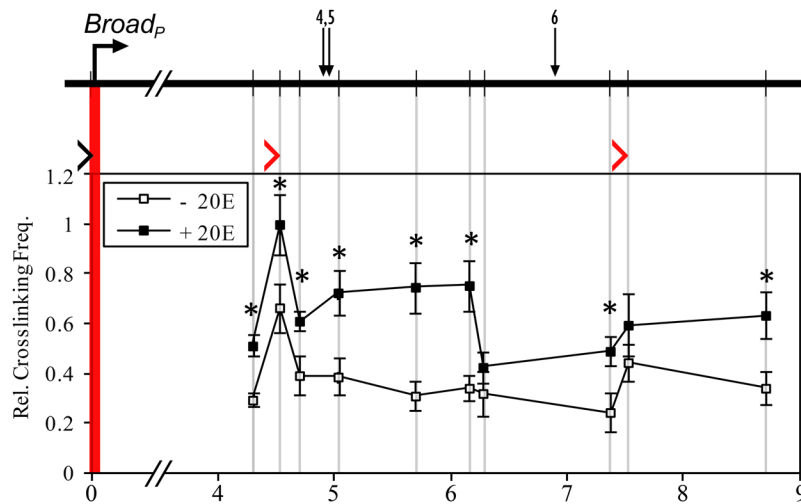
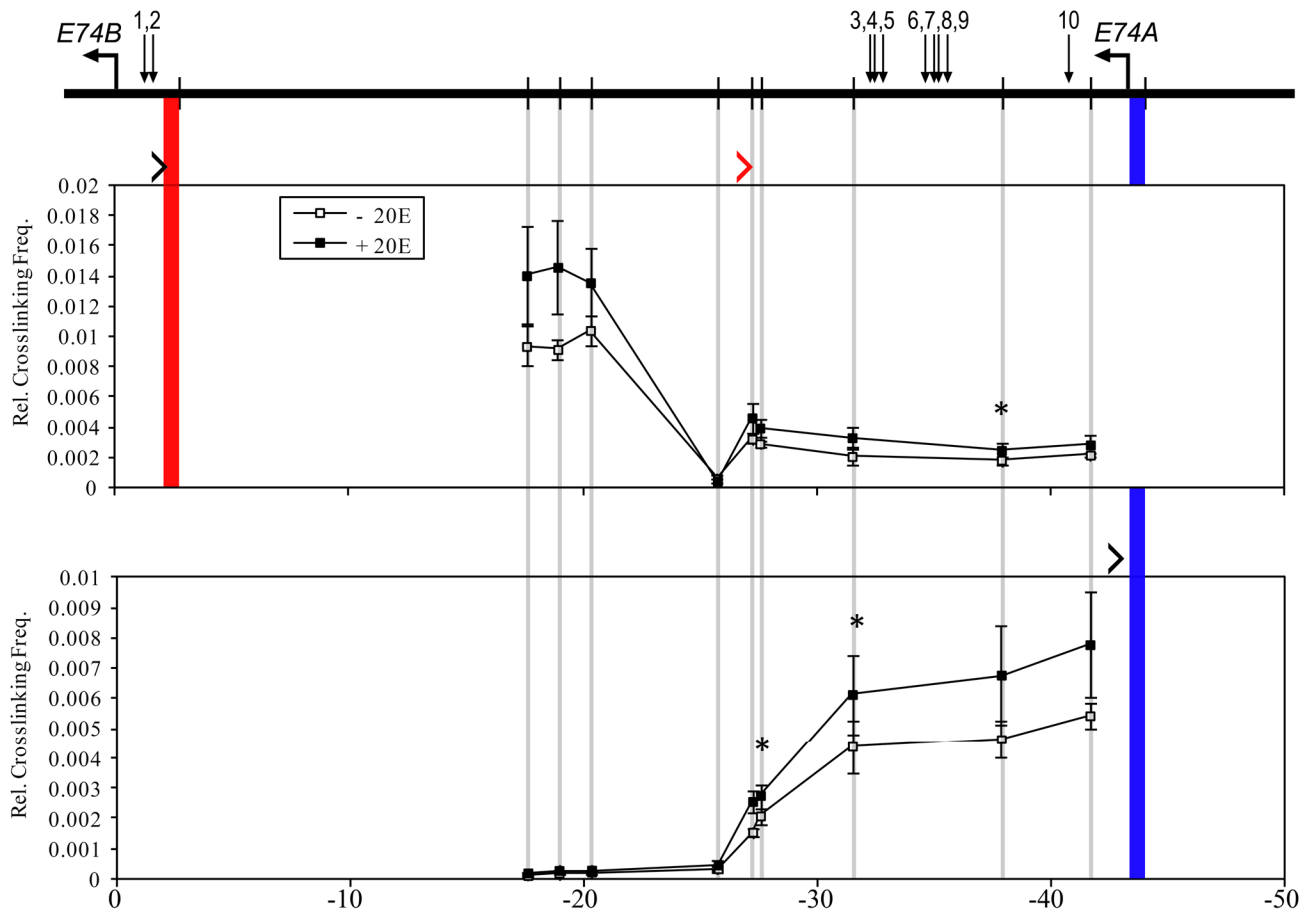


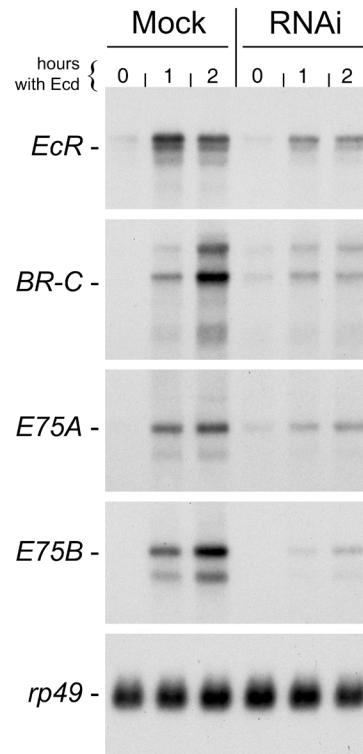
**Figure S1. High resolution 3C interaction profiles at the *E75* gene.** Schematic of the *E75* locus is shown at the top, with horizontal arrows indicating transcription start sites, vertical bars indicating DpnII sites and numbered arrows indicating EcREs. Crosslinking frequencies (y axis) between the fixed DpnII anchor for *E75B* (upper panels), *E75A* (middle panels) or *E75C* (lower panel) and several EcRE-containing regions were measured in S2 cells in the absence or presence of ecdysone as indicated. Location of fixed anchor sites are marked by red (*E75B*), blue (*E75A*), and green (*E75C*) bars, and test sites are marked by gray bars. Black angle brackets (">") above each interaction profile indicate the location and direction of anchor primers. Color-coded angle brackets indicate the location and direction of test primers in fragments that interact with the *E75B* (blue), *E75A* (red), and *E75C* (green) promoters, either in the presence or absence of ecdysone. Coordinates are given along the x axis relative to the *E75A* transcription start site. Asterisk indicates significant difference between ecdysone and control samples (Paired Student's *t*-test,  $P < 0.05$ ). Mean  $\pm$  S.E.M. is shown from three independent experiments.



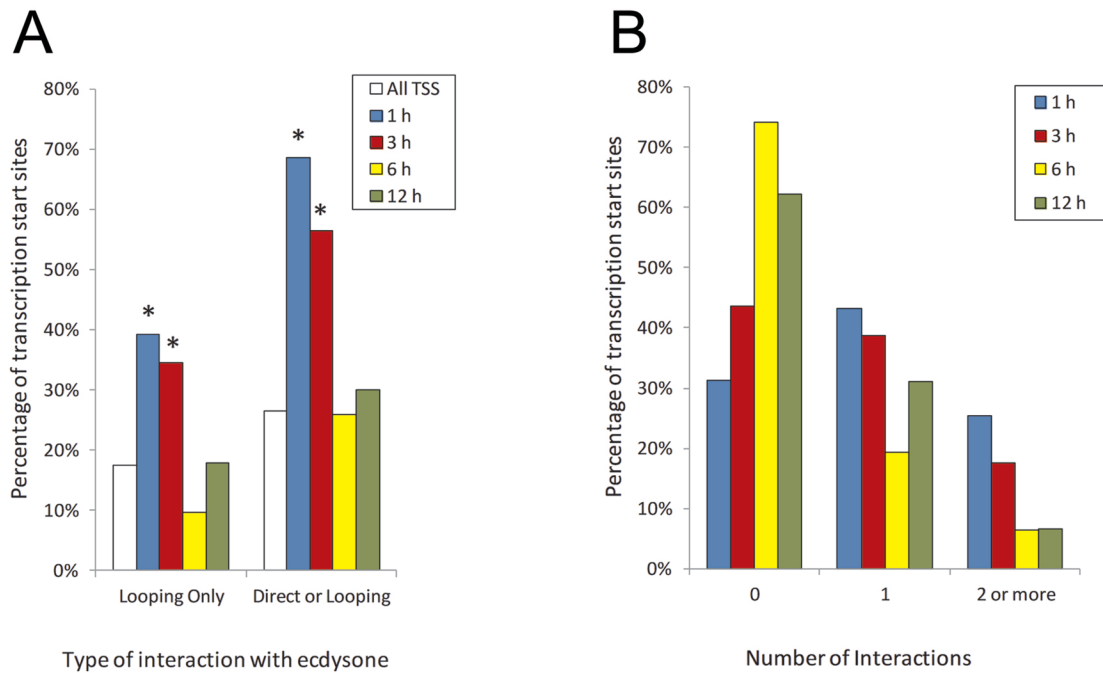
**Figure S2. High resolution 3C interaction profile at the *Broad* locus.** Schematic is shown for the region 0 to 9 kilobases downstream from the proximal promoter, indicated by a horizontal arrow. Vertical bars indicate DpnII sites and numbered arrows indicate EcREs. Crosslinking frequencies (y axis) between the fixed DpnII anchor and the downstream EcRE-containing region were measured in S2 cells in the absence or presence of ecdysone as indicated. Locations of fixed anchor site and test sites are marked by red and gray bars, respectively. Black angle bracket (" $>$ ") indicates the location and direction of the anchor primer. Red angle brackets indicate the location and direction of test primers in fragments that interact with the *Broad* promoter, either in the presence or absence of ecdysone. Coordinates are given along the x axis relative to the proximal transcription start site. Asterisk indicates significant difference between ecdysone and control samples (Paired Student's *t*-test,  $P < 0.05$ ). Mean  $\pm$  S.E.M. is shown from three independent experiments.



**Figure S3. Long-distance 3C interactions at the *E74* locus.** Schematic of *E74* is shown at the top. Horizontal arrows indicate transcription start sites, vertical bars indicate HindIII sites, and numbered arrows designate EcREs. Crosslinking frequencies (y axis) between the fixed HindIII anchor for *E74B* (upper panel) or *E74A* (middle panel) and the rest of the locus were measured in S2 cells in the absence or presence of ecdysone as indicated. Location of fixed anchor sites are marked by red (*E74B*) or blue (*E74A*) bars, and test sites are marked by gray bars. Black angle brackets (" $>$ ") indicate the location and direction of anchor primers. The red angle bracket indicates the location and direction of the test primers in a fragment that interacts with the *E74B* promoter. Coordinates are given along the x axis relative to the *E74B* transcription start site. Asterisk indicates significant difference between ecdysone and control samples (Paired Student's *t*-test,  $P < 0.05$ ). Mean  $\pm$  S.E.M. is shown from three independent experiments.



**Figure S4. Knockdown of *EcR* transcript by RNAi.** S2 cells were transfected with dsRNA targeting *EcR* or with mock dsRNA as indicated. After 3 days of incubation, cells were treated with  $1 \times 10^{-6}$  M ecdysone for 0, 1, or 2 h. Cells were collected, and total RNA was purified for Northern blot hybridization. Probes are designated on the left. The *EcR* panel confirms reduction of *EcR* transcripts. Panels *BR-C*, *E75A*, and *E75B* demonstrate that reduction of *EcR* by RNAi substantially disrupts ecdysone-induced activation of the early genes. The bottom panel shows the *rp49* loading control.



**Figure S5. Promoter-EcRE interactions correspond to rapid ecdysone response.** (A) Publicly available data in Kc167 cells describing the genome-wide distribution of ecdysone receptor binding sites (GEO accession GSE9156), ecdysone-responsive transcripts (accession GSE11625), and the genome-wide interactome (accession GSE38468) was used to identify direct and looping interactions as indicated between the ecdysone receptor and all transcription start sites as well as transcription start sites from genes activated by ecdysone after 1, 3, 6, or 12 h. The percentage of interacting transcription start sites from each category is shown on the y axis. Asterisk (\*) indicates significantly greater frequency of interaction compared to all transcription start sites (Fisher's exact test,  $P < 0.05$ ). (B) Same as in (A), except TSS are categorized by the number of EcRE interactions.

**Table S1.** Chromatin immunoprecipitation amplicon coordinates and primer sequences

Gene	Candidate EcRE(s)	Coordinates <sup>a</sup>	Amplicon	Primer Sequences (5'-3')
E75	+26726/26714, +26626/26614	+26752/+26616	TGTGCGATTTTGTAGCTCTG ATTTCCTTGAACGCATTTTCG	
	+26385/26373	+26416/+26291	CTCCGCTTCTCTCTCAGC AGAGCAGGCTATGGGTATGG	
	+19778/19766, +19768/19756	+19813/+19694	GACATGTGCCTCGATGTGAG CATGCTCGTTGACTTCGTTG	
	+17808/17796	+17880/+17736	TTTTCCATCGCTGTTTGTGTC AGTCGCATGAAAAGCAAAGG	
	+17477/17465	+17551/+17429	ACACACACCAAGCACTCAC TTCACITGAGAGGCGAAGAC	
	+16475/16463	+16599/+16438	TTCAGTTCGGTTAGGCTTGG CGCAGCACGTACCTTGTTAG	
	-	+15055/+14878	CGCACTTACGCACAATTTTC TGCCCATTTATATCGACTACACATC	
	+12788/12776	+12809/+12690	AACTCGTTGCGTTTGTGTC TTTCTCCGCTGCAATCTAGC	
	+10099/10087	+10125/+9990	GCAGTCAGTCGTTCAAGTGC AAGGAAAAGCCAGGGAAGAAAC	
	+9389/9377	+9427/9278	GCGTACGCTGCTTCTGTTTC TCGGCAGTTGTTGTGTATGC	
	+7601/7589, +7592/7580	+7698/+7562	CAAATACAAGGACCGCCAAG CAAGCGAGTCGGGTTAATG	
	+7349/7337	+7393/+7253	AACGATCGCTGGACTCTGTC TTGTGCAGTGTTCCTTGG	
	+5575/5563, +5552/5540	+5685/+5540	CGATCACGATCATGAGCAAC AAGTGCAACGATCTGCAGTG	
	+1796/1784	+1819/+1675	GGACTTGAACCGTTGCTC TAACCTCAGCACTCGCCATC	
Broad	-50025/-50037	-49935/-50104	AGCCGCAGAAATCTGAAAAC CTCGAATCTCGATCCCAAAG	
	-46108/-46120, -45973/-45985	-45909/-46147	ATTTGTGTGCGCTGTGTTG GCAGCAGGCTTTTATAATTTTC	
	-35135/-35147	-35041/-35205	TTCACGCAGTCTCTCGAATG GGGCTTTTGTGTTGGATTTCAG	
	-34375/-34387	-34280/-34419	CGGACAAGTATTCTTCGATGC GCCGTATGGAAAAGAGTTTCG	
	-33932/-33944	-33910/-34081	CTTCATACCCACACCTTGC GTTTCGCTCGAGTCGTTGG	
	-28807/-28819	-28748/-28912	ACCAGGCAGAGCACTACCAC CCCAGTAGTGGGATGTTGTTG	
	-25989/-26001	-25956/-26030	CGGGTAGCATCTTTCTCAGC GCAAAGACGGAAATGGGTAG	
	-20870/-20882	-20770/-20906	AACTTTGTGTAGTGCGCCATC ATTTTCAGCTGCTGCTACCG	
	-	-18691/-18858	GGGATAGTCGCTGCCAATAC TAGTTCGTTGCCGCTGTTC	
	-316/-328	-276/-424	CAGGCAGCGCTGAAACTG ACTTCTCGTGTGCGATTGTG	
	+4905/+4893, +4923/+4911	+4839/+5003	GCTCTCTCTCGTGGTTGC CGCACGCTGCAATGTATTAG	
	+6984/+6972	+6918/+7062	CGTATGGATTGAAGCTTTTCG TCGCTCTTTTCGCTTAGTTG	
	E74	-1773/-1785	-1662/-1807	GCAGGCAGAAGAAGGAGAAG TGTGAGTGCACCCCTAGTTG
		-2392/-2404	-2311/-2430	ACAGCTCCTTGACTCCTTCG ACCAGTGTGCAGCATTGTGTC
-7601/-7613		-7563/-7680	TCGTTGTCTCGCTCAGTGTG GGCGGACTCACAAATAGGTTG	
-		-11661/-11771	AATTTGCGAGGAAGGAGAAC TTCGCAGATTGCAGTTCG	
-16753/-16765		-16660/-16805	AGAGGGGGCTTTCAGAGTTG AGCCAGCAAACGAACTGAAC	
-23394/-23406		-23354/-23499	TTTCGGTTTTCGCGATTGTG AAATTTGTGCCGACGTC	
-31543/-31555		-31505/-31619	ATTGAAAATTCGCGTCGTTG GCGCTAGTTGTTGTTGTTG	
-31560/-31572, -31754/-31766		-31705/-31807	AGCGCCACAACACTCACTTAC TCTGGTCTGTGAACTCTGC	
-32549/-32561, -32589/-32601		-32516/-32633	CACCGTTTGCATTCACTCAC AGTTCAGCGTGTGTTGTGCTG	
-35044/-35056, -35237/-35249, -35255/-35267		-35034/-35277	TGATTGTTTGGGGTAATTGAAC TGGTTATCGAAGGTCTTTCACC	
-35533/-35545		-35502/-35646	TGAGAGCCTGCTAAGAGCTG TTTGATGTGTGTGCGTATG	
-40447/-40459		-40410/-40513	GTAGTGCCCGTTATCGGAAG GATCGTCGGAGAGGAGAGTG	

<sup>a</sup> Shown relative to the *E75A*, *Broad proximal*, and *E74B* transcription start sites.

**Table S2.** 3C HindIII coordinates and primer sequences

Gene	HindIII Coordinates <sup>a</sup>	Primer Sequences (5'-3')
E75	+31166 (E75B Anchor)	GTGTGAGGCAATGGGAATG
	+23422	TCTTTTCGTGCTAAAACATTTATGG
	+22199	CAACCATGGTATCCCAAATTC
	+18295	AACGGAAATATTGTGGGCTAAAC
	+15868	CAGCAGGCAAGCCGTATC
	+13767	AAGTAGCCTTCCAAACGGTTC
	+230 (E75A Anchor)	TGTGAAGAAAACATGTAAACTTGG
	-2587	TTTACCTTACCCCTGCGATG
	-4448	GCAAAAATTCTGACGACCTTG
	-6847	GCGAATTCGGTGTGTAGC
	-11550	GCCACCTAAGGCTAAAATTTCC
	-11845	CGGCGATAAGATCGAAATTG
	-14536	CGAGAAGCAAGTTCAAATTAC
	-17646	AGTGATCGCAATACCCCTTGC
	-20249	GAGTTGCTGAACCGTCCAC
	-22134	TTCTACTTTCAGGTAAGTCAACTGC
	-26700	AGTGGCACTGAGATTCAAACC
	-29646	AATCCTGATAAGCAAAGAAGAACG
	-31306	GCACAGTTGTGACCTAATGACC
	-33336	TGTCTAATGCAACTGGAAAATTG
-33446	TTTCGTGCGGAACTTTAG	
-39360	TTCGTTGTGAATTTTTTCAGAGTC	
-42318	GGTCACAATTCTCTCCTTTACAGTG	
-58321 (E75C Anchor)	GACATCTGTAGCTTAGTGTAAGAAGTGG	
Broad	+7479	AAAGGCGAAGAGCGAGAAC
	+6930	ACACAGCCCACTCACGTTC
	+2667 (Broad Anchor)	ATTGTTGCTGCCACAGATTG
	-7706	AGTAGGTGGAGCAACCAATTTTC
	-8667	CAACGAGTGAGTAGGGCAGAG
	-19008	TTACTATCGGTGGCATTCTCG
	-22874	CATCAGCTGCGGTGGAG
	-31124	ACGAGGTTGAGAACGGGAATG
	-33572	GCTCTCCGATCCACACTTTC
	-52023	TGCCCTCTACAACGACTGG
E74	-2283 (E74B Anchor)	TGAAGAGGAAGCGCATTAGG
	-17573	TGATGAGCAGCAATGAATAACA
	-18908	AATGCTGTGCATGCTTAAAGG
	-20303	TTGTTAAATCAAAAACATTCCTTC
	-25727	AGTGAGCGGTACTCAGTGCTC
	-27206	TTTACATTTCCCGAACACC
	-27540	TCTCACACTGTCATGGCTACG
	-31481	CGAAAATATGGAGGAGCAAAAC
	-37862	TGAAGCCGATTTTGATTTCC
	-41662	CGTTTCGCACATTTTCTTTG
-43162 (E74A Anchor)	GCGGAACTCATTAGCAAG	

<sup>a</sup> Shown relative to the *E75A*, *Broad proximal*, and *E74B* transcription start sites.

**Table S3. 3C DpnII coordinates and primer sequences**

Gene	DpnII Coordinates <sup>a</sup>	Primer Sequences (5'-3')
E75	+27902 (E75B Anchor)	GCGACACTCTGATTTCTCAGG
	+26208	AAGCGAGTATGACAGGCCTAAG
	+25975	TGTTCTCCCTGACGTGCTC
	+25975	TGAAACCGCCTACCTGTTG
	+25856	TGTTTCGGTTCTTTGTTTTGC
	+13061	CAACATCTAGACCTGCCACTTG
	+11101	AGATGGCGCCAGTATGTTATG
	+8710	GGAACATTTTCGAGCATAACTCAC
	+8347	CAATAGACAAGACGAAGGTCCAC
	+7772	CAATTTTCTACCAGCCTTGAC
	-132 (E75A Anchor)	CTTTGCACTCTCTCGCACTTC
	-1148	GACAAATGCGTCTAACACAAAGTG
	-1337	CCGGTACCCTTGAATCAAATC
	-2032	TCTGCCAATTTCCCAATGAG
	-2265	CCTGGCTGGCTGTTACTTAGTC
	-2394	GCAAAACTCGCCTTCAACTC
	-2505	GTTGAAGGCTTCCGTGCTAC
	-3138	TTCATGATGTCATGGCGAAG
	-4927	CCAGCACTAACTCGAATTTTCC
	-22360	CAAGATATCGCTAGCATCCAAAG
-22557	GCACAGCAGCAACGACTG	
-23041	GCCTTACGCCCTTGACATAC	
-25880	TCCAATCAAACGTTCCGGTATC	
-26011	CAGATGTGGTATTACCAGTCTTTC	
	-60206 (E75C Anchor)	TGGAAAGTTGCGTTTGTGTTG
Broad	-6 (Broad Anchor)	TGATTCAATTGGGCAGACG
	+4294	ACTGTGATGGTAAGCGACCTAAC
	+4526	GCATTGACATTCGGTATCCAC
	+4697	GGAAATTCGCACACACGAG
	+5029	GCAGAGAGCAGCTTTTGTATG
	+5694	GCAGTTTACTTAGCCTCCCTCTC
	+6157	GCTTGCCAAGTTCAAAGACC
	+6275	ATCCGTGGAGGGATAGGAAC
	+7369	GCGAGACAAAAGTGGTGAGG
	+7524	TTCTAACGCTCTCTCGCTCTG
+8706	GGTGGTGCAGCAGATATGG	
E74	-768 (E74B Anchor)	GCATGCGAGAAATATGTTTAATTG
	-845	TTACCACATTTTCCCCGATG
	-1072	TTCTCCGCGATTCTCTGG
	-2123	AGTAACAAAGGAAAGGGTTTGC
	-2734	TTAGGATTACTTTTAGCGCACAAAC
	-2771	TAGCATTCGACGAGGGAAG
	-29477	CCGAGAGCTGTCGTCTGATAC
	-30448	ACACACGGACATTCTGCAAG
	-31913	AAGTGCCCGTTATCACATGG
	-32549	CACCGTTGCATTTCATTCAC
	-32979	AGCAAATGCAAATGCTTAGAAC
	-33590	AACTGCCGATTGGTGGTG
	-33798	AAGGCTAAAGTTCAATGGAAACC
	-34183	TTCCAAAGGGAAAAGTGCATC
	-35948	CGGCGAAGGACAATCAAC
	-36559	AGAATCGCCCACGTAATCTG
-36795	GAATGTACGCGACGTCAATG	
-38017	CGAACCAAATGCACTGAATG	
	-43257 (E74A Anchor)	ACCTGTCTGCAAGCTCACC

<sup>a</sup> Shown relative to the *E75A*, *Broad proximal*, and *E74B* transcription start sites.



**Table S4.** Transcript induction by ecdysone

Early gene transcript	Hour of Peak Expression	Fold induction at Peak
E75A	1	13.8
E75B	4	89.0
E75C	2	29.3
E74A	<sup>a</sup>	20.3
E74B	2	9.7
Broad	2	21.3
hsp27	<sup>a</sup>	3.7

<sup>a</sup>. Expression does not peak within four hours.

**Table S5. Summary of 3C interactions**

Promoter	No. of interacting EcREs
E75B	8
E75A	4
E75C	4
E74B	6
E74A	4
Broad <sub>prox</sub>	5

Table S6. TSS of early (1, 3 h) and late (6, 12 h) ecdysone-inducible genes in Kc167 cells							
1 h		3 h		6 h		12 h	
Gene ID	TSS	Gene ID	TSS	Gene ID	TSS	Gene ID	TSS
FBgn0000210	X:1469165	FBgn0000250	2L:16325444	FBgn0000454	3R:9608012	FBgn0000045	3L:21978310
FBgn0000210	X:1469182	FBgn0000250	2L:16325656	FBgn0000454	3R:9608362	FBgn0000071	3R:2588315
FBgn0000210	X:1502902	FBgn0000250	2L:16326102	FBgn0001224	3L:9374982	FBgn0000071	3R:2589128
FBgn0000210	X:1504545	FBgn0000448	2R:6098853	FBgn0001225	3L:9370476	FBgn0003447	X:7858057
FBgn0000210	X:1506341	FBgn0000448	2R:6115459	FBgn0003890	3R:23814090	FBgn0003447	X:7863738
FBgn0000210	X:1506374	FBgn0000448	2R:6120897	FBgn0010039	3R:8198790	FBgn0003870	3R:27539591
FBgn0000210	X:1507690	FBgn0000448	2R:6120907	FBgn0014427	3R:25026174	FBgn0003870	3R:27550731
FBgn0000210	X:1516765	FBgn0000448	2R:6124854	FBgn0029853	X:6155202	FBgn0003870	3R:27551503
FBgn0000546	2R:2019347	FBgn0000566	2R:14515399	FBgn0030993	X:19082068	FBgn0003870	3R:27552124
FBgn0000546	2R:2019428	FBgn0001168	3L:8668859	FBgn0031220	2L:120743	FBgn0004237	3R:9486242
FBgn0000546	2R:2044635	FBgn0001168	3L:8669055	FBgn0031220	2L:121752	FBgn0004449	3L:22400964
FBgn0000546	2R:2056593	FBgn0001226	3L:9377163	FBgn0031220	2L:121975	FBgn0004456	X:13151205
FBgn0000565	3L:15504152	FBgn0002526	3L:6211123	FBgn0031717	2L:5547086	FBgn0004456	X:13152524
FBgn0000565	3L:15504663	FBgn0004606	3R:26591648	FBgn0031717	2L:5547203	FBgn0004838	2L:6920704
FBgn0000565	3L:15504989	FBgn0004606	3R:26608980	FBgn0031717	2L:5547290	FBgn0004838	2L:6920966
FBgn0000567	3L:17569421	FBgn0005612	2R:19866744	FBgn0031988	2L:8127024	FBgn0004838	2L:6921260
FBgn0000567	3L:17569727	FBgn0005612	2R:19867418	FBgn0031992	2L:8162390	FBgn0004838	2L:6921639
FBgn0000567	3L:17612426	FBgn0010300	2L:19133816	FBgn0036194	3L:11689599	FBgn0010382	2L:15731781
FBgn0000568	3L:17954915	FBgn0010300	2L:19158540	FBgn0036194	3L:11690064	FBgn0010382	2L:15746610
FBgn0000568	3L:17964540	FBgn0010395	2L:21053033	FBgn0037206	3L:22903591	FBgn0010382	2L:15748156
FBgn0000568	3L:17992922	FBgn0011766	3R:17458745	FBgn0037206	3L:22903933	FBgn0010909	3L:2586255
FBgn0000568	3L:18052699	FBgn0011766	3R:17463828	FBgn0038110	3R:8837011	FBgn0010909	3L:2586541
FBgn0000568	3L:18054554	FBgn0011766	3R:17486128	FBgn0038516	3R:13360537	FBgn0013772	2R:10776515
FBgn0000568	3L:18057797	FBgn0011785	3R:20144741	FBgn0038516	3R:13360655	FBgn0015778	3R:9472726
FBgn0001257	3L:4225951	FBgn0013984	3R:17415914	FBgn0038516	3R:13361409	FBgn0015778	3R:9474539
FBgn0001257	3L:4227303	FBgn0013984	3R:17417844	FBgn0039129	3R:19759253	FBgn0023407	2L:13547772
FBgn0001257	3L:4236148	FBgn0013984	3R:17444759	FBgn0039130	3R:19759637	FBgn0023407	2L:13549329
FBgn0004865	3L:21219709	FBgn0013984	3R:17445044	FBgn0039130	3R:19759718	FBgn0025574	3R:19715284
FBgn0004865	3L:21220019	FBgn0014002	3L:15134589	FBgn0051363	3R:7417967	FBgn0025574	3R:19715753
FBgn0004865	3L:21233447	FBgn0014002	3L:15135693	FBgn0051363	3R:7430474	FBgn0025574	3R:19715798
FBgn0014469	2R:4332217	FBgn0014037	3L:19902511	FBgn0051363	3R:7445629	FBgn0026084	X:4000148
FBgn0016076	2L:5299647	FBgn0020309	2L:11808973			FBgn0026084	X:4000868
FBgn0016076	2L:5305708	FBgn0020309	2L:11809434			FBgn0026084	X:4002982
FBgn0016076	2L:5305722	FBgn0020386	3L:129450			FBgn0027660	3L:17396835
FBgn0020445	2L:3338448	FBgn0020386	3L:131812			FBgn0027660	3L:17399622
FBgn0020445	2L:3355001	FBgn0020386	3L:132733			FBgn0027660	3L:17408912
FBgn0027578	3R:24709415	FBgn0020386	3L:141090			FBgn0027660	3L:17414615
FBgn0031913	2L:7433517	FBgn0020386	3L:141096			FBgn0029903	X:6697720
FBgn0032482	2L:13203768	FBgn0020386	3L:141321			FBgn0029903	X:6697737
FBgn0032482	2L:13209266	FBgn0020389	3L:19838393			FBgn0029903	X:6697930
FBgn0034045	2R:11734862	FBgn0020389	3L:19840409			FBgn0029903	X:6699018
FBgn0034045	2R:11734866	FBgn0020389	3L:19841265			FBgn0029903	X:6699161
FBgn0035173	3L:882014	FBgn0023215	X:3565834			FBgn0029903	X:6699418
FBgn0036956	3L:20224769	FBgn0023215	X:3582270			FBgn0030797	X:16722176
FBgn0038074	3R:8500332	FBgn0023215	X:3582288			FBgn0030797	X:1672217
FBgn0038981	3R:18354384	FBgn0023215	X:3583398			FBgn0030797	X:16729108
FBgn0039613	3R:24706525	FBgn0024734	2L:16251789			FBgn0030797	X:16729548
FBgn0050203	2R:8198194	FBgn0024734	2L:16256156			FBgn0031489	2L:3018094
FBgn0262867	2R:2083513	FBgn0024734	2L:16257607			FBgn0031489	2L:3018309
FBgn0262867	2R:2083733	FBgn0024921	3L:6186580			FBgn0032350	2L:11155717
FBgn0262867	2R:2083868	FBgn0024921	3L:6186846			FBgn0033051	2R:1910712
		FBgn0025390	X:1512189			FBgn0034180	2R:12903392
		FBgn0025693	2R:1670361			FBgn0035282	3L:1855310
		FBgn0026160	3L:10850989			FBgn0035282	3L:1855592
		FBgn0026160	3L:10851504			FBgn0035338	3L:2379062
		FBgn0026160	3L:10862928			FBgn0035338	3L:2388078
		FBgn0026263	3L:8049476			FBgn0035338	3L:2400509
		FBgn0026263	3L:8051814			FBgn0035424	3L:3224838
		FBgn0026404	3L:9961579			FBgn0035424	3L:3226053
		FBgn0027561	2R:5019307			FBgn0035526	3L:4283851
		FBgn0027561	2R:5019325			FBgn0035772	3L:7361291
		FBgn0027561	2R:5019487			FBgn0035772	3L:7362361
		FBgn0027865	3R:21707163			FBgn0035904	3L:8511985
		FBgn0029924	X:6863950			FBgn0036007	3L:9490467
		FBgn0031745	2L:5879482			FBgn0036007	3L:9498429

Table S6, continued							
1 h		3 h		6 h		12 h	
Gene ID	TSS	Gene ID	TSS	Gene ID	TSS	Gene ID	TSS
		FBgn0031897	2L:7220030			FBgn0036534	3L:15817305
		FBgn0033310	2R:4487938			FBgn0036534	3L:15819524
		FBgn0033476	2R:5936124			FBgn0037636	3R:4646379
		FBgn0033638	2R:7520018			FBgn0037636	3R:4646387
		FBgn0033638	2R:7520076			FBgn0040091	2R:18531421
		FBgn0033638	2R:7528419			FBgn0041092	2L:9166778
		FBgn0033999	2R:11103192			FBgn0041092	2L:9176358
		FBgn0034368	2R:14519404			FBgn0051122	3R:14235583
		FBgn0034647	2R:17549750			FBgn0051122	3R:14238597
		FBgn0035558	3L:4542051			FBgn0051462	3R:4213925
		FBgn0035558	3L:4542120			FBgn0052594	X:14918467
		FBgn0035558	3L:4550689			FBgn0052654	X:12490080
		FBgn0035693	3L:6193573			FBgn0052654	X:12490709
		FBgn0037007	3L:20510405			FBgn0052654	X:12490983
		FBgn0037007	3L:20512494			FBgn0053208	3R:5847198
		FBgn0037010	3L:20519275			FBgn0053208	3R:5847400
		FBgn0038071	3R:8473782			FBgn0053208	3R:5847573
		FBgn0038535	3R:13504426			FBgn0053208	3R:5850873
		FBgn0038535	3R:13505010			FBgn0053208	3R:5868544
		FBgn0038535	3R:13507966			FBgn0053208	3R:5868618
		FBgn0038535	3R:13508235			FBgn0064123	X:20964871
		FBgn0039612	3R:24705964			FBgn0085436	2R:5465516
		FBgn0039972	2L:22311931			FBgn0085436	2R:5466513
		FBgn0039972	2L:22311954			FBgn0086686	3R:10480913
		FBgn0039972	2L:22354355			FBgn0086686	3R:10481437
		FBgn0040398	X:945557			FBgn0262735	X:10700890
		FBgn0040493	3R:8787358			FBgn0262735	X:10708127
		FBgn0040493	3R:8793101			FBgn0262735	X:10709658
		FBgn0040493	3R:8793435			FBgn0262735	X:10715745
		FBgn0041182	2L:7701586			FBgn0262735	X:10715818
		FBgn0043884	3R:20056284			FBgn0262735	X:10716816
		FBgn0051324	3R:21932590			FBgn0262937	3L:1295913
		FBgn0051324	3R:21954526			FBgn0262975	3R:19014852
		FBgn0052218	3L:19889492			FBgn0262975	3R:19015003
		FBgn0052479	3L:871899			FBgn0262975	3R:19016142
		FBgn0052479	3L:872911			FBgn0262975	3R:19016877
		FBgn0052479	3L:888018			FBgn0262975	3R:19026373
		FBgn0052486	3L:3070840			FBgn0262975	3R:19040280
		FBgn0052699	X:9465342			FBgn0262975	3R:19047684
		FBgn0052702	X:9474009			FBgn0262975	3R:19052431
		FBgn0085370	2L:18563173			FBgn0263416	3R:4303316
		FBgn0085370	2L:18571649				
		FBgn0085443	X:10387770				
		FBgn0085443	X:10404676				
		FBgn0085443	X:10440788				
		FBgn0085443	X:10449493				
		FBgn0085443	X:10479456				
		FBgn0086902	2L:221963				
		FBgn0086902	2L:250824				
		FBgn0243514	3R:22925402				
		FBgn0259984	2L:13550141				
		FBgn0259984	2L:13550164				
		FBgn0260486	2L:7496308				
		FBgn0260794	3R:627658				
		FBgn0260794	3R:637784				
		FBgn0260794	3R:637789				
		FBgn0261239	2L:21237250				
		FBgn0261258	3L:21375601				
		FBgn0261618	3R:24151964				
		FBgn0261618	3R:24151987				
		FBgn0261618	3R:24154006				
		FBgn0261618	3R:24156941				
		FBgn0261618	3R:24161264				

Table S6, continued							
1 h		3 h		6 h		12 h	
Gene ID	TSS	Gene ID	TSS	Gene ID	TSS	Gene ID	TSS
		FBgn0261618	3R:24162163				
		FBgn0261674	3L:12783292				
		FBgn0261674	3L:12784318				
		FBgn0261885	3R:13544060				
		FBgn0262127	3R:10549979				
		FBgn0262160	2L:13060116				
		FBgn0262579	3L:8056974				
		FBgn0262579	3L:8064052				
		FBgn0262579	3L:8067406				
		FBgn0262579	3L:8070492				
		FBgn0262579	3L:8089622				
		FBgn0262579	3L:8094119				
		FBgn0264562	X:1835973				
		FBgn0264562	X:1886333				

**Table S7.** Early gene promoter-EcRE interactions in Kc167 cells

Gene	TSS Coordinates <sup>a</sup>	EcR/Usp binding site <sup>b</sup>	Type of Interaction <sup>c</sup>
E75A	3L:17992921	3L:17992222-17998221	Direct
		3L:18002222-18012221	Looping
E75B	3L:17964539	3L:17908222-17922221	Looping
		3L:17924222-17928221	Looping
		3L:17956222-17976221	Direct
E75C	3L: 18052698	-	-
E75D	3L:17954914	3L:17908222-17922221	Looping
		3L:17924222-17928221	Looping
		3L:17956222-17976221	Looping
Broad <sub>Dist</sub>	X:1469181	-	-
Broad <sub>Prox</sub>	X:1506373	X:1507262-1515261	Looping
E74A	3L:17612408	3L:17604222-17610221	Looping
		3L:17612222-17618221	Direct
E74B	3L: 17569726	3L:17568222-17574221	Direct

<sup>a</sup> Coordinates are based on published reports (1-4). Note that additional TSS annotations have been predicted.

<sup>b</sup> See (5).

<sup>c</sup> Direct, binding site overlaps TSS; Looping, binding site interacts with TSS through a Hi-C loop (6).

**Table S8.** 1 and 3 h ecdysone-activated genes exhibiting multiple EcRE-promoter interactions in Kc167 cells

Ecdysone response	Gene	TSS Coordinate	EcRE-promoter interactions
1 h	CG5958	2L:7433516	2
	Eip71CD	3L:15504151	2
		3L:15504662	2
	EcR	3L:15504988	2
		2R:2019346	2
	vri	2R:2019427	2
		2L:5299646	2
		2L:5305707	2
3 h	brat	2L:5305721	2
		2L:19133815	3
	CG31324	2L:19158539	2
		3R:21932589	2
	CG5059	3R:21954525	2
		3L:20510404	2
	grsm	3L:20512493	2
		3R:8787357	2
		3R:8793100	2
	InR	3R:8793434	2
		3R:17415913	2
	kuz	3R:17417843	2
		2L:13550140	2
	Pdi	2L:13550163	2
		3L:15134588	3
	PRL-1	3L:15135692	3
2L:16251788		2	
2L:16256155		2	
Su(Tpl)	2L:16257606	2	
	3L:19902510	2	
	X:10440787	2	
spri	X:10449492	2	
	X:10479455	2	

**Table S9.** EcR binding sites in early genes at the onset of metamorphosis

Gene	EcR binding site coordinates <sup>a, b</sup>		EcR Designation <sup>c</sup>	EcRE No. <sup>d</sup>
	L3	WPP (0-0.5 h APF)		
E75	+27410/+27075	+28813/+26078	-	8,9,10
	-	+25013/+24678	-	-
	-	+24413/+22478	-	-
	-	+22113/+21078	-	-
	-	+18413/+17878	-	-
	-	+11613/+9378	-	15
	-	+6013/+5678	-	-
	-	+4313/+3678	-	-
	-	+213/-622	A	-
	-	-1287/-2222	B	1
	-	-5487/-6322	C	-
	-	-7687/-8122	D	-
	-	-8787/-9622	E	-
	-	-10687/-10922	F	-
	-	-11687/-12822	G	-
	-	-13287/-13922	H	3
-	-16587/-17022	I	-	
-	-19287/-19522	J	-	
-	-29787/-30122	K	-	
-	-37687/-38422	L	-	
Broad	-	-35262/-34927	A	1
	-	-34262/-32927	B	2
	-	-2362/-1927	C	-
	-27362/-27227	-	-	-
	-	+4238/+5173	D	4,5
-	+5438/+6473	E	-	
E74	-	-31182/-32917	-	3,4,5
	-	-34382/-35817	-	6,7,8,9
	-	-36582/-37017	-	-

<sup>a</sup> Coordinates shown relative to *E75A*, *Broad proximal*, and *E74B* transcription start sites. Only includes intragenic sequences studied in S2 cells (see Figure 2).

<sup>b</sup> Data retrieved from modENCODE, ID 2640 (L3), 2641 (WPP).

<sup>c</sup> See Figures 8-10.

<sup>d</sup> EcREs identified in S2 cells. Number designations are from this study and (7).



## Supplementary References

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