**Additional File 2 (Figures): Supporting information for**

**LncRNA IGFL2-AS1 mediates NSCLC chemoresistance via YBX1-induced HSPA1A/RAP1 activation**

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**Fig. S1. LncRNA and mRNA high-throughput screening with DDP-resistant A549 cells.**

**A** Resistance index of resistant cells (A549/DDP) and parental A549 cells to cisplatin (DDP), as measured using CCK8 and IC50 calculation. **B** KEGG analysis of pathways upregulated in both DDP primary resistant cells (PR) and DDP secondary resistant cells (SR) compared to parental A549 cells (PC). **C** Potential peptide coding of *IGFL2-AS1*, as predicted using the Lncipedia database. All plots are presented as the mean ± SD. Statistical analyses: two-way ANOVA with Dunnett’s multiple comparison test (A). \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001.

**Fig. S2. IGFL2-AS1 prohibits the invasiveness and stemness of lung cancer cells and associated markers.**

**A** Wound healing assay and quantification of A549 (left) and H520 (right) 48 h after *IGFL2-AS1* overexpression. Scale bar, 50 μm. **B** Transcription levels of EMT-related genes (*Snail*, *Twist*, *Vimentin*, and *E-cadherin*) in lung cancer cells following *IGFL2-AS1* overexpression, as detected using qRT-PCR**. C** Transcription level of stemness-related genes (*ABCG2*, *OCT4*, and *NANOG*) in lung cancer cells following IGFL2-AS1 overexpression, as detected using qRT-PCR. **D** Wound healing assay and quantification of A549 (upper) and H520 (lower) expression 48 h following *IGFL2-AS1* knockdown. Scale bar, 50 μm. **E** Transcription levels of EMT-related genes (*Snail*, *Twist*, *Vimentin*, *and E-cadherin*) in lung cancer cells following *IGFL2-AS1* knockdown, as detected using qRT-PCR. All plots are presented as the mean ± SD. Statistical analyses: two-tailed unpaired Student’s *t-*test (A, B and C), one-way ANOVA with Dunnett’s multiple comparison test (D, E). \**P* < 0.05, \*\**P* < 0.01, \*\*\**P*< 0.001

**Fig. S3. IGFL2-AS1 inhibition sensitizes A549 cells to chemotherapy *in vivo*.**

**A** Luminescent signal of subcutaneously implanted luciferase-tagged A459 cells following IGFL2-AS1 inhibition or in combination with chemotherapy (n = 6 mice per group). **B** IHC staining of Ki67, Vimentin, and SOX2 across different groups. Scale bar, 50 μm. **C** Changes in the body weight of mice in various groups (n = 6 mice per group).

**Fig. S4. IGFL2-AS1 promotes invasion via HSPA1A.**

**A** KEGG analysis of the signaling cascade stimulated in both A549 and H520 IGFL2-AS1 overexpressing cells compared to corresponding control cells infected with the empty vector backbone. **B** Venn diagram displaying genes upregulated in both A549 and H520 cells following *IGFL2-AS1* overexpression. **C** Immunoblot assay for the validation of genetic manipulation of *HSPA1A* in H520 cells. **D** Transcription levels of EMT-related markers (*E-cadherin*, *Twist*, and *Snail*) following *HSPA1A* knockdown in H520 cells, as detected using qRT-PCR. **E** Transcription levels of EMT-related markers (*E-cadherin*, *Twist,* and *Snail*) following HSPA1A overexpression in H520 cells, as detected using qRT-PCR. **F** Wound healing assay and histogram quantification of lung cancer cells following *IGFL2-AS1* overexpression alone or together with *HSPA1A* downregulation. **G** Wound healing assay and histogram quantification of lung cancer cells following *IGFL2-AS1* knockdown either alone or together with *HSPA1A* overexpression. All plots are depicted as the mean ± SD. Statistical analyses: two-tailed unpaired Student’s *t-*test (E), one-way ANOVA with Dunnett’s multiple comparison test (D, F and G). \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001

**Fig. S5. IGFL2-AS1 and HNRNPA1 do not interact in lung cancer cells.**

**A** Interaction domain between HNRNPA1 and IGFL2-AS1, as predicted by the catRAPID database. **B** Binding sequences between *HNRNPA1* and *HSPA1A* mRNA, as predicted by the catRAPID database. **C** Negative interaction between HNRNPA1 and IGFL2-AS1 in A549 cells, as demonstrated by an RNA pulldown assay followed by immunoblotting. **D** Negative interaction between HNRNPA1 and IGFL2-AS1 in A549 cells, as demonstrated by RIP followed by qRT-PCR. **E** Negative effect of *IGFL2-AS1* knockdown on *HNRNPA1* transcription, as detected using qRT-PCR. **F** Negative effect of *IGFL2-AS1* knockdown on HNRNPA1 protein level, as detected using immunoblot. All plots are depicted as the mean ± SD. Statistical analyses: two-tailed unpaired Student’s *t-*test (D), one-way ANOVA with Dunnett’s multiple comparison test (E). \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001

**Fig. S6. YBX1 positively modulates the expression of** **IGFL2-AS1 and HSPA1A.**

**A** RNA pulldown precipitate by the *IGFL2-AS1* sense strand compared to the antisense strand in lung cancer cells. **B** Interaction domain between YBX1 and IGFL2-AS1 predicted by the catRAPID database. **C** Transcription levels of IGFL2-AS1 and HSPA1A in lung cancer cells following *YBX1* overexpression, as detected using qRT-PCR. **D** Transcription levels of *IGFL2-AS1* and *HSPA1A* in lung cancer cells following *YBX1* knockdown, as detected using qRT-PCR. **E** Regulatory effect of YBX1 on HSPA1A protein level in lung cancer cells, as detected using immunoblot. **F** Negative effect of IGFL2-AS1 knockdown on *YBX1* mRNA expression, as detected using qRT-PCR. **G** Negative effect of *IGFL2-AS1* knockdown on YBX1 protein level, as detected using immunoblotting. All plots are presented as the mean ± SD. Statistical analyses: two-tailed unpaired Student’s *t-*test (C) and one-way ANOVA with Dunnett’s multiple comparison test (D, F). \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001

**Fig. S7. Signaling cascades affected by *HSPA1A* upregulation in lung cancer cells.**

**A** Heatmap of oncogenic pathways (Rap1, AMPK, Hippo, Hedgehog, cAMP and mTOR) changed in response to *HSPA1A* upregulation. **B** KEGG assay of RNA sequencing demonstrating pathways downregulated upon *HSPA1A* upregulation. **C** Heatmap of anti-cancer cascades (p53 and apoptosis pathways) upon HSPA1A upregulation. **D** Gene set enrichment analysis of anti-cancer cascades (p53 and apoptosis pathways) following *HSPA1A* upregulation. **E** Flow cytometry detection of changes in apoptosis capacity in A549 and H520 cells after knockdown of *IGFL2-AS1* or *HSPA1A.* All plots are presented as the mean ± SD. Statistical analyses: one-way ANOVA with Dunnett’s multiple comparison test (E). \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001

**Fig. S8. *In vivo* therapeutic intervention of HSPA1A sensitizes lung cancer to chemotherapies.**

**A** Images of luciferase indicated subcutaneously implanted A549 tumor bulk upon monotherapy or combinational therapy (n = 5 mice per group). **B** IHC staining of Ki67 in mice following VER155008 monotherapy or in combination with chemotherapy. Scale bar, 50 μm. **C** IHC staining of HSPA1A in mice following VER155008 monotherapy or combination with chemotherapy. Scale bar, 50 μm.

**Fig. S9. Images of luciferase indicated lung metastatic A549 tumor bulk upon distinct therapy.**

Images of luciferase indicated lung metastatic A549 tumor bulk upon monotherapy or combinational therapy (n = 3 mice per group).