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Supplemental Information

Prolonged Exposure to Microgravity Reduces Cardiac Contractility and Initiates Remodeling in *Drosophila*

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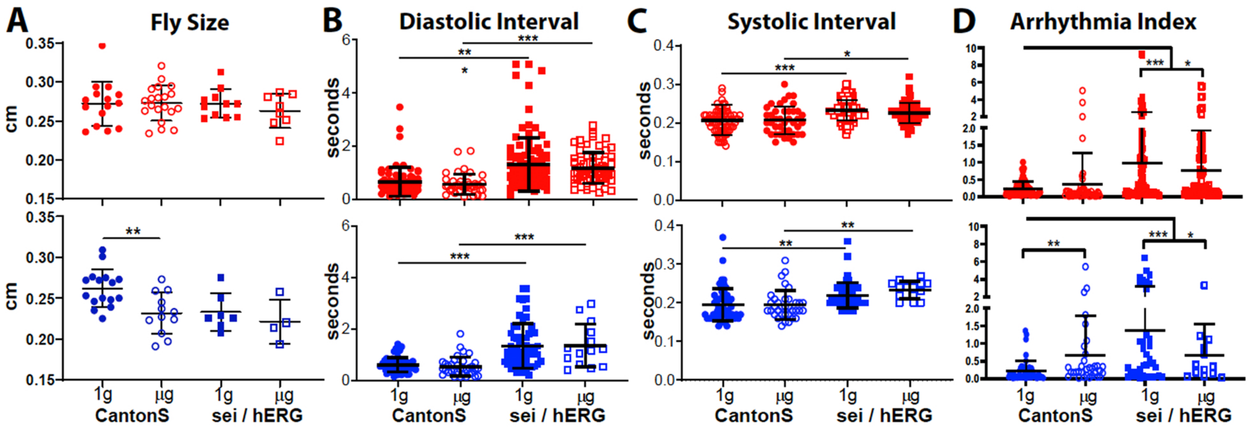
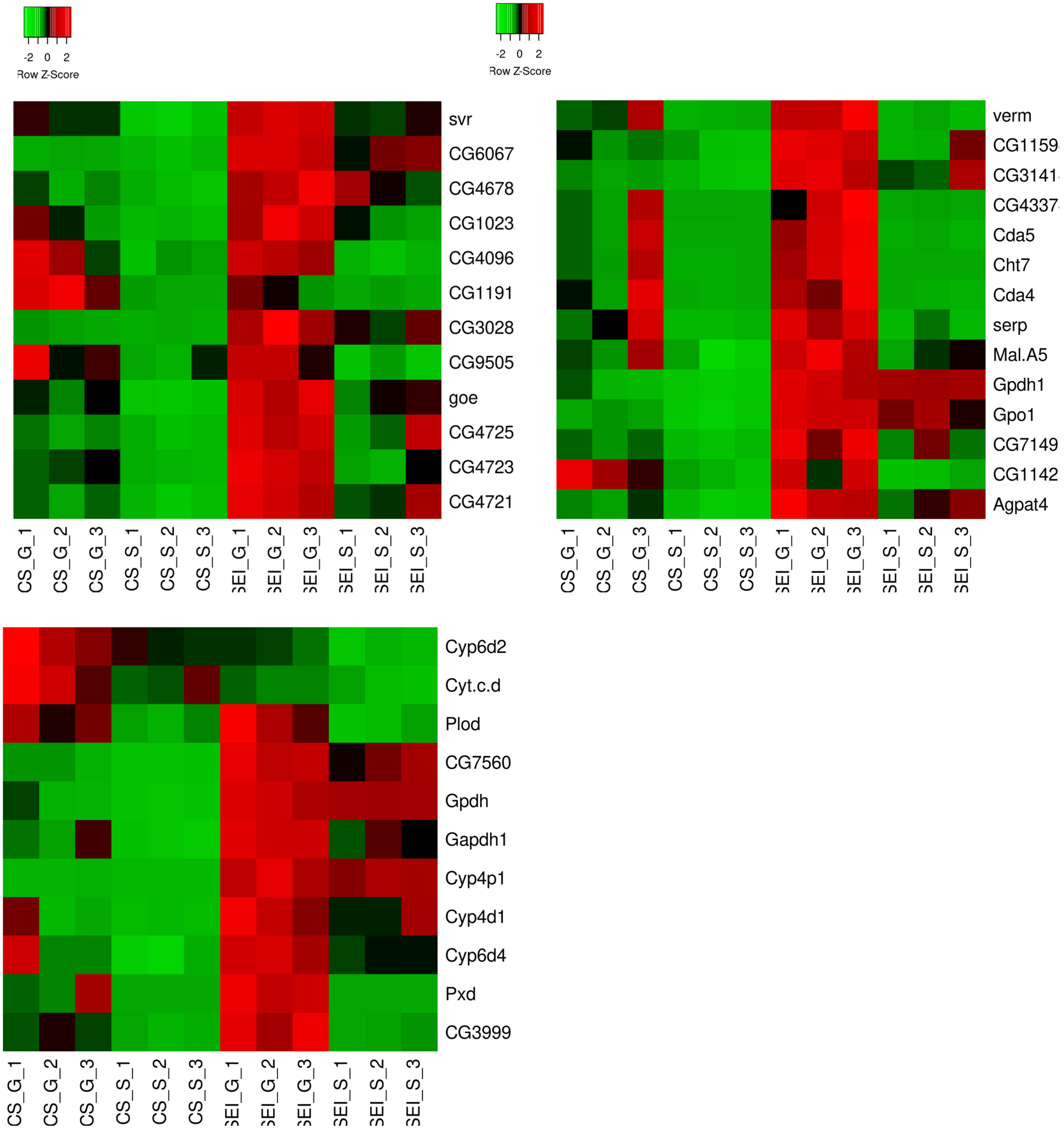
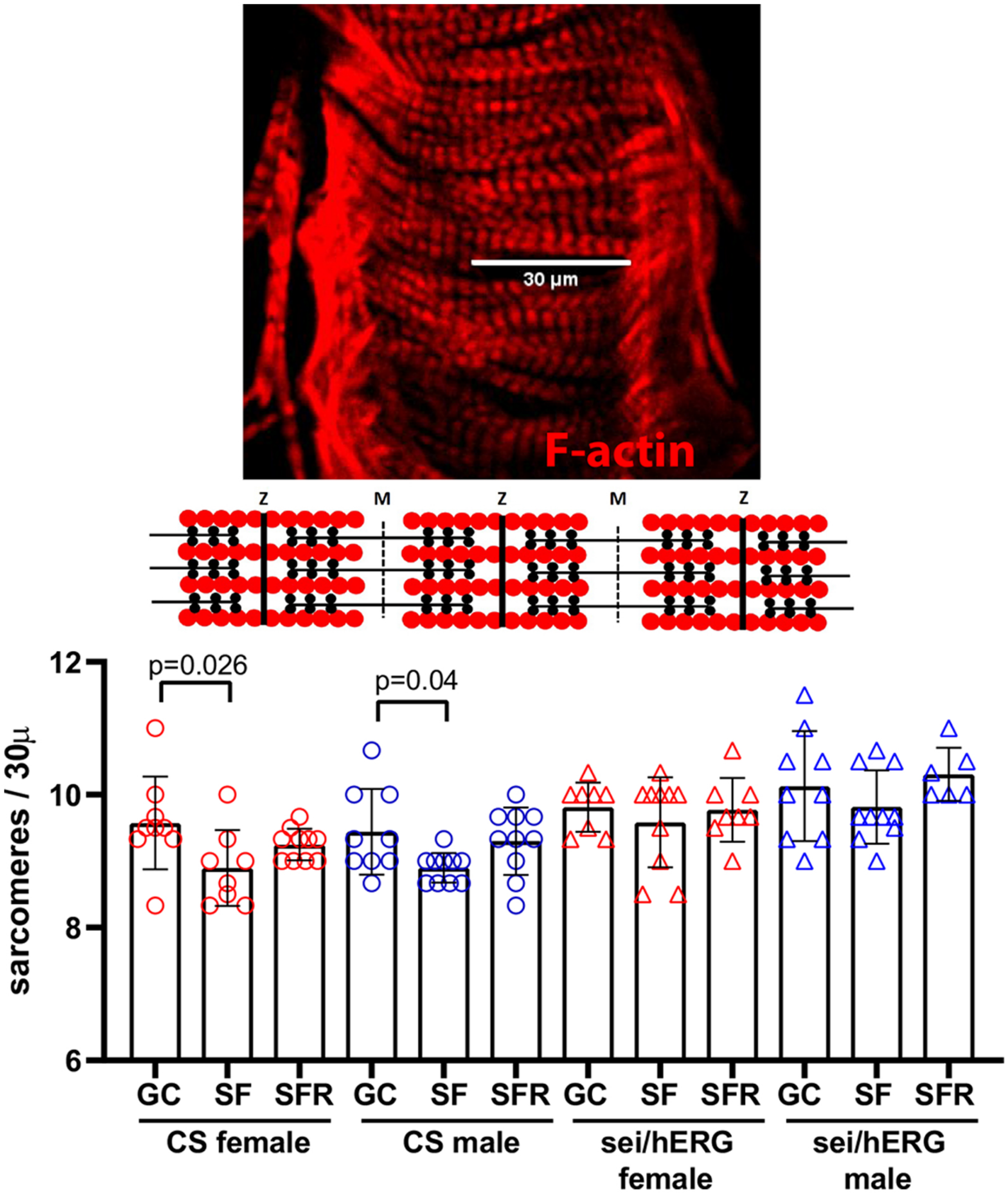


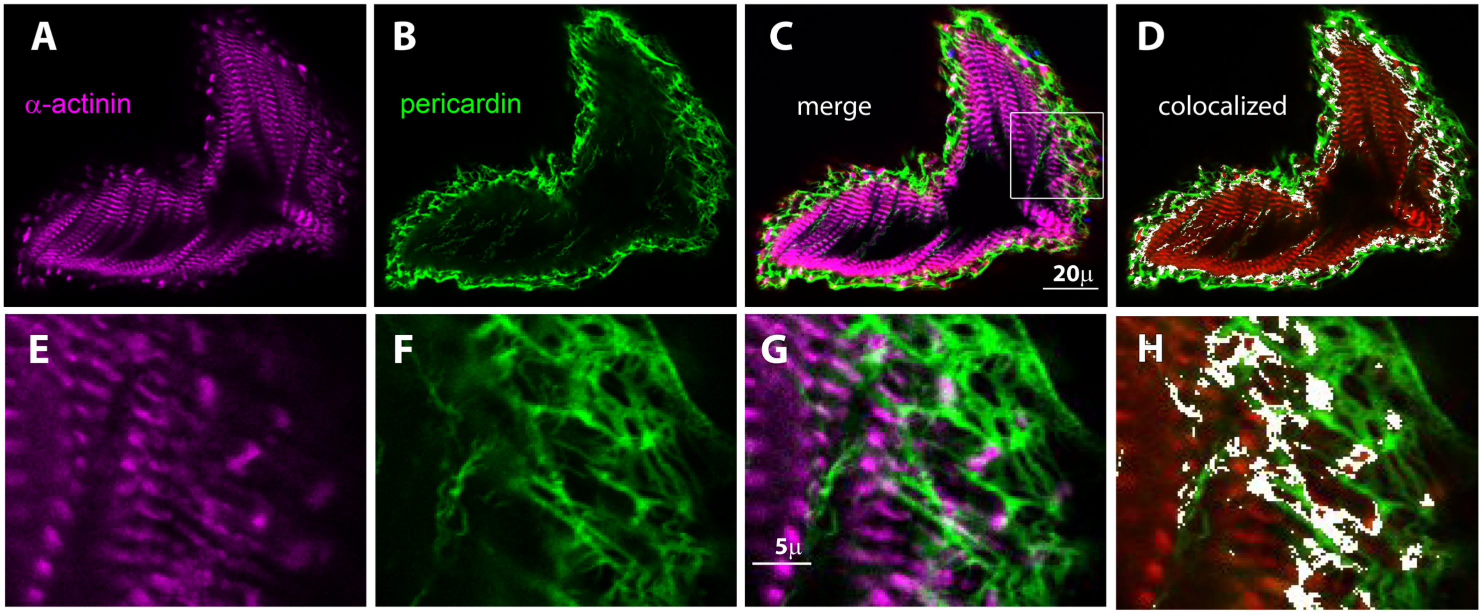
Supplemental Figure 1 (related to Figure 1). Effects of lifetime microgravity exposure on fly size and chronotropy. (A) Fly size was measured from still images of flies in climbing assay videos. For all panels females are in red and males are in blue. **(B)** Mean Systolic and **(C)** Diastolic intervals were unchanged by µg exposure in both Canton S controls and *sei/hERG* mutants although mutants under both gravity conditions showed the expected prolongation of SI and DI. **(D)** Rhythmicity was not significantly altered by µg. (2-way ANOVA with Tukey's multiple comparison posthoc test; *p<0.05, **p<0.01, **p<0.001)



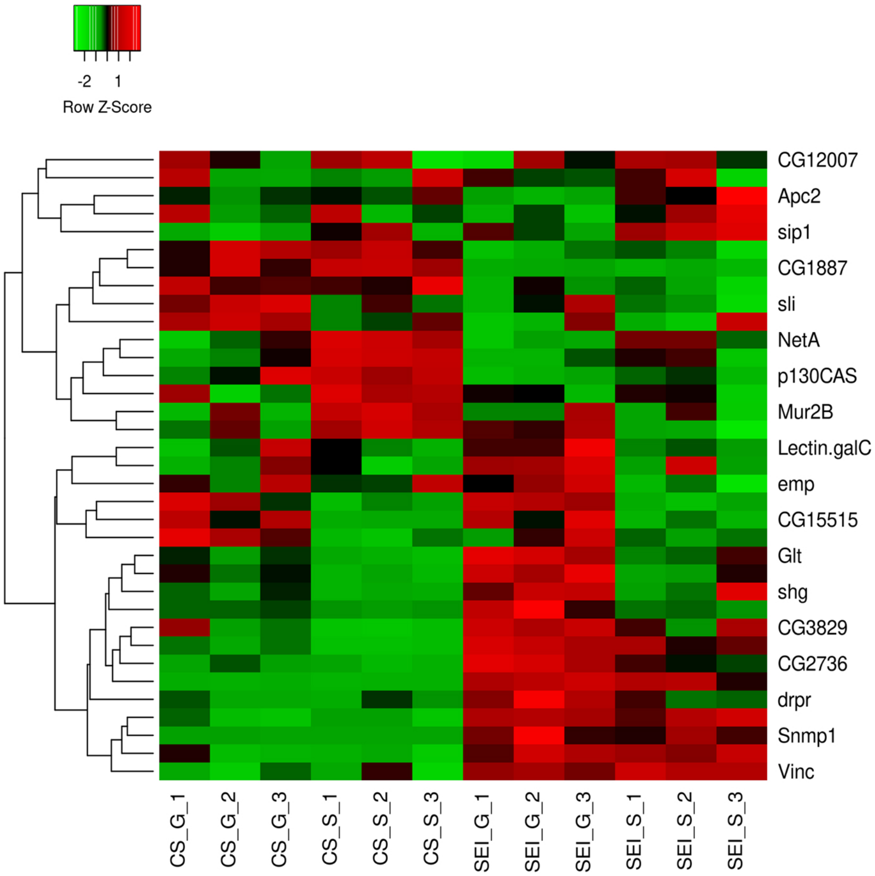
Supplemental Figure 2 (related to Figure 2). Heatmap of differentially expressed gene subsets in CS and *sei/hERG* hearts at 1g and µg. Heatmap comparison of comparing CantonS ground controls (CS_G) CantonS microgravity exposed (CS_S), *sei/hERG* ground controls (*SEI/HERG*_G) and *sei/hERG* space flown (*SEI/HERG*_S) fly hearts. Data was normalized to the average expression across row for each gene (Z-score). "Cool" green colors denote low relative expression, "Warm" red colors denote high relative expression while black denotes average expression.



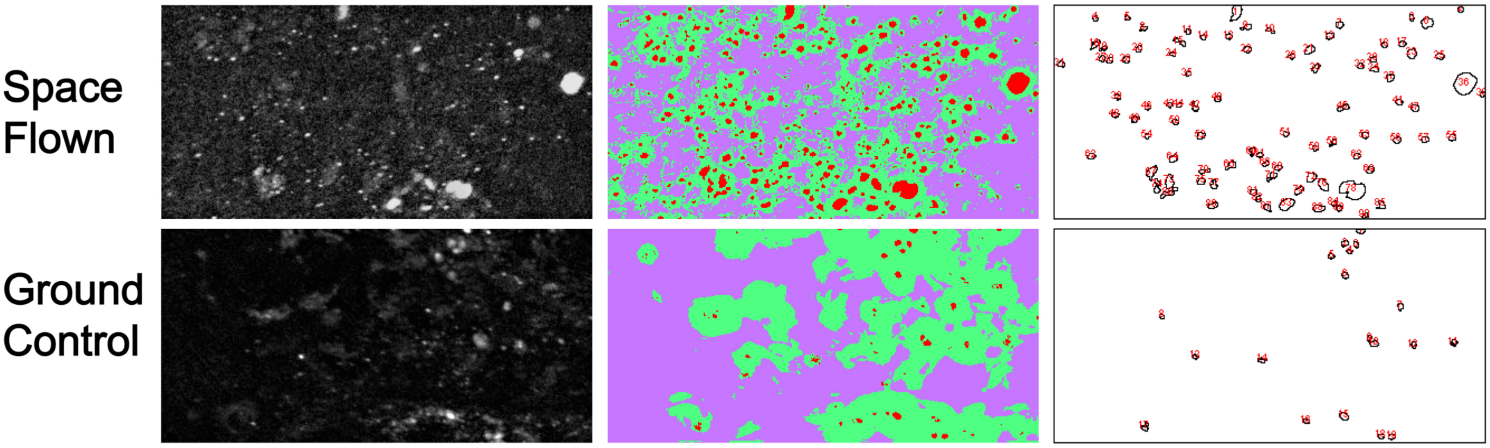
Supplemental Figure 3 (related to Figure 3). Sarcomere length decreased under ug. (A) Z-stack image of myofibrillar structure in myocardial cells from a ground control, CantonS female. Myofilaments were stained with phalloidin to detect F-actin (red), scale bar is 30 microns. (B) Schematic showing how the F-actin staining in A is arranged within sarcomeres. (C) Mean sarcomere count per 30 micron stretch of individual myofibrils showed a significant decrease in wildtype CS flies when reared under ug. Significance was determined by one way ANOVA and Tukey's multiple comparisons post-hoc test.



Supplemental Figure 4 (related to Figure 3). Pericardin network interacts with cardiac myofibrils at Z Bands (A&B) Confocal images of a fly heart stained for the Z band protein α-actinin (magenta) and pericardin (green), a fly ortholog of collagen IV. Images show a single, 1 u slice from a Z stack through a cardiac chamber. **(C)** Merged images with a box showing the area of detail in (E-H). **(D)** Images in A and B were analyzed using a colocalization plugin (JACoP) in Image J; regions where α-actinin and pericardin co-localize within this 1 μ slice are shown in white. **(E&F)** Enlarged image showing a-actinin stained Z bands (magenta) and the collagen IV network (green). **(G)** Merged images. **(H)** Colocalized α-actinin and collagen IV shown in white.



Supplemental Figure 5 (related to Figure 2). Heatmap of differentially expressed cell adhesion related genes in CS and *sei/hERG* at 1g and µg. Heatmap comparison of comparing CantonS ground controls (CS_G), CantonS µg exposed (CS_S), *sei/hERG* ground controls (*SEI/HERG*_G) and *sei/hERG* (*SEI/HERG*_S) fly hearts. Data was normalized to the average expression across row for each gene (Z-score). "Cool" green colors denote lower relative expression, "Warm" red colors denote higher relative expression while black denotes average expression.



Supplemental Figure 6 (related to Figure 4). Quantification of proteasome punctae. (Left panels) Z-stack images of hearts stained for proteosome subunits (left panels). (Middle Panels) Output from the trainable weka segmentation tool in ImageJ showing proteosome punctae identified by the algorithm in red. The punctae could be differentiated from the more diffuse staining haze (green) and true background (purple). (Right Panels) identified punctae were counted and outlined regions were used to calculate the total area in Image J.

Supplemental Table 1. 38 upregulated genes commonly shared between CS and sei under ug

Fly Symbol	Human Symbol	DIOPT	Description
CG5380	POLR3F	15	DNA-directed RNA Polymerase III Subunit RPC6
mst	MSTO1	15	Mitchondrial Dist.& Morphology Regulator
Npl4	NPLOC4	14	Nuclear Protein Localization 4
CG1218	HPF1	14	Histone Parylation Factor 1
ERR	ESRRG	14	Estrogen-Related Receptor Gamma
Fem-1	FEM1B	14	FEM1-Like Protein in Apoptotic Pathway
htl	FGFR3	14	Fibroblast Growth Factor Receptor 3
CG15771	NANP	13	N-acetylneraminic acid phosphatase
Keap1	KEAP1	13	Kelch-like ECH-Associated Protein
Shaw	KCNC2	13	Shaw-Related Voltage Gated Potassium Channel
Prosalpha6	PSMA1	13	Proteasome Subunit Alpha Type I
CG5846	RFXANK	13	Regulatory Factor X
Rpn13	ADRM1	13	Adhesion-Regulating Molecule 1
CG4572	CPVL	13	Vitellogenic-like Carboxypeptidase
NetA	NTN1	12	Netrin 1
Elk	KCNH8	12	Voltage-Gated Potassium Channel H8 Family
B9d1	B9D1	12	B9-Domain Containing Protein 1
MED30	MED30	12	Mediator Complex Subunit 30
Lnk	SH2B2	11	SHB2 Adaptor Protein
CG5445	ILRUN	11	Chromosome 6 ORF 106
neb	KIF14	10	Kinesin Family Member 14
Rh6	OPN4	9	Opsin 4
Cyp6a8	CYP3A43	6	Cytochrome P450 Subfamilly IIIA
Neto	NETO2	6	Neuropilin and Tolloid-like 2
Cyp6a8	CYP3A7-CYP3A51P	5	Cytochrome P450 Subfamilly IIIA
CG3223	UBL7	5	Ubiquitin-like 7
CG3308	TATDN2	5	Putative Deoxyribonuclease
Tsf1	MELTF	5	Melanoma-associated antigen p97
CG42554	NRBF2	4	Nuclear Receptor Binding Factor 2
dome	PTPRQ	2	Proteinptyrosine phosphatase
CG2046	PSMG1	2	Proteasome Assembly Chaperone 1
CG34149	GPD1L	1	Glycerol-3-Phosphate Dehydrogenase 1-like
CG8852	LRRC3	1	Leucine-Rich Repeat-Containing Protein 3
Sry-beta	ZNF576	1	Zinc Finger Protein 576
CG10479	SH2D5	1	SH2 Domain Containing Protein 5
CG11398	ZNF576	1	Zinc Finger Protein 576
lncRNA:CR40469	N.O.I	0	N/A
CG5612	N.O.I	0	N/A
Fie	N.O.I	0	N/A
CG32428	N.O.I	0	N/A

Supplemental Table 1 (related to Figure 2). 38 upregulated genes commonly shared between CS and *sei/hERG* under µg. Table lists fly genes, corresponding human orthologs, DIOPT orthology scores and description of human gene function.

Supplemental Table 2. Differentially expressed gene subsets commonly shared between CS and sei under ug

TERM	Dir.	DESCRIPTION	COUNT	FOLD	FDR
BP_GO:0040003	dwn	chitin-based cuticle development	12	6.34	7.9E-4
BP_GO:0005975	dwn	carbohydrate metabolic process	10	5.29	0.005
BP_GO:0006508	dwn	proteolysis	12	6.34	0.02
CC_GO:0005615	dwn	extracellular space	23	12.1	1.4E-4
MF_GO:0042302	dwn	structural constituent of cuticle	13	6.87	2.2E-6
KEGG_ dme00564	dwn	Glycerophospholipid metabolism	5	2.64	0.002

Supplemental Table 2 (related to Figure 2). Differentially expressed gene subsets commonly shared between CS and *sei/hERG* under µg. Table lists gene ontology term, direction (dir.) either upregulated (up) or downregulated (dwn) genes, (BP-biological process, MF-molecular function, CC-cellular localization, KEGG- KEGG pathway ID), ontological description, fold enrichment and false discovery rate (FDR) statistics.