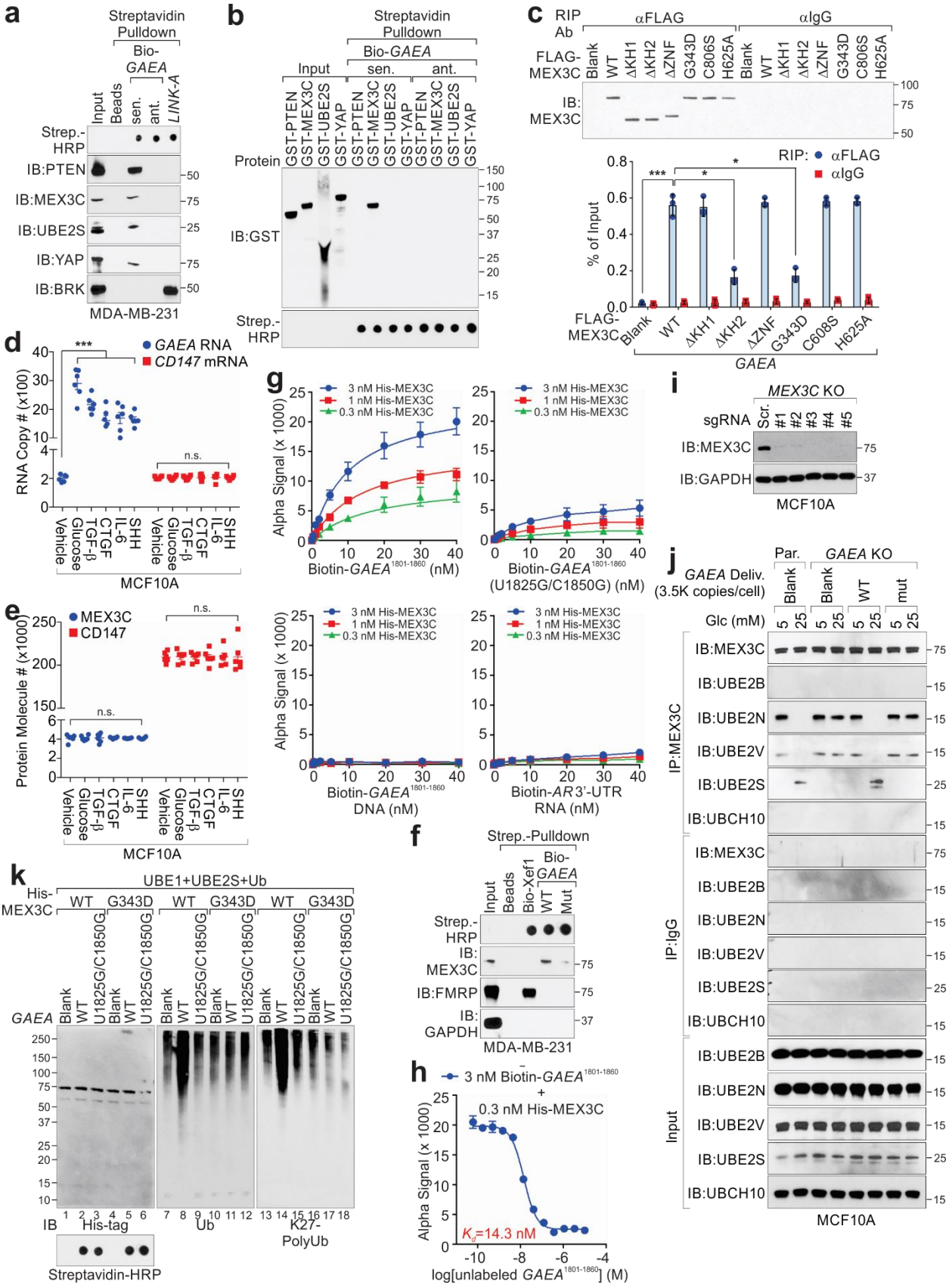
a
Streptavidin Pulldown
Bio-GAEA
Input
Beads
sen.
ant.
LINK-A
Strep.-HRP
IB:PTEN
IB:MEX3C
IB:UBE2S
IB:YAP
IB:BRK
MDA-MB-231
b
Streptavidin Pulldown
Bio-GAEA
Input
sen.
ant.
Protein
GST-PTEN
GST-MEX3C
GST-UBE2S
GST-YAP
IB:GST
Strep.-HRP
c
RIP Ab
αFLAG
αIgG
FLAG-MEX3C
Blank
WT
ΔKH1
ΔKH2
ΔZNF
G343D
C806S
H625A
IB: MEX3C
RIP: αFLAG
αIgG
% of Input
GAEA
d
GAEA RNA
CD147 mRNA
RNA Copy # (x100)
n.s.
Vehicle
Glucose
TGF-β
CTGF
IL-6
SHH
MCF10A
e
MEX3C
CD147
Protein Molecule # (x1000)
n.s.
f
Strep.-Pulldown
Bio-GAEA
Bio-Xef1
WT
Mut
IB: MEX3C
IB:FMRP
IB: GAPDH
MDA-MB-231
g
3 nM His-MEX3C
1 nM His-MEX3C
0.3 nM His-MEX3C
Alpha Signal (x 1000)
Biotin-GAEA1801-1860 (nM)
Biotin-GAEA1801-1860 (U1825G/C1850G) (nM)
Biotin-GAEA1801-1860 DNA (nM)
Biotin-AR3'-UTR RNA (nM)
h
3 nM Biotin-GAEA1801-1860
+
0.3 nM His-MEX3C
Kd=14.3 nM
log[unlabeled GAEA1801-1860] (M)
i
MEX3C KO
sgRNA
Scr.
#1
#2
#3
#4
#5
IB:MEX3C
IB:GAPDH
MCF10A
j
Par.
GAEA KO
GAEA Deliv. (3.5K copies/cell)
Blank
WT
mut
Glc (mM)
IP:MEX3C
IP:IgG
Input
IB:UBE2B
IB:UBE2N
IB:UBE2V
IB:UBE2S
IB:UBCH10
MCF10A
k
UBE1+UBE2S+Ub
His-MEX3C
WT
G343D
U1825G/C1850G
GAEA
IB
His-tag
Ub
K27-PolyUb
Streptavidin-HRP

Figure S2: *GAEA* modulates the E3 ligase activity of MEX3C.

(**a, b**) IB detection of proteins retrieved by *in vitro*-transcribed biotinylated *GAEA* from MDA-MB-231 cell lysates (a) or indicated recombinant proteins (b). (**c**) IB detection using indicated antibodies (top) or RT-QPCR detection of *GAEA* (bottom) retrieved by indicated antibodies in MDA-MB-231 cells transfected with MEX3C or indicated mutants. Error bars, SD, n=3 independent experiments. Student's t-test. (**d, e**) RNA copy number (d) or protein molecule number measurement (e) of indicated RNA/protein molecules in MCF10A cells subjected to indicated stimuli. Error bars, SD, n=6 independent experiments, one-way ANOVA. (**f**) Immunoblotting (IB) detection of proteins retrieved by indicated in vitro-transcribed biotinylated RNA from MDA-MB-231 cell lysates. **(g)** Saturation binding experiments showing the binding capacity of MEX3C to biotinylated-$GAEA^{1801\text{-}1860}$ RNA (WT and U1825G/C1850G mutant, top left and right), biotinylated-$GAEA^{1801\text{-}1860}$ DNA (bottom left) and *AR* 3-'UTR RNA (bottom right) in Alpha format. Error bars, SD, n=3 independent experiments. (**h**) Competition binding assay to determine K_d for interaction between $GAEA^{1801\text{-}1860}$ and MEX3C, with unlabeled $GAEA^{1801\text{-}1860}$ RNA titrated from 10 μM to 0.1 nM. Error bars, SD. n=3 independent experiments. (**i, j**) IB detection using indicated antibodies of MCF10A cells harboring scramble or *MEX3C* sgRNAs (i) or *GAEA* sgRNAs and indicated expression vectors followed by glucose stimulation (5, 25 mM) (j). (**k**) In vitro ubiquitination assay were performed with recombinant ubiquitin-UBE1-UBE2S-MEX3C (WT or indicated mutants) in the presence of *in vitro*-transcribed biotinylated *GAEA*, and immunoblotted with indicated antibodies (n.s. $p>0.05$, *$p<0.05$, **$p<0.01$, and ***$p<0.001$).