**The BAF A12T mutation reduces BAF affinity to lamin A/C, preventing its recruitment to nuclear ruptures in Nestor Guillermo Progeria Syndrome cells.**

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## Abstract

The premature aging disorder Nestor Guillermo Progeria Syndrome (NGPS) is caused by a homozygous Alanine to Threonine mutation at position 12 (A12T) in Barrier-to-Autointegration Factor (BAF). BAF is a small essential protein binding to DNA and to various proteins, thereby playing a role in various cellular processes including transcription regulation and nuclear envelope reformation after mitosis. More recently, BAF was identified as an important factor for nuclear envelope repair upon rupture in interphase. However, the mechanism by which the BAF A12T mutation causes NGPS has remained unclear. To investigate the effects of this mutation on nuclear envelope integrity, we used NGPS-derived patient cells and engineered an isogenic cell line by reversing the BAF A12T homozygous mutation using CRISPR/Cas9. Using a combination of cellular models, structural data and in vitro assays, we identified that the A12T mutation reduces the binding affinity of BAF to lamin A/C by tenfold. As a result, BAF A12T is unable to recruit lamin A/C to sites of nuclear envelope rupture. This leads to the persistence of lamina gaps at sites of ruptures that could contribute to nuclear fragility in NGPS patients. Overexpression of wild-type BAF in a NGPS context rescues lamin A/C recruitment to sites of nuclear rupture, which could explain why the heterozygous A12T mutation does not cause premature ageing.

## Introduction

The nuclear envelope (NE) is a critical double membrane structure that surrounds and encloses the nucleus, maintaining the organisation of the chromatin (Dechat et al., 2009; Taddei et al., 2004), controlling nucleocytoplasmic transport and allowing the transduction of mechanical signals from the cytoplasm into the nucleus (Crisp et al., 2006; Lombardi et al., 2011). The NE is made of the inner and outer nuclear membranes (INM and ONM). The lamina, which is a meshwork of intermediate filaments of A-type (lamins A and C) and B-type (lamin B1 and B2) lamin proteins, lies at the nucleoplasmic side of the INM (Gruenbaum et al., 2005). It interacts with the chromatin, with LEM domain proteins which are embedded in the INM, and with other nuclear proteins. These interactions play critical roles in maintaining the structural integrity of the nucleus (Dechat et al., 2008).

The importance of the lamina is evident by the numerous diseases arising from mutations in the *LMNA* gene (encoding for lamin A/C proteins) or in associated NE proteins. These mutations compromise the integrity of the lamina and of the NE, causing a range of

laminopathies (Cohen et al., 2008; Worman and Bonne, 2007) including premature ageing syndromes, muscular dystrophies and neuropathies (De Sandre-Giovannoli et al., 2002; De Sandre-Giovannoli et al., 2003; Goldman et al., 2002). One of the consequences of NE destabilisation is the appearance of NE ruptures that cause loss of nuclear compartmentalization. This has been observed in laminopathy patient cells and animal models (De Vos et al., 2011; Kim et al., 2021; Muchir et al., 2004), in cells undergoing a viral infection (Cohen et al., 2011; de Noronha et al., 2001) or lacking specific components of the lamina (De Vos et al., 2011; Earle et al., 2020). Additionally, mechanical stress can cause NE rupture, for example in vivo when cells are migrating through dense tissues (Denais et al., 2016; Raab et al., 2016; Xia et al., 2018), or in vitro when cells are cultured in 2D on stiff substrates (Tamiello et al., 2013). A rupture of the NE is typically preceded by the formation of a gap in the nuclear lamina, generating a weak point at the NE, more prone to deformation by mechanical stress (Hatch and Hetzer, 2014; Le Berre et al., 2012). This allows the formation of a protrusion of the nuclear membrane that under continued mechanical stress will grow and eventually rupture (Deviri et al., 2019; Raab et al., 2016; Xia et al., 2018). The exposure and leakage of the nuclear content - including the chromosomal DNA - to the cytoplasmic compartment can cause DNA damage (Denais et al., 2016; Pfeifer et al., 2018; Raab et al., 2016) and activation of innate immune signalling pathways, such as cGAS/STING that can trigger inflammation (Ablasser and Chen, 2019; Denais et al., 2016; Guey et al., 2020; Raab et al., 2016). Importantly, the cells are able to detect and reseal the NE rupture within minutes, through the recruitment of several proteins to the site of rupture. These include Barrier-to-autointegration factor (BAF), LEM domain proteins (including emerin) and ESCRT-III components (Denais et al., 2016; Halfmann et al., 2019; Raab et al., 2016; Robijns et al., 2016; Young et al., 2020a). Finally, lamin A/C accumulates at the sites of ruptures, leaving behind a lamin "scar" believed to stabilise the NE and prevent further ruptures (Denais et al., 2016; Le Berre et al., 2012; Xia et al., 2018).

BAF is a small (89 amino acid) protein that localizes to the nucleus, at the NE and in the cytoplasm. BAF dimerization allows for its binding to LEM domain proteins and the Ig-fold domain of lamin A/C (Cai et al., 2007; Samson et al., 2018), while each individual subunit can bind dsDNA (Bradley et al., 2005; Zheng et al., 2000). BAF has been previously involved in the regulation of transcription, viral defense and postmitotic nuclear reassembly (de Oca et al., 2011; Gruenbaum et al., 2005; Haraguchi et al., 2001; Jamin et al., 2014). More recently, BAF was found to play a direct role in repairing ruptures of the NE by recruiting LEM domain proteins and membrane to the sites of rupture (Halfmann et al., 2019; Young et al., 2020a).

The interest around the function of BAF at the NE has grown since an Alanine to Threonine homozygous point mutation at position 12 in BAF (Ala12Thr – BAF A12T) was identified to be the cause of a new premature aging disorder termed Nestor-Guillermo Progeria Syndrome (NGPS) (Cabanillas et al., 2011; Fisher et al., 2020; Puente et al., 2011). So far, only three NGPS patients have been identified and they all carry the same homozygous A12T mutation, inheriting one mutated copy from each of their parents, both carriers of a heterozygous BAF A12T mutation and devoid of disease. On the contrary to the classic Hutchinson-Gilford Progeria Syndrome (HGPS), caused by heterozygous *LMNA* mutations (De Sandre-Giovannoli et al., 2003; Eriksson et al., 2003), NGPS patients live over 30 years and do not display any vascular or cardiovascular dysfunction. Instead, in addition to aging phenotypes including alopecia, lipodystrophy and joint stiffness, they present with severe osteolysis and bone deformation that is the primary cause of concern in these patients.

The mechanism by which the BAF A12T mutation causes these detrimental effects remains unclear. Therefore, we set out to characterize the effect of the mutation in NGPS-derived patient cells. In order to identify specific cellular phenotypes caused by the A12T mutation, we engineered isogenic cell lines using CRISPR-Cas9 mediated genome editing to reverse the homozygous BAF A12T mutation in an NGPS patient cell line. We found that reversion of the mutation restored emerin localization to the NE, a phenotype previously described in NGPS cells. Furthermore, we identified that while not affecting BAF structure, the BAF A12T mutation reduces the affinity of BAF for lamin A/C by about 10 times *in vitro*. As a consequence, the recruitment of lamin A/C to NE rupture sites is greatly affected in NGPS patient cells, in accordance with recent reports using different cell models (Sears and Roux, 2022). This leads to the persistence of lamina gaps at sites of ruptures, and absence of lamin "scars" that could contribute to nuclear fragility in NGPS patient cells.

**Results**

Whilst the first NGPS patients were described just over ten years ago, the mechanism by which the BAF A12T mutation causes NGPS has remained unclear. Cells from NGPS patients show nuclear abnormalities including the presence of nuclear blebs and the delocalisation of emerin from the NE to the cytoplasm (Loi et al., 2016; Puente et al., 2011). To characterize NGPS patient cell phenotypes, we used hTERT immortalized skin fibroblasts of a healthy age-matched donor (Control) and two NGPS patients (NGPS1 and NGPS2) (gift from C. Lopez-Otin). Microscope visualization of the nuclei in both NGPS patient cells showed clear differences between the cell lines. NGPS1 cells displayed smaller nuclei with nuclear membrane folding (white arrowheads), while NGPS2 cells had bigger nuclei, and discontinuity of the lamin A/C network (magenta arrowheads) without the presence of membrane folds (Figure 1a, b). The control cells also showed some nuclear shape abnormalities with areas of lamin A/C accumulation at the NE (green arrowheads). We then assessed several NE constituents known to be altered in HGPS, senescence or in normal aging. NGPS cells did not show reduction in lamin B1 levels (Figure 1c, d), which has been observed in HGPS (Scaffidi and Misteli, 2005; Taimen et al., 2009) and is associated with senescence (Freund et al., 2012). Similarly, we did not observe any change in the expression levels of lamin A/C or emerin (Figure 1d). In HGPS cells, Sun1 accumulates at the NE and in the golgi, contributing to HGPS pathogenicity (Chen et al., 2012). On the contrary, Sun1 appeared unaffected in NGPS cells (Figure 1c). DNA damage accumulation has been previously associated with various age-related diseases and is also observed in HGPS (Gonzalo and Kreienkamp, 2015). Maybe surprisingly, we did not find increased DNA damage in NGPS cells – as assessed by yH2AX staining, a marker of DNA double-strand breaks (Figure S1). We also assessed the level of H3K9me3, a marker for transcriptionally silent heterochromatin, loss of which is another hallmark of HGPS and aging. The level of H3K9me3 was not affected in NGPS patient cells (Figure 1d). One caveat here is the use of immortalized cells, which could impact on the expression level of some of these markers, however primary NGPS cells could not be obtained due to their inability to grow in culture.

Through these initial studies, and using the only available patient cell lines (derived from the two originally identified NGPS patients), we were thus unable to find highly consistent phenotypes when looking at NE associated readouts in the two NGPS patient cells. Therefore, we decided to engineer an isogenic cell line using CRISPR-Cas9 gene editing by reversing the BAF A12T mutation in NGPS cells. We used NGPS2 as they grew clones more easily from single cells, and used an all-in-one plasmid strategy containing a Cas9 nickase, two sgRNA targeting BAF around the mutation site, and GFP for cell sorting (Figure 2a) (Chiang et al., 2016). The supplied ssODN template for homologous recombination contained the wild type

*BANF1* sequence to correct the mutation and silent mutations in the PAM motifs to prevent recutting after recombination had occurred. In addition, another silent mutation which causes loss of the NcoI restriction site was introduced to facilitate clone screening. After screening ~150 clones, by PCR amplification and sequencing, we identified two separate clones with a homozygous reversion of the A12T mutation (NGPS2 WT clone1-2) (Figure 2b, Figure S2). Using these isogenic cell lines, we confirmed that reversing the BAF A12T mutation improves emerin nuclear/cytoplasmic ratio (Figure 2c-d).

Interestingly, *BANF1* sequencing revealed the presence of an additional and similar deletion in intron 2 in both NGPS patient cell lines (Figure S3a). To check whether this deletion might affect RNA splicing and therefore result in an altered protein, we isolated RNA from both patient cell lines. Sequencing of the generated cDNA showed that this deletion did not affect the mRNA sequence (Figure S3b).

In addition to the previously described delocalization of emerin from the nucleus to the cytoplasm, we found that both NGPS cell lines showed a decrease in nuclear BAF enrichment as observed by immunofluorescence which was rescued by the mutation reversion (NGPS2 WT clone1) (Figure 3a, b). This suggested that BAF levels might be decreased in NGPS patient cells. However, immunoblot analysis instead showed that BAF levels are increased in both NGPS patient cell lines, going down upon mutation reversion (Figure 3c). Phosphorylation of BAF is known to affect its subcellular localization and to regulate its interaction with different protein partners. The decrease of nuclear BAF we observed in NGPS patient cells could thus suggest altered BAF phosphorylation upon A12T mutation. Since BAF is phosphorylated by VRK1 on Ser4 and Thr3 (Marcelot et al., 2021; Nichols et al., 2006), both being close to the mutated A12T site, we speculated that phosphorylation of BAF could be affected by the NGPS mutation.

Using Nuclear Magnetic Resonance (NMR) spectroscopy, we monitored the phosphorylation of BAF WT and BAF A12T *in vitro* over time (Figure 3d-e and Figure S4). On $^{1}$H-$^{15}$N HSQC NMR spectra, each peak reports on the chemical environment of one residue. A change of this residue's chemical environment, due to a phosphorylation for example, will change the position of the corresponding NMR peak on the spectrum. Peaks of phosphorylated serine and threonine appear on the very left side of the spectrum: in general, at more than 9 ppm in the hydrogen dimension. Therefore, we focused our analysis on this area, where very little difference was observed between BAF WT and BAF A12T before VRK1 kinase addition (Figure 3e, top panel). After 15 minutes of *in vitro* phosphorylation reaction of BAF by VRK1, the signal corresponding to phosphorylated Ser4 was visible on both spectra (Figure 3e, middle panel). Similarly, after 8 hours of phosphorylation, the signal corresponding to the phosphorylated Thr3 was visible on both spectra (Figure 3e, bottom panel). This phosphorylation caused a global change in both spectra (Figure S4). Finally, no signal corresponding to another phosphorylated threonine appeared on the BAF A12T spectrum at the end of the reaction and thus the introduced Thr12 is not phosphorylated by VRK1. Altogether, this showed that BAF WT and BAF A12T are phosphorylated in a similar way by VRK1 *in vitro*, i.e. that they are both phosphorylated on Ser4 and Thr3 only, with similar kinetics, and that phosphorylation of Thr3 triggers a global change in their $^{1}$H-$^{15}$N HSQC NMR spectra.

In order to characterize the effect of the A12T mutation on BAF three-dimensional (3D) structure, we solved for the first time the structure of BAF A12T at a resolution of 1.6 Å by X-ray crystallography. In this structure, BAF A12T is bound to the lamin A/C Ig-fold domain (Figure 4a). We compared the obtained structure to that of BAF WT (PDB code: 6GHD) and

found that the structures of BAF WT and BAF A12T overlapped perfectly (CαRMSD = 0.4 Å) with Ala12 or Thr12 being largely exposed to the solvent (Figure 4a). We thus concluded that the A12T mutation does not affect BAF 3D structure. Therefore, we reasoned that it is unlikely to affect other interactions than the ones in direct contact with residue 12. The mutated residue is located at the binding interface with the lamin A/C Ig-fold domain (Figure 4a) and it has therefore been speculated to perturb the interaction between the Ig-fold domain of lamin A/C and BAF (Samson et al., 2018). Additionally, lamin A/C has been reported to be important for retention of BAF in the nucleus (Kone et al., 2022; Lin et al., 2020).

We thus investigated the effect of the BAF A12T mutation on the binding affinity to the lamin A/C Ig-fold domain *in vitro*. Despite an apparent similarity in both complexes , prediction of the binding energies from the 3D structures using the PDBePISA server (Krissinel and Henrick, 2007) suggested that the A12T mutation changes the thermodynamical equilibrium of the system, with the mutant binding with a free energy ΔG that is 1.8 kcal/mol less favourable compared to the WT (Figure S5a). To confirm this prediction experimentally, we first verified by NMR that the A12T mutation causes a significant decrease in BAF affinity for lamin A/C Ig-fold domain (Figure S5d-g), and then determined the dissociation constant ($K_D$) of these interactions using Isothermal Titration Calorimetry (ITC). We fitted our data assuming a stoichiometry of two BAF molecules for one lamin A/C Ig-fold domain, consistent with the crystal structure. We obtained $K_D$ values of 2.7 +/- 1.2 µM and 33 +/- 4 µM for the interactions involving BAF WT and BAF A12T, respectively (Figure 4b and Figure S5b,c), showing that the A12T mutation decreases the affinity of BAF for lamin A/C Ig-fold by about tenfold. The experimental ΔG value is 1.4 kcal/mol, close to that predicted using the PDBePISA server.

To assess whether the reduced interaction between lamin A/C Ig-fold and BAF A12T *in vitro*, was also translated in a cellular context, we used a Proximity Ligation Assay (PLA) which involves the initial recognition of two proteins of interest by antibodies. Upon close proximity of these two proteins, DNA fragments present on secondary antibodies can hybridize and can be detected by an amplification reaction and a specific DNA tagged fluorophore. The PLA foci visualized by microscopy therefore reflect protein interaction in cells. While each of the BAF or laminA/C antibodies on their own gave very little PLA signal as expected, specific PLA foci were detected upon combining the two antibodies, confirming the interaction between the two proteins in control cells. Furthermore, we observed a strong reduction in the number of PLA foci in NGPS2 cells, reflecting a decreased interaction between BAF-laminA/C, which was rescued by reversion of the mutation (Figure 4c, d). Even though BAF and lamin A/C are both found throughout the nucleus, we observed a clear enrichment of foci at the nuclear periphery. Indeed, by measuring the number of PLA foci in concentric rings within the nucleus, starting from the nuclear periphery, we confirmed that most of the PLA foci accumulated in the outer nuclear ring (Figure 4e), indicating that these proteins mostly interact at the NE. In addition, we noticed enrichment of PLA foci in nuclear blebs of a subset of control cells, indicating that blebs could be a site of increased interaction between laminA/C and BAF (Figure 4f).

Nuclear blebs can be an early step in nuclear envelope rupture and recruitment of lamin A/C by BAF at sites of ruptures has recently been shown to be an early event in the repair of these ruptures (Kone et al., 2022; Sears and Roux, 2022). Initially, a mobile A-type lamin population is recruited to rupture sites which becomes stabilized, leaving behind a lamin “scar”. Indeed, we observed these lamin scars at the NE of Control cells (Figure 1a, green arrowheads) but in contrast, often observed gaps in the lamina of NGPS patient cells (Figure 1a, magenta arrowheads). Gaps in the lamina can generate weak spots in the NE leading to bleb formation

and NE rupture. We therefore investigated the effect of the BAF A12T mutation on the recruitment of lamin A/C to nuclear blebs, which can be recognized on immunofluorescence images by a lack of B-type lamins (Denais et al., 2016). In Control cells, we observed a clear accumulation of lamin A/C to a subpopulation of blebs (Figure 5a, green arrowheads). NGPS cells on the other hand didn't show lamin A/C recruitment to blebs (Figure 5a, magenta arrowheads). Automated quantification using a CellProfiler based pipeline (Janssen et al., 2022) of either lamin A/C intensity at blebs or enrichment of lamin A/C at blebs when compared to the lamin A/C staining intensity in the rest of the nucleus, confirmed a lack of lamin A/C recruitment in NGPS cells (Figure 5b). Reversion of the A12T mutation in NGPS2 cells was able to rescue the lamin A/C recruitment defect (Figure 5a (green arrowheads) and 5b, Figure S6a-b), demonstrating that this is a specific phenotype caused by the BAF mutation. Additionally, we examined the recruitment of emerin to blebs, as emerin is also known to accumulate at rupture sites in the NE repair process, and because emerin localization is altered in NGPS patient cells. We indeed found a decrease in emerin intensity at NE blebs in NGPS cells which could be partially explained by an overall decrease in emerin levels at the NE (Figure 5c-d, Figure S6c-d).

As an additional model to confirm the effect of the BAF A12T mutation, we generated control and NGPS patient cells stably expressing FLAG-BAF or FLAG-BAF A12T. We used these cells to assess whether overexpression of WT BAF in NGPS cells could rescue the observed defects in recruitment of lamin A/C or emerin to blebs. This system should mimic a situation where BAF A12T is present as a heterozygous mutation, as it occurs in NGPS patients' parents, who are devoid of any disease phenotypes. First, we assessed FLAG-BAF localization in these cells and observed that FLAG-BAF(A12T) localized to the nucleus as expected (Figure 6a). We additionally checked whether BAF A12T recruitment to rupture sites was affected, as contradictory results on BAF A12T affinity for DNA were published (Marcelot et al., 2021; Paquet et al., 2014). We observed that FLAG-BAF WT and A12T localized to blebs identified by lack of lamin B in both Control and NGPS2 background (Figure 6a-b). Around 30% of all blebs showed FLAG-BAF enrichment for both the WT and the A12T protein (Figure 6c). Together, these results suggest that the recruitment of FLAG-BAF to DNA at nuclear rupture sites is not affected by the A12T mutation, consistent with our *in vitro* data (Marcelot et al., 2021) and recent reports by others in cells (Sears and Roux, 2022). Emerin nuclear localization was rescued by overexpression of FLAG-BAF WT in NGPS patient cells (Figure S7a, b). Finally, looking at recruitment of lamin A/C and emerin to NE blebs, we identified that overexpression of wild type FLAG-BAF in NGPS patient cells was sufficient to rescue lamin A/C and emerin recruitment (Figure 6d-f). Similarly, overexpression of BAF A12T in a control background did not cause defects in recruitment of these proteins, which is consistent with the lack of disease phenotypes in NGPS parents who carry a BAF A12T heterozygous mutation (Cabanillas et al., 2011; Puente et al., 2011).

## Discussion

Using a combination of *in vitro* and cell-based experiments, we showed that while the A12T mutation causing Nestor-Guillermo Progeria Syndrome did not affect BAF three-dimensional structure, it reduces the binding affinity of BAF to the Ig-fold of A-type lamins. As a consequence, we found that the accumulation of A-type lamins at nuclear rupture sites - known to be dependent on BAF (Young et al., 2020b) - was strongly reduced in NGPS patient cells. Upon reversing the BAF A12T homozygous mutation using CRISPR-Cas9, we confirmed that this was indeed a specific effect of the mutation in BAF. This is consistent with recent reports showing that recruitment of A-type lamins to laser-induced nuclear ruptures is impaired in fibroblasts expressing BAF A12T (Sears and Roux, 2022). Although the function of the lamin

A/C recruitment to sites of nuclear envelope ruptures remains unclear, it is tempting to speculate that the accumulation of lamins allows the stabilization of the nuclear envelope after it has ruptured, thereby protecting its integrity. The downstream consequences of the lack of A-type lamin recruitment to nuclear ruptures in NGPS patient cells remains to be explored. One hypothesis is that it might cause repetitive ruptures of the nuclear envelope at the same site due to the persistence of a lamina gap. Alternatively, as the presence of lamin A has been shown to be important for the accumulation of BAF at the sites of rupture (Kone et al., 2022), the repair process could be delayed or impaired although we and others (Sears and Roux, 2022) did not find an obvious defect in BAF A12T recruitment to sites of nuclear ruptures. It was also shown that cells depleted of A-type lamins are still able to repair the NE (Halfmann et al., 2019). The ER-associated exonuclease TREX1 was recently shown to translocate into the nucleus and induce DNA damage upon NE rupture (Nader et al., 2021). Surprisingly, we did not find evidence of increased DNA damage in NGPS cells so far which leaves the question open as to what the effects of the impaired A-type lamin recruitment to rupture sites might be and how this might contribute to disease phenotypes in NGPS patients. Recently, several mutations in the Ig-fold domain of A-type lamins found in laminopathies have been shown to decrease their binding affinity for BAF (Samson et al., 2018) leading to reduced recruitment of A-type lamins to rupture sites (Kone et al., 2022; Sears and Roux, 2022) thereby suggesting a common mechanism for these diseases.

Phosphorylation of BAF is important for regulating BAF activity as it modifies it ability to bind to various proteins. Phosphorylation has been shown to reduce the interaction of BAF with DNA while not affecting its ability to bind to the Ig-fold domain of A-type lamins (Marcelot et al., 2021). Therefore, in the nucleus, the non-phosphorylated BAF population is thought to be mainly bound to the DNA while the phosphorylated BAF population is more soluble and binds to various proteins including NE proteins. In the cytoplasm, BAF is less phosphorylated (Zhuang et al., 2014) and this allows for the non-phosphorylated, freely available cytoplasmic BAF to bind to the exposed DNA upon rupture of the nucleus and to accumulate at the rupture sites. It remains unclear how BAF is subsequently released from the rupture sites. One possibility is that VRK2A, a NE localized BAF kinase that binds A-type lamins (Birendra KC et al., 2017), accumulates at the rupture site when the NE reforms and A-type lamins accumulate. This would lead to BAF phosphorylation and decreased DNA binding affinity reminiscent of the role of VRK1 kinases in the release of BAF from the reforming NE after mitosis (Gorjánácz et al., 2007; Molitor and Traktman, 2014). Although we showed that BAF A12T can still be phosphorylated in vitro by VRK1, reduced interaction with A-type lamins and delocalization into the cytoplasm in NGPS cells could lead to a decrease in BAF phosphorylation by VRK2A kinases residing at the NE. Recently, loss of lamin A or C was shown to reduce BAF phosphorylation and accumulation in the nucleus (Kone et al., 2022).

Lamin A/C is important for BAF nuclear retention and similarly BAF is important for proper lamin A localization (Haraguchi et al., 2008). The decrease in interaction between these proteins thereby likely has additional subtle effects on the organization of the nuclear lamina which remain to be explored. This could also explain why the A12T mutation causes mislocalization of emerin and impaired emerin recruitment to nuclear blebs in NGPS cells even though the mutation is not expected to impair BAF-emerin binding directly. Both BAF and lamin are important for nuclear enrichment of emerin (Haraguchi et al., 2008 JCS). Interestingly, NGPS cells show an increase in BAF levels which could be a mechanism to compensate for a decrease in BAF – lamin A/C interaction. This compensation mechanism could have consequences for the many other cellular processes involving BAF.

## Material and Methods

Cell culture

Cells were cultured in Dulbecco's modified Eagle's medium containing 10% foetal calf serum and Penicillin/Streptomycin. Cells were maintained at 37 °C and 5% $CO_2$. Control human fibroblast cell line was derived from AG10803 immortalized with SV40LT and TERT, NGPS1 and NGPS2 were derived from NGPS5796, and NGPS5787 respectively and were immortalized with SV40LT and TERT. These immortalised cell lines were a gift from Carlos López-Otín.

Generation of stable cell lines

Stable cell lines expressing FLAG-BAF WT and FLAG BAF A12T were generated using a PiggyBac system. First, BAF WT and BAF A12T sequence were PCR-amplified from GFP-BAF vectors (kind gift from Cristina Capanni) and inserted into a FLAG vector. Piggybac vectors containing FLAG-BAF WT and FLAG BAF A12T were subsequently assembled by PCR amplification followed by Gibson assembly using HiFi DNA assembly cloning kit (New England Biolabs, #E5520S). FLAG-BAF was cloned into a BamHI and AfeI digested piggybacV1_CMV, a custom piggyback vector containing a CMV promotor and hygromycin resistance gene (kind gift from Jonathon Nixon-Abell). Cells were transfected with the piggybacV1 plasmid together with a second plasmid containing the PiggyBac transposase (Jonathon Nixon-Abell) under an EF1alpha promotor using lipofectamine 3000 or Transit2020 according to manufacturer's protocol. Cells were grown on hygromycin selection (100 ug/ml) the next day and were maintained in hygromycin containing medium.

Reversion of the BAF A12T mutation using CRISPR-Cas9 gene editing

To reverse the A12T mutation in *BANF1* in NGPS2 cells, a previously described strategy was used that combines an All-in-One Cas9$^{D10A}$ nickase vector with enrichment by fluorescence-activated cell sorting to enrich for transfected cells (Chiang et al., 2016). A pair of guide RNAs (sgRNAs) was designed to target the DNA on opposing strands surrounding the *BANF1* mutation in intron 1. The sgRNAs were then cloned into the All-in-One Cas9$^{D10A}$ nickase vector using DNA oligos (Sigma-Aldrich) using the BsaI and BbsI recognition sites. Several sgRNA pairs were originally designed using the CRISPR design tool in Benchling. After optimization, the sgRNA sequences: GCCCATGGGGGAGAAGCCAG and GAAGTCTCGGTGCTTTTGGG were used for CRISPR targeting in combination with a PAGE-purified ssODN of 200bp long (Integrated DNA Technologies). The ssODN contains the BANF1 WT genomic sequence to reverse the A12T mutation and in addition has silent mutations to mutate the PAM sites to prevent Cas9 recutting and a mutation in an NcoI restriction site to facilitate clone screening by digestion of PCR products. Full ssODN sequence:AGAAGTTCCAGGTCTTCAGCCCTAATCTGCCTTTTTTTTGGGATTCCTAG ATTAAGCCTGATCAAGATGACAACATCCCAAAAGCACCGAGACTTCGTGGCAGA GCCTATGGGGGAGAAGCCAGTCGGGAGCCTGGCTGGGATTGGTGAAGTCCTGGG CAAGAAGCTGGAGGAAAGGGGTTTTGACAAGGTGTGGGGTGG.

Cells were transfected with the All-in-One vector and the ssODN using Transit2020. GFP positive cells were sorted the next day either directly into 96 well plates or as a polyclonal population for manual seeding into 96 well plates. Genomic DNA of expanded clones was isolated using QuickExtract DNA extraction solution (Lucigen, QE0905T). PCR was performed by amplifying a 241bp area surrounding the mutation site, followed by restriction digestion using NcoI to screen for positive clones.

RNA isolation and cDNA sequencing

RNA isolation was performed starting with a confluent 10cm dish and using the Monarch total RNA miniprep kit for RNA isolation (NEB, #T2010S). RNA was stored at -80 °C. RT-PCR was performed using a One-Step RT-PCR kit (Qiagen, #210210) using the following primers Forward: AAAGCACCGAGACTTCGTGG and Reverse: AAGGCATCCGAAGCAGTCC. The reaction was set up according to manufacturer's protocol. The generated cDNA products were gel purified using Qiaquick Gel Extraction kit (Qiagen, #28704) and sent for sequencing.

Immunoblotting

Cells were washed with ice-cold PBS, lysed in Laemmli buffer (4% SDS, 20% glycerol, and 120 mM Tris-HCl (pH 6.8)) and then incubated for 5 min at 95°C. The DNA was sheared by syringing the lysates 10 times through a 25-gauge needle. Absorbance at 280 nm was measured (NanoDrop; Thermo Fisher Scientific) to determine protein concentration. Samples were prepared using Protein Sample Loading Buffer (LI-COR, #928-40004) and DTT (final concentration 50 mM) and heated at 95°C for 5 min. Proteins were separated using NuPAGE 4-12% Bis-Tris gels (ThermoFisher) and NuPAGE MES SDS running buffer (Thermo Fisher, #NP0002) and transferred to nitrocellulose membranes for immunoblotting. Membranes were blocked in 5% milk PBS and incubated overnight at 4°C with primary antibodies. Next day, membranes were incubated for 1h at room temperature with IRDye-conjugated secondary antibodies (LI-COR) and scanned on an Odyssey imaging system. The following primary antibodies were used: mouse anti-Tubulin (Sigma Aldrich, #T9026, 1/2000), mouse anti-lamin A/C (Santa Cruz, #sc-7292, 1/500), mouse anti-lamin B1 (Santa Cruz, #sc-365214, 1/1000), rabbit anti-emerin (Proteintech, #10351-1-AP, 1/1000), rabbit anti-H3K9me3 (Abcam, #ab8898, 1/1000), rabbit anti-BAF (ProSci, #4019, 1/500).

Immunofluorescence

Cells were fixed at room temperature for 10 min with 4% PFA. Cells were washed in PBS, permeabilized using 0.2% Triton-X100, and blocked using 3% bovine serum albumin (BSA) in PBS for 30min. Cells were incubated overnight at 4 °C or for 1hr at RT in 3% BSA PBS containing primary antibody. Cells were washed using PBS and incubated for 1 h at room temperature with secondary antibody in 3% BSA PBS. Cells were washed in PBS and mounted using Prolong Gold (Thermo Fischer). The following primary antibodies were used: rabbit anti-BAF (Abcam, ab129184, 1/200), mouse anti-lamin A/C (Santa Cruz, sc-376248, 1/500) and mouse anti-lamin A/C (Santa Cruz, sc-7292, 1/500), rabbit anti-emerin (Proteintech, 10351-1-AP, 1/500), mouse anti-lamin B1 (Santa Cruz, sc-365214, 1/500), mouse anti-yH2AX (Millipore, 05-636-I, 1/200), rabbit anti-sun1 (Abcam, ab124770, 1/100), mouse anti-FLAG (Sigma Aldrich, F1804, 1/1000). Images were taken on Zeiss Axio Imager Z2 using a 63x oil immersion objective (Plan APO, Na 1.4, Zeiss).

Image processing and analysis

Quantitative analysis of nuclear/cytoplasmic ratio's, nuclear size and recruitment of proteins to blebs was performed using CellProfiler V4.1.3. Custom pipelines will be made available on request.

Nuclear/cytoplasmic ratio: Nuclear object was identified based on DAPI channel. The cytoplasm object was subsequently generated by expansion of the nuclear object by 50 pixels and subtraction of the nucleus to create a cytoplasmic ring. The intensity of additional channels in the nuclear and cytoplasmic object were measured. In addition, the area of the nuclear object was measured. Data was transferred to Microsoft Excel for calculation of the nuclear/cytoplasmic ratio and subsequently plotted and analysed using GraphPad Prism 9.

Bleb analysis (Janssen et al., 2022): Chromatin object was identified based on DAPI staining. A lamin B1 object was identified and subtraction of both areas identifies the bleb object as it is devoid of lamin B1. Subsequently, bleb areas are filtered to exclude small identified regions or pixels at the edge of the nuclei. Size, shape and number of bleb areas are measured as well as intensity of any additional channels at the bleb regions.

Proximity ligation assays (PLA)

PLA was used to detect interaction between endogenous lamin A/C and BAF in control, NGPS2 and NGPS2 clone1 cells. Cells were seeded on 12mm coverslips and fixed for 10 min with 4% PFA at room temperature and permeabilized using 0.2% Triton-X100. Cells were blocked for 1.5 h at room temperature using manufacturers blocking solution and primary antibody incubation, DuoLink Probe incubation, ligation and amplification steps were all carried out according to manufacturer's protocol (DuoLink PLA assay kit, #DUO92008, Sigma Aldrich). Primary antibodies used were rabbit anti-BAF (Abcam, ab129184, 1/200) and mouse anti-lamin A/C (Santa Cruz, sc-376248, 1/1000). Duolink In Situ PLA probe anti-rabbit PLUS, Duolink In Situ PLA probe anti-mouse MINUS and Duolink Amplification Red were used. Cells were mounted in Duolink Mounting Media with DAPI. Confocal microscopy image acquisition was performed using LSM880 Laser scanning microscope (Zeiss). Single plane images were taken except for analysis of PLA foci location were Z-stacks were recorded and the middle slice was eventually used for analysis.

Quantitative analysis of PLA foci was performed using CellProfiler V4.1.3. In brief, first the nuclear object was detected based on DAPI signal after which PLA foci were detected in the nuclear object using thresholding. The number of PLA foci per nucleus were plotted for three independent experiments and the mean was indicated. Statistical analysis was performed using GraphPad PRISM 9. For analysis of the location of PLA foci the middle slice from the recorded Z-stack was used. In brief, the nucleus was detected based on DAPI signal after which 2 outer rings were identified by shrinking the nucleus by 10 pixels. The defined outer nuclear circles and inner objects were used to identify the number of PLA foci in these areas. These numbers were then normalized for the surface area and the fraction of foci in the outer rings and the centre was calculated.

Protein constructs and expression vectors

For protein purification, we used our BAF WT construct that codes for an N-terminal tag containing 8 histidines, a TEV cleavage site, and the human BAF sequence from which all cysteines were mutated into alanines to allow for protein resistance to oxidation and thus, aggregation (Samson et al., 2018). After cleavage of the tag, the purified protein corresponds to human BAF containing the following mutations: M1G, C67A, C77A, C80A and C85A. The gene coding for BAF WT was synthetized by Genscript after codon optimisation for expression in *E. coli* and cloned in a pETM13 vector providing kanamycin resistance to bacteria. The vector used for BAF A12T expression was obtained by mutagenesis of the BAF WT expression vector using the Quikchange Site-Directed Mutagenesis kit (Agilent). The lamin A/C Ig-fold construct codes for a GST tag, a thrombin cleavage site, and the human lamin A/C fragment from aa 411 to aa 566. It was cloned in a pGEX vector providing an ampicillin resistance. The VRK1 expression vector is a gift from John Chodera, Nicholas Levinson and Markus Seeliger (Addgene plasmid #79684 (Albanese et al., 2018)). All expression vectors were purified using the New England BioLabs kit (reference #T1010L) from 5 mL of bacteria culture.

Protein expression and purification

All vectors were transformed in E. coli BL21* (DE3). The transformations were carried out by adding 100 ng of plasmid, onto about one billion of bacteria (30 µL). Vectors entry in the cells

was triggered by a heat shock at 42°C during 45 s. Finally, the cells were spread on LB agar medium containing the appropriated antibiotic resistance.

Bacteria grew either in LB (Lysogeny Broth) or M9 (Minimum 9) medium depending on the needs. The M9 media were supplemented with either $^{15}NH_4Cl$ and natural abundance glucose. Precultures of bacteria containing the vector coding for the protein of interest were prepared in LB with antibiotic and incubated overnight at 37°C under agitation (180 rpm). Then, 20 mL of preculture were used to inoculate 800 mL of culture (LB or M9). When the $OD_{600nm}$ reached 0.8 +/- 0.1, protein expression was triggered using IPTG (Isopropyl-β-D-thiogalactoside). All proteins were expressed overnight at 20°C. Cells were finally harvested by centrifugation, flashed frozen in 30 mL of lysis buffer (50 mM Tris HCl pH 8, 300 mM NaCl, 5 % glycerol, 0.1 % Triton X-100, 1 mM PMSF) and stored at -20°C during maximum 1-2 months.

Both BAF constructs are insoluble after overexpression in *E. coli*, so purification was performed in urea and followed by a refolding step. After sonication in lysis buffer (50 mM Tris pH 8, 300 mM NaCl, 5% glycerol, 0.1% Triton 100X), and centrifugation at 50 000 g for 15 minutes at 4°C, the pellet was resuspended in urea purification buffer (50 mM Tris pH 8.0, 150 mM NaCl, 8 M urea), for 20 min. Then, the sample was centrifuged again and the soluble fraction was incubated on Ni-NTA beads preequilibrated with urea purification buffer, for 30 min at room temperature. Ni-NTA beads were washed with the purification buffer and the protein was eluted in 50 mL of the same buffer supplemented with 1 M imidazole. Proteins were then refolded by dialysis in BAF buffer (50 mM Tris pH 8, 150 mM NaCl). After concentration, the histidine-tag was cleaved by the TEV protease (from a Batch of TEV purified in the lab) overnight at 4°C. The protein was separated from the TEV protease (containing a histidine tag), and its Tag by Ni-NTA affinity chromatography. Finally, a gel filtration was performed using a Superdex 200 pg HiLoad 16/600 column (GE healthcare). The final yield was typically about 0.6 mg (LB) or 0.1 mg (M9) of purified protein per liter of bacterial culture for BAF WT and twice more for BAF A12T.

For the lamin A/C Ig-fold domain, after sonication at 10°C, the supernatant was incubated 20 min at room temperature with benzonase and centrifuged at 50 000 g for 15 min at 4°C. The soluble extract was then supplemented with 5 mM DTT and loaded onto glutathione beads. After 1 h of incubation at 4°C, glutathione beads were washed first with 1 M NaCl buffer and then with the purification buffer (50 mM Tris pH 7.5, 150 mM NaCl, 5 mM DTT). The GST tag was cleaved with thrombin (commercial thrombin, Sigma Aldrich), at 200 units per mL for 2h at room temperature, then the protein was recovered in the flow-through and separated from thrombin and last contaminants using gel filtration (Superdex 200 pg HiLoad 16/600 column, GE healthcare). The final yield was typically 20 mg (LB) or 6mg (M9) of purified protein per liter of bacterial culture.

In the case of VRK1, after sonication, the soluble extract was incubated with benzonase for 20 min at 20°C (room temperature). The lysate was then centrifuged at 50 000 g for 15 min at 4°C and loaded onto a 5 ml Ni-NTA column (FF crude, GE-Healthcare). The column was washed with washing buffer (50 mM Tris pH 8.0, 1M NaCl), re-equilibrated with purification buffer (50 mM Tris pH 8.0, 150 mM NaCl), and eluted with an imidazole gradient (0 to 500 mM). After concentration to 5 ml, the histidine tag was cleaved by the TEV protease (from a batch of TEV purified in the lab) during 1h30 at room temperature. Proteins were separated from the TEV protease (containing a histidine tag) by affinity chromatography, using Ni-NTA beads. Finally, last contaminants were removed by gel filtration (Superdex-200 HiLoad 16/600

column). The final yield was typically 28 mg (LB) of purified protein per liter of bacterial culture.

X-ray crystallography

The BAF A12T-lamin A/C Ig-fold complex was recovered after ITC experiments (in 50 mM Hepes pH 7.4, 150 mM NaCl) and concentrated to about 20 mg/ml. Crystallization experiments were carried out at the HTX Lab (EMBL Grenoble) (Dimasi et al., 2007). Crystals were obtained by sitting drop vapor diffusion at room temperature against reservoir containing 0.1 M bicine pH 9 and 3 M ammonium sulfate. They were flashed-freezed in liquid nitrogen and prepared for X-ray diffraction experiments using the CrystalDirect technology (Zander et al., 2016). Diffraction data were collected on the MASSIF-1 beamline (ESRF synchrotron, Grenoble, France). The 3D structure was solved by molecular replacement with Molrep software in CCP4 using the 6GHD.pdb coordinates file as starting model (Vagin and Teplyakov, 1997; Winn et al., 2011). The resulting model was iteratively improved by alternating manual reconstruction with the COOT software (Emsley et al., 2010) and refinement with the BUSTER (Bricogne et al., 2020) and PHENIX REFINE softwares (Adams et al., 2010); ( Table S1). Structure coordinates were deposited to the PDB, with entry 7Z21. All structure representations and Cα RMSD calculations were performed with PyMOL (Schrodinger, LLC).

Liquid-state Nuclear Magnetic Resonance spectroscopy

NMR experiments were performed on 600 MHz and 700 MHz spectrometers equipped with triple resonance cryogenic probes. The data were processed using Topspin v. 4.0.2 to v. 4.0.8 (Bruker), and analyzed using Topspin 4.1.3 (Bruker) and CCPNMR 2.4 (Vranken et al., 2005). Sodium trimethylsilylpropanesulfonate (DSS) was used as a chemical shift reference.

For monitoring phosphorylation of BAF WT and BAF A12T by NMR, 2D $^{1}$H-$^{15}$N HSQC spectra were recorded at 303 K on a 700 MHz spectrometer. The 3 mm-diameter NMR sample tube contained 150 µM of BAF (either WT or A12T) in kinetics 40 mM HEPES pH 7.2, 150 mM NaCl, 5 mM ATP, 5 mM MgSO4, 1 mM TCEP, 1X antiproteases (Roche), 95:5 H2O:D2O, and 150 nM of VRK1 kinase (molar ratio relatively to BAF: 0.1 %). 2D $^{1}$H-$^{15}$N NMR spectra were acquired every 15-25 min and 1D $^{1}$H spectra were recorded in between to report for potential pH drifts.

To detect an interaction between the lamin A/C Ig-fold domain and BAF (either WT or A12T), 2D $^{1}$H-$^{15}$N HSQC spectra were recorded on a sample containing 80 µM of $^{15}$N labelled lamin A/C Ig-fold and non-labeled BAF (dimers) at different ratios. For the interaction with BAF WT, two spectra were acquired by adding 40 µM or 80 µM of BAF WT (dimer) corresponding to molar ratios of 1:0.5 and 1:1, respectively. For the interaction with BAF A12T, two spectra were acquired by adding 80 µM of BAF A12T (dimer), corresponding to a ratio of 1:1. Then, another sample containing the lamin A/C Ig-fold domain concentrated at 160 µM and BAF WT (dimer) at 80µM was prepared, corresponding to a ratio of 2:1. The number of scans during the NMR acquisition was adjusted to 12 to obtain a signal-to-noise similar to the previous experiments. All these experiments were performed in 50 mM HEPES pH 7.4, 150 mM NaCl, 95:5 H2O:D2O, DSS, in 3-mm-diameter tubes, at 293K. Control experiments were carried out with the labelled protein alone, in the same experimental conditions.

Isothermal Titration Calorimetry (ITC) binding assays

The interaction between lamin A/C Ig-fold domain and BAF (either WT or A12T) was assessed by ITC using a VP-ITC calorimetry system (MicroCal-Malvern Panalytical, Malvern, UK).

Calorimetric titrations were performed with either 100 or 200 µM lamin A/C Ig-fold domain in the injecting syringe and either 20 µM BAF WT or 40 µM BAF A12T in the calorimetric cell, all in 50 mM Hepes pH 7.4 and 150 mM NaCl. All measurements were performed at 288 K in order to increase the signal-to-noise ratio. For each titration, a sequence of 29 times 10 µl injections was programmed, with reference power of 10 mcal/s and spacing between injections of 180 s. Each experiment was performed twice. Data were analyzed using Origin (OriginLab, Northampton, MA) by setting the stoichiometry to 0.5 (assuming that a dimer of BAF binds to a monomer of lamin A/C Ig-fold domain, as observed by X-ray crystallography).

Statistics

Statistical analysis was done using GraphPad Prism v9. Individual data points are plotted from 3 independent experiments. Mean or median is indicated by the black bar as indicated in the figure legends. Post-testing was performed as indicated to correct for multiple comparison. Details of statistical tests are included in the figure legends. Sample size was not predetermined and experiments were not randomized.

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**Author Contributions**

Conceptualization: A.F.J.J. and D.L.; formal analysis: A.F.J.J., A.M., P.L.; investigation: A.F.J.J., S.Y.B., A.M., P.L.; writing: original draft preparation, A.F.J.J.; writing: review and editing: A.F.J.J., A.M., S.Z.J., D.L.; visualization: A.F.J., A.M.; supervision: S.Z.J., D.L.; project administration: D.L.; funding acquisition: A.F.J.J., A.M., S.Z.J., D.L. All authors have read and agreed to the published version of the manuscript.

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## Figures

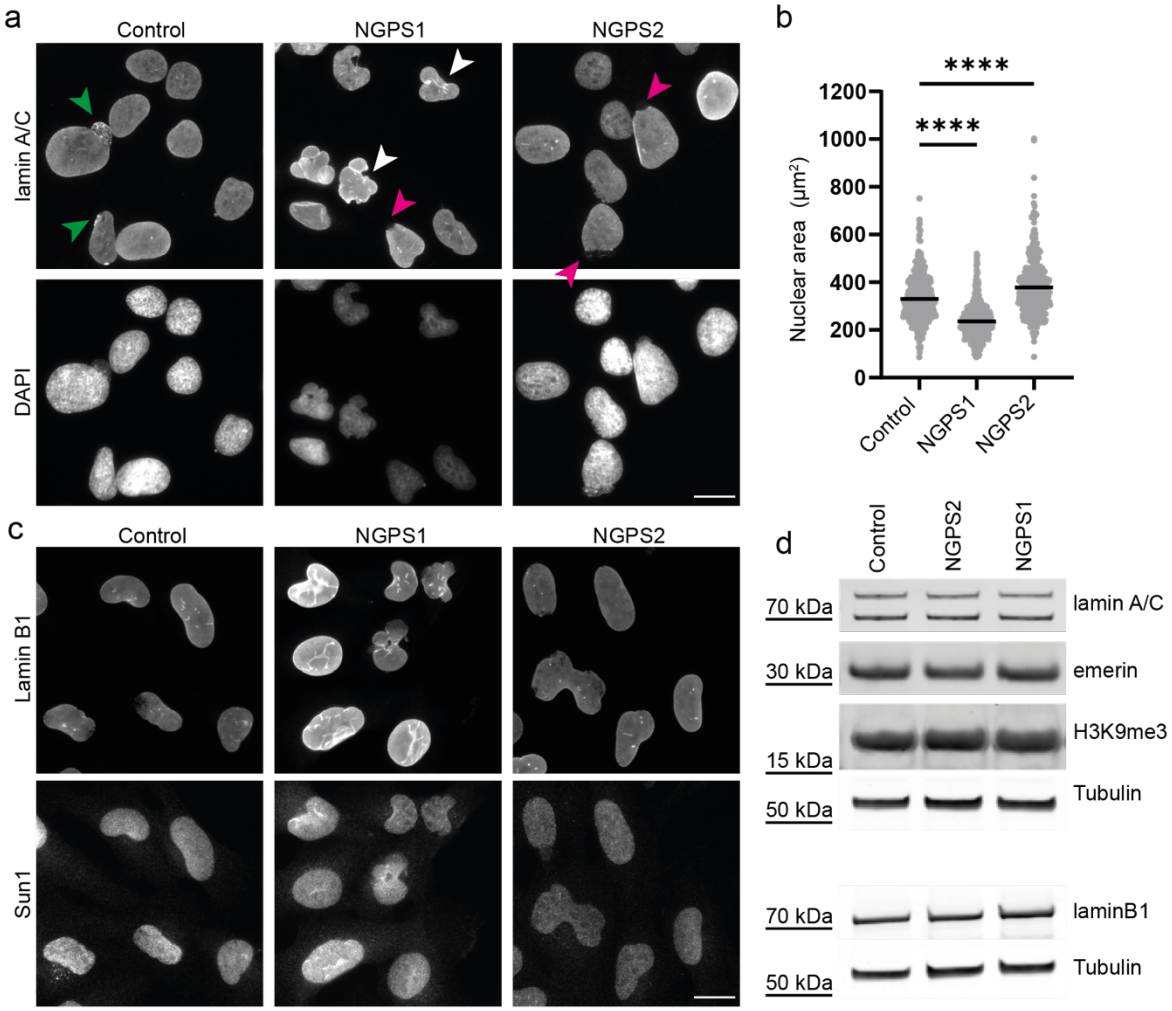


**Figure 1: Characterisation and comparison of fibroblast cell lines derived from two Nestor Guillermo Progeria patients. a)** Representative lamin A/C immunofluorescence images of immortalized fibroblasts obtained from an unrelated healthy donor (Control) or from two Nestor Guillermo Progeria Syndrome (NGPS) patients (NGPS1 and NGPS2). The arrows indicate area's of increased lamin intensity (green), nuclear envelope folding (white) and lamina gaps (magenta). Scale bar, 20 µm. **b)** Quantification of nuclear area based on DAPI images as presented in a). Mean from n=469, 563 and 413 nuclei for Control, NGPS1 and NGPS2 respectively. The data were collected in 3 independent experiments and analysed using a one-way ANOVA analysis with Šídák's multiple comparisons test (****P<0.0001). **c)** Representative immunofluorescence images of immortalized NGPS patient and control cell fibroblasts stained for lamin B1, and Sun1. Scale bar, 20 µm. **d)** Immunoblot analysis of the indicated proteins from whole-cell lysate in Control and NGPS cells. Tubulin was used as a loading control.

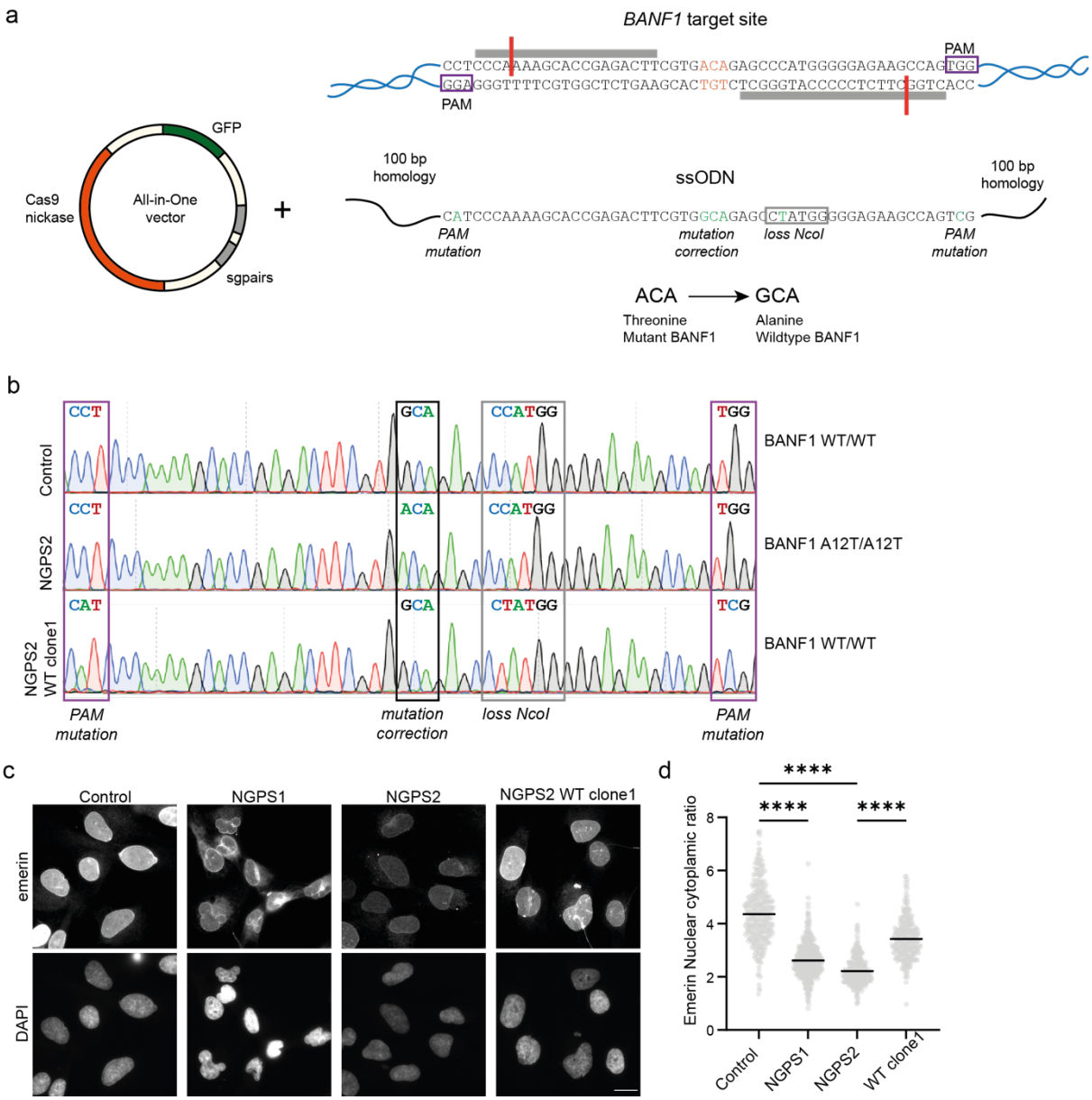


**Figure 2: Reversion of the homozygous BAFA12T mutation in NGPS patient cells using CRISPR/Cas9. a)** Graphical representation of the CRISPR-Cas9 strategy showing: (top) *BANF1* sequence around the A12T mutated codon (orange), the PAM sites for Cas9 recognition (purple boxes) and the annealing sites of the single guide RNAs (sgRNAs - grey bars). Bottom: the all-in-one vector carrying the Cas9 nickase (orange), the sgRNA pairs targeting the *BANF1* mutation site (grey) and the Green Fluorescent Protein (GFP - used for cell sorting) was used in combination with an ssODN carrying the correct wild-type *BANF1* sequence as a template for mutation correction through homologous recombination. **b)** DNA sequencing traces of the *BANF1* gene around the mutation site showing the wild-type GCA codon (Alanine) in control cells, the mutated ACA (Threonine) codon in NGPS2 cells and the corrected mutation (GCA – Alanine) in one of the successfully NGPS2 derived wild-type clones (NGPS2 WT clone1). Boxes indicate the A12T mutation site (black) and additional silent mutations introduced in the PAM motifs (purple) and in the restriction enzyme NcoI site for screening (grey). **c)** Representative immunofluorescence images of emerin in Control, NGPS patient cells and NGPS2 WT clone1. Scale bar, 20 µm. **d)** Quantification of the mean emerin nuclear to cytoplasmic ratio measured in 345, 465, 334 and 301 cells for Control,

NGPS1, NGPS2 and NGPS2 WTclone1 respectively. Data is presented from 3 independent experiments and the p value (****P<0.0001) was calculated using a one-way ANOVA analysis with Šídák's multiple comparisons test.

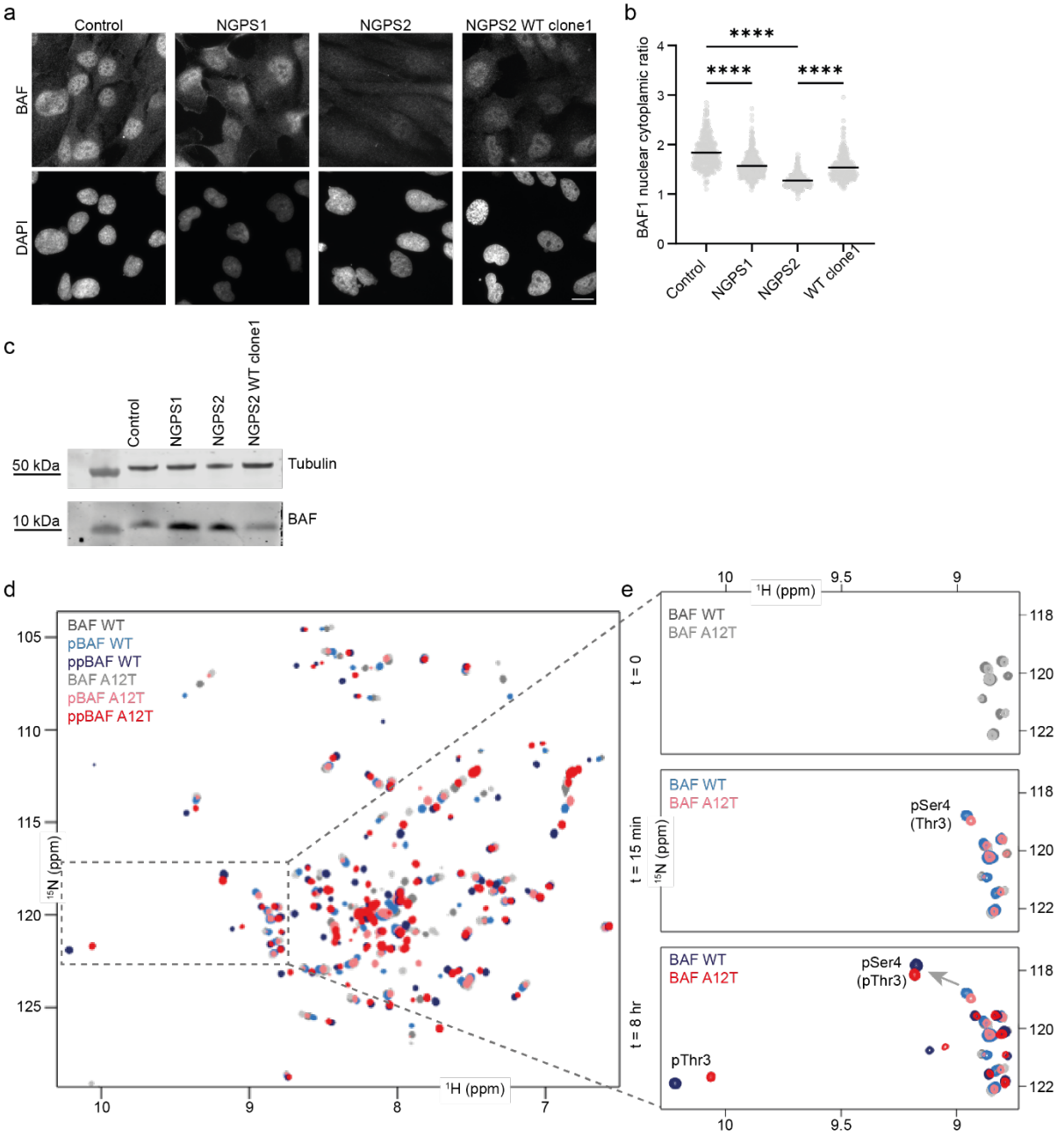


**Figure 3: The BAF A12T mutation in NGPS patient cells causes BAF mislocalization without affecting its phosphorylation state *in vitro*. a)** Representative immunofluorescence images showing endogenous BAF staining in Control, NGPS patient cells and NGPS2 WT clone1. Scale bar, 20 µm. **b)** Quantification of the mean BAF nuclear to cytoplasmic ratio in 425, 495, 401 and 363 cells for Control, NGPS1, NGPS2 and NGPS2 WTclone1 respectively. Data was obtained from 3 independent experiments and quantified using a one-way ANOVA analysis with Šídák's multiple comparisons test (****P<0.0001). **c)** Representative immunoblot showing the expression level of BAF in whole cell lysates from the indicated cell lines. Tubulin was used as a loading control. **d-e)** Using Nuclear Magnetic Resonance (NMR), 2D $^{1}$H-$^{15}$N Heteronuclear Single Quantum Coherence (HSQC) spectra were recorded on purified BAF WT (in shades of blue) and A12T (in shades of red) upon phosphorylation by the

VRK1 kinase *in vitro*. Six spectra are superimposed, corresponding to: non-phosphorylated BAF WT and A12T (t = 0 min), in dark and light grey respectively, mono-phosphorylated BAF WT and A12T (t = 15 min), in light blue and pink respectively, and di-phosphorylated BAF WT and A12T (t = 8 h), in dark blue and red respectively. The zoom in (e) shows the spectral regions of the $^{1}H$-$^{15}N$ HSQC of BAF WT and A12T spectra where signals of phosphorylated serines and threonines are commonly observed. The signals of the two BAF residues phosphorylated by VRK1 (Ser4 and Thr3) are annotated and the arrow shows the chemical shift perturbation of the phosphorylated Ser4 upon Thr3 phosphorylation.

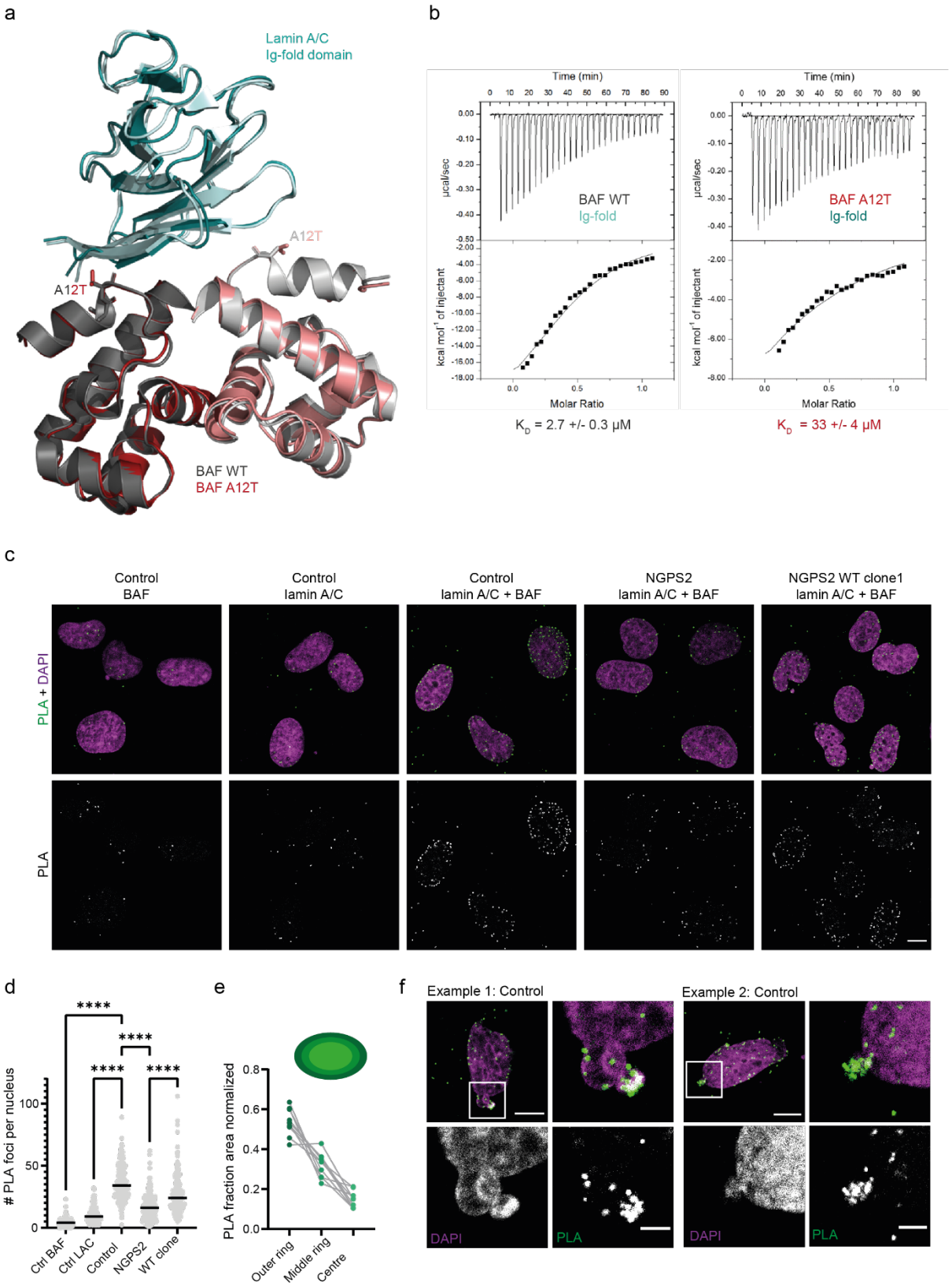


**Figure 4: The BAF A12T mutation disrupts BAF binding to lamin A/C *in vitro* and in NGPS cells. a)** Superimposition of the three-dimensional structure of BAF A12T (in light pink) bound to the lamin A/C Ig-fold domain (in light blue), onto the previously reported structure of BAF WT bound to this same lamin A/C domain (in grey; PDB code: 6GHD). Binding free energies calculated from these 3D structures are indicated in Fig. S5a. **b)** ITC curves reporting binding of BAF (either WT or A12T) to the lamin A/C Ig-fold domain. The experiments were

performed twice and the mean dissociation constant ($K_D$) values are shown under one representative curve. The duplicated experiments are shown in Fig. S5b. The thermodynamic parameters deduced from all experiments are summarized in Fig. S5c. **c)** Representative confocal images of the signal obtained by Proximity Ligation Assay (PLA) using either BAF or lamin A/C antibodies alone as negative controls or as a combination in the indicated cell lines. The Top row shows merged pictures of the PLA signal (green) and DAPI (magenta). Scale bar, 10 µm. **d)** Quantification of the number of PLA foci per nucleus. The data represent the mean PLA signal measured in individual nuclei in n=214 (Control BAF), 187 (Control lamin A/C), 229 (Control lamin A/C+BAF), 213 (NGPS2 lamin A/C +BAF) and 219 cells (NGPS2 WT clone1, lamin A/C + BAF). One-way ANOVA analysis with Šídák's multiple comparisons test (****P<0.0001). **e)** Analysis of the nuclear distribution of PLA foci in the middle slice of a z-stack obtained from confocal images in control cells using CellProfiler. The nucleus was subdivided in concentric circles going from the nuclear periphery to the nuclear interior and the fraction of PLA foci in each of these circles was counted per nucleus (n=10 nuclei). **f)** Example confocal images (from images as shown in c) showing the BAF-lamin A/C PLA signal accumulation at nuclear bleb sites in Control cells. Scale bar 10 µm and 3 µm (zoom).

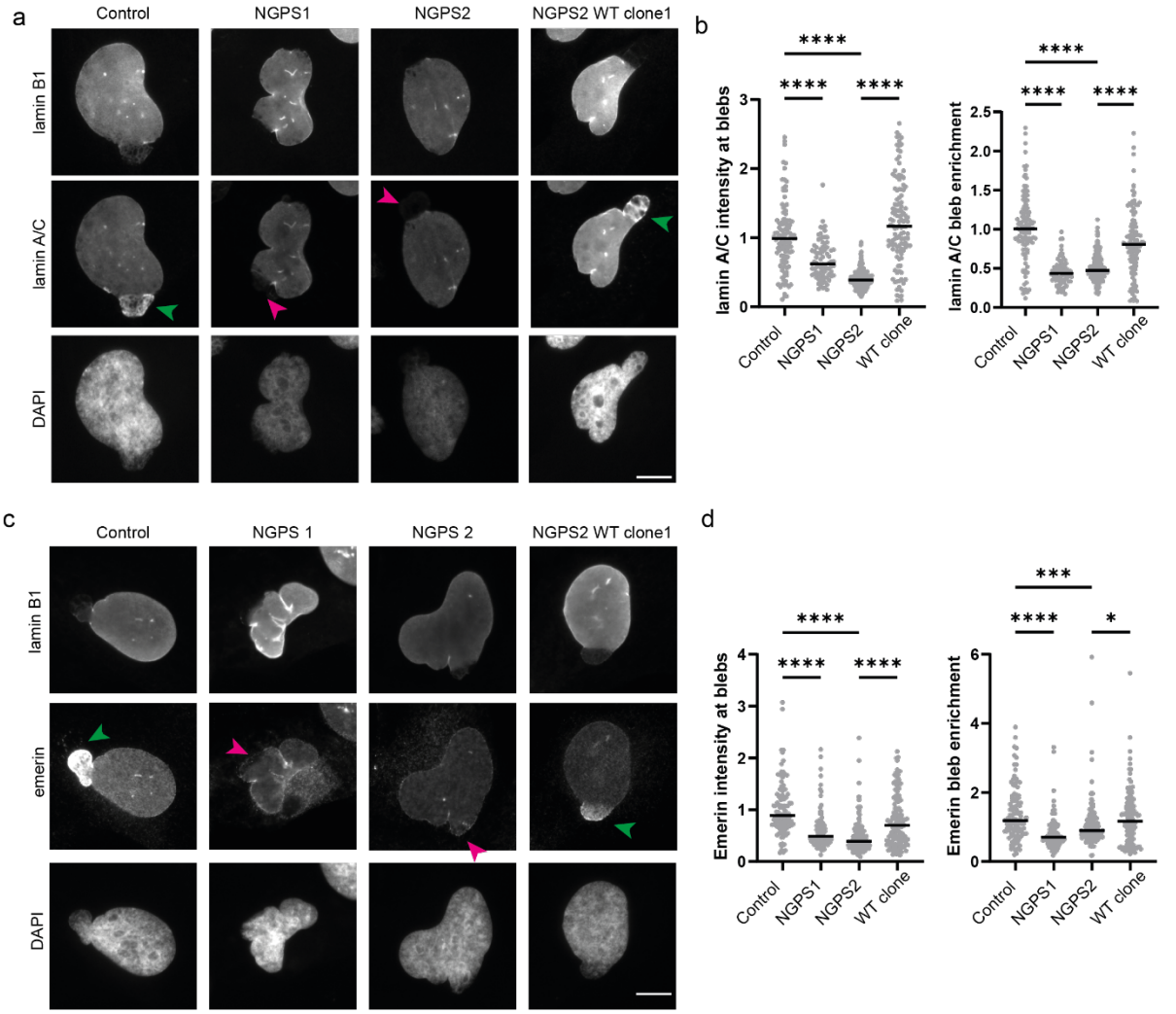


**Figure 5: The BAF A12T mutation in NGPS patient cells prevents the recruitment of lamin A/C and emerin to nuclear blebs. a)** Representative immunofluorescence images of endogenous lamin B1, and lamin A/C at nuclear blebs in Control, NGPS patient cells and NGPS2 WT clone1 cells. Arrows indicate the enrichment (green) or lack of (magenta) lamin A/C at blebs. Scale bar 10 µm. **b)** Quantification of the normalized lamin A/C intensity at blebs and of the enrichment of lamin A/C at nuclear bleb regions compared to the levels measured in the rest of the nucleus. Blebs were identified by the lack of lamin B1 staining. Data points represent 117 (Control), 81 (NGPS1), 235 (NGPS2), and 122 (WT clone1) individual blebs from 3 independent experiments, the median is indicated. One-way ANOVA analysis with Šídák's multiple comparisons test (****P<0.0001). **c)** Representative immunofluorescence images of lamin B1 and emerin in nuclear blebs of Control, NGPS patient cells and NGPS2 WT clone1 cells. Arrows indicate the enrichment (green) or lack of (magenta) emerin at blebs. Scale bar 10 µm. **d)** Quantification of the normalized emerin intensity at blebs and the enrichment of emerin at bleb regions compared to levels in the rest of the nucleus. Data points represent 113 (Control), 87 (NGPS1), 163 (NGPS2) and 134 (WT clone1) individual blebs from 3 independent experiments, the median is indicated. The data was analysed with a one-way ANOVA analysis with Šídák's multiple comparisons test (****P<0.0001, ***P<0.005, *P<0.05).

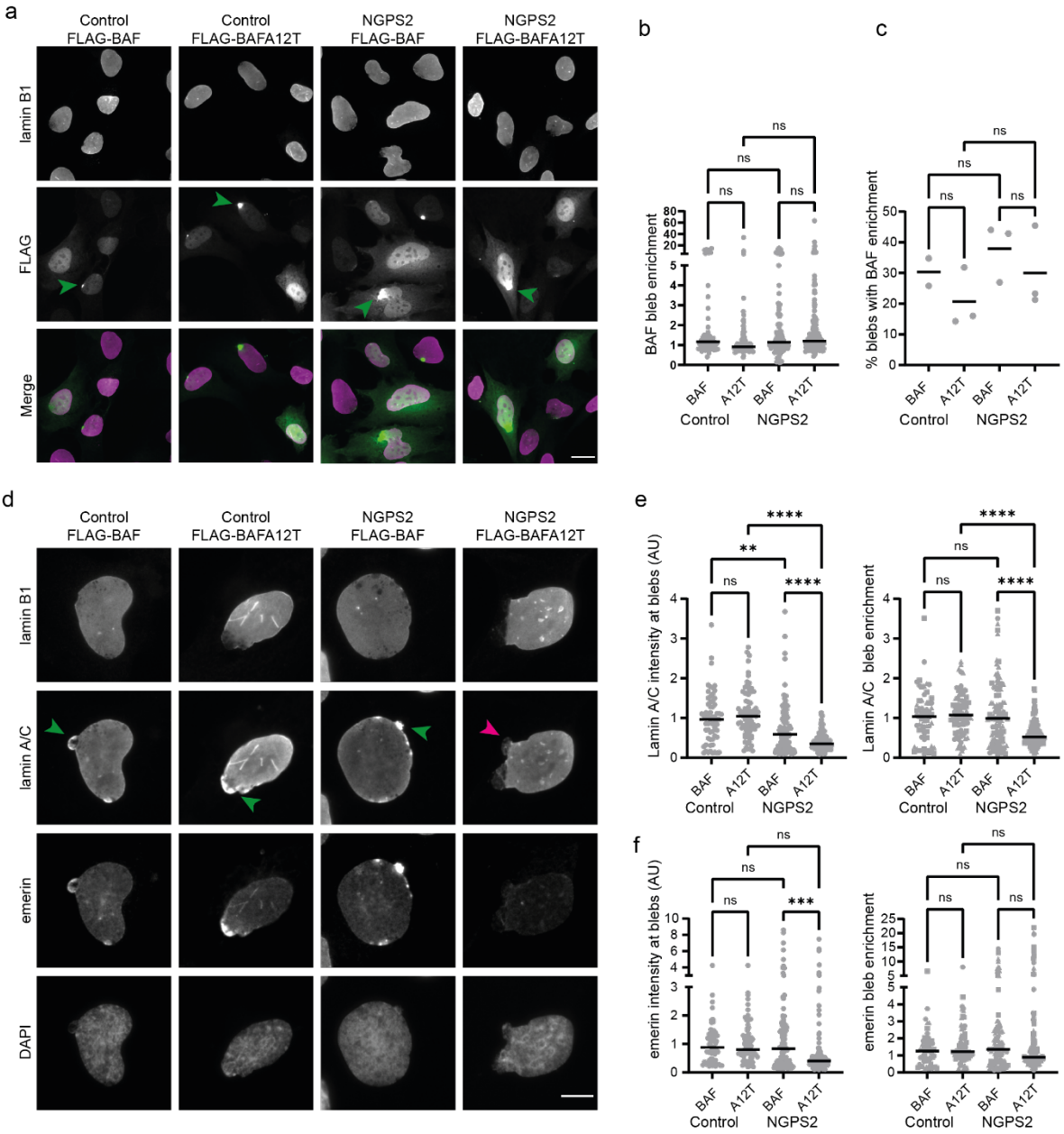


**Figure 6: The defective recruitment of lamin A/C to nuclear blebs is restored in NGPS cells by BAF wild-type overexpression. a)** Representative immunofluorescence images of FLAG and lamin B1 staining in Control and NGPS2 cells stably expressing FLAG-BAF WT or A12T. Green arrows indicate FLAG-BAF accumulation at nuclear blebs. Scale bar 20 µm. **b)** Quantification of FLAG-BAF WT and A12T enrichment at bleb regions compared to the levels measured in the rest of the nucleus. Blebs were identified by the lack of lamin B1 staining. Data points represent n=56 (Control FLAG-BAF), 82 (Control FLAG-BAFA12T), 112 (NGPS2 FLAG-BAF) and 156 (NGPS2 FLAG-BAFA12T) individual blebs from 3 independent experiments, median is indicated. One-way ANOVA analysis with Šídák's multiple comparisons test showed no significant (ns) differences. **c)** For each experiment in b) the percentage of cells showing >1.5 FLAG-BAF enrichment at blebs was calculated. Mixed-effects analysis with Šídák's multiple comparisons test showed no significant (ns) differences, mean is indicated. **d)** Representative immunofluorescence images of lamin B1, lamin A/C and emerin staining in Control and NGPS2 cells stably expressing FLAG-BAF WT or A12T. Arrows point to nuclear blebs with accumulation (green arrowheads) or lack of (magenta arrowheads) lamin A/C. Scale bar 10 µm. **e)** Quantification of the normalized lamin A/C

intensity at blebs and of the enrichment of lamin A/C at bleb regions compared to the levels measured in the rest of the nucleus. Blebs were identified by the lack of lamin B1 staining. Data points represent n=61 (Control FLAG-BAF), 79 (Control FLAG-BAFA12T), 108 (NGPS2 FLAG-BAF) and 153 (NGPS2 FLAG-BAFA12T) individual blebs from 3 independent experiments, median is indicated. One-way ANOVA analysis with Šídák's multiple comparisons test (****P<0.0001, **P<0.01). **f)** Quantification of the normalized emerin intensity at blebs and of the enrichment of emerin at bleb regions compared to the levels measured in the rest of the nucleus. Data points represent n=61 (Control FLAG-BAF), 79 (Control FLAG-BAFA12T), 108 (NGPS2 FLAG-BAF) and 153 (NGPS2 FLAG-BAFA12T) individual blebs from 3 independent experiments. One-way ANOVA analysis with Šídák's multiple comparisons test (***P<0.001).