

**Table S1 Composition and macronutrient energy distribution of the normal diet (NC) and Ketogenic diet (KD) used in this study.**

| Main components       | Normal Diet (g/kg) | Main components                             | Ketogenic Diet (g/kg) |
|-----------------------|--------------------|---------------------------------------------|-----------------------|
| Moisture              | 100                | Casein                                      | 173.3                 |
| Crude Protein         | 180                | DL-Methionine                               | 2.6                   |
| Crude Fat             | 40                 | Vegetable Shortening, hydrogenated (Crisco) | 586.4                 |
| Crude Fiber           | 50                 | Corn Oil                                    | 86.2                  |
| Crude Ash             | 80                 | Cellulose                                   | 87.97                 |
| Calcium (Ca)          | 10-18              | Vitamin Mix, Teklad (40060)                 | 13.0                  |
| Total Phosphorus (P)  | 6-12               | Choline Bitartrate                          | 2.5                   |
| Ca:P Ratio            | 1.2-1.7:1          | TBHQ, antioxidant                           | 0.13                  |
| Lysine                | 8.2                | Mineral Mix, Ca-P Deficient (79055)         | 20.0                  |
| Methionine+Cystine    | 5.3                | Calcium Phosphate, dibasic                  | 19.3                  |
|                       |                    | Calcium Carbonate                           | 8.2                   |
|                       |                    | Magnesium Oxide                             | 0.4                   |
| Protein (% kcal)      | 21.7               | Protein (% kcal)                            | 9.2                   |
| Carbohydrate (% kcal) | 66.5               | Carbohydrate (% kcal)                       | 0.3                   |
| Fat (% kcal)          | 11.8               | Fat (% kcal)                                | 90.5                  |

Diet composition data were provided by the manufacturer. Values are expressed as grams per kilogram of diet (g/kg). Macronutrient energy distribution is presented as the percentage of total caloric intake (% kcal).

**Table S2 Fatty acid profile of Ketogenic diet (KD) used in this study.**

| Profile (Unit)                        | Content (g/100g) |
|---------------------------------------|------------------|
| Capric acid (C10:0)                   | 0.0809           |
| Lauric acid (C12:0)                   | 0.0754           |
| Myristic acid (C14:0)                 | 1.04             |
| Pentadecanoic acid (C15:0)            | 0.0245           |
| Palmitic acid (C16:0)                 | 19.3             |
| Palmitoleic acid (C16:1)              | 1.28             |
| Heptadecanoic acid (C17:0)            | 0.152            |
| Stearic acid (C18:0)                  | 11.6             |
| Elaidic acid (C18:1n9t)               | 0.0387           |
| Oleic acid (C18:1n9c)                 | 25.4             |
| Linoleic acid (C18:2n6c)              | 13.3             |
| Arachidic acid (C20:0)                | 0.195            |
| Gamma-linolenic acid (C18:3n6)        | 0.021            |
| Gadoleic acid (C20:1)                 | 0.471            |
| Alpha-linolenic acid (C18:3n3)        | 0.477            |
| Eicosadienoic acid (C20:2)            | 0.326            |
| Behenic acid (C22:0)                  | 0.0138           |
| Dihomo-gamma-linolenic acid (C20:3n6) | 0.0508           |
| Erucic acid (C22:1)                   | 0.0357           |
| Eicosatrienoic acid (C20:3n3)         | 0.042            |
| Arachidonic acid (C20:4n6)            | 0.138            |
| Tetracosanoic acid (C24:0)            | 0.0194           |
| Docosahexaenoic acid (C22:6n3)        | 0.0104           |
| Total Fatty Acid Content              | 74.1             |

Fatty acid composition data were obtained from the manufacturer's specification sheet. Individual fatty acid contents are reported as grams per 100 g of diet (g/100 g). Total fatty acid content represents the sum of all detected fatty acids in the ketogenic diet formulation.

**Table S3 Sequence information of genes.**

| Sequence name  | Sequence-Sense           |
|----------------|--------------------------|
| rHOPX siRNA-1  | GCUUCUGUUGAGAAGUUAATT    |
| rHOPX siRNA-2  | AAAUUAUAUUCUAAAUAAATT    |
| rHOPX siRNA-3  | GUGUAUUUGAGUCAUUAATT     |
| rHCAR2 siRNA-1 | GGAUGGUCCUUCACCGCUATT    |
| rHCAR2 siRNA-2 | CGGACAUGAUGACCCGAAATT    |
| rHCAR2 siRNA-3 | CUGUGUGUCCGAGAUAATT      |
| rFFAR3 siRNA-1 | GCUCACUGUAGUGUGAUUUUAUTT |

| Sequence name  | Sequence-Sense          |
|----------------|-------------------------|
| rFFAR3 siRNA-2 | AGCACCCUCAACUCUUGUAUUTT |
| rFFAR3 siRNA-3 | UUUGCUUAACCUGACCAUUUCTT |

The small interfering RNA (siRNA) oligonucleotides were designed and synthesized for gene silencing experiments in H9C2 cells. Three candidate siRNA sequences were generated for each target gene and subsequently screened for knockdown efficiency. Only sense strand sequences are shown.

**Table S4 Information of antibodies used in this study.**

| Antibody name   | Dilution ratio | Catalog number | Company information |
|-----------------|----------------|----------------|---------------------|
| ACTC1           | 1:5000         | 66125-1-Ig     | Proteintech, China  |
| cTNI            | 1:30000        | 21652-1-AP     | Proteintech, China  |
| cMyBPC3         | 1:1000         | 19977-1-AP     | Proteintech, China  |
| SERCA2 $\alpha$ | 1:10000        | 27311-1-AP     | Proteintech, China  |
| RYR2            | 1:2000         | 19765-1-AP     | Proteintech, China  |
| MYH6            | 1:10000        | 22281-1-AP     | Proteintech, China  |
| PLN             | 1:1000         | 15873-1-AP     | Proteintech, China  |
| mCherry         | 1:1000         | 26765-1-AP     | Proteintech, China  |
| GFP tag         | 1:2000         | 50430-2-AP     | Proteintech, China  |
| Ffar3           | 1:1000         | 66811-1-Ig     | Proteintech, China  |
| Ffar3           | 1:500          | A02437-2       | Boster, China       |
| Hcar2           | 1:2000         | A21974         | Abclonal, China     |
| Hopx            | 1:200          | sc-398703      | Santa, USA          |
| Vinculin        | 1:5000         | 26520-1-AP     | Proteintech, China  |
| GAPDH           | 1:100000       | 60004-1-Ig     | Proteintech, China  |

This table summarizes the primary antibodies used for Western blotting (WB) in this study, including antibody dilution ratios, catalog numbers, and supplier information.

**Table S5 The primers used in the present study.**

| Gene            | Forward primer (5'-3') | Reverse primer (5'-3') |
|-----------------|------------------------|------------------------|
| NPPA            | GACCCCTCCGATAGATCTGC   | GTCAATCCTACCCCGAAGC    |
| NPPB            | GAGTCCTTCGGTCTCAAGGC   | ACAACCTCAGTGCGTTACAGC  |
| MYH6            | GGACCAGGCCAATGAGTACC   | TCCATCTGCTGGGTGTAGGA   |
| MYH7            | CGACTGTGGCCTCCATTGAT   | TCTTCAGTGCCGTCTGGTTC   |
| Hopx            | ACAAGGTCAACAAGCACCCG   | GGCGCTGCTTAAACCATTTCT  |
| ACTC1           | GCCTTACCCCATGCCATCAT   | AGGGCGACGTAACACAGTTT   |
| cTNI            | CTCGGGTGGACAAAGTGGAT   | ACTTGCCACGGAGGTCATAG   |
| cMyBPC3         | TTCGAGGCATGCTGAAGAGG   | AGCCAGTTCCACAGTAAGCC   |
| SERCA2 $\alpha$ | TGACCCCCGAGCTGTTAATC   | GTTGCCACCATTTTCATCCCG  |
| RYR2            | CATCTTGGTCAGCGTGTCTCT  | CACCGCCAATGAGATAGCCT   |
| PLN             | TACCTCACTCGCTCGGCTAT   | ATGCAGATCAGCAGCAGACA   |
| HMGCS2          | AGTCCAGCTACTGGGATGGT   | ACGCGTTCTCCATGTGAGTT   |
| UCP1            | CACGGGGACCTACAATGCTT   | ACAGTAAATGGCAGGGGACG   |
| PDK4            | AGCTGCTGGACTTTGGTTCA   | CGGTCAGGCAGGATGTCAAT   |
| GCK             | GTACGACCGGATGGTGGATG   | AGCACAAAGTCGTACCAAGTC  |
| ACOT1           | CCAAGGATGGCCTCAAGGATG  | TGGTCGTCTGACCTACGAG    |
| CAR3            | ATTGCCAAAGGGGACAACCA   | ACCCCTCAGCATAGACCTGT   |
| PTGDS           | TGGTTCCGGGAGAAGAAAGC   | GGGCTGCTGTAGGTGTAGTG   |
| BDH1            | CGCGTCGTTAACATCAGCAG   | ATCTCATAGCGCAGGCAGTC   |
| ACTA1           | CCATCGGCAATGAGCGTTTC   | CCTGATGTGATGTGCGCACT   |
| Hcar2           | CAGGTGGCACGATGCTATGT   | GGCCCTCTTGATCTTGGCAT   |
| Ffar3           | TTTCTGAGCGTGGCCTATCC   | ACACTACAGTGAGCCGATGC   |
| GAPDH           | GCTGCCCAGAACATCATCCCT  | TGAAGTCGCAGGAGACAACC   |

The table lists the forward and reverse primer sequences used for quantitative real-time PCR (qRT-PCR) analysis of genes associated with cardiac development, calcium handling, ketone body metabolism and receptor signaling. All primer sequences are presented in the 5'-3' orientation.

**Table S6 Hopx Primer Information.**

| Primer Name  | Primer sequence (5'-3')    | Fragment length (bp) |
|--------------|----------------------------|----------------------|
| M-HOPX(M)-F2 | ATTTTCGGAGGTAGTATTTGAGGC   | 217                  |
| M-HOPX(M)-R2 | AAACCAAATAACCCGAATAAACG    |                      |
| M-HOPX(U)-F2 | GGATTTTTGGAGGTAGTATTTGAGGT | 222                  |
| M-HOPX(U)-R2 | AAAAAACCAAATAACCCAAATAAACA |                      |

Primer pairs were designed to amplify bisulfite-converted DNA for assessment of DNA methylation within the Hopx promoter region. Amplified fragment lengths are indicated in base pairs (bp).

**Table S7 Representative HOPX-interacting proteins identified by Co-immunoprecipitation coupled with mass spectrometry (Co-IP/MS).**

| Protein Accessions | Gene Name | Coverage (%) | Peptides | Sequest HT |
|--------------------|-----------|--------------|----------|------------|
| A6HP76             | ACTC1     | 63           | 23       | 532.36     |
| A0A0G2JUA5         | Ahnak     | 33           | 36       | 246.55     |
| P29457             | Serpinh1  | 48           | 16       | 245.56     |
| P30427             | Plec      | 16           | 51       | 240.90     |
| M0R843             | Tubb4a    | 43           | 14       | 202.54     |
| P82995             | Hsp90aa1  | 24           | 16       | 146.98     |
| Q9QXQ0             | Actn4     | 32           | 20       | 135.25     |
| Q6AYZ1             | Tuba1c    | 49           | 16       | 128.36     |
| F1LNF0             | Myh14     | 4            | 8        | 116.21     |
| A6JYZ9             | Tuba4a    | 38           | 13       | 107.63     |
| A0A8I6G4G4         | Anxa6     | 19           | 11       | 70.62      |
| A0A8I6AKB2         | Pfn1      | 33           | 6        | 47.07      |

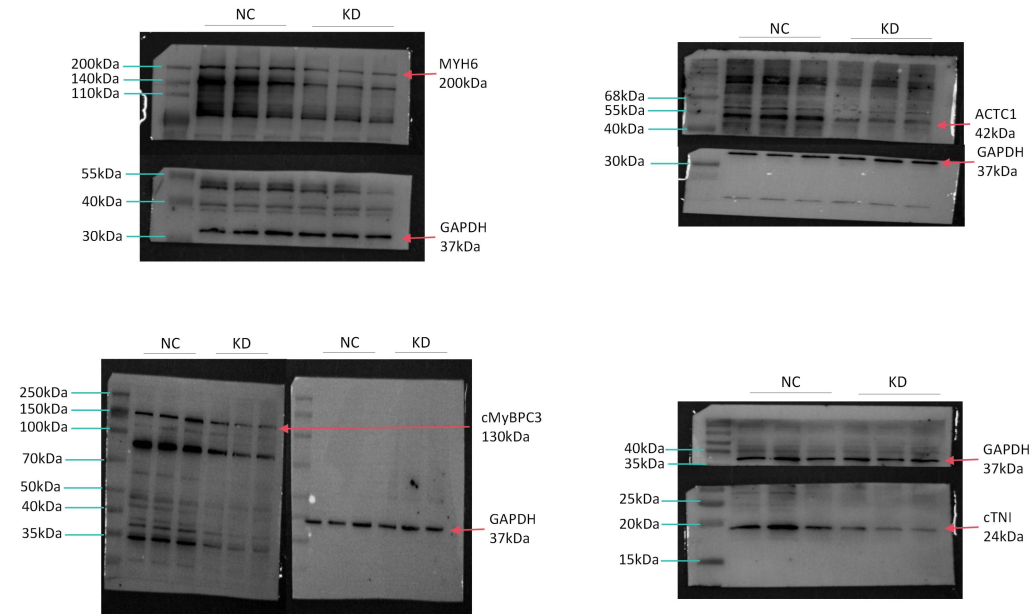
Proteins were identified by LC-MS/MS analysis following HOPX immunoprecipitation. Representative candidate proteins with potential relevance to cardiac structure, contractile function, and cytoskeletal organization are shown. Protein identification confidence is indicated by sequence coverage, peptide number, and protein score. ACTC1 was selected for subsequent validation based on its established role in cardiac muscle structure and function.



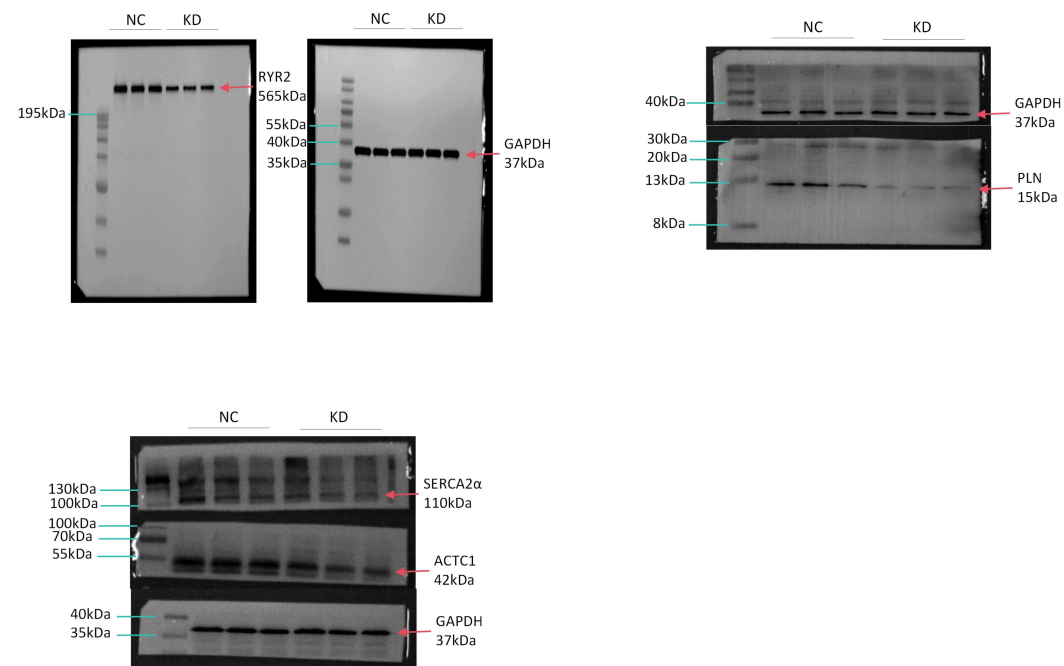
**Figure.S1** Identification and functional characterization of HOPX-interacting proteins by Co-immunoprecipitation coupled with mass spectrometry (Co-IP/MS). (A) An overview of protein identification. (B) Statistics of protein identification results. (C) Gene Ontology (GO) enrichment analysis. (D) Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis. (E) Domain analysis. (F) Subcellular localization analysis.



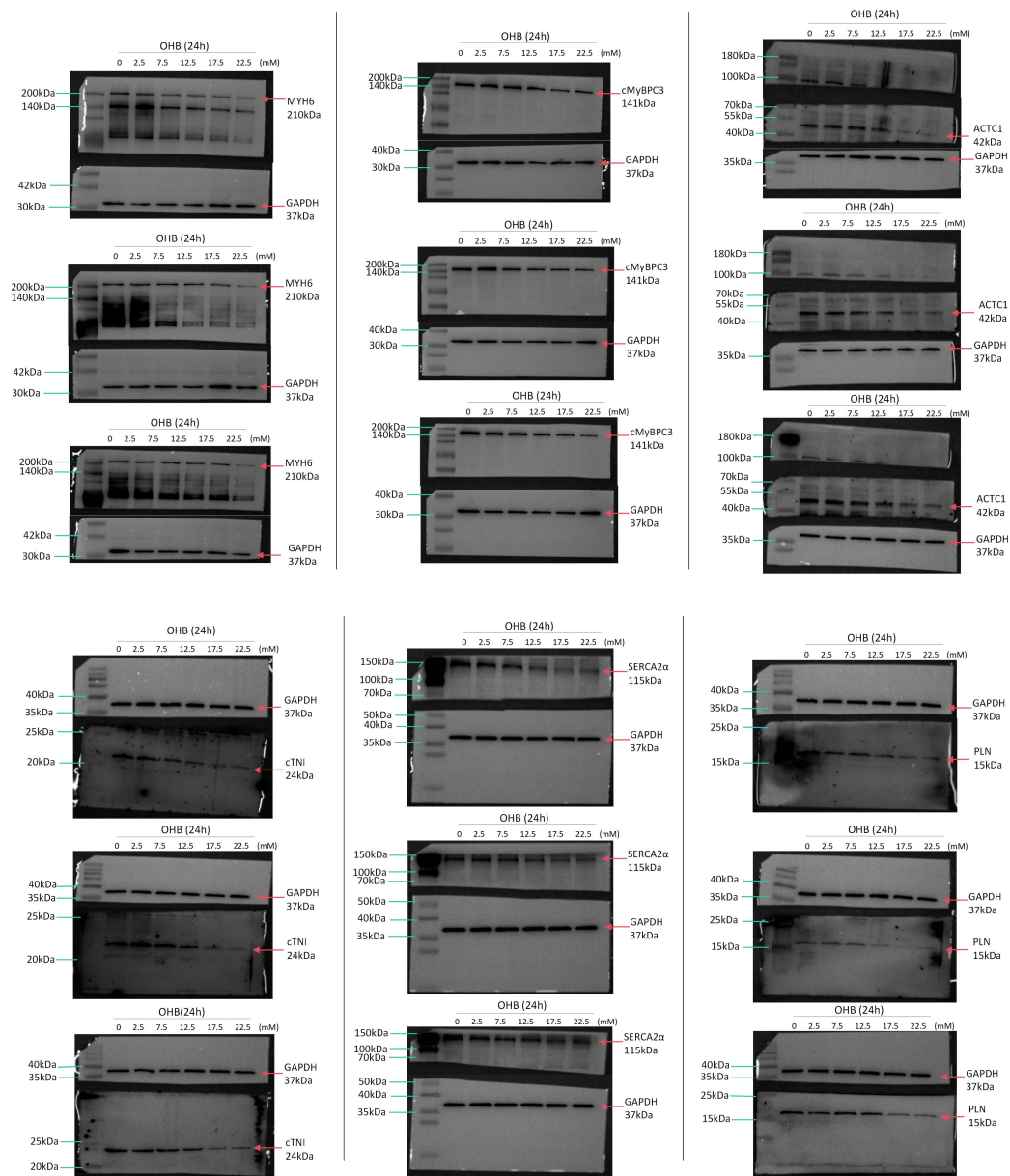
**Figure S2.** Original uncropped blots for Figure 3E.



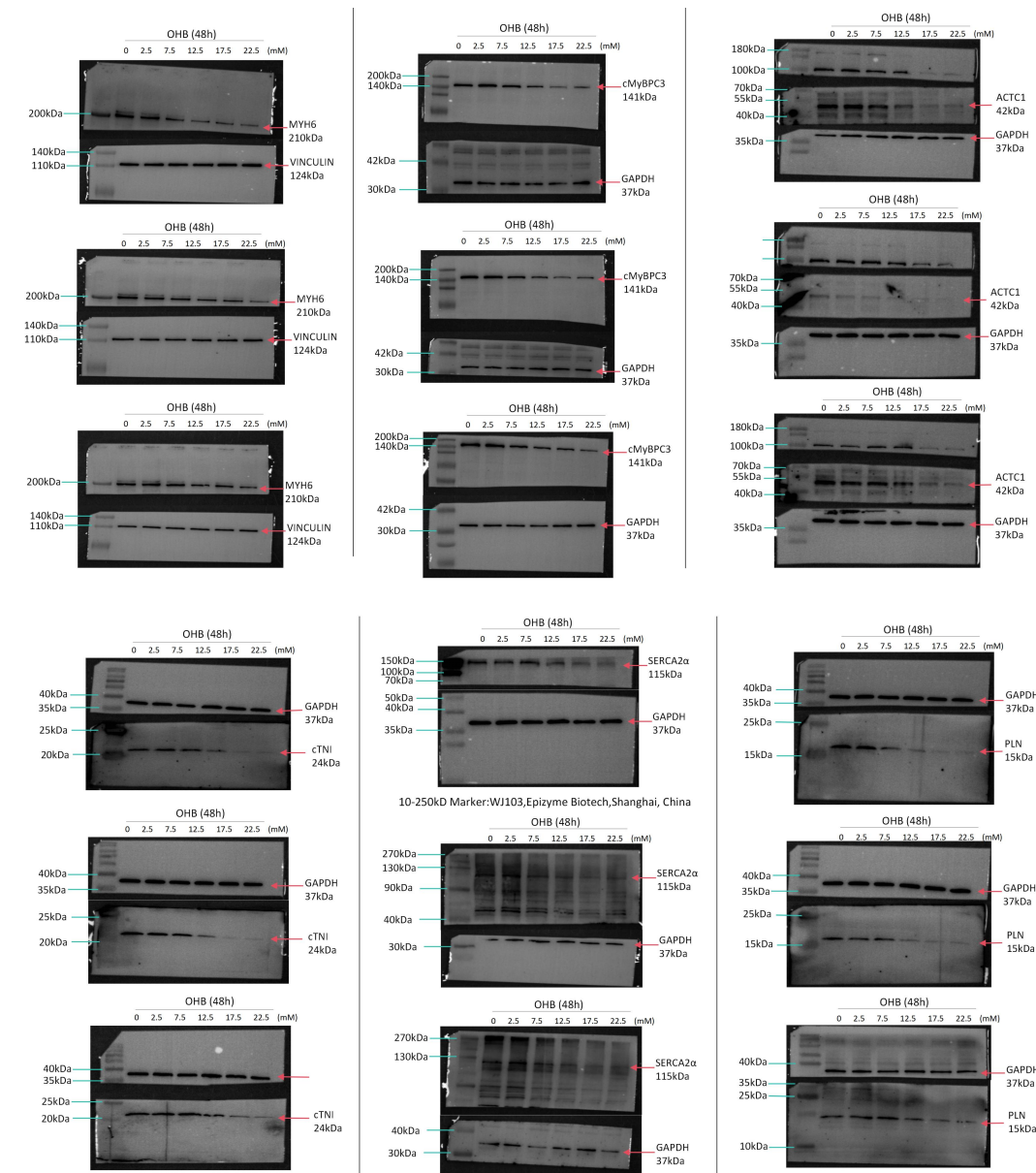
**Figure S3.** Original uncropped blots for Figure 3F.



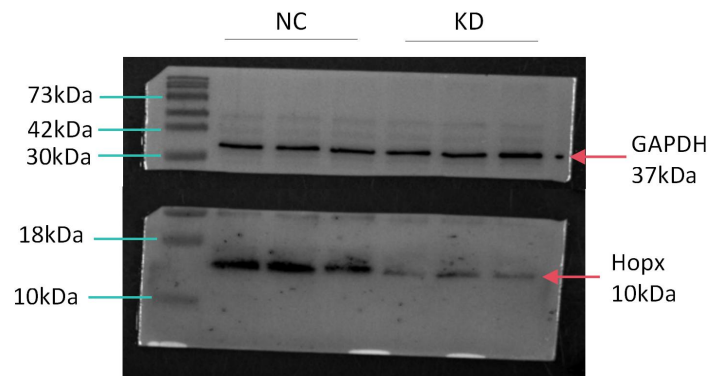
**Figure S4.** Original uncropped blots for Figure 4C.



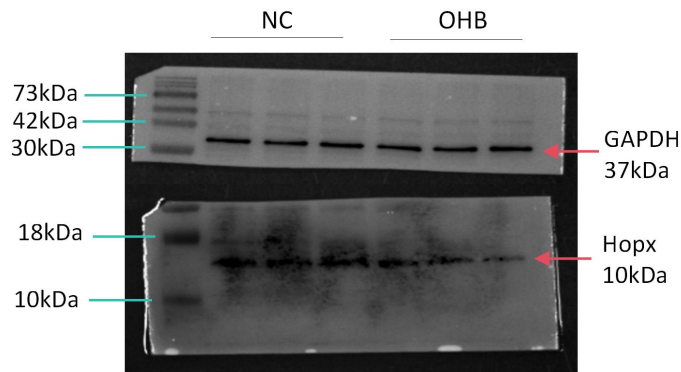
**Figure S5.** Original uncropped blots for Figure 4D.



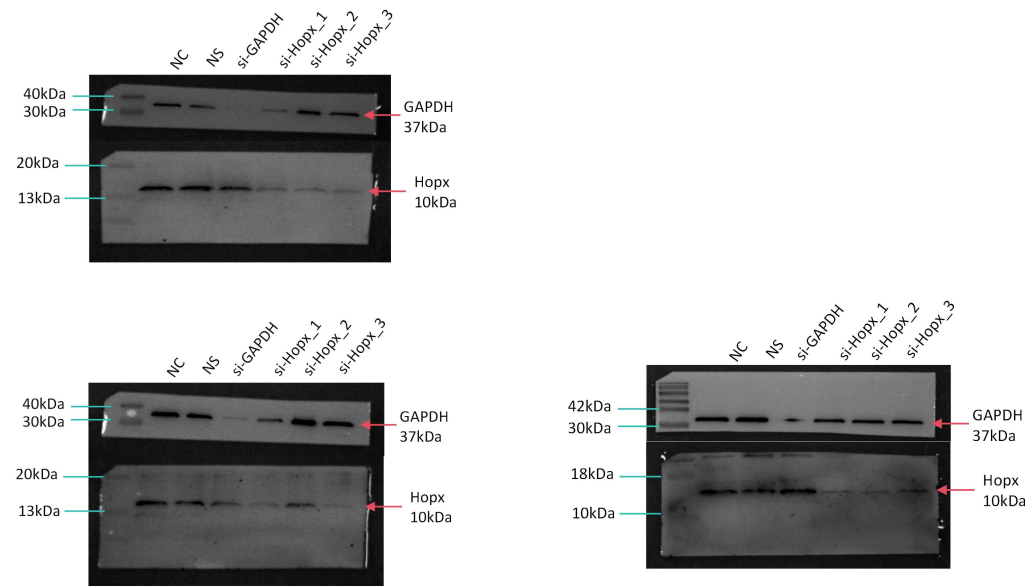
**Figure S6.** Original uncropped blots for Figure 6F.



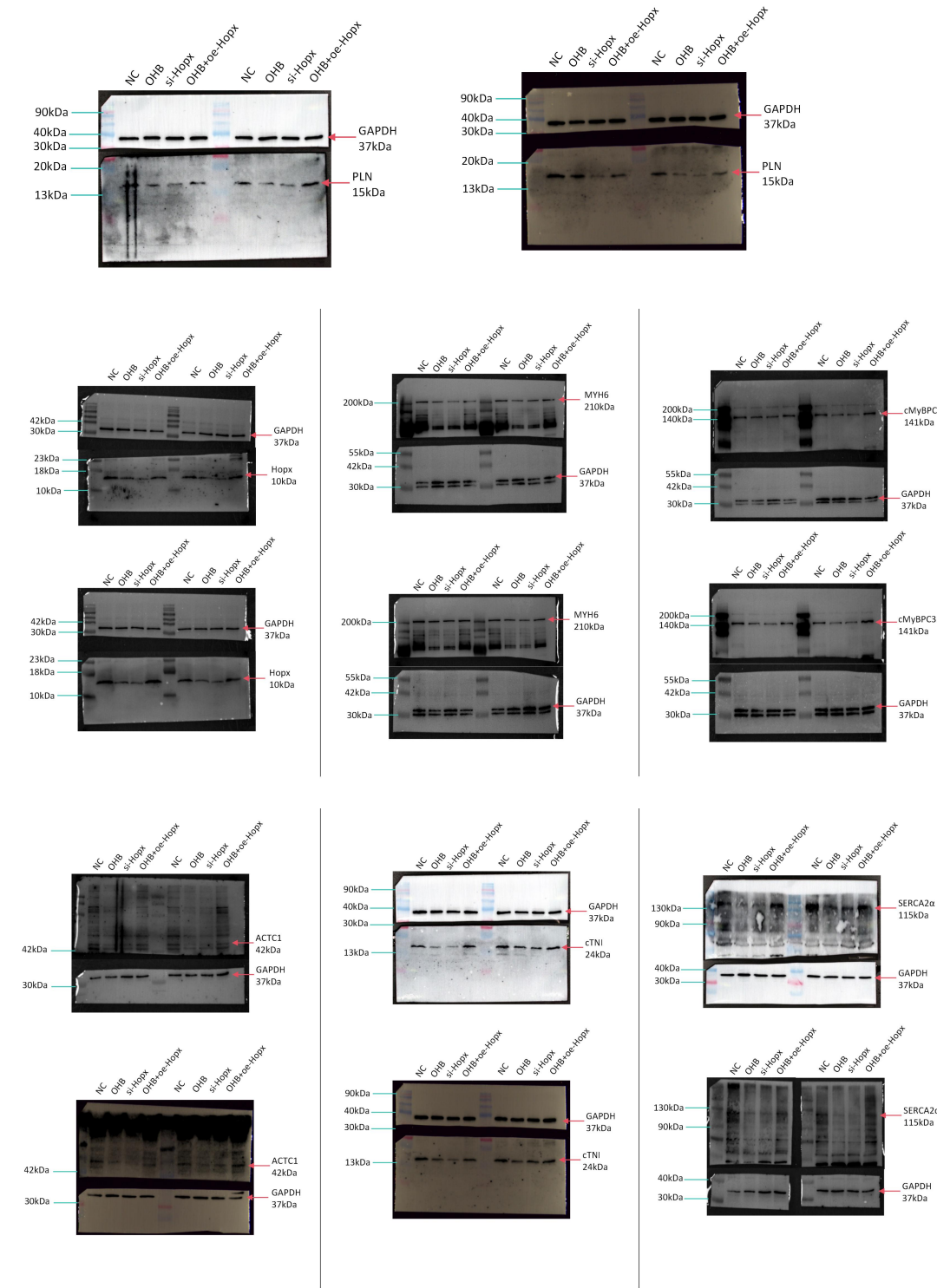
**Figure S7.** Original uncropped blots for Figure 7B.



**Figure S8.** Original uncropped blots for Figure 7C.

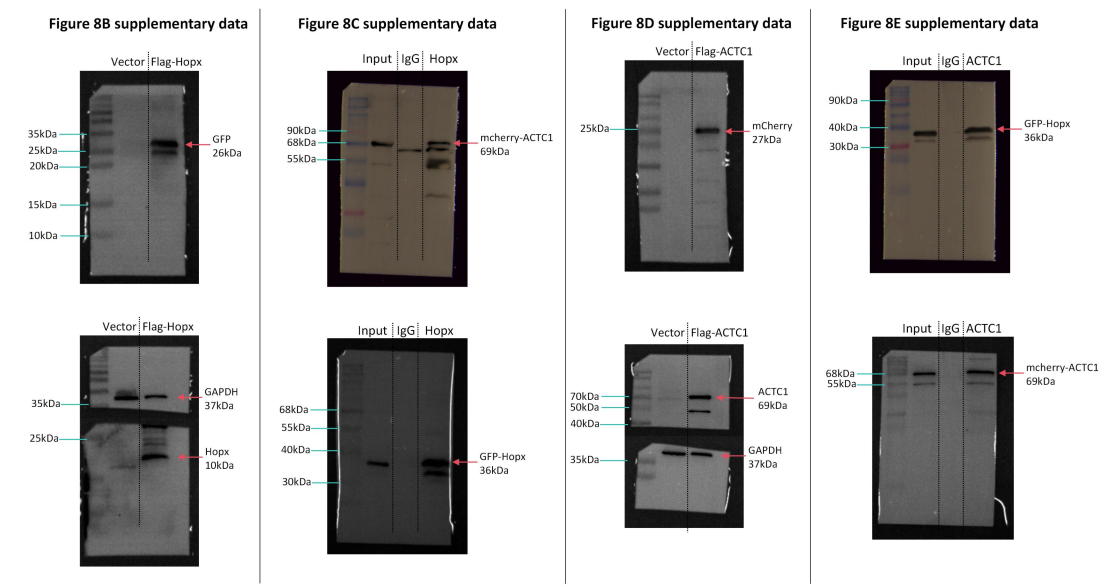


**Figure S9.** Original uncropped blots for Figure 7D.

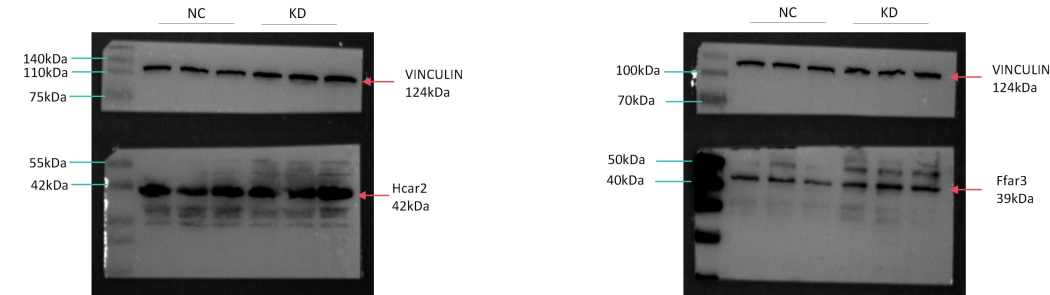




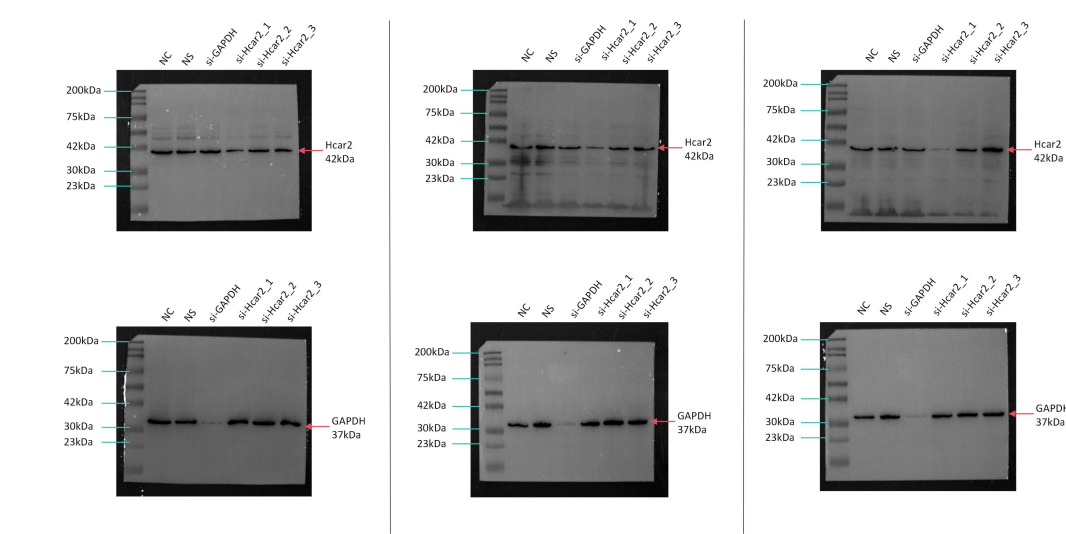
**Figure S10.** Original uncropped blots for Figures 8B-8E.



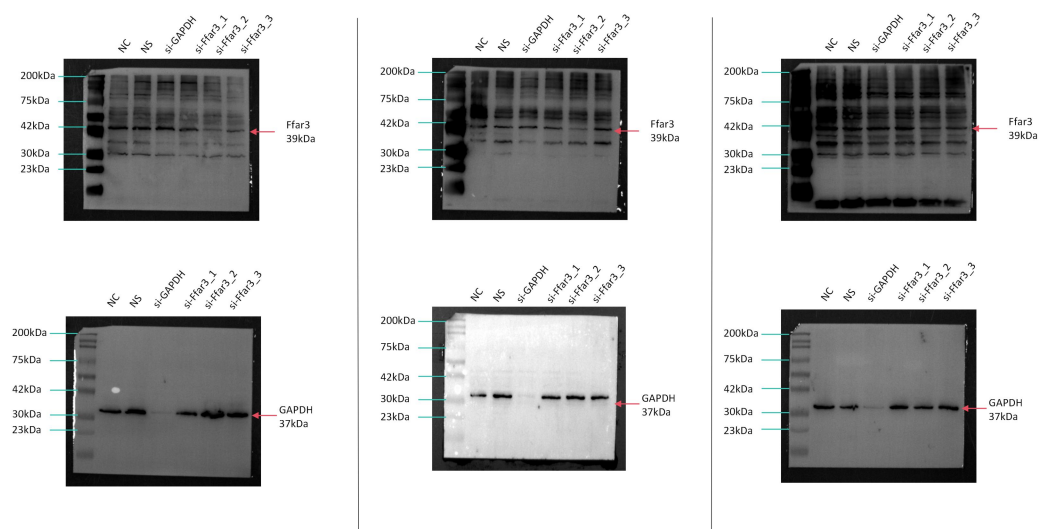
**Figure S11.** Original uncropped blots for Figure 9C.



**Figure S12.** Original uncropped blots for Figure 9D.



**Figure S13.** Original uncropped blots for Figure 9E.



**Figure S14.** Original uncropped blots for Figure 9F.

