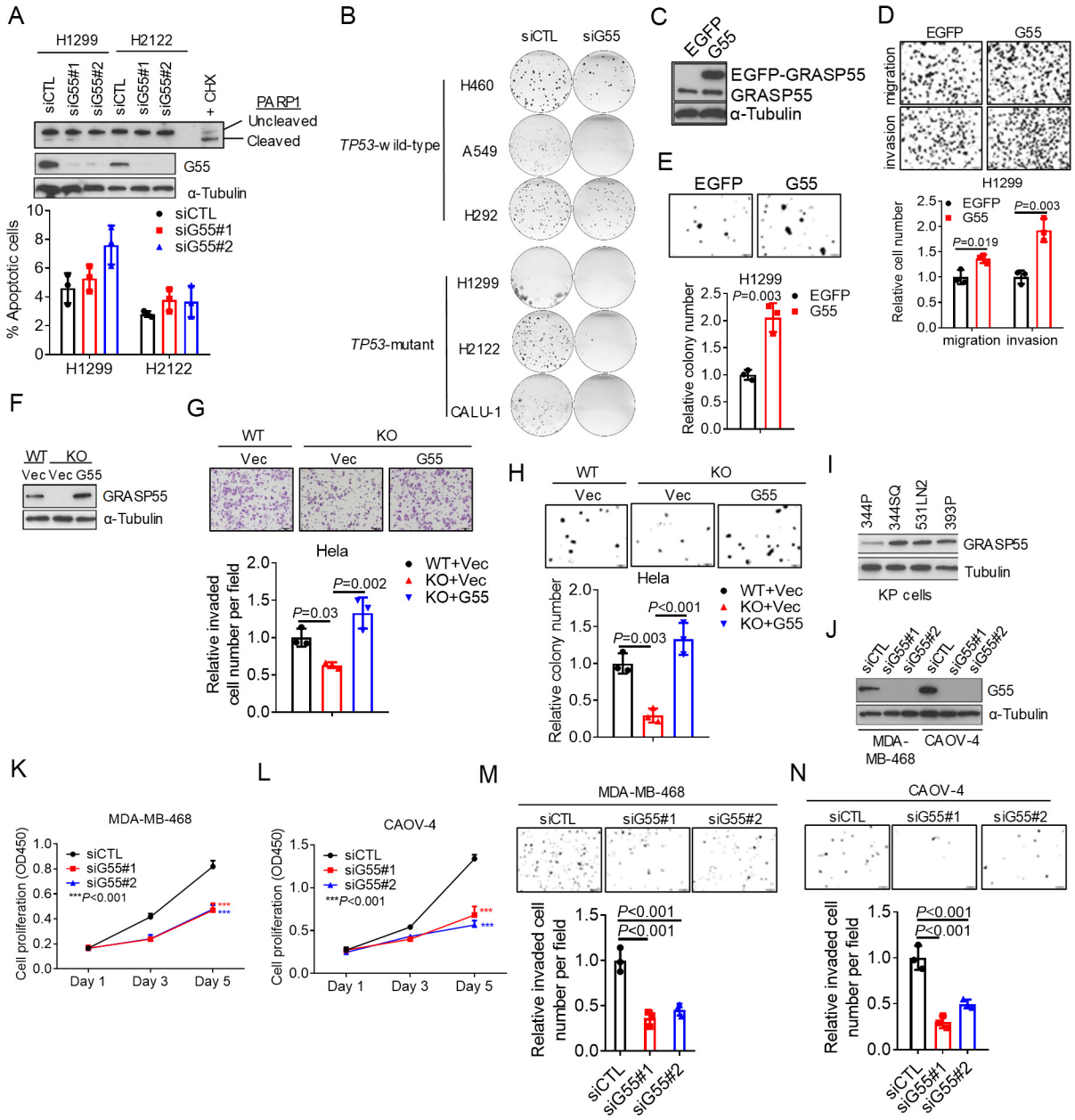
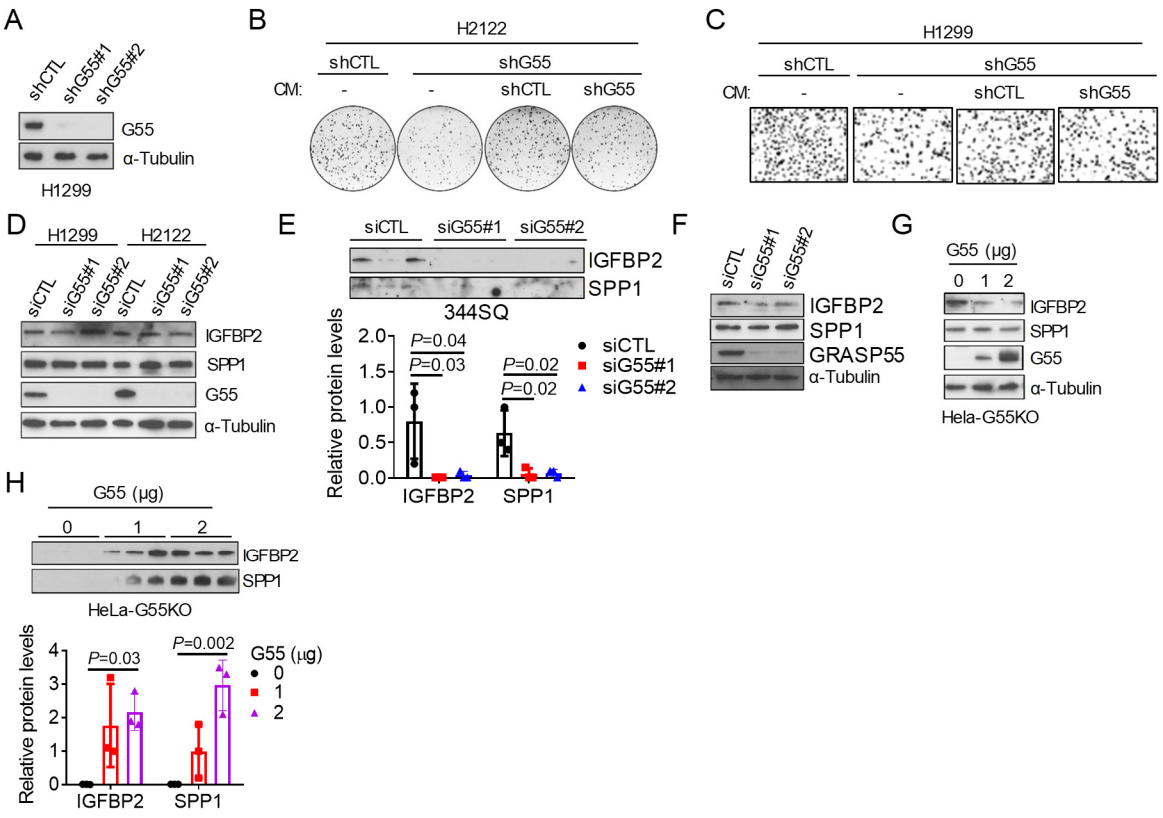
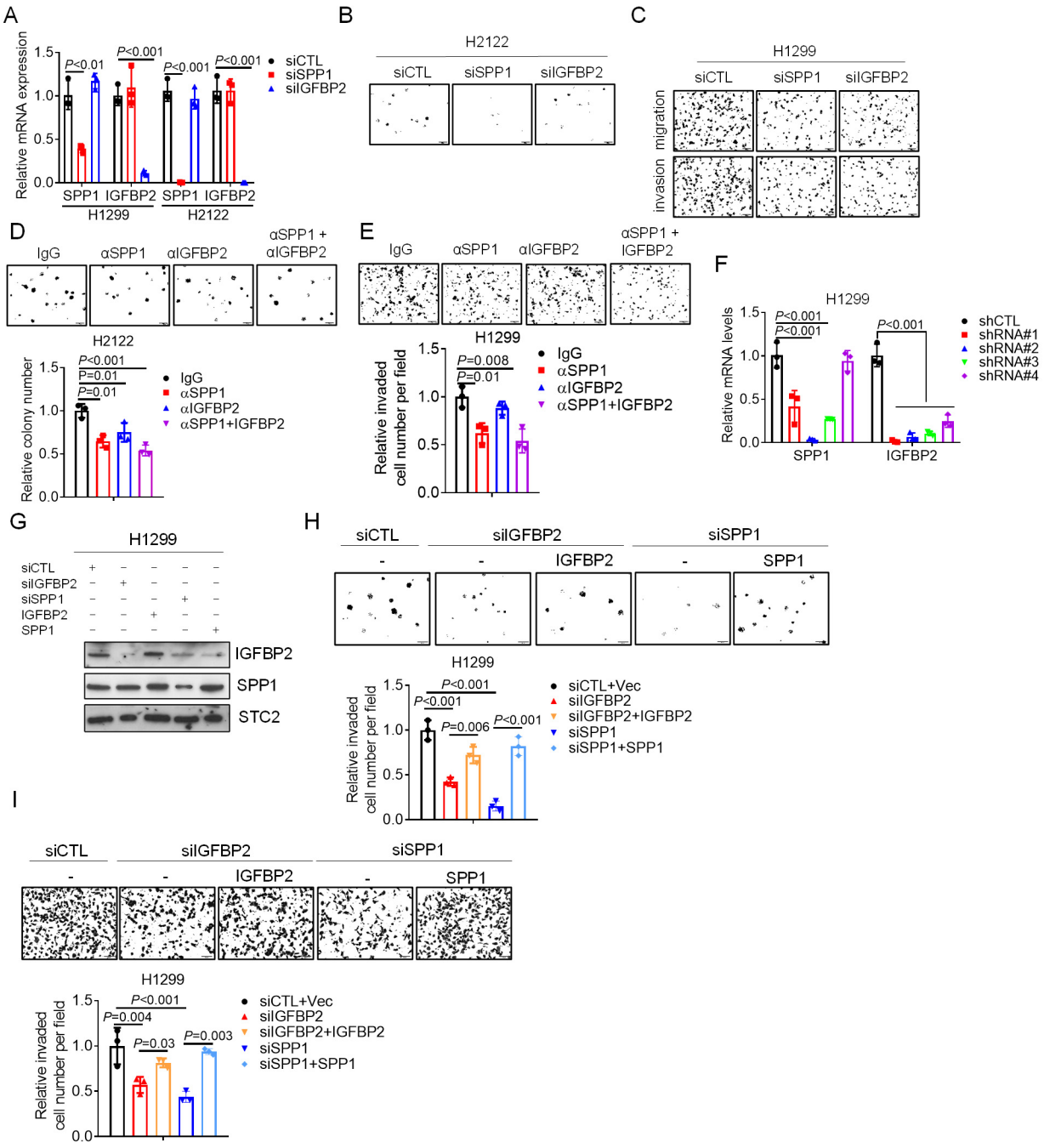


Supplemental Figure 1. High G55 drives *TP53*-mutant LUAD progression. (A) WB analysis of G55 (G55) and PARP1 in H1299 and H2122 cells transfected with G55 or control siRNAs. Cycloheximide treatment (+ CHX) included as a positive control for PARP cleavage. α-tubulin included as loading control. Apoptotic cells were quantified by Annexin V/propidium iodide flow cytometry (bar graph). (B) Anchorage-independent colonies formed in soft agar by *TP53*-mutant and –wild-type human LUAD cell lines transfected with G55 (G55) or control (CTL) siRNAs. (C)

WB analysis of H1299 cells transfected with EGFP-tagged G55 (G55) or empty (EGFP) expression vectors. Ectopic (EGFP-G55) and endogenous (G55) G55-specific bands indicated. (D, E) Migration and invasion (D) and anchorage-independent colony formation (E) assays on H1299 cells in (C). (F) WB analysis of G55 protein levels in parental (WT) and *GORASP2* KO HeLa cells transfected with G55 or empty (Vec) expression vectors. (G, H) Invasion (G) and anchorage-independent colony formation (H) assays on HeLa cells in (F). (I) WB analysis of G55 protein levels in a panel of LUAD cell lines (KP cells) derived from mice that co-express K-ras^{G12D} and p53^{R172H}. (J) WB analysis of G55 in MDA-MB-468 breast cancer cells and CAOV-4 ovarian cancer cells transfected with control (siCTL) or G55 (siG55) siRNAs. (K, L) Cell proliferation assays on MDA-MB-468 cells (K) and CAOV-4 cells (L) using WST-1. (M, N) Invasion assays on MDA-MB-468 cells (M) and CAOV-4 cells (N) in Boyden chambers. Results expressed relative to siCTL. Results represent mean values ± SD. n = 3, unless otherwise indicated. P values, two-way t-test (D, E), ANOVA (all others).

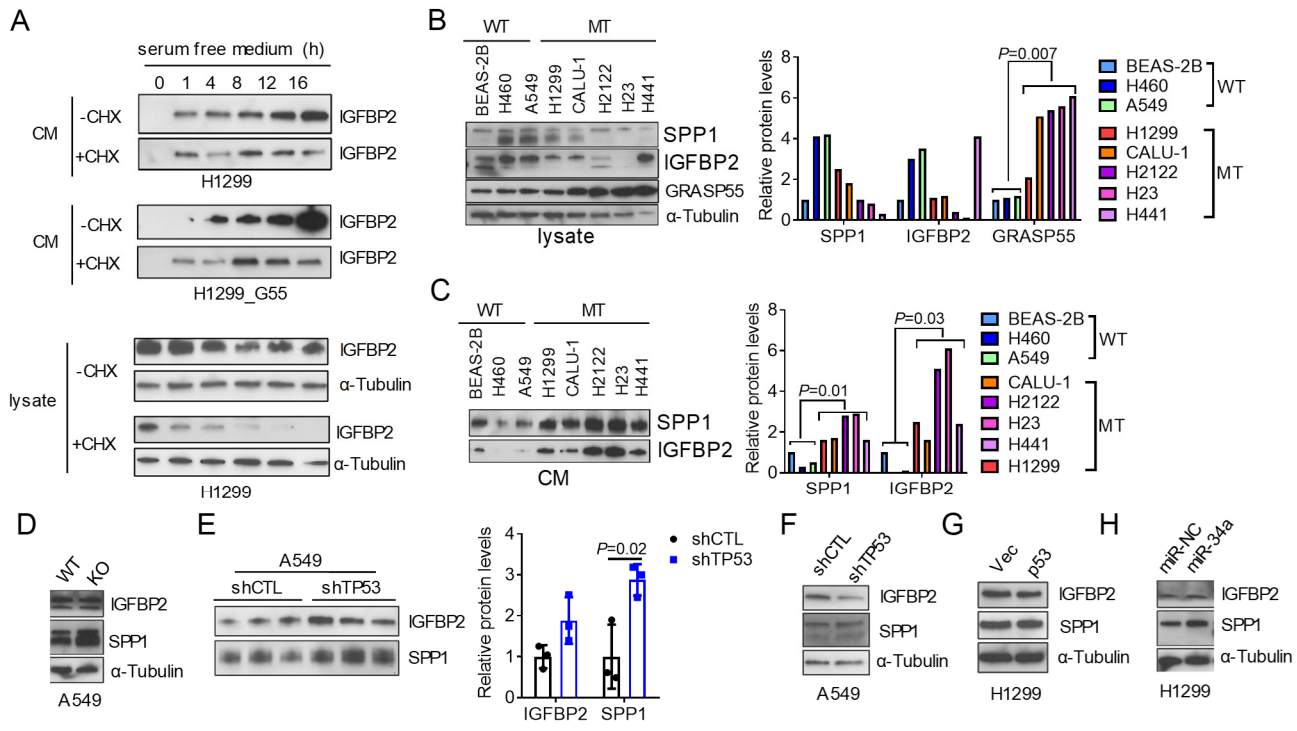


Supplemental Figure 2. G55 activates the secretion of IGFBP2 and SPP1. (A) WB analysis of H1299 cells stably transfected with indicated shRNAs. (B) Anchorage-independent colonies in soft agar treated for 10 d with or without (-) conditioned medium (CM) samples. (C) Invasive cells in Boyden chambers treated for 24 h with or without CM as described in (B). (D) WB analysis of cell lysates. (E, F) WB analysis of CM samples (E) and cell lysates (F) from 344SQ cell transfectants. Densitometric results expressed relative to siCTL (bar graph). (G, H) WB analysis of cell lysates (G) and CM samples (H) from *GORASP2* KO (G55KO) HeLa cells transfected with indicated amounts of G55 or empty (0 μg) expression vectors. Densitometric analysis of WB (bar graph). Results represent mean values ± SD. n = 3, unless otherwise indicated. P values, ANOVA.

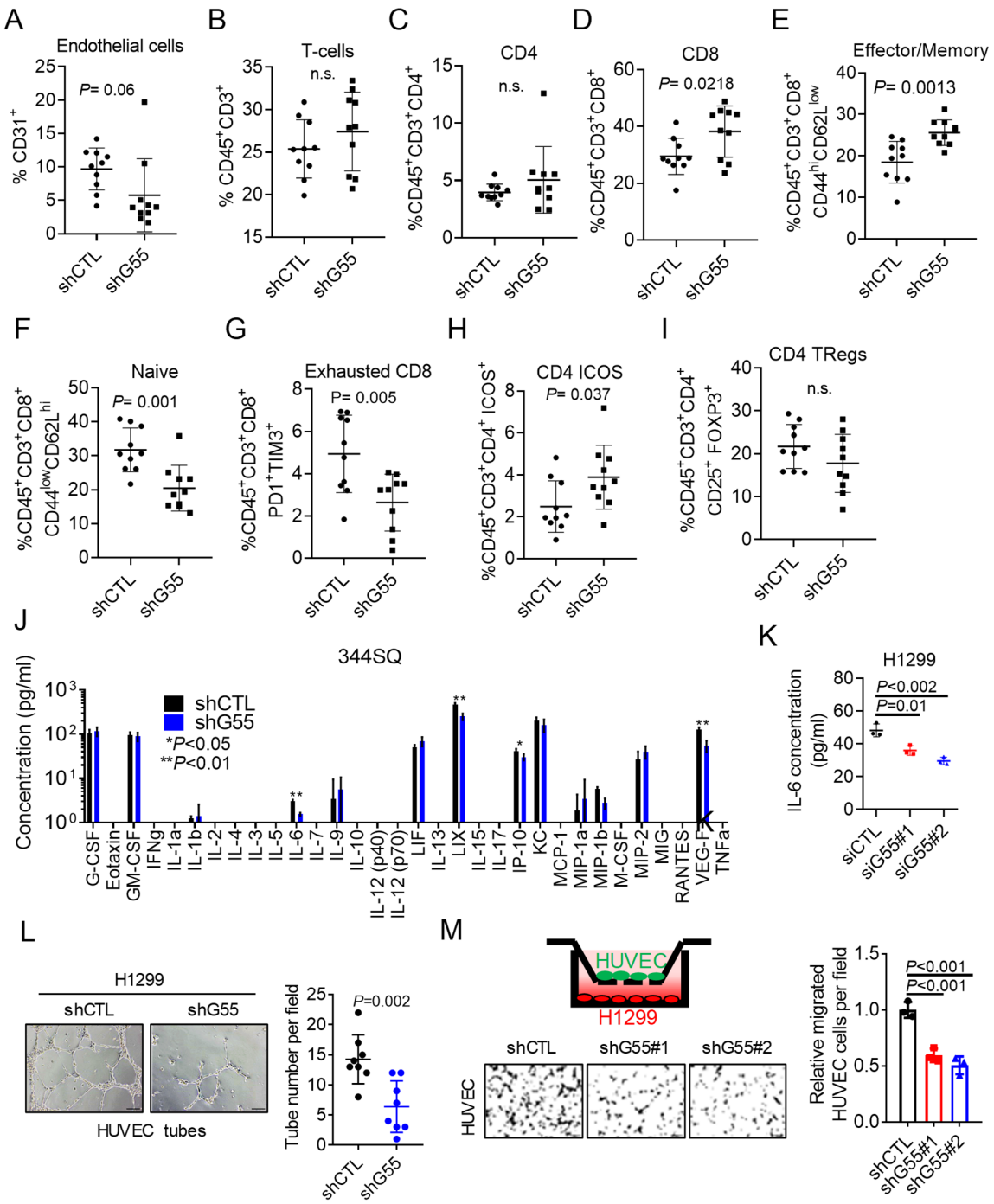


Supplemental Figure 3. IGFBP2 and SPP1 are pro-tumorigenic in *TP53*-mutant LUAD. (A) qPCR analysis to confirm siRNA-mediated target gene silencing in H1299 cells and H2122 cells. Results expressed relative to siCTL. (B, C) Anchorage-independent colonies in soft agar (B) and invasive and migratory cells in Boyden chambers by cells in (A) are quantified in Figures 3E and 3F, respectively. (D, E) Anchorage-independent colony formation assays on H2122 cells (D) and

invasion assays in Boyden chambers on H1299 cells (E) in the presence of anti-IGFBP2 (αIGFBP2) or –SPP1 (αSPP1) neutralizing antibodies or IgG. (F) Quantitative real-time PCR (qPCR) assays to confirm target gene depletion in the indicated short hairpin RNA (shRNA) transfectants. (G) WB analysis of CM from H1299 cells transfected with or without (-) siIGFBP2 or siSPP1 and treated with or without (-) soluble proteins (100 ng/ml IGFBP2 or SPP1, respectively). Stanniocalcin 2 (STC2) included as a loading control. (H, I) Anchorage-independent colony formation assays in soft agar (H) and invasion assays in Boyden chambers (I) performed on H1299 cells that were treated as described in (G). Results represent mean values ± SD. n = 3, unless otherwise indicated. P values, ANOVA.

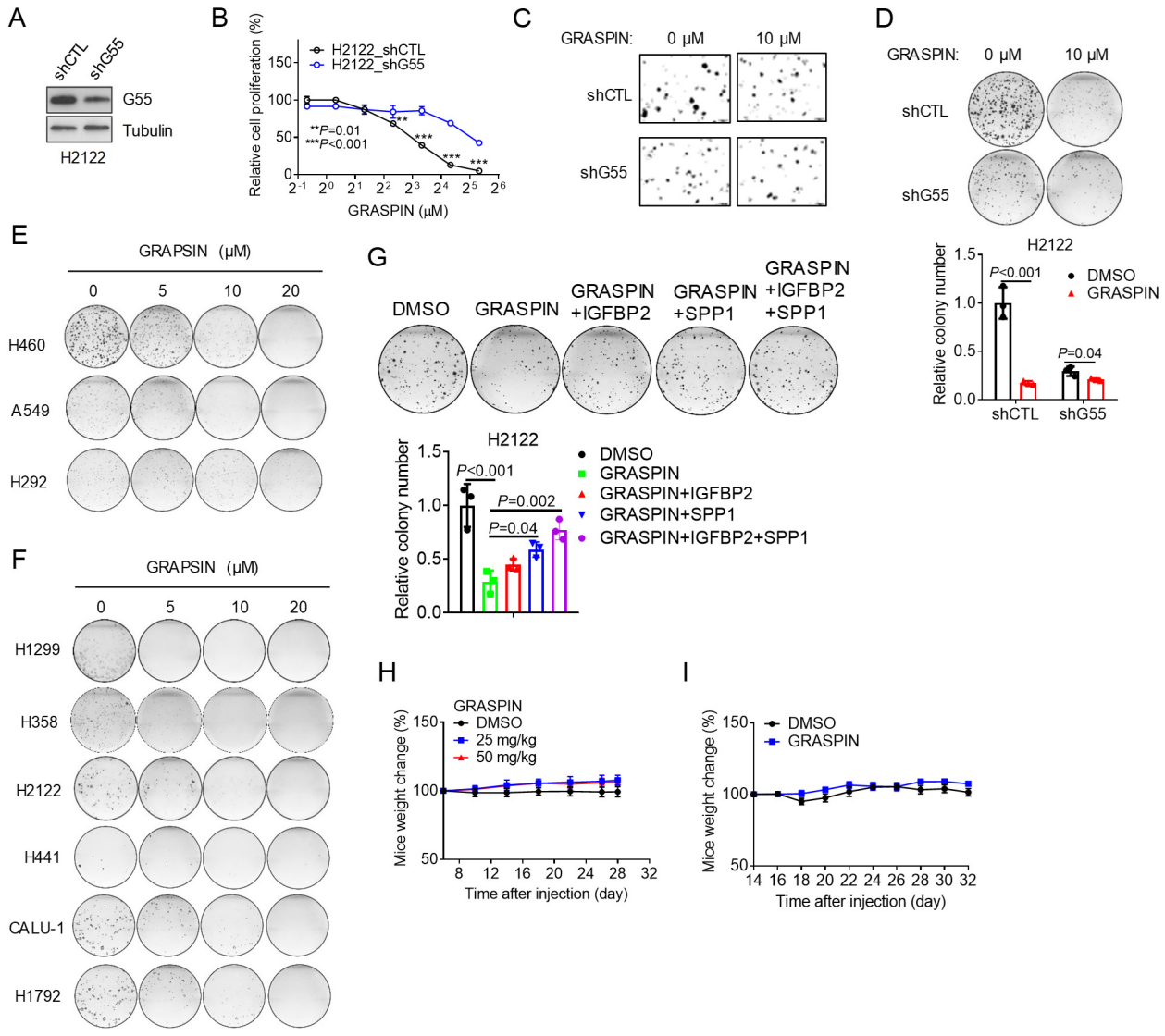


Supplemental Figure 4. p53 regulates the secretion, but not the intracellular levels, of IGFBP2 and SPP1. (A) WB analysis of IGFBP2 in CM and lysates from H1299 cells and H1299 cells that have ectopic G55 expression (H1299_G55) after treatment for the indicated durations with (+) or without (-) cycloheximide (CHX). (B, C) WB analysis of lysates (B) and CM (C) samples from *TP53*-wild-type (WT) or –mutant (MT) human LUAD cell lines. Densitometric analysis (bar graph) to compare protein levels in WT and MT cells. (D) WB analysis of lysates from parental (WT) and *TP53* KO (KO) A549 cells. (E) WB analysis of CM samples from A549 cells transfected with indicated shRNAs. Densitometric analysis (bar graph). Results expressed relative to shCTL. (F-H) WB analysis of lysates from A549 cells transfected with indicated shRNAs (F) or H1299 cells transfected with p53 or empty (Vec) expression vectors (G) or miR-34a or negative control (miR-NC) (H). Results represent mean values ± SD. n = 3, unless otherwise indicated. P values, two-way t-test.



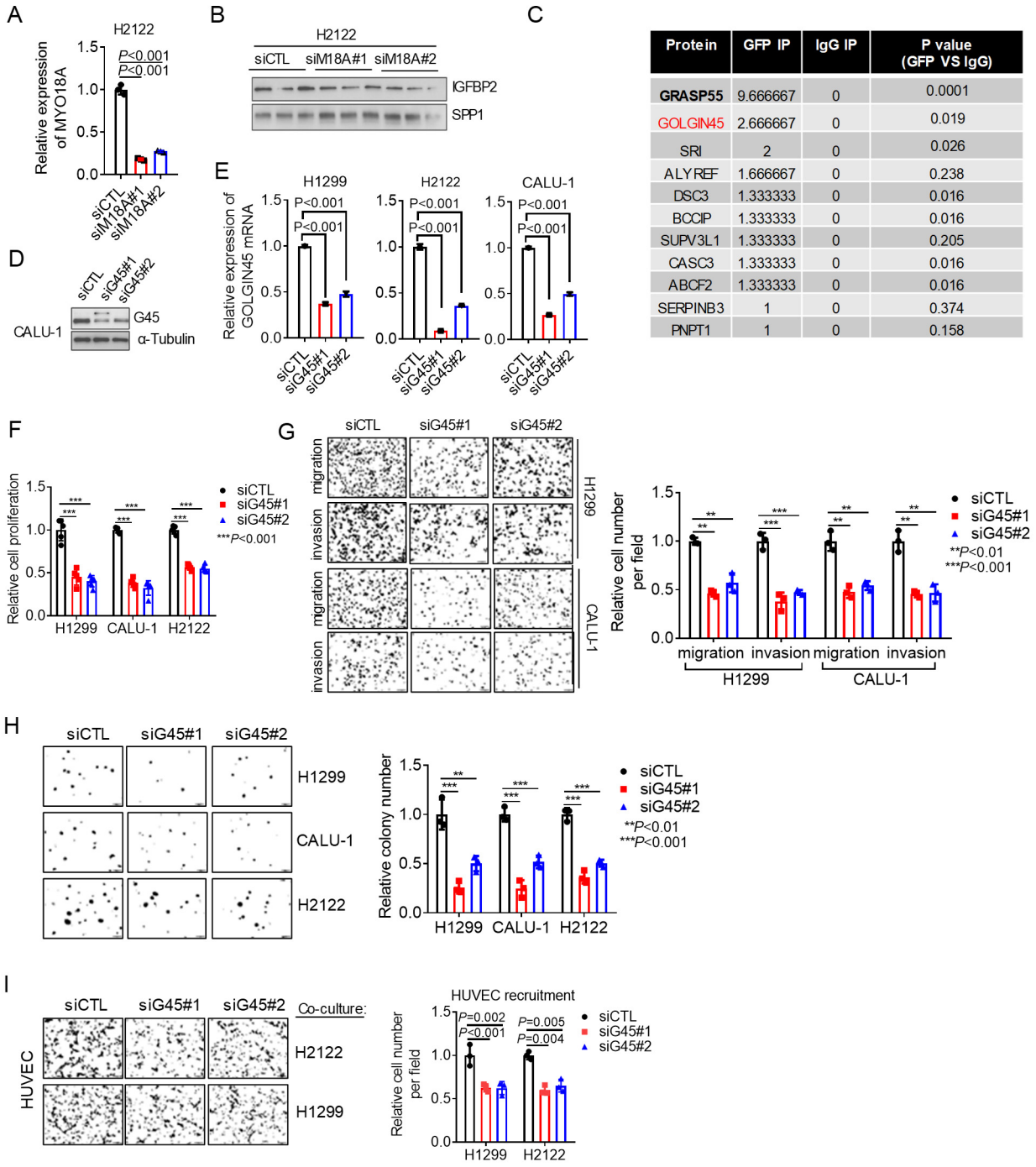
Supplemental Figure 5. G55-dependent secretion promotes tumor microenvironment remodeling. (A-I) Flow cytometric analysis of G55-deficient (shG55) and –replete (shCTL) 344SQ flank tumors isolated from syngeneic, immunocompetent mice (n=8 tumors per cohort). Percentages of $CD31^+$ endothelial cells (A), total T cells (B), $CD4^+$ T cells (C), $CD8^+$ T cells (D), $CD44^{hi}CD62L^{low}$

effector/memory $CD8^+$ T cells (E), $CD44^{low}CD62L^{hi}$ naïve $CD8^+$ T cells (F), $PD1^+TIM3^+$ exhausted $CD8^+$ T cells (G), $ICOS^+$ $CD4^+$ T cells (H), and $CD25^+$ $FOXP3^+$ regulatory T cells (I) were quantified. (J) Cytokine concentrations in CM samples from G55-deficient (shG55) and –replete (shCTL) 344SQ cells. Values determined from triplicate samples by multiplexed antibody bead assays. (K) IL-6 concentrations by enzyme-linked immunosorbent assay in H1299 cells transfected with indicated siRNAs. (L, M) HUVEC tube formation (L) and migration (M) quantified in co-culture with siCTL- or siG55-transfected H1299 cells in Boyden chambers. Results represent mean values ± SD. n = 3, unless otherwise indicated. P values: ANOVA (K, M), two-sided t-test (all others).



Supplemental Figure 6. GRASPIN inhibits the proliferative and colony forming activities of *TP53*-mutant LUAD cells. (A) WB analysis of the indicated H2122 transfectants. (B) Cell proliferation by WST-1. Results expressed relative to values from shCTL transfectants treated with lowest GRASPIN dose. n=5. (C) Anchorage-independent colonies generated by H1299 cells in soft agar quantified in Figure 4D. (D) Anchorage-dependent colony formation assays on shG55- and shCTL-transfected H2122 cells treated with or without (0 μg) GRASPIN. Results expressed relative to controls (shCTL/DMSO) (bar graph). (E, F) Anchorage-dependent colonies formed by *TP53*-wildtype (E) and –mutant (F) LUAD cells in the presence or absence (0 μg) of GRASPIN

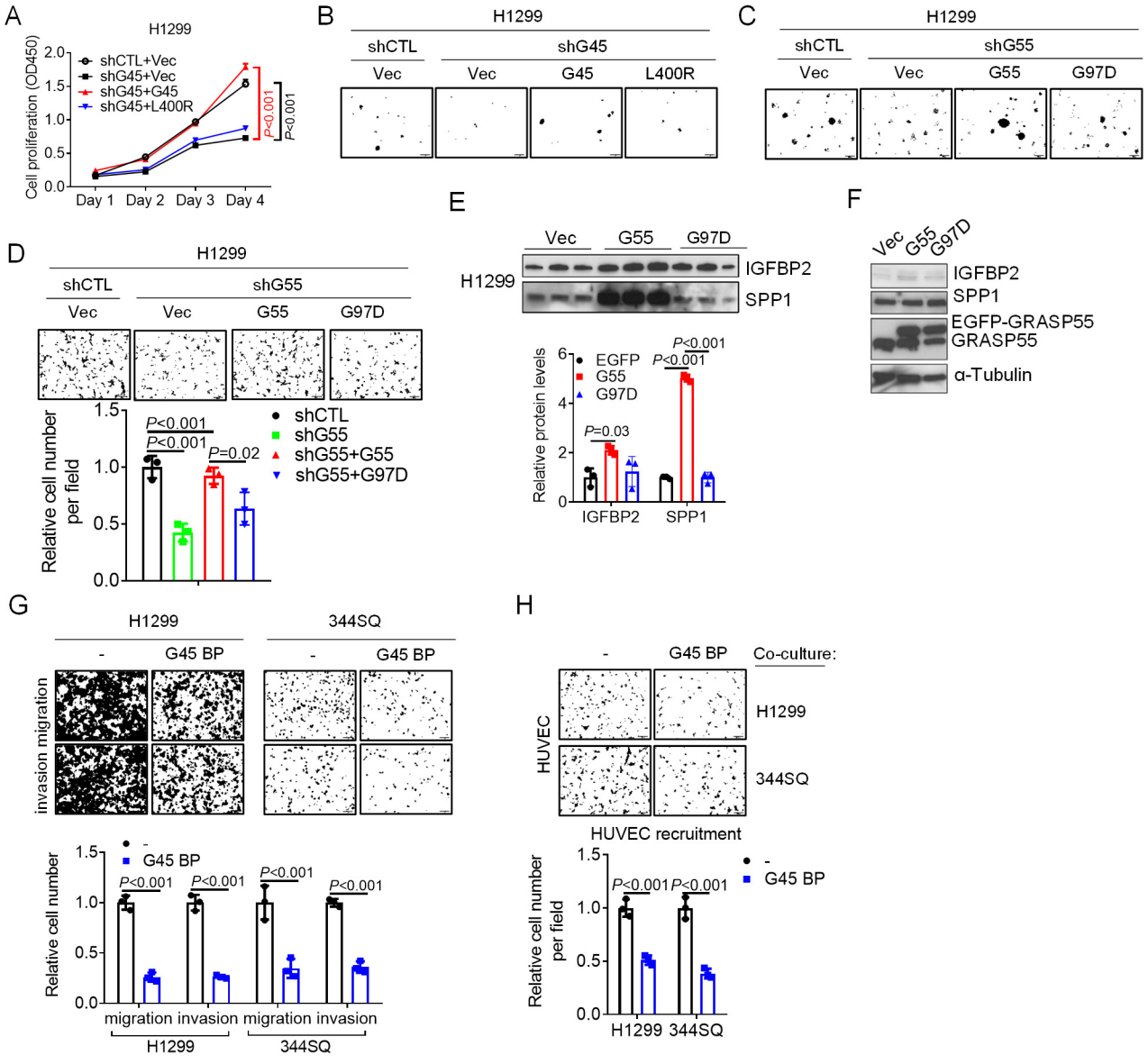
were quantified in Figure 4E. (G) Anchorage-dependent colony formation by H2122 cells treated with vehicle (DMSO) or GRASPIN alone or in combination with soluble IGFBP2 or SPP1 proteins, singly and in combination. Results expressed relative to DMSO control (bar graph). (H, I) Body weights of 344SQ (H) and H1299 (I) orthotopic tumor-bearing mice on the indicated days after tumor cell injection. n=10 mice per cohort. Results represent mean values ± SD. n = 3, unless otherwise indicated. P values, ANOVA (G), two-way t-test (all others).



Protein	GFP IP	IgG IP	P value (GFP VS IgG)
GRASP55	9.666667	0	0.0001
GOLGIN45	2.666667	0	0.019
SRI	2	0	0.026
ALYREF	1.666667	0	0.238
DSC3	1.333333	0	0.016
BCCIP	1.333333	0	0.016
SUPV3L1	1.333333	0	0.205
CASC3	1.333333	0	0.016
ABCF2	1.333333	0	0.016
SERPINB3	1	0	0.374
PNPT1	1	0	0.158

Supplemental Figure 7. GOLGIN45 (G45) is a key mediator of G55. (A) qPCR analysis to quantify MYO18A mRNA levels in MYO18IIA siRNA (siM18A)- and siCTL-transfected H2122 cells. (B) WB analysis of CM samples from cells in (A). (C) Proteins identified by LC-MS (≥ 2 peptides per protein, 1% false discovery rate). (D) WB analysis to quantify G45 levels in CALU1 cells

transfected with siG45 or siCTL (E) qPCR analysis of G45 mRNA levels in siG45- and siCTL-transfected human LUAD cell lines. (F) Cell proliferation determined using WST-1. (G) Migration and invasion in Boyden chambers. (H) Anchorage-independent colony formation in soft agar. (I) HUVEC migration in co-culture with siG45- or siCTL-transfected H2122 cells or H1299 cells. Results expressed relative to siCTL and represent mean values ± SD. n = 3, unless otherwise indicated. P values, ANOVA.

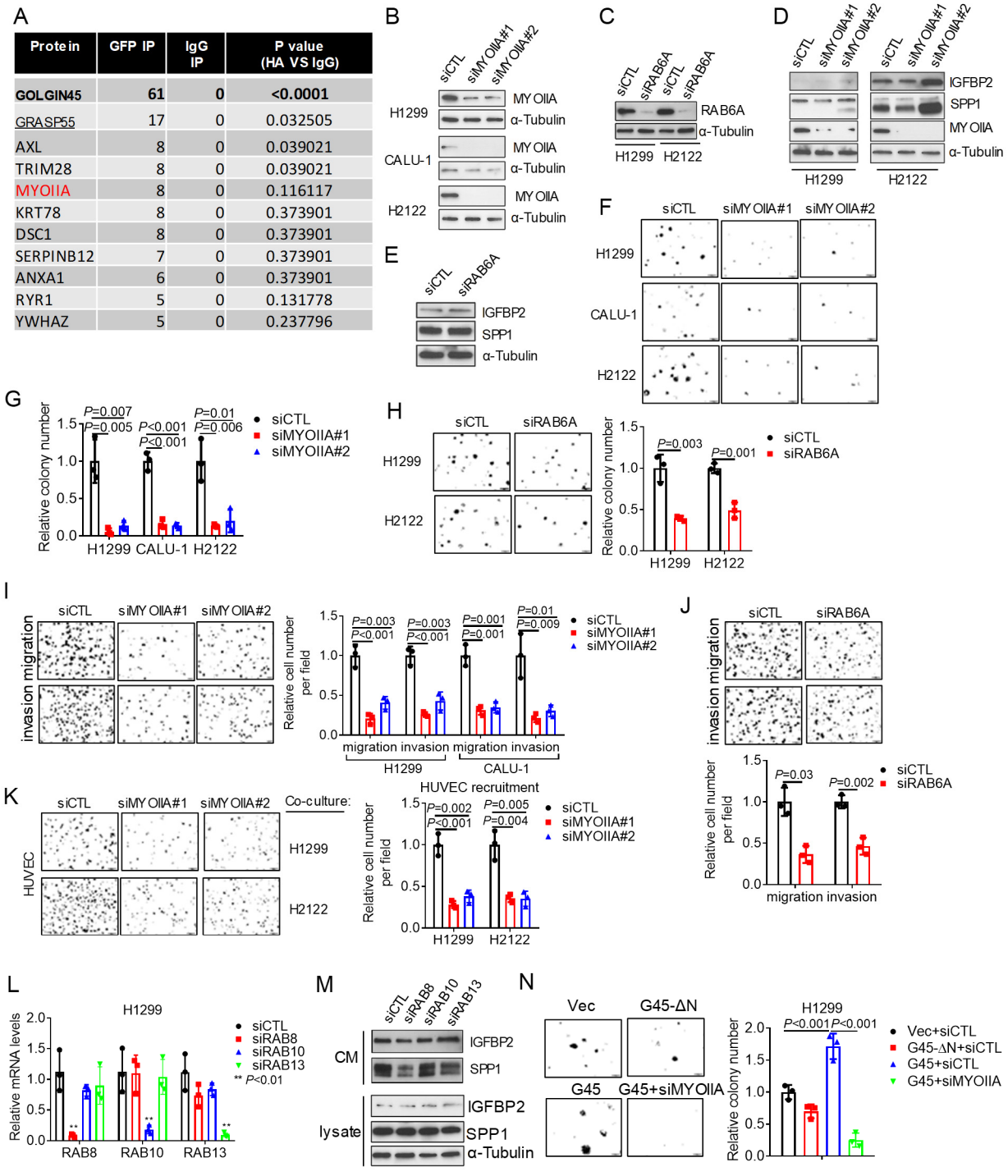


Supplemental Figure 8. G55/G45 interactions govern LUAD cell properties. (A) Proliferation of shG45-transfected H1299 cells reconstituted with wild-type (G45) or L400R-mutant G45 that cannot interact with G55. Empty vector (Vec). Control shRNA (shCTL). Results expressed relative to shCTL/Vec on day 1. n=5. (B) Anchorage-independent colonies formed by shG45-transfected H1299 cells reconstituted with wild-type (G45) or mutant (L400R) G45. (C, D) Anchorage-independent colony formation assays (C) and invasion assays (D) on shG55-transfected H1299 cells reconstituted with wild-type (G55) or G97D-mutant G55 that cannot interact with G45. (E, F)

WB analysis of CM samples (E) and lysates (F) from cells in (D). (G) Migration and invasion assays on H1299 cells and 344SQ cells that ectopically express a 30-amino acid G45 peptide (G45 BP) that blocks G55/G45 interactions. Empty vector (-). (H) HUVEC migration in co-culture with H1299 cells or 344SQ cells that ectopically express G45 BP or empty vector (-). Results expressed relative to siCTL and represent mean values ± SD. n = 3, unless otherwise indicated. P values, ANOVA (A, D, and E), two-way t-test (G, H).

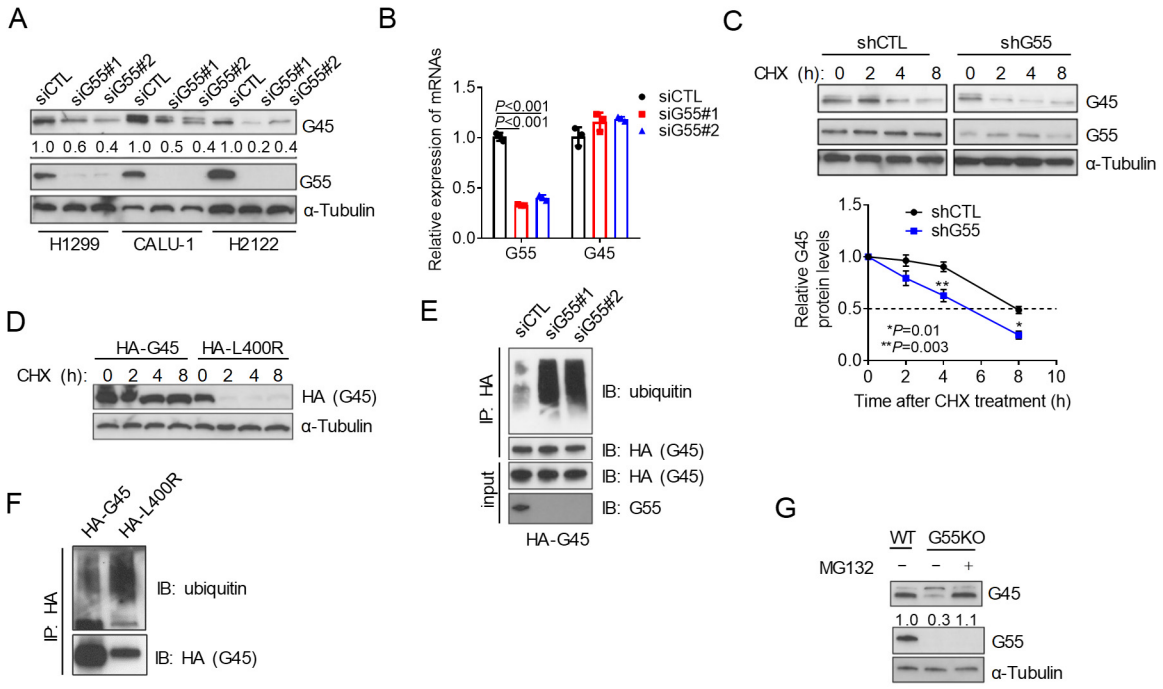
A

Protein	GFP IP	IgG IP	P value (HA VS IgG)
GOLGIN45	61	0	<0.0001
GRASP55	17	0	0.032505
AXL	8	0	0.039021
TRIM28	8	0	0.039021
MYOIIA	8	0	0.116117
KRT78	8	0	0.373901
DSC1	8	0	0.373901
SERPINB12	7	0	0.373901
ANXA1	6	0	0.373901
RYR1	5	0	0.131778
YWHAZ	5	0	0.237796



Supplementary Figure 9. MYOIIA and RAB6A mediate G55-dependent LUAD cell properties. (A) Proteins identified by LC-MS (≥ 2 peptides per protein, 1% false discovery rate) using HA-G45 as bait. (B, C) WB analysis to quantify target gene expression in LUAD cells transfected with

siMYOIIA (B) or siRAB6A. (D, E) WB analysis of lysates from cells transfected with siMYOIIA (D) or siRAB6A (E). (F-H) Anchorage-independent colony formation in soft agar by LUAD cells transfected with siMYOIIA (F, G) or siRAB6A (H). (I, J) Migration and invasion assays on cells transfected with siMYOIIA (I) or siRAB6A (J). (K) HUVEC migration in co-culture with H1299 cells or H2122 cells transfected with siMYOIIA or siCTL. (L) qPCR to quantify target gene mRNA levels in siRNA-transfected H1299 cells. (M) WB analysis of CM and lysates from cells in (L). (N) Anchorage-independent colony formation by H1299 cells co-transfected with full-length (G45) or N-terminally truncated G45 (G45-ΔN) and siMYOIIA. Empty vector (Vec). Results expressed relative to siCTL and represent mean values ± SD. n = 3, unless otherwise indicated. P values, ANOVA (G, I, K, L and N), two-way t-test (all others).



Supplemental Figure 10. G55 increases G45 protein stability. (A) WB analysis of G55 and G45 in siG55 and siCTL-transfected LUAD cell lines. G45 densitometric values expressed relative to siCTL. (B) qPCR analysis of G45 and G55 in siG55- and siCTL-transfected H1299 cells. Results expressed relative to siCTL. (C) WB analysis of G55 and G45 protein levels in shG55- and shCTL-transfected H1299 cells treated with 20 μM cycloheximide (CHX) for the indicated time periods. Densitometric analysis of G45 protein levels over time (line graph). (D) WB analysis of H1299 cells transfected with hemagglutinin (HA)-tagged wild-type (G45) or mutant (L400R) G45 expression vectors and treated with 20 μM CHX for the indicated time periods. (E) WB analysis of H1299 cells co-transfected with HA-tagged G45 and G55 or siCTL siRNAs and treated with 20 μM MG-132. (F) Immunoprecipitation (IP)/WB analysis of H1299 cells transfected with HA-tagged wild-type (G45) or mutant (L400) G45 and treated with 20 μM MG-132. Proteins immunoprecipitated from cell lysates using anti-HA antibody were subjected to WB using anti-ubiquitin or anti-HA antibodies. (G) WB analysis of parental (WT) and G55 KO HeLa cells treated

with (+) or without (-) 20 μM MG132. Results represent mean values ± SD. n = 3, unless otherwise indicated. P values, ANOVA (B), two-way t-test (C).