

Construct	Purpose for developing construct	Outcome
hMRAP α	Functional comparison of untagged and tagged hMRAP α	Good for functional testing
hMRAP α -FLAG	Fixed confocal microscopy analysis of hMRAP α subcellular localisation	Good for functional testing and fixed confocal microscopy
hMRAP α -FLAG-FLN	Fixed confocal microscopy to determine that hMRAP α -FLN subcellular localisation is similar to hMRAP α -FLAG	Good for fixed confocal microscopy
hMRAP α -FLN	Dual biarsenical imaging of hMRAP α and hMC4R	Good for functional testing but not for biarsenical imaging
hMC4R	Functional comparison of untagged and tagged hMC4R	Good for functional testing
HA-hMC4R	Fixed confocal microscopy analysis of hMC4R subcellular localisation	Good for functional testing and fixed confocal microscopy
hMC4R-PG	Dual biarsenical imaging of hMC4R and hMRAP α	Not good for functional testing nor for biarsenical imaging
hMC4R-eGFP	Live cell imaging of hMC4R	Good for functional testing and live cell imaging although subcellular localisation differs from HA-hMC4R
eGFP-hMC4R	Live cell imaging of hMC4R	Not good for functional testing

S1 Table: Summary of constructs, their purpose and outcomes of functional testing.