Expanded View Figures

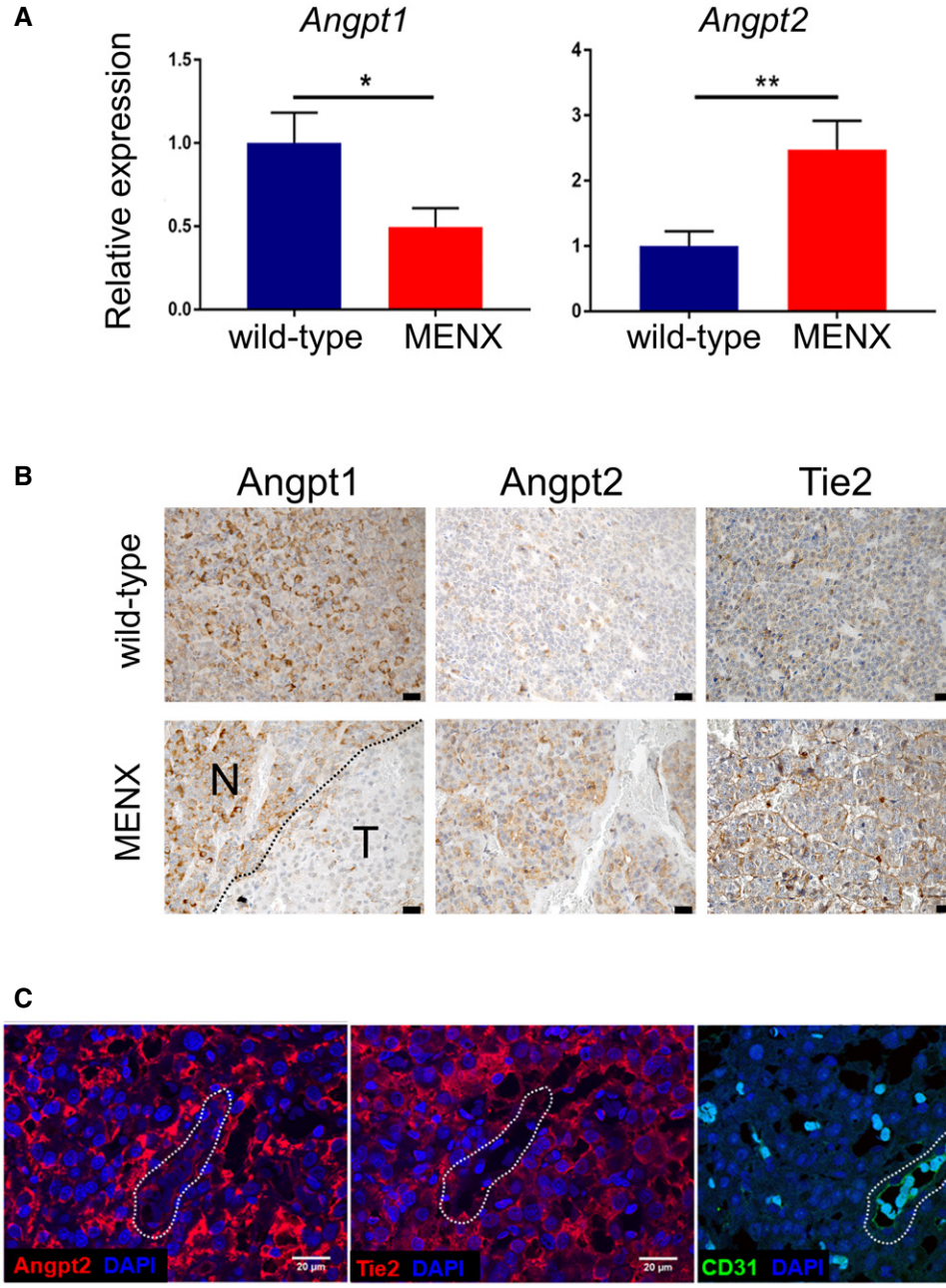

Figure EV1. Angpt2 is expressed in rat primary NF-PitNETs together with Tie2 and Angpt1.

A qRT–PCR for *Angpt1* and *Angpt2* was performed on pituitary samples from tumor-bearing MENX mutant rats (n = 10, 12, respectively) and wild-type control rats (n = 5, 8). Data are expressed as mean ± SEM. *P = 0.049; **P = 0.008 by t-test.

B IHC was performed on pituitary tissues from wild-type (n = 5) and MENX mutant rats (n = 10) using antibodies against Angpt1, Angpt2. Representative stainings are shown. Original magnification: 400×; scale bar: 20 µm. N, normal area; T, tumor area.

C Expression of Angpt2, Tie2, and CD-31 in rat NF-PitNETs and associated ECs (used as positive control). Consecutive tissue sections of rat NF-PitNETs (n = 4) were stained with the indicated antibodies. One representative tumor is shown. The dashed shape indicates the vessel present in the consecutive slides. Original magnification: 200×; scale bar: 20 µm.

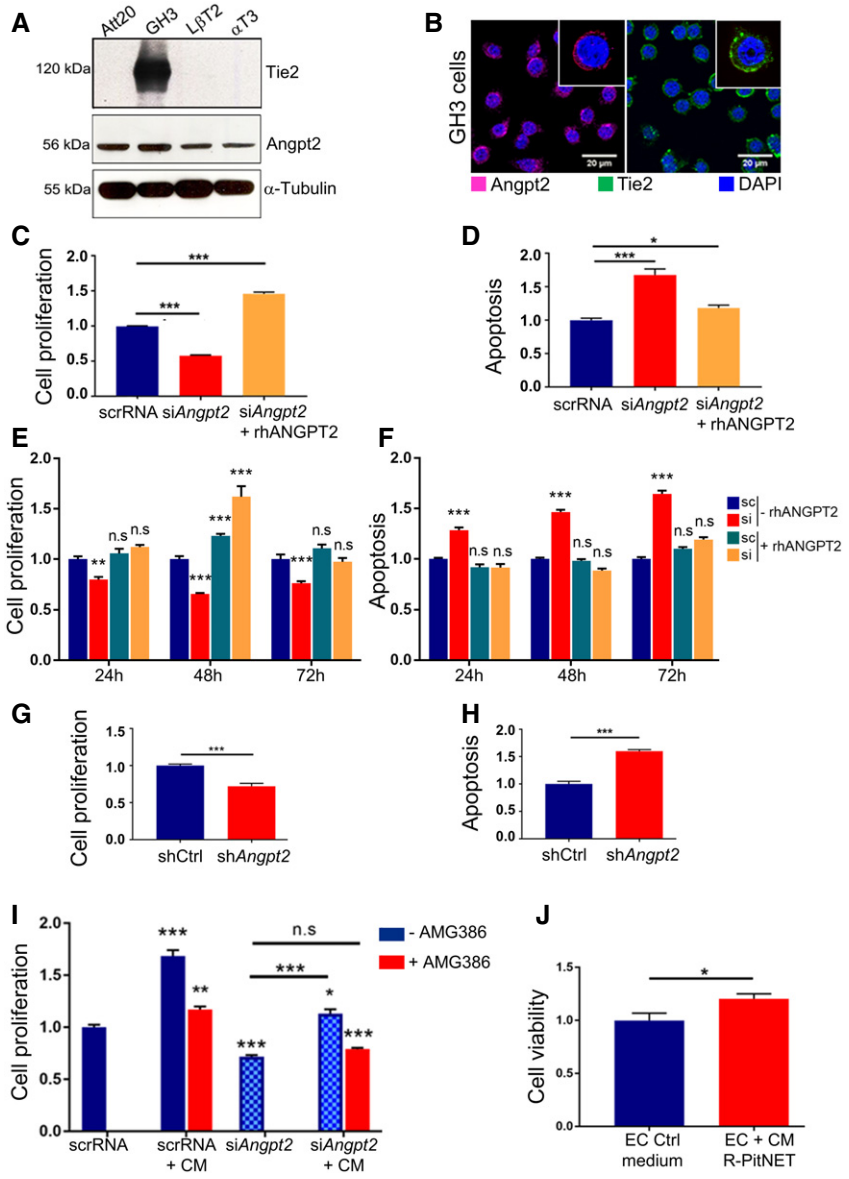

Figure EV2. PitNET cells express Angpt2 and Tie2, and intrinsic Angpt2 promotes PitNET cell survival *in vitro*.

A Expression of Angpt2 and Tie2 was assessed in Att20, GH3, LβT2 and αT3 cells by western blotting (WB) using specific antibodies. α-Tubulin was included as loading control.

B Immunofluorescence (IF) of GH3 cells for Angpt2 (red) and Tie2 (green). Nuclei were counterstained with DAPI (blue). Original magnification: 400x; scale bar: 20 μm. Panels shown are representative of 3 independent experiments.

C Cell proliferation of GH3 cells transfected with si*Angpt2* or scrRNA POOLs and incubated with rhANGPT2 (+rhANGPT2) or left untreated (−rhANGPT2) normalized against scrRNA-transfected cells. ***$P < 0.0001$ (one-way ANOVA).

D In samples parallel to (C), activated caspase-3/7 was measured to assess for apoptosis. ***$P < 0.0001$; *$P = 0.0411$ (one-way ANOVA).

E Cell proliferation of GH3 cells transfected with the individual siAngpt2 or scrRNA and incubated with rhANGPT2 (+rhANGPT2) or left untreated (−rhANGPT2) normalized against scrRNA-transfected, untreated (scr-rhANGPT2) cells. n.s., not significant; ***$P < 0.0001$; **$P < 0.002$ (one-way ANOVA).

F In samples parallel to (E), activated caspase-3/7 was measured to assess for apoptosis. n.s., not significant; ***$P < 0.0001$ (one-way ANOVA).

G Cell proliferation of GH3-sh*Angpt2* (#2) cells or cells transduced with shCtrl was measured 24h later and normalized against shCtrl. ***$P < 0.0001$ (*t*-test).

H In samples parallel to (G), activated caspase-3/7 was measured to assess for apoptosis. ***$P < 0.0001$ (*t*-test).

I GH3 cells transfected with si*Angpt2* or scrRNA POOLs were incubated with conditioned medium (CM) from rat primary PitNET cells for 24 h. CM was pre-incubated with AMG386 (red bars), or left untreated (blue bars). Cell proliferation was measured and normalized against that of scrRNA-transfected cells. n.s., not significant; ***$P < 0.0001$; **$P = 0.00116$; *$P = 0.0192$ by two-way ANOVA.

J Cell viability of isolated rat primary EC cells incubated with CM from isolated rat primary PitNET cells. The experiments was performed independently 2 times, each with 3 technical replicates. Results are expressed as mean ± SEM. *$P < 0.0368$ (*t*-test). (C–I) Data are expressed as the mean ± SEM of three biological replicates, each with 3–6 technical replicates.

Source data are available online for this figure.

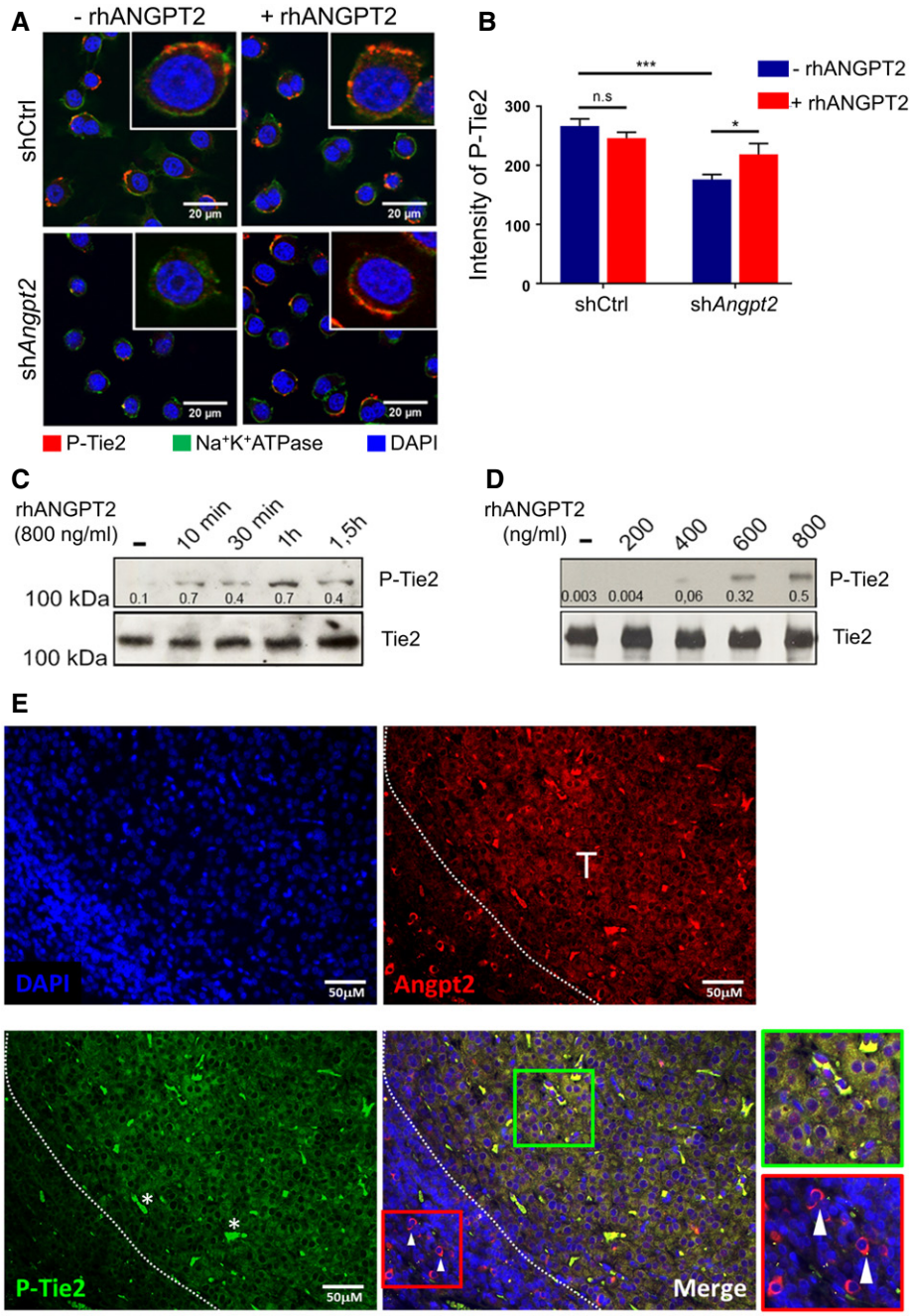

Figure EV3. Tie2 on PitNET cells is phosphorylated and can be stimulated by Angpt2.

A Co-IF for both P-Tie2 (Tyr 1102/1108; red) and for Na^+K^+ ATPase (green), used as plasma membrane marker, of GH3 cells transduced with unspecific shRNA (shCtrl) and sh*Angpt2* (#2) and incubated with rhANGPT2 or left untreated. Original magnification: 400×; scale bar: 20 µm.

B Quantification of P-Tie2 immunostaining intensity in cells shown in (A) shCtrl-transduced GH3 cells (266.71 ± 12.12); in sh*Angpt2* cells (176.04 ± 8.46); in control cells treated with rhANGPT2: shCtrl + rhANGPT2: 246.33 ± 9.79 (versus shCtrl, not significant by *t*-test); in treated knockdown cells: sh*Angpt2* + rhANGPT2: 218.68 ± 18.16 (versus shC2; **P* = 0.0421 by *t*-test). The experiment was performed twice each with three technical replicates. Intensities are expressed as arbitrary units ± SEM. ****P* < 0.0001 by *t*-test.

C Tie2 and P-Tie2 expression in serum-starved GH3 cells transfected with siAngpt2 POOLs and stimulated with rhANGPT2 for the indicated times.

D Tie2 and P-Tie2 expression in cells as in (C) stimulated with the indicated doses of rhANGPT2 for 30 min.

E Co-IF for Angpt2 (red) and P-Tie2 (green) of a representative rat primary NF-PitNET. Nuclei were counterstained with DAPI. White arrows point to Angpt2-positive (P-Tie2 negative) cells in adjacent non-tumor area. Scale bars: 50 µm. T, tumor area.

Data information: (C, D) Blots shown in all panels are representative of three independent experiments. The numbers represent the ratio phospho/total proteins.
Source data are available online for this figure.

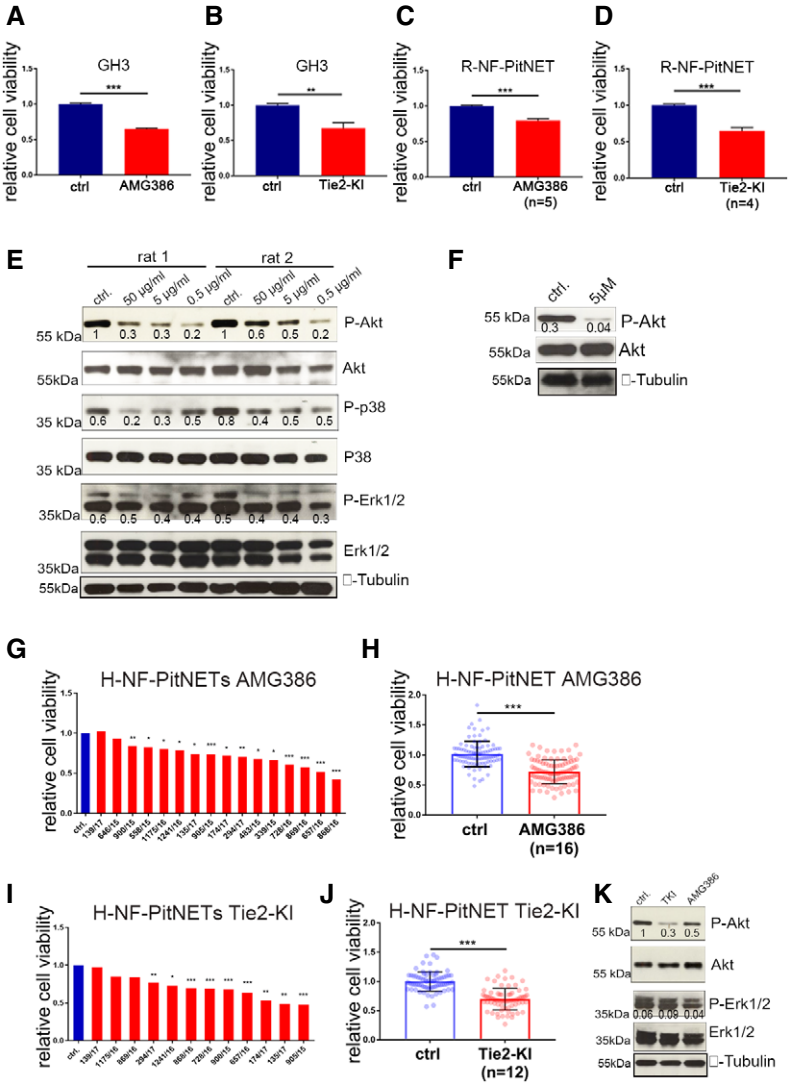

Figure EV4. Pharmacological Ang/Tie2 pathway inhibition reduces the viability of PitNET cell lines, and of rat/human primary cultures *in vitro*.

A, B Cell proliferation of GH3 cells treated with 5 µg/ml AMG386 (A) or 5 µM Tie2-KI (B) or left untreated (ctrl) normalized against untreated cells. ***$P < 0.0001$; **$P = 0.0072$ (*t*-test). Results are expressed as mean ± SEM of three biological replicates with 6 technical replicates.

C, D Cell viability of rat primary PitNET cells (R-PitNET) treated with AMG386 ($n = 5$ rats) (C) or Tie2-KI ($n = 4$ rats) (D) or left untreated (ctrl) normalized against untreated cells. Primary cultures were treated, each with 6 technical replicates. Results are expressed as mean ± SD. ***$P < 0.0001$ (*t*-test).

E Expression of phosphorylated (P) and total Akt, p38 and Erk1/2 in two R-PitNET cultures with enough cells upon treatment with AMG386.

F Expression of phosphorylated (P) and total Akt in one R-PitNET culture treated with Tie2-KI or left untreated (ctrl).

G–J Cell viability of human primary PitNET cultures treated with (G, H) AMG386 ($n = 16$) or (I, J) Tie2-KI ($n = 12$) normalized against untreated control cells for each patient (set to 1). Significance in (G, I): *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ (by *t*-test, comparing each treated sample with its untreated control). Results in (H) and (J) are expressed as mean ± SEM of the normalized values of 6 technical replicates for each samples. Each dot represents the relative cell viability of one technical replicate. Significance in (H, J) ***$P < 0.0001$ (*t*-test).

K Example of responsive H-PitNET treated with AMG386 or Tie2-KI. Total proteins were extracted and probed for P- and total Akt and Erk1/2. Anti-α-tubulin antibody as used to check for equal loading. The blot shown is representative of three independent cultures.

Data information: (E, F) Blots shown are representative of 3 independent experiments. (E, F, K) The numbers below the bands represent the ratio phospho/total proteins.
Source data are available online for this figure.