

Author reply to: RUNX3 is expressed in the epithelium of the gastrointestinal tract

We appreciate Dr. Ito's comments (Ito, 2012) about our discovery (Levanon et al, 2011) that Runx3 is not expressed in gastrointestinal tract (GIT) epithelium, thus challenging the data reported by Li et al (2002).

Li et al (2002, Fig 1E therein) showed dark LacZ-stained GIT of a mouse in which the β -gal gene was knocked into the *Runx3* locus (Li-Runx3-LacZ mice) (Fig reproduced in this response, Fig 1A) and further described in the results of Li et al (2002): 'Strong β -gal activity was found in gastrointestinal organs, including stomach, small and large intestines from 14.5 dpc through to adulthood'. These observations represent the foundation of Li et al (2002) report and its conclusion that *Runx3* is a tumour suppressor gene involved in GIT cancer. Our report (Levanon et al, 2011) re-examined these findings using a variety of biochemical and genetic techniques and questions the Li-2002 data and take-home message.

Using Li-Runx3-LacZ mice bred in our institute in Rehovot (Rehovot-bred Li-Runx3-LacZ mice), we could not detect LacZ in the GIT of these mice (Levanon et al, 2011). Similarly, Dr. Ito himself conceded recently that his group cannot

replicate the GIT LacZ staining in their mice (Normile, 2011). To provide a potential explanation for the lack of LacZ staining in GIT epithelium of these mice, Dr. Ito suggests a breeding schedule-mediated exon-skipping in his correspondence. However, the purity of the isolated GIT epithelium samples used in the polymerase chain reaction (after reverse transcription) (RT-PCR) assay shown in the correspondence (Ito, 2012, Fig 1B therein) remains unclear. Using purified-fluorescence-activated cell sorting (FACS)-sorted GIT epithelium, no Runx3 ribonucleic acid (RNA) was detected using stringent TaqMan-qRT-PCR (Levanon et al, 2011, Fig 5I). Furthermore, rigorous analyses of Rehovot-bred Li-Runx3-LacZ mice showed efficient LacZ staining in the known Runx3-expressing sites comparable to the originally reported levels (Li et al, 2002) in these mice (Levanon et al, 2011, Fig 6 and response, Fig 1B and C), excluding general exon skipping as an explanation for the failure to detect LacZ in GIT of Li-Runx3-LacZ mice.

To verify our findings, we generated additional reporter mouse strains and investigated the expression of Runx3 in their GIT (Levanon et al, 2011). Specifi-

cally and as mentioned by Dr. Ito, we also assessed Runx3 expression in mouse GIT epithelial cells by flow cytometry of tdTomato-positive cells isolated from R26-tdTomato/Runx3^{Cre} E16.5 embryos (not adult mice) as well as R26-LacZ/Runx3^{Cre} embryos. In these lineage-tracing experiments, LacZ or tdTomato expression is switched on by the Cre recombinase placed under the control of the Runx3 promoter. Thus, once Runx3-mediated Cre is expressed, the cell population and its progeny remain permanently labelled at high and fixed levels dictated by the activity of ROSA26 locus. The small peak mentioned by Dr. Ito could not 'suggest the existence of a small population of epithelial cells expressing high Runx3 level', because this presumed population does not accumulate in adult mice (Levanon et al, 2011, Fig 5H) and is not observed in cells from R26-LacZ/Runx3^{Cre} mice (Levanon et al, 2011, Fig 5E). Furthermore, Fig 1E in Li et al (2002) depicted an embryonic GIT intensely stained by LacZ, which persists 'throughout adulthood'.

While the focus of our work was mouse tissue, we included supplementary data derived from normal human GIT epithelium where we failed to detect epithelial RUNX3 (Levanon et al, 2011). In addition, two independent reports that analyzed more than 100 patients have also failed to detect RUNX3 mRNA or

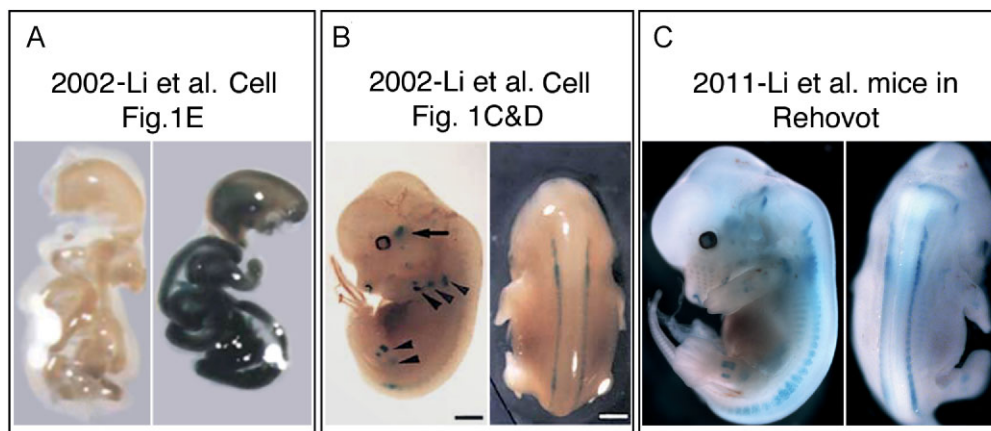


Figure 1. Analysis of the original (Li et al, 2002) mice either in Singapore or in Rehovot failed to reproduce the (Li et al, 2002) data.

- A.** LacZ staining of E14.5 GIT of Runx3-LacZ mice (right) compared to WT mice (left) (Li et al, 2002, E). The figure is reproduced with permission.
B. E12.5 Li-Runx3-LacZ mice stained and published in 2002 (Li et al, 2002, C and D). Shown are lateral and dorsal views. The figure is reproduced with permission.
C. E12.5 Li-Runx3-LacZ mice stained in Rehovot in 2011. Shown are lateral and dorsal views, demonstrating comparable LacZ level to the original 2002 level shown in B. Hence, no reduction in LacZ staining of Li-Runx3-LacZ mice is observed.

protein in gastric epithelium (Carvalho et al, 2005; Friedrich et al, 2006). Since we concentrated on mouse tissue, Ito et al (2005), which concerns RUNX3 expression in human GIT, was not directly cited in the text but listed in the Supplementary material section (Levanon et al, 2011, Table S1).

The p33 issue and Raveh et al (2005) are not relevant here and are thus addressed elsewhere.

In summary, using eight different anti-Runx3 antibodies, *in situ* hybridization, TaqMan qRT-PCR, three different reporter mouse strains (Runx3-GFP-KI, R26-LacZ/Runx3^{Cre} and R26-tdTomato/Runx3^{Cre}) and rigorous re-analysis of the original Li-Runx3-LacZ mice, we are unable to demonstrate Runx3 expression in GIT epithelium although it is readily demonstrable in a series of adjacent tissues. Thus, we maintain that these data show that normal GIT epithelium lacks

detectable Runx3 expression, which is in contradiction with Li et al (2002) report.

The authors declare that they have no conflict of interest.

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