Genetic determinants of phosphate response in Drosophila.

Supporting Information

Table S1.
Annotations of 146 validated genes.
doi:10.1371/journal.pone.0056753.s010
(XLSX)

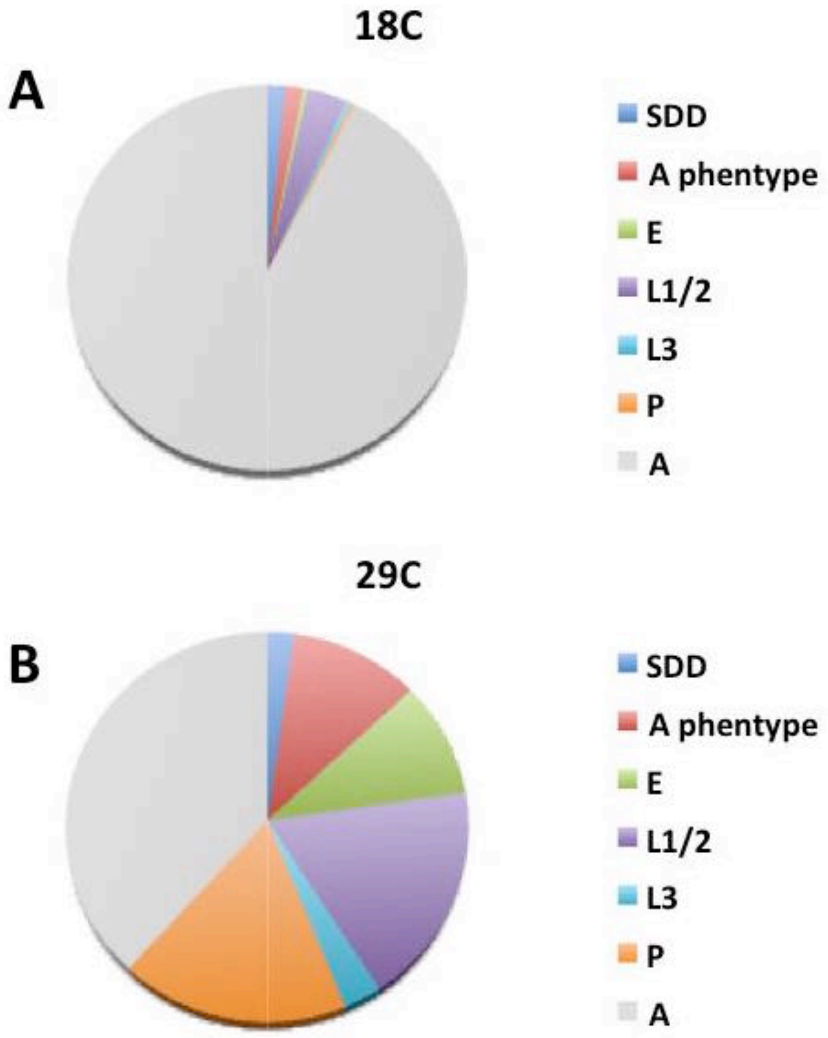

Figure S1.
Developmental phenotypes. A: Latest larval stage observed for F1 offspring generated in matings between 268 UAS-RNAi males and virgin *w-;tub-Gal80^{ts20};da-Gal4* females when cultured on standard medium at 18°C (non-inducing temperature). **B:** Latest larval stage observed for F1 offspring generated as described for (A) with the same genetic crosses on standard medium at 29°C (inducing temperature).
doi:10.1371/journal.pone.0056753.s001
(TIF)

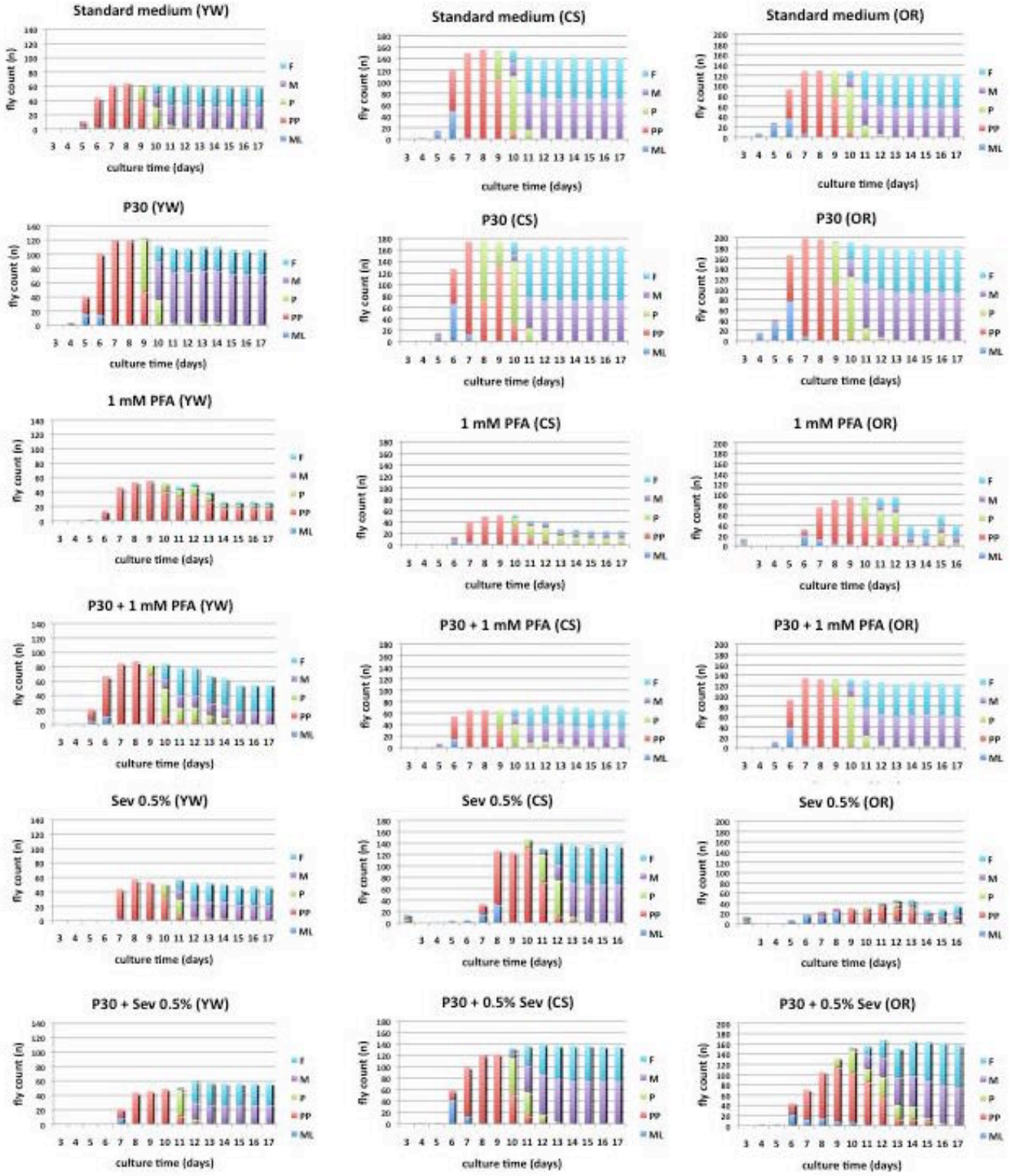

Figure S2.
The effect of sevelamer and PFA on larval development by developmental stage. Shown is larval development of three wild-type strains: *y w* (YW), Oregon R (OR) *and* Canton S (CS) on control (C), P30, Sev1% and PFA 1 mM medium. Abbreviations as follows: ML, migrating instar 3 larva; PP, prepupa; P, pupa; M, adult male; F, adult female. Shown is one representative experiment with cumulative fly counts, means of three vials per condition.
doi:10.1371/journal.pone.0056753.s002
(TIF)

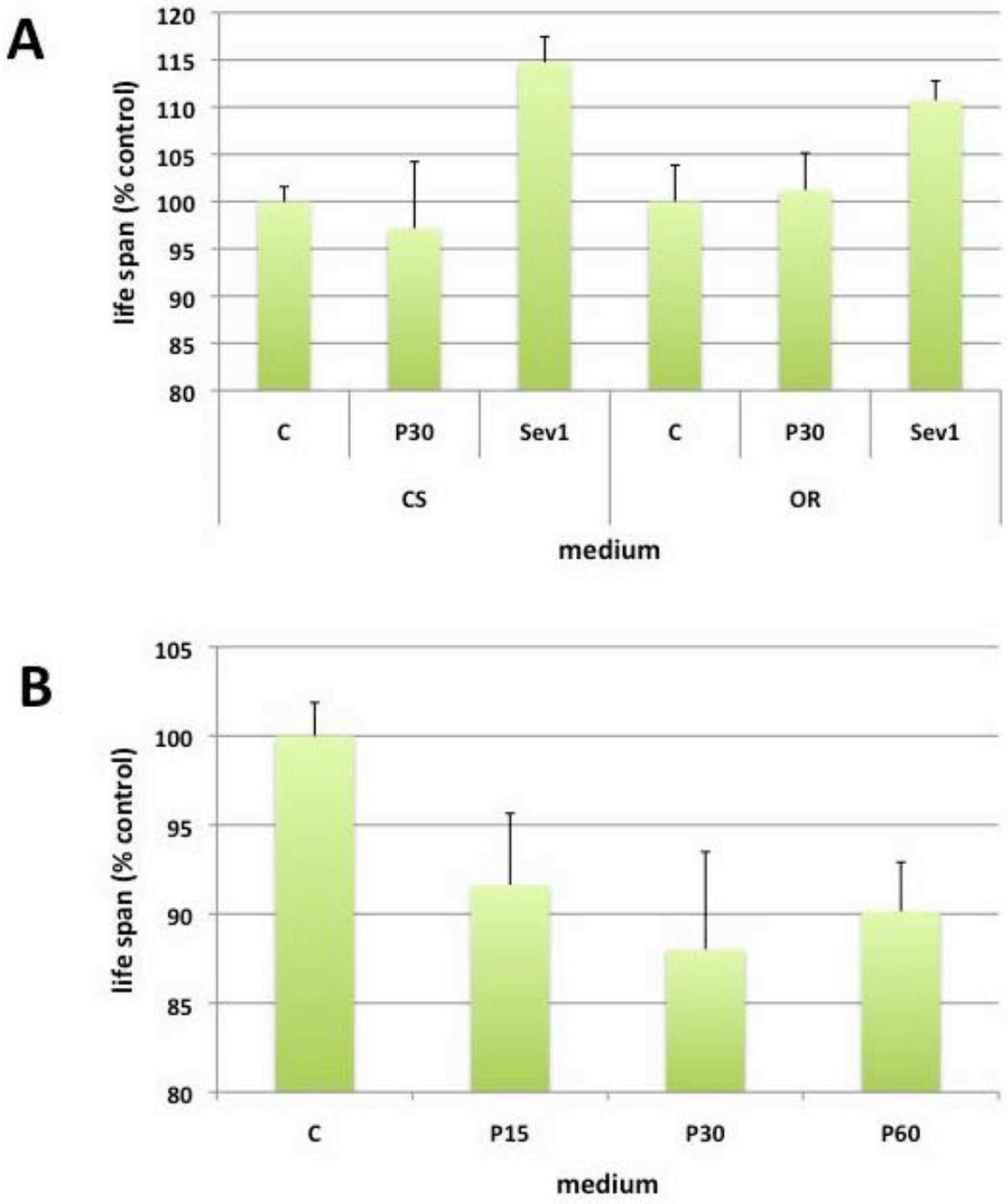

Figure S3.
Effect of phosphate, sevelamer and PFA on adult life span. A: Median life span of Canton S (CS) *and* Oregon R (OR) wild type males on standard medium or SM with 30 mM sodium phosphate or 1% sevelamer (CS: C, n=155; P30, n=62; Sev1, n=58, OR: C, n=117; P30, n=52; Sev1, n=59). **B:** Median life span of *y w* males on standard medium, supplemented with 15, 30 and 60 mM sodium phosphate (C, n=550; P15, n=282; P30, n=465; P60, n=115). To correct for the influence of osmolarity, life spans for P15, P30, and P60 are displayed as % of life spans for 15, 30, and 60 mM sodium sulfate, respectively.
doi:10.1371/journal.pone.0056753.s003
(TIF)

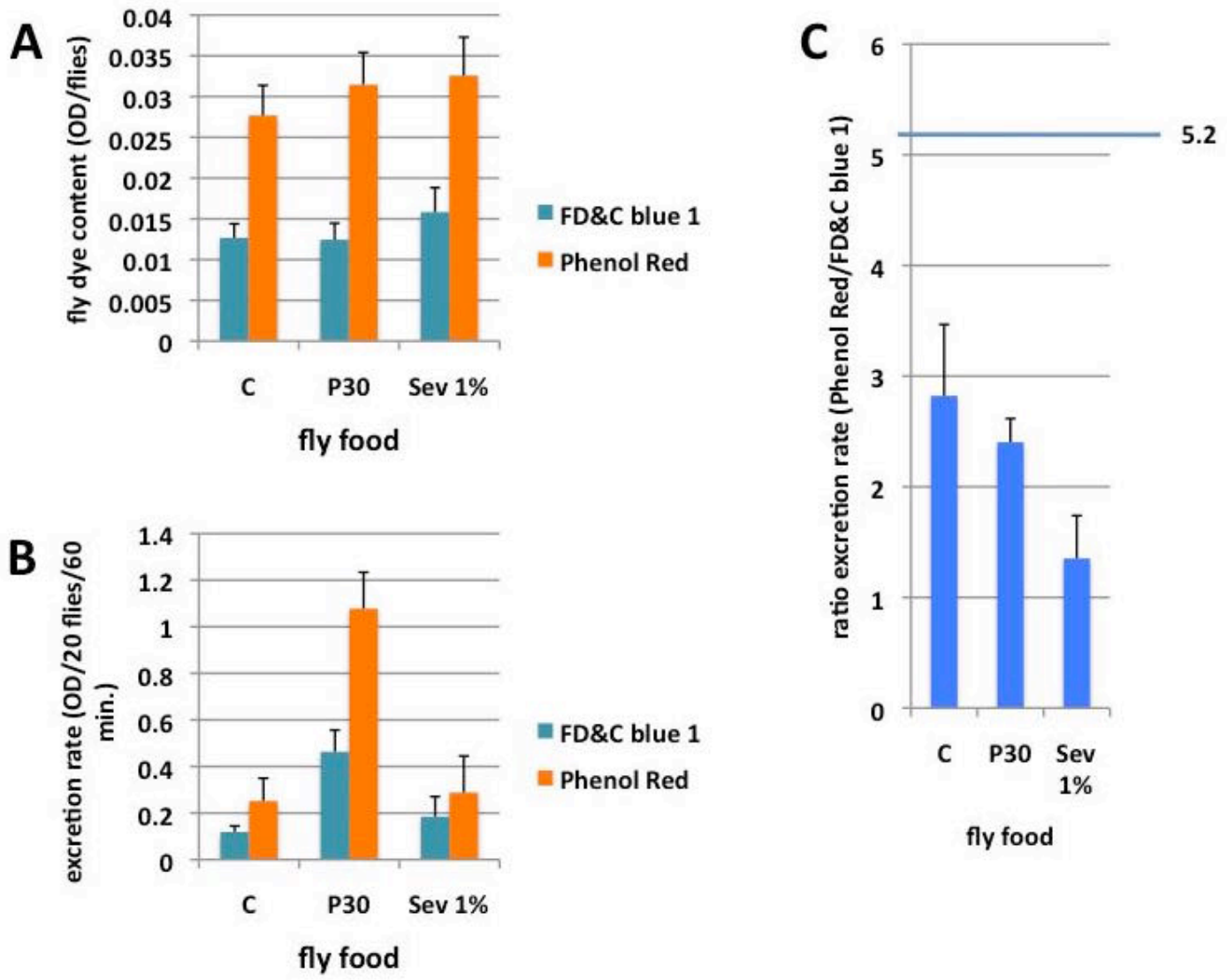

Figure S4.
Dye uptake and excretion. A: Uptake of food dyes within 60 min. **B:** Dye excretion over 60 min. after flies were loaded with food dyes over night. **C:** Ratio of amount of dyes present in excretions (theoretical ratio 5.2 from fresh food is indicated by blue line).
doi:10.1371/journal.pone.0056753.s004
(TIF)

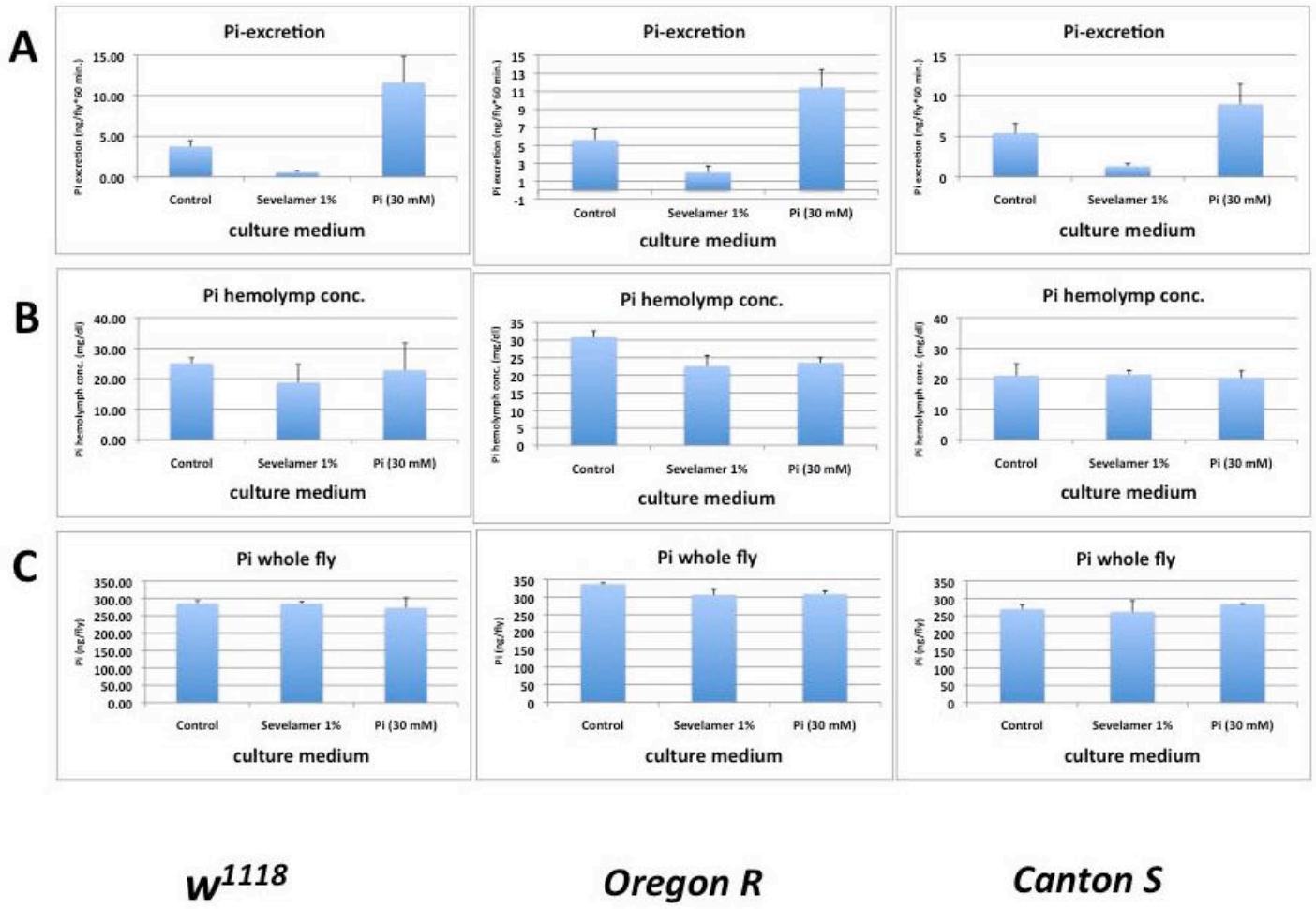

Figure S5.
Adult hemolymph Pi, Phosphate excretion and whole fly Pi. Young adult *w*^{1118}, Canton S or Oregon R females were cultured on standard medium alone or SM supplemented with 30 mM sodium phosphate (P30), or 1% sevelamer. Following culture for 5 days at 25°C, phosphate excretion was determined **(A**)(n=3, 20 flies each), and hemolymph phosphate **(B)** (n=3) and whole fly phosphate **(C)** (n=10 individual flies) were measured.
doi:10.1371/journal.pone.0056753.s005
(TIF)

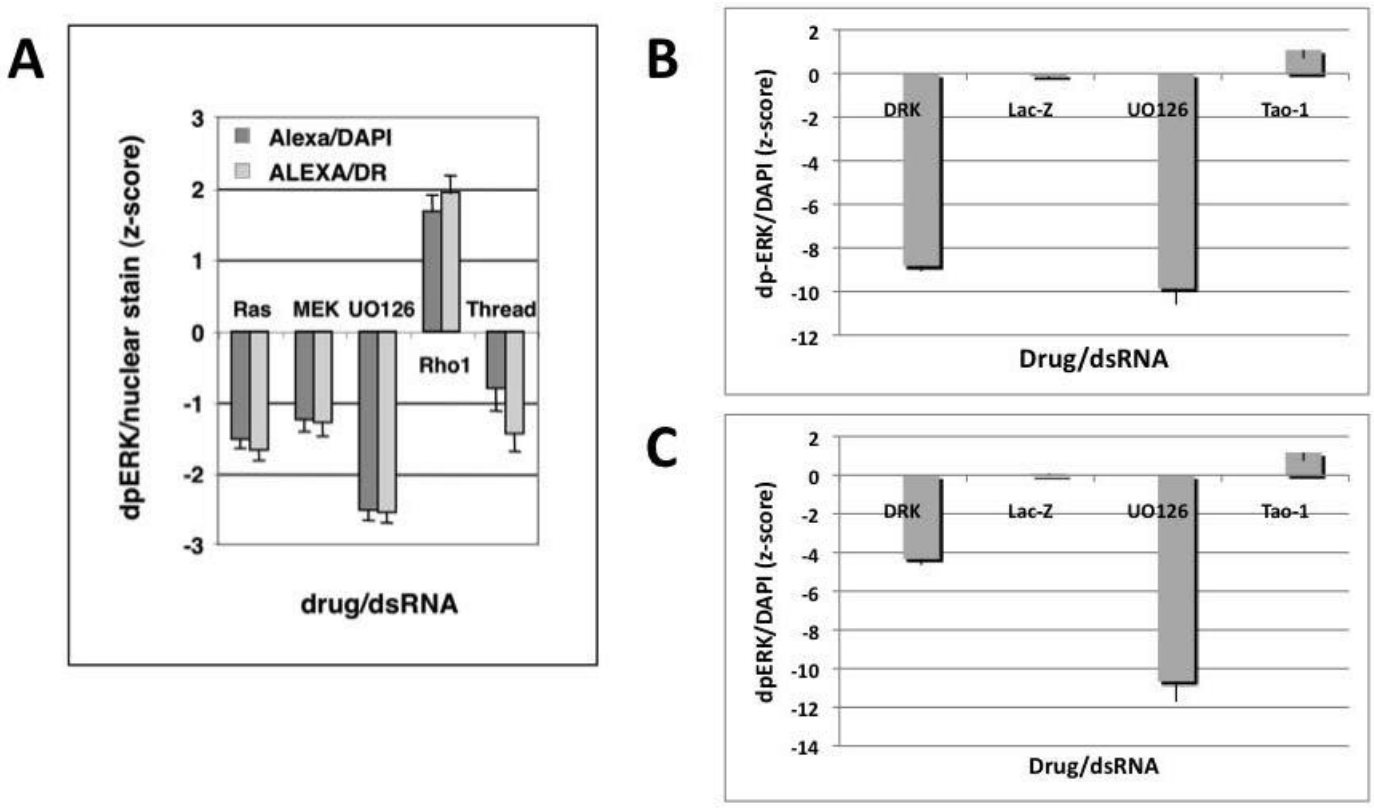

Figure S6.
Genome-wide RNAi screen (Controls). 20,000 S2R+ cells/384 well were treated with 0.375 ug dsRNA targeting Lac-Z, Ras85D, Dsor1/MEK, Rho1 and thread (A, primary screen) or LacZ, drk/GRB2, and Tao-1 **(B,** secondary screen)/well as described in materials and methods. After four days 10 mM phosphate (P10) **(A, B),** 50 ug/ml human insulin **(C)** or 30 uM of the MEK-inhibitor UO126 (UO126) were added for 10 min., followed by fixation and antibody staining for dpERK and cellular staining with Dead Red or DAPI and detection of total fluorescence with the appropriate filter sets. Ratios of dpERK signal over cell Dead Red or nuclear DAPI stain were expressed after subtraction of well background and wandering-median correction across each 384-well plate as z-scores.
doi:10.1371/journal.pone.0056753.s006
(TIF)

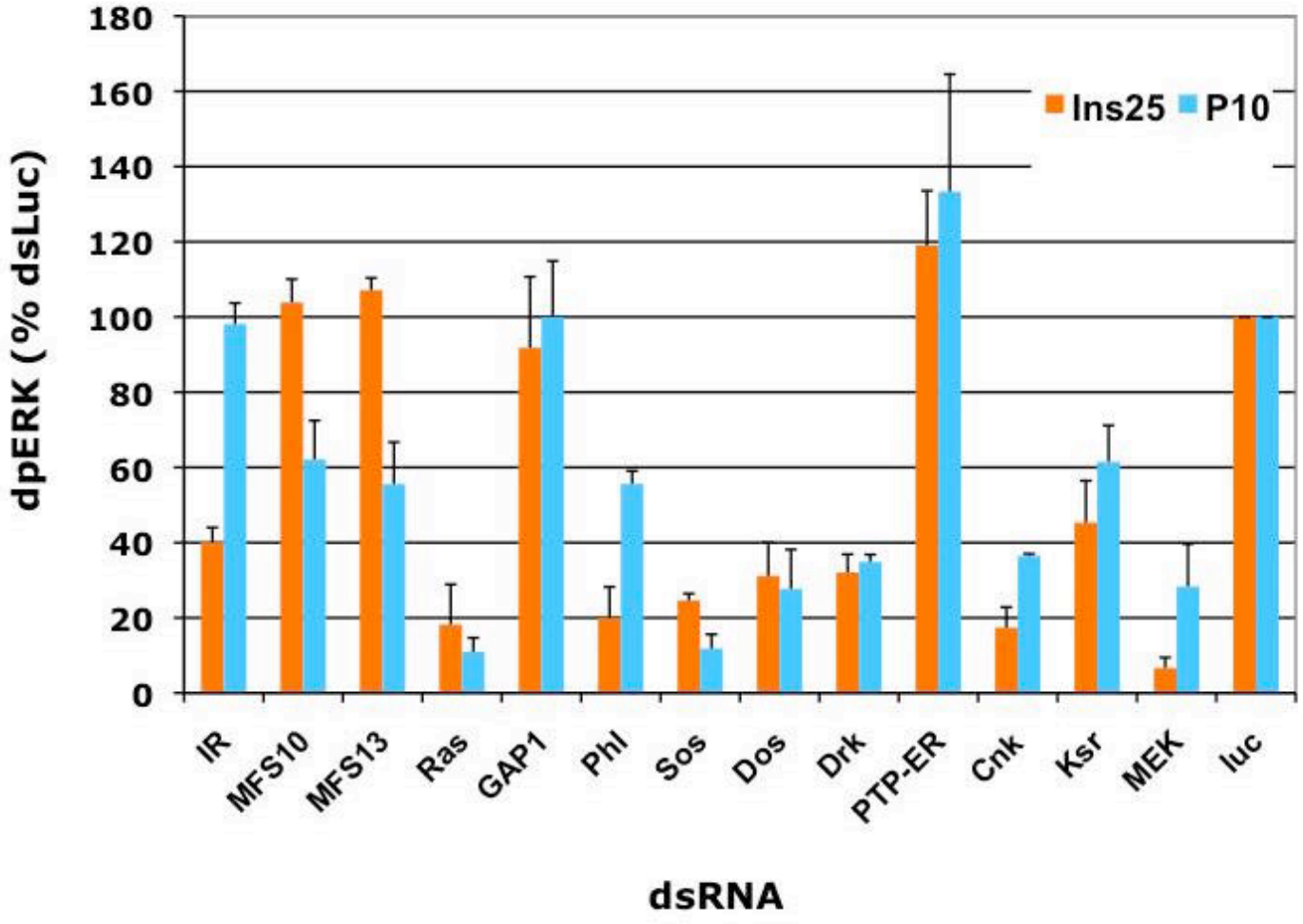

Figure S7.
P-induced ERK activation in murine and *Drosophila* cells is blocked by RNAi-knockdown of sodium-phosphate co-transporters and members of the canonical MAPK pathway. RNAi knockdown in S2R+ cells using dsRNA targeting luciferase (luc), insulin receptor (IR), two sodium-phosphate co-transporters (MFS10 and MFS13), or various components of the canonical MAPK pathway was performed for three days prior to challenge with 10 mM sodium phosphate (pH7.4) or 25 ug/ml Insulin for 3 min. Immunoblot analysis of cell lysates was performed with anti-dpERK antibody, converted into percent-stimulation (mean+/− SD of three independent experiments).
doi:10.1371/journal.pone.0056753.s007
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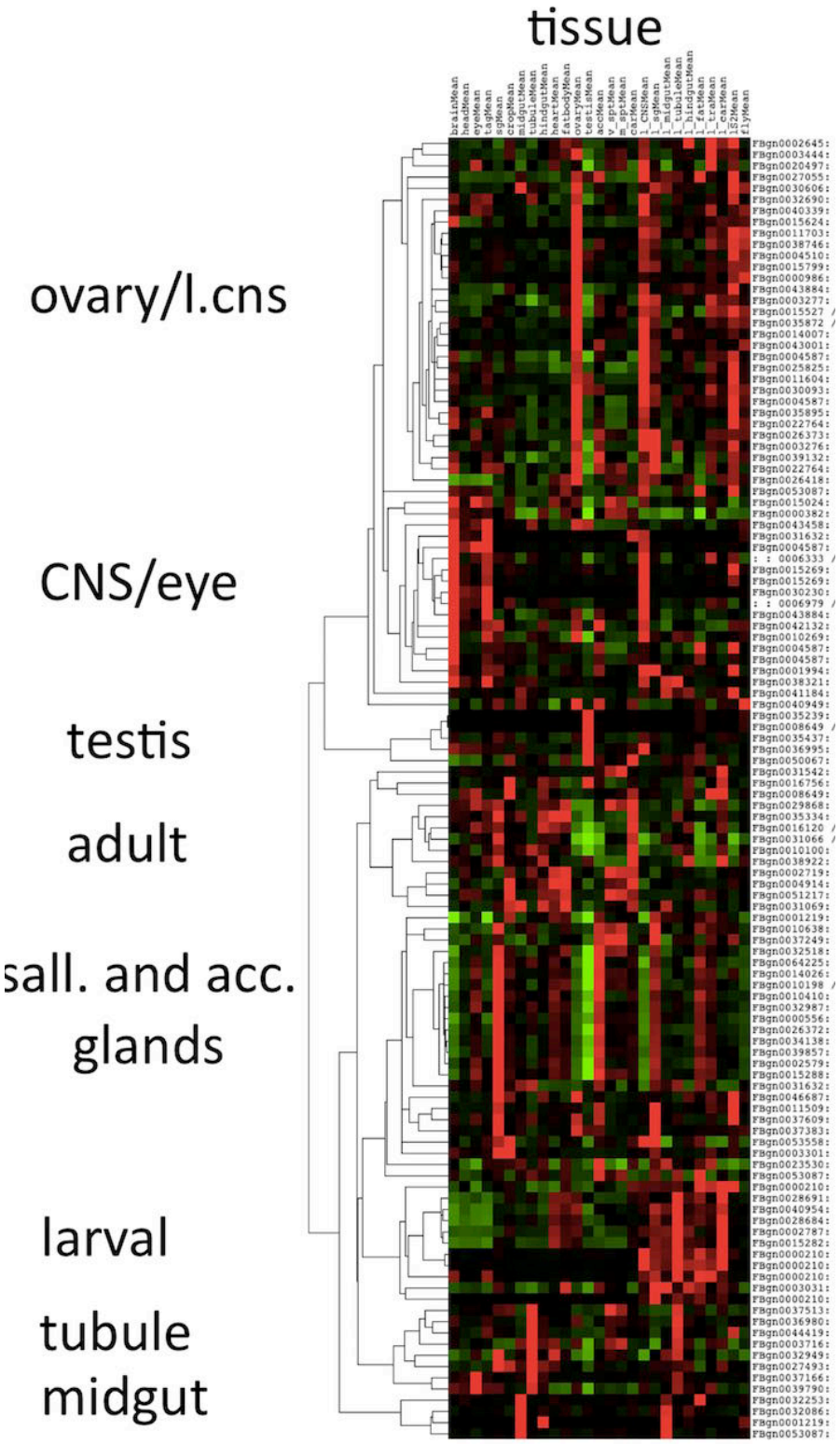

Figure S8.
Tissue distribution of genes indentified in the primary screen. Available expression data for all 146 genes were downloaded from Fly Atlas [44], normalized by gene and hierarchically clustered using Cluster 3.0 [32] and displayed using Java TreeView 1.1.6 [33]. Red indicates high, green low expression.
doi:10.1371/journal.pone.0056753.s008
(TIF)

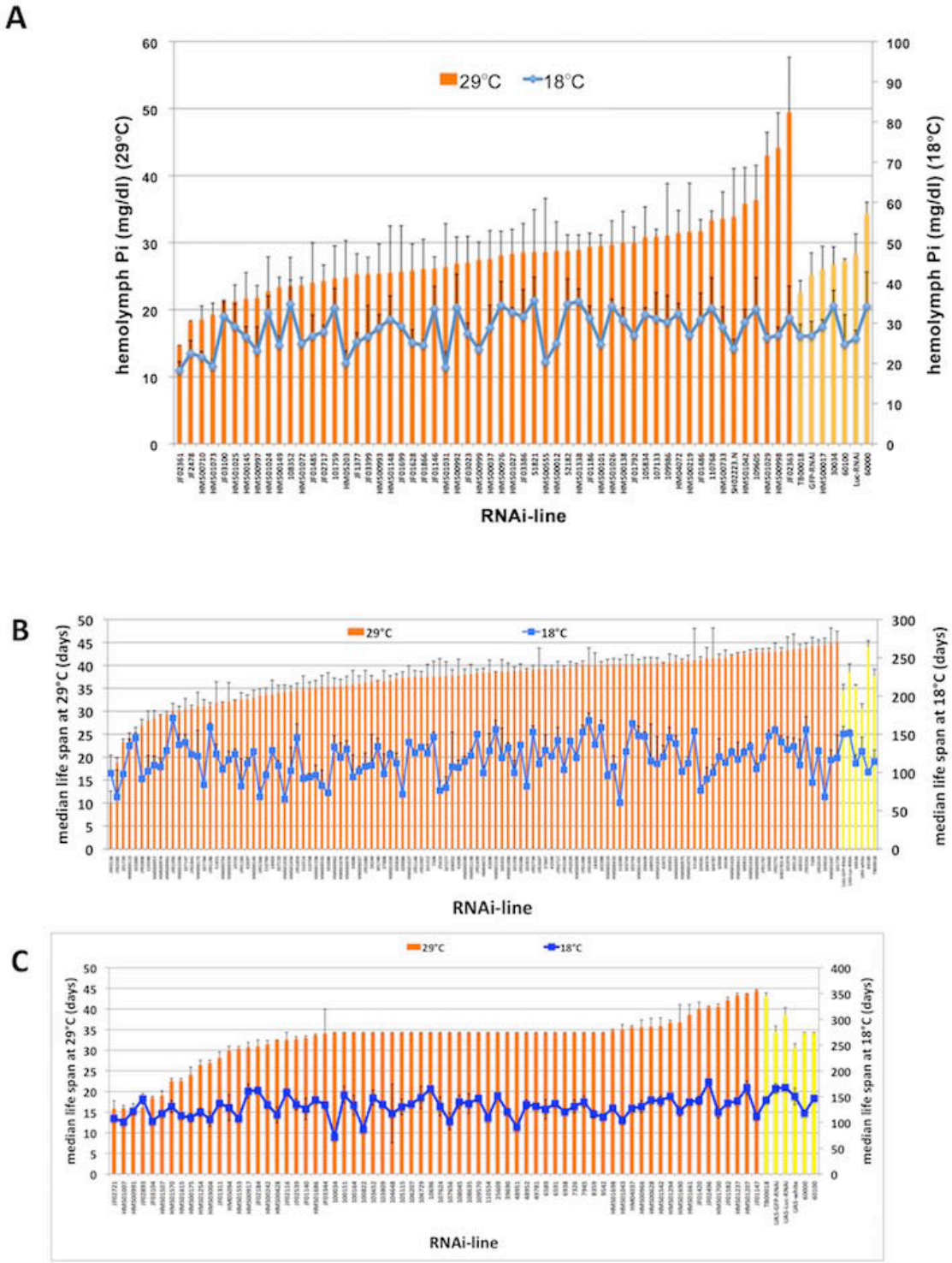

Figure S9.
Temperature dependence of RNAi-effects on hemolymph Phosphate and adult life span. A: Hemolymph phosphate after culture of F1 offspring at inducing temperature 29°C (orange bars), and 18°C (blue line) for 63 RNAi-lines, control hairpins are shown in light orange, mean+/−SEM. **B, C:** Median life-spans of 118 and 68 RNAi-lines at inducing temperature 29°C (orange bars) and 18°C (blue line)
doi:10.1371/journal.pone.0056753.s009
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