Figure S1

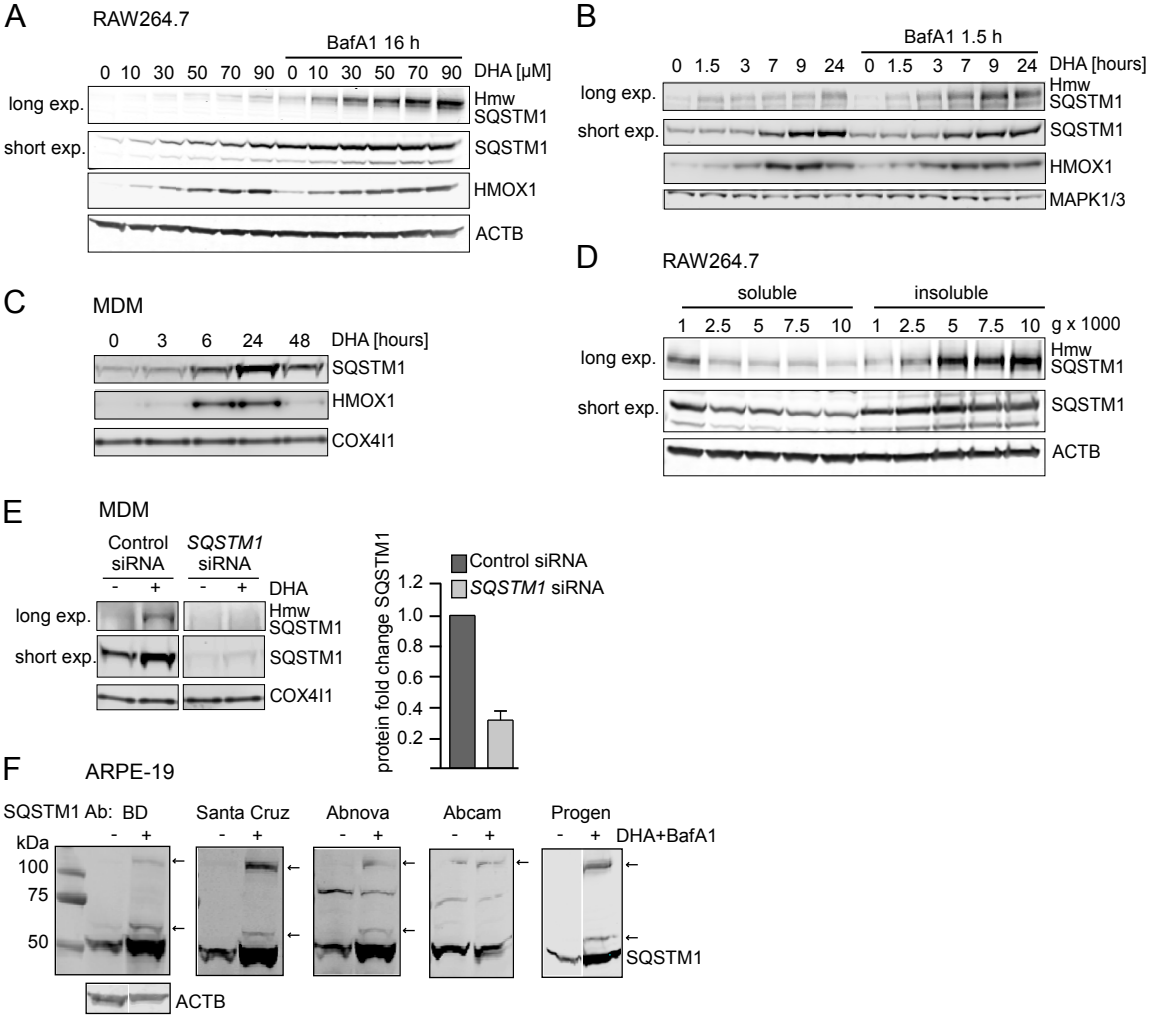


Figure S1. SQSTM1 protein level and modifications are induced by DHA. Immunoblot (IB) analysis of RAW 264.7 cells treated with (**A**) the indicated concentrations of DHA with and without BafA1 (100 nM) for 16 h or (**B**) DHA (70 μM) for the indicated time points and BafA1 for the last 1.5 h. Hmw SQSTM1 was visualized by long exposure (exp). (**C**) IB analysis of MDMs treated with DHA (70 μM) for the indicated time points. (**D**) IB analysis of SQSTM1 in RAW264.7 cells treated with DHA and BafA1 for 16 h. Soluble and insoluble fractions were separated with increasing centrifugation speed. (**E**) IB analysis of SQSTM1 in MDMs transfected with *SQSTM1* siRNA and stimulated with DHA for 16 h. Hmw SQSTM1 was detected by long exposure (exp). Quantification of n=3 (right). (**F**) IB analysis with the indicated SQSTM1 antibodies in ARPE-19 cells treated with DHA and BafA1 for 16 h. Arrows represent slow migrating SQSTM1. Related to Figure 1 and 2.

Figure S2

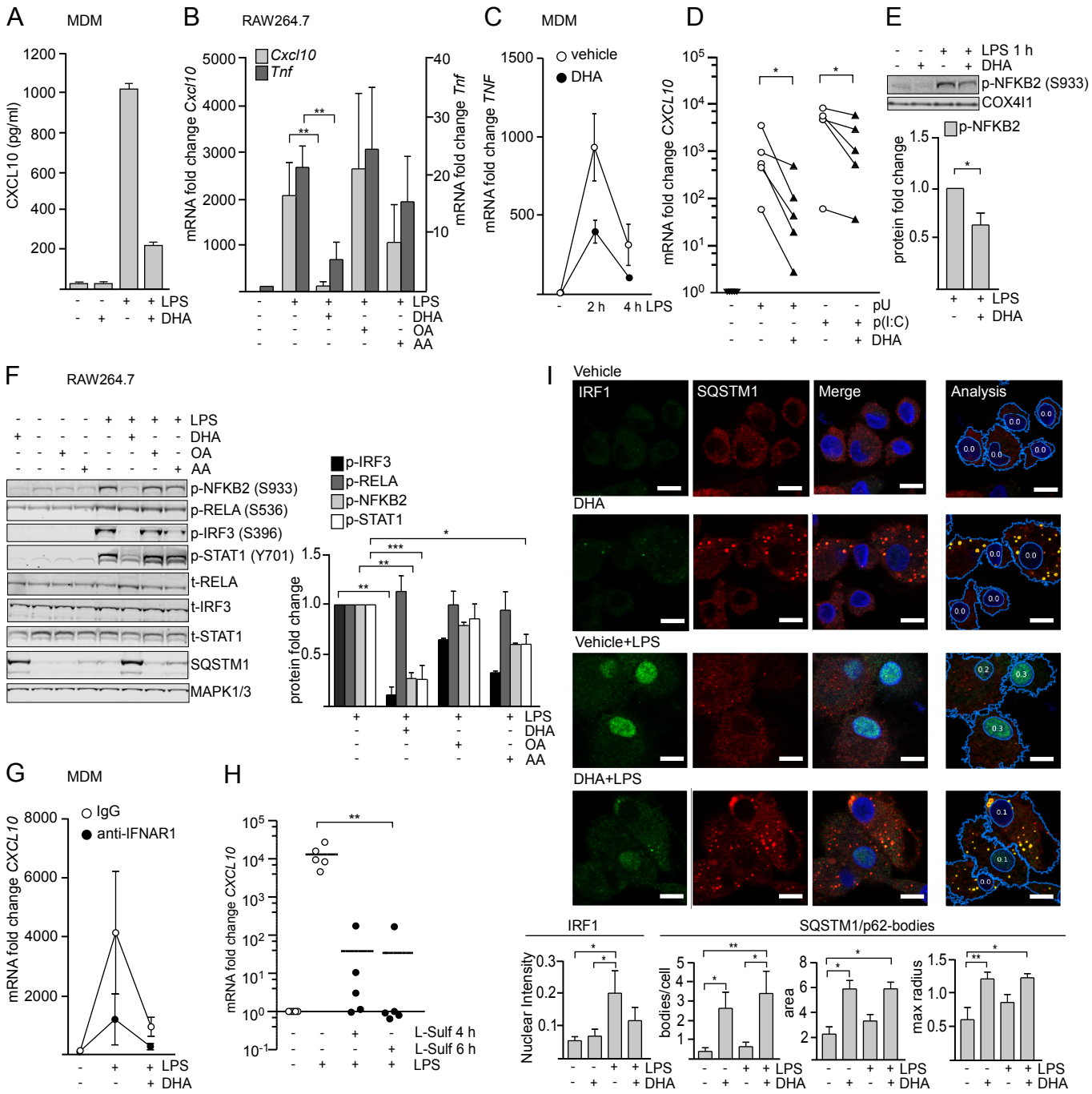


Figure S2. DHA affects IRF signaling. (**A**) ELISA of CXCL10 levels in MDMs treated with DHA for 16 h and LPS for 3 h. Shown is a representative result of 3 independent experiments (mean ± SD of duplicates). (**B**) QRT-PCR-analysis of *Cxcl10* or *Tnf* in RAW264.7 cells treated with DHA, OA or AA for 16 h and LPS for 3 h, mean fold change ± SEM, n=3 (**p<0.01, repeated-measures ANOVA with Dunnett's). (**C**) QRT-PCR analysis of *TNF* in MDMs treated with DHA for 16 h and LPS for 2 h or 4 h, mean fold change ± SEM, n=5. (**D**) QRT-PCR analysis of *CXCL10* in MDMs treated with DHA for 16 h and pU (1 µg/ml, complexed to pL-Arg) or p(I:C) (1 µg/ml, transfected) for 3 h, mean fold change ± SEM, n=5 (Wilcoxon matched-pairs signet rank test, one-tailed). (**E**) IB analysis of phosphorylated NFKB2 (Ser933) in MDMs treated with DHA for 16 h and LPS for 1 h, mean fold change ± SEM, n=5 (Wilcoxon matched-pairs signet rank test, one-tailed). (**F**) IB analysis and quantification (right) of phosphorylated IRF3, RELA and STAT1 in RAW264.7 cells treated with DHA, OA or AA for 16 h and LPS for 3 h, mean fold change ± SEM, n=3 (**p<0.01, repeated-measures ANOVA with Dunnett's). (**G**) QRT-PCR analysis of *CXCL10* in MDMs treated with anti-IFNAR1 (1 µg/ml) or control antibody, DHA for 16 h and LPS for 3 h, mean fold change ± SEM, n=4. (**H**) QRT-PCR analysis of *CXCL10* in MDMs treated with L-sulforaphane (L-Sulf; 25 µM) for 4 or 6 h and LPS for the last 4 h, mean fold change ± SEM, n=5 (**p<0.01,Friedman test with Dunn's) (**I**) Immunostaining for IRF1 and SQSTM1 in MDMs treated with DHA for 16 h and LPS for 2 h (scale bars: 10 µm). Images were analyzed by CellProfiler 2.2 (panel below) for mean intensity of nuclear IRF1 staining, mean number of SQSTM1/p62-bodies per cell, mean area of SQSTM1/p62-bodies and maximum radius of SQSTM1/p62-bodies, >150 cells/condition/donor (n=6), mean ± SEM (**p<0.01, *p<0.05, Friedman test with Dunn's). Related to Figure 3 and 4.

Figure S3

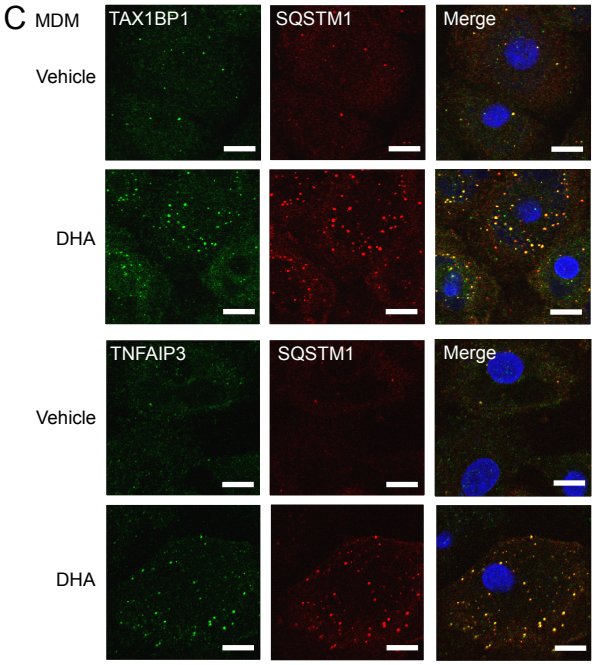
A

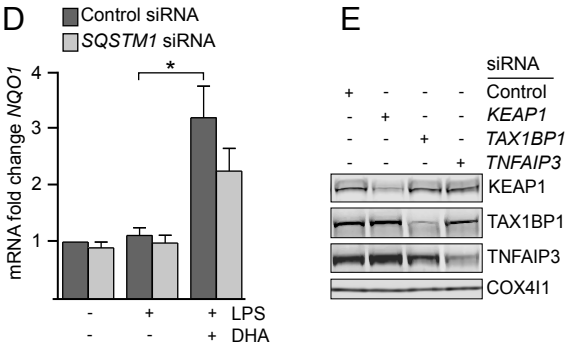
RAW 264.7

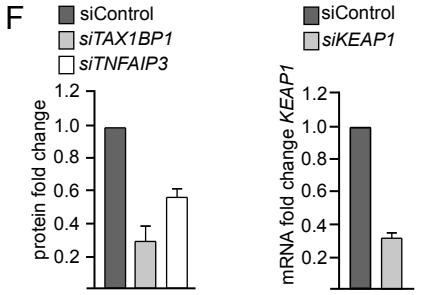
	control		BafA1	
Proteins	**vehicle**	**DHA**	**vehicle**	**DHA**
SQSTM1	477.2	2564	8787.9	18286.9
TAX1BP1	-	23.8	2522.7	2803.4
KEAP1	-	84.7	1287.6	1695.6
2AAA	46.5	143.8	427.7	865.2
ANGL2	-	20.4	199.8	714.8
FRIH	-	37.2	412.9	633.3
TCPD	72.6	320.7	423.4	490.4
ATPB	-	52.8	144	257.3
FUS	62.6	310.1	52.3	205
MYO5A	108.3	446.9	111.3	188.7
PK1IP	80.7	383.7	-	184.2
ABCF1	28.3	87.3	53.5	146.7
CDC42	-	114.5	39.6	135.3
IMA3	-	61	-	128.7
CPSF2	-	22.1	-	123.9
UBP14	-	31.9	84.9	116.4
SC61B	-	24.9	67.9	111.5
PSD12	-	52.7	69.3	101.9
SHIP1	-	32.3	-	85
AFAD	24	75	66.2	75.4
ARHG7	-	137.6	-	59.2
PSMD5	-	29.1	-	52.3
TUT7	-	133.7	-	50.1
SYWC	-	38.9	44.2	50
UFL1	-	30.9	-	46.3
LIMA1	297	992	24.1	44.9
ABCD3	-	22.7	-	36.7
WDR1	27.1	216.2	-	36.4
MYPT1	38.3	135.5	-	36.1
GSH0	-	39.6	-	31.8
TRM2A	-	26.7	-	31.7
IKZF1	-	40.5	-	29.7
RAVR1	-	39	-	26.3
SVIL	88.4	615.4	-	23.3
TDRD3	-	28.8	-	21.3

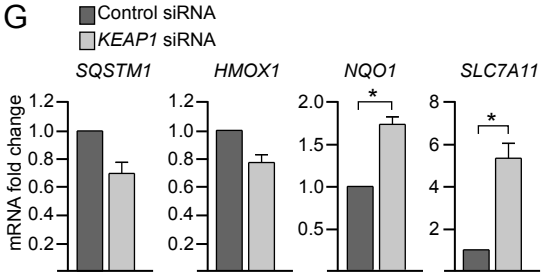
B

	Vehicle			DHA		
Protein	**Score**	**Peptides**	**PSMs**	**Score**	**Peptides**	**PSMs**
SQSTM1	8767	22	283	26372	25	785
KEAP1	2124	29	71	5291	34	161
MIC60	1358	24	48	4117	34	135
SAM50	176	7	9	650	14	18
GBB4	47	2	2	59	2	2
TAX1BP1	-	-	-	507	14	17









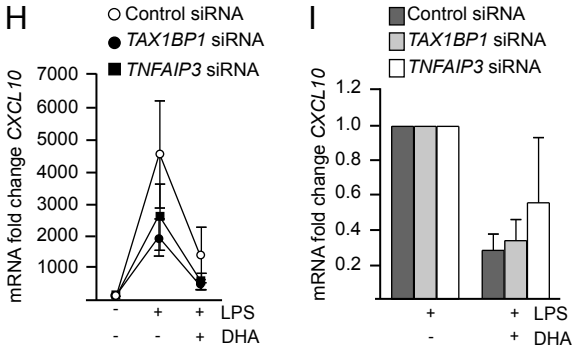


Figure S3. Binding partners in SQSTM1/p62-bodies. (**A**) Insoluble fractions were prepared from RAW264.7 cells treated with DHA with or without BafA1 for 16 h and subjected to MS analysis. Proteins that increased by DHA supplementation in both control cells and BafA1-treated cells were considered as DHA specifically enriched. Cut-off was set at a 3-fold increase by DHA in control cells. The proteins are displayed according to maximum levels in DHA- and BafA1-treated samples. Score values are shown. (**B**) IP of SQSTM1 from RAW264.7 cells treated with DHA for 16 h and analysis by MS. Proteins shown were detected 10 times over background and increased by DHA in 3 independent experiments. Score values of 1 representative experiment are shown, (PSM, peptide-spectrum match). (**C**) Immunostaining for TAX1BP1 and TNFAIP3 in MDMs treated with vehicle or DHA for 24 h (scale bars: 10 µm). (**D**) QRT-PCR analysis of *NQO1* mRNA in control or *SQSTM1* knockdown MDMs with vehicle or DHA and LPS, n=5 (*p<0.05, Friedman test with Dunn's). (**E**) IB analysis and (**F**) quantification of MDMs treated with *TAX1BP1* or *TNFAIP3* siRNA (protein levels) and *KEAP1* siRNA (mRNA levels). (**G**) QRT-PCR analysis of *NFE2L2* target gene mRNA in control or *KEAP1* knockdown MDMs, n=3 (*p<0.05, one-sample t-test). (**H**) QRT-PCR analysis of *CXCL10* in *TAX1BP1* or *TNFAIP3* siRNA-transfected MDMs treated with DHA for 16 h and LPS for 3 h, n=5 (*p<0.05, Friedman test with Dunn's). (**I**) Comparison of the downregulating effect of DHA in control or *TAX1BP1* or *TNFAIP3* siRNA-treated MDMs from (H) (Mann Whitney test). Related to Figure 5.