Supplementary Figure Legends

Figure S1. Mutational distribution of ERBB2, ERBB3, ERBB4 and EGFR genes obtained by mutation mapper tool of cBioPortal.

Figure S2. HER inhibitors are specific targeted drugs in cervical cancer cell lines. **(A)** To assess the IC_{50} values of the drugs, the cells were incubated with increasing concentrations of all drugs for 72 hours, and the cell viability was assessed by MTS. All experiments were done at least three times in triplicate and the graphs are presented as the mean ± SD relative to DMSO alone (100% viability). **(B)** Western blot analysis for HER receptors and ERK/AKT intracellular pathways in all cell lines treated with 2.5 μM of all drugs for a period of 2 hours. **(C)** At Control conditions (without treatment) all the cell lines where treated with or without 10ng/ml EGF for 15 minutes and analyzed by western blotting for ERK/AKT intracellular pathways. **(D)** Cells were treated for 2 hours with 2.5 μM of selumetinib (SE) and MK2206 (MK) to confirm their ability to inhibit MAPK and AKT pathways, respectively by Western blotting,. Combination studies were done in the two cells lines treated with lapatinib and 2.5 μM of MK and SE for 72 hours. To assess the IC_{50} values, the cellular viability was assessed by MTS.

Figure S3. *In vivo* effect of drugs on cervical cancer growth and angiogenesis. **(A)** Representative pictures (16X magnification) of CAM assay *in ovo* at days 13 and 17 and *ex ovo* and day 17, for SiHa and C-33A induced tumors. The experiment was done as described in Materials and Methods with or without treatment with AST1306 (AST) or lapatinib (LA) and 2-DG or both. **(B)** Quantification of vessels area, as described in Materials and Methods. **(C)** Graphic representation of the scores obtained in IHC analysis of *in vivo* tumors for HER4 (C-33A induced tumors), EGFR (SiHa induced tumors), and KI-67 in SiHa- (orange) and C-33A (blue)-induced tumors. The horizontal lines represent the mean score obtained in the samples for each condition.

Figure S4. Effective combinations to improve lapatinib therapy response *in vitro*. **(A)** Representative pictures of the immunohistochemistry (IHC) analysis for HIF1-α in C-33A induced tumors (200X magnification). **(B)** Graphic representation of the scores obtained in IHC analysis of *in vivo* tumors for HIF1-α. The horizontal lines represent the mean score obtained in the samples for each condition. **(C)** Quantification of extracellular lactate levels *in vitro*, in C-33A cell line after 24 hours of treatment with 1.5 μM of AST, LA, 1.5 mM of 2-DG or LA in combination with 2-DG. **(D)** Viability effect of two concentrations of 2-DG was evaluated by MTS assay after 72 hours of treatment. **(E)** Cellular viability was measured *in vitro* by MTS after 72 hours of treatment to determine the half maximal inhibitory concentrations (IC_{50}) for combinations of 2-DG with lapatinib (C-33A cell line) or AST1306 **(F)** in both cell lines. All experiments were done at least three times in triplicate and the graphs are presented as the mean ± SD relative to DMSO alone (100% viability).

Figure S1

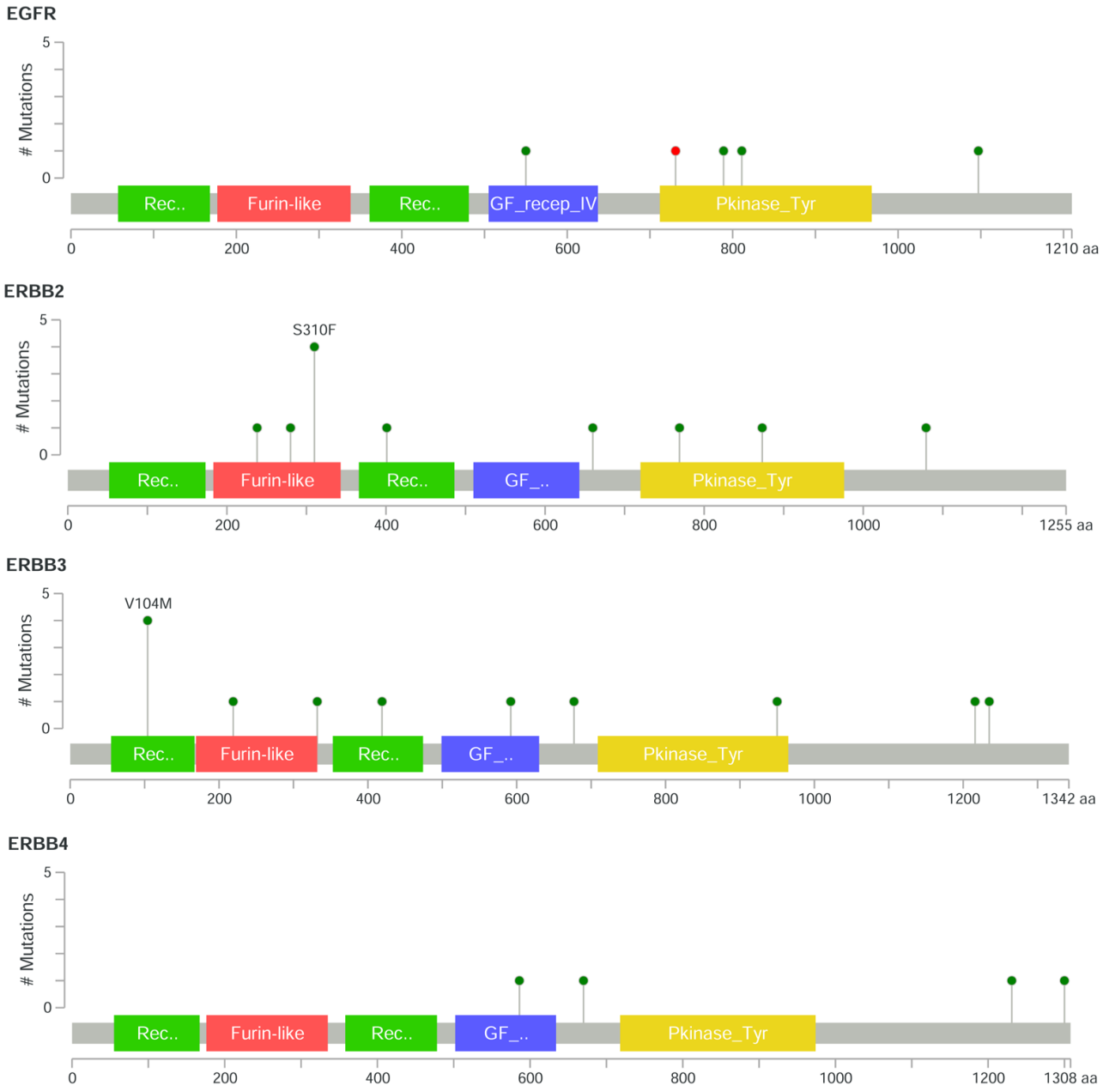
EGFR
Mutations
5
0
Rec..
Furin-like
Rec..
GF_recep_IV
Pkinase_Tyr
0
200
400
600
800
1000
1210 aa
ERBB2
S310F
Mutations
5
0
Rec..
Furin-like
Rec..
GF_..
Pkinase_Tyr
0
200
400
600
800
1000
1255 aa
ERBB3
V104M
Mutations
5
0
Rec..
Furin-like
Rec..
GF_..
Pkinase_Tyr
0
200
400
600
800
1000
1200
1342 aa
ERBB4
Mutations
5
0
Rec..
Furin-like
Rec..
GF_..
Pkinase_Tyr
0
200
400
600
800
1000
1200
1308 aa

Figure S2

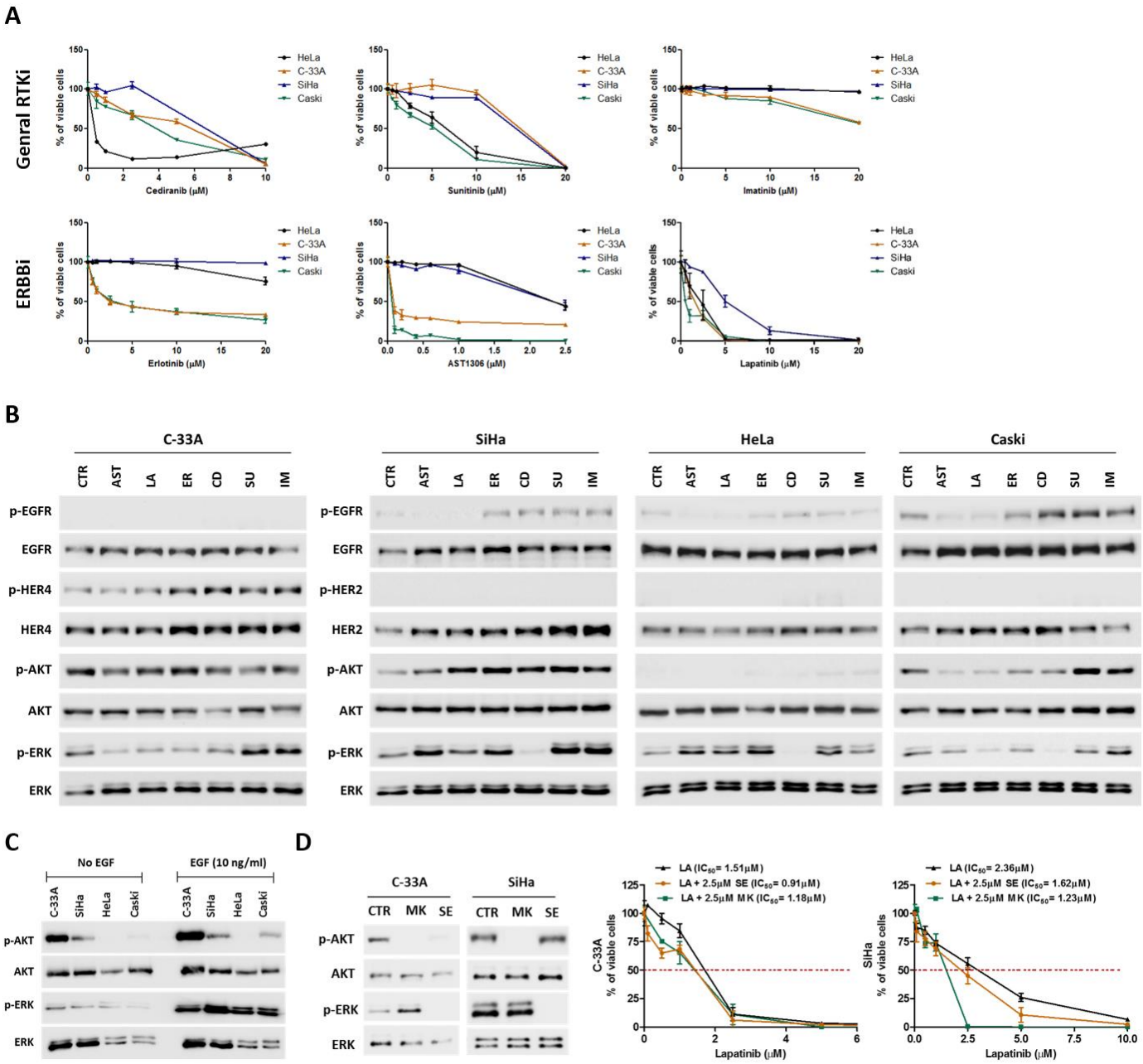
A
Genral RTKi
ERBBi
% of viable cells
HeLa
C-33A
SiHa
Caski
Cediranib (μM)
Sunitinib (μM)
Imatinib (μM)
Erlotinib (μM)
AST1306 (μM)
Lapatinib (μM)
B
C-33A
SiHa
HeLa
Caski
CTR
AST
LA
ER
CD
SU
IM
p-EGFR
EGFR
p-HER4
p-HER2
HER4
HER2
p-AKT
AKT
p-ERK
ERK
C
No EGF
EGF (10 ng/ml)
D
MK
SE
LA (IC50= 1.51μM)
LA + 2.5μM SE (IC50= 0.91μM)
LA + 2.5μM MK (IC50= 1.18μM)
LA (IC50= 2.36μM)
LA + 2.5μM SE (IC50= 1.62μM)
LA + 2.5μM MK (IC50= 1.23μM)

Figure S3

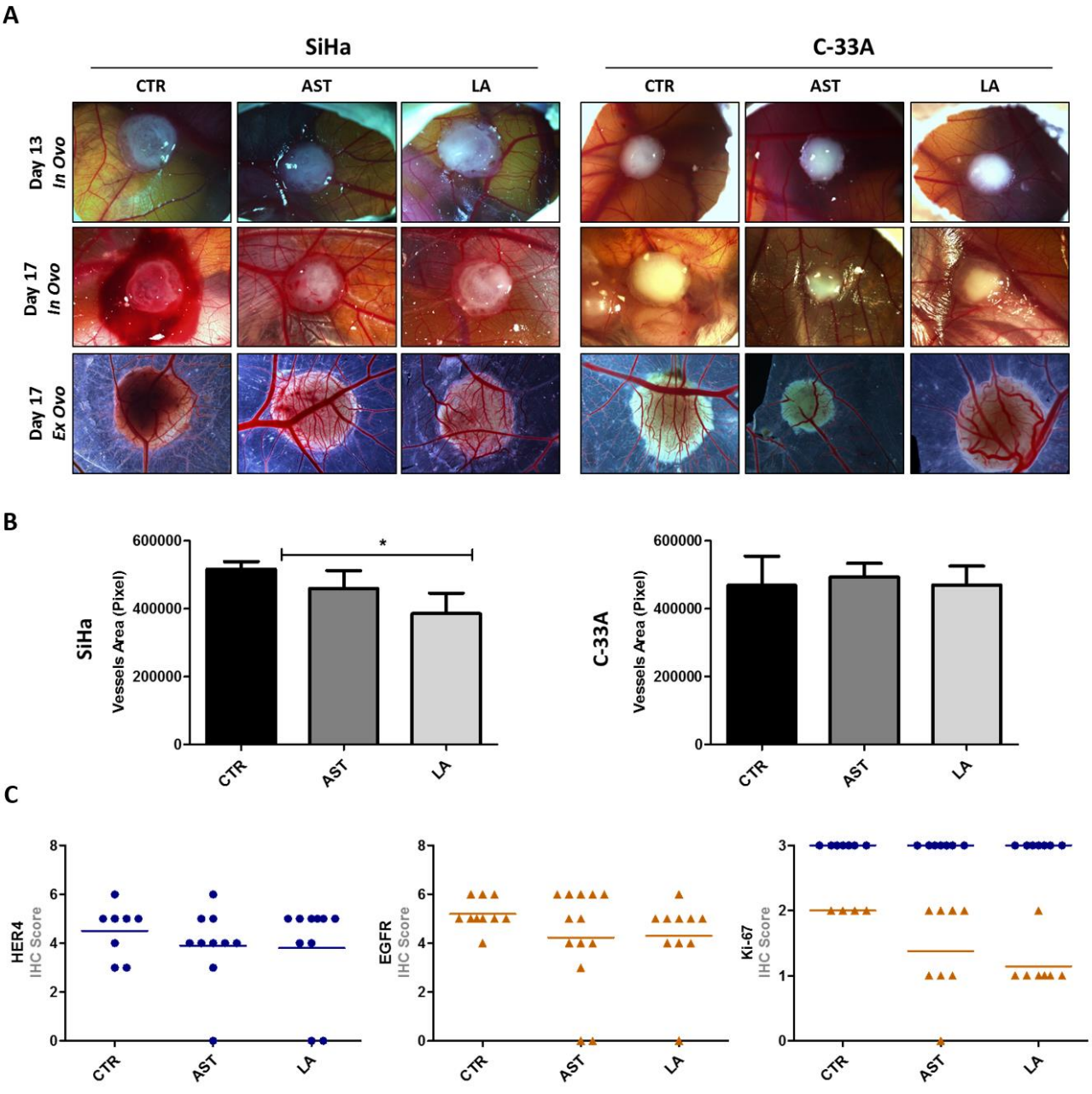
A
SiHa
C-33A
CTR
AST
LA
Day 13 In Ovo
Day 17 In Ovo
Day 17 Ex Ovo
B
SiHa
C-33A
Vessels Area (Pixel)
600000
400000
200000
0
*
C
HER4 IHC Score
EGFR IHC Score
Ki-67 IHC Score

Figure S4

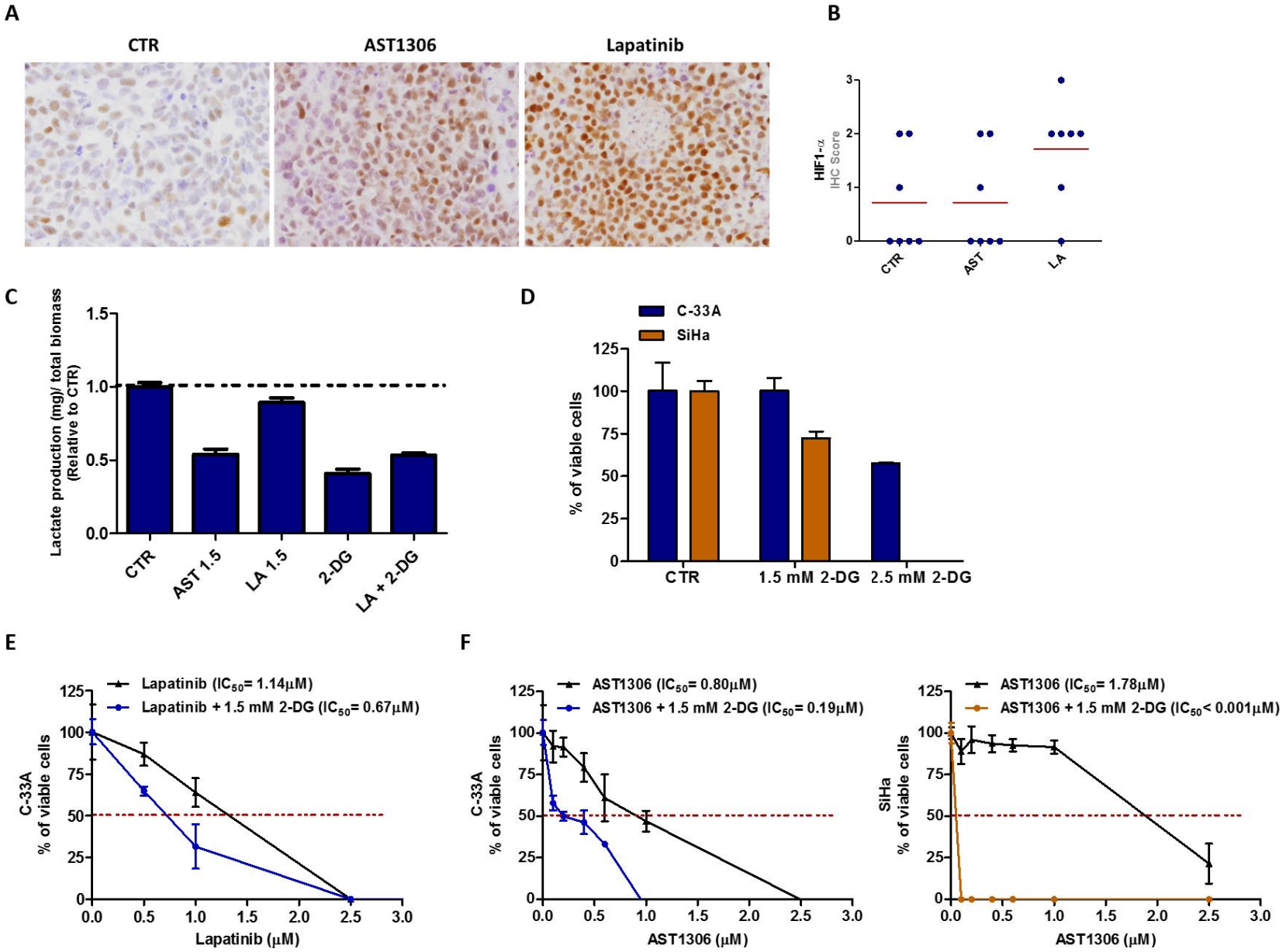
A
CTR
AST1306
Lapatinib
B
HIF1-α IHC Score
CTR
AST
LA
C
Lactate production (mg)/ total biomass (Relative to CTR)
CTR
AST 1.5
LA 1.5
2-DG
LA + 2-DG
D
C-33A
SiHa
% of viable cells
CTR
1.5 mM 2-DG
2.5 mM 2-DG
E
Lapatinib (IC50= 1.14μM)
Lapatinib + 1.5 mM 2-DG (IC50= 0.67μM)
C-33A % of viable cells
Lapatinib (μM)
F
AST1306 (IC50= 0.80μM)
AST1306 + 1.5 mM 2-DG (IC50= 0.19μM)
C-33A % of viable cells
AST1306 (μM)
AST1306 (IC50= 1.78μM)
AST1306 + 1.5 mM 2-DG (IC50< 0.001μM)
SiHa % of viable cells
AST1306 (μM)

Table S1: Expression analysis of HER family receptors in relation to patient's clinical features.

Parameter	EGFR Expression				HER2 Expression				HER3 Expression				HER4 Expression			
	N	Negative (%)	Positive (%)	*p*	N	Negative (%)	Positive (%)	*p*	N	Negative (%)	Positive (%)	*p*	N	Negative (%)	Positive (%)	*p*
Age (years)																
≤47	68	57 (83.8)	11 (16.2)	0,282	108	74 (68.5)	34 (31.5)	0.198	108	64 (59.3)	44 (40.7)	**0.017**	108	89 (82.4)	19 (17.6)	0.809
>47	68	52 (76.5)	16 (23.5)		92	55 (59.8)	37 (40.2)		92	39 (42.4)	53 (57.6)		92	77 (83.7)	15 (16.3)	
Histology																
Adenocarcinomas	130	111 (85.4)	19 (14.6)	**0.001**	194	123 (63.4)	71 (36.6)	0.951	194	102 (52.6)	92 (47.4)	0.290	194	165 (85.1)	29 (14.9)	0.450
Adenosquamous	27	15 (55.6)	12 (44.4)		35	22 (62.9)	13 (37.1)		35	15 (42.9)	20 (57.1)		35	28 (80)	7 (20)	
Disease recurrence																
Present	3	3 (100)	0	0.414	4	3 (75)	1 (25)	0.649	4	3 (75)	1 (25)	0.280	4	3 (75)	1 (25)	0.758
Absent	82	67 (81.7)	15 (18.3)		122	78 (63.9)	44 (36.1)		122	58 (47.5)	64 (52.5)		122	99 (81.1)	23 (18.9)	
Metastasis																
Present	17	12 (70.6)	5 (29.4)	0.164	22	13 (59.1)	9 (40.9)	0.654	22	4 (18.2)	18 (81.8)	**0.003**	22	18 (81.8)	4 (18.2)	0.934
Absent	61	52 (85.2)	9 (14.8)		95	61 (64.2)	34 (35.8)		95	51 (53.7)	44 (46.3)		95	77 (81.1)	18 (18.9)	
Differentiation grade																
Low	90	76 (84.4)	14 (15.6)	0.578	133	87 (65.4)	46 (34.6)	0.365	133	71 (53.4)	62 (46.6)	0.735	133	113 (85)	20 (15)	0.879
Medium/High	42	37 (88.1)	5 (11.9)		63	37 (58.7)	26 (41.3)		63	32 (50.8)	31 (49.2)		63	53 (84.1)	10 (15.9)	
Tumor size																
>4 mm	4	3 (75)	1 (25)	0.659	7	2 (28.6)	5 (71.4)	0.127	7	3 (42.9)	4 (57.1)	0.863	7	6 (85.7)	1 (14.3)	1.000
≤4 mm	19	16 (84.2)	3 (15.8)		28	17 (60.7)	11 (39.3)		28	11 (39.3)	17 (60.7)		28	24 (85.7)	4 (14.3)	
Parametrial invasion																
Present	42	34 (81)	8 (19)	0.829	60	38 (63.3)	22 (36.7)	0.821	60	27 (45)	33 (55)	0.823	60	47 (78.3)	13 (21.7)	0.922
Absent	35	29 (82.9)	6 (17.1)		49	30 (61.2)	19 (38.8)		49	21 (42.9)	28 (57.1)		49	38 (77.6)	11 (22.4)	