Cell Reports Methods, Volume *2*

Supplemental information

A versatile viral toolkit for functional discovery in the nervous system

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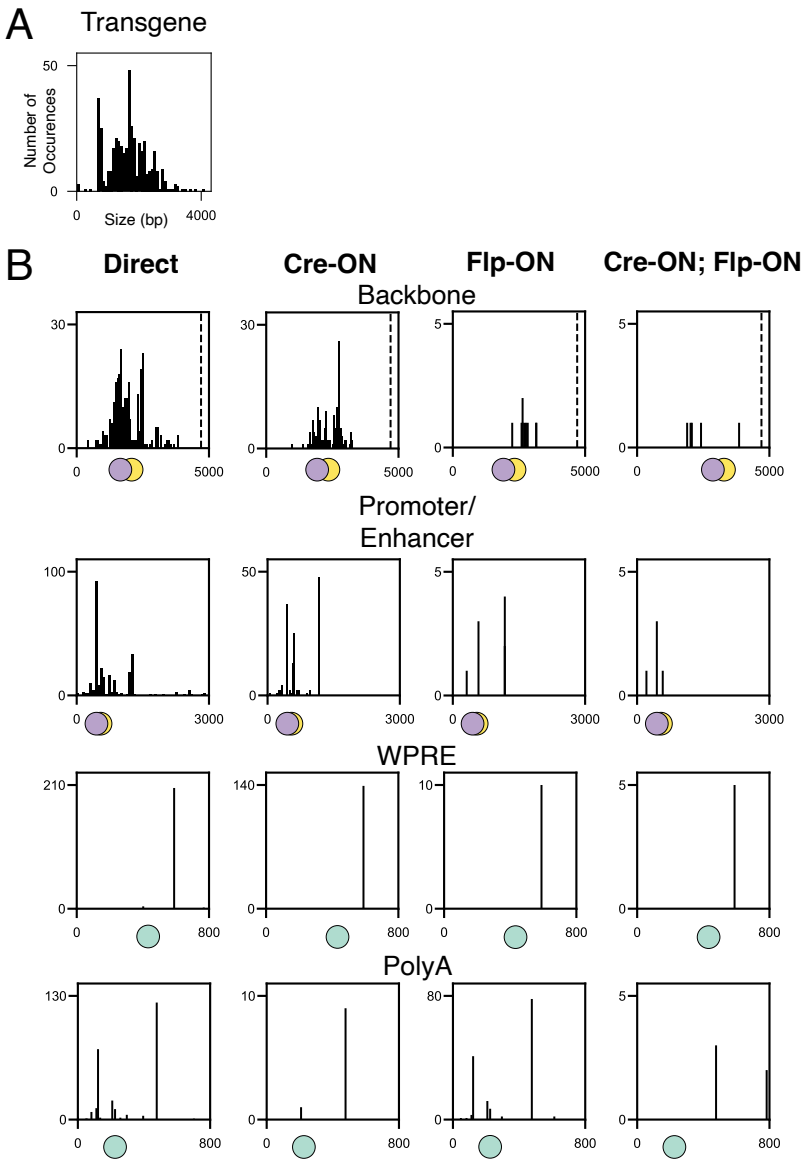
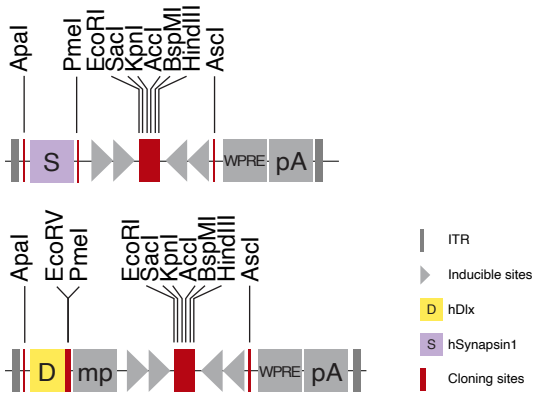
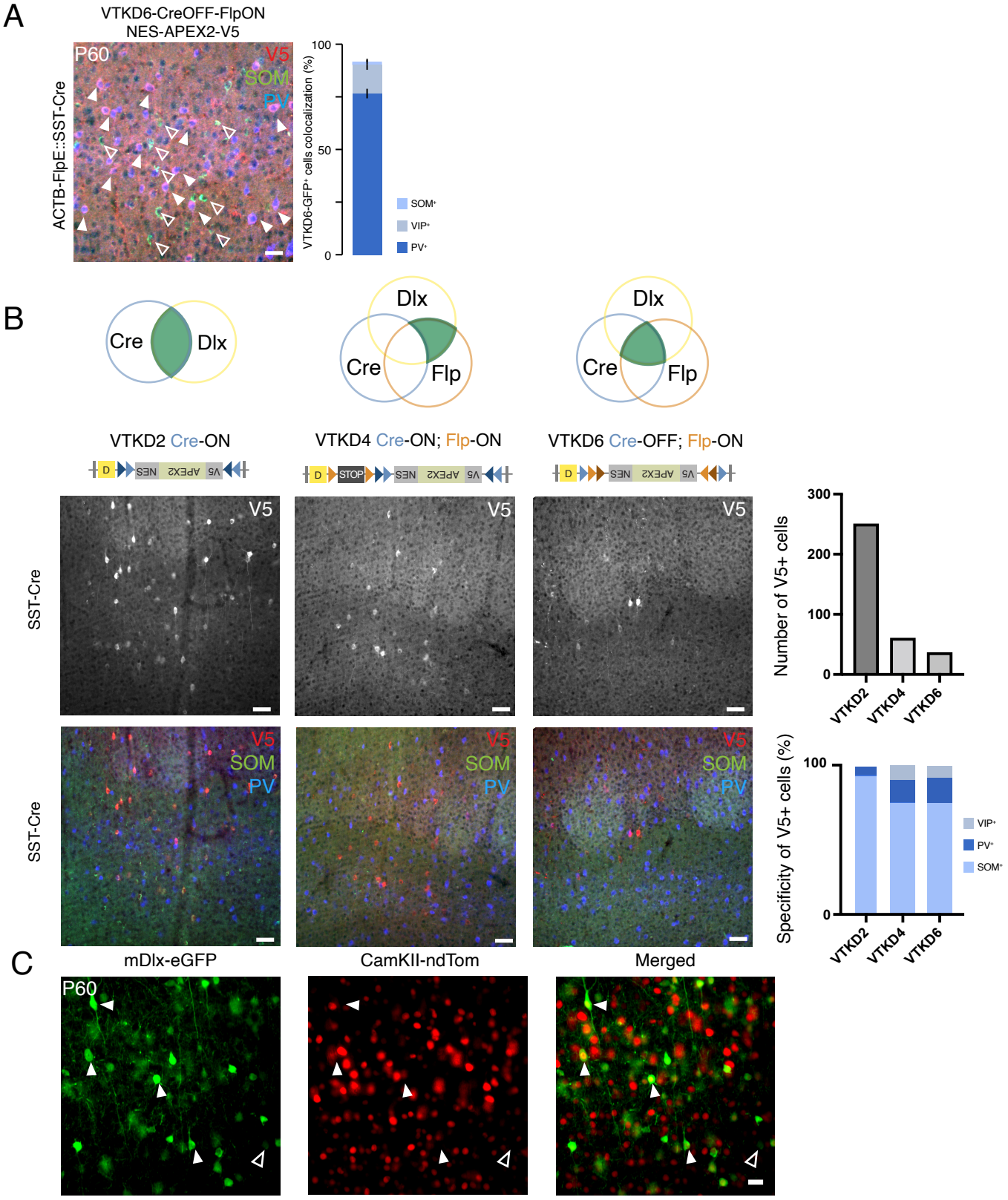


Supplementary Figure 1. AAV vector composition, related to Figure 1. vA) Number of occurrences of the top Addgene plasmids sorted by the size of transgenes. (B) Number of occurrences of backbones, promoter/enhancers, WPREs and polyAs for each Boolean strategy, sorted by size (base pairs, bp). The dotted line shows the 4.7kb payload limit. The colored dots on the x-axis indicate the size of the corresponding elements used in VTK vectors. Green dots for common elements to all VTKs (WPRE and polyA), purple dots for VTKS1–6 and yellow dots for VTKD1–6.

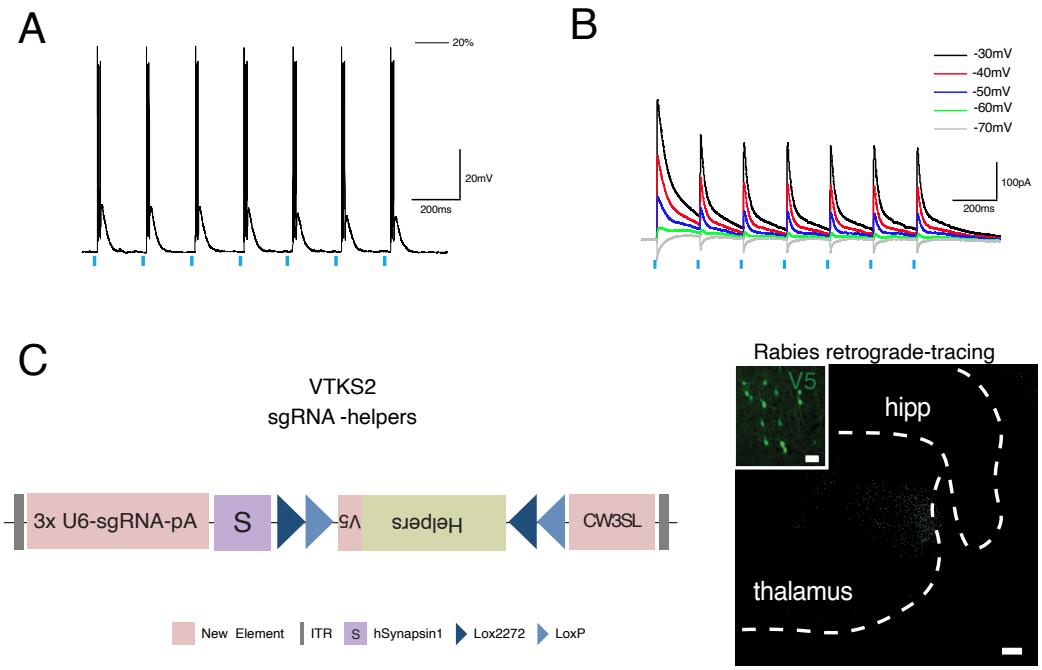


Supplementary Figure 2. VTK multiple cloning sites, related to Figure 2.
DNA-synthesis of VTK backbones allowed for the strategic insertion of cloning sites, represented in red, in the backbone diagrams for VTKS and VTKD. mp: minimal promoter; pA: polyA.mato expression.



Supplementary Figure 3. Validation of constructs and co-injection of AAVs, related to Figure 3

(A) Validation of VTKD6. Left, colocalization of V5 labeling with parvalbumin (PV, full arrows), but not SOM (empty arrows) in somatosensory cortex. Scale bar 100 µm. Data are shown as mean ± s.e.m. Right, quantification of V5 colocalization with PV 76.6% ± 2.3; VIP 13.6 % ± 2.2; SOM 1.5 % ± 1.5 (N=3 sections) showing the recombination of VTKD6-NES-APEX2 in GABAergic interneurons and primarily in PV+ cells. (B) Assessment of VTKD4 and VTKD6 expression in SSTcre animals. Top: Number of V5+ cells per area of 200 mm^2 around the injection site upon bilateral local injection of VTKD2, VTKD4 and VTKD6 in the somatosensory cortex. Note that the number of cells in VTKD2 is higher than VTKD4 or VTKD6 (250, 60 and 36, respectively), demonstrating the utility of the Frt-Stop-Frt cassette to prevent expression in the absence of Flp-recombinase. The non-null level of expression needs to be taken into consideration by users for specific experimental strategies. Bottom: Specificity of V5+ cells for indicated markers. Out of the 250 cells expressing the reporter with VTKD2, 94% of them colocalize with SST and only 6% with PV. Out of the 60 cells expressing VTKD4, 75% colocalize with SST, 15% with PV and 10% with VIP. Out of the 36 cells expressing VTKD6, 75% colocalize with SST, 17% with PV and 8% with VIP. (C) Co-injection of the indicated AAVs leads to unexpected co-localization of mDlx-driven eGFP expression with CamKII-driven nls-dTomato expression (full arrows: colocalization; empty arrows: mDlx-eGFP+ cells only) (N=3). Scale bar 100 µm.



Supplementary Figure 4. Optogenetic and retrograde tracing, related to Figure 4.
(A) Depolarization and burst firing of VTKD2-ChR2-mCherry infected interneuron upon blue light stimulation at 20% intensity. (B) Representative example traces of light evoked IPSC current-voltage relationship (IV) showing reversal potential around -60mV in a CA1 pyramidal cell. (C) Test of W3SL with large transgenes comprising rabies helpers and CRIPSR guides. Left : plasmid composition indicating the position of W3SL replacing the WPRE+pA; Right: V5 staining in the cortex (inset) and corresponding retrograde projections in the thalamus. Scale bar 200 µm.