Supplemental figure legends

Supplemental Figure 1: Microarray-based expression of GPCRs in AIMAH compared with adenomas and with normal and Cushing’s disease adrenal cortex.

Each dot represents a GPCR. The X axis shows the expression ratio between AIMAH and the adrenal adenomas (n=13). The Y axis shows the expression ratio between AIMAH (n=18) and the normal (n=3) or Cushing’s disease (n=4) adrenal cortex. GPCRs showing a significant difference between AIMAH and adenomas are presented as point-up triangles (two-sided Wilcoxon p<0.01). Those with a significant difference between AIMAH and normal or Cushing’s disease adrenal cortex are presented as point-down triangles (two-sided Wilcoxon p<0.01). Those with a significant difference between AIMAH and both normal and Cushing’s adrenal cortex and adenomas are presented as stars. The X and Y axes are expressed in scaled fold (log-scales).

Supplemental Figure 2: Expression of ADRA2A protein in AIMAH tissue and in normal adrenocortical cell cultures.

A, Western blot analysis of ADRA2A in protein extracts (30 µg) from 3 AIMAH samples (left panel). In the right panel, the specificity of the immunoreactive bands was assessed by pre-incubation of anti-ADRA2A antibody with the ADRA2A antigenic peptide (ns: non-specific signal). Mouse brain (mBrain) was used as a positive control for ADRA2A expression. β-actin was used for standardization of protein loading. B, comparison of ADRA2A expression in AIMAH 2 (20 µg) and in human normal adrenocortical cell culture (NA1, 20 µg).

Supplemental Figure 3: Effect of clonidine on basal and forskolin-induced stimulation of cortisol production in NCI H295R cells.

A, Cortisol production of NCI H295R cells incubated with increasing concentrations of clonidine, in the absence or in the presence of yohimbine (10^-6 M). B, Cortisol production of NCI H295R cells incubated with increasing concentrations of yohimbine, in the presence of clonidine (10^-6 M); C,
Cortisol production of NCI H295R cells stimulated with forskolin (FSK, 10 µM) in the absence or in the presence of increasing concentrations of clonidine. BL: basal cortisol level. Measurements were performed in triplicate and values are means ± SE from triplicate samples. *, significantly different from basal with p<0.05; §, significantly different from FSK with p<0.05, ###, significantly different from clonidine with p<0.01.

**Supplemental Figure 4:** Effect of clonidine on cAMP production in NCI H295R cells.

A, Intracellular cAMP levels in NCI H295R cells incubated with increasing concentrations of clonidine, in the absence or in the presence of yohimbine (10^{-6} M); BL: basal cAMP level; B, Intracellular cAMP levels in NCI H295R cells stimulated with forskolin (FSK, 10 µM) in the absence or in the presence of increasing concentrations of clonidine. Measurements were performed in duplicate and values are means ± SE from triplicate samples. **, significantly different from basal with p<0.01; ##, #### significantly different from FSK with p<0.01 and p<0.001, respectively.

**Supplemental Figure 5:** Effect of pertussis toxin and H89 on clonidine-elicited cortisol production in NCI H295R cells.

NCI H295R cells were pretreated with pertussis toxin (PTX) or H89 for 2 h or 30 min, respectively, and then subsequently incubated with serum-free medium alone, 10^{-6} M clonidine in the absence (A) or in the presence (B) of forskolin (FSK) for 24 h as stated in Supplemental Material and Methods. BL: basal cortisol level. Measurements were performed in triplicate and values are means ± SE from triplicate samples.