

## Expanded View Figures

**Figure EV1. Comprehensive karyotypic characterization of the late passage ICL lines.**

A–D Karyotypic analysis of later passage, control and ICL of a replicate (#2) of Ch12 (A), Ch10 (B), Ch14 (C) and Ch9 (D) lines. All chromosomes are shown for 20 metaphases. Target chromosomes are boxed in red.

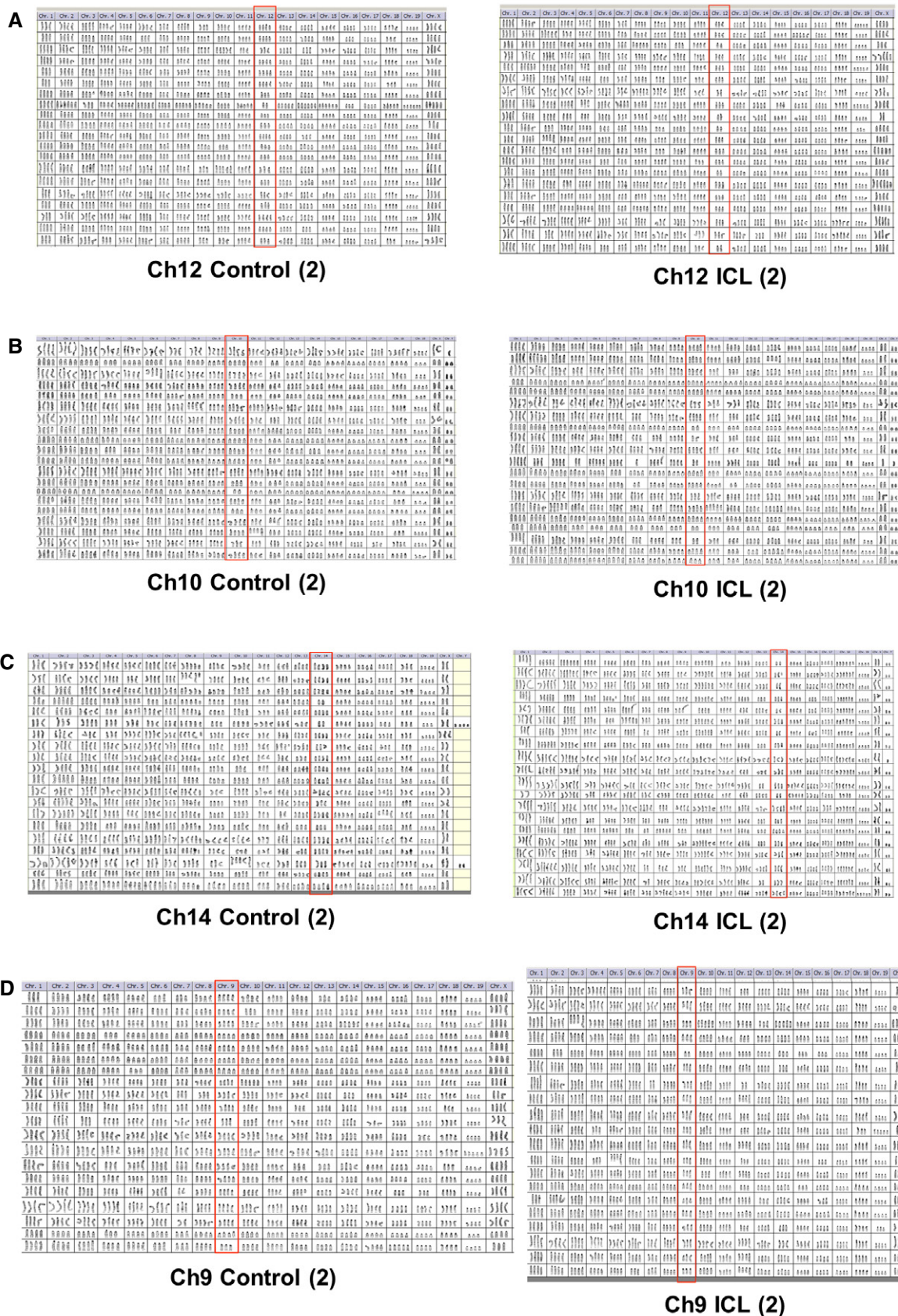
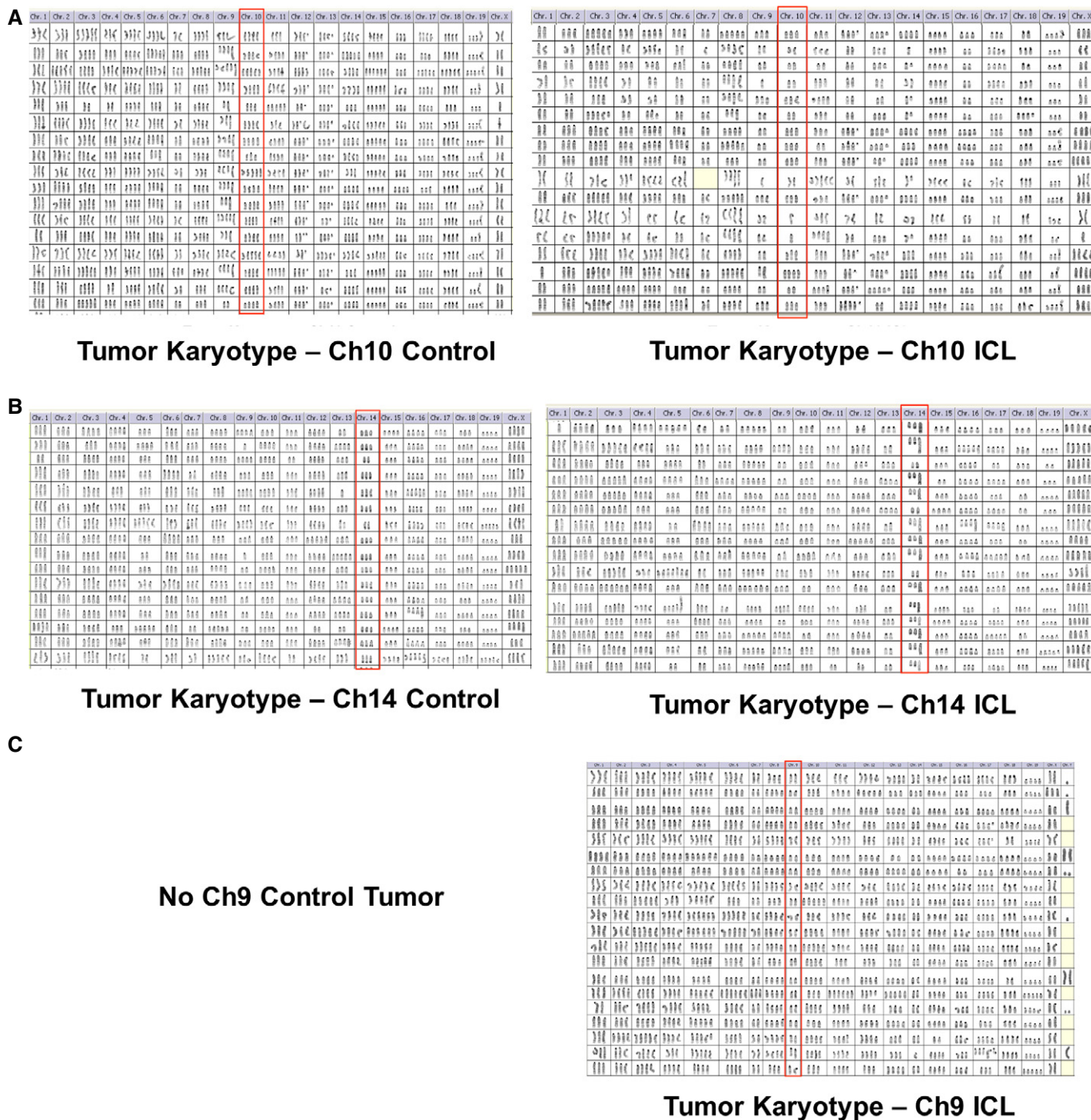
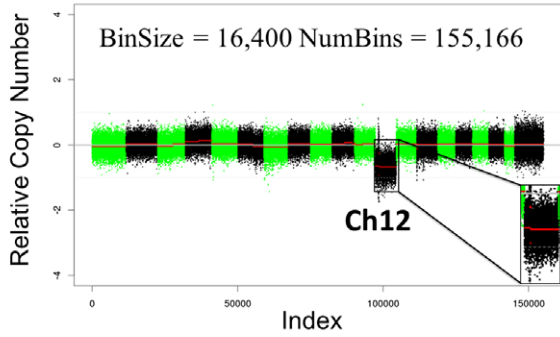


Figure EV1.

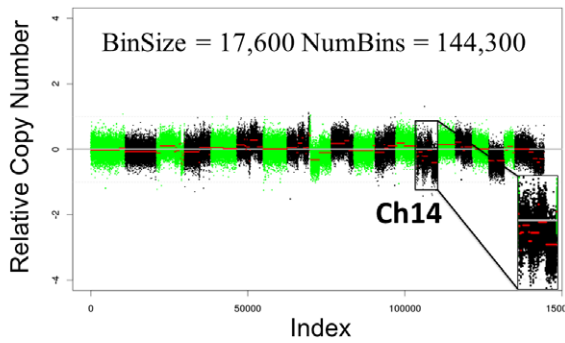


**Figure EV2. Comprehensive karyotypic characterization of the late passage ICL tumor explants.**  
 A–C Karyotypic analysis of later passage, control, and ICL tumor explants derived from the injecting the late passage control and ICL cells of Ch10 (A), Ch14 (B), and Ch9 (C). All chromosomes are shown for 20 metaphases. Target chromosomes are boxed in red. No karyotypes are shown for the Ch9 controls, as they did not form tumors in nude mice.

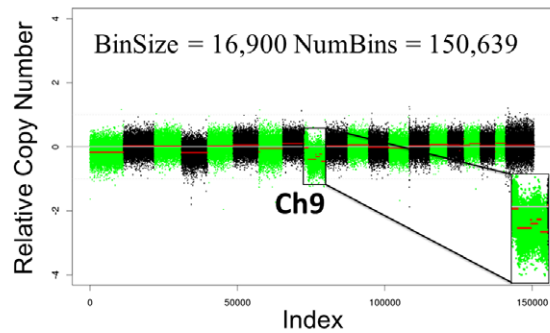
**Late Passage – Ch12 ICL normalized to Ch12 Control**



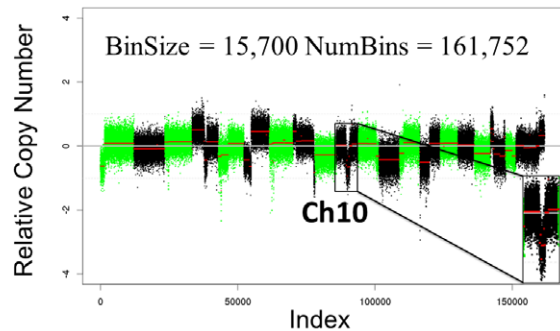
**Late Passage – Ch14 ICL normalized to Ch14 Control**



**Late Passage – Ch9 ICL normalized to Ch9 Control**

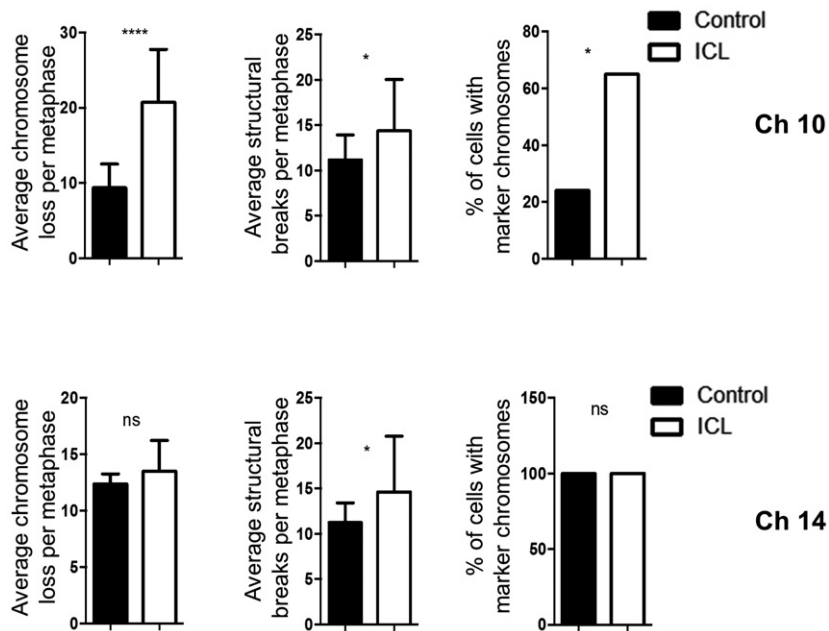


**Late Passage – Ch10 ICL normalized to Ch10 Control**



**Figure EV3. Global copy number profiles of the late passage ICL MEFs.**

A–C Shallow whole-genome sequencing (copy number profiles) of large T antigen immortalized, late passage MEFs after exposure to Cre recombinase and serially sorted for control (hCD2 Plus) and ICL (hCD2 Minus) cells for chromosomes 12, 14 and 9 and 10.



**Figure EV4. Increased chromosomal instability in ICL tumors.**

Chromosomal instability readouts, of tumor lines, assessed by average chromosomes loss per metaphase, average number of structural rearrangements per metaphase and percentage of metaphases with marker chromosomes ( $n = 20$  for each data set and error bars denote SD, average chromosome loss: \*\*\*\* $P < 0.0001$  for Ch10; average structural rearrangements: \* $P < 0.05$  for Ch10 and Ch14; percentage of metaphases with marker chromosomes: \* $P < 0.05$  for Ch10). Two-tailed unpaired t-test was used to determine statistical significance for average chromosome loss and structural breaks. Two-tailed Z-test was used to determine statistical significance for the percentage of cells with marker chromosomes.