The Effects of Chronic Exposure to Ambient Traffic-Related Air Pollution on Alzheimer’s Disease Phenotypes in Wildtype and Genetically Predisposed Male and Female Rats

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Figure S1. Photo montage of the various systems comprising the Tunnel Exposure Facility.
Figure S2. Effects of TRAP on cued fear conditioning and neuronal cell loss in the hippocampus. (A-B) Cued fear conditioning was performed on-site at 9.5 or 14.5 month old animals for the 10 and 15 month-old cohort. An increased average motion index indicates impaired cognitive behavior. (C-D) To assess neuronal cell loss, brain sections were immunostained for NeuN, a biomarker of neurons, and the number of NeuN-immunopositive cells per mm² were counted in the CA1. (E) Densitometric analyses of total tau relative to GAPDH in the crude pellet fraction of cortical tissue. All data presented as the mean ± SD (n=10-12 animals per group for A-D; n=5-6 animals per group for E). Data were analyzed by three-way ANOVA using sex, genotype, and exposure as factors (A-B, E) or two-way ANOVA using genotype and exposures as factors (C, D) with post-hoc Sidak’s test; *p<0.05. Circles represent individual animals (for C-E, each circle is an average of 4 technical replicates). M=male; F=female; WT=wildtype; Tg=TgF344-AD. Summary values are available in Table S6.

Figure S3. Effects of TRAP on Aβ deposition by brain region, and on guanidine-HCL soluble brain extracts. (A) Analyses of ThioS+ plaques by brain regions in TgF344-AD rats (DG=dentate gyrus; EC=entorhinal cortex; Thal=thalamus; Cer=cerebellum) (B) Guanidine-HCL-soluble ratios of Aβ42:40, as measured by ELISA in cortical samples and normalized to Aβ levels in 3-month-old WT female rats. All data presented as the mean ± SD (n=5-6 animals per group). Circles represent individual animals. Four brain sections were measured per animal in A, and two technical replicates were performed for each animal in B. M=male; F=female. Data were analyzed by three-way ANOVA using sex, genotype, and exposure as factors, with post-hoc Sidak’s test. *p<0.05. Summary data are available in Table S7.
Materials

**Tunnel Exposure Facility:** This is a core exposure facility approved by the U.C. Davis IACUC office and capable of accommodating acute and chronic studies of real-time exposure to traffic related air pollution (TRAP) drawn directly from a major freeway tunnel system. The facility is located on a 3200 sq. ft. Caltrans-owned vacant lot immediately adjacent to the eastbound exit of tunnel bores 1 and 2. The bores are approximately 1.1 km long with a 4.2% incline and service the evening commute (15:00-19:00) from urban centers to the west to suburbs in the east at a rate of ~ 4000 vehicles/hour. It is fully enclosed by a 6-foot-high chain link perimeter fence with privacy windscreens to restrict visibility. There is onsite parking so supplies and animals can be offloaded while shielded from public view. A photo montage of the facility is shown in Figure S1. A systems-level synopsis follows.

**Onsite Office Trailer:** As shown in Figure S1, this is a 3-room 400 sq. ft. mobile office trailer that houses (1) an instrumentation room for all air sampling and measurement instrumentation, sampling ports, sampling train, and computer systems, (2) a vivarium with two large exposure chambers and (3) general lab space for supply storage, animal care and other activities. There is a fully integrated electrical power system, HVAC system with programmable temperature controls, and fully programmable vivarium lighting system.

**TRAP Supply Lines:** Independent sampling ports are situated immediately above the exits of bores 1 and 2 on top of a honeycomb-style shade grating, as shown in the upper left-hand panel of Figure S1. The ports are plumbed across the shade grating, up the sides of the parapet wall, and through holes at the top of the wall to the air flow control systems beneath the office trailer. The outlet of the air flow control system is plumbed up the trailer wall and to the interior through flanged ports secured to the boarded-up window of the instrumentation room. These supply lines are then split into multiple sampling ports: (1) the exposure chambers in the vivarium, (2) the PM samplers in the instrumentation room and (3) the air sampling train for air monitoring instrumentation.

**Filtered Air (FA) Supply Lines:** Clean filtered air for negative control exposure groups originates in a storage shed immediately adjacent to the office trailer and is subjected to a series of emissions control technologies before being plumbed to the air flow control systems beneath the trailer and then through the window to the interior sampling ports, identical to the TRAP supply lines. Emission controls include coarse filtration for removing large debris and dust, inline activated carbon for removing volatile organic compounds (VOCs), barium oxide-based catalytic converters for removing nitrogen oxides (NOx) and a 6-port ultrahigh-efficiency particle filtering system for removing ultrafine, fine, and coarse mode particulate matter (PM), as shown in Figure S1.

**Air Flow Control Systems:** Facility-level air flow and control is achieved via a combination of blowers, variable frequency drives (VFDs), flowmeters and custom made sound dampening mufflers. In brief, VFDs control motor speed through a proportional-integral-derivative (PID) control loop with a continuous flowrate monitor to maintain a constant flowrate setpoint that satisfies IACUC specifications for air exchange rates through the exposure chambers. Blowers are placed upstream of the exposure chambers, ultrahigh-efficiency particle filtering system, and sampling ports to maintain positive pressure throughout the system so that any leaks are outward, not inward which would dilute TRAP and contaminate FA supply lines. Air flow through all other instrumentation is achieved via independent pumps and a centralized air sampling train.
**Air Sampling Train:** This is a custom-built sampling train designed to handle all the various air flow control needs of the instrumentation suite and sampling package associated with characterizing the TRAP and FA exposure atmospheres. Multiple atmospheres cannot be monitored simultaneously with a single instrument, so a system of computer-controlled solenoid valves has been implemented to switch between the various supply lines sequentially on a preprogrammed sampling schedule to allow different atmospheres to be measured in cyclical series by individual instruments. Furthermore, the sampling train is immediately upstream of the exposure chambers to avoid any contaminating PM and gases from animal activity in their cages.

**Exposure Chambers:** Two custom-built, state-of-the-art, airtight, IACUC-approved exposure chambers are housed in the office trailer vivarium, as shown in Figure S1. Each exposure chamber is further divided into three fully isolated sub-chambers with 36-cage capacities for a total facility capacity of six exposure groups at 36 cages per group. Exposure atmospheres are pumped through custom-built, fully automated air cooling and heating systems prior to delivery to the animals to ensure IACUC temperature specifications are maintained. Air delivery is through a series of orifice plates and diffusers at the top of the exposure chambers to ensure evenly balanced and well-mixed flows and then exhausted through the bottom of the chambers to a series of coarse PM and activated carbon filters prior to being vented back over the parapet wall. Environmental variables inside the exposure chambers are continuously monitored and archived, including pressure, temperature, flow rates, and relative humidity. Chamber doors and cage racking systems are fully removable for easy cleaning/disinfection. Emergency shutoff systems and pressure relief valves are integrated into the chambers for animal safety.

**Computer, Security and Software Systems:** A fully integrated computer and custom software system with remote access and control capabilities provides data acquisition and archiving for continuous monitoring of gas and PM concentrations, exposure chamber environmental variables, and air flow control parameters. An emergency email notification system has been implemented to alert investigators of sustained periods of out-of-range variables and parameters. A 4-point security camera system provides a continuous 360° view of the facility. Mirrored monitors are in the vivarium for real-time monitoring during animal care.
Table S1: Antibodies and conditions used in immunohistochemistry (IHC) analyses

| Antibody | Species | Clonality | Source | Number | Dilution | Indication |
|----------|---------|-----------|--------|--------|----------|------------|
| OC       | Rabbit  | Polyclonal | Millipore | Ab2286 | 1:1000   | Amyloid-beta fibrils, IHC |
| IBA1     | Rabbit  | Polyclonal | Wako   | 19-19741 | 1:1000   | Microglia/monocytes, IHC |
| CD68     | Mouse   | Monoclonal | Abd Serotec | MCA341R | 1:300    | Lysosomal marker, IHC   |
| NeuN     | Mouse   | Monoclonal | Millipore | MAB377 | 1:500    | Neuronal marker, IHC   |
| PHF1     | Mouse   | Monoclonal | P. Davies, Albert Einstein College of Medicine | PHF1 | 1:50    | Hyperphosphorylated tau, IHC |
| GFAP     | Mouse   | Monoclonal | Cell Signaling | 3670S | 1:1000   | Astrocyte marker, IHC   |
| Anti-mouse IgG H&L AlexaFluor 488 | Goat | N/A | Invitrogen | A11029 | 1:750    | Used with CD68, NeuN, GFAP primary antibodies |
| Anti-mouse IgG H&L AlexaFluor 568 | Goat | N/A | Invitrogen | A11031 | 1:1000   | Used with PHF1 primary antibody |
| Anti-rabbit IgG H&L AlexaFluor 568 | Goat | N/A | Invitrogen | A11036 | 1:1000   | Used with OC, IBA1 primary antibodies |

IBA1: ionized binding adapter molecule 1; CD68: cluster of differentiation 68; GFAP: glial fibrillary acidic protein; H&L: Heavy and light chain; IgG: Immunoglobulin G; NeuN: neuronal nuclei; PHF1: paired helical fragment 1.

Table S2: Summary of regional analysis of ThioS staining in 15 month-old TgF344-AD rats

| Region     | Main Effect Sex | Main Effect Exposure | Interaction | Post-hoc result                      |
|------------|-----------------|----------------------|-------------|--------------------------------------|
| DG         | ns              | F (1, 19) = 13.65, p=0.0015 | ns          | Sidak; F TRAP > F FA; p = 0.0109     |
| CA1        | F (1, 19) = 8.203, p=0.0099 | F (1, 19) = 21.07, p=0.0002 | ns          | Sidak; F TRAP > F FA; p = 0.0006     |
| CA3        | ns              | F (1, 19) = 4.657, p=0.0439 | ns          | ns                                   |
| EC         | ns              | F (1, 19) = 4.669, p=0.0437 | ns          | Sidak; F TRAP > F FA; p = 0.0445     |
| Thalamus   | ns              | ns                   | ns          | ns                                   |
| Cerebellum | ns              | ns                   | ns          | ns                                   |

DG: dentate gyrus; EC: entorhinal cortex; F: female; M: male; FA: filtered air; TRAP: Traffic-related air pollution; ns: not significant
Table S3: Summary of major effects of TRAP by age and endpoint.

| Age (mo) | Aβ area | NeuN count in EC | PHF1 IHC | Contextual FC | %IBA1+ cells expressing CD68 |
|----------|----------|-----------------|----------|--------------|-----------------------------|
| 3        | >100x, F/Tg | N/A             | N/A      | N/A          | 1.13x, F/WT                 |
| 6        | 2.7x, M/Tg | N/A             | N/A      | N/A          | 1.17x, F/Tg; 1.37x, M/Tg    |
| 10       | ns        | ns              | N/A      | N/A          | N/A                         |
| 15       | 1.4-1.5x, F/Tg; 1.3-1.4x, M/Tg; 0.89x, WT; 0.85x, Tg | >100x, M/WT | 2.27x, M/WT | N/A                      |

*TRAP vs. FA unless otherwise noted. Differences that only occurred between TgF344-AD and WT are not indicated. IHC: Immunohistochemistry; FC: fear conditioning; N/A: not applicable; ns: not significant; M: male; F: female; WT: wildtype F344; Tg: TgF344-AD

Table S4. Summary of numeric data in Figure 1.

| Figure panel | Age (mo) | Values (mean ± S.D.) |
|--------------|----------|----------------------|
| 1C           | N/A      | FA: 0.25 ± 0.11; TRAP: 15.6 ± 3.7 |
| 1D           | N/A      | FA: 33 ± 4; TRAP: 46 ± 7 |
| 1F           | 3        | WT/FA: 0.36 ± 0.43; WT/TRAP: 4.17 ± 4.50; TgF344-AD/FA: 0.48 ± 0.68; TgF344-AD/TRAP: 5.39 ± 3.32 |
|              | 6        | WT/FA: 0.58 ± 0.56; WT/TRAP: 2.46 ± 1.45; TgF344-AD/FA: 0.58 ± 0.93; TgF344-AD/TRAP: 8.85 ± 5.01 |
|              | 10       | WT/FA: 1.04 ± 1.39; WT/TRAP: 3.25 ± 2.98; TgF344-AD/FA: 1.42 ± 1.43; TgF344-AD/TRAP: 5.63 ± 3.18 |
|              | 15       | WT/FA: 2.23 ± 2.71; WT/TRAP: 3.75 ± 2.49; TgF344-AD/FA: 3.125 ± 3.52; TgF344-AD/TRAP: 7.83 ± 2.38 |
| 1G           | All      | FA: 29.85 ± 2.11; WT/TRAP: 32.13 ± 1.73; TgF344-AD/FA: 27.66 ± 1.67; TgF344-AD/TRAP: 31.51 ± 2.92 |
| 1H           | All      | FA: 5803 ± 2354; WT/TRAP: 10363 ± 2635; TgF344-AD/FA: 6911 ± 2495; TgF344-AD/TRAP: 11132 ± 2864 |

Values correspond to data shown in Figure 1. N/A: not applicable; WT: wildtype F344; FA: filtered air; TRAP: traffic-related air pollution.
### Table S5: Summary of numeric data in Figure 2.

| Figure panel | Age (mo) | Values (mean ± S.D.) |
|--------------|----------|----------------------|
| **2B (FC)**  |          |                      |
|              | 3        | WT/FA: 0.18 ± 0.40;  |
|              |          | WT/TRA: 0.29 ± 0.62; |
|              |          | TgF344-AD/FA: 0.31  |
|              |          | ± 0.49; TgF344-AD/TRA: 0.22 ± 0.39 |
|              | 6        | WT/FA: 0.75 ± 0.69;  |
|              |          | WT/TRA: 0.58 ± 0.76; |
|              |          | TgF344-AD/FA: 0.44  |
|              |          | ± 0.43; TgF344-AD/TRA: 1.17 ± 0.88 |
|              | 10       | WT/FA: 0.92 ± 0.60;  |
|              |          | WT/TRA: 0.63 ± 0.48; |
|              |          | TgF344-AD/FA: 1.33  |
|              |          | ± 0.78; TgF344-AD/TRA: 1.18 ± 0.70 |
|              | 15       | WT/FA: 0.91 ± 0.42;  |
|              |          | WT/TRA: 1.30 ± 0.86; |
|              |          | TgF344-AD/FA: 1.13  |
|              |          | ± 0.47; TgF344-AD/TRA: 1.96 ± 1.50 |
| **2B (Thal)** |          |                      |
|              | 3        | WT/FA: 0.05 ± 0.15;  |
|              |          | WT/TRA: 0.25 ± 0.50; |
|              |          | TgF344-AD/FA: 0.32  |
|              |          | ± 0.49; TgF344-AD/TRA: 0.27 ± 0.41 |
|              | 6        | WT/FA: 0.35 ± 0.41;  |
|              |          | WT/TRA: 0.67 ± 0.72; |
|              |          | TgF344-AD/FA: 0.58  |
|              |          | ± 0.76; TgF344-AD/TRA: 1.04 ± 1.00 |
|              | 10       | WT/FA: 0.68 ± 0.51;  |
|              |          | WT/TRA: 0.88 ± 0.53; |
|              |          | TgF344-AD/FA: 1.18  |
|              |          | ± 0.70; TgF344-AD/TRA: 0.75 ± 0.75 |
|              | 15       | WT/FA: 1.06 ± 0.48;  |
|              |          | WT/TRA: 1.88 ± 2.32; |
|              |          | TgF344-AD/FA: 0.83  |
|              |          | ± 0.44; TgF344-AD/TRA: 1.31 ± 1.16 |
| **2B (Cer)** |          |                      |
|              | 3        | WT/FA: 0.09 ± 0.30;  |
|              |          | WT/TRA: 0.17 ± 0.33; |
|              |          | TgF344-AD/FA: 0.40  |
|              |          | ± 0.42; TgF344-AD/TRA: 0.75 ± 0.69 |
|              | 6        | WT/FA: 0.42 ± 0.36;  |
|              |          | WT/TRA: 0.57 ± 0.74; |
|              |          | TgF344-AD/FA: 0.46  |
|              |          | ± 0.49; TgF344-AD/TRA: 0.65 ± 0.61 |
|              | 10       | WT/FA: 0.46 ± 0.45;  |
|              |          | WT/TRA: 0.64 ± 0.48; |
|              |          | TgF344-AD/FA: 0.42  |
|              |          | ± 0.42; TgF344-AD/TRA: 0.38 ± 0.43 |
|              | 15       | WT/FA: 0.36 ± 0.55;  |
|              |          | WT/TRA: 0.56 ± 0.43; |
|              |          | TgF344-AD/FA: 0.33  |
|              |          | ± 0.39; TgF344-AD/TRA: 0.50 ± 1.71 |
| **2C**       |          |                      |
|              | 3        | WT/FA: 30.95 ± 15.56; |
|              |          | WT/TRA: 28.88 ± 11.88; |
|              |          | TgF344-AD/FA: 33.46  |
|              |          | ± 7.55; TgF344-AD/TRA: 29.30 ± 10.79 |
|              | 6        | WT/FA: 34.22 ± 17.31; |
|              |          | WT/TRA: 37.67 ± 8.73; |
|              |          | TgF344-AD/FA: 34.36  |
|              |          | ± 19.47; TgF344-AD/TRA: 38.58 ± 13.48 |
|              | 10       | WT/FA: 35.03 ± 17.81; |
|              |          | WT/TRA: 31.80 ± 13.09; |
|              |          | TgF344-AD/FA: 43.13  |
|              |          | ± 22.71; TgF344-AD/TRA: 39.87 ± 11.54 |
|              | 15       | WT/FA: 32.25 ± 16.95; |
|              |          | WT/TRA: 41.67 ± 16.36; |
|              |          | TgF344-AD/FA: 37.39  |
|              |          | ± 17.37; TgF344-AD/TRA: 33.17 ± 16.00 |
| **2D**       |          |                      |
|              | 3        | WT/FA: 6943.5 ± 1178.3; |
|              |          | WT/TRA: 9303.4 ± 1757.1; |
|              |          | TgF344-AD/FA: 8123.6  |
|              |          | ± 1627.6; TgF344-AD/TRA: 12372.1 ± 2346.8 |
|              | 6        | WT/FA: 4379.8 ± 2187.2; |
|              |          | WT/TRA: 11289.4 ± 3620.7; |
|              |          | TgF344-AD/FA: 5985.4  |
|              |          | ± 3980.2; TgF344-AD/TRA: 10251.2 ± 3572.8 |
|              | 10       | WT/FA: 5785.1 ± 2865.0; |
|              |          | WT/TRA: 10873.7 ±2565.0; |
|              |          | TgF344-AD/FA: 5441.0  |
|              |          | ±1681.8; TgF344-AD/TRA: 11382.6 ± 3167.1 |
|              | 15       | WT/FA: 6566.1 ± 2164.9; |
|              |          | WT/TRA: 9934.5 ± 2030.9; |
|              |          | TgF344-AD/FA: 8049.8  |
|              |          | ±1878.7; TgF344-AD/TRA: 10522.6 ± 1935.0 |

Values correspond to data shown in Figure 2. WT: wildtype F344; FA: filtered air; TRAP: Traffic-related air pollution.
Table S6. Summary of numeric data in Figure 3 and Figure S2.

| Figure panel | Age (mo) | WT values (mean ± S.D.) | TgF344-AD values (mean ± S.D.) |
|--------------|---------|-------------------------|-------------------------------|
| 3A           | 10      | F/FA: 111.00 ± 80.46; F/TRAP: 172.00 ± 189.20; M/FA: 209.20 ± 159.80; M/TRAP: 175.00 ± 184.00 | F/FA: 149.50 ± 131.80; F/TRAP: 66.55 ± 54.98; M/FA: 195.80 ± 97.07; M/TRAP: 145.20 ± 128.40 |
| 3B           | 15      | F/FA: 153.00 ± 160.40; F/TRAP: 163.90 ± 124.30; M/FA: 98.61 ± 104.60; M/TRAP: 223.90 ± 154.50 | F/FA: 108.10 ± 97.35; F/TRAP: 141.60 ± 122.30; M/FA: 231.30 ± 163.80; M/TRAP: 169.80 ± 182.50 |
| 3C           | 10      | FA: 769.2 ± 70.17; TRAP: 719.2 ± 78.4 | FA: 660.7 ± 62.17; TRAP: 641.7 ± 81.58 |
| 3D           | 15      | FA: 737.2 ± 54.11; TRAP: 657.9 ± 67.82 | FA: 656.1 ± 72.90; TRAP: 597.5 ± 41.93 |
| 3F           | 15      | F/FA: 0.0 ± 0.0; F/TRAP: 0.21 ± 0.51; M/FA: 1.00 ± 0.03; M/TRAP: 1.26 ± 0.12 | F/FA: 9.39 ± 2.54; F/TRAP: 10.40 ± 4.05; M/FA: 8.97 ± 1.47; M/TRAP: 9.98 ± 3.96 |
| 3H           | 15      | F/FA: 1.00 ± 0.02; F/TRAP: 1.08 ± 0.04; M/FA: 1.00 ± 0.03; M/TRAP: 1.26 ± 0.12 | F/FA: 1.00 ± 0.02; F/TRAP: 1.06 ± 0.02; M/FA: 1.10 ± 0.04; M/TRAP: 1.12 ± 0.05 |
| S2-A         | 10      | F/FA: 20.13 ± 13.78; F/TRAP: 22.66 ± 11.94; M/FA: 31.73 ± 18.04; M/TRAP: 33.01 ± 32.81 | F/FA: 37.83 ± 31.12; F/TRAP: 39.84 ± 40.53; M/FA: 41.59 ± 34.57; M/TRAP: 22.74 ± 24.08 |
| S2-B         | 15      | F/FA: 21.31 ± 15.83; F/TRAP: 29.77 ± 20.50; M/FA: 14.01 ± 22.30; M/TRAP: 19.96 ± 24.75 | F/FA: 41.68 ± 27.91; F/TRAP: 36.00 ± 22.19; M/FA: 42.56 ± 39.66; M/TRAP: 34.29 ± 33.78 |
| S2-C         | 10      | FA: 397.3 ± 60.72; TRAP: 349.55 ± 50.88 | FA: 333.4 ± 51.65; TRAP: 310.7 ± 51.59 |
| S2-D         | 15      | FA: 267.3 ± 19.93; TRAP: 238.7 ± 33.33 | FA: 233.8 ± 42.32; TRAP: 209 ± 28.69 |
| S2-E         | 15      | F/FA: 1.01 ± 0.04; F/TRAP: 1.02 ± 0.01; M/FA: 1.00 ± 0.03; M/TRAP: 1.36 ± 0.16 | F/FA: 1.07 ± 0.06; F/TRAP: 1.08 ± 0.12; M/FA: 1.12 ± 0.06; M/TRAP: 1.09 ± 0.09 |

Values correspond to data shown in Figure 3 and Figure S1. F: female; M: male; WT: wildtype F344; FA: filtered air; TRAP: Traffic-related air pollution.
### Table S7. Summary of numeric data in Figure 4 and Figure S3.

| Figure panel | Age (mo) | TgF344-AD values (mean ± S.D.) | WT values (mean ± S.D.) |
|--------------|---------|--------------------------------|-------------------------|
| 4C 3         | F/FA: 0.00 ± 0.00; F/TRAP: 0.18 ± 0.10; M/FA: 0.00 ± 0.00; M/TRAP: 0.00 ± 0.00 | F/FA: 0.00 ± 0.00; F/TRAP: 0.00 ± 0.00; M/FA: 0.00 ± 0.00; M/TRAP: 0.00 ± 0.00 |
| 4C 6         | F/FA: 0.34 ± 0.07; F/TRAP: 0.36 ± 0.11; M/FA: 0.22 ± 0.19; M/TRAP: 0.61 ± 0.25 | F/FA: 0.00 ± 0.00; F/TRAP: 0.00 ± 0.00; M/FA: 0.00 ± 0.00; M/TRAP: 0.00 ± 0.00 |
| 4C 10        | F/FA: 3.86 ± 2.42; F/TRAP: 4.32 ± 2.37; M/FA: 4.23 ± 1.40; M/TRAP: 3.00 ± 1.23 | F/FA: 0.00 ± 0.00; F/TRAP: 0.00 ± 0.00; M/FA: 0.00 ± 0.00; M/TRAP: 0.00 ± 0.00 |
| 4C 15        | F/FA: 8.06 ± 0.66; F/TRAP: 11.41 ± 1.84; M/FA: 9.05 ± 1.64; M/TRAP: 13.05 ± 3.70 | F/FA: 0.00 ± 0.00; F/TRAP: 0.00 ± 0.00; M/FA: 0.00 ± 0.00; M/TRAP: 0.00 ± 0.00 |
| 4D 3         | F/FA: 0.00 ± 0.00; F/TRAP: 0.25 ± 0.30; M/FA: 0.00 ± 0.00; M/TRAP: 0.00 ± 0.00 | F/FA: 0.00 ± 0.00; F/TRAP: 0.25 ± 0.30; M/FA: 0.00 ± 0.00; M/TRAP: 0.00 ± 0.00 |
| 4D 6         | F/FA: 0.60 ± 0.17; F/TRAP: 0.68 ± 0.27; M/FA: 0.36 ± 0.16; M/TRAP: 0.76 ± 0.15 | F/FA: 0.00 ± 0.00; F/TRAP: 0.25 ± 0.30; M/FA: 0.00 ± 0.00; M/TRAP: 0.00 ± 0.00 |
| 4D 10        | F/FA: 5.49 ± 1.12; F/TRAP: 4.75 ± 1.23; M/FA: 5.12 ± 0.91; M/TRAP: 4.97 ± 1.11 | F/FA: 0.00 ± 0.00; F/TRAP: 0.25 ± 0.30; M/FA: 0.00 ± 0.00; M/TRAP: 0.00 ± 0.00 |
| 4D 15        | F/FA: 8.98 ± 1.34; F/TRAP: 13.99 ± 3.78; M/FA: 8.22 ± 0.94; M/TRAP: 11.12 ± 1.17 | F/FA: 0.00 ± 0.00; F/TRAP: 0.25 ± 0.30; M/FA: 0.00 ± 0.00; M/TRAP: 0.00 ± 0.00 |
| 4E 15        | F/FA: 371.70 ± 23.87; F/TRAP: 430.30 ± 20.86; M/FA: 370.70 ± 22.33; M/TRAP: 380.50 ± 43.04 | F/FA: 371.70 ± 23.87; F/TRAP: 430.30 ± 20.86; M/FA: 370.70 ± 22.33; M/TRAP: 380.50 ± 43.04 |
| 4F 15        | F/FA: 247.54 ± 43.14; F/TRAP: 279.20 ± 60.02; M/FA: 194.50 ± 22.06; M/TRAP: 245.90 ± 20.99 | F/FA: 247.54 ± 43.14; F/TRAP: 279.20 ± 60.02; M/FA: 194.50 ± 22.06; M/TRAP: 245.90 ± 20.99 |
| 4G 3         | F/FA: 0.95 ± 0.16; F/TRAP: 0.99 ± 0.12; M/FA: 0.89 ± 0.22; M/TRAP: 1.16 ± 0.22 | F/FA: 0.95 ± 0.16; F/TRAP: 0.99 ± 0.12; M/FA: 0.89 ± 0.22; M/TRAP: 1.16 ± 0.22 |
| 4G 6         | F/FA: 1.17 ± 0.32; F/TRAP: 1.12 ± 0.42; M/FA: 1.54 ± 0.27; M/TRAP: 1.61 ± 0.67 | F/FA: 0.95 ± 0.16; F/TRAP: 0.99 ± 0.12; M/FA: 0.89 ± 0.22; M/TRAP: 1.16 ± 0.22 |
| 4G 10        | F/FA: 3.27 ± 0.40; F/TRAP: 3.29 ± 0.91; M/FA: 3.03 ± 4.56; M/TRAP: 4.56 ± 1.18 | F/FA: 3.27 ± 0.40; F/TRAP: 3.29 ± 0.91; M/FA: 3.03 ± 4.56; M/TRAP: 4.56 ± 1.18 |
| 4G 15        | F/FA: 4.83 ± 1.08; F/TRAP: 8.25 ± 2.06; M/FA: 8.45 ± 3.29; M/TRAP: 11.64 ± 1.53 | F/FA: 4.83 ± 1.08; F/TRAP: 8.25 ± 2.06; M/FA: 8.45 ± 3.29; M/TRAP: 11.64 ± 1.53 |

Values correspond to data shown in Figure 4 and Figure S2. F: female; M: male; WT: wildtype F344; FA: filtered air; TRAP: Traffic-related air pollution; DG: dentate gyrus; EC: entorhinal cortex; Thal: thalamus; Cer: cerebellum.

### Table S8. Summary of numeric data in Figure 5.

| Figure panel | Age (mo) | WT values (mean ± S.D.) | TgF344-AD values (mean ± S.D.) |
|--------------|---------|-------------------------|-------------------------|
| 5B 3         | F/FA: 15.66 ± 2.24; F/TRAP: 15.58 ± 3.02; M/FA: 12.06 ± 2.91; M/TRAP: 15.03 ± 1.69 | F/FA: 15.66 ± 2.24; F/TRAP: 15.58 ± 3.02; M/FA: 12.06 ± 2.91; M/TRAP: 15.03 ± 1.69 |
| 5B 6         | F/FA: 17.30 ± 2.32; F/TRAP: 17.31 ± 0.87; M/FA: 15.38 ± 2.34; M/TRAP: 14.93 ± 2.41 | F/FA: 17.30 ± 2.32; F/TRAP: 17.31 ± 0.87; M/FA: 15.38 ± 2.34; M/TRAP: 14.93 ± 2.41 |
| 5B 10        | F/FA: 17.52 ± 2.58; F/TRAP: 19.24 ± 4.41; M/FA: 16.01 ± 2.37; M/TRAP: 16.49 ± 1.86 | F/FA: 17.52 ± 2.58; F/TRAP: 19.24 ± 4.41; M/FA: 16.01 ± 2.37; M/TRAP: 16.49 ± 1.86 |
| 5B 15        | F/FA: 19.34 ± 2.63; F/TRAP: 22.16 ± 3.00; M/FA: 20.09 ± 3.18; M/TRAP: 19.14 ± 1.66 | F/FA: 19.34 ± 2.63; F/TRAP: 22.16 ± 3.00; M/FA: 20.09 ± 3.18; M/TRAP: 19.14 ± 1.66 |

Values correspond to data shown in Figure 5. F: female; M: male; WT: wildtype F344; FA: filtered air; TRAP: Traffic-related air pollution.
| Figure panel | Age (mo) | WT values (mean ± S.D.) | TgF344-AD values (mean ± S.D.) |
|--------------|---------|-------------------------|-------------------------------|
| 6B           | 3       | F/FA: 1.00 ± 0.04; F/TRA: 1.13 ± 0.09; M/FA: 1.01 ± 0.03; M/TRA: 1.06 ± 0.05 | F/FA: 1.02 ± 0.05; F/TRA: 1.15 ± 0.16; M/FA: 1.01 ± 0.03; M/TRA: 1.15 ± 0.05 |
|              | 6       | F/FA: 1.08 ± 0.09; F/TRA: 1.27 ± 0.15; M/FA: 1.21 ± 0.09; M/TRA: 1.40 ± 0.12 | F/FA: 1.28 ± 0.13; F/TRA: 1.50 ± 0.20; M/FA: 1.48 ± 0.14; M/TRA: 2.03 ± 0.29 |
|              | 10      | F/FA: 2.66 ± 0.31; F/TRA: 3.54 ± 0.06; M/FA: 2.15 ± 0.66; M/TRA: 2.73 ± 0.29 | F/FA: 3.54 ± 0.06; F/TRA: 2.94 ± 1.04; M/FA: 3.40 ± 0.38; M/TRA: 3.83 ± 0.37 |
|              | 15      | F/FA: 3.65 ± 0.78; F/TRA: 2.65 ± 0.60; M/FA: 3.39 ± 1.07; M/TRA: 4.58 ± 0.90 | F/FA: 5.17 ± 0.59; F/TRA: 4.20 ± 0.55; M/FA: 4.98 ± 0.23; M/TRA: 6.15 ± 0.76 |
| 6C           | 3       | F/FA: 228.23 ± 8.71; F/TRA: 238.19 ± 16.95; M/FA: 240.94 ± 29.86; M/TRA: 228.67 ± 13.20 | F/FA: 240.94 ± 29.86; F/TRA: 228.67 ± 13.20; M/FA: 223.67 ± 17.12; M/TRA: 246.90 ± 25.40 |
|              | 6       | F/FA: 252.53 ± 16.01; F/TRA: 272.01 ± 31.82; M/FA: 249.61 ± 19.28; M/TRA: 261.70 ± 15.33 | F/FA: 262.86 ± 18.52; F/TRA: 263.68 ± 27.54; M/FA: 259.84 ± 18.03; M/TRA: 246.61 ± 15.22 |
|              | 10      | F/FA: 370.10 ± 57.69; F/TRA: 506.99 ± 29.92; M/FA: 439.46 ± 67.24; M/TRA: 417.15 ± 64.86 | F/FA: 436.81 ± 36.91; F/TRA: 550.32 ± 68.67; M/FA: 416.50 ± 51.79; M/TRA: 508.58 ± 24.78 |
|              | 15      | F/FA: 425.96 ± 53.06; F/TRA: 472.03 ± 94.89; M/FA: 440.07 ± 45.64; M/TRA: 433.31 ± 27.70 | F/FA: 552.57 ± 70.14; F/TRA: 591.11 ± 64.71; M/FA: 627.41 ± 39.20; M/TRA: 628.74 ± 44.69 |

Values correspond to data shown in Figure 6. F: female; M: male; WT: wildtype F344; FA: filtered air; TRAP: Traffic-related air pollution.
Figure S1. Photo montage of the various systems comprising the Tunnel Exposure Facility.
Figure S2. Effects of TRAP on cued fear conditioning and neuronal cell loss in the hippocampus. (A-B) Cued fear conditioning was performed on-site at 9.5 or 14.5 month old animals for the 10 and 15 month-old cohort. An increased average motion index indicates impaired cognitive behavior. (C-D) To assess neuronal cell loss, brain sections were immunostained for NeuN, a biomarker of neurons, and the number of NeuN-immunopositive cells per mm² were counted in the CA1. (E) Densitometric analyses of total tau relative to GAPDH in the crude pellet fraction of cortical tissue. All data presented as the mean ± SD (n=10-12 animals per group for A-D; n=5-6 animals per group for E). Data were analyzed by three-way ANOVA using sex, genotype, and exposure as factors (A-B, E) or two-way ANOVA using genotype and exposures as factors (C, D) with post-hoc Sidak’s test; *p<0.05. Circles represent individual animals (for C-E, each circle is an average of 4 technical replicates). M=male; F=female; WT=wildtype; Tg=TgF344-AD. Summary values are available in Table S6.
Figure S3. Effects of TRAP on Aβ deposition by brain region, and on guanidine-HCL soluble brain extracts.

(A) Analyses of ThioS+ plaques by brain regions in TgF344-AD rats (DG=dentate gyrus; EC=entorhinal cortex; Thal=thalamus; Cer=cerebellum) (B) Guanidine-HCL-soluble ratios of Aβ42:40, as measured by ELISA in cortical samples and normalized to Aβ levels in 3-month-old WT female rats. All data presented as the mean ± SD (n=5-6 animals per group). Circles represent individual animals. Four brain sections were measured per animal in A, and two technical replicates were performed for each animal in B. M=male; F=female. Data were analyzed by three-way ANOVA using sex, genotype, and exposure as factors, with post-hoc Sidak’s test. *p<0.05. Summary data are available in Table S7.