A novel podocyte phenotype of TRPC6 KO mice and the importance of the protein interaction between TRPC6 and calpain in health and disease.

Background  
Mutations in TRPC6 have been shown to cause FSGS and this was thought to be due to an increased calcium conductance of this membrane channel. However, several disease-causing mutations of TRPC6 have been identified which have decreased calcium conductance and indeed some which show no change at all suggesting that there are other mechanisms for the disease pathogenesis of these mutations. We have established a cohort of TRPC6 knockout mice and have used these to develop TRPC6 knockout podocytes. We have used these unique resources to study the effect of TRPC6 knockout both in vivo and in vitro and to identify novel binding partners of TRPC6 using proteomic techniques.

Methods  
A novel ex vivo glomerular albumin permeability assay was performed on young (12-16 weeks) and old (10-14 month) WT and TRPC6 KO C57Bl/6 mice. Kidneys were harvested from these mice and electron microscopy (EM) performed. Conditionally immortalised podocyte cell lines were generated from the TRPC6 KO mice. GFP tagged WT/disease causing TRPC6 mutants were stably reintroduced into the KO cell line using lentiviral transduction. Cell lines were then characterised to determine motility and adhesion using scratch and adhesion assays. GFP TRAP beads were used to pull down GFP TRPC6 and novel binding partners identified by mass spectrometry and confirmed through immunoprecipitation. Calpain assays were performed using a commercially available kit. TIRF, confocal and electron microscopy were done at the Wolfson bioimaging facility, University of Bristol.

Results  
Although, as previously reported, the TRPC6 knockout mice had no increase in albuminuria we found that aged TRPC6 KO mice (10-14 months) had a significantly increased glomerular albumin permeability compared to age matched control mice. EM of the kidneys of these animals demonstrated an increased foot process width and glomerular basement membrane (GBM) thickness in both young (12-14 weeks) and old TRPC6 KO mice (10-14 months) compared to age matched controls.

TRPC6 KO (T6K) podocytes developed from these animals are less motile and more adhesive than wild type podocytes and knockout cells into which WT TRPC6 had been reintroduced. Calpain 1 and 2, ERK 1/2 and caldesmon were identified as novel TRPC6 binding partners using GFP-TRAP, proteomics and these interactions were verified through co-immunoprecipitation of endogenous proteins. Calpain is a protease with targets including focal adhesion kinase (FAK), talin-1 and caldesmon. T6K cells have decreased protein cleavage compared to cells containing WT TRPC6. There was a loss of calpain activity in T6K cells, suggesting that TRPC6 is responsible for calpain activation. The disease causing mutant form of TRPC6, K874\*, has normal calcium conductance, however cells expressing this mutant also have decreased calpain activity and decreased cleavage of calpain targeted proteins. Co-IP experiments have shown that TRPC6 K874\* does not bind to calpain. Confocal and TIRF microscopy showed a loss of calpain from the membrane in T6K and K874\* podocytes compared to WT.

Conclusions  
TRPC6 KO mice have altered glomerular structure and increased glomerular albumin permeability compared to WT mice. TRPC6 mediated activation of calpain plays an important role in podocyte motility and detachment. This is achieved through a physical interaction between TRPC6 and calpain, which keeps calpain anchored at the membrane. Loss of this interaction leads to podocytes which have increased adhesion and decreased motility.