Structurally novel antiestrogens elicit differential responses from constitutively active mutant estrogen receptors in breast cancer cells and tumors

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Supplementary Figure Legends

Fig. S1 Dose-dependent inhibition of the growth of T47D cells by antiestrogen compounds with WT or mutant-ERα in tissue culture medium with 5% fetal bovine serum and $3x10^{-9}$ M E2.

T47D cells with WT, Y537S- or D538G-ERα were cultured in E2-containing medium, and were treated with E2 ($3x10^{-9}$ M) and compounds (**A,** Fulv; **B,** 4-OHT; **C,** K-07; **D,** K-09; **E,** K-62**)** at the concentrations indicated ($3x10^{-11}$ to $3x10^{-6}$ M) for 6 days. Cell viability values are mean ± SD of 3 determinations from 3 separate experiments.

Fig. S2 Antiestrogen compounds K-07, K-09, K-62 and trans-hydroxy-tamoxifen (TOT) suppress the binding of SRC3 to wild type ER, Y537S-ER, and D538G-ER, in a dose-dependent fashion. Site-specific labeled biotin-streptavidin/terbium ER-ligand binding domain constructs (donor) were primed to ~50% activity with estradiol and were then incubated with a fluorescein labeled SRC3 (acceptor). When the donor and acceptor fluorophores are in close proximity, they interact, and the resonance energy transferred from terbium to fluorescein is measured by time-resolved Fluorescence Resonance Energy Transfer (FRET). Increasing concentrations of E2 increase interaction of ER and SRC3, and show increased FRET, whereas increasing concentrations of K-07, K-09, K-62 and TOT reduce interaction of E2-ER and SRC3

and show reduced FRET. WT and mutant ERs required different E2 concentrations to be primed to ca. 50% activity, reflecting their differing levels of constitutive activities and differing affinities for E2; the priming concentration required for each receptor is given in square brackets in each panel.

Fig. S3 Antiestrogen compounds suppress proliferation of breast cancer cells with heterozygous levels of mutant Y537S or D538G ERα

MCF7 or T47D cells containing 50% mutant ERα and 50% wild type ERα (**panels A,B**) were treated with E2 or compounds alone for 6 days at the concentrations indicated, and cell viability was measured. Values are mean ± SD from 3 determinations from 3 separate experiments.

Fig. S4 Compounds induce down-regulation of ERα in MCF7 and T47D breast cancer cells containing half mutant and half wild type ERα

ICW assay of ERα protein was performed in breast cancer cells treated for 24 h with Control Vehicle, E2, or compounds alone (**panels A,B**) at the concentrations indicated.

Fig. S5 Suppression of ERα-regulated gene expressions in MCF7 and T47D breast cancer cells containing half mutant and half wild type ERα

After 24 h of Control Vehicle, E2 or compound treatment, cells were harvested and processed for qPCR analysis of the ERα-regulated genes GREB1 and PGR. Fold change of mRNA level was calculated relative to the vehicle treated cell samples, set at 1.

Fig. S6 Treatment with antiestrogen compounds in vivo does not affect animal body weights

Mice received daily Vehicle or 80 mg/kg of the compounds indicated by **(A)** sc injection or **(B)** oral gavage, and body weights of the mice bearing MCF7 xenograft tumors were monitored over time (2-way ANOVA, Bonferroni post-test, $P>0.05$, n=8 per group).

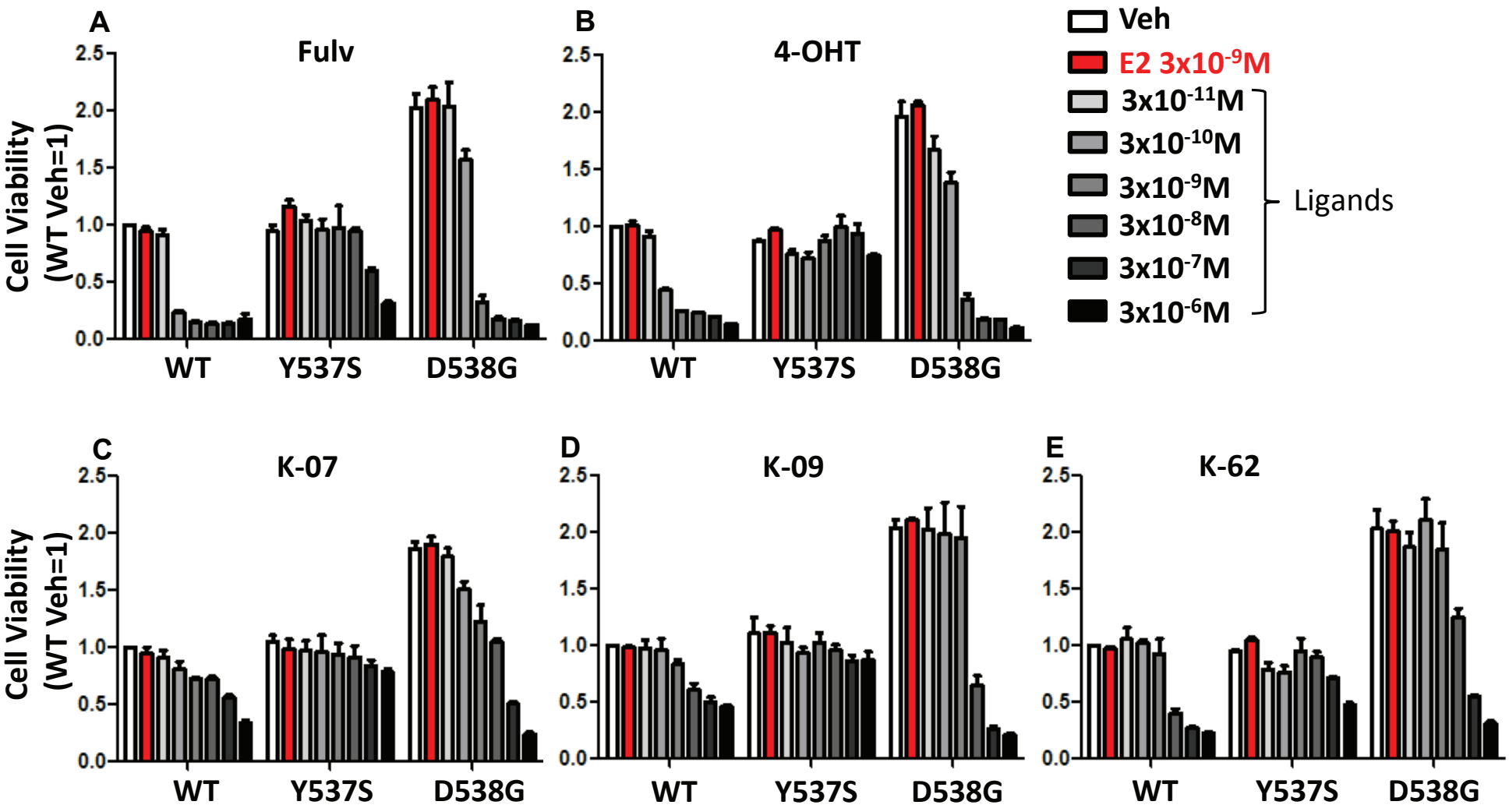
A
Fulv
B
4-OHT
Veh
E2 3x10-9M
3x10-11M
3x10-10M
3x10-9M
3x10-8M
3x10-7M
3x10-6M
Ligands
Cell Viability (WT Veh=1)
2.5
2.0
1.5
1.0
0.5
0.0
WT
Y537S
D538G
C
K-07
D
K-09
E
K-62

Figure S1

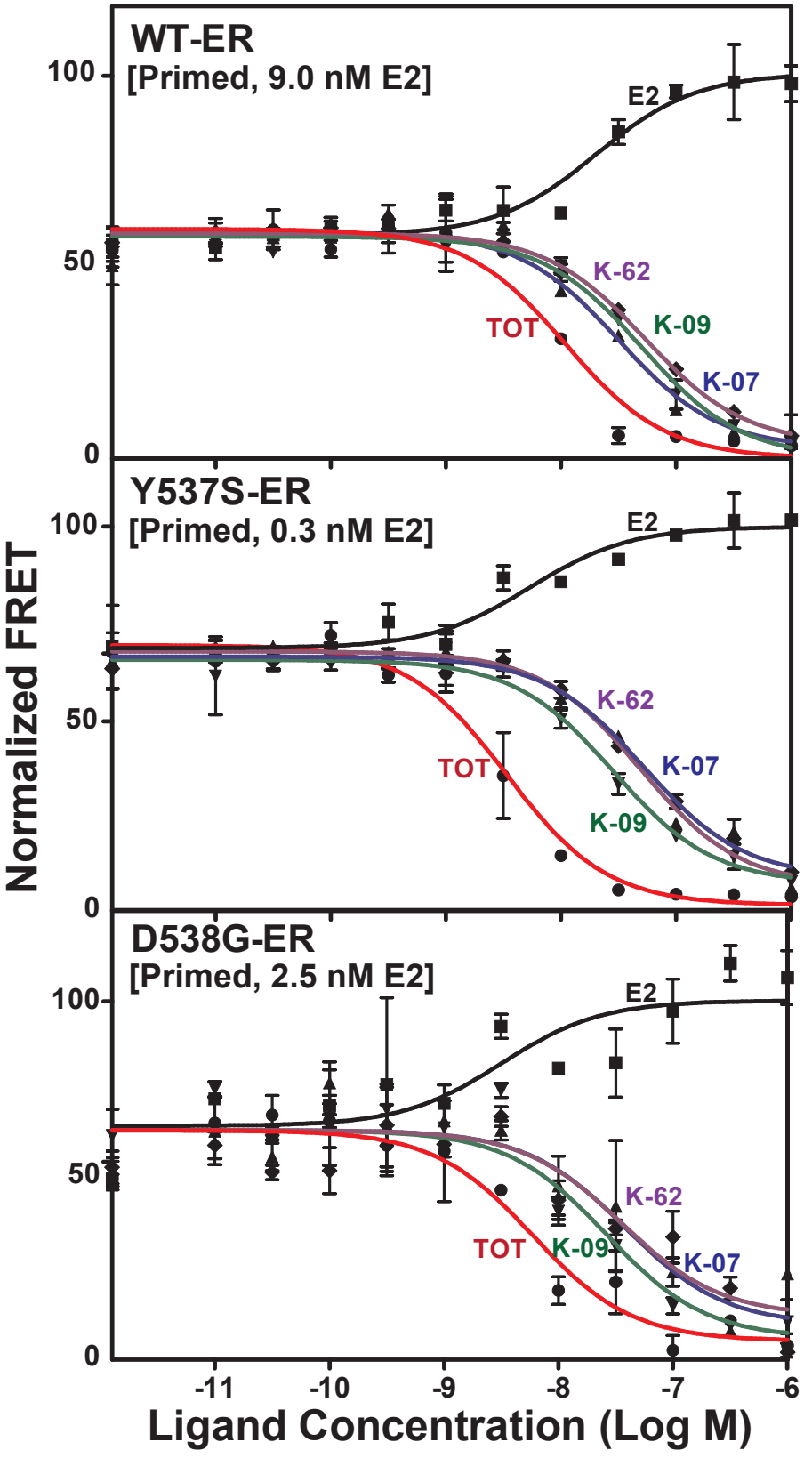
WT-ER
[Primed, 9.0 nM E2]
Y537S-ER
[Primed, 0.3 nM E2]
D538G-ER
[Primed, 2.5 nM E2]
E2
K-62
K-09
K-07
TOT
Normalized FRET
100
50
0
-11
-10
-9
-8
-7
-6
Ligand Concentration (Log M)

Figure S2

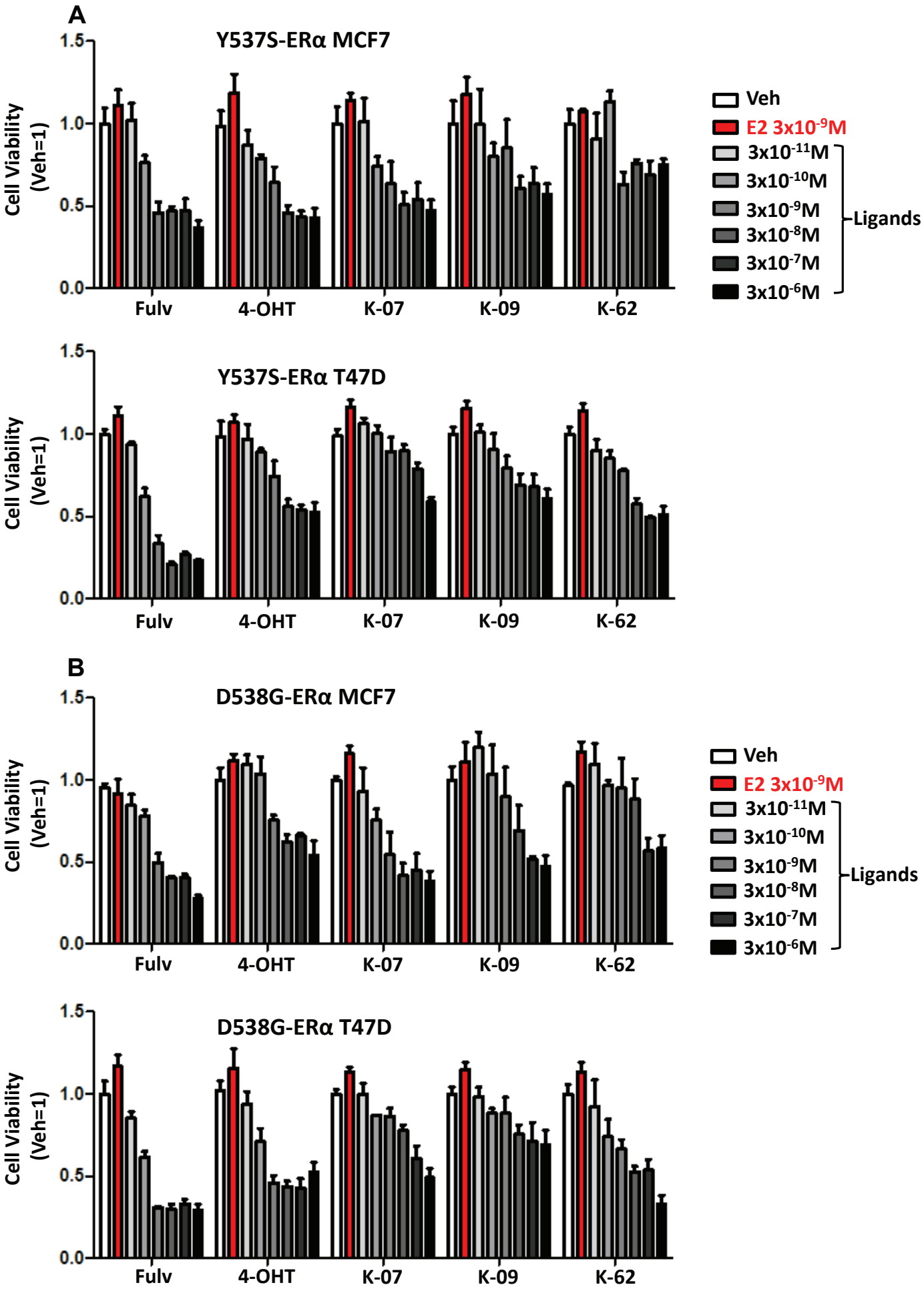
A
Y537S-ERα MCF7
Cell Viability (Veh=1)
1.5
1.0
0.5
0.0
Fulv
4-OHT
K-07
K-09
K-62
Veh
E2 3x10-9M
3x10-11M
3x10-10M
3x10-9M
3x10-8M
3x10-7M
3x10-6M
Ligands
Y537S-ERα T47D
B
D538G-ERα MCF7
D538G-ERα T47D

Figure S3

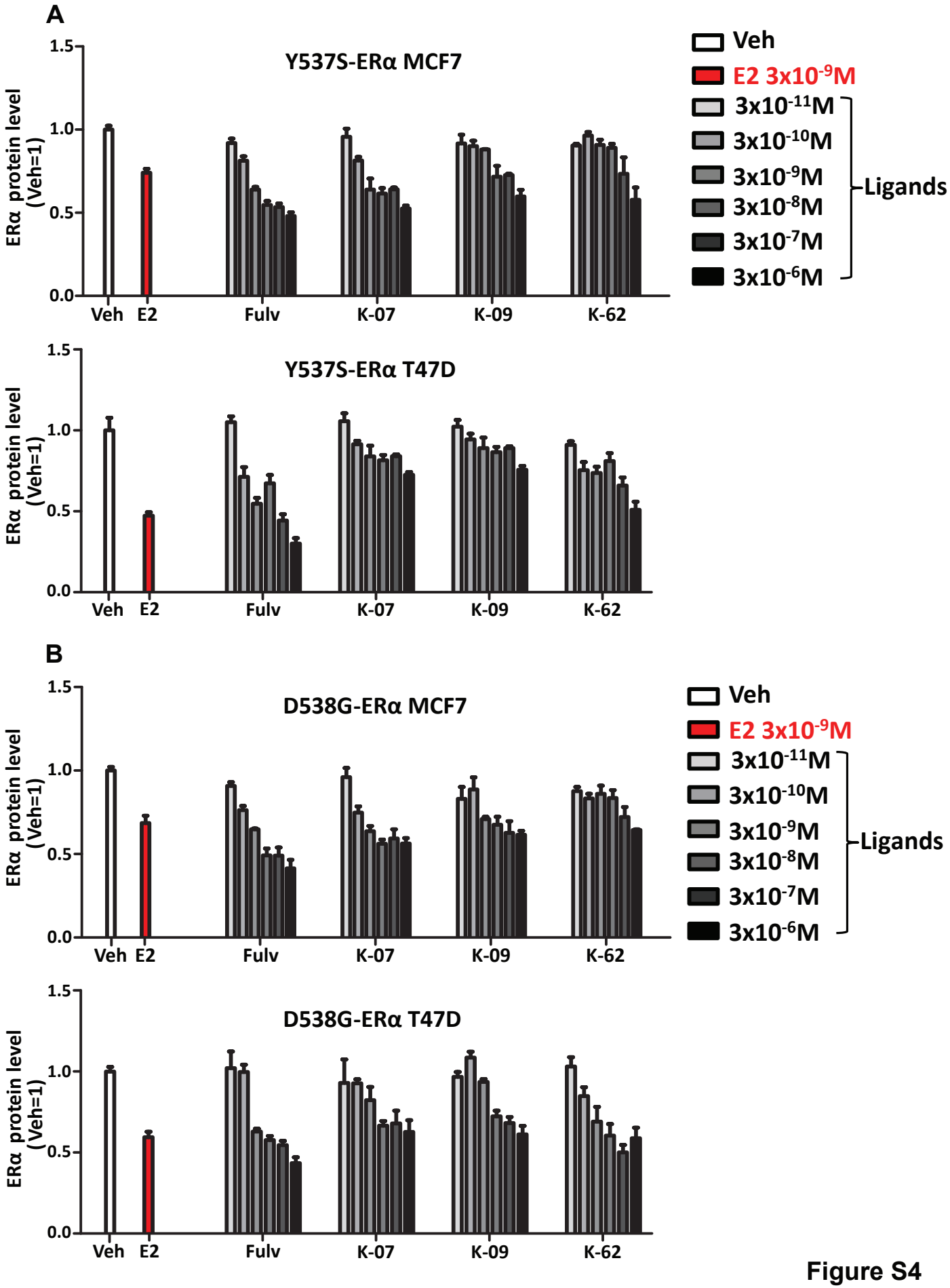
A
Y537S-ERα MCF7
ERα protein level (Veh=1)
1.5
1.0
0.5
0.0
Veh
E2
Fulv
K-07
K-09
K-62
Y537S-ERα T47D
Veh
E2 3x10-9M
3x10-11M
3x10-10M
3x10-9M
3x10-8M
3x10-7M
3x10-6M
Ligands
B
D538G-ERα MCF7
D538G-ERα T47D

Figure S4

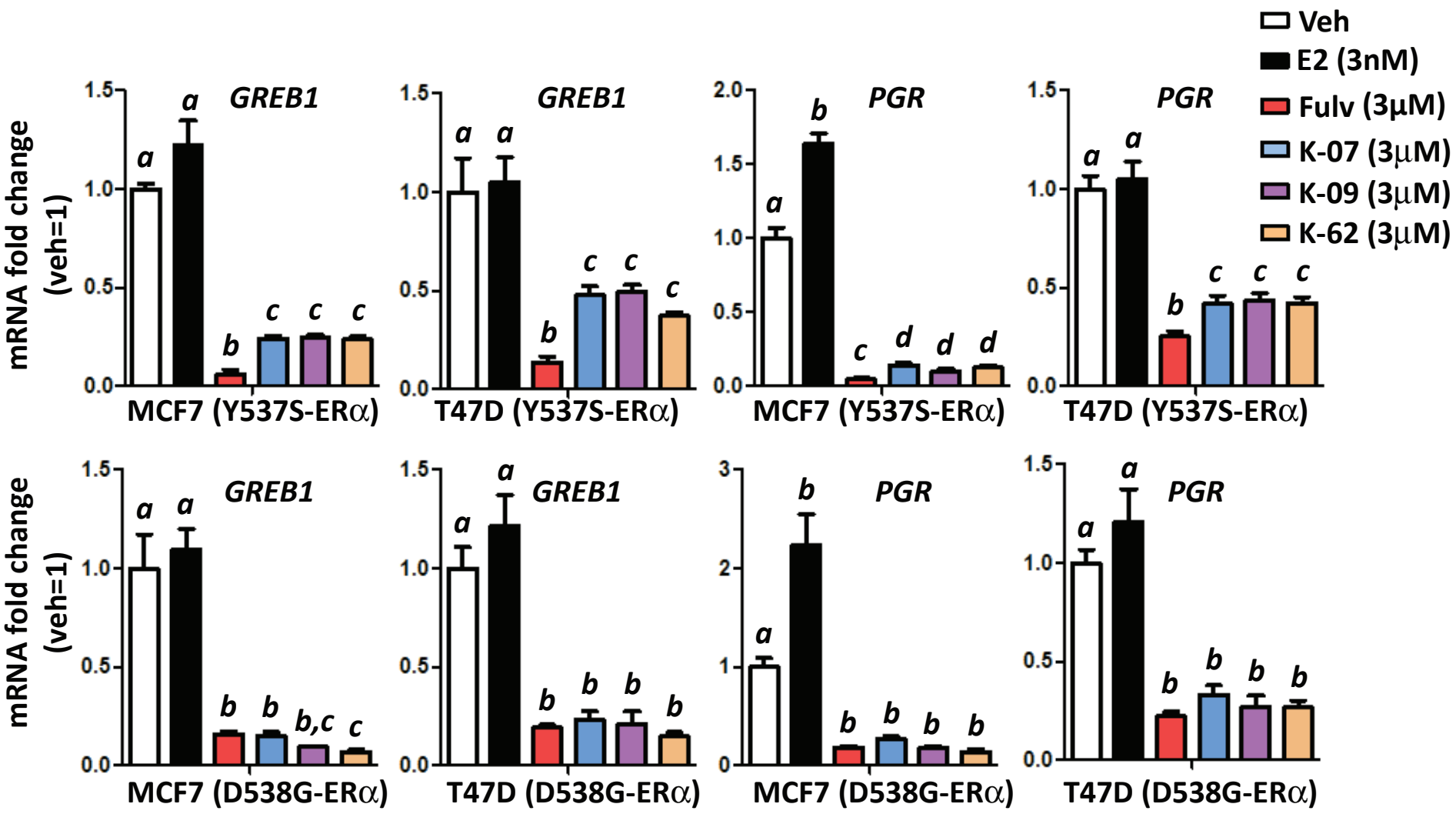
Veh
E2 (3nM)
Fulv (3μM)
K-07 (3μM)
K-09 (3μM)
K-62 (3μM)
mRNA fold change (veh=1)
GREB1
PGR
MCF7 (Y537S-ERα)
T47D (Y537S-ERα)
MCF7 (D538G-ERα)
T47D (D538G-ERα)

Figure S5

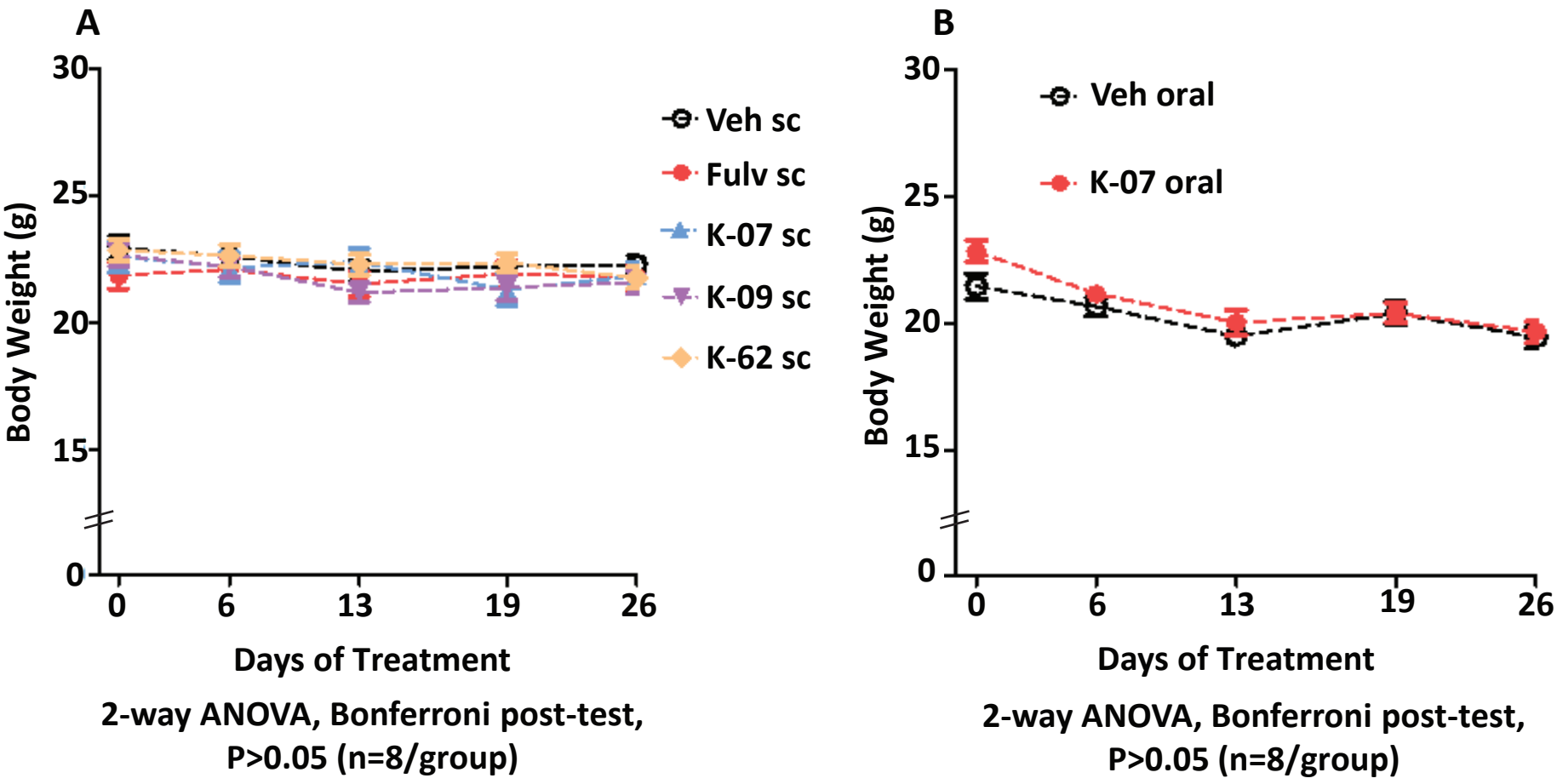
A
Body Weight (g)
30
25
20
15
0
0
6
13
19
26
Days of Treatment
Veh sc
Fulv sc
K-07 sc
K-09 sc
K-62 sc
2-way ANOVA, Bonferroni post-test,
P>0.05 (n=8/group)
B
Body Weight (g)
30
25
20
15
0
0
6
13
19
26
Days of Treatment
Veh oral
K-07 oral
2-way ANOVA, Bonferroni post-test,
P>0.05 (n=8/group)

Figure S6