

## Smooth muscle Hgs deficiency leads to impaired esophageal motility

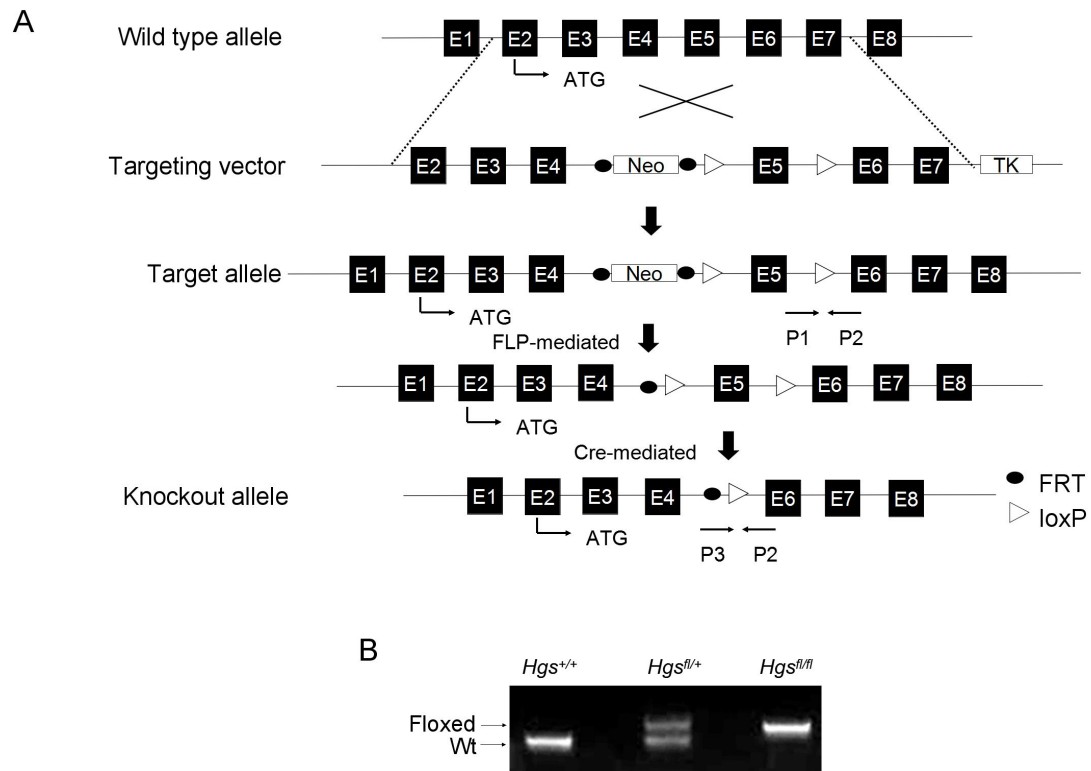
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Zeng<sup>2</sup>, Rui Li<sup>2</sup>, Wei Wang<sup>3</sup>, Xiao Yang<sup>1,2,†</sup>, and Yu Lan<sup>2,†</sup>

**Supplementary Table.1. The primer sequences for real-time PCR**

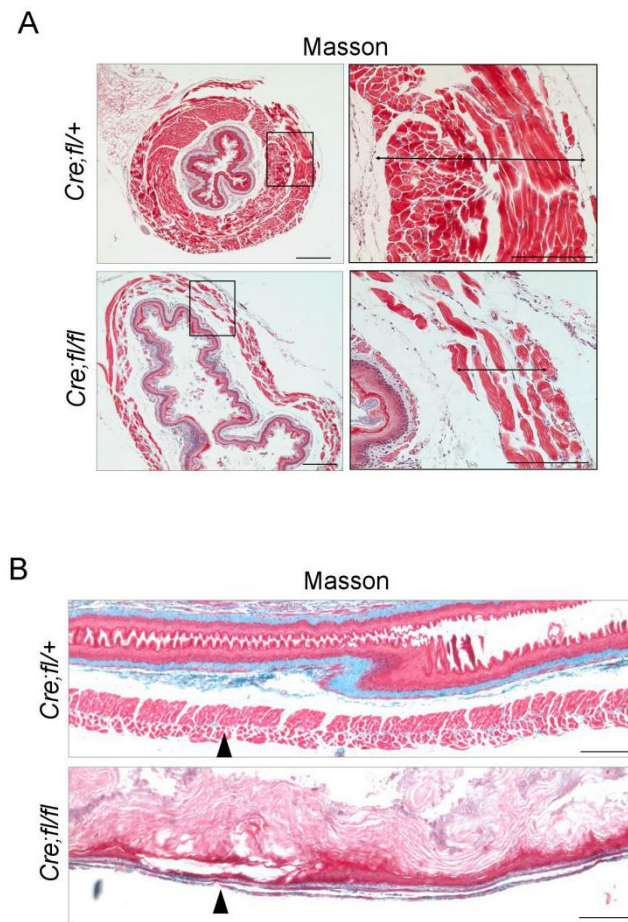
|          |                         |           |                         |
|----------|-------------------------|-----------|-------------------------|
| nNOS-S   | AGACACGGCAAGCCTCCA      | Il-1b-S   | TGACGGACCCCAAAAGAT      |
| nNOS-AS  | GCCAAGACGACTCCCACAG     | Il-1b-AS  | GTGATACTGCCTGCCTGA      |
| ACHE-S   | ACCTGTGGGCTCACGTAGATT   | Il-6-S    | TAGTCCTTCCTACCCCAATTTCC |
| ACHE-AS  | CCACGTAAGTGGTAGCAGACATT | Il-6-AS   | TTGGTCCTTAGCCACTCCTTC   |
| VIP-S    | TGCTGTTCTCTCAGTCGCTG    | TNF-a-S   | CAGGCGGTGCCTATGTCTC     |
| VIP-AS   | GCTCCTTCAAACGGCCTCCT    | TNF-a-AS  | CGATCACCCCGAAGTTCAGTAG  |
| ChAT-S   | GCCAGTGGAAGAATCGTCAT    | Cxcl9-S   | ATAAGGAATGCACGATGC      |
| ChAT-AS  | TTGTGCATGTGAGTGTGTGG    | Cxcl9-AS  | TCTTCACATTTGCCGAGT      |
| NGF-S    | TGATCGGCGTACAGGCAGA     | Cxcl10-S  | GAGCCTATCCTGCCACG       |
| NGF-AS   | GCTGAAGTTTAGTCCAGTGGG   | Cxcl10-AS | GGAGCCCTTTTAGACCTT      |
| GDNF-S   | CTTGGGTTTGGGCTATGAAA    | Cxcl16-S  | CGCAGGGTACTTTGGATC      |
| GDNF-AS  | ACAGGAACCGCTGCAATATC    | Cxcl16-AS | CTCGTGTCCGAAGGTGTC      |
| HPRT1-S  | ATTAGCGATGATGAACCA      |           |                         |
| HPRT1-AS | AGCAGGTCAGCAAAGAAC      |           |                         |

Supplementary Figure 1



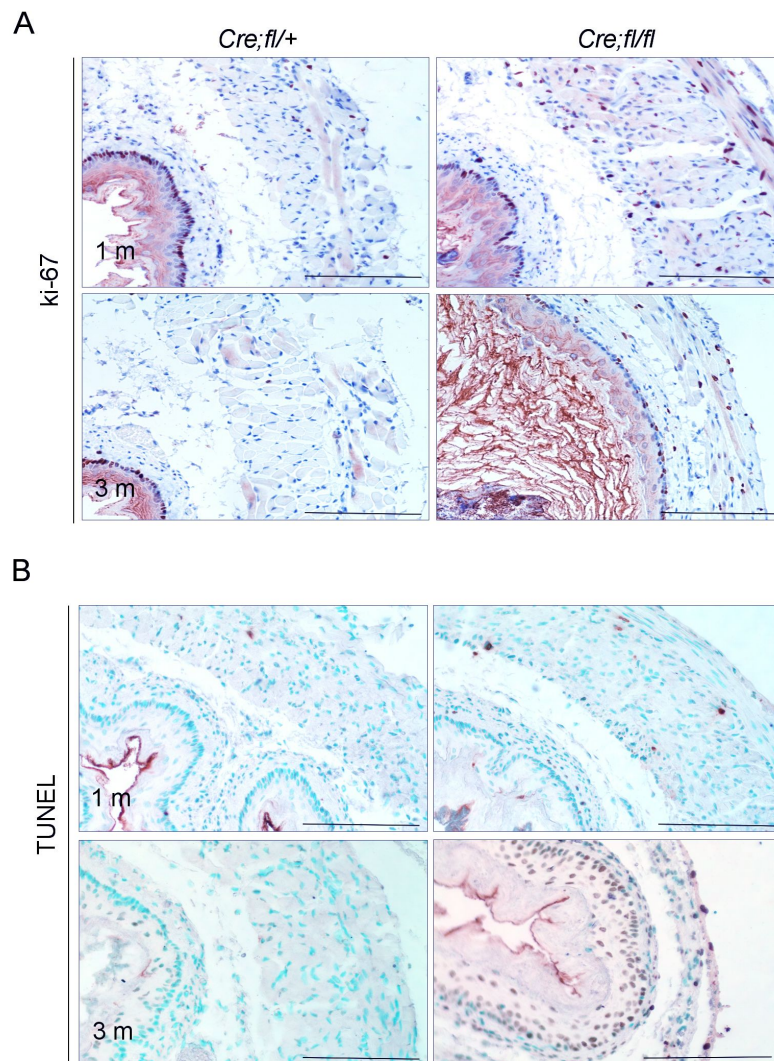
**Supplementary Fig.1. Generation of *Hgs*-floxed mice.** (A) Schematic representation of the *Hgs* genomic locus, targeting vector, and *Hgs* mutated locus. The targeting vector was designed to replace exon 2 (E2) to E7. A length of 124 bp in E5 was depleted in the knockout allele. (B) Genotyping analysis of wild-type, *Hgs*<sup>fl/+</sup> and *Hgs*<sup>fl/fl</sup> mice using primers P2: 5'-GCCTGTATTCTCGCCTGTG-3' and P3: 5'-TTCCGTGCTTCCTCCTGTTT-3'.

Supplementary Figure 2



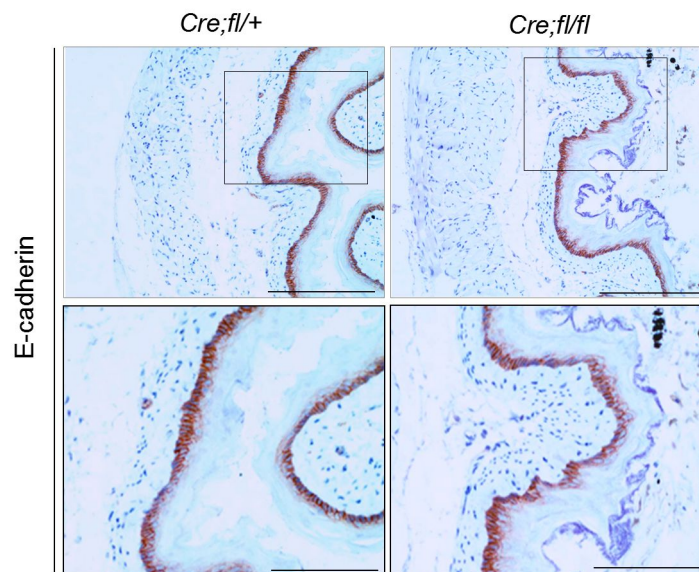
**Supplementary Fig.2. Thinning muscle layer of the mutant esophagus revealed by Masson trichrome staining.** (A) Masson trichrome staining of the esophagus from 3-month-old  $\alpha$ -SMA-Cre;Hgs<sup>fl/+</sup> (Cre;fl/+) and  $\alpha$ -SMA-Cre;Hgs<sup>fl/fl</sup> (Cre;fl/fl) mice, showing a remarkable thinning muscle layer and collagenous fibrosis in the mutants. (B) Masson trichrome staining on the longitudinal sections of the esophagus from 3-month-old  $\alpha$ -SMA-Cre;Hgs<sup>fl/+</sup> and  $\alpha$ -SMA-Cre;Hgs<sup>fl/fl</sup> mice. Scale bars: 200  $\mu$ m.

Supplementary Figure 3



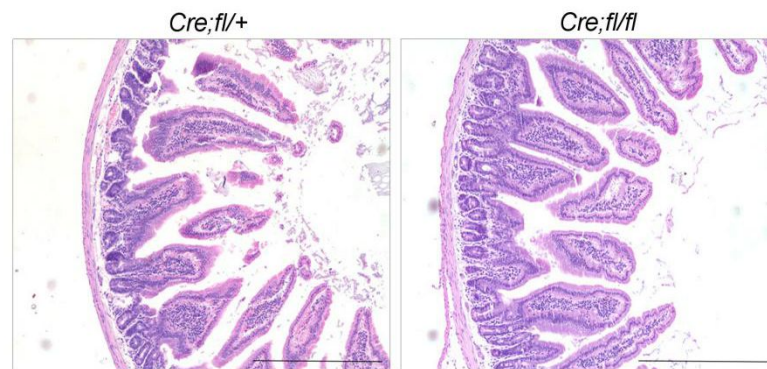
**Supplementary Fig.3. Both proliferation and apoptosis were increased in the esophageal muscle layer of the *Hgs* mutants. (A) Ki-67 immunohistochemistry staining of the esophagus from 1-month and 3-month-old  $\alpha\text{-SMA-Cre};Hgs^{fl/+}$  ( $Cre;fl/+$ ) and  $\alpha\text{-SMA-Cre};Hgs^{fl/fl}$  ( $Cre;fl/fl$ ) mice. (B) TUNEL staining of the esophagus from 1-month and 3-month-old  $\alpha\text{-SMA-Cre};Hgs^{fl/+}$  and  $\alpha\text{-SMA-Cre};Hgs^{fl/fl}$  mice. Scale bars: 200  $\mu\text{m}$ .**

Supplementary Figure 4



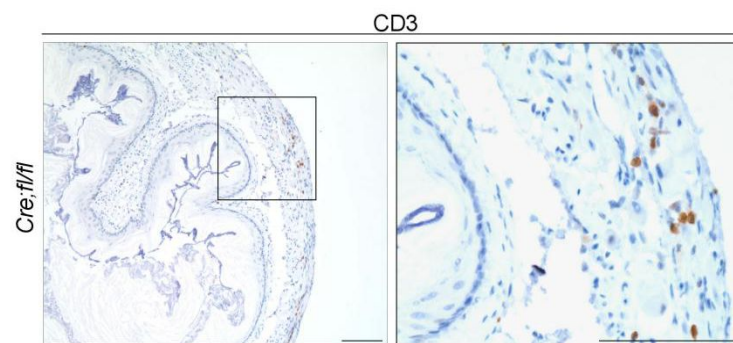
**Supplementary Fig.4. Normal expression and location of E-cadherin in the esophageal epithelium of the *Hgs* mutants.** E-cadherin immunohistochemistry staining of the esophagus from 1-month-old  $\alpha$ -SMA-Cre;*Hgs*<sup>fl/+</sup> (*Cre;fl/+*) and  $\alpha$ -SMA-Cre;*Hgs*<sup>fl/fl</sup> (*Cre;fl/fl*) mice. Scale bars: 200  $\mu$ m (upper), 80  $\mu$ m (lower).

Supplementary Figure 5



**Supplementary Fig.5. No obvious morphological abnormality in the small intestine of the *Hgs* mutants.** H&E staining of the small intestine from 3-month-old  $\alpha$ -SMA-Cre;*Hgs*<sup>fl/+</sup> (*Cre;fl/+*) and  $\alpha$ -SMA-Cre;*Hgs*<sup>fl/fl</sup> (*Cre;fl/fl*) mice. Scale bars: 200  $\mu$ m.

Supplementary Figure 6



**Supplementary Fig.6. Increased T lymphocyte infiltration in the esophageal muscle layer of the *Hgs* mutants.** Immunohistochemistry staining of CD3 in the esophagus from 6-week-old  *$\alpha$ -SMA-Cre;Hgs<sup>fl/fl</sup> (Cre;fl/fl)* mice. Scale bars: 200  $\mu$ m.