

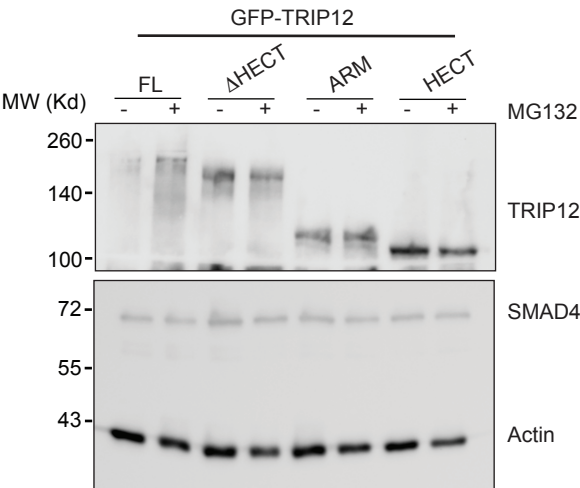
**Control of TGF β signalling by ubiquitination independent function of
E3 ubiquitin ligase TRIP12**

Kripa S. Keyan et al

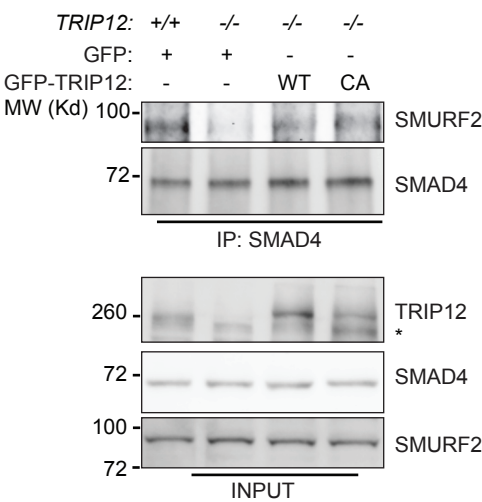
Supplementary Material (Supp Figures 1 to 5)

Supplementary Fig 1

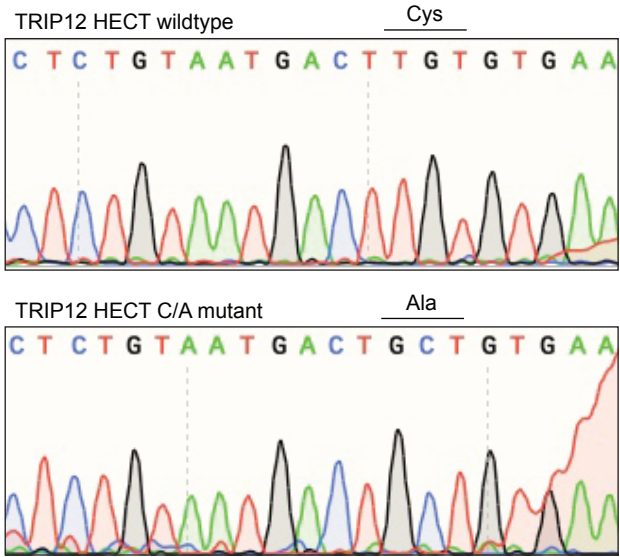
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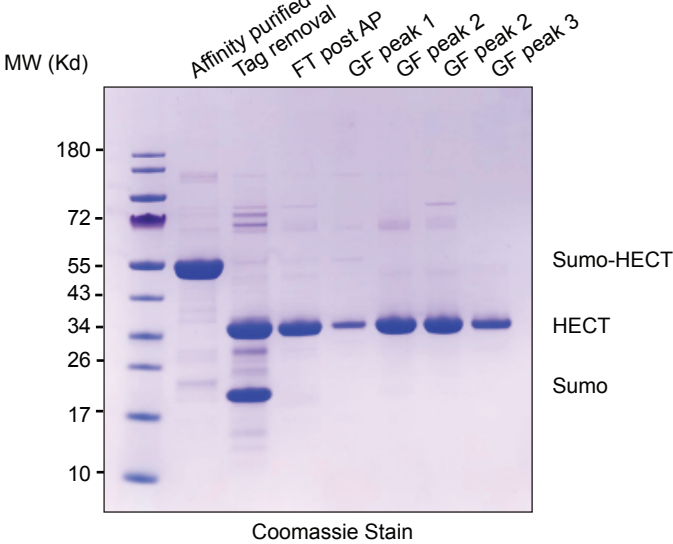
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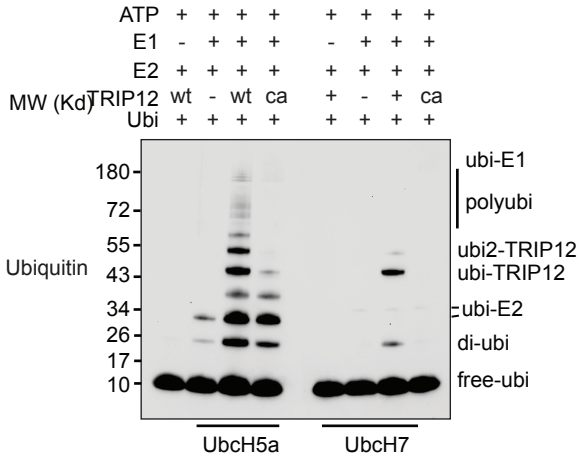
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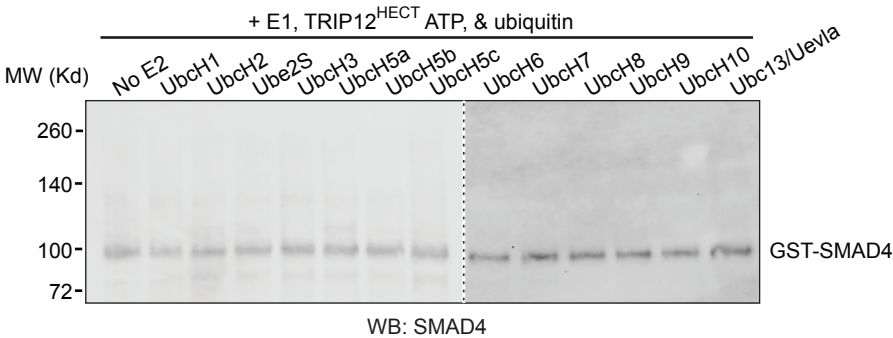
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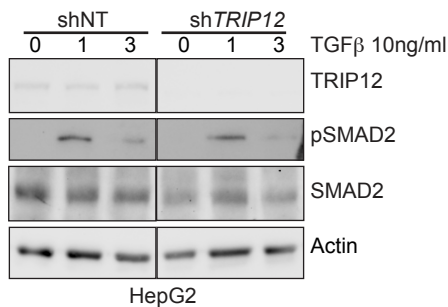


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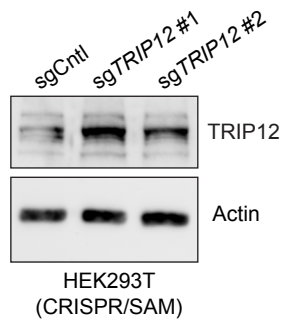


Supplementary Fig 2

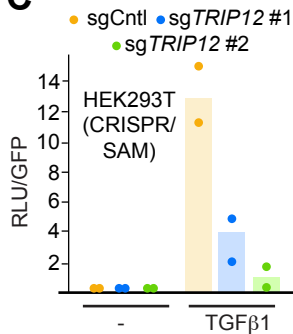
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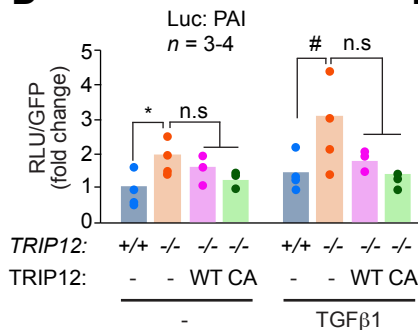
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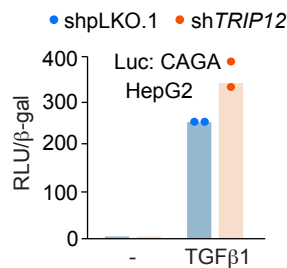
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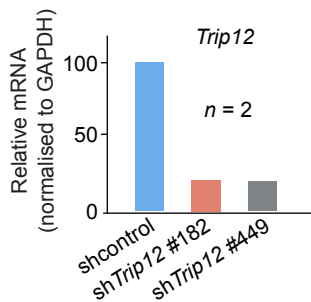
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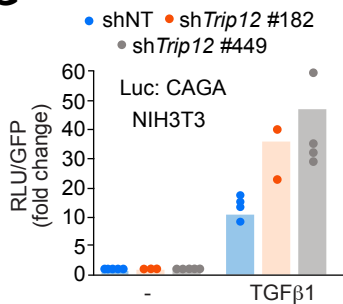
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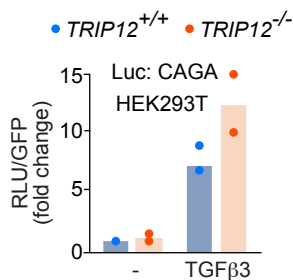
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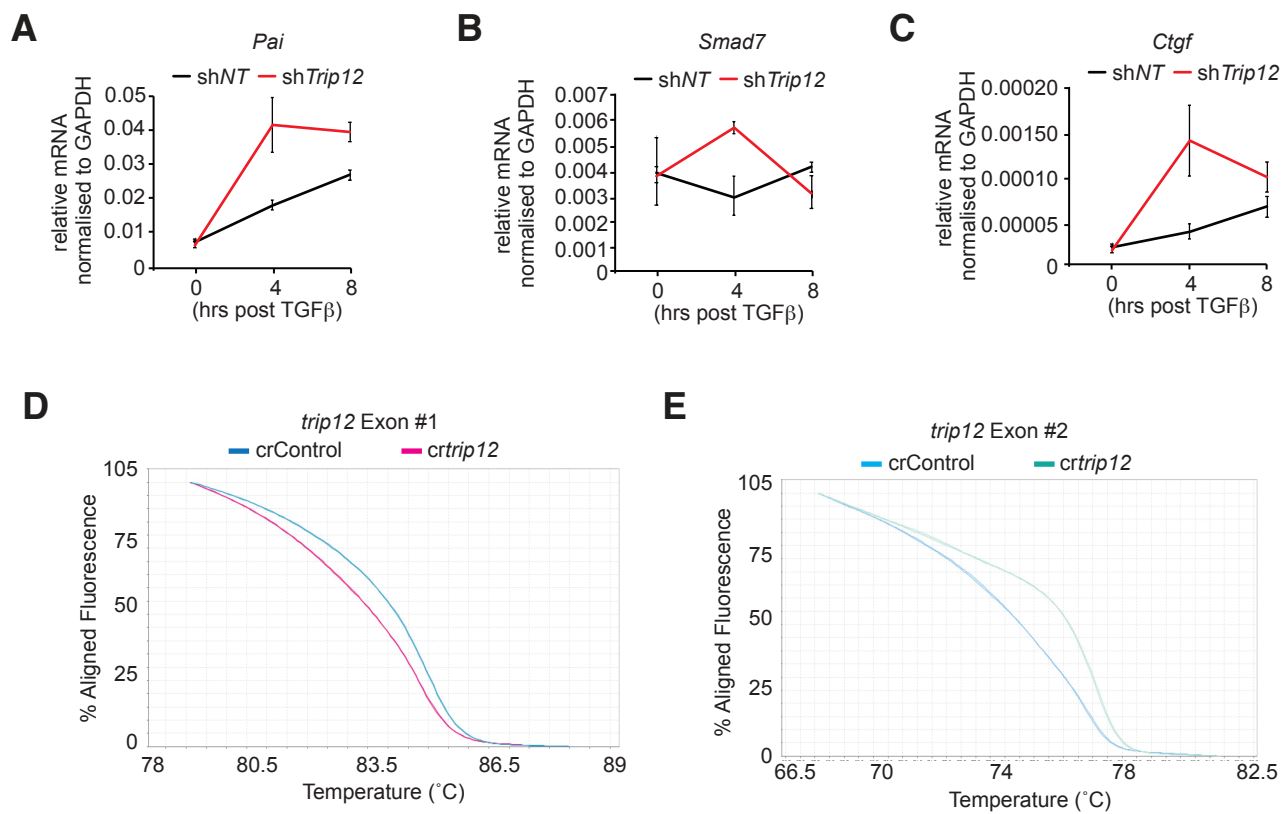
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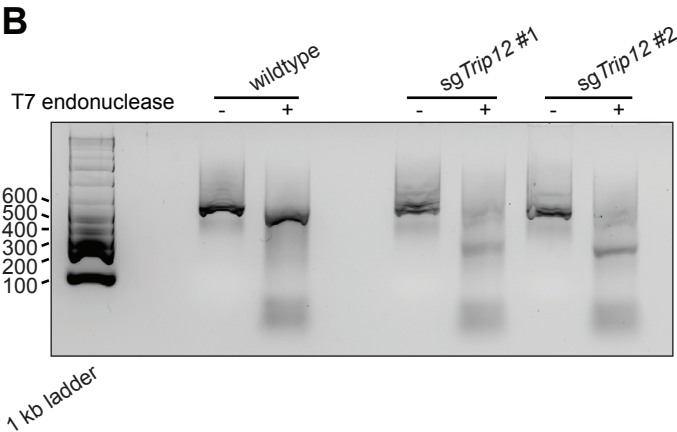
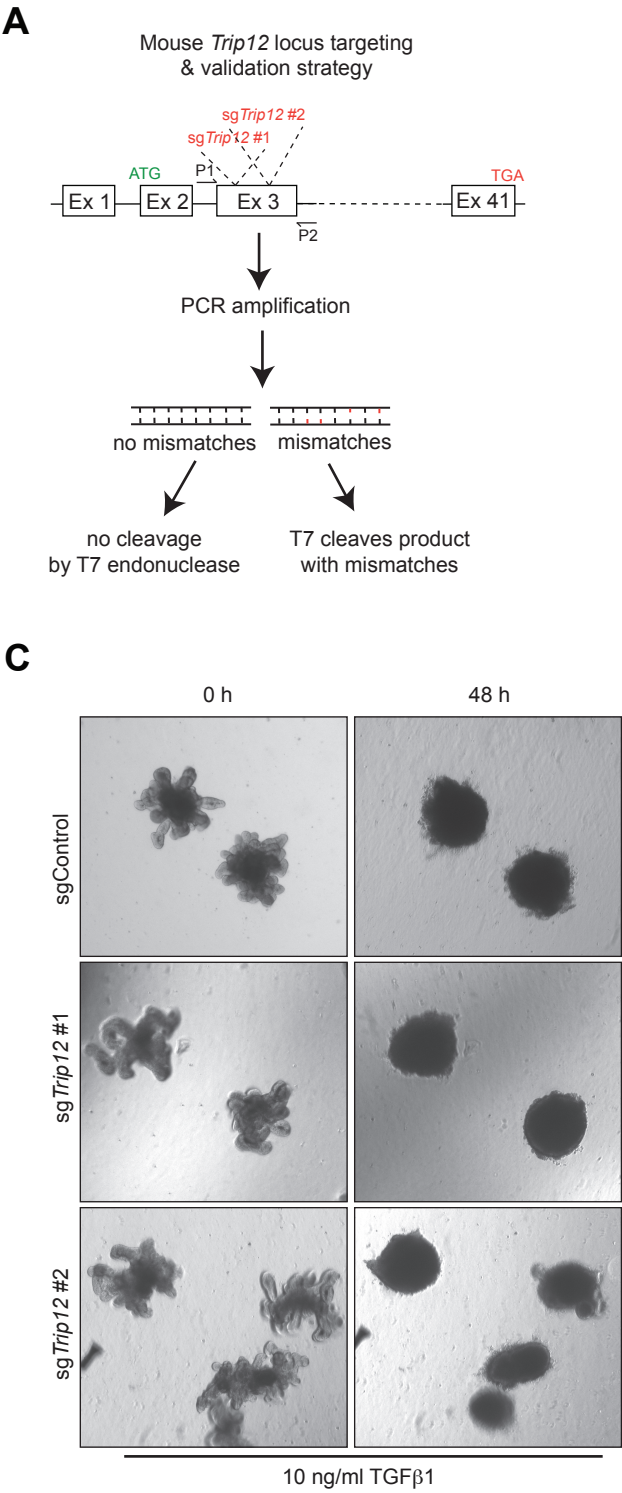
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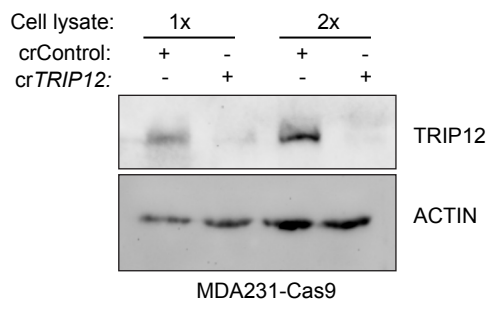
Supplementary Fig 3



Supplementary Fig 4



Supplementary Fig 5



Supplementary Figure Legends

Supplementary Figure 1 (Related to Figure 2). **A**, Western blot showing endogenous SMAD4 levels in HEK293T cells overexpressing indicated constructs. **B**, SMAD4 IP followed by Western blots against indicated proteins. Lower panel shows Western blots against indicated proteins in 10 % input. **C**, Sanger sequencing validation of TRIP12 wildtype and C1959A construct. **D**, Coomassie confirmation of bacterial overexpression of TRIP12 HECT domain. **E**, *In vitro* ubiquitination assay in presence of indicated reagents. **F**, *In vitro* GST-SMAD4 ubiquitination assay in presence of indicated reagents.

Supplementary Figure 2 (Related to Figure 3). **A**, Western blot showing endogenous p-SMAD2 levels in HepG2 cells expressing indicated shRNAs. **B**, Western blot showing endogenous TRIP12 levels in HEK293T cells stably expressing CRISPR SAM sgcontrol (Cntl) or sg*TRIP12*. **C**, Luc:CAGA transcriptional reporter assay in HEK293T cells stably expressing CRISPR SAM sgcontrol or sg*TRIP12*. **D**, TGF β Luc:PAI transcriptional reporter assay performed in presence or absence of indicated TRIP12 plasmids. **E**, Luc:CAGA transcriptional reporter assay in HepG2 cells expressing indicated shRNAs. **F**, qRT-PCR validation of *Trip12* knockdown expressing indicated shRNA in NIH3T3 cells. **G**, Luc:CAGA reporter assay in NIH3T3 cells expressing indicated shRNAs. **H**, Luc:CAGA transcriptional reporter assay in HEK293T cells with indicated genotypes in response to TGF β 3.

Supplementary Figure 3 (Related to Figure 4). **A–C**, qRT-PCR on indicated genes from NIH3T3 cells expressing a control and sh*Trip12*, bars are standard deviation. **D**, High-resolution melting (HRM) validation of *trip12* exon 1 genetic manipulation in zebrafish embryos **E**, High-resolution melting (HRM) validation of *trip12* exon 2 genetic manipulation in zebrafish embryos.

Supplementary Figure 4 (Related to Figure 7). **A**, CRISPR/Cas9 strategy for targeting mouse *Trip12* gene in intestinal organoids. **B**, T7 restriction endonuclease validation of *Trip12* locus manipulation in intestinal organoids expressing indicated sgRNAs. **C**, Bright field images of intestinal organoids with indicated genotypes and treated with 10 ng/ml TGF β for 48 h.

Supplementary Figure 5 (Related to Figure 8). Western blot validation of *TRIP12* knockout in MDA-MB-231 cr*TRIP12* targeted cells.