

A

human TMEM175 knock-out (KO) cell lines generation:

S K E R V E A F S D G V Y A IIS1

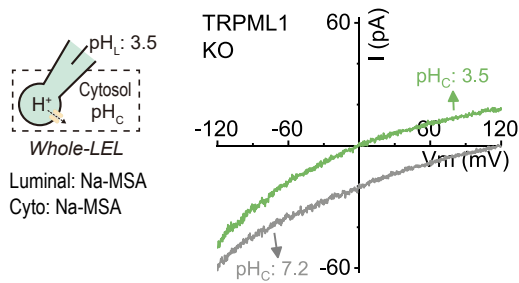
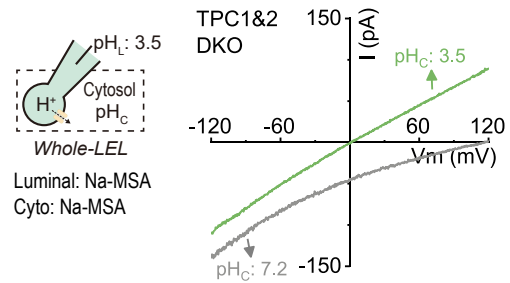
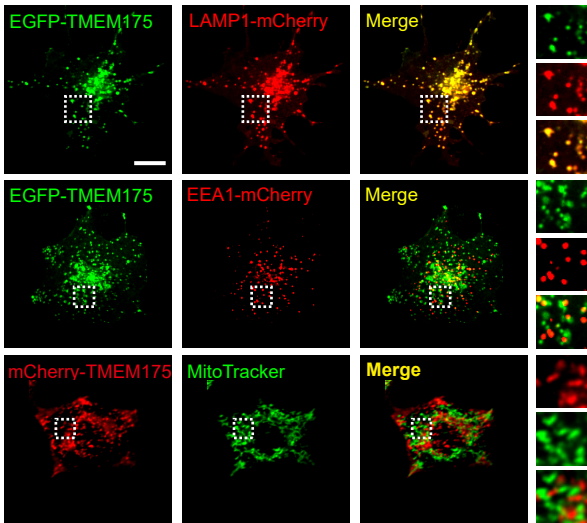
5' AGCAAGGAGCGCGTGGAAAGCCTTCAGCGACGGAGTCTACGCC 3' Wildtype

Hap1 KO AGCAAGGAGCGCGTG-----C-----CTACGCC (19 bp deletion)

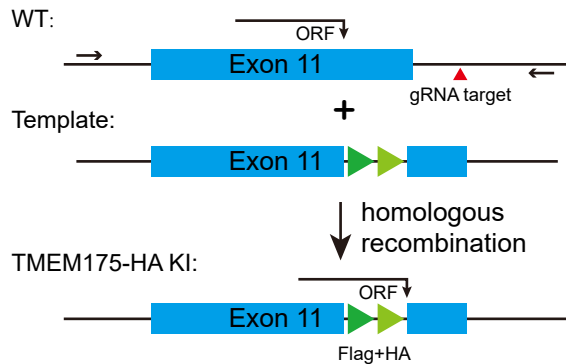
HeLa KO-1 AGCAAGGAGCG-----GAGTCTACGCC (20 bp deletion)
AGCAAGGAGCGCGTGGAAAGCCTTCAG-----GAGTCTACGCC (5 bp deletion)

HeLa KO-2 AGCAAGGAGCGCGTGGAAAGCCTTC--CGACGGAGTCTACGCC (2 bp deletion)
AGCAAGGAGCGCGTGGAAAGCCTT-----ACGGAGTCTACGCC (5 bp deletion)

HEK293T KO AGCAAGGAGCGCGT-----GGAGTCTACGCC (16 bp deletion)
AGCAAGGAGCGCGTGG-----CGCC (22 bp deletion)

B**C****D****E**

human TMEM175-HA knock-in (KI) cell line generation:

**Figure S2**