interpretations must be considered. Data were not presented to show which cells express Krt5 or Krt6 in the absence of flu infection; thus, DASCS may be the progeny of an earlier stem or progenitor cell that does not express Krt5 or Krt6. It should also be noted that some of the markers that the authors used to define type 1 pneumocytes, such as PDPN and Aqpn5, are expressed in other cell types in the lung, including basal cells. Other type 1 pneumocyte markers were not examined or were not expressed (e.g., Hopx, Ager)[6]; lack of expression of expected markers may be a sign not only of immature differentiation of a progenitor but of epithelial metaplasia. Moreover, in models of specific cell-type ablation, one should consider the possible effects of a disrupted local cellular microenvironment, which might preclude activation of another critical cell type or prevent activation of molecular pathways and complicate any conclusions about the role of a specific cell type. This question could be addressed by a mechanistic approach, such as knocking out a key regulatory component in DASCs.

The second report, by Vaughan *et al.*[3], provides evidence for an important alternative interpretation. The authors examined the cellular origins of Krt5-expressing pods formed after H1N1 infection using multiple mouse endogenous locus knock-in strains with lineage-specific, tamoxifen-dependent Cre activity driving fluorescent reporters (Spc to interrogate the contribution of type 2 pneumocytes[7], CC10(Scgb1a1) for club (Clara) cells[8] and Krt5 for basal cells and DASCs[9]). Only the Krt5 lineage was detected in Krt5-expressing pods, and even then only 13% of the pod cells carried the lineage trace. Because the majority of pod cells did not fall into any of the tested lineages, their cell of origin was called a lineage-negative epithelial progenitor (LNEP) cell.

The authors found that integrin-Beta4, a marker of alveolar progenitors that they had discovered previously[7], marks, but is not sufficient to isolate, LNEP cells. They therefore used single-cell RNA-seq to identify surface markers and derived a sorting algorithm to enrich LNEP cells (Scgb1a1$^-$; integrin β4$^+$; CD200$^+$; CD14$^+$). In an exciting result, LNEP cells transplanted into the nose of flu-damaged mice gave rise to more-specialized, lineage-committed lung epithelial cell types in the alveoli, including cell clusters expressing Krt5 or Spc. Vaughan *et al.*[3] propose a repair model in which Krt5-negative LNEPs migrate and differentiate into the Krt5-expressing cells of the post-flu pods.

Although both studies[2,3] investigated the response of stem cells to influenza infection in the mouse lung, they have notable differences. Zou *et al.*[2] were able to trace 100% of distal Krt5 pod cells from the Krt5 lineage, whereas Vaughan *et al.*[3] found that only 13% of Krt5 pod cells originated from the Krt5 lineage. Consequently, it is still unclear whether a single cell type, such as LNEPs or DASCs, is responsible for the formation of Krt5 pods after influenza infection. The differences in the lineage tracing results between the two reports could be attributable to differences in the reporter systems (Zuo *et al.*[2] used a transgene carrying the bovine Krt5 promoter, whereas Vaughan *et al.*[3] used a knock-in at the Krt5 locus), differences in the timing of tamoxifen delivery and/or differences in the timing of lineage analyses. Only by developing new genetic tools that more specifically mark and trace LNEP and DASC populations will we clearly understand the role of these cells in the flu response.

Remarkably, both reports suggest an epithelial cell (LNEPs, DASCs or another progenitor) capable of migrating from the distal airway into the inflamed interstitial space. This observation raises important questions for future studies. How do the cells migrate to sites of inflammation? Can DASCs and LNEPs detach from or navigate the existing basal membrane before accumulating in the parenchyma? What is the impact on the airway epithelium of the cytokine cascade that occurs after flu infection? What signals orchestrate the response to flu, which could involve differentiation of progenitor cells or restructuring processes? And most important, is there *de novo* repair in the lung after influenza infection, or does proliferation and/or metaplasia of epithelial cells sustain the flu response?

Another notable advance in the two studies is the transplantation model. In the field of hematopoiesis, transplantation assays are the gold standard for demonstrating stem cell function. Now the lung field has a similar system for testing lung epithelial cell types. Previous transplantation attempts have shown very low engraftment rates, possibly due to the remaining presence of an intact basement membrane that precludes cell retention[10]. In using flu infection to precondition the lungs, and coupling that with delivery of the right type of putative progenitor cells (cultured DASCs or primary LNEPs), the authors have found a recipe for successful transplantation. This development gives researchers a critically important tool to home in on lung cells that effect repair in a variety of lung injuries and to investigate underlying mechanisms. A thorough understanding of alveolar repair in the mouse lung will in turn set the stage for the development of therapies for human distal lung diseases.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

1. Hogan, B.L.M. *et al. Cell Stem Cell* **15**, 123–138 (2014).
2. Zuo, W. *et al. Nature* Nov. 12 10.1038/nature13903 (2014).
3. Vaughan, A.E. *et al. Nature* Dec. 24 10.1038/nature14112 (2014).
4. Kumar, P.A. *et al. Cell* **147**, 525–538 (2011).
5. Sell, S. *et al. Viral Immunol.* **27**, 484–496 (2014).
6. Barkauskas, C.E. *et al. J. Clin. Invest.* **123**, 3025–3036 (2013).
7. Chapman, H.A. *et al. J. Clin. Invest.* **121**, 2855–2862 (2011).
8. Rawlins, E.L. *et al. Cell Stem Cell* **4**, 525–534 (2009).
9. Van Keymeulen, A. *et al. Nature* **479**, 189–193 (2011).
10. Lau, A.N., Goodwin, M., Kim, C.F. & Weiss, D.J. *Mol. Ther.* **20**, 1116–1130 (2012).

Research Highlights

Papers from the literature selected by the Nature Biotechnology editors. (Follow us on Twitter, @NatureBiotech #nbtHighlight)

Massively parallel single-amino-acid mutagenesis
Kitzman, J.O. *et al. Nat. Methods* doi:10.1038/nmeth.3223 (5 January 2015)

The human splicing code reveals new insights into the genetic determinants of disease
Xiong, H.Y. *et al. Science* **347**, 1254806 (2015)

Genome-scale transcriptional activation by an engineered CRISPR-Cas9 complex
Konermann, S. *et al. Nature* doi:10.1038/nature14136 (10 December 2014)

Engineering complex synthetic transcriptional programs with CRISPR RNA scaffolds
Zalatan, J.Z. *et al. Cell* doi:10.1016/j.cell.2014.11.052 (18 December 2014)

A new antibiotic kills pathogens without detectable resistance
Ling, L.L. *et al. Nature* doi:10.1038/nature14098 (7 January 2015)

Genetic diagnosis of developmental disorders in the DDD study: a scalable analysis of genome-wide research data
Wright, C.F. *et al. Lancet* doi:10.1016/S0140-6736(14)61705-0 (17 December 2014)