Expanded View Figures

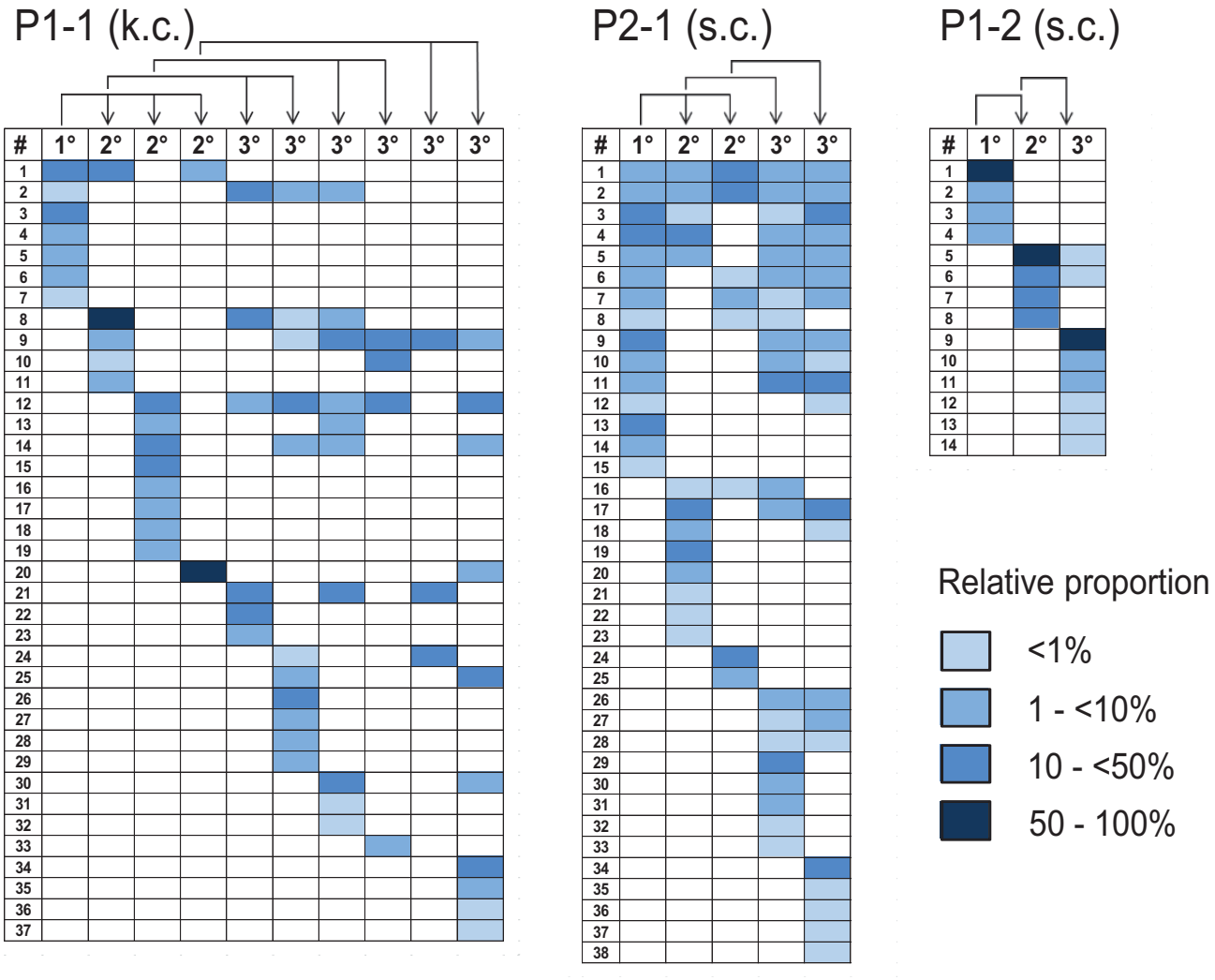

Figure EV1. Long-term tumor growth is maintained by clonal succession of pancreatic TICs.
High-throughput sequencing of lentiviral insertion sites (IS) shows that distinct sets of marked clones formed serial tumors derived from the same individual primary xenograft. Columns indicate distinct mouse generations (1°/2°/3°). Rows indicate distinct lentiviral integration sites. Arrows indicate serial transplantation steps; PX-Y: X = patient number and Y = experiment number.

Figure EV2. Proliferation of TIC cultures *in vitro* is driven by clonal succession.

A Patient-derived adherent TIC cultures were lentivirally marked and passaged 8 times *in vitro*.

B Lentivirally marked TIC cultures were transplanted directly into NSG mice. After tumor purification, cells were taken into culture for several passages (*ex vivo*). IS analysis was done by LAM-PCR.

Data information: In serial passaging, distinct sets of tumor cells drive proliferation of *in vitro* (A) and *ex vivo*-cultured lentivirally marked xenografts (B). Blue fields indicate the relative contribution of individual clones as depicted in the color legend. Columns indicate distinct passages (1–8°). Rows indicate distinct lentiviral integration sites. Arrows indicate passaging steps; PX-Y: X = patient number and Y = experiment number.

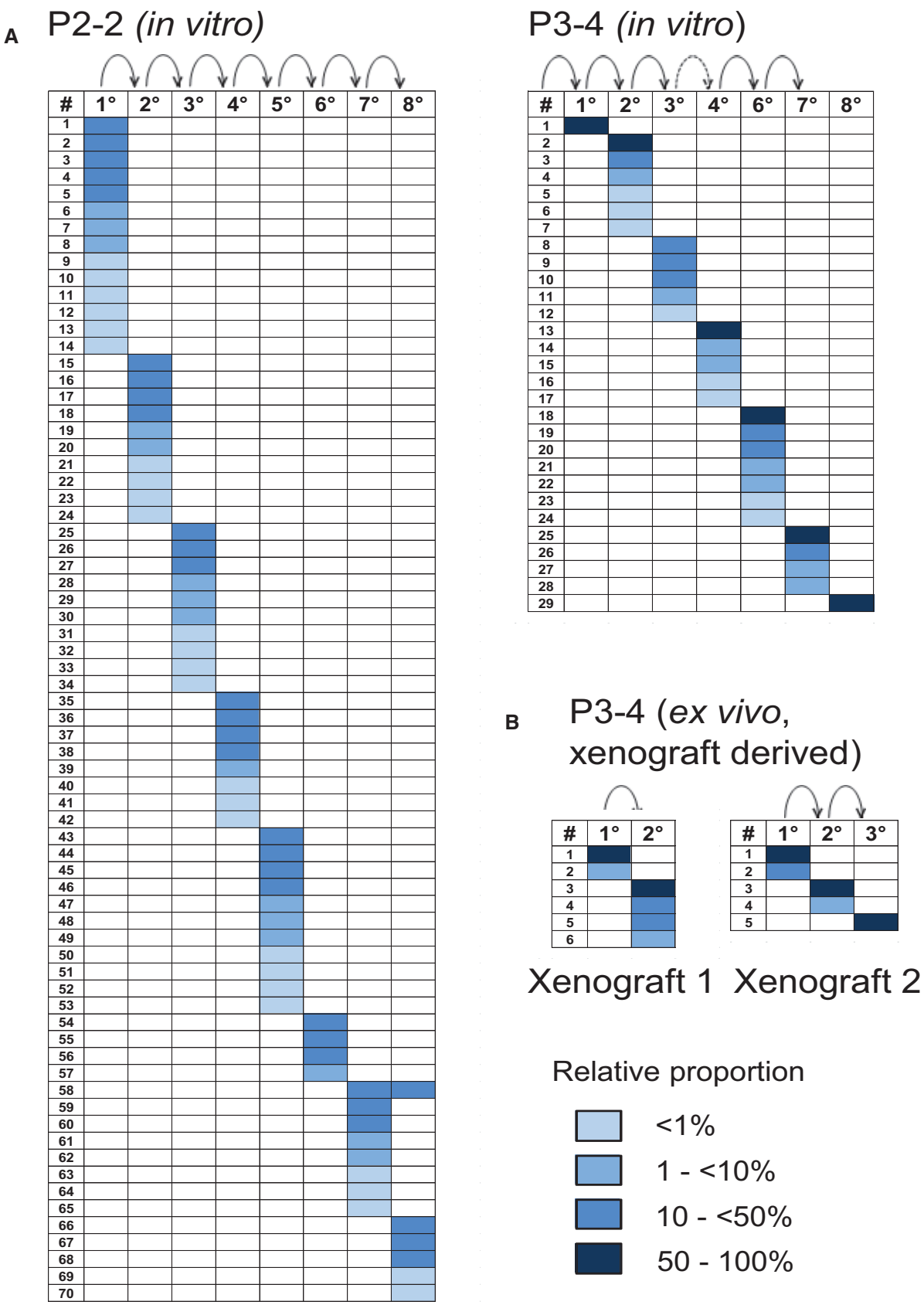
A
P2-2 (in vitro)
P3-4 (in vitro)
B
P3-4 (ex vivo, xenograft derived)
Xenograft 1
Xenograft 2
Relative proportion
<1%
1 - <10%
10 - <50%
50 - 100%

Figure EV2.