Supplementary Figures for

Synthetic Alkyl-Ether-lipid promotes TRPV2 Channel trafficking trough PI3K/Akt-Girdin Axis in cancer cells and increases mammary tumor volume.

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FigureS1

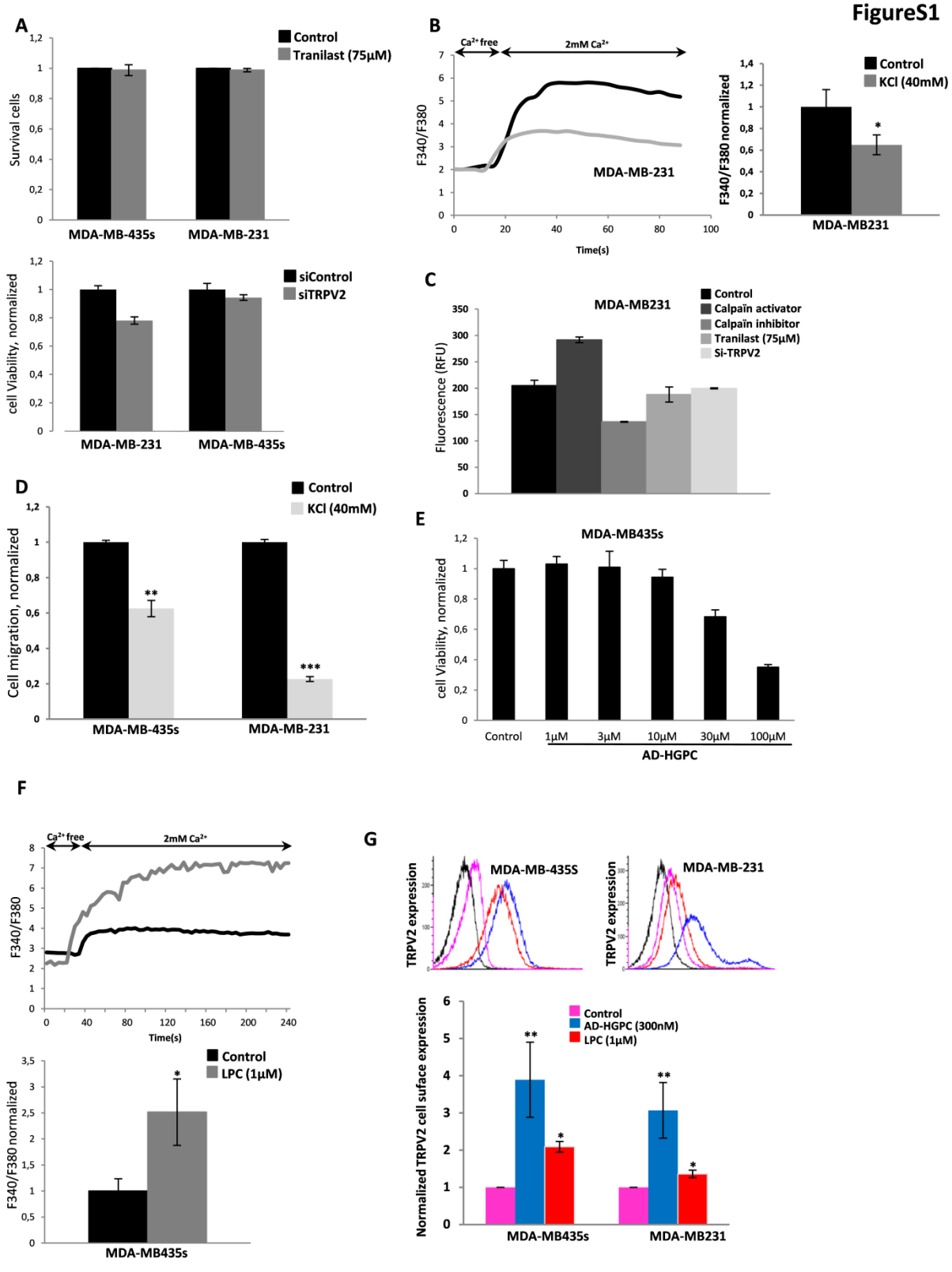
A
Control
Tranilast (75µM)
Survival cells
MDA-MB-435s
MDA-MB-231
siControl
siTRPV2
cell Viability, normalized
MDA-MB-231
MDA-MB-435s
B
Ca2+ free
2mM Ca2+
F340/F380
MDA-MB-231
Time(s)
Control
KCl (40mM)
F340/F380 normalized
MDA-MB231
C
MDA-MB231
Control
Calpaïn activator
Calpaïn inhibitor
Tranilast (75µM)
Si-TRPV2
Fluorescence (RFU)
D
Control
KCl (40mM)
Cell migration, normalized
MDA-MB-435s
MDA-MB-231
E
MDA-MB435s
cell Viability, normalized
Control
1µM
3µM
10µM
30µM
100µM
AD-HGPC
F
Ca2+ free
2mM Ca2+
F340/F380
Time(s)
Control
LPC (1µM)
F340/F380 normalized
MDA-MB435s
G
MDA-MB-435S
MDA-MB-231
TRPV2 expression
Control
AD-HGPC (300nM)
LPC (1µM)
Normalized TRPV2 cell suface expression
MDA-MB435s
MDA-MB231

Figure S1: TRPV2 has no effect on cancer cell viability and induces calcium cell migration independently of calpain activity. Induction of TRPV2 plasma membrane translocation by LPC and AD-HGPC in cancer cells**. A**. Tranilast (75 µM) and siTRPV2 have no effect on MDA-MB435s and MDA-MB231 cell viability. Histograms showing mean +/- SEM. Data were normalized to control condition (*N=2,n=4).* **B.** Fluorescence measurement and relative fluorescence to Ca^{2+} entry in MDA-MB-231 cells cultured in medium with 40 mM KCl and 40 mM NaCl. Histograms show mean +/- SEM of ratiometric fluorescence**.** Data were normalized to conditions obtained with cells cultured in medium with 40mM NaCl (*N=3, n=6 *p < 0.05*). **C.** Tranilast treatment (75µM) or TRPV2 knock-down are no effect on calpaïn activity, in MDA-MB231 cell line *(N=3;n=6).* **D.** Depolarisation induced by 40 mM KCl is involved in breast cancer cell migration. Histograms showing MDA-MB-231 cell migration in 40 mM of NaCl (control) or 40 mM KCl (*mean +/- SEM, N=3,n=6 ***p < 0.001*). **E.** AD-HGPC has no effect on MDA-MB-435s cell viability until 10 µM (*N=3*). Cell viability is affected from 30µM **F.** LPC promoted constitutive Ca^{2+} entry in MDA-MB-435 cells. Fluorescence measurement was preformed to characterize this Ca^{2+} entry in cells treated by LPC (1 µM) and DMSO (control). Histograms show mean +/- SEM of ratiometric fluorescence. Data were normalized to conditions obtained with control condition (N=4, *p < 0.05). **G.** AD-HGPC and LPC induced translocation of TRPV2 to the plasma membrane in MDA-MB-231 cells (Black line represents isotype used as negative control). Histograms show TRPV2 surface expression in MDA-MB-231 cells after acute application of LPC (1 µM) or AD-HGPC (300 nM) (*mean +/- SEM , N=4 *p <0.05, **p < 0.01*).

Figure S2

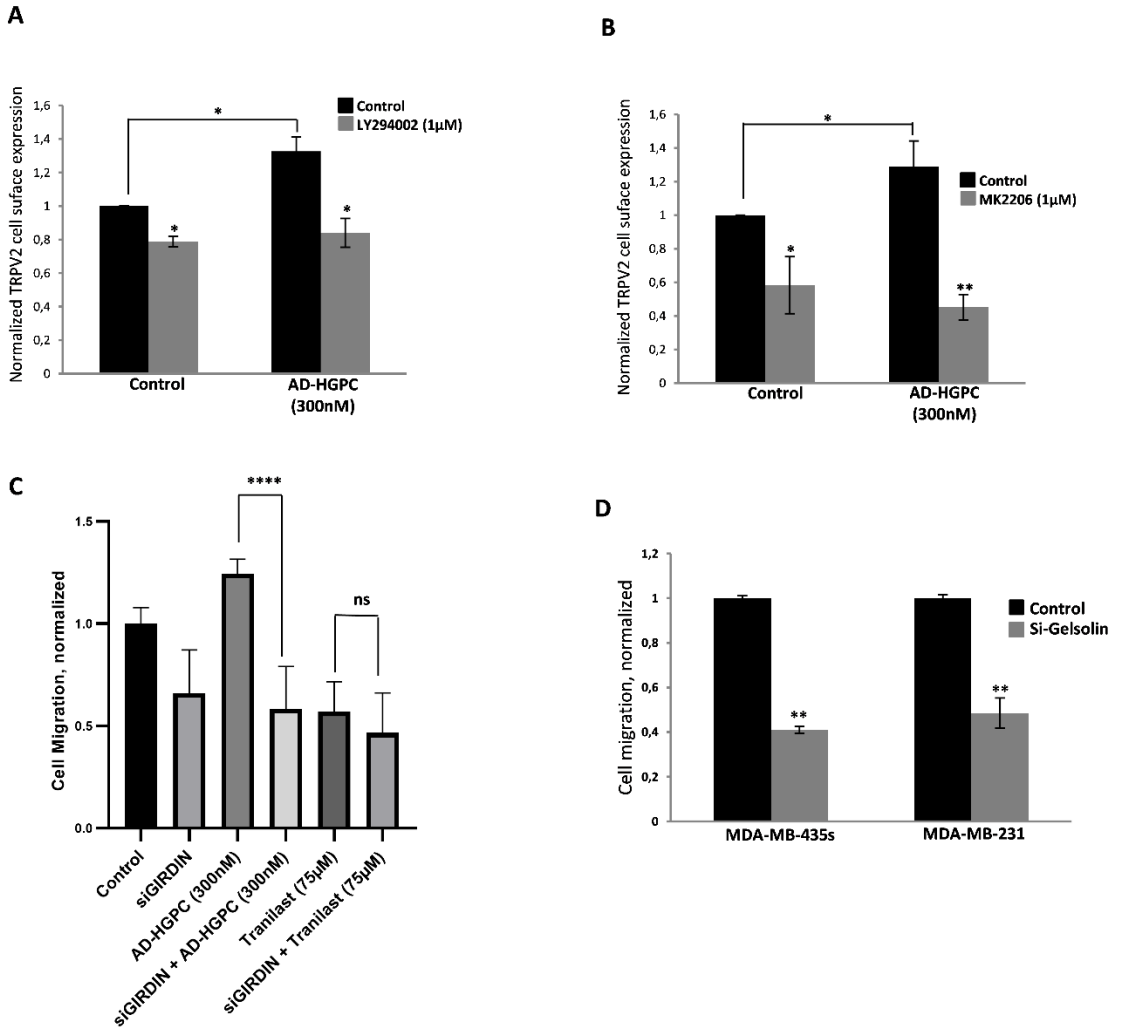

Figure S2: **AD-HGPC induces cells migration by stimulating PI3K/Akt/girdin pathway and effect of Gelsolin's *knock-down* on cancer cells migration**. **A**. Inhibition of PI3K blocks the translocation of TRPV2 to the plasma membrane of HEK-hTRPV2 cells. The TRPV2 plasma membrane translocation induced by acute application of AD-HGPC is abolished by a pre-treatment with LY294002) (*mean +/- SEM, N=4 *p <0.05).* **B.** The increase of recruitment of TRPV2 is inhibited by pharmacological inhibition of Akt signaling pathway by MK2206 (1 µM) on HEK-hTRPV2 cells (*mean +/- SEM, N=3, *p < 0.05, **p < 0.01*). **C**.Silencing of girdin inhibits cell migration effect induce by AD-HGPC and and do not modify Tranilast effect. Histograms represent the migration of MDA-MB-435s and MDA-MB-231 cells for 24 h transfected with siGirdin (*mean +/- SEM, N=3, n=8-9 ****p < 0.0001*).**D.** Gelsolin is involved in breast cancer cell migration. Histograms represent the migration of MDA-MB-435s and MDA-MB-231 cells for 24 h transfected with siGelsolin (*mean +/- SEM, N=4, n=9 **p < 0.01*).