Supplementary Data for

Embryonic Lethality in Mice Lacking Trim59 Due to Impaired Gastrulation Development

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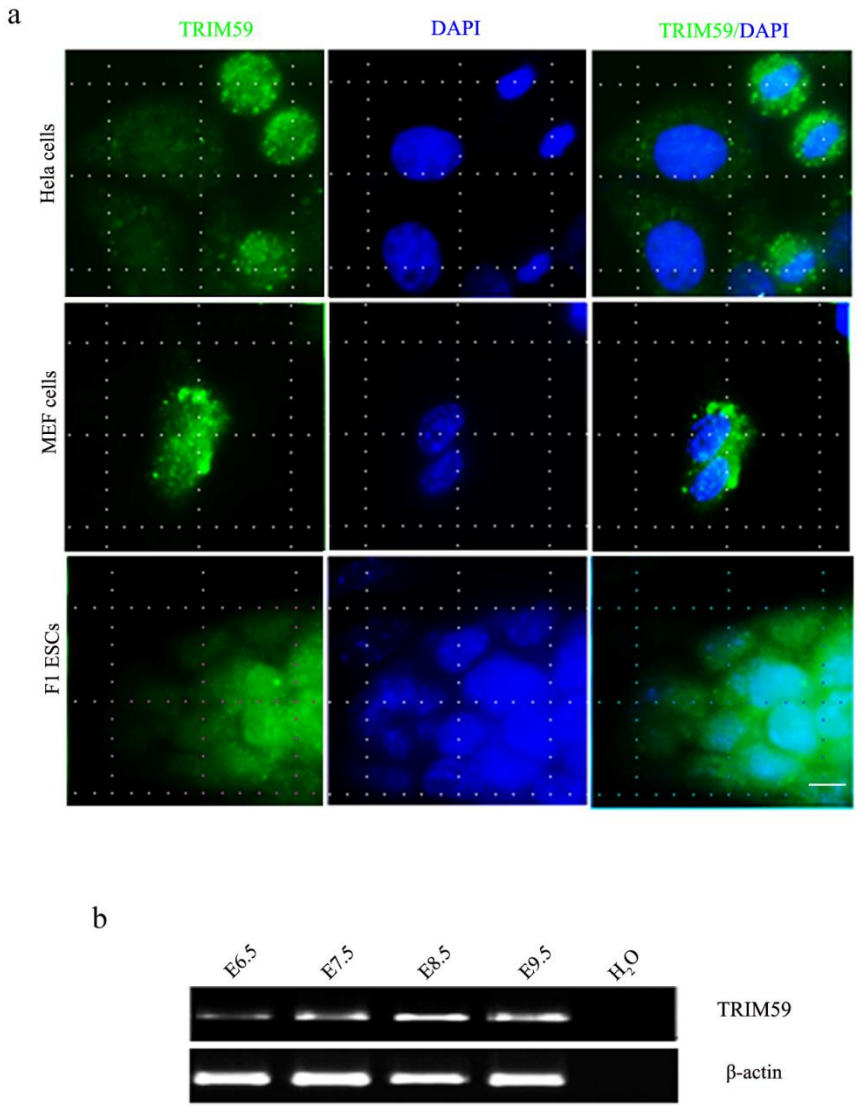
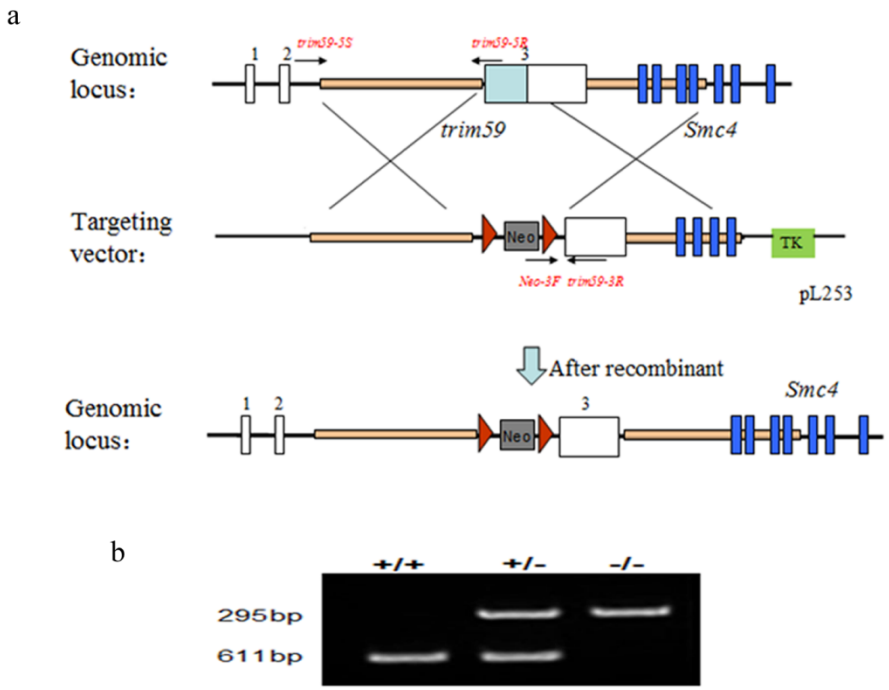
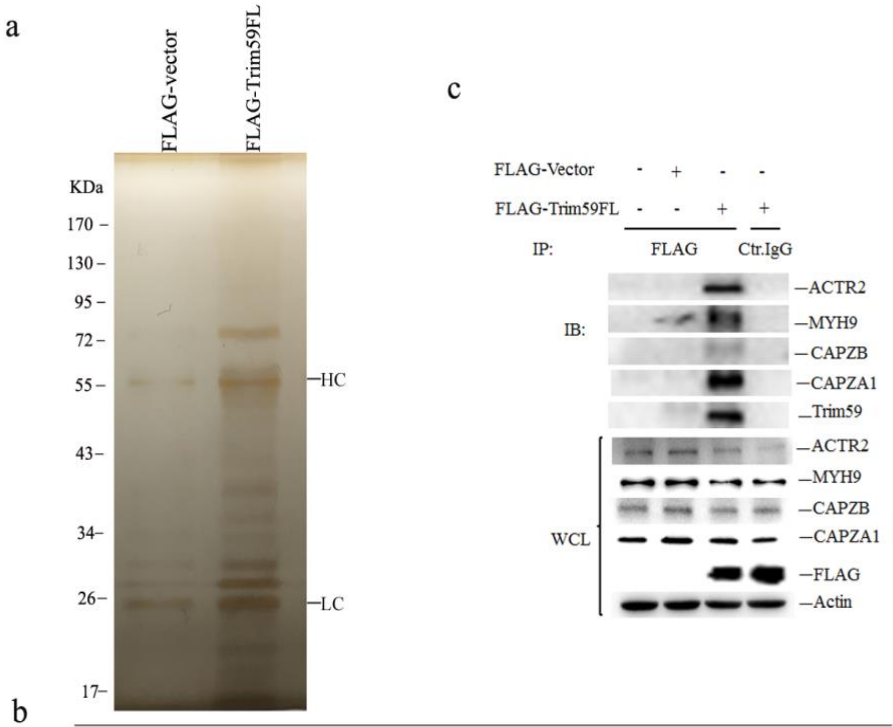


Supplementary Figure S1 Expression of Trim59 in different cell lines and mouse early embryos. a Immunofluorescence assay of Trim59 in Hela cells (human cervical cancer cell), MEF (murine Embryonic Fibroblast) cells and F1 ESCs. Trim59 (green) and chromatin (blue). Images were acquired using a Bio-Rad Radiance 2100 confocal microscope with a Zeiss 63× oil immersion objective. Scale bar, 10 μm. **b** RT-PCR of Trim59 in mouse early embryos from E6.5 to E9.5. β-actin, a loading control.



Supplementary Figure S2 Generation and identification of *Trim59* knockout mice. a Strategy for generating *Trim59* KO mice. *Trim59* KO mice were generated according to the described protocol in materials and methods. **b** PCR of mouse tail genome DNA. Total tail genome DNA was extracted using a DNA kit and PCR was performed. +/+, wild-type mouse; +/–, heterozygote mouse; –/–, *Trim59* KO mouse.

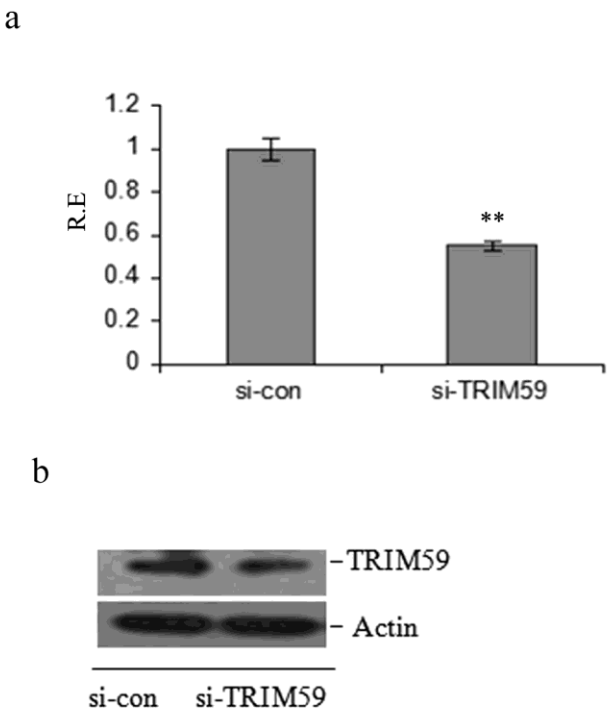


b

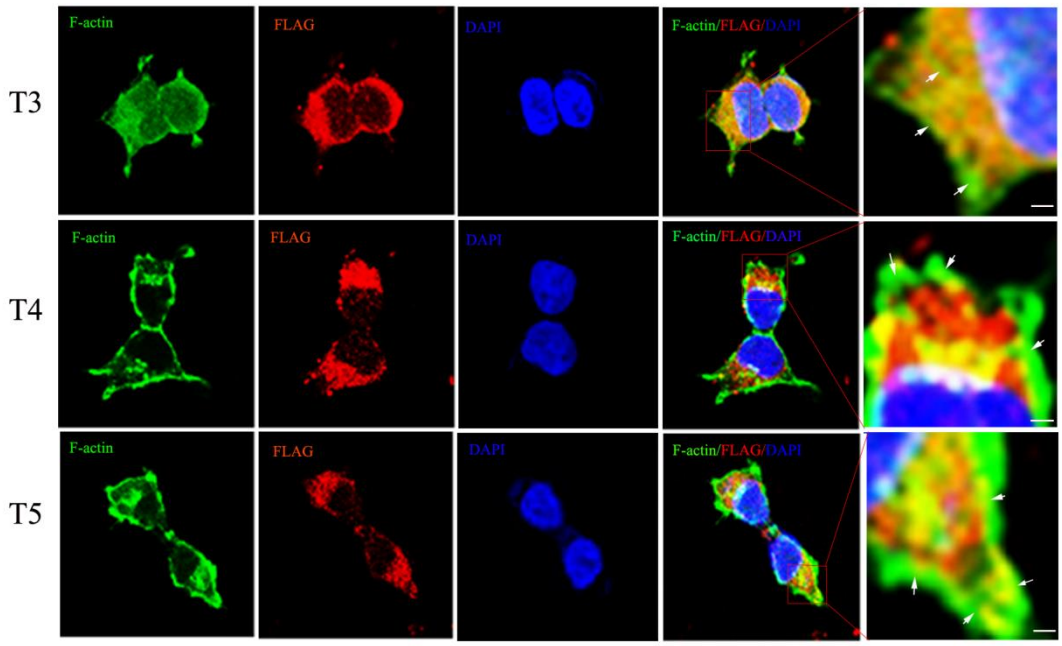
Name	Accession number	Description
CAPZB	NM_001206540.2	*Homo sapiens* capping actin protein of muscle Z-line beta subunit
CAPZA1	NM_006315.2	*Homo sapiens* capping actin protein of muscle Z-line alpha subunit 1
ACTR2	NM_001005386.2	*Homo sapiens* ARP2 actin related protein 2 homolog
MYL6	NM_021019.4	*Homo sapiens* myosin light chain 6
MYH9	NM_002473.5	*Homo sapiens* myosin heavy chain 9
MYH10	NM_001256012.1	*Homo sapiens* myosin heavy chain 10

Supplementary Figure S3 Interaction of Trim59 and actin-associated proteins. **a** Immunoprecipitation analyses. HEK293T cells were transfected with FLAG-vector or FLAG-Trim59. After immunoprecipitation by anti-FLAG, proteins were separated by SDS-PAGE, and then stained using silver staining kit. Asterisks indicate proteins which only appeared in Trim59 overexpressing group. HC, antibody heavy chain; LC, antibody light chain. **b** Proteins which are relative to the functions of cytoskeleton which only appeared in FLAG-Trim59 overexpressing group. The gel lanes containing the immunopurified samples were excised for Liquid Chromatography-

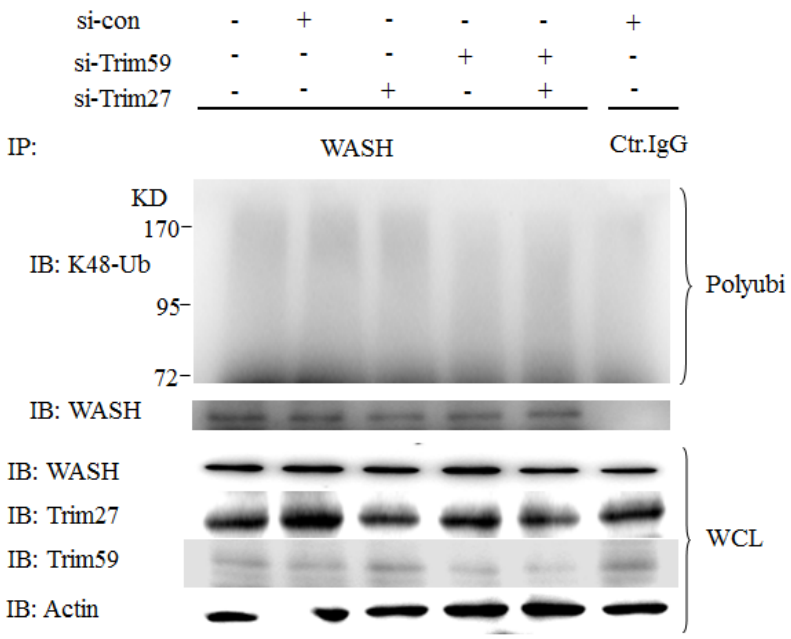
tandem Mass (LC-MS/MS) analysis. **c** Immunoprecipitation analyses. HEK293T cells were transfected with FLAG-vector (+) or FLAG-Trim59FL (+). After immunoprecipitation by anti-FLAG, Trim59 was detected by anti-Trim59 antibody. Trim59 binding protein ACTR2, MYH9, CAPZA1 and CAPZB were detected by anti- ACTR2, anti-MYH9, anti-CAPZA1 and anti-CAPZB. IP, immunoprecipitation; IB, immunoblot assay; WCL, whole cell lysate; Actin, a loading control; Ctr.IgG, isotype contro IgG.



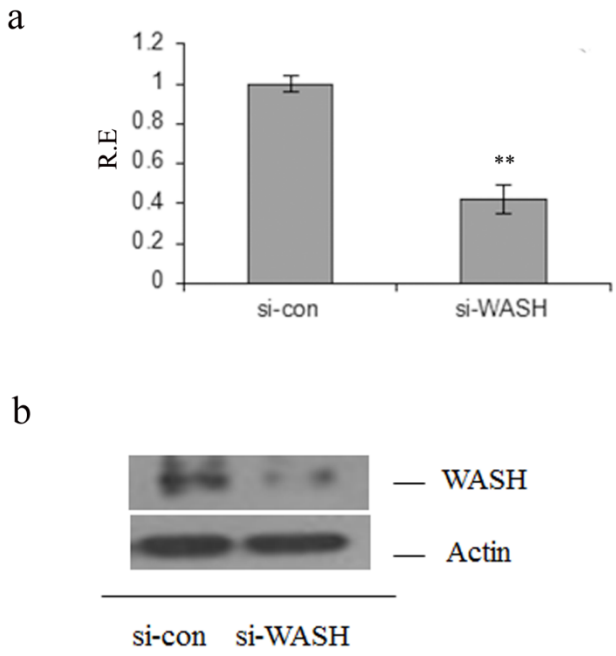
Supplementary Figure S4 Expression of Trim59 in siRNA silencing mouse embryonic stem cells. a qRT-PCR of Trim59 in the F1 ESCs with (si-TRIM59) or without (si-Con) mouse Trim59 siRNA treatment. *p<0.05 and **p<0.01(t-test). **b** Immunoblot of Trim59 in the F1 ESCs with (si-TRIM59) or without (si-Con) mouse Trim59 siRNA treatment. Actin, a loading control.



Supplementary Figure S5 Trim59 RING-finger domain is required for F-actin assembly. HEK293T cells were transfected with expression vectors encoding FLAG-tagged Trim59 domain mutants for 24hrs. The expression of FLAG-tag Trim59 domain deleted mutants (T3, T4, T5) and the F-actin assembly were analyzed by immunofluorescence. F-actin (green), Trim59 mutants (red) and chromatin (blue). Images were acquired using a Bio-Rad Radiance 2100 confocal microscope with a Zeiss 63× oil immersion objective; scale bar, 10 μm; zoom: ×4.



Supplementary Figure S6 Immunoblot of K48-linked ubiquitination of endogenous WASH. Immunoblot analysis of K48-linked ubiquitination (K48-Ub) of endogenouse WASH which was immunoprecipitated in F1 ESCs after treated with mouse Trim59 siRNA (top). Trim59, WASH and actin (below) were detected in the same cells without immunoprecipitation. Polyubi., polyubiquitination; IP, immunoprecipitation; IB, immunoblot assay; Actin, a loading control; WCL, whole cell lysates; Ctr.IgG, isotype control IgG.



Supplementary Figure S7 Expression of WASH after siRNA treatment. a qRT-PCR of WASH in F1 ESCs with (si-WASH) or without (si-Con) mouse WASH siRNA.*p<0.05 and **p<0.01(t-test). **b** Immunoblot of WASH in F1 ESCs with (si-WASH) or without (si-Con) mouse WASH siRNA. Actin, a loading control.

Supplementary Table S1

Table S1 Proteins that potentially interact with Trim59 (data from yeast two-hybrid)

Name	Accession number	Description
ACTN1	NM_001102	*Mus musculus* actinin, alpha 1
ARNT	NM_178427	*Mus musculus* aryl hydrocarbon receptor nuclear translocator
CD74	NM_004355	*Mus musculus* CD74 antigen
HOMER3	NM_004838	*Mus musculus* homer homolog 3
PLSCR1	NM_021105	*Mus musculus* phospholipid scramblase 1
RPS2	NM_002952	*Mus musculus* ribosomal protein S2
TRIOBP	NM_007032	*Mus musculus* TRIO and F-actin binding protein

Supplementary Table S2

Table S2a Oligoes used in the identification of mouse genotype

Oligo name	Sequence (5'to 3')	Description
Trim59-WGF3	GCATTACAAATTAGGGATTTTGTCG	for genotype
Trim59-WGR3	CCTCAAAATTGTGCATTTCCTGTC	
ES-1R3	AAGGGTTATTGAATATGATCGGA	

Table S2b Primers used in cloning Trim59 full-length and mutants

Oligo name	Sequence (5'to 3')	Description
Trim59 FL-F	GGGGTACCCATGCACAATTTTGAGGAAGAG	For cloning Trim59 full-length
Trim59 FL-R	CGGGATCCCGTCAATGGGAAACTATTTTCCA	
Trim59 T2-F	GGGGTACCCGAAATTGCTCCAACTGGCA	For cloning Trim59 RING domain deleted fragment
Trim59 T2-R	CGGGATCCCGTCAATGGGAAACTATTTTCCA	
Trim59 T3-F	GGGGTACCCGATGATGTACGCCAGCATG	For cloning Trim59 RING, B-box, and CC domains deleted fragment
Trim59 T3-R	CGGGATCCCGTCAATGGGAAACTATTTTCCA	
Trim59 T4-F	GGGGTACCCATGCACAATTTTGAGGAAGAG	For cloning Trim59 TM domain deleted fragment
Trim59 T4-R	CGGGATCCCGTCACTTTTCATCCTTACCAGGCC	
Trim59 T5-F	GGGGTACCCATGCACAATTTTGAGGAAGAG	For cloning Trim59 B-box domain deleted fragment
Trim59 T5-R	CGGGATCCCGTCAATGGGAAACTATTTTCCA	

Table S2c Oligoes used in RT-PCR

Oligo name	Sequence (5'to 3')	Description
Trim59-F	ATGCACAATTTTGAGGAGGAG	Mouse Trim59 detection
Trim59-R	TCAACGAGAAACTATTTTCC	
β-actin-F	GCCGGGACCTGACAGACTAC	Mouse β-actin detection
β-actin-R	CGGATGTCAACGTCACACTT	

Table S2d Oligoes used in qPCR

Oligo name	Sequence (5'to 3')	Description
Trim59-F	CATATGGAGGCCTCTGCGAA	Murine Trim59 detection
Trim59-R	GGTGACAACATCTGGGTGGT	
WASH-F	CTCCCTGCTGCTCTTCAACA	Murine WASH detection
WASH-R	GACAGAGGGGCATCGAACAA	
GAPDH-F	TCAACGGCACAGTCAAGG	Murine GAPDH detection
GAPDH-R	TACTCAGCACCGGCCTCA	

Table S2e SiRNA sequences used in this study

Oligo name	Sequence (5'to 3')
Mouse Trim59	CCATGTTCCTGGTCTGATA GACACACATTGGACAGATA
Mouse Trim27	CCACCTAAGAAGAGTGAAA GGGCTGAAAGAATCAGGAT
Mouse WASH	GGAGGAGAAATTGTTCGAT CCATCTTTACTGGCGCCTT

Table S2f Probes used in *in situ* hybridization

Oligo name	Sequence (5'to 3')	Targeted sequence
Murine Trim59-1 Murine Trim59-2	AGCACTGCTTGCTGCTTGTGG TCCCACCTGCTAACACTGGCAC	Murine Trim59
Murine Oct4-1 Murine Oct4-2	GAGGTAATCCACCCCCGGCTCGG TGCCCTTCTGGCGCCGGTTA	Murine Oct4
Murine T gene-1 Murine T gene-2	CCACCGAACGCGAACTGCGA ACAAGCCACCCCCATTGGGAAT	Murine T gene
Murine BMP4-1 Murine BMP4-2	AGCCATGAGCTCCTGCGGGA GACCAGGAGGGGCCGGAGTT	Murine BMP4
Murine Wnt3-1 Murine Wnt3-2	CGGGAATTTGCGGATGCGCG GTCCTCGTGTTGTGGCCCCG	Murine Wnt3
Murine Cer1-1	TTGCCTCTGGGGAAGGCAGACC	Murine Cer1

Murine Cer1-2	GCAATGGTCTGGTTGAAGGGCACA	
Murine Lefty2-1 Murine Lefty2-2	TCGACTCTAGGCTCGTGTCC GGAGACTACAGATCTATCCCCCTG	Murine Lefty2
Murine Otx2-1 Murine Otx2-2	GCCAGAATCCAGGGTGCAGGTAT GGACCCTTCTAAGGCCCCTTCCT	Murine Otx2
Mock probe	Offered by company	