

oG	1	ATG GTCCCA CAGGCTCTC CTCTCGTCCCTCTGCTGCTTCCCACTCTGCTTTGGT A	oG	1021	CTGATGAGGGCAGACGCCATTATAAATCTGTG AGAAC CTGGAATGAAATCATTCTAGT
PBG	1	ATGGTTCTCAGGCTCTCCTGTTGTACCCCTTCTGGTTTTCCATTGTGTTTTGGGAAA	PBG	1021	TTGATGGAAGCCGATGCTCACTACAAGTCAGTCAGA ACTT GGAATGAGATCATCCCTCA
oG	61	TTCCCTAT CTACACTATCCCC GACAAG CTGGGC CCCTGG AGTCTATCGATATCCACAT	oG	1081	AAGGGATGCCTGCGAGTC GGGG CTCGATGT C CCCCA CT GTG AACGG GTCTCTTTAAT
PBG	61	TTCCCTATTTACAGATACCAGACAAGCTTGGTCCCTGGAGCCGATTGACATACATCAC	PBG	1081	AAAGGGTGTAAAGAGTTGGGGGAGGTGTCTATCTCATGAAACGGGGTATTTTCAAT
oG	121	CTGTCA TGC CCTA ACAAT CTCGTGGT CGAGGACGA AGGCTGT ACCAAC CTGAGCGGATTC	oG	1141	GGTAT CATTCTGGGGCAGATGT AAC CTGTGAT CCCCGAGAT GCAGTCTAGTCTCTC
PBG	121	CTCAGCTGCCAAACAATTTGGTAGTGGAGGACGAAGGATGCACCAACCTGTCAAGGTTTC	PBG	1141	GGTATAATATTAGGACCTGACGGCAATGTCTTAATCCAGAGATGCAATCATCCCTCCTC
oG	181	TCCTACAT GGAGT GAAA GTGGGCTATATCTCTGCTATTAAG ATGAACGGG TTCA TATGC	oG	1201	CAGCAG CACATGGA ACT GTCTGTCAAGCGT CATTCC CTGATGCATCTCTCG CAGAC
PBG	181	TCCTACATGGAAC TTAAAG TTGGATACATCTCAGCCATAAAAATGAACGGG TTCACTTGC	PBG	1201	CAGCAACATATGGAGTTGGTATCTCGGTTATCCCTTATGCACCCCTGGCAGAC
oG	241	ACTG GT GTGGT CACCGAG GCA GAAACCTAC ACAAATTTTGGGGA TATGTC ACCACA ACT	oG	1261	CCAAGC AG GTGTTCAA AAC GGC GACGAG GCCGA GATTT GTGGAGT CAC CTGCC
PBG	241	ACAGGCGTGTGACGGAGGCTGAACCTACACTAACTTCGTTGGTTATGTCACAACCACG	PBG	1261	CCGCTACCGTTTTCAAGAACGGTGACGAGGCTGAGGATTTTGTGAAGTTACCTTCCC
oG	301	TT CAAG AGA AAACACTTT CGG CCA ACACCA GACG CT GTGGG CAGCTTACAAC CTGGA AG	oG	1321	GAC GTGCACGAACGGAT CAGTGGCTCGATCTCGGACTGCCTAAT TTGGG CAAGTACG TG
PBG	301	TTCAAAAGAAAGCATTTCGCGCCAACACAGATGCATGTAGAGCCGCTACAAC CTGGA AG	PBG	1321	GATGTGCACGAACGGATCTCAGGAGTGTACTGGGTTCTCCGAAC CTGGGGAAGTATG TA
oG	361	AT GGCTGGC GACCC CGCTAT GAGGAA AGCCTG CACAAT CCTTACCCAGAC TAT CATTGG	oG	1381	CTGCTCTCCG TGGAG CACTGACTGCTCTC AT GTGATC ATTTTCTGAT ACCTG CTGG
PBG	361	ATGGCCGGTGACCCAGATATGAAGAGTCTCTACACAATCCGTACCTGACTACCACTGG	PBG	1381	TTACTGAGTGACGGGCCCTGACTGCCCTTGATGTTGATAATTTTCTGATGACATGCTGG
oG	421	CTGCGC ACT GTGA AGACC ACAAA GAG AGCCTGGT CAT TTTCC CCAT CTGTGCGCCGAC	oG	1441	CGACGAGTGA ACCG CTCCGACCACTCAG CACAAT CTGAGG GGG ACCGCTAGAGA AGTG
PBG	421	CTTCGAAC TGTA AAAAC CAAGGAGTCTCTCGTTATCATATCTCCA AGTGTGGCAGAT	PBG	1441	AGAAGAGTCAATCGATCAGAACCTACGCAACACAATCTCAGAGGGACAGGGAGGGAGTG
oG	481	CTG GACCC TACG CCG ATCTCTCC ACAG TCCAGT TT TTCC GGC GGA ACT GTCC GGGA	oG	1501	TCTGT CACACCC CAGAGCGGTA AAAT CATCAGCAGT TTGGAA AGTCATAAATCT GGGGC
PBG	481	TTGGACCATATGACAGATCCCTTCACTCGCCGGTCTTCCCTGGCGGAATTGCTCAGGA	PBG	1501	TCA GTCACT CCCCAAAGCGGGAAGATCATATCTTCA TTGGAATCACACA AGAGTGGGGT
oG	541	GTGGCCGT CAG CTC CACTTACT GTTC ACC AA CTGAT TATACA ACTGGAT GCCTG AG	oG	1561	GAG ACACGGCT CTAA
PBG	541	GTAGCGGTGCTTCTACTACTGTCTC ACTA ACCA GCAG ATTAGACCA TTTGGATGCC CGAG	PBG	1561	GAGACCAGACTGTAA
oG	601	AAT CCAAGGCT GGGGAT GTCTGCG GACAT TTCA CC AA CAGCAGGGG CAAGAGGGC CTCA			
PBG	601	AATCCGAGACTAGGGATGCTTGTGACATTTTACCAATAGTAGAGGGAAGAGAGCATCC			
oG	661	AAA GGCAGC GAGACT TTGGATTT GTGGATGAAAG GGGCTG TATAAG AGCCTCAA AGGT			
PBG	661	AAAGGGAGTGAGACTTGGCGCTTTGTAGATGAAAGAGGCCTATATAAGTCTTTAAAGGA			
oG	721	GCCTGCAAGT GAA ACTTGT GGC GTGCTGGGA CT CGGCTG AT GGAC GGAA ACTTGGG TC			
PBG	721	GCATGCAAACTCAAGTTATGTGGAGTTCTAGGACTTAGACTTATGGATGGAACATGGGTC			
oG	781	GCTATGCAGAC ACT CTAACG AGACTAAGTGGTGC CCCCCTGGG CAG CTCGTGAATCTG CAC			
PBG	781	GCATGCAAAACATCAAATGAAACCAATGGTGCCTCCCGTCAAGTGGTGAATTTGCAC			
oG	841	GACT TTCCGAAGCGAT GAGAT CGAACATCTGGTGTG GAG GA ACT CGTGA AGAAA AGGGAG			
PBG	841	GACTTTCGCTCAGACGAAATTGAGCACCTTGTGTAGAGGAGTTGGTCAAGAAAGAGAGAG			
oG	901	GAA TG CTCGATGCTCTG GAG TCAATTATGACT ACCAAG AGCTGAGCTT CAGGAG GGCTG			
PBG	901	GAGTGTCTGGATGCACTAGAGTCCATCATGACCACCAAGTCAAGTTCAGACGTCTC			
oG	961	AGCCACCTGAGGAAGCT CGTCC CTGGCTT GGAAAAG CATAC ACC ATCTTTAACA AGACA			
PBG	961	AGTCATTTAAGAAA ACTTGTCCCTGGGTTTGGAAA AGCATATACCATATTCAACAAGACC			

Figure S1. DNA alignment between oG and PBG, Related to Figure 1. Base pairs oG optimized for *Mus musculus* are colored red.

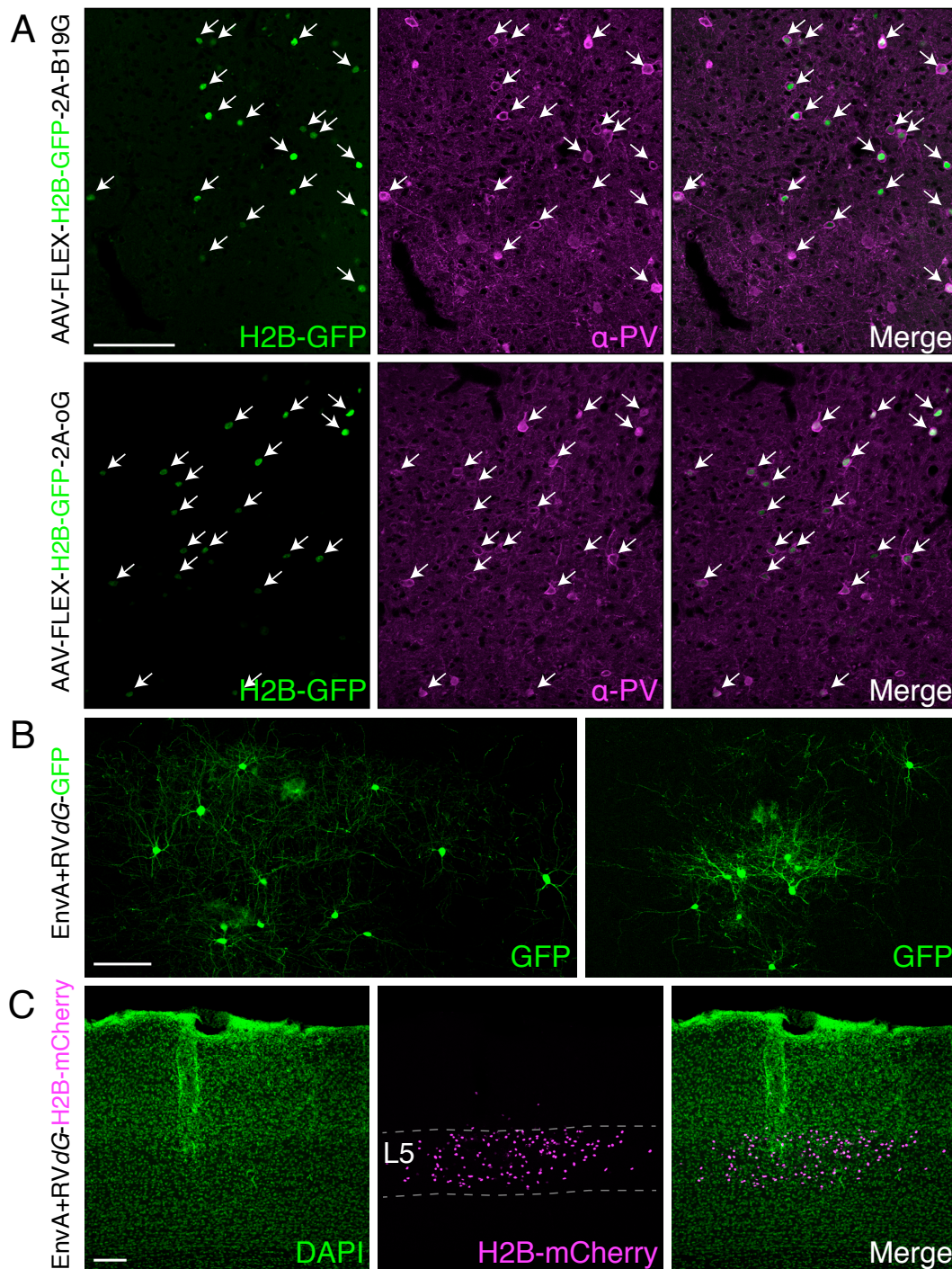


Figure S2. Tight control of transgene expression from AAV-FLEX-H2B-GFP-2A-G viral vectors and $R26^{LSL-TVA/+}$ mice, and selectivity of EnvA-RVdG infection, Related to Figure 3. (A) Coronal sections of V1 in $PV^{IRES-Cre/+};R26^{LSL-TVA/+}$ mouse showing that H2B-GFP expression from AAV-FLEX-H2B-GFP-2A-B19G (top panels) or AAV-FLEX-H2B-GFP-2A-oG (bottom panels) is co-localized with PV antibody staining (magenta) in PV-Cre neurons. These data demonstrate selective expression of glycoproteins from these AAV constructs only in PV⁺ neurons and no leak expression in other cell types. (B) Following injection of EnvA-RVdG-GFP into the visual cortex of $PV^{IRES-Cre/+};R26^{LSL-TVA/+}$ mouse, GFP is expressed only in neurons with PV inhibitory neuron morphologies; no pyramidal neurons are seen. These data demonstrate a lack of TVA expression in cells that do not express Cre in the $R26^{LSL-TVA/+}$ mice. (C) Following injection of EnvA+RVdG-H2B-mCherry in the V1 cortex of $Tlx3-Cre/+;R26^{LSL-TVA/+}$ mouse, mCherry (magenta) is seen exclusively in the nuclei of layer 5 cells, as expected from the exclusive expression of Cre within layer 5 of $Tlx3-Cre$ mice. These data further demonstrate a lack of TVA expression in cells that do not express Cre in the $R26^{LSL-TVA/+}$ mice. Scale bars = 100 μ m (A-C).

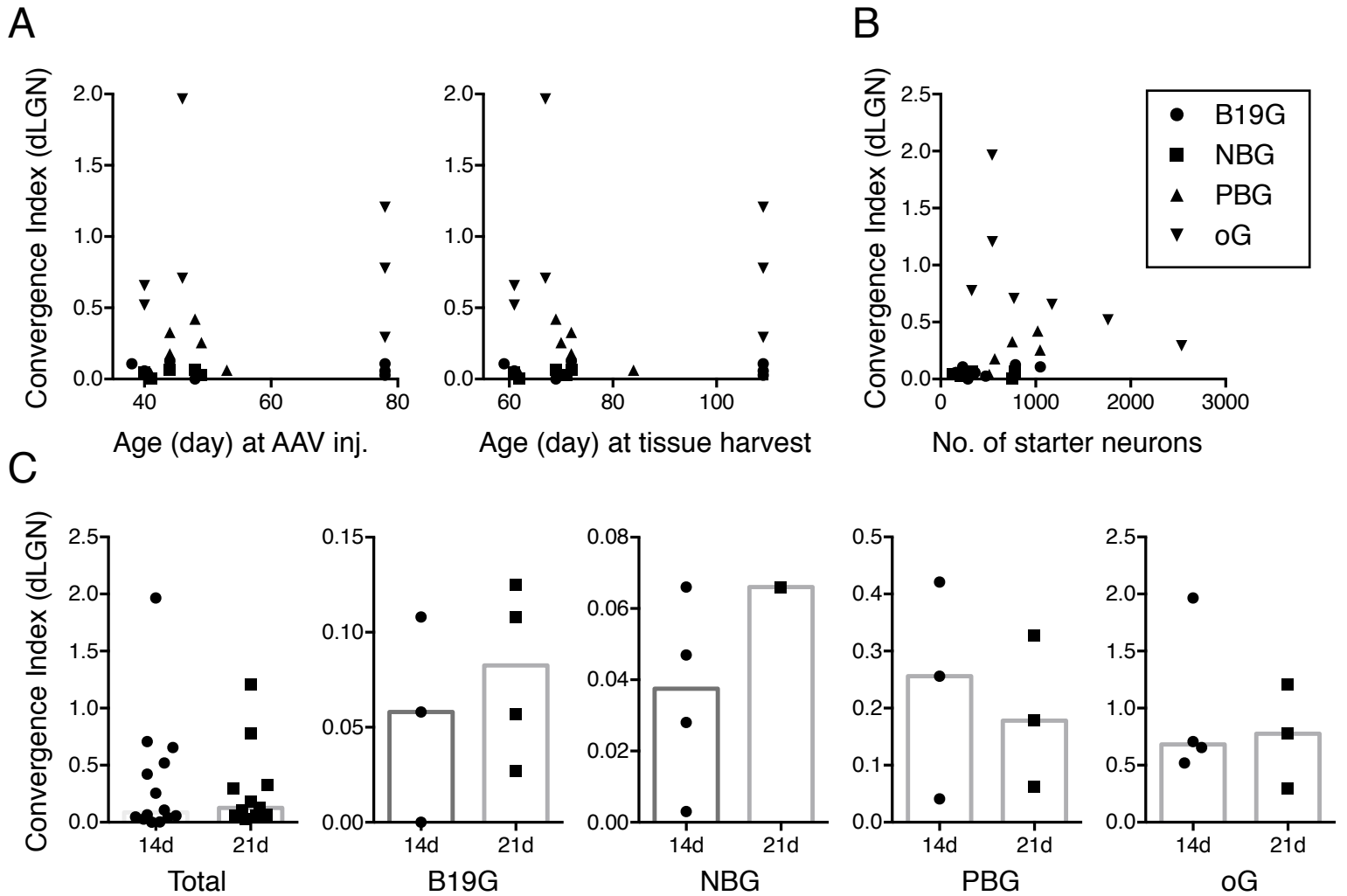


Figure S3. Animal ages, numbers of starter neurons, and duration of AAV expression before rabies infection do not affect virus tracing efficiency, Related to Figure 4. (A) dLGN convergence indices plotted against the age of animals when AAV helper was injected (left panel) or when the tissues were harvested (right panel). Each data point indicates the convergence index from a single animal and symbols indicate different glycoproteins used for complementation according to the legend in B. (B) dLGN convergence indices plotted against the numbers of starter neurons for each animal, as in A. (C) dLGN convergence indices plotted against duration of AAV expression (14 or 21 days) prior to EnvA+RVdG-dsRed injection. Each data point corresponds to a single animal and gray bars indicate medians. Circular symbols correspond to 14 days survival and squares to 21 days. Data are plotted separately for all experiments combined (left panel) as well as independently sorted according to the glycoprotein variant tested (B19G, NBG, PBG, or oG). There is no correlation between dLGN convergence index and time between AAV and RV injections.

Glycoprotein	Sex	Age	AAV	# Starter	# dLGN	CI dLGN	# LP	CI LP
B19G	M	109	21	157	9	0.057	2	0.013
B19G	M	109	21	231	25	0.108	2	0.009
B19G	M	69	14	285	0	0	0	0
B19G	F	61	14	361	21	0.058	6	0.017
B19G	M	109	21	474	13	0.027	0	0
B19G	M	72	21	786	98	0.125	14	0.018
B19G	F	59	14	1049	113	0.108	5	0.005
NBG	F	61	14	128	6	0.047	0	0
NBG	F	71	14	211	6	0.028	0	0
NBG	F	72	21	333	22	0.066	0	0
NBG	M	62	14	755	2	0.003	0	0
NBG	M	69	14	775	51	0.066	6	0.008
PBG	F	84	21	162	10	0.062	1	0.006
PBG	M	62	14	512	21	0.041	1	0.002
PBG	F	72	21	568	101	0.178	22	0.039
PBG	M	72	21	754	247	0.328	21	0.028
PBG	M	69	14	1021	430	0.421	32	0.031
PBG	F	70	14	1045	268	0.256	13	0.012
oG	F	109	21	327	254	0.777	18	0.055
oG	M	67	14	540	1061	1.965	216	0.400
oG	F	109	21	543	655	1.206	208	0.383
oG	M	67	14	770	544	0.707	149	0.194
oG	F	61	14	1170	768	0.656	80	0.068
oG	F	61	14	1762	914	0.519	162	0.092
oG	M	109	21	2536	741	0.292	153	0.060

Table S1. List of $PV^{IRES-Cre/+};R26^{LSL-TV\Delta/+}$ mice for quantification of rabies tracing, Related to Figures 3 and 4. Age indicates when tissues were harvested (day). AAV indicates duration of AAV expression (14 or 21 days) prior to EnvA+RVdG-dsRed injection. Abbreviation: CI, Convergence Index.