SUPPLEMENTARY INFORMATION

Piezo1 integration of vascular architecture with physiological force

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VIDEO LEGENDS

Video S1| *In vivo* ultrasound to detect E9.5 embryonic heart beat. Wild-type embryo (EC-wt.). The embryo is on the left.

Video S2| *In vivo* ultrasound to detect E9.5 embryonic heart beat. Embryo with endothelial-specific deletion in *Piezo1* (EC-del.). The embryo is on the right.

Relevant peptides that were significantly different (P<0.05) in P1.si.1 compared with sc.si. in HUVECs	
Peptides were detected by mass spectrometry after titanium dioxide trapping	
Static condition	
Peptide sequence	**Protein**
KLQGRPSPGPPAPEQLLSQAR	Tax_Id=9606 Gene_Symbol=NOS3 Nitric oxide synthase, endothelial
KESSNTDSAGALGTLR	Tax_Id=9606 Gene_Symbol=NOS3 Nitric oxide synthase, endothelial
Shear stress condition (orbital shaker)	
PSPGPPAPEQLLSQAR	Nitric oxide synthase, endothelial n=2 Tax=Homo sapiens RepID=NOS3_HUMAN
TYVQDILR	Nitric oxide synthase, endothelial n=2 Tax=Homo sapiens RepID=NOS3_HUMAN
VEDPPAPTEPVAVEQLEK	Nitric oxide synthase, endothelial n=2 Tax=Homo sapiens RepID=NOS3_HUMAN
AQSYAQQLGR	Nitric oxide synthase, endothelial n=2 Tax=Homo sapiens RepID=NOS3_HUMAN
LLQLGQGDELCGQEEAFR	Nitric oxide synthase, endothelial n=2 Tax=Homo sapiens RepID=NOS3_HUMAN
ESSNTDSAGALGTLR	Nitric oxide synthase, endothelial n=2 Tax=Homo sapiens RepID=NOS3_HUMAN
EQQELEALSQDPR	Nitric oxide synthase, endothelial n=2 Tax=Homo sapiens RepID=NOS3_HUMAN
APASLLPPAPEHSPPSSPLTQPPEGPK	Nitric oxide synthase, endothelial n=2 Tax=Homo sapiens RepID=NOS3_HUMAN
TELAAEVHR	Nitric oxide synthase, endothelial n=2 Tax=Homo sapiens RepID=NOS3_HUMAN
SVAQEPGPPCGLGLGLGLGLCGK	Nitric oxide synthase, endothelial n=2 Tax=Homo sapiens RepID=NOS3_HUMAN
LPPDPSLPCILVGPGTGIAPFR	Nitric oxide synthase, endothelial n=2 Tax=Homo sapiens RepID=NOS3_HUMAN

Table S1| Differential eNOS peptides identified by proteomics in HUVECs. Shown are specific peptides detected by mass spectrometry as significantly different between three populations of HUVECs transfected with control siRNA (sc.si.) and three populations of HUVECs transfected with Piezo1 siRNA (P1.si.1). The experiment was repeated in static (upper) and shear stress conditions on the orbital shaker (lower).

Displayed are calpain-substrate and calpain peptides that were significantly different (P<0.05) in Piezo1-/- compared with Piezo1+/+ at E10.5
Peptides were detected by mass spectrometry after titanium dioxide trapping

Peptide	Protein
ETADTDTADQVMASFK	Alpha-actinin-1 OS=Mus musculus GN=Actn1 PE=1 SV=1
VLAVNQENEQLMEDYEK	Alpha-actinin-1 OS=Mus musculus GN=Actn1 PE=1 SV=1
HRPELIDYGK	Alpha-actinin-1 OS=Mus musculus GN=Actn1 PE=1 SV=1
KDDPLTNLNTAFDVAER	Alpha-actinin-1 OS=Mus musculus GN=Actn1 PE=1 SV=1
GISQEQMNEFR	Alpha-actinin-1 OS=Mus musculus GN=Actn1 PE=1 SV=1
RDQALTEEHAR	Alpha-actinin-1 OS=Mus musculus GN=Actn1 PE=1 SV=1
AGTQIENIEEDFR	Alpha-actinin-1 OS=Mus musculus GN=Actn1 PE=1 SV=1
ILAGDKNYITEDELR	Alpha-actinin-1 OS=Mus musculus GN=Actn1 PE=1 SV=1
CQLEINFNTLQTK	Alpha-actinin-1 OS=Mus musculus GN=Actn1 PE=1 SV=1
ICDQWDNLGALTQK	Alpha-actinin-1 OS=Mus musculus GN=Actn1 PE=1 SV=1
TINEVENQILTR	Alpha-actinin-1 OS=Mus musculus GN=Actn1 PE=1 SV=1
AGTQIENIEEDFR	Actinin alpha 2 n=1 Tax=Mus musculus RepID=Q8K3Q4_MOUSE
CQLEINFNTLQTK	Actinin alpha 2 n=1 Tax=Mus musculus RepID=Q8K3Q4_MOUSE
ISSSNPYSTVTMDELR	Actinin alpha 2 n=1 Tax=Mus musculus RepID=Q8K3Q4_MOUSE
MLDAEDIVNTARPDEK	Alpha-actinin-4 n=4 Tax=Mus musculus RepID=ACTN4_MOUSE
QGDAEFNR	Alpha-actinin-4 n=4 Tax=Mus musculus RepID=ACTN4_MOUSE
KDDPVTNLNNAFEVAEK	Alpha-actinin-4 n=4 Tax=Mus musculus RepID=ACTN4_MOUSE
ETTDTDTADQVIASFK	Alpha-actinin-4 n=4 Tax=Mus musculus RepID=ACTN4_MOUSE
LSGSNPYTTVTPQIINSK	Alpha-actinin-4 n=4 Tax=Mus musculus RepID=ACTN4_MOUSE
VQQLVPK	Alpha-actinin-4 n=4 Tax=Mus musculus RepID=ACTN4_MOUSE
ICDQWDNLGSLTHSR	Alpha-actinin-4 n=4 Tax=Mus musculus RepID=ACTN4_MOUSE
GISQEQMQEFR	Alpha-actinin-4 n=4 Tax=Mus musculus RepID=ACTN4_MOUSE
RDHALLEEQSK	Alpha-actinin-4 n=4 Tax=Mus musculus RepID=ACTN4_MOUSE
VEQIAAIAQELNELDYYDSHNVNTR	Alpha-actinin-4 n=4 Tax=Mus musculus RepID=ACTN4_MOUSE
CQLEINFNTLQTK	Alpha-actinin-4 n=4 Tax=Mus musculus RepID=ACTN4_MOUSE
MAPYQGPDAAPGALDYK	Alpha-actinin-4 n=4 Tax=Mus musculus RepID=ACTN4_MOUSE
HRPELIEYDK	Alpha-actinin-4 n=4 Tax=Mus musculus RepID=ACTN4_MOUSE
QLETIDQLHLEYAK	Alpha-actinin-4 n=4 Tax=Mus musculus RepID=ACTN4_MOUSE
VLAGDKNFITAEELR	Alpha-actinin-4 n=4 Tax=Mus musculus RepID=ACTN4_MOUSE
TINEVENQILTR	Alpha-actinin-4 n=4 Tax=Mus musculus RepID=ACTN4_MOUSE
KAGTQIENIDEDFRDGLK	Alpha-actinin-4 n=4 Tax=Mus musculus RepID=ACTN4_MOUSE
MDCQECPEGYR	Filamin, alpha OS=Mus musculus GN=Flna PE=4 SV=1
VTAQGPGLEPSGNIANK	Filamin, alpha OS=Mus musculus GN=Flna PE=4 SV=1
VATVPQHATSGPGPADVSK	Filamin, alpha OS=Mus musculus GN=Flna PE=4 SV=1
EEGPYEVEVTYDGVPVPGSPFPLEAVAPTKPSK	Filamin, alpha OS=Mus musculus GN=Flna PE=4 SV=1
VQVQDNEGCSVEATVK	Filamin, alpha OS=Mus musculus GN=Flna PE=4 SV=1
VHSPSGALEECYVTEIDQDKYAVR	Filamin, alpha OS=Mus musculus GN=Flna PE=4 SV=1
LVSIDSK	Filamin, alpha OS=Mus musculus GN=Flna PE=4 SV=1
SPFEVYVDK	Filamin, alpha OS=Mus musculus GN=Flna PE=4 SV=1
AEISFEDR	Filamin, alpha OS=Mus musculus GN=Flna PE=4 SV=1
AFGPGLQGGNAGSPAR	Filamin, alpha OS=Mus musculus GN=Flna PE=4 SV=1
SAGQGEVLVYVEDPAGHQEEAK	Filamin, alpha OS=Mus musculus GN=Flna PE=4 SV=1
IPEISIQDMTAQVTSPSGK	Filamin, alpha OS=Mus musculus GN=Flna PE=4 SV=1
VANPSGNLTDTYVQDCGDGTYK	Filamin, alpha OS=Mus musculus GN=Flna PE=4 SV=1
SPYTVTVGQACNPAACR	Filamin, alpha OS=Mus musculus GN=Flna PE=4 SV=1
IVSPSGAAVPCK	Filamin, alpha OS=Mus musculus GN=Flna PE=4 SV=1
IANLQTDLSDGLR	Filamin, alpha OS=Mus musculus GN=Flna PE=4 SV=1
AWGPGLEGGIVGK	Filamin, alpha OS=Mus musculus GN=Flna PE=4 SV=1
ALGALVDSCAPGLCPDWDSWDASKPVNNAR	Filamin, alpha OS=Mus musculus GN=Flna PE=4 SV=1
VEPGLGADNSVVR	Filamin, alpha OS=Mus musculus GN=Flna PE=4 SV=1
DGSCGVAYVVQEPGDYEVSVK	Filamin, alpha OS=Mus musculus GN=Flna PE=4 SV=1
ANLPQSFQVDTSK	Filamin, alpha OS=Mus musculus GN=Flna PE=4 SV=1
GTVEPQLEAR	Filamin, alpha OS=Mus musculus GN=Flna PE=4 SV=1
DAGEGGLSLAIEGPSK	Filamin, alpha OS=Mus musculus GN=Flna PE=4 SV=1
VPVHDVTDASK	Filamin, alpha OS=Mus musculus GN=Flna PE=4 SV=1
VTYTPMAPGSYLISIK	Filamin, alpha OS=Mus musculus GN=Flna PE=4 SV=1
IQQNTFTR	Filamin, alpha OS=Mus musculus GN=Flna PE=4 SV=1
RLTVSSLQESGLK	Filamin, alpha OS=Mus musculus GN=Flna PE=4 SV=1
AGNNMLLVGVHGPR	Filamin, alpha OS=Mus musculus GN=Flna PE=4 SV=1
YGGPYHIGGSPFK	Filamin, alpha OS=Mus musculus GN=Flna PE=4 SV=1
SAGQGEVLVYVEDPAGHQEEAK	Filamin, alpha OS=Mus musculus GN=Flna PE=4 SV=1
LDVQFSGLAK	Filamin, alpha OS=Mus musculus GN=Flna PE=4 SV=1
EAGAGGLAIAVEGPSK	Filamin, alpha OS=Mus musculus GN=Flna PE=4 SV=1
NDNDTFTVK	Filamin, alpha OS=Mus musculus GN=Flna PE=4 SV=1
DGSCSVEYIPYEAGTYSLNVTYGGHQVPGSPFK	Filamin, alpha OS=Mus musculus GN=Flna PE=4 SV=1

Table S2

Displayed are calpain-substrate and calpain peptides that were significantly different (P<0.05) in Piezo1-/- compared with Piezo1+/+ at E10.5	
VLFASQEIPASPFR	Filamin-B n=2 Tax=Mus musculus RepID=FLNB_MOUSE
IEYDDQNDGSCDVK	Filamin-B n=2 Tax=Mus musculus RepID=FLNB_MOUSE
APAVATVGSICDLNLK	Filamin-B n=2 Tax=Mus musculus RepID=FLNB_MOUSE
SGCTINNPAEFIVDPK	Filamin-B n=2 Tax=Mus musculus RepID=FLNB_MOUSE
GAGIGGLGITVEGPSESK	Filamin-B n=2 Tax=Mus musculus RepID=FLNB_MOUSE
IGNLQTDLSDGLR	Filamin-B n=2 Tax=Mus musculus RepID=FLNB_MOUSE
AQITNPSGASTECFVK	Filamin-B n=2 Tax=Mus musculus RepID=FLNB_MOUSE
VGEPGILCVDCSEAGPGTLGLEAVSDSGAK	Filamin-B n=2 Tax=Mus musculus RepID=FLNB_MOUSE
SPFEVQVGPEAGMQK	Filamin-B n=2 Tax=Mus musculus RepID=FLNB_MOUSE
LVSPGSANETSSILVESVTR	Filamin-B n=2 Tax=Mus musculus RepID=FLNB_MOUSE
ILAQDGEGQPIDIQMK	Filamin-B n=2 Tax=Mus musculus RepID=FLNB_MOUSE
LSPEELLLR	Plastin-3 OS=Mus musculus GN=Pls3 PE=2 SV=1
QFVTPADVVSGNPK	Plastin-3 OS=Mus musculus GN=Pls3 PE=2 SV=1
YTLNVLEDLGEGQK	Plastin-3 OS=Mus musculus GN=Pls3 PE=2 SV=1
AVAGNISDPGLQK	Vinculin n=1 Tax=Mus musculus RepID=VINC_MOUSE
ALASQLQDSLK	Vinculin n=1 Tax=Mus musculus RepID=VINC_MOUSE
MTGLVDEAIDTK	Vinculin n=1 Tax=Mus musculus RepID=VINC_MOUSE
VAMANIQPQMLVAGATSIAR	Vinculin n=1 Tax=Mus musculus RepID=VINC_MOUSE
QVATALQNLQTK	Vinculin n=1 Tax=Mus musculus RepID=VINC_MOUSE
TNISDEESEQATEMLVHNAQNLMQSVK	Vinculin n=1 Tax=Mus musculus RepID=VINC_MOUSE
MSAEINEIIR	Vinculin n=1 Tax=Mus musculus RepID=VINC_MOUSE
LLAVAATAPPDAPNREEVFDER	Vinculin n=1 Tax=Mus musculus RepID=VINC_MOUSE
SLGEIAALTSK	Vinculin n=1 Tax=Mus musculus RepID=VINC_MOUSE
TNLLQVCER	Vinculin n=1 Tax=Mus musculus RepID=VINC_MOUSE
QNLLQAAGNVGQASGELLQQIGESDTDPHFQDVLMQLAK	Talin-1 n=2 Tax=Mus musculus RepID=TLN1_MOUSE
VGAIPANALDDGQWSQGLISAAR	Talin-1 n=2 Tax=Mus musculus RepID=TLN1_MOUSE
AGALQCSPSDVYTK	Talin-1 n=2 Tax=Mus musculus RepID=TLN1_MOUSE
TMQFEPSTMVYDACR	Talin-1 n=2 Tax=Mus musculus RepID=TLN1_MOUSE
ALDGDFTEENR	Talin-1 n=2 Tax=Mus musculus RepID=TLN1_MOUSE
ASAGPQPLLVQSCK	Talin-1 n=2 Tax=Mus musculus RepID=TLN1_MOUSE
AVASAAAALVLK	Talin-1 n=2 Tax=Mus musculus RepID=TLN1_MOUSE
AQEACGPLEMDSALSVVQNLEK	Talin-1 n=2 Tax=Mus musculus RepID=TLN1_MOUSE
VVAPTISSPVCQEQLVEAGR	Talin-1 n=2 Tax=Mus musculus RepID=TLN1_MOUSE
TMLESAGGLIQTAR	Talin-1 n=2 Tax=Mus musculus RepID=TLN1_MOUSE
LAQAAQSSVATITR	Talin-1 n=2 Tax=Mus musculus RepID=TLN1_MOUSE
MVAAATNNLCEAANAAVQGHASQEK	Talin-1 n=2 Tax=Mus musculus RepID=TLN1_MOUSE
GLAGAVSELLR	Talin-1 n=2 Tax=Mus musculus RepID=TLN1_MOUSE
LGAASLGAEDPETQVVLINAVK	Talin-1 n=2 Tax=Mus musculus RepID=TLN1_MOUSE
VGDDPAVWQLK	Talin-1 n=2 Tax=Mus musculus RepID=TLN1_MOUSE
SNTSPEELGPLANQLTSDYGR	Talin-1 n=2 Tax=Mus musculus RepID=TLN1_MOUSE
AVTQALNR	Talin-1 n=2 Tax=Mus musculus RepID=TLN1_MOUSE
VSQMAQYFEPLTLAAVGAASK	Talin-1 n=2 Tax=Mus musculus RepID=TLN1_MOUSE
EADESLNFEEQILEAAK	Talin-1 n=2 Tax=Mus musculus RepID=TLN1_MOUSE
ASVPTIQDQASAMQLSQCAK	Talin-1 n=2 Tax=Mus musculus RepID=TLN1_MOUSE
VSQMAQYFEPLTLAAVGAASK	Talin-1 n=2 Tax=Mus musculus RepID=TLN1_MOUSE
ERIPEALAGPPNDFGLFLSDDDPK	Talin-1 n=2 Tax=Mus musculus RepID=TLN1_MOUSE
LNEAAAGLNQAATELVQASR	Talin-1 n=2 Tax=Mus musculus RepID=TLN1_MOUSE
SKDHFGLEGDEESTMLEDSVSPK	Talin-1 n=2 Tax=Mus musculus RepID=TLN1_MOUSE
LLGEIAQGNENYAGIAAR	Talin-1 n=2 Tax=Mus musculus RepID=TLN1_MOUSE
GDVTAEEAAGASPAK	MARCKS-related protein n=1 Tax=Mus musculus RepID=MRP_MOUSE
GEGESPPVNGTDEAAGATGDAIEPAPPSQEAEAK	MARCKS-related protein n=1 Tax=Mus musculus RepID=MRP_MOUSE
AAATPESQEPQAK	MARCKS-related protein n=1 Tax=Mus musculus RepID=MRP_MOUSE
LQAFLQDLDDFK	Spectrin beta 1 n=2 Tax=Mus musculus RepID=B2RX08_MOUSE
MLTAQDMSYDEAR	Spectrin beta chain, non-erythrocytic 1 n=5 Tax=Mus musculus RepID=SPTB2_MOUSE
AELFTQSCADLDK	Spectrin beta chain, non-erythrocytic 1 n=5 Tax=Mus musculus RepID=SPTB2_MOUSE
VQAVVAVAR	Spectrin beta chain, non-erythrocytic 1 n=5 Tax=Mus musculus RepID=SPTB2_MOUSE
TQTAIASEDMPNTLTEAEK	Spectrin beta chain, non-erythrocytic 1 n=5 Tax=Mus musculus RepID=SPTB2_MOUSE
VIESTQDLGNDLAGVMALQR	Spectrin beta chain, non-erythrocytic 1 n=5 Tax=Mus musculus RepID=SPTB2_MOUSE
EAEKLESEHPDQAQAILSR	Spectrin beta chain, non-erythrocytic 1 n=5 Tax=Mus musculus RepID=SPTB2_MOUSE
HRPDLIDFDK	Spectrin beta chain, non-erythrocytic 1 n=5 Tax=Mus musculus RepID=SPTB2_MOUSE
VLDNAIETEK	Spectrin beta chain, non-erythrocytic 1 n=5 Tax=Mus musculus RepID=SPTB2_MOUSE
LVSQDNFGFDLPAVEAATK	Spectrin beta chain, non-erythrocytic 1 n=5 Tax=Mus musculus RepID=SPTB2_MOUSE
LAEISDVWEEMK	Spectrin beta chain, non-erythrocytic 1 n=5 Tax=Mus musculus RepID=SPTB2_MOUSE
ITDLYTDLR	Spectrin beta chain, non-erythrocytic 1 n=5 Tax=Mus musculus RepID=SPTB2_MOUSE
EVDDLEQWIAER	Spectrin beta chain, non-erythrocytic 1 n=5 Tax=Mus musculus RepID=SPTB2_MOUSE

Table S2

Displayed are calpain-substrate and calpain peptides that were significantly different (P<0.05) in Piezo1-/- compared with Piezo1+/+ at E10.5	
LTTPTYGDLNHLVSATMSGVTTCLR	Tubulin beta-2A chain OS=Mus musculus GN=Tubb2a PE=1 SV=1
GHYTEGAELVDSVLDVVR	Tubulin beta-2A chain OS=Mus musculus GN=Tubb2a PE=1 SV=1
INVYYNEAAGNK	Tubulin beta-2A chain OS=Mus musculus GN=Tubb2a PE=1 SV=1
TAVCDIPPR	Tubulin beta-2A chain OS=Mus musculus GN=Tubb2a PE=1 SV=1
EVDEQMLNVQNK	Tubulin beta-2A chain OS=Mus musculus GN=Tubb2a PE=1 SV=1
ESESCDCLQGFQLTHSLGGGTGSGMGTLLISK	Tubulin beta-2A chain OS=Mus musculus GN=Tubb2a PE=1 SV=1
IMNTFSVMPSPK	Tubulin beta-2A chain OS=Mus musculus GN=Tubb2a PE=1 SV=1
EIVHIQAGQCGNQIGAK	Tubulin beta-2A chain OS=Mus musculus GN=Tubb2a PE=1 SV=1
ALTVPELTQQMFDSK	Tubulin beta-2A chain OS=Mus musculus GN=Tubb2a PE=1 SV=1
NMMAACDPR	Tubulin beta-2A chain OS=Mus musculus GN=Tubb2a PE=1 SV=1
AILVDLEPGTMDSVR	Tubulin beta-2A chain OS=Mus musculus GN=Tubb2a PE=1 SV=1
LAVNMVPFPR	Tubulin beta-2A chain OS=Mus musculus GN=Tubb2a PE=1 SV=1
AILVDLEPGTMDSVR	Tubulin beta-2A chain OS=Mus musculus GN=Tubb2a PE=1 SV=1
ALTVPELTQQMFDSK	Tubulin beta-2A chain OS=Mus musculus GN=Tubb2a PE=1 SV=1
FPGQLNADLR	Tubulin beta-2A chain OS=Mus musculus GN=Tubb2a PE=1 SV=1
PDNFVFGQSGAGNNWAK	Tubulin beta-2A chain OS=Mus musculus GN=Tubb2a PE=1 SV=1
EIVHIQAGQCGNQIGAK	Tubulin beta-2A chain OS=Mus musculus GN=Tubb2a PE=1 SV=1
KESESCDCLQGFQLTHSLGGGTGSGMGTLLISK	Tubulin beta-2A chain OS=Mus musculus GN=Tubb2a PE=1 SV=1
LTTPTYGDLNHLVSATMSGVTTCLR	Tubulin beta-2B chain OS=Mus musculus GN=Tubb2b PE=1 SV=1
GHYTEGAELVDSVLDVVR	Tubulin beta-2B chain OS=Mus musculus GN=Tubb2b PE=1 SV=1
INVYYNEATGNK	Tubulin beta-2B chain OS=Mus musculus GN=Tubb2b PE=1 SV=1
TAVCDIPPR	Tubulin beta-2B chain OS=Mus musculus GN=Tubb2b PE=1 SV=1
EVDEQMLNVQNK	Tubulin beta-2B chain OS=Mus musculus GN=Tubb2b PE=1 SV=1
ESESCDCLQGFQLTHSLGGGTGSGMGTLLISK	Tubulin beta-2B chain OS=Mus musculus GN=Tubb2b PE=1 SV=1
IMNTFSVMPSPK	Tubulin beta-2B chain OS=Mus musculus GN=Tubb2b PE=1 SV=1
EIVHIQAGQCGNQIGAK	Tubulin beta-2B chain OS=Mus musculus GN=Tubb2b PE=1 SV=1
ALTVPELTQQMFDSK	Tubulin beta-2B chain OS=Mus musculus GN=Tubb2b PE=1 SV=1
NMMAACDPR	Tubulin beta-2B chain OS=Mus musculus GN=Tubb2b PE=1 SV=1
AILVDLEPGTMDSVR	Tubulin beta-2B chain OS=Mus musculus GN=Tubb2b PE=1 SV=1
LAVNMVPFPR	Tubulin beta-2B chain OS=Mus musculus GN=Tubb2b PE=1 SV=1
AILVDLEPGTMDSVR	Tubulin beta-2B chain OS=Mus musculus GN=Tubb2b PE=1 SV=1
ALTVPELTQQMFDSK	Tubulin beta-2B chain OS=Mus musculus GN=Tubb2b PE=1 SV=1
FPGQLNADLR	Tubulin beta-2B chain OS=Mus musculus GN=Tubb2b PE=1 SV=1
PDNFVFGQSGAGNNWAK	Tubulin beta-2B chain OS=Mus musculus GN=Tubb2b PE=1 SV=1
EIVHIQAGQCGNQIGAK	Tubulin beta-2B chain OS=Mus musculus GN=Tubb2b PE=1 SV=1
KESESCDCLQGFQLTHSLGGGTGSGMGTLLISK	Tubulin beta-2B chain OS=Mus musculus GN=Tubb2b PE=1 SV=1
LTTPTYGDLNHLVSATMSGVTTCLR	Tubulin beta-4B chain OS=Mus musculus GN=Tubb4b PE=1 SV=1
GHYTEGAELVDSVLDVVR	Tubulin beta-4B chain OS=Mus musculus GN=Tubb4b PE=1 SV=1
EAESCDCLQGFQLTHSLGGGTGSGMGTLLISK	Tubulin beta-4B chain OS=Mus musculus GN=Tubb4b PE=1 SV=1
TAVCDIPPR	Tubulin beta-4B chain OS=Mus musculus GN=Tubb4b PE=1 SV=1
EAESCDCLQGFQLTHSLGGGTGSGMGTLLISK	Tubulin beta-4B chain OS=Mus musculus GN=Tubb4b PE=1 SV=1
AVLVDLEPGTMDSVR	Tubulin beta-4B chain OS=Mus musculus GN=Tubb4b PE=1 SV=1
EVDEQMLNVQNK	Tubulin beta-4B chain OS=Mus musculus GN=Tubb4b PE=1 SV=1
INVYYNEATGGK	Tubulin beta-4B chain OS=Mus musculus GN=Tubb4b PE=1 SV=1
EIVHLQAGQCGNQIGAK	Tubulin beta-4B chain OS=Mus musculus GN=Tubb4b PE=1 SV=1
NMMAACDPR	Tubulin beta-4B chain OS=Mus musculus GN=Tubb4b PE=1 SV=1
IMNTFSVVPSPK	Tubulin beta-4B chain OS=Mus musculus GN=Tubb4b PE=1 SV=1
LAVNMVPFPR	Tubulin beta-4B chain OS=Mus musculus GN=Tubb4b PE=1 SV=1
ALTVPELTQQMFDAK	Tubulin beta-4B chain OS=Mus musculus GN=Tubb4b PE=1 SV=1
FPGQLNADLR	Tubulin beta-4B chain OS=Mus musculus GN=Tubb4b PE=1 SV=1
PDNFVFGQSGAGNNWAK	Tubulin beta-4B chain OS=Mus musculus GN=Tubb4b PE=1 SV=1
EIVHLQAGQCGNQIGAK	Tubulin beta-4B chain OS=Mus musculus GN=Tubb4b PE=1 SV=1
IMNTFSVVPSPK	Tubulin beta-4B chain OS=Mus musculus GN=Tubb4b PE=1 SV=1
LEICNLTPDTLTCDSYK	Calpain-2 catalytic subunit OS=Mus musculus GN=Capn2 PE=2 SV=4
RPTEICADPQFIIGGATR	Calpain-2 catalytic subunit OS=Mus musculus GN=Capn2 PE=2 SV=4

Table S2| Differential calpain and calpain-substrate peptides identified by proteomics in embryos and HUVECs. Shown are specific peptides detected by mass spectrometry as significantly different between three Piezo1+/+ and three Piezo1-/- E10.5 embryos. Only peptides relevant to the follow-up studies of Fig 4 are shown. Associated with the Table is an Excel spreadsheet (Source Data) that includes the same peptides as seen in Table S2 and related peptides differentially detected in studies of three populations of HUVECs transfected with control siRNA (sc.si.) and three populations of HUVECs transfected with Piezo1 siRNA (P1.si.1) in shear stress conditions on the orbital shaker. CAPNS1 is also included in the HUVEC peptide list because it is an important regulatory subunit of calpain-2. It was not detected in the embryo study.

Gene	PCR primer 5'-3'	Predicted amplicon (bp)	siRNA sequence (5'-3')
PIEZO1	F AGATCTCGCACTCCAT R CTCCTTCTCACGAGTCC	182	
Piezo1 5'	F GCTTGCTAGAACTTCACG R GTACTCATGCGGGTTG	224	
Piezo1 3'	F CACAAAGTACCGGGCG R AAAGTAAATGCACTTGACG	370	
PIEZO1			P1.si.1 GCCUCGUGGUCUACAAGAUtt
PIEZO1			P1.si.2 AGAAGAAGAUCGUCAAGUAtt
PIEZO1			P1.si.3 (SMARTpool) GCAGCAUGACAGACGACAU CUGGAGCAGUUCAGCGUAU UGGAGUAUGCCAACGAGAA UGGCUGAUGUUGUCGACUU
NOS3			eNOS.si.1 GAACAGCACAAGAGUUAUAtt
NOS3			eNOS.si.2 GGAGGUGACAAGCCGCAUAtt
NOS3			eNOS.si.3 CCUCGUCCCUGUGGAAAGAtt
LacZ	F AAT GGT CTG CTG CTG CTG AAC R GGC TTC ATC CAC CAC ATA CAG	225	
LoxP-spanning	F GGAGGGTTGCTTGTTGGATA R ACTCATCTGGGTGAGGTTGC	155, 189	
Tie2	F GCG GTC TGG CAG TAA AAA CTA TC R GTG AAA CAG CAT TGC TGT CAC TT	102	
cre deletion-spanning	F ACCACCTGAGAAGTTGTCCC R ACTCATCTGGGTGAGGTTGC	379	

Table S3| Sequence information for Piezo1 and genotyping PCR primers and siRNAs.