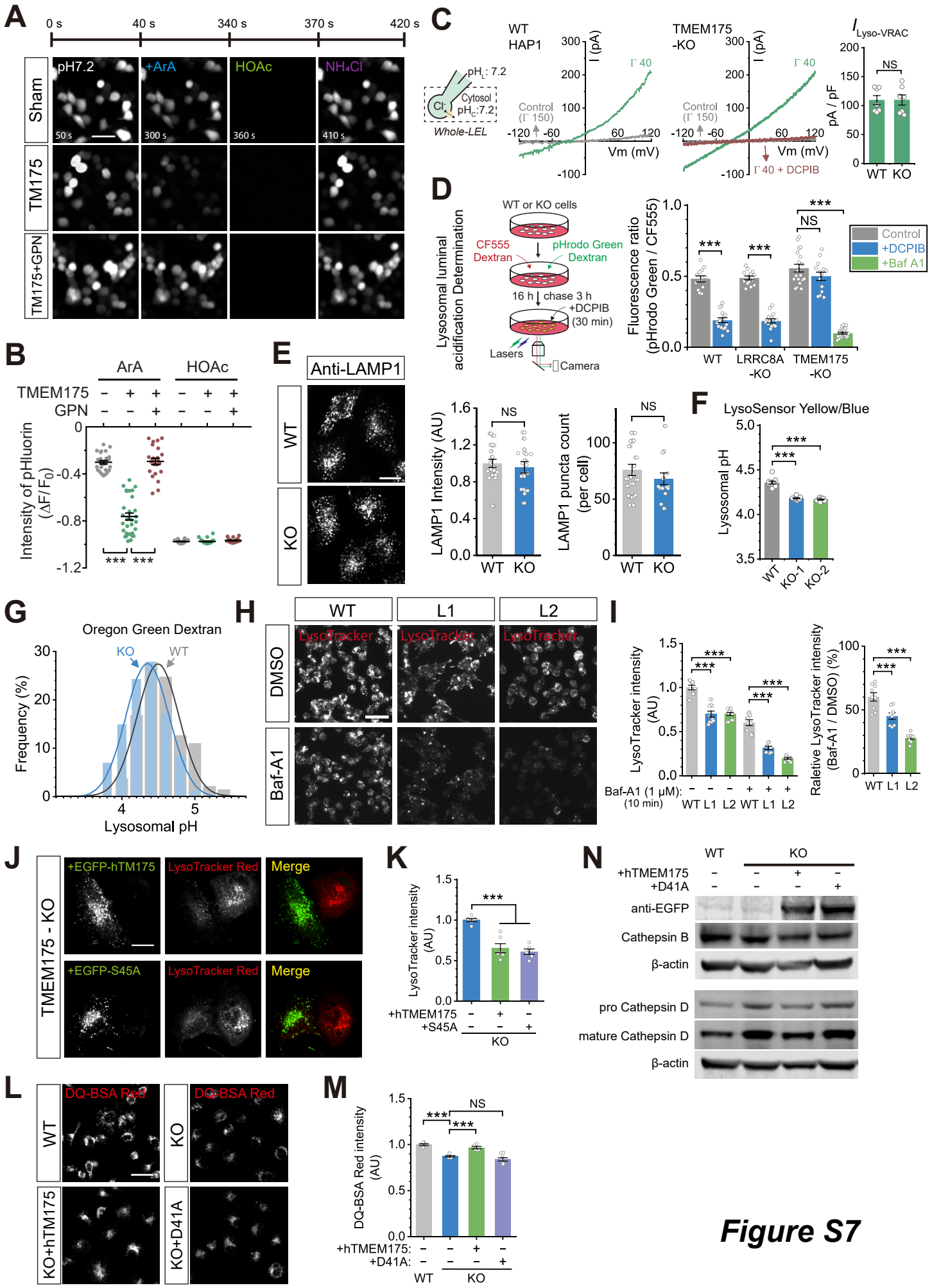
A
0 s
40 s
340 s
370 s
420 s
Sham
TM175
TM175+GPN
pH7.2
+ArA
HOAc
NH4Cl
50 s
300 s
360 s
410 s
B
ArA
HOAc
TMEM175
GPN
Intensity of pHluorin (ΔF/F0)
C
WT HAP1
TMEM175 -KO
Whole-LEL
Control (Γ 150)
Γ 40
Γ 40 + DCPIB
I (pA)
Vm (mV)
I Lyso-VRAC
pA / pF
NS
D
WT or KO cells
CF555 Dextran
pHrodo Green Dextran
16 h chase 3 h
+DCPIB (30 min)
Lasers
Camera
Lysosomal luminal acidification Determination
Fluorescence ratio (pHrodo Green / CF555)
Control
+DCPIB
+Baf A1
WT
LRRC8A -KO
TMEM175 -KO
E
Anti-LAMP1
WT
KO
LAMP1 Intensity (AU)
LAMP1 puncta count (per cell)
F
LysoSensor Yellow/Blue
Lysosomal pH
KO-1
KO-2
G
Oregon Green Dextran
KO
WT
Frequency (%)
Lysosomal pH
H
L1
L2
DMSO
Baf-A1
LysoTracker
I
LysoTracker intensity (AU)
Baf-A1 (1 μM): (10 min)
Raletive LysoTracker intensity (Baf-A1 / DMSO) (%)
J
TMEM175 - KO
+EGFP-hTM175
+EGFP-S45A
LysoTracker Red
Merge
K
LysoTracker intensity (AU)
+hTMEM175
+S45A
KO
N
+hTMEM175
+D41A
anti-EGFP
Cathepsin B
β-actin
pro Cathepsin D
mature Cathepsin D
L
DQ-BSA Red
KO+hTM175
KO+D41A
M
DQ-BSA Red intensity (AU)

Figure S7