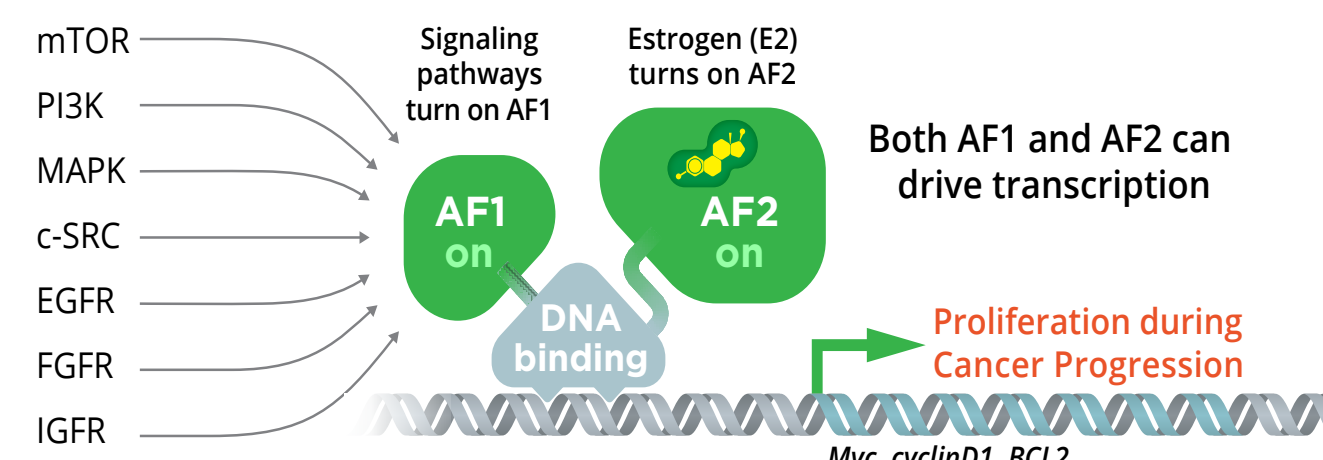
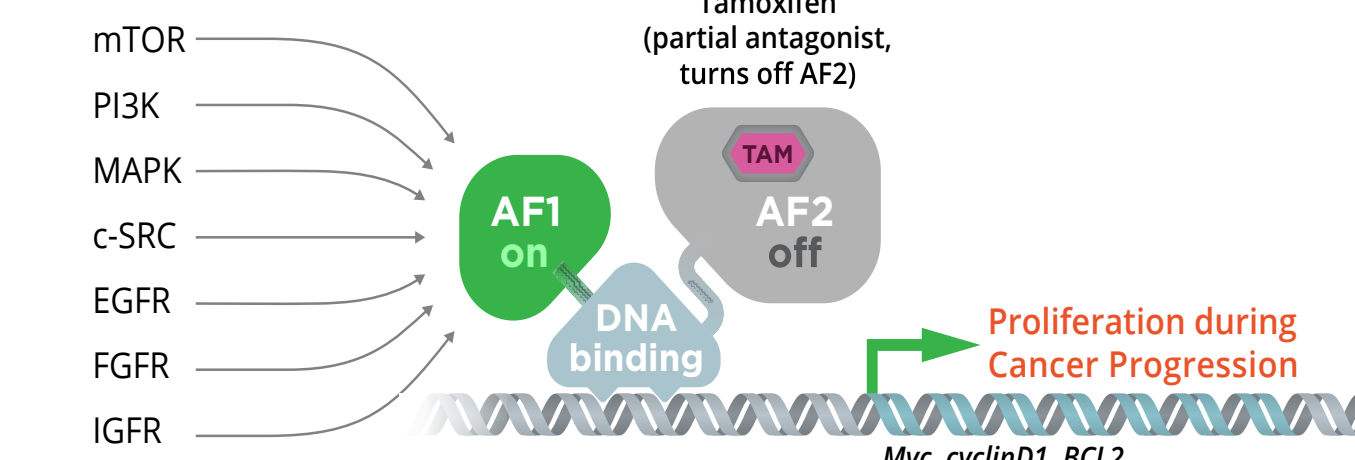


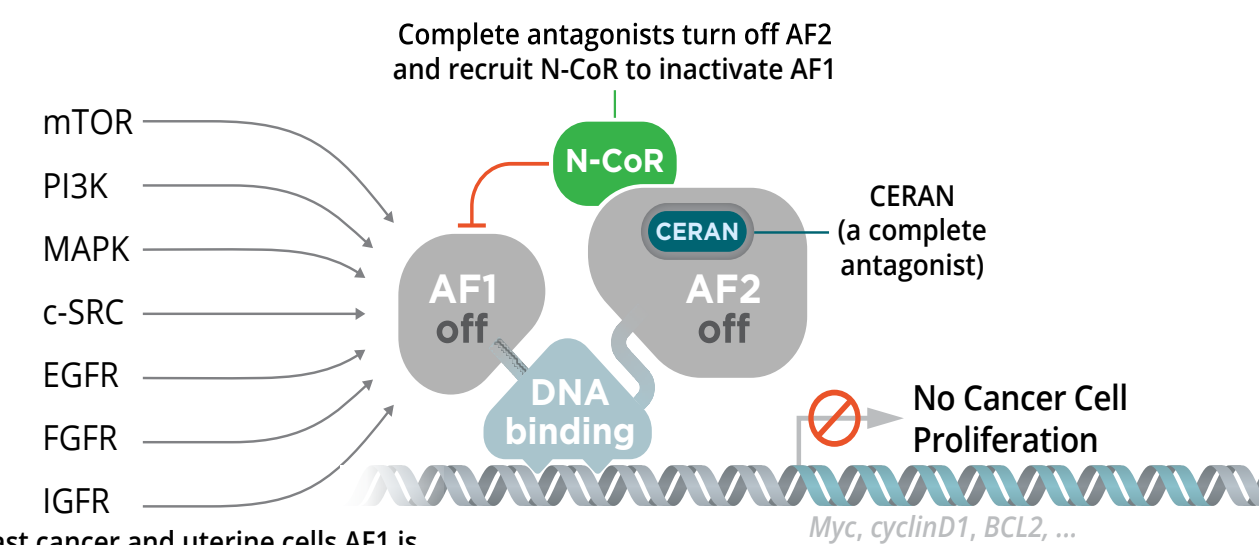
**The Estrogen Receptor (ER) is a tripartite protein with two distinct transcriptional activation functions (AF1 and AF2)**



**Partial antagonists (such as tamoxifen) have a short duration of response for treatment of breast cancer**



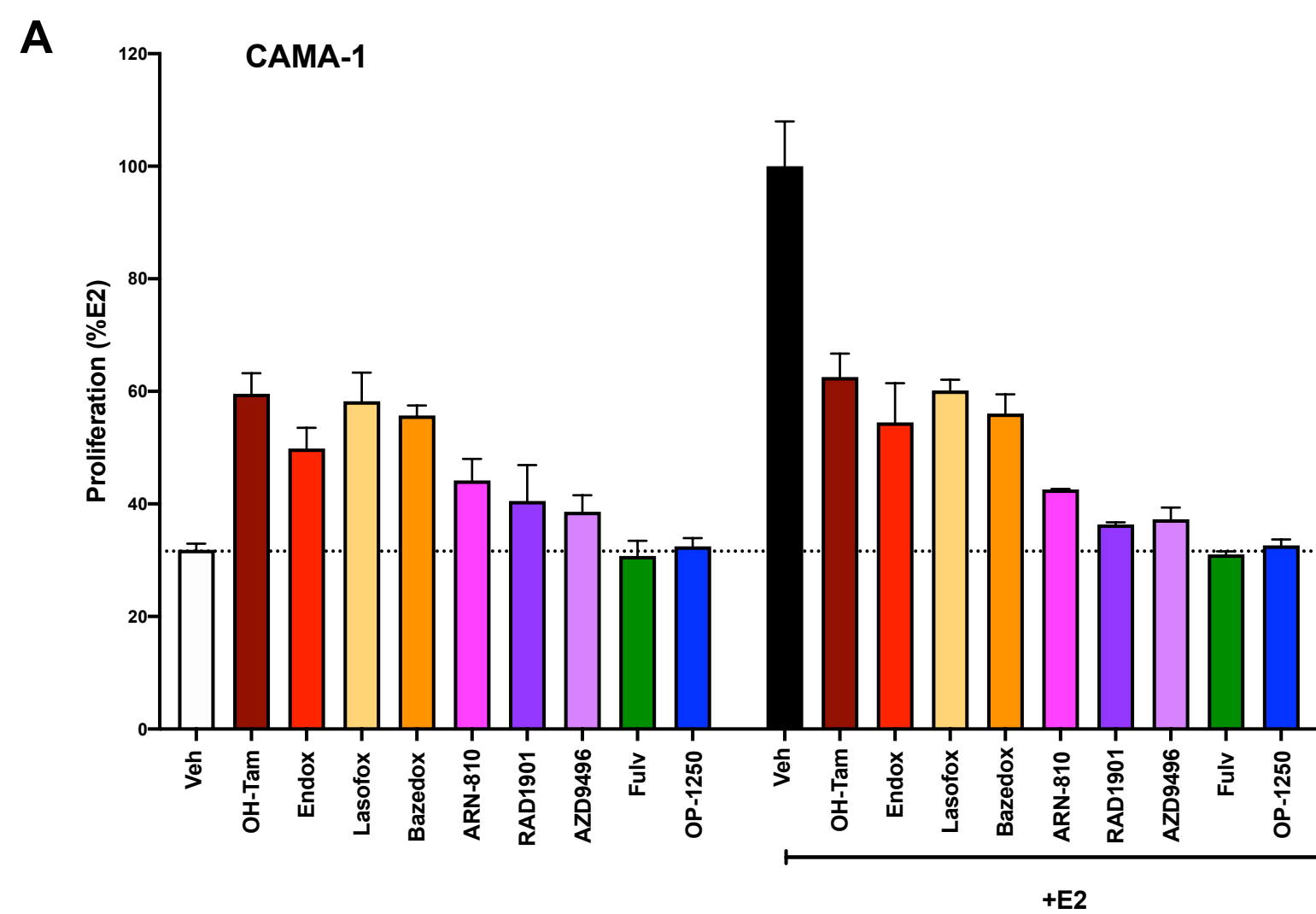
**CERANs shut down both activation functions (AF1 and AF2) of the ER**



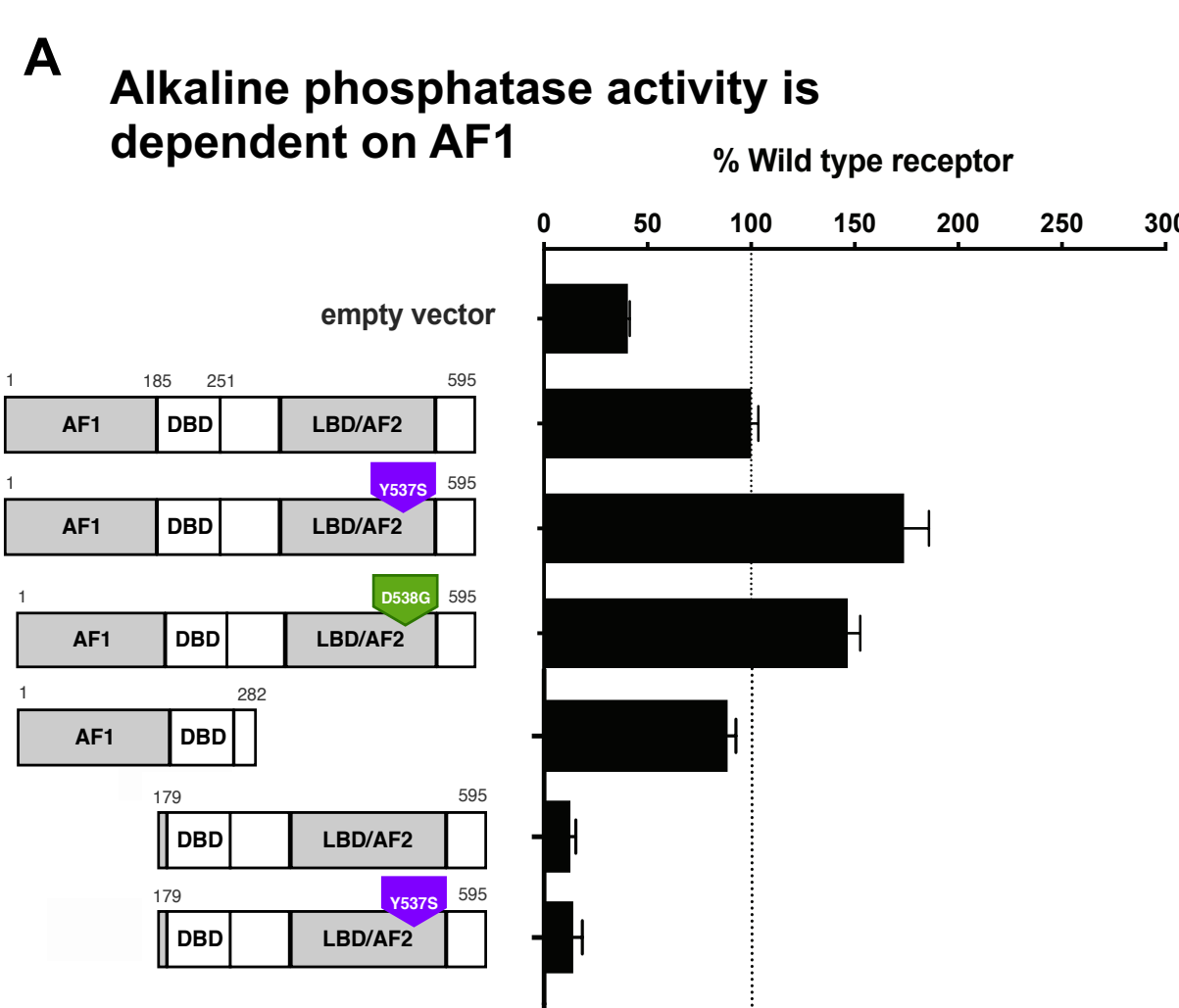
CERANs block AF1 activity, even in the presence of signaling, preventing cell proliferation

**References:** 1) Shang and Brown, *Science*, 29 Mar 2002: Vol. 295, Issue 5564, pp. 2465-2468. 2) Webb, Nguyen, and Kushner, *JBC*, Vol. 278, 28 Feb 2003, pp. 6912–6920. 3) Fanning, et al., *Nature Comm.*, Jun 2018, Vol. 9:2268. 4) Hodges-Gallagher, *SABCS 2019 (poster)*.

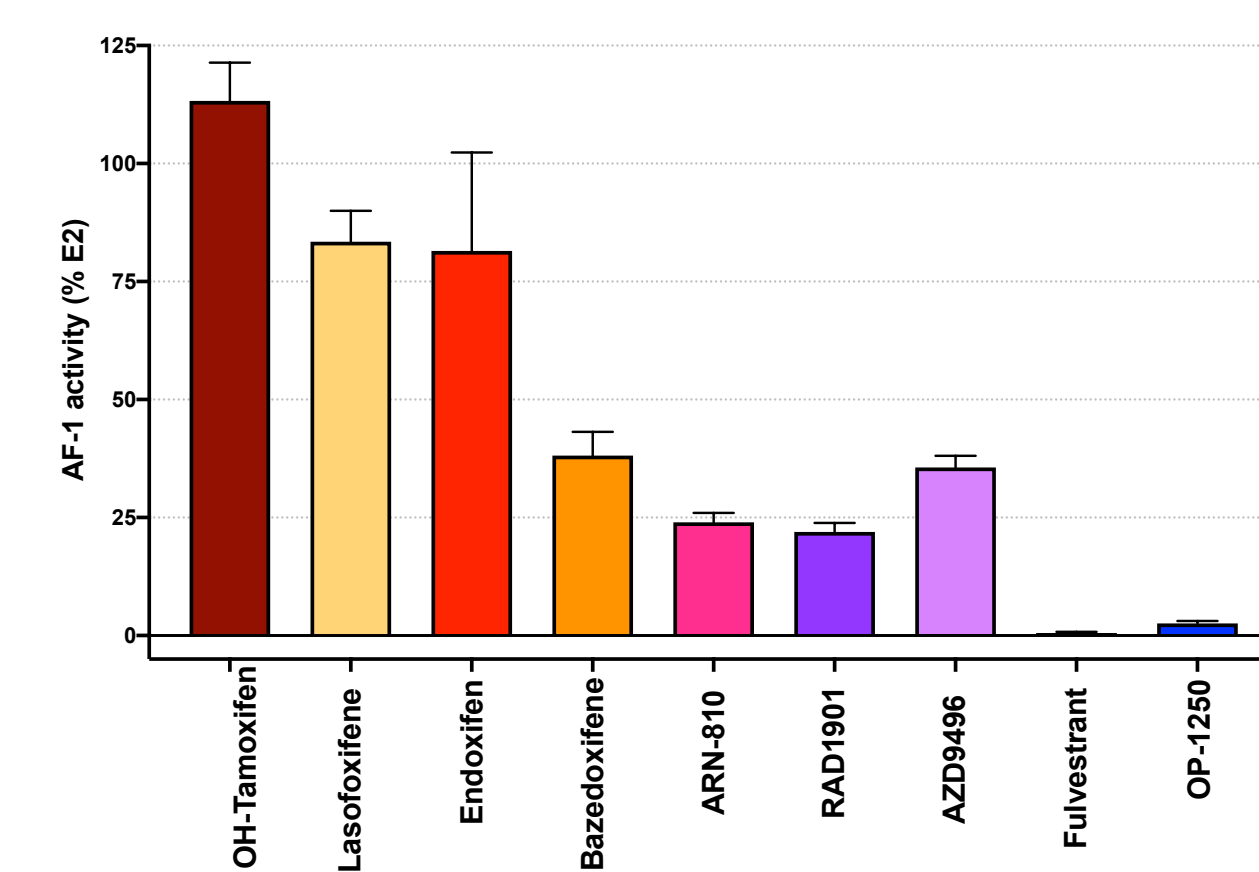
## Partial agonists increase, or incompletely block, breast cancer cell proliferation



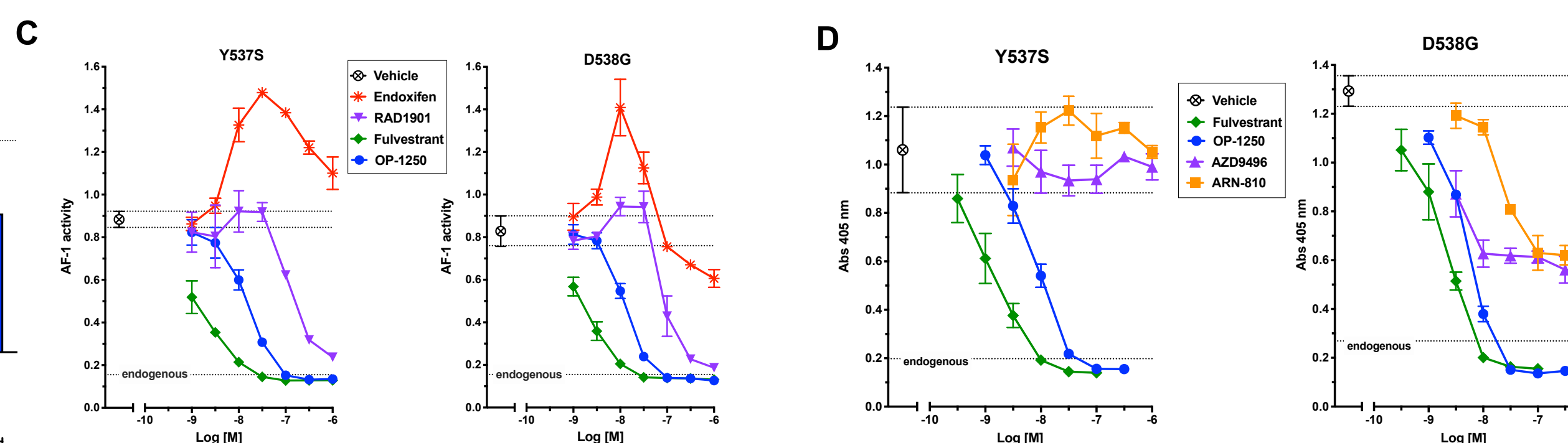
**OP-1250 is a complete antagonist that turns off both AF1 and AF2 of the wild type and mutant ER**



### B Partial agonists, but not CERANs, activate AF1

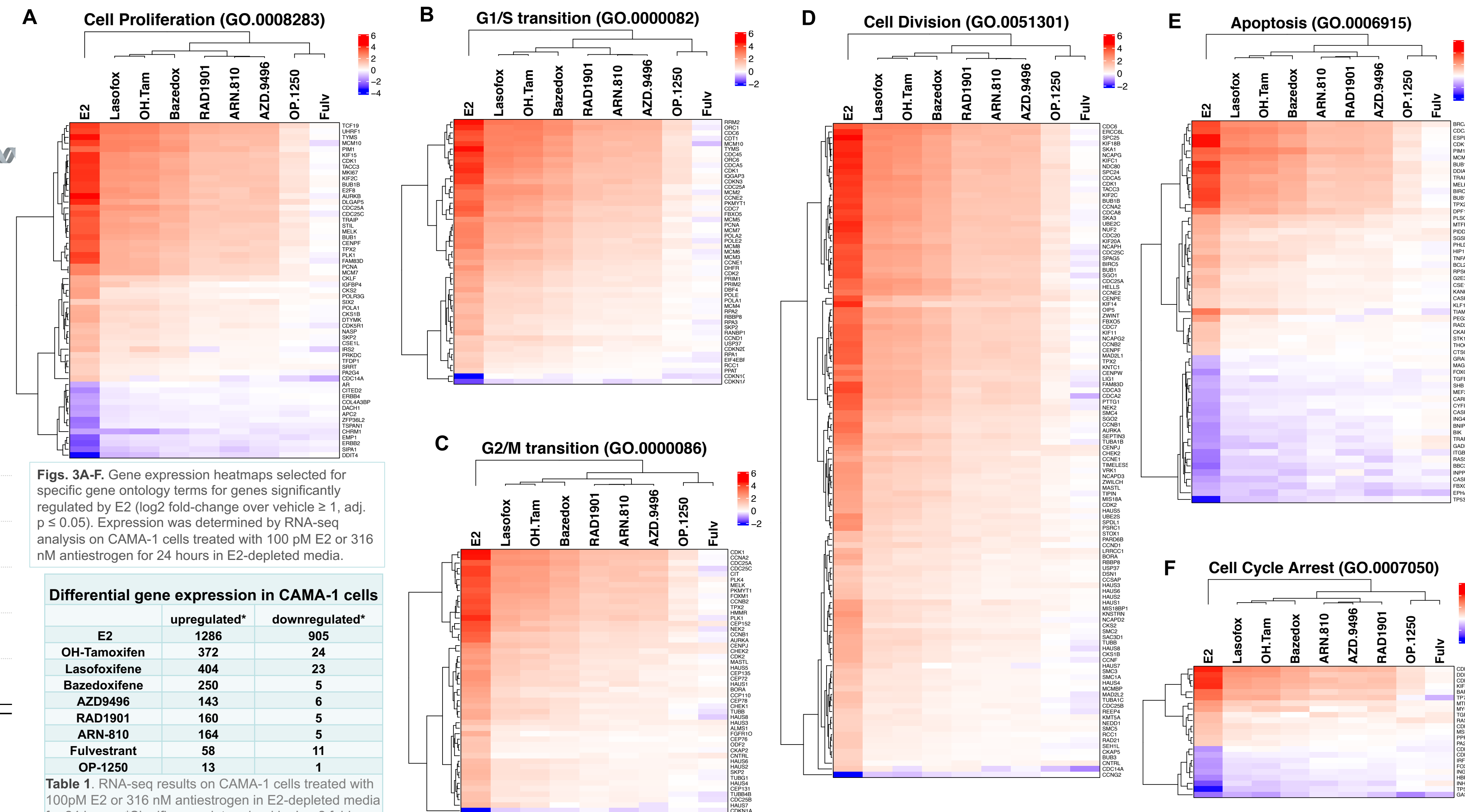


**CERANs like OP-1250 and Fulvestrant completely antagonize AF1 activity mediated by mutant ER $\alpha$**



**Fig. 2.** Alkaline phosphatase activity was used as a measure of AF1 activity. Ishikawa endometrial cells were treated with ligands in E2-depleted media for 3 days. Absorbance was read after incubation with a chromogenic substrate. Except panel B, cells were transfected with the indicated expression plasmid. Line labeled endogenous denotes endogenous AP activity of cells treated with empty expression vector. Figures 2A and 2D were adapted from previous poster<sup>4</sup>.

## OP-1250 lacks agonist activity on estrogen-regulated pro-proliferative, anti-apoptotic genes in breast cancer cells



**Figs. 3A-F.** Gene expression heatmaps selected for specific gene ontology terms for genes significantly regulated by E2 (log2 fold-change over vehicle  $\geq 1$ , adj.  $p \leq 0.05$ ). Expression was determined by RNA-seq analysis on CAMA-1 cells treated with 100 pM E2 or 316 nM antiestrogen for 24 hours in E2-depleted media.

Differential gene expression in CAMA-1 cells		
	upregulated*	downregulated*
E2	1286	905
OH-Tamoxifen	372	24
Lasofloxifene	404	23
Bazedoxifene	250	5
AZD9496	143	6
RAD1901	160	5
ARN-810	164	5
Fulvestrant	58	11
OP-1250	13	1

**Table 1.** RNA-seq results on CAMA-1 cells treated with 100pM E2 or 316 nM antiestrogen in E2-depleted medium for 24 hours. \*Significance determined by Log2 fold-change vs. vehicle  $\geq 1$  and adj. p  $\leq 0.05$ .

## Conclusions and Clinical Significance of Complete ER Antagonism by OP-1250

- Recent experiments conducted by us and third parties in nonclinical models of breast cancer suggest that ER degradation, as achieved by many SERDs, on its own is not sufficient to effectively treat tumors and that the ability to completely inhibit ER function is best achieved through complete antagonism. Here we show in nonclinical studies that OP-1250 is a potent complete ER-antagonist (CERAN) that completely inhibited breast cancer cell proliferation in multiple ER+ breast cancer cell lines.
- The expression of many genes that regulate proliferation, such as cyclin D1, are dependent on activation of AF1 of the ER $\alpha$ . Unlike partial agonists, OP-1250 completely turned off signaling through AF1 of ER $\alpha$ . Consequently, OP-1250 lacked agonist activity on the expression of genes involved in regulating a pro-proliferative and anti-apoptotic response.
- We previously reported that OP-1250 shrinks tumors in the HCI-013 PDX model of mutant ER $\alpha$  that models endocrine resistance.<sup>4</sup> Here we show that OP-1250 was a complete antagonist of AF1 on Y537S and D538G mutants of ER $\alpha$ .
- OP-1250 is a potential new orally bioavailable agent for ER+ breast cancer. We have recently initiated a phase I/II dose escalation and expansion trial of OP-1250 in patients with recurrent, locally advanced or metastatic ER+/HER2- breast cancer whose disease has progressed on endocrine therapy.