

SUPPLEMENTAL INFORMATION

Hsp90 α forms condensate engaging client proteins with RG motif repeats

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a

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.1M Citric acid pH 3.5	0.1M Citric acid pH 3.5, 50% (w/v) PEG 500	0.1M Citric acid pH 3.5, 20% (w/v) PEG 3,350	0.1M Citric acid pH 3.5, 20% (w/v) PEG 8,000	0.1M Citric acid pH 3.5, 20% (w/v) Ficoll 400	0.1M Citric acid pH 3.5, 20% (w/v) Dextran 10	0.1M Citric acid pH 3.5, 20% (w/v) Dextran 40	0.1M Citric acid pH 3.5, 20% (w/v) Dextran 70	0.1M Citric acid pH 3.5, 3 M Sodium chloride	0.1M Citric acid pH 3.5, 3 M Ammonium sulfate	50 μ M CR7	50 μ M CR20
B	0.1M Citric acid pH 4.5	0.1M Citric acid pH 4.5, 50% (w/v) PEG 500	0.1M Citric acid pH 4.5, 20% (w/v) PEG 3,350	0.1M Citric acid pH 4.5, 20% (w/v) PEG 8,000	0.1M Citric acid pH 4.5, 20% (w/v) Ficoll 400	0.1M Citric acid pH 4.5, 20% (w/v) Dextran 10	0.1M Citric acid pH 4.5, 20% (w/v) Dextran 40	0.1M Citric acid pH 4.5, 20% (w/v) Dextran 70	0.1M Citric acid pH 4.5, 3 M Sodium chloride	0.1M Citric acid pH 4.5, 3 M Ammonium sulfate	200 μ M CR7	200 μ M CR20
C	0.1M Bis-Tris pH 5.5	0.1M Bis-Tris pH 5.5, 50% (v/v) PEG 500	0.1M Bis-Tris pH 5.5, 20% (w/v) PEG 3,350	0.1M Bis-Tris pH 5.5, 20% (w/v) PEG 8,000	0.1M Bis-Tris pH 5.5, 20% (w/v) Ficoll 400	0.1M Bis-Tris pH 5.5, 20% (w/v) Dextran 10	0.1M Bis-Tris pH 5.5, 20% (w/v) Dextran 40	0.1M Bis-Tris pH 5.5, 20% (w/v) Dextran 70	0.1M Bis-Tris pH 5.5, 3 M Sodium chloride	0.1M Bis-Tris pH 5.5, 3 M Ammonium sulfate	1000 μ M CR7	1000 μ M CR20
D	0.1M Bis-Tris pH 6.5	0.1M Bis-Tris pH 6.5, 50% (v/v) PEG 500	0.1M Bis-Tris pH 6.5, 20% (w/v) PEG 3,350	0.1M Bis-Tris pH 6.5, 20% (w/v) PEG 8,000	0.1M Bis-Tris pH 6.5, 20% (w/v) Ficoll 400	0.1M Bis-Tris pH 6.5, 20% (w/v) Dextran 10	0.1M Bis-Tris pH 6.5, 20% (w/v) Dextran 40	0.1M Bis-Tris pH 6.5, 20% (w/v) Dextran 70	0.1M Bis-Tris pH 6.5, 3 M Sodium chloride	0.1M Bis-Tris pH 6.5, 3 M Ammonium sulfate	200 μ M Heparin	200 μ M Heparin 20% (w/v) PEG 3,350
E	0.1M HEPES pH 7.5	0.1M HEPES pH 7.5, 50% (v/v) PEG 500	0.1M HEPES pH 7.5, 20% (w/v) PEG 3,350	0.1M HEPES pH 7.5, 20% (w/v) PEG 8,000	0.1M HEPES pH 7.5, 20% (w/v) Ficoll 400	0.1M HEPES pH 7.5, 20% (w/v) Dextran 10	0.1M HEPES pH 7.5, 20% (w/v) Dextran 40	0.1M HEPES pH 7.5, 20% (w/v) Dextran 70	0.1M HEPES pH 7.5, 3 M Sodium chloride	0.1M HEPES pH 7.5, 3 M Ammonium sulfate	1000 μ M Heparin	1000 μ M Heparin 10% (w/v) PEG 3,350
F	0.1M Tris pH 8.5	0.1M Tris pH 8.5, 50% (v/v) PEG 500	0.1M Tris pH 8.5, 20% (w/v) PEG 3,350	0.1M Tris pH 8.5, 20% (w/v) PEG 8,000	0.1M Tris pH 8.5, 20% (w/v) Ficoll 400	0.1M Tris pH 8.5, 20% (w/v) Dextran 10	0.1M Tris pH 8.5, 20% (w/v) Dextran 40	0.1M Tris pH 8.5, 20% (w/v) Dextran 70	0.1M Tris pH 8.5, 3 M Sodium chloride	0.1M Tris pH 8.5, 3 M Ammonium sulfate	2 μ g/ μ l PolyU	2 μ g/ μ l PolyU 20% (w/v) PEG 3,350
G	0.1M Zinc acetate	0.1M Calcium chloride	0.1M Magnesium acetate	0.1M Magnesium sulfate	0.1M Manganese chloride	0.1M Sodium chloride	0.1M Potassium acetate	0.1M Sodium acetate	0.1M Ammonium sulfate	0.1M Lithium chloride	5% (w/v) PEG 3,350	12% (w/v) PEG 3,350
H	0.1M Zinc acetate 20% (w/v) PEG 3,350	0.1M Calcium chloride 20% (w/v) PEG 3,350	0.1M Magnesium acetate 20% (w/v) PEG 3,350	0.1M Magnesium sulfate 20% (w/v) PEG 3,350	0.1M Manganese chloride 20% (w/v) PEG 3,350	0.1M Sodium chloride 20% (w/v) PEG 3,350	0.1M Potassium acetate 20% (w/v) PEG 3,350	0.1M Sodium acetate 20% (w/v) PEG 3,350	0.1M Ammonium sulfate 20% (w/v) PEG 3,350	0.1M Lithium chloride 20% (w/v) PEG 3,350	18% (w/v) PEG 3,350	25% (w/v) PEG 3,350

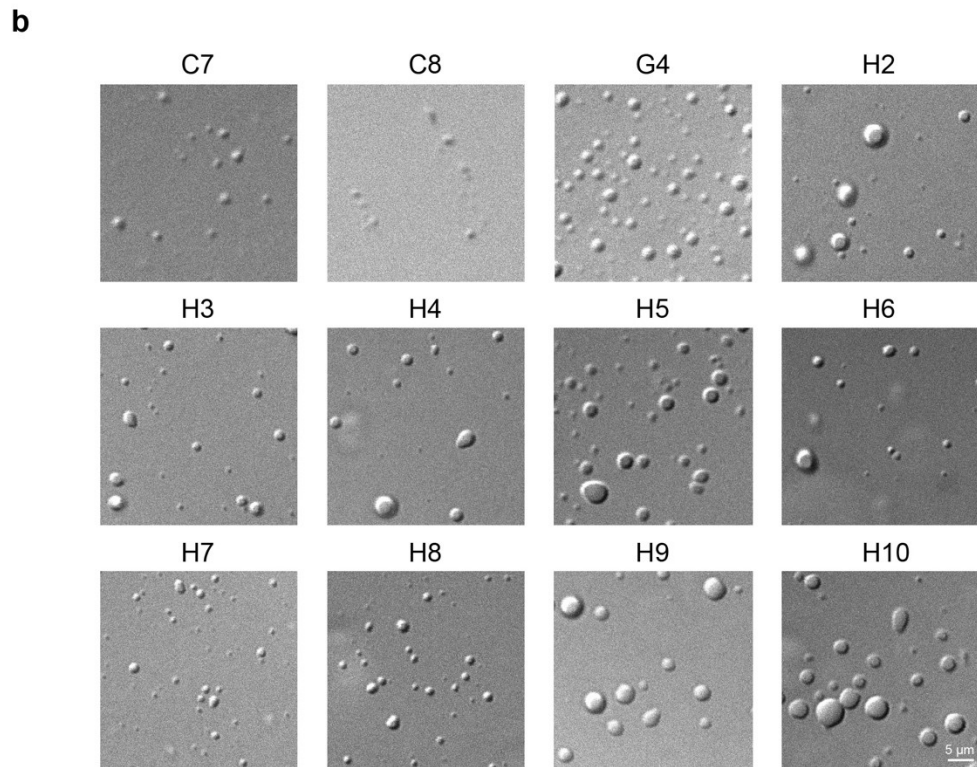


Figure S1. The conditions of HiPPS and Hsp90 α LLPS in HiPPS profiling.
 (a) Conditions included in HiPPS profiles.
 (b) DIC images for wells C7, C8, G4, and H2-H9.

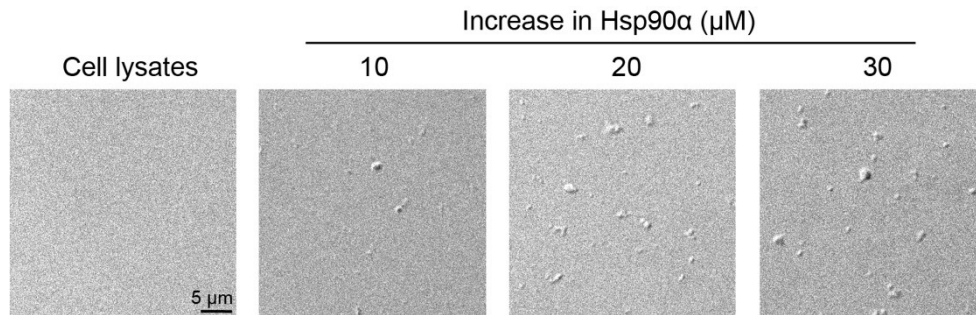


Figure S2. Addition of Hsp90α in cell lysates to induce Hsp90α granules.

DIC images of varying concentrations of Hsp90α LLPS in cell lysates (1 mg/ mL) after induction with 10% PEG 8,000 for 30 min.

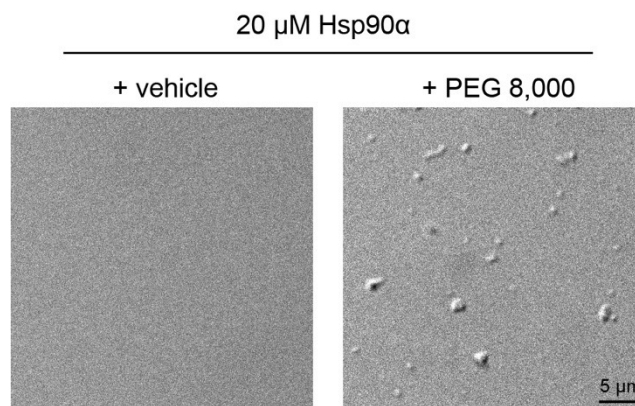


Figure S3. The formation of Hsp90α condensation in cell lysates.

DIC images of 20 μM Hsp90α LLPS in cell lysates (1 mg/ mL) with or without the addition of 10% PEG 8,000.

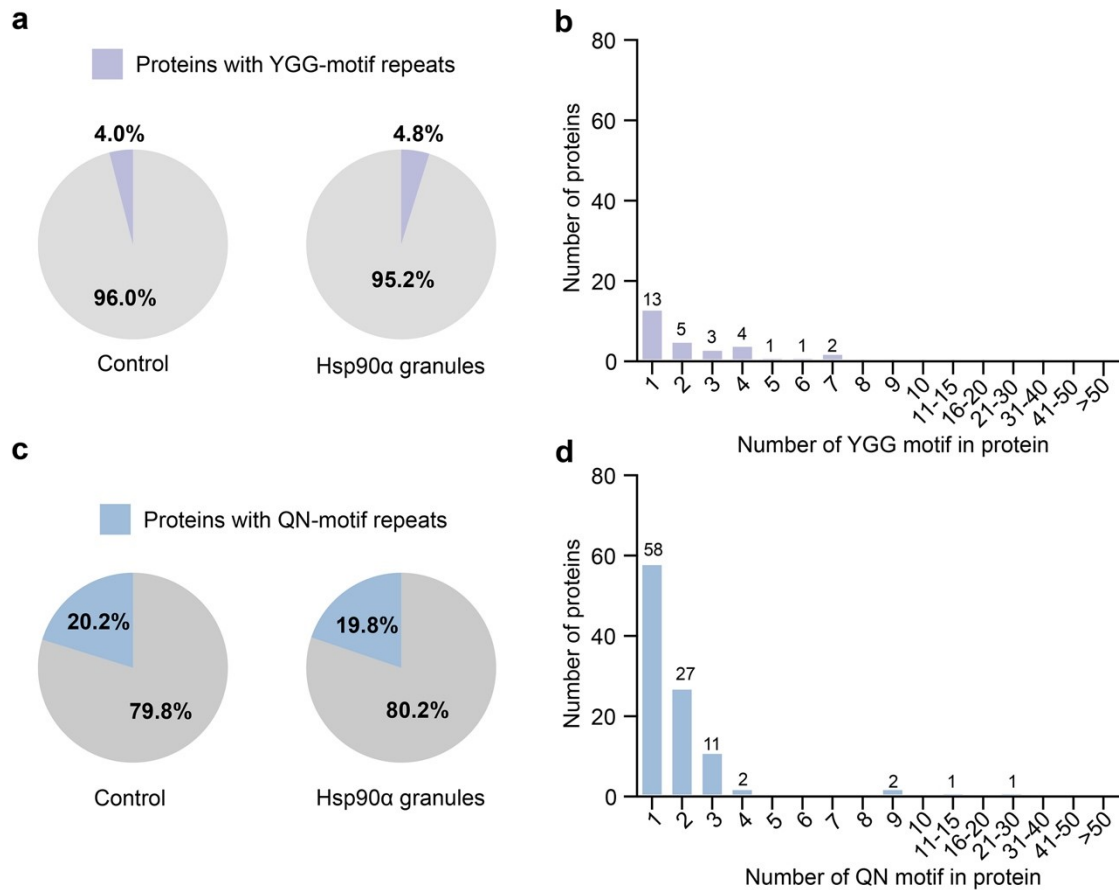


Figure S4. Analysis of YGG and QN motif in proteins from Hsp90α granules and control group.

(a) Percentage of YGG motif-containing proteins within the Hsp90α granules compared to percentage of YGG motif-containing proteins in the control.

(b) The number of YGG motifs in the client proteins of Hsp90α granules.

(c) Percentage of QN motif-containing proteins within the Hsp90α granules compared to percentage of QN motif-containing proteins in the control.

(d) The number of QN motifs in the client proteins of Hsp90α granules.

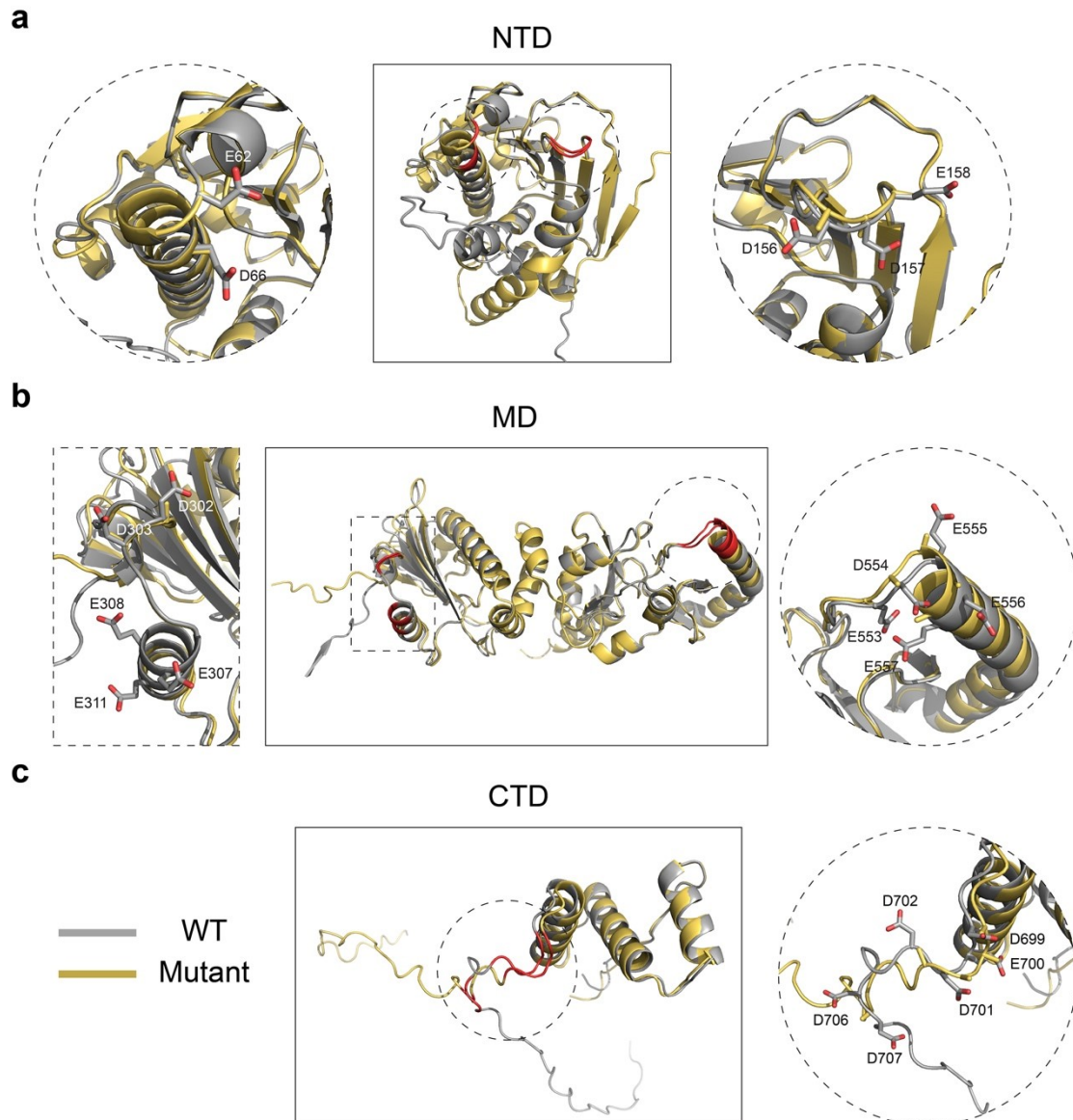


Figure S5. Structure comparison of NTD, MD, CTD and mutants using AlphaFold2. Comparison of NTD (a), MD (b) and CTD (c) constructs prediction models. WT constructs are colored with gray, while mutant constructs are colored with yellow. The mutating sites are colored with red with zoom-in views and residues labeled.

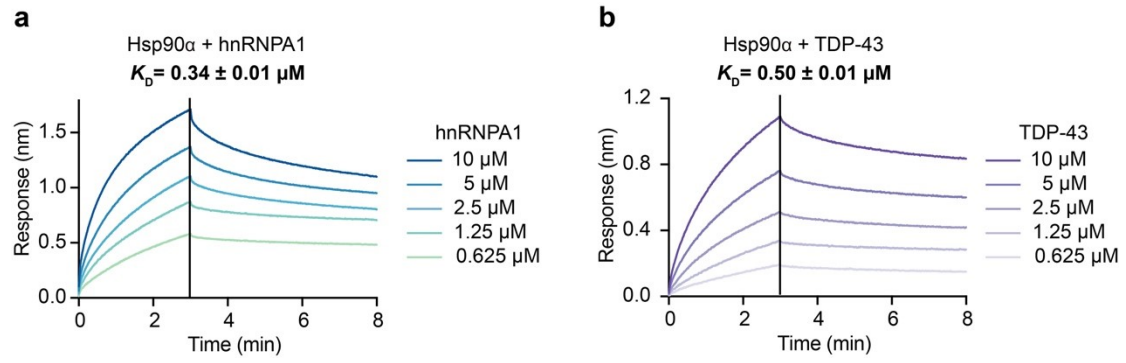


Figure S6. The binding affinities of hnRNPA1 and TDP-43 to Hsp90α measured by the BLI assay.

The binding affinities of Hsp90α to hnRNPA1 (a) and TDP-43 (b) were measured by BLI. The association and dissociation profiles are divided by a vertical black line.

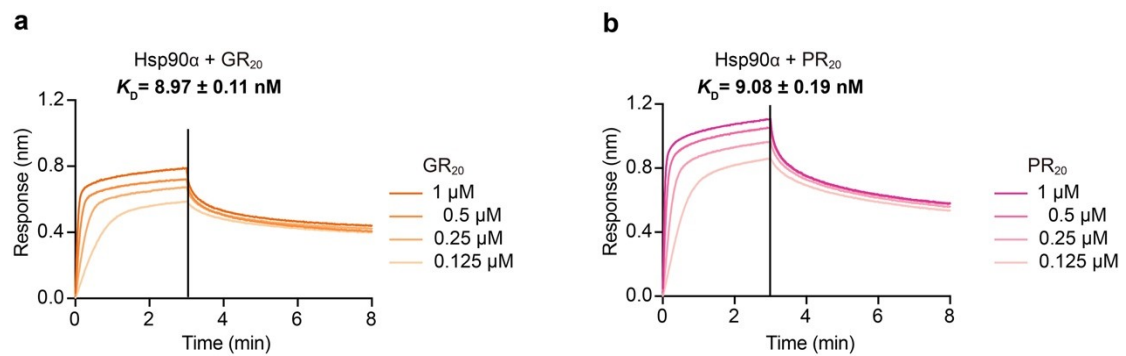


Figure S7. The binding affinities of GR₂₀ and PR₂₀ to Hsp90α measured by the BLI assay.

The binding affinities of Hsp90α to GR₂₀ (a) and PR₂₀ (b) were measured by BLI. The association and dissociation profiles are divided by a vertical black line.