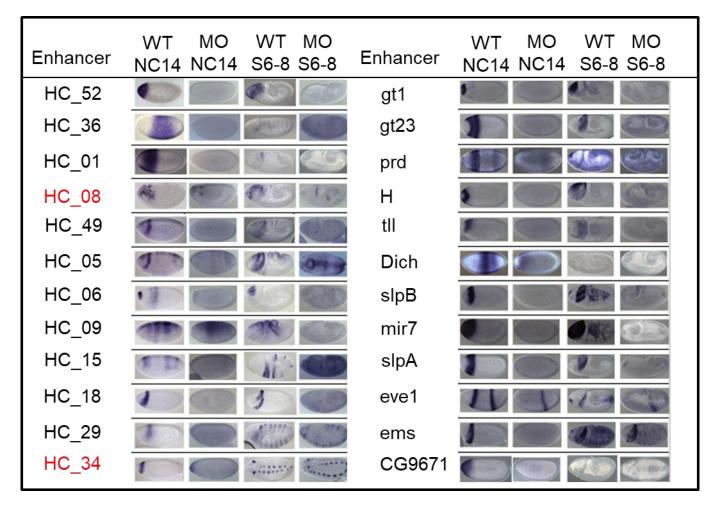


Supplemental Figure 1. (A) Maternal RNA expression. RNA is localized to the anterior tip for all constructs tested. Construct name and RNA detected are in the bottom left corner. (B) Expression of Cad in wild type, *bcd*⁻, and embryos containing maternal transgenes. Genotypes are indicated in the bottom left corner of the panel.



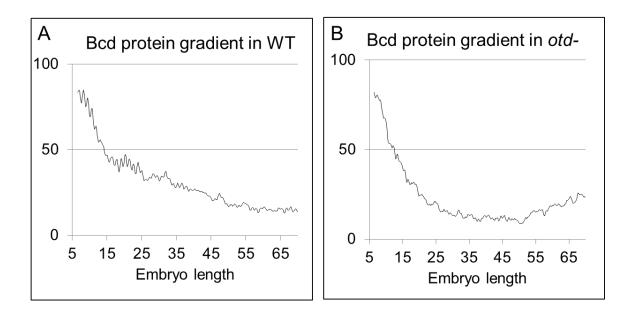
Supplemental Figure 2. The 24 Bcd-dependent enhancers tested in the Mat>Otd (MO) genetic background. The two enhancers activated by Mat>Otd are shown in red.

A	Bound by: Bcd S5 + (Otd S5) + Otd S6-8	Other name*	S5 Expression	S6-8 Expression	Bound by: Bcd S5 + (Otd S5) + Otd S6-8	Other name*	S5 Expression	S6-8 Expression	Bound by: Bcd S5 + (Otd S5) + Otd S6-8	Other name*	S5 Expression	S6-8 Expression
	BcdOtd ^{EL1}	eve1	11		BcdOtd ^{EL11}	HC_18	-	3	BcdOtd ^{EL21}	HC_02	1	
	BcdOtd ^{EL2}	HC_16		9	BcdOtd ^{EL12}	HC_05	1	200	BcdOtd ^{EL22}	slpB		C.S.
	BcdOtd ^{EL3}	HC_27	Ċ	0	BcdOtd ^{EL13}	HC_23		Due	BcdOtd ^{EL23}	HC_06		
	BcdOtd ^{EL4}	HC_01		3	BcdOtd ^{EL14}	HC_25			BcdOtd ^{EL24}	HC_17		- Fite
SS 1	BcdOtd ^{EL5}	HC_34	10	1	BcdOtd ^{EL15}	HC_29	CED .	all and	BcdOtd ^{EL25}	HC_49		
CLASS	BcdOtd ^{EL6}	otd_EHE	1		BcdOtd ^{EL16}	HC_15	T	ALS.	BcdOtd ^{EL26}	HC_14		20
	BcdOtd ^{EL7}	HC_03	1	90	BcdOtd ^{EL17}	prd_OE		13	BcdOtd ^{EL27}	HC_19		2000
	BcdOtd ^{EL8}	gt1		-	BcdOtd ^{EL18}	HC_36		C D	BcdOtd ^{EL28}	tll_OE	1	
	BcdOtd ^{EL9}	HC_12			BcdOtd ^{EL19}	HC_04		-Alline	BcdOtd ^{EL29}	HC_33		10
	BcdOtdEL10	HC_35		C	BcdOtd ^{EL20}	HC_08	Nin O	125	BcdOtd ^{EL30}	HC_26		

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-	5	
_	,	

Enhancer	WТ	Otd-	Enhancer	WT	Otd-	Enhancer	WT	Otd ⁻	Enhancer	WT	Otd
BcdOtd ^{EL1}	11		BcdOtd ^{EL3}	1		BcdOtd ^{EL5}	1		BcdOtd ^{EL7}	1	
BcdOtd ^{EL1}	ID	(III)	BcdOtd ^{EL3}			BcdOtd ^{EL5}			BcdOtd ^{EL7}	1	
BcdOtd ^{EL1}	11	(LD	BcdOtd ^{EL3}			BcdOtd ^{EL5}		0	BcdOtd ^{EL7}	10	ID
BcdOtd ^{EL1}	10	TD	BcdOtd ^{EL3}			BcdOtd ^{EL5}	Ó		BcdOtd ^{EL7}	1	100
BcdOtd ^{EL1}	11		BcdOtd ^{EL3}			BcdOtd ^{EL5}		0	BcdOtd ^{EL7}	1	T
BcdOtd ^{EL2}			BcdOtd ^{EL4}			BcdOtd ^{EL6}	1.80	3	BcdOtd ^{EL8}	1	
BcdOtd ^{EL2}			BcdOtd ^{EL4}			BcdOtd ^{EL6}	1		BcdOtd ^{EL8}		
BcdOtd ^{EL2}			BcdOtd ^{EL4}			BcdOtd ^{EL6}	1	1	BcdOtd ^{EL8}		
BcdOtd ^{EL2}			BcdOtd ^{EL4}		0	BcdOtd ^{EL6}			BcdOtd ^{EL8}		
BcdOtd ^{EL2}			BcdOtd ^{EL4}			BcdOtd ^{EL6}		1	BcdOtd ^{EL8}		

Supplemental Figure 3 (A) *lacZ* expression patterns of a subset of "relay" enhancers (Class 1). (B) Representative embryos from relay enhancer lines showing loss of expression in *Otd* mutants.



Supplemental Figure 4. (A) Bcd protein gradient in WT and (B) otd- embryos.

А					В				С					
	Bound by: Bcd S5	Other name*	S5 Expression	S6-8 Expression		Bound by: Otd S6-8	Late S5 Expression	S6-8 Expression	Bound by: Otd S5 + Otd S6-8	S5 Expression	S6-8 Expression	Bound by: Otd S5 + Otd S6-8	S5 Expression	S6-8 Expression
	Bcd ^{E1}	bl/Mir7_O-E		CD.	CLASS 3	Otd ^{L1}		-	Otd ^{EL1}	Ì	CIED	Otd ^{EL4}		639
	Bcd ^{E2}	HC_46		2		Otd ^{L2}			Otd ^{EL2}	\bigcirc	(ME)	Otd ^{EL5}		(14)
	Bcd ^{E3}	HC_57		20		Otd ^{L3}			Otd ^{EL3}		0	Otd ^{EL6}		Cho.
S 2	Bcd ^{E4}	HC_07				Otd ^{L4}		20						
CLASS 2	Bcd ^{E5}	HC_58	U	(TRSD)		Otd ^{L5}		20	Bound by: Otd S5	S5 Expression	S6-8 Expression	Bound by: Otd S5	S5 Expression	S6-8 Expression
	Bcd ^{E6}	HC_09		ME		Otd ^{L6}		1	Otd ^{E1}			Otd ^{E3}		OD
	Bcd ^{E7}	HC_52				Otd ^{L7}		20	Otd ^{E2}	0		Otd ^{E4}		CE S
	Bcd ^{E8}	HC_11	0	100		Otd ^{L8}		1						
	*as described in O	choa-Espinosa et al.	2008 and Chen et al	2012		Otd ^{L9}	0	23						

Supplemental Figure 5. *lacZ* expression patterns of Bcd and Otd bound enhancers. (A) Class 2 enhancers are bound by Bcd and expressed at both timepoints. (B) Class 3 enhancers are bound by Bcd and Otd and are expressed at both timepoints. (C) Otd-bound fragments that are not bound by Bcd do not show any expression.