Figure S1

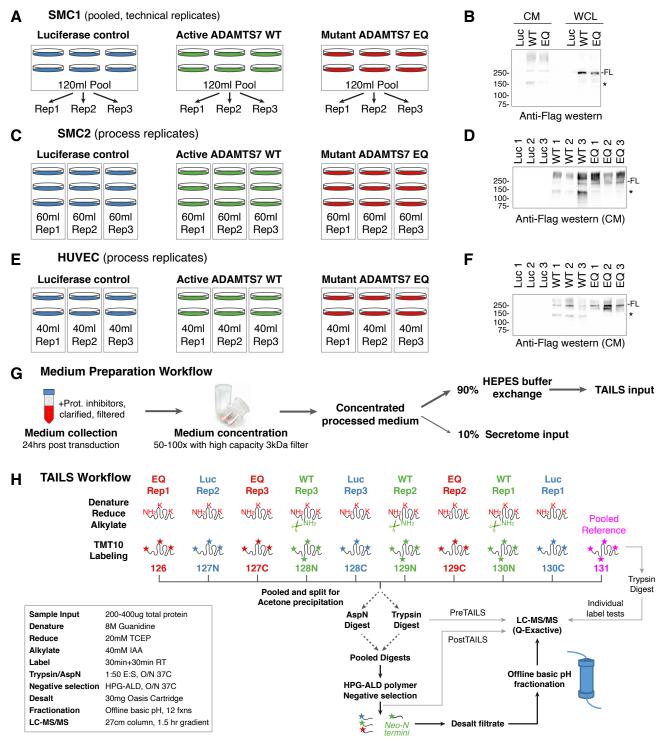


Figure S1 Proteomics sample input, medium processing and TAILS workflow. Medium collected from human coronary artery smooth muscle cells (SMC) or human umbilical vein endothelial cells (HUVEC) expressing control Luciferase (Luc), active mouse ADAMTS7 (WT) or catalytic mutant mouse ADAMTS7 E373Q (EQ) from three separate experiments. 20ml of medium collected from each 15cm tissue culture dish. *A*, SMC1 media was pooled for each condition and split into technical replicates after concentration to generate technical replicates. *B*, expression of full-length (FL) ADAMTS7-3xFLAG constructs was verified in the conditioned medium (CM) and whole cell lysate (WCL) by direct anti-Flag HRP western blot detection. * indicates mucin domain cleaved degradation product detected by c-terminal Flag tags. *C*, Replicates from SMC2 were collected from 3 dishes and processed separately. *D*, expression in the medium from SMC2 replicates was verified by western blot. *E*, Replicates from HUVEC were collected from 2 dishes and processed separately. *F*, expression in the medium from HUVEC replicates was verified by western blot. *G*, medium preparation workflow for each replicate to generate input for total secretome and TAILS proteomics experiments. *H*, sample processing for TMT10 TAILS proteomics to identify neo-N-termini from the active ADAMTS7 enzyme condition. SMC1 TAILS experiment was digested with Trypsin only. SMC2 and HUVEC were digested with AspN or Trypsin before negative selection.