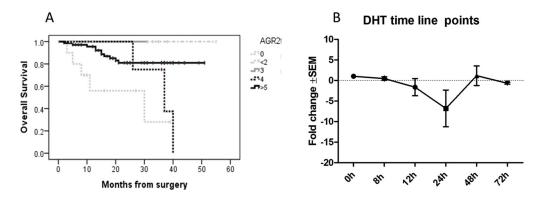
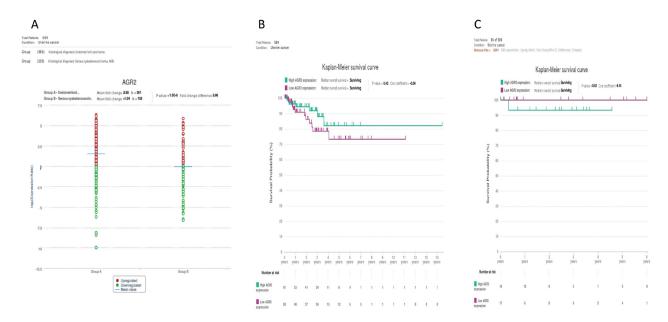
High AGR2 protein is a feature of low grade endometrial cancer cells

SUPPLEMENTARY MATERIALS

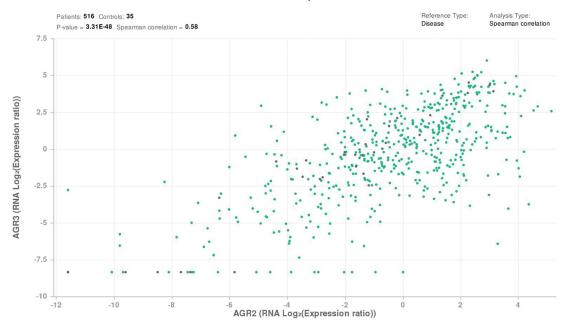


Supplementary Figure 1: (A) Kaplan–Meier curve with different AGR2 immunostaining quickscore cut off points; (B) AGR2 mRNA level after treatment with 1 μ M 5α -dihydrotestosterone (DHT; D-073-1ML) at different time points.



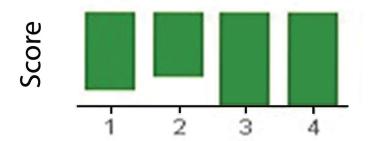
Supplementary Figure 2: Validations from TCGA Uterine cancer cohort data. (A) The expression of AGR2 gene in Group A = endometrioid EC Group B = Serous EC. (B) Kaplan–Meier survival curves for the correlation between AGR2 gene expression and overall survival in the entire endometrial cancer cohort (n = 589); (C) Kaplan-Meier survival curves for the correlation between AGR2 gene expression and overall survival in EC patients with high ERSI (ER) RNA expression (n = 86). Data produced using Illumina's BaseSpace Cohort Analyzer application.

Correlation plot



Supplementary Figure 3: Correlation of AGR2 and AGR3 RNA expression in TCGA cohort of uterine cancer samples. TCGA uterine cancer samples had RNA expression data for AGR2 and AGR3 in 516 endometrial cancer samples (green dots) and 35 control (black dots). The analysis demonstrated a significant positive correlation between the two genes (r = 0.58, p = 3.31E-48).

Gene-Bioset Correlations for AGR2



Supplementary Figure 4: External validation with 3 published EC microarray datasets. *AGR2* gene expression in 3 independent sample sets, using Illumina's BaseSpace Correlation Engine application (software; https://www.illumina.com/informatics/research/biological-data-interpretation/nextbio.html; Illumina, San Diego, CA, USA) to include 126 grade 1 endometrioid cancers, and 131 grade 3 endometrioid and serous EC from the following publically available microarray datasets; (1) GSE17025, 26 grade 3 versus 30 grade 1 endometrioid, (2); GSE24537, 11 serous versus 11 grade 1 (3); GSE2109, 31 grade 3 versus 22 grade 1 endometrioid, (4); GSE2109, 63 endometrioid grade 3 versus 22 endometrioid grade 1. The height of each vertical bar in the score matrix graph represents the score of the correlation between the analysed biosets and the *AGR2* gene. The correlation's score is based on the significance of the measurement made for the *AGR2* gene in the biosets analysed. The bar is green and appears below the midline depicting the *AGR2* gene was significantly down-regulated or deleted in in grade 3 and serous ECs compared with grade 1 ECs.

Supplementary Table 1: Primary antibodies and their immunohistochemistry conditions

Primary	Type	Clone	Supplier	HIAR*	Dilution	Incubation conditions	
Ab				(min)		Time (hour)	Temp (°C)
AGR2	Monoclonal	EPR3278	Abcam ²	2	1:1500	20	4
AR	Monoclonal	441	$DAKO^1$	2	1:50	20	4
PR	Monoclonal	PgR 636	DAKO	2	1:1000	1	18
$ER\alpha$	Polyclonal		Abcam ²	2	1:50	2	18
ERβ	Monoclonal	PPG5/10	Serotec ³	2	1:50	20	4
Ki67	Monoclonal	MM1	Leica ⁴	4	1:200	20	4

^{*}Heat induced antigen retrieval by pressure cooking in citrate buffer pH 6 (Hapangama *et al*, 2012). ¹Ely, Cambridgeshire, UK; ²Cambridge, UK; ³Oxford, UK; ⁴Newcastle upon Tyne, UK; Dorset, UK.

Supplementary Table 2: Genes that perturb *AGR2* **when mutated or knocked down.** The genes that influence *AGR2* expression when either mutated or knocked down were examined with Illumina's BaseSpace Knockout atlas application (software;https://www.illumina.com/informatics/research/biological-data-interpretation/nextbio.html; Illumina, San Diego, CA, USA). See Supplementary_Table_2