Supplementary Figures

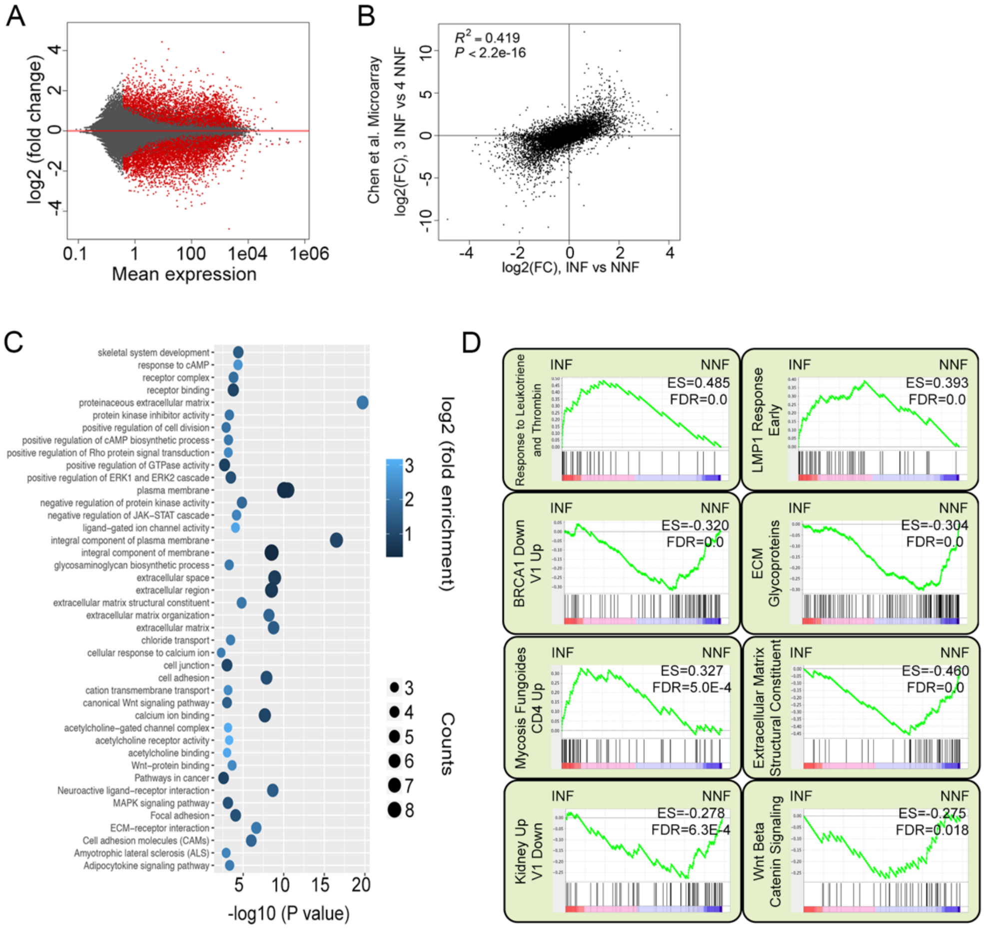

Figure S1. Differential gene expression and pathway analysis in NNF- vs. INF-PitNEts.

(A) MA plot of all genes identified by RNA-seq between NNF- vs. INF-PitNEts. (B) Independent replication analysis of NNF- vs. INF-PitNEt gene fold changes between previously reports from ref. 22 by microarray and data from this study by RNA-seq. The RNA-seq data show a replication signal between previous study and results from this study. (C) GO analysis of differential expression genes in NNF- vs. INF-PitNEt. (D) Gene set enrichment analysis (GSEA) showing characteristic data set from the NNF vs. INF group.

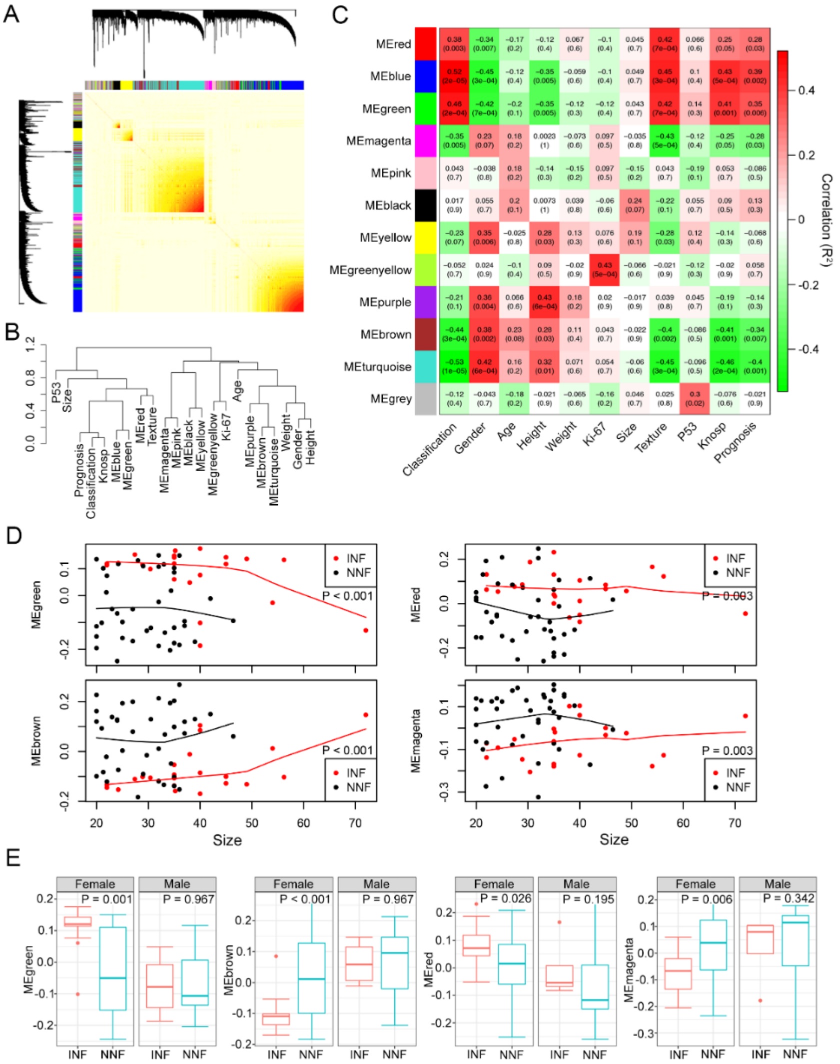

Figure S2. Weighted gene co-expression network analysis of NF-PitNEts.

(A) Heatmap of topological overlap matrix combined with the corresponding hierarchical clustering dendrogram and module colors. (B) Clustering dendrogram of the relationships among the module eigengenes and the clinical traits. (C) Correlation analysis based on the expression of the transcriptomic signature in two independent expression datasets with NNFs and INFs. (D) Plot of green, brown, red and magenta module eigengenes across tumor size. (E) Eigengene expression between sex in green, brown, red and magenta modules. *P* values are for the difference between temporal trajectories for NNFs and INFs by permutation test.

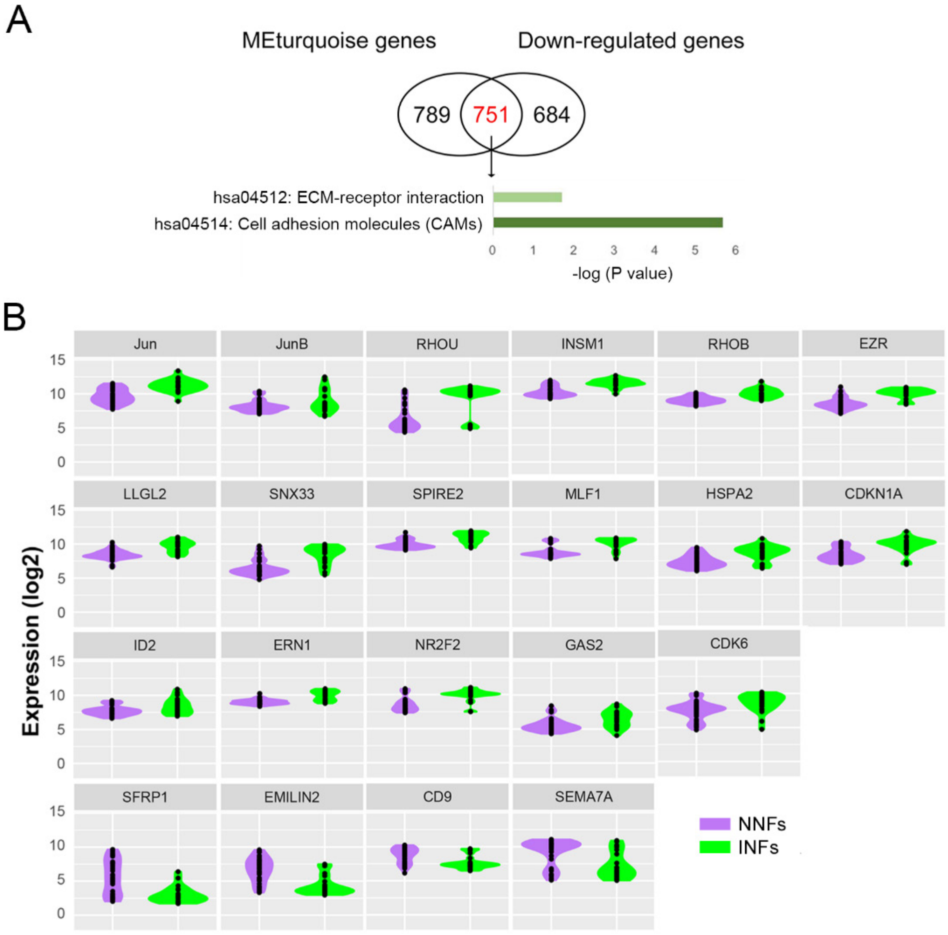

Figure S3. Significantly modulated pathways and genes between NNF- vs. INF-PitNETs.

(A) Significantly changed pathways enriched by the overlapped DEGs of turquoise module and down-regulated genes in INFs. (B) Violin plots representing the expression of DEGs that selected for qPCR validation in NNF vs. INF group.

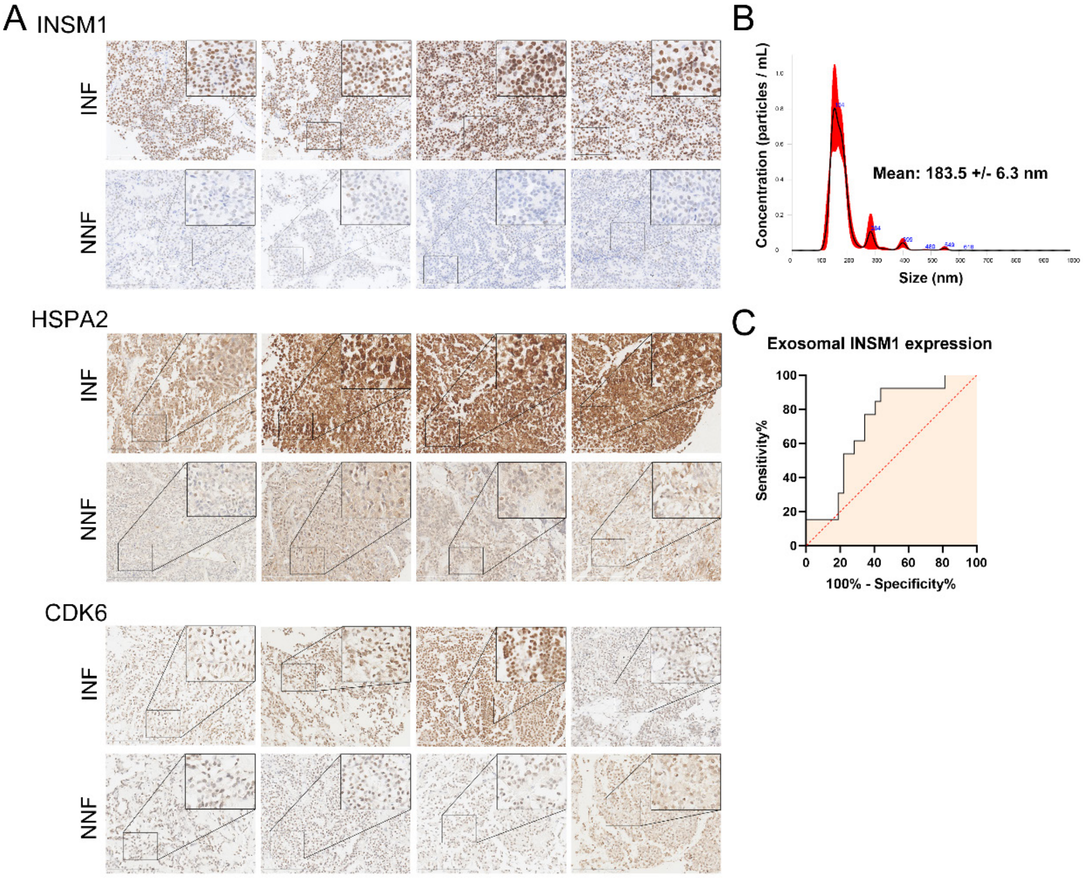

Figure S4. The histological and serum-exosomal INSM1 expression in NF-PitNEts.

(A) Additional figures for TMA analyzing INSM1, HSPA2 and CDK6 expression in NF-PitNEts. Original magnification, 200 x or 400 x; Bar, 200 µm. (B) Size distribution and concentration of exosomes analyzed by the NanoSight NS300 instrument. (C) ROC curve for exosomal INSM1 mRNA. At a cutoff value of 0.104, sensitivity was 56.3% and specificity was 92.3%. AUC, area under the curve.

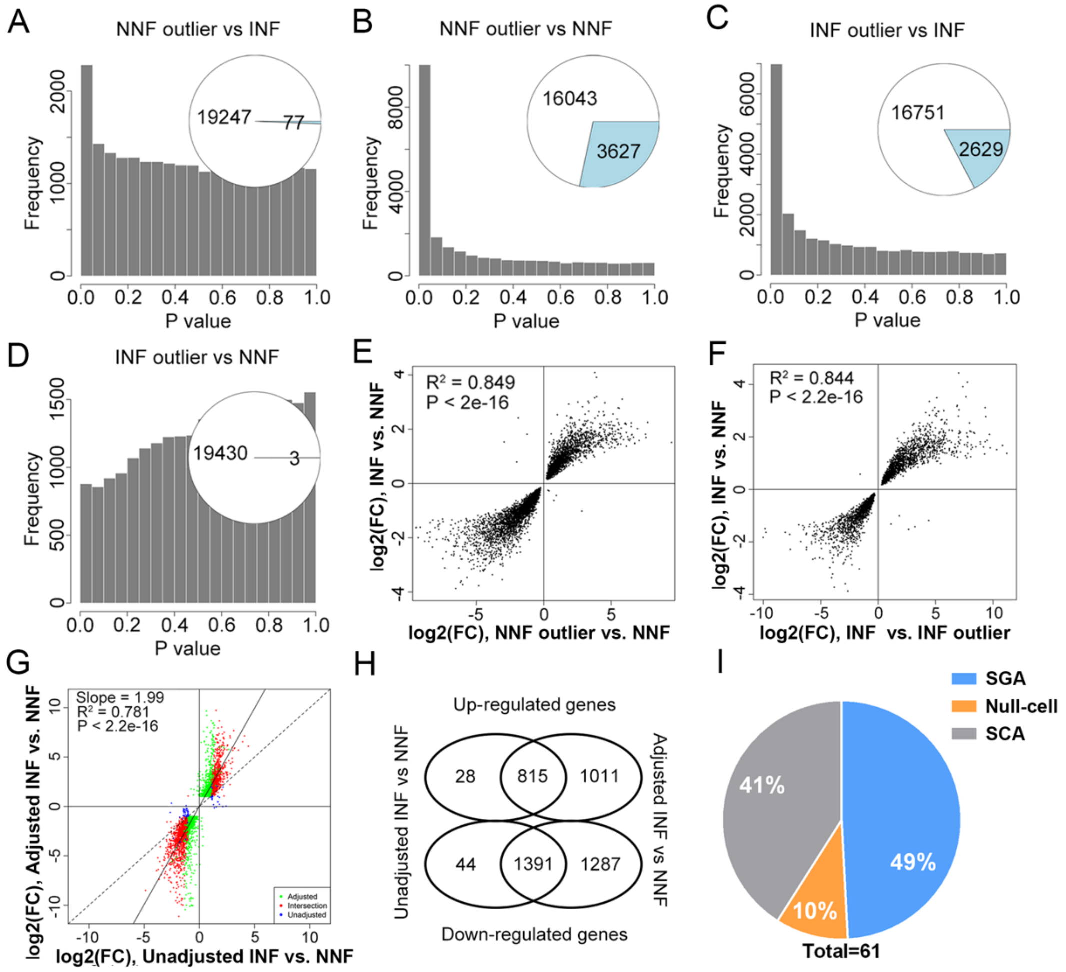

Figure S5. NF-PitNEts were divided into three subtypes.

(A-D) The distribution of *P* values and numbers of gene expression in NNF outliers vs. INFs, NNF outliers vs. NNFs, INF outliers vs. INFs, INF outliers vs. NNFs. (E) Replication of DGEs between NNF outliers vs. NNF and INF vs. NNF. (F) Replication of DGEs between INF outliers vs. INF and INF vs. NNF. (G) Replication of DEGs between outlier-unadjusted INF vs. NNF and outlier-adjusted INF vs. NNF. Red, DEGs in both; green, DEGs only in outlier-adjusted INF vs. NNF; blue, DEGs only in outlier-unadjusted INF vs. NNF. (H) Venn diagram of DEGs in outlier-unadjusted INF vs. NNF and outlier-adjusted INF vs. NNF was presented below. (I) NF-PA subtype distribution.

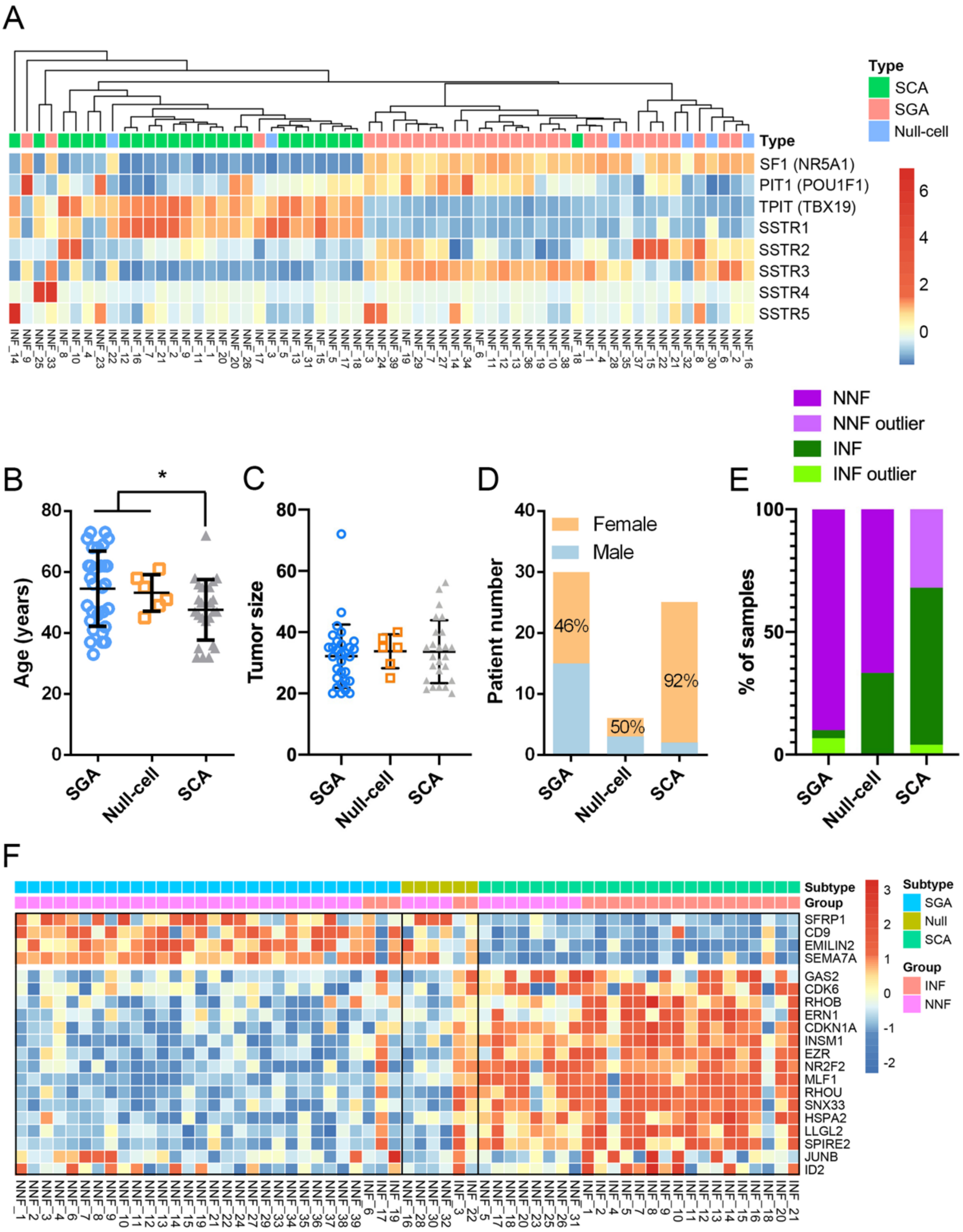

Figure S6. The clinical characteristics of NF-PAs with different cell lineages.

(A) Expression of transcription factors and somatostatin receptors (SSTRs) genes in NF-PA samples from RNA-seq data. (B-E) Identification of the age (B), tumor size (C), gender (D) and invasiveness (E) distribution among gonadotroph, null cell and corticotroph NF-PAs. (F) Expression of 20 invasion-related markers in SGA, null cell adenoma and SCA. *$P<0.05$, **$P<0.01$, ***$P<0.001$, ***$P<0.0001$, Mann-Whitney U test.

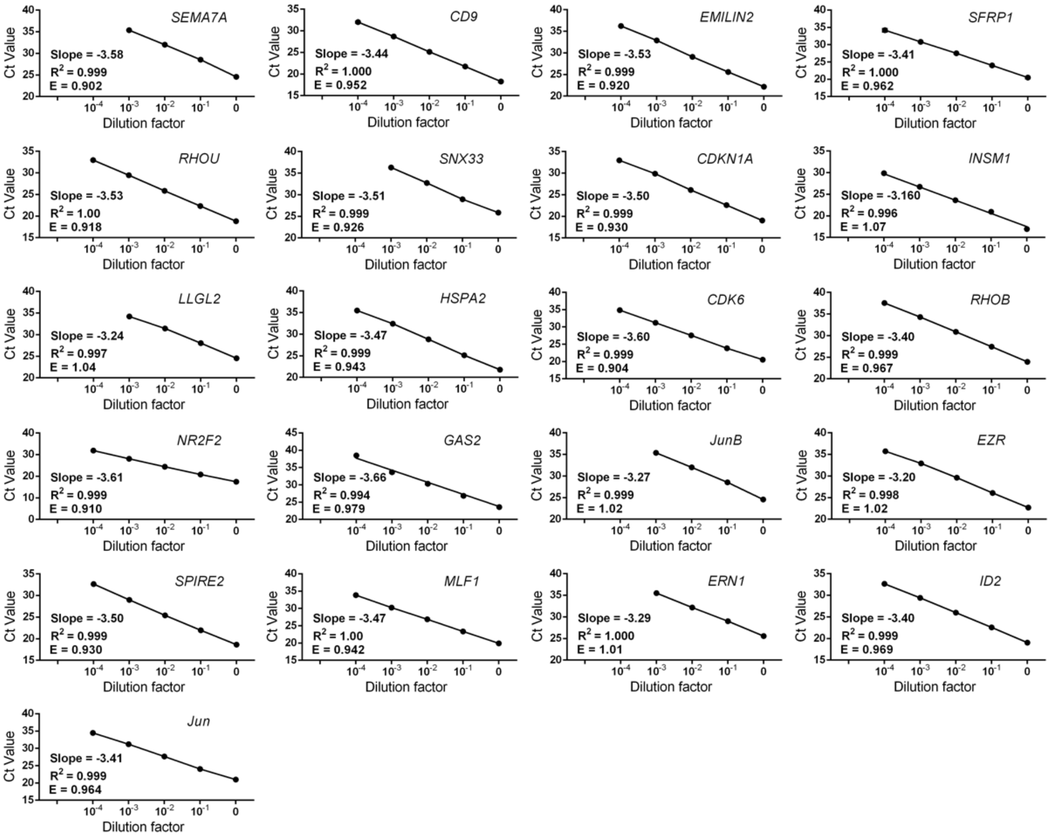

Figure S7. Standard curves for all the validated genes.

Quantification of the pituitary cDNA was performed using specific primer pairs for each gene. Calibration curves generated using 4- or 5-fold dilutions of cDNA samples (triplicates). The Cq values are plotted against the concentration of the nucleic acid target as copies/reaction. To determine the reaction efficiency (E), the slope of each calibration curve was determined from the following equation: $E = 10^{-1}/\text{slope} - 1$.

Supplementary Tables

Table S1. Classification of NF-PitNEts for RNA-seq according to pituitary hormones and transcription factors.

Cell lineage	Pituitary Hormones by IHC	Transcription Factors by IHC	No. NNF	No. INF	Total
Corticotroph Adenomas	ACTH	TPIT	1	2	3
	None	TPIT	7	15	22
Gonadotroph Adenomas	FSHβ and/or LHβ	SF1	7	0	7
	FSHβ and/or LHβ	None	20	3	23
Null cell adenomas	None	None	4	2	6
Total			39	22	61

Table S2. Primers and probes used in qRT-PCR.

Gene name	Gene ID	Forward primer (5'-3')	Reverse primer (5'-3')	Probe (5'-3')
SFRP1	6422	GAGGAGGTTTGCCTGTGATTT	TCCCAGGACTCTATCCAGAATG	AATTATCGCTAGGGCCAAGGTGGG
EMILIN2	84034	CAGCAGCAGTGTGATGACTAT	CGGGCTCGGAGGTTATTTATT	TCTATCACTCCCAGGTAGCTGCTCC
CD9	928	CGGCTTCCTCTTGGTGATATT	GCTTGTTGTAGGTGTCCTTGTA	ATGGCCGCAGCTATTTCAATGGC
SEMA7A	8482	TTCCGTACCTCCTCACTCAA	TGGAAGGTCTCTGTGGGTAT	CTACCACTCAAGCCTTCCCAACCC
Jun	3725	CACAGAGAGACAGACTTGAGAAC	ACTTGGATACCCTTGGCTTTAG	CTTGACAAGTTGCGACGGAGAGAA
JunB	3726	CCATCAACATGGAAGACCAAGA	CTTGAGCGTCTTCACCTTGT	CATCAAAGTGGAGCGCAAGCGGC
RHOU	58480	GCAGGGAGGTGAATACTCTTG	GAAGTACATCTTGGCCGACTTA	AAGGCCAACAGCAAGTGTTTGTGGGA
INSM1	3642	GTATGTAGCGTACGGGTTGTT	CAGCAGTTCACAAGCCATAATAAA	AGGCTGTAAATTGAACTTCCCACACGAT
RHOB	388	TTCGGAGCTAAGATGGTGTTAT	GGAAAGATGGTCAAGTCCTGT	TAAGGGTGGTGATGGGTGAGCGC
EZR	7430	GATCGAGGATACTCACCAGAAA	CCAGTTGTATTTGGCTGGATTG	CACCATGGATGCAGAGCTGGAGTTT
LLGL2	3993	TGATCTCACCCTCGGAGTTT	GCGGTGGTTCTTGGTTTCT	CACTTGGTGGAGAGAGAGAAGCGC
SNX33	257364	CCGTAACCTCAACCGTTTCT	GGAGTATGTCTCAGCGATCTTG	ATGCTTTGTGCGTTCTGGAGTGGA
SPIRE2	84501	GTCTGCACTTCCTGTAGCATAA	GATACCCTCTGAGGACTCTCAA	CACATCCCTGTCTACACACTGGGC
MLF1	4291	CTGGCTCCCGAGA	GCCTTTGATGTGG	AGGGAGAAACCTCAA

		ACTTAAA	AGTTTGTC	CAAAGTCCAGC
HSPA2	3306	CCCTGGAGTCCTATACCTACAA	GACACTTGTCGAGGATCTTGTT	AAGCAGACGGTGGAAGACGAGAAA
CDKN1A	1026	CGGAACAAGGAGTCAGACATT	AGTGCCAGGAAAGACAACTAC	AGATGGTGGCAGTAGAGGCTATGGA
ID2	3398	CAAGAAGGTGAGCAAGATGGA	GGTGATGCAGGCTGACAATA	TGCAGCACGTCATCGACTACATCTTG
ERN1	2081	CCCATCAACCTCTCTTCTGTATC	AGGCCGCATAGTCAAAGTAG	TGGTGATGGTGTATTCTGTTCGCCC
NR2F2	7026	CGTCTCCTCCTCAGTCATAGA	TGCCATATACGGCCAGTTAAA	TGCTGCCGGACAGTAACATATCCC
GAS2	2620	GATTGCAGCCAGGTATGGT	GAAGGAGAAGGTGAAGGAGAAG	TGGAGCCTCCTGGTTTGATAAAGCTG
CDK6	1021	CTAGCAACCATCCCTCCATTAC	CTCAGAGCATTCTGAAGACAGTAG	ACACAGAAAGCCCTCTTGAAGCAA
GAPDH	2597	GGTGTGAACCATGAGAAGTATGA	GAGTCCTTCCACGATACCAAAG	AGATCATCAGCAATGCCTCCTGCA
UBC	7316	GTACCCTGTCTGACTACAACATC	GTCTTGCCAGTGAGTGTCTT	AGAAAGAGTCCACCTTGCACCTGG

Table S3. Differential expressed genes between NNF- and INF-PAs.

This table is uploaded as excel file.

Table S4. The statistical significance of modules obtained from WGCNA.

Modules	**P**	**BH adjusted P**	**log10(adj. P)**
MEred	0.003	0.0072	2.1426675
MEblue	2.00E-05	0.00012	3.92081875
MEgreen	2.00E-04	0.0008	3.09691001
MEmagenta	0.005	0.01	-2
MEpink	0.7	0.7636364	0.11711338
MEblack	0.9	0.9	0.04575749
MEyellow	0.07	0.12	-0.9208188
MEgreenyellow	0.7	0.7636364	-0.1171134
MEpurple	0.1	0.15	-0.8239087
MEbrown	3.00E-04	0.0009	-3.0457575
MEturquoise	1.00E-05	0.00012	-3.9208188

Table S5. Clinical characteristics of NNF- and INF-PitNEt patients for validation.

Clinicopathological feature	**RT-PCR cohort**		*P* Value
	NNF	**INF**	
	92	51	

Age (years)			0.154 ^{a}
Average	52.8	46.4	
SD	14.2	14.0	
Gender			**0.007** ^{b}
Male	59	20	
Female	33	31	
P53			1.000 ^{c}
Negative	89	49	
Positive	3	2	
Ki-67			0.0869 ^{c}
≤3%	86	43	
＞3%	6	8	
Largest diameter			**0.001**^{a}
average	28.6	36.4	
SD	7.4	12.6	

^{a} Unpaired t test; ^{b} Chi-square test; ^{c} Fisher's exact test.

Boldface type indicates statistical significance.

Table S6. Extent of INSM1 staining in PA subtypes.

Subtypes	Invasion	INSM1 moderate & strong / positive (percentage)	Percentage of positive tumor cell nuclei				P value (H-score)
			<40%	≥40 and <60%	≥60 and <80%	≥80	
SGA	NNF-PA	15/40 (37.50%)	8	5	20	7	0.4085
	INF-PA	6/8 (75%)	1	1	2	2	
Nul cell adenomas	NNF-PA	2/8 (25%)	2	2	2	2	***0.0497***
	INF-PA	3/5 (60%)	0	0	3	2	
SCA	NNF-PA	5/5 (100%)	0	0	0	5	0.8651
	INF-PA	13/14 (92.86%)	0	0	3	11	

Table S7. Extent of HSPA2 staining in PA subtypes.

Subtypes	Invasion	HSPA2 positive / total (percentage)	Percentage of positive tumor cells				P value (H-score)
			<40%	≥40 and <60%	≥60 and <80%	≥80	
SGA	NNF-PA	17/39 (43.59%)	4	3	8	2	0.7532
	INF-PA	5/8 (62.50%)	2	2	1	0	
Null cell adenomas	NNF-PA	4/10 (40%)	0	0	2	2	0.8228
	INF-PA	3/5 (60%)	0	1	2	0	
SCA	NNF-PA	3/5 (60%)	0	0	1	2	0.7374
	INF-PA	14/14 (100%)	3	1	5	5	

Table S8. Extent of CDK6 staining in PA subtypes.

Subtypes	Invasion	CDK6 positive / total (percentage)	Percentage of positive tumor cells				P value (H-score)
			<40%	≥40 and <60%	≥60 and <80%	≥80	
SGA	NNF-PA	34/42 (80.95%)	11	6	14	3	***0.0435***
	INF-PA	4/6 (66.67%)	3	1	0	0	
Null cell adenomas	NNF-PA	6/9 (66.67%)	3	0	1	2	0.1528
	INF-PA	5/5 (100%)	1	2	1	1	
SCA	NNF-PA	4/5 (80%)	2	2	0	0	0.0684
	INF-PA	14/15 (93.33%)	2	2	8	2	