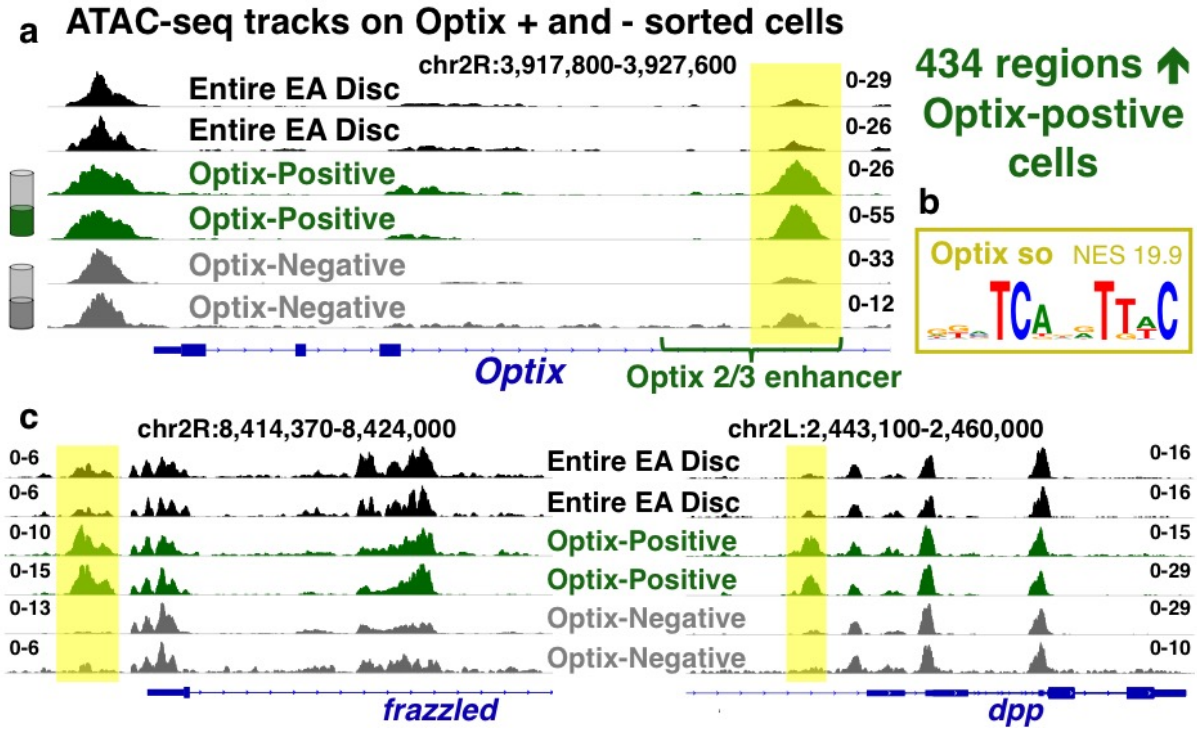
Supplementary note 1: Sorted subpopulations from the eye-antennal disc

To further investigate the decoupling of accessibility and activity of the Grh-enhancers, we performed ATAC-seq on FACS isolated progenitor cells that were marked by GFP expression driven by enhancers of the *optix* gene[1]. These cells constituted about half of the cells in eye-antennal discs at the time of harvesting (Fig. 4d). Reassuringly, we found that the Optix2/3 enhancer is mainly accessible in the GFP positive (Optix expressing) sorted cells and not accessible across the GFP-negative tissue (Supplementary note Fig. 1a). Additionally, we identified 434 regions that were significantly more accessible (DESeq2 log2FC>0.5, Padj<0.05) in the Optix positive subpopulation, when compared to the rest of the tissue. The Optix motif (SIX family, Supplementary note Fig. 1b) was the top enriched motif (i-cisTarget, NES 19.9) found in 171 of these regions and the Sine-Oculis-ChIP-seq track was the most enriched track (NES 8.4). This clearly indicates that these regions are indeed specific to the Optix subpopulation, allowing us to identify potential Optix bound enhancers and their target genes, like for example enhancers near *frazzled* and *dpp* (Supplementary note Fig. 1c). All potential Optix target enhancers with their target genes can be found in Supplementary Table 4. Their area restricted accessibility is in sharp contrast to the Grh bound enhancers, which are similarly accessible in the sorted subpopulation compared to the rest of the tissue (Fig. 4e).



Supplementary note Figure 1: ATAC-seq on sorted subpopulations of the eye-antennal disc (a) Accessible chromatin profile of the *Optix2/3* enhancer for the entire discs (similar for 30 biological replicates), *Optix2/3* positive sorted bulk (2 biological replicates), single-cell aggregate and *Optix2/3* negative sorted bulk (2 biological replicates). (b) The top enriched motif (NES 19.9) annotated for Optix or Sine Oculis, in the 434 regions specifically more accessible in the Optix pos cells. (c) ATAC tracks with two examples of potential Optix target regions near *frazzled* and *dpp*.

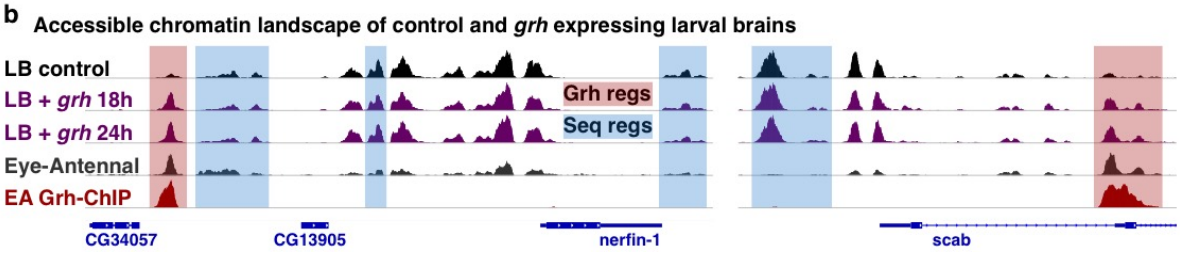
Supplementary note 2: Accessible chromatin landscape of larval brains

Intriguingly, except for the Grh target regions, the original accessible chromatin landscape of larval brains remains intact upon ectopic *grh* expression (ρ = 0.96). The exact same motifs are enriched in the entire accessible chromatin landscapes of control and *grh* expressing larval brains, with very similar normalized enrichment scores (Supplementary note Fig. 2a). Specifically, motifs for the more general Trithorax-like[2,3] factor are again the strongest associated with open chromatin, followed by motifs linked to the pan-neuronal transcription factors Lola[4], Tramtrack[5] and Sequoia[6], which may play a similar role in neuronal tissues as Grh in epithelia. As an example, Supplementary note Fig. 2b shows the chromatin landscapes around *nervous fingers 1,* a gene coding for a protein involved in axon guidance[7], and *scab*, a gene coding for a protein associated with cell adhesion and memory[8]. We observed no particular changes in chromatin accessibility, except for the Grh regions that became ectopically accessible.

a

TF	Motif	LB	18h grh	24h grh	reg up
Trl		7.9	7.9	8.2	/
Seq		5.8	5.6	5.5	/
m1		5.2	5.0	5.0	/
Lola		4.3	4.3	4.2	/
Grh		/	3.6	3.7	26.6

b



Supplementary note Figure 2: Accessible chromatin landscape of control and *grh* expression larval brains (a) Normalized enrichment scores from i-cisTarget[9] for motifs (Trithorax-like, Sequoia, motif1, Lola and Grh) enriched in the open chromatin landscape of control 3rd instar larval brains, 3rd instar larval brains with ectopic *grh* expression (18h and 24h) and regions that became accessible due to the ectopic *grh* expression (log2FC>1, pval<0.05). (b) Tracks visualizing ATAC-seq on control larval brains (black), larval brains with 18h and 24h *grh* expression (purple) (ATAC-seq experiments were done once for each time point) and eye-antennal discs (dark grey, 30 biological replicates), and Grh-ChIP on eye-antennal discs (dark-red, 3 biological replicates). Genomic loci near the *neuronal nerfin-1* and *scab* genes are shown with predicted Sequoia target regions (blue) and Grh target region (red).

Supplementary note 3: Acute DNA damage-response enhancers in epithelial tissues

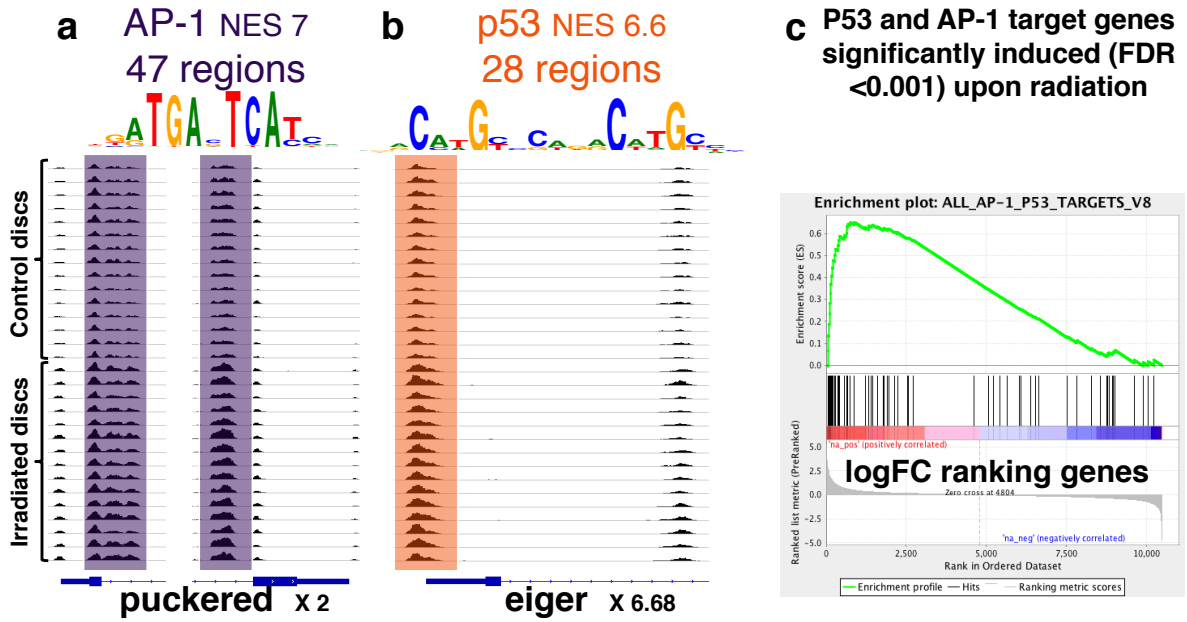
We showed in this work that lineage factors, like Grh, are responsible for setting up a large part of the accessible chromatin landscape of a tissue. Lineage factor target regions were mainly accessible "primed" throughout the entire tissue, made available for other factors to bind to generate tissue specific enhancer activity. There are however also other "types" of enhancers that are, or can be, activated in the cells of the epithelial tissues. To put our study concerning Grh as a lineage/pioneer factor into more perspective, we also investigated how the accessible chromatin of the cells in our tissue changed in response to acute damage, caused by ionizing radiation.

We irradiated (40 Grey) 3rd instar larvae of 15 different DGRP strains and performed 2 hours later ATAC-seq on the eye-antennal discs. We then compared the ATAC-seq profiles of eye-antennal discs after irradiation with the profiles of eye-antennal discs without radiation (Supplementary note Fig. 3a,b). Using DESeq2 we identified a relatively low number of regulatory regions that gained accessibility upon acute DNA damage. In total, we identified only 186 regions in the open chromatin landscape of eye-antennal discs that reproducibly gained accessibility (log2FC > 0.5, Padj < 0.01) after ionizing radiation.

Using the motif enrichment tool i-cisTarget[9], we identified some interesting motifs that were specifically enriched in these DNA damage responsive enhancers. The most enriched motif belonged to the AP-1 family of transcription factors (Supplementary note Fig. 3a), known to be generally activated in cells when they experience stress. The arguably most interesting motif, identified in 28 damage induced enhancers, was for the well-studied transcription factor and tumour suppressor protein p53 (Supplementary note Fig. 3b). Using public microarray data[10], we confirmed that the expression of p53 was also significantly upregulated upon irradiation (log2FC=1.1, Padj<0.03). Interestingly, these AP-1 and p53 bound enhancers were strongly associated with genes that are upregulated upon acute DNA damage caused by ionizing radiation[10] (Supplementary note Fig. 3c), suggesting that they are active enhancers.

Recent studies have demonstrated that for p53 responsive enhancers, p53 binding alone is sufficient to activate the enhancers without the requirement of other TFs to bind[11] and that p53 functions as a pioneer factor, capable of directly engaging and opening its target regions[12]. We can recapitulate the functions of p53 in the irradiated cells of our epithelial tissue. Though the large majority (>96%) of accessible regions in our tissues did not change upon acute DNA damage, p53 target regions became specifically accessible and activated the expression of nearby target genes.

Compared to the Grh target enhancers, these p53 responsive enhancers function differently. Both transcription factors have pioneering capacities, yet p53 is also capable of directly activating its target enhancers (fast response) while Grh is rather empowering a large group of regulatory regions (lineage factor), that seem to mostly require additional TFs to bind for activation. Investigating the effect of acute DNA damage on chromatin accessibility has allowed us to identify, in the same tissue, different types of regulatory regions that function and are activated by different mechanisms.



Supplementary Note Figure 3: Open chromatin changes in response to acute damage, caused by ionizing radiation (a) Motif for the AP-1 transcription factor with two induced AP-1 target intronic enhancers of *puckered*, a damage response gene that gets induced 2-fold. (b) Motif for the transcription factor p53 with an induced p53 target enhancer upstream of *eiger*, a gene upregulated 6.68-fold upon irradiation. (c) Gene Set Enrichment Analysis of the 62 potential target genes (black bars) of the p53 and AP-1 target regions (5kb upstream or intronic) on the gene ranking (10484 genes ranked by Log2FoldChange) of irradiated discs versus their controls. Genes that are upregulated upon irradiation are on the left (red) and genes that are down-regulated are on the right (blue).

Supplementary Notes Bibliography

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Supplementary Tables

Supplementary table 1: Origin of the 18832 motifs

Source	Reference	Organism(s)	Type of motif	# motifs in v1.4
Bergman	(Down et al., 2007)	Drosophila	Curated (FlyReg)	62
C2H2-ZFs	(Greenblatt et al., 2015)	Human	B1H	64
CIS-BP	(Weirauch et al., 2014)	Multiple species	Experimental	6523 *(CIS-BP v1.02)*
Elemento	(Elemento and Tavazoie, 2007) (Elemento and Tavazoie, 2005)	Drosophila, Human, Mous	Predicted (conserved) om	619
FactorBook	(Wang et al., 2012)	Human	ENCODE ChIP-seq	82
FlyFactorSurvey	(Enuameh et al., 2013) (Zhu et al., 2011)	Drosophila	B1H, others (e.g., FlyReg)	688 *(includes Wolfe)*
hPDI	(Xie et al., 2010) (Hu et al., 2009)	Human	Experimental	437
HOCOMOCO	(Kulakovskiy et al., 2016) (Kulakovskiy et al., 2013)	Human and mouse	ChIP-seq, HT-SELEX, curated	426 *(HOCOMOCO v9)*
HOMER	(Heinz et al., 2010)	Multiple species	ChIP-seq, others (e.g. ENCODE	307
iDMMPMM	(Kulakovskiy and Makeev, 2009)	Drosophila	DNase I footprints, SELEX, B1H	39
JASPAR	(Mathelier et al., 2016) (Lim et al., 2014) (Portales-Casamar et al., 2010)	Multiple species	Curated	593 *(JAPSAR 2014 nr)*
Neph	(Neph et al., 2012)	Human	Predicted (from DHS) om	683
PreDREM	(Zheng et al., 2015)	Human	Predicted	538
ScerTF	(Spivak et al., 2012)	Saccharomyces	Curated plus ChIP-seq	196
Stark	(Stark et al., 2007)	Drosophila	Predicted (conserved) om	232
SwissRegulon	(Pachkov et al., 2013) (Pachkov et al., 2007)	Multiple species	Curated	538
Taipale	(Jolma et al., 2013)	Human	HT-SELEX	581
Taipale TF pairs	(Jolma et al., 2015)	Human	CAP-SELEX	664
TF dimers	(Jankowski et al., 2013)	Human	Predicted dimers	603
Tiffin	(Down et al., 2007)	Drosophila	Predicted (gene sets) om	120
TRANSFAC® PRO	(Matys et al., 2006)	Multiple species	Curated, ChIP-chip	5145 *(TFP2015.3)*
TRANSFAC® 7.0 Public 2005	(Matys et al., 2006)	Multiple species	Curated, ChIP-chip	398
YeTFaSCo	(de Boer and Hughes, 2012)	Saccharomyces	Uniprobe, Curated, ChIP-chip	244
Total				21586 18832 motifs

Supplementary table 2: Gene Ontology terms for the 1786 potential Grh target genes.

GO term on 1786 genes near Grh target regions (flymine.org)	p-Value (H	Matches	ImaGO Term (flymine.org)	p-Value (Hyp	Matches
organ development [GO:0048513]	2,85E-74	425	embryonic dorsal epidermis	2,00E-90	345
multicellular organismal development [GO:0007275]	1,78E-65	679	embryonic ventral epidermis	5,54E-75	304
system development [GO:0048731]	5,82E-64	583	embryonic head epidermis	2,91E-51	223
anatomical structure morphogenesis [GO:0009653]	1,08E-62	487	dorsal epidermis primordium	8,21E-40	168
tube development [GO:0035295]	4,32E-62	292	embryonic epipharynx	1,25E-37	156
organ morphogenesis [GO:0009887]	4,63E-62	298	embryonic foregut	1,96E-33	177
morphogenesis of an epithelium [GO:0002009]	8,30E-59	259	ventral epidermis primordium	1,47E-29	139
tissue development [GO:0009888]	9,43E-58	373	dorsal ectoderm primordium	7,80E-29	134
tissue morphogenesis [GO:0048729]	1,13E-57	261	embryonic esophagus	1,44E-26	101
epithelium development [GO:0060429]	1,74E-57	345	dorsal ectoderm anlage in statu nascendi	4,56E-23	114
single-organism developmental process [GO:0044767]	7,56E-55	742	embryonic/larval posterior spiracle	4,81E-22	104
post-embryonic organ development [GO:0048569]	1,33E-54	221	ventral ectoderm anlage in statu nascendi	2,86E-21	104
developmental process [GO:0032502]	1,64E-53	745	embryonic/larval tracheal system	4,19E-21	122
tube morphogenesis [GO:0035239]	2,18E-53	227	clypeo-labral primordium	1,10E-15	70
post-embryonic development [GO:0009791]	5,36E-53	248	procephalic ectoderm anlage in statu nascendi	5,05E-15	94
anatomical structure development [GO:0048856]	1,53E-52	691	ventral ectoderm primordium	1,18E-14	108
imaginal disc development [GO:0007444]	4,54E-52	247	tracheal primordium	1,43E-14	78
epithelial tube morphogenesis [GO:0060562]	3,69E-51	216	foregut primordium	5,34E-14	129
single-multicellular organism process [GO:0044707]	2,76E-49	729	ventral ectoderm primordium P2	4,98E-13	124
imaginal disc morphogenesis [GO:0007560]	3,17E-49	197	procephalic ectoderm anlage	5,67E-12	110
post-embryonic organ morphogenesis [GO:0048563]	3,17E-49	197	embryonic hindgut	7,57E-12	228
post-embryonic morphogenesis [GO:0009886]	6,24E-49	203	anlage in statu nascendi	1,10E-10	75
instar larval or pupal morphogenesis [GO:0048707]	1,00E-48	200	posterior spiracle specific anlage	1,28E-10	40
metamorphosis [GO:0007552]	3,70E-48	206	embryonic proventriculus	8,27E-09	110
instar larval or pupal development [GO:0002165]	1,06E-47	224	procephalic ectoderm primordium	2,19E-08	125
appendage development [GO:0048736]	2,54E-44	177	clypeolabrum primordium P2	3,78E-08	27
appendage morphogenesis [GO:0035107]	5,59E-44	175	head epidermis primordium P1	1,05E-07	36
imaginal disc-derived appendage morphogenesis [GO:0035114	8,29E-44	174	clypeolabrum	1,32E-07	26
imaginal disc-derived appendage development [GO:0048737]	8,41E-44	175	hindgut proper primordium	1,62E-07	153
post-embryonic appendage morphogenesis [GO:0035120]	6,12E-43	171	clypeolabrum anlage	1,96E-07	22
wing disc development [GO:0035220]	5,66E-41	186	embryonic/larval visceral branch	5,79E-07	27
biological regulation [GO:0065007]	3,69E-40	729	clypeolabrum anlage in statu nascendi	7,21E-07	19
neuron differentiation [GO:0030182]	1,43E-39	260	hindgut anlage in statu nascendi	7,68E-07	35
locomotion [GO:0040011]	4,80E-38	209	head epidermis dorsal	2,83E-06	38
generation of neurons [GO:0048699]	1,04E-37	273	ventral epidermis primordium P2	4,34E-06	17
imaginal disc-derived wing morphogenesis [GO:0007476]	2,86E-37	150	embryonic/larval dorsal trunk	4,87E-06	37
wing disc morphogenesis [GO:0007472]	6,57E-37	151	atrium primordium	1,53E-05	28
multicellular organismal process [GO:0032501]	2,12E-36	763	proventriculus primordium	2,13E-05	35
pattern specification process [GO:0007389]	2,70E-36	191	visual primordium	2,15E-05	37
regulation of cellular process [GO:0050794]	2,96E-34	626	visual anlage	2,66E-05	27
regulation of biological process [GO:0050789]	1,33E-33	665	visual anlage in statu nascendi	7,46E-05	26
single-organism process [GO:0044699]	1,01E-32	1128	embryonic anal pad	7,47E-05	96
neuron development [GO:0048666]	3,32E-31	221	mesectoderm primordium	1,27E-04	31
sensory organ development [GO:0007423]	1,29E-30	172	amnioserosa	1,44E-04	83
cell morphogenesis involved in differentiation [GO:0000904]	4,47E-29	175	gap	2,88E-04	34
regionalization [GO:0003002]	5,51E-29	168	midline primordium	3,11E-04	38
cell development [GO:0048468]	7,89E-29	368	amnioserosa anlage in statu nascendi	4,80E-04	25
movement of cell or subcellular component [GO:0006928]	2,67E-28	197	segmentally repeated	5,48E-04	24
cell projection organization [GO:0030030]	4,50E-28	215	sensory system head	6,83E-04	83
taxis [GO:0042330]	1,36E-26	128	dorsal ectoderm anlage	7,36E-04	15
embryo development [GO:0009790]	1,99E-26	175	embryonic optic lobe primordium	8,94E-04	26
nervous system development [GO:0007399]	3,54E-26	379	embryonic/larval dorsal vessel	0,001252	39
cell morphogenesis involved in neuron differentiation [GO:004	4,89E-26	160	embryonic optic lobe	0,001848	26
neuron projection development [GO:0031175]	5,49E-26	183	embryonic dorsal apodeme	0,001872	21
chemotaxis [GO:0006935]	1,89E-25	112	amnioserosa anlage	0,002119	23
cellular component morphogenesis [GO:0032989]	7,41E-25	243	hypopharynx primordium P1	0,002723	16
cell morphogenesis [GO:0000902]	8,30E-25	220	dorsal epidermis anlage	0,004789	15
neuron projection morphogenesis [GO:0048812]	9,11E-25	177	ectoderm anlage in statu nascendi	0,005313	24
respiratory system development [GO:0060541]	9,87E-25	101	mesoderm anlage in statu nascendi	0,006267	34
regulation of transcription, DNA-templated [GO:0006355]	1,12E-24	242	stomatogastric nervous system primordium	0,009153	21
regulation of nucleic acid-templated transcription [GO:190350	1,12E-24	242	inclusive hindgut primordium	0,011744	76
regulation of RNA biosynthetic process [GO:2001141]	1,12E-24	242	pars intercerebralis primordium	0,012454	16
cell differentiation [GO:0030154]	1,68E-24	479	embryonic ventral apodeme	0,014839	18
cellular developmental process [GO:0048869]	3,69E-24	494	gnathal lobes anlage	0,02094	10
axon guidance [GO:0007411]	1,82E-23	104	embryonic stomatogastric nervous system	0,021006	22
neuron projection guidance [GO:0097485]	2,65E-23	106	embryonic leading edge cell	0,025184	13
open tracheal system development [GO:0007424]	1,02E-22	95	embryonic rectum	0,025397	21
axon development [GO:0061564]	1,12E-22	119	embryonic foregut sensory structure	0,031641	18
regulation of RNA metabolic process [GO:0051252]	2,83E-22	251	hypopharynx anlage	0,034925	9
response to stimulus [GO:0050896]	6,80E-22	549	amnioserosa primordium	0,035079	25
cell projection morphogenesis [GO:0048858]	2,00E-21	183	head epidermis lateral primordium P2	0,041676	10
axonogenesis [GO:0007409]	4,22E-21	114	antennal primordium1	0,043735	16

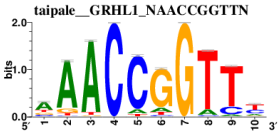
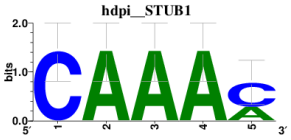
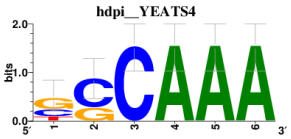
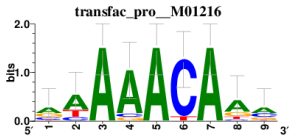
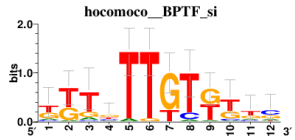
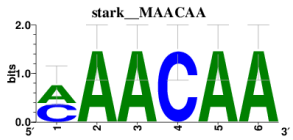
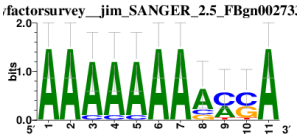
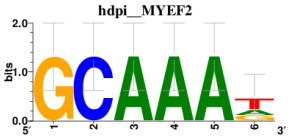
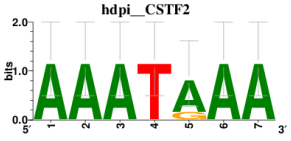
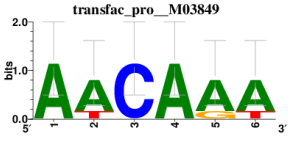
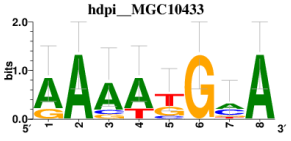
Supplementary table 3: Tested Grh bound enhancers

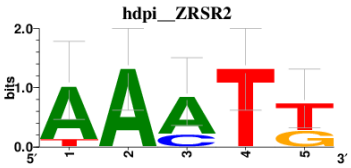
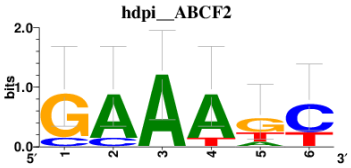
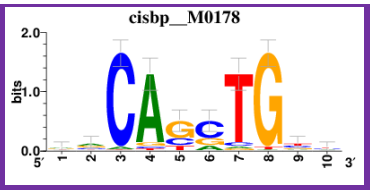
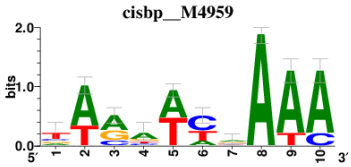
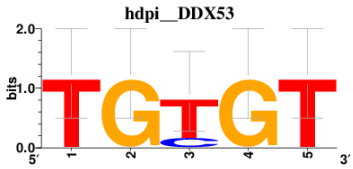
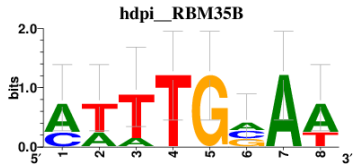
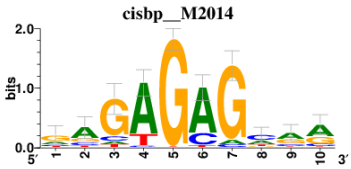
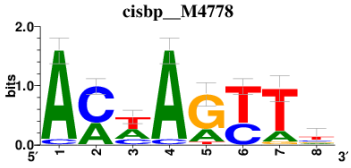
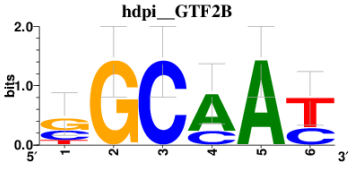
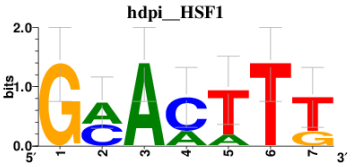
Bloominton number	Active in EA	position_dm3	closest gene
38727	yes	chr2L:17266203-17270147	*beat-IIIc*
38758	yes	chr2R:20898026-20898872	*Zip*
40432	yes	chr2R:7074512-7077884	*shn*
40436	yes	chr2R:7081197-7084847	*shn*
45151	yes	chr2L:8277657-8281321	*Btk29A*
45275	no	chr2R:13692354-13695691	*grh*
45531	yes	chr2R:6021498-6023006	*gem*
45723	yes	chr3R:16367700-16370556	*Stat92E*
45745	yes	chr3R:6411558-6415522	*hth*
46823	yes	chr3L:10993794-10997681	*klu*
47361	no	chr3R:6419287-6423188	*hth*
47530	yes	chrX:6876110-6879886	*ogre*
47714	no	chr3L:16630877-16634655	*abl*
47890	no	chr2L:6536075-6538679	*eya*
48037	yes	chr2R:17439112-17442366	*Egfr*
48457	yes	chr2L:5294138-5297840	*vri*
48944	no	chr3R:20121542-20125543	*crb*
49542	no	chr2R:9826145-9829599	*shot*
49785	yes	chr4:79419-83379	*ci*
50129	yes	chr2L:21242267-21245864	*Hr39*
50233	yes	chr3R:19157220-19160884	*pnt*

Supplementary table 4: Potential Optix target enhancers (selectively accessible + motif)

chr	start	end	potential target gene	chr	start	end	potential ta
chr2L	16470889	16471855	*dac*	chr2R	14457912	14460206	*CG30115*
chr2L	15457257	15458263		chr2R	15917206	15919494	
chr2L	2446779	2447902	*dpp*	chr2R	14785308	14786687	*sano snoRi*
chr2R	8414916	8416403	*fra*	chr3L	17968313	17970080	*Eip75B*
chrX	4472146	4473898		chr2L	21971546	21972144	
chr2R	5547784	5548876	*Mmp2 tRNA:M3:46A*	chr3R	20984237	20986626	
chrX	5890195	5891426		chr2R	15721484	15722613	
chr2L	14431040	14432054		chr3R	27512865	27513729	
chr3L	6303429	6305541		chr3R	14201327	14203344	*gl*
chrX	4477268	4478390		chr3L	19418437	19418995	*CR32205*
chr2L	8851927	8852855		chr2R	19551045	19553143	*Pde8*
chr3R	23052297	23053428	*CG42813*	chr2L	18381469	18382635	*Fas3*
chrX	17429553	17430600	*CG12432*	chr3R	9612482	9614066	
chr2R	3931209	3931878		chr3R	15140203	15141727	*Dl*
chr3L	18223443	18224703		chr3R	23378816	23379810	*Klp98A*
chr2R	11786454	11790005	*CG33463 sli*	chr2L	9592105	9595065	
chr2L	14429565	14431039	*Cpr35B*	chr3L	8005102	8005606	*nmo*
chr2R	3925690	3927150	*Optix*	chr3L	22150918	22151819	*olf413*
chr2R	19553558	19554775	*Pde8*	chr2R	20991183	20991836	*CG16778*
chr2L	19216521	19217610		chr3L	20087382	20088182	
chr3R	4712706	4713321	*pyd*	chr3L	15702497	15703916	
chr3L	14185008	14186191	*nuf*	chr2R	17983153	17985722	*CG11073*
chr2L	14514797	14515778	*tRNA:G3:35Ba*	chr3R	17651151	17652227	*CASK*
chr3L	10395857	10396490		chr3R	25130763	25131838	
chr3R	26393147	26393800	*tmod*	chr2L	2430126	2431137	*dpp*
chr2L	20020018	20020681	*CG13966*	chr3R	685505	686724	*opa*
chr3L	13401397	13402644		chr3L	7539322	7540485	*CG32376*
chr2L	7309636	7312092	*wg*	chr3L	14427628	14428825	*bbg*
chr2R	17413790	17414354	*Egfr*	chr3L	15705761	15707835	*CG42571*
chr3L	14952805	14954318		chr3R	24902930	24905176	*CG11873*
chr3R	9796351	9796673	*rdx*	chr2L	676290	677417	*ds*
chr3R	12162999	12164383	*Sulf1*	chr2L	2479903	2481112	
chr2R	6006047	6006983	*CG11866*	chr3R	21470765	21472589	*jigr1*
chr3L	12423362	12425175	*toe*	chr3R	17749240	17750037	
chr2R	5652902	5654069	*CG1688 trpl*	chr3L	1367754	1368355	*ru Ptp61F*
chr2R	17805760	17806436	*Fili*	chr2L	12607871	12609559	*nub*
chrX	13803381	13804542		chr3L	5345899	5347136	*lama*
chr3L	20928615	20929765	*fng*	chrX	17463168	17464552	*ppk23*
chr3L	22390952	22393902	*Ten-m*	chr3L	18622487	18623301	*MYPT-75D*
chr3R	12345258	12349333	*CG42342*	chr3L	14998162	14998928	*Reck*
chr2L	15340986	15342260		chr3L	1591278	1592404	*CG13917*
chr3R	5325301	5326919	*RhoL*	chr3L	8215941	8217105	*CG13678*
chr2L	636706	637539		chrX	13814278	13816189	
chr3L	3430902	3432203	*eIF5B*	chrX	16818597	16819944	*CG4928*
chr2L	4055663	4056929	*ed*	chr2R	16057175	16058629	
chr3L	22402850	22403968	*Ten-m*	chr2R	15724428	15726237	
chr3L	21121373	21122444	*ko*	chr3R	6388869	6390199	*hth*
chr3R	5894294	5895466	*nerfin-2*	chr2R	10317480	10319412	*phyl*
chr3R	4108630	4109603	*CG11671*	chr2R	3428644	3429569	*Corin CG15*
chr2L	3848811	3849903		chr3L	18191009	18192026	*CG7320*
chr3L	13390554	13392484	*sens*	chr3R	19163708	19164962	*pnt*
chr2L	16441421	16442467	*CG5888 ldgf1*	chr3R	929591	930522	
chrX	3480966	3481711	*AlstR*	chr3R	20235929	20237705	*nAcRalpha*
chr3L	8907768	8909625	*mfr Tsp66E*	chr3R	2519180	2521186	*Ccp84Ac C*
chr3L	6429469	6430429	*CG42747*	chr2L	16461199	16463237	
chrX	17236082	17236710		chr2R	16058630	16059655	
chr3L	12091166	12092166		chr3R	23607351	23609705	*CG34353*
chr2R	8849528	8850222		chr3R	25131839	25134257	
chr2R	3901825	3903497	*CG14762*	chr3R	2048371	2049818	*Osi5 Osi4*
chr3R	491056	492218	*CG31531*	chr3L	2414558	2415719	*CG42669*
chr2R	7717941	7719056	*Sobp*	chr3L	17927589	17928655	*CG43174 C*
chr3L	13135344	13136253		chrX	10432701	10433332	*spri*
chr2R	4625100	4628545	*CG8740 CG42615*	chr2R	18936907	18938229	*CG30194*
chrX	7248773	7249494	*CG1999 CG1677*	chr2R	17259706	17260794	*cv-2*
chr2R	18210530	18212167	*Gp150*	chrX	13131758	13132733	*CG32639 m*
chr3R	19145518	19146845	*pnt*	chrX	4499002	4500280	
chr2L	7326571	7327893		chr2L	5234468	5235628	*tkv*
chr3R	27308883	27319419		chr2L	14215650	14217850	*smi35A*
chr2R	15991469	15995776	*18w*	chr3L	20919973	20920808	*fng*
chrX	18894876	18896035	*CG42506 CG32541*	chr2L	10886086	10887153	
chr3R	4849567	4850223	*neur*	chr3L	7095378	7097265	*form3*
chr3R	13387092	13389664	*Hmx*	chrX	4338311	4339563	*bi*
chrX	18841084	18842265	*CG42506 CG32541*	chr3R	9084867	9085753	*Ace CG116*
chr3L	15799717	15801517	*CG6244*	chr2R	13208217	13209937	*mbl*
chr3L	7607547	7608640	*CG33275*	chr2R	10307177	10308543	*CG17386*
chr3L	7128581	7129348	*melt*	chr3R	26391854	26393146	*tmod*
chr3R	10319815	10322304		chr2L	18556216	18557427	*Pde11*
chr2R	15985377	15986851		chr3R	22049724	22051698	*CG12290*
chr2R	12276701	12280114	*CG33960*	chr3R	6642834	6646482	*CG4089*
chr3R	19123471	19125359	*pnt*	chrX	15441071	15442654	
chr3L	18443846	18445152		chr2R	5584849	5586283	*Uba1*
chr3R	17268122	17270806	*lbe*	chr2L	16210401	16211587	*CG31819*
chr3R	9645435	9647520		chr3L	10382051	10383538	
chr3L	3742875	3744940	*CG32264*	chr3R	19285089	19289218	*CG4374*
chr3R	12322167	12323064	*Cad89D*	chr2R	17412577	17413789	*Egfr*
chr2L	15929049	15929718	*beat-Ib*				

Supplementary table 5: Co-conserved motifs using the Branch Length Score

Motif		potential TF	BLS	BLS_shuffled	Delta_BLS
taipale_GRHL1NAACCGGTTN		grh	3499,2	128.197	3371
hdpi__STUB1		STUB1	1.867	251.001	1615.72
hdpi__YEATS4		Gas41	1.772	311.729	1460.44
transfac_pro__M01216		foxo slp2	1.619	212.037	1406.72
hocomoco__BPTF_si		E(bx)	1.529	133.4	1395.79
stark__MAACAA		Aef1	1556,8	231.784	1324.98
flyfactorsurvey_jim_SANGER_2.5_FBgn0027339		jim	1.288	93.16	1194.5
hdpi__MYEF2		rump	1.356	220.157	1135.84
hdpi__CSTF2		CstF-64	1.161	112.537	1048.06
transfac_pro__M03849		Sox14	1.082	95.596	986.673
hdpi__MGC10433		CG2931	1.130	169.677	960.574

hdpi__ZRSR2		CG3294	1.059	110.648	947.903
hdpi__ABCF2		CG9281	990	107.387	882.672
cisbp__M0178		ato	1.043	163.614	879.413
cisbp__M4959		exd	941	109.429	832.062
hdpi__DDX53		CG7878	925	100.24	825.018
hdpi__RBM35B		fus	959	165.567	793.256
cisbp__M2014		Trl	924	137.079	787.267
cisbp__M4778		br	1.003	246.683	756.403
hdpi__GTF2B		TfIIB	938	220.096	717.448
hdpi__HSF1		Hsf	959	242.41	716.729

Supplementary table 6: Primers for genomic PCR of Grh target enhancers with caQTLs

Regions	chr	Start	End	Accessible	Closed	Forward Primer	Reverse Primer
Region 1	chr3R	25441420	25442766	28123	25208	GTTTAAGGAGCTTCTGCTGCAT	GGCGCCATCCATTCCATCA
Region 2	chr2L	7,699,491	7,700,491	28123	25208	TCCGACCCATCGAATGATGC	TGTCCACAAGGCAGATGGTC
Region 3	chr3L	12908700	12909400	28123	25208	TCTCAGGATCCCTGCCATTA	ATTAATTGGCACACGCCGC
Region 4	chr2L	20114900	20115700	28222	25208	ACAAATAACACCGACGTTTTCACT	CGAGGTTCGACCCCAACTAT