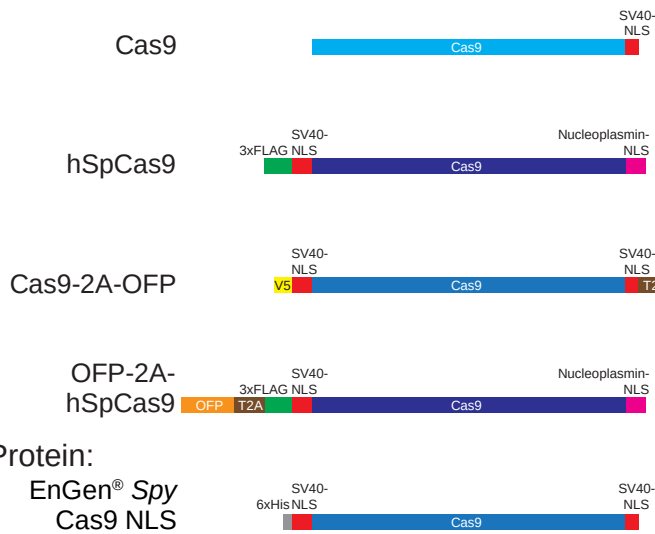


**S7.**

**Activity of distinct Cas9 RNAs and protein for *tyrosinase* gene knockout in *X. laevis* embryos**

**A**  
RNA:



Protein:

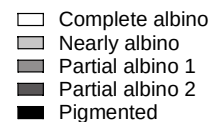
EnGen® *Spy*  
Cas9-NLS

Cas9 (Addgene:47322) and Cas9-2A-OFP (GeneArt:A21174) share 77% Cas9 nucleotide sequence conservation

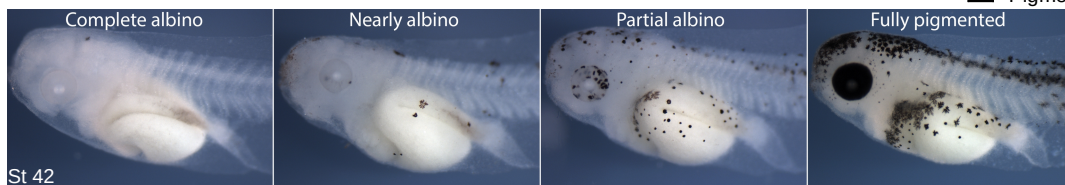
**B**

Source	Cas9 Nucleotide Sequence Conservation	<i>Tyrosinase</i> gene knockout efficiency										
Addgene: 47322	80%	<table border="1"> <tr><td>Complete albino</td><td>0.80%</td></tr> <tr><td>Nearly albino</td><td>5.60%</td></tr> <tr><td>Partial albino 1</td><td>69.60%</td></tr> <tr><td>Partial albino 2</td><td>24.00%</td></tr> <tr><td>Pigmented</td><td>0.00%</td></tr> </table> Total embryos=125	Complete albino	0.80%	Nearly albino	5.60%	Partial albino 1	69.60%	Partial albino 2	24.00%	Pigmented	0.00%
Complete albino	0.80%											
Nearly albino	5.60%											
Partial albino 1	69.60%											
Partial albino 2	24.00%											
Pigmented	0.00%											
Addgene: 51815	100%	<table border="1"> <tr><td>Complete albino</td><td>24.24%</td></tr> <tr><td>Nearly albino</td><td>22.51%</td></tr> <tr><td>Partial albino 1</td><td>43.94%</td></tr> <tr><td>Partial albino 2</td><td>3.25%</td></tr> <tr><td>Pigmented</td><td>6.06%</td></tr> </table> Total embryos=462	Complete albino	24.24%	Nearly albino	22.51%	Partial albino 1	43.94%	Partial albino 2	3.25%	Pigmented	6.06%
Complete albino	24.24%											
Nearly albino	22.51%											
Partial albino 1	43.94%											
Partial albino 2	3.25%											
Pigmented	6.06%											
GeneArt: A21174	86%	<table border="1"> <tr><td>Complete albino</td><td>6.28%</td></tr> <tr><td>Nearly albino</td><td>69.96%</td></tr> <tr><td>Partial albino 1</td><td>12.11%</td></tr> <tr><td>Partial albino 2</td><td>11.66%</td></tr> <tr><td>Pigmented</td><td>0.00%</td></tr> </table> Total embryos=223	Complete albino	6.28%	Nearly albino	69.96%	Partial albino 1	12.11%	Partial albino 2	11.66%	Pigmented	0.00%
Complete albino	6.28%											
Nearly albino	69.96%											
Partial albino 1	12.11%											
Partial albino 2	11.66%											
Pigmented	0.00%											
Produced in house	100%	<table border="1"> <tr><td>Complete albino</td><td>0.99%</td></tr> <tr><td>Nearly albino</td><td>6.95%</td></tr> <tr><td>Partial albino 1</td><td>71.52%</td></tr> <tr><td>Partial albino 2</td><td>14.57%</td></tr> <tr><td>Pigmented</td><td>5.96%</td></tr> </table> Total embryos=302	Complete albino	0.99%	Nearly albino	6.95%	Partial albino 1	71.52%	Partial albino 2	14.57%	Pigmented	5.96%
Complete albino	0.99%											
Nearly albino	6.95%											
Partial albino 1	71.52%											
Partial albino 2	14.57%											
Pigmented	5.96%											
NEB: M0646M	NA	<table border="1"> <tr><td>Complete albino</td><td>72.92%</td></tr> <tr><td>Nearly albino</td><td>8.33%</td></tr> <tr><td>Partial albino 1</td><td>17.71%</td></tr> <tr><td>Partial albino 2</td><td>1.04%</td></tr> <tr><td>Pigmented</td><td>0.00%</td></tr> </table> Total embryos=192	Complete albino	72.92%	Nearly albino	8.33%	Partial albino 1	17.71%	Partial albino 2	1.04%	Pigmented	0.00%
Complete albino	72.92%											
Nearly albino	8.33%											
Partial albino 1	17.71%											
Partial albino 2	1.04%											
Pigmented	0.00%											

**Key to albino severity**



**C** Example albino tadpoles



Class definitions are equivalent to Guo *et al* (2014) Development 141(3):707-14, figure 5O-R.

Complete albino: 100% albino.	No pigmented cells present in stage 42 tadpole.
Nearly albino: >90% albino.	No pigmented cells in the eyes (retinal pigment epithelium).
Partial albino 1: ~51-90% albino	Few pigmented cells (melanocytes) in the tail.
Partial albino 2: ~5-50% albino	Some pigmented cells in the eyes and tail.
Fully pigmented: 0% albino.	Mostly pigmented. Few bare patches visible within the eyes' pigment.
	Fully pigmented. No bare patches visible within the eyes' pigment.

The 'partial albino' definition encompasses a broad range of pigment-loss phenotypes and hence was divided into two groups.

**D** Timed series during a single injection experiment with Cas9 protein

