File S1 Construction of Fc-Mychis, EFN-1-Fc-Mychis and EFN-4-Fc-Mychis expression plasmids

pMH180 (Fc-Mychis expression vector) was built by PCR amplifying the Fc open reading frame from plasmid pCXFc-KAL (Bulow et al. 2002) using the primers below, digesting the PCR fragment with HindIII and EcoRI, then ligating into HindIII/EcoRI-digested pSecTagA (Life Technologies):

Forward primer: ATAAAGCTTCGACAAAACTCACACATG

Reverse primer: TATGAATTCTTTACCCGGAGACAGG

pMH873 (EFN-1ΔSecΔGPI-Fc-MycHis) was built by PCR amplifying the EFN-1 open reading frame from plasmid pLC708 (kind gift of Lihsia Chen) using the following primers:

Forward primer: AGGCGCGCCGTACGAAATCCCCTAGTGGAACGATATG

Reverse primer: GATCTTCccGAATTCTGCAAGcttCGACAAAACTCACACA

pMH874 (EFN-4ΔSecΔGPI-Fc-MycHis) was built by PCR amplifying the EFN-4 open reading frame from plasmid pMH828 (*P-unc-119-efn-4* cDNA) using the following primers:

Forward primer: AGGCGCGCCGTACGAAGcttAGACGAGCACATTGTCTAC

Reverse primer: AAAATCCTTGGAATATTtAAGcttCGACAAAACTCACACA

The *efn-1* and *efn-4* cDNAs PCR products were inserted into *Hind*III - linearized pMH180 using Gibson assembly (New England BioLabs), according to the manufacturer's protocol. All expression plasmids were sequenced then validated by transient transfection into 293T cells and Western blotting 48 hours later, using the anti-myc 9E10 antibody to detect the Myc tags.