Novel modulation of autophagy and intestinal homeostasis via mRNA binding protein IMP1

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University of Pennsylvania
What regulates homeostatic balance in the intestinal epithelium?

Normal homeostasis

Small Intestine crypt

Proliferation  Cell death

Wnt3a

Paneth cell

‘Quiescent’

‘Active’

Position 4/5 (+4) stem cell

Crypt base columnar (CBC) stem cell
What regulates homeostatic balance in the intestinal epithelium?

- **Normal homeostasis**
- **Stress/disease**
- **Restored homeostasis**

- Cell damage
- Cell death

- Paneth cell
- Wnt3a
- Niche-mediated repair

- ‘Active’ stem cell division
- Activation of ‘quiescent’ stem cells

- ‘Quiescent’
- ‘Active’
- Position 4/5 (+4) stem cell
- Crypt base columnar (CBC) stem cell

Diagram showing the transition from normal homeostasis to stress/disease and back to restored homeostasis, with key cellular and molecular events indicated.
RNA binding proteins are post-transcriptional coordinators of homeostasis

DNA → RNA → Protein

**IMP1**
(lgf2 mRNA binding protein 1)

Control  Imp1−/

Splicing  Transport  Stability  Localization

Keene J, Nat Rev Genetics., 2007
Kechavarzi and Janga, Genome Biology, 2014

Hansen TV et al, Mol Cell Biol. 2004
Hypothesis: IMP1 is required for normal homeostasis in the intestine

VillinCre;Imp1^{fl/fl} or Imp1^{ΔIEC}

What is the functional consequence of deleting IMP1 in the intestine?
*Imp1$^{ΔIEC}$* mice exhibit diffuse lysozyme staining and increased crypt autophagy

![Immunofluorescence images of Imp1WT and Imp1$^{ΔIEC}$](image)

**Lysozyme score in Imp1$^{ΔIEC}$ mice**

![Bar chart showing lysozyme expression](image)

**Western blots**

- LC3-I
- LC3-II
- p62
- GAPDH

**Autophagic Vessicles via CytoID**

![Fold change graph](image)

Hamilton KE et al, unpublished
Autophagy is defective in (a subset of) Crohn’s patients

Ileum in Crohn’s

- A reduction in Paneth cells or abnormal synthesis of granules (Lewin, *Gut*, 1969)

Control

<table>
<thead>
<tr>
<th>Atg16L1 risk allele</th>
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<tbody>
<tr>
<td>a</td>
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<tr>
<td>b</td>
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</table>

(Cadwell K et al *Nature*, 2008)

Does Imp1 loss confer protection or regenerative capacity (due to increased autophagy)?
Imp1 loss promotes enhanced crypt regeneration following irradiation

12Gy 4 days Evaluate

Imp1WT Imp1ΔIEC

EdU E-cadherin

Microcolonies per high-powered field, 4 days post-irradiation

Imp1WT Imp1ΔIEC

2.4 ± 0.15

3.2 ± 0.23

Hamilton KE et al, unpublished
IMP1 binds directly multiple autophagy transcripts

Hamilton KE et al, unpublished
Imp1 loss promotes crypt autophagy and increased regeneration following injury

Ongoing Experiments

1) Evaluate Imp1ΔIEC mice with genetic silencing of autophagy (Atg7fl/fl mice) to “rescue” protective effect of Imp1 loss during injury
2) Evaluate IMP1 expression in patient samples (epithelium)

Hamilton KE et al, unpublished
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Molecular Biology Core
Electron Microscopy Core
Cell Culture Core
Transgenic and Chimeric Mouse Core

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HHMI Research Fellows Program (ETL)
Imp1 loss promotes enhanced enteroid growth post-irradiation

12Gy 4 days Analyze Day 1-4

Simple Complex

Enteroid growth on Day 4 post-plating

% survival after plating

Enteroid complexity on Day 4 post-plating

% budding after plating

Hamilton KE et al, unpublished

* p<0.05 by 2-way ANOVA for effect of treatment; n=3-4
**Imp1** is expressed in intestinal epithelial crypts of adult mice

![Diagram of intestinal epithelial crypts]

**Imp1 expression in Sox9<sup>EGFP</sup> cell sub-populations**

- Differentiated cells
- Transit amplifying cells
- Crypt base stem cells
- +4/5 cells

**Imp1 expression in Lgr5<sup>+</sup> and Paneth cells compared to total crypt base**

<table>
<thead>
<tr>
<th>Reporter Model</th>
<th>% of total SI epithelium</th>
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<tbody>
<tr>
<td>Lgr5&lt;sup&gt;EGFP&lt;/sup&gt;</td>
<td>1.7</td>
</tr>
<tr>
<td>HopX&lt;sup&gt;CreERT2&lt;/sup&gt;</td>
<td>0.7</td>
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<tr>
<td>Bmi1&lt;sup&gt;CreERT2&lt;/sup&gt;</td>
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</tr>
<tr>
<td>Lrig1&lt;sup&gt;CreERT2&lt;/sup&gt;</td>
<td>2.4</td>
</tr>
<tr>
<td>Imp1&lt;sup&gt;EGFP&lt;/sup&gt;</td>
<td>0.4</td>
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</tbody>
</table>

Hamilton KE et al, unpublished
Crypt cells from \textit{Imp1}^{ΔIEC} mice exhibit increased Paneth and stem cell gene expression

Position 4/5 (+4) stem cell
Crypt base columnar (CBC) stem cell
Paneth cell

qPCR Paneth cell genes

\textbf{Lyz1}

\begin{figure}
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\includegraphics[width=\textwidth]{lyz1}
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Fold change vs Gapdh

Imp1^{floxP/floxP} vs Imp1^{ΔIEC}

* Significant difference

qPCR intestinal stem cell genes

\textbf{Lgr5}

\begin{figure}
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\end{figure}

Fold change vs Gapdh

Imp1^{floxP/floxP} vs Imp1^{ΔIEC}

* Significant difference

\textbf{Olfm4}

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Fold change vs Gapdh

Imp1^{floxP/floxP} vs Imp1^{ΔIEC}

* Significant difference

\textbf{Ascl2}

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Fold change vs Gapdh

Imp1^{floxP/floxP} vs Imp1^{ΔIEC}

* Significant difference

\textbf{Bmi1}

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Fold change vs Gapdh

Imp1^{floxP/floxP} vs Imp1^{ΔIEC}

* Significant difference

\textbf{Hoxp}

\begin{figure}
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\end{figure}

Fold change vs Gapdh

Imp1^{floxP/floxP} vs Imp1^{ΔIEC}

\textit{p}=0.052

\textbf{Imp1}

\begin{figure}
\begin{center}
\includegraphics[width=\textwidth]{imp1}
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Fold change vs Gapdh

Imp1^{floxP/floxP} vs Imp1^{ΔIEC}

* Significant difference

Hamilton KE et al, unpublished
Is the increase in autophagy associated with Imp1 loss protective in the epithelium following injury?

* $p<0.01$ vs Imp1$^{loxP/loxP}$; **$p<0.01$ vs Imp1$^{loxP/loxP}$ 12Gy; ne3
IMP1 binds directly several autophagy transcripts

RNA-IP-Seq

<table>
<thead>
<tr>
<th>Target</th>
<th>Risk Allele</th>
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<tbody>
<tr>
<td>Atg16l</td>
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<tr>
<td>Atg5</td>
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<tr>
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<tr>
<td>Map1lc3b</td>
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</table>