Table S1. List of primer sequences:

Gene	Forward (5’-3’)	Reverse (5’-3’)
M1-AChR	GCTCAAAGTGGGTGCCCTTG	GGAGGGAGGAAGGGAAAGAGAG
M2-AChR	CATGCCTGGTGGTGATGGTG	GGCCCAGGGAAGTGGAAAC
M3-AChR	TTATGAACCGCTGGGCTCTG	AATCATCACACCGGCTCGTT
M4-AChR	GCTAGTTCCGCCGTCTGTCC	CAGGTGGTTGTGGGCTGTTG
M5-AChR	GCATGGCTGGTCTCCTTCATC	CCCGGTAGATCCGGCAGTAG

Figure S1. pM1shRNA enables Cre-dependent knockdown of M1-AChR, but did not influence mRNA levels of other mAChRs

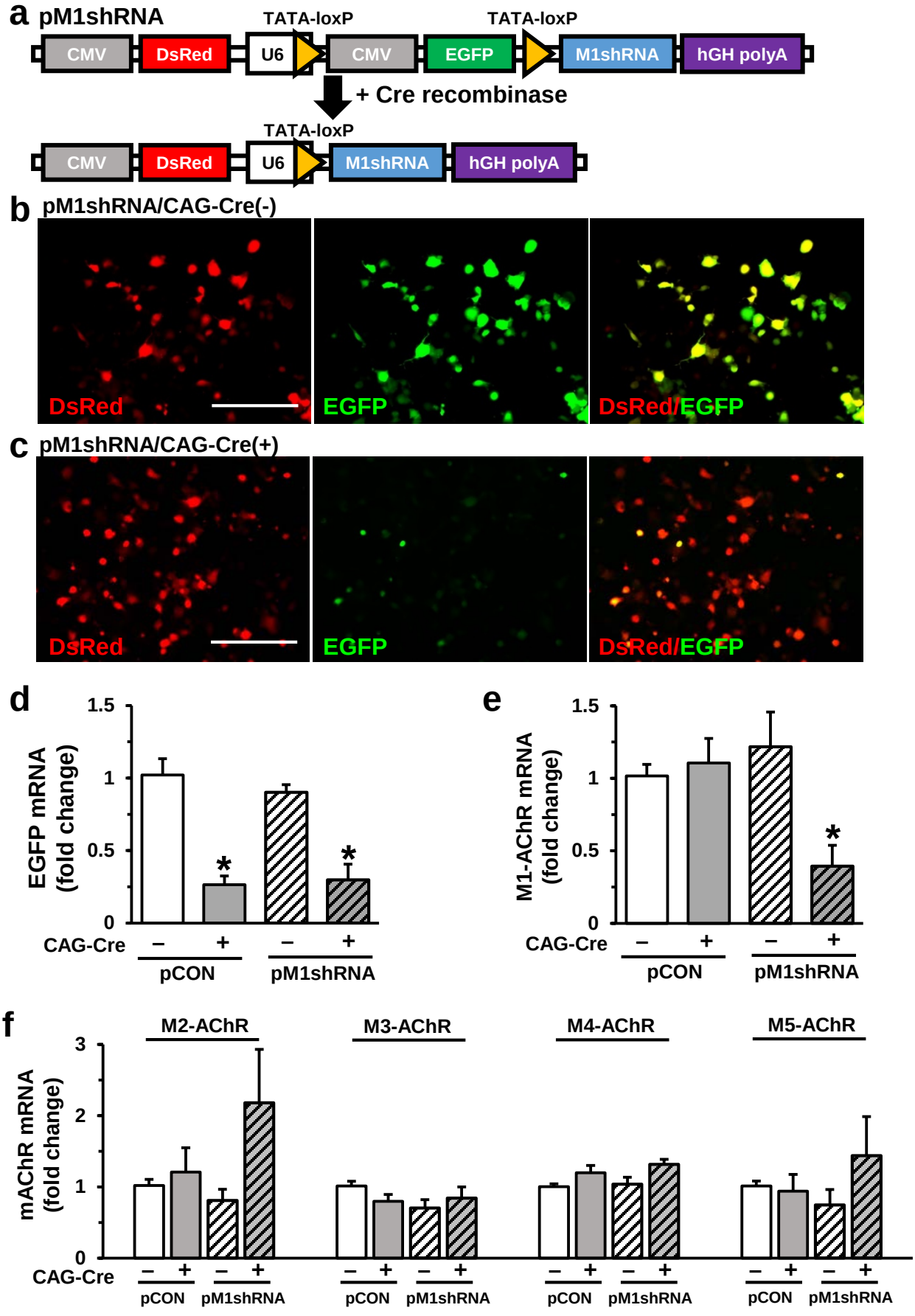


Figure S2. M1-AChR knockdown in CaMKII^{CRE} and SST^{CRE} mice in a neuron-specific manner.

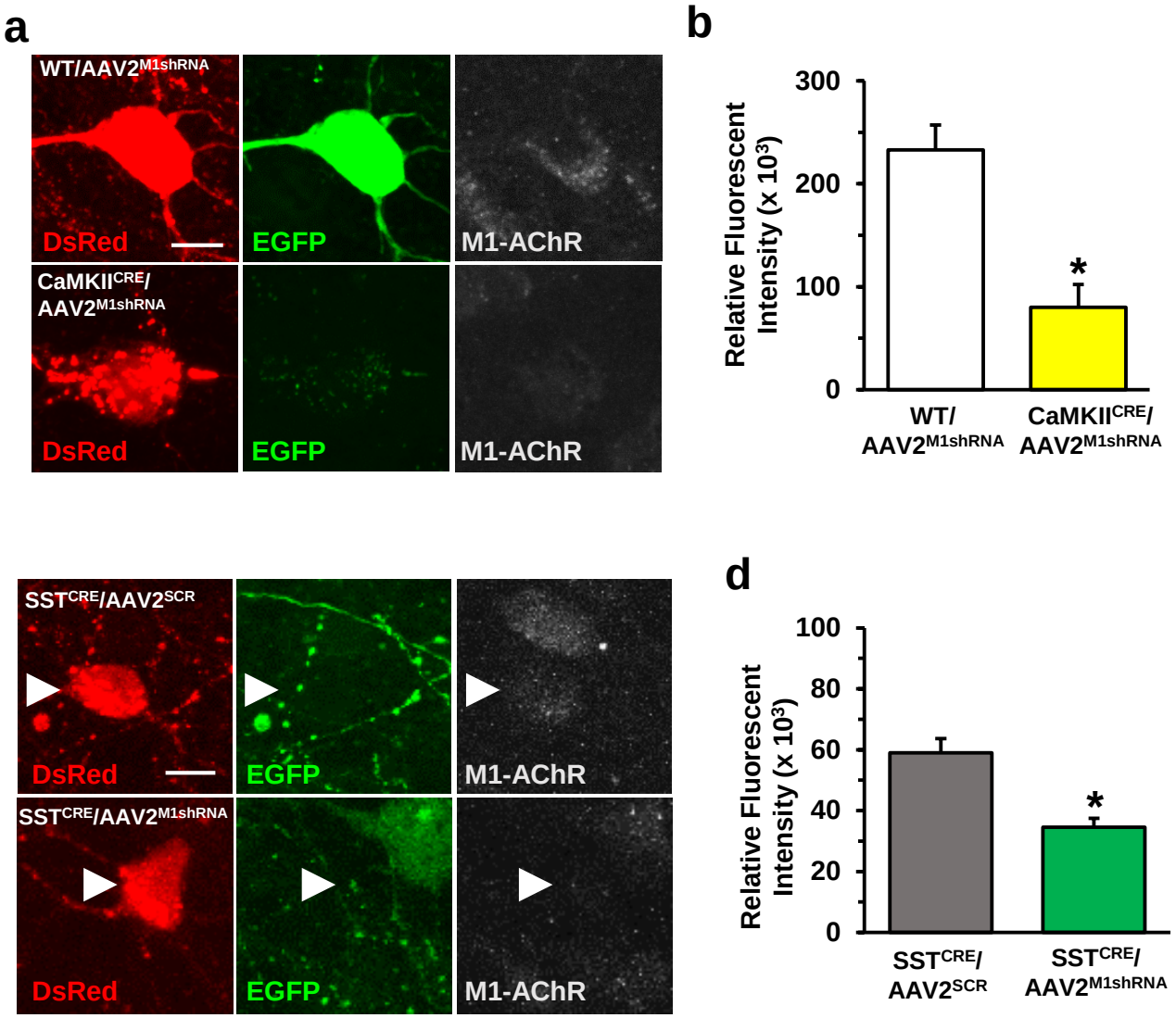


Figure S3. M1-AChR knockdown in CaMKII^{CRE} mice did not affect FosB activation following scopolamine treatment.

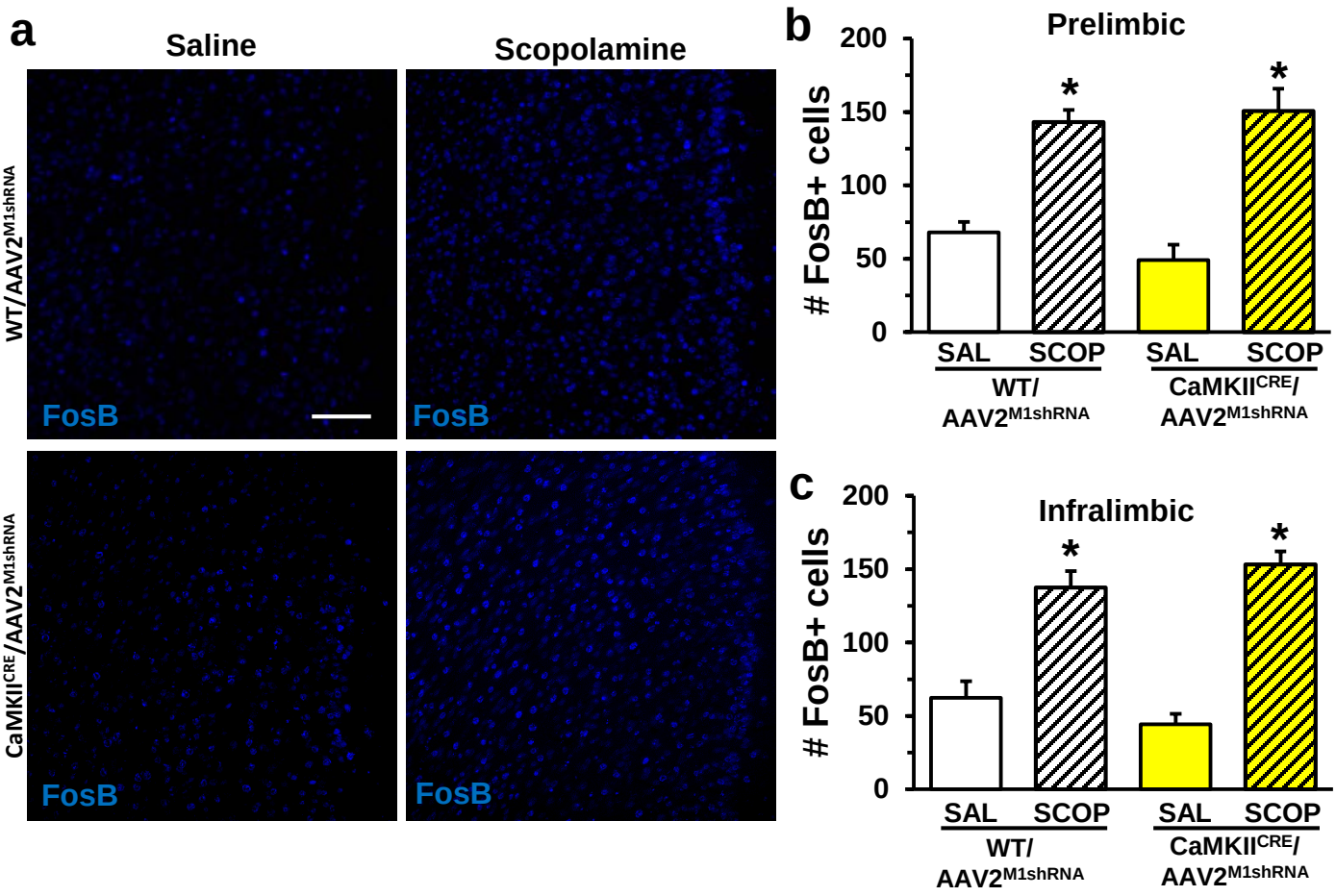


Figure S4. Somatostatin and parvalbumin interneurons have varied distribution in the mPFC.

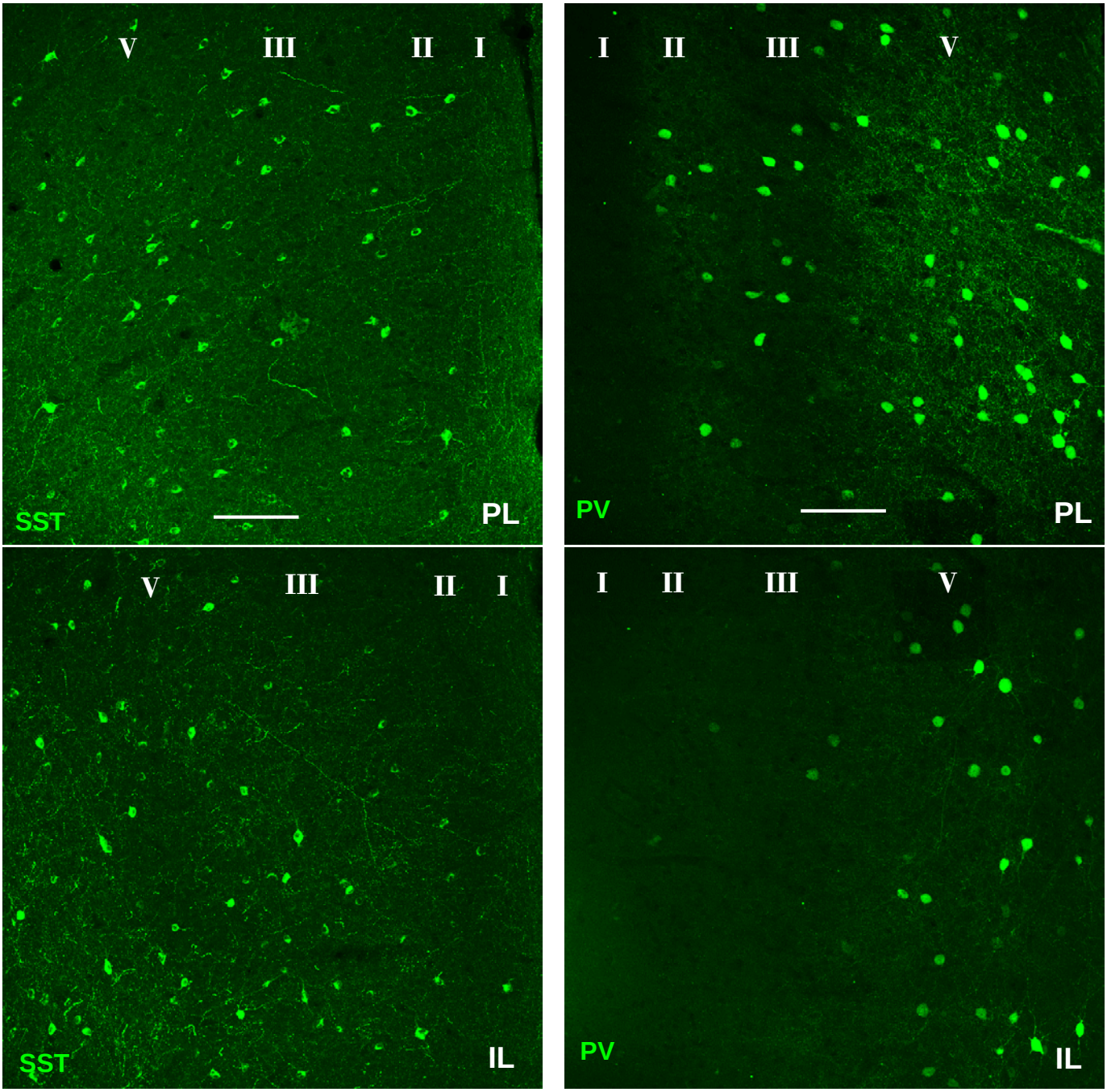


Figure S5. AAV2^{M1shRNA} infusion in the mPFC of SST^{CRE} or PV^{CRE} mice did not influence baseline behavior.

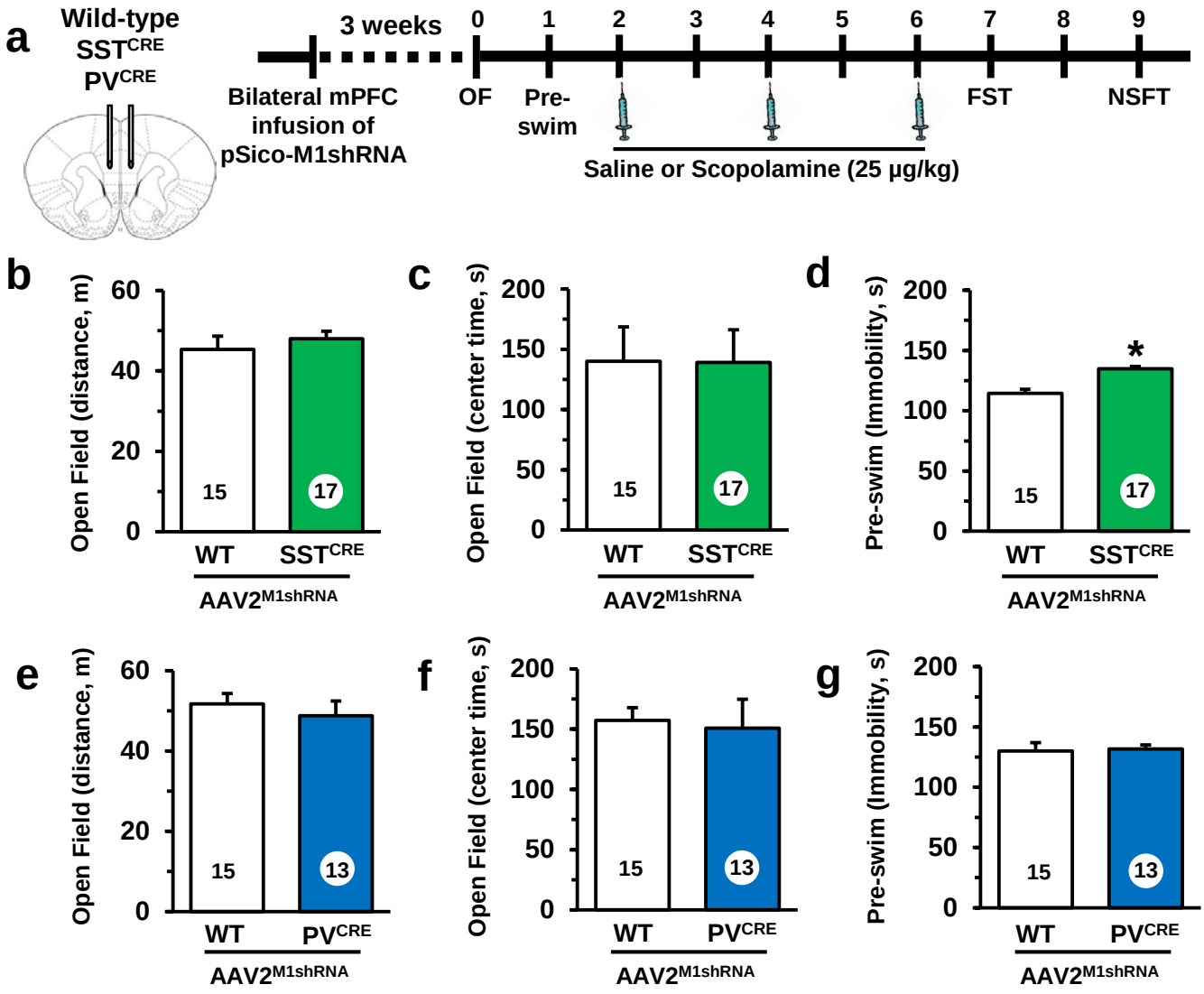
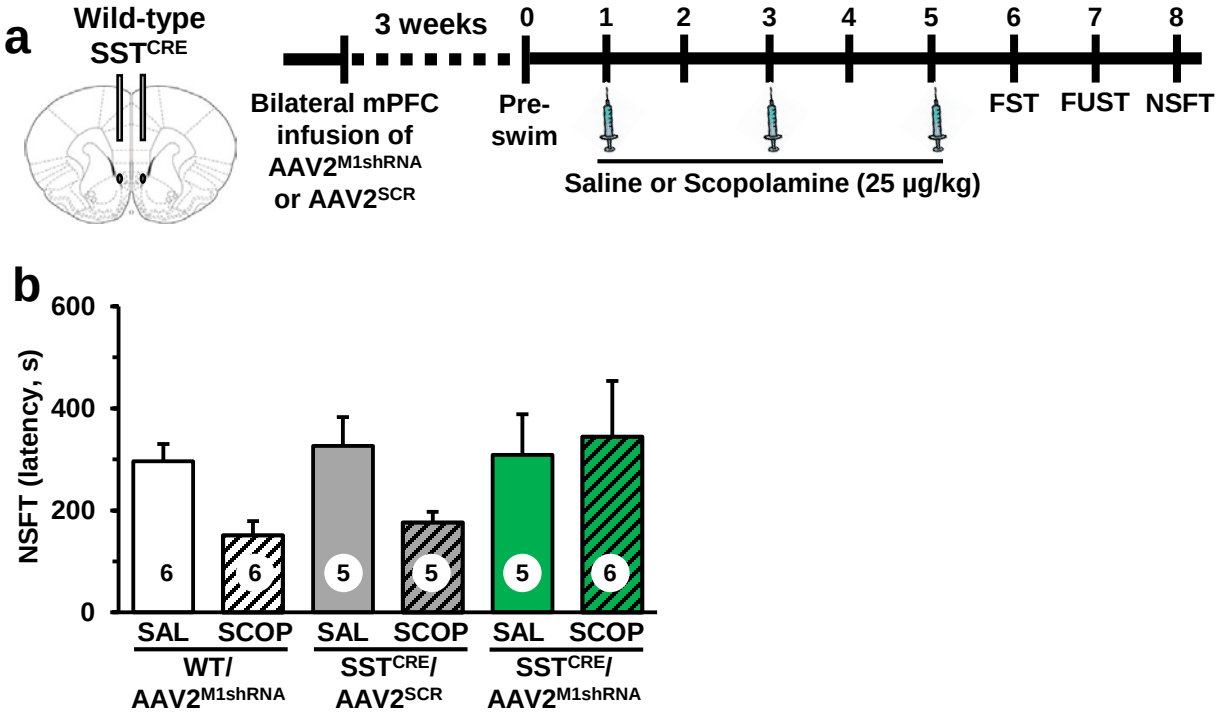


Figure S6. AAV2^{M1shRNA}, but not AAV2^{SCR}, infusion in the mPFC of SST^{CRE} mice blocked scopolamine antidepressant-like effects in NSFT.



Supplemental Figure Legends:

Table S1. List of primer sequences.

Figure S1. pM1shRNA enables Cre-dependent knockdown of M1-AChR, but did not influence mRNA levels of other mAChRs. a) Layout of pM1shRNA construct and Cre-induced recombination enabling U6 driven expression of M1shRNA. **b)** Representative images of N2a transfection with pM1shRNA alone (no CAG-Cre) showing expression of DsRed and EGFP expression. **c)** Representative images of N2a co-transfection with pM1shRNA and CAG-Cre showing DsRed expression and limited EGFP after recombination. Relative gene expression of EGFP **(d)**, M1-AChR **(e)**, and other mAChRs **(f)** in transfected N2a cultures.

Figure S2. M1-AChR knockdown in CaMKII^{CRE} and SST^{CRE} mice in a neuron-specific manner. a) Representative images of DsRed, EGFP, and M1-AChR in pyramidal neurons from wild-type (WT) or CaMKII^{CRE} mice infused with AAV2^{M1shRNA}. **b)** Quantification of M1-AChR relative fluorescent intensity in WT and CaMKII^{CRE} mice. **c)** Representative images of DsRed, EGFP, and M1-AChR in recombined interneurons from SST^{CRE} mice infused with AAV2^{SCR} or AAV2^{M1shRNA}. **d)** Quantification of M1-AChR relative fluorescent intensity in SST^{CRE} mice. Means that are significantly different than respective control group based on *T*-test are noted by ($*$, $p < 0.05$).

Figure S3. M1-AChR knockdown in CaMKII^{CRE} mice did not affect FosB activation following scopolamine treatment. a) Represenative images of FosB immunolabeling in the prelimbic mPFC of wild-type (WT) or CaMKII^{CRE} mice infused with AAV2^{M1shRNA} and treated with saline or scopolamine. White scale bar represents 100 μm. Quantification of FosB+ cells in the prelimbic **(b)** and infralimbic **(c)** mPFC. Means that are significantly different than respective saline group based on ANOVA are noted by ($*$, $p < 0.05$).

Figure S4. Somatostatin and parvalbumin interneurons have varied distribution in the mPFC. Representative images of somatostatin (SST) and parvalbumin (PV) immunolabeling in the prelimbic (PL) and infralimbic (IL) regions of the mPFC. Approximate laminar regions (I-V) are noted. White scale bar represents 100 μm.

Figure S5. AAV2^{M1shRNA} infusion in the mPFC of SST^{CRE} or PV^{CRE} mice did not influence baseline behavior. As in Fig.7, wild-type (WT) littermates and SST^{CRE} or PV^{CRE} mice were infused with AAV2^{M1shRNA} and treated with saline or scopolamine **(a)**. In SST^{CRE} mice baseline open-field distance traveled **(b)**, time in center of open-field **(c)**, and immobility in pre-swim test **(d)** are shown. In PV^{CRE} mice baseline open-field distance traveled **(e)**, time in center of open-field **(f)**, and immobility in pre-swim test **(g)** are shown.

Figure S6. AAV2^{M1shRNA}, but not AAV2^{SCR}, infusion in the mPFC of SST^{CRE} mice attenuated scopolamine antidepressant-like effects in NSFT. As in Fig.9, wild-type (WT) littermates and SST^{CRE} mice were infused with either AAV2^{M1shRNA} or scrambled control (AAV2^{SCR}) and treated with saline or scopolamine **(a)**. Following saline or scopolamine treatment, latency to feed in the NSFT is shown **(b).**