

Ferritin coupling location	Other features	Survival	Calcium imaging
C-terminus ( $\Delta$ 760-871)	N/A	Yes	Yes
N-terminus ( $\Delta$ 1-201)	N/A	Yes	No
C-terminus (full channel)	N/A	Yes	No
S4/5 loop: YFTRGLKLTG...ferritin...YSIMIQKILF	"Arg" linker 1X	No	
	"Gly" linker 1X	No	
	"Arg" linker 2X	No	
	"Gly" linker 2X	No	
	"Arg" linker 3X	No	
	"Gly" linker 3X	No	
	"Arg" linker 4X	No	
	"Gly" linker 4X	No	
	"Arg" linker 5X	No	
	"Gly" linker 5X	No	
	"Arg" linker 6X	No	
	"Gly" linker 6X	No	
	"Arg" linker 7X	No	
	"Gly" linker 7X	No	
	"Arg" linker 8X	No	
	"Gly" linker 8X	No	
	"Arg" linker 9X	No	
	"Gly" linker 9X	No	

**\*Arg linker: TRPV4-RRLLSGCP...ferritin...RPRERRRRLRR-TRPV4**

**\*Gly linker: TRPV4-RRLLSGCP...ferritin...RGGGGGSGGY-TRPV4**

### Supplementary Table 1: Description of TRPV4-ferritin fusion proteins tested

For insertions into the TRPV4 S4-S5 intracellular loop, the amino acid sequence of TRPV4 precedes and succeeds the intervening ferritin protein in the “ferritin coupling location” column. “Arg” and “Gly” are separate linkers used to expand the S4/S5 intracellular loop where ferritin was inserted and refer to the predominant amino acids expressed on the C-terminus of ferritin reconnecting to the S5 transmembrane domain of TRPV4. Specific amino acid sequences for the linkers used to expand the S4-S5 intracellular loop are shown at the bottom, which were sequentially inserted (e.g. 1X, 2X, 3X, etc.). The “Survival” column indicates whether cells survived following transfection as measured by expression of the mCherry gene under control of an IRES cassette. The “Calcium imaging” column refers to whether calcium transients were detectable following stimulation with magnetic fields.