SUPPLEMENTARY FIGURES

MAPK15 controls cellular responses to oxidative stress by regulating NRF2 activity and expression of its downstream target genes

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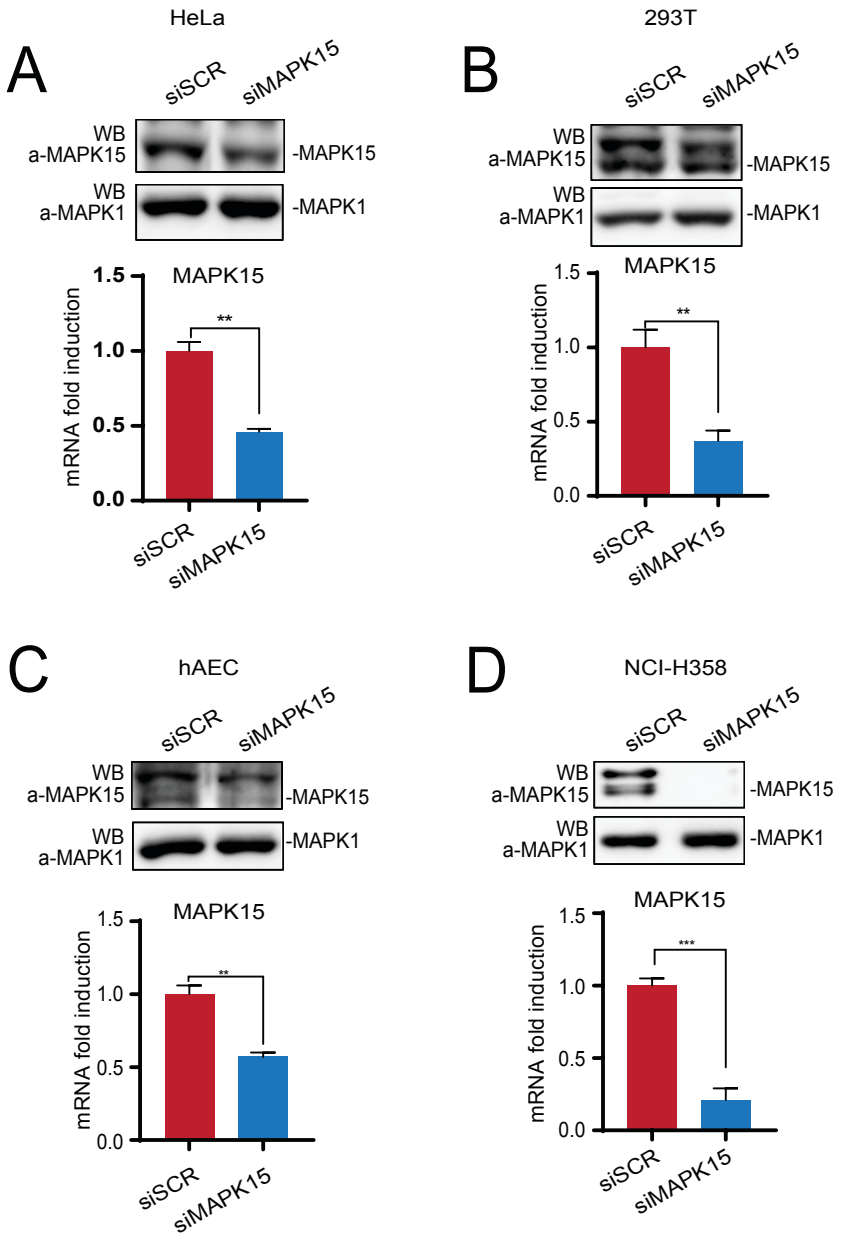

Fig. S1. Efficacy of endogenous MAPK15 knockdown by specific siRNA, in different cell lines. Cells were transfected with siSCR or siMAPK15. Seventy-two hours after siRNA transfection, cells were collected and subjected to western blot analysis, to monitor endogenous MAPK15 protein levels or to RT-qPCR, to monitor mRNA expression in HeLa (**A**), 293T (**B**), hAEC (**C**), and NCI-H358 (**D**) cells.

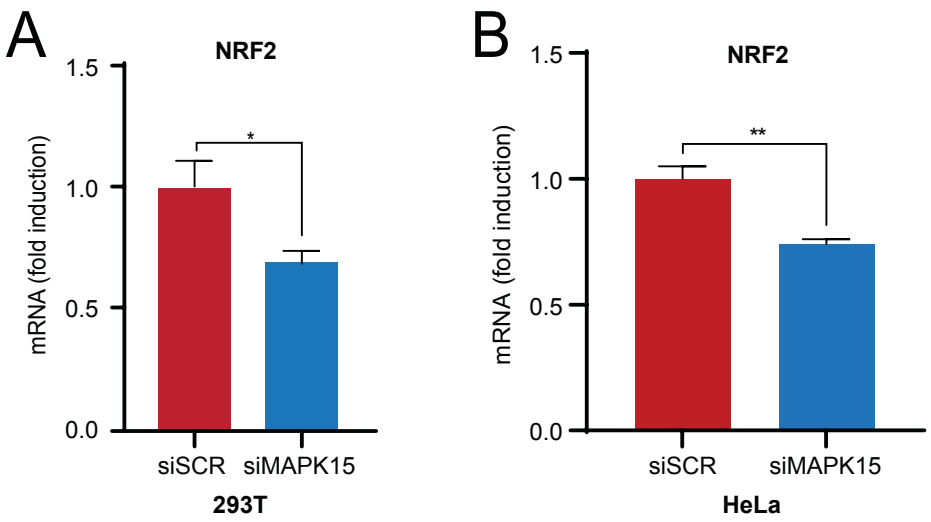

Fig. S2. Effect of MAPK15 knockdown on NRF2 mRNA expression. 293T (**A**) and HeLa (**B**) cells were transfected with siSCR or siMAPK15. Seventy-two hours after siRNA transfection, cells were collected and subjected to RT-qPCR, to monitor NRF2 mRNA expression.

	Peptide Sequence	Modification	Site	Delta (ppm)	RT
NRF2+MAPK15	TPVHSSGDMVQPLSPSQGQS**T**HVHDAQCENTPEK	Phosphorylation	**T415**	0	31,51
	TPVHSSGDMVQPLSPSQGQSTHVHDAQCEN**TP**EKE	Phosphorylation	**T425**	-0,35	38,67
	QDIDLGV**S**REVFDFSQRR	Phosphorylation	**S33**	2,17	72,76
	GENDK**S**LHLLKK	Phosphorylation	**S549**	-2,39	21,73
	EKGENDK**S**LHLLKK	Phosphorylation	**S549**	2,95	29,2
	SLHLLKK	Phosphorylation	**S549**	0,99	76,67
	SKKPDVKK	Phosphorylation	**S597**	-1,18	13,43
	TPVHSSGDMVQPLSPSQGQSTHVHDAQCENTPEKELPVSPGHRK	Phosphorylation	**T395**	-3,7	40,86
	TPFTKDK	Phosphorylation	**T439**	-0,76	49,55
NRF2	TPVHSSGDMVQPLSPSQGQSTHVHDAQCEN**TP**EKE	Phosphorylation	**T425**	1,24	38,71
	SLHLLK	Phosphorylation	**S549**	1,38	22,86
	TPFTKDK	Phosphorylation	**T439**	0,24	49,85

Fig. S3. List of phosphorylation sites identified in NRF2 in control and MAPK15-treated samples. The first column shows the peptide sequence in which a phosphorylation is found. The phosphorylated amino acid residues are reported in the column "Site". "Delta (ppm)" indicates the mass difference between the theoretical mass of the peptide and the experimental measured mass computed by using the following formula: Delta (ppm) = 1.000.000 x ([Mono Mass Exp. – Mono Mass Theo.] x Mono Mass Theo.). "RT" shows the retention time range with the most abundant MS area. The only phosphorylation sites showing different amounts (MS areas) between the control and the MAPK15-containing kinase reactions are T425 and T439, while phosphorylation of T395 was detected only in presence of MAPK15 (see corresponding **Fig. 3C**). Proline-directed threonine phosphorylation sites are indicated in red. No proline-directed serine phosphorylation site has been detected.

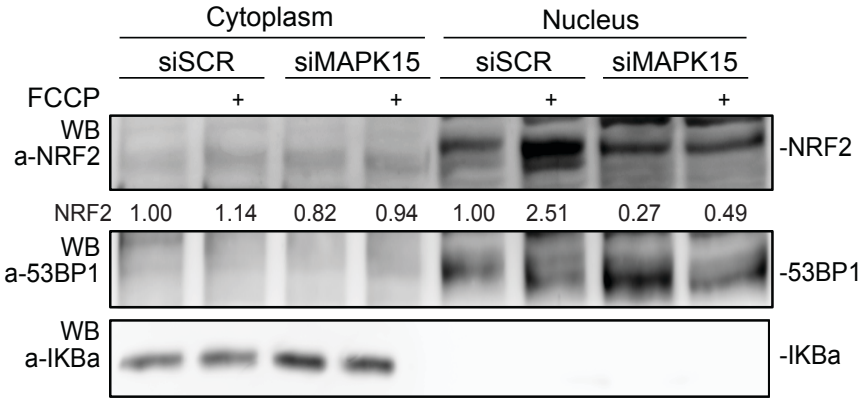

Fig. S4. MAPK15 regulates NFR2 nuclear translocation. 293T cells were transfected with siSCR or siMAPK15. After 72 h, cells were treated with 15 μM FCCP for 1 h and then subjected to cell fractionation, using the NE-PER fractionation kit. Lysates were analyzed by WB. Cytosolic NRF2 were normalized with IKBα, while nuclear NRF2 was normalized with 53BP1. One experiment, representative of 3 independent experiments is shown. Densitometric analysis of bands is shown.

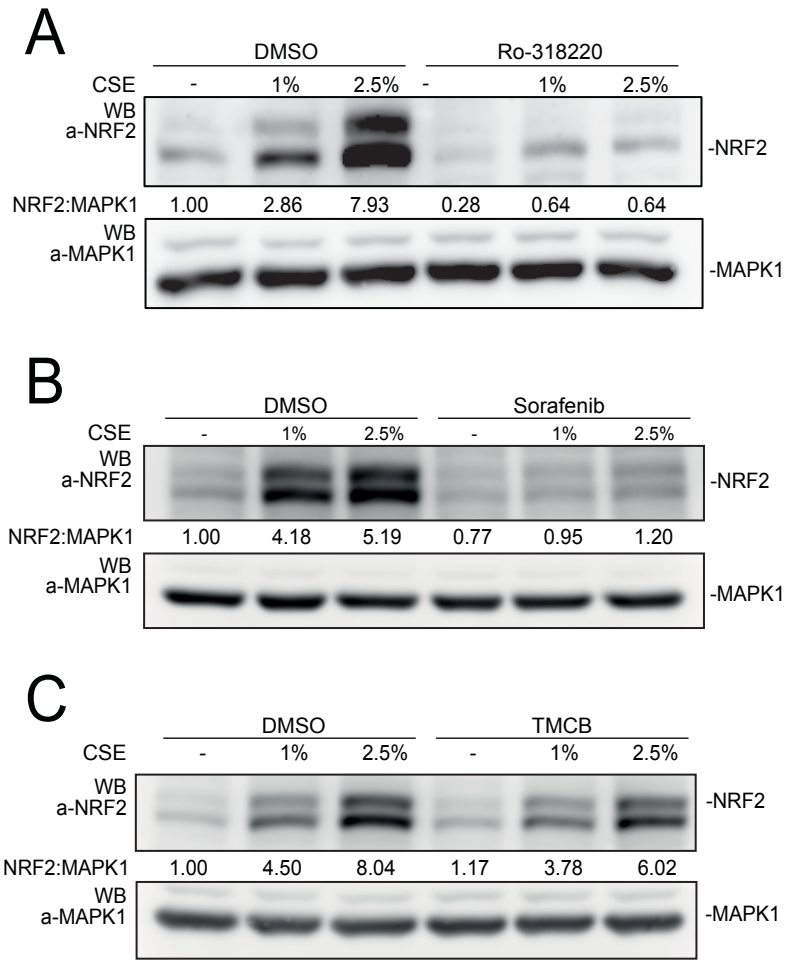

Fig. S5. Pharmacological inhibition of MAPK15 decreases NRF2 protein expression. 293T cells were treated with vehicle or 2 μM Ro-318220 for 6 h (**A**), 10 μM Sorafenib for 6 h (**B**) or 10 μM TMCB for 24 h (**C**). During the last 4 hour of drug treatment, samples were also treated with 1% or 2.5% CSE. Then samples were collected and analyzed by WB. One experiment, representative of 3 independent experiments is shown. Densitometric analysis of bands is shown.

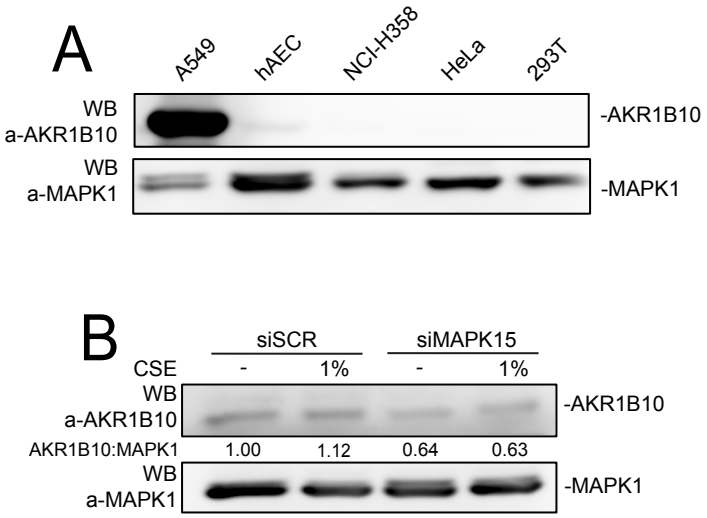

Fig. S6. AKR1B10 is regulated by MAPK15 in hAEC cells. (**A**) AKR1B10 protein expression evaluation by WB analysis, in the different cell lines used throughout the study. A549 cells were used as positive control. (**B**) hAEC cells were transfected with siSCR or siMAPK15. After 72 h, cells were first treated for 4 h with 1% CSE, and then subjected to SDS-PAGE followed by WB. One experiment, representative of 3 independent experiments, is shown. Densitometric analysis of bands is shown.

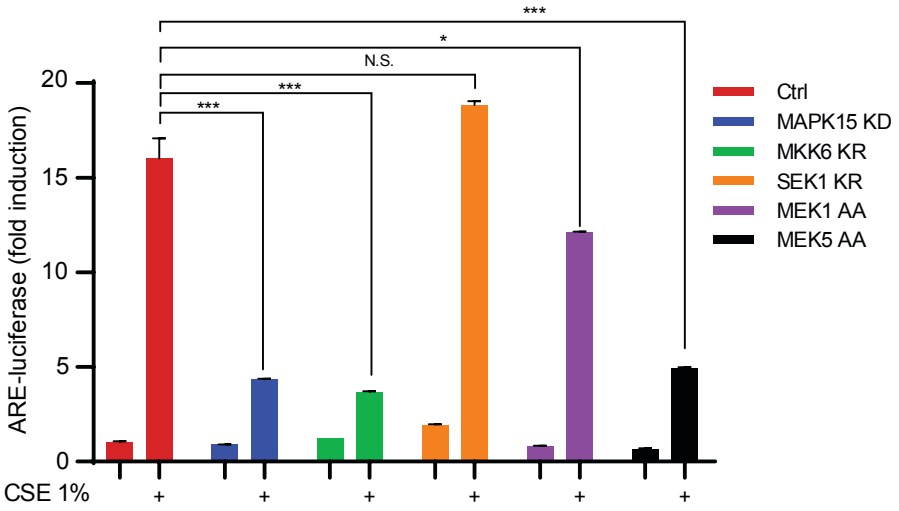

Fig. S7. Comparative analysis of the regulation of NRF2 transactivation by different members of the MAP kinase family. 293T cells were transiently co-transfected with the ARE luciferase reporter vector and the indicated dominant negative mutants for the different MAP kinase pathways. After 24 h, cells were treated for 4h with 1% CSE. Samples were next lysed and luciferase activity measured in cell extracts. Data are presented as fold induction of the normalized luciferase activity compared to control cells transfected with GFP. All luciferase results represent the average ± S.D. of three independent experiments. All samples were measured in triplicate.

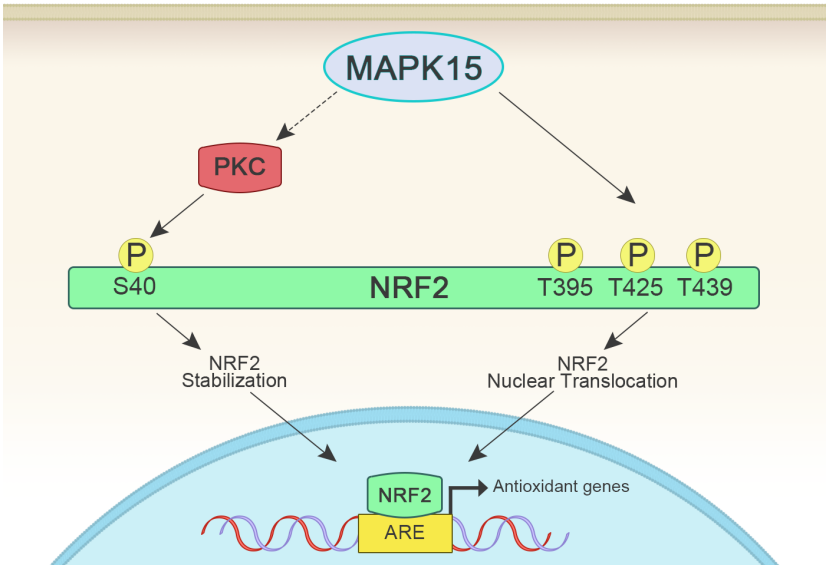

Fig. S8. Proposed model for the control of NRF2 phosphorylation by MAPK15. MAPK15 is able to directly phosphorylate different residues (T395; T425 and T439) at the C-terminus of NRF2 while contemporarily stimulating the activity of PKC which, in turn, phosphorylates Ser40 of NRF2. These two converging mechanisms allow to increase NRF2 nuclear translocation and protein stabilization, finely modulating the extent and accuracy of NRF2 responses.