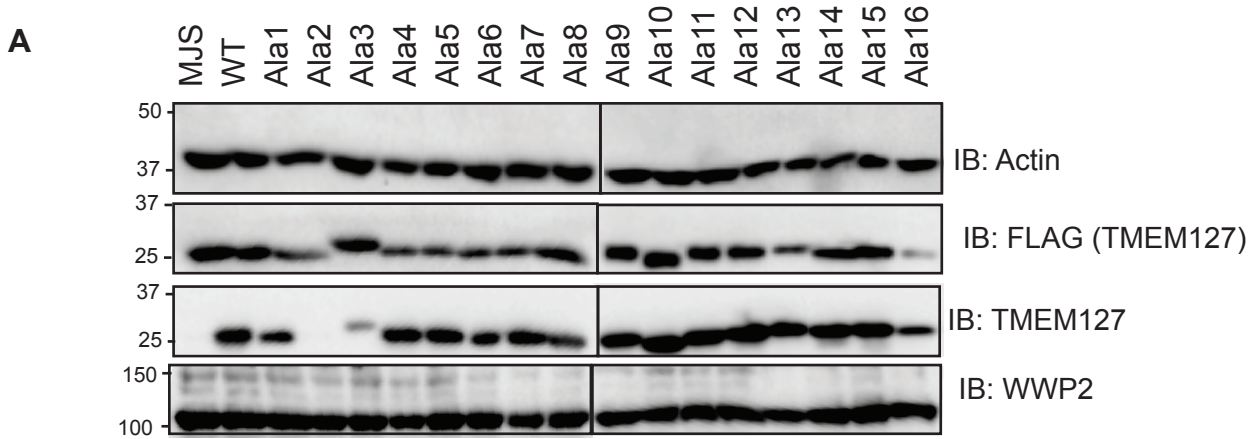


Wild type 140 ALETDSSPPP YSSITVEVPT TSDTEVYGEF YPVPPPYSSVA TSLPTYDEAE 190
PY1(Y151A) 140 ALETDSSPPP ASSITVEVPT TSDTEVYGEF YPVPPPYSSVA TSLPTYDEAE 190
PY2 (Y177A) 140 ALETDSSPPP YSSITVEVPT TSDTEVYGEF YPVPPPA SVA TSLPTYDEAE 190
PY3 (Y186A) 140 ALETDSSPPP YSSITVEVPT TSDTEVYGEF YPVPPPYSSVA TSLPTADEAE 190
PY12 (YY151,177AA) 140 ALETDSSPPP ASSITVEVPT TSDTEVYGEF YPVPPPA SVA TSLPTYDEAE 190
PY13 (YY151,186AA) 140 ALETDSSPPP ASSITVEVPT TSDTEVYGEF YPVPPPYSSVA TSLPTADEAE 190
PY23 (YY177,186AA) 140 ALETDSSPPP YSSITVEVPT TSDTEVYGEF YPVPPPA SVA TSLPTADEAE 190
PY123(YYY151,177,186AAA) 140 ALETDSSPPP ASSITVEVPT TSDTEVYGEF YPVPPPA SVA TSLPTADEAE 190

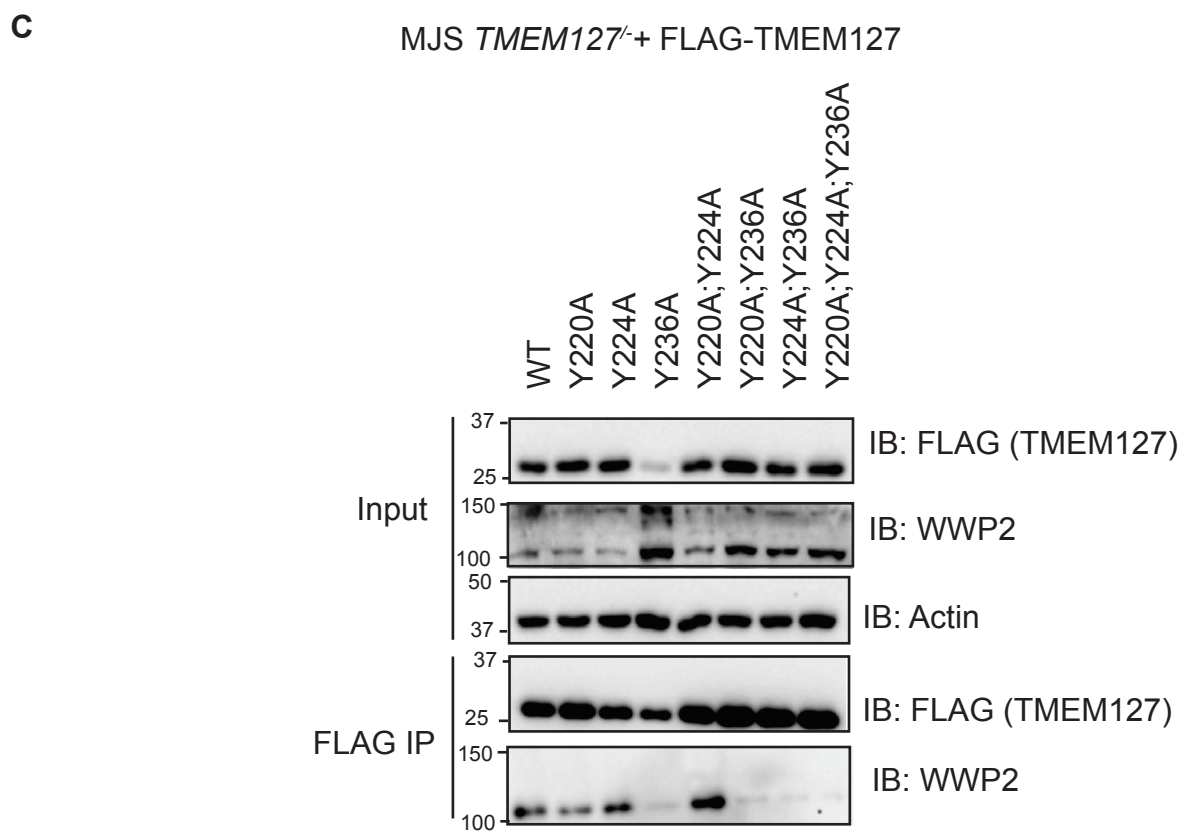
S1 NDFIP2

Amino acid sequence of NDFIP2 showing PY mutants highlighted in red.



B

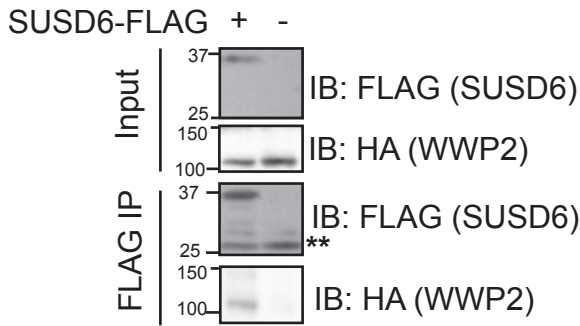
WT 210 LSEMEENE**PY** PAE**Y**EVINQF QPPPA**Y**TP 238
Y220A 210 LSEMEENE**P** PAE**Y**EVINQF QPPPA**Y**TP 238
Y224A 210 LSEMEENE**PY** PAE**A**EVINQF QPPPA**Y**TP 238
Y236A 210 LSEMEENE**PY** PAE**Y**EVINQF QPPPA**A**TP 238
Y220A;Y224A 210 LSEMEENE**P** PAE**A**EVINQF QPPPA**Y**TP 238
Y220A;Y236A 210 LSEMEENE**PY** PAE**A**EVINQF QPPPA**A**TP 238
Y224A;Y236A 210 LSEMEENE**PY** PAE**A**EVINQF QPPPA**A**TP 238
Y220A;Y224A;Y236A 210 LSEMEENE**P** PAE**A**EVINQF QPPPA**A**TP 238



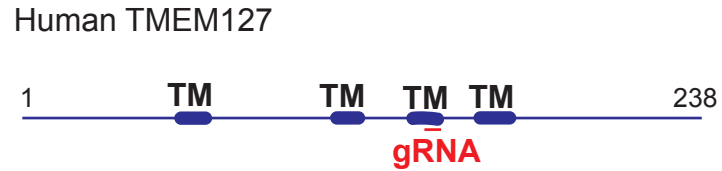
S2 TMEM127

A. Mel JuSo cells or mutant TMEM127 alanine mutant (as indicated) cell line levels of FLAG-TMEM127 as assessed by immunoblotting. B. Amino acid sequence of TMEM127 and PY mutants created from 210 to 238 with tyrosines within possible PY motifs indicated in red and mutations created indicated in orange. C. FLAG immunoprecipitation of TMEM127 PY mutant cell line (as indicated) and analysis of immunoprecipitate by immunoblotting. The blot shown is representative of the three independent experiments.

A



B

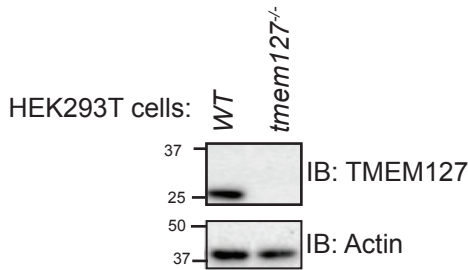


C

tmem127 allele 1 TTCTGCAGTGTGCCA|**CCGT**|CATTGGCTTTTCTTATTGGGCTTCTGAACTCATCTTGGC
4bp deletion at 11895 CCAGCAGCAGCAGCATAAGAAGTACCATGGATCCAGGTCTATGTCACCTTCGCCGT**TAG**

tmem127 allele 2 GTTCTGCAGT|**GTGCCACCGTCATTGGCTT**|TTCTTATTGGGCTTCTGAACTCATCTTGGC
19bp deletion at 11889 CCAGCAGCAGCAGCATAAGAAGTACCATGGATCCAGGTCTATGTCACCTTCGCCGT**TAG**

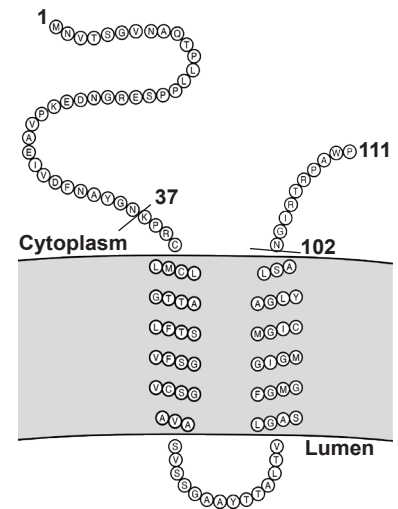
D



E

SteD truncation	Amino acids	Subcellular localisation
Full length	1-111	Normal (Golgi and endosomes)
N-terminal cytoplasmic region	1-41	Cytoplasmic
N-terminal truncation	37-111	Plasma membrane, Golgi and endosomes
C-terminal truncation	1-102	Normal (Golgi and endosomes)
N- & C-terminal truncation	37-102	Plasma membrane, Golgi and endosomes

F



S3 SUS D6, generation of TMEM127^{-/-} HEK293T cells and SteD

A. HEK293T cell transfection with/without SUS D6-FLAG and HA-WWP2 followed by FLAG-immunoprecipitation and immunoblot of immunoprecipitate (IP)**indicates light chain. **B.** Cartoon of TME127 with position of gRNA shown. **C.** HEK 293T pX330 *tmem127*^{-/-} sequencing results with deletions outline in blue and with strike through and premature stop codons in bold and red. **D.** Immunoblot of WT and TMEM127^{-/-} cells. **E.** A summary of GFP-SteD truncations used in Figure 5, truncations previous published in¹⁵. **F.** Schematic of truncations of SteD in relation to SteD topology. IB, immunoblot.