

Figure- S1

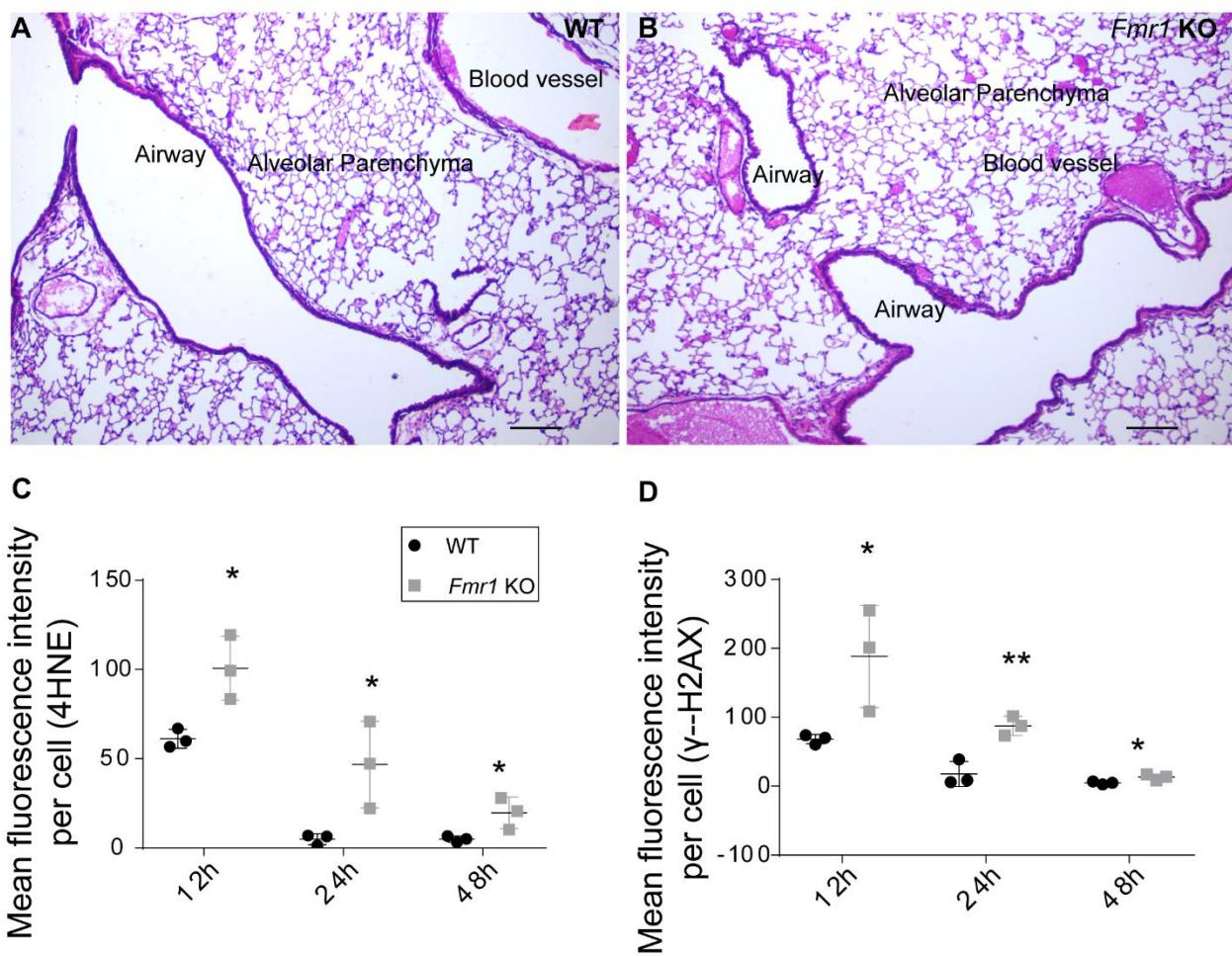
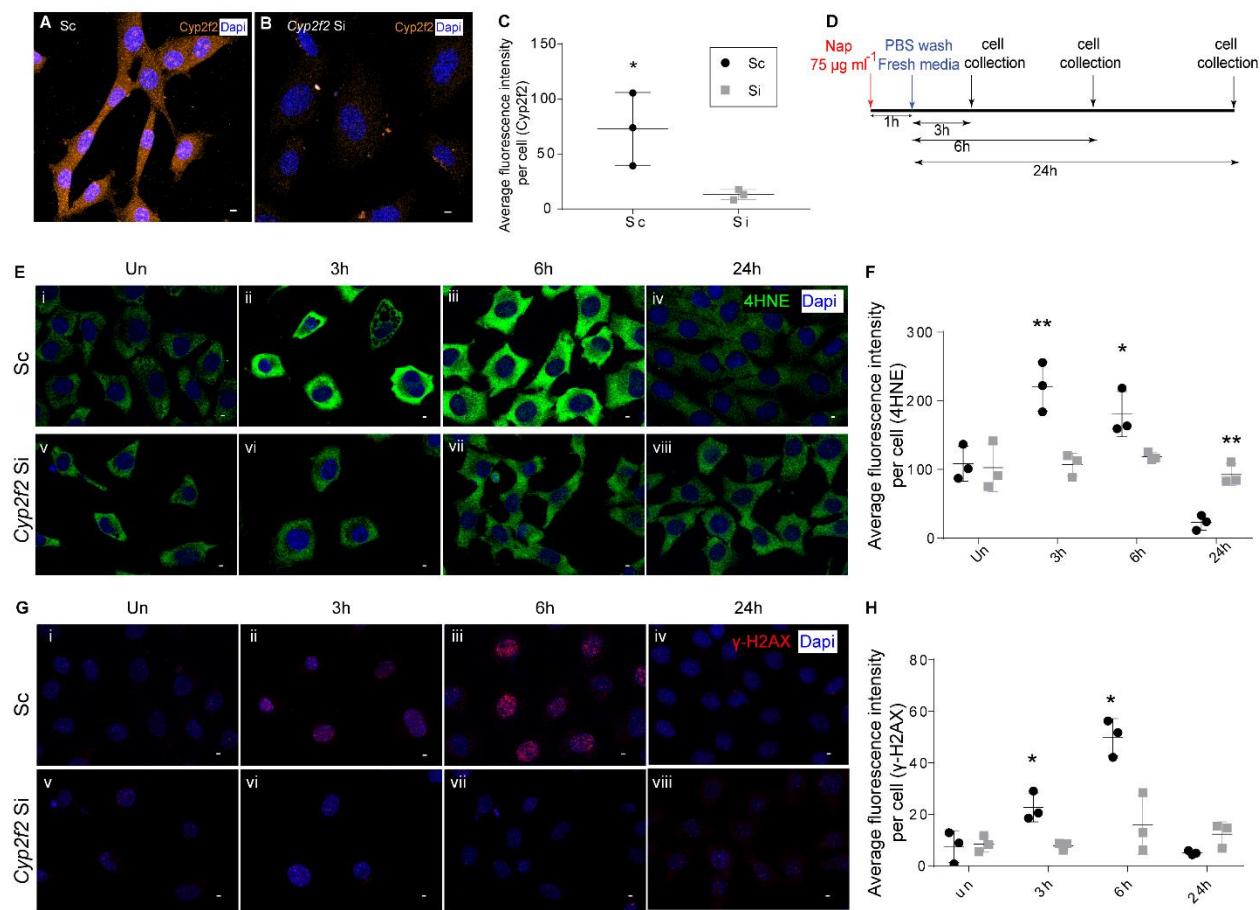


Fig. S1. FMR KO animals express markers of oxidative and genotoxic stress in the airways in response to Nap. (A-B) Lung morphology in control and *Fmr1* KO animals. H&E stained lung sections from control (A) and *Fmr1* KO (B) ($n=2$ animals each). Scale Bar=200 μ m. (C-D) Quantitation of levels of expression of 4HNE and γ -H2AX in CCs in wild-type and *Fmr1* KO lungs post Nap. (C) Quantitation of total 4HNE immunofluorescence per CC at different timepoints (≥ 50 cells were analysed per timepoint/per animal, $n=3$ animals). Data represents mean \pm SEM. (D) Quantitation of total γ -H2AX immunofluorescence per CC at different timepoints (≥ 50 cells were analysed per timepoint/per animal, $n=3$ animals). Unpaired two-tailed t-test ($p< .05^*$, $p< .01^{**}$, $p< .001^{***}$). For normality test and two-way ANOVA see Table S6.

Figure-S2

**Fig. S2. Validation of Cyp2f2 dependent Nap injury in C22 cells.**

(A-C) Cyp2f2 immunostaining and quantitation. **(A-B)** Cyp2f2 (orange) immunostaining in scrambled siRNA-treated (Sc) **(A)** and Cyp2f2 siRNA-treated (Si) **(B)** C22 cells. **(C)** Quantitation of Cyp2f2 immunofluorescence per cell in Sc and Si treated cells. **(D-G)** Cyp2f2 siRNA-treated (Si) C22 cells fail to respond to Nap induced stress. **(D i- D viii)** 4HNE immunostaining (green) in Sc and Si cells prior to and post Nap. **(E)** Quantitation of 4HNE immunofluorescence per cell in Sc and Si cells prior to and post Nap. Note the level of 4HNE in Si treated cells remains unchanged prior to and post Nap. **(F i- F viii)** γ -H2AX immunostaining (red) in Sc and Si cells prior to and post Nap. **(G)** Quantitation of γ -H2AX immunofluorescence per cell in Sc and Si cells post Nap. Black circle (Sc), Grey square (Si). (≥ 25 cells were analysed per timepoint/per experiment, n=3 experiments). Data represents mean \pm SEM Unpaired two-tailed t-test ($p < .05^*$, $p < .01^{**}$, $p < .001^{***}$). For normality test and two-way ANOVA see Table S7. Scale Bar=5 μ m.

Figure- S3

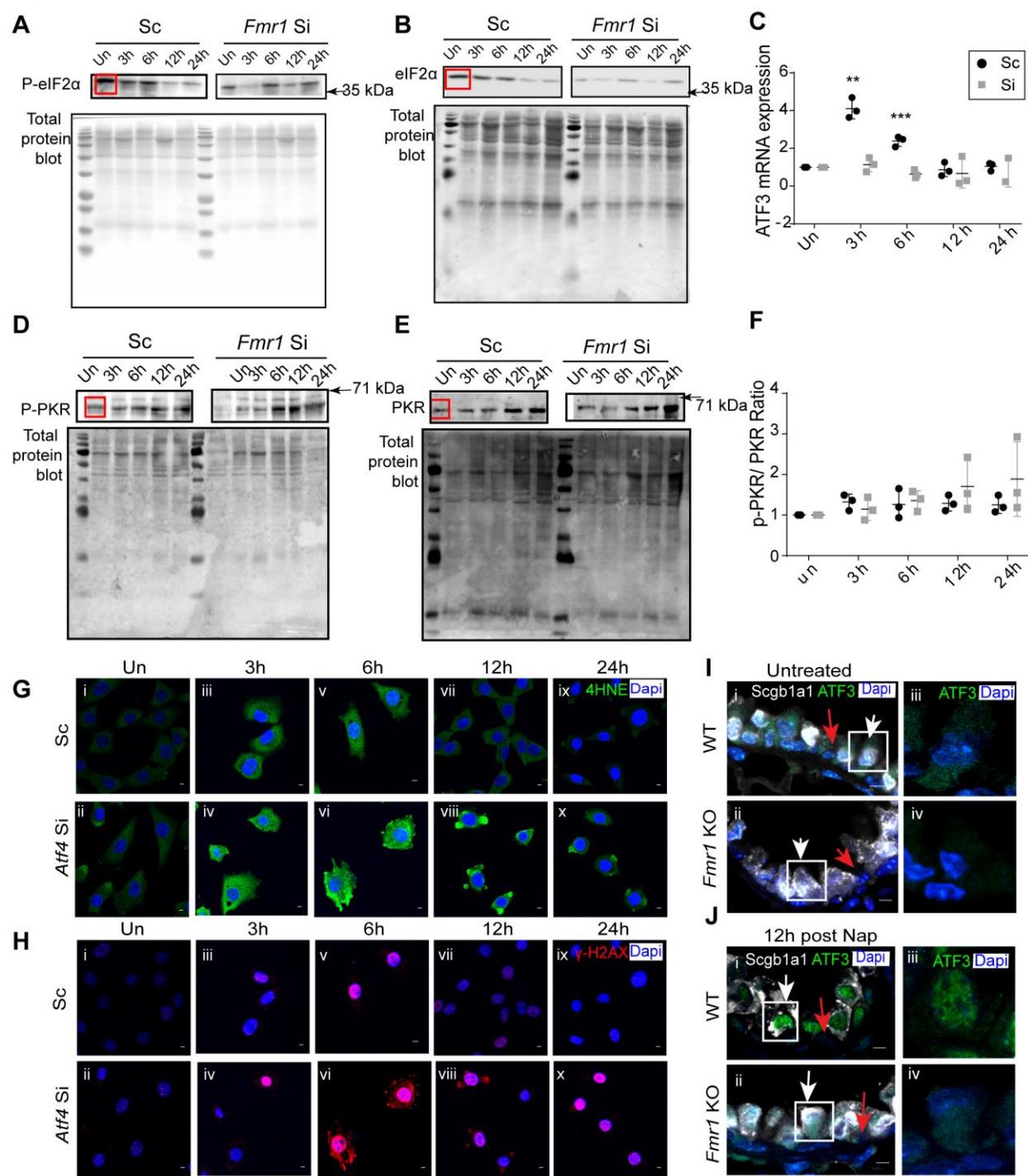


Fig. S3. Regulation and requirement of the Integrated Stress Response post Nap.

(A-B) Blots used for ratiometric quantitation of phospho-eIF2 α **(A)** and eIF2 α **(B)** in Sc and Si cells prior to and post Nap. Upper panels show blots probed with either anti-phosphorylated eIF2 α **(A)** or with anti-eIF2 α **(B)** antibodies. Red boxes indicate the area sampled for quantitation of band intensity. Lower panels show respective MemCode stained membranes used for quantitation of total protein/well. **(C)** Quantitative RT-PCR-based analysis of ATF4 target gene *ATF3* in Sc and Si cells prior to and post Nap (n=3 experiments). **(D-E)** Blots used for ratiometric quantitation of phospho-PKR **(D)** and PKR **(E)** in Sc and Si cells prior to and post Nap. Upper panels show blots probed with anti-phospho-PKR **(D)** or anti-PKR **(E)** antibodies. Red box indicates the area sampled for quantitation of band intensity. Lower panels show respective MemCode stained membranes used for quantitation of total protein/well. **(F)** Western blot-based quantitation of phospho-PKR/ PKR ratios in Sc and Si cells prior to and post Nap treatment (n=3 experiments). **(G i - G x)** Immunostaining for 4HNE (green) in control (Sc) and ATF4-deficient (*Atf4* Si) cells prior to and post Nap. **(H i-H x)** Immunostaining for γ -H2AX (red) Sc and Si cells prior to and post Nap. **(I-J)** Expression of ATF3 in the airways in wild type and *Fmr1* KO mice prior to and post Naphthalene exposure. **(I i – I iv)** ATF3 (green) and Scgb1a1 (white) immunostaining in uninjured wild type **(i, iii)** and *Fmr1* KO **(ii, iv)** mice. **(J i – J iv)** ATF3 (green) and Scgb1a1 (white) immunostaining in wild type **(i, iii)** and *Fmr1* KO **(ii, iv)** mice 12 h post Nap. Note increased nuclear ATF3 expression in CCs in wild type but not *Fmr1* KO (white arrow showing Scgb1a1 $^{+}$ cell, red arrow showing ciliated cell). Unpaired two-tailed t-test (p< .05*, p< .01**, p< .001***). For normality test and two-way ANOVA see Table S8. Scale Bar=5 μ m

Figure- S4

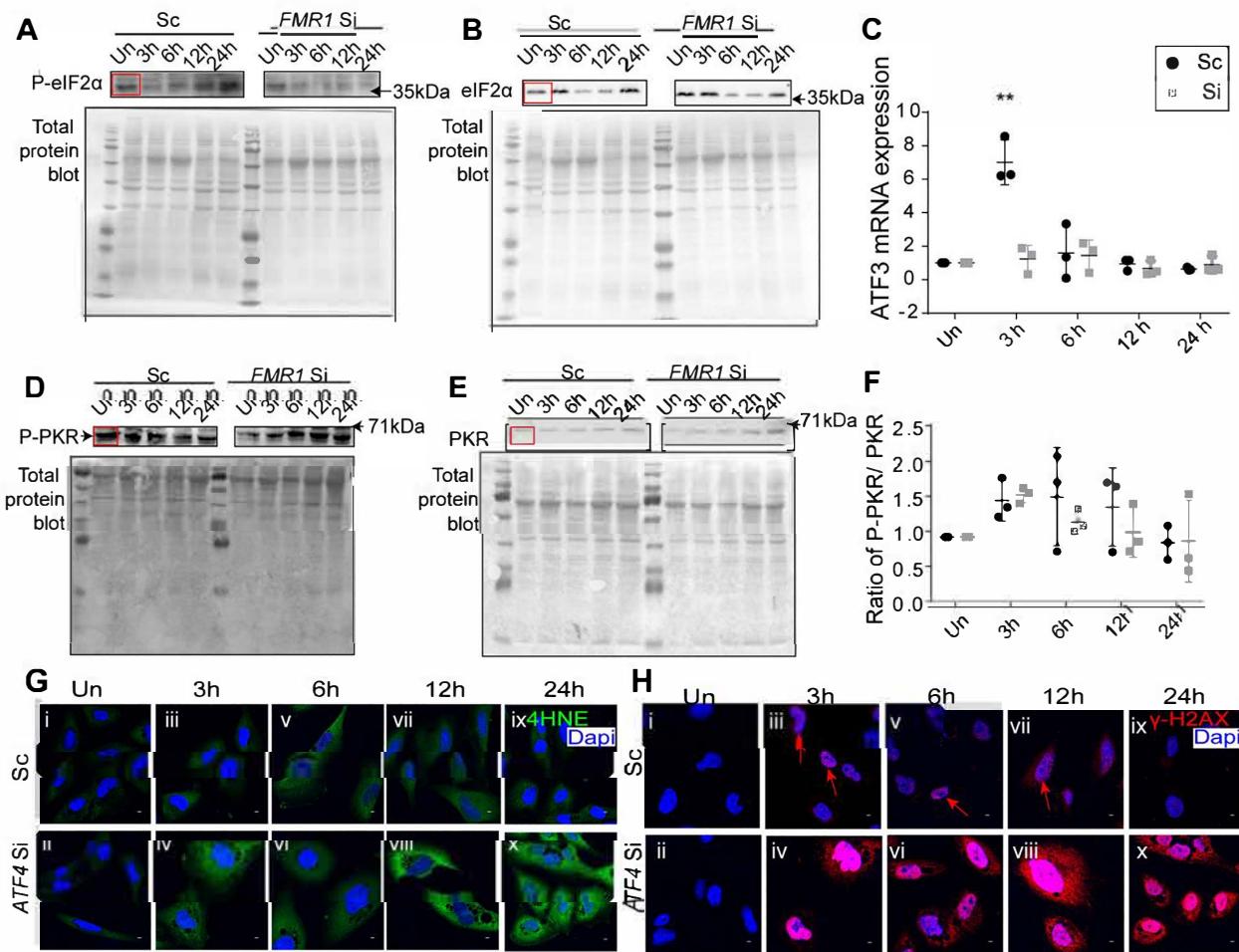


Fig. S4. Regulation and requirement of the Integrated Stress Response post PQ in BEAS-2B cells. (A-B) Blots used for ratiometric quantitation of phospho-eIF2 α (A) and eIF2 α (B) in Sc and Si cells prior to and post PQ. Upper panels show blots probed with either anti-phospho-eIF2 α (A) or anti-eIF2 α (B) antibodies. Red boxes indicate the area sampled for quantitation of band intensity. Lower panels show respective MemCode stained membranes used for quantitation of total protein/well. (C) Quantitative RT-PCR-based analysis of expression of ATF3 in Sc and Si cells prior to and post PQ (n=3 experiments). (D-E) Blots used for ratiometric quantitation of phospho-PKR (D) and PKR (E) in Sc and

Si cells prior to and post PQ. Upper panels show blots probed with anti-phospho-PKR (**D**) or anti-PKR (**E**) antibodies. Red boxes indicate the area sampled for quantitation of band intensity. Lower panels show respective MemCode stained membranes used for quantitation of total protein/well. (**F**) Western blot-based quantitation of phospho-PKR/PKR ratios in Sc and Si cells prior to and post PQ treatment (n=3 experiments). (**G i – G x**) Immunostaining for 4HNE (green) in control (Scrambled siRNA treated, Sc) and ATF4-siRNA treated (Si) cells prior to and post PQ. (**H i - H x**) Immunostaining for γ -H2AX (red) Sc and Si cells prior to and post PQ. Unpaired two-tailed t-test ($p < .05^*$, $p < .01^{**}$, $p < .001^{***}$). For normality test and two-way ANOVA see Table S9. Scale Bar=5 μ m

Figure-S5

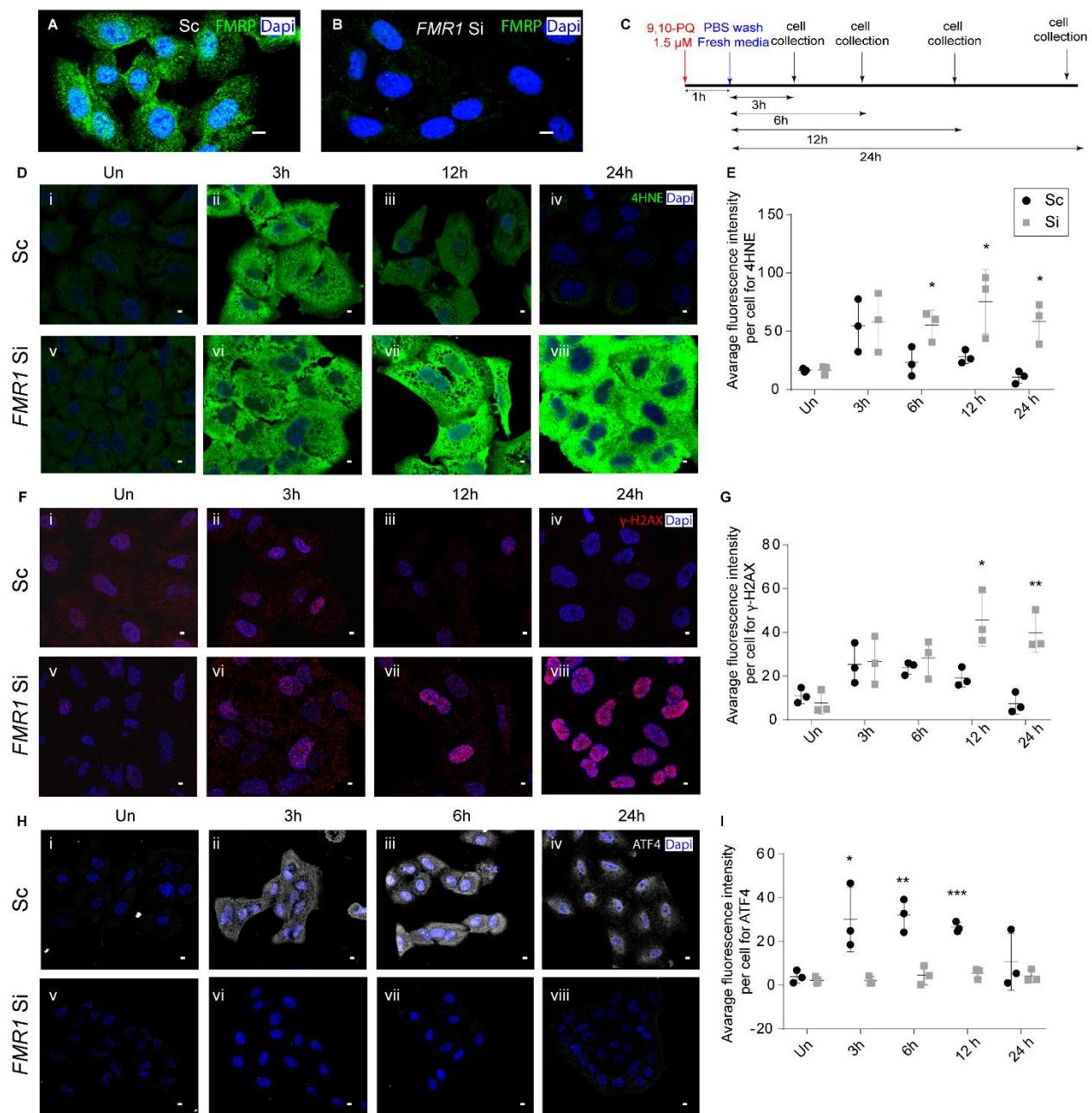


Fig. S5. FMRP is expressed in human A549 cells and protects it from 9, 10-Phenanthrenequinone-induced stress. (A-B) FMRP expression in the A549 cells. (A) FMRP immunostaining of A549 cells. (B) FMRP immunostaining of *FMR1* siRNA-treated A549 cells. (C) Schematic showing regimen for PQ injury. (D-I) Susceptibility of FMRP-deficient A549 cells to PQ injury. (D-G) Expression of markers of oxidative (4HNE) and genotoxic (γ -H2AX) stress in (Control,Scrambled siRNA treated) Sc and (*FMR1* siRNA treated) Si cells prior to and post PQ. (D i - D viii) 4HNE immunostaining (green) in Sc and Si cells prior to and post PQ. (E) Quantitation of 4HNE immunofluorescence per cell in Sc and Si cells post PQ. (F i- F viii) γ -H2AX immunostaining (red) in Sc and Si cells prior to and post PQ. (G) Quantitation of γ -H2AX immunofluorescence per cell in Sc and Si cells post PQ. (H i - H viii) Immunostaining of ATF4 levels (white) in Sc and Si cells prior to and post PQ treatment. (I) Quantitaion of ATF4 immunofluorescence per cell in Sc and Si cells prior to and post Nap. ≥ 25 cells were analysed per timepoint/per experiment, n=3 experiments. Black circle (Sc), Grey square (Si). Graphical data represents mean \pm SEM. Unpaired two-tailed t-test ($p < .05^*$, $p < .01^{**}$, $p < .001^{***}$). For normality tests and two-way ANOVA see Table S10. Scale Bar=5 μ m.

Figure- S6

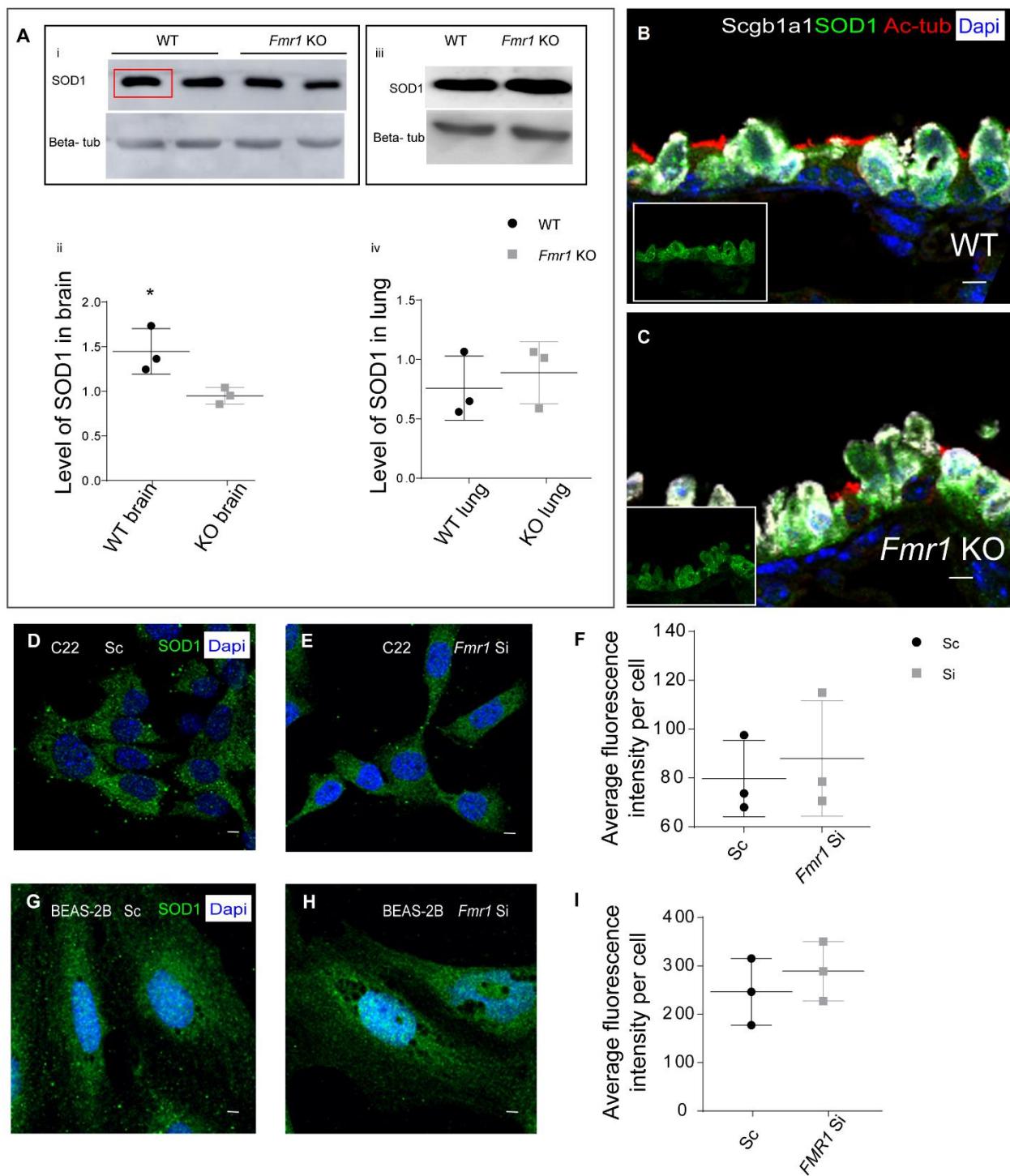


Fig. S6. FMRP does not regulate SOD1 expression in lung and bronchial cell lines

(A i- A iv) Western blot-based quantitation of SOD1 in WT and *Fmr1* KO brain and lung (n=3 experiments). Blots used for quantitation of SOD1 from brain **(A i)** and lung **(A iii)** tissue lysate in WT and *Fmr1* KO mice. Upper panels show blots probed with anti-SOD1 and lower panels show blots probed with anti- β -tubulin **(A i, Aiii)** antibodies. Red boxes indicate the area sampled for quantitation of band intensity. **(B-C)** SOD1 expression in the murine lung. SOD1 immunostaining (green) in airway epithelial cells. Shown here are CCs (white, Scgb1a1) and ciliated cells (red, acetylated-tubulin) in wild-type **(B)** and *Fmr1* KO **(C)**. **(D-F)** SOD1 expression in the C22 cell line with or without FMRP. SOD1 immunostaining (green) in control (Scrambled siRNA-treated, Sc) **(D)** and *Fmr1* siRNA-treated (Si) cells **(E)**. **(F)** Quantitation of SOD1 immunofluorescence per cell in Sc (black bars) and Si (grey bars). **(G-I)** SOD1 expression in the BEAS-2B cell line with or without FMRP. SOD1 immunostaining (green) in control (Scrambled siRNA-treated, Sc) **(G)** and *Fmr1* siRNA-treated (Si) cells **(H)**. **(I)** Quantitation of SOD1 immunofluorescence per cell in Sc (black bars) and Si (grey bars). Graphical data represents mean \pm SEM. Unpaired two-tailed t-test ($p < .05^*$, $p < .01^{**}$, $p < .001^{***}$). For normality test see Table S9. Scale bar= 5 μ m.

Table S1. ANOVA table and Normality test for Fig. 1 graphical data.

Figure number	Variables	Sum of Squares	F statistic	P value	Normality (Shapiro-Wilk normality test)
Figure 1F	Interaction Time Genotype	3746 32659 6825	F (3, 12) = 8.862 F (3, 12) = 77.27 F (1, 4) = 64.9	P=0.0023 P<0.0001 P=0.0013	Passed

Table S2. ANOVA table and Normality test for Fig. 2 graphical data.

Figure number	Variables	Sum of Squares	F statistic	P value	Normality (Shapiro-Wilk normality test)
Figure 2G	Interaction Time Genotype	5525054195 22221659115 14690673188	F (4, 16) = 5.999 F (4, 16) = 24.13 F (1, 4) = 10.73	P=0.0038 P<0.0001 P=0.0306	Passed
Figure 2I	Interaction Time Genotype	872823942 2103967406 2326731004	F (4, 16) = 8.556 F (4, 16) = 20.62 F (1, 4) = 408.7	P=0.0007 P<0.0001 P<0.0001	Passed

Table S3. ANOVA table and Normality test for Fig. 3 graphical data.

Figure number	Variables	Sum of Squares	F statistic	P value	Normality (Shapiro-Wilk normality test)
Figure 3A	Interaction Time Genotype	3.844 1.437 10	F (4, 32) = 12.66 F (4, 32) = 4.733 F (1, 8) = 57.8	P<0.0001 P=0.0041 P<0.0001	Passed
Figure 3C	Interaction Time	1227296089 1496657239	F (4, 32) = 21.54 F (4, 32) = 26.27	P<0.0001 P<0.0001	Passed
	Genotype	1452261497	F (1, 8) = 33.42	P=0.0004	
Figure 3E	Interaction Time Genotype	1629028646 8957959343 6959867445	F (4, 16) = 4.11 F (4, 16) = 22.6 F (1, 4) = 11.66	P=0.0177 P<0.0001 P=0.0269	Passed
Figure 3F	Interaction Time Genotype	2412881952 9546744357 6562205063	F (4, 16) = 9.973 F (4, 16) = 39.46 F (1, 4) = 271.4	P=0.0003 P<0.0001 P<0.0001	Passed

Table S4. ANOVA table and Normality test for Fig. 4 graphical data.

Figure number	Variables	Sum of Squares	F statistic	P value	Normality (Shapiro-Wilk normality test)
Figure 4F	Interaction	8452531801	$F(4, 16) = 12.1$	$P=0.0001$	Passed
	Time	16492731939	$F(4, 16) = 23.62$	$P<0.0001$	
	Genotype	16448237473	$F(1, 4) = 72.34$	$P=0.0010$	
Figure 4H	Interaction	606376703	$F(4, 16) = 3.198$	$P=0.0414$	Passed
	Time	825663994	$F(4, 16) = 4.354$	$P=0.0143$	
	Genotype	1730144538	$F(1, 4) = 76.47$	$P=0.0009$	

Table S5. ANOVA table and Normality test for Fig. 5 graphical data.

Figure number	Variables	Sum of Squares	F statistic	P value	Normality (Shapiro-Wilk normality test)
Figure 5A	Interaction	2.693	$F(4, 32) = 4.493$	$P=0.0054$	Passed
	Time	2.027	$F(4, 32) = 3.381$	$P=0.0204$	
	Genotype	6.657	$F(1, 8) = 30.61$	$P=0.0006$	
Figure 5C	Interaction	671166420	$F(4, 32) = 18.27$	$P<0.0001$	Passed
	Time	865757156	$F(4, 32) = 23.57$	$P<0.0001$	
	Genotype	833533615	$F(1, 8) = 108.7$	$P<0.0001$	
Figure 5E	Interaction	2140882670	$F(4, 16) = 3.972$	$P=0.0200$	Passed
	Time	3078466752	$F(4, 16) = 5.711$	$P=0.0047$	
	Genotype	6767050455	$F(1, 4) = 22.36$	$P=0.0091$	
Figure 5F	Interaction	3271361264	$F(4, 16) = 7.662$	$P=0.0012$	Passed
	Time	3918054177	$F(4, 16) = 9.177$	$P=0.0005$	
	Genotype	7807658529	$F(1, 4) = 10.24$	$P=0.0329$	

Table S6. ANOVA table and Normality test for Fig. S1 graphical data.

Figure number	Variables	Sum of Squares	F statistic	P value	Normality (Shapiro-Wilk normality test)
Figure S1C	Interaction	6786923	$F(2, 8) = 1.428$	$P=0.2948$	Passed
	Time	158536285	$F(2, 8) = 33.37$	$P=0.0001$	
	Genotype	46216894	$F(1, 4) = 115.1$	$P=0.0004$	
Figure S1D	Interaction	93896384	$F(2, 8) = 3.971$	$P=0.0634$	Passed
	Time	437685349	$F(2, 8) = 18.51$	$P=0.0010$	
	Genotype	196523094	$F(1, 4) = 29$	$P=0.0057$	

Table S7. ANOVA table and Normality test for Fig. S2 graphical data.

Figure number	Variables	Sum of Squares	F statistic	P value	Normality (Shapiro-Wilk normality test)
Figure S2F	Interaction	277282829	$F(3, 12) = 16.47$	$P=0.0001$	Passed
	Time	413013067	$F(3, 12) = 24.53$	$P<0.0001$	
	Genotype	46317078	$F(1, 4) = 5.944$	$P=0.0714$	
Figure S2H	Interaction	15397110	$F(3, 12) = 14.84$	$P=0.0002$	Passed
	Time	24262281	$F(3, 12) = 23.39$	$P<0.0001$	
	Genotype	6287973	$F(1, 4) = 15.82$	$P=0.0164$	

Table S8. ANOVA table and Normality test for Fig. S3 graphical data.

Figure number	Variables	Sum of Squares	F statistic	P value	Normality (Shapiro-Wilk normality test)
Figure S3C	Interaction Time Genotype	9.668 14.04 8.417	F (4, 16) = 17.56 F (4, 16) = 25.49 F (1, 4) = 22.54	P<0.0001 P<0.0001 P=0.0090	Passed
Figure S3F	Interaction Time Genotype	1.925 3.227 3.401	F (4, 16) = 1.813 F (4, 16) = 3.039 F (1, 4) = 72.97	P=0.1758 P=0.0484 P=0.0010	Passed

Table S9. ANOVA table and Normality test for Fig. S4 graphical data.

Figure number	Variables	Sum of Squares	F statistic	P value	Normality (Shapiro-Wilk normality test)
Figure S4C	Interaction Time Genotype	39.81 48.27 10.59	F (4, 16) = 14.69 F (4, 16) = 17.81 F (1, 4) = 18.4	P<0.0001 P<0.0001 P=0.0128	Passed
Figure S4F	Interaction Time Genotype	0.2878 1.675 0.1134	F (4, 16) = 0.9184 F (4, 16) = 5.346 F (1, 4) = 0.2715	P=0.4772 P=0.0063 P=0.6298	Passed

Table S10. ANOVA table and Normality test for Fig. S5 graphical data.

Figure number	Variables	Sum of Squares	F statistic	P value	Normality (Shapiro-Wilk normality test)
Figure S5E	Interaction	3210672601	$F(4, 16) = 6.877$	$P=0.0020$	Passed
	Time	5874063911	$F(4, 16) = 12.58$	$P<0.0001$	
	Genotype	5128674934	$F(1, 4) = 6.088$	$P=0.0691$	
Figure S5G	Interaction	15464281	$F(4, 16) = 5.435$	$P=0.0059$	Passed
	Time	17602971	$F(4, 16) = 6.187$	$P=0.0033$	
	Genotype	11380216	$F(1, 4) = 63.39$	$P=0.0013$	
Figure S5I	Interaction	889643528	$F(4, 16) = 3.84$	$P=0.0226$	Passed
	Time	1043483050	$F(4, 16) = 4.505$	$P=0.0125$	
	Genotype	2155934395	$F(1, 4) = 125.8$	$P=0.0004$	

Table S11. Primer sequences for RT-PCR experiment

Gene	Sequence
ATF3 Human Forward primer	GTACCCAGGCTTAGCATTA
ATF3 Human Reverse primer	TTAATAGACAGTAGCCAGCG
Beta actin Human Forward primer	AAAACTGGAACGGTGAAGGT
Beta actin Human Reverse primer	ACAACGCATCTCATATTGGAA
ATF3 Mouse Forward primer	GAGATGTCAGTCACCAAGTC
ATF3 Mouse Reverse primer	TCCAGTTCTCTGACTCTTC
Beta actin Mouse Forward primer	CTTCCAGCAGATGTGGATCAG
Beta actin Mouse Reverse primer	AAAACGCAGCTCAGTAACAGT