1 **Supplementary Materials for**

2 **Modulation of the Proteostasis Network Promotes Tumor Resistance to Oncogenic KRAS**
3 **Inhibitors**

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13 **The PDF file includes:**

14 Figs. S1-S18

15 Tables S1-S5

16 **Other Supplementary Materials for this manuscript include the following:**

17 MDAR Reproducibility Checklist

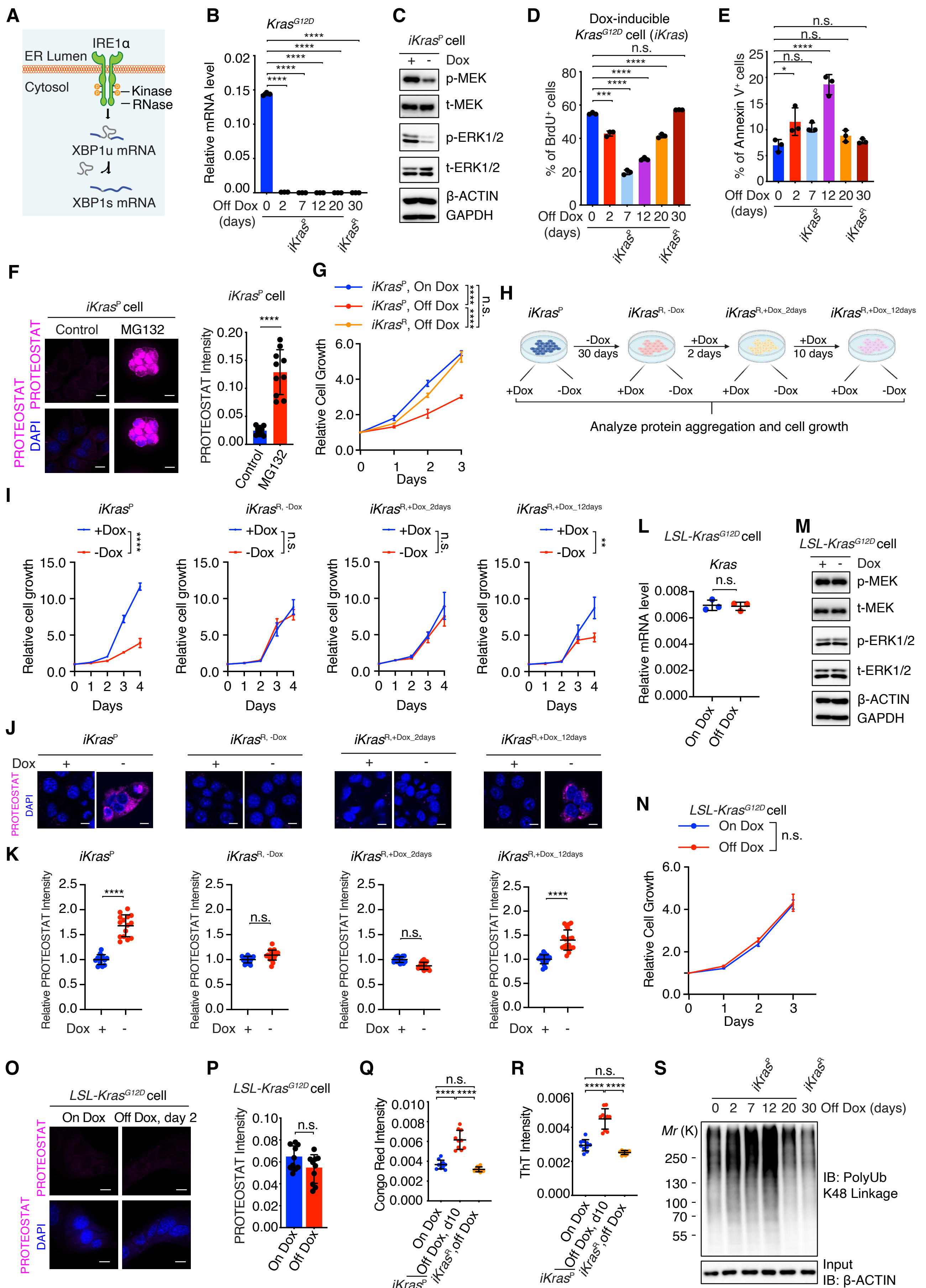

Figure S1. Characterization of mutant KRAS inhibition resistant *iKras*^{R} cells

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Figure S1. Characterization of mutant KRAS inhibition resistant *iKras*^{R} cells

A. Schematic illustration of the IRE1α/XBP1 pathway. **B.** RT-qPCR analysis of *Kras*^{G12D} expression in *iKras* cells at different time points after doxycycline (Dox) withdrawal (Off Dox) until the cells acquired resistance to *Kras*^{G12D} inactivation (*iKras*^{R} cell). *iKras*^{P}: parental Dox-inducible *Kras*^{G12D} PDAC cells. Dox: 1 µg/mL. Data are presented as mean ± SD relative to *Actb*, *n*=3. The data are representative of 3 independent experiments. **C.** Immunoblot of total and phosphorylated MEK (Ser217/221, p-MEK) and ERK1/2 (Thr202/Tyr204, p-ERK1/2) in whole-cell lysates of *iKras*^{P} cells cultured in the presence or absence of doxycycline (Dox, 1 µg/mL) for 2 days. **D.** Quantification of BrdU^{+} *iKras* cells at different time points after *Kras*^{G12D} inactivation by doxycycline (Dox)-withdrawal (Off Dox) until the cells acquired resistance to *Kras*^{G12D} inactivation (*iKras*^{R} cell). Data are presented as mean ± SD, *n*=3. **E.** Quantification of Annexin V^{+} apoptotic *iKras*^{P} cells at different time points after *Kras*^{G12D} inactivation by Dox-withdrawal (Off Dox) until the cells acquired resistance to *Kras*^{G12D} inactivation (*iKras*^{R} cell). Data are presented as mean ± SD, *n*=3. **F.** Representative images (**left**) and quantification (**right**) of PROTEOSTAT (magenta) and DAPI (blue) staining in *iKras*^{P} cells treated with DMSO or 5µM MG132 for 16h. Data represent the average fluorescence intensity of PROTEOSTAT /cell from each image acquired and are presented as mean ± SD from *n*=10 images. Scale bar: 20µm. **G.** Quantification of cell growth of *iKras*^{P} or *iKras*^{R} cells in the presence or absence of Dox for indicated time points using CCK-8 assay. Data are normalized to the OD450 at day 0 and presented as mean ± SD, *n*=3. **H.** *iKras*^{P} cells cultured in the absence of Dox for 30 days acquired resistance to *Kras*^{G12D} inhibition and was designated as *iKras*$^{R,-Dox}$ cells. Dox was added back to *iKras*$^{R,-Dox}$ cells for 2 days (*iKras*$^{R,+Dox_2days}$) or 12 days (*iKras*$^{R,+Dox_12days}$), and then removed to test the resistance phenotypes by measuring cell growth and proteostasis. **I.** Quantification of cell growth of *iKras*^{P}, *iKras*$^{R,-Dox}$, *iKras*$^{R,+Dox_2days}$ or *iKras*$^{R,+Dox_12days}$ cells in the presence or absence of Dox for indicated time points using CCK-8 assay. Data are normalized to the OD450 at day 0 and presented as mean ± SD, *n*=3. **J-K.** Representative images (**J**) and quantification (**K**) of PROTEOSTAT and DAPI staining in*iKras*^{P}, *iKras*$^{R,-Dox}$, *iKras*$^{R,+Dox_2days}$ or *iKras*$^{R,+Dox_12days}$ cells cultured in the presence or absence of Dox for 2 days. Data represent the average fluorescence intensity of PROTEOSTAT/cell from each image acquired and presented as mean ± SD relative to '+Dox' group from *n*=15 images. Scale bar: 20µ m. **L.** RT-qPCR analysis of *Kras* expression in *LSL-Kras*^{G12D} cells cultured in the presence or absence of Dox for 2 days. Data are presented as mean ± SD relative to *Actb*, *n*=3. The data are representative of 3 independent experiments. **M.** Immunoblot of total and phosphorylated MEK and ERK1/2 in whole-cell lysates of *LSL-Kras*^{G12D} cells cultured in the presence or absence of Dox for 2 days. **N.** Quantification of cell viability of *LSL-Kras*^{G12D} cells cultured in the presence or absence of Dox for indicated time using CCK-8 assay. Data are normalized to the OD450 at day 0, and presented as mean ± SD, *n*=3. **O-P.** Representative images (**O**) and quantification (**P**) of PROTEOSTAT (magenta) and DAPI (blue) staining in *LSL-Kras*^{G12D} cells cultured in the presence or absence of Dox for 2 days. Data represent the average fluorescence intensity of PROTEOSTAT/cell from each image acquired and are presented as mean ± SD from *n*=10 images. Scale bar: 20µm. **Q-R.** Quantification of Congo Red (CR) (**Q**) and ThT (**R**) fluorescence intensity in *iKras*^{P} cells cultured in the presence or absence of doxycycline (Off Dox) for 10 days, or in the cells that acquired resistance to *Kras*^{G12D} inactivation (*iKras*^{R}). Data represent the average fluorescence intensity/cell from each image acquired and are presented as mean ± SD from *n*=10 images. **S.** Immunoblot of polyubiquitin (K48 linkage) in detergent-insoluble aggregates fractionated from *iKras*^{P} cells at different time points after *Kras*^{G12D} inactivation by Dox-withdrawal (Off Dox) until the cells acquired resistance to *Kras*^{G12D} inactivation (*iKras*^{P} cell). β-ACTIN in whole-cell lysates served as loading control. IB: immunoblot. Ordinary one-way ANOVA (**B, D, E, Q** and **R**), two-way ANOVA (**G, I** and **N**), or 2-tailed, unpaired Student's t test with Fisher's exact test (**F, L, K** and **P**) was used to calculate *P* values. n.s., not significant, * P<0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001.

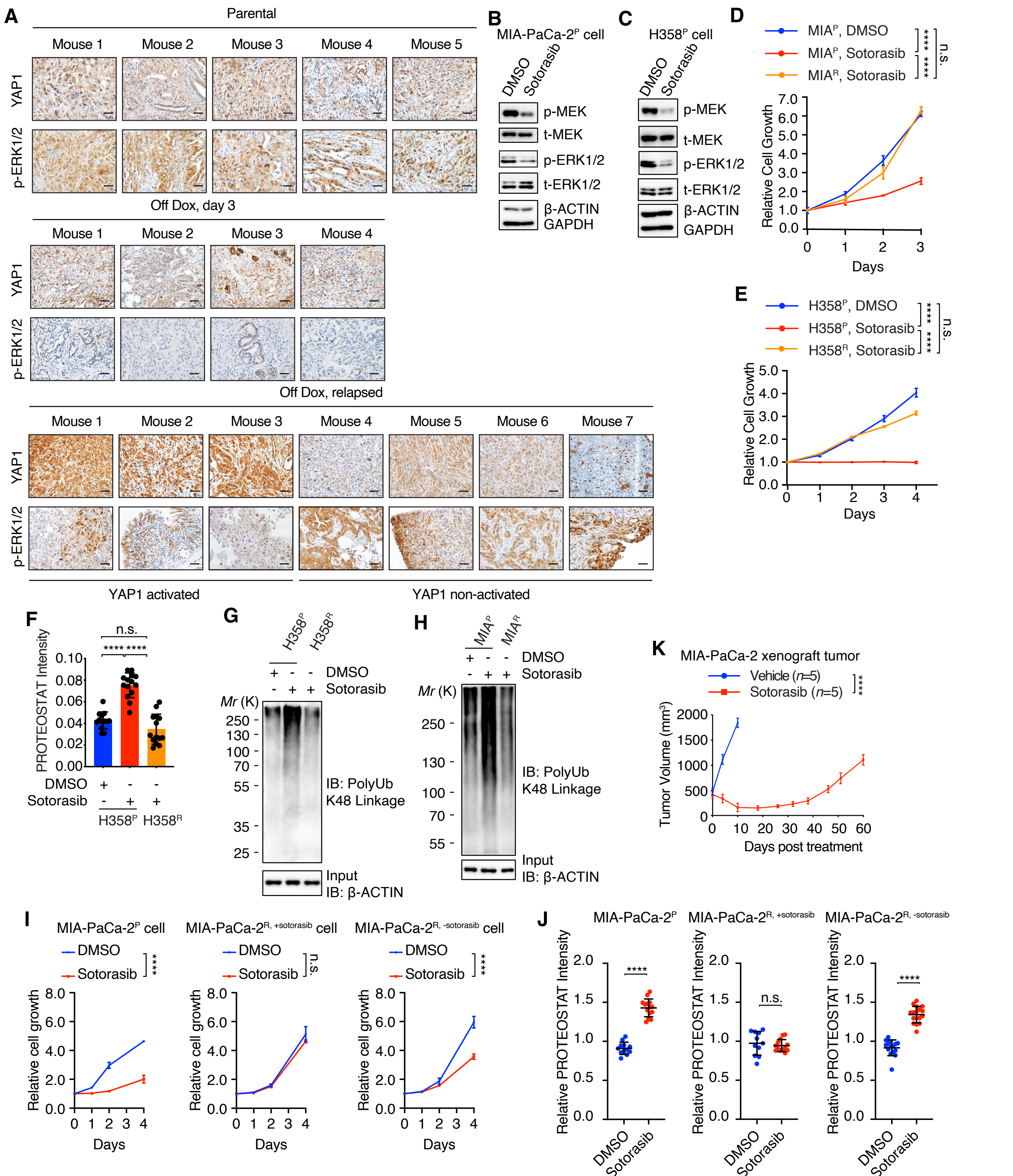

Figure S2. Characterization of sotorasib-resistant models

A. IHC staining of phospho-ERK1/2 and YAP1 in *iKras* GEMM tumors treated with doxycycline (On Dox), Dox withdrawal for 3 days (Off Dox), or relapsed after 30 weeks of Dox-withdrawal (Off Dox, relapsed) as in **Fig. 1D-E**. Scale bar: 40µm. **B.** Immunoblot of total and phosphorylated MEK and ERK1/2 in whole-cell lysates of parental MIA-PaCa-2 (MIA-PaCa-2^{P}) cells treated with DMSO or 30 nM sotorasib for 2 days. **C.** Immunoblot of total and phosphorylated MEK and ERK1/2 in whole-cell lysates from parental H358 (H358^{P}) cells treated with DMSO or 30 nM sotorasib for 2 days. **D.** Quantification of cell growth of parental (MIA^{P}) and *in vitro* generated sotorasib-resistant MIA-PaCa-2 cells (MIA^{R}) treated with DMSO or 30 nM KRAS^{G12C} inhibitor sotorasib for indicated time using CCK-8 assay. Data are normalized to the OD450 at day 0, and presented as mean ± SD, n=3. **E.** Quantification of cell growth of parental (H358^{P}) and sotorasib-resistant H358 cells (H358^{R}) treated with DMSO or 30 nM sotorasib for indicated time using CCK-8 assay. Data are normalized to the OD450 at day 0 and presented as mean ± SD, n=3. **F.** Quantification of PROTEOSTAT intensity in H358^{P} cells treated with DMSO or 30nM sotorasib for 2 days or in the sotorasib-resistant H358^{R} cells. Data represent the average fluorescence intensity of PROTEOSTAT/cell from each image acquired (>10 cells per image) and are presented as mean ± SD from n=14 images. **G.** Immunoblot of polyubiquitin (K48 linkage) in detergent-insoluble aggregates fractionated from H358^{P} cells treated with DMSO or 30nM sotorasib for 2 days or from the sotorasib-resistant H358^{R} cells. β-ACTIN from whole-cell lysates served as loading control. IB, immunoblot. **H.** Immunoblot of polyubiquitin (K48 linkage) in detergent-insoluble aggregates fractionated from parental MIA-PaCa-2 (MIA^{P}) cells treated with DMSO or 30nM sotorasib for 2 days or from MIA^{R} cells treated with 30nM sotorasib. β-ACTIN in whole-cell lysates served as loading control. IB, immunoblot. **I.** Quantification of cell growth of MIA-PaCa-2^{P}, MIA-PaCa-2$^{R,+sotorasib}$ and MIA-PaCa-2$^{R,-sotorasib}$ cells treated with DMSO or 30 nM sotorasib for indicated time using CCK-8 assay. MIA-PaCa-2$^{R,+sotorasib}$ cell is the sotorasib-resistant MIA-PaCa-2^{R} cells cultured constantly in the presence of 30 nM sotorasib *in vitro*. Sotorasib was removed from the culture medium of MIA-PaCa-2$^{R,+sotorasib}$ cell for 14 days (MIA-PaCa-2$^{R,-sotorasib}$) and then added back to test the resistance phenotypes. Data are normalized to the OD450 at day 0, and presented as mean ± SD, n=3. **J.** Quantification of PROTEOSTAT intensity in MIA-PaCa-2^{P}, MIA-PaCa-2$^{R,+sotorasib}$ and MIA-PaCa-2$^{R,-sotorasib}$ cells treated with DMSO or 30 nM sotorasib for 2 days. Data represent the average fluorescence intensity of PROTEOSTAT/cell from each image acquired and presented as mean ± SD relative to DMSO group from n=12 images. **K.** Tumor volume quantification of established MIA-PaCa-2 xenograft tumors treated with vehicle (n=5) or sotorasib (30mg/kg, n=5). Data are presented as mean ± SEM. Ordinary one-way ANOVA (**F**), Two-way ANOVA test (**D, E, I** and **K**), or 2-tailed, unpaired Student's t test (**J**) was used to calculate P values. n.s., not significant, * P<0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001.

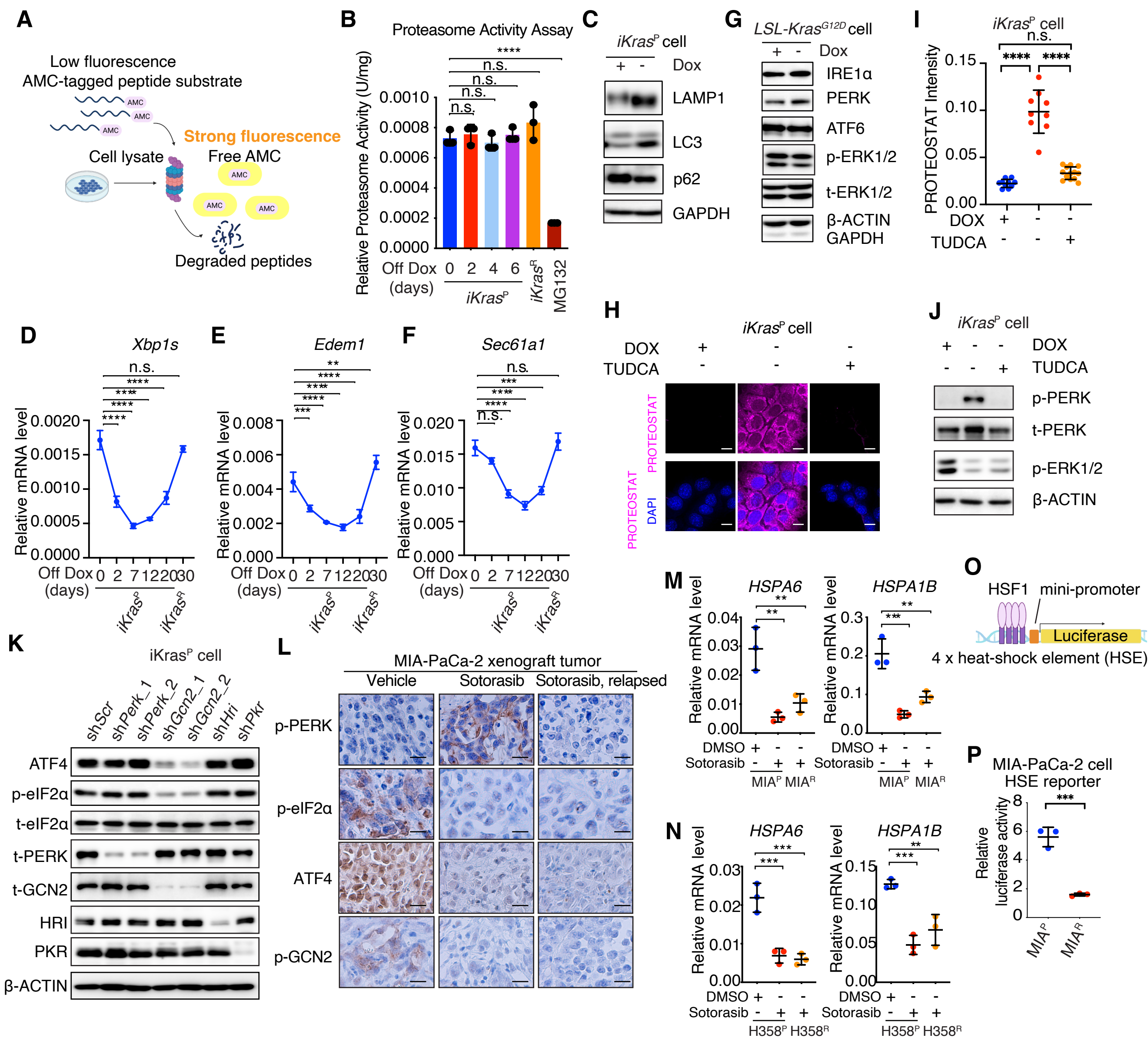

Figure S3. Mutant KRAS inactivation reprograms proteostasis network

A. Diagram showing the fluorometric assay that measures the chymotrypsin-like protease activity of the proteasome. In the presence of proteolytic activity of the proteasome, an AMC-tagged peptide substrate (low fluorescence) is degraded, leading to the release of free and highly fluorescent AMC. **B.** Quantification of proteasome activity of parental *iKras* cells (*iKras*^{P}) at different time points after *Kras*^{G12D} inactivation by Dox withdrawal (Off Dox) or the cells that acquired resistance to *Kras*^{G12D} inactivation (*iKras*^{R}). MG132 inhibits proteasome activity and was used as a positive control at a concentration of 10 µM. Average proteasome activities normalized against total protein abundance are presented as mean ± SD, *n*=3. **C.** Immunoblot of LAMP1, LC3 and P62 in whole-cell lysates of *iKras*^{P} cells cultured in the presence or absence of doxycycline (Dox) for 2 days. **D-F.** RT-qPCR analysis of spliced *Xbp1* (*Xbp1s*), IREα/XBP1 targets *Edem1* and *Sec61a1* expression in *iKras*^{P} cells at different time points after Dox withdrawal (Off Dox) until the cells acquired resistance to *Kras*^{G12D} inactivation (*iKras*^{R} cell). Data are presented as mean ± SD relative to *Actb*, *n*=3. The data are representative of 3 independent experiments. **G.** Immunoblot of UPR markers in *LSL-Kras*^{G12D} cells cultured in the presence or absence of Dox for 2 days. **H-I.** Representative image (**H**) and quantification (**I**) of PROTEO-STAT (magenta) and DAPI (blue) staining in *iKras*^{P} cells cultured in the presence or absence of Dox for 4 days. Cells were also treated with 1.0 mM TUDCA dissolved in water for 2 days before collection as indicated. Data represent the average fluorescence intensity of PROTEOSTAT/cell from each image acquired (>10 cells per image) and presented as mean ± SD from *n*=10 images. Scale bar: 20µm. **J.** Immunoblot of t-PERK, p-PERK and p-ERK1/2 in whole-cell lysates of *iKras*^{P} cells treated as in (**H-I**). β-ACTIN in whole-cell lysates served as loading control. **K.** Immunoblot of ATF4, total-eIF2α (t-eIF2α), phospho-eIF2α (p-eIF2α), PERK, GCN2, HRI and PKR in whole-cell lysates of *iKras*^{P} cells infected with lentiviruses encoding *scramble* shRNA (sh*Scr*), *Perk* shRNAs (sh*Perk*_1 and sh*Perk*_2), *Gcn2* shRNAs (sh*Gcn2*_1 and sh*Gcn2*_2), *Hri* shRNA (sh*Hri*) or *Pkr* shRNA (sh*Pkr*). β-ACTIN in whole-cell lysates served as loading control. **L.** Immunohistochemical staining of p-PERK, p-eIF2α, ATF4 or p-GCN2 in MIA-PaCa-2 xenograft tumors treated with vehicle, sotorasib (100mg/kg for 4 days), or relapsed after 9 weeks of sotorasib treatment (100mg/kg). Scale bar: 40µm. **M-N.** RT-qPCR analysis of HSF1 targets *HSPA6* and *HSPA1B* in parental MIA-PaCa-2 (MIA^{P}) (**M**) or H358 (H358^{P}) (**N**) cells treated with DMSO or 30 nM sotorasib for 2 days or in the sotorasib-resistant MIA^{R} or H358^{R} cells. Cells were heat shocked for 1 h at 43 °C and recovered for 4 h before harvest. Data are presented relative to *ACTIN* and shown as mean ± SD, *n*=3. **O.** Schematic of the heat shock element (HSE) luciferase reporter. **P.** The MIA-PaCa-2^{P} or MIA-PaCa-2^{R} cells were transfected with the HSE luciferase reporter. After selection, cells were then heat shocked for 1 h at 43 °C and recovered overnight before luciferase activities were measured. Data are presented as relative *Firefly* luciferase activities normalized against *Renilla* luciferase activities and shown as mean ± SD, *n*=3. Ordinary one-way ANOVA (**B, D, E, F, I, M** and **N**) and 2-tailed, unpaired Student's t test (**P**) were used to calculate *P* values. n.s., not significant, * $P<0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

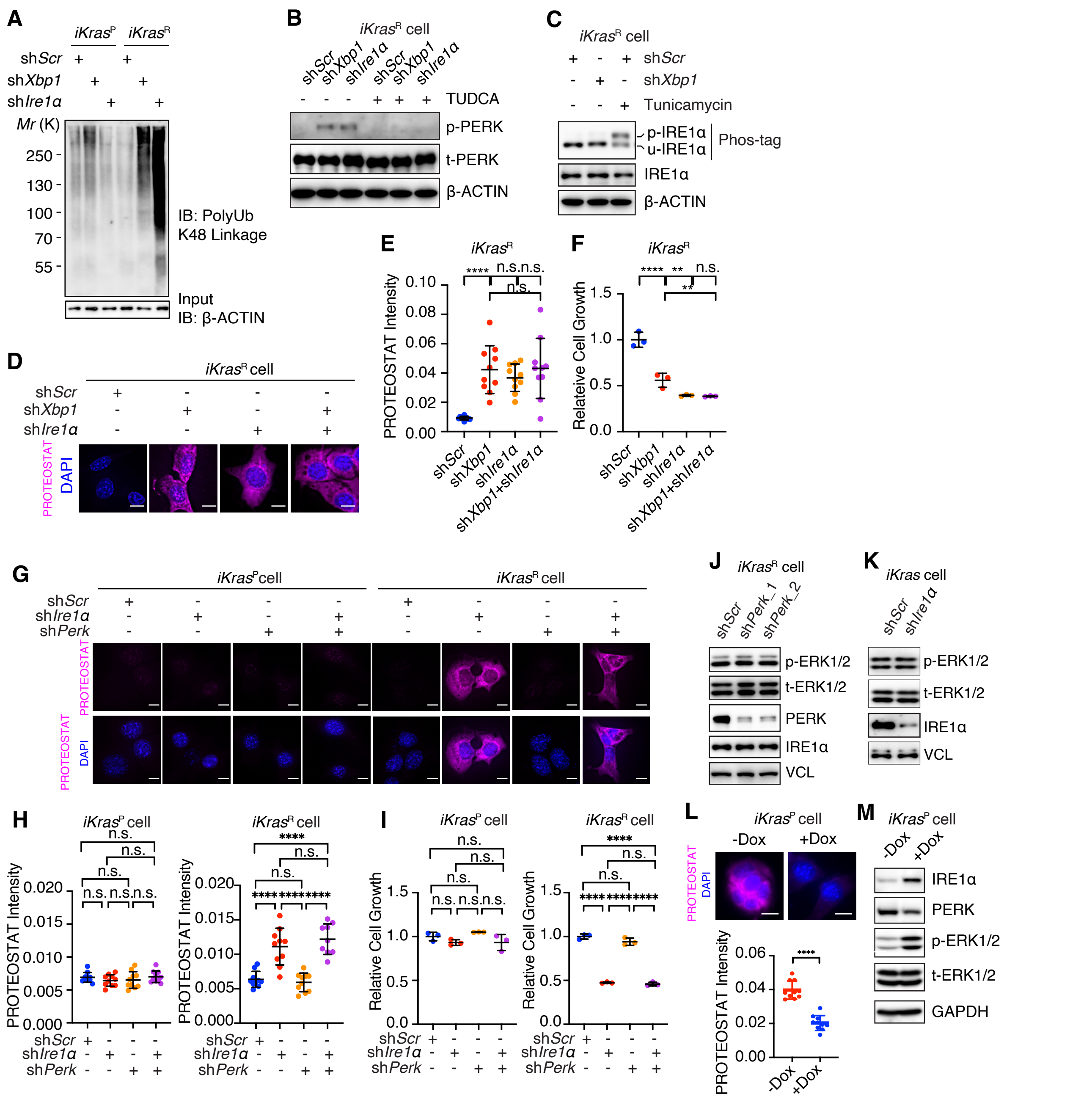

Figure S4. IRE1α, but not PERK, sustains proteostasis in KRASi resistant cells

A. Immunoblot of polyubiquitin (K48 linkage) in detergent-insoluble aggregates fractionated from *iKras*^{P} or *iKras*^{R} cells infected with lentiviruses encoding sh*Scr*, sh*Xbp1* or sh*Ire1α*. β-ACTIN in whole protein lysates served as loading control. IB, immunoblot. **B.** Immunoblot of total-PERK and phospho-PERK in *iKras*^{R} cells infected with lentiviruses encoding *scramble* shRNA (sh*Scr*), *Xbp1s* shRNA (sh*Xbp1*) or *Ire1α* shRNA (sh*Ire1α*) and treated with or without 2.5mM TUDCA dissolved in water for 2 days. β-ACTIN in whole-cell lysates served as loading control. **C.** Phos-tag SDS-PAGE analysis of IRE1α activation in whole-cell lysates of *iKras*^{R} cells infected with lentiviruses encoding *scramble* shRNA (sh*Scr*) or *Xbp1s* shRNA (sh*Xbp1*). The *iKras*^{R} cells treated with tunicamycin (5 µg/mL) for 6 hours served as positive control. Regular SDS-PAGE was used to monitor IRE1α total protein level. β-ACTIN in whole-cell lysates served as loading control. **D-E.** Representative images (**D**) and quantification (**E**) of PROTEOSTAT (magenta) and DAPI (blue) staining in *iKras*^{R} cells infected with lentiviruses encoding *scramble* shRNA (sh*Scr*), *Ire1α* shRNA (sh*Ire1α*), *Xbp1s* shRNA (sh*Xbp1*) or sh*Ire1α* plus sh*Xbp1*. Data represent the average fluorescence intensity of PROTEOSTAT/cell from each image acquired (>10 cells per image) and presented as mean ± SD from *n*=10 images. Scale bar: 20µm. **F.** Impact of *Ire1α* or *Xbp1s* silencing on the viability of *iKras*^{R} cells. CCK-8 assay was used to quantify cell viability of *iKras*^{R} infected with lentiviruses encoding *scramble* shRNA (sh*Scr*), *Ire1α* shRNA (sh*Ire1α*), *Xbp1s* shRNA (sh*Xbp1*) or sh*Ire1α* plus sh*Xbp1*. Data are presented as mean ± SD relative to scramble control (sh*Scr*), *n*=3. **G-H.** Representative images (**G**) and quantification (**H**) of PROTEOSTAT (magenta) and DAPI (blue) staining in *iKras*^{P} and *iKras*^{R} cells infected with lentiviruses encoding *scramble* shRNA (sh*Scr*), *Ire1α* shRNA (sh*Ire1α*) and/or *Perk* shRNA (sh*Perk*). Data represent the average fluorescence intensity of PROTEOSTAT/cell from each image acquired (>10 cells per image) and presented as mean ± SD from *n*=10 images. Scale bar: 20µm. **I.** Impact of *Ire1α* or *Perk* silencing on the viability of *iKras*^{P} or *iKras*^{R} cells. CCK-8 assay was used to quantify cell viability of *iKras*^{P} or *iKras*^{R} infected with lentiviruses encoding sh*Scr*, sh*Perk* or sh*Ire1α*. Data are presented as mean ± SD relative to *scramble* control (sh*Scr*), *n*=3. **J.** Immunoblot of IRE1α, phospho-ERK1/2, total-ERK1/2 and PERK in whole-cell lysates of *iKras*^{R} cells infected with lentiviruses encoding *scramble* shRNA (sh*Scr*) or *PERK* shRNA (sh*PERK*). VCL in whole-cell lysates served as loading control. **K.** Immunoblot of IRE1α, phospho-ERK1/2 and total-ERK1/2 in whole-cell lysates of *iKras*^{R} cells infected with lentiviruses encoding *scramble* shRNA (sh*Scr*) or *IRE1α* shRNA (sh*IRE1α*). VCL in whole-cell lysates served as loading control. **L.** Representative images (**upper panels**) and quantification (**lower panel**) of PROTEOSTAT (magenta) and DAPI (blue) staining in *iKras*^{P} cells. The *iKras*^{P} cells were cultured in the absence of Dox for 2 days. After that, the cells were further cultured in the presence or absence of Dox for 2 days followed by PROTEOSTAT staining. Data represent the average fluorescence intensity of PROTEOSTAT/cell from each image acquired (>10 cells per image) and presented as mean ± SD from *n*=10 images. Scale bar: 20µm. **M.** Immunoblot of IRE1α, PERK, p-ERK1/2 and t-ERK1/2 in *iKras*^{P} cells. The *iKras*^{P} cells were cultured in the absence of Dox for 2 days. After that, the cells were further cultured in the presence or absence of Dox for 2 days followed by western blot analysis. GAPDH in whole-cell lysates served as loading control. Ordinary one-way ANOVA (**E, F, H** and **I**) and 2-tailed, unpaired Student's t test (**L**) were used to calculate *P* values. n.s., not significant, * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$.

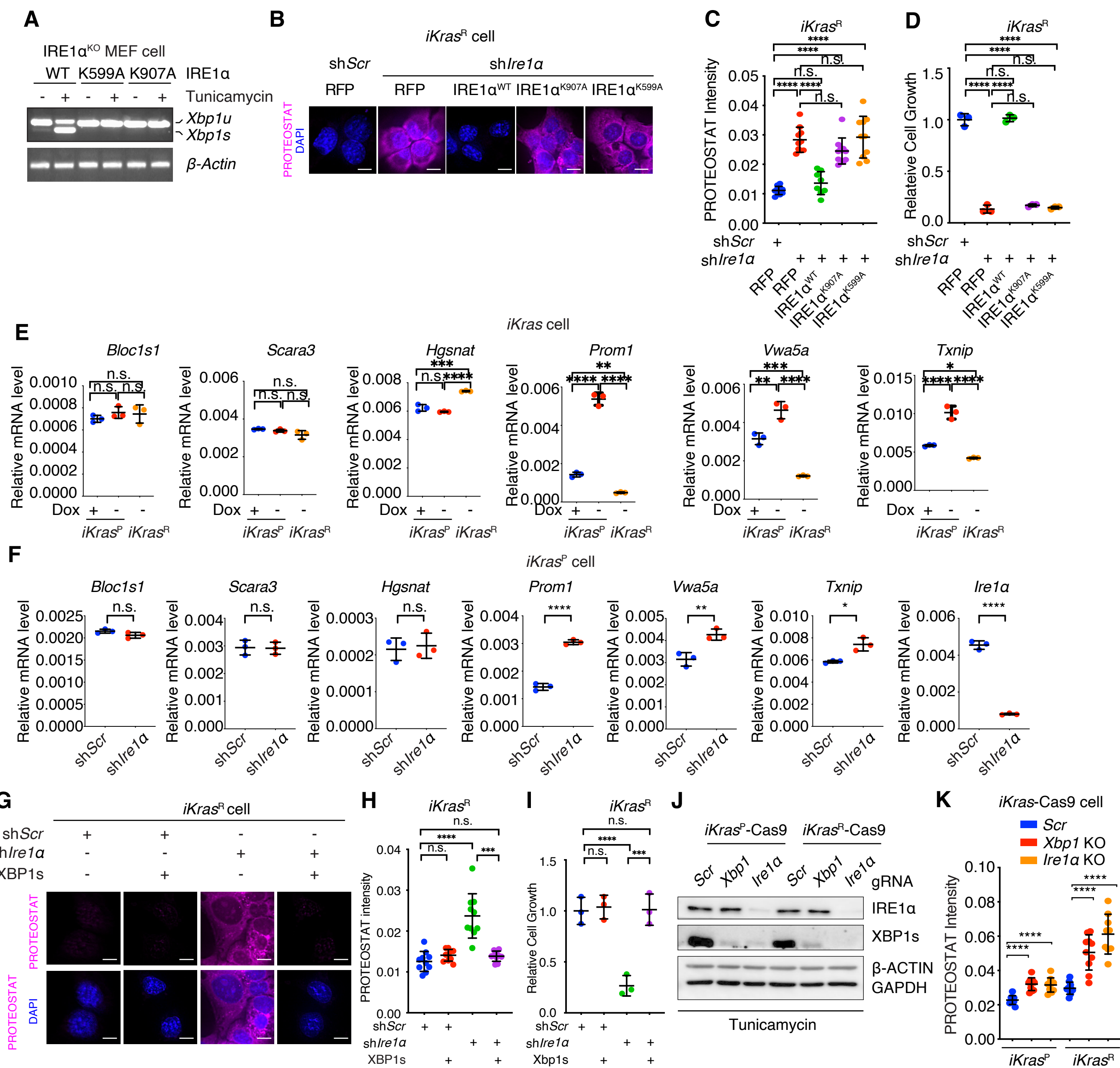

Figure S5. IRE1α RNase-dependent *XBP1* splicing sustains proteostasis in KRASi resistant cells

A. RT-PCR analysis of *Xbp1* splicing in *IRE1α* knock out MEF cells expressing IRE1α^{WT}, IRE1α^{K599A} or IRE1α^{K907A} and treated with DMSO or tunicamycin (TM, 5 μg/mL) for 6 hours as indicated. β-ACTIN served as loading control. **B-C.** Representative images (**B**) and quantification (**C**) of PROTEOSTAT (magenta) and DAPI (blue) staining in *iKras*^{R} cells infected with lentiviruses encoding *scramble* shRNA (sh*Scr*), *Ire1α* shRNA (sh*Ire1α*) and shRNA-resistant IRE1α^{WT}, IRE1α^{K599A} or IRE1α^{K907A}. Data represent the average fluorescence intensity of PROTEOSTAT/cell from each image acquired (>10 cells per image) and presented as mean ± SD from *n*=8 images. Scale bar: 20μm. **D.** Colony formation assay was used to quantify the growth of control (sh*Scr*) *iKras*^{R} cells or *Ire1α*-knockdown *iKras*^{R} cells reconstituted with shRNA-resistant IRE1α^{WT}, IRE1α^{K599A} or IRE1α^{K907A}. Data are presented as mean ± SD relative to *scramble* control, *n*=3. **E.** RT-qPCR analysis of *Bloc1s1, Scara3, Hgsnat, Prom1, Vwa5a* and *Txnip* expression in *iKras*^{P} cells in the presence (+Dox) or absence (- Dox) of *Kras*^{G12D} for 2 days or in *iKras*^{R} cell cultured without doxycycline (Dox). Data are presented as mean ± SD relative to *Actb*, *n*=3. **F.** RT-qPCR analysis of *Bloc1s1, Scara3, Hgsnat, Prom1, Vwa5a* and *Txnip* expression in *iKras*^{P} cells infected with lentiviruses encoding *scramble* shRNA (sh*Scr*) or *Ire1α* shRNA (sh*Ire1α*). Data are presented as mean ± SD relative to *Actb*, *n*=3. **G-H.** Representative images (**G**) and quantification (**H**) of PROTEOSTAT (magenta) and DAPI (blue) staining in *iKras*^{R} cells infected with lentiviruses encoding *scramble* shRNA (sh*Scr*) or *Ire1α* shRNA (sh*Ire1α*) in the presence or absence of XBP1s. Data represent the average fluorescence intensity of PROTEOSTAT/cell from each image acquired (>10 cells per image) and presented as mean ± SD from *n*=10 images. Scale bar: 20μm. **I.** Colony formation assay in *iKras*^{R} cells infected with lentiviruses encoding *scramble* shRNA (sh*Scr*) or *Ire1α* shRNA (sh*Ire1α*) in the presence or absence of XBP1s. Data are presented as mean ± SD, *n*=3. **J.** Immunoblot of IRE1α or spliced XBP1 protein (XBP1s) in whole-cell lysates of *iKras*^{P} and *iKras*^{R} cells expressing Cas9 (*iKras*-Cas9) and infected with lentiviruses encoding double gRNA targeting the same exon of *Xbp1* or *Ire1α*. Cells were treated with tunicamycin (5 μg/mL) for 6 h before harvest. **K.** Quantification of PROTEOSTAT intensity in *Ire1α* or *Xbp1* knock-out (KO) *iKras*^{P} or *iKras*^{R} cells. Data represent the average fluorescence intensity of PROTEOSTAT/cell from each image acquired (>10 cells per image) and are presented as mean ± SD from *n*≥9 images. Ordinary one-way ANOVA (**C, D, E, H, I** and **K**) and 2-tailed, unpaired Student's t test (**F**) was used to calculate *P* values. n.s., not significant, * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$.

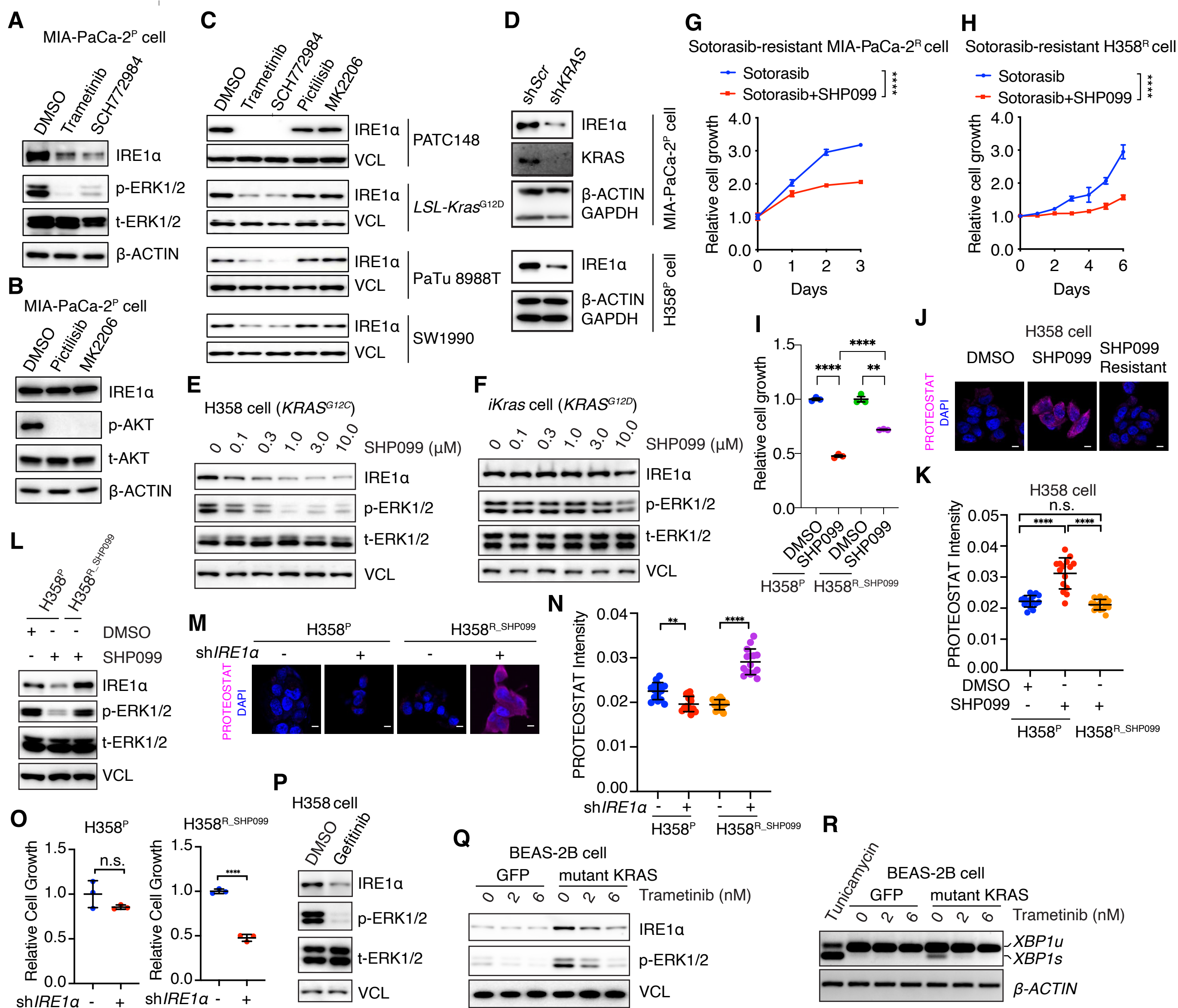

Figure S6. Regulation of IRE1α by KRAS-MAPK signaling and its upstream regulators
A. Immunoblot of IRE1α, t-ERK1/2, and p-ERK1/2 in whole-cell lysates of MIA-PaCa-2^{P} cells treated with DMSO, 20 nM trametinib, or 0.3 μM SCH772984 for 2 days. β-ACTIN in whole-cell lysates served as loading control. **B.** Immunoblot of IRE1α, t-AKT, and p-AKT in whole-cell lysates of MIA-PaCa-2^{P} cells treated with DMSO, 1 μM pictilisib, or 2 μM MK2206 for 2 days. β-ACTIN in whole-cell lysates served as loading control. **C.** Immunoblot of IRE1α in whole-cell lysates of PATC148, *LSL-Kras*^{G12D}, PaTu 8988T, and SW1990 cells treated with DMSO, 20 nM trametinib, 1 μM SCH772984, 2 μM MK2206 or 1 μM pictilisib for 2 days. VCL in whole-cell lysates served as loading control. **D.** Immunoblot of IRE1α in whole-cell lysates of MIA-PaCa-2^{P} or H358^{P} cells infected with lentiviruses encoding sh*Scr* or sh*KRAS*. β-ACTIN and GAPDH in whole-cell lysates served as loading control. **E.** Immunoblot of IRE1α, total ERK1/2 (t-ERK1/2), and phospho-ERK1/2 (p-ERK1/2) in whole-cell lysates of H358 cells treated with DMSO, or different doses of SHP2 inhibitor SHP099 (0.1-10 μM) for 2 days. VCL in whole-cell lysates served as loading control. **F.** Immunoblot of IRE1α, total ERK1/2 (t-ERK1/2), and phospho-ERK1/2 (p-ERK1/2) in whole-cell lysates of *iKras*^{G12D} cells treated with DMSO, or different doses of SHP099 (0.1-10 μM) for 2 days. VCL in whole-cell lysates served as loading control. **G-H.** Quantification of cell growth of sotorasib-resistant MIA-PaCa-2^{R} (**G**) or H358^{R} (**H**) cells treated with 30 nM sotorasib plus DMSO or 1 μM SHP099 for indicated time points using CCK-8 assay. Data are normalized to the OD450 at day 0 and presented as mean ± SD, n=3. **I.** Quantification of cell growth of parental (H358^{P}) and SHP099-resistant H358 cells (H358^{R_SHP099}) treated with DMSO or 25 μM SHP099 for 4 days using CCK-8 assay. Data are normalized to the OD450 of DMSO treated H358^{P} group and presented as mean ± SD, n=3. **J-K.** Representative images (**J**) and quantification (**K**) of PROTEOSTAT intensity in H358^{P} cells treated with DMSO or 0.3μM SHP099 for 4 days or in the SHP099-resistant H358^{R_SHP099} cells. Data represent the average fluorescence intensity of PROTEOSTAT/cell from each image acquired (>10 cells per image) and are presented as mean ± SD from n=15 images. **L.** Immunoblot of IRE1α, total ERK1/2 (t-ERK1/2), and phospho-ERK1/2 (p-ERK1/2) in whole-cell lysates of H358^{P} treated with DMSO or SHP099 (0.3 μM) for 2 days or SHP099-resistant H358^{R_SHP099} cells treated with 0.3 μM SHP099. VCL in whole-cell lysates served as loading control. **M-N.** Representative images (**M**) and quantification (**N**) of PROTEOSTAT intensity in H358^{P} or SHP099-resistant H358^{R_SHP099} cells infected with lentiviruses encoding sh*Scr* or sh*IRE1α*. Data represent the average fluorescence intensity of PROTEOSTAT/cell from each image acquired (>10 cells per image) and are presented as mean ± SD from n=15 images. **O.** Colony formation assay was used to quantify the growth of H358^{P} or SHP099-resistant H358^{R_SHP099} cells infected with lentiviruses encoding sh*Scr* or sh*IRE1α*. Data are presented as mean ± SD relative to sh*Scr* control, n=3. **P.** Immunoblot of IRE1α, total ERK1/2 (t-ERK1/2), and phospho-ERK1/2 (p-ERK1/2) in whole-cell lysates of H358 cells treated with DMSO or gefitinib (EGFR inhibitor, 1 μM) for 2 days. VCL in whole-cell lysates served as loading control. **Q.** Immunoblot of IRE1α and p-ERK1/2 in whole-cell lysates of BEAS-2B cells expressing GFP or mutant KRAS treated with DMSO or increasing doses of trametinib for 2 days. VCL in whole-cell lysates served as loading control. **R.** RT-PCR analysis of *XBP1* splicing in BEAS-2B cells expressing GFP or mutant KRAS treated with DMSO or increasing doses of trametinib for 2 days. 293T cells treated with tunicamycin (5 μg/mL) for 6 hours served as positive control. *β-Actin* served as loading control. Ordinary one-way ANOVA (**I, K** and **N**), Two-way ANOVA test (**G** and **H**), and 2-tailed, unpaired Student's t test (**O**) was used to calculate P values. n.s., not significant, * P<0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001.

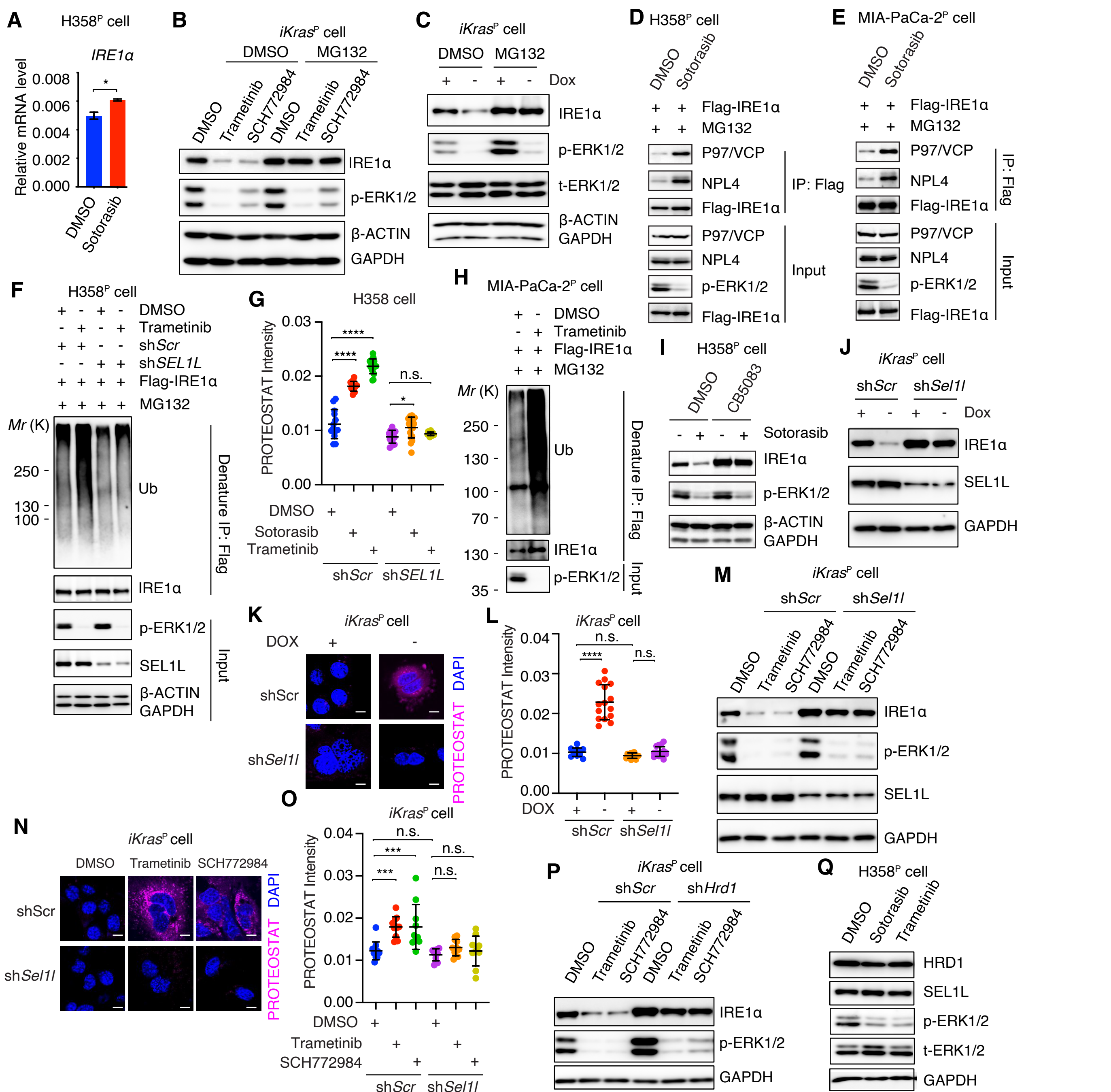

Figure S7. Oncogenic KRAS-MEK-ERK pathway regulates IRE1α protein stability

A. RT-qPCR analysis of IRE1α in H358^P cells treated with DMSO or 30nM sotorasib for 2 days. Data are presented relative to *ACTIN* and shown as mean ± SD, *n*=3. The data are representative of 3 independent experiments. **B.** Immunoblot of IRE1α and p-ERK1/2 in whole-cell lysates of *iKras*^{P} cells treated with DMSO, 20 nM trametinib or 1 μM SCH772984 for 2 days. Cells were treated with DMSO or 3 μM MG132 for 12h before harvest. β-ACTIN and GAPDH in whole-cell lysates served as loading control. **C.** Immunoblot of IRE1α in whole-cell lysates of *iKras*^{P} cells cultured in the presence or absence of Dox (Off Dox) for 2 days. Cells were treated with DMSO or 3 μM MG132 12h before collection. β-ACTIN and GAPDH in whole-cell lysates served as loading control. **D.** Whole-cell lysates of H358 cells expressing Flag-IRE1α and treated with DMSO or sotorasib (30 nM) for 2 days were subjected to immunoprecipitation (IP) with anti-Flag M2 agarose beads, followed by immunoblot to detect interaction between P97/NPL4 and IRE1α. MG132 (1 μM) was added into the culture medium 12 h before harvest. **E.** Whole-cell lysates of MIA-PaCa-2 cells expressing Flag-IRE1α and treated with DMSO or sotorasib (30 nM) for 2 days were subjected to immunoprecipitation (IP) with anti-Flag M2 agarose beads, followed by immunoblot to detect interaction between P97/NPL4 and IRE1α. MG132 (1 μM) was added into the culture medium 12 h before harvest. **F.** Trametinib promotes SEL1L-dependent IRE1α ubiquitination. H358^P cells expressing Flag-IRE1α were infected with lentivirus encoding sh*SCR* or sh*SEL1L* and treated with DMSO or 20 nM trametinib for 2 days. Cells were treated with 1 μM MG132 overnight before harvest. Denature immunoprecipitation (IP) was performed with anti-Flag M2 agarose beads followed by immunoblot to detect IRE1α ubiquitination. **G.** Quantification of PROTEOSTAT intensity in H358 cells infected with lentiviruses encoding sh*Scr* or sh*SEL1L* and treated with DMSO, 30 nM sotorasib or 20 nM trametinib for 2 days. Data represent the average fluorescence intensity of PROTEOSTAT/cell from each image acquired and presented as mean ± SD from *n*=13 images. **H.** Trametinib promotes IRE1α ubiquitination in MIA-PaCa-2 cells expressing empty vector or Flag-IRE1α and treated with DMSO or 20 nM trametinib for 2 days. Cells were treated with 3 μM MG132 12h before collection. Denature immunoprecipitation (IP) was performed with anti-Flag M2 agarose beads followed by immunoblot to detect IRE1α ubiquitination. **I.** Immunoblot of IRE1α and p-ERK1/2 in whole-cell lysates of H358 cells treated with DMSO or 30 nM sotorasib for 2 days. Cells were treated with DMSO or 1 μM CB5083 (P97 inhibitor) for 12h before harvest. β-ACTIN and GAPDH in whole-cell lysates served as loading control. **J.** Immunoblot of IRE1α in whole-cell lysates of *iKras*^{P} cells infected with lentiviruses encoding sh*Scr* or sh*Sel1l* and cultured in the presence or absence of Dox (Off Dox) for 2 days. **K-L.** Representative images (**K**) and quantification (**L**) of PROTEOSTAT and DAPI staining in *iKras*^{P} cells infected with lentiviruses encoding sh*Scr* or sh*Sel1l* and cultured in the presence or absence of Dox for 2 days. Data represent the average fluorescence intensity of PROTEOSTAT/cell from each image acquired and presented as mean ± SD from *n*=11 images. Scale bar: 20μm. **M.** Immunoblot of IRE1α in whole-cell lysates of *iKras*^{P} cells infected with lentiviruses encoding sh*Scr* or sh*Sel1l* and treated with DMSO, 20 nM trametinib or 1 μM SCH772984 for 2 days. GAPDH in whole-cell lysates served as loading control. **N-O.** Representative images (**N**) and quantification (**O**) of PROTEOSTAT and DAPI staining in *iKras*^{P} cells infected with lentiviruses encoding sh*Scr* or sh*Sel1l* and treated with DMSO, 20 nM trametinib or 1 μM SCH772984 for 2 days. Data represent the average fluorescence intensity of PROTEOSTAT/cell from each image acquired and presented as mean ± SD from *n*=10 images. Scale bar: 20μm. **P.** Immunoblot of IRE1α in whole-cell lysates of *iKras*^{P} cells infected with lentiviruses encoding sh*Scr* or sh*Hrd1* and treated with DMSO, 20 nM trametinib or 1 μM SCH772984 for 2 days. *iKras*^{P} cells were cultured in the presence of 1μg/mL Dox to maintain *Kras*^{G12D} expression. **Q.** Immunoblot of SEL1L, HRD1, t-ERK1/2 and p-ERK1/2 in whole-cell lysates of parental H358 cells treated with DMSO, 30 nM sotorasib, or 20 nM trametinib for 2 days. GAPDH in whole-cell lysates served as loading control. 2-tailed, unpaired Student's t test (**A**), or ordinary one-way ANOVA (**G**, **L** and **O**) was used to calculate *P* values. n.s., not significant, * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001.

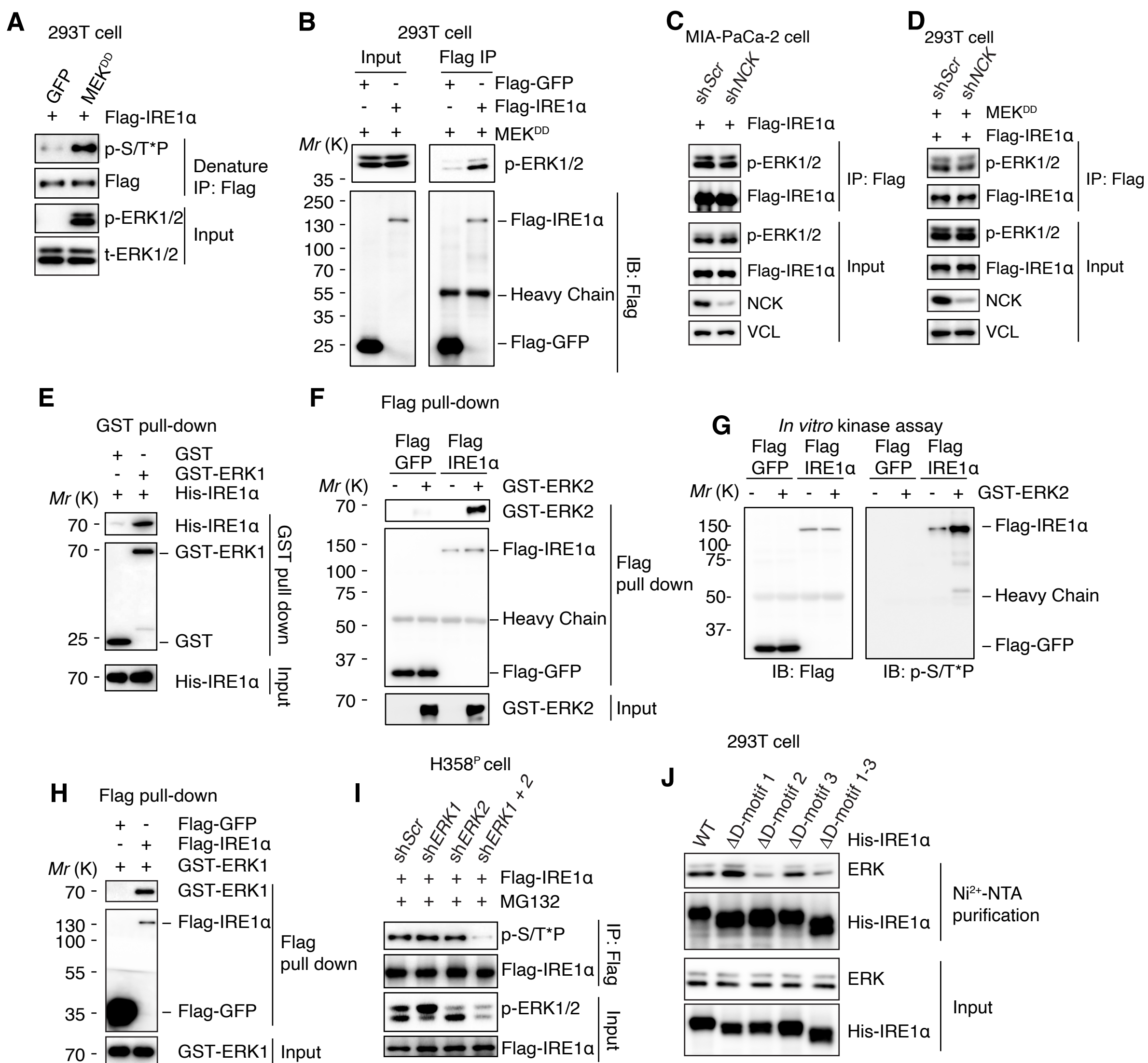

Figure S8. ERK directly interacts with and phosphorylates IRE1α

A. Constitutively activated MEK (MEK^{DD}) promotes IRE1α phosphorylation. 293T cells expressing Flag-IRE1α were transfected with GFP or MEK^{DD} and subjected to denature immunoprecipitation (IP) with anti-Flag M2 agarose beads, followed by immunoblot with anti-phospho-MAPK substrates motif (S/T*P) antibody (pS/T*P) to detect IRE1α phosphorylation. **B.** Whole-cell lysates of 293T cells transfected with MEK^{DD} together with Flag-GFP or Flag-IRE1α plasmids were subjected to immunoprecipitation (IP) with anti-Flag M2 agarose beads, followed by immunoblot to detect interaction between phospho-ERK1/2 and IRE1 α. IB, immunoblot. **C.** Depletion of *NCK* had no impact on phospho-ERK1/2 levels and the interaction between IRE1α and ERK1/2 in MIA-PaCa-2 cells. MIA-PaCa-2 cells expressing Flag-IRE1α were infected with lentiviruses encoding *scramble* shRNA (sh*Scr*) or *NCK* shRNA (sh*NCK*). The whole cell lysates were subjected to immunoprecipitation (IP) with anti-Flag M2 agarose beads. VCL in whole-cell lysates served as loading control. **D.** Depletion of *NCK* had no impact on phospho-ERK1/2 levels and the interaction between IRE1α and MEK^{DD}-activated ERK1/2 in 293T cells. 293T cells expressing Flag-IRE1α and MEK^{DD} were infected with lentiviruses encoding *scramble* shRNA (sh*Scr*) or *NCK* shRNA (sh*NCK*). The whole cell lysates were subjected to immunoprecipitation (IP) with anti-Flag M2 agarose beads. VCL in whole-cell lysates served as loading control. **E.** GST pull-down assay was performed using recombinant His-tagged IRE1α protein purified from Sf9 cells and GST-ERK1 protein purified from *E. coli.* Immunoblot was performed to detect the interaction between GST-ERK1 and His-IRE1α. **F.** Flag pull-down assay using Flag-GFP or Flag-IRE1α purified from 293T cells and recombinant GST-ERK2 protein. **G.** *In vitro* kinase assay using Flag-GFP or Flag-IRE1α proteins purified from 293T cells and recombinant GST-ERK2 protein in the presence of ATP. Denature IP was performed to purify Flag-GFP or Flag-IRE1α, followed by immunoblot to detect phosphorylation using anti-phospho-MAPK substrates motif (S/T*P) antibody (p-S/T*P). IB, Immunoblot. **H.** Flag pull-down assay using Flag-GFP or Flag-IRE1α purified from 293T cells and recombinant GST-ERK1 protein. Immunoblot was performed to detect the interaction between GST-ERK1 and Flag-IRE1α. **I.** H358^{P} cells expressing Flag-IRE1α were infected with lentiviruses encoding *scramble* shRNA (sh*Scr*), *ERK1* shRNA (sh*ERK1*), *ERK2* shRNA (sh*ERK2*), or sh*ERK1* plus sh*ERK2*. The whole lysates were then subjected to denature IP with anti-Flag M2 agarose beads. The immunoblot was probed with anti-phospho-MAPK substrates motif (S/T*P) antibody (p-S/T*P) to detect IRE1α phosphorylation. MG132 (1 μM) was added into the culture medium 12h before harvest. **J.** Whole-cell lysates of 293T cells transfected with MEK^{DD} together with His-tagged WT or mutant IRE1α (with indicated D-motif deletion) were subjected to immunoprecipitation (IP) with Ni^{2+}-NTA agarose, followed by immunoblot to detect interaction between ERK1/2 and IRE1α.

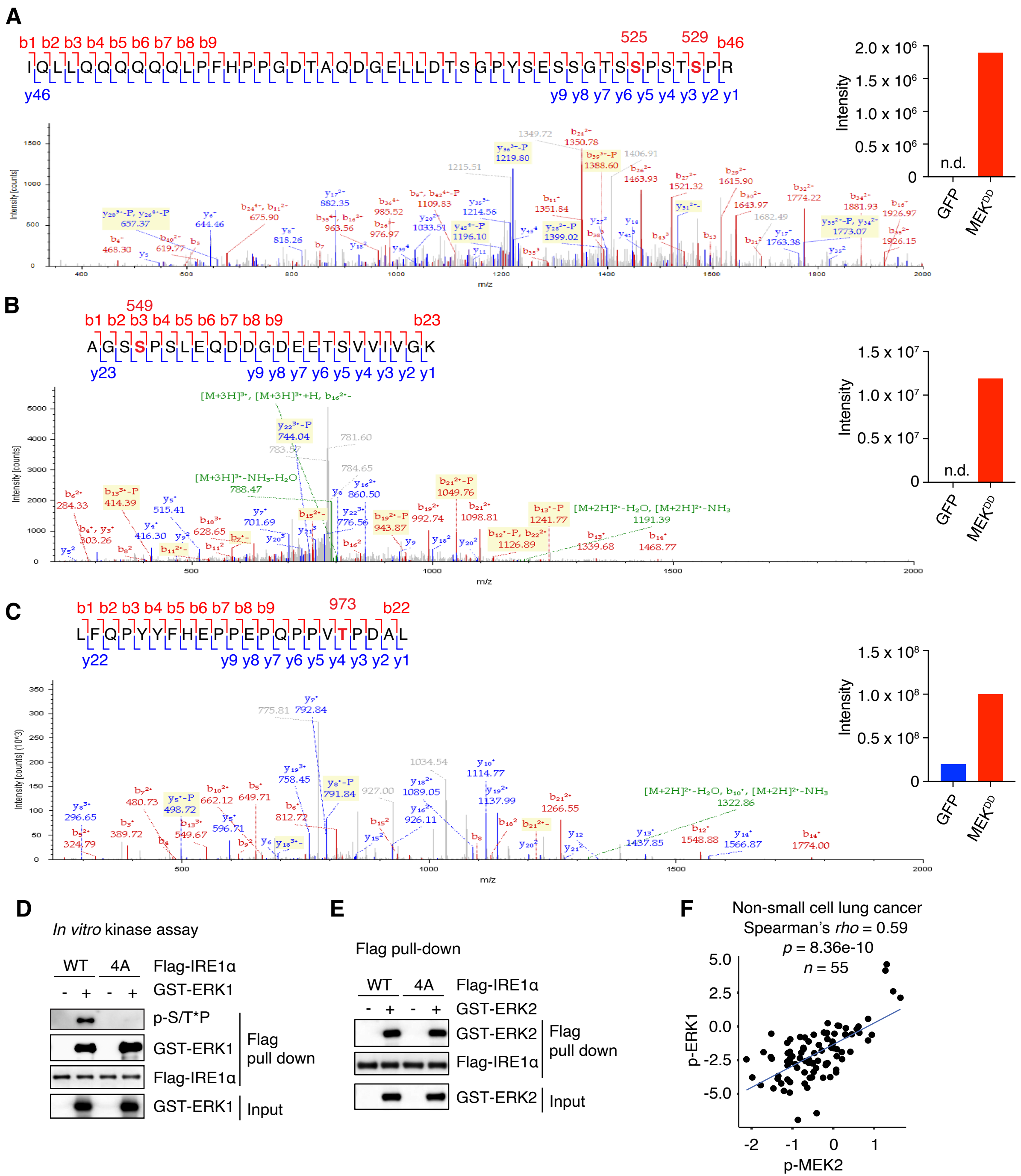

Figure S9. ERK phosphorylates IRE1α at S525, S529, S549 and T973

A-C. Liquid Chromatography with tandem mass spectrometry (LC-MS/MS) spectra and intensity of phosphor-peptides of IRE1α covering S525, 529 (**A**), S549 (**B**) and T973 (**C**) in 293T cells overexpressing GFP or MEK^{DD}. **D.** *In vitro* kinase assay using equal amount of Flag-tagged WT or phospho-deficient IRE1α mutant proteins (4A: S525A, S529A, S549A, T973A) purified from 293T cells and recombinant GST-ERK1 in the presence of ATP. Denature IP was performed to purify Flag-IRE1α, followed by immunoblot to detect IRE1α phosphorylation with anti-phospho-MAPK substrates motif (S/T*P) antibody (p-S/T*P). **E.** Flag pull-down assay using equal amount of Flag-tagged WT or phospho-deficient (4A) IRE1α mutant proteins purified from 293T cells and recombinant GST-ERK2 protein to detect interaction between IRE1α and ERK2. **F.** Spearman correlation between p-ERK1 and p-MEK2 in 55 patients with non-small cell lung cancer, Spearman *rho* = 0.59, $p = 8.36 \times 10^{-10}$, $n = 55$. Phosphoproteomics data are from PDC000149.

Figure S10. Biological function of the identified IRE1α phosphorylation sites

A. Schematic illustration showing the *in vitro* assay to detect the interaction between IRE1α and SEL1L/HRD1. **B.** Equal amount of unphosphorylated or *in vitro* phosphorylated Flag-IRE1α proteins by ERK2 were subjected to Flag-pull down assay with whole cell lysates of 293T cells expressing HA-HRD1 and Myc-SEL1L to detect the interaction between IRE1α and SEL1L/HRD1. Three independent experiments data shown. **C.** Equal amount of unphosphorylated or *in vitro* phosphorylated Flag-tagged WT or phospho-deficient IRE1α proteins (4A) purified from 293T cells were subjected to Flag-pull down assay with whole cell lysates of 293T cells expressing HA-tagged HRD1 to detect the interaction between IRE1α and HRD1. **D.** Quantification of the levels of HRD1 pulled down by IRE1α as shown in **B** and **C**. The intensities of HA-HRD1 pulled down by unphosphorylated or phosphorylated IRE1α were quantified using Image J and normalized against input. Data are presented as mean ± SD relative to HA-HRD1 pulled-down by unphosphorylated IRE1α, n=4. **E.** Quantification of the levels of SEL1L pulled down by IRE1α as shown in **B**. The intensities of Myc-SEL1L pulled down by unphosphorylated or phosphorylated IRE1α were quantified using Image J and normalized against input. Data are presented as mean ± SD relative to Myc-SEL1L pulled-down by unphosphorylated IRE1α, n=3. **F.** 293T cells expressing HA-HRD1 together with Flag-tagged WT or phospho-mimetic (SDTE) IRE1α in the presence or absence of MEK^{DD} were subjected to immunoprecipitation with anti-Flag M2 agarose beads to detect IRE1α interaction with HRD1. **G.** Immunoblot of Flag-IRE1α levels in MIA-PaCa-2^{P} cells expressing Flag-tagged WT or phospho-mimetic (SDTE) IRE1α and treated with DMSO, 30 nM sotorasib or 1 μM SCH772984 for 2 days. **H.** Quantification of PROTEOSTAT intensity in MIA-PaCa-2^{P} cells expressing WT or phospho-mimetic (SDTE) IRE1α and treated with DMSO or 30 nM sotorasib for 5 days. Data represent the average fluorescence intensity of PROTEOSTAT/cell from each image acquired (>10 cells per image) and are presented as mean ± SD from n=10 images. **I-J.** Representative images (**I**) and quantification (**J**) of PROTEOSTAT (magenta) and DAPI (blue) staining in *iKras*^{R} cells infected with lentiviruses encoding *scramble* shRNA (sh*Scr*) or *Ire1α* shRNA (sh*Ire1α*) together with shRNA-resistant WT IRE1α, loss-of-function IRE1α^{4A} mutant, or gain-of-function IRE1α^{SDTE} mutant. Data represent the average fluorescence intensity of PROTEOSTAT/cell from each image acquired (>10 cells per image) and presented as mean ± SD from n=5 images. Scale bar: 20μm. **K.** Immunoblot of phospho-PERK and total-PERK in whole-cell lysates of *iKras*^{R} cells infected with lentiviruses encoding *scramble* shRNA (sh*Scr*) or *Ire1α* shRNA (sh*Ire1α*) together with shRNA-resistant Flag-tagged WT or phospho-mimetic mutant IRE1α. β-ACTIN in whole-cell lysates served as loading control. **L.** Colony formation assay was used to quantify the growth of control (sh*Scr*) or *Ire1α*-knockdown (sh*Ire1α*) *iKras*^{R} cells expressing shRNA-resistant WT IRE1α or various phosphorylation sites-mutated IRE1α as indicated. Data are presented as mean ± SD relative to control (sh*Scr*) *iKras*^{R} cells, n=3. **M-N.** Representative images (**M**) and quantification (**N**) of PROTEOSTAT (magenta) and DAPI (blue) staining in *iKras*^{R} cells infected with lentiviruses encoding *scramble* shRNA (sh*Scr*) or *Ire1α* shRNA (sh*Ire1α*) together with shRNA-resistant Flag-tagged WT IRE1α or various phospho-deficient IRE1α mutants. Data represent the average fluorescence intensity of PROTEOSTAT/cell from each image acquired (>10 cells per image) and presented as mean ± SD from n=10 images. Scale bar: 20μm. **O.** Immunoblot of p-PERK, t-PERK and Flag-IRE1α in whole-cell lysates of *iKras*^{R} cells infected with lentiviruses encoding *scramble* shRNA (sh*Scr*) or *Ire1α* shRNA (sh*Ire1α*) together with shRNA-resistant Flag-tagged WT IRE1α or phospho-deficient IRE1α mutants as indicated. β-ACTIN in whole-cell lysates served as loading control. 2-tailed, unpaired Student's t test (**D** and **E**) or ordinary one-way ANOVA (**H, J, L** and **N**) was used to calculate P values. n.s., not significant, * P<0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001.

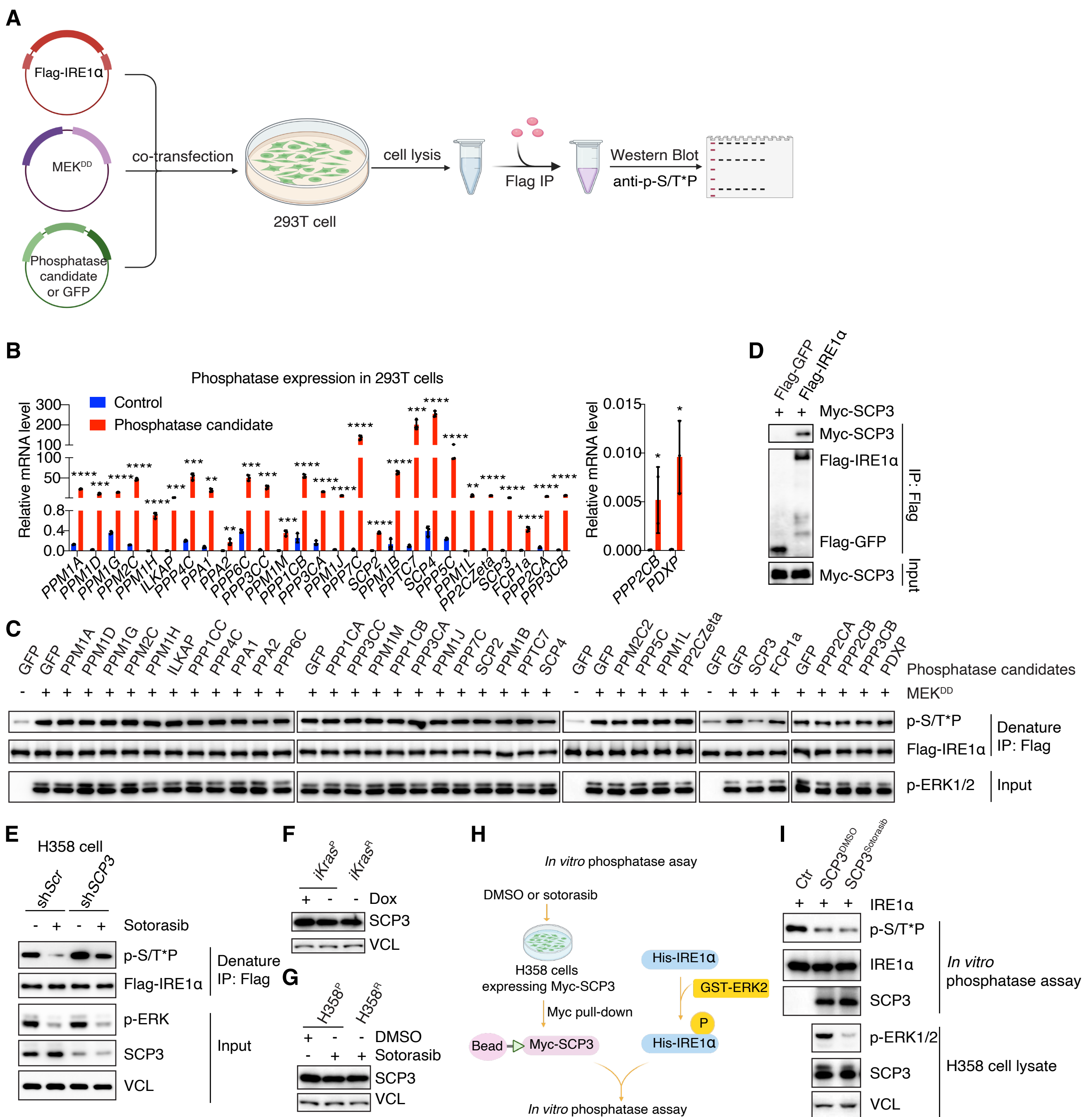

Figure S11. Identification of SCP3 as the phosphatase regulating IRE1α phosphorylation
A. Schematic illustration of the screen assay to identify phosphatase regulating ERK-mediated IRE1α phosphorylation. 293T cells expressing Flag-IRE1α were transfected with GFP or MEK^{DD} plus individual serine/threonine phosphatase candidate. The whole cell lysates were then subjected to denature immunoprecipitation (IP) with anti-Flag M2 agarose beads, followed by immunoblot with anti-phospho-MAPK substrates motif (S/T*P) antibody (pS/T*P) to detect IRE1α phosphorylation. **B.** RT-qPCR analysis individual serine/threonine phosphatase candidate expression as described in **A**. RNAs extracted from GFP-expressing 293T cells was used as control. Expression was normalized to *β-ACTIN*. Data are presented as mean ± SD, n=3. **C.** Impact of individual phosphatase candidates on ERK-mediated IRE1α phosphorylation. **D.** Whole-cell lysates of 293T cells transfected with Myc-SCP3 together with Flag-GFP or Flag-IRE1α plasmids were subjected to immunoprecipitation (IP) with anti-Flag M2 agarose beads, followed by immunoblot to detect interaction between Myc-SCP3 and IRE1α. **E.** Control (sh*Scr*) or SCP3-knockdown (sh*SCP3*) H358 cells expressing Flag-IRE1α were treated with DMSO or 30 nM sotorasib for 2 hours and subjected to denature IP with anti-Flag M2 agarose beads. The immunoblot was probed with anti-phospho-MAPK substrates motif (S/T*P) antibody (p-S/T*P) to detect IRE1α phosphorylation. **F.** Immunoblot of SCP3 in whole-cell lysates of *iKras*^{P} cells in the presence (+Dox) or absence (-Dox) of *Kras*^{G12D} for 2 days or in *iKras*^{R} cells cultured in the absence of Dox. VCL served as loading control. **G.** Immunoblot of SCP3 in whole-cell lysates of parental H358 (H358^{P}) cells treated with DMSO or 30 nM sotorasib for 2 days or in the sotorasib-resistant H358^{R} cells treated with 30nM sotorasib. VCL served as loading control. **H-I.** Schematic illustration (**H**) and results (**I**) of *in vitro* phosphatase assay using Myc-SCP3 purified from H358 cells treated with DMSO or sotorasib (30 nM) for 2 days. Recombinant IRE1α protein phosphorylated by recombinant ERK2 in vitro were subjected to in vitro phosphatase assay with equal amount of Myc-SCP3 purified from H358 cells treated with DMSO or sotorasib (30 nM) for 2 days, followed by immunoblot with anti-phospho-MAPK substrates motif (S/T*P) antibody (p-S/T*P) to detect IRE1α phosphorylation. 2-tailed, unpaired Student's t test (**B**) was used to calculate P values. * P<0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001.

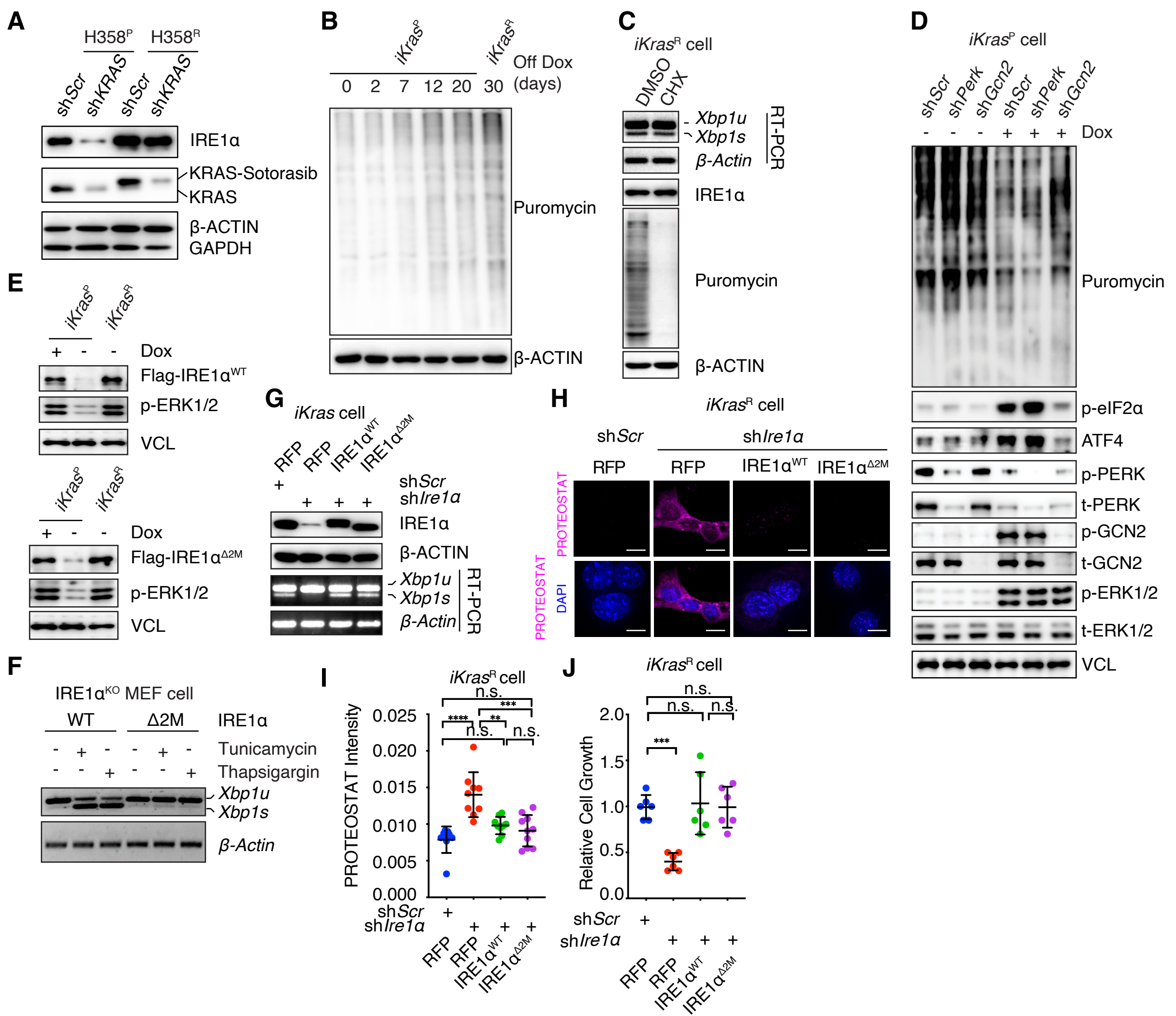

Figure S12. IRE1α is reactivated in an ER stress independent manner.
A. Immunoblot of IRE1α and KRAS in whole-cell lysates of $H358^{P}$ and $H358^{R}$ cells infected with lentiviruses encoding sh*SCR* or sh*KRAS*. β-ACTIN and GAPDH in whole-cell lysates served as loading control. **B.** Immunoblot of puromycin in whole-cell lysates of parental *iKras* cells ($iKras^{P}$) at different time points after $Kras^{G12D}$ inactivation by Dox-withdrawal (Off Dox) until the cells acquired resistance to $Kras^{G12D}$ inactivation ($iKras^{R}$ cell). Cells were treated with 2 μg/mL puromycin 15 min before collection. β-ACTIN in whole-cell lysates served as loading control. **C.** RT-PCR analysis of *XBP1* splicing and immunoblot of IRE1α and puromycin in whole-cell lysates of $iKras^{R}$ cells treated with cycloheximide (CHX, 5μg/ml). β-ACTIN in whole-cell lysates served as loading control. **D.** Immunoblot of phospho-PERK, total-PERK, phospho-GCN2, total-GCN2, phospho-ERK1/2, total-ERK1/2, phospho-eIF2α, ATF4, and puromycin in whole-cell lysates of $iKras^{P}$ cells infected with lentiviruses encoding *scramble* shRNA (sh*Scr*), *Perk* shRNA (sh*Perk*), or *Gcn2* shRNA (sh*Gcn2*). The $iKras^{P}$ cells were cultured in the absence of Dox before adding Dox for 2 days prior to western blot analysis. Cells were treated with 2 μg/mL puromycin 15 min before collection. VCL in whole-cell lysates served as loading control. **E.** Immunoblot of Flag-$IRE1\alpha^{WT}$ or Flag-$IRE1\alpha^{\Delta 2M}$ in whole-cell lysates of parental $iKras^{P}$ cells in the presence (+Dox) or absence (-Dox) of $Kras^{G12D}$ for 2 days or in $iKras^{R}$ cell cultured without doxycycline (Dox). Vinculin (VCL) in whole-cell lysates served as loading control. **F.** Validation of ER stress-sensing deficient $IRE1\alpha^{\Delta 2M}$ mutant. RT-PCR analysis of *Xbp1* splicing in *IRE1α* knock out MEF cells expressing $IRE1\alpha^{WT}$ or $IRE1\alpha^{\Delta 2M}$ (with aa 19-112 deleted) and treated with DMSO, tunicamycin (TM, 5 μ g/mL) or thapsigargin (TG, 0.5 μM) for 6 hours as indicated. β -ACTIN served as loading control. **G.** Examination of IRE1α protein levels and *Xbp1* splicing in control (sh*Scr*) *iKras* cells or *Ire1α*-knockdown *iKras* cells reconstituted with shRNA-resistant $IRE1\alpha^{WT}$ or $IRE1\alpha^{\Delta 2M}$. β-ACTIN served as loading control. **H-I.** Representative images (**H**) and quantification (**I**) of PROTEOSTAT (magenta) and DAPI (blue) staining in control (sh*Scr*) $iKras^{R}$ cells or *Ire1α*-knockdown $iKras^{R}$ cells reconstituted with shRNA-resistant $IRE1\alpha^{WT}$ or $IRE1\alpha^{\Delta 2M}$. Data represent the average fluorescence intensity of PROTEOSTAT/cell from each image acquired (>10 cells per image) and presented as mean ± SD from $n \geq 9$ images. Scale bar: 20μm. **J.** Colony formation assay was used to quantify the growth of control (sh*Scr*) $iKras^{R}$ cells or *Ire1α*-knockdown $iKras^{R}$ cells reconstituted with shRNA-resistant $IRE1\alpha^{WT}$ or $IRE1\alpha^{\Delta 2M}$. Data are presented as mean ± SD relative to scramble control, n=6. Ordinary one-way ANOVA (**I** and **J**) were used to calculate P values. n.s., not significant, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

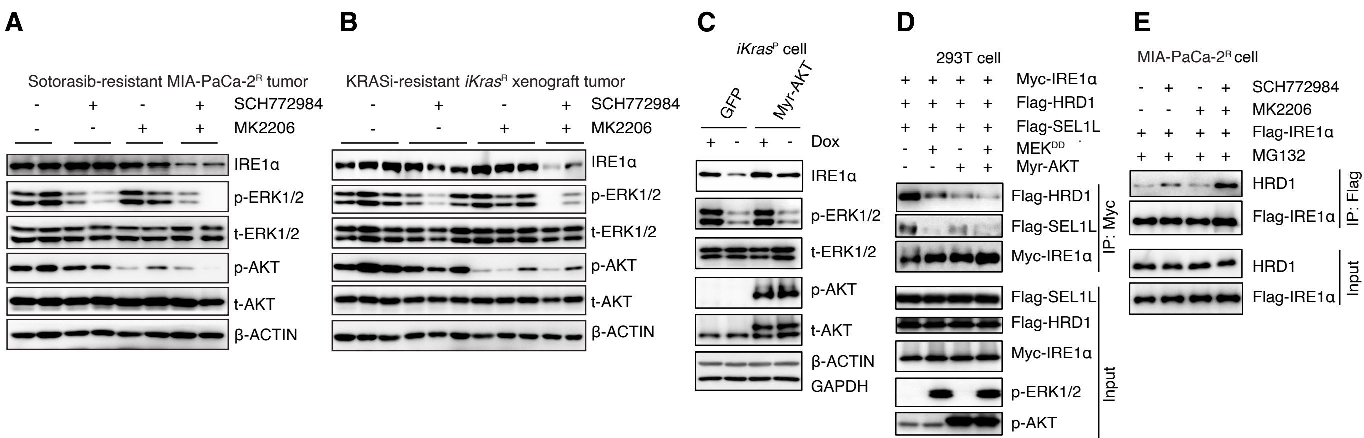

Figure S13. Multiple resistance mechanisms converge on IRE1α

A. Immunoblot with indicated antibodies in sotorasib-resistant MIA-PaCa-2^{R} tumors treated with SCH772984 (50mg/kg) and/or MK2206 (120mg/kg) for 4 days. β-ACTIN in whole-cell lysates served as loading control. **B.**Immunoblot with indicated antibodies in KRASi-resistant *iKras*^{R} xenograft tumors treated with SCH772984 (50mg/kg) and/or MK2206 (120mg/kg) for 9 days. β-ACTIN in whole-cell lysates served as loading control. **C.** Immunoblot of IRE1α in whole-cell lysates of *iKras*^{P} cells expressing GFP or constitutively activated AKT (myr-AKT) in the presence or absence of Doxycycline (Dox) for 2 days. **D.** 293T cells expressing Myc-IRE1α, Flag-HRD1, and Flag-SEL1L in the presence or absence of MEK^{DD} or myr-AKT were subjected to immunoprecipitation with anti-Myc agarose beads followed by immunoblot to detect the interaction between IRE1α and SEL1L or HRD1. **E.** MIA-PaCa-2^{R} cells expressing Flag-IRE1α were treated with DMSO, 2μM MK2206, and/or 1 μM SCH772984 for 2 days and subjected to immunoprecipitation with anti-Flag M2 agarose beads followed by immunoblot to detect the interaction between IRE1α and HRD1. Cells were treated with 3μM MG132 for 12h before harvest.

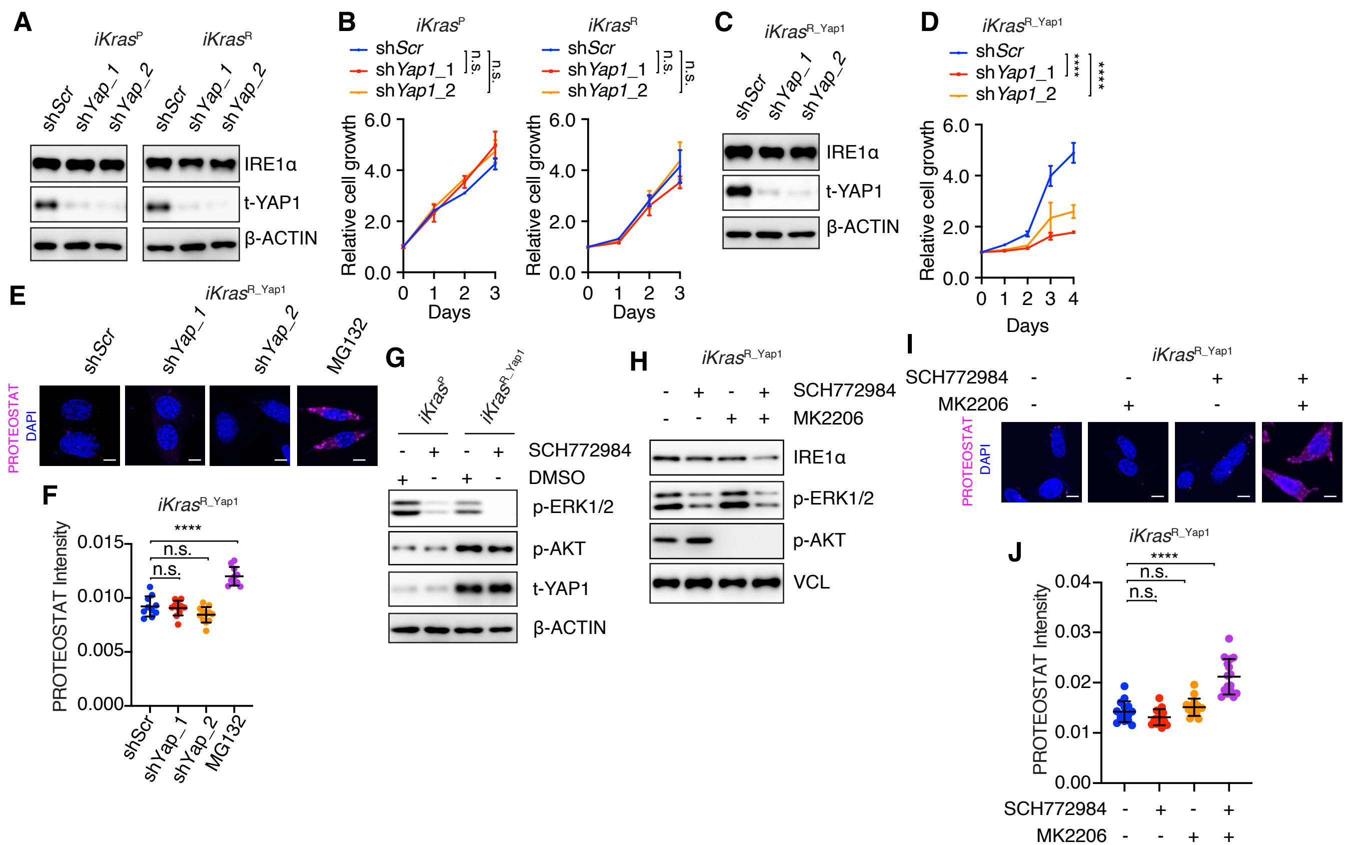

Figure S14. YAP1 is not involved in proteostasis in *iKras* model.

A. Immunoblot of IRE1α and YAP1 in whole-cell lysates of *iKras*[P] or *iKras*[R] cells infected with lentiviruses encoding *scramble* shRNA or *Yap1* shRNA. **B.** Quantification of cell growth of *iKras*[P] or *iKras*[R] cells infected with lentiviruses encoding *scramble* shRNA or *Yap1* shRNA for indicated time points using CCK-8 assay. Data are normalized to the OD450 at day 0 and presented as mean ± SD, *n*=3. **C.** Immunoblot of IRE1α and YAP1 in whole-cell lysates of control or *Yap-1* knockdown *iKras*[R_YAP1] cells derived from *iKras* GEMM tumor relapsed after Dox withdrawal. Cells were cultured in the absence of Dox. **D.** Quantification of cell growth of *iKras*[R_YAP1] cells infected with lentiviruses encoding *scramble* or *Yap1* shRNA for indicated time using CCK-8 assay. Data were normalized to the OD450 at day 0 and presented as mean ± SD, *n*=3. **E-F.** Representative images (**E**) and quantification (**F**) of PROTEOSTAT and DAPI staining in control or *Yap-1* knockdown *iKras*[R_YAP1] cells. Cells treated with 5μM MG132 for 16h were used as positive control. Data represent the average fluorescence intensity of PROTEOSTAT/cell from each image acquired and presented as mean ± SD from *n*=10 images. Scale bar: 20μm. **G.** Immunoblots in whole-cell lysates of *iKras*[P] (cultured with Dox) or *iKras*[R_YAP1] cells (cultured without Dox) treated with DMSO or 1μM SCH772984 for 2 days as indicated. **H.** Immunoblots in whole-cell lysates of *iKras*[R_YAP1] cells cultured without Dox and treated with DMSO, 1μM SCH772984, and/or 2μM MK2206 for 2 days as indicated. **I-J.** Representative images (**I**) and quantification (**J**) of PROTEOSTAT and DAPI staining in *iKras*[R_YAP1] cells treated as in (G). Data represent the average fluorescence intensity of PROTEOSTAT/cell from each image acquired and presented as mean ± SD from *n*=15 images. Scale bar: 20μm. All *iKras*[R_YAP1] cells were cultured in the absence of Dox. Ordinary one-way ANOVA with Dunnett's multiple comparisons test (**F** and **J**) or two-way ANOVA with Bonferroni's multiple comparisons test (**B** and **D**) was used to calculate *P* values. n.s., not significant, * P<0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001.

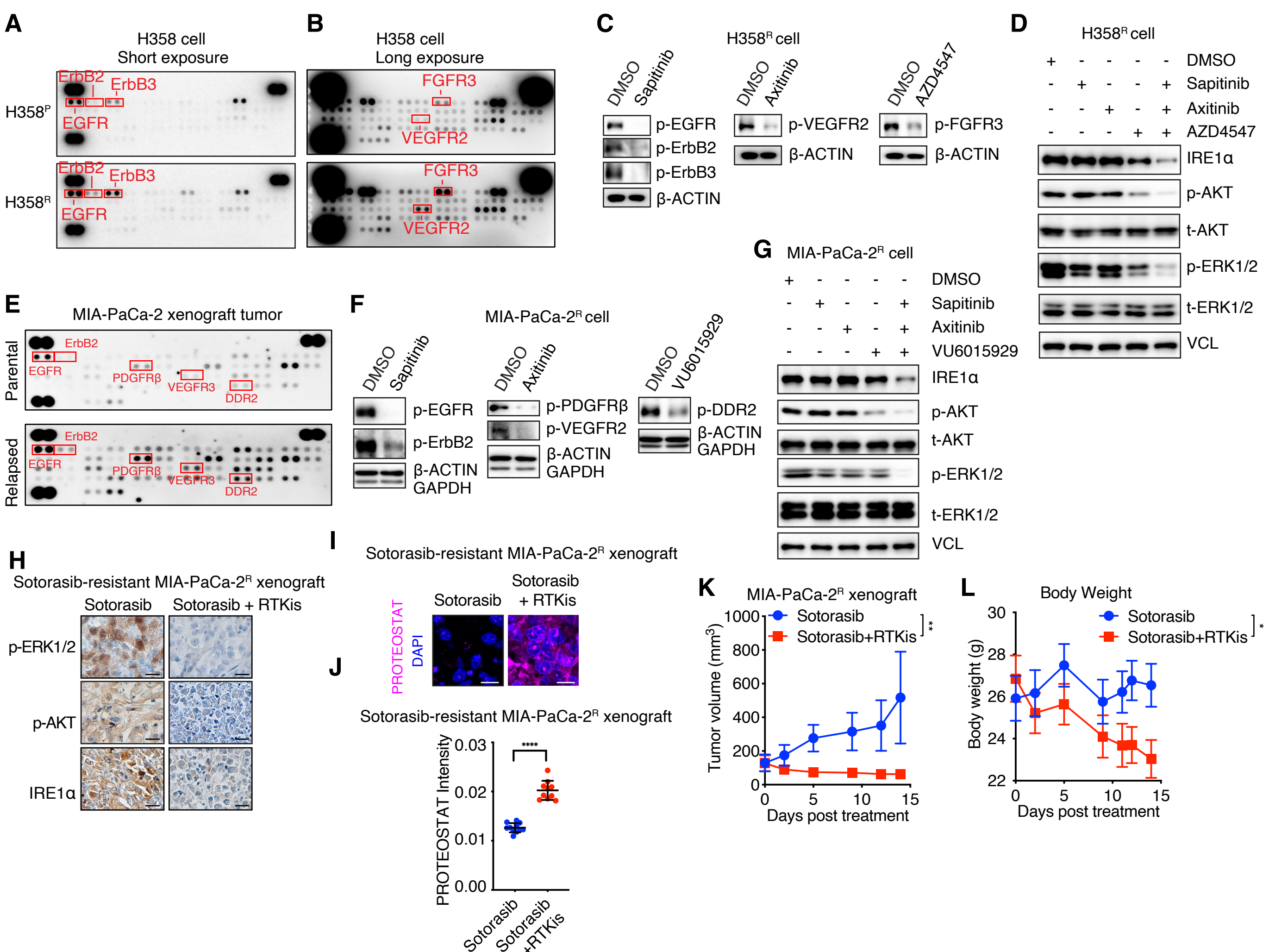

Figure S15. RTKs drives ERK and AKT activation in sotorasib-resistant tumors.
A-B. Phospho-RTK array of whole-cell lysates from parental H358 cells (H358[P]) or sotorasib-resistant H358 cells (H358[R]) treated with 30nM sotorasib. Short (**A**) and long (**B**) exposure images are shown. **C.** Immunoblot of phospho-EGFR (p-EGFR), phospho-ErbB2 (p-ErbB2), phospho- ErbB3 (p-ErbB3), phospho-VEGFR2 (p-VEGFR), and phospho-FGFR3 (p-FGFR) in whole-cell lysates of H358[R] cells treated with sapitinib (pan-EGFR inhibitor, 1 nM), axitinib (VEGFR inhibitor, 0.3 µM), or AZD4547 (FGFR inhibitor, 1 µM) for 2 days. β-ACTIN in whole-cell lysates served as loading control. **D.** Immunoblot of IRE1α, total ERK1/2 (t-ERK1/2), phospho-ERK1/2 (p-ERK1/2), total AKT (t-AKT), and phospho-AKT (p-AKT) in whole-cell lysates of H358[R] cells treated with DMSO, sapitinib (pan-EGFR inhibitor, 1 nM), axitinib (VEGFR inhibitor, 0.3 µM), AZD4547 (FGFR inhibitor, 1 µM) or combination of three inhibitors for 2 days. VCL in whole-cell lysates served as loading control. **E.** Phospho-RTK array of parental MIA-PaCa-2 xenograft tumors or sotorasib-resistant MIA-PaCa-2[R] xenograft tumors relapsed after 9 weeks of 100mg/kg sotorasib treatment. **F.** Immunoblot of phospho-EGFR (p-EGFR), phospho-ErbB2 (p-ErbB2), phospho-PDGFRβ (p- PDGFRβ), phospho-VEGFR2 (p-VEGFR), and phospho-DDR2 (p-DDR2) in whole-cell lysates of MIA-PaCa-2[R] cells treated with DMSO, sapitinib (pan-EGFR inhibitor, 1 nM), axitinib (VEGFR inhibitor, 0.3 µM), or VU6015929 (DDR inhibitor, 1 µM) for 2 days. β-ACTIN and GAPDH in whole-cell lysates served as loading control. **G.** Immunoblot of IRE1α, total ERK1/2 (t-ERK1/2), phospho-ERK1/2 (p-ERK1/2), total AKT (t-AKT), and phospho-AKT (p-AKT) in whole-cell lysates of MIA-PaCa-2[R] cells treated with DMSO, sapitinib (pan-EGFR inhibitor, 1 nM), axitinib (VEGFR inhibitor, 0.3 µM), VU6015929 (DDR inhibitor, 1 µM) or combination of three inhibitors for 2 days. Vinculin (VCL) in whole-cell lysates served as loading control. **H.** Immunohistochemical staining of p-ERK1/2, IRE1α and p-AKT in sotorasib-resistant MIA-PaCa-2[R] xenograft tumors treated with sotorasib (100mg/kg) alone, or sotorasib (100mg/kg) combined with multiple RTK inhibitors (sapitinib, 20mg/kg, axitinib, 50mg/kg, and VU6015929, 3mg/kg) for 3 days. Scale bar: 40µm. **I-J.** Representative images (**I**) and quantification (**J**) of PROTEOSTAT (magenta) and DAPI (blue) staining in sotorasib-resistant MIA-PaCa-2[R] xenograft tumors treated with sotorasib (100mg/kg), or sotorasib (100mg/kg) plus multiple RTK inhibitors (sapitinib, 20mg/kg, axitinib, 50mg/kg, and VU6015929, 3mg/kg) for 3 days. Data represent average fluorescence intensity of PROTEOSTAT/cell from each image acquired and are presented as mean ± SD from *n*=12 images. Scale bar: 20µm. **K.** Tumor volume quantification of established sotorasib-resistant MIA-PaCa-2[R] tumors treated with sotorasib (100mg/kg, *n*=5), or sotorasib (100mg/kg) plus multiple RTK inhibitors (sapitinib, 20mg/kg, axitinib, 50mg/kg, and VU6015929, 3mg/kg, *n*=6). **L.** Body weight of sotorasib-resistant MIA-PaCa-2[R] tumor-bearing SCID/beige mice treated with sotorasib (100mg/kg, *n*=5), or sotorasib (100mg/kg) plus multiple RTK inhibitors (sapitinib, 20mg/kg, axitinib, 50mg/kg, and VU6015929, 3mg/kg, *n*=6). Student's t test (**J**) or Two-way ANOVA with Bonferroni's multiple comparisons test (**K** and **L**) was used to calculate *P* values. n.s., not significant, * $P<0.05$, ** $P<0.01$, **** $P<0.0001$.

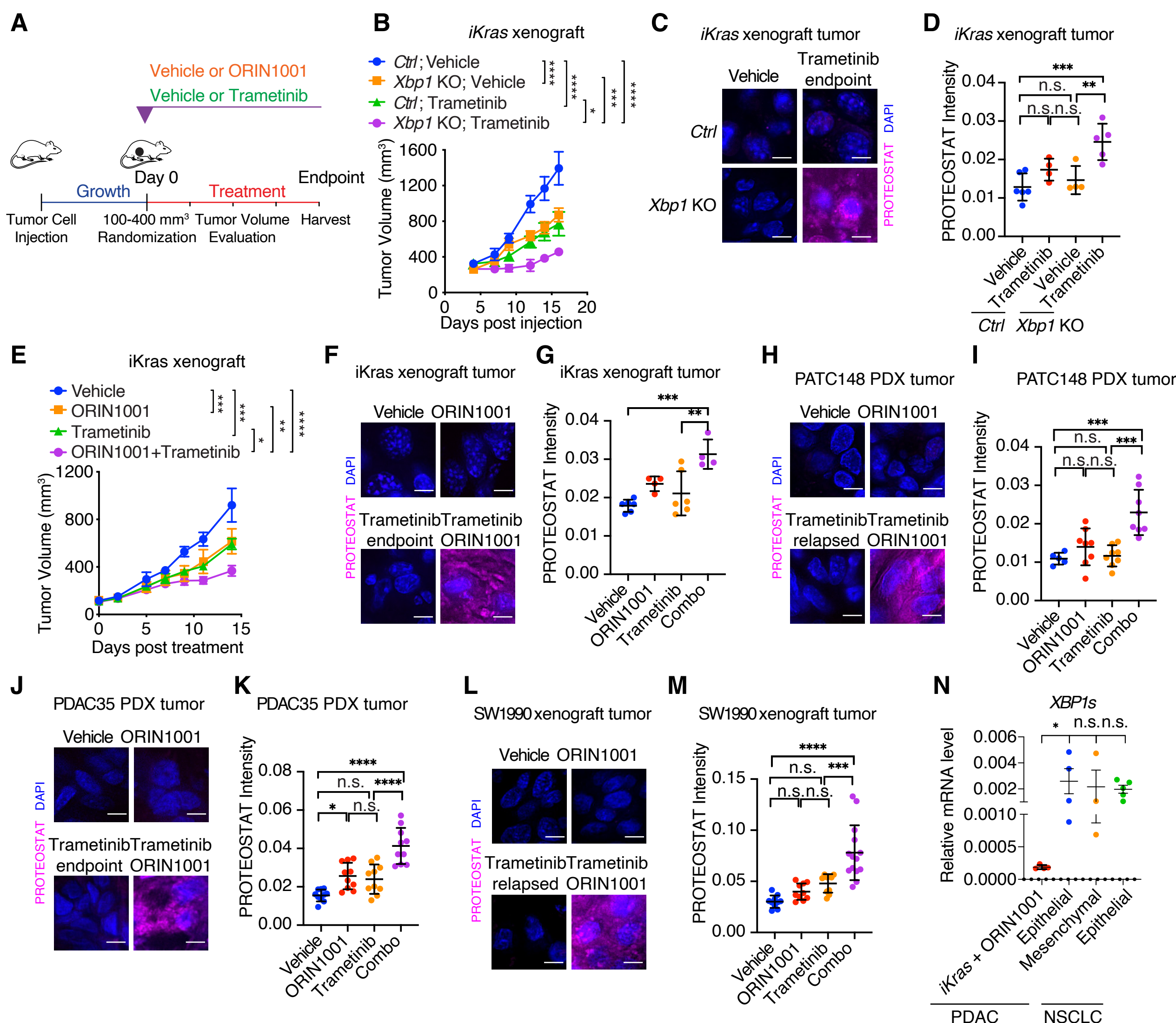

Figure S16. IRE1α inhibition sensitize KRAS-mutant tumors to MEK inhibitor

A. Schematic showing treatment strategy with IRE1α RNase inhibitor ORIN1001 and/or MEK inhibitor trametinib. **B.** Tumor volume quantification of established *scramble* control (*Ctrl*) or *Xbp1* knock-out (*Xbp1* KO) *iKras* xenograft tumors treated with vehicle or 1mg/kg trametinib (*n*=6/group) starting from day 4 post injection. **C-D.** Representative images (**C**) and quantification (**D**) of PROTEOSTAT (magenta) and DAPI (blue) staining in endpoint *scramble* control (*Ctrl*) or *Xbp1* knock-out (*Xbp1* KO) *iKras* xenograft tumors treated with vehicle or MEK inhibitor trametinib (1mg/kg). Data represent average fluorescence intensity of PROTEOSTAT/cell from each tumor acquired and are presented as mean ± SD from *n*=4 (*Ctrl* with trametinib, and *Xbp1* KO with vehicle) or 6 (*Ctrl* with vehicle, and *Xbp1* KO with trametinib) tumors. Scale bar: 20μm. **E.** Tumor volume quantification of established *iKras* xenograft tumors in SCID/beige mice treated with vehicle (*n*=5), ORIN1001 (150 mg/kg, *n*=6), trametinib (1 mg/kg, *n*=5), or ORIN1001 plus trametinib (*n*=5). **F-G.** Representative images (**F**) and quantification (**G**) of PROTEOSTAT (magenta) and DAPI (blue) staining in endpoint *iKras* xenograft tumors treated with vehicle, IRE1α RNase inhibitor ORIN1001 (150mg/kg), MEK inhibitor trametinib (1mg/kg), or ORIN1001 plus trametinib. Data represent average fluorescence intensity of PROTEOSTAT/cell from each tumor acquired and are presented as mean ± SD from *n*=4 (ORIN1001 and combo) or *n*=6 (vehicle and trametinib) tumors. Scale bar: 20μm. **H-I.** Representative images (**H**) and quantification (**I**) of PROTEOSTAT (magenta) and DAPI (blue) staining in endpoint PATC148 xenograft tumors treated with vehicle, IRE1α RNase inhibitor ORIN1001 (150mg/kg), MEK inhibitor trametinib (1mg/kg), or ORIN1001 plus trametinib. Data represent average fluorescence intensity of PROTEOSTAT/cell from each tumor acquired and are presented as mean ± SD from *n*=5 (vehicle) or 8 (ORIN1001, trametinib and combo) tumors. Scale bar: 20μm. **J-K.** Representative images (**J**) and quantification (**K**) of PROTEOSTAT (magenta) and DAPI (blue) staining in endpoint PDAC35 xenograft tumors treated with vehicle, IRE1α RNase inhibitor ORIN1001 (150mg/kg), MEK inhibitor trametinib (1mg/kg), or ORIN1001 plus trametinib. Data represent average fluorescence intensity of PROTEOSTAT/cell from each image acquired and are presented as mean ± SD from *n*=10 independent images. Scale bar: 20μm. **L-M.** Representative images (**L**) and quantification (**M**) of PROTEOSTAT (magenta) and DAPI (blue) staining in endpoint SW1990 xenograft tumors treated as in (**Fig 6H**). Data represent average fluorescence intensity of PROTEOSTAT/cell from each image acquired and are presented as mean ± SD from *n*=10 independent images. Scale bar: 20μm. **N.** RT-qPCR analysis of *XBP1* splicing in epithelial or mesenchymal-like KRAS-driven PDAC and NSCLC preclinical models. Epithelial PDAC models: *iKras*, PATC53, SW1990 and PDAC19. The *iKras* model (epithelial PDAC model) treated with ORIN1001 served as negative control. Mesenchymal PDAC models: MIA-PaCa-2, PATC148, and PDAC35. Epithelial NSCLC models: J000096652, TM00186, TC303AR, J000093018, and TM00192. Data were presented as mean ± SEM (**B** and **E**) or mean ± SD (**D, G, I, K, M** and **N**). Two-way ANOVA (**B** and **E**) and Ordinary one-way ANOVA (**D, G, I, K, M** and **N**) were used to calculate *P* values. n.s., not significant, * P<0.05, ** P< 0.01, *** P< 0.001, **** P < 0.0001.

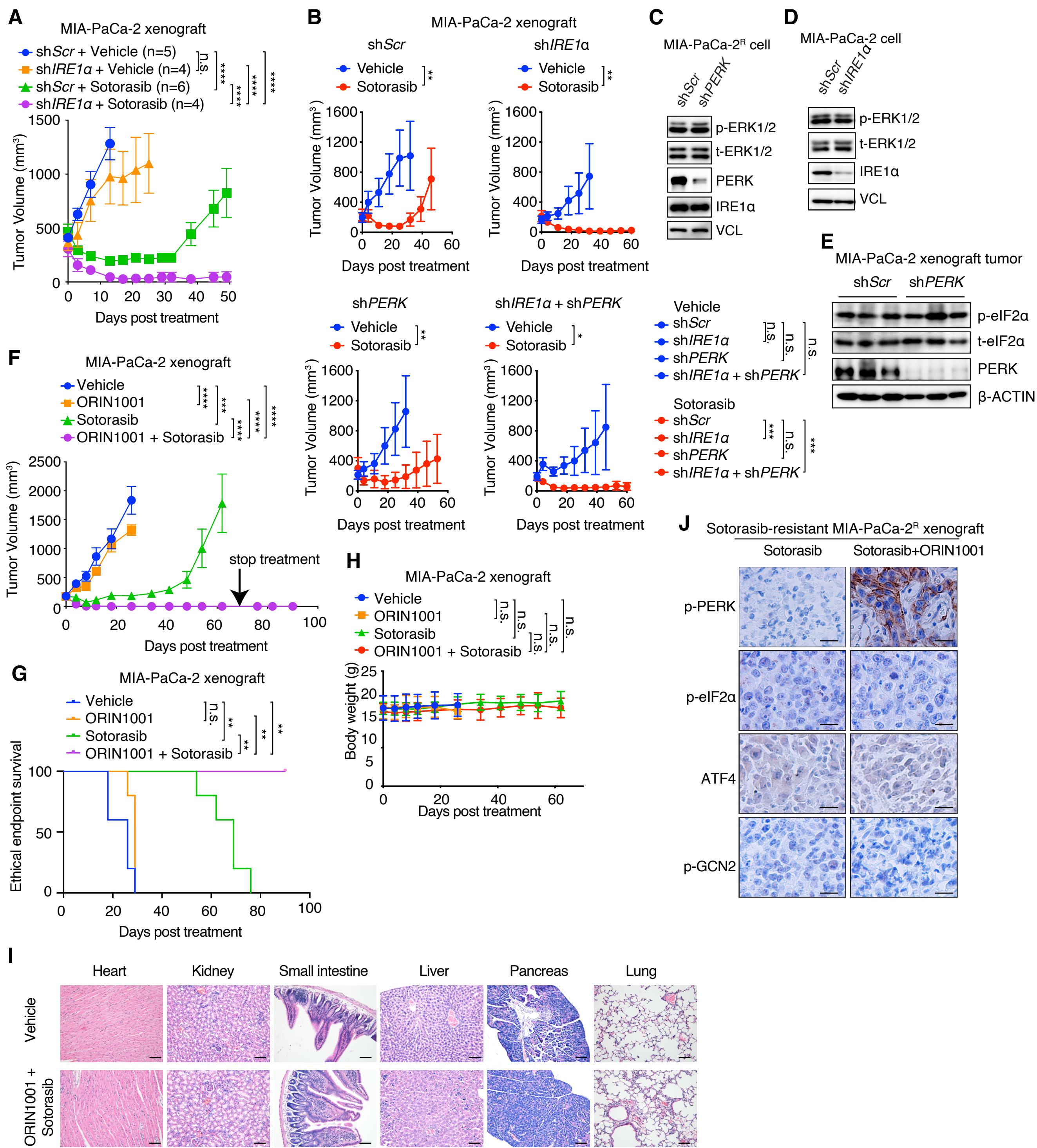

Figure S17. IRE1α inhibition sensitizes *KRAS*^{G12C}-mutant tumors to KRAS inhibitor.
A. Tumor volume quantification of established MIA-PaCa-2 tumors infected with lentiviruses encoding doxycycline-inducible sh*SCR* or sh*IRE1α* and treated with vehicle or sotorasib (30mg/kg). When tumor volume reached 350-500 mm³, tumor-bearing mice were randomized and treated with 2g/L of doxycycline in drinking water (to induce expression of shRNA) and vehicle or sotorasib. **B.** Tumor volume quantification of established MIA-PaCa-2 tumors expressing doxycycline-inducible sh*Scr*, sh*IRE1α*, sh*PERK* or sh*IRE1α* plus sh*PERK*. When tumor volume reached 200 mm³, tumor-bearing mice were randomized and treated with 2g/L of doxycycline in drinking water (to induce expression of shRNA) and vehicle or sotorasib (100 mg/kg). shScr and shIRE1 data in (**B**) and the data in **Fig.7A** are from the same experiments. **C.** Immunoblot of IRE1α, phospho-ERK1/2, total-ERK1/2 and PERK in whole-cell lysates of MIA-PaCa-2R cells infected with lentiviruses encoding *scramble* shRNA (sh*Scr*) or *PERK* shRNA (sh*PERK*). VCL in whole-cell lysates served as loading control. **D.** Immunoblot of IRE1α, phospho-ERK1/2 and total-ERK1/2 in whole-cell lysates MIA-PaCa-2R cells infected with lentiviruses encoding *scramble* shRNA (sh*Scr*) or *IRE1α* shRNA (sh*IRE1α*). VCL in whole-cell lysates served as loading control. **E.** Immunoblot of t-PERK, phospho-eIF2α and total-eIF2α in MIA-PaCa-2 tumors expressing doxycycline-inducible sh*Scr* or sh*PERK*. β-ACTIN served as loading control. **F.** Tumor volume quantification of established MIA-PaCa-2 tumors in SCID/beige mice treated with vehicle (*n*=4), ORIN1001 (300mg/kg, *n*=5), sotorasib (50mg/kg, *n*=5), or ORIN1001 plus sotorasib (*n*=5). Treatment was stopped at day 64. **G.** Kaplan-Meier survival curve of MIA-PaCa-2 tumor-bearing mice under different treatments as indicated in (**F**) from treatment start time. Treatment was stopped at day 64. **H.** Body weight of MIA-PaCa-2 tumor-bearing SCID/beige mice treated with vehicle (*n*=4), ORIN1001 (300mg/kg, *n*=5), sotorasib (50mg/kg, *n*=5), or ORIN1001 plus sotorasib (*n*=5) as indicated in **F** and **G**. **I.** H&E staining of heart, kidney, small intestine, liver, pancreas and lung of MIA-PaCa-2 tumor-bearing SCID/Beige mice treated as in **F**. Scale bar: 80µm. **J.** Immunohistochemical staining of phospho-PERK, phospho-eIF2α, ATF4 or phospho-GCN2 in sotorasib-resistant MIA-PaCa-2R xenograft tumors treated with sotorasib (100 mg/kg) or sotorasib (100 mg/kg) plus ORIN1001 (300 mg/kg) for 4 days. Scale bar: 40µm. Data are presented as mean ± SEM (**A, B** and **F**) or mean ± SD (**H**). Two-way ANOVA (**A, B, F** and **H**) or log-rank (Mantel-Cox) test (**G**) was used to calculate *P* values. n.s., not significant, * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$.

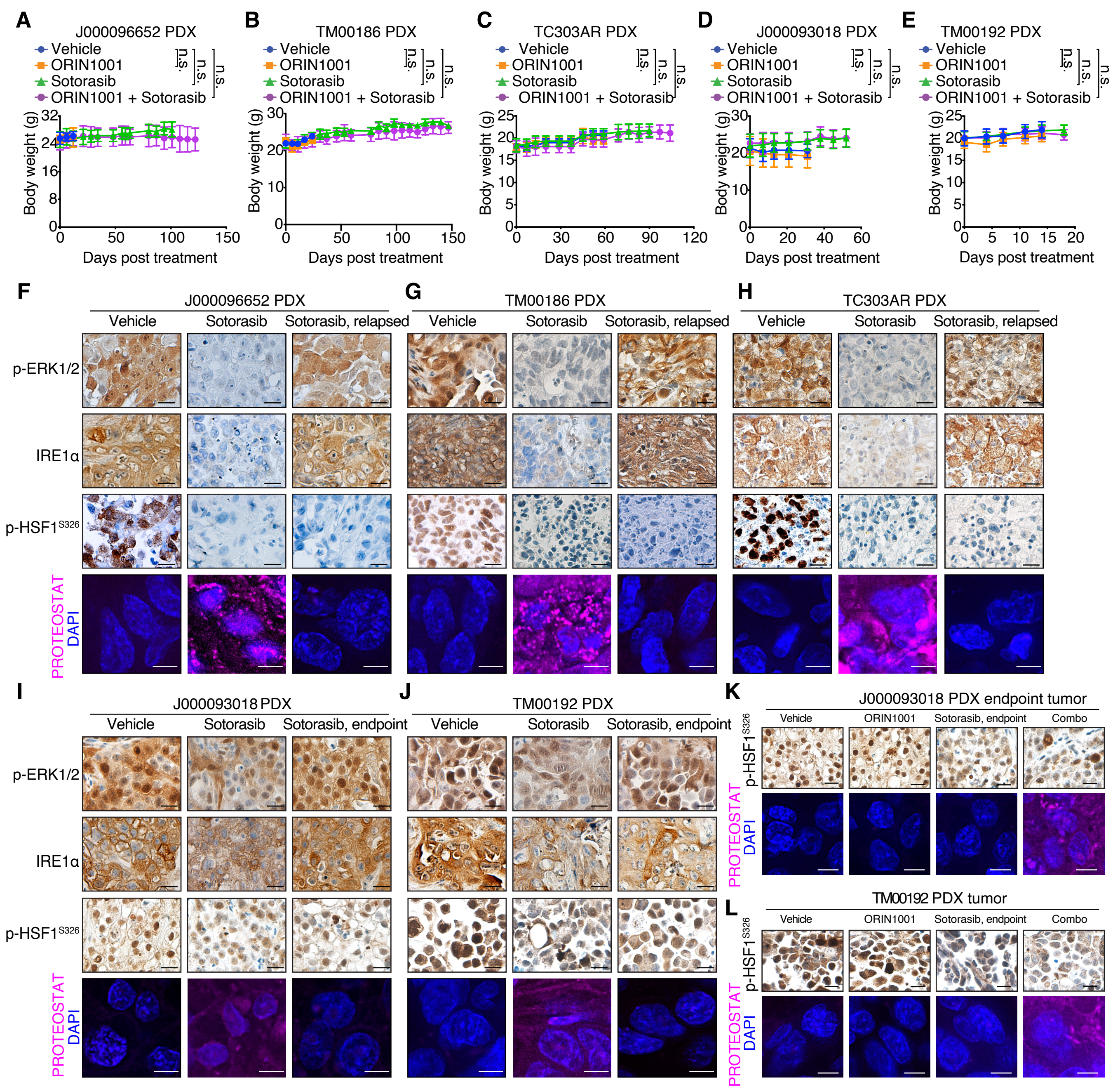

Figure S18. Proteostasis reprogramming upon KRAS inhibition in NSCLC PDX models.
A-E. Body weight of PDX tumor-bearing NSG mice treated with vehicle, ORIN1001, sotorasib, or ORIN1001 plus sotorasib as shown in **Figure 8, C-G**. **F-J. Upper panels:** Immunohistochemical staining of p-ERK1/2, IRE1α or p-HSF1 (Ser326) in established J000096652 (**F**), TM00186 (**G**), TC303AR (**H**), J000093018 (**I**), or TM00192 (**J**) PDX tumors treated with vehicle or sotorasib for 2 days, or until endpoint. Scale bar: 40µm. **Lower panels:** Representative images of PROTEOSTAT (magenta) and DAPI (blue) staining in PDX tumors treated as described above. Scale bar: 20µm. **K-L.** Immunohistochemical staining of p-HSF1 (Ser 326) (**Upper panels**) or representative images of PROTEOSTAT (magenta) and DAPI (blue) staining (**Lower panels**) in endpoint J000093018 (**K**) and TM00192 (**L**) PDX tumors treated with vehicle, IRE1α RNase inhibitor ORIN1001, sotorasib, or ORIN1001 plus sotorasib. Scale bar: 20µm. Data are presented as mean ± SD (**A** to **E**). Two-way ANOVA test with Bonferroni's multiple comparisons test was used to calculate *P* values. n.s., not significant.

Supplementary Table 1. Cell lines used in this study

Cell line	Species	Catalog	Identifier (RRID)	Supplier	Reference
MIA-PaCa-2	*Homo sapiens*	CRM-CRL-1420	CVCL_0428	ATCC	
SW1990	*Homo sapiens*	CRL-2172	CVCL_1723	ATCC	
293T	*Homo sapiens*	CRL-3216	CVCL_0063	ATCC	
H358	*Homo sapiens*	CRL-5807	CVCL_1559	ATCC	
BEAS-2B	*Homo sapiens*	CRL-9609	CVCL_0168	ATCC	
PATC53	*Homo sapiens*		CVCL_VR69	Dr. Michael Kim at The University of Texas MD Anderson Cancer Center	90
PATC148	*Homo sapiens*			Dr. Michael Kim at The University of Texas MD Anderson Cancer Center	90
iKras	*mus musculus*			Derived from GEMM (*tetO_LSL-Kras* ^{G12D} */p53* $^{flox/+}$ */p48-Cre/ROSA26-LSL-rtTA-IRES-GFP*)	55
LSL-Kras ^{G12D}	*mus musculus*			Derived from GEMM (*LSL-Kras* ^{G12D} */p53* $^{flox/+}$ */p48-Cre*)	55
iKras ^{R_YAP1}	*mus musculus*			Derived from GEMM tumor relapsed in the absence of Dox (*tetO_LSL-Kras* ^{G12D} */p53* $^{flox/+}$ */p48-Cr*	37

Supplementary Table 2. Reagents and Kits used in this study

1. Reagents

Name	Catalog	Supplier
DMEM W/GLN GLU PYR	45000-306	VWR/Corning
RPMI 1640 WITH L-GLU	45000-398	VWR/Corning
Gibco Fetal Bovine Serum (FBS)	10437028	Life Technology
Charcoal stripped FBS	97065-304	VWR
Penicillin and streptomycin	15140163	Life Technologies
TrypLE™ Express Enzyme (1X), no phenol red	12604021	Life Technologies
Puromycin	P8833	MilliporeSigma
Doxycycline	AAJ60579-22	VWR
Blasticidin S HCl solution	3513-03-09	Santa Cruz
Lipofectamine 3000	L300008	Thermo Fisher
Polybrene	TR1003G	Sigma
Power SYBR Green PCR Master Mix	4367659	Thermo Fisher
DAPI (4',6-Diamidino-2-Phenylindole, Dihydrochloride)	D1306	Thermo Fisher
TRIzol™ LS Reagent	10296028	Thermo Fisher
MKC8866 (ORIN1001)	HY-104040	MedChemExpress
Sotorasib	AMG510	Chemgood
Trametinib	HY-10999	MedChemExpress
SCH772984	HY-50846	MedChemExpress
MK2206	HY-10358	MedChemExpress
Pictilisib	S1065	Selleckchem
MG132	HY-13259	MedChemExpress
Tunicamycin	76102-666	VWR
Tauroursodeoxycholic Acid, Sodium Salt (TUDCA)	580549	Millipore
Agarose, Ultra-low Gelling Temperature	A5030	Sigma
Congo Red	234610	Sigma
Thioflavin T	T3516	Sigma
Crystal Violet	97061-850	VWR
Methanol	179337	Sigma
EDTA	45001-122	VWR/CORNING
Triton X-100	T8787	Sigma
NP-40	97064-918	VWR
Paraformaldehyde	P6148	Sigma
Hydrogen peroxide 30% stabilized ACS	BDH7690-1	VWR
Goat Serum	G9023	Sigma
Hematoxylin solution (Mayer)	100504-658	VWR
Tris-HCl pH 6.8	NP21-112	SignalChem
Tris-HCl pH7.5	15567027	Life Technologies
Sodium chloride solution	S5150	Sigma
Sodium deoxycholate	30970	Sigma
Sodium dodecyl sulfate (SDS)	L3771	Sigma
N-Ethylmaleimide	E3876	Sigma
Urea	9510	Sigma
DTT	646563	Sigma
Glycerol	G5516	Sigma
β-mercaptoethanol	M3148	Sigma
Bromophenol blue	114391	Sigma
EZview™ Red ANTI-FLAG® M2 Affinity Gel	F-2426	Sigma
EZview™ Red Anti-c-Myc Affinity Gel	E-6654	Sigma
GSH-Sepharose	17075601	GE Healthcare
Ni-NTA Agarose	30210	QIAGEN

GST-ERK2	M28-10G-20	SignalChem
Kinase assay buffer I	K01-09	SignalChem
ATP, [γ-32P]	NEG002A100UC	PerkinElmer
cOmplete™ Protease Inhibitor Cocktail	14826500	Roche
PhosSTOP	4906845001	Sigma
One-Step Blue Protein Gel Stain	21003	Biotium
SHP099	HY-100388	MedChemExpress
Gefitinib	HY-50895	MedChemExpress
Osimertinib	HY-15772	MedChemExpress
CB-5083	HY-12861	MedChemExpress
GST-ERK1	M29-10G-10	Signalchem
Sapitinib	HY-13050	MedChemExpress
Axitinib	HY-10065	MedChemExpress
VU6015929	HY-135401	MedChemExpress
AZD4547	HY-13330	MedChemExpress

2. Kits

Name	**Catalog**	**Supplier**
PROTEOSTAT® Aggresome detection kit	ENZ-510350K100	Enzo Life Sciences
FITC BrdU Flow Kit	559619	BD Bioscnences
Dual-Luciferase® Reporter Assay System	E1910	Promega
Proteasome Activity Fluorometric Assay Kit	K245	BioVision
Q5® Site-Directed Mutagenesis Kit	E0554	New England Biolabs
Cell Counting Kit-8 (CCK-8)	K1018	APExBIO
Pierce™ BCA Protein Assay Kit	23225	Thermo Fisher Scientific
High-Capacity cDNA Reverse Transcription Kit	4368813	Thermo Fisher Scientific
ImmPRESS Excel HRP Goat Anti-Rabbit Polymer Reagent	MP-7451-15	Vector labs
Dako Liquid DAB+ Substrate Chromogen System	K3468	Dako
Proteome Profiler Human Phospho-RTK Array Kit	ARY001B	R&D systems

Supplementary Table 3. Primers used in this study

1. shRNA targeting region (5'-3')

sh*Scr*	CCTAAGGTTAAGTCGCCCTCG
sh*Xbp1*	CCAGGAGTTAAGAACACGCTT
sh*Ire1 α*	GGAATTACTGGCTTCTCAT
sh*Perk.1*	CCATGAGTTCATCTGGAACAA
sh*Perk.2*	GCCACTTTGAACTTCGGTATA
sh*Gcn2.1*	GTTGGTGAAGTATAGCTTAAA
sh*Gcn2.2*	TGTCGAATGAAAGTGTTAATT
sh*Hri*	ACAAACGTCACGCTACTTAAA
sh*Pkr*	GGAGTAGCCATTACGTATAAA
sh*Scp3*	AGTGATCATCGTGGACAATTC
sh*PERK*	ATCATAGCAACAACGTTTATT
sh*NCK*	GCGAGTTCGAAATTCCATGAA
sh*IRE1 α*	GCAGGACATCTGGTATGTTAT
sh*SEL1L*	GCAAAGGCTCTTGTATATTAT
sh*Yap1* .1	TGAGAACAATGACAACCAATA
sh*Yap1* .2	CCACCAAGCTAGATAAAGAAA

2. sgRNA targeting region (5'-3')

sg*Scr*	GCACTACCAGAGCTAACTCA
sgXbp1.1	ACGCTTGGGAATGGACACGC
sgXbp1.2	GGACACGCTGGATCCTGACG
sgIre1 α.1	CTTGTTGTTTGTCTCGACCC
sgIre1 α.2	AGAGGACGGGCTCCATCAAG

3. 4 X Heatshock Response Element reporter (5'-3')

4 X HSE + minimal promoter	CCTGGAAGATTCTAGAACGTTCTGGAAGATTCTAGAACGTTCAGAGGGTATATAATGGAAGCTCGACTTCCAGA

4. Primers for RT-qPCR (5'-3')

	Forward	**Reverse**
Actin	TACCACCATGTACCCAGGCA	CTCAGGAGGAGCAATGATCTTGAT
KrasG12D	GCTTATCGATACCGTCGATCG	GGTCATACTCATCCACAAAGTG
Kras	ACGAAACAGGCTCAGGAGTTAG	GCATCGTCAACACCCTGTCT
Xbp1s	CTGAGTCCGCAGCAGGTG	TCCAACTTGTCCAGAATGCC
Edem	AATAACTGATTCCAAACAGCCC	TGTCTTCAGATTCACCCGAG
Sec61a1	CTGGCGGTAGAATGCCTCT	TGAGACCATTGTGTGGAAGG
Xbp1 splicing	ACACGCTTGGGAATGGACAC	CCATGGGAAGATGTTCTGGG
ACTIN	GCGAGAAGATGACCCAGATC	CCAGTGGTACGGCCAGAGG
HSPA6	CGGTTCTCTCCATTGACGCT	CCGGTTGTCGAAGTCCTCTC
HSPA1B	GGGTCAGGCCCTACCATTGA	TTGAGTCCCAACAGTCCACCT
IRE1 α	AGTATGTGGAGCAGAAGGAC	GTTGTGTGGCTTTAGGTCTC
Bloc1s1	AGCCAAGGCATAGTCCAGTTC	CTTCTCTCCTCCTCTTCTCCTGC
Scara3	GTGCTGGCCTCTCTGGTTTTT	GACCTTCCTGATCTCTTGCCC
Hgsnat	TCTCCGCTTTCTCCATTTTG	CGCATACACGTGGAAAGTCA
Prom1	ACTGTGCCCATGAAAAATTTGGAA	AGCTTCAACTCCAGTTGTCAGTATC
Vwa5a	AGCTTTGAAGCCTTGGTGGAT	ACTCGCTGTGAGCCTTCATC
Txnip	CCGACACACTTACCTTGCCA	GGACACTGACGTAGATCAGCAA
NCK	GACCTCAACATGCCCGCTTA	CCATTGTAGCTACCACGCCA
PPM1A	GTCCCCGACTTAGTCCATGTG	CTCTTGCTTGCCAATTCACCC
PPM1D	GAGTGAGCGTCTTCTCCGAC	TTCGGGCTCCACAACGATTT
PPM1G	TCATTTGCTTCAAGCCCCGA	CCCCTCAGTAGAGAGCACCT
PPM2C	GAAGAGGACGGCTCATGGTC	TGCCCTAAATGGCATCAGCA
PPM1H	CCAATTTCATGGGCGGCATC	TCCACCTCGTCCTGAGACAG
ILKAP	TCGGGCAATCTTGTGTCGTT	CCCATCCCTGACGTTTCCTC

PPP4C	GTGCGCTAAGGCCAGAGAGAT	CGCTATAGAAGCCACGGTCC
PPA1	TTGACGAAGGGGAAACCGAC	AAGTAGCCAGGTTTCAGCCG
PPA2	CTGTACCACACTGAGGAGCG	CTTCCAGGTAACCCGGTTTGAA
PPP6C	ACCATCGAACGGAATCAGGAAA	TGCTCCTCGGGGACTGATAG
PPP3CC	TTTACGGAACCTCCCGCCTT	CCTCGGACAGTGTTGTGGG
PPM1M	ACATCCAGCTCAAGCCCCTTC	TGAGAACCTGTGTGGGTCCT
PPP1CB	AGGTACGAGGATGTCGTCCA	TCAGCGGTGCTTCCAATTCC
PPP3CA	TGGTCCCTTCCATTTGTTGGG	ACACTCTGGCCATTTTGCCT
PPM1J	AACAGATGGCCTGTGGGATG	ACCTGCTGTGGTCATTAGGC
PPP7C	GCCAGACAACACTATGCCCT	CCCTCATGTCCTGTTCGCTT
SCP2	AAGGTGCAACGCTGGTTGAT	GCAGGAATGTCCTCCTGTAGC
PPM1B	GTCATGCGCATCTTGTCTGC	GCCTGAGAGCTTCCACCAAT
PPTC7	CTGACGGCAACAGATGGACT	ACATGCAAACTGTGCAAAAGGT
SCP4	AAAGCAACTGGTCAGGCACC	AAGGCTTGTGGTGAGTTGTCA
PPP5C	ACCGGAAATGTGCCTACCAG	TGAGTGTGGTTTCCACGAGC
PPM1L	CTGAGACCCGAGACGCTTTT	GCTACCTTGCCCTTCACCAT
PP2CZeta	AACAGATGGCCTGTGGGATG	ACCTGCTGTGGTCATTAGGC
SCP3	CCCATACCAAGTCCACCAGC	CCGCTTCAGCACATACACCT
FCP1a	AGCGACAGCCCAGTATGTCT	CTGCCTTCTCCAAGGATGTCG
PPP2CA	CGTTGTGGTAACCAAGCTGC	TGCTGGGTCAAACTGCAAGA
PPP2CB	TGCCAATGGTCTCACACTGG	GCCTGGTTCCCACAACGATA
PPP3CB	AGTTTGCCTCTTGCTGCACT	GTCCAAATGCAGGTGGCTCT
PDXP	CTGTTCGACTGTGACGGGG	TGTTGTTGCTCACAAACAGAGC
PPM1L	GATGAAGCAGGCACAACGTG	CTTTGTCACACAGGACCCCG
ACTIN	GCGAGAAGATGACCCAGATC	CCAGTGGTACGGCCAGAGG

5. Primers for cloning (5'-3')

	Forward	**Reverse**
IRE1 α	AGAATTCATGCCGGCCCGGCGGCTGCT	CCCAAGCTTTCAGAGGGCGTCTGGAGTCA
IRE1 α_shRNA resistant	ggtacgtcatTGACCTCCTGACCGGAGA	atatatcttgtTTTTTACCCATGTAGAGGATTCC
IRE1 α_S525A	GGGCACCAGCgcCCCCAGCACG	GAGCTCTCTGAGTACGGG
IRE1 α_S529A	CCCCAGCACGgCCCCCAGGGC	CTGCTGGTGCCCGAGCTCTCTGAGTACG
IRE1 α_S549A	GGCTGGCAGCgcCCCCTCCCTG	TTGGAGGCAGAGCTGCCG
IRE1 α_T973A	GCCCCCAGTGgCTCCAGACGC	TGGGGCTCTGGGGGCTCG
IRE1 α_K599A	CGTGGCCGTGgcGAGGATCCTC	TCGCGGTTGTCAAACATG
IRE1 α_K907A	CATGAGAAATgcGAAGCACCACTACCG	GCTCGGAGGAGATCTCTG
IRE1 α_S525D	GGGCACCAGCgaCCCCAGCACG	GAGCTCTCTGAGTACGGG
IRE1 α_S529D	CCCCAGCACGgaCCCCAGGGCC	CTGCTGGTGCCCGAGCTC
IRE1 α_S549D	GGCTGGCAGCgaCCCCTCCCTG	TTGGAGGCAGAGCTGCCG
IRE1 α_T973E	GCCCCCAGTGgaaCCAGACGCCC	TGGGGCTCTGGGGGCTCG
IRE1 α_V918F	GCCTGCAGAGttcCGGGAGACGC	AGCTCCCGGTAGTGGTGC
IRE1 α_∆2M	GATGGAATCCTCTACATG	TTTATCATCATCATCTTTATAATCC
IRE1 α_∆D-motif 1	CTGCAAGAGTATGTGGAGC	CTCCGTGCAGAAGTAGCG
IRE1 α_∆D-motif 2	CACGGCAAGATCAAGGCC	GTGAACGATGTTGAGGGAG
IRE1 α_∆D-motif 3	GGGTCCCTCCCCGACGAC	ATTTCTCATGGCTCGGAGGAGATCTC

6. Primers for XBP1 splicing (5'-3')

	Forward	**Reverse**
Xbp1 splicing	ACACGCTTGGGAATGGACAC	CCATGGGAAGATGTTCTGGG
XBP1 splicing	CCTGGTTGCTGAAGAGGAGG	CCA TGGGGAGA TGTTCTGGAG

Supplementary Table 4. Plasmids used in this study

Gene symbol	Catalog	Supplier	Note
*IRE1*α	NA	Generated in this study	shRNA resistant
*IRE1*α_K599A	NA	Generated in this study	shRNA resistant
*IRE1*α_K599A,S525A,T973A	NA	Generated in this study	shRNA resistant
*IRE1*α_K599A,S529A,T973A	NA	Generated in this study	shRNA resistant
*IRE1*α_K599A,S549A,*T973A*	NA	Generated in this study	shRNA resistant
*IRE1*α_K599A,T973A	NA	Generated in this study	shRNA resistant
*IRE1*α_K599A,4A	NA	Generated in this study	shRNA resistant
*IRE1*α_S525A	NA	Generated in this study	shRNA resistant
*IRE1*α_S529A	NA	Generated in this study	shRNA resistant
*IRE1*α_S549A	NA	Generated in this study	shRNA resistant
*IRE1*α_T973A	NA	Generated in this study	shRNA resistant
*IRE1*α_4A	NA	Generated in this study	shRNA resistant
*IRE1*α_S525D	NA	Generated in this study	shRNA resistant
*IRE1*α_S529D	NA	Generated in this study	shRNA resistant
*IRE1*α_S549D	NA	Generated in this study	shRNA resistant
*IRE1*α_T973E	NA	Generated in this study	shRNA resistant
*IRE1*α_SDTE	NA	Generated in this study	shRNA resistant
*IRE1*α_V918F	NA	Generated in this study	shRNA resistant
*IRE1*α_Δ2M	NA	Generated in this study	shRNA resistant
*IRE1*α_K907A	NA	Generated in this study	shRNA resistant
*IRE1*α_ΔD-motif 1	NA	Generated in this study	Cytosolic portion
*IRE1*α_ΔD-motif 2	NA	Generated in this study	Cytosolic portion
*IRE1*α_ΔD-motif 3	NA	Generated in this study	Cytosolic portion
*IRE1*α_ΔD-motif 1-3	NA	Generated in this study	Cytosolic portion
BRAF^{V600E}	NA	Generated as described previously	91
MEK^{DD}	NA	Generated as described previously	91
PIK3CA^{H1047R}	NA	Generated as described previously	91
Flag-HA-HRD1	NA	Generated previously in our lab	91
ILKAP	PLOHS_100005430	Precision LentiORF collection	
PPM1M	PLOHS_100067193	Precision LentiORF collection	
PPA1	PLOHS_100006227	Precision LentiORF collection	
PPA2	PLOHS_100066443	Precision LentiORF collection	
PPM1A	PLOHS_100004396	Precision LentiORF collection	
PPM1D	PLOHS_100003677	Precision LentiORF collection	
PPM1G	PLOHS_100004780	Precision LentiORF collection	
PPM1H	PLOHS_100064031	Precision LentiORF collection	
PPM2C	PLOHS_ccsbBEn_03447	Precision LentiORF collection	
PPP1CC	PLOHS_100067022	Precision LentiORF collection	
PPP4C	PLOHS_100004577	Precision LentiORF collection	
SCP4	PLOHS_100006354	Precision LentiORF collection	
PP2Ceta	PLOHS_100073830	Precision LentiORF collection	
PPM1B	PLOHS_100072155	Precision LentiORF collection	
PPP1CA	PLOHS_ccsbBEn_01258	Precision LentiORF collection	

PPP1CB	PLOHS_100002296	Precision LentiORF collection
PPP3CA	PLOHS_100002562	Precision LentiORF collection
PPP3CC	PLOHS_100071792	Precision LentiORF collection
PPP6C	PLOHS_ccsbBEn_01270	Precision LentiORF collection
PPP7C	PLOHS_100066919	Precision LentiORF collection
SCP2	PLOHS_100071501	Precision LentiORF collection
PPTC7/TA-PP2C	PLOHS_ccsbBEn_05108	Precision LentiORF collection
PP2CZeta	PLOHS_100067287	Precision LentiORF collection
shMAPK1.1	V2LHS_47254	Dharmacon Reagents
shMAPK1.2	V2LHS_217986	Dharmacon Reagents
shMAPK3.1	V3LHS_634594	Dharmacon Reagents
shMAPK3.2	V3LHS_634592	Dharmacon Reagents
PPP2CA	10689	Addgene
PPP2CB	67923	Addgene
PPP3CB	179135	Addgene
Myr-AKT	46969	Addgene
FCP1a	NA	Generated in this study
PDXP	NA	Generated in this study
SCP3	NA	Generated in this study
PPM2C2	NA	Generated in this study
PPP5C	NA	Generated in this study
PPM1L	NA	Generated in this study
shS*cr*	NA	Generated in this study
sh*Xbp1*	NA	Generated in this study
sh*Ire1* α	NA	Generated in this study
sh*Perk.1*	NA	Generated in this study
sh*Perk.2*	NA	Generated in this study
sh*Gcn2.1*	NA	Generated in this study
sh*Gcn2.2*	NA	Generated in this study
sh*Hri*	NA	Generated in this study
sh*Pkr*	NA	Generated in this study
sh*Scp3*	NA	Generated in this study
sh*PERK*	NA	Generated in this study
sh*NCK*	NA	Generated in this study
sh*IRE1* α	NA	Generated in this study
sh*SEL1L*	NA	Generated in this study
Myc-SEL1L	NA	Generated in this study

Supplementary Table 5. Antibodies used in this study

1. Antibodies used in WB

Antibody	Catalog	Supplier	Dilution	Host	Identifier (RRID)	Website
Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204)	4370	Cell Signaling	1:1000	Rabbit	AB_2315112	https://www.cellsignal.com/products/primary-antibodies/phospho-p44-42-mapk-erk1-2-thr202-tyr204-d13-14-4e-xp-rabbit-mab/4370#pdpCiteABCitations
p44/42 MAPK (Erk1/2) (Thr202/Tyr204)	4695	Cell Signaling	1:1000	Rabbit	AB_390779	https://www.cellsignal.com/products/primary-antibodies/p44-42-mapk-erk1-2-137f5-rabbit-mab/4695#pdpCiteABCitations
Phospho-MEK1/2 (Ser217/221)	9154	Cell Signaling	1:1000	Rabbit	AB_2138017	https://www.cellsignal.com/products/primary-antibodies/phospho-mek1-2-ser217-221-41g9-rabbit-mab/9154#pdpCiteABCitations
MEK1/2	9122	Cell Signaling	1:1000	Rabbit	AB_823567	https://www.cellsignal.com/products/primary-antibodies/mek1-2-antibody/9122#pdpCiteABCitations
ACTIN	4970	Cell Signaling	1:3000	Rabbit	AB_2223172	https://www.cellsignal.com/products/primary-antibodies/b-actin-13e5-rabbit-mab/4970#pdpCiteABCitations
GAPDH	sc-32233	Santacruz	1:10000	Mouse	AB_627679	https://www.scbt.com/p/gapdh-antibody-6c5
IRE1α	3294	Cell Signaling	1:1000	Rabbit	AB_823545	https://www.cellsignal.com/products/primary-antibodies/ire1a-14c10-rabbit-mab/3294#pdpCiteABCitations
VINCULIN	A1758	ABclonal	1:1000	Rabbit	AB_2763802	https://abclonal.com/catalog-antibodies/VinculinRabbitpAb/A1758
HSF1	4356	Cell Signaling	1:1000	Rabbit	AB_2120258	https://www.cellsignal.com/products/primary-antibodies/hsf1-antibody/4356#pdpCiteABCitations
Phospho-HSF1 (Ser326)	BSM-52166R	Life Technologies	1:1000	Rabbit		https://www.thermofisher.com/antibody/product/Phospho-HSF1-Ser326-Antibody-clone-37E4-Monoclonal/BSM-52166R
LC3	2775	Cell Signaling	1:1000	Rabbit	AB_915950	https://www.cellsignal.com/products/primary-antibodies/lc3b-antibody/2775#pdpCiteABCitations
PERK	3192	Cell Signaling	1:1000	Rabbit	AB_2095847	https://www.cellsignal.com/products/primary-antibodies/perk-c33e10-rabbit-mab/3192#pdpCiteABCitations
Phospho-PERK(Thr980)	3179	Cell Signaling	1:1000	Rabbit	AB_2095853	https://www.cellsignal.com/products/primary-antibodies/phospho-perk-thr980-16f8-rabbit-mab/3179#pdpCiteABCitations
ATF6	65880	Cell Signaling	1:1000	Rabbit	AB_2799696	https://www.cellsignal.com/products/primary-antibodies/atf-6-d4z8v-rabbit-mab/65880#pdpCiteABCitations
XBP1s	619502	Biolegend	1:1000	Rabbit	AB_315908	https://www.labome.com/product/BioLegend/619502.html
Phospho-AKT(Ser473)	4060	Cell Signaling	1:1000	Rabbit	AB_2315049	https://www.cellsignal.com/products/primary-antibodies/phospho-akt-ser473-d9e-xp-rabbit-mab/4060#pdpCiteABCitations
K48-linkage Specific Polyubiquitin	A-101	BostonBiochem	1:1000	Mouse	AB_2894847	https://www.rndsystems.com/products/ubiquitin-k48-linkage-antibody-1001c_a-101#product-citations
AKT	4691	Cell Signaling	1:1000	Rabbit	AB_915783	https://www.cellsignal.com/products/primary-antibodies/akt-pan-c67e7-rabbit-mab/4691#pdpCiteABCitations
Myc-tag	sc-40	Santacruz	1:3000	Mouse	AB_2857941	https://www.scbt.com/p/c-myc-antibody-9e10
Flag-tag	F1804	Sigma	1:3000	Mouse	AB_262044	https://www.sigmaaldrich.com/US/en/product/sigma/f1804
His-tag	AE028	Abclonal	1:1000	Mouse	AB_2769867	https://abclonal.com/catalog-antibodies/HRPconjugatedMouseantiHisTagmAb/AE028#Publication
SEL1L	PA5-88333	Thermo Fisher Scientific	1:1000	Rabbit	AB_2804837	https://www.thermofisher.com/antibody/product/SEL1L-Antibody-Polyclonal/PA5-88333
HRD1	14773	Cell Signaling	1:1000	Rabbit	AB_2798607	https://www.cellsignal.com/products/primary-antibodies/syvn1-d3o2a-rabbit-mab/14773#pdpCiteABCitations
pS/T*P	9391	Cell Signaling	1:200	Mouse	AB_331801	https://www.cellsignal.com/products/primary-antibodies/phospho-threonine-proline-mouse-mab-p-thr-pro-101/9391#pdpCiteABCitations
KRAS	H00003845-MO1	Abnova	1:500	Mouse	AB_425519	http://www.abnova.com/products/products_detail.asp?catalog_id=H00003845-M01
Phospho-HSF1(Ser121)	OASG03625	Aviva	1:1000	Rabbit		https://www.avivasysbio.com/hsf1-antibody-phospho-ser121-oasg03625.html
eIF2a	11386	Santacruz	1:1000	Rabbit	AB_640075	https://www.scbt.com/p/eif2alpha-antibody-fl-315
Phospho-eIF2a (Ser51)	9721	Cell Signaling	1:1000	Rabbit	AB_330952	https://www.cellsignal.com/products/primary-antibodies/phospho-eif2a-ser51-antibody/9721
ATF4	11815	Cell Signaling	1:1000	Rabbit	AB_2616025	https://www.cellsignal.com/products/primary-antibodies/atf-4-d4b8-rabbit-mab/11815
GCN2	3302	Cell Signaling	1:1000	Rabbit	AB_2277617	https://www.cellsignal.com/products/primary-antibodies/gcn2-antibody/3302
Phospho-GCN2 (Thr899)	94668	Cell Signaling	1:1000	Rabbit		https://www.cellsignal.com/products/primary-antibodies/phospho-gcn2-thr899-e1v9m-rabbit-mab/94668
Phospho-EGFR (Tyr1068)	3777	Cell Signaling	1:1000	Rabbit	AB_2096270	https://www.cellsignal.com/products/primary-antibodies/phospho-egf-receptor-tyr1068-d7a5-xp-rabbit-mab/3777
Phospho-ErbB2 (Tyr1221/1222)	2243	Cell Signaling	1:500	Rabbit	AB_490899	https://www.cellsignal.com/products/primary-antibodies/phospho-her2-erbb2-tyr1221-1222-6b12-rabbit-mab/2243
Phospho-ErbB3 (Tyr1222)	AP1052	Abclonal	1:500	Rabbit	AB_2863925	https://abclonal.com/catalog-antibodies/PhosphoErbB3HER3Y1222RabbitmAb/AP1052
Phospho-VEGFR2 (Tyr1175)	2478	Cell Signaling	1:500	Rabbit	AB_331377	https://www.cellsignal.com/products/primary-antibodies/phospho-vegf-receptor-2-tyr1175-19a10-rabbit-mab/2478
Phospho-FGFR (Tyr653/654)	3471	Cell Signaling	1:500	Rabbit	AB_331072	https://www.cellsignal.com/products/primary-antibodies/phospho-fgf-receptor-tyr653-654-antibody/3471
Phospho-PDGFRb (Tyr751)	4549	Cell Signaling	1:500	Rabbit	AB_1147704	https://www.cellsignal.com/products/primary-antibodies/phospho-pdgf-receptor-b-tyr751-c63g6-rabbit-mab/4549
Phospho-DDR1/2 (Tyr796/Tyr740)	MAB25382-SP	R&D systems	1:500	Rabbit		https://www.rndsystems.com/products/human-phospho-ddr1-ddr2-ddr1-y796-ddr2-y740-antibody-1119d_mab25382
Goat anti-mouse HRP	31430	Thermo Fisher Scientific	1:3000	Goat	AB_228307	https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Secondary-Antibody-Polyclonal/31430
Goat anti-rabbit HRP	31460	Thermo Fisher Scientific	1:3000	Goat	AB_228341	https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Secondary-Antibody-Polyclonal/31460
YAP (D8H1X)	14074	Cell Signaling	1:1000	Rabbit	AB_2650491	https://www.cellsignal.com/products/primary-antibodies/yap-d8h1x-xp-rabbit-mab/14074
Phospho-YAP (Ser127)	4911	Cell Signaling	1:1000	Rabbit	AB_2218913	https://www.cellsignal.com/products/primary-antibodies/phospho-yap-ser127-antibody/4911

2. Antibodies used in IHC

Antibody	Catalog	Supplier	Dilution	Host	Identifier (RRID)	Website
IRE1α	3294	Cell Signaling	1:20	Rabbit	AB_823545	https://www.cellsignal.com/products/primary-antibodies/ire1a-14c10-rabbit-mab/3294#pdpCiteABCitations
Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204)	4376	Cell Signaling	1:200	Rabbit	AB_331772	https://www.cellsignal.com/products/primary-antibodies/phospho-p44-42-mapk-erk1-2-thr202-tyr204-20g11-rabbit-mab/4376#pdpCiteABCitations
Phospho-HSF1 (Ser326)	BSM-52166R	Life Technologies	1:200	Rabbit		https://www.thermofisher.com/antibody/product/Phospho-HSF1-Ser326-Antibody-clone-37E4-Monoclonal/BSM-52166R
Phospho-PERK(Thr980)	3179	Cell Signaling	1:1000	Rabbit	AB_2095853	https://www.cellsignal.com/products/primary-antibodies/phospho-perk-thr980-16f8-rabbit-mab/3179#pdpCiteABCitations
Phospho-AKT(Ser473)	4060	Cell Signaling	1:1000	Rabbit	AB_2315049	https://www.cellsignal.com/products/primary-antibodies/phospho-akt-ser473-d9e-xp-rabbit-mab/4060#pdpCiteABCitations
Phospho-GCN2 (Thr899)	PA5-105886	Thermo Fisher	1:200	Rabbit		https://www.thermofisher.com/antibody/product/Phospho-GCN2-Thr899-Antibody-Polyclonal/PA5-105886
Phospho-eIF2a (Ser51)	9721	Cell Signaling	1:50	Rabbit	AB_330952	https://www.cellsignal.com/products/primary-antibodies/phospho-eif2a-ser51-antibody/9721
ATF4	390063	Santa Cruz	1:50	Mouse	AB_2910206	https://www.scbt.com/p/atf-4-antibody-b-3
YAP (D8H1X)	14074	Cell Signaling	1:400	Rabbit	AB_2650491	https://www.cellsignal.com/products/primary-antibodies/yap-d8h1x-xp-rabbit-mab/14074

Materials Design Analysis Reporting (MDAR)
Checklist for Authors

The MDAR framework establishes a minimum set of requirements in transparent reporting applicable to studies in the life sciences (see Statement of Task: doi:10.31222/osf.io/9sm4x.). The MDAR checklist is a tool for authors, editors, and others seeking to adopt the MDAR framework for transparent reporting in manuscripts and other outputs. Please refer to the MDAR Elaboration Document for additional context for the MDAR framework.

For all that apply, please note where in the manuscript the required information is provided.

Materials:

Newly created materials	indicate where provided: page no/section/legend)	n/a
The manuscript includes a dedicated "materials availability statement" providing transparent disclosure about availability of newly created materials including details on how materials can be accessed and describing any restrictions on access.	Newly generated plasmids are described in **Materials and Methods, “Plasmids, virus production , and infection” section** and listed in **Supplementary Table 4.**	

Antibodies	indicate where provided: page no/section/legend)	n/a
For commercial reagents, provide supplier name, catalogue number and RRID, if available.	**Supplementary Table 5.**	

DNA and RNA sequences	indicate where provided: page no/section/legend)	n/a
Short novel DNA or RNA including primers, probes: Sequences should be included or deposited in a public repository.	**Supplementary Table 3.**	

Cell materials	indicate where provided: page no/section/legend	n/a
Cell lines: Provide species information, strain. Provide accession number in repository **OR** supplier name, catalog number, clone number, **OR** RRID.	**Materials and Methods, “Cell culture and treatment section” section** and listed in **Supplementary Table 1.**	
Primary cultures: Provide species, strain, sex of origin, genetic modification status.		N/A

Experimental animals	indicate where provided: page no/section/legend)	n/a
Laboratory animals or Model organisms: Provide species, strain, sex, age, genetic modification status. Provide accession number in repository **OR** supplier name, catalog number, clone number, **OR** RRID.	**Materials and Methods, “Tumor inoculation and treatment” section.**	
Animal observed in or captured from the field: Provide species, sex, and age where possible.		N/A

Plants and microbes	indicate where provided: page no/section/legend)	n/a
Plants: provide species and strain, ecotype and cultivar where relevant, unique accession number if available, and source (including location for collected wild specimens).		N/A
Microbes: provide species and strain, unique accession number if available, and source.		N/A

Human research participants	indicate where provided: page no/section/legend) or state if these demographics were not collected	n/a
If collected and within the bounds of privacy constraints report on age, sex and gender or ethnicity for all study participants.		N/A

Design:

Study protocol	indicate where provided: page no/section/legend)	n/a
If study protocol has been pre-registered, provide DOI. For clinical trials, provide the trial registration number **OR** cite DOI.		N/A

Laboratory protocol	indicate where provided: page no/section/legend)	n/a
Provide DOI **OR** other citation details if detailed step-by-step protocols are available.	No citation for protocol. All the detailed protocols are provided in **Materials and Methods.**	

Experimental study design (statistics details)		
For in vivo studies: State whether and how the following have been done	**indicate where provided: page no/section/legend. If it could have been done, but was not, write not done**	**n/a**
Sample size determination	**Materials and Methods "Statistics and reproducibility" section.**	
Randomisation	**Materials and Methods "Tumor inoculation and treatment" section.**	
Blinding	**Materials and Methods "Statistics and reproducibility" section.**	
Inclusion/exclusion criteria	**Materials and Methods "Statistics and reproducibility" section.**	

Sample definition and in-laboratory replication	indicate where provided: page no/section/legend	n/a
State number of times the experiment was replicated in laboratory.	**Materials and Methods "Statistics and reproducibility" section and figure legends.**	
Define whether data describe technical or biological replicates.	**Materials and Methods "Statistics and reproducibility" section and figure legends.**	

Ethics	indicate where provided: page no/section/legend	n/a
Studies involving human participants: State details of authority granting ethics approval (IRB or equivalent committee(s), provide reference number for approval.		N/A
Studies involving experimental animals: State details of authority granting ethics approval (IRB or equivalent committee(s), provide reference number for approval.	**Materials and Methods, "Study approval" section.**	
Studies involving specimen and field samples: State if relevant permits obtained, provide details of authority approving study; if none were required, explain why.		N/A

Dual Use Research of Concern (DURC)	indicate where provided: page no/section/legend	n/a
If study is subject to dual use research of concern regulations, state the authority granting approval and reference number for the regulatory approval.		N/A

Analysis:

Attrition	indicate where provided: page no/section/legend	n/a
Describe whether exclusion criteria were preestablished. Report if sample or data points were omitted from analysis. If yes report if this was due to attrition or intentional exclusion and provide justification.		N/A

Statistics	indicate where provided: page no/section/legend	n/a
Describe statistical tests used and justify choice of tests.	**Materials and Methods "Statistics and reproducibility" section and figure legends.**	

Data availability	indicate where provided: page no/section/legend	n/a
For newly created and reused datasets, the manuscript includes a data availability statement that provides details for access or notes restrictions on access.		N/A
If newly created datasets are publicly available, provide accession number in repository **OR** DOI **OR** URL and licensing details where available.		N/A
If reused data is publicly available provide accession number in repository **OR** DOI **OR** URL, **OR** citation.	**Materials and Methods "Modeling of the IRE1α-ORIN1001 complex" section, and "Clinical Proteomic Tumor Analysis Consortium (CPTAC) data analysis" section.**	

Code availability	indicate where provided: page no/section/legend	n/a
For all newly generated custom computer code/software/mathematical algorithm or re-used code essential for replicating the main findings of the study, the manuscript includes a data availability statement that provides details for access or notes restrictions.		N/A
If newly generated code is publicly available, provide accession number in repository, **OR** DOI **OR** URL and licensing details where available. State any restrictions on code availability or accessibility.		N/A
If reused code is publicly available provide accession number in repository **OR** DOI **OR** URL, **OR** citation.		N/A

Reporting

MDAR framework recommends adoption of discipline-specific guidelines, established and endorsed through community initiatives. Journals have their own policy about requiring specific guidelines and recommendations to complement MDAR.

Adherence to community standards	indicate where provided: page no/section/legend	n/a
State if relevant guidelines (e.g., ICMJE, MIBBI, ARRIVE) have been followed, and whether a checklist (e.g., CONSORT, PRISMA, ARRIVE) is provided with the manuscript.	**ICMJE criteria have been followed.**	