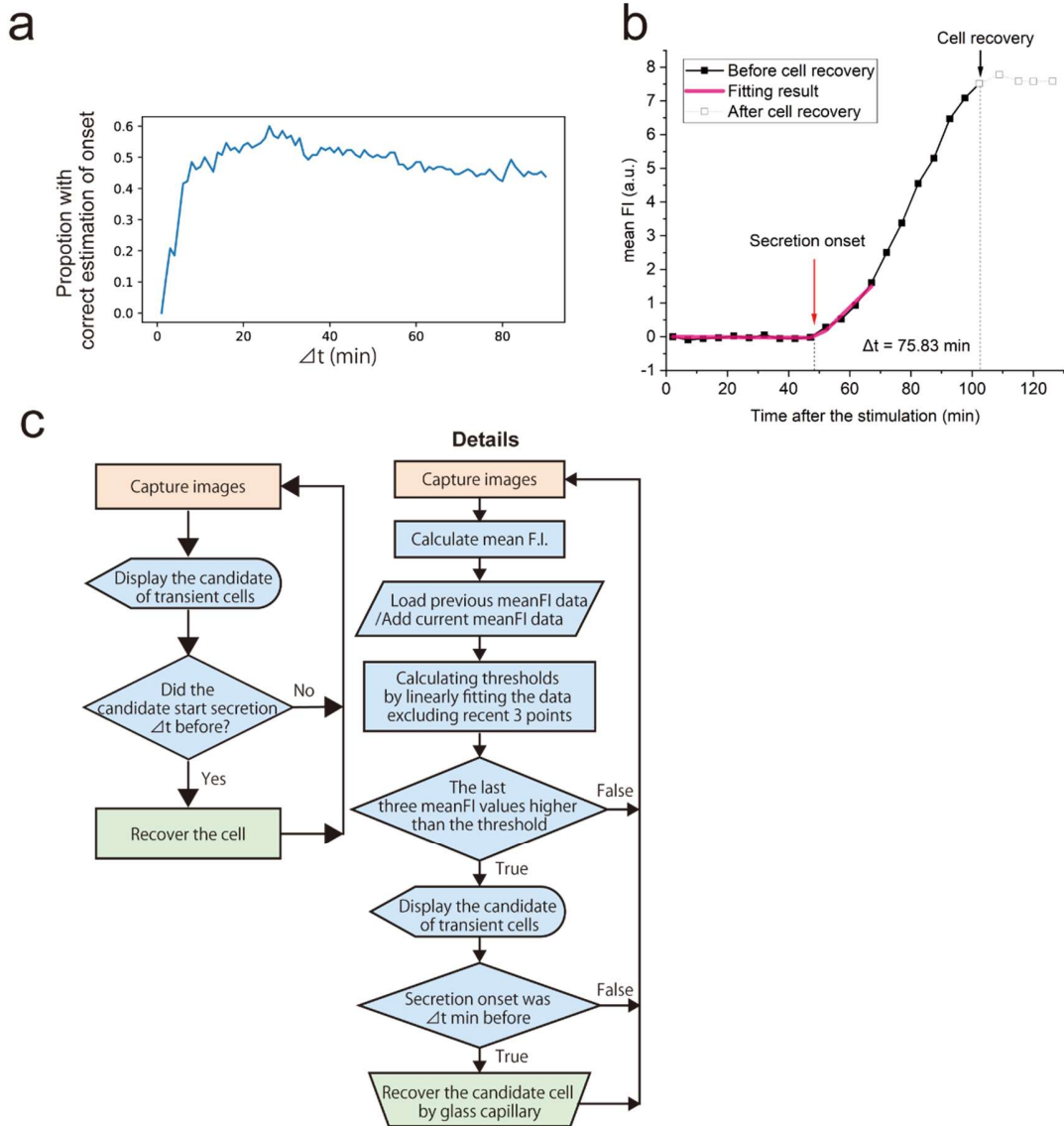


1 **Supplementary information**

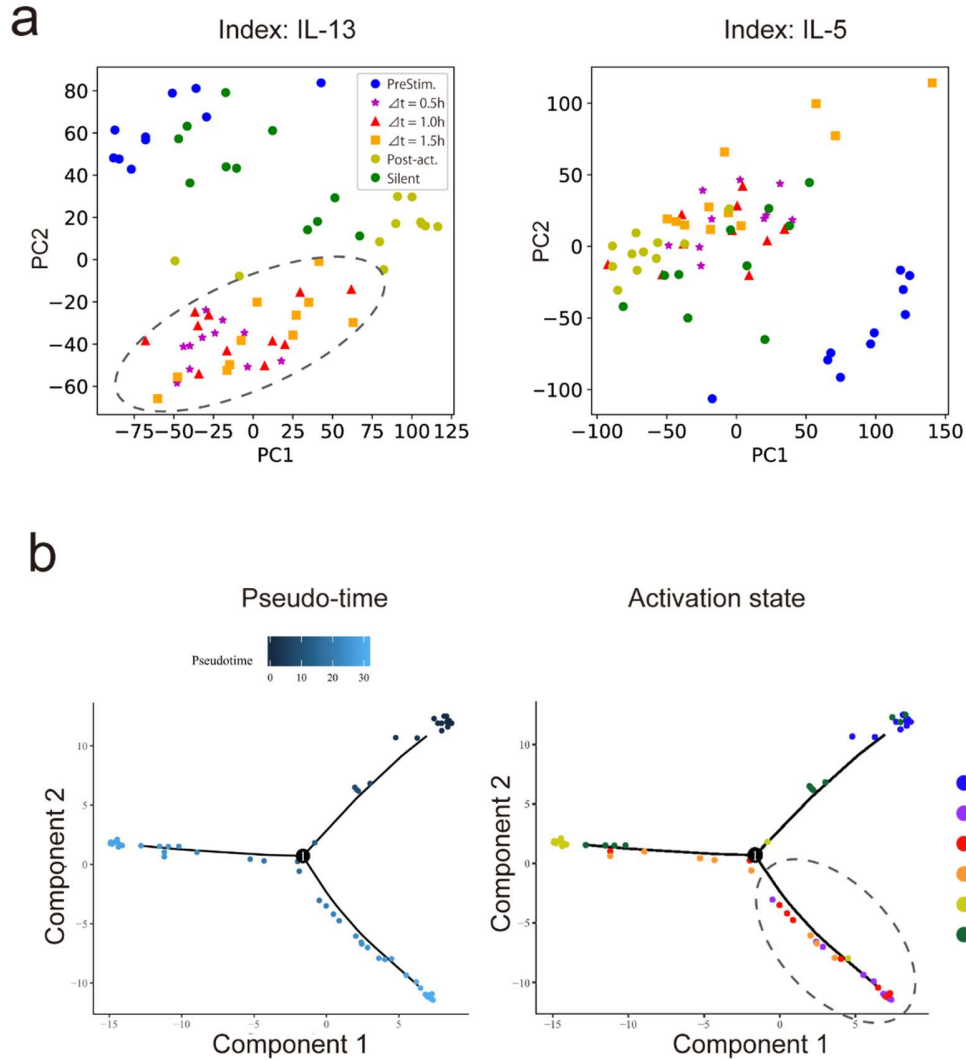


2 **Extended data figure 1| Detection of secretion onset**

- 3 (a) Trade-off between time from secretion onset to recovery flag (Δt) and accuracy of index determination.
- 4 The vertical axis shows the proportion of traces whose standard deviation is lower than 10 when the

5 secretion onset is determined by fitting traces from the start of measurement to Δt minutes after the
6 secretion onset (see Extended data Fig. 1c and Methods). (b) How to determine the timing of secretion
7 onset using post-analysis. The secretion signal trace was fitted to an elbow-shaped function (see Methods).
8 (c) Simplified (left) and detailed (right) flowcharts showing how the TDCSS technique operates. Red, blue
9 and green correspond to the operations of measurement, analysis and recovery, respectively.

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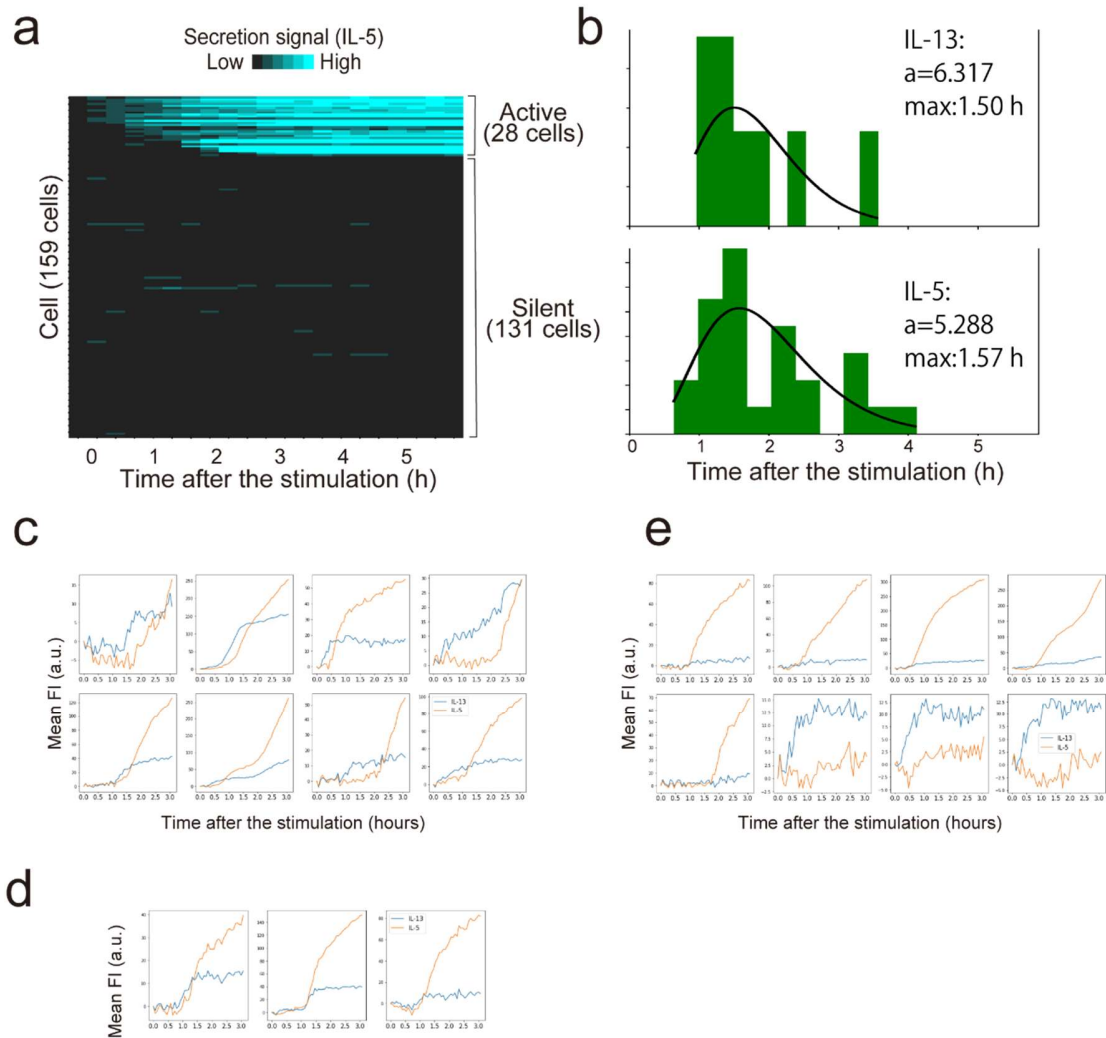
11 **Extended data figure 2| Overview of the similarity between activation states**

12 (a) Principal component analysis of expression data from cells recovered using the secretion onset of IL-

13 13 or IL-5 as an index. (b) Trajectory inference using Monocle of expression data from cells recovered

14 using the secretion onset of IL-13 as an index. The area where cells in the switching period are clustered

15 is circled (dashed line).



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17 Extended data figure 3| Secretion dynamics of IL-13 and IL-5 from stimulated mILC2s

18 (a) Heatmap of IL-5 secretion signal of 159 single-mILC2s. (b) Histograms of IL-13 or IL-5 secretion

19 onset. Data were fit using a Gamma distribution; and the acquired shape parameters and time reaching

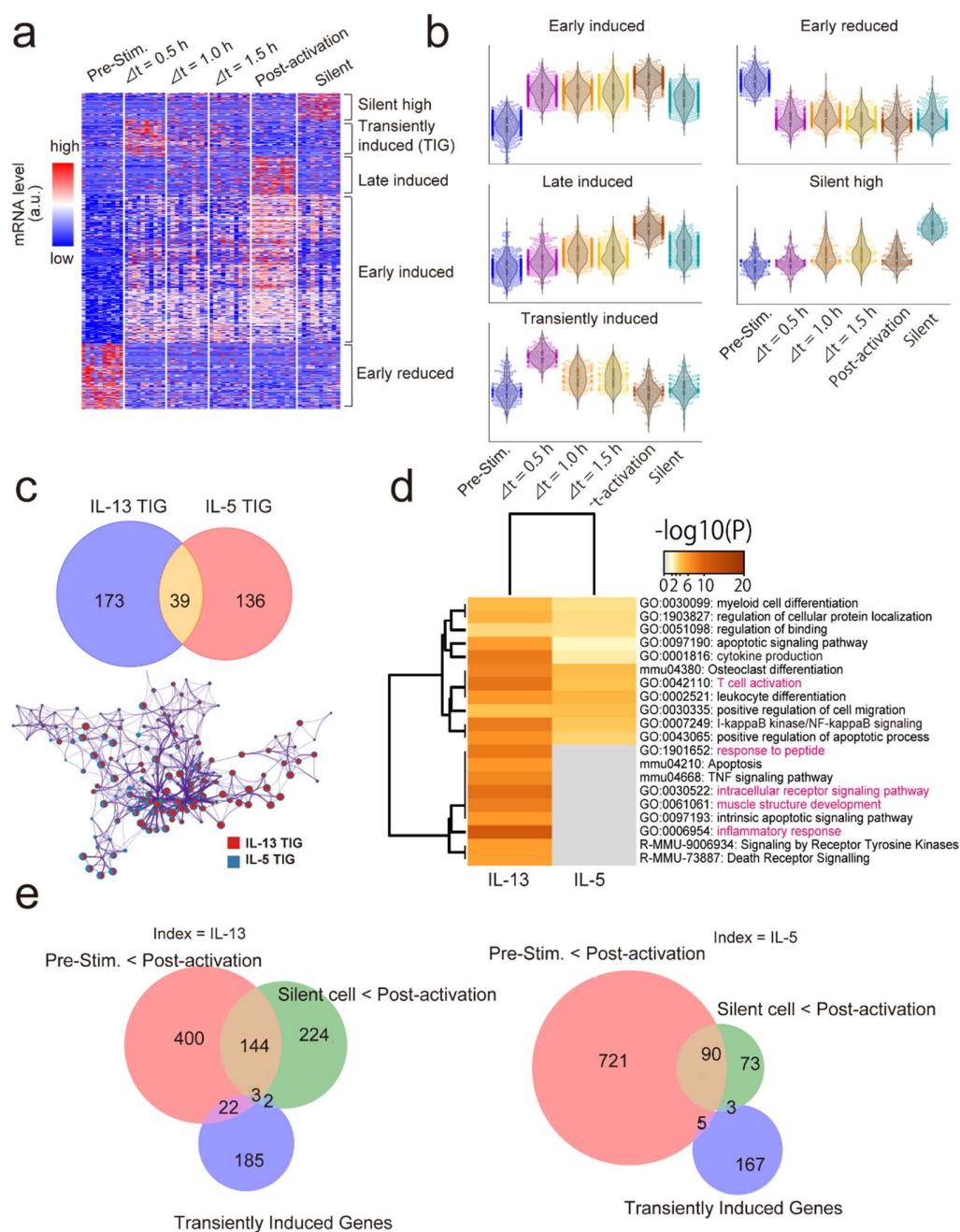
20 maximum value are shown. (c, d, e) Representative data of the secretion dynamics of IL-5 and IL-13 from

21 the same cell showing secretion of IL-13 preceded that of IL-5 (c), both starting at the same time (d), or
22 secreting only one of them (e).

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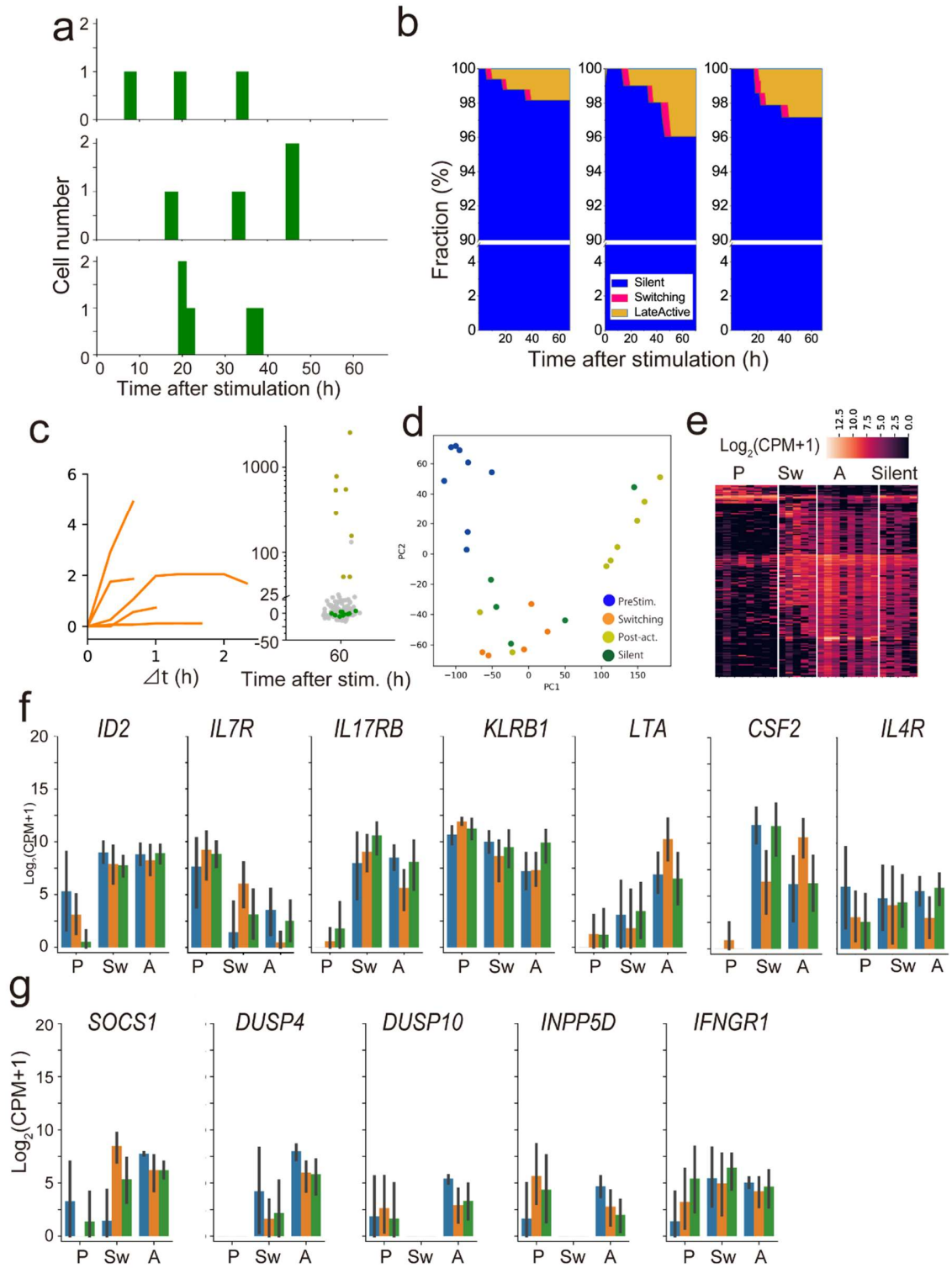
26 Extended data figure 4| Changes in information obtained when the index was changed

27 (a) Heatmap showing the relative expression of 1,505 highly variably expressed genes in cells recovered

28 using IL-5 as an index. (b) Violin plots showing the relative average expression levels within each state of

the genes classified into each class. Different from when IL-13 is used as an index, there is no transiently repressed class of genes but there is a class of genes whose expression is up-regulated only in silent cells.

(c) Venn diagram showing the overlap of TIGs obtained using IL-13 or IL-5 as an index and the respective enrichment ontology clusters. (d) Heatmap of accumulative hypergeometric p-values of each enrichment term. The terms that were particularly enriched in the IL-13 TIG in Figure 3d are shown in magenta. (e) Venn diagram showing the overlap of TIGs with significantly upregulated genes in post-activation compared to pre-stimulation and significantly up-regulated genes in post-activation compared to silent cells.



Extended data figure 5| Information obtained using real-time recovery of hILC2

(a) Histogram of IL-13 secretion onset of single-ILC2s from three individuals different from those who provided the ILC2s used in the real-time recovery experiment. (b) Changes in the percentage of cells in the pre-activation, switching period ($\Delta t < 3$ hours), and post-activation states measured using LCI-S. (c) IL-13 signal traces of hILC2s recovered in real-time (left) and signal intensity distribution of hILC2s 60 hours after stimulation (right). The signal intensity of cells recovered as post-activation is shown in yellow, and that of cells collected as silent is shown in green. (d) PCA results of the expression data from an individual. (e) Heatmap showing the expression levels of differentially expressed genes at the single cell/colony level. Data from the same individuals as in (d) are shown. (f) Expression levels of genes known to be associated with ILC2. (g) Expression levels of genes whose products are known to repress ILC2's activation.

Abbreviations

ILC2: Group 2 innate lymphoid cell

TDCSS: Time-Dependent Cell-State Selection

LCI: Live-Cell Imaging

56 LCI-S: Live-Cell Imaging of Secretion activity

57 IL: Interleukin

58 mILC2: mouse ILC2

59 hILC2: human ILC2

60 RNA: Ribonucleic Acid

61 mRNA: messenger RNA

62 PCR: Polymerase Chain Reaction

63 RT-PCR: Reverse Transcription PCR

64 RNA-seq: RNA sequencing

65 DEG: Differential Expression Gene

66 GO: Gene Ontology

67 TIG: Transiently Induced Gene

68 IFN- γ : Interferon gamma

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