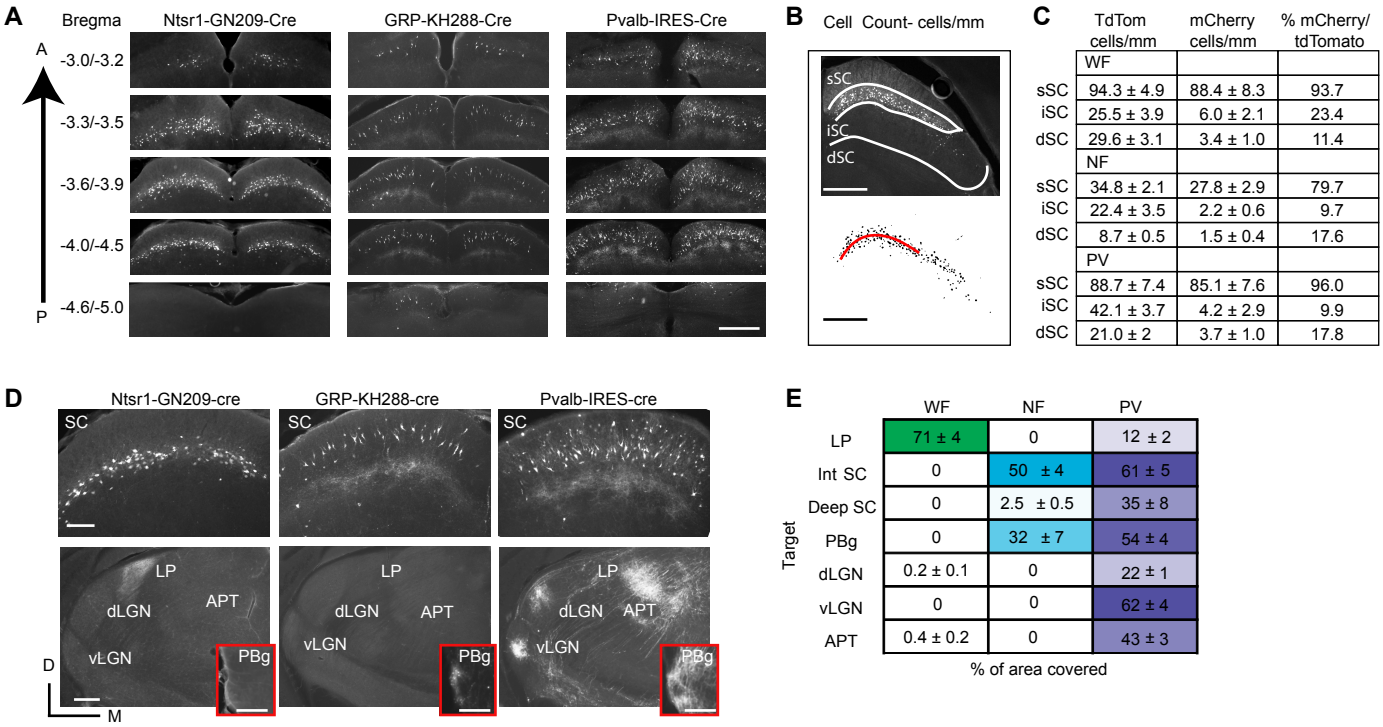

Figure S1. Histological characterization of expression and projection patterns in three Cre lines. Related to Figure 1.

(A) Representative distribution of mCherry expression in the superficial Superior Colliculus of all three cre lines from mice injected with AAV8-hSyn-DIO-hM4Di-mCherry (iDREADDs). A = Anterior and P = Posterior. Scale bar = 500 µm. (B) Schematic of SC laminar divisions and cell count procedure cells/mm were based on counting the number of cells labeled along the length of the red line. Scale bar = 300 µm. C) Quantification of the number of fluorescently labeled cells in the different lamina of SC in virus injected mice versus Ai14 reporter mice crossed to the cre lines. sSC= superficial Superior Colliculus, iSC= intermediate Superior Colliculus and dSC= deep Superior Colliculus. Error bars are +/- SEM and n=10, 12 & 9 or 3, 4, 3 for virus injected mice versus Ai14 reporter mice, respectively. (D) Representative images of mCherry fluorescence in the SC of virus injected mice (top), the thalamus (bottom) and ipsilateral PBg (inset). Scale bars = 200 µm. (E) Quantification of axonal projection density to different target regions for each Cre line. Fluorescence was quantified in all structures where significant fluorescence was observed in at least one of the three lines. LP = Lateral Posterior Nucleus, dLGN = dorsal Lateral Geniculate Nucleus, vLGN = ventral Lateral Geniculate Nucleus, APT = Anterior Pretectal Nucleus, and PBg = Parabigeminal Nucleus. Error bars are +/- SEM and n=10, 12 & 9 mice.

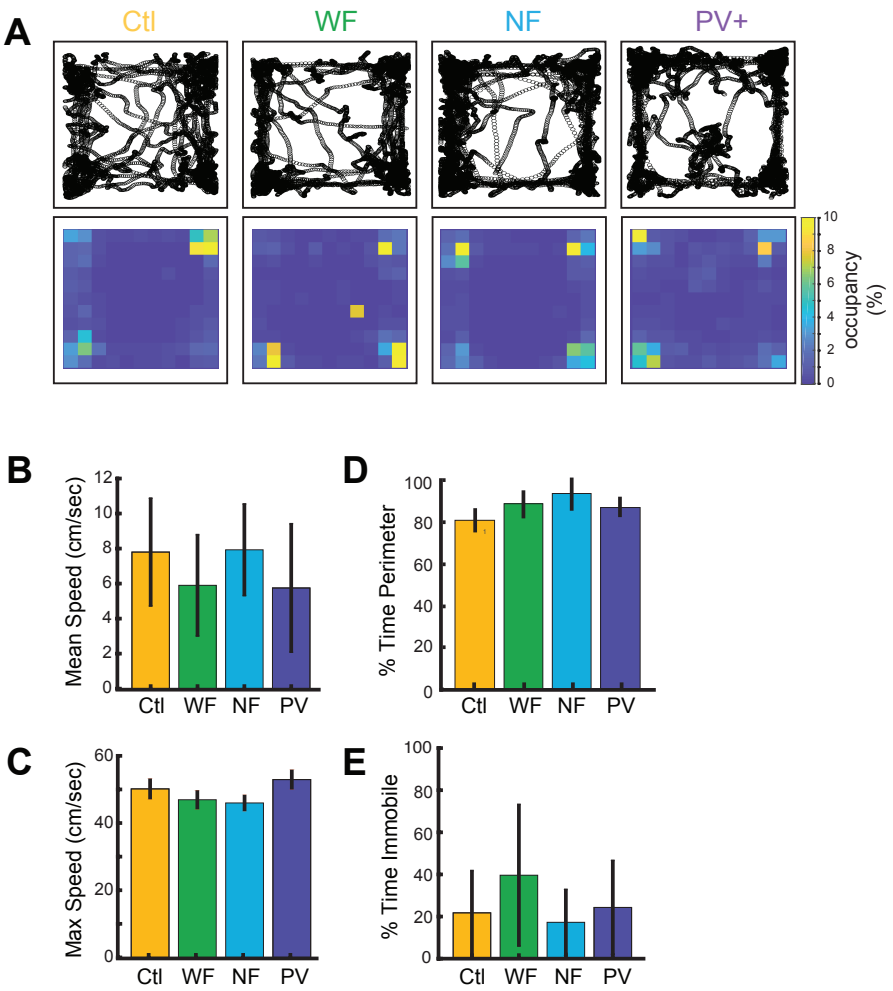

Figure S2. Spontaneous locomotion and exploratory behavior are unaffected by iDRE-ADD-mediated suppression. Related to Figure 2.

(A) Representative tracks (top) and occupancy maps (bottom) for all four groups of experimental animals during free exploration of the arena in the absence of prey following CNO injection. (B) Mean and (C) maximum locomotion speed during free exploration following CNO injection. (D) Mean percent time spent in the perimeter of the arena and (E) percent time immobile following CNO injection. Data are trial averaged per mouse, n=10, 10, 12, 9. Significance tested by Kruskal-Wallis, =0.05. Error bars are +/- SEM.

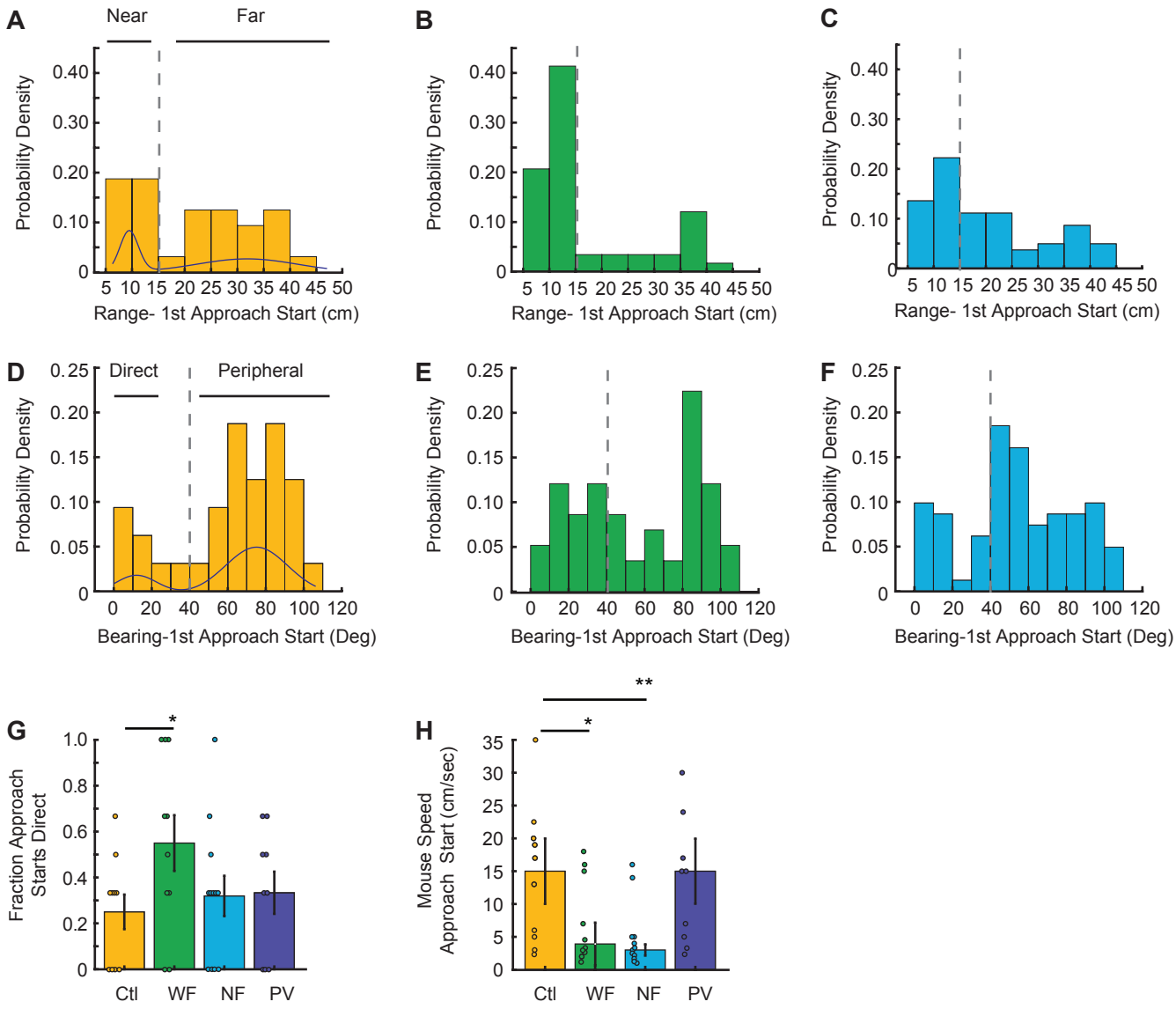

Figure S3. Behaviors preceding approach starts related to prey capture success. Related to Figure 3. (A) Distribution of ranges where first approach starts in control mice (orange). Dark blue trace is the fit to the control distribution highlighting two separate peaks in the distribution of the data above and below 15 cm. (B) Approach start ranges for mice with WF cells inhibited (green) and (C) for mice where NF cells are inhibited (blue). Grey dashed line in A-C is the threshold used segregate near versus far approaches, based on the fitted data from control mice (see Methods). Histograms were generated from all first approaches in each group, 2-3 trials for each mouse. (D) Distribution of mean head angle relative to cricket (bearing) in the 500 ms prior to approach starts for the control mice (orange). There are two modes in the control distribution, one corresponding to the cricket nearly in front (direct) and another one corresponding to the cricket more peripheral. (E,F) Distribution of mean head angles prior to approach for WF mice (E) and NF mice (F). Grey dashed line in D-F represents the threshold to divide the distribution into direct and peripherally centered head angles, based on fitted data from control mice (see Methods). (G) Mean fraction of approaches started from direct head angles per subject. Error bars are ± SEM. (H) Trial averaged median speed of each mouse prior to successful approaches 500ms prior to approach. Error bars are standard error of the median. Significance tested using Kruskal-Wallis, =0.05, followed by Mann-Whitney-U and Dunn-Sidak correction for multiple comparisons. Number of trials=29, 29, 35 and 25, n = 10, 10, 12, 9, control, WF, NV and PV mice, respectively. **=p<0.01, *=p<0.05.