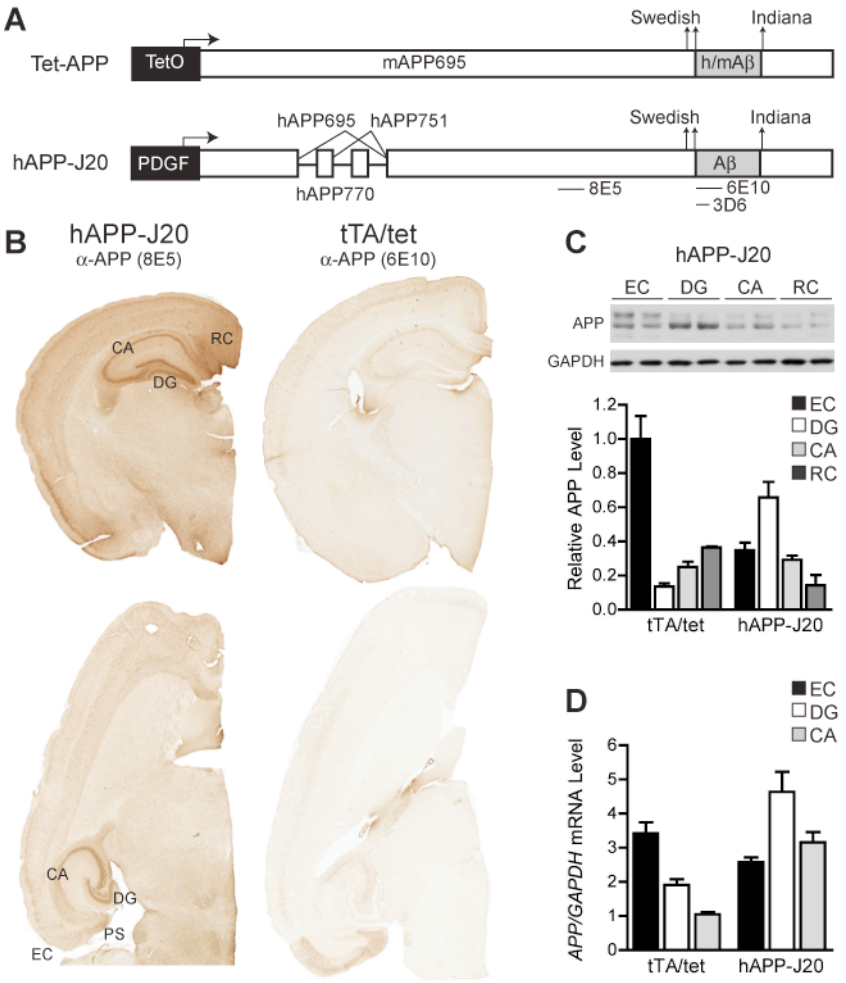
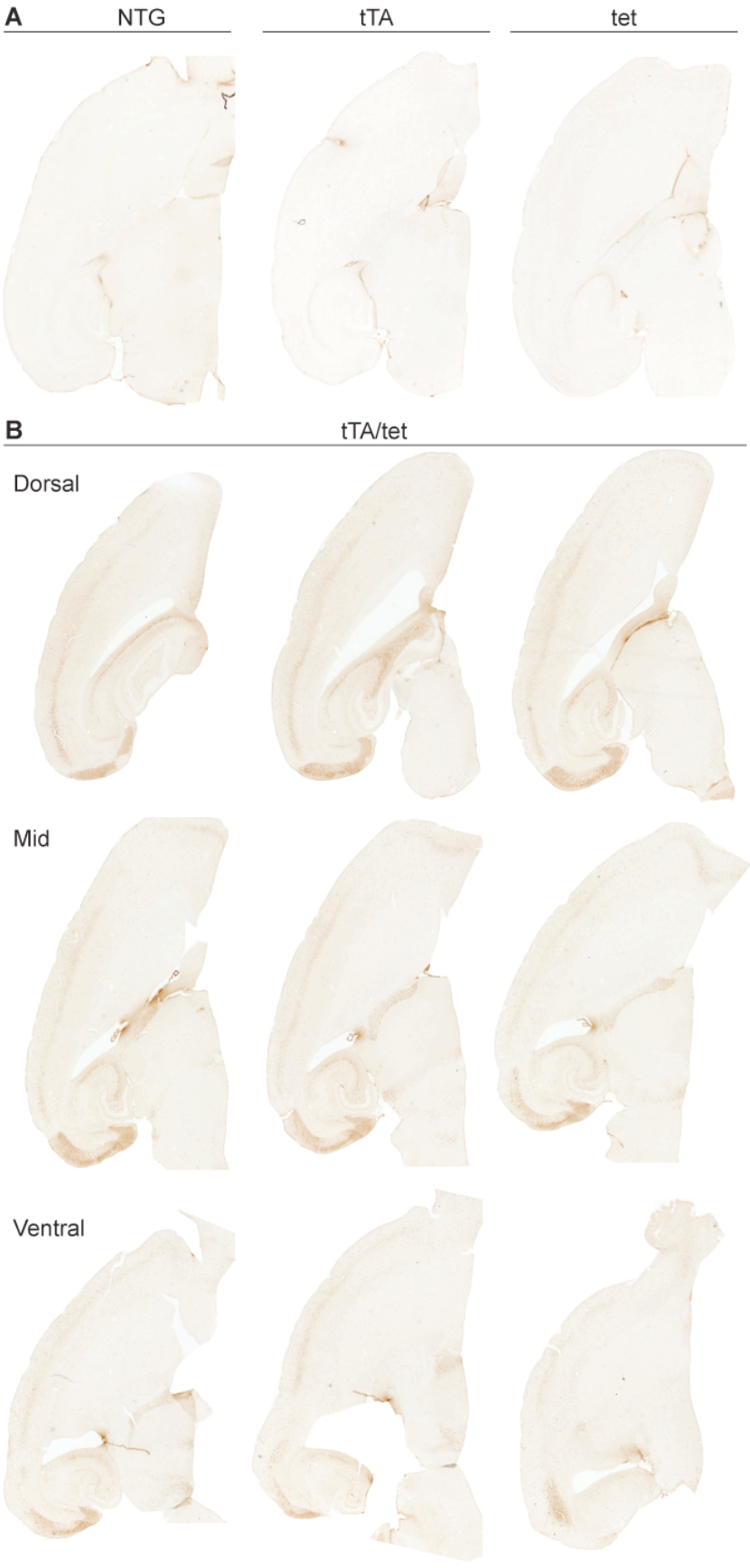
Supplemental Figures

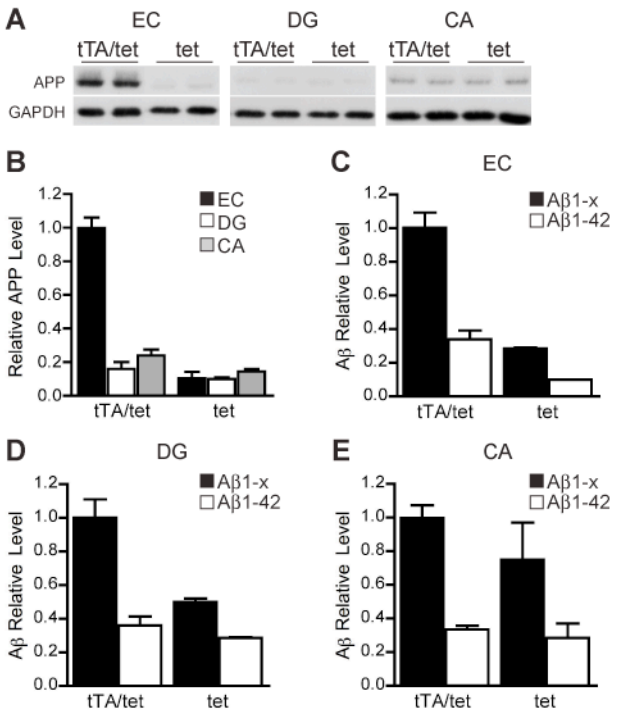


Supplementary Figure 1. Comparison of APP/Aβ expression in EC-APP mice and hAPP-J20 mice. (A) Transgenes used to generate tet-APP mice (top) and hAPP-J20 mice (bottom) (Rockenstein et al., 1995; Mucke et al., 2000; Jankowsky et al., 2005). The tet-APP transgene encodes mouse APP695 with a humanized Aβ domain, i.e., the mouse Aβ domain was mutated at key nucleotides to encode the amino acid sequence of human Aβ. The hAPP-J20 transgene is an alternatively spliced minigene that encodes hAPP695, hAPP751, and hAPP770. Both transgenes carry the Swedish and Indiana familial AD mutations. The anti-APP/Aβ antibodies 6E10 and 3D6 recognize both transgenes, but do not cross-react with murine APP/Aβ. tTA, which is required to

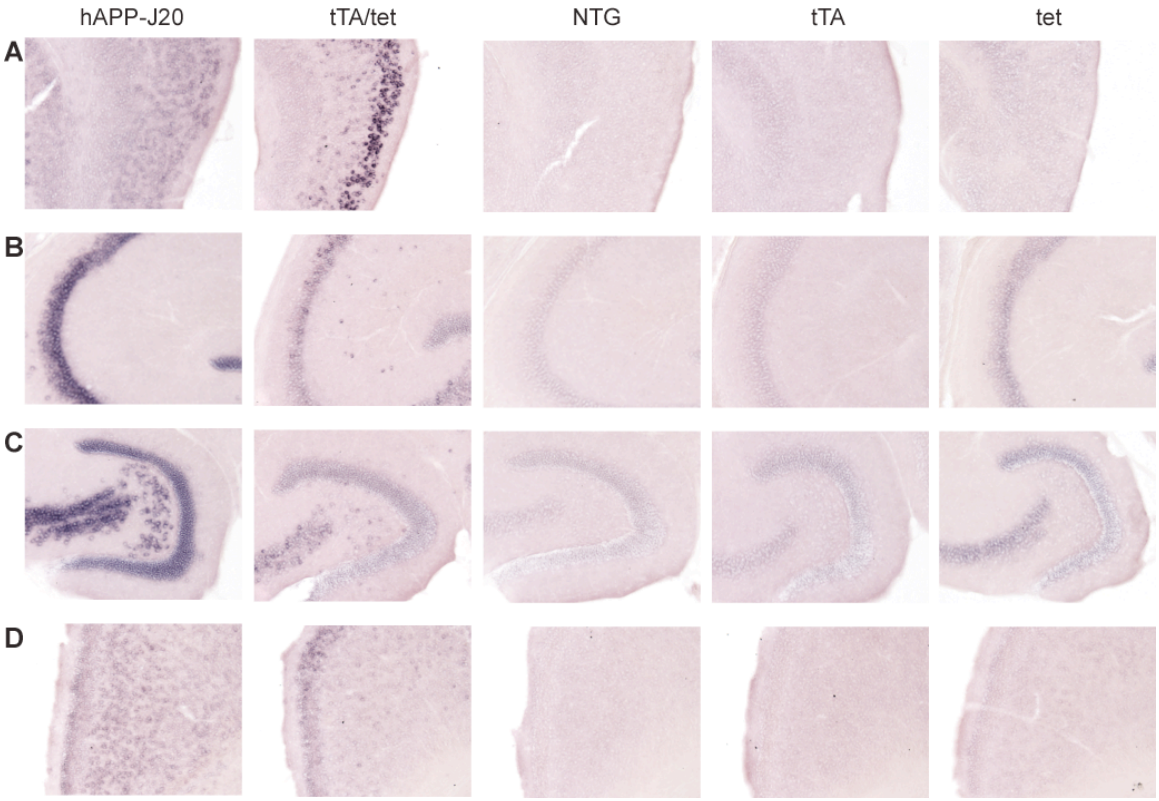
transactivate the expression of the tet-APP transgene, is directed by another transgene consisting of tTA placed under the regulatory control of the neuropsin promoter (see Figure 1). hAPP expression in hAPP-J20 mice is directed by the PDGF β-chain promoter. (B) Coronal (top) and horizontal (bottom) brain sections immunostained for hAPP (hAPP-J20) or for the humanized Aβ domain of chimeric m/hAPP (tTA/tet). APP expression is widespread in hAPP-J20 mice and restricted in the EC-APP (tTA/tet) mice. (C) Levels of transgene-derived APP in different brain regions were determined by western blot analysis with the 6E10 antibody. GAPDH was used as a loading control. The highest level of transgene-derived protein was found in the EC in EC-APP (tTA/tet) mice and in the DG in hAPP-J20 mice. n=3 mice/genotype. (D) Similar differences in transgene expression were detected at the mRNA level by quantitative RT-PCR. n=3-7 mice/genotype.


A
NTG
tTA
tet
B
tTA/tet
Dorsal
Mid
Ventral

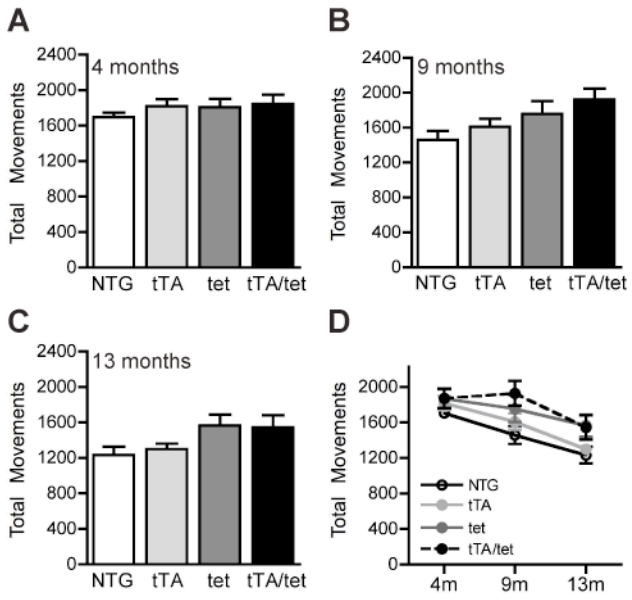
Supplementary Figure 2. Transgene-derived APP is expressed primarily in the EC in EC-APP mice. (A) Representative horizontal brain sections stained for the humanized Aβ domain with the 6E10 antibody revealed no significant labeling in NTG mice and in neuropsin-tTA or tet-APP singly transgenic mice. (B) Every 10^{th} section through the dorsal-ventral extent of the EC of an EC-APP (tTA/tet) mouse was immunostained as above. Transgene expression was highest in the medial EC, which is located in the more dorsal sections. There was, however, also some expression in the lateral EC, as seen in the more ventral sections.



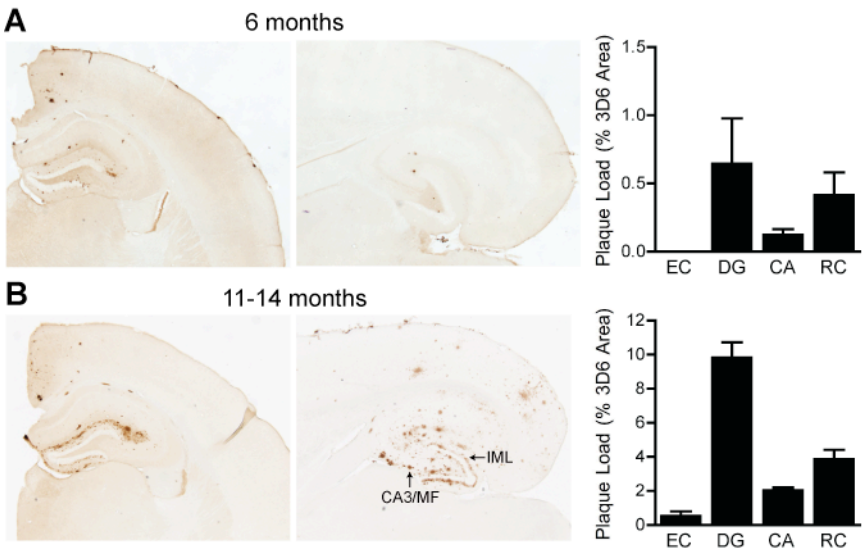
Supplementary Figure 3. Low levels of transgene-derived APP and Aβ expression in tet-APP singly transgenic mice. (A) Levels of transgene-derived APP in the EC, DG, and CA regions of tet-APP singly transgenic mice and EC-APP (tTA/tet) mice were determined by western blotting with the 6E10 antibody, which recognizes human Aβ. GAPDH served as a loading control. (B) Quantitation of western blot signals. APP levels in the EC were roughly 10-fold higher in tTA/tet-APP mice than in tet-APP mice. (C-E) Soluble Aβ1-x and Aβ1-42 levels were measured in lysates of EC (C), DG (D), and CA1 (E) by ELISA in 3-month-old EC-APP (tTA/tet) and singly transgenic tet-APP mice. EC-APP mice had higher levels of Aβ1-x and Aβ1-42 in the EC and DG. In the CA region, EC-APP and tet-APP mice had similar Aβ1-x and Aβ1-42 levels, which were much lower than those seen in hAPP-J20 mice and likely reflect some leakiness of tet-APP expression in CA (Figure 8).



Supplementary Figure 4. Distribution of transgene-derived APP mRNA detected by in situ hybridization with an LNA oligonucleotide probe. Mice of the indicated genotypes were analyzed at 3-6 months of age. In hAPP-J20 mice (first column), hAPP mRNA was detected throughout the brain, including EC (A), CA (B), DG (C), and RC (D). In EC-APP mice (tTA/tet, second column), expression of transgene-derived APP mRNA was highest in the superficial layers of the EC (A). Consistent with the distribution of transgene-derived protein (Figure 1), scattered cells expressing chimeric h/mAPP mRNA were also seen in CA1, CA3, and RC (B, C, D), but not in the DG (C).



Supplementary Figure 5. Locomotor activity of mice in the open field at 4, 9, and 13 months of age. (A-C) Total movements (measured as the number of beam breaks) in the open field were not significantly increased in EC-APP (tTA/tet) mice at any of the ages analyzed. (D) All groups of mice showed habituation to the open field with repeated exposures ($p<0.0001$ effect of age by 2-way ANOVA). n=10-13 mice per group. Graphs show mean ± SEM.



Supplementary Figure 6. Aβ deposition in hAPP-J20 mice at 6 (A) and 11-14 (B) months of age was detected in coronal (left) and horizontal (middle) brain sections and quantitated as percent area covered by 3D6 immunoreactivity (right). At 6 months, the DG had the highest plaque loads, but no deposits were detected in the EC. Similar to EC-APP mice (Figure 8A,B), hAPP-J20 mice also showed a substantial amount of Aβ deposition in the RC. At 11-14 months, Aβ deposits in hAPP-J20 mice had increased in several regions, with the largest amount still seen in the DG. In the hippocampus, plaques were localized predominantly in the inner molecular layer of the DG (IML) and the CA3/MF regions (B, middle). Number of mice analyzed: at 6 months, n=5 (coronal; DG, CA, RC) and 2 (horizontal; EC); at 13 months, n=5 (coronal) and 3 (horizontal). All graphs show mean ± SEM.

Supplemental Experimental Procedures

Open Field. Spontaneous locomotor activity in an open field arena was measured in an automated Flex-Field/Open Field Photobeam Activity System (San Diego Instruments, San Diego, CA). Before testing, mice were transferred to the testing room and acclimated for at least 1 h. Mice were tested in a clear plastic chamber (41 x 41 x 30 cm) for 15 min with two 16 x 16 photobeam arrays detecting horizontal and vertical movements. The apparatus was cleaned with 70% alcohol after testing of each mouse. Total movements in the open field were recorded for further data analysis.

Quantitative real time RT PCR. Total RNA was isolated from microdissected brain regions with the RNeasy Mini kit (Qiagen, Valencia, CA). After treatment with RNase-free DNase (Ambion) for 30 min at 37°C, total RNA was reverse transcribed with random hexamers and oligo(dT) primers. The expression levels of hAPP and chimeric m/hAPP relative to GAPDH was determined by SYBR green dye chemistry and an ABI Prism 7700 sequence detector (Applied Biosystems, Foster City, CA), as recommended by the manufacturer. The quality of primers and amplification reactions was verified by analysis of dissociation curves, slopes of standard curves, and reactions without RT. Primers were designed to detect both the hAPP and m/hAPP transgenes without detecting endogenous mouse APP. The primer sequences used were: forward, 5' TGCAGAATTCCGACATGACT-3' and reverse, 5'-GCCTTTGTTTGAACCCACAT-3'. Mouse *GAPDH* was detected with the following primer sequences*:* forward, 5'-GGGAAGCCCATCACCATCTT-3'; reverse, 5'-GCCTTCTCCATGGTGGTGAA-3'.

In situ Hybridization. The Locked Nucleic Acid (LNA)-modified oligonucleotide probe to detect transgene-derived APP mRNA in EC-APP mice was custom designed and obtained from Exiqon (Woburn, MA). The LNA technology improves specific

hybridization to short target sequences (Koshkin et al., 1998; Vester and Wengel, 2004). The 19-nucleotide probe sequence was 5'-gaacttcatatcctgagtc-3', which contains 3 mismatches to the endogenous mouse sequence. The probe was labeled on both 5' and 3' ends with digoxygenin. *In situ* hybridization was performed on floating coronal and horizontal sections (30 µm) essentially as described (Palop et al., 2005). Sections were fixed in 4% paraformaldehyde, treated for 15 min with proteinase K (0.005%), and acetylated with acetic anhydride (0.25%) for 10 min. Sections were washed with PBS and 0.5% Tween after each step. Sections were then incubated with hybridization buffer containing: 50% formamide, 5x SSC, 5x Denhardt's solution, salmon sperm DNA, and yeast tRNA for 4 h at room temperature. The LNA oligoprobe was then diluted to 40 nM in hybridization buffer, heated to 65°C for 5 minutes, placed on ice and added to the sections. Hybridization was done at 58°C for 16 h. Sections were then washed once with 5x SSC, followed by washes in 0.2x SSC at 58°C for 4 h. Non-specific binding was blocked with 10% heat-inactivated sheep serum, and sections were incubated overnight with sheep anti-digoxigenin-alkaline phosphatase (1:5000; Roche Molecular Biochemicals). The signal was visualized with nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate (1:50; Roche Molecular Biochemicals) in NTMT (100 mM NaCl, 100 mM Tris-HCl, pH 9.5, 50 mM $MgCl_2$, 0.1% Triton X-100) for 2 hours.

Supplemental Material References

Jankowsky JL, Slunt HH, Gonzales V, Savonenko AV, Wen JC, Jenkins NA, Copeland NG, Younkin LH, Lester HA, Younkin SG, Borchelt DR (2005) Persistent amyloidosis following suppression of Aβ production in a transgenic model of Alzheimer disease. PLoS Med 2:e355(1318)–e1355(1333).

Koshkin AA, Nielsen P, Meldgaard M, Rajwanshi V, Singh S, Wengel J (1998) LNA (Locked Nucleic Acid): An RNA Mimic Forming Exceedingly Stable LNA: LNA Duplexes. J Am Chem Soc 120:13252–13253.

Mucke L, Masliah E, Yu G-Q, Mallory M, Rockenstein EM, Tatsuno G, Hu K, Kholodenko D, Johnson-Wood K, McConlogue L (2000) High-level neuronal expression of $A\beta_{1-42}$ in wild-type human amyloid protein precursor transgenic mice: Synaptotoxicity without plaque formation. J Neurosci 20:4050–4058.

Palop JJ, Chin J, Bien-Ly N, Massaro C, Yeung BZ, Yu G-Q, Mucke L (2005) Vulnerability of dentate granule cells to disruption of Arc expression in human amyloid precursor protein transgenic mice. J Neurosci 25:9686–9693.

Rockenstein EM, McConlogue L, Tan H, Gordon M, Power M, Masliah E, Mucke L (1995) Levels and alternative splicing of amyloid β protein precursor (APP) transcripts in brains of transgenic mice and humans with Alzheimer's disease. J Biol Chem 270:28257–28267.

Vester B, Wengel J (2004) LNA (locked nucleic acid): high-affinity targeting of complementary RNA and DNA. Biochemistry 43:13233–13241.