Appendix A. Supplementary material

Title: MLKL inhibits intestinal tumorigenesis by suppressing STAT3 signaling pathway

Qun Zhao \(^1,^3\), Xinran Cheng \(^1\), Jian Guo \(^1\), Yun Bi \(^1\), Li Kuang \(^2\), Jianhua Ren \(^2\), Jing Zhong \(^1\), Longrui Pan \(^1\), Xudong Zhang \(^1\), Yang Guo \(^1\), Yongqiang Liu \(^4\), Shu Jin \(^5\), Yan Tan \(^1\), Xianjun Yu \(^1,^*\)

Supplemental Data

Figure S1, Related to Figure 1
Figure S2, Related to Figure 2
Figure S3, Related to Figure 3
Figure S4, Related to Figure 4
Figure S5, Related to Figure 5
Figure S1, Related to Figure 1. Clinical scores of disease from the chimeric mice in the AOM/DSS models were calculated at days 12, 31 and 50. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ versus WT → WT groups.
Figure S2, Related to Figure 2. MLKL is dispensable for the self-renewal under physiological conditions

(A) H&E staining of representative intestines from 12-month-old WT and Mlkl−/− mice. (B) qRT-PCR analysis of gene expression in the intestines of WT and Mlkl−/− mice as indicated.
Figure S3, Related to Figure 3. Loss of MLKL stimulates STAT3 signaling

(A) Heat map analysis of differentially mRNA in 6-week-old Apc\textsuperscript{min/+} mice and Apc\textsuperscript{min/+} Mlkl\textsuperscript{-/-} intestinal tissues. (B) Upregulated and downregulated genes in intestinal tissues of Apc\textsuperscript{min/+} mice and Apc\textsuperscript{min/+} Mlkl\textsuperscript{-/-} mice was shown. (C) The expression of CD44 and SOX 9 the intestine of WT and Mlkl\textsuperscript{-/-} mice during regeneration (days 0 and 3). (D) The expression of pSTAT3 and STAT3 target gene in intestinal tissues during regeneration (days 0 and 3) in Apc\textsuperscript{min/+} and Apc\textsuperscript{min/+} Mlkl\textsuperscript{-/-} mice. (E) Protein lysates were isolated from intestine polyps from four groups of AOM/DSS-treated chimeric mice. Lysates were analyzed by western blotting to detect the expression of pSTAT3 and STAT3 target genes.

* p < 0.05, ** p < 0.01 and *** p < 0.001.
Figure S4, Related to Figure 4. MLKL deficiency exacerbates IL-6/STAT3 activation

HT-29 cells were treated with IL-6 for the indicated time interval in which MLKL was knocked down. Cell lysates collected at indicated time points were analyzed for pSTAT3. * $p < 0.05$. 
Figure S5, Related to Figure 5. Blocking IL-6 signaling suppresses intestinal tumorigenesis

(A) Images of colons isolated from $Apc^{min/+}$ and $Apc^{min/+}Mlkl^{-/-}$ animals after 10 weeks of anti-IL6R therapy. (B-C) Hematocrit (B) and thymus weight (C) of $Apc^{min/+}$ and $Apc^{min/+}Mlkl^{-/-}$ mice after anti-IL6R therapy for 10 weeks. ** $p < 0.01$. 
Supplemental tables

Table S1. Related to Fig. 1. The stool and bleeding score

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<th>Day 50</th>
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</tr>
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<td>2</td>
<td>1</td>
</tr>
<tr>
<td>WT→ Mlkl−/−</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Mlkl−/−→ Mlkl−/−</td>
<td>4</td>
<td>4</td>
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