

Hepatocyte nuclear factor 1 alpha influences pancreatic cancer growth and metastasis

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Supplementary materials and methods:

Antibodies and Reagents

The primary antibodies obtained and used for the experiments in this study were: *HNF1 α* (12425), Notch 2 (4530), Snail (3879), Zeb (3396), Vimentin (5741), Slug (9585), N-Cadherin (4016), E-Cadherin (3195), pPI3K (4228), PI3K (4292), pmTOR (2974), mTOR (4517), pp70S6 kinase (9206), p70S6 kinase (9202), and Bim (2933), Bak (3814), Bax (2772), Cleaved Caspase 3 (9661), P53, pP53 (Cell Signaling Technology, Boston, MA, USA); Cleaved PARP (sc-8007), pAKT (sc-101629), AKT (sc-5298), pERK (sc-101760), and ERK (sc-94) (Santa Cruz, CA, USA); Caspase 8 (ab61755) and Bcl-2 (ab73985) (Abcam, Cambridge, MA, USA); β -actin (Sigma Aldrich, St. Louis, MO, USA). Respective anti-mouse and anti-rabbit secondary antibodies were acquired from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The Transwell (6.5 mm) with 8.0 μ m pore polycarbonate membrane inserts were purchased from Corning Incorporated (Corning, NY, USA). Both BD Matrigel (Bedford, MA, USA) and BD Pharmingen Annexin V-FITC Apoptosis Detection Kit I were obtained from BD Biosciences (San Diego, CA, USA). MTS reagent [3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] was purchased from Promega (Madison, WI, USA). Mammalian protein extraction reagent (mPER) was acquired from Thermo Scientific (Rockford, IL, USA).

Sequences for *HNF1 α* siRNA subtypes “A”, “B”, and “C”

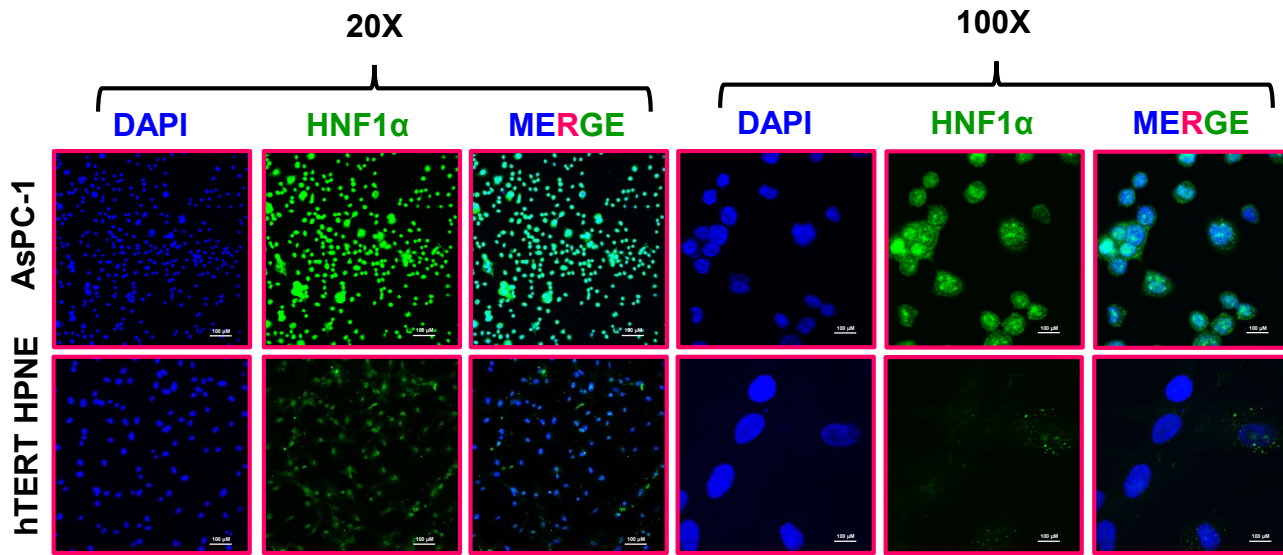
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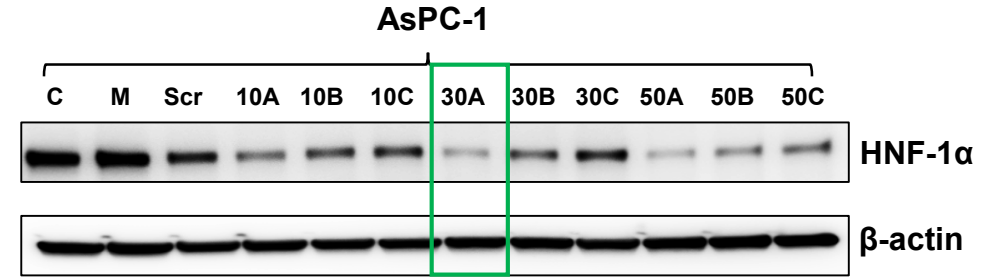
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Supplementary Figure S1

A

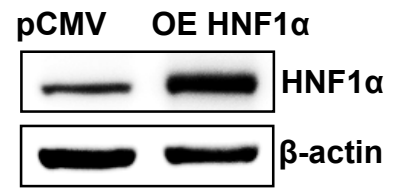


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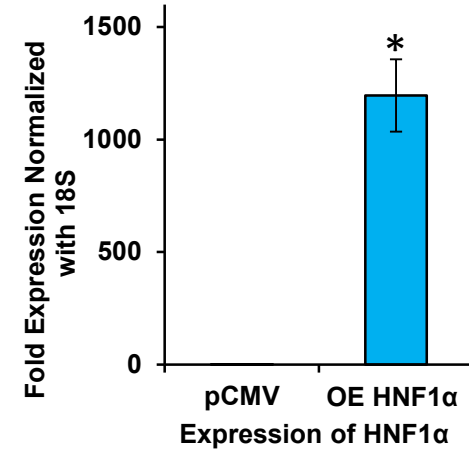


Supplementary Figure S2

A

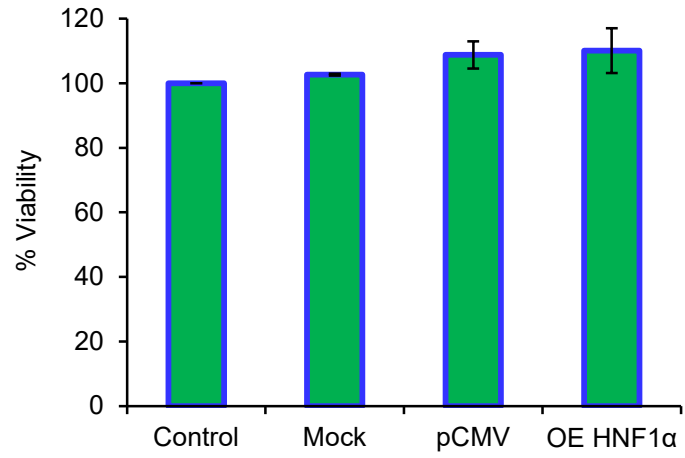


B

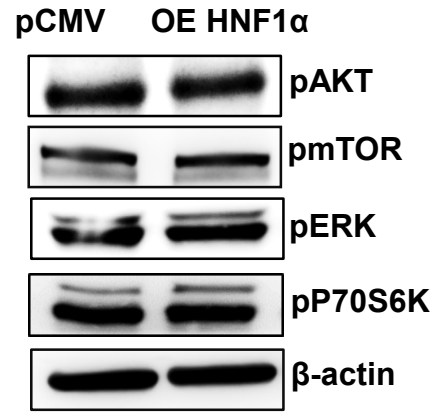


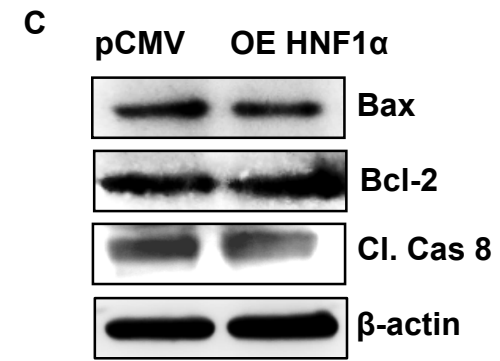
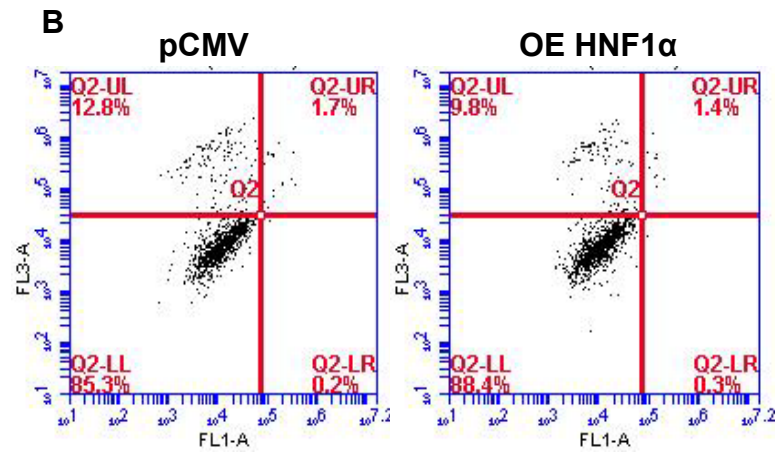
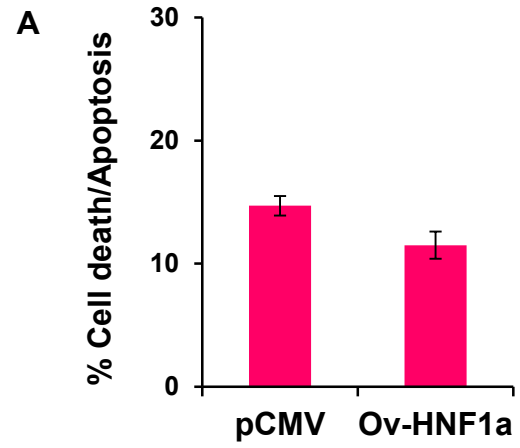
Supplementary Figure S3

A

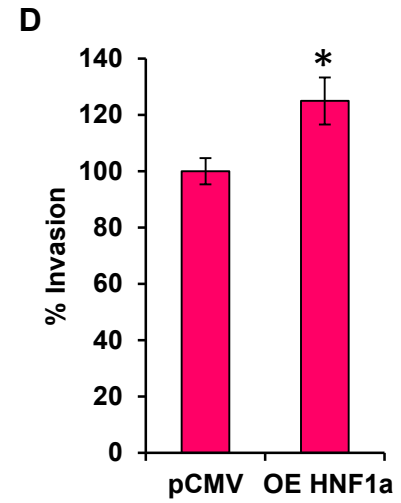
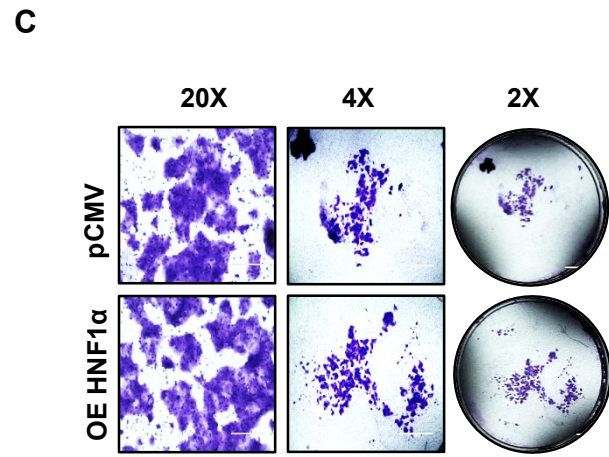
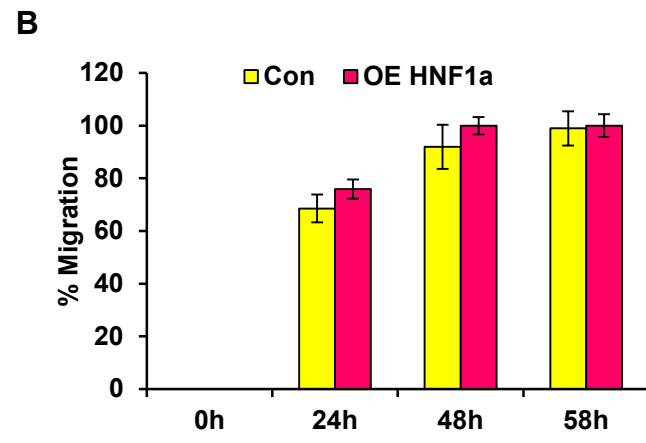
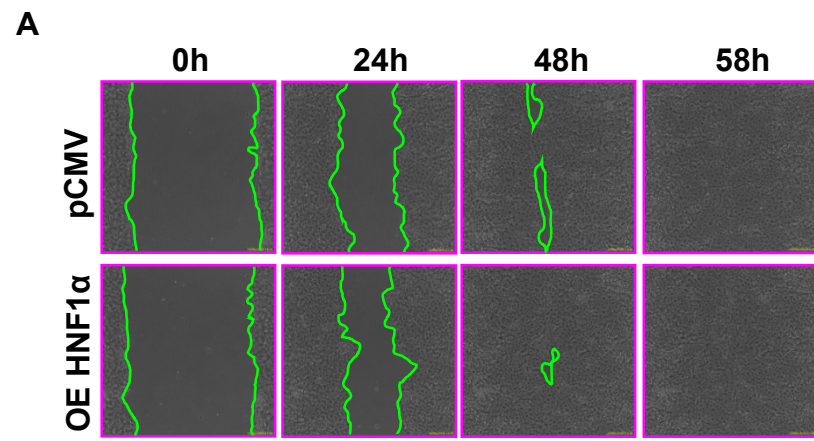


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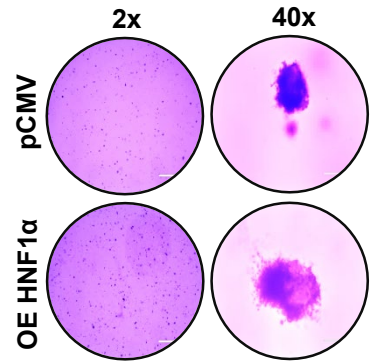


Supplementary Figure S5

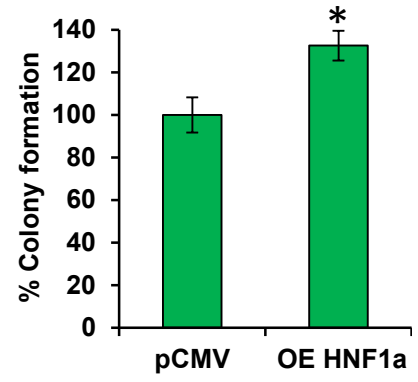


Supplementary Figure S6

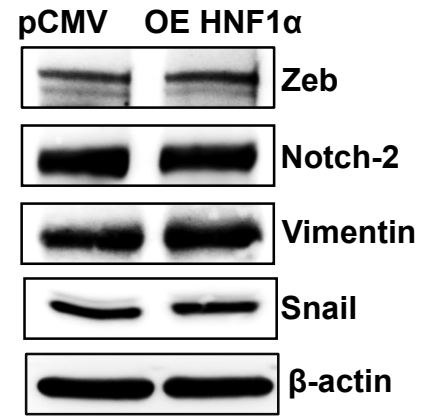
A



B



C



1 **Supplementary Figure Legends:**

2 **Supplementary Fig. S1**

3 **A.** Immunofluorescence of HNF1 α expression in AsPC-1 and hTERT HPNE cells (20X and 100X
4 magnification). **B.** AsPC1 cells were transfected with three different predesigned HNF1 α siRNAs
5 at different concentrations.

6 **Supplementary Fig. S2**

7 **A.** Western blot analysis of HNF1 α protein expression in overexpressed HNF1 α HPAC pancreatic
8 cancer cells. **B.** Relative mRNA expression level of HNF1 α in overexpressing HNF1 α HPAC cells.
9 Data shown as mean +/- SEM. Experiments (n=3) were repeated three times in triplicates.
10 *p<0.05.

11 **Supplementary Fig. S3**

12 **A.** MTS assay was used to measure cell viability in response to HNF1 α overexpression in HPAC
13 cells. **D.** Expression levels of pAKT, pmTOR, pERK, mTOR, pP70S6K, and β -actin were
14 determined by Western Blot in overexpressed HNF1 α HPAC cells. Data shown as mean +/- SEM.
15 Experiments (n=3) were repeated three times in triplicates.

16 **Supplementary Fig. S4**

17 **A & B.** Apoptosis analysis of overexpressed HNF1 α HPAC cells measured by flow cytometry.
18 **C.** Western blot analysis of apoptotic markers in siHNF1 α AsPC-1 cells and overexpressed HPAC
19 cells. Data shown as mean +/- SEM. Experiments (n=3) were repeated three times in triplicates.

20

21

22 **Supplementary Fig. S5**

23 **A & B.** Wound-healing assay was performed in HNF1 α overexpressed HPAC cells; migration was
24 analyzed using Nikon Biostation Ct at 2h intervals for up to 96h at 4X magnification. **C & D.**
25 Invasiveness of OVHNF1 α HPAC cells were observed using a Matrigel invasion assay, captured
26 using Nikon Eclipse TS 100 microscope. Data shown as mean +/- SEM. Experiments (n=3) were
27 repeated three times in triplicates. *p<0.05.

28 **Supplementary Fig. S6**

29 **A & B.** Colony formation assay was performed in HNF1 α overexpressed HPAC cells. **C.** EMT
30 markers analyzed by Western blot in overexpression HNF1 α in HPAC cells. Data shown as mean
31 +/- SEM. Experiments (n=3) were repeated three times in triplicates. *p<0.05

Fig. 2A

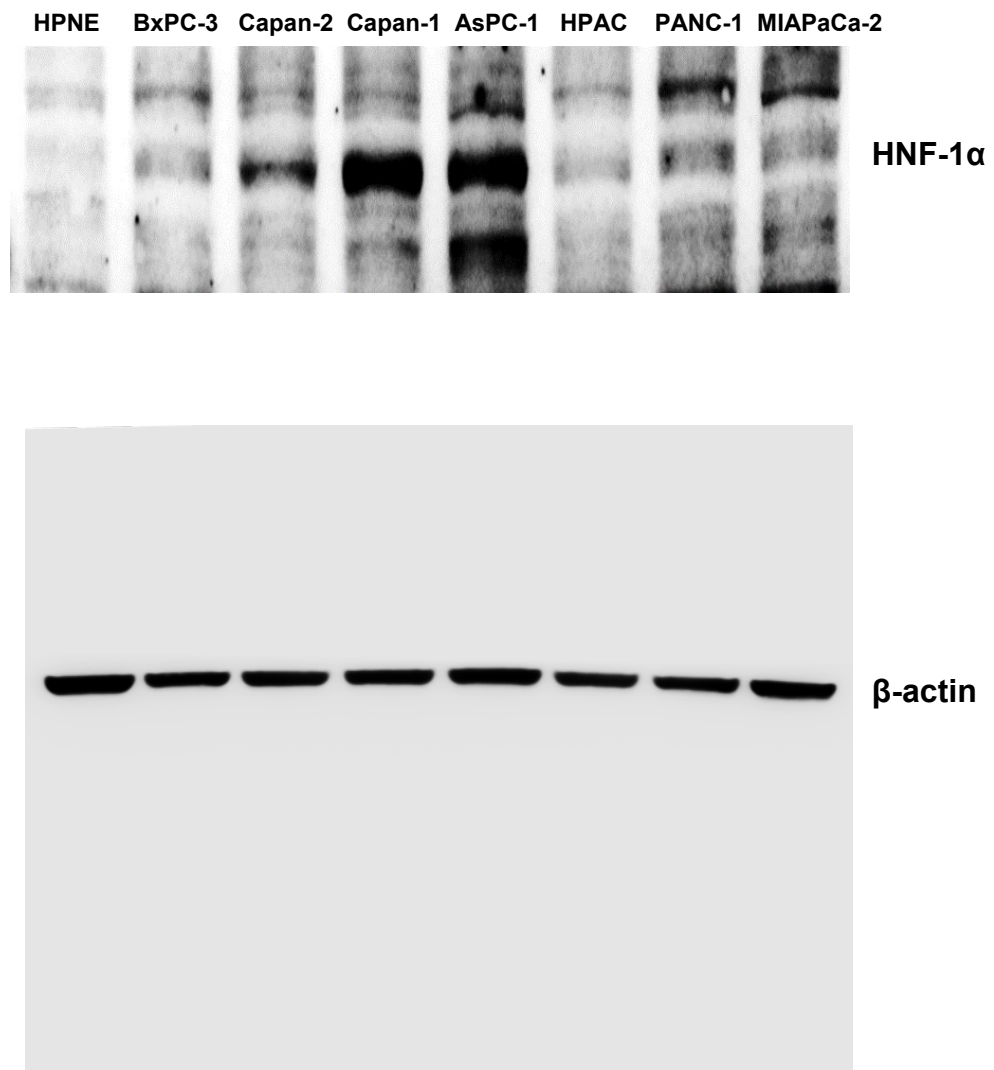


Fig. 2C

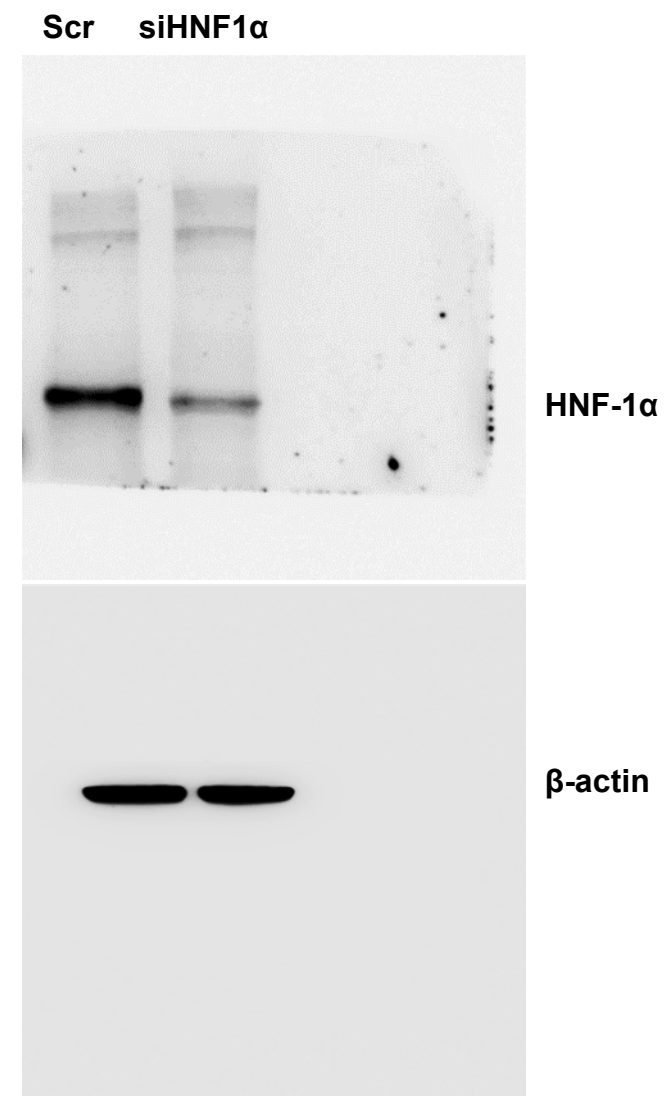


Fig. 3A

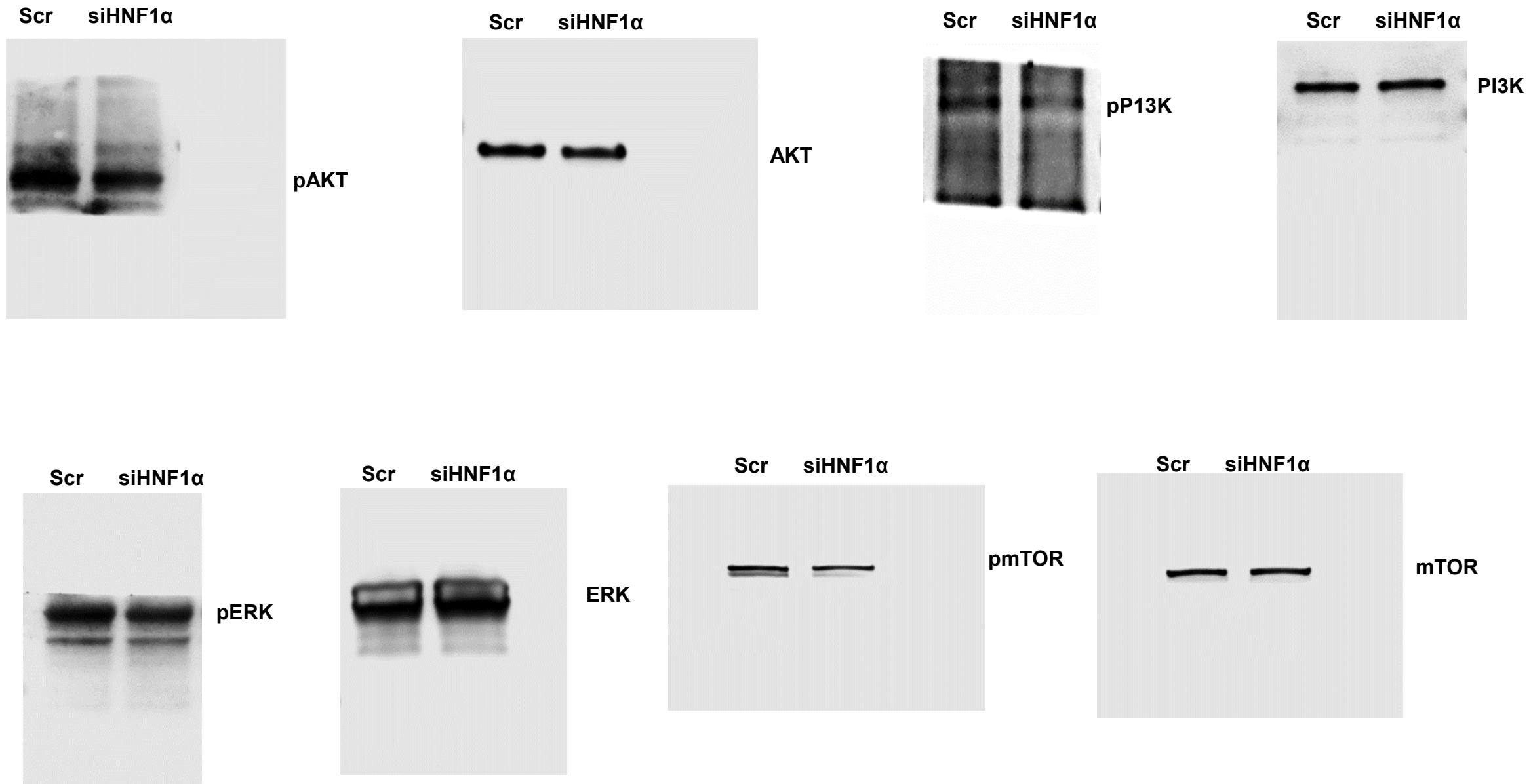


Fig. 3E

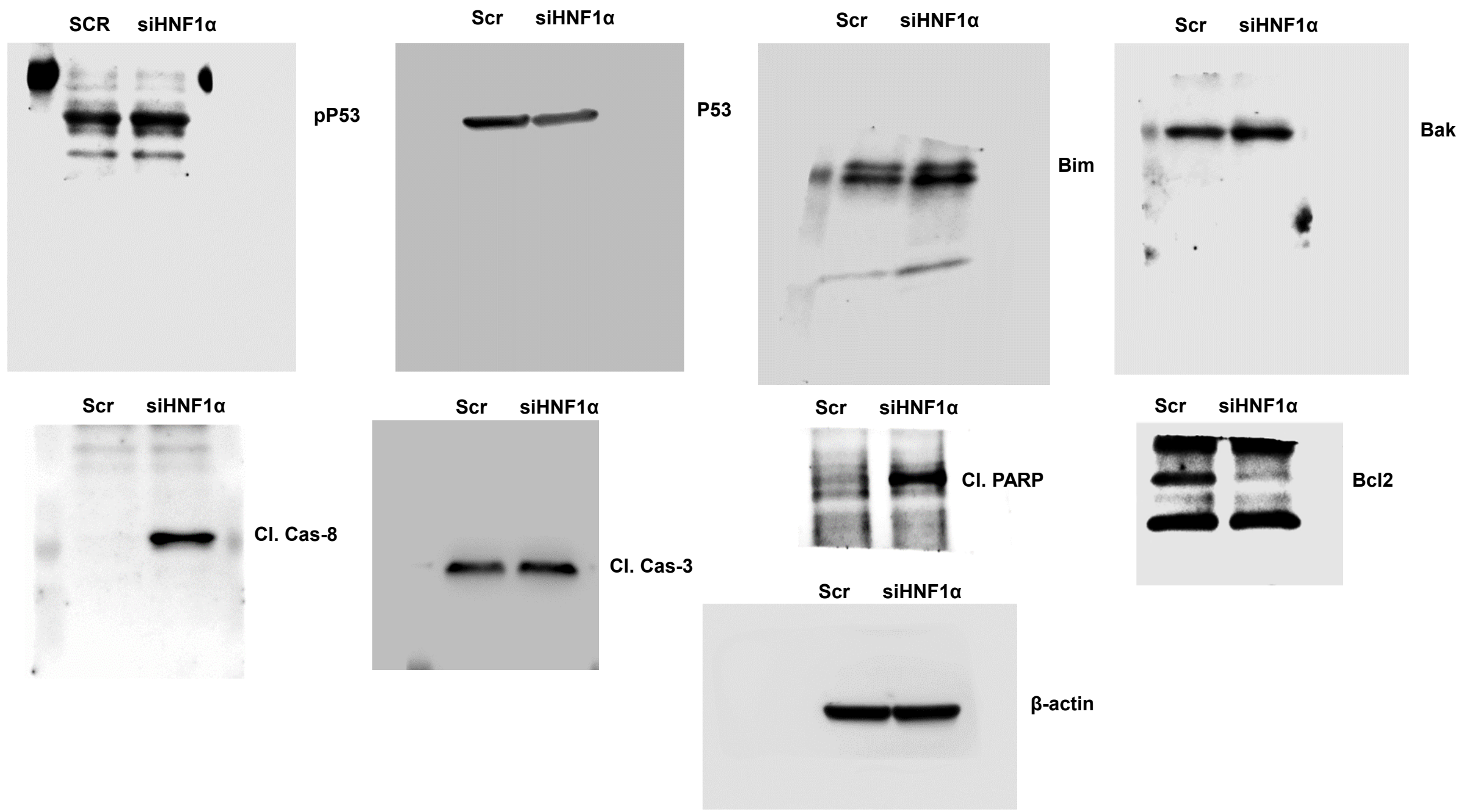


Fig. 4G

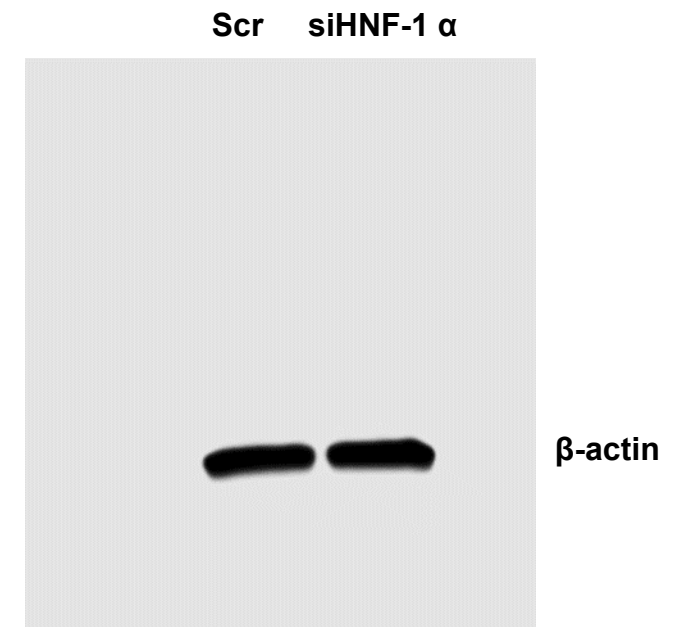
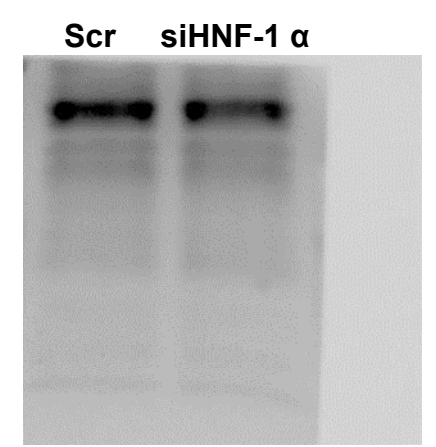
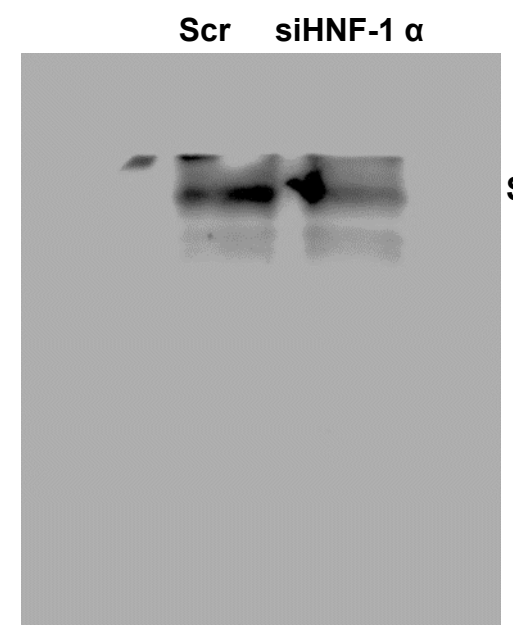
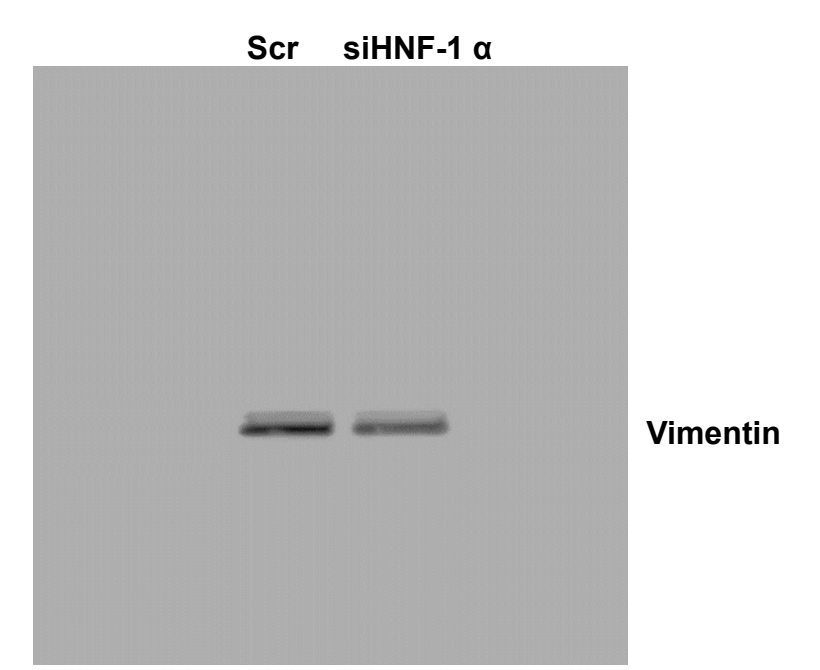
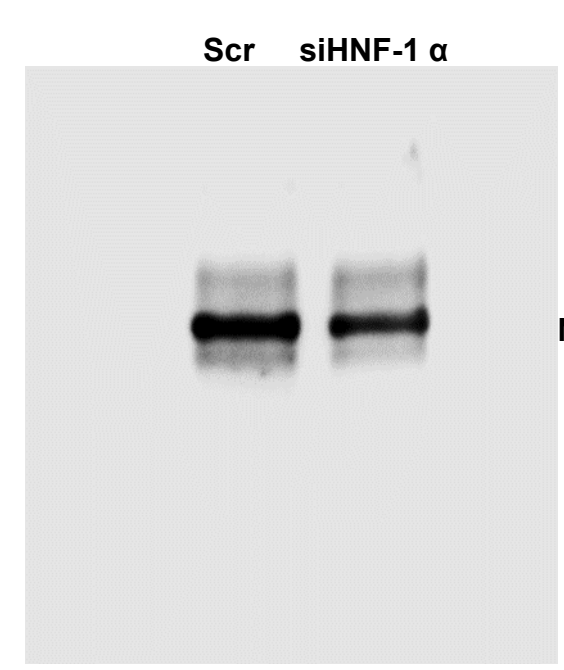
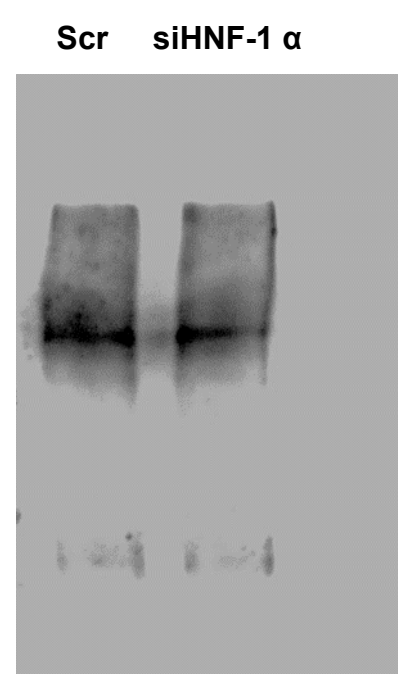


Fig. 5D

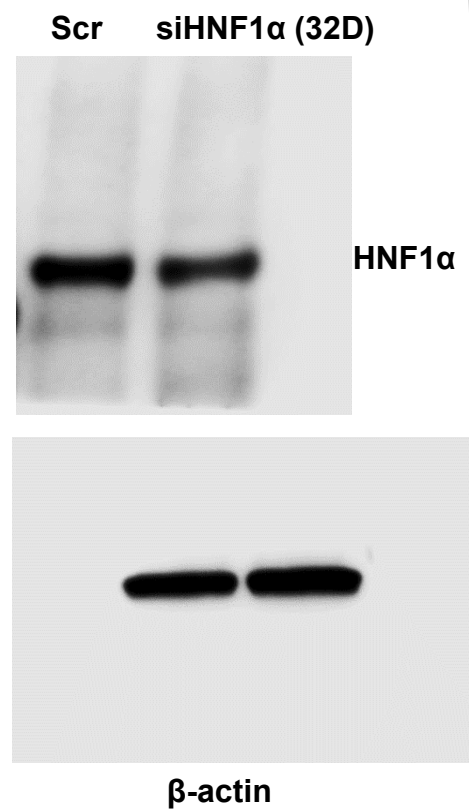


Fig. 5E Scr si-HNF1α

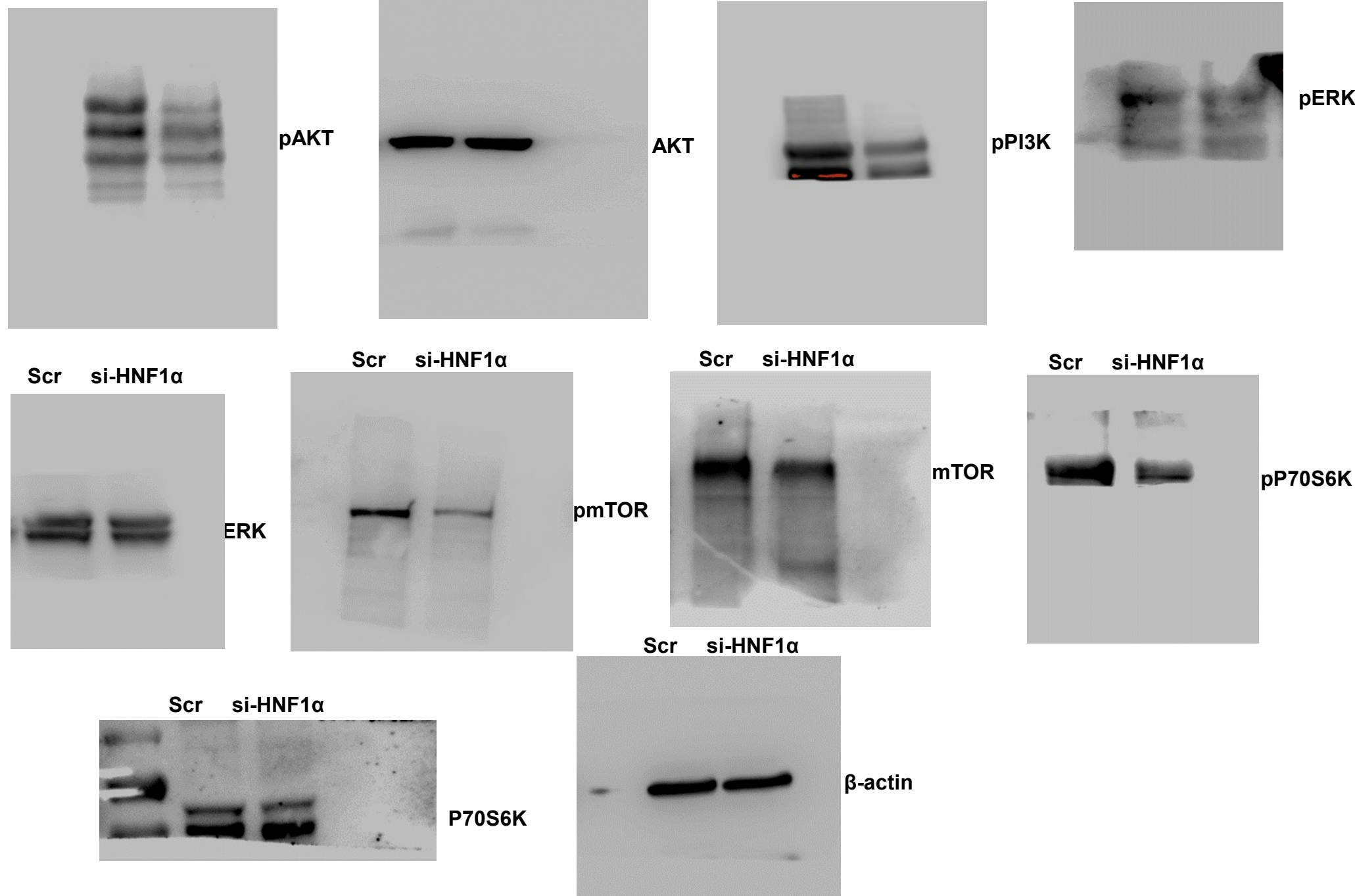


Fig. 5F

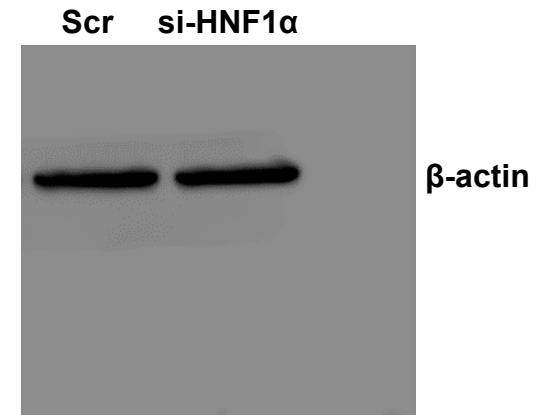
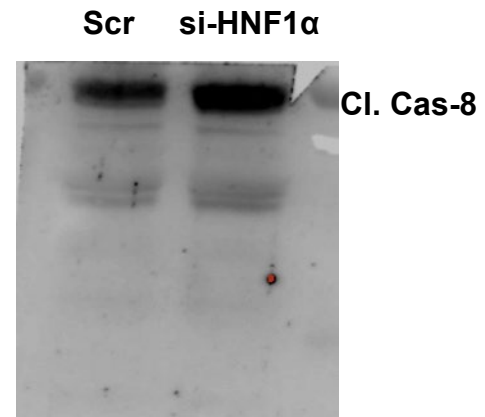
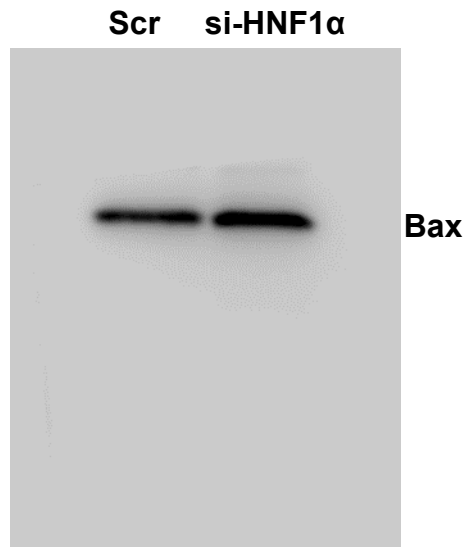
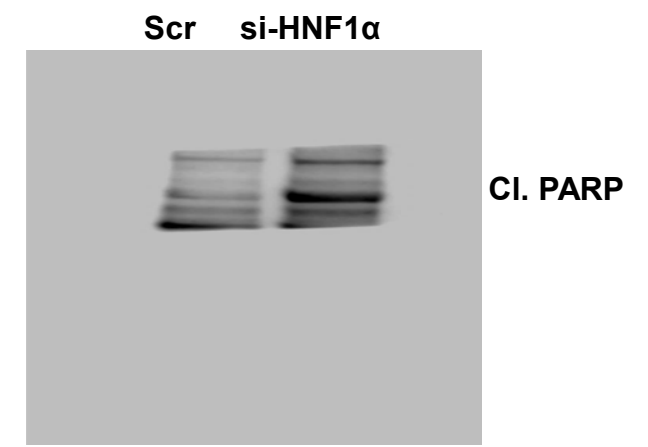
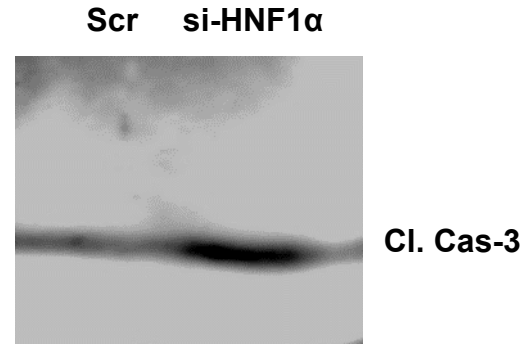
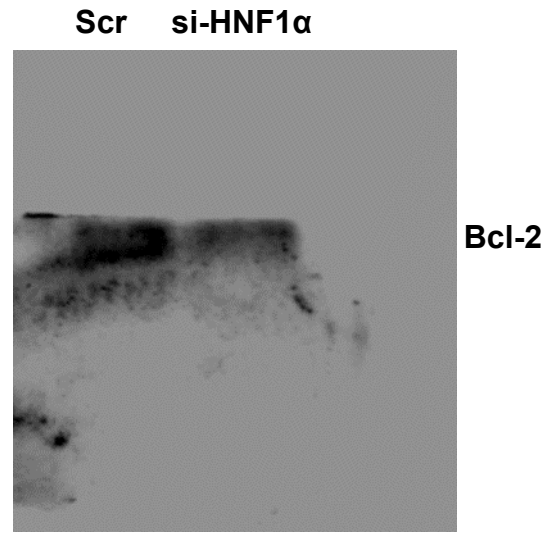
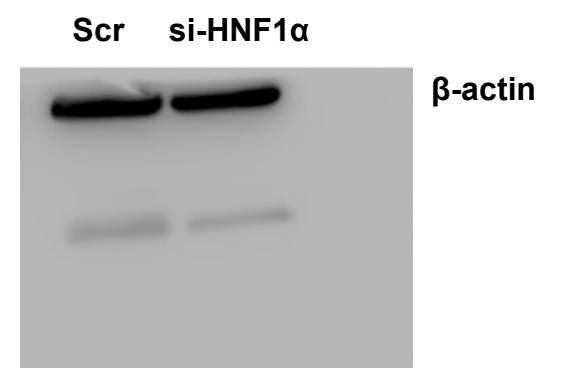
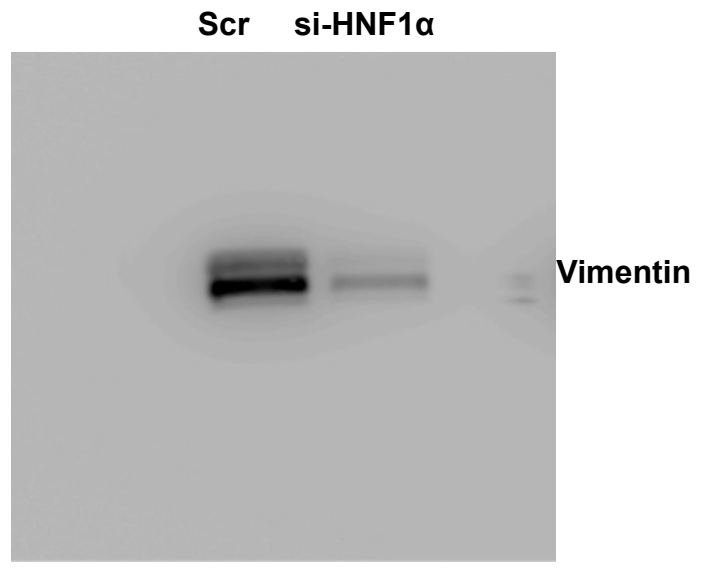
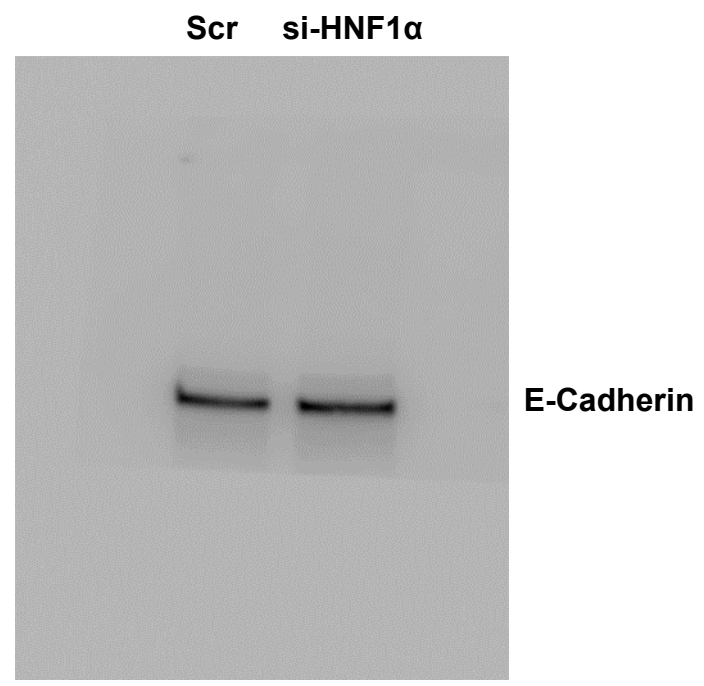
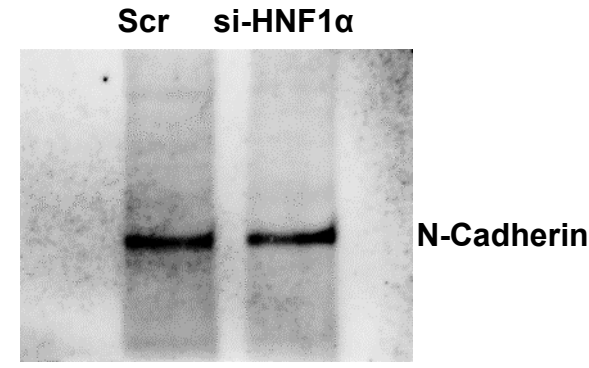
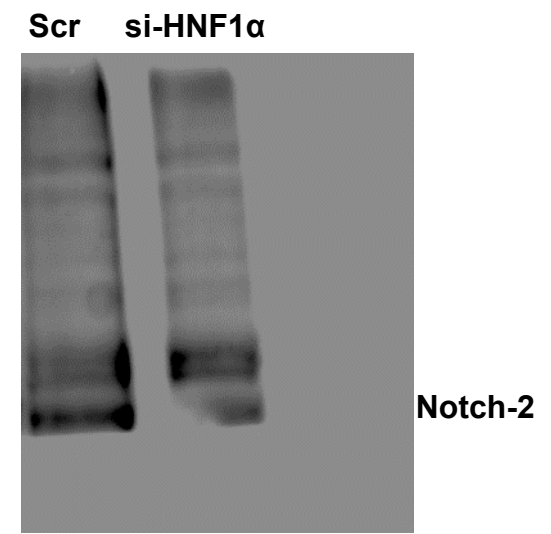
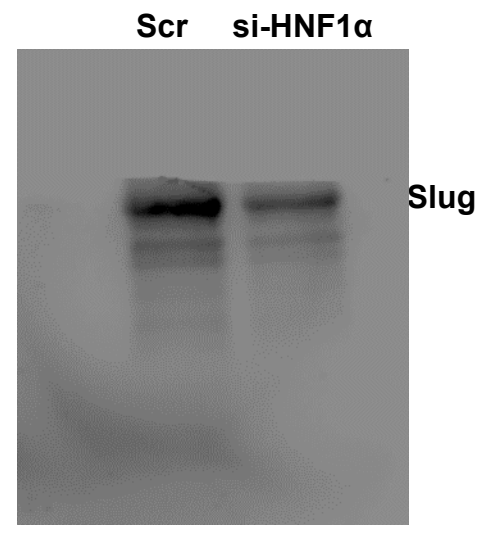
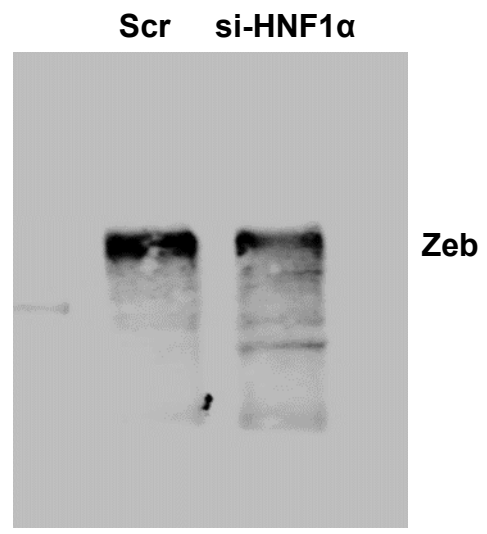
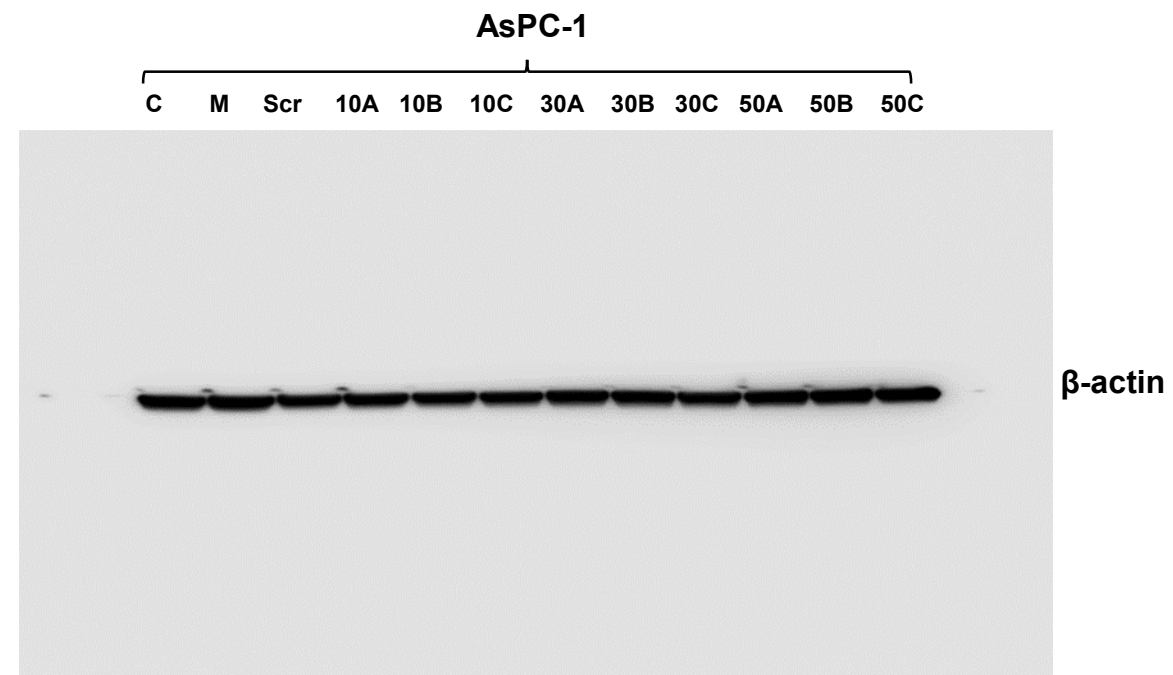
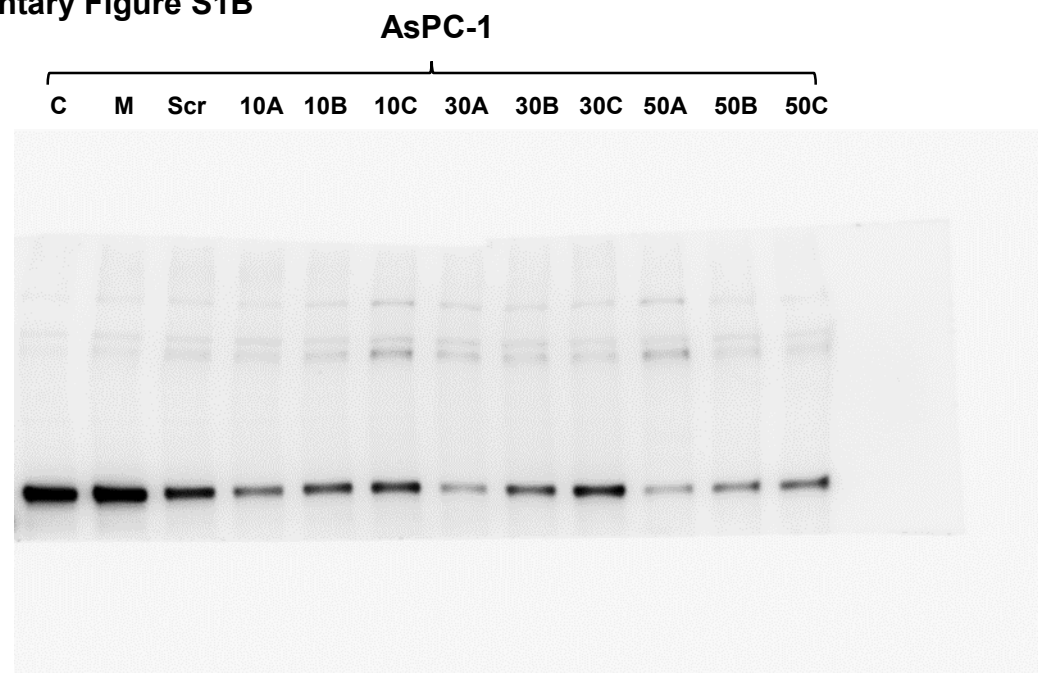


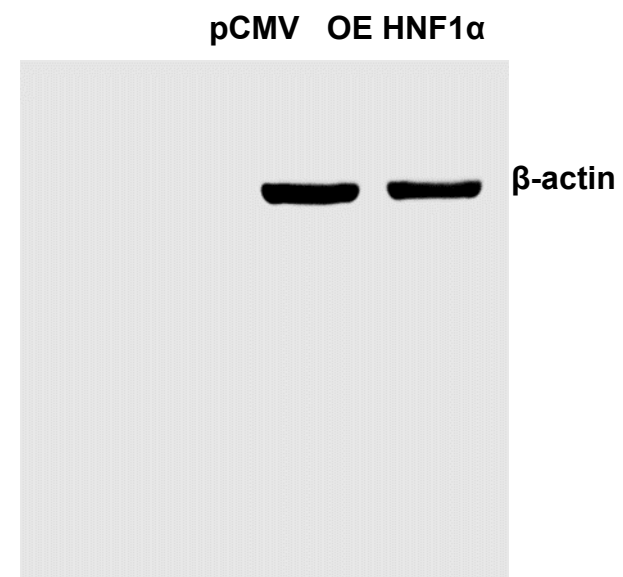
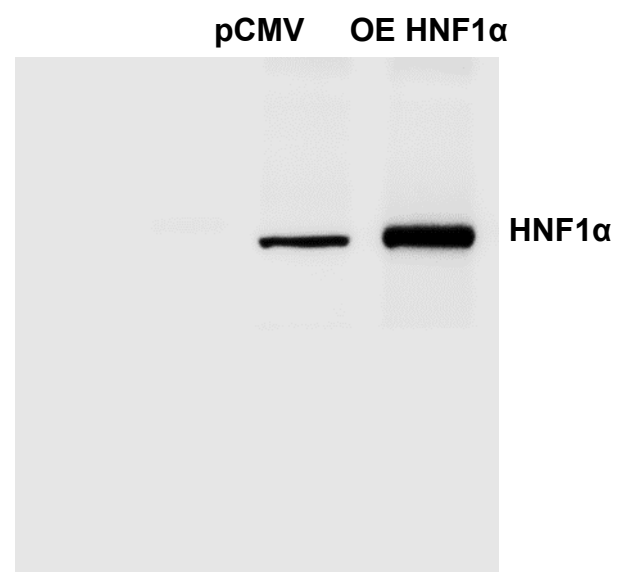
Fig. 5G



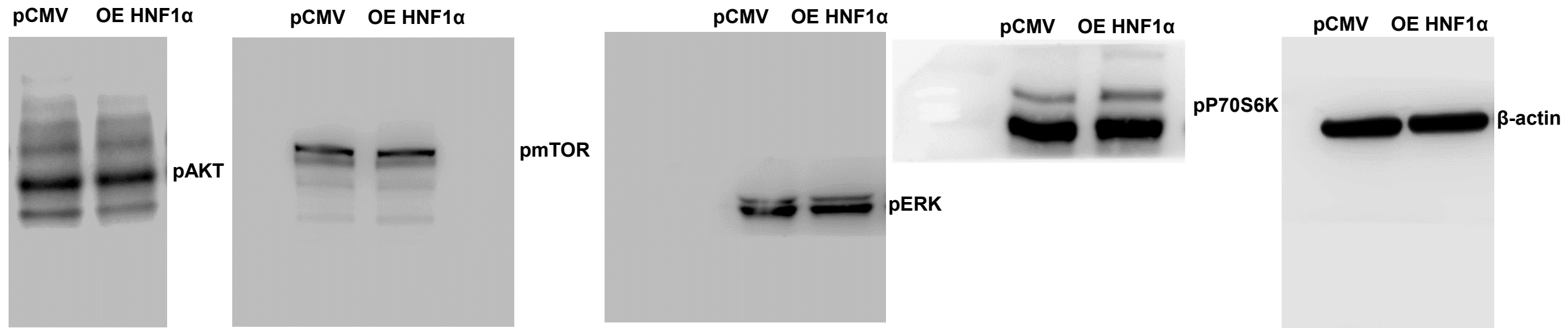
Supplementary Figure S1B



Supplementary Figure S2A



Supplementary Figure S3B



Supplementary Figure S4C

