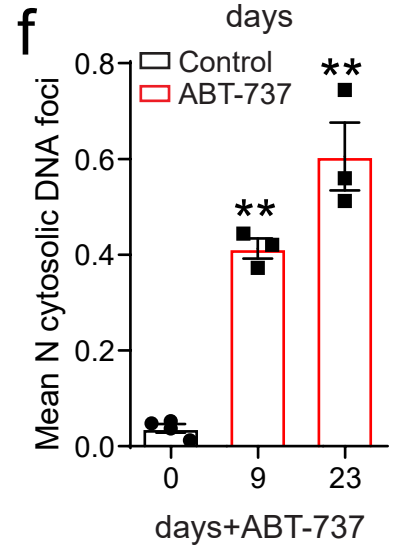
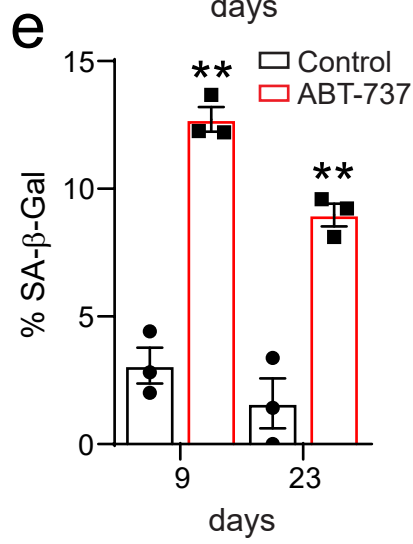
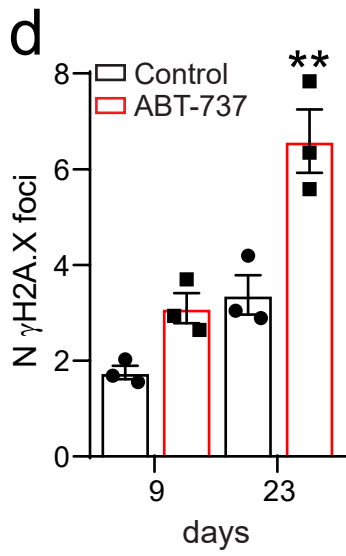
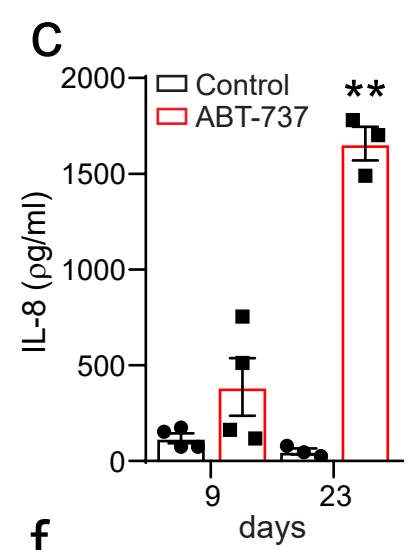
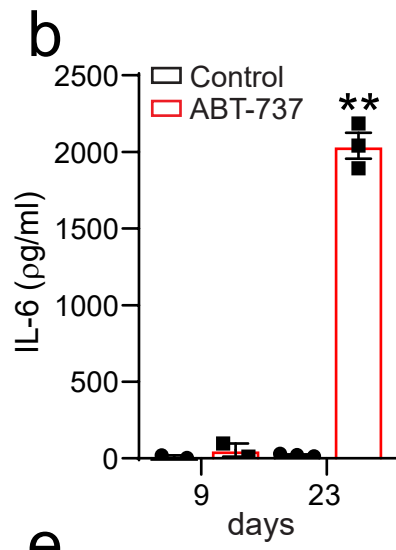
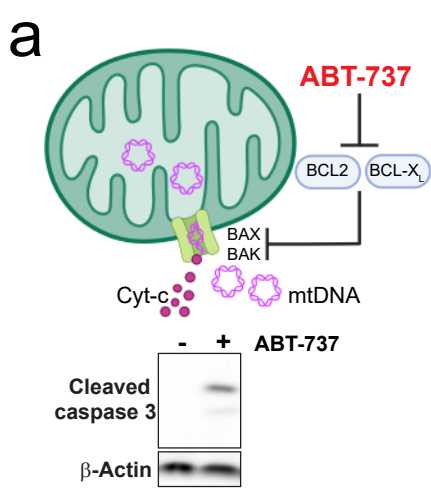
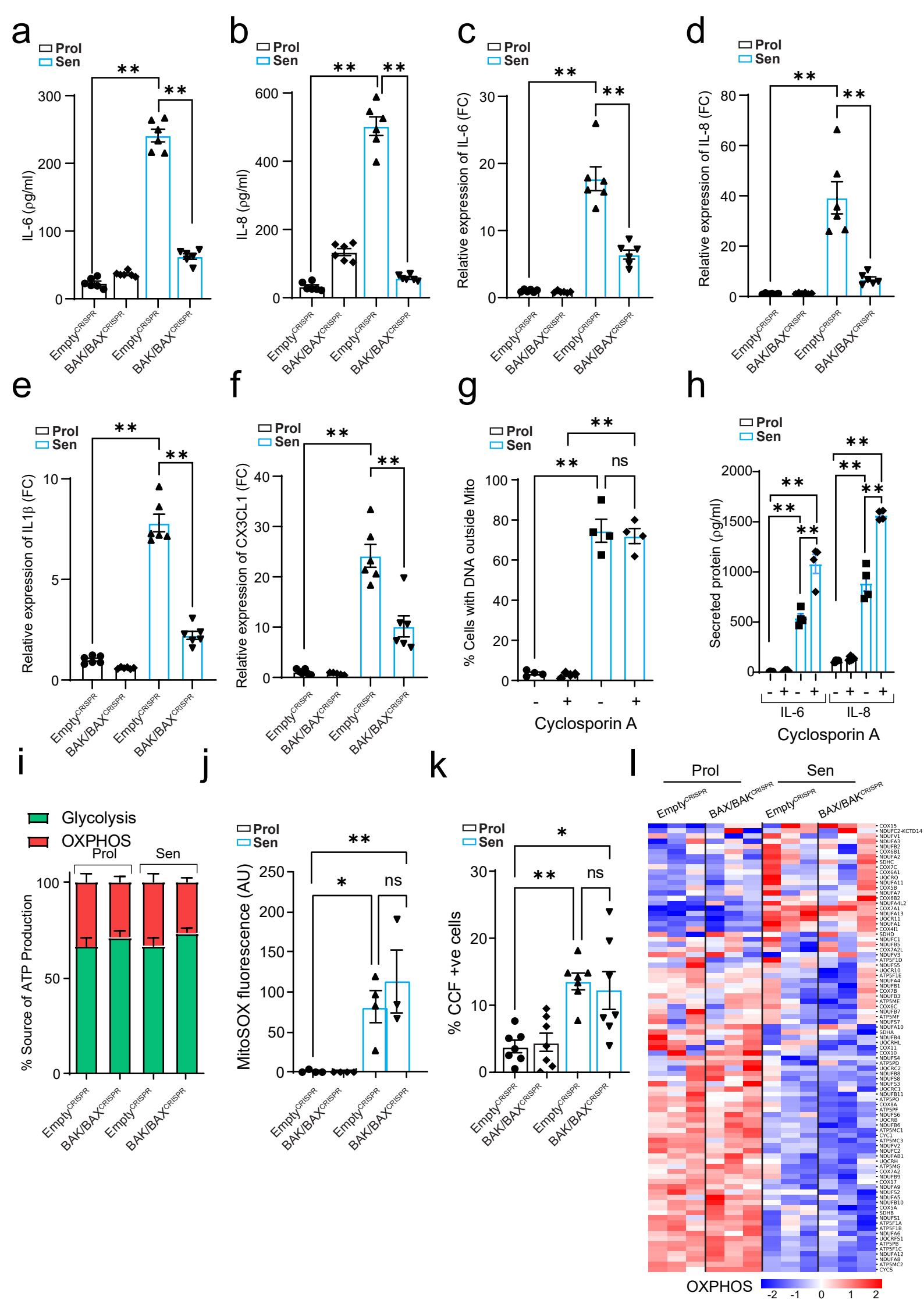
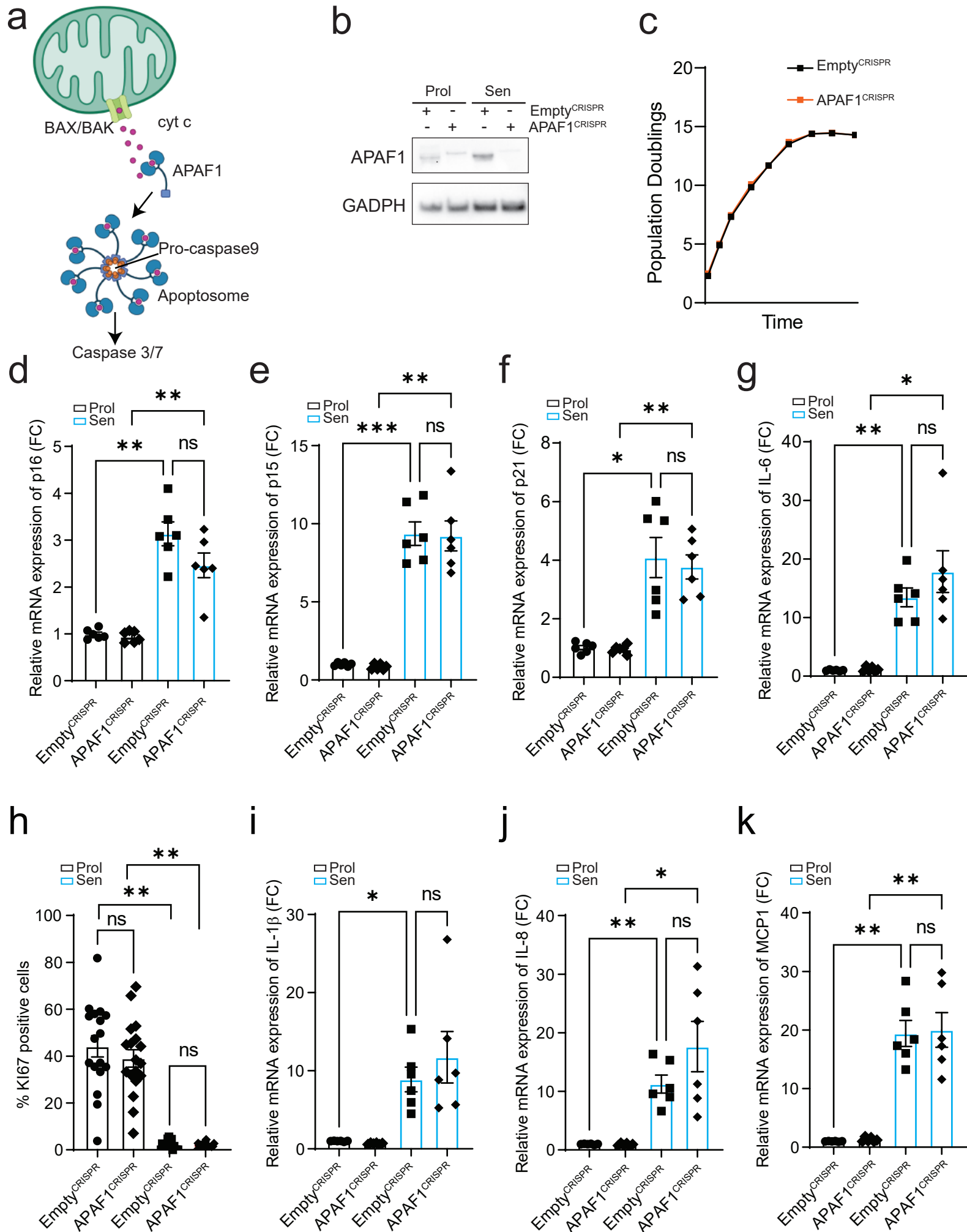




Supplementary Figure 1 – ABT-737 treatment induces miMOMP and drives senescence and the SASP. (a) Scheme representing the mechanism by which ABT-737 induces miMOMP. Below, representative Western blot showing cleaved caspase 3 in proliferating and ABT-737-treated cells. Levels of secreted (b) IL-6 and (c) IL-8 in control and cells treated with ABT-737 for 9 and 23 days. Quantification of (d) mean number of γ H2AX foci, (e) percentage of Sen- β -Gal-positive cells and (f) mean number of DNA nucleoids located outside of the mitochondrial network in control and cells treated with ABT-737 for 9 and 23 days. Data are mean of n=3 independent experiments $\pm$ S.E.M. Statistical significance (one-way ANOVA) is indicated (**P<0.01).

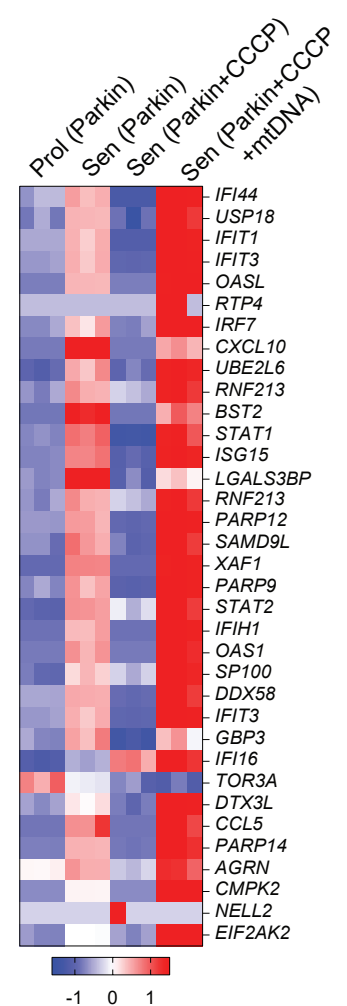


Supplementary figure 2 – BAX and BAK mitochondrial pores regulate the SASP without the involvement of the MTPT. Levels of secreted **(a)** IL-6 and **(b)** IL-8 in proliferating and senescent EV and BAX/BAK^{-/-} cells. Quantification of mRNA levels of **(c)** IL-6, **(d)** IL-8, **(e)** IL1 β and **(f)** CX3CL1. Data are expressed as fold change to proliferating EV cells. **(g)** Percentage of cells containing DNA foci located outside of mitochondrial network, and **(h)** Levels of secreted IL-6 and IL-8 in proliferating and senescent cells treated with DMSO or 1 μ M Cyclosporin A. Data are mean of n=4-6 independent experiments $\pm$ S.E.M. Statistical significance (one-way ANOVA) is indicated (**P<0.01). **(i)** Graph showing the energy production by glycolysis or oxidative phosphorylation normalized to total ATP production and expressed as a percentage. Data are mean of n=5 independent experiments $\pm$ S.E.M. Statistical significance was assessed using a one-way ANOVA. **(j)** Quantification of MitoSOX fluorescence intensity in proliferating and senescent EV and BAX/BAK^{-/-} cells. **(k)** Quantification of the percentage of EV and BAX/BAK^{-/-} cells displaying CCF. Data are mean of n=4-7 independent experiments $\pm$ S.E.M. Statistical significance (one-way ANOVA) is indicated (*P<0.05; **P<0.01). **(l)** Column clustered heatmap of OXPHOS genes that are differentially expressed in senescent cells and are not changed by BAX/BAK deletion. The color intensity represents column Z-score, where red and blue indicate high and low expression, respectively.

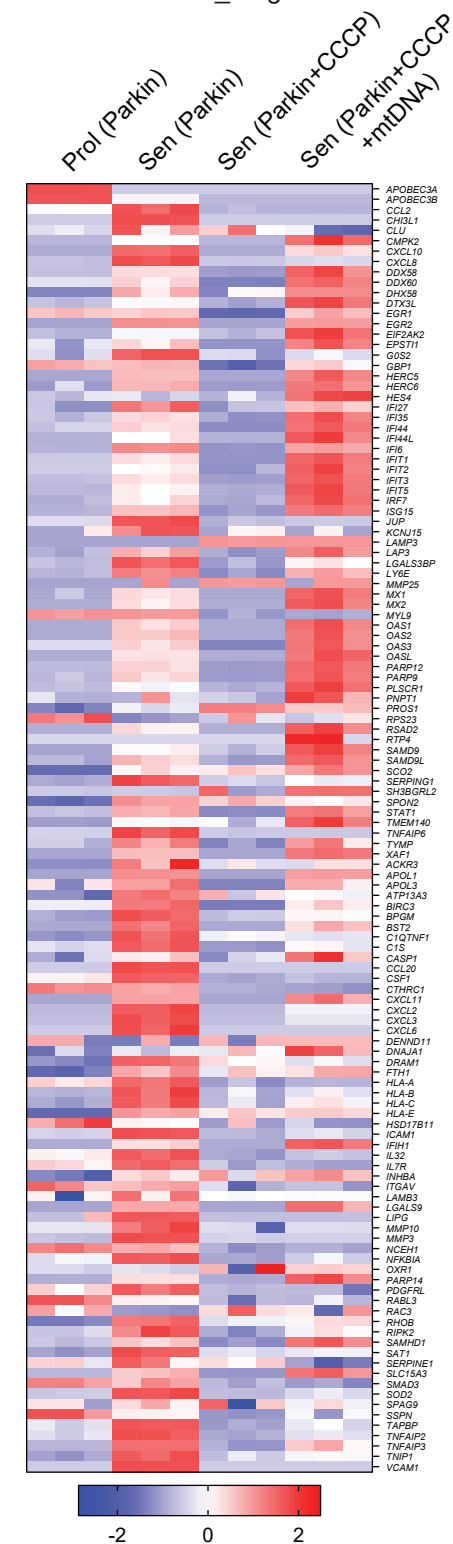


Supplementary figure 3 – Caspase activation is not involved in SASP regulation in senescent cells. (a) Scheme showing the mechanism of miMOMP-induced caspase activation. (b) Western blot showing successful CRISPR/Cas9 deletion of APAF1 in proliferating and senescent cells. (c) Graph showing population doublings of EV and APAF1-deficient cells. Data are representative of n=2 independent experiments. Quantification of mRNA levels of (d) p16, (e) p15, (f) p21 and (g) IL-6 in proliferating and senescent EV and APAF1-deficient cells. Data are mean of n=6 independent experiments $\pm$ S.E.M. Statistical significance (one-way ANOVA) is indicated (*P<0.05; **P<0.01, ***P<0.001). (h) Quantification of the percentage of EV and APAF1-deficient cells positive for Ki67. Quantification of mRNA levels of (i) IL-1 β , (j) IL-8, (k) MCP1 in proliferating and senescent EV and APAF1-deficient cells. Data are mean of n=6 independent experiments $\pm$ S.E.M. Statistical significance (one-way ANOVA) is indicated (*P<0.05; **P<0.01).

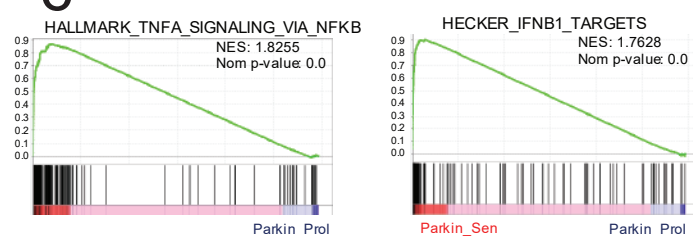
a mtDNA stress signature
(West *et al.* Nature 2015)



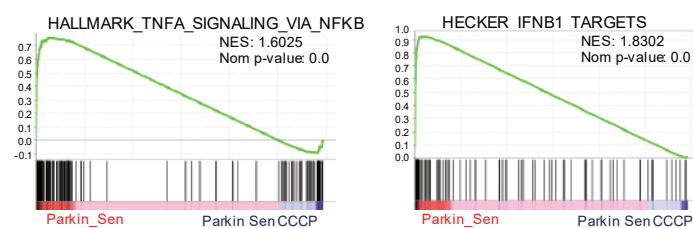
b TNFA_signaling_via-NFKB
IFNB1_Targets



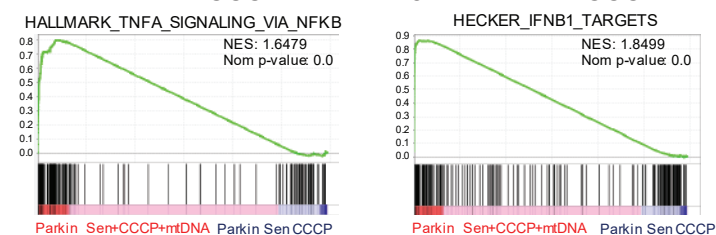
c Sen Parkin vs. Prol Parkin



Sen Parkin vs. Sen Parkin+CCCP



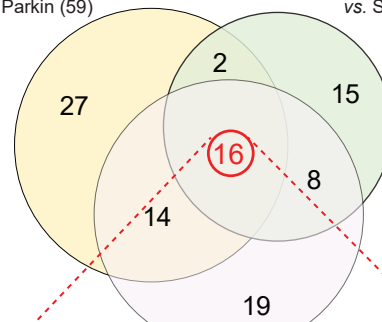
Sen Parkin+CCCP+mtDNA vs. Sen Parkin+CCCP



d

High in SEN Parkin
vs. Prol Parkin (59)

High in Sen Parkin + CCCP + mtDNA
vs. Sen Parkin + CCCP (41)

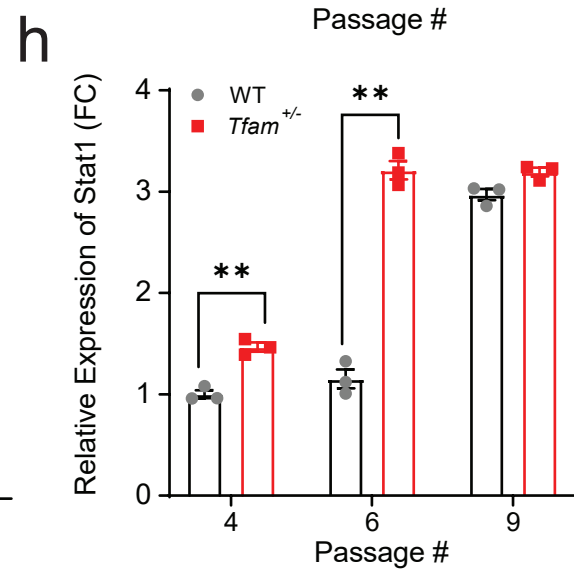
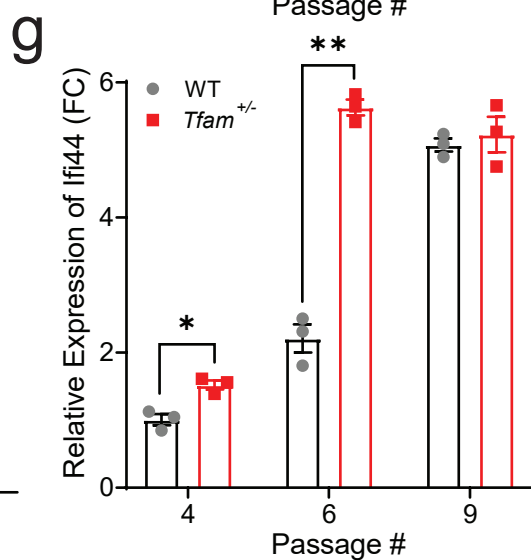
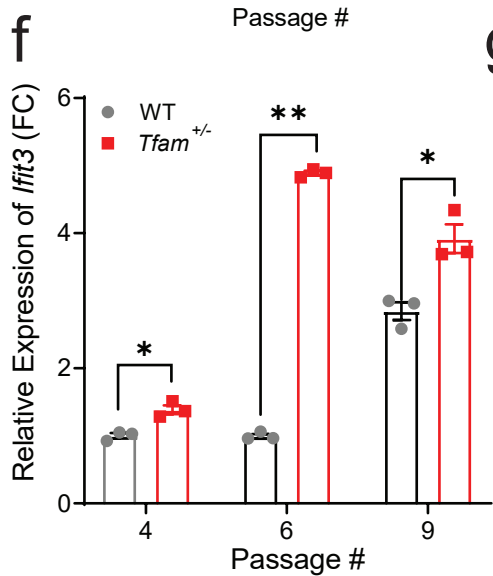
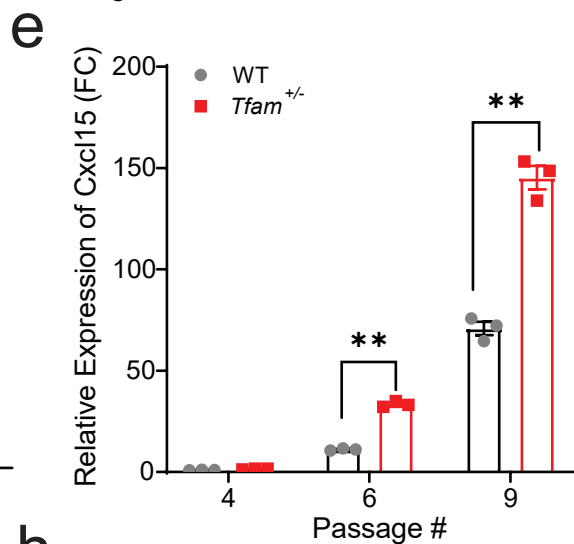
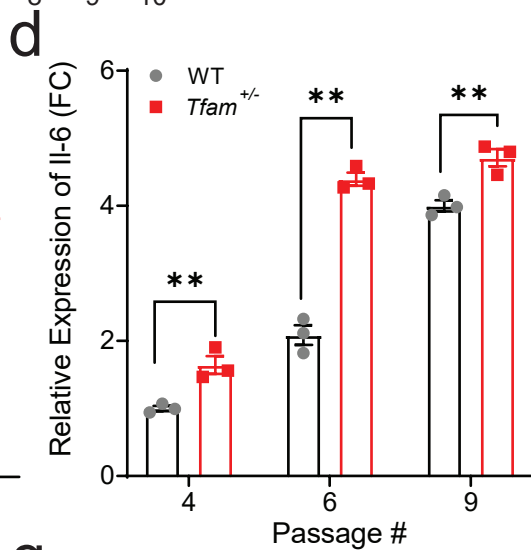
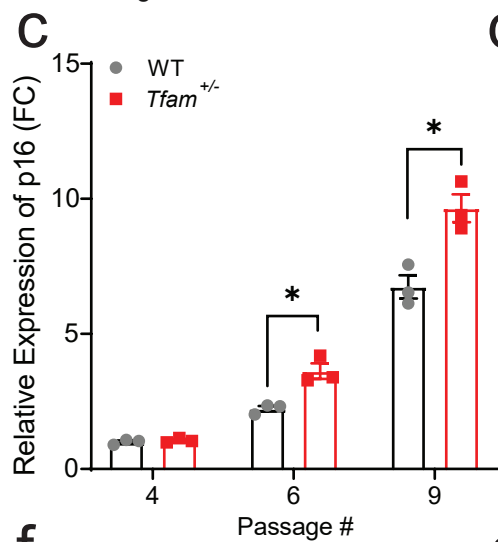
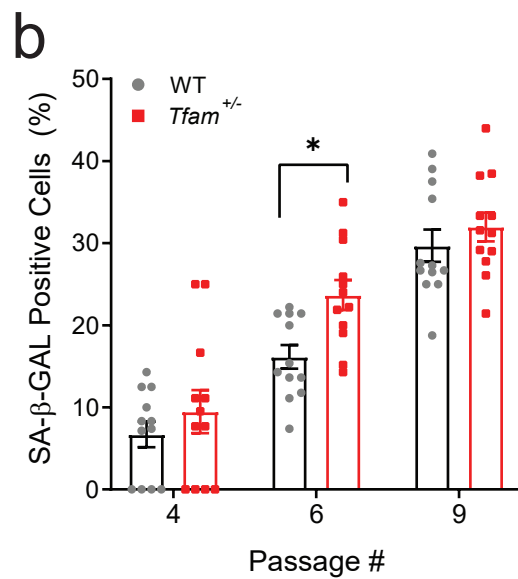
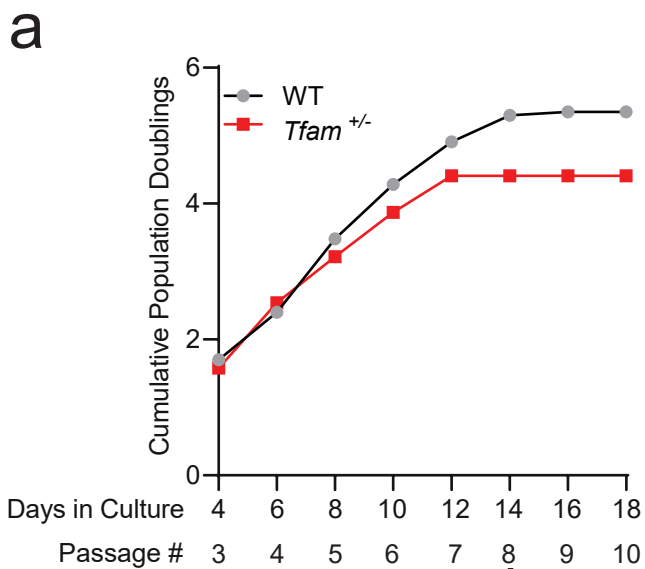


High in Sen Parkin vs.
Sen Parkin + CCCP (57)

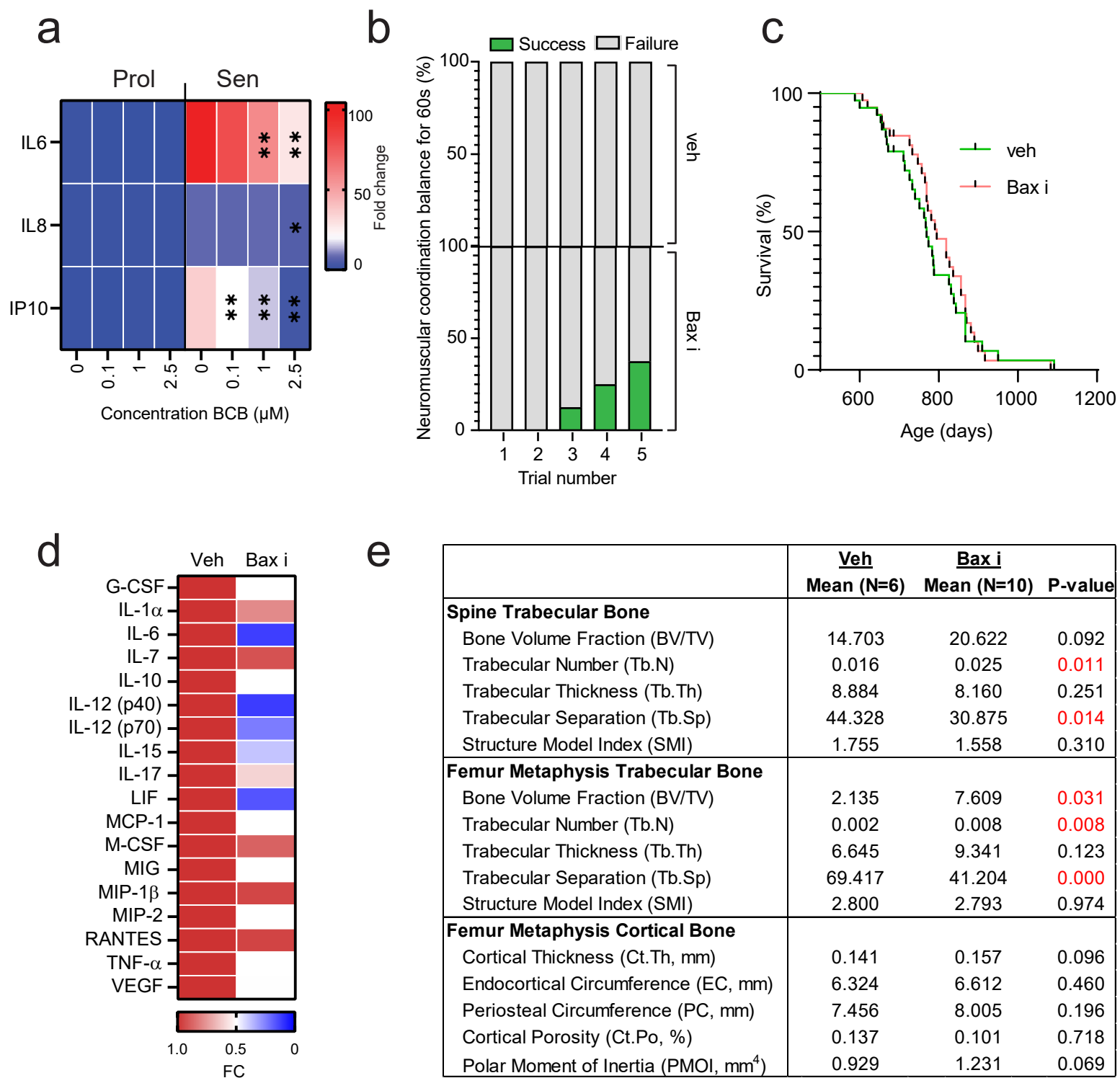
HECKER_IFNB1_TARGETS
SANA_TNF_SIGNALING_UP
REACTOME_CHEMOKINE_RECEPTORS_BIND_CHEMOKINES
REACTOME_INTERFERON_ALPHA_BETA_SIGNALING
SANA_RESPONSE_TO_IFNG_UP

Supplementary figure 4 – Widespread mitochondrial clearance rescued mtDNA stress signature and NF- κ B and IFN-gene expression, while the addition of mtDNA restored it.

(a) Heatmap revealing that the mtDNA stress signature according to West *et al.* 2015 was induced on mRNA level in senescent cells while the addition of CCCP reversed this phenotype. Additional mtDNA treatment was able to restore the stress signature. **(b)** Gene members of two hallmark signatures, TNFA-signaling via NF κ B (M5890) and Hecker IFNB1 targets (M3010), were upregulated in senescent cells and downregulated after CCCP. Subsequent addition of mtDNA rescued this phenotype. **(c)** The GSEA plots for Parkin Sen vs. Parkin Prol displayed an enrichment for the TNFA-signaling via NF κ B (M5890) and Hecker IFNB1 targets (M3010) pathways in the senescent cell population. Addition of mtDNA led to a significant enrichment compared to CCCP alone in senescent cells. **(d)** Venn diagram depicting a substantial overlap of enriched pathways in all three conditions (FWER p-value<0.25).



Supplementary Figure 5 – *Tfam*^{+/-} MEFs display an accelerated onset of replicative senescence. (a) Cumulative population doublings of MEFs derived from WT and *Tfam*^{+/-} mice during the course of 10 serial passages. Senescence was confirmed by (b) quantification of SA- β -GAL positive cells (mean $\pm$ SD; 12 images per condition from 2 independent experiments; unpaired *t*-test, **P*≤0.05) and (c) RT-qPCR assessment of *p16* expression, as well as (d) RT-qPCR assessment of SASP genes (*CXCL15* and *IL6*) relative to *ACTIN* levels. (e) RT-qPCR assessment of ISGs (*STAT1*, *IFIT3*, and *IFI44*) relative to *ACTIN* levels. Error bars indicate the mean $\pm$ SD of technical triplicates and results (c-e) are representative of 2 independent experiments (unpaired *t*-test, **** *P*<0.0001, ****P*<0.001, ***P*<0.01 and **P*≤0.05).



Supplementary figure 6 – Pharmacological Bax inhibition improves healthspan without affecting lifespan of aged mice. **(a)** Heatmap showing secreted levels of IL-6, IL-8 and IP-10 in proliferating and senescent cells treated with different concentrations of the Bax inhibitor BCB. Values are shown as fold change to proliferating controls. Blue denotes low expression and red indicates high expression. Data are mean of n=4 independent experiments $\pm$ S.E.M. Statistical significance (one-way ANOVA) is indicated (*P<0.05; **P<0.01). **(b)** Neuromuscular coordination shown as a percentage number of successful attempts (green) to remain on a straight rod for 60 s (n=7-8 per group). **(c)** Kaplan-Meier survival curves of animals treated with vehicle (n=38) or Bax inhibitor (n=39) from 18-20 months old until death. **(d)** Heatmap showing levels of cytokines found in plasma from mice treated with vehicle or Bax inhibitor. Values are shown as fold change compared to vehicle-treated animals. Red denotes high expression and blue indicates low expression. **(e)** Table summarizing μ CT-derived parameters obtained for the spine and femur from vehicle- and Bax inhibitor-treated mice (n=6-10 per group). Data are mean $\pm$ S.E.M. Statistical significance (unpaired t-test) is indicated.

Supplemental tables:

Table 1: – Antibodies used for immunocytochemistry and immunohistochemistry

Primary antibodies	Source	Product number	Concentration
Anti-TOMM20 rabbit polyclonal antibody	Millipore Sigma	HPA011562	1:200
Anti-Cytochrome c mouse monoclonal antibody	BioLegend	612301	1:1000
Anti-Bax(6A7) mouse monoclonal antibody	Santa Cruz	sc-23959	1:100
Anti-Phospho-Histone H2A.X (Ser139) (20E3) rabbit monoclonal antibody	Cell Signaling	9718	1:1000
Anti-DNA mouse monoclonal antibody	Millipore Sigma	CBL186	1:100
Anti-TFAM rabbit monoclonal antibody	Cell Signaling	8076S	1:100
Anti-p16 (E6H4) mouse monoclonal antibody	Roche	705-4713	1:5
Anti p21 Waf1 Cip1 (12D1) rabbit monoclonal antibody	Cell Signaling	2947S	1:1000
Anti-Ki67 rabbit polyclonal antibody	Abcam	ab15580	1:1000
Anti-Bax (D3R2M) rabbit monoclonal antibody	Cell Signaling	14796	1:100
Anti-Cleaved Caspase-3 (Asp175) Antibody	Cell Signaling	9661	1:50
Secondary antibodies	Source	Product number	Concentration
Goat anti-Rabbit IgG (H+L), Superclonal™ Recombinant Secondary Antibody, Alexa Fluor 647	Thermo Fisher Scientific	A-27040	1:1000
Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	Thermo Fisher Scientific	A-11008	1:1000
Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 594	Thermo Fisher Scientific	A-11012	1:1000
Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 594	Thermo Fisher Scientific	A-11032	1:1000
Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 647	Thermo Fisher Scientific	A-21235	1:1000
Goat anti-Mouse IgG (H+L), Superclonal™ Recombinant Secondary Antibody, Alexa Fluor	Thermo Fisher Scientific	A-28175	1:1000

488			
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Table 2: – Antibodies used for Western blotting.

Primary antibodies	Source	Catalog number	Concentration
Anti-cytochrome c (D18C7) rabbit monoclonal antibody	Cell Signaling	11940S	1:1000
Anti-UQCR2 rabbit monoclonal antibody	Abcam	ab103616	1:1000
Anti-Cleaved Caspase 3 (D175 hAIE) rabbit monoclonal antibody	Cell Signaling	9664S	1:500
Anti- β -actin mouse monoclonal antibody	Abcam	ab8226	1:10000
Anti-TFAM rabbit monoclonal antibody	Cell Signaling	8076S	1:200
Anti-Bak (D4E4) rabbit monoclonal antibody	Cell Signaling	12105	1:1000
Anti-Bax (D2E11) rabbit monoclonal antibody	Cell Signaling	5023	1:1000
Anti-Bax (6A7) mouse monoclonal antibody	Santa Cruz	sc-23959	1:1000
Anti- β -Tubulin rabbit polyclonal antibody	Cell Signaling	2146S	1:10000
Anti-Lamin B1 rabbit polyclonal antibody	Abcam	ab16048	1:1000
Anti-HMGB1 rabbit monoclonal antibody	Cell Signaling	6893S	1:1000
Anti-NDUFB8 mouse monoclonal antibody	Abcam	ab110242	1:1000
Anti-GAPDH rabbit monoclonal antibody	Cell Signaling	5174S	1:1000
Anti-cGAS (D1D3G) rabbit monoclonal antibody	Cell Signaling	15102	1:1000
Anti-STING (D2P2F) rabbit monoclonal antibody	Cell Signaling	13647S	1:1000
Anti-APAF1 rabbit monoclonal antibody	Cell Signaling	8723S	1:500
Secondary antibodies	Source	Catalog number	Concentration
Goat Anti-Rabbit HRP Conjugated	Sigma Aldrich	A0545	1:5000
Goat Anti-Mouse HRP Conjugated	Sigma Aldrich	A2554	1:5000

Table 3: – qPCR Primer assays used.

qPCR Primer Assay	Source	Product number
IL-6	IDT	Hs.PT.58.40226675
IL-8	IDT	Hs.PT.58.39926886.g
RSP16	IDT	Hs.PT.58.366374
CDKN1a	IDT	Hs.PT.58.38492863.g
CDKN2a (Fig. 3e, Supp 3)	IDT	Hs.PT.58.40743463.g
IL-1 β (Supp. 2e, Supp. 3)	IDT	Hs.PT.58.1518186
CX3CL1 (Supp. 2f)	IDT	Hs.PT.58.19601997
CDKN2b (Supp. 3)	IDT	Hs.PT.58.4919581
MCP1 (Supp. 3)	IDT	Hs.PT.58.45467977

Table 4: – Sybr Green primers used for qPCR.

Gene name	Forward and reverse PCR primer sequences
hMT-Dloop	CATCTGGTTCCTACTTCAGGG CCGTGAGTGGTTAATAGGGTG
mMT-Dloop	AATCTACCATCCTCCGTGAAACC TCAGTTTAGCTACCCCCAAGTTTAA
mActinb	ATGCTCCCCGGGCTGTAT CATAGGAGTCCTTCTGACCCATTC
mCdkn2a	GAAGTCTTTTCGGTCGTACCC CGAATCTGCACCGTAGTTGA
mCxcl15	GTCCTTAACCTAGGCATCTTCG TCTGTTGCAGTAAATGGTCTCG
mIl6	TGATGCACTTGCAGAAAACA ACCAGAGGAAATTTTCAATAGGC
mStat1	CGCGCATGCAACTGGCATATAACT ATGCTTCCGTTCCACGTAAGCTT
mIfit3	TTCCAGCAGCACAGAAAC AAATTCCAGGTGAAATGGCA
mIfi44	CTGATTACAAAAGAAGACATGACAGAC AGGCAAAACCAAAGACTCCA
mIL6	CTACCAAAGTGGATATAATCAGGA CCAGGTAGCTATGGTACTCCAGAA
mIL1a	AGGAGAGCCGGGTGACAGTA TCAGAATCTTCCCGTTGCTTG
mIL-1b	CCAAAAGATGAAGGGCTGCT TCATCAGGACAGCCCAGGTC
mCXCL1	CAATGAGCTGCGCTGTCAGT TTGAGGTGAATCCCAGCCAT
mMMP3	TGGAGCTGATGCATAAGCCC TGAAGCCACCAACATCAGGA
mIFNA	GGACTTTGGATTCCCGCAGGAGAAG GCTGCATCAGACAGCCTTGACGGTC

mOAS1	GCCTGATCCCAGAATCTATGC GAGCAACTCTAGGGCGTACTG
mInhbinA	GATCATCACCTTTGCCGAGT TGGTCCTGGTTCTGTTAGCC
m18s	GTAACCCGTTGAACCCCAT CCATCCAATCGGTAGTAGCG
mCcl2	GTCTGTGCTGACCCCAAGAAG TGGTTCCGATCCAGGTTTTTA
mCcl3	TCCCAGCCAGGTGTCATTTT TTGGAGTCAGCGCAGATCTG
mCcl5	GCCCACGTCAAGGAGTATTTCT ACAAACACGACTGCAAGATTGG
mCcl7	CCCTGGGAAGCTGTTATCTTCA CTGATGGGCTTCAGCACAGA
mCsf1	ATTGCCAAGGAGGTGTCAGAA GGACCTTCAGGTGTCCATTCC
mCxcl1	CCGAAGTCATAGCCACACTCAA CAAGGGAGCTTCAGGGTCAAG
mCxcl2	TCAAGGGCGGTCAAAAAGTT CAGTTAGCCTTGCCTTTGTTCA
mCxcl15	TCCATGGGTGAAGGCTACTGT TTCATTGCCGGTGGAAATTC
mHmgb1	TCCTTCGGCCTTCTTCTTGTT AGGATGCTCGCCTTTGATTTT
mIcam1	GTGGCGGGAAAGTTCCTGTT GTCCAGCCGAGGACCATACA
mIgfbp2	GCCCCCTGGAACATCTCTACT GTTGTACCGGCCATGCTTGT
mIgfbp4	GCAACTTCCACCCCAAACAGT CCTGTCTTCCGATCCACACA
mIl1a	AAGAGACCATCCAACCCAGATC CCTGACGAGCTTCATCAGTTTG
mIl1b	TCAGGCAGGCAGTATCACTCA CACGGGAAAGACACAGGTAGCT
mIl6	ACCACGGCCTTCCCTACTTC TTGGGAGTGGTATCCTCTGTGA
mIl17a	GGACTCTCCACCGCAATGAA GCACTGAGCTTCCCAGATCAC
mInhba	CAGGAAGACACTGCACTTTGA TTCAGGAAGAGCCACACTTCT
mMmp3	TTGACGATGATGAACGATGGA GAGCAGCAACCAGGAATAGGTT
mMmp9	TGAGTCCGGCAGACAATCCT CCCTGGATCTCAGCAATAGCA
mMmp12	GTGCCCCGATGTACAGCATCTT GGTACCGCTTCATCCATCTTG
mMmp13	TGAGGAAGACCTTGTGTTTGCA GCAAGAGTCGCAGGATGGTAGT
mNfkb1	GGCTTTGCAAACCTGGGAAT TCCGTGCTTCCAGTGTTTCA

mPappa	CATCTCAGGTGTGTCGAACCA TGCAAGGATACCAAGCATGCT
mSerpine1	GGACACCCTCAGCATGTTCA CGGAGAGGTGCACATCTTTCT
mSerpinb2	TTCCGCATACTGGAAACATCAG GGATGCGTCCTCAATCTCATC
mTnf	GTTCTGCAAAGGGAGAGTGG GCACCTCAGGGAAGAGTCTG
mActb	AATCGTGCGTGACATCAAAGAG GCCATCTCCTGCTCGAAGTC
mHprt	CGTGATTAGCGATGATGAACCA TCCAAATCCTCGGCATAATGA
mTuba1	GGTTCCCAAAGATGTCAATGCT CAAACCTGGATGGTACGCTTGGT