

Figure S1. Cloning efficiency of representative Level≥1 GBelements. (a) Restriction analysis of 24 plasmids obtained from randomly selected colonies to test the accuracy of gRNA assemblies under different conditions (HindIII expected bands: 6345-374). (b) Restriction analysis of GB binary reactions combining gRNAs with each other and/or with the Cas9-encoding TU. All tested plasmids resulted in the expected restriction patterns. GB1198 BamHI expected bands: (6674-728); GB1202 EcoRI expected bands: (6345-1242); GB1107 EcoRV expected bands: (6674-4620-1236-194); GB1064 EcoRV expected bands: (6674-5117-1236); GB1108 EcoRI expected bands: (6987-6345-828).

(a)



**Figure S2.** Schema of the architecture of the dead Cas9 transcriptional units tested on the repression and activation experiments.