

Data presented in figures 2 and 6 to 9

Fig 2: Birthdating of horizontal cells

Expressed as % immunolabelled cells also positive for 3H-dT (analysed at st35)

Injection stage	Prox1	± s.d.	Lim1	± s.d.	Difference (=Isl1+ HC)	t-test p<0.05
19	34.8	8.1	29.9	3.9	4.9	n
20	48.4	6.7	40.5	4.3	7.9	n
21	58.8	11.5	44.2	3.3	14.6	y
22	57.2	9.5	33.8	4.7	23.4	y
23	52.9	8.5	21.0	4.3	31.9	y
24	50.9	5.5	15.4	3.8	35.5	y
25	41.7	6.6	9.3	2.8	32.4	y
26	43.2	3.8	8.6	3.0	34.6	y
27	27.9	6.4	5.0	3.8	22.9	y
28	26.6	3.3	6.3	2.8	20.3	y
29	14.9	3.9	6.2	2.0	8.7	y
30	11.2	2.2	4.0	2.1	7.2	y
31	7.0	2.3	2.5	2.6	4.5	y
32	2.6	3.0	0.8	1.0	1.8	n
33	1.6	2.0	0.4	0.8	1.2	n

Fig 6A: qRT-PCR

Stage	Relative values	
	Follistatin	FSTL1
st14	2689.24	3.10
st16	593.64	3.36
st18	246.28	3.85
st21	63.87	4.78
st23	22.77	4.30
st25	5.68	4.99
st28	1.00	4.54
st30	1.43	3.36
st35	7.45	1.82
st38	157.44	1.14
st42	145.83	1.00
st45	110.76	1.30

Fig. 6E: Lim1+ cells 22h after follistatin injection

Expressed as Lim1+cells/mm²

Stage	st21		st22		st23	
	Control	Follistatin	Control	Follistatin	Control	Follistatin
n animals	4	5	6	6	4	7
Median	245.0	192.4	274.0	192.4	305.6	236.3
p-value (MW)	0.0159		0.0022		0.0424	
Mean values ± s.d.						
Mean value	248.7	185.2	271.8	199.0	293.2	234.0
± pooled s.d.	36.9	24.2	33.4	27.1	40.6	21.9
Expressed as % of average mean value						
Mean value	100.0	74.5	100.0	73.2	100.0	79.8
± s.d.	6.9	6.0	4.4	11.8	14.1	8.8

Fig. 7C: BrdU+ cells 22h after activin injection at st23Expressed as BrdU+cells/mm²

Treatment	Control	Activin
n animals	4	5
Median	1443.9	366.2
p-value (MW)	0.0159	
Mean values ± s.d.		
Mean value	1468.8	420.9
± pooled s.d.	153.9	78.7

Fig. 8G-I: Number of horizontal cells at st35 after follistatin injection at E3

Expressed as cells/ 40x visual field

8G: Prox1						
Treatment	Control		Follistatin			
Stage	st21	st23	st20	st21	st22	st23
n animals	5	4	5	4	4	6
Median	53.5	58.9	71.1	70.6	71.4	75.5
Mann-Whitney p-value (vs. ctrl st21)			0.0079	0.0159	0.0159	0.0043
Mann-Whitney p-value (vs. ctrl st23)			0.0159	0.0286	0.0286	0.0095
Mean values ± s.d.						
Mean value	53.0	59.2	73.3	72.3	71.9	79.3
± pooled s.d.	4.6	6.3	7.7	6.9	8.0	9.9
Expressed as % of average mean value						
Mean value	100.0		131.5	129.5	128.9	142.2
± s.d.	6.6		12.2	14.3	4.9	16.0

8H: Lim1						
Treatment	Control		Follistatin			
Stage	st21	st23	st20	st21	st22	st23
n animals	5	4	5	4	4	6
Median	26.0	25.6	36.0	36.9	36.8	39.4
Mann-Whitney p-value (vs. ctrl st21)			0.0079	0.0159	0.0159	0.0043
Mann-Whitney p-value (vs. ctrl st23)			0.0159	0.0286	0.0286	0.0190
Mean values ± s.d.						
Mean value	26.1	26.7	35.0	36.6	36.4	38.3
± pooled s.d.	2.6	2.4	4.0	3.5	4.4	5.0
Expressed as % of average mean value						
Mean value	100.0		133.0	139.1	138.4	145.4
± s.d.	6.2		8.8	19.5	5.2	22.0

8I: Isl1						
Treatment	Control		Follistatin			
Stage	st21	st23	st20	st21	st22	st23
n animals	5	4	5	4	4	6
Median	25.8	29.6	40.3	37.3	43.8	44.4
Mann-Whitney p-value (vs. ctrl st21)			0.0079	0.0159	0.0159	0.0043
Mann-Whitney p-value (vs. ctrl st23)			0.0159	0.0286	0.0286	0.0095
Mean values ± s.d.						
Mean value	26.4	29.3	43.2	39.1	41.1	44.8
± pooled s.d.	2.6	4.0	6.2	4.7	5.6	7.6
Expressed as % of average mean value						
Mean value	100.0		156.0	141.2	148.4	161.8
± s.d.	7.2		33.9	19.3	11.5	28.8

Fig. 9G-I: Number of various cell types at st35 after follistatin injection at st23
Expressed as cells/ 40x visual field

9G: Photoreceptors				
Cell type	Isl1 PRs		Lim3 PRs	
Treatment	Control	Follistatin	Control	Follistatin
n animals	4	6	3	4
Median	96.6	99.0	115.3	108.0
p-value (MW)	0.914		0.400	
Mean values ± s.d.				
Mean value	97.9	104.2	111.4	105.2
± pooled s.d.	7.6	14.2	7.4	9.8

9H: Bipolar cells				
Cell type	Isl1 BPs		Lim3 BPs	
Treatment	Control	Follistatin	Control	Follistatin
n animals	4	6	3	4
Median	121.4	128.5	228.8	231.5
p-value (MW)	0.610		0.857	
Mean values ± s.d.				
Mean value	121.5	115.0	234.4	234.1
± pooled s.d.	11.8	15.9	14.9	21.8

9I: Ganglion cells and amacrine cells				
Cell type	Isl1 RGCs/ACs		Ap2α ACs	
Treatment	Control	Follistatin	Control	Follistatin
n animals	4	6	3	4
Median	109.6	119.4	242.0	244.5
p-value (MW)	0.257		0.629	
Mean values ± s.d.				
Mean value	107.6	117.6	238.9	245.5
± pooled s.d.	10.2	19.2	14.3	17.5

Note on "pooled s.d.-values":

The "± pooled s.d."- values were calculated by pooling the variances of all individuals adhering to a specific stage and treatment, using the standard formula:

$$PooledSD = \sqrt{\frac{((n_1 - 1)s_1^2 + (n_2 - 1)s_2^2 + \dots)}{((n_1 - 1) + (n_2 - 1) + \dots)}}$$

n= number of observations for each individual (eg. Individual 1= 4 observations, individual 2= 8 observations)
s= s.d. for those 4 or 8 observations, respectively

Thus, each s.d. value that belongs to an individual is weighed based on the number of observations that gave the value. (e.g. A s.d.-value based on 4 observations is given less weight than a s.d.-value based on 8 observations).

P-values were calculated in Prism using either the Student's t-test (Fig. 2), or the Mann-Whitney (Figs. 6-9, indicated by MW).