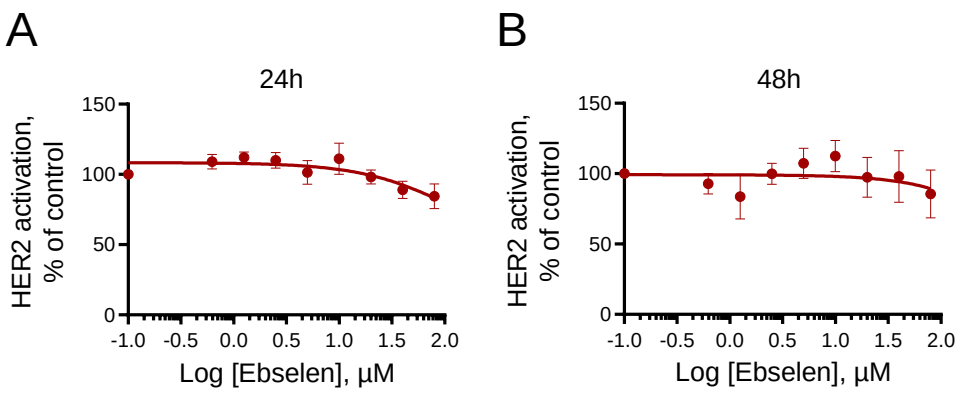
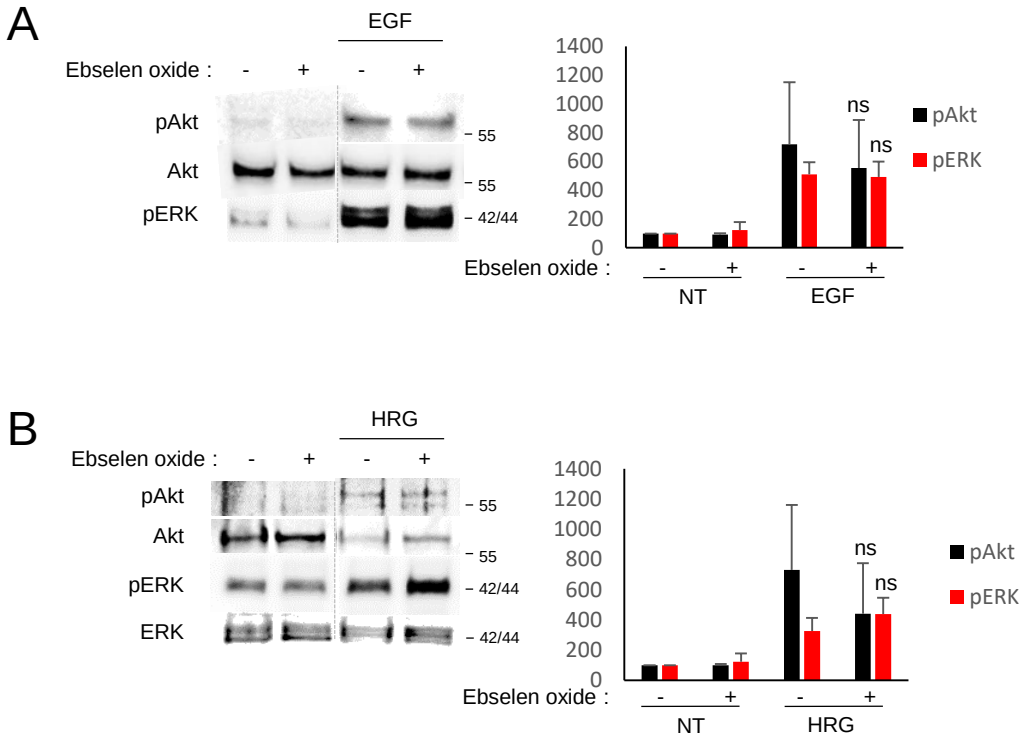


Supplementary figure 1 : Ebselen does not block HER2 activation

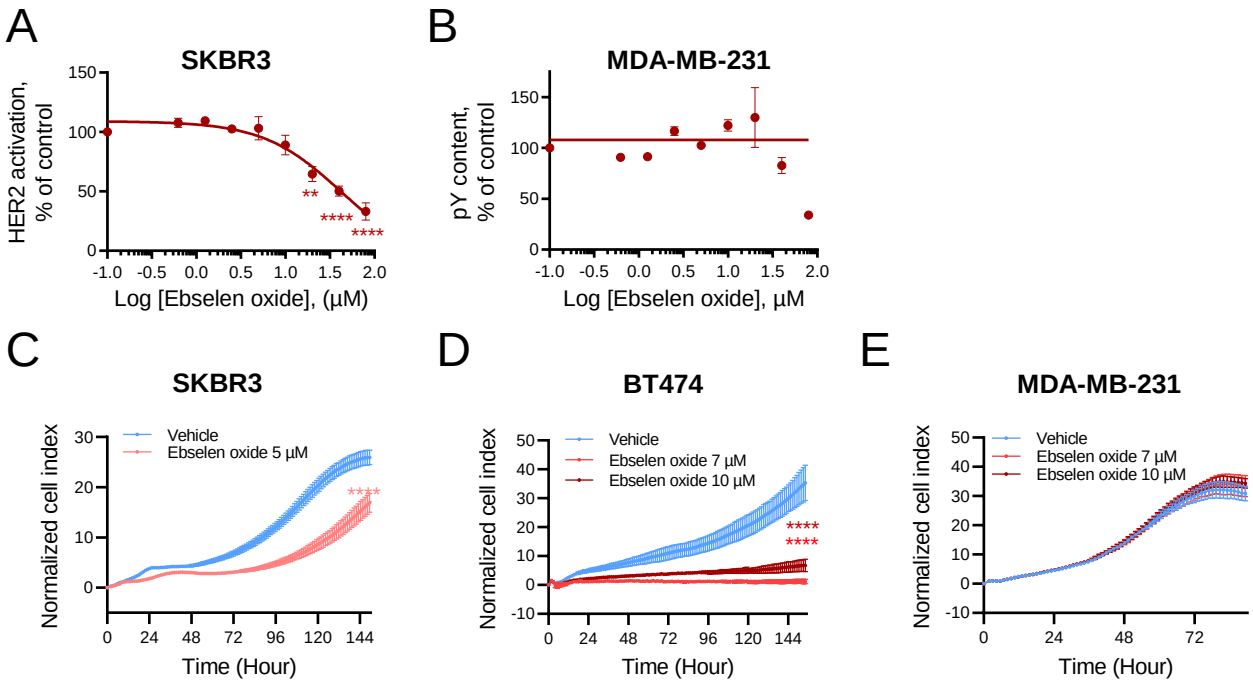
(A-B) Quantification of HER2 activation (pY1248-HER2) in HER2+ breast cancer cells (SKBR3) treated with increasing concentrations of Ebselen (0-80 µM) for 24h (A) or 48h (B) measured by dot blots.



Supplementary figure 2 : Ebselen oxide does not block HER2 ligand-dependent activation in heterodimers with the other HER family members.

(A) HBMECs were treated with or without 10 µM Ebselen oxide before 5 min stimulation with 50 ng/ml EGF. Whole cell lysates were analysed by western blot using anti-pAkt, Akt and pERK antibodies. Data are mean ± sem (n=3); 2-way anova followed by Bonferroni's multiple comparison test. Comparison of Ebselen oxide/EGF-treated cells to EGF-stimulated cells is shown, (ns, not significant).

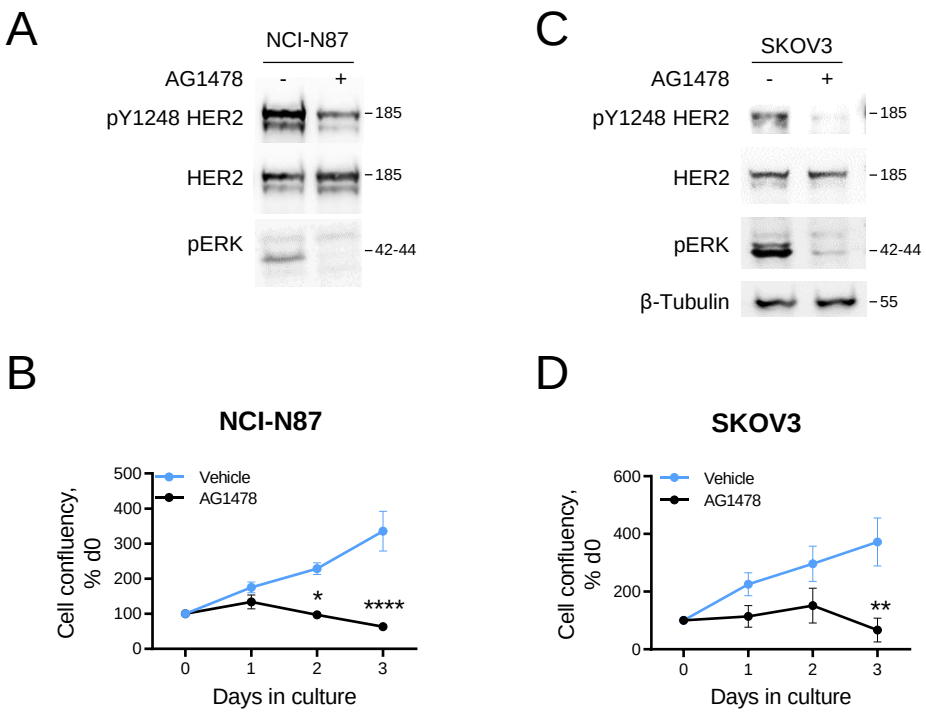
(B) HBMECs were treated with or without 10 µM Ebselen oxide before 5 min stimulation with 100 ng/ml Heregulin-1β (HRG). Whole cell lysates were analysed by western blot using anti-pAkt, Akt, pERK and ERK antibodies. Data are mean ± sem (n=3). Comparison of Ebselen oxide/HRG-treated cells to HRG-stimulated cells is shown, (ns, not significant).



Supplementary figure 3: Ebselen oxide inhibits HER2 activation and proliferation of HER2+ breast cancer cell

(A-B) Quantification of HER2 activation (pY1248-HER2) in HER2+ (SKBR3, A) or in HER2-negative (MDA-MB-231, B) breast cancer cells treated with increasing concentrations of Ebselen oxide (0-80 µM) measured by dot blots. Data are mean ± sem (n=3); statistical analysis is Two-way ANOVA followed by multiple comparison test, **** P<0.0001, ** P<0.01

(C-E) Monitoring of the proliferation by real time impedance-based cell assay of HER2-positive SKBR3 (C) and BT474 (D) or HER2-negative MDA-MB-231 (E) breast cancer cell lines treated with vehicle or with 5-10 µM Ebselen oxide, as indicated. A representative experiment is shown (n=3-4) where data are mean ± SD (n=2-5); statistical analysis are paired t test or Two-way ANOVA followed by Dunnett's multiple comparison test, **** P<0.0001.



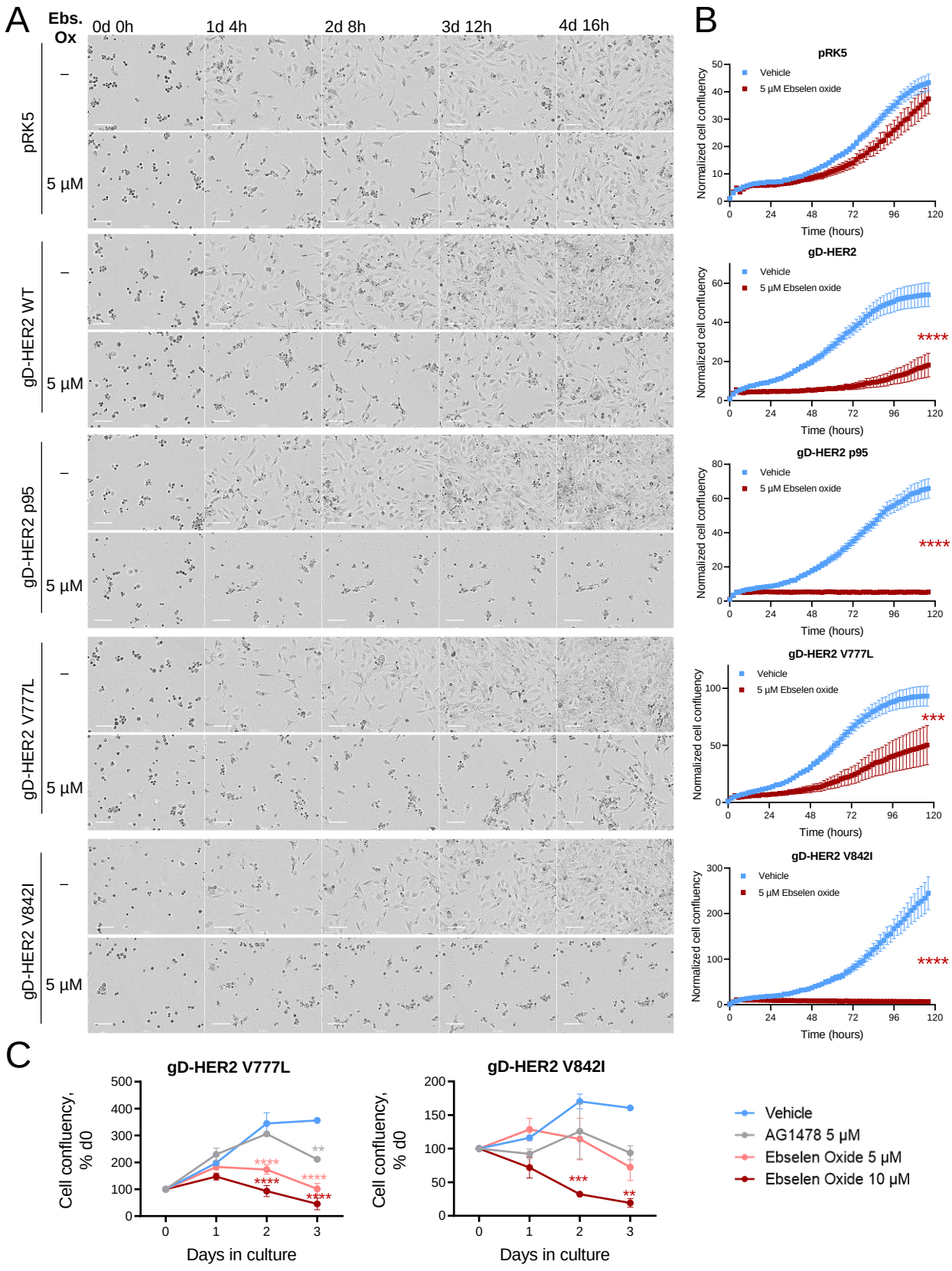
Supplementary figure 4: NCI-N87 and SKOV3 cancer cell lines are addicted to the HER2 pathway

(A) NCI-N87 cells were treated (+) or not (-) with a non-specific HER2 inhibitor AG1478 (5 µM). Whole cell lysates were analyzed by western blot with pY1248 HER2, HER2 and pERK antibodies.

(B) SKOV3 cells were treated (+) or not (-) with a non-specific HER2 inhibitor AG1478 (5 µM). Whole cell lysates were analyzed by western blot with pY1248 HER2, HER2, pERK or beta-tubulin antibodies.

(C) Proliferation curves of NCI-N87 treated or not with AG1478 (5 µM). Data are mean ± sem (n=4-6). 2-way Anova (mixed effect analysis) followed by Bonferroni's multiple comparison test, * P<0.05, **** P<0,0001.

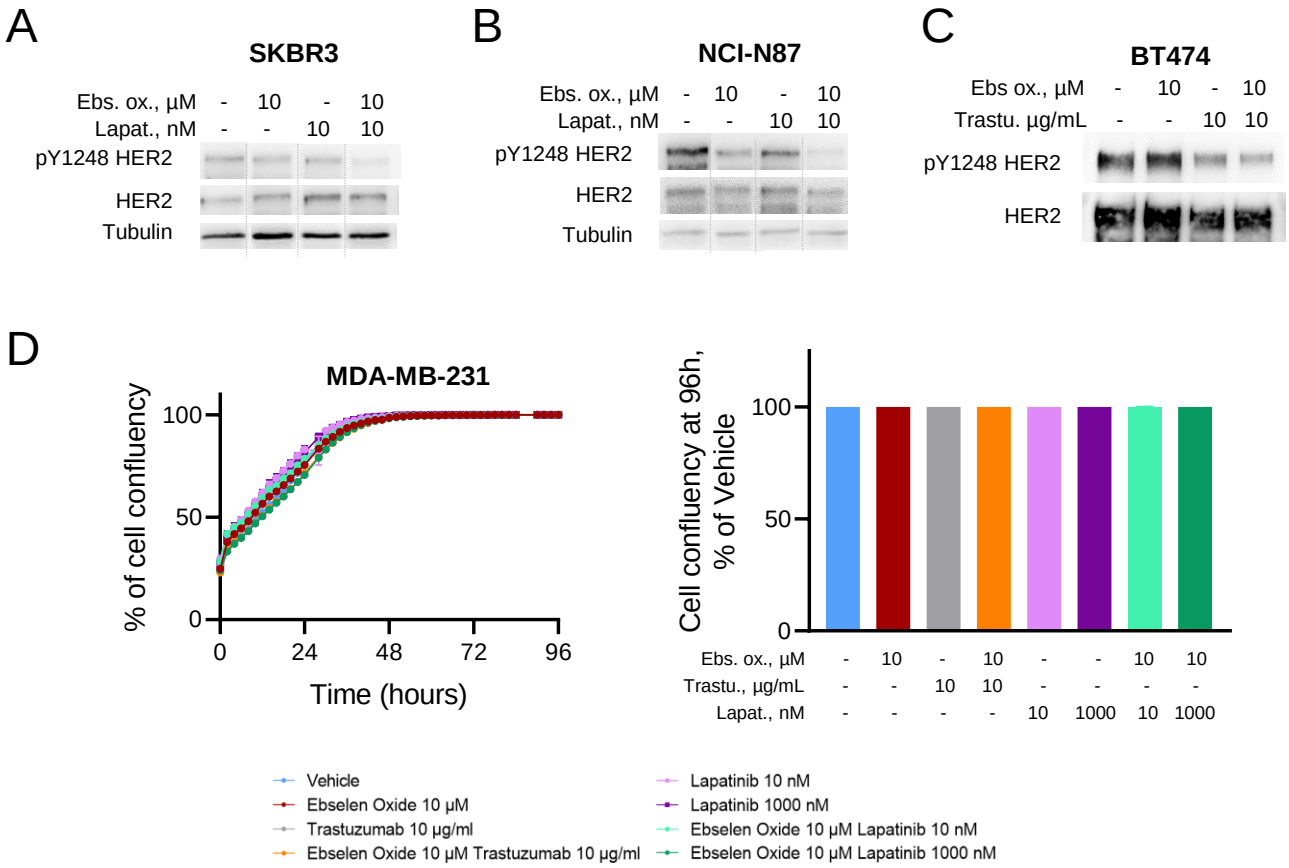
(D) Proliferation curves of SKOV3 cells treated or not with AG1478 (5 µM). Data are mean ± sem (n=4). 2-way Anova (mixed effect analysis) followed by Bonferroni's multiple comparison test, ** P<0,01.



Supplementary figure 5: Ebselen oxide inhibits the cell proliferation induced by WT and mutant forms of gD-HER2

(A-B) HBMECs transfected with empty vector (pRK5) or vectors encoding gD-HER2 WT, the truncated gD-HER2 p95, or the mutated gD-HER2-V777L or V842I forms were treated or not with 5 µM Ebselen oxide. Proliferation was assessed by Incucyte during 5 days. Representative micrographs are shown (A). Representative quantification of the proliferation (B) is shown where data are mean ± sem (n=8-12). Two-way ANOVA followed by Bonferroni's multiple comparison test, *** P<0.001, **** P<0.0001. Scale bars are 100 µM.

(C) HBMECs transfected with vector encoding V777L or V842I HER2 mutants, as indicated, were treated with vehicle, 5-10 µM Ebselen oxide or 5 µM AG1478. Proliferation was assessed by MTT assay during 3 days. Data are mean ± sem (n=3). Two-way ANOVA followed by Bonferroni's multiple comparison test, * P<0.05 ** P<0.01 *** P<0.001 and**** P<0.0001.



Supplementary figure 6: Ebselen oxide potentiates HER2 inhibition induced by anti-HER2 agents in HER2-positive but not in HER2-negative breast cancer cells

(A-C) HER2-positive breast cancer cells (SKBR3, A; NCI-N87, B; BT474, C) treated or not with Ebselen oxide (10 µM), lapatinib (10-1000 nM), trastuzumab (10 µg/mL) or combination of these agents, as indicated, were analyzed by western blot to evaluate HER2 activation status using antibodies directed against pY1248 HER2 and HER2.

(D) Proliferation curve of HER2-negative (MDA-MB-231) breast cancer cell lines treated or not with Ebselen oxide (10 µM), lapatinib (0.01-1 µM), trastuzumab (10 µg/mL) or combination of these agents, as indicated. Proliferation was assessed by Incucyte during 4 days. Representative curves and quantification are shown (n=2-3) where data are mean ± SD (n=8-12).