Expanded View Figures

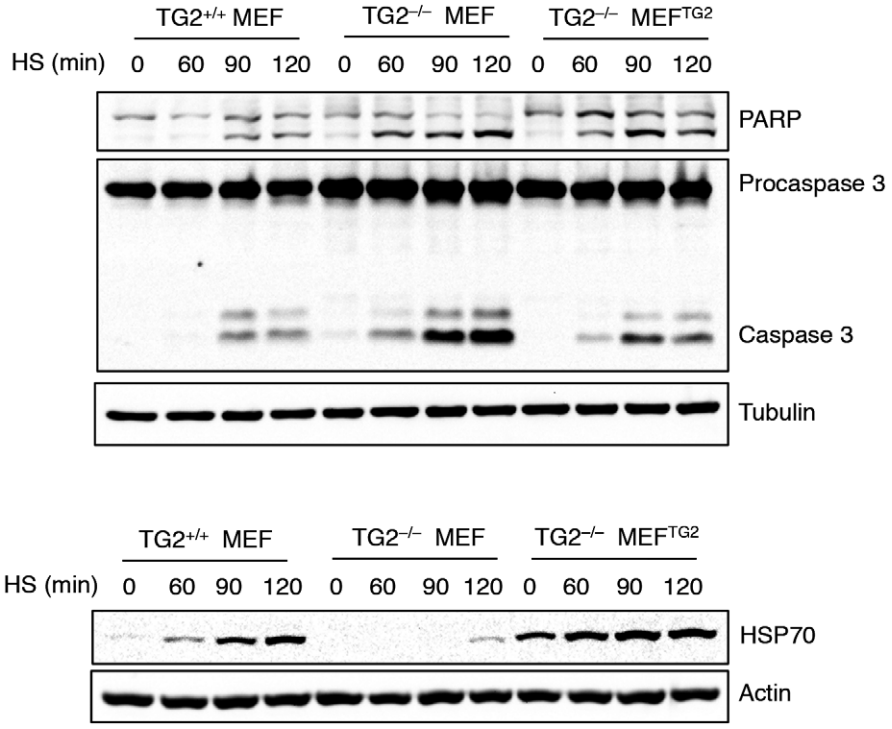

Figure EV1. Long-term exposure to HS fails to induce HSP70 in TG2$^{-/-}$ MEF, leading to cell death.

Western blot analysis of cleaved caspase 3 and PARP, as markers for apoptotic cell death induction, and HSP70 in protein extracts of TG2$^{+/+}$ and TG2$^{-/-}$ MEFs as well as TG2$^{-/-}$ MEF^{TG2} after heat shock for 60, 90 and 120 min. Tubulin and actin were used as loading control.

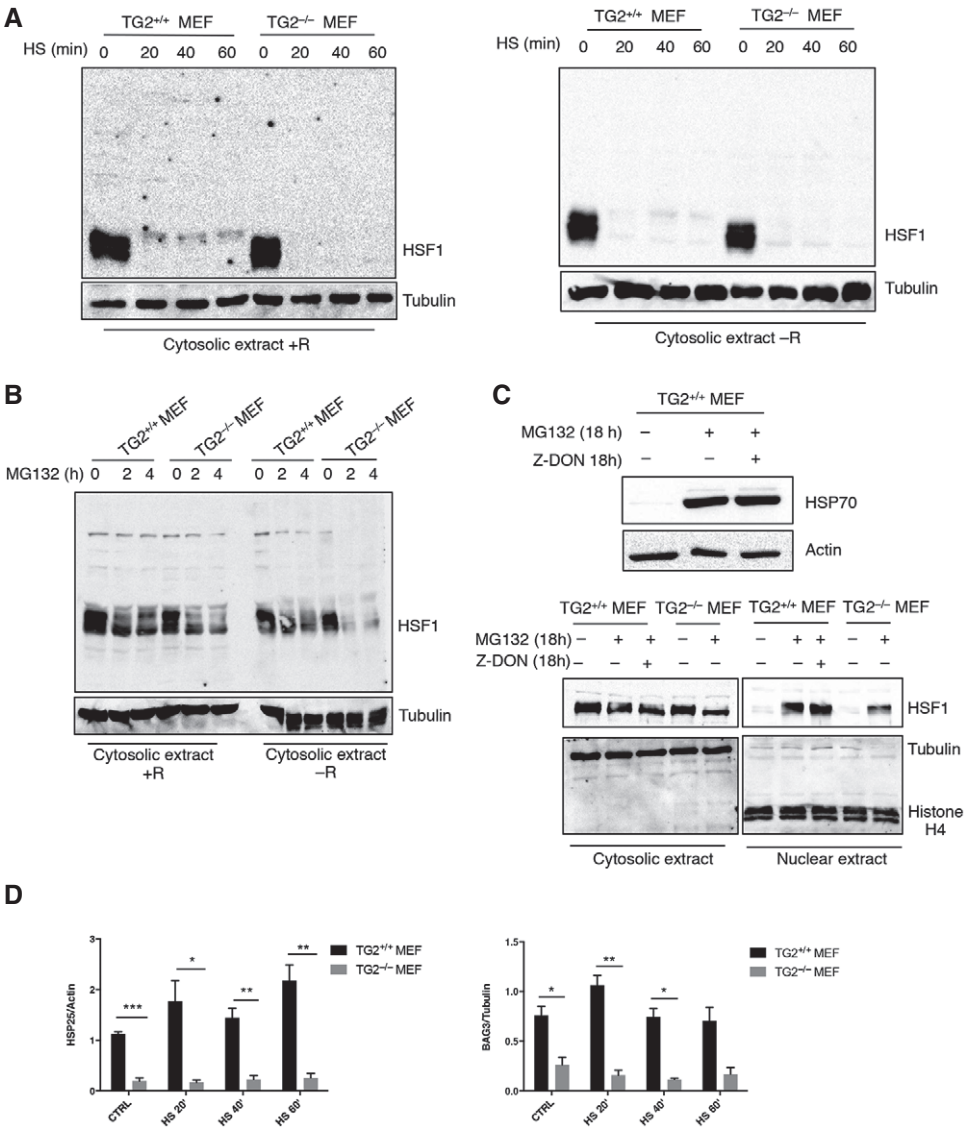

Figure EV2. TG2-dependent HSF1 trimerization occurs in the nucleus and not in the cytosolic compartment.

A, B Western blot analysis of HSF1 in cytosolic extracts of $TG2^{+/+}$ and $TG2^{-/-}$ MEF after heat shock for 20, 40 and 60 min (A) and proteasome inhibition by treating with MG132 for 2 and 4 h (B). The analysis was performed in presence (+R) and absence (−R) of reducing agents to detect the formation of disulphide bonds. Tubulin was used as loading control.

C HSF1 nuclear translocation and HSP70 induction do not depend on TG2 transamidating activity. Western blot analysis of HSP70 expression (upper) and HSF1 nuclear translocation (lower) in $TG2^{+/+}$ and $TG2^{-/-}$ MEF after proteasome inhibition with MG132 for 18 h in presence or not of Z-DON, a specific inhibitor of TG2 transamidating activity. Tubulin was used as loading control for cytosolic extract. Histone H4 was used as loading control for nuclear extract. Actin was used as loading control for whole protein extract.

D Densitometric analysis of HSP25 and BAG3 protein in $TG2^{+/+}$ and $TG2^{-/-}$ MEF after heat shock for 20, 40 and 60 min. Tubulin and actin were used as loading control.

Data information: Results are mean ± SEM of three independent experiments; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ (one-way ANOVA).

$CFTR^{wt/F508del}/TG2^{+/+}$ x $CFTR^{wt}/TG2^{-/-}$	expected %	born on 46	obtained %
$CFTR^{wt}/TG2^{+/-}$	50	19	41,3
$CFTR^{wt/F508del}/TG2^{+/-}$	50	27	58,7

$CFTR^{wt/F508del}/TG2^{+/-}$ x $CFTR^{wt/F508del}/TG2^{+/-}$	expected %	born on 237	obtained %
$CFTR^{wt}/TG2^{+/+}$	6,0	18	7,6
$CFTR^{wt}/TG2^{+/-}$	12,5	37	15,6
$CFTR^{wt}/TG2^{-/-}$	6,0	19	8,0
$CFTR^{wt/F508del}/TG2^{+/+}$	12,5	25	10,5
$CFTR^{wt/F508del}/TG2^{+/-}$	25,0	63	26,6
$CFTR^{wt/F508del}/TG2^{-/-}$	12,5	33	13,9
$CFTR^{F508del}/TG2^{+/+}$	6,0	15	6,3
$CFTR^{F508del}/TG2^{+/-}$	12,5	19	8,0
$CFTR^{F508del}/TG2^{-/-}$	6,0	8	3,4

$CFTR^{wt/F508del}/TG2^{-/-}$ X $CFTR^{wt/F508del}/TG2^{-/-}$	expected %	born on 153	obtained %
$CFTR^{wt}/TG2^{-/-}$	25	35	22,9
$CFTR^{wt/F508del}/TG2^{-/-}$	50	84	54,9
$CFTR^{F508del}/TG2^{-/-}$	25	34	22,2

Figure EV3. Mice strains crossed to generate $CFTR^{F508del}/TG2^{-/-}$ mice.

C57Bl/6 $TG2^{-/-}$ transgenic mice have been crossed with 129/FVB $CFTR^{wt}/^{F508del}$ transgenic ones. The wild-type littermates have been also produced. The presence of TG2 and F508del CFTR has been determined by PCR. The new mouse model has been characterized in terms of survival and number of littermates.

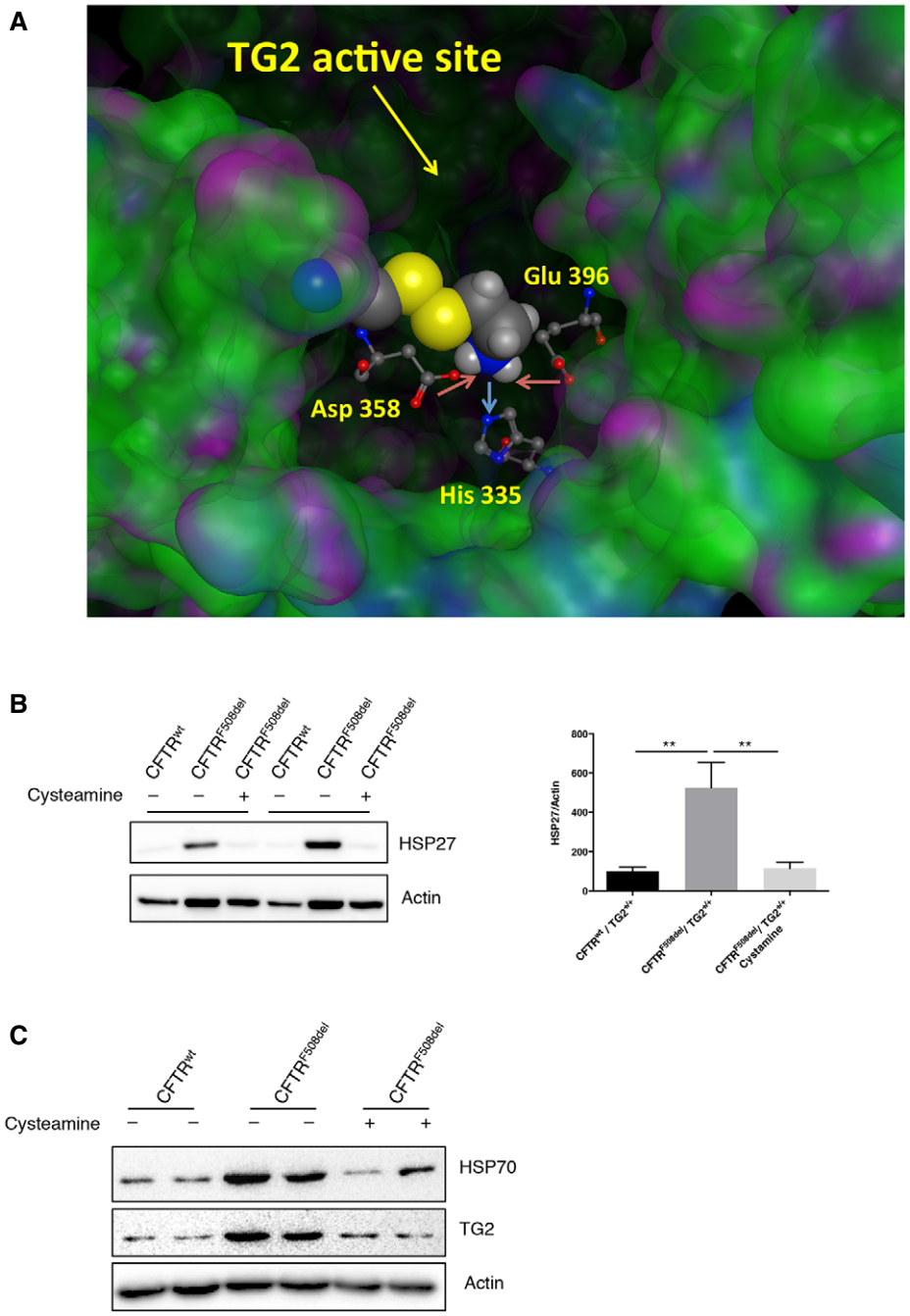

Figure EV4. Cysteamine inhibition of TG2's PDI activity rescues HSP27 and HSP70 protein levels.

A Docking analysis of cysteamine binding to TG2.

B Western blot and densitometric analysis of HSP27 protein levels in nasal epithelial cells, from CF patients (n = 6), cultured *ex vivo* with cysteamine for 18 h.

C Western blot of HSP70 in brushed nasal epithelial cells freshly collected from F508del CFTR homozygous patients (n = 2) underwent a phase II clinical trial with cysteamine bitartrate. Actin was used as loading control.

Data information: Results are mean ± SEM; $^{**}P < 0.01$ (one-way ANOVA).

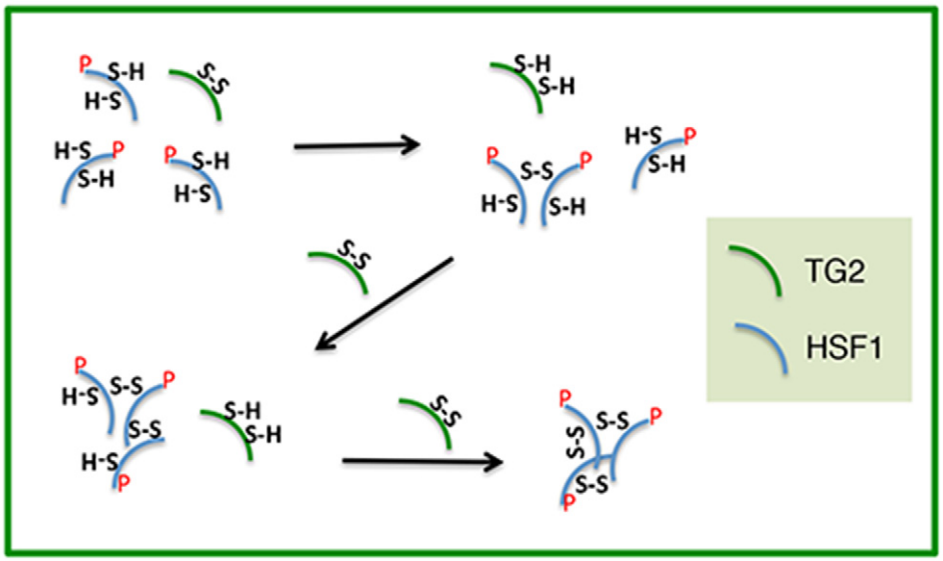

Figure EV5. Scheme of TG2 dependent trimerization of HSF1.

It is showed the hypothetical mechanism of action of TG2's PDI activity on HSF1.