1 cells were grown in DMEM-F12 (Sigma-Aldrich) supplemented with 10% FBS (BioSera), 2 mM L-glutamine, penicillin (100 U/ml), and streptomycin (100 µg/ml). For serum starvation experiments, the same medium was used without any FBS. The following siRNA duplexes were purchased from Life Sciences: NAT10 stealth RNA interference (RNAi), GAGCAUGGACCUCUCUGAAUA-CAUA; and stealth RNAi negative control duplexes. TNPO1 siRNAs were from Sigma-Aldrich: GCAAAGAUGUACUCGUAAG, GUAUAGAGAUGCAGCCUUA, and GUAAAUACCAGCAUAAGAA. SMARTpool NUP153 and NTF2 siRNA oligonucleotides were purchased from Dharmacon. siRNA transfections were carried out using Lipofectamine RNAiMAX (Life Sciences), following the manufacturer's instructions. Cells were analyzed 48 to 72 hours after transfection. U2OS human osteosarcoma cells were grown in DMEM supplemented as above. A plasmid encoding the TNPO1 inhibitor Myc-MBP-M9M (41) was transfected using Lipofectamine 2000 (Life Sciences), following the manufacturer's instructions. Cells were analyzed 24 hours after transfection.

Cell proliferation

Nontransformed human RPE-1 cells were plated at equivalent low densities and transfected with siCT or siTNPO1 as explained above. Six days later, cells were fixed and stained with crystal violet staining solution (0.5% crystal violet, 20% methanol).

Cell growth inhibition

RPE-1 cells or control fibroblasts were kept in either normal medium supplemented with 10% FBS or in medium without serum to arrest cell proliferation. After 4 days of serum deprivation, cells were processed for immunofluorescence.

Drug treatments

Remodelin was synthesized as described previously (21) and added to cell cultures to a final concentration of 1 µM for at least 5 days,

replacing the medium every 3 days. For immunofluorescence assays, nocodazole and tubacin were added to the medium for 16 hours at 100 ng/ml and 10 µM, respectively.

Senescence assay

Senescence was assessed 72 hours after siRNA transfection using the Senescence β-Galactosidase Staining Kit no. 9860 from Cell Signaling Technology. Blue cells were counted using the Cell Counter plugin from ImageJ.

Immunoblotting

Total cell extracts were prepared by scraping cells from culture plates in SDS lysis buffer [4% SDS, 20% glycerol, and 120 mM tris-HCl (pH 6.8)], boiling for 5 min at 95°C, followed by 10 strokes through a 25-gauge needle. Before loading, lysates were diluted with a solution of 0.01% bromophenol blue and 200 mM dithiothreitol and boiled for 5 min at 95°C. Proteins were resolved by SDS-polyacrylamide gel electrophoresis on 4 to 12% gradient gels (NuPAGE, Life Sciences) and transferred onto nitrocellulose membrane (Protran, Whatman). Secondary antibodies conjugated to IRDye 800CW were from LI-COR Biosciences and used at 1:10,000. Detection was performed with an imager (Odyssey, LI-COR Biosciences).

Subcellular fractionation

Cells were harvested with trypsin-EDTA and centrifuged at 500g for 5 min. Cells were washed once with 1 ml of ice-cold phosphate-buffered saline (PBS) and centrifuged at 500g for 5 min. Supernatant was removed, and 20 µl of packed cell volume was processed for subcellular fractionation using the kit for cultured cells from Thermo Fisher Scientific (catalog no. 78840) using the manufacturer's instructions. Subcellular fractions were then processed for immunoblotting as described above. Protein compartmentalization in chromatin and in cytoskeletal fractions was assessed by detection of the histone H3 and α-tubulin proteins.

Immunofluorescence

Cells were washed with PBS and fixed for 20 min with 2% paraformaldehyde in PBS. Cells were permeabilized for 5 min with PBS/0.2% Triton X-100 and blocked with PBS/0.2% Tween 20 containing 5% bovine serum albumin. Coverslips were incubated for 1 hour with primary antibodies and for 30 min with appropriate secondary antibodies coupled to Alexa Fluor 488, 594, or 647 fluorophores (1:1000; Life Technologies) before being incubated with DAPI (2 µg/ml). Pictures were acquired with a Fluoview 1000 confocal microscope (Olympus) using the same laser power for matching images and antibodies. All the immunofluorescence experiments were performed at least three times independently, and the pictures shown in the figures are representative images of the three experiments. Image analyses were carried out with Volocity software (PerkinElmer) using the following protocol: find objects (DAPI); subpopulation, fill holes in objects; exclude touching edge of image; exclude objects by size, <100 µm²; measure. The fluorescence intensity in each channel was then recorded.

Proximity ligation assay

The PLAs were carried out using the Duolink In Situ Red Starter Kit Mouse/Rabbit (DUO9101, Sigma-Aldrich) following the manufacturer's instructions. Image analysis was carried out with Volocity software (PerkinElmer) using the following protocol: find objects (DAPI);

exclude objects >1000 and $<20 \mu\text{m}^2$; fill holes in objects; exclude touching edge of image; find spots using intensity; compartmentalize, divide spots—between nuclei; select region of interest; and record the number of PLA spots.

Primary antibodies

All of the antibodies used in this study and the concentrations at which they were used for immunofluorescence and Western blotting are summarized in table S6.

MNase digestion sensitivity assay

Because this assay requires a lot of cells, we could not perform it with HGPS cells and thus used human osteosarcoma U2OS cells instead. Cells (1×10^6) were trypsinized, harvested, and washed once with 1 ml of 1× RSB buffer [10 mM tris (pH 7.6), 15 mM NaCl, and 1.5 mM MgCl₂]. After centrifugation (300g), the cell pellet was resuspended in 1 ml of 1× RSB buffer with 1% Triton-X 100 and homogenized by five strokes with a loose-fitting glass pestle to release nuclei. Nuclei were collected by centrifugation (13,000g) and washed twice with 1 ml of buffer A [15 mM tris (pH 7.5), 15 mM NaCl, 60 mM KCl, 0.34 M sucrose, 0.5 mM spermidine, 0.15 mM spermine, 0.25 mM phenylmethylsulfonyl fluoride, and 0.1% β -mercaptoethanol]. Nuclei were resuspended in buffer A and aliquoted into 100 μl of aliquots. CaCl₂ (1.2 μl of 0.1 M) was added to each aliquot, and nuclei were digested by addition of 0.25 μl of MNase (200 U/ml; Sigma-Aldrich) and incubated at 30°C. Each aliquot was put back on ice at different time points, and digestion was immediately stopped by addition of 3 μl of EDTA. DNA was purified using the Qiagen PCR Purification Kit, and 1500 ng of DNA was analyzed on a 1.5% agarose gel. Digestion profiles were analyzed using ImageJ, and values were adjusted relative to the global intensity of each lane to compensate for DNA loading variations.

Microarray analysis

Long-term remodelin treatment for microarray experiments was performed by keeping and passaging cells in medium containing 1 μM remodelin or DMSO only for 12 population doublings. Three independent biological replicates were used for each condition. RNA extracted from cells was purified using a Direct-zol RNA MiniPrep kit (catalog no. R2072, Zymo Research) and treated with deoxyribonuclease I (Zymo Research), according to the manufacturer's instructions. RNA quality was assessed using the TapeStation (Agilent Technologies) and Qubit (Invitrogen). Quality analysis of total RNA, hybridization, and data capture were performed at the Cambridge Genomic Services. Illumina HumanHT-12 v4 beadchips were used for gene expression screening. Results were analyzed using the *Lumi* (55) Bioconductor package in R. Probe-level data were combined to give gene-level data by taking the mean of all probes for a single gene. Differentially expressed genes were identified in each cell line using *limma* (56), using a two-way factorial design with disease state as the first factor (HPGS versus control fibroblasts) and treatment as the second factor (remodelin versus DMSO or siNAT10/siTNPO1/ both versus siCT). Differentially expressed genes were calculated by performing pairwise comparisons between the four groups (treated normal, untreated normal, treated HPGS, and untreated HPGS) for each treatment. All resulting *P* values were corrected for multiple testing by using the Benjamini and Hochberg false discovery rate correction (52). Genes were defined as showing significantly altered expression if they showed a fold change greater than some threshold

value [2-fold (up or down) for remodelin, 1.5-fold (up or down) for siRNA] compared to control, with an adjusted *P* value lower than 0.05.

SUPPLEMENTARY MATERIALS

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Fig. S1. NAT10 inhibition rescues impaired TPR nuclear localization in cells from aged individuals.

Fig. S2. Nuclear import is affected in HPGS.

Fig. S3. TNPO1-dependent protein cargos are affected in HPGS cells.

Fig. S4. NAT10 nuclear import does not depend on TNPO1.

Fig. S5. Cell proliferation inhibition does not affect the TNPO1 pathway.

Fig. S6. NAT10 localizes both in the nucleus and in the cytoskeleton.

Fig. S7. TNPO1 accumulates at the microtubule network of HPGS cells.

Fig. S8. Microtubule destabilization rescues TNPO1 pathway defects in HPGS cells and in cells from aged individuals.

Fig. S9. NUP153 depletion does not affect the TNPO1 pathway.

Fig. S10. Efficiency of protein depletions.

Table S1. List of genes modified by remodelin treatment.

Table S2. List of genes modified by NAT10 depletion.

Table S3. List of genes modified by depletion of TNPO1 and NAT10.

Table S4. List of genes modified by TNPO1 depletion.

Table S5. Gene ontology analysis of siNAT10-only genes.

Table S6. Primary antibodies.

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received consulting fees over the past 3 years from Carrick Therapeutics, which is engaged in activities related to the subject matter of this study. All other authors declare that they have no competing interests. **Data and materials availability:** The small molecule remodelin used in this study was synthesized by R. Rodriguez (Curie Institute, Paris) and obtained under a material transfer agreement through the CNRS. The raw data and processed differential expression tables from the microarray are available from ArrayExpress under accession no. E-MTAB-6651 (www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-6651/).

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Inhibition of the acetyltransferase NAT10 normalizes progeric and aging cells by rebalancing the Transportin-1 nuclear import pathway

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Rescuing premature aging

Cells from patients with Hutchinson-Gilford progeria syndrome (HGPS) have defects in nuclear architecture and function that lead to premature cellular senescence, aging, and early death. Larrieu *et al.* (see the Focus by Wilson) found that inhibition or depletion of the acetyltransferase NAT10 rescued many of the phenotypes of HGPS patient cells by destabilizing microtubules, which reversed the abnormal cytoplasmic accumulation of the nuclear importer Transportin-1 (TNPO1). This restored proper assembly of the nuclear pore complex, import of nuclear proteins, chromatin organization, and gene expression patterns. Together, these effects delayed the premature senescence of HGPS cells. Cells from aged individuals also showed cytoplasmic retention of TNPO1, suggesting that targeting NAT10 might be a clinical strategy for treating both HGPS and age-related pathologies.

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