SUPPLEMENTARY METHODS

Hematoxylin and eosin staining

Hematoxylin and eosin staining were performed using MMI H&E Staining Kit Plus (MMI, cat.no. 70302) according to the manufacturer's instructions. Briefly, after in turn dunk in xylene and propanol for 45 s, sections were stained with hematoxylin and eosin for 45 s and 30 s, respectively. After each staining, rinsed sections in water for 45 s. At last, dunk slices in propanol for 45 s and in xylene for another 45 s.

Nuclear fast red staining

Sections were rinsed in water for 30 s, then stained with nuclear fast red solution (1 mg/ml) for 5 min. After wash with water for 1 min, stained sections were dehydrated with 70% alcohol, 95% alcohol and absolute ethanol.

Whole-mount in situ hybridization

Whole-mount in situ hybridization on E7.0 mouse embryo was performed as described previously\(^1\). The primers for hybridization probes are listed below (a T7 promoter sequence (5`-taatcgcactataggaga3`) was added to the respective reverse primer):

Sall2-Forwards: TGAAGAAGGA TCCAGGAGAGAG
Sall2-Reverse:taatcgcactataggagaGGAAAGGGAGAAGGGAGAGAGAGAG
Utf1-Forwards: AGAGACGGAGCTACTTTCTGGG
Utf1-Reverse: taatcgcactataggagaGGTCGAAGGAACCTCGTAGAT
Uchl1-Forwards: AGCTGAAGCCGA TGGAGA
Uchl1-Reverse: taatcgcactataggagaCACTCCAGGGCCGTGAGAT
Sp5-Forwards: ACGTGAAGACGCAACAAA
Sp5-Reverse: taatcgcactataggagaAGGAGACCTTGGGAATGAGTA
Ccnd2-Forwards: CCAGATAAGGTCGTGGTGA
Ccnd2-Reverse: taatacgcatactagggagaCCAGGGAAGGAGCTAAATGAA

REFERENCES