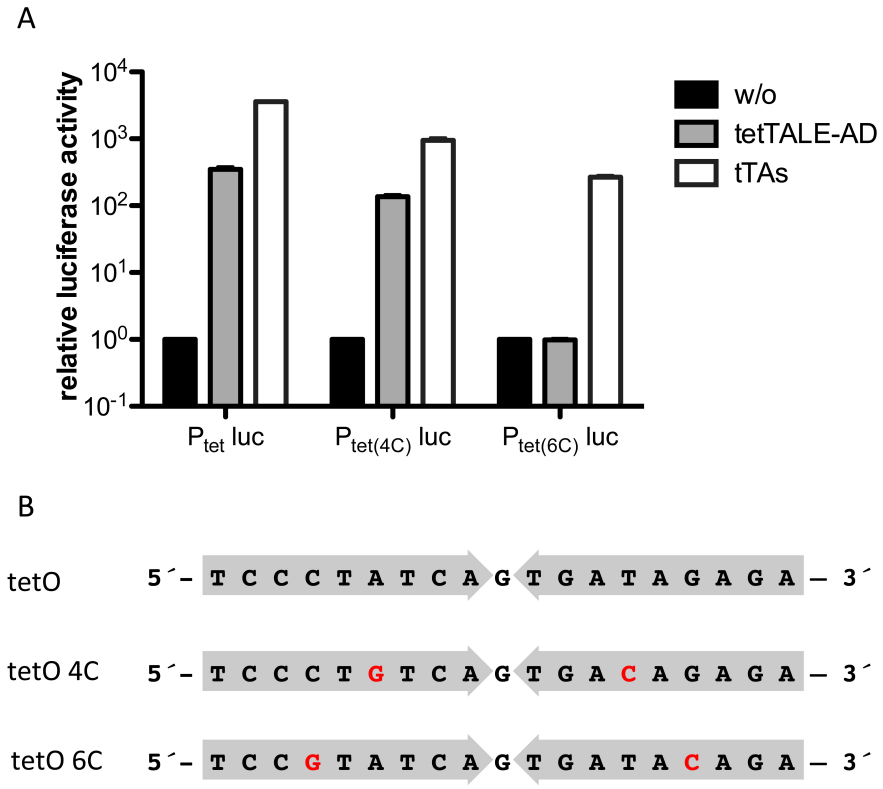
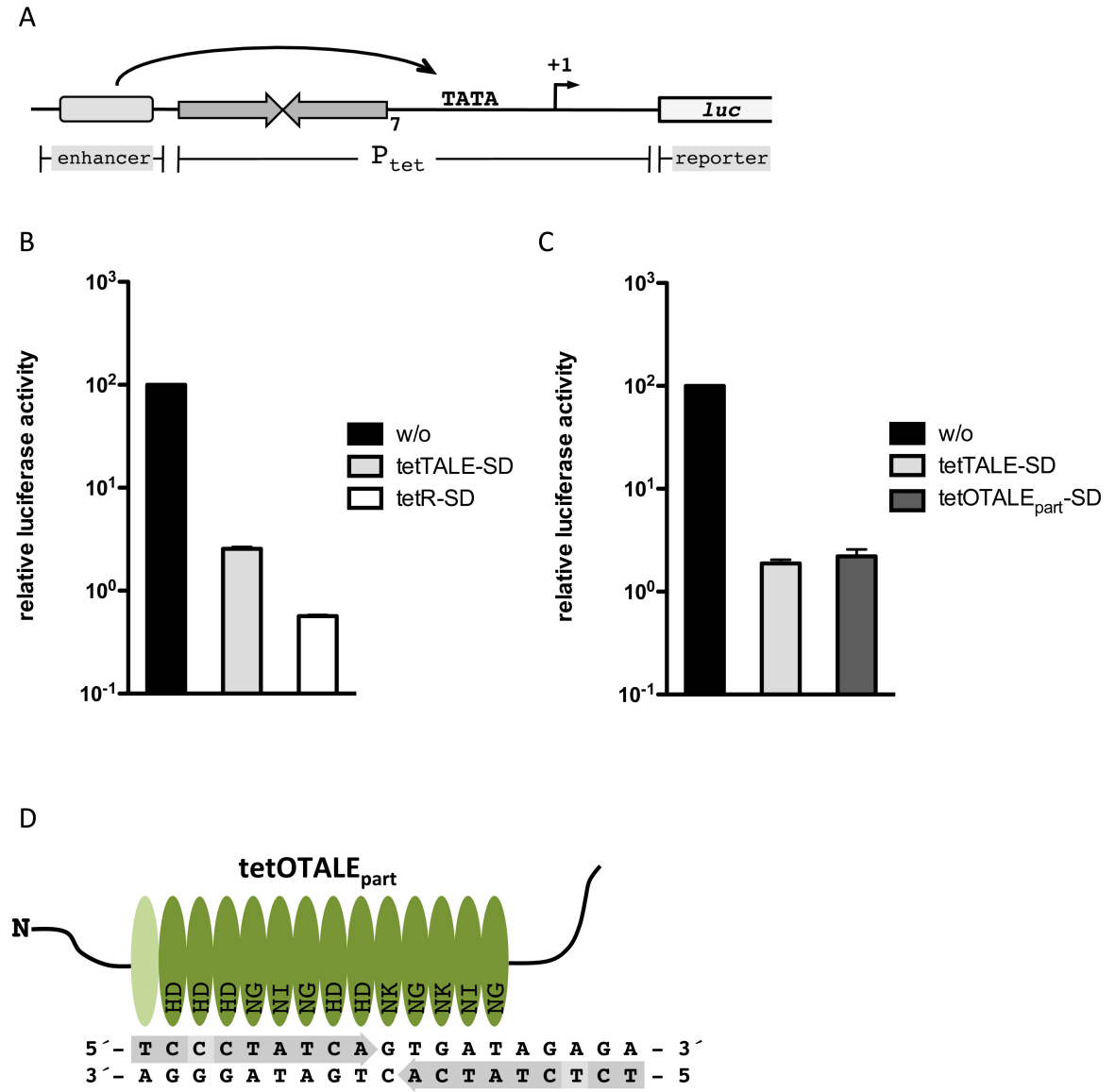
Supplementary Figure 1



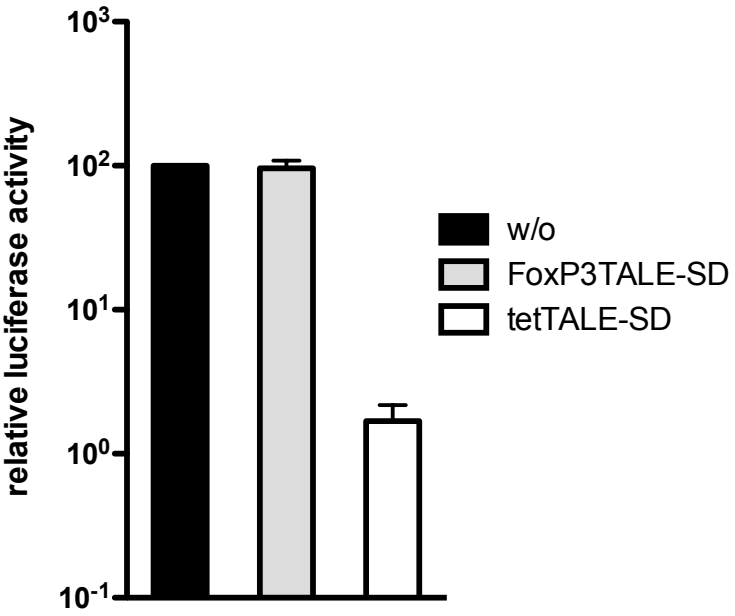
Supplementary Figure 1. Activation capacity of tetTALE-AD compared to tet *trans*-activator (tTAs) using different *tet*O variants. (A) HeLa cells were co-transfected with activation reporter constructs carrying variants of P_{tet} with mutations in the *tet*O sequence and either a tTAs or tetTALE-AD expression construct. A Renilla luciferase expression construct was included for internal standardization. Luciferase activity resulting from P_{tet} luc transfected without activator was set to 1. Shown are mean values of three independent experiments with standard deviation. (B) Schematic representation of the P_{tet} variants differing in the *tet*O sequence.

Supplementary Figure 2



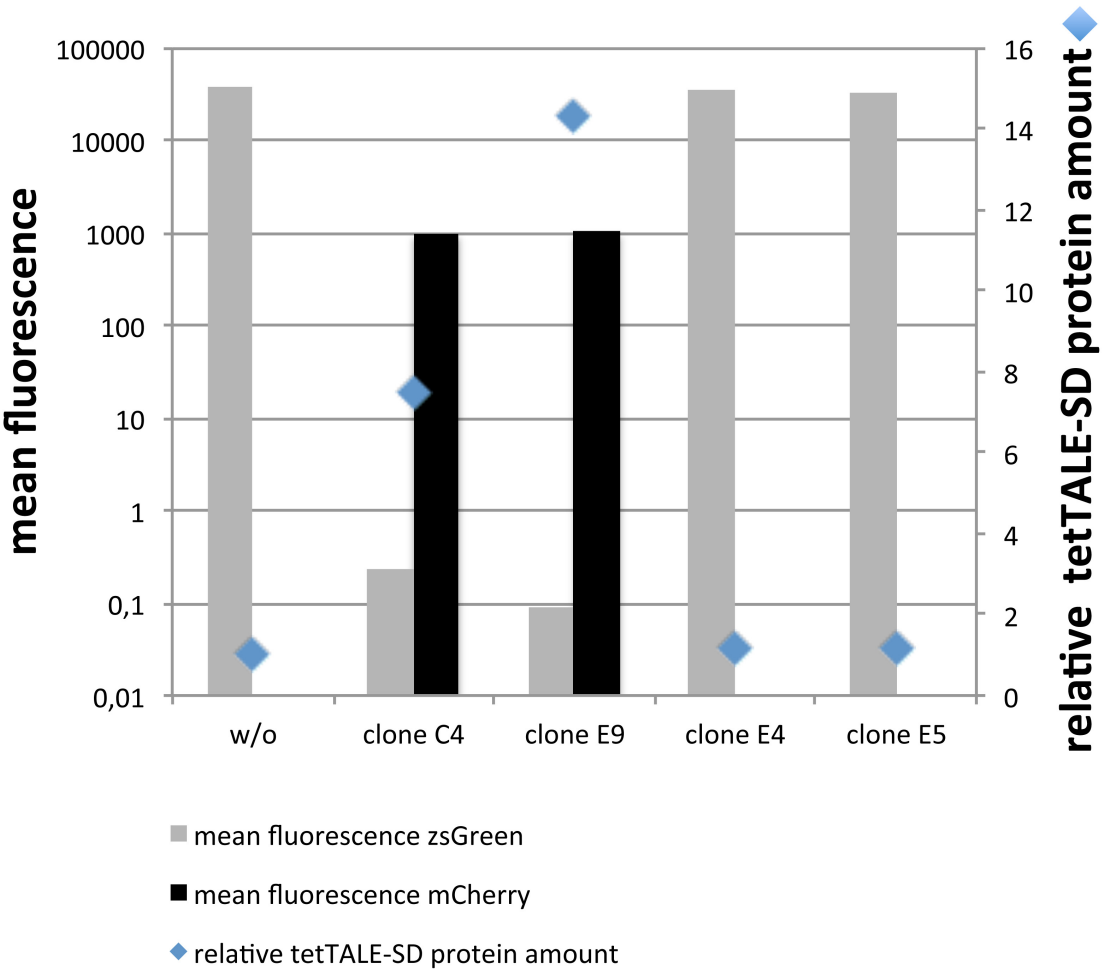
Supplementary Figure 2. Silencing capacity of tetTALE-SD compared to a tet trans-silencer (tetR-SD) and another tet promoter-targeting TALE-SD. (A) Schematic presentation of the reporter constructs for transient silencing experiments. A tet-responsive promoter with an upstream enhancer that renders it constitutively active in driving a luciferase reporter. (B) HeLa cells were co-transfected with the reporter construct carrying heptameric *tet*O sequences and either a tetR-SD or tetTALE-SD expression construct. A Renilla luciferase expression construct was included for internal standardization. Reporter activity in the absence of a silencer was set to 100. Shown are mean values of three independent experiments with standard deviation. (C) HeLa cells were co-transfected with the reporter construct carrying heptameric *tet*O sequences and either tetTALE-SD or another tet promoter-binding TALE-SD (tetOTALE$_{part}$-SD), partially covering the tetO sequence as described by Li et al. (25). A Renilla luciferase expression construct was included for internal standardization. Reporter activity in the absence of a silencer was set to 100. Shown are mean values of three independent experiments with standard deviation. (D) Target sequence of the DNA binding domain of tetOTALE$_{part}$ with the target sequence according to Li et al. (25) and the used RVDs. Light grey boxes mark positions deviating from the operator symmetry.

Supplementary Figure 3



Supplementary Figure 3. TALE-SD mediated silencing depends on the presence of a TALE target sequence. HeLa cells were co-transfected with the reporter construct carrying heptameric *tet*O sequences (shown in Supplementary Figure 2) and either a tetTALE-SD or a TALE-SD targeting the human FoxP3 promoter. A Renilla luciferase expression construct was included for internal standardization. Reporter activity in the absence of a silencer was set to 100. Shown are mean values of three independent experiments with standard deviation.

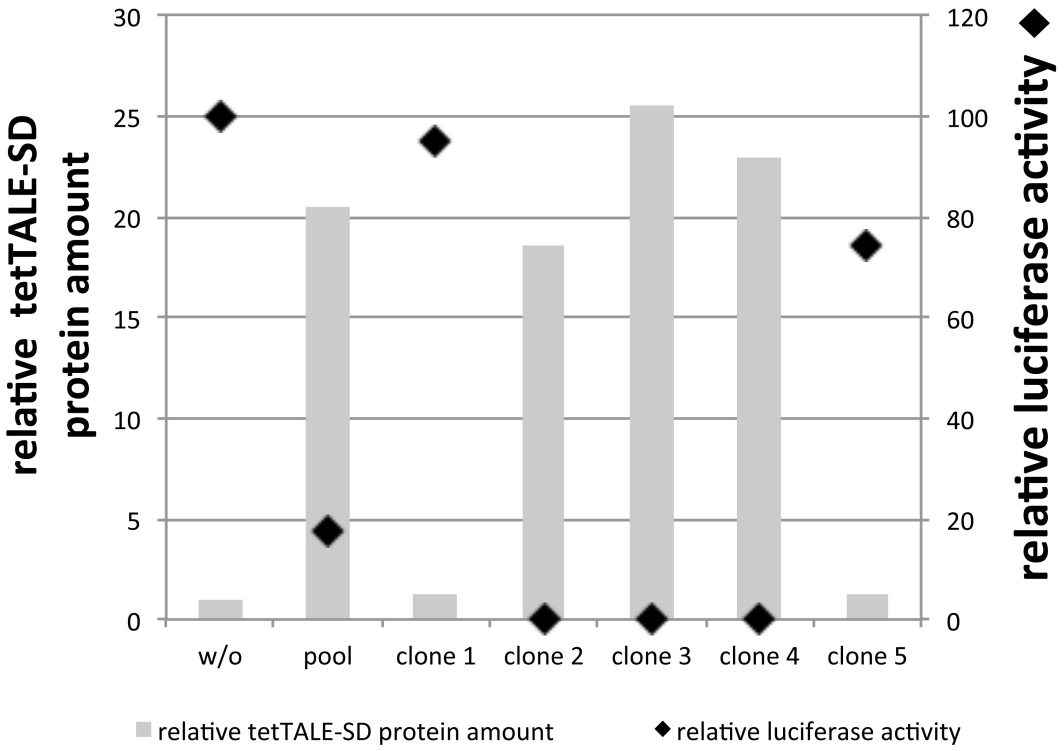
Supplementary Figure 4



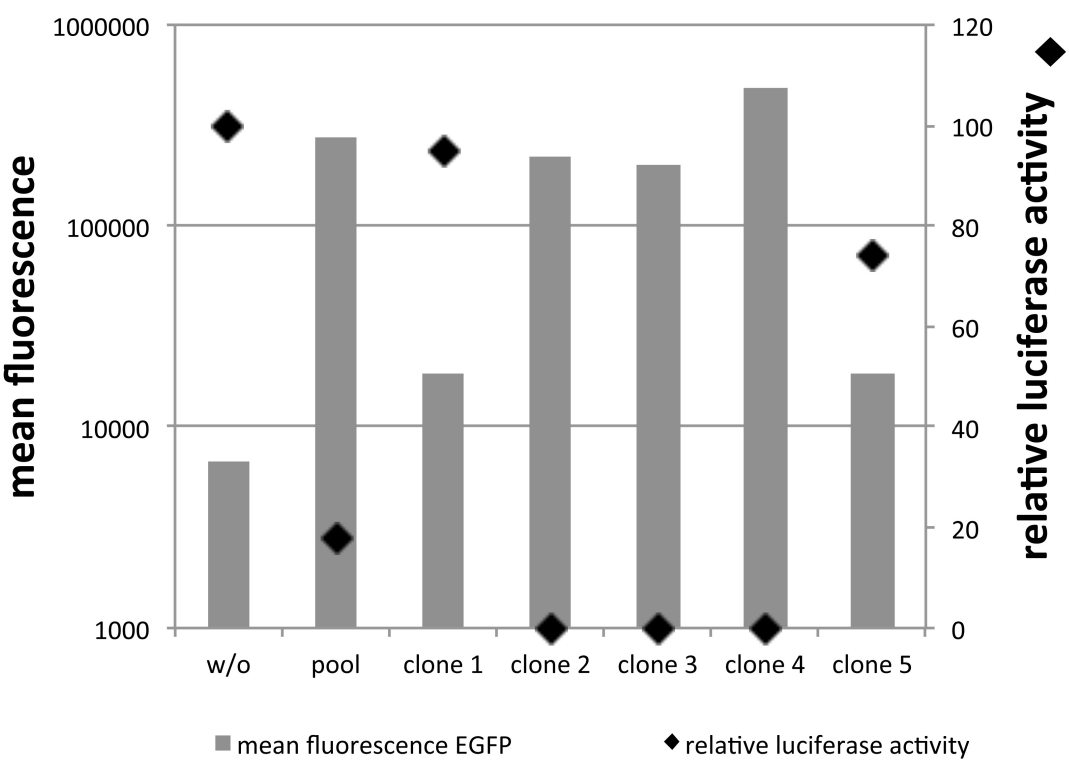
Supplementary Figure 4. Quantification of zsGreen, mCherry and tetTALE-SD protein levels in HAFTL tetEF ZsGreen cells before transfection (w/o) and single clones isolated from the tetTALE-SD+ pool. Fluorescence protein expression levels were measured by analytical FACS. Protein expression was quantified by a fluorescence based immunoblot system. Signal intensity for tetTALE protein expression before transfection was set to 1. Quantification results shown refer to Figure 2.

Supplementary Figure 5

A

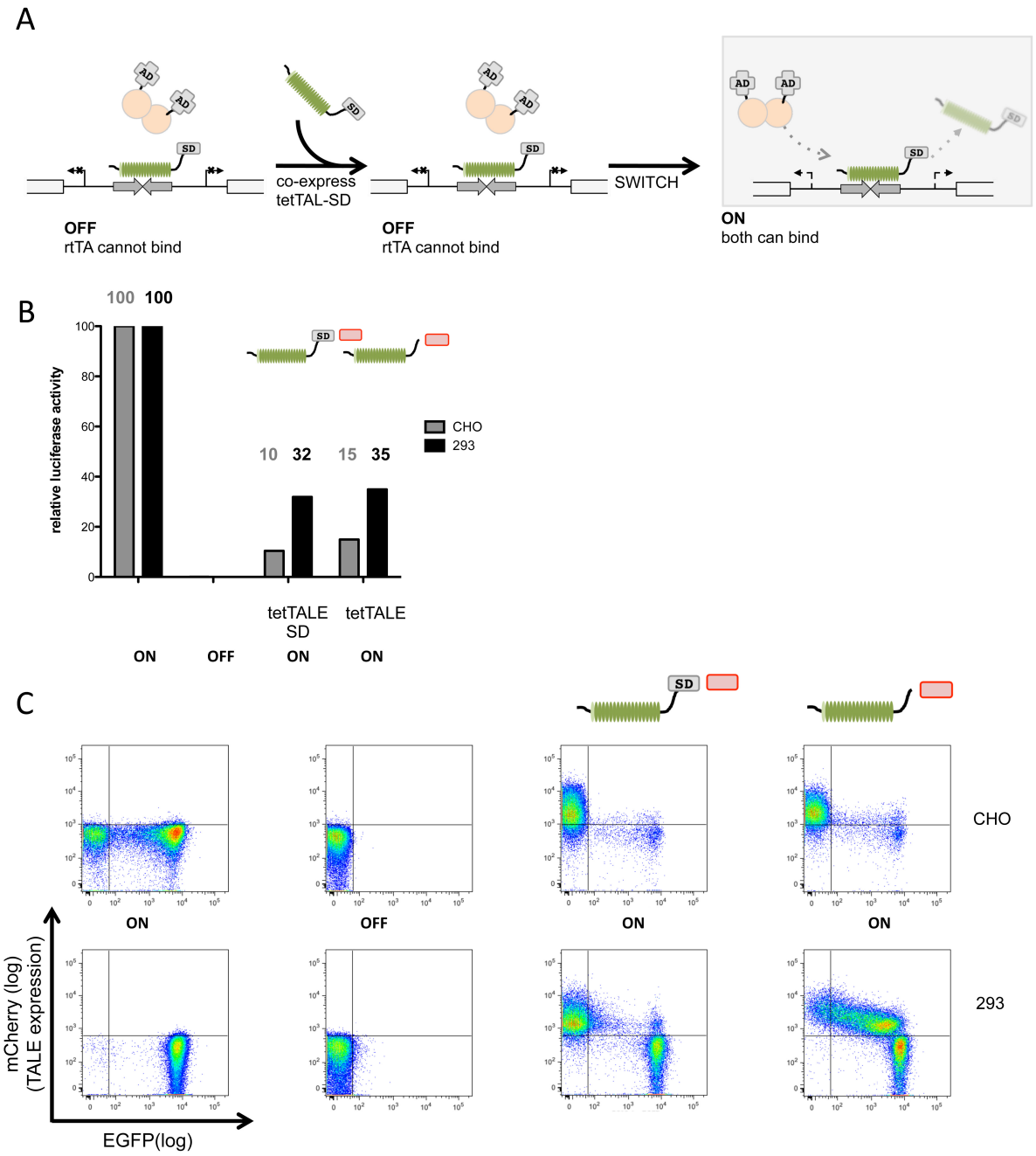


B



Supplementary Figure 5. Correlation between luciferase reporter activity in competion experiments and TALE protein levels as monitored in either FACS quantification (A) or immunoblot analysis(B)
(A) Quantification of tetTALE linked EGFP levels and luciferase reporter activity a in X1/5 cells before transfection (w/o) and single clones isolated from the tetTALE-SD+ pool. Fluorescence protein levels were measured by analytical FACS. Luciferase activity of X1/5 cells before transfection was set to 100. (B) Quantification of tetTALE-SD protein levels and luciferase reporter activity a in X1/5 cells before transfection (w/o) and single clones isolated from the tetTALE-SD+ pool. Protein expression was quantified by a fluorescence based immunoblot system. Signal intensity for tetTALE protein expression before transfection was set to 1. Luciferase reporter activity of X1/5 cells before transfection was set to 100. Quantification results shown refer to Figure 5C, D and E.

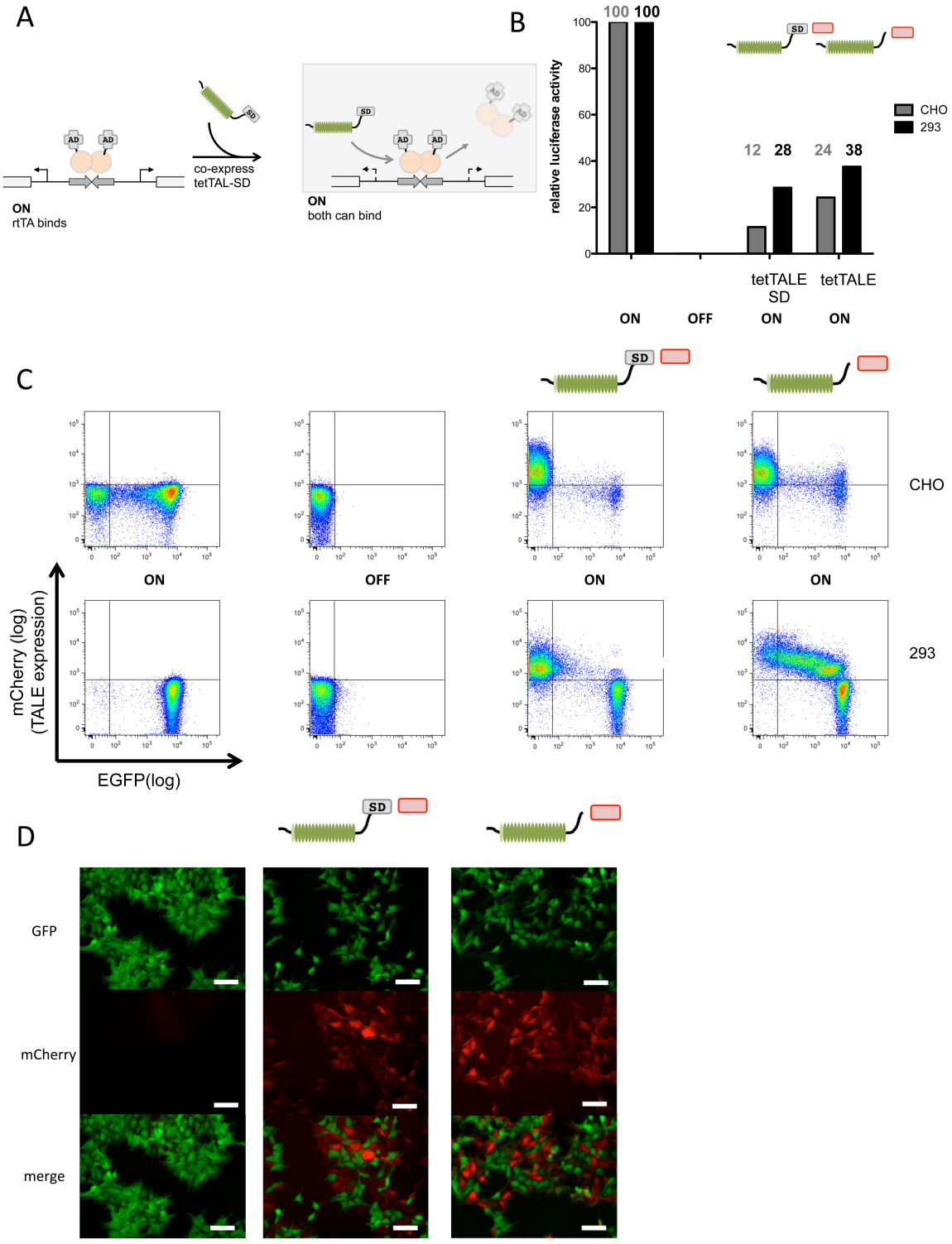
Supplementary Figure 6



Supplementary Figure 6. Competition of tetTALE/ tetTALE-SD with a reverse tTA for the same binding site (A) Experimental setup. CHO K1 Tet-On and HEK293 Tet-On cells containing a bidirectional P_{tet}, driving a luciferase and a EGFP reporter, and a rtTA expression cassette stably integrated were cultured under OFF conditions. Cells were stably transfected with either tetTALE or tetTALE-SD expression construct containing a T2A linked mCherry marker. In the OFF condition only the tetTALE(-SD) can occupy the *tet*O. The Tet system was subsequently switched from OFF to ON. (B) Cell pools stably transfected with either tetTALE or tetTALE-SD were harvested 7 days after the switch of doxycycline conditions and luciferase activity was analyzed P_{tet} mediated luciferase activation of the TALE negative parental cell lines with bound tet activator only was set to 100. (C) Cell pools stably transfected with either tetTALE or tetTALE-SD were harvested 7 days after the

switch of doxycycline conditions. Expression of EGFP and mCherry (coupled to TALE via T2A) was analyzed by FACS.

Supplementary Figure 7



Supplementary Figure 7. Competition between *tet*O bound rtTA and tetTALE/ tetTALE-SD (A) Experimental setup. CHO K1 Tet-On and HEK293 Tet-On cells containing a bidirectional P_{tet} , driving a luciferase and a EGFP reporter, and a rtTA expression cassette stably integrated were cultured under ON conditions where rtTA is bound to *tet*O. Cells were stably transfected with either a tetTALE

or tetTALE-SD expression construct containing a T2A linked mCherry marker (B) Analysis of luciferase activity of cell pools (ON) transfected with either tetTALE or tetTALE-SD. P_{tet} mediated luciferase activation of the TALE negative parental cell lines with bound tet activator only was set to 100. (C) Expression of EGFP and mCherry (coupled to TALE via T2A) was analyzed by FACS in cell pools stably transfected with either tetTALE or tetTALE-SD (D) Microscopic picture of HEK293 Tet-On cells containing a bidirectional P_{tet} , driving a luciferase and a EGFP reporter, and a rtTA expression cassette before and after stable transfection with tetTALE/ tetTALE-SD: EGFP (top), mCherry (middle), merge (bottom) Scale bar: 50 µm