Supplementary Materials for

**STAT1 drives the interferon-like response and aging hallmarks in progeria**

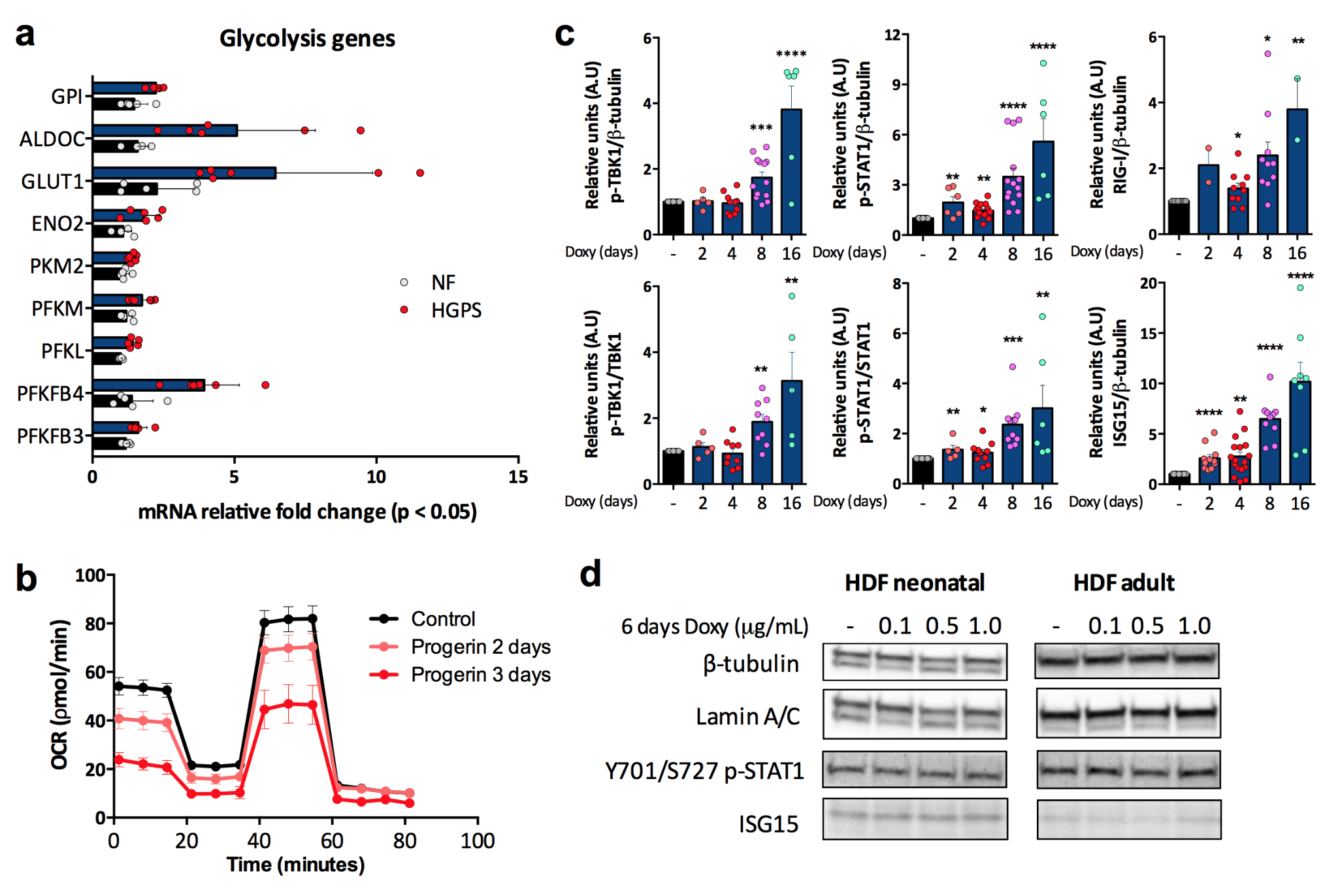
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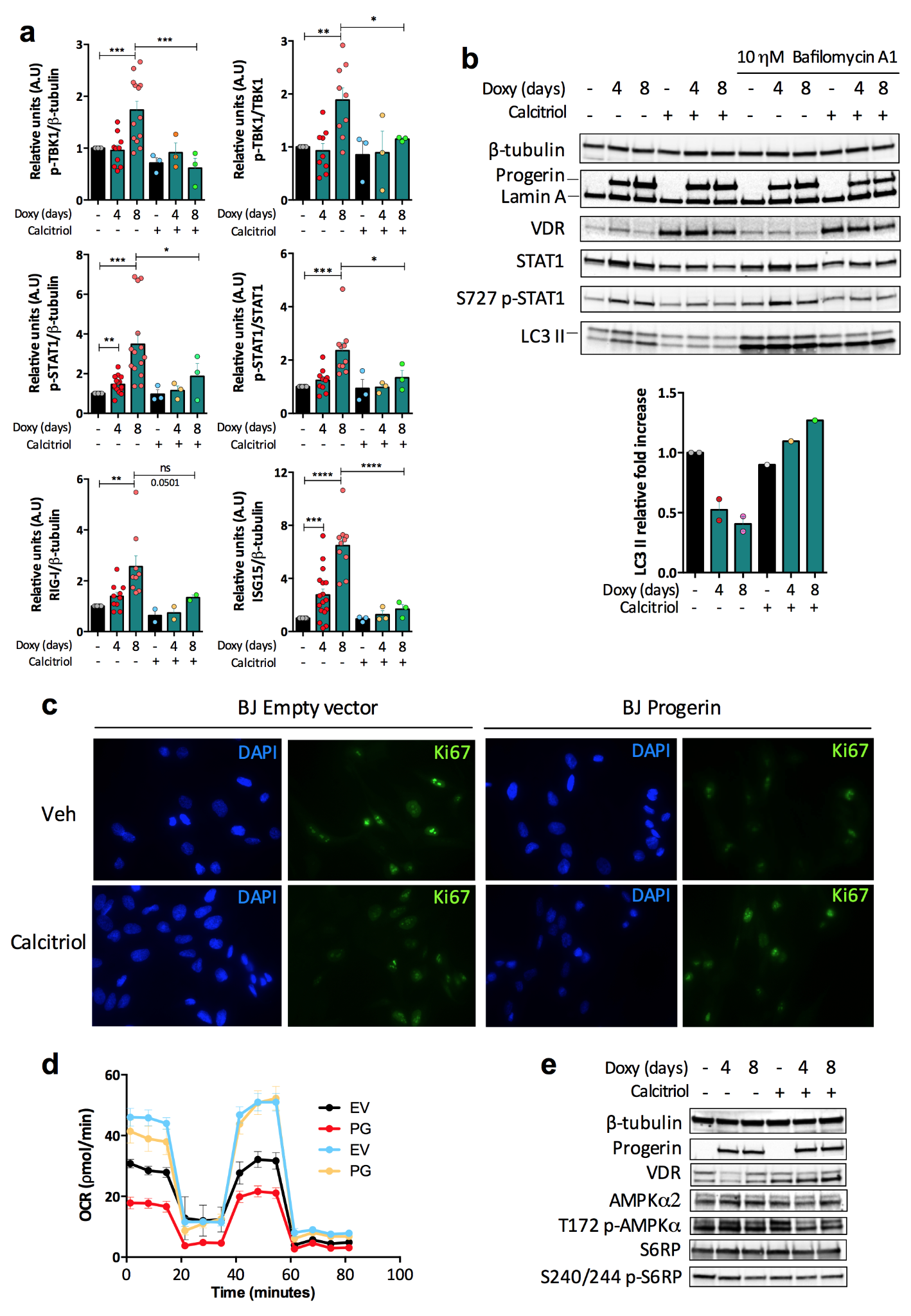
**This PDF file includes:**

Figs. S1 to S5

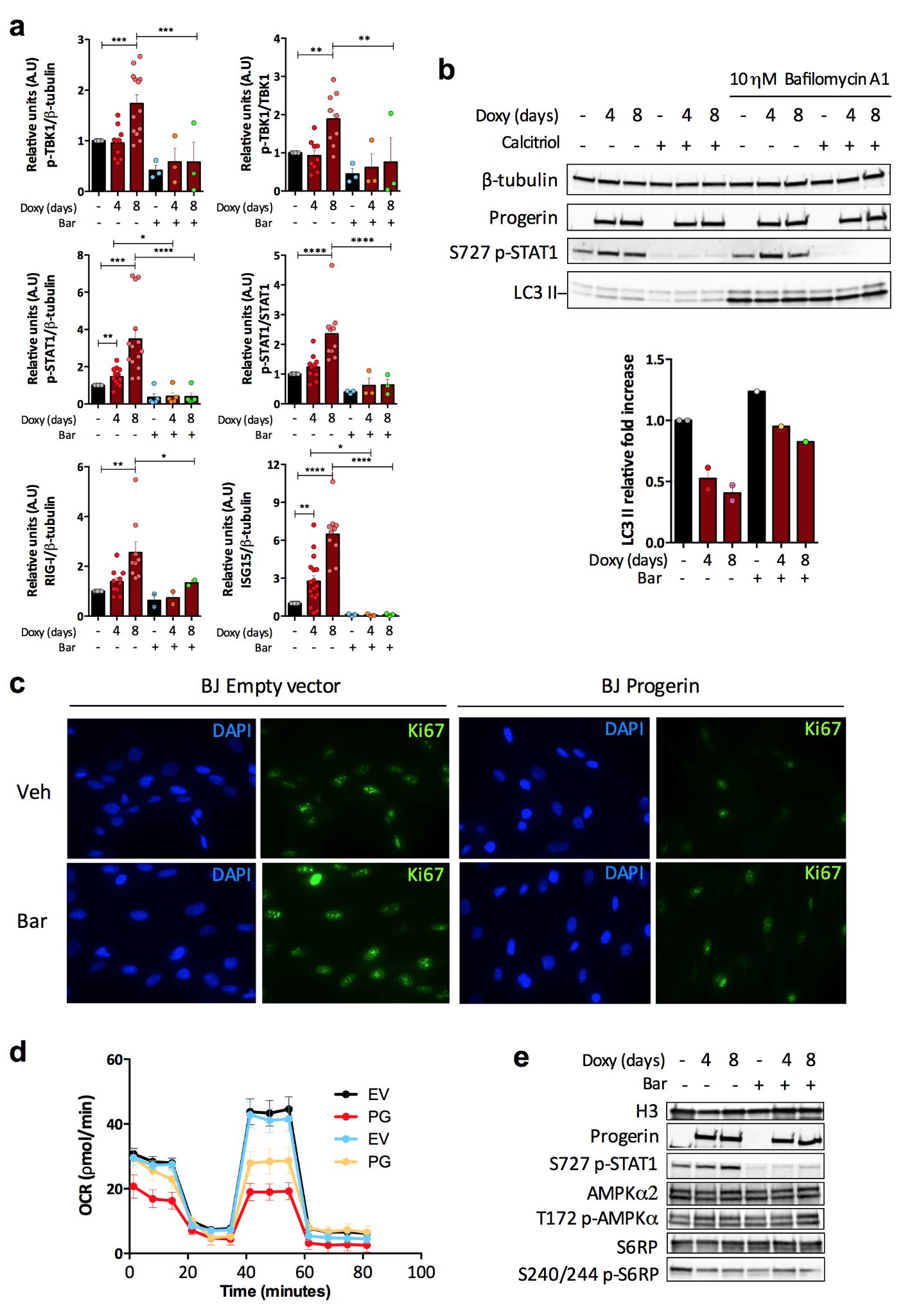
Tables S1 to S4



**Fig. S1. Relative to Figure 1. Progerin induces metabolic changes and sterile inflammation in fibroblasts.** (a) Graph show a re-analysis of published RNA seq data on five lines of normal fibroblasts (NF) and three lines of HGPS patient-derived fibroblasts (accession # GSE97986). Expression of glycolytic enzymes are significantly upregulated in HGPS cells (p > 0.05). (b) Densitometry of immunoblots from sterile inflammation markers (Figure 1g) showing average ± SEM from three to twelve biological repeats. (c) Respiration curve obtained in Seahorse experiments of HDFs treated with doxy for 1 or 2 days and 24 h of doxy release, resulting in 2 or 3 days of progerin expression, respectively. (d) Immunoblot of a control experiment to detect p-STAT1 and ISG15 in HDFs primary cells (neonatal and adults) without GFP-Progerin construct. Cells were treated for 6 days with different doxy concentrations. No activation of STAT1-ISG15 axis occurs upon doxy treatment (\*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, \*\*\*\*p < 0.0001).



**Fig. S2.** **Relative to Figure 2. Calcitriol mitigates aging phenotypes in progerin-expressing fibroblasts and extends HGPS mouse longevity**. (a) Densitometry of immunoblots from sterile inflammation markers (Figure 2A) with calcitriol or vehicle treatment (n=3-12). Graphs show average ± SEM. (b) Autophagy measurement using immunoblot of LC3-II marker. Cells were treated with 10 nM bafilomycin A1 for 24 h to block autophagy flux. β-tubulin was used as loading control. (c) Immunofluorescence pictures of Ki67 staining (green) in BJ EV (empty vector) and BJ PG (constitutive expression of progerin) treated with calcitriol or vehicle. DAPI (blue) was used for DNA staining. (d) Respiration curve obtained in Seahorse experiments of BJ EV or BJ PG. Samples were treated with calcitriol or vehicle for 2 days prior to the assay. (e) Immunoblot of doxy-induced GPF-progerin expression in HDFs for 4 and 8 days treated with calcitriol or vehicle (n=3). Proteins detected: GFP-progerin, nutrient sensing proteins (AMPKα and p-AMPKα), mTORC1 target proteins (S6RP and p-S6RP) and autophagy markers (LC3-II and p62). VDR was used to validate calcitriol treatment and β-tubulin as loading control. \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, \*\*\*\*p < 0.0001.

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**Fig. S3. Relative to Figure 3.** **JAK-STAT inhibition using baricitinib improves cellular health in progerin-expressing fibroblasts.** (a) Densitometry of immunoblots from sterile inflammation markers (Figure 3A) with baricitinib or vehicle treatment (n=3-12). Graphs show average ± SEM. (b) Autophagy measurement using immunoblot of LC3-II marker. Cells were treated with 10 nM bafilomycin A1 for 24 h to block autophagy flux. β-tubulin was used as loading control. (c) Immunofluorescence pictures of Ki67 staining (green) in BJ EV (empty vector) and BJ PG (constitutive expression of progerin) treated with baricitinib or vehicle. DAPI (blue) was used for DNA staining. (d) Respiration curve obtained in Seahorse experiments of BJ EV or BJ PG. Samples were treated with baricitinib or vehicle for 2 days prior to the assay. (e) Immunoblot of doxy-induced GPF-progerin expression in HDFs for 4 and 8 days treated with baricitinib or vehicle (n=3). Proteins detected: GFP-progerin, nutrient sensing proteins (AMPKα and p-AMPKα), mTORC1 target proteins (S6RP and p-S6RP) and autophagy markers (LC3-II and p62). VDR was used to validate calcitriol treatment and β-tubulin as loading control. \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, \*\*\*\*p < 0.0001.

Diagram

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**Fig. S4. Relative to Figure 4. Targeting Stat1 provides significant benefits to progeria mice health and lifespan.** (a) Body weight percentage and (b) Kaplan–Meier survival curves of chow-fed male G609G, *Stat1+/+* (n=7), G609G, *Stat1+/-* (n=8) and G609G, *Stat1-/-* (n=5). Mouse body weight was monitored three times per week in all experiments. Median survival of male G609G, *Stat1+/+* mice is 119 days, 141 days for G609G, *Stat1+/-* mice (p=0.0674) and 120 days for G609G, *Stat1-/-* (p=0.0979). (c) Body weight percentage and (d) Kaplan–Meier survival curves of chow-fed female G609G, *Stat1+/+* (n=11), G609G, *Stat1+/-* (n=5) and G609G, *Stat1-/-* (n=5). Median survival of female G609G, *Stat1+/+* mice is 132 days, 158 days for G609G, *Stat1+/-* mice (p=0.0406) and 119 days for G609G, *Stat1-/-* (0.0879). (e) Body weight percentage and (f) Kaplan–Meier survival curves of HFD-fed male G609G, *Stat1+/+* (n=12), G609G, *Stat1+/-* (n=8) and G609G, *Stat1-/-* (n=6). Median survival of male G609G, *Stat1+/+* mice is 149.5 days, 167.5 days for G609G, *Stat1+/-* mice (p=0.6256) and 159.5 days for G609G, *Stat1-/-* (p=0.4868). (g) Body weight percentage and (h) Kaplan–Meier survival curves of HFD-fed female G609G, *Stat1+/+* (n=13), G609G, *Stat1+/-* (n=6) and G609G, *Stat1-/-* (n=6). Median survival of female G609G, *Stat1+/+* mice is 170, 185 days for G609G, *Stat1+/-* mice (0.1139) and 157 days for G609G, *Stat1-/-* (0.0518). (i) Histology of aortic arch with H&E staining of HFD‐fed G609G mice treated with vehicle or baricitinib (120 days of age) and chow‐fed G609G mice (90 days of age) treated with vehicle, baricitinib or *Stat1+/-* (25 μm scale). Quantification of the amount of VSMC nuclei is shown in Figure 4k and represents approximately 5 random areas in each mouse sample (n=3). \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, \*\*\*\*p < 0.0001.



**Fig. S5. Relative to Figure 5. Targeting Stat1 improves adipocyte health and delays lipodystrophy in progeria mice.** (a) NMR measurements of percentage of fat accumulation in male and female G609G, *Stat1+/+* and G609G, *Stat1+/-* mice fed standard chow (n=4-8) or (b) HFD (n=6-8). (c) Measurement of mitochondrial respiration using Oroboros instrument of skeletal muscle (soleus) from mice experimental groups fed a standard chow or (d) HFD. Graphs show average ± SEM of oxygen flux in permeabilized soleus samples after sequential additions of octanoyl-l-carnitine (OC); pyruvate and malate (P/M); glutamate (G); adenosine diphosphate (ADP), succinate (Suc), and FCCP (n = 4-5). \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, \*\*\*\*p < 0.0001.

**Table S1. Antibodies and dilutions employed in this study.**

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| **Antibody** | **Dilution** | **Company** | **Catalog number** |
| β-tubulin | 1-5,000 | Origene | AP31823PU-N |
| Lamin A | 1-3,000 | Abcam | ab26300 |
| H3 | 1-60,000 | Abcam | ab1791 |
| ISG15 | 1-1,000 | Santa Cruz | sc-166755 |
| LC3 | 1-5,000 | NOVUS | NB100-2220 |
| p62 | 1-5,000 | NOVUS | NBP1-48320 |
| Ki67 | 1-700 | Cell Signaling | 9129S |
| VDR | 1-1,000 | Cell Signaling | 12550S |
| AMPKα | 1-1,000 | Cell Signaling | 2757S |
| T172 p-AMPKα | 1-1,000 | Cell Signaling | 50081S |
| S6 | 1-5,000 | Cell Signaling | 2217S |
| S240/244 p-S6 | 1-5,000 | Cell Signaling | 2215S |
| S757 p-ULK1 | 1-1,000 | Cell Signaling | 14202T |
| TBK1 | 1-1,000 | Cell Signaling | 3013S |
| S172 p-TBK1 | 1-1,000 | Cell Signaling | 5483S |
| STAT1 | 1-1,000 | Cell Signaling | 14994S |
| Y701 p-STAT1 | 1-1,000 | Cell Signaling | 7649S |
| S727 p-STAT1 | 1-1,000 | Cell Signaling | 9177S |
| RIG-I | 1-1,000 | Cell Signaling | 3743S |

**Table S2. Reagents, diets and resources employed in this study.**

|  |  |  |
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| **Reagent** | **Company** | **Catalog number** |
| ADP | Milipore | 117105 |
| Baricitinib (JAK-STAT inhibitor) | TargetMol | T2485 |
| Blebbistatin | Cayman Chemical | 13013 |
| BSA | Sigma-Aldrich | A7906 |
| Calcitriol (1𝛼,25-dihydroxyvitamin D3) | Cayman Chemical | 71820 |
| Chloroquine | Sigma-Aldrich | C6628 |
| Chow diet | LabDiet | 5L0B |
| Chow diet + cholecalciferol-D3 10,000 IU kg-1 | LabDiet | 9GEQ |
| Creatine | Sigma-Aldrich | C0780 |
| DAPI | Vectashield | H-1800 |
| DMSO | Sigma-Aldrich | D2650 |
| Doxycycline | Sigma-Aldrich | D9891 |
| Dulbecco’s modified Eagle’s medium (DMEM) | Sigma-Aldrich | D5796 |
| FCCP | Cayman Chemical | 15218 |
| Fetal Bovine Serum (FBS) | Atlas Biologicals | EF-0500-A |
| Formaldehyde solution | Sigma-Aldrich | F8775 |
| Gel cream hair remover | Veet | 8329150 |
| Glucose | Agilent | 103577-100 |
| Glutamate | Sigma-Aldrich | G1251 |
| Glutamine | Agilent | 103579-100 |
| High-fat diet | Research Diet | D12492 |
| Octanoyl Carnitine | TOCRIS | 605 |
| Polyethylene glycol, PEG 300, | Sigma-Aldrich | 90878 |
| Power SYBR Green PCR Master Mix | Applied Biosystems | 4367659 |
| Pyruvate | Agilent | 103578-100 |
| Saponin | Sigma-Aldrich | 57900 |
| Sodium pyruvate | Sigma-Aldrich | P2256 |
| Succinate | Sigma-Aldrich | S3674 |
| Triton X-100 | Fisher | BP151-500 |
| Tween 20 | Fisher | BP337500 |
| Tween 80 | Sigma-Aldrich | P1754 |
| UREA buffer | Sigma-Aldrich | 51457 |
| XF Base Assay media | Agilent | 103335-100 |

**Table S3. Assays and kits used in this study.**

|  |  |  |
| --- | --- | --- |
| **Kit** | **Company** | **Catalog Number** |
| L-lactate assay | Sigma-Aldrich | MAK329 |
| Mitochondrial Stress Test | Agilent | 103015-100 |
| SuperScript VILO cDNA Synthesis | Invitrogen | 11754-050 |
| Direct-zol RNA Mini prep | Zymo Research | R2052 |

**Table S4. Primer sequences used for SYBR Green qPCR.**

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| --- | --- |
| **Human Gene** | **Primer sequence (5' → 3')** |
| *IFNB* | F - GTCAGAGTGGAAATCCTAAG |
| R - ACAGCATCTGCTGGTTGAAG |
| *IL1B* | F - TGCACGCTCCGGGACTCACA |
| R - CATGGAGAACACCACTTGTTGCTCC |
| *IL6* | F - CCTTCCAAAGATGGCTGAAA |
| R - TTTCACCAGGCAAGTCTCCT |
| *GAPDH* | F - GCATGGCCTTCGGTGTCC |
| R - AATGCCAGCCCCAGCGTCAAA |
| *STAT1* | F - ATCAGGCTCAGTCGGGGAATA |
| R - TGGTCTCGTGTTCTCTGTTCT |
| *ISG15* | F - CGCAGATCACCCAGAAGATCG |
| R - TTCGTCGCATTTGTCCACCA |