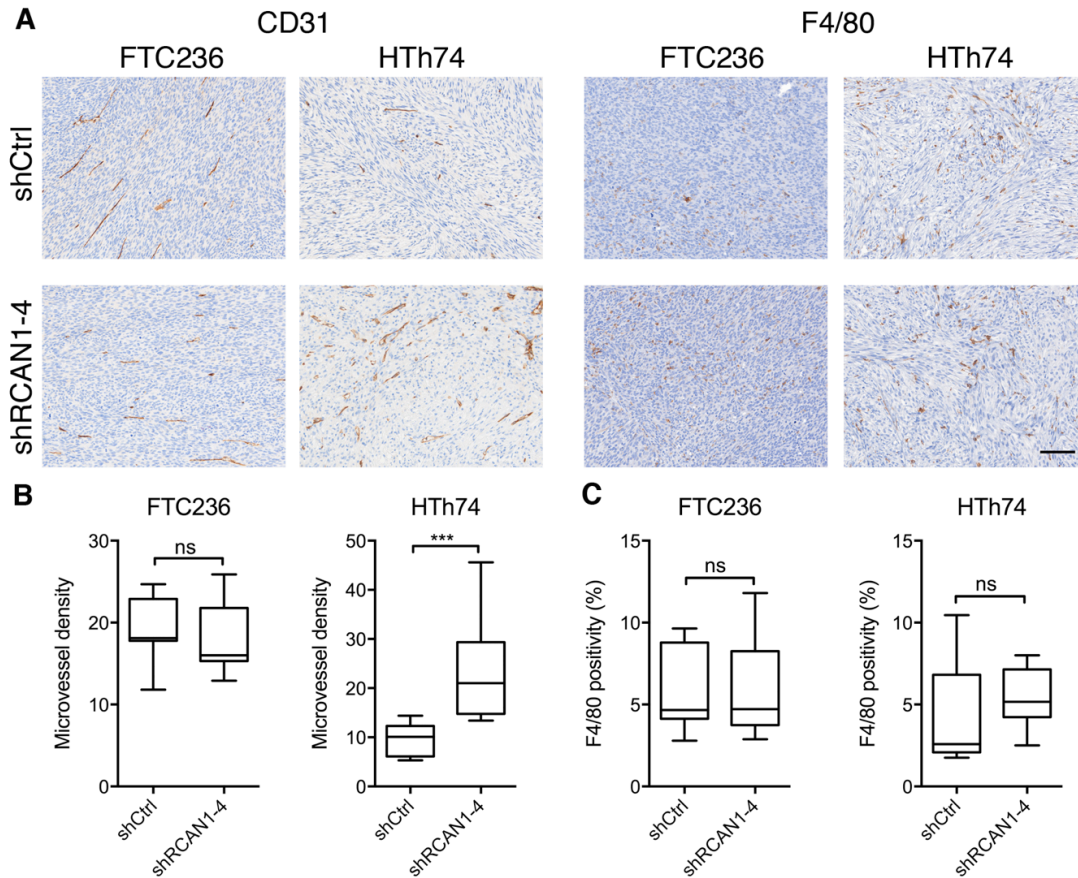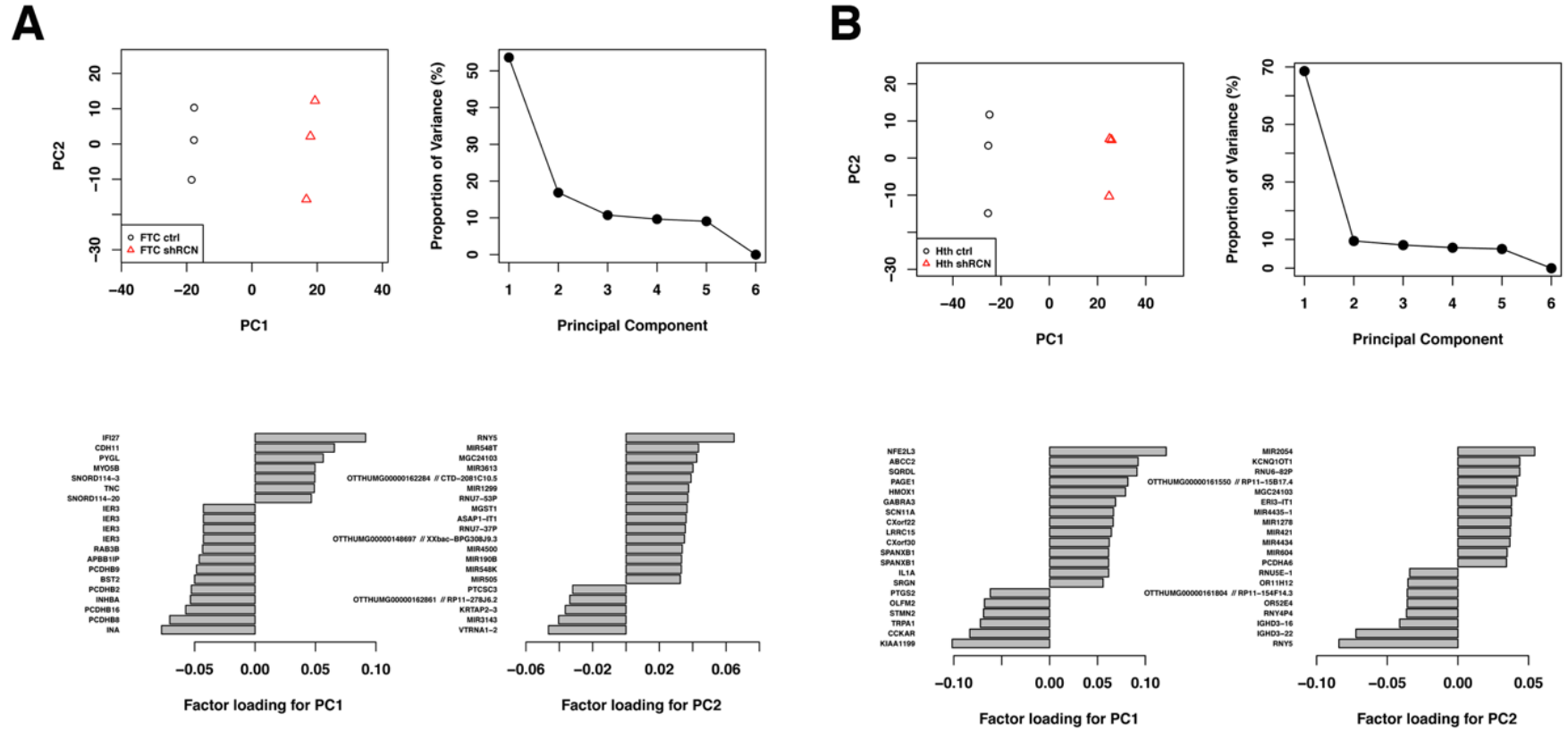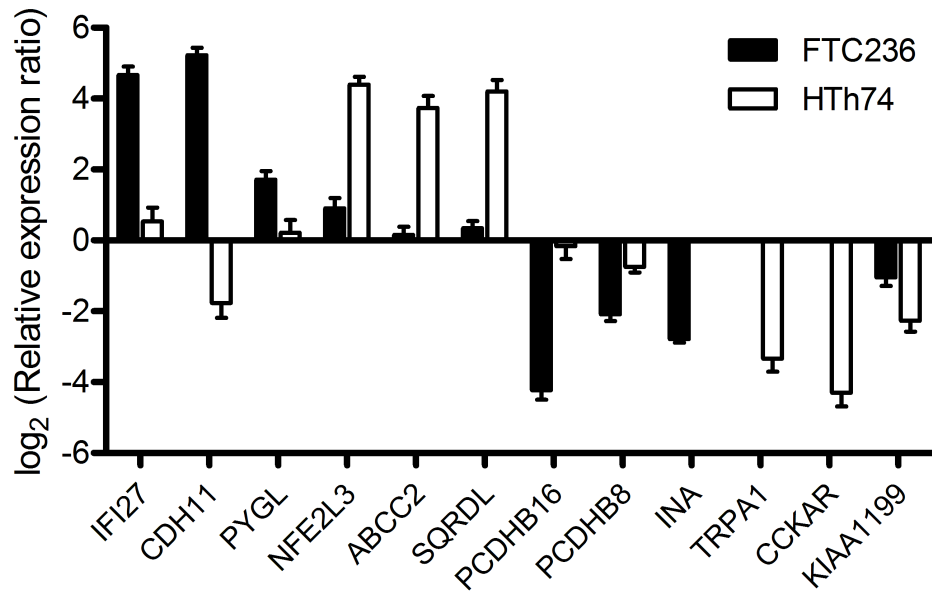**Supplemental Figures**

Supplemental Figure S1

Supplemental Figure S1. CD31 and F4/80 IHC staining of subcutaneous xenograft tumors. (**A**) Representative CD31 and F4/80 IHC staining images of the tumors. (**B and C**) Boxplots of CD31 staining (**B**) and F4/80 staining (**C**) for all tumors. The boxplots were generated using the Tukey method. Medline in the box represents the median value. The box boundaries are the Q1 (25%) and Q3 (75%) respectively (n = 7 - 9). Linear mixed models were used to perform all the statistical analyses. ***P < 0.001, ns: not significant.
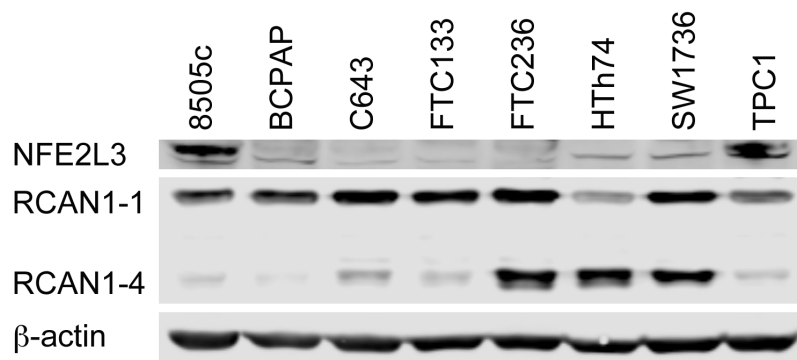
Supplemental Figure S2

Supplemental Figure S2. Principal component analysis of the microarray data. (**A and B**) The shRCAN1-4 and shCtrl cells were well separated by the first principal components (PC1) for both FTC236 cells (**A**) and HTh74 cells (**B**).
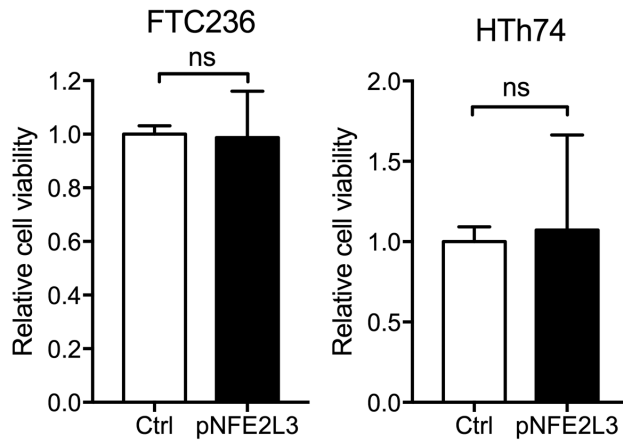
Supplemental Figure S3. Expression of Principal Component genes from both cell lines. qRT-PCR was performed for the top three overexpressed genes from FTC236 (IFI27, CDH11, PYGL) and HTh74 cells (NFE2L3, ABCC2, SQRDL) and the top three downregulated genes from FTC236 (PCDHB16, PCDHB8, INA) and HTh74 cells (TRPA1, CCKAR, KIAA1199). NFE2L3 was the most consistently overexpressed genes for both cell lines. The relative expression ratio was log2 transformed. 18S ribosomal RNA was used as control for quantitation. Each experiment was repeated three times with duplicate samples. Data were expressed as mean ± SEM.

Supplemental Figure S4

Supplemental Figure S4. The inverse correlation between RCAN1-4 and NFE2L3 in thyroid cancer cell lines. NFE2L3 and RCAN1-4 expression in eight different thyroid cancer cell lines were analyzed using Western blot. Actin was the loading control.

Supplemental Figure S5

Supplemental Figure S5. NFE2L3 overexpression does not affect cell 3D spheroid growth. The shCtrl cells were transfected with control plasmid (Ctrl) or NFE2L3 plasmid (pNFE2L3) and subjected to 3D spheroid assay. Cell viability was quantified and normalized to the control plasmid-transfected cells. A linear mixed model was used to determine the statistical significance. ns: not significant.

Supplemental Table 1. Clinical sample pathological information and the IHC scores for each available sample. The IHC score is given a 0-3 scale based on intensity. − : 0, ± : 1, +: 2, ++: 3. Representative score from one researcher was shown. Empty spaces indicate the corresponding samples are not available. PTC: Papillary thyroid cancer; FTC: Follicular thyroid cancer; ATC: Anaplastic thyroid cancer.

Supplemental Table 1

ID	Histology	Normal	Tumor Center	Tumor Invasive Front	Metastasis	Metastasis Sites
OSU3	PTC	-				
OSU6	PTC	-	+	++	+	Lung
OSU9	PTC				+	Lung
OSU10	PTC				+	Lung
OSU14	PTC		±	+	+	Kidney
OSU16	PTC				+	Lung
OSU19	PTC				+	Lung
OSU21	PTC	-	±	+		
OSU32	PTC	±	+	++		
OSU1	FTC	-	-	++	++	Soft tissue
OSU2	FTC	-	-	+	++	Bone
OSU7	FTC	-	+		+	Bone
OSU15	FTC	-	±	-	±	Bone
OSU31	FTC	-	±	+	+	Pancreas
OSU18	ATC	-	±	+		

Full unedited gel figure 1A beta actin

Full unedited gel figure 1A RCAN 1
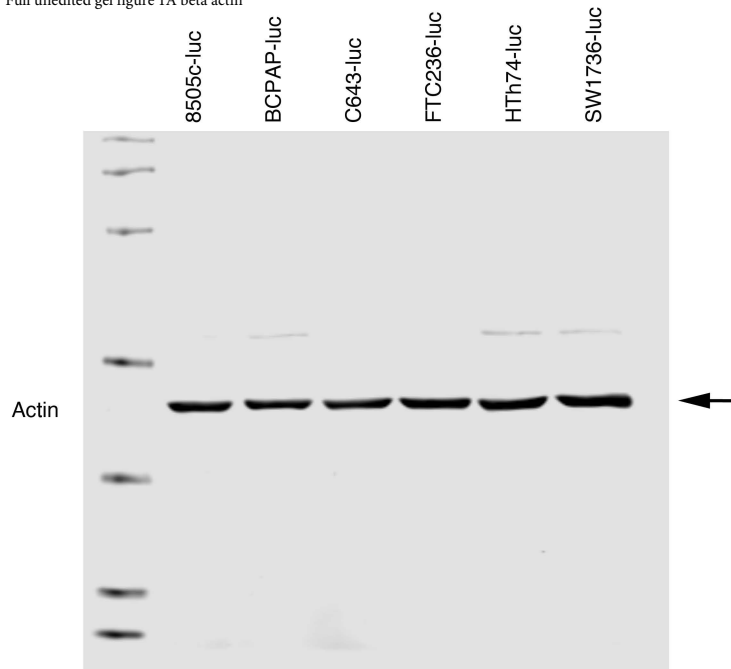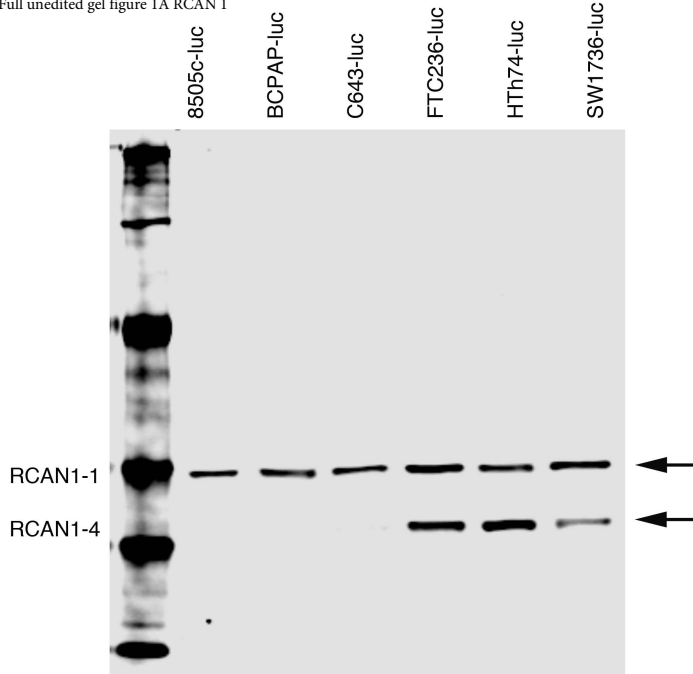
Full unedited gel
figure 1B beta actin

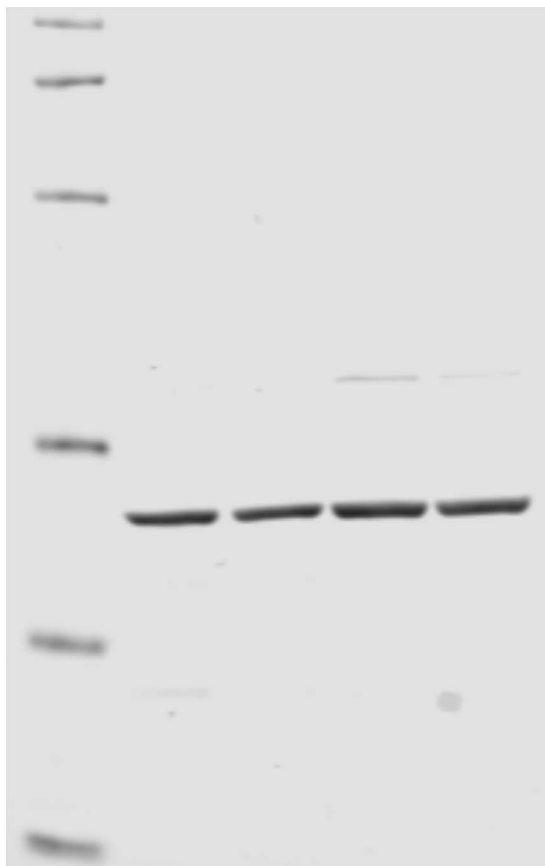FTC236   HTh74

shCtrl   shRCAN1-4   shCtrl   shRCAN1-4

Actin

FTC236   HTh74

Full unedited gel figure 1B
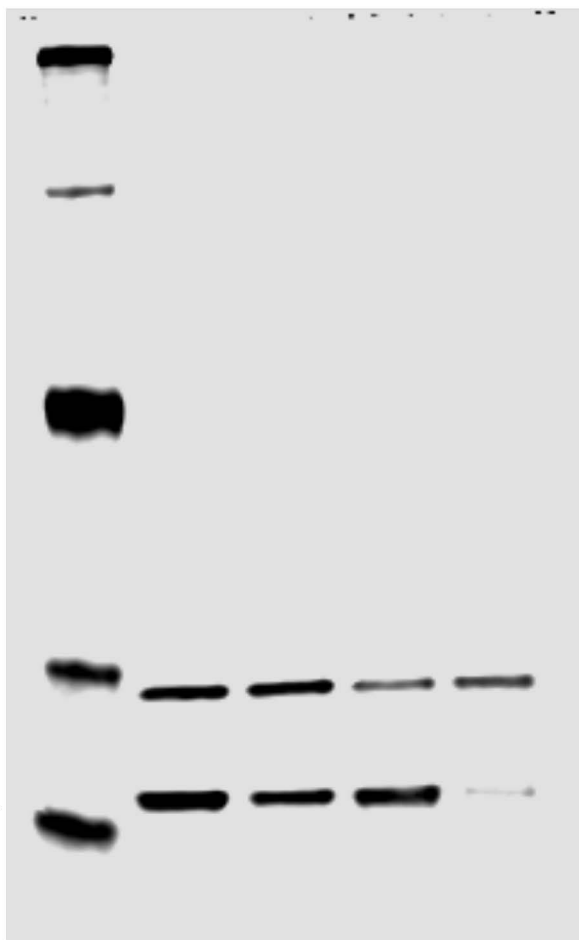RCAN1 long Exposure

shCtrl   shRCAN1-4   shCtrl   shRCAN1-4

RCAN1-1

RCAN1-4

Full unedited gel figure 1B
RCAN1 Short Exposure
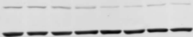
FTC236　　HTh74
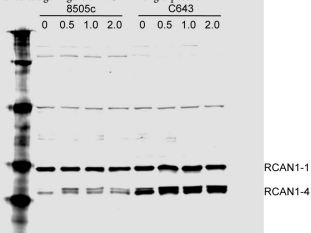
shCtrl　shRCAN1-4　shCtrl　shRCAN1-4

RCAN1-1
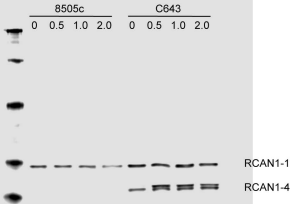
RCAN1-4

Full unedited gel Figure 5A beta actin

Actin

Full unedited gel Figure 5A RCAN1 long exposure

Full unedited gel Figure 5A RCAN1 short exposure

	8505c				C643			
0	0.5	1.0	2.0		0	0.5	1.0	2.0

RCAN1-1

RCAN1-4

FTC236   HTh74   HEK

Full unedited gel Figure 6D beta actin
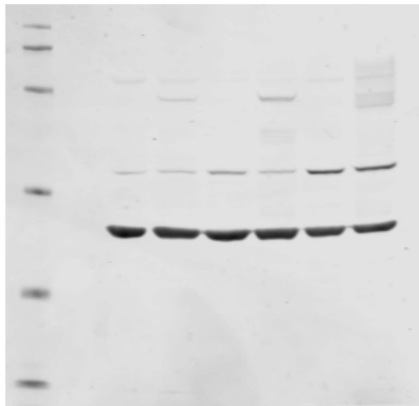
shCtrl · shRCAN1-4 · shCtrl · shRCAN1-4 · Ctrl · NFE2L3

Actin

Full edited gel
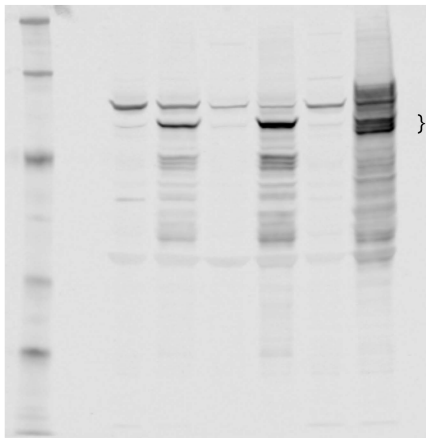Figure 6D NFE2L3

FTC236   HTh74   HEK

shCtrl   shRCAN1-4   shCtrl   shRCAN1-4   Ctrl   NFE2L3

NFE2L3

Full unedited gel
figure 7B beta-Actin

HEK   FTC236   HTh74

pCtrl   pNFE2L3   Marker   siCtrl   siNFE2L3   siCtrl   siNFE2L3

Actin

Full unedited gel figure
7B NFE2L3 long exposure
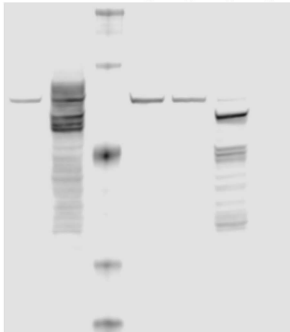
HEK   FTC236   HTh74

pCtrl
pNFE2L3
Marker
siCtrl
siNFE2L3
siCtrl
siNFE2L3

NFE2L3 {

HEK   FTC236   HTh74

Full unedited gel figure 7B
NFE2L3 short exposure

pCtrl
pNFE2L3
Marker
siCtrl
siNFE2L3
siCtrl
siNFE2L3

NFE2L3 {

FTC236 HTh74 HEK

Full unedited gel Figure 8B
beta actin

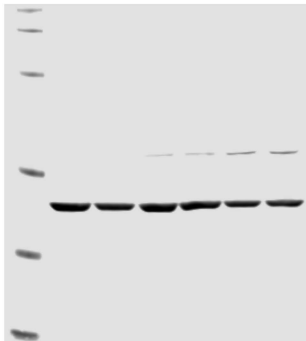Ctrl pNFE2L3 Ctrl pNFE2L3 Ctrl pNFE2L3

Actin

FTC236   HTh74   HEK

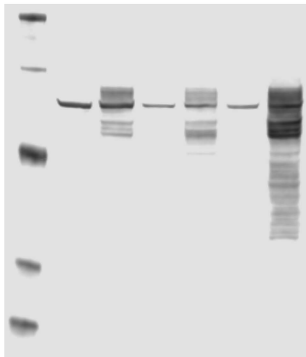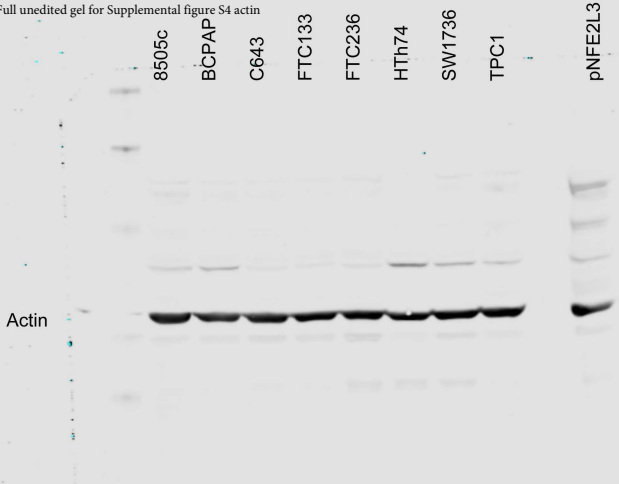Full unedited gel Figure 8B
NFE2L3

Ctrl pNFE2L3 Ctrl pNFE2L3 Ctrl pNFE2L3
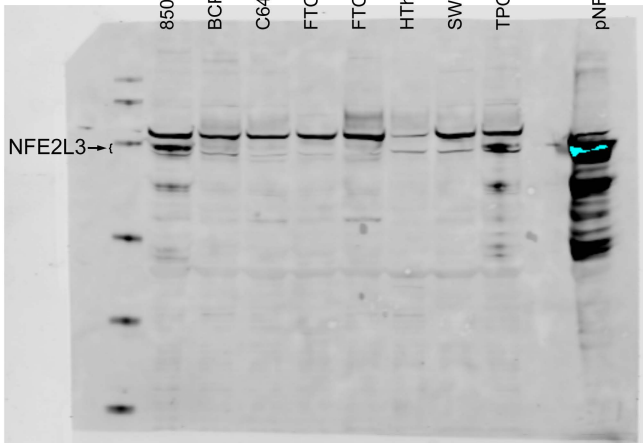
NFE2L3

Full unedited gel for Supplemental figure S4 actin

8505c BCPAP C643 FTC133 FTC236 HTh74 SW1736 TPC1 pNFE2L3

Actin

Full unedited gel for Supplemental figure S4 NFE2L3

Full unedited gel for Supplemental figure S4 RCAN1