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Supplementary Materials for

Induced degradation of lineage-specific oncoproteins drives the therapeutic vulnerability of small cell lung cancer to PARP inhibitors

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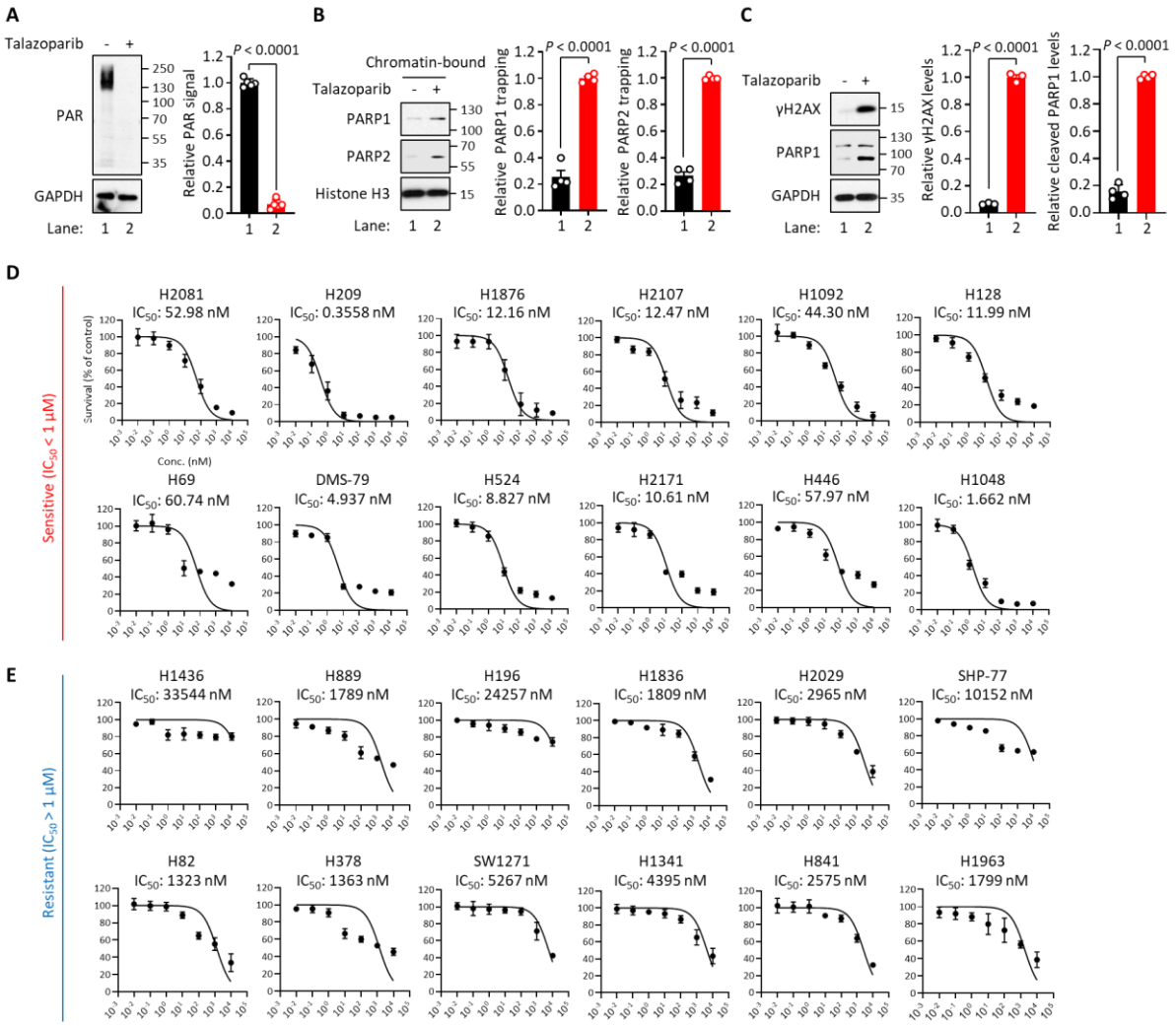
Legends for tables S1 to S7

Other Supplementary Material for this manuscript includes the following:

Tables S1 to S7

Supplementary figures



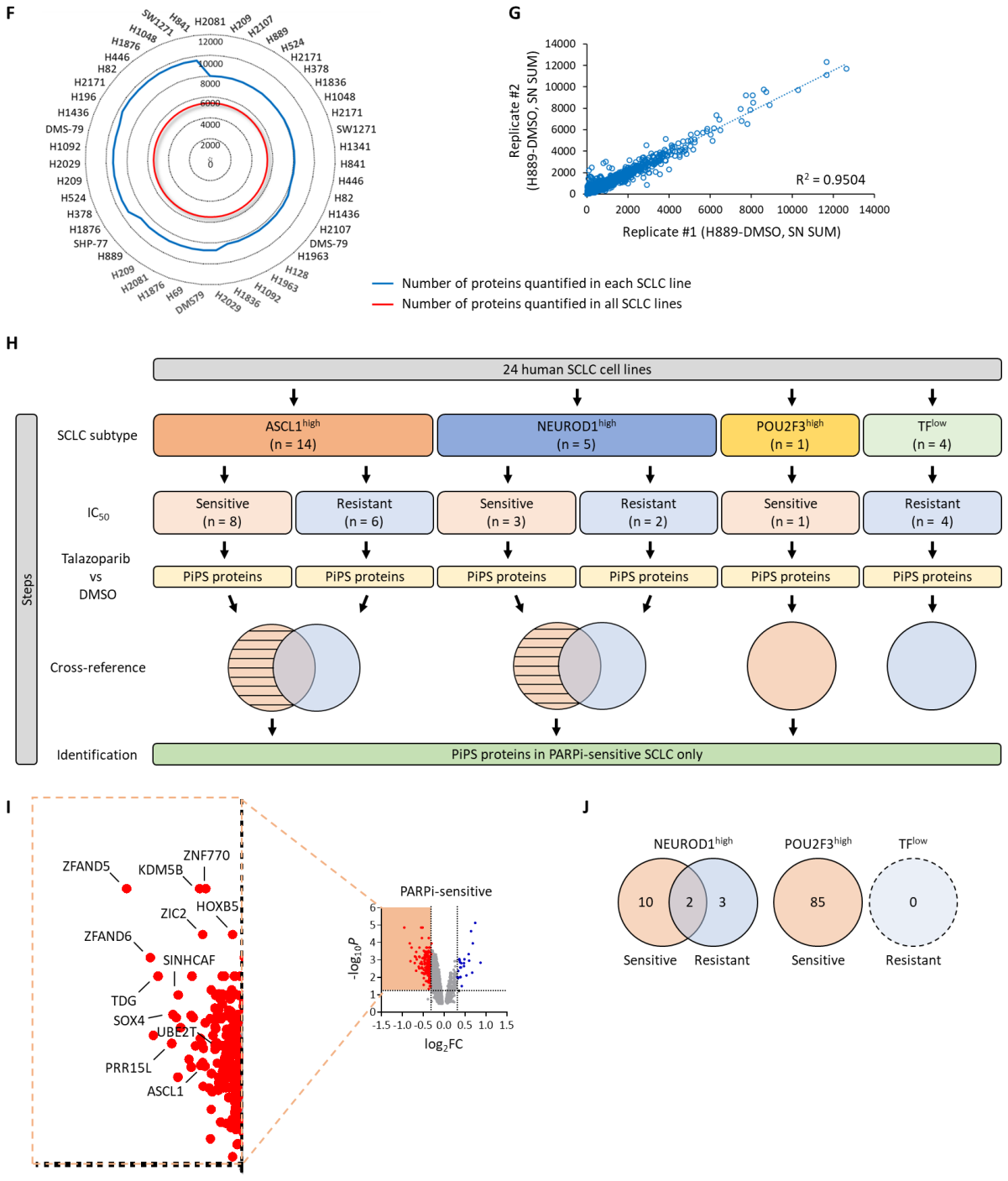


Fig. S1. Biochemical and proteomic analyses of PARPi treatment in SCLC. (**A**) PAR levels in talazoparib treatment. H2081 cells were treated with talazoparib (1 μM for 48 hrs) and the cell lysates were subjected to immunoblot analysis using the indicated antibodies. Values were presented as means ± SD (n = 5). (**B**) The levels of PARP1/2 trapping in talazoparib treatment. H2081 cells were treated with or without talazoparib (1 μM for 48 hrs). Chromatin-bound fractions were isolated and subjected to immunoblot analysis using the indicated antibodies.

Values were presented as means ± SD (n = 4). (**C**) The extent of DNA damage and PARP1 cleavage in talazoparib treatment. H2081 cells were treated with talazoparib (1 μM for 48 hrs) and cell lysates were subjected to immunoblot analysis using the indicated antibodies. Values were presented as means ± SD (n = 3-4). (**D**) The concentration-response curves and IC_{50} values in PARPi-sensitive SCLC cell lines (IC_{50} < 1 μM). **(E)** The concentration-response curves and IC_{50} values in PARPi-resistant SCLC cell lines (IC_{50} > 1 μM). (**F**) Radar chart showing the number of proteins quantified in this study. (**G**) Reproducibility of biological replicates in H889 cells. (**H**) Steps of the identification of PiPS proteins in PARPi-sensitive and -resistant SCLC cell lines of each SCLC subtype. TF^{low}, low expression of all three transcription factors. (**I**) Volcano plot of differentially expressed proteins in $ASCL1^{high}$/PARPi-sensitive SCLC cells. Left, several significantly down-regulated top proteins are highlighted as specific target proteins in $ASCL1^{high}$/PARPi-sensitive SCLC cells. (**J**) PiPS proteins identified in PARPi-sensitive and -resistant SCLC of $NEUROD1^{high}$, $POU2F3^{high}$, and TF^{low} subtype.

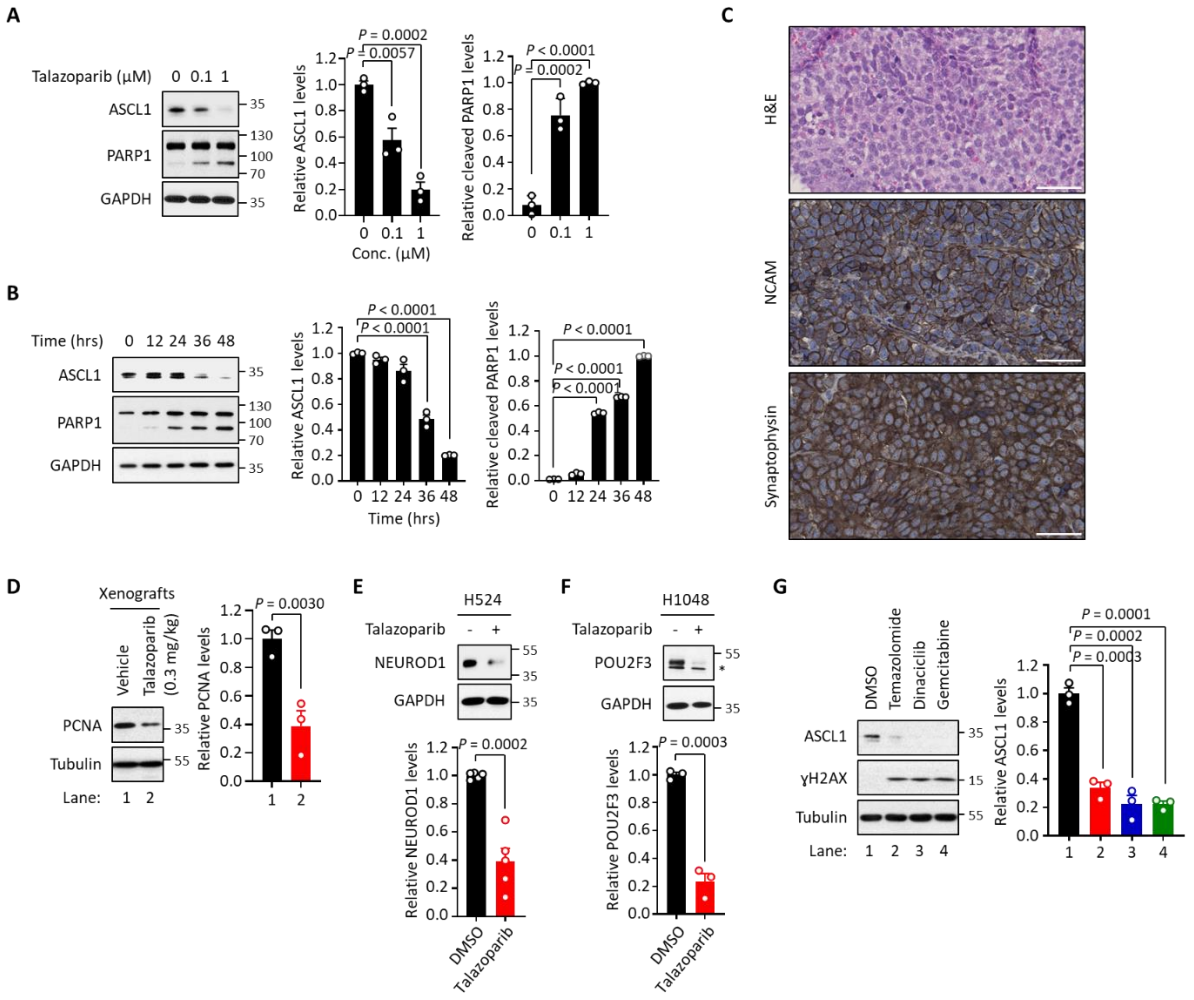
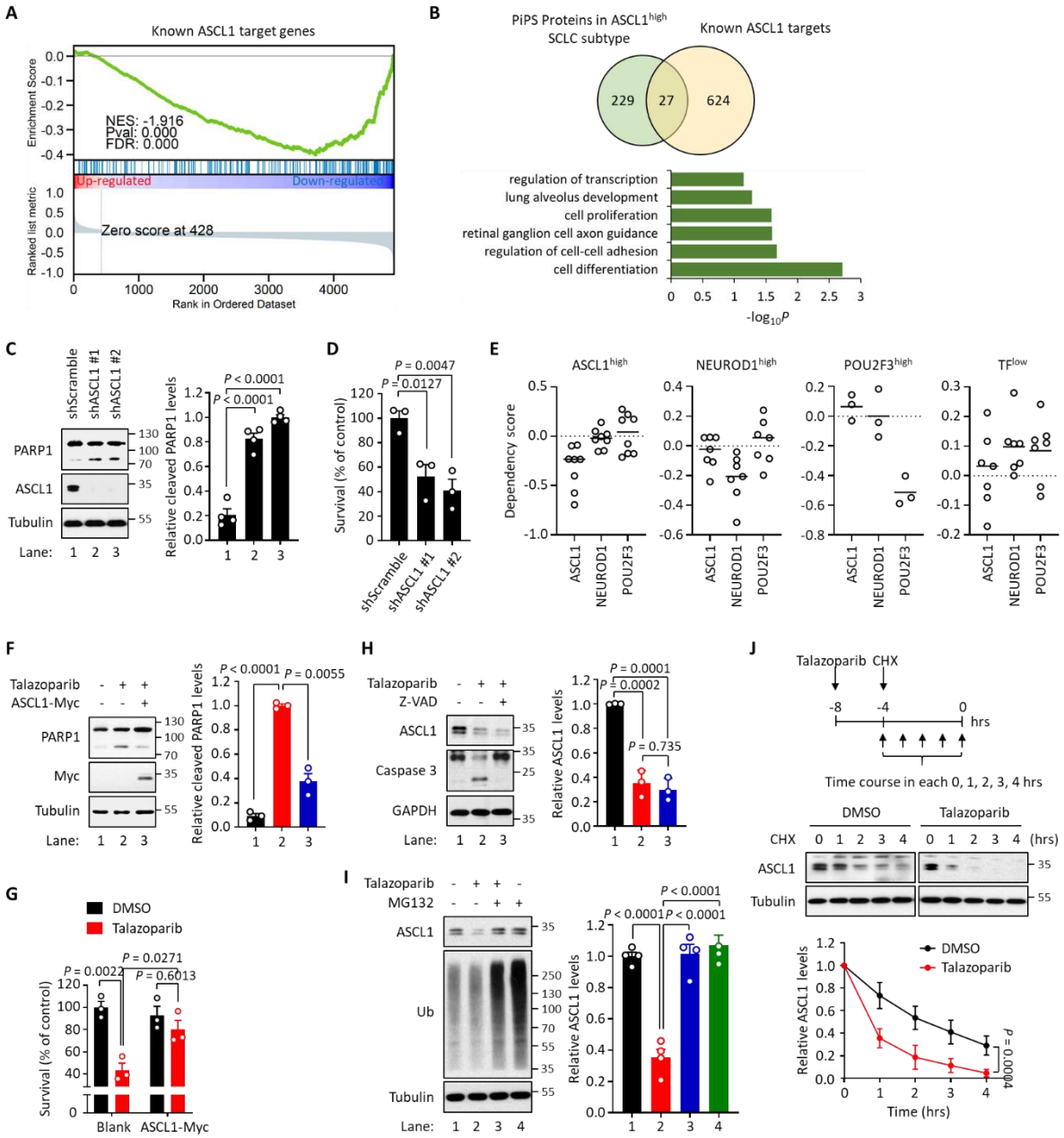


Fig. S2. PARPi-induced degradation of lineage-specific oncoproteins in SCLC. (**A**) The levels of ASCL1 in a concentration-dependent manner. H2081 cells were treated with talazoparib (for 48 hrs) in a concentration-dependent manner, and the cell lysates were subjected to immunoblot analysis using the indicated antibodies. Values were presented as means ± SD (n = 3). (**B**) The levels of ASCL1 in a time-dependent manner. H2081 cells were treated with talazoparib (1 μM) in a time-dependent manner, and the cell lysates were subjected to immunoblot analysis using the indicated antibodies. Values were presented as means ± SD (n = 3). (**C**) Histological analysis of H2081-implanted xenograft tumors. Tissue sections were collected from H2081-derived mouse xenograft tumors. Hematoxylin and eosin (H&E) staining and immunohistochemistry (IHC) for NCAM and synaptophysin were performed. Scale bars represent 50 μm. (**D**) The levels of PCNA *in vivo*. H2081-implanted xenograft tumors were treated with or without talazoparib (0.3 mg/kg for 30 days), and the tumor extracts were subjected to immunoblot analysis using the indicated antibodies. Values were presented as means ± SD (n = 3). (**E**) The levels of NEUROD1 in talazoparib treatment. H524 cells were

treated with talazoparib (1 μM for 48 hrs) and cell lysates were subjected to immunoblot analysis using the indicated antibodies. Values were presented as means ± SD (n = 5). (**F**) The levels of POU2F3 in talazoparib treatment. H1048 cells were treated with talazoparib (1 μM for 48 hrs), and the cell lysates were subjected to immunoblot analysis using the indicated antibodies. Values were presented as means ± SD (n = 3). The asterisk indicates a non-specific band. **(G)** The levels of ASCL1 in the treatment of DNA damaging agents. H2081 cells treated with temazolomide (1 μM), dinaciclib (1 μM), or gemcitabine (1 μM) for 48 hrs, and the cell lysates were subjected to immunoblot analysis using the indicated antibodies. Values were presented as means ± SD (n = 3).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Down-regulated
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Known ASCL1 targets
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27
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hrs
Time course in each 0, 1, 2, 3, 4 hrs
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Talazoparib
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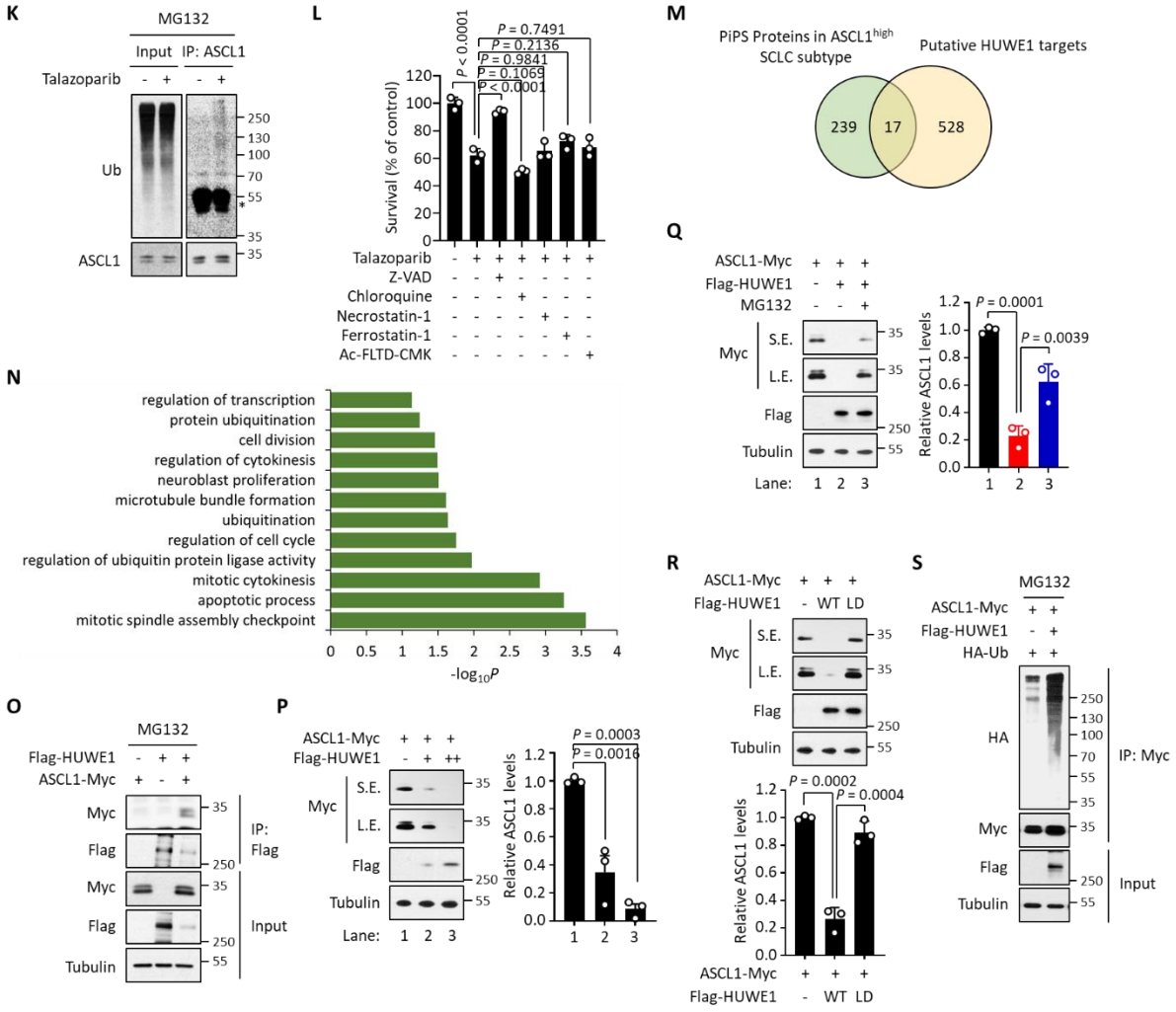


Fig. S3. Biochemical experiments for ASCL1 ubiquitination and degradation by talazoparib-induced HUWE1 activation. (**A**) The gene set enrichment analysis (GSEA) indicates that known ASCL1 target genes are positively enriched in the down-regulated PiPS proteins identified from the PARPi-sensitive/ASCL1^{high} SCLC subtype. **(B)** Top, Venn diagram showing the common candidates between PiPS proteins in ASCL1^{high} SCLC subtype and known ASCL1 targets. See also table S4.; Bottom, gene ontology (GO) analyses of commonly identified 27 targets. Biological processes were analyzed using the ToppGene database (https://toppgene.cchmc.org/). (**C**) The levels of cleaved PARP1 in depletion of ASCL1. H2081 cells were depleted with ASCL1 (shASCL1 #1 or #2), and the cell lysates were subjected to immunoblot analysis using the indicated antibodies. Values were presented as means ± SD (n = 4). (**D**) Viability assays in depletion of ASCL1. H2081 cells were depleted with ASCL1 (shASCL1 #1 or #2), and viability was measured using a CellTiter-Glo assay. Values were presented as means ± SEM (n = 3). (**E**) DepMap analysis for the requirement of ASCL1,

NEUROD1, and POU2F3 in each SCLC subtype. (**F**) The levels of PARP1 cleavage in ectopic expression of ASCL1. H2081 cells expressing ASCL1 (ASCL1-Myc) were treated with talazoparib (1 μM for 48 hrs), and the cell lysates were subjected to immunoblot analysis using the indicated antibodies. Values were presented as means ± SD (n = 3). (**G**) Viability assays in ectopic expression of ASCL1. H2081 cells expressing ASCL1 (ASCL1-Myc) were treated with talazoparib (1 μM for 48 hrs), and viability was measured using a CellTiter-Glo assay. Values were presented as means ± SEM (n = 3). (**H**) The levels of ASCL1 in the inhibition of caspase activity. H2081 cells pre-treated with Z-VAD (50 μM for 1 hr) were treated with talazoparib (1 μM for 48 hrs), and the cell lysates were subjected to immunoblot analysis using the indicated antibodies. Values were presented as means ± SD (n = 3). (**I**) Regulation of ASCL1 degradation with proteasome inhibition. H2081 cells were treated with talazoparib (1 μM for 48 hrs) and then, proteasome inhibitor (MG132, 10 μM) was further treated for last 6 hrs. Values were presented as means ± SD (n = 4). **(J)** Half-life of ASCL1. H2081 cells pre-treated with or without talazoparib (1 μM) were treated with cycloheximide (CHX, 10 μg/ml) as indicated. The cell lysates were subjected to immunoblot analysis using the indicated antibodies. Top, schematic flow; Bottom, the normalized levels of ASCL1. (**K**) Ubiquitination of endogenous ASCL1. H2081 cells were treated with or without talazoparib (1 μM for 48 hrs) and then, MG132 (10 μM) was further treated for last 6 hrs. The cell lysates were subjected to immunoprecipitation analysis using anti-ASCL1 antibody. Immunoprecipitates or inputs were resolved by SDS-PAGE and subjected to immunoblot analysis using the indicated antibodies. The asterisk indicates IgG heavy chains. (**L**) Viability assays with diverse cell death inhibitors in PARPi treatment. H2081 cells pre-treated with Z-VAD (50 μM), chloroquine (50 μM), necrostatin-1 (20 μM), ferrostatin-1 (10 μM), and Ac-FLTD-CMK (10 μM) were treated with talazoparib (1 μM for 48 hrs), and viability was measured using a CellTiter-Glo assay. Values were presented as means ± SEM (n = 3). (**M**) Venn diagram showing the common candidates between PiPS proteins in ASCL1^{high} SCLC subtype and putative HUWE1 targets. See also table S5. **(N)** Gene Ontology (GO) analyses of commonly identified 17 targets in fig. S3M. Biological processes were analyzed using the ToppGene database (https://toppgene.cchmc.org/). (**O**) The interaction of ASCL1 with HUWE1. HEK293T cells transfected with Flag-HUWE1 or ASCL1-Myc alone or in combination were treated with MG132 (10 μM for 6hr), and the cell lysates were subjected to co-immunoprecipitation using anti-Flag M2 affinity gel beads. Immunoprecipitates or inputs were resolved by SDS-PAGE and subjected to immunoblot analysis using the indicated antibodies. (**P**) The levels of ASCL1

in ectopic expression of HUWE1. HEK293T cells were transfected with ASCL1-Myc alone or in combination with Flag-HUWE1, and the cell lysates were subjected to immunoblot analysis using the indicated antibodies. Values were presented as means ± SD (n = 3). S.E., short exposure; L.E., long exposure. (**Q**) The levels of ASCL1 with HUWE1 in proteasome inhibition. HEK293T cells transfected with ASCL1-Myc alone or in combination with Flag-HUWE1 were treated with MG132 (10 μM for 6hr). The cell lysates were subjected to immunoblot analysis using the indicated antibodies. Values were presented as means ± SD (n = 3). S.E., short exposure; L.E., long exposure. (**R**) The levels of ASCL1 in HUWE1 E3 ligase activity. HEK293T cells transfected with ASCL1-Myc alone or in combination with Flag-HUWE1 wild-type (WT) or ligase-dead (LD) mutant were subjected to immunoblot analysis using the indicated antibodies. Values were presented as means ± SD (n = 3). S.E., short exposure; L.E., long exposure. (**S**) Ubiquitination of ASCL1 by HUWE1. HEK293T cells transfected with ASCL1-Myc alone or in combination with Flag-HUWE1 were treated with MG132 (10 μM for 6hr), and the cell lysates were subjected to immunoprecipitation using anti-Myc antibody. Immunoprecipitates or inputs were resolved by SDS-PAGE and subjected to immunoblot analysis using the indicated antibodies.

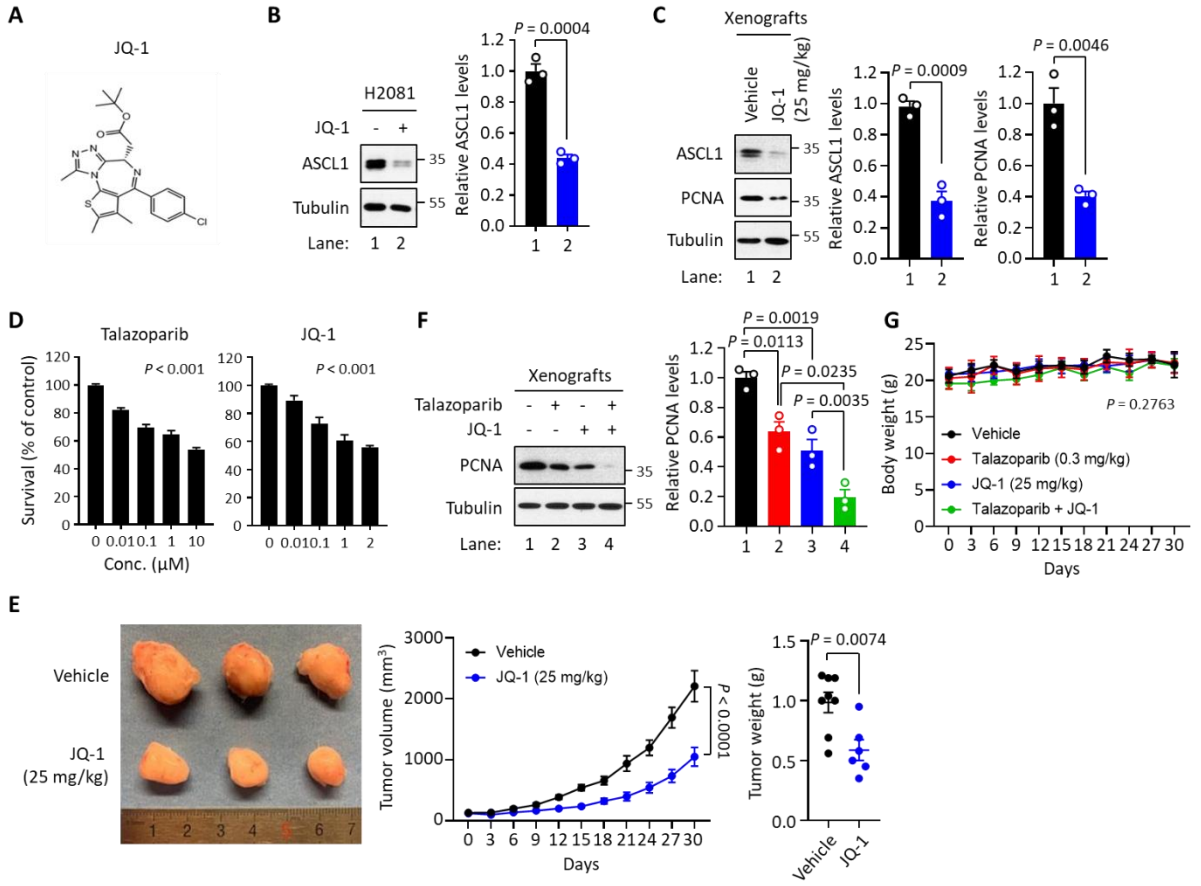


Fig. S4. The role of JQ-1 in ASCL1 degradation and SCLC growth inhibition. (**A**) The structure of JQ-1. (**B**) The effect of JQ-1 in ASCL1 downregulation. H2081 cells were treated with JQ-1 (0.1 μM for 48 hrs), and the cell lysates were subjected to immunoblot analysis using the indicated antibodies. Values were presented as means ± SD (n = 3). (**C**) The levels of ASCL1 and PCNA *in vivo*. H2081-implanted xenograft tumors were treated with or without JQ-1 (25 mg/kg for 30 days), and the tumor extracts were subjected to immunoblot analysis using the indicated antibodies. Values were presented as means ± SD (n = 3). (**D**) The cytotoxic effects of talazoparib or JQ-1 in a concentration-dependent manner. H2081 cells were treated with talazoparib or JQ-1 for 48 hrs in a concentration-dependent manner and viability was measured using a CellTiter-Glo assay. Values were presented as means ± SEM (n = 3). (**E**) Toxicity of JQ-1 *in vivo*. Mice implanted with H2081 cells were treated with or without JQ-1 (25 mg/kg for 30 days). Left, the image of representative tumors; Right, tumor volume and weight Values were presented as means ± SD (n = 6-10). **(F)** The effect of talazoparib with JQ-1 in PCNA regulation *in vivo*. Mice implanted with H2081 cells were treated with talazoparib (0.3 mg/kg), JQ-1 (25 mg/kg), or talazoparib + JQ-1 as indicated, and the tumor extracts were

subjected to immunoblot analysis using the indicated antibodies. Values were presented as means ± SD (n = 3). (**G**) Body weight of H2081-implanted xenograft tumors to talazoparib (0.3 mg/kg for 30 days), JQ-1 (25 mg/kg for 30 days), and talazoparib + JQ-1. Values were presented as means ± SD (n = 6-10).

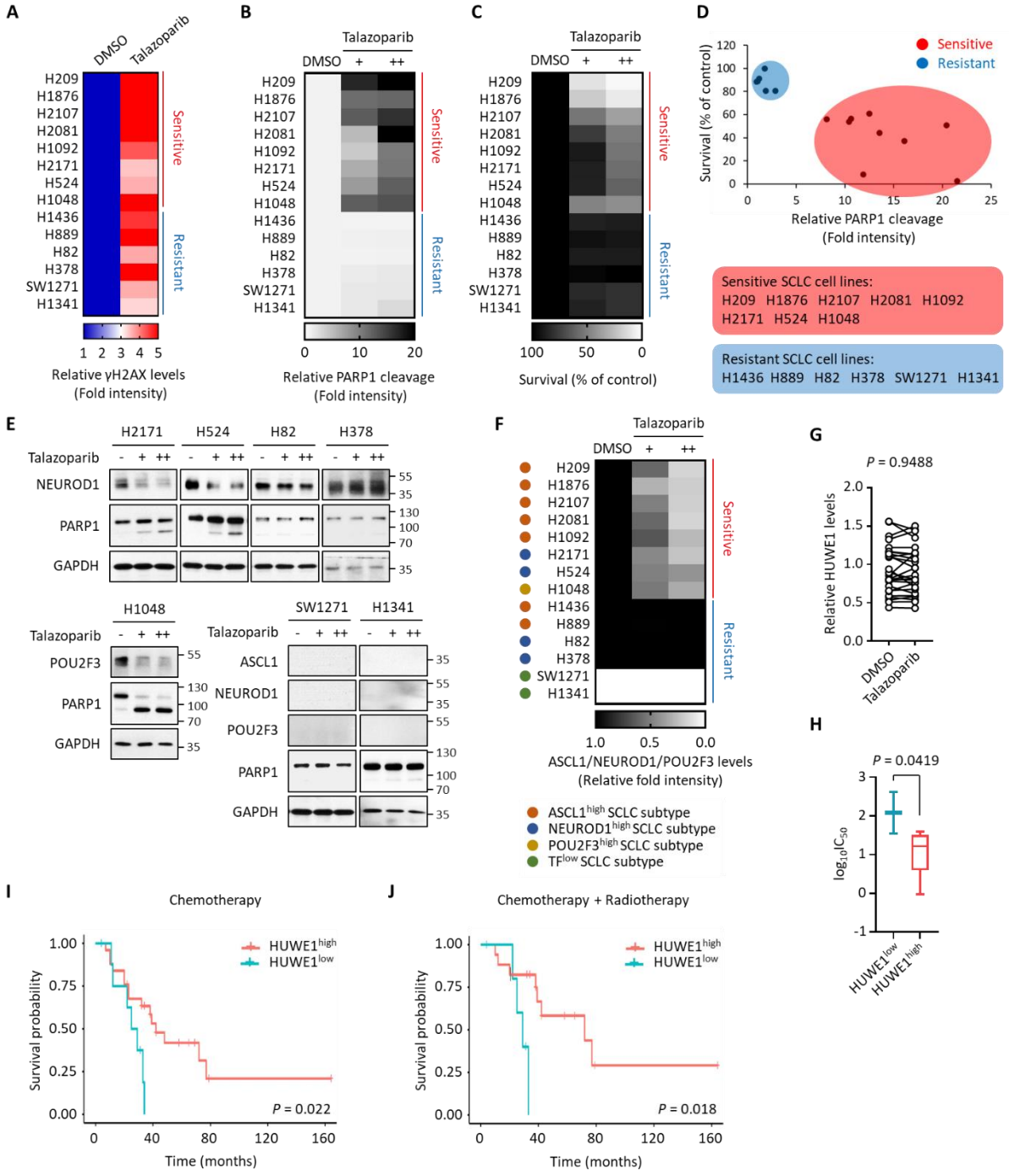
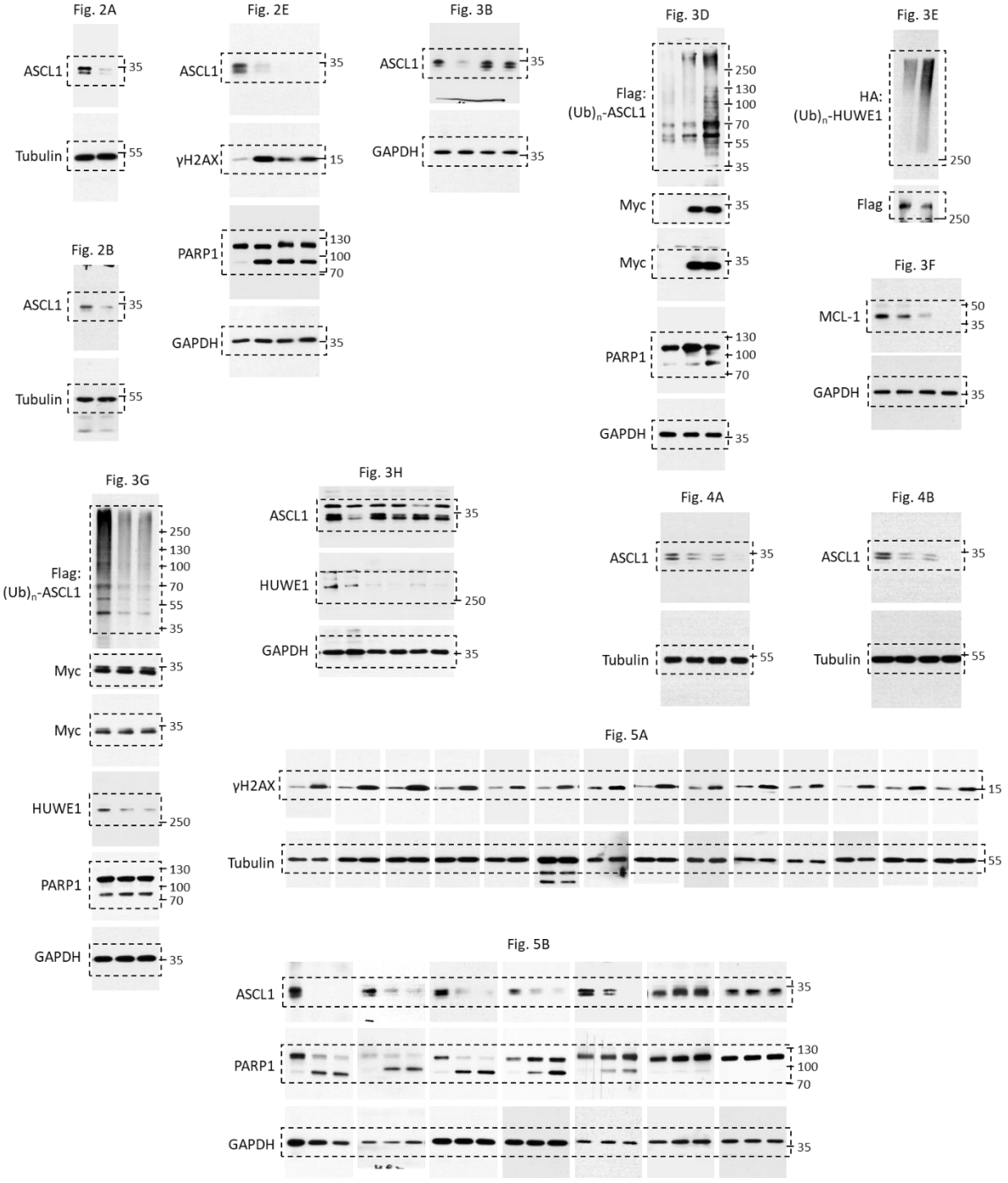


Fig. S5. The unequal degradation of the lineage-specific oncoproteins and selective sensitivity to PARPi in SCLC. (**A**) The extent of DNA damage in a panel of SCLC cell lines treated with talazoparib. Quantification of the immunoblots presented in Fig. 5A was shown. Red: sensitive SCLC cell lines; Blue: resistant SCLC cell lines. (**B**) Cell death as measured by PARP1 cleavage in a panel of SCLC cells treated with Talazoparib. Red: sensitive SCLC cells; Blue: resistant SCLC cells. (**C**) Cell death as measured by the CellTiter-Glo assays in a panel

of SCLC cells treated with Talazoparib. Red: PARPi-sensitive SCLC cells; Blue: PARPi-resistant SCLC cells. (**D**) The distribution of SCLC cells that are sensitive or resistant to PARPi based on PARP1 cleavage and cell survival ratio from fig. S5, B and C, respectively. Red: PARPi-sensitive SCLC cells; Blue: PARPi-resistant SCLC cells. (**E**) The levels of ASCL1, NEUROD1, and POU2F3 in a panel of SCLC cell lines treated with talazoparib. A panel of SCLC cell lines were treated with talazoparib (1 (+) and 10 (++) μM for 48 hrs), and the cell lysates were subjected to immunoblot analysis using the indicated antibodies. (**F**) The levels of ASCL1, NEUROD1, and POU2F3 in a panel of SCLC cell lines. The levels of ASCL1, NEUROD1, and POU2F3 in a panel of SCLC cells treating with talazoparib (1 (+) and 10 (++) μM for 48 hrs) were normalized to GAPDH. Red: sensitive SCLC cell lines; Blue: resistant SCLC cell lines. (**G**) The relative levels of HUWE1 in $ASCL1^{high}$ SCLC cell lines from TMT-based proteomic datasets. The values of HUWE1 from all $ASCL1^{high}$ SCLC cell lines used in this study were collected, normalized and presented as means ± SD. **(H)** Boxplots showing the sensitivity of a panel of $ASCL1^{high}$ SCLC cell lines to PARPi. $ASCL1^{high}$ SCLC cell lines were grouped by the relative levels of HUWE1 expression, and the sensitivity was determined by the analysis of the GDSC database measuring IC_{50} against olaparib treatment. Values were presented as means ± SD. **(I)** Kaplan-Meier survival curves of SCLC patients treated with chemotherapy according to HUWE1 expression. **(J)** Kaplan-Meier survival curves of SCLC patients co-treated with chemotherapy and radiotherapy according to HUWE1 expression.

Fig. S6. Raw images of all blots



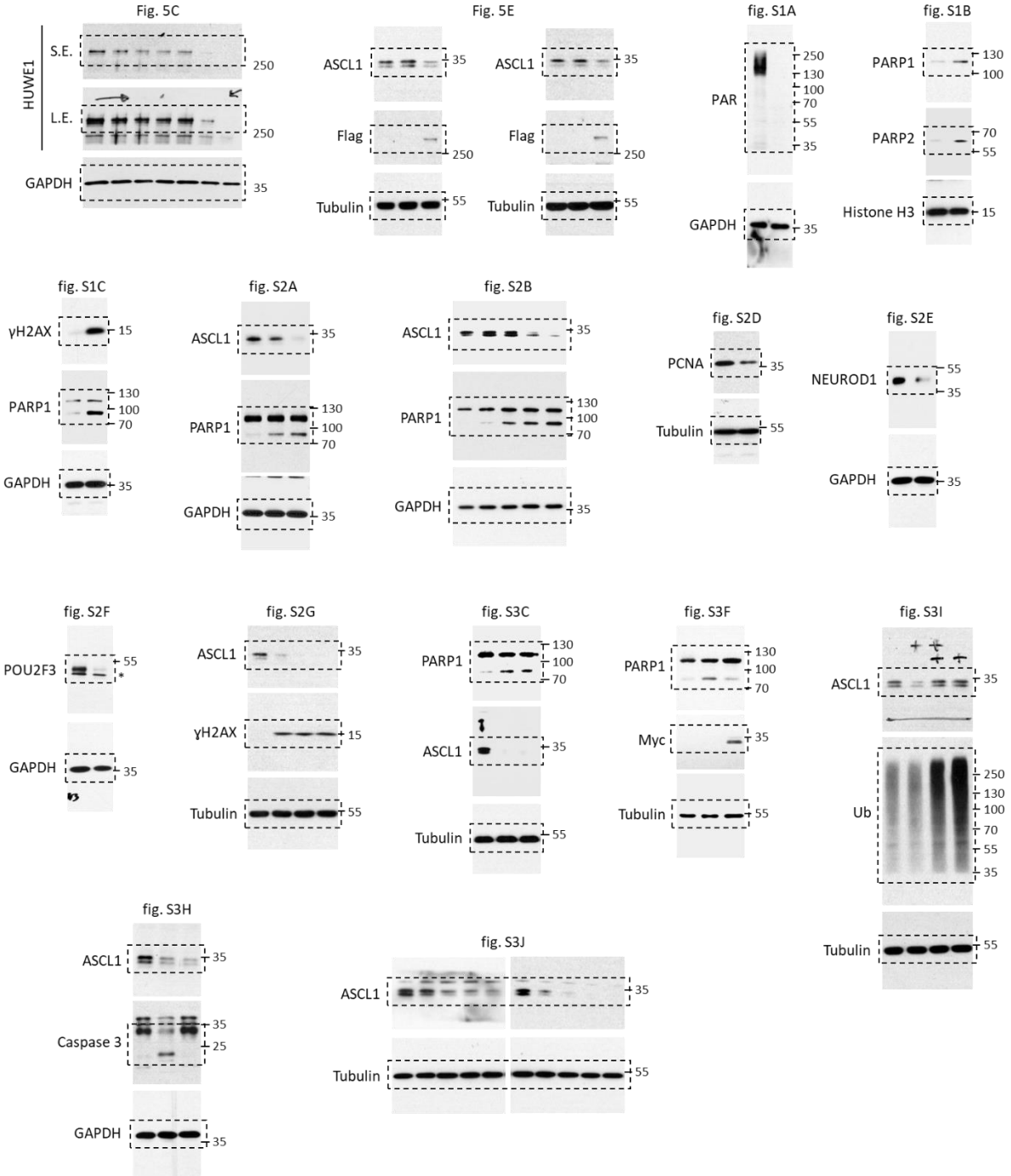

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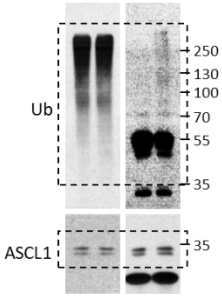
fig. S3K



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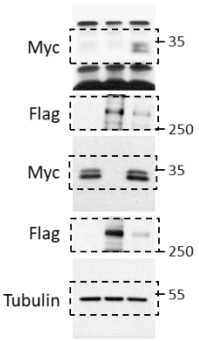

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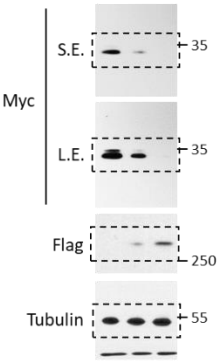

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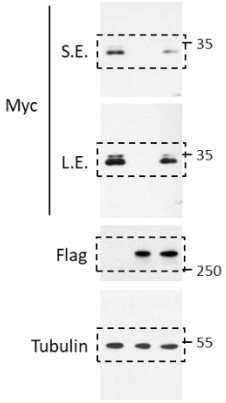

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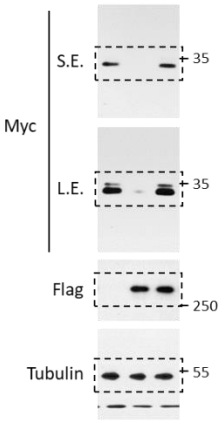

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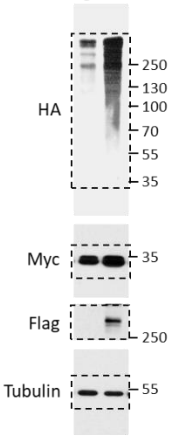

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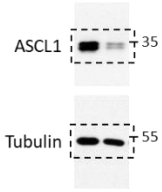

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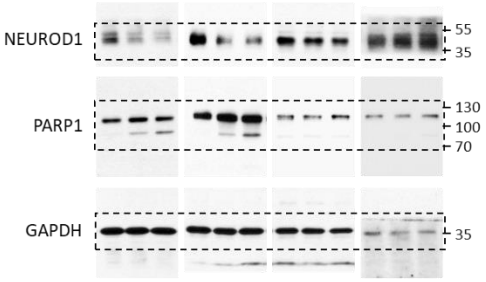

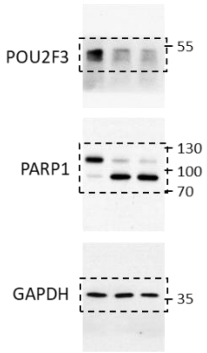

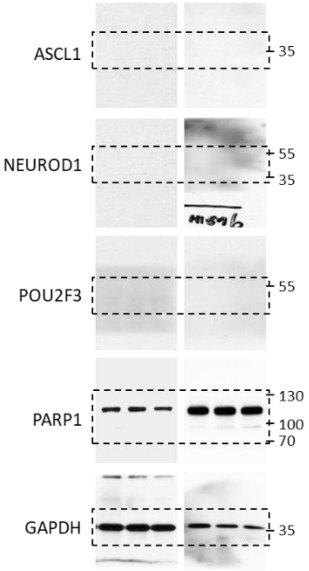

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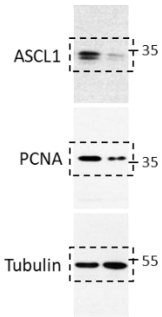

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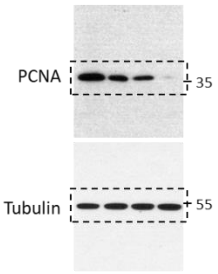

Tables.