pharmaceuticals

MDPI

Supplementary Materials

Impact of MnTBAP and Baricitinib treatment on Hutchinson-Gilford progeria fibroblasts

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Supplementary Materials.

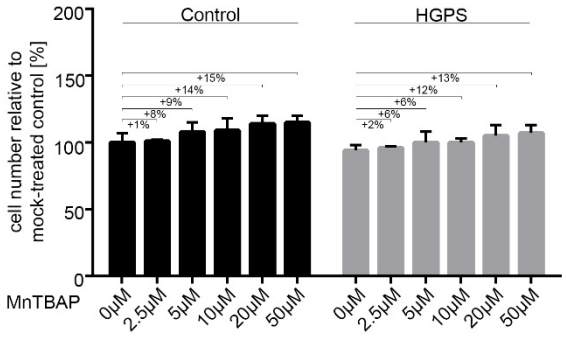


Figure S1. Cell cytotoxicity of MnTBAP at different concentrations. The cell numbers of control and HGPS cells after 4 days treatment with a mock and different concentrations of MnTBAP (2.5µm – 50µm) (n =3).

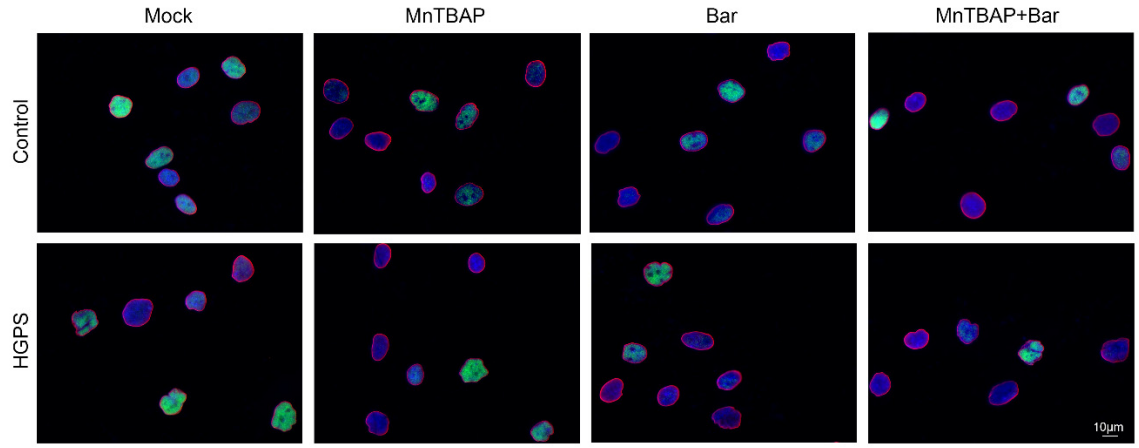


Figure S2. Anit-p21-immunostaining in control fibroblasts after treatment with the different regimens. Representative immunofluorescence images of control fibroblasts after 9 d of treatment with a mock solution, 5 µM MnTBAP, 1 µM Bar or the MnTBAP/Bar combination. The cells were stained with anti-p21 (green) and anti-lamin A/C (red) antibodies and counterstained with DAPI. At least 900 nuclei were counted for each condition. (scale bar 10µm, n=4).

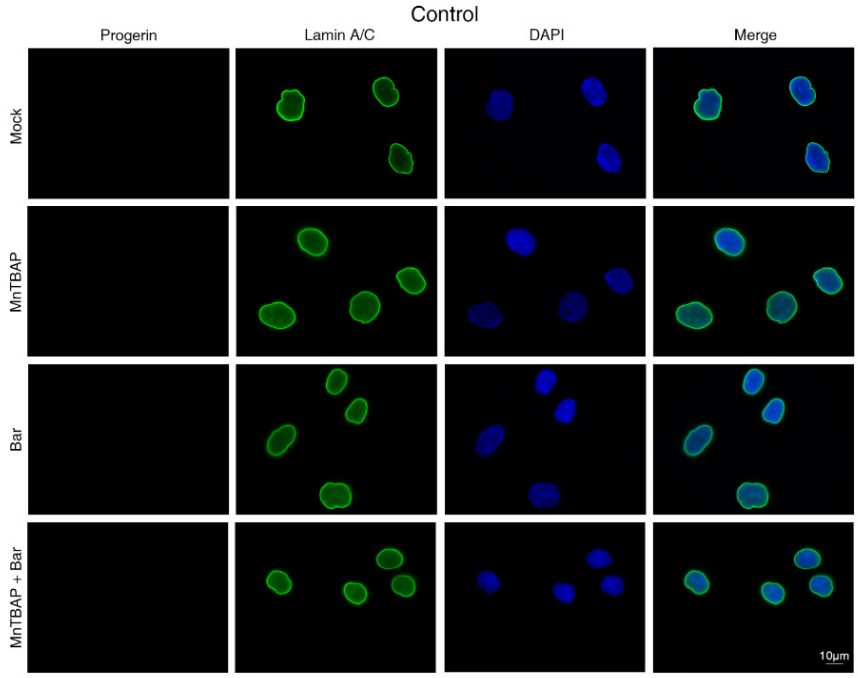


Figure S3. Progerin immunostaining in control fibroblasts after the different regimens. Representative immunofluorescence images of control fibroblasts after 9 d of treatment with a mock solution, 5 µM MnTBAP, 1 µM Bar or the MnTBAP/Bar combination. The cells were stained with anti-progerin (red) and anti-lamin A/C (green) antibodies and counterstained with DAPI (scale bar 10µm, n=4).

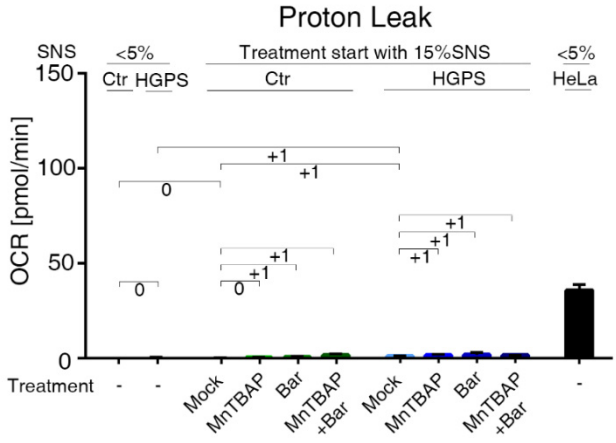


Figure S4. Proton leakage measurements. Proton leakage determined using Seahorse XF96 Flux analyzer after treatment of the control and HGPS cells with mock, 5 μM MnTBAP, 1 μM Bar or with the MnTBAP/Bar combination for 9 d, (n=3).

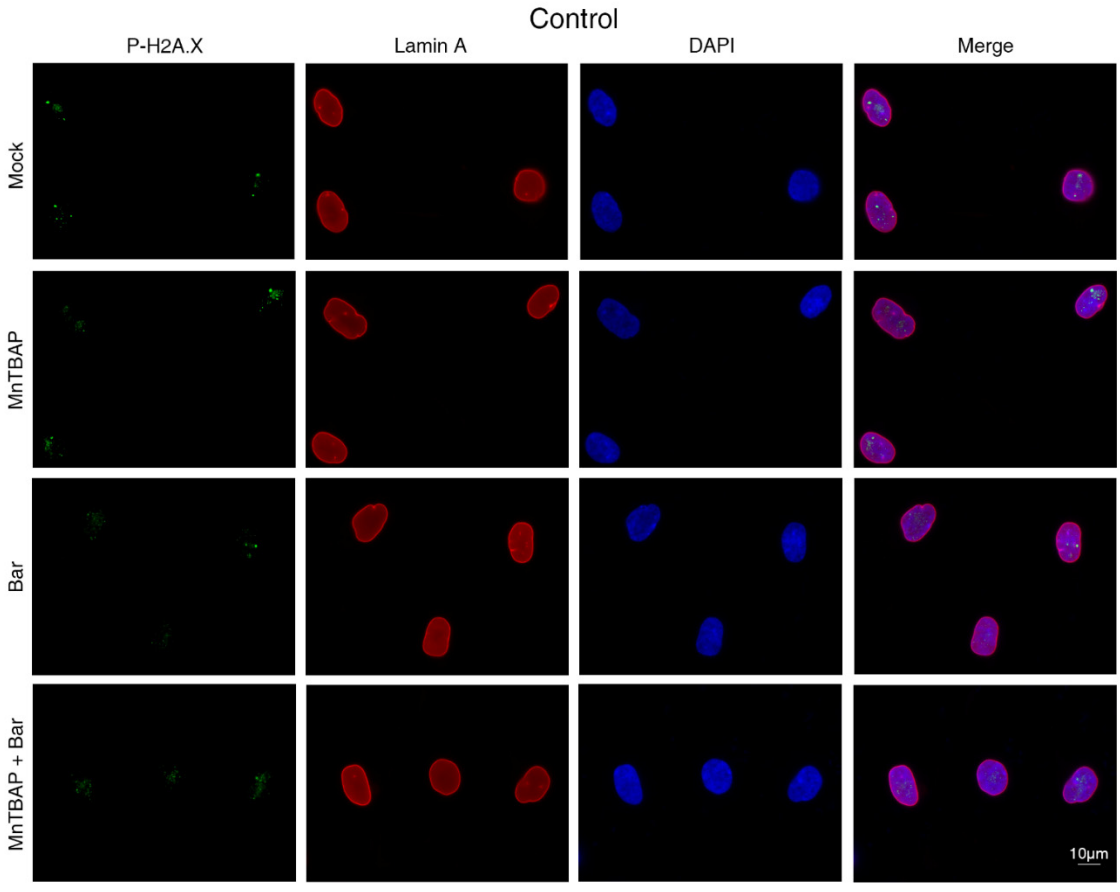
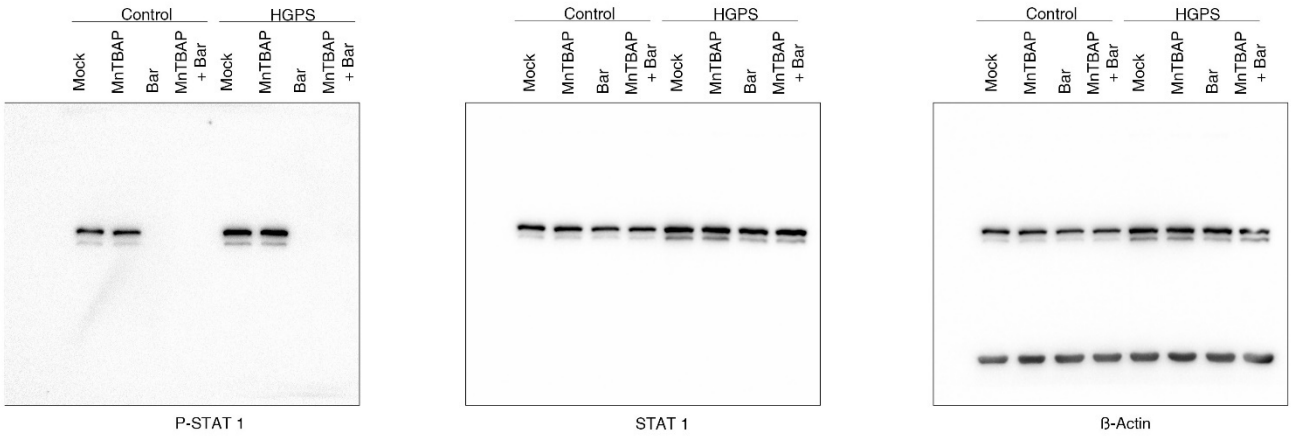
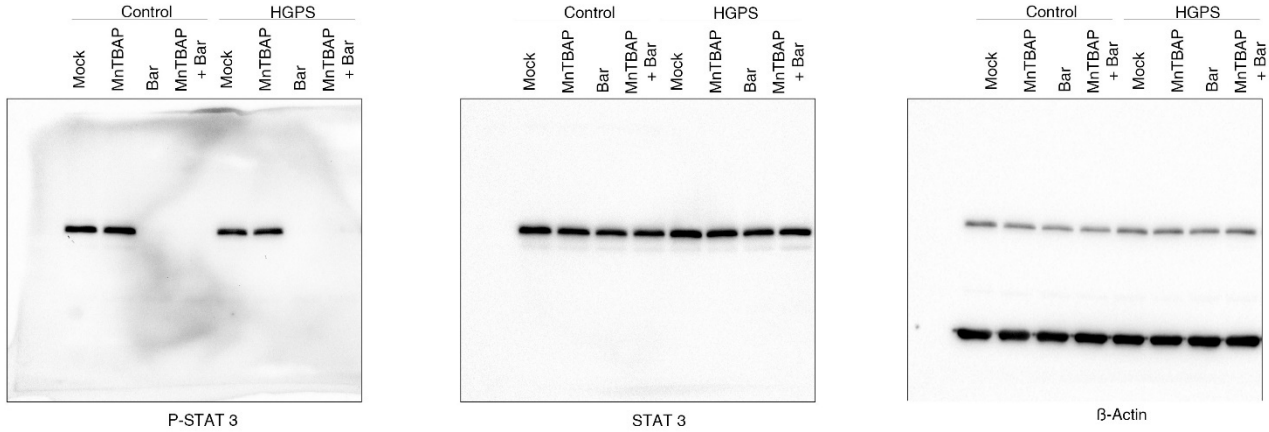


Figure S5. p-H2A.X immunostaining in control fibroblasts after treatments. Representative immunofluorescence images of control fibroblasts after 9 d of treatment with a mock solution, 5 μM MnTBAP, 1 μM Bar or the MnTBAP/Bar combination. The cells were stained with anti-p-Histone H2A.X (Ser139) (green) and anti-lamin A (red) antibodies and counterstained with DAPI (scale bar 10μm, n=4).

Full-length scan of western blot in Figure 2A



Full-length scan of western blot in Figure 2B



Full-length scan of western blot in Figure 3A

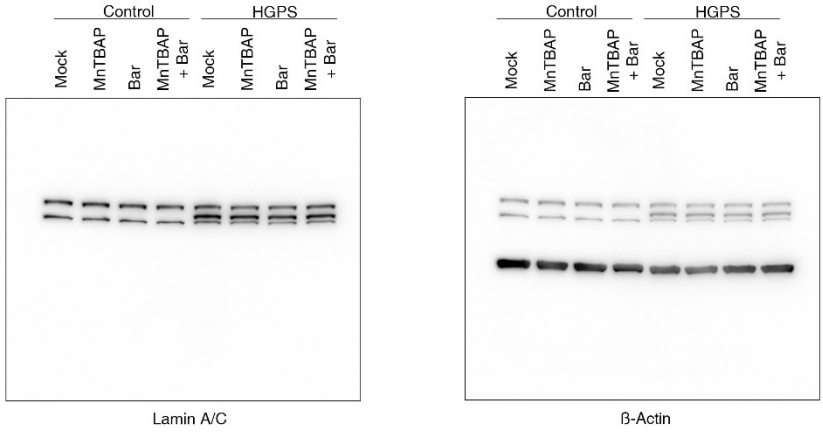


Figure S6. Full-length scan of western blots from Figure 2A, 2B, Figure 3A.

Primer sequence	Target Gene	Product size (bp)	Melting Temp	Info
FW:5'-CGCCAATGACTCAGAGGAAGA-3' REV:5'-AGGGCGTCATTCAGGATGAA-3'	IL-1	120	59.79 ℃ 59.09 ℃	doi.org/10.1371/journal.pone.0002301
FW:5'-AGCATGAAAGTCTCTGCCGC-3' REV:5'-GGCATTGATTGCATCTGGCTG-3'	CCL2	93	62.9 ℃ 66.0 ℃	doi.org/10.1172/jci.insight.87023
FW:5'-GGTACATCCTCGACGGCATCT-3' REV:5'-GTGCCTCTTTGCTGCTTTCAC-3'	IL-6	81	63.6 ℃ 62.4 ℃	doi.org/10.1152/ajpendo.00255.2002
FW:5'-CTGGCCGTGGCTCTCTTG-3' REV:5'-CCTTGGCAAAACTGCACCTT-3'	IL-8	69	63.2 ℃ 62.5 ℃	doi.org/10.1172/jci.insight.87023

Table S1. Primers used for real-time quantitative PCR analysis.