Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information for

Analysis of the Effects of Aryl Hydrocarbon Receptor Expression on Cancer Cell Invasion via Three-Dimensional Microfluidic Invasion Assays

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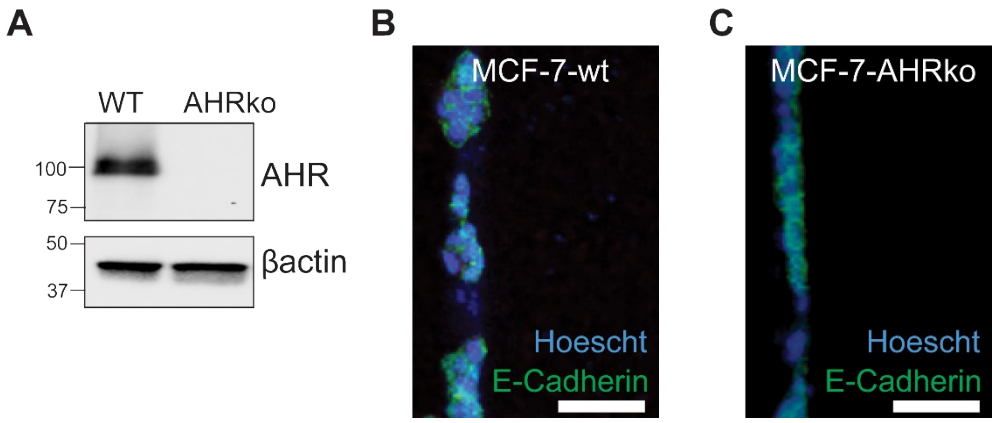

Figure S1. (A) Western blot for AHR protein in MCF-7-wt (left) and MCF-7-AHRko (right) cells. Top-view fluorescent confocal microscope images of MCF-7-wt (B) and MCF-7-AHRko (C) cells on CIMMS devices on day 4 after seeding on microgels, immunofluorescently labeled for Nucleus (blue) and E-cadherin (green). Scale bar A,B = 100 µm.

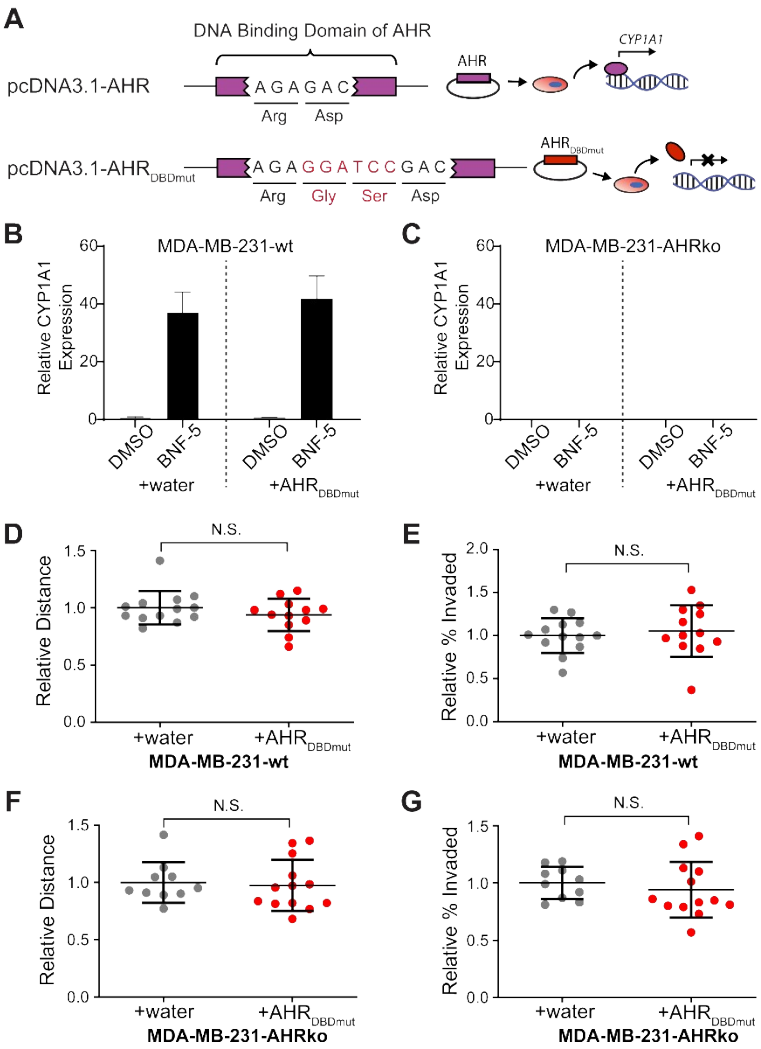

Figure S2. Overexpression of AHR with DNA-binding-domain mutation (AHR_{DBDmut}) does not affect invasiveness. (A) Cartoon showing the differences between transfection plasmids. The AHR_{DBDmut} plasmid has a 2 amino acid insertion into the DNA-binding-domain which inactivates its transcriptional function. (B,C) ddPCR results for CYP1A1 mRNA expression following 36 hours incubation with vehicle (DMSO, left/grey) or 1 μM beta-naphthoflavone (BNF-5, right/black) for MDA-MB-231-wt cells (B) and MDA-MB-231-AHRko cells (C). Cells were cultured in well-plates and transfected with vehicle (+water) or AHR_{DBDmut} plasmid (+AHR_{DBDmut}), with error bars = 1 std. dev from n = 3 replicates per condition. Expression values were first normalized to the expression level of housekeeping gene GAPDH, then normalized to the expression level of CYP1A1 in MDA-MB-231-wt cells transfected with water and treated 36 hours with DMSO. (D-G) Plots generated from CIMMS experiments illustrating invasion characteristics of cells transfected with vehicle (grey, +water) or AHR_{DBDmut} plasmid (red, + AHR_{DBDmut}), including relative invasion distance and relative percentage of cells invaded for wild-type cells (D,E), and AHRko cells (F,G). Measurements were made on day 4 after seeding, and error bars represent ±1 std. deviation for n = 10-13 microgels per condition. (N.S. $p > 0.2$)

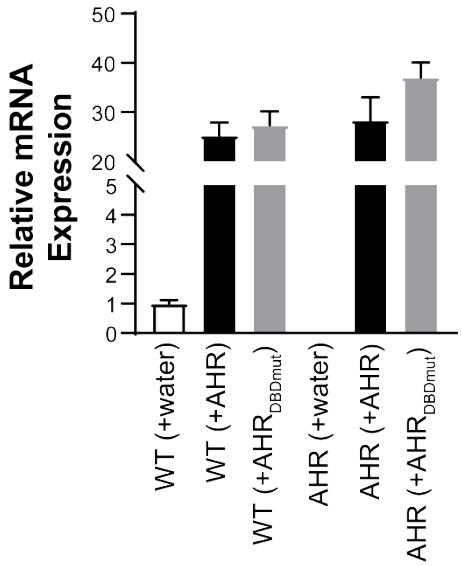

Figure S3. ddPCR results for AHR mRNA expression following 36 hours incubation with DMSO for MDA-MB-231-wt cells and MDA-MB-231-AHRko cells. Cells were cultured in well-plates and transfected with vehicle (+water, white), AHR plasmid (+AHR, black) or AHR_{DBDmut} plasmid (+AHR_{DBDmut}, grey), with error bars = 1 std. dev from n = 3 replicates per condition. Expression values were first normalized to the expression level of housekeeping gene GAPDH, then normalized to the expression level of AHR in MDA-MB-231-wt cells transfected with water. The primers used in this experiment amplify a region of the AHR gene that does not contain the DBD mutation, and thus this measurement does not discriminate between the transcripts originating from the AHR or AHR_{DBDmut} plasmids.

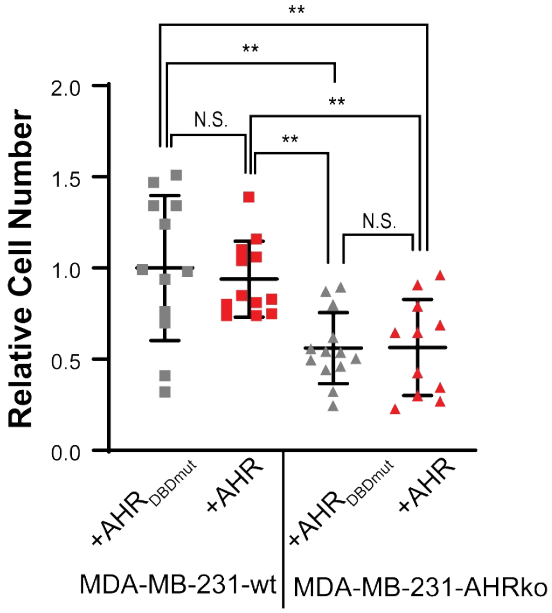

Figure S4. Total cell count generated from CIMMS experiments on Day 4 for wild-type (square) and AHRko (triangle) cells transfected with AHR_{DBDmut} plasmid (+AHR_{DBDmut}, grey) or AHR plasmid (+AHR, red). Error bars represent ±1 std. deviation for n = 11-13 microgels per condition. (** $p < 0.01$, and N.S. not significant).

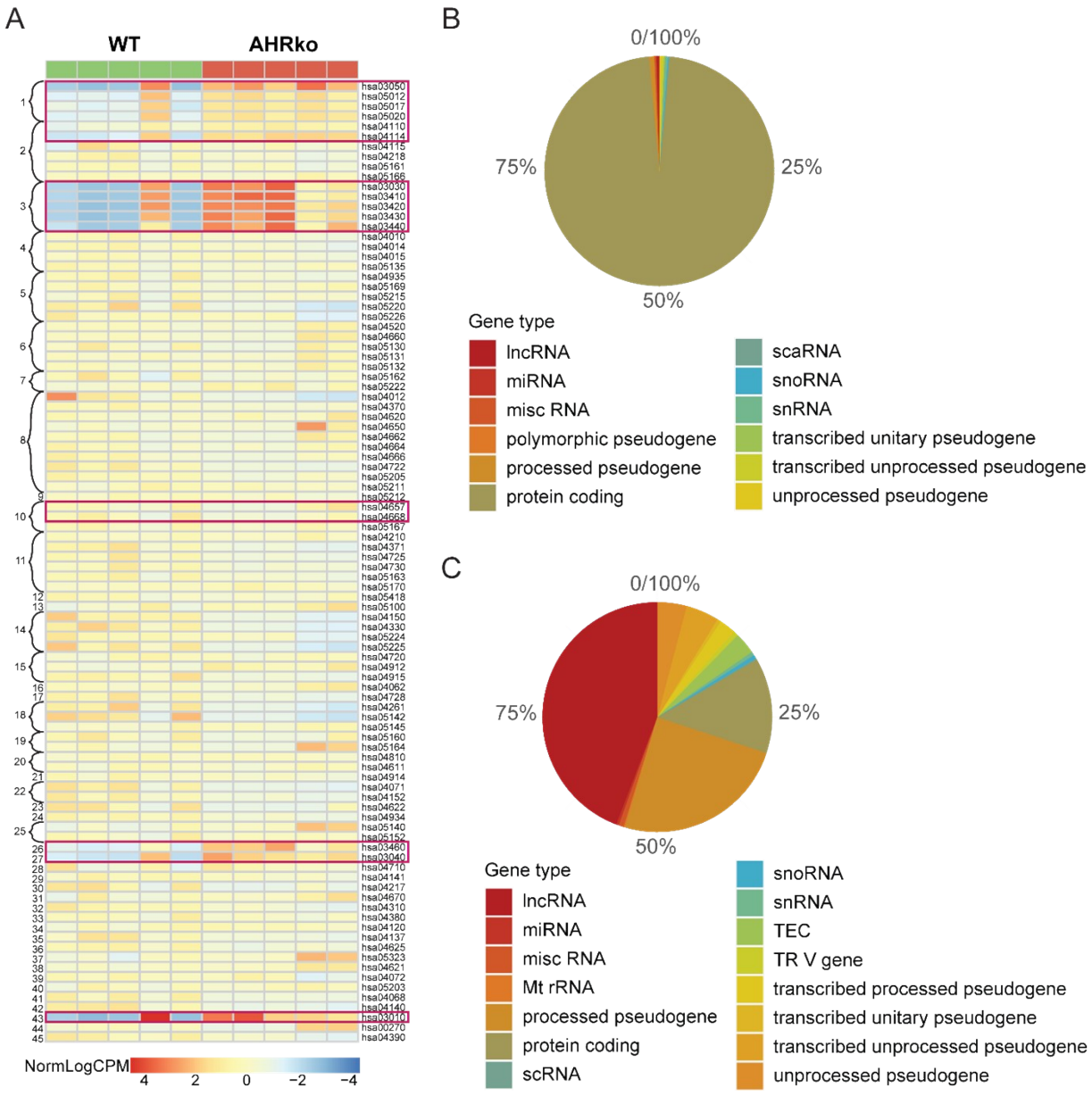

Figure S5. PathfindR analysis of 1809 differentially expressed genes between invaded MDA-MB-231-wt cells and invaded MDA-MB-231-AHRko cells. (A) Heat map of pathway expression [shown as variance-stabilized log of read count per million (CPM), normalized by expression values between columns, where red = high and blue = low] of the 96 pathways (rows, labels on right, Table S6) that were enriched in a pathfindR analysis of transcriptomes of invaded MDA-MB-231-AHRko cells (red header, five columns on right) relative to those of invaded MDA-MB-231-wt cells (green header, five columns on left). The 96 pathways cluster into 45 functional groups (numbers on left). Pink boxes identify the 16 pathways of interest probed in more detail in Figure 4C of the main text (and Table S7). Pie charts of gene type compositions of (B) the 1287 DEGs for invaded AHRko cells vs. invaded wild-type cells (out of 1809 total, Table S3) assigned to pathways by pathfindR and (C) the 659 DEGs not assigned to pathways.

Supplementary Table S1 (external file) List of 65 differentially expressed genes (DEGs) observed in invaded vs. non-invaded sub-populations of MDA-MB-231-AHRko cells in CIMMS experiments.

Supplementary Table S2 (external file) List of 2 groups of pathways and gene ontologies determined using ClueGO that contained genes that were up-regulated in non-invaded sup-populations of MDA-MB-231-AHRko cells (relative to invaded AHRko cells) among the 65 DEGs in Table S1.

Supplementary Table S3 (external file) List of 1809 differentially expressed genes (DEGs) observed in invaded sub-populations of MDA-MB-231-wt cells vs. invaded sub-populations of MDA-MB-231-AHRko cells in CIMMS experiments.

Supplementary Table S4 (external file) List of 3 groups of pathways and gene ontologies determined using ClueGO that contained genes that were up-regulated in invaded sup-populations of MDA-MB-231-AHRko cells (relative to invaded wild-type cells) among the 1809 DEGs in Table S3.

Supplementary Table S5 (external file) List of 8 groups of pathways and gene ontologies determined using ClueGO that contained genes that were up-regulated in invaded sup-populations of MDA-MB-231-wt cells (relative to invaded AHRko cells) among the 1809 DEGs in Table S3.

Supplementary Table S6 (external file) List of 96 pathways determined using pathfindR that were enriched in the 1809 DEGs in Table S3.

Supplementary Table S7 (external file) List of 159 genes associated with the 16 of the pathways selected from the list of 96 pathways determined by pathfinder (Table S6) of the 1809 DEGs in Table S3.