



**Figure S4** ASUs and CSUs interact, as shown at the phenotypic level with the co-over expression system. (A) and (B) Examples of spot assays for growth on sorbose of the cells carrying, respectively, plasmid pEA238 (*SOU1 CSU51 ASU51*) or pEA162 (*SOU1 CSU53 ASU53*). Each of these plasmids co-over expresses three essential genes: (i) *SOU1*; (ii) *CSU* in a natural configuration *CSU5/ASU5*; and (iii) *ASU*. Notice that pEA238 carries an entire region A encompassing *CSU51* in a natural configuration *CSU51/ASU51*, while pEA162 carries a portion from region 135 encompassing *CSU53* in a natural configuration *CSU53/ASU53*, which is designated *CSU53\*\**. For the plasmid types, see, respectively, cartoons 8 and 9 in Figure 2. Note that either one of natural configurations *CSU/ASU* confers the Sou<sup>-</sup> repressive phenotype, because of the dominant *CSU* (Results). Also included is the general negative and positive control for growth due to the plasmid pRC2312 and pCA88, respectively. Also included is growth due to co-over expression with *SOU1* of a single element, either a *CSU/ASU* or an *ASU*, as indicated in parenthesis. The corresponding plasmids serve as the additional repressive Sou<sup>-</sup> (pAK65 in A or pEA105 in B) or the additional Sou<sup>++</sup> (pEA209 in A or pEA104 in B) controls. Note that the presence of either an extra *ASU51* or an extra *ASU53* diminished the Sou<sup>-</sup> repressive phenotype of a corresponding *CSU*. Specifically, the plasmid pEA238 in A conferred either the intermediate confluent growth designated Sou<sup>+/-</sup> (A, top) or the high frequency Sou<sup>+</sup> colonies designated Sou<sup>-\*</sup> (A, bottom), instead of the repressive Sou<sup>-</sup> phenotype. See also section “*CSUs* and *ASUs* interact among themselves at the phenotypic and transcriptional levels”. Furthermore, the plasmid pEA162 in B conferred the confluent growth, which is almost equal to the Sou<sup>+</sup> growth due to control pCA88 and is designated Sou<sup>+/-</sup>. It should be noted that sorbose plates in A are presented after five days of incubation. This enabled the control growth due to pCA88 to catch up with enhanced growth due to pEA238 (see section “Identification of new elements, *ASUs*, which are associated with *CSUs*, and which act opposite to *CSUs* by enhancing the growth on sorbose” for explanations). Also shown is growth of the control spots on glucose medium.