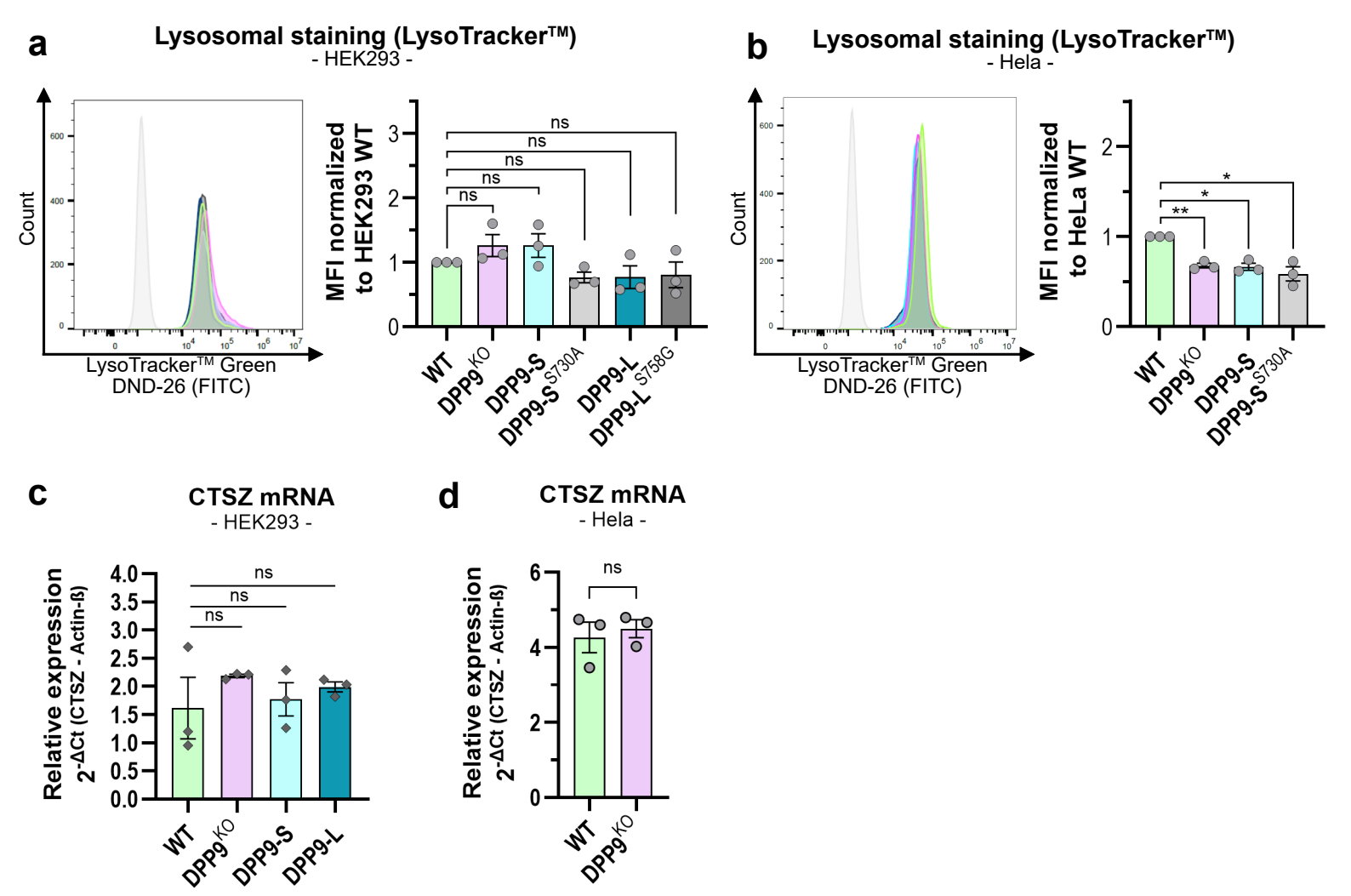
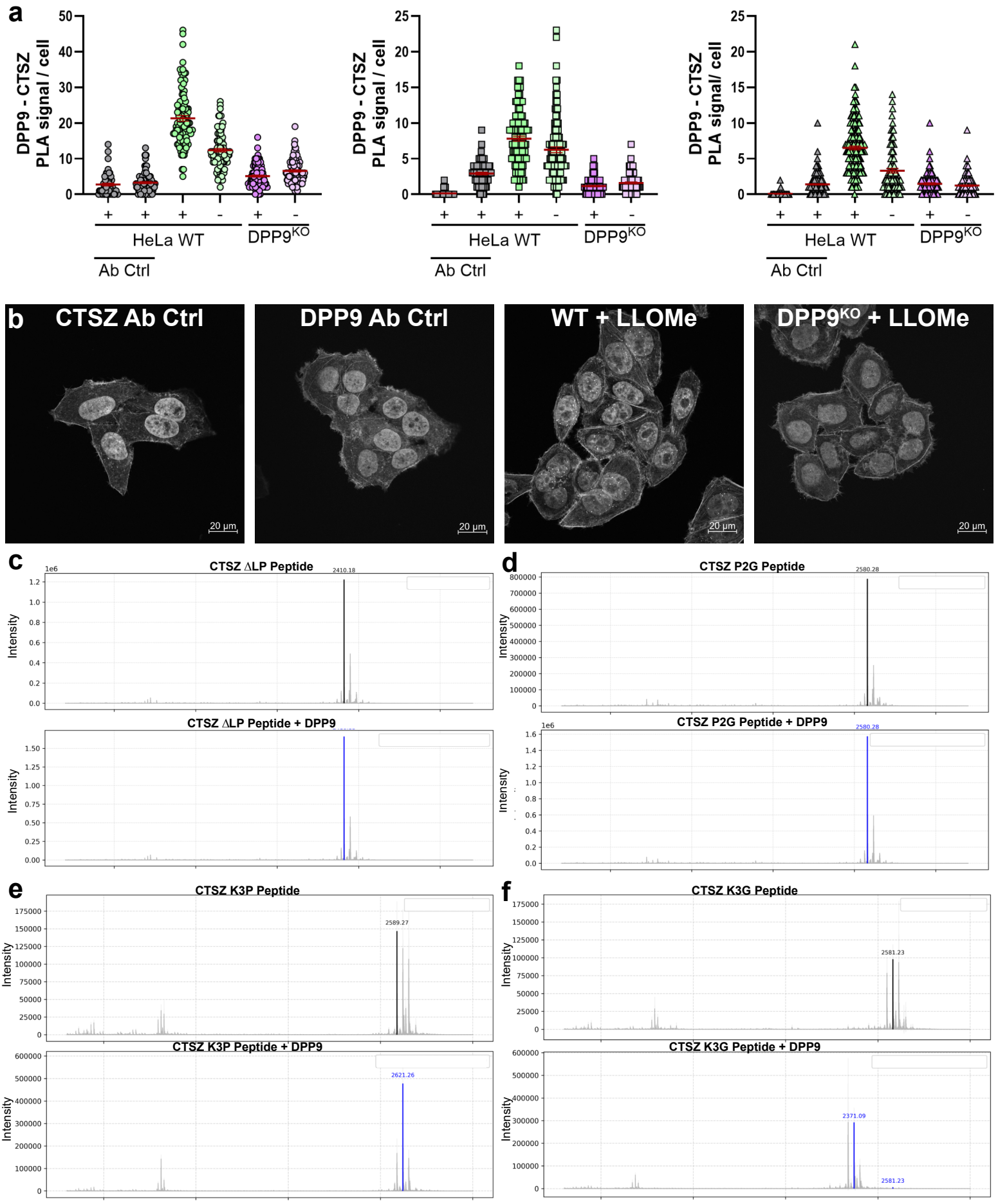

a
Lysosomal staining (LysoTracker™)
- HEK293 -
Count
LysoTracker™ Green
DND-26 (FITC)
MFI normalized
to HEK293 WT
ns
WT
DPP9 KO
DPP9-S
DPP9-S S730A
DPP9-L
DPP9-L S758G
b
Lysosomal staining (LysoTracker™)
- Hela -
Count
LysoTracker™ Green
DND-26 (FITC)
MFI normalized
to HeLa WT
*
**
WT
DPP9 KO
DPP9-S
DPP9-S S730A

c
CTSZ mRNA
- HEK293 -
Relative expression
2-ΔCt (CTSZ - Actin-ß)
ns
WT
DPP9 KO
DPP9-S
DPP9-L
d
CTSZ mRNA
- Hela -
Relative expression
2-ΔCt (CTSZ - Actin-ß)
ns
WT
DPP9 KO

Sup. Fig. 1

Supplementary Fig.1

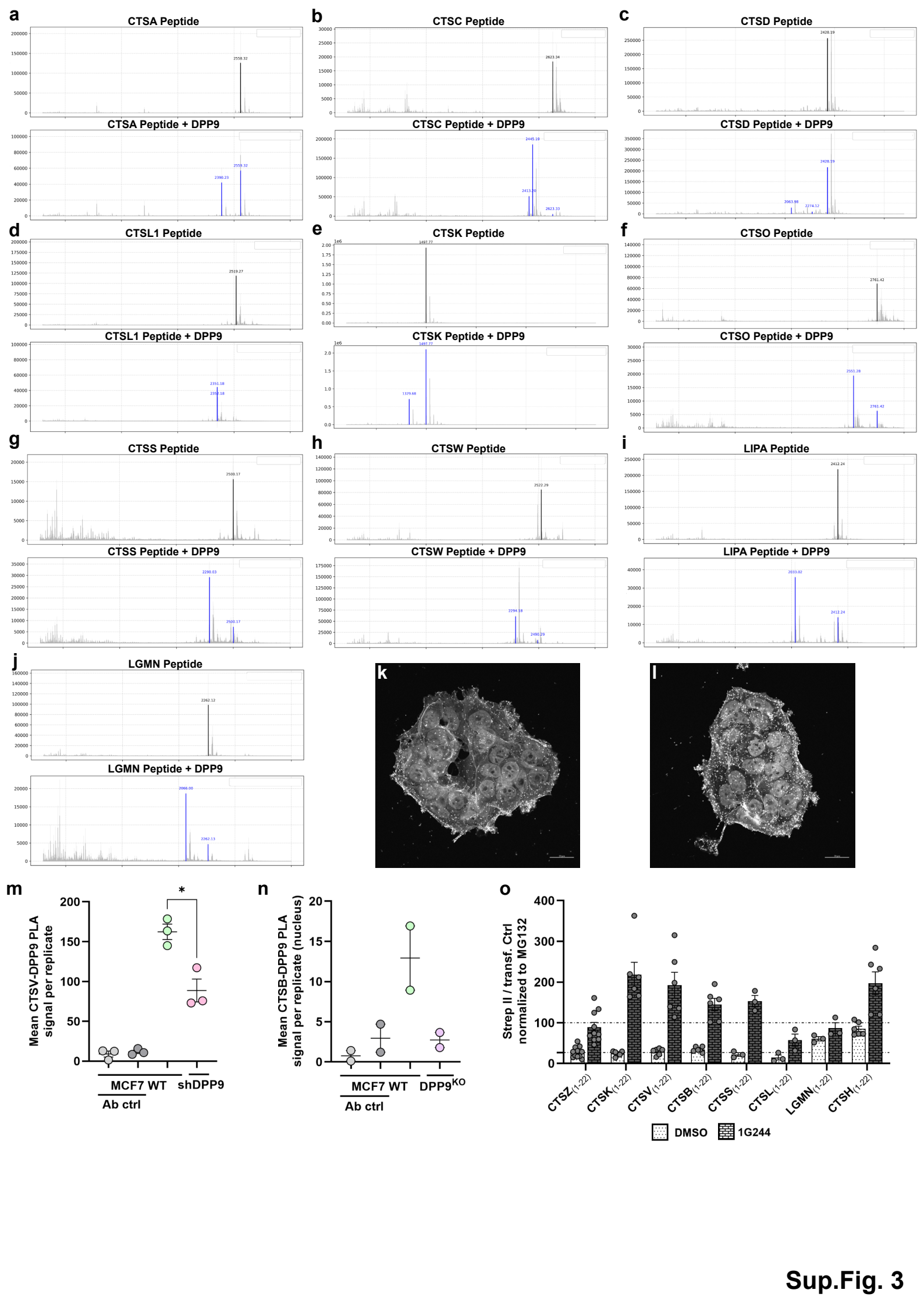
a, b, Flow cytometry analysis of lysosomal staining with LysoTracker™ in indicated HEK293 **(a)** and Hela **(b)** cell-lines with doxycycline induced DPP9 variants, exemplary histogram comparison (left) and quantification (right). Shown are mean ± SEM, data were analyzed by t-test comparisons (ns (not significant), $p > 0.05$; *, $p \leq 0.05$; **, $p \leq 0.01$). **c, d,** Relative CTSZ mRNA expression in HEK293 **(c)** and Hela **(d)** cell-lines with doxycycline induced DPP9 variants. Expression was measured via qRT-PCR. Each symbol refers to an average of a biological replicate, each of three technical replicates, normalized to ß-Actin (ΔCt method). Shown are mean ± SEM, data were analyzed by t-test comparisons (ns, $p > 0.05$; *, $p \leq 0.05$)**.**


a
DPP9 - CTSZ PLA signal / cell
HeLa WT
DPP9KO
Ab Ctrl
DPP9 - CTSZ PLA signal/ cell
b
CTSZ Ab Ctrl
DPP9 Ab Ctrl
WT + LLOMe
DPP9KO + LLOMe
20 µm
c
CTSZ ΔLP Peptide
2410.18
CTSZ ΔLP Peptide + DPP9
d
CTSZ P2G Peptide
2580.28
CTSZ P2G Peptide + DPP9
e
CTSZ K3P Peptide
2589.27
CTSZ K3P Peptide + DPP9
2621.26
f
CTSZ K3G Peptide
2581.23
CTSZ K3G Peptide + DPP9
2371.09
Intensity

Sup. Fig. 2

Supplementary Fig.2

a Single replicate quantification of DPP9-CTSZ PLA events in $Hela^{WT}$ and $DPP9^{KO}$ cells. Where indicated, cells were treated with 300 µM LLOMe for 3 h. Each dot represents the number of PLA events in a single cell. **b** Exemplary microscopy images of A. **c-f** *In-vitro* peptide cleavage assays in absence (up) and presence (below) of recombinant DPP9, showing that DPP9 is not able to process the $CTSZ_{1-22}$ ΔLP (**c**), $CTSZ_{1-22}$ P2G (**d**) and $CTSZ_{1-22}$ K3P peptide (**e**), while the $CTSZ_{1-22}$ K3G peptide is still processed (**f**)**.** 100 ng DPP9 were incubated with 1 µg peptide for 16 h RT.


a
CTSA Peptide
CTSA Peptide + DPP9
b
CTSC Peptide
CTSC Peptide + DPP9
c
CTSD Peptide
CTSD Peptide + DPP9
d
CTSL1 Peptide
CTSL1 Peptide + DPP9
e
CTSK Peptide
CTSK Peptide + DPP9
f
CTSO Peptide
CTSO Peptide + DPP9
g
CTSS Peptide
CTSS Peptide + DPP9
h
CTSW Peptide
CTSW Peptide + DPP9
i
LIPA Peptide
LIPA Peptide + DPP9
j
LGMN Peptide
LGMN Peptide + DPP9
k
l
m
Mean CTSV-DPP9 PLA signal per replicate
MCF7 WT
shDPP9
Ab ctrl
n
Mean CTSB-DPP9 PLA signal per replicate (nucleus)
MCF7 WT
DPP9ko
Ab ctrl
o
Strep II / transf. Ctrl normalized to MG132
CTSZ(1-22)
CTSK(1-22)
CTSV(1-22)
CTSB(1-22)
CTSS(1-22)
CTSL(1-22)
LGMN(1-22)
CTSH(1-22)
DMSO
1G244

Sup.Fig. 3

Supplementary Fig.3

a-j *In-vitro* PCA in absence (up) and presence (below) of recombinant DPP9 for $CTSA_{1-22}$ (**a**), $CTSC_{1-22}$ (**b**), $CTSD_{1-22}$, (**c**), $CTSL1_{1-22}$ (**d**), $CTSK_{1-22}$ (**e**), $CTSO_{1-22}$ (**f**), $CTSS_{1-22}$ (**g**), $CTSW_{1-22}$ (**h**), $LIPA_{1-22}$ (**i**), and $LGMN_{1-22}$ (**j**). **k, l,** Exemplary microscopy images showing PLA events in MCF7 shDPP9 (**k**) and $MCF7^{WT}$ cells (**l**). **m, n**: Single replicate quantification of DPP9-CTSV PLA events in $MCF7^{WT}$ and DPP9 silenced cells (**m**) as well as DPP9-CTSB PLA events in the nuclei of $MCF7^{WT}$ and $DPP9^{KO}$ cells (**n**). Data were analyzed by unpaired t-test comparisons (*, $p \leq 0.05$). **o** Summarized quantification of cathepsin reporter assays (shown in **Figure 4g-m**). The StrepII signal of each reporter was normalized to a transfection control and its own MG132 sample. Each dot refers to a single measurement. Constructs were tested in at least three independent experiments. Shown are mean ± SEM.