Supp. Fig. 1

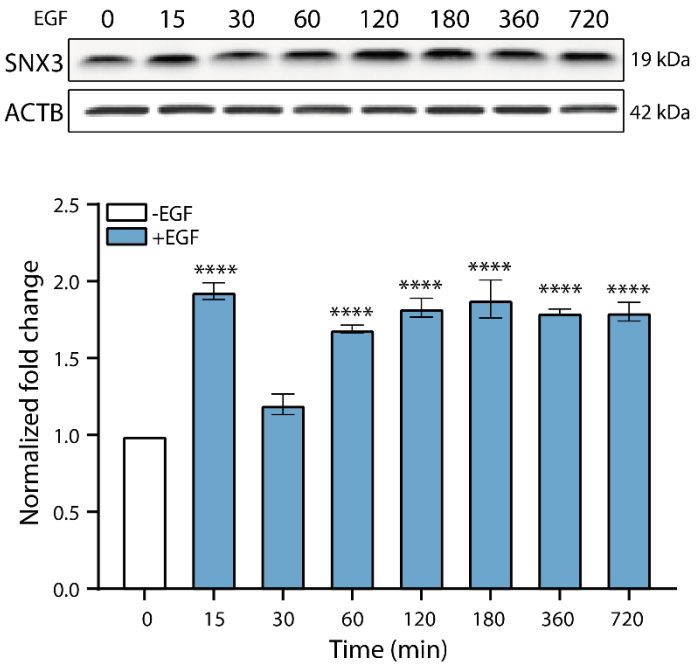


Fig. S1. EGF induction of SNX3 protein in HEK293 cells. HEK293 cells were serum-starved in DMEM for 6 hours and were treated with 50 ng/ml EGF for the indicated time points (n=2 independent treatments). Bars show densitometric quantification of bands using Image Lab software. For statistical analysis, one-way ANOVA with Tukey's multiple comparison test was used. **** indicates $p<0.0001$.

Supp. Fig. 2

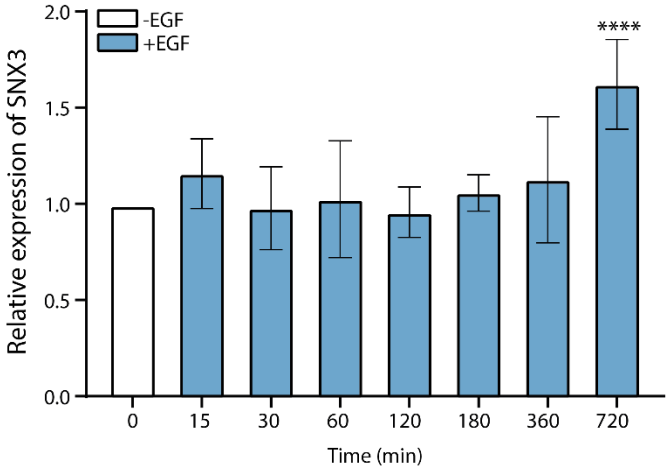
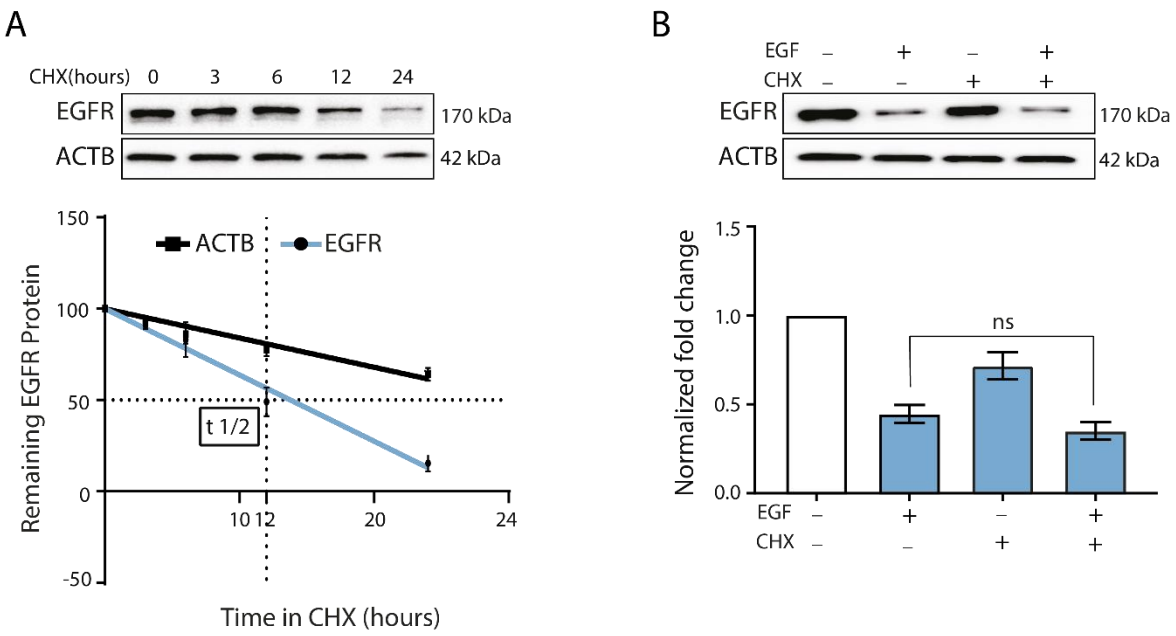


Fig. S2. Effect of EGF stimulation on *SNX3* mRNA levels. MCF10A cells were treated with 20 ng/ml EGF for the indicated time points. Expression of *SNX3* mRNA was determined by RT-qPCR and normalized against the reference gene, *RPLP0*. Treated samples were normalized to the untreated samples. n= 3 independent treatments. For statistical analysis, one-way ANOVA with Dunnett's multiple comparison test was applied. **** indicates $p<0.0001$.

Supp. Fig. 3



Supp. Fig. 3. EGFR levels in CHX treated cells. **(A)** MCF10A cells were treated with CHX (30 µg/ml) for the indicated time points. Cell lysates were immunoblotted for EGFR and ACTB. Graph shows remaining EGFR protein in CHX treated cells. Bands were quantified by densitometry to determine the half-life (t ½) of EGFR protein, **(B)** Western blot for EGFR levels. MCF10A cells were treated with CHX (30 µg/ml) and with EGF (20 ng/ml) for 6 hours. Cell lysates were immunoblotted for EGFR and ACTB as the loading control. The data represent the mean (SD) of 3 independent treatments. Unpaired t-test with Welch's correction was performed for statistical significance, ns: non-significant.

Supp.Fig. 4.

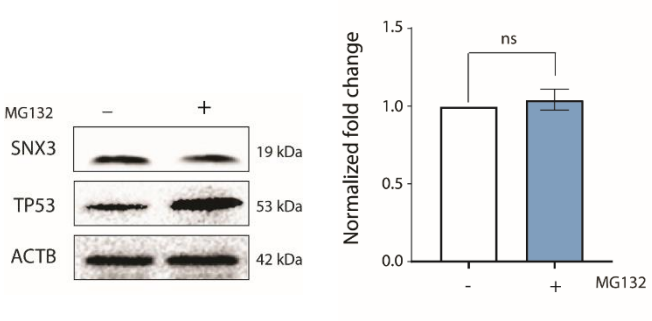
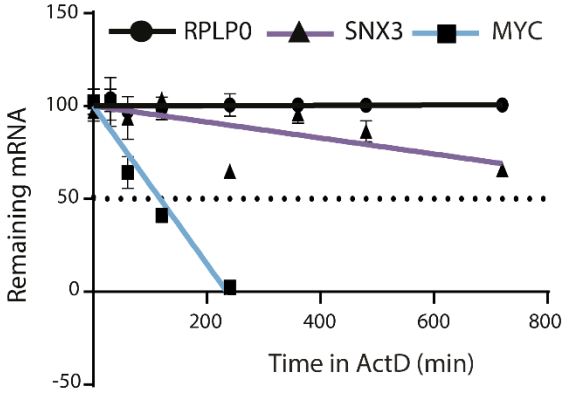


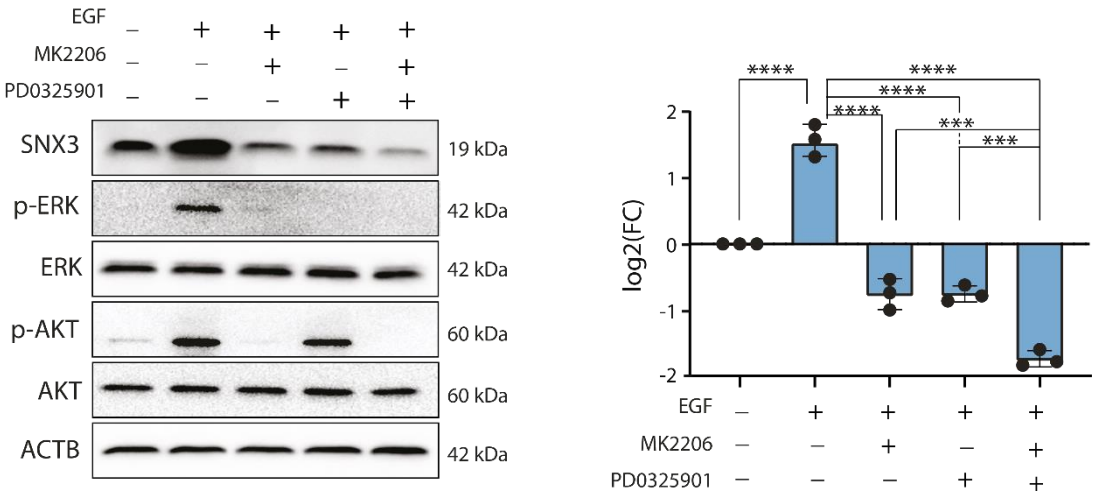
Fig. S4. Inhibition of proteasomal degradation. MCF10A cells were treated with MG132 (1 μM) for 6 hours, and lysates were collected for western blotting with SNX3 antibody. TP53 (p53) antibody was used to test MG132 activity. The same blot was hybridized to ACTB antibody to confirm equal loading. The graph shows densitometric quantification of SNX3 bands. The data represent the mean (SD) of three experiments. Unpaired t-test was performed for statistical significance, ns: not significant.

Supp. Fig. 5



Supp. Fig. 5. The decay rate of *SNX3* mRNA. Transcription was blocked by actinomycin D (10 µg/mL) treatments (n=3) for 12 hours in MCF10A cells. RNA was isolated at indicated time points to perform RT-qPCR. *RPLP0* and *MYC* have long and short decay rates, respectively.

Supp. Fig. 6



Supp. Fig. S6. MK2206 and PD0325901 co-treatment. MCF10A cells were pre-treated with MK2206 (1 μM), with PD0325901 (1 μM), and with both inhibitors for 4 hours. Following the pre-treatment with the inhibitors, cells were stimulated with EGF (20 ng/ml) for 30 minutes. Western blots show SNX3, p-AKT, p-ERK, AKT and ERK protein levels. The same blots were hybridized to ACTB antibody to confirm equal loading. Blots are representative of 3 independent experiments. Bars show densitometric quantification of SNX3 protein levels (mean (SD) of 3 experiments). One-way ANOVA with Tukey's multiple comparison test was used, ***p<0.001, ****p<0.0001.

Supp. Fig. 7

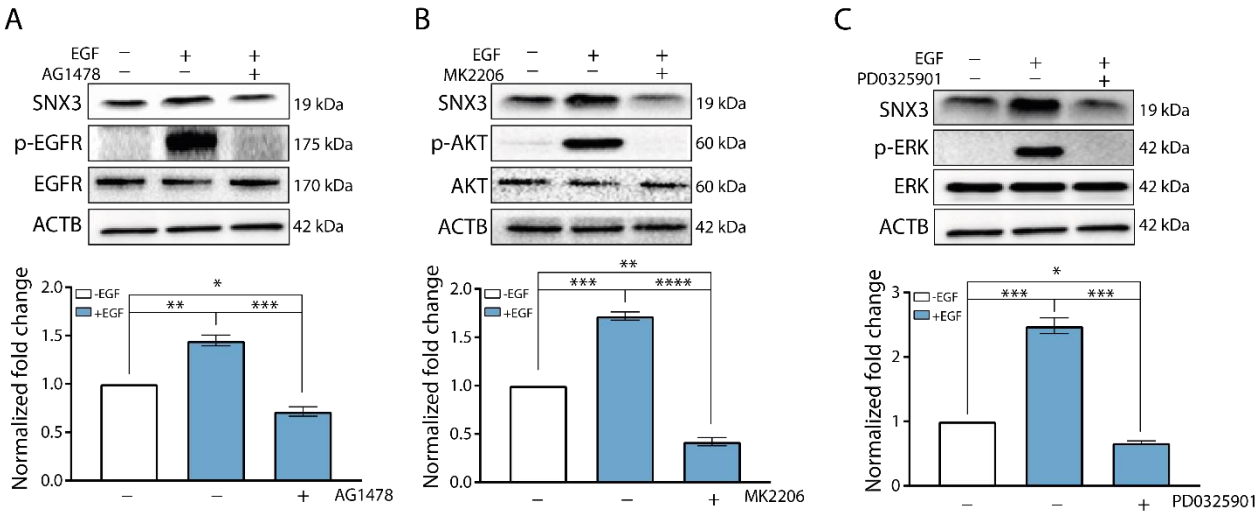


Fig. S7. Effect of EGFR blockade on SNX3 protein levels in HEK293 cells. HEK293 cells were serum-starved in DMEM for 6 hours and were pre-treated with **(A)** AG1478 (25 μM), (**B)** MK2206 (1 μM), (**C)** PD0325901 (1 μM) for 4 hours. Cells were then stimulated with EGF (50 ng/ml) for 15 minutes, and lysates were collected and blotted for p-EGFR, p-AKT, p-ERK, EGFR, total AKT, and total ERK levels. ACTB was used as the loading control. Bars show densitometric quantification of SNX3 protein bands in three independent treatments. For statistical analysis, one-way ANOVA with Tukey's multiple comparison test was used. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Supp. Fig. 8

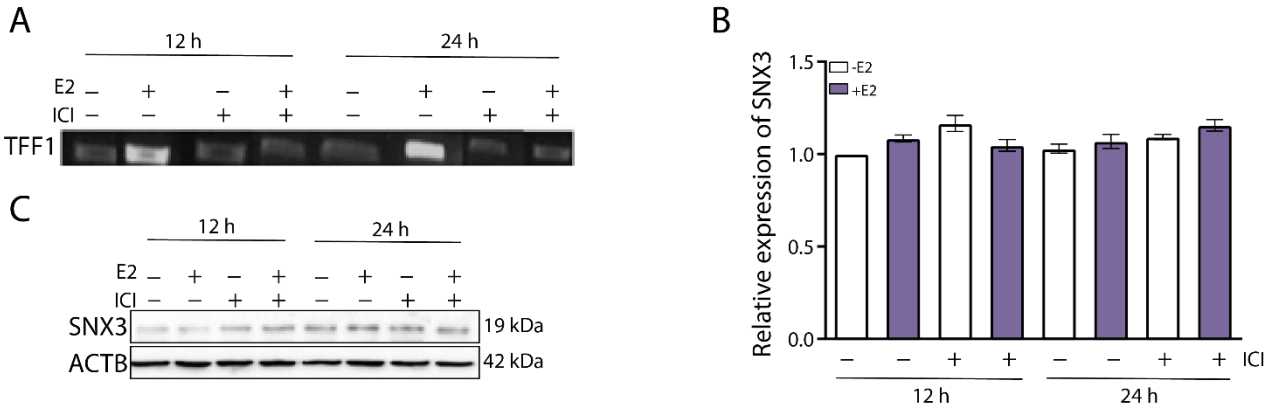


Fig. S8. Effect of E2 induction on SNX3 in MCF7 cells. MCF7 cells were grown in phenol red-free media supplemented with 10% dextran-coated charcoal-stripped FBS for 48 hours. Then, cells were pre-treated with ICI 182,780 (Fulvestrant, Faslodex) (1 μM), an estrogen receptor antagonist, for 1 hour and/or with 10 nM E2 for 12 and 24 hours, along with the ethanol-treated samples. ICI pre-treated samples were also treated with 10 nM E2 for 12 and 24 hours. (**A)** *TFF1* was used as a positive control for E2 treatments. (**B)** *SNX3* mRNA expression was quantified by RT-qPCR upon E2 treatment. (**C)** Western blot for SNX3 protein levels upon E2 and ICI treatment in MCF7 cells. ACTB antibody was used to show equal loading of proteins.

Supp. Fig. 9

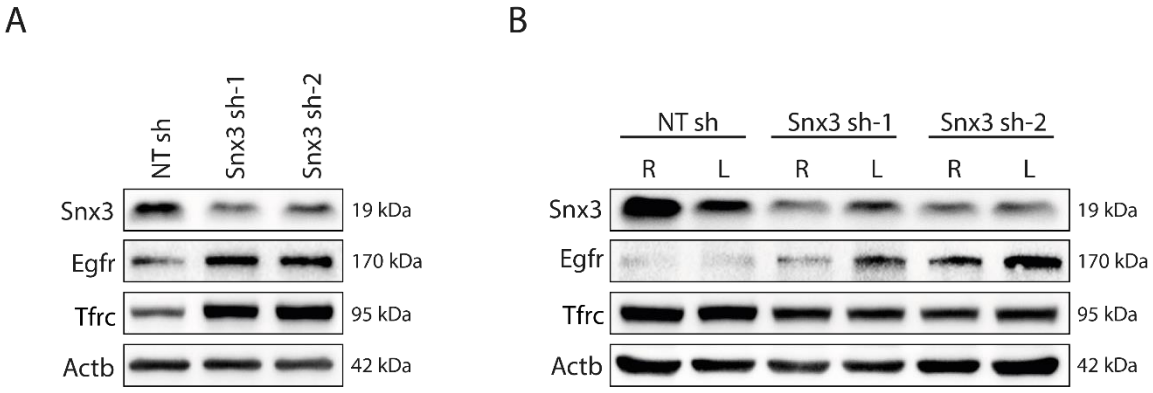


Fig. S9. Transferrin receptor (Tfrc) levels in SNX3 silenced cells. Western blots with the total cell lysates of **(A)** 4T1 cells (shNT, shSnx3-1, shSnx3-2), and **(B)** primary tumors. The same blots were hybridized with Snx3, Egfr, and Tfrc antibodies. Actb antibody was used as a control for equal loading. (R: right side, L:left side)