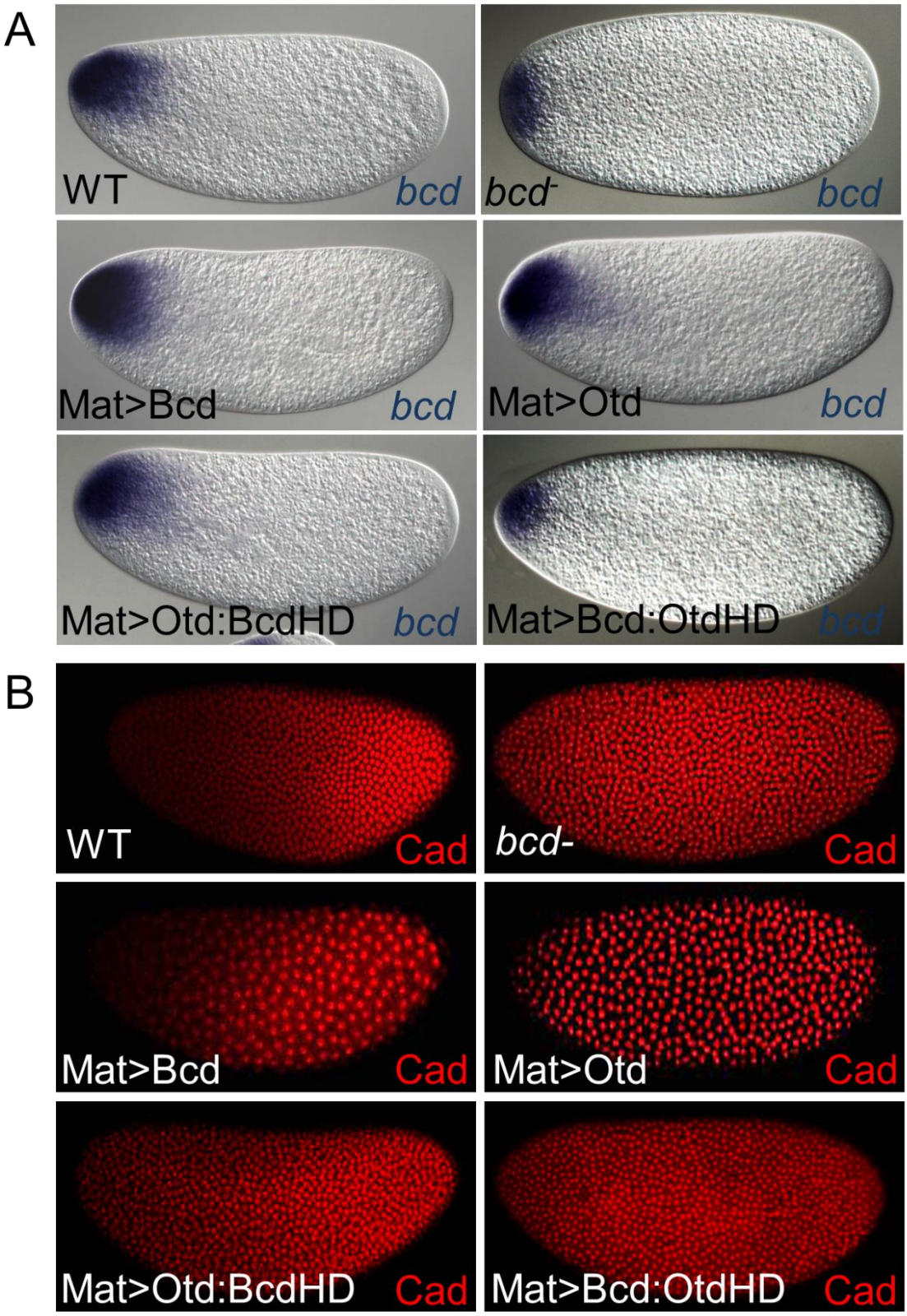
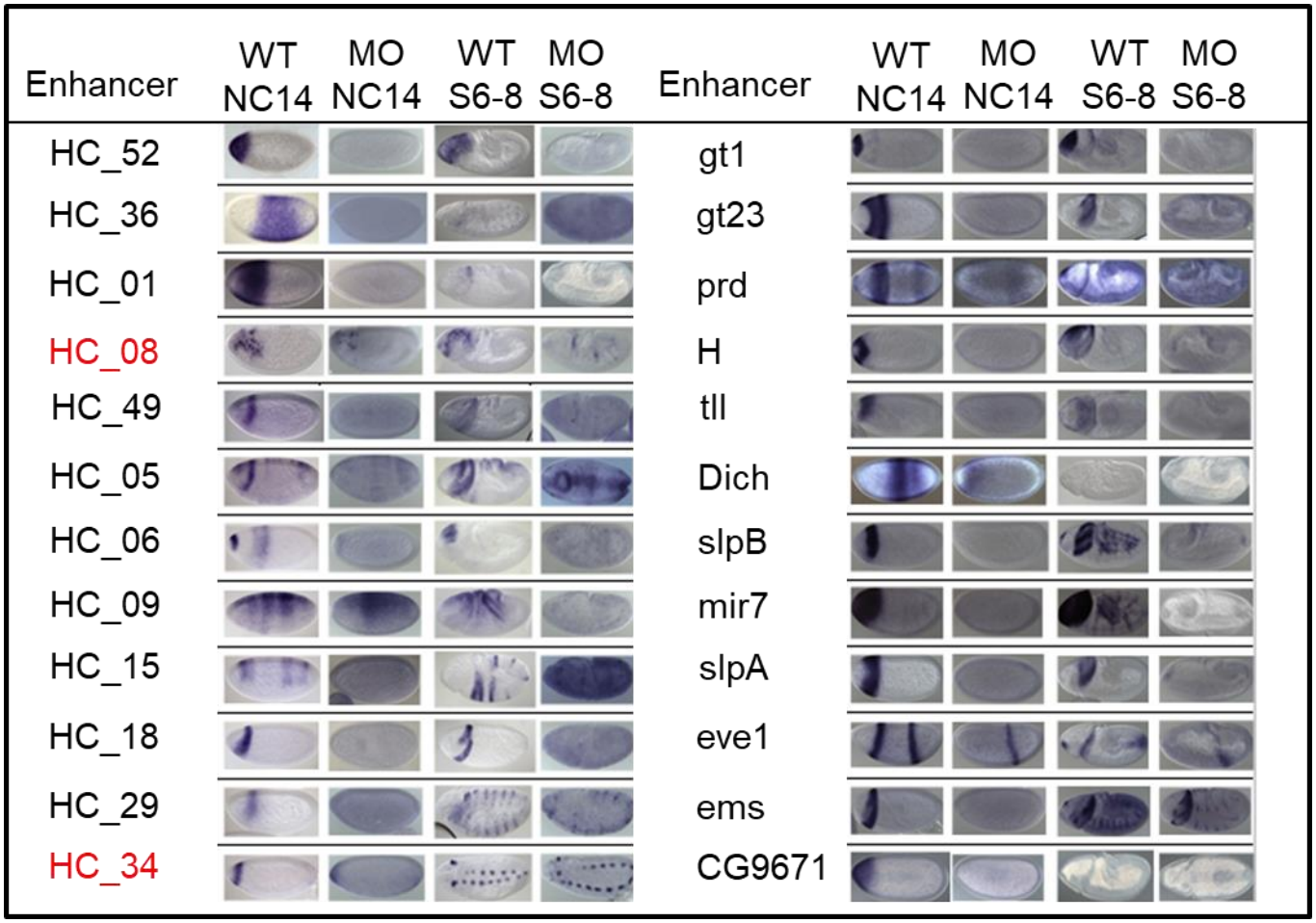
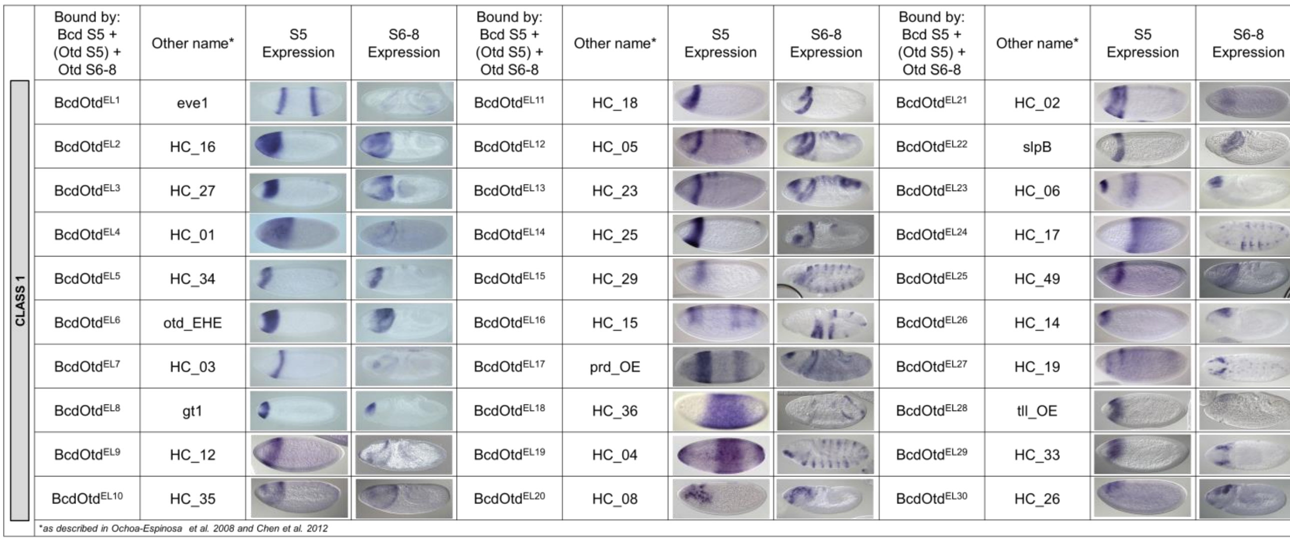


Supplemental Figure 1. (A) Maternal RNA expression. RNA is localized to the anterior tip for all constructs tested. Construct name and RNA detected are in the bottom left corner. (B) Expression of Cad in wild type, *bcd*$^{-}$, and embryos containing maternal transgenes. Genotypes are indicated in the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

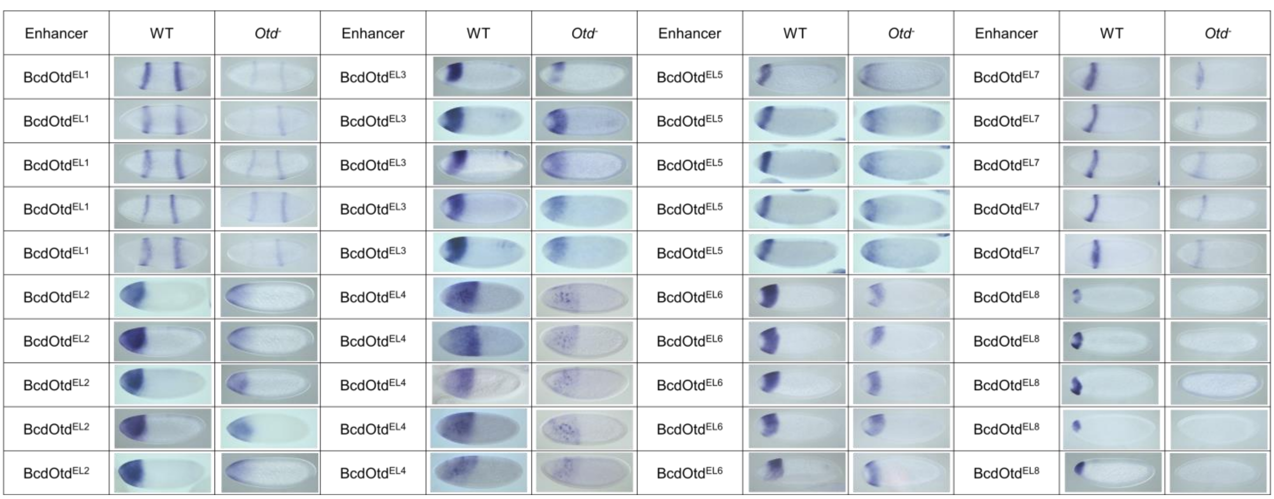


Bound by: Bcd S5 + (Otd S5) + Otd S6-8	Other name*	S5 Expression	S6-8 Expression	Bound by: Bcd S5 + (Otd S5) + Otd S6-8	Other name*	S5 Expression	S6-8 Expression	Bound by: Bcd S5 + (Otd S5) + Otd S6-8	Other name*	S5 Expression	S6-8 Expression
BcdOtd^{EL1}	eve1			BcdOtd^{EL11}	HC_18			BcdOtd^{EL21}	HC_02		
BcdOtd^{EL2}	HC_16			BcdOtd^{EL12}	HC_05			BcdOtd^{EL22}	slpB		
BcdOtd^{EL3}	HC_27			BcdOtd^{EL13}	HC_23			BcdOtd^{EL23}	HC_06		
BcdOtd^{EL4}	HC_01			BcdOtd^{EL14}	HC_25			BcdOtd^{EL24}	HC_17		
BcdOtd^{EL5}	HC_34			BcdOtd^{EL15}	HC_29			BcdOtd^{EL25}	HC_49		
BcdOtd^{EL6}	otd_EHE			BcdOtd^{EL16}	HC_15			BcdOtd^{EL26}	HC_14		
BcdOtd^{EL7}	HC_03			BcdOtd^{EL17}	prd_OE			BcdOtd^{EL27}	HC_19		
BcdOtd^{EL8}	gt1			BcdOtd^{EL18}	HC_36			BcdOtd^{EL28}	tll_OE		
BcdOtd^{EL9}	HC_12			BcdOtd^{EL19}	HC_04			BcdOtd^{EL29}	HC_33		
BcdOtd^{EL10}	HC_35			BcdOtd^{EL20}	HC_08			BcdOtd^{EL30}	HC_26		

CLASS 1

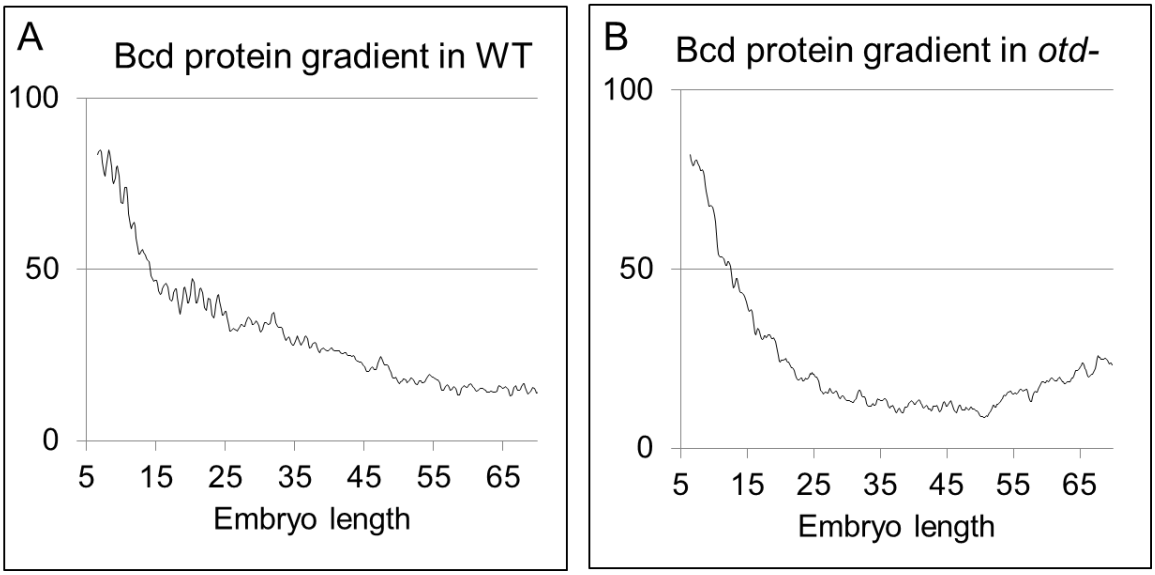
*as described in Ochoa-Espinosa et al. 2008 and Chen et al. 2012

B

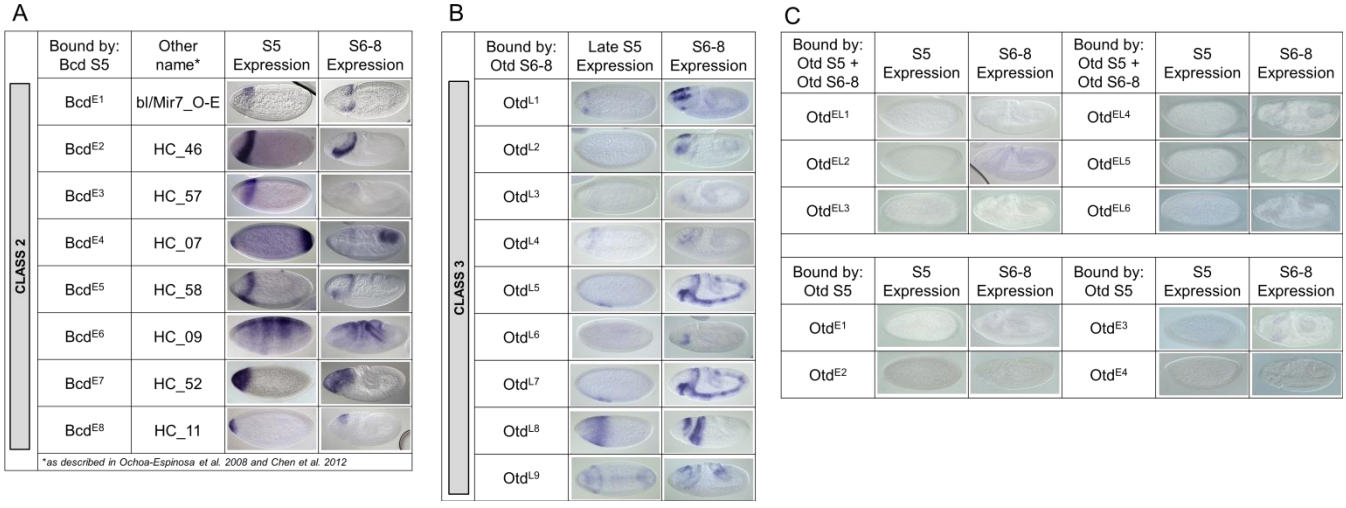


Enhancer	WT	*Otd*$^-$	Enhancer	WT	*Otd*$^-$	Enhancer	WT	*Otd*$^-$	Enhancer	WT	*Otd*$^-$
BcdOtd^{EL1}			BcdOtd^{EL3}			BcdOtd^{EL5}			BcdOtd^{EL7}		
BcdOtd^{EL1}			BcdOtd^{EL3}			BcdOtd^{EL5}			BcdOtd^{EL7}		
BcdOtd^{EL1}			BcdOtd^{EL3}			BcdOtd^{EL5}			BcdOtd^{EL7}		
BcdOtd^{EL1}			BcdOtd^{EL3}			BcdOtd^{EL5}			BcdOtd^{EL7}		
BcdOtd^{EL1}			BcdOtd^{EL3}			BcdOtd^{EL5}			BcdOtd^{EL7}		
BcdOtd^{EL2}			BcdOtd^{EL4}			BcdOtd^{EL6}			BcdOtd^{EL8}		
BcdOtd^{EL2}			BcdOtd^{EL4}			BcdOtd^{EL6}			BcdOtd^{EL8}		
BcdOtd^{EL2}			BcdOtd^{EL4}			BcdOtd^{EL6}			BcdOtd^{EL8}		
BcdOtd^{EL2}			BcdOtd^{EL4}			BcdOtd^{EL6}			BcdOtd^{EL8}		
BcdOtd^{EL2}			BcdOtd^{EL4}			BcdOtd^{EL6}			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**.**



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.