Supplementary Information: Experimental Procedures & Supplemental Figures 1-6

Experimental Procedures

Molecular biology and strains

The ASE YC2.12 expression plasmid was constructed in the following manner: a 2680 bp 5-UTR region of the *flp-6* gene was PCR amplified from *C. elegans* genomic DNA using the proof-reading enzyme Pwo-polymerase (Roche) and primers CGCTGCAGCGCTTGACTTCTGATGACATAT and GCGTCGACATTCTGGAATAATCATATTGTTTTCAAAAATAATG. The promoter fragment was digested by Pst I and Sal I, and cloned into a Fire Lab '97 Vector kit plasmid L3613 cleaved by Pst I and EcoRI together with a YC2.12 cameleon cDNA obtained by Sal I/ EcoRI digestion of a *mec-4*::YC2.12 expression vector[1]. The expression construct was injected into *lin-15(n765)* worms along with a rescuing *lin-15(+)* marker[2] by standard methods[3]. AQ1216 *lin-15(n765); ljEx135[flp-6::YC2.12; lin-15(+)]* was integrated and outcrossed four times, yielding XL76 *ntIs13[flp-6::YC2.12, lin-15(+)]*. XL76 was used for all wild-type imaging experiments except for Supplemental Fig. 2 where AQ1216 was used. Fluorescence in the head region was detectable in these lines only in ASEL and ASER, although in a previous report a GFP reporter construct with a similar promoter fragment was also expressed in AFD[4]. Possible explanations for the different expression pattern are: (1) YC2.12 cDNA contained 18bp upstream of the initiation Met to optimize expression in mammalian tissues, (2) a slightly shorter promoter region was used for YC2.12, (3) in our experience, cameleon emission tends to be weaker than GFP emission and to diminish in some tissues during development. All four calcium binding sites in cameleon were mutated (E31Q, E67Q, E104Q and E140Q) to generate the calcium-insensitive cameleon. When we imaged mechanosensory neurons[1] using the mutated probe, we found that ratio changes normally present after a touch stimulus were absent. *flp-6*::YC2.12 was introduced into mutant backgrounds by crossing mutants with XL76. Mutants were provided by the *Caenorhabditis* Genetics Center, which is funded by the NIH National Center for Research Resources (NCRR). List of strains used in this study: AQ1216 *lin-15(n765); ljEx135[flp-6::YC2.12; lin-15(+)],* XL76 *ntIs13[flp-6::YC2.12, lin-15(+)],* AQ1470 *lin-15(n765); ljE136x[flp-6::YC2.12-EallQ; lin-15(+)],* XL128 *unc-13(e51); ntIs13[flp-6::YC2.12, lin-15(+)]*, XL134 *unc-31(e928); ntIs13[flp-6::YC2.12, lin-15(+)]*, XL135 *snb-1(md247); ntIs13[flp-6::YC2.12, lin-15(+)],* XL139 *tax-2(p671); ntIs13[flp-6::YC2.12, lin-15(+)]*, XL139 *tax-4 (p678); ntIs13[flp-6::YC2.12, lin-15(+)],* XL136 *egl-4(n479); ntIs13[flp-6::YC2.12, lin-15(+)],* AQ1467 *lin-15(n765); ljEx138[flp-6::YC2.12, gcy-7::TRPV1, lin-15(+)]*, AQ1468 *lin-15(n765); ljEx139[flp-6::YC2.12, gcy-5::TRPV1, lin-15(+)].*

References

1 Suzuki, H. *et al. In vivo* imaging of *C. elegans* mechanosensory neurons demonstrates a specific role for the MEC-4 channel in the process of gentle touch sensation. *Neuron.* **39**, 1005-1017 (2003).

2 Huang, L.S., Tzou, P. & Sternberg, P.W. The lin-15 locus encodes two negative regulators of Caenorhabditis elegans vulval development. *Mol Biol Cell.* **5**, 395-411 (1994).

3 Mello, C. & Fire, A. DNA transformation. *Methods Cell Biol.* **48**, 451-482 (1995).

[4] Li, C., Kim, K. & Nelson, L.S. FMRFamide-related neuropeptide gene family in Caenorhabditis elegans. *Brain Res.* **848**, 26-34 (1999).

Supplementary Figure Legends

Figure S1. Response of ASEL and ASER to NaCl concentration steps

a. The individual calcium transients that underlie the average traces shown in Fig. 1a,b. **b**. Average calcium transients in ASE neurons in response to NaCl concentration steps of ± 40 mM from baselines of 0 and 80 mM, rather than from a baseline of 40 mM as in Fig. 1a,b (see also Fig. 4a for another example of responses from different NaCl baselines). The gray band represents ± 1 standard error of the mean (SEM). In both panels, ASEL is shown on the left and ASER is shown on the right and the concentration step is indicated below the calcium traces.

Figure S2. Ratio changes with Ca^{2+} insensitive cameleon and representative responses to other salts or non-ionic compounds

a. Average ratio traces in ASE neurons expressing Ca^{2+} insensitive cameleon. Traces are the response to NaCl concentration steps of ± 40 mM from a baseline of 40 mM. The gray band represents ± 1 standard error of the mean. The concentration step is indicated below the ratio traces. The transients observed here were markedly smaller and opposite in sign to the transients observed with Ca^{2+} sensitive cameleon, indicating that the transients observed in Text Figures 1-4 reflect increases in Ca^{2+} in ASE neurons. **b**-**d**. Representative calcium traces in ASE neurons in response to brief pulses of the indicated compound (horizontal lines below the traces) at 40 mM. Note that ASE responds to 40 mM ammonium acetate in **b**. In contrast, ASE does not respond to 10 mM ammonium acetate (Fig. 1e, Supplemental Fig. 3a). Thus ASE neurons have a higher threshold for ammonium acetate than for NaCl.

Figure S3. Differential sensitivity of ASEL and ASER to Na^+ and Cl^-

a-d. Average ASE calcium transients in response to ± 10 mM concentration steps of the indicated compound from a baseline of 40 mM. The concentration step is indicated above each column of traces. The gray band represents ± 1 standard error of the mean (SEM); $n \geq 5$ five recordings, with one recording per worm. In **a**-**c**, the ASER downstep column and the ASEL upstep column show SEM for the data in Text Fig. 1e.

Figure S4. Effects of additional synaptic and signal transduction mutants on ASE sensory responses

Average ASE calcium transients in two mutant strains in response to NaCl concentration steps of ± 40 mM from a baseline of 40 mM. **a**. A mutant with a defect in synaptic vesicle release. **b**. A mutant with a defect in cGMP-dependent signaling. Excess noise in **b** is due to low levels of cameleon expression in this mutant. In each panel: traces indicate average percent change in ratio; the gray band represents ± 1 standard error of the mean (SEM); ASEL is shown on the left and ASER is shown on the right; the concentration step is indicated below each calcium trace; and $n \geq 5$ five recordings, with one recording per worm.

Figure S5. Control experiments for unilateral activation of ASEL and ASER

a-d. The time course of ratio changes in ASE neurons in strains expressing the capsaicin receptor (TRPV1) exclusively in either ASEL or ASER. Dashed lines indicate the onset of maintained application of 25μM capsaicin or vehicle (0.25% ETOH) as indicated on the left. The gray band represents ± 1 standard error of the mean with $n \geq 4$. Panels **a** and **c** show that calcium transients are detected only in the neuron expressing TRPV1; panels **b** and **d** show that calcium transients are not elicited by vehicle alone.

Figure S6. Effects of unilateral and bilateral ASE ablations on the behavioural response to large NaCl concentration steps

a-f. Probability of forward locomotion is plotted against time relative to the step. Statistical significance (ablation vs. sham) was assessed via repeated measures ANOVA over the indicated time window (horizontal lines above traces) following the step (shown above **a** and **b**). As in the case of small NaCl steps (Text Fig. 4b-g), ASER is required for normal responses to upsteps and downsteps (**a**, **b**, **e**, **f**), whereas ASEL is required for normal responses to upsteps but not downsteps (**c** vs. **d**). Effects of ablations here are generally the same or smaller than in the case of small NaCl steps, consistent with the likelihood of greater neuronal redundancy when stronger stimuli are used. Notation and symbols: *ASEL(-)*, ASEL ablation; *ASER(-)*, ASER ablation; *ASE(-)*, bilateral ASE ablation; *solid horizontal line*, ANOVA significant at $p < 0.05$ or less; *dashed horizontal line*, ANOVA not significant; *stars*, time points at which there was a significant difference between means after correcting for multiple comparisons (*t* test; $p < 0.05$); *pluses*, time points at which there were significant differences in uncorrected *t* tests ($p < 0.05$). The gray band represents ± 1 standard error of the mean with $n \geq 20$ in each panel.

Figure S1

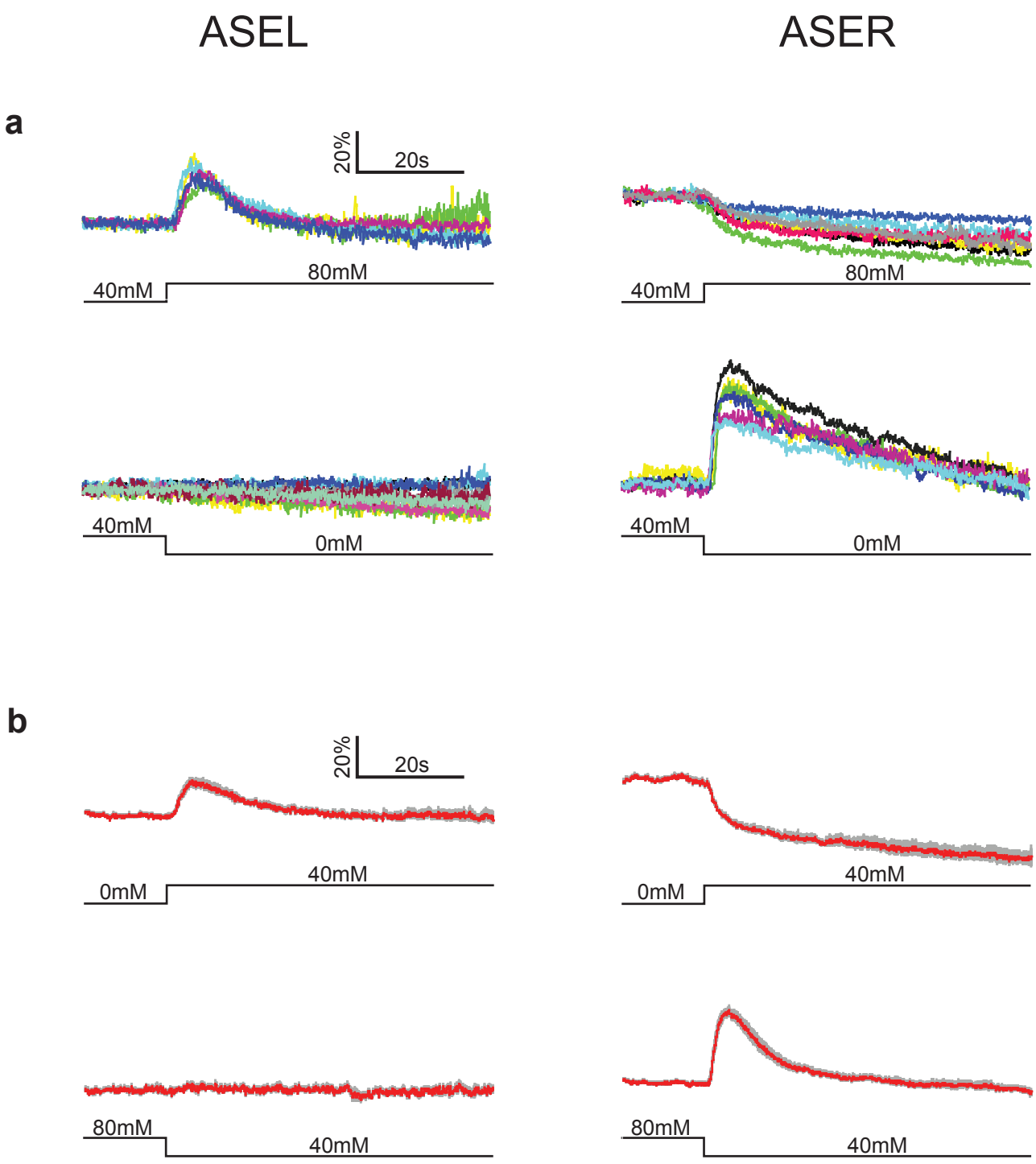
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Figure S2

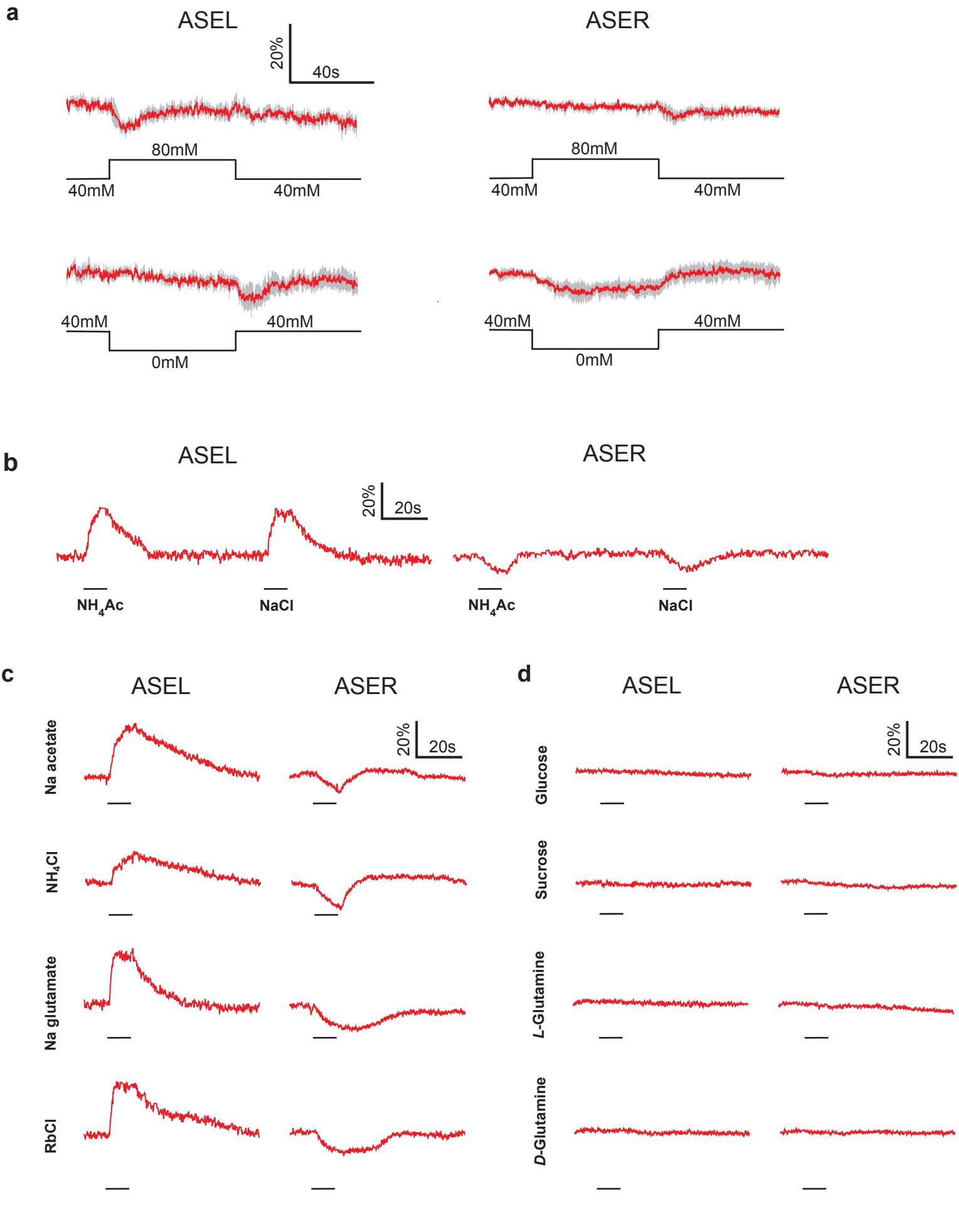
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Figure S3

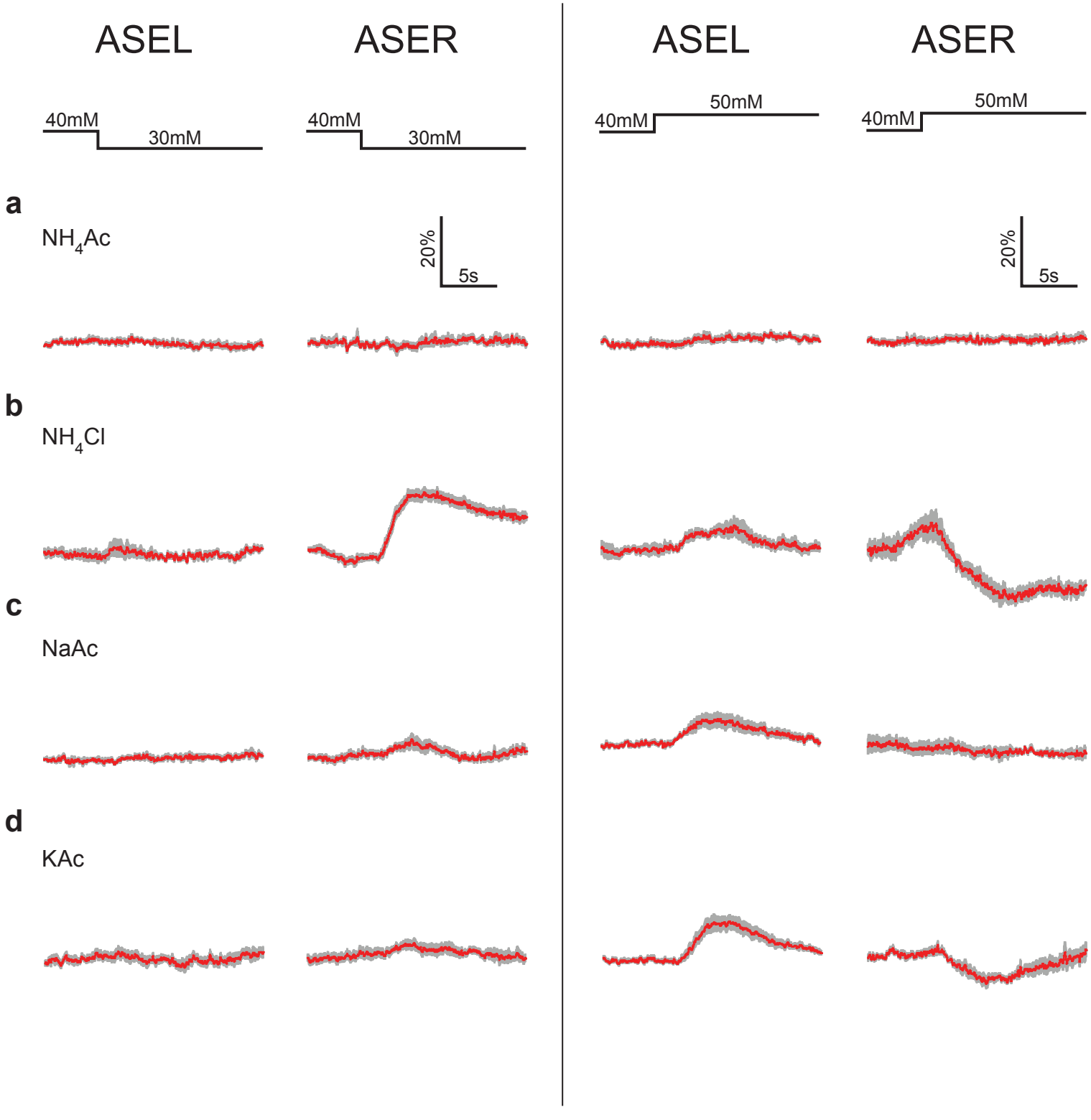
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Figure S4

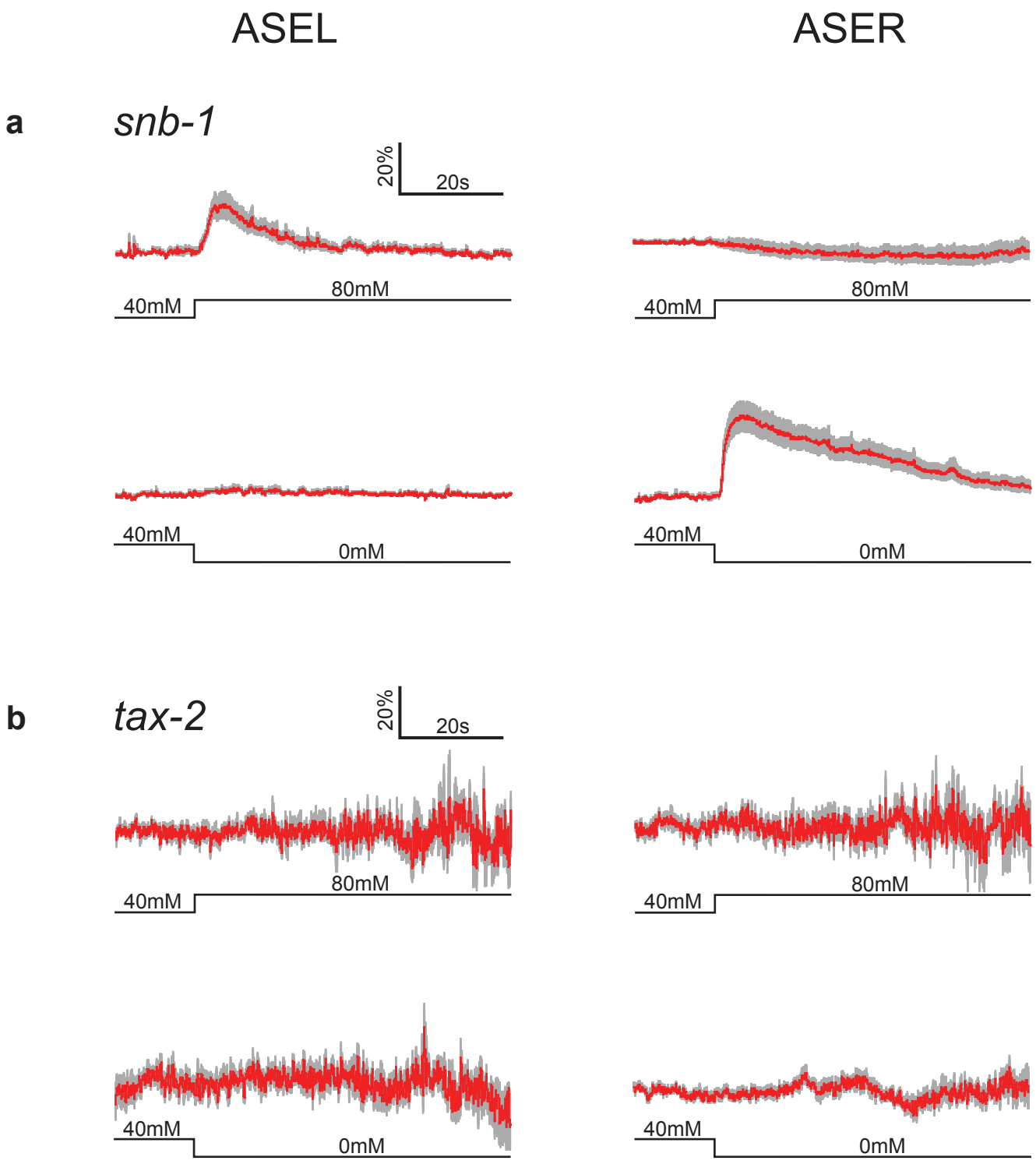
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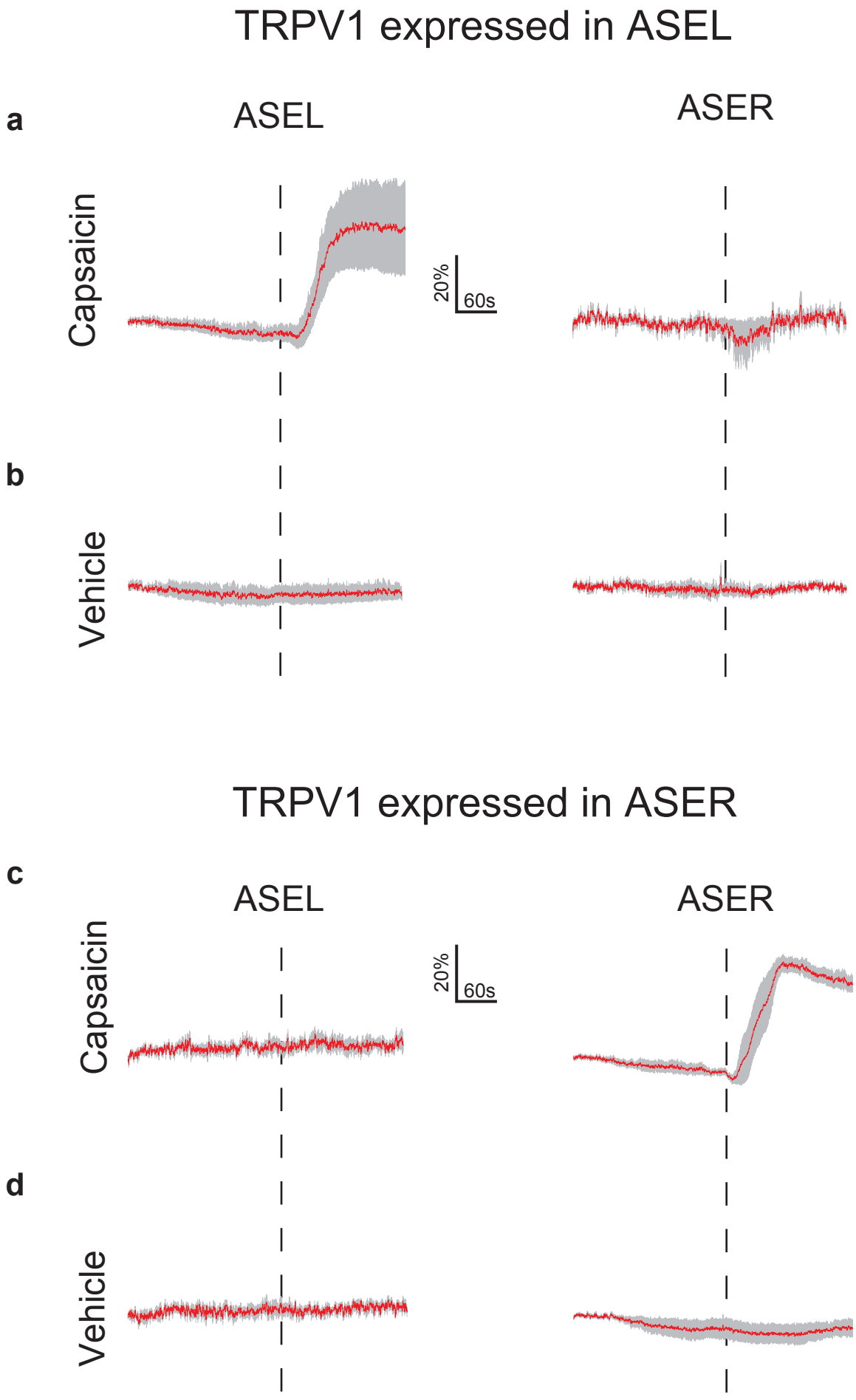
Figure S5


TRPV1 expressed in ASEL
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ASER
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b
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c
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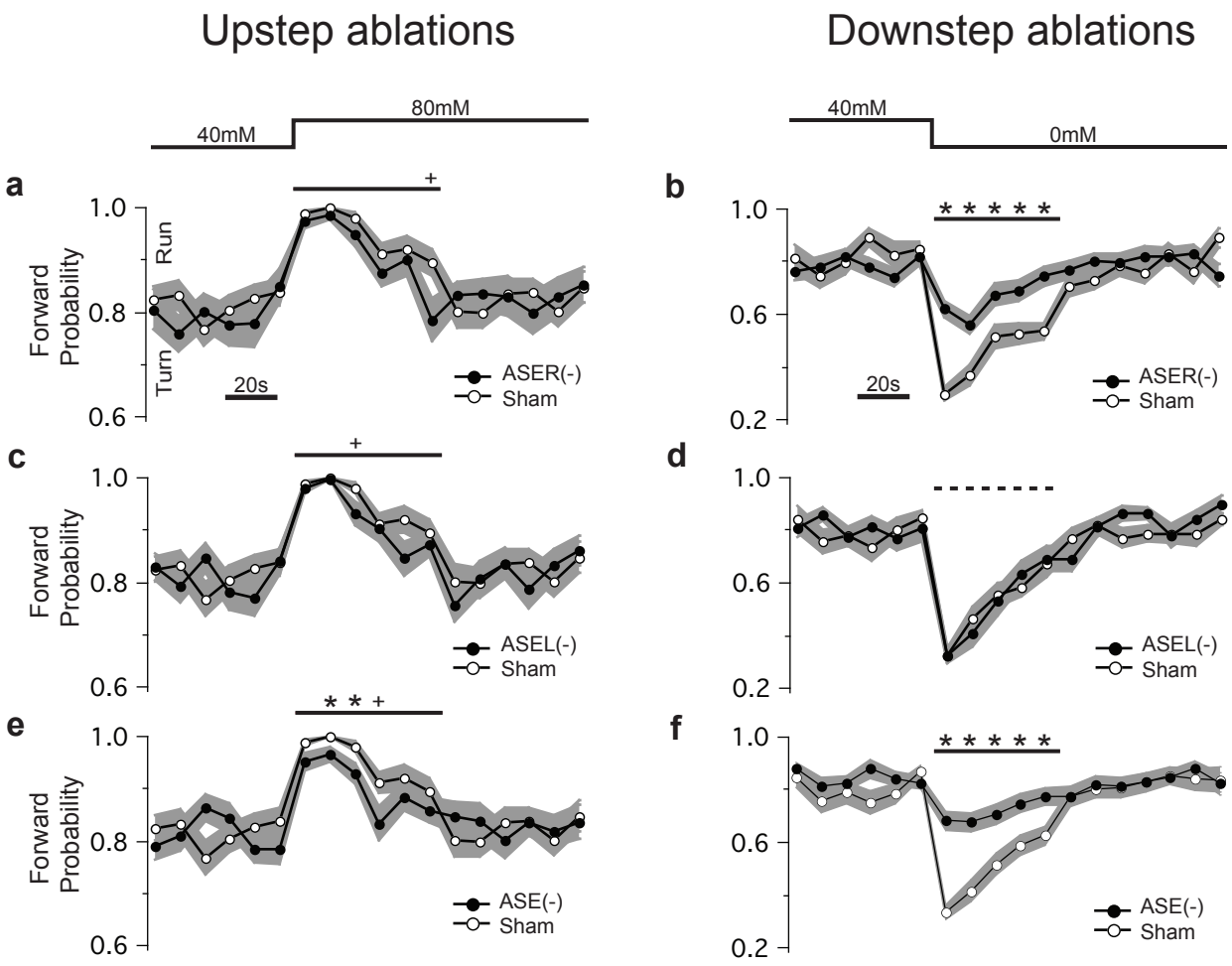
20%
60s
d
Vehicle

Figure S6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